Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary Information for: Free Energy Landscapes of Sodium Ions Bound to DMPC-Cholesterol Membrane Surfaces at Infinite Dilution

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System equilibration

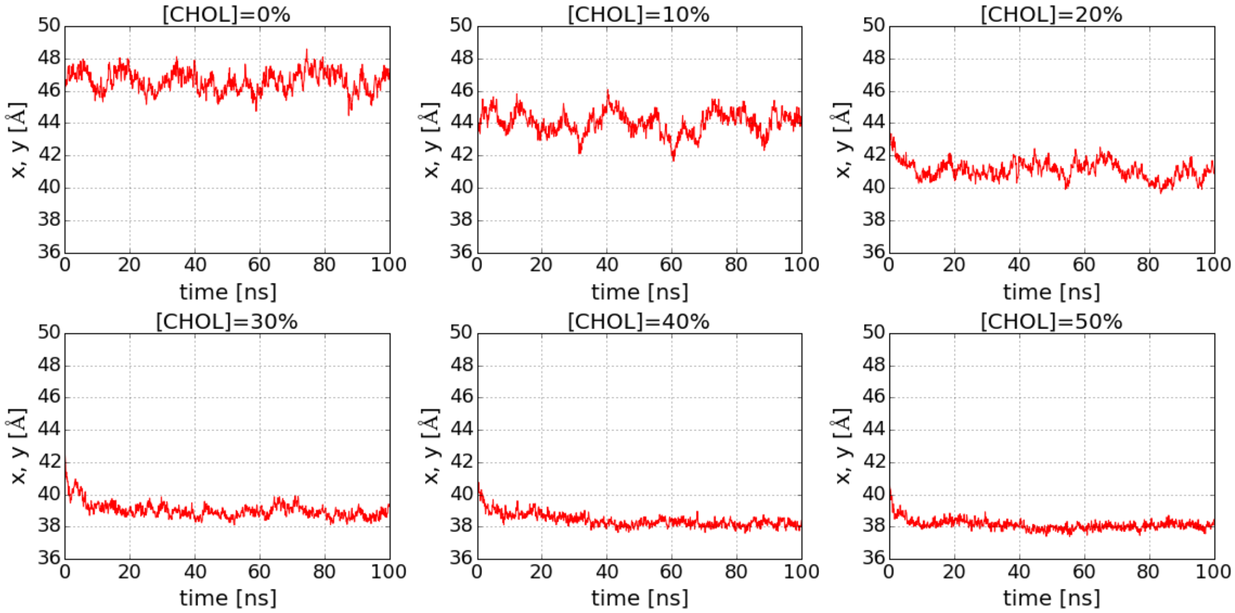

Figure S1: Lateral plane size x and y as a function of time during equilibration. The lengths of x and y are kept the same.

Table S1: After equilibration for 100 ns in the NPT ensemble, the system sizes are fix to perform well-tempered metadynamics in the NVT ensemble. The system sizes for membranes with cholesterol concentration [CHOL] varying between 0% and 50% are listed below.

cholesterol concentration	x [Å]	y [Å]	z [Å]	area per molecule [Å^2]
0%	46.7696	46.7696	84.8519	60.76
10%	44.2460	44.2460	92.8678	54.38
20%	40.7959	40.7959	107.122	46.23
30%	39.2401	39.2401	112.749	42.77
40%	38.3779	38.3779	115.639	40.91
50%	38.4103	38.4103	113.922	40.98

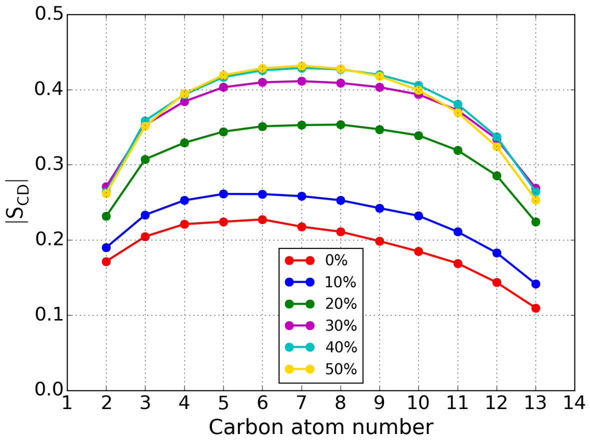

Figure S2: Order parameter $|S_{CD}|$ for the acyl tails of DMPC with cholesterol concentration varying between 0% and 50%. Data are averaged from the last 20 ns of equilibration.

