

thermoscientific

# Pushing the leading edge in protein analysis



Thermo Scientific Q Exactive HF-X hybrid  
quadrupole-Orbitrap mass spectrometer

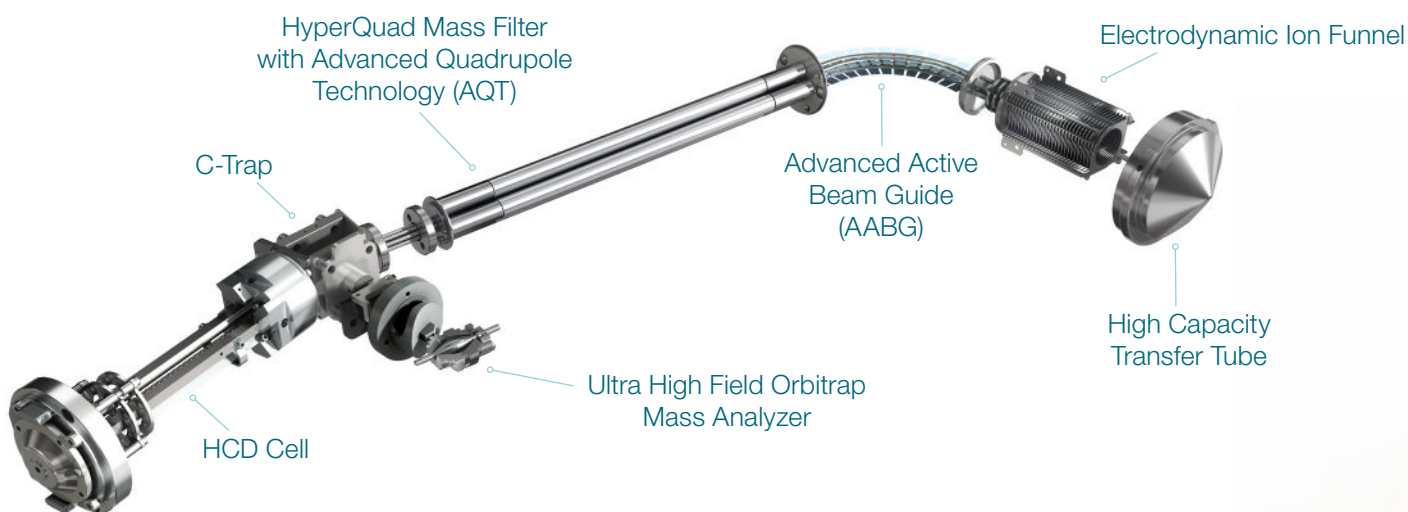
**ThermoFisher**  
SCIENTIFIC

# Accelerating insights, from discovery to verification

Understanding biomolecules as drug targets, disease markers, and therapeutic agents demands highly sensitive analysis of complex biological samples, without sacrificing robustness and speed. The Thermo Scientific™ Q Exactive™ HF-X hybrid quadrupole-Orbitrap™ mass spectrometer rises to the challenge as a versatile platform, setting new standards in sensitivity, performance and productivity for any workflow. Building on the proven performance of the Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap™ MS family of instruments, the new Q Exactive HF-X mass spectrometer couples a high field Thermo Scientific™ Orbitrap™ mass analyzer with a high capacity transfer tube and electrodynamic ion funnel that maximize ion loading and transmit ions over a broad mass range.

