**Detection of Late Intermediates in Virus Capsid Assembly by Charge Detection Mass Spectrometry**

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Supplementary Figures

Figure S1.   Charge detection signal (a) from a single HBV capsid. The signal has been passed
through at 5 kHz high pass filter. The transient at early times is in response to the
switching of the end cap voltages in order to trap the ion. After FFT processing
(b), the ion's fundamental frequency is easily observed at ~9.5 kHz along with the
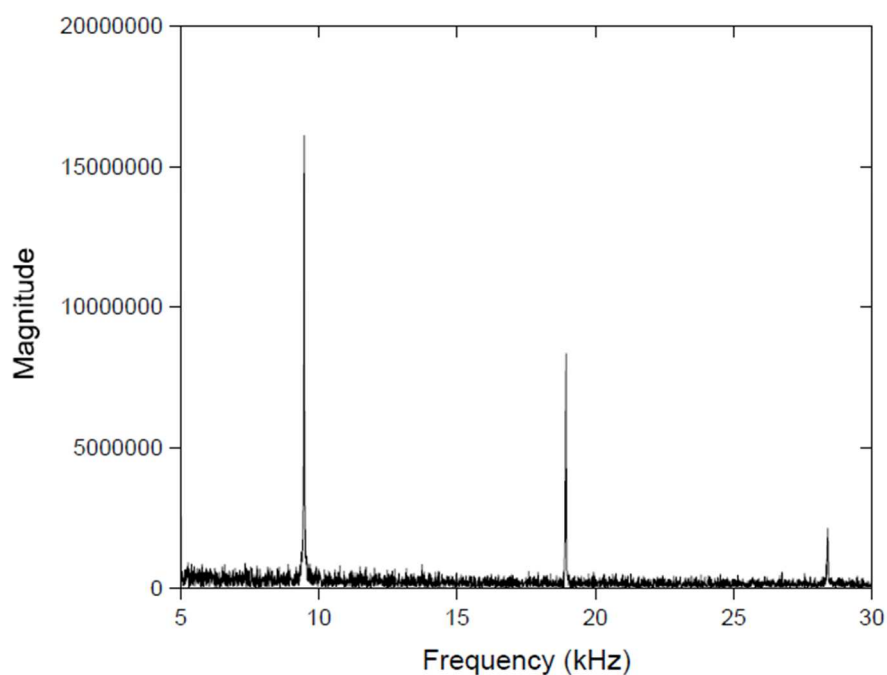first and second harmonics.

a)
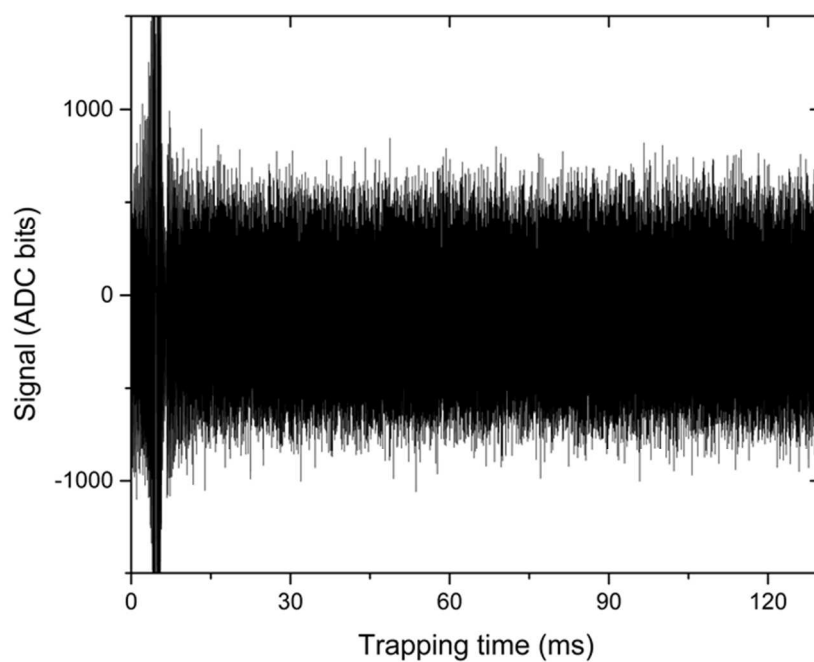
b)

Figure S2. Mass spectrum measured by CDMS for HBV capsids assembled under mild conditions (300 mM NaCl). The spectrum consists of 9767 ions sorted into 20 kDa bins and then smoothed with a five point Savitsky-Golay algorithm. The red line shows an expanded view (x8) of the region between the $T=3$ and $T=4$ peaks.
