Supporting Information:

NMRlipids IV: Headgroup & glycerol backbone structures, and cation binding in bilayers with PS lipids

O. H. Samuli Ollila[*,†] and NMRlipids collaboration Authorship query to be sent soon.[¶]

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S1   Simulated systems

S1.1   CHARMM36

To be written by Piggot, Madsen and Ollila

S1.2   CHARMM36ua

To be written by Piggot

S1.3   Slipids

To be written by Piggot and Favela

S1.4   Berger

To be wiritten by Piggot and Ollila Simulations with sodium were taken directly from Ref. ?   and simulations with calcium directly from 1. Simulation of POPC at 310 K was taken directly from Ref. 2.

S1.5   GROMOS-CKP

To be written by Piggot

S1.6   Lipid17

To be written by Kav and Miettinen

S1.7   MacRog

To be written by Javanainen and Piggot

S2 Electrometer concept in PC lipid bilayers mixed with negatively charged lipids

The electrometer concept is based on the empirical observations that the order parameters of α and β carbons in PC lipid headgroup decrease (increase) proportionally to the bound positive (negative) charge.[3–6] The concept is also valid for lipid mixtures where PC order parameters increase when mixed with anionic lipids, in contrast to the mixtures with neutral or cationic surfactants[5,7] (Fig. S1).
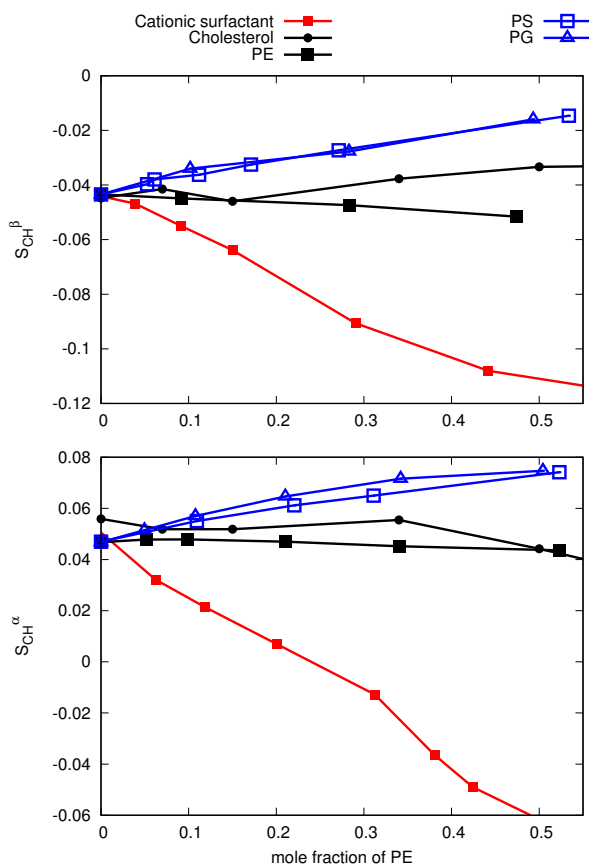

Figure S1: PC headgroup order parameters from experiments of mixtures with PE, PS, PG and cholesterol.[6–8] Signs are determined as discussed in.[9,10]

Based on the electrometer concept, the headgroup order parameters of PC lipids can be used to measure the ion binding affinity to lipid bilayers.[3–5] Changes of the headgroup order parameters of negatively charged PS and PG lipids with bound charge are also systematic,

