UPPSALA 2017

14th Uppsala Conference on Electron Capture and Transfer Dissociation Mass Spectrometry

July 12-15, 2017

Cornell University, Ithaca, NY

Organizers

Ying Ge

Joseph A. Loo

UPPCON 2017

July 3, 2017

Dear Colleagues and Friends,

It is our great pleasure to welcome you to the 14th Uppsala Conference on Electron Capture (ECD) and Transfer Dissociation (ETD) and related radical directed dissociation techniques (UPPCON 2017), at Cornell University (the birthplace of ECD) in Ithaca, NY, from Wed., July 12 through Sat., July 15, 2017. We hope that you will enjoy both the scientific program and the social activities. They should make for a stimulating and fun atmosphere to learn the latest developments in mass spectrometry!

UppCon, started by Professor Roman Zubarev in 2003, has developed into a major international meeting in the field of electron-based dissociation techniques (e.g., ECD, ETD, UVPD). The impact of these methods on peptide and protein analysis has been increasingly recognized. This conference provides a venue for speakers from around the world to discuss new insights into radical ion chemistry, the latest developments in methodology and instrumentation, as well as novel applications for biomolecule characterization. New features for UppCon 2017 are a session on characterization of antibodies and antibody-drug conjugates and a special session to feature young investigators/rising stars. A poster session will feature additional contributions for in-depth discussion. This conference celebrates the 20th anniversary of ECD. After the main conference, there is a special session to celebrate the legacy of Professor Fred McLafferty.

We are very grateful to our “local facilitator” Jack Henion (Cornell and Advion), Melissa Manning and Drew Hager from Cornell Conference Services, and Michael Lenetsky, Barbara Baird, and Hening Lin from Cornell Chemistry Department for providing essential local support in organizing this conference. We also thank Yiwen Gu from UW-Madison for the design and maintenance of the conference website. We would like to acknowledge our advisory board members, Jon Amster, Kathrin Breuker, Neil Kelleher, Yuri Tsybin and Roman Zubarev. Many thanks to all the speakers, session chairs, and poster presenters.

The generous contributions of our sponsors have kept the meeting costs affordable for participants. Please take an opportunity to thank our participating sponsors during the conference at their display tables.

Thank you for joining us! We look forward to a splendid conference! (And to the many Cornell Alumni attending, we hope that returning back to Ithaca brings back many fond memories.)

Sincerely,

Ying Ge
Department of Cell and Regenerative Biology
Department of Chemistry
University of Wisconsin-Madison

Joseph A. Loo
David Geffen School of Medicine at UCLA
Department of Chemistry and Biochemistry
University of California, Los Angeles

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NEW OBJECTIVE

TOP DOWN PROTEOMICS

- Please plan to wear your name tag lanyard while participating in all conference related events as this will help us identify who is associated with the group.

- This is a non-smoking facility. Smoking is not permitted inside the buildings or within 25' of any entrances.

- There is wireless available in most of our buildings on campus. Please register on our visitor network. Due to heightened security please know that you will need to register each day.

- Parking is located in the CC lot, located adjacent to the Robert Purcell Community Center. Parking permits are required twenty four hours a day, seven days a week. Guests will need to purchase a parking permit at $6.00 plus tax per day through the Parkmobile app, https://transportation.fs.cornell.edu/parking/campusparking/visitors/parkmobile.cfm.

- All events on July 12 (Wed.), including reception and opening talks, will be held at the Robert Purcell Community Center (RPCC), which is across St. from the townhouse apartments.

- All oral and poster presentations from July 13 (Thurs.) to July 15 (Sat.) will be held in the Cornell Department Chemistry, Physical Sciences Building. The Oral presentations will be held in Physical Sciences Building room 120 and poster sessions will be held along the hallway outside of the room. Please see conference program agenda for location of meals and breaks.

GUIDELINES FOR ORAL PRESENTERS

- There will be a shared computer provided by Cornell Conference Services to upload your talks. Speakers may review your talks prior to session. Please see Kyle Brown (a graduate student from Ge group) as early as you can to upload your presentation (no later than one session break prior to your scheduled talk). If you are the first talk in the morning, please see Kyle Brown the evening prior. You could also choose to email Conference mailbox (uppcon2017@gmail.com) your presentation before July 10, 2017.

- The LCD projector will be provided in Physical Sciences Building room 120.

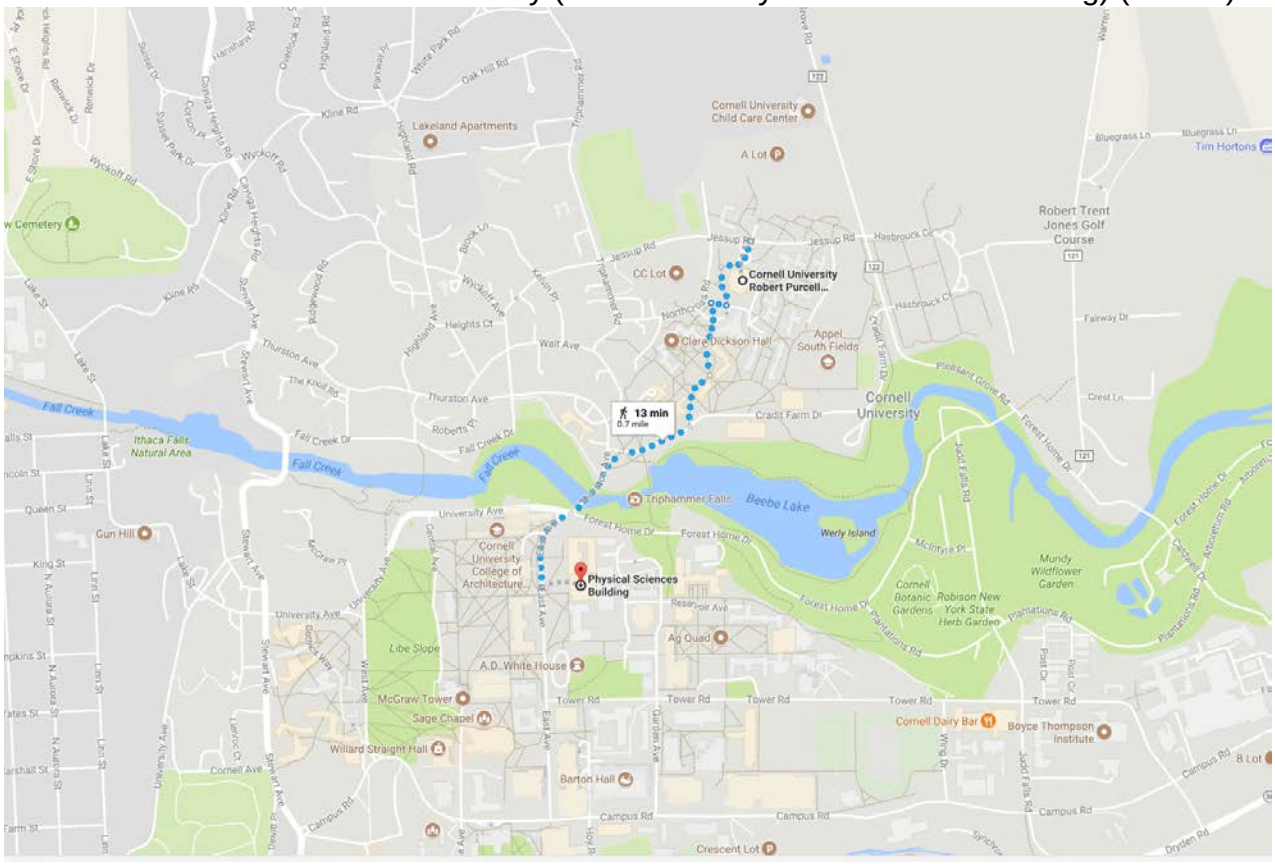
GUIDELINES FOR POSTER PRESENTERS

- **POSTER BOARDS**. Each author will be provided one board 69" (H) x 49" (W).

