SUPPORTING INFORMATION: Towards atomistic resolution structure of phosphatidylcholine headgroup and glycerol backbone at different ambient conditions†

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Simulation details

Berger based models

For the Berger based models we use here the following naming convention: Berger - {*molecule name*} - {*year when model published first time*} {*citation*}. The reason is that there are several different molecular topologies which are using the non-bonded parameters originally developed by Berger et al.[1] Thus the common factor in the Berger based models are the non-bonded parameters, while the molecule specific parameters might somewhat vary. However, the majority of the molecular level topologies are relying (especially for the glycerol backbone and headgroup) on the parameters originally introduced by Marrink et al.[2] This is the case for all the Berger based simulations discussed in this work.

POPC simulations at full hydration at 298 K and simulations studying the effect of cholesterol are the same as in previous publications.[3,4] In these simulation the POPC parameters introduced by Ollila et al[5] are used, which are using the non-bonded parameters of Berger[1] and a molecular topology from Tieleman et al.[6] with improved double bond dihedrals by Bachar et al.[7] Thus they are called Berger-POPC-07.[5] The cholesterol model is based on the parameters by Höltje et al.[8] with the exception that the atom types were changed from CH2/CH3 to LP2/LP3 to avoid overcondensation of the bilayer as suggested in ref.[9] Since this modification was introduced by Ferreira et al.,[3] we call the used cholesterol model as

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Höltje-CHOL-13.[3]

For the POPC at 323 K and POPC in low hydration the same force field parameters are used. For DPPC the implementation of Berger parameters[1] by Peter Tieleman et al. are used.[2] For all of these simulations a timestep of 2 fs was used with a leapfrog integrator. Covalent bond lengths were constrained with the LINCS algorithm.[10,11] Coordinates were written every 10 ps. PME[12,13] with real space cut-off of 1.0 nm was used for electrostatics. Plain cut-off was used for the Lennard-Jones interactions with a 1.0 nm cut-off. The neighbor lists with cut-off of 1.0 nm were updated every 5 steps. Temperature was coupled separately for lipids and water to 298 K using the velocity-rescale method[14] with coupling constant 0.1 ps. Pressure was semi-isotropically coupled to the atmospheric pressure with the Berendsen method.[15]

CHARMM36

DPPC and POPC with 72 lipids. The starting structures in PDB format were downloaded from the NIH/NHLBI Laboratory of Computational Biology Membrane Biophysics Section website (`http://www.lobos.nih.gov/mbs/coords.shtml`), which refers to these as the final structures (for DPPC after 40 ns, POPC after 35 ns) of the NPT lipid bilayer trajectories used in the original CHARMM36 publication.[16] The TIP3P[17] water model was used to solvate the system. The publicly available CHARMM36 parameters in Gromacs format (September 2013 update: `charmm36_gmx_format_sep13.tgz`) from the MacKerell Lab website (`http://mackerell.umaryland.edu/CHARMM_ff_params.html`) were used. Timestep of 1 fs was used with the leapfrog integrator. Covalent bonds with hydrogens were constrained with LINCS algorithm.[10,11] Coordinates were written every 5 ps. PME[12,13] with real space cut-off of 1.4 nm was used for electrostatics. Lennard-Jones interactions were switched to zero between 0.8 nm and 1.2 nm. The neighbour lists with a cut-off of 1.4 nm were updated every 5 steps. Temperature was coupled separately for lipids and water to 303 K using the velocity-rescale method[14] with coupling constant of 0.2 ps. Pressure was semi-isotropically

coupled to the atmospheric pressure with the Berendsen method.[15]

