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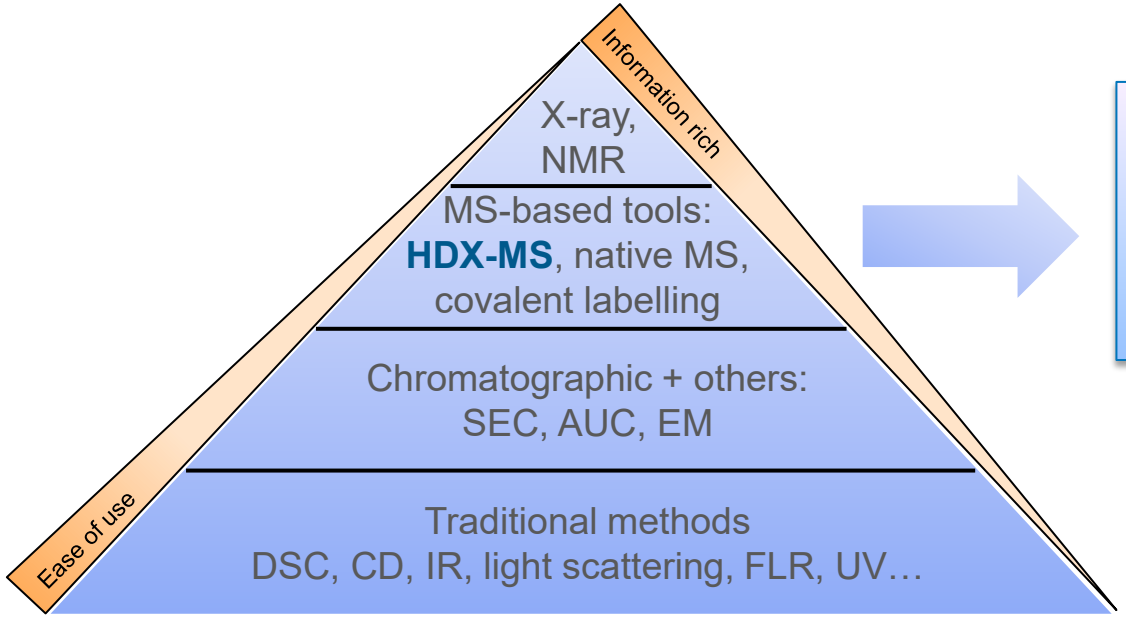
Characterising Protein Structure with HDX-MS: Improving Resolution with IMS-MS and Electron-based Fragmentation

Suraj Dhungana, Ph.D.

Director
Americas Pharmaceutical Market Segment

Biophysical Tools for Characterizing Higher Order Structure



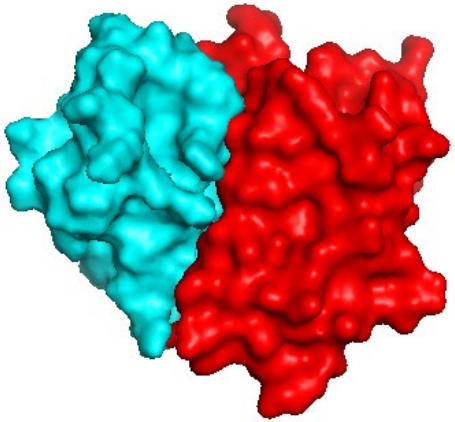
HDX-MS:
Changes in protein structure and dynamics
Solvent accessibility and hydrogen bonding

Why HDX?

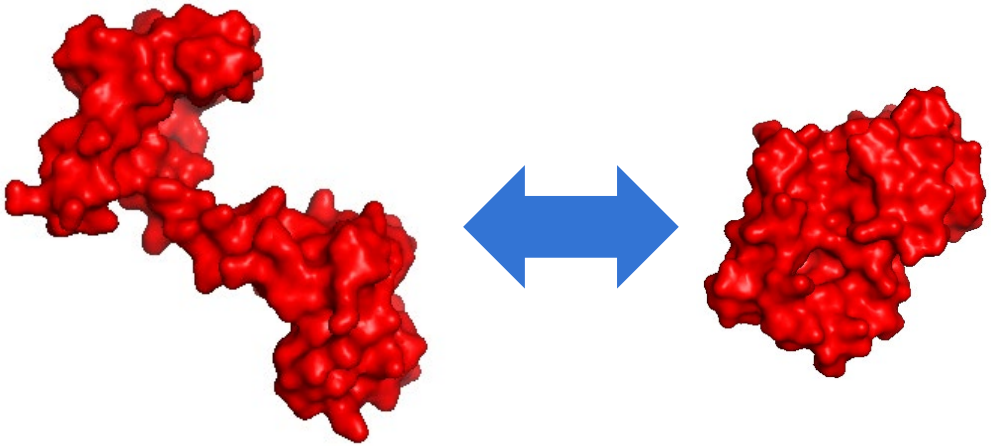
- ✓ Small protein amounts
- ✓ No chemical labeling of protein
- ✓ Limit unwanted structural perturbations
- ✓ Relatively quick turnaround time vs other techniques

Adapted from: J. Ahn, K. Yu and S. J. Skilton, *LCGC Europe.* 2013, 26 (6), 344-353.

Uses for HDX-MS



Protein-protein / protein-ligand interactions



Changes in protein structure/dynamics

Confirming higher order structure

Target protein binding site identification / specificity

Mode of Action (screening)

Binding affinity / dynamics

Epitope mapping—design and mechanism of action

Structural impact of post-translational modifications

Biosimilar structural analysis

Characterizing reversible self-association/aggregation

Stability (PTM effects)

HDX to Characterize the Architecture and Self-assembly of Sars-CoV-2 Nucleocapsid Protein

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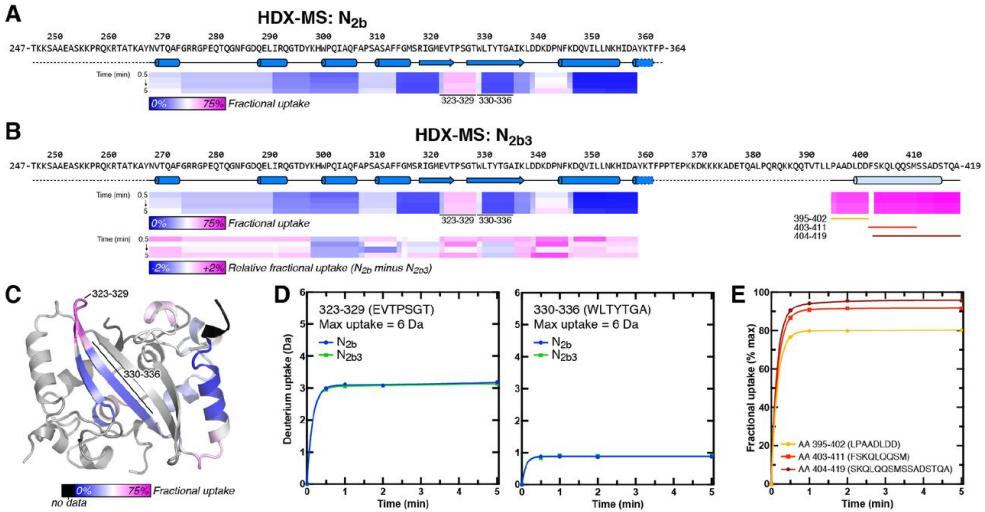

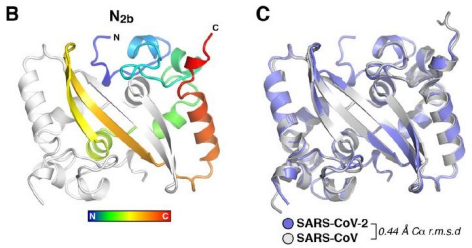

Comparative Analysis with Previously Characterized Viral Proteins

- Secondary structure and solvent accessibility across an entire protein
- Loop regions showed increased exchange rates, consistent with their flexibility
- “systematically examine the epitopes”

Received: 6 June 2020 | Revised: 2 July 2020 | Accepted: 3 July 2020
DOI: 10.1002/pro.3909

FULL-LENGTH PAPERS

THE PROTEIN SOCIETY WILEY

Architecture and self-assembly of the SARS-CoV-2 nucleocapsid protein

Qiaozhen Ye[1] | Alan M. V. West[1] | Steve Silletti[2] | Kevin D. Corbett[1,2,3]

HDX for SARS-CoV-2 S protein:ACE2—Reveals Novel Allosteric Targets

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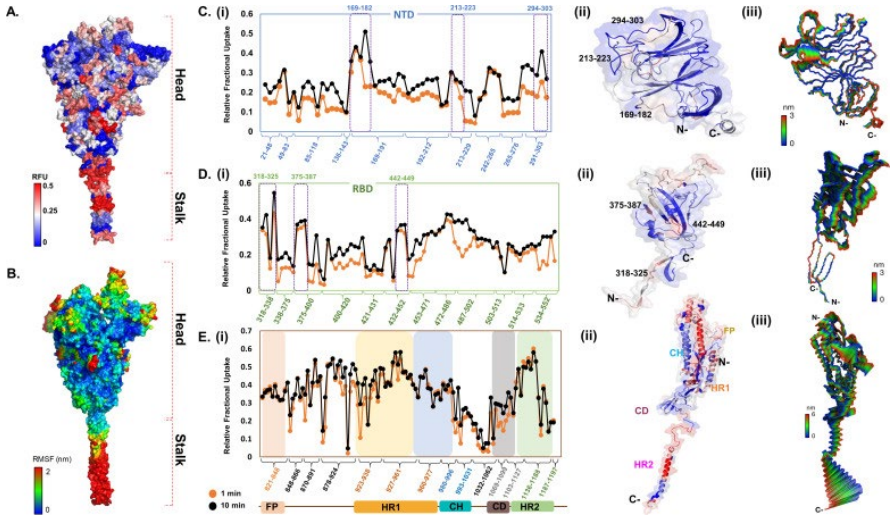
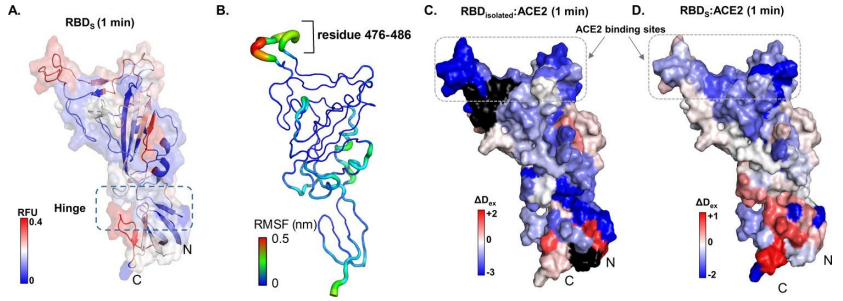


