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Carol Robinson obtained her PhD in mass spectrometry from the University of Cambridge. She is currently a Royal Society Research Professor at the University of Oxford. Her research interests include all aspects of mass spectrometry applied to the elucidation of the3D architecture and structure of macromolecular complexes. Recent highlights from her work include the discovery that membrane protein complexes can be liberated from micelles in the gas phase while retaining their subunits interactions, lipid binding properties and overall topology.

Brian T. Chait is currently Camille and Henry Dreyfus Professor at Rockefeller University in New York, where he is Head of the Laboratory for Mass Spectrometry and Gaseous Ion Chemistry. He also directs the NIH-funded National Resource for the Mass Spectrometric Analysis of Biological Macromolecules. Dr. Chait's laboratory specializes in the development and use of mass spectrometry as a tool for investigating a variety of biological and biochemical phenomena. He has co-authored some 334 publications (cited >33,000 times) and has been awarded 23 US patents.

Speakers – please arrive ½ hour before your session begins to load your presentation.

THURSDAY, JANUARY 19

6:00 – 7:00 pm **Registration**, *Outside of the Tarpon/Sawyer Ballroom*

7:00 – 8:00 pm
Opening Session
Session Chair, Brandon T. Ruotolo, *University of Michigan*
Tarpon/Sawyer Ballroom

7:00 - 7:10 pm **Opening Remarks**
7:10 – 8:10 pm **Opening Lecture: Carol Robinson**, *University of Oxford*
Mass Spectrometry and its Role in Structural Biology

8:00 - 10:00 pm
Reception
setup all posters
Banyan Breezeway

FRIDAY, JANUARY 20

8:30 – 11:20 am
Structural Characterization by Ion Mobility
Session Chair: Matt Bush, *University of Washington*
Tarpon/Sawyer Ballroom

8:30 – 9:00 am **David Clemmer**, *Indiana University*
Conformations of Peptides and Proteins Emerging from Solution into the Gas Phase

9:00 – 9:30 am **Julie Leary**, *UC Davis*
Protein-Carbohydrate Interactions: Ion Mobility and RAPTOR Analysis for Isomeric Differentiation

9:30 - 9:50 am **Coffee Break,** *Banyan Breezeway*

9:50 – 10:20 am **Joseph Loo**, *UCLA*
Utility of Mass Spectrometry/Ion Mobility for Defining Protein-Ligand and Protein-Protein Interactions Important in Biology and Medicine

10:20 – 10:50 am **Albert Heck**, *Utrecht University*
Structural Characterization of Virus Assembly using Native Ion Mobility Mass Spectrometry

10:50 – 11:20 am **Brandon T. Ruotolo**, *University of Michigan*
Collision Induced Unfolding (CIU) Applied to Frontier Challenges in Structural Biology and Protein-Ligand Binding

11:20 – 11:40 am **Coffee Break,** *Banyan Breezeway*

FRIDAY, JANUARY 20

11:40 am - 12:40 pm
Structural Characterization by Ion Activation/Fragmentation I
Session Chair: Frank Sobott, *University of Antwerp*
Tarpon/Sawyer Ballroom

11:40 am - 12:10 pm **Justin Benesch**, *University of Oxford*
Determining the Likely Structures of Polydisperse Proteins

12:10 - 12:40 pm **Vicki Wysocki**, *University of Arizona*
SID/IM and IM/SID: Useful Approaches for Characterization of Non-Covalent Complexes

12:40 – 2:00 pm **Group Lunch,** *Garden Courtyard*

2:00 - 3:30 pm
Structural Characterization by Ion Activation/Fragmentation II
Session Chair: Frank Sobott, *University of Antwerp*
Tarpon/Sawyer Ballroom

2:00 - 2:30 pm **John Klassen**, *University of Alberta*
Structure and Stability of Protein-Ligand Complexes in the Gas Phase

2:30 - 3:00 pm **Nick Polfer**, *University of Florida*
Unscrambling the CID Fragmentation Chemistry of Peptides

3:00 - 3:30 pm **Evan Williams**, *UC Berkeley*
Supercharging Proteins from Native Solutions: Mechanisms and Applications

7:00 - 8:00 pm
Promoted Talks I
Session Chair: Justin Benesch, *University of Oxford*
Tarpon/Sawyer Ballroom

7:00 - 7:15 pm **Frank Sobott**, *University of Antwerp*
Poster 7 - What do Ion Mobility Measurements tell us about the Conformational Space of Proteins and Protein Complexes?

7:15 - 7:30 pm **A. E. Ashcroft**, *University of Leeds*
Poster 9 - Exploring Lactococcal Phage Baseplate Assembly

7:30 - 7:45 pm **Esther van Duijn**, *Utrecht University*
Poster 18 - Structural Characterization of CRISPR-Associated Immunity Complexes

7:45 - 8:00 pm **Owen Nadeau**, *University of Kansas Medical Center*
Poster 26 - Mass Spectrometry Reveals Different Subunit Interaction Maps for the Native and Phosphorylated Conformers of the (abgd)4 Phosphorylase Kinase Complex

8:00 - 10:00 pm
Poster Session I
posters 1 - 29
Banyan Breezeway

SATURDAY, JANUARY 21

8:30 - 10:30 am
Structural Characterization by Hydrogen/Deuterium Exchange
Session Chair: Thomas Wales, *Northeastern University*
Tarpon/Sawyer Ballroom

8:30 - 9:00 am	**John Engen**, *Northeastern University* Protein Flexibility and Plasticity
9:00 - 9:30 am	**Michael Fitzgerald**, *Duke University* Covalent Labeling Strategy for the Large Scale and High-Throughput Analysis of Protein-Ligand Binding
9:30 - 10:00 am	**Igor Kaltashov**, *University of Massachusetts* Protein Dynamics and Interactions: Using Ion-Electron Interactions to Enhance Quality and Resolution of Native ESI MS and HDX MS Measurements
10:00 - 10:30 am	**Alan Marshall**, *NHMFL/Florida State University* H/D Exchange for Mapping Contact Surfaces in Large Protein Complexes
10:30 - 11:00 am	**Coffee Break,** *Banyan Breezeway*

11:00 am - 12:30 pm
Labeling Strategies for Structural Characterization
Session Chair: Nick Polfer, *The University of Florida*
Tarpon/Sawyer Ballroom

11:00 - 11:30 am	**Lars Konermann**, *University of Western Ontario* Oxidative Labeling and HDX-MS As Complementary Tools for Probing Membrane Protein Structure and Function
11:30 - 12:00	**Mark Chance**, *Case Western Reserve University* Structural Mass Spectrometry: Dynamics of Membrane Proteins
12:00 - 12:30 pm	**Ryan Julian**, *UC Riverside* Examination of Surface Structure in Highly Homologous Proteins with SNAPP-MS
12:30 - 12:45 pm	**Alexander Leitner**, *ETH Zurich* *Poster 47* - Probing the Topology of Large Protein Complexes Using Chemical Cross-Linking and Mass Spectrometry
12:45 - 1:00 pm	**Gavin Reid**, *Michigan State University* *Poster 52* - Oxidation Induced Conformational Changes in Calcineurin Studied by 'Fixed Charge' Chemical Derivatization and Data Dependant Multistage Tandem Mass Spectrometry
1:00 - 7:00 pm	**Afternoon Free**

SATURDAY, JANUARY 21

7:00 - 8:00 pm
Promoted Talks II
Session Chair: Natalia Carulla, *Institut de Recerca Biomèdica (IRB)*
Horizons Ballroom

7:00 - 7:15 pm	**Kerry M. Hassell**, *Northeastern University* *Poster 37* - Solution-Phase Hydrogen Exchange Followed by Electron Transfer Dissociation for Analysis of Conformational Features of Proteins
7:15 - 7:30 pm	**Matthias P. Mayer**, *Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH)* *Poster 43* - Conformational Dynamics of Hsp90 Chaperones Analyzed by Hydrogen Exchange Mass Spectrometry
7:30 - 7:45 pm	**Romina Hofele**, *Max Planck Institute for Biophysical Chemistry* *Poster 51* - DEAH Box Helicase Prp43-Ntr1 Complex: Insights into Structure Combining Oxidative Footprinting with Protein-RNA Cross-Linking and Mass Spectrometry
7:45 - 8:00 pm	**John O'Brien**, *University of Texas at Austin* *Poster 57* - Evaluating Protein Solvent Accessibility via Electron Transfer Dissociation and 351 nm Ultraviolet Photodissociation Mass Spectrometry

8:00 - 10:00 pm
Poster Session II
posters 30 - 65
Banyan Breezeway
remove all posters by 10:30 pm

SUNDAY, JANUARY 22

9:00 - 10:00 am
Emerging Directions and Techniques
Session Chair: Esther van DuJin, *Utrect University*
Tarpon/Sawyer Ballroom

9:00 - 9:30 am	**David Russell**, *Texas A&M University* Enhanced Sampling MDS and IM-MS Studies of Environment-Dependent Conformer Preferences of Amphipathic Membrane-Active Peptides
9:30 - 10:00 am	**Scott McLuckey**, *Purdue University* Ion/Ion Reactions for Protein Cross-linking of Gaseous Polypeptide Ions
10:00 - 10:15 am	**Coffee Break,** *Lobby*
10:15 - 10:45 am	**Richard Smith**, *Pacific Northwest National Laboratory* Advances in Applications of Separations with Mass Spectrometry for the Broad Characterization of Complex Biological Systems
10:45 - 11:15 am	**John A. McLean**, *Vanderbilt University* Structurally-Based Discovery Strategies for Complex Biological Systems
11:15 - 11:30 am	**Coffee Break,** *Lobby*

11:30 am - 12:45 pm
Closing Session
Session Chair, John A. McLean, *Vanderbilt University*
Tarpon/Sawyer Ballroom

11:30 - 12:30 pm	**Closing Lecture: Brian Chait**, *Rockefeller University* On the Role of Mass Spectrometry in Structural Biology: The Present and Future
12:30 - 12:45 pm	**Closing Remarks**

Poster List

All posters are located in the Banyan Breezeway
posters 1 - 29 will be presented on Friday from 8:00 - 10:00 pm
posters 30 - 65 will be presented on Saturday from 8:00 - 10:00 pm
All posters should be set up by 7:00 pm on Thursday
Remove all posters by 10:30 pm on Saturday

Poster Topic
Structural Characterization by Ion Mobility

1 **Trapped Ion Mobility Spectrometry (TIMS) as a Tool for Gas Phase Ion Structure Studies;** Mark E. Ridgeway[1], Desmond Kaplan[1], Goekhan Baykut[2], Melvin Park[1]; *[1] Bruker Daltonics, 40 Manning Road, Billerica, MA and [2] Bruker Daltonik GmbH, Bremen, Germany*

2 **Study of Oligomeric Forms of the Alzheimer's Disease-Related Amyloid-β (Aβ) Protein;** Rosa Pujol-Pina,[1] Ernest Giralt,[1,2] Natàlia Carulla[1] ;*[1]Institute for Research in Biomedicine (IRB Barcelona), Barcelona, Spain. and [2]Department of Organic Chemistry, University of Barcelona, Barcelona, Spain.*

3 **Conformational Dynamics Study of Prolyl Oligopeptidase by Ion Mobility Mass Spectrometry;** Abraham López[1,2], Teresa Tarragó[1], Marta Vilaseca[3], Sergio Madurga[4], Ernest Giralt[1,2]; *[1] Institute for Research in Biomedicine (IRB-Barcelona). Baldiri Reixach, 10, 08028, Barcelon, [2] Department of Organic Chemistry, University of Barcelona (UB), [3] Mass Spectrometry Core Facility, IRB-Barcelona, [4] Department of Physical Chemistry, University of Barcelona (UB).*

4 **Characterization of Structural Changes of Metallothionein by Ion Mobility-Mass Spectrometry (IM-MS): Metal-Free vs. Metallated Forms;** Shu-Hua Chen and David H. Russell; *Department of Chemistry, Texas A&M University, College Station, TX*

5 **A Simple Damping Factor Links Periodic Focusing and Uniform Field Ion Mobility Measurements for Accurate Determination of Collision Cross Sections;** Joshua A. Silveira, Chaminda M. Gamage, Junho Jeon, Pei-Jing Pai, Kyle L. Fort, David H. Russell; *Texas A&M University, Department of Chemistry, Laboratory for Biological Mass Spectrometry, College Station, TX*

6 **Protein Interactions with Synthetic Polymers: Do Structural Changes Occur?;** Vincenzo Scionti and Chrys Wesdemiotis; *The University of Akron, Department of Chemistry, Akron, OH*

7 *Promoted poster 15-minute talk during the Friday Promoted Talks I session* - **What do Ion Mobility Measurements tell us about the Conformational Space of Proteins and Protein Complexes?;** Frank Sobott; *University of Antwerp, CFP-CeProMa and Chem. Dept., Antwerp, Belgium*

8 **Ion Mobility Mass Spectrometry of DNA/*Sgr*AI Nuclease Oligomers;** Xin Ma, Mowei Zhou, Chad K. Park, Santosh Shan, Vicki H. Wysocki and Nancy C. Horton; *Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ*

9 *Promoted poster 15-minute talk during the Friday Promoted Talks I session* - **Exploring Lactococcal Phage Baseplate Assembly;** D. A. Shepherd[1], D. Veesler[2], J. Lichière[2], C. Cambillau[2], A. E. Ashcroft[1]*, *[1] Astbury Centre for Structural Molecular Biology & Faculty of Biological Sciences, University of Leeds, Leeds, UK and [2] Architecture et Fonction des Macromolécules Biologiques, UMR 6098 CNRS & Universités Aix-Marseille I & II, Marseille, France*

Poster Topic
Structural Characterization by Ion Mobility

10 **A Tale of a Tail: Structural Insights into Ataxin-3 Gained by Ion Mobility Spectrometry - Mass Spectrometry;** Charlotte A. Scarff, Alessandro Sicorello, Sheena E. Radford, Alison E. Ashcroft; *The Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK*

11 **A Conformational Study of the Hepatitis B Core Antigen by Non-Covalent Mass Spectrometry and Ion Mobility Spectrometry- Mass Spectrometry;** Dale A. Shepherd, Kris K. Holmes, Nicola J. Stonehouse, Alison E. Ashcroft; *The Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK*

12 **Gas-phase Multiprotein Complexes: Preserving Native-like Structures via Optimized Buffer Conditions;** Linjie Han, Suk-Joon Hyung, and Brandon T. Ruotolo; *Department of Chemistry, University of Michigan, Ann Arbor*

13 **An Integrated Structural Biology Dataset Reveals Metal-Protein and Protein-Protein Interactions of Critical Importance for Amyloid Formation;** Suk-Joon Hyung[1]; Molly Soper[1]; Mi Hee Lim[1,2]; Brandon Ruotolo[1]; *Department of Chemistry[1] and Life Science Institute[2], University of Michigan, Ann Arbor, MI*

14 **Collision Induced Unfolding of Multi-Protein Ligand Complexes: Using Ion Mobility-Mass Spectrometry to Study the Conconavalin A –Sugar Binding System;** Shuai Niu, Suk-Joon Hyung, Brandon T. Ruotolo; *University of Michigan, Ann Arbor, MI*

15 **Developing Ion Mobility-Mass Spectrometry as a Novel High Throughput Screening Method for Protein Tyrosine Kinase Inhibitors**; Jessica Rabuck, Sukjoon Hyuang, Brandon Ruotolo; *Department of Chemistry, University of Michigan, Ann Arbor, MI*

16 **Ion Mobility-Mass Spectrometry Screening Reveals Neuropeptide Interactions with Amyloid b Peptides and their Oligomers;** Molly Soper[1], Suk-Joon Hyung[1], Mi Hee Lim[1,2], and Brandon T. Ruotolo[1]; *Department of Chemistry[1], Life Science Institute[2], University of Michigan*

17 **Development of Ion Mobility-Mass Spectrometry as a High-throughput Approach for Structural Proteomics;** Yueyang Zhong, Suk-Joon Hyung and Brandon Ruotolo; *Department of Chemistry, University of Michigan, Ann Arbor, MI*

18 *Promoted poster 15-minute talk during the Friday Promoted Talks I session* - **Structural Characterization of CRISPR-Associated Immunity Complexes;** Esther van Duijn[1,2#*], Ioana M. Barbu[1,2#], Arjan Barendregt[1,2], Matthijs M. Jore[3], Blake Wiedenheft[4], Magnus Lundgren[3,5], Edze. R. Westra[3], Stan J.J. Brouns[3], Jennifer A. Doudna[4], John van der Oost[3] and Albert J.R. Heck[1,2*]; *[1]Biomolecular Mass Spectrometry and Proteomics Group, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, University of Utrecht, Utrecht, The Netherlands, [2]Netherlands Proteomics Centre, Utrecht, The Netherlands, [3], Wageningen University, Wageningen, The Netherlands [4] Department of Molecular and Cell Biology, University of California, Berkeley, CA and [5] Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden*

19 **Characterization of Carbohydrates and Carbohydrate Natural Products by LC- Ion Mobility-Mass Spectrometry;** Nichole M. Lareau, Larissa S. Fenn, Cody R. Goodwin, Jody C. May, Brian O. Bachmann and John A. McLean; *Department of Chemistry, Vanderbilt Institute of Chemical Biology and Vanderbilt Institute for Integrative Biosystems Research and Education Vanderbilt University, Nashville TN*

Poster Topic
Structural Characterization by Ion Mobility

20 **Using Distance Geometry with Ion Mobility-Mass Spectrometry Data to Study the Conformational Space of Natural Products;** Sarah M. Stow,[1,2,3,4] Cody R. Goodwin,[1,2,4] Michal Kliman,[1,2,4] Brian O. Bachmann,[1,2] John A. McLean[1,2,4]; *1. Department of Chemistry at Vanderbilt University, 2. Vanderbilt Institute of Chemical Biology, 3. Center for Structural Biology, 4. Vanderbilt Institute for Integrative Biosystems Research and Education,Vanderbilt University, Nashville, TN*

Poster Topic
Structural Characterization by Ion Activation/Fragmentation

21 **Characterization of New Fatty Acid from *Maytenus* Speices using Fragmentaion Pattern and Mass Behaviour;** Mohamed F. AlAjm*, PhD.[1] and Maged Saad Abdel-Kader[2], *[1]Prince Sultan Prince College for emergency medical services, King Saud University, Riyadh, Saudi Arabi and [2]College of Pharmacy, Prince Salman University, Kharj, Saudi Arabia*

22 **Density Functional Theoretical Studies of Charge-Remote Fragmentations: Toward the Development of Predictive Reactions Mechanisms for Steroids Mass Spectrometry;** Yassin A. Jeilani[1] and Victor M. Ibeanusi[2]; *[1]Department of Chemistry, Spelman College, Atlanta, GA and [2]Environmental Science and Studies Program, Spelman College, Atlanta, GA*

23 **Modeling Gas-Phase Anion-Molecule Complexes of 1,3,5-Trinitroperhydro-1,3,5-Triazine (RDX);** Domnique Newallo, Nripendra K Bose, Albert Thompson, Yassin Jeilani; *Spelman College*

24 **Effect of Charge State on Gas-phase Dissociation Behavior of Non-Covalent Protein Complexes Examined by Ion Mobility-Mass Spectrometry;** Mowei Zhou, Royston Quintyn, Shai Dagan, Vicki Wysocki; *University of Arizona, Tucson, AZ*

25 **IRMPD Spectroscopy of b_n (n=4-12) Fragment Ions "How Large Can Macrocycles Become?";** Marcus Tirado,[a] Josipa Grzetic,[b] Jos Oomens,[b] and Nick C. Polfer [a]; *[a]Department of Chemistry, University of Florida, Gainesville, FL and [b]FOM Institute "Rijnhuizen", Nieuwegein, The Netherlands*

26 *Promoted poster 15-minute talk during the Friday Promoted Talks I session* - **Mass Spectrometry Reveals Different Subunit Interaction Maps for the Native and Phosphorylated Conformers of the $(\alpha\beta\gamma\delta)_4$ Phosphorylase Kinase Complex;** Owen W. Nadeau[1], Laura A. Lane[2,3], Jessica Sage[1], Gerald M. Carlson[1] and Carol V. Robinson[4]; *[1]Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS , .A., [2]Department of Chemistry, University of Cambridge, Cambridge, U.K., [3]Department of Chemistry, Chemistry Research Laboratory, Oxford, U.K. and [4]Department of Chemistry, the Physical and Theoretical Chemistry Laboratory, University of Oxford, Oxford, U.K.*

27 **Twenty-Seven Years of Neutral Gas Collisions of Carbohydrate Samples; Where are We Now?;** Vern Reinhold, David Ashline, Andy Hanneman, and Hailong Zhang; *The Glycomics Center, University of New Hampshire, Durham, NH*

28 **Comparison of Peptoid Fragmentation under CID and IRMPD Conditions;** Jianhua Ren*, Kiran Morishetti and Bogdan Bogdanov; *University of the Pacific, Stockton, CA*

Poster Topic
Structural Characterization by Ion Activation/Fragmentation

29 **Evidence for Hydrogen/Deuterium Scrambling in Electron Capture Dissociation – Fourier Transform Ion Cyclotron Resonance Mass Spectrometry;** Teerapat Rojsajjakul, Thomas P. Neis, and Fred L. King*, *C. Eugene Bennett Department of Chemistry, West Virginia University, Morgantown, WV*

Poster Topic
Structural Characterization by Hydrogen/Deuterium Exchange

30 **Structural Evidence for Cooperative Microtubule Stabilization by Taxol and the Endogenous Dynamics Regulator MAP4;** Hui Xiao[§], Hui Wang[¶], Xuechun Zhang[¶], Zongcai Tu[¶], Chloë Bulinski[‡], Marina Khrapunovich-Baine[#], Ruth Hogue Angeletti[€], Susan Band Horwitz[#,], *[§]Department of Pathology, [#]Department of Molecular Pharmacology, [€]Laboratory of Macromolecular Analysis and Proteomoics, Albert Einstein College of Medicine, Bronx, NY, [¶]State Key Laboratory of Food Science & Technology, Nanchang University, China and [‡]Department of Biological Sciences, Columbia University, New York, NY*

31 **Impact of Site-Specific Oxidation on the Higher-Order Structure of Interferon β-1a;** George M. Bou-Assaf, Marek Kloczewiak, Damian Houde, and Steven A. Berkowitz; *Biogen Idec, Inc., Cambridge, MA*

32 **Fragment Antibody Binding to a Highly Conserved Influenza Epitope - an HDX Study;** Joomi Ahn*, Eveline Sneekes-Vriese[¶], Otto Diefenbach[¶], Ying Qing Yu*, Alex Muck*, St John Skilton*, Els C.M. Brinkman van der Linden[¶], Robert H. Friesen[¶] and Adrian C. Apetri[¶,&], * *Waters Corporation, Milford, MA, [¶] Crucell BV, Archimedesweg 4, Leiden, 2333CN, The Netherlands and [&] To whom correspondence should be addressed*

33 **Identification of Residues that Mediate the Assembly of COPII Cages by H/D Exchange Coupled with FT-ICR MS;** Qian Zhang[2], Scott M. Stagg[2,3], Christopher L. Hendrickson[1,2] and Alan G. Marshall[1,2]; [1] *Ion Cyclotron Resonance Program, National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL,* [2] *Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL and* [3] *Department of Biological Science and Institute of Molecular Biophysics, Florida State University, Tallahassee, FL*

34 **High Resolution Hydrogen/Deuterium Exchange Mass Spectrometry Gives Insight to the Mechanism of Action of Hepatitis B Virus Capsid Assembly Effectors;** Navid Movahed [1], Dewey Brooke [1], Adam Zlotnick [2], and Brian Bothner[1]; *1 Dept. Chem. Biochem., Montana State University, Bozeman, MT ; 2 Dept. Biochem., Indiana University, Bloomington, IN*

35 **Changes in a Monoclonal Antibody upon Dimerization as Revealed by HX MS;** Roxana E. Iacob[1], Damian Houde[2], Steven Berkowitz[2] and John R. Engen[1]; *[1]Department of Chemistry & Chemical Biology, Northeastern University, Boston, MA, [2]Biogen Idec, Inc., Cambridge, MA*

36 **Conformational Locking upon Cooperative Assembly of Notch Transcription Complexes;** Sung Hee Choi[1], Thomas E. Wales[2], Stephen C. Blacklow[1,3], and John R. Engen[2]; *[1]Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA; [2]Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA; [3]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA*

Poster Topic
Structural Characterization by Hydrogen/Deuterium Exchange

37 *Promoted poster 15-minute talk during the Saturday Promoted Talks III session* - **Solution-Phase Hydrogen Exchange Followed by Electron Transfer Dissociation for Analysis of Conformational Features of Proteins;** Kerry M. Hassell[1], William B. Smith[1], Sean Marcisin[1], Elaine M. Marzluff[1], Jeffery M. Brown[2], Michael Morris[2], Jay Bradner[3], John R. Engen[1]*; *[1] Department of Chemistry and Chemical Biology and The Barnett Institute of Chemical and Biological Analysis, Northeastern University, Boston, MA, [2] Waters MS Technologies Centre, Micromass UK Ltd., Wythenshawe, Manchester, UK and [3] Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA*

