

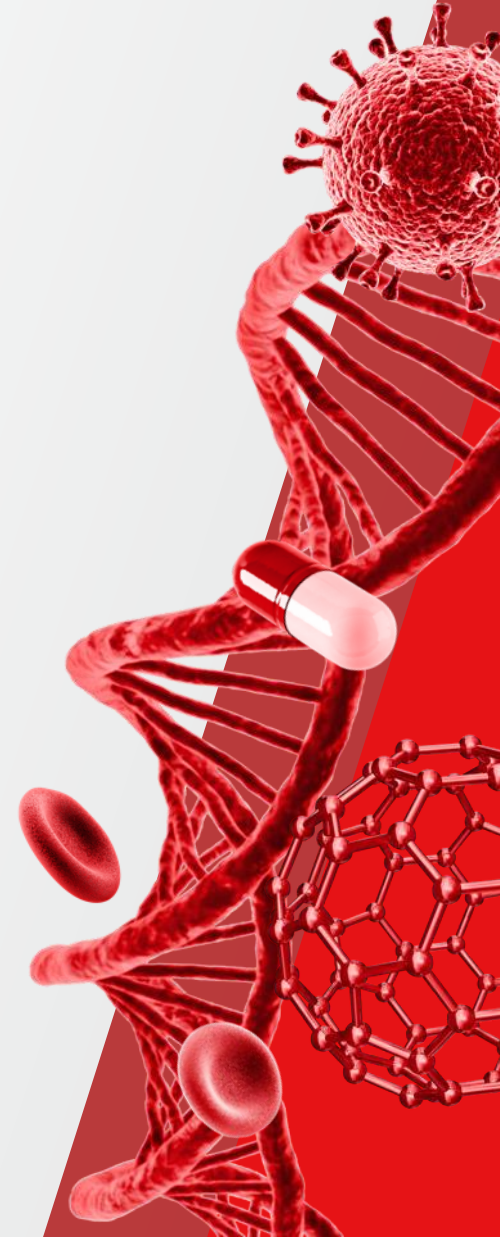
Introduction to common ESI-MS systems and their data. Choosing the Best MS system for your experiment.

Steve Danielson

LCMS Technical Sales Specialist

Feb. 13th, 2024

 The world leader in serving science



MS Data Quality

What do you need from your MS Experiment?

- Basic Types of MS Experiments
 1. Identify Compounds
 - A. Unknowns/Discovery
 - I. DDA
 - II. DIA
 - B. Knowns/Targeted
 - I. HRAM (Full Scan, SIM, PRM/Targeted MS2)
 - II. Triple Quad (SRM/MRM)
 2. Quantify Compounds
 - A. Absolute
 - B. Relative (LFQ, DIA and TMT)
- Data Quality/Confidence
- How do we know our results are good?
 - Mass Accuracy
 - Resolution (Distinguish Isobaric Compounds)
 - Repeatable (Technical/Sample Replicates) CV, RSD
 - False Discovery Rates
 - Matrix Effects (Contaminants, Ion Suppression)
 - Retention Time Variability
 - Missing Data
 - Internal Standards, Standard Curve

Liquid chromatography (LC) – mass spectrometry (MS)

Fit-for-purpose LC-MS instrumentation with experimental flexibility

Triple Quadrupole

Sensitive Quantitative Performance

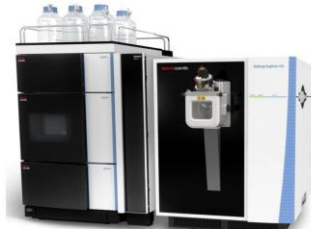


- Pharma QA/QC
- BioPharma
- Food Safety
- Clinical
- Environmental

Thermo Scientific™
TSQ+ Triple Quadrupole MS

Quadrupole-Orbitrap Hybrid

Market Leading Qual/Quan HRAM



- Omics
- Pharma/BioPharma
- Food Safety
- Clinical
- Environmental

Thermo Scientific™
Orbitrap™ Exploris MS

Quadrupole-Orbitrap-Ion Trap Tribrid

Ultimate Versatility HRAM



- Omics
- Pharma/BioPharma
- Food Safety
- Clinical
- Environmental

Thermo Scientific™
Orbitrap™ Tribid™ MS

Profile



Biofluids



Oligo



Omics



SCP



DIA



Unknowns



Native



Ab



Rethink what is possible with ground-breaking innovation



Thermo Scientific™
Orbitrap™ Astral™
Mass Spectrometer



Thermo Scientific™ Triple
Quadrupole
Mass Spectrometers



Thermo Scientific™ Hybrid
Orbitrap Mass Spectrometers

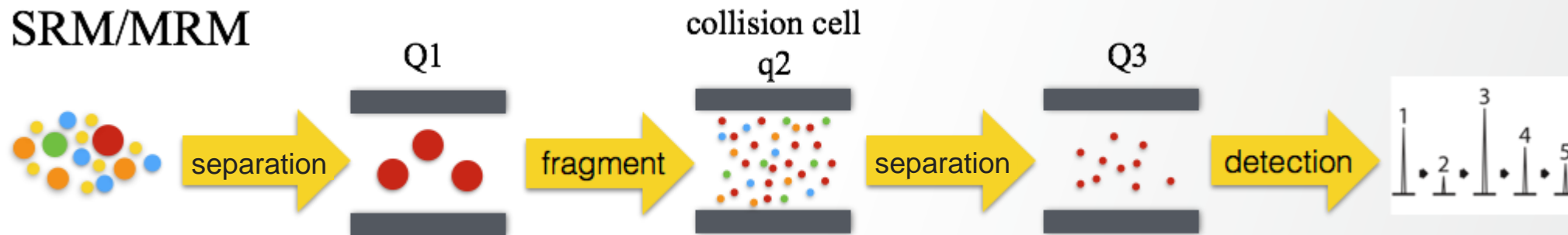


Thermo Scientific™ Orbitrap™
Tribid™ Mass Spectrometers

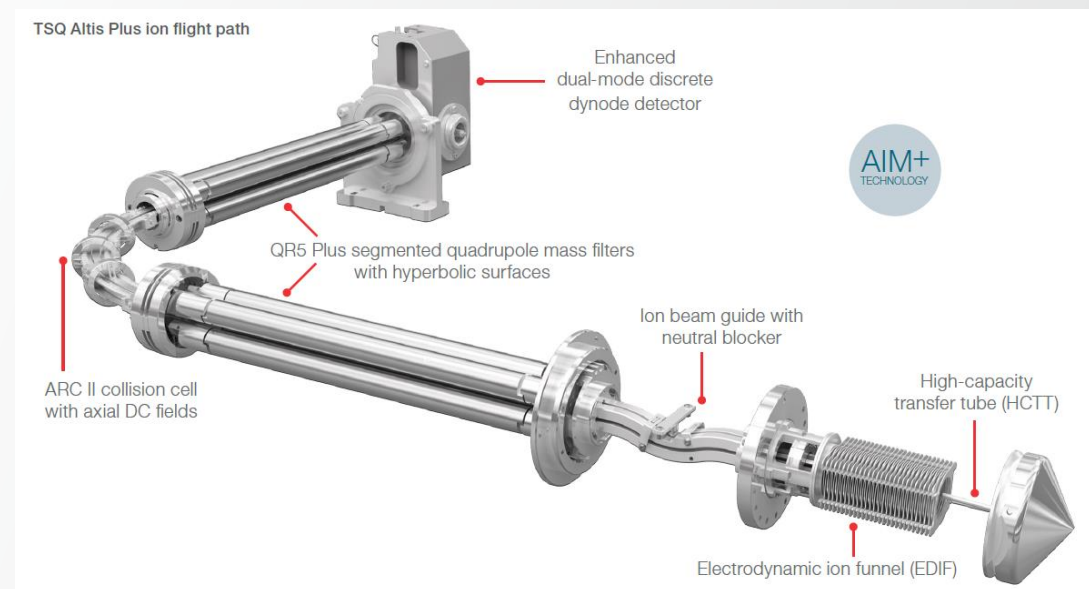
Revolutionary platforms to tackle the unmet needs in mass spectrometry

QqQ Triple Quadrupole

Overview



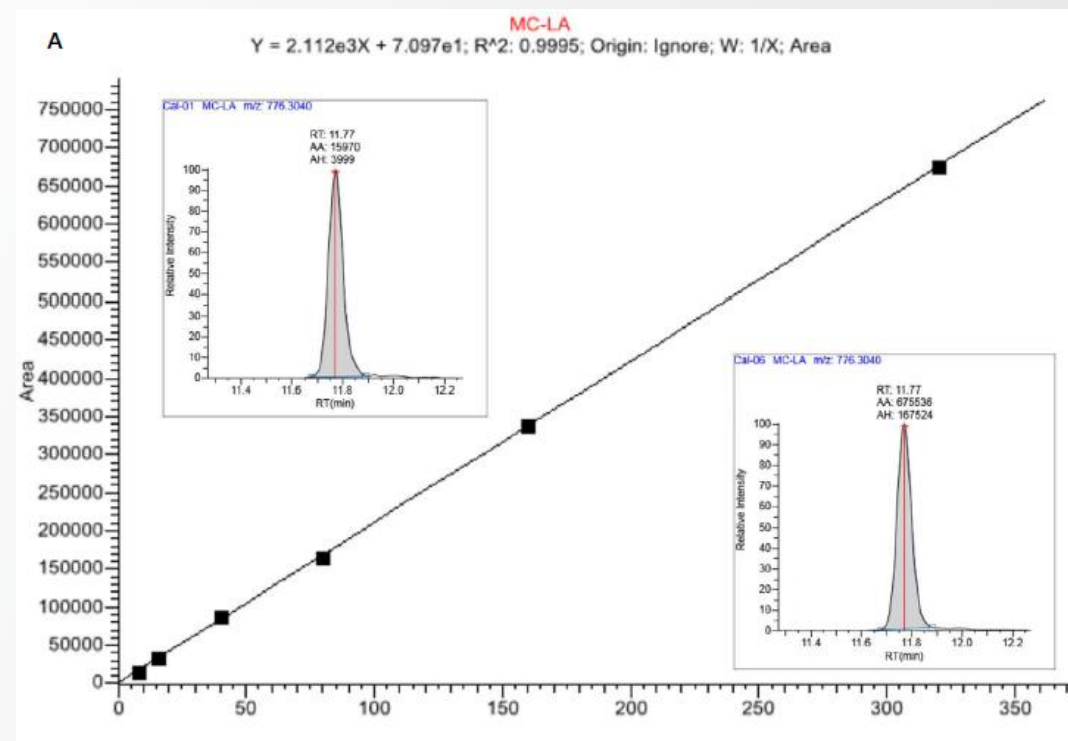
TSQ Altis Plus triple quadrupole mass spectrometer



Ideal Applications for Triple Quadrupole MS

- Targeted Quan
 - ID/Quan specific known targets.
 - Not for Discovery (unknown ID/Quan)
- Benefits of QqQ MS
 - Fast (600 SRM/sec)
 - Sensitive
 - Robust
 - Relative Low Cost
- Keep in Mind
 - Data on targets only, no unknowns.
 - Nominal Mass (+/- 0.5 Da Mass Accuracy)
 - Ideally 2 or more SRM/target.
 - 7-15 scans across the peak for good quan.

- Example Applications
 - Pesticides
 - Drugs of Abuse
 - PFAS



Description of sensitivity across triple quadrupole instrumentation can be measured by signal-to-noise (S/N)

TSQ Fortis Plus

Environmental
Food Safety
Clinical Research
Pharma QA/QC



Mass Range m/z 2 – 3000
Max Resolution **0.4 FWHM**
Polarity Switching 5 msec
Linear Dynamic range >5
Excellent Mass Stability**
150,000:1 S/N*
3-year factory warranty

TSQ Quantis

Food Safety
Pharma
Clinical Research
Forensic Toxicology



Mass Range m/z 2 – 3000
Max Resolution **0.4 FWHM**
Polarity Switching 25 msec
Linear Dynamic range 6
Excellent Mass Stability**
150,000:1 S/N*
1-year factory warranty

TSQ Quantis Plus

Food Safety
Pharma
Clinical Research
Forensic Toxicology



Mass Range m/z 2 – 3000
Max Resolution **0.4 FWHM**
Polarity Switching 5 msec
Linear Dynamic range >6
Excellent Mass Stability**
500,000:1 S/N*
3-year factory warranty

TSQ Altis Plus

Pharma/Biopharma
Environmental
Food Safety
Clinical Research
Targeted Omics



Mass Range m/z 2 – 2010
Max Resolution **0.2 FWHM**
Polarity Switching 5 msec
Linear Dynamic range >6
Excellent Mass Stability**
1,500,000:1 S/N*
3-year factory warranty

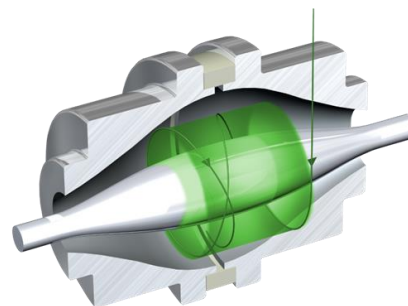
Performance

*The current signal to noise specification highlights the minimum performance specification required at installation

**For lockout specifications, please contact the Product Management Team

Orbitrap HRAM-High Resolution Accurate Mass

- Applications for any Orbitrap.
 - Proteomics ID and Quan (DDA and DIA)
 - Intact Proteins
 - Oligonucleotides
 - Metabolomics
 - Lipidomics
 - Small Molecules
 - SCP
 - And more
- Orbitrap Data Quality
 - Mass Accuracy (1-3ppm)
 - Resolution (120,000 up to 1,000,000*)
 - Sensitivity
 - Dynamic Range
 - Ease of Use



Thermo Scientific™ Hybrid Orbitrap Mass Spectrometers



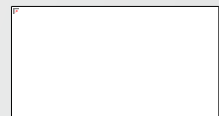
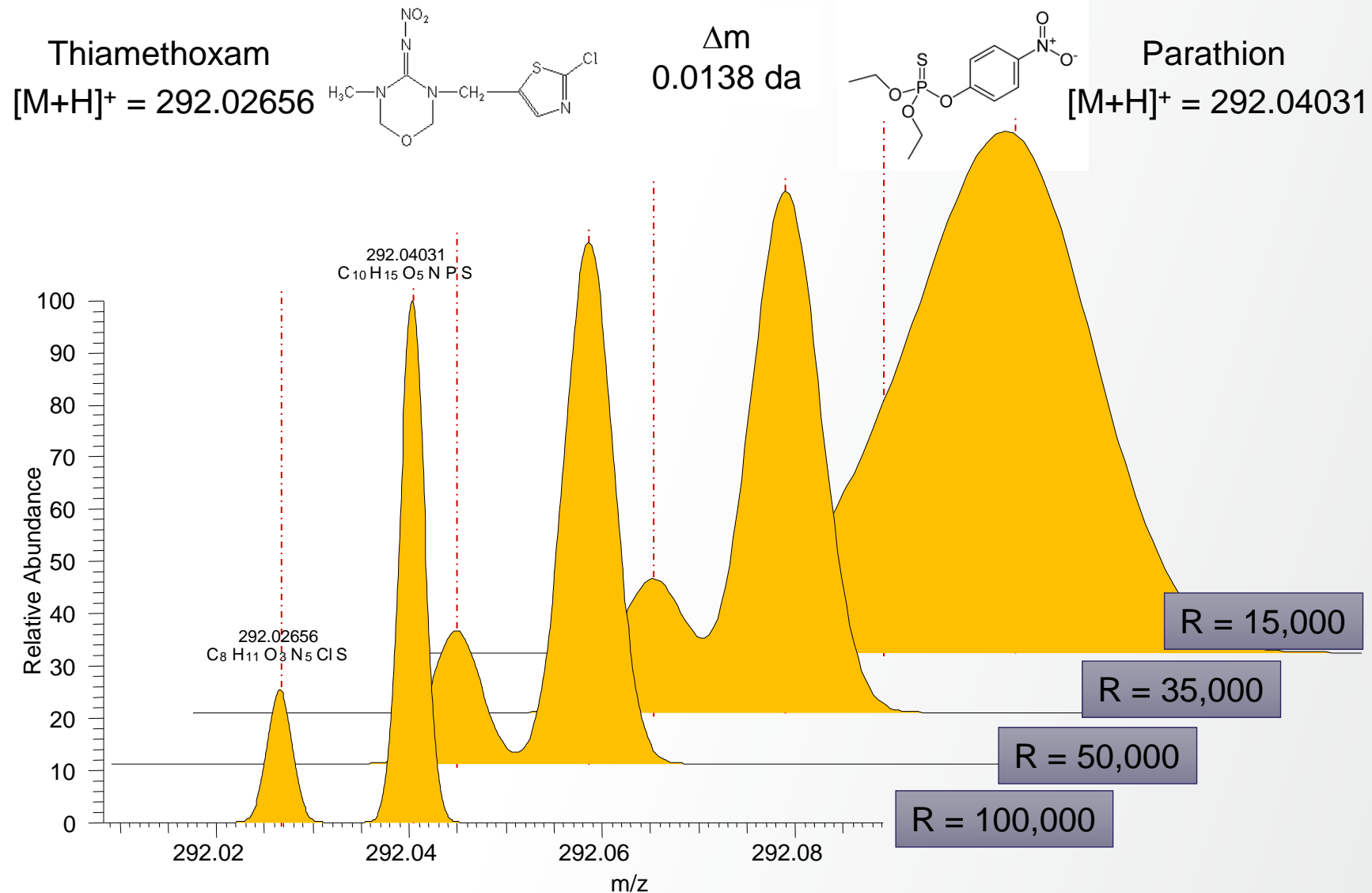
Thermo Scientific™ Orbitrap™ Astral™ Mass Spectrometer



