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Expansion of the Bacteriophage ϕ6 Procapsid Revealed by Electron Cryo-Microscopy

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Bacteriophage ϕ6 is a spherical enveloped dsRNA virus (diameter 860 Å) that infects the bacterium *Pseudomonas syringae* and shares many properties with viruses of the *Reoviridae* family [1]. The first assembly product is the icosahedral procapsid composed of the proteins P1, P2, P4 and P7, and with deeply recessed 5-fold vertices. During packaging of the 3-segment genome, the P1 shell expands to a near-spherical shape. The P1 subunits must undergo significant conformational changes to achieve the expansion. The P2 protein (the RNA-dependent RNA polymerase) was identified inside the unexpanded procapsid shell on the 3-fold axis [2], and should remain in close proximity to the 5-fold vertex on expansion for minus RNA strand synthesis. To shed more light on the mechanics of procapsid expansion, we imaged empty, expanded, and RNA-packaged procapsids.

Vitrified specimens were imaged at 50,000× magnification with a CM200 FEG (FEI) microscope operating at 120 keV (expanded and packaged procapsids), or with a Tecnai F30He (FEI) microscope operating at 300 keV (LN_2 temperature, empty procapsids). Single particle analysis was done with the package, Bsoft [3]. Packaged procapsids treated with salt exhibited filled, empty, and partially empty states. These particles were classified with multiple reference maps through several iterations where small classes of particles (<100) were eliminated and larger classes retained and refined.

It was our experience that the empty procapsid (Fig 1A) expands to some degree spontaneously and with buffer manipulations, retaining some of the recessed nature at the 5-fold vertices (Fig 1B). The procapsid packaged with two segments of RNA (Fig 1F), the S segment with a kanamycin gene insert (4.8 kb) and the L segment (6.4 kb), releases various amounts of RNA in the presence of higher levels of salt, giving rise to three distinct particles (Fig 1C-E). The empty particle (Fig 1C) appears very similar to the expanded procapsid (Fig 1B), suggesting it is the same stable intermediate state. The other two particles (Fig 1D,E) may represent intermediate states further along the packaging sequence, although the procapsid could adopt different conformations during loss of RNA. Also, the location of P2 during expansion and in the packaged procapsid is an open question, while some densities inside the five-fold vertices may be likely candidates.

References

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[2] A. Sen et al., *J Biol Chem* 283 (2008) 12227.
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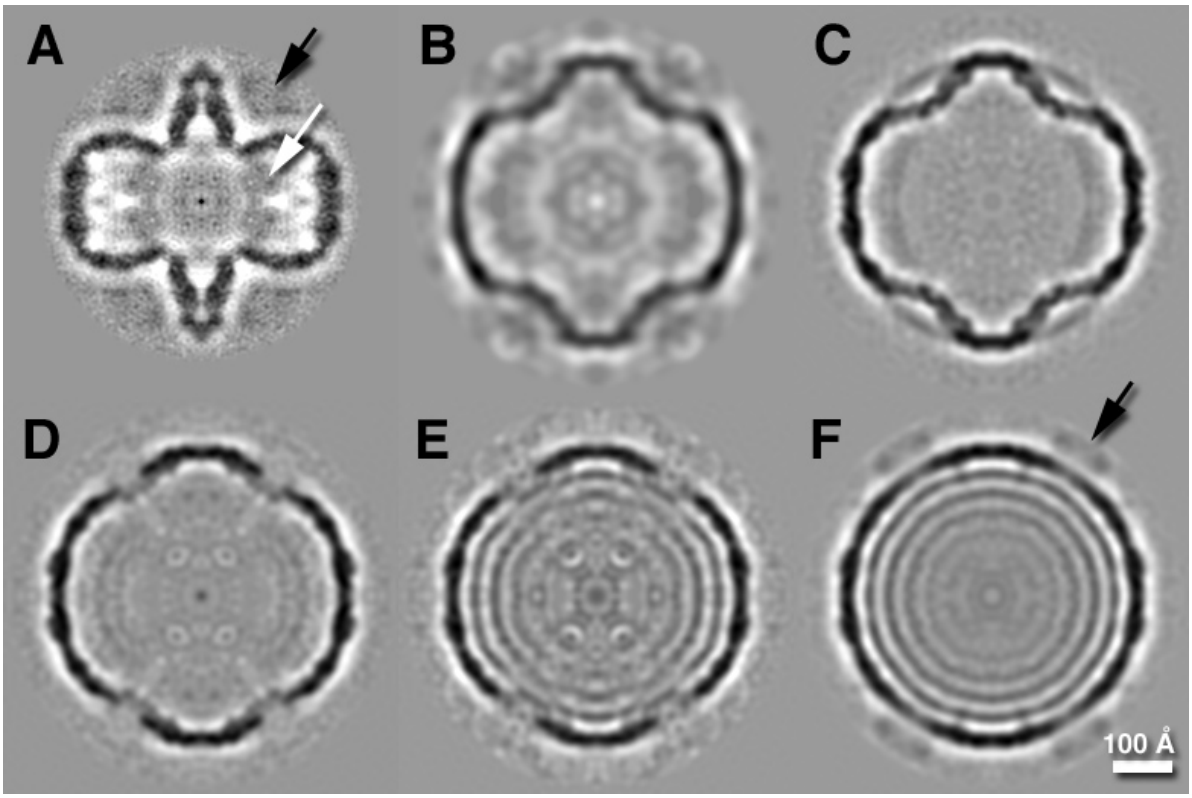

