Top reasons to upgrade to a Q Exactive HF-X hybrid quadrupole-Orbitrap mass spectrometer

Keywords

Proteomics, protein identification, protein ID, protein quantiation, relative quantitation, protein multiplexing, TMT, high throughput mass spectrometry, Q Exactive MS, Q Exactive Plus MS, Q Exactive HF MS, Q Exactive HF-X MS, BioPharma Option, monoclonal antibodies

Goal

This document is intended to address the technical and workflow benefits of using the Q Exactive HF-X mass spectrometer (MS) system, specifically providing conclusive arguments for laboratories looking to enhance their MS capabilities to an advanced hybrid quadrupole-Orbitrap mass spectrometer.

Summary

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 together 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 to quantitation.

Introduction

The Thermo Scientific Q Exactive HF-X mass spectrometer is the latest and most advanced MS instrument in the Q Exactive MS product portfolio. Orbitrap mass analyzer-based mass spectrometers have been well known for high resolution and accurate mass detection capabilities. Benchtop Q Exactive MS instruments are widely used by many laboratories and have evolved into a proteomics analysis workhorse, being highly versatile for multiple proteomics applications and workflows, be they qualitative or quantitative. Researchers constantly face issues with sample complexities and dynamic range, which cannot be addressed fully by up-front fractionation or optimal chromatographic resolution alone. In trying to resolve these problems (e.g., abundance differences of peptides), it is imperative that mass spectrometric technologies be refined in terms of scan speeds, resolution and sensitivity.

The release of the Q Exactive HF-X MS introduced several proven hardware innovations and novel acquisition methods driven by instrument control improvements implemented on this platform. These features are targeted towards increasing experimental efficiency, providing multiple modes of analysis and addressing common analytical challenges associated with highly complex, low abundant or challenging samples, while improving ease of instrument operations for MS users. Aimed at comprehensive analysis of proteins present in complex biological samples, the Q Exactive HF-X MS is a breakthrough, as well as an essential MS tool for discovery proteomics, translational research, precision medicine and clinical applications.

Hardware benefits

The Q Exactive HF-X MS has the following capabilities:

- Consistency in analytical performance to achieve quantitative accuracy and reproducibility with uncompromised sensitivity for highly rigorous research workflows, suited for large-scale studies involving challenging sample types
- Improved scan rates up to 40 Hz for faster scan acquisitions allowing for increased instrument throughput and productivity
- New intelligent data-dependent acquisition workflow for intact proteins that facilitates deeper insights from top-down proteomics
- Parallel reaction monitoring (PRM) and high-resolution data-independent acquisition (HR-DIA) modes, providing flexibility and delivering robust workflows for all quantitative experimental needs

The Q Exactive HF-X MS is equipped with the following:

- 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
- Advanced Quadrupole Technology (AQT) that enhances selectivity in precursor isolation and improvement of transmission at narrow isolation widths
- Advanced Active Beam Guide (AABG) for greater sensitivity and maximum robustness
- Advanced Peak Determination (APD) feature coupled with increased acquisition speed dramatically increases peptide sequencing speed and coverage depth
- A C-trap that stores and compresses ion populations before injection into the ultra-high field Orbitrap mass analyzer
- Ultra-high field Orbitrap mass analyzer providing resolution at 240,000 FWHM at *m/z* 200 and scan speeds up to 40 Hz at 7,500 FWHM at *m/z* 200

In previous generations of hybrid Orbitrap mass spectrometers (e.g., Thermo Scientific™ Orbitrap™ Velos™ and Orbitrap Elite™ MS), precursor selection and fragmentation typically occurred in a linear ion trap. For these MS systems, the linear ion trap provided sufficient MS/MS scan rates for most qualitative proteomics experiments. However, as the MS community evolved to seek quantitative information, faster acquisition rates, faster chromatographic separations and higher sample throughputs became the basic requirements for routine quantitative analysis.[1] The combination of a quadrupole mass filter with an Orbitrap mass analyzer provides a configuration for virtually instantaneous ion selection along with similarly fast Higher-energy Collisional Dissociation (HCD) fragmentation, to satisfy the requirements of speed and sensitivity.

Figure 1. Hardware schematic of the Q Exactive HF-X MS.

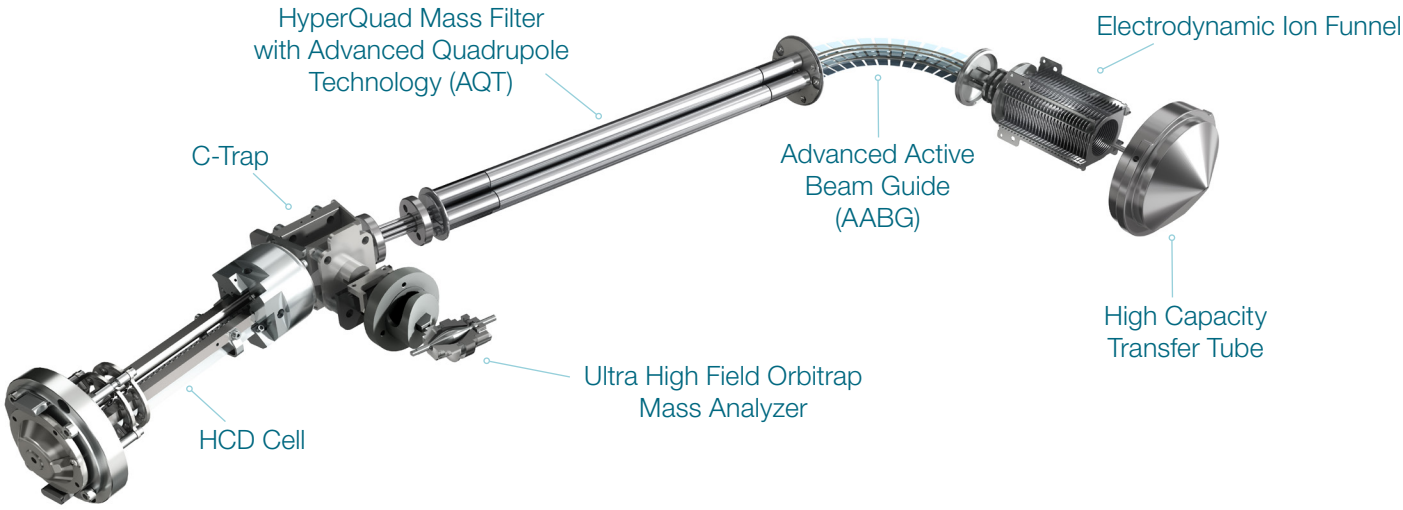

Table 1. Fundamental features and benefits of Q Exactive hybrid quadrupole-Orbitrap MS technology.

