Electronic Supplementary Material (ESI) for Analyst.

Supplemental Material

Expanding the structural analysis capabilities on an Orbitrap-based mass spectrometer for large macromolecular complexes

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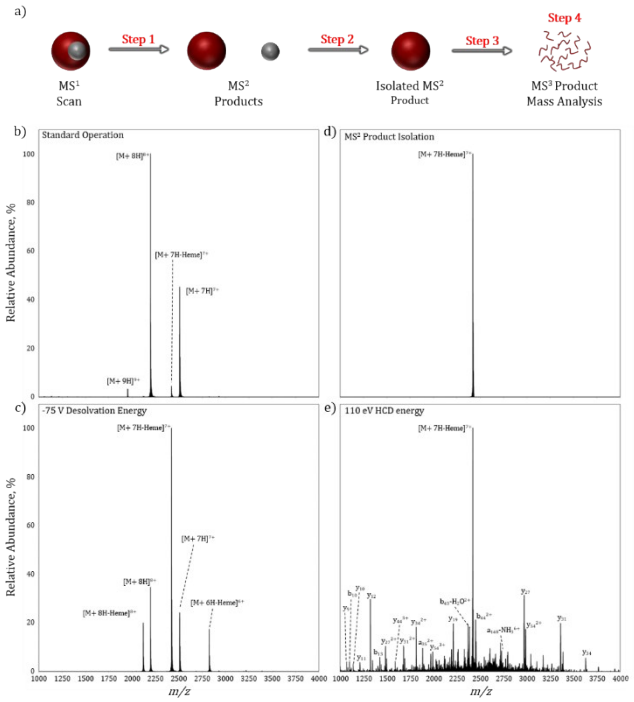
MS^3 Capabilities on Small and Large Protein Assemblies

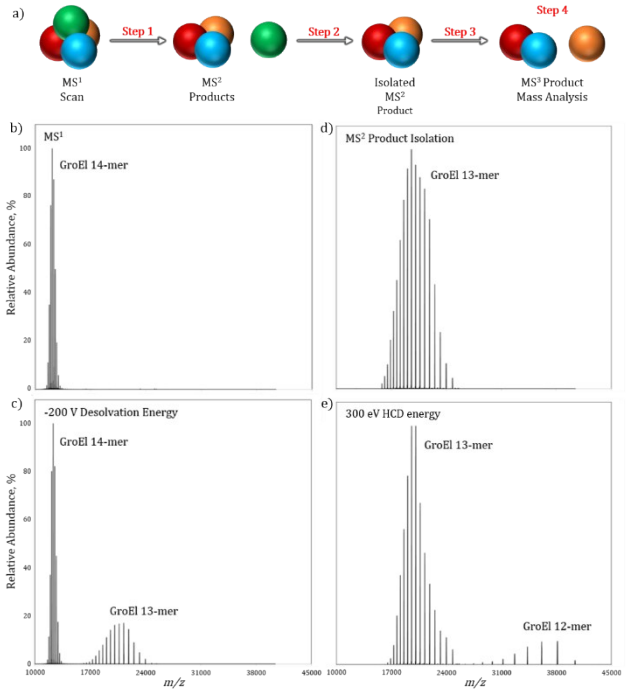
For the analysis of protein complexes, the ability to systematically investigate the higher-order structure of the macromolecular complex can be beneficial. With traditional CID-based fragmentation, MS^2 activation of protein complexes, on conventionally used Q-ToF platforms, often leads to the ejection of a single subunit and provides little other information as additional fragmentation steps are unavailable. On the platform described here, MS^3 analysis is enabled through increasing the desolvation voltage to a point where protein complex dissociation or covalent bond fragmentation occurs. The MS^3 workflow on a simple protein-ligand noncovalent model system, *e.g.,* myoglobin (Supplemental Figure 1a), illustrates the added capability of the modification. The full MS scan of myoglobin (Supplemental Figure 1b) shows three charge states for intact myoglobin with the heme group, the holo- form of myoglobin. With a desolvation voltage of -75 V, the mass spectra change substantially. The spectrum contains ion signals for both the holo- and apo- form, the form where the heme group has been ejected from the protein; however, the intensity for apo- has increased to the most abundant species present (Supplemental Figure 1c). Isolation of the apo- 7+ charge state (Supplemental Figure 1d) confirms that this ion is formed in the source due to the desolvation voltage. Finally, this isolated product can be fragmented in the HCD cell (Supplemental Figure 1e) and the mass spectrum shows a range of covalent bond dissociation products from the protein backbone, attaining sequence information leading to protein identification.