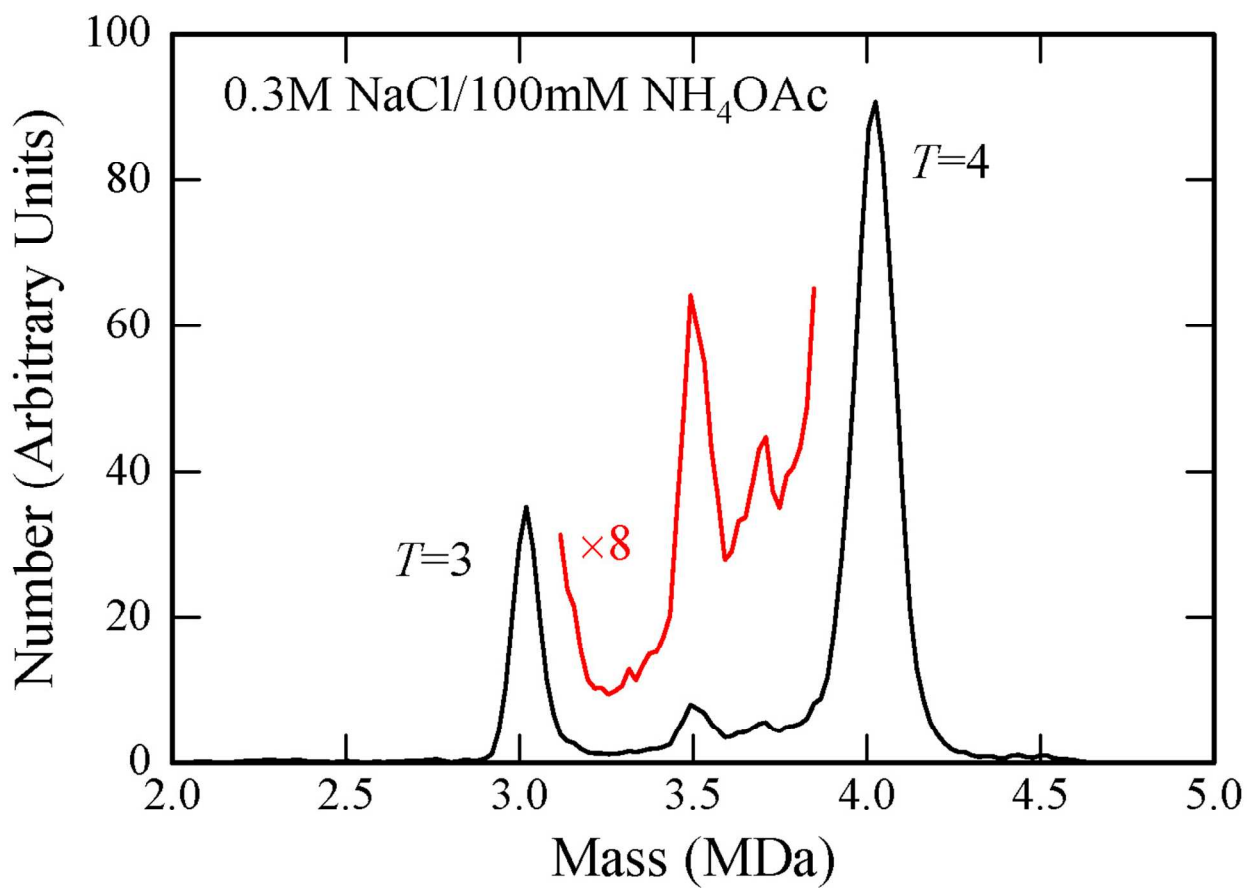
Figure S3. Mass spectrum measured by CDMS for HBV capsids assembled under mild conditions (300 mM NaCl). The spectrum was collected 3 days after dialysis into low ionic strength (100 mM) ammonium acetate. Almost all of the intermediates between the T=3 and T=4 capsid peaks have annealed, presumably by a process where some dissociate to provide free subunits for the remainder to be completed. The spectrum contains 4210 ions sorted into 20 kDa bins and then smoothed with a five-point Savitsky-Golay algorithm. The red line shows an expanded view (x8) of the region between the T=3 and T=4 peaks. This spectrum shows that essentially pure, complete capsids generate the expected mass spectrum.
