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NEW on Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ MS

Stephane Houel, Ph.D.
BioPharma Vertical Marketing

NEW On Thermo Scientific Orbitrap Fusion Lumos MS in 2017

ADVANCED PEAK DETERMINATION ALGORITHM RESULTS IN SIGNIFICANT IMPROVEMENT IN PROTEOMICS EXPERIMENTS

APD

STANDARD ON NEW SYSTEMS
OPTIONAL ON EXISTING SYSTEMS

NEW
30 Hz
OT MS²

UVPD

UNIQUE FRAGMENTATION FOR TOP-DOWN AND SMALL MOLECULES CHARACTERIZATION

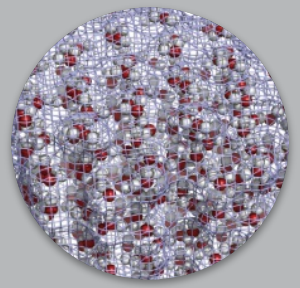
OPTIONAL ON NEW AND EXISTING SYSTEMS

1M

ULTRA-HIGH RESOLUTION FOR FINE ISOTOPE STRUCTURE

OPTIONAL ON NEW AND EXISTING SYSTEMS

Challenges In Life Science Mass Spectrometry



Complex samples, insufficient depth of analysis

$C_{28}H_{35}NO_7S$
vs.
$C_{30}H_{32}N_3O_4P$

Isobaric analytes, confirmation of elemental composition

Comprehensive sequence characterization of protein drugs

Structural elucidation of lipids, metabolites, xenobiotics and others

Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ Mass Spectrometer is the most sensitive and versatile MS system

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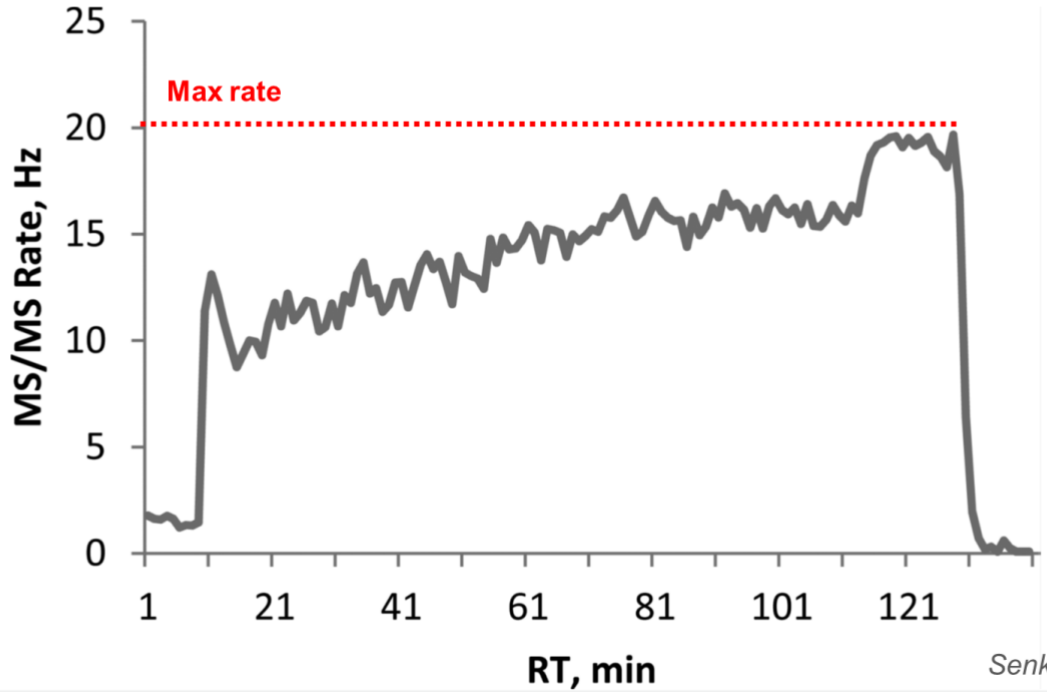
Advanced Peak Determination

Challenge

Achieving maximal depth of analysis in complex samples

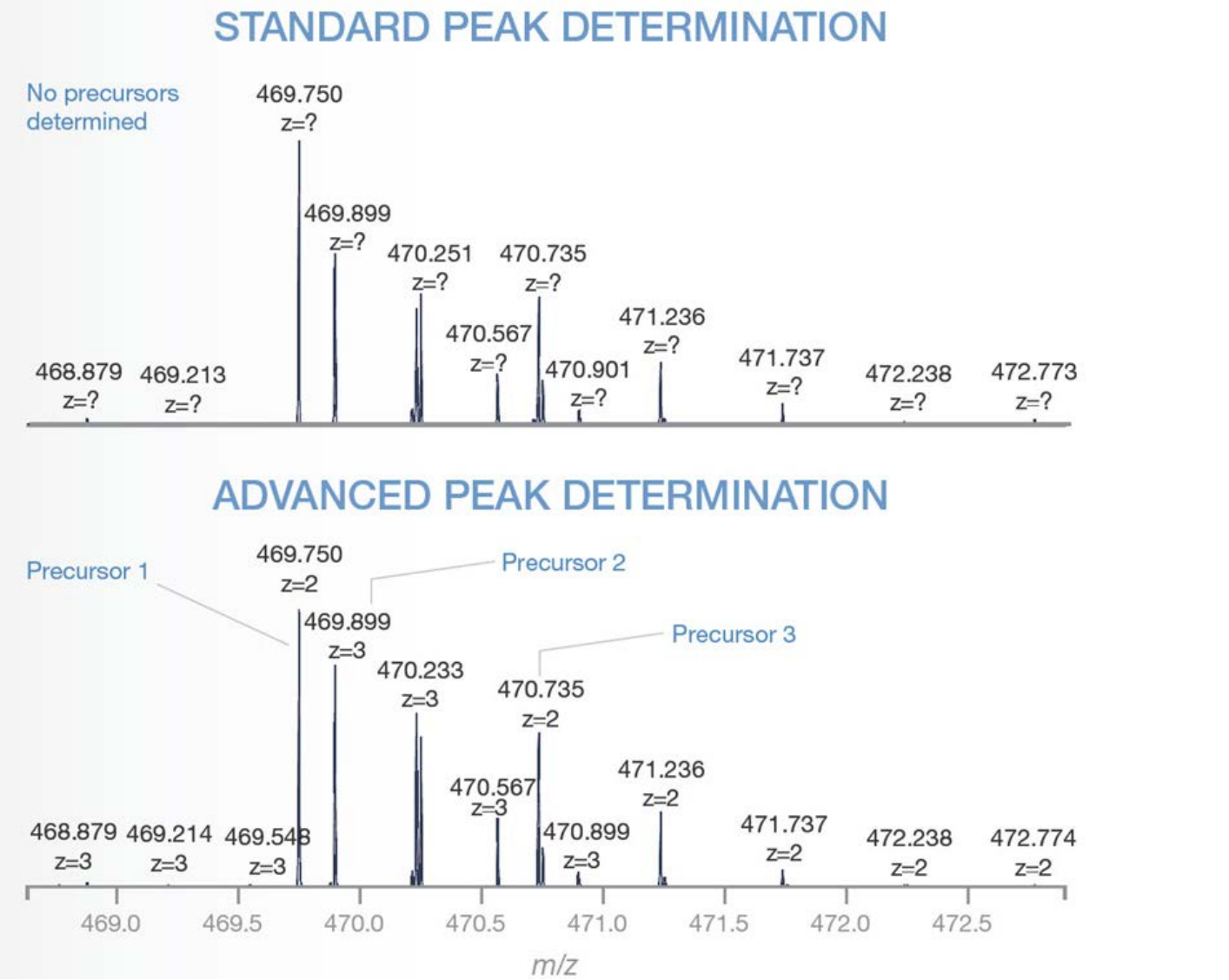
- MS spectra are complex
- Fast & sensitive instrumentation run out of data-dependent targets
- Can we determine more unique peaks for MS/MS analysis?

Typically, MS/MS rate in a DDA experiment is well below its maximum



Senko at al., 2013

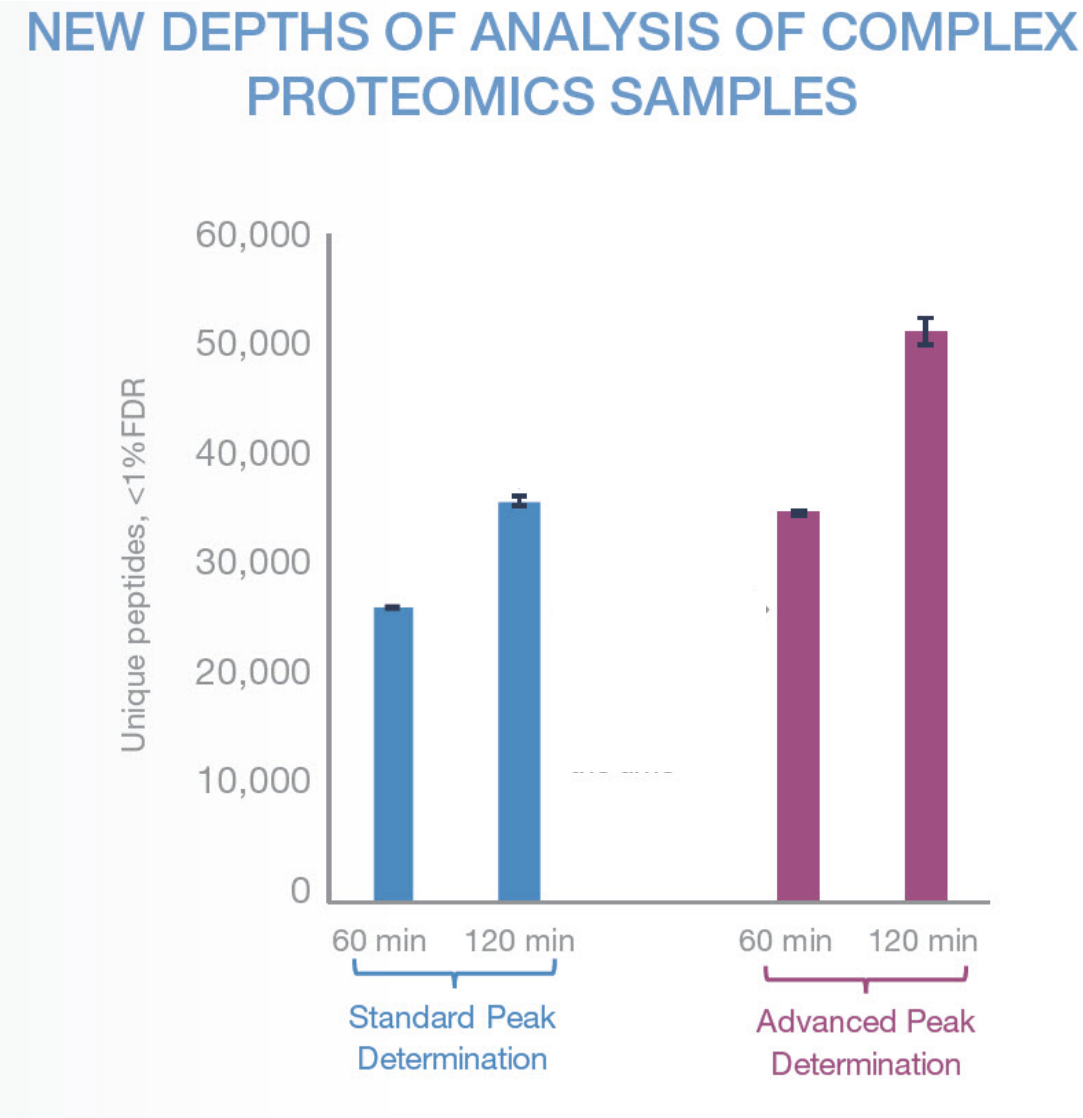
Advanced Peak Determination: *Reading Between The Lines*



Details

- Advanced Peak Determination (APD) algorithm identifies the **charge states and monoisotopic *m/z*** values of isotopic envelopes at greatly improved peak depths **in real-time**
- This significantly increases the population of precursors available for data-dependent analysis, which in turn results in more MS^n spectra, PSMs, and unique peptide identifications
- APD has been evaluated for peptides/proteins only. Improvements for small molecule analysis are still under investigation

