

The Structure of a T=169d Algal Virus, PBCV-1, at 15Å Resolution

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Paramecium bursaria chlorella virus 1 (PBCV-1) (genus *Chlorovirus*, family *Phycodnaviridae*), infects certain unicellular, exsymbiotic, chlorella-like green algae [1]. The PBCV-1 virion ($\sim 1 \times 10^9$ Da) has a linear dsDNA genome (330kbp) and contains at least 100 different proteins [1, unpublished data]. A published cryo-electron microscopy and three-dimensional (3D) image reconstruction of PBCV-1 (26Å resolution) revealed that the outer icosahedral glycoprotein capsid, with a maximum diameter of 1,900Å along the five-fold axis, consists of 1680 trimeric capsomers (trimer) and 12 pentameric capsomers (pentamer) [2]. These capsomers are arranged on a T=169d ($h=7, k=8$) quasi-equivalent lattice. The trimers and pentamers are organized into 20 trisymmetrons (66 trimers) and 12 pentasymmetrons (30 trimers plus one central pentamer). In the present study, we have recorded new images, which have been used to compute a 3D image reconstruction of PBCV-1 at 15Å resolution.

PBCV-1 sample preparation and microscopy were carried out as described [2]. Of the 94 micrographs recorded in an FEI/Philips CM200 FEG microscope (defocus level 1.44 ~ 3.64µm, x38,000), 45 were digitized at 7µm intervals on a Zeiss Phodis scanner. The obtained images were binned and interpolated to give a pixel size of 4.0Å. Particle origins and orientations were determined by model-based procedures with the 26Å map of PBCV-1 serving as the initial model [2, 3]. Corrections were made to each image to compensate for the effects of the microscope contrast transfer function. The effective resolution of the final reconstruction was determined by Fourier Shell Correlation analysis to be 15Å [4]. Of the 1262 boxed particle images, 1000 were used to compute the final density map.

The latest reconstruction (Fig. 1A-D) reveals a more detailed view of the morphological features in the PBCV-1 virion compared to that observed in the 26Å map. The bilayer membrane is now more prominent and uniform. Also, each trimer, formerly seen as a simple, doughnut-like structure, now exhibits a hexagonal base that is topped with three, tower-like protrusions. The higher resolution PBCV-1 map permitted more accurate fitting of the atomic model for the Vp54 trimer into the virion density map and this has provided a more detailed view of the organization of the trisymmetron and pentasymmetron structures, and also may provide clues about the locations of minor capsid proteins [5]. One of the 28 trimers in each asymmetric unit is unique in that it contains a fiber-like structure (Fig. 1.B, black arrow) and these fibers (60/virion) may be involved in host recognition and attachment [6].

References

- [1] J. L. Van Etten, *Annu. Rev. Genet.* 37 (2003) 153.
- [2] X. Yan et al., *Nat. Struct. Biol.* 7 (2000) 101.
- [3] T. S. Baker and R.H. Cheng, *J. Struct. Biol.* 116 (1996) 120.
- [4] T. S. Baker et al., *Microbiol. Mol. Biol. Rev.* 63 (1999) 862.
- [5] N. Nandhagopal et al, *Proc. Natl. Acad. Sci.* 99 (2002) 14758.