Finite size effect

To discard a finite size effect in our results obtained from systems containing 72 lipids. We have performed regular MD simulations on DMPC-Cholesterol systems containing 72 and 128 lipids in contact with a NaCl solution at the concentration of $\sim$ 200 mM. We have run the simulations in the NPT ensemble for 200 ns and analyzed the binding of the ions to the membrane and water from the last 100 ns trajectories. Doing this we have been able to characterize the lower free energy states of the ion with lipids (characterized by CLP) and water (given by CWT). The results showing the frequency of each of the (CLP, CWT) binding states are provided in Figure S3. The results, for the cases with [CHOL] = 0%, 20%, and 30%, show that the lower binding states are the same (within the statistical error of these simulations) for both systems containing 72 and 128 lipids.

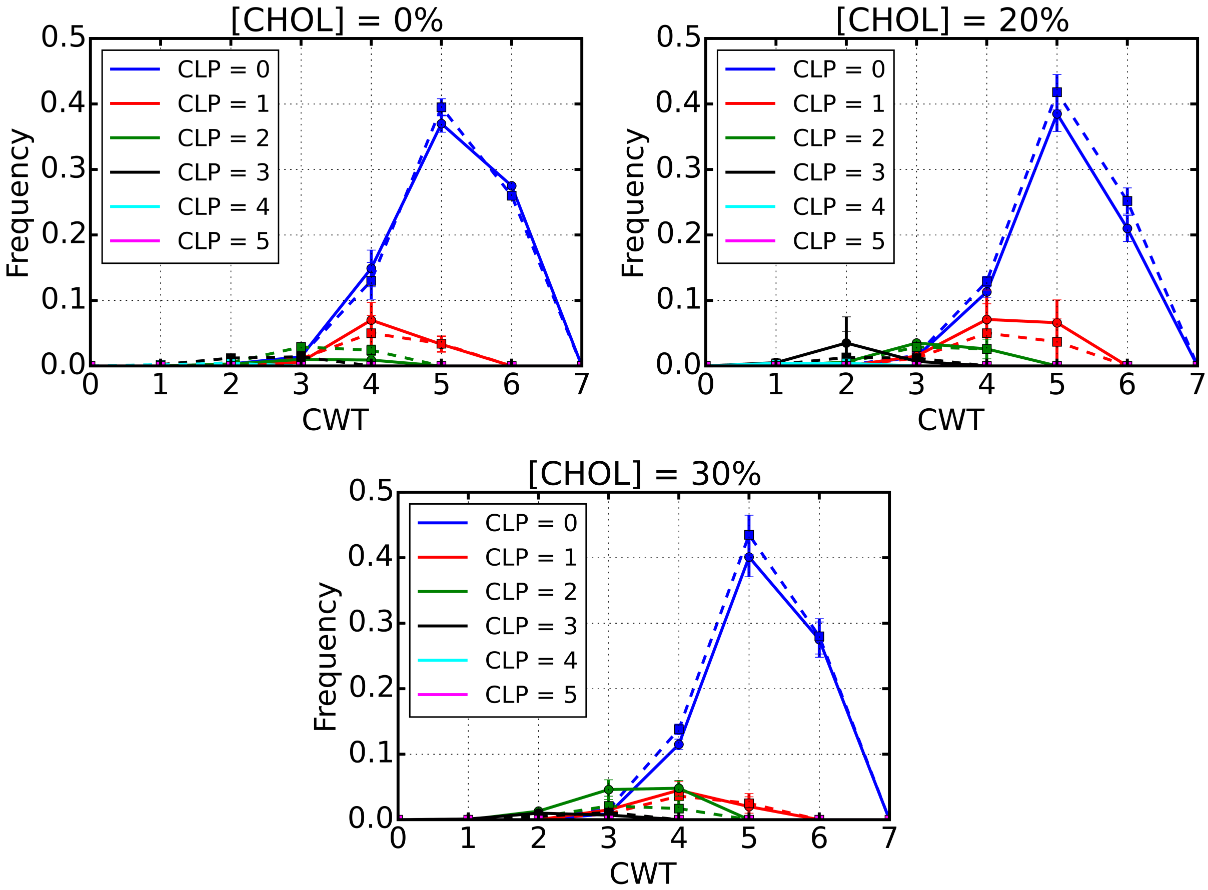

Figure S3: Frequency of binding states of Na$^+$ ion with the binding sites in lipids (CLP) and water molecules (CWT). The dots connected by solid lines correspond to the cases with 72 lipids and the squares connected by dashed lines correspond to the cases with 128 lipids.

