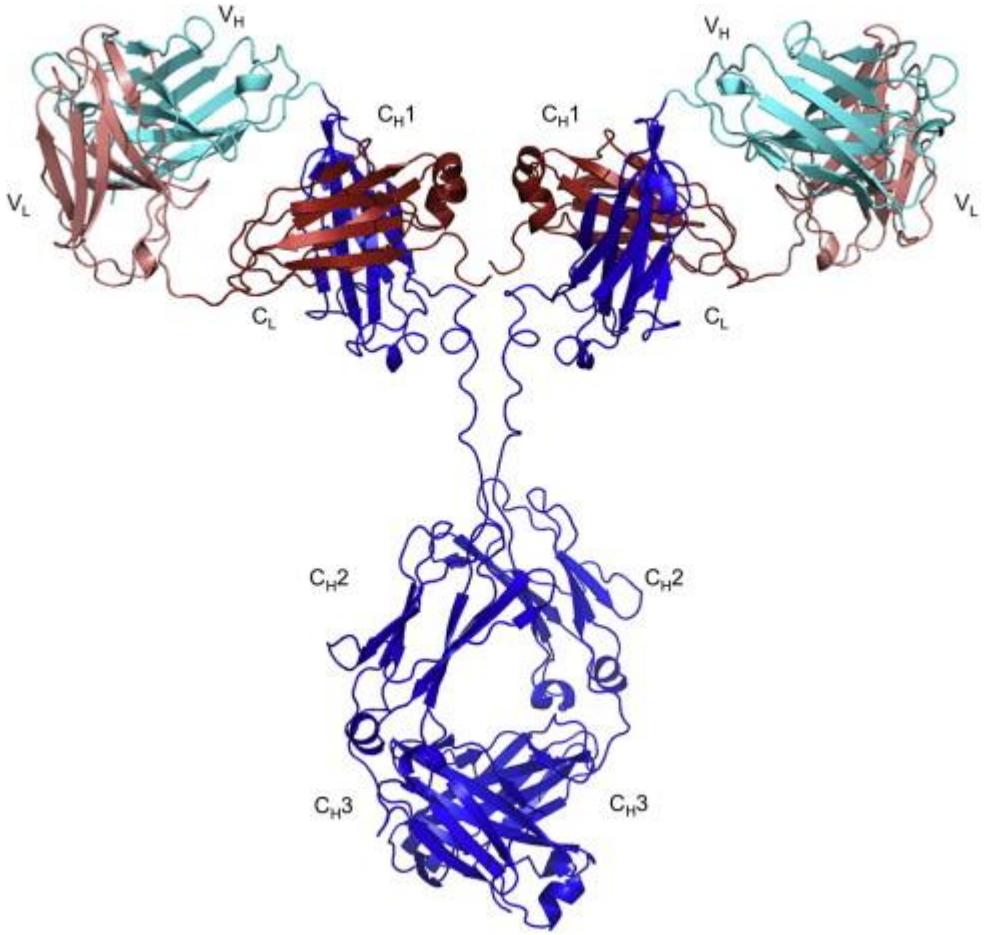
MOBILion SYSTEMS, INC.

Revealing What Others Leave Unseen

SLIM Based High-Resolution Ion Mobility for Large Biomolecule Characterization

Tom Doherty PhD | Sr. Product Manager MOBIE

Why Characterization Matters

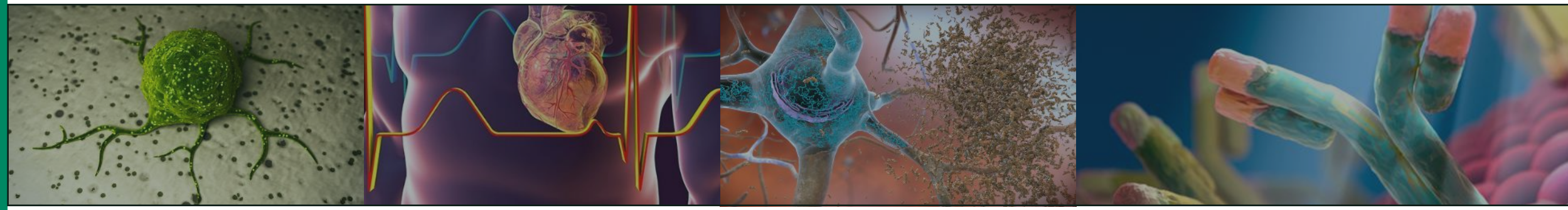


- Small structural changes can have dramatic effects on product **safety and efficacy**
- Post-translational modifications (PTMs) can be induced by environmental factors during **production, formation, and storage**
- Manufacturers are required to demonstrate **full control** over their production process and **thoroughly characterize the protein sequence and PTMs**

Image: Rouet, et al. Stability engineering of the human antibody repertoire. FEBS Letters. 588 (2), 21 Jan 2014 https://doi.org/10.1016/j.febslet.2013.11.029

The Problem

Classical analytical techniques are too slow, too complex and/or not powerful enough to address molecular and structural characterization challenges in biopharma drug development, early disease detection and clinical diagnostic markets.



RESOLUTION
Instruments are not powerful enough to separate and identify challenging molecules with minimal differences

THROUGHPUT
Analysis takes too long, component changes and complex method development reduces instrument uptime

EASE-OF-USE
A lack of practical utility prevents adoption in routine test labs or requires hiring expensive, skilled operators

TRADE-OFFS
Incumbent instruments might provide adequate speed, resolution or ease of use, but not all at the same time

The Solution: Digitizing Separations with High Resolution Ion Mobility

Easier methods, better resolution, faster analysis, more reproducible results

CURRENT SEPARATION TECHNIQUE

Liquid Chromatography

MOBILion PCB-BASED INSTRUMENT

HRIM (High Resolution Ion Mobility)

A Brief History of MOBILion Systems, Inc.

