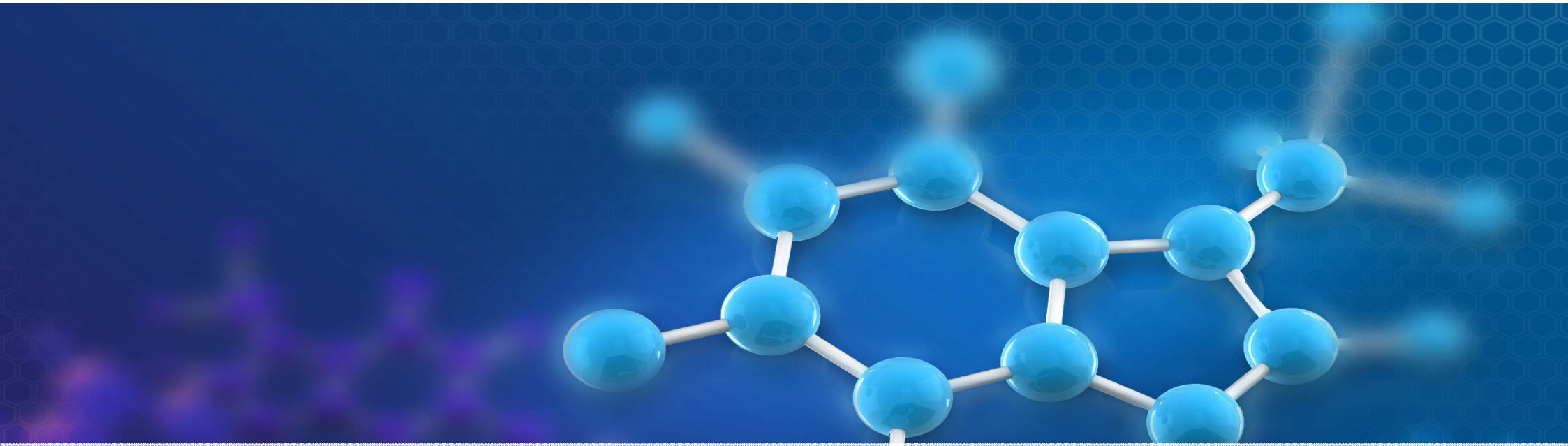
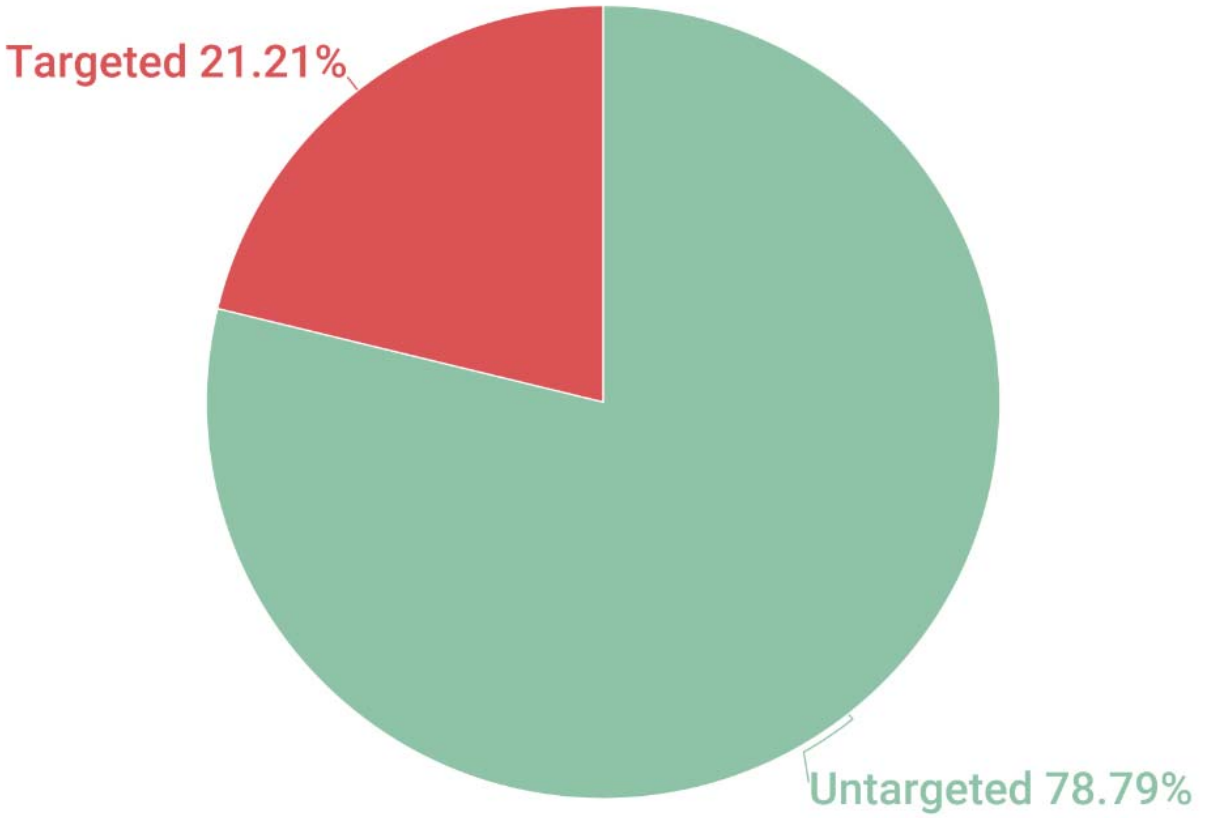


ThermoFisher SCIENTIFIC

Metabolomica e Lipidomica Untarget,

The world leader in serving science

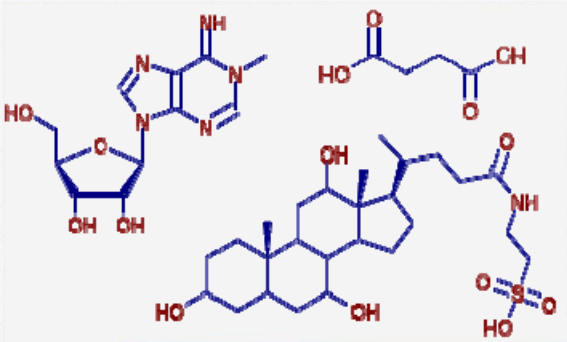
Untargeted Metabolomics Takes the Lead



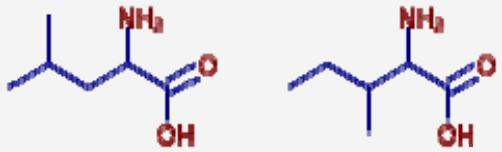
Thermo Scientific™ MS instruments included: *Thermo Scientific™ Q Exactive™ MS, Thermo Scientific™ Q Exactive™ Plus MS, Thermo Scientific™ Q Exactive™ HF MS, Thermo Scientific™ Orbitrap ™ Fusion Tribrid MS, Thermo Scientific™ Orbitrap™ Fusion Tribrid MS, Thermo Scientific™ TSQ Quantiva™ Quadrupole MS*

ThermoFisher SCIENTIFIC

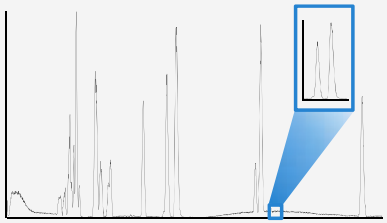
Untargeted Metabolomics: Challenges



Structural and physical diversity



Isomeric / isobaric species



Dynamic range

Large sample sets

ThermoFisher SCIENTIFIC

High Quality Data for High Quality Results

- Complex matrix
- Differentiate similar masses
- Fine isotopic pattern

High Resolution

- Identification of unknowns
- Narrow mass tolerance
- Mass stability from peak to peak and run to run

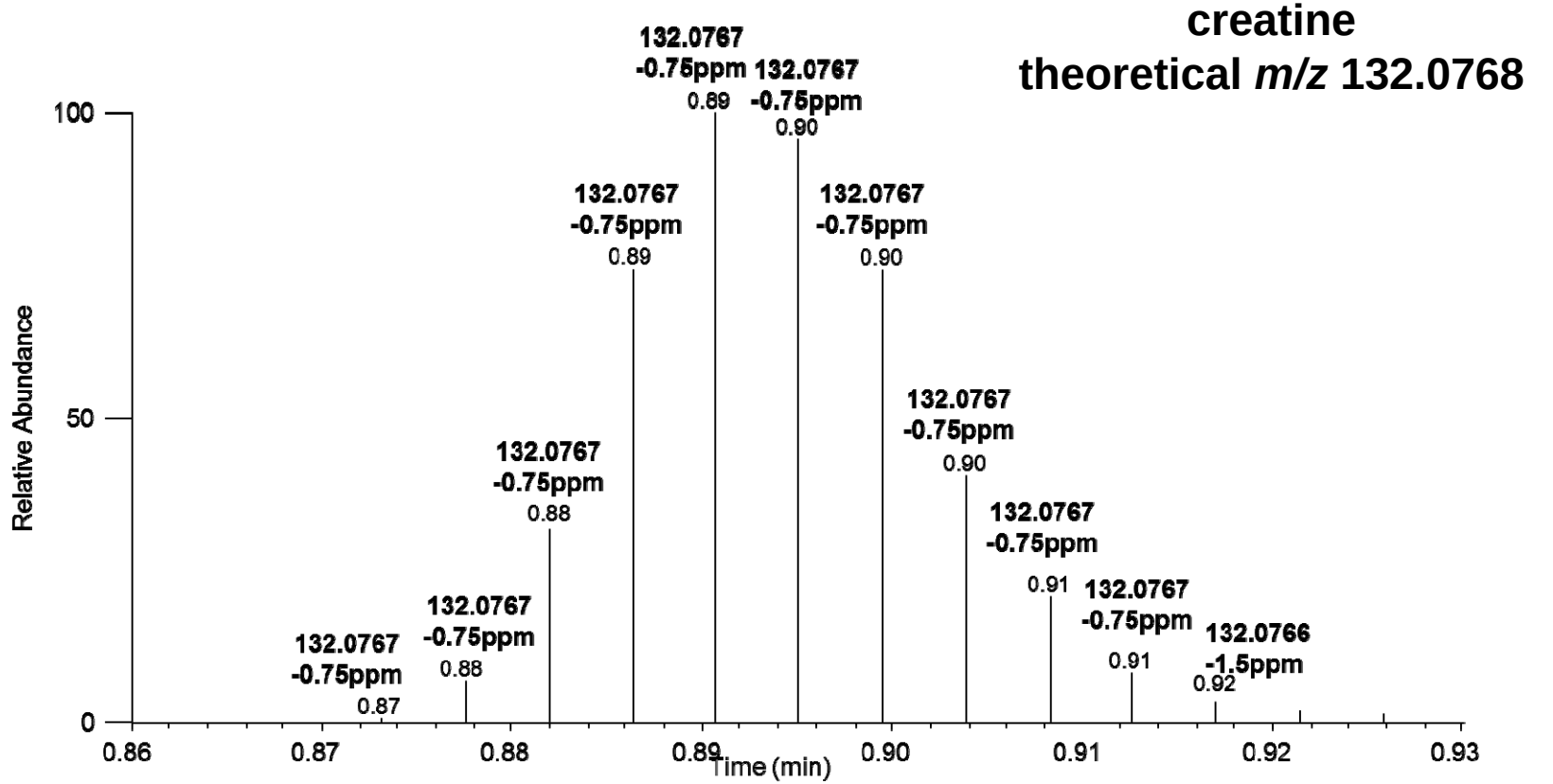
Mass Accuracy

- Scan-to-scan consistency
- Injection-to-injection reproducibility
- Robustness over extended time periods

Instrument Performance

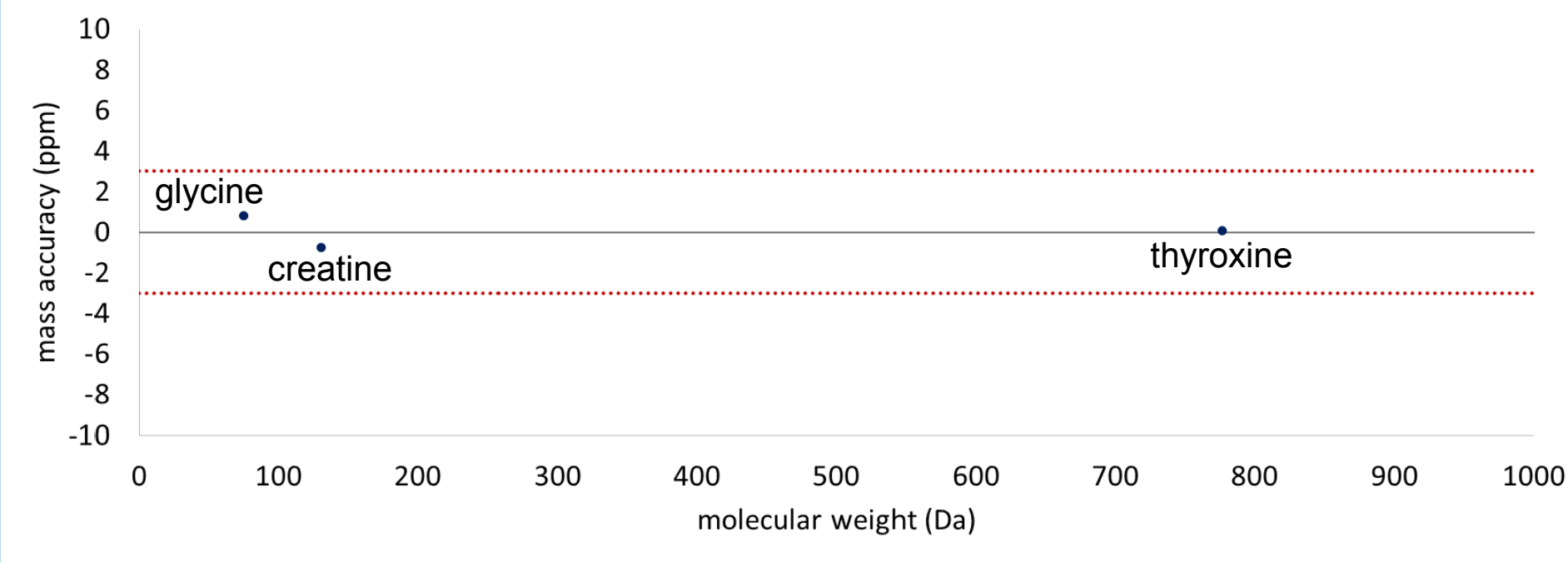
Stable Mass Accuracy from Scan to Scan Across the Peak

Orbitrap MS provides confidence in peak detection



ThermoFisher SCIENTIFIC

Excellent Mass Accuracy Across the Molecular Weight Range

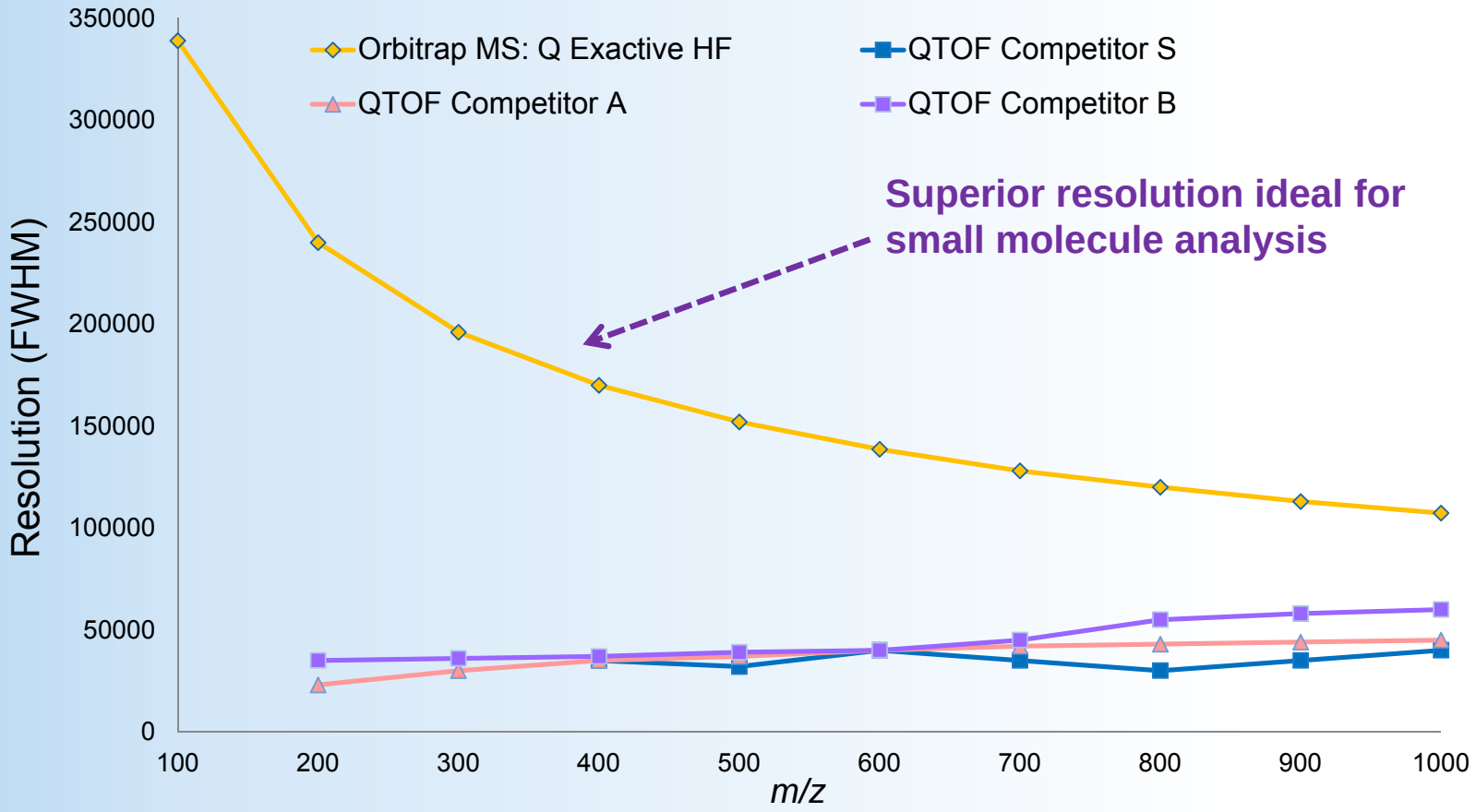


Metabolites identified from human plasma and verified against an authentic standard

Orbitrap MS delivers accurate mass measurements for all metabolites

ThermoFisher SCIENTIFIC

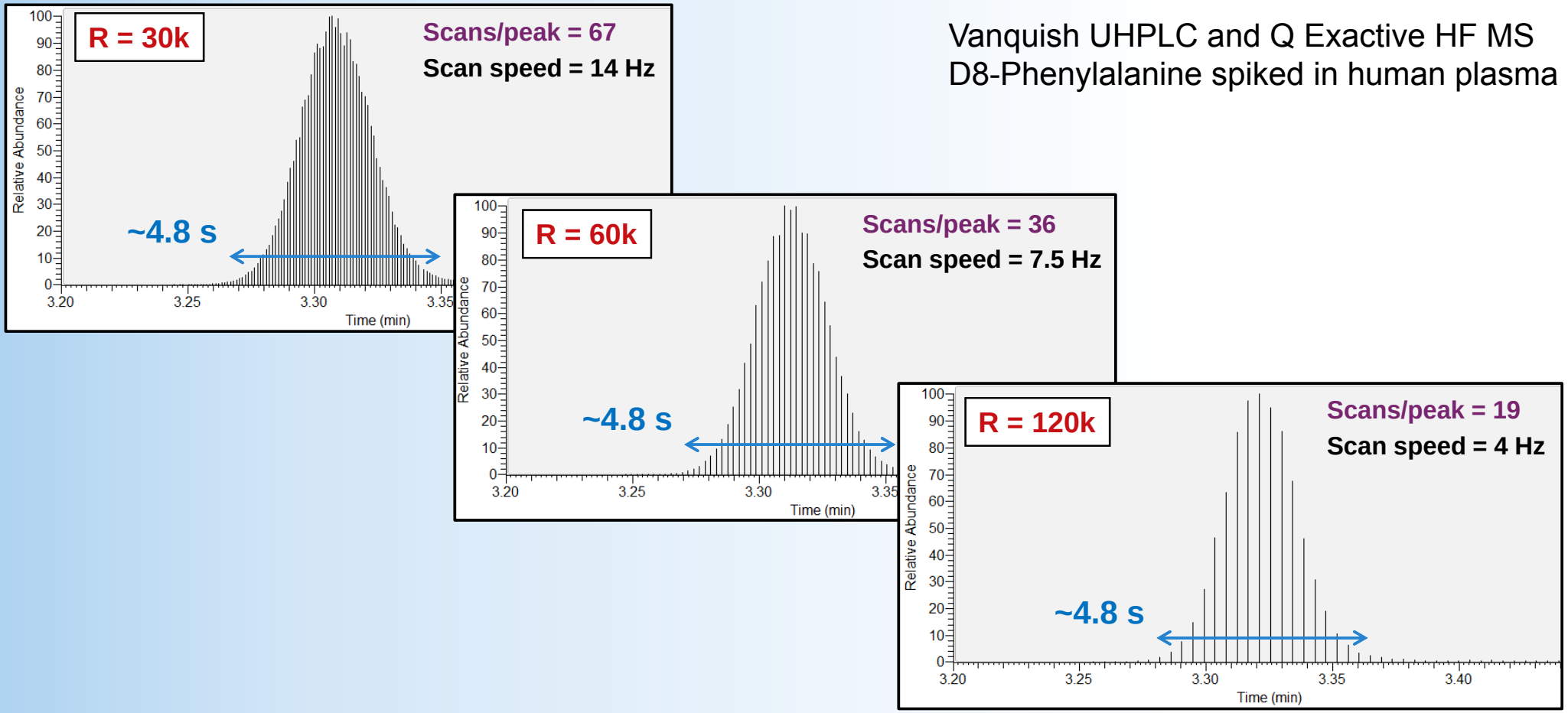
Orbitrap MS: Unmatched Resolution



High Orbitrap MS resolving power for correct identification of isomeric and isobaric species

ThermoFisher SCIENTIFIC

Mass Resolution and Scan Speed

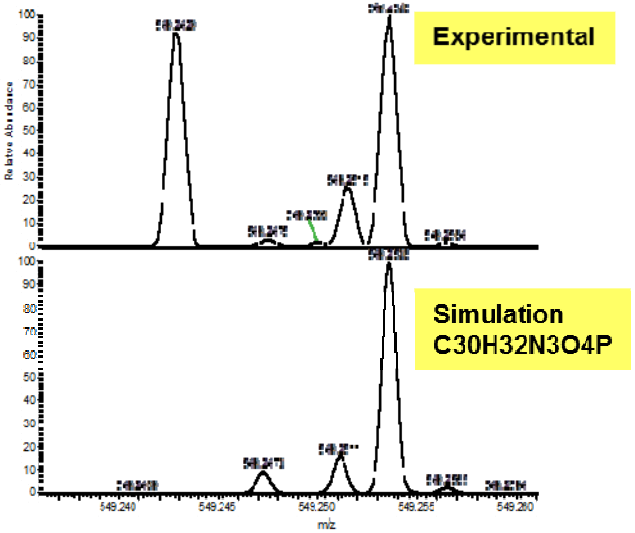


Vanquish UHPLC and Q Exactive HF MS
D8-Phenylalanine spiked in human plasma

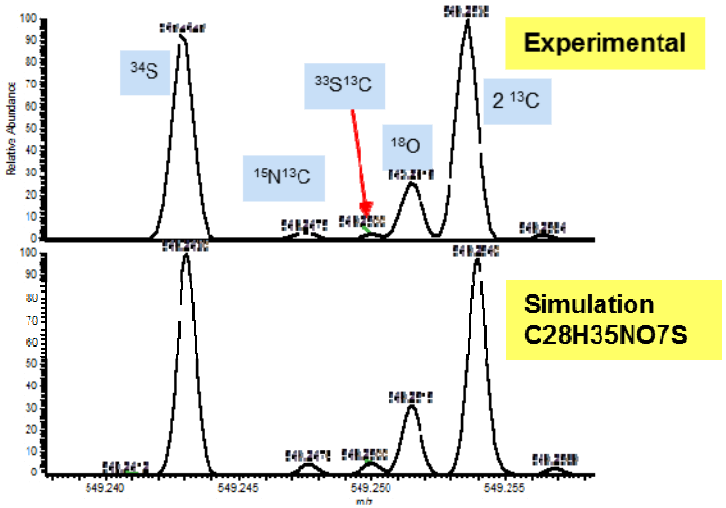
ThermoFisher SCIENTIFIC

Higher Confidence For Unknown Extractable & Leachable Analysis

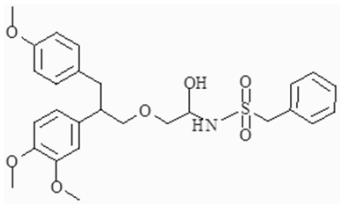
Predicted composition does not agree with the observed fine isotope structure



Predicted composition matches the observed fine isotope structure



Proposed Structure



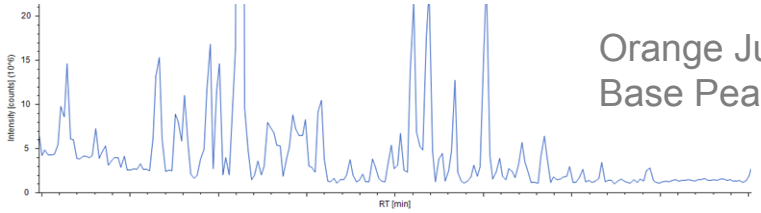
Experiment vs. Theory for M+2

- Fine isotope structure data was compared with simulated spectrum for the predicted elemental compositions
- Only one out of the 6 predicted elemental compositions for M0 also matched M+2 fine isotope structure with the data
- The selected composition along with MS/MS fragment analysis allowed structure elucidation for this unknown extractable.

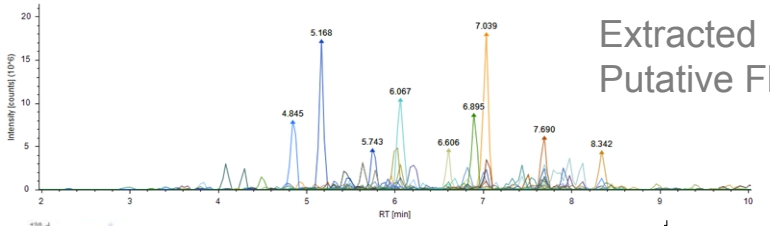
ThP 362, Comstock et al.

ThermoFisher SCIENTIFIC

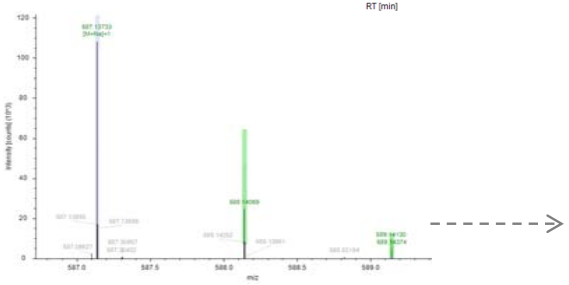
Selective Component Identification In Complex Matrices

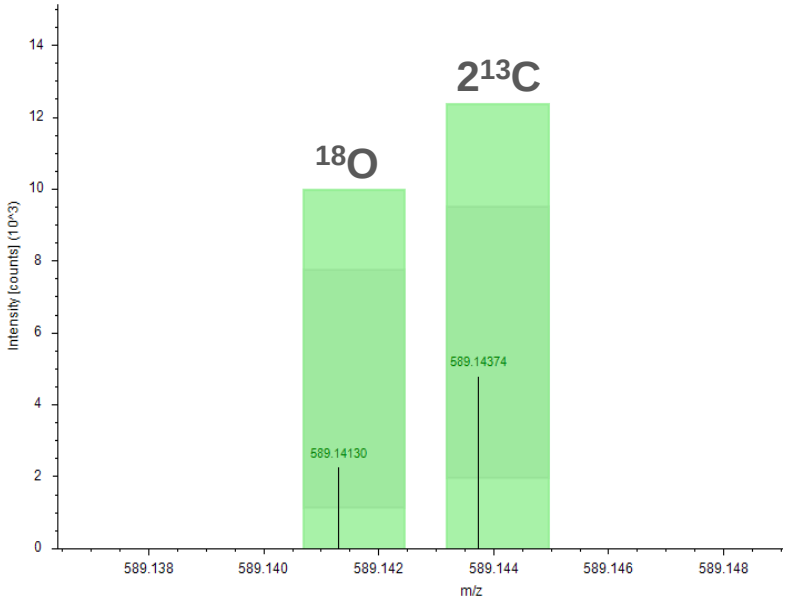


Orange Juice Matrix
Base Peak Chromatogram



Extracted Ion Chromatogram for
Putative Flavonoid Conjugates





WP 457, T. Stratton

LC-MS Analysis Of Flavonoid Conjugates In Orange Juice

- The fine isotope structure obtained was utilized as a fingerprint for selecting compounds having C/O ratio between 1.5 and 3.
- The detected peak lists were exported as an inclusion list for subsequent MS^n characterization.
- 128 putative flavonoid conjugates were detected in orange juice matrix containing over 4,000 compounds.

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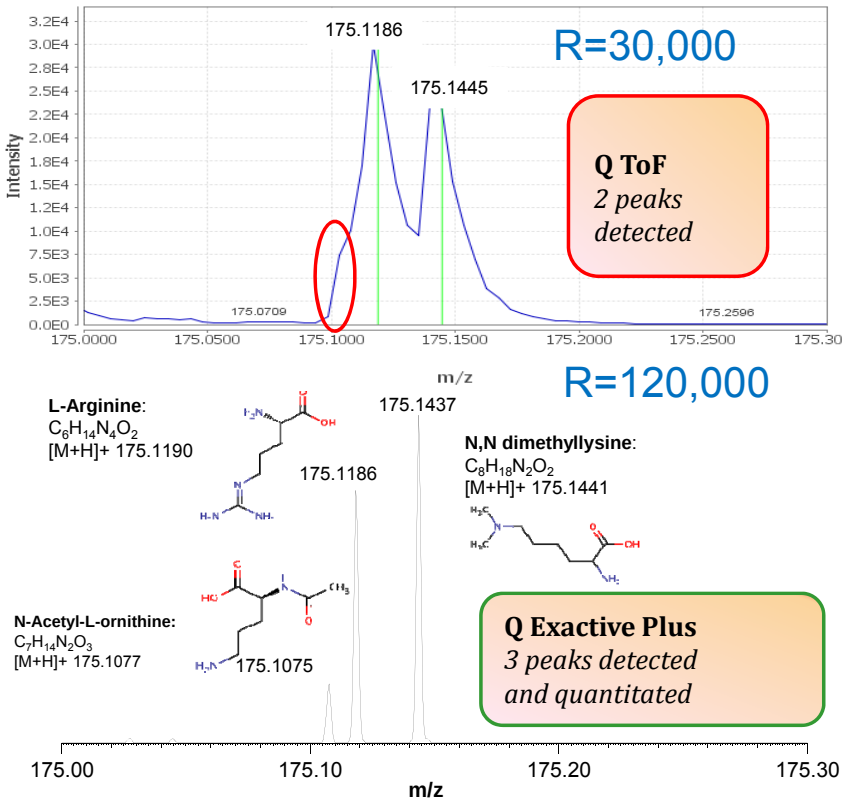
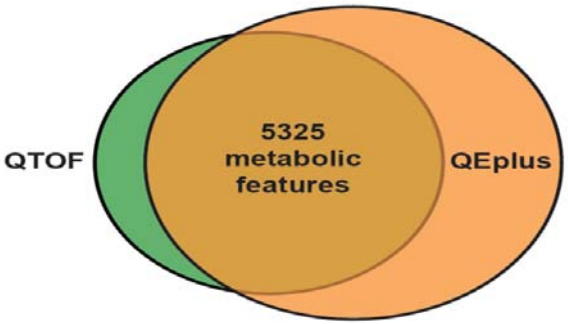
Why Orbitrap Is Naturally A Better Tool

Higher resolution can view more metabolic peaks



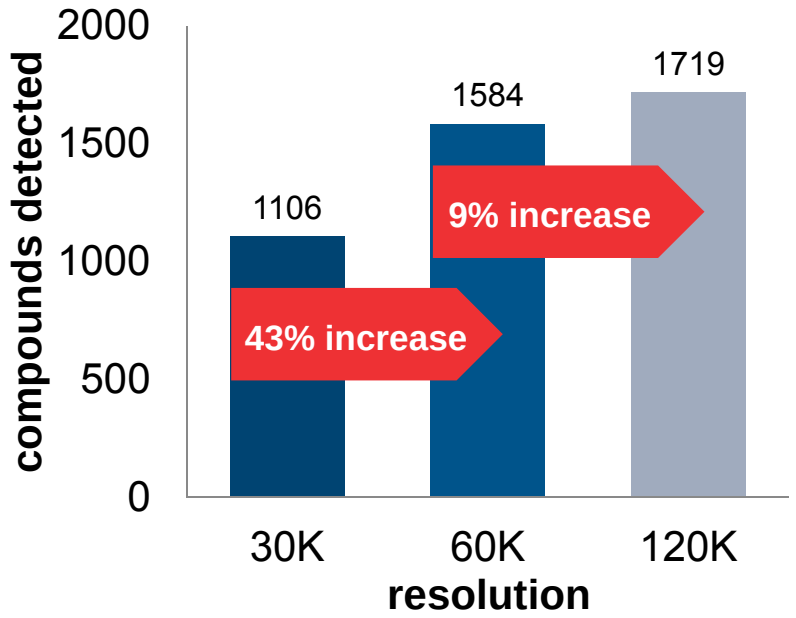


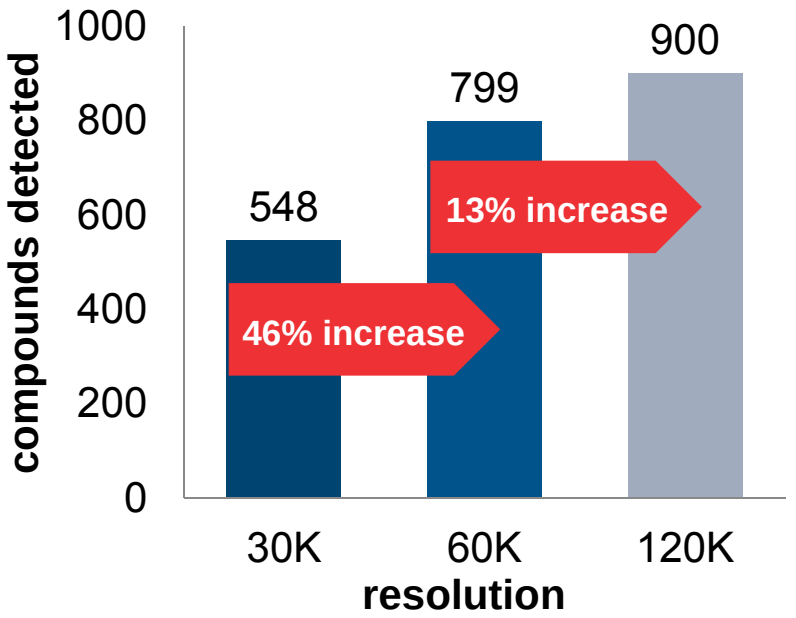
Q TOF: 6294 features

Q E Plus: 8960 features → >42% more

Data courtesy Stanford University

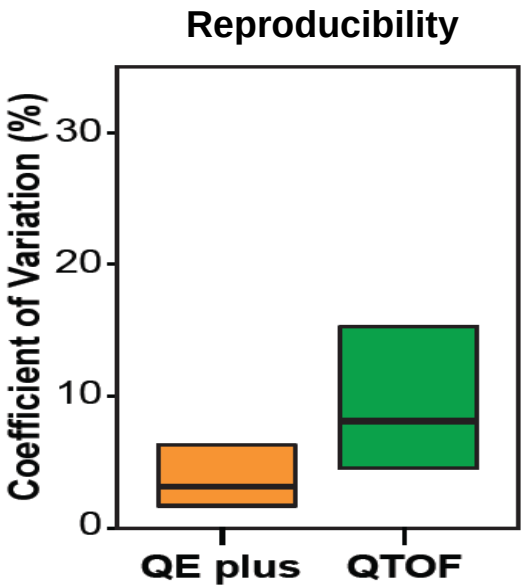
High Resolving Power Increases Metabolome Coverage

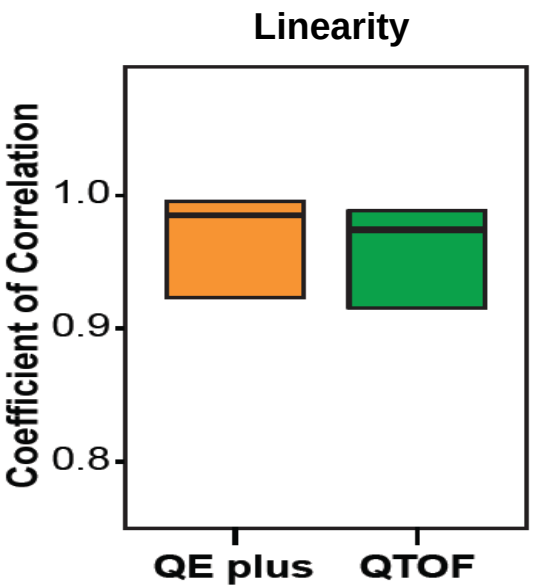


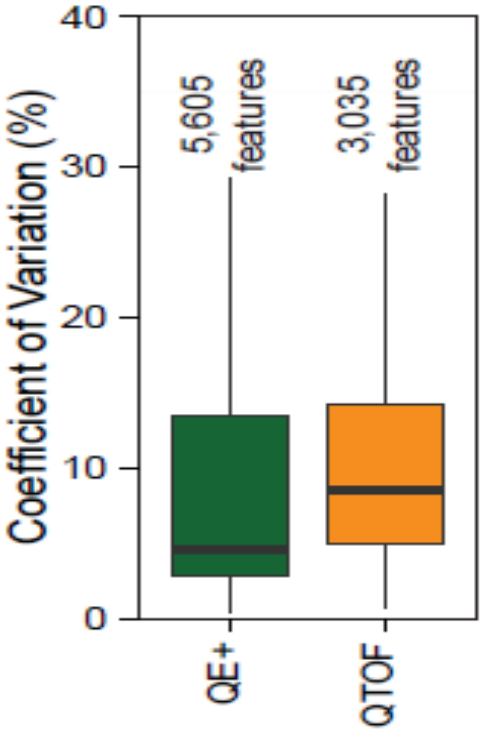


Why Orbitrap Is Naturally A Better Tool (5)

Great reliability and linearity is a must for quantitation

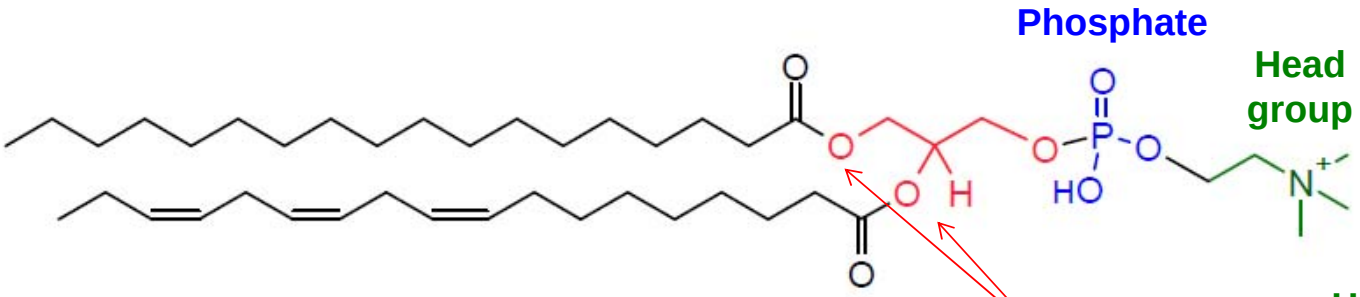






Data courtesy Stanford University

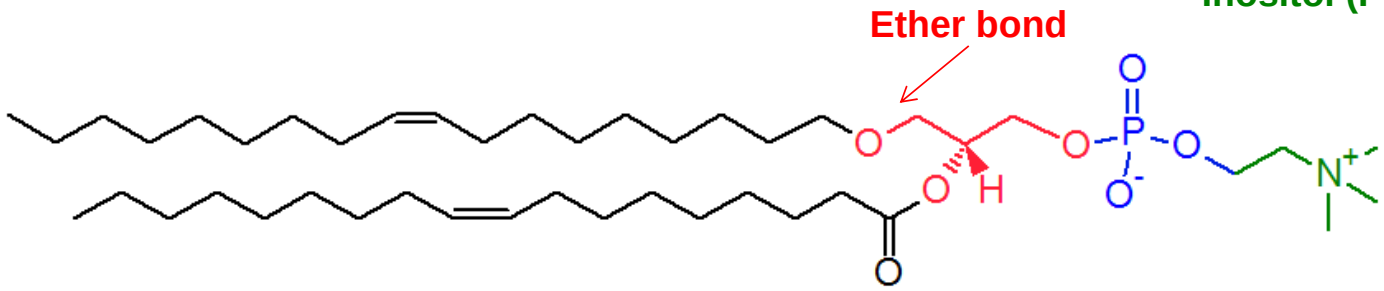
Lipid Nomenclature: Glycerophospholipids



Molecular species composition:
PC 18:0/18:3

Sum composition:
PC 36:3

Hydrogen (PA)
Choline (PC)
Ethanolamine (PE)
Serine (PS)
Glycerol (PG)
Inositol (PI)