Features	Benefits
Ultra-High Resolution	High resolving power for confident structural confirmation
Sub ppm Mass Accuracy	High selectivity and confidence in molecular formula
Optimized Scan Matrix	Improved transient times advantageous for efficient, high quality MS/MS acquisitions
Speed	Faster scan rates for improving protein identification rates, achieving quantitation accuracy
Easy-to-Use Software	Build complex methods using the intuitive method editor
Experimental Flexibility	DDA, DDA+, PRM, HR-DIA acquisition modes

Key improvements have been made to various hardware components on the Q Exactive HF-X mass spectrometer to give enhanced analytical performance:

- Novel front-end architecture similar to the proven high-end research grade Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer and high-end triple quadrupole Thermo Scientific™ TSQ™ series mass spectrometers to provide increased sensitivity. The ion source is comprised of the High Capacity Transfer Tube (HCTT) and Electrodynamic Ion Funnel (EDIF) to achieve better ion transmission, ion stability and reach lower limits of detection ranging from small molecule analytes to native antibodies.
- Accelerated Higher-Energy Collisional Dissociation (aHCD) enables faster MS/MS acquisition and compatibility with capillary-flow rates, producing reliable and high quality MS^2 spectra

Table 2. Specifications.

Features	Q Exactive HF-X MS
Resolution	240,000 (FWHM) at *m/z* 200
Mass Range	50 to 6,000 *m/z* (up to 8,000 *m/z*)*
Scan Rate	Up to 40 Hz at resolution setting of 7,500 at *m/z* 200
Quad Isolation	Down to 0.4 Da
Mass Accuracy	<3 ppm RMS (external); <1 ppm RMS (internal)
Dissociation	Source CID, HCD
Multiplexing	Up to 10 precursors/scan
Polarity Switching	One full cycle in <1 sec (one full positive mode scan and one full negative mode scan at resolution setting of 60,000)
Sensitivity	MS/MS: 100 fg reserpine on column S/N 150:1; SIM 50 fg reserpine on column S/N 150:1
Dynamic Range	>5,000:1
Scan Functions	Full Scan (FS), All Ion Fragmentation (AIF), Selected Ion Monitoring (SIM), Parallel Reaction Monitoring (PRM), Data Independent Acquisition (DIA), data dependent HCD (ddHCD)

*With BioPharma option

Detailed product specifications for the Q Exactive HF-X MS can be found on www.thermofisher.com.

For sole source specifications, kindly contact your local sales representative or contact us at Grant Central.

Experimental benefits of the Q Exactive HF-X MS

- Analytical robustness for routine applications, delivering reproducibility and high throughput
- High-resolution accurate-mass and sensitivity
- Fast scan speeds for shorter gradient times
- Highest data quality, in comparison with QTOFs
- Innovative instrument control features enable unrivaled data-dependent performance
- Push the limits of discovery proteomics with faster and deeper sequencing
- Empowers standardized label-free quantification workflows, providing robust and reproducible results for translational proteomics studies

Reason 1: Innovative instrument control features enable unrivaled data-dependent performance

The Q Exactive HF-X MS is equipped with a suite of novel instrument control optimizations permitting improved precursor selection, more efficient scanning matrices and faster Orbitrap mass analyzer acquisition speeds. Collectively, the following innovations ultimately allow superior sensitivity and productivity to meet the demands at various experimental stages (from discovery, to verification and quantitation), throughput levels and depth of analysis, regardless of your application.

Advanced Peak Determination (APD)

Traditional data-dependent MS acquisition relies on robust and accurate ion charge determination as the first step to initiate MS^n analysis and allow molecular identification. Conventional versions of peak detection algorithms have significant limitations including inefficient identification of all isotopic clusters in a complex spectrum, limited identification of adjacent or overlapping isotopic clusters, and an inability to assign poorly resolved spectra. FTMS MS^1 scans have been shown to contain many more precursors than what is typically selected for MS/MS during a data-dependent run. This is due to limited scan rates and sensitivity on legacy MS systems impacting the number of MS/MS events triggered. Today, higher performance mass spectrometers like the Orbitrap Fusion Lumos Tribrid MS and Q Exactive HF-X MS, are engineered with extreme sensitivity and resolution, and can generate high quality MS/MS spectra from low signal-to-noise components in the shortest possible time, driving the need for improved on-the-fly spectral analysis and peak assignment. The APD algorithm fulfills this need by identifying the charge states and monoisotopic *m/z* values of isotopic envelopes at greatly improved peak depths in complex MS spectra (Figure 2). For conventional bottom-up shotgun experiments of complex proteomes, this algorithm dramatically increases the population of precursors available for data-dependent analysis, which in turn results in more MS/MS spectra, PSMs and unique peptide identifications.

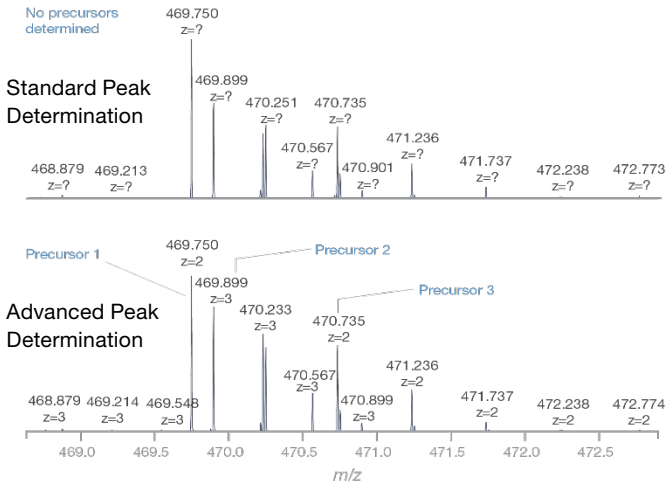

Figure 2. Enhancements to on-the-fly charge state detection leads to the identification of more precursor candidates in complex spectra resulting in more data-dependent triggers in proteomics samples, and higher number of identifications.

While providing an obvious advantage to complex bottom-up sample analysis, APD can benefit other applications in an equal manner. Top-down analysis of intact molecules is often complicated by complex charge envelope profiles, resulting in redundant fragmentation events that ultimately limit the dynamic range of identification and result in incomplete characterization of proteins in a sample. Utilizing the Protein Mode within the BioPharma option, top-down workflows will benefit from APD's ability to perform real-time, robust charge state assignments of unresolved or resolved intact proteins. In addition, smart APD algorithms automatically select the dominant charge proteoform along with optimal collision energy for the fragmentation of each selected precursor to achieve complete and confident identification of proteins in the sample (Figure 3).

On-the-fly deconvolution based on charge envelope to select a single charge state of each dominant proteoform.

MS/MS analysis of each proteoform fragmented with optimal collision energy and detected at a resolution setting of 120,000.

