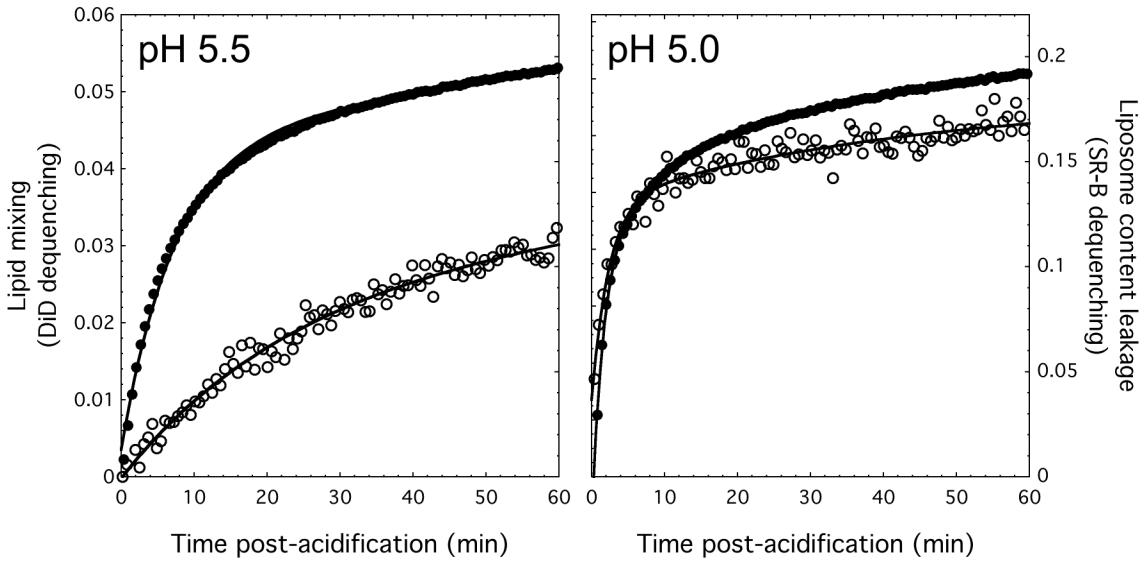
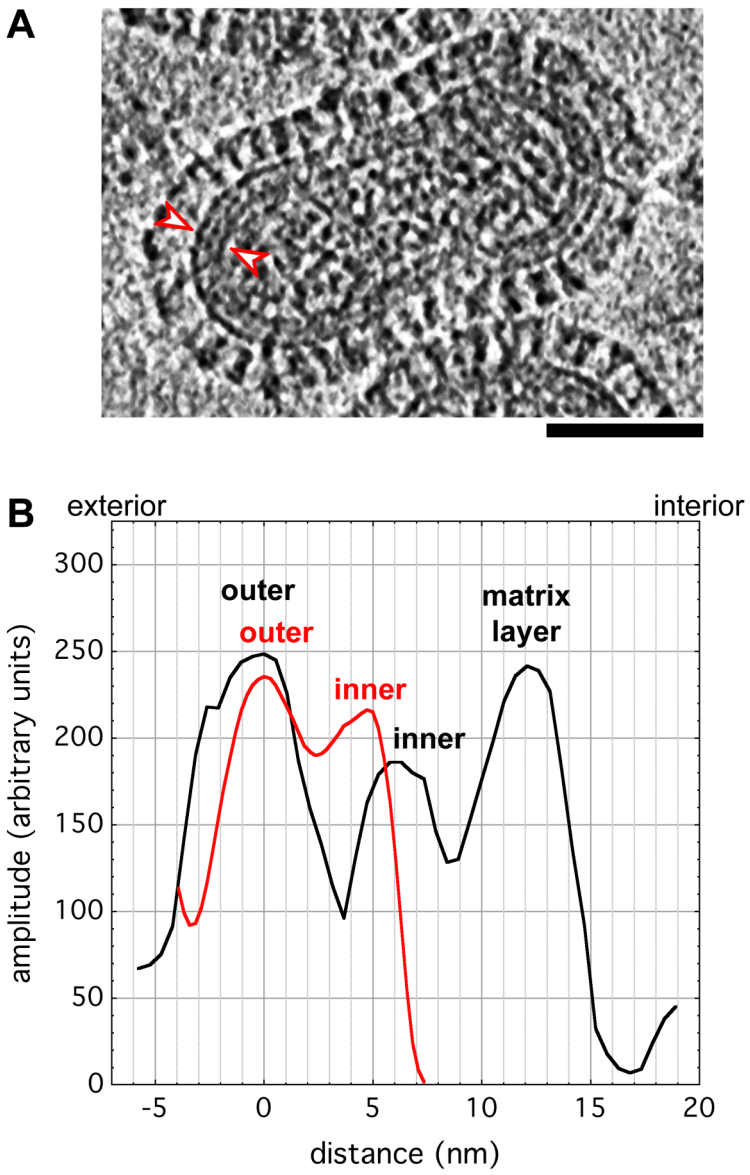
SUPPLEMENTARY MATERIAL