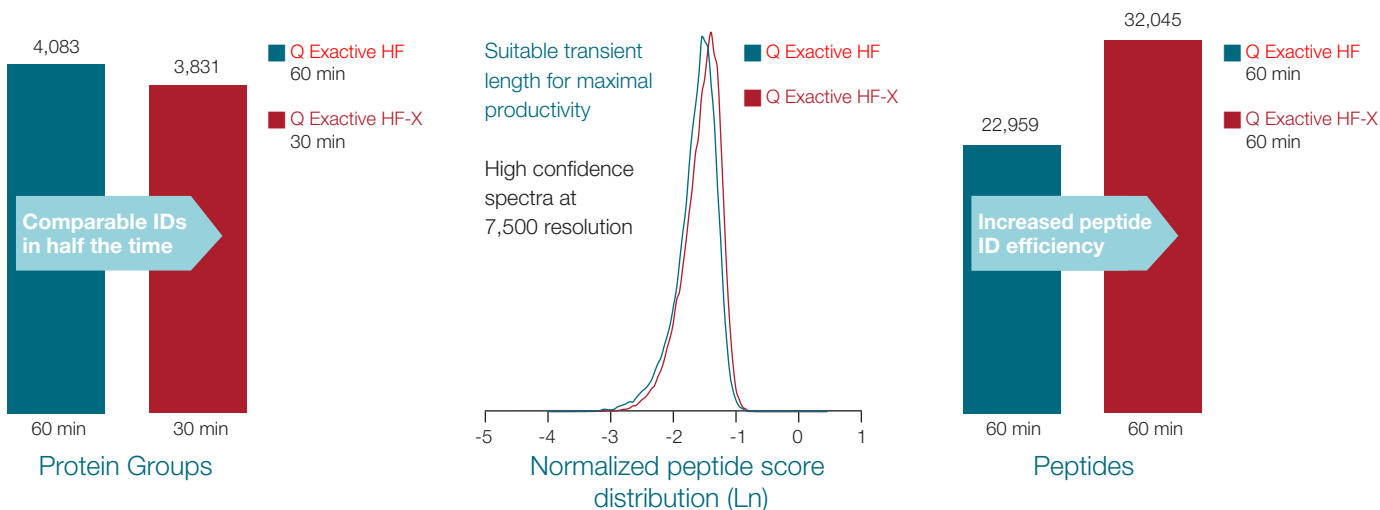
As a result, the instrument delivers a bright, focused and ion-rich beam, which translates to high sensitivity and excellent stability in analysis quality from run to run. Smart instrument control features match the superior ion transmission with increased acquisition speed and even more sophisticated data-dependent acquisition and analysis strategies to meet data quality needs at every stage of your work, from discovery to verification and quantitation.

- Novel architecture with a high capacity transfer tube (HCTT) and electrodynamic ion funnel that increases ion transmission and boosts sensitivity for all analytes, from small molecules to native antibodies.
- Increased acquisition speed and advanced precursor determination (APD) dramatically improves peptide sequencing speed and depth.
- Robust quantitative workflows, including parallel reaction monitoring (PRM) and high-resolution data-independent acquisition (HR-DIA).
- Available with the powerful BioPharma option to support all workflows in the characterization of biotherapeutics with enhanced sensitivity.
- Superior consistency and performance set new standards in quantitative accuracy, sensitivity and reproducibility for rigorous research workflows in large-scale studies of challenging protein samples.



# Proteins identified faster than ever

The Q Exactive HF-X mass spectrometer boosts productivity in protein identification, while maintaining the superb accuracy of the high field Orbitrap mass analyzer. Superior ion transmission, together with new scan rates up to 40 Hz, drive more efficient use of the Orbitrap mass analyzer. As a result, the Q Exactive HF-X mass spectrometer takes half as long to perform a typical proteomic analysis as the Thermo Scientific™ Q Exactive™ HF hybrid quadrupole-Orbitrap™ mass spectrometer. This results in up to 40% more unique peptide identifications per unit time, so you can shorten your time-to-results at any throughput scale and at any depth of analysis.