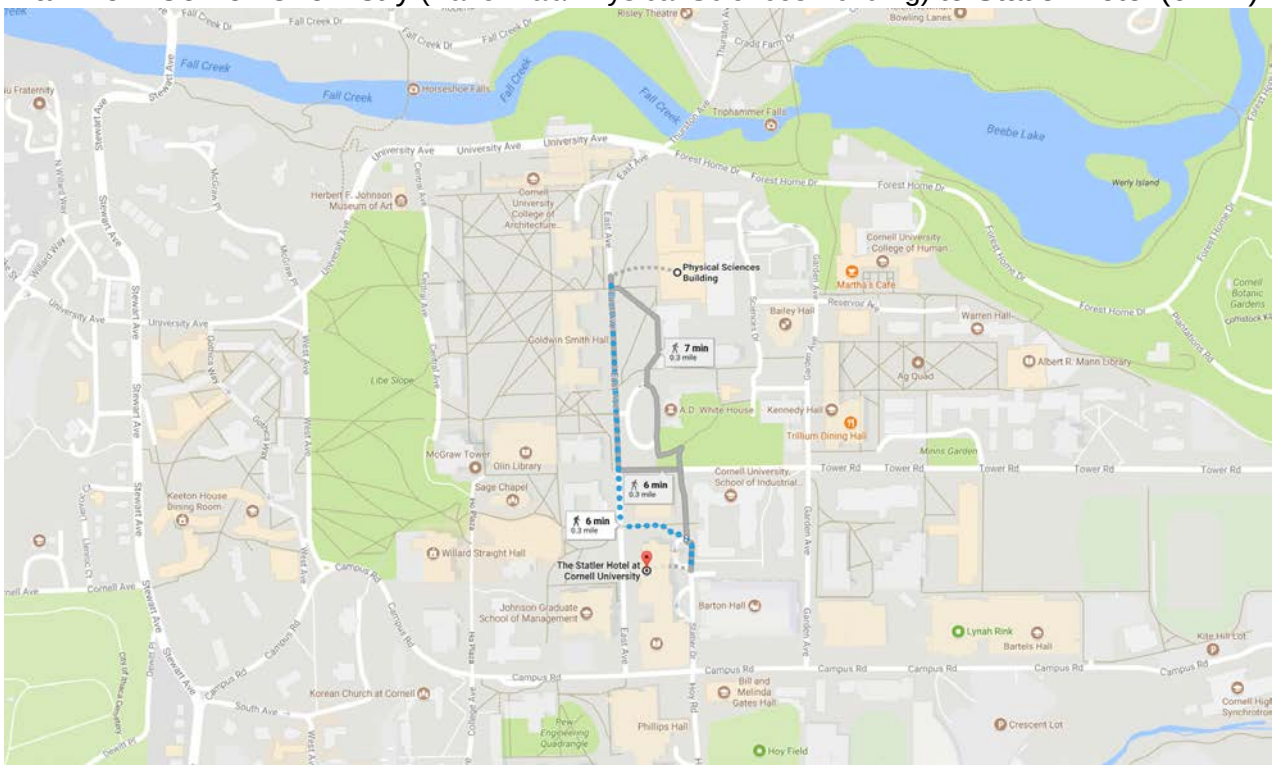
- Posters need to be set up on Thurs., July 13 before 9 am, and removed no later than Sat., July 15 before12:00 p.m. Poster presenters will present their posters during the poster session (Fri., July 14, 3:30-5 p.m.).

Map and Directions:

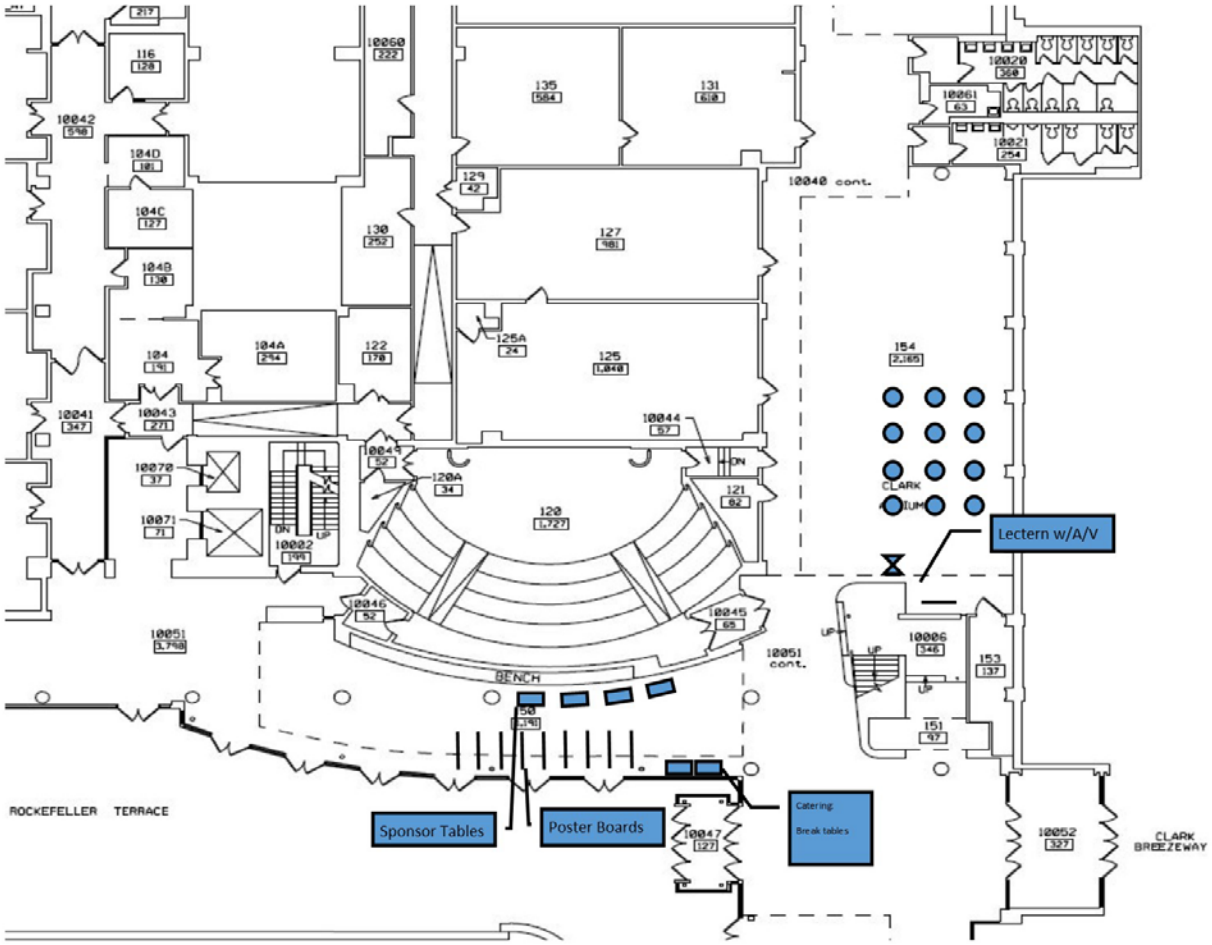
Walk from RPCC to Cornell Chemistry (Baker Lab/Physical Sciences Building) (13 min)



Walk from Cornell Chemistry (Baker Lab/Physical Sciences Building) to Statler Hotel (6 min)



Physical Sciences Building Diagram (Level 1)



14th Uppsala Conference (UPPCON)

on Electron Capture and Transfer Dissociation Mass Spectrometry and Related Radical Directed Dissociation Techniques

Cornell University, Ithaca, NY, USA

July 12 – 15, 2017

Wednesday, July 12

5 - 8 pm **Registration**
Robert Purcell Community Center (RPCC) 2nd floor, Conference Registrations Desk

7:30 - 9:30 pm **Welcome Reception**
Wendy Purcell Lounge in RPCC
Includes:

8:25 - 8:35 pm **Opening remarks (Ying Ge and Joseph Loo)**
RPCC Auditorium

8:35 - 9:00 pm **Neil Kelleher**
Northwestern University
"From the Invention of ECD to Top Down Proteomics and the Cure for Emeritis"
RPCC Auditorium

Thursday, July 13

7:30 - 8:40 a.m. **Breakfast**
RPCC Marketplace (3rd floor)

Session I. **Chairs: Ying Ge and Joseph Loo (Physical Sciences Room 120)**

9:00 am **Welcome from Cornell: Barbara Baird**

9:05 am **Introduction of ExD: Roman Zubarev**

9:10 am **Fred McLafferty**
Cornell University
"Protein folding: anhydrous is anomalous"

9:35 am **Cathy Costello**
Boston University School of Medicine
"Combinations of IMS and ExD MS/MS"

10:00 am **Alan Marshall**
Florida State University
"Top-Down 21 Tesla FT-ICR MS/MS: Instrumentation and applications"

10:25 am Coffee Break (Physical Sciences Clark Atrium)

Session II. **Chair: John Syka (Physical Sciences Room 120)**

10:45 am **Scott McLuckey**
Purdue University
"Toward selective cleavage of proteins: A new trick for radical cations"

11:10 am **Chrys Wesdemiotis**
University of Akron
"ETD of synthetic polymers and bioconjugates"

11:35 am **Frank Sobott**
University of Leeds (UK)
"Higher-order structure in ETD and CID fragmentation"

12:00 – 1:00 pm **Lunch** (Physical Sciences Clark Atrium)

Session III. **Chair: Neil Kelleher (Physical Sciences Room 120)**
Special session on "Antibody-drug conjugate"

1:00 pm **Yuri Tsybin**
Spectroswiss Sàrl (Switzerland)
"Asking more from FTMS for electron capture and transfer dissociation applications"

1:25 pm **Sonja Hess**
MedImmune, a member of the AstraZeneca group
"Bottom-up and middle-down ETD for antibody sequencing"

1:50 pm **Jeremy Wolff**
Bruker Daltonics
"Comprehensive characterization of antibodies by ultra-high resolution mass spectrometry with ECD/ETD"

2:30 – 6:30 pm **Bus tour of Ithaca (sponsored by Advion)**

Group 1 Waterfall
- Pickup at Cornell
- Botanical Gardens, & FR Newman Arboretum for photos
- Cascadilla Gorge Hike (downhill only)
- Purity Ice Cream
- Advion Tour
- Back to Cornell

Group 2 Wine
- Pickup at Cornell
- Purity Ice Cream
- Drive to Ithaca Falls for pictures
- Advion Tour
- Ports of New York Wine Tasting
- Back to Cornell

7 – 10 pm **Statler Hotel, Dinner, "Birthday celebration for FWM/CEC"**

Friday, July 14

7:30 am – 8:40 am Breakfast (RPCC Dining Hall)

Session IV. Chair: Cheng Lin (Physical Sciences Room 120)

9:00 am **Albert Heck**
Utrecht University (Netherlands)
"Exploring the dark side of the proteasome by EThcD"

9:25 am **Frank Turecek**
University of Washington
"UV photodissociation action spectroscopy of biological radicals"

9:50 am **Hanbin Oh**
Sogang University (S. Korea)
"TEMPO-assisted free radical initiated peptide sequencing mass spectrometry"

