

Orbitrap Exploris 120 mass spectrometer

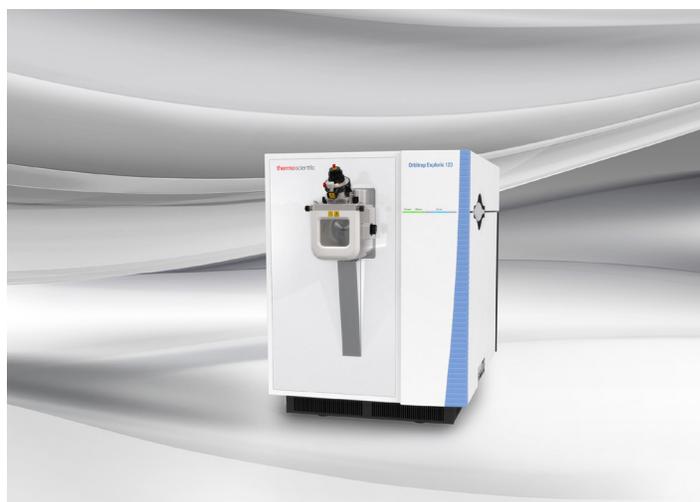
Versatile performance in a compact, multi-purpose, high-resolution mass spectrometer for the analysis of peptides, proteins, and oligonucleotides

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The Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer has demonstrated its unmatched performance in many small molecule application labs around the world. It has redefined productivity in applications such as veterinary drug screening, pesticide analysis, drugs of abuse and horse doping analysis.¹⁻⁶ Now, the full power of Thermo Scientific™ Orbitrap™ mass spectrometers can be truly unleashed enabling the analysis of peptides, intact proteins, and oligonucleotides, making this instrument ideal for labs that need to perform every-day targeted and non-targeted work with the absolute confidence that a high resolution, accurate mass instrument can deliver.

The Orbitrap Exploris 120 MS enabled with next-generation instrument control software and modern method editor provides a fast jump start that permits a walk-up system for users that covers typical HRAM applications in food safety,



toxicology, environmental, drugs of abuse, metabolomics, medium size proteins and general proteomics applications. The broad selection of predefined acquisition methods tailored to each application and the confidence behind thoroughly tested ready-to-go methods ensures researchers rapid success and consistent results. In addition, the Method Editor and the Tune applications are fully shared across the Thermo Scientific™ Orbitrap™ Exploris MS series and with other Thermo Scientific mass spectrometers such as the Thermo Scientific™ TSQ™ series triple quadrupole MS and Thermo Scientific™ Tribrid™ Orbitrap™ mass spectrometers reducing the learning curve when transitioning from one analytical platform to another.

Discovery and quantitation features

The main feature sets on the Orbitrap Exploris 120 MS for both discovery and quantitation are listed in Table 1 and

Table 2. The comparison clearly shows the improvements and capabilities of the new instrument.

Table 1. Discovery comparison—Orbitrap Exploris 120 MS vs Thermo Scientific™ Q Exactive™ Plus MS and Thermo Scientific™ Q Exactive HF™ MS.

Feature	Orbitrap Exploris 120 MS	Q Exactive Plus MS	Q Exactive HF MS
Orbitrap	High-Field Orbitrap	Standard Orbitrap	High-Field Orbitrap
EASY-IC	Standard	n/a	n/a
DDA speed	14 MS ² per sec	10 MS ² per sec	18 MS ² per sec
Charge state recognition	Yes	Yes	Yes
NCE per charge state	Yes	Yes	Yes
MS ² m/z range for DDA	Yes	Yes	Yes
Charge state filter for DDA TopN experiments	Trigger-all, 1+, undetermined	Trigger-all, 1+, multiple charged, undetermined	Trigger-all, 1+, multiple charged, undetermined
DIA	Yes	Yes	Yes

* Orbitrap Exploris MS comes with system templates for all scan types serving different types of applications

Table 2. Quantitation comparison—Orbitrap Exploris 120 MS vs Q Exactive Plus MS and Q Exactive HF MS.