Figure 1: Central sections through reconstructions of ϕ6 procapsids. (A) Empty procapsid composed of P1, P2 (white arrow), P4 (black arrow) and P7. (B) Expanded procapsid. (C-E) Packaged procapsids treated with salt, classified into 3 structures with increasing amounts of RNA. (F) Packaged procapsid under lower salt conditions. Resolution ($FSC_{0.3}$) and particle numbers: (A) 13 Å, 1019; (B) 36 Å, 77; (C) 19 Å, 2059; (D) 20 Å, 899; (E) 22 Å, 708; (F) 20 Å, 4288.

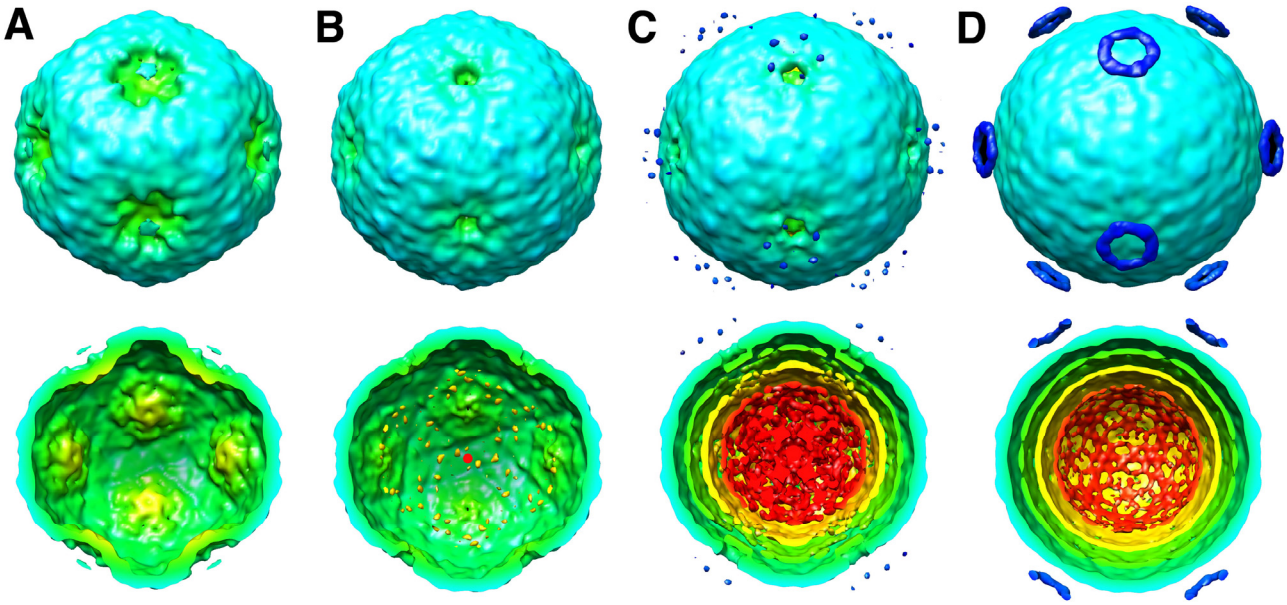

Figure 2: Isosurface renderings of the reconstructions from Fig 1 (A-D correspond to C-F), showing the outward movement of the five-fold vertex and the RNA shells in (C,D).

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