Conformational dynamics of prefusion spike (S) protein trimer



Map of receptor binding domain (RBD) isolated:angiotensin-converting enzyme 2 (ACE2) interactions


eLife

RESEARCH ARTICLE

Raghuvamsi, Tulsian, *et al.* eLife 2021;10:e63646. DOI: https://doi.org/10.7554/eLife.63646

SARS-CoV-2 S protein:ACE2 interaction reveals novel allosteric targets

Palur V Raghuvamsi[1,2†], Nikhil K Tulsian[1,3†], Firdaus Samsudin[2], Xinlei Qian[4], Kiren Purushotorman[4], Gu Yue[4], Mary M Kozma[4], Wong Y Hwa[5], Julien Lescar[5], Peter J Bond[1,2*], Paul A MacAry[4*], Ganesh S Anand[1,6*]

HDX-MS for mAbs and Covid-19 Target Protein Binding

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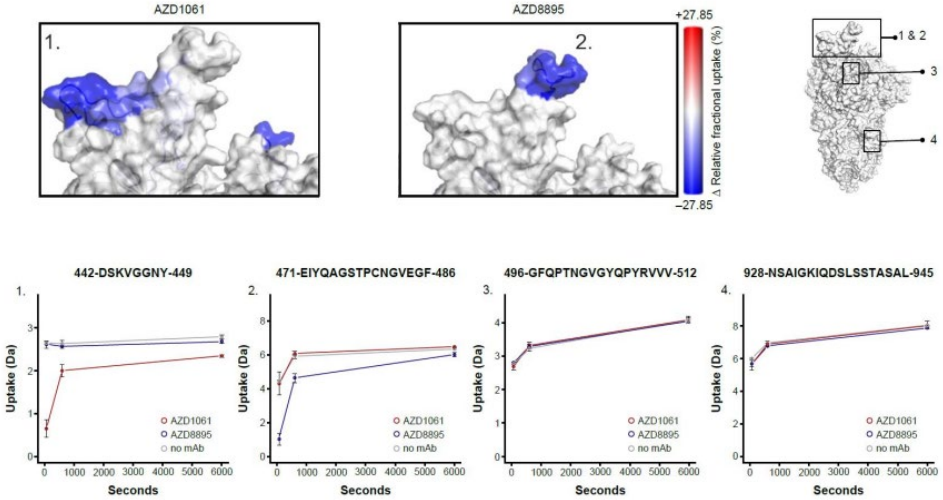

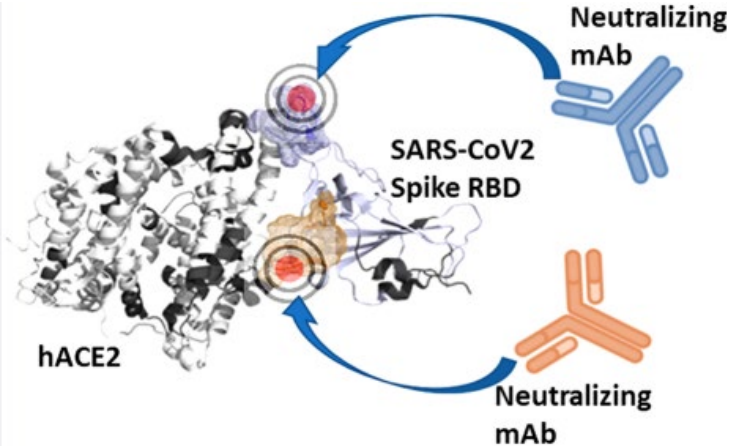

The SARS-CoV-2 monoclonal antibody combination, AZD7442, is protective in nonhuman primates and has an extended half-life in humans

YUEH-MING LOO, PATRICK M. MCTAMNEY, ROSALINDA H. ARENDS, MICHAEL E. ABRAM, ANASTASIA A. AKSYUK, SEME DIALLO, DANIEL J. FLORES, ELIZABETH J. KELLY, KUISHU REN, [...] MARK T. ESSER +22 authors Authors Info & Affiliations

Protein Footprinting, Conformational Dynamics, and Core Interface-Adjacent Neutralization "Hotspots" in the SARS-CoV-2 Spike Protein Receptor Binding Domain/Human ACE2 Interaction

Dominic Narang, D. Andrew James,* Matthew T. Balmer, and Derek J. Wilson*

HDX Useful for Biotherapeutics

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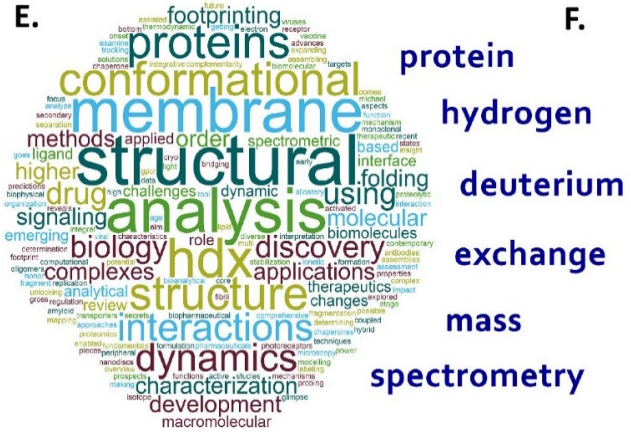
"Developments in Hydrogen/Deuterium Exchange Mass Spectrometry" J Engen et al. Analytical Chemistry. Oct 28, 2020



- Vaccines, therapeutics
- HOS, Epitope mapping

HDX use in New Modalities

© American Society for Mass Spectrometry, 2018 | CrossMark | J. Am. Soc. Mass Spectrom. (2018) 29:1524–1531 DOI: 10.1007/s13361-018-1966-2

RESEARCH ARTICLE

Conformational Assessment of Adnectin and Adnectin-Drug Conjugate by Hydrogen/Deuterium Exchange Mass Spectrometry

Richard Y.-C. Huang,[1] Steven R. O'Neil,[2] Daša Lipovšek,[2] Guodong Chen[1]

analytical chemistry

pubs.acs.org/ac | Article

Integrated Approach for Characterizing Bispecific Antibody/Antigens Complexes and Mapping Binding Epitopes with SEC/MALS, Native Mass Spectrometry, and Protein Footprinting

Richard Y.-C. Huang,* Feng Wang, Matthew Wheeler, Yun Wang, Robert Langish, Bryant Chau, Jia Dong, Winse Morishige, Natalie Bezman, Pavel Strop, Arvind Rajpal, Olafur Gudmundsson, and Guodong Chen*

pharmaceuticals | MDPI

Article

State-of-the-Art Native Mass Spectrometry and Ion Mobility Methods to Monitor Homogeneous Site-Specific Antibody-Drug Conjugates Synthesis

Evolène Deslignière [1,2], Anthony Ehkirch [1,2], Bastiaan L. Duivelshof [3,4], Hanna Toftevall [5], Jonathan Sjögren [5], Davy Guillarme [3,4], Valentina D'Atri [3,4], Alain Beck [6], Oscar Hernandez-Alba [1,2] and Sarah Cianférani [1,2,*]

analytical chemistry | Article | pubs.acs.org/ac

Antibody Structural Integrity of Site-Specific Antibody-Drug Conjugates Investigated by Hydrogen/Deuterium Exchange Mass Spectrometry