Feature	Orbitrap Exploris 120 MS	Q Exactive Plus MS	Q Exactive HF MS
Orbitrap	High-Field Orbitrap	Standard Orbitrap	High-Field Orbitrap
EASY-IC	Standard	n/a	n/a
Polarity Switching	>1.6 Hz	1 Hz	1 Hz
Speed at 120k/140k* res setting	3 Hz	1 Hz*	3 Hz
Scan speed PRM/tMS ²	22 Hz @ 15 k res	12 Hz @ 17.5 k res	18 Hz @ 15 k res
MS ¹ speed at DDA TopN (60k/70k res MS ¹ ; 15 k/17.5 k res MS ²)	4 Hz MS ¹	1 Hz MS ¹	2 Hz MS ¹
Spectral multiplexing	20 msx tSIM	10 msx tSIM	10 msx tSIM
DIA	Yes	Yes	Yes
Mild trapping	Yes	n/a	n/a

* Orbitrap Exploris MS comes with system templates for all scan types

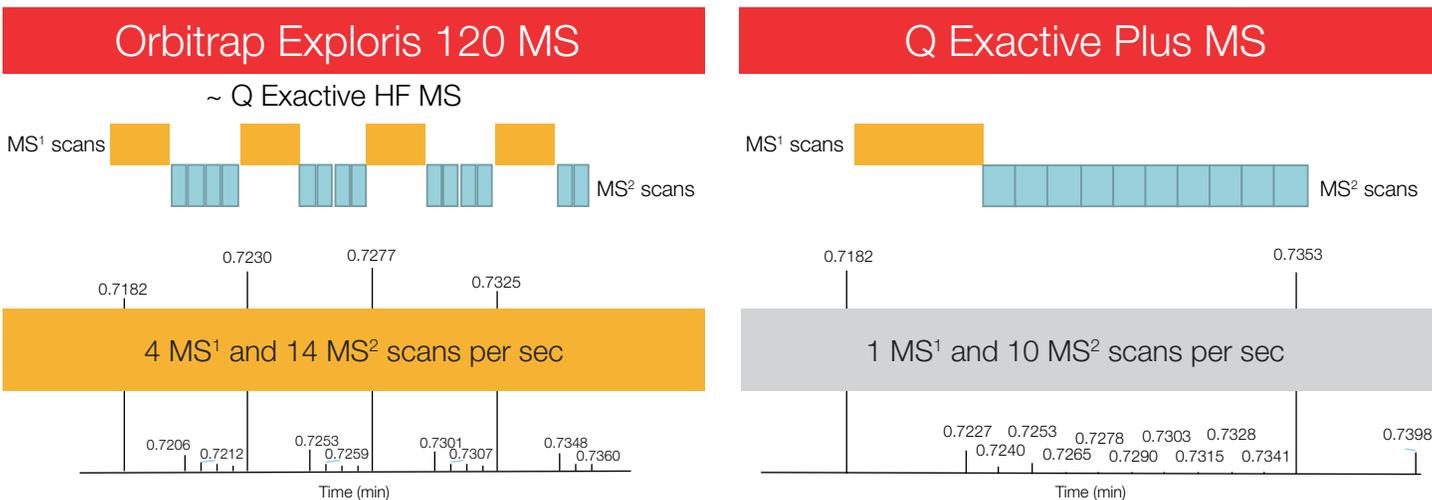


Figure 1. Scan speed comparison demonstrates how the Orbitrap Exploris 120 MS achieves a higher rate of MS/MS events per second than the Q Exactive Plus MS. Average over one minute acquisition.

Performance features

The Orbitrap Exploris 120 MS, in addition to its outstanding capabilities for small molecule characterization, is a versatile mass spectrometer capable of tackling challenging analytical workflows such as shotgun proteomics, intact protein analysis, as well as oligonucleotide analyses. Here are some examples demonstrating the performance of such capabilities.

