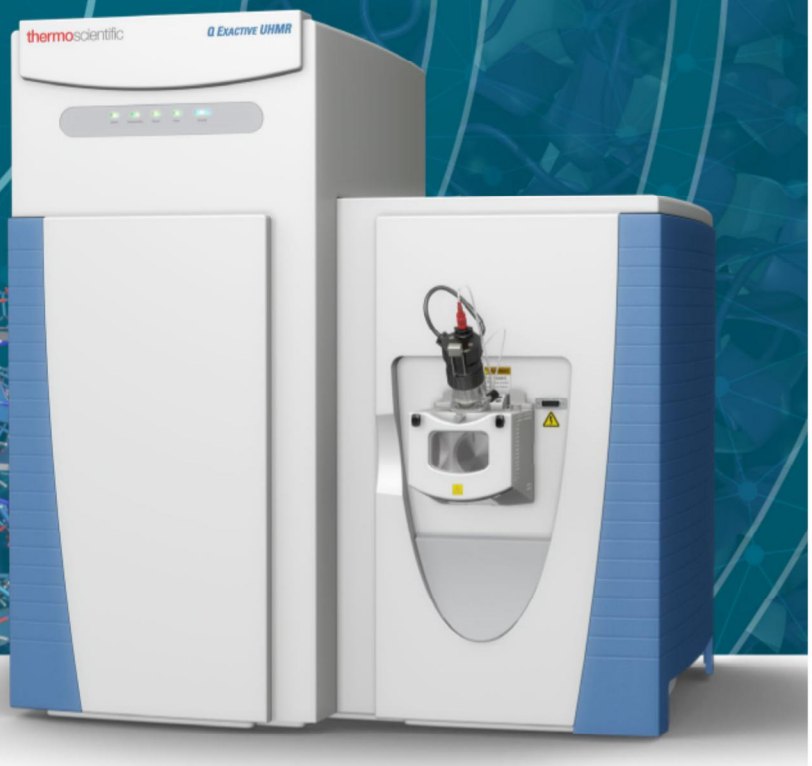
thermoscientific

Go beyond what you thought possible with **native MS**



Thermo Scientific Q Exactive UHMR Hybrid Quadrupole-Orbitrap mass spectrometer

ThermoFisher SCIENTIFIC

Gain deeper insight into **native proteins**

Native mass spectrometry (MS) is a powerful technique for studying the structure of large protein complexes, protein-protein, and protein-ligand interactions. The approach relies on maintaining a biomolecule's natural folded state and associated non-covalent interactions for MS analysis. Technology limitations have prevented native MS from achieving its full potential—that is until now.

The Thermo Scientific™ Q Exactive™ UHMR Hybrid Quadrupole-Orbitrap™ mass spectrometer is the first to provide the unique combination of unprecedented ultra-high mass range resolution, highest sensitivity, and MS^2 and pseudo-MS^3 capabilities needed to go beyond what's possible with today's native MS experiments.

Confidently resolve small mass differences

The Q Exactive UHMR mass spectrometer resolves the small differences in masses needed to characterize large and heterogeneous intact biomolecular assemblies. The detail obtained is an excellent complement to other biophysical characterization methods such as x-ray crystallography or single-particle electron microscopy.

Conserve precious sample

Samples are often precious and only available in limited amounts. With the sensitivity of the Q Exactive UHMR mass spectrometer, you can make high-confidence measurements, even if you have very little sample to work with.

Rapidly perform native top-down analysis

With the Q Exactive UHMR mass spectrometer, you can perform top-down analysis of protein complexes using a combination of novel in-source trapping, high mass quadrupole ion selection, and efficient HCD fragmentation.

Characterize intact native protein assemblies, including membrane proteins

The Q Exactive UHMR mass spectrometer enables the release of intact membrane proteins from a variety of detergent micelles and membrane mimetics into its ion source. By varying the in-source trapping energy, the instrument can release protein subunits for top-down sequencing or, with gentle activation, retain membrane proteins bound to multiple ligands allowing whole complex analysis.

"In the analysis of large protein assemblies like viruses, ribosomes, and proteasomes, the Q Exactive UHMR mass spectrometer has made things possible that we couldn't do before. The major benefits of the system are the substantial increase in sensitivity and resolution that we get for very large protein assemblies, together with the ability to do MS/MS experiments."

—Professor Albert Heck, Utrecht University

"Thermo Fisher Scientific has made a major contribution to native mass spectrometry in recent years. In the past we couldn't get resolution of very large protein complexes with few charges. This was a real stumbling block for us. We have benefited from recent developments in native high-resolution mass spectrometry. We didn't realize what we weren't seeing before. It gives us a new view of our molecules and this is an exciting transformation. I'm very excited where it will take us in the future."

—Professor Dame Carol Robinson, University of Oxford, Founder and Chief Scientific Consultant, OMass Technologies

Proven Q Exactive Hybrid Quadrupole-Orbitrap technology solves native MS challenges

As analytes get larger and more complex, MS system capabilities need to advance to keep pace. Built on proven Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ mass spectrometer technology, the Q Exactive UHMR mass spectrometer addresses the analytical challenges inherent to native MS. A combination of Orbitrap technology enhancements come together to allow direct detection of large, intact proteins and protein complexes with unprecedented resolution and orders of magnitude more sensitivity.

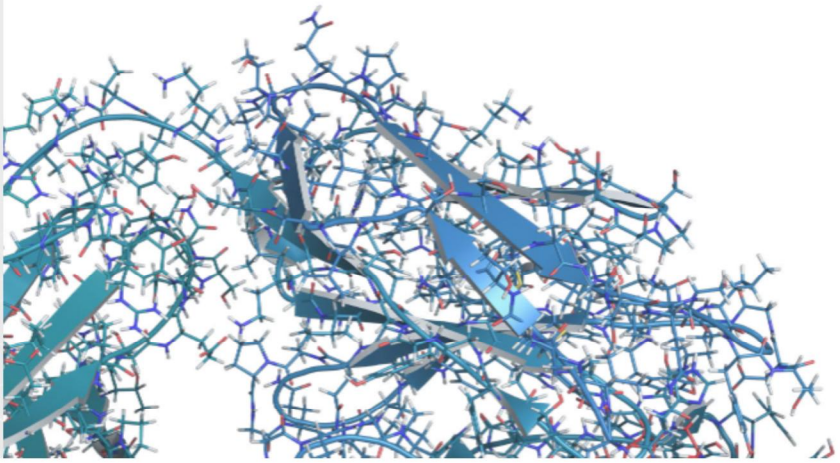
"For researchers analyzing protein complexes that have never or rarely been measured, the Q Exactive UHMR instrument provides orders of magnitude higher sensitivity. The instrument also provides much higher resolving power, which delivers unprecedented detail in resolving antigen interactions and protein-protein binding—detail that is not always seen when using other methods such as crystallography or single-particle electron microscopy. Now native mass spectrometry can become complementary to these tools of structural biology."