Thermo Scientific™ Orbitrap™ Tribid™ Mass Spectrometers



Resolution Ex. Isobaric Pesticides: Mix 1:3 Simulated



Mass Accuracy and Resolution Role in Compound ID

Possible Chemical Formulas

Mass Tolerance Window	± 20 ppm	± 10 ppm	± 5 ppm
Monoisotopic	35	18	9
At least 1 N, 1 S, no Cl, no Br	6	2	1

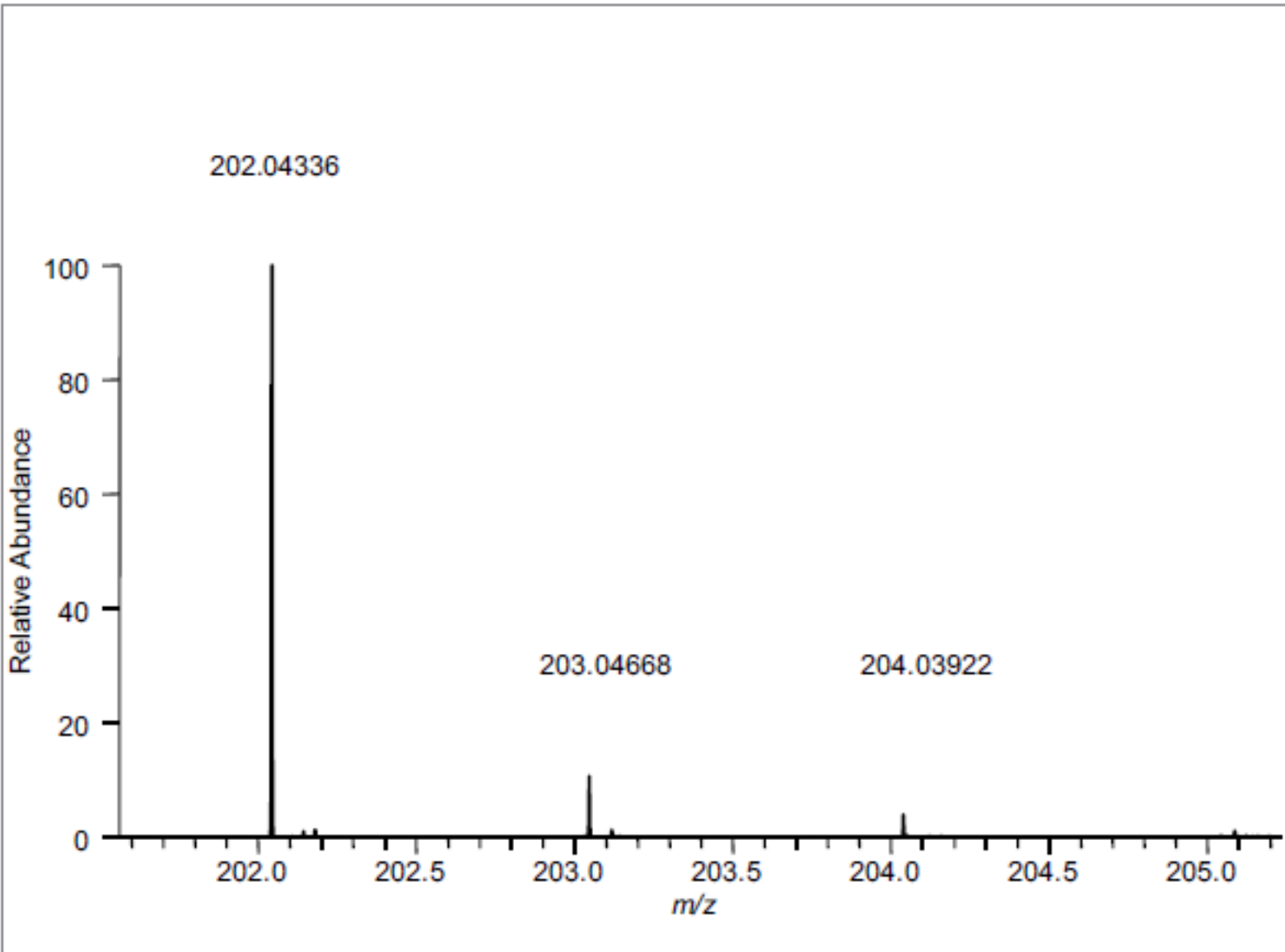
QE (External) < 5 ppm

QE (Internal) < 3 ppm

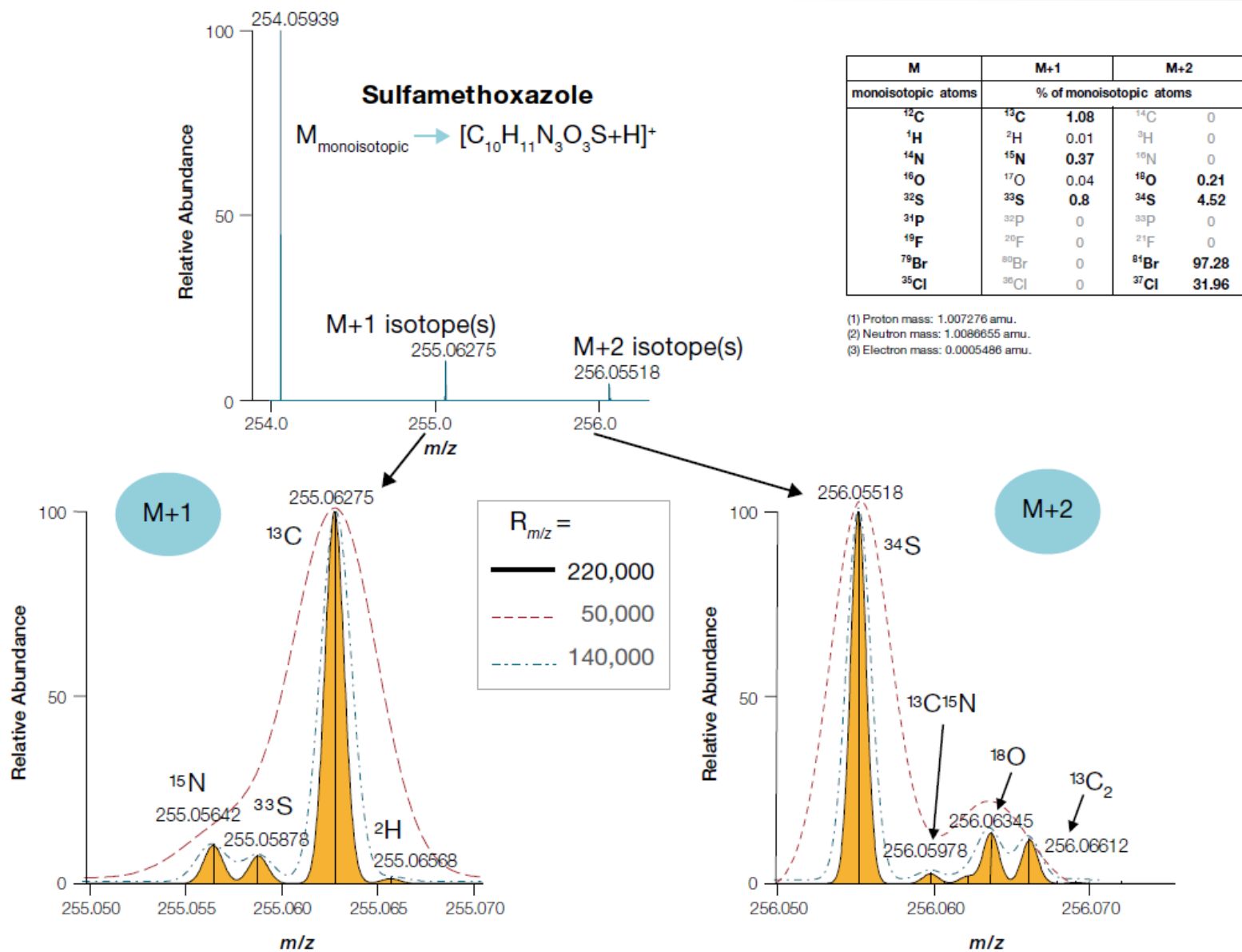
Exploris (IC on) sub 1ppm

1) Software Scores Isotope Patterns

2) Software looks for Fine isotope patterns if high enough resolution



Fine Isotope Patterns



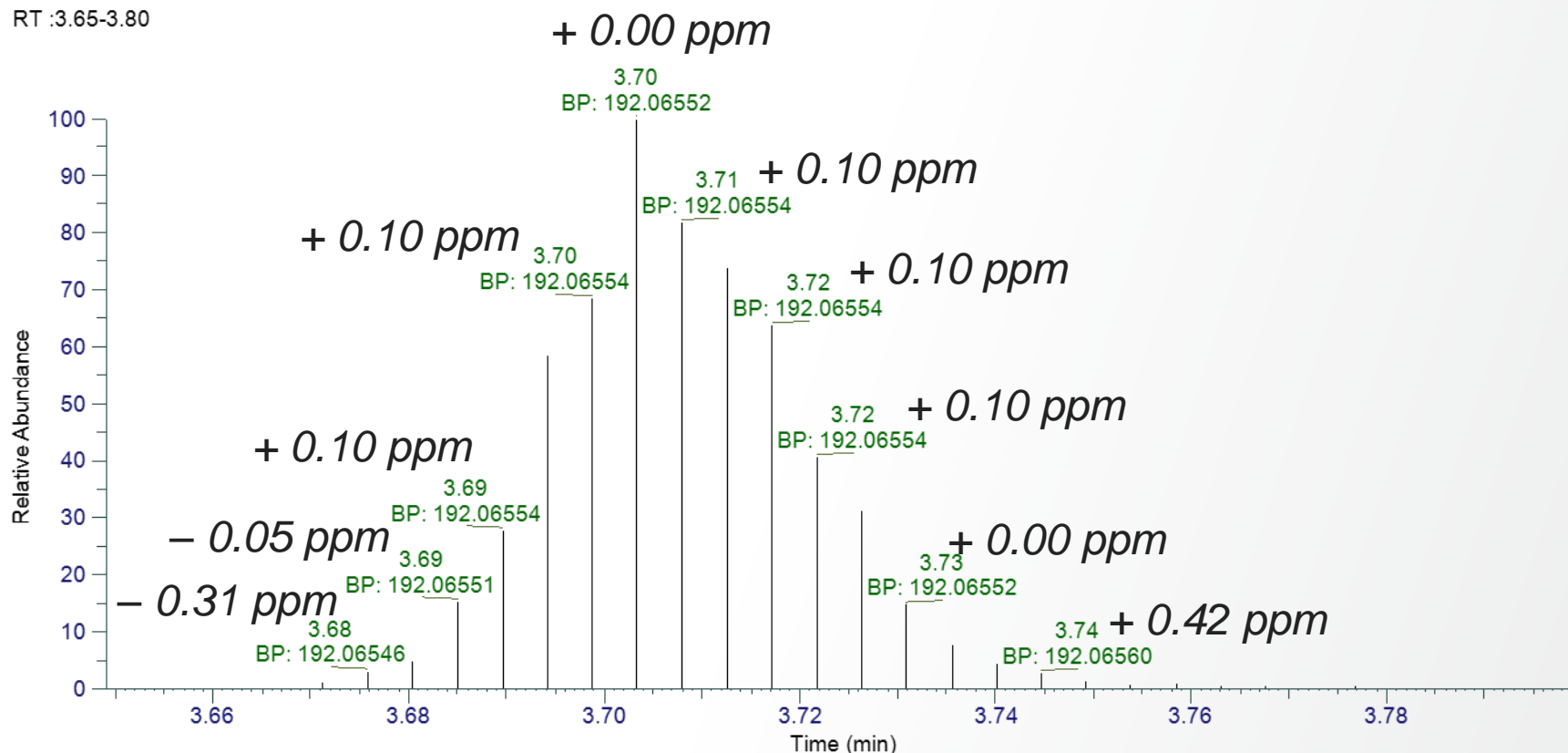
Excellent mass accuracy for every scan

5-HIAA $C_{10}H_9NO_3$

$M+H^+$ m/z 192.06552

RT :3.65-3.80

Orbitrap Exploris 240 MS
mouse plasma QC pool

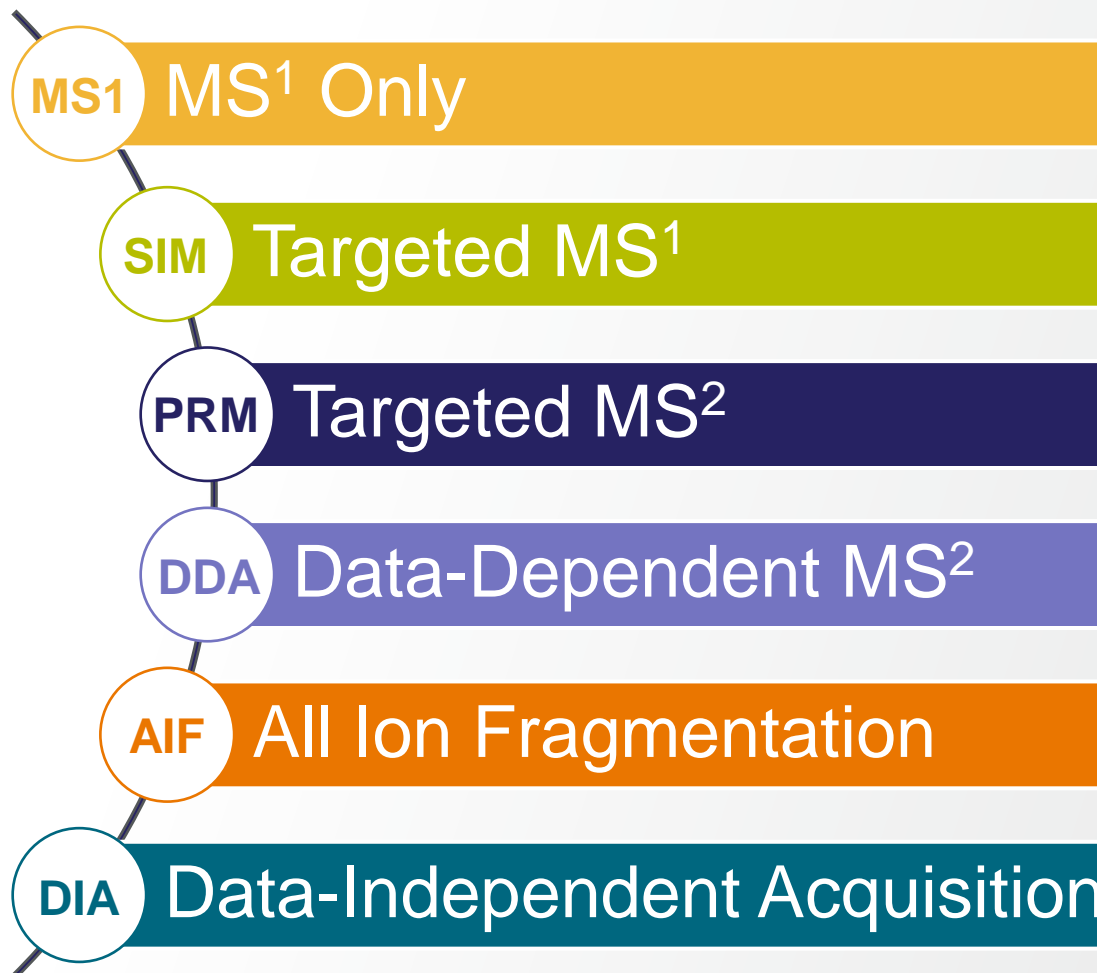
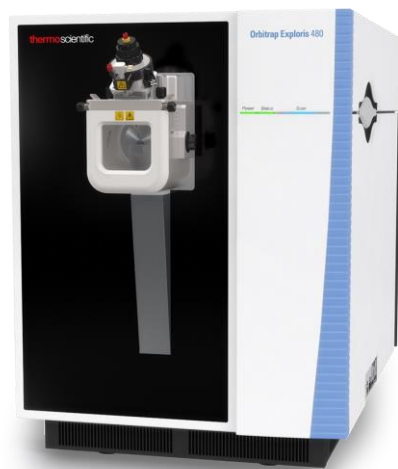


NL: 1.51E6
C10H9NO3: m/z = 192.0646-
192.0665 MS F: FTMS + p
ESI Full ms
[67.0000-1000.0000]
03_PP1_QC

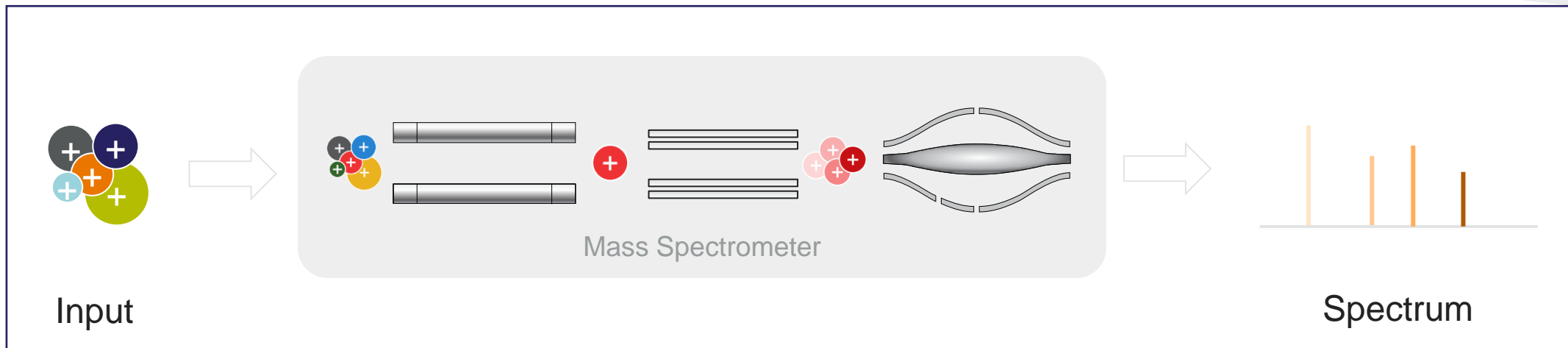
EASY-IC provides sub-1 ppm mass accuracy for every single mass spectrum across the peak

Note: A single spectrum (MS, MS²) can provide high quality data – no spectral averaging is needed.