10:15 am Coffee Break (Physical Sciences Clark Atrium, with poster viewing and vendor tables)

Session V. Chair: Chris Hendrickson (Physical Sciences Room 120)

10:45 am **Ron Heeren**
Maastricht University (Netherlands)
"Imaging Ions and Electrons: organization based on structure"

11:10 am **Ryan Julian**
University of California, Riverside
"Zwitterions, zwitterions everywhere! A case for ubiquitous charge separation"

11:35 am **Peter O'Connor**
University of Warwick (UK)
"2-Dimensional mass spectrometry using electron capture dissociation"

12:00 - 1:00 pm **Lunch (**Physical Sciences Clark Atrium)

Session VI. **Chair: Vlad Zabrouskov (Physical Sciences Room 120)**

1:00 pm **Kathrin Breuker**
Universität Innsbruck (Austria)
"Top-Down mass spectrometry of ribonucleic acids"

1:25 pm **Newman Sze**
Nanyang Technological University (Singapore)
"Carcinogenic dietary proteins in instant food products discovered by top-down LC-MS/MS"

1:50 pm **Joe Beckman**
Oregon State University
"Using ECD to understand how copper incorporation affects superoxide dismutase in ALS"

2:15 pm Break (Physical Sciences Clark Atrium)

Session VII. **Chair: Jack Henion (Physical Sciences Room 120)**

2:30 - 3:30 pm **Short talks (15 minute each, selected from abstracts)**

Cheng Lin
Boston University
"Unleashing the Power of Electronic Excitation Dissociation for de novo Glycan Sequencing through Machine Learning"

Steven Patrie
UT Southwestern Medical Center
"A User-Friendly Bioinformatics Resource for Automated Continuous Elution Proteoform Analysis"

John Tran
Genentech
"Studying Therapeutic Antibody Degradation *In Vivo* Using High Throughput Affinity Capture Mass Spectrometry for Peptide and Intact Protein Level Analyses"

Nick Riley
University of Wisconsin-Madison
"Mapping the Glycoproteome with Activated Ion Electron Transfer Dissociation"

3:30 – 4:30 pm **Posters, Coffee and vendor tables**
Posters to be set along Hallway south of 120

5:00 – 11:00 pm **Cruise dinner**
Bus picks up delegates at Cornell chemistry at 5 pm and drop off before 11:00 pm (~3 hr Seneca Lake Dinner Cruise at Watkins Glen plus ~2 hr round trip from Cornell to Watkins Glen).

Saturday, July 15

7:30 am – 8:40 am **Breakfast (RPCC Dining Hall)**

Session VIII. **Chair: Mike Senko (Physical Sciences Room 120)**

9:00 am **Julian Whitelegge**
University of California, Los Angeles
"Effective application of ECD to integral membrane protein analysis"

9:25 am **Chris Mullen**
Thermo Fisher Scientific
"Improved top-down sequence coverage on an Orbitrap Fusion Lumos by ion-ion proton transfer (IIPT) reactions subsequent to ETD and UVPD"

9:50 am **Xianglei Kong**
Nankai University (China)
"Large blue-shift of O-H vibration mode in hydrated scandium oxide ions: Investigated by IRPD spectroscopy and theoretical calculations"

10:15 am Coffee Break

Session IX. **Chair: Klaas Van Wijk (Physical Sciences Room 120)**
"Young investigator/Rising star session"

10:35 am **Nick Young**
Baylor College of Medicine
"Electron Transfer Dissociation-Enabled High Throughput Quantitative Top Down Proteomics for the Study of Proteoform Temporal Dynamics"

10:55 am **Leslie Hicks**
University of North Carolina at Chapel Hill
Natural product bioactive peptide discovery using PepSAVI-MS

11:15 am **Si Wu**
University of Oklahoma
Top-down ACPP: an untargeted high-throughput active enzyme characterization platform

11:35 am **Owen Skinner**
Harvard Medical School

"Revisiting Native Electron Capture Dissociation to Map Iron Binding in Equine Ferritin"

11:55 am **Conference wrap up**

12:00 - 1:20 pm **Lunch** (Physical Sciences Clark Atrium)

Special Session (Chairs: Tom Brenna and Bing Wang, Physical Science Room 120)
***Open to Cornell faculty & students, friends, and family of Fred McLafferty.**

1:30 pm **Mike Gross**
Washington University at St. Louis
"MS for Understanding the Proteins in Alzheimer's Disease: Abeta and ApoE"

1:55 pm **Evan Williams**
University of California, Berkeley
"Electrochemistry in the gas phase and in solution"

2:20 pm **Roman Zubarev**
Karolinska Institutet (Sweden)
"OMNI-Trap - the ultimate device for studying ion-electron reactions?"

2:45 pm Coffee Break

3:10 pm **Jon Amster**
University of Georgia
"Electron-based methods for the structural analysis of glycosaminoglycans"

3:35 pm **Joseph Loo**
University of California, Los Angeles
"Top-Down MS, 1982-2017: From 1 kDa to 500 kDa"

4:00 pm **Ying Ge**
University of Wisconsin, Madison
"Top-down proteomics: bridging the silos between chemistry and biology/medicine"

4:25 pm **Short speeches by alumni and friends of Fred McLafferty; pictures and memories of the "good-old days at Cornell".**

5:00 - 9:00 pm Dinner Banquet
Reception- Physical Sciences Room 401
Dinner- Physical Sciences Clark Atrium

Abstracts

Protein Folding: Anhydrous Is Anomalous

Owen S. Skinner[a], Sergio Castro[a], Xianglei Kong[a,b], Kathrin Breuker[c], and Fred W. McLafferty[a]

[a]Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853-1301; [b] Nankai University, Tianjin, China; [c]Institute of Organic Chemistry and Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innrain 80/82, 6020, Innsbruck, Austria.

Despite an extensive understanding of protein folding in solution, there still is little agreement on the folding of anhydrous protein cations, the central species in intact ("top-down") protein characterization by mass spectrometry. For the well-studied ubiquitin, electrospray ionization produces highly-charged gaseous ions, but of the conformations proposed from their subsequent folding (e.g., native-related, helices), none clearly reflect their specific reactivity. Here further detailed studies (e.g., H/D exchange, deprotonation kinetics, charge site mass spectra) now show that each ion's folding also involves (Step 1) formation of characteristic proton distributions nearby each charged residue, modifying its proton affinity to be Type-characteristic (e.g., α, β, γ). Step 2 local folding of the residue (H^+-bonding to a nearby amide carbonyl) produces a local conformer(s) whose HDX reactivity is characterized by its Step 1 distribution. Fortunately for proteomics, ECD shows no such selectivities for interresidue dissociations.

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Top-Down 21 Tesla FT-ICR MS/MS: Instrumentation and Applications

Alan G. Marshall[1,2], Lissa C Anderson[1]; David Barnidge[3], Greg T Blakney[1], Lidong He[2], Tingting Jiang[2], David L. Murray[4], John P. Quinn[1], Ryan P Rodgers[1,2,5], Donald F Smith[2]; Chad R Weisbrod[2]; and Christopher L Hendrickson[1,2]

[1]Ion Cyclotron Resonance Program, National High Magnetic Field Laboratory, Tallahassee, FL
[2]Department of Chemistry & Biochemistry, Florida State University, Tallahassee, FL
[3]The Binding Site, Rochester, MN
[4]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN
[5]Future Fuels Institute, Florida State University, Tallahassee, FL

The NHMFL 21 T Fourier transform ion cyclotron resonance mass spectrometer [1] offers nonpareil boadband mass resolution and mass accuracy, both of which are essential for top-down MS/MS for identifying and sequencing proteoforms Here, we shall describe various design and performance features: front-end MS/MS by collision-induced dissociation and/or electron transfer dissociation[2], internal MS/MS by UV photodissociation, multiple-fill external quadrupole ion storage for enhanced dynamic range, broadband ion transfer to the ICR cell, dynamically harmonized ICR cell with 120-degree excitation/detection with the same electrodes for improved S/N ratio and mass accuracy, tripled-frequency detection (for tripled mass resolving power), and stored-waveform selection of precursor ions to within less than 5 mDa. These features enable top-down proteomics with unprecedented sequence coverage and upper mass limit at an on-line LC time scale. Applications include petroleomics and identification of clinically diagnostic sequence variants and multiple glycoforms in monoclonal antibodies produced by multiple myeloma cells [3].

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References:

[1] Hendrickson, C. L.; Quinn, J. P.; Kaiser, N. K.; Smith, D. F.; Blakney, G. T.; Chen, T.; Marshall, A. G.; Weisbrod, C. R.; Beu, S. C. "21 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer: A National Resource for Ultrahigh Resolution Mass Analysis," J. Am. Soc. Mass Spectrom. 2015, 26, 1626-1632.

[2] Weisbrod, C. R.; Kaiser, N. K.; Syka, J. E. P.; Early, D.; Mullen, C.; Dunyach, J.-J.; English, A. M.; Anderson, L. C.; Blakney, G. T.; Shabanowitz, J.; Hendrickson C. L.; Marshall, A. G.; Hunt, D. F. "Front-End Electron Transfer Dissociation Coupled to a 21 Tesla FT-ICR Mass Spectrometer for Intact Protein Sequence Analysis," J. Am. Soc. Mass Spectrom, 2017, 28, 000-000.

[3] He, L.; Anderson, L. C.; Barnidge, D. R.; Murray, D. L.; Hendrickson, C. L.; Marshall, A. G. "Analysis of Monoclonal Antibodies in Human Serum as a Model for Clinical Monoclonal Gammopathy by Use of 21 Tesla FT-ICR Top-Down and Middle-Down MS/MS, J. Am. Soc. Mass Spectrom. **2017**, 28, 827-838. Erratum: DOI 10.1007/s13361-017-1652-9.