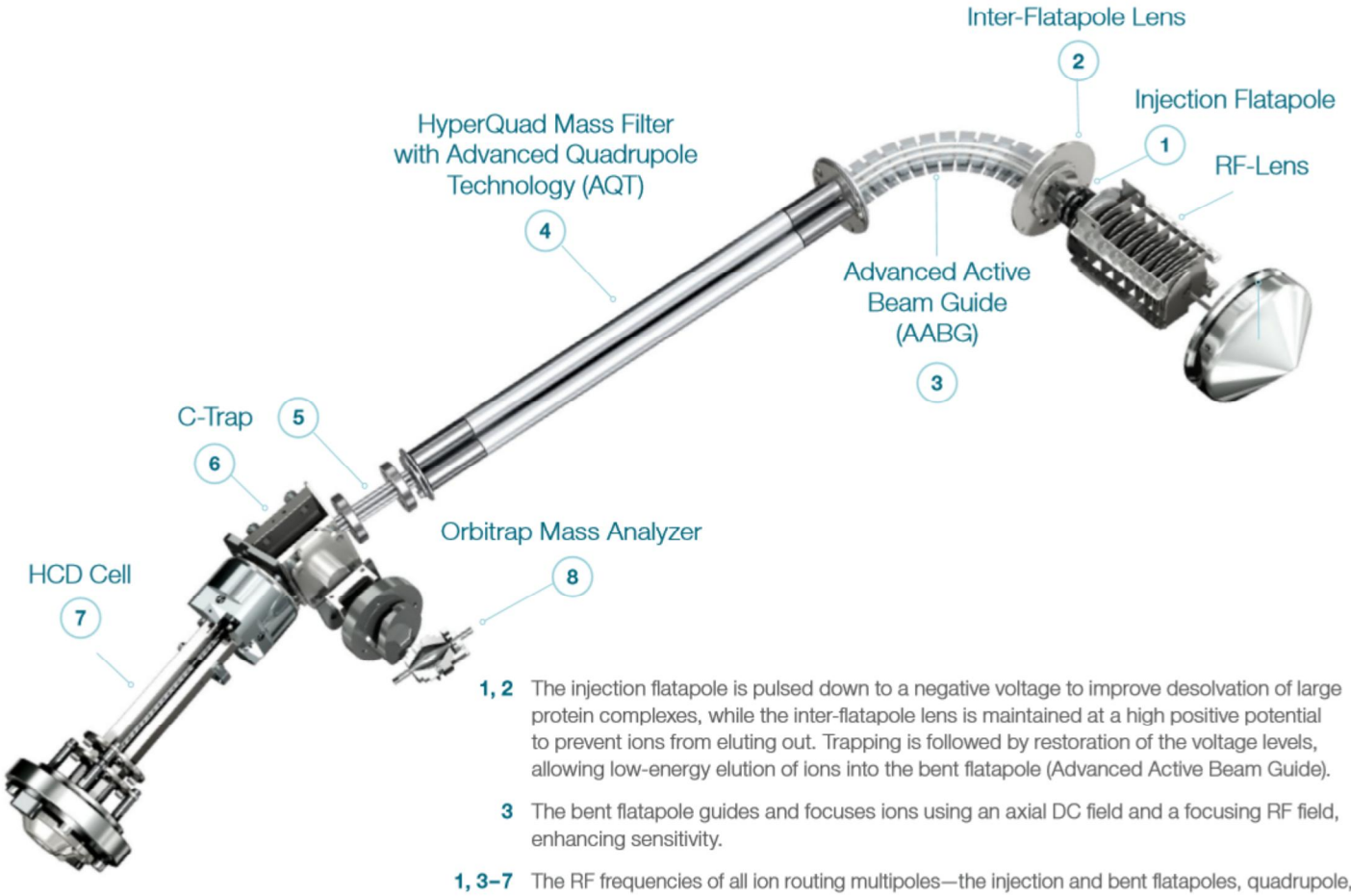
—Alexander Makarov, Director of Research, Life Science Mass Spectrometry, Thermo Fisher Scientific

Orbitrap-technology provides fundamental advantages, now optimized to address native MS challenges

The Q Exactive UHMR mass spectrometer takes full advantage of the intrinsic trapping capability of Orbitrap mass spectrometer technology, where ion source processes can be decoupled from downstream mass analysis. The newly introduced 'in-source trapping' feature allows highly efficient desolvatation of large ions as well as optional application of additional energy to dissociate ions prior to their release for subsequent mass analysis, including pseudo-MS^3 analysis.

Ion transfer and utilization are highly efficient, delivering orders of magnitude higher sensitivity, resulting in much lower sample volume requirements. Multiply charged ions are detected with such sensitivity that individual ions can be detected within milliseconds, providing the ultimate analytical sensitivity needed for minute sample volumes.





1, 2 The injection flatapole is pulsed down to a negative voltage to improve desolvation of large protein complexes, while the inter-flatapole lens is maintained at a high positive potential to prevent ions from eluting out. Trapping is followed by restoration of the voltage levels, allowing low-energy elution of ions into the bent flatapole (Advanced Active Beam Guide).

3 The bent flatapole guides and focuses ions using an axial DC field and a focusing RF field, enhancing sensitivity.

1, 3–7 The RF frequencies of all ion routing multipoles—the injection and bent flatapoles, quadrupole, transport multipole, C-Trap and HCD cell—are reduced to improve ion transmission.

8 High mass ions are efficiently injected into the Orbitrap mass analyzer by adjusting the slew rate of the high-voltage pulse that captures ions in the analyzer.

Challenge: Native MS must retain the structure and optimally the function of a protein or protein complex while it is measured.

Solution: In-source trapping—pulsed trapping of ions in the injection flatapole—gently desolvates non-covalent protein complexes and efficiently removes detergent micelles for the analysis of intact membrane proteins and protein complexes.

Challenge: Top-down sequencing of native protein complexes has been limited by poor fragmentation into subunits.

Solution: Ultra-high mass quadrupole selection up to 25k *m/z* and higher fragmentation efficiency in the injection flatapole and HCD cell region allow native top-down analysis. A protein complex can be fragmented in the front end of the instrument, and the subunits fragmented downstream in HCD cell for high-resolution accurate-mass sequencing.

Challenge: Native MS has suffered from low transmission efficiency at high *m/z*, which has limited obtainable sensitivity and resolution.

Solution: Reduction of the frequency of RF voltages applied to the injection and bent flatapoles, quadrupole, transfer multipole, C-trap and HCD cell, and adjustment of the voltage ramp rate on the central Orbitrap electrode, significantly improve transmission of high *m/z* ions with no known limit.

