Figures and figure supplements

All-atom molecular dynamics of the HBV capsid reveals insights into biological function and cryo-EM resolution limits

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Figure 1. Capsid size, stability, and morphology. (a) The T = 4 HBV capsid is composed of 120 copies of Cp homodimer, where A (red) and B (beige) quasi-equivalent chains comprise AB dimers, and C (cyan) and D (blue) quasi-equivalent chains comprise CD dimers. (b) A polyhedron of 240 triangular faces was used to estimate capsid inner volume and sphericity; see Materials and methods. Polyhedron shown within a cross-section of the capsid, with fivefold (red spheres), sixfold (beige spheres), and threefold (blue spheres) vertices indicated. (c) Capsid inner volume increased and stabilized within 0.05 μs. (d) Capsid sphericity remained high throughout the simulation. DOI: https://doi.org/10.7554/eLife.32478.002
RMSD of complete capsid. Cα RMSD of the capsid (Å), measured pairwise between sampled conformations, converged to within 5 Å around 0.1 µs. This indicates the total simulation time necessary for the capsid to relax from its crystallographic state and equilibrate to a stable configuration under physiological conditions.

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Figure 1—figure supplement 1.
Figure 2. Flexibility of Cp dimers. (a) Schematic of Cp dimer, with helices 1 through 5 indicated. (b) Average Cα RMSF (Å) over 60 copies of AB dimers. (c) Average Cα RMSF (Å) over 60 copies of CD dimers. CD dimers are more flexible than AB dimers, with greater mobility in the spike tips. Calculations are based on internal alignment of dimers extracted from the capsid simulation, totaling 60 µs of conformational sampling for each dimer group. Error bars are given as standard deviation. (d) 3D histogram of AB dimer spike tip motions in the xy plane, given alignment of dimers along the z-axis. (e) 3D histogram of CD dimer spike tip motions in the xy plane, given alignment of dimers along the z-axis. CD dimer spike tips sample a larger spatial area than AB dimer spike tips. Histograms are based on 60 µs of conformational sampling for each dimer group, or a total of 3 M conformations. See Materials and methods for more information on dimer alignments and spike tip definitions.

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Figure 3. RMSF of complete capsid. (a) Average Cα RMSF (Å) of Cp dimers. Calculations are based on internal alignment of dimers extracted from the capsid simulation (local dynamics, bottom curve) and alignment of the full capsid (global dynamics, top curve), totaling 120 μs of conformational sampling. The increase in apparent dimer flexibility in the latter case arises from spatial movements of dimers relative to each other within the capsid assembly. (b) Projection of RMSF values onto their corresponding locations in the capsid model instead of averaging over dimer copies reveals clear asymmetry in global dynamics. Fivefold vertices are highlighted with stars.

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Figure 3—figure supplement 1. Deviations in transformations between dimers of neighboring subunits. (a) Schematic showing the relationship of capsid chains around a fivefold symmetry axis. A given set of Euler angles $\theta_1$, $\theta_2$, and $\theta_3$ transform the dimers composed of chains AB and CD to their neighbors A'B' and C'D'. (b) The Euler angles required to transform the chains AB and CD to their neighbors A'B' and C'D' show deviations in excess of ±8° relative to the idealized structure. These altered Euler angles produce deviations in displacements of neighboring chains up to ±8 Å compared to a symmetric structure.

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Figure 4. Spatial fluctuations of pentamers with respect to the capsid center. (a–f) Pentamers in the upper hemisphere (red) of the capsid are shown paired with their counterparts across the twofold symmetry axis in the lower hemisphere (blue). Icosahedral schematics indicate the relative locations of the measured distances (Å) within the capsid. Distances are measured between the center of mass of pentamers and the center of mass of the capsid. Variations in distance profiles indicate asymmetric distortions in capsid shape over time.
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Figure 5. Essential dynamics of the capsid. (a–f) Views of the first mode from PCA, which comprises only 6% of total variance, illustrate the striking complexity and intrinsic asymmetry of capsid dynamics. PCA calculation based on Cα trace of 50,000 conformations sampled over 1 μs of simulation. Mode representation shown as a porcupine plot projected onto a polyhedral surface with pentamers highlighted in red; eigenvalue scaling increased by five for visual clarity.
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Figure 5—figure supplement 1. Scree plot for PCA modes. Scree plot showing percent contribution to total variance for the first 100 modes determined by PCA of the capsid.
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Figure 6. Theoretical density maps. (a) A representative density map calculated for a single conformer sampled during simulation. The map necessarily shows atomic detail, with individual side chains clearly visible. (b) An averaged map calculated as the mean of 1,000 individual maps, such as that in panel a, based on conformers extracted from the simulation at 1-ns intervals. All maps used in the calculation share a common orientation, based on alignment on the capsid’s Cα trace. Averaging clearly reduces molecular detail. (c) A map calculated by icosahedral averaging of the map in panel b. With icosahedral averaging, a technique commonly used in experimental structure determination, molecular detail is further reduced and features at low spatial frequency become more apparent. For map calculations, individual atoms were treated as Gaussian density distributions with a width of 1 Å at half maximal density and pixel size of 0.75 Å.