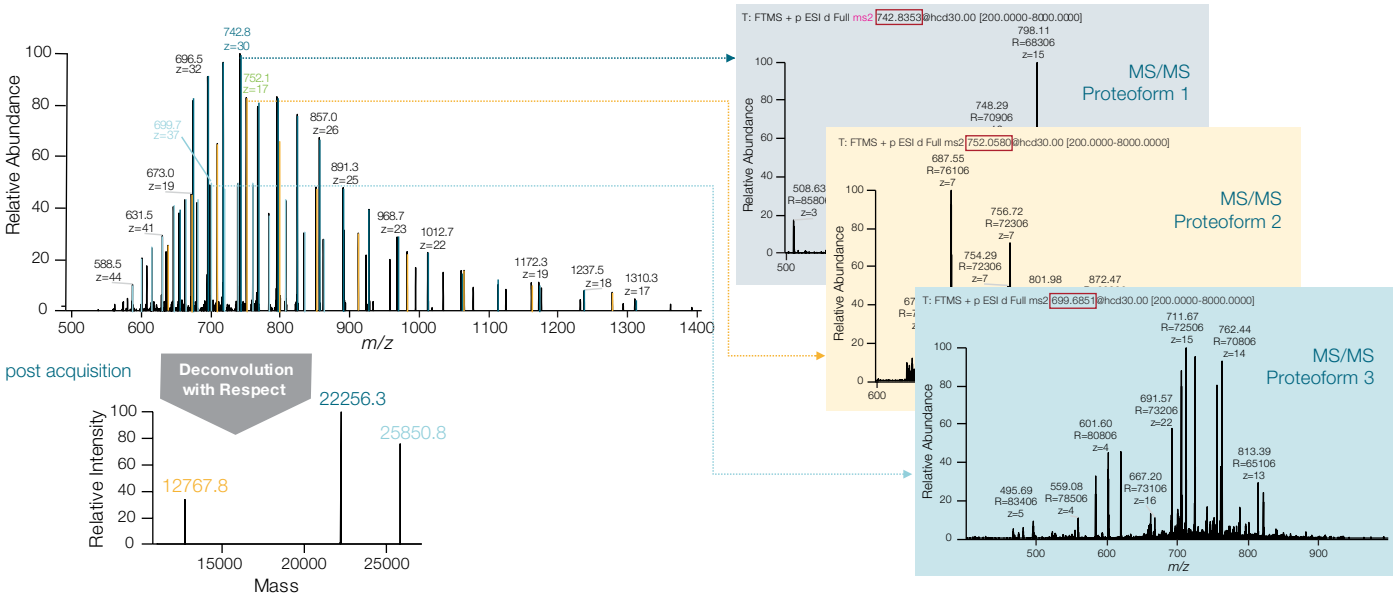

Figure 3. APD facilitates improved top-down analysis in an intact protein mixture. Separation of a mixture of *E.coli* ribosomal proteins using a 40 min method, 7,500 resolution setting on the Q Exactive HF-X MS can be achieved with confident deconvolution of the charge envelope and selection of a single charge state for each dominant proteoform (shown for 3 proteoforms in the mixture).

Optimized Scan Matrix and Accelerated Higher-Energy Collisional Dissociation (aHCD)

With the Q Exactive HF-X MS, we introduce a 16 ms transient length to allow up to a 40 Hz scan rate at 7,500 resolution. In addition, the reduction of inter-scan and intra-scan overhead times deliver an extra 10 ms ion injection time (IT) into the Orbitrap mass analyzer in MS/MS mode for the 16 ms transient (Figure 4). This modified aHCD timing maximizes ion accumulation time within a shorter transient time and ensures the highest MS^2 spectral quality even at rapid 40 Hz scan rates. This is demonstrated in a triplicate analysis where a HeLa cell digest ran on the Thermo Scientific™ Q Exactive HF hybrid quadrupole-Orbitrap™ mass spectrometer and Q Exactive HF-X MS at two different MS^2 resolution settings of 15,000 and 7,500 respectively (Figure 5). From the peptide score distribution (>80,000 peptides), the Q Exactive HF-X MS clearly achieves the perfect balance between resolution and reliable MS^2 spectra without sacrificing spectral quality. Thus, the Orbitrap mass analyzer delivers the same high-quality, high-confidence data but at roughly twice the scan rate of previous Q Exactive MS platforms.

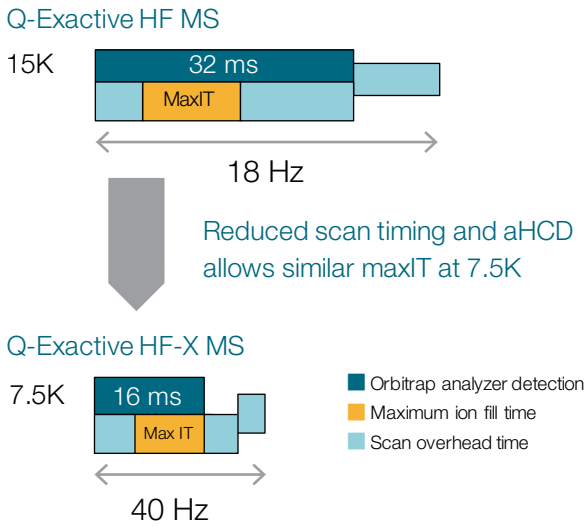

Figure 4. Optimized scan matrix now enables a novel 7,500 resolution Orbitrap mass analyzer setting for maximal scanning speed.

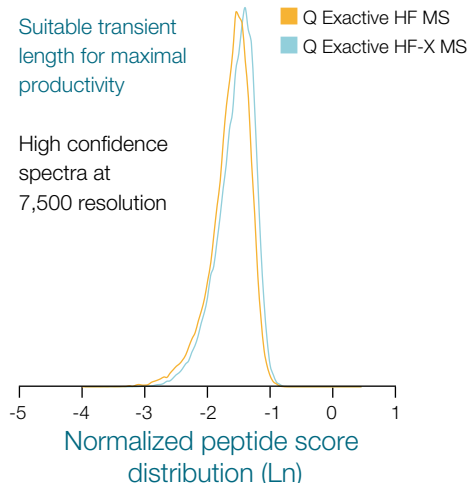

Figure 5. Maximal productivity can be achieved with the new 7,500 resolution setting while still retaining highest data quality.

To highlight the performance benefit of these features with respect to a bottom-up analysis, 1 µg of HeLa cell line digest was analyzed on the Q Exactive HF-X MS using the 7,500 resolution setting under various LC gradient durations. This same experiment was also performed on the Q Exactive HF MS at 15,000 resolution. The short 16 ms transient time combined with aHCD resulted in the detection and identification of ~1,100 peptides per minute under conditions where sample complexity is maximal. Identification of more unique peptides on the Q Exactive HF-X MS is especially evident at shorter gradient times. This highlights the potential to maximize insights from your peptide ID experiments, by improving productivity without sacrificing data quality (Figure 6).