38 **Structural Insight into the Mechanism of Regulation of *Neisseria meningitidis* NadA Expression by the Small Natural Ligand 4-HPA;** Sébastien Brier[1], Luca Fagnocchi[1,2], Danilo Donnarumma[1,2], Maria Scarselli[1], Rino Rappuoli[1], Mikkel Nissum[1], Isabel Delany[1] and Nathalie Norais[1]; *[1]Novartis Vaccines, Microbial Molecular Biology, Via Fiorentina 1, 53100 Siena, Italy and [2]Recipient of a Novartis fellowship from the PhD program in Functional Biology of Molecular and Cellular Systems of the University of Bologna.*

39 **Beyond Structure Characterization: Structure Dynamics (Hydrogen Deuterium Exchange) Guided Biocatalyst Improvement**; Ugur Uzuner[1], Weibing Shi[1], Quentin Johnson[2], Hong Guo[2], Joshua S. Yuan[1] and Susie Y. Dai[3]; *[1] Department of Plant Pathology and Microbiology, [2] University of Tennessee Knoxville, TN and Oak Ridge National Laboratory, Oak Ridge, TN,[3] Office of the Texas State Chemist, Department of Veterinary Pathobiology, Texas A&M University, College Station, TX*

40 **Identification of Regions of Rabbit Muscle Pyruvate Kinase Important for Allosteric Regulation by Phenylalanine, Detected by H/D Exchange Mass Spectrometry;** Charulata B. Prasannan, Maria T. Villar , Antonio Artigues, and Aron W. Fenton; *[a]Department of Biochemistry and Molecular Biology, The University of Kansas Medical Center, Kansas City, KS*

41 **Evidence for an Allosteric Auto-Inhibitory Phosphorylation Mechanism Regulating the Endosomal Localization of MTMR2 using Hydrogen/Deuterium Exchange Mass Spectrometry;** Christopher A. Bonham and Panayiotis O. Vacratsis; *Dept. of Chemistry and Biochemistry, University of Windsor, Windsor ON, Canada*

42 **Use of Ion Mobility and Fragment Ions to Increase Coverage in Hydrogen-Deuterium Exchange;** [1]Martha Stapels, [2]Thomas Wales, [1]Keith Fadgen, [1]Michael Eggertson, and [2]John Engen; *Waters Corporation[1] and Northeastern University[2]*

43 *Promoted poster 15-minute talk during the Saturday Promoted Talks III session* - **Conformational Dynamics of Hsp90 Chaperones analyzed by Hydrogen Exchange Mass Spectrometry**; Chung-Tien Lee, Christian Graf, Franz-Josef Mayer, Minh Nguyen and Matthias P. Mayer; *Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), Heidelberg, Germany*

Poster Topic
Labeling Strategies for Structural Characterization

44 **Characterization of Proinsulin and Diabetes Mellitus-Associated Insulin Mutants by Hydroxyl-radical Footprinting and Molecular Modeling;** Janna G. Kiselar[1], Manish Datt[1], Nelson Phillips[3], Mark R. Chance[1, 2] and Michael A. Weiss[3].; *[1]Center for Proteomics and Bioinformatics, [2]Center for Synchrotron Biosciences, [3]Department of Biochemistry Case Western Reserve University, Cleveland, OH*

45 **A Mass Spectrometry-based Carboxyl Footprinting Enrichment Strategy for Detection of Low Abundant Derivitized Peptides;** Erik J. Soderblom, J. Will Thompson and M. Arthur Moseley; *Proteomics Core Facility, Institute for Genome Science & Policy, Duke Univ School of Medicine, Durham, NC*

46 **Protein Fluorescence in the Gas Phase: the Green Fluorescent Protein and Protein-Dye Conjugates for Probing the Structure of Gaseous Protein Ions;** Konstantin Barylyuk, Vladimir Frankevich, Pavel Sagulenko, Robert Steinhoff, and Renato Zenobi; *Department of Chemistry and Applied Biosciences, ETH Zurich, Switzerland*

47 *Promoted poster 15-minute talk during the Saturday Promoted Talks II session* - **Probing the Topology of Large Protein Complexes using Chemical Cross-Linking and Mass Spectrometry;** Alexander Leitner, Franz Herzog, Thomas Walzthoeni, Ruedi Aebersold; *Institute of Molecular Systems Biology, ETH Zurich, 8093 Zurich, Switzerland*

48 **Detection of Non-covalent Interactions of Single Stranded DNA with Escherichia Coli Single-Stranded DNA-Binding Protein by MALDI-MS;** Fan Chen, Stefanie Mädler, Simon Weidmann, Renato Zenobi; *Department of Chemistry and Applied Biosciences, ETH Zürich, Zürich, Switzerland*

49 **Concatenated Protein Oligomers for High-Mass MALDI-MS Calibration;** Simon Weidmann, Konstantin Barylyuk, Stefanie Mädler, Renato Zenobi; *Department of Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland*

50 **Observing the Binding Site and Dynamics of Protein S1 on a Bacterial Ribosome using Cross-Linking and Mass Spectrometry;** Matthew A. Lauber[1], Juri Rappsilber[2], and James P. Reilly[1]; *[1]Department of Chemistry, Indiana University, Bloomington, IN, [2] Wellcome Trust Centre for Cell Biology, Institute of Cell Biology, The University of Edinburgh, Edinburgh, UK*

51 *Promoted poster 15-minute talk during the Saturday Promoted Talks III session* **- DEAH Box Helicase Prp43-Ntr1 Complex: Insights into Structure Combining Oxidative Footprinting with Protein-RNA Cross-Linking and Mass Spectrometry;** Romina Hofele[1], Henning Christian[2], Ralf Ficner[2], Henning Urlaub[1,3]; *[1]Max Planck Institute for Biophysical Chemistry, Göttingen, Germany; [2]Institute for Microbiology and Genetics, Georg August Universität Göttingen, Germany; [3]University Medical Center Göttingen*

52 *Promoted poster 15-minute talk during the Saturday Promoted Talks II session* - **Oxidation Induced Conformational Changes in Calcineurin Studied by 'Fixed Charge' Chemical Derivatization and Data Dependant Multistage Tandem Mass Spectrometry;** Gavin E. Reid; *Department of Chemistry, Department of Biochemistry, Michigan State University, East Lansing, MI*

53 **Studying Structures and Conformation Dynamics of Peptides and Proteins Using Host-Guest Chemistry in the Gas Phase**; Hugh I. Kim; *Department of Chemistry, Pohang University of Science and Technology (POSTECH), Pohang, S. Korea*

Poster Topic
Labeling Strategies for Structural Characterization

54 **Understanding the Protein-Protein Interactions of the Human Fe-S Cluster Complex using Hydroxyl Radical Footprinting;** Michaella J. Levy, Pei-Jing Pai, David H. Russell, David P. Barondeau; *Texas A&M University*

55 **A Computational Data Analysis Platform for Identifying Zero-Length Chemical Crosslinks using Tandem Mass Spectrometry;** Sira Sriswasdi[1,2], Sandra Harper[1], Hsin-Yao Tang[1], and David W. Speicher[1,2]; *[1]The Wistar Institute, Philadelphia, PA, [2]Genomics and Computational Biology Graduate Group, University of Pennsylvania, Philadelphia, PA*

56 **Xlink-Identifier 2.0: Identifying Modified Cross-linked Peptides using Tandem Mass Spectrometry and Parallel Computing;** *Wenchao Zhang,[†] Kyle Suttlemyre,[†] Peter Pham,[†] ,James Rorie,[†] Xiuxia Du[†*], [†] Department of Bioinformatics & Genomics, University of North Carolina at Charlotte, NC*

57 *Promoted poster 15-minute talk during the Saturday Promoted Talks III session* - **Evaluating Protein Solvent Accessibility via Electron Transfer Dissociation and 351 nm Ultraviolet Photodissociation Mass Spectrometry;** John O'Brien, Jennifer Brodbelt; *Department of Chemistry and Biochemistry, University of Texas at Austin*

Poster Topic
Emerging Directions and Techniques

58 **Does the Whole Influence the Parts in an Fc Fusion Protein?;** Damian Houde and Steven A. Berkowitz; *Biogen Idec, Inc., Cambridge, MA*

59 **Strong Metal Ions Bind Peptides in the Iminol Conformation;** Robert C. Dunbar,[†*] Jeffrey D. Steill,[‡^] Nicolas C. Polfer,[#] Giel Berden[‡] and Jos Oomens[‡§]; *†Chemistry Department, Case Western Reserve University, Cleveland, OH, #Chemistry Department, University of Florida, Gainesville, FL, ‡FOM-Institute for Plasma Physics Rijnhuizen, NL-3439 MN Nieuwegein, The Netherlands, §University of Amsterdam, Science Park 904, 1098XH Amsterdam, The Netherlands*

60 **In Situ Enrichment of Phosphopeptides on MALDI Plates Functionalized by Ambient Ion Landing;** Petr Pompach[1], Lukas Krásný[1,2], Marcela Strnadova[1], Petr Novak[1], Martin Strohalm[1], Vladimir Havlicek[1], Frantisek Tureček[3], Karel Lemr[4], Oldrich Benada[1], and Michael Volný[1]; *[1]Institute of Microbiology AS CR, v.v.i., Prague, Czech Republic, [2]Institute of Chemical Technology, Prague, Czech Republic, [3]Department of Chemistry, University of Washington, Seattle, WA and [4]Department of Analytical Chemistry, Palacky University, Olomouc, Czech Republic*

61 **Characterizing Self-Assembled Extracellular Matrix Fibrillar Films with TOF-SIMS;** Christopher R. Anderton, Kiran Bhadriraju, Frank W. DelRio, and Anne L. Plant ; *[a]National Institute of Standards and Technology, Gaithersburg, MD*

62 **PCA of TOF-SIMS Data to Elucidate Lipid Distribution Within Biological Membranes;** Christopher R. Anderton,[a,b]* Bita Vaezian,[a] Kaiyan Lou,[a] and Mary L. Kraft[a] ; *[a]School of Chemical Sciences, University of Illinois at Urbana-Champaign, Urbana, IL and [b]Current institution: National Institute of Standards and Technology, Gaithersburg, MD*

63 **Custom QIT Mass Spectrometer for Infrared Photodissociation Spectroscopy of Biomolecular Ions;** Corey N. Stedwell,[†] Kerim Gulyuz,[†] Nick C. Polfer[†]; [†]*Department of Chemistry, University of Florida, Gainesville, FL*

Poster Topic
Emerging Directions and Techniques

64 **Vibrational Spectroscopy of b_2 Fragment Ions from Peptides in the Hydrogen Stretching Region;** Kerim Gulyuz; Corey N. Stedwell; Da Wang; Nick C. Polfer; *University of Florida, Gainesville, FL*

65 **Analysis of Human SLOS Fibroblast Cholesterol and 7-Dehydrocholesterol via Silver Sputtering Laser Desorption Ionization – Ion Mobility – Mass Spectrometry and Computational Modeling;** Michal Kliman, Libin Xu, Sarah M. Stow, Ned A. Porter, John A. McLean; *Department of Chemistry, Vanderbilt University, Nashville, TN*

Poster 1

Trapped Ion Mobility Spectrometry (TIMS) as a Tool for Gas Phase Ion Structure Studies

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Trapped ion mobility spectrometry (TIMS) is a new technology which offers reduced instrument size, decreased operating voltage and pressure when compared to conventional ion mobility spectrometry (IMS) while providing similar analytical capabilities, time scales, and resolution. Fundamentally, TIMS differs from conventional IMS in that ions are held stationary in a moving gas rather than being forced by an electric field through a stationary gas. In TIMS, ions are radially confined in a quadrupolar ion funnel by RF voltages while axially trapped by the balance of the frictional force in a gas flow and the electric force generated by a DC electric field that varies as a function of position within the TIMS device. Reducing the DC electric field strength over time allows trapped ions to elute in order of mobility from low to high mobility. With the use of calibrants, K_0 can be determined from the elution potential and compared directly with results from conventional IMS systems. Previously, this analyzer has demonstrated an ability to separate biological conformations with a resolving power exceeding 100 for some analytes[1]. A second TIMS device has been constructed and installed into a prototype bench top quadrupole-orthogonal time of flight mass analyzer which provides improvements in both mobility and mass resolution relative to the previous generation TIMS.

To demonstrate the potential of a prototype TIMS for analysis of biological gas phase ion structures, unfolding of ubiquitin ions generated by electrospray from a solution composed of water (49.9% in vol.), methanol (49.9% in vol.) and acetic acid (0.2% in vol) was monitored over the time range 10 ms to 1 s. Results agree well with previously published observation of unfolding of ions in the gas phase generated from similar solvents conditions using conventional IMS for low charge states[2], but also suggest structural changes for high charge states as well which have not previously been reported. In addition reduced mobilities measured in nitrogen gas using TIMS correlate well when compared to literature data acquired with traditional IMS drift tubes using helium gas[3] (Figure 1).

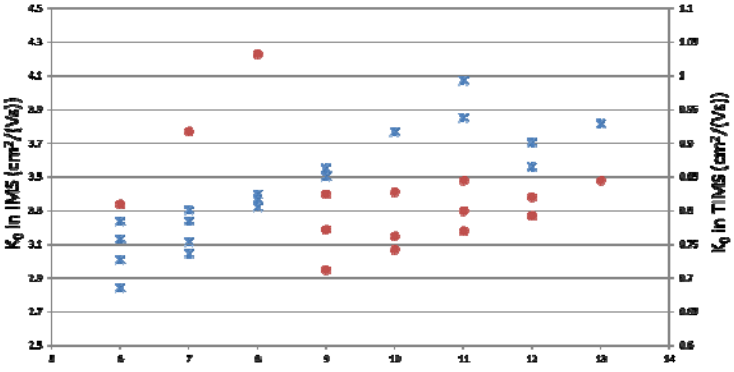

Figure 1 Ubiquitin reduced mobilities acquired with conventional IMS using helium drift gas (red circles)[2] and TIMS using nitrogen drift gas (blue asterisks).

References:

1. Fernandez-Lima, F.; Kaplan, D. A.; Suetering, J.; Park, M. A.; *Int. J. Ion Mobility Spectrom.* **2011**, *14*, 93-98

2. Myung, S.; Badman, E.; Lee, Y. J.; Clemmer, D. E. *J. Phys. Chem. A* **2002**, *106*, 9976-9982.

3. Wyttenbach, T.; Bowers, M. T. *J. Phys. Chem. B*, **2011**, *115*, 12266–12275

Poster 2

Study of Oligomeric Forms of the Alzheimer's Disease-Related Amyloid-β (Aβ) Protein

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Soluble amyloid-β protein (Aβ aggregates, known as Aβ oligomers, are now considered the pathogenic molecular form of Aβ in Alzheimer's disease (AD). The strong association of Aβ oligomers with AD demands for studies to elucidate the structure of Aβ oligomers. The low physiological concentration of tissue-derived Aβ oligomers has prevented researchers from obtaining the large quantities of highly pure homogeneous samples required for *in vitro* structural studies. Regarding synthetic Aβ oligomer forms, their dynamic and metastable nature has also complicated their structural study. Photo-induced cross-linking of unmodified proteins (PICUP) is an established chemical reaction that is able to provide snapshots of dynamic oligomeric mixtures[1]. Moreover, the size of the oligomers formed using PICUP is in the range of those extracted from AD human[2] and transgenic mice[3] brain samples.

In this study, we use ion mobility mass spectrometry (IM-MS) to determine the oligomer distributions as well as the cross-section of each detected oligomer for low molecular weight (LMW) and PICUP cross-linked Aβ40 and Aβ42 samples, of 40 and 42 residues, respectively. LMW Aβ preparations are the earliest Aβ aggregation state that can be studied. Furthermore, we have compared these results to those obtained by analysis of the same samples by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions and by size-exclusion chromatography (SEC) under native conditions. The results of this investigation do not reveal differences in the type of early oligomers sampled for Aβ40 and Aβ42 while report on differences in the size of Aβ40 and Aβ42 oligomers. Moreover, they suggest caution in deriving conclusions from oligomer distributions obtained by SDS-PAGE.

1 Bitan, G. & Teplow, D. B. Rapid photochemical cross-linking--a new tool for studies of metastable, amyloidogenic protein assemblies. *Acc Chem Res* **37**, 357-364, (2004).

2 Shankar, G. M. *et al.* Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* **14**, 837-842, (2008).

3 Lesne, S. *et al.* A specific amyloid-beta protein assembly in the brain impairs memory. *Nature* **440**, 352-357, (2006).

Poster 3

Conformational Dynamics Study of Prolyl Oligopeptidase by Ion Mobility Mass Spectrometry

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Prolyl Oligopeptidase (POP, 80 KDa) is a serine peptidase that cleaves post-proline bonds in small peptides. Structurally, this enzyme is formed by a cylindrical □-propeller structural domain and a catalytic □/□ hydrolase domain. The linker region between them is flexible and allows an equilibrium between an open and a closed conformers. This equilibrium probably drives the substrate/products circulation and the enzymatic mechanism. In addition, it might modulate the POP protein-protein interactions. POP inhibitors are suggested to stabilize the closed form, consequently altering the catalytic activity and POP interactomics. The elucidation of the inhibition mechanism is an area of great interest since POP is pointed as a therapeutic target for the treatment of cognitive dysfunctions.

Ion Mobility Mass Spectrometry (IM/MS) is an emerging technique in the study of biomacromolecules. Ions obtained under soft conditions could be separated according not only to their mass to charge ratio, but also by their collision cross section (CCS) to charge ratio. The objective of the present study is to observe this conformational equilibrium by IM/MS.

Specific charged POP ion species were separated by IM under controlled conditions, yielding up to three groups of conformers. The more compact conformer would correspond with the closed form, and the intermediate group with the open form. Assignations were done by converting experimental drift time data CCS using native standard protein ions of known CCS for calibration, and results were compared with the theoretical CCS values of the X-ray closed form (1H2W) and the X-ray opened form (3IUJ). The more extended group of conformers corresponded with partially unfolded forms, as it was confirmed by forcing collision induced unfolding.

The equilibrium in solution was reflected in the early injected ions. The conformational population was highly dependent on the trapping time, since ions rapidly undergo to a compaction of the tertiary structure more stable in the gas phase. Another marked effect over the populations was observed if a covalent POP inhibitor was present. As expected, the population of the opened forms decreased.

In conclusion, it was possible to separate different POP conformers by IM. CCSs of the conformers were in agreement with the theoretical calculations derived from the crystal structures. The behavior of the conformational population suggested that the transient equilibrium between an open and a closed form in solution was transferred to the gas phase. Future experiments with different inhibitors and interacting proteins will confirm this hypothesis.

Poster 4

Characterization of Structural Changes of Metallothionein by Ion Mobility-Mass Spectrometry (IM-MS): Metal-Free vs. Metallated Forms

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Metal ions play important roles in many chemical and biochemical processes, such as oxidation, oxygen transport, and electron transfer, and almost half of the known enzymes require a specific metal to function. Metallothionein (MT) is one of the metalloenzymes that controls cellular trafficking of a large number of metal ions. MTs are a family of low molecular weight (6–7 kDa), cysteine-rich (~30%) proteins with unusual metal-reactivity. X-ray crystallography and NMR studies of fully cadmium-metallated human MT-2A revealed two distinct metal-binding clusters: the N-terminal β domain $Cd_3(Cys)_9$ (residues 1-30) and the C-terminal α domain $Cd_4(Cys)_{11}$ (residue 31-61), with metals tetrahedrally coordinated to the cysteinyl thiols. Although the structure of Cd_7MT was published 30 years ago, the structures of metal-free and partially-metallated MTs are difficult to determine and the metal-binding mechanism remain largely unknown.

ESI-MS has considerable potential for studies of how metal binding influences structure of human MT-2A. Here, we used ESI-MS to isolate partially metallated MT-2A species, Cd_iMT (i = 1 – 7), and then used tandem MS and IM-MS and to determine metal ion binding sites and structural diversity of each species. IM-MS arrival time distributions (ATD) clearly show that the structure of the Cd_iMT^{5+} ion changes as the numbers of metal ions increase, and in some cases multiple conformers are observed. This data is consistent with metal-dependent folding, *i.e.,* increasing numbers of metal ions yield more compact ion structures. Although a single component ATD is observed for the 5+ ion of apoMT (1050 Å), the ATD of the metallated intermediates is broadened as the number of Cd^{2+} ion increased, which suggests a high degree of conformer diversity among the ion population. Very different ATDs are observed for Cd_4MT, *i.e.,* two distinct ATDs (CCS of 980 $Å^2$ and then 890 $Å^2$) are present, whereas Cd_7MT has two distinct populations at 750 $Å^2$ and 890 $Å^2$. These studies illustrate the potential utility of IM-MS for understanding the influence of metal ion binding on conformer preferences for this biologically important class of protein. We are currently using molecular dynamics simulations (MDS) to gain additional insight into the 3-D conformations of the various metallated protein species.

Poster 5

A Simple Damping Factor Links Periodic Focusing and Uniform Field Ion Mobility Measurements for Accurate Determination of Collision Cross Sections

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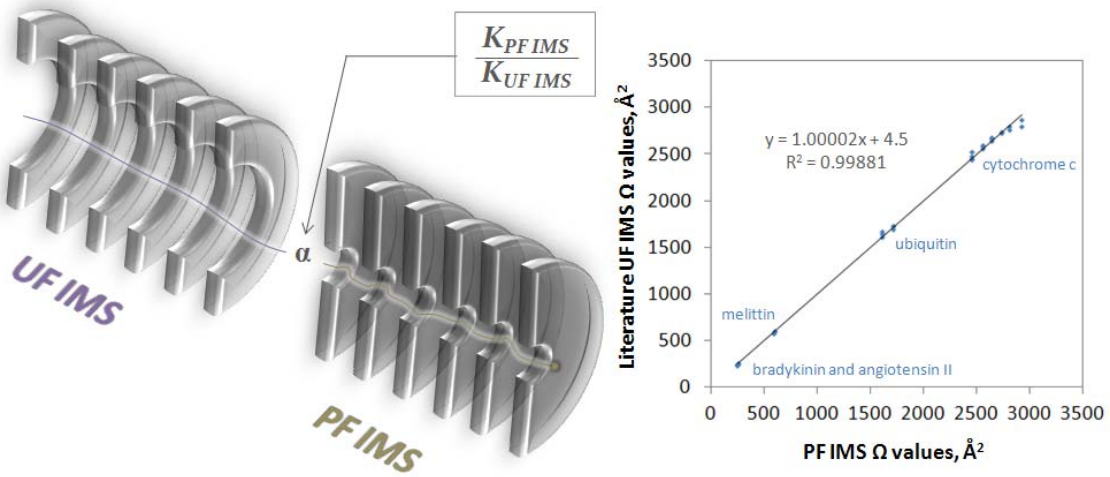
Ion mobility-mass spectrometry has recently emerged as a complementary platform for empirically studying the molecular dimensions of peptides, proteins, and their complexes. While traveling wave ion mobility spectrometry (TW IMS, Synapt G1 and G2) has developed into a robust and sensitive biophysical tool with an expanding user-base, TW IMS requires complex electronic design for the applied dynamic voltages that limit the overall drift length and subsequent IMS resolving power (typically R ~40). Moreover, determination of collision cross section (Ω) values by TW IMS deviates from the first-order uniform field IMS (UF IMS) principles thereby demanding careful calibration methods because Ω is exponentially proportional to TW IMS drift time.

Alternatively, we present the methodology for obtaining accurate Ω values for peptides and proteins using periodic focusing ion mobility spectrometry (PF IMS), which has previously demonstrated high ion transmission and $R > 100$ without the use of RF voltages. In this work, a mobility damping factor (represented by the term α) is introduced to account for the relative increase in ion-neutral collisions in PF IMS compared to UF IMS for equivalent operating conditions. The results show that α may be easily quantified both theoretically and empirically for a specific PF IMS design operating at a given pressure based upon the charge state of the analyte. By simply incorporating an $\alpha(z)$ term into traditional UF IMS expressions, accurate Ω values were obtained with excellent agreement (≤ 4% difference) compared to all available UF IMS measurements found in the current literature. The results presented herein suggest that charge state-dependence is not only an important consideration for PF IMS drift tubes, but also for calibration of TW IMS and other ion mobility devices that utilize radial focusing strategies.



Poster 6

Protein Interactions with Synthetic Polymers: Do Structural Changes Occur?

Vincenzo Scionti and Chrys Wesdemiotis
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A significant number of synthetic polymers and materials are engineered for various biomedical applications, including the manufacture of stents, implants, drug delivery systems, and corrective devices [1]. In these functions, the synthetic products come in contact with biological fluids, where they can develop binding interactions with biomolecules. Of particular interest is non-covalent attachment of proteins to the inserted material, which may be beneficial (for example, by promoting cell growth for wound repair) or detrimental (for example, by triggering adverse immune system response or by interfering with enzyme function) [2]. It has remained largely unknown whether proteins interacting in vivo with polymers or polymer surfaces undergo structural changes. To address this emergent issue, we probed, in vitro, the conformational and aggregation changes occurring in small proteins that interact non-covalently with synthetic polymers.

The polymer system investigated initially are polyhedral oligomeric silsesquioxane (POSS) nanoparticles, which are either used or being tested in a number of biomedical areas, such as drug delivery, dental composites, biosensors, and biomedical devices [3]. The effect of tri-silanol phenyl-POSS (tri-POSS) on physiological solutions of insulin and cytochrome C was examined by electrospray ionization mass spectrometry (ESI-MS), tandem mass spectrometry (MS^2) fragmentation, and ion mobility mass spectrometry (IM-MS) using a Waters Synapt HDMS quadrupole time-of-flight mass spectrometer. Aqueous solutions of the proteins (20 pmol/mL) were analyzed, mixed with POSS solutions (20 pmol/mL) in the ratio 2:1 (v/v), and reanalyzed after a short (~10 min) incubation period.