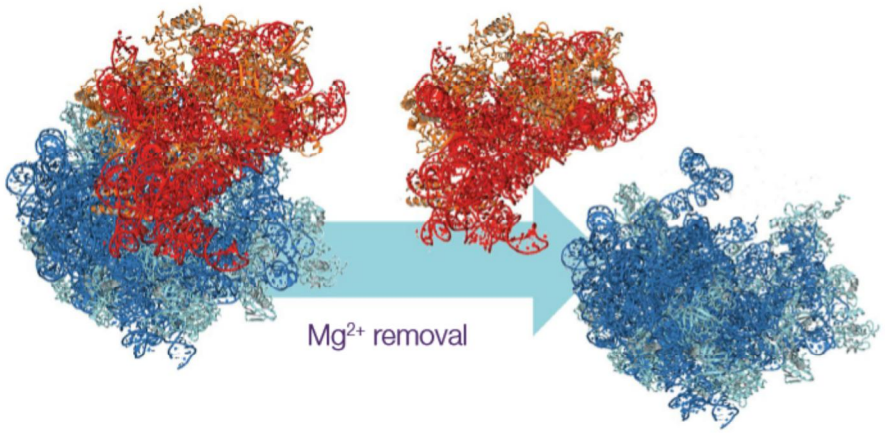
Remarkable sensitivity and mass resolution

at high *m/z* enable investigation of structure and heterogeneity

Use of the Q Exactive UHMR can reveal previously unknown details about biomolecular structures. Investigation of biomolecular structures such as ribosomes are challenging not only because of their size and complexity, but also because of their heterogeneity. For example, some ribosomal proteins may be present substoichiometrically, whereas other ribosome-interacting proteins may be recruited at different stages of translation. Additionally, small modifications have been mapped to both the ribosomal proteins and rRNA fragments. An additional challenge is the use of Mg^{2+} ions to stabilize subunits, which causes peak broadening and signal suppression in mass spectra.

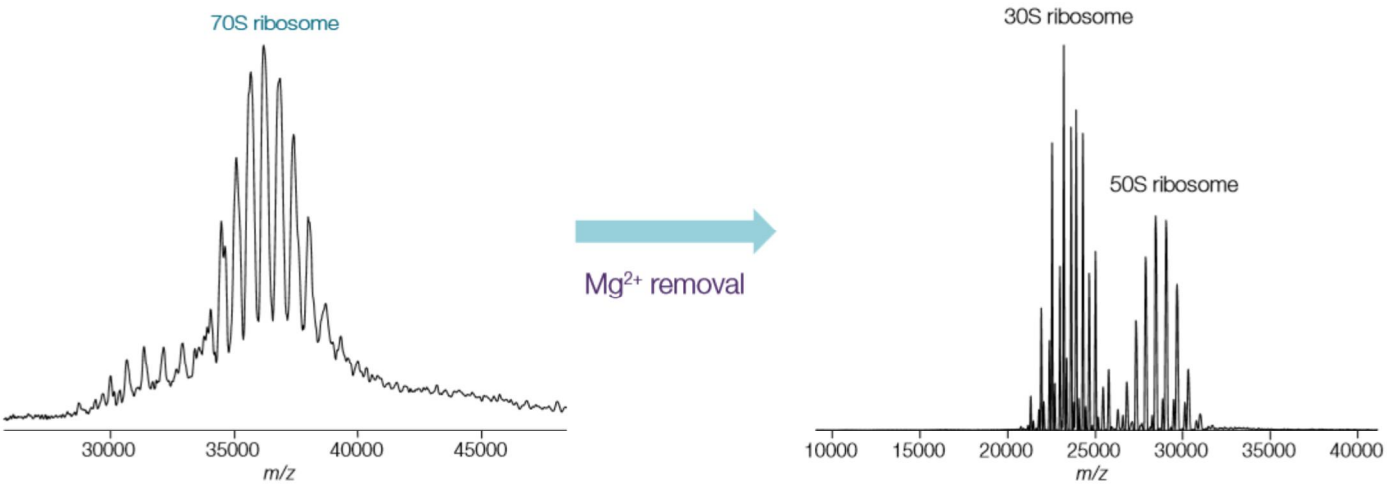
Native MS analysis of 2.3 MDa *E. coli* 70S, 50S, and 30S ribosome particles

The *E. coli* 70S ribosome consists of the 50S and 30S particles, which are generated as the Mg^{2+} concentration is lowered.

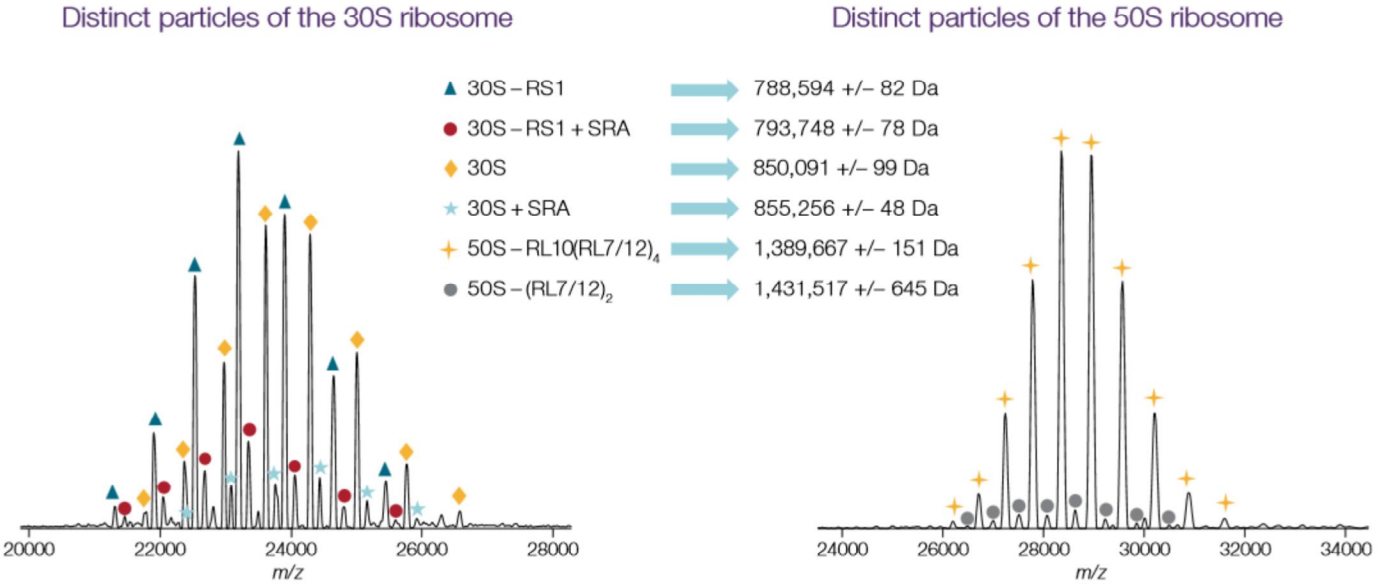


"The Q Exactive UHMR mass spectrometer enables native MS experiments with substantially increased sensitivity and mass resolution at high m/z. This instrument enabled high-fidelity, hypothesis-free mass analysis of E. coli ribosome particles, revealing the substoichiometric association of the elusive small protein SRA."