Parameters for CLP

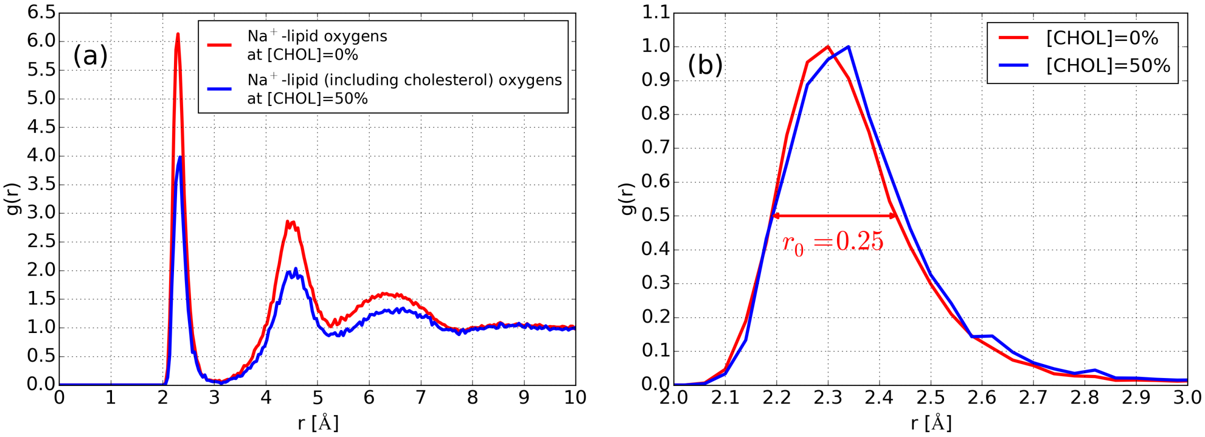

Figure S4: The determination of d_0 and r_0 for CLP at cholesterol concentration [CHOL] of 0% and 50%. Figure S4a shows the radial distribution function g(r) between Na$^+$ and lipid oxygens at [CHOL]=0% and that between Na$^+$ and lipid (including cholesterol) oxygens at [CHOL]=50%. Figure S4b is a close look on the normalized first coordination shell of Figure S4a. For [CHOL]=0%, the peak of g(r) is located at $d_0 = 2.3$ Å, and the width at half maximum of the peak is $r_0 = 0.25$ Å. For [CHOL]=50%, the values of d_0 and r_0 are very similar with the case of [CHOL]=0%. Therefore, we choose the same values of $d_0 = 2.3$ Å and $r_0 = 0.25$ Å for CLP at different cholesterol concentrations.

Free energy convergence

Free energy convergence is estimated by the diffusion behaviors of CVs (Figure S5 and S6), decay of HILLS Height (Figure S7), and free energy difference between unbound states (CLP < 0.5) and bound states (CLP > 0.5)(Figure S8).