Molecular species composition:
PC 18:1p/18:1e

Sum composition:
PC 36:2e

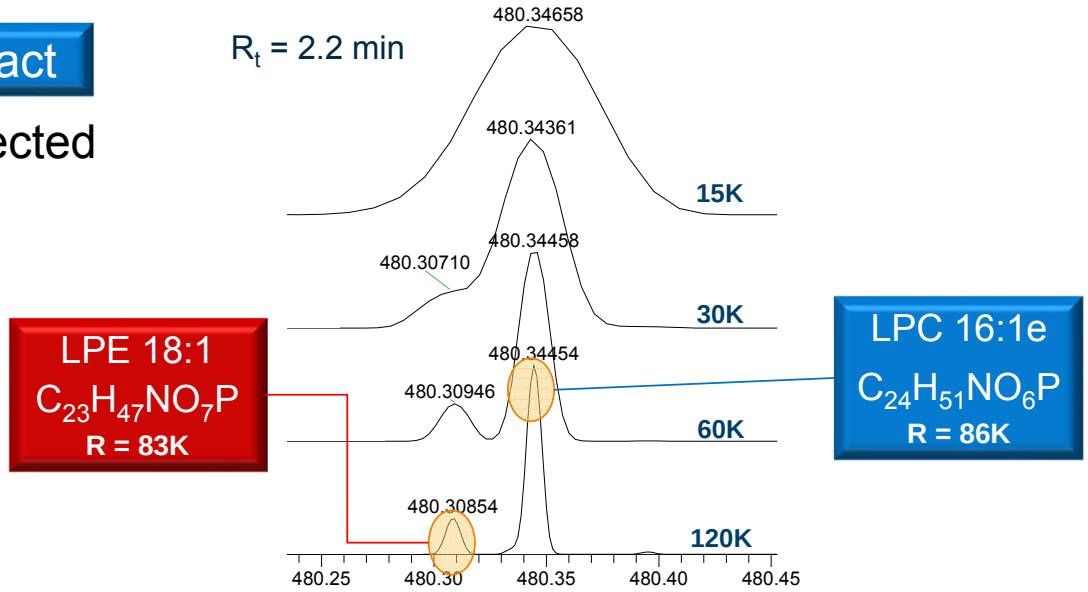
Resolving Isobaric Species Improves ID and Quan

Bovine Heart Extract

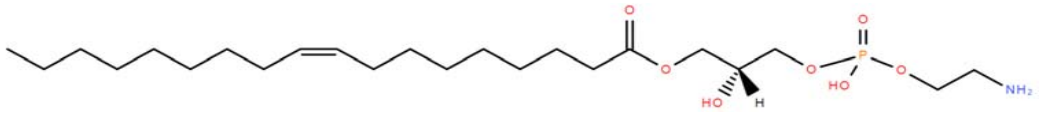
500ng/µL x 2µL injected

30min LC-MS run

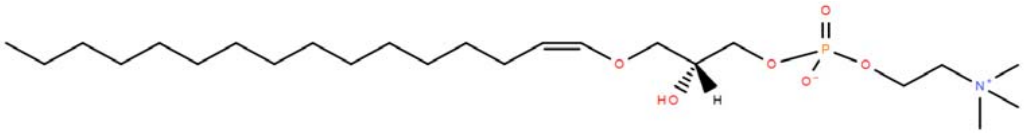
Q Exactive HF



Lyso PE 18:1



LPC 16:1e/16:0p



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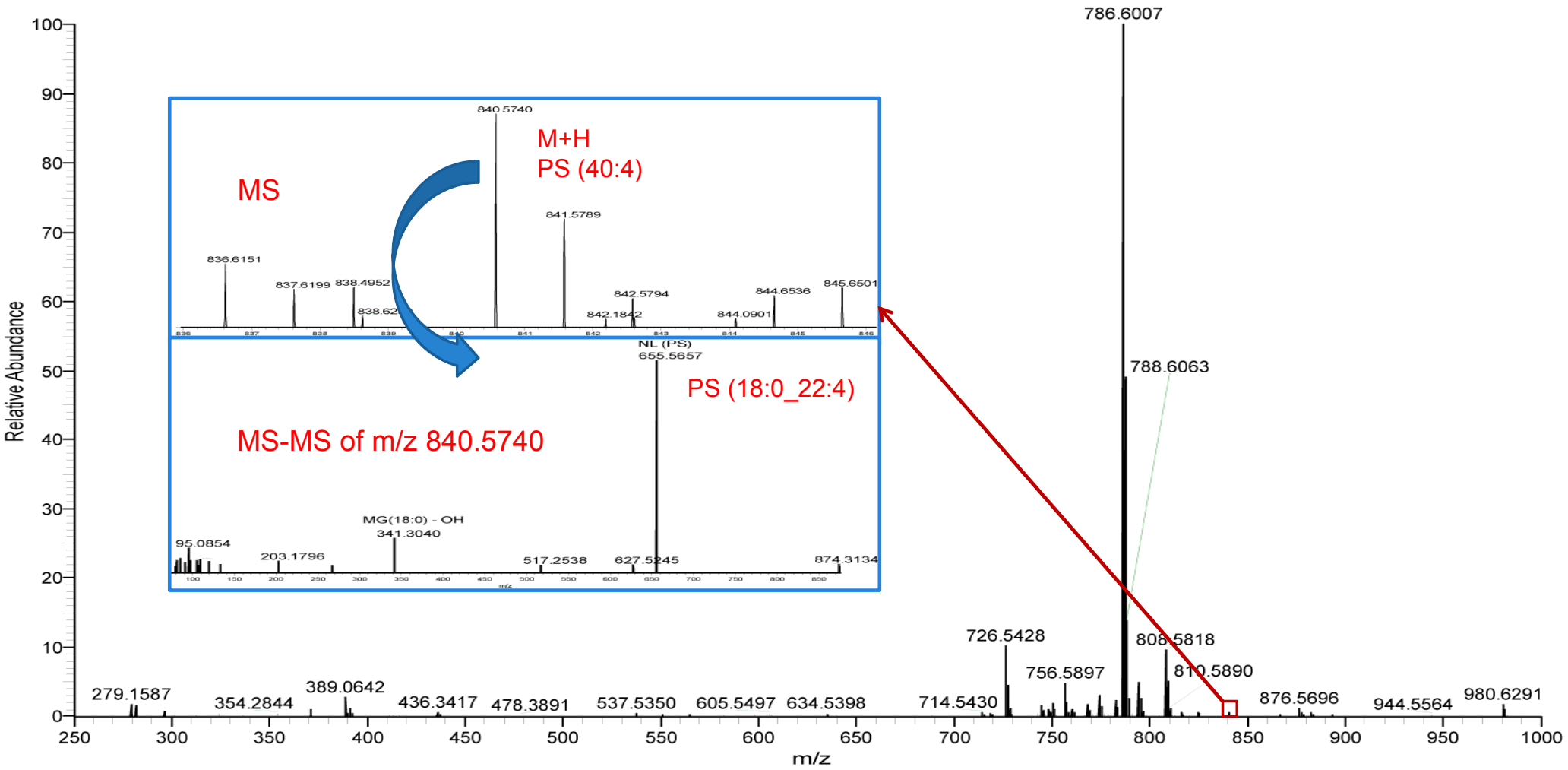
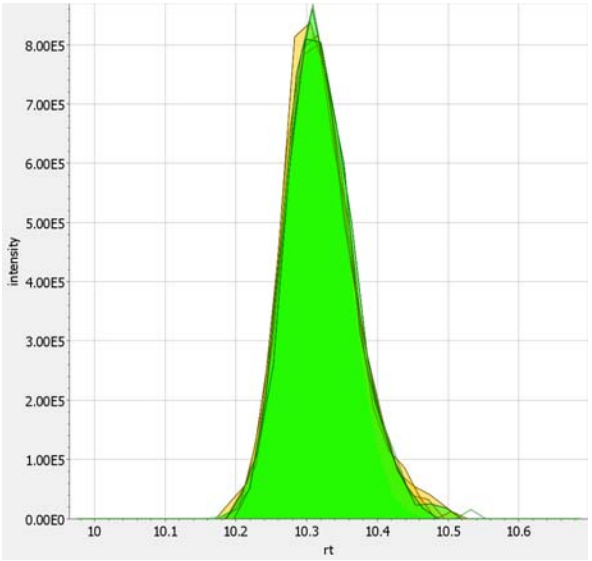
Confident Identification of Low Abundant PS Species



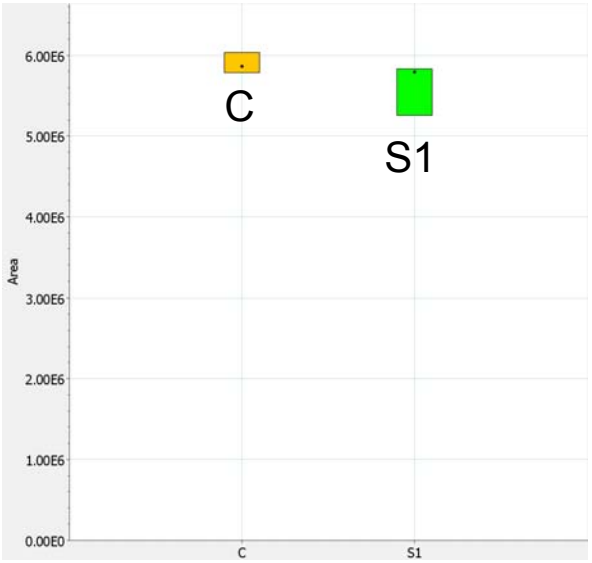
ThermoFisher SCIENTIFIC

LipidSearch Batch Data Processing and Quantitation

d_7-18:1/15:0 PE, 830 ng/mL



Control vs. Diabetic



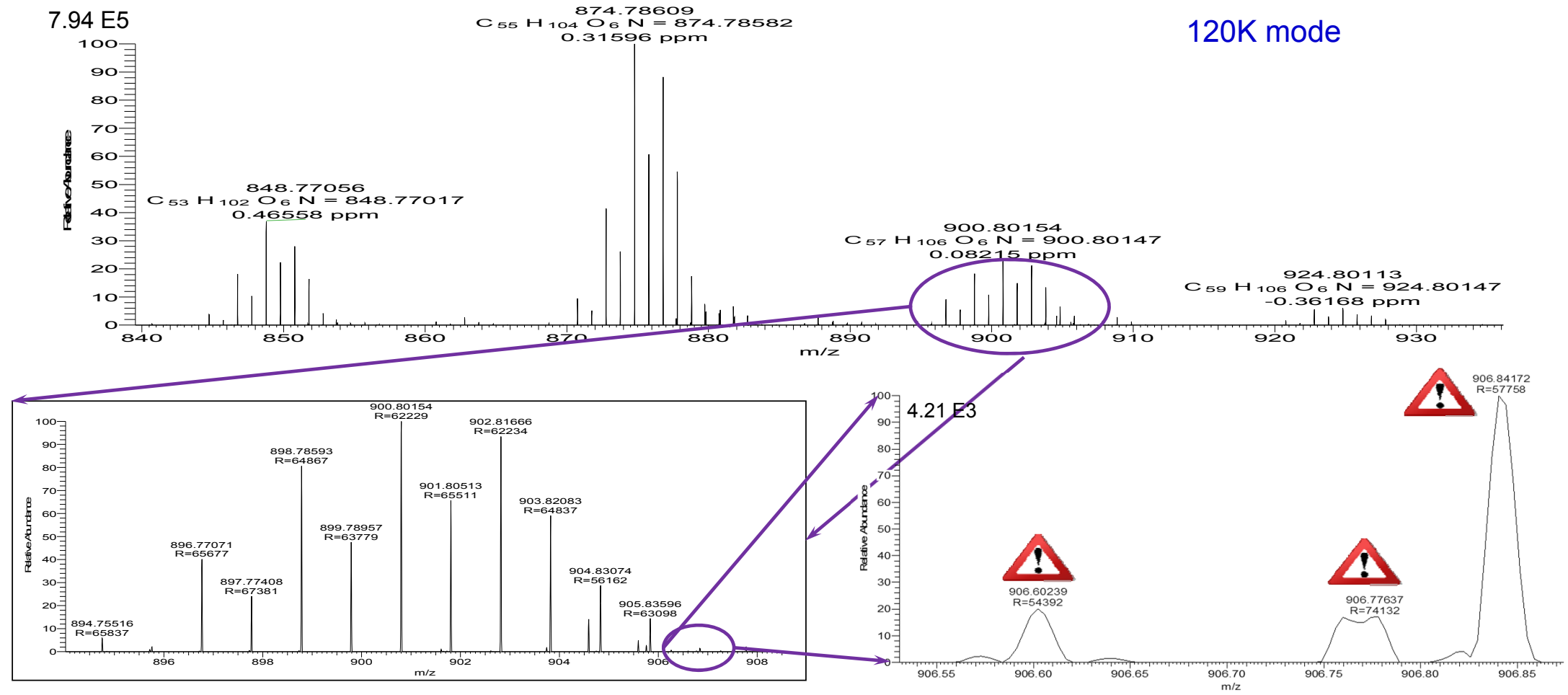
Lipid Molec key	Ion key	Grade	Polarity	BaseRt	Obs Mz	Delta(m/z)	Delta(ppm)	Ion Formula	Area	Area RSD
PE(18:1D7/15:0)	-H	A	N	10.3064	709.5532	0.0013	1.8211	C38 H66 O8 N1 P1 D7	5.629E06	5.753E0
PE(18:1D7/15:0)	-H	A	N	10.3064	709.5537	0.0018	2.5224	C38 H66 O8 N1 P1 D7	5.894E06	2.177E0
PE(18:1D7/15:0)	**+H**	**B**	**P**	**10.3064**	**711.5661**	**-0.0003**	**-0.4384**	**C38 H68 O8 N1 P1 D7**	**2.599E07**	**3.960E0**
PE(18:1D7/15:0)	**+H**	**C**	**P**	**10.3064**	**711.5655**	**-0.0010**	**-1.3456**	**C38 H68 O8 N1 P1 D7**	**2.429E07**	**4.482E0**

Processing Steps:

1. Search each data file
2. Merge the search results
   Pos. and Neg. ion
3. Report includes ID's
   a) Estimated Quan (IS) or
   b) Rel. Amounts (no IS)

ThermoFisher SCIENTIFIC

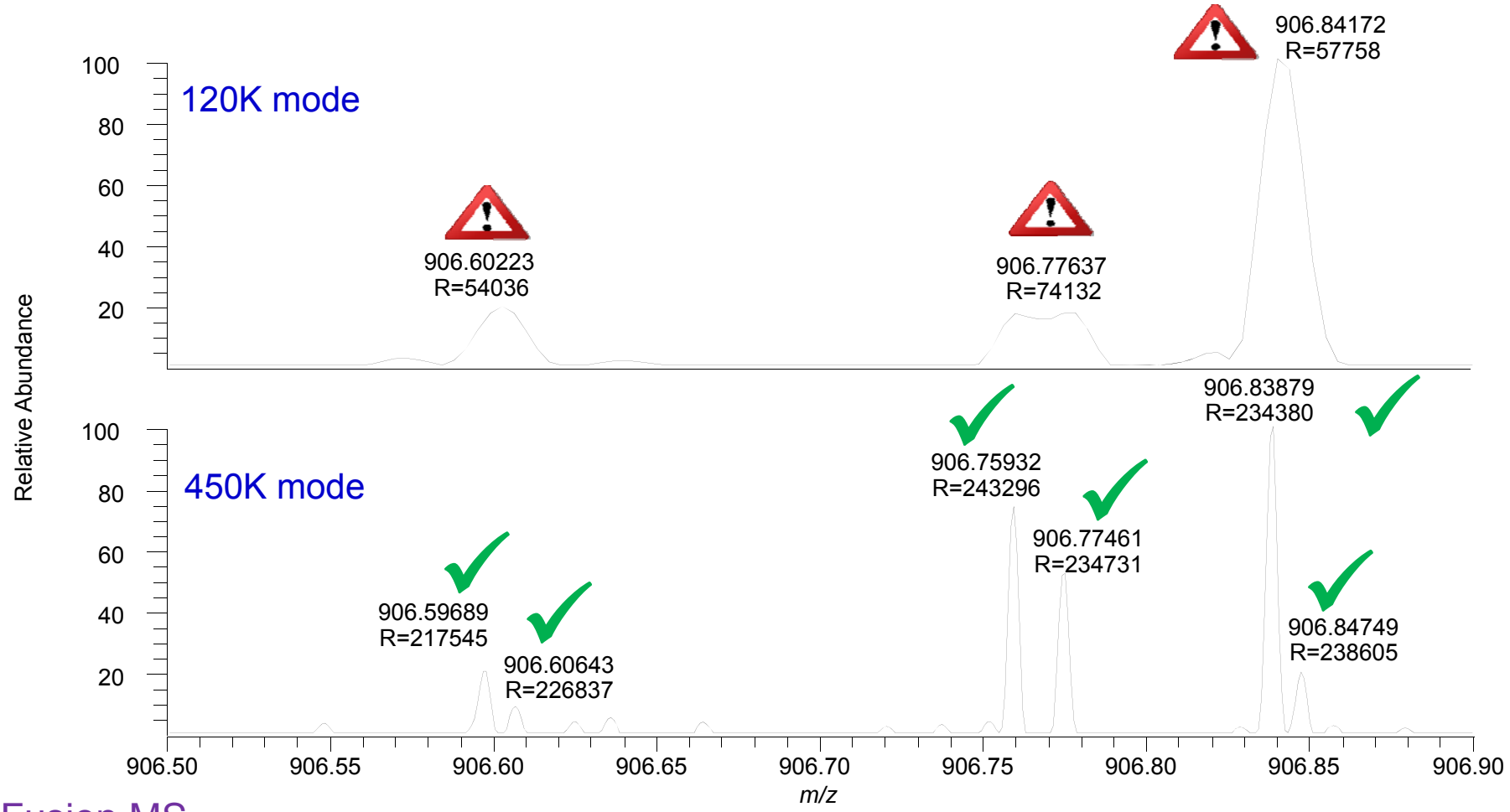
Triacylglycerol Lipids in Control Non-Diabetic Human Serum – Orbitrap Fusion MS



Data courtesy of Prof. Gavin Reid, Michigan State University

ThermoFisher SCIENTIFIC

Resolving Isobaric TAGs with Ultra-high Resolution

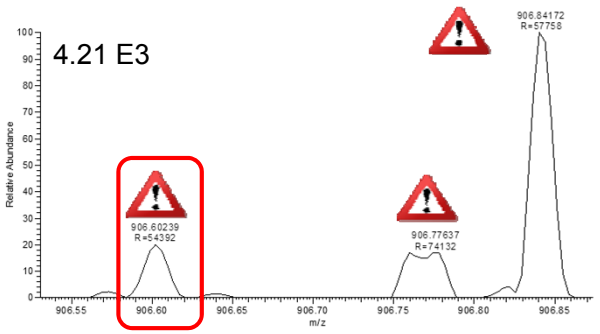


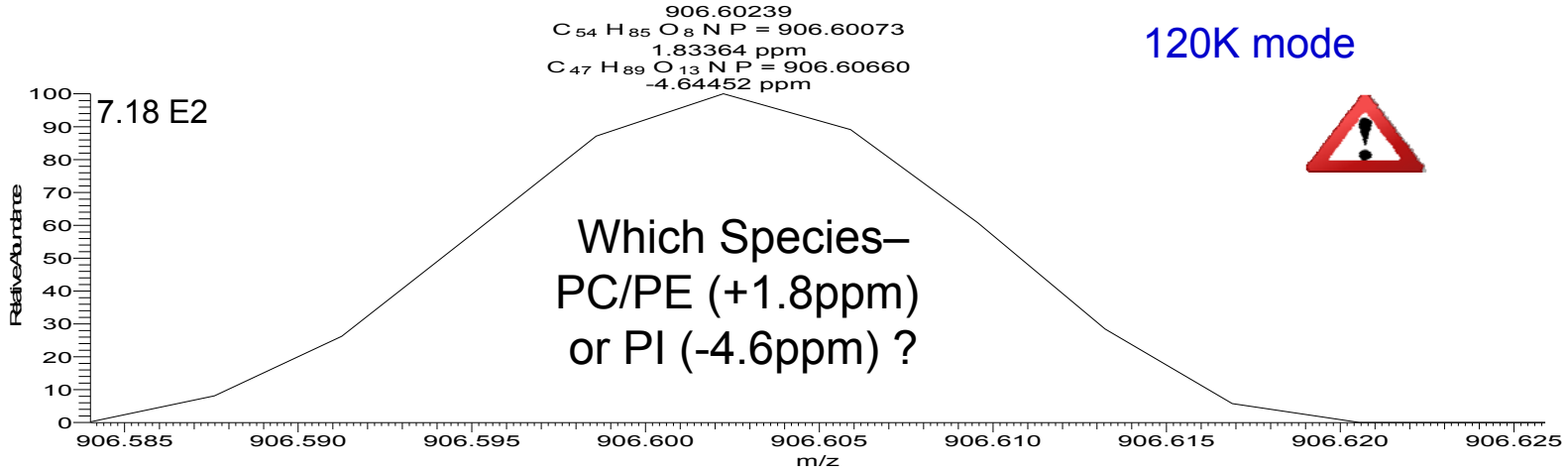
Orbitrap Fusion MS

Data courtesy of Prof. Gavin Reid, Michigan State University

ThermoFisher SCIENTIFIC

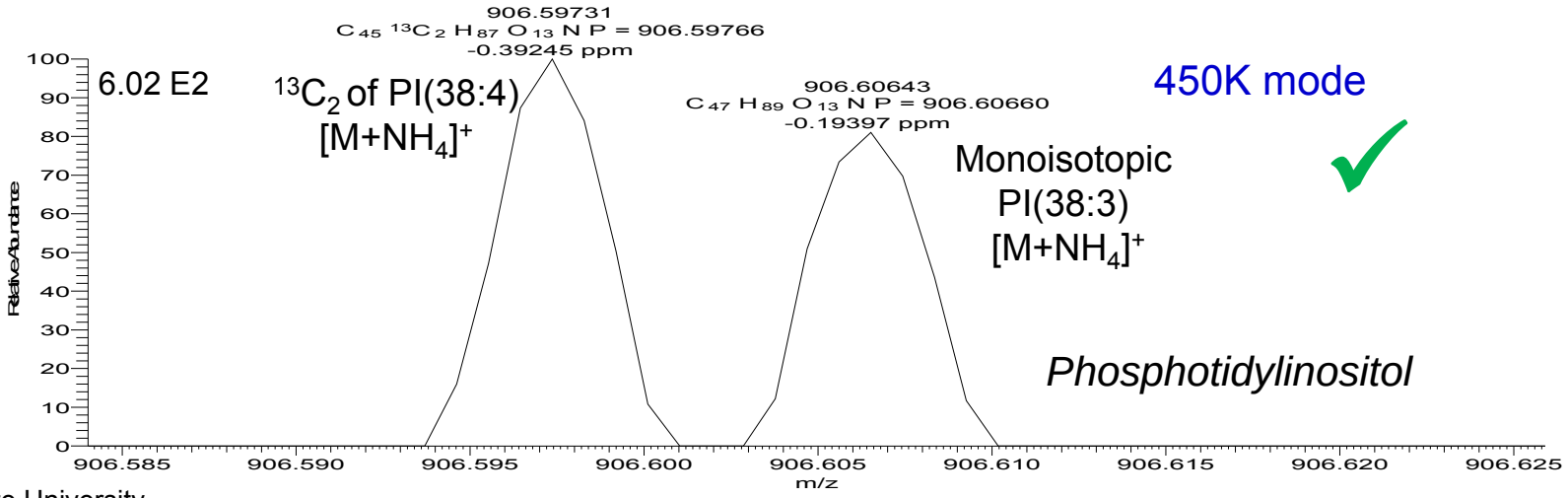
Accurate ID and Quan Made Possible by Ultra-high Resolution





120K mode

Which Species–
PC/PE (+1.8ppm)
or PI (-4.6ppm) ?



450K mode

Monoisotopic
PI(38:3)
[M+NH$_4$]$^+$

Phosphotidylinositol

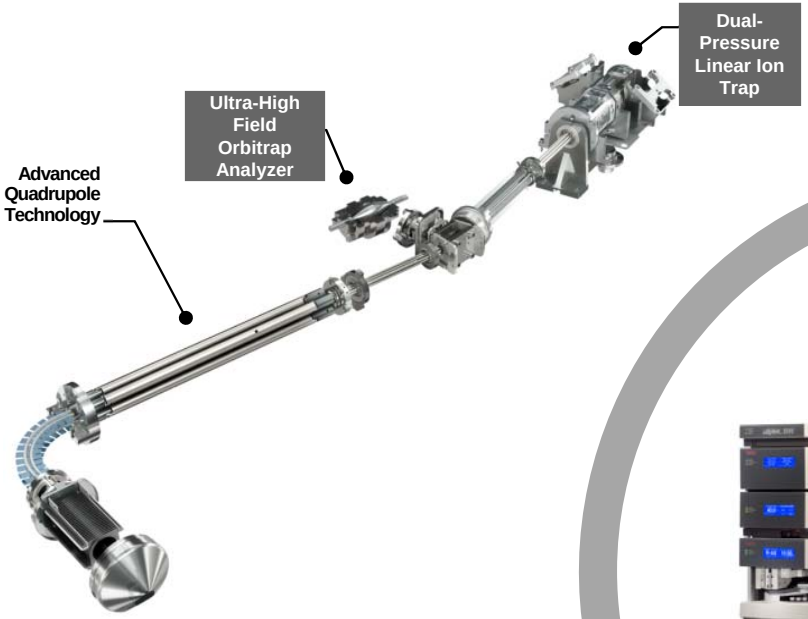
Orbitrap Fusion MS

Data courtesy of Prof. Gavin Reid, Michigan State University

ThermoFisher SCIENTIFIC

Unique Features of Orbitrap Fusion Lumos MS – A Tribrid Orbitrap Mass Spectrometer

new



HCD MS/MS

CID MS^n

- LIPID IDENTIFICATION REQUIRES MORE THAN ONE TYPE OF DISSOCIATION TECHNIQUE
- ION TRAP MS^N EXPERIMENTS PROVIDE MORE DEFINITIVE STRUCTURAL INFORMATION

UVPD

- UNIQUE FRAGMENTATION FOR LIPIDOMICS CHARACTERIZATION
- OPTIONAL ON NEW AND EXISTING SYSTEMS

1M

- ULTRA-HIGH RESOLUTION FOR FINE ISOTOPE STRUCTURE
- OPTIONAL ON NEW AND EXISTING SYSTEMS

ThermoFisher SCIENTIFIC

UVPD Implementation (Class 1 Laser System)

UVPD Source

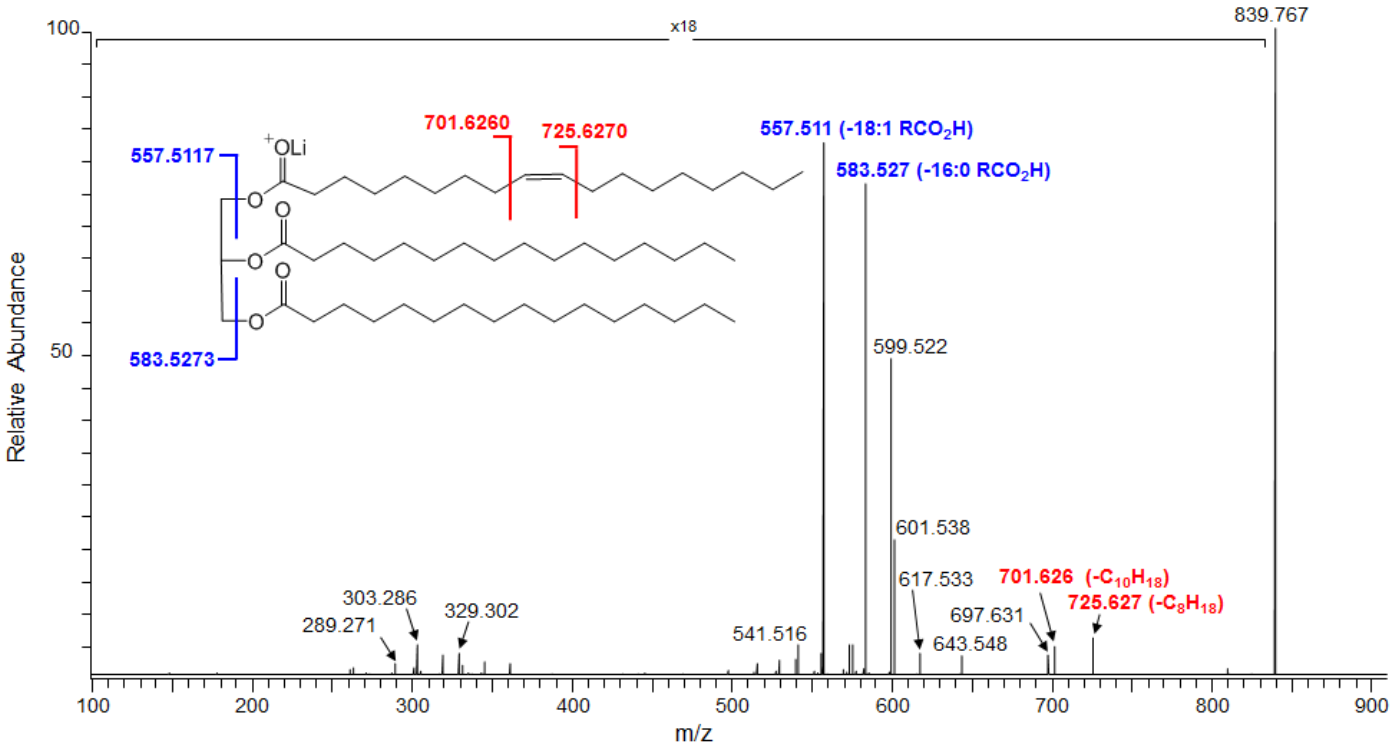
The UVPD MS^n fragments are generated in the linear ion trap and can be detected by either the ion trap or Orbitrap

new

Compact Footprint

- UVPD source is embedded inside the instrument, directly connected to the dual-pressure linear ion trap
- UVPD source employs a 213 nm laser with 2.5 kHz repetition rate delivering >1.2 µJ/pulse
- UVPD is a field upgradable option

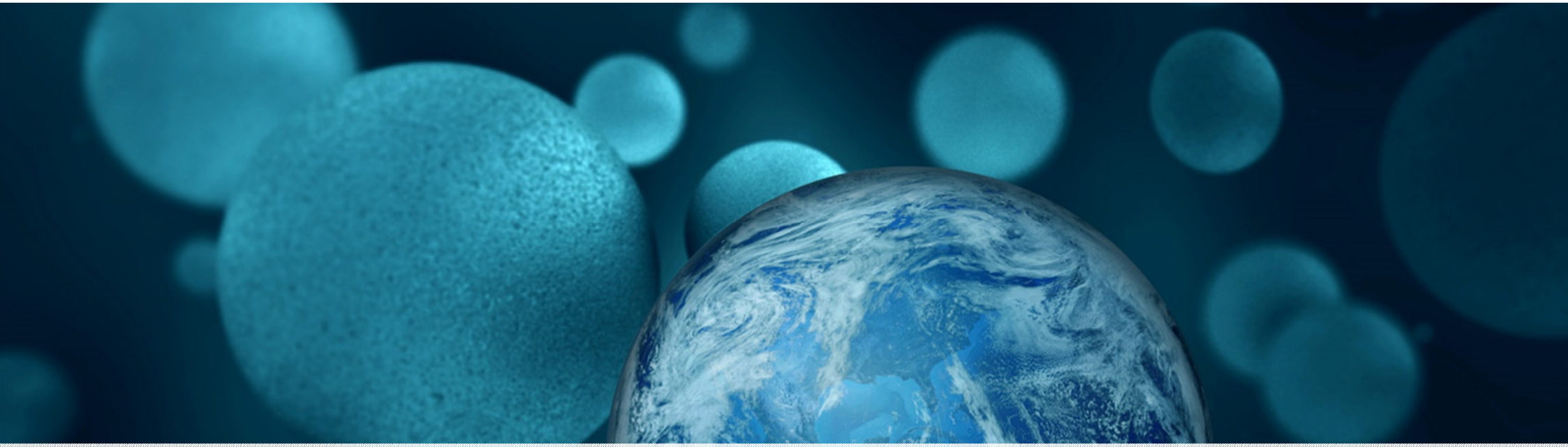
UVPD For Comprehensive Lipid Characterization



Locating Double Bonds

- HRAM UVPD MS^2 spectrum of $[M+Li]^+$ precursor ions of TG 16:0/16:0/18:1
- Fragments identify acyl chains
- UVPD unique fragments identify location of double bonds within the acyl chains

ASMS 2017, WOD 03:10 pm : Reid G.et al.

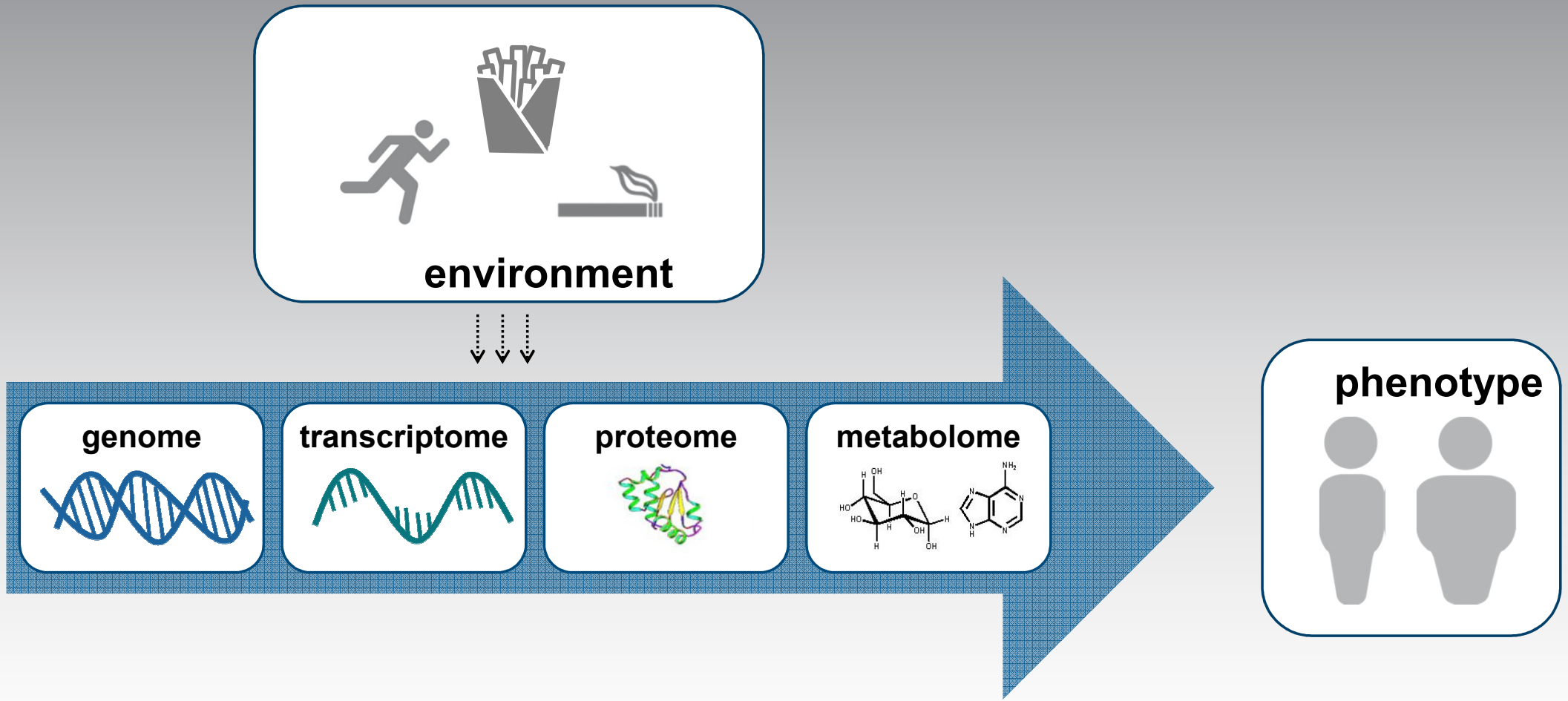


ThermoFisher SCIENTIFIC

Proteomics Analysis with Orbitrap

Leopoldo Dimiziani
Thermo Fisher Scientific
Verona 12/12/2017

Omics Studies – the Link between Genotype and Phenotype



Proteomics is Not Genomics

- Proteomics is not genomics where a single, whole genome sequencing experiment provides a relatively accurate picture of the genomic aspects of biology
- Proteomics is (was?) complicated, expensive and time-consuming, we have been forced to limit the number of samples we process and restrict ourselves to a static view of biology
- We have been looking at *snapshots*, when we really want to see *dynamics* as biology changes over time, across many samples
- We want to see the important differences between closely related biological states, such as the stages of cellular development and differentiation or the cellular response to therapeutic intervention at a protein, PTM, or even proteoform level. And we want to quantify them

What Are Biologists Researching?

- Molecular changes in the life cycle of plants and animals
- Comparison of normal and diseased tissues in animal and plants
- Study of molecular changes associated with particular genotypes
- Understanding the impact of the environment on species separated by, or located in, different geographies
- Understanding the evolution of species

All of these require the ability to measure multiple conditions or in other words measure over a time

What Are Biologists Researching?

Taking molecular snapshots does not provide the answers

What Are Biologists Researching?