Pacific Northwest National Laboratory publishes first SLIM research in 2014

MOBILion has exclusive license to the SLIM technology

Dr. Richard D. Smith
Photo Courtesy of Pacific Northwest National Lab

MOBILion Partners with Agilent to Develop First SLIM-MS Product

Technical information exchange and planning begins

MOBILion Launches Beta Units in the Field

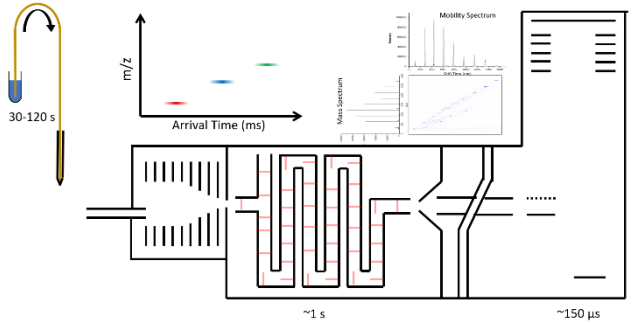
Beta units deployed to collaborators

Commercial Launch of MOBIE Product

2014 | 2017 | 2018 | 2019 | 2020 | 2021

MOBILion Begins its Journey

The first team members join and start to build out proof of concept

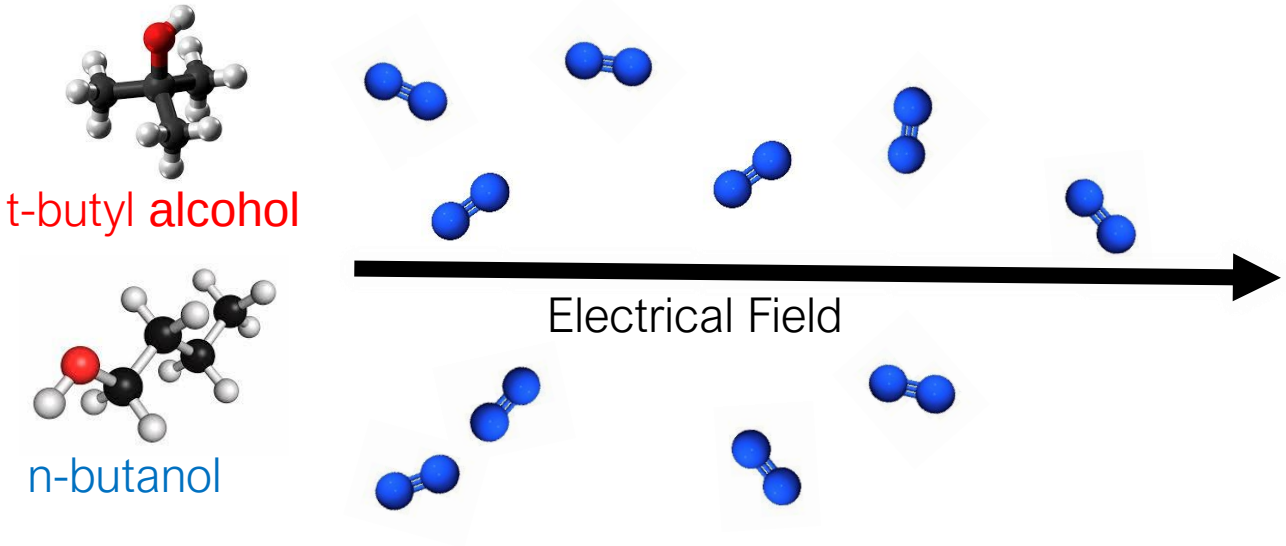


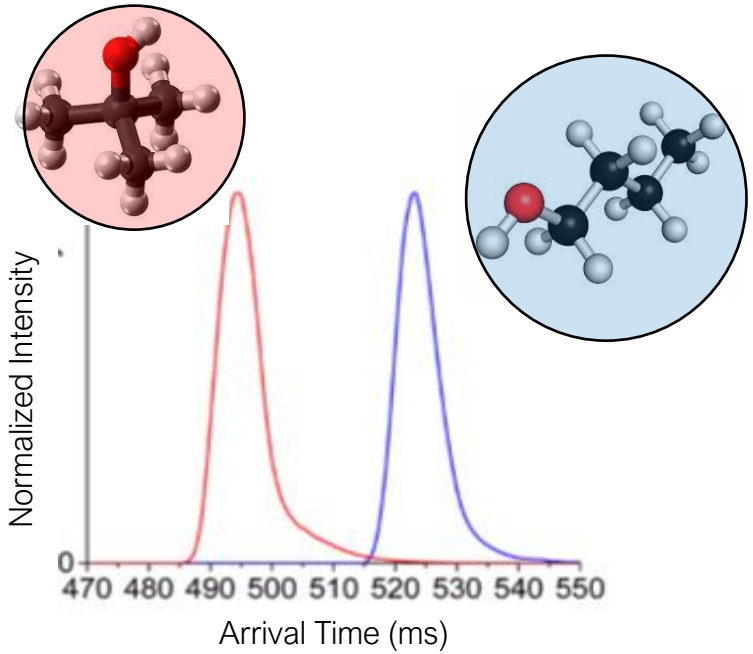
MOBILion Product Development Accelerates

Research prototype redesigned for manufacturability, robustness, and reliability to bring SLIM technology to the masses

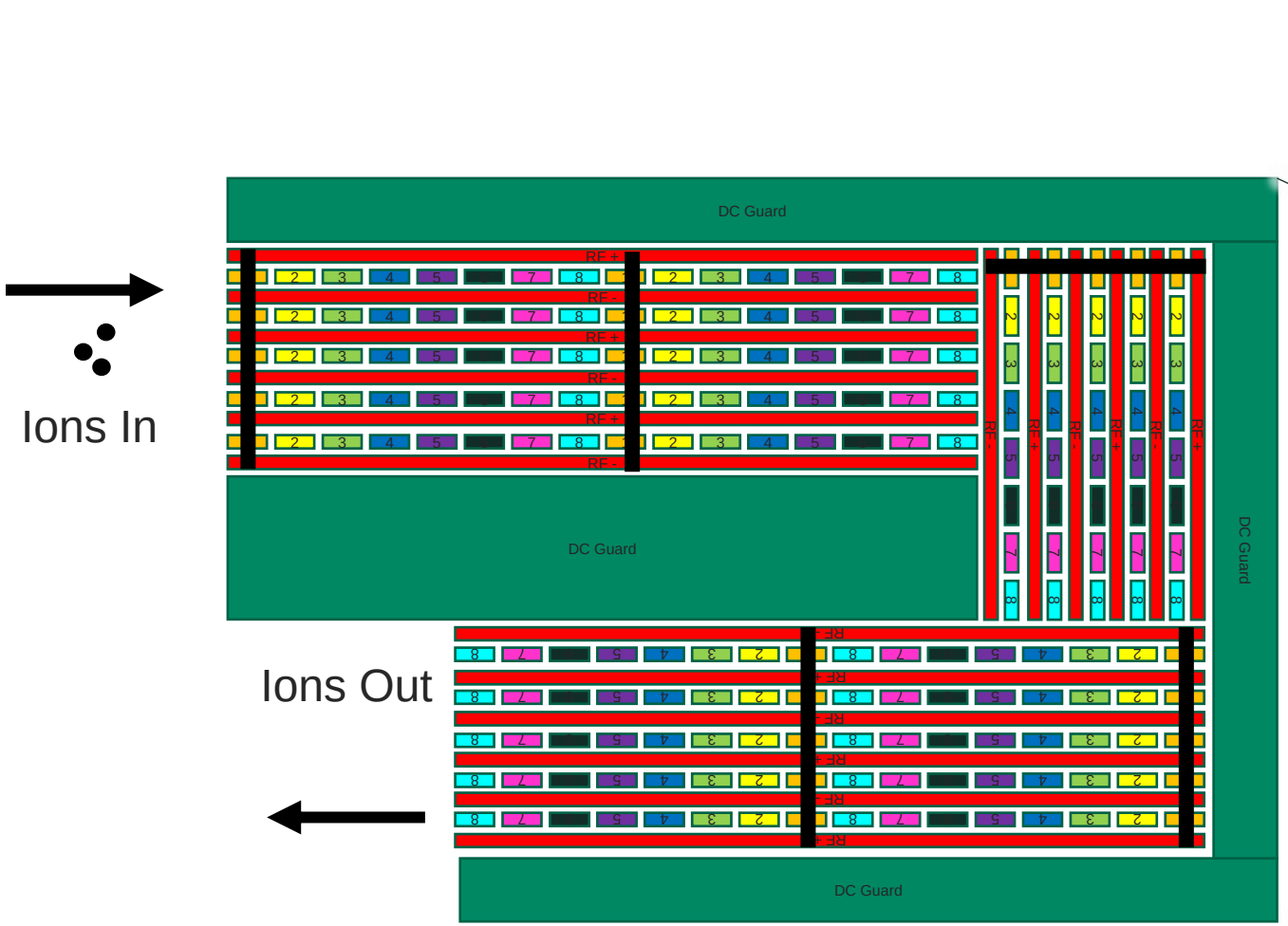
What is Ion Mobility?

- Gas Phase Size-based Separation
  - The time it takes ions to travel through a gas depends on their size/charge, pressure and electric field strength
  - Challenging chemical separations can be achieved in less than a second

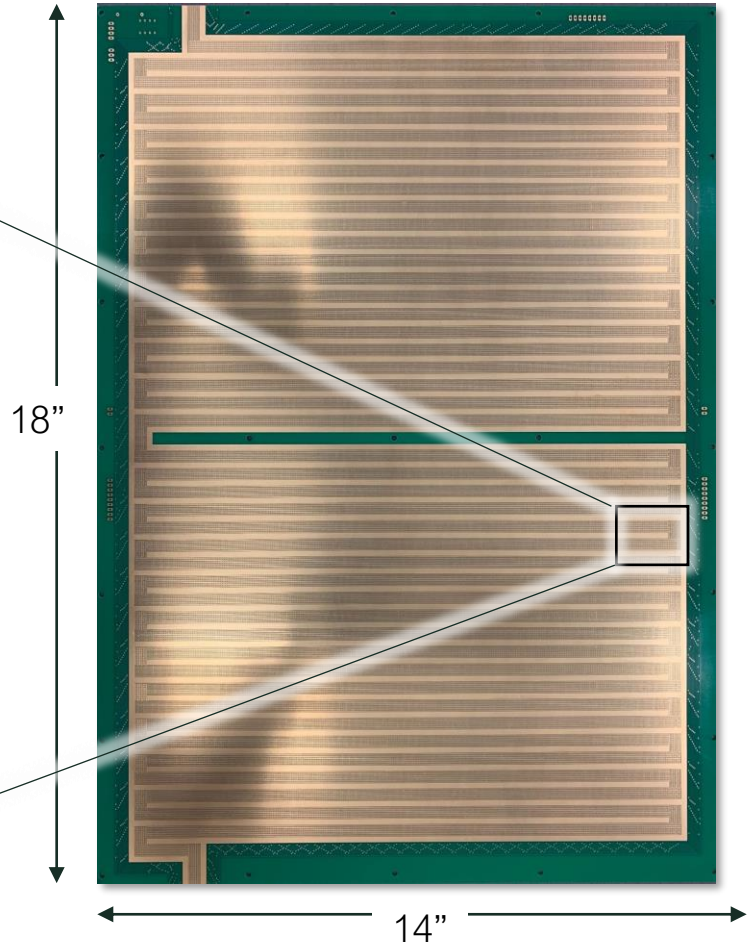




Unparalleled Resolution within a Small Footprint



13-meter SLIM Path

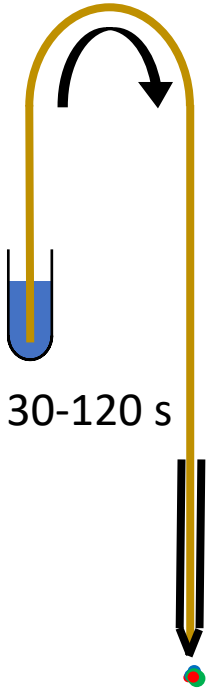


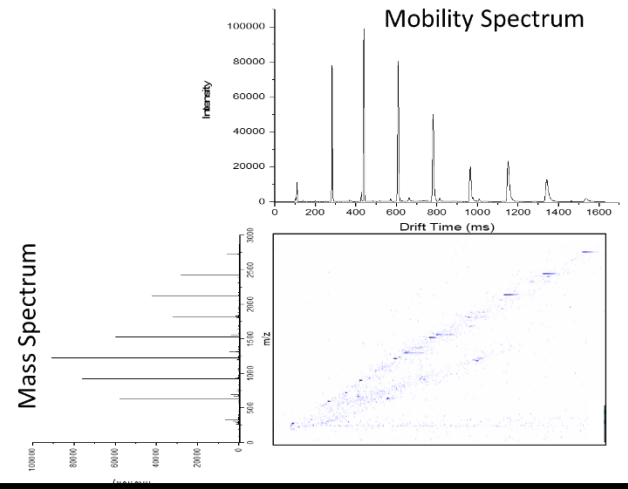
MOBILion is the exclusive licensee of the SLIM technology for commercialization purposes