## Same protein identifications in half the analysis time

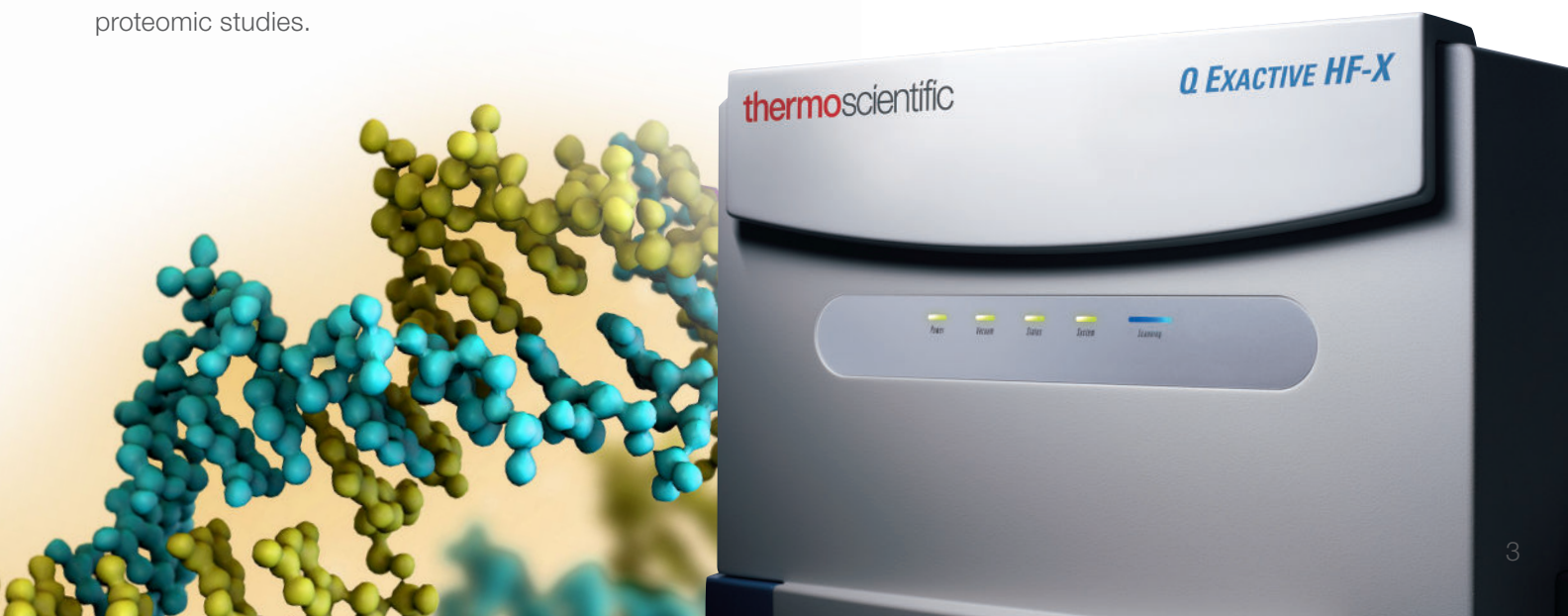
The same cell line digest was run in triplicate using a 30 minute separation on a Q Exactive HF-X MS and a 60 minute separation on a Q Exactive HF MS. The Q Exactive HF-X MS identified almost the same number of protein groups in half the time, achieving an almost 2-fold improvement in analysis time. This represents a tremendous potential throughput advantage for large scale proteomic studies.

## Faster, with same high quality results

In a similar triplicate analysis, a cell line digest was run on the Q Exactive HF-X MS at two different MS<sup>2</sup> resolution settings: 7,500 and 15,000. The peptide score distribution (>80,000 peptides), demonstrates that the Q Exactive HF-X MS achieves the perfect balance between resolution and reliable MS<sup>2</sup> spectra without sacrificing spectral quality.

## Increased peptide ID efficiency

In a similar triplicate analysis, a cell line digest was run on both the Q Exactive HF-X MS and Q Exactive HF MS systems, this time using a 60 minute separation on both systems. Results show that the Q Exactive HF-X MS was able to identify significantly more peptides in the same amount of time, resulting in improved proteome depth and protein coverage.