POPC with 128 lipids The starting structures for the pure POPC simulations was taken from the Slipids[18] website (`http://mmkluster.fos.su.se/slipids/Downloads.html`). The starting structures for mixed POPC/Cholesterol simulations were constructed with the CHARMM-GUI website.[19] They contained 100 POPC/24 cholesterol molecules and 80 POPC/80 cholesterol molecules for the simulations of 20% cholesterol and 50% cholesterol respectively. The TIP3P water model[17] was used to solvate the system. The publicly available CHARMM36 forcefield parameters (`http://www.gromacs.org/@api/deki/files/184/=charmm36.ff\\\_4.5.4\_ref.tgz`) by Piggot et al.[20] were used. Cholesterol parameters came from Lim et al.[21] and were converted into GROMACS format with the PyTopol tool.[22] Single point energy calculation was done to assess the conversion. Simulations were performed for 200 ns and the last 100 ns was used for the calculations. Timestep of 2 fs was used with leapfrog integrator. All bond lengths were constrained with LINCS.[10,11] Temperature was maintened at 303 K with the velocity-rescale method[14] and a time constant of 0.2 ps. Pressure was maintained semiisotropically at 1 bar using the Parrinello–Rahman algorithm[23] with a time constant of 1.0 ps. The neighbour list with a cut-off of 1.2 nm was updated every 10 steps. Lennard-Jones interactions were switched to zero between 0.8 nm and 1.2 nm. PME[12,13] with real space cut-off of 1.2 nm was used for electrostatics.

POPC with 512 lipids. POPC The starting structures for simulations were constructed with the CHARMM-GUI website.[19] 512 POPC lipids (256 per leafleat) were used for the initial 0% concentration and they were subsequently substituted by cholesterol molecules to fulfil the desired concentration. The TIP3P[17] water model was used to solvate the system. The publicly available port of the CHARMM36 forcefield parameters (`mackerell.umaryland.edu/CHARMM_ff_params.html`) was used for both POPC and cholesterol. Simulations were performed for 170 ns and the last 100 ns was used for the calculations. Timestep of 1 fs was used with the leapfrog integrator. Covalent bonds with hydrogen were constrained with LINCS algorithm. Coordinates were written every 10 ps. PME with real space cut-off

of 1.2 nm was used for electrostatics. Lennard-Jones interactions were switched to zero between 0.8 nm and 1.2 nm. The neighbour lists with a cut-off of 1.2 nm were updated every 5 steps. Temperature was coupled separately for cholesterol, lipids and water to 298 K using the velocity-rescale method with coupling constant of 0.2 ps. Pressure was semi-isotropically coupled to the atmospheric pressure with the Berendsen method.

MacRog

The lipid force field parameters were obtained from the developers and the parameters are presented in Refs. 24,25. A bilayer with 288 POPC lipids was hydrated with 12600 TIP3P water[17] molecules (∼44/lipid) and simulated for 100 ns with a time step of 2 fs. Data was saved every 10 ps and the first 20 ns of the trajectory was discarded from the analysis. All bond lengths were constrained with LINCS.[10,11] The temperatures of the lipids and the solvent were separately coupled to the Nosé–Hoover thermostat[26,27] with a target temperature of 310 K and a time constant of 0.4 ps. Semi-isotropical pressure coupling to 1 bar was obtained with the Parrinello–Rahman barostat[23] with a time constant of 1 ps. PME[12,13] was employed to calculate the long-range electrostatic interactions. Lennard-Jones interactions were cut off at 1 nm and the dispersion correction was applied to both energy and pressure. A neighbour list with a radius of 1 nm was updated every step.

Identical parameters were employed for both full hydration and for the dehydration simulations. The dehydration simulations were also run for 100 ns with data saved every 10 ps.

The initial structures for the simulations with 10, 40, 50 and 60 mol% of cholesterol were obtained by replacing 14, 56, 64 or 72 POPC molecules with cholesterol molecules in the initial structure containing 128 POPC molecules. These systems were simulated for 400 ns and the first 200 ns was discarded from analysis. Data was saved every 100 ps.

GAFFLipid

The initial structure in Lipidbook[28] had different glycerol backbone isomers in different leaflets. To generate the initial structure we took the structure delivered by Slipids developers.[18] Also this structure had one lipid with different glycerol backbone isomer. This lipid and one lipid from opposite leaflet were removed after the system was equilibrated.