UV Photodissociation Action Spectroscopy of Biological Radicals

František Tureček

Department of Chemistry, University of Washington, Seattle, WA

Electron capture and transfer are among the new methods of generating transient and stable radicals from biomolecules using reduction-oxidation intra- and intermolecular reactions. Electron transfer to multiply charged peptide ions triggers radical-induced dissociations but also can produce metastable and stable hydrogen-rich peptide radical-ions [1]. Structures of both non-dissociating radicals and their dissociation products are of prime importance for our understanding of the electron transfer process. Recently, action spectroscopy has been applied to elucidate the structure of several peptide cation radicals [2] and fragment ions [3,4], and the developed methodology is now being applied to other biological radicals. The lecture will present new results with action spectroscopy applied to structure elucidation of DNA cation radicals and RNA chimeras. UV-VIS action spectra can be interpreted by comparison with absorption spectra obtained by time-dependent density functional theory calculations [5,6], including vibronic band shifts and broadening. The combination of action spectroscopy of radical species and theory provides a powerful tool for structure elucidation of gas-phase ions formed by electron-based methods of mass spectrometry.

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References:

[1] Pepin, R.; Layton, E. D.; Liu, Y.; Afonso, C.; Tureček, F.: Where Does the Electron Go? Stable and Metastable Peptide Cation Radicals Formed by Electron Transfer. J. Am. Soc. Mass Spectrom. **2017**, 28, 164-181.

[2] Viglino. E.; Shaffer, C. J.; Tureček, F.: UV-VIS Action Spectroscopy and Structures of Tyrosine Peptide Cation Radicals in the Gas Phase. Angew. Chem. Int. Ed. **2016**, 55, 7469-7473.

[3] Martens, J.; Grzetic, J.; Berden, G.; Oomens, J. Structural Identification of Electron Transfer Dissociation Products in Mass Spectrometry Using Infrared Ion Spectroscopy. Nature Commun. **2016**, 7, 11754.

[4] Nguyen, H. T. H.; Shaffer, C. J.; Pepin, R.; Tureček, F.: UV Action Spectroscopy of Gas-Phase Peptide Radicals. J. Phys. Chem. Lett. **2015**, 6, 4722-4727.

[5] Riffet, V.; Jacquemin, D.; Cauet, E.; Frison, G. Benchmarking DFT and TD-DFT Functionals for the Ground and Excited States of Hydrogen-Rich Peptide Radicals. J. Chem. Theory Comput. **2014**, 10, 3308−3318.

[6] Turecek, F. Benchmarking Electronic Excitation Energies and Transitions in Peptide Radicals. J. Phys. Chem. A **2015**, 119, 10101-10111.

Asking more from FTMS for electron capture and transfer dissociation applications.

Yury Tsybin

Spectroswiss, EPFL Innovation Park, Lausanne, Switzerland

Electron capture and transfer dissociation (ECD/ETD), with or without collisional activation before or after ion-electron (AI-ECD) or ion-ion reactions (AI-ETD, EThcD), continue to demonstrate the power of complementarity to collision-induced dissociation (CID) and its variants, including high energy collision dissociation (HCD). Particularly important are the applications of ECD/ETD techniques to top-down mass spectrometry and proteomics, as well as to the analysis of peptides with labile modifications, especially glycopeptides. The ability of ECD/ETD to generate cleavages between almost any pair of amino acids also means that the total precursor ion signal is divided into many product ion channels. Therefore, improved workflows are required to increase the sensitivity of product ion detection. Here, we will discuss our recent advances in FTMS data acquisition and data processing for improving performance of ECD/ETD for analysis of peptides and proteins, including for the structural analysis of monoclonal antibodies.

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2-Dimensional Mass Spectrometry using Electron Capture Dissociation

Maria van Agthoven,[1] Christopher A. Wootton,[1] Pui Yiu (Yuko) Lam,[1] Meng Li,[1] Tomos Morgan,[1] Federico Floris,[1] Marc-Andre Delsuc,[2] Peter B. O'Connor[1]

Department of Chemistry, University of Warwick, Coventry, UK,[1] IGMBC, University of Strasbourg, France[3]

Two dimensional mass spectrometry is an old technique, initially studied by the groups of Tino Gaumann and Alan Marshall in the late 1980's and early 1990's. At the time, the limited computational power available made the technique impractical. Today, with modern cluster computing and advanced denoising algorithms, 2D mass spectrometry is a reality.

We have published a series of papers over the last few years exploring the utility of 2-Dimensional mass spectrometry for a range of molecular systems, but generally focusing on proteins and peptides due to easy access to samples. In this presentation, we will show the modern state-of-the art using 2-Dimensional mass spectrometry for bottom-up and top-down proteomics using electron capture dissociation as well as infrared multiphoton dissociation.

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References:

[1] Agthoven, M. A.; O'Connor, P. B., Two-dimensional mass spectrometry in a linear ion trap, an in silico model. Rapid Commun. Mass Spectrom. **2017,** 31 (8), 674-684.
[2] van Agthoven, M. A.; Wootton, C. A.; Chiron, L.; Coutouly, M.-A.; Soulby, A.; Wei, J.; Barrow, M. P.; Delsuc, M.-A.; Rolando, C.; O'Connor, P. B., Two-Dimensional Mass Spectrometry for Proteomics, a Comparative Study with Cytochrome c. Anal Chem **2016,** 88 (8), 4409-4417.
[3] Simon, H. J.; van Agthoven, M. A.; Lam, P. Y.; Floris, F.; Chiron, L.; Delsuc, M. A.; Rolando, C.; Barrow, M. P.; O'Connor, P. B., Uncoiling collagen: a multidimensional mass spectrometry study. Analyst **2016,** 141 (1), 157-165.
[4] Maria van Agthoven, P. B. O. C. 2 Dimensional Mass Spectrometry in a Linear Ion Trap UK Patent Pending GB1615469. 2016.
[5] Floris, F.; Agthoven, M. A. v.; Chiron, L.; Soulby, A.; Wootton, C. A.; Barrow, M. P.; Delsuc, M.-A.; Rolando, C.; O'Connor, P. B., 2-Dimensional IRMPD/FTICR MS of Calmodulin: A Top-Down and Bottom-Up Approach. J Am Soc Mass Spectrom **2016,** 27 (9), 1531-1538.

TEMPO-assisted Free Radical Initiated Peptide Sequencing Mass Spectrometry

Han Bin Oh

Sogang University, Seoul, Korea

Radical-directed peptide dissociation mass spectrometry is a tandem mass spectrometry method complementary to collision-activated dissociation mass spectrometry. Generally, a radical-directed dissociation MS method consists of two consecutive steps. In the first step, a radical site is generated from radical precursor-containing peptides. In the second step, radical-directed peptide sequencing is ensued. In many radical-directed peptide dissociation MS methods, the second step usually follows similar dissociation pathways. On the other hand, different radical-directed peptide dissociation MS methods make use of different radical generation mechanism. In our laboratory, we have introduced a TEMPO-Bz- moiety as a radical precursor and have taken advantage of the extraordinary thermodynamic stability of a TEMPO radical species for facile generation of a radical site attached to the peptides of interest: the TEMPO-assisted free radical initiated peptide sequencing (FRIPS) mas spectrometry. The generated peptide radical ions with a benzyl radical site have shown that it is very effective in inducing extensive peptide backbone dissociations, leading to production of a-, c-, x- and z-type peptide backbone fragments. Furthermore, the TEMPO-assisted FRIPS MS is shown to be a good tool for characterization of post-translational modifications (PTMs) of proteins, such as phosphorylation and o-glycosylation. In the conference, I will show recent updates of the TEMPO-assisted FRIPS MS, with an emphasis on PTM analyses.

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Using ECD to understand how copper incorporation affects superoxide dismutase in ALS

Joseph S Beckman, Nathan I. Lopez and Valery G. Voinov.

Linus Pauling Institute, Oregon State University and e-MSion, Inc. Corvallis, Oregon

Mutations to copper, zinc superoxide dismutase (SOD) are the first identified cause of ALS (motor neuron disease or Lou Gehrig's disease). Transgenic expression of human mutant SOD in mice results in the disease developing at ~90 days and terminal disease occurring by 130 days. After 25 years of testing, only the copper delivery ligand CuATSM has been shown to be reproducibly protective in this model. We have been able to extend survival of these mice from four months to nearly two years. Because SOD is overexpressed in transgenic mice, SOD could be extracted directly from spinal cord using a C4 ZipTip (TM Millique) and eluted using 25% acetonitrile with 100 uM formic acid. This leaves copper and zinc in place in the SOD monomer. We used this approach with native mass spectrometry to show that CuATSM works by increasing the maturation of SOD. Here, we report how the ECD cell developed by Voinov and Barofsky with a modified Agilent 6545 Q-ToF can be used to assess how CuATSM affects copper incorporation *in vivo* and to assess the turnover of SOD. The ECD cell was inserted directly between the first quad and the collision cell. With the modified Q-ToF, we were able to sequence SOD directly from 100 ug of ventral spinal cord. Furthermore, the fragmentation with this ECD cell was gentle enough to leave copper and zinc bound to their native ligand sites. Approximately half of SOD in transgenic mice was found to be deficient in copper, but still contained zinc. Treatment with CuATSM over several weeks restored copper to this large fraction of SOD protein in mice, converting most of the SOD to mature Cu,Zn SOD over a period of two weeks. The use of native mass spectrometry of metal delivery to SOD has helped identify alternative ligands to CuATSM that are be more effective at maturing SOD. The use of native mass spectrometry combined with ECD fragmentation can provide powerful new insights into how SOD causes ALS.