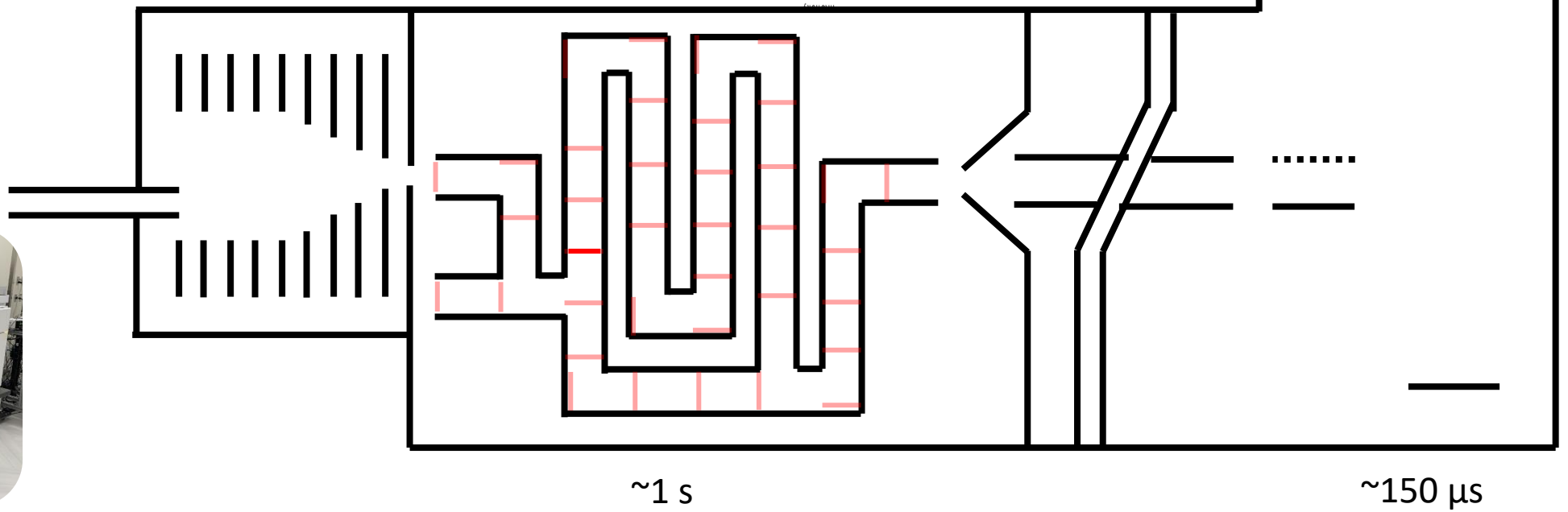
MOBIE – MOBILion's High-Resolution Ion Mobility Platform

- Best-in-class High-Resolution Ion Mobility (HRIM) Platform across a wide mass range
- Based on SLIM – Structures for Lossless Ion Manipulation
- Compatible with Agilent Q-TOF Models
  - 6545 LC/Q-TOF
  - 6545XT AdvanceBio LC/Q-TOF
  - 6546 LC/Q-TOF
- Integrated with Protein Metrics Byos®

HRIM-TOF Data Acquisition Methodology





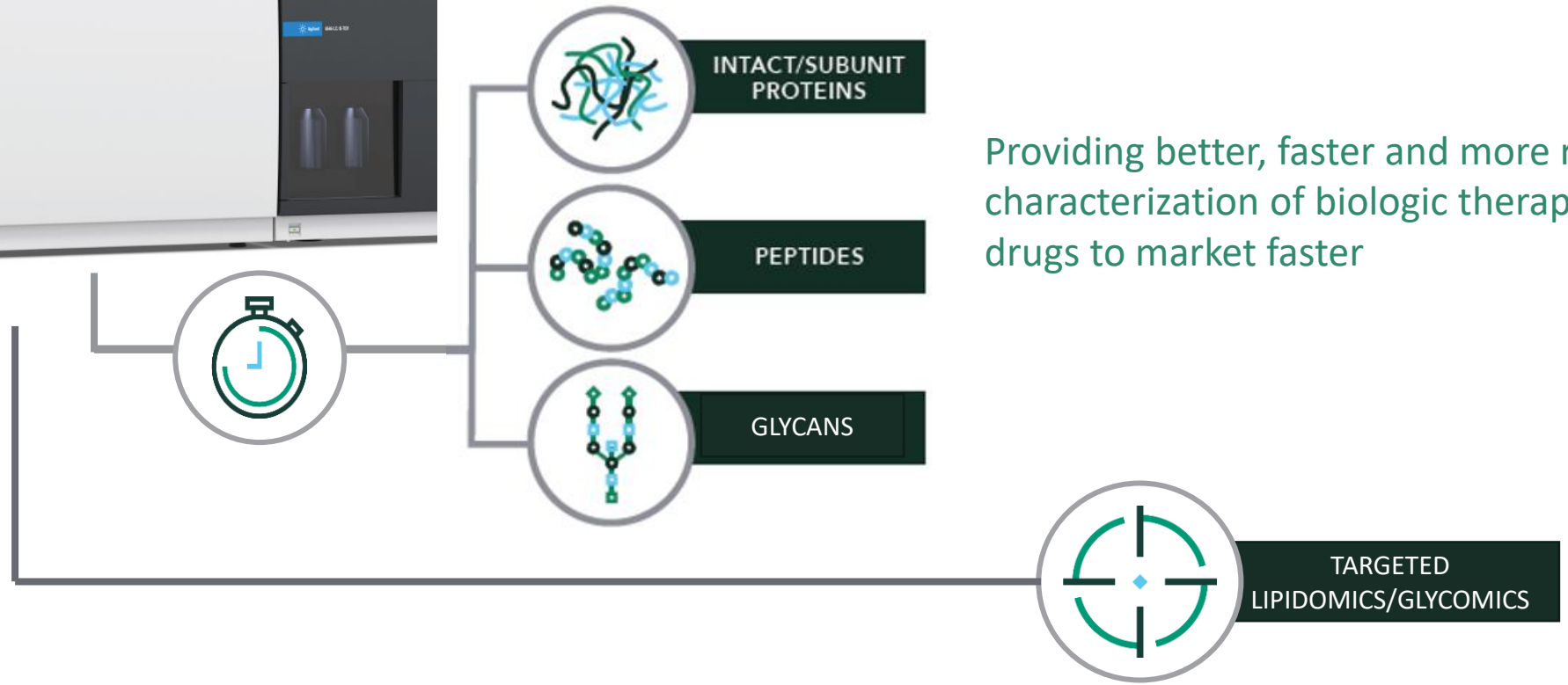


Improve Resolution, Throughput and Ease of Use with HRIM

Lengthy chromatographic methods are required for PTM identification and quantitation	HRIM separations occur in gas-phase with peak capacities rivaling UHPLC
Some PTMs are poorly resolved, and at worst, go undetected	HRIM resolves isomeric structural variants without additional hardware modification
Decreasing method complexity often comes at the sacrifice of method performance	HRIM *reduces method time by 3 – 4.5X*[1] while maintaining method performance

Higher quality data in less time = safer, more efficacious drugs to market faster