The force field parameters were generated using files obtained from the Lipidbook website (`http://lipidbook.bioch.ox.ac.uk/package/show/id/150.html`).[28] The conversion to GROMACS compatible formats was performed using the acpype tool.[29] The accuracy of the conversion was checked by calculating the total energy of a single POPC lipid molecule using the sander program which is part of the AmberTools14 package[30] and version 4.6.5 of GROMACS. A difference of 0.002 kcal/mol was obtained between the two programs.

Timestep of 2 fs was used in Langevin dynamics with zero friction term and collision frequency of 1.0 ps^{-1}. Covalent bonds with hydrogens were constrained with the LINCS algorithm.[10,11] Coordinates were written every 10 ps. PME[12,13] with a real space cut-off at 1.0 nm was used for electrostatics. Plain cut-off with 1 nm was used for Lennard-Jones interactions. The neighbour lists with a cut-off of 1.0 nm were updated every 5 steps. Pressure was semi-isotropically coupled to a pressure of 1 bar with the Berendsen method.[15]

It should be noted that the area per molecule with these settings for the GAFFlipid model was 61.6 $Å^2$, while the original publication reported 63.9 $Å^2$.[31] However, the same parameters and Amber to Gromacs conversion reproduced the area per molecule from original publication for the lipid14 model (see next section).

Lipid14

The initial structure was taken directly from the Lipidbook.[28] The Amber compatible force field parameters were generated using the tleap program which is integrated in the AmberTools14 package.[30] A workflow similar to the one used previously for the conversion and validation of the GAFFLipid parameters was followed here. As before, a negligible energy

difference of 0.003 kcal/mol was obtained between the two programs.

Timestep of 2 fs was used in Langevin dynamics with zero friction term and collision frequency of 1.0 ps^{-1}. Covalent bonds with hydrogens were constrained with LINCS algorithm.[10,11] Coordinates were written every 10 ps. PME[12,13] with real space cut-off of 1.0 nm was used for electrostatics. Plain cut-off with 1 nm was used for Lennard-Jones interactions. Dispersion correction was applied for both energy and pressure. The neighbor lists with a cut-off of 1.0 nm were updated every 5 steps. Pressure was semi-isotropically coupled to a pressure of 1 bar with the Berendsen method.[15]

The area per molecule with these settings was 65.4 $Å^2$ which is in agreement with the value reported in the original publication 65.6±0.5 $Å^2$.[32]

Poger et al.

The Poger lipids are derived from GROMOS G53A6[33] and were initially coined 53A6-L (L for lipids). They are now part of GROMOS G54A7[34] and parametrized to work with the SPC water model.[35] The initial hydrated bilayer structure of 128 DPPC and 5841 water molecules as well as force field parameters were downloaded from David Poger's web site (`http://compbio.chemistry.uq.edu.au/~david/`) on April 2012. We noticed that the same files downloaded in October 2013 appear to lack two dihedral angles in the choline headgroup (only one dihedral of type gd_29 allowing the rotation of the 3 choline methyls) compared to the April 2012 version (3 dihedrals of type gd_29 for the 3 choline methyls). This should not affect the bilayer structure and only change the kinetics of the choline methyls rotation. However the October 2013 version has not been tested in this study.

MD Simulations (two repetitions with independent initial velocities) were run for 100 ns using a 2 fs time step and the analysis was performed on the last 50 ns. Coordinates were saved every 50 ps for analysis. All bond lengths were constrained with the LINCS algorithm.[10,11] Temperature was kept at 323 K employing the velocity-rescale[14] thermostat with a time constant of 0.1 ps (DPPC and water coupled separetly). Pressure was maintained

semi-isotropically at 1 bar using the Parrinello–Rahman barostat[23] using a 4 ps time constant and a compressibility of 4.5e-5 bar^{-1}. For non-bonded interactions, two conditions were tested: i) A 0.8–1.4 nm twin-range cut-off with the neighbor list updated every 5 steps for both electrostatics and Lennard-Jones (LJ) interactions (simulation files available at[36,37]). For the former the generalized reaction field (RF) with a dielectric permittivity of 62 was used beyond the 1.4 nm cut-off.[38] This is the original setup that Poger et al.[33] used. ii) PME[12,13] electrostatics with a real space cut-off of 1.0 nm, a Fourier spacing of 0.12 nm and an interpolation order of 4, LJ interactions computed with a 1.0–1.4 nm twin-range cut-off, neighbor list updated every 5 steps (simulation files available at[39,40]). Note that Poger and Mark tested the effect of PME vs RF in ref.,[34] but used a 1.0 nm cut-off with PME and 1.4 nm with RF for LJ interactions. Since 0.8–1.4 nm twin-range cut-off for LJ interations is used in the parametrization of GROMOS force field, we decided to use that also in the simulations with PME.