Lucy Yan Pan,* Oscar Salas-Solano, and John F. Valliere-Douglass

Typical HDX-MS workflow

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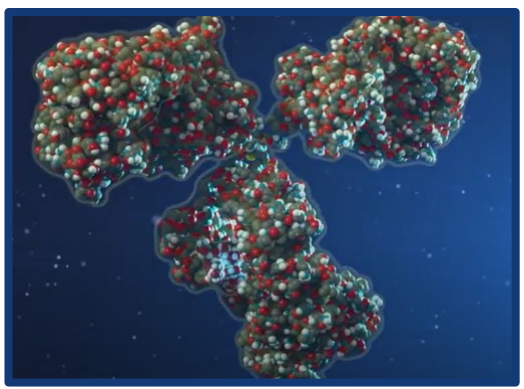
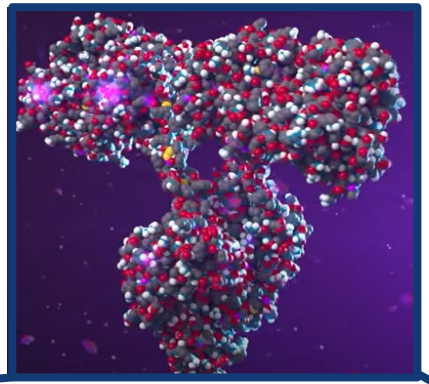
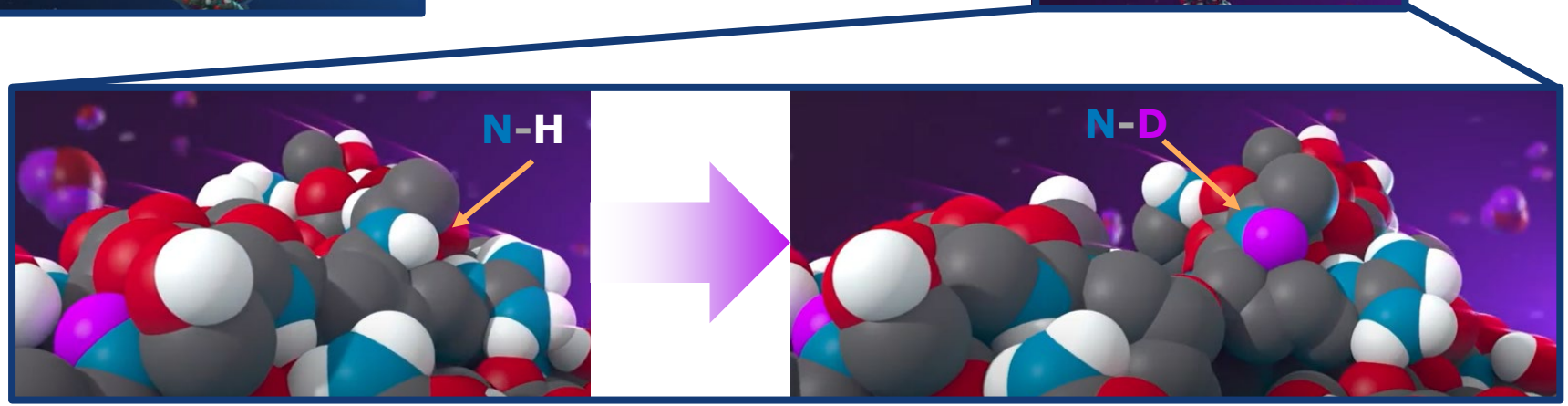


Dilute sample into D_2O





Typical HDX-MS workflow

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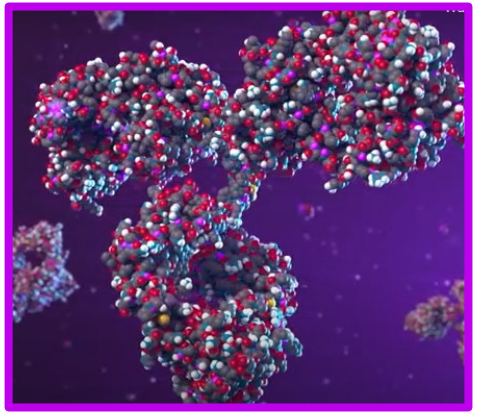
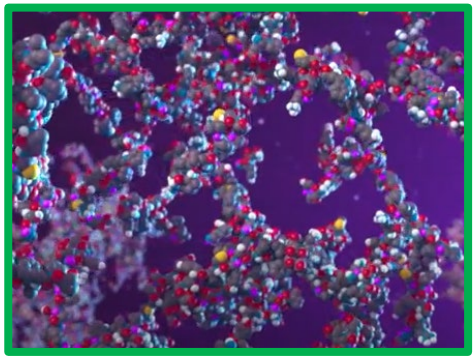
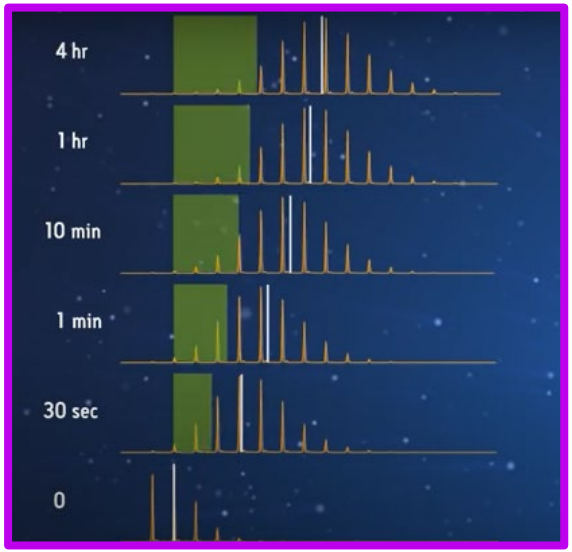
Quench



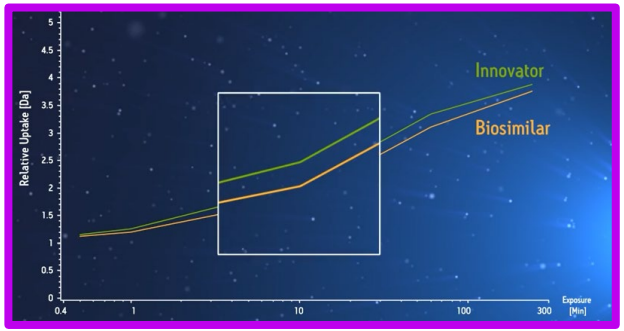
Digest



LC-MS



Compare



HDX: Pausing for Fundamentals

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Goal: Structural Resolution

Most information from the sample better the structural resolution

How?

- Reversable Isotopic labeling of accessible H with D
- Use rate of exchange to measure accessibility/flexibility in structure

Challenge

Scrambling, back-exchange

Fast Analysis to Capture all Relevant Information

- Striving for high separation in fast chromatography
- Add ion mobility to increase analytical peak capacity
- Sensitivity for capture of low abundant species
- Isotopic fidelity
- Complementary fragmentation techniques

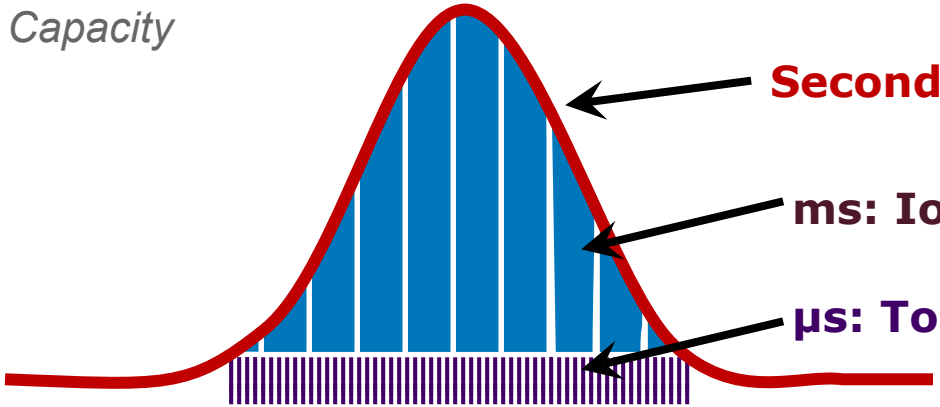
Alternate

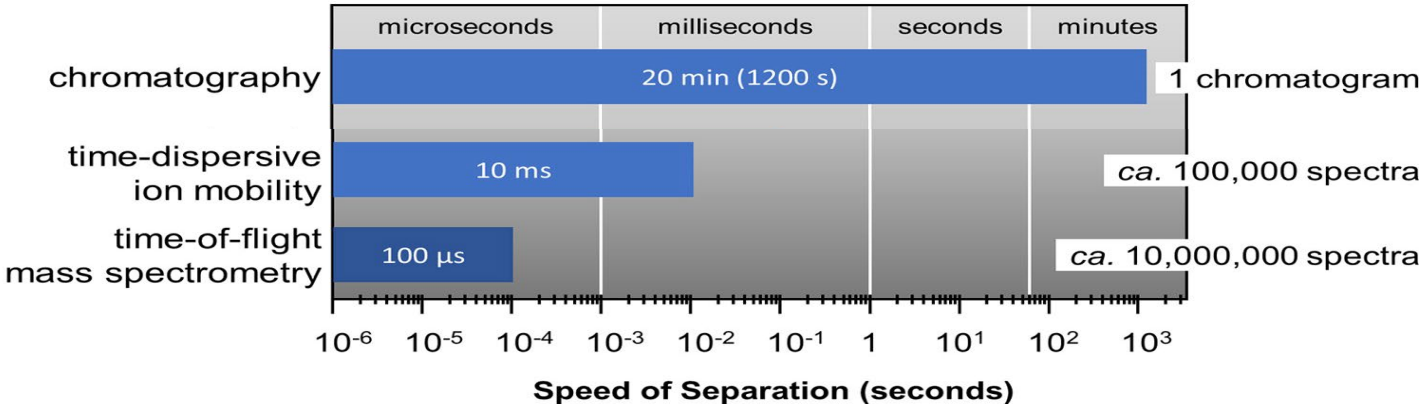
Covalent modification

✓ Risk of unwanted structural perturbations

Taking Advantage of Nested Analytical Timescales

Increase Analytical Peak Capacity





Adapted from May, et al. *Anal. Chem.* **2015,** 87, 1422-1436.

