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A molecular view of cholesterol flip-flop and chemical potential in different membrane environments

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Hydrogen Bonds

We have determined the average number of hydrogen bonds formed between cholesterol and the rest of the system for the AA bilayers (Fig S1). In bulk water, cholesterol forms on average ~2 hydrogen bonds. Moving cholesterol from water to equilibrium, the average number of hydrogen bonds drops to 1.5. As we move cholesterol from equilibrium to the center of the bilayer, the number of hydrogen bonds drops to zero, corresponding to the plateau of the PMF. No hydrogen bonds are observed when the hydroxyl group of the cholesterol is located at the center of the membrane.

CG Equilibrium Flip-flop

Using the CG model allowed equilibrium simulations on the time-scale we predict cholesterol flip-flop to occur. By calculating the density of the hydroxyl of cholesterol (ρ_{OH}) normal to the bilayer throughout the equilibrium simulations, we can calculate a PMF directly using,

$$\Delta G = -RT \ln(\rho_{OH})$$

Fig S2 shows cholesterol PMFs for a POPC bilayer calculated using equilibrium simulations and umbrella sampling with both small and larger bilayers. The three PMFs are in good agreement and demonstrate that the free energy barrier for flip-flop from umbrella sampling and equilibrium simulations are the same, and that system size has little effect on the PMF.

To count the number of flip-flops observed in the equilibrium CG simulations we have determined the angle formed by the z-axis and a vector from the hydroxyl of cholesterol to the carbon joining its rings and tail, and the position of the hydroxyl as a function of simulation time. We define a flip-flop as the combination of the long axis of cholesterol flipping from < -45˚to > 45˚ and the hydroxyl moving from < -0.5 nm to > 0.5 nm or vice versa. The extremely fast flip-flop of cholesterol in the poly-unsaturated DAPC

bilayer, and the appreciable amount of time spent in the bilayer interior necessitated this strict definition.

Force field Issues

An initial assumption would be that the AA model is more accurate, due to the CG model's simplified representation. Recent neutron scattering data showed that cholesterol prefers the center of poly-unsaturated (DAPC) bilayers [1], and was previously supported by MARTINI simulations [2, 3]. This agreement suggests the AA DAPC model overestimates the free energy barrier for cholesterol flip-flop. Better parameterized double bonds in lipid acyl chains might improve the agreement.

The chemical potentials of cholesterol in the CG model are similar for all the bilayers, except the DAPC-0%C bilayer. The AA model predicts an increase in the chemical potential for the DPPC-0%C bilayer compared to the DPPC-40%C bilayer, while the CG model does not. The precise structure of cholesterol is important for many of its effects on lipid bilayers. Very similar sterol molecules, such as lanosterol [4] and desmosterol [5], have different biophysical effects on bilayers compared to cholesterol. The CG model might be missing the detail required to reproduce accurate phase behavior of high cholesterol bilayers. However, it is also possible that current AA force fields lack the accuracy to describe lipid phase behavior correctly. Our method provides the possibility of investigating lipid thermodynamics on a molecular scale, which could be used in parameterizing lipid force fields.

Cholesterol water to octane transfer free energies and decompositions

We have determined PMFs for cholesterol transfer from water to an octane slab. Using octane allowed us to decompose the PMF at a range of temperatures: 293K, 303K, 313K, 323K, 333K, and 343K. For octane, we used the GROMOS87 parameters, as a control for combining GROMOS87 cholesterol with Berger lipids. Where appropriate we have used the same procedure and parameters as for the DPPC AA