The ESI mass spectrum of pure insulin shows monomer ions in charge states +3 to +6 as well as dimer ions in charge states +5 to +7. The monomer and dimer species are clearly discernible in IM-MS plots. After incubation with tri-POSS, the dimer disappears, while the distribution and relative intensities of the monomeric charge states are not affected significantly. At the same time, complexes of insulin and up to three tri-POSS particles are detected in charge states +3 to +5. MS^2 spectra confirm the adducts' non-covalent nature; moreover, insulin released from the complexes shows the same ion mobility as native insulin. Overall, these results provide strong evidence that association equilibria of the protein with the oligomeric nanoparticle disrupt the weak interactions within the insulin dimer, but do not change markedly the conformation of the strongly bound insulin monomer. Similar experiments with cytochrome C show that this protein is not denatured by interactions with tri-POSS. These studies are currently extended to larger proteins, known to denature easily or to exist in distinct conformations, in order to ascertain the structural determinants that make protein folding and tertiary structure sensitive to encounters with polymeric materials that can form intermolecular hydrogen bonds.

1. Mahapatro, A.; Kulshrestha, A.S. (Eds.). Polymers for biomedical applications; American Chemical Society: Washington, DC, 2008.
2. Mahmoudi, M.; Lynch, I.; Ejtehadi, M.R.; Monopoli, M.P.; Baldelli Bombelli, F.; Laurent, S. Chem. Rev. 2011, 111, 5610–5637.
3. Ghanbari, H.; de Mel, A.; Seifalian, A.M. Int. J. Nanomedicine 2011, 6, 775-786.

Poster 7

What do Ion Mobility Measurements tell us about the Conformational Space of Proteins and Protein Complexes?

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While established techniques in structural biology such as x-ray crystallography can reveal biomolecular structure in unsurpassed detail, they only in part represent the different conformational and association states of a protein. Other methods such as NMR are either not well suited to large, dynamic structures, or they lack the detail necessary to resolve individual species in heterogeneous equilibria (e.g. EM).

Native MS as a 'single molecule technique' can cope well with such heterogeneity, and in combination with ion mobility spectrometry different conformational states can now also be resolved reasonably well. But what does a measured collision cross section really tell us about the conformational space of a protein or the subunit arrangement of a complex? How important is the absence of bulk solvent, and how much do the measured size and the 'flexibility' depend on the internal energy of the ions?

We are using native nano-ESI MS coupled with T-wave ion mobility spectrometry (Waters Synapt G1 and G2) to assess the structure and conformational space of a couple of well understood proteins and complexes, and correlate this data with results from other biophysical methods such as size-exclusion chromatography, surface plasmon resonance and small-angle x-ray and neutron scattering (SAXS and SANS). In side-by-side comparison of closely related protein sequences (e.g. with and without mutations, truncations or post-translational modifications; fig. 1), such subtle changes are frequently reflected in the position and width of the drift time profile (i.e. 'size' and 'flexibility' of the ion).

The binding of a ligand or counter-ions however can cause a considerable compaction of the gas-phase structure, as will be demonstrated with an example. For multiprotein complexes it is an often reported phenomenon that a systematic variation of instrumental parameters such as voltage offsets leads to an intial gas-phase collapse of hollow structures such as in the hexameric ring discussed here (fig. 2) before further increase of the internal energy leads to extended, but still hexameric conformations of the complex. Taken together these data highlight the importance of controlling the internal energy of ions carefully in the experiment, and raise some critical points regarding the measurement of absolute (rather than relative) sizes by IMS.

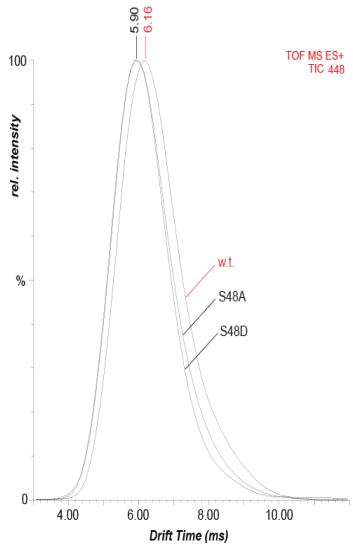

Fig. 1: Drift time profile ofα-parvin (involved in cell adhesion to the extracellular matrix) and mutants
(Collab. M. Noble, Newcastle Univ.)

Fig. 2: Mobilograms and drift time profiles of hexameric E.coli Hfq protein
(an snRNA chaperone) at different ion internal energies (A to C), showing first the gas-phase collapse and then sequential unfolding of the hexamer.
(all Data Synapt G1; collab. with A. Callaghan, Portsmouth Univ., U.K.)

Poster 8

Ion Mobility Mass Spectrometry of DNA/*Sgr*AI Nuclease Oligomers

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*Sgr*AI is a type II F restriction endonuclease that cuts an unusually long recognition sequence and exhibits self-modulation of DNA activity and sequence specificity. Previous research has shown that the oligomers of DNA-bound *Sgr*AI (i.e., high molecule weigh substance or HMWS) are the active form of the enzyme. The structure, composition and the basic block of the HMWS are not clear. To figure out the information, ion mobility mass spectrometry (IM-MS) was employed to analyze *Sgr*AI/DNA compelxes. The results showed that the basic block of HWMS is the DNA-bound *Sgr*AI dimer. The HWMS are a heterogeneous mixture of species with different number of DNA bound *Sgr*AI dimer and the number can be as large as 19. The collision cross section (CCS) of the HMWS has a linear relationship with the number of DNA bound *Sgr*AI dimer. Some models of HMWS with different orientations of *Sgr*AI dimer were built and their CCSs were calculated to compare with the experimental data. The result suggested a head to tail orientation is the most possible structure of HMWS.

Poster 9

Exploring Lactococcal Phage Baseplate Assembly

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Two siphophages, p2 and TP901-1, have been shown to infect different strains of the gram+ lactic acid bacterium *Lactococcus lactis*. They possess a large multi-protein organelle (1-2 MDa) at their distal tail end, termed the baseplate, which is responsible for specific host recognition, attachment and initiation of infection. However, despite X-ray crystallography and/or electron microscopy of the final baseplate, the pathways leading to these large complexes remain ill-defined. Here we have used ESI-(IMS)-MS to study the mass and stoichiometry of the intact, non-covalently bound p2 and TP901-1 baseplate complexes, as well as several significant structural sub-complexes generated by a "block cloning" strategy[1,2].

Baseplate complexes and sub-complexes were dialysed against 200 mM aqueous ammonium acetate (pH 6.8) prior to analysis. NanoESI-(IMS)-MS data were acquired on a Synapt HDMS instrument (Micromass UK Ltd/Waters). For ESI-IMS-MS, a ramped travelling wave height of 8-25 V was used for complexes >1 MDa, and a fixed wave height of 10 V for complexes <1 MDa. The wave velocity was 250 ms^{-1} in both cases.

The masses of the intact phage baseplates p2 and TP901-1, and a range of associated sub-complexes, were determined within an error of ≤0.2 %. Thus, the stoichiometry of the intact baseplates was confirmed and the identities of the multi-protein sub-complexes unambiguously assigned (e.g. Figure 1). The characterisation of these sub-complexes has provided valuable insights into the assembly of the organelles and we have been able to propose plausible baseplate assembly pathways for the p2 and TP901-1 lactococcal phages.

The collision cross-sectional areas measured by ESI-IMS-MS were compared with solution-phase dynamic light scattering data to support the notion that the structure of a protein complex can be maintained in the gas-phase. Together the data illustrate the value of ESI-(IMS)-MS for studying heterogeneous, megaDalton, macromolecular baseplate complexes.

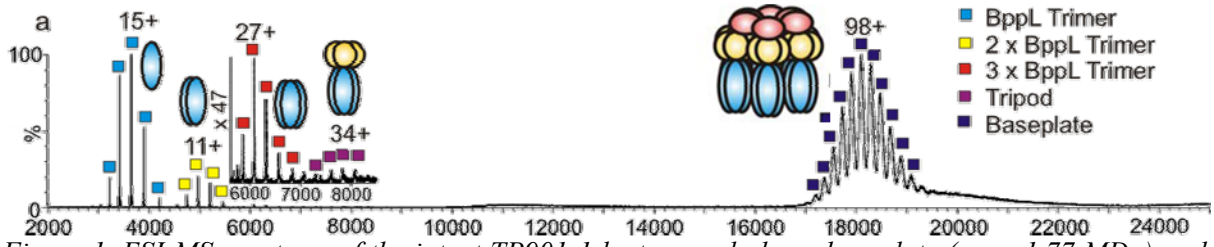

Figure 1. ESI-MS spectrum of the intact TP901-1 lactococcal phage baseplate (mass 1.77 MDa) and associated sub-complexes.

References.

1. Unraveling lactococcal phages baseplates assembly through mass spectrometry, D. A. Shepherd, D. Veesler, J. Lichière, A. E. Ashcroft, C. Cambillau, *Molecular & Cellular Proteomics*, 10, M111.009787 (2011).

2. Solution and electron microscopy characterization of lactococcal phage baseplates expressed in *Escherichia coli*, V. Campanacci, D. Veesler, J. Lichiere, S. Blangy, G. Sciara, S. Moineau, D. van Sinderen, P. Bron, C. Cambillau, *J. Struct. Biol.,* 172, 75-84 (2010).

Poster 10

A Tale of a Tail: Structural Insights into Ataxin-3 Gained by Ion Mobility Spectrometry - Mass Spectrometry

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Ataxin-3 is the protein responsible for the neurodegenerative polyQ disease Spinocerebellar ataxia type 3 or Machado-Joseph disease. PolyQ diseases result from expansions of polyglutamine stretches in their causative proteins, which induce protein aggregation and the formation of neuronal intranuclear inclusions. These in turn cause cell death. Protein misfolding and aggregation are thought to be key in the pathogenesis of these disorders and so structural characterisation of the causative proteins is sought.

Ataxin-3 is a 42 kDa protein that contains an N-terminal Josephin domain (JD) followed by two ubiqutin interacting motifs (UIMs), a polyQ stretch and a variable C-terminal region. Whilst the structure of the JD has been characterised in detail, little is known about the conformational properties of the C-terminal domain, which is loosely termed “the tail”.
Studies of ataxin-3 fibril formation have shown previously that protein aggregation initially involves Josephin self-association.

Here, ion mobility spectrometry-mass spectrometry and limited proteolysis studies were used to provide insights into the structure and dynamics of the tail of ataxin-3 and the JD. Oligomeric species formed in the initial stages of fibril formation were also probed.
Our results suggest that the tail is extremely dynamic and does not interact significantly with the JD. Limited proteolysis studies suggest that the core of the JD is very stable but that the α2/α3 hairpin is flexible, in agreement with previous NMR experiments. Proteolysis studies of the dimer indicate that the self-association of ataxin-3 monomers into dimers is likely mediated by the JD. These results demonstrate how mass spectrometry can be a powerful technique in the characterisation of proteins with large structurally-disordered regions.

Poster 11

A Conformational Study of the Hepatitis B Core Antigen by Non-Covalent Mass Spectrometry and Ion Mobility Spectrometry- Mass Spectrometry

Dale A. Shepherd, Kris K. Holmes, Nicola J. Stonehouse, Alison E. Ashcroft
The Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK LS2 9JT

Hepatitis B Virus is a major cause of liver disease and can lead to liver cancer. It chronically infects 400 million people worldwide. The infectious virion consists of a partially double-stranded DNA genome, a protein capsid shell, and a protein and lipid envelope. The capsid protein (HBcAg) has been shown to self-assemble *in vitro* at high ionic strength to form particles identical to those observed in the blood of infected individuals, and has been studied extensively as a model system for virus capsid assembly.

In this work we aimed to study the capsid protein building block in order to identify structural transitions that might be important for capsid assembly.

Ion mobility spectrometry - mass spectrometry (IMS-MS) can be used to study protein conformations in the gas phase, by observing charge state distributions and by measuring collision cross-sections (Ω). However, it is still not clear if the conformations seen in the gas phase are functional and representative of the protein's fold in solution.

The ESI mass spectrum of HBcAg displayed two charge state distributions, indicative of two structural families of the protein in solution, each with different degrees of solvent accessible surface area. These conformers can be separated by IMS-MS and their collision cross-sections (Ω) were estimated. The Ω of the lowest charge state is consistent with the theoretical Ω calculated from its X-ray structure, suggesting the lower charged conformers adopt a native-like structure in the gas phase. Methods used to try to elucidate the structures of the less compact conformer include limited proteolysis of flexible regions, gas-phase unfolding and probing with assembly affectors.

A Synapt HDMS mass spectrometer equipped with a Travelling Wave Ion Mobility Spectrometry (TWIMS) device (Waters UK Ltd., Manchester, UK) was used to measure the mass and collision cross-sections of the HBcAg protein under various conditions.

Poster 12

Gas-phase Multiprotein Complexes: Preserving Native-like Structures via Optimized Buffer Conditions

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Ion mobility-mass spectrometry (IM-MS) is a powerful tool for structural biology with the ability to simultaneously assess the structure, topology, dynamics, and composition of large heterogeneous multiprotein complexes at low micromolar concentrations. However, one of the challenges in using IM-MS is that the structures of protein complexes in the gas phase can differ from those in solution as a consequence of ionization, desolvation, transport and anlalysis over a range of time scale and energies. Thus, a general means must be identified to stabilize protein structures in the absence of bulk solvent. Our approach to this problem involves the protection of protein complex structure through the addition of salts in solution prior to ionization/desolvation. We have screened a series of Hofmeister-type cations and anions for their ability to increase the structural stability of multiprotein complexes in the absence of bulk solvent. By assessing the collision induced dissociation (CID) and collision induced unfolding (CIU) profiles of several multiprotein systems in the gas phase upon addition of cations and anions respectively on the Synapt G2 IM-MS platform (Waters Corp. Milford MA, USA), the stability afforded to the multiprotein complexes by ions was determined quantitatively, leading to the mechanistic understanding of stabilization. Our current data shows that cations and anions stabilize gas-phase protein structures through different mechanisms. For example, cations tend to tightly bind protein complexes and act to reduce Coulombic unfolding. In stark contrast to cations, anion-protein complexes exhibit primarily a 'dissociative cooling' type mechanism characterized by the dissociation of protein-bound anions upon collisional activation. Despite these differences, both cations and anions feature ion-protein binding as an important factor in protein structural stabilization in the absence of bulk solvent. Further, we have used our data to rank anions and cations with respect to their ability to stabilize gas-phase protein structure, and this rank order is substantially different from the known Hofmeister salt series in solution. While this is an expected outcome of our work, due to the diminished influence of ion and protein solvation by water, our data correlates well with expected ion binding in solution and highlights the fact that both hydration layer and ion-protein binding effects are critical for Hofmeister-type stabilization. This presentation will present our most recent data and mechanistic insights surrounding protein ion structure stabilization, as measured by IM-MS.

Poster 13

An Integrated Structural Biology Dataset Reveals Metal-Protein and Protein-Protein Interactions of Critical Importance for Amyloid Formation

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Amyloidosis is a pathogenic form of protein aggregation associated with a number of diseases including Alzheimer's and Parkinson's disease. While studies using conventional technologies have yielded important structural insights and some understanding of the molecular processes underlying aggregation, the application of these insights to amyloidogenesis at the cellular level has been less successful, suggesting a discrepancy between our molecular-level understanding and amyloidosis *in vivo*. Our approach involves the integration of ion mobility-mass spectrometry (IM-MS) and other chemical biology-derived datasets in an attempt to assess the complete interaction network involved in amyloid formation. In this study, we focus on the interactions between Amyloid Beta (Aβ) with metals and proteins.

Ions are generated from an aqueous solution buffered at pH 7. The IM-MS instrumentation consists of a nanoESI source, a quadrupole mass analyzer, travelling-wave (T-wave) drift cells and a time-of-flight mass analyzer. Ion mobility separation is carried out utilizing a T-wave drift cell, which replaces a standard RF ion guide collision cell (Synapt G2, Waters, Milford, MA). Ions are pulsed into the device and separated based on their ability to traverse the drift cell maintained at 1-3 mBar with N2, under the influence of a series of low-voltage "waves". Collision cross-sections are externally calibrated using protein ions of known ion mobility. Data analysis and model generation is carried out using DriftscopeTM (Waters) and Mobcal (developed by Jarrold and co-workers) software packages.

We have begun work in this field by focusing on amyloid β polypeptides (Aβ, Alzheimers disease). Aβ typically exists in two forms: Aβ40 and Aβ42, which differ by the addition of two amino acids to the C-terminus. Aβ42 is significantly more toxic than Aβ40, is the primary component of Alzheimer's amyloid plaques, and forms larger oligomeric species than Aβ40. When the course of aggregation of Aβ40 was monitored by IM-MS, the result shows insignificant changes in the arrival time distributions (ATDs) over time, indicating that no new oligomers are forming over the course of the experiment. The result was also independently verified by observing a lack of interaction between Aβ and Thioflavin T during the experiment. In contrast, the polyphenol (-)epigallocatechin gallate binds directly to Aβ40, and IM results indicate that the molecule binds preferentially to compact forms of the peptide only. The complex was shown to bind further with copper (II), which enhances the amyloidogenic property of Aβ40. A survey of binding interaction between neuropeptides and Aβ40 revealed an interaction with a small opioid peptide neurotransmitter which resulted in a complex at 1:1 stoichiometry, with sub-micromolar affinity.

Poster 14

Collision Induced Unfolding of Multi-Protein Ligand Complexes: Using Ion Mobility-Mass Spectrometry to Study the Conconavalin A –Sugar Binding System

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Currently, mass spectrometry (MS) based assays play a vital role within the drug development pipeline as both a method of screening potential ligands for protein binding and assessing relative or, in some cases, the absolute binding strength of a protein-ligand complex. However, obtaining final confirmation of ligand binding and structurally characterizing the newly-formed complex still remains a significant challenge. This situation frames a critical technology gap in the development phase of pharmaceuticals, where many potential drug candidates are screened by time-consuming or inefficient analytical methods leading to long lead compound development times and, eventually, cost inefficient pharmaceutical products. In this presentation, we develop ion mobility-mass spectrometry (IM-MS) and collision induced unfolding (CIU), an IM-MS methodology that allows us to track the gas-phase unfolding and stability of various protein-ligand complexes, as a means of providing a screening step for protein-ligand complexes prior to high-resolution structural biology tools (e.g., NMR or X-ray).

Following on from preliminary data published for the TTR-thyroxine binding system which demonstrated that unfolding and dissociation energetics can be uncoupled and used as independent metrics for protein-ligand stability[1], we have initiated proof of principle experiments employing the Conconavalin A (ConA) system. ConA is a manosyl sugar-binding tetrameric protein complex, with one sugar binding site per protein. While the biological unit of the complex is a tetramer, the assembly has an established pH-dependant equilibrium with a dimeric form. We have screened the binding of various manosyl sugars by ConA using ESI-MS to both the dimeric and tetrameric forms of the protein, and have recorded preliminary IM data that indicate the stability of the complex is much enhanced upon ligand binding. These stability differences are not detectable using CID or other MS-only methods for the protein ligand complexes studied here. We have chosen to study a range of poly-manosyl ligands binding to ConA, that differ in both in terms of their known protein binding constants and in their total molecular mass, in order to assess the role that such factors may play in the CIU response.

For example, data from Mannopentaose-di-(N-acetyl-D-glucosamine), Oligomannose-5 glycan (Ligand A) and Mannotriose-di-(N-acetyl-D-glucosamine), Oligomannose-5 glycan (Ligand B) bound Con A tetramer complexes over a range of stoichiometries (from 1:1 to 4:1) indicates that while both ligands offer branched mannose structures (5 and 3 mannose respectively) for protein binding, the stability responses recorded by IM-MS based CIU differ significantly. Furthermore, our preliminary IM data shows that while the complex is, in general, additionally stabilized upon each additional ligand binding event, we observed a non-linear/cooperative response in our CIU data that is absent in our CID datasets. Our CIU measurements indicate that the 2^{nd} and 4^{th} ligand binding events stabilize the complex disproportionately when compared with the 1^{st} and 3^{rd} tetramer binding events. Here, we will summarize our most recent CIU and CID results for selected ConA-manosyl sugar binding systems, and use this data to project the universality and utility of the CIU approach for high-throughput ligand screening.

References:

1. Hyung, SJ, Robinson CV, Ruotolo BT *Chem. Biol.* **2009**, *16*, 382-390.

Poster 15

Developing Ion Mobility-Mass Spectrometry as a Novel High Throughput Screening Method for Protein Tyrosine Kinase Inhibitors

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Protein tyrosine kinases (PTKs) are of interest in drug development efforts worldwide because they are aberrantly active in cancer cells. However, the search for small molecules that inhibit abnormal PTK function is stymied by lack of high-throughput screening (HTS) processes for potent inhibitors. This study aims to develop more-sensitive screening routes for different types of PTK inhibitors using ion mobility separation coupled to mass spectrometry (IM-MS), a technology capable of assessing size, shape, binding stoichiometry, stability and structure of protein-ligand complexes at low concentrations within the context of complex mixtures. Here, we present our latest data describing the utility of IM-MS in differentiating between inhibitors of the Abl kinase variants found in chronic myeloid leukemia. The goal of this study is to determine if the type of inhibition can be revealed by investigating the inhibitor-drug complexes with IM-MS, and to demonstrate the potential of IM-MS as a novel HTS method.

Poster 16

Ion Mobility-Mass Spectrometry Screening Reveals Neuropeptide Interactions with Amyloid β Peptides and their Oligomers

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Amyloidosis is a pathogenic form of protein aggregation associated with a number of diseases including Alzheimer's disease (AD), where the amyliod β peptide (Aβ) is the primary constituent of disease-associated plaques. While studies using conventional technologies have yielded important structural insights and some understanding of the molecular processes underlying aggregation, the application of these insights to amyloidogenesis at the cellular level has been less successful, suggesting a discrepancy between our molecular-level understanding and amyloidosis *in vivo*. While our general approach to this problem continues to revolve around integrating many different structural biology and synthetic approaches, we also feel that ion mobility-mass spectrometry (IM-MS), with its capabilities to measure oligomer size and structure from potentially complex mixtures at relatively low concentrations, can play a pre-emptive, screening role in efforts to develop potential therapeutics and small molecule binders for Aβ disaggregation, fibril formation disruption, and tagging applications. Further, IM-MS can play an important role in defining the interaction partners bound to Aβ in *in vivo* environments. To this end, we have begun iteratively screening for interactions with proteins and peptides that either co-localize or have been theorized to interact with Aβ. Here, we present our most recent IM-MS data on such interactions, which primarily include IM-based structural characterization and MS-based K_d measurements of Aβ-neuropeptide interactions.

Recently, we performed an ESI-MS-based binding interaction survey between neuropeptides and $A\beta_{1\text{-}40}$ which revealed an interaction between the small opioid peptide neurotransmitter leucine enkephalin (LE). While many other neuropeptides have now been screened for Aβ interactions, LE has so far proved to be the strongest binder from all ESI-MS screening data collected. While initial surveys indicated a 1:1 stoichiometry, further work has revealed LE binding to the $A\beta_{1\text{-}40}$ dimer. In addition, at higher LE concentrations, additional binding stoichiometries can be observed that range from the previously-observed 1:1 and 2:1 Aβ:LE ratios, to encompass 1:4 and 2:5 complexes at the highest LE concentrations studied here (~80 μM). Measured collision cross-section (CCS) values for these peptides on the Synapt G2 (Waters Corp, Milford MA, USA) indicate a relatively tight interaction between Aβ and LE. In contrast, the K_d values recorded for the Aβ:LE interaction are relatively weak (~60 μM), although the interaction is stronger than other small molecule binders that have been pursued as natural product-based therapeutics for AD. Critically, LE binding appears to converts Aβ multimers into a more compact state upon binding (or, LE selects the more-compact configuration for binding), similar to our previous observations with other potential AD theraputic targets. Here, we will present our most-recent structural data surrounding this system, present a more-complete mechanism of action, and compare LE binding to other potential AD therapeutics studied in our laboratory.

Poster 17

Development of Ion Mobility-Mass Spectrometry as a High-throughput Approach for Structural Proteomics

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Proteins act as the molecular machinery of cells, executing critical functions in the life cycle of every organism. To perform their biological function, individual proteins associate, often in a transient manner, to form complexes. Understanding the way such structures function and interact is a far-reaching scientific goal for structural genomics. One of the chief bottle-necks in this endeavor is the technology available for determining the structures or architectures of protein assemblies. Ion Mobility separation coupled with mass spectrometry (IM-MS), has received interests recently for its ability to determine the topology of protein complexes from small amounts of material within heterogeneous mixtures. Here, we discuss novel methods for generating high-throughput structure determinations using ion mobility coupled to mass spectrometry (IM-MS).

