



# The Dynamic Binding of Cholesterol to the Multiple Sites of C99: Revealed by Coarse-Grained and All-Atom Simulations

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# Supporting information

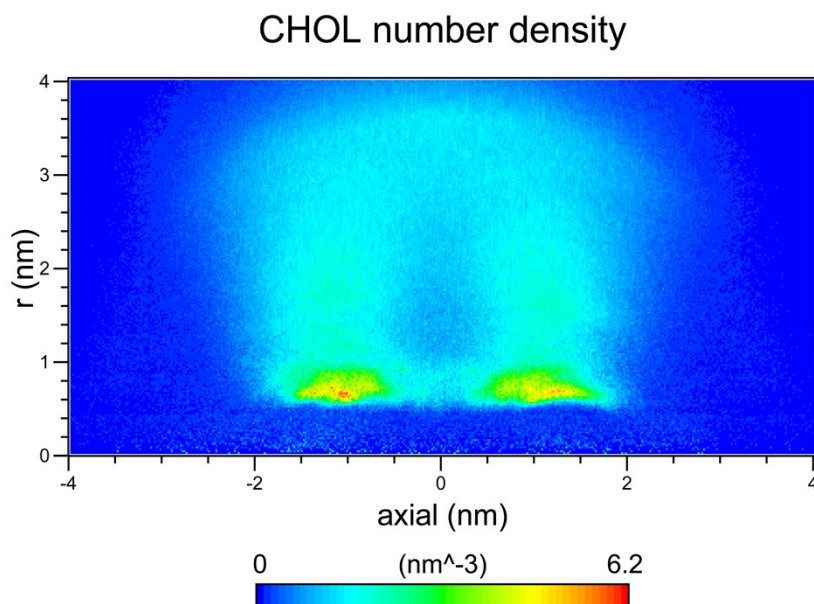
**Table S1.** Summary of simulations

Number of Proteins	Membrane Composition	Initial protein structure	Cholesterol (20%)	Simulation type	Sample number
1	DPPC	2LP1	YES	3 $\mu$ s CG	10
1	DPPC	EbrB2 TM	YES	3 $\mu$ s CG	10
1	DPPC	equilibrated	YES	3 $\mu$ s	10
		2LP1		restraint CG	
1	DPPC	2LP1	YES	1 $\mu$ s AT	1

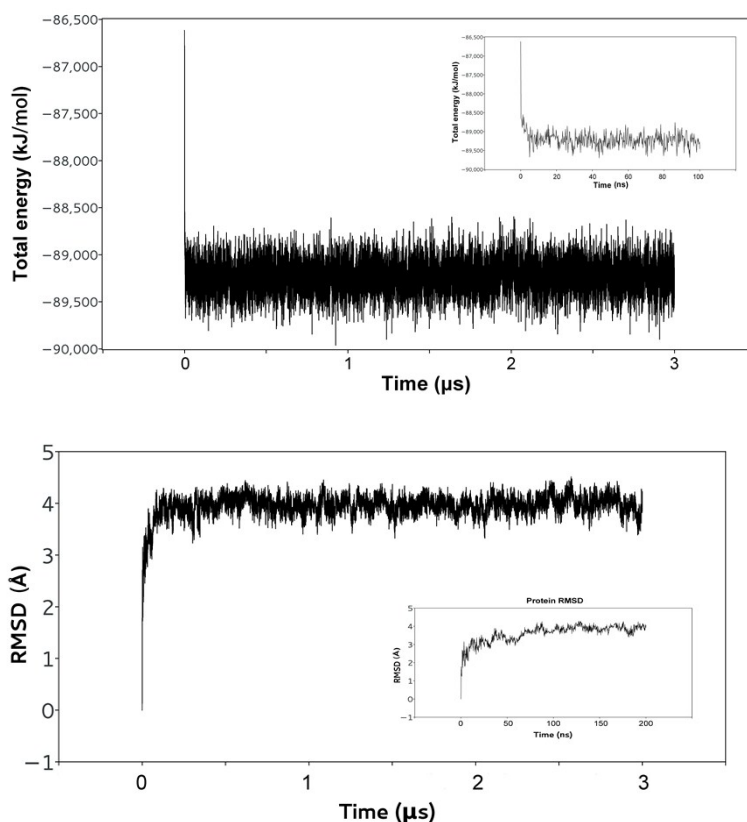
**Table S2.** The hydrogen bonds of each binding site characterized by VMD.

Site	Hydrogen bond
<b>No. 0</b>	ALA <sub>701</sub> -Main 0.30%, PHE <sub>690</sub> -Side 0.05%, GLY <sub>700</sub> -Main 0.04%, LYS <sub>687</sub> -Main 2.66%, VAL <sub>689</sub> -Main 0.08%, LYS <sub>687</sub> -Side 0.03%, GLN <sub>686</sub> -Main 0.01%
<b>No. 1</b>	PHE <sub>690</sub> -Main 23.25%, ALA <sub>701</sub> -Main 0.20%
<b>No. 1</b>	SER <sub>697</sub> -Main 24.00%
<b>No. 2</b>	LYS <sub>699</sub> -Side 2.00%, LYS <sub>699</sub> -Main 0.01%
<b>No. 3</b>	THR <sub>719</sub> -Side 0.02%
<b>No. 4</b>	LYS <sub>725</sub> -Side 2.59%
<b>No. 5</b>	GLN <sub>727</sub> -Side 0.24%, LYS <sub>726</sub> -Main 0.67%
<b>No. 6</b>	None

Note: **No. 1** is for the tightly binding state, and **NO. 1** is for the loosely binding state at site1.



**Figure S1.** The chol number density from the simulation of ebrB2 in DPPC bilayer. To ensure that our conclusions are robust and to explore the common features of the association, we performed the CG simulations of another proteins ebrB2 (the members of the family of epidermal growth factor receptors) in DPPC bilayer. The method of the axial-radial number-density map is consistent with that in Figure 1B legend.



**Figure S2.** The top panel is total energy analysis. The total energy of system has been convergent and reached the platform phase in 100 ns.

The bottom panel is the RMSD analysis of protein, which shows that the conformation of protein has been convergent and reached the platform phase in 100 ns. The data was calculated from the free 3 $\mu$ s CG simulation of 2LP1 in DPPC bilayer.