—Professor Albert Heck, Utrecht University



The sensitivity and resolution of Q Exactive UHMR mass spectrometer at high *m/z* enables analysis of intact *E.coli* 70S ribosome in the presence of 10 mM Mg^{2+} with ions centered around 36,000 *m/z* (left). The well-resolved charge states allow determination of the intact mass of the assembled 70S ribosome of 2,316 ± 1 kDa, which matches well with the expected mass (2,302 kDa). The mass deviation of 0.6% from the theoretical mass is mainly due to nonspecific adduction of the Mg^{2+} ions. With Mg^{2+} removal 70S particles disassemble into 30S and 50S particles (right).



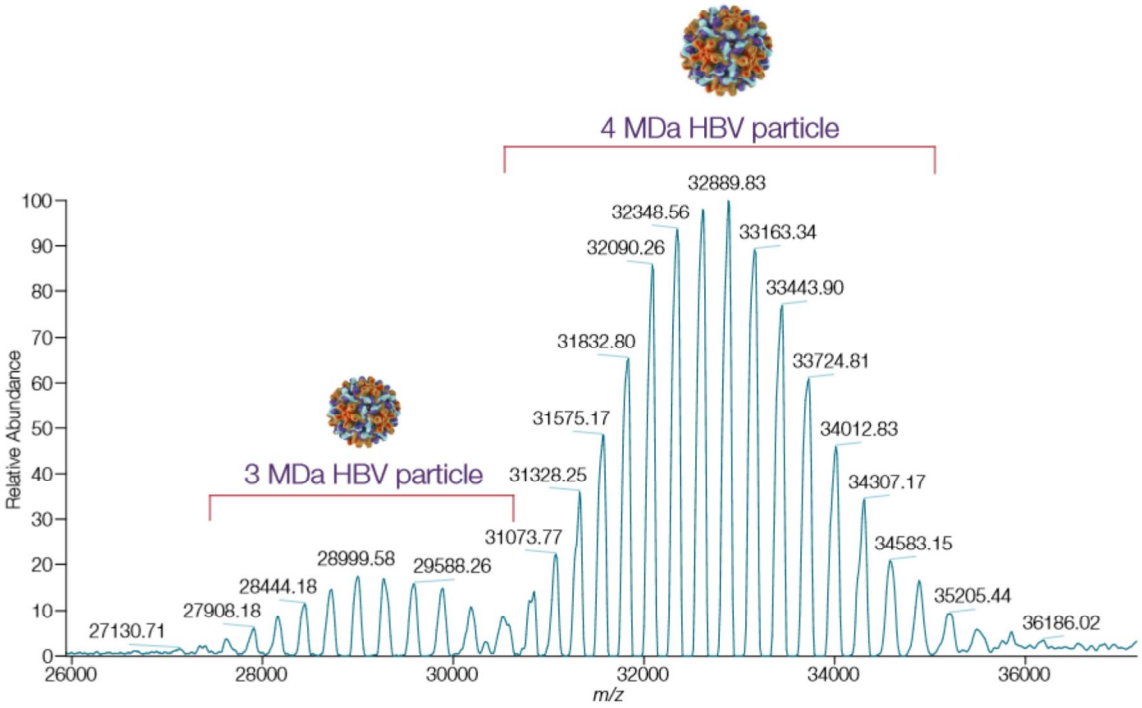
High-resolution MS data produced by the Q Exactive UHMR mass spectrometer reveals the heterogeneity of the ribosomal particles and the substoichiometric association of stationary phase–induced ribosomal-associated protein (SRA).

The two most abundant 30S ribosome assemblies (788.59 ± 0.08 kDa and 850.1 ± 0.10 kDa) have a mass difference of ~61.50 kDa. The mass of 850.09 kDa corresponds with the expected mass for complete 30S particles (847.54 kDa). The mass at 788.59 kDa likely originates from the absence of the RS1 protein (expected mass loss = 61.2 kDa), which is loosely associated to 30S particles and often removed before crystallization. Therefore, it is absent in high-resolution cryo-EM reconstructions. The 5.16-kDa mass difference between the sets of assemblies indicates the presence of SRA. Stoichiometric measurements indicated that ~22% of the 30S particles in the preparation were bound to SRA protein. The majority of 50S ribosome particles had a mass of 1,389.7 ± 0.15 kDa, in agreement with the expected molecular weight of a 50S particle without the stalk complex. A less-abundant form of a 50S particle with a mass of 1,431.5 ± 0.65 kDa was also detected.

Ultra-high mass quadrupole selection and ultra-high mass range **exceed previous limits**

