

Cholesterol level affects surface charge of lipid membranes in physiological environment

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Supplementary Information

A: All atom molecular simulation systems

Cholesterol Content	0%	16.66%	20%	25%	33.33%	50%
DSPC/POPC	288	240	230	216	192	144
Cholesterol	0	48	58	72	96	144
Water	12600	12600	12600	12600	12600	12600
Na Ions	30	30	30	30	30	30
Cl Ions	30	30	30	30	30	30

Table S1: Composition of the lipid bilayers used in the molecular dynamics simulations
The table shows the simulation system's molecular content used in this study.

B: Surface area per lipid calculations

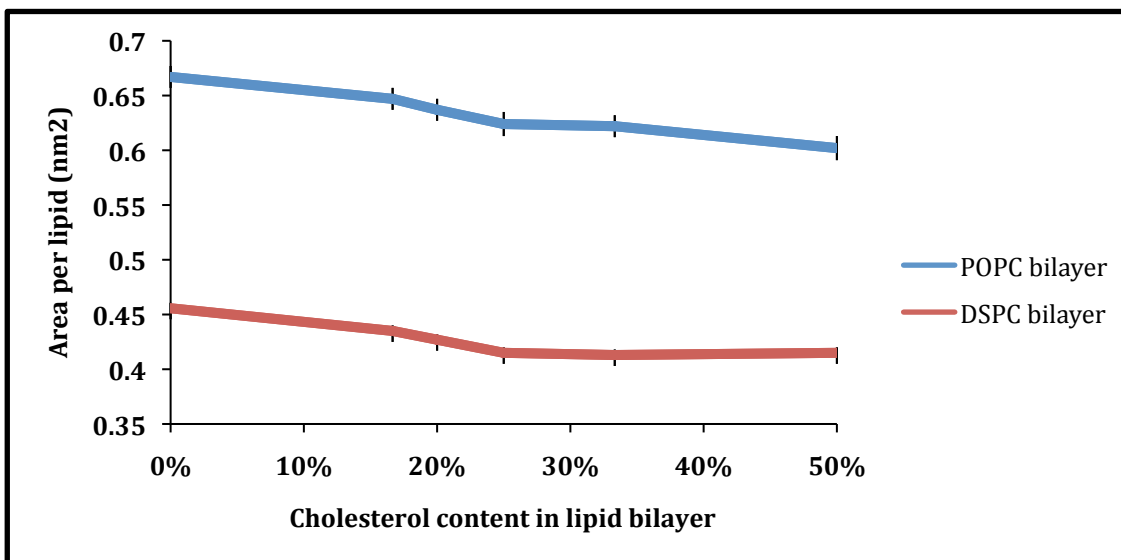


Figure S2: Area per lipid: The figure shows that as the cholesterol content increases the area per lipid decreases that is the membrane bilayer contracts. This is in accordance with the previous studies. (The area per lipid was calculated with the following formula: Area per lipid = (Total area of membrane bilayer - (0.39* number of cholesterol molecules))/ number of PC molecules.

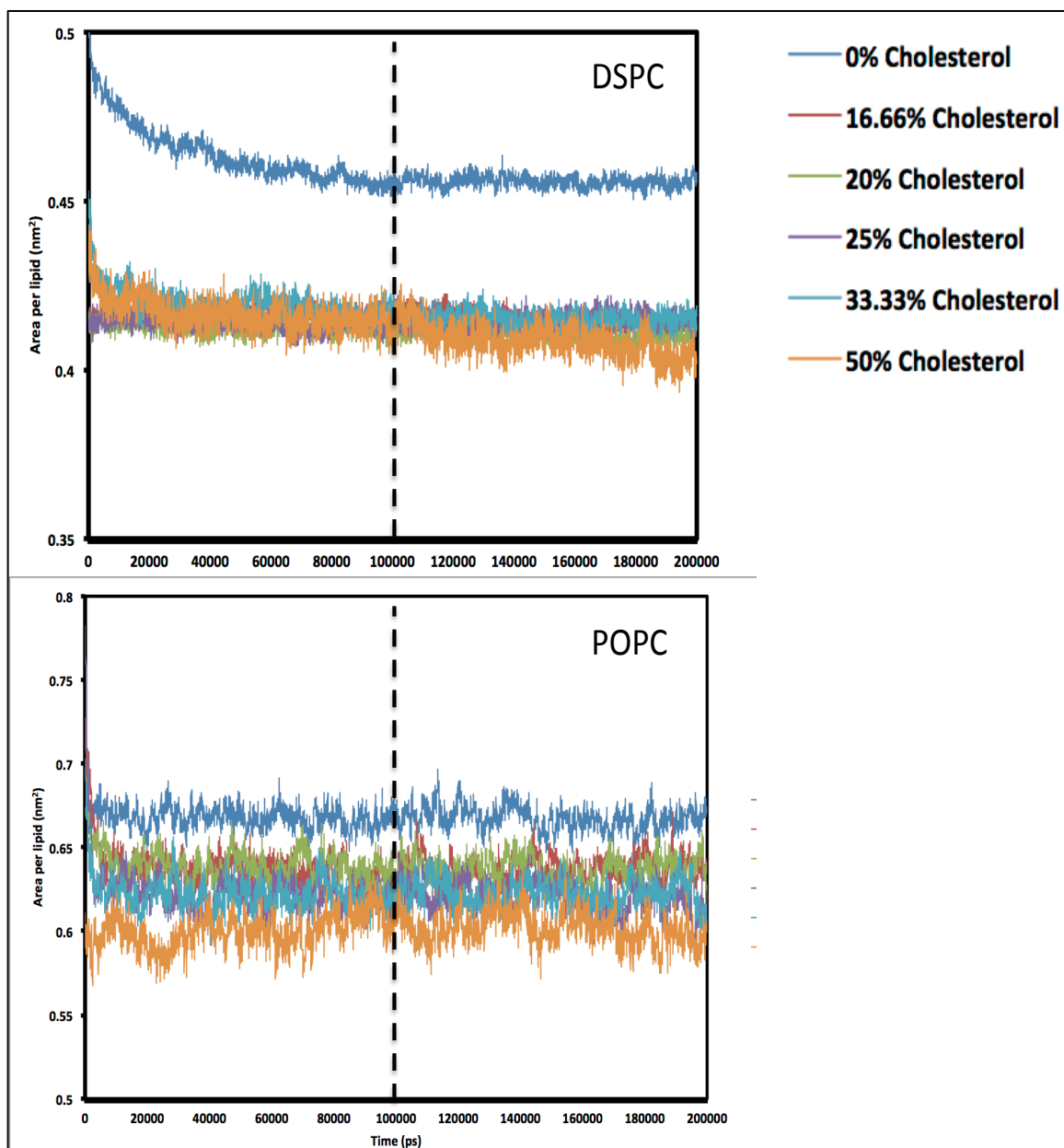


Figure S3: The figure shows area per lipid as a function of time of DSPC/POPC lipid bilayer containing cholesterol. The trajectories post 100 ns (marked by dashed line) were considered as equilibrated and thus used for the analysis.

