# STRUCTURAL ANALYSIS OF VIRUS ASSEMBLY BY CRYO-ELECTRON TOMOGRAPHY: MEASLES VIRUS AND RESPIRATORY SYNCYTIAL VIRUS

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**Supplementary Material, Movie legends:**

**Movie 2.1 Cryo-ET of MeV-infected HeLa cells at the assembly site.** A MeV assembly site from the frozen-hydrated Edm MeV-infected HeLa cell demonstrates the viral structural organization in the context of whole-cell tomography.

**Movie 2.2 Cryo-ET of MeV-infected MRC-5 cells at the assembly site.** A MeV assembly site from the frozen-hydrated Edm MeV-infected MRC-5 cell demonstrates the viral structural organization in the context of whole-cell tomography. Scale bar is 200 nm.

**Movie 2.3 Cryo-ET of a released MeV particle from MeV-infected cells.** A released MeV particle from Edm MeV-infected HeLa cells demonstrates the viral structural organization. Note the M lattice and RNP are in register inside the native MeV particle.

**Movie 2.4 Cryo-ET of MeV-infected cells at the assembly site.** A MeV assembly site from the frozen-hydrated recMeV-(H-118▽41×) infected HeLa cell demonstrates the viral structural organization in the context of whole-cell tomography. Note the F lattice on the surface of the native MeV particle.

**Movie 2.5 Subvolume average of recMeV-(H-118▽41×) F-M lattice.** Tomographic slices going from the bottom to the top (round trip) of the averaged recMeV-(H-118▽41×) F-M overlaid lattice structure. Black is density in the tomographic slices. In the segmentation view, blue is F and cyan is M. Scale bar is 10 nm.

**Movie 2.6 Subvolume average of Edm F-M lattice.** Tomographic slices going from the bottom to the top of the averaged Edm F-M overlaid lattice structure. Black is density. Scale bar is 10 nm.

**Movie 2.7 Model of MeV structural proteins organization.** Segmentation of the F-M lattice in conjunction with the RNP complex demonstrating the 3 layers of structural protein organization.

**Movie 2.8 Clonemodel view of RNP classes within a tomogram.** The subvolumes extracted from the tomogram were placed back into the original volume. The color scheme is the same as **Figure 2.5**. Scale bar is 100 nm.

**Movie 2.9 RNP length quantification of a released MeV particle.** Tomographic slices going from the bottom to the top (round trip) of a released MeV particle from MeV-infected cells. The color scheme is the same as **Figure 2.16**. The blue lines are coated RNP while the pink lines are uncoated RNP. Scale bar is 200 nm.

**Movie 2.10 RNP length quantification of a released MeV particle.** Tomographic slices going from the bottom to the top (round trip) of a released MeV particle from MeV-infected cells. The color scheme is the same as **Figure 2.16**. The pink lines indicate the presence of the uncoated RNP. Scale bar is 200 nm.

**Movie 2.11 Tilt series of MeV.** Releases MeV particles from MeV-infected HeLa cells collected using SerialEM software and images were aligned using IMOD software. Tilt range is -62º to 62º at 2-degree step increment. Scale bar is 200 nm.

**Movie 2.12 Reconstructed tomographic data from tilt series (MeV).** The tilt series from **Movie 2.11** was further reconstructed using back-projection algorithm in IMOD software package and the 3D tomographic data was generated. Movies showing tomographic slices from top to bottom of the volume. Scale bar is 200 nm.

**Movie 3.1 Tilt series of RSV.** Releases RSV particles from RSV-infected A549 cells collected using SerialEM software and images were aligned using IMOD software. Tilt range is -62º to 62º at 2-degree step increment. Scale bar is 500 nm.

**Movie 3.2 Reconstructed tomographic data from tilt series (RSV).** The tilt series from Movie 3.1 was further reconstructed using back-projection algorithm in IMOD software package and the 3D tomographic data was generated. Movies showing tomographic slices from top to bottom of the volume. Scale bar is 500 nm.

**Movie 3.3 RSV assembly at the plasma membrane (A2-infected MRC-5 cells).** Volume rendering using Amira software to show different components of RSV-infected MRC-5 cells at the assembly site. The color schemes are: membrane is cyan, glycoproteins are yellow, the RNPs are red, the ring-like structures are blue, actin filaments are green, microtubules are gold, and ribosomes are magenta.

**Movie 3.4. Cryo-ET of RSV assembly (Early Assembly).** Tomographic reconstruction and its corresponding segmentation of a RSV assembly site captured by cryo-ET, revealing the initiation, elongation, and scission stages. The color scheme is the same as **Figure 3.8**, Green is (viral) membrane, red is glycoprotein, and magenta is the RNP complex.

**Movie 4.1 Cryo-ET of immunolabeled RSV F glycoproteins at the site of virus assembly on a HeLa cell.** This movie is of a z-slice progression through a tomographic reconstruction and the segmentation of the site of RSV assembly on the surface of a HeLa cell and corresponds to the data presented in Figure 5. The cell membrane is presented in cyan. The filamentous actin network is noted in green. The matrix protein is depicted in blue. The glycoprotein densities are highlighted in magenta. Red tubular densities correspond to the RNP. Gold densities are the 6 nm gold particles conjugated to the secondary antibody. Gold fiducial markers 20 nm in diameter were added to the sample and used as image alignment aids during the 3D tomographic reconstruction process.