The IM-MS technology for structural proteomics relies heavily on measurements of intact protein complexes, as well as subcomplexes and subunits, for the determination of multiprotein topology. Despite the proliferation of gas-phase dissociation and disruption methodologies for multiprotein assemblies, such technologies often do not provide sufficient information on their own to deduce a complete protein network map. As such, those studies that have reported high-confidence level protein contact diagrams have optimized conditions in solution prior to nESI in order to generate subcomplexes having largely orthogonal compositions to those produced by the gas-phase methodologies. Such disruption experiments often involve small additions of organic solvent, alterations in ionic strength and solution pH in order to elicit the formation of topologically-informative subcomplexes. In addition, other solution parameters are often sought that provide the best mass measurement accuracy, signal intensity, or information content in the form of sub-assembly generation. While sometimes such solution conditions overlap considerably, often the optimal solution conditions that provide all of the relevant information to form a topology model for a multi-protein complex represent several distinct states that must be discovered after an extensive period of trial and error.

Here, we present the first semi-automated IM-MS screens of protein complex solution disruption using a robotically sampled nano-electrospray interface (Advion Nanomate coupled to a Waters Synapt G2). We use this system to deploy an extensive screen of solution conditions for protein complex disruption in order to derive a set of best-practices and basic rules for selecting appropriate solution conditions for high-throughput IM-MS data collection and protein topology determination. Screens are performed as a series of two-dimensional surveys of solution parameters (i.e., organic content and ionic strength) so as to be compatible with robotic sampling of a 96-well plate. For each protein complex studied in this way, the solution conditions are typically altered to include variations in pH, ionic strength and/or addition of organic solvents (i.e., Acetonitrile or DMSO) and compared to a control sample prepared in ammonium acetate. Preliminary data for the Avidin and TTR homotetramers screened in this fashion indicates a dramatically different dependence on solution conditions for protein complex disruption, and we interpret these observed differences in terms of the chemical nature (i.e., hydrophobicity) of the non-covalent protein-protein interfaces known to exist in these tetramers. Finally, we will focus this presentation on evaluating the ability of an automated IM-MS screen to accurately estimate the relative areas of protein-protein interfaces within multiprotein complexes, and will report solution conditions that both preserve and distort interface geometries for a wide range of known systems.

Poster 18

Structural Characterization of CRISPR-Associated Immunity Complexes

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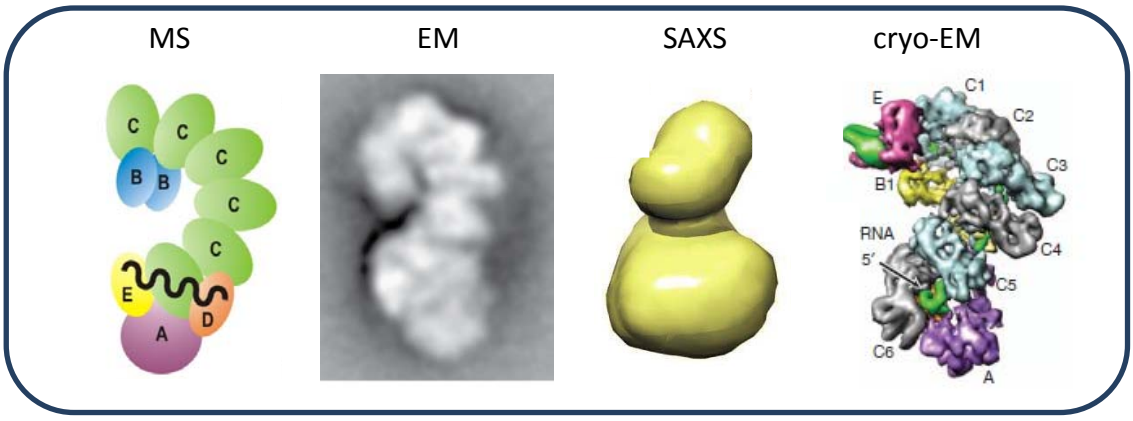
Prokaryotic CRISPR/cas-associated complexes (clustered regularly interspaced short palindromic repeats/CRISPR associated genes) play a vital role in the immune system of bacteria. They provide acquired resistance against viruses and plasmids by a strategy analogous to RNA-interference.

In this presentation I will focus on the structural characterisation of two CRISPR/cas-associated complexes, Cascade and the Csy-complex, originating from *Escherichia coli* K12 and *Pseudomonas aeruginosa* respectively. Despite their similar biological activity both CRISPR/cas assemblies are comprised of a distinct set of proteins and nucleic acid constituents. Interestingly, each of the two ribonucleoprotein complexes have a similar stoichiometry and morphology. Thereby, these immunity systems perfectly illustrate the direct relation between a structure of an assembly and its biological activity.

Structural models for Cascade and the Csy-complex were generated using a combination of native (ion mobility) mass spectrometry, electron microscopy and small angle X-ray scattering analysis. I will summarize the stepwise construction of the Cascade topology model based on mass spectrometry data. This model served as an ideal starting point for complementary structural biology, resulting in a Cascade structure at higher resolution. An identical approach was followed to examine the three dimensional organization of the Csy-complex. Both systems share a spiral shaped hexameric structure, flanked by associating proteins and one CRISPR RNA (crRNA).

Mass spectrometry not only provides information about the intact complexes. Also numerous intermediate states of the CRISPR/cas complexes were identified from which so called subunit connectivity diagrams can be derived. These diagrams allow speculations about homologous proteins between the two systems, information that is not evident from their amino acid sequences.



Structural models for E. coli Cascade as determined by several structural biology methods (left to right: mass spectrometry, electron microscopy, small angle X-ray scattering, cryo-electron microscopy).[1,2]

References: [1] Jore et al., NSMB 2011, 18(5), 529-537; [2] Wiedenheft et al., Nature 2011, 477(7365), 486-489

Poster 19

Characterization of Carbohydrates and Carbohydrate Natural Products by LC- Ion Mobility-Mass Spectrometry

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The characterization of carbohydrates and carbohydrate decorated natural products in biological systems is challenging due to the high level of heterogeneity of glycan structural isomers and the corresponding difficulty of separation. Liquid chromatography (LC) and mass spectrometry (MS) are commonly used for rapid structural characterization of complex glycan mixtures, though differentiating isomeric glycans is still challenging and oftentimes requires complementary fragmentation spectra derived from tandem MS experiments in order to arrive at confident structural identities. One promising approach for glycan characterization is the use of ion mobility-mass spectrometry (IM-MS). IM-MS addresses several of the analytical limitations resulting from high isomeric heterogeneity and sample complexity by performing rapid, structurally selective separations, which are complimentary to the MS analysis. Quantitative structural information can also be derived from these mobility measurements that are specific to isomeric species. This report presents the development of methodology for analysis of glycan isomers by LC-IM-MS.

Simultaneous glycomic and proteomic analysis was demonstrated for model glycoproteins by subjecting them to sequential proteolytic digestion (trypsin) followed by enzymatic release of the N-linked glycans (PNGase F). Digested samples were directly analyzed by a commercial LC-IM-MS (Waters Synapt G2) operated with both MALDI and ESI ionization sources. Ion signals were isolated by chemical class (glycan or peptide) based upon their partitioning in 2-dimensional IM-MS conformational space. Resulting *m/z* values of the glycan fragments were identified by Glycosuite DB (Tyrian Diagnostics, Sydney, Australia) characterization tool on the ExPASY Proteomics Server. Glycans with multiple isomeric structures were separated by optimization of both LC and IM parameters. This method offers rapid resolution of carbohydrate isomers, relevant to carbohydrate natural products, based on collision cross-sections (effectively molecular size). Structural assignments were confirmed with fragmentation data obtained in a parallel IM/MS experiment whereby all ions were separated by IM and subjected to high and low energy activation in order to generate interleaved precursor and high/low energy fragmentation spectra. Additionally, theoretical conformations were generated using a molecular dynamics approach for conformational space exploration. Data were discriminated using experimentally derived collision cross sections. This two-pronged approach to isomer determination adds confidence to our methods. Implications of this analysis are discussed in terms of high throughput characterization of carbohydrate natural products with minimal sample workup.

Poster 20

Using Distance Geometry with Ion Mobility-Mass Spectrometry Data to Study the Conformational Space of Natural Products

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Ion mobility-mass spectrometry (IM-MS) allows us the separation of ionized molecules based on their structural properties such as size and shape, in addition to their mass-to-charge ratio. The drift time data that is obtained from IM-MS is used to calculate the collision cross section of the ionized molecule, which is representative of the conformation of the ion.[1] Studying the conformational landscape of these ionized molecules computationally provides further insight into the three dimensional structures that these collision cross sections represent. In the past, simulated annealing has often been paired with IM-MS data to describe the conformational space, but the high kinetic energies used in simulated annealing can cause damaging distortions to the molecules and does not ensure sampling of all conformational space. Distance geometry creates conformations based on all the distances between the atoms within the molecule.[2] Our preliminary results suggest that distance geometry is a better-suited computational approach due to its ability to sample *all* conformational space without subjecting the compounds to high kinetic energies. Also, the calculation is less computationally expensive and it allows us to work in force fields that are designed for our drug-like molecules of interest. We are currently using distance geometry to study a small set of natural products for which we have measured collision cross sections and there are known three-dimensional conformations. The majority of the conformations that distance geometry produces are within the experimental collision cross section range we obtained from IM-MS measurements as illustrated in Figure 1.

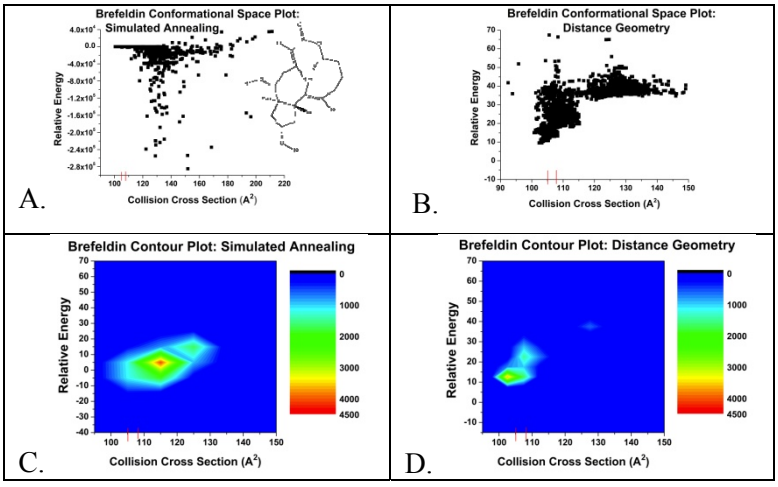

Figure 1 The simulated annealing and distance geometry results for brefeldin are presented here. The experimental collision cross section for brefeldin measured with MALDI-IM-TOFMS is 106.96 $\pm$ 1.29 A^2 and is noted with red marks in the plots. **A. & B.** Scatter plot for simulated annealing (with brefeldin structure) and for distance geometry respectively. **C. & D.** Contour plot for simulated annealing and for distance geometry respectively. The scatter plots show the conformational space that is covered and the contour plots are used to show the density of the conformation space.

[1] Mason EA, McDaniel EW. *Transport Properties of Ions in Gases.* John Wiley and Sons; Indianapolis, IN, USA: 1988.

[2] Havel, T., Distance Geometry: Theory, Algorithms and Chemical Applications. In: Encyclopedia of Computational Chemistry, J. Wiley & Sons (1998).

Poster 21

Characterization of New Fatty Acid from *Maytenus* Speices using Fragmentaion Pattern and Mass Behaviour

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The genus *Maytenus* is widely spread in Africa, South America and Asia. It is present in north and south Saudi Arabia and is being used as medicinal remedy for many illnesses such as anticancer, insecticide, anti-inflammatory, antispasmodic and others. Successive fractionation of *Maytenus obscura* yielded highly non-polar (n-hexane soluble) fraction from which a pure compound was isolated. IR, 1D and 2D NMR provided many structural clues which contain one double bond. However, all the spectrometric means fail to determine unambiguously the exact position of the double. Mass spec analysis was done and careful analysis of the fragmentation pattern with the application of the theories of the fatty acids behavior upon the bombardment with the electron beam provided indispensable information that lead to the identification of the exact structure of the molecule. Detailed analysis of the mass fragmentation and its relation to the parent M^+ and odd fragmentation of some fatty acids is explained with different molecular ions fragments' structures up to the parent compound.
Keywords: Fatty acids. Mass spec. *Maytenus.* Fragmentation patterns.

Poster 22

Density Functional Theoretical Studies of Charge-Remote Fragmentations: Toward the Development of Predictive Reactions Mechanisms for Steroids Mass Spectrometry

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Current advancements in both computational and mass spectrometric approaches allow the design of new fragmentation mechanisms with details of reaction thermochemistry that is unprecedented. Many classical mechanisms need further studies with new theoretical evidence. A good example is mass spectrometry (MS) of steroids that represents one of the best group of compounds that have been studied by MS in the past 50 years. Steroids are credited to our current understanding of gas-phase reactions in many aspects. Despite the large collections of MS data, interpretation of spectra and development of fragmentation mechanisms of many common steroids are not trivial. MS of steroids including progesterone and estradiol were selected for the current study. All steroids were studied by triple quadrupole MS and density functional theory. The results show that charge-remote-fragmentation reactions of the selected steroids such as progesterone can be extended to predict the fragmentation mechanisms within steroids-family.

Poster 23

Modeling Gas-Phase Anion-Molecule Complexes of 1,3,5-Trinitroperhydro-1,3,5-Triazine (RDX)

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Spelman College

RDX (1,3,5-trinitroperhydro-1,3,5-triazine) is an explosive cyclic nitramine that is used for both military and industrial applications. Negative chemical ionization (NCI) mass spectrometry of RDX shows important structural information. The initial ionization step is generation of a negatively charged RDX. The structure of RDX and its anion have been the focus of many previous studies. To better understand fragmentation mechanisms, it is necessary to determine the correct structure of the anion. Both RDX and its anion were studied using density functional theory (DFT) methods. Heterocyclic six-membered ring conformations of RDX were modeled: AAA, AAE, AEE, and EEE. The goal was to identify geometry of the optimized anion and the conformation of the energetically favored RDX anion. B3LYP functional with 6-311G (d,p) basis sets were used to optimize all conformers. The results were compared with those obtained using O3LYP, X3LYP, and B3PW91 functionals. The effects of basis sets were also studied using 6-311++G (d, p) basis sets. The results show that RDX anion under mass spectrometric conditions is an anion-molecule complex. Only AAE and AEE conformers were found as anion-molecule complexes; both AAE and AEE were more stable than other conformers. The use of B3LYP and X3LYP functionals were important in finding the anion molecule complexes. These results show important structural considerations that are needed in designing sound fragmentation mechanisms.

Poster 24

Effect of Charge State on Gas-phase Dissociation Behavior of Non-Covalent Protein Complexes Examined by Ion Mobility-Mass Spectrometry

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The charge state of protein ions generated from electrospray has been shown to affect their conformation. Recently, the use of charge manipulation reagents and counter-ion solution additives in electrospray has been reported, and is intended to better preserve their native solution structure as they are transferred into the gas phase. Activation of non-covalent protein complexes is usually achieved through collision induced dissociation (CID) in order to gain more information about the subunit arrangements, and it has been reported that at "normal" charge states, protein complexes typically dissociate into highly charged monomers and complementary (n-1)-mers in CID. In contrast, studies from our group with surface induced dissociation (SID) show a symmetric dissociation pattern for protein complexes where the charge is approximately evenly distributed among the fragments. In the present work, dissociation and unfolding behavior of several tetrameric (transthyretin, concanavalin A) and pentameric (C-reactive protein, serum amyloid P) protein complexes by CID/SID are examined at their "normal" charge states (in ammonium acetate) and reduced charge states (addition of alkyl ammonium salts, organic molecules with high basicity such as guanidine and diazabicycloundecene) in a modified quadrupole/ion mobility/time-of-flight mass spectrometer. A custom SID device was inserted before the ion mobility cell to allow collisional cross section (CCS) measurements of subunit fragments from SID, in addition to those from CID for direct comparison in the same instrument.

A significant difference in the behavior of protein complexes upon activation with different charge states produced by different solution additives is observed, although the CCS of the precursor does not seem to change with charge state. In most cases, the proteins unfold into certain intermediates upon activation. Precursor unfolding during dissociation is largely suppressed at low charge states, with certain additives showing a better preservation of folded conformation. Subunit dissociation of the complexes is also affected by charge states of precursor ions. For charge-reduced precursors, CID shows a pattern typically observed for "normally" charged precursors but with reduced fragmentation efficiency. However, with SID subcomplex fragments with various oligomeric states, most of which exhibit compact conformations, are distinctively detected. The data suggests that more information about the subunit contacts may be obtained with SID when the charge of the precursor ion is reduced. Charge repulsion within the precursor ion could play a role in the stability of the complex thus affecting unfolding/dissociation of non-covalent protein complexes.

Poster 25

IRMPD Spectroscopy of b_n (n=4-12) Fragment Ions "How Large Can Macrocycles Become?"

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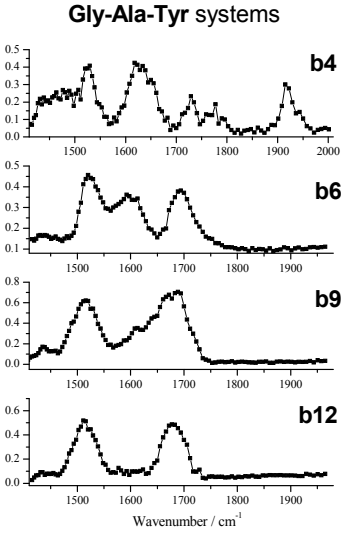
Earlier work by our group has demonstrated that for a series of oligoglycine b fragments (b_2-b_8) generated by collision-induced dissociation (CID), the larger b fragments exhibited a progressively higher propensity to form a macrocycle by "head-to-tail" cyclization.[1] As macrocycles are intimately involved in sequence permutation processes, this trend is worrisome for mass spectrometry-based sequencing. Here, a series of b_n (n=4-12) fragments , based on the repeating sequence motifs TyrAlaGly, AlaTyrGly, and GlyAlaTyr, are investigated. The presence of oxazolone *vs.* macrocycle fragment structures was validated by infrared multiple-photon dissociation (IRMPD) spectroscopy, using the free electron laser FELIX. While the smaller b_4 ions display sizeable oxazolone bands at ~1900 cm^{-1}, the larger b ions (b_6, b_9 and b_{12}) are characterized by an absence of this diagnostic band (see Figure to the left). A control experiment for a synthetically made cyclic peptide confirms the observation of the macrocycle structure for b_6, and strongly suggests the exclusive presence of macrocycle structures for larger b ions (up to 12 amino acid residues). These results, in addition to previous findings, indicate that oxazolone b fragment structures are mainly confined to smaller b ions, where as larger b fragments more readily adopt macrocycles.



[1] Chen et al. *JACS* **2009**, 131, 18272. Chen et al. *JASMS* **2010**, 21, 1313.

Poster 26

Mass Spectrometry Reveals Different Subunit Interaction Maps for the Native and Phosphorylated Conformers of the $(\alpha\beta\gamma\delta)_4$ Phosphorylase Kinase Complex

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Phosphorylase kinase (PhK) is a 1.3 MDa enzyme complex that regulates glycogenolysis in skeletal muscle. It is composed of four copies of four distinct subunits, termed α, β, γ and δ. The catalytic kinase subunit within this complex is γ and its activity is regulated by the three remaining subunits, each of which is targeted by allosteric activators from neuronal, metabolic and hormonal signaling pathways. Although this regulation of PhK activity has been studied extensively, less is known about the interactions of its subunits, particularly in non-activated versus activated forms of the complex. Using nano-electrospray mass spectrometry and partial denaturants to disrupt PhK, the subunit dissociation patterns of non-activated and phospho-activated (autophosphorylation) conformers were compared. In so doing, we have established a network of subunit contacts that complements prior evidence of subunit interactions in these complexes using chemical cross-linking and immunochemical analyses. We have modelled the network of contacts for each conformer within the context of a known three-dimensional reconstruction of PhK from particles of the complex frozen in vitreous ice and have localized these networks, based on the approximate location determined for each subunit by electron microscopic methods. Our analyses show that the network of contacts between subunits differs significantly between the non-activated and phospho-activated conformers of PhK, with the latter revealing new contact patterns for the β subunit, which is predominate in the activation of PhK by phosphorylation. Partial disruption of the phosphorylated conformer yields several novel subcomplexes containing the β subunits that when considered together demonstrate their self-association and location in the quaternary structure of PhK, as well as interaction with the endogenous calmodulin (δ) subunit.

Poster 27

Twenty-Seven Years of Neutral Gas Collisions of Carbohydrate Samples; Where are We Now?

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For a number of years selective strategies and instrumental approaches have been coalescing gradually, leading to a better understanding of oligosaccharide structures. The earlier developments of collisional activation (CA) MS, introduced as CID by Jennings and by Haddon and McLafferty, and tandem MS were interesting adjunct techniques in MS, but the value to biopolymer analysis could not be appreciated until the advent of advances in high mass ionization. Field desorption, chemical ionization, and fast atom bombardment (FAB) were interim strategies that provided some direction for solid sample analysis and avoided the pyrolysis of heat vaporization and high energy of electron bombardment. Both electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) produced mainly protonated and deprotonated molecules with very little fragmentation; thus, CID became a major strategy for the characterization of biopolymers. The first indications of the combined power of intact ionization with gas-phase disassembly for carbohydrates were reported in 1985 using FAB ionization and two coupled analyzers (BE-EB) (Fig. 1). FAB ionization and the first analyzer (BE) provided precursor (or parent) ions free from matrix contaminants, and structural details of these precursors were observed after collision and product ion analysis in a second coupled analyzer (EB). Starting from complex mixtures, this instrumental approach, BE-CID-EB, contributed significantly to glycan analyses. These exciting structure-functional relationships heralded the prospects of gas-phase structural analysis in the absence of chromatography, complete with the sensitivity expected from electron multiplier detection. The developments, however, which have brought the most significant impact to carbohydrate analysis, were those leading to the dynamic stabilization of ions in three-dimensional radiofrequency quadrupole fields by Wolfgang Paul. These achievements led to the subsequent developments of the quadrupole mass filter and the quadrupole IT. Thus, with the ability to ionize samples at high mass and to observe fragments by CID in Paul traps, all the components were in place for a full and detailed investigation of carbohydrate structure. This **spatial resolution**, vis-à-vis **resolution in time**, has been shown to be largely a result of two features, stereospecific metal ion binding (which yields unique collision products), and methylation, which places components of structure in a glycan array. In direct applications, metastatic tissues exhibit component up regulation, (m/z 2982.6, Fig. 2) in colorectal cancer cells. Disassembly, MS^{3-5} following the pathway (right, Fig. 2) defines two isomers, one possessing antennal fucosylation; disassembly, m/z 1021.6, 646.4, (MS^2) indicate Lex/sLex epitopes, (Fig. 2). Serum biomarkers, HIV and stem cell applications will be presented with comprehensive structural details as contemporary product goals.