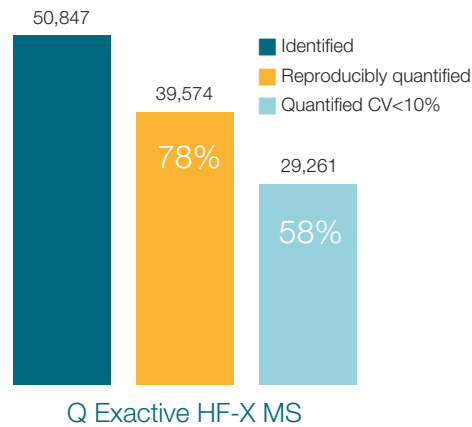


# A new level of sensitivity to detect and quantify proteins

Verification of protein biomarkers requires quantitation of low-level proteins in large cohort studies. Analytical consistency from sample-to-sample and lab-to-lab is paramount for reliable results from large-scale studies. Precursor ion-based MS<sup>1</sup> quantitation is a proven approach, delivering confident and reliable protein determinations. Taking advantage of the high-resolution, accurate mass (HRAM) capability of the Q Exactive HF-X MS and a HR-DIA workflow, excellent peptide quantitation with high reproducibility is achieved. By further minimizing missing peptide values with the DDA+ workflow, peptide reproducibility is maximized and precise protein quantitation achieved.



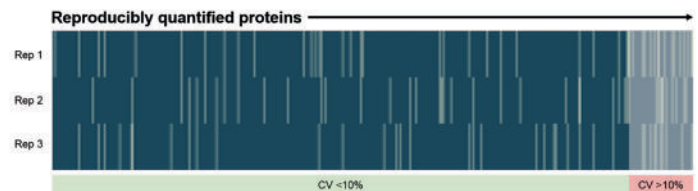
## Excellent peptide quantitation reproducibility



4 µg of HeLa digest was analyzed on the Q Exactive HF-X MS instrument coupled to a capillary LC system using the HR-DIA method. A total of 50,847 peptides were identified providing high sample coverage. MS<sup>1</sup> quantitation at 120,000 resolution enabled reproducible quantitation for 39,574 of these peptides across all experiments. 29,261 of the quantified peptides had a CV of <10%.

## Maximizing protein quantitation precision

The Q Exactive HF-X MS in combination with Thermo Scientific™ Proteome Discoverer™ software enables the DDA+ workflow for increased protein coverage and quantitation precision. 1 µg of HeLa digest was analyzed at 120,000 resolution and MS<sup>1</sup>-based analysis yielded 4,383 proteins with 87% of the proteins quantified at a CV of <10%.



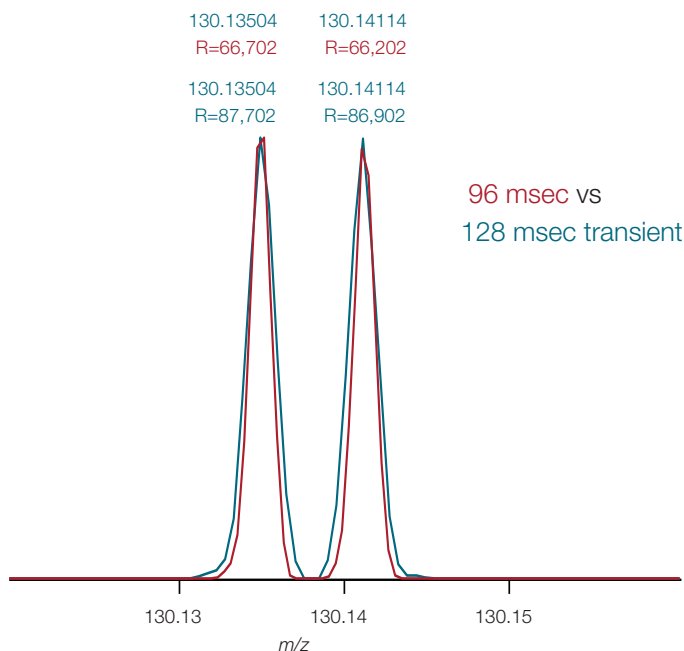
**Eliminating missing values in protein quantitation.** Three sample replicates were analyzed with the DDA+ workflow on the Q Exactive HF-X MS. Protein quantitation performed in Proteome Discoverer software eliminated missing values by mapping identifications across replicates (light green) achieving 100% quantitative reproducibility for 87% of proteins in the sample at a precision of <10% CV.