APD Leads To Increased Number Of IDs In Proteomics Analyses

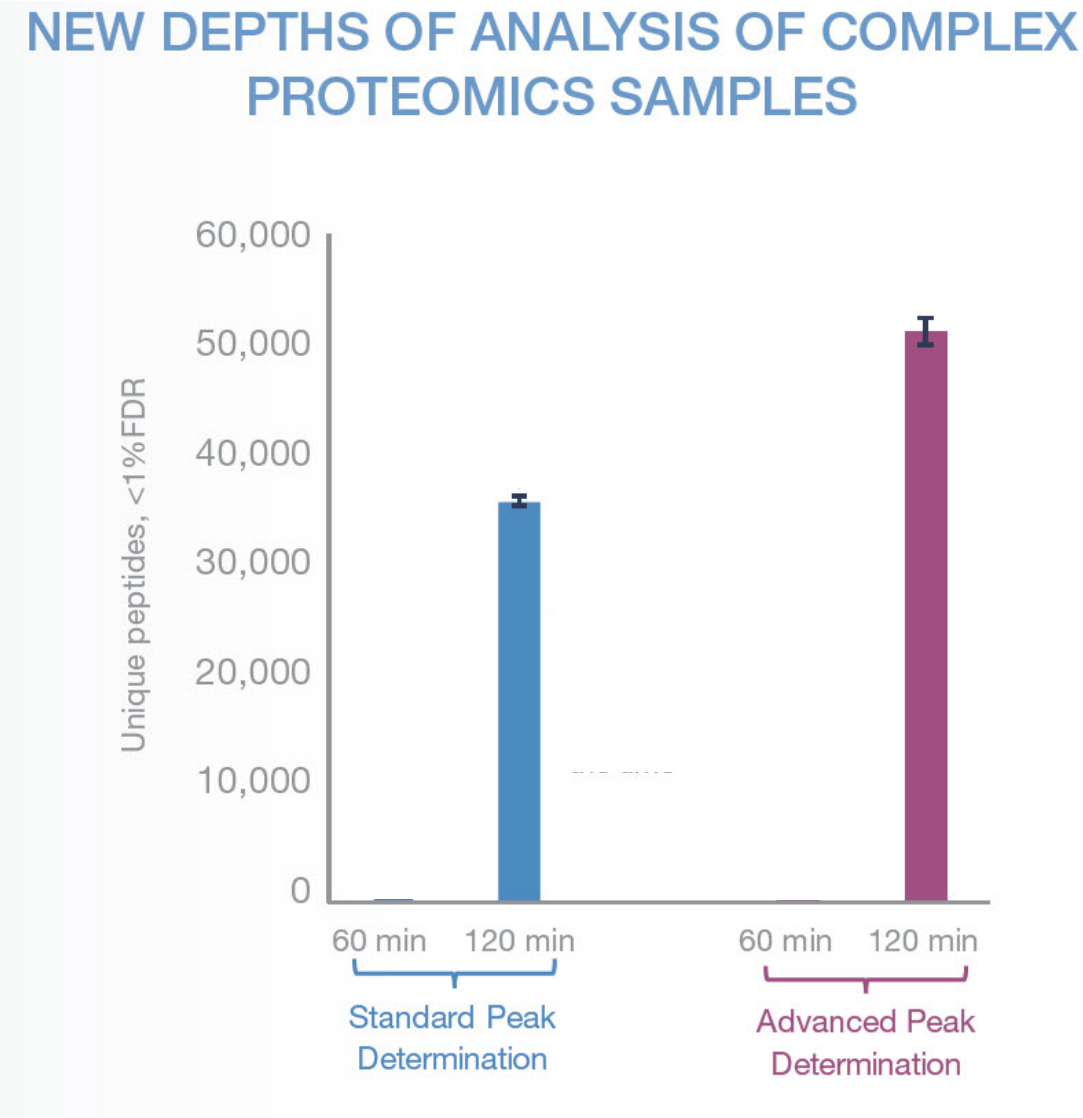


APD Performance On A Tribrid

- APD leverages the low detection limits of the FTMS, and the incredibly high ion trap MS/MS spectral acquisition rate, to significantly boost the rate of unique peptide identifications during data-dependent LC-MS/MS
- We analyzed1 µg of a HeLa digest by data-dependent LC-MS/MS
- Results that used to take 2 hours can be obtained in 1 hour
- Expected improvements for 1 µg HeLa standard (Pierce)*:
  - >25% unique peptide level
  - >5% protein level

*2 h gradient, OT MS at 240K FWHM, LT MS/MS, 20 ms max IT

APD Leads To Increased Number Of IDs In Proteomics Analyses



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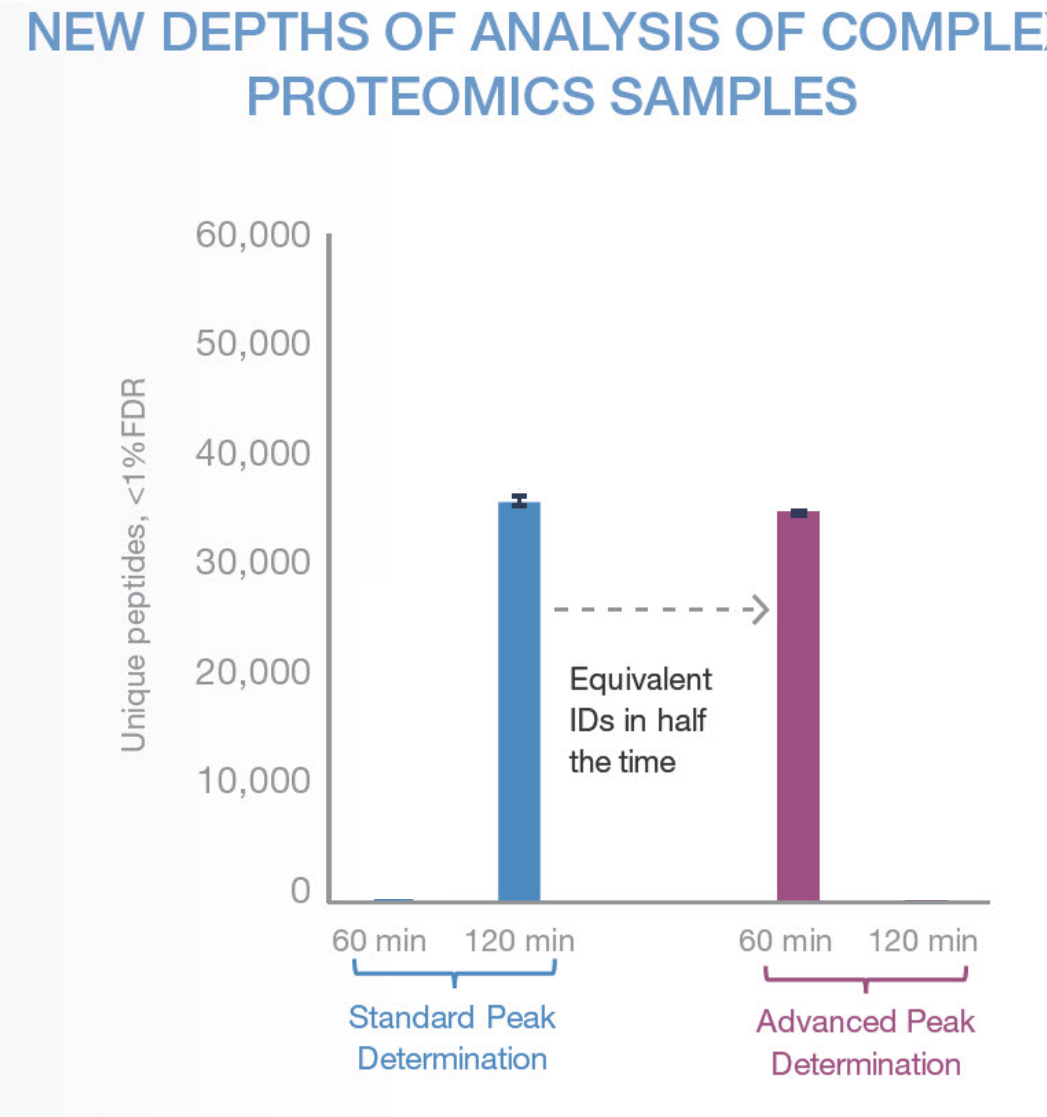

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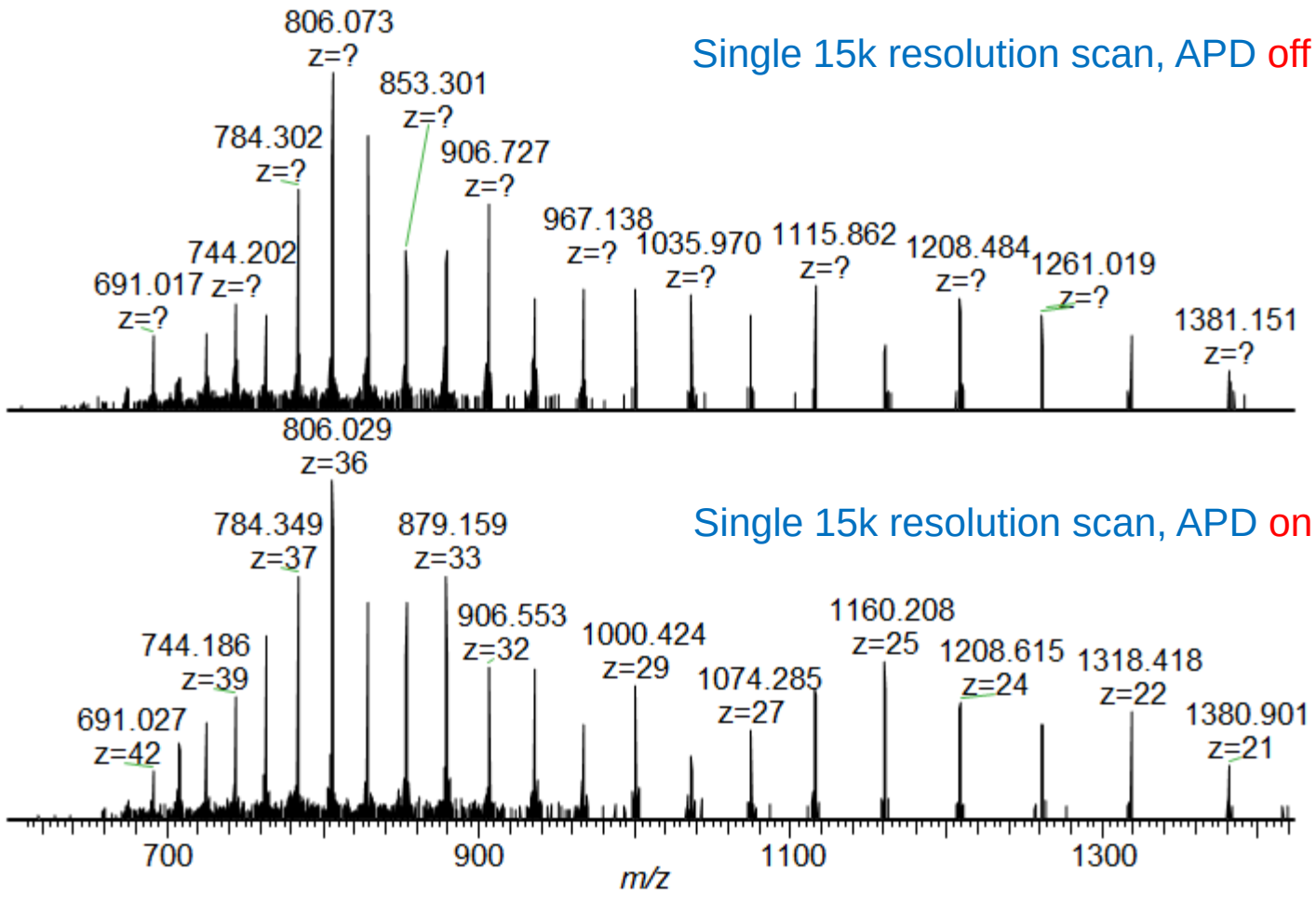
Intact Protein Analysis With APD



On-the-fly charge state assignment of isotopically unresolved charge states of carbonic anhydrase