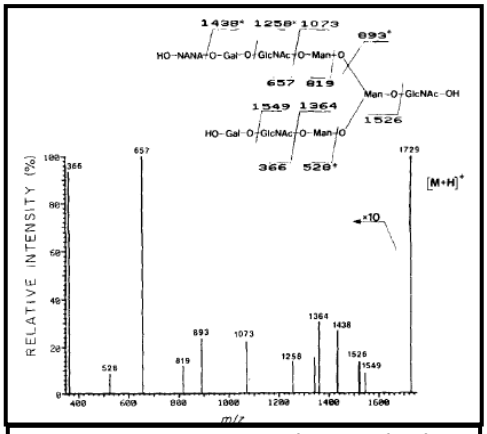

Figure 1. **Positive ion FAB ionized BE-C-EB daughter ion spectrum. VG ZAB-4F HF (VG Analytical, Manchester, UK). Carr, Reinhold, Hass, Biomedical Mass Spectrom., 1985.**

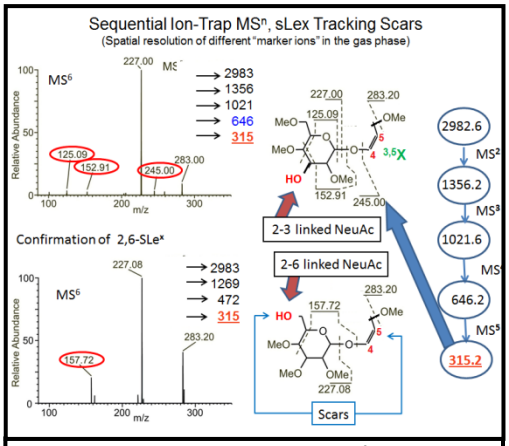

Figure 2. **Isolation of the product ion m/z 315.2 (following the pathway, right) provided the top spectrum (MS^6) with cross-ring cleavages indicating a 3-position open hydroxyl, which indicates a former 2-3Sial linkage to the Hex. The cross-ring $^{3,5}X$ ion can only be accounted for by a former 1-4HexNAc with a 3-linked fucose in the precursor ion m/z 646.2; 2012.**

Poster 28

Comparison of Peptoid Fragmentation under CID and IRMPD Conditions

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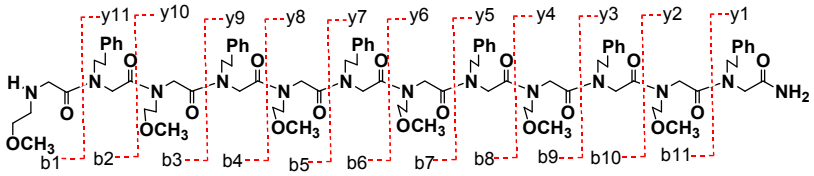
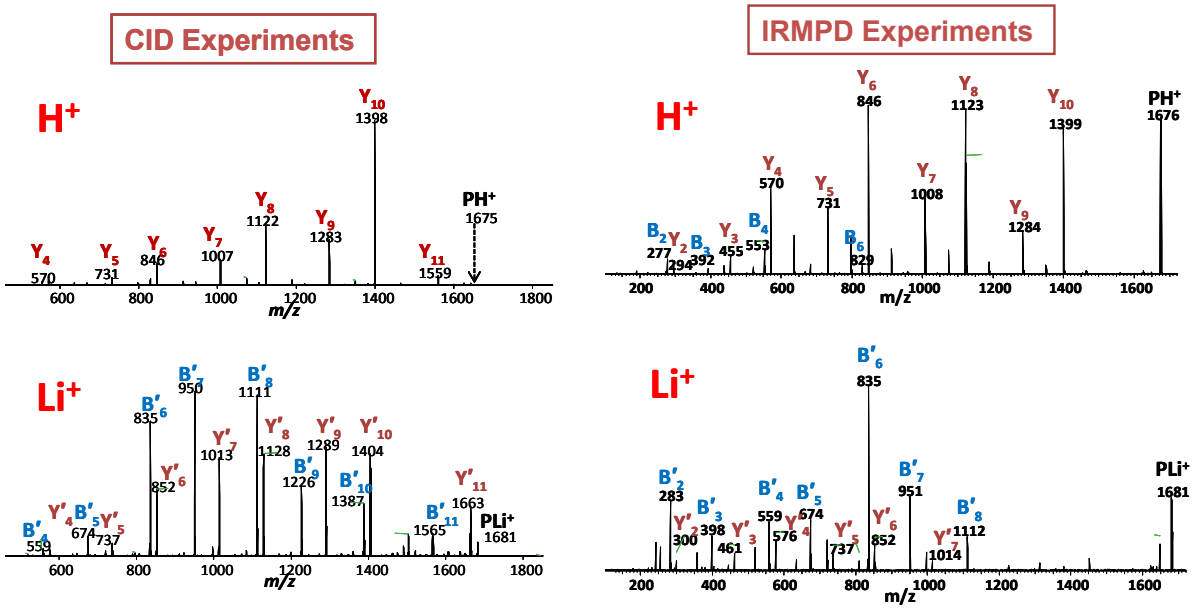
Peptoids are peptide-mimicking polymers. Peptoids with certain constructs can fold into peptide-like secondary structures. Folded peptoids have the potential to mimic biological functions of peptides and proteins and have been thought to be promising therapeutic agents. The fast growing interest in peptoids and the increasingly diverse structures in peptoid libraries require efficient analytical methods to analyze the sequences and the structural changes of peptoids. We are investigating peptoid fragmentation patterns under different tandem mass spectrometry conditions. We have studied a group of model peptoids. The charged peptoids were generated by the MALDI and ESI processes via protonation and alkali metal cation (Li^+, Na^+, K^+, Rb^+, and Cs^+) addition. The peptoid ions were fragmented via collision-induced dissociation (CID) and infrared multiphoton dissociation (IRMPD) experiments. Highly abundant and characteristic fragment ions were observed. The protonated peptoids fragmented by producing predominantly Y-type ions in both the CID and the IRMPD experiments. All alkali metalated peptoids fragmented by producing both Y'- and B'-type ions. For the same peptoid with the same charge type, the fragmentation patterns resulting from the CID experiments are quite different from those from the IRMPD experiments. Examples of CID and IRMPD spectra are shown below. Molecular modeling suggests that the isomeric peptoids with different charge locations may exist in different conformations.

Acknowledgements: The peptoid samples were provided by David Robinson (Sandia National Laboratory) and Ronald Zuckermann (The Molecular Foundry, LBNL).





Poster 29

Evidence for Hydrogen/Deuterium Scrambling in Electron Capture Dissociation – Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

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Amide hydrogen/deuterium exchange (HDX) coupled with mass spectrometry (MS) is widely used for the study of protein dynamics. This report regards the use of ECD FTICR MS to investigate H/D scrambling in HDX the neuropeptide, Substance P. The potential improvement of results arising from the use of HPLC separation to remove the influence of deuterium exchange with amino acid side chains and the termini to focus on exchange with the amide backbone was examined. ECD FTICR MS of Substance P ions generated by ESI examined the 22 possible protons available for HDX (8 backbone amide protons, 10 side chain protons, and 4 termini protons) in solution. The results from triplicate experiments with and without HPLC showed the maximum of exchange occurs respectively for 9 and 22 proton sites. The maximum number of amide protons exchanged with deuterium up to c10 for both experiments was 8. The results obtained with the exclusion of influence from proton sites at side chains and N-termini positions yielded better fit. The plot for measurements without the side chain influence exhibited an exponential rise to the maximum and less deuterium migration within the peptide chain. The mass spectrometric data showed different solvent accessibilities of cleavage sites on Substance P, suggesting that the structural information of this peptide can be studied by Top-Down ECD FTICR MS. The incorporation of HPLC separation, using a C18 peptide trap, provided superior results with less H/D scrambling and minimal amide back exchange.

References:

1. Zhang Z., Smith D. L.: Determination of amide hydrogen exchange by mass spectrometry: a new tool for protein structure elucidation. *Protein Sci.* , **1993**, 2, 522-531.
2. Rand D. K., Adams M. C., Zubarev A., Joergensen J. D. T.: Electron Capture Dissociation Proceeds with a Low Degree of Intramolecular Migration of Peptide Amide Hydrogens. *J. Am. Chem. Soc.*, **2008**, *130*, 1341–1349.
3. Pan J., Han J., Borchers H. C., Konermann L.: Hydrogen/Deuterium Exchange Mass Spectrometry with Top-Down Electron Capture Dissociation for Characterizing Structural Transitions of a 17 kDa Protein. *J. Am. Chem. Soc.*, **2009**, *131*, 12801–12808.

Poster 30

Structural Evidence for Cooperative Microtubule Stabilization by Taxol and the Endogenous Dynamics Regulator MAP4

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Microtubules composed of αβ-tubulin heterodimers are highly dynamic polymers, whose stability can be regulated by numerous endogenous and exogenous factors. The anti-mitotic drug, Taxol, and microtubule-associated proteins (MAPs) stabilize this dynamicity by binding to and altering the conformation of the microtubules (MTs). In the current study, amide hydrogen/deuterium exchange coupled with mass spectrometry (HDX-MS) was used to examine the structural and dynamic properties of the MT complex with the microtubule binding domain of MAP4 (MTB-MAP4) in the presence and absence of Taxol. The changes in the HDX levels indicate that MTB-MAP4 may bind to both the outside and the luminal surfaces of the microtubules, and that Taxol reduces both of these interactions. Despite this effect, enhancement of stabilization of the intradimer and lateral interfaces observed in the presence of Taxol suggests that endogenous effectors of MT dynamics may cooperate with MSAs in stabilizing MTs and regulating mitotic activity of the cells (Table 1). The MTB-MAP4 binding induces conformational rearrangements of α- and β-tubulin that promote an overall stabilization of the microtubules.

Table 1. Quantitative summary of MT-stabilizing trends for MAP4 in the presence and absence of Taxol.

Interface	Total ΔHDX (α)		Pα	Total ΔHDX (β)		Pβ
	MAP4	MAP4+Taxol		MAP4	MAP4+Taxol	
*Interdimer	-136 ± 38	-118 ± 40	0.6	-270 ± 18	-246 ± 18	0.1
Intradimer	-108 ± 43	-292 ± 54	0.002	-184 ± 24	-441 ± 36	0.000
Lateral	-142 ± 15	-17 ±21	0.001	-81 ± 41	-298 ± 43	0.001

Poster 31

Impact of Site-Specific Oxidation on the Higher-Order Structure of Interferon β-1a

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Interferon Beta-1a (IFN) is a protein biopharmaceutical used to treat the neurodegenerative disorder, multiple sclerosis (1). A relatively small protein (~ 23 kDa), IFN is composed of 166 residues, contains a single N-linked glycosylation site (N80), one disulfide bond (C31-C141), and one free cysteine residue (C17). Although IFN is an extensively characterized protein, recent studies that employed hydrogen/deuterium exchange monitored by mass spectrometry (H/DX-MS) demonstrated that various non-enzymatic posttranslational modifications (PMTs), specifically methionine and cysteine oxidation, can significantly alter IFN structure (2-3). Because oxidation is relatively common in protein biopharmaceuticals and is linked to their instability and immunogenicity, understanding the impact of this modification is essential. In the present work, we investigate the oxidation implication on the higher-order structure of IFN. We differentiate between oxidation of susceptible methionines and the free cysteine combined, and the oxidation of the susceptible methionines alone. We implemented specific reaction conditions that enable us to selectively oxidize susceptible methionines without modifying the free cysteine residue. Modified IFN species were characterized and compared to non-oxidized IFN with H/DX-MS as well as other classical biophysical tools (CD, fluorescence). Preliminary results indicate that the combined oxidation of the susceptible methionines and free cysteine induces global changes in the higher-order structure of IFN compared to its non-oxidized counterpart. However, selective oxidation (methionines only) has little to no impact on IFN's structure. Although the current work focuses on oxidation, the implementation of H/DX-MS could be expanded to other PTMs, thus providing helpful insights into the design and development of protein biopharmaceuticals.

Reference

1. J.H. Noesworthy, et al. *N. Engl. J. Med*., 2000, 343 (13), p938.
2. D. Houde, et al. *J. Pharm. Sci.*, 2010, 100 (6), p2071.
3. I. Kaltashov, et al. *J Am. Soc. Mass Spectrom*., 2009, 21 (3), p323.

Poster 32

Fragment Antibody Binding to a Highly Conserved Influenza Epitope - an HDX Study

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Influenza represents a persistent threat to public health. Vaccination is the most common way of prevention but it provides protection only against virus strains which are similar to the isolates from the vaccine. High-affinity binding monoclonal antibodies directed against a conserved epitope on the stem region hemagglutinin could provide a powerful solution against a wider range of virus strains. The major surface antigen (hemagglutinin, HA) epitope recognized by the broadly neutralizing human antibody CR6261 was previously identified by cocrystallization of Fab-CR6261 in complex with the 1918 H1N1 influenza pandemic and a recent lethal case of H5N1 avian influenza, respectively (Ekiert 2009, Science, 246-251).

Here we show an alternative method to probe the interaction between Fab-CR6261 and the HA associated with the SC 1918 H1N1 influenza pandemic by hydrogen/deuterium exchange mass spectrometry (HDX MS). In the HDX system, a fully automated pepsin online digestion and UPLC separation at 0 °C were coupled with ESI-interfaced Q-Tof MS to conduct measurements of deuterium uptake at peptide level. Based on differences in the level of HDX of the amide hydrogens of Fab-CR6261, in the free form and the HA bound state, we could identify the IgG regions that are involved in binding to HA. Our experiments identified four regions in the Fab-CR6261 that display a significantly lower level of deuterium uptake when the antibody is bound to HA (Figure 1). Furthermore, these regions contain all contact residues of the Fab-CR6261 that were previously identified in the cocrystallization experiments. In sum, the HDX technology constitutes a reliable alternative method for epitope-paratope mapping especially when there are difficulties encountered in protein crystallization.

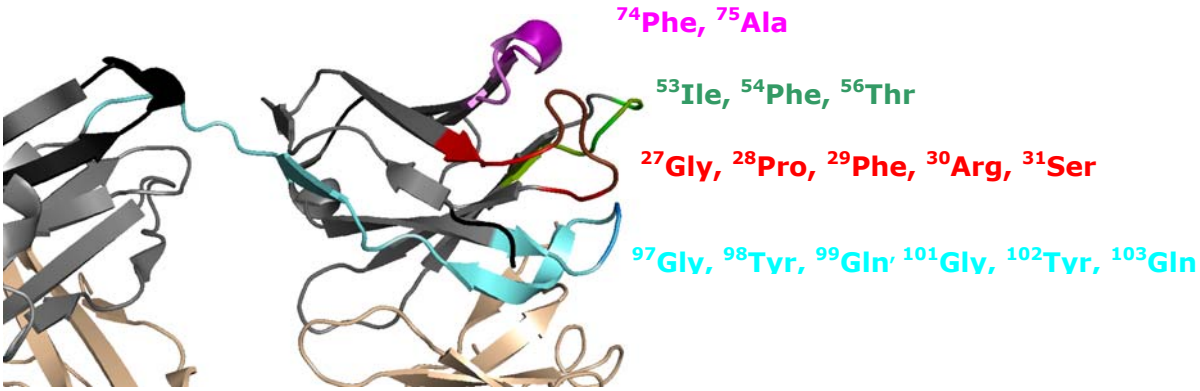

Figure 1. Fab-CR6261 displaying the four regions that show protection in HDX uptake upon binding to SC 1918 HA. The contact residues identified by crystallography are highlighted in the colors corresponding to the protected regions.

Poster 33

Identification of Residues that Mediate the Assembly of COPII Cages by H/D Exchange Coupled with FT-ICR MS

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When eukaryotic membrane proteins or other secreted proteins are synthesized, they are cotranslationally translocated into the endoplasmic reticulum (ER). They are then transported from the ER to the Golgi apparatus, in a process mediated by COPII coated vesicles [1]. COPII vesicles are assembled from the association of the COPII proteins: Sar1, Sec23/24, and Sec13/31. The COPII cage is the outermost layer of the COPII vesicles constructed by Sec13/31 building blocks. Our goal is to identify the residues in Sec13/31 whose solvent accessibility differs in the non-assembled and assembled states. We label the full cage (calculated (34+127)*2*24 = 7.728 MDa; experimental electron microscopy (EM) estimate = 5.4-9.6 MDa) with D_2O and disassemble the cage under H/D exchange (HDX)-quenching conditions by denaturants such as urea. The denatured complex will be proteolytically digested, separated by LC, and analyzed by FT-ICR MS. The information will be combined with a cryo-EM structure to better describe how the COPII cage assembles.

The entire HDX experiment was optimized and automated with an HTC Pal autosampler (Eksigent Technologies, Dublin, CA) [2]. A 5 μL stock of Sec13/31 edge or cage (~10 μM in monomer) was mixed with 45 μL of HEPES buffer in D_2O, pH meter reading 7.5, to initiate each H/D exchange period. Each HDX reaction was quenched by rapid mixing with 25 μL of 200 mM TCEP, 6 M urea solution in 1.0% formic acid and 25 μL 5-fold dilution of saturated protease type XIII in 1.0% formic acid. The final pH was ~2.3 and protease digestion was performed for 3 min at ~1 ˚C. The digested sample was then injected for LC-MS analysis.

References

1. Lee, M.C. and E.A. Miller, *Molecular mechanisms of COPII vesicle formation. Semin Cell. Dev. Biol.* **2007**, *18(4)*, 424-434.
2. Zhang, Q., Willison, L. N.; Tripathi, P.; Sathe, S. K.; Roux, K. H.; Emmett, M. R.; Blakney, G. T.; Zhang, H.-M.; Marshall, A. G. *Epitope mapping of a 95 kDa antigen in complex with antibody by solution-phase amide backbone hydrogen/deuterium exchange monitored by Fourier transform ion cyclotron resonance mass spectrometry. Anal. Chem.* **2011**, *83(18)*. 7129-7136.

Poster 34

High Resolution Hydrogen/Deuterium Exchange Mass Spectrometry Gives Insight to the Mechanism of Action of Hepatitis B Virus Capsid Assembly Effectors

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Hydrogen deuterium exchange mass spectrometry (HDX-MS) provides valuable insight into solution-phase protein conformation and structure. The resolution of protein structural information in HDX-MS measurements is primarily limited by the peptide coverage of the on-line pepsin proteolysis. We have realized near single amino acid resolution coverage maps by combining online proteolysis with rapid reverse-phase chromatography of highly rich peptide mixtures.

We use Hepatitis B virus (HBV) as a model system for investigating the principles of icosahedral capsid assembly. Viral capsids are not simply a static container for the viral genome. Rather, they are highly functional molecular machines critical for virus life cycle. The assembly process of the HBV capsid involves the concerted assembly of 120 homodimeric subunits to form a T=4 icosahedron, which has been shown to be affected by temperature, ionic strength, and small molecules in a manner consistent with models of allosteric regulation.

We have already completed rigorous measurements of the conformational equilibria for HBV protein using enzyme-mediated kinetic hydrolysis coupled to liquid chromatography mass spectrometry (LC-MS), where we investigated the role of potential molecular switches in capsid assembly. These studies have now been complimented with HDX-MS approaches. We have also developed high-throughput drug-binding measurements coupled to multidimensional LC-MS as a part of our framework to screen potential anti-viral candidates and thermodynamically profile the binding of high affinity drugs.

We analyzed the differential HDX of capsid protein upon binding to anti-viral drugs which thermodynamically or kinetically targets the capsid assembly. High resolution HDX-MS was used to investigate protein dynamics and binding sites of assembly effectors. This has allowed us to elucidate the mechanism of drug-action. We also investigated other capsid-assembly effectors such as temperature, single mutations, and ionic strength on the dynamics of the capsid protein. Together these results indicate that the conformational landscape of HBV can be remodeled by a range of factors. The ability to map the landscape and specifically select conformational states has profound implications in revealing quasi-equivalent subunit associations and the design of antiviral therapies.

Poster 35

Changes in a Monoclonal Antibody upon Dimerization as Revealed by HX MS

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Antibodies are among the most important protein therapeutic agents in the pharmaceutical industry. Their structural characterization has always been very challenging due to their large size (150 kDa). Therefore, there is a continuous need to develop and implement methods to be able to characterize antibody higher-order structure in great detail in order to understand their folding, stability, solubility and aggregation. Recently structural characterization of a therapeutic monoclonal antibody in solution was achieved by hydrogen exchange mass spectrometry (HX MS) (1). In the present work, we investigated a dimer interface of a glycosylated antibody. Hydrogen exchange experiments were performed essentially as described in Houde et al. (1) where deuterium labeling in solution was performed for purified forms of a known monomeric or dimeric version of the same IgG1. The levels of deuteration of both species were measured with electrospray MS and the data were analyzed with Waters DynamX™ Software. The results show that in most regions of the antibody there were no changes in conformation or dynamics upon dimerization. However, in several specific regions we detected subtle changes in hydrogen exchange in the dimer relative to the monomer sample. The differences observed were located in a specific region of the CH2 domain and the hinge region between CH1 and CH2 domains. The implications of these changes on the antibody structure will be discussed.

References

1.Houde, D., Arndt, J., Domeier, W., Berkowitz, S., and Engen, J. R. (2009) *Anal Chem* **81**, 2644-2651

Poster 36

Conformational Locking upon Cooperative Assembly of Notch Transcription Complexes

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Notch proteins are receptors in a highly conserved signaling pathway that play an essential role in both development and adult tissue homeostasis. The effector of Notch signaling is its intracellular domain (NICD), which is released from the membrane upon activating proteolysis. On entering the nucleus, NICD forms a complex with the transcription factor CSL (gene name RBPJ) and a transcriptional co-activator of the Mastermind family (MAML), directly regulating the transcription of Notch-responsive genes. The RAM (RBPJ-associated molecule) region of NICD recruits Notch to CSL, which enables the binding of MAML to a composite binding groove created at the interface between the ankyrin (ANK) repeat domain of NICD and CSL.

Here, we used hydrogen exchange mass spectrometry (HX-MS) to investigate changes in the backbone dynamics of MAML1, RAMANK, and CSL upon complexation through the use of both intact and peptide level experiments. Proteins were expressed as previously described [1, 2]. To prepare protein complexes, the individual purified components were mixed and the complex purified by size exclusion chromatography to eliminate uncomplexed subunits. Deuterium exchange was performed and labeled samples were analyzed using a custom Waters nanoACQUITY [3] coupled to a Waters QTof Premier with an ESI source. All samples were digested offline prior to RP-UPLC separation at 0.1 °C. Peptides were identified using Waters ProteinLynx Global Server, and deuterium incorporation data were analyzed using HX-Express and other software. Data were processed through a comparison of deuterium uptake data for the proteins alone and in complexes.

Results of both intact and peptide level experiments with MAML show that the protein alone was maximally deuterated at the initial 10 second labeling time point and protection from exchange occurred only upon formation of the ternary complex. Within the isolated RAM and ANK domains (RAMANK), ANK repeats 4-6 were the most resistant to exchange. Association of RAMANK with CSL slows exchange in the RAM region, but exerted little effect on the deuterium incorporation of the ANK domain. MAML binding greatly retarded exchange into ANK repeats 2-3 and further protected the interface between RAM and CSL. These exchange patterns identify critical features contributing to the cooperative assembly of Notch transcription complexes (NTCs), and highlight the importance of MAML recruitment in rigidifying the ANK domain and stabilizing its interface with CSL. The data also rationalize the known requirement for MAML in enabling the cooperative dimerization of NTCs on paired site DNA.

1. Nam, Y., et al., *Structural basis for cooperativity in recruitment of MAML coactivators to Notch transcription complexes.* Cell, 2006. **124**(5): p. 973-83.
2. Moellering, R.E., et al., *Direct inhibition of the NOTCH transcription factor complex.* Nature, 2009. **462**(7270): p. 182-U57.
3. Wales, T.E., et al., *High-speed and high-resolution UPLC separation at zero degrees Celsius.* Anal Chem, 2008. **80**(17): p. 6815-20.

Poster 37

Solution-Phase Hydrogen Exchange Followed by Electron Transfer Dissociation for Analysis of Conformational Features of Proteins

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Gas-phase reactions within a mass spectrometer can provide useful information about proteins and protein conformation. Using the traveling wave ion guide technology of the Waters G2 Synapt, two ions of opposite polarity can be reacted in the gas-phase to yield a product ion that differs from the ionized ion.[1] Performing electron transfer dissociation (ETD) on peptides generated after solution-phase deuterium exchange (HDX) labeling of proteins allows for the determination of deuterium incorporation at individual amino acid residues. In the work presented, we will illustrate some technical details of the ion selection process, data processing, and challenges of making deuterium incorporation measurements using a nanoACQUITY UPLC with HDX technology coupled to a Waters Synapt G2 with ETD. The analysis of deuterium incorporation using the system described above in two model systems will be described. First, the conformational changes associated with binding of the small molecule inhibitor to several bromodomains (BRDs) will be detailed. Bromodomains have previously been shown to bind a small molecule drug JQ1,[2] which inhibit cell proliferation in cancer cells. The exact effects of JQ1 binding to isoforms of BRD3 will be compared by deuterium incorporation measures of the single amino acid level. In a second example, the analysis of deuteration through cooperative unfolding will be explained.

References

1. Rand, K.D.; Pringle, S.D.; Morris, M.; Engen, J.R.; Brown, J. M. *J. Am. Soc. Mass Spectrom.* **2011**, *22*, 1784-1793.
2. Bradner, J.E. et. al. *Nature* **2010**, *468*, 1067-1073.

Poster 38

Structural Insight into the Mechanism of Regulation of *Neisseria meningitidis* NadA Expression by the Small Natural Ligand 4-HPA

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The *Neisseria* adhesin A (NadA) is a surface exposed trimeric protein present in most hypervirulent meningococcal strains and involved in epithelial cell adhesion and colonization. The expression of NadA is controlled by NadR, a member of the MarR family, which binds to the *nadA* promoter and strongly represses the transcription of *nadA*. It was recently demonstrated that the DNA-binding activity of NadR was attenuated by the 4-hydroxphenylacetic acid (4-HPA), a natural molecule released in human saliva, thus leading to the derepression of NadA *in vivo*. To elucidate the mechanism of regulation of NadR by 4-HPA, we used hydrogen-deuterium exchange mass spectrometry in association with *in silico* docking and site-directed mutagenesis. We show here that 4-HPA binds at the interface between the dimerization and the DNA-binding domains and stabilizes the homodimeric state of NadR. The residues predicted to be in contact with 4-HPA were further selected for mutagenesis to assess their in vitro and in vivo functions in 4-HPA binding. Our results indicate that Arg40 is critical for DNA-binding and reveal that Tyr115 interacts with 4-HPA. In addition, our data revealed a cross-talk between the HPA-binding pocket and the DNA-binding lobes which suggests that occupancy of the HPA-binding pocket might be communicated to the DNA-binding lobes. We thus propose that 4-HPA regulates the DNA-binding activity of NadR likely by repositioning and stabilizing the DNA-binding lobes in an orientation incompatible with DNA interactions.

Poster 39

Beyond Structure Characterization: Structure Dynamics (Hydrogen Deuterium Exchange) Guided Biocatalyst Improvement

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Enzyme dynamics has been shown to be crucial for functionality in recent years. Hydrogen deuterium exchange (HDX) mass spectrometry emerged as a relatively higher throughput technology to probe enzyme structure dynamics, as compared to NMR. Despite the progresses, fewer studies have actually used the dynamic information revealed by HDX mass spectrometry to guide the rational design of biocatalysts. We hereby demonstrate that dynamic information as revealed by HDX mass spectrometry can be used as an alternative for rational design of xylanase, an enzyme important for hemicelluose degradation in the second generation biofuel production. One desirable feature that has been improved in this work is the expression compatibility because most of the highly efficient fungal enzymes including xylanase have very low activity when expressed in fermentative bacteria or yeast, which makes it difficult to consolidate fermentation and saccharification using engineered microbes. In this research, we have used a *T. reesei* xylanase as a model to prove the concept of dynamics-guided rational design. Furthermore, we have carried out comparative analysis of multiple cellulytic enzymes to investigate the mechanism of the dynamics to enzyme function.

