

## Dissecting the Structure and Function of the Orthoreovirus $\mu$ 1 ('Penetrin') Protein at 7.0-Å and Higher Resolution

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Non-fusogenic mammalian orthoreoviruses (reoviruses), from the genus *Orthoreovirus* (family *Reoviridae*), are a classic system for studies of virus-cell and virus-host interactions [1-3]. A poorly understood step in cell entry by reovirus and other non-enveloped viruses is membrane penetration, by which the virus crosses the endosomal or lysosomal membrane barrier and enters the cytoplasm to initiate replication. The ~850 Å diameter reovirus virion comprises two concentric, icosahedral protein capsids that surround a ten-segmented, double-stranded RNA genome. One of the major outer-capsid proteins,  $\mu$ 1 (76-kDa, 600 copies), has been implicated in membrane destabilization [4]. The destabilization activity is thought to allow a partially uncoated, transcriptionally activated form of the virus particle to be freed into the cytoplasm. How  $\mu$ 1 effects this function remains a subject of ongoing study, but recent results implicate autolytic release of a myristoylated, amino (N)-terminal fragment of  $\mu$ 1 as one key step [5].

We recently used electron cryo-microscopy and three-dimensional image reconstruction methods to obtain a density map of the reovirus virion at 7.6-Å resolution, which enabled us to locate the  $\lambda$ 3 polymerase inside the viral core [6]. Nearly 8,000 particle images, recorded with low dose techniques in FEI/Philips CM200 and CM300 FEG microscopes, were used to obtain the map. Further refinement of these data has now yielded a map of the viral capsid at 7.0-Å resolution (Figure 1). At this resolution clear and interpretable density is seen for all of the large, secondary structural features of the viral structural proteins (342  $\alpha$ -helices of 5 or more residues and 92  $\beta$ -sheets of 3 or more strands for the 27 total viral subunits in the asymmetric unit of the structure). In addition, local averaging of seven of the ten copies of the  $\mu$ 1 and  $\sigma$ 3 proteins in each asymmetric unit of the icosahedron has improved the resolution of these components (to ~6.7-Å for  $\mu$ 1). In conjunction with atomic modeling experiments, the higher resolution reovirion structure has enabled us to visualize new details of the outer capsid. For example, several new features such as the one illustrated in Figure 2 are evident in the N-terminally myristoylated, outer-capsid  $\mu$ 1 protein that were not seen in a previously reported crystal structure of the  $\mu$ 1/ $\sigma$ 3 hetero-hexamers [7]. These features appear important for stabilizing the outer capsid, regulating the conformational changes in  $\mu$ 1 that accompany destabilization of target membranes, and/or contributing directly to membrane penetration during cell entry by reovirus [8].

## References

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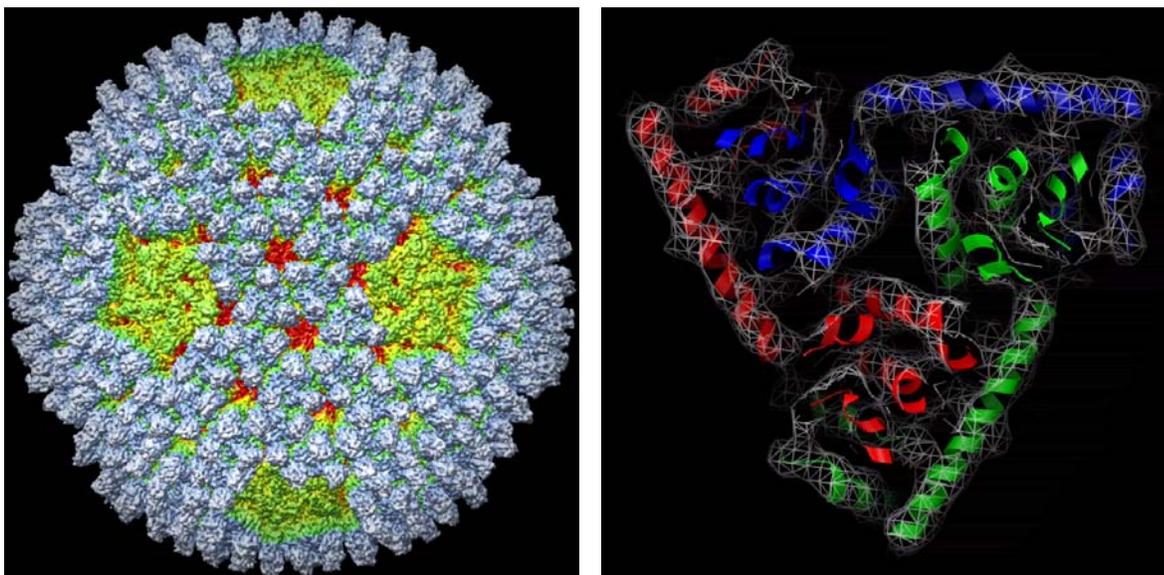


Fig.1 Reovirus T3D reconstruction at 7.0-Å resolution. (Left) Radially color-coded surface view of T3D virion in two-fold orientation. (Right) Close-up view of a thin section through the T3D density map (grey cage) in a region near the bottom of a  $(\mu 1\sigma 3)_3$  heterohexamer. The red, blue and green ribbon traces show the fit of the  $(\mu 1\sigma 3)_3$  atomic model into the experimental density map.

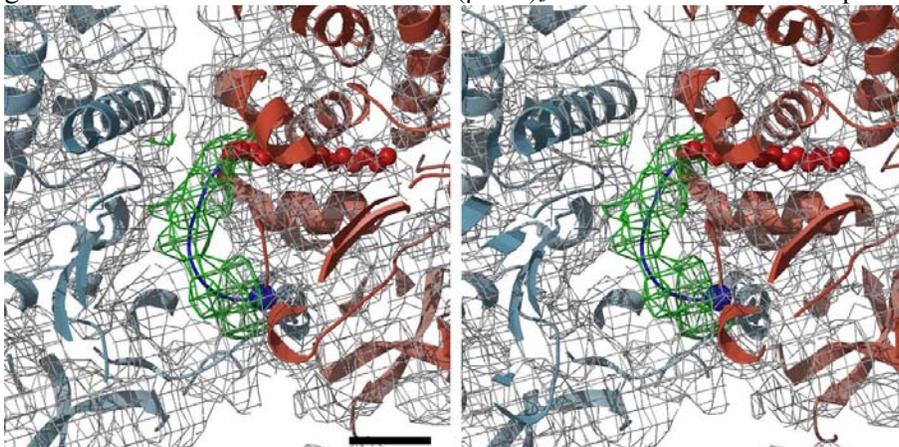


Fig.2 Close-up stereo view of the cryoEM densities (gray net) and fitted X-ray crystal structures (cyan and orange ribbon traces) at the interface between two  $\mu 1$  trimers. The extra cryo-EM densities (green net) have been tentatively assigned to the N-terminal eight residues of one  $\mu 1$  subunit from the orange trimer. A blue sphere marks the position of residue Thr10, residues Gly2 to Gln9 are modeled as a blue loop, and the N-terminal myristoyl group is represented as a space-filling model (red) occupying a hydrophobic pocket. Scale bar is 10Å.

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