Charge Assignment Of Unresolved Charge States

- We analyzed the Thermo Scientific™ Pierce™ Intact Protein Standard Mix with a data-dependent LC-MS/MS method
- FTMS1 spectra were collected at 15k resolving power, and were the sum of 5 microscans
- With APD *on*, the instrument can accurately identify highly charged and complex protein envelopes on-the-fly
- It is now possible to execute more advanced top-down experiments including, **MS/MS fragmentation of one charge state per precursor**

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Advanced Peak Determination Benefits Bottom Up and Top Down Proteomics

BOTTOM UP PROTEOMICS

Data-dependent experiments with complex peptide mixtures

TOP DOWN PROTEOMICS

Identification and characterization of intact proteins by MS

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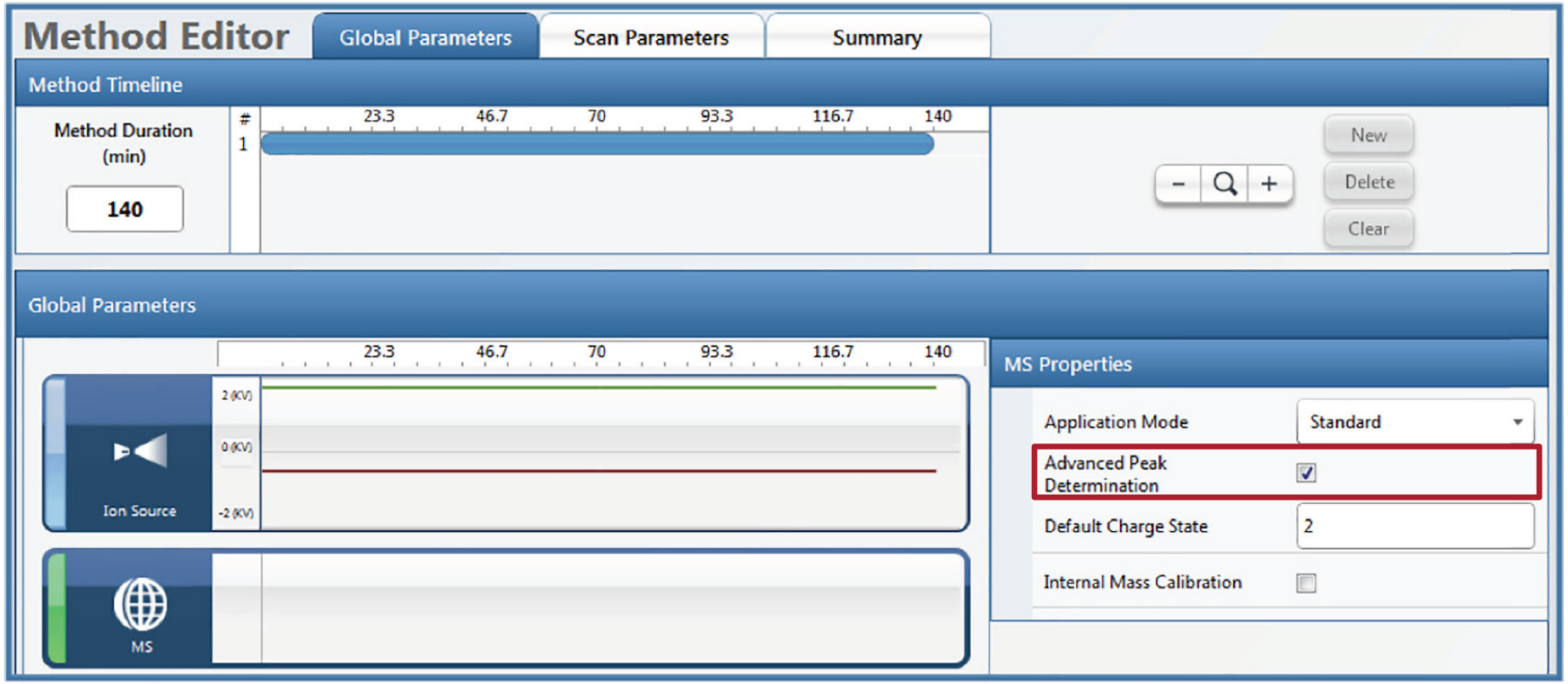
Turn APD On And Off In The Method Editor



Advanced Peak Determination is an optional Global MS property of the Method Editor.

Josh Coon, University of Wisconsin

The new APD algorithm provides new peptide precursors to go after and amounts to a 25-35% identification bonus. I have no doubt this will be a key technology in achieving deeper coverage and higher throughput for proteome analyses.

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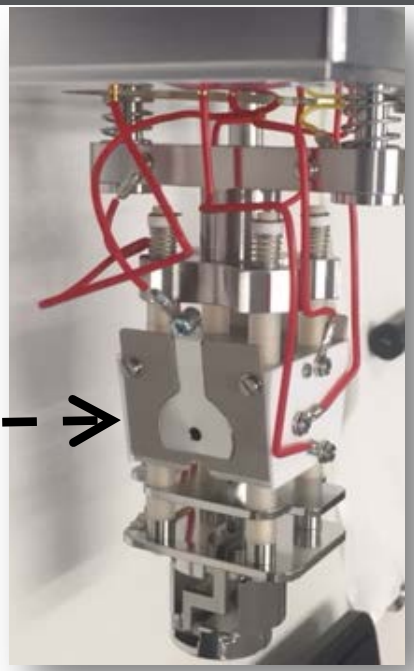
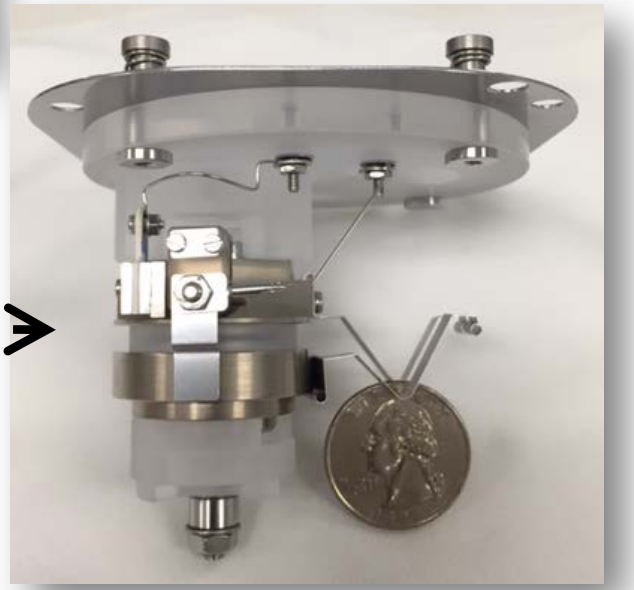
Technology: What Is 1M?

Matching C-trap



1M Orbitrap



A Kit Comprising An Orbitrap And A C-trap Assembly

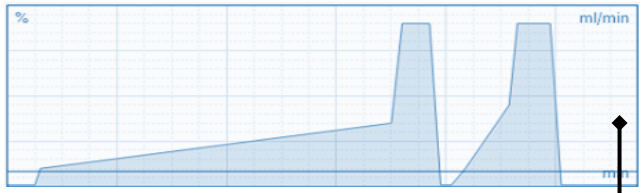
- Ultra high resolution (1,000,000 at *m/z* 200, 2 sec transient) while maintaining isotope ratio specs
- A matching C-trap and a high-performance Thermo Scientific™ Orbitrap™ mass analyzer, available for factory installations or field upgrades

1M option for peptide mapping: Do we need it?

Question

Does the lack of resolution prevent the detection/quantitation of almost isobaric components.

NISTmAb Digest Peptide Map



3 ug Trypsin Digested
Nist mAb

Thermo Scientific™ Vanquish™ UHPLC
70 minute Gradient 250 uL/min
0.1% FA in H2O and ACN
50 C Column Temp

Thermo Scientific™ Orbitrap Fusion™ Lumos™ MS
120k, 240k, 500k, and 1 M Resolution @ 200 m/z
Mass range: 300 to 1800 m/z

Thermo Scientific™Xcalibur™ Software and Chromeleon™ 7.2 SR5 CDS

Sample

Column

uHPLC

MS

Processing

Thermo Scientific™ Accucore™
Vanquish™ C18+
2.1 x 150 mm 1.5 uM

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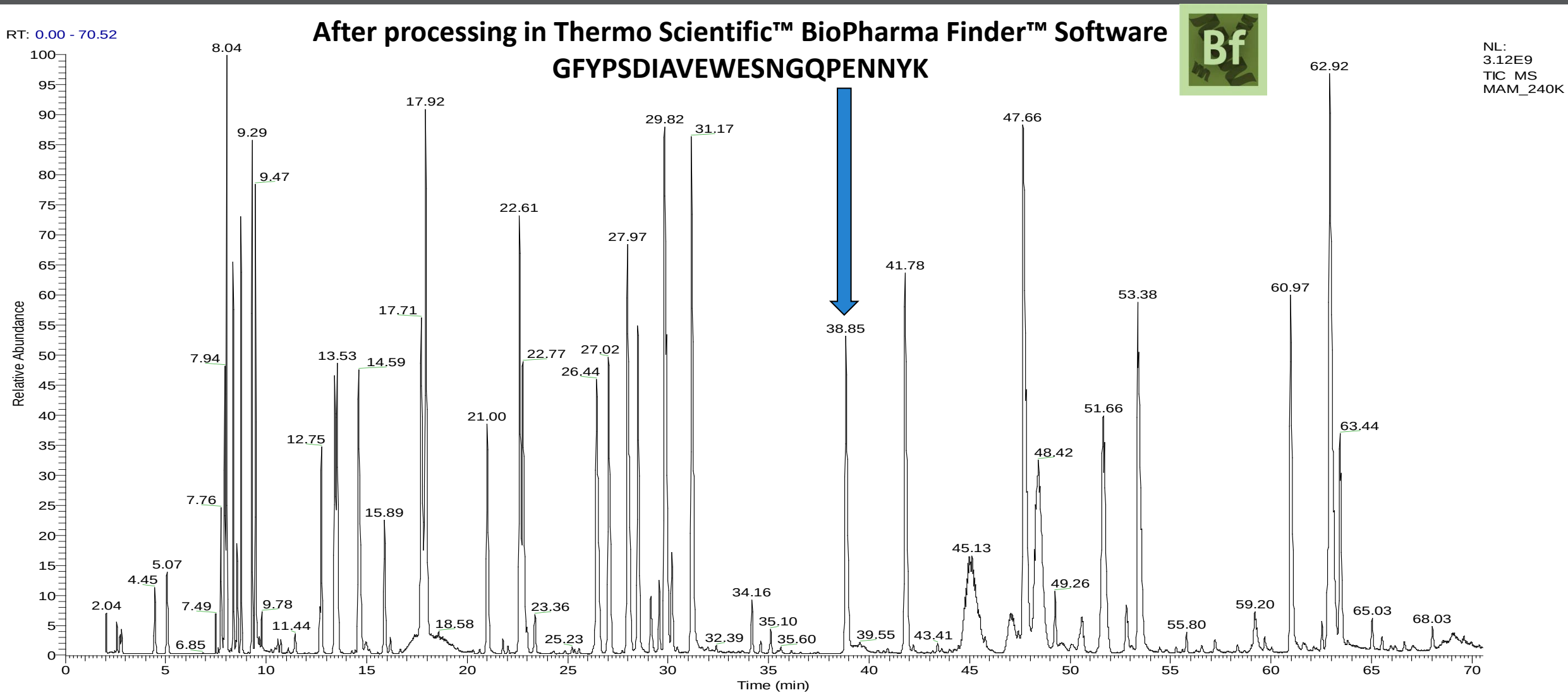
NISTmAb Digest Peptide Map Chromatogram