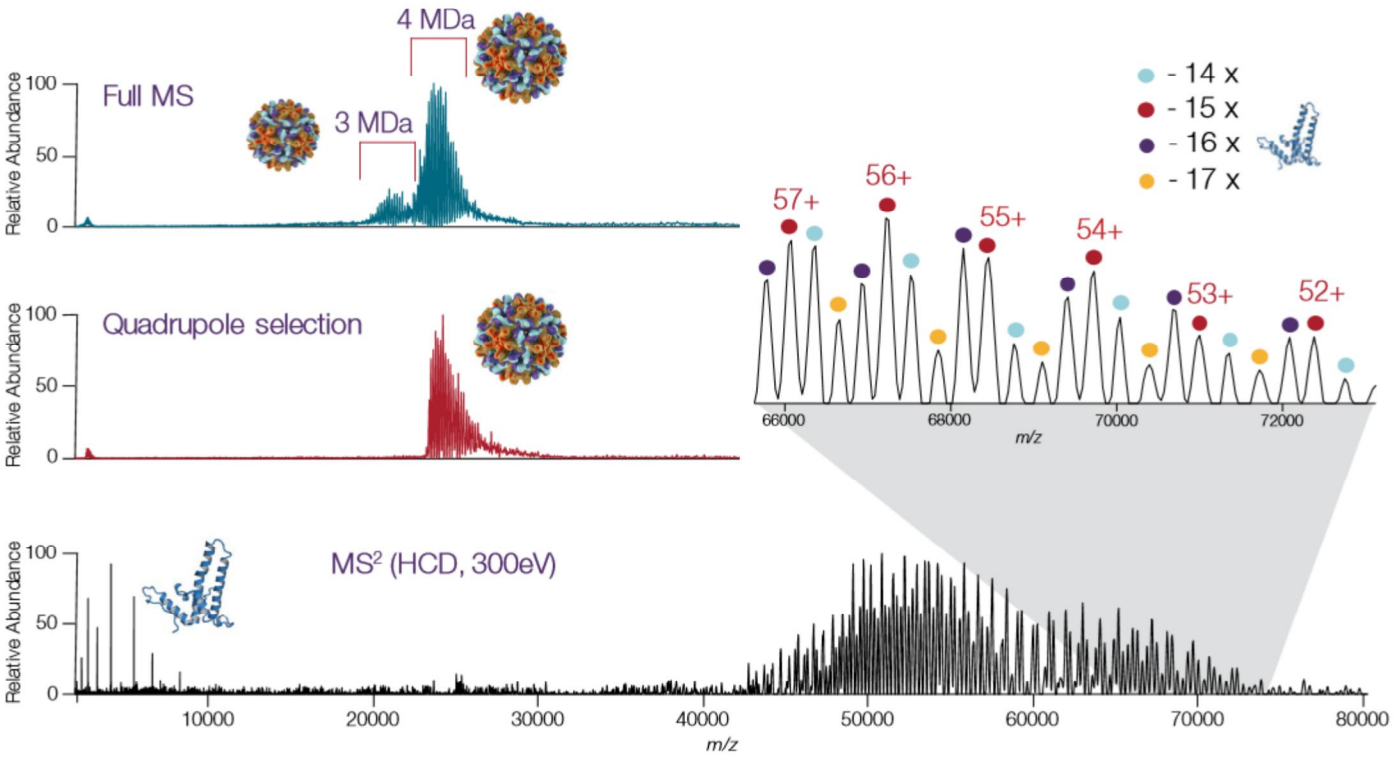
The optimized Q Exactive UHMR instrument efficiently transmits and detects ions up to 80,000 *m/z* with unprecedented sensitivity. The spectra obtained are well resolved, allowing characterization of mixtures of protein particles, providing insights into their composition, ligand binding, and structure that are not easily obtained using other approaches.

Native MS analysis of hepatitis B virus capsids under charge reducing conditions



Mixtures of 3 MDa and 4 MDa hepatitis B virus capsids analyzed under charge reducing conditions span a wide mass range between 27,000 and 36,000 *m/z*. The spectrum contains well-resolved charge states for both particles and shows no bias for the 25% smaller 3 MDa particle.

Native MS/MS analysis of hepatitis B virus capsids

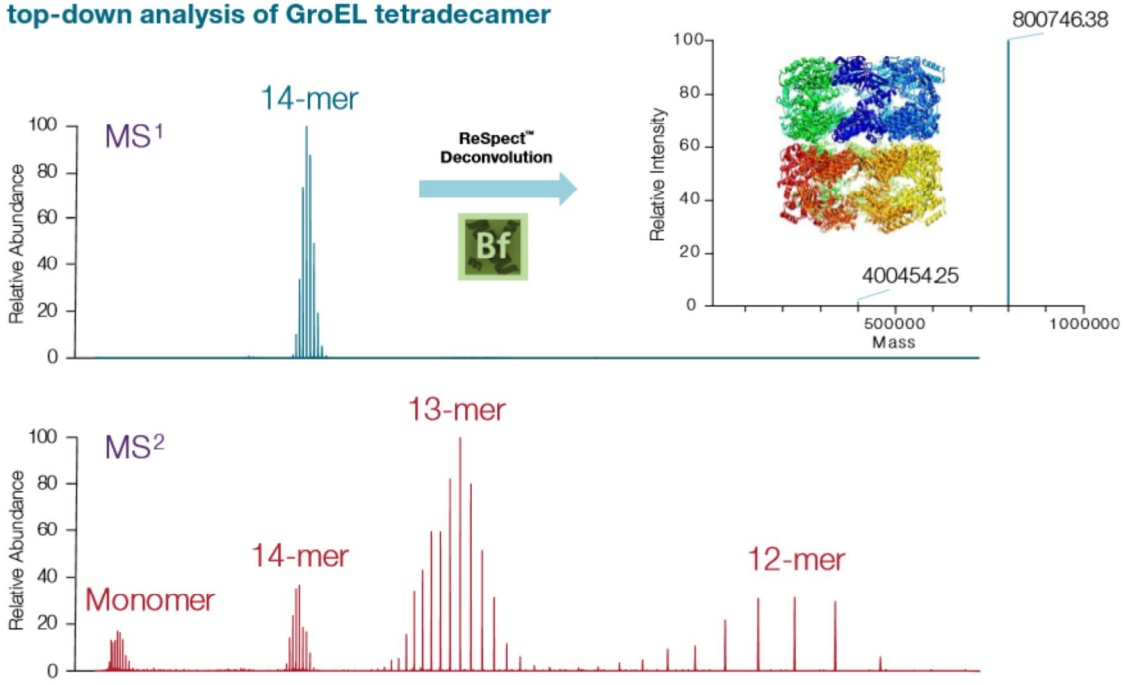


The ultra-high mass selection quadrupole can be used to isolate the 4 MDa HBV particle at *m/z* 25,000 for subsequent fragmentation. The Q Exactive UHMR instrument confidently detects ions with *m/z* values up to 80,000 *m/z*. Here, the tandem MS spectrum of the 4 MDa HBV capsids at 300 eV HCD energy shows sequential ejection of up to 17 out of the 240 copies of the capsid protein. The ejected monomers appear at low *m/z* while the product ions appear at increasingly higher *m/z*. The inset shows an enlargement of the spectrum at 70,000 *m/z*, which contains baseline resolved ions of HBV capsids that have lost between 14 and 17 capsid proteins (6.5% of the original mass) and 68% of the original charge.

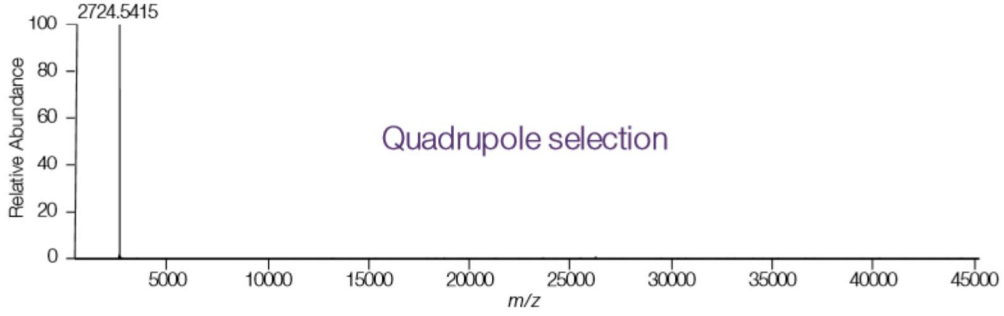
Powerful native MS and native top-down analysis, **in one instrument**

