# Advances in Orbitrap Instrumentation for Native Top-Down Analysis of Non-Covalent Protein Complexes

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## ABSTRACT

**Purpose:** Evaluate the performance of the new Thermo Scientific™ Q Exactive™ UHMR mass spectrometer for native MS and native top-down analysis of large protein complexes

**Methods:** Native MS and native top-down analysis using a Q Exactive UHMR mass spectrometer

**Results:** Demonstrated excellent performance of the new Q Exactive UHMR mass spectrometer for structural characterization of homomeric and heteromeric protein assemblies

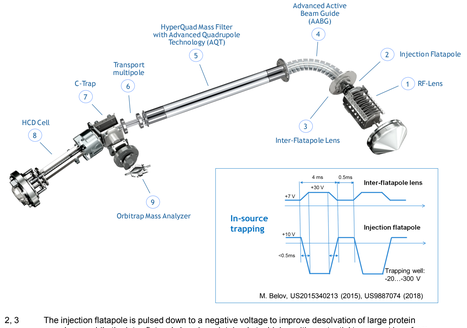
## INTRODUCTION

Native mass spectrometry has emerged as a powerful technique to study protein-ligand interactions and elucidate the structure of macromolecular assemblies, including both soluble and membrane protein complexes. Top-down studies of intact protein complexes have been reported since the early 1990s, but their characterization using $MS^2$ has only recently been reported[1,2] and most work has been done on homomeric assemblies. However, poor fragmentation into subunits and stripped complexes in the front end of the MS limits the use of current MS instrumentation for native top-down analysis using a pseudo-$MS^3$ approach. In this work we examine this limitation and explore new ways for extending native top-down performance to allow interrogation of heteromeric protein assemblies like proteasome by top-down pseudo-$MS^3$.

## MATERIALS AND METHODS

Experiments were performed using a Thermo Scientific™ Q Exactive™ UHMR (Ultra-High Mass Range) $MS^{3-5}$ (Figure 1) in which the ability to perform pseudo-$MS^3$ scans for native top-down analysis and transmission of high *m/z* ions were improved by implementing several hardware and software modifications. The most important of these are pulsed trapping of ions in the injection flatapole region ('in-source trapping') and reduction of the frequency of RF voltages applied to injection and bent flatapoles, quadrupole, transfer multipole, C-trap, and HCD cell. Other modifications include gas pressure control, adjustment of the voltage ramp rate of the Orbitrap™ mass analyzer, and the increase of the maximum HCD energy from 200 to 300 V. GroEL, rabbit 20S proteasome, LmrP membrane protein, and AmtB membrane protein complex were used as model systems.

**Figure 1. Schematic of the Q Exactive UHMR Hybrid Quadrupole-Orbitrap MS system**




2, 3 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).

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

2, 4–8 The RF frequencies of all ion routing multipoles—the injection and bent flatapoles, quadrupole, transport multipole, and HCD cell—are reduced to improve ion transmission.

9 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.

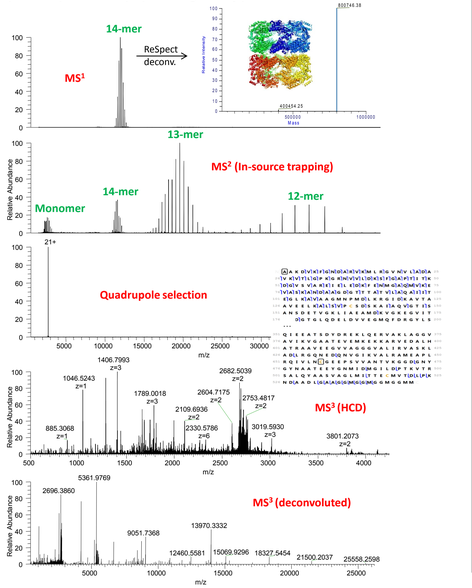
## RESULTS

Advances towards native MS and native top-down analysis of heteromeric protein complexes became possible only after addressing several major technical challenges. First, implementation of "in-source trapping" capability addressed the insufficient or poorly controllable desolvation issue and allowed significantly improved fragmentation into subunits and stripped complexes in the inject flatapole region. Second, the reduction of RF frequencies on all RF guides and the mass filter was aimed at increasing the transmission of high *m/z* ions. Third, the adjustment of the voltage ramp rate on the central Orbitrap electrode facilitated successfully transmitting the high *m/z* ions from the C-trap into the Orbitrap analyzer.

The improvements in performance afforded by these modifications were demonstrated in a series of pseudo-$MS^3$ experiments performed for several non-covalent protein complexes. During such analysis the intact protein complex is initially transferred through the mass spectrometer without fragmentation using only moderate desolvation energy, yielding the $MS^1$ spectrum. Dissociation of the protein complex into its composing subunits in the inject flatapole region gives the $MS^2$ spectrum, and it is followed by quadrupole selection and fragmentation of individual subunits in the HCD cell enabling sequence analysis through the $MS^3$ spectrum.

Using "in-source trapping", very efficient desolvation and fragmentation of the GroEL 14-mer complex into monomer and stripped complexes (13-mer and 12-mer) has been observed (Figure 2). This was followed by quadrupole selection of the monomer and its fragmentation in the HCD cell that resulted in the identification of a total number of 112 b and y ions, which represents 21% residue cleavages.