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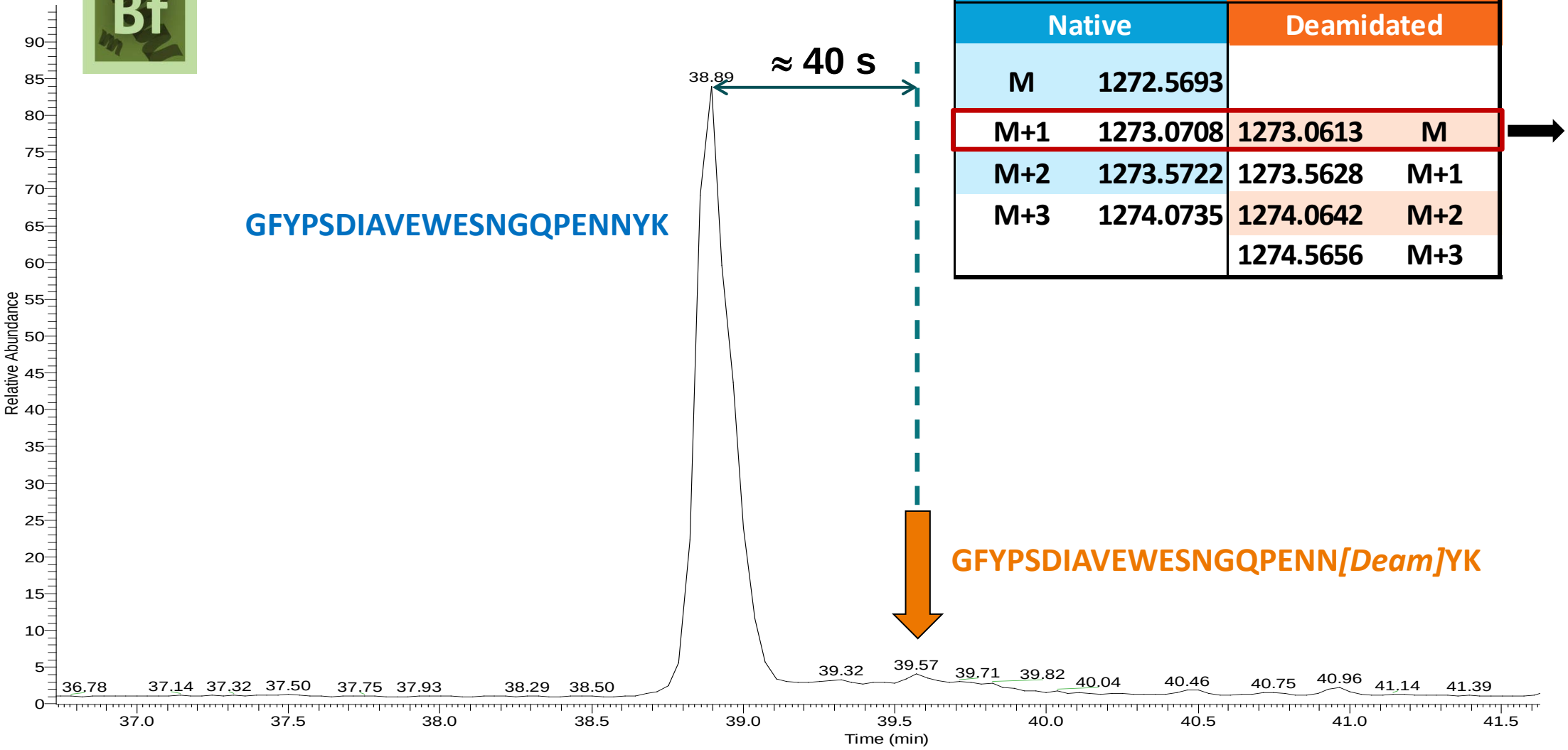
Retention time of GFYPSDIAVEWESNGQPENNYK Peptides



GFYPSDIAVEWESNGQPENNYK (+2) m/z			
Native		Deamidated	
M	1272.5693		
M+1	1273.0708	1273.0613	M
M+2	1273.5722	1273.5628	M+1
M+3	1274.0735	1274.0642	M+2
		1274.5656	M+3

→ Δ m/z = 0.0095

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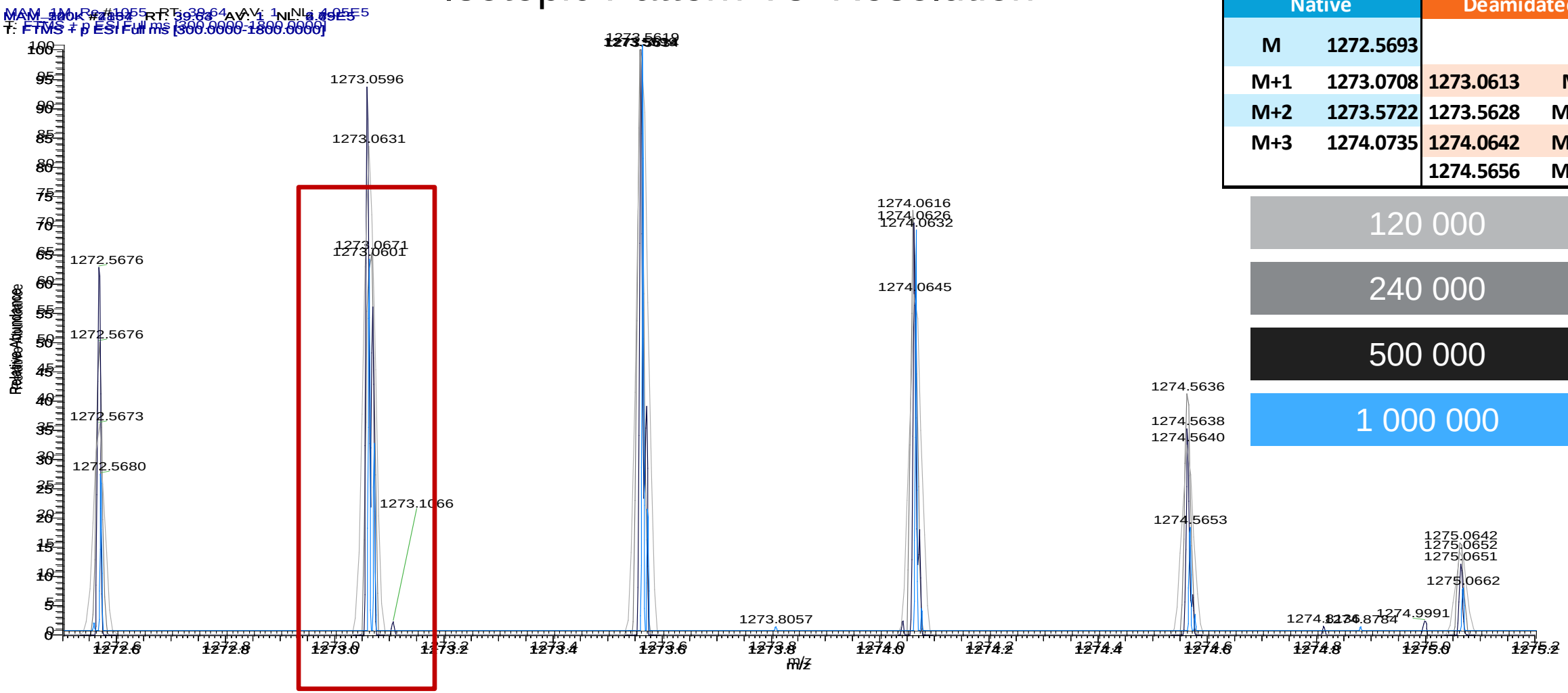
Spectrum at the Apex of the Deamidated PENNYK Peptide

Isotopic Pattern Vs. Resolution



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M+2	1273.5722	1273.5628	M+1
M+3	1274.0735	1274.0642	M+2
		1274.5656	M+3

120 000

240 000

500 000

1 000 000

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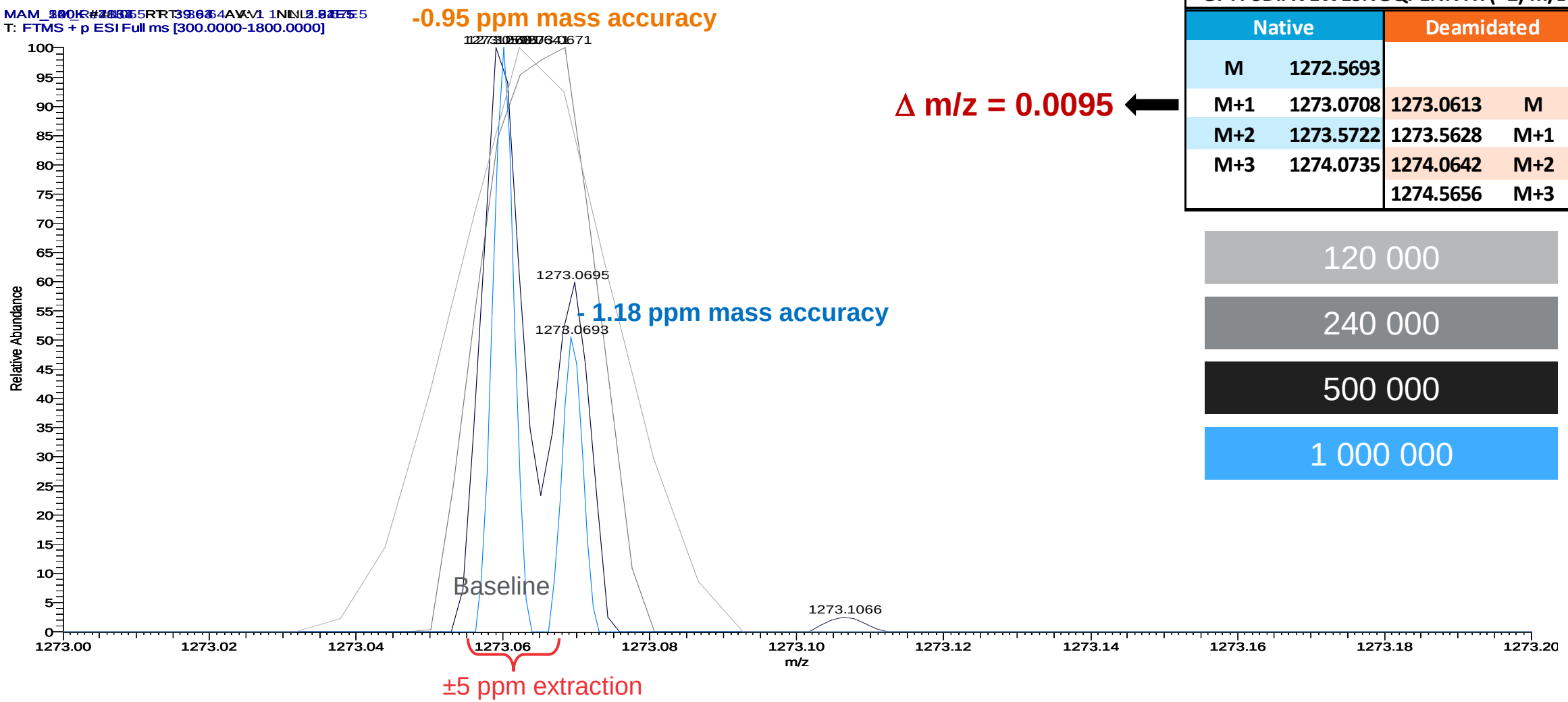
PENN[Deam]YK Monoisotopic Peak vs. Resolution



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		1274.5656	M+3

Δ m/z = 0.0095

120 000

240 000

500 000

1 000 000

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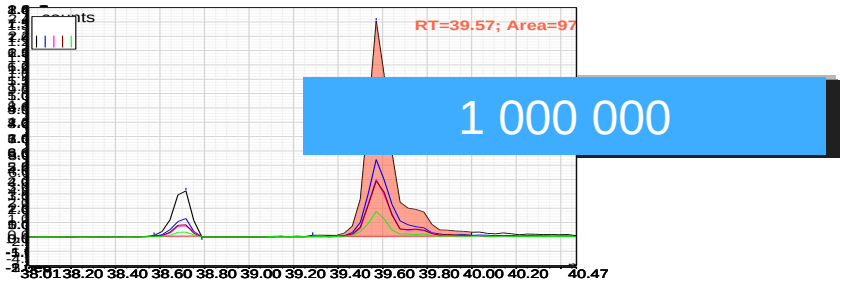
PENN[Deam]YK (+2 and+3): XIC of First 3 Isotopic Peaks for Quantitation



1 000 000

No.	Injection Name	Area counts*min GFYPSDIAVEWESNGQPENNYK[IsoDeamid]	Area counts*min GFYPSDIAVEWESNGQPEN[Deamid]NYK	Area counts*min GFYPSDIAVEWESNGQPENNYK	Relative Abundance % PENNYK-IsoDeam	Relative Aundance % PENNYK Deam
1	MAM_1M_Re	1.53E+05	9.71E+05	7.09E+07	0.212	1.348
2	MAM_500K	2.53E+05	1.61E+06	1.14E+08	0.219	1.396
3	MAM_240K	2.93E+05	1.93E+06	1.41E+08	0.204	1.342
4	MAM_120K	6.70E+05	3.48E+06	1.67E+08	0.391	2.028