C: Partial mass density profile of ions in simulation systems

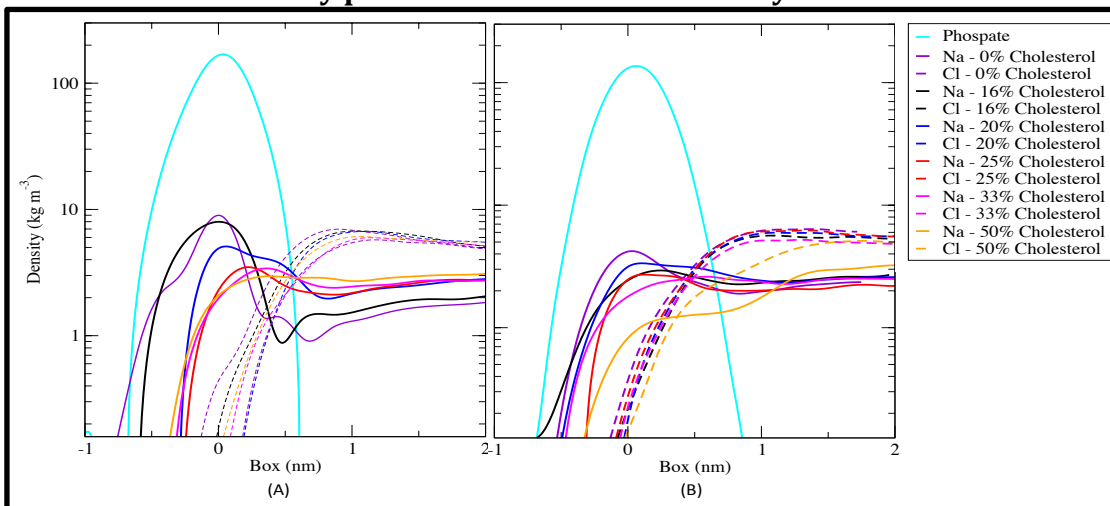


Figure S4: Detailed mass density profile of the Phosphate head groups of DSPC (A) and POPC (B) with Na⁺ and Cl⁻. In the absence of cholesterol, the Na⁺ are localized with membrane head groups. But as the cholesterol content is increased, association of Na⁺ ions with membrane head groups decreases.

D: Na⁺ interactions with phospholipid bilayers

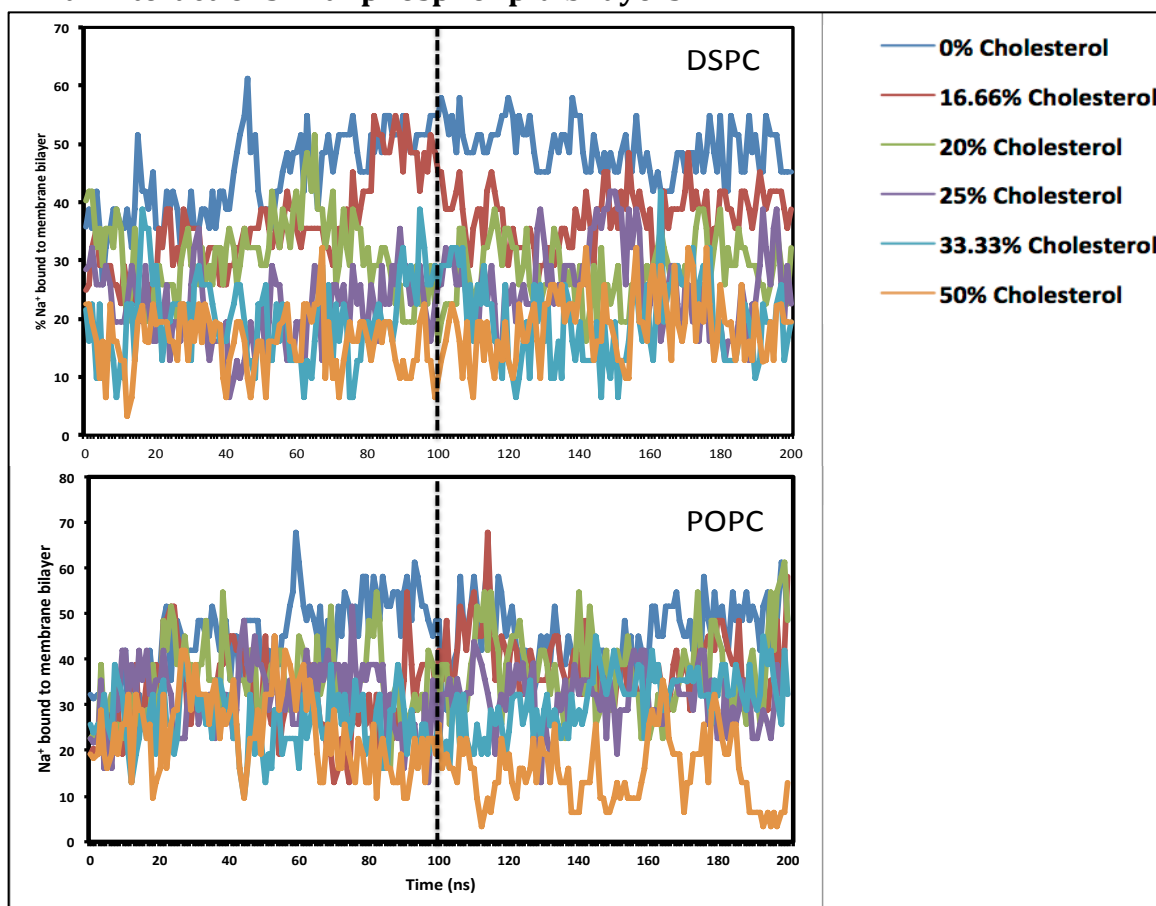


Figure S5: % Bound Na⁺ to membrane headgroups vs. time. The figure represents the % amount of Na⁺ ions bound to DSPC and POPC membrane containing cholesterol. The trajectories post 100 ns (marked by dashed line) were considered as equilibrated and thus used for the analysis.

