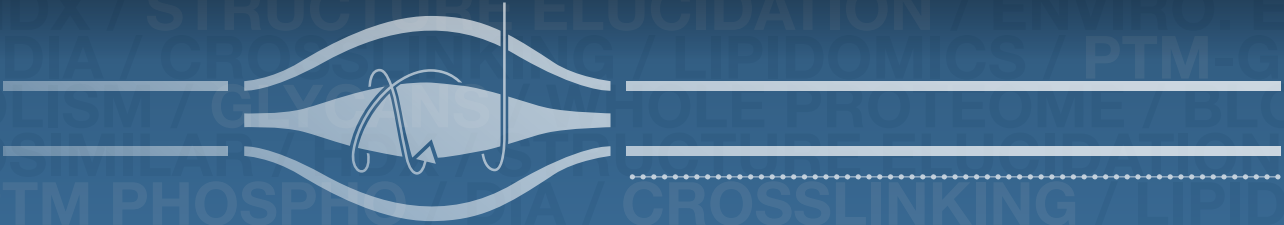


DESIGNED TO ADVANCE YOUR SCIENTIFIC PURSUITS



**Orbitrap Fusion Lumos Tribrid  
Mass Spectrometer**



**ThermoFisher**  
SCIENTIFIC

# PUSHING NEW FRONTIERS IN SCIENCE

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Breakthroughs in science rely on great scientists making rapid progress answering challenging questions.

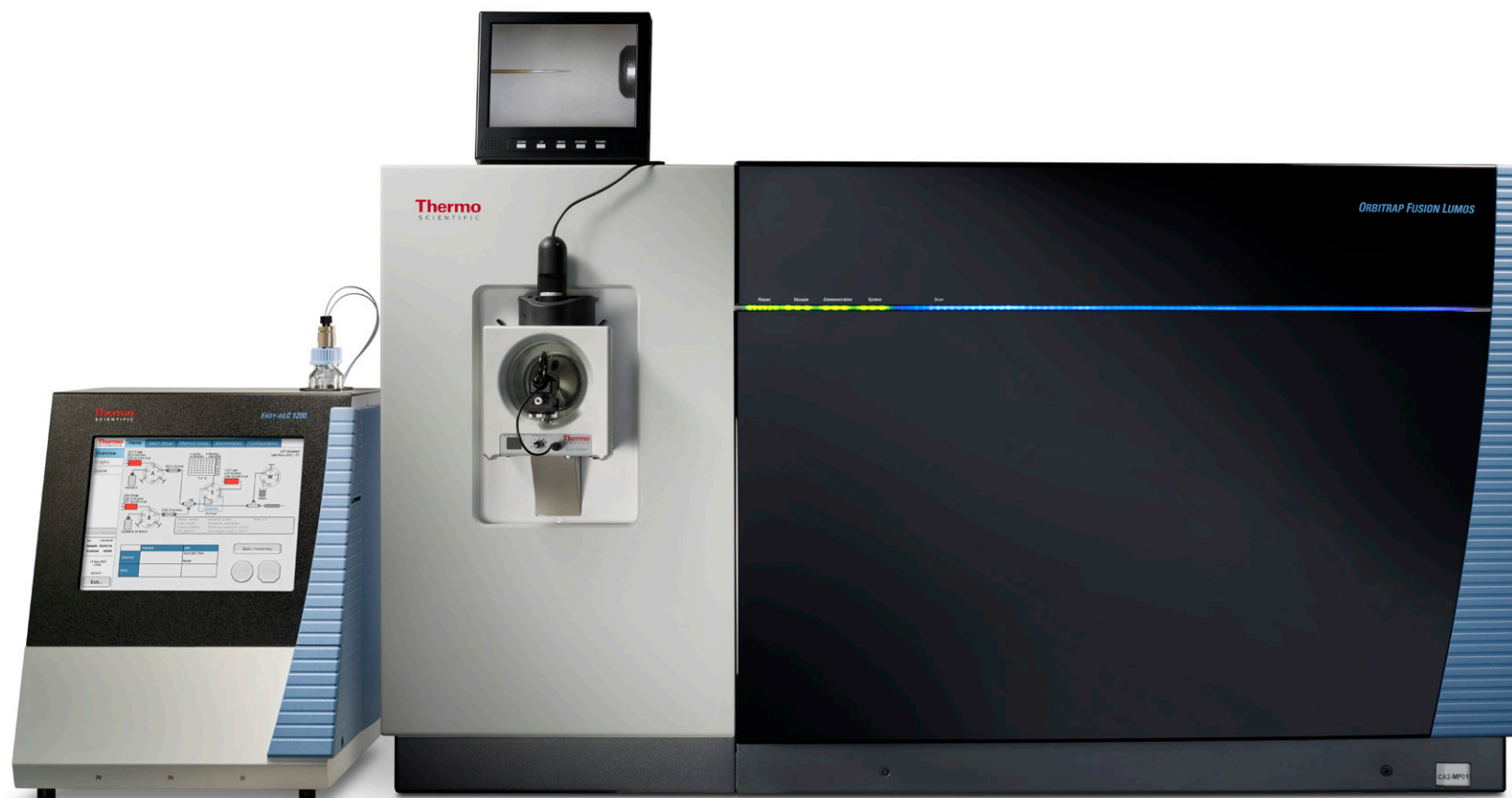
**“How does lipid metabolism affect cancer?”**

**“How do non-cancerous cells mutate?”**

**“What protein modifications are indicative of a disease state?”**

As a leading scientist, you need to be equipped with the latest innovations in analytical technology.

**With the Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribid™ mass spectrometer, we are committed to keeping pace with your scientific pursuits through continuous innovations.**