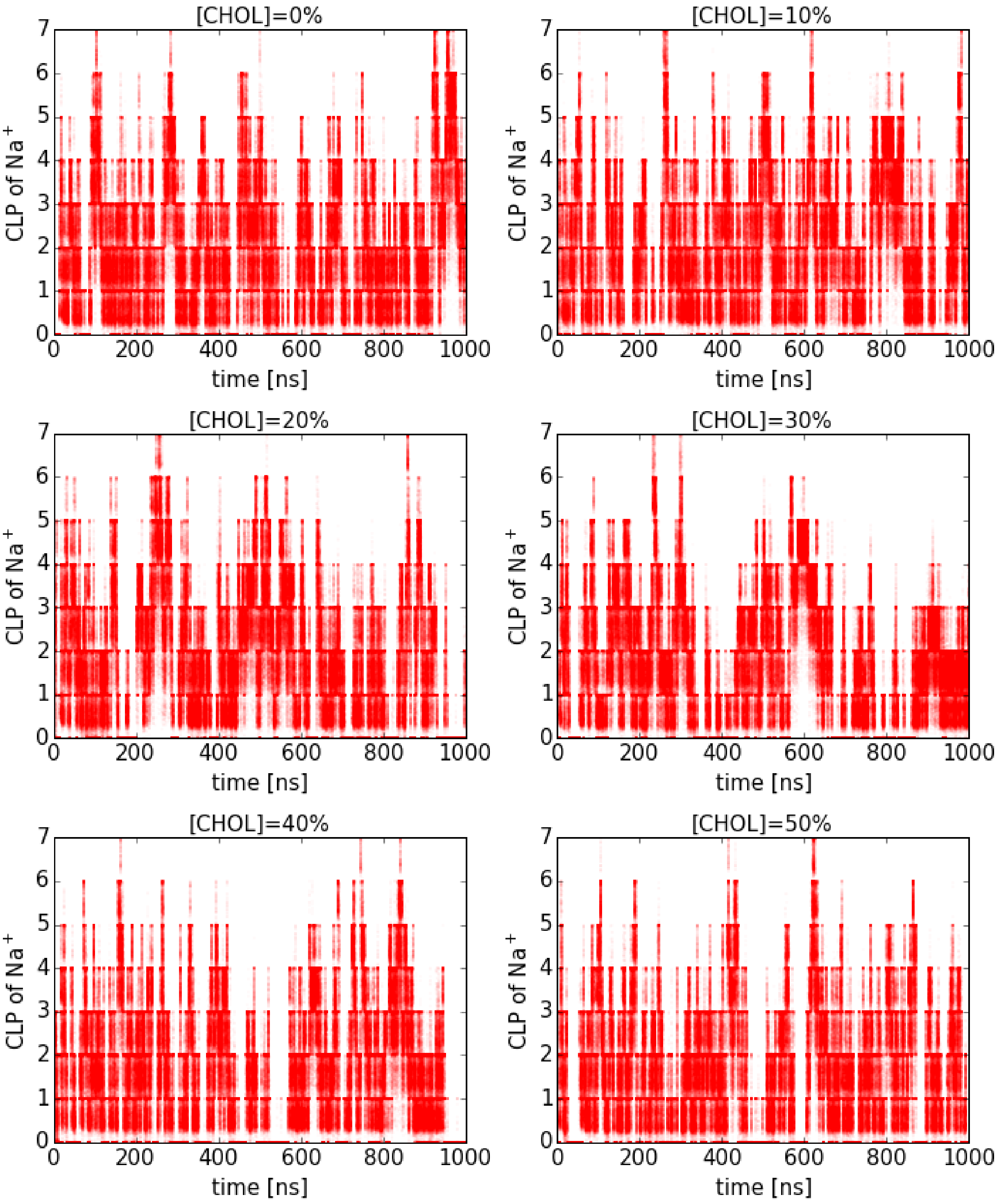

Figure S5: Coordination number between a Na$^+$ ion and lipid (including cholesterol) oxygens (CLP) as a function of time.