Since Poger lipids come from the GROMOS force field, it is important to note that GROMOS uses the RF scheme for computing electrostatics (this is the method used for the force field parameterization). Using setup i) based on RF, we were able to reproduce the results (i.e. area per lipid value of 0.63 nm^2) from the original work only with GROMACS versions 4.0.X and earlier (the original authors[33] used GROMACS version 3.3.3). When switching to versions 4.5.X and above, the area per lipid dropped to below 0.58 nm^2. The GROMACS developers were contacted and a redmine issue opened (`http://redmine.gromacs.org/issues/1400`). The difference comes from the new Trotter decomposition introduced in versions 4.5.X. A fix has been introduced in version 4.6.6 that allows a recovery of an area per lipid value of 0.615 nm^2. The results in terms of area per lipid using the different GROMACS versions are available at.[37] Thus we decided to use only the PME setup ii) for computing the order parameter since it gives stable results regardless of the GROMACS version. We obtained an area per lipid of 0.615 nm^2, below 0.648 nm^2 found by the original authors with their PME setup (see[34]). We explained that by the fact

that we used a 1.4 nm for the LJ cut-off whereas a value of 1.0 nm was used in the original publication.

Slipids

Initial coordinates for a hydrated DPPC (at 323 K) and POPC (at 310 K) bilayers (30 and 40 waters/lipid, respectively) were taken directly from the Slipids home page `http://mmkluster.fos.su.se/slipids/Downloads.html`. The Slipids force field[18,41] was used for the the all atom descriptions of DPPC and POPC, and water was described with the TIP3P water model.[17] Simulations were performed within the NPT ensemble using the GROMACS 4.6.X simulation package.[42] The Nosé–Hoover thermostat[26,27] was used with reference temperatures of 323 K (DPPC) and 310 K (POPC) and a relaxation time constant of 0.5 ps. Water and lipids were coupled separately to the heat bath. Pressure was kept constant at 1.013 bar using a semi–isotropic Parrinello–Rahman barostat[23] with a time constant of 10.0 ps. Equations of motion were integrated with the leapfrog algorithm using a timestep of 2 fs. Long range electrostatic interactions were calculated using the PME method,[12,13] with a fourth order smoothing spline. A real space cut-off of 1.0 nm was employed with grid spacing of 0.12 nm in the reciprocal space. Lennard-Jones potentials were cut off at 1.4 nm, with a dispersion correction applied to both energy and pressure. All covalent bonds in lipids were constrained using the LINCS algorithm,[10] whereas water molecules were constrained using SETTLE.[43] Twin-range cutoffs, 1.0 nm and 1.6 nm, were used for the neighbor lists with the longrange neighbor list updated every 10 steps. This simulation protocol corresponds to the protocol used in Ref.[44]

Kukol

A bilayer patch with 512 POPC lipids was constructed and hydrated with ∼40 SPC water molecules per lipid. The force field parameters were obtained from Lipidbook.[28] This bilayer was simulated with a 2 fs time step for a total of 50 ns and coordinates were saved every

100 ps. All bonds were constrained with LINCS.[10,11] PME[12,13] was employed for the long-range electrostatics. Lennard-Jones interactions were cut off at 1.4 nm. A neighbour list with a radius of 0.8 nm was updated every 5 steps. The constant temperature of 298 K was maintained with the Berendsen thermostat[15] with a time constant of 0.1 ps. The Berendsen barostat[15] was employed for semi-isotropical pressure coupling at 1 bar.

Chiu et al.