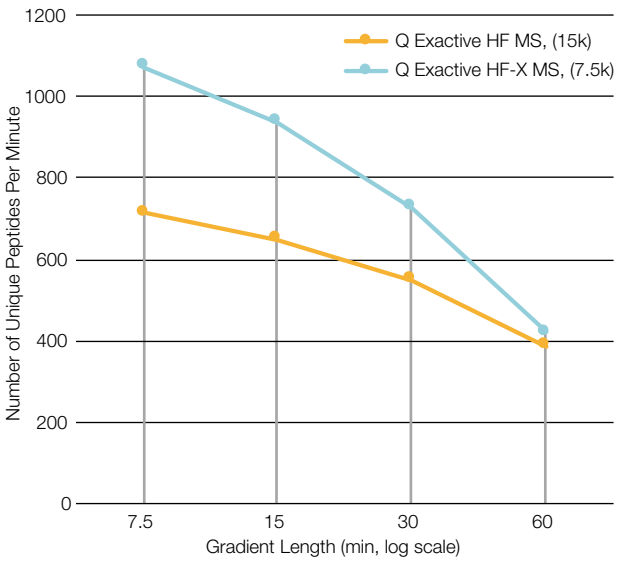

Figure 6. The Q Exactive HF-X MS delivers rapid, deep proteome sequencing results with maximal productivity. Data provided courtesy of Jesper Olsen.[2]

Reason 2: Push the limits of discovery proteomics with faster and deeper sequencing

Basic research as well as biomedical studies increasingly rely on advanced technology for rapid, wide scale, comparative analysis of proteomes. Taking proteomics discovery to the next level of insight and productivity requires innovative advances in MS technology. The Q Exactive HF-X MS sets new standards in speed and sensitivity building on the well-known Orbitrap mass analyzer attributes of providing the highest confidence and selectivity. Deep proteome analysis should be completed in hours rather than days. The new Q Exactive HF-X MS is faster and has improved sensitivity with the enhanced HCTT and EDIF front-end design architecture, allowing you to probe deeper and more efficiently. The Q Exactive HF-X mass spectrometer boosts productivity in protein identification, while maintaining the proven mass accuracy of the high field Orbitrap mass analyzer. Superior ion transmission, together with aHCD and new scan rates up to 40 Hz, drive more efficient use of the Orbitrap mass analyzer.

The same, or better, protein identifications in half the analysis time

HeLa cell line digest was analyzed in triplicate using a 30-minute liquid chromatographic (LC) separation on the Q Exactive HF-X MS and a 60 minute LC separation on both a Q Exactive HF MS and Q Exactive MS. Almost the same number of protein groups were identified on the Q Exactive HF-X MS in half the time, achieving a nearly 2-fold improvement in analysis time as the previous generation instrument. Users with past experiences using previous generation Q Exactive series mass spectrometers can perform the same experiments in half the time and expect to obtain even more significant data than before (Figure 7). The faster scan rates on this instrument, while preserving spectral quality, present a tremendous potential throughput advantage for large-scale proteomic studies as the productivity in large-cohort analyses can be effectively doubled. Such an improvement is beneficial for labs that have limited instrument access time to accommodate increasing sample quantities and drive experimental efficiency.

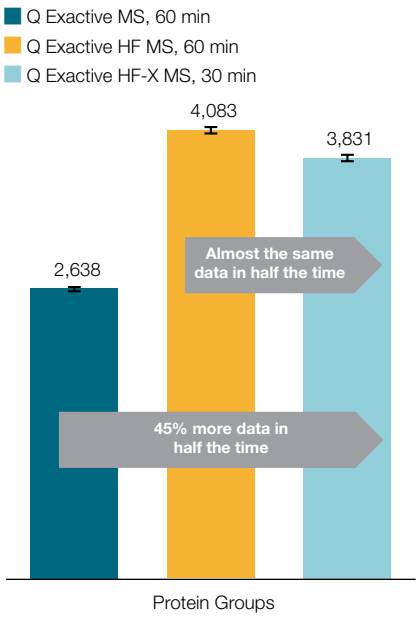

Figure 7. Proteins identified faster than ever.

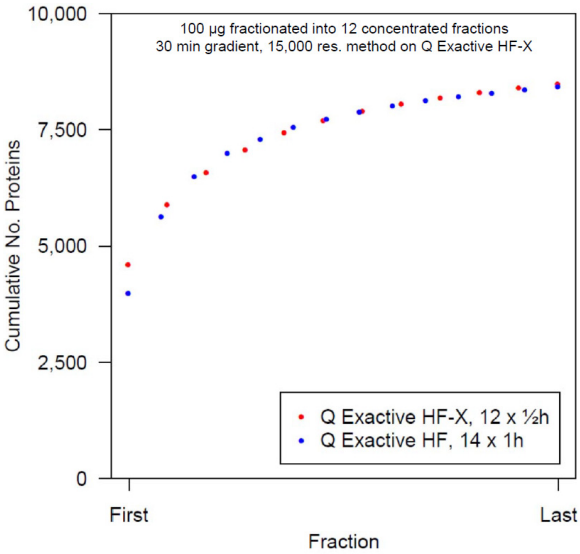

Figure 8. Deep proteome fractionation studies are completed in 50% less time. Data with courtesy from J. Olsen, Novo Nordisk Foundation, Center for Protein Research, University of Copenhagen.[2]

To further illustrate the improved identification efficiency for peptide sequencing shotgun experiments, Jesper Olsen's group at the University of Copenhagen performed an analysis of in-depth proteome profiling of HeLa cell line using a typical offline peptide fractionation scheme followed by analysis on the Q Exactive HF-X MS. This experiment provided the ultimate performance test of this instrument and the results from this deep proteome sequencing study were overlaid with previously published data collected on a Q Exactive HF MS using comparable acquisitions settings.[3] Impressively, the enhanced speed of the Q Exactive HF-X MS produced over 8,000 protein identifications in less than half the time and with fewer fractions than their previous best analysis (Figure 8). In addition, proteins from the first fraction alone contained approximately 50% of proteins found across all fractions which could prove useful for experiments where maximal protein annotation needs to be achieved in the shortest amount of time.