1. Peptide and Protein Groups Identification

In classic shotgun proteomics applications, identifying and quantifying the highest number of proteins and peptides is highly important to enable drawing conclusions from the biological system under study. For this application, the Orbitrap Exploris 120 MS delivers exceptional results in terms of peptides and proteins identified. The utilization of the Thermo Scientific™ FAIMS Pro interface further improves the performance identifying over 20% more proteins and gains are observed at the protein level for 60 min and 120 min gradients, representing the most typical gradient lengths in proteomics.

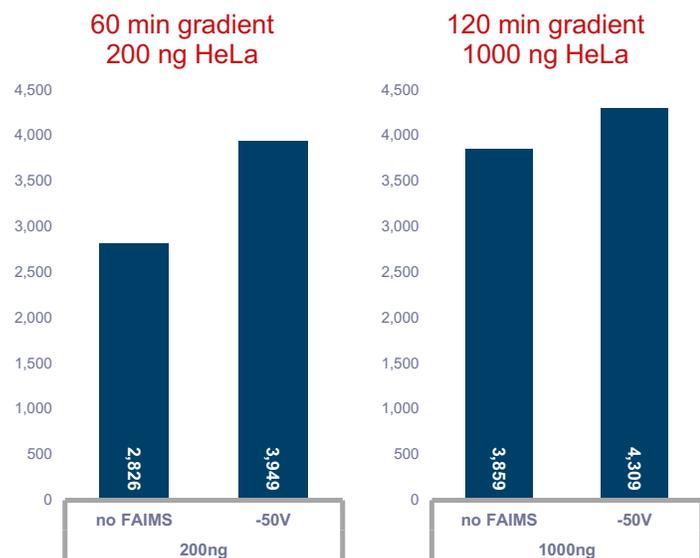


Figure 2. Orbitrap Exploris 120 MS offers solid performance in standard bottom up peptide ID experiments. Using the FAIMS Pro interface with Single CV greatly enhances proteome coverage independently of the gradient length and the sample load.

2. FAIMS Pro Boosts Proteome Coverage Using Gas-Phase Fractionation with Multiple CVs

As an alternative to high pH reverse phase peptide fractionation to minimize sample handling steps and reduce overall the time to answer while still obtaining very high proteome coverage, the FAIMS Pro source can be used to perform gas phase fractionation. In the same way, fractions are collected during the high pH chromatography separation, the compensation voltages can be tuned to collect only specific peptides within a certain mobility fraction. This way, the instrument injects the sample several times while for each of them changes the compensation voltage in the FAIMS source reducing the time of tedious bench-work and extra LC time. This strategy increases the number of protein IDs by 50% compared to three replicates of the same sample without FAIMS, and the gain at the peptide level is greater than 60% (Figure 2). The extra increase on peptides directly translate into greater protein coverage, as it is highlighted in Figure 3, where the protein coverage for mTor protein kinase increases by over 10 peptides. Demonstrating the accessibility to the low abundant proteome and high dynamic range.