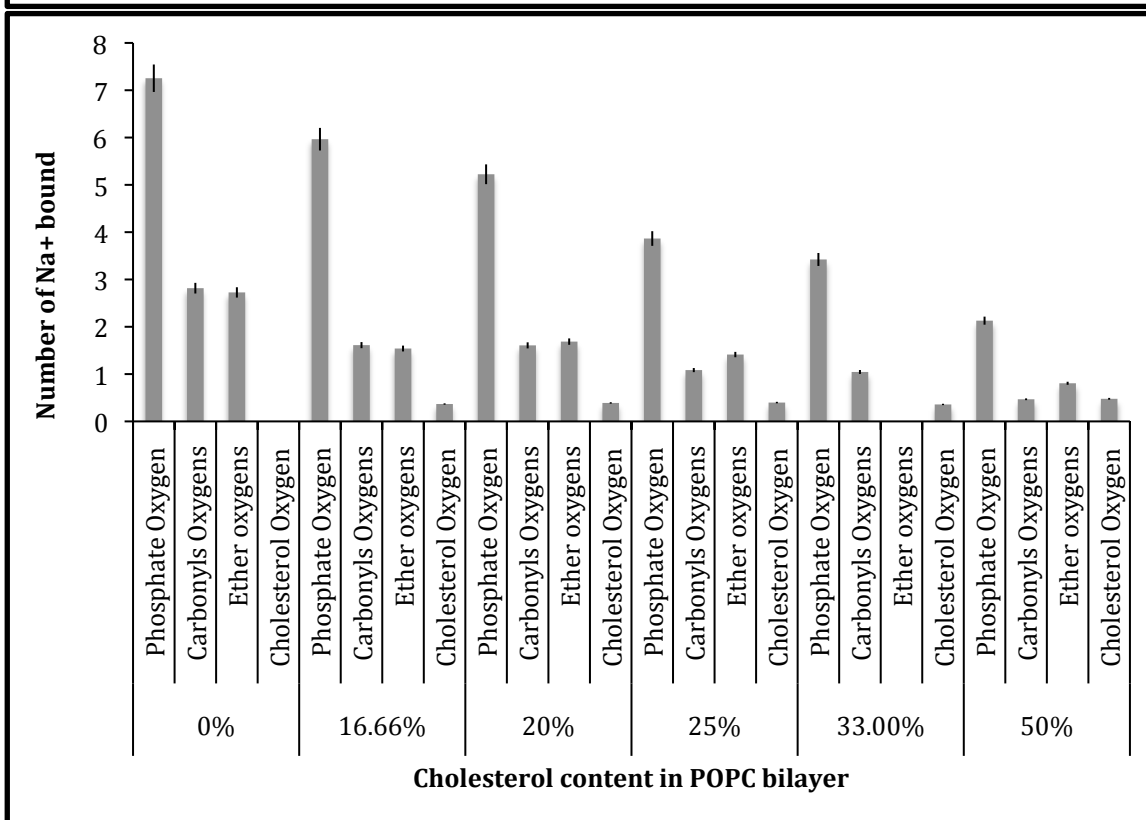
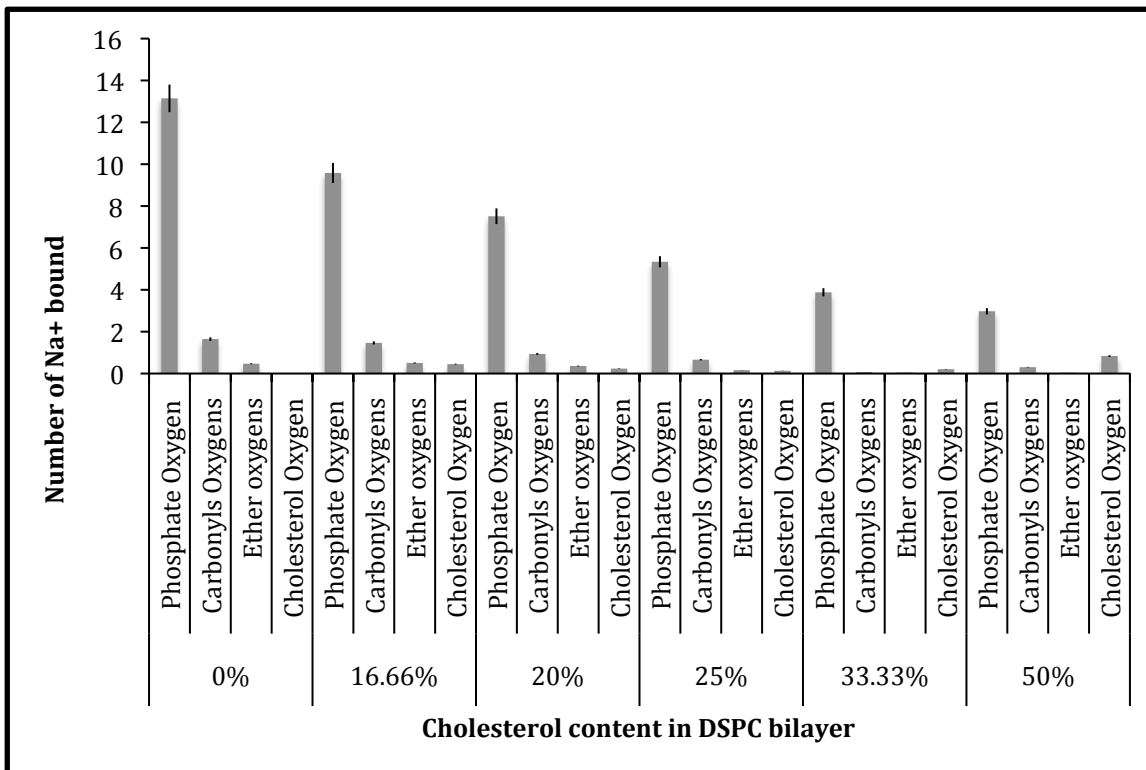