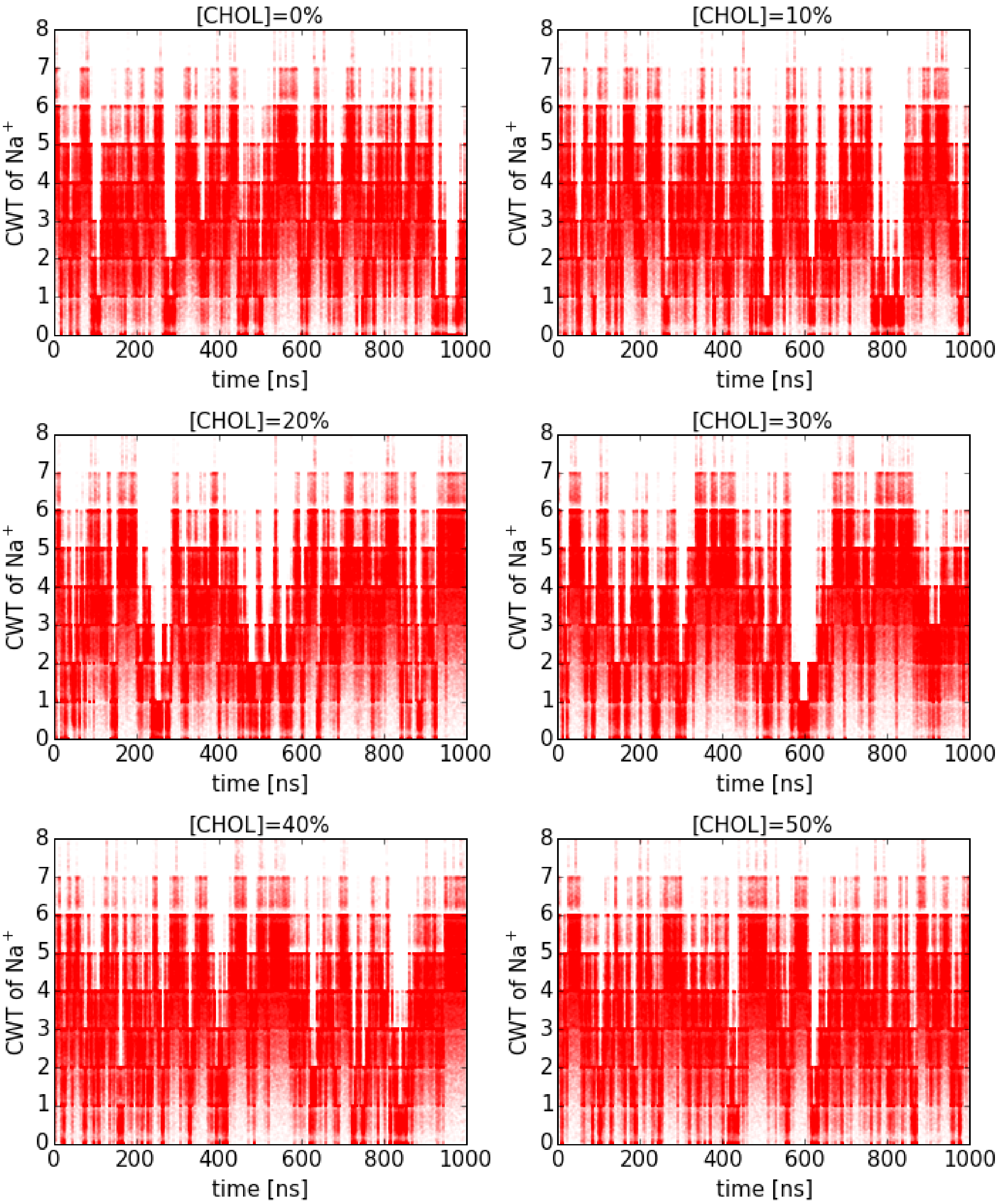

Figure S6: Coordination number between a Na$^+$ ion and water oxygens (CWT) as a function of time.