3. Peptide and Protein Quantitation

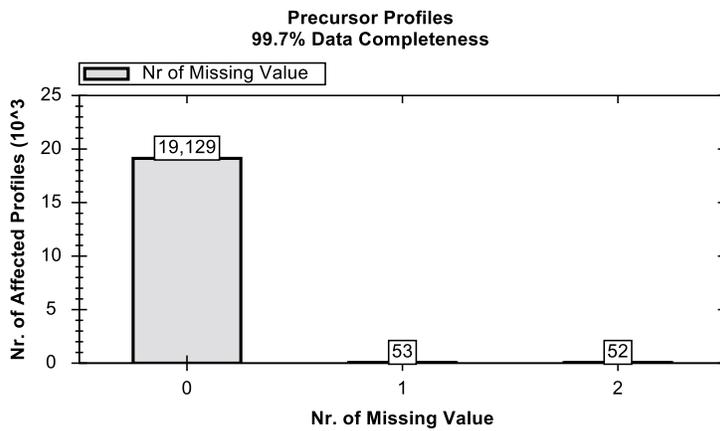
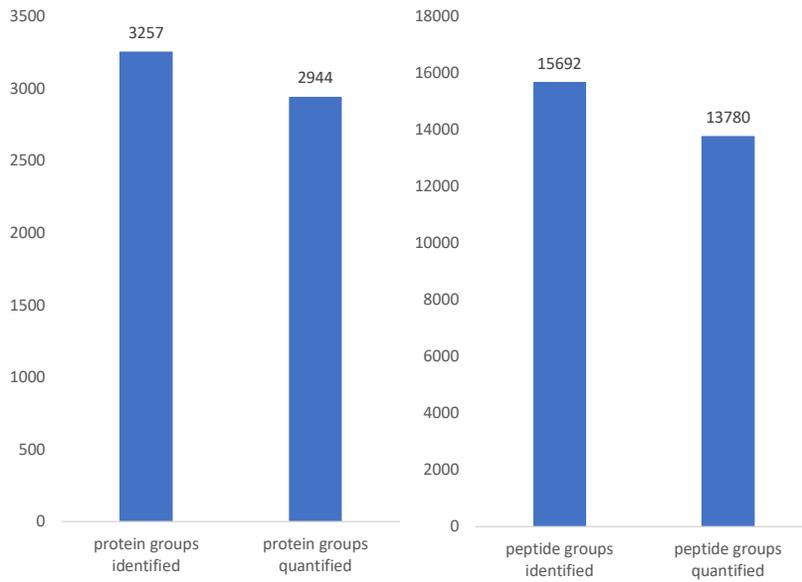
The Orbitrap Exploris 120 MS enabled for MS¹-based DIA quantification with pre-configured DIA method templates for operational simplicity and reproducibility (Figure 4). As it is demonstrated, the method offers great proteome coverage as well as very high data completeness and very high precise measurements with a median coefficient of variation of 5%

4. Identification of Intact Proteins Analysis

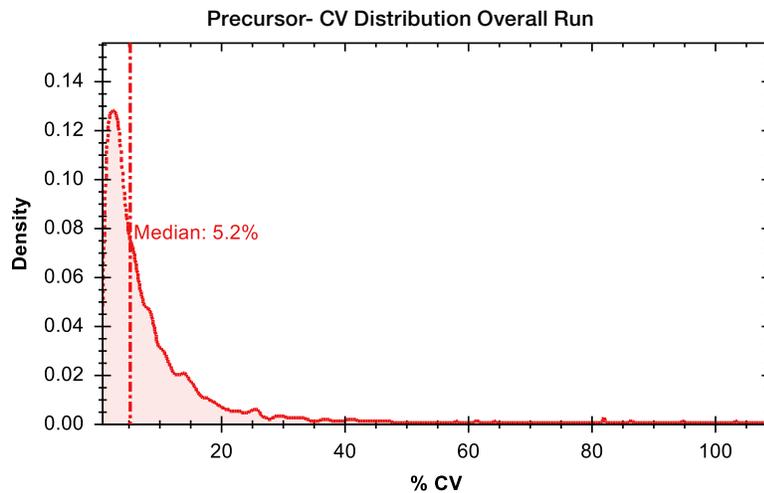
The Orbitrap Exploris 120 MS is also enabled for the routine high-resolution analysis molecular weight of small intact proteins <30 kDa as exemplified here with Carbonic Anhydrase II (Figure 5).

5. Sequence Analysis of Intact Oligonucleotides

For the analysis of oligonucleotides, the Orbitrap Exploris 120 MS delivers excellent data quality with accurate mass assignment. The LC-MS data is acquired in a negative mode using a full scan resolution of 120000 @ *m/z* 2000. The data is processed using Thermo Scientific™ BioPharma Finder™ software version 4.0 (Figure 6).



Data Completeness: Three technical replicates



CV Distribution: Precursor Profiles show low variability in three technical replicates

Figure 4. DIA Quantification, 90% of identified proteins are quantified, 88% of identified peptides are quantified (with CV<20%), and the median CV for all the quantified peptides is 5.2% 120 min gradient, 1000 ng HeLa.