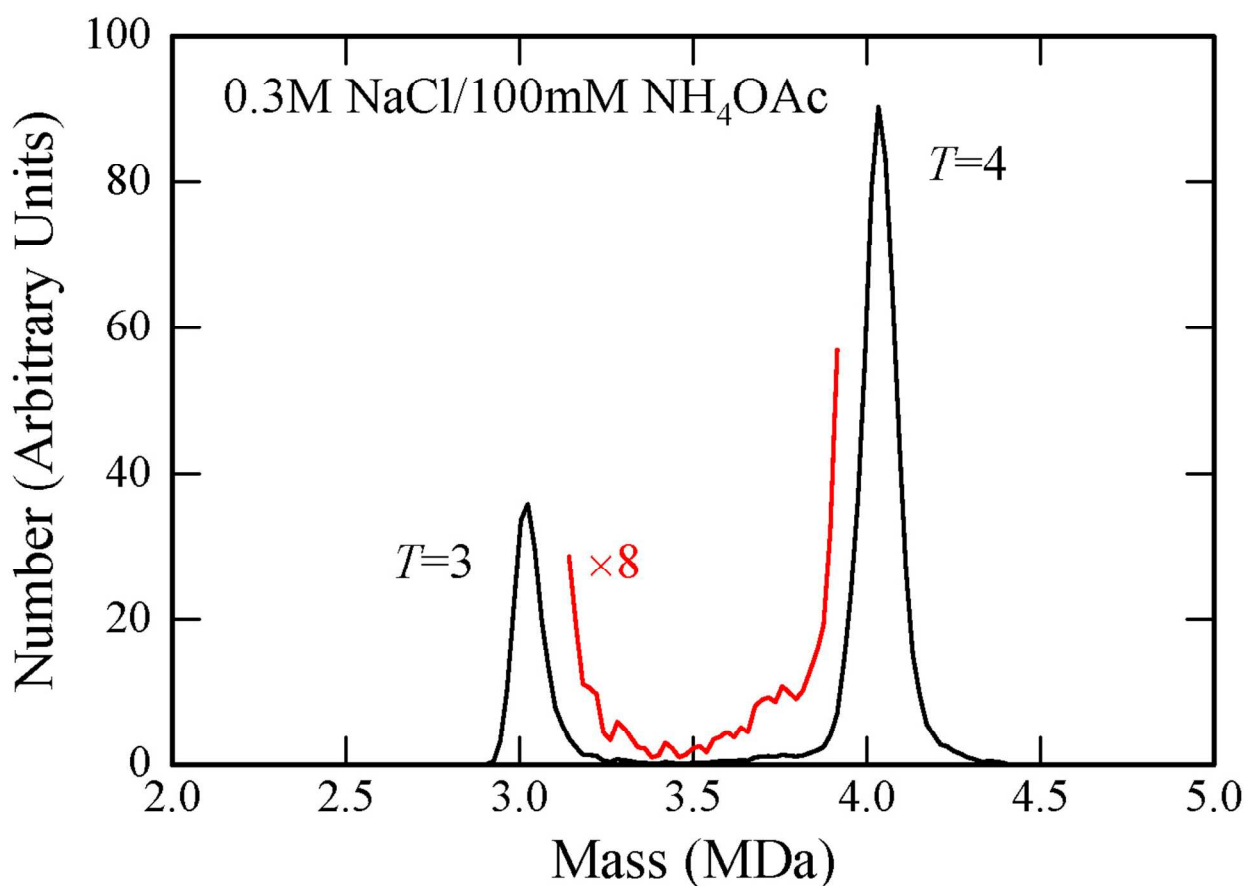
Figure S4. Representative Cryo-EM micrograph of HBV capsids. Black arrowhead, *T*=3 capsid. White arrowhead, *T*=4 capsid. A *T*=4 capsid with defects, a partial capsid in an arc shape, and a doublet of half capsids are indicated. Scale bar, 100 nm.