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Reference:

Williams, J. R., Trias, E., Beilby, P. R., Lopez, N. I., Labut, E. M., Bradford, C. S., Roberts, B. R., McAllum, E. J., Crouch, P. J., Rhoads, T. W., Pereira, C., Son, M., Elliott, J. L., Franco, M. C., Estevez, A. G., Barbeito, L., Beckman, J. S., 2016. Copper delivery to the CNS by CuATSM effectively treats motor neuron disease in SOD(G93A) mice co-expressing the Copper-Chaperone-for-SOD. Neurobiol Dis. 89**,** 1-9.

Effective application of ECD to integral membrane protein analysis

Julian Whitelegge[1], Chris Gisriel[2], Raimund Fromme[2,3]

The Pasarow Mass Spectrometry Laboratory, NPI-Semel Institute, David Geffen School of Medicine, University of California Los Angeles[1]
School of Molecular Sciences, Arizona State University, Tempe, Arizona[2]
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Membrane proteins have two principal secondary structure motifs, namely transmembrane alpha helices and beta sheets. Given the similar dielectric properties of the hydrophobic interior of the bilayer membrane and a vacuum it is likely that considerable secondary structure is preserved in the mass spectrometer even when acidic/organic mixtures are used for sample preparation. Such structure has implications for both collisional and electron activated dissociations and the appearance of product ions.

Membrane proteins are purified under non-denaturing conditions and then prepared for electrospray ionization in acidic aqueous organic solvent mixtures that preserve their solubility. Fractions are collected during chromatography on a low-resolution mass analyzer (LC-MS+) allowing for extended static nanospray experiments by FT-ICR mass spectrometry (7 T LTQFT Ultra). Data from the primary low-resolution analysis is used to steer high-resolution experiments. Secondary structure of the analyte protein is manipulated using chemical denaturants prior to ionization and ion activation techniques within the mass spectrometer.

It was previously shown that ion activation was necessary for effective ECD analysis of a two-transmembrane helix membrane protein (Zabrouskov and Whitelegge, 2007). The rational is that while extensive ECD may be occurring, hydrogen bonding within alpha helices prevents release of product ions. Use of an IR laser irradiation within the ICR cell allowed thermal activation of ions, breakage of hydrogen bonds and appearance of ECD product ions within the mass spectrum. Another way to improve efficacy of ECD is to work with more highly charged ions. Consequently we investigated the use of a supercharging reagent, propylene carbonate, in ECD analysis of a single transmembrane helix protein. While no supercharging was observed there was a clear improvement in yield of product ions during ECD, presumably due to denaturation of the protein prior to ionization.

In conclusion, membrane protein structure modulates productive ECD.

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Reference:

Zabrouskov V, Whitelegge JP. Increased coverage in the transmembrane domain with activated-ion electron capture dissociation for top-down Fourier-transform mass spectrometry of integral membrane proteins. *J Proteome Res.* 2007 6(6):2205-10. PMID: 17441748

Improved top-down sequence coverage on an Orbitrap Fusion Lumos by ion-ion proton transfer (IIPT) reactions subsequent to ETD and UVPD.

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Electron and photon based activation methods have proven to be indispensable tools for the analysis of peptides and proteins.[1] Electron Transfer Dissociation (ETD) and Ultra Violet Photo-dissociation (UVPD) have been demonstrated to provide unique sequence information, and, in conjunction with recent advances in proteome fractionation and top-down informatics, have facilitated intact protein characterization.[2,3] However, as the molecular weight of protein precursor ions increases, the number of possible product ions increases. Further, because charge

distribution is roughly uniform on protein ions, most all of these product ions have m/z values that are in the neighborhood of the m/z of the protein precursor ion. Hence the product ion spectra from proteins exhibit a significant amount of spectral congestion in the vicinity of the precursor ion m/z. In this region, the combination of low product ion signal-to-noise ratios and overlapping isotopic clusters greatly complicate both manual and computerized charge state and sequence ion assignment. Anderson et. al. were able to reduce product ion spectral density and demonstrate improved sequence coverage for apomyoglobin by dispersing the fragment ion population resulting from multiple ETD reactions over a greater m/z range using IIPT.[4] Here we demonstrate the potential for IIPT to increase top-down sequence coverage by exposing 10-30 m/z windows of UVPD or ETD product ion populations to varying degrees of IIPT. The discussion will cover specifics the implementation of UVPD and IIPT on an Orbitrap Fusion Lumos as well as data from such MS^3 analyses of infused apomyoglobin and carbonic anhydrase standards.

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References:

[1] Broadbelt JS. “Ion Activation for Peptides and Proteins” Anal Chem 2016;88:30-51.

[2] Shaw JB, Li W, Holden DD, Zhang Y, et. al. “Complete protein characterization using top-down mass spectrometry and ultraviolet photodissociation” J. Am. Chem. Soc. 2013;135:12646-12651.

[3] Cleland TP, DeHart CJ, Fellers RT, et. al. “High-throughput analysis of intact human proteins using UVPD and HCD on an Orbitrap mass spectrometer” J. Proteome Research 2017;15(5):2072-2079.

[4] Anderson LC, English MA, Wang WH, et. al. “Protein derivatization and sequential ion/ion reactions to enhance sequence coverage produced by electron transfer dissociation mass spectrometry” IJMS 2015;377:717-624.

ETD of synthetic polymers and bioconjugates

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Electron transfer dissociation (ETD) of synthetic polymers causes less consecutive backbone fragmentation than collisionally activated dissociation (CAD), thus providing more conclusive information about chain end groups and copolymer sequences. This advantage generally applies to polyester copolymers.[1,2] A completely different reactivity is often observed for polymers functionalized in the side chains, but not the backbone, such as poly(acrylamide)s. Now, single-stage ETD (or CAD) only causes small neutral losses from the side chains or end groups. In such cases, primary structure determination can be achieved by sequential CAD on ETD products that promote backbone cleavages; the charge-reduced precursor and ETD fragments with either a radical site or an anionic site within a salt bridge fulfill this requirement, as will be demonstrated for homo- and copolymeric poly(N-isopropylacrylamide)s.[3] Sequential ETD-CAD is particularly useful for the characterization of polypeptide-polymer conjugates. For a covalently bonded bioconjugate composed of a bioactive peptide functionalized with a bone-binding polymer, ETD causes extensive cleavages within the peptide chain, revealing its sequence and the conjugation site; whereas the consecutive CAD step helps to identify the structure and branching of the polymer segment. For supramolecular polyelectrolyte complexes containing poly(lysine) and poly(styrene sulfonate), ETD mainly leads to losses of small units from either electrolyte; consecutive CAD on these primary fragments provides the subunit connectivity of the noncovalent assembly and also renders a more complete sequence coverage of the polypeptide. ETD of the vast majority of synthetic macromolecules has involved multiply sodiated or multiply protonated species. In certain cases, the use of different or mixed cations can have a profound effect on the ensuing fragmentation pattern, as will be illustrated for an ethoxylated polysaccharide, which is found to undergo structure-indicative cross-ring cleavages only when ammoniated-sodiated, but not when doubly sodiated.[4]

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References:

[1] V. Scionti and C. Wesdemiotis, J. Mass Spectrom. 47 (2012) 1442-1449.

[2] B.C. Katzenmeyer, L.R. Cool, J.P. Williams, K. Craven, J.M. Brown, and C. Wesdemiotis, Int. J. Mass Spectrom. 378 (2015) 303-311.

[3] S. Gerislioglu and C. Wesdemiotis, Int. J. Mass Spectrom. 413 (2017) 61-68.

[4] X. Liu and C. Wesdemiotis, Eur. J. Mass Spectrom. 21 (2015) 713-723.

Unleashing the Power of Electronic Excitation Dissociation for de novo Glycan Sequencing through Machine Learning

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A major challenge in glycomics is the characterization of complex glycan structures that are essential for understanding their diverse roles in many biological processes. Although electronic excitation dissociation (EED) and other electron activation dissociation (ExD) tandem mass spectrometry methods are capable of delivering unprecedented rich structural information for glycans, ExD tandem mass spectra of glycans are often very complex and difficult to interpret manually, especially for unknown structures.

Here, we present a novel efficient computational approach, named GlycoDeNovo, for accurate elucidation of the topologies from the tandem mass spectra of glycans. Given a spectrum, GlycoDeNovo first builds an interpretation graph specifying how to interpret each peak using preceding interpreted peaks. It then reconstructs the topologies of peaks that contribute to interpreting the precursor ion. We theoretically prove that GlycoDeNovo is highly efficient. A major innovative feature added to GlycoDeNovo is a data-driven IonClassifier which can be used to effectively rank candidate topologies. IonClassifier is automatically learned from experimental spectra of known glycans to distinguish B- and C-type ions from all other ion types. Application of GlycoDeNovo to analysis of EED spectra of a variety of glycan standards shows that it is robust and accurate for topology reconstruction of glycans from their tandem mass spectra.

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Separation and Characterization of Isomeric Glycans by TIMS-ExD MS/MS and LC-ExD MS/MS

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Carbohydrates, or glycans, are the most abundant biopolymers found in nature. The many important roles glycans play in biological systems are closely related to their complex and diverse structures. Complete characterization of naturally occurring glycan mixtures remains a challenging task due to the many structural variables of a glycan, where the presence of structural isomers significantly enhances the sample complexity. The structural heterogeneity of glycans arises from differences in not only their saccharide composition and topology, but also their linkage and stereochemical configurations. Although tandem mass spectrometry has emerged as a powerful tool for isomer differentiation, characterization of linkage and stereochemical glycan isomers is still a major analytical challenge because of their similarity in chemical properties and fragmentation behaviors.

