Hemagglutinin Spatial Distribution Shifts in Response to Cholesterol in the Influenza Viral Envelope

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Supporting Information

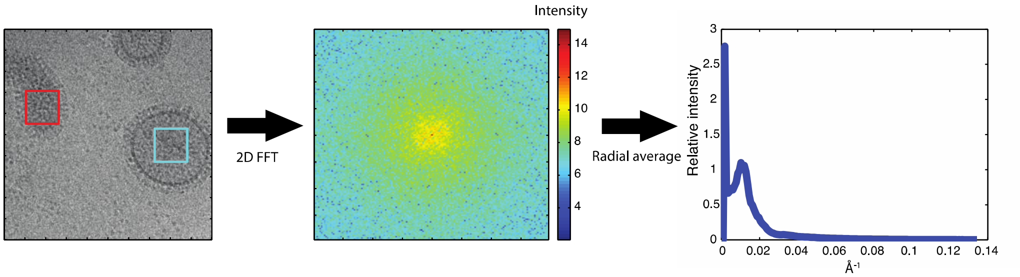

Figure S1. Stages in processing a cryomicrograph to extract spatial distributions. Panels show a cryomicrograph region with two virion interiors boxed (of 25 for the entire micrograph), the 2D FFT image of the cyan region, and the spectrum obtained via radial averaging.

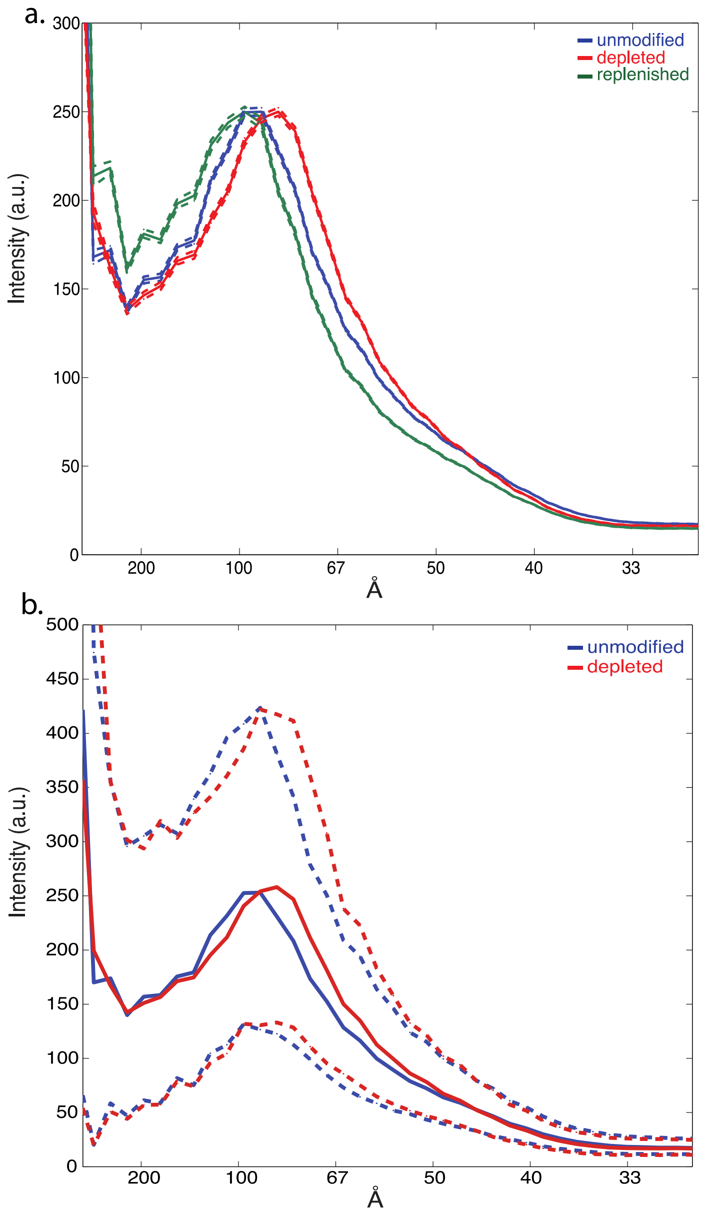

Figure S2. Error analysis of radial distribution functions. 95% confidence intervals for the average radial distribution function computed via bootstrap resampling are plotted in dashed lines for panel (a); these overlay the average data and are visible only on close inspection, indicating that the average radial distributions are extremely robust over the large (~4000) number of virions analyzed in each sample. In panel (b), 95% confidence intervals are plotted in dashed lines for individual virion images. As is typical for single-particle electron microscopy data, the individual images show substantial variation in intensity and are typically analyzed only in average form. However, the confidence "envelope" even for single particles clearly shows a lateral shift in the peak corresponding to hemagglutinin distribution from unmodified virus to virus where cholesterol has been extracted with 20 mM MβCD.