The force field parameters and the initial configuration were available through the Lipidbook.[28] Timestep of 2 fs was used with leapfrog integrator. Covalent bond lengths were constrained with LINCS algorithm.[10,11] Coordinates were written every 10 ps. PME[12,13] with real space cut-off of 1.0 nm was used for electrostatics. Twin range cut-off was used for the Lennard-Jones interactions with short and long cut-offs of 1.0 nm and 1.6 nm, respectively. The neighbour lists with a cut-off of 1.0 nm were updated every 5 steps. Temperature was coupled separately for lipids and water to 298 K with the velocity-rescale method[14] with a coupling constant 0.2 ps. Pressure was semi-isotropically coupled to the atmospheric pressure with the Parrinello–Rahman method.[23]

Ulmschneider

The initial structure containing 128 POPC molecules with 3328 TIP3P water[17] molecules (26 per lipid) was downloaded from Lipidbook[28] together with the topologies. This bilayer was simulated for 100 ns with a time step of 2 fs and the data was saved every 10 ps. The bonds involving hydrogen atoms were constrained with LINCS.[10,11] The temperature was kept at 298 K with the Berendsen thermostat.[15] The pressure was semi-isotropically coupled to the Berendsen barostat[15] with a time constant of 1 ps and a target pressure of 1 bar. PME[12,13] was employed for long range electrostatics and a cut-off of 1 ns was employed for the Lennard-Jones interactions. A neighbour list with a radius of 1 nm was updated every 10 steps.

Additionally, the simulations were repeated with the dispersion correction applied to pressure and temperature. Even though the area per lipid decrease d slightly, the headgroup order parameters were only slightly affected.

Tjörnhammar et al.

The gel phase DPPC bilayer structure delivered by Tjörnhammar and Edholm[45] was ran for 5 ns at 343 K in order to destroy the ordered gel configuration. This was followed by a 200 ns simulation at 323 K, i.e. in the fluid phase. The last 100 ns of this simulation was used for analysis. The same mdp file as in the Supplementary Information section of the original paper [45] was used except for the simulation temperature.

Lee-CHARMM36-UA

A hydrated bilayer consisting of 72 DLPC lipids and 2189 water molecules is constructed using the model by Lee et al. [46] This model describes the important all-atom CHARMM36 character of the lipid headgroup but reduces the details of the lipid chains into a united-atom model. The initial equilibrated structure was downloaded from the web page of J. Klauda, Department of Chemical and Biomolecular Engineering University of Maryland. The parameter files are taken from the Supplementary Material of Ref. 46. This bilayer was equilibrated for 20 ns and the production run was 50 ns long, with data was saved every 20 ps. The equations of motion were integrated using the multiple time step Verlet r-RESPA algorithm with a time step of 2 fs, and a calculation of the electrostatic forces only every two timesteps. Covalent bonds between heavy and hydrogen atoms were constrained using SHAKE/RATTLE algorithm.[43]

The temperature was kept at 323 K with a Langevin thermostat with a damping coefficient of 5 ps. The modified NAMD version of the Nose-Hoover barostat with Langevin dynamics (piston period of 0.1 ps and piston decay time of 0.05 ps) was used semi-isotropically to reach the averaged target pressure of 1 bar and an averaged zero surface tension. PME[12,13]

was employed for long range electrostatics. A cut-off of 1.2 nm was employed for the Lennard-Jones interactions, with a force-based switching function for distances beyond 1 nm. A neighbour list with a radius of 1.4 nm was updated every 10 timesteps.

NAMD was developed by the Theoretical and Computational Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign.[47]

Botan-CHARMM36-UA

This model is similar to the Lee-CHARMM36-UA model with a few differences. A hydrated bilayer consisting of 128 DLPC lipids and 3840 water molecules is described by a model derived from the CHARMM27-UA model by Hénin et al.[48]

The distribution into all-atom (AA) and united-atom (UA) parts within the lipids is the same as in the original CHARMM27-UA model by Hénin et al.[48] This distribution differs from the one by Lee-CHARMM36-UA model solely for the first methylene groups of the two acyl chains (C22 and C32 in CHARMM36 topology): their hydrogens are merged into united atoms, whereas in Lee's model their hydrogens are described explicitely. The united-atom Berger model [1] was used for the tails and the UA-AA interactions as in Ref. 48. The difference between this model and the original one by Hénin[48] is the replacement of the AA parameters for the heads by the parameters of the all-atom CHARMM36 force-field.[16] Contrarily to the model by Lee et al.,[46] no reparametrization was done.