- Requires 100 quantitative protein comparisons (10 development stages x 2 mutants x 5 biological replicates/flies)
- Observing 3500 proteins quantitatively using label-free technique requires: 100 injections with 180 min gradients = **13 days instrument time**
- Results: 350,000 protein detection events with 80% reproducibility between runs results in an overlap of <<2000 proteins that are reproducibly observed

High Quality Data for High Quality Results

- Complex matrix
- Differentiate similar masses
- Isobaric species
- Fine isotopic pattern

High Resolution

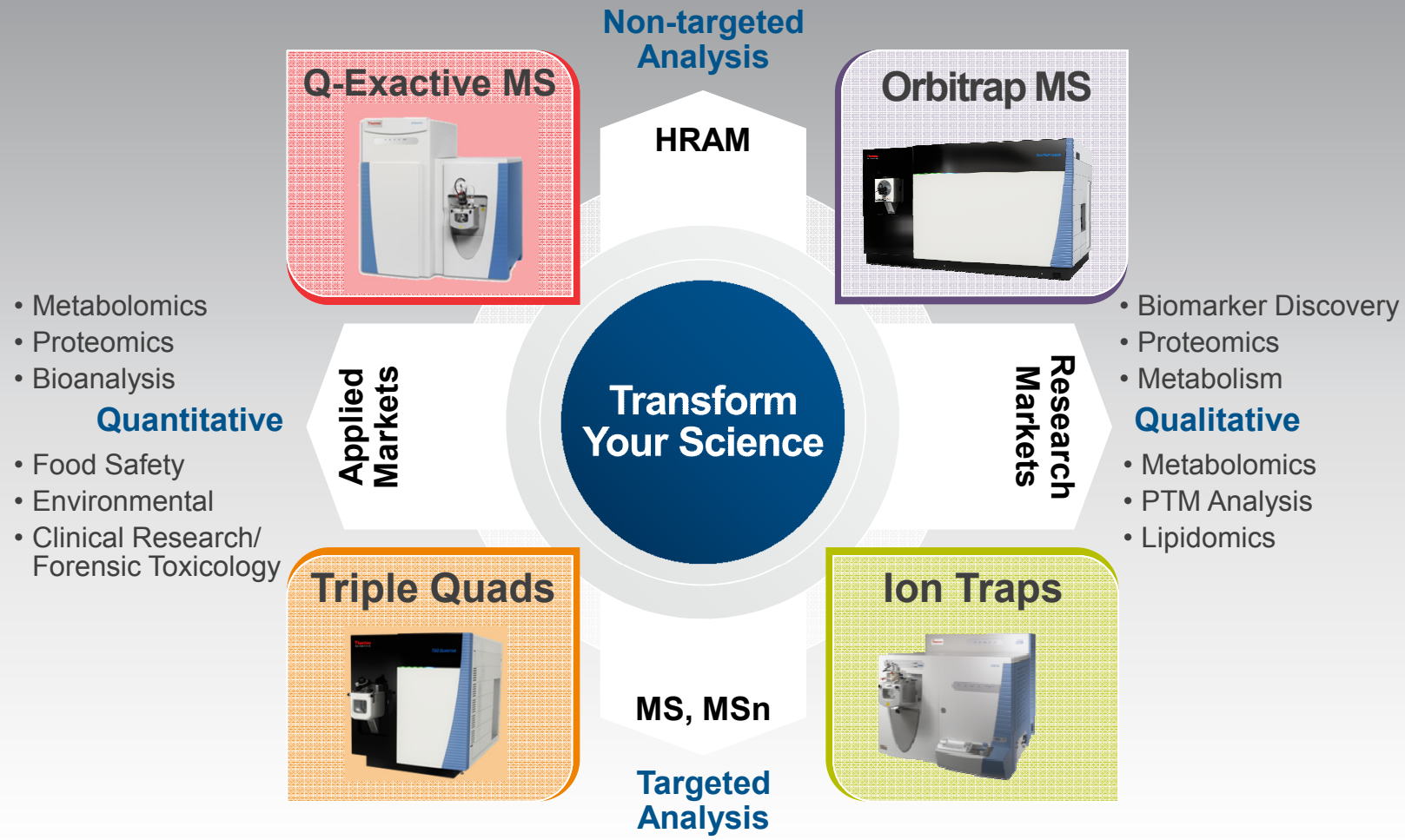
- Identification of unknowns
- Narrow mass tolerance
- Mass stability from peak to peak and run to run

Mass Accuracy

- Scan-to-scan consistency
- Injection-to-injection reproducibility
- Robustness over extended time periods

Instrument Performance

The Industry's Leading Portfolio of MS Solutions



ThermoFisher SCIENTIFIC

Thermo Scientific™ Q Exactive™ Series Portfolio for Proteomics

PERFORMANCE

Thermo Scientific™ Q Exactive™ MS

- Orbitrap analyzer
- Mass Range *m/z* 50 - 6000
- Mass Accuracy <1ppm
- Max. Mass Resolution >140,000
- Scan speed up to 12Hz
- Spectral Multiplexing
- Polarity switching <1 sec

Thermo Scientific™ Q Exactive™ Plus MS

- Orbitrap analyzer
- Mass Range *m/z* 50 - 6000
- Mass Accuracy <1ppm
- Max. Mass Resolution >140,000
- Scan speed up to 12Hz
- Spectral multiplexing
- Polarity switching <1 sec
- Advanced Quadrupole Technology (AQT)
- Advanced Active Beam Guide (AABG)
- Opt. Enh Res.Mode (280k)

Thermo Scientific™ Q Exactive™ HF MS

- Ultra High Field Orbitrap analyzer
- Mass Range *m/z* 50 - 6000
- Mass Accuracy <1ppm
- Max. Mass Resolution >240,000
- Scan speed up to 18Hz
- Advanced Quadrupole Technology (AQT)
- Advanced Active Beam Guide (AABG)
- Spectral Multiplexing
- Polarity switching <1 sec

Thermo Scientific™ Q Exactive ™ HF-X MS

- Ultra High Field Orbitrap analyzer
- Mass Range *m/z* 50 - 6000
- Mass Accuracy <1ppm
- Max. Mass Resolution >240,000
- Scan speed up to 40Hz
- High capacity transfer tube
- Electrodynamic ion funnel
- Advanced Quadrupole Technology (AQT)
- Advanced Active Beam Guide (AABG)
- Spectral Multiplexing
- Polarity switching <1 sec
- Advanced ddHCD algorithm

VALUE

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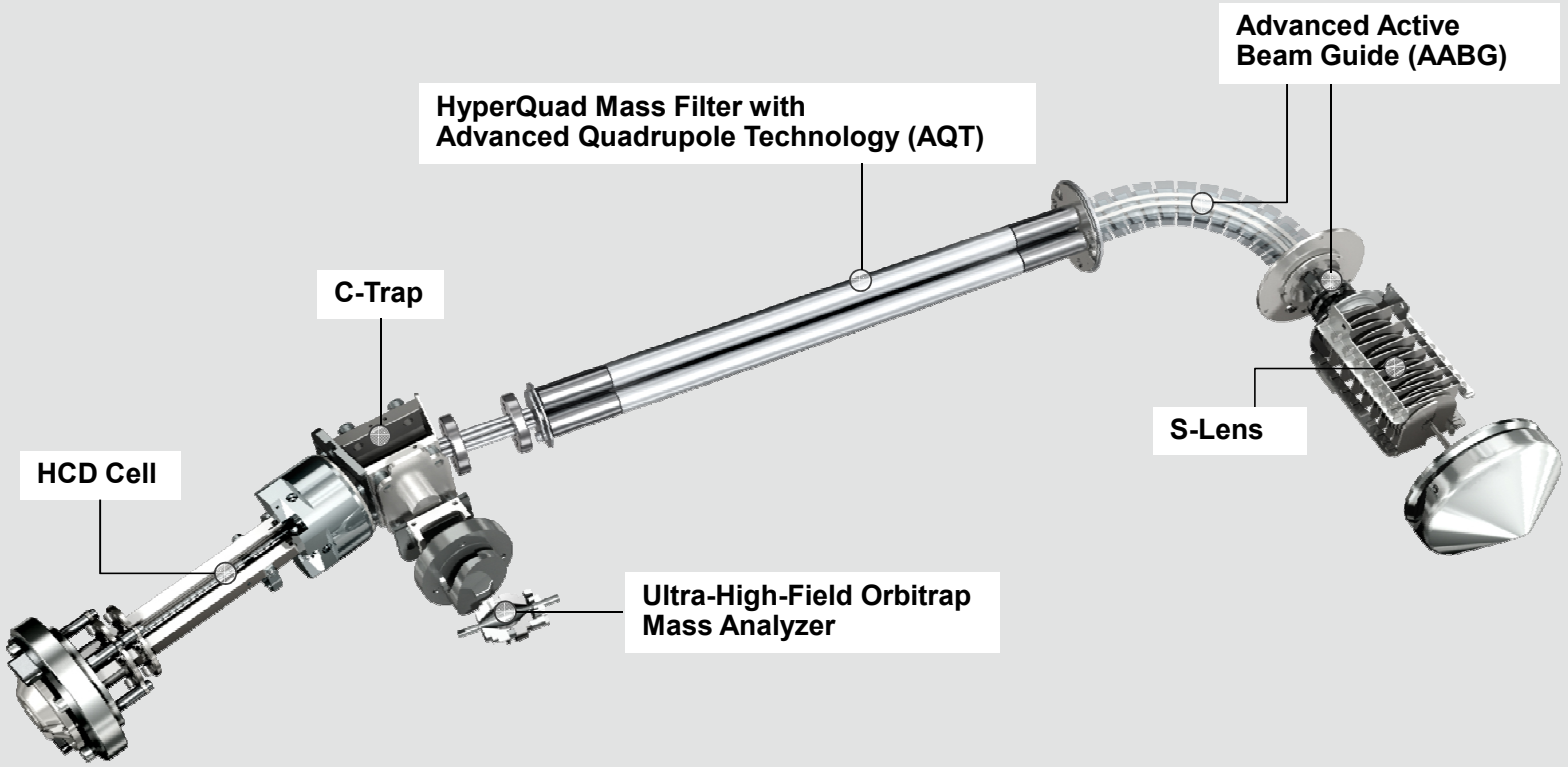
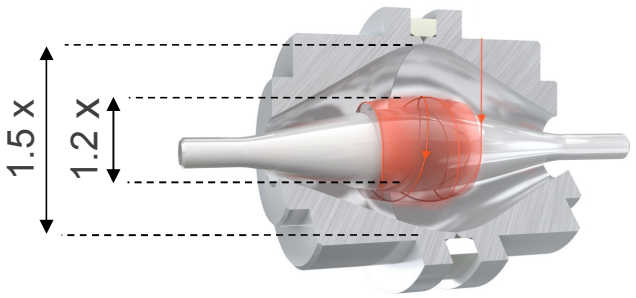
Q Exactive Plus/HF Mass Spectrometer



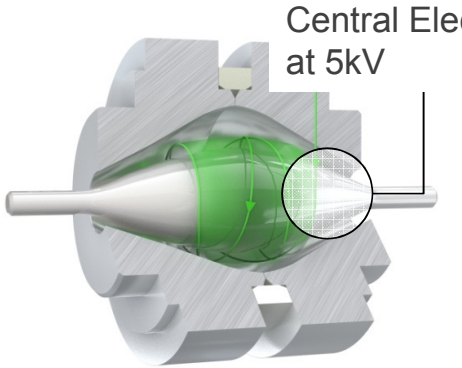
ThermoFisher SCIENTIFIC

Key Technologies of Q Exactive HF MS

Standard Orbitrap



Ultra-High Field Orbitrap



Q Exactive HF MS

Key Technologies

- Ultra-High-Field Orbitrap
  - Up to 18 Hz and standard 240k resolution
- Smaller Size
  - 1.8x frequency at the same voltage
  - 1.8x higher resolution over standard Orbitrap
  - New lenses for focusing ions in to the Orbitrap entrance
- 32 msec transient for fastest MS/MS data and max scan speed

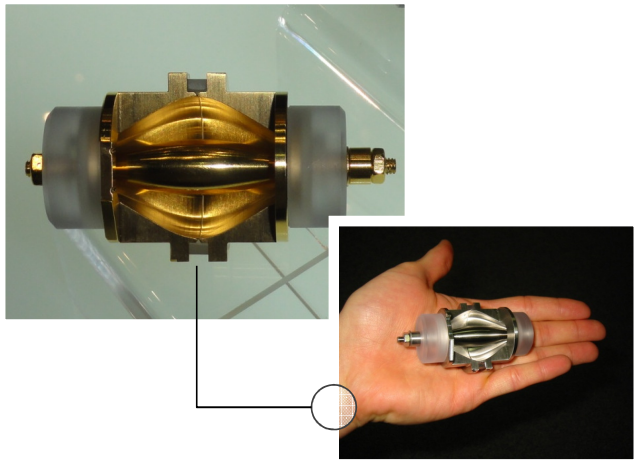
Standard Orbitrap Analyzer & Ultra-High-Field Orbitrap Analyzer- Real Size Cutaways

STANDARD Orbitrap™ Analyzer

Thermo Scientific™ LTQ Orbitrap Classic/XL/ Discovery /Velos (Pro) MS

Thermo Scientific™(Q)Exactive Plus™ MS

Thermo Scientific™ Exactive Plus EMR MS



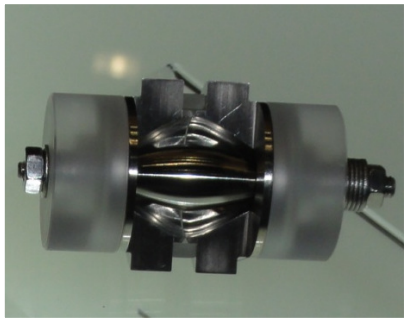
HIGH FIELD Orbitrap Analyzer

Thermo Scientific™ Orbitrap Elite™ MS

ULTRA-HIGH-FIELD Orbitrap Analyzer

Thermo Scientific™ Orbitrap Fusion™/Lumos ™ MS

Thermo Scientific™ **Q Exactive HF™/HF-X ™ MS**



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Q Exactive Plus MS: Specifications

Scan rate	12 Hz
Max resolution	140K at m/z 200 (280k optional)
Quad isolation	Step-less from full mass range down to 0.4 amu
Mass Accuracy	3 ppm external, 1 ppm internal
Dissociation	Source CID, HCD
Multiplexing	Up to 10 precursor ions
Detectors	Orbitrap
Polarity Switching	1 sec cycle time (@ RES 35k)
Scan Functions	FS: Full Scan, AIF: All Ion Fragmentation, SIM: Selected Ion Monitoroing, PRM: Parallel Reactin Monitoring, DIA: Data Independent Acquisition, ddHCD: data dependent HCD
Options	Intact Protein Mode
	Enhanced resolution

Q Exative HF MS: Specification

Mass Range	50 < m/z < 6,000
Resolution @ m/z 200	15,000 at 18Hz 30,000 at 12 Hz 60,000 at 7 Hz 120,000 at 3 Hz 240,000 at 1.5 Hz
Mass Accuracy	< 1ppm RMS, Internal Calibration < 3ppm RMS, External Calibration
Polarity Switching	one full cycle in <1 sec (one full positive mode scan and one full negative mode scan at a resolution setting of 60,000)

Unmatched Analytical Performance

240,000 Resolution for best selectivity

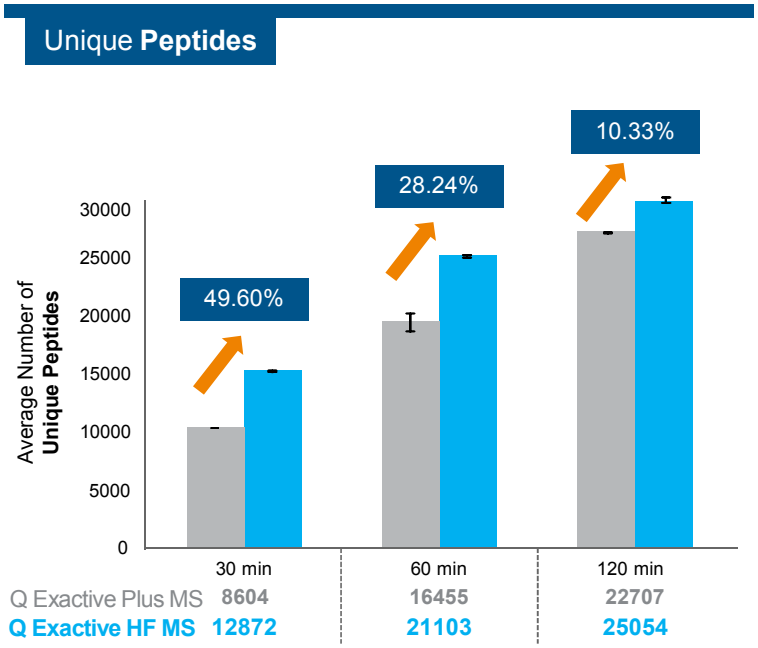
18 Hz for maximum MS/MS scan speed

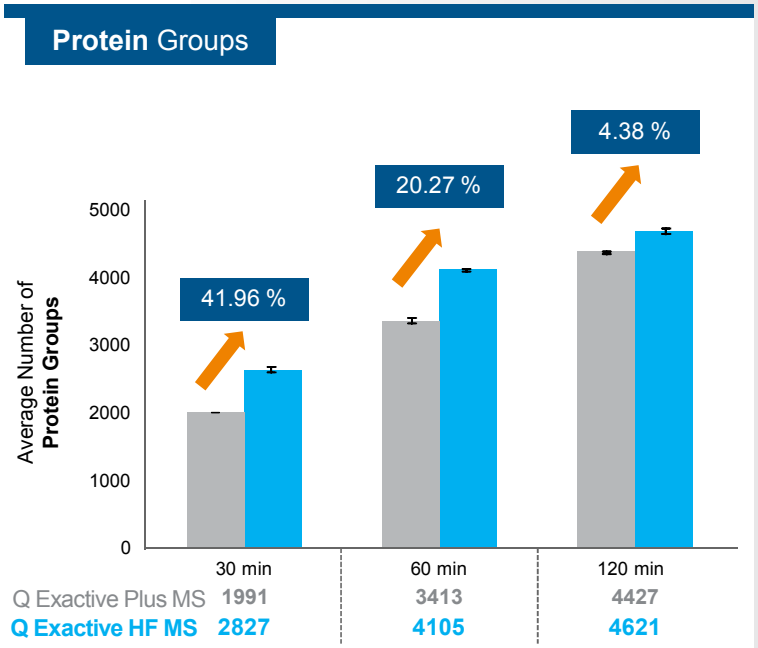
Intact Protein Mode for best
S/N of intact proteins

Comparison of Q Exactive Plus MS and Q Exactive HF MS

ASMS 2014
Poster M186
Tabiwang Arrey

HeLa – 1 µg sample load





More IDs with shorter Gradients

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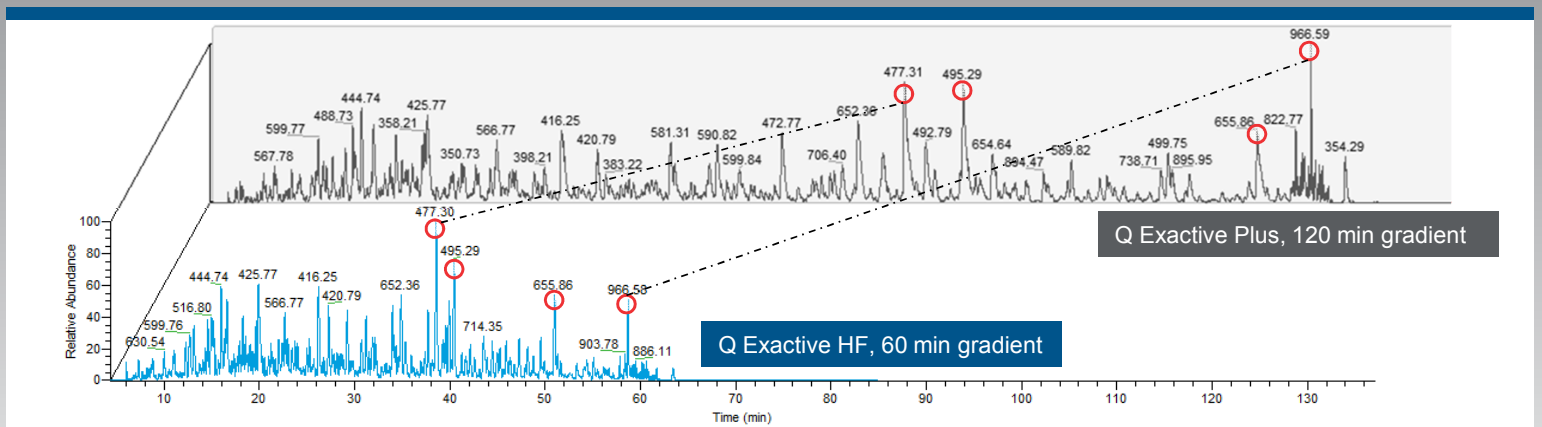
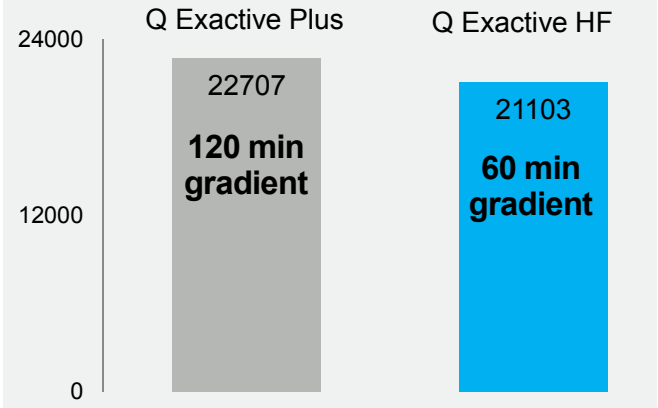
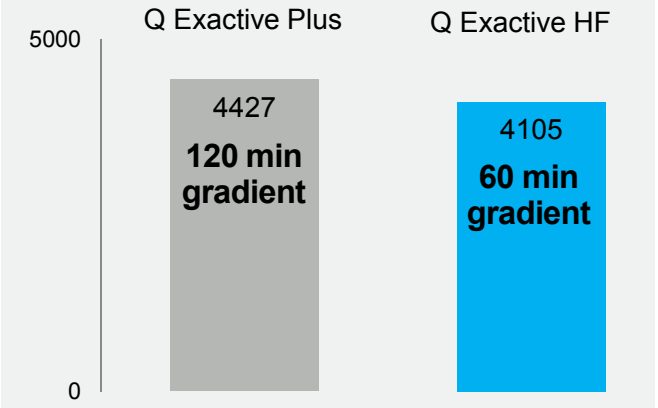
Same Identifications with **Half the Time**



Unique Peptides



Protein Groups



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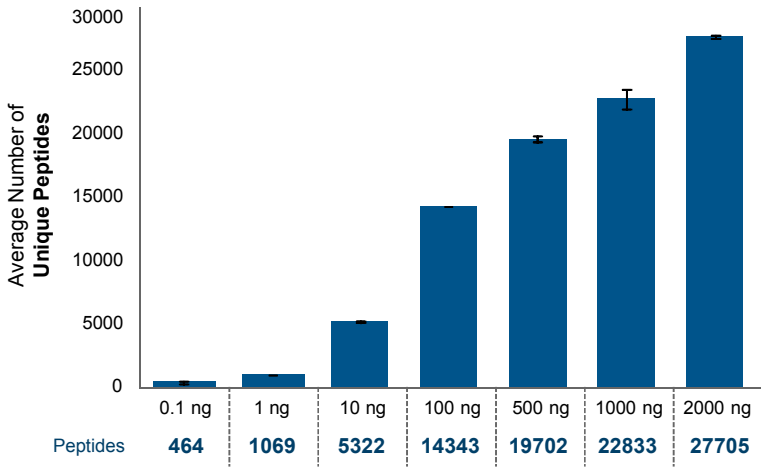
More Identifications with Less Sample

What if I am sample limited?

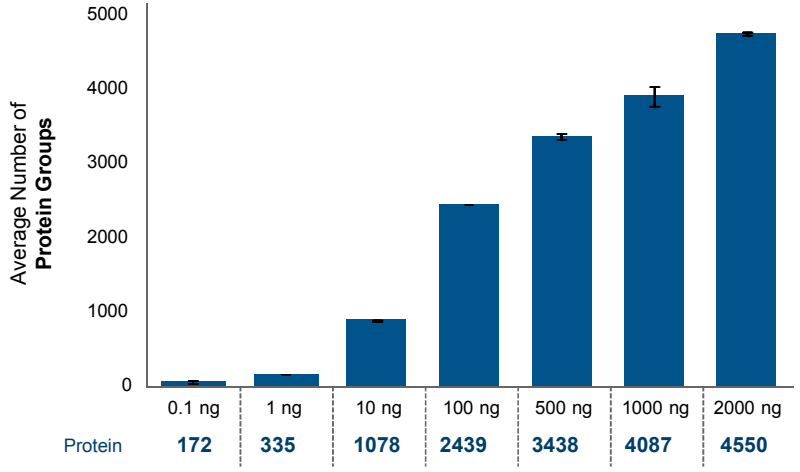
Q Exactive HF™

60 min gradient

Unique **Peptides**



Protein Groups



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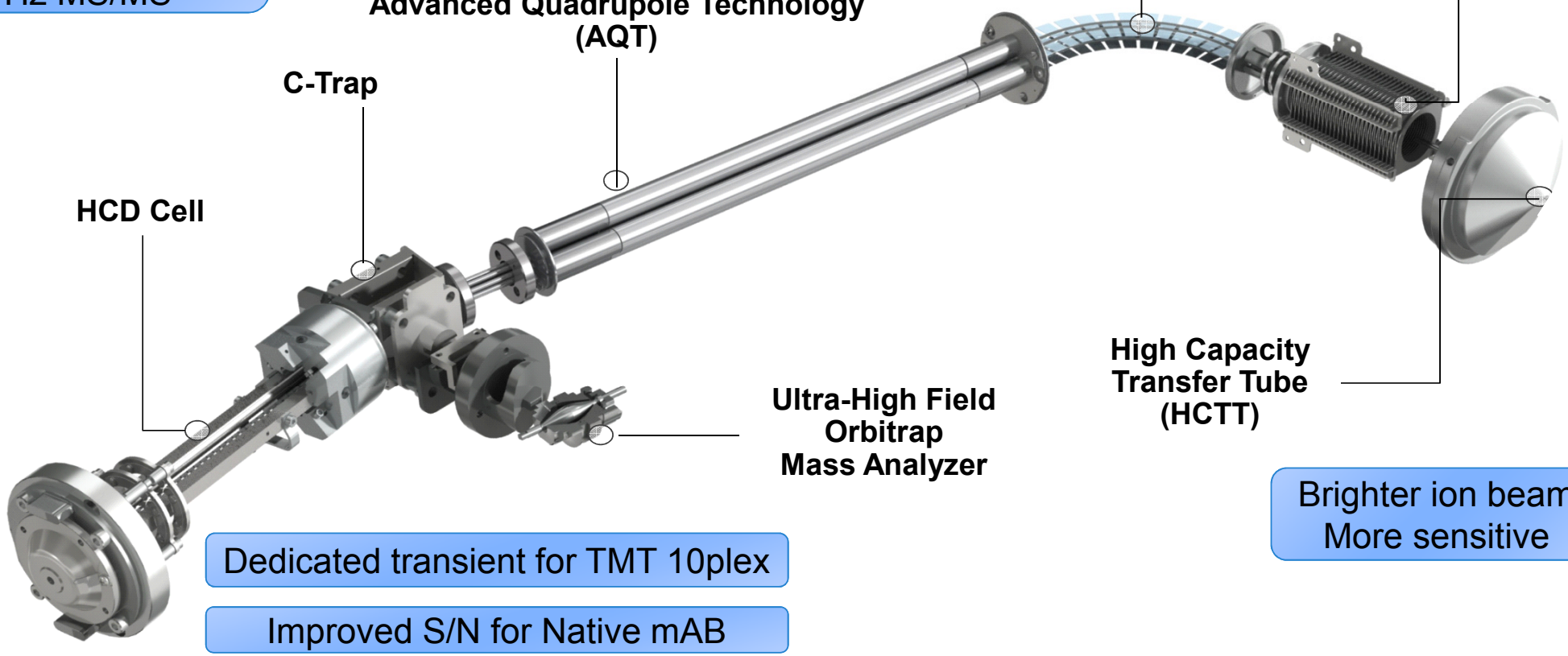
Q Exactive HF-X – new architecture

Optimized Scan Matrix with accelerated HCD 40 Hz MS/MS

Advanced DDA for bottom-up and top-down



Brighter ion beam
More sensitive

Dedicated transient for TMT 10plex

Improved S/N for Native mAB

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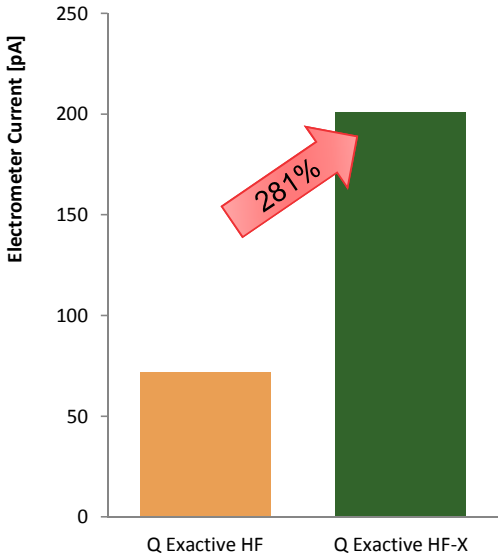
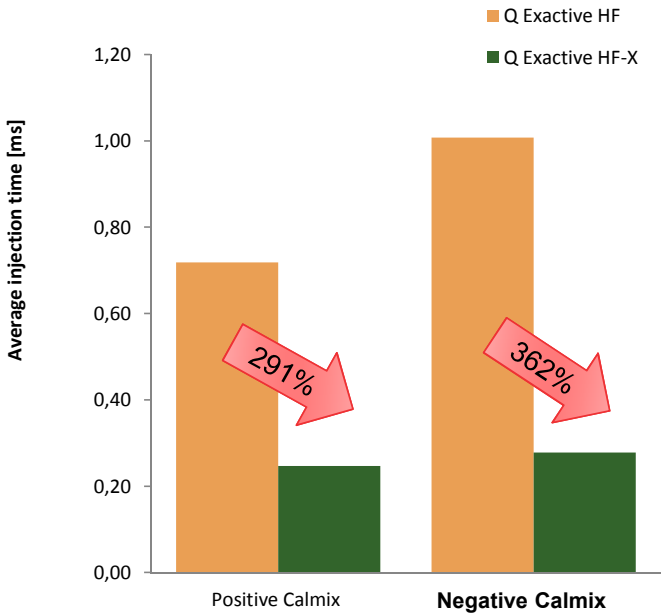
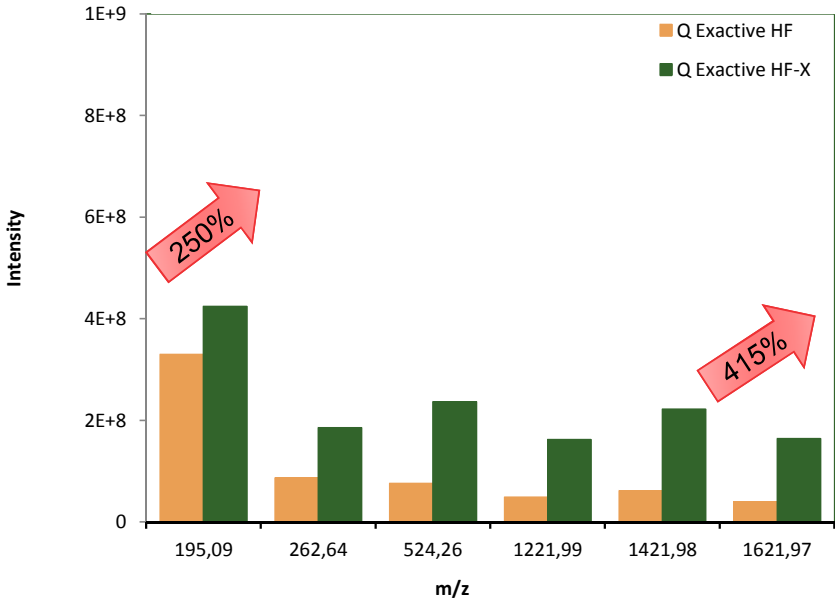
Improvement of sensitivity – direct infusion







Electrometer current **Injection times** **Ion intensities**

ASMS'17: TP 389, T.N. Arrey et al. New innovations implemented on the Q Exactive HF mass spectrometer.

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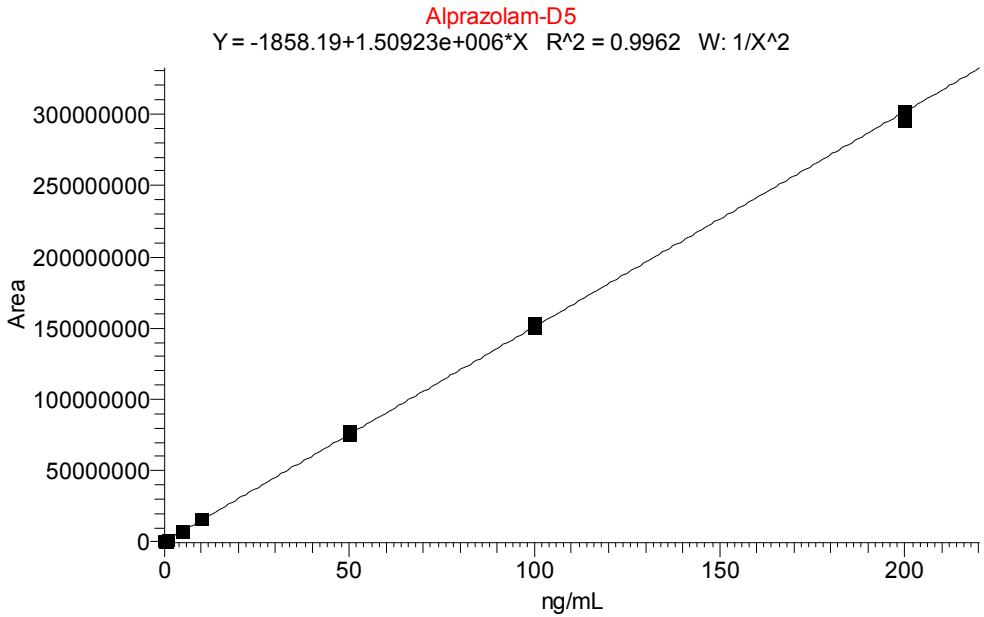
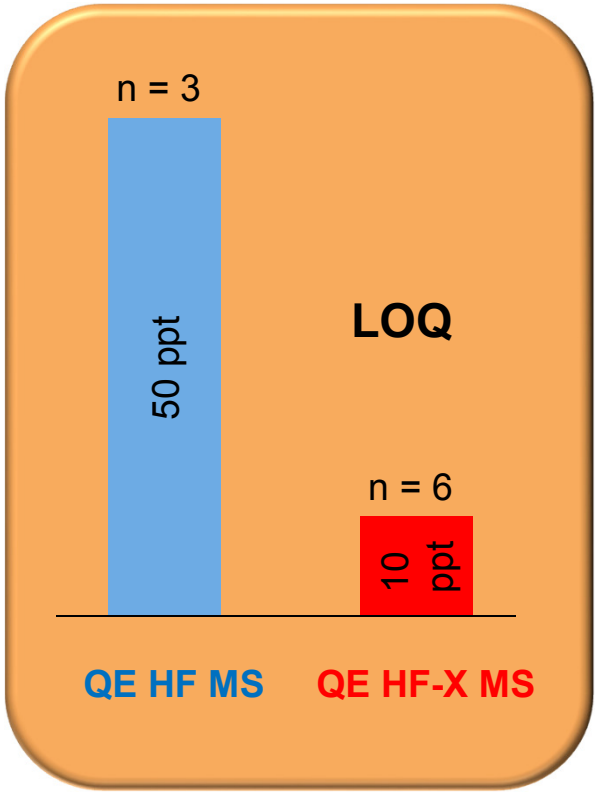
Sensitivity and Linear Dynamic Range in Quantitation





- tSIM, Resolution 60K, maxIT 119ms, Iso 8 amu
- 3 replicates
- Sample: **Alprazolam** spiked into crashed plasma
- LOQ = 10 fg/µL = 10 ppt
- Range = 10 - 200,000 pg/mL

>10^4 linear dynamic range - LOQ at low ppt level

ASMS'17: TP 389, T.N. Arrey et al. New innovations implemented on the Q Exactive HF mass spectrometer.

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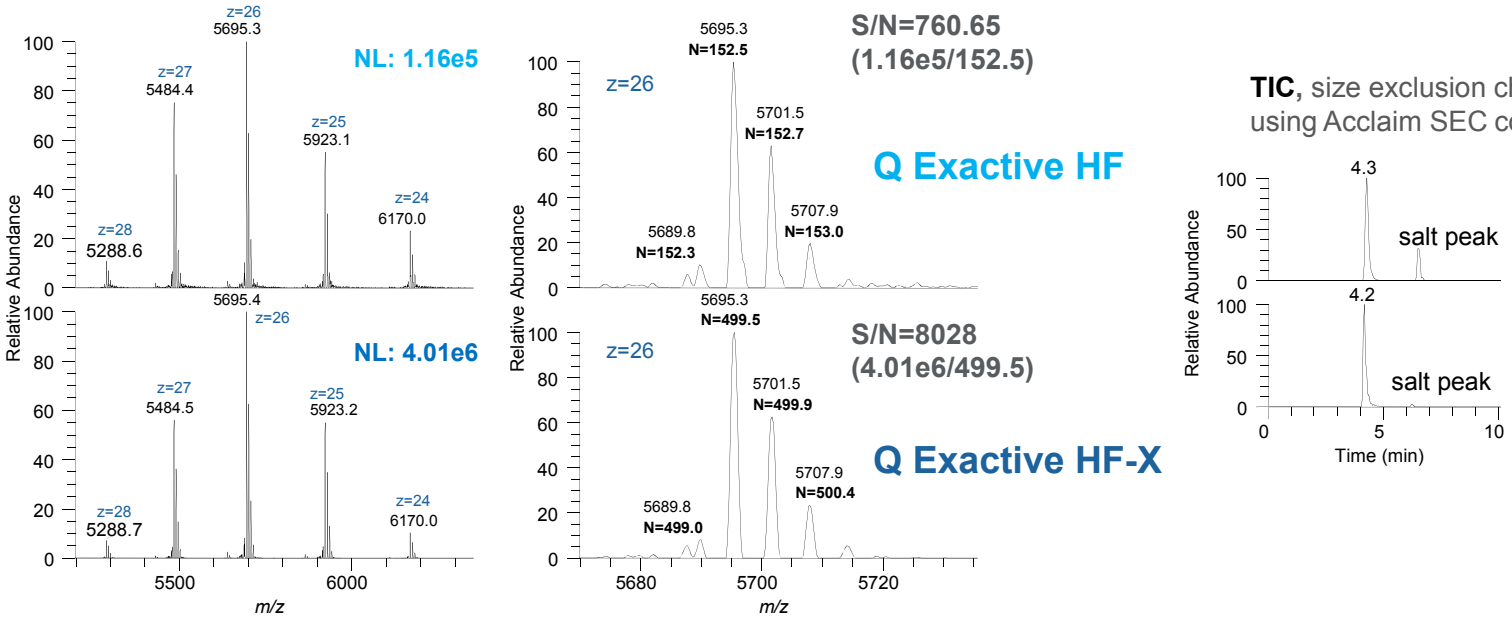
Analysis of Intact Trastuzumab under Native Conditions in HMR Mode

Improved S/N ratio on the Q Exactive HF-X by a factor of ~5-10.



TIC, size exclusion chromatography using Acclaim SEC column

SEC-LC/MS analysis of intact Trastuzumab monoclonal antibody using Acclaim SEC column, 4.6 x 300 mm, 300 µl/min flow rate, 50 mM ammonium acetate. Full MS, HMR mode, m/z 2500–8000, resolution setting 30k, 10 µscans. Spectra show an average of 3 scans (10 µscans each).

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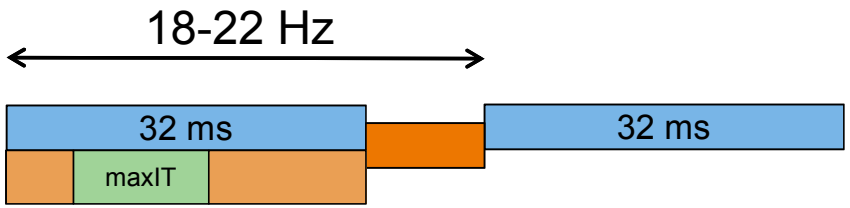
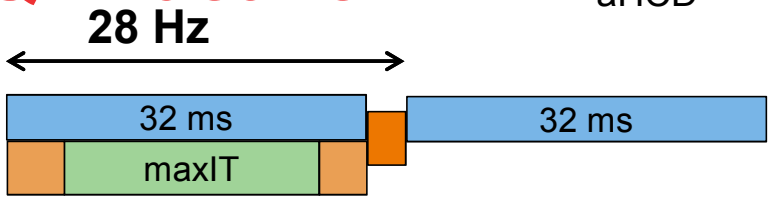
Optimized Scan Matrix

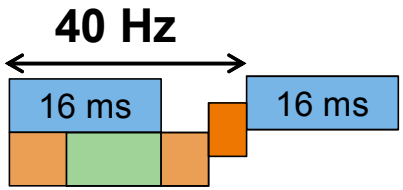
Q Exactive HF MS/MS



Longer fill time
Reduced scan overhead
aHCD

Q Exactive HF-X





Comparable fill time
Reduced scan overhead
aHDC

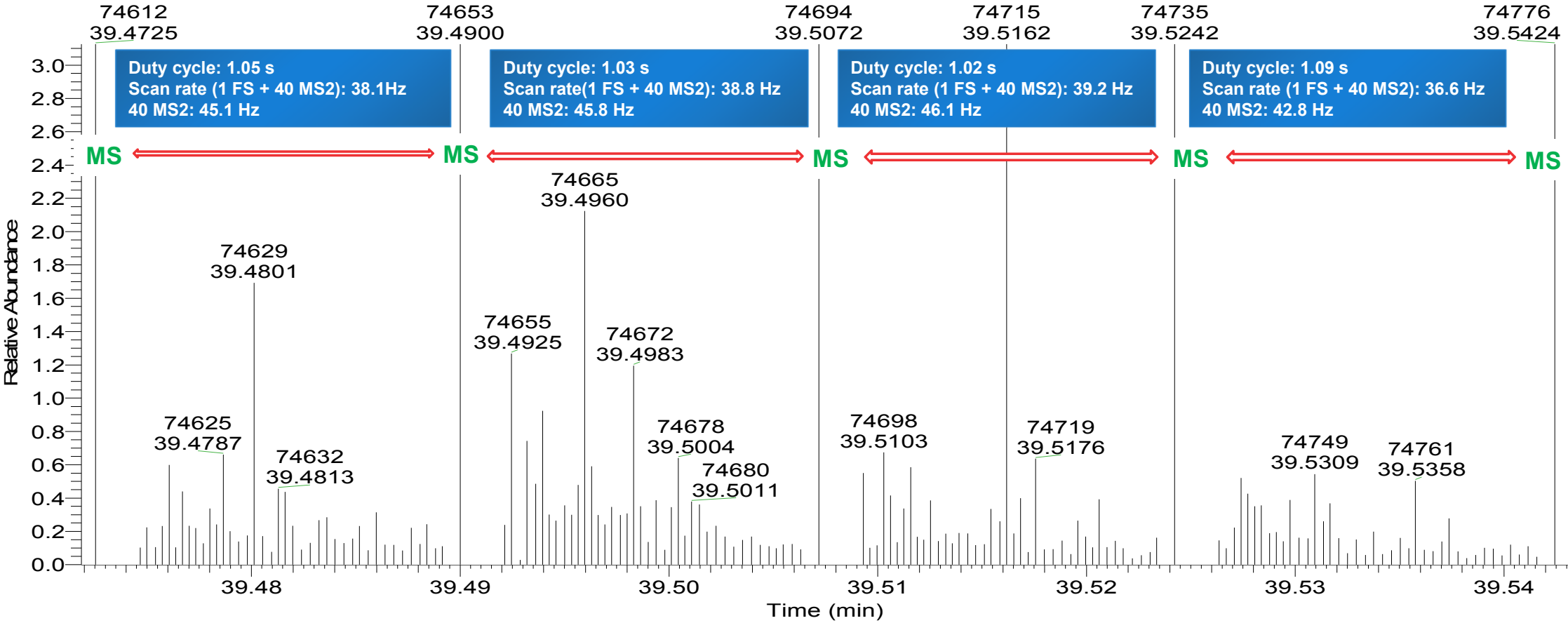
- Brighter ion beam, reduced scan overhead, and accelerated HCD (aHCD) is boosting acquisition speed
- Advantage for both MS and MS/MS mode
- Fast and high quality MS/MS acquisition up the 40 Hz with new 16 msec transient (7,500 resolution setting)

Orbitrap detection

Maximum fill time with precursor ions

Inter and intra scan overheads

Ultra Fast MS/MS Scan Speed > 40 Hz



1 full scan (60,000 @ m/z 200) and 40 MS^2 scans 7,500@ m/z 200) at LC time scale in 1 second.
30 min gradient, MS2 max IT: 11 ms

ASMS'17: TP 389, T.N. Arrey et al. New innovations implemented on the Q Exactive HF mass spectrometer.

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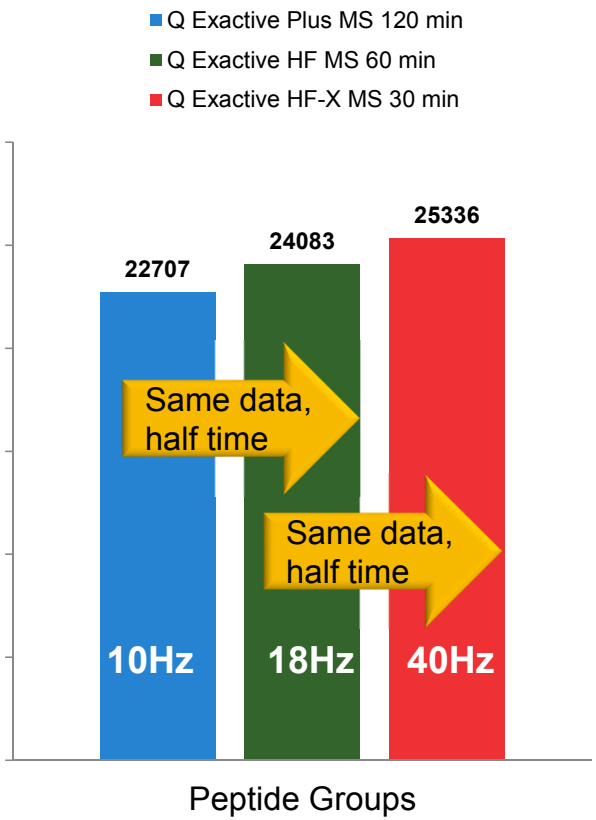
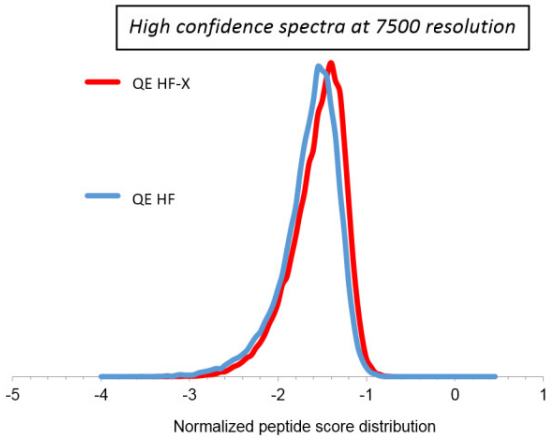
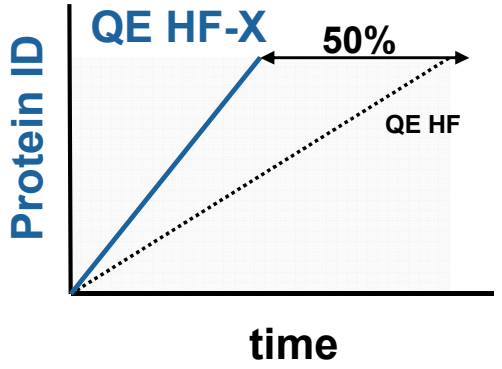
Protein identification faster than ever



Sample: 1 ug Pierce HeLa digest

- Maximizing protein identifications
- Same protein identifications in half the analysis time
- Faster, with same high quality results





ASMS'17: TP 389, T.N. Arrey et al. New innovations implemented on the Q Exactive HF mass spectrometer.

