

Dr Brandon Ruotolo, Cambridge, UK

Determining Protein Quaternary Structures using Constraints from Gas-phase Measurements

Structural biology is ultimately concerned with determining high-resolution structures of all the functional macromolecules within living cells and tissues. While high-detail structural information can be obtained by X-ray diffraction analysis, this experiment requires the availability of a sufficient quantity of homogenous material and definition of suitable crystallization parameters. Both conditions are often difficult to meet and the number of structures of multi-subunit complexes deposited in structural databases remains relatively low.

Alternative methodologies such as electron microscopy (EM) and small angle X-ray scattering (SAXS) allow the determination of the surface envelope of complexes of sufficient dimensions but interpretation of these data is aided by detailed knowledge of complex composition, and is limited to homogeneous complexes. Consequently there is a need to apply new approaches that define the subunit stoichiometry, composition, and shape of heterogeneous macromolecular complexes of biological importance.

Over the past several years, we have been developing ion mobility-mass spectrometry (IM-MS) methods for the analysis of large protein assemblies. IM separates ions based on their ability to traverse a chamber filled with inert neutral molecules under the influence of a weak electric field. Ions that are large undergo a greater number of collisions with neutral molecules and thus take more time to elute from the chamber than smaller, more compact molecules. Ion size is, therefore, the primary information content of IM separation and computational approaches can be used in conjunction with this information and MS data to assign the overall topology and structure to the assembly. This presentation will focus a number of heteromeric and homomeric protein complexes currently under investigation to illustrate this approach. A focal point of these examples will be recent data on the DNA replisome, where we have identified assembly intermediates in the formation of the complex, refined the position of many interacting sub-complexes, and identified other new structural features of this multi-protein system which were previously unknown.