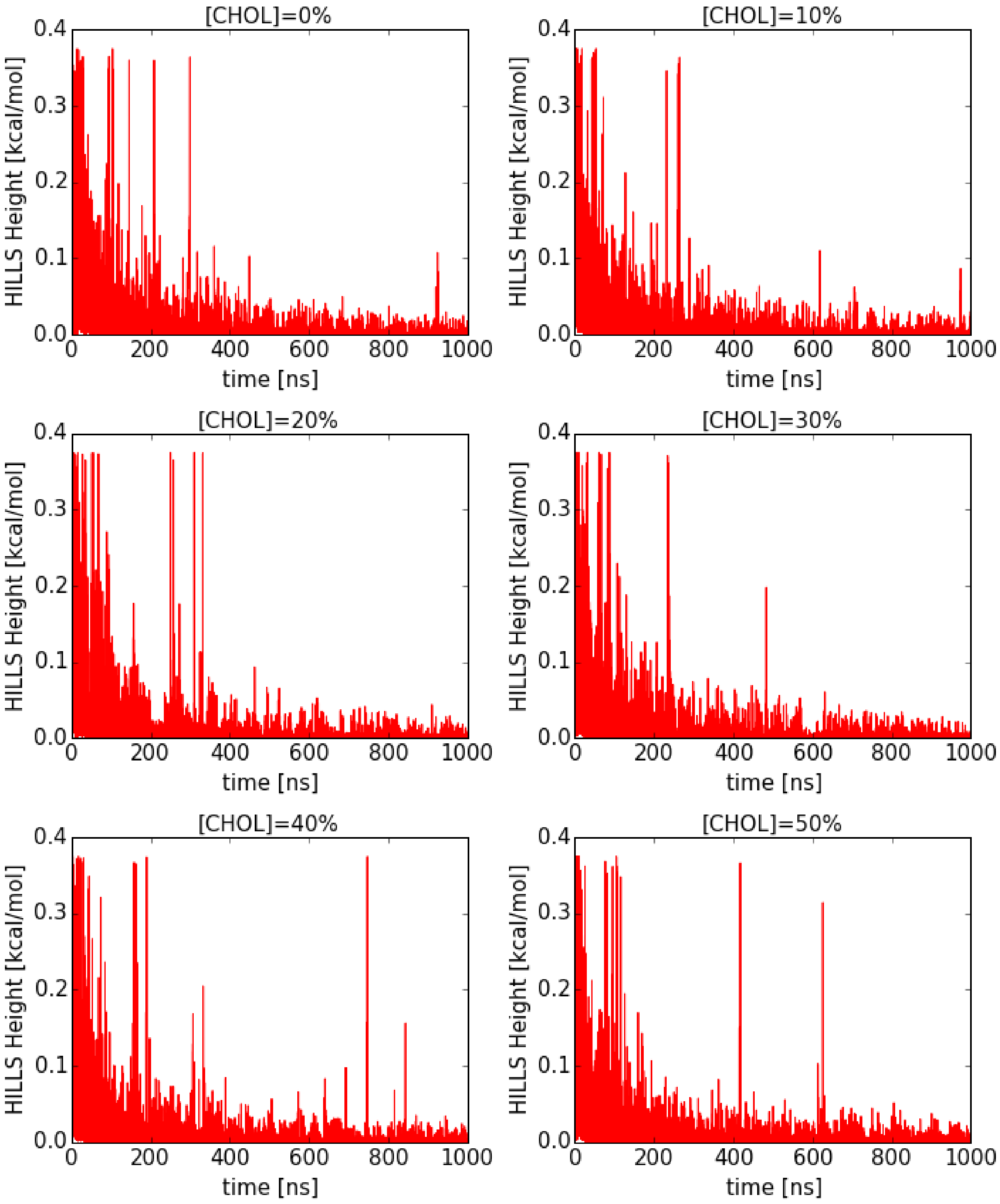

Figure S7: Well-tempered metadynamics HILLS Height as a function of time.