The non-bonded interactions are calculated using an atom-based switching function with short and long cut-offs of 0.8 and 1.2 nm. Long range electrostatic interactions are implemented using the particle-particle particle-mesh solver with a relative accuracy of 10^{-4}. The system is first equilibrated for 30 ns in the NPγT ensemble (Nosé–Hoover[26,27] style thermostat and barostat with anisotropic pressure coupling) at 323 K and 1 bar with timestep of 1 fs. The following 20 ns of dynamics are taken for calculation of configurational averages. Simulations were carried out by using the LAMMPS package.[49]

Details of the rough subjective force field ranking (Figure 4)

The assessment was based fully on the Fig. 2. First, for each carbon (the columns in Fig. 4) in each force field (the rows), we looked separately at deviations in magnitude and forking.

Magnitude deviations, i.e., how close to the experimentally obtained C–H order parameters (OPs) the force-field-produced OPs were. For each carbon, the following 5-step scale was used:

0 ( ): More than half of all the calculated OPs (that is, of all different hydrogens in all different lipids) were within the *subjective sweet spots* (SSP, blue-shaded areas in Fig. 2).

1 (ᴍ): All the calculated OPs were < 0.03 units away from the SSP.

2 (M): All the calculated OPs were < 0.05 units away from the SSP.

3 (M): All the calculated OPs were < 0.10 units away from the SSP.

4 (M): Some of the calculated OPs were > 0.10 units away from the SSP.

Forking deviations, i.e., how well the difference in order parameters of two hydrogens attached to a given carbon matched that obtained experimentally. Note that this is not relevant for g_2, which has only one hydrogen. For the β and α carbons, for which no forking is experimentally seen, the following 5-step scale was used:

0 ( ): The distance D between the dots (that mark the measurement-time-weighted averages in Fig. 2) was < 0.005 units for all the calculated OPs (that is, for all different lipids).

1 (ꜰ): $D < 0.015$.

2 (F): $0.015 < D < 0.030$.

3 (F): $0.030 < D < 0.050$.

4 (F): $0.050 < D$.

For the g_3 carbon, for which a small forking of 0.02 is indicated by experiments, the following 5-step scale was used:

0 ( ): $0.010 < D < 0.030$.

1 (F): $(D < 0.010)$ OR $(0.030 < D < 0.040)$.

2 (F): $0.040 < D < 0.055$.

3 (F): $0.055 < D < 0.070$.

4 (F): $0.070 < D$.

For the g_1 carbon, for which a considerable forking of 0.15 is experimentally seen, the following 5-step scale was used:

0 ( ): $0.13 < D < 0.17$.

1 (F): $(0.11 < D < 0.13)$ OR $(0.17 < D < 0.19)$.

2 (F): $(0.09 < D < 0.11)$ OR $(0.19 < D < 0.21)$.

3 (F): $(0.07 < D < 0.09)$ OR $(0.21 < D < 0.23)$.

4 (F): $(D < 0.07)$ OR $(0.23 < D)$.

Based on these assessments of magnitude and forking deviations, each carbon was then assigned to one of the following groups: "within experimental error" (magnitude and forking deviations both on step 0 of the scales described above), "almost within experimental error" (sum of the magnitude and forking deviation steps 1 or 2), "clear deviation from experiments" (sum of magnitude and forking deviation steps from 3 to 5), and "major deviation from experiments" (sum of magnitude and forking deviation steps from 6 to 8). These groups

are indicated by colors in Fig. 2. (Note that for g_2, for which there can be no forking, the corresponding group assigment limits were: 0, 1, 2, and 3.)