# Designed for High Impact Discoveries

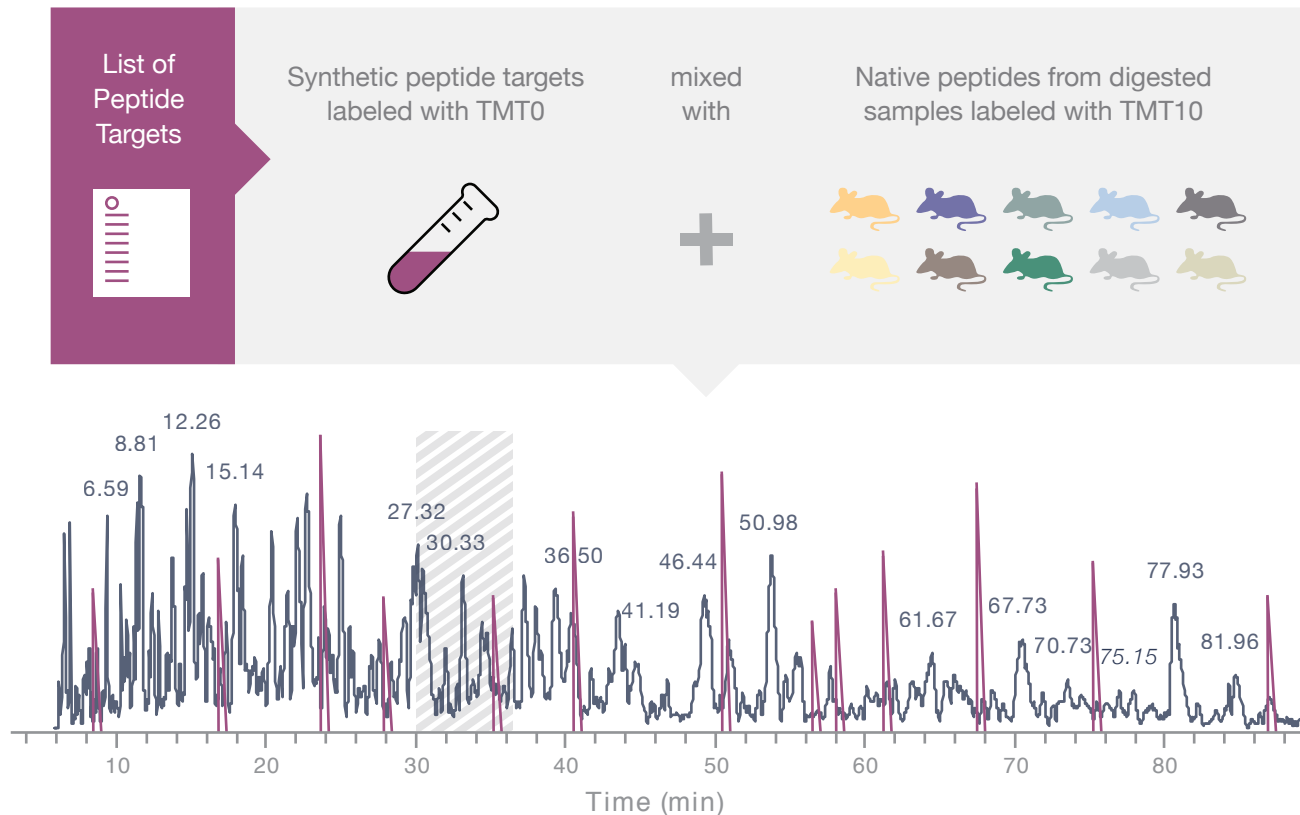
The Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribid™ MS delivers superior sensitivity, selectivity and versatility to enable life scientists to obtain the highest quality data. It is designed to pursue difficult analyses, including multiplexed quantitation of low-abundance peptides in complex matrices, characterization of positional isoforms of intact proteins, resolution of isobaric metabolites, protein structure characterization using chemical crosslinking and deep mining of challenging post-translational modifications.

You trusted us to make the versatile and powerful mass spectrometer a reality.  
We now trust you with groundbreaking science and high impact discoveries.

# Multiplexing Targeted Analysis

## Accurately Quantifying Hundreds of Proteins in 90 Minutes

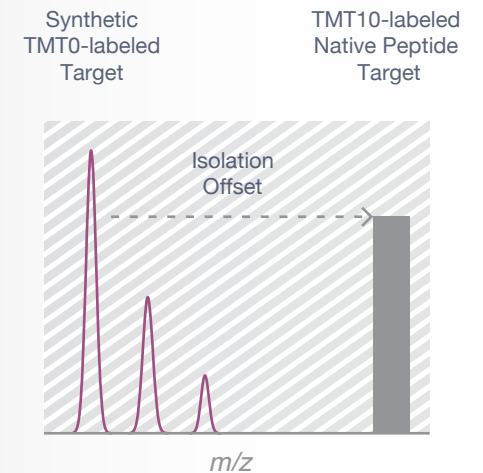
Multiplexed analyses using isobaric mass tags are widely utilized for high throughput quantitative comparisons of protein abundances. The TMT SPS MS<sup>3</sup> workflow available on the Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ MS is a proven method which enables the simultaneous analysis of 10 samples with improved quantitative accuracy. Pushing the multiplexing frontiers even further, targeted assays incorporating this technique can now be built to detect and quantify very low levels of target peptides even from undetected precursors. This new TMT SPS tMS<sup>3</sup> workflow further benefits from the enhanced sensitivity of the Orbitrap Fusion Lumos MS, which boosts the number of quantifiable peptides present at low levels.



Base peak Extracted Ion Chromatogram (XIC). Purple peaks are XICs of TMT0-labeled peptides.

Everley et al. WP 663, ASMS 2016.

## MS DETECTION

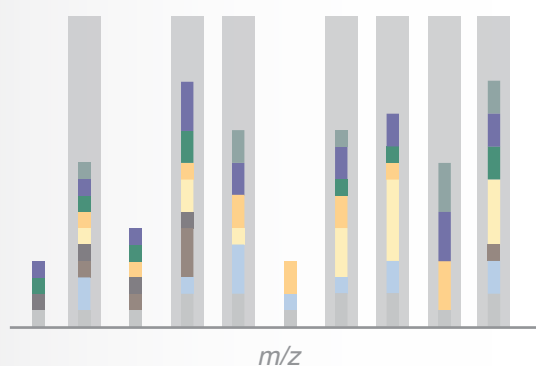


The synthetic TMT0-labeled peptide is detected in the full MS scan. The native TMT10-labeled peptides are isolated by using an offset ( $m/z$  difference between TMT0 and TMT10).

The Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ MS now supports targeted proteomics assays with multiplexing in two dimensions: targets and samples. These experiments make use of triggered-by-offset-in-mass peptides that allow for quantitation, even from undetected precursors. Using this method, we monitored 131 target peptides corresponding to 69 proteins across the NCI-60 cell line in biological triplicate, analyzing 180 samples in only 48 hours (16 min/sample). We found that accurate and reproducible TMT SPS tMS<sup>3</sup> quantitation elucidated a correlation between expression of key proteins and cellular response to drug treatment.

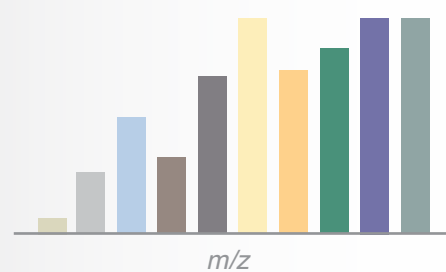
—Steven Gygi, Professor, Harvard Medical School

### CID MS<sup>2</sup>



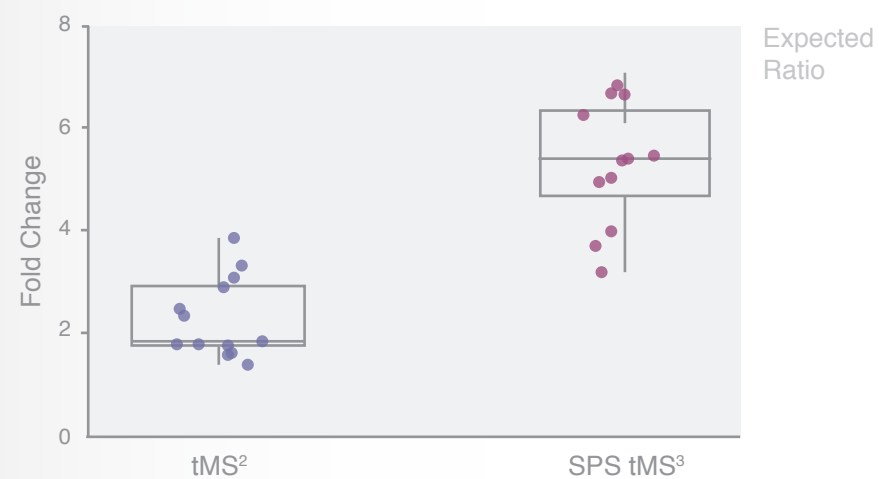
CID MS<sup>2</sup> spectrum of native peptide target contains multiplexed fragments. Gray bars indicate the predicted fragment target ions that are selected for the following SPS tMS<sup>3</sup> step.