MOBIE Target Applications

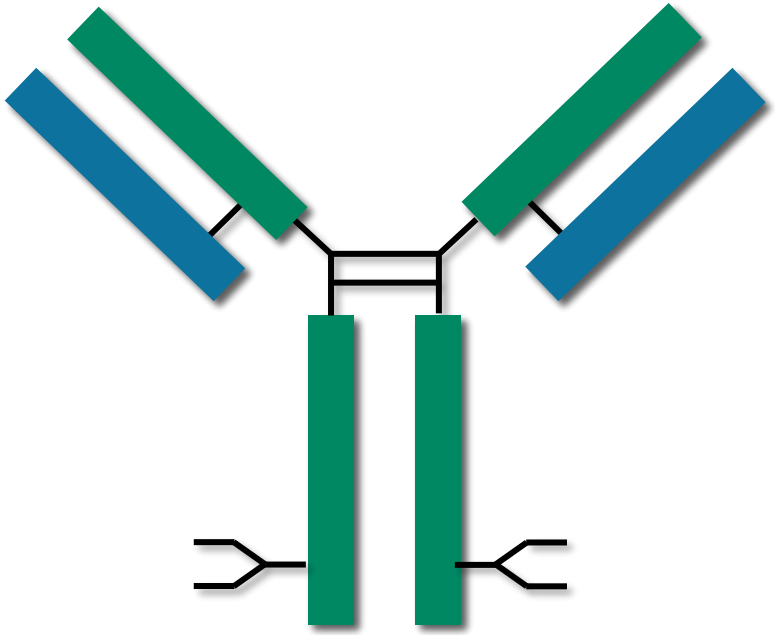


Providing better, faster and more reproducible CQA characterization of biologic therapeutics to bring drugs to market faster

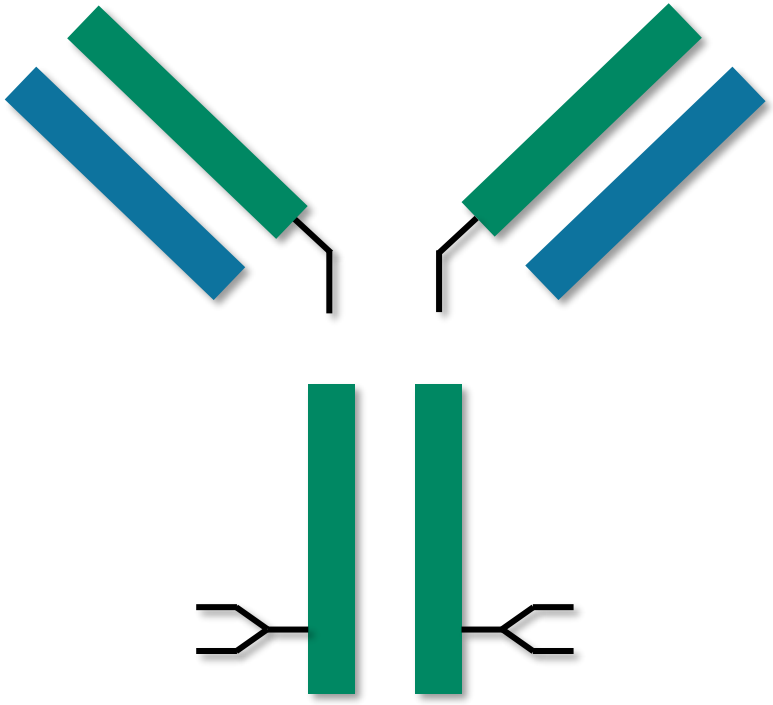
Highest resolution for best-in-class separation

HRIM-MS for Characterization of Biotherapeutics

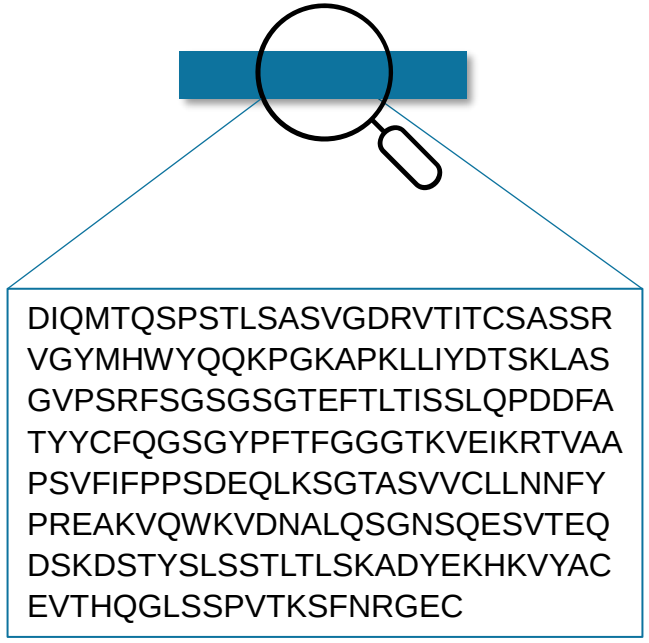
Intact Analysis



Subunit Analysis

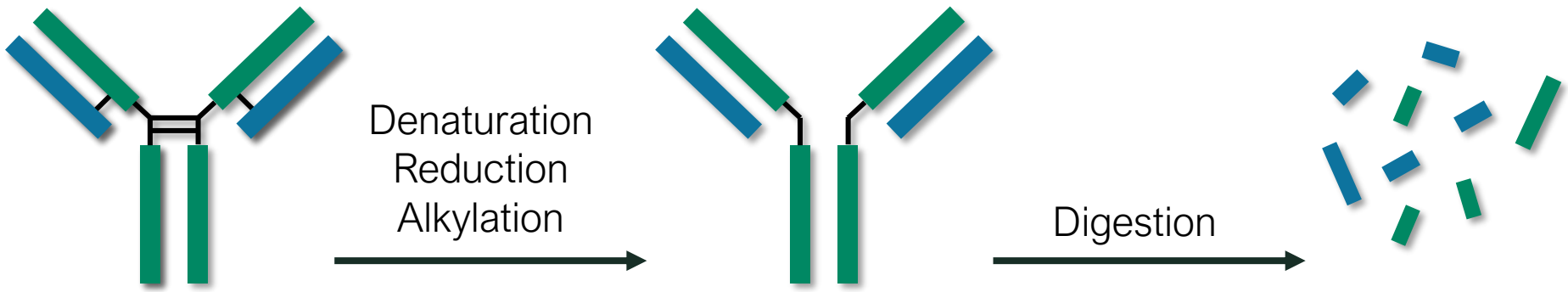


Peptide Mapping



High-Resolution Ion Mobility Peptide Mapping

HRIM Decreases Overall Analysis Time



- Peptide Mapping to Identify and Monitor CQAs
- Conventional LC-MS peptide mapping workflows take 60-90 minutes
- Data sets are complex and timely to process

Implementing **HRIM** into this workflow can **decrease overall analysis time** and provide an orthogonal mode of peptide identification for **increased accuracy**

Integrated Data Processing Software - BioPharma

Exclusive Ion Mobility-Enabled Data Processing With Protein Metrics

Control and Acquisition	Preprocessing (Optional)	Data Analysis

MOBIE EyeOn
Control Acquisition Software (with real time display GUI) integrated with Agilent Mass Hunter Control Software