Ion Mobility Increase Peak Capacity

Increased Separation and Clean Detection of Deuterium Mass Shifts

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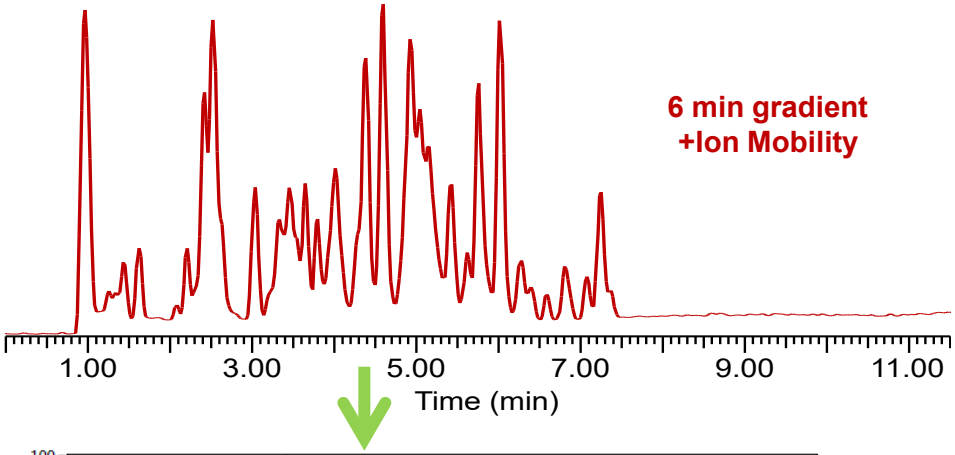
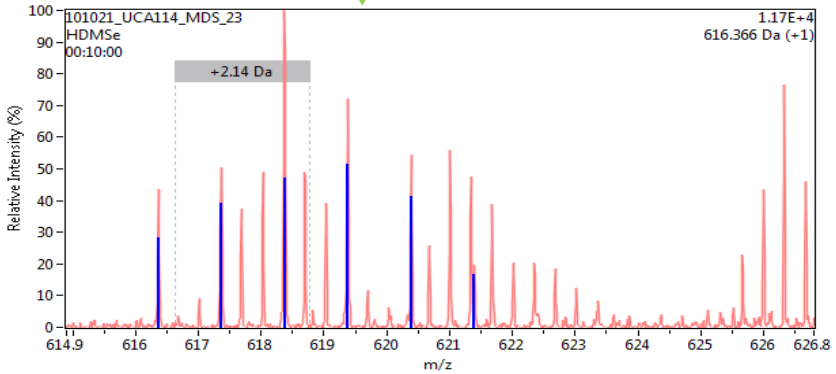
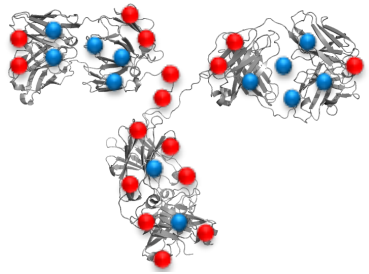
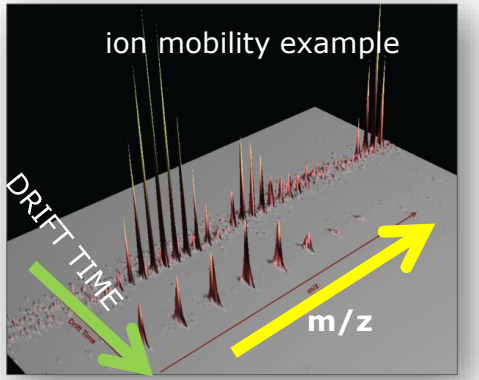




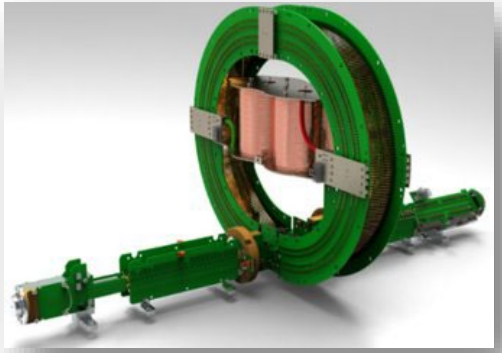
mAb 150 kDa

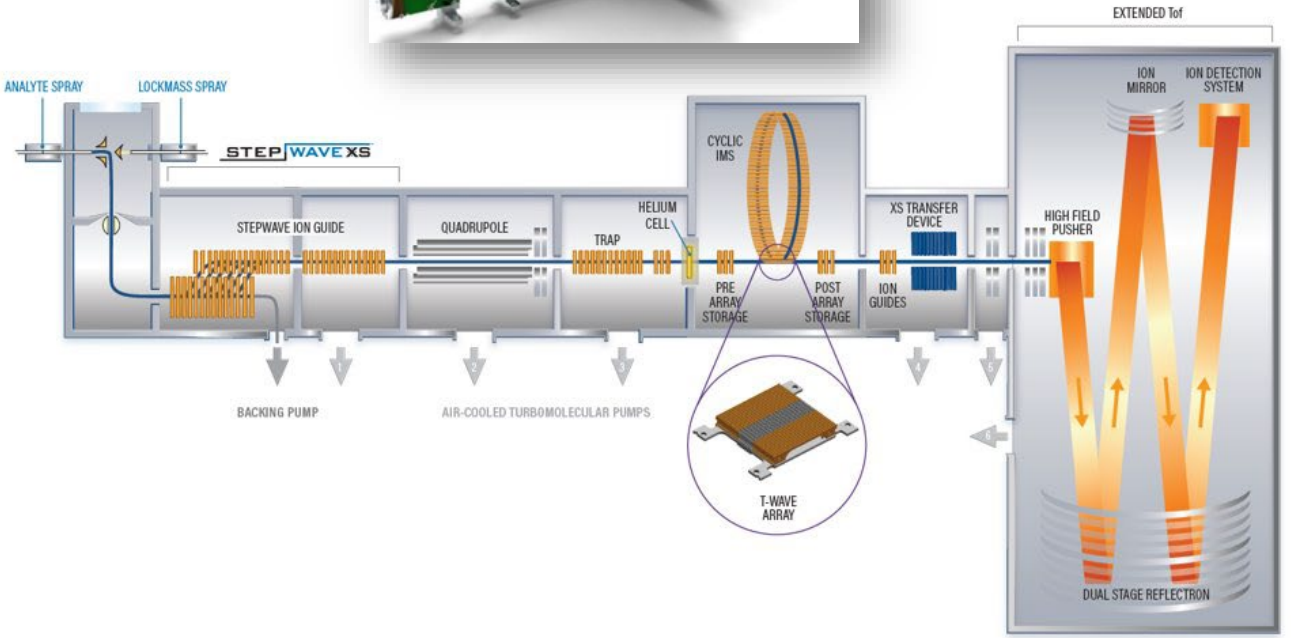




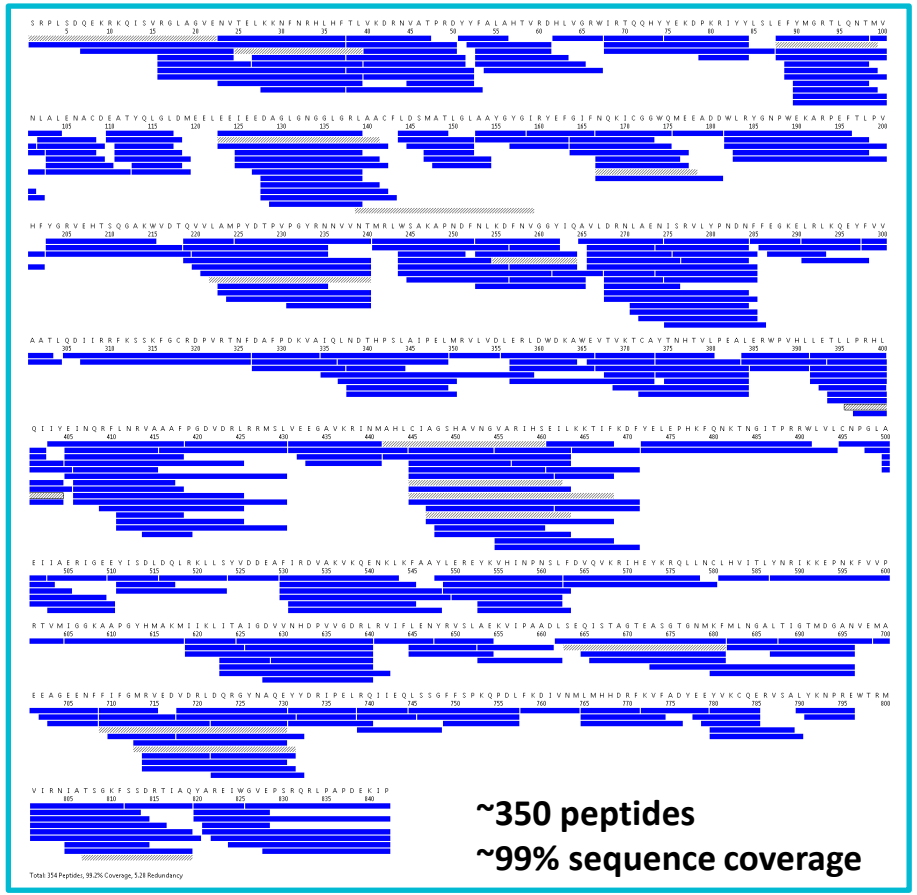
HDX on Cyclic IMS-MS

- Step Wave XS
  - Improved sensitivity
- Improved IMS resolution
  - Multiple passes
- Dual-gain Detector
  - Enhanced mass resolution & accuracy
    - > 60K V-mode
    - > 100K W-mode
  - Extended dynamic range
- Multiple Fragmentation Options
  - CID and ECD





HDX on Cyclic IMS



- Increased peptide ID
- Increase sequence coverage
- Reduced back exchange

Cyclic IMS data collected in HDMS^E mode (Ion Mobility enabled DIA—default mode for peptide analysis)