### FTMS HCD SPS tMS<sup>3</sup>



Targeted SPS MS<sup>3</sup> of the predicted MS/MS fragments generates ten reporter ions used for quantitation.

### IMPROVED QUANTITATIVE ACCURACY AT LOW LEVELS

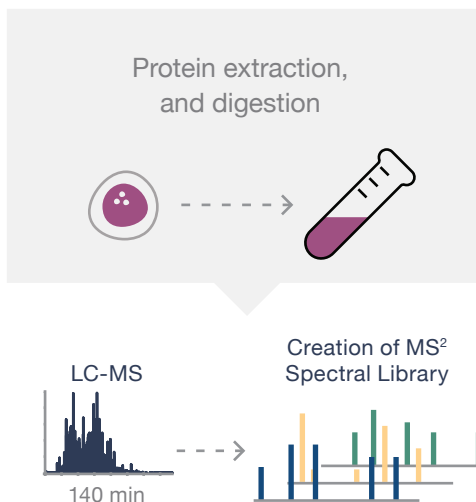


Comparison of results for the TMT tMS<sup>2</sup> and TMT SPS tMS<sup>3</sup> quantification methods shows improved accuracy of the tMS<sup>3</sup> method. Each point represents the fold change of a single quantification event for each of the targeted peptides present at 100 amol level in matrix. The expected ratio is 8:1.

# Advancing Peptide Quantitation

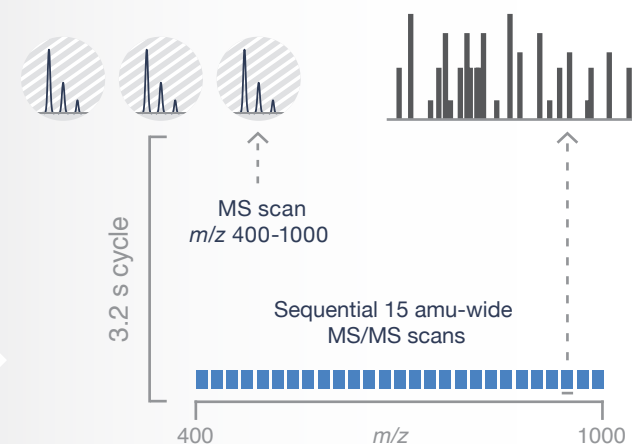
## Large-Scale Quantitation with Data-Independent Acquisition

Data-Independent Acquisition (DIA) is widely used today for the global identification and quantification of thousands of peptides in complex mixtures. Step-wise isolation and MS/MS fragmentation of all ions in a defined  $m/z$  window cover the targeted mass range and provide ultra-high resolution MS and MS/MS data for all components in the sample, allowing accurate quantitation of peptides, and the unique opportunity for retrospective analysis of unknowns and new targets of interest in the future. The Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ MS provides speed, selectivity and enhanced sensitivity to obtain exceptional performance while maintaining high reproducibility of quantitation for low abundance analytes.



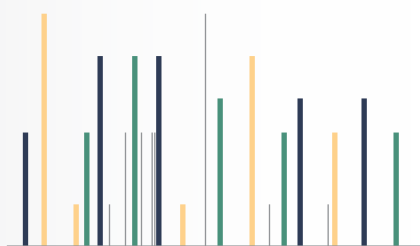
HeLa digest standard was introduced into the Orbitrap Fusion Lumos MS using RP-HPLC. Data-Dependent experiments were used to generate high resolution accurate mass (HRAM) MS<sup>2</sup> spectra. The data was searched using SEQUEST™ HT in order to generate a spectral library for the following DIA data analysis.

### DATA-INDEPENDENT HRAM HCD MS<sup>2</sup>



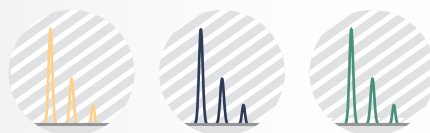
DIA acquisition was performed using a 120,000 FWHM resolution MS scan followed by 15 amu-wide MS/MS windows at 30,000 FWHM resolution with 60 ms maximum injection time.

## MATCHING DIA SPECTRUM TO THE SPECTRAL LIBRARY



DIA MS<sup>2</sup> spectra were searched against the library built from Sequest HT search results. Fragment peak detection was performed with Biognosys Spectronaut™ software.

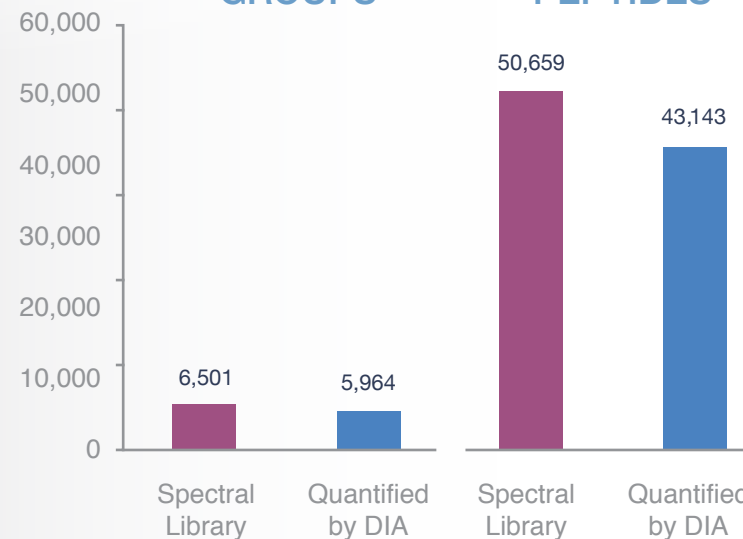
## QUANTITATION USING FULL MS



Peptide quantitation took into account all confidently identified isotopes from consecutive MS full scans, correcting for interference and using cross-run normalization.