but less well characterized.[11–14] Therefore, the ion binding affinity to negatively charged bilayers can be better characterized measuring the PC headgroup order parameters from mixed bilayers. The PC headgroup order parameters in mixtures with anionic lipids are larger than in pure PC lipid because addition of negative charges increase the order parameters (Figs. S1 and S2). Upon addition of $CaCl_2$, the PC headgroup order parameters in the mixed bilayers decrease and reach the values of pure PC bilayer close to the $CaCl_2$ concentrations of $\sim$ 50-300mM, depending on the amount of negatively charged lipids in the mixture (Fig. S2). At this point the charge of bound Ca^{2+} probably cancels the charge of anionic negative lipids and the bilayer has neutral charge. The overcharging then occurs with larger concenterations. As expected, the more pronounced order parameter decrease is observed in systems with more negatively charged lipids (Fig. S3) indicating higher binding affitiny of cations into the negatively charged membranes. In conclusion, the consistency of the experimental results suggests that the electrometer concept can be used to determine the cation binding also to the lipid bilayers containing negatively charged lipids.
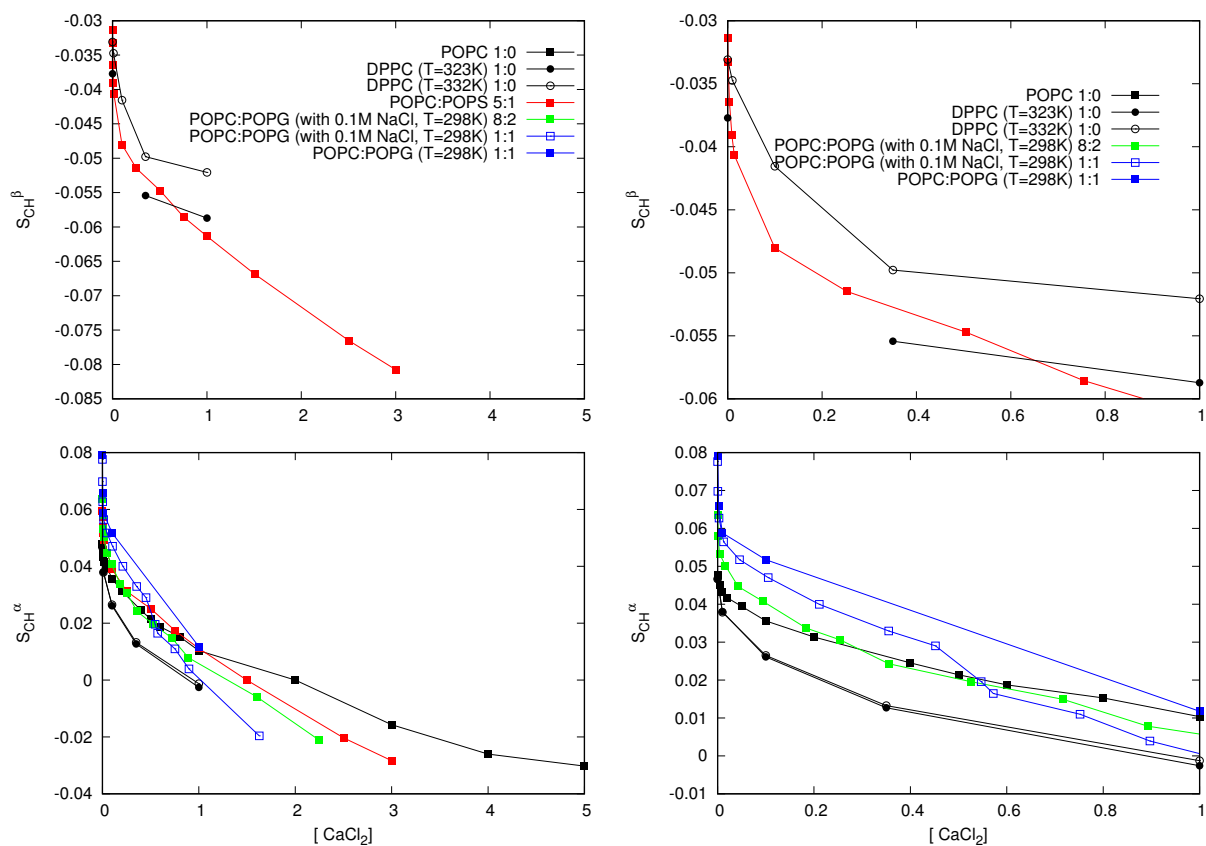
Figure S2: PC headgroup order parameters as a function of CaCl concentration from experiments different mole fractions of negatively charged lipids. Pure DPPC data from,[3] pure POPC data from,[4] POPC:POPS mixture data from,[14] POPC:POPG mixture data with 0.1M NaCl from[12] and POPC:POPG mixture data without NaCl from.[11] The right column shows the results zoomed to concentrations below 1M.
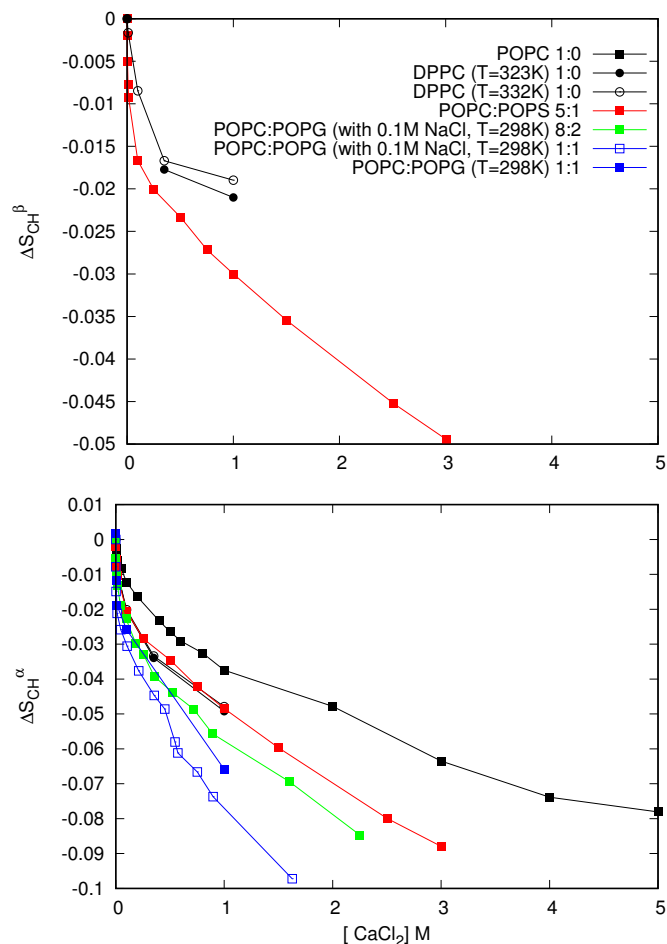
Figure S3: The change of PC headgroup order parameters as a function of $CaCl_2$ measured from bilayers containing different amount of negatively charged lipids. The values are taken from