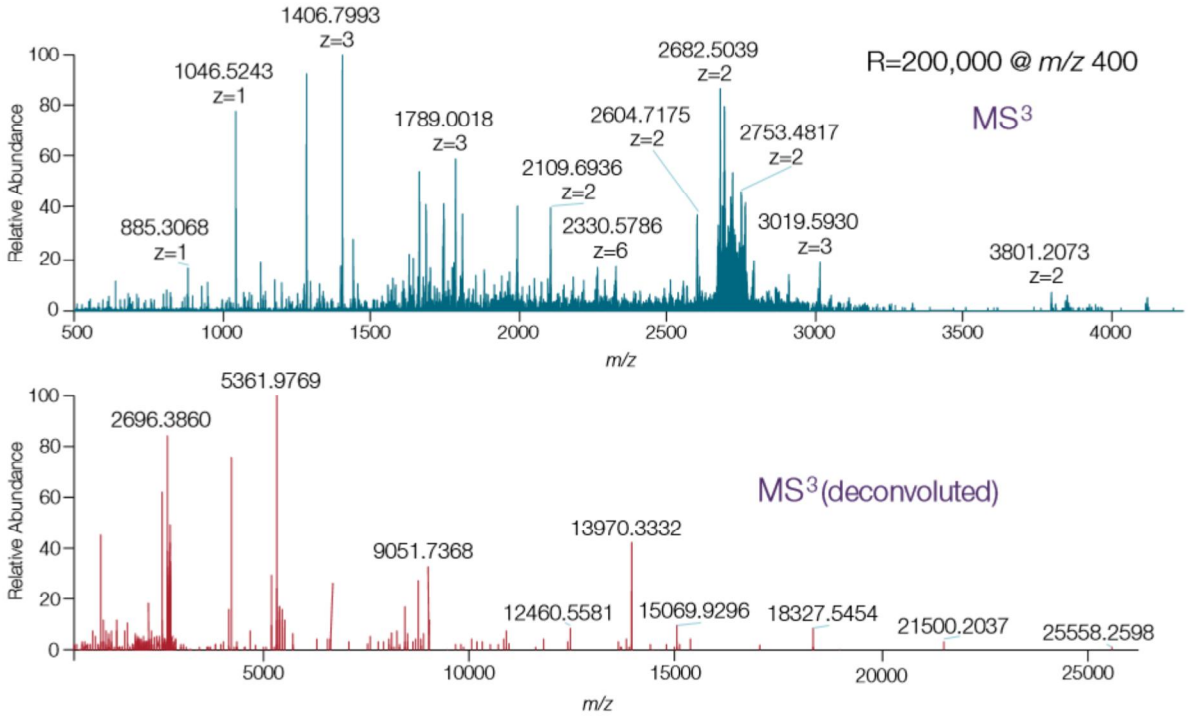
Though top-down sequencing of native protein complexes has been reported, poor fragmentation into subunits and stripped complexes in the front end of the MS have limited previous MS instruments' ability to perform this work. The Q Exactive UHMR mass spectrometer overcomes these limitations via in-source trapping and pseudo-MS^3. First, the intact protein complex is desolvated gently with moderate energy and transferred through the mass spectrometer without fragmentation, producing an MS^1 spectrum. Applying more in-source energy dissociates the protein complex into its subunits, producing an MS^2 spectrum. Quadrupole selection and fragmentation of the subunits in the HCD cell produces MS^3 spectra for sequence analysis.

Native MS and native top-down analysis of GroEL tetradecamer



Native MS1 analysis of the GroEL tetradecamer protein complex, a molecular chaperone required for the proper folding of many proteins in cells. Using in-source trapping, very efficient desolvation and fragmentation of the GroEL 14-mer into monomer and stripped complexes (13-mer and 12-mer) is achieved.





Q Exactive UHMR quadrupole selection of the monomer (shown at the bottom of the previous page), followed by fragmentation in the HCD cell for pseudo-MS³ analysis.