Modes of Instrument Function



- With Data Dependent MS² (DDA), both MS¹ and MS² scans are acquired. Data from the MS¹ scans determine which ions are selected for MS² fragmentation.



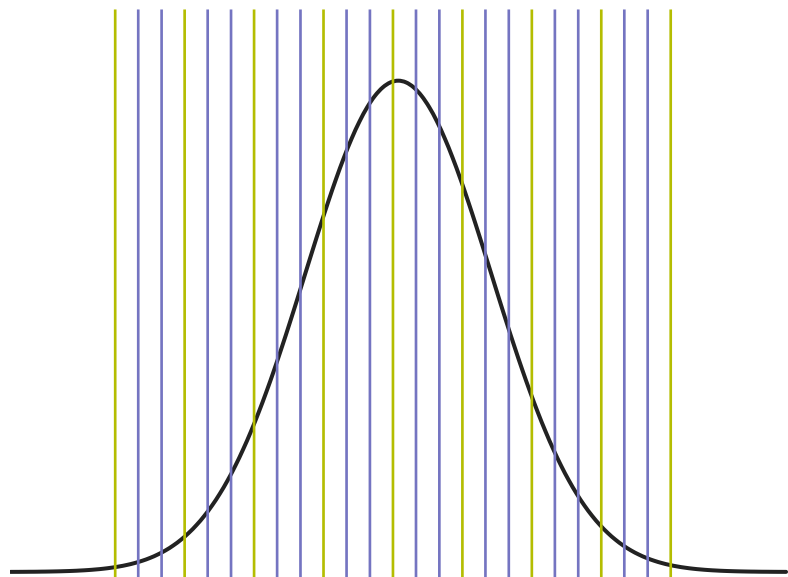
1. The 'Top N' number selected will affect how many MS¹ scans are acquired

MS¹

MS²

MS²

MS¹



9 MS¹ Scans Across Peak

MS¹

MS²

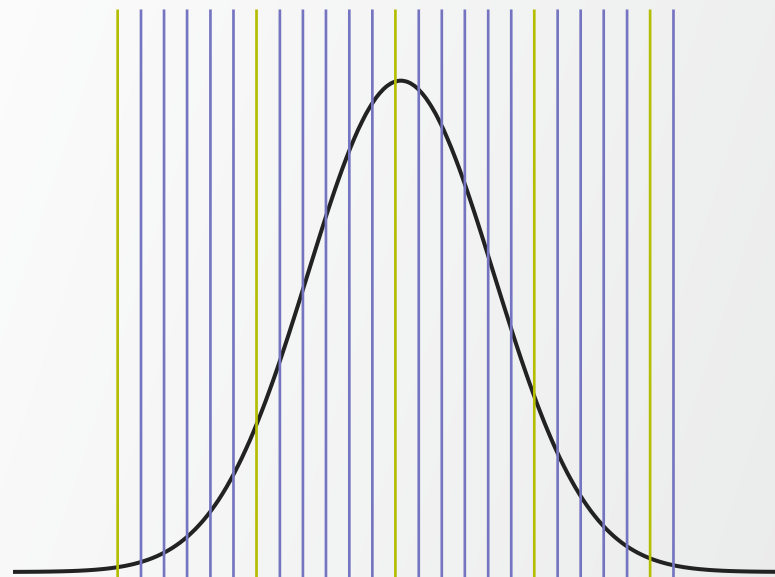
MS²

MS²

MS²

MS²

MS¹



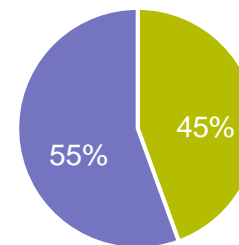
5 MS¹ Scans Across Peak

- 2. It's important to balance the amount of duty cycle that is used for collecting MS¹ and MS² scans
- 2.1. Orbitrap resolutions also must be carefully considered to allow appropriate cycle times and ensure sufficient MS² scans are acquired



MS1 High Res Method

MS1 Resolution: **120k**
MS2 Resolution: **15k**
Top N: **10**

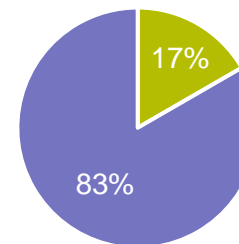


1.7 MS¹ scans/sec



Identification Focused Method

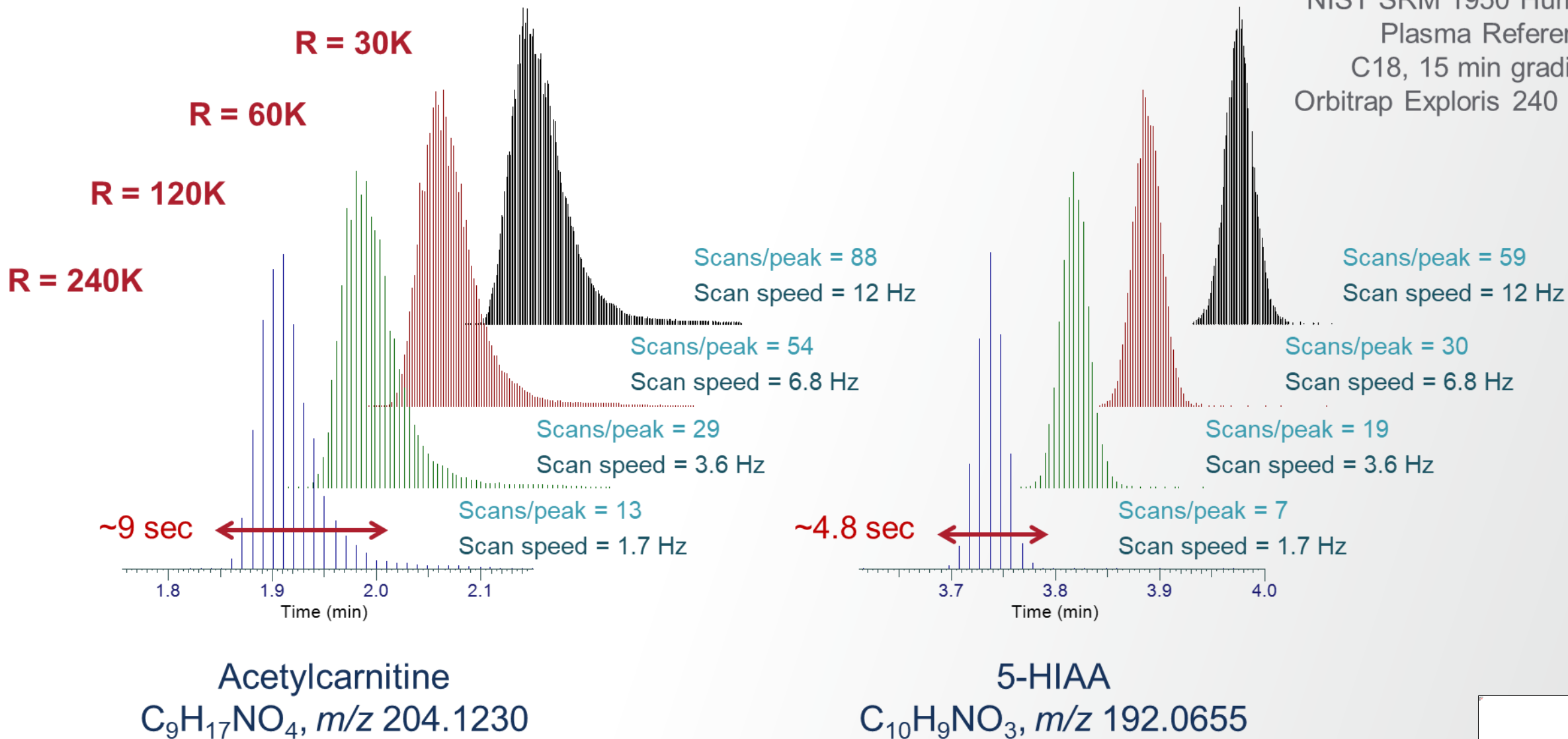
MS1 Resolution: **60k**
MS2 Resolution: **15k**
Top N: **20**



1.3 MS¹ scans/sec

Resolution vs. scan speed

NIST SRM 1950 Human
Plasma Reference
C18, 15 min gradient
Orbitrap Exploris 240 MS

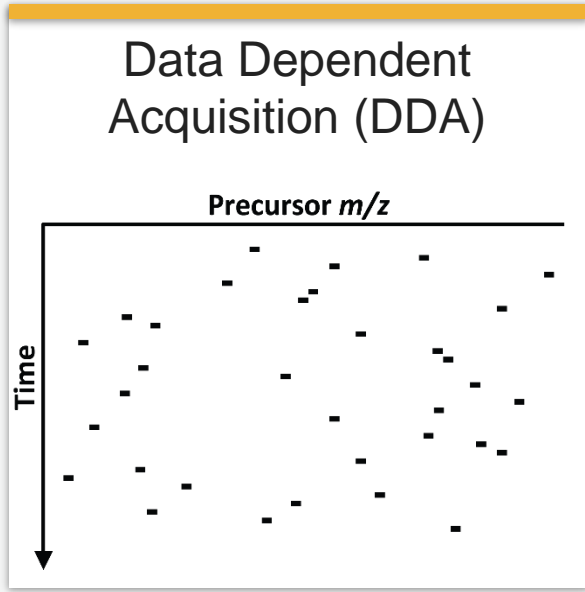


DDA Method Considerations

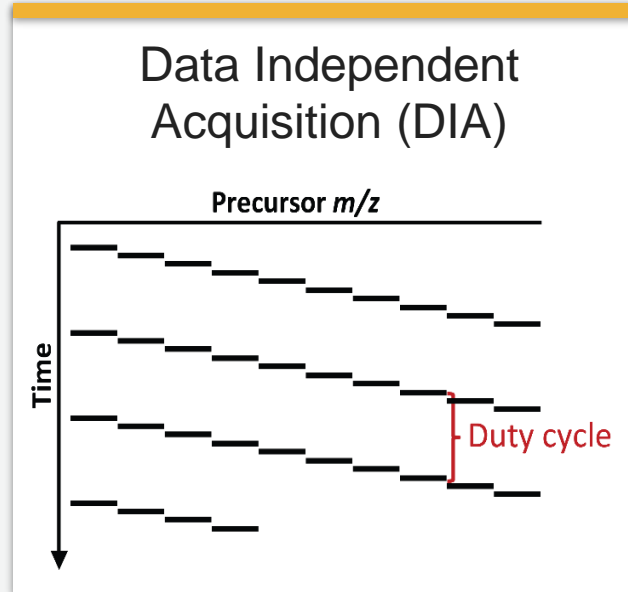
- Throughput/Samples per Day
- Sample Complexity and Coverage
- LC Gradient and Peak Shape
- Ideal TopN experiment
 - MS1 and MS2 Resolution needed
 - # of MS2 per cycle loop/TopN
 - Cycle time
- Match AGC, and Max IT to experiment
- Match Max IT to Resolution used (no wasted time)
- Match Dynamic Exclusion to Peak Shape

Exploris		
Res@ m/z 200	Transient length (ms)	Scan speed (Hz)
7,500*	16	40
15,000	32	22
30,000	64	12
45,000	96	10
60,000	128	7
120,000	256	3
240,000*	512	1.5
480,000*	1024	0.7

*Some Resolution Settings not available on all Exploris Models



Data Dependent Acquisition (DDA)



Data Independent Acquisition (DIA)

Article | [Published: 29 September 2004](#)

Automated approach for quantitative analysis of complex peptide mixtures from tandem mass spectra

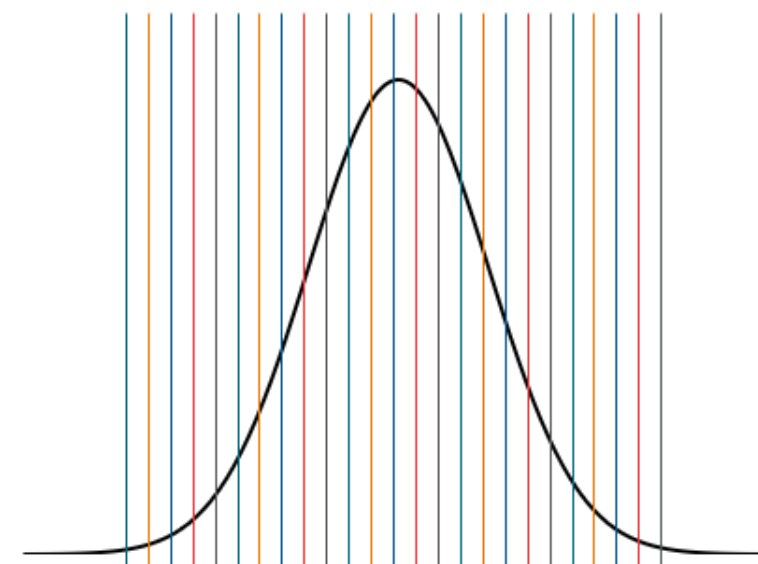
[John D Venable](#), [Meng-Qiu Dong](#), [James Wohlschlegel](#), [Andrew Dillin](#) & [John R Yates III](#)

[Nature Methods](#) **1**, 39–45 (2004) | [Cite this article](#)

DIA Considerations

- Throughput/Samples per Day
- Sample Complexity and Coverage
- LC Gradient
- m/z range
- # windows/size of windows
 - Wider Windows **→** Faster Methods
 - Wider Windows can increase variability
 - Window Overlap
- Scans across the peak depends on scan speed, window size and # of windows.
- Direct DIA vs Library
- PTMs

Five Windows



5 Scans Across Peak

Velocity DIA workflow for quantitative measurements without sacrificing scope

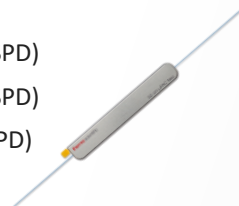


HeLa Protein Digest Standard (P/N 8829), 200 ng/run



30-minute gradient (36 SPD)
60-minute gradient (20 SPD)
9-minute gradient (80 SPD)

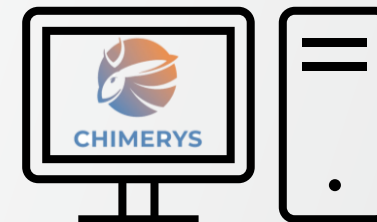
Thermo Scientific™ Vanquish™
NEO UHPLC System



Thermo Scientific™ μPAC™
NEO 50cm HPLC Column



Thermo Scientific™ Orbitrap
Exploris™ 480 Mass Spectrometer
with Thermo Scientific™ FAIMS Pro™
Interface



DIA processing software,
e.g. Proteome Discoverer
with Chimerys

Sample
preparation

Data
acquisition

Data
analysis

Software influences ID Numbers



CHIMERYYS

Proteome Discoverer software with
CHIMERYYS intelligent search algorithm

Chimerys DIA: Spectrum-
centric search that utilizes
predicted combinations of
spectra