PMFs, for ease of comparison. One exception was the use of constant area, which is necessary, due to the surface tension at the interface. Using octane allowed us to determine PMFs at a broad range of temperatures, including room temperature, which the DPPC bilayer system prohibited. As well, the homogeneity of the octane - water system allowed shorter simulations. Fig S4 B shows PMFs for the cholesterol water – octane transfers, determined by umbrella sampling. As expected, the octane PMF has a similar shape as the lipid bilayer PMF. There is a trough at the octane – water interface. There is a large favorable free energy of transfer from water to the octane slab. In Fig S4 C, we show the $-T\Delta S$ of transfer for a range of temperatures, again using the centered difference method. In equation (1), we have used a ΔT of 10 K, for ease of comparison to our DPPC decomposition. We note that using a ΔT of 20 K resulted in a similar decomposition, within the statistical error. At 333 K, the transfer from water to octane has a large unfavorable $-T\Delta S$ component, in agreement with our DPPC decomposition. At the lower temperatures, $-T\Delta S$ is near zero, and favorable at 313 K. The decomposition at 303 K does not follow the trend observed for the other temperatures, possibly due to worse sampling at lower temperatures. From these control simulations, we show $-T\Delta S$ is largely temperature dependant. Combining GROMOS87 cholesterol with Berger lipids likely does not have a major effect on the PMF decompositions, as the octane PMFs used the GROMOS87 parameters, and were qualitatively similar.

Cholesterol without a hydroxyl head group

As cholesterol is slightly amphipathic, we wanted to investigate the effect of the hydroxyl on the PMF and the entropy and enthalpy of transfer. We have determined a PMF for an analog of cholesterol, which did not have a hydroxyl head group, in a DPPC bilayer (Fig S5). The PMF has a similar shape as the cholesterol PMF, except there is no barrier for flip-flop. From the shape of the PMF, we expect the transfer of the analog to the DPPC bilayer center would also be enthalpy driven. It is interesting to note that the hydroxyl being desolvated appears to be the only barrier to flip-flop, while the bulky

hydrophobic body and tail of cholesterol being exposed to water is responsible for the large free energy of desorption.

References:

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Figure Legends

Figure S1: Cholesterol hydrogen bonds. The average number of hydrogen bonds to the pulled cholesterol. We define a hydrogen bond using a geometric criterion with a distance cut-off of 0.35 nm and an angle of 30˚.

Figure S2: Equilibrium PMFs compared to umbrella sampling. CG PMFs for cholesterol flip-flop in a POPC bilayer calculated using umbrella sampling (Umb.) for bilayers with 64 and 152 POPC lipids and from the density of the cholesterol hydroxyl from an equilibrium simulation with 152 POPC lipids (Equil.). The black curve diverges as cholesterol is removed from the bilayer, due to less water in the simulation set-up.

Figure S3: Snapshots of cholesterol in AA and CG simulations. Snapshots of cholesterol in 0% DPPC bilayers using both atomistic (A,C) and CG (B,D) models. Water particles are blue spheres, the DPPC cholines are red spheres, their phosphates are yellow spheres, and their tails are grey lines. The body of cholesterol is brown licorice, and its hydroxyl is a green sphere. (A and B) The hydroxyl of cholesterol restrained at the bilayer center, with its body parallel with the plane of the bilayer. In both models, cholesterol can rotate a full 180˚ with respect to the bilayer normal. (C and D) The hydroxyl of cholesterol restrained at 3.2 nm from the bilayer center, with its tail still interacting with the bilayer. Moving the hydroxyl 0.1 nm further out causes it to stop interacting with the bilayer.

Figure S4: Cholesterol PMFs in a water - octane system. (A) Partial density profile for the water – octane system. (B) PMFs for cholesterol transfer from bulk water to an octane slab. PMFs were set equal to zero in bulk water. Error bars are the standard error from the mean of the two leaflets cholesterol PMFs. (C) The $-T\Delta S$ component of the free energy at various temperatures. We have used the centered difference method (See Methods). (D) The ΔH component of the free energy at the various temperatures.

Figure S5: PMFs for cholesterol without a head group. A PMF for a cholesterol analog missing the hydroxyl head group (No-OH) partitioning in a DPPC bilayer was calculated using umbrella sampling. The black curve is the blue curve subtracted from the red curve.