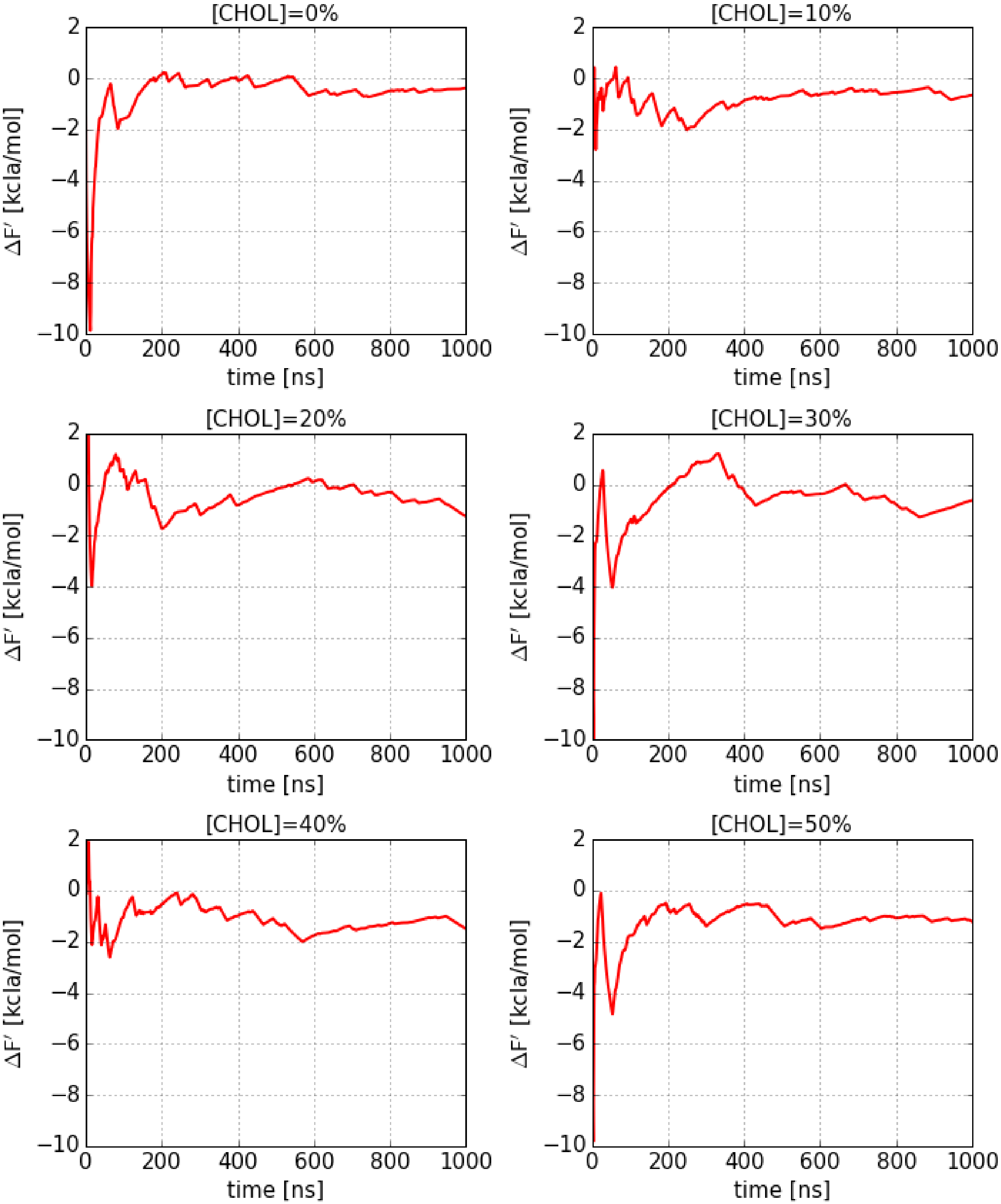

Figure S8: We monitor the convergence of the free energy as a function of CLP (Figure 3 in the paper) by calculating ΔF', the free energy difference between unbound states (CLP < 0.5) and bound states (CLP > 0.5) as a function of time. The average values and standard deviations of ΔF' calculated from the last 100 ns are shown in Figure 4 in the paper.