

MODELLING THE HK97 CAPSID MATURATION PROCESS BY CRYO-ELECTRON MICROSCOPY IN COMBINATION WITH OTHER BIOPHYSICAL METHODS

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The capsid of bacteriophage HK97 has been studied by a variety of biophysical techniques, including cryo-EM, X-ray crystallography, calorimetry, and low angle X-ray scattering. Data from each of these offer particular insights into the dynamic process of capsid assembly and maturation that involves ~420 copies of the major capsid protein and leads to a highly stable, extensively crosslinked particle of ~550Å diameter.¹ An atomic resolution model of the mature capsid has not only provided important insights into the capsid structure and topology of interlinked subunit rings,² but has also provided a subunit atomic model of the mature capsid protein for modelling lower resolution cryoEM density maps of precursor structures. We have studied the pre-expansion capsid, Prohead II, in this way³ and more recently the intermediates in the expansion transition allowing a trajectory of backbone atoms to be proposed that includes a partly defined sequence for the pattern of covalent cross-linking between subunits.^{4, 5} Current work is focused on the initial complete capsid state, Prohead I, which includes the 102-amino acid N-terminal Δ -domain of the major capsid protein that is subsequently proteolysed to form Prohead II. This domain is proposed to aid capsid assembly, for which no separate scaffolding protein exists, and is located on the interior surface of the capsid grouped beneath the hexameric and pentameric capsomers. According to comparison of cryo-EM density maps at ~9Å resolution, proteolysis of the Δ -domains is accompanied by a reorganization of the new N-terminals that appears to enhance inter-capsomeric interactions by sealing the "cracks" between them. As with most other steps in the maturation pathway, this process is progressive and irreversible, leading inexorably towards the end-product.

References

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