# The next level in state-of-the-art proteomics

A single platform that can span the complete workflow spectrum of today's quantitative proteomics must integrate high sequencing speed with maximum sensitivity and selectivity. Confidently discover and quantify proteins of interest with label-free, SILAC, or isotopically labeled tandem mass tags (TMT) quantitation strategies, and validate with parallel reaction monitoring (PRM). The enhanced sensitivity of the Q Exactive HF-X mass spectrometer enables unparalleled workflow versatility to accommodate every stage of proteomic analysis.

## Optimal separation of TMT reporter ions

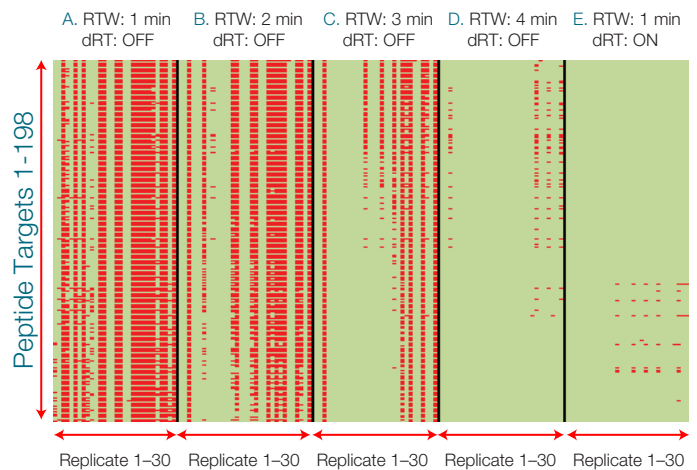
A dedicated scan mode of the Q Exactive HF-X mass spectrometer with a resolution setting of 45,000 (FWHM) at  $m/z$  200 optimally resolves reporter ions of the Thermo Scientific™ TMT10plex™ Isobaric Mass Tag Labeling Kit, and frees up scan time to quantify 10–20% more peptides in discovery experiments.



**Improved TMT Performance.** An MS<sup>2</sup> spectrum showing baseline resolution of TMT10-130N and TMT10-130C reporter ions. A dedicated TMT scan mode enables the Q Exactive HF-X MS to effectively resolve TMT reporter ions in only 96 msec versus the previous 128 msec method. This assures accurate quantitative results and allows more scans per run for deeper levels of analysis.

## Smart corrections to target more peptides for quantification

Parallel reaction monitoring (PRM) is further enhanced with a new dynamic retention time correction (dRT) technique. The Q Exactive HF-X mass spectrometer compensates for run-to-run retention time variation by adjusting scheduled retention time windows (RTWs) on the fly. Thus, the instrument assigns retention times with greater accuracy and captures precursors within narrower RTWs. Consequently, a greater number of peptides can be scheduled for quantitation in a run while maintaining a sufficient acquisition sampling rate for reliable and precise quantification.



**30 replicate injections with 4 different RTW applied and dramatically improved peptide targeting with the use of dynamic retention time correction (dRT).** The same 198 scheduled peptide precursors were monitored by 3 fragments each. To mimic common challenges in nano HPLC approaches, the gradients were randomly switched between 6 slightly different gradients. Graphs A to D show the results when dRT is switched off with increasing RTWs from 1 to 4 minutes. With a narrow 1 minute RTW, the red marks show where many targeted peptides were not sampled, likely because shifts in retention time caused them to elute outside the programmed RTW. As we increase the RTW from 1 to 4 minutes, the wider RTW is better able to accommodate for RT shifts from run-to-run. The last graph E demonstrates that dynamic retention time adjustment is able to compensate for shifts in retention time, such that there are very few missing measurements.