Figure S6: Identity of oxygens to which Na⁺ ions are bound. For all systems we see that binding (see text for definition of binding) to the phosphate oxygens dominates

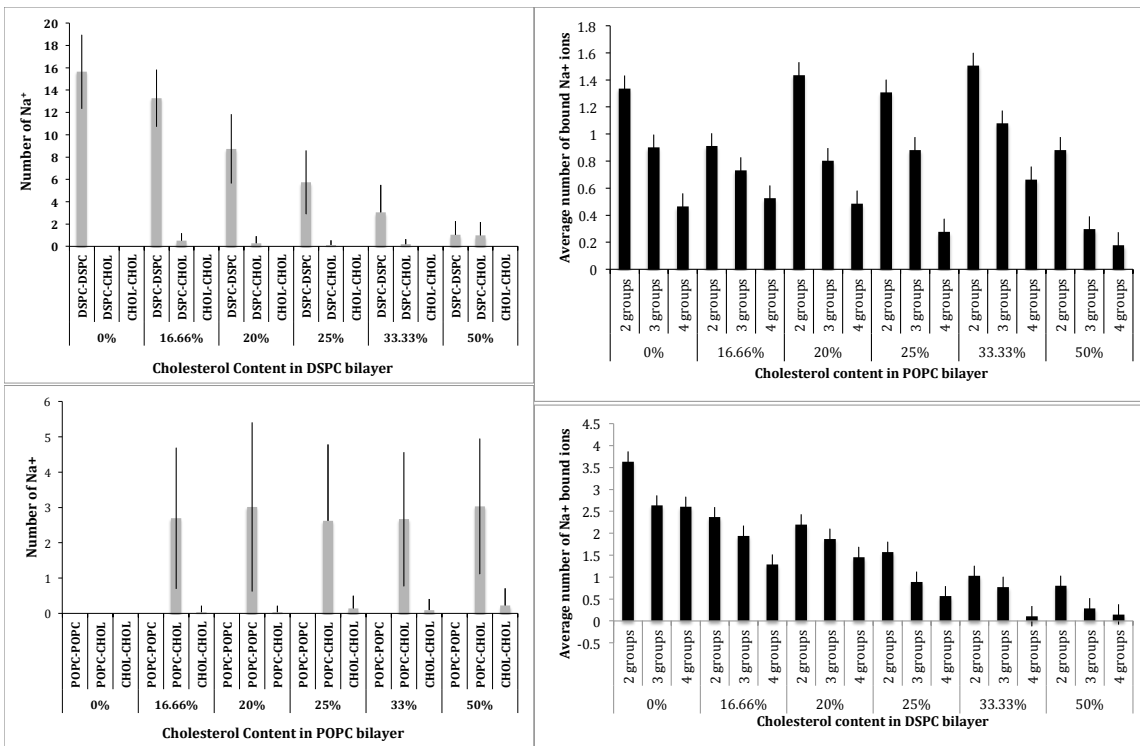
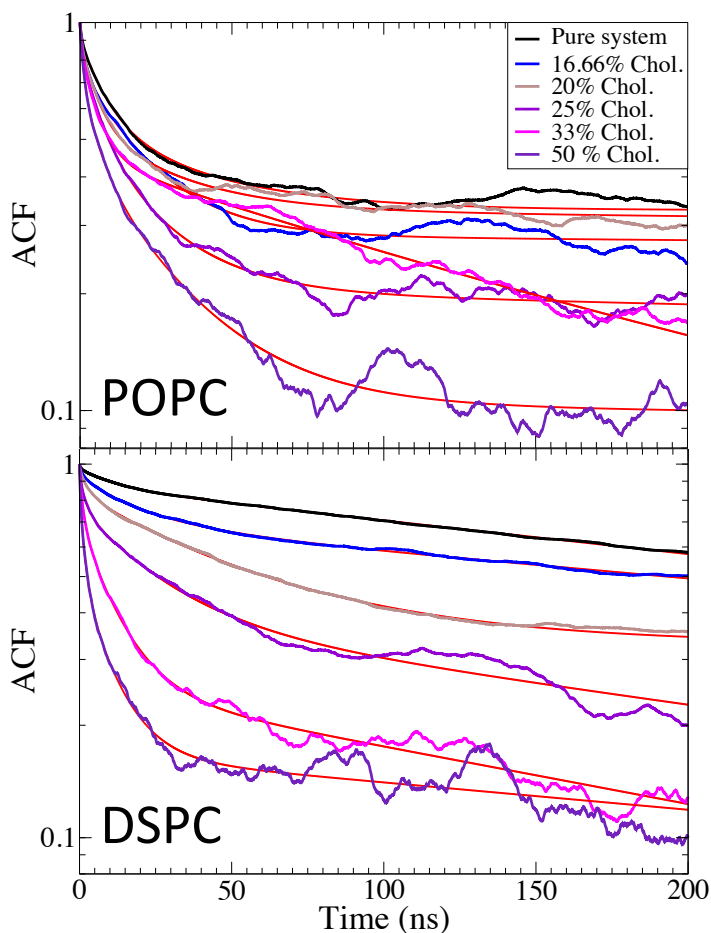


Figure S7 Plots showing stoichiometry of number of sodium ions that bridge different headgroup binding sites.