## PROTEIN GROUPS

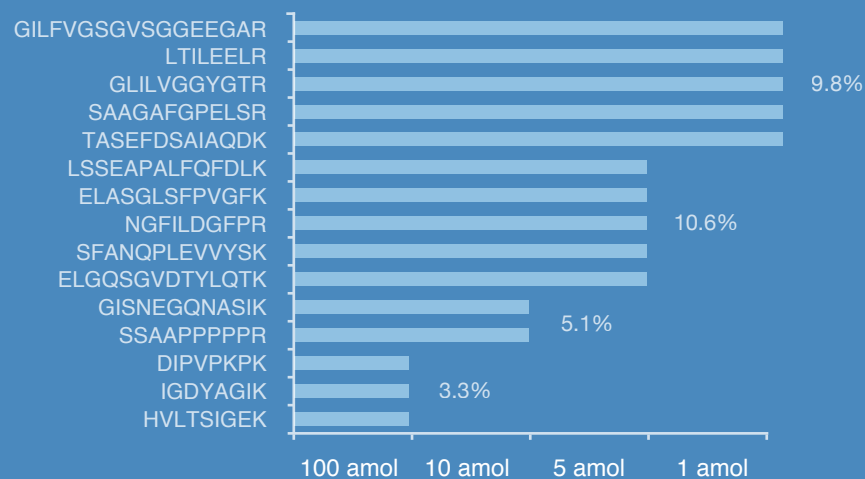
## UNIQUE PEPTIDES



Nearly 92% of the protein groups and over 85% of the unique peptides were confidently matched to the spectral library and quantified with <6.9% CV when using 500 ng of HeLa digest.

## Confident Low-Attomole Limit Peptide Quantitation Using Parallel Reaction Monitoring

Parallel Reaction Monitoring (PRM) is uniquely designed for quantifying hundreds of targeted proteins in complex matrices. Using this approach, precursor ions are isolated and selectively fragmented, with the resulting product ions analyzed in the Orbitrap. This approach benefits from the brighter ion source and Advanced Quadrupole Technology of the Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ MS, routinely achieving attomole-level limits of quantification (LOQ) in matrix.



Fifteen PRTC peptides were spiked into 200 ng of HeLa digest and analyzed by LC-MS (30 min run). The Orbitrap Fusion Lumos MS provides accurate quantitation of all 15 PRTC peptides with some down to 1 attomole levels. Average CV% for each LOQ level is shown.

# New Horizons in Intact Protein Analysis

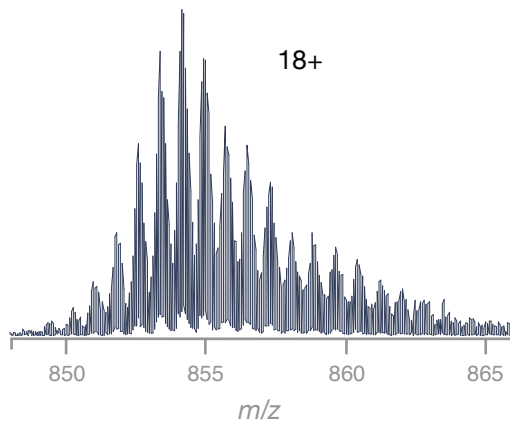
## Highly Selective Analysis of Protein Isoforms

Top-down mass spectrometry is commonly utilized to characterize intact proteins and their modifications. The Advanced Vacuum Technology, first pioneered on the Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ MS, provides conditions optimized for improved performance in intact protein analysis. The high selectivity of Advanced Quadrupole Technology allows for isolation of precursors and detection of fragments with very high resolving power in the Orbitrap analyzer. Combined, the new system efficiently delivers the high quality data for the characterization of protein isoforms and their post-translational modifications.

“The Lumos proved capable of high selectivity precursor selection in MS but retained exceptionally high sequence coverage for MS<sup>2</sup>. This enabled the decoding of the most complicated core histone H3 to detect trivalent proteoforms uniquely detected in a cellular model of the B-cell cancer, multiple myeloma.”

—Neil Kelleher, Professor, Northwestern University

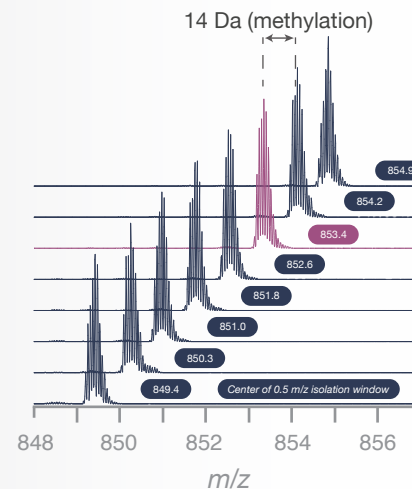
### INTACT HISTONE H3 SPECTRUM, 18+



Advanced Vacuum Technology increases ion transmission, improving the quality of intact protein spectra acquired in the high resolution accurate mass Orbitrap analyzer.

Zheng, Y et al. *Mol Cell Proteomics* 2016, 15,776-90.

### HIGH RESOLUTION ISOLATION OF METHYLATED FORMS OF HISTONE H3



With improved ion transmission provided by the Advanced Quadrupole Technology, it is now possible to efficiently enrich for individual isobaric protein forms for a subsequent top-down analysis.

### FRAGMENTATION MAPS OF HISTONE H3 FROM ETD HD™ TOP-DOWN EXPERIMENTS



ETD HD identification of isomeric forms of histone H3, differing in the site of trimethylation (K9 vs. K27) from the precursors at  $m/z$  853.4.

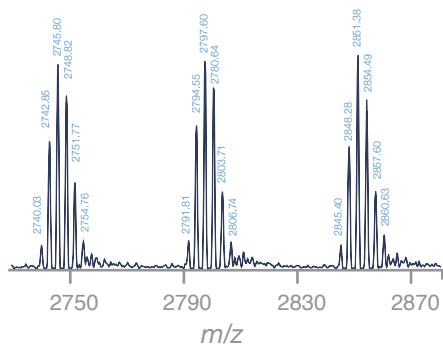
ETD HD enhances the dynamic range of ETD spectra by increasing the precursor ion storage capacity. The higher efficiency of ETD HD experiments provides greater sequence coverage at faster acquisition rates.



# Comprehensive Characterization of Intact Monoclonal Antibodies

Recent advances in protein and antibody-based therapeutics have led to a demand for mass spectrometers capable of comprehensive characterization of antibody heterogeneity in addition to providing accurate mass measurements. The Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ MS allows high accuracy mass analysis of the intact monoclonal antibody with isotopic resolution of the heavy and light chains. The combination of the various fragmentation methods available on the Orbitrap Fusion Lumos MS and the improved efficiency provided by ETD HD enables users to obtain high sequence coverage for the light and heavy chains.

