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Electron Cryo-Tomography Demonstrates Variable Distributions of the Viral NTPase and RNA Polymerase in Bacteriophage ϕ6 Procapsids

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The double-stranded (ds) RNA bacteriophage ϕ6 that infects *Pseudomonas syringae* exhibits similar structural and functional properties as eukaryotic viruses of the *Reoviridae* family [1]. The inner shell of the triple-layered virion matures from an icosahedral precursor (procapsid) upon RNA packaging and consists of four viral proteins. The three segments of the RNA genome are translocated into the procapsid shell, which consists of proteins P1 and P7 by a hexameric NTPase (P4) located at the 5-fold vertices [2]. Several copies of the RNA-dependent RNA polymerase (P2) are enclosed in the procapsid at the 3-fold axes and replicate the minus strand of the packaged ssRNA segments [3]. The procapsid has multiple binding sites for P2 monomers and P4 hexamers that, typically, are only partially occupied. To better understand the mechanism of RNA packaging and replication during bacteriophage ϕ6 assembly, we have investigated occupancy of the P2 and P4 binding sites by electron cryo-tomography.

Procapsids were isolated from the wildtype strain [3]. Samples for cryo-tomography were mixed with fiducial markers (10-nm nanogold particles) and imaged on a Tecnai 12 (FEI) microscope operating at 120 keV. Tomograms were reconstructed and individual particles extracted and rotated to standard icosahedral orientation using *Bsoft* (Fig 1). A new program was written to quantitate selected densities within the oriented particles and determine the distribution of P2 and P4 molecules in the procapsids.

The P2 molecules occur at 20 possible locations on the 3-fold axis within the procapsids [3]. On average, only half of the positions are occupied by P2 based on our analysis (Fig 2A). These data are consistent with previous quantitations by biochemical assay and from averaged density in cryo-EM reconstructions [3]. We also observe other densities inside the procapsid not at the expected P2 location that may be detached P2 molecules or other entrapped substances. Similar measurements revealed that only a part of the 12 possible binding sites for P4 hexamers were occupied in this preparation of procapsids (Fig 2B), significantly fewer than were reported for packaged nucleocapsids of the related bacteriophage ϕ8 (~10 P4 hexamers per particle) [4].

References

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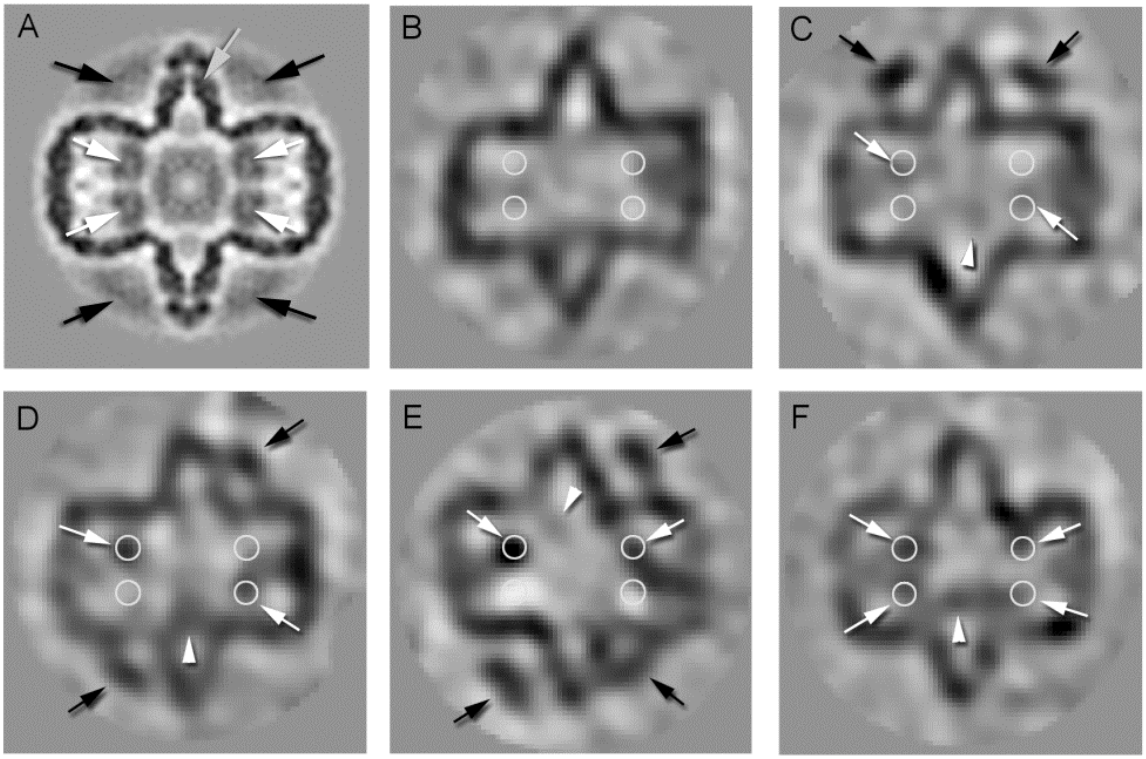