MOBILion HRIM Data Processor

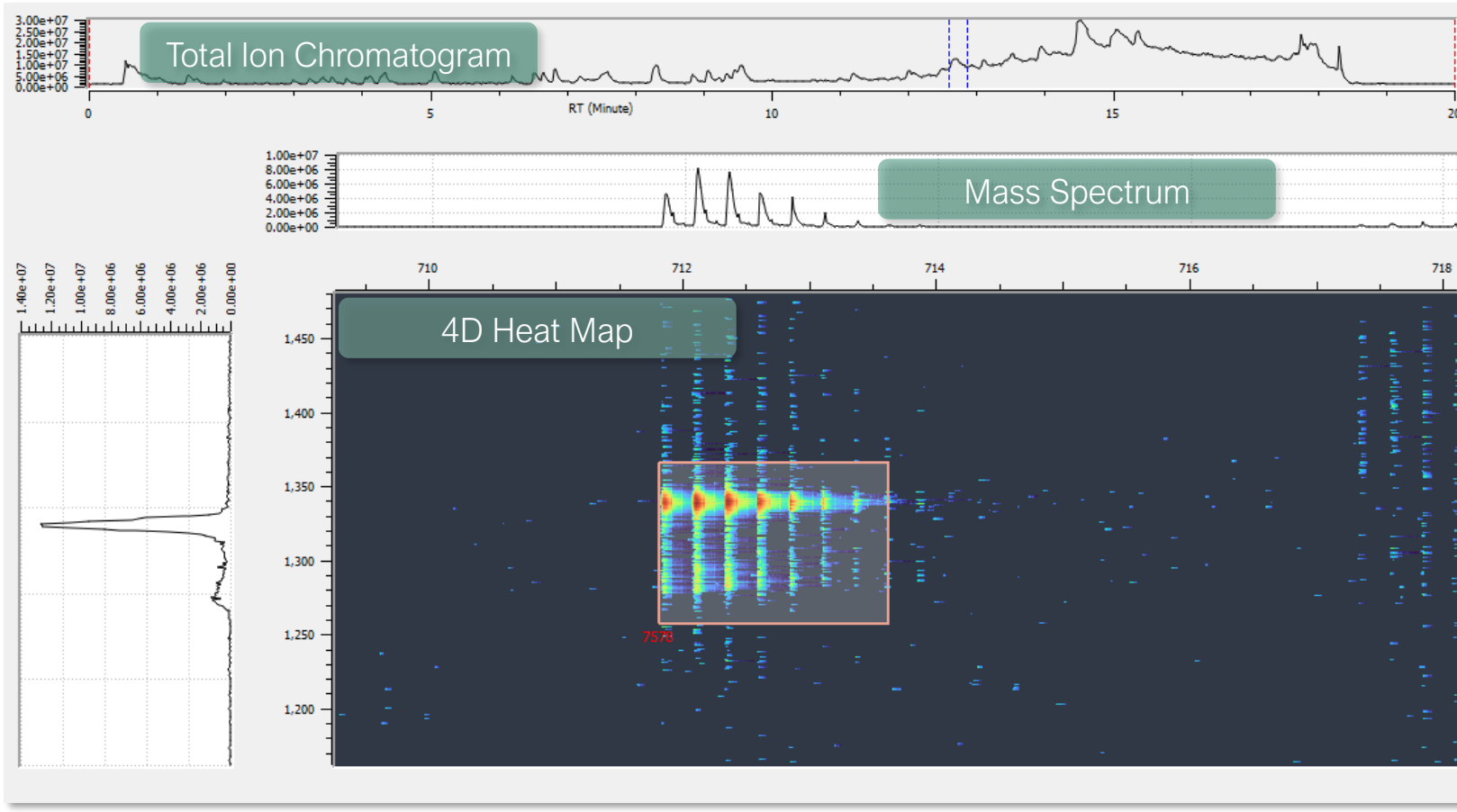
PROTEIN METRICS

Peptide Mapping WF
Intact & Subunit WF
Released Glycans WF

HRIM HRIM HRIM

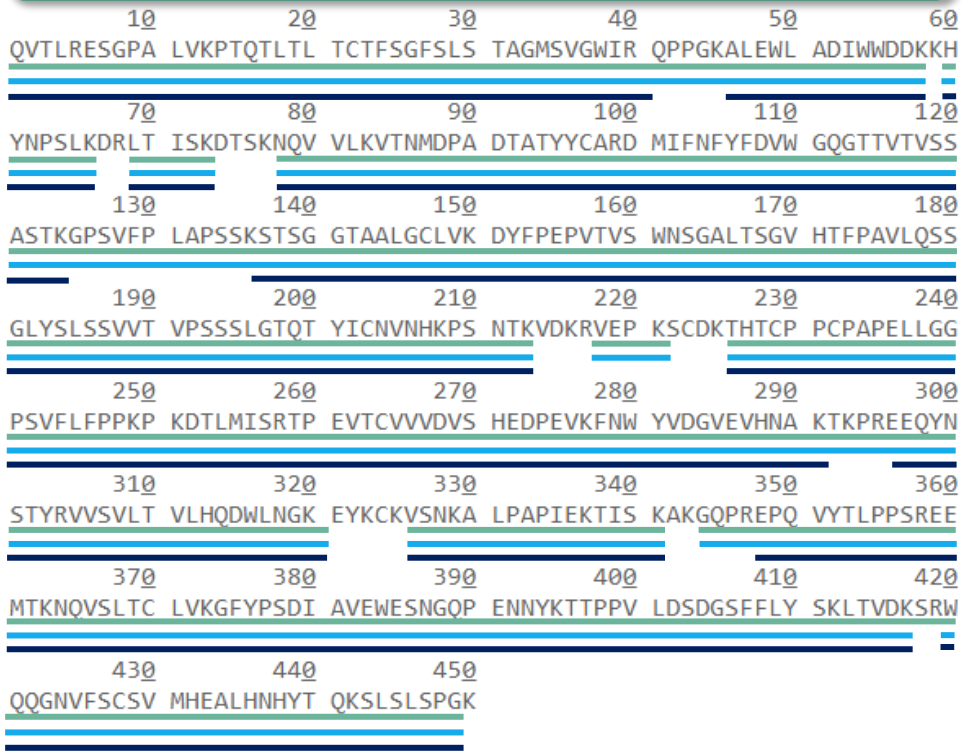
Protein Metrics Byos® Workflow for HRIM-MS

- Fully integrated with MOBIE
- Identification and quantification of protein modifications
- Analyze 4D data with simple interface

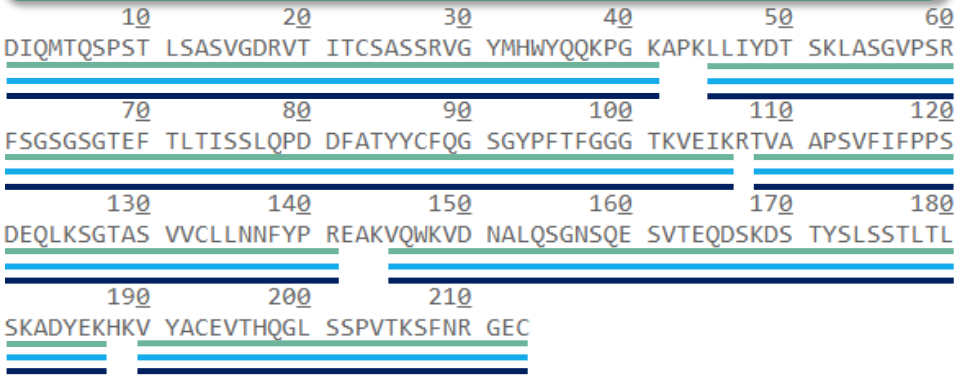


HRIM Reduces Dependency on LC for Sequence Confirmation

NIST mAb Heavy Chain



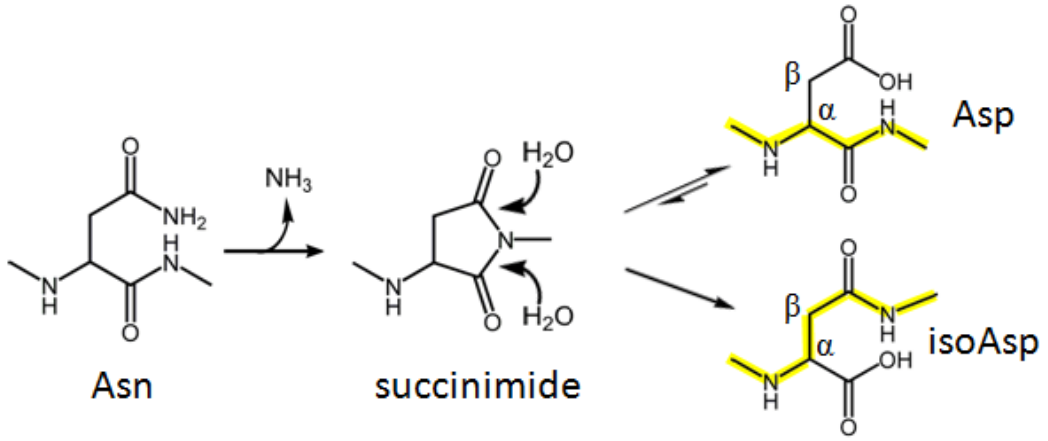
NIST mAb Light Chain

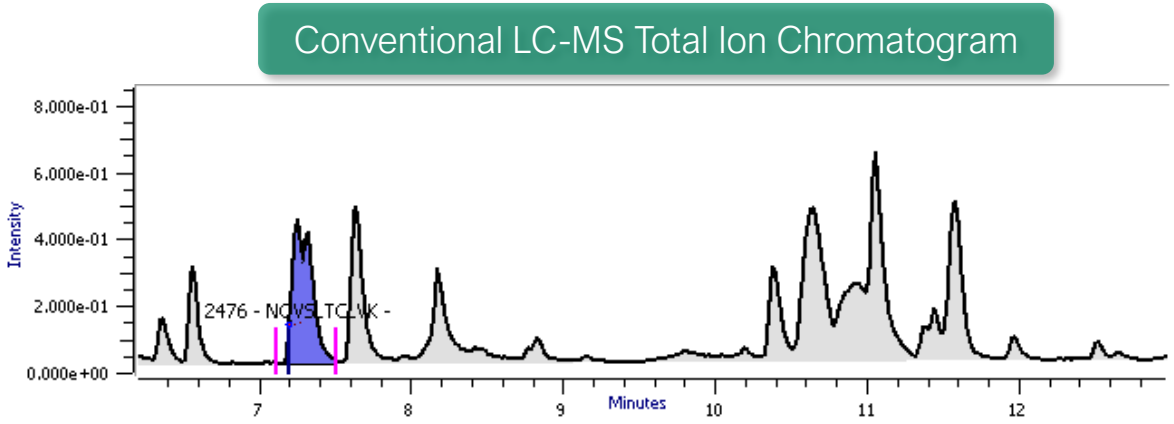


- 20-minute LC-HRIM-MS1 gradient, *95.3%* coverage
- 2-minute LC-HRIM-MS1 gradient, *95.0%* coverage
- Guard Column Trap and Elute HRIM-MS1, *90.6%* coverage

Increase Throughput for Targeted CQAs

Isomerization of aspartic acid is a spontaneous, non-enzymatic PTM that can cause changes to protein structure, and therefore function



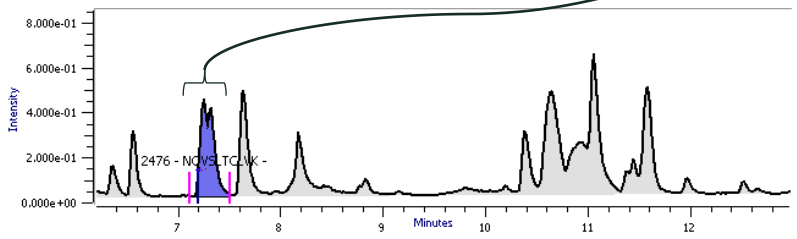


Asp and isoAsp have the same mass and similar physiochemical properties, making them difficult to separate and identify by LC-MS. What about HRIM?