Native peptide

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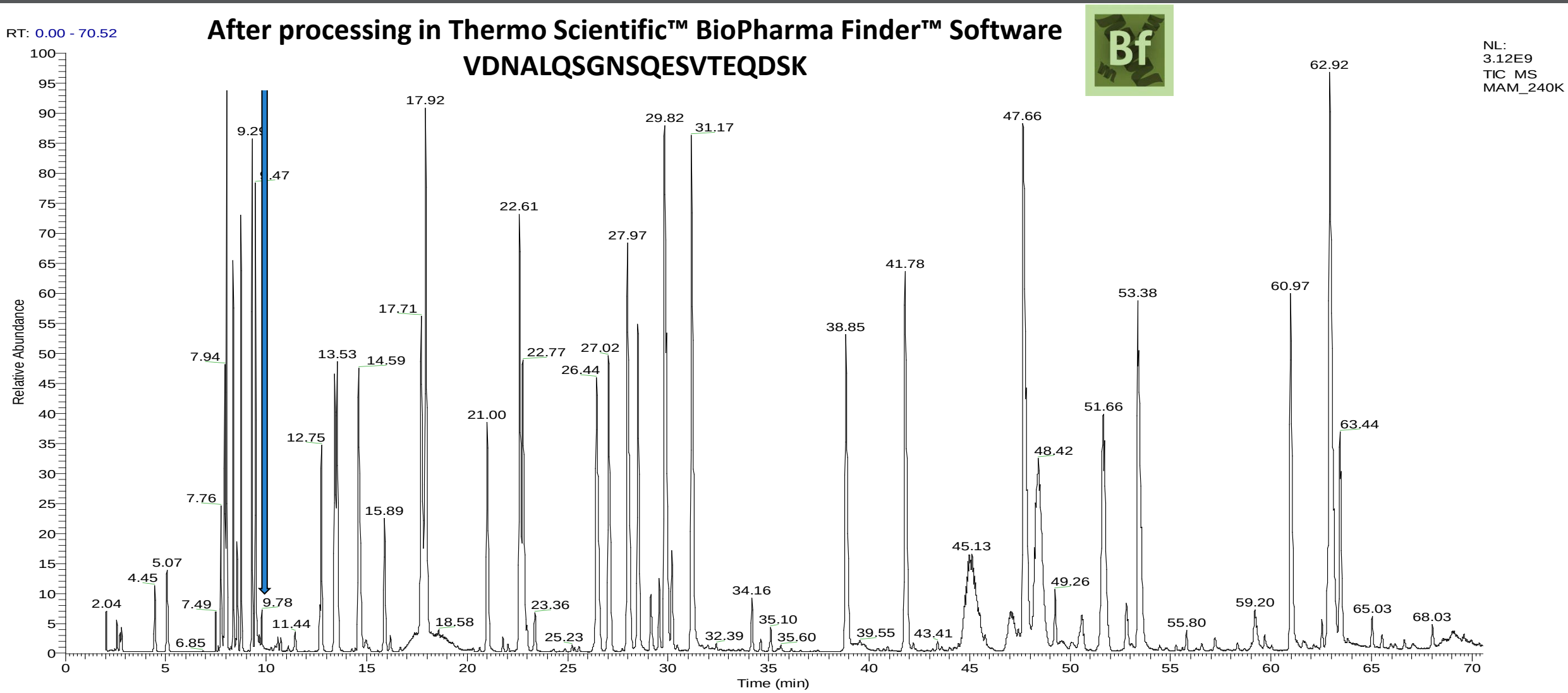
NISTmAb Digest Peptide Map Chromatogram



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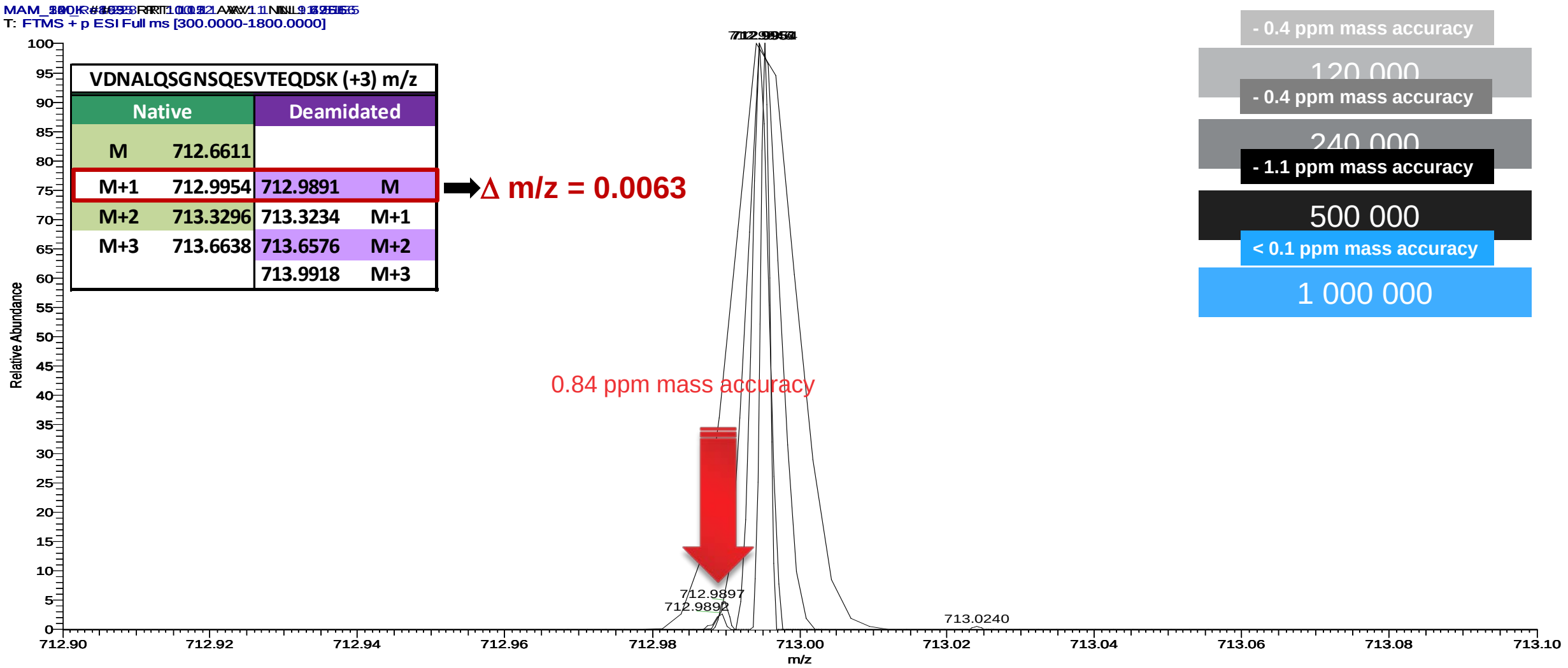
VDNALQSGN[Deam]SQESVTEQDSK Monoisotopic Peak



- 0.4 ppm mass accuracy

120 000

- 0.4 ppm mass accuracy

240 000

- 1.1 ppm mass accuracy

500 000

< 0.1 ppm mass accuracy

1 000 000

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Summary

- 1M Resolution can spectrally baseline resolve PENNYK deamidation from the native peptide for both +2 and +3 with <1 ppm mass accuracy
- Higher resolutions can benefit quantitation by preventing over integration of coeluting native+deamidated isotopes
- 1M resolution enables the detection of low level peptides obscured beneath their coeluting native counterparts

1M Ultra-High Resolution For Lipid Flux Analysis

> The 1 million resolution Orbitrap is a unique tool to allow fluxomics analysis of lipids with sensitivity comparable to radioactivity tracing.

Matt Mitsche, The University of Texas

To know more, please watch recorded webinar on c&en WEBINARS website:
https://cen.acs.org/media/webinar/thermo_090717.html

c&en | WEBINARS

Let's Chew the Fat with Orbitrap MS - Enabling Lipid Metabolic Flux Studies and Structural Elucidation of Lipids with Ultra High Resolution and UVPD Fragmentation

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Challenge

Dissociation techniques currently available (CID, HCD and ETD) can be insufficient for comprehensive characterization of analytes of interest

UV Photodissociation (UVPD) option

- Provides unique fragments vs. other dissociations increasing sequence coverage of proteins
- Available only on Thermo Scientific™ Orbitrap Fusion™ Lumos™ MS

UVPD Implementation (Class 1 Laser System)

UVPD Source

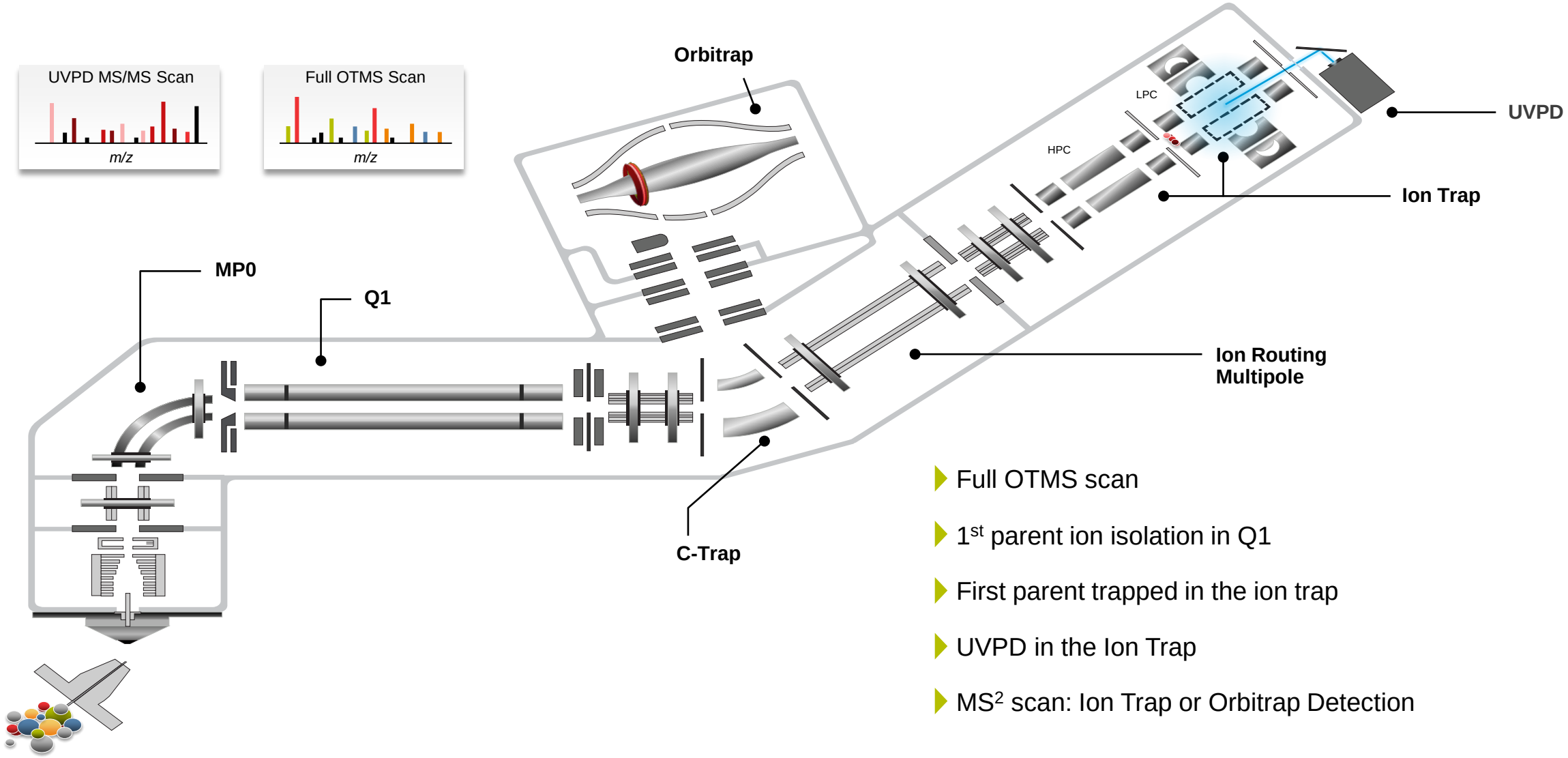
The UVPD MS^n fragments are generated in the linear ion trap and can be detected by either the ion trap or Orbitrap