Advances in Ion Mobility Enabled HRMS and HDX

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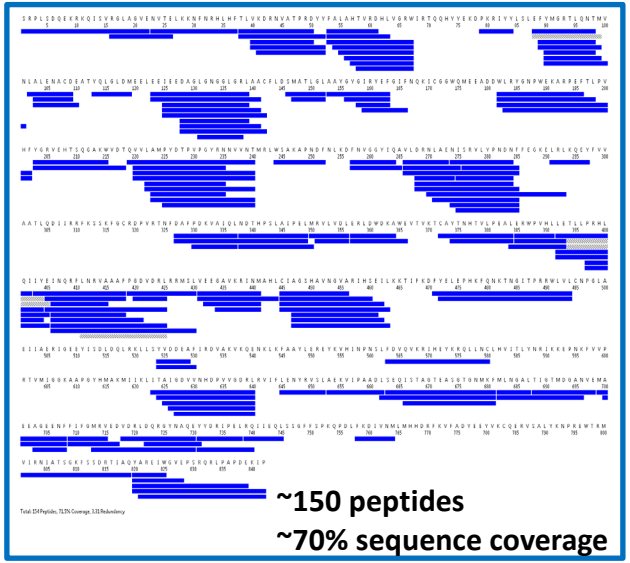
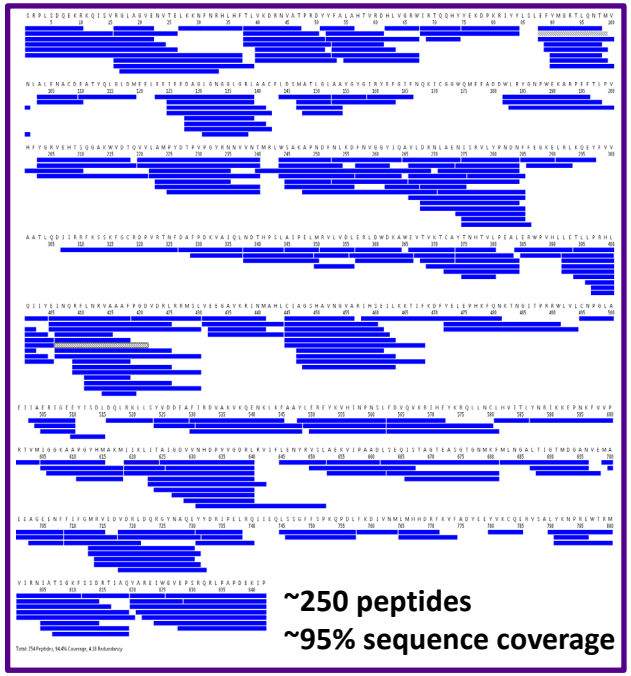
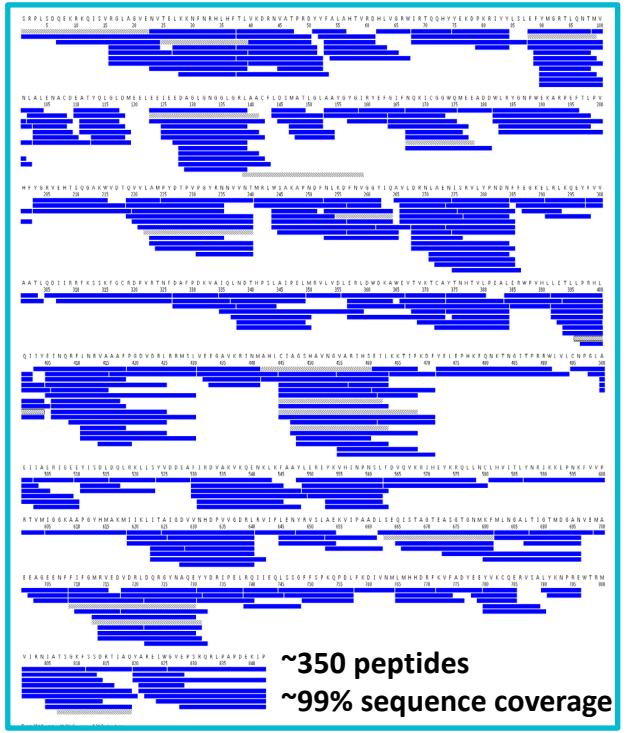
Improved HDX MS Performance



~150 peptides
~70% sequence coverage



~250 peptides
~95% sequence coverage



~350 peptides
~99% sequence coverage

Cyclic IMS: Additional Sequence Coverage

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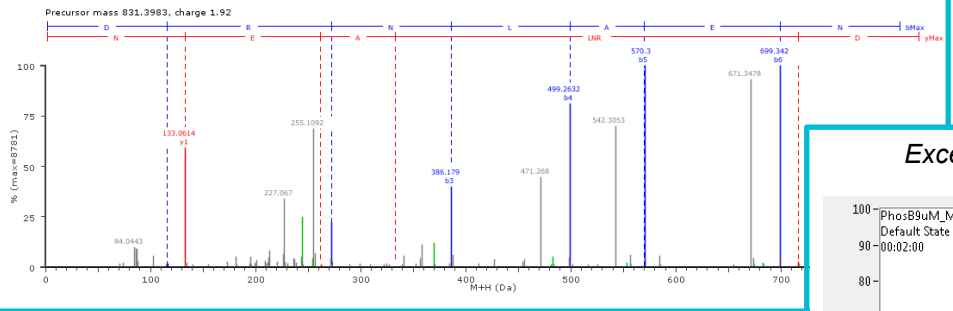
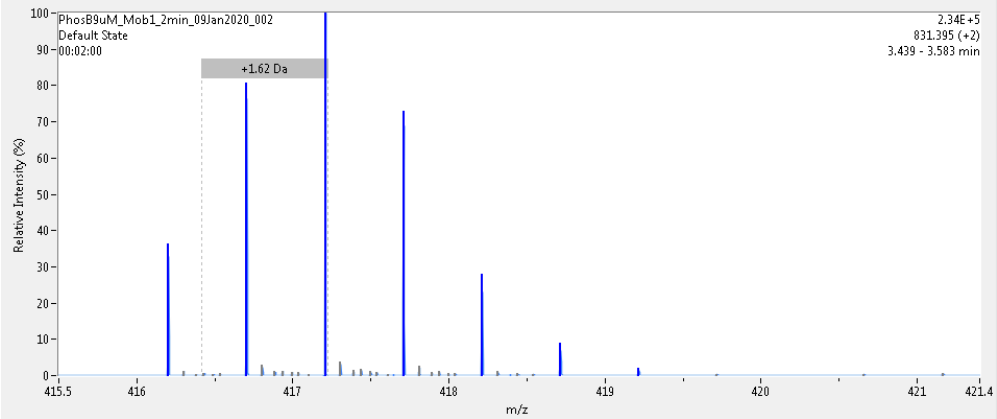
Improved Detection of Low Intensity Peptide

High quality spectra with peptide identification only obtained by the SELECT SERIES Cyclic IMS

High quality fragment ion spectrum and sequence assignment:



Excellent quality deuterated spectra enable confident uptake measurement:



High Ion Mobility Resolutions for Separating Deuterated Peptides in a Mixture

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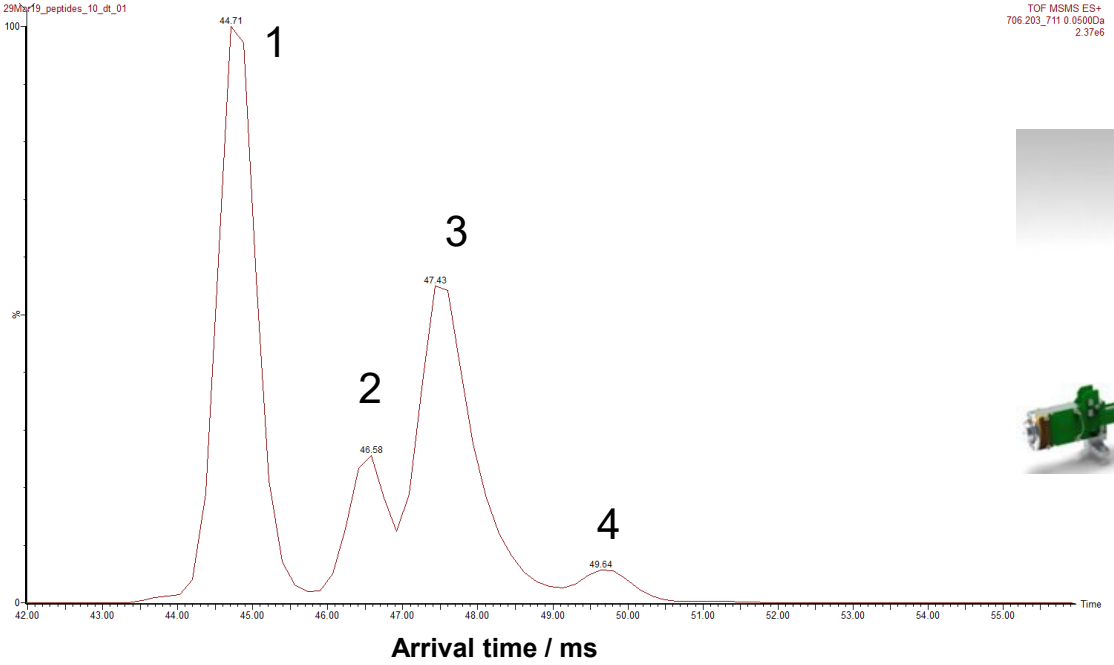
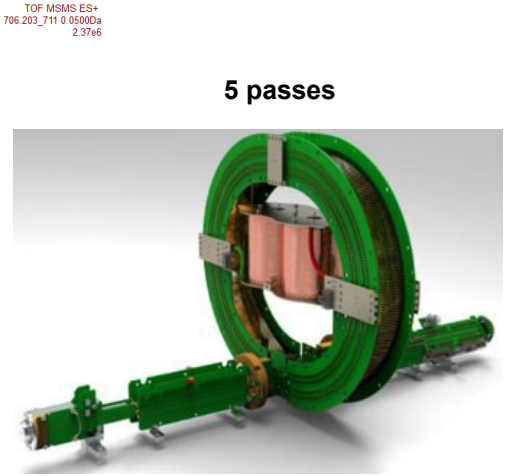
After five passes, four species were observed in the Arrival Time Distribution (ATD)