Figure S3. Binding Site 0

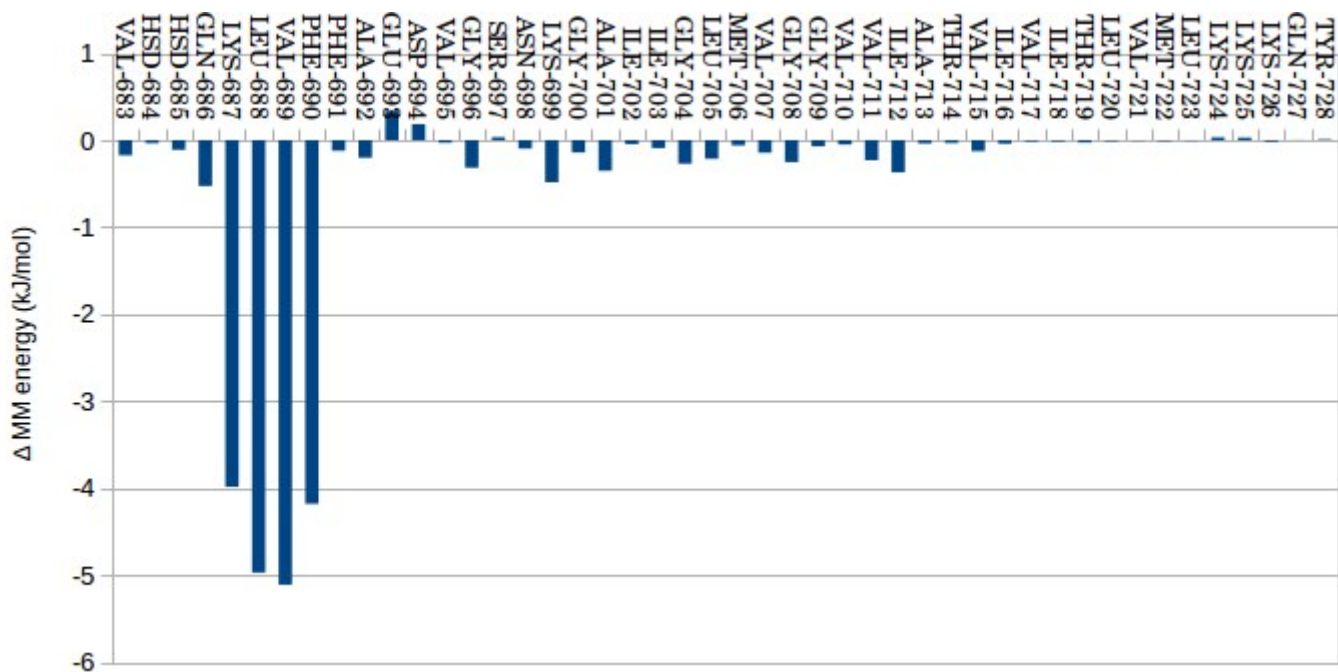
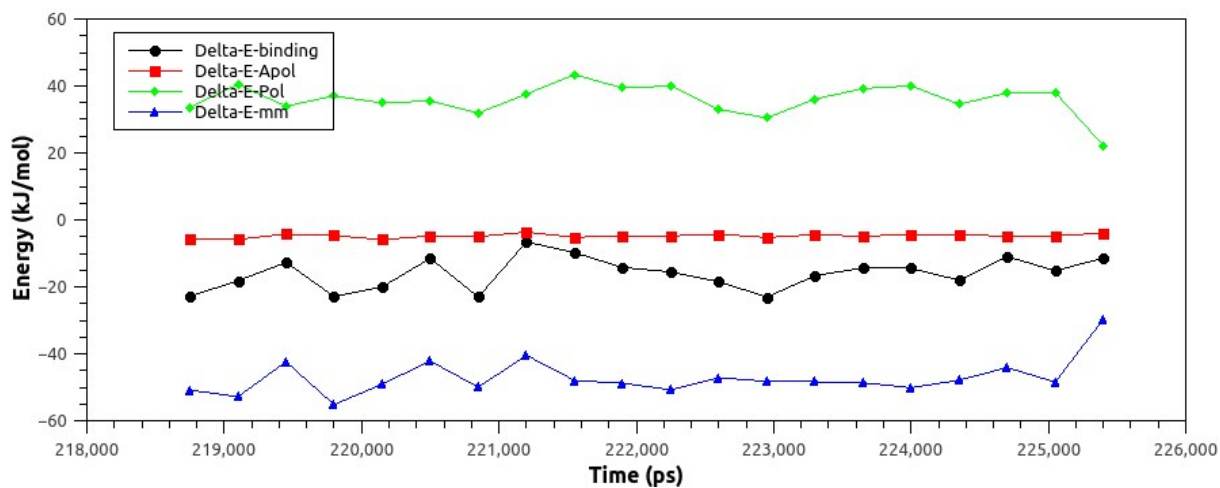
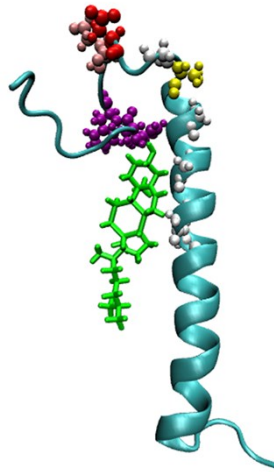
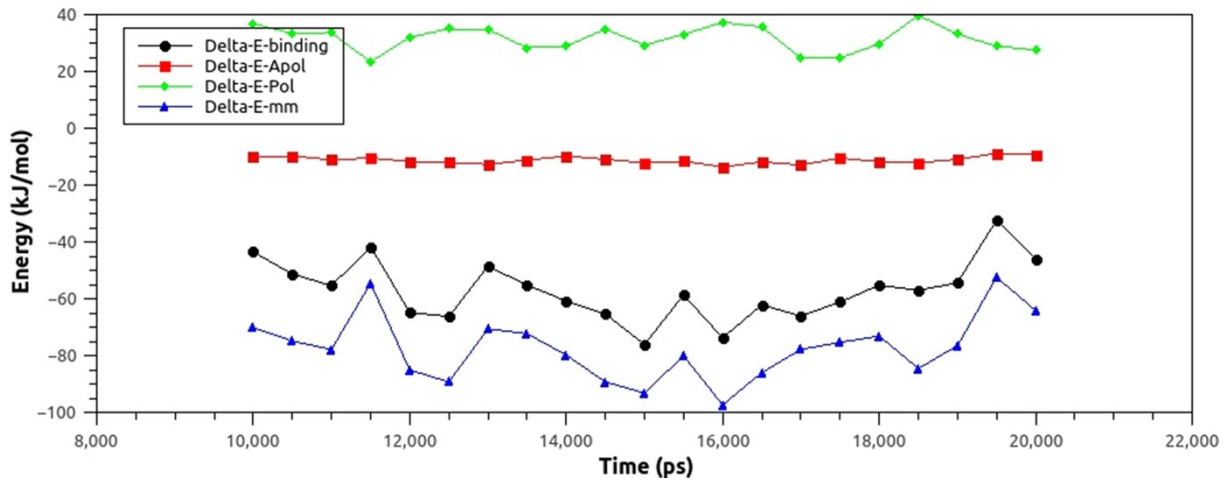