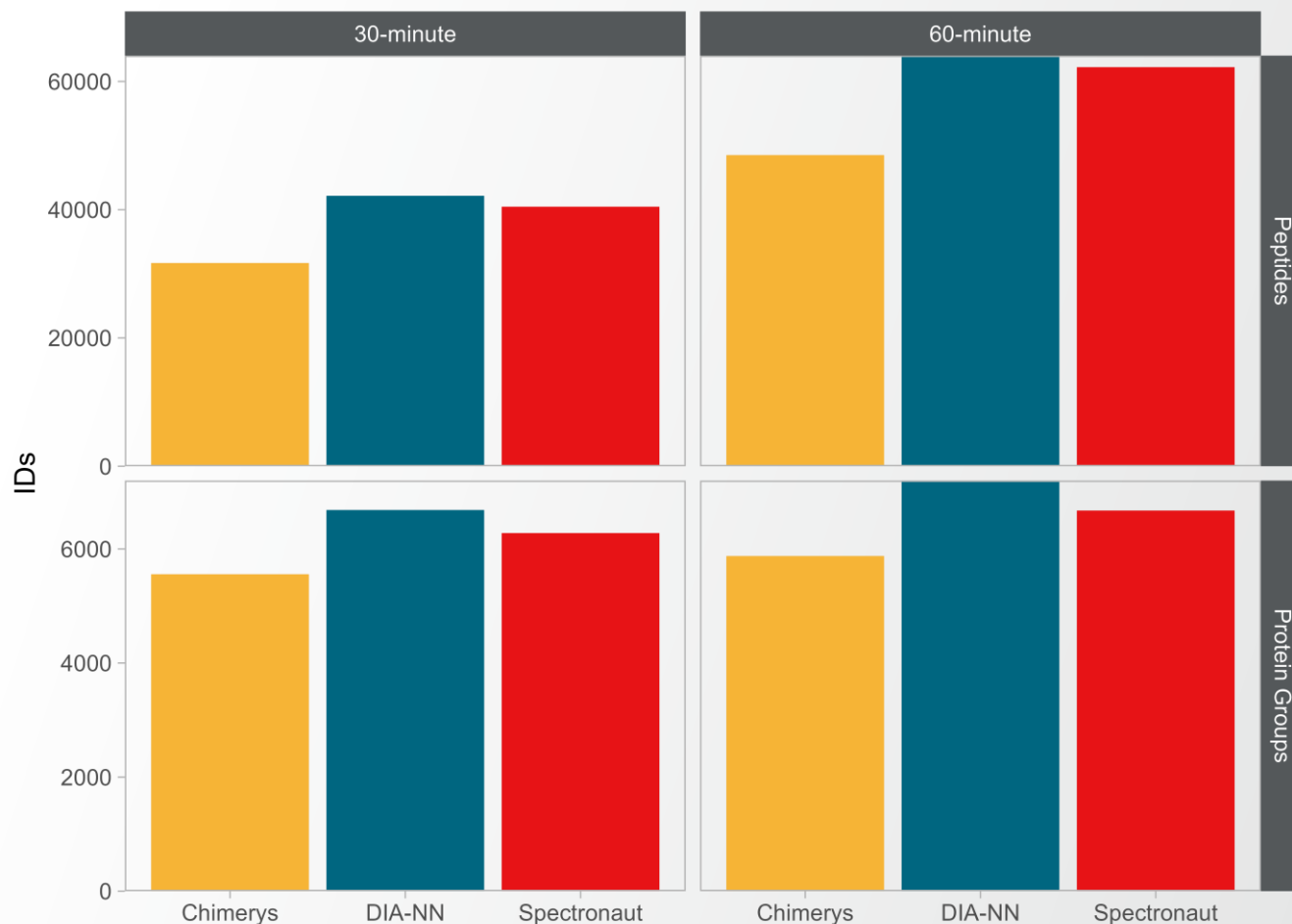
DIA-NN software

DIA-NN: Peptide-centric
library free search



Spectronaut® software

Spectronaut Direct-DIA:
Spectrum-centric library free
search



DIA-NN library free search and Spectronaut Direct-DIA search were used. Chimerys results were obtained in Proteome Discoverer software V 3.1 using Inferys 3.0.0 fragmentation model. All results were filtered to 1% FDR at the peptide and protein level.

DIA Data Independent Acquisition

Method

The screenshot displays the software interface for configuring a DIA method. On the left, a sidebar contains 'Global Lists' (Lock Masses, Inclusion, Exclusion, Neutral Loss, Tag Masses), 'Tune Files', 'External Hardware', 'Chromatogram', and 'Scan Groups'. A chromatogram shows a single peak labeled 'DIA' from 0 to 10 minutes. Below this is a list of 'Experiments' including Full MS - SIM, AIF, Full MS / AIF, Full MS / dd-MS² (TopN), Targeted-SIM, PRM, Targeted-SIM / dd-MS², Full MS / AIF / NL dd-MS², DIA, and TMT. The 'Properties' panel on the right is divided into 'Properties of the method' (Use lock masse: best, Chrom. peak wi 15 s, Time: Method duration 10.00 min) and 'Properties of DIA' (General: Runtime 0 to 10 min, Polarity positive, Default charge: 2; DIA: Resolution 30,000, AGC target 2e5, Maximum IT auto, Loop count 1, MSX count 1, MSX isochronou on, Isolation window 3.0 m/z, Fixed first mass -, (N)CE / stepped nce: 30).



Q Exactive

The screenshot displays the software interface for configuring a DIA method. At the top, it shows 'Experiment # 1 0-60' with 'SWITCH', 'CLEAR', and a trash icon. A 'DIA' button is highlighted in the center. On the right, the 'Data-Independent Acquisition Properties' panel is shown with various settings: Precursor Mass Range (100-1100), Isolation Window (m/z) (100), Window Overlap (m/z) (0), Number Of Scan Events (10), Collision Energy Type (Normalized), Collision Energy (%) (30), Orbitrap Resolution (30000), Scan Range Mode (Auto), RF Lens (%) (50), and Polarity (Positive). At the bottom, a table lists 'DIA m/z wii' with columns for 'ADD', 'DELETE', 'IMPORT', and 'EXPORT'. The table contains four rows of calculated m/z windows.

	Calculated m/z Window
1	100-200
2	200-300
3	300-400
4	400-500



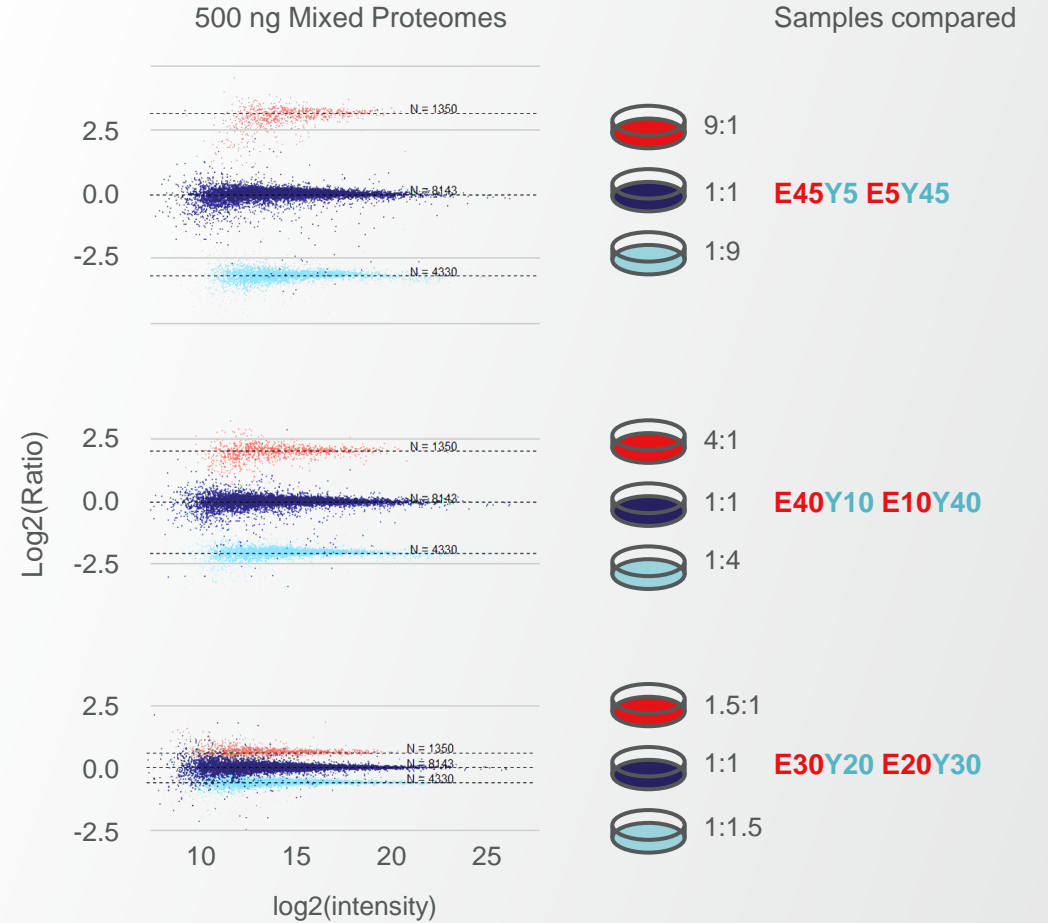
Exploris

Orbitrap Data Quality: Exploris, Orbitrap Ascend and Orbitrap Astral

**Accurate and
Precise Quantitation**

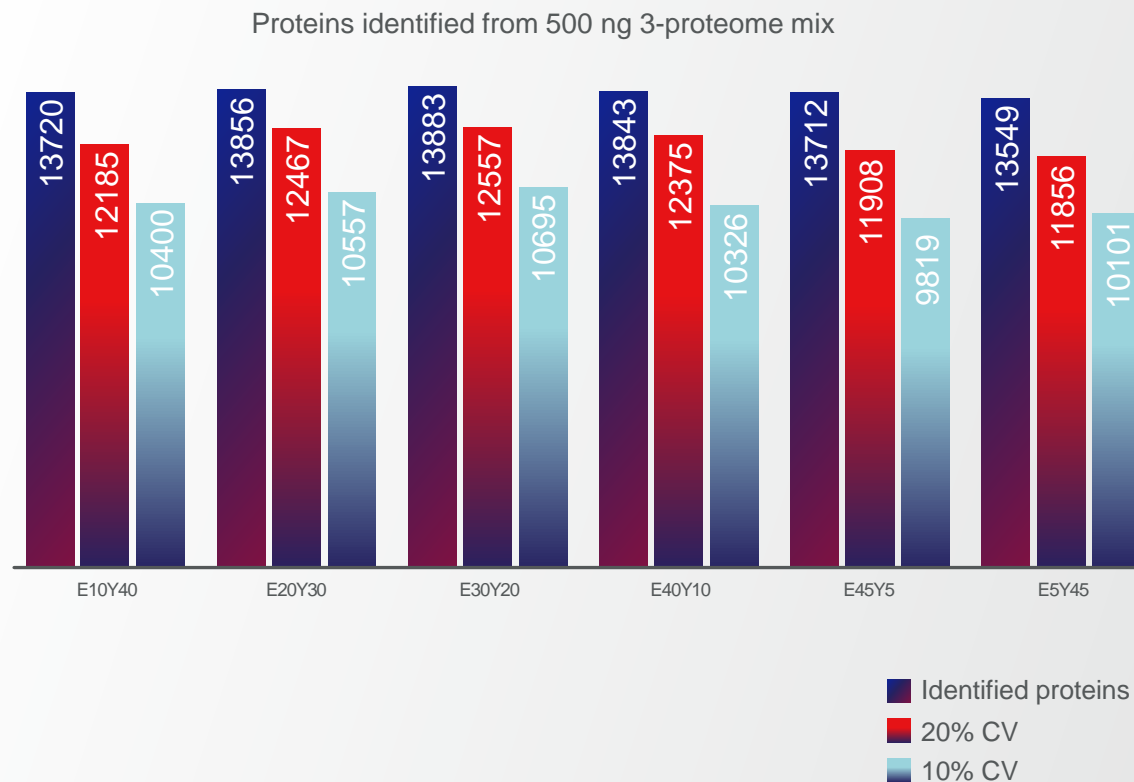
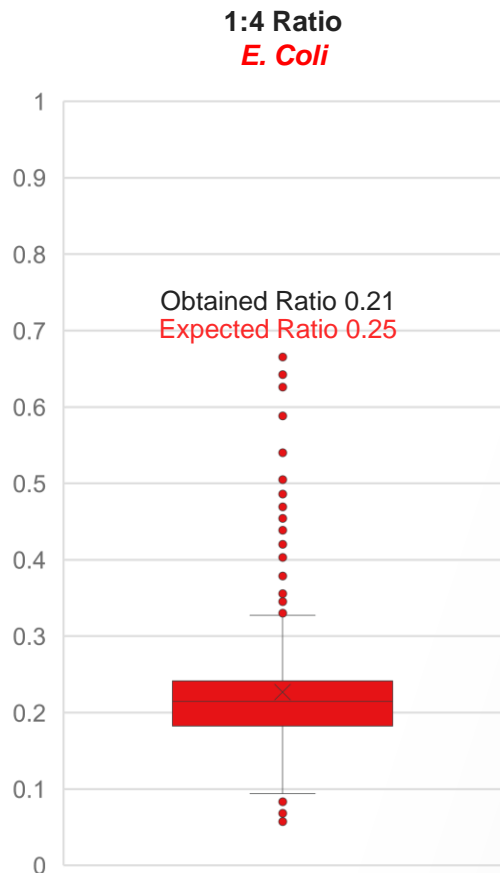
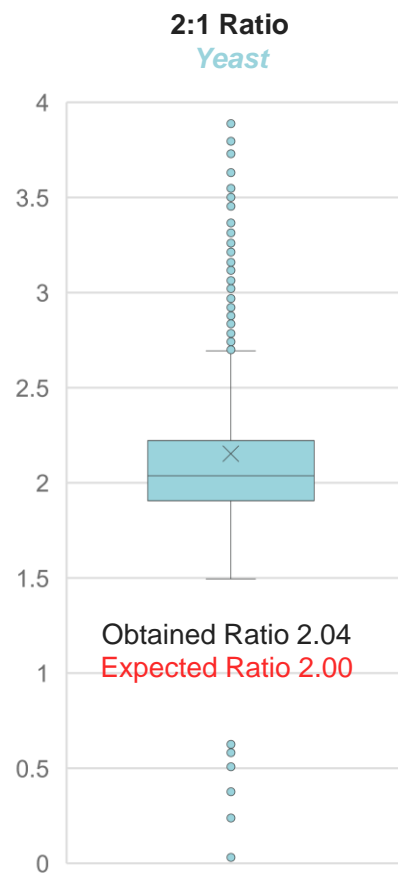
Discover more biomarkers with a larger dynamic range

Accurate and precise label-free quantitation with data-independent acquisition for greater statistical power



Accurate and precise label-free quantitation

Correct mixing ratios with highly reproducible measurements

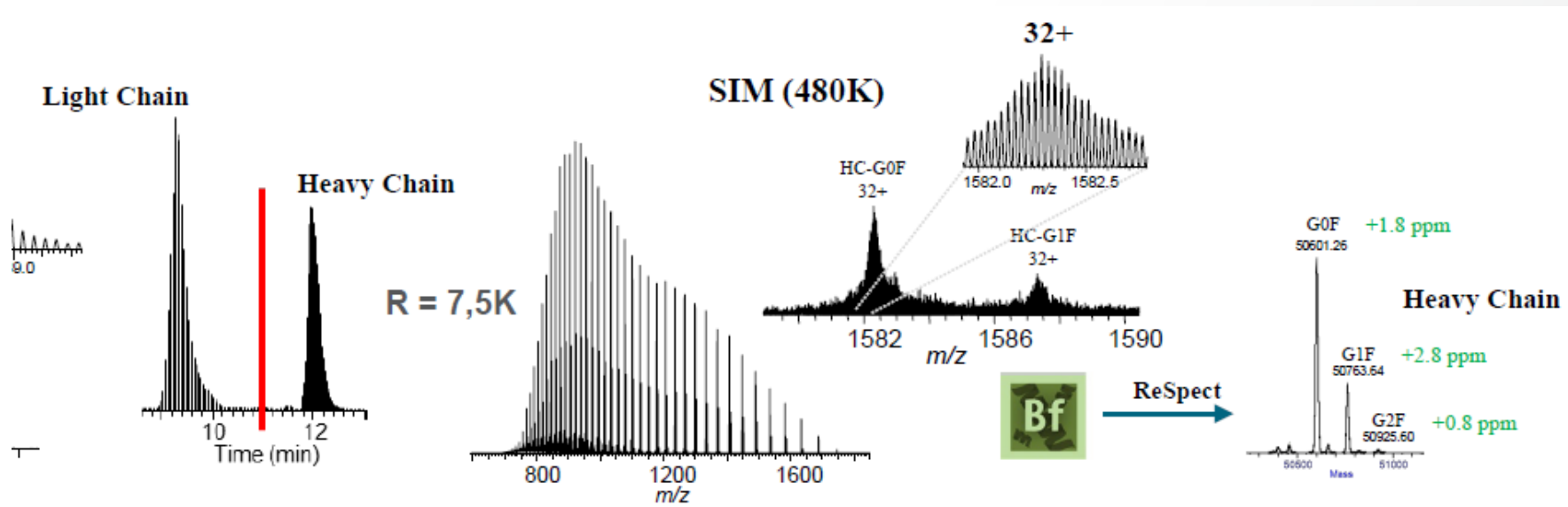


Intact Protein, mAb/ADC Analysis

- **Therapeutic Protein Challenges**
 - Sample Heterogeneity/Complexity
 - Size >150kD

- **High Resolution Accurate Mass Orbitrap**
 - Superior Resolution and accurate mass can separate contaminants and variability in complex samples.
 - Provides confident mAb/ADC characterization.

- High Resolution Thermo Scientific Orbitrap Exploris 480 Herceptin Heavy Chain



- Orbitrap isotopically resolved HC peaks.
- G0F identified with 2 ppm Mass Accuracy for Herceptin HC.