IM Separated Deuterated Peptides in a Mixture

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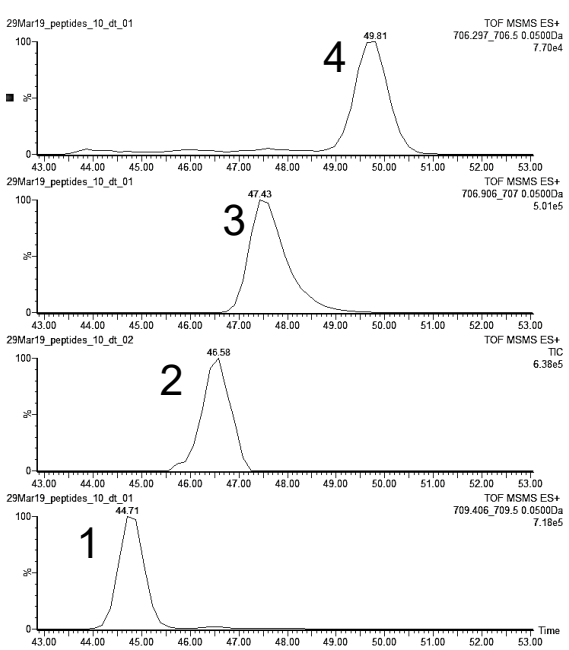
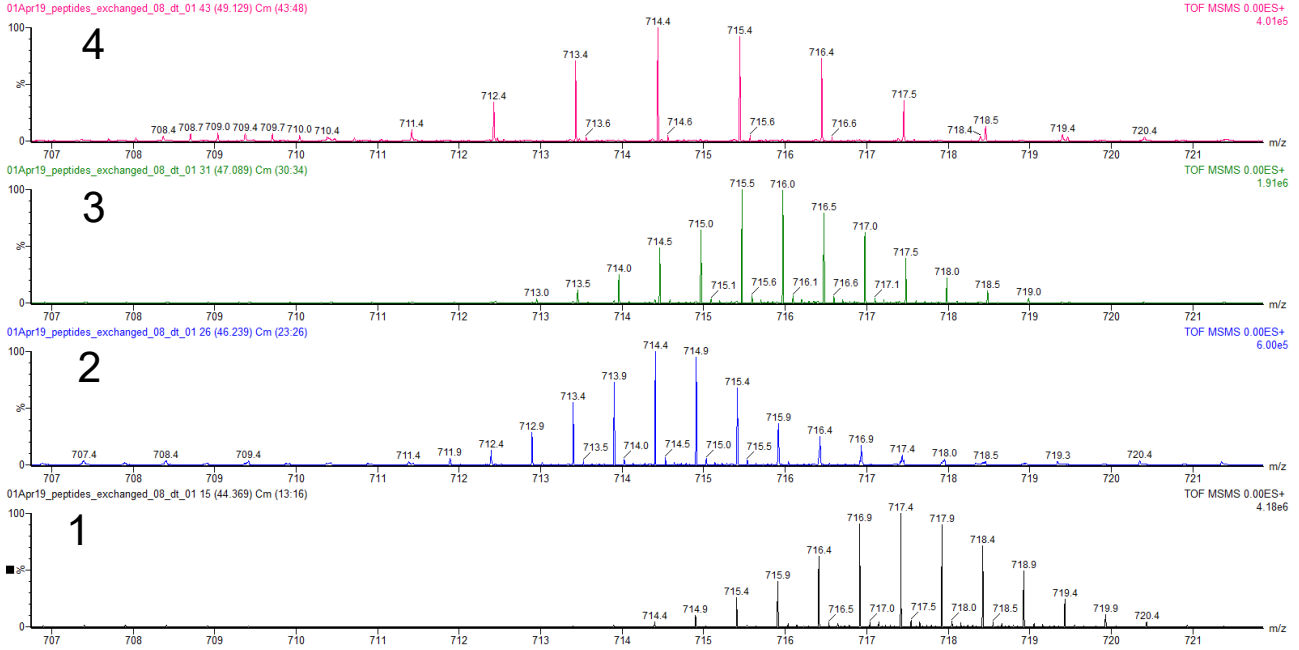
Improved separation provides information rich clearer spectra

5 passes



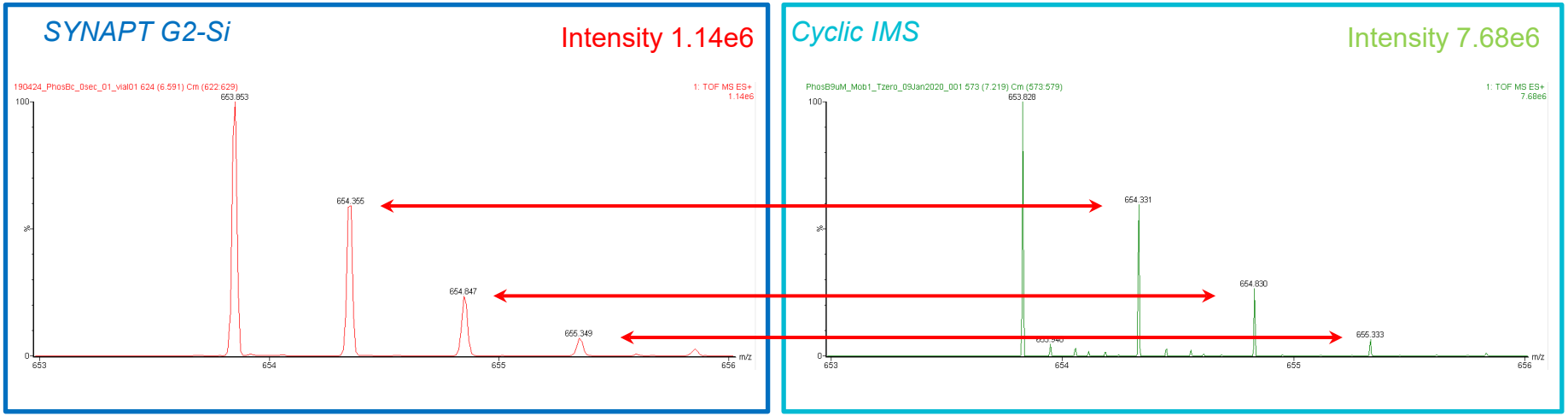
Arrival time / ms



Cyclic IMS: Detector Dynamic Range

Example: An intense peptide found in both SYNAPT G2-S*i* data and Cyclic IMS data

Zoomed-in spectra of isotopic peaks:



Dual gain ADC offers extended dynamic range and ensures correct peak intensities in the output spectrum