**Figure 2. Native MS and native top-down analysis of the GroEL protein complex, a molecular chaperone required for the proper folding of many proteins in cells**




Figures 3–5 show the results obtained from the native MS and native top-down analysis of the rabbit 20S proteasome. The 20S proteasome complex consists of four stacked rings composed of alpha/beta/beta/alpha subunits. There are seven different alpha non-catalytic subunits and seven different beta subunits from which three have catalytic activity. The measured molecular mass of the 28 subunits complex obtained after ReSpect™ deconvolution was 717,023.187 Da, which is consistent with the expected theoretical mass (deviation from expected mass < 0.082%). Native MS/MS analysis of the intact 20S proteasome complex shows dissociation of six out of seven alpha subunits. Native top-down pseudo-$MS^3$ experiments enabled unambiguous identification of alpha-6, alpha-2, and alpha-5 subunits. Three more alpha subunits (alpha-7, alpha-4, and alpha-3 (phosphorylated and unphosphorylated forms) were identified based on the accurate monoisotopic masses obtained from the high-resolution native MS/MS analysis.

**Figure 3. Native MS and MS/MS (HCD) analysis of the rabbit 20S proteasome complex**

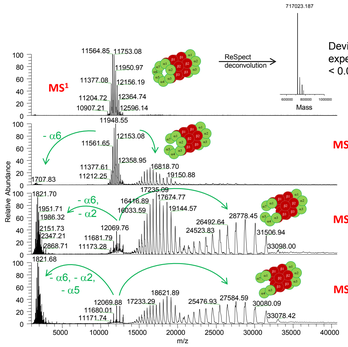



**Figure 4. Native MS/MS (in-source-trapping) analysis of the rabbit 20S proteasome complex**

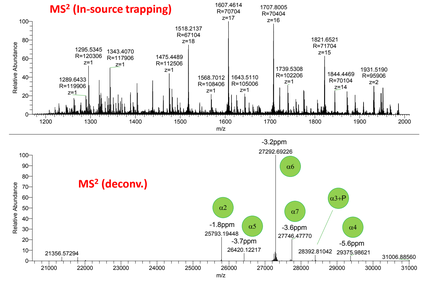
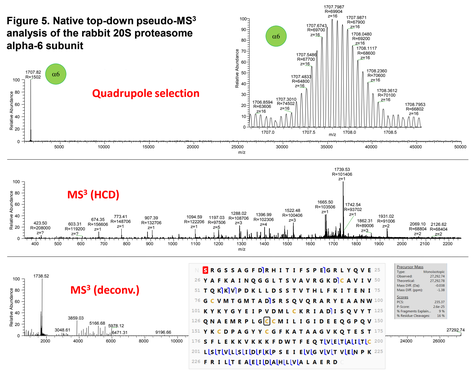
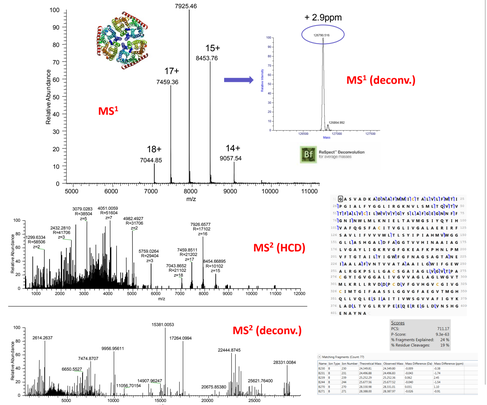



**Figure 5. Native top-down pseudo-$MS^3$ analysis of the rabbit 20S proteasome alpha-6 subunit**



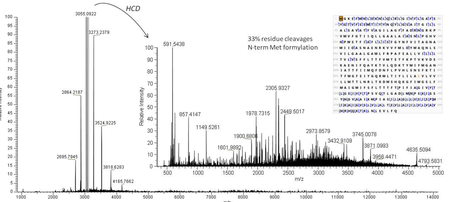

Membrane protein complexes are generally hydrophobic, requiring highly heterogeneous micelle assemblies for solubilization. This makes them extremely challenging for many biophysical analytical methods, including native MS. With in-source trapping, the Q Exactive UHMR mass spectrometer can release intact membrane protein assemblies from a variety of detergent micelles and membrane mimetics directly into the ion source. In the example shown in Figure 6, in-source trapping allowed efficient removal of detergent micelles for accurate intact mass determination of the AmtB trimer. Native top-down analysis of this membrane protein complex resulted in very confident identification of AmtB protein with 75 b and y matched ions representing 19% residue cleavages.

**Figure 6. Native MS and native top-down analysis of the AmtB membrane protein complex**




Furthermore, "in-source trapping" allowed very efficient removal of detergent micelles and top-down analysis of the multidrug transporter membrane protein LmrP from *L. lactis*, which resulted in the identification of a total number of 104 b and y ions, which represent 25% residue cleavages (Figure 7).

**Figure 7. Native MS and native top-down analysis of the multidrug transporter membrane protein LmrP from *L. lactis***




## CONCLUSIONS

In this work we demonstrated outstanding performance of the new Q Exactive UHMR mass spectrometer for structural characterization of homomeric and heteromeric protein assemblies using native MS and native top-down analysis .

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## TRADEMARKS/LICENSING



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