Increased peptide ID efficiency

In certain scenarios, productivity is not the key analytical criteria, but rather depth of insight is desired. Studies involving PTM analysis, deep proteome investigation, or spectral library building can benefit from the advanced technology of the Q Exactive HF-X MS. To evaluate the efficiency potential of the Q Exactive HF-X MS, 1 µg of HeLa cell line digest was analyzed in triplicate on the Q Exactive HF-X MS, Q Exactive HF MS and Q Exactive MS systems. A 60-minute LC separation was used on all systems. Results show that the Q Exactive HF-X MS identified significantly more unique peptides in the same amount of time, resulting in improved proteome depth and protein coverage (Figure 9A). Alternatively, with significantly more unique peptide identifications per unit time, you can shorten your time-to-results at any throughput scale and at any depth of analysis (Figure 9B 60 min vs 30 min).

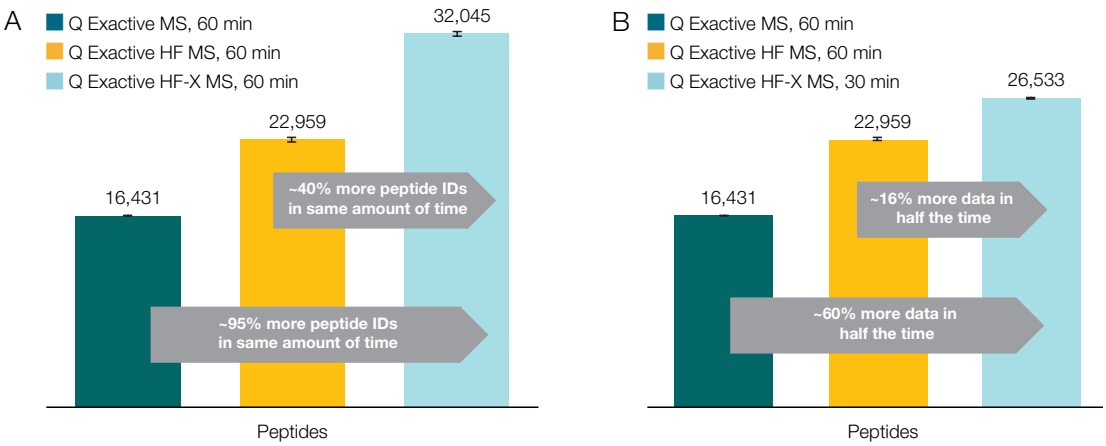

Figure 9. Increased peptide ID efficiency with the Q Exactive HF-X MS allows improved depth and coverage (A) or higher productivity to be achieved (B).

Reason 3: The Q Exactive HF-X MS empowers standardized label-free quantification workflows, providing robust and reproducible results for translational proteomics studies

Precision medicine and translational proteomics studies require implementation of highly robust analytical platforms to deliver accurate and reproducible peptide/protein quantitation, along with productive throughput. Verification of protein biomarkers requires confident quantitation of low-level proteins in large cohort studies. To this end, the superior consistency and performance of the Q Exactive HF-X MS sets new standards in quantitative accuracy, sensitivity and reproducibility for rigorous research workflows in large-scale studies of challenging protein samples. Label-free precursor ion-based MS[1] quantitation is a proven approach, delivering confident and reliable protein measurements. New DDA+ and HR-DIA workflows for label-free quantitation eliminate missing values across samples, delivering reproducibility and precision for large sample cohorts.

DDA+ is a new label-free quantitation workflow on the Q Exactive HF-X MS optimized for analytical robustness and reproducible precursor-based protein quantitation when the highest level of data precision is required. In traditional data-dependent workflows, consistent precursor and thereby protein quantitation can be challenging to achieve due to the stochastic sampling nature of the mass spectrometer. Since a subset of all possible precursor ions is selected for fragmentation and subsequent identification, reliable and consistent precursor quantitation is routinely accomplished for only a fraction of proteins in a sample. The remaining unselected precursors, and proteins by extension, are undetected and unquantified even if they were in fact present within the sample. This creates limitations for experiments where researchers want to compare protein levels across many samples, such as biomarker discovery and validation studies, because data for some proteins will be missing in some samples, leading to poor reproducibility and the so-called 'missing value' problem. The newly developed DDA+ quantitation workflow addresses this limitation by providing a sensitive, standardized, robust approach for all stages of the analytical process.

DDA+ builds on the proven, trusted approach of data-dependent MS acquisition and database-mediated identification utilized in peptide ID studies and applies the necessary analytical components to facilitate reproducible precursor-based protein quantitation. The DDA+ workflow leverages the capillary-flow Thermo Scientific™ UltiMate™ 3000 RSLCnano system and new 150 µm I.D. Thermo Scientific™ EASY-Spray™ columns in combination with the performance attributes of the next-generation Q Exactive HF-X MS to maximize robustness and up-time without sacrificing sensitivity (Figure 10). The UltiMate 3000 RSLCnano capillary-flow solution with direct flow-control delivers application versatility ideal for high-throughput large-sample cohort discovery profiling (3–5 µL/min flow rate) as well as highly sensitive, targeted validation experiments (1.2 µL/min flow rate). The integrated microflow loading pump permits fast sample loading and column washing at increased flow rates whether operating in pre-concentration or direct inject mode.

Figure 10. DDA+ provides a standardized, robust, sensitive solution for label-free quantitation.

The 150 µm ID x 150 mm EASY-Spray column was designed to ensure the optimal balance between sensitivity found on a nano-LC separation scale, while providing the throughput benefits of analytical flow LC-MS. Increased loading capacity of this capillary column allows higher sample loading amounts to be analyzed and higher peptide and protein identification rates to be achieved while preserving the robustness and analytical stability necessary for demanding translational studies. In fact, retention time stability of RSD <1% along with peak area stability of RSD <10% can be achieved across hundreds of injections, spanning many days delivering consistent and reliable data that you can trust (Figure 11). In addition, improvements in column consistency and robustness ensure that the 150 µm EASY-Spray column will provide the dependable long-term performance required for demanding applications such as large-cohort analysis (Figure 12).

The Q Exactive HF-X MS is the perfect complement to the DDA+ workflow—with the brighter ion beam, APD, optimized scan matrix with aHCD, and higher MS/MS scan speed, allowing it to achieve a higher level of sensitivity while retaining the throughput benefits of capillary flow and workflow robustness. This enhanced sensitivity essentially allows the 150 µm EASY-Spray column to operate in cap flow mode and achieve quantitative results similar to a traditional nano-LC setup (Figure 13).