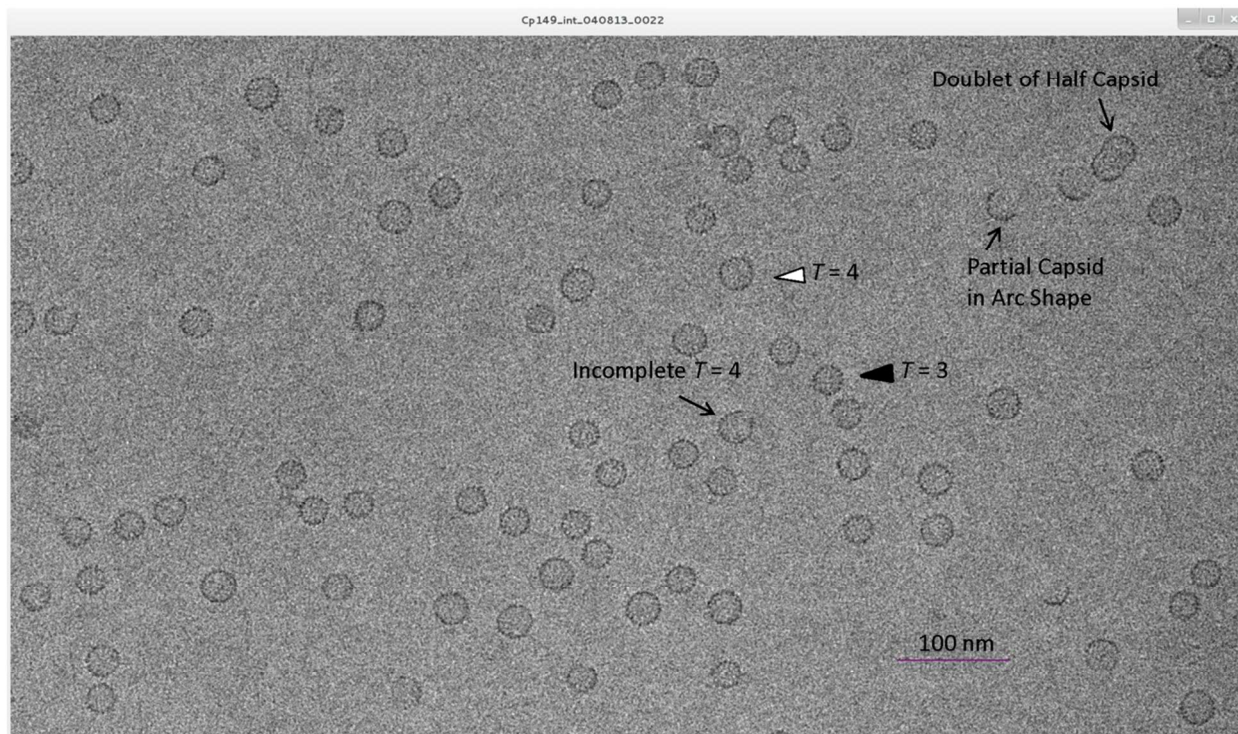
Figure S5. Class averages from the 2-D image clustering and classification of HBV capsids assembled in 1M NaCl. 5681 images were subjected to CL2D classification using XMIPP software. The final number of classes was limited to 28 as this left an average of 200 particles per class and further division of the data set revealed no further unique features. a) Shows the resulting class averages from the initial 4 classes. Class #0 and #1 represent T=3 capsids, #2 represents T=4 capsids, and #3 represents capsids with defects. b) Shows the final result of 28 class averages. The yellow numbers in the lower right hand corners show the assignment of the classes to complete T=3 and T=4 capsids. The incomplete capsids are circled.