Optimal Fragmentation for Structural Resolution

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Long peptides

Larger groups of backbone amides

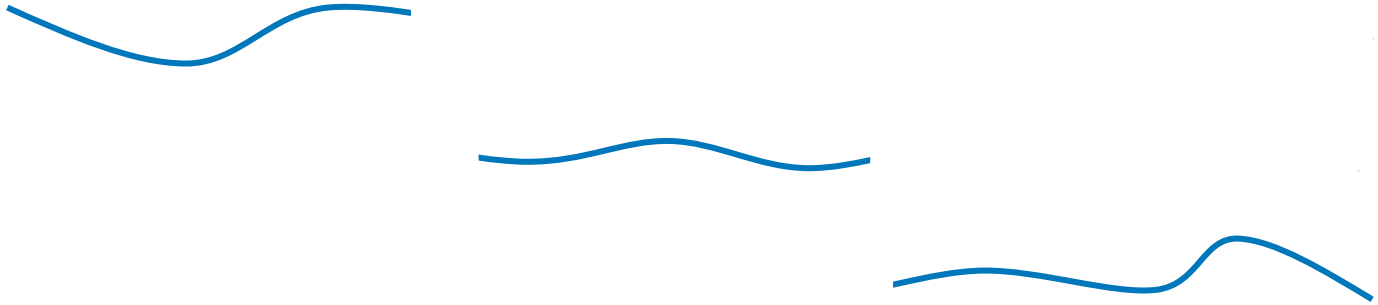
Lower structural resolution

fragmentation

Shorter peptides

Smaller groups of backbone amides

Higher structural resolution



Improving Peptide Fragmentation and Limiting Deuterium Scrambling

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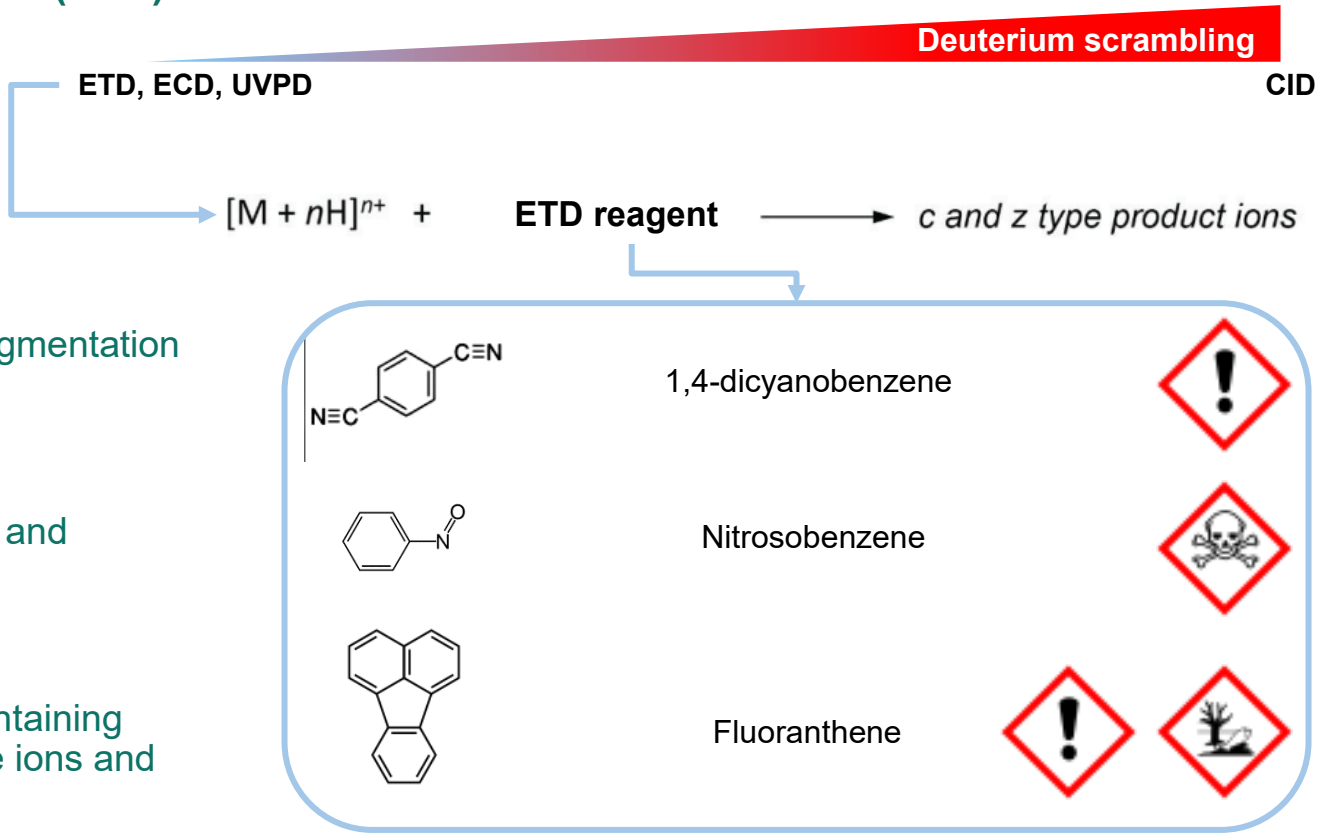
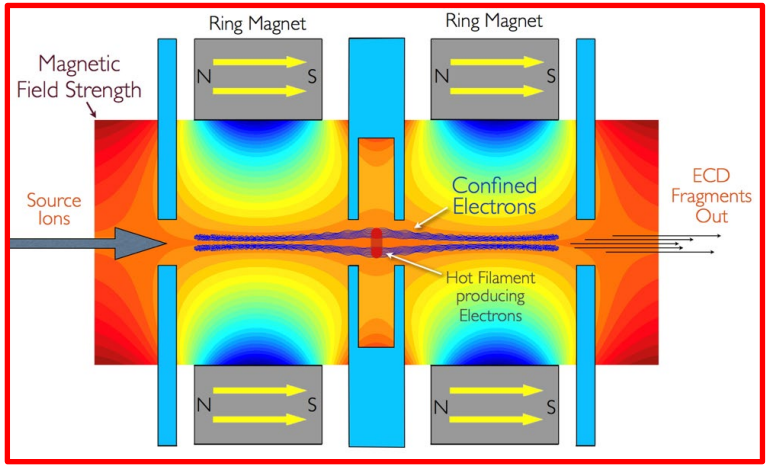
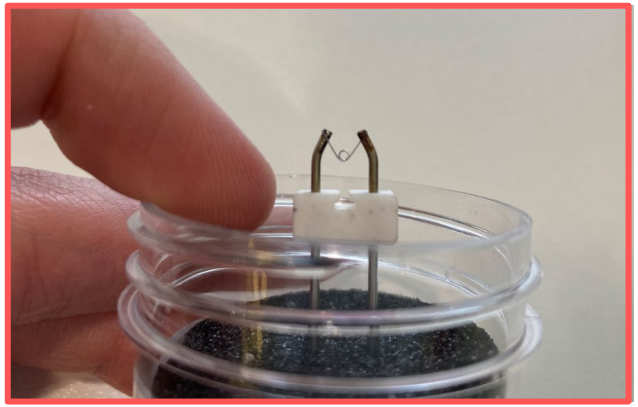
Electron transfer dissociation (ETD)



- ETD is advantageous for the fragmentation of longer peptides
- Leaves labile PTMs intact
- Electron transfer between anion and analyte induces fragmentation
- ETD reagents are often toxic
- Challenges associated with maintaining transmission of both +ve analyte ions and –ve ETD reagent

Electron Capture Dissociation (ECD)





Rhenium ECD filament

ECD unit from eMSion

Heated Rhenium filament produces electrons, which are captured by analyte ions as they pass through causing fragmentation

Peptide P1 ECD Fragmentation

No IMS

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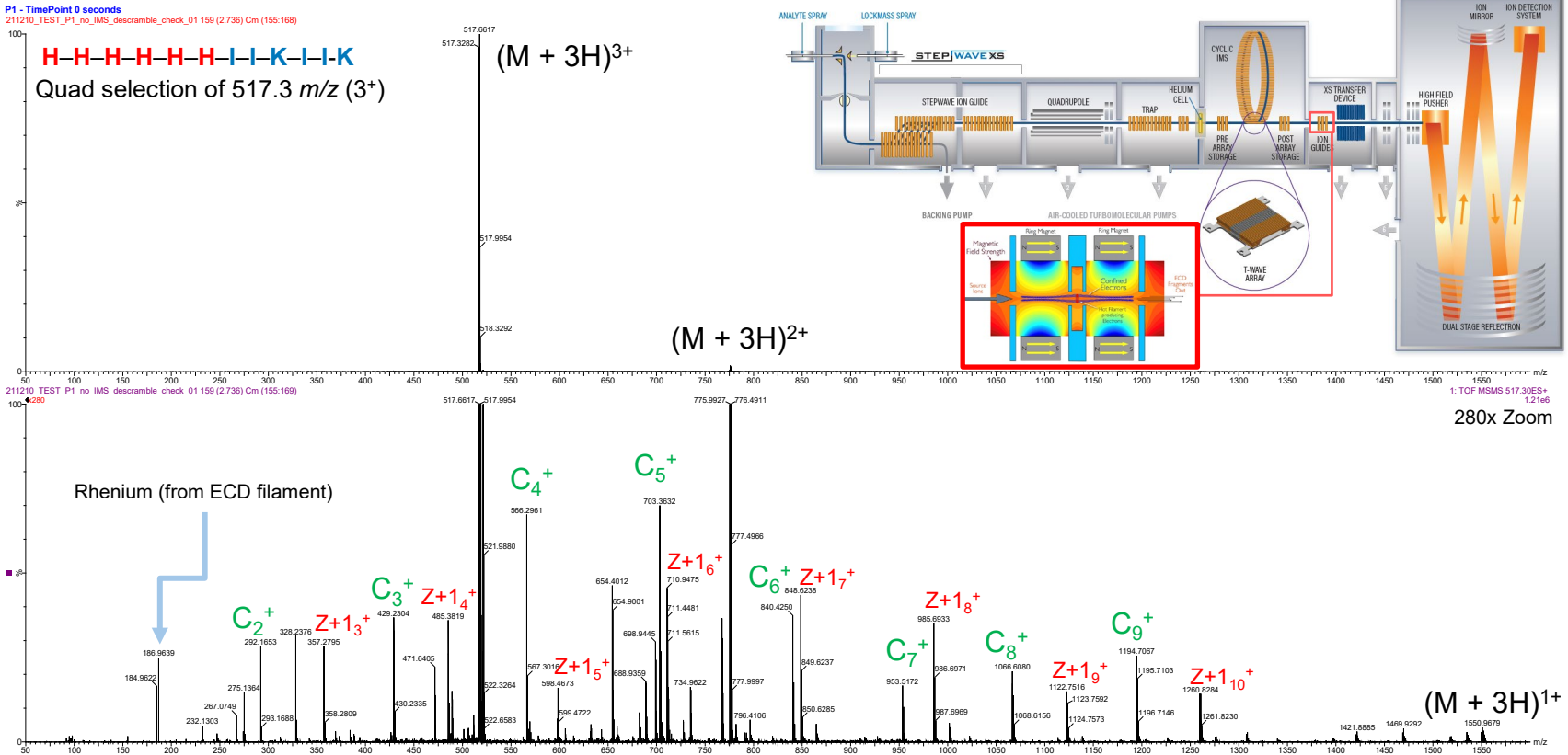