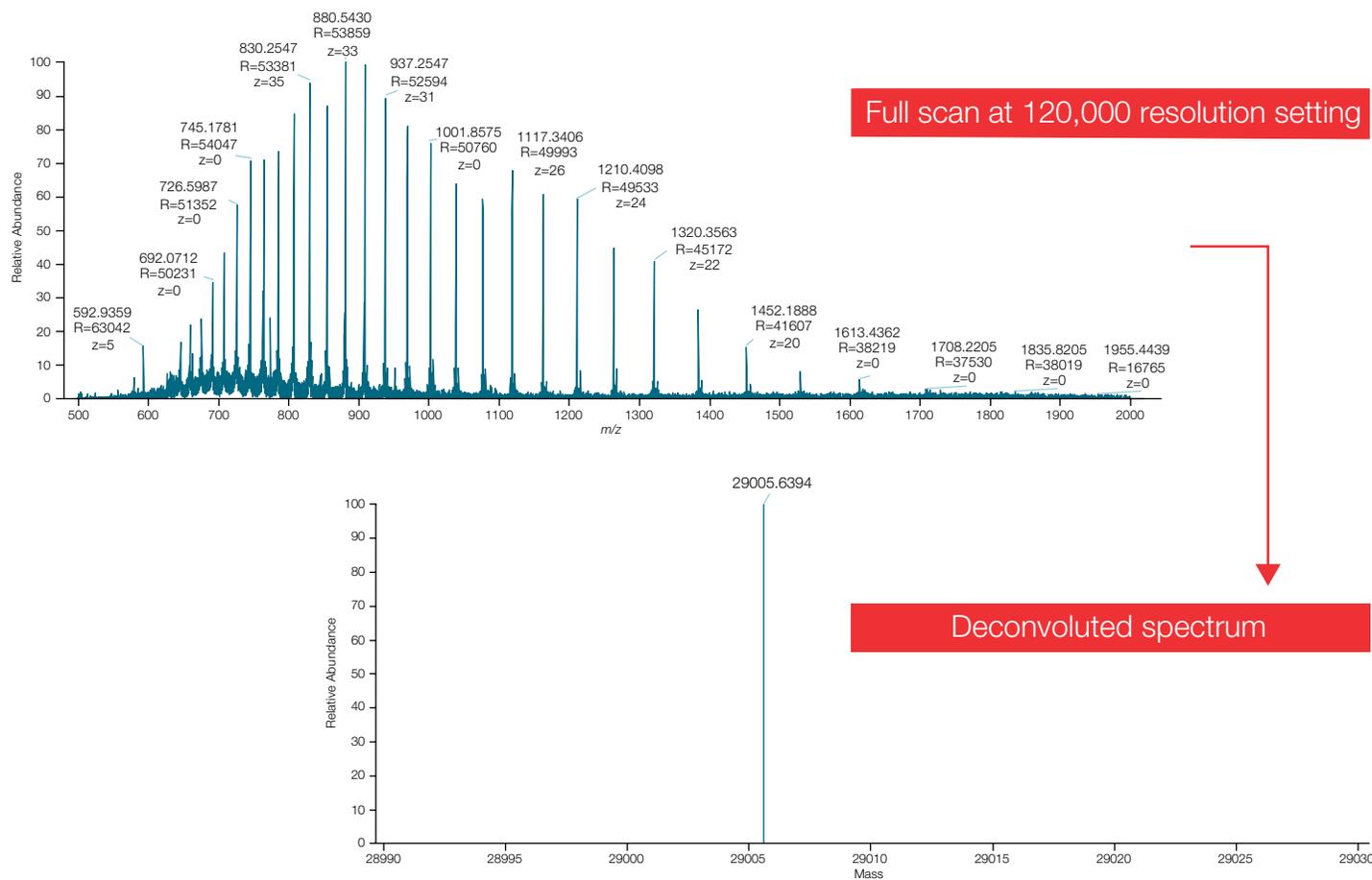


Figure 5. Orbitrap Exploris 120 MS | intact proteins. Carbonic Anhydrase II, 29 kDa i.e. approximately the size of a light chain of a mAb. Full scan at 120,000 resolution setting, accumulation time of 30 sec. The correct charge envelope and charge states of CA II are determined. Accurate mass is determined after deconvolution and is completely enabled for routine MW assessment of intact proteins. The full benefit of Orbitrap mass accuracy translates into accurate protein MW.