## INTACT mAb GLYCOFORMS

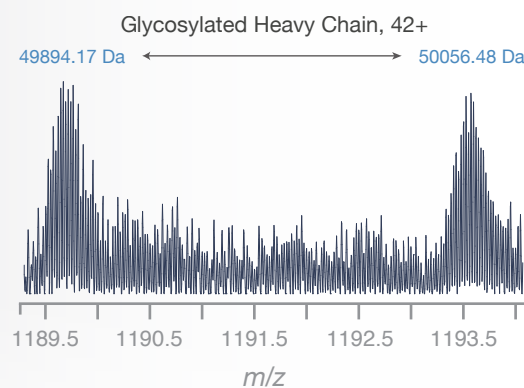
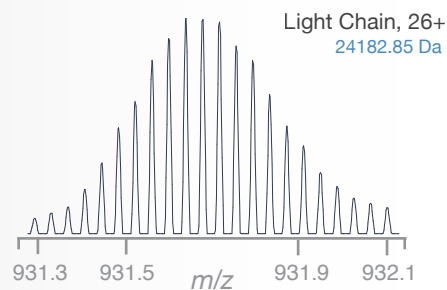


148541.91	7.0	G1F/G2F	38.6165
148380.82	0.0	G1F/G1F	84.1024
148219.21	3.6	G0F/G1F	100.0000
148058.29	11.8	G0F/G0F	68.152
147909.08	9.2	G0/G0F	10.2611

Average Mass	Matched Delta Mass (ppm)	Matched Glycoforms	Relative Abundance
148541.91	7.0	G1F/G2F	38.6165
148380.82	0.0	G1F/G1F	84.1024
148219.21	3.6	G0F/G1F	100.0000
148058.29	11.8	G0F/G0F	68.152
147909.08	9.2	G0/G0F	10.2611

Raw spectrum showing baseline resolution of major intact mAb glycoforms. Deconvolution results are shown in the table above.

## ISOTOPIC RESOLUTION OF mAb LIGHT AND HEAVY CHAINS



Isotopic resolution of light chain (top panel), and heavy chain (bottom panel), showing the detection of glycosylated forms.

## TOP-DOWN SEQUENCE COVERAGE FOR mAb LIGHT AND HEAVY CHAINS

```

N 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 25
26]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 50
51]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 75
76]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 100
101]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 125
126 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 150
151]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 175
176 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 200
201]A]T]H]K]T]S]T]S]P]I]V]K]S]F]N]R N E]C C
    
```

```

N Q V Q L]K]E]S]G]P]G]L]V]A]P]S]Q]S]L]S]I]T]C]T]V]S 25
26]G]F]S]L]L]G]Y]G V]N]W]V]R]Q]P]P G]Q G]L]E]W]L]M]G 50
51 I]W]G]D]G S]T]D Y]N S]A L]K]S]R]I]S I]T]K]D]N]S]K 75
76]S]Q]V]F]L]K]M]N]S]L]Q T]D]D]T]A]K]Y]Y]C T]R]A P]Y 100
101]G]K]Q]Y]F]A]Y]W]G]Q]G]T]L]V]T]V]S]A]A]K]T]T]P]S 125
126]V]Y]P L]A]P]G]S]A]A]Q]T]D]S M]V]T L]G C L]V]K]G]Y 150
151]F]P]E]P V]T V]T]W]N]S G]S L]S]S]G]V]H]T]F]P]A]V]L 175
176 Q]S]D]L]Y]T]L]S]S]V]T]V]P]S]S]T]W]P]S]E]T]V]T C 200
201 N]V]A]H]P]A]S]S]T]K]V]D]K]K]I]V]P]R]D]C]G C]K]P]C 225
226]I]C]T]V]P E]V]S]S]V]F]I]F]P]P]K]P]D]V]L]T]I]T]L 250
251 T]P]K]V]T C]V]V]V]D]I]S]K]D]D]P]E]V]Q]F]S]W]F]V]D 275
276 D]V]E]V]H]T]A]H]T]Q]P]R]E]E]Q]F]N]S]T]F]R]S]V]S]E 300
301]L]P]I]M]H]Q]D]W]L]N]G]K]E]F]K C]R]V]N]S]A]A]F]P]A 325
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351]E]Q]M]A]K]D]K]V]S]L]T]C]M]I]T]D]F]F]P]E]D]I]T]V]E 375
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401]V]Y]S]K]L]N]V]Q]K]S]N]W]E]A]G]N]T]F]T]C]S]V]L]H]E 425
426]G]L]H]N]H]H]T]E]K]S]L]S]H]S]P]G]C
    
```

Combination of ETD HD, CID and HCD fragmentation modes provided 91% sequence coverage for the light chain (top panel) and 62% sequence coverage for the heavy chain (bottom panel).

# Revolutionizing Glycoproteomics

## Simplified Glycopeptide Analysis

Analysis of post-translational modifications (PTMs) is often central to biological research because of their roles in cellular function and disease states. Some PTMs, such as glycosylation, are particularly challenging to analyze because they require information to both accurately determine the composition of the modification as well as correctly identify the modification site. The multiple fragmentation capabilities (CID, HCD, ETD and ETHcD) on the Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ MS are essential for high-throughput, comprehensive glycopeptides analysis.

The high dynamic range of ETD and ETHcD HD yields high-quality MS/MS spectra of glycopeptide backbone fragmentation, resulting in higher sequence coverage and accurate localization of the modification site. Accompanying HCD spectra provide complementary information about glycan composition. Combination of fragmentation techniques, in addition to intelligent instrument control workflows for glycopeptide analysis available on the Orbitrap Fusion Lumos MS, enables deeper mining of glycosylation modifications in a variety of biological samples.

