New structural insights from high-efficiency ion mobility and tandem mass spectrometry

The Waters® Synapt™ High Definition Mass Spectrometry™ (HDMS™) System is a powerful tool for the analysis of biomolecules and macromolecular complexes, allowing them to be differentiated by size, shape and charge, as well as mass.

Electrospray mass spectrometry (ESI-MS) is widely used for the analysis of macromolecular structures such as proteins, antibodies and non-covalently assembled supramolecular protein complexes. Precise mass measurement (using high-resolution mass spectrometry) can reveal the presence of amino acid substitutions—post-translational modifications, protein-subunit and protein-ligand stoichiometry—providing valuable insight into the molecular mechanisms involved in biological pathways and disease processes.

Tandem mass spectrometry (MS/MS) can also provide evidence of noncovalent interactions and binding affinities, but, like many other biophysical techniques, even MS/MS cannot be used to directly probe molecular conformation. The ability to study macromolecular conformation is not only important in advancing functional studies, but also in the development and manufacture of biopharmaceuticals.

The role of IMS
Ion-mobility spectrometry (IMS) is an increasingly popular technique that allows separation of molecules based on a combination of their size, shape and charge. Recently, an IMS approach was used to demonstrate that gas-phase structures of intact multiprotein complexes are essentially equivalent to native solution structures and highlight the potential of ion mobility separation for defining the shapes of heterogeneous macromolecular assemblies1.

Synapt™ High Definition MS system
Here we introduce a new instrument, Waters Synapt™ High Definition Mass Spectrometry (HDMS) System (Fig. 1), which combines high-efficiency ion-mobility measurements and separations with a high-performance orthogonal acceleration time-of-flight (oa-TOF) tandem mass spectrometer2. We demonstrate how the system allows molecules to be separated on the basis of their size, shape and charge (mobility), as well as mass-to-charge (m/z), to provide increased specificity and sample definition beyond conventional mass spectrometry. We also highlight its application to the analysis of macromolecular conformation, for which it offers unique structural insights not possible with any other structural probe.

Probing molecular conformation
In its simplest form of operation, the Synapt HDMS system separates...
ions by their mobility (IMS) in the Triwave™, followed by mass measurement (MS) in the oa-TOF mass analyzer. The conventional multiply charged mass spectrum for native lysozyme reveals that the enzyme is present in several different charge states (Fig. 2a). Closer investigation of the data from the IMS-MS analysis of lysozyme reveals the presence of two distinct conformations (compact and open) for the [M+8H]8+ ion, which were not visible in the initial MS analysis (Fig. 2b). This is in agreement with results of previously reported IMS studies of lysozyme. A powerful feature of the Driftscope software is the ability to reconstruct mass spectra or mobility-based mass chromatograms from selected regions of the IMS-MS plot. Figure 2c shows drift time data for the highlighted region in Figure 2b, confirming the separation of two [M+8H]8+ conformers of lysozyme, and illustrates the ability of the Synapt HDMS System to separate macromolecules of the same m/z based upon subtle differences in conformation. It is possible to correlate such measurements with the protein’s cross-sectional area.

‘Top-down’ sequencing
Further investigations have demonstrated the ability of the Synapt HDMS system to enhance the information content for protein sequencing experiments (Pringle, S. et al. 54th ASMS Conference on Mass Spectrometry. Poster MP22:478; 2006). In this study, the [M+13H]13+ ion of intact human ubiquitin was isolated (quadrupole) and fragmented (T-Wave TRAP) with the resulting protein fragments analyzed by IMS-MS (Fig. 2d). The predominant y588+ fragment (peptide) ion observed was then isolated and subjected to fragmentation analysis in a second experiment, generating a data set of IMS-separated fragment (sequence) ions (Fig. 2d). From the resulting IMS/MS data, individual MS spectra (Fig. 2e) were reconstructed by selecting groups of ions from discrete regions of the plot. In some cases, ion mobility appears to separate specific fragment ion types; for example, y7-type and b-type ions, for a given charge state. Using this approach, it was possible to directly sequence the majority of the intact protein.

Summary
We have described a new analytical system incorporating Triwave technology, which uses highly efficient and reproducible ion mobility-based measurements and separations to greatly enhance sample definition over traditional tandem mass spectrometry approaches.

The IMS separations considerably reduce spectral congestion associated with the mass spectra, making it possible to define shapes (cross-sectional area) of heterogeneous macromolecular assemblies, and to observe and identify many fragment ions that would not be observed with MS/MS alone. The additional dimension of separation obtained in the Synapt HDMS system maximizes the amount of information that can be extracted from a sample and defines a new category of mass spectrometry.


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