ECD of PhosB Peptides from Online Pepsin Digests

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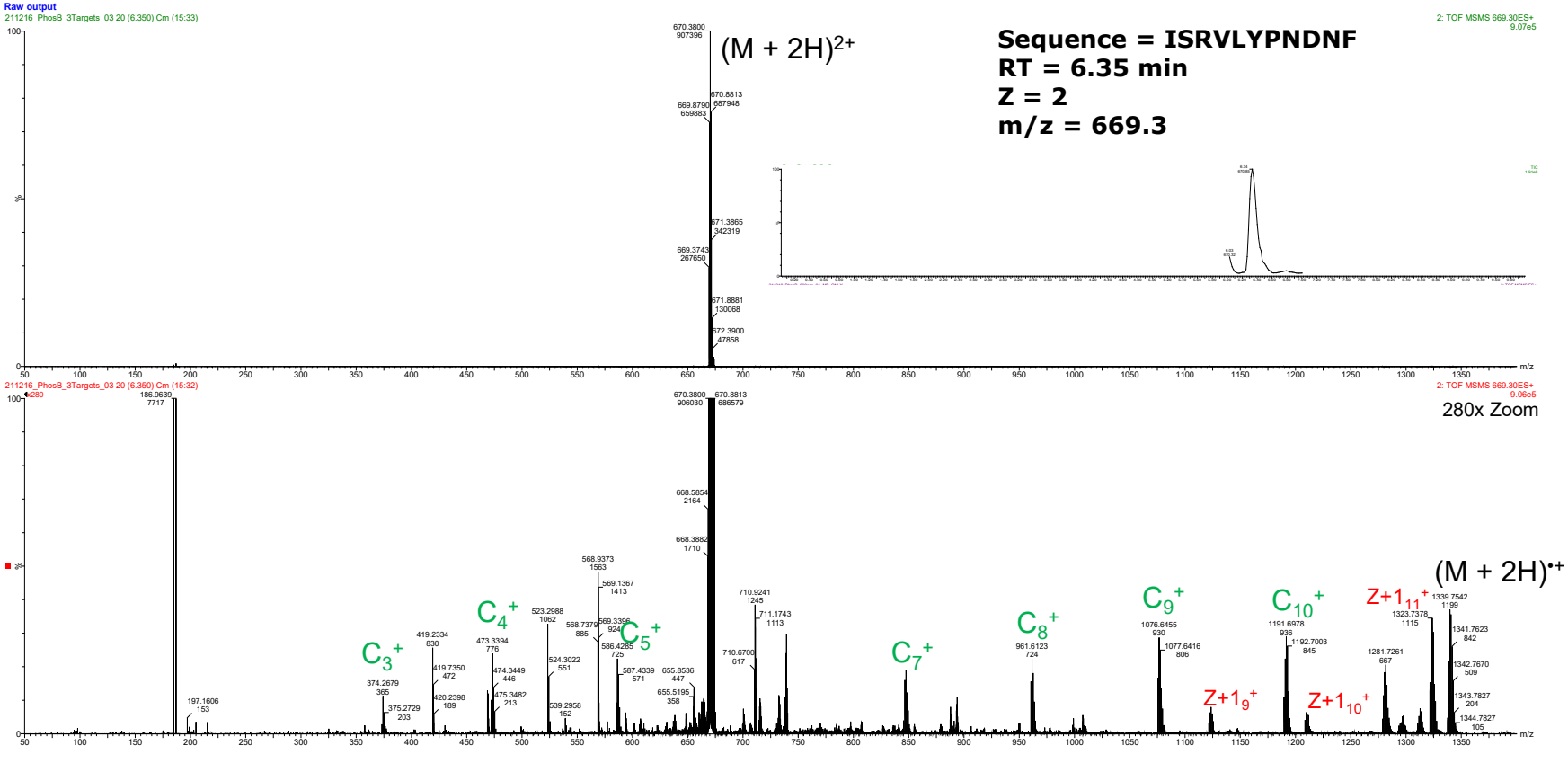
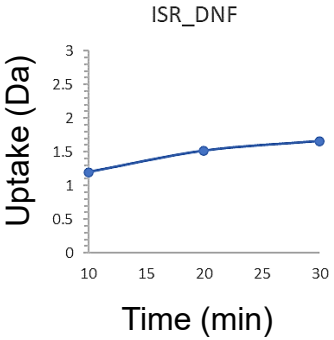
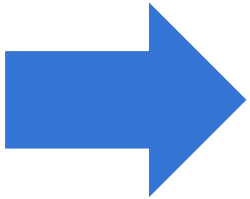
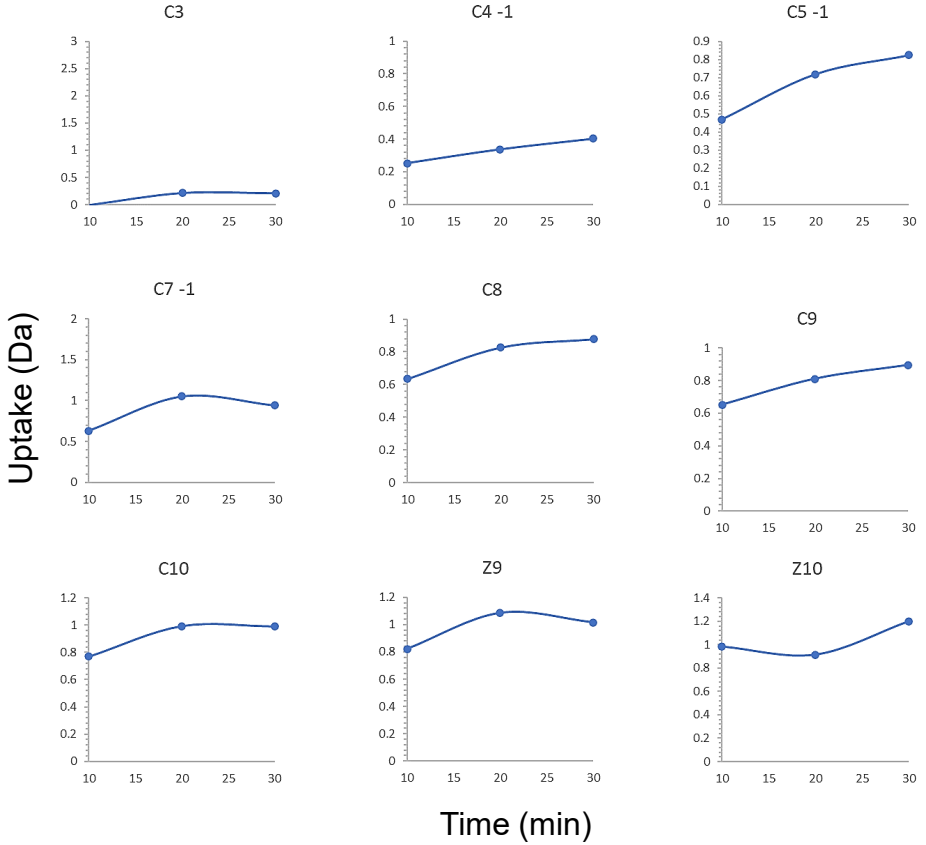


Higher Structural Resolution From ECD







Conclusions

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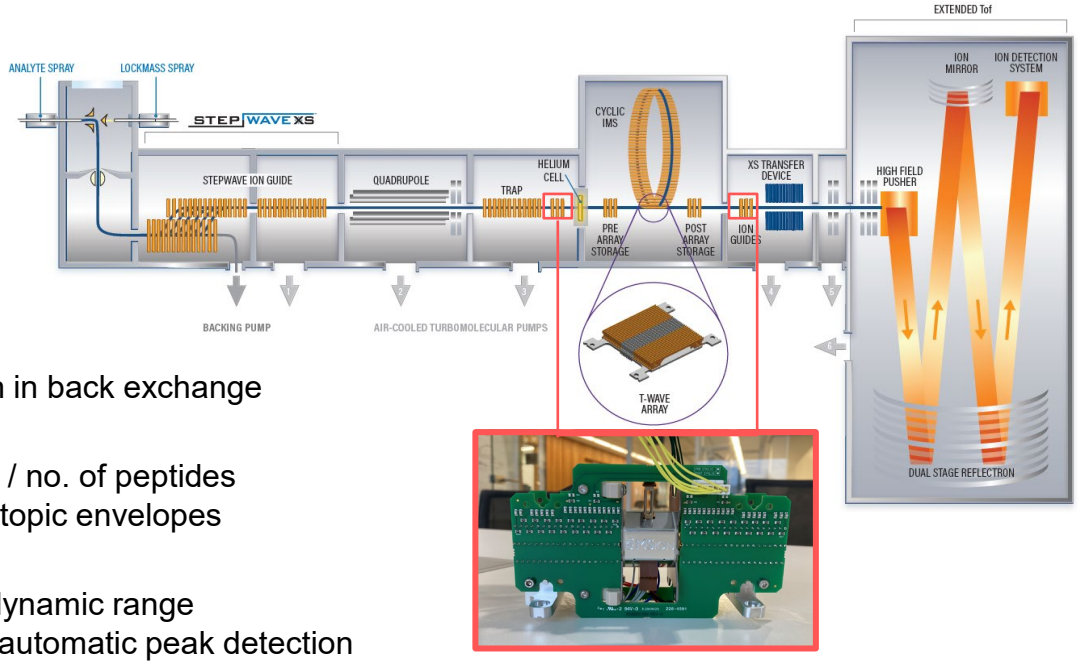
Cyclic IMS provides significantly improved structural resolution

- Increased sequence coverage / number of peptides / redundancy for HDX



Stepwave XS

- Improved sensitivity and suggested reduction in back exchange

Higher Mobility Resolution

- Contributes to increased sequence coverage / no. of peptides
- Resolving differently charged overlapping isotopic envelopes

Increased detector dynamic range

- Dual gain ADC detector provides increased dynamic range
- Improvement in isotopic fidelity for improved automatic peak detection
- Increased range of useable peptide intensities

ECD

- High quality fragmentation of deuterated peptides on the LC timescale
- Provide higher Structural Resolution

Acknowledgement

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