Figure 1: (A) Central section the ϕ6 procapsid derived from icosahedral reconstruction [3]: P2 density (white arrows); P4 density (black arrows); P1 icosahedral shell (grey arrow). (B-F) Central sections of individual tomographic particles with P2 locations depicted by white circles. P2 sites deemed to be occupied are marked with white arrows. P4 hexamers are present at only some of its binding sites at the procapsid's five-fold vertices (black arrows).

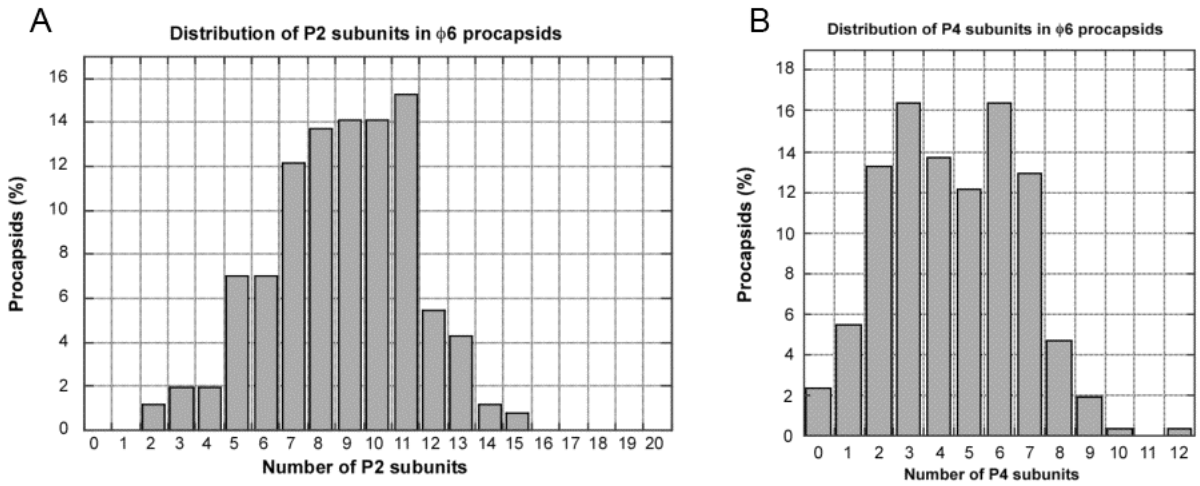

Figure 2: Distribution of numbers of occupied P2 (A) and P4 (B) binding sites in individual ϕ6 procapsids. The data derived from 256 aligned particles indicate that P2 molecules occupy on average about half of the 20 binding sites at the 3-fold axes and P4 hexamers occupy about half or less of the 12 icosahedral vertices per procapsid.

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