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Figure 6—figure supplement 1. FSC analysis of theoretical density maps. (a) Two average maps, each based on 500 randomly selected maps from the ensemble of 1,000 maps, were calculated with a width of 1 Å at half.
maximal density. The FSC between the two maps falls below the cutoff of 0.143 (dotted line) at 0.44, the inverse of 2.3 Å, indicating the resolution of the map based on the full ensemble. (b) Repeating the analysis from panel a with maps calculated with widths of 0.5 Å and 1.5 Å at half maximal density produces an equivalent FSC curve, demonstrating that the selection of this parameter does not introduce bias. (c) Repeating the analysis from panel a with an icosahedrally averaged map shows increased correlation at lower spatial frequencies, but does not alter the predicted resolution.

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Figure 7. Comparison of Ca RMSF with theoretical local resolution and experimental B-factors. (a) Per-residue comparison of RMSF and local resolution; average RMSF is 2.03 Å, while average local resolution is 2.65 Å. For reference, the global FSC value of the theoretical density map is 2.3 Å. (b) Per-residue comparison of normalized RMSF and normalized local resolution with normalized experimental B-factors, reported as an average based on the two available apo-form HBV capsid crystal structures. To place metrics on the same scale for comparison, values \((x)\) minus their average \((\mu)\) are normalized in units of standard deviation from the mean \((\sigma)\). (c) Relative changes between normalized RMSF and normalized local resolution are highly correlated. (d) Relative changes between normalized RMSF and normalized experimental B-factors are also highly correlated, although noisier.

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Figure 8. Exchange rates for water and ions crossing the capsid surface. Cumulative numbers of solvent species moving inward (blue) and outward (red) across the capsid surface over a given segment of simulation time are plotted versus that simulation time, and the slope of the linear fits give the exchange rates, which are reported as averages ± standard deviations. (a) Water molecules exchange at a rate of $4.7 \times 10^3 \pm 0.03 \text{ ns}^{-1}$ inward and...
Figure 8 continued

4.7 × 10³±0.04 ns⁻¹ outward. (b) Sodium ions exchange at a rate of 8.4±0.7 ns⁻¹ inward and 8.5±0.6 ns⁻¹ outward.
(c) Chloride ions exchange at a rate of 1.8±0.4 ns⁻¹ inward and 1.7±0.3 ns⁻¹ outward.

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Figure 8—figure supplement 1. Exchange rates for water and ions crossing a spherical surface. Cumulative numbers of solvent species moving inward (blue) and outward (red) across the spherical surface of radius 145.4 Å over a given segment of simulation time are plotted versus that simulation time, and the slope of the linear fits give the exchange rates, which are reported as averages ± standard deviations. (a) Water molecules exchange at
an average rate of $233.1 \times 10^3 \pm 2.1 \text{ ns}^{-1}$. (b) Sodium ions exchange at an average rate of $412.3 \pm 93.3 \text{ ns}^{-1}$. (c) Chloride ions exchange at an average rate of $516.6 \pm 121.1 \text{ ns}^{-1}$.

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Figure 9. Sodium ion occupancy. (a) View of two AB and three CD dimers from the capsid exterior, illustrating sodium ion localization (yellow) within the pores at the center of trimers of dimers, where three copies each of D2.
Figure 9 continued

D4, E14, D40, and E43 (black spheres) aggregate. Isosurface contour level at ΔG = -1.0 kcal mol⁻¹. Calculation based on alignment of 60 asymmetric units with surrounding solvent, totaling 60 μs of conformational sampling.

(b) Cross-section of the capsid showing sodium ion localization (yellow) in a shell along the interior and in arcs above the spike tips. Isosurface contour level at ΔG = -0.5 kcal mol⁻¹. Calculation based on alignment of full capsid with surrounding solvent, totaling 1 μs of conformational sampling.

(c) View of the asymmetric unit from the capsid interior, illustrating sodium binding (yellow) at E117, adjacent to E113 (black spheres). Isosurface contour level at ΔG = -1.4 kcal mol⁻¹. Calculation based on alignment of 60 asymmetric units with surrounding solvent, totaling 60 μs of conformational sampling. Inset: Schematic showing sodium binding locations within extended capsid lattice. ΔG error estimates are all within 4 x 10⁻⁵ kcal mol⁻¹.

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Figure 9—figure supplement 1. Chloride ion occupancy. View of an AB dimer from the capsid exterior illustrating chloride binding (cyan), which mediates a contact between R28 and R39 from one Cp and the positively charged N-terminal M1 from its partner. Equivalent interaction occurs in the CD dimer. Isosurface contour level at $\Delta G = -0.8$ kcal mol$^{-1}$. Error estimates for $\Delta G$ are within $4 \times 10^{-5}$ kcal mol$^{-1}$.

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