HDX mass spectrometry analysis was performed on both the Apo enzyme and substrate binding enzyme. Site-directed mutagenesis (SDM) was designed based on the dynamic region in the HDX analysis and the hypothesis that stabilizing the highly dynamic regions for substrate binding will increase enzyme stability and performance. The modified protein was expressed in *E. Coli* and purified with FPLC. Enzyme activity assay was carried out to compare the wild type xylanase expressed in Fungi, the wild type xylanase in *E. Coli*, and the mutated xylanase in *E. Coli*.

Figure 1 shows regions that have been analyzed in the HDX experiment for xylanase and the red region indicated the regions that have been significantly changed upon substrate binding. Combined with sequence alignment, site-directed mutagenesis has been carried out to derive mutants with higher activity when expressed in *E. Coli*. Two mutants have been designed and expressed based on the HDX analysis. Figure 2 shows the xylanase activity was very low when heterologously expressed in *E. coli*, and the dynamics-guided mutagenesis has restored the enzyme activity to a high level. If the *E. coli* expressed mutant and wild-type xylanase were compared, the enzyme activity has increased over 30 folds. The mutated enzymes are further being transformed into yeast for consolidated biomass processing. Meanwhile, our comparative HDX analysis highlighted new mechanistic information and differential dynamics among different types of enzymes.

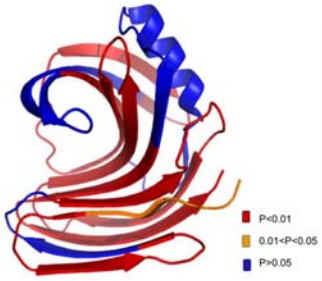

Figure 1. HDX exchange of xylanase by HDX mass spectrometry

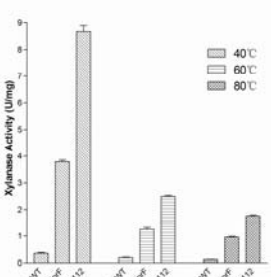

Figure 2. Enzyme activities of wild type and mutants

Conclusions: Differential HDX analysis revealed important and new structure determinants for downstream mutation study of xylanase enzyme based on structure dynamics information. The study implicates the important application of HDX dynamics information toward enzyme engineering. The study opens a new chapter for structure dynamics-based rational design of biocatalysts in biofuel applications.

Poster 40

Identification Of Regions Of Rabbit Muscle Pyruvate Kinase Important For Allosteric Regulation By Phenylalanine, Detected By H/D Exchange Mass Spectrometry

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Abstract: Mass spectrometry has been used to determine the number of exchangeable backbone amide protons and the associated rate constants that are different when rabbit muscle pyruvate kinase (rM_1-PYK) binds either the allosteric inhibitor, phenylalanine, or a non-allosteric analogue of the inhibitor. Alanine is used as the non-allosteric analogue since it binds competitively with phenylalanine, but elicits a negligible allosteric inhibition, i.e. a negligible reduction of the affinity of rM_1-PYK for the substrate, phosphoenolpyruvate (PEP). This experimental design is expected to distinguish changes in the protein caused by effector binding (i.e. those changes common upon the addition of alanine vs. phenylalanine) from changes associated with allosteric regulation (i.e. those elicited by the addition of phenaylalanine binding, but not alanine binding). High quality peptic fragments covering 98% of the protein were identified. Change in both the number of exchangeable protons per peptide and in the rate constant associated with exchange highlight regions of the protein with allosteric roles. Several of allosterically relevant peptides identified by this technique include residues previously identified by mutagenesis studies to have roles in the allosteric regulation by phenylalanine

Poster 41

Evidence for an Allosteric Auto-Inhibitory Phosphorylation Mechanism Regulating the Endosomal Localization of MTMR2 using Hydrogen/Deuterium Exchange Mass Spectrometry

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The controlled membrane distribution of distinct phospholipid species defines the biophysical and biochemical characteristics of membrane dynamics and lipid second messenger signaling. Inositide modification of phospholipids is not as prevalent as that of choline, serine or ethanolamine, however, it provides a moiety capable of poly-phosphorylation to form seven unique species. Variable isoforms of phosphatidylinositol phosphates (PIPs) are localized to specific vesicular structures of the cytosol, serving as functionalized membrane organelle markers. PI phosphorylation is regulated by PI kinases and phosphatases to govern membrane docking interactions and recruitment of effector signaling proteins containing appropriate PIP binding module(s). PIP lipid second messengers and their effectors enable coordination of complex fundamental processes such as receptor signaling and vesicular trafficking mechanisms through translation of this dynamic PIP code.

Endosomal vesicular membranes vary considerably in their PIP complement depending on their maturation state. Rates of early endosome maturation are dependent on levels of PI(3)P, a substrate of the lipid phosphatase myotubularin related protein 2 (MTMR2). The exact roles of MTMR2 in early endosomal processing are slowly emerging with increased understanding of PIP binding domains and MTM spatial regulation. Recent studies from our lab have identified an N-terminal phosphorylation site that potently inhibits access of MTMR2 to PI(3)P rich early endosomes. This site is found adjacent to a speculated PIP binding module within MTMR2, however functional evidence for this domain has been limited by the inability to localize this phosphatase to endosomes, presumably due to the high stoichiometry of this phosphorylation event. Notably, strong evidence exists for phosphorylation mediated regulation of other lipid phosphatases, suggesting that phosphorylation is equally important in the regulation of MTMR2.

On this framework, we have employed hydrogen/deuterium exchange mass spectrometry (H/DX MS) to examine conformational changes in protein solvent accessibility upon MTMR2 phosphorylation. We have identified regions in the putative PIP binding domain which exhibit decreased accessibility upon phosphorylation, suggesting an allosteric regulatory role of this event. Modeling data with the crystal structure of MTMR2 shows that the three dimensional folding of these regional targets form a pocket of positive electrostatic potential on the membrane proximal face of the enzyme. As many phosphate and PIP binding modules utilize electrostatic interactions to stabilize the electronegative charge density of phosphorylated biomolecules, this regulatory event could sequester MTMR2 from substrate/membrane association by preventing key residues within the PIP binding domain accessibility to membrane phospholipid ligands.

The H/DX MS data has provided a targeted-structural approach for mutagenic studies of candidate positively charged residues potentially involved in phosphate and/or PIP interactions which may influence sub-cellular localization of MTMR2. In conjunction to the H/DX MS studies, hetero-bifunctional crosslinkers in combination with mass spectrometry will permit analysis of the structural and spatial characteristics of phosphate interactions within this regulatory domain. Thus, structural mass spectrometry coupled with traditional cell biology techniques have provided evidence of a sophisticated mechanism mediating MTMR2 sub-cellular targeting and subsequent temporal regulation of endosomal maturation and signaling.

Poster 42

Use of Ion Mobility and Fragment Ions to Increase Coverage in Hydrogen-Deuterium Exchange

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Peptide signals in H/D exchange mass spectrometry experiments are often overlapping on the m/z scale due to the nonspecific digestion, fast chromatographic conditions, and cold temperatures required to preserve the deuterium label. Co-eluting peptides which are well resolved when undeuterated can overlap and become impossible to analyze with increasing time in deuterium. It has been shown that the addition of ion mobility into the LC-MS analysis of deuterium labeled samples enables an orthogonal separation in the gas phase. This added separation power is poised nicely between chromatographic and mass spectral analyses (1). The use of ion mobility in H/D experiments resolves many peptides that show spectral overlap (Figure 1). This increases the number of peptides that can be followed in an H/D exchange experiment as well as increases the overlapping linear sequence coverage. This becomes more important with increasing sample complexity, protein and protein complex molecular weight, and importantly with a desire for an increase in backbone resolution to 1 or 2 amino acids.

Previously, fragment ions have been used in data-dependent experiments to measure deuterium uptake (2). In this study, fragment ions that were generated in a data-independent manner (3) were used to measure deuterium uptake. In this example, the precursor ion is obstructed under both MS^E and $HDMS^E$ conditions (Figure 2, bottom, inset). However, a y8 ion from this specific peptide showed no interference (Figure 2). The use of fragment ions that have had the additional specificity of ion mobility alignment improved the measurement over those that only used mass and retention time to associate precursors with product ions. Comparisons of uptake data for peptides and their fragment ions will be demonstrated.

1. Iacob, R.E., *et. al. Rapid Commun Mass Spectrom.* 2008, 22(18):2898-904.
2. Percy, A.J., *et. al. Anal Chem.* 2009, 81(19):7900-7.
3. Geromanos, S.J., *et. al. Proteomics.* 2009, 9(6):1683-95.

Figure 1:

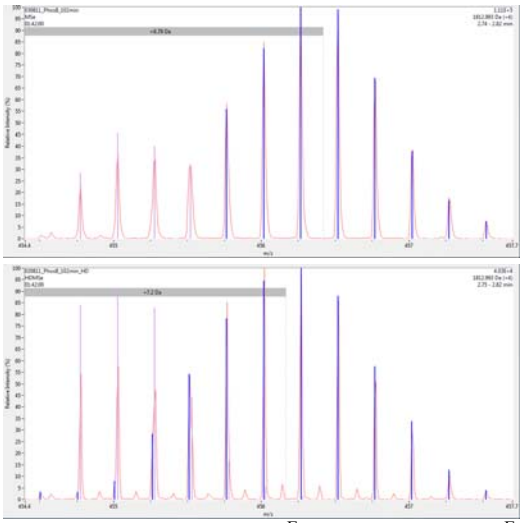
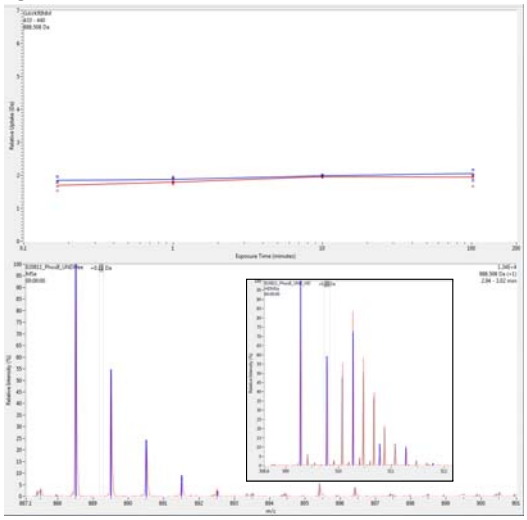


Figure 2:



1. Spectra of a peptide, with MS^E (top) and $HDMS^E$ (bottom). The assigned ions are shown in blue. Due to overlap in MS^E, the lower isotopes were unresolved. 2. Uptake curve and spectrum of a spectrally resolved fragment ion, where the precursor was interfered with even in $HDMS^E$ (inset).

Poster 44

Characterization of Proinsulin and Diabetes Mellitus-Associated Insulin Mutants by Hydroxyl-radical Footprinting and Molecular Modeling.

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Insulin, central to the hormonal control of metabolism, has long provided a model for the development of biophysical techniques. Renewed interest in the folding of insulin and its biosynthetic precursor (proinsulin) has been stimulated by the discovery of clinical mutations associated with permanent neonatal-onset diabetes mellitus (DM). Although such patients are heterozygous, the remaining wild-type insulin allele fails to enable metabolic homeostasis. A variety of evidence suggests that such DM-associated mutations impair the nascent folding of the variant proinsulin and block in *trans* the biosynthesis of the wild-type hormone. Disulfide-coupled protein misfolding disrupts trafficking from the endoplasmic reticulum, leading to beta-cell dysfunction and eventual cell death. This syndrome (which accounts for almost half of the cases of DM presenting within the first year of life) has motivated structural studies of proinsulin. Whereas an extensive crystallographic database of insulin structures has been obtained over the past four decades, proinsulin has proven refractory to crystallization. We therefore sought to establish an alternative approach that combines synchrotron-based hydroxyl-radical footprinting and molecular modeling to the biophysical characterization of proinsulin and disease-associated mutants. We also anticipate that these studies will provide a foundation for comparative studies of disease-associated mutant proinsulins.

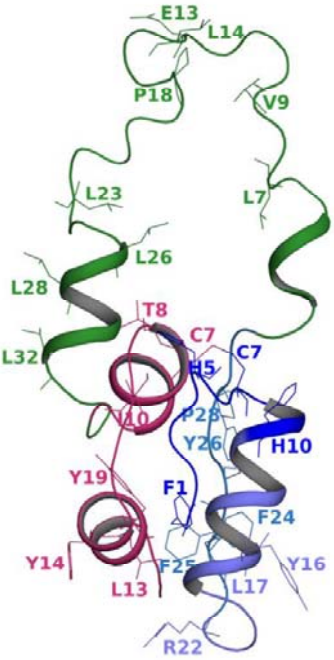

Figure 1. Modeled structure of wild-type proinsulin showing the amino acid residues undergoing modification in protein footprinting experiments. The A-chain is in *magenta* and C-chain in *green*. The three peptide fragments in B-chain are rendered in different shades of *blue*.

To test the feasibility of this approach, we have analyzed the differences between the footprints of insulin and proinsulin, defining a "shadow" of the connecting (C) domain. This structural information was used to refine proinsulin model (Figure1.). Our results demonstrate that in its monomeric form (i) proinsulin contains a native-like insulin moiety and (ii) the C-domain footprint resides within an adjoining segment (residues B23-B29) that is accessible to modification in insulin but not proinsulin. Corresponding oxidation rates were observed within core insulin moieties of insulin- and proinsulin hexamers, suggesting that the proinsulin hexamer retains an A/B structure similar to that of insulin. Further similarities in rates of oxidation between the respective C domains of proinsulin monomers and hexamers suggest that this loop in each case flexibly projects from an outer surface. Although dimerization or hexamer assembly would not be impaired, an ensemble of predicted C-domain positions would block hexamer-hexamer stacking as visualized in classical crystal lattices. In the next step, we have analyzed insulin mutants including B23Val human insulin where Gly-23 was mutated to Val-23 in B-chain, and B23Val and B25Leu double mutant of engineered monomer insulin (DKP) where Gly-23 and Phe-25 were replaced with Val-23 and Leu-25, respectively. Peptides B11-22 and B23-29 in the B-chain of B23Val mutant insulin showed significant increase in oxidation rate relative to native human insulin indicating that a significant conformational shift occurs in B-chain of B23Val compared to native insulin. In addition, no significant changes were observed for A1-21 peptide in A-chain. To further understand the effect of mutations on the conformation of the human insulin molecule, we will model of these mutants using footprinting constrains.

Poster 45

A Mass Spectrometry-based Carboxyl Footprinting Enrichment Strategy for Detection of Low Abundant Derivitized Peptides

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Mass spectrometry based protein footprinting is a powerful approach for characterizing changes in surface accessibility of various amino acid side chains as a function of ligand, denaturant, time, etc. One of the more commonly used protein footprinting techniques involves the irreversible derivatization of Asp and Glu sidechains through the addition of 1-ethyl-3-(dimethylaminopropyl)-carbodiimide (EDC) and glycine ethyl ester (GEE) (Gross et.al). In an effort to not disrupt native protein conformation during the labeling procedure, the conditions are kept such that the overall stoichiometry of the modifications are very low. One consequence of this, especially in more complex peptide mixtures from larger protein systems, is a reduced number of qualitatively identified labeled peptides during LC-MS/MS. We have developed a strategy in which protein carboxyl groups are labeled with EDC and pyridine dithioethylamine (PTEA) and the majority of the sample is subjected to enrichment on a sulfhydryl containing resin (**Figure 1**). After LC-MS/MS analysis of both fractions, the additional qualitative identifications obtained from enriched sample are then projected back to the non-enriched sample based on accurate-mass and retention time (AMRT). Ratios of labeled vs unlabeled peptides are then calculated from extracted ion chromatogram (EIC) peak intensities, regardless if the modified peptide is qualitatively identified in the non-enriched sample, overcoming the challenge of low stoichiometry modifications necessary to maintain native protein conformation during labeling. Preliminary data was acquired using 15 uM of purified human hemoglobin in pH 7.5 PBS labeled in the presence or absence of urea at a ratio of 1:6500:163 protein:PTEA:EDC. Trypsin digests from both enriched and non-enriched samples were analyzed by nanoscale LC-MS/MS (Waters NanoAcquity, 75 um ID x 250 mm 1.7 um BEH C18 UPLC column; Waters Synapt G2 HDMS). Spectra were searched (Mascot Server v2.2, SwissProt_Human DB) at 10 ppm precursor and 0.04 Da product ion tolerances allowing for a dynamic mass modification on DE residues corresponding to the final reduced/alkylated PTEA moiety. Results indicated that under these specific labeling conditions, PTEA modified peptides were exclusively qualitatively identified in the enriched sample LC-MS runs. However, in all cases the derivatized peptides could indeed be found through EICs in the non-enriched sample (average of 756-fold lower intensity). An example enrichment is illustrated in **Figure 2.** A description of the workflow, including automated AMRT using Rosetta Elucidator, will be presented in addition to the results from urea induced protein denaturation.

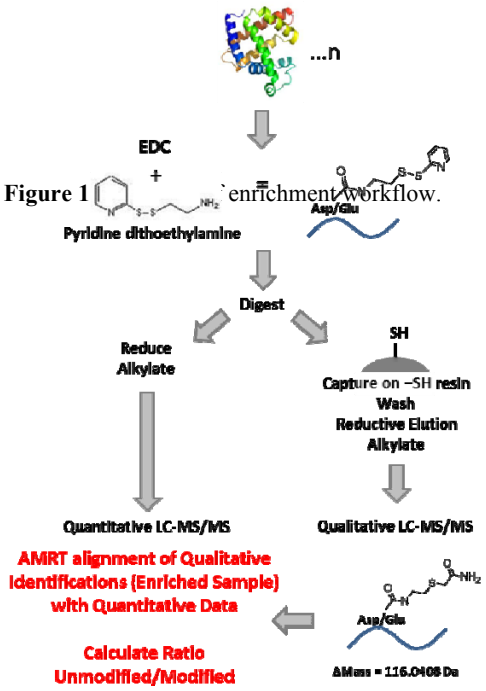

Figure 1 enrichment workflow.

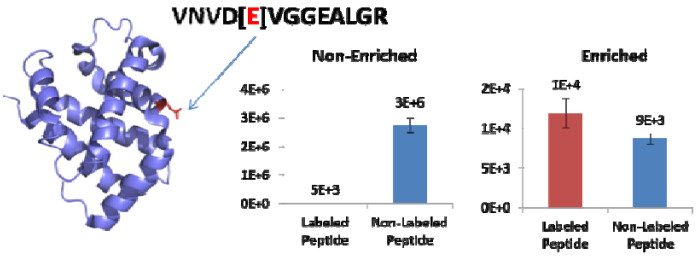

Figure 2. VNVDEVGGEALGR from hemoglobin labeled with PTEA.

Poster 46

Protein Fluorescence in the Gas Phase: the Green Fluorescent Protein and Protein-Dye Conjugates for Probing the Structure of Gaseous Protein Ions

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Electrospray ionization mass spectrometry (ESI-MS) has emerged as a powerful tool for structural biology. Information on supramolecular assembly of biomolecular non-covalent complexes, such as the stoichiometry, subunit composition, topography, and relative binding strength can be accessed. Despite increasing evidence suggesting that the quaternary structure features are correctly reflected by ESI mass spectra little is known on the tertiary structure of biomolecular gaseous ions.

We utilize a combination of Fourier-transform ion cyclotron (FTICR) mass spectrometry and fluorescence spectroscopy to probe the photophysical properties of the green fluorescent protein (GFP) in the gas phase. GFP is a 27 kDa globular protein that is strongly fluorescent in the folded state thanks to the presence of covalently bound natural chromophore. The fluorescence of GFP is completely lost upon unfolding – the property allowing for probing the protein tertiary structure in the gas phase by our method.

In our preliminary experiments on electrosprayed GFP ions, an exceptional stability and compactness of the protein conformation was demonstrated: *i*) the protein exhibited the lack of dissociation in sustained off-resonance irradiation collision-induced dissociation (SORI-CID) experiments; *ii*) no laser-induced dissociation was found; *iii*) the collision cross-section of native-like GFP ions measured by traveling wave ion mobility spectroscopy (TWIMS) was in reasonable agreement with the value predicted based on available high-resolution 3D structures of GFP variants. In contrast to these findings, no laser-induced fluorescence of GFP ions was detected inside the FTICR cell up to now, which can be attributed either to a gas-phase conformational rearrangement of GFP or to insufficient sensitivity of our setup.

In the present work, we suggest to use covalent conjugates between fluorescent dyes and different globular proteins including GFP and bovine ubiquitin to rule out the aforementioned sensitivity issue. Recently, rhodamine dyes, free and covalently linked to peptides, were shown to retain their strong fluorescence in the gas phase. We used an amino-reactive N-hydroxysuccinimidyl (NHS) ester of 5,6-carboxyrhodamine 6G (cR6G) to prepare the protein-dye conjugates. Several protein-to-dye ratios (1:1, 1:3, 1:10, 1:20, and 1:50) were tested yielding the desired degree of labeling. At low molar ratios the attachment of one or two dye molecules per protein was favored, while up to 5 labels were found on ubiquitin at high excess of cR6G-NHS. The selectivity of labeling can be increased by lowering the pH of reaction mixture from 8.3 to 7.0-7.5 or by using activated dyes with a different reactivity, e.g. thiol-reactive maleimides. The gas-phase optical spectroscopy experiments on different variants of protein-dye conjugates including laser-induced fluorescence and photofragmentation complement our studies of the bare GFP and allow us to rule out low sensitivity of the optical setup.

Poster 47

Probing the Topology of Large Protein Complexes Using Chemical Cross-Linking and Mass Spectrometry

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The concept of chemical cross-linking (XL), which uses the formation of covalent bonds between two functional groups within a protein or between two interacting proteins, has been applied to the analysis of (small) protein complexes for more than a decade. More recently, the combination of cross-linking with advanced mass spectrometry techniques has enabled the application of this technique to larger protein assemblies. Still, the number of published reports remains limited to date, and widespread use is hampered by the complex MS analysis of cross-linked peptides and the lack of dedicated software for high-throughput data analysis. In order to make XL-MS more generally applicable and an accepted tool for structural biologists, we are developing an integrated workflow for the routine generation of structural information from protein complexes.

We have opted for a workflow in which protein samples react with two differentially isotope-labeled forms of a cross-linking reagent (e.g. the amine-reactive disuccinimidyl suberate) so that all modified peptides obtain a characteristic isotope pattern. After the cross-linking step, samples are enzymatically digested, enriched for cross-linked peptides and analyzed by LC-MS/MS. xQuest, a dedicated search engine developed in our group, is then used for identification of cross-linked peptides from tandem mass spectra.

We demonstrate that an optimized workflow now allows the identification of up to several hundred nonredundant cross-links in large protein complexes, including proteasomes and RNA polymerases. As we will show, the concept may be extended to the study of protein interaction networks by obtaining cross-links from affinity purified complexes. The increased complexity of such samples also requires careful control of false discovery rates in the resulting data sets. Accordingly, the integration of quality control measures in the xQuest pipeline will also be discussed.

Poster 48

Detection of Non-covalent Interactions of Single Stranded DNA with Escherichia Coli Single-Stranded DNA-Binding Protein by MALDI-MS

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The Escherichia coli single-stranded DNA binding (SSB) protein selectively binds single-stranded (ss) DNA and participates in the process of DNA replication, recombination and repair. Different binding modes have previously been observed in SSB□ssDNA complexes, due to the four potential binding sites of SSB. Here, chemical cross-linking, combined with high-mass matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS), was applied to explore the interactions in the complex. SSB forms a stable homotetramer in solution, but only the monomeric species (*m/z* 19,100) can be detected with standard MALDI-MS. With chemical cross-linking, the quaternary structure of SSB is conserved and the tetramer (*m/z* 79,500) was observed. By comparing the results generated with and without cross-linking, we could define the stoichiometry and subunit assembly of various SSB□ssDNA complexes. Besides the cross-linker, ssDNA also functions as a stabilizer to conserve the quaternary structure of SSB, as evidenced by the detection of a SSB□ssDNA complex at *m/z* 94,200 even in the absence of cross-linking. This observation is attributed to electrostatic interactions that are enhanced in the gas phase, which was further verified by applying a series oligonucleotides of different length. The capability to simultaneously observe different species in mixture sample by applying high mass MALDI suggests the possibility to analyze the interactions between SSB and ssDNA in vivo.