### ORBITRAP FUSION LUMOS MS CAN PRODUCE:

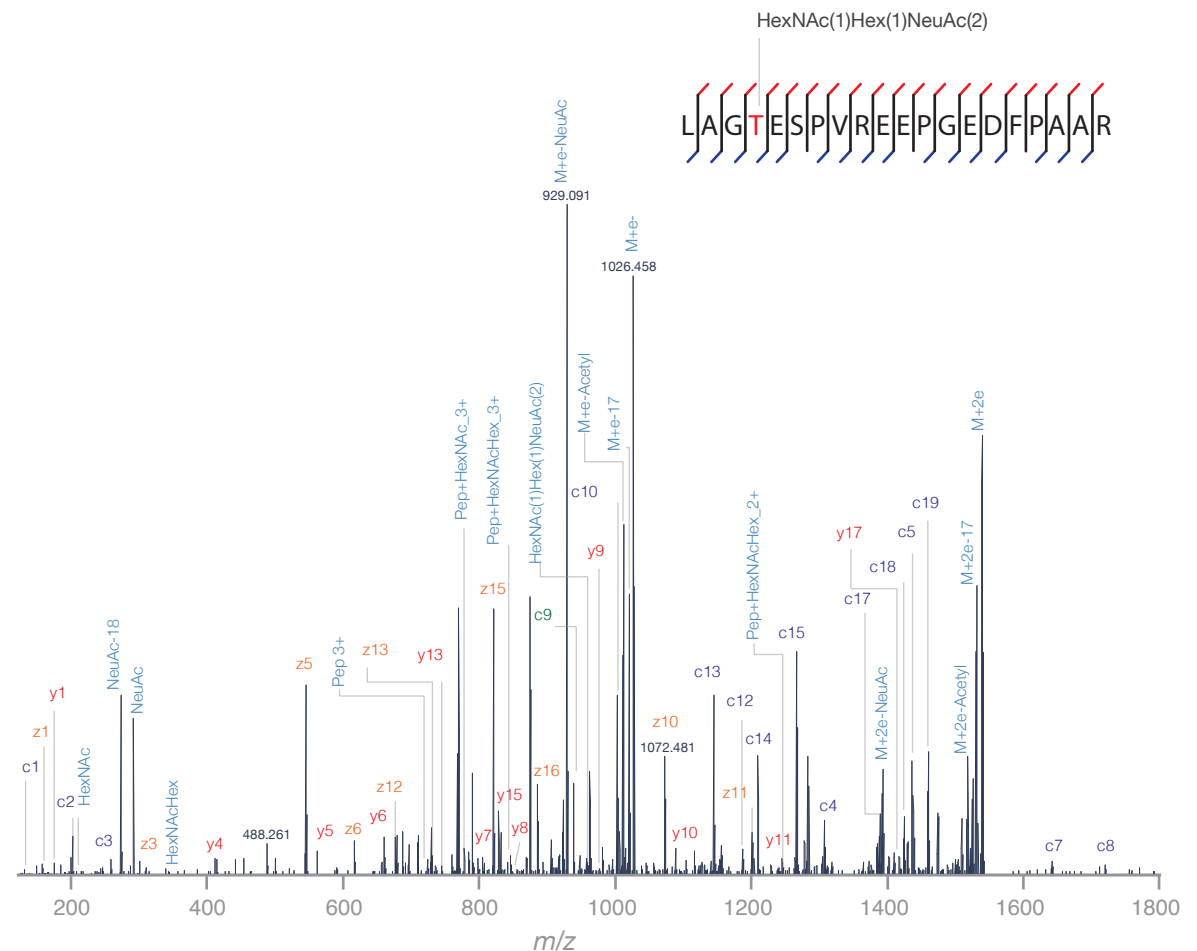
Large scale LC-MS intact  
glycopeptide identification

Intelligent acquisition strategies  
for complete characterization of intact  
glycopeptide structure using HCD-  
product ion dependent-ETHcD/CID

Increased peptide sequence coverage  
and confidence in glycosylation site  
localization with ETHcD

Quantitation of intact glycopeptides  
using isobaric tags via SPS MS<sup>3</sup>

### ETHcD MS/MS spectrum of O-linked glycopeptide



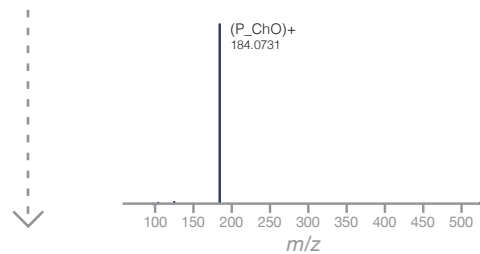
# Comprehensive Lipid Profiling

## Enhancing Productivity with Fast Polarity Switching

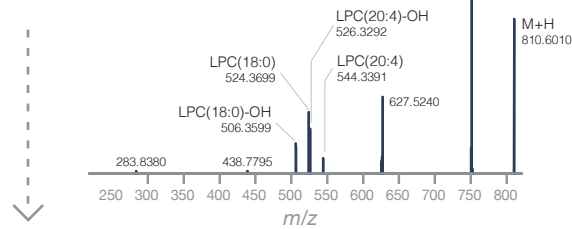
For specific lipid sub-classes such as phosphatidylcholine (PC), both positive and negative HCD MS/MS data are required for full characterization of the individual molecular species. The Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ MS allows fast polarity switching and intelligent acquisition of HCD and CID MS<sup>n</sup>. This provides complementary fragmentation information for identifying PC molecular species and elucidating co-eluting triacylglycerol (TG) isomers within a single LC-MS run, yielding higher throughput, increased sensitivity and more confident analysis of lipid molecular species.

POSITIVE MODE 0-18 MIN

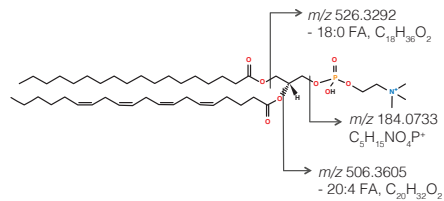
HCD MS/MS  
of *m/z* 810.5997



CID MS/MS  
of *m/z* 810.5997

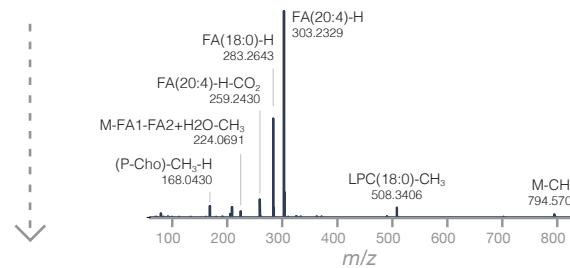


PC (18:0\_20:4)

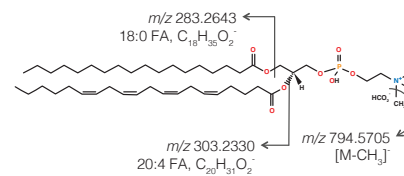


NEGATIVE MODE 0-18 MIN

HCD MS/MS  
of *m/z* 854.5917

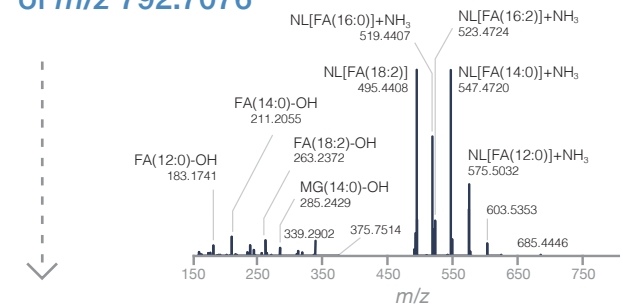


PC (18:0\_20:4)