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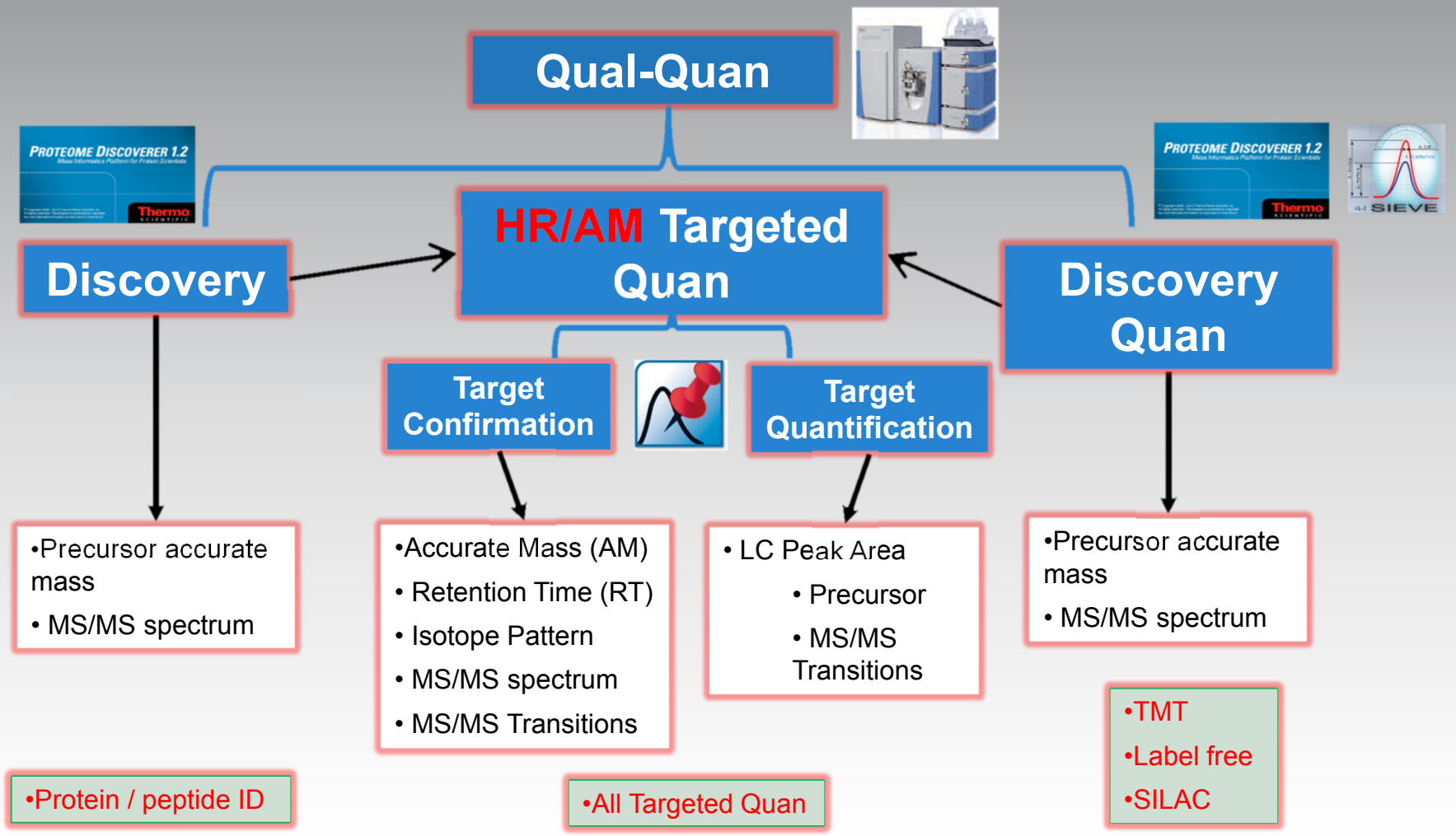
From Discovery to Quantification - do it all with a Q Exactive



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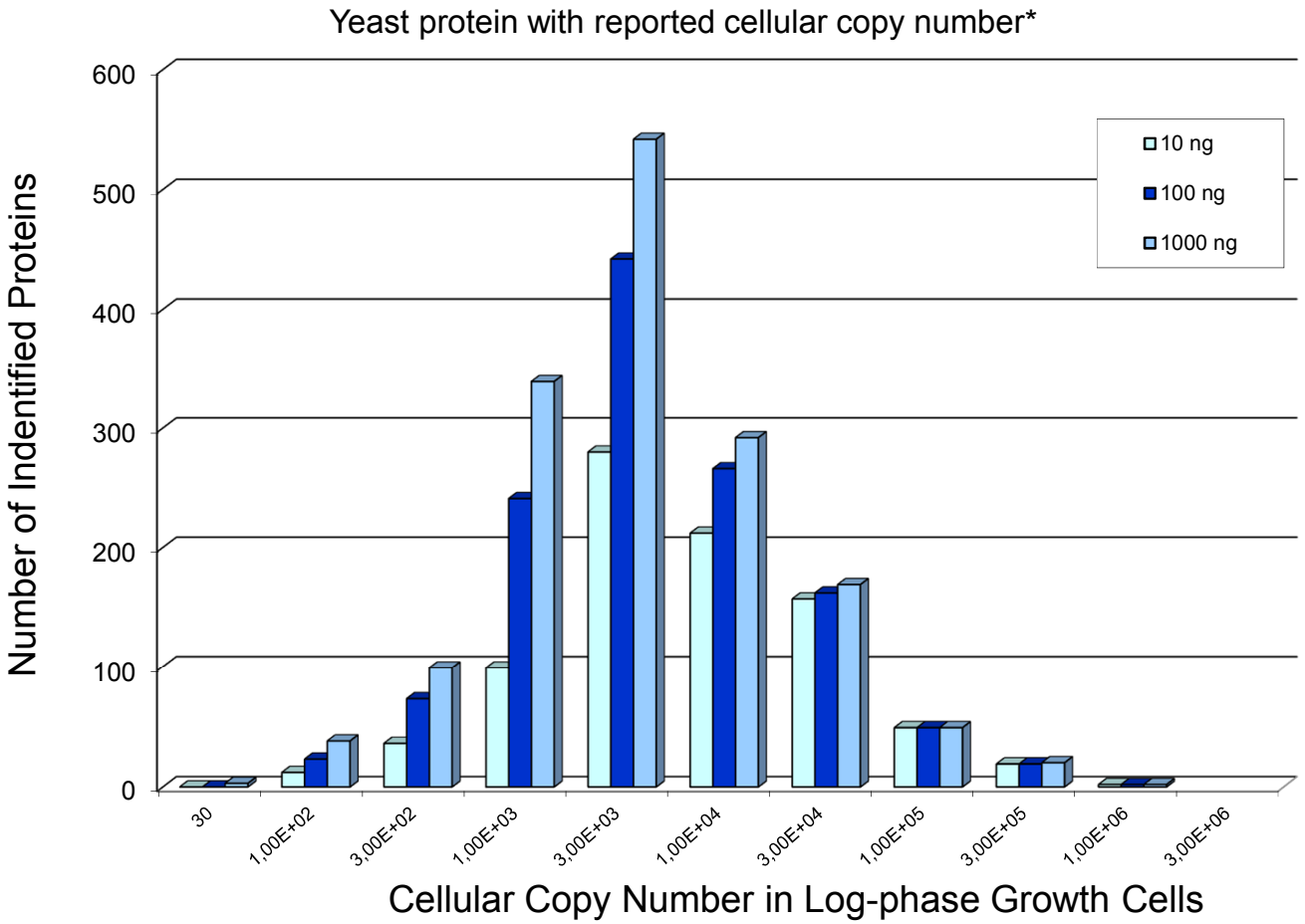
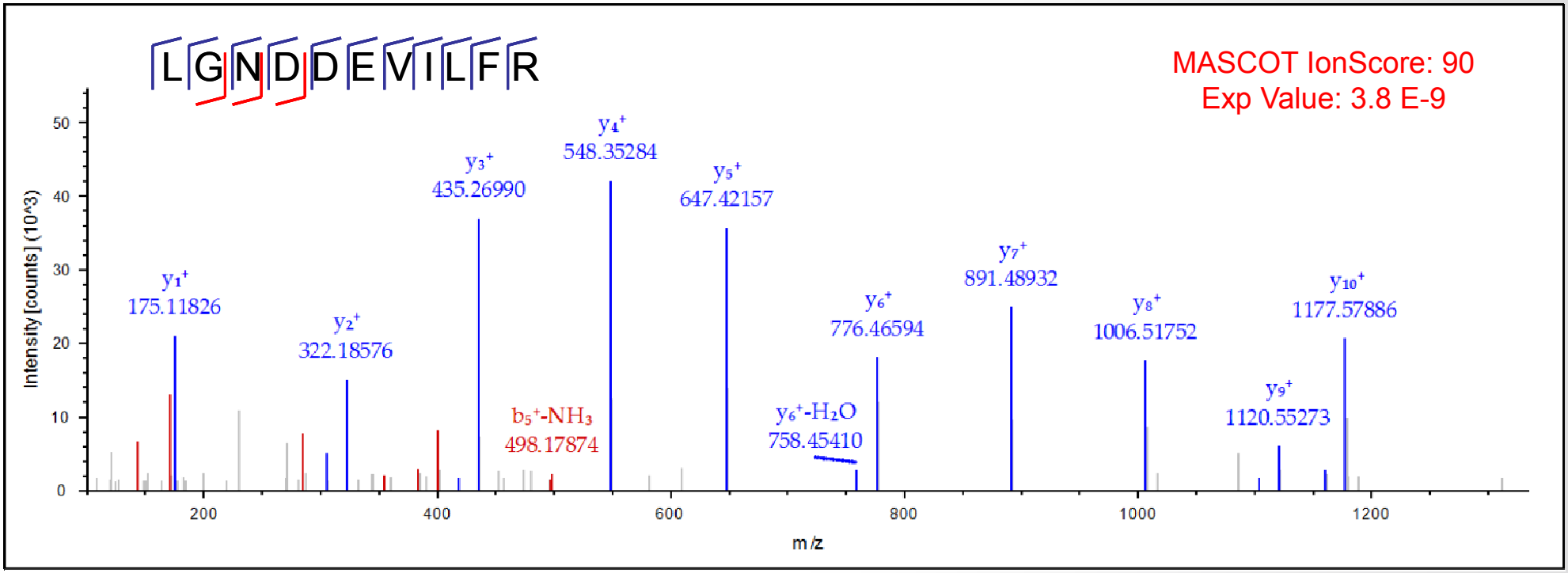
The Power of Q Exactive to Access the Low-abundant Proteins



*Yeast cellular protein copy numbers are from Weissman and co-workers, Nature, 2003, 16, 737-41.

Low-abundant Protein Identified from Low Sample Load

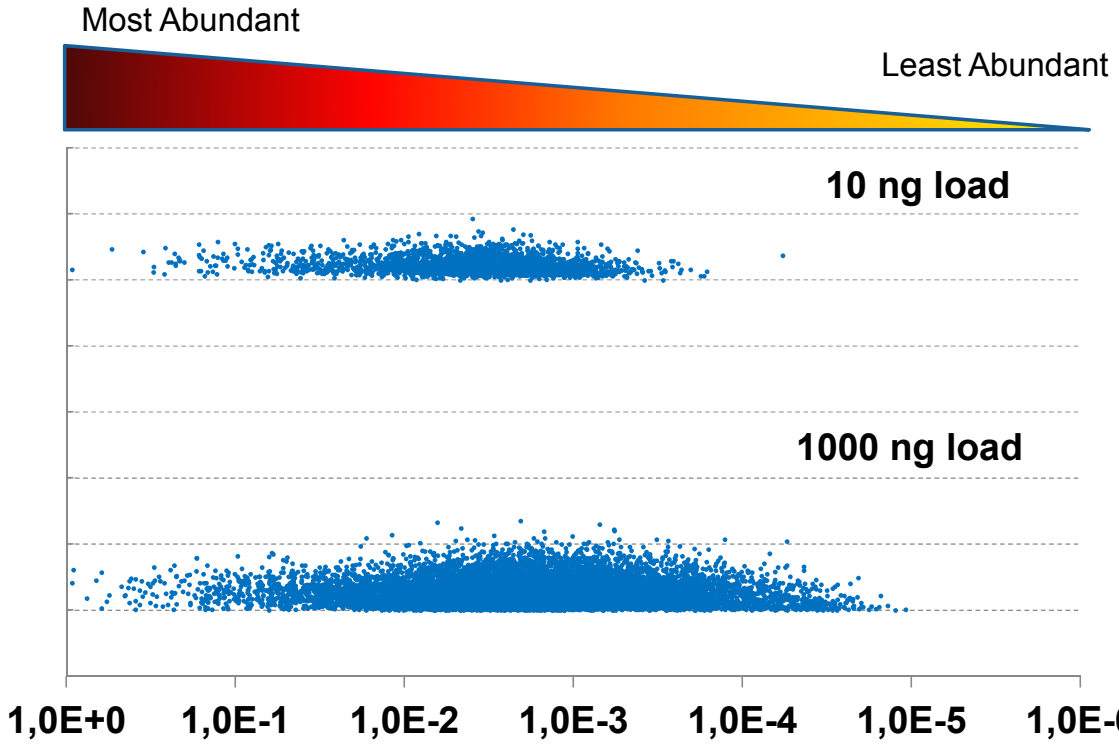
High confidence identification from 10 ng of Yeast Digest



Peptide of YOR020C, **149** copy number, identified from **10 ng** yeast digest

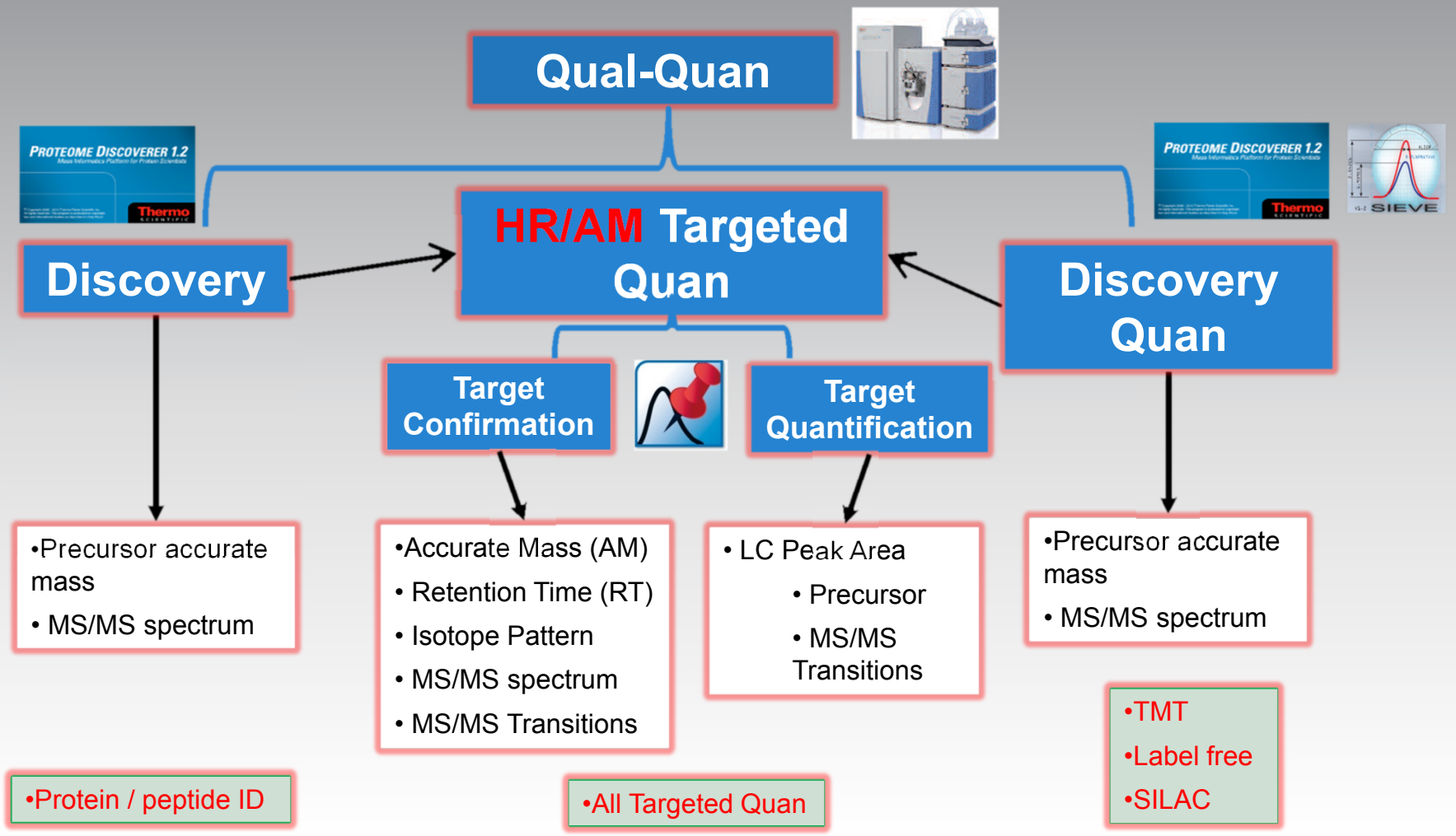
Larger dynamic range leads to deeper sequencing

Q Exactive raises the challenge in discovery proteomics to the next level – identifying the proteins that matter.



Precursor intensity of identified peptides (normalized to the most intense peak)

From Discovery to Quantification - do it all with a Q Exactive



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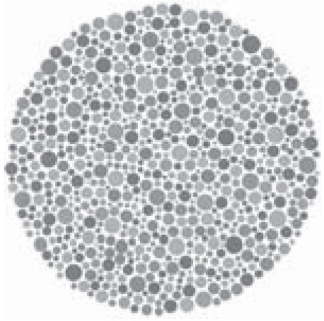
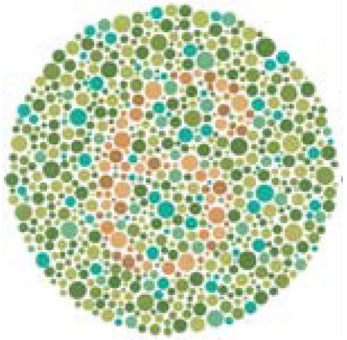
Moving Beyond Qualitative Proteomics

Problem: Quantitative information about expression level of a protein is essential to understanding its biological role in response to change or disease.

Qualitative

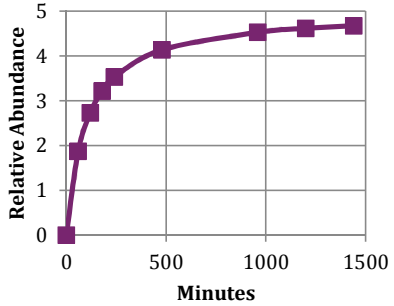


Quantitative

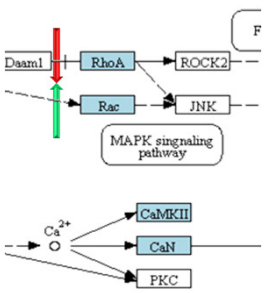


Add another dimension to any experiment by determining the relative abundance of each identified protein

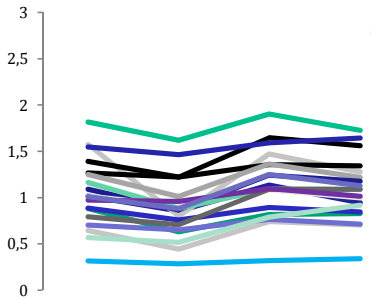
Alterations in expression can reveal a meaningful biological pattern not apparent in a pure identification experiment, which provides only a list of detected proteins



Changes with time



Changes with treatment



Changes with cell line

ThermoFisher SCIENTIFIC

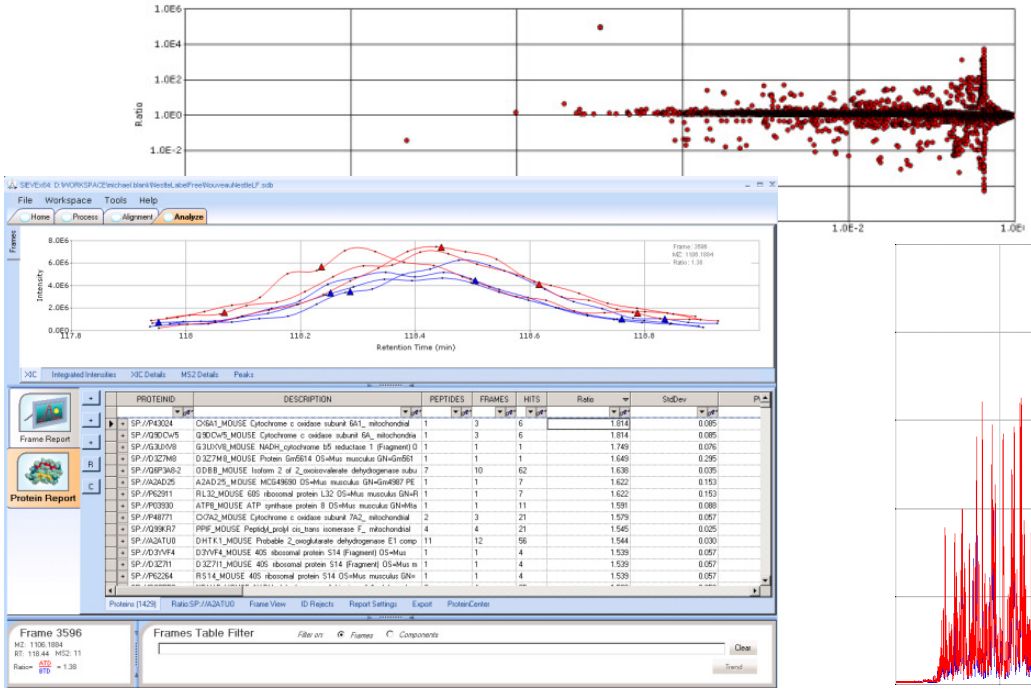
Label Free Quantitation

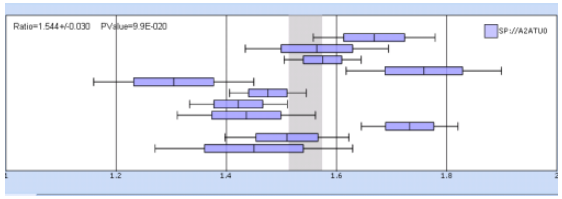
Several well established pipelines for the quantitation of label-free data from a data dependent (or DDA informed DIA experiment) exist. Among these:

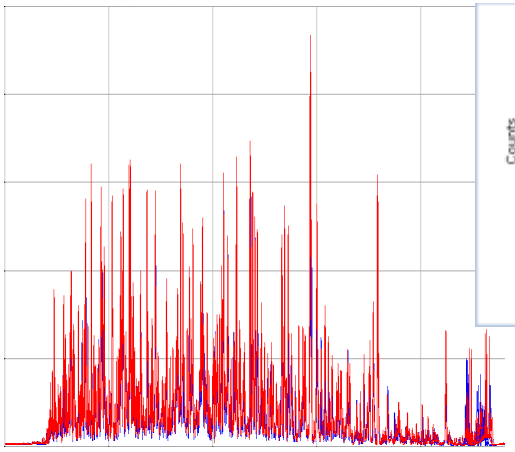
Label Free

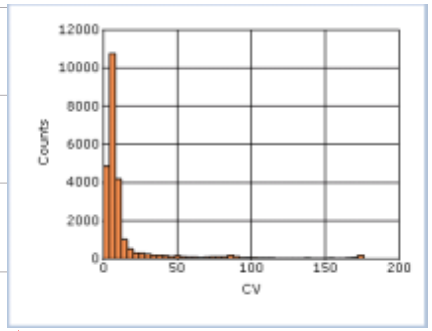
- Multiple LC/MS Runs
- Compare a few conditions
- Requires replicate sample material

SIEVE 2.2









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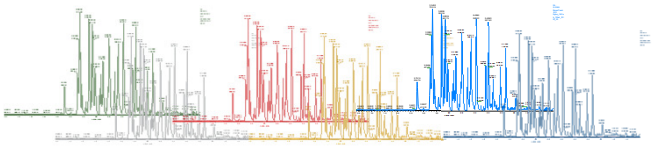
Label Free Quantitation

Problem: Requires multiple LC/MS analyses and is thus sample intensive

A differential analysis of 2 biological conditions with 3 technical replicates each would require **six** LC/MS injections and analyses:

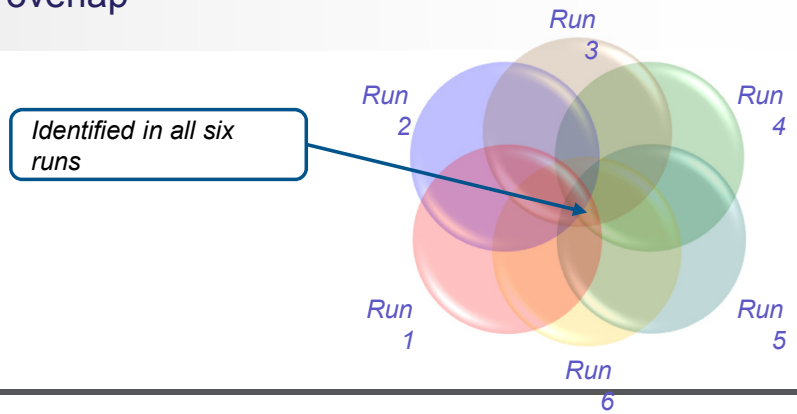


Problem: Substantial instrument time to compare only a few conditions simultaneously

Comparing just two conditions with a two hour gradient would take more than 14 hours of instrument time

Problem: Irreproducibility due to less than 100% sample overlap

Even with 85% overlap run to run
AND
4000 proteins identified in each run

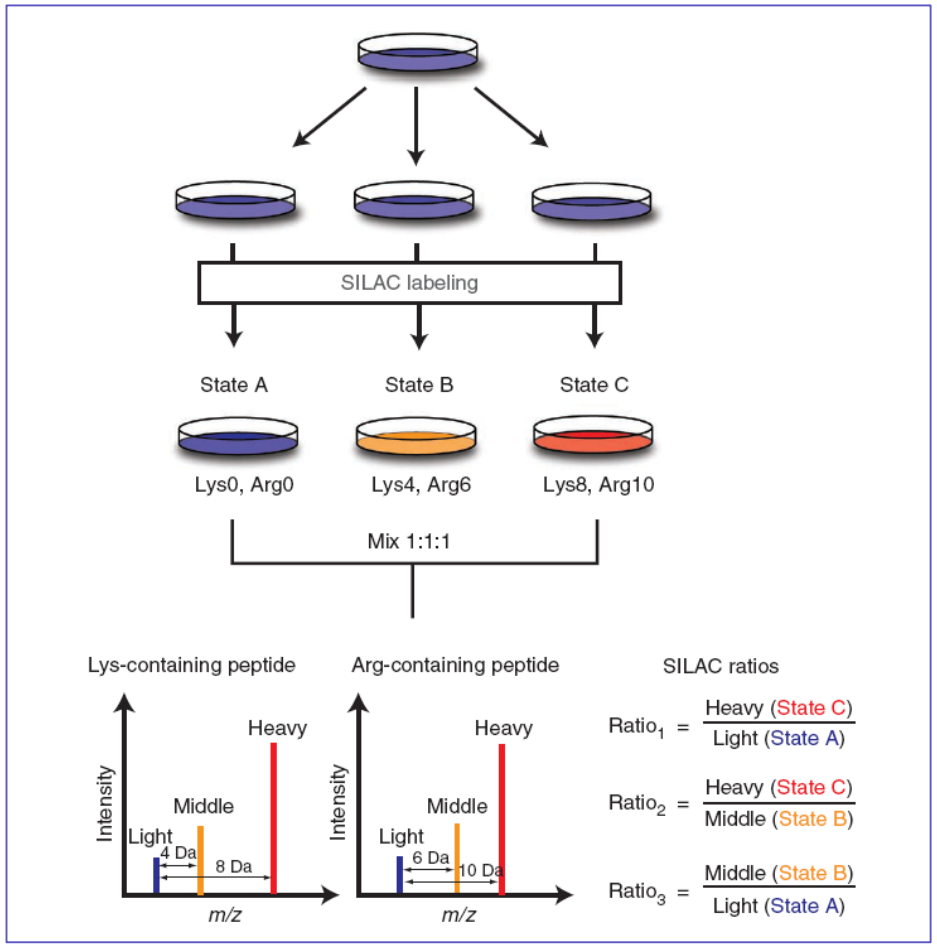


…less than 2500 common proteins

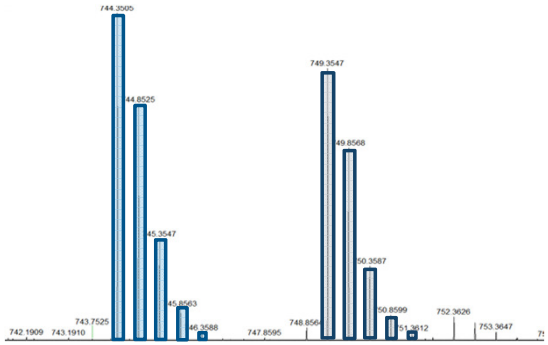
ThermoFisher SCIENTIFIC

Improving Quantitation Throughput: SILAC

SILAC Workflow



SILAC MS1 Quantitation



Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC)

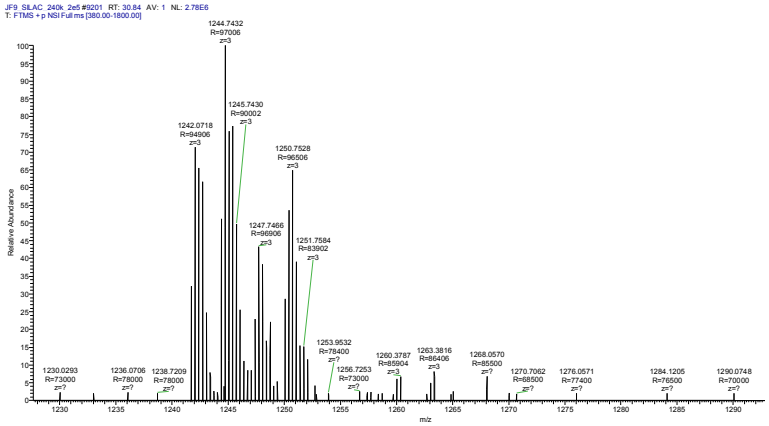
- Low variation between samples
- Requires Hi-Res Mass Spectrometry
- Compare up to 3 conditions
- Applicable to cell culture
- Peptide ID not required

Geiger T., *et al*, Nature protocols(2011):147-157

SILAC Quantitation

Problem: Increases MS1 Spectral Complexity

High resolution and intelligent precursor selection (i.e. selection of only one SILAC labeled peptide per pair or triad) is required for best quantitative results



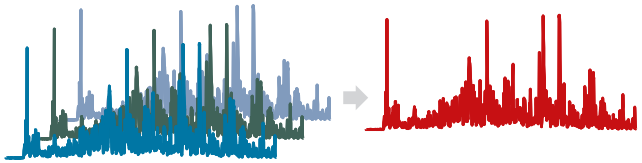
Problem: Requires cell labeling in culture

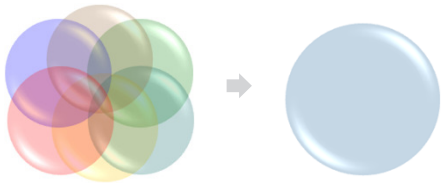
Proteins must be able to be metabolically labelled and thus is not suitable for all organisms/conditions

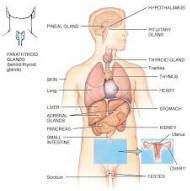
With SILAC began a trend towards increased multiplexing…

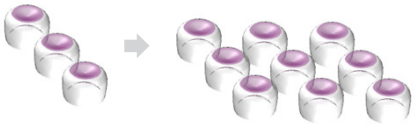
A Better Multiplexing Method– Isobaric Mass Tagging

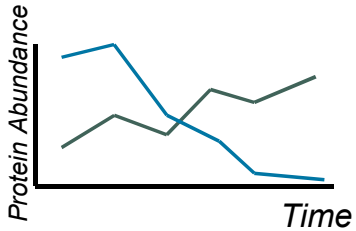
- Less MS1 Complexity
- Increased Throughput
  - Concurrent MS analysis of multiple samples
  - Less consumed samples and less instrument time
- Fewer Missing Values
  - Identification and quantification achieved in a single run
  - No worries about irreproducibility
- Sample Origin Flexibility
  - Samples can be derived from cells, tissues or biological fluids
- Increased Multiplexing
  - Compare more than 3 conditions
- Multiple Comparisons and Improved Statistics
  - Incorporate replicates with multiple conditions: dose-response, time-course, multiple tissues, subcellular fractions, etc









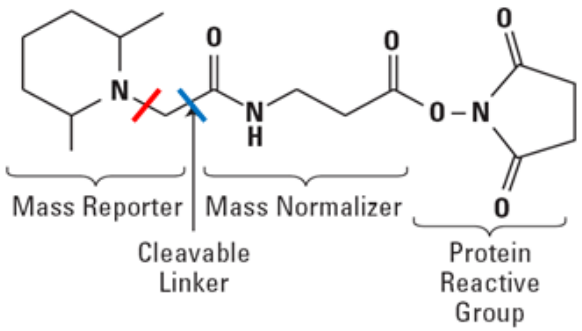


ThermoFisher SCIENTIFIC

Thermo Scientific Tandem Mass Tag (TMT) Isobaric Tag Family

TMT^0

Method Development & SRM

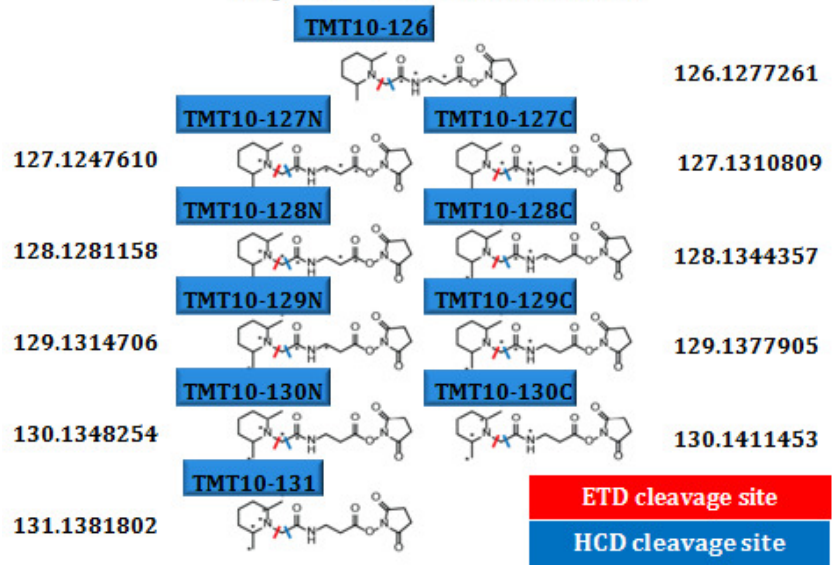


- 13C and 15N labeled reporter
- Isotopes balanced between linker region and reporter region keeping all tags exactly isobaric
- Fragments by ETD or HCD
- Up to 10 different tags
- Other reactive tags :Iodo TMT and Aminoxy TMT

TMT
Duplex Quantitation

TMT
Six Plex Quantitation

TMT
10plex Quantitation



ThermoFisher SCIENTIFIC

The Multiplexing Revolution –Not Only Consumables…

SILAC

Compare 3 Conditions

Ong SE, Blagoev B, et al.
Mol Cell Proteomics. 2002 May;1(5):376-86

TMT6plex

Compare 6 Conditions in MS^2 with amine reactive tags

Andrew Thompson , Juergen Schaefer , Karsten Kuhn, et al. *Anal. Chem.*, **2006**, *78* (12), pp 4235–4235

iTRAQ8plex

Label and compare 8 Conditions

Choe, L., D'Ascenzo, M., Relkin, et al. (2007),. Proteomics, 7: 3651–3660

TMT8 and TMT10

Concurrently quantify up to 10 sample conditions

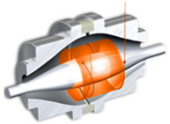
McAlister, G., Huttlin, E.L.; Haas, W.; et. al. Anal Chem. 2012. 84, 7469-7478.