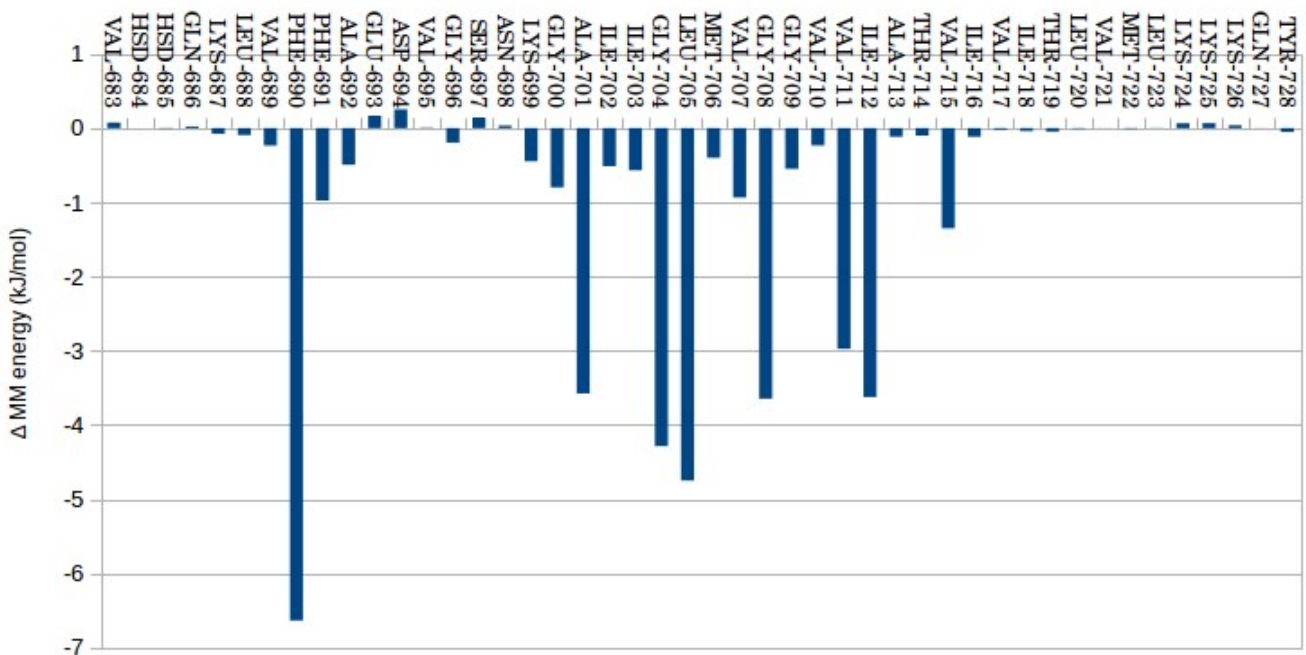
Figure S4. Binding Site 1



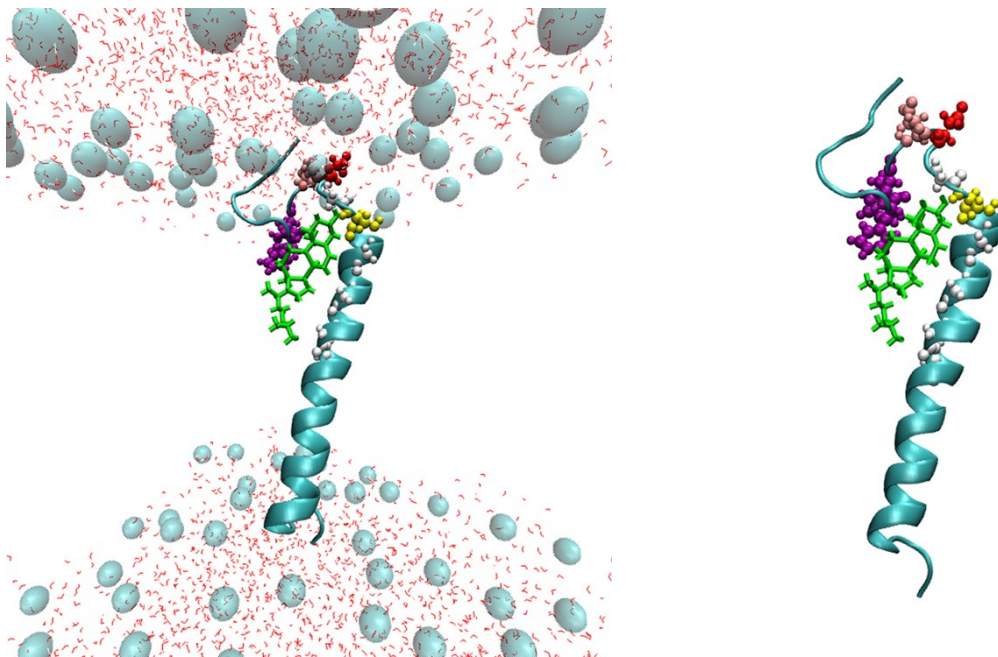
The loosely binding state of site1. Protein is shown in cyan, cholesterol in green. Residues GLY is shown in white, SER<sub>697</sub> in yellow, GLU<sub>693</sub> in pink, ASP<sub>694</sub> in red and PHE<sub>690&691</sub> in purple.



