Native Mass Spectrometry in Viral Structural Biology and Molecular Systems Biology

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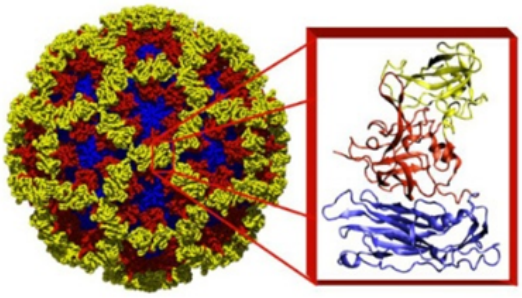
The development of electrospray ionization coupled to mass spectrometry has enabled the analysis of very large intact protein complexes even when they are held together by non-covalent interactions. Together with equally spectacular advances in mass spectrometric instrumentation a new field has emerged, termed native protein mass spectrometry that focuses on the structural and functional analysis of the dynamics and interactions occurring in protein complexes. Native mass spectrometry allows the topological investigation of intact protein complexes with high sensitivity and a theoretically unrestricted mass range. This unique tool provides complementary information to established technologies in structural biology.

Using such mass spectrometry based technologies we have begun to study the biophysical properties of virus structure and assembly, focusing on the important HBV and norovirus human pathogens. The masses of HBV (3-4 million Da) and the noro virus (over 10 million Da) create significant challenges on any structural method to characterize these particles with considerable resolution, are some of the largest particles ever analyzed by high resolution mass spectrometry. For these viruses we have been able to probe several distinct assembly intermediates, which are generally low abundant and therefore difficult to monitor by other techniques. Several biophysical parameters such as self-assembly, stability and shape of the virus particles were monitored as well as their dependence on pH, ionic strength and temperature.



Moreover, we used native mass spectrometry to probe the regulation of a cyano-bacterial circadian clock, which is regulated by the interplay of protein assembly and protein phosphorylation, involving three proteins; KaiA, KaiB and KaiC. Native mass spectrometry was used to measure the real-time assembly properties of this self-organizing system and provided the missing link needed to construct a full theoretical molecular model to describe the rhythm, stability and regulation of this clock.