High-Resolution Accurate-Mass Mass Spectrometry Enabling In-Depth Characterization of *in Vivo* Biotransformations for Intact Antibody-Drug Conjugates

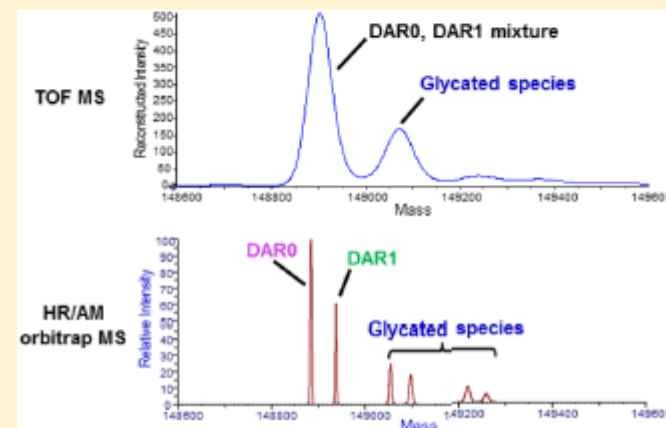
Jintang He,^{*,†} Dian Su,[†] Carl Ng,[†] Luna Liu,[†] Shang-Fan Yu,[†] Thomas H. Pillow,[†] Geoffrey Del Rosario,[†] Martine Darwish,[†] Byoung-Chul Lee,[†] Rachana Ohri,[†] Hongxiang Zhou,[‡] Xueji Wang,[‡] Jiawei Lu,[‡] Surinder Kaur,[†] and Keyang Xu^{*,†}

[†]Genentech Inc., 1 DNA Way, South San Francisco, California 94080, United States

[‡]Wuxi Apptec, 288 Fute Zhong Road, Waigaoqiao Free Trade Zone, Shanghai, 200131, China

Supporting Information

ABSTRACT: Antibody-drug conjugates (ADCs) represent a promising class of therapeutics for the targeted delivery of highly potent cytotoxic drugs to tumor cells to improve bioactivity while minimizing side effects. ADCs are composed of both small and large molecules and therefore have complex molecular structures. *In vivo* biotransformations may further increase the complexity of ADCs, representing a unique challenge for bioanalytical assays. Quadrupole-time-of-flight mass spectrometry (Q-TOF MS) with electrospray ionization has been widely used for characterization of intact ADCs. However, interpretation of ADC biotransformations with small mass changes, for the intact molecule, remains a limitation due to the insufficient mass resolution and accuracy of Q-TOF MS. Here, we have investigated *in vivo* biotransformations of multiple site-specific THIOMAB antibody-drug conjugates (TDCs), in the intact form,



Orbitrap HRAM-High Resolution Accurate Mass

Thermo Scientific™ Hybrid
Orbitrap Mass Spectrometers



- Exploris 240 and 480
 - Proteomics (DDA and DIA)
 - Intact Proteins, Protein Complexes
 - Top Down
 - SCP
 - Oligonucleotides
 - Metabolomics
 - Lipidomics
 - Small Molecules

Thermo Scientific™ Orbitrap™
Tribid™ Mass Spectrometers



- Orbitrap Ascend (Tribid)
 - All applications for Exploris +
 - TMT (SPS)
 - MS_n
 - Multiple Fragmentation (HCD, CID, ETD, EThcD, UVPD, PTCR)
 - Real Time Search (TMT, Lipid, Met ID)

Thermo Scientific™
Orbitrap™ Astral™
Mass Spectrometer



- Orbitrap Astral
 - All Application for Exploris +
 - Higher Throughput
 - Deeper Coverage
 - Higher Sensitivity

Differences across Orbitrap Exploris 240 and Orbitrap Exploris 480 are demonstrated by increases in sensitivity and mass resolution capability

Orbitrap Exploris 240 MS

- S-Lens
- Segmented quadrupole with configuration switching
- FS-MS, t-MS², DDA Scan Rate: 22 Hz
- Mass Range m/z 40-6,000 (opt. 8,000)
- Max. Mass Resolution 240,000



Orbitrap Exploris 480 MS

- High Capacity Transfer Tube (HCTT)
- Electrodynamic Ion Funnel (EDIF)
- Segmented quadrupole with configuration switching
- FS-MS, t-MS², DDA Scan Rate: 40 Hz
- Mass Range m/z 40-6,000 (opt. 8,000)
- Max. Mass Resolution 480,000

Options: Intact Protein (Thermo Scientific™ BioPharma option), Thermo Scientific™ FAIMS Pro™ interface

New Orbitrap Ascend Tribrid MS system



Unmatched Analytical Performance and Versatility

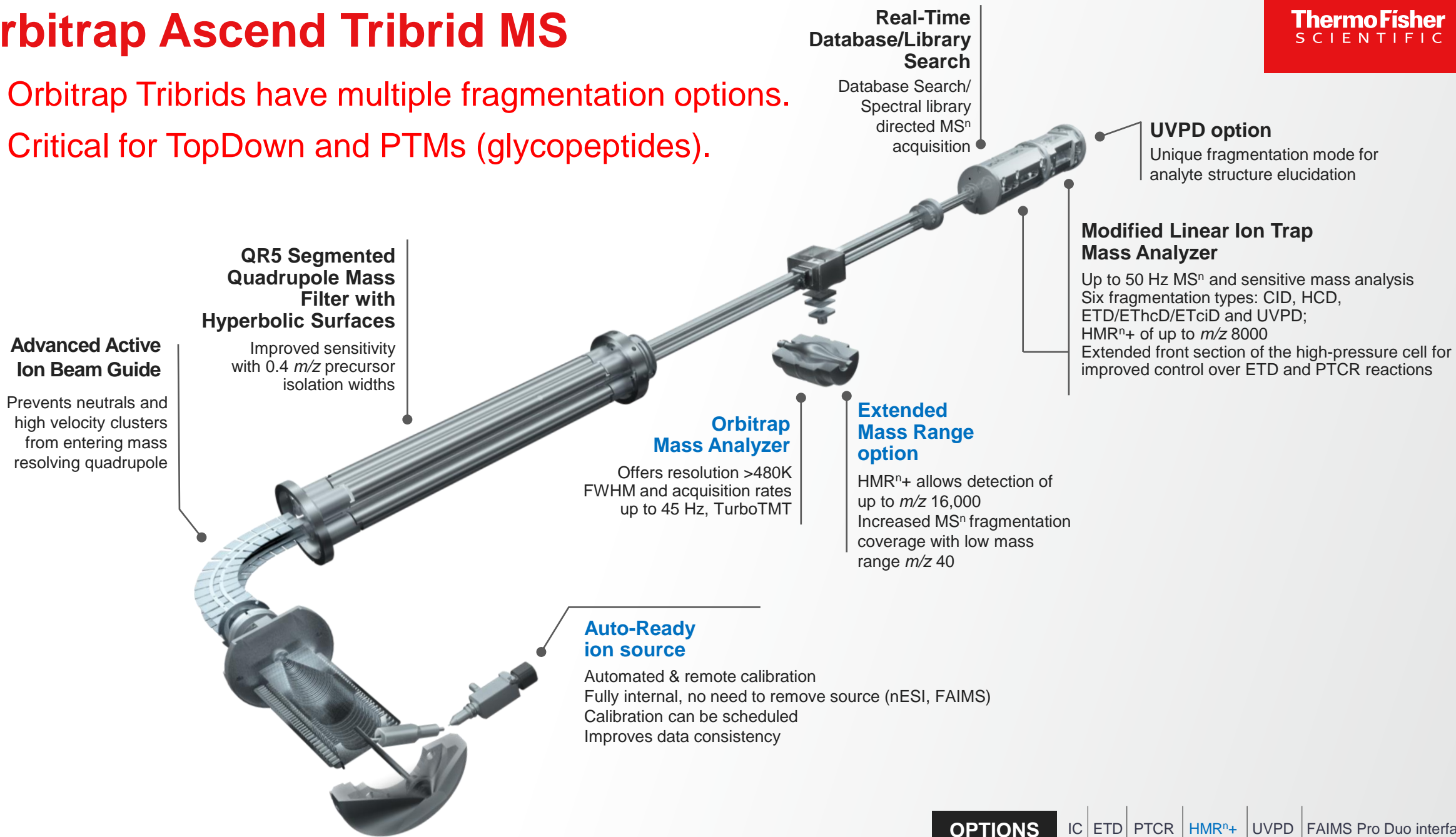
- **Improved ion introduction and transfer** for greater sensitivity and acquisition rates
- **QR5 Segmented Quadrupole Mass Filter** for outstanding precursor selectivity and sensitivity
- **Real-Time Database/Library Search** for exceptional depth and accuracy for TMT analysis
- **Higher Mass Range MSⁿ (HMRⁿ⁺) option** for structural analysis of native protein complexes
- **Proton Transfer Charge Reduction (PTCR) option** for simplification of complex spectra and improved top-down data interpretation
- **The fully integrated Auto-Ready ion source** for complete, unattended system calibration, without the need for manual hardware reconfiguration
- **Full Customization** with a range of optional capabilities:

EASY-IC | ETD | UVPD | HMRⁿ⁺ | PTCR | FAIMS Pro interface
- **Common interface** with Orbitrap Exploris 480 MS and TSQ Triple Quadrupole MS

PARAMETER	CHARACTERISTICS
Acquisition rate OTMS²	45 Hz
Acquisition rate ITMS²	50 Hz
Maximum resolution	480K FWHM at m/z 200
Quadrupole minimum isolation width	0.4 m/z
Mass range	m/z 50-6,000, up to m/z 16,000 with HMR ⁿ⁺
Mass Accuracy	3 ppm external, 1 ppm internal
Dissociation / Ion Activation	CID, HCD, ETD, ETHcD, ETciD, UVPD, PTCR
MSⁿ	Up to MS ¹⁰ with the ion trap or Orbitrap mass analyzer
Analyzers	Q, OTMS, ITMS
Detectors	Ion Trap, Orbitrap mass analyzer
Size	1270 × 805 × 703 mm (w, d, h)

Orbitrap Ascend Tribrid MS

- Orbitrap Tribbrids have multiple fragmentation options.
- Critical for TopDown and PTMs (glycopeptides).



OPTIONS

IC

ETD

PTCR

HMRⁿ⁺

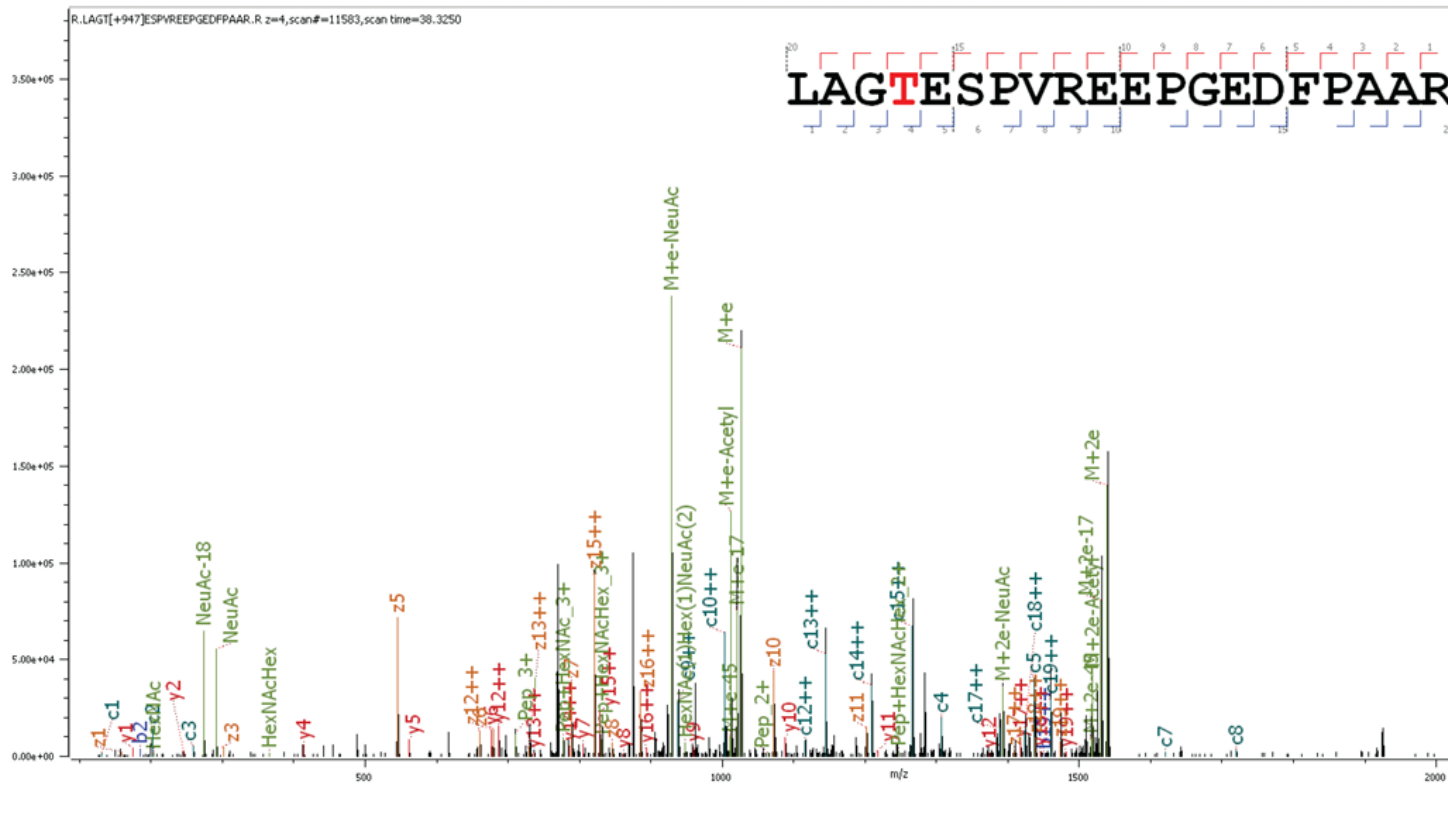
UVPD

FAIMS Pro Duo interface

Advantages of Multiple Fragmentation Options

mAb Glycopeptide Analysis with EThcD*

Figure 8. EThcD FT-MS/MS spectrum of O-linked glycopeptide



- Sugar-Peptide Linkage often weakest bond and preferentially cleaved during MS/MS fragmentation.
- Fragmenting with EThcD (combination of ETD and HCD) preserves sugar-peptide bond, allowing PTM site localization as well as peptide backbone fragmentation for sequencing.

- *EThcD only possible in Thermo Scientific Tribrid Orbitraps: Ascend, Eclipse and Lumos.

Combination of Multiple Fragmentation Options Provide best TopDown Sequence Coverage (mAb LC)

HCD 10: 22% Seq Cov

N	Q	S	A	L	T	Q	P	R	S	V	S	G	S	P	G	Q	S	V	T	I	S	C	T	G	T	25	
26	S	S	D	I	G	G	Y	N	F	V	S	W	Y	Q	Q	H	P	G	K	A	P	K	L	M	I	50	
51	Y	D	A	T	K	R	P	S	G	V	P	D	R	F	S	G	S	K	S	G	N	T	A	S	L	75	
76	T	I	S	G	L	Q	A	E	D	E	A	D	Y	Y	C	C	S	Y	A	G	D	Y	T	P	G	100	
101	V	V	F	G	G	G	T	K	L	T	V	L	G	Q	P	K	A	A	P	S	V	T	L	F	P	125	
126	P	S	S	E	E	L	Q	A	N	K	A	T	L	V	C	L	L	I	S	D	F	Y	P	G	A	V	150
151	L	T	V	A	W	K	A	D	S	S	P	V	K	A	G	V	E	T	T	T	P	S	K	Q	S	N	175
176	N	K	Y	A	A	S	S	Y	L	S	L	L	T	P	E	Q	W	K	S	H	R	S	Y	S	C	Q	200
201	V	T	H	E	G	S	T	V	E	K	T	V	A	P	T	E	C	S	C								

CID 30: 22% Seq Cov

N	Q	S	A	L	T	Q	P	R	S	V	S	G	S	P	G	Q	S	V	T	I	S	C	T	G	T	25	
26	S	S	D	I	G	G	Y	N	F	V	S	W	Y	Q	Q	H	P	G	K	A	P	K	L	M	I	50	
51	Y	D	A	T	K	R	P	S	G	V	P	D	R	F	S	G	S	K	S	G	N	T	A	S	L	75	
76	T	I	S	G	L	Q	A	E	D	E	A	D	Y	Y	C	C	S	Y	A	G	D	Y	T	P	G	100	
101	V	V	F	G	G	G	T	K	L	T	V	L	G	Q	P	K	A	A	P	S	V	T	L	F	P	125	
126	P	S	S	E	E	L	Q	A	N	K	A	T	L	V	C	L	L	I	S	D	F	Y	P	G	A	V	150
151	L	T	V	A	W	K	A	D	S	S	P	V	K	A	G	V	E	T	T	T	P	S	K	Q	S	N	175
176	N	K	Y	A	A	S	S	Y	L	S	L	L	T	P	E	Q	W	K	S	H	R	S	Y	S	C	Q	200
201	V	T	H	E	G	S	T	V	E	K	T	V	A	P	T	E	C	S	C								

ETD 15ms: 46% Seq Cov

N	Q	S	A	L	T	Q	P	R	S	V	S	G	S	P	G	Q	S	V	T	I	S	C	T	G	T	25	
26	S	S	D	I	G	G	Y	N	F	V	S	W	Y	Q	Q	H	P	G	K	A	P	K	L	M	I	50	
51	Y	D	A	T	K	R	P	S	G	V	P	D	R	F	S	G	S	K	S	G	N	T	A	S	L	75	
76	T	I	S	G	L	Q	A	E	D	E	A	D	Y	Y	C	C	S	Y	A	G	D	Y	T	P	G	100	
101	V	V	F	G	G	G	T	K	L	T	V	L	G	Q	P	K	A	A	P	S	V	T	L	F	P	125	
126	P	S	S	E	E	L	Q	A	N	K	A	T	L	V	C	L	L	I	S	D	F	Y	P	G	A	V	150
151	T	V	A	W	K	A	D	S	S	P	V	K	A	G	V	E	T	T	T	P	S	K	Q	S	N	175	
176	N	K	Y	A	A	S	S	Y	L	S	L	L	T	P	E	Q	W	K	S	H	R	S	Y	S	C	Q	200
201	V	T	H	E	G	S	T	V	E	K	T	V	A	P	T	E	C	S	C								

