Supporting Information

Competition between Normative and Drug-Induced Virus Self-Assembly Observed with Single-Particle Methods

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Control Experiments

Effect of size exclusion chromatography (SEC) and resistive-pulse measurements on the particle size distributions. Resistive-pulse measurements were made in 1 M NaCl for higher signal-to-noise ratio due to the greater number of displaced ions per unit volume. Because high salt drives assembly, the reaction solutions at different salt concentrations were purified with SEC to remove the unassembled dimer and excess drug. To test if SEC filters larger particles present in the samples, DLS measurements were taken before and after this purification step. The assembly reaction was initiated at 20 μM dimer, 80 μM HAP-TAMRA (4:1 molar ratio of HAP-TAMRA to dimer), and 50 mM NaCl to ensure sufficient concentration of particles after purification. **Figure S2a** illustrates that SEC does not significantly alter the size distribution of the particles.

To confirm that resistive-pulse sensing does not selectively pass smaller particles relative the larger ones, the purified sample from assembly of 20 μM dimer with 80 μM HAP-TAMRA (4:1 HAP-TAMRA to dimer molar ratio) and 50 mM NaCl diluted to 1 M NaCl was run for few minutes on devices with two different pore-sizes until >100 particles were measured. As seen in **Figure S2b**, the histograms from resistive-pulse measurements of particles on a 3-pore device with 100 nm wide by 100 nm deep pores and a 3-pore device with 120 nm wide by 120 nm deep pores can be fitted by Gaussian distributions and have similar relative standard deviations. These data show minimal bias, if any, toward selectively passing smaller particles relative to larger particles from the assembly reactions.

Effect of dilution into high salt to the purified assembly products. To ensure that dilution to high salt (1000 mM NaCl) before the resistive-pulse measurements does not alter the size-distribution of the purified particles, histograms from measurements immediately after the dilution to high salt and one day after the dilution were compared. These measurements were collected

after overnight assembly of 5 μM dimer with 20 μM HAP-TAMRA (4:1 HAP-TAMRA to dimer molar ratio) and 300 mM NaCl and SEC purification. As seen in **Figure S3**, the histograms overlap well, which indicates that dilution of the purified reaction solutions to a high salt solution does not significantly alter the size-distributions of the particles on the timescale of the measurements.

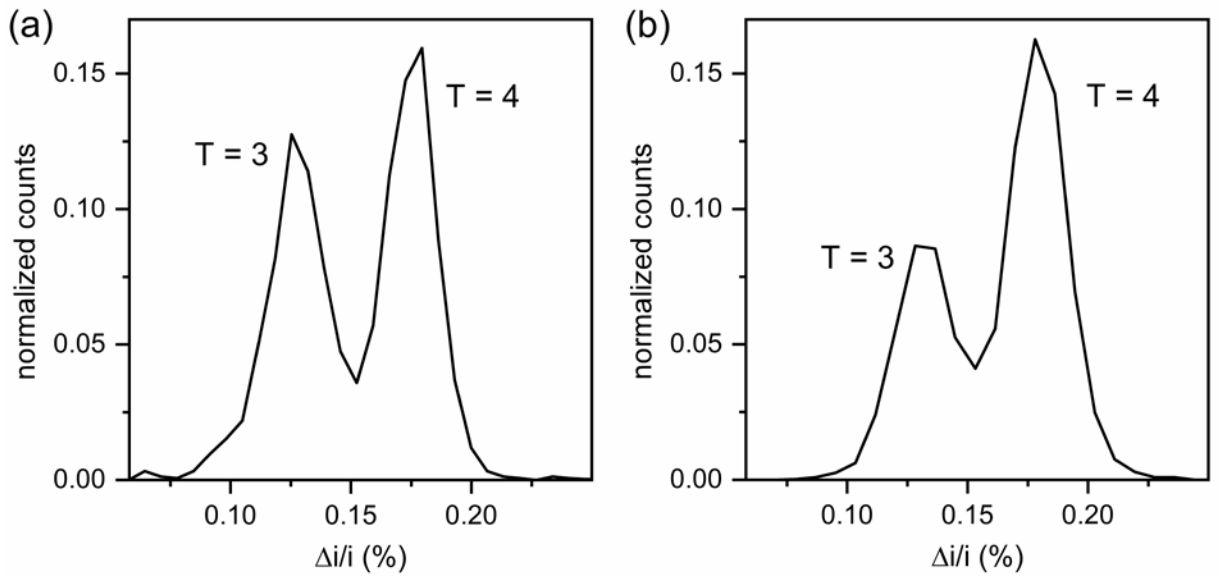

Figure S1. Resistive-pulse measurements of calibration standards and capsid assembly without HAP-TAMRA. (a) Histogram of a 1:1 mixture of T = 3 and T = 4 capsid standards (1 nM each) from resistive-pulse measurements on a 3-pore device used to study HBV assembly in the presence of HAP-TAMRA. T = 3 and T = 4 capsids are easily resolved, and the nanopore device does not discriminate against T = 3 or T = 4 capsids. Also, the ratio of the mean normalized pulse amplitude (Δi/i) distributions of T = 3 to T = 4 capsids equaled 0.73, which compares well to the mass ratio of 0.75 for T = 3 to T = 4 capsids. (b) Histogram of assembly of 5 μM dimer with 1000 mM NaCl in 50 mM HEPES without HAP-TAMRA. As expected, T = 3 capsids are clearly detected in a relative abundance of 20% - 30%.