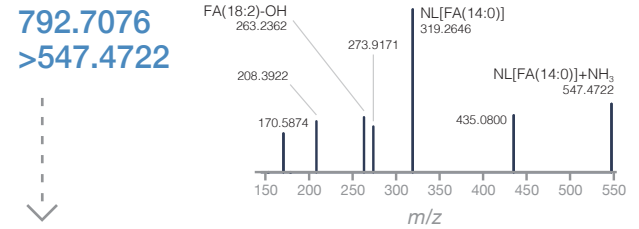


POSITIVE MODE 18-28 MIN

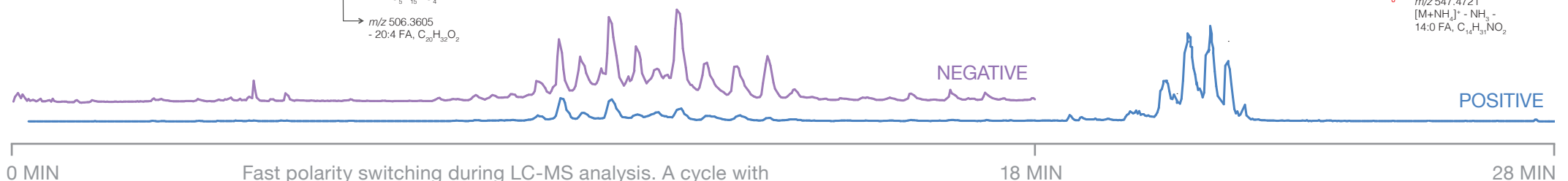
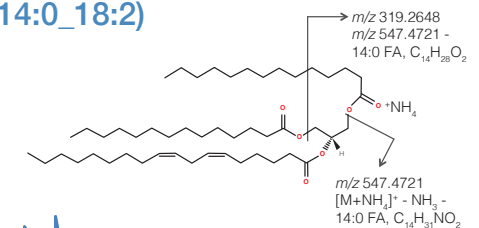
HCD MS/MS  
of *m/z* 792.7076



CID MS<sup>3</sup>  
of *m/z* 792.7076  
>547.4722



TG (14:0\_14:0\_18:2)



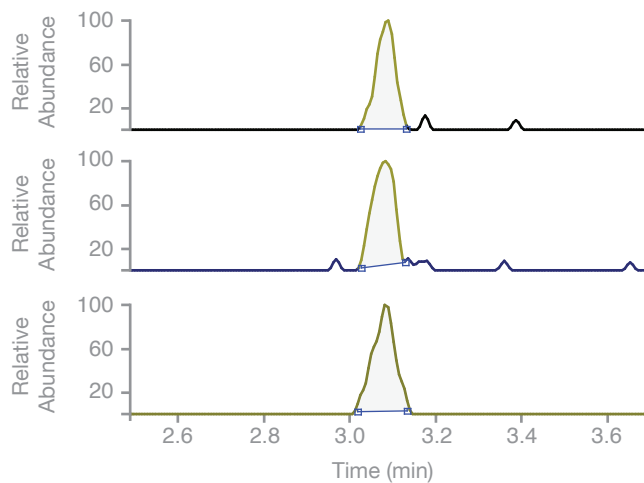
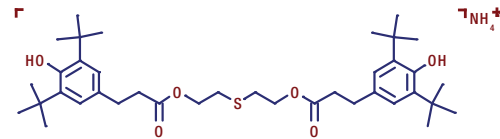
Fast polarity switching during LC-MS analysis. A cycle with one positive and one negative Orbitrap MS scan at 120,000 resolving power takes less than 2 seconds to complete.

# Small Molecule Analysis

## High Confidence in Identification and Quantitation

The high sensitivity and high resolution of the Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ MS makes it a powerful instrument for the identification and quantitation of small molecules. Due to the instrument's very high resolution capabilities, fine isotopic structure can be observed, enabling the determination of highly accurate molecular formulae. This direct measurement removes the ambiguity of pattern matching estimations and is critical in cases where monoisotopic elements like fluorine or phosphorous may be present in the compound, as shown in the example of norfloxacin below. Furthermore, the enhanced sensitivity of the Orbitrap Fusion Lumos MS enables far lower levels of quantitation.

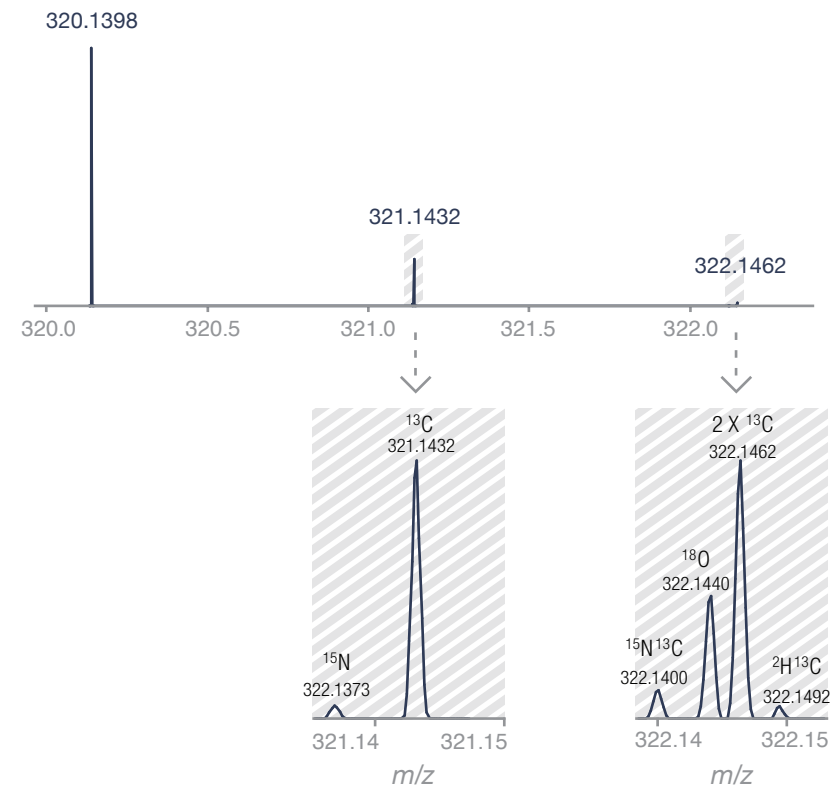
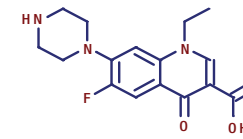
### Improved Low Limit Of Quantitation



LOQ	Standard Ion Source	New Ion Source on Orbitrap Fusion Lumos MS
	500 fg	100 fg

Extracted ion chromatogram of Irganox 1035 ( $M+NH_4$ )<sup>+</sup> ion at  $m/z$  660.429 (100 fg on column). Irganox is a plasticizer known to leach into foods stored in plastic and must be quantified at very low levels. The Orbitrap Fusion Lumos MS operated in SIM mode was able to quantify Irganox 1035 in food simulant matrix, achieving an LOQ of 100 fg with linear dynamic range of 5 orders and <10% CV for all levels. This LOQ is 5x lower than previously achieved on an Orbitrap instrument equipped with a standard ion source.

### Resolving Isobaric Interferences



High resolution MS<sup>2</sup> spectra of norfloxacin. The direct observations of fine isotopes of the drug are essential for determining elemental composition.