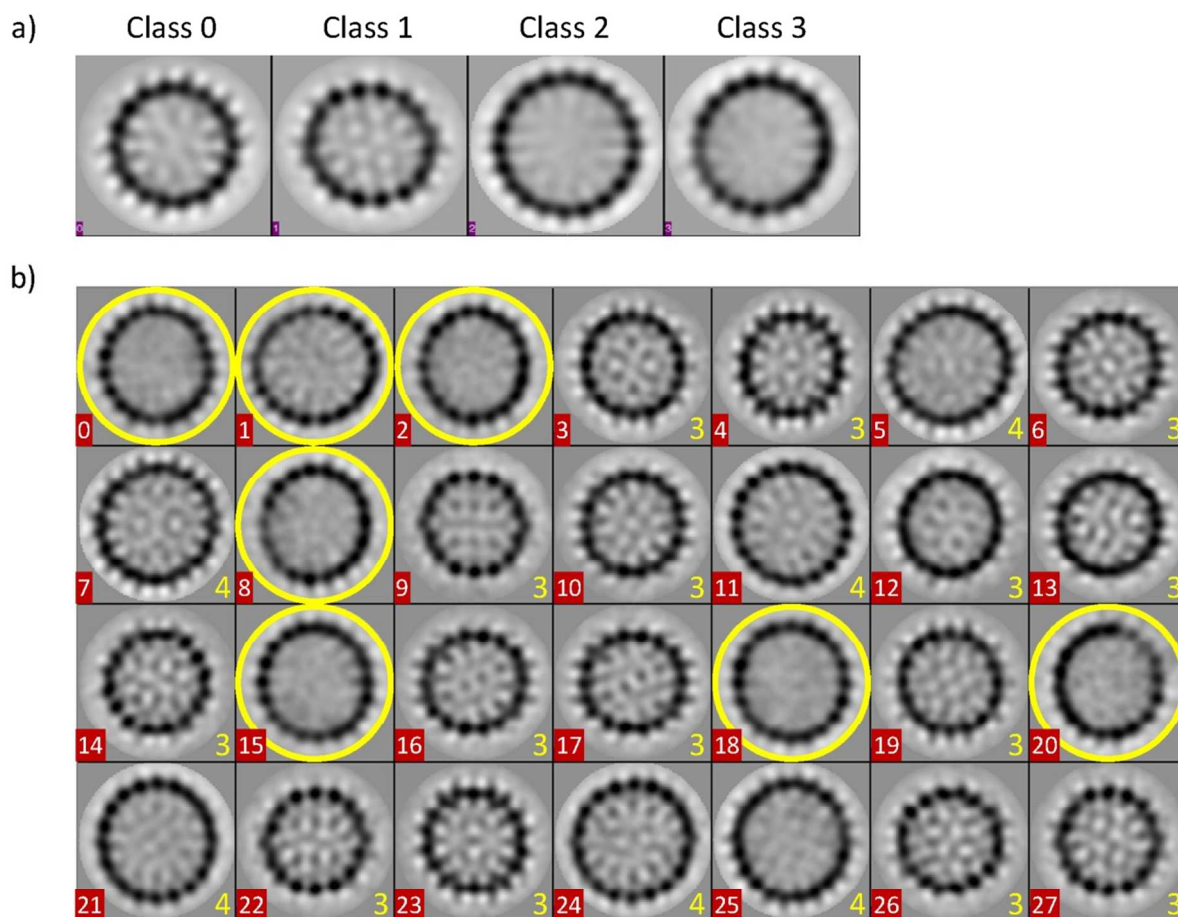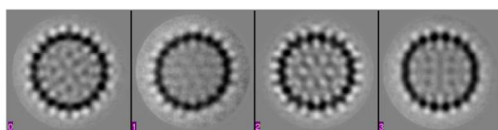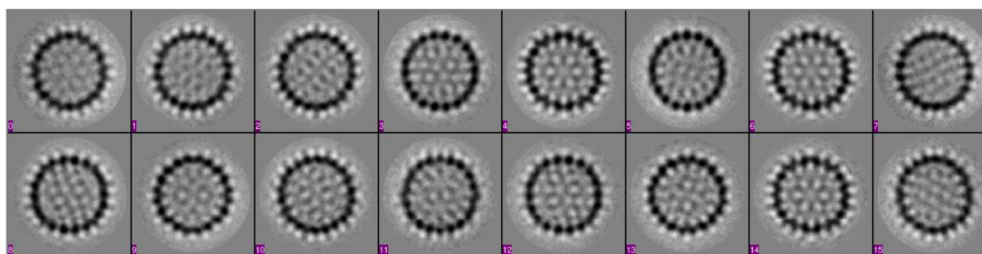
Figure S6. Class averages from the 2-D image clustering and classification of HBV capsids assembled in 0.15 M NaCl. 8064 images of *T*=4 capsids were subjected to CL2D classification using XMIPP software. a) The initial 4 classes from the first level classification. b) Shows the result from the third level of classification (16 class averages). c) Shows the final result of 40 class averages. No classes with a missing wedge of protein were observed and the capsomer organization was well defined in all class averages. This demonstrates that the incomplete capsids were not an artifact of the classification scheme.

a)

b)

c)