2002
2005
2006
2007
2009
2011
2013
2015

Orbitrap Classic

High Resolution Orbitrap Mass Analyzer

Hu, Q., Noll, R. J., Li, H., Makarov, A., et al. (2005), J. Mass Spectrom., 40: 430–443



Orbitrap Velos

New Axial Field HCD Cell for Improved MS^2

Olsen, JV; Schwartz, JC, et al.
Mol Cell Proteomics. 2009 December; 8: 2759-2769

Orbitrap Elite

Hybrid; Single Notch MS^3; PT

Wenger CD, Lee MV, Hebert AS, McAlister Phanstiel DH, Westphall MS, Coon JJ.
Nat Methods. 2011 Oct 2;8(11):933-5

Orbitrap Fusion

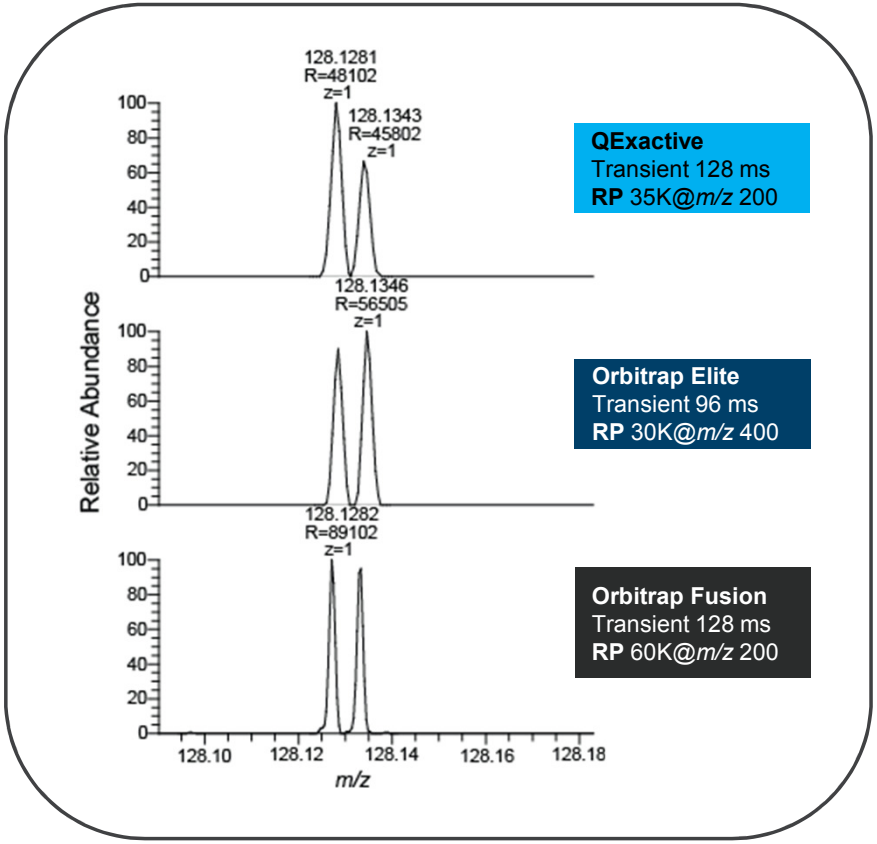
Tribrid, Parallelized Analysis, Multinotch

Erickson BK, Jedrychowski MP, McAlister GC, Everley RA, Kunz R, Gygi SP.
Anal Chem 2015 Jan 20;87(2):1241-9

Orbitrap Fusion Lumos

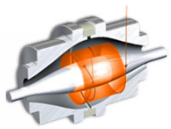
Newest Tribrid, highest sensitivity and selectivity

High Performance Depends Upon High Resolution Instruments



HIGH RESOLVING POWER IS ESSENTIAL FOR ACCURATE QUANTIFICATION OF THE TMT10PLEX REAGENTS

Result: Get accurate quantitation using the high resolution of **Orbitrap Mass Analyzer**



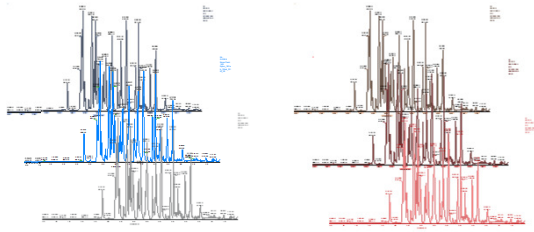
ThermoFisher SCIENTIFIC

A Real Example

Sample: Mouse mitochondrial extract untreated or treated with phosphatase inhibitor

Orbitrap Elite
- *75 um x 50 cm PepMap C18*
- *210 min gradient: 250 min run*
- *1 ug of sample on column*

LABEL FREE



Untreated
- Three Analyses
- 750 minutes
- 3 ug

Treated
- Three Analyses
- 750 minutes
- 3 ug

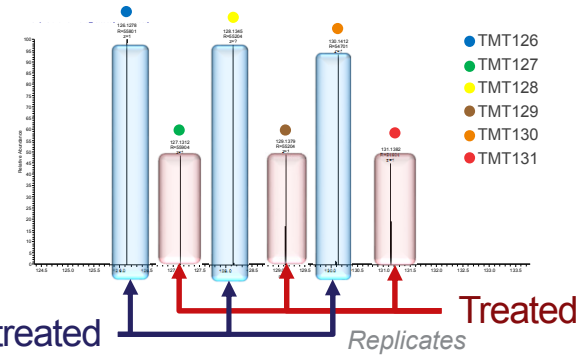
Quantified

1423 protein groups

in **1.04 days**

using **6 ug** material

TMT6



Single Analysis | 250 minutes | 1 ug

Quantified

1310 protein groups

in **4.16 hours**

using **1 ug** material

Thermo Poster Note : Liver Mitochondria Proteomics Employing High –Resolution MS Technology; J.Ho. et al

ThermoFisher SCIENTIFIC

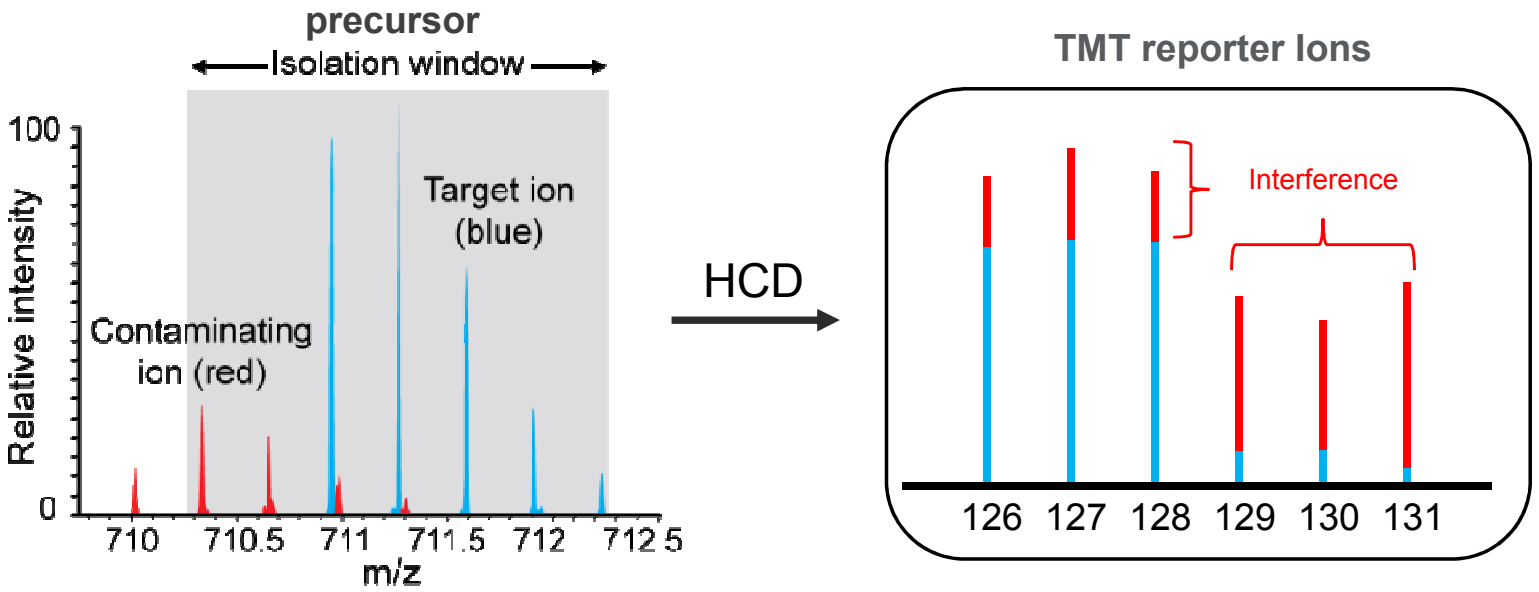
Ratio Distortion with Isobaric Multiplexing

Problem: Quantitation of low-abundance proteins in a complex background is distorted by co-isolated interfering precursor ions

research articles Journal of proteome research

iTRAQ Underestimation in Simple and Complex Mixtures: "The Good, the Bad and the Ugly"

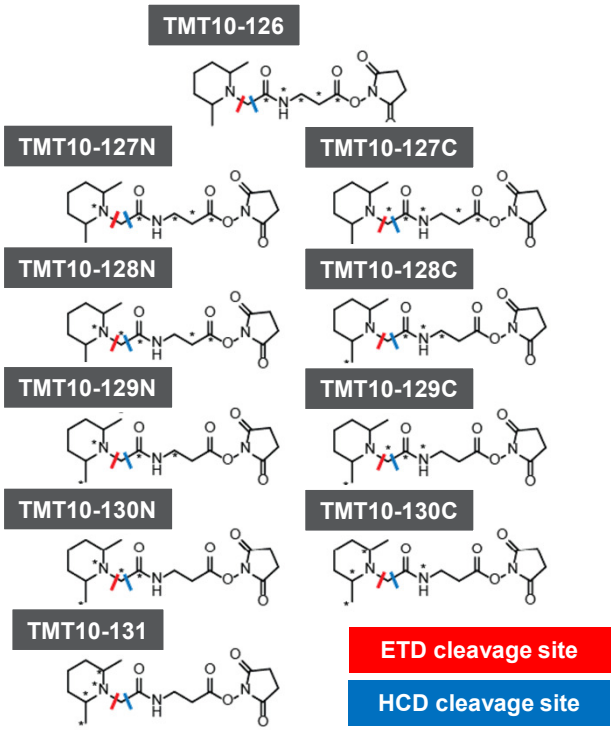
Saw Yen Ow,† Malinda Salim,† Josselin Noirel,† Caroline Evans,†,‡ Ishtiaq Rehman,‡ and Phillip C. Wright*,†

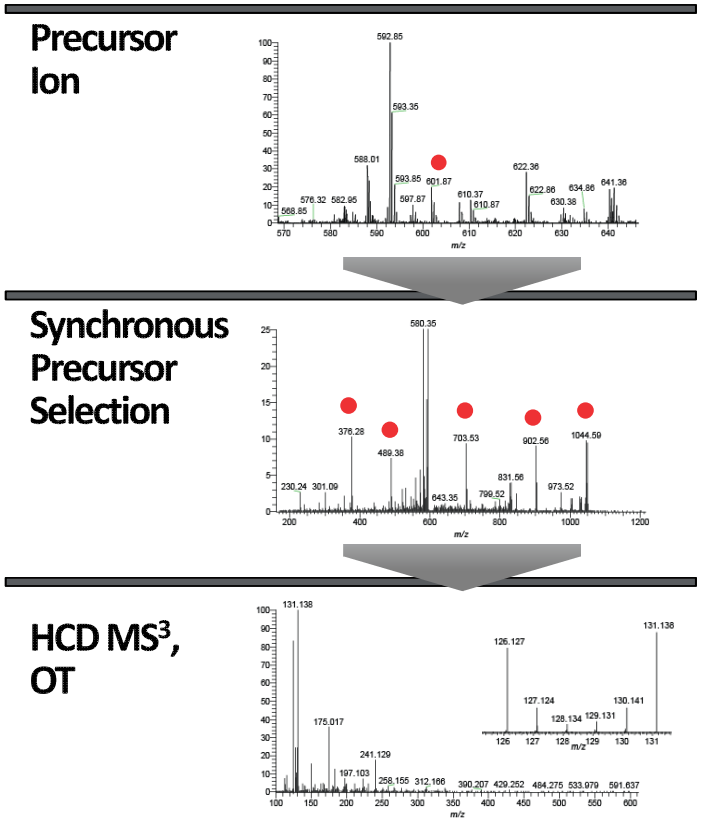


Ow, S.Y. *et al.* 2009. *JPR* 5347-5355 Ting, L. *et al.* 2011. *Nature Methods* 8: 937-940

ThermoFisher SCIENTIFIC

TMT10plex and SPS MS^3 for Quantitative Proteomics





[Achieving accurate and precise quantitation using SPS MS^3]

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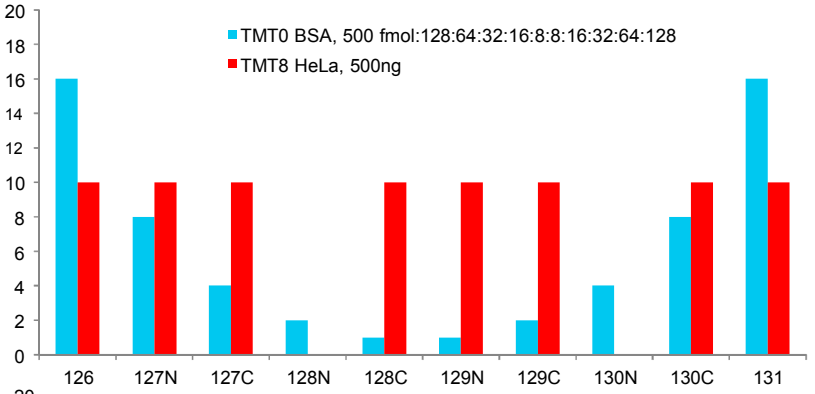
Co-isolation of Interfering Ions Affects Accuracy

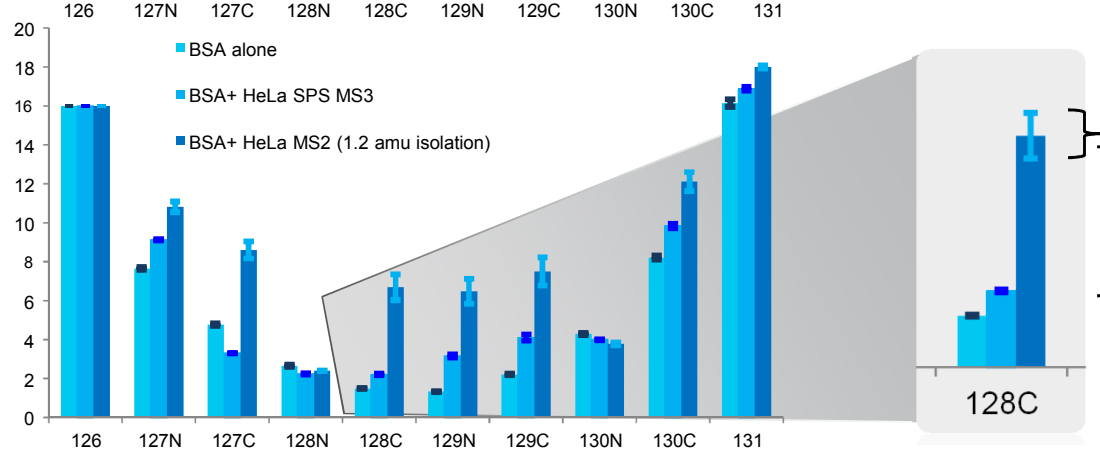
Expected



- 500fmol of BSA in 500ng HeLa digest
- Narrow precursor isolation with 4 hour gradient

Constant background

Observed



Less precision

Less accuracy (ratio distortion)

Results: Best possible accuracy and precision by reducing co-isolated interfering ions.

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Orbitrap Fusion Tribrid Mass Spectrometer

Tribrid (trī-brəd) n. three Mass Analyzers working together to produce unmatched analytical results

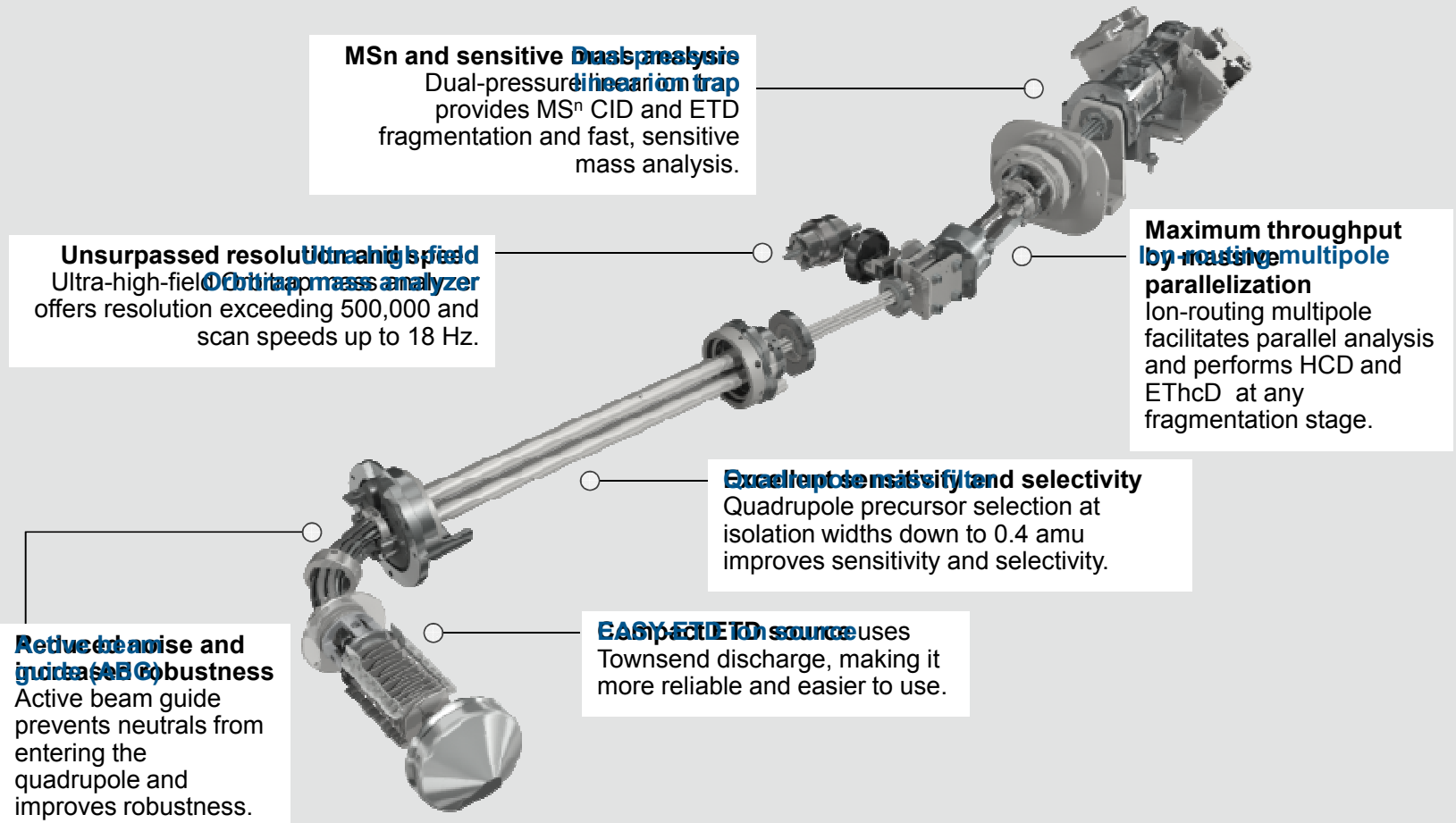
Unmatched Analytical Performance

Revolutionary
Performance

Exceptional
Versatility

Unprecedented
Usability

Orbitrap Fusion Tribrid Mass Spectrometer



MSn and sensitive mass analysis
Dual-pressure linear ion trap provides MS^n CID and ETD fragmentation and fast, sensitive mass analysis.

Unsurpassed resolution and speed
Ultra-high-field Orbitrap mass analyzer offers resolution exceeding 500,000 and scan speeds up to 18 Hz.

Maximum throughput by massive parallelization
Ion-routing multipole facilitates parallel analysis and performs HCD and EThcD at any fragmentation stage.

Excellent sensitivity and selectivity
Quadrupole precursor selection at isolation widths down to 0.4 amu improves sensitivity and selectivity.

Reduced noise and increased robustness
Active beam guide prevents neutrals from entering the quadrupole and improves robustness.

EASY-ETD source
Compact ETD source uses Townsend discharge, making it more reliable and easier to use.

ThermoFisher SCIENTIFIC

Orbitrap Fusion Tribrid Mass Spectrometer

Scan rate OTMS^2	18 Hz
Scan rate ITMS^2	20 Hz
Max resolution	500, 000 at m/z 195
Quad isolation	down to 0.4 amu
Ion trap isolation	down to 0.2 amu
Mass Accuracy	3 ppm ext, 1 ppm int
Dissociation	CID, HCD, ETD, EThcD
MSn	Up to **MS^{10}** in ion trap or Orbitrap analyzer
Analyzers	Q, OTMS, ITMS
Detectors	Ion Trap, Orbitrap
Compact	1186 x 674 x 650 mm (w, d, h)

Unmatched Analytical Performance

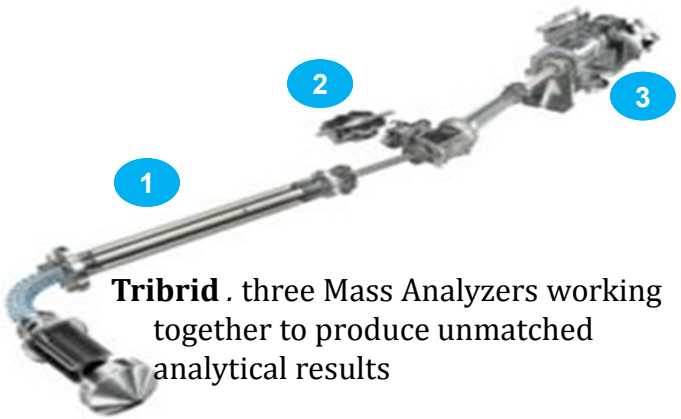
500,000 Resolution to remove spectral interferences

CID/HCD/ETD/EThcD detected by the **Ion Trap or Orbitrap** analyzer at any level of MS^n for maximum experimental flexibility

Powered by the new **Dynamic Scan Management** architecture that ensures efficient operation of the mass spectrometer

Orbitrap Fusion Lumos Tribrid Mass Spectrometer

2015



Tribrid . three Mass Analyzers working together to produce unmatched analytical results

Unmatched Analytical Performance

Revolutionary performance

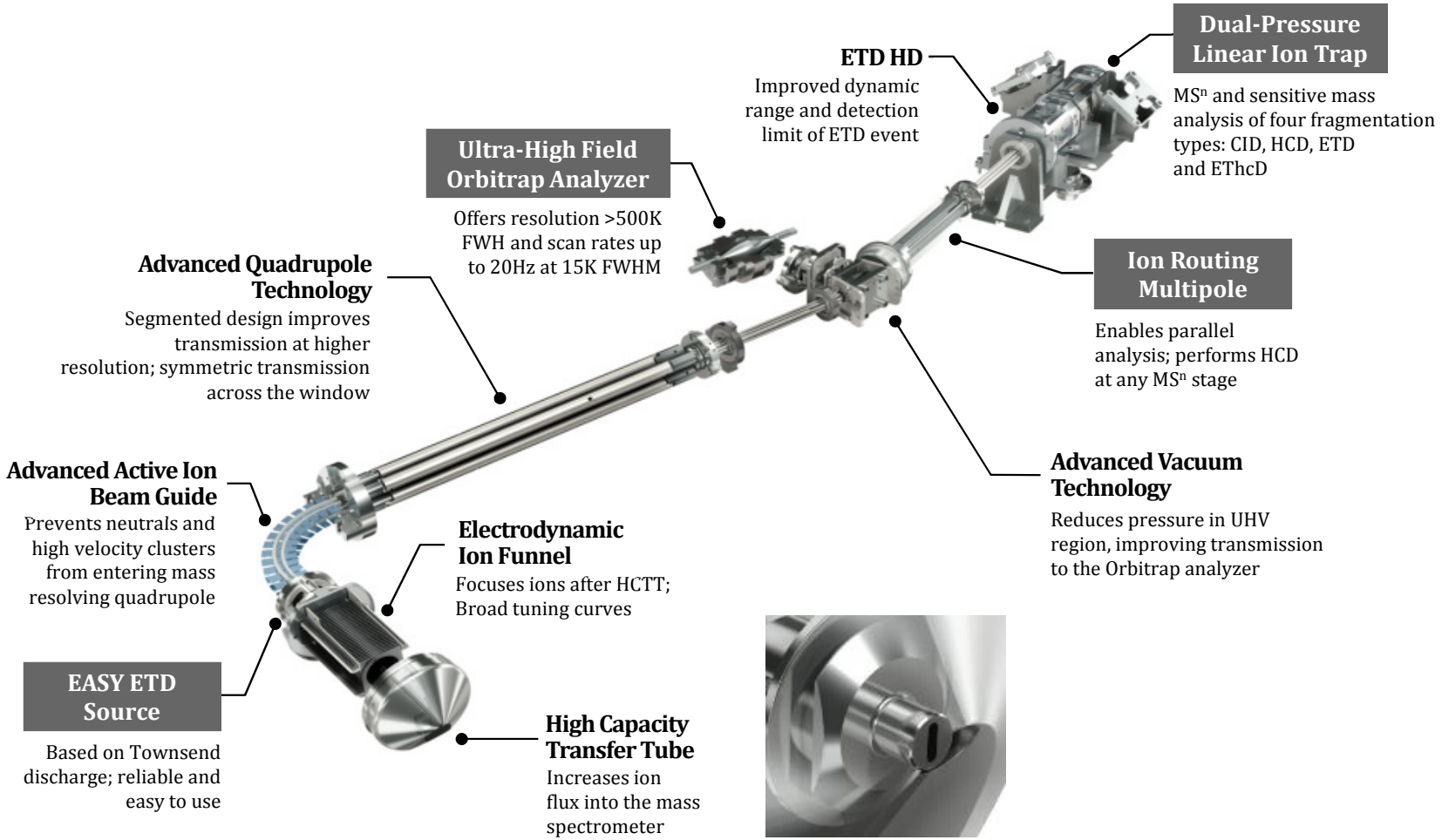
Exceptional versatility

Unprecedented usability

Highest sensitivity

ThermoFisher SCIENTIFIC

Orbitrap Fusion Lumos Tribrid Mass Spectrometer



Dual-Pressure Linear Ion Trap

MS^n and sensitive mass analysis of four fragmentation types: CID, HCD, ETD and EThcD

ETD HD

Improved dynamic range and detection limit of ETD event

Ultra-High Field Orbitrap Analyzer

Offers resolution >500K FWH and scan rates up to 20Hz at 15K FWHM

Ion Routing Multipole

Enables parallel analysis; performs HCD at any MS^n stage

Advanced Quadrupole Technology

Segmented design improves transmission at higher resolution; symmetric transmission across the window

Advanced Vacuum Technology

Reduces pressure in UHV region, improving transmission to the Orbitrap analyzer

Advanced Active Ion Beam Guide

Prevents neutrals and high velocity clusters from entering mass resolving quadrupole

Electrodynamic Ion Funnel

Focuses ions after HCTT; Broad tuning curves

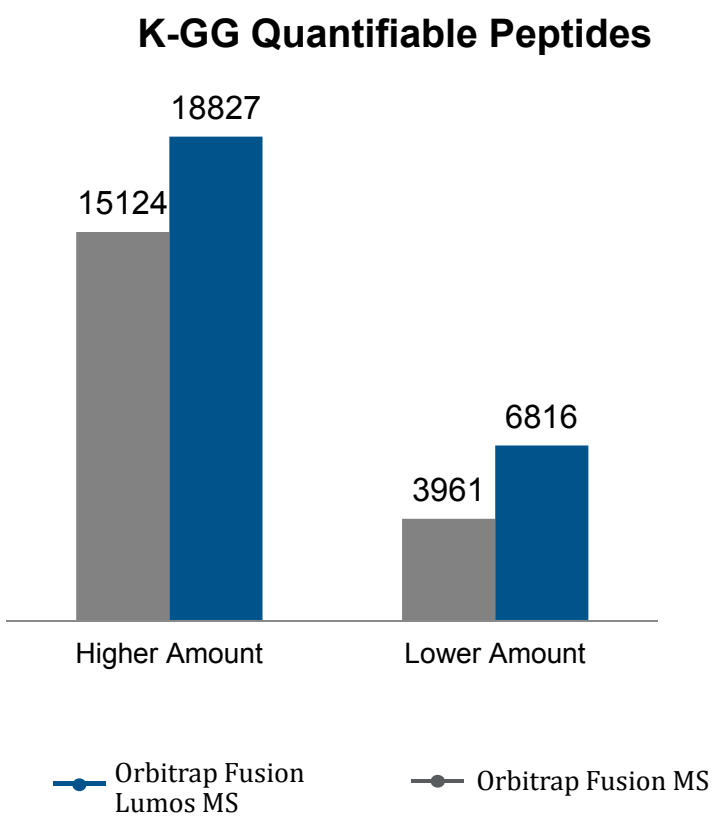
EASY ETD Source

Based on Townsend discharge; reliable and easy to use

High Capacity Transfer Tube

Increases ion flux into the mass spectrometer

Improved Low Level Quan: Ubiquitinated Peptides



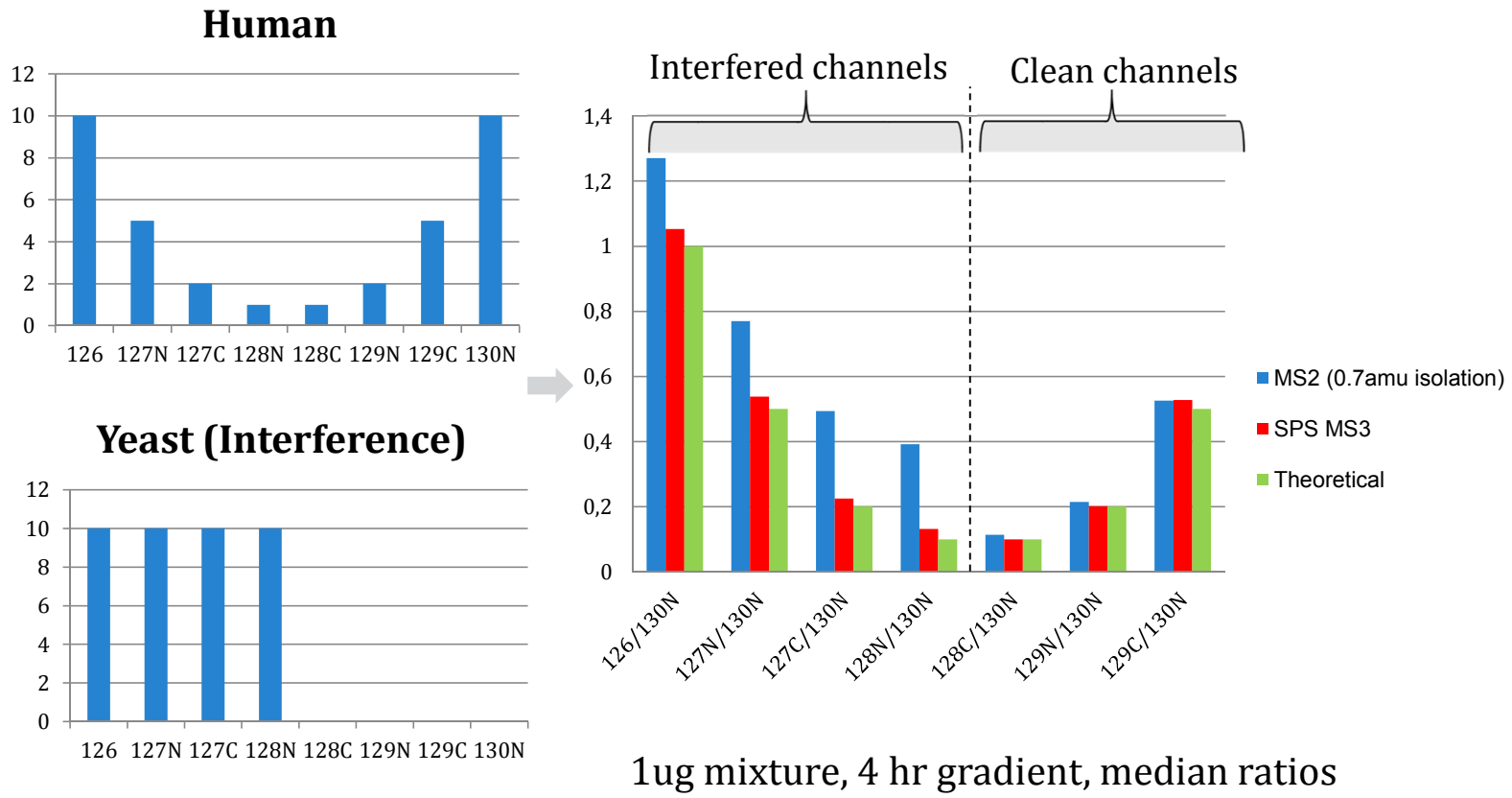
TMT10 Quantitation of Ubiquitinated Peptides

- Human HTC116 cells were treated with a proteasome inhibitor (Bortezomib) for 16 h and analyzed with TMT 10-plex (5 treated vs. 5 untreated)
- Two fractions were prepared
  - With higher amount
  - With lower amount
- 25-73% more quantifiable peptides

ASMS Lecture: Rose et al. Isobaric labeling enables 10-Plex quantitative analysis of ubiquitylated peptides: A diagnostic ion to improve identification and quantification

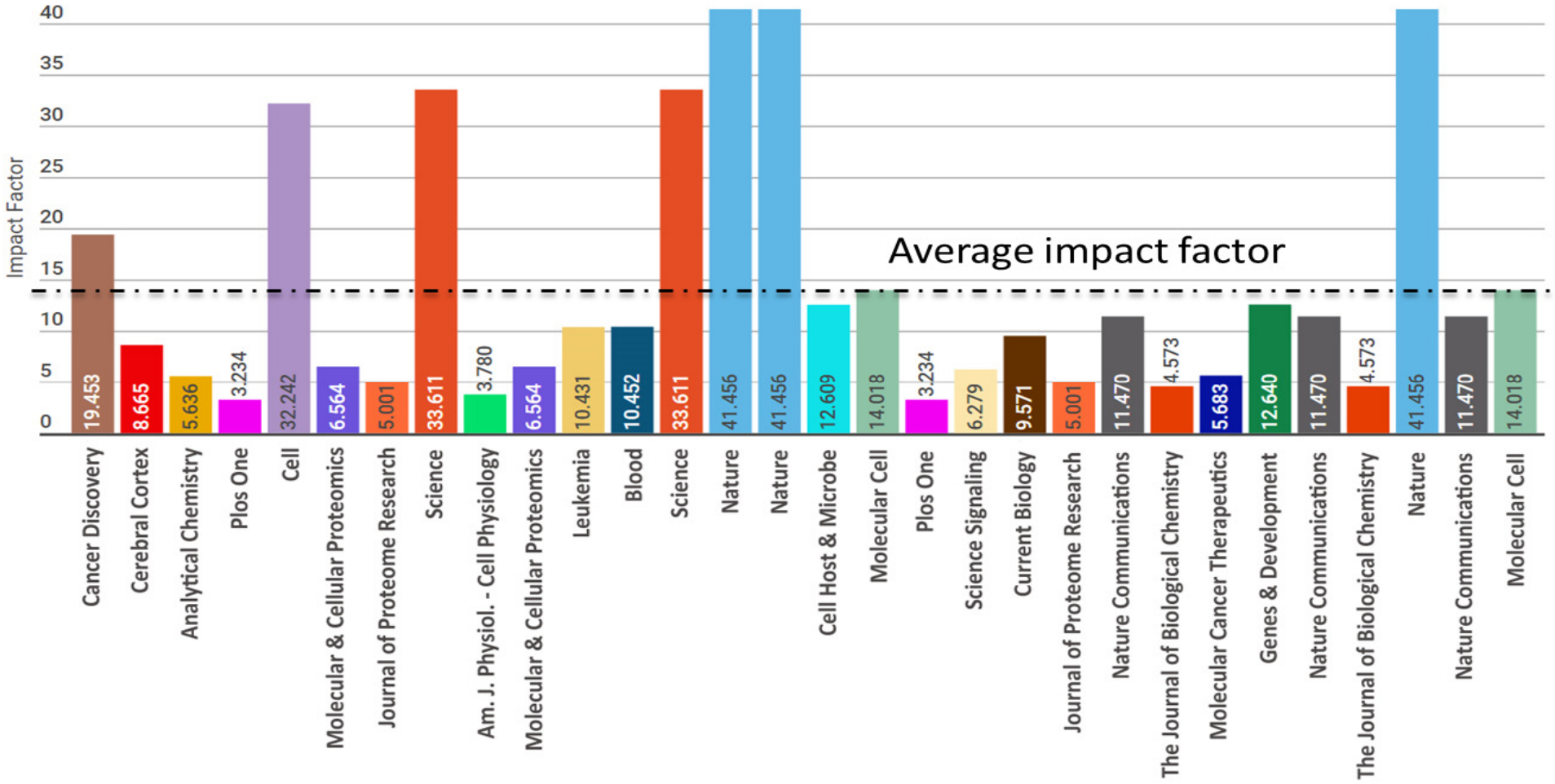
SPS MS^3 Quantification on Orbitrap Fusion Lumos MS

Results: Best possible accuracy by reducing co-isolated interferences.



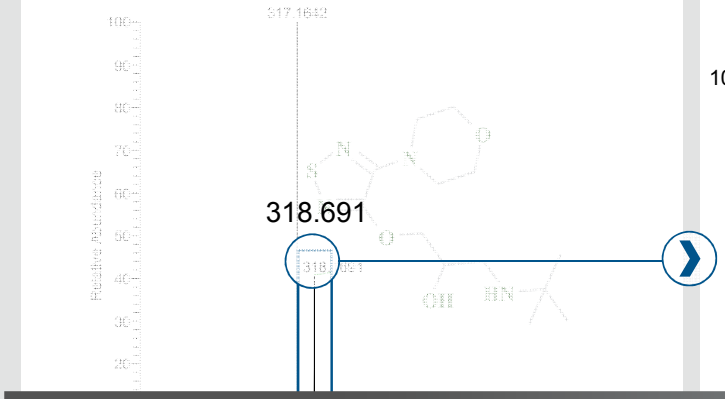
1ug mixture, 4 hr gradient, median ratios

TMT SPS MS^3 Publications Have Very High Impact

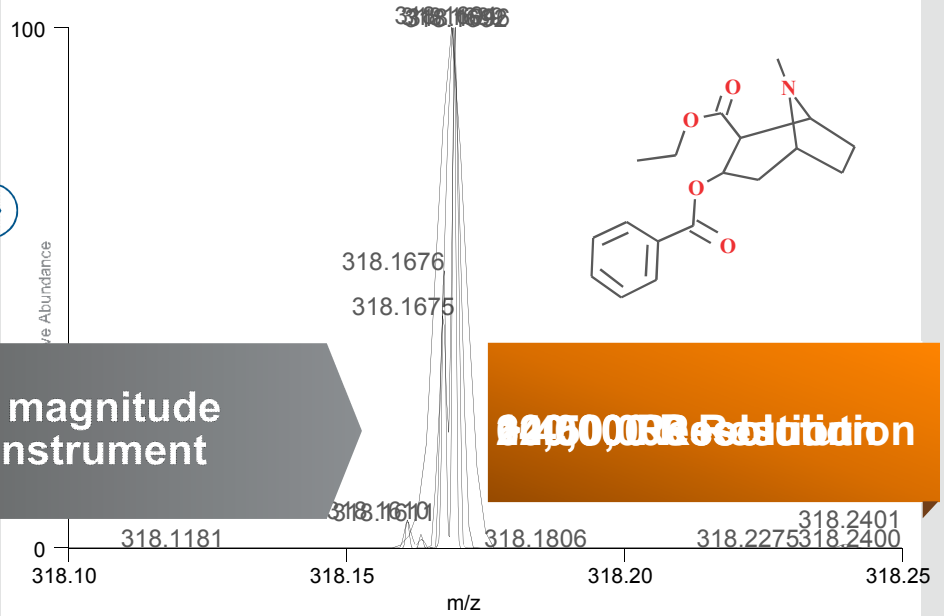


>500,000 Resolution on Orbitrap Fusion MS

Timolol: glaucoma drug,
beta blocker 0.3 ppm



Cocaethylene: Ethanol + Cocaine
1.6 ppm



This resolution is an order of magnitude better than any competitive instrument

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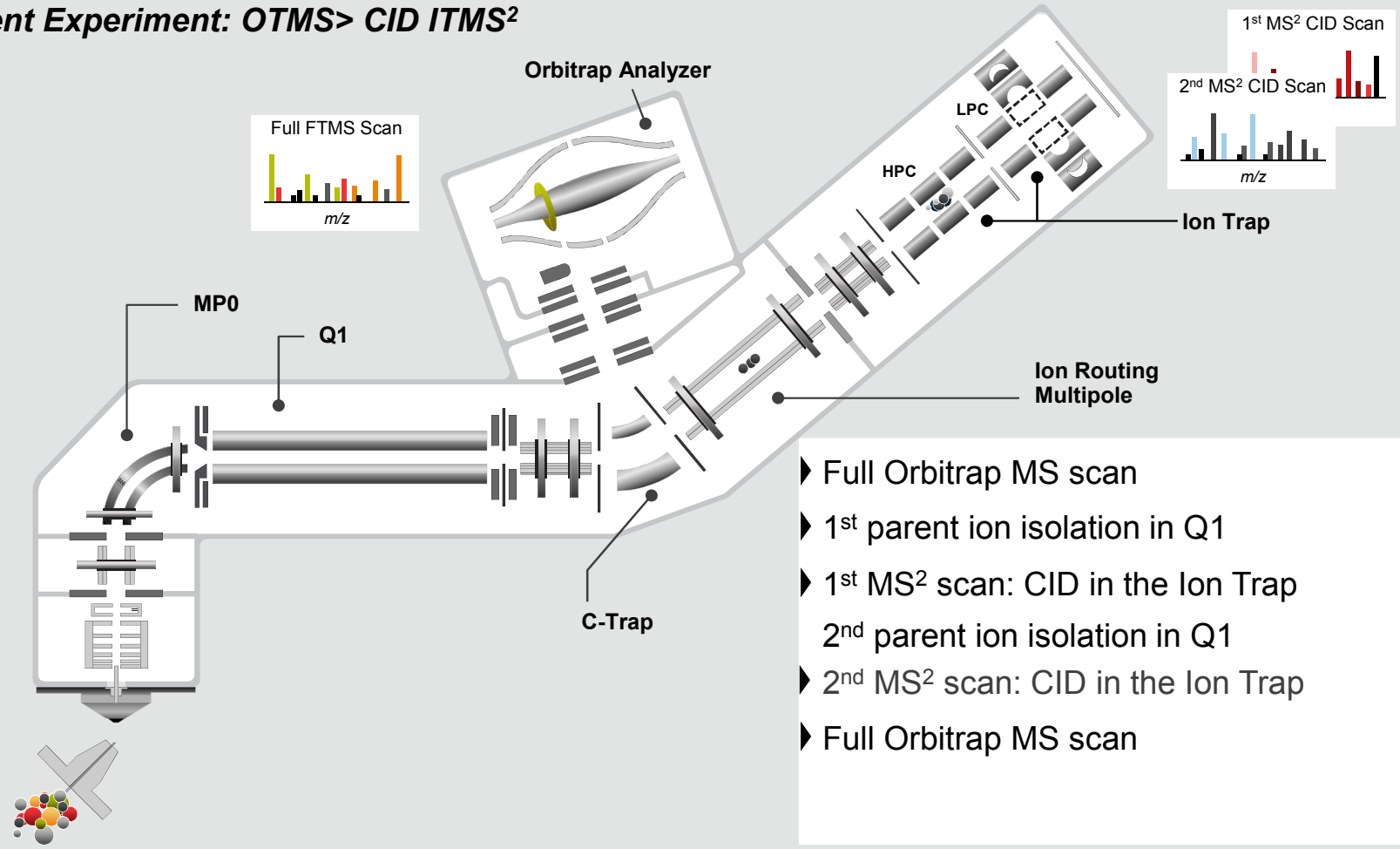
Dynamic Scan Management Ensures Efficiency

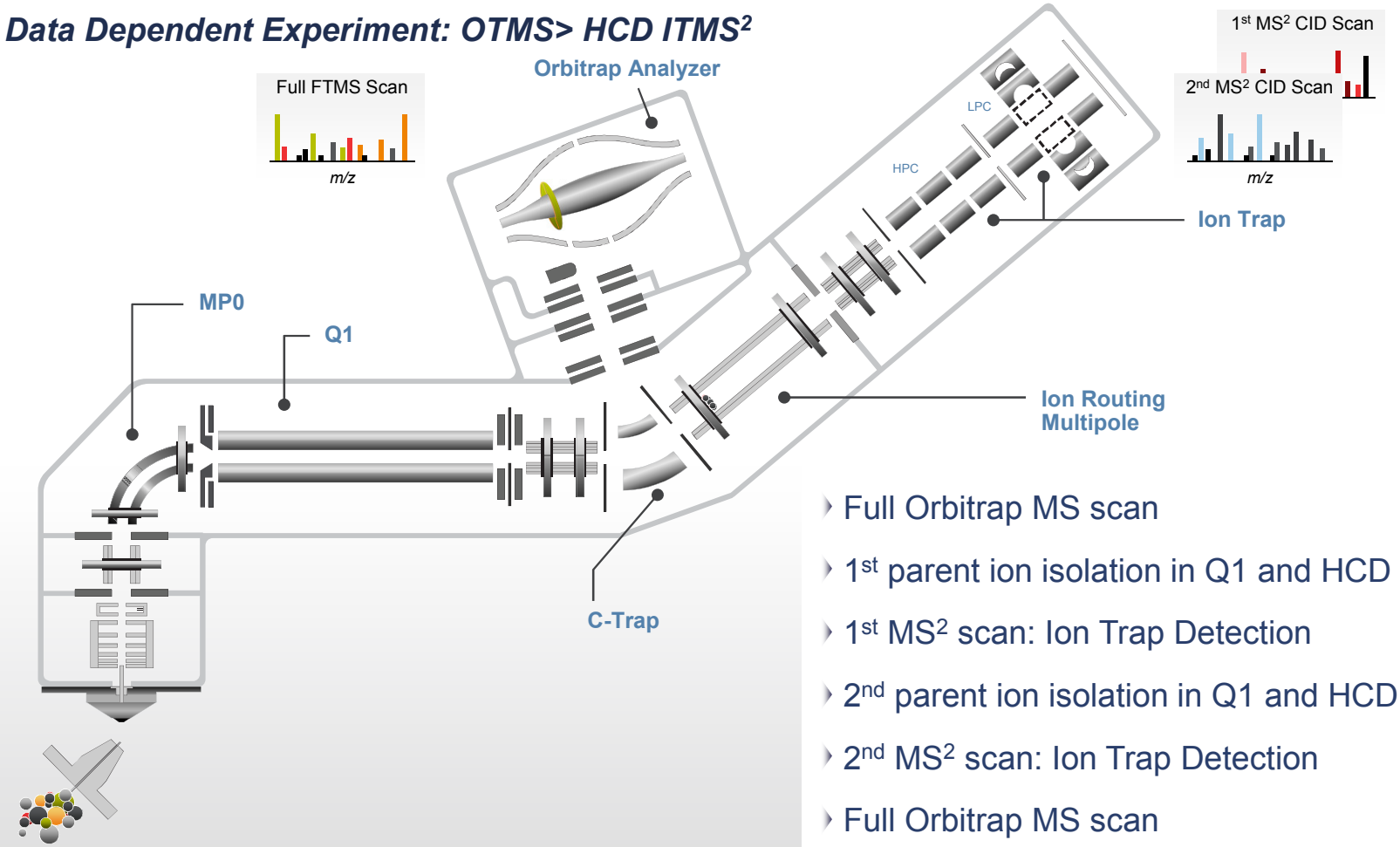
Data Dependent Experiment: OTMS> CID ITMS²



