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In situ Structure of Viral RNA by Cryo Electron Tomography with Volta Phase Plate, Energy Filtering and Direct Electron Counting

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The most significant limiting factor of biology electron microscopy is that only low electron dose (about 25 to 100 electrons/Å^2 depending on the sample and the target resolution) is available for imaging prior to sample damage. As a result, cryo electron microscopy (cryoEM) images of biological complexes are characterized by poor contrast and low signal/noise ratio. Single-particle cryoEM relies on the power of averaging low-contrast, high-resolution images of as many as one million purified particles of the same homogeneous complex to overcome this low contrast problem to obtain structures suitable for functional interpretation. This approach was first demonstrated at near atomic resolution for proteins in an icosahedral virus [1] and now has become a method of choice for structure determination of protein complexes in general. For non-homogeneous structures, such as nucleic acid molecules inside many viruses, however, cryo electron tomography (cryoET) must be used. Without the power of averaging, resolving nucleic acid structures within viruses by cryoET has not been possible.

Recently, Volta phase plate (VPP) has shown great promise for enhancing the contrast of biological samples at low dose condition [2-5]. To explore the potential of VPP in resolving *in situ* nucleic acid structures inside viruses, we set up to image a median-sized RNA virus, rotavirus. As a prototypical member of the *Reoviridae* family, rotavirus is the most common cause of severe vomiting and diarrhoea among children. Infectious rotavirus is a triple-layered spherical particle with an 11-segment, double-strand RNA genome. Upon entry into the host cell, the virus become a double-layered particle (DLP), which is capable of endogenous RNA transcription and capping. High resolution structures of the viral proteins are previously known from both X-ray crystallography and single-particle cryoEM. But the structure of the viral RNA genome and its *in situ* organization within DLP have not been obtained though models have been suggested based average structures from single-particle cryoEM [6].

We recorded cryoET tilt series of rotavirus DLPs in a Titan Krios instrument operated at 300kV accelerating voltage (Fig. 1a). The instrument is equipped with a VPP, a Gatan imaging filter (GIF) and a post-GIF K2 summit direct electron counting camera, thus providing one of the most powerful combination of cutting-edge technologies. Tilt series were collected with the *SerialEM* software package in electron counting mode using the tilt range of –66° to +60°, an angular step of 2°, total accumulated electron dose of 120 electrons/Å^2, defocus value of 1.0μm, and a pixel size of 2.6 Å on the specimen. Tilt series alignment and 3D reconstruction with statistical image reconstruction techniques (SIRT) were performed using IMOD software package [7]. In 2D slices of the 3D tomogram, structures of both protein and RNA molecules are resolved (Fig. 1b,c). The RNA molecule appear as density threads distinctively different from the globular structures of the protein molecules, which are similar to those resolved in single-particle cryoEM [6], as confirmed by subtomogram averaging of some DLPs (Fig. 1d). The organization of RNA density threads gives rise to parallel striations with somewhat variable

inter-rod distances around 30 Å (Fig. 1c), suggesting that each thread is likely a dsRNA duplex. Some dsRNA duplex extend across much of the interior space of the inner capsid layer, indicating that individual segments of the RNA genome do not spiral locally around a viral polymerase complex as previously suggested from single-particle cryoEM [6][8].

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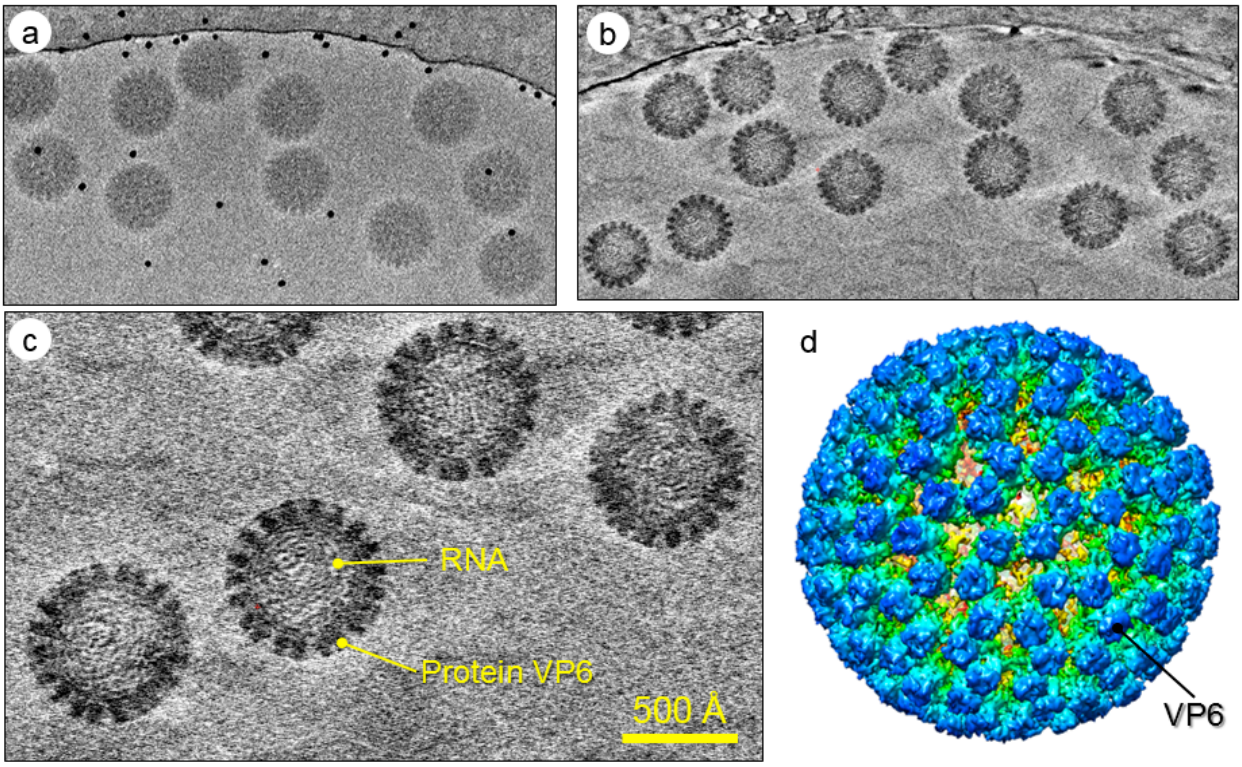

Figure 1. CryoET of the rotavirus double-layered particle with a Volta phase plate. (a) One tilt image from a tilt series. The black dots are 5 nm fiducial gold particles used for alignment. (b) A central slice in the tomogram obtained from the tilt series shown in (a). (c) A close-up view of the four rotavirus particles near the bottom left corner of (b) showing the organization of dsRNA duplex inside the protein capsid. (d) Radially colored surface representation of the subtomogram average of 187 DLPs by the software PEET.

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