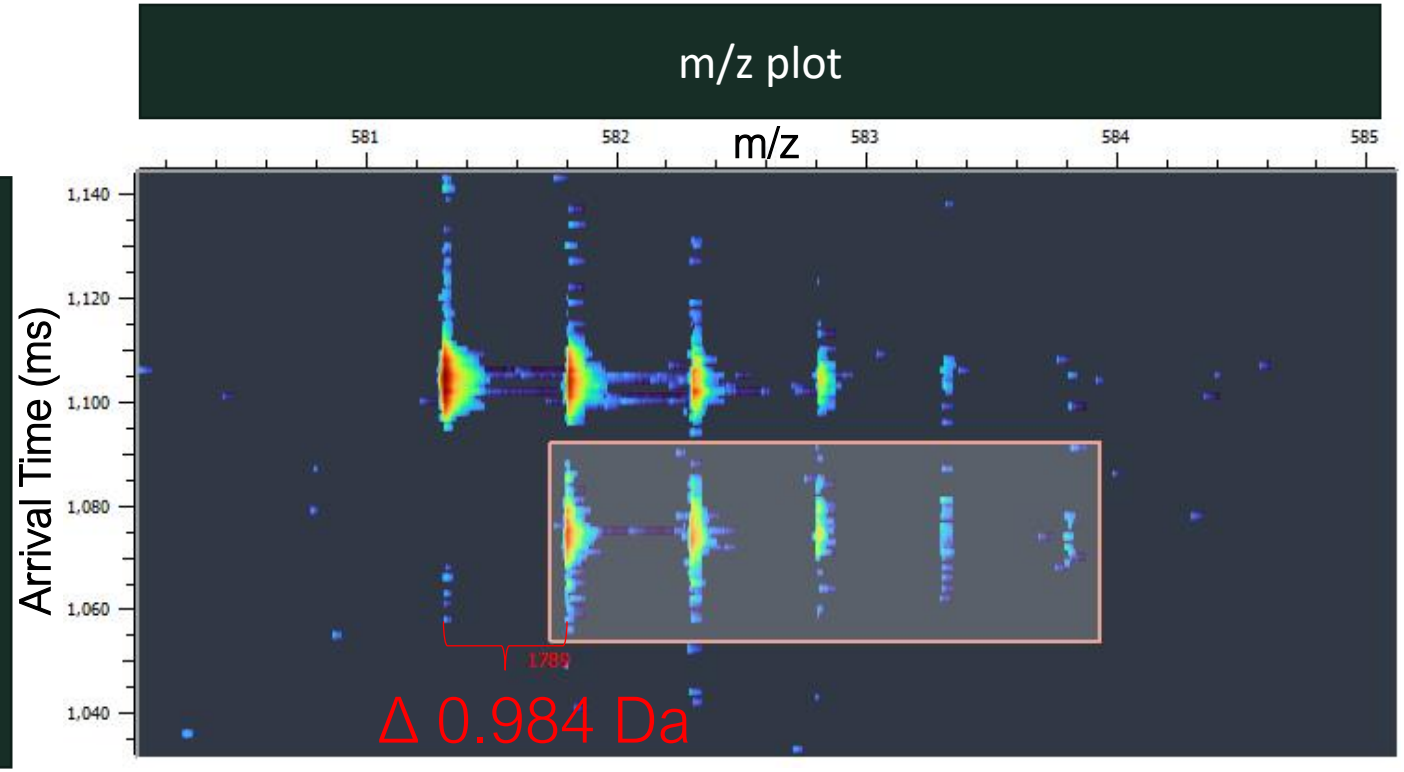
Pathway Image from: Boston University, Fourier Transform Mass Spectrometry Lab (https://www.bumc.bu.edu/ftms/research/isoaspartome/)