- Full Orbitrap MS scan
- 1st parent ion isolation in Q1
- 1st MS^2 scan: CID in the Ion Trap
- 2nd parent ion isolation in Q1
- 2nd MS^2 scan: CID in the Ion Trap
- Full Orbitrap MS scan

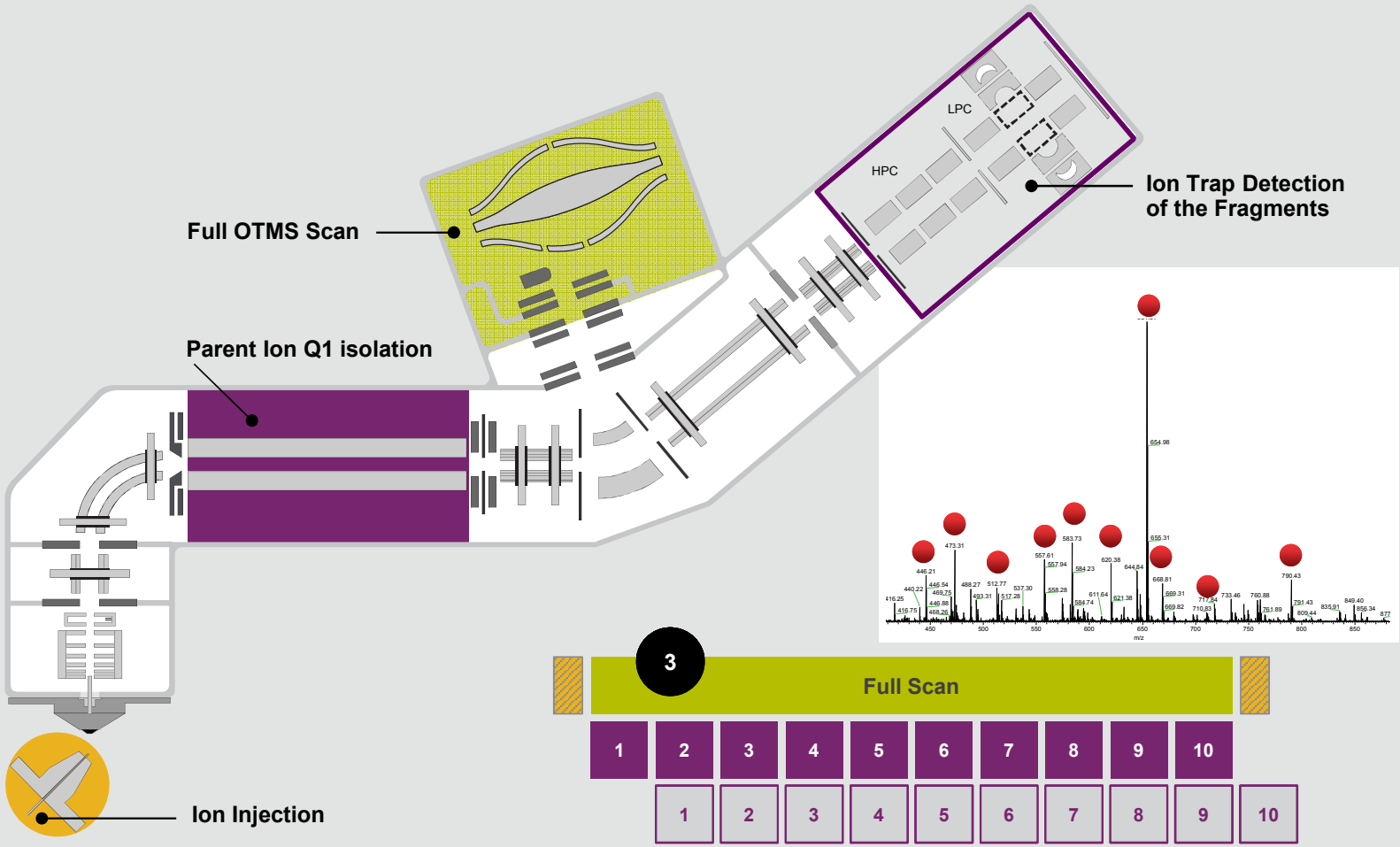
Dynamic Scan Management Ensures Efficiency

Data Dependent Experiment: OTMS> HCD ITMS²



- Full Orbitrap MS scan
- 1st parent ion isolation in Q1 and HCD
- 1st MS^2 scan: Ion Trap Detection
- 2nd parent ion isolation in Q1 and HCD
- 2nd MS^2 scan: Ion Trap Detection
- Full Orbitrap MS scan

Ion Trafficking and Dynamic Scan Management



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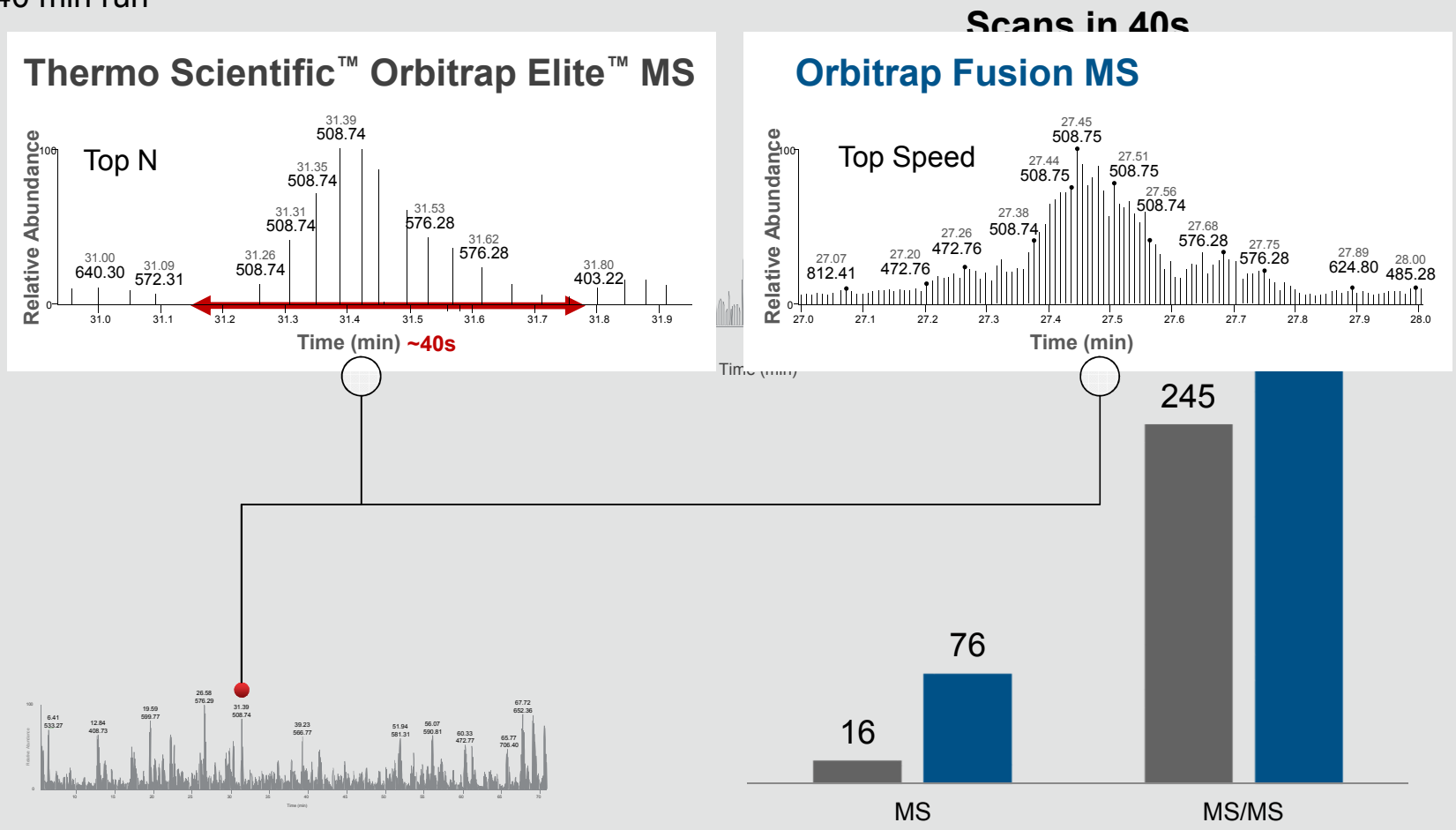
Speed = Many More Points Across LC Peak

1 ug HeLa, 140 min run



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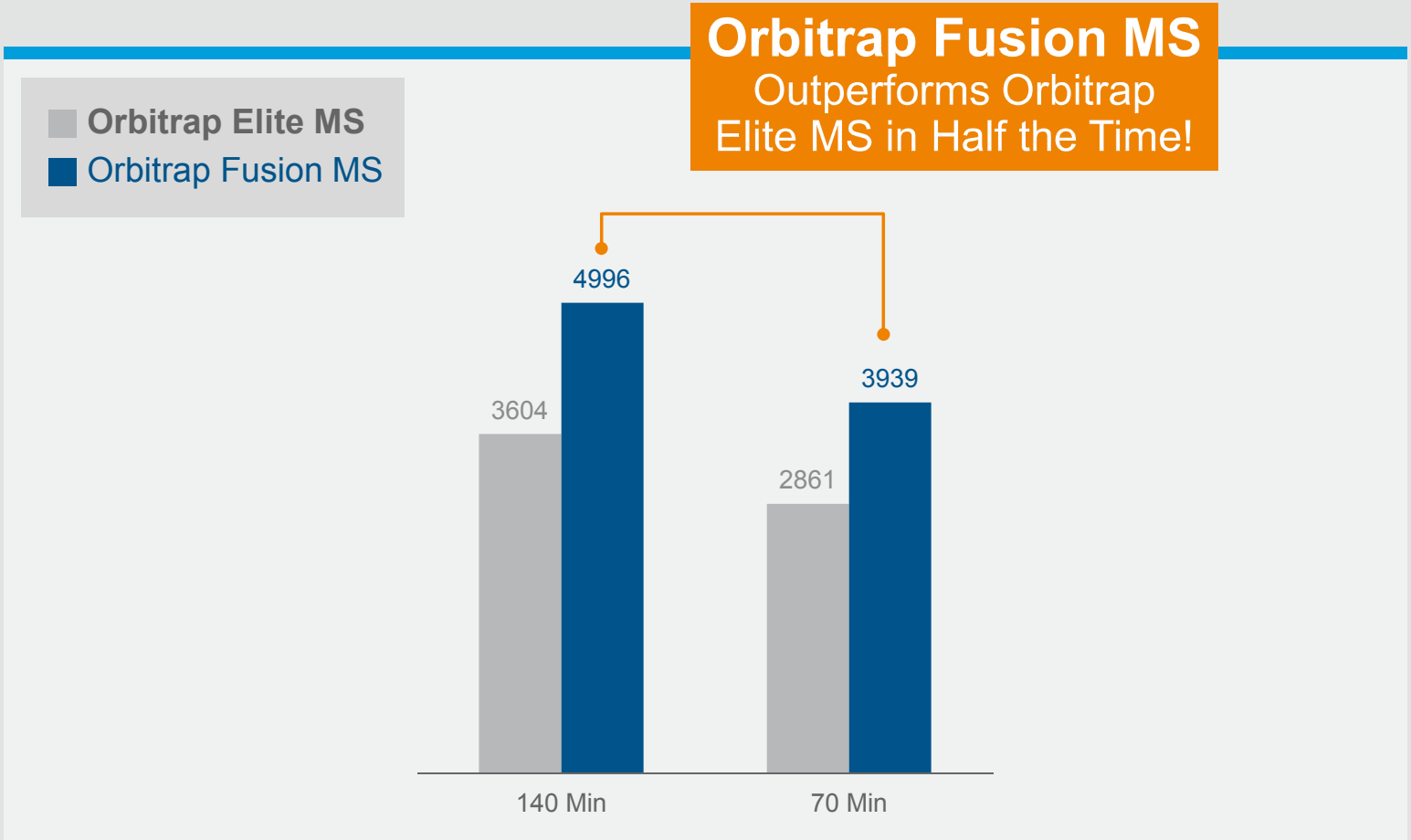
Protein Groups

1 ug HeLa



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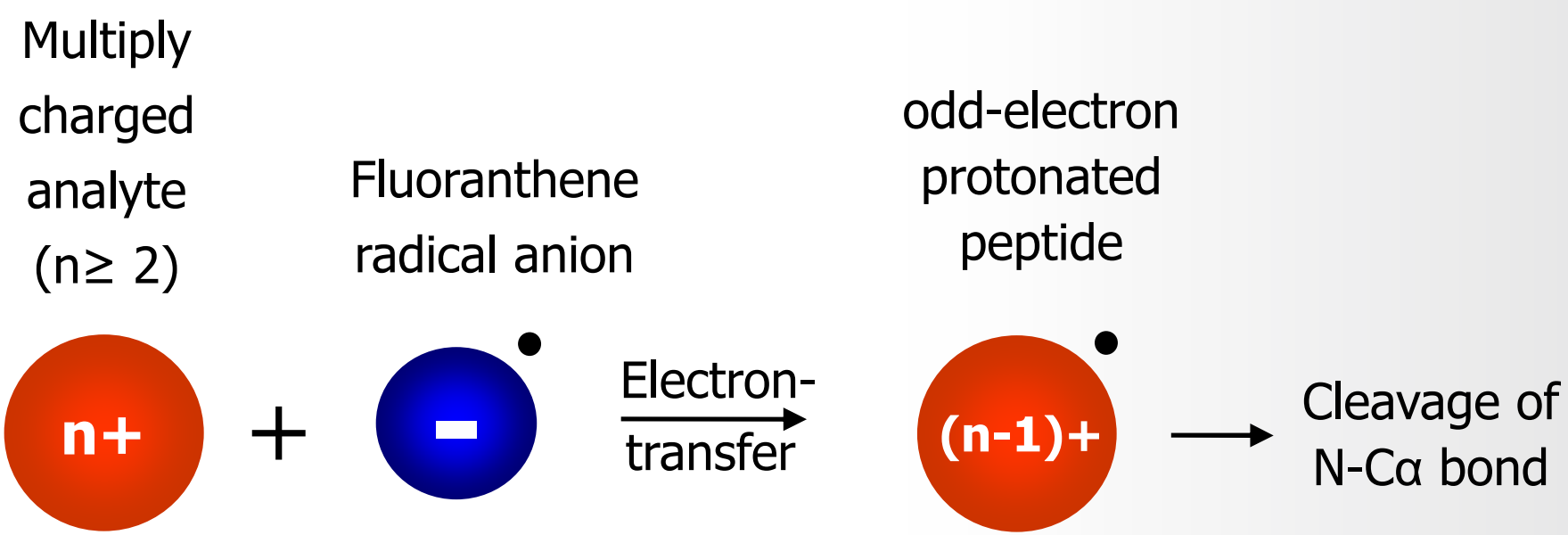
Principle of ETD



ETD versus CID

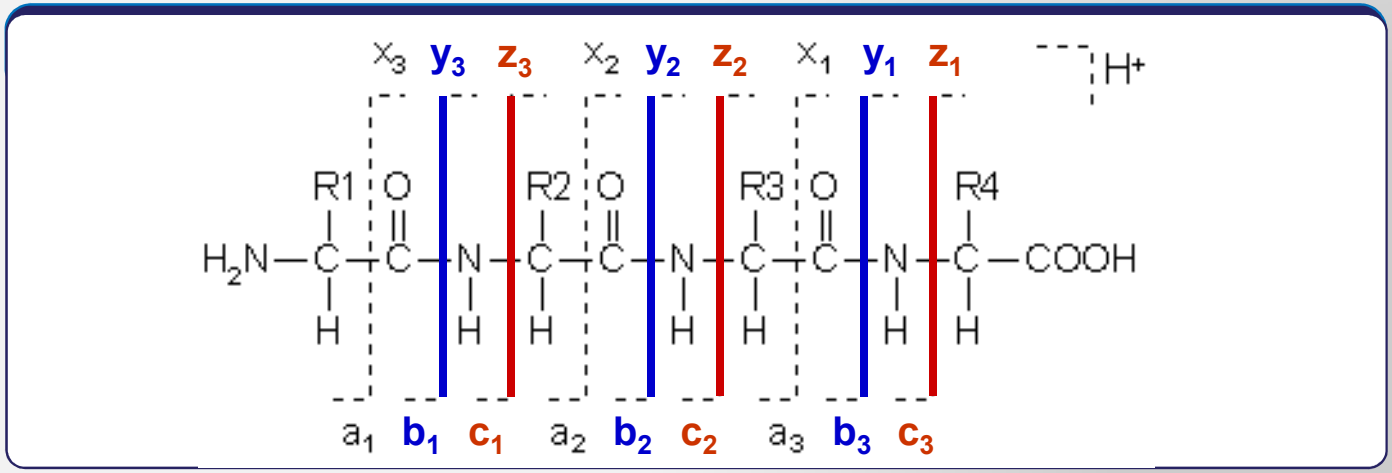
ETD

- electron transfer surpasses internal heating
- rapid bond cleavage (no energy dissipation)
- random fragmentation of peptide backbone
- leaves labile bonds like from PTMs intact

- N-Cα bond cleavage yields c- and z-ion
- preferable charge state z > 2

Conventional **(resonant) CID**

- via several collisions with Helium
  precursor ion is internally heated
- preferences for weak bond cleavages
- nearby selected amino acids (E, D, P)
  backbone cleavage is preferred
- b- and y-ions (and internal fragments)
- best fragment spectra from 2+ ions



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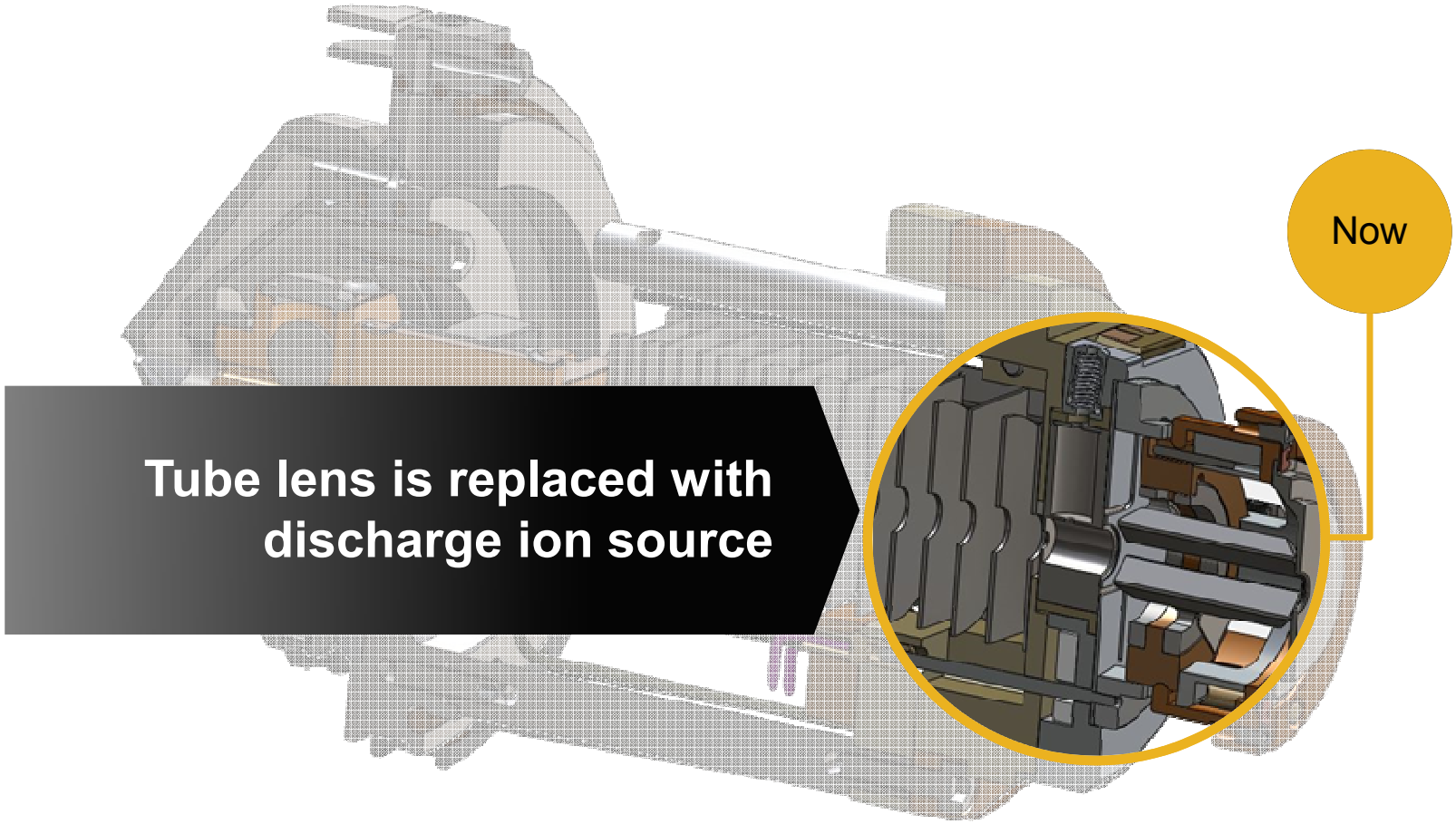
Reagent/Internal Calibrant Source



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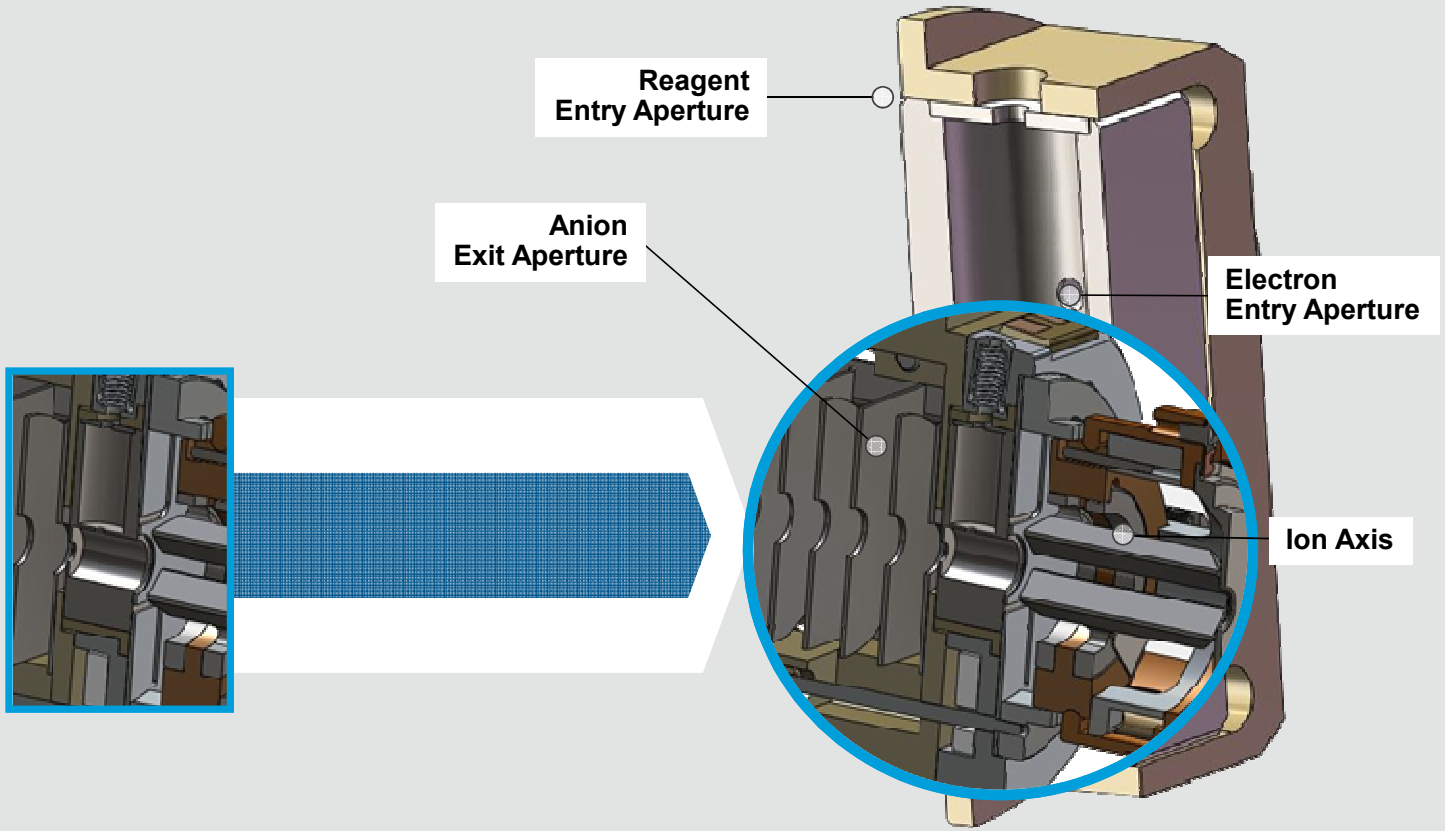
Discharge Ion Source Detail



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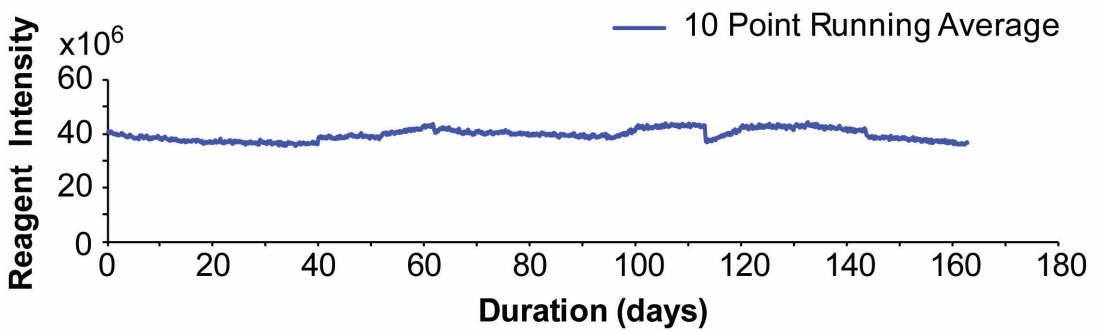
New Front Reagent Source: ETD and Internal Calibration



Compact

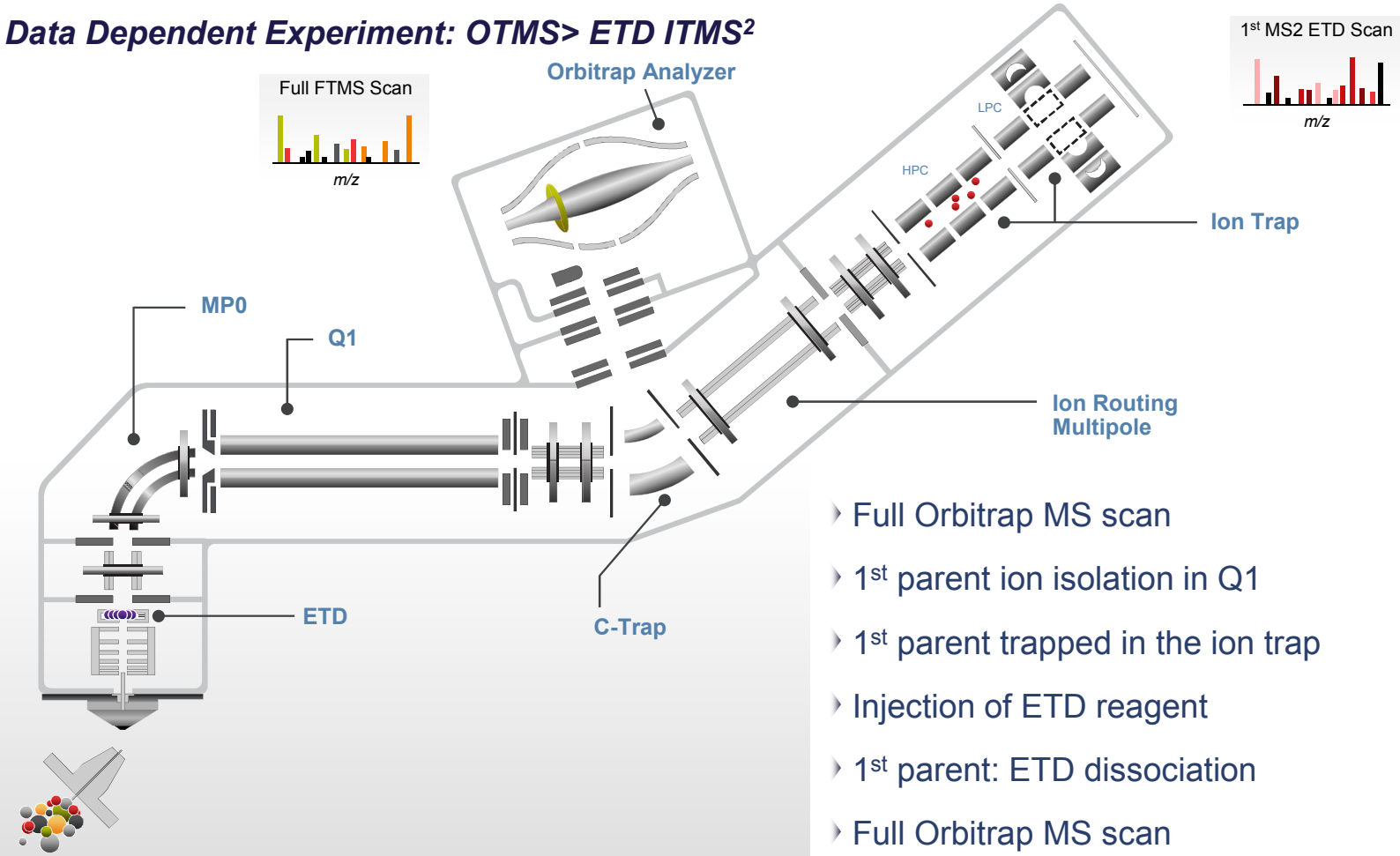
Townsend Discharge Ionization, No Filament

Stable Reagent Ion Flux

ThermoFisher SCIENTIFIC

Electron Transfer Dissociation

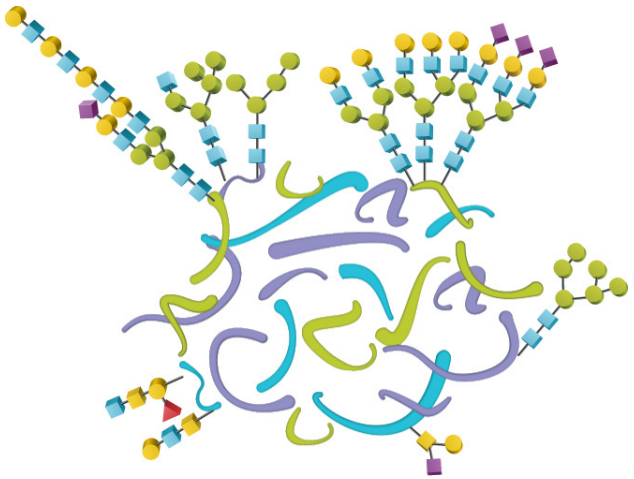
Data Dependent Experiment: OTMS> ETD ITMS²



- Full Orbitrap MS scan
- 1st parent ion isolation in Q1
- 1st parent trapped in the ion trap
- Injection of ETD reagent
- 1st parent: ETD dissociation
- Full Orbitrap MS scan

Advanced PTM Analysis

Challenges Associated with Glycopeptide Analysis

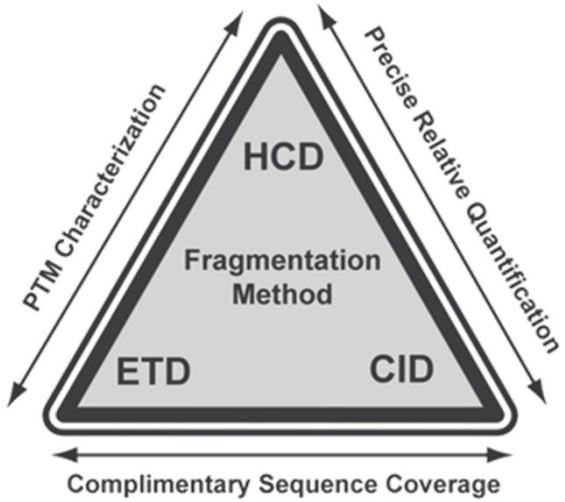


- More than one glycan attached at a single site.
  Can be up to 100 glycans.
- Difficult to detect by MS in the presence of non-glycopeptides

Huge glycopeptide impact:
Four publications, including PNAS, JBC, and Anal. Chem.

Glycosylation profile and site occupancy cannot be predicted!

Modes of MS Operation for Glycoproteomics



FT ITMS^n (CID)
General Glycan Sequencing

FT ITMS^n (ETD)
Glycopeptide Sequencing and Glycosite ID

Unmatched Analytical Performance

Glycan/Glycopeptide Sequencing

FT ITMS^n (HCD)

Glycopeptide Detection/Sequencing

FT FTMS^2 (HCD) pd-CID/ETD/HCD

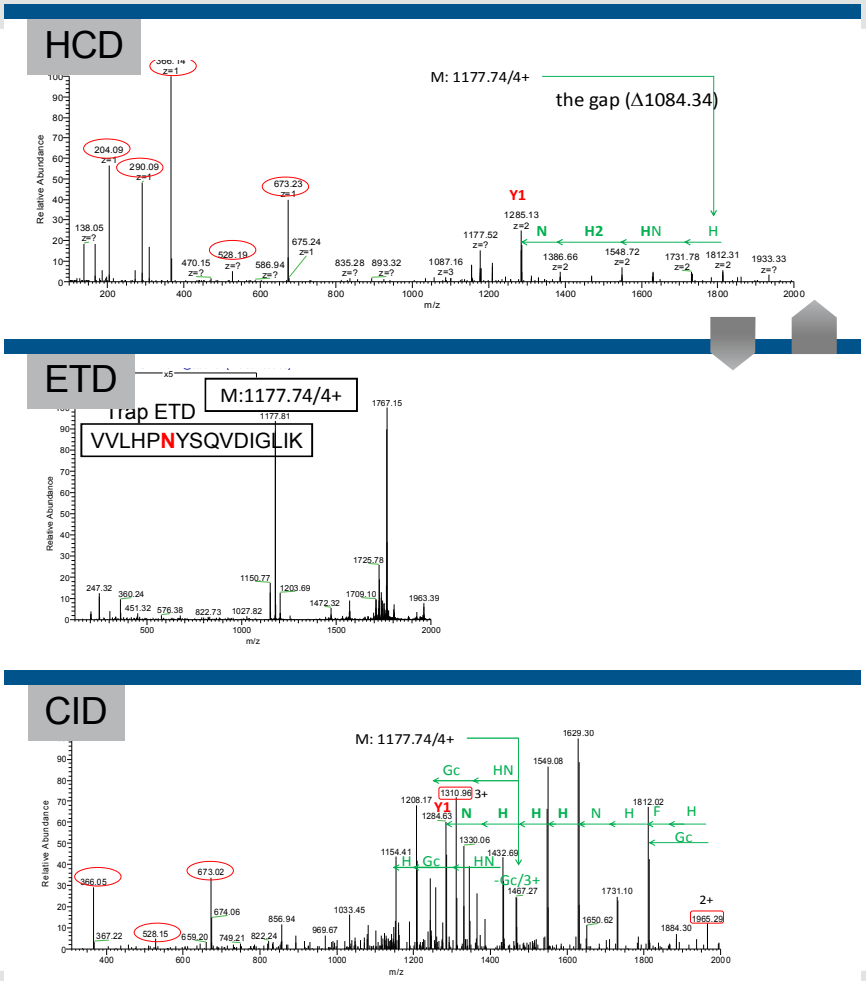
Isobaric Glycopeptide Quantification

FT ITMS^2 (ETD) SPS MS^3 HCD

Glycopeptide Sequencing Using Y1 Ion

FT FTMS^2 (HCD) ITMS^3 CID

Ultimate in Flexibility: HCDpd “Any MS2”



HCD-pd-(CID+ETD)

HCD for selective trigger

ETD for peptide sequencing

CID for glycan sequencing

Wu *et al., Anal. Chem.*,
Just Accepted

Unique to Orbitrap Fusion MS

ThermoFisher SCIENTIFIC

analytical chemistry

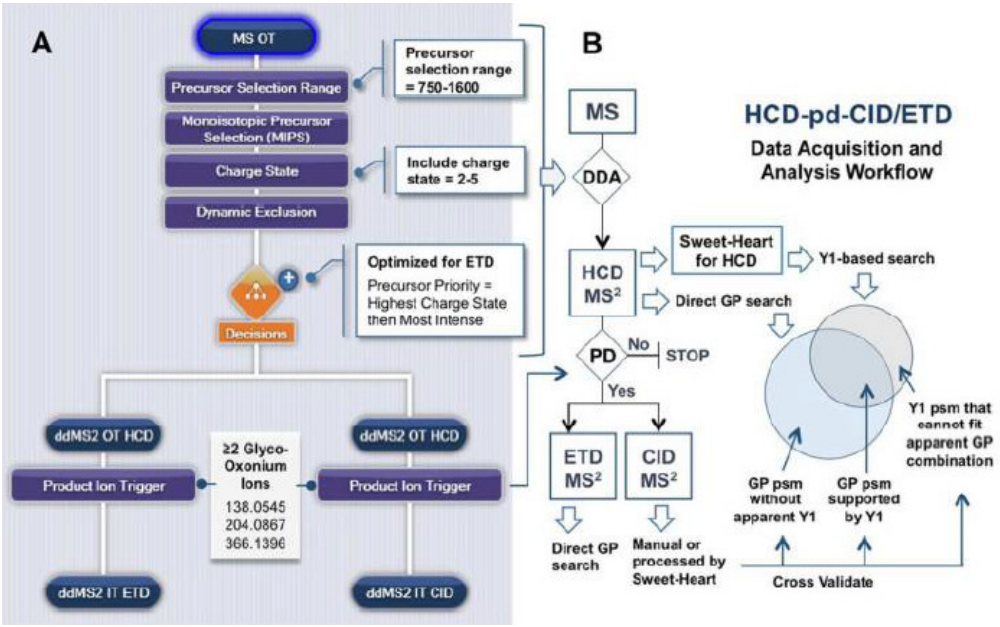
Subscriber access provided by HEALTH CANADA

Article

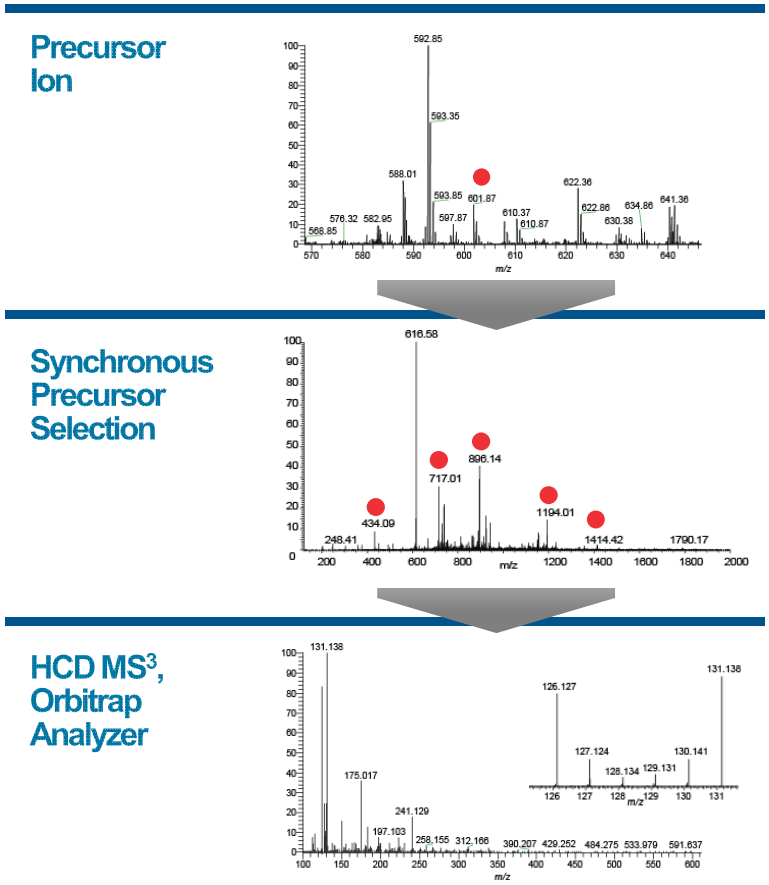
A novel LC-MS product dependent parallel data acquisition function and data analysis workflow for sequencing and identification of intact glycopeptides

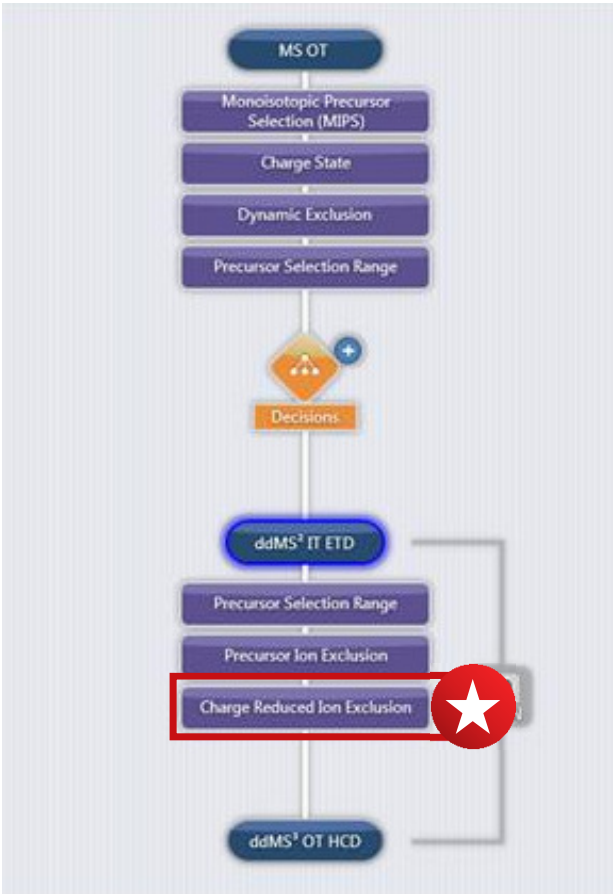
Sz-Wei Wu, Tsung-Hsien Pu, Rosa Viner, and Kay-Hooi Khoo

