3D Repertoire – Complex Validation by Mass Spectrometry

Objectives

Mass spectrometry of non-covalent complexes as a first validation approach can be used in this project to: (1) check the integrity and homogeneity; (2) establish the stoichiometry of the purified complexes; (3) detect sub-stoichiometric binding of components, and (4) decipher interaction network of the complexes on the basis of the combined information from sub-complexes generated through controlled gas-phase and solution phase dissociation.

The use of native mass spectrometry for studying macromolecular complexes is advantageous compared with conventional methods in several aspects: It can tolerate heterogeneous systems such as RNA and protein mixtures; Dynamic complexes in which some components are only interacting transiently can be readily detected; The speed of the analysis is of the order of a few seconds; and it can provide valuable information about interacting partners, which can lead to the elucidation of structural organisation of the whole complexes.

Previous Experience

We have developed and optimised our protocol for analysing TAP purified complexes by nanospray mass spectrometry. Using this approach we have established subunit stoichiometry and identified sub-stoichiometric binding of components for two yeast protein assemblies (scavenger decapping and nuclear cap-binding complexes). We have also identified internal subunit interaction patterns for yeast NatC complex. More importantly, we have successfully deduced a complete subunit interaction map for the yeast exosome, a nuclease with ten different subunits, through controlled solution and gas phase dissociation of the complex followed by mass spectrometry of the intact sub-complexes. The success of our approach with the yeast exosome has demonstrated its utility as a structural probe for large, heterogeneous complexes.

Methods and Facilities

We have several modified quadrupole time-of-flight mass spectrometers in our lab for the analysis of non-covalent complexes. The Q-TOF instruments incorporate two major modifications: (1) They incorporate additional collisional cooling by introducing collision gas at various stages of the flight path of the ions to reduce their translational energy and hence the internal energy. (2) The m/z range of the quadrupole is extended to 32,000 for the transmission and isolation of high mass

complexes, as compared to the cut-off of 4,000 of a standard instrument. With nanoflow electrospray ionisation and our modified Q-TOF instruments, non-covalent macromolecular complexes can survive the transition from solution to gas phase and remain intact during their flight in the mass spectrometer, which enables us to directly detect interacting subunits of complexes.

Sample Requirements

(1) Purity: The complex for MS analysis should be the major component as shown by native-PAGE gel.
(2) Quantity: Concentration of the complex should be no less than 1μM. A sample volume of 20μL is normally required for our MS analysis.
(3) Shipping: Samples in their optimal buffers can be shipped frozen with dry ice.

Time Needed for MS Analysis

It normally takes 1 to 2 hours for the MS analysis. Longer time up to a few weeks may be required for determination of subunit organisation of large complexes, as we have to carry on multiple nanospray experiments.

People Involved

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