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 Is Unique to the Thermo Scientific Orbitrap Fusion Lumos MS

Data Dependent Experiment: OTMS>UVPD OTMS^2



- Full OTMS scan
- 1st parent ion isolation in Q1
- First parent trapped in the ion trap
- UVPD in the Ion Trap
- MS^2 scan: Ion Trap or Orbitrap Detection

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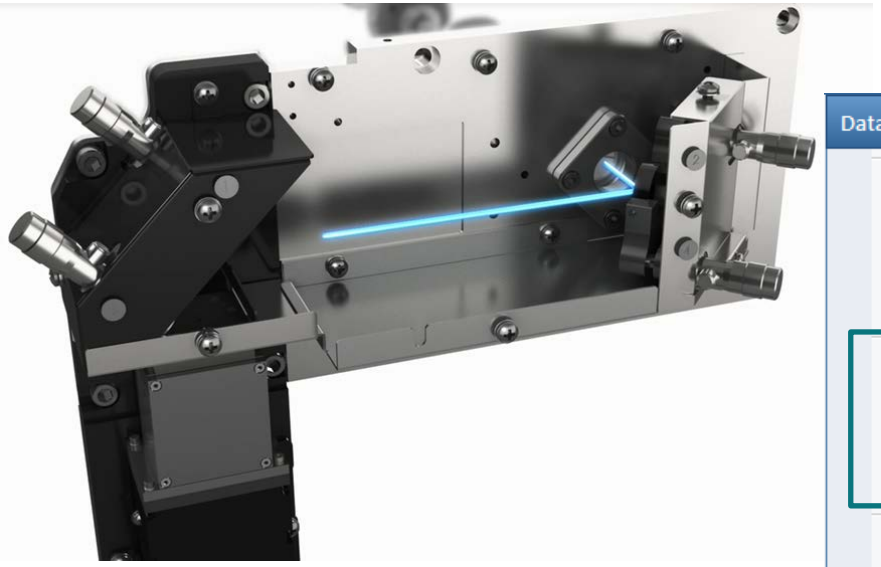
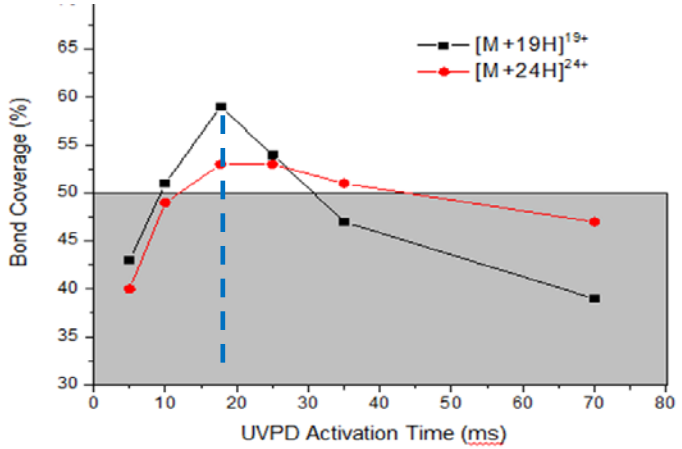
UVPD Is Easy To Use





UVPD activation time is *m/z* independent:
The highest coverage is achieved at the same activation time (17 ms) for two different charges states of apomyoglobin

Data-Dependent MSⁿ Scan Properties

Isolation Mode	Quadrupole
Isolation Window (m/z)	1.6
Isolation Offset	Off
Activation Type	UVPD
Use Calibrated Molecular Weight-Dependent UVPD Activation Time	☐
UVPD Activation Time (ms)	10
Detector Type	Orbitrap
Scan Range Mode	Auto: m/z Normal
Orbitrap Resolution	60000
AGC Target	2.0e5
Inject Ions for All Available Parallelizable Time	☐
Maximum Injection Time (ms)	118
Microscans	1
Data Type	Profile
Use EASY-IC™	☐
Scan Description	

User-Friendly Implementation

- The UVPD source is embedded inside the mass spectrometer for optimal performance and reliability
- Minimal set-up required by user
- User defined UVPD activation time (ms)
- Calibrated Molecular Weight-Dependent Activation Time
  - UVPD Activation time multiplier (%)

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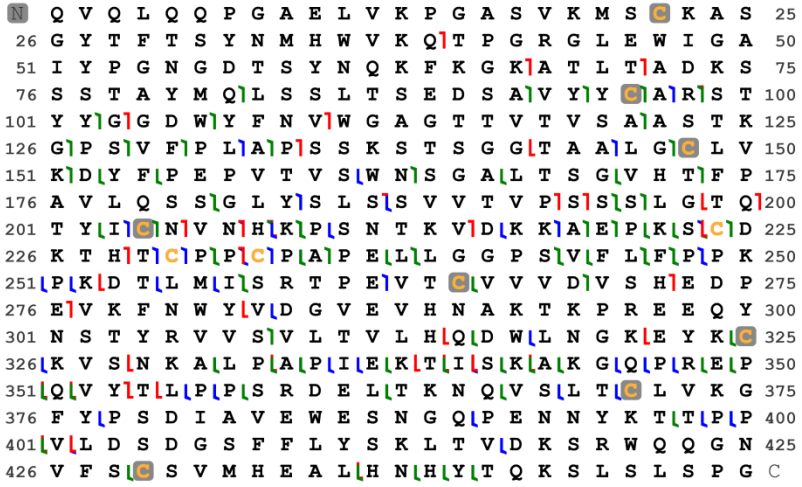
UVPD Top-Down Of Intact IgG

ETD HD

26%

(2 LC-MS runs combined)



UVPD

30%

(single LC-MS run)

Unique Sequence Coverage

- LC-MS analysis of intact Rituximab
- ETD HD of the Heavy Chain provides up to 26% sequence coverage
- UVPD yields up to 30% sequence coverage
- UVPD provides more large fragments than ETD HD confirming the sequence in the middle
- Both ETD HD and UVPD combined provide up to 43% sequence coverage within 1 hour

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UVPD Top-Down Of Intact IgG

ETD HD 26%

(2 LC-MS runs combined)

UVPD 30%

(single LC-MS run)

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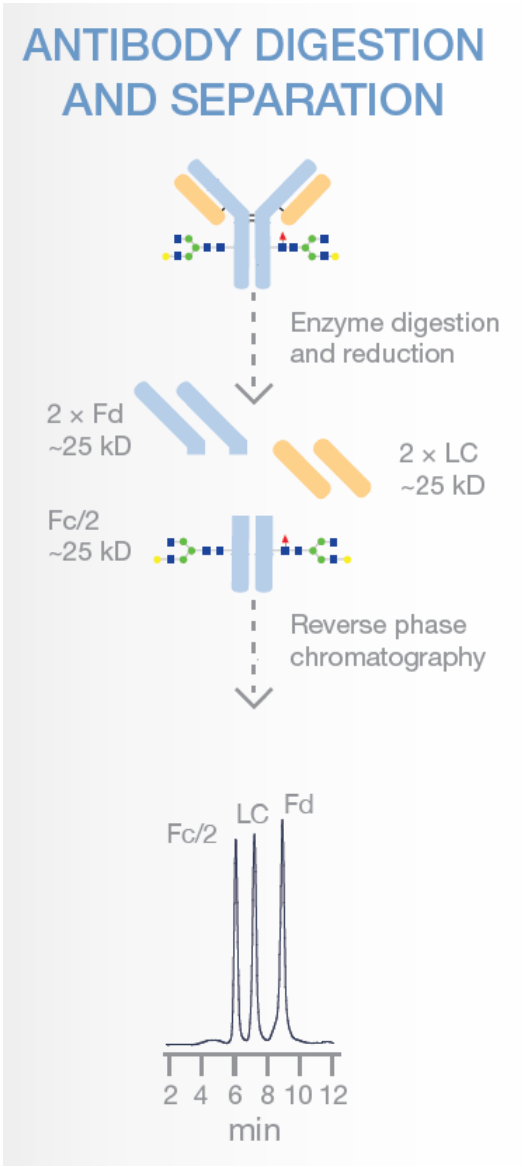
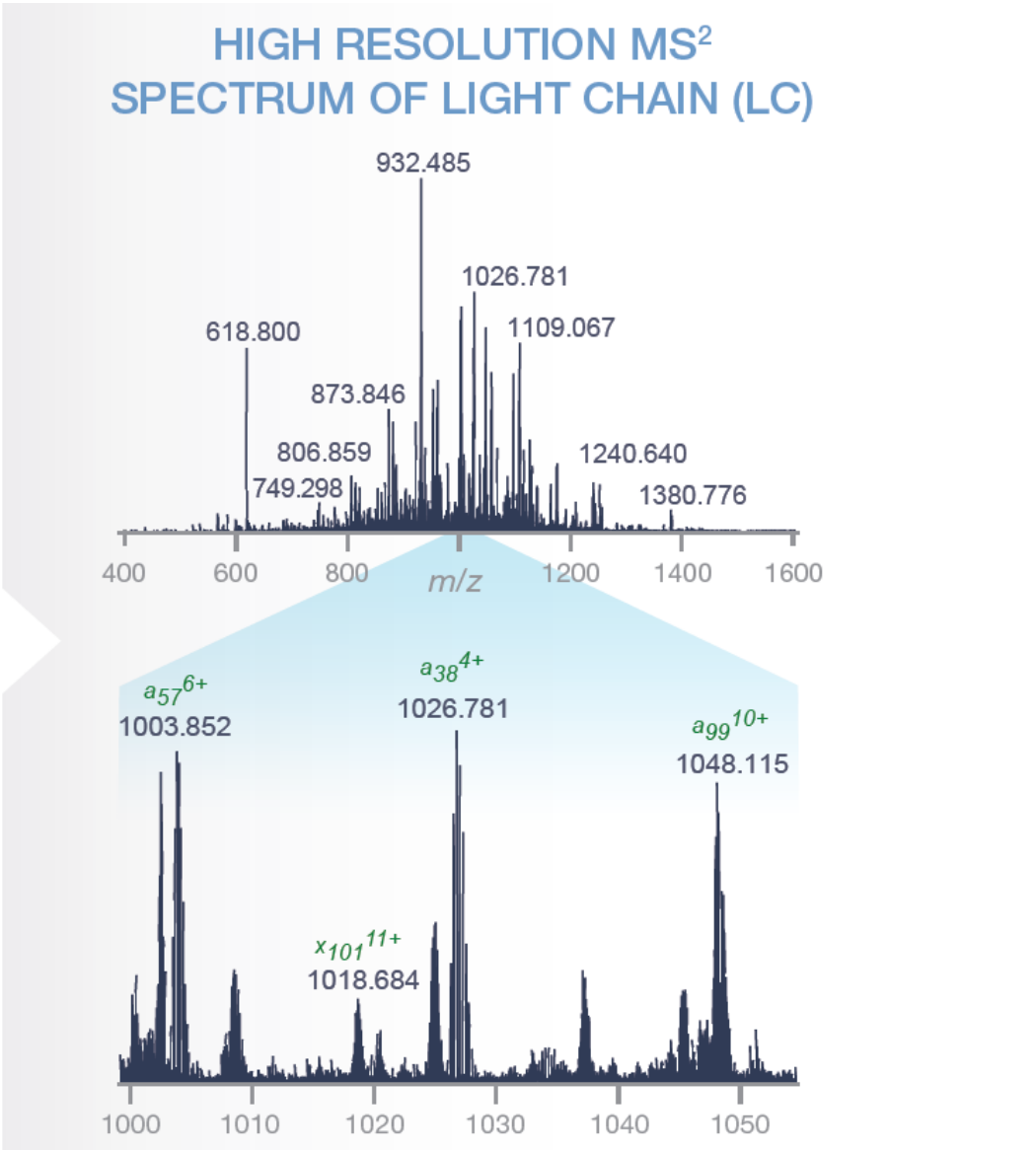
UVPD Middle-Down Of Rituximab





Unique Sequence Coverage

- New UVPD fragmentation complements existing fragmentation techniques
- UVPD provides 20% unique sequence coverage vs. ETD/EThcD
- Unambiguous glycosylation site localization and extensive coverage of the antigen-binding complementarity determining regions