DSPC	A0	A1	A2	A3	A4	A5	A6	A7	A8	A9
pure	0.87	2.1E-3	0.10	0.078			0.26	1.54	0.0023	33.5
16% Chol.	0.69	1.7E-3	0.23	0.045	0.036	0.27	0.041	1.29	0.0074	19.2
20% Chol.	0.33	1.0E-5	0.50	0.018	0.12	0.28	0.041	1.54	0.0097	17.6
25% Chol.	0.38	2.6E-3	0.38	0.039	0.15	0.47	0.072	2.52	0.0098	29.5
33% Chol.	0.25	3.5E-3	0.46	0.084	0.2	0.66	0.077	3.09	0.0121	35.5
50% Chol.	0.17	1.7E-3	0.35	0.099	0.30	0.59	0.15	2.67	0.033	18.1
POPC	A0	A1	A2	A3	A4	A5	A6	A7	A8	A9
pure	0.32	1.7E-6	0.23	0.026	0.35	0.12	0.088	1.61	0.0080	37.3
16% Chol.	0.28	1.6E-4	0.48	0.049	0.17	0.51	0.062	3.47	0.0072	91.5
20% Chol.	0.33	1.8E-4	0.23	0.035	0.35	0.18	0.088	2.57	0.0080	59.2
25% Chol.	0.20	4.2E-4	0.35	0.044	0.30	0.16	0.14	1.78	0.013	39.6
33% Chol.	0.28	8.1E-3	0.17	0.0027	0.41	0.18	0.12	1.83	0.015	34.9
50% Chol.	0.10	2.0E-4	0.36	0.036	0.27	0.18	0.23	0.86	0.036	10.75

Figure S8: Auto-correlation functions (ACF) of Na⁺ binding with the interface in the POPC (upper panel) and DSPC (lower panel) bilayers. The autocorrelation function was defined as: $ACF(t') = \langle hw(t+t') hw(t) \rangle / \langle hw(t) \rangle$, where the function $hw(t)$ is 1 if the

Na^+ ion is bonded with lipids, and 0 otherwise (J. Phys. Chem. B *114*, 11784–11792). The ACF curves show multiexponential fits in all cases, for the pure DSPC system 4 exponentials, and for the POPC systems 5 exponentials. The fit parameters are included in the above table, where the parameters correspond to a fit of the decay to the function $y = a_0 e^{-a_1 x} + a_2 e^{-a_3 x} + a_4 e^{-a_5 x} + a_6 e^{-a_7 x} + a_8 e^{-a_9 x}$. In all cases the curves show clear multiexponential fits, for the pure DSPC system four exponentials, and for the POPC systems five exponentials, thus four decay modes for all systems and an extra mode is absent in the gel system. The fit parameters of the multi exponential fit are included. While interpreting this data one can discard the fastest mode that is both small amplitude, and decays over the first few data points, thus is neither well sampled nor important. This fast time scale can be associated with fast local oscillations. The slowest decaying exponential is likely the result of finite system size effects. This leaves us with two decay modes for the gel phase DSPC system three decay modes for all other systems (all DSPC-Chol POPC). Unambiguous mechanistic interpretation of which specific interactions the different decay rates correspond to is not possible. As cholesterol content increases the ion mobility increases and the mechanism for the increase can be seen to be increased strength of the faster decay modes, while the rates of the different decay modes seem to remain approximately constant. The characteristic binding time can be approximated as the time taken for the case of all POPC bilayers the initial decay in the ACF is completed in ~ 40 ns time, indicating full exchange of ions between interface and water. For the case of DSPC only in the two highest cholesterol concentration curves is the constant value reached. These results indicate that cholesterol increases the rate of ion exchange between water and membrane, and our results for the pure DSPC system are in agreement with our previously published results (Stepniewski et al., J. Chem. Phys. B *2010*).

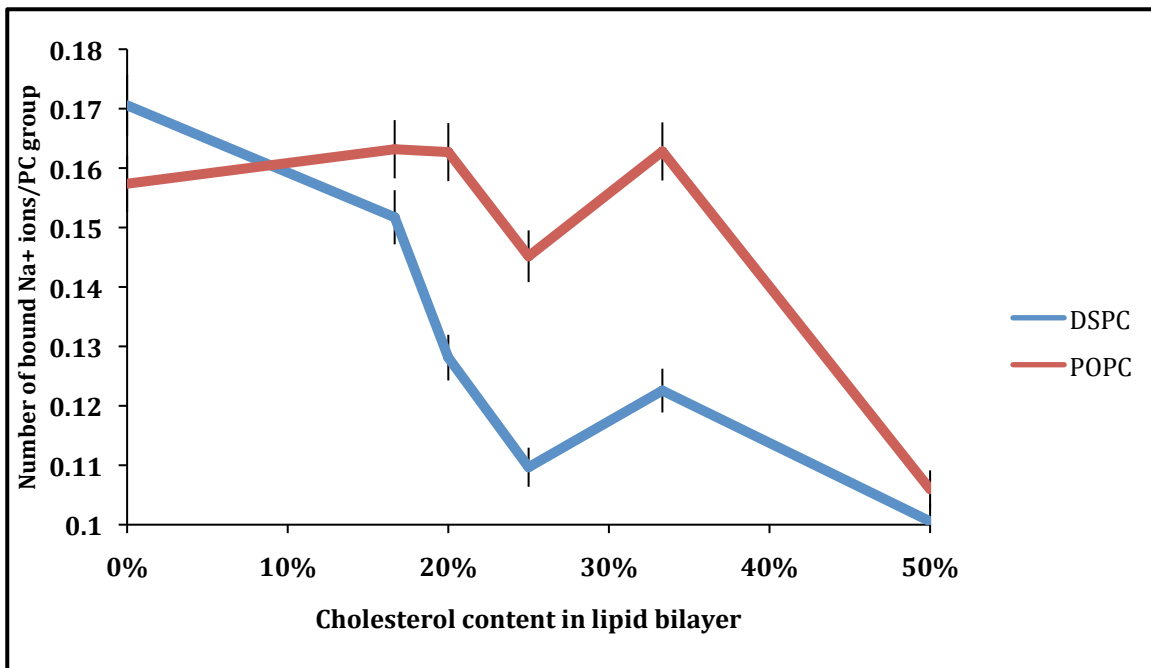


Figure S9: Number of bound Ions per PC headgroup present. For both DSPC and POPC these decline with increasing cholesterol content.