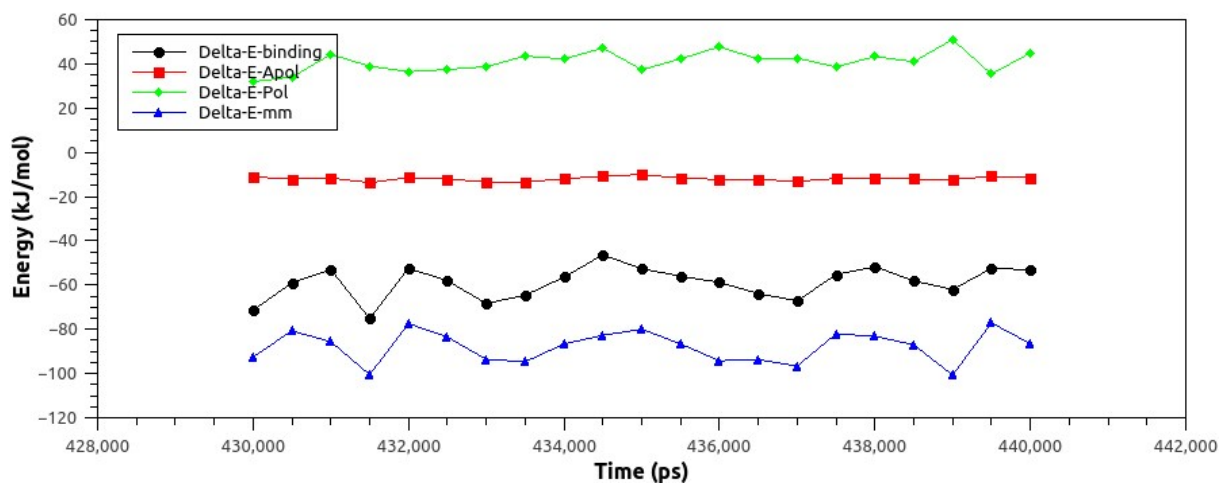
Loosely binding state



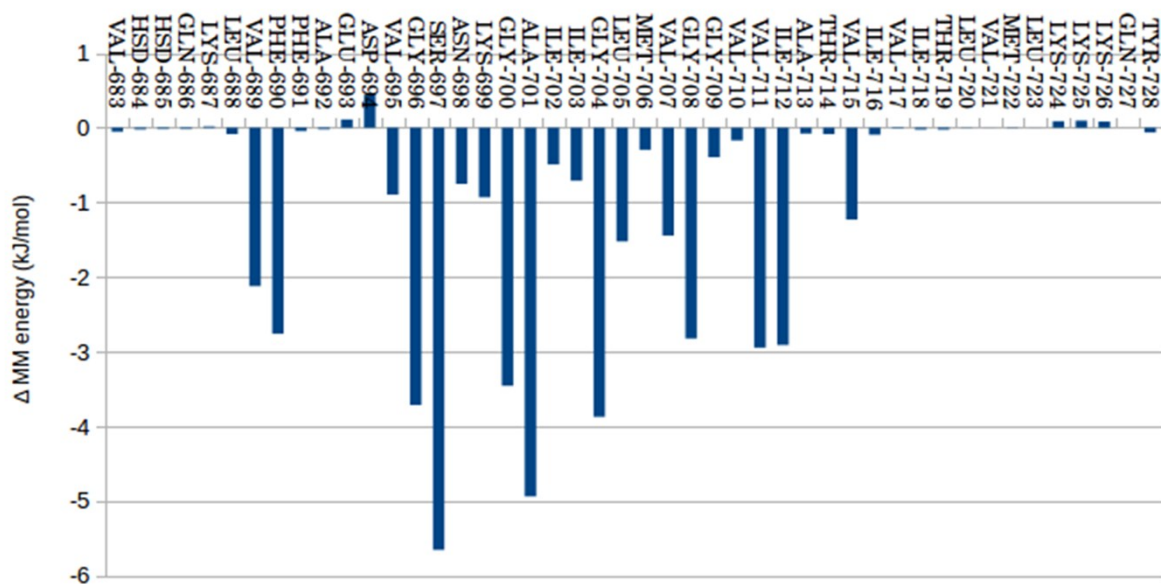
Loosely binding state



The tightly binding state of site1. The left shows the view in membrane. Protein is shown in cyan, cholesterol in green. Residues GLY is shown in white, SER<sub>697</sub> in yellow, GLU<sub>693</sub> in pink, ASP<sub>694</sub> in red and PHE<sub>690&691</sub> in purple.

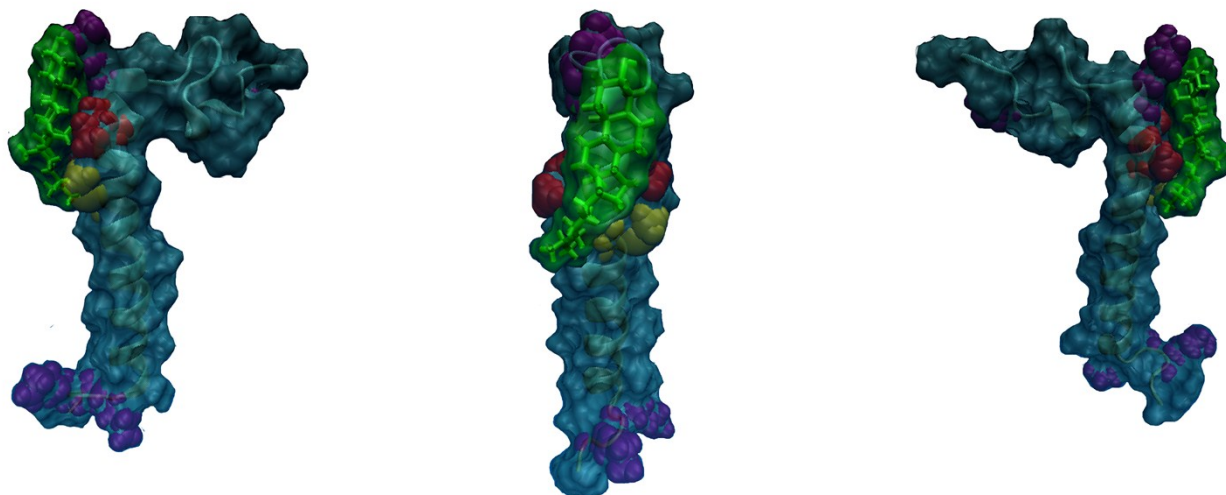


Tightly binding state



Tightly binding state

Figure S5. Binding Site 2



The binding conformations of site2. Protein is shown in cyan, cholesterol in green. Residues LYS is shown in purple, ILE<sub>702&703</sub> in red and MET<sub>706</sub> in yellow.

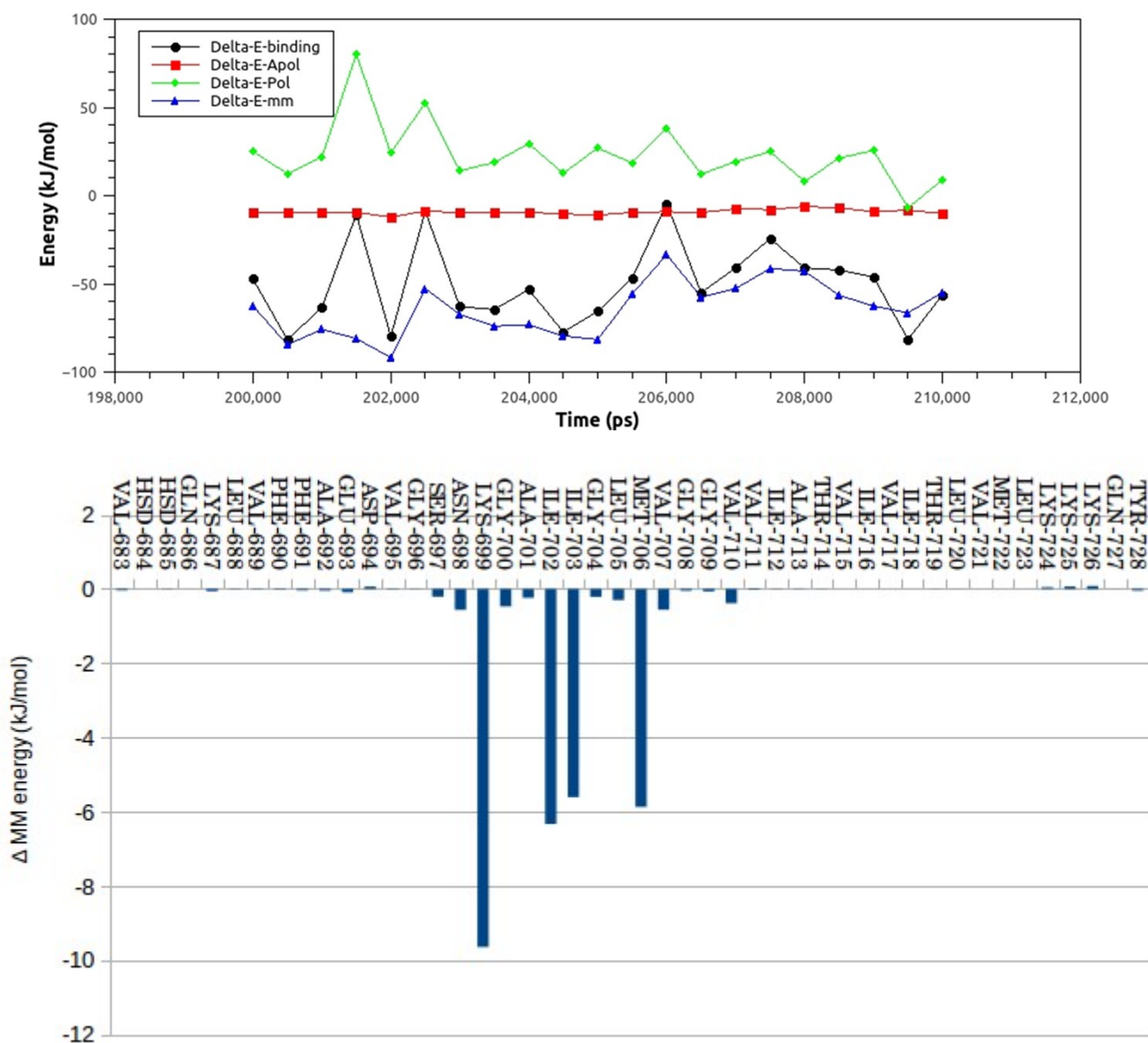
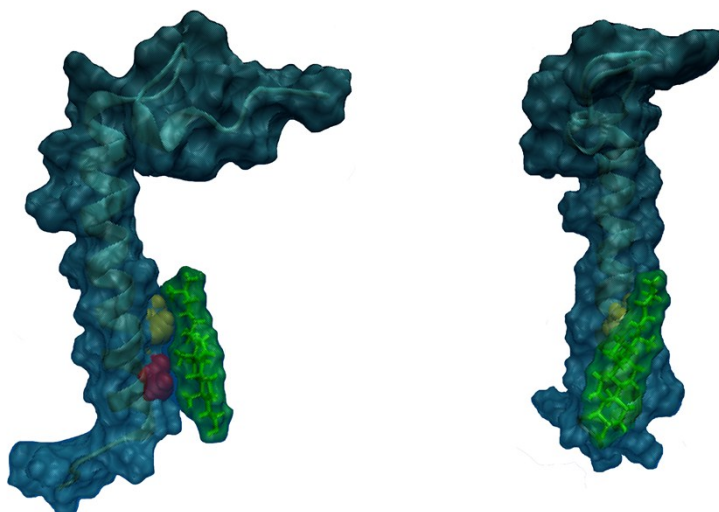