Here, trapped ion mobility spectrometry (TIMS) and reversed phase liquid chromatography (RPLC) were used to separate isomeric glycans, which were then analyzed on line by electron activated dissociation tandem mass spectrometry methods. Both electronic excitation dissociation (EED) and negative electron transfer dissociation (NETD) were utilized in conjunction with gated-TIMS Fourier transform ion cyclotron mass spectrometry for the first time. Mixture of human milk oligosaccharide isomers and heparan sulfate isomers were successfully analyzed by TIMS-ExD MS/MS or RPLC-ExD MS/MS, showing great potentials of these approaches for characterization of isomeric glycans.

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The Impact of Phosphorylation on Electron Capture Dissociation of Proteins

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Electron capture dissociation (ECD) is well suited for the characterization of phosphoproteins, with which labile phosphate groups are generally preserved during the fragmentation process. Previous studies have shown deleterious effect on bond cleavages in doubly charged phosphopeptides. However, the impact of phosphorylation on ECD fragmentation of intact proteins remains unclear. Here, we have performed a systematic investigation of the phosphorylation effect on ECD of intact proteins by comparing the ECD cleavages of mono-phosphorylated α-casein, multi-phosphorylated β-casein, and immunoaffinity-purified phosphorylated cardiac troponin I with that of their unphosphorylated counterparts, respectively. In contrast to phosphopeptides, phosphorylation has significantly reduced deleterious effects on the fragmentation of intact proteins during ECD. On a global scale, the fragmentation patterns are highly comparable between unphosphorylated and phosphorylated precursors under the same ECD conditions, despite a slight decrease in the number of fragment ions observed for the phosphorylated forms. On a local scale, single phosphorylation of intact proteins imposes minimal effects on fragmentation near the phosphorylation sites, but multiple phosphorylations in close proximity results in a significant reduction of ECD bond cleavages.

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Reference:

Chen, B.; Guo, X.; Tucholski, T.; Lin, Z.; McILwain, S.; Ge, Y. The Impact of Phosphorylation on Electron Capture Dissociation of Proteins: A Top-down Perspective. JASMS. Accepted.

Electron Transfer Dissociation-Enabled High Throughput Quantitative Top Down Proteomics for the Study of Proteoform Temporal Dynamics

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Complete comprehension of biology will ultimately require proteoform level analytics. To date relatively little top down proteomics work has been quantitative or high throughput: qualities required to properly address biological questions. Here we demonstrate a top down proteomics method that is quantitative, reproducible, sensitive and

high-throughput. It uses novel proteoform resolving on-line separations, electron transfer dissociation (ETD) and custom in-house data analysis approaches. These methods have exceptionally low sample requirements (hundreds of replicates per tissue culture dish). Our chromatography resolves and allows detection of previously unreported and low abundance proteoforms and sequence variants (as low as 10ppm). We have exceptionally high reproducibility (Pearson Correlation of ~0.90) with unparalleled variance (average SD of ±0.3% abundance) of up to ~300 proteoforms, even for biological replicates cultured, prepared and analyzed weeks apart. With the use of ETD we are able to reach a coverage that can reliably localize K31ac, a mark not analyzed by alternative methods; we have found exceptional reproducibility in the quality of fragment spectra generated through ETD. With a throughput of 90 minutes per sample, we have generated massive quantitative data sets on the fast temporal dynamics of histone proteoforms in response to multiple stimuli. Despite limited response at the discrete PTM level, there are fast, dramatic and reproducible changes to specific proteoforms. The effects resolve over hours with distinct dynamic response curves for each proteoform. For example, inhibition of the histone methyltransferases SUV420H1/2 results in rapidly decreased H4K20me2 but only for unacetylated proteoforms. Surprisingly, inhibition of SUV420H1/2 results in a rise in histone H4 acetylation, most dramatically at K5 and K31, but specifically for hyper-acetylated and dimethylated proteoforms. Overall, proteoform dynamics are significantly faster, proteoform specific and more complex than previously understood.

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Unpublished work.

Mapping the Glycoproteome with Activated Ion Electron Transfer Dissociation

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Protein glycosylation is a prevalent, chemically complex, and biologically diverse post-translational modification (PTM) involved in a wide array of intra- and inter-cellular functions. Approximately half of all expressed proteins undergo glycosylation, and this heterogeneous modification accounts for the greatest proteome diversity over any other PTM. Changes in protein glycosylation are associated with cellular proliferation, inter-cellular communication, and metabolic processes, making the characterization of the cellular landscape of protein glycosylation integral to advancing our understanding of cell biology. Glycan microheterogeneity, i.e., different glycans modifying the same glycosite, makes glycan identity at a given site crucial to the biological context of the modification. This unique feature of glycosylation makes analysis of intact glycopeptides imperative for glycoproteome characterization, but current analytical tools are ill-suited for this task.

Tandem mass spectrometry (MS) is an ideal platform to advance glycoproteomic technology, but current dissociation methods are often suitable only for characterization of either peptide or glycan moieties. This mandates multiple analyses of the same precursor ions that limit throughput and challenge data interpretation. We have developed a tandem MS dissociation method called activated ion-electron transfer dissociation (AI-ETD) that addresses several of challenges of intact glycopeptide analysis. Through the use of concurrent ion-ion reactions and infrared photo-activation, AI-ETD can access glycan and peptide information from intact glycopeptides in a single MS/MS scan, and with this AI-ETD provides (1) improved product ion generation for peptide backbone sequencing, (2) higher MS/MS success rates to sequence more glycopeptides per experiment, and (3) valuable fragmentation for glycan composition determination. Here we showcase AI-ETD for large-scale intact glycopeptide characterization on the newest generation of Orbitrap instruments. We show that >2000 localized N-glycosites can be confidently identified from approximately 30,000 localized N-linked glycopeptide spectral matches (>6,500 unique) in mouse brain tissue. This represents a more than 3-fold increase over recent studies in the number of

glycopeptides and glycosites that can be identified via intact glycopeptide analyses, and it rivals, if not outmatches, glycoproteomic studies that analyze only deglycosylated peptides.

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Implementation of Activated Ion Electron Transfer Dissociation on a Quadrupole-Orbitrap-Linear Ion Trap Hybrid MS System

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Abstract Text:
Electron transfer dissociation (ETD) and related technologies have benefited a wide variety of analytical approaches for characterizing biomolecules with tandem mass spectrometry (MS/MS). A major challenge in ETD fragmentation, however, is non-dissociative electron transfer (ETnoD), where non-covalent interactions prevent formation of sequence-informative product ion generation. ETD can effectively fragment high charge density precursor ions, but as charge density decreases, more compact secondary gas-phase structures contribute to higher degrees of non-covalent interactions that hold product ions together even when an electron transfer event and subsequent backbone cleavage occur. Several approaches have been explored to mitigate ETnoD in both ETD and its ion-electron counterpart, electron capture dissociation, including collisional- and photo-activation, elevated temperatures of reaction cells, and higher energy electrons. Our group has focused on implementing and exploring the utility of activated ion electron transfer dissociation (AI-ETD), where infrared photo-activation is used concurrent to the ETD reaction to disrupt non-covalent interactions, improve dissociation efficiency of the reaction, and ultimately promote formation of sequence-informative product ions.

Previous work with AI-ETD has largely been performed on standalone linear ion trap instruments or on highly modified ion trap-Orbitrap MS systems. Here we showcase our most recent work to implement AI-ETD on a quadrupole-Orbitrap-ion trap Tribrid MS system (Orbitrap Fusion Lumos). We show that a 60 Watt CO_2 continuous wave laser (10.6 μm) for AI-ETD reactions can be introduced to the Lumos system with straightforward, yet robust instrument modifications. Furthermore, we demonstrate the distinct advantage of AI-ETD over traditional ETD and ETD with other modes of supplemental activation for **(1)** peptide fragmentation and bottom-up proteomics experiments, **(2)** phosphopeptide characterization and global phosphoproteomic analyses, **(3)** top down analysis of intact phosphoproteins, **(4)** intact protein dissociation for proteins up to ~50 kDa, a mass range which has not yet been reported for the technique, and **(5)** top down fragmentation of proteins with multiple intact disulfide bonds. In all, we highlight the ability of AI-ETD to increase c- and z-type fragment ion production by ten-fold over ETD for peptides, double MS/MS success rates and peptide identifications in bottom-up proteomic and phosphoproteomic experiments, and provide optimum fragmentation of intact proteins, including those with PTMs, for precursors spanning the entire *m/z* range.

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Top-down ACPP: an untargeted high-throughput active enzyme characterization platform

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Functional enzyme characterization remains one of the major challenges for proteomics. To address this challenge, we have developed an activity-correlated quantitative proteomics platform (ACPP) that systematically correlates protein-level enzymatic activity patterns with hundreds of protein elution profiles from the high performance

chromatography using a label-free quantitative proteomics approach. The ACPP was successfully applied to characterize active biomass degrading enzymes in fungal secretome using the bottom-up approach. Our published results demonstrate that the ACPP is an unbiased and high-throughput enzyme characterization platform.