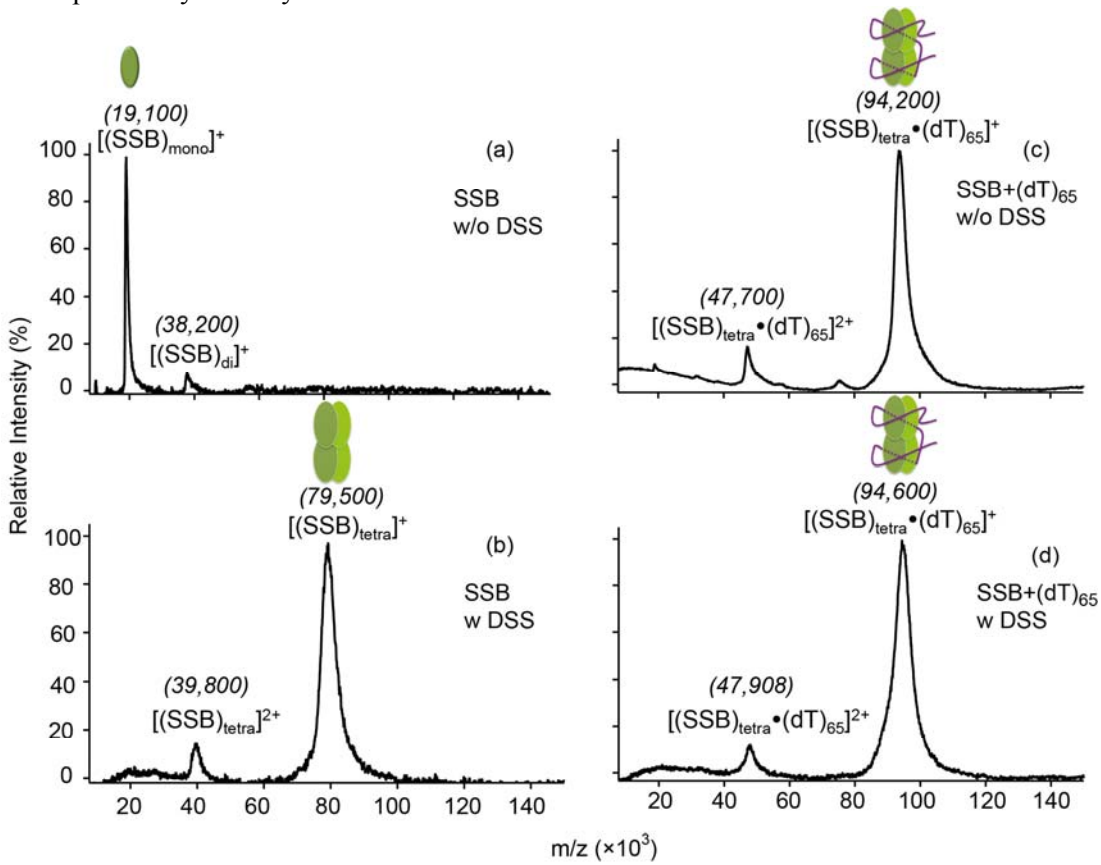

Figure 1. High-mass MALDI mass spectra of SSB (a) without cross-linking and (b) after cross-linking; SSB interacting with (dT)65 at a protein-to-DNA molar ratio of 4:1 (c) without cross-linking and (d) after cross-linking.

Poster 49

Concatenated Protein Oligomers for High-Mass MALDI-MS Calibration

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When studying protein-protein interactions, detecting interaction sites of protein complexes, or defining stoichiometric composition of protein assemblies, mass spectrometry is one of the methods of choice in systems biology. Stabilization of these interactions with chemical crosslinking enables analysis by matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS). Compared to spectra recorded with electrospray ionization (ESI) MS, MALDI-MS leads to simpler spectra containing direct information about the mass and the stoichiometry of the system under investigation. The ions generated in the MALDI process usually possess low charge states. Therefore, when dealing with intact proteins and their complexes, the m/z ratio becomes rather large. A calibration to determine the exact mass of the sample is therefore crucial. Up to now, there are no suitable calibrants for the m/z range above 100 kDa available.

To obtain a sample with a well-known sequence and therefore known mass, we propose to use a genetically engineered concatamer of maltose binding protein (MBP). Between the concatenated proteins, specific recognition sites are introduced, allowing for orthogonal proteolytic cleavage. These oligomers and their fragments can also be crosslinked covalently, thus allowing the synthesis of monomeric, dimeric, tetrameric, and hexameric species. These MBP oligomers can be used as calibrants to an m/z range up to 250 kDa. Separation of the different oligomers and mixing in well-known ratios allows careful tuning of the required calibration-range as well.

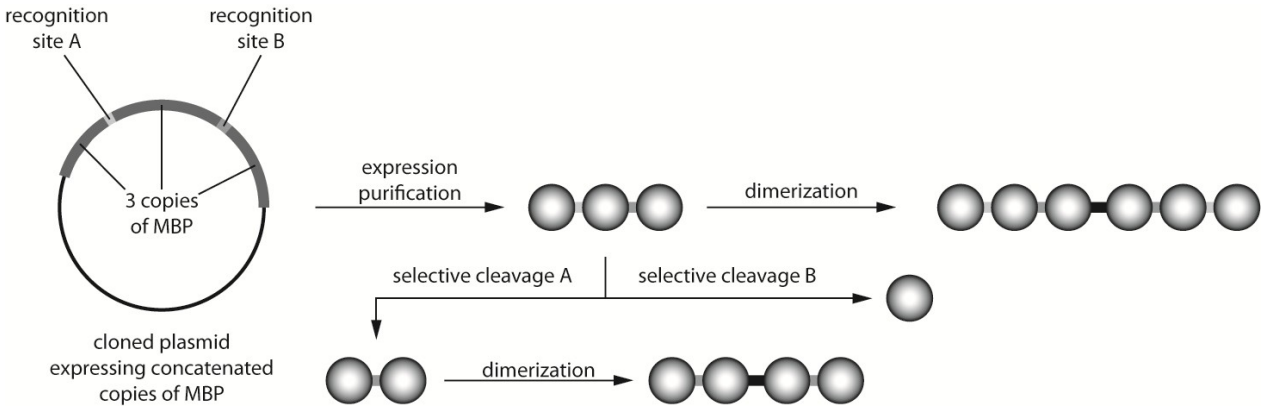

Figure 1: Expression of concatenated maltose binding protein separated by specific recognition sites allows orthogonal cleavage of the expressed protein. Covalent crosslinking of the complete MBP oligomer or its subunits gives access to a broad range of calibrants in a high m/z range.

Poster 50

Observing the Binding Site and Dynamics of Protein S1 on a Bacterial Ribosome Using Cross-Linking and Mass Spectrometry

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Although ribosomal protein S1 has been shown to be a significant effector of prokaryotic translation, structural insights into this process have remained elusive as S1 is recalcitrant to traditional techniques of structural analysis. Nevertheless, through the application of protein cross-linking and high resolution mass spectrometry, we have detailed the ribosomal binding site of S1 and have observed evidence of its dynamics. Intact 30S ribosome subunits were isolated from *E. coli* cells and exposed to our diethyl suberthioimidate cross linker. Proteins were extracted from the modified subunits, fractionated by size-exclusion chromatography and digested with trypsin. The resulting peptides were analyzed by ion exchange and reversed phase chromatography and LTQ-orbitrap mass spectrometry. Mass spectra were interpreted using the algorithm *Xi*. A total of 23 interprotein linkages involving S1 and other ribosomal proteins were identified, many of which are displayed in Figure 1. S1 is known to have six beta barrel domains. Those that are believed to bind the ribosome were found to cross-link to specific regions of the 30S subunit. Others, that are believed to bind mRNA, cross-link to residues spread far across the subunit, suggesting that they are structurally flexible and highly dynamic. Our results are thus consistent with a previous hypothesis that S1 acts as the mRNA catching arm of the prokaryotic ribosome.

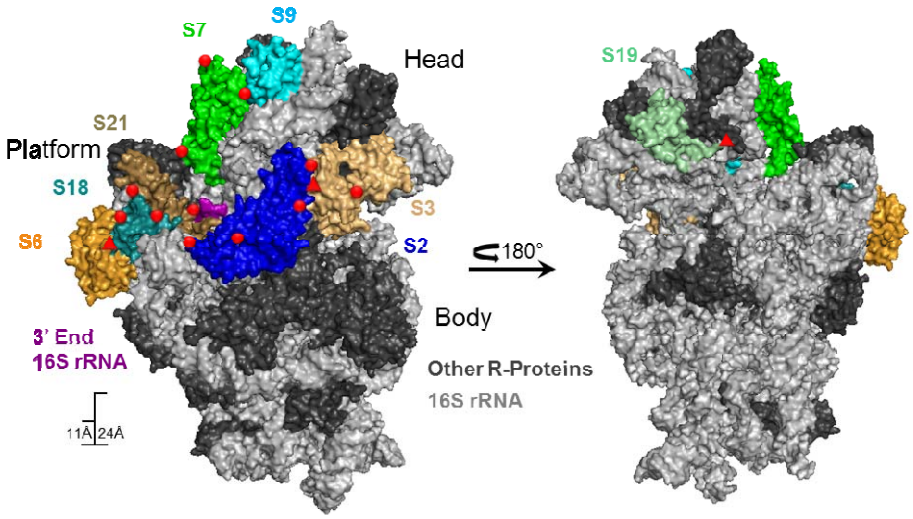

Figure 1. Cross-linking of S1 to the 30S subunit. Residues found cross-linked to S1 are marked with red circles. Triangles denote residues that could not be explicitly shown. The triangles on S6 and S19 show the approximate positions of residues not present in the crystal structure, and the triangle on S3 shows the location of a residue found to be cross-linked to S1 but not solvent accessible in the crystal structure .

Poster 51

DEAH Box Helicase Prp43-Ntr1 Complex: Insights into Structure Combining Oxidative Footprinting with Protein-RNA Cross-Linking and Mass Spectrometry

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The spliceosome is a complex RNP (ribonucleoprotein) machinery that catalyses pre-mRNA splicing. Once the second step of catalysis is completed, and the mature mRNA is released, the spliceosomal particles need to be disassembled to begin a new cycle of catalysis. Prp 43 is an ATP dependent DEAH box helicase with post splicing activity which is required for the disassembly of the spliceosome and release of the excised intron. The disassembly of RNPs occurs in an active process for which Ntr1 is needed. In order to gain molecular insight into this phenomenon several high resolution structural determination studies were attempted. Due to the fully unstructured nature of Ntr1 conclusive X-Ray results remain elusive. In this context we attempt to obtain information on the structure of this complex by combining oxidative footprinting and protein-RNA cross-linking experiments with high resolution mass spectrometry. We subjected the Prp43-Ntr1[1-120] complex, as well as its individual components, to hydroxyl radicals. These covalently modify the side chains of solvent accessible amino acid residues. Proteins were digested with trypsin and analyzed by LC-coupled ESI-MS/MS. A data-analysis strategy that involves the identification and label-free quantification of modified peptides on the MaxQuant platform was developed. By comparing the levels of modification in both individual proteins and protein complex, we could derive information on the binding interface and changes associated to the formation of the complex. Since the activity of the complex is that of an RNA helicase, the contact sites of Prp43 to RNA are of special interest. A UV cross-linking approach was utilized to covalently link reactive bases on RNA with proximal amino acid residues. Samples were hydrolyzed with RNAses and trypsin and the obtained mixture was subjected to desalting and titanium dioxide columns in order to selectively enrich for cross-linked fragments. Finally, the cross-linked heteroconjugates were identified by LC-coupled tandem mass spectrometry.

Using hydroxyl radical footprinting studies we identified residues in the C-terminal OB-fold domain of Prp34 which show stark solvent accessibility changes, indicating the binding site of Ntr1. The N-term domain of Prp43 connects the top and bottom of the structure with a loop; changes in solvent accessibility in this region suggest a coordinated movement of the N-term and C-term OB fold domain upon Ntr1 binding. With the protein-RNA cross-linking studies several contact sites in both the RNA and peptide moieties were identified. These contact sites give new insights into the molecular players that define the functional role of the Prp43-Ntr1 complex. This structural analysis approach, in combination with molecular modelling, can succeed where traditional structural determination techniques are unsuitable.

Poster 52

Oxidation Induced Conformational Changes in Calcineurin Studied by 'Fixed Charge' Chemical Derivatization and Data Dependant Multistage Tandem Mass Spectrometry

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Calcineurin (CN) is a Ca^{2+}/Calmodulin (CaM) activated serine/threonine phosphatase that is widely distributed in mammalian tissues. CN functions in a signal transduction pathway to regulate gene expression and participates in a wide variety of physiological processes. Besides being regulated by CaM, CN is also reported to be inactivated by H_2O_2- or superoxide-induced oxidation both *in vivo* and *in vitro* [1,2]. Here, in order to obtain further insights into oxidation induced CN inactivation from a structural perspective, the relative rates of H_2O_2 induced oxidative modification of methionine residues within CN were first determined using a multi enzyme (trypsin, Lys-C and Glu-C) digestion strategy coupled with triplicate analysis using capillary HPLC-ESI-MS and CID-MS/MS. Then, a 'fixed charge' sulfonium ion containing amine-specific protein modification reagent, S,S'-dimethylthiobutanoylhydroxysuccinimide (DMBNHS) coupled with data dependant multistage tandem mass spectrometry was applied to identify changes in CN conformation before and after oxidation. After being incubated with H_2O_2 for various times (0, 15, 30, 45 and 60 min), CN was modified by using different molar excesses of DMBNHS, followed by Glu-C digestion. The resulting samples were then analyzed by capillary HPLC-ESI-MS, CID-MS/MS and data dependant neutral loss mode MS^3, similar to that reported previously [3]. CID-MS/MS of DMBNHS modified peptides results in the formation of characteristic product ions via the exclusive neutral loss(es) of dimethylsulfide (DMS), thereby enabling the simple identification of modified peptides from within complex mixtures. The observation of these characteristic neutral losses was then used to automatically 'trigger' the acquisition of an MS^3 spectrum to allow the peptide sequence and the site(s) of modification to be characterized. Importantly, the extent of DMBNHS modification of several CN lysine residues was found to increase upon incubation with H_2O_2, indicating a conformational change of CN after oxidation. These changes are correlated with different functional domains of CN, and are suggestive of a role for oxidation induced conformation change in CN as a possible cause of CN inactivation by inhibiting CN/CaM interactions.

1. Rusnak, F.; Mertz, P. Calcineurin: form and function. *Physiol. Rev.*, **2000**, *80,* 1483-1521.
2. Carruthers, N.J.; Stemmer, P.M. Methionine oxidation in the calmodulin-binding domain of calcineurin disrupts calmodulin binding and calcineurin activation. *Biochem.*, **2008**, *47,* 3085-3095.
3. Zhou, X.; Lu, Y.; Wang, W.; Borhan, B.; Reid, G.E. 'Fixed charge' chemical derivatization and data dependant multistage tandem mass spectrometry for mapping protein surface residue accessibility. *J. Am. Soc. Mass Spectrom.*, **2010**, *21,* 1339-1351.

Poster 53

Studying Structures and Conformation Dynamics of Peptides and Proteins Using Host-Guest Chemistry in the Gas Phase

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We present studies of structures of peptides and small proteins using host-guest chemistry in the gas phase. Cucurbit[6]uril (CB[6]) and 18-crown-6 (18C6) are neutral cyclic molecules, which are efficient host molecules in molecular recognition and have high affinity for positively charged or cationic compounds, especially, in the gas phase. We have investigated the host-guest chemistry of CB[6] and 18C6 to lysine residues in gas phase and its applications to study structures of peptides and proteins using mass spectrometry and ion mobility mass spectrometry combined with electrospray ionization. For example, the propensity of the alanine based peptides Ac-Ala_{9-n}-$LysH^+$-Ala_n (n = 0, 1, 3, 5, 7, and 9) to form a helix in the gas phase is improved via host-guest chemistry with 18C6.[1] Low energy collision induced dissociation of a CB[6]-protein complex yields highly selective fragments and additional MS^n spectra reveal details of the CB[6] binding sites, which allow us to deduce the protein structures in the solution phase.[2] Further applications of host-guest chemistry related to controlling protein structure dynamics are discussed.

References

(1)Ko, J. Y.; Heo, S. W.; Lee, J. H.; Oh, H. B.; Kim, H.; Kim, H. I. *J. Phys. Chem. A* **2011**. Article ASAP, DOI: 10.1021/jp208045a

(2)Heo, S. W.; Choi, T. S.; Park, K. M.; Ko, Y. H.; Kim, S. B.; Kim, K.; Kim, H. I. *Anal. Chem.* **2011**, *83*, 7916-7923.

Poster 54

Understanding the Protein-Protein Interactions of the Human Fe-S Cluster Complex using Hydroxyl Radical Footprinting

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Texas A&M University

Iron-sulfur (FeS) clusters are integral components in many biological processes. In humans, FeS clusters are assembled by a complex of four proteins before transfer to apo acceptor proteins. Although the identity of the four proteins is known, the spatial orientation of the four proteins has remained elusive. Standard structural techniques such as NMR spectroscopy and protein crystallography are unable to be used for the human FeS cluster complex due to its size and instability. Therefore, we have decided to employ hydroxyl radical footprinting to determine the surfaces of binding for these four proteins.

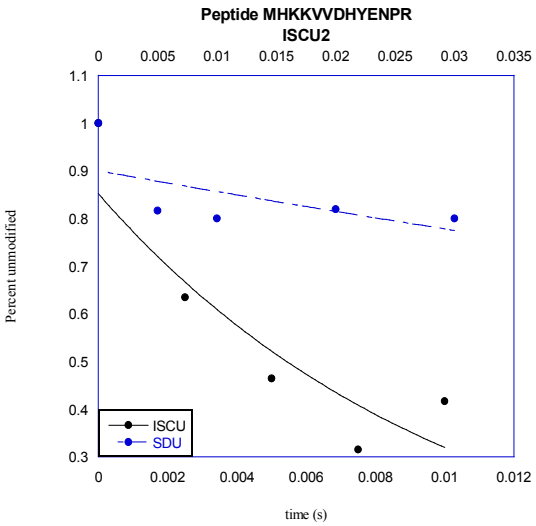

Figure 1. Change in oxidation rate between unbound and bound ISCU2 to NFS1/ISD11. Black is the protein alone in solution. Blue is ISCU2 bound to NFS1/ISD11.

Individual proteins were labeled in buffer and then added sequentially to form the complex. The area of protection upon the addition of different subunits indicates where that protein binds. After exposure to X-ray radiation and quenching with methionine, the samples were trypsin digested and analyzed by MALDI-TOF MS. Rates of oxidation were compared between proteins in solution and in the complex. The modifications were mapped onto homologous crystal structures from bacteria and compiled to give an overview of the four protein complex.

Initial data analysis of the ISCU2 scaffold protein binding to the NFS1/ISD11 cysteine desulfurase protein complex indicate two regions of protection compared to the ISCU2 protein alone in solution (Figure 1). For ISCU2 alone in solution, the peptide corresponding to the N-terminus had a rate of oxidation of 98 min^{-1}. When ISCU2 was bound to NFS1/ISD11, this peptide oxidation rate decreased to 5 min^{-1}. The change in rate for the specific peptides has been mapped onto a homologous protein structure from E. coli (Figure 2).

Future experiments include mapping the areas of protection on the NFS1/ISD11 protein when ISCU2 is bound. The fourth protein, FXN, will then be added to the complex and the binding surface will be determined for NFS1/ISD11 and the protein-protein interactions with ISCU2.

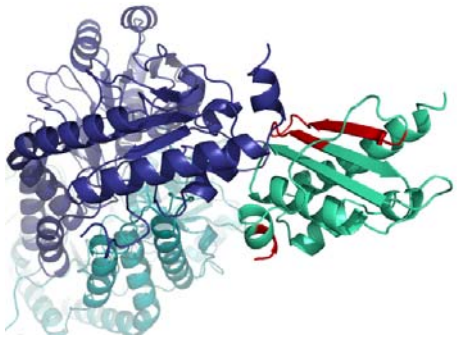

Figure 2. Homologous crystal structure of IscS and IscU2 interactions from *E. coli*. The peptides in red show had decreased rates of oxidation for human ISCU2 when bound to human NFS1/ISD11.

Acknowledgements: We would like to thank R. D'Mello and S. Gupta at Brookhaven National Lab for performing hydroxyl radical experiments.

Poster 55

A Computational Data Analysis Platform for Identifying Zero-Length Chemical Crosslinks using Tandem Mass Spectrometry

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Identifying protein-protein interactions and solving structures of large macromolecular complexes are crucial steps in understanding diverse biological systems. Although the combination of powerful techniques such as X-ray crystallography and NMR allows us to determine high-resolution structures of modest sized proteins and small complexes, solving structures of large protein complexes remains impractical.

Chemical cross-linking coupled with modern mass spectrometry (MS) methods offers a unique opportunity to help address these issues since the technique provides distance constraints for inter-molecular and intra-molecular interactions, allowing accurate assembly of detailed structural data of individual, smaller components. Furthermore, intra-molecular distance constraints are useful for refining predictions made by molecular modeling of proteins with unknown structures.

Zero-length crosslinkers such as EDC yield the most stringent distance constraints of roughly 10 Angstrom between the two crosslinked residues and therefore, they are best-suited for refining protein structure predictions. However, because no external atoms are added to the crosslink products by zero-length crosslinkers, techniques such as stable isotope labeling and MS cleavage cannot be employed to reduce the complexity of the resulting MS data.

We have developed a high-resolution MS data acquisition strategy and label-free data analysis pipeline together with new computational software tools to identify crosslinks, especially zero-length crosslinks, with high throughput and accuracy. Our strategy relies on an initial high-resolution LC-MS label-free comparison to identify candidate signals unique to the crosslinked sample and subsequent rounds of targeted high-resolution LC-MS/MS to obtain high mass accuracy MS/MS fragmentation patterns for every potential crosslink candidate. To narrow down the list of crosslink candidates and increase efficiency, our software tool compares the distributions of sequence-spectrum correlation scores for MS/MS data from crosslinked and control samples, using the control samples as negatives. Lastly, our software tool automatically summarizes and annotates MS/MS spectra of high-confidence crosslink candidates, allowing facile manual review and verification.

Poster 57

Evaluating Protein Solvent Accessibility via Electron Transfer Dissociation and 351 nm Ultraviolet Photodissociation Mass Spectrometry

John O'Brien, Jennifer Brodbelt

Department of Chemistry and Biochemistry, University of Texas at Austin

NMR and x-ray crystallography have been the most popular methods for determination of protein conformation, but several new mass spectrometric strategies have been developed for evaluation of protein conformation and solvent accessibility. The solvent accessible surface area (SASA) provides insight into protein folding and regions of proteins that are significantly influenced upon protein-ligand interactions. SASA can be evaluated by covalent modification of surface accessible residues, followed by mass spectrometric analysis of the protein after modification. Highly exposed residues (those on the surface) are more reactive than buried residues, thus allowing the relative SASAs to be mapped based monitoring individual residues and their extent of chemical modification. This approach typically entails enzymatic digestion of the proteins after modification, followed by conventional bottom-up LCMS/MS analysis of the peptides . One of the most significant hurdles of this strategy is the ability to track and identify the modified peptides relative to the numerous unmodified peptides in the complex digests.

We have developed a new lysine-specific reagent (**Figure 1**) to probe the surface accessibility of proteins. The reagent was designed to incorporate a selectively ETD-cleavable hydrazone bond, as well as a strong UV chromophore. This facilitates the use of ETD and UV photodissociation to track the modified peptides. ETD promotes N-N bond cleavage, resulting in a characteristic neutral loss of the amino pyridine moiety and thus facilitating a data-dependent MS/MS mode for identification of the modified peptides. In addition, the modified peptides are labeled with a strong UV chromophore that endows them with high photoabsorptivity at 351 nm, providing an easy way to differentiate modified and unmodified peptides. The relative reactivities of the lysine-containing peptides based on their abundances in the LC-MS/MS runs are correlated with their surface accessibilities. This strategy is demonstrated for the protein complex eIF4F, a novel initiation factor involved in plant protein translation, and eIF4E, a single subunit of the protein complex eIF4F.

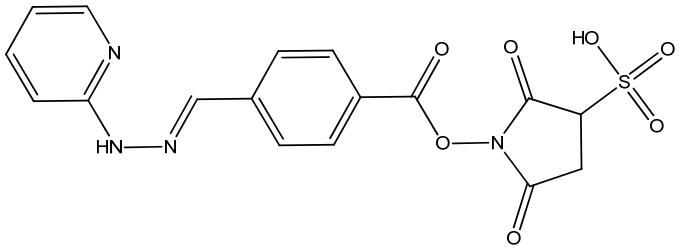

Figure 1. Structure of the lysine-selective chemical probe used for evaluation of surface accessibility.

Poster 58

Does the Whole Influence the Parts in an Fc Fusion Protein?

Damian Houde and Steven A. Berkowitz

Biogen Idec, Inc., Cambridge, MA

Fusion proteins involve the recombinant coupling of different gene products with diverse functions into a single protein. Those fusion proteins that involve the coupling of an immunoglobulin gamma (IgG) Fc to a native protein are called Fc fusion proteins and offer a specific advantage over the traditional biopharmaceutical drugs. The Fc fusion protein presents longer circulation life, which may ultimately limit the need for reoccurring treatments and/or provide additional functionality that further improves their therapeutic value. In developing such a fusion protein, a key concern that arises is the impact of chemical complexion on the higher-order structure of the two proteins being coupled. This work focuses on showing the comparability of a commercial recombinant factor IX (rFIX) (a calcium dependent serine protease critical for blood coagulation) with an innovative and promising recombinant therapeutic fusion protein, factor IX-Fc (rFIX-Fc), which is being evaluated clinically for the treatment of the blood clotting disorder hemophilia B (1). We studied and compared the conformations of rFIX-Fc, rFIX and Fc fragment (isolated from rFIX-Fc) with and without Ca^{2+} and in so doing, we have made significant progress in understanding the influence these individual protein components have on the other.