Figure S6. Binding Site 3



The binding conformations of site3. Protein is shown in cyan, cholesterol in green. Residue VAL<sub>715</sub> is shown in yellow and ILE<sub>719</sub> in red.

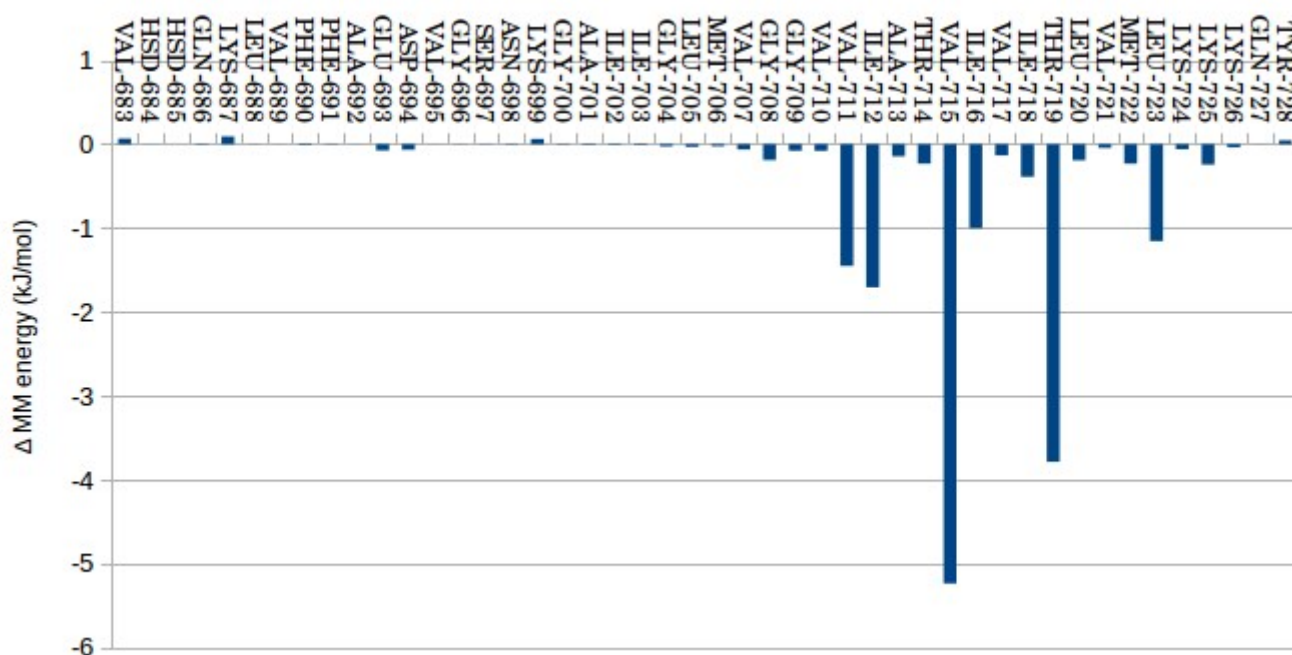
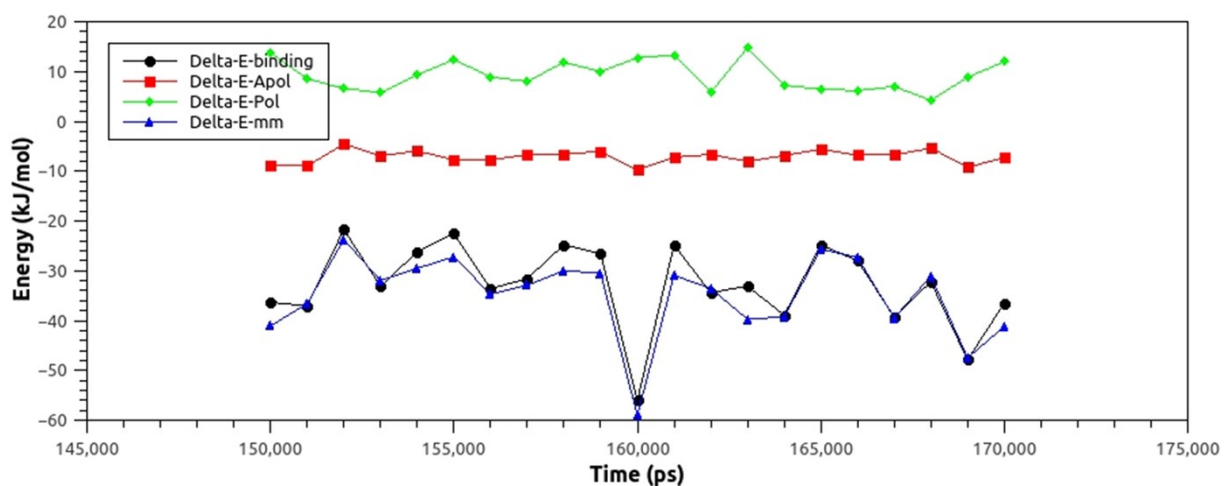
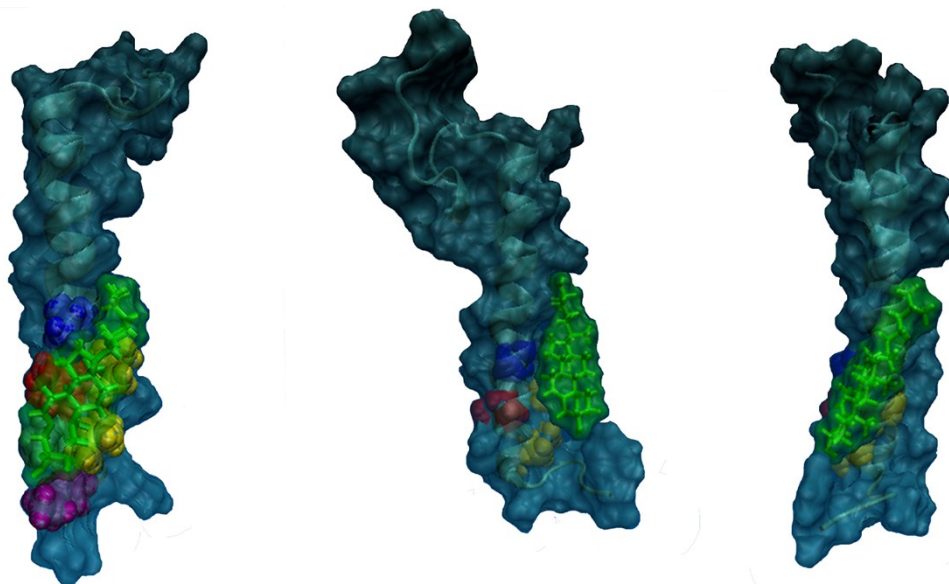