ETD 25ms: 44% Seq Cov

N	Q	S	A	L	T	Q	P	R	S	V	S	G	S	P	G	Q	S	V	T	I	S	C	T	G	T	25	
26	S	S	D	I	G	G	Y	N	F	V	S	W	Y	Q	Q	H	P	G	K	A	P	K	L	M	I	50	
51	Y	D	A	T	K	R	P	S	G	V	P	D	R	F	S	G	S	K	S	G	N	T	A	S	L	75	
76	T	I	S	G	L	Q	A	E	D	E	A	D	Y	Y	C	C	S	Y	A	G	D	Y	T	P	G	100	
101	V	V	F	G	G	G	T	K	L	T	V	L	G	Q	P	K	A	A	P	S	V	T	L	F	P	125	
126	P	S	S	E	E	L	Q	A	N	K	A	T	L	V	C	L	L	I	S	D	F	Y	P	G	A	V	150
151	T	V	A	W	K	A	D	S	S	P	V	K	A	G	V	E	T	T	T	P	S	K	Q	S	N	175	
176	N	K	Y	A	A	S	S	Y	L	S	L	L	T	P	E	Q	W	K	S	H	R	S	Y	S	C	Q	200
201	V	T	H	E	G	S	T	V	E	K	T	V	A	P	T	E	C	S	C								

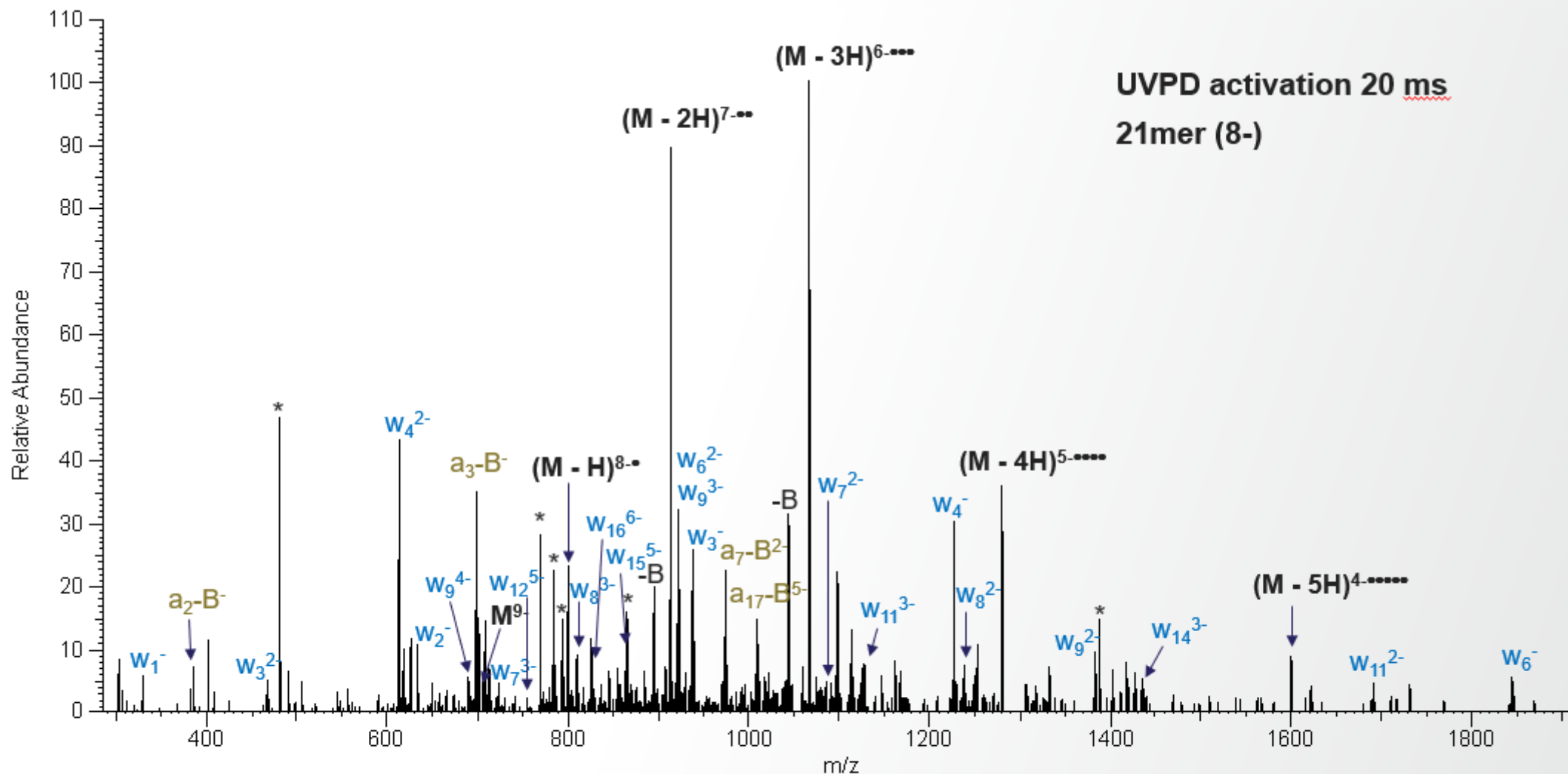
mAb LC Combined Sequence Coverage ETD, HCD, CID

70% Sequence Coverage

- ETD 15ms, 25ms
- HCD 10
- CID 30

N **Q** S A L T Q P R S V S G S P G Q S V T I S C T G T 25
26 S S D I G G Y N F V S W Y Q Q H P G K A P K L M I 50
51 Y D A T K R P S G V P D R F S G S K S G N T A S L 75
76 T I S G L Q A E D E A D Y Y C C S Y A G D Y T P G 100
101 V V F G G G T K L T V L G Q P K A A P S V T L F P 125
126 P S S E E L Q A N K A T L V C L L I S D F Y P G A V 150
151 T V A W K A D S S P V K A G V E T T T P S K Q S N 175
176 N K Y A A S S Y L S L L T P E Q W K S H R S Y S C Q 200
201 V T H E G S T V E K T V A P T E C S C

UVPD fragmentation of oligonucleotides



UVPD of 21mer leads to abundant charge reduced species and a series of w and a-B ions for sequencing

* = internal fragments. Not all UVPD fragments are annotated in the spectrum above.

Characterizing lipids using HCD/UVPD and MS3 scans

Pinpointing Double Bond and *sn*-Positions in Glycerophospholipids via Hybrid 193 nm Ultraviolet Photodissociation (UVPD) Mass Spectrometry

Peggy E. Williams, Dustin R. Klein, Sylvester M. Greer, and Jennifer S. Brodbelt*[✉]

Department of Chemistry, University of Texas at Austin, Austin, Texas 78712, United States

[✉] Supporting Information

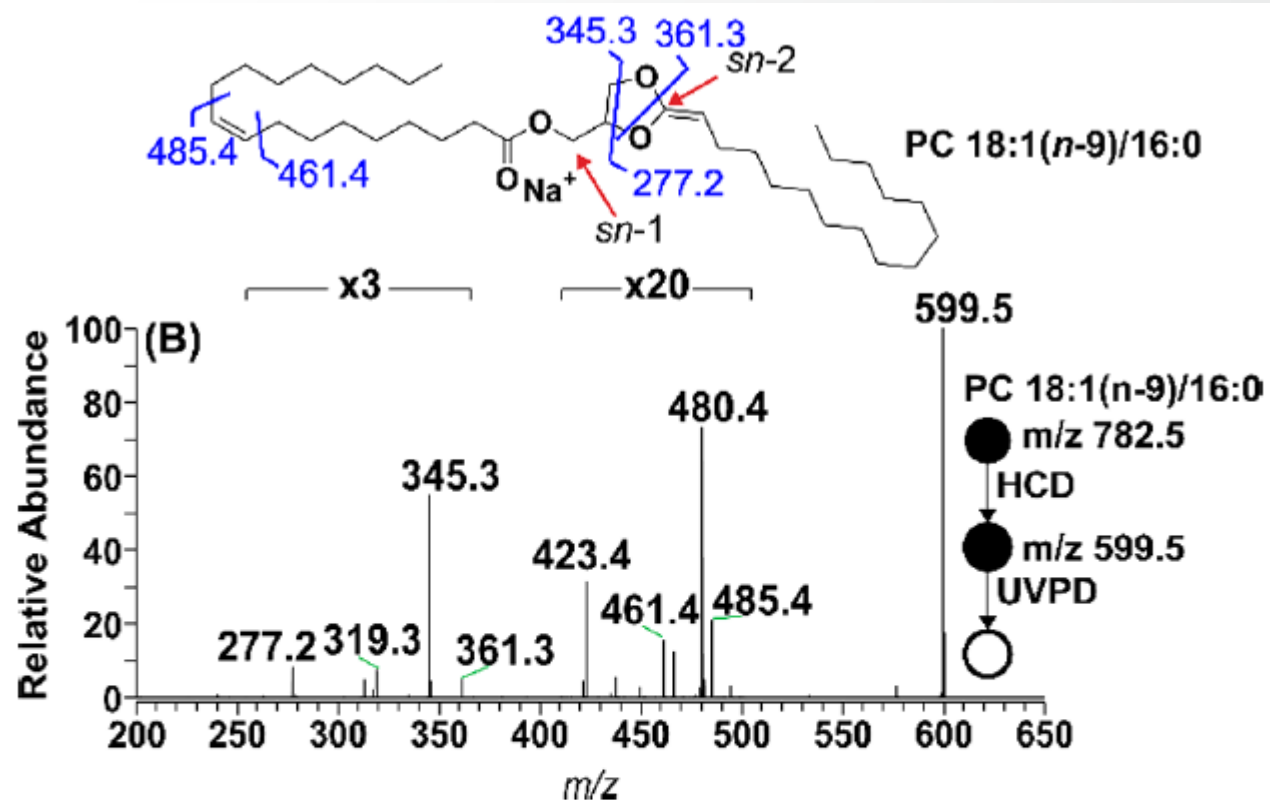
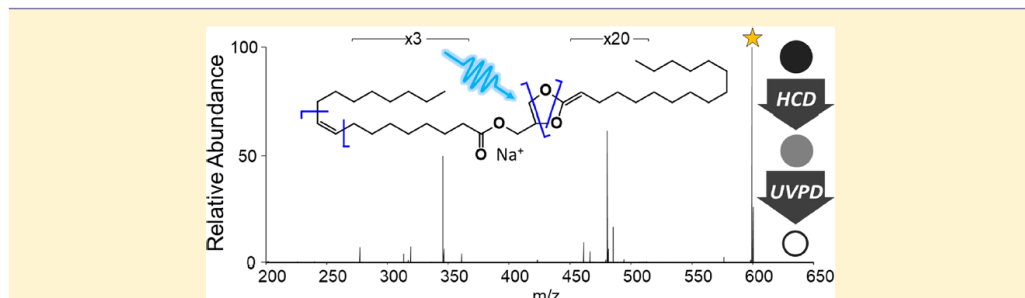


Figure 1. HCD/UVPD spectra of two sodium-cationized *sn*-regioisomeric PCs (A) PC 16:0/18:1(*n*-9) and (B) PC 18:1(*n*-9)/16:0. These spectra were obtained by isolating the headgroup loss ions (*m/z* 599.5) generated by HCD and subjecting them to 10 laser pulses (193 nm) with 4 mJ per pulse.

Real-time Search and
Intelligent MS³ data
acquisition aid Quan
and Identification

Increasing Accuracy and Depth for TMT SP3



TMTpro 18plex reagents

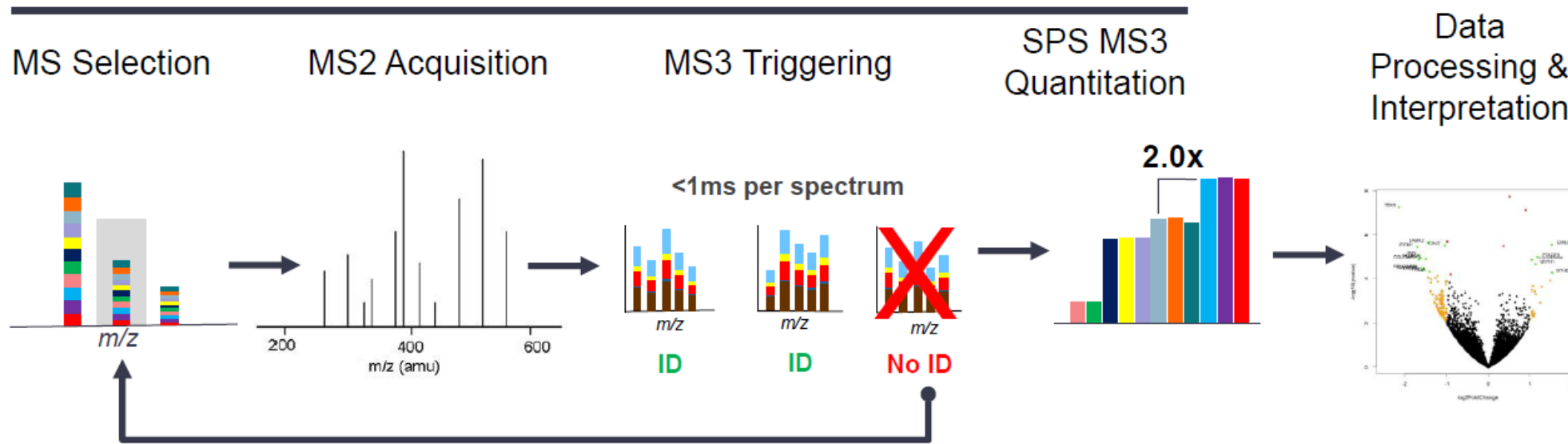
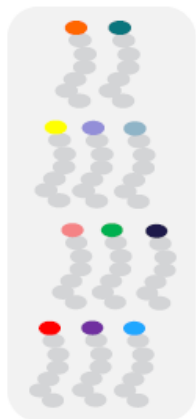