HRIM enables separation of deamidated species

- 20-minute LC-HRIM-MS1 Method
- HRIM Peptide Byos workflow



Mobiligram



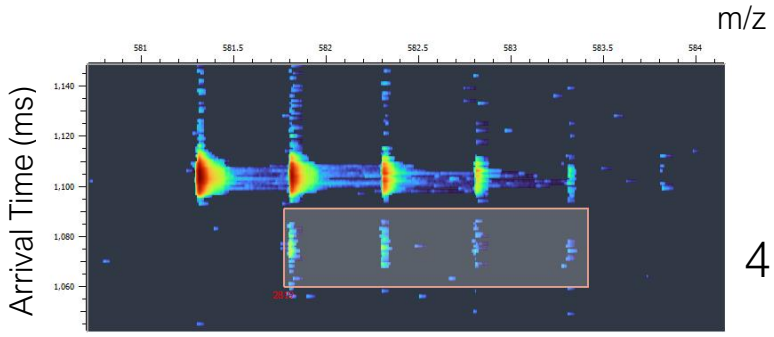
NIST mAb HC 363-374 **NQVSLTCLVK**

N1 Asn → Asp 0.984 Da mass difference

HRIM rapidly characterizes downstream CQAs

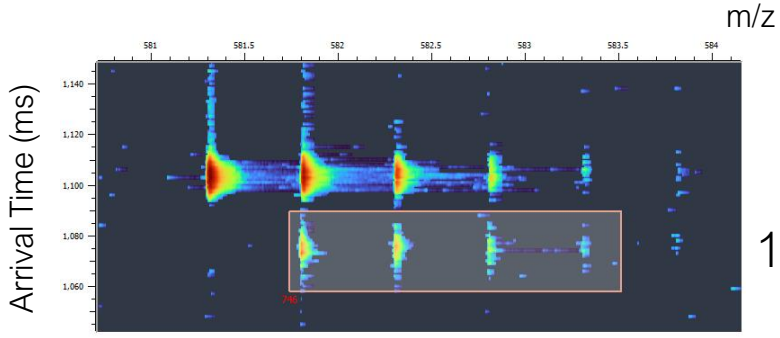
NIST mAb HC 364-373
NQVSLTCLVK

pH 3.5, 37 ℃



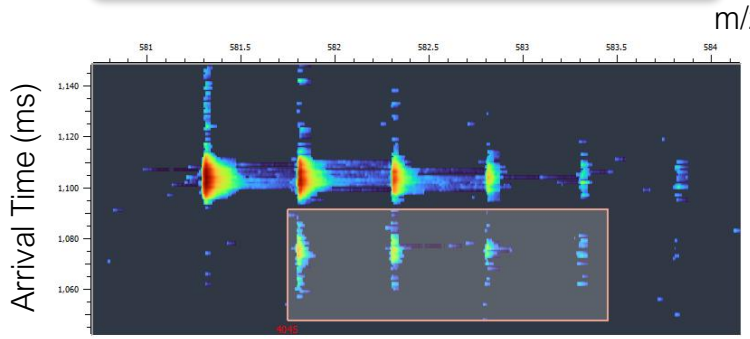
4.5 %

pH 8.5, 37 ℃



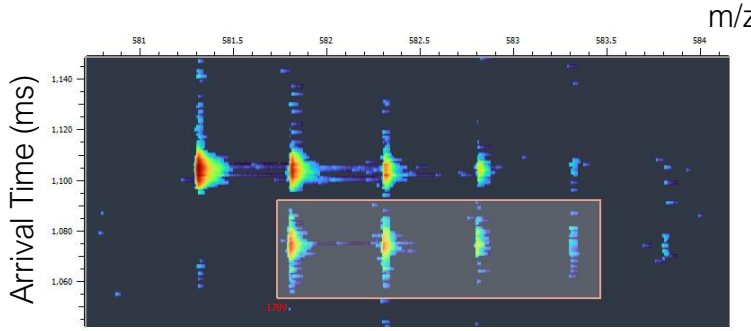
14.1 %

pH 3.5, 50 ℃



8.5 %

pH 8.5, 50 ℃

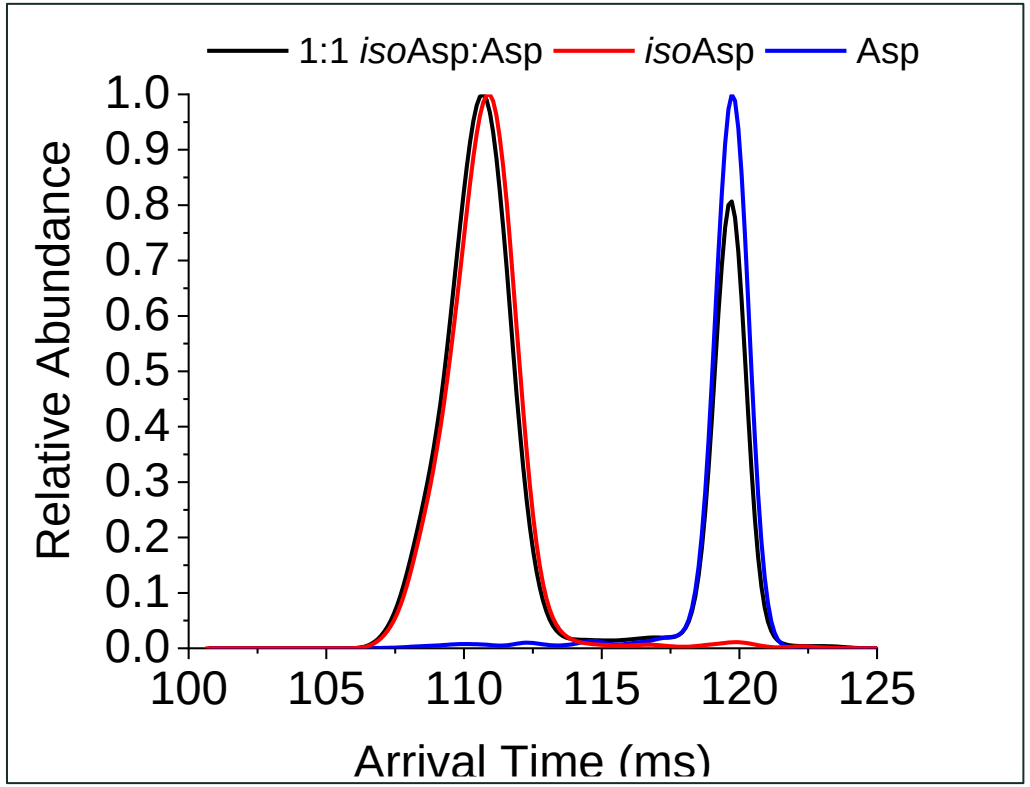


27.3 %

HRIM enables rapid, high-resolution separation of isomers

- Reduce dependency on LC
- 2-minute FIA-HRIM-MS method
- Baseline resolution of Asp/isoAsp

Separation of 14 Residue Asp/isoAsp Peptide Standards

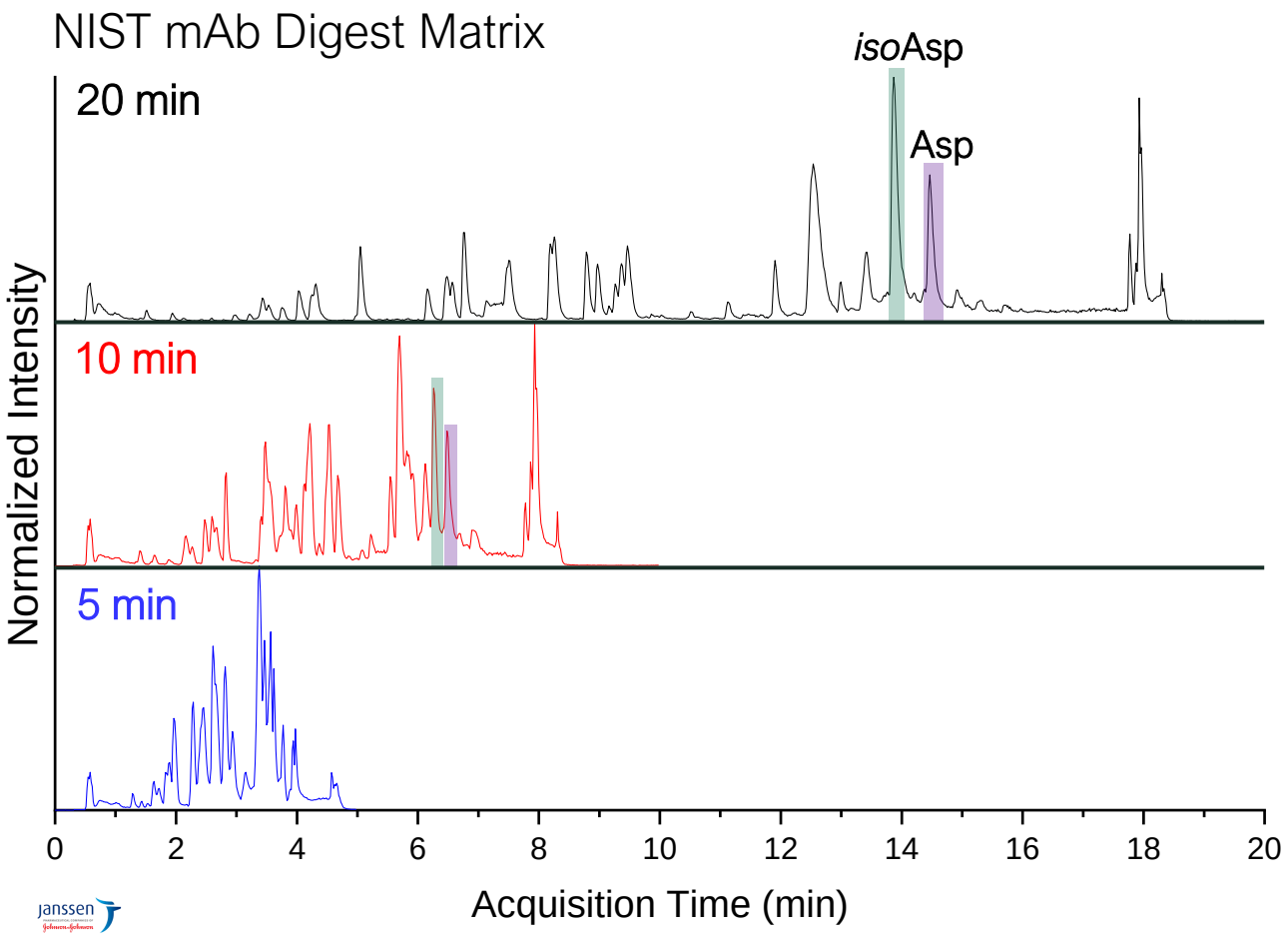


Ion Mobility Distributions

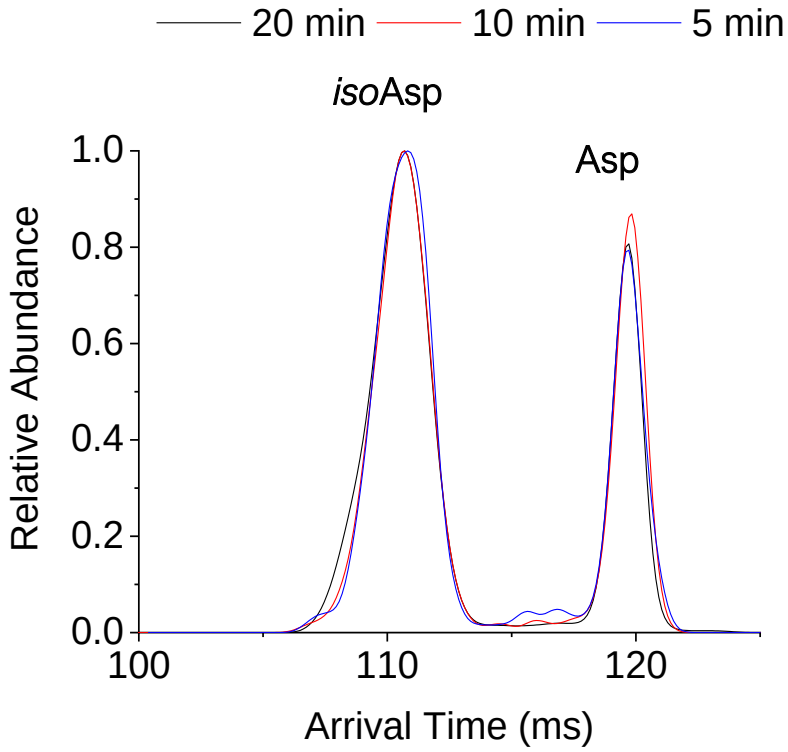
janssen PHARMACEUTICAL COMPANIES OF Johnson & Johnson