accompanying "Architecture of a Nascent Viral Fusion Pore" by K. K. Lee

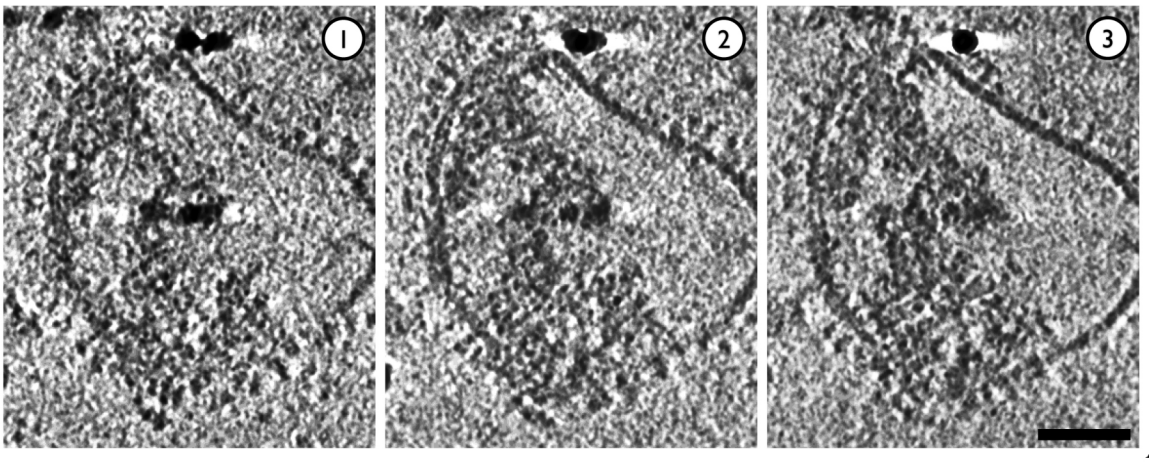
SUPPLEMENTARY FIGURES



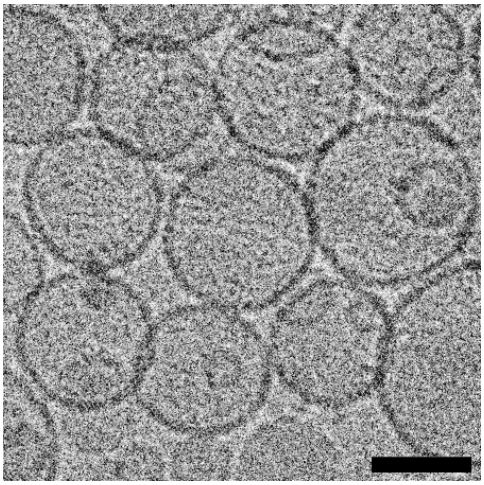
Supplementary Figure S1. Fluorescence spectroscopy of DiD-labeled virions mixed with sulforhodamine-B containing DOPC liposomes at pH 5.5 and 5.0. Fluorescence dequenching of the water-soluble (SRB, solid circles) and lipophilic (DiD, open circles) dyes was monitored as a function of time. As in the case sulforhodamine-B/DiD doubly labeled DOPC liposomes fusing with unlabeled virions (Figure 2), leakage of liposomal contents appear to take place more rapidly than lipid mixing. Extent of dequenching was calculated as $[F(t)-F(0)]/[F_{TX\text{-}100}-F(0)]$, where $F_{TX\text{-}100}$ was the fluorescence measured in the presence of 0.1% w/v TritonX-100 detergent. Fitted curves are only meant to guide the eye and do not reflect a particular kinetic model.