Thermo Scientific™ Orbitrap Eclipse™
Tribrid™ Mass Spectrometer



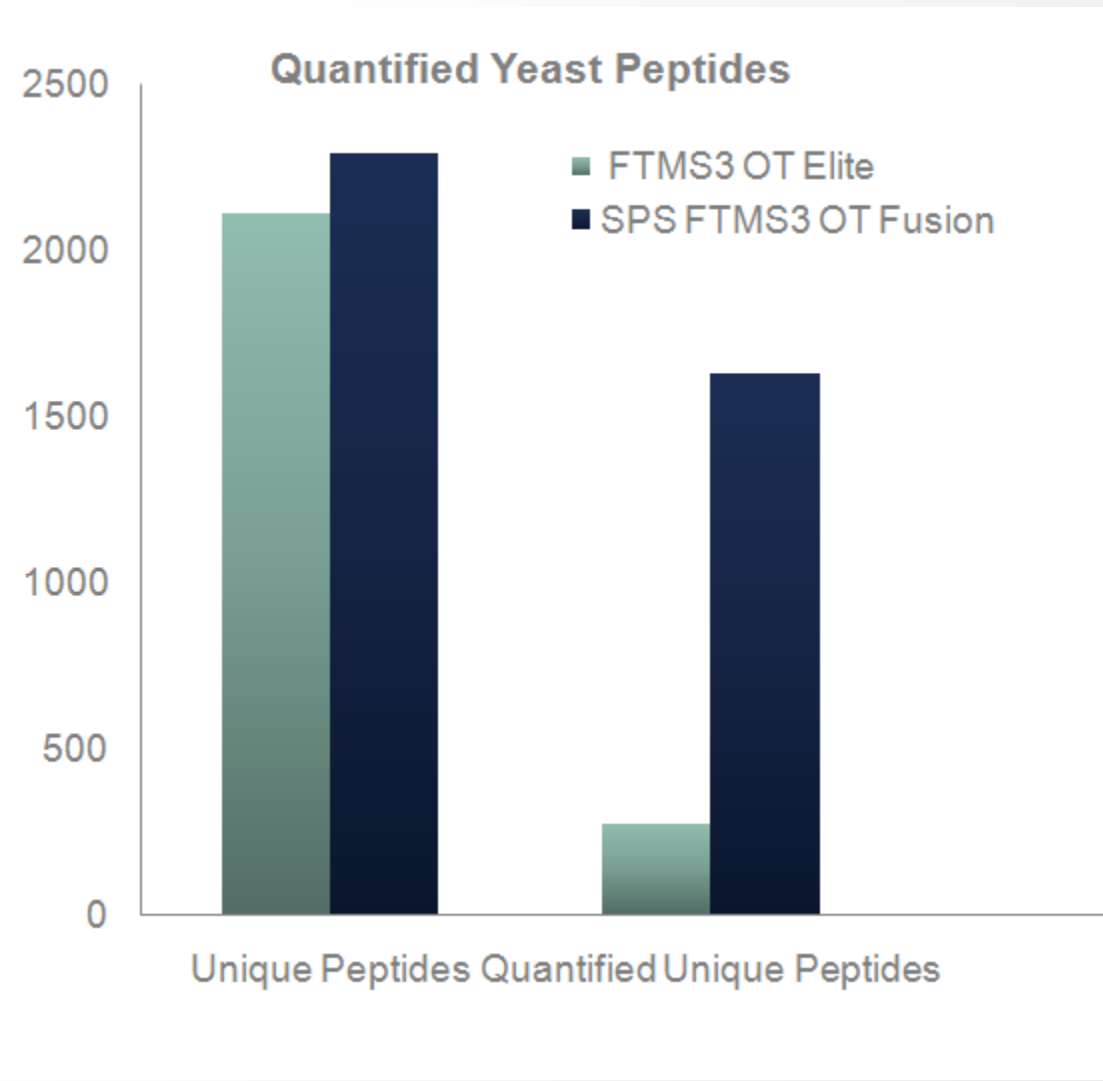
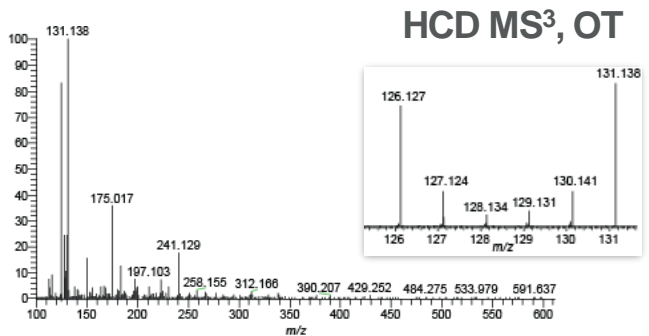
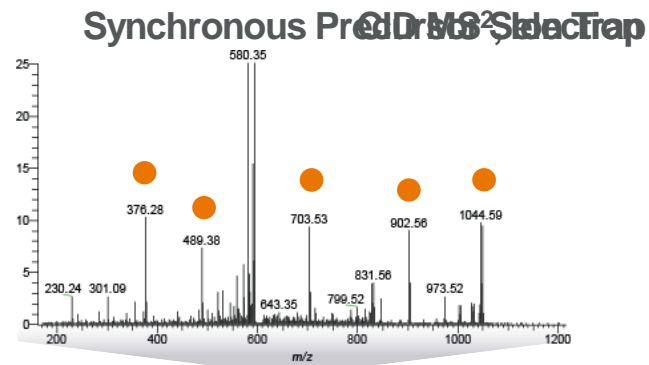
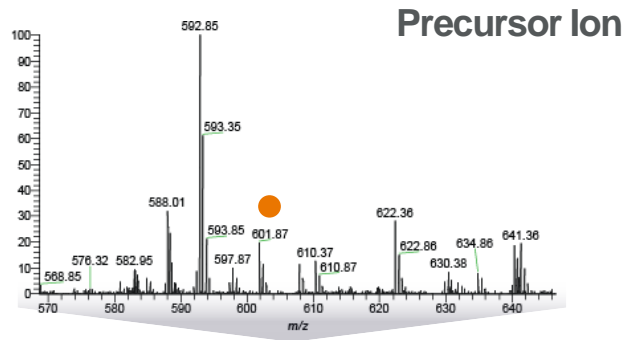
Proteome Discoverer
Software 3.0

TMT Labeled
Peptides



Real-Time Search

Synchronous Precursor Selection - Accurate and Sensitive TMT Quantitation

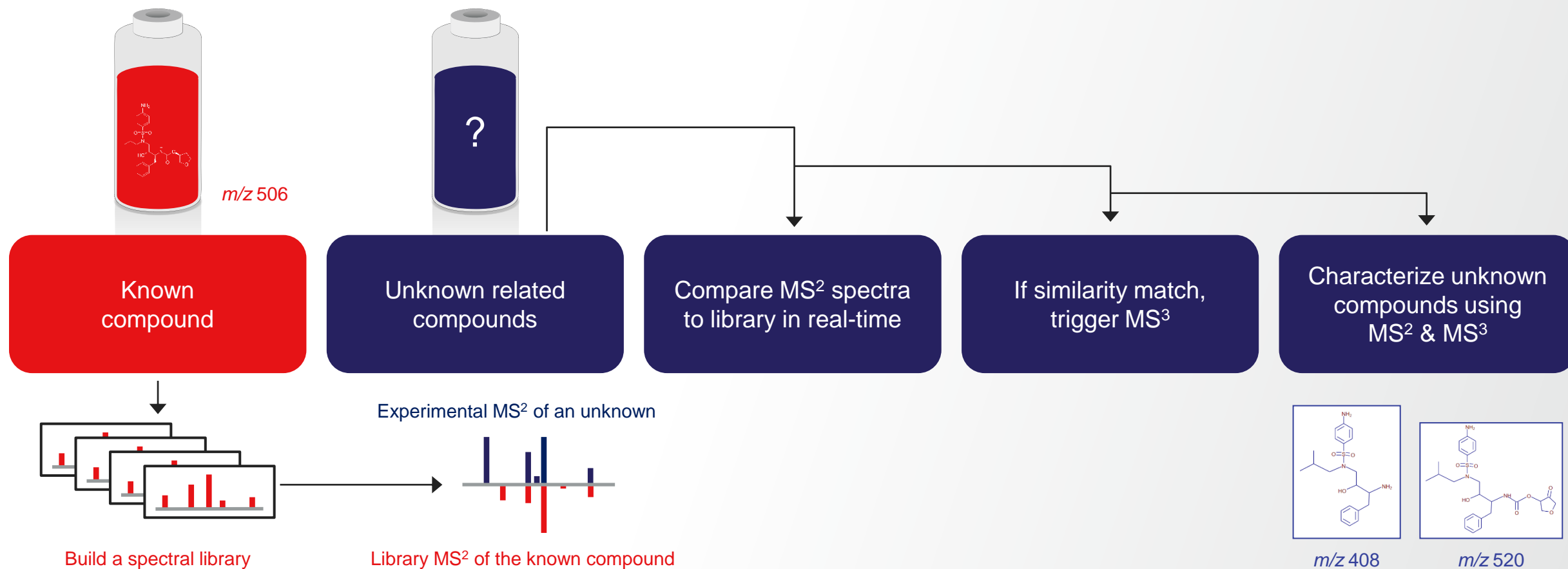


Source: Data courtesy Graeme McAllister

MET-IQ: Intelligent mass spectrometry for small molecules

Challenge: Identification of unknown metabolites, degradants, or transformation products

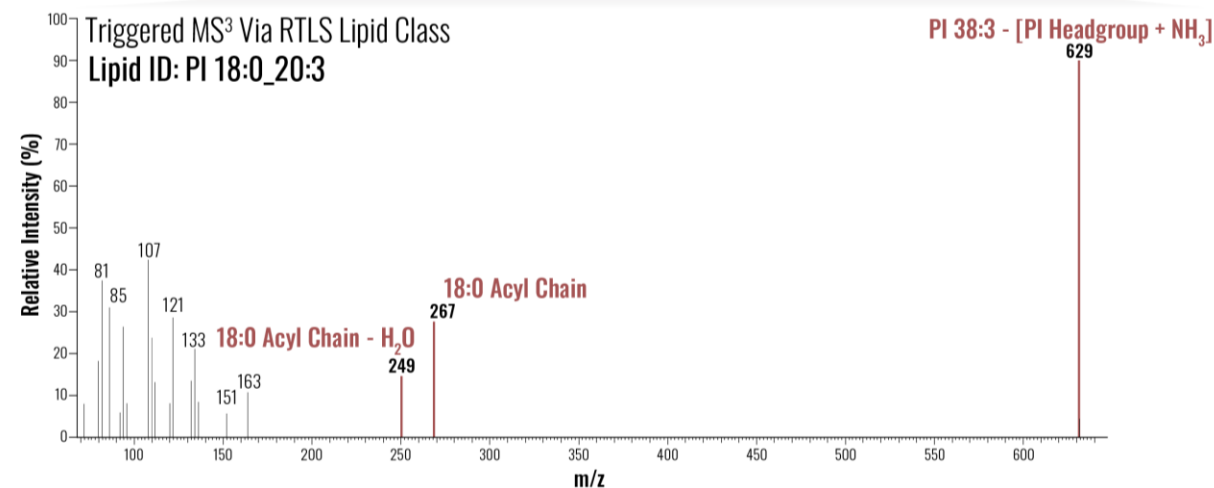
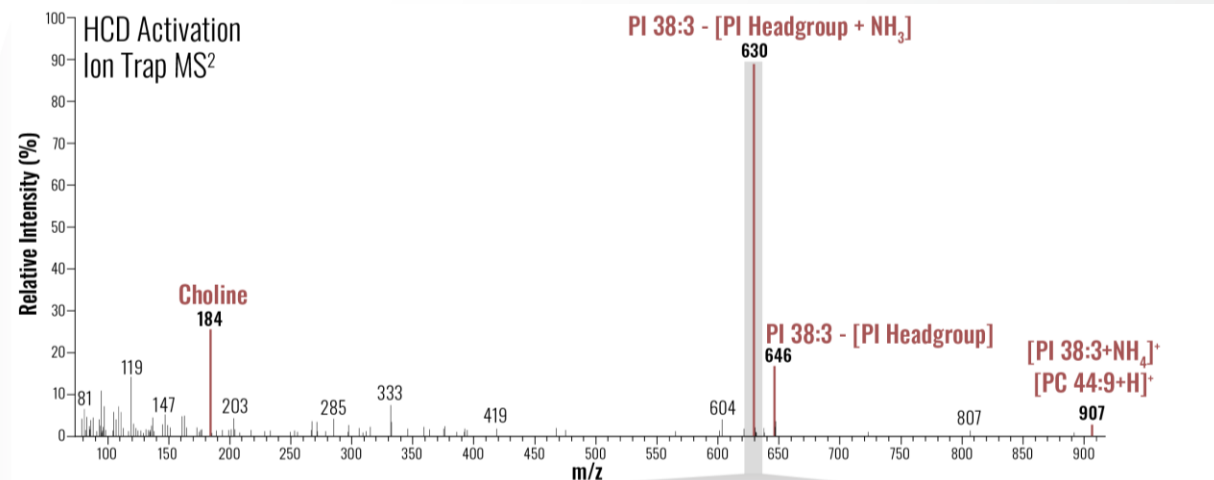
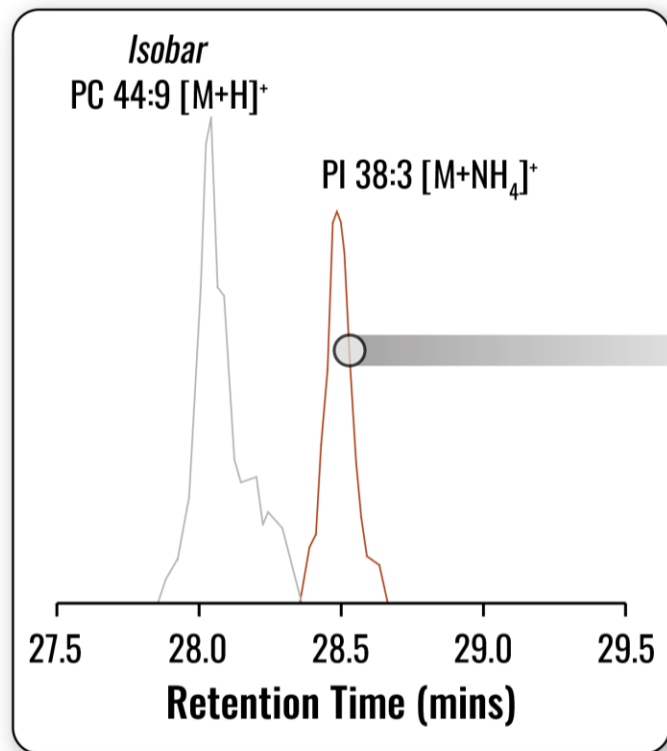
Solution: Intelligent MS³ data acquisition enables annotation and characterization of unknown compounds



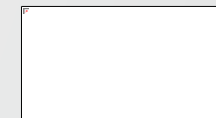
Real-Time Library Search triggered MS³ reveals Phosphatidylinositol (PI) Acyl chain composition

Extracted Ion Chromatogram

m/z: 906.5985 - 906.6075



Data courtesy of Dr. Joshua Coon,
University of Wisconsin-Madison



Introducing the

Thermo Scientific™ Orbitrap™ Astral™

MASS SPECTROMETER



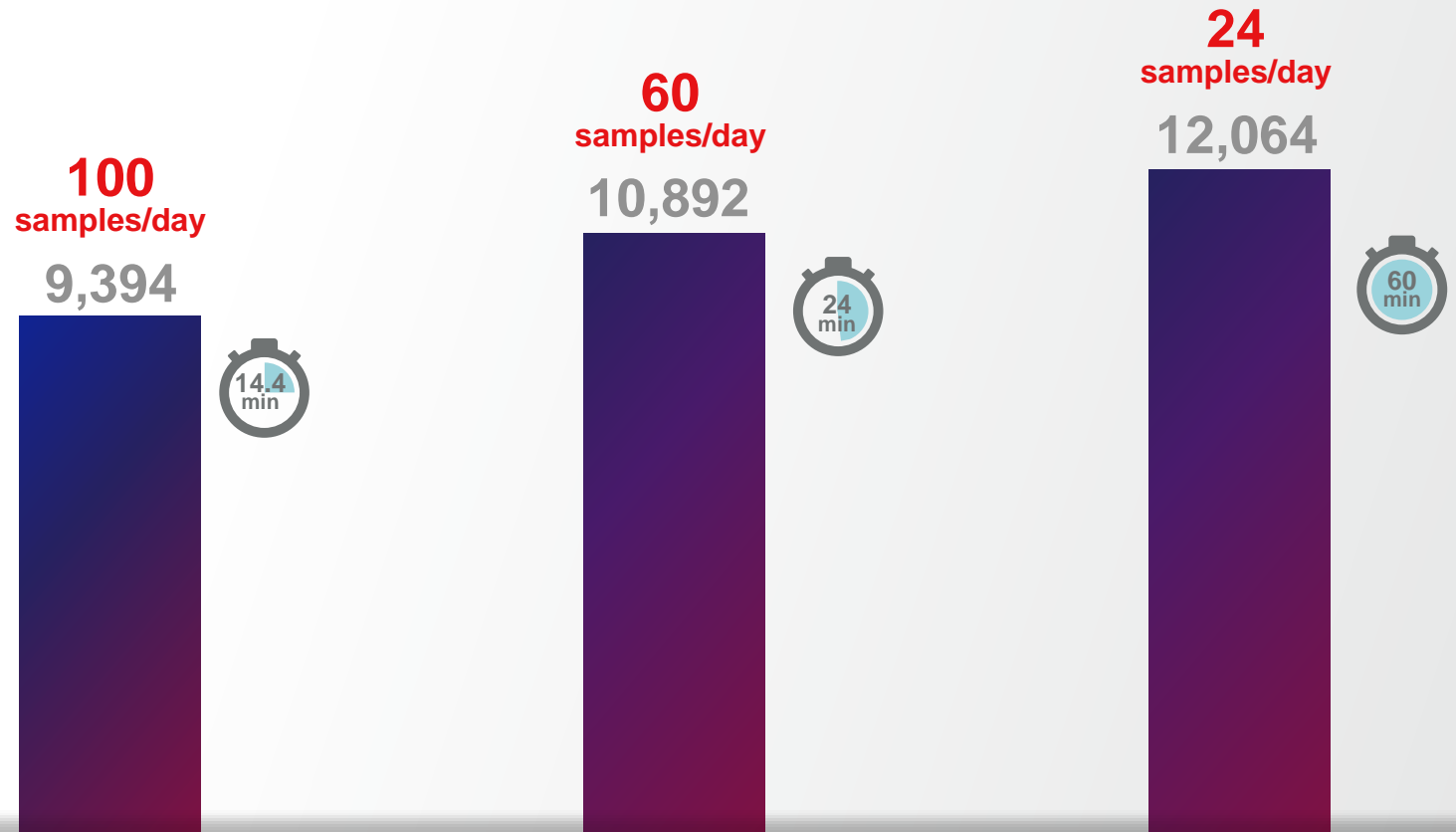
**Faster
throughput**

**Deeper
coverage**

**Higher
sensitivity**

Accurate and precise quantitation

Incredible flexibility
to deliver high
coverage at
high throughput
or unprecedented
depth in 1 hour