HRIM Increases Throughput for Targeted CQA Monitoring

Decreasing Chromatographic Run Time

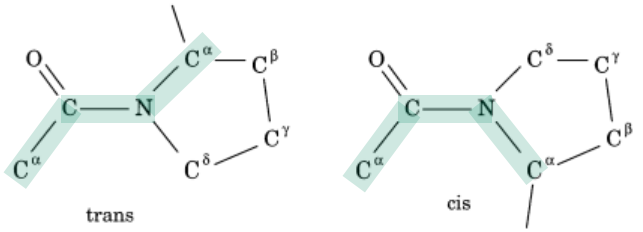


HRIM Separation Retained at 5-minute Run Time



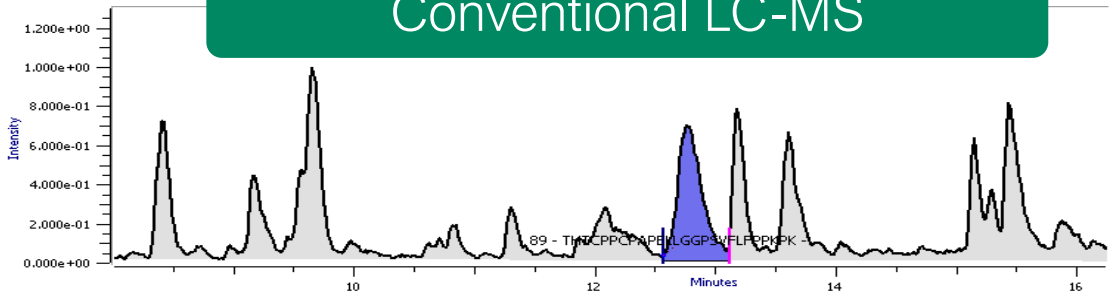
Arrival times remain consistent as LC-acquisition time is decreased from 20- to 5- minutes

HRIM Reveals Peptide Isomers Not Identified With LC



Biochemistry, 2002, 41, 5014637-14644.

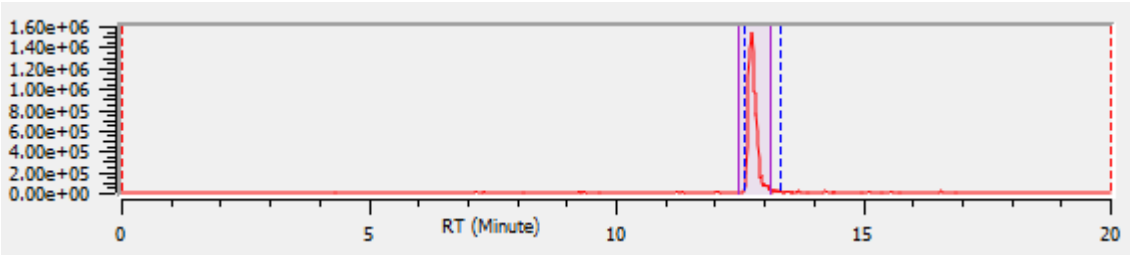
Conventional LC-MS



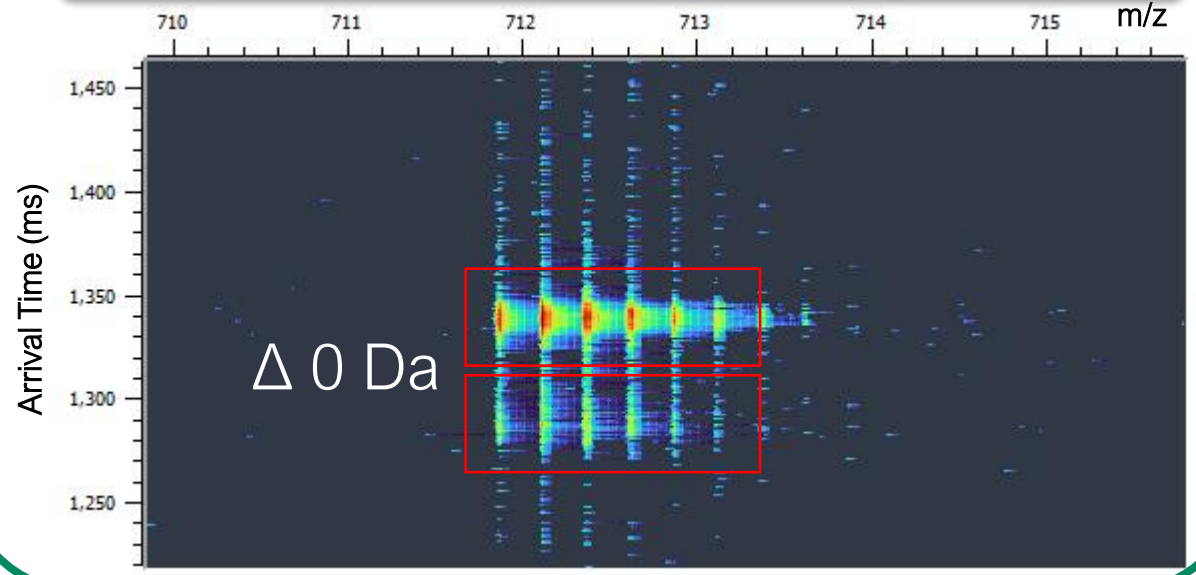
NIST mAb HC 226-249
THTCPPCPAPELLGGPSVFLFPPK

HRIM

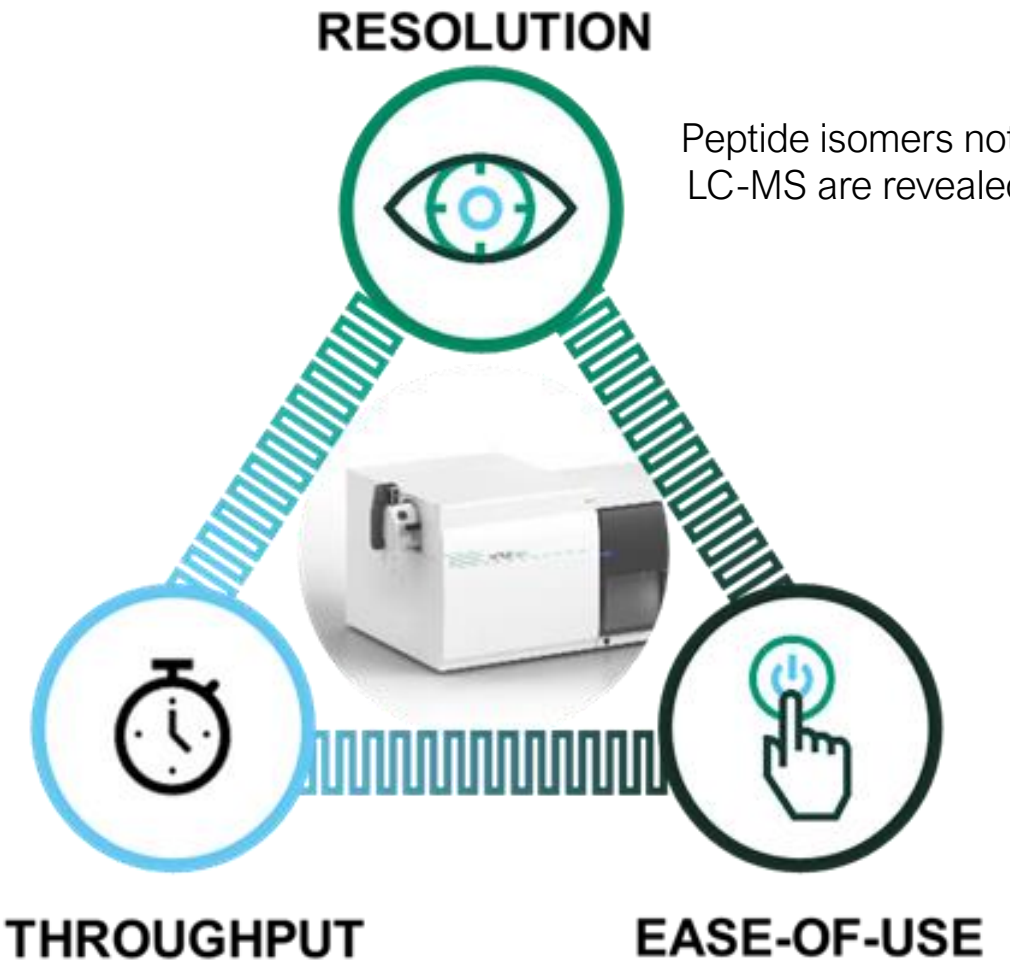
Extracted Ion Chromatogram



HRIM-MS Heat Map



Faster, More Efficient, and Deeper Characterization with HRIM-MS



Peptide isomers not detected in LC-MS are revealed with HRIM

Implementation of HRIM decreases analysis time and can perform rapid targeted analysis

Integration with Protein Metrics HRIM data processing modules leads to a streamlined process

Thank You

- Tom Doherty PhD

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