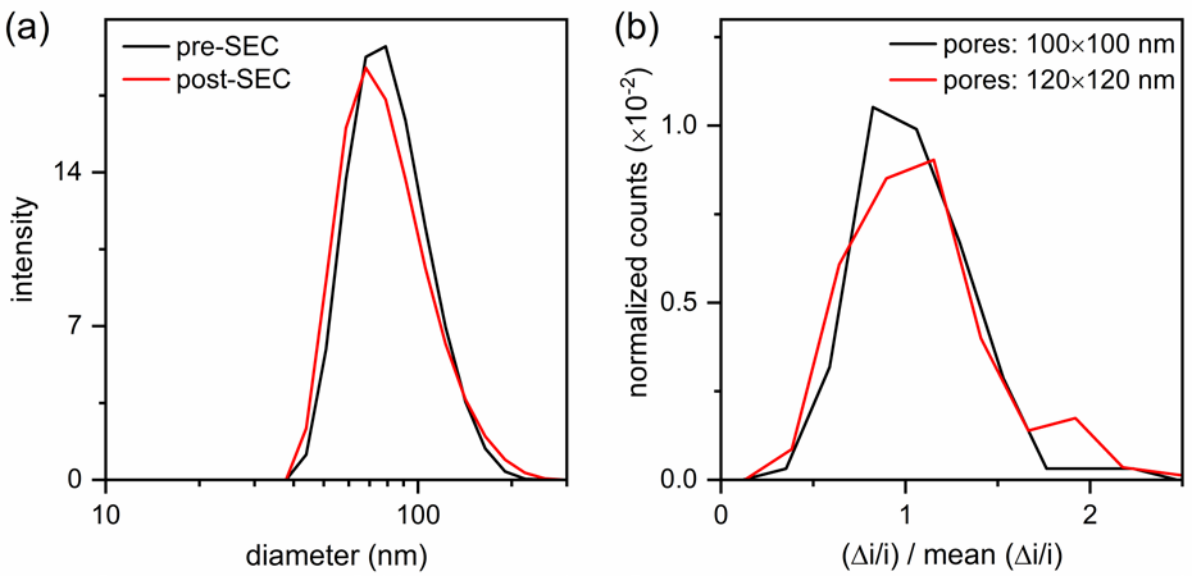

Figure S2. Impact of size exclusion chromatography and resistive-pulse measurements on particle size distributions. (a) DLS measurements of virus particles assembled from 20 μM dimer with 80 μM HAP-TAMRA and 50 mM NaCl in 50 mM HEPES before and after purification with size exclusion chromatography (SEC). The two size distributions overlap well, which indicates that SEC does not filter larger structures. (b) Resistive-pulse measurements of the purified reaction solution on devices with pores 100 nm wide and 100 nm deep and devices with pores 120 nm wide and 120 nm deep. To normalize the x-axis, the signal was divided by the mean of each distribution. Both histograms fit Gaussian distributions with similar relative standard deviations.

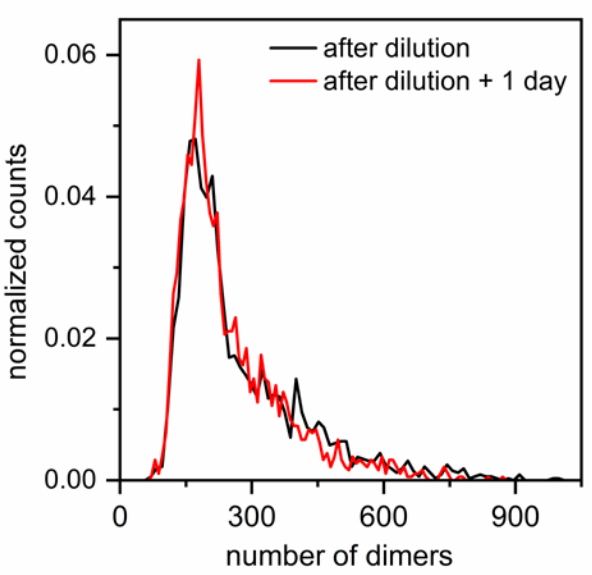

Figure S3. Size distribution of purified products after dilution into high ionic strength buffer. Histograms of purified products from an assembly reaction of 5 μM dimer with 20 μM HAP-TAMRA and 300 mM NaCl in 50 mM HEPES. The histogram represented with the black line was generated by resistive-pulse measurements taken immediately after the dilution to 1000 mM NaCl and 50 mM HEPES. The histogram represented with the red line was taken 1 day after the dilution to 1000 mM NaCl and 50 mM HEPES. This control experiment verifies that dilution of reactions in low salt into a high salt buffer for analysis does not significantly alter the product distribution even after 1 day.