Figure S1

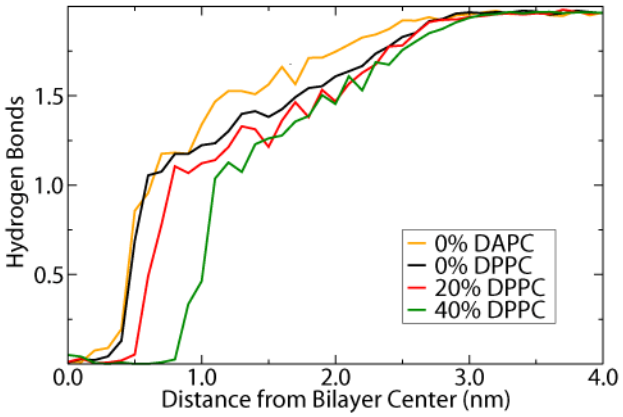

Hydrogen Bonds
1.5
1.0
0.5
0.0
1.0
2.0
3.0
4.0
Distance from Bilayer Center (nm)
0% DAPC
0% DPPC
20% DPPC
40% DPPC

Figure S2

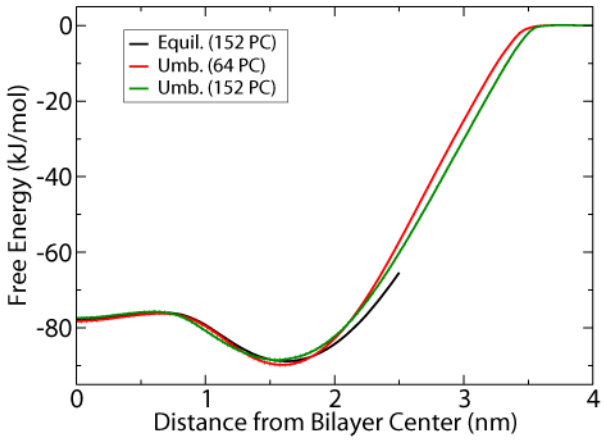

Equil. (152 PC)
Umb. (64 PC)
Umb. (152 PC)
Free Energy (kJ/mol)
0
-20
-40
-60
-80
0
1
2
3
4
Distance from Bilayer Center (nm)