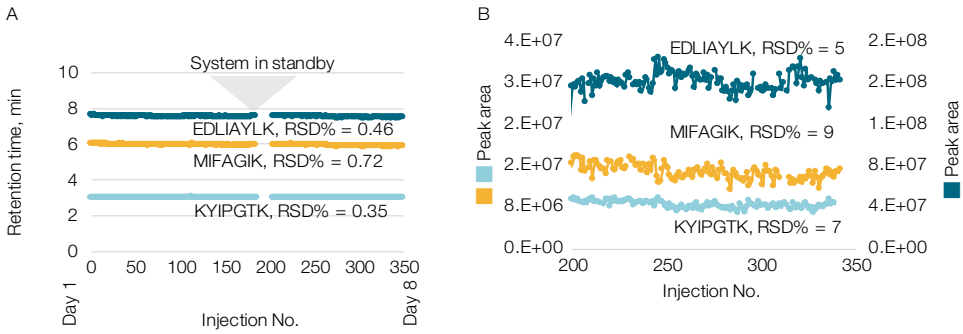

Figure 11. Analytical stability of capLC MS analysis of Cytochrome C protein digest. (A) Excellent retention time stability was observed (RSD <1%) for 350 injections. (B) Peak area stability was evaluated on MS^1 level for 150 consecutive injections conducted between day 5 and 8. Source data "Pushing the leading edge in protein quantitation: Integrated, precise, and reproducible proteomic workflows".

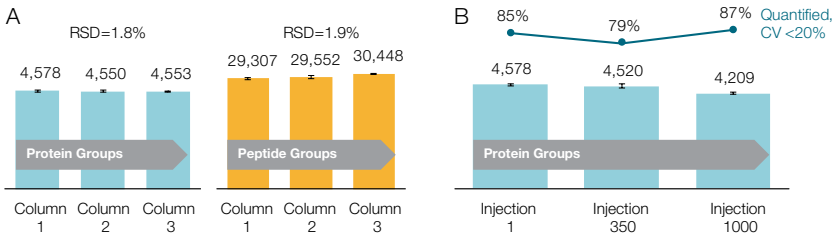

Figure 12. Dependable, robust LC performance for demanding applications. (A) Inter-column consistency with RSD <2% RSD, and (B) long-term quantitative performance are possible with the new 150 µm EASY-Spray column. (Analysis of 4 µg HeLa, 60 min method, UltiMate 3000 system, 1.2 µL/min, Q Exactive HF-X MS, 120 K/7.5 K, Top 40). Source data "Pushing the leading edge in protein quantitation: Integrated, precise, and reproducible proteomic workflows".

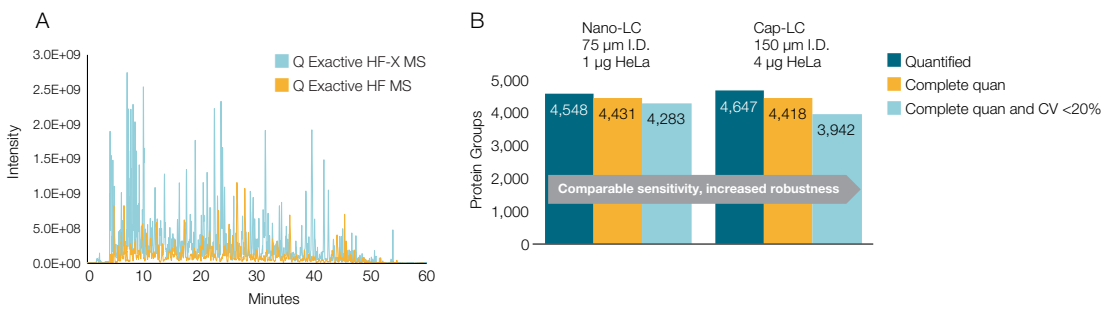

Figure 13. Q Exactive HF-X MS combined with capLC-MS demonstrates enhanced MS^1 sensitivity and robustness. (A) 1 µg HeLa digest was analyzed using the 150 µm EASY-Spray column and 60 min LC-MS analysis. The MS^1 base peak chromatograms on each system are overlaid and shown. (B) The improved sensitivity of the Q Exactive HF-X MS allows quantitative results comparable to nano-LC (300 nL/min flow) to be obtained using the 150 µm EASY-Spray column and cap-LC (1.2 µL/min flow). Source data "Pushing the leading edge in protein quantitation: Integrated, precise, and reproducible proteomic workflows".

The DDA+ workflow on the Q Exactive HF-X MS is empowered by novel data processing and analysis features in Thermo Scientific™ Proteome Discoverer™ software. The optimized, label-free quantitation processing pipeline including, retention-time alignment and feature linking across datasets, can extract more information across replicates, increasing quantitation, and virtually eliminating the missing value issue associated with DDA analysis. This delivers more robust, highly reproducible and precise protein quantification for virtually all the proteins identified (Figure 14 and 15). This precision becomes important when monitoring very subtle biological changes that require very high fidelity quantitation.

Taking advantage of the high-resolution, accurate mass (HRAM) capability of the Q Exactive HF-X MS and a high-resolution data independent acquisition (HR-DIA) workflow, excellent peptide quantitation with high reproducibility can also be achieved. The HR-DIA approach is uniquely suited for large scale studies due to its ability to rapidly identify and to reproducibly quantify all ions within an LC-MS analysis. HR-DIA presents a complementary workflow for users undertaking quantitative studies as the high resolving power of the Orbitrap mass analyzer really benefits label-free precursor quantitation. HR-DIA, optimally applied on Orbitrap mass analyzer instruments like the Q Exactive HF-X MS, utilizes high resolution full MS^1 data for quantification and MS^2 information for identification based on spectral libraries. The Q Exactive HF-X MS provides the requisite high-resolving power (120 K or 240 K) on the MS^1 scan to reduce interferences to a minimum, and the brighter ion source and optimized scan matrix offer unparalleled depth of coverage and dynamic range. In addition, the scan speed and sensitivity enhancements on the Q Exactive HF-X MS maximize productivity and quantitative insight (Figure 16A). For users with limited or precious sample, the improved performance attributes of the Q Exactive HF-X MS permit equivalent quantitative results to be generated with only half the amount of sample as before (Figure 16B).

The Q Exactive HF-X MS has emerged as the instrument of choice for its HRAM capabilities while retaining the flexibility to provide robust, standardized, reproducible quantification workflows no matter your experimental needs.

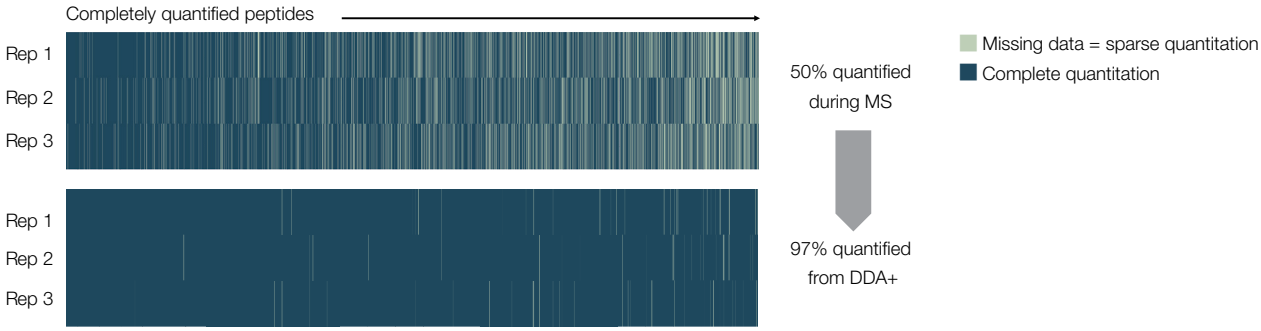

Figure 14. Maximize quantitative reproducibility and reduce missing data points. A triplicate analysis of 4 µg HeLa protein digest was analyzed using the Q Exactive HF-X MS and DDA+. The heat maps indicate peptides that were commonly quantified across replicates (dark green) and those that were not quantified in one or more replicates (light green). The improved label free quantitation algorithm of DDA+ boosts quantitative information resulting in a more comprehensive analysis.