# A powerful, single platform for the analytical demands of BioPharma

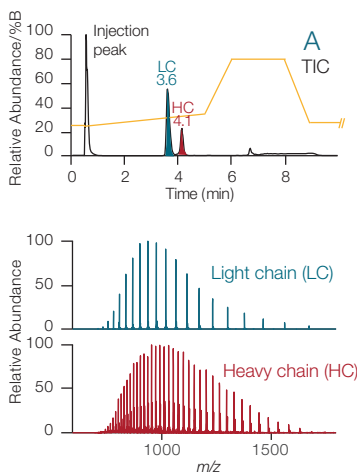
The enhanced sensitivity of the Q Exactive HF-X mass spectrometer across all BioPharma workflows provides a complete high performance analysis platform for biotherapeutic characterization. The BioPharma option increases the mass range and transmission for sensitive analysis of heterogeneous mAbs and ADCs under native conditions. In addition, it provides optimized conditions for subunit analysis and middle-down sequencing.

## Greater sensitivity reveals more details about proteins

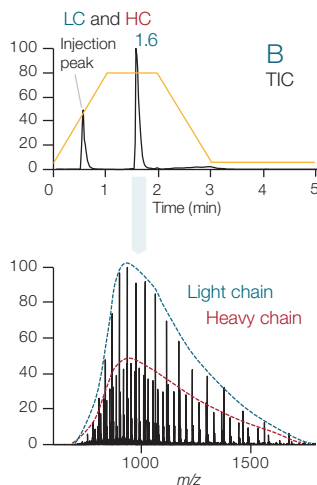
The High Mass Range mode of the BioPharma option was designed to enable the analysis of intact therapeutic proteins under native conditions detected above  $m/z$  6,000 with high transmission. The superior ion transmission of the Q Exactive HF-X MS allows for using high resolution settings to better resolve protein modifications, such as glycosylations and conjugations. This instrument achieves unprecedented quality and detail in the analysis of large, complex analytes. In the case of intact Antibody-Drug Conjugates (ADC), drug-to-antibody ratios can be accurately determined without deglycosylation.

In Protein mode, fast scan rates (16 msec transient) and superior sensitivity produce top-quality data to reveal antibody subunits that are typically difficult to resolve without chromatographic separation. Antibody heavy and light chains are clearly distinguishable in baseline-resolved spectra.