Reference

1.Peters, R. T., Low, S. C., Kamphaus, G. D., Dumont, J. A., Amari, J. V., Lu, Q., Zarbis-Papastoitsis, G., Reidy, T. J., Merricks, E. P., Nichols, T. C., and Bitonti, A. J. (2010) *Blood* **115**, 2057-2064

Poster 59

Strong Metal Ions Bind Peptides in the Iminol Conformation

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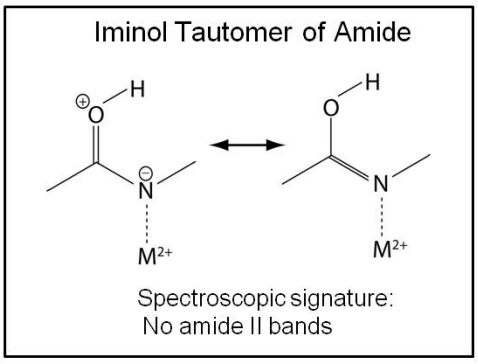
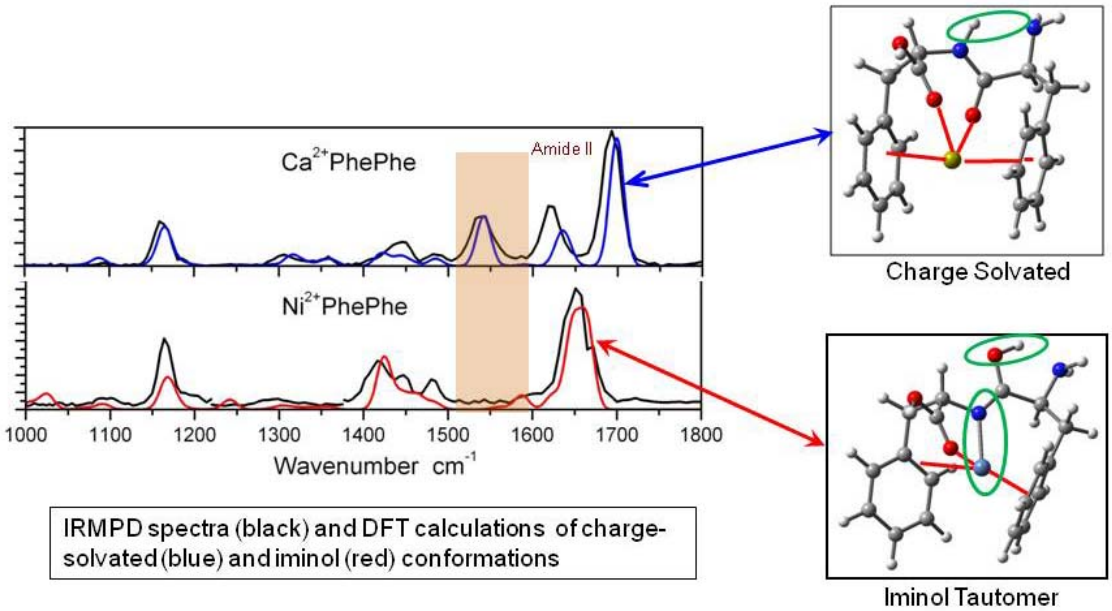
Metal ion complexes with small gas-phase peptides have frequently been observed in the charge-solvated binding mode. The metal ion is anchored to backbone carbonyls and to Lewis-basic chelation points on the chain termini and side chains. In some cases rearrangement to the zwitterion, with metal ion binding to the carboxylate, has also been found as a possible alternative.



Using IRMPD (infrared multiple photon dissociation) spectroscopy at the FELIX free electron laser facility, we have recently uncovered a new binding mode, termed the ***iminol*** mode (left, showing two resonance forms), adopted by strongly binding divalent metal ions including Mg^{2+} and Ni^{2+}. The metal ion displaces the amide nitrogen. A spectroscopic signature of the tautomerization is the disappearance of the characteristic Amide II band normally seen in peptide ion spectra.

Iminol binding is considered rare or non-existent in condensed phase metal-ion complexation to peptides. If binding to the backbone amide nitrogens takes place, it is normally associated with simple deprotonation, not tautomerization of the amide group. The poster will discuss recent results exploring the prevalence and characteristics of this new binding mode in the gas phase.



IRMPD spectra (black) and DFT calculations of charge-solvated (blue) and iminol (red) conformations

Poster 60

In Situ Enrichment of Phosphopeptides on MALDI Plates Functionalized by Ambient Ion Landing

Petr Pompach[1], Lukas Krásný[1,2], Marcela Strnadova[1], Petr Novak[1], Martin Strohalm[1], Vladimir Havlicek[1], Frantisek Tureček[3], Karel Lemr[4], Oldrich Benada[1], and Michael Volný[1]

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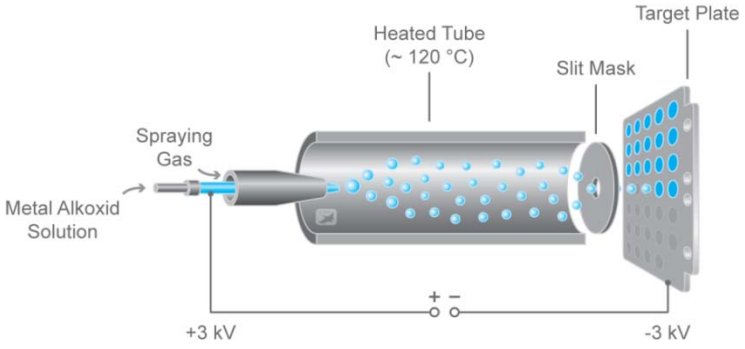
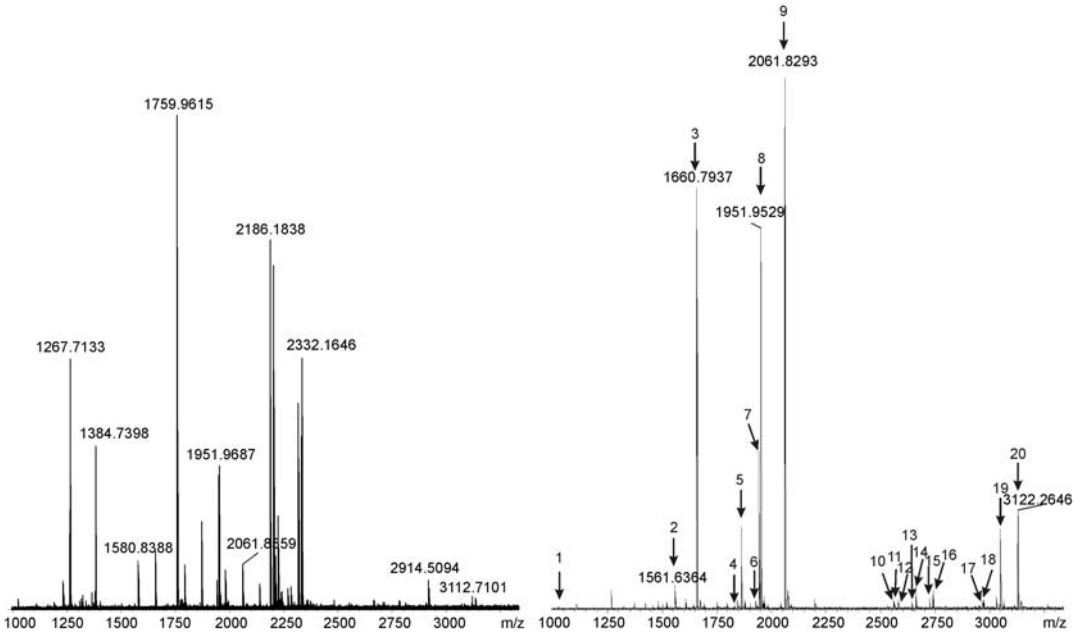
[3]*Department of Chemistry, University of Washington, Seattle, Washington, USA*

[4]*Department of Analytical Chemistry, Palacky University, Olomouc, Czech Republic*

We report substantial *in situ* enrichment of phosphopeptides in peptide mixtures using titanium and zirconium oxide coated MALDI plates prepared by recently reported ambient ion landing technique. The approach was based on electronebulization (electrospray) of solutions of organometalic compounds related to elements of 4B class of periodic table of elements. The generated charged electrospray was dried on the fly by passing the heated compartment and focused to a surface forming a stable oxide layer there (top figure). The surface modified this way has been used for phosphopeptide enrichment from peptide mixtures prior to desorption/ionization mass spectrometry. The surface modification process itself is cheap and provides rugged surface surviving multiple sample loading and washing. Phosphopeptides from the whole casein peptide digest (bottom left) were successfully enriched (bottom right) using prepared spots and optimized washing procedure protocol. Spots were examined by MALDI-FTICR mass spectrometry. The proof of concept was also verified on enrichment of phosphopeptides derived from trehalase (data not shown). The modified surfaces can, in principle, be used in LC-MALDI mode.





Acknowledgement: This work was supported by Ministry of Education, Youth and Sports of the Czech Republic (ME10013) and Czech Science Foundation (P206/10/P018, 204/09/H084, P206/12/1150).

Poster 61

Characterizing Self-Assembled Extracellular Matrix Fibrillar Films with TOF-SIMS

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Cell interactions with the extracellular matrix (ECM) initiate an array of biophysical and biochemical signals that drive biological processes ranging from embryonic development to cancer progression. Consequently, developing model ECMs and analytical techniques to determine their properties, as well as the resulting cellular responses, is vital to understanding how the ECM regulates cell state, and for engineering such structures as bioscaffold for tissue repair and regeneration. Adsorbed protein monolayers are often used as model ECMs, but are not analogous to what is experienced *in vivo*, where ECM proteins often self-assemble into higher order structures. Decellularized ECM scaffolds derived from tissue or cell cultures offer a biologically relevant model, but the complexity of these systems often makes it difficult to understand how specific components influence cell function. We have previously developed a model ECM comprised of films of fibrillar Type 1 collagen, which are robust and highly reproducible in the cell behaviors they evoke [1]. The mechanical properties of these films strongly influence cell responses such as proliferation, cytoskeleton assembly and activation of specific signalling molecules [1]. In this study, we employ TOF-SIMS and principal component analysis (PCA) to examine if there are chemical or orientation differences that may be responsible for differences in cell responses to mechanically compliant or mechanically rigid collagen fibril matrices. Our results establish the feasibility of this method as a label-free approach to identify chemical differences in ECM preparations, and allow us to distinguish between more complex binary and ternary self-assembled ECMs. We show that TOF-SIMS is likely to be a valuable technique in the analytical toolbox for characterizing biomanufactured ECMs.

[1] A. L. Plant, K. Bhadriraju, T. A. Spurlin, J. T. Elliott, *BBA-Mol. Cell Res.*, **2009**, 1793, *893-902*

Poster 62

PCA of TOF-SIMS Data to Elucidate Lipid Distribution Within Biological Membranes

Christopher R. Anderton,[a,b]* Bita Vaezian,[a] Kaiyan Lou,[a] and Mary L. Kraft[a]

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[b]*Current institution: National Institute of Standards and Technology, Gaithersburg, MD 20899*

Current theory suggests that the non-random distribution of membrane components is responsible for many vital cellular functions such as signalling, transport, and sorting. With the benefit of immunohistochemisty and fluorescent protein constructs methods, protein aggregation within the cell membrane, and its role in cell processes is well established. However, elucidating the lateral composition of lipids within the cell membrane is more challenging, due in part to analytical and physiological constraints. Here, we demonstrate the utility of PCA to discriminate between structurally similar phosphatidylcholine species, which differ only in fatty acid chain length, based upon spectral variances in the positive-ion TOF-SIMS data [1]. By employing PCA on TOF-SIMS images of phase separated supported lipid membranes made from these structurally similar phosphatidylcholine species, we demonstrate the ability to reveal the lateral distribution of the lipids with higher contrast than that of individual TOF-SIMS ion images. In further studies, we identify the positive-ion TOF-SIMS fragments relating to phosphatidylcholine lipid headgroups and tail groups [2]. By using restricted peak sets in PCA, which only relate to lipid content, we reduce the variability that can be attributed to contamination and increase the probability that discrimination is based on chemical differences related to lipid composition. Overall, we expect PCA of TOF-SIMS data, coupled with a comprehensive lipid-related peak set, to be useful in analyzing complex biological membrane systems.

[1] B. Vaezian, C. R. Anderton, M.L. Kraft, *Anal. Chem.*, **2010**, *24*. 10006-10014.
[2] C. R. Anderton, B. Vaezian, K. Lou, J. F. Frisz, M.L. Kraft, *Surf. Interface Anal.*, **2011**, **doi:** 10.1002/sia.3806

Poster 63

Custom QIT Mass Spectrometer for Infrared Photodissociation Spectroscopy of Biomolecular Ions

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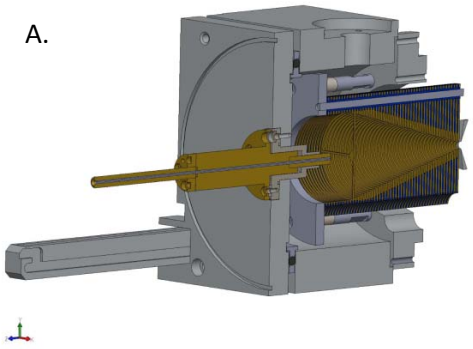
Infrared multiple-photon dissociation (IRMPD) spectroscopy is a technique that combines the ultra-low sensitivity of mass spectrometry with the wealth of structural information from the IR measurements. IRMPD experiments require bright, tunable radiation sources to induce dissociation via the absorption of tens to hundreds of photons. While free electron lasers (FELs), such as FELIX and CLIO, are well suited to this task, recently it has been shown that bench-top tunable oscillating parametric oscillator/amplifiers (OPO/As) can readily dissociate small, covalently-bound systems.[1] We present recent developments of a laboratory-constructed mass spectrometer, coupled to an OPO and a CO_2 laser, where IRMPD of biomolecular ions is carried out in a reduced pressure quadrupole ion trap (QIT).

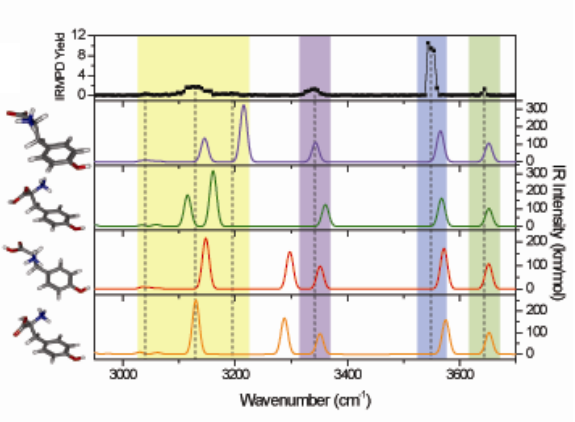
Ions of interest are generated via a commercial electrospray ionization source, mass selected via a quadrupole mass filter, and stored in a rectilinear ion guide. Ions are ejected out of the rectilinear ion guide following a pulsed gas event, momentarily raising the pressure to ~10^{-3} torr, within the QIT.[2] Trapped ions are irradiated with the output from a tunable OPO (LINOS Photonics OS 4000) and a continuous wave CO_2 laser (Apollo lasers). Subsequently, ions are pulsed out of the ion trap and analyzed via a time-of-flight (ToF) mass analyzer.

Recently, much attention has been focused on instrumental development, and these efforts have culminated in the addition of an electrodynamic ion funnel and heated, metal capillary (model shown in Figure A.) to the source region of the custom-built mass spectrometer. These developments have increased signal intensity by an order of magnitude, and allowed IRMPD spectroscopy experiments to be performed on ions that are inherently low in abundance. The increased sensitivity facilitates the routine collection of IRMPD spectra in the hydrogen-stretching region, which is informative on proton location, such as carbonyl oxygen atoms, amines (e.g. N-terminus) or other nitrogen locations (e.g. secondary amines or the imine N). We have compiled a database of diagnostic vibrations, proceeding from smaller to larger molecular systems. The methodology described has been employed in the structural characterization of gas-phase protonated tyrosine (Figure B.), and the a_4 fragment from Leu-Enkephalin.

A.



B.



ACKNOWLEDGEMENTS
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REFERENCES

[1] Mino Jr., W. K. et al. *J. Phys. Chem. Lett.*, **2011**, 2, 299-304.
[2] Gulyuz, K. et al. *Rev. Sci. Instrum.*, **2011**, 82, 054101.

Poster 64

Vibrational Spectroscopy of b_2 Fragment Ions from Peptides in the Hydrogen Stretching Region

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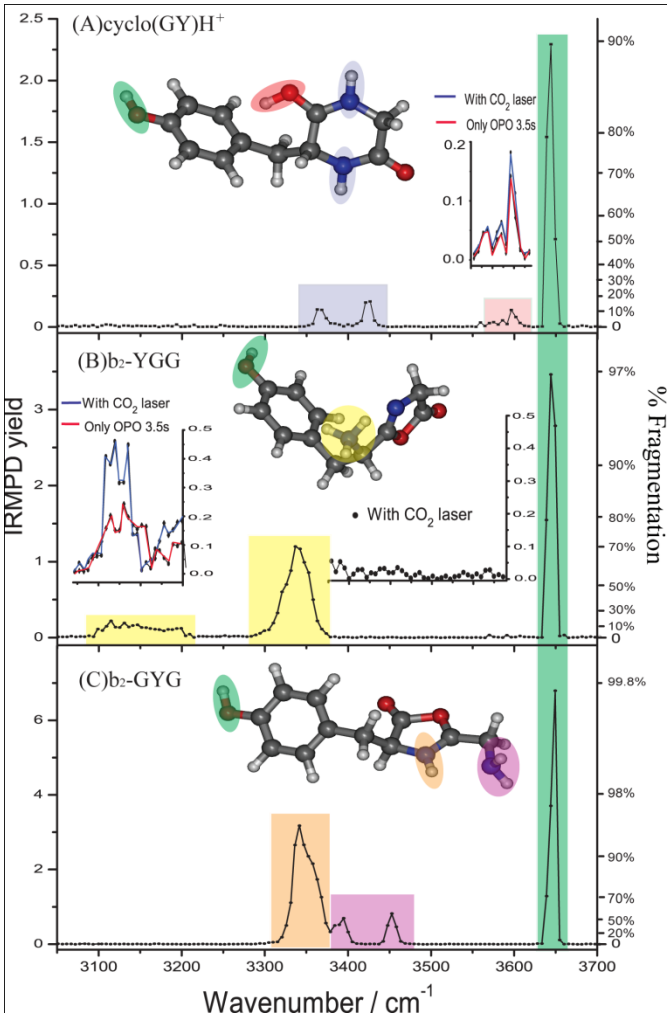
Infrared multiple photon dissociation (IRMPD) spectroscopy is a powerful technique that couples mass spectrometers with tunable lasers to collect vibrational spectra of ions for characterization of peptide fragmentation products from collision-induced dissociation. For example, structural identification of 'b' fragments, based on oxazolone CO stretch modes, have been performed in the mid-infrared region (500-2000 cm^{-1}) at free electron laser facilities [1]. Recently, we have extended this approach to the hydrogen stretching region (3000-4000 cm^{-1}) [2,3], which is particularly relevant in verifying proton/charge location.



A custom-built mass spectrometer equipped with an electrospray ionization (ESI) source, a quadrupole mass filter, and a quadrupole ion trap coupled to a time-of-flight (TOF) drift tube is employed to record IRMPD spectra of b_2 ions from protonated peptides. The trapped ions are irradiated by a continuous wave optical parametric oscillator to induce fragmentation, and the remaining precursor and photofragments are then mass analyzed with a TOF spectrometer.

It is recently shown that the IRMPD spectrum of b_2 from triglycine exhibits a strong NH stretch band, ascribed to an oxazolone ring NH stretch. On the other hand, a control experiment on protonated cylo(Gly-Gly), which is identical to the diketopiperazine structure, shows a strong OH mode due to the protonated carbonyl O-H^+ stretch, which is not observed in the experimental spectrum for b_2 [3]. In the on-going experiments, we recorded IRMPD spectra on other b_2 motifs, like YGG and GYG (shown in figure), and GEG, EGG, GPPG, PGPG. A comparison to theoretical IR absorption spectra computed at the density functional theory level will help us to determine the effect of side-chains on the reaction chemistry and the diagnostic vibrational modes.

[1] N.C.Polfer, et al., JACS, 2005, **127**, 17154, and J.Oomens, et al., JASMS, 2009, **20**, 334.
[2] W.K.Mino,Jr., et al., JPCL, 2011, **2**, 299.
[3] D.Wang, et al., JASMS, 2011, **22**, 1197.

Poster 65

Analysis of Human SLOS Fibroblast Cholesterol and 7-Dehydrocholesterol via Silver Sputtering Laser Desorption Ionization – Ion Mobility – Mass Spectrometry and Computational Modeling

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In human Smith-Lemli-Opitz Syndrome (SLOS), mutations in the gene encoding 7-dehydrocholesterol reductase (Dhcr7) enzyme, which catalyzes the conversion of 7-dehydrocholesterol (7-DHC) to cholesterol, lead to elevated levels of 7-DHC and reduced levels of cholesterol in tissues and fluids of affected individuals. The phenotypes of SLOS include multiple congenital malformation, incomplete myelination, developmental defects, and autism-like mental impairment. Recently, oxidative metabolites of 7-DHC (*i.e.*, oxysterols), which were identified in the nervous tissue of mouse SLOS model and in cultured fibroblasts from SLOS patients, and were shown to be potentially critical to the pathophysiological changes seen in SLOS. These advances may lead to more effective therapeutic strategies for SLOS and the understanding of autism-like symptoms on the molecular level. Several animal SLOS models exist, but the ideal approach for high-throughput small molecule intervention studies are cell lines that posses the SLOS genetic makeup. In this work we introduce a strategy for the detection and relative quantitation of cholesterol and 7-DHC directly from plated and trypsinized cells using silver sputtering assisted laser desorption ionization–ion mobility–mass spectrometry (LDI-IM-MS). Ion mobility structure-based separation allows the removal of isobaric chemical noise that prevents reproducible detection and quantitation of cholesterol and 7-DHC in traditional LDI-MS platforms. We have determined the optimal silver sputter coating thicknesses and detection limits for standards and trypsinized cells, and have used this approach to obtain images of plated cells that were grown directly on ITO-coated glass plates (Figure 1.Left). We have also initiated computational modeling studies of 7-DHC gas phase structural conformers, as IM-MS analysis of pure 7-DHC standards shows two distinct mobility distributions for this compound when coordinated with the stable isotopes of the silver cation (Figure 1. Right). Understanding the mobility separation profile of 7-DHC will inform our ability to accurately estimate the ratio of 7-DHC to cholesterol in upcoming small molecule SLOS intervention studies.

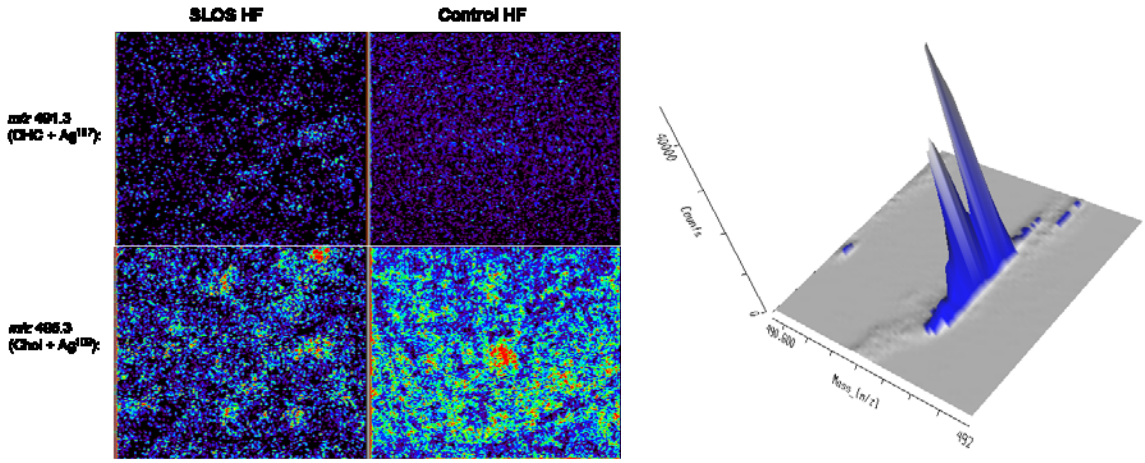

Figure 1. (Left) Silver sputtering assisted LDI-IM-MS, mobility selected images of cholesterol (Chol) and 7-dehydrocholesterol (DHC) in SLOS and control human fibroblast (HF) cells plated on indium tin oxide coated glass slides. At the same contrast settings, DHC appears at a higher concentration in SLOS cells, whereas cholesterol concentration is higher in control cells. **(Right)** The 3D IM-MS spectrum of the $(7\text{-DHC} + Ag^{107})^{+}$ ion signal. The y-axis corresponds to the drift time distribution of the 7-DHC signal.