The screenshot displays the 'Method Editor' interface with three tabs: 'Global Parameters', 'Scan Parameters', and 'Summary'. The 'Method Timeline' at the top shows a duration of 120 minutes. Below this, the 'Experiment 1' section shows a time range of 0-120 minutes. The main workflow area contains a vertical sequence of steps: MS OT, MIPS, Charge State, Dynamic Exclusion, Intensity, ddMS<sup>2</sup> IT CID, Precursor Selection Range, Precursor Ion Exclusion, Isobaric Tag Loss Exclusion, and ddMS<sup>3</sup> OT HCD. A '3 sec' box is connected to the start of the workflow, and a '10 SPS' box is connected to the 'ddMS<sup>2</sup> IT CID' step. On the left, 'System Templates' include Glycans, Metabolites, Peptides-ID, Peptides-Quan, and PTM. 'Custom Templates' include My Experiments. Two callout boxes are present: one for 'MS Scan Properties' and another for 'Data-Dependent MS<sup>n</sup> Scan Properties'. The 'MS Scan Properties' panel shows parameters like Orbitrap Resolution (120000), Scan Range (380-1500), RF Lens (60%), AGC Target (2.0e5), and Maximum Injection Time (50). The 'Data-Dependent MS<sup>n</sup> Scan Properties' panel shows parameters like Synchronous Precursor Selection (checked), Number of Precursors (10), MS Isolation Window (2), MS2 Isolation Window (2), Activation Type (HCD), HCD Collision Energy (65%), Detector Type (Orbitrap), Orbitrap Resolution (60000), AGC Target (1.0e5), Inject Ions for All Available Parallelizable Time (unchecked), and Maximum Injection Time (120). A tooltip for the HCD Collision Energy field indicates a 'Recommended value is 30'.

The list of the viewable parameters in the scan and filter properties can now be customized

Run advanced experiments effortlessly with validated template methods for a variety of applications

Edit parameters in easily accessible properties panels, featuring recommended defaults for each experiment

# Focus on Your Science, Not on Instrument Set Up

The highly intuitive method editor features a user-friendly interface that includes optimized pre-designed templates for a wide range of applications.



# Building on Revolutionary Tribrid Architecture

The Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer further amplifies the power and versatility of the innovative Tribrid design, first pioneered on the Orbitrap Fusion MS. The Orbitrap Fusion Lumos mass spectrometer incorporates many of the latest technologies and groundbreaking innovations in ion transmission, dissociation and detection. The combination of these improvements makes it one of the most sensitive, most selective and most versatile mass spectrometer to date.

With the Orbitrap Fusion Lumos MS, we are true partners, committed to innovations to advance your scientific pursuits.

## ADVANCED ACTIVE ION BEAM GUIDE

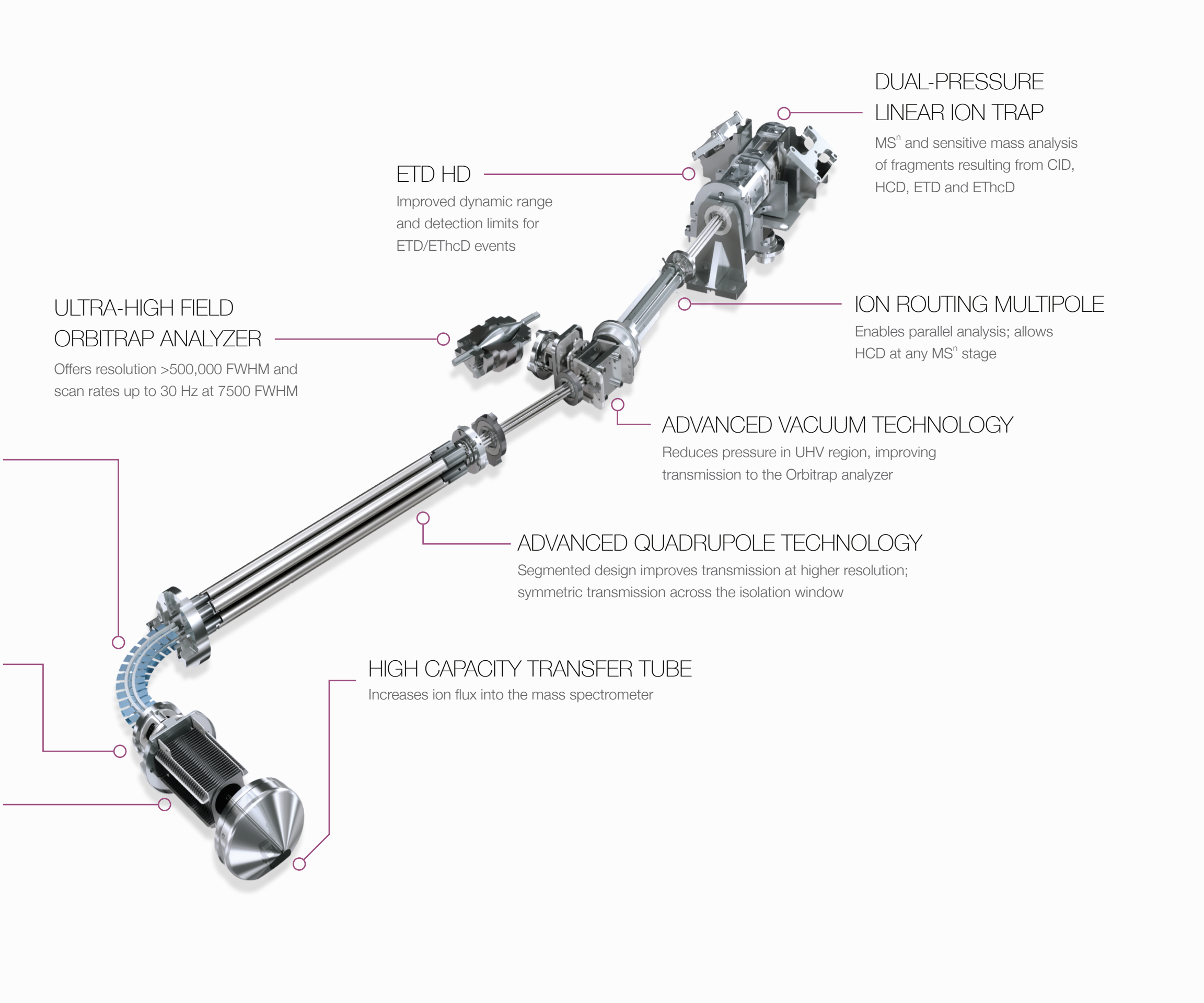
Prevents neutrals and high velocity clusters from entering mass resolving quadrupole

## EASY ETD SOURCE

Based on Townsend discharge; reliable and easy to use

## ELECTRODYNAMIC ION FUNNEL

Focuses ions after High Capacity Transfer Tube; broad tuning curves



### DUAL-PRESSURE LINEAR ION TRAP

MS<sup>n</sup> and sensitive mass analysis of fragments resulting from CID, HCD, ETD and EThcD

### ETD HD

Improved dynamic range and detection limits for ETD/EThcD events

### ION ROUTING MULTIPOLE

Enables parallel analysis; allows HCD at any MS<sup>n</sup> stage

### ULTRA-HIGH FIELD ORBITRAP ANALYZER

Offers resolution >500,000 FWHM and scan rates up to 30 Hz at 7500 FWHM

### ADVANCED VACUUM TECHNOLOGY

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

### ADVANCED QUADRUPOLE TECHNOLOGY

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

### HIGH CAPACITY TRANSFER TUBE

Increases ion flux into the mass spectrometer



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