We further expanded the ACPP capability to characterize intact "active" enzyme proteoforms through a "top-down" ACPP. We combined the complementary fragmentation approaches such as HCD, CID and ETD to improve the sequence coverage through a new data analysis framework. Our previous results on the proteome level analysis of E. coli using top-down MS showed that combining HCD and ETD significantly increases protein sequence coverage (average coverage increases from about 30-35% to 66%) of top-down tandem mass spectra. With these developments, we successfully applied the top-down ACPP and confirmed that the "active" proteoforms of the A. niger cellulose hydrolysis enzymes are C-term degraded proteoforms with N-glycosylations, and the glycosylation pattern does not change with the correlated activities.

Overall, the top-down ACPP is a novel high-throughput method that can directly characterize intact "active" enzyme proteoforms in complex protein samples. By correlating the active proteoforms with their functions rather than abundances, the top-down ACPP holds great potentials on the identifications of novel disease targets.

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Reference:

Ma, H.; Delafield, D. G.; Wang, Z.; You, J.; Wu, S., Finding Biomass Degrading Enzymes Through an Activity-Correlated Quantitative Proteomics Platform (ACPP). *J Am Soc Mass Spectrom* **2017,** *28* (4), 655-663.

Electronic Excitation Dissociation Characterization of Native Glycans Derivatized with a Reducing-End Fixed Charge

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De novo glycan sequencing by tandem mass spectrometry can be complicated by gas-phase structural rearrangements and/or loss of one or more terminal residues. These undesirable processes may be minimized by avoiding the presence of a mobile proton by utilizing metal cation adduction or fixed charge derivatization to provide the required positive charge to the analyte. In this study, we are investigating the electronic excitation dissociation (EED) fragmentation behavior of glycans derivatized with a reducing-end fixed charge, and exploring the potential of this approach for detailed glycan structural characterization. All tandem MS analyses were performed on a Bruker 12-T solariX Fourier transform ion cyclotron resonance mass spectrometer with EED as the fragmentation mode.

EED of glycans with a reducing end fixed charge produced predominantly reducing-end fragments. In particular, complete series of Z-, Y- and ^{1,5}X-ions were observed in all tandem mass spectra. They form predicted triplet patterns with characteristic spacing that can be easily identified and used for determination of the glycan topology. Additional radical-driven dissociation pathways generated several linkage-specific cross-ring or secondary fragments. For example, formation of a ^{0,4}X ion is characteristic of the 1→6 linkage, whereas a ^{0,2}X ion may be produced anywhere, except at 1→2 linked residues. Furthermore, two linkage-specific secondary fragment ions, Z•–OH and Z•–CH_2OH, were generated, likely deriving from radical Z• ions via β-elimination of the substituent at an adjacent carbon. The presence and relative ratios of these fragments could be used to differentiate 1→3 and 1→4 linkages. At HexNAc residues, Z•–CH_2CO and Z•–CH_3CO fragments were observed (in addition to Z•–OH and Z•–CH_2OH ions), for 1→3 and 1→4 linkages, respectively, due to the presence of CH_3CONH at the C2 position. These ions provide additional information for linkage determination on HexNAc residues. Similarly, identification of a branching site can be made from the Z/Z-type ion produced via the loss of an entire nonreducing-end branch connected to the neighboring carbon of a Z• ion. We found irradiation by 16-eV electrons generated the most

informative spectra, as it produced more linkage-specific, cross-ring and secondary fragments than lower-energy EED.

We have developed a computer algorithm named GlycoDeNovo for *de novo* glycan topology reconstruction from tandem mass spectra. Preliminary results show that GlycoDeNovo can accurately elucidate the glycan topology based on the EED spectra of glycans with a reducing-end fixed charge, with the correct topology consistently ranked as the top candidate by a data-driven IonClassifier that utilizes the peak context information.

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Natural product bioactive peptide discovery using PepSAVI-MS

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As current methods for antibiotic drug discovery are being outpaced by the rise of antimicrobial resistance, new methods and innovative technologies are crucial to replenish our dwindling arsenal of antimicrobial therapeutics. While natural products are a well-studied source of biologically active small molecules, peptidyl factors contributing to their medicinal properties remain largely unexplored. To this end, we have developed the PepSAVI-MS (Statistically-guided bioactive peptides prioritized via mass spectrometry) pipeline[1] to identify bioactive peptide targets from complex biological samples. MS/MS techniques such as CID and ETD are implemented for de novo characterization. To validate this pipeline, we have demonstrated successful detection and identification of a known antimicrobial peptide, cycloviolacin O2 (cyO2), from the botanical species *Viola odorata*. Additionally, we have widened the known antimicrobial spectrum for *V. odorata* cyclotides, including antibacterial activity of cyO2 against *A. baumannii* and novel anticancer activities for cycloviolacins by their cytotoxicity against ovarian, breast and prostate cancer cell lines. The developed platform is highly versatile as it is adaptable to any natural product source of peptides and can test against diverse physiological targets, including bacteria, fungi, viruses, protozoans, and cancer cells for which there is a developed bioassay. As such, we demonstrate extension of this pipeline to fungal and bacterially-sourced AMPs through the identification of the killer toxin KP4 from *Ustilago maydis*[2] and the bacteriocin bac-21 from *Enterococcus faecalis* harboring pPD1[3]. Bac-21 is identical in nucleotide sequence to another enterococcal bacteriocin, AS-48[4], but herein we have experimentally validated the protein sequence of bac-21 for the first time. Additionally, we begin to probe the vast array of botanical natural product sources to prioritize highly active species for downstream analysis.

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Characterization of Large Proteins (>100 kDa) Enabled by Serial Size Exclusion Chromatography and Electron Capture Dissociation FT ICR Mass Spectrometry

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Top-down mass spectrometry (MS) analysis of large proteins is significantly challenging due to an exponential decrease in S/N with increasing molecular weight (MW) and co-elution with low-MW and high-abundance proteins in a mixture. Signal suppression and interference, especially from low-MW species, hinders the MS detection and characterization of high-MW proteins. Thus, size-based fractionation prior to MS analysis is essential for detection and characterization of high-MW proteins. Herein, we developed serial size exclusion chromatography (sSEC), an MS-compatible technique for size-based fractionation of intact proteins from complex mixtures. We have achieved

high-resolution size-based fractionation for proteins over a broad MW range (10-223 kDa) using sSEC with three columns connected in series. sSEC successfully separated large (>60 kDa) and intermediate (35 – 60 kDa) size proteins from small (<35 kDa) proteins from human heart tissue lysate. Additionally, we have shown that 2D sSEC-RPC allowed for detection of large proteins up to 223 kDa using q-TOF MS, with a 15-fold increase in detection of proteoforms >60 kDa. Furthermore**,** sSEC fractionation prior to RPC-MS enabled detection of charge state envelopes of large proteins, allowing the efficient isolation of precursor ions of high MW proteins for online MS/MS fragmentation. In addition, we have achieved high-quality online LC/MS/MS for the large cardiac phosphoprotein, nebulette (116 kDa). Notably, sSEC fractions can be directly analyzed using ultra-high resolution top-down FT ICR MS with electron capture dissociation (ECD) for characterization off high-MW proteins, highlighting the MS compatibility of sSEC. sSEC-FT ICR MS/ECD was used to identify and characterize large metabolic enzymes extracted from swine heart tissue. We have shown high sequence coverage for two endogenous metabolic enzymes, creatine kinase (43 kDa) and malate dehydrogenase (33 kDa), using sSEC-FT ICR MS/ECD. We are using this strategy to characterize other large proteins (>100 kDa) from complex protein mixtures.

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Reference:

Cai, W.; Tucholski, T.; Chen, B.; Alpert, A.J.; McIlwain, S.; Kohmoto, T.; Jin, S.; **Ge, Y**. Top-Down Proteomics of Large Proteins up to 223 kDa Enabled by Serial Size Exclusion Chromatography Strategy, *Anal. Chem.*, **2017**, *89***;** 5467–75.

Statistical Fragmentation Pattern of Intact Proteins based on Their Top-down MS/MS ETD/ECD Spectra

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Database searching based on experimental mass spectra is the most popular method to identify intact proteins, in which fragmentation pattern will play an increasingly important role. It will be helpful to design a knowledge-based search engine in top-down proteomics. Based on our previous research results on analyzing peptide ETD spectra (1), we comprehensively analyzed the fragmentation events of ETD/ECD from intact proteins, including their tendency, using the publicly available data sets from the perspective of statistics (2). All ETD/ECD mass spectra were firstly preprocessed by pParseTD, a software tool for deconvolution and deisotoping of the raw mass spectral data sets. Then these deconvoluted spectra were searched against the protein databases by pTop (3), a new search engine specifically designed for intact protein identification. After the false discovery rate control by the target-decoy strategy, a relatively confident spectra have been selected and manually validated. The fragmentation frequency and fragmentation intensity were analyzed and visualized. According to the statistical results, the fragmentation is related to the position of charge, i.e. the position of basic amino acids. To our best knowledge, this is the first discovery on ETD/ECD fragmentation patterns of intact proteins based on the large scale mass spectra, which will provide the fundamental knowledge to design a high-performance search engine for intact protein identification and quantification, and help to interpret ETD/ECD spectra in depth as well.

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[1] Rui-Xiang Sun, Meng-Qiu Dong, Chun-Qing Song, Hao Chi, Bing Yang, Li-Yun Xiu, Li Tao, Zhi-Yi Jing, Chao Liu, Le-Heng Wang, Yan Fu, and Si-Min He. Improved Peptide Identification for Proteomic Analysis Based on Comprehensive Characterization of Electron Transfer Dissociation Spectra. *Journal of Proteome Research*. 2010, 9(12):6354–6367.