Finally, the total ability of the force field to describe the headgroup and glycerol structure was estimated. To this end, the groups were given the following weights: 0 (within experimental error), 1 (almost within experimental error), 2 (clear deviation from experiments), 4 (major deviation from experiments), and the weights of the five carbons were summed up. The sum, given in the Σ-column of Fig. 2, was then used to (roughly and subjectively, as should be clear from the above description) rank the force fields.

The effect of cholesterol on glycerol backbone structure

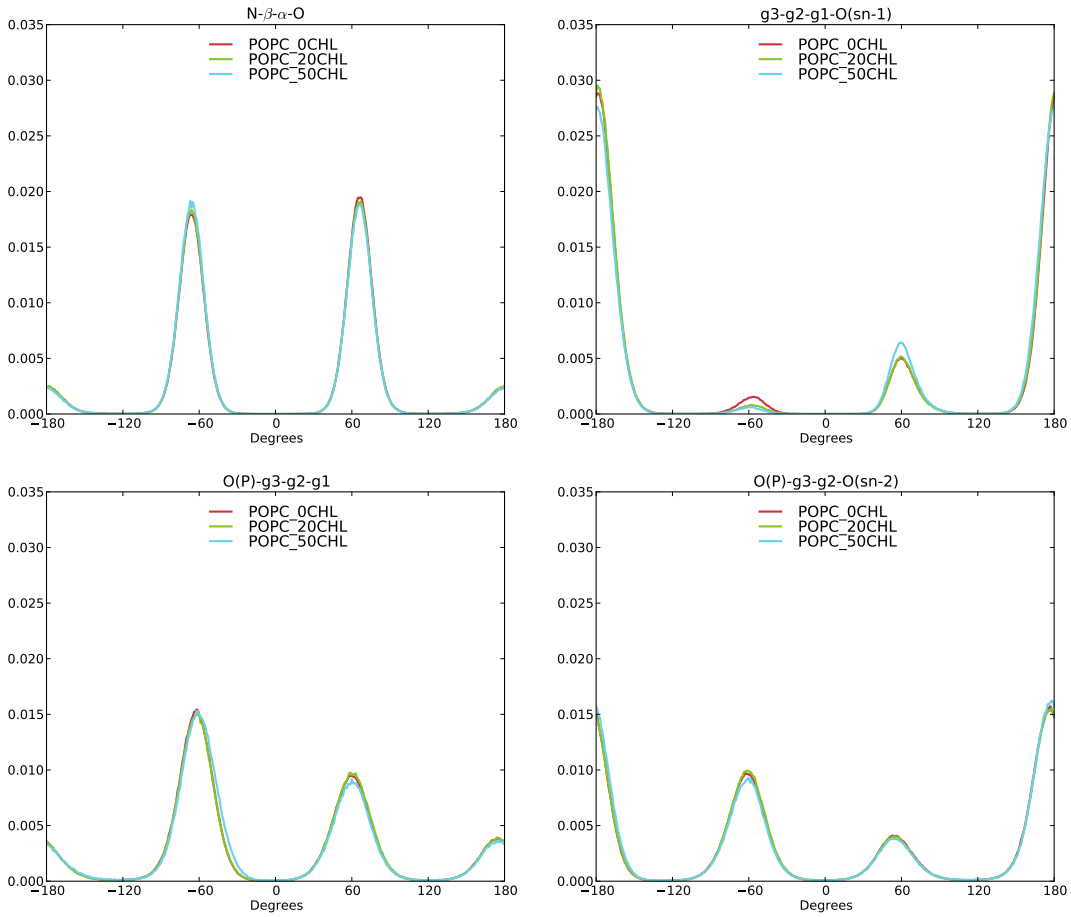

Figure S1: The effect of cholesterol content on the POPC glycerol backbone and choline dihedral angles in CHARMM36 model (T=303 K).