Anal. Chem., **Just Accepted Manuscript** • Publication Date (Web): 05 May 2014



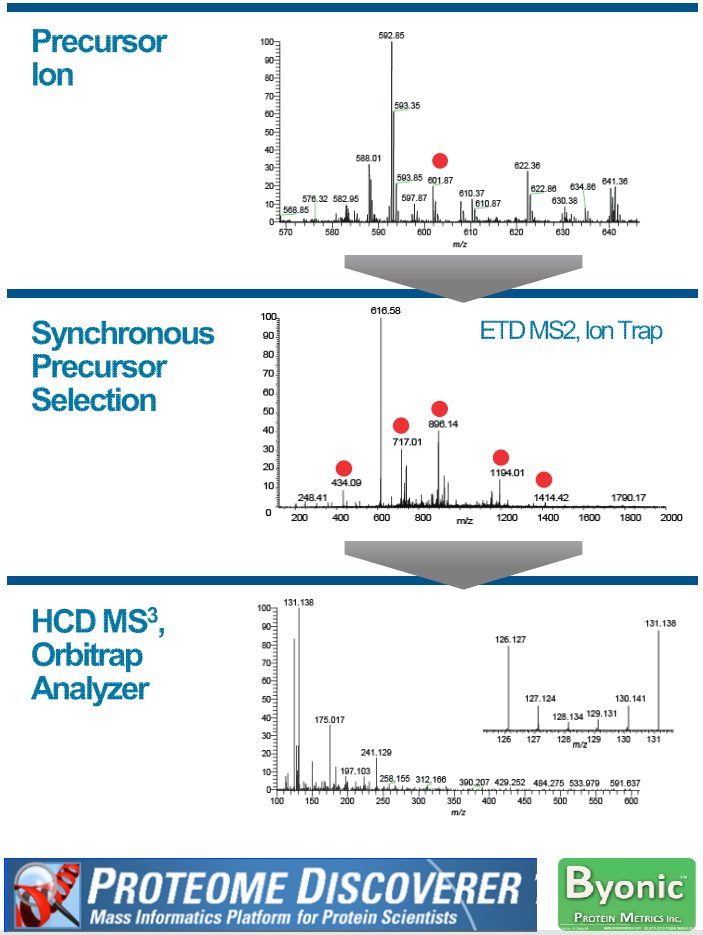
Improving ETD-SPS Quantification of Glycopeptides

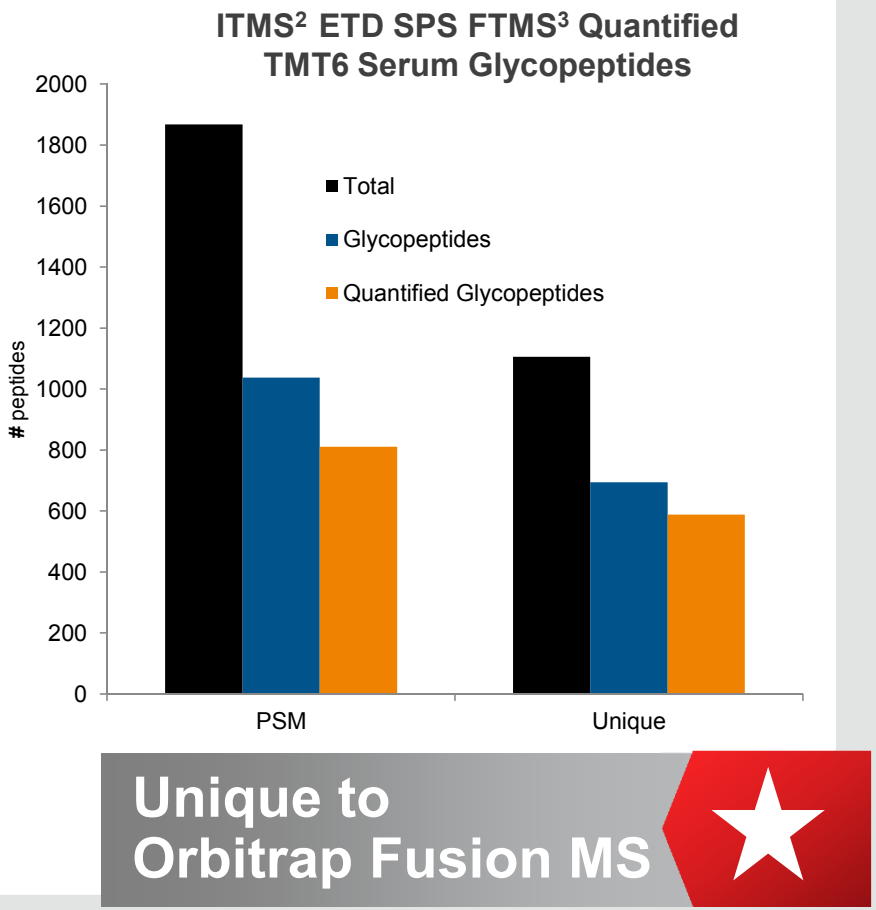




ThermoFisher SCIENTIFIC

Synchronous Precursor Selection

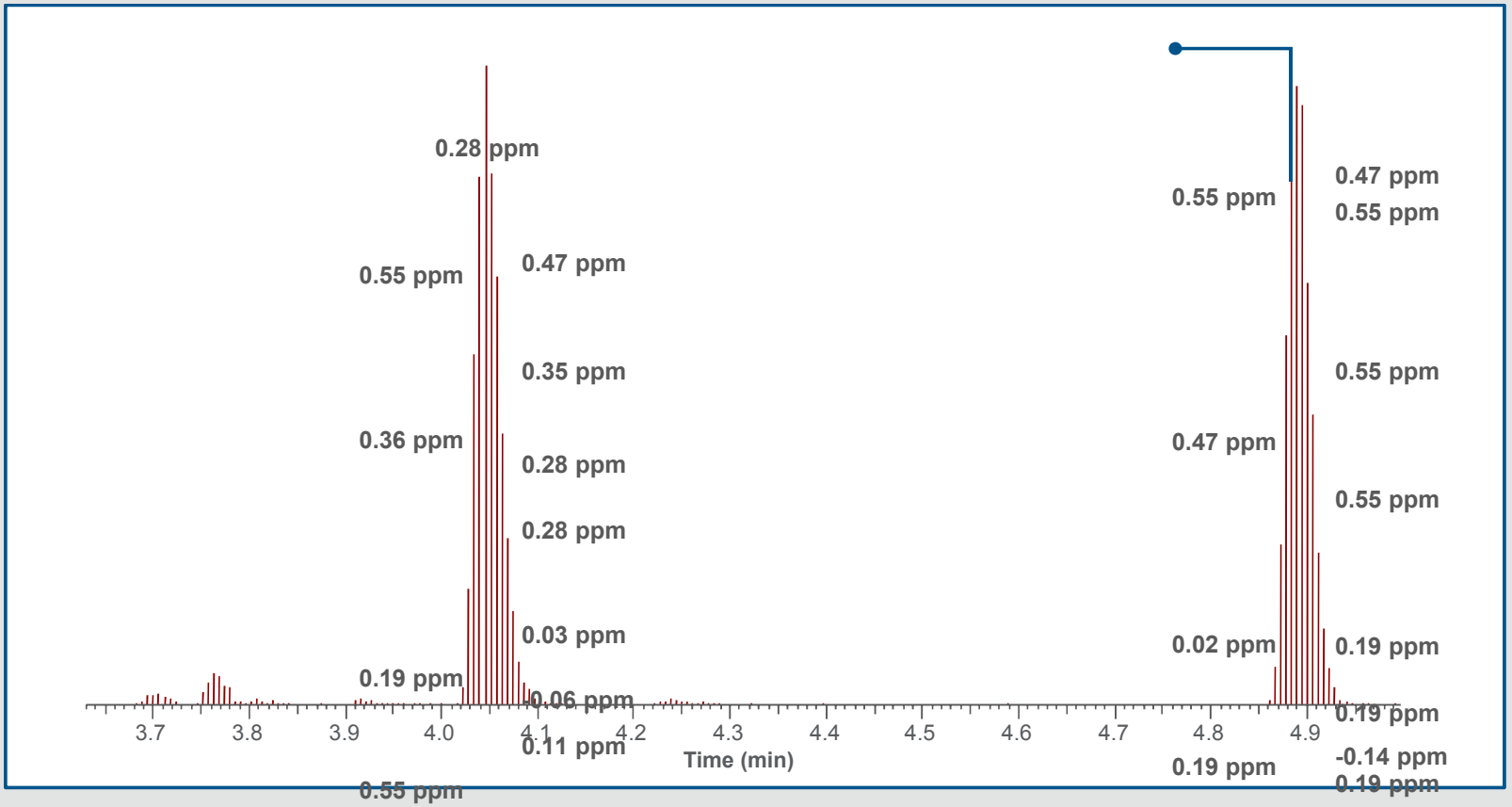




Unique to Orbitrap Fusion MS

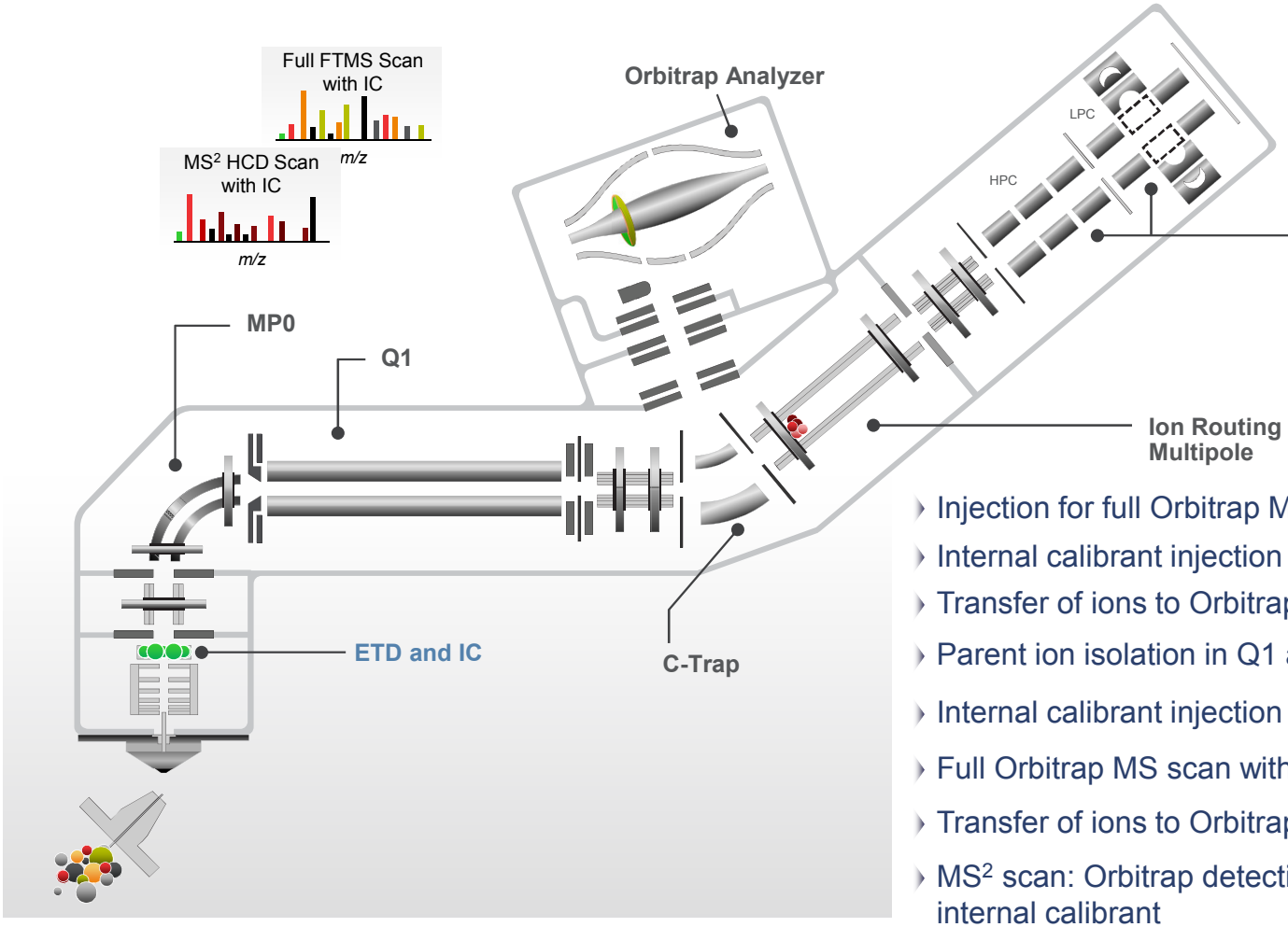
ThermoFisher SCIENTIFIC

Internal Calibration: LC/MS of Omeprazole Metabolites



ThermoFisher SCIENTIFIC

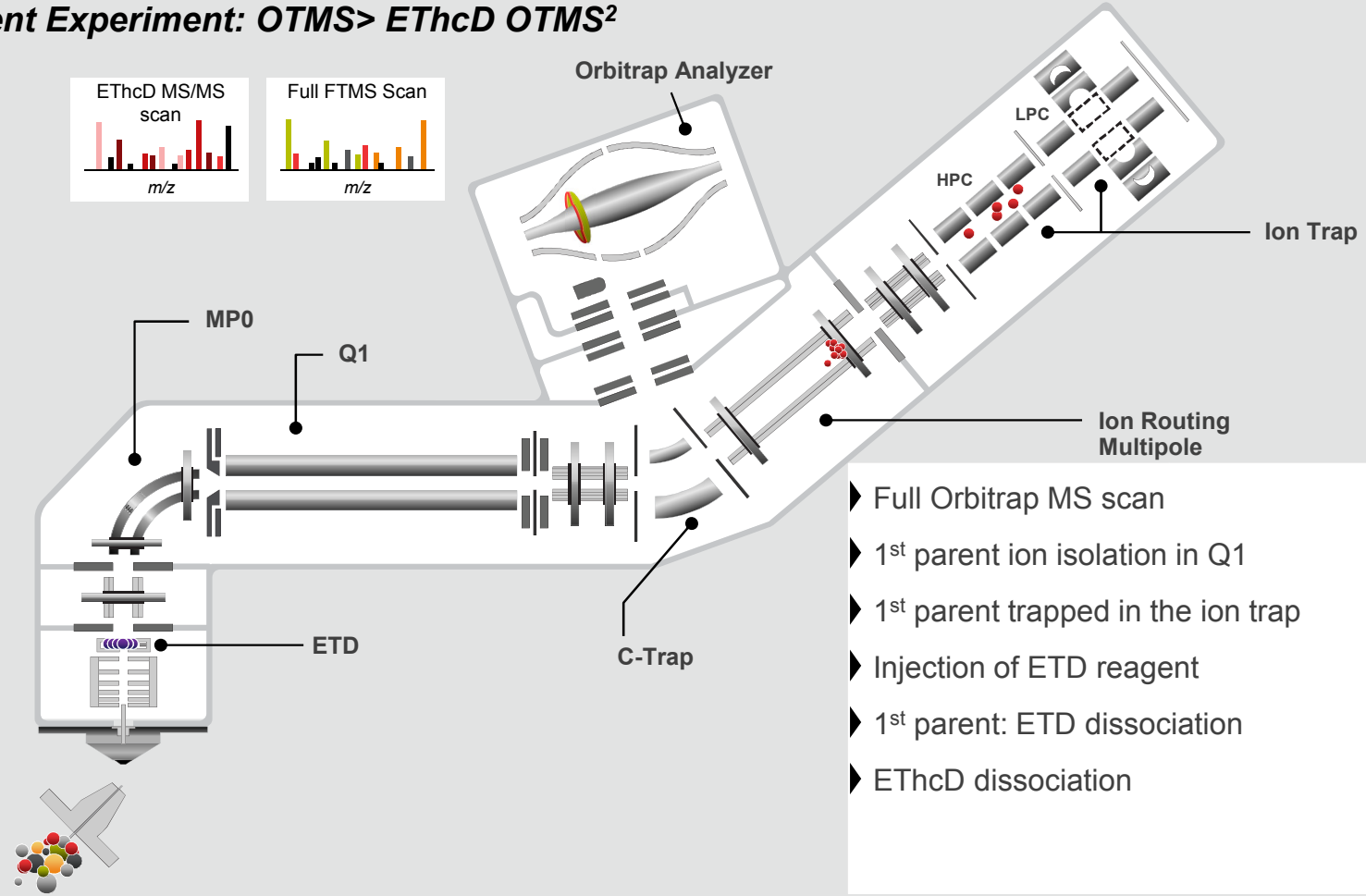
Internal Calibration of MS and MS^2 scan



- Injection for full Orbitrap MS scan
- Internal calibrant injection
- Transfer of ions to Orbitrap analyzer
- Parent ion isolation in Q1 and HCD
- Internal calibrant injection
- Full Orbitrap MS scan with internal calibrant
- Transfer of ions to Orbitrap analyzer
- MS^2 scan: Orbitrap detection with internal calibrant

EThcD

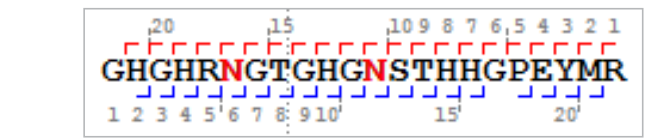
Data Dependent Experiment: OTMS> EThcD OTMS²

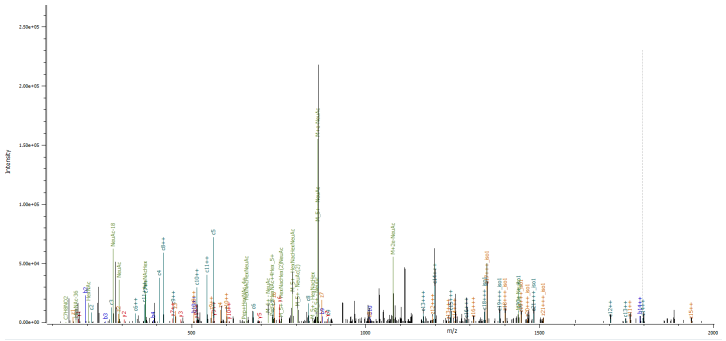


- Full Orbitrap MS scan
- 1st parent ion isolation in Q1
- 1st parent trapped in the ion trap
- Injection of ETD reagent
- 1st parent: ETD dissociation
- EThcD dissociation

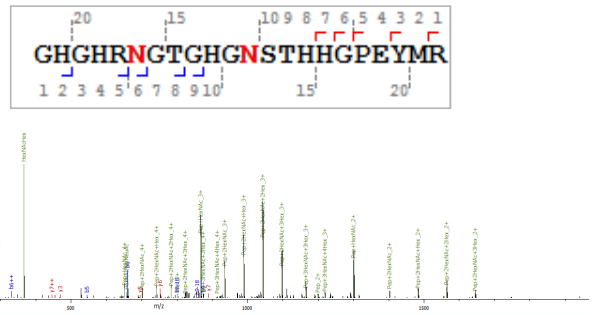
Glycopeptide Sequencing Using EThcD

EThcD

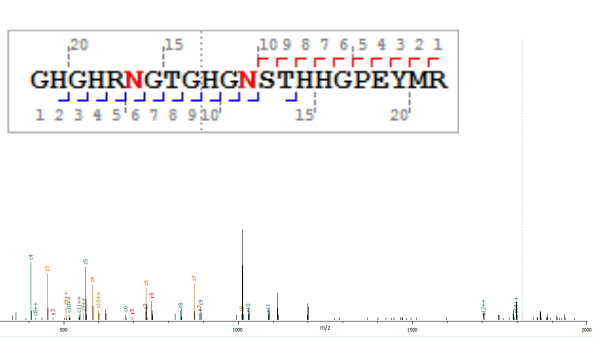




HCD



ETD

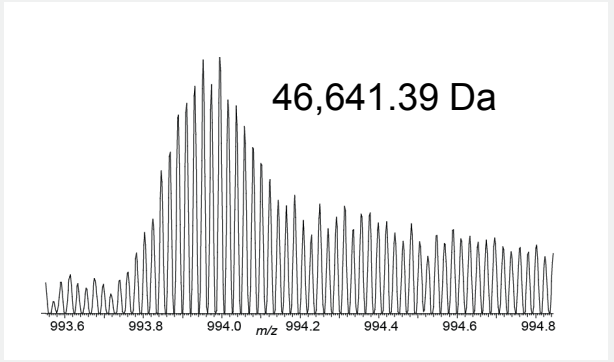


ThermoFisher SCIENTIFIC

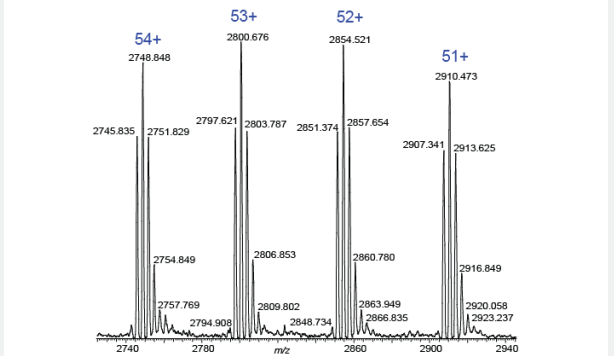
Intact Protein Mode Principles of Operation

- Reduced pressure in the IRM>CTRAP>Orbitrap region
- Calibration of ion transfer at reduced pressure
- Push-button operation once calibrated
- Can be used via Tune or in the Method (Global Parameter)
- Good for signal conservation with longer transients necessary to obtain isotopic resolution of large intact proteins 25-50kDa
- Unnecessary for Intact IgGs analyzed at low resolution

Intact Protein Mode Enolase, 47+

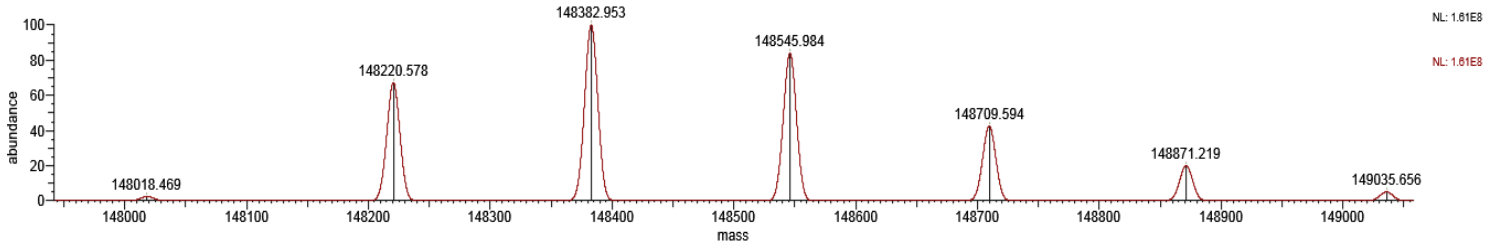


Standard Pressure Mode

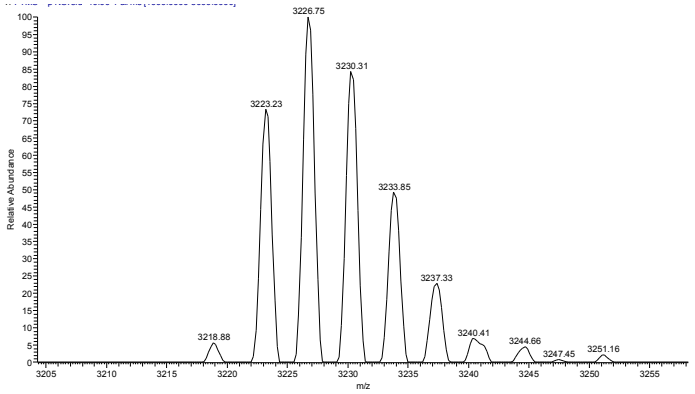


ThermoFisher SCIENTIFIC

Intact IgG: Seven Major Glycosylated Forms



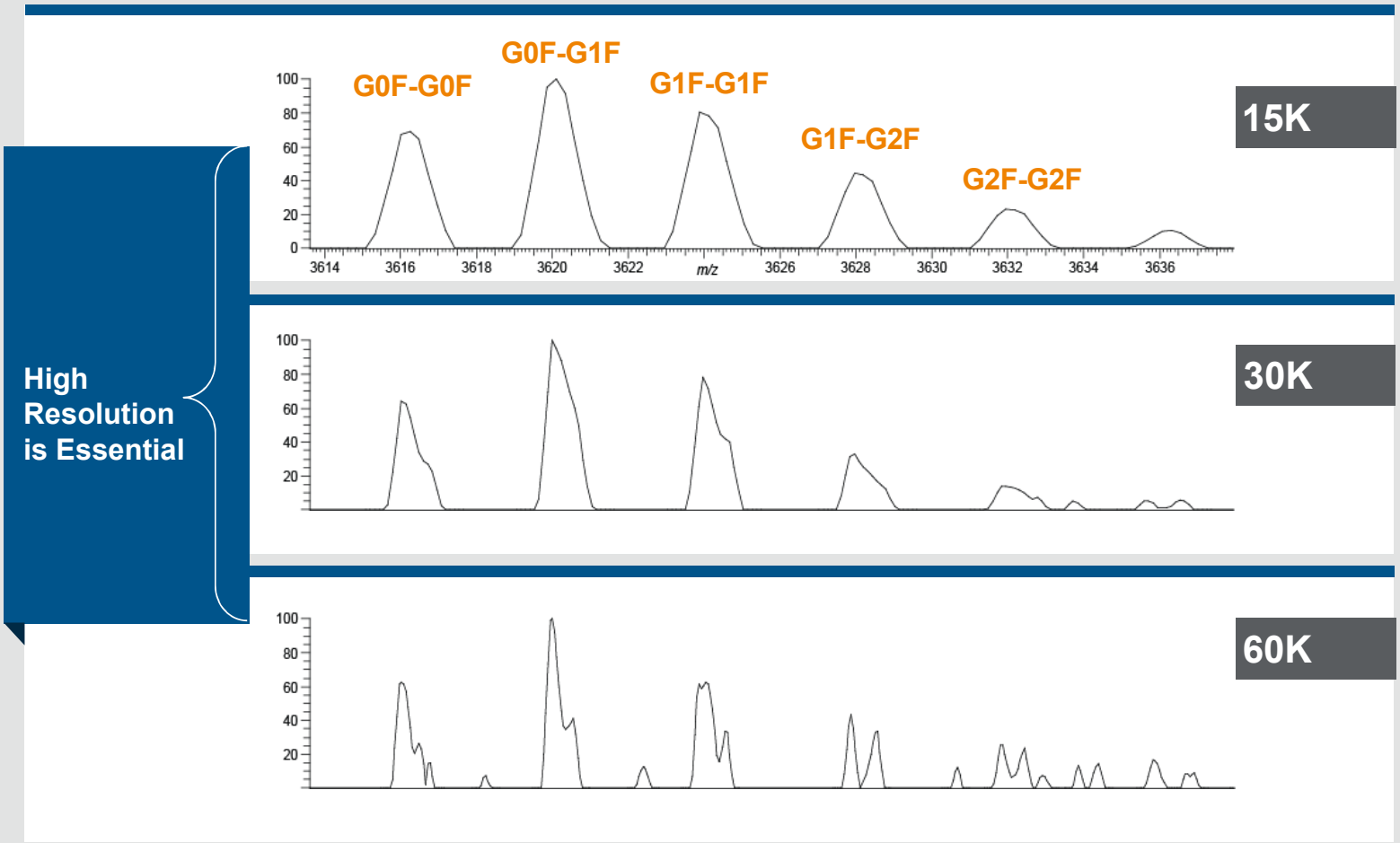
Deconvoluted Mass, Da	Reported Mass, Da
148018.5	
148220.6	148220.4
148383.0	148382.5
148546.0	148544.6
148709.6	148706.7
148871.2	148868.8
149035.7	



Why mass shifts?

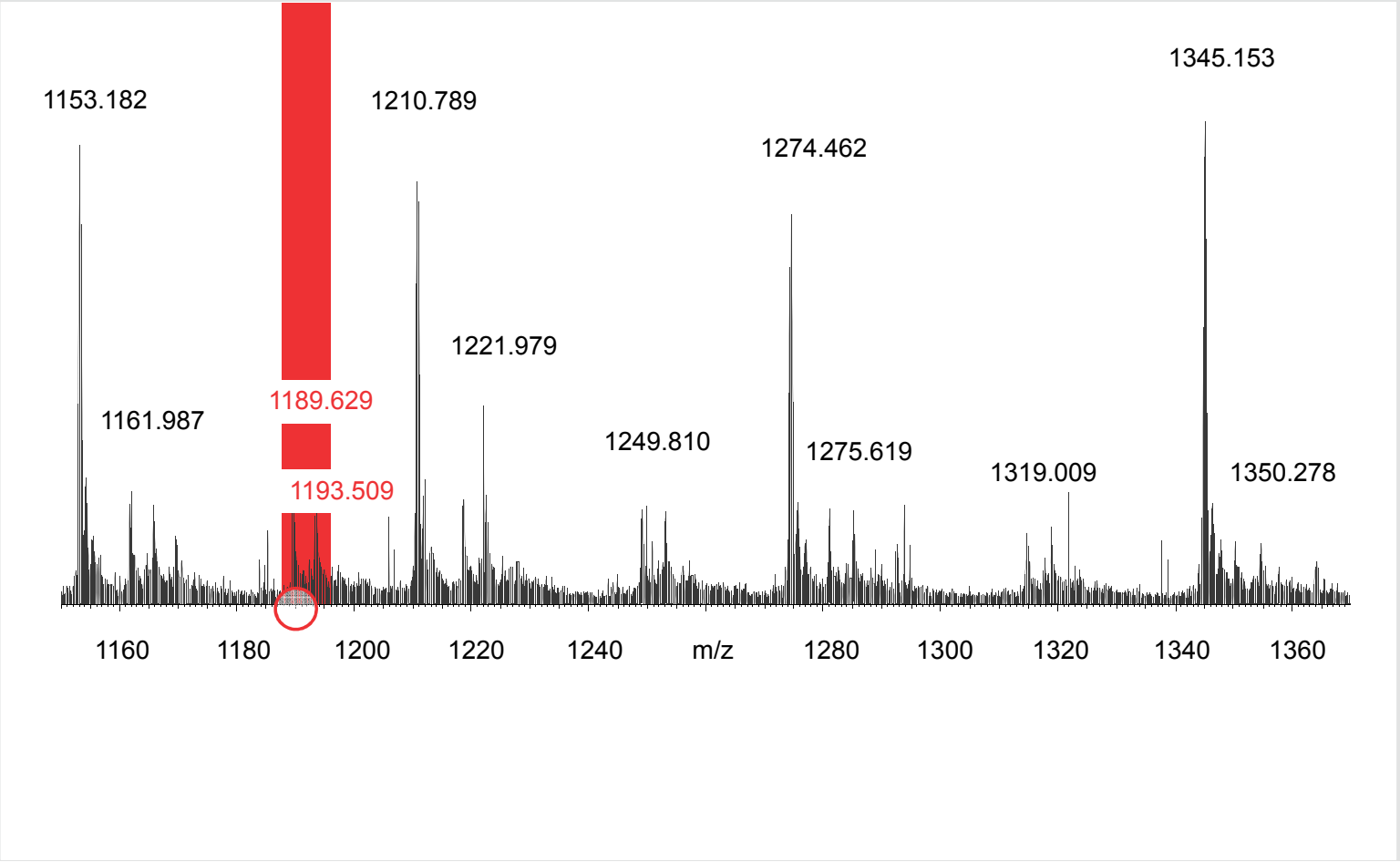
ThermoFisher SCIENTIFIC

41+: Higher Resolution Reveals Multiple Isoforms



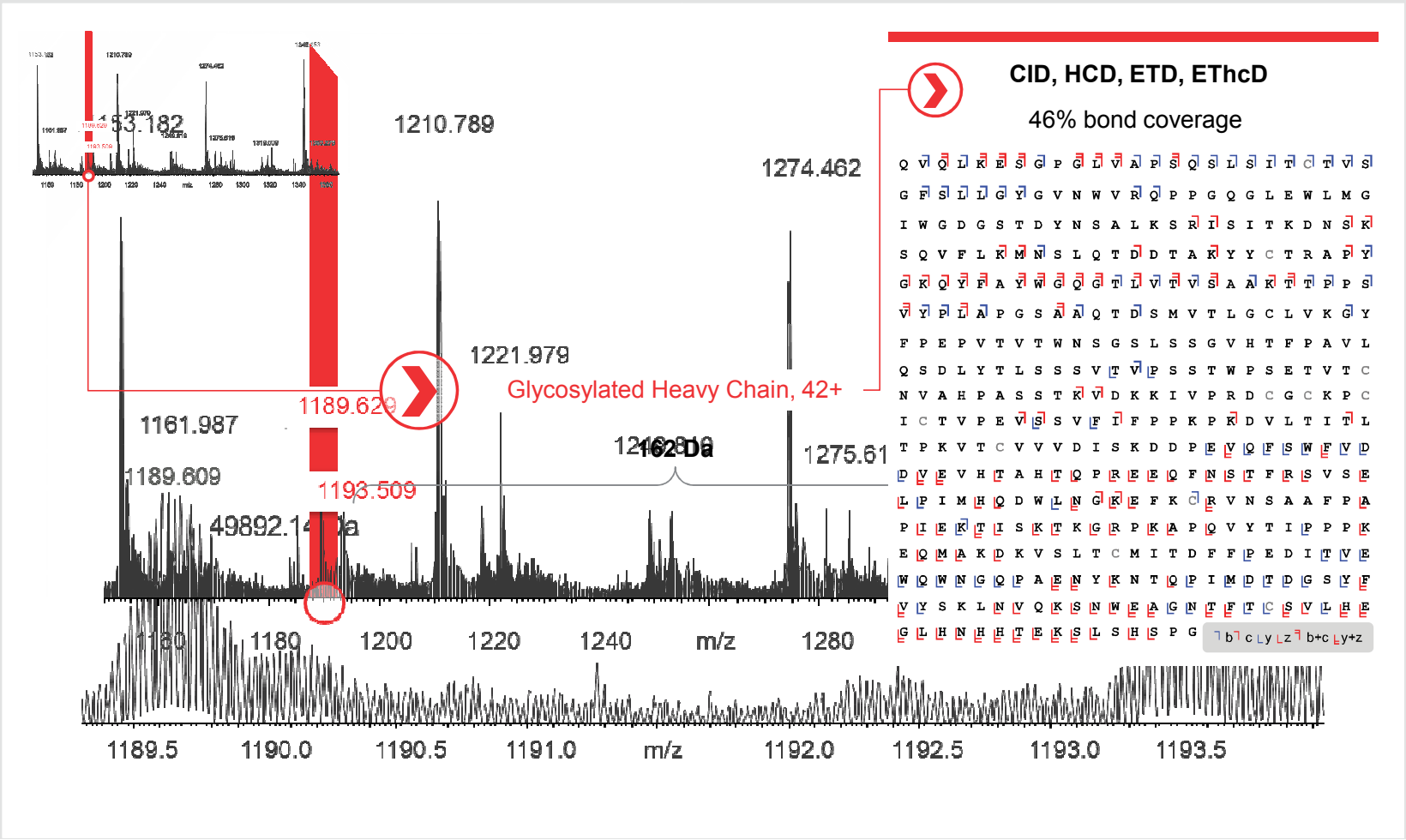
ThermoFisher SCIENTIFIC

Top Down of IgG: ETD, EThcD, CID, HCD



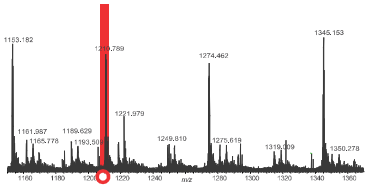
ThermoFisher SCIENTIFIC

Top Down of IgG: ETD, EThcD, CID, HCD



ThermoFisher SCIENTIFIC

Top Down of IgG: ETD, EThcD, CID, HCD



Light Chain, 20+

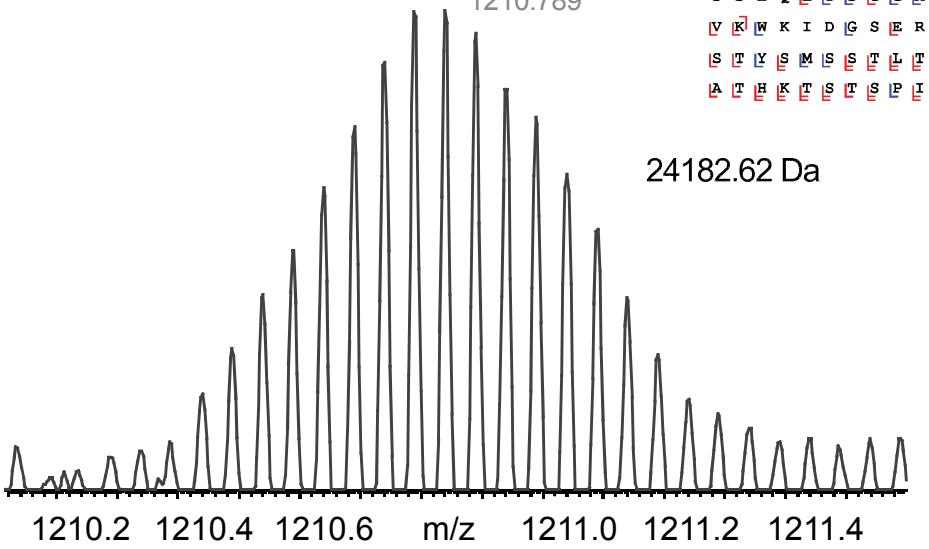
CID, HCD, ETD

77% bond coverage

D V L M T Q T P L S L P V S L G D Q A S I S C R S
S Q Y I V H S N G N T Y L E W Y L Q K P G Q S P K
L L I Y K V S N R F S G V P D R F S G S G S G T D
F T L K I S R V E A E D L G V Y Y C F Q G S H V P
L T F G A G T K L E I K R A D A A P T V S I F P P
S S E Q L T S G G A S V V C F L N N F Y P K D I N
V K W K I D G S E R Q N G V L N S W T D Q D S K D
S T Y S M S S T L T L T K D E Y E R H N S Y T C E
A T H K T S T S P I V K S F N R N E C

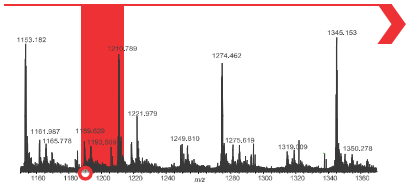
1210.789

24182.62 Da



ThermoFisher SCIENTIFIC

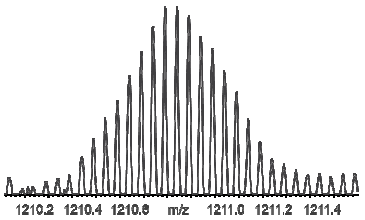
Top Down of IgG: ETD, EThcD, CID, HCD



High Bond Coverage with Orbitrap Fusion MS

CID, HCD, ETD

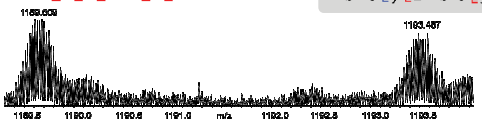
77% bond coverage



Light Chain, 20+

CID, HCD, ETD

77% bond coverage



Glycosylated Heavy Chain, 42+

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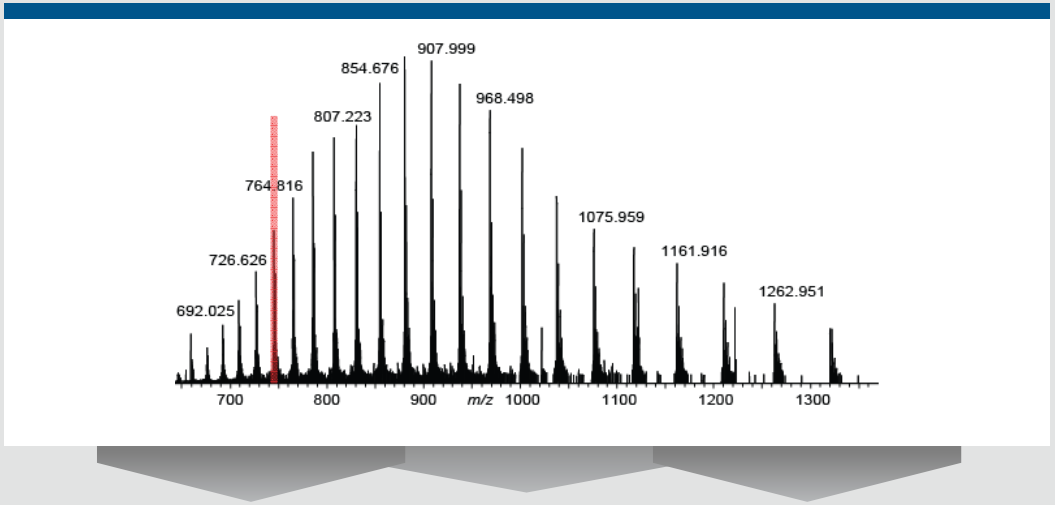
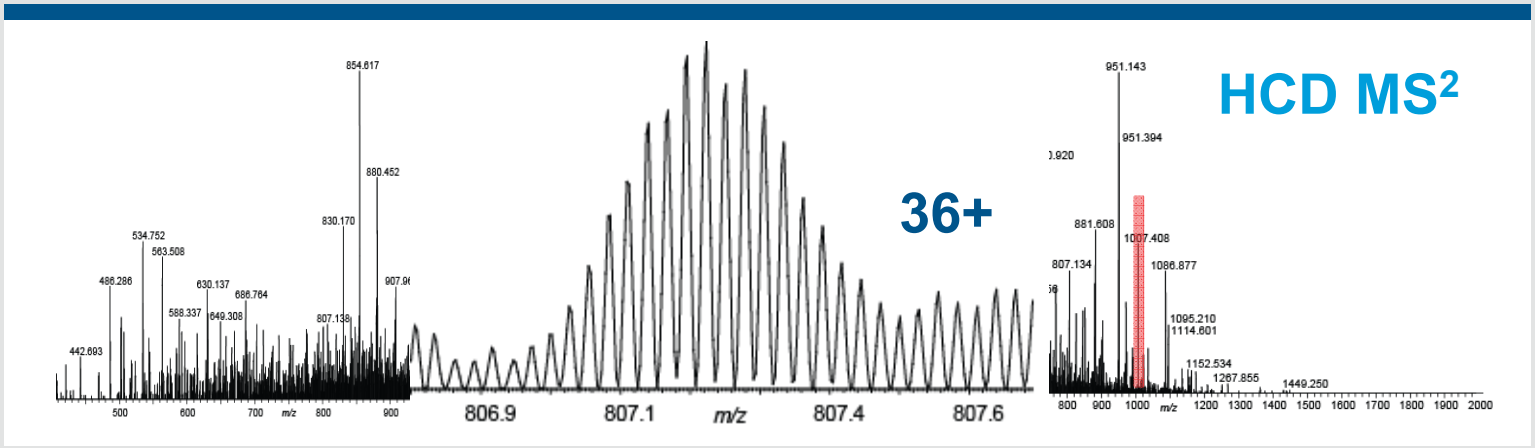
Top Down MS^n of Carbonic Anhydrase





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Combined Sequence Coverage

ETD HCD ETD MS^3

(only unique z fragments are shown)

ThermoFisher SCIENTIFIC

Innovation Applied

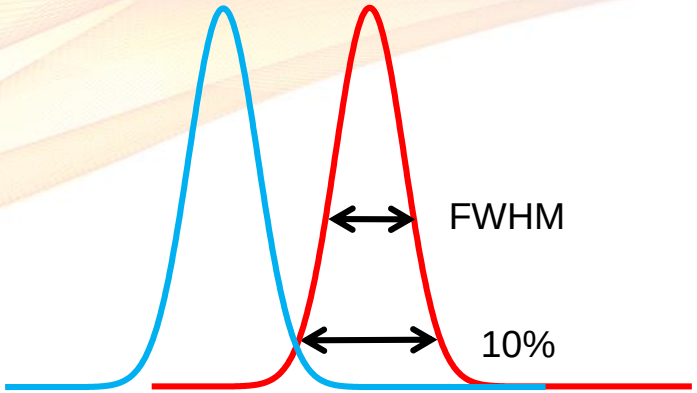
Pharma & Tox

Alla ricerca della Massa esatta

Resolution and Mass Accuracy

- Risoluzione

$$R = \frac{m}{\Delta m}$$

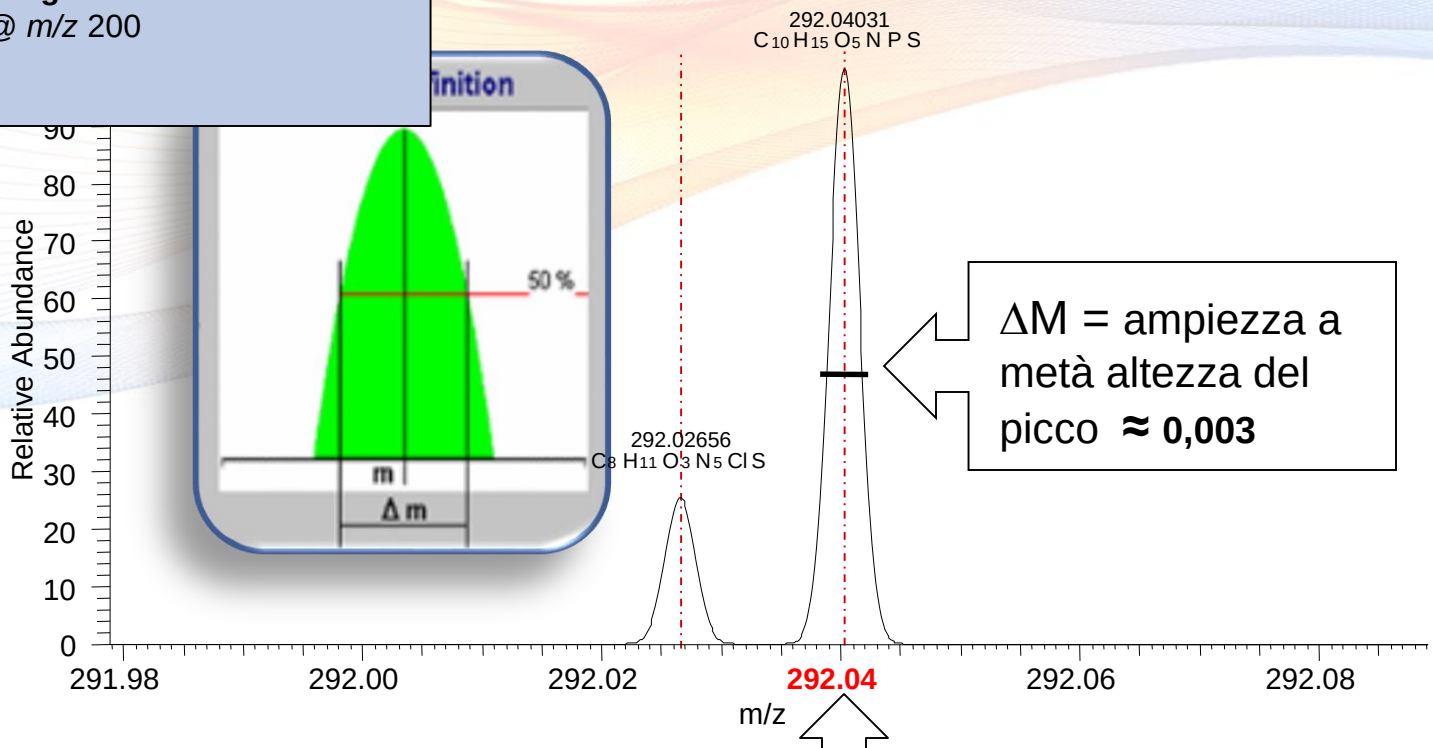


Definita come la capacità di riuscire a distinguere due ioni aventi rapporti m/z diversi.

