Supplemental Information

SANS:

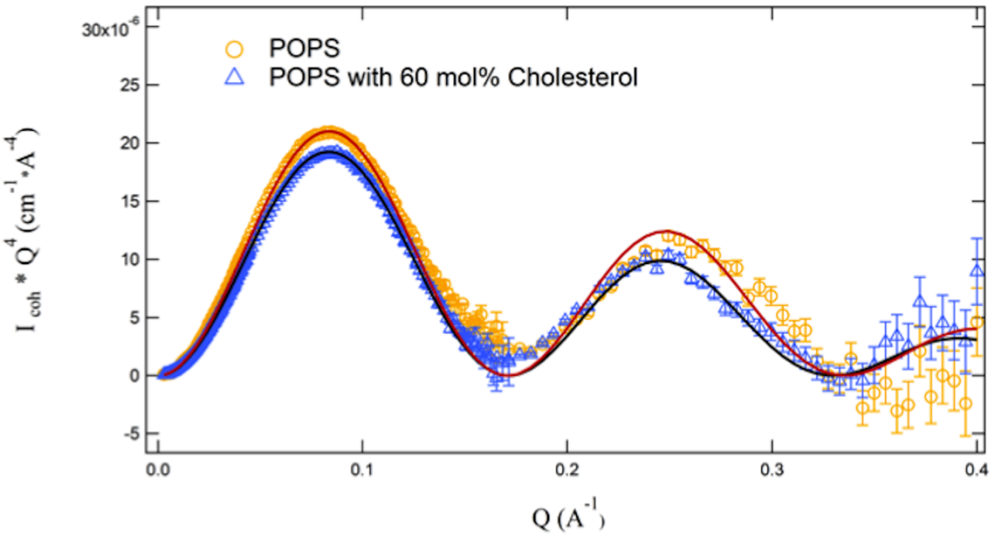

Figure S1. IQ^4 vs Q curves for 100nm vesicles made of hydrogenated POPS (with no cholesterol) and 60 mol% cholesterol. This high Q region shows a minima for both curves at around Q=0.17 $Å^{-1}$ and Q=0.33 $Å^{-1}$. Fits to the data were done with the lamellar form factor including tail and headgroup regions. The thicknesses were 40.3 ± 0.3 Å and 40.8 ± 0.3 Å for POPS with 0% and 60% mole fraction of cholesterol respectively.

Molecular Dynamics Simulations:

Standard simulation parameters associated with the MARTINI force field and polarizable water model were used. The Lennard-Jones potential was shifted from 0.9 to 1.2 nm and cut off after 1.2 nm. The Coulomb potential was shifted from 0 to 1.2 nm with a relative dielectric constant of 2.5. A 20 fs integration time step was used in all simulations with neighbor list updates every 10 steps. Atoms overlaps were removed using the steepest descent algorithm. The system was coupled to a pressure bath (1 atm, τp=3 ps) using the semi-isotropic coupling scheme. Different molecule types were coupled separately to a heat bath at 303 K using the Berendsen temperature coupling (τT = 1 ps). The simulated boxes ranged in sizes from 12 nm to 20 nm and the molecular composition of the simulated systems is described in tables 1 and 2. The simulation times were in the range of 500 ns.

% mol Chol	# POPC lipids	# Cholesterols	# Waters
10	460	52	1760
20	410	102	4096
30	358	154	2800
40	307	205	2800
50	256	256	2800
60	200	300	2800
65	194	374	3300
70	94	234	2400
75	100	300	2000
80	94	400	3000

Table 1. Composition of simulated POPC-CHOL systems

% mol Chol	# POPS lipids	# Cholesterols	# Waters
10	460	52	4096
20	410	102	4096
30	358	154	2800
40	307	205	3584
50	256	256	3584
60	205	307	3584
65	200	374	3300
70	100	234	2000
75	100	300	2000
80	92	400	2500

Tables 2. Composition of simulated POPS-CHOL systems.