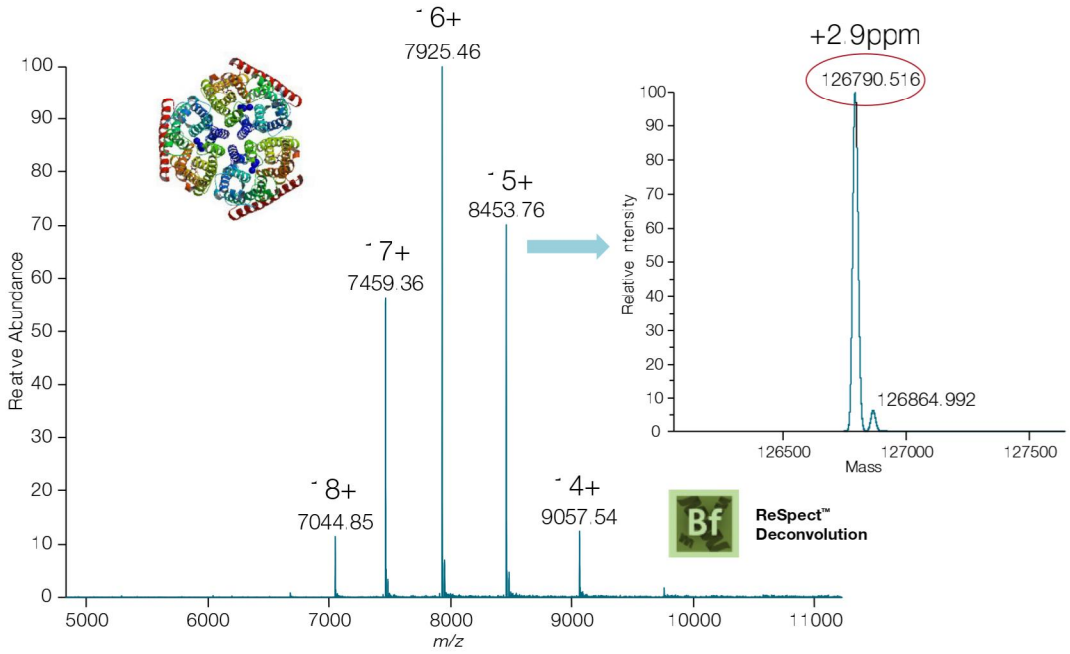
21% residue cleavages

Novel in-source trapping permits native MS and native top-down analysis

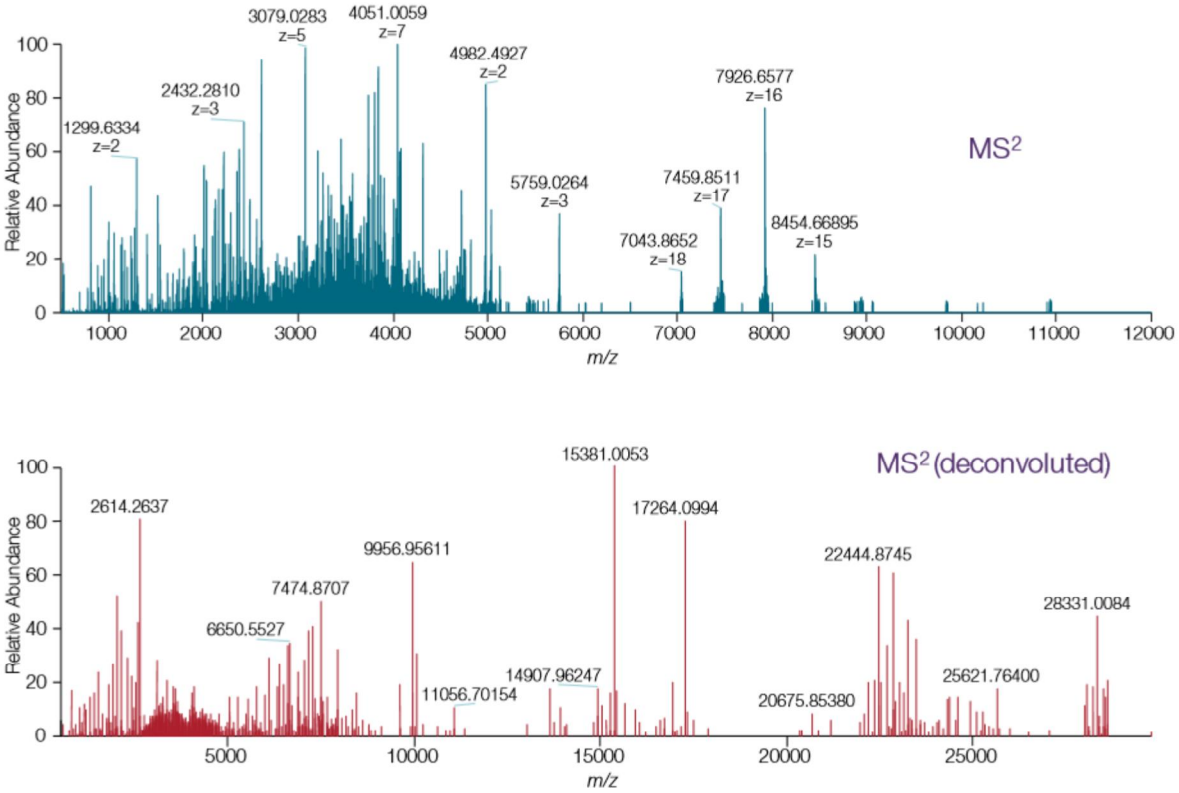
of membrane proteins and membrane protein complexes

Membrane proteins are generally hydrophobic requiring highly heterogeneous micelle assemblies for solubilization. This makes them extremely challenging for many biophysical analytical methods, including native MS. With in-source trapping, the Q Exactive UHMR mass spectrometer can release intact membrane protein assemblies from a variety of detergent micelles and membrane mimetics directly into the ion source. By varying the in-source trapping energy, membrane proteins bound to multiple ligands can be retained or subunits can be released for top-down sequencing.

Native MS and native top-down analysis of the AmtB membrane protein complex

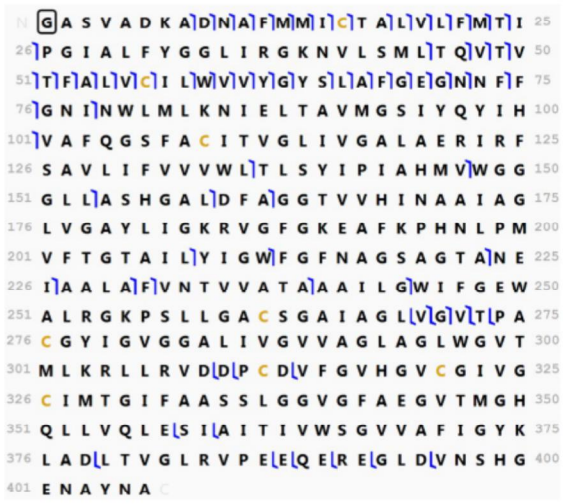


In-source trapping allowed efficient removal of detergent micelles for accurate native MS analysis.



Native top-down analysis of the AmtB membrane protein complex enabled by high mass quadrupole selection and efficient HCD fragmentation.

19% residue cleavages



Scores	
PCS:	711.17
P-Score:	9.30E-63
% Fragments Explained:	24%
% Residue Cleavages:	19%

Matching Fragments (Count 77)						
Name	Ion Type	Ion Number	Theoretical Mass	Observed Mass	Mass Difference (Da)	Mass Difference (ppm)
B230	B	230	24,349.81	24,349.80	-0.009	-0.38
B231	B	231	24,496.88	24,496.83	-0.043	-1.74
B239	B	239	25,252.29	25,252.36	0.062	2.45
B244	B	244	25,677.56	25,677.52	-0.040	-1.54
B270	B	270	28,330.98	28,331.01	0.031	1.10
B271	B	271	28,388.00	28,387.97	-0.026	-0.91

Low-ppm mass accuracy allowed confident identification of AmtB protein. Seventy-five b and y ions were identified, representing 19% residue cleavages.

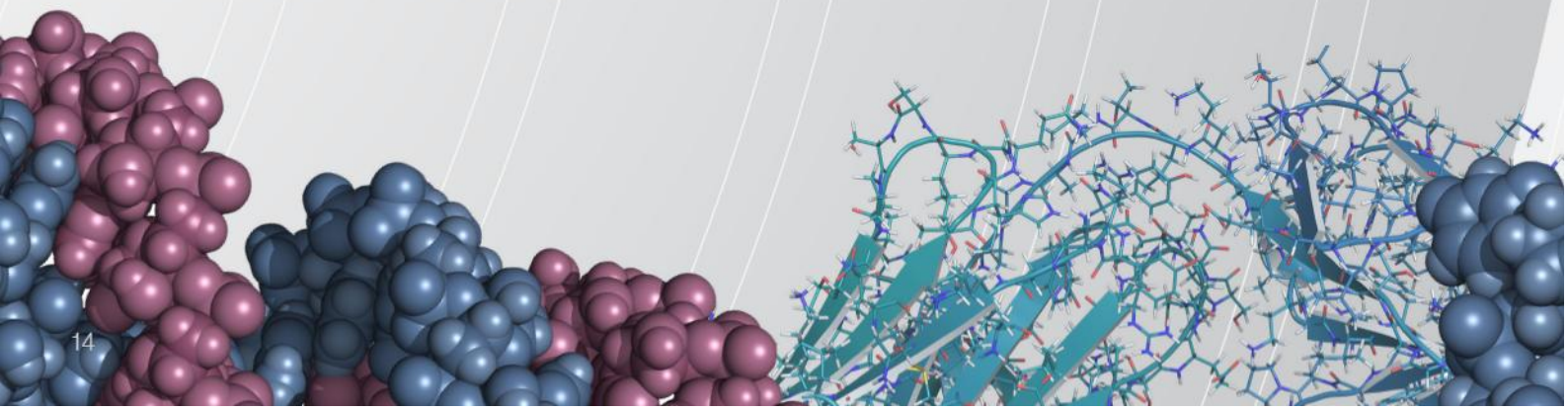
"Membrane proteins represent an extremely challenging class of drug targets for all biophysical techniques but are of essential interest to current and future drug discovery."

