**Characterization of Large Intact Protein Complexes by Native Mass Spectrometry**

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**Abstract** Conventional top-down proteomics analysis allows the entire sequence of intact protein chains to be observed, providing information on integrity of the whole sequence and on co-occupancies of PTMs. However, in the traditional top-down method, intact proteins are routinely measured under denaturing conditions, destroying noncovalent protein assemblies and substrate bound complexes. Fully active protein assemblies can be studied under native conditions, providing rich information on stoichiometry of complexes and on binding strength of components and/or substrates. Native mass spectrometry (ionization of protein complexes in their native states) experiments are challenging due to the limited surface area of protein complexes for protonation at physiological pH. Ions of large protein complexes have high m/z values and can only be detected by specifically designed FTICR, TOF, or Orbitrap instruments. Using the native MS method, we have analyzed several classes of protein complexes, including a membrane embedded complex and a DNA/protein complex. Association constants for an inhibitor binding to a large protein assembly can be obtained by directly measuring the relative abundances of substrate/protein complex assemblies. Charge state deconvolution software was developed to analyze low signal-to-noise ratio and overlapping native MS spectra.

**Key words:** protein complexes, native mass spectrometry, relative abundance