UVPD Middle-Down Of Rituximab

Rituximab (Fc/2) total bond coverage by LC-MS middle-down

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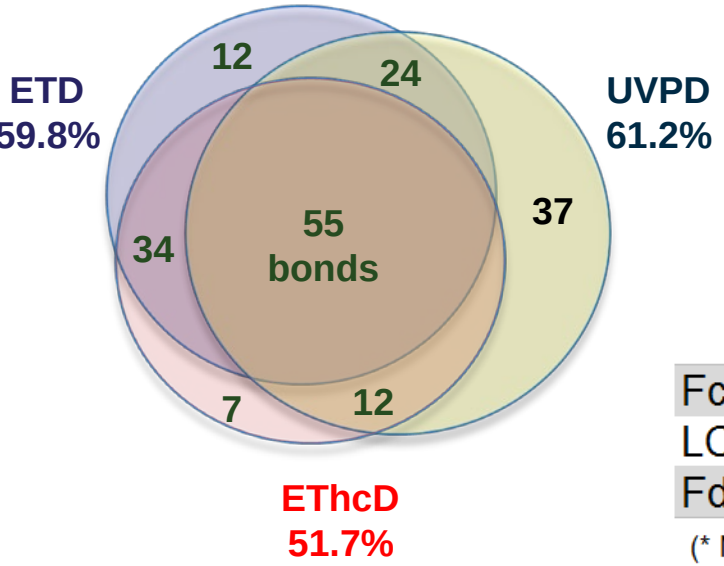


Rituximab Fc/2 (6 runs)

	% Sequence coverage*	% Unique matched fragment		
		UVPD	ETD	EThcD
Fc/2	87	20	7	4
LC	80	21	12	5
Fd	72	19	18	4

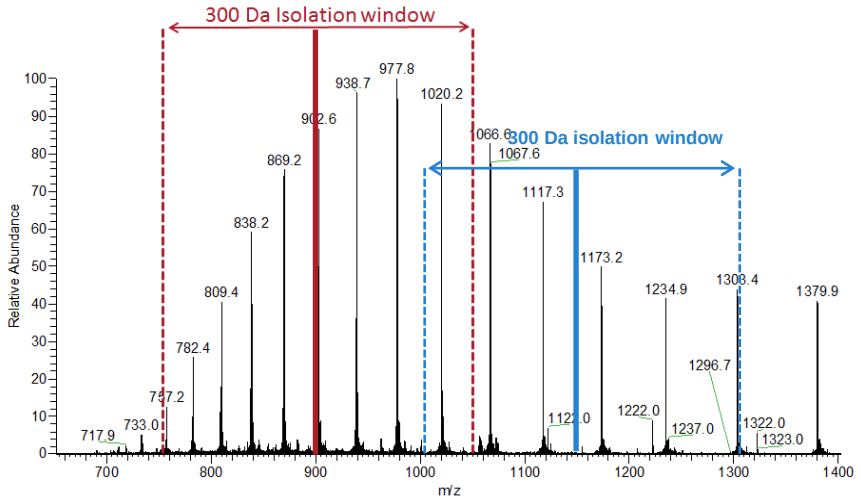
(* Number of LC-MS/MS runs used: 3 ETD, 1 EThcD and 2 UVPD)

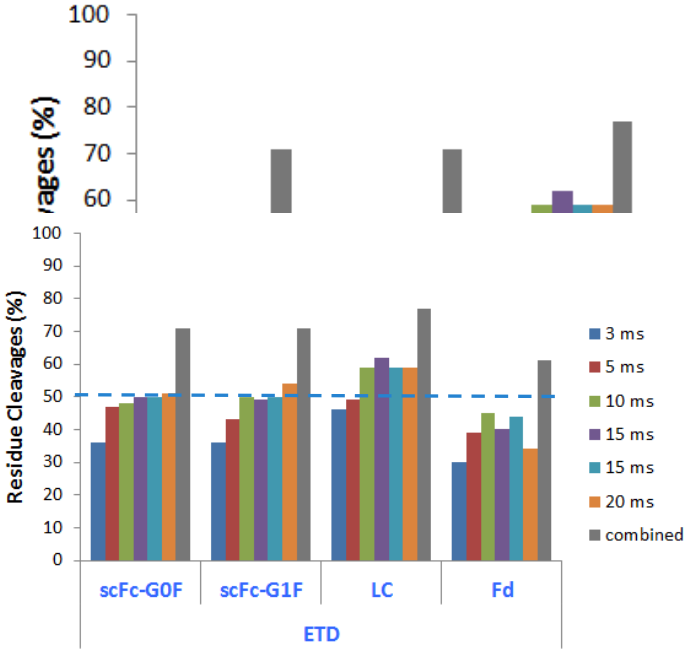
Unique Sequence Coverage

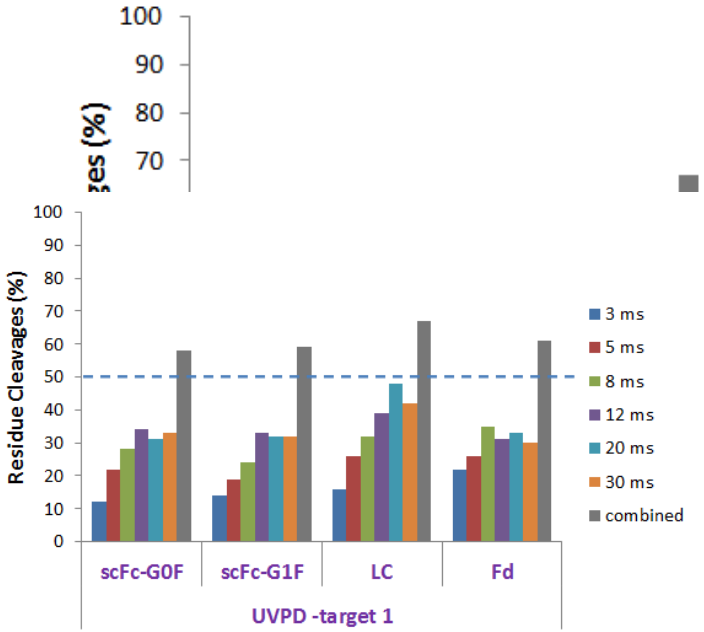
- New UVPD fragmentation complements existing fragmentation techniques
- UVPD provides 20% unique matched fragments vs. ETD/EThcD
- Unambiguous glycosylation site localization and extensive coverage of the antigen-binding complementarity determining regions

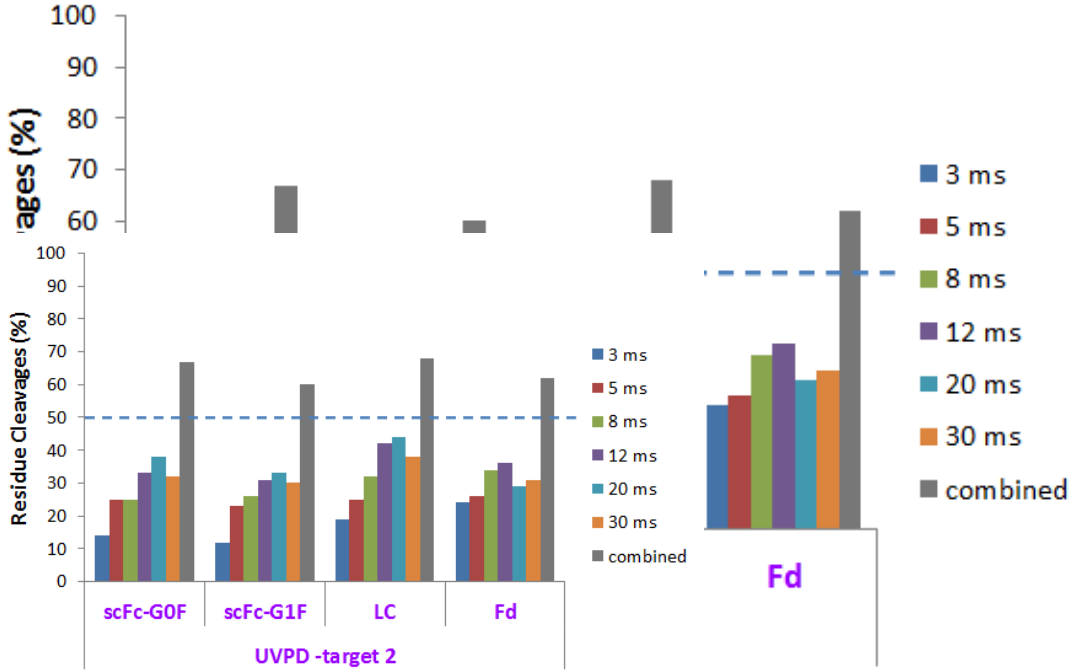
Middle-Down of NIST mAb: ETD and UVPD

Wide isolation window









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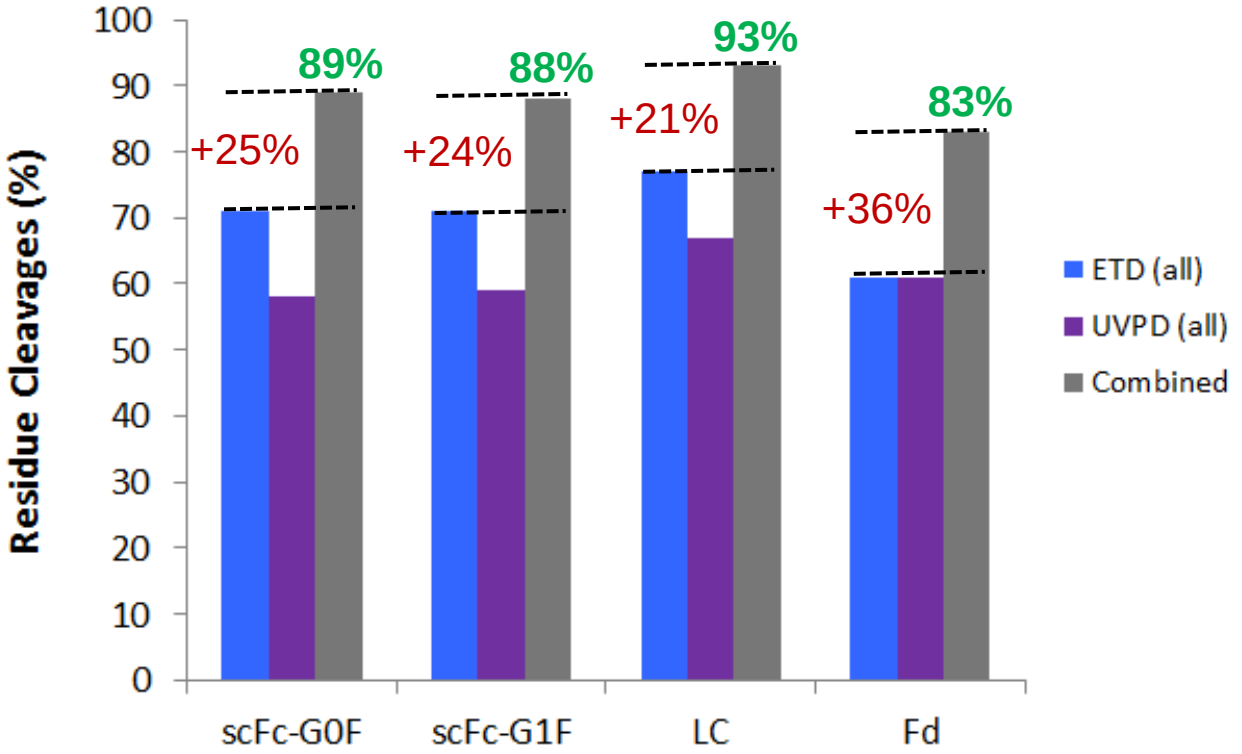
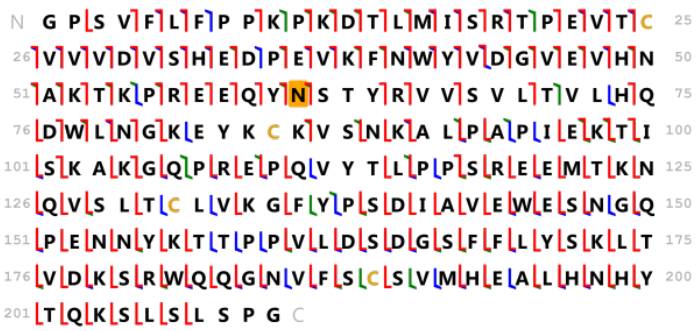
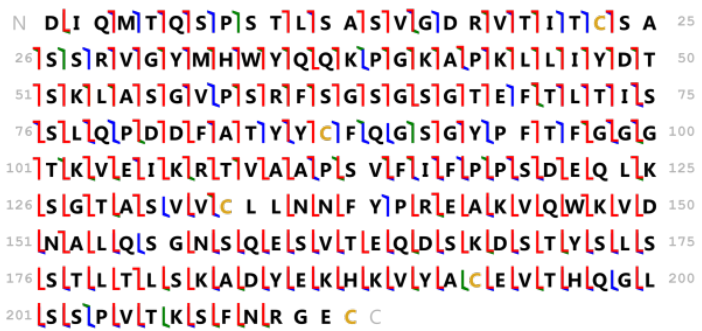
Combining ETD and UVPD: All Runs