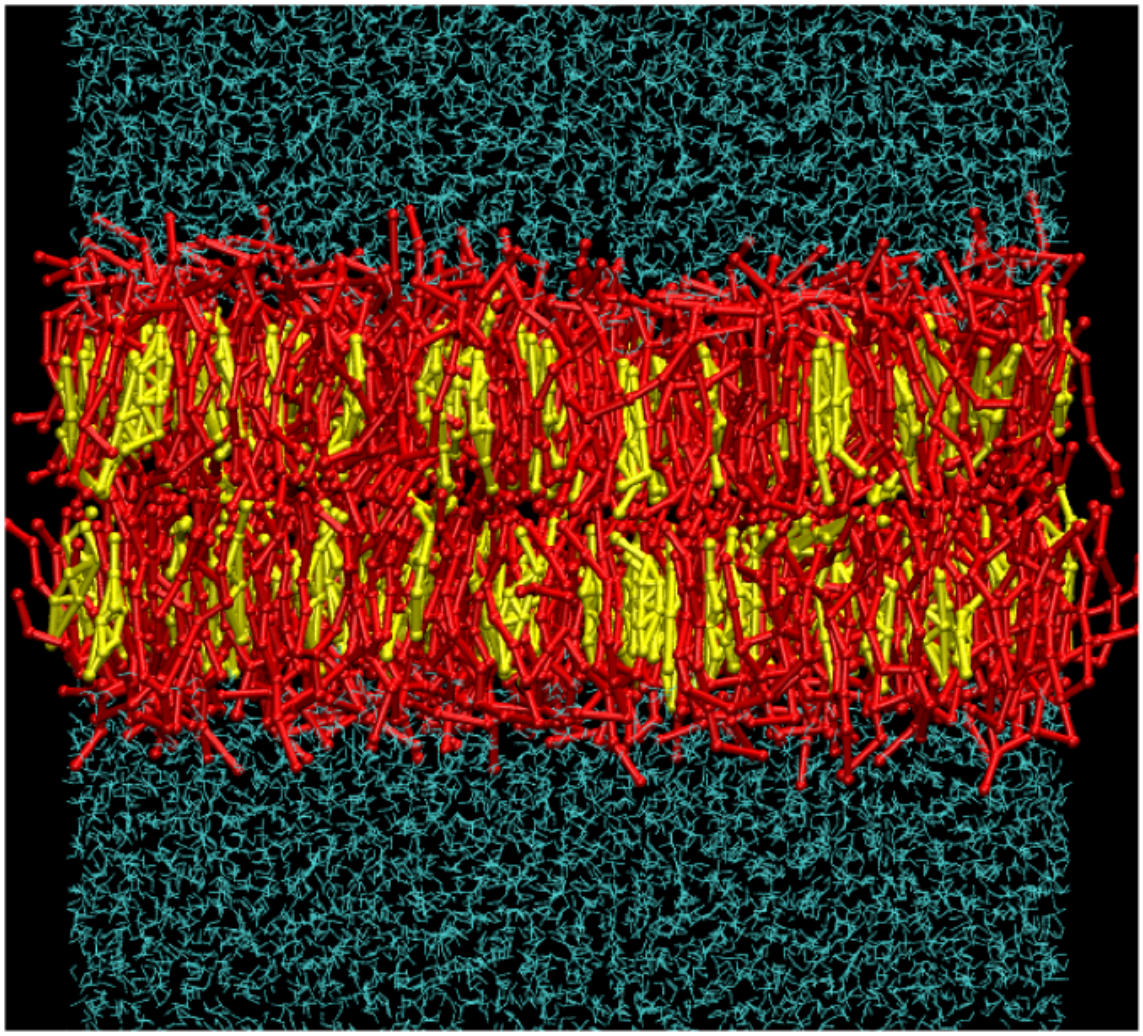

Figure S2.1. Snapshot of a simulated POPC system at 40% mol chol showing a typical self- assembled bilayer with cholesterol at the usual location, parallel to the bilayer normal. POPC lipids are represented in red, cholesterol in yellow and water in cyan.