All'aumentare della risoluzione aumenta la capacità di distinguere e misurare ioni con segnali m/z anche molto vicini

Per una miglior comprensione sarebbe utile riferire il valore
di risoluzione ad una massa di riferimento

Max Resolving Power
140,000 @ *m/z* 200



ΔM = ampiezza a metà altezza del picco ≈ **0,003**

M = la massa

R = M / ΔM

Full **W**idth **H**alf **M**aximum

FWHM = 292,04 / 0,003 = 97.3460

Definita come la capacità di riuscire a distinguere due ioni aventi rapporti m/z diversi. All'aumentare della risoluzione diminuisce la differenza che deve esistere tra due ioni affinchè diano due segnali m/z distinti

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Resolution and Mass Accuracy

- Massa accurata

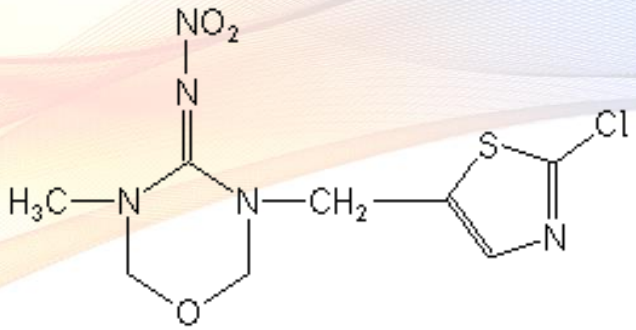
$$(ppm) = \frac{m_{true} - m_{measured}}{m_{true}} \cdot 10^6$$

L'ACCURATEZZA
nella misura di massa, ovvero la differenza tra la massa ottenuta sperimentalmente e quella teorica

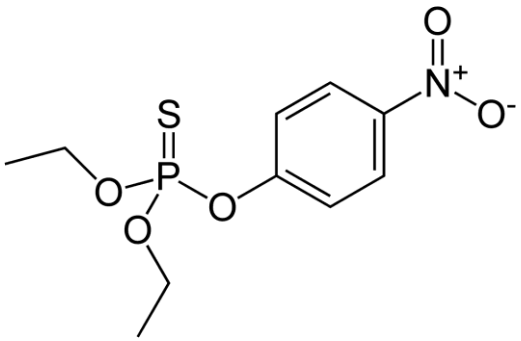
Esempio: Molecole Isobariche

Thiamethoxam

$[M+H]^+ = 292.02656$



Δm
0.0138 Da

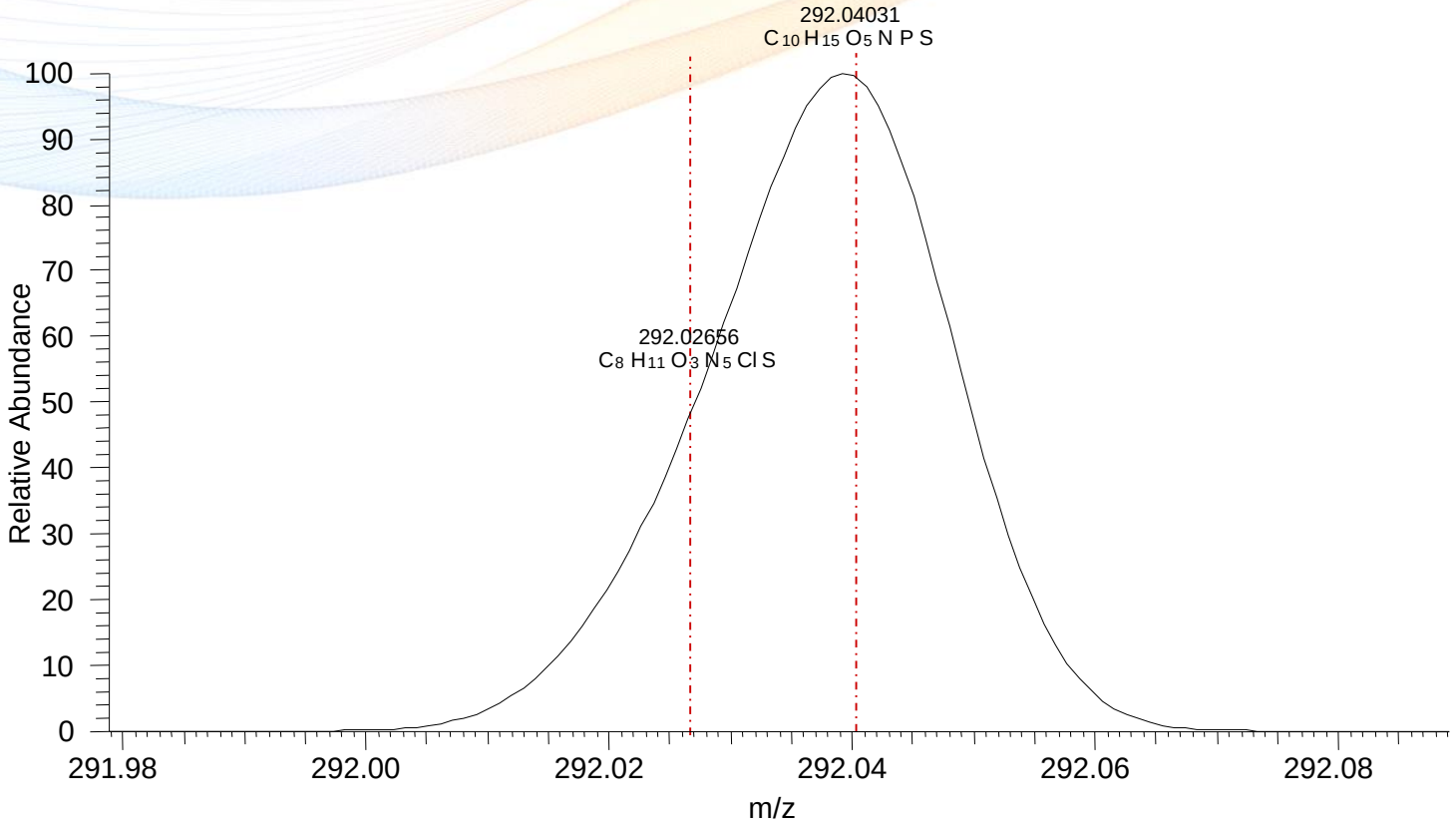


Parathion

$[M+H]^+ = 292.04031$

15,000 (Mix 1:3)

Resolution 15,000



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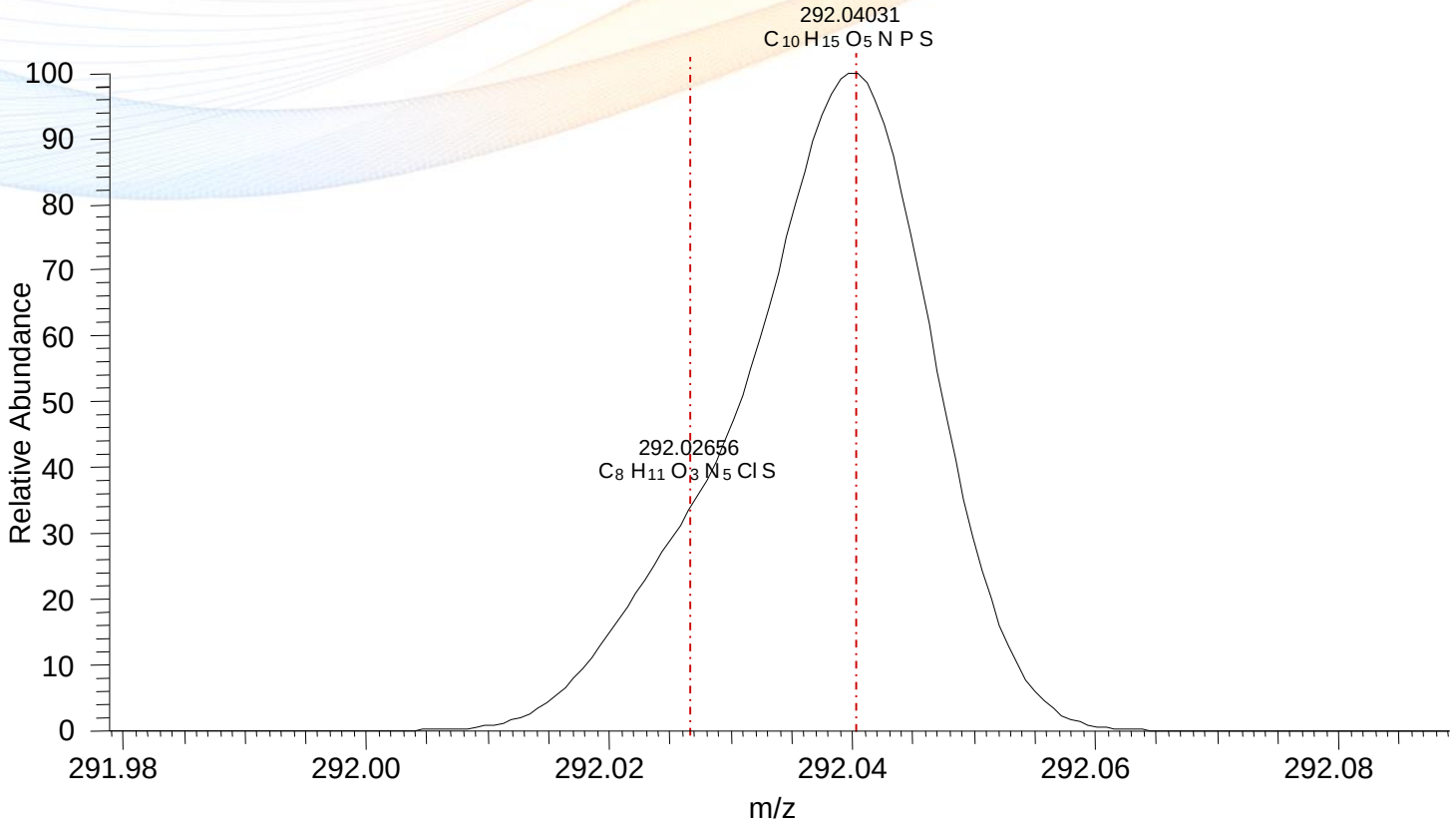
20,000 (Mix 1:3)

Resolution 20,000



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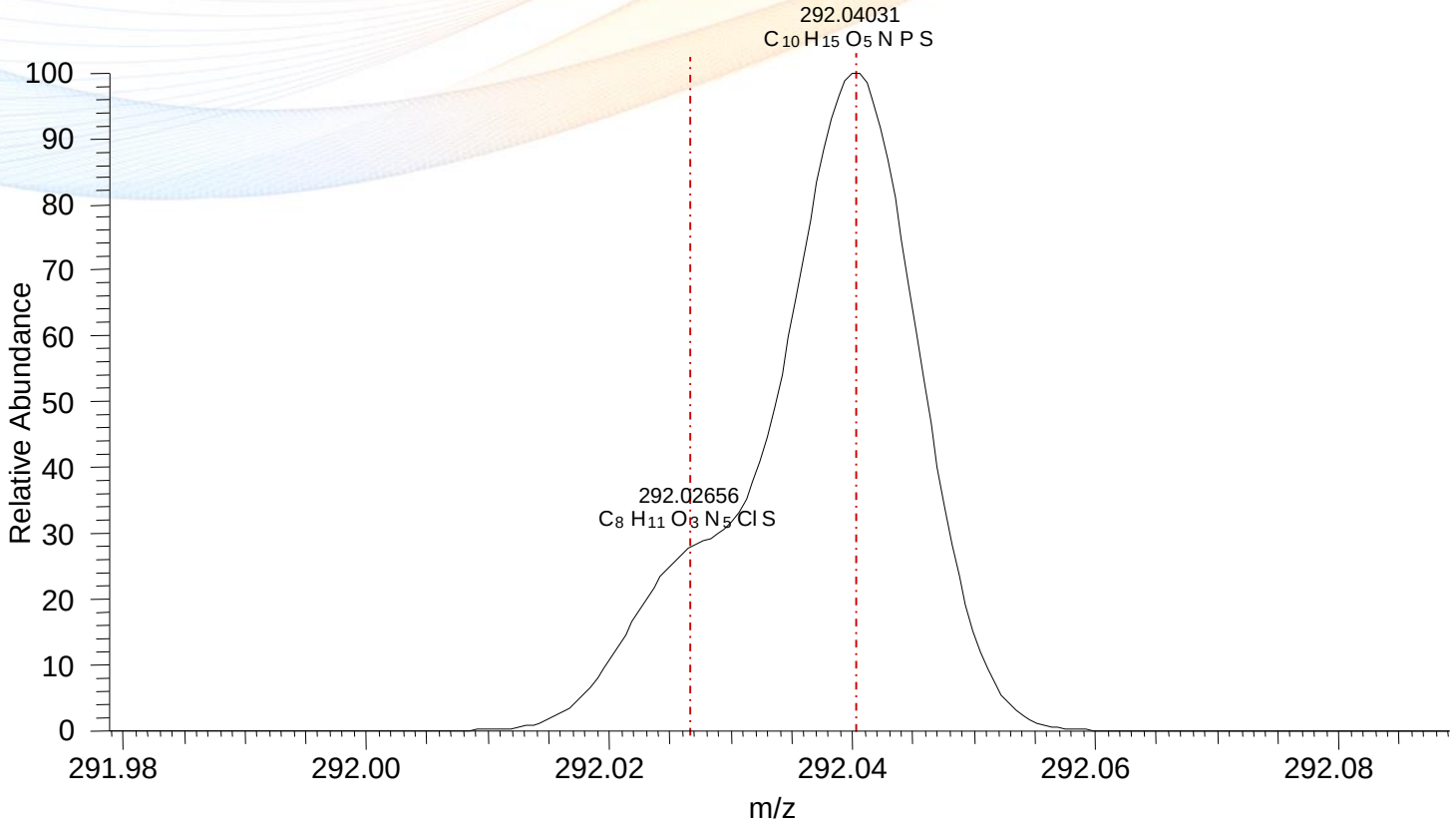
25,000 (Mix 1:3)

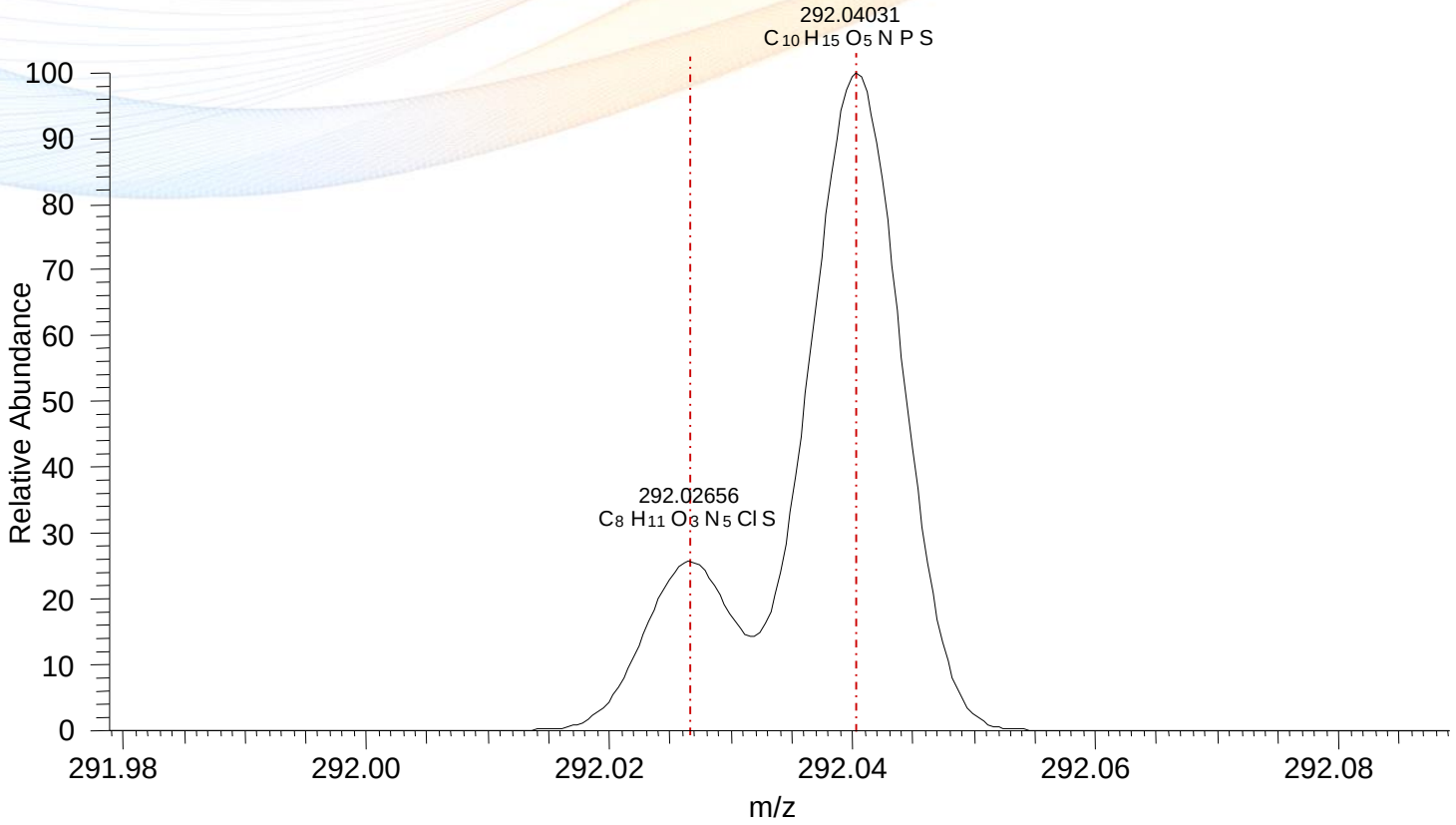
Resolution 25,000



ThermoFisher SCIENTIFIC

35,000 (Mix 1:3)

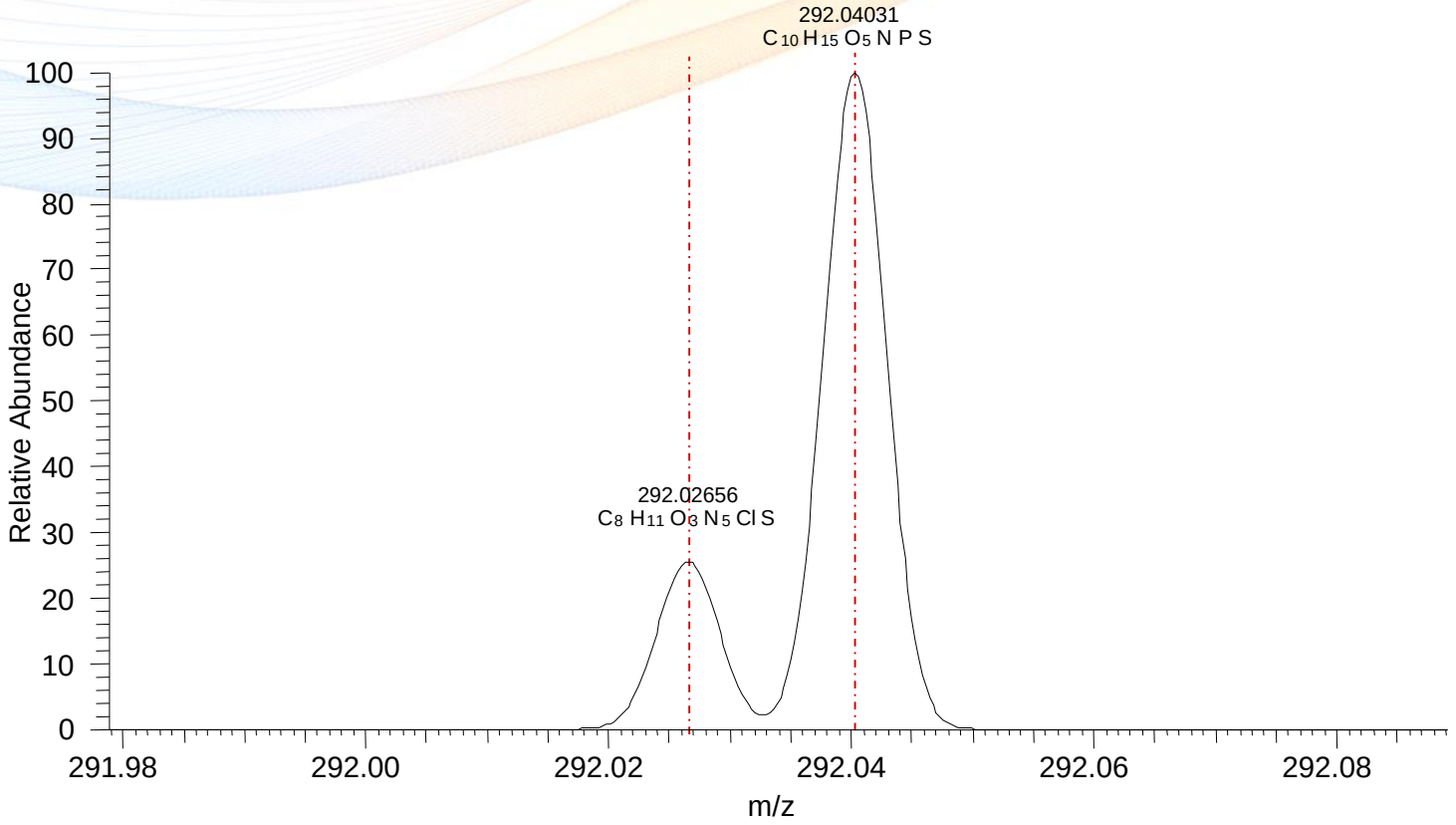
Resolution 35,000



ThermoFisher SCIENTIFIC

50,000 (Mix 1:3)

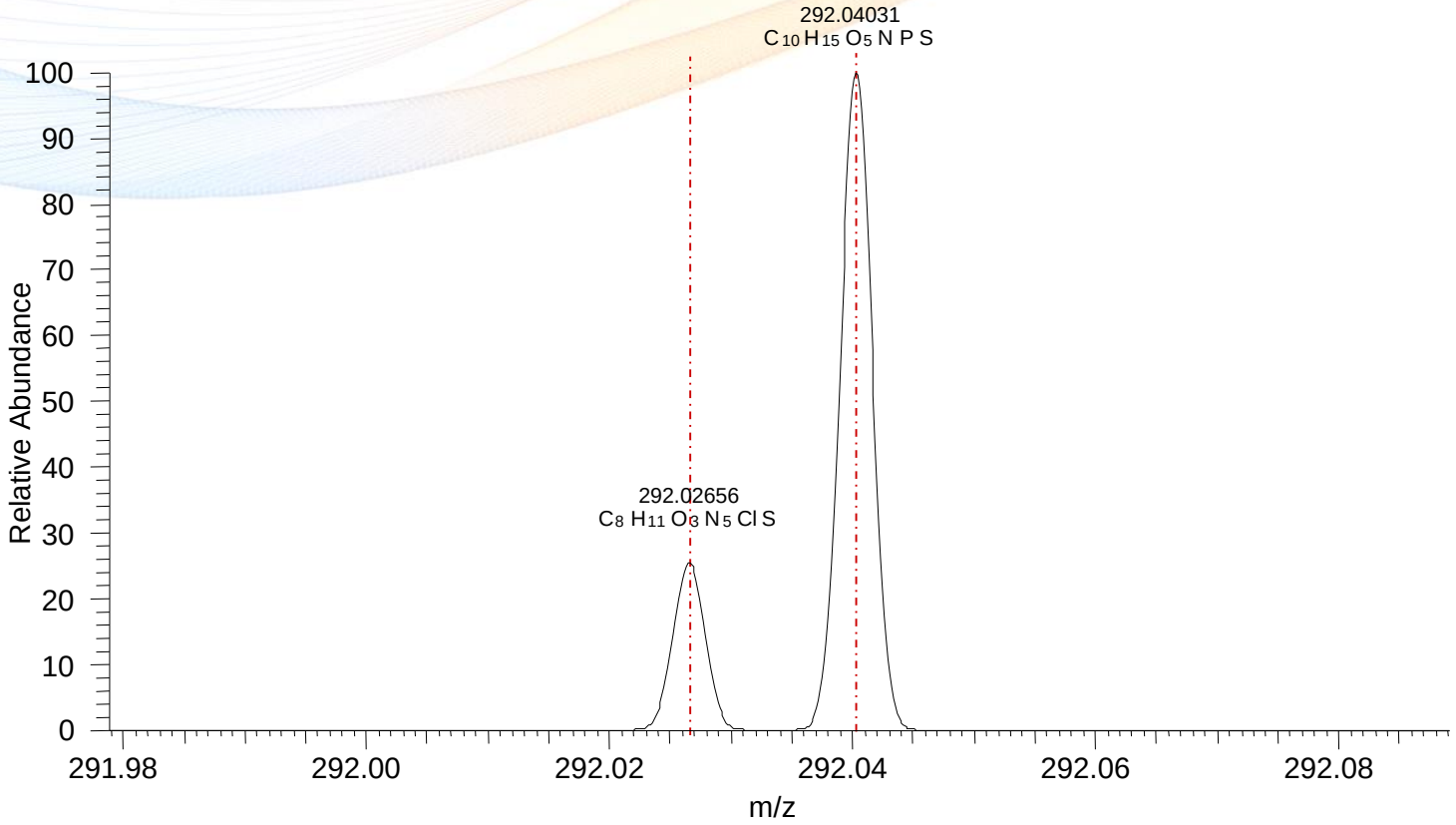
Resolution 50,000



ThermoFisher SCIENTIFIC

100,000 (Mix 1:3)

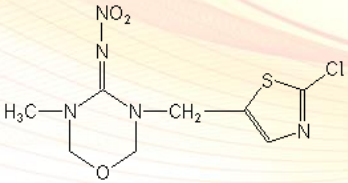
Resolution 100,000



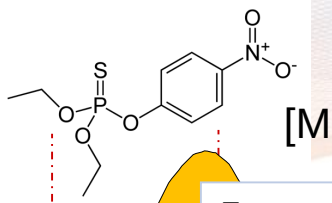
ThermoFisher SCIENTIFIC

Risoluzione e accuratezza

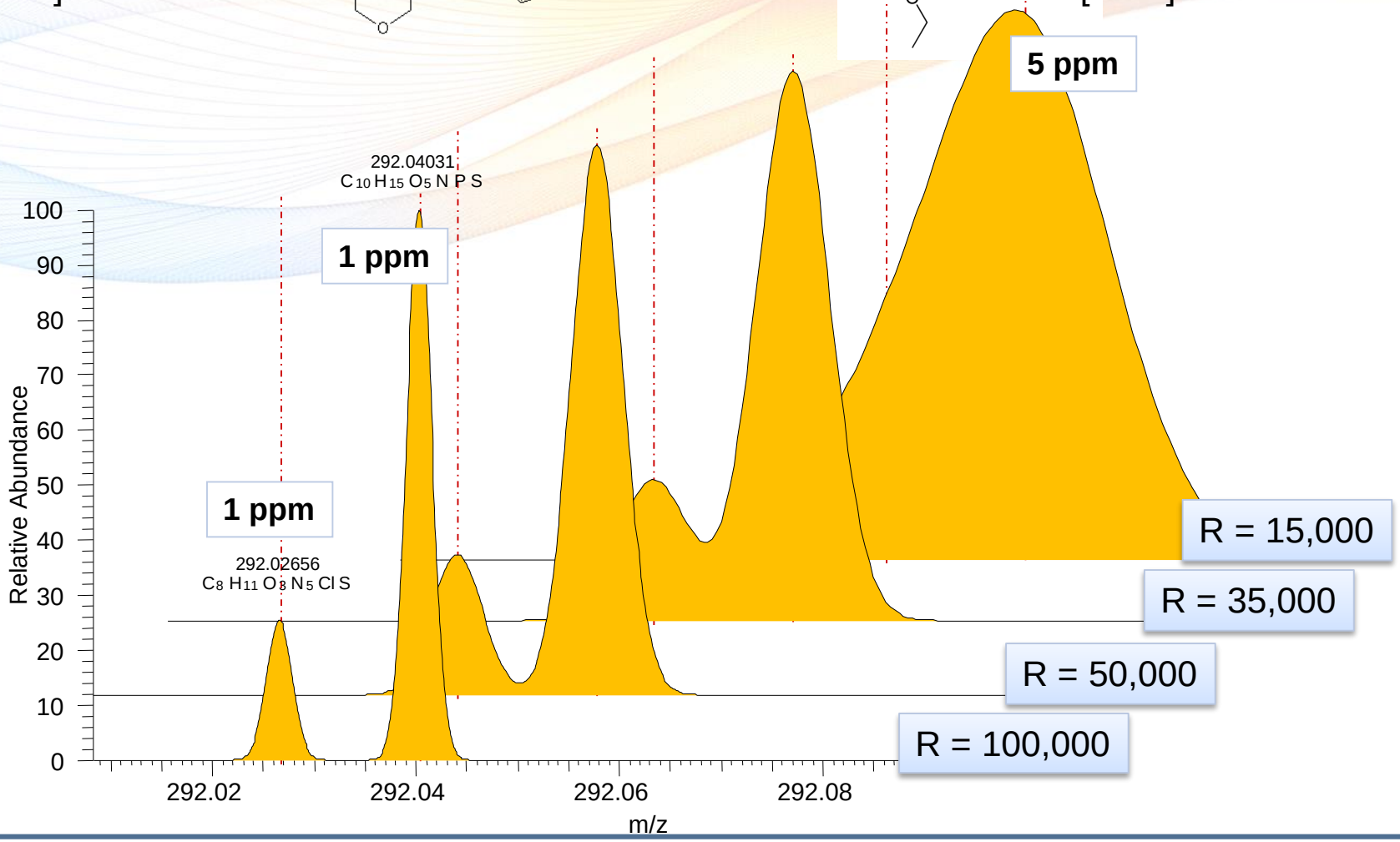
Thiamethoxam
$[M+H]^+ = 292.02656$



Δm
0.0138 Da



Parathion
$[M+H]^+ = 292.04031$



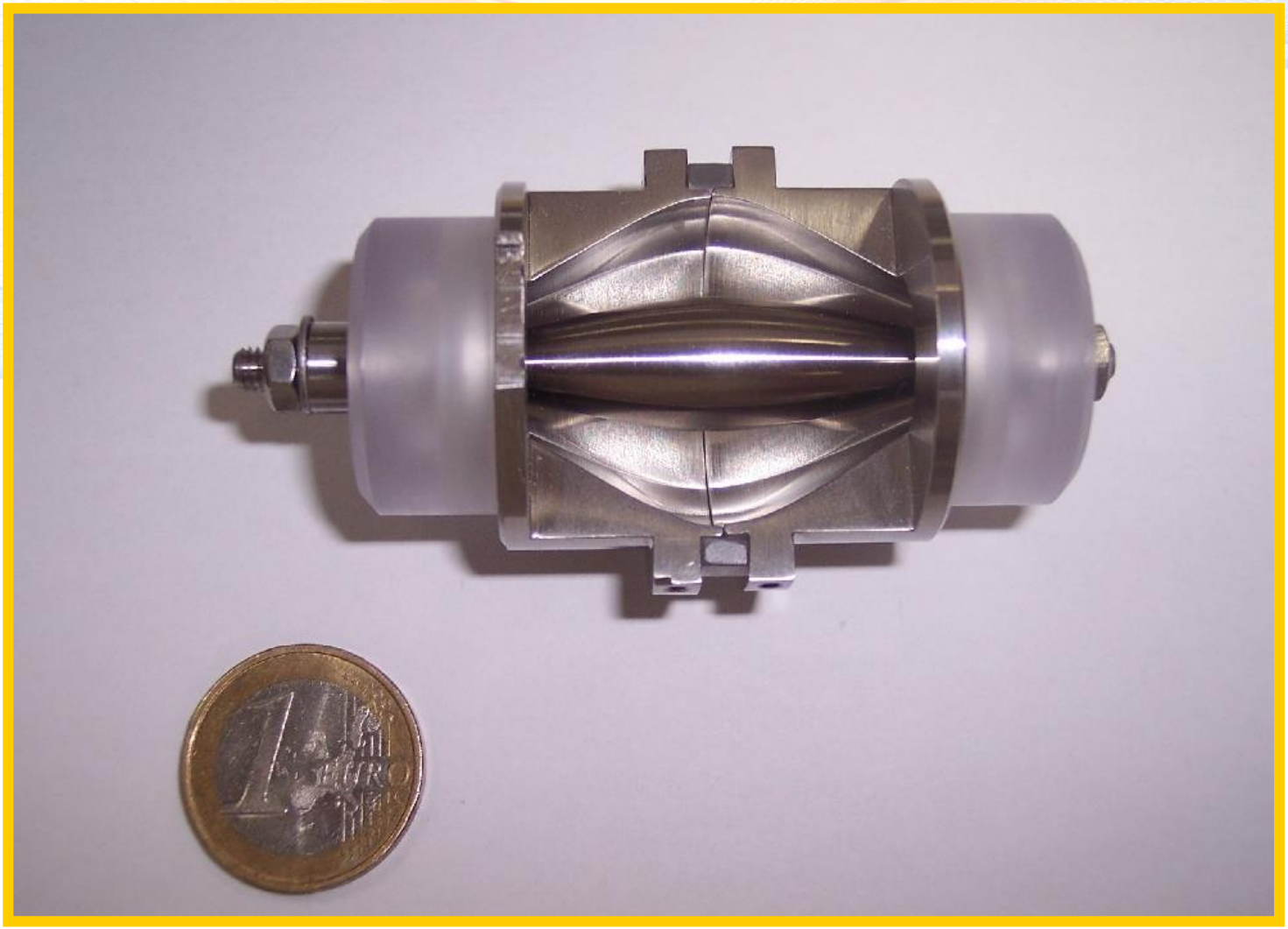
ThermoFisher SCIENTIFIC

ThermoFisher SCIENTIFIC

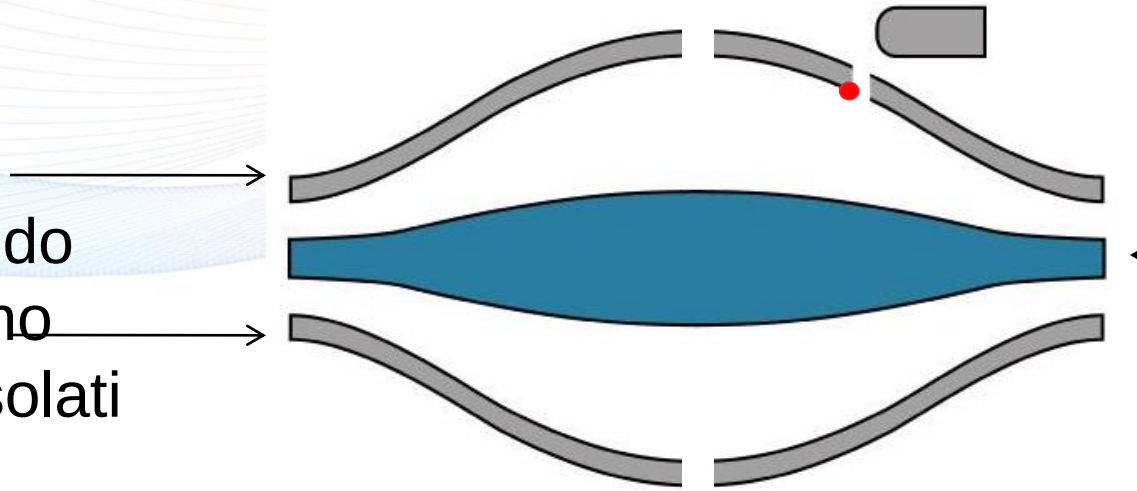
Innovation Applied

La tecnologia Orbitrap™ applicata alla LCMS

nello sito produttivo di Brema venne quindi prodotto
l’ Orbitrap



Dove gli ioni ruotano in un campo elettrico…



“Fast” Injection

Elettrodo esterno

2 coni isolati

Elettrodo interno

...ed il segnale è elaborato dalla Fourier-transform

In breve Orbitrap significa:

- Altissima Risoluzione
- Accuratezza di massa e sua stabilità
- Sensibilità
- Range dinamico
- Acquisizioni Full Scan
- Analisi retrospettiva