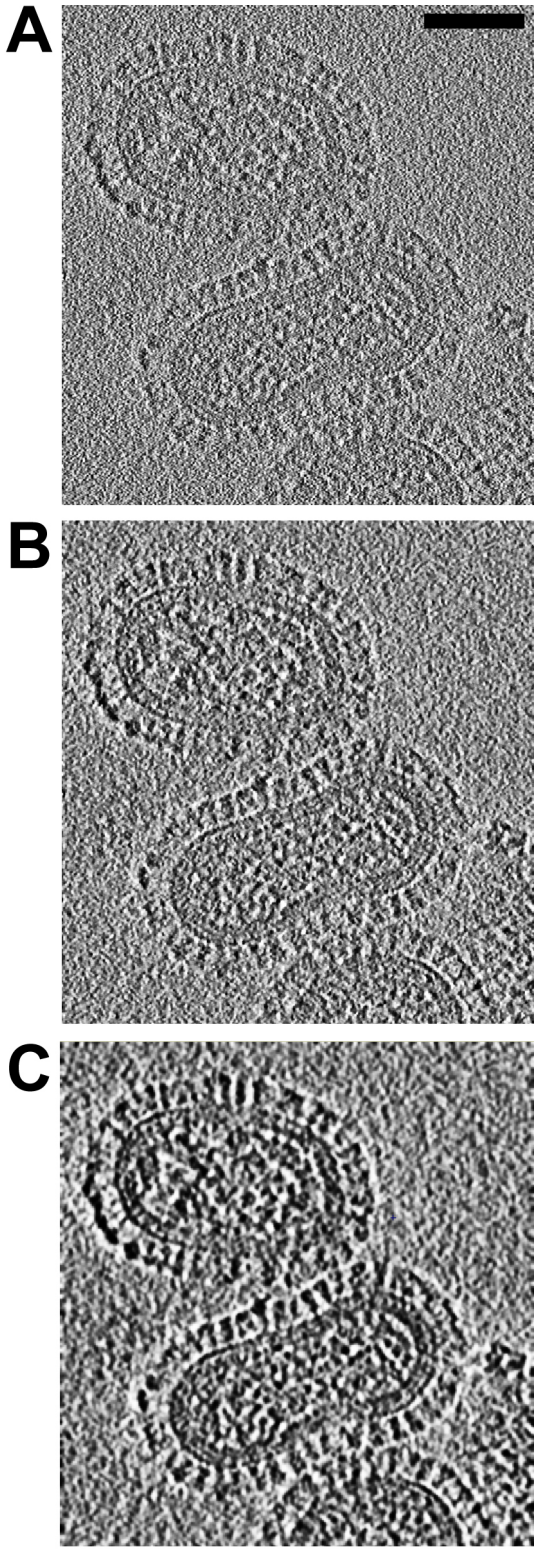
Supplementary Figure S2. Electron density plot through a typical virion envelope and through a DOPC liposome bilayer. (A) Most influenza virions exhibit a 3-layered envelope. Microscope defocus 3 µm. 50 nm scalebar. **(B)** A typical density distribution plot taken orthogonally through the virus envelope (position in panel A indicated by arrowheads) is shown below. 3 distinct peaks are seen with ~6 nm relative separations. The intermediate layer is generally observed to have the weakest density. A density plot for DOPC liposome bilayer (red curve), ~4 nm leaflet spacing, is shown for comparison.



Supplementary Figure S3. Representative post-fusion particle. Due to the clumped interior density, it is not clear whether these entities resulted from the fusion of one of the dominant matrix-bearing virus particles with a liposome or one of the matrixless particles. At least a portion of the membrane on the lefthand side of the fused entity appears to be a standard bilayer, seen in slice 2 for example. Sample acidified for 5 min at pH 5.5. 5.3 nm virtual serial sections shown. Microscope defocus 3 μm. Scalebar 50 nm.



Supplementary Figure S4. Low-dose cryo-EM image of DOPC liposome substrate. Scalebar 100 nm. Microscope defocus 4 μm. The liposomes, produced by 5 rounds of freeze-thawing and 21 extrusions through a polycarbonate membrane with 100 nm pores, are predominantly unilamellar. Relatively few small vesicles are found inside the liposomes (~15%), a number of the small vesicles are located outside of the liposomes and may have been generated by interaction of the phospholipid with the plasma-cleaned carbon-coated grids. By contrast, nearly every liposome in contact with a virus particle exhibits one or more ~20 nm internal vesicles (see main text). Scalebar 50 nm.



Supplementary Figure S5. Tomogram filtering. Comparison of unfiltered tomogram of 2 viruses **(A)** with 3-D median filtering **(B)**, and median plus gaussian (width 1.5σ) filtering **(C)**. A 5.3 nm z-slice through the tomographically reconstructed density is shown. Microscope defocus 3 μm. 50 nm scalebar. Filtering or denoising brings out features such as the three membrane strata and individual glycoprotein spikes.

SUPPLEMENTARY MOVIES

Supplementary Movie 1. Reconstructed tomogram showing a DOPC liposome being pinched by an influenza virion **<Lee_SupplementaryMovie1.mov>**

Supplementary Movie 2. Tomogram showing a complex of DOPC liposome and virus that exhibits a prefusion funnel-shaped feature **< Lee_SupplementaryMovie2.mov >**

Supplementary Movie 3. Tomogram showing a complex of DOPC liposome and virus that exhibits the prefusion funnel-shaped feature **< Lee_SupplementaryMovie3.mov >**