[2] Anderson LC, DeHart CJ, Kaiser NK, Fellers RT, Smith DF, Greer JB, LeDuc RD, Blakney GT, Thomas PM, Kelleher NL, Hendrickson CL. Identification and Characterization of Human Proteoforms by Top-Down LC-21 Tesla FT-ICR Mass Spectrometry.J Proteome Res. 2017,16(2):1087-1096.

[3] Rui-Xiang Sun, Lan Luo, Long Wu, Rui-Min Wang, Wen-Feng Zeng, Hao Chi, Chao Liu, Si-Min He. pTop 1.0: a High-accuracy and High-efficiency Search Engine for Intact Protein Identification. *Analytical Chemistry*. 2016, 88(6):3082–3090.

Studying Therapeutic Antibody Degradation In Vivo Using High Throughput Affinity Capture Mass Spectrometry for Peptide and Intact Protein Level Analyses

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The importance of antibody therapeutic has grown exponentially but challenges associated with antibody degradation during blood circulation can lead to decreased drug activity, as well as undesirable changes of pharmacokinetics and immunogenicity. Further, degradations of antibody drug conjugates (ADC) can lead to increased toxicity. Therefore, thorough characterization of both antibodies and ADCs in vivo is essential for optimal drug design. Due to the complexity of serum samples, immuno-affinity capture is typically used for specific enrichment of antibody prior to LC-MS analysis. This presentation will focus on our developed methods to characterize and quantitate antibody biotransformations using affinity capture on tips followed by LC-MS at both the peptide and intact protein level. Bottom-up MS was used for studying degradations on the antibody backbone including deamidation, and oxidation. In particular, for isomerization stability, electron transfer dissociation was shown to be effective in differentiating the isomeric species. We have optimized the on-tip affinity capture for rapid digestion (2 hr for capture, digestion and elution) to minimize artificial modifications introduced through sample handling.1 Using the automated platform (intra and inter assay CVs < 8%), we successfully quantified the levels of deamidation of a humanized monoclonal antibody in cynomolgus monkeys over a time period of twelve weeks after administration (RSD <1%). As an alternative to bottom-up MS, intact protein analysis was used on applications for quantitating the stoichiometry of combinatorial modifications and degradations of the linked drugs observed with ADC stability studies. We compared the stability profiles of several ADCs from four different animal species over a 24 hour period and observed similar MS responses from affinity capture by both magnetic beads and affinity tips. However, the on-tip approach has much simpler automation, significantly lower samples volumes and higher throughput.

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Reference:

[1] Tran et al. Anal. Chem., 2016, 88, 11521

Revisiting Native Electron Capture Dissociation to Map Iron Binding in Equine Ferritin

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Native electron capture dissociation (NECD) is a process during which proteins undergo fragmentation similar to that from radical dissociation methods, but without the addition of exogenous electrons. However, after three initial reports of NECD from the cytochrome c dimer complex, no further evidence of the effect has been published. Here, we report NECD behavior from a 24-meric ferritin complex, ~20-fold larger than the previously studied cytochrome

c dimer. Application of front-end infrared excitation (FIRE) in conjunction with low and high-m/z quadrupole isolation and collisionally activated dissociation (CAD) provides new insights into the NECD mechanism, showing that activation of the intact complex in either the electrospray droplet or the gas phase can produce c-fragments with asymmetrically partitioned net charges. Similar to the previously reported results on cytochrome c, these fragments appear to form near residues that noncovalently interact with iron atoms. By mapping the location of backbone cleavages onto the crystal structure, we are able to characterize two distinct iron binding channels that facilitate iron ion transport from the cytosol to the core of the complex. The resulting pathways, in good agreement with previously reported results for iron binding in overexpression model systems, provide the first direct mapping of iron binding sites in mammalian ferritin from an endogenous sample.

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A User-Friendly Bioinformatics Resource for Automated Continuous Elution Proteoform Analysis

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Bioinformatics tools for top-down proteoform characterization are largely intended for data-dependent LC-MS/MS experiments. While this method has the benefit of increased sensitivity, it is not adequate for highly heterogeneous proteins that exhibit several proteoforms with different intact masses present within any single scan. Data-independent acquisition (DIA) utilizing fragmentation techniques such as ETD, CID, or nozzle skimmer dissociation (NSD) has the potential to increase proteoform identification because of simultaneous fragmentation. We have created a user-friendly bioinformatics tool to automate DIA proteoform assignment. A novel aspect of is resource is continuous elution scoring that facilitates detection of novel proteoforms not predicted in databases, as well as distinguishing related chromatographically-resolved proteoforms. We will highlight the workflow on myelin basic protein (MBP) which plays an important structural and functional role in the neuronal myelin sheath. MBP exhibits extreme microheterogeneity with numerous alternative splice variants (ASVs) and post translational modifications (PTMs) reportedly tied to central nervous system maturation, myelin stability, and the pathobiology of various de- and dys-myelinating disorders. Conventional bioanalytical tools cannot efficiently examine ASV and PTM events simultaneously which limits understanding of the role of MBP microheterogeneity in human physiology and disease. To address this need, we report on a top-down pipeline that combines superficially porous reversed-phase liquid chromatography (SPLC), Fourier transform mass spectrometry (FTMS), DIA, and aligned bioinformatics resources to rapidly characterize abundant MBP proteoforms. The three tier identification workflow resolved 4 known MBP ASVs and hundreds of differentially modified states from a single 90 min run on ~0.5 µg of material. This included 323 proteoforms for 14.1 kDa ASV alone. We also identified two novel ASVs from an alternative transcriptional start site (ATSS) of the MBP gene as well as a never before characterized S-acylation events linking palmitic acid, oleic acid and stearic acid at C78 of the 17.125 kDa ASV.

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Free Radical Induced Peptide Sequencing for the Site Localization of Sulfation and Phosphorylation

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Tandem mass spectrometry (MS/MS) is one of the primary methods for discovering, identifying, and localizing post-translational modifications in proteins.[1] Conventional positive ion mode collision induced dissociation (CID)-based MS/MS, however, often fails to identify site specific information for an important subset of labile and acidic modifications. This inability is due to the predisposition of these modifications to be lost as neutrals and their low positive ion mode ionization efficiency. In fact, it has been suggested that only 20-40% of phosphopeptides are detected using standard proteomics methods.[2] This impotence is only magnified when examining the extremely labile tyrosine sulfation.[3] While a number of methods to combat this problem have been developed, most require

special instrumentation or cumbersome sample handling. In this work, we present tempo-based free radical peptide sequencing (FRIPS) in both positive and negative ion mode for the analysis of phosphorylated and sulfated peptides. We demonstrate that FRIPS-based fragmentation is capable of producing sequence informative ions for both phosphorylated and sulfated peptides with limited loss of the PTM. Furthermore, we investigated how FRIPS-based fragmentation compares to ETD, ECD, CID, and HCD, both in terms of sequence coverage and fragmentation efficiency for phospho- and sulfo-peptides. The lack of special instrumentation requirements and the ability to limit PTM neutral loss makes FRIPS-based fragmentation a promising alternative to current techniques for the analysis of labile and acidic PTMs.

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References:

[1] Olsen, J. V; Mann, M. Mol. Cell. Proteomics 2013, 12, 3444–3452.

[2] Hsu, C.; Xue, L.; Arrington, J. V; Wang, P.; Sebastian, J.; Paez, P.; Zhou, Y.; Zhu, J.; Tao, W. A. J. Am. Soc. Mass Spectrom. 2017, 28, 1127–1135.

[3] Monigatti, F.; Hekking, B.; Steen, H. Biochim. Biophys. Acta - Proteins Proteomics 2006, 1764, 1904–1913.

Structure Refinement of the VDR-RXR-MED1 Nuclear Receptor Co-regulator Complex using Integrated Structural Mass Spectrometry

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Nuclear receptors (NRs) are transcription factors that regulate gene expression through the ligand controlled recruitment of a diverse group of proteins known as co-regulators. Most NR co-regulators function in large multi-protein complexes that modify chromatin and thereby regulate the transcription of target genes. Our functional understanding of NRs has relied greatly on structural studies, involving either isolated DNA binding domain (DBD) and ligand binding domain (LBD). Structural information about full-length NRs are beginning to reveal how these complexes are assembled to mediate multiple functionalities. However, structural information on bound co-activators and co-repressors is so far limited to peptides containing and LXXLL motif. These observations highlight the need for further investigations of the structural of full-length NRs co-regulator complexes in different functional states in order to unravel the relevant architectures.

The vitamin D receptor (VDR) is a member of the NR superfamily, it functions as a heterodimer in complex with retinoid X receptor (RXR) to regulate the expression of target genes through binding to specific sequence in the promoter or enhancer regions. Previously, the structure of the DNA-bound full-length VDR-RXR has been constructed based on cryo-EM, SAXS, isolated DBS and LBD structures (Rochel, Ciesielski et al. 2011, Orlov, Rochel et al. 2012). However, there is only SAXS data available for the VDR-RXR heterodimer in complex with mediator (MED1). It is therefore a growing need to integrate the structural and molecular information obtained from different approaches for the investigations of biological macromolecular complexes. Mass spectrometry (MS) has an expanding role in integrated structural biology and servers as a go-between. Here, we couple different structural MS approaches including native top-down MS, cross-linking, limited proteolysis, and surface labeling to fill the missing structural information by other biophysical approaches.

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Reference:

Orlov, I., et al. (2012). "Structure of the full human RXR/VDR nuclear receptor heterodimer complex with its DR3 target DNA." The EMBO Journal **31**(2): 291-300.

Rochel, N., et al. (2011). "Common architecture of nuclear receptor heterodimers on DNA direct repeat elements with different spacings." Nat Struct Mol Biol **18**(5): 564-570.