—Professor Dame Carol Robinson, University of Oxford, Founder and Chief Scientific Consultant, OMass Technologies

Powerful Thermo Scientific software
empowers biological research

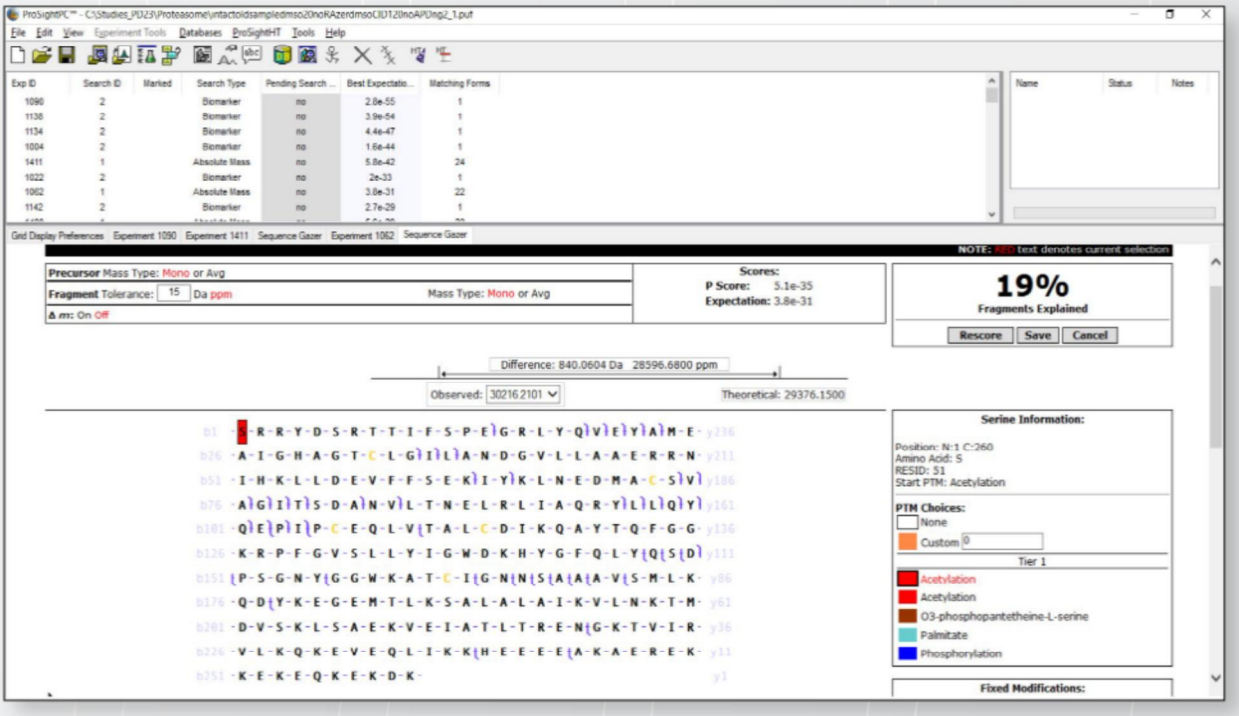
Thermo Scientific BioPharma Finder mass informatics platform—intact and top down analysis

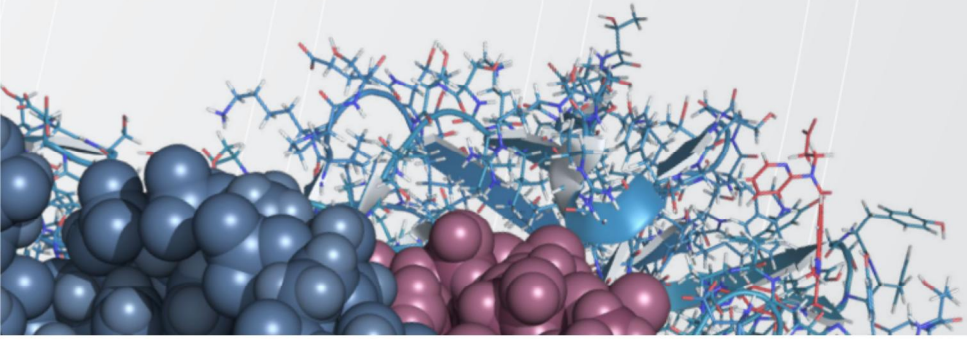
Screen, identify, and characterize intact proteins with higher productivity and confidence using Thermo Scientific™ BioPharma Finder™ software. All BioPharma Finder software workflows take full advantage of the high-quality, HRAM data produced by Thermo Scientific™ Orbitrap™ mass spectrometers. Confirm intact molecular weight of proteins, oligonucleotides and protein complexes. Perform efficient sequence verification, along with identification and localization of modifications by using the automated top down workflow.



Thermo Scientific ProSightPC software

Perform quick analysis of top-down proteomics data with Thermo Scientific™ ProSightPC™ software, the first stand-alone software that also characterizes proteins with known PTMs. ProSightPC software accommodates data generated by multiple fragmentation techniques and processes all accurate-mass MS/MS data. It is the only proteomics software that allows the user to search MS/MS data against proteome warehouses containing the biological complexity present in UniProt.





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Thermo Fisher Cloud

Monitor your system, access your data and system remotely and securely from anywhere in the world. Leverage real-time monitoring of your instrument with automatic email notifications anytime, anywhere. Store data in secure accounts and share with colleagues and collaborators around the world.

Technical support: peak performance for your instruments

Helping you keep your instruments running at peak performance is our goal. Whether you're looking for an instrument manual or spare parts or want to submit a repair request or check on the status of your warranty or service contract, we have every support option you're looking for.

Protecting your investments: unparalleled laboratory services

Unity™ Lab Services provides a single source for integrated lab service, support, and supply management. Our customized service offerings and world-class service experts have the flexibility and experience to effectively address your laboratory's business needs. We provide a complete portfolio of services and support solutions designed to help you improve productivity, reduce total cost of ownership, and ensure performance throughout your laboratory—from instrument and equipment acquisition to disposition.

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

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