For the much larger GroEl, the utility of this MS^3 capability combined with high *m/z* transmission efficiency for the analysis of protein complexes are also demonstrated (Supplemental Figure 2a). The full MS spectrum shows a range of charge states corresponding to the intact 14-mer (Supplemental Figure 2b). Application of maximum desolvation voltage causes ejection of a single GroEl subunit and a charge state envelope for the 13-mer (Supplemental Figure 2c). Owing to the *m/z* isolation capabilities of the quadrupole, this envelope can be isolated (Supplemental Figure 2d) and then further activated in the HCD-cell to form the 12-mer (Supplemental Figure 2e). Collectively,

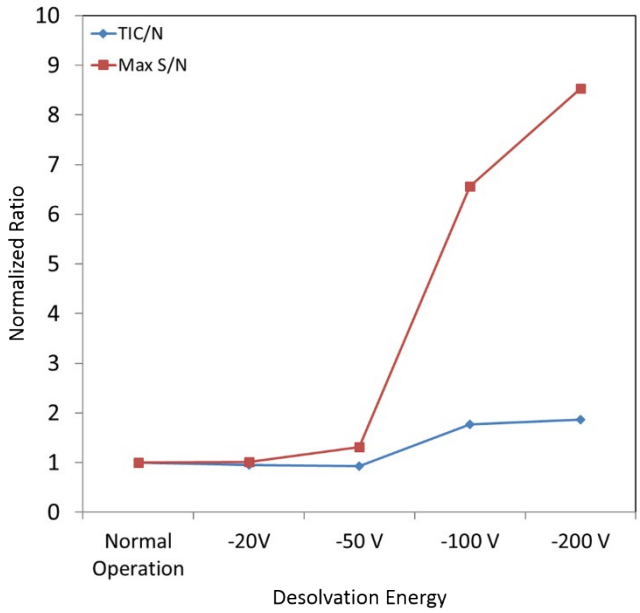
these data on GroEl and myoglobin demonstrate the ability to disrupt a protein complex and either subject a monomer protein or a (n-1)-mer protein complex to further dissociation, providing sequence information on the monomer protein or topographical information on the complex. From these proof of principle experiments, it can be reasoned that an uncharacterized protein complex can be analyzed by first dissociating the complex into individual subunits, followed by sequencing and identifying these subunits individually, allowing for full protein complex characterization.



Supplemental Figure 1: A depiction of the MS^3 workflow of the modified instrument for myoglobin (a), where each step of MS^3 is shown in red. The full MS scan (b) shows a charge state envelope for the holo- form of myoglobin and a small signal for the 7+ charge state of apo-myoglobin. Application of -75 V desolvation voltage (c, step 1) gives rise to the disruption of the native fold of myoglobin and the formation of apo-myoglobin. Isolation of the 7+ charge state (d, step 2) of apo-myoglobin demonstrates the formation of this ion in the source region of the mass spectrometer. MS^3 of this ion (e, step 3 and 4) shows the resulting covalent bond cleavage products.



Supplemental Figure 2: A depiction of the MS^3 workflow of the modified instrument for the GroEl protein complex (a), where each step of MS^3 is shown in red. The full MS scan (b) shows a charge state envelope for the 14-mer GroEl complex. Application of -200 V desolvation voltage (c, step 1) gives rise to the ejection of a single subunit from the native complex and the formation of the 13-mer. Isolation of the 13-mer charge state envelope (d, step 2) demonstrates the formation of this ion in the source region of the mass spectrometer. MS^3 of this distribution (e, step 3 and 4) shows the further ejection of subunits and the production of GroEl 12-mer.



Supplemental Figure 3: The normalized ratio (with respect to the values of Normal Operation) of total ion current to noise (TIC/N) and base peak signal to noise (Max S/N) for the antibody Herceptin with various desolvation voltages. With the application of increasingly high desolvation voltages, the ion transmission increases slightly; however, significant gains are realized in the base peak S/N.