E: Cl⁻ interactions with phospholipid bilayers

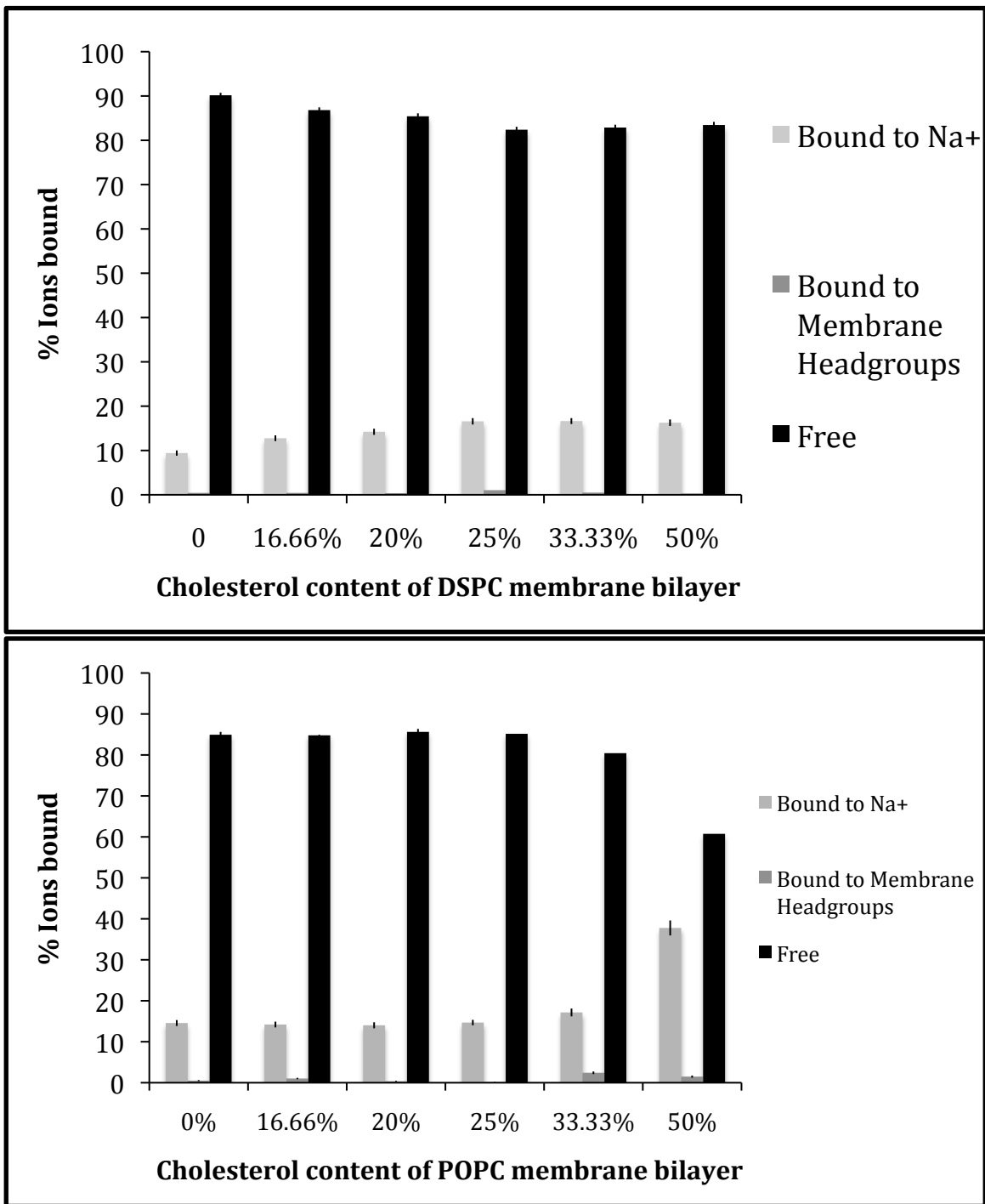


Figure S10: Cl⁻ ion binding, (see text for definition of binding) in the DSPC and POPC bilayer simulations. We see no significant binding of Cl⁻ ions to lipid headgroups.