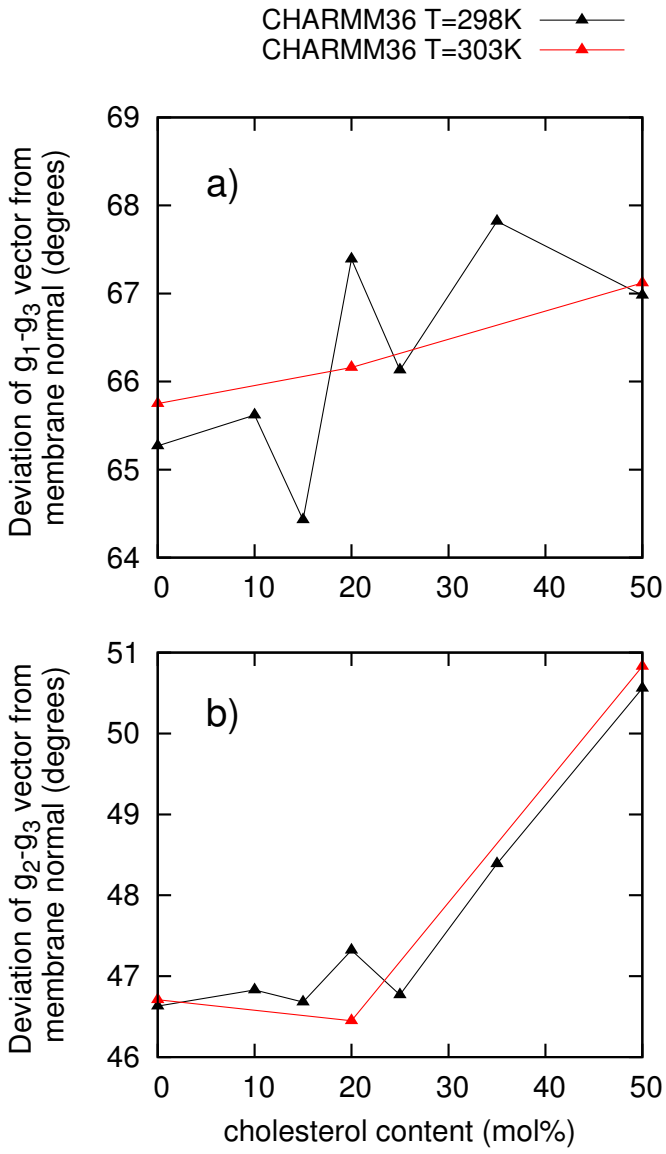

Figure S2: Orientation of g_1-g_3 (a) and g_2-g_3 (b) respect to membrane normal as a function of cholesterol content calculated from CHARMM36 simulations in 298K and 303K.

Author Contributions

Alexandru Botan provided simulation results for charmm36-UA

Fernando Favela prepared, performed and analyzed simulations with popc and cholesterol for the CHARMM36 All-Atom FF.

Patrick F. J. Fuchs ran and analyzed the Poger simulations. Provided scientific information which significantly advanced the project (signs and forking of order parameters).

Matti Javanainen prepared and performed simulations with multiple lipid models and analyzed the results. Supervised the work of JT.

Matej Kanduc provided simulation results for the Berger DLPC model

Waldemar Kulig prepared the MD simulations with cholesterol for the MacRog FF

Antti Lamberg prepared and performed simulations with Berger and its variants to show the importance of the signs and stereospecific labeling of the order parameters.

Claire Loison provided simulation results for charmm36-UA.

Alexander Lyubartsev provided simulation results for Hgberg08 force field.

Markus S. Miettinen co-designed the project with OHSO and supported in the work management. Provided Berger DMPC trajectory; prepared parameter files for 72 lipids CHARMM36. Made Figures 2–4 and 7–9.

Luca Monticelli: Critical discussions in all phases of the project. Collaboration with Jukka Määttä.

Jukka Määttä prepared and performed simulations with Berger and Slipids models and analyzed the results.

O. H. Samuli Ollila co-designed the project with MSM and managed the work. Ran and analyzed several simulations. Wrote the manuscript.

Marius Retegan prepared and validated the GROMACS compatible parameter files for GAFFlipid and Lipid14 force fields.

Tomasz Rog provided the topologies for the MacRog model and also the full hydration simulation data with this model.

Hubert Santuz prepared and performed the cholesterol simulations with CHARMM36 and analyzed the results. Made significant contribution in the data management of the project. *Joona Tynkkynen* prepared and performed the dehydration simulations with the MacRog FF.

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