Figure S3

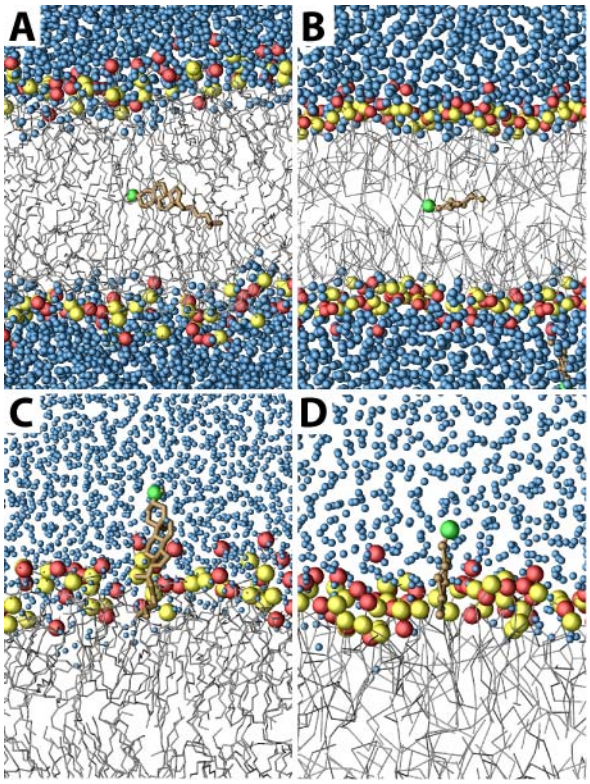


Figure S4

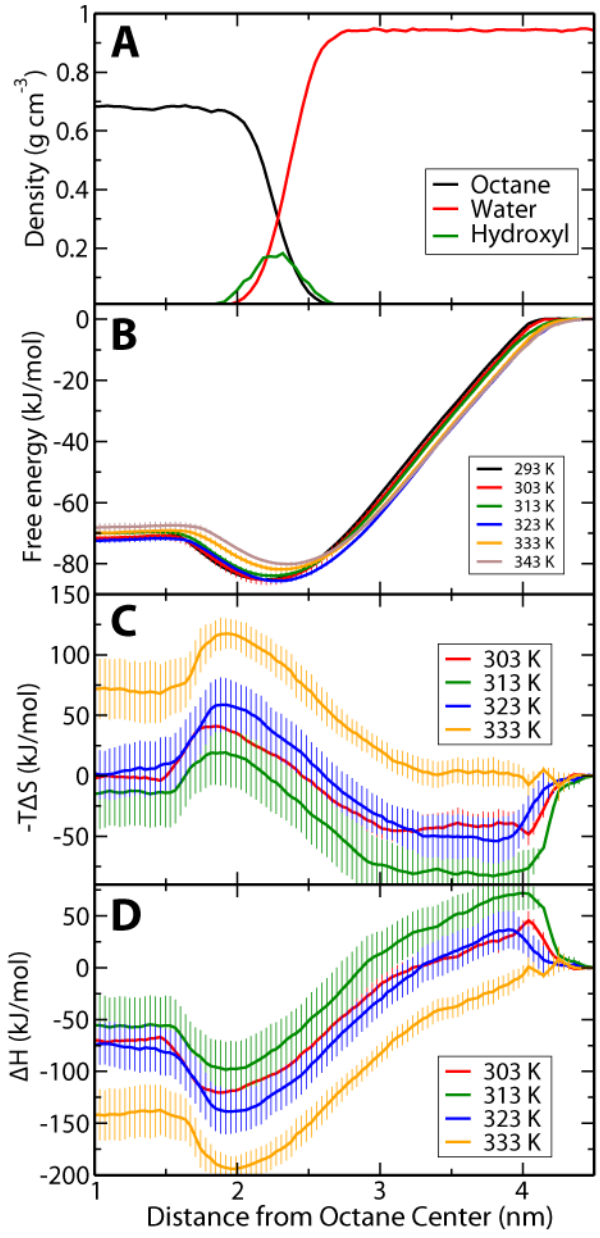
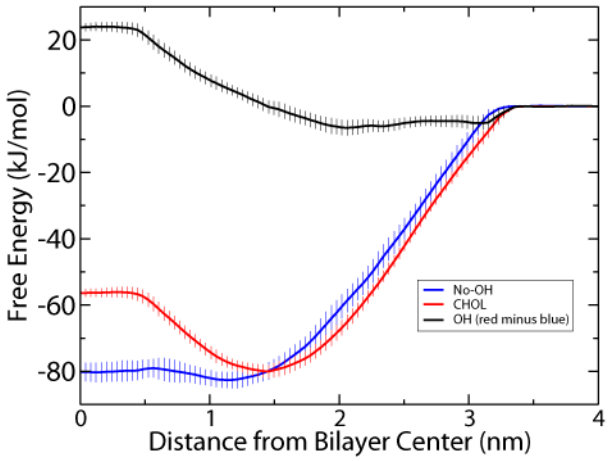

A
Density (g cm^{-3})
Octane
Water
Hydroxyl
B
Free energy (kJ/mol)
293 K
303 K
313 K
323 K
333 K
343 K
C
-TΔS (kJ/mol)
303 K
313 K
323 K
333 K
D
ΔH (kJ/mol)
303 K
313 K
323 K
333 K
Distance from Octane Center (nm)

Figure S5


20
0
-20
-40
-60
-80
Free Energy (kJ/mol)
0
1
2
3
4
Distance from Bilayer Center (nm)
No-OH
CHOL
OH (red minus blue)