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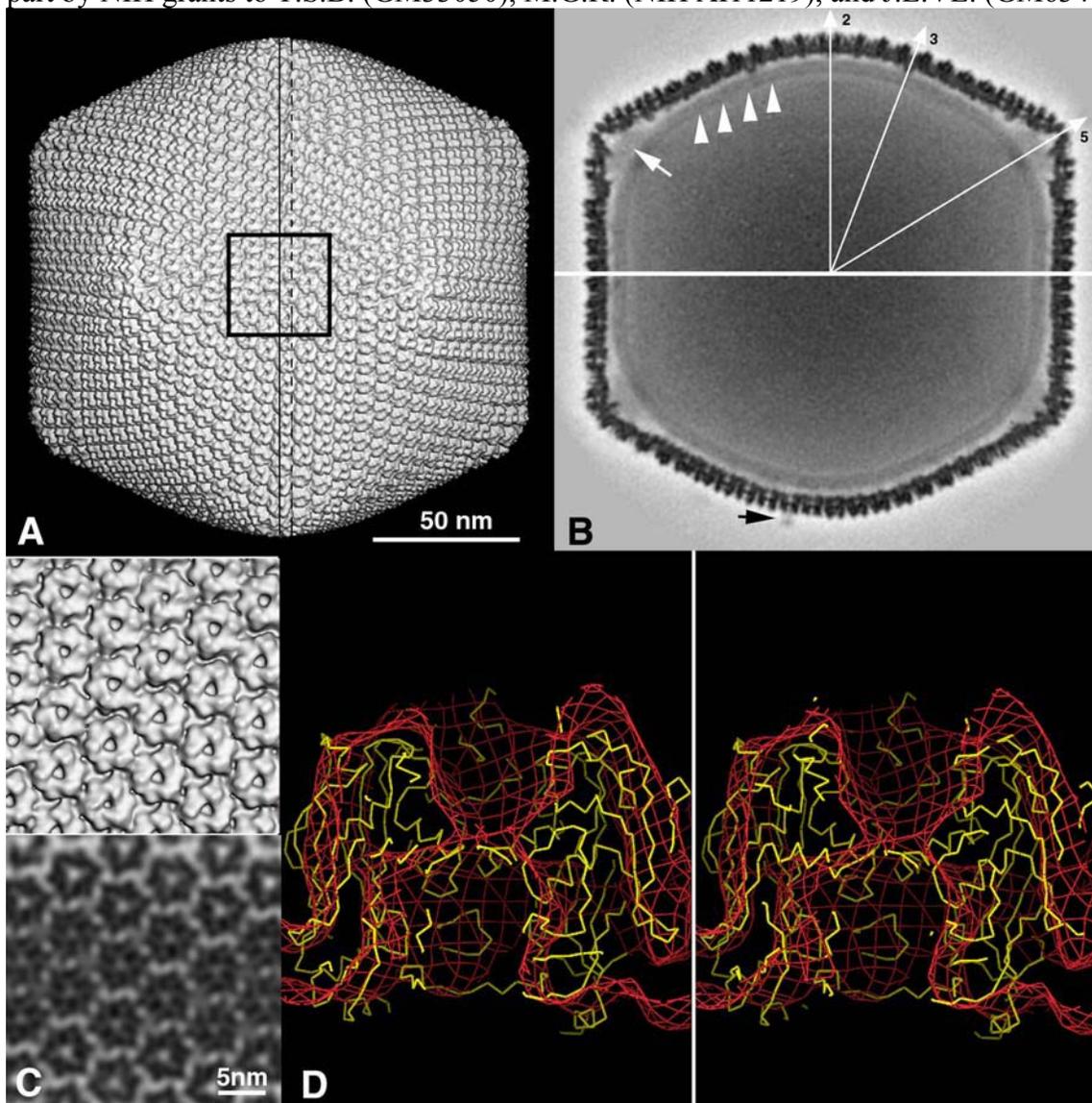


FIG. 1. A. Shaded surface view of PBCV-1 3D reconstruction at 15Å resolution, viewed along a 2-fold axis. B. Density sections of PBCV-1 reconstruction (densest regions appear darkest). Top half: section obtained from region marked by solid line in (A). Two-, three- and five-fold symmetry axes are identified (thin white lines and numbers). Bottom half: section from region marked by dashed line in (A). The lipid membrane (white arrowheads) appears as a uniform, diffuse band of density that closely follows the contour of capsid shell everywhere except near the icosahedral 5-fold vertices. The trimers and pentamers form the bulk of the densest and high radii portion the PBCV-1 virion. Extensive yet diffuse density (white arrow) is observed just below the outer capsid and near the 5-fold axes. One fiber-like structure (black arrow) protrudes away from the virion surface. C. Close-up views of the boxed region in (A), shows both surface-shaded (top) and planar section (bottom) representations. Some density features (best viewed in the planar section) appear to interconnect neighboring trimers. D. Stereo view of one half of a trimer (caged density) with an atomic model (solid lines for $C\alpha$ backbone) of Vp54 superimposed to show its excellent fit into the reconstructed density map.