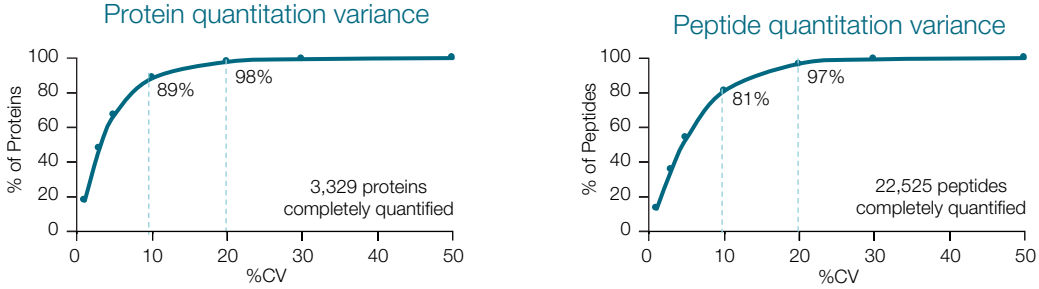

Figure 15. DDA+ enables quantitative confidence through high precision measurements. A triplicate analysis of 4 µg HeLa protein digest was analyzed using the Q Exactive HF-X MS and DDA+. The distribution of the coefficient of variation for all the proteins and peptides that were quantified in the experiment are plotted.

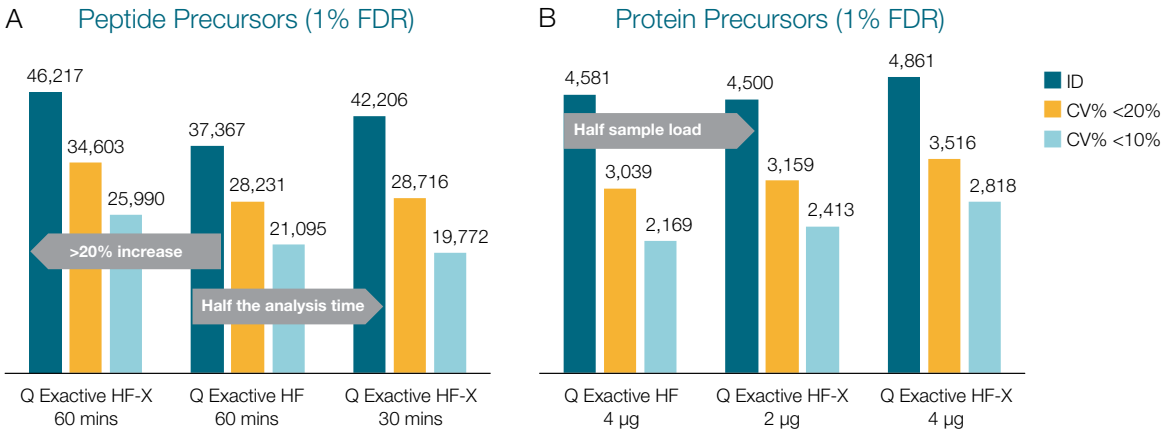

Figure 16. HR-DIA provides unparalleled proteome coverage and reproducibility. A triplicate analysis of HeLa protein digest was analyzed with a Q Exactive HF-X MS and Q Exactive HF MS using an optimized HR-DIA method (120 K MS[1], 30 K DIA, 10 *m/z* x 80 windows). Data was analyzed using Spectronaut™. (A) Peptide groups quantified from 4 µg HeLa protein digest. (B) Protein groups quantified from a 60 minute MS analysis.

Why choose Orbitrap mass analyzer technology?

Changing research trends and analytical needs have driven mass spectrometry innovation, especially in the past decade. Today's mass spectrometers must be equipped with superior performance features such as high resolution, mass accuracy, dynamic range and fast scanning capabilities in order to fulfill rigorous experimental demands and handle extremely complex samples. In today's research, these same instruments have to provide the flexibility to carry out a variety of analytical techniques including multiplexing and multiple acquisition modes, in addition to being highly robust with consistent performance for high throughput analysis to suit a variety of experimental workflows and varying laboratory needs. Since its introduction in 2005, Orbitrap mass analyzer technology has revolutionized mass spectrometry-based research to meet these various challenges across multiple application fields of interest. The exceptional value of Orbitrap mass analyzer systems in delivering uncompromising analytical performance and achieving greater experimental possibilities has been well recognized by the scientific community. Adoption of Orbitrap mass analyzer technology has grown over the years with the proven increase in numbers of Nature and Science family publications (Figure 17).

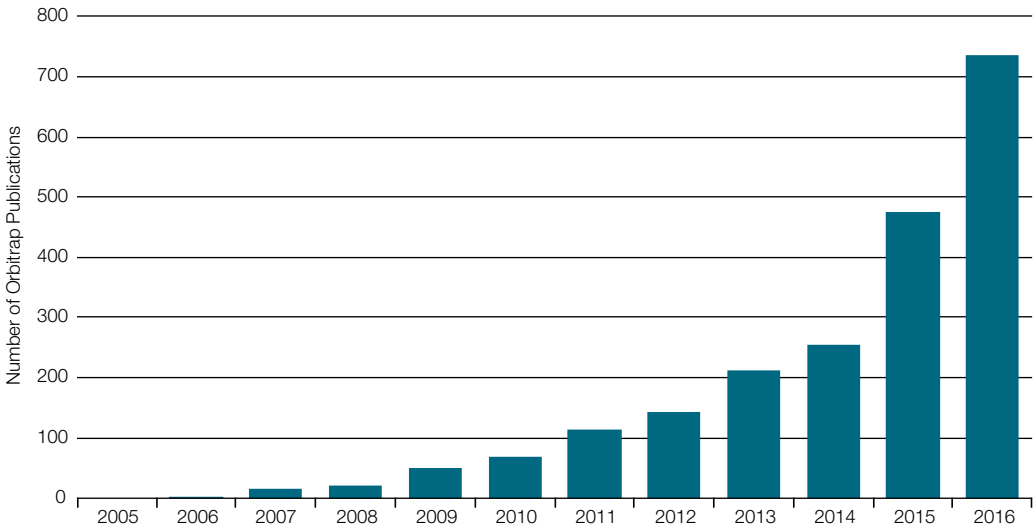

Figure 17. Rising trend in number of Orbitrap mass analyzer-based research publications in Nature and Science journals since its introduction in 2005. Source data "Nature Family of Journals and Science Journals AAAS".