Protein groups

See more in less time

or

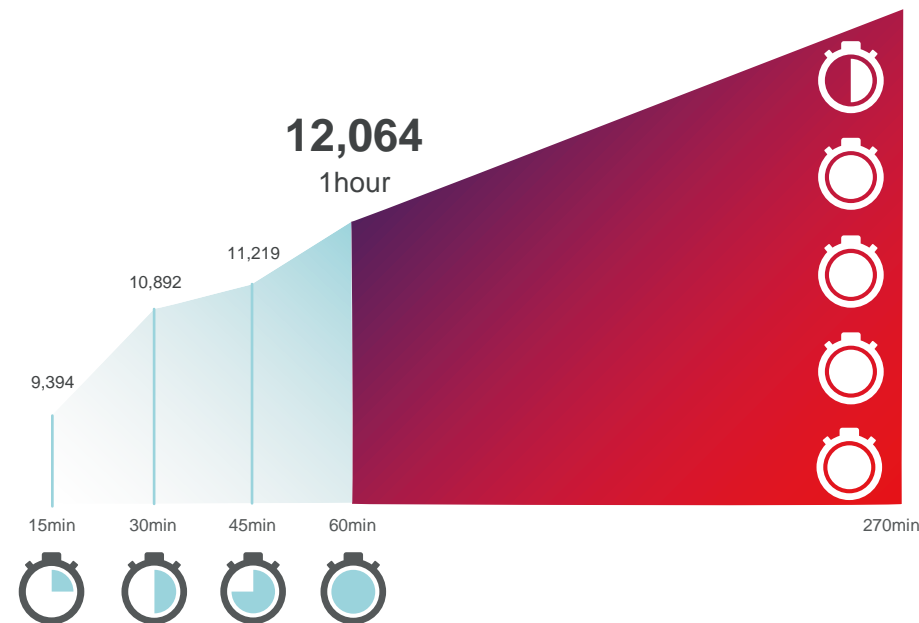
Spend more time

and go even deeper **15,147**

In 4.5 hours

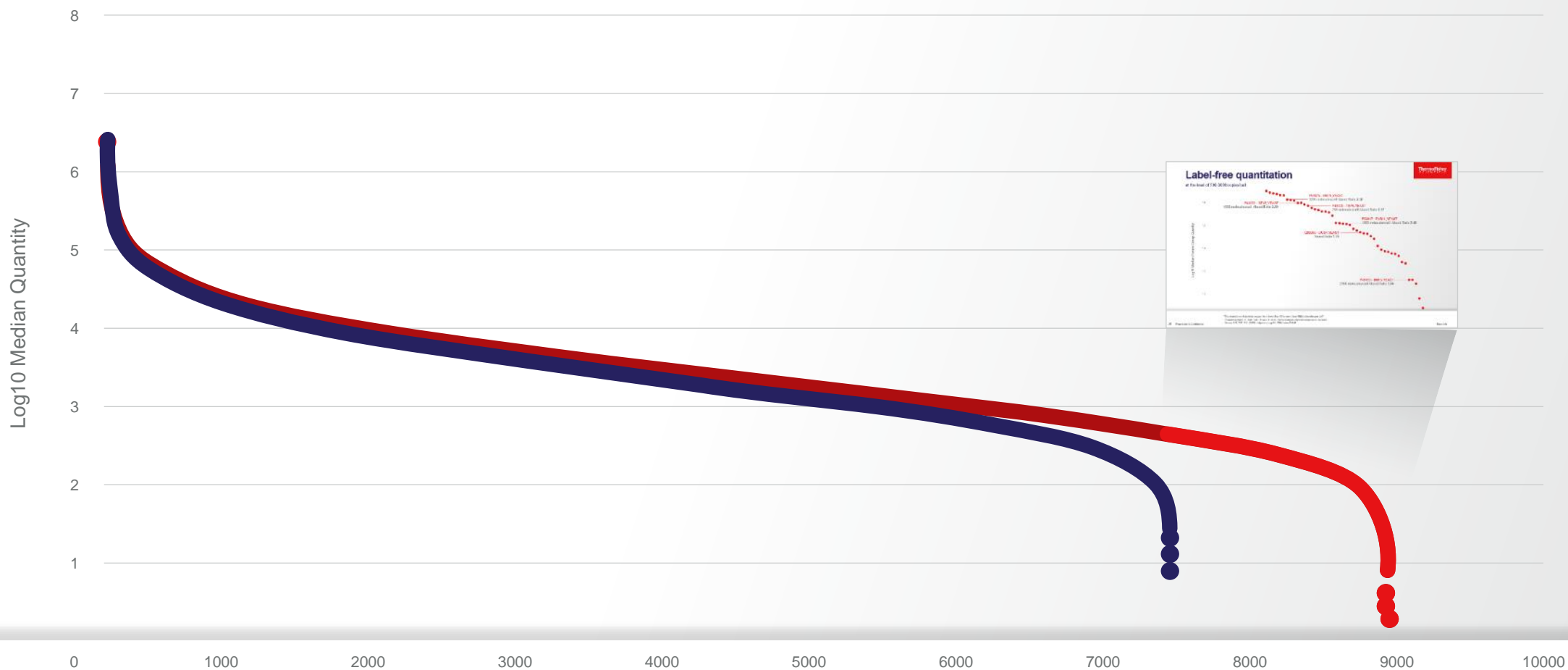
Now identify over

15,000
proteins



Discover more biomarkers with a larger dynamic range

Comparison of LFQ DIA performance between state-of-the-art Orbitrap MS and Orbitrap Astral MS

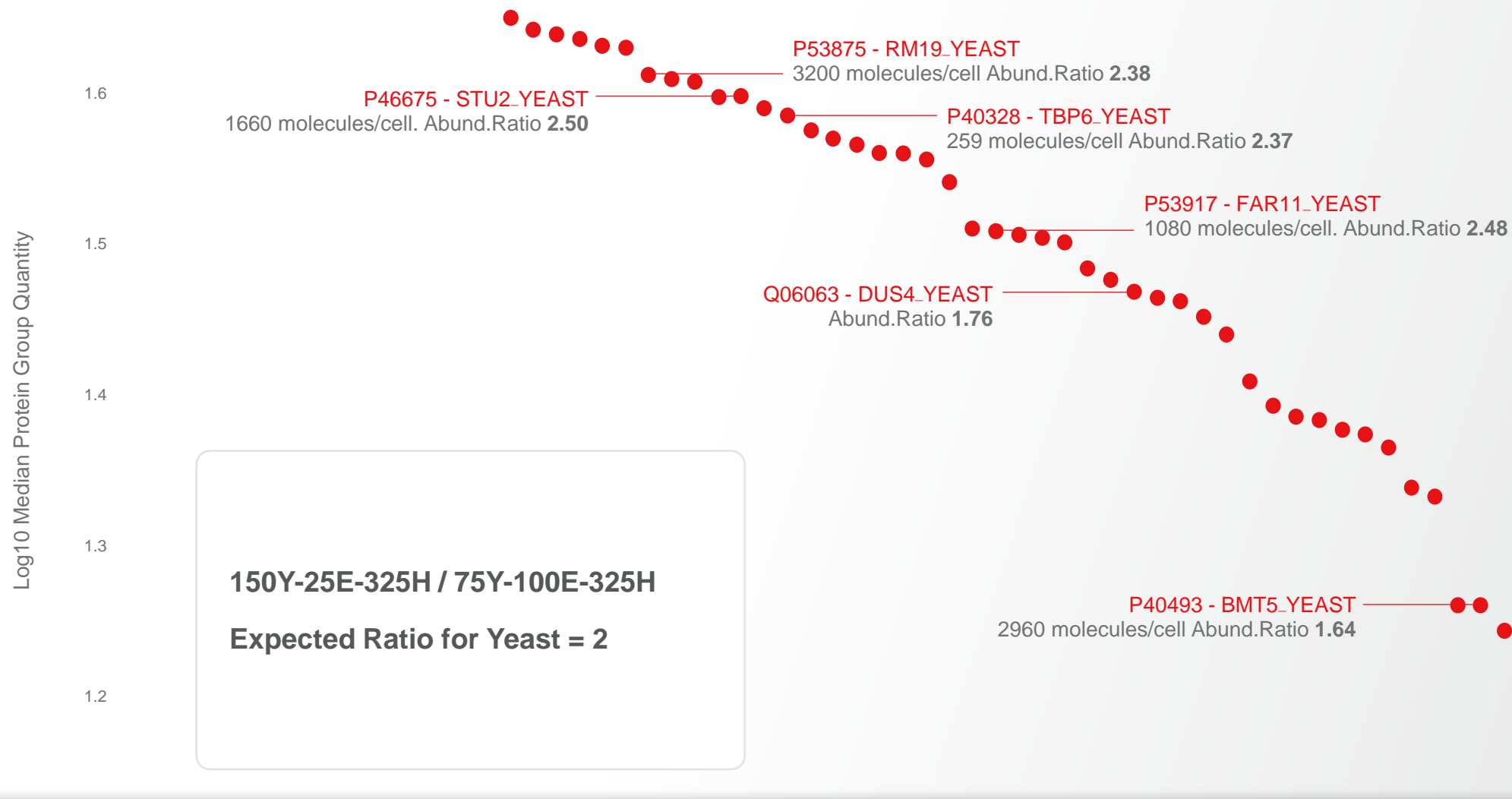


Ranked order of identified
protein groups

- State-of-the-art Orbitrap MS
- Orbitrap Astral MS

High sensitivity label-free quantitation

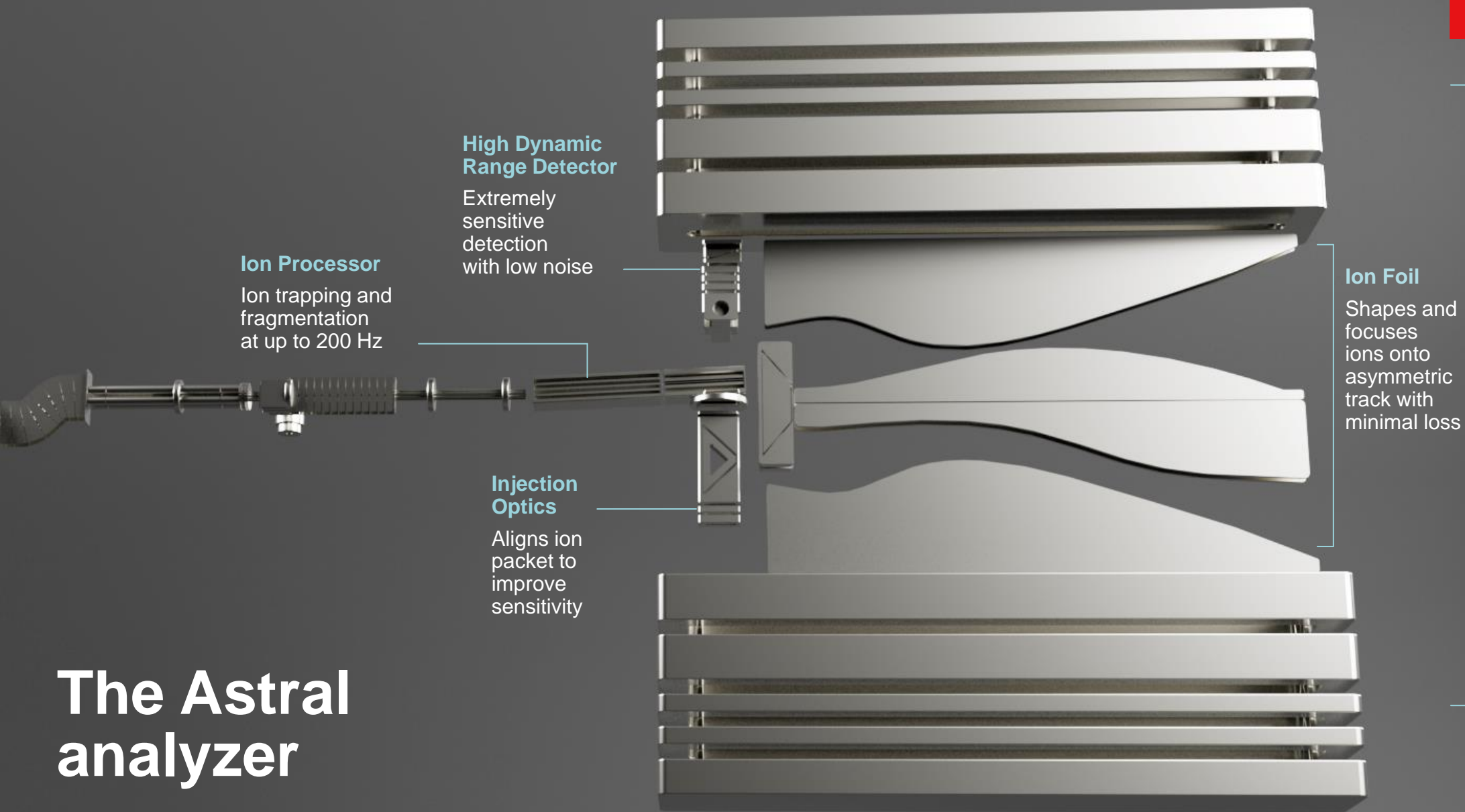
Accurate and precise quantitation at the level of 200-3000 copies/cell



“The abundance of proteins ranges from fewer than 50 to more than 10(6) molecules per cell”

Ghaemmaghami, S., Huh, WK., Bower, K. et al. Global analysis of protein expression in yeast. Nature 425, 737–741 (2003). <https://doi.org/10.1038/nature02046>

• Orbitrap Astral MS



Ion Processor

Ion trapping and fragmentation at up to 200 Hz

High Dynamic Range Detector

Extremely sensitive detection with low noise

Injection Optics

Aligns ion packet to improve sensitivity

Ion Foil

Shapes and focuses ions onto asymmetric track with minimal loss

Asymmetric Ion Mirrors

Elongated ion track for up to 80,000 resolution

The Astral analyzer

Q Exactive™ UHMR Instrumentation for native MS

1. Ultra-High Mass Range,
 m/z 350-80,000
2. High Mass Quadrupole
Isolation up to m/z 25,000
3. Desolvation and Dissociation
In-source trapping or CID, and HCD
4. Direct Mass Technology
Enabling Charge Detection MS⁰



Thermo Scientific™ Q Exactive™ UHMR Hybrid
Quadrupole-Orbitrap™ Mass Spectrometer

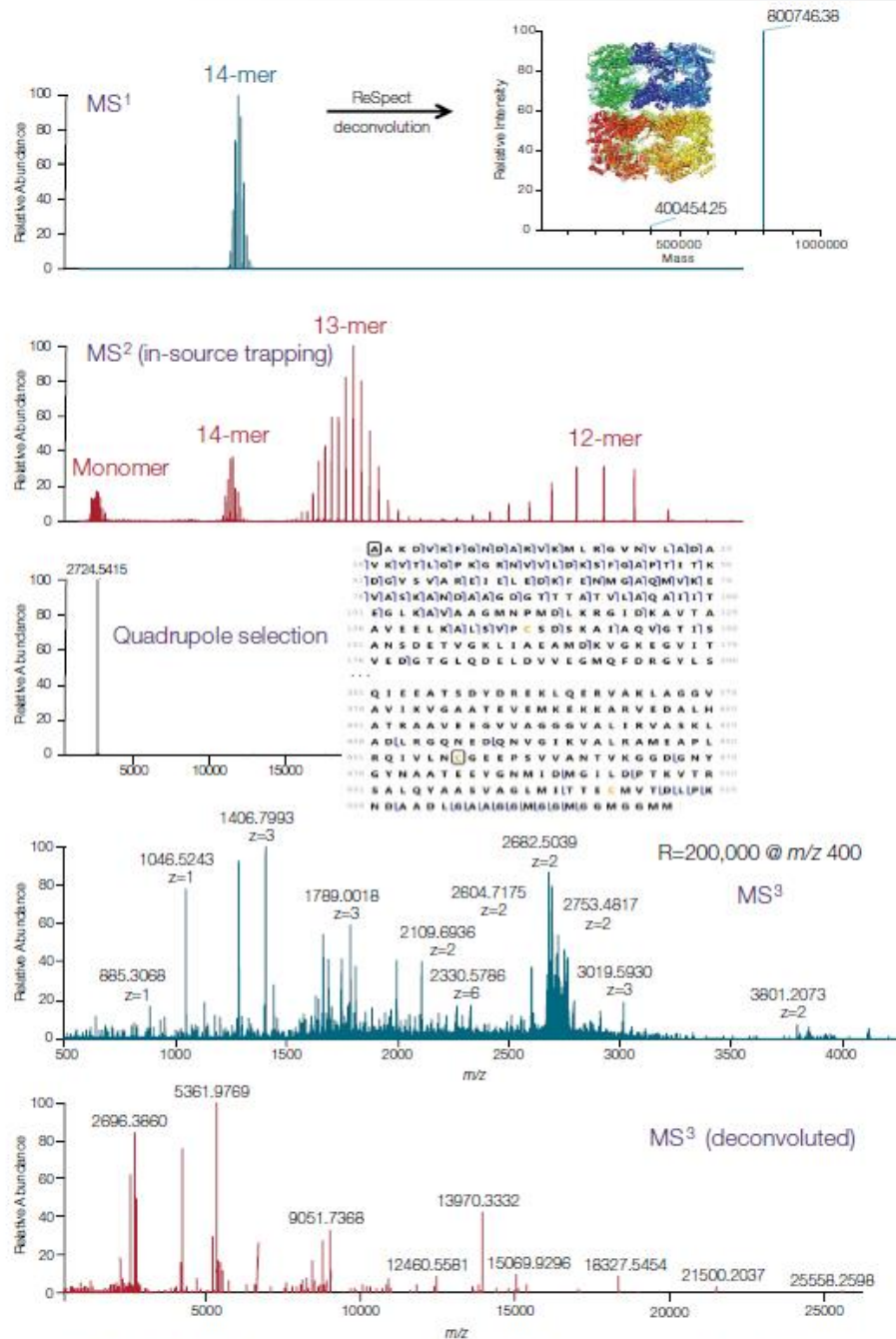


Figure 2. The native MS and native top-down analysis of the GroEL protein complex enabled identification of a total of 112 b and y ions, representing 21% of the residue cleavages.

Learn more at www.thermofisher.com/massspec

Contact Information

For HPLC, GC, IC and LCMS products

Navette Shirakawa

navette.shirakawa@thermofisher.com

Thank you