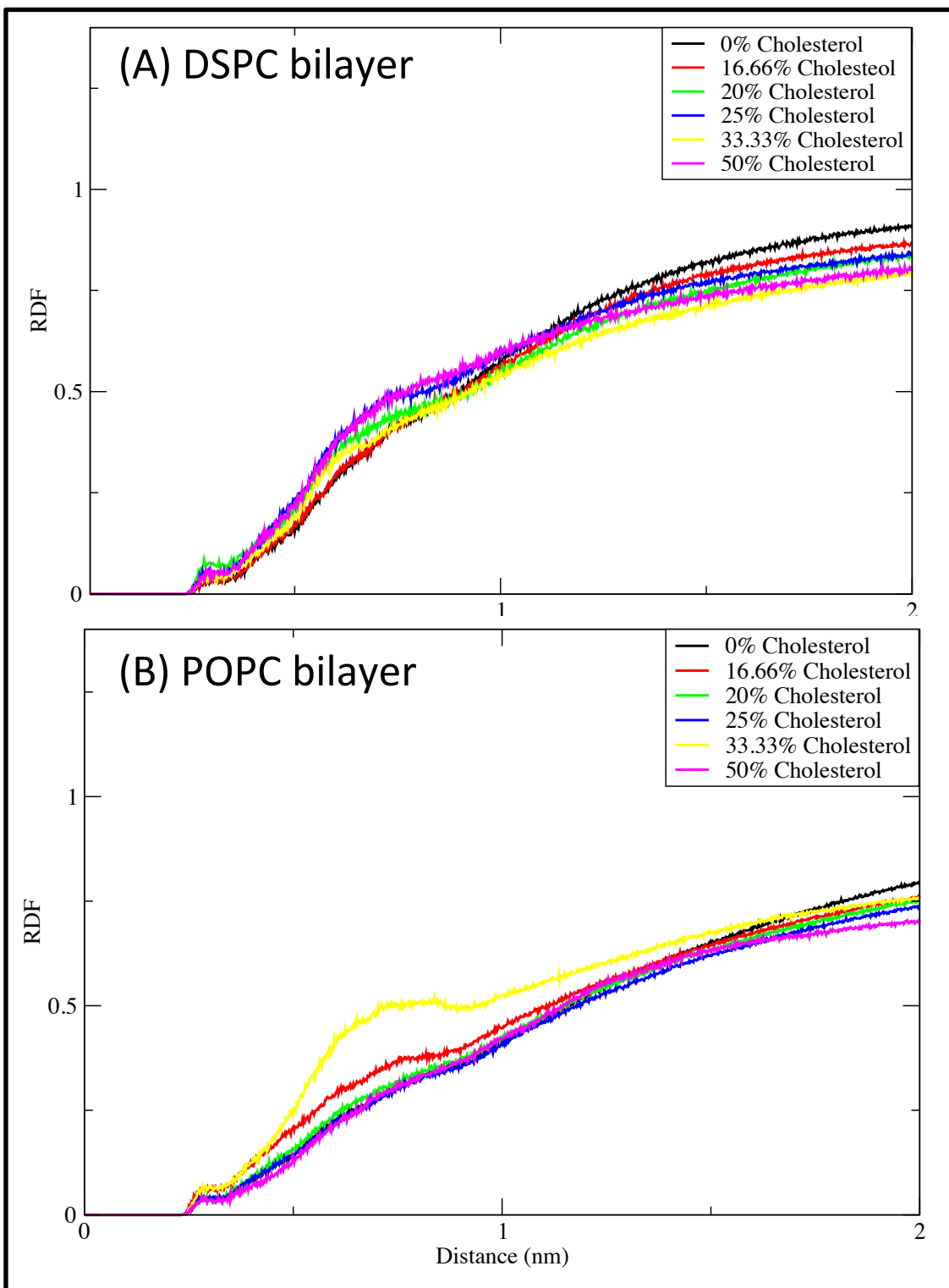


Figure S11: Radial distribution function of distance between Cl⁻ ions and choline group of DSPC/POPC. The figure shows no sharp peak indicating there is no strong interaction between them.

F: interactions of cholesterol with phospholipids

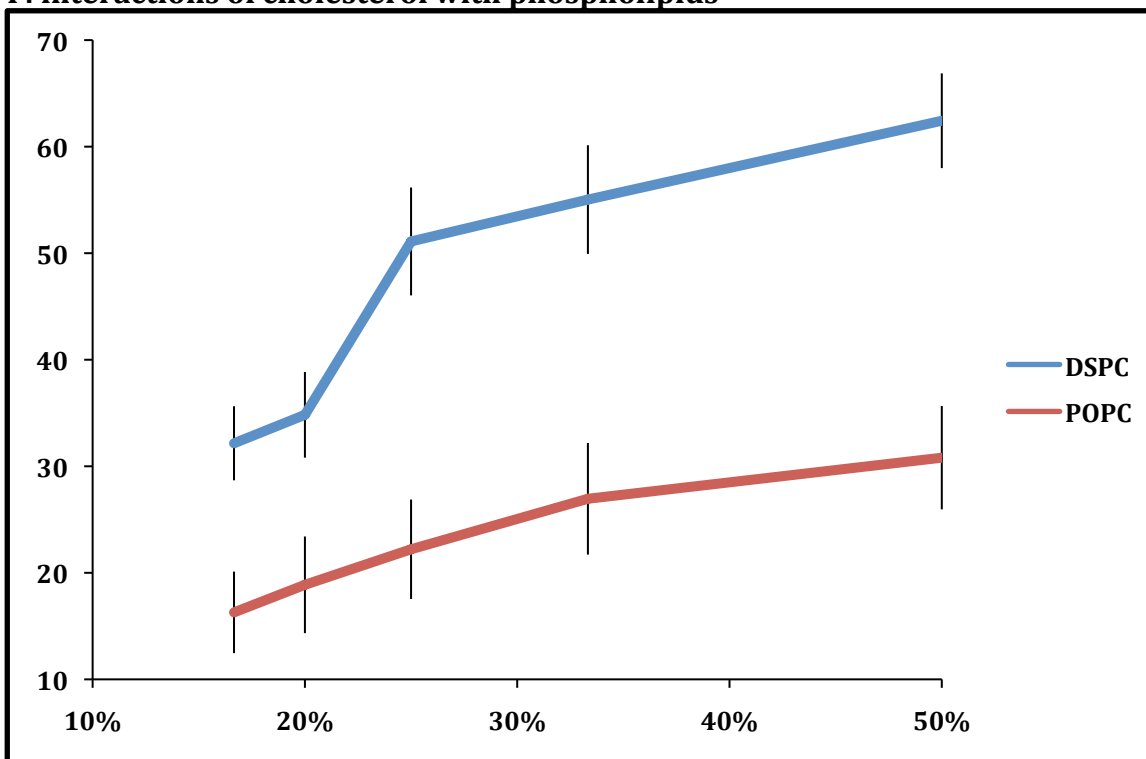


Figure S12A: Number of hydrogen bonds between hydroxyl group of cholesterol and phospholipids. This shows as the cholesterol content in the lipid bilayer increases, the number of hydrogen bonds between the hydroxyl group of cholesterol and the phospholipid head group.