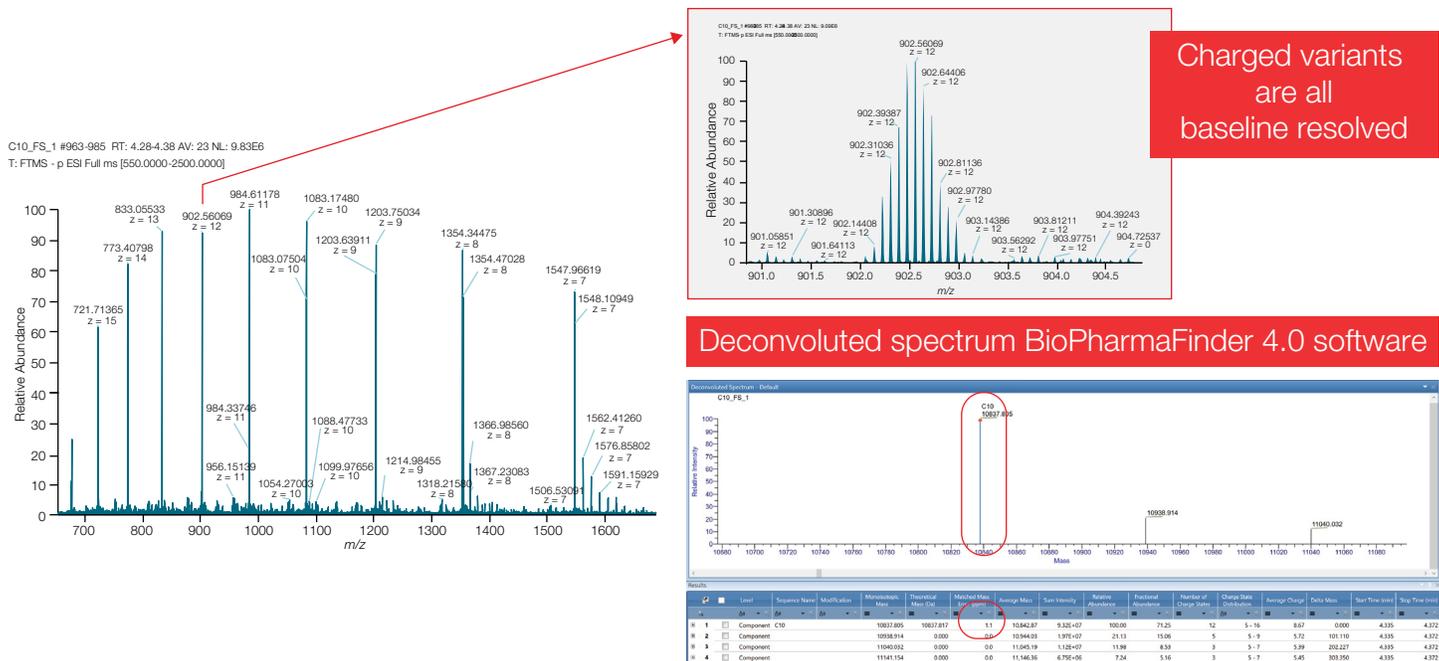


Figure 6. LC-MS sequence analysis of intact 35-mer oligonucleotide (TACTGGACCACCTGGGTGGGTTTAAAGCGGCAGTA) in negative ion mode. High resolution data leads to the identification of oligonucleotides with high mass accuracy; for the 35-mer shown the mass accuracy is 1.1 ppm.