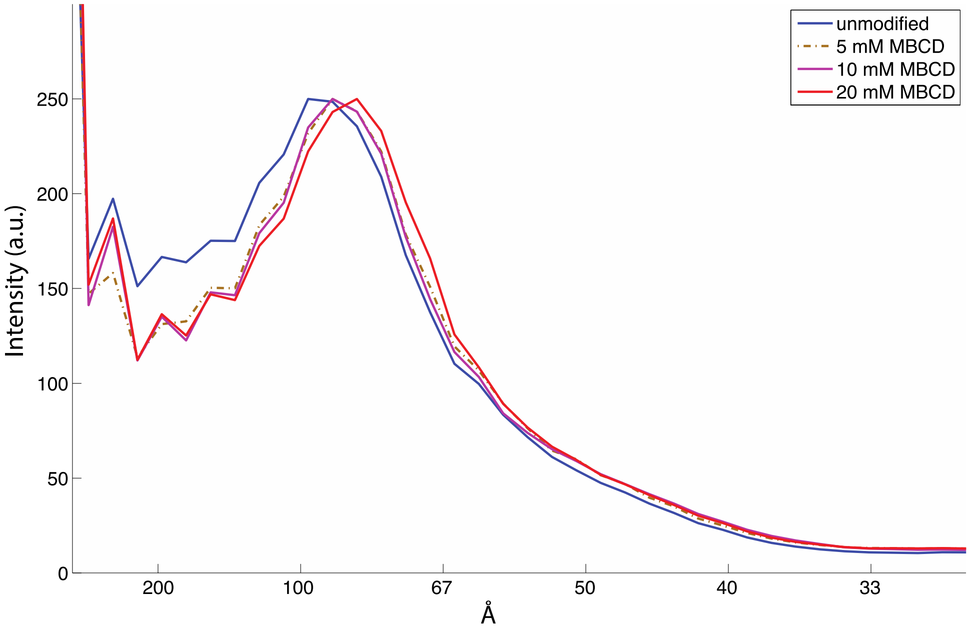

Figure S3. **Radial distribution functions showing hemagglutinin spatial distribution in response to cholesterol**. Average radial distributions of electron density are plotted for unmodified virus and cholesterol-depleted virus with 5 mM, 10, or 20 mM MβCD, using a different viral lot from that analyzed in Figure 2. Approximately 400-600 virions were analyzed per condition, resulting in somewhat higher errors than Figure 2, which used ~4000 virions per condition. Cholesterol levels in these samples were 63% of unmodified virus for 5 mM MβCD, 12% of unmodified virus for 10 mM MβCD, and 4% of unmodified virus for 20 mM MβCD. Modes of the HA-HA distribution peaks are 97 Å for unmodified virus, 90 Å for virus that was cholesterol-extracted using either 5 mM or 10 mM MβCD, and 84 Å for virus that was cholesterol-extracted using 20 mM MβCD.

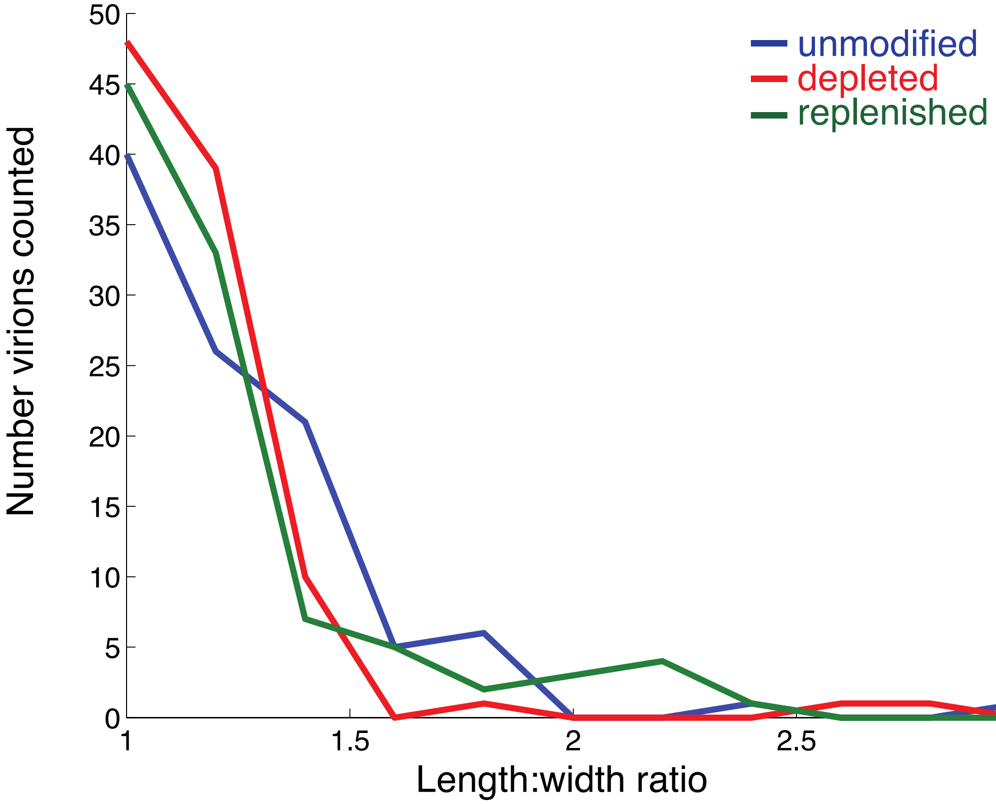

Figure S4. Length-to-width ratios of virions do not change significantly under cholesterol depletion and re-addition. Ratios were calculated using elliptical fits to the virions measured in Figure 4 (100 virions per condition). Statistical comparisons were performed using a 2-sample Kolmogorov-Smirnov test with a Bonferroni multiple hypothesis correction.