Figure S7. Binding Site 4



The binding conformations of site4. Protein is shown in cyan, cholesterol in green. Residue Thr<sub>714</sub> is shown in blue, Ile<sub>718</sub> in red, Val<sub>717</sub>&721 in yellow, and Lys<sub>725</sub> in purple.

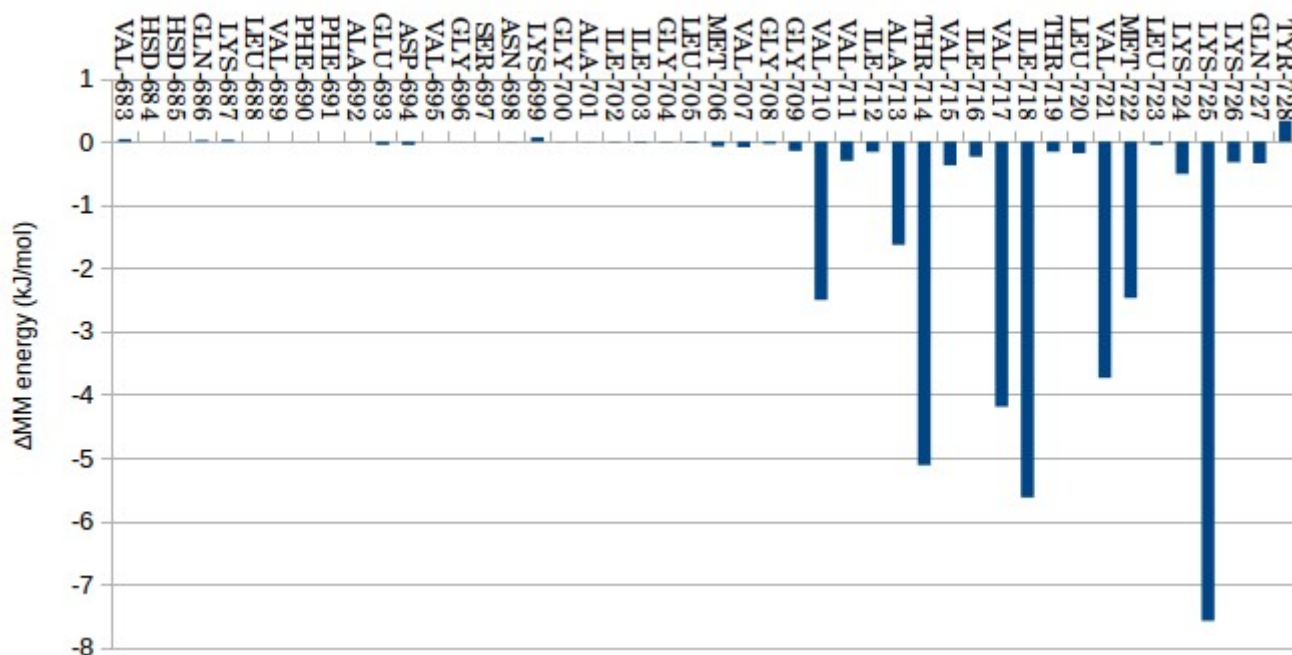
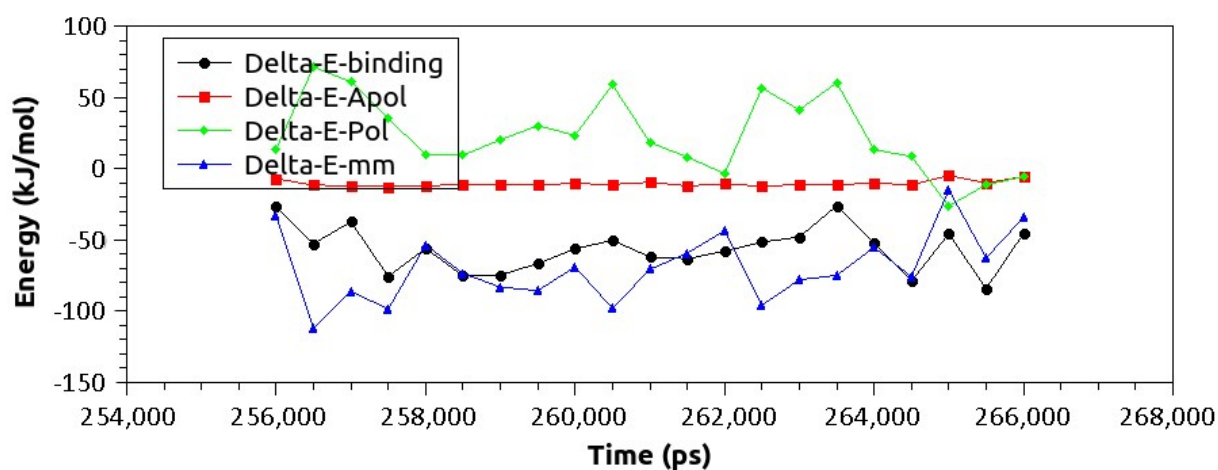
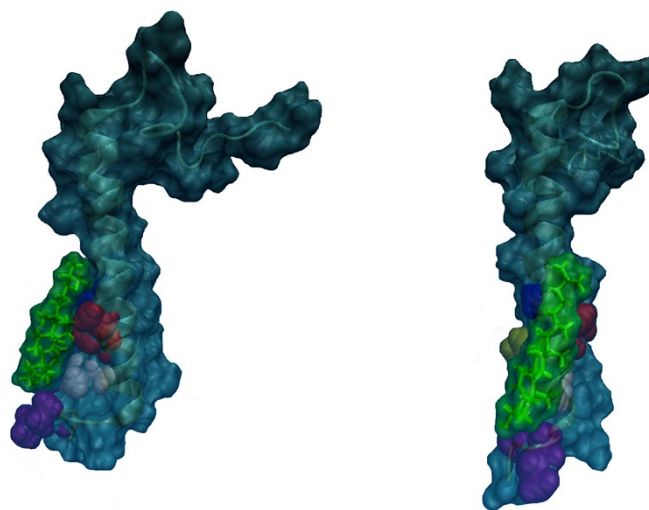


Figure S8. Binding Site 5



The binding conformations of site5. Protein is shown in cyan, cholesterol in green. Residue ALA<sub>713</sub> is shown in blue, ILE<sub>716</sub> in red; VAL<sub>717</sub> in yellow, LEU<sub>720</sub> in white, and LYS<sub>726</sub> in purple.