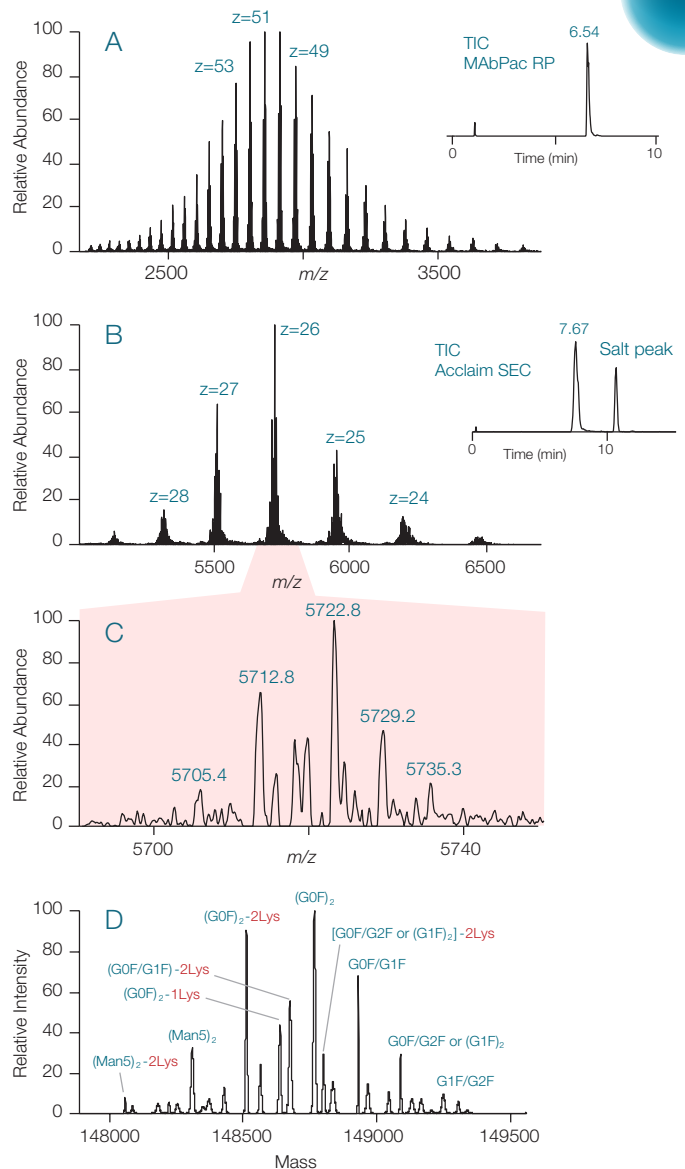
### Separated LC & HC



### Unseparated LC & HC



Speed and sensitivity support superb quality detection of antibody light and heavy chains (A), even without chromatographic separation (B). The Q Exactive HF-X MS produces distinct, high-quality baseline-resolved spectra of antibody subunits with superior quality.



### In-depth characterization of the intact mAb infliximab

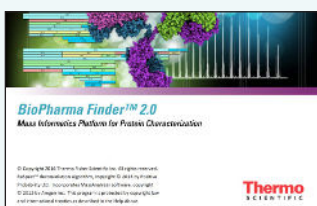
- Full MS spectrum and total ion chromatogram obtained upon analysis under denaturing conditions at a resolution setting of 60,000 with automated charge state annotation of isotopically unresolved peaks
- Full MS spectrum and total ion chromatogram obtained upon analysis under native conditions
- Spectrum excerpt highlighting the baseline resolved detection of a complex glycoform pattern in the zoom of charge state 26
- Deconvoluted spectrum under native/denaturing conditions

# Accelerating productivity with software solutions



## Proteome Discoverer Software

Identify and quantify proteins in complex biological samples using Proteome Discoverer software. Proteome Discoverer software simplifies a wide range of proteomics workflows, from protein and peptide identification to PTM, analysis to isobaric mass tagging and both SILAC and label-free quantitation. It supports multiple database search algorithms (SEQUEST<sup>®</sup>, Z-Score, Mascot<sup>™</sup>, and Byonic<sup>™</sup>).



## BioPharma Finder Software

Save time and identify more with Thermo Scientific<sup>™</sup> BioPharma Finder<sup>™</sup> integrated software. Screen, identify and characterize intact proteins with higher productivity and confidence using Intact Protein workflow. Two deconvolution algorithms take full advantage of the high-quality, HRAM data produced by Orbitrap mass spectrometers. Confirm amino acid sequence, identify site and type of known and unknown PTMs while providing relative amounts using Peptide Mapping workflow.



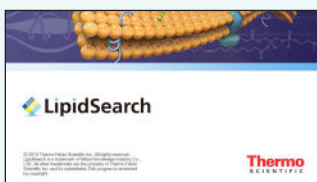
## Compound Discoverer Software

The complete small molecule identification and characterization solution, Thermo Scientific<sup>™</sup> Compound Discoverer<sup>™</sup> software is a flexible solution that offers a full suite of powerful tools to address mass spectrometry-based (MS) small molecule differential analysis, identification, and pathway mapping.



## TraceFinder Software

Thermo Scientific<sup>™</sup> TraceFinder<sup>™</sup> software provides a complete quantitation and screening workflow for routine applications across multiple markets for GC- and LC-MS systems. TraceFinder software allows users to develop comprehensive assays with multiple levels of confirmations that can be refined to simplified workflows for technician-level operators.



## LipidSearch Software

Thermo Scientific<sup>™</sup> LipidSearch<sup>™</sup> software processes LC-MS data, including high-resolution accurate-mass data generated by Orbitrap mass spectrometers, to make provide accurate lipid identification. It automatically integrates complex data into reports and dramatically reduces data analysis time.

Find out more at [thermofisher.com/QExactiveHFX](http://thermofisher.com/QExactiveHFX)

**ThermoFisher**  
SCIENTIFIC