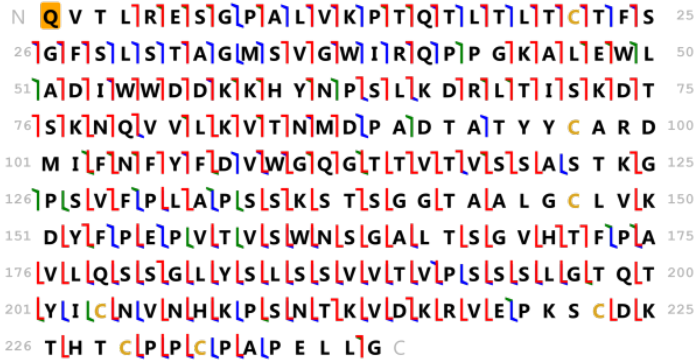
scFc – G0F



LC



Fd



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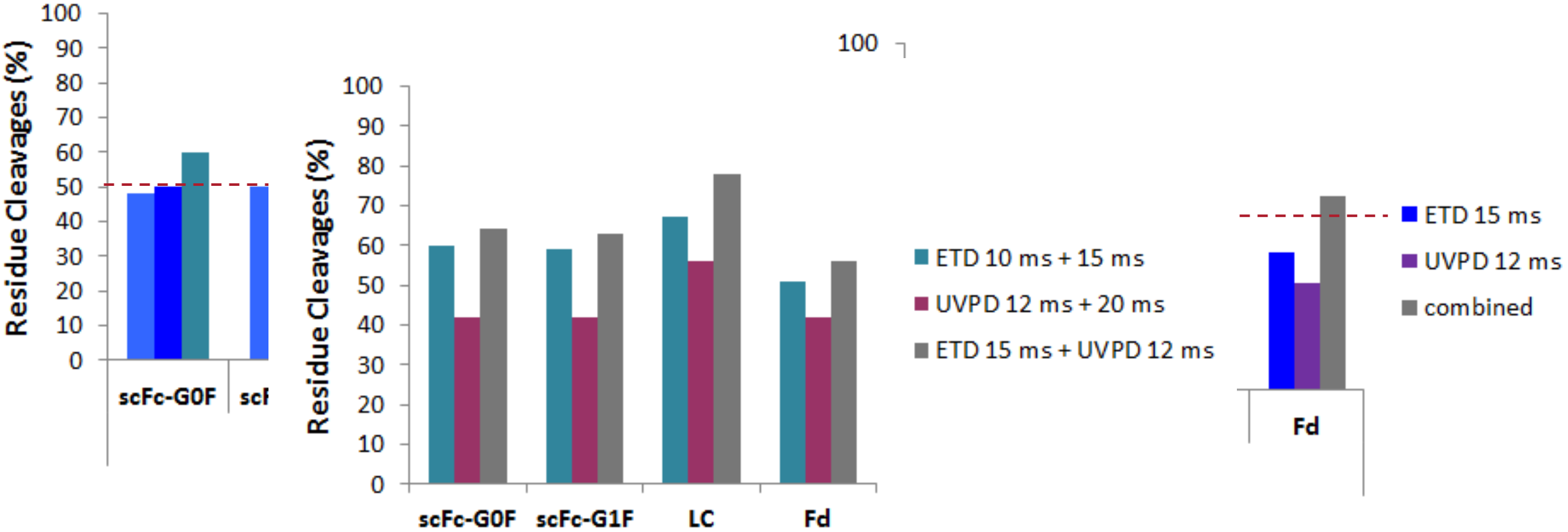
Combining ETD and UVPD: Only Two Runs



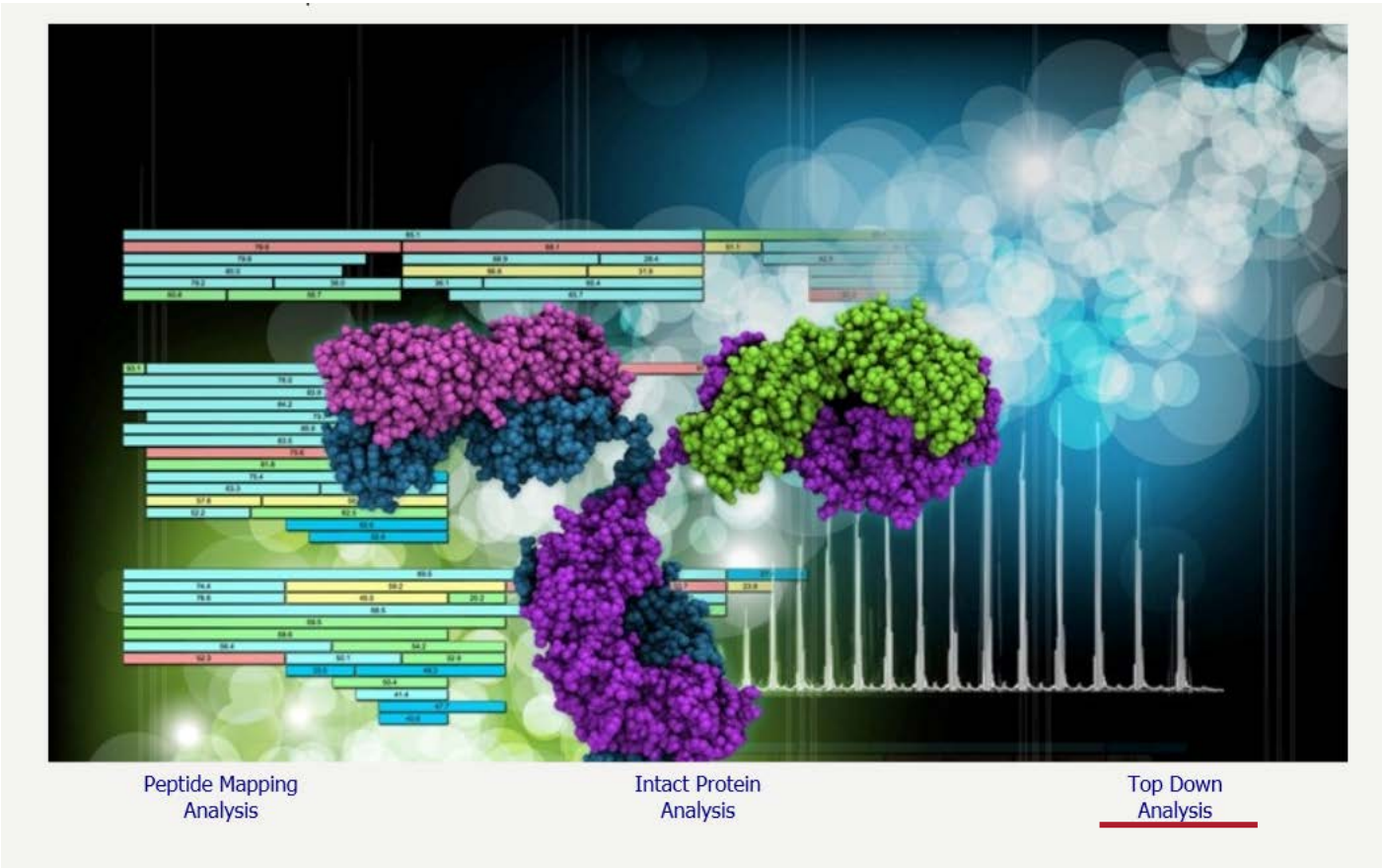
Even with only 2 runs: the combination of ETD and UVPD gives more sequence coverage

Thermo Scientific BioPharma Finder 3.0 Software Preview (End of 2017)

Experiment Types

- Protein Sequence Manager
- Peptide Mapping Analysis
- Intact Protein Analysis
- Top Down Analysis

(Time defined data processing)



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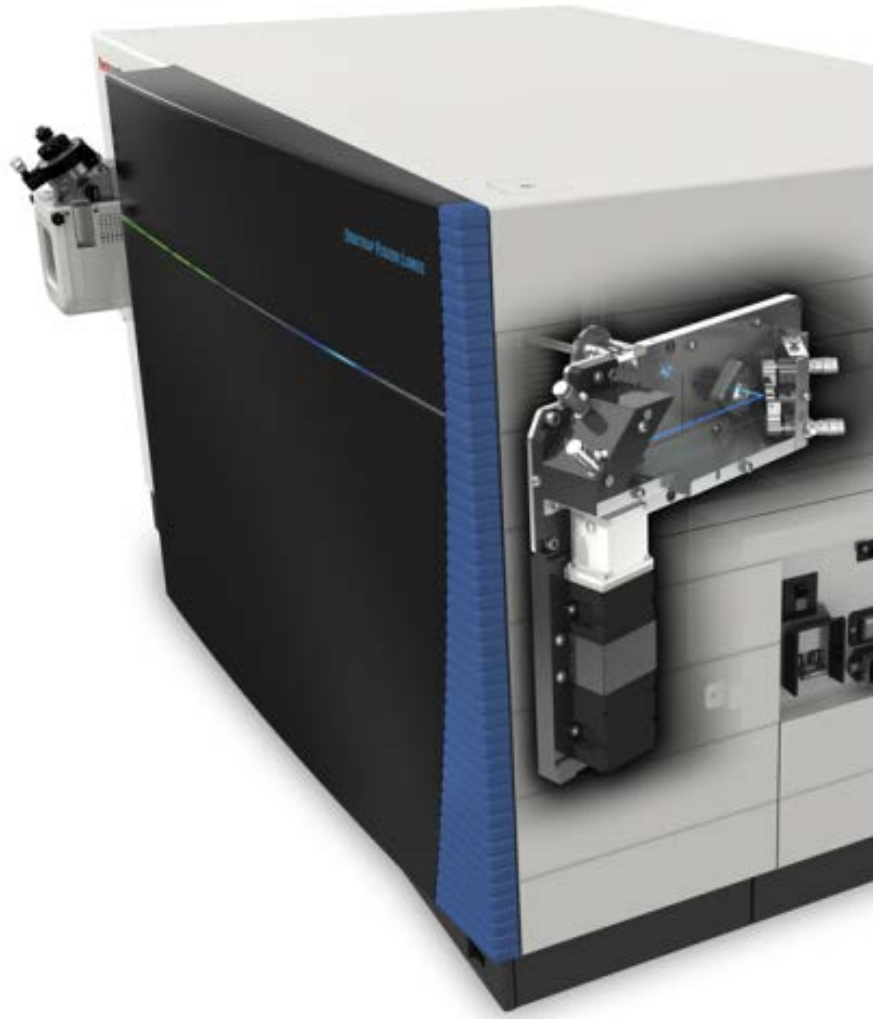
Example Applications Of UVPD

Comprehensive sequence characterization/confirmation of protein drugs

Identification and characterization of intact proteins by MS



Thermo Scientific™ Orbitrap Fusion™ Lumos™ MS with UVPD provides a unique fragmentation mode for unmatched large and small molecule structure determination for Proteomics, Metabolomics, and Biopharma applications.

Neil Kelleher, Northwestern University

> UVPD is unique because of the different fragmentation channels and there are so many of them which leads to complete molecular characterization for proteins and proteoforms. The depth of analysis afforded by UVPD, combined with the speed of the OT Fusion Lumos and proper software is very impressive.

UVPD For Comprehensive Lipid Characterization

> The Orbitrap Fusion Lumos with UVPD allows us to more comprehensively characterize complex mixtures of lipids that are present in a given biological sample, in order to understand their functional roles in the progression of disease.

Gavin Reid, The University Of Melbourne

To know more, please watch recorded webinar on c&en WEBINARS website:
https://cen.acs.org/media/webinar/thermo_090717.html

To Find Out MORE

planetorbitrap.com/orbitrap-fusion-lumos

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