The ability to walk-up to an Orbitrap HRAM mass spectrometer and analyze a broad range of molecules with ready-to-go system templates makes the Orbitrap Exploris 120 MS convenient for users at all levels. In this example, we illustrate predefined templates for peptide IDs with the choice of selecting between analyzing high as well as low concentrated samples. Ready-to-go method templates make the lab personnel productive and successful within a short period of time with minor training while retaining the flexibility for method customization (Figure 7 and 8).

The Orbitrap Exploris 120 mass spectrometer coupled to either a nanoLC or high flow UHPLC provides an excellent platform for identification and quantitation of small and large compounds. With the FAIMS Pro interface coupled to the Orbitrap Exploris 120 MS, the number of protein and peptide IDs are increased up to 40%. The Orbitrap Exploris 120 mass spectrometer is completely enabled for routine MW assessment of intact proteins with shown examples of bovine Carbonic Anhydrase II. Excellent data quality of sequence analysis with accurate mass assignment of a

35-mer oligonucleotide processed with BioPharma Finder software is shown. Installed and ready to use system templates help to make lab personnel productive and successful for a broad range of applications.

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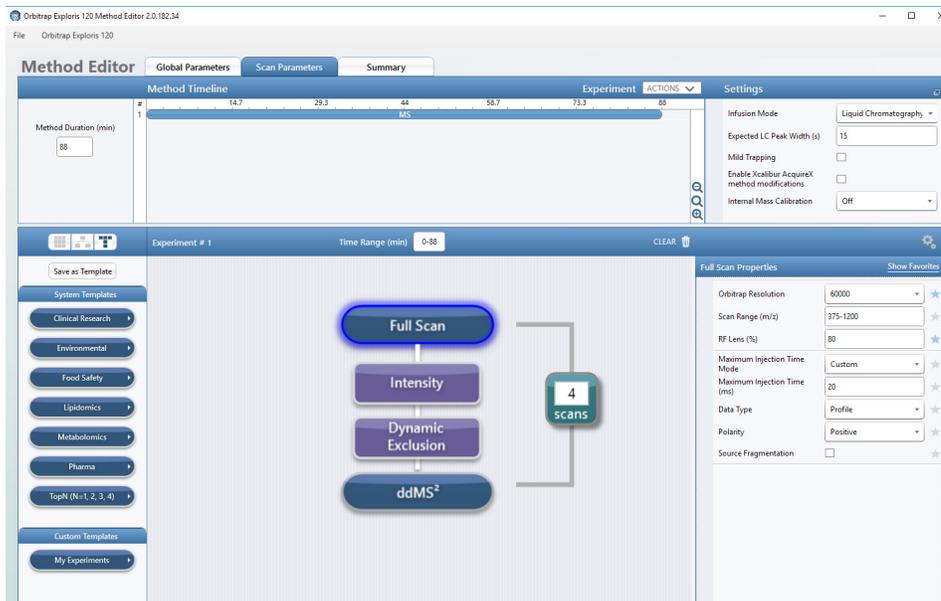


Figure 7. Orbitrap Exploris 120 MS Instrument Control Software features easy-to-use drag-n-drop method creation allowing flexibility to customize parameters quickly.

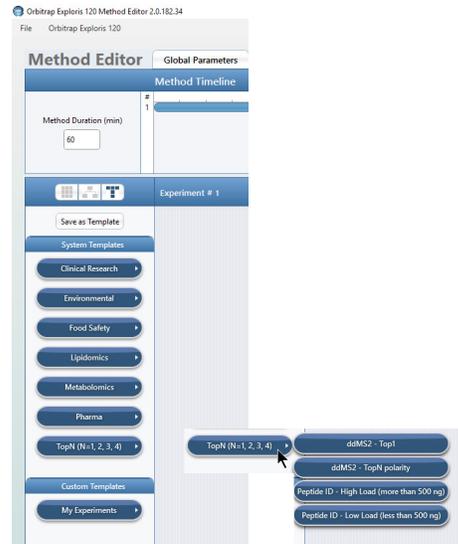


Figure 8. Orbitrap Exploris 120 MS Instrument Control Software includes pre-made system templates for multiple application areas that are ready to use.

Find out more at thermofisher.com/OrbitrapExploris120

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