

OF9 Probing the Structure and Assembly of Large Protein-Protein Complexes by Ion Mobility-Mass Spectrometry

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IM-MS identifies protein subunit interactions and defines protein complex architecture.

The study of protein interaction networks and stable protein assemblies is becoming increasingly important. However, the structural diversity and often transient nature of macromolecular complexes means their topology can be difficult to characterise using traditional methods. Here we present an IM-MS approach capable of defining subunit interactions and overall structural architecture for protein complexes.

IM-MS separates ions based on mass-to-charge ratio and cross-section, yielding an added dimension of information over traditional MS. Our approach involves IM-MS measurements of a protein assembly, both intact and in various disrupted states. Intact measurements establish identity, stoichiometry and overall size, with charge-dependant size information often providing critical clues to the topology. Partial disruption by manipulation of solvent conditions then gives rise to an ensemble of sub-complexes for analysis, which is of critical importance for defining the structure and connectivity of the intact assembly.

As an example, we present results for two model homo-dodecameric complexes, namely ornithine carbamoyl transferase (PDB 1pvv) and glutamine synthetase (PDB 1hto). Despite having the same stoichiometry, we show these assemblies to be formed by distinct sub-complexes related to the comparative strength of subunit interfaces. For all intact and sub-complexes, extensive structural modelling was employed to describe topological arrangements consistent with IM-MS measurements, and protein-protein disassembly pathways were extracted. Our data is most consistent with a process which retains the native interactions between subunits within the sub-complexes, and where gross rearrangement is not occurring in either solution or gas phases.