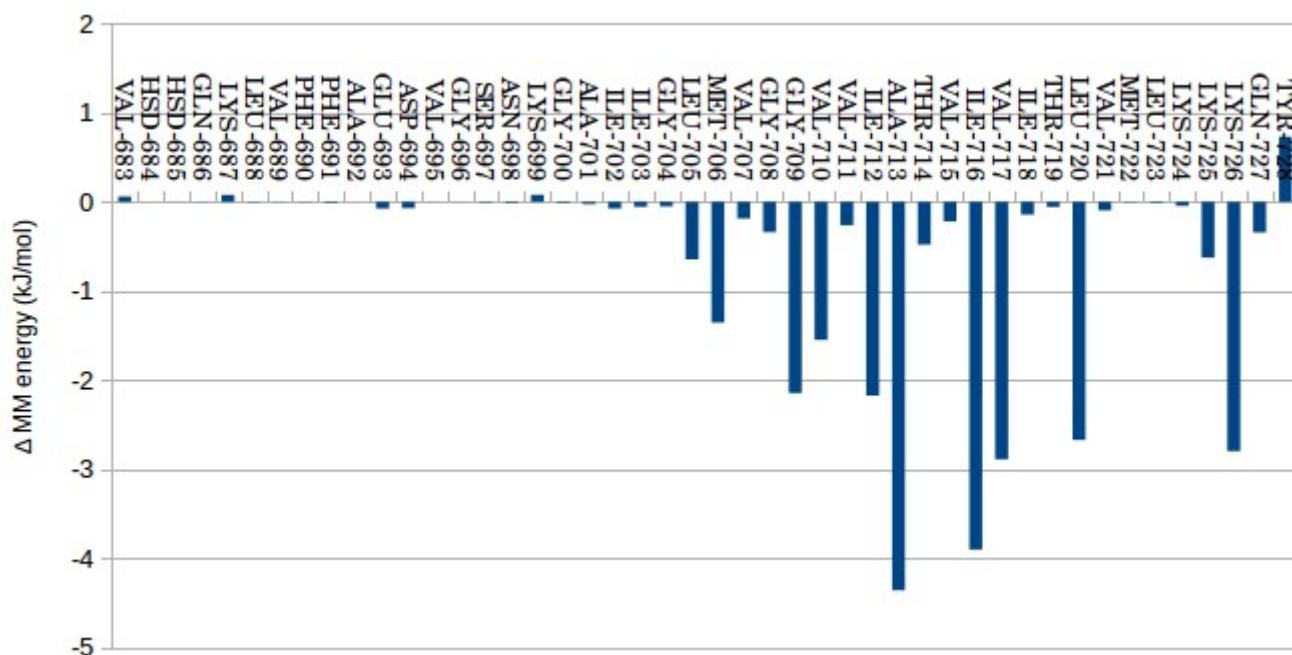
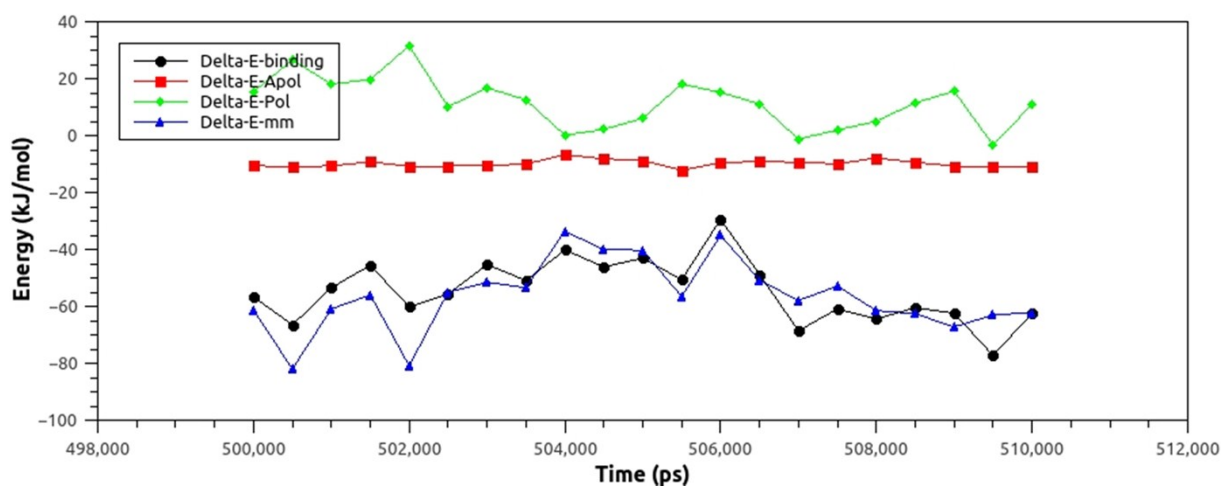
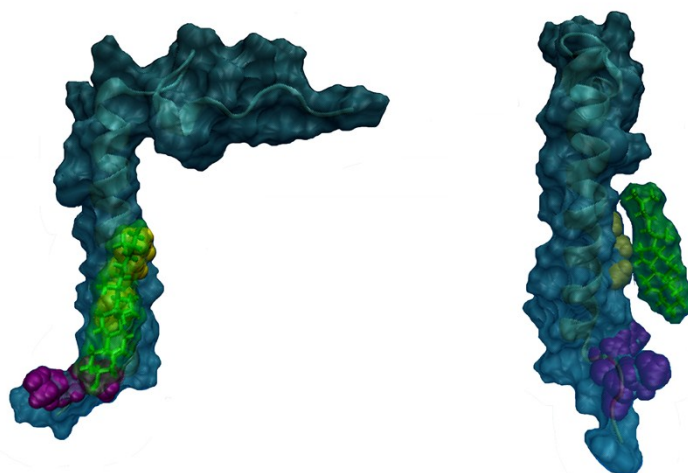
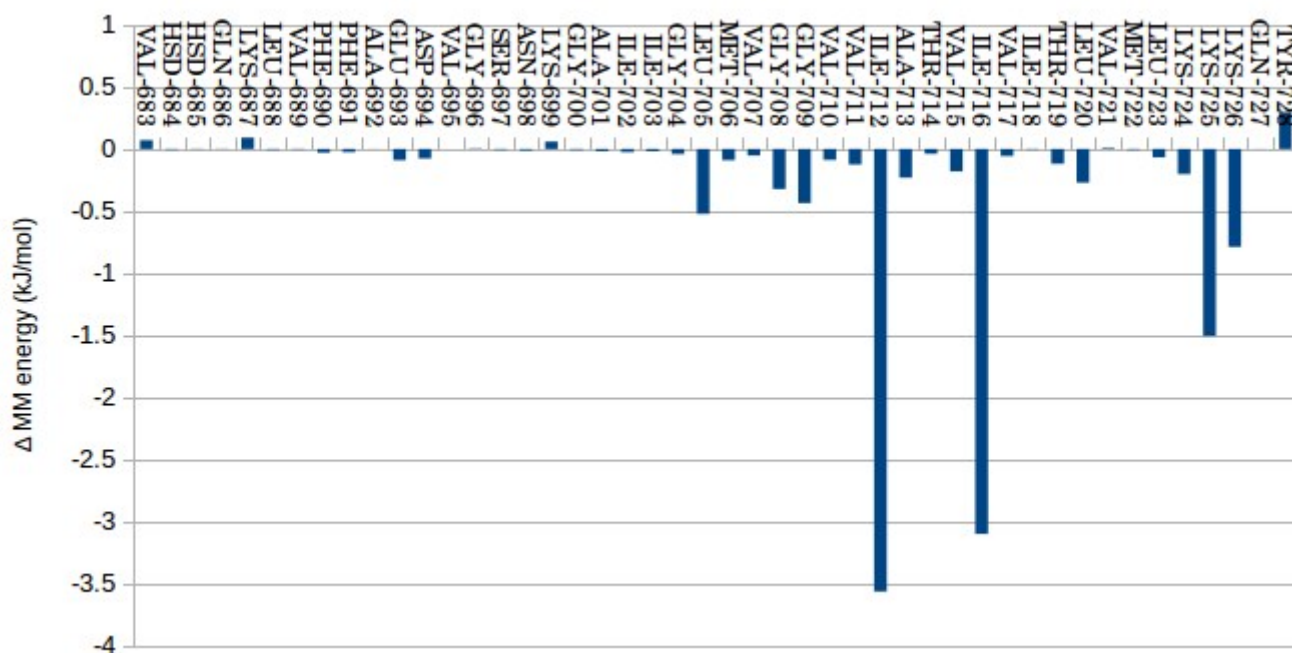
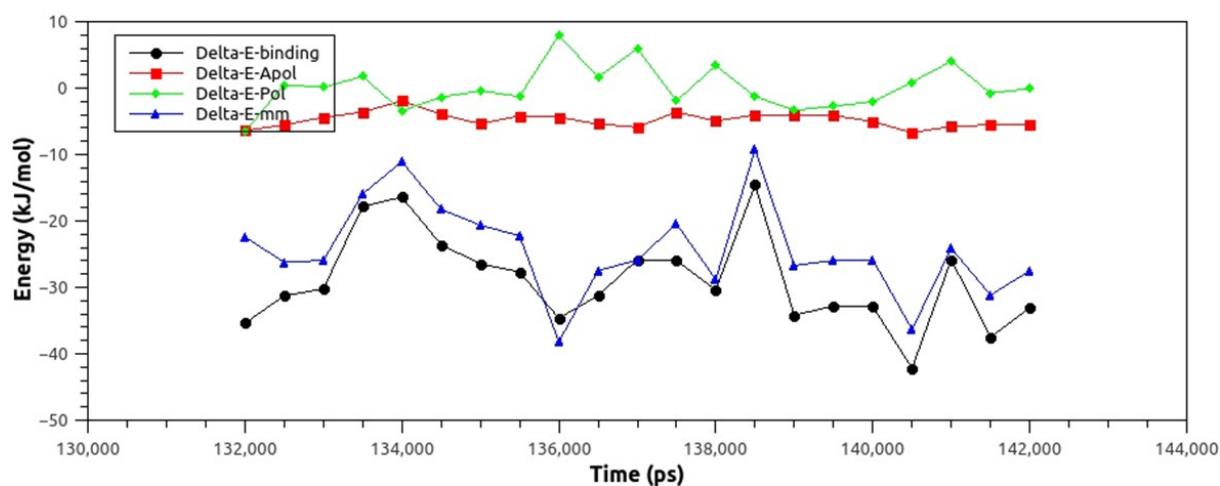
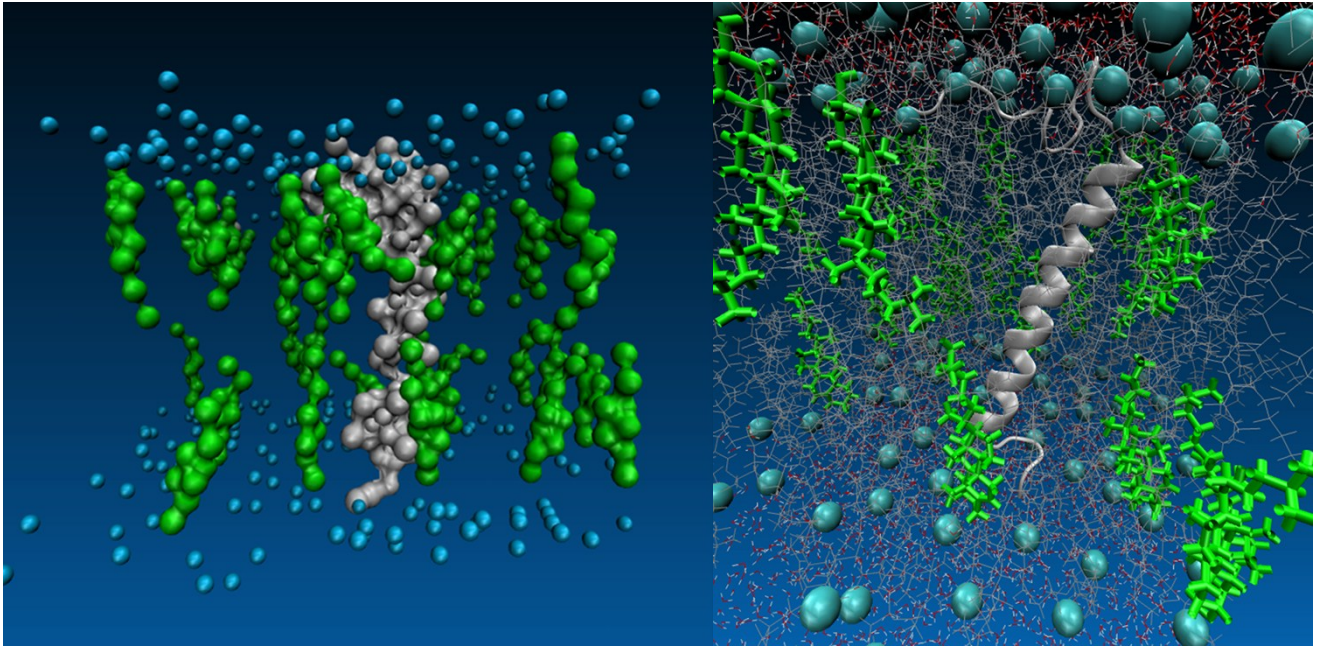


Figure S9. Binding Site 6



The binding conformations of site6. Protein is shown in cyan, cholesterol in green. Residue ILE<sub>712&716</sub> are shown in red and LYS<sub>725</sub> in purple.





**Figure S10.** The systems embedded in membrane. The left is for CG system and the right for AT system. Protein is shown in white, cholesterol in green. Phospholipid head is shown in cyan, tail is in gray lines and the water is in red point.