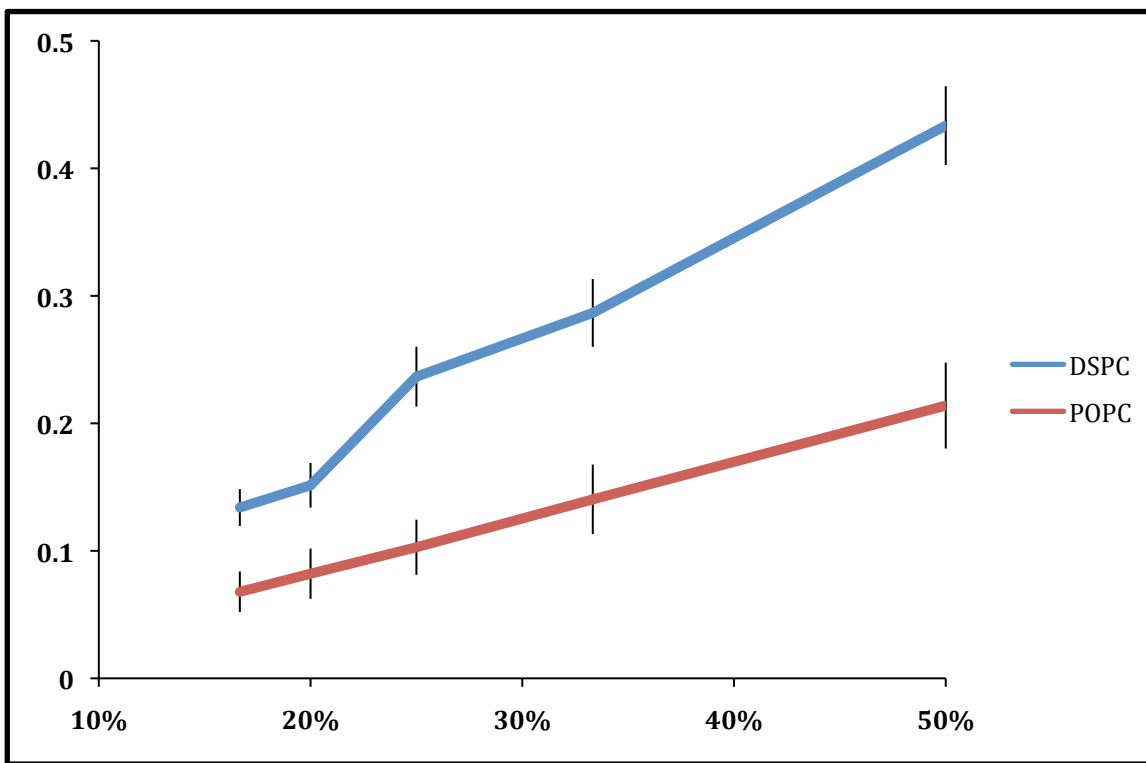


Figure S12B: Number of hydrogen bonds between hydroxyl group of cholesterol and phospholipids per DSPC/POPC molecules. This shows as the cholesterol content in the lipid bilayer increases, the likelihood that the DSPC/POPC lipid is bonded to cholesterol increases.

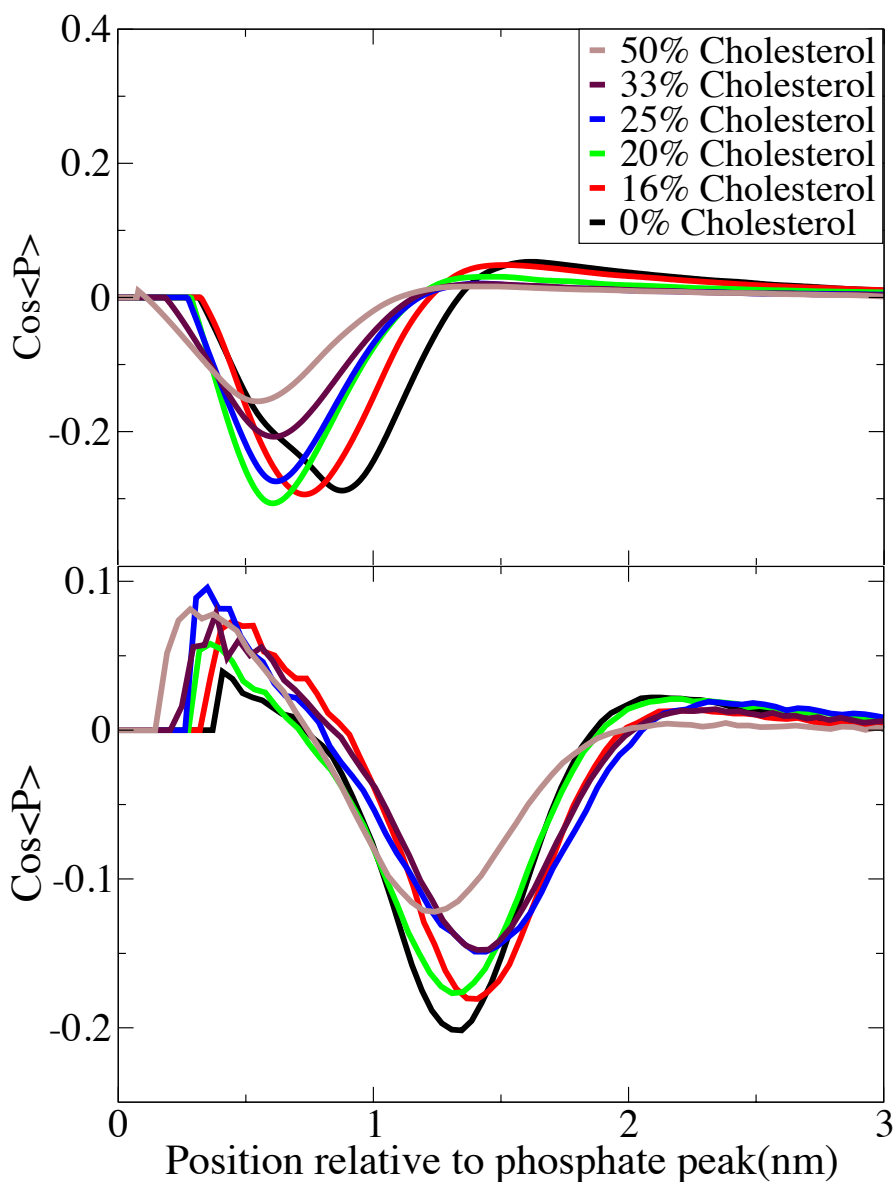


Figure S13 Water ordering plots normal to the membrane surface. We see in both the case of POPC (upper panel) and DSPC (lower panel) that the qualitative form of the water ordering is unchanged from previous computational results (*J. Phys. Chem. B.* **114**, 11784-11792 (2010)). For the case of DPPC the bilayer water ordering is systematically decreased with increasing cholesterol concentration while for the case of POPC for smaller cholesterol concentrations (0-25mol%) water ordering remains relatively constant and decreases for higher concentrations. Water ordering at the lipid/water interface originates from strong interactions between lipid headgroups and water, e.g. hydrogen bonds. The addition of cholesterol into the ordered DSPC bilayer increases the area of the whole membranes systematically with increasing cholesterol concentration (spacing effect) thus the area per individual headgroup increases and effectively it is seen as a decrease of the water ordering. For the case of POPC in lower concentrations the total area does not increase as a result of the condensing cholesterol effect which rich saturation at 20-30mol% (condensing effect does not occur in DSPC as lipids in the gel phase are already condensed), after this point the total area of the membrane becomes larger which again manifests itself in a decreased degree of water ordering. Thus the whole effect might be attributed to cholesterol spacing vs condensing effect.