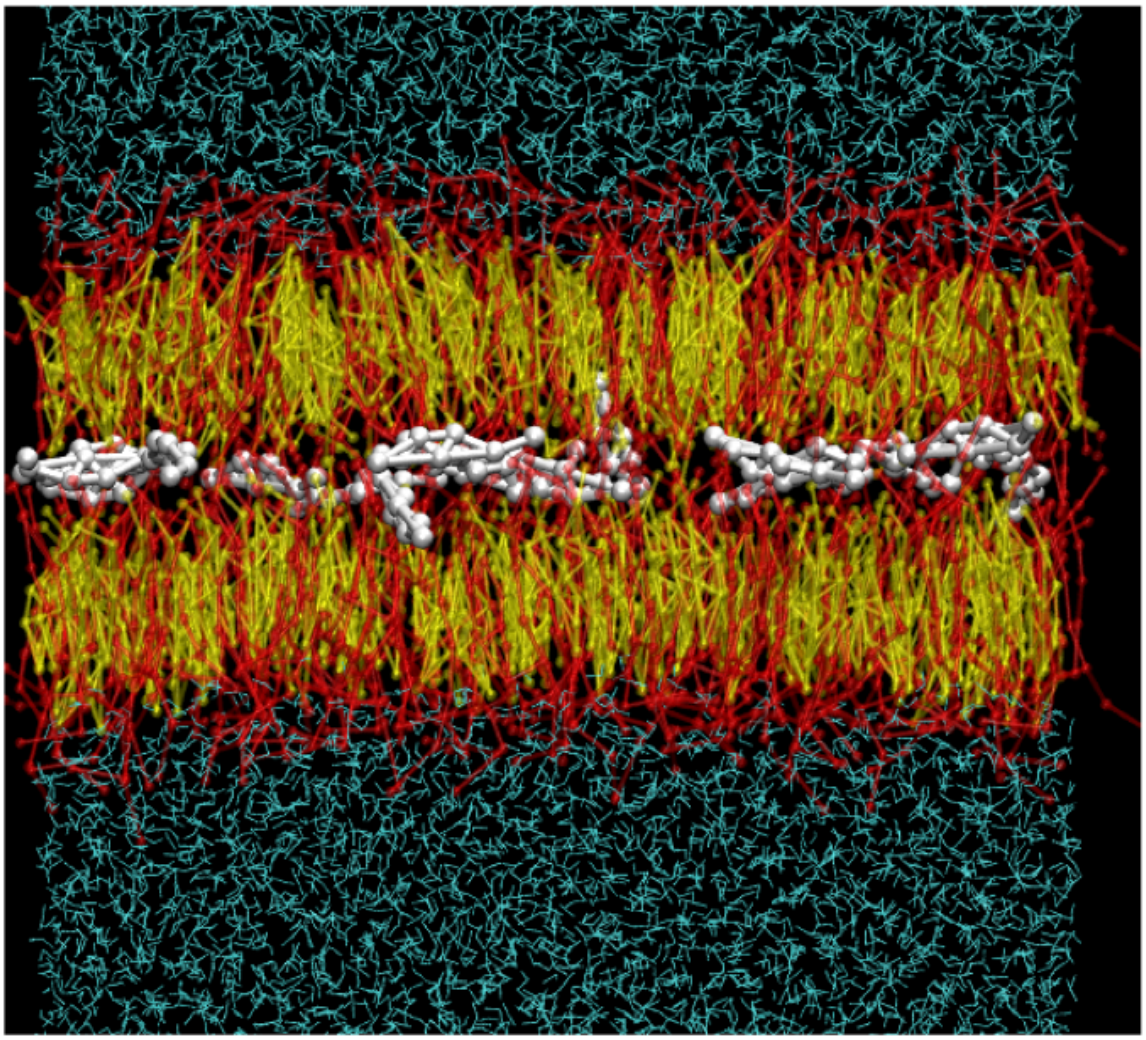

Figure S2.2 Snapshot of a simulated POPC system at 60% mol chol with some cholesterol accumulated in the center of the bilayer and adopting horizontal molecular configurations. POPC lipids are represented in red, cholesterol in yellow and water in cyan. Cholesterol molecules located at the center of the bilayer are shown in white.