Which Orbitrap mass analyzer system is right for my life science research?

The Q Exactive series mass spectrometers have multiple products to suit your research requirements.

Table 3. Orbitrap selection guide.

Instrument Attributes	Q Exactive Focus MS	Q Exactive MS	Q Exactive Plus MS	Q Exactive HF MS	Q Exactive HF-X MS
Analyzer	Orbitrap	Orbitrap	Orbitrap	Ultra-High Field Orbitrap	Ultra-High Field Orbitrap
Mass Range	*m/z* 50–3,000	*m/z* 50–6,000	*m/z* 50–6,000; up to 8,000 *m/z* with BioPharma option	*m/z* 50–6,000; up to 8,000 *m/z* with BioPharma option	*m/z* 50–6,000; up to 8,000 *m/z* with BioPharma option
Maximum Resolution at *m/z* 200	70,000	140,000	140,000; 280,000 with Enhanced Resolution Mode	240,000	240,000
Maximum Scan Speed	12 Hz	12 Hz	12 Hz	18 Hz	40 Hz
Top N/MS^n	Top 3 ddMS^2	Top 10 ddMS^2	Top 10 ddMS^2	Top 20 ddMS^2	Top 40 ddMS^2
Mass Accuracy—Internal Calibration	<1 ppm	<1 ppm	<1 ppm	<1 ppm	<1 ppm
Polarity Switching	<1 sec	<1 sec	<1 sec	<1 sec	<1 sec
Multiplex	SIM only (up to 10 precursors)	Yes, up to 10 precursors	Yes, up to 10 precursors	Yes, up to 10 precursors	Yes, up to 10 precursors
Intact Protein Mode	No	No	Yes	Yes	N/A
BioPharma Option	No	No	Yes	Yes	Yes
Collision Energy	NCE and CE	Normalized CE	Normalized CE	Normalized CE	Normalized CE
Dissociation	HCD	HCD	HCD	HCD	HCD

Which LC should I select for my Orbitrap mass analyzer system?

UHPLC portfolio for LC-MS applications. Detailed product specifications can be found on www.thermofisher.com

Table 4. UHPLC selection guide.

Instrument Attributes	Nano-flow LC	Nano-, capillary-, micro-flow LC	Micro- and analytical flow LC	Analytical flow LC	
	EASY-nLC™ 1200	UltiMate 3000 RSLCnano	Vanquish™ Horizon	Vanquish™ Flex Quaternary	Vanquish™ Flex Binary
Settable Flow-Range	20–2,000 nL/min	0–50 µL/min* and 0–2,500 µL/min**	0.001–5 mL/min	0.001–8 mL/min	0.001–8 mL/min
Recommended Flow Range	100–1,000 nL/min	50 nL/min-50 µL/min and 5–2,500 µL/min** (isocratic, gradient formation from 50 µL/min)	50 µL/min–5 mL/min	100 µL/min–8 mL/min	100 µL/min–8 mL/min
System Pressure	1,200 bar (17,500 psi)	860 bar*** (12,500 psi)	1,500 bar (22,000 psi)	1,000 bar (15,000 psi)	1,000 bar (15,000 psi)
Pump	Binary syringe (high-pressure gradient)	1. Binary serial dual-piston (high-pressure gradient); 2. Integrated ternary serial dual-piston (low pressure gradient)	Binary parallel dual-piston (high-pressure gradient)	Quaternary serial dual-piston (low-pressure gradient)	Binary serial dual-piston (high-pressure gradient)
Autosampler Type	Pulled loop, zero-loss sample pick up	Pulled loop, zero-loss sample pick up	Split loop	Split loop	Split loop
System Gradient Delay Volume GDV	<1 µL	<350 nL in preconcentration configuration	Adjustable; default: 175 µL (with 35 µL mixer)	Adjustable; default: 620 µL (with 200 µL mixer)	Adjustable; default: 175 µL (with 35 µL mixer)
Sample Capacity	1 sample rack	3 sample racks	4 sample racks****	4 sample racks****	4 sample racks****
Column Temperature	N/A	Room temperature, +7 °C up to 75 °C	5–120 °C	5–120 °C	5–120 °C
Column Heating Mode	N/A	Forced air	Still air/forced air	Still air/forced air	Still air/forced air
Column Pre-Heating	N/A	N/A	Active and passive	Active and passive	Active and passive

* using dedicated nano-, capillary-, or micro-flow meters

** in integrated micro pump of the NCS-3500RS module

*** 800 bar (11,600 psi) for capillary- and micro-flow range

**** Thermo Scientific™ Vanquish™ Charger module is available to increase capacity up to 12 high racks or 23 shallow well-plates

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Table 5: Which Q Exactive hybrid quadrupole Orbitrap system best suits my experimental requirements?

Performance Features	Q Exactive Focus MS	Q Exactive MS	Q Exactive Plus MS	Q Exactive HF MS	Q Exactive HF-X MS
Resolution					
Sensitivity					
Speed					
Dynamic Range					
Mass Accuracy					
Multiplexing					

Table 6: Which Q Exactive hybrid quadrupole Orbitrap system best suits my area of life science research?

Application	Q Exactive Focus MS	Q Exactive MS	Q Exactive Plus MS	Q Exactive HF MS	Q Exactive HF-X MS
Peptide IDs					
TMT Quantitation					
SILAC					
Label Free Quantitation					
Top Down					
Intact Analysis					
PTM Phosphorylation					

Least Fit **Best Fit**

References

1. Scheltema RA, et al. The Q Exactive HF, a Benchtop Mass Spectrometer with a Pre-filter, High-performance Quadrupole and an Ultra-high-field Orbitrap Analyzer. 2014 *Molecular & Cellular Proteomics,* 13, 3698-3708.
2. Kelstrup, CD et al. Performance evaluation of the Q Exactive HF-X for shotgun proteomics. *Journal of Proteome Research.* 2018 Jan 5;17(1):727-738.
3. Kelstrup, CD et al. Rapid and Deep Proteomes by Faster Sequencing on a Benchtop Quadrupole Ultra-High-Field Orbitrap Mass Spectrometer. *Journal of Proteome Research.* 2014 Dec 5;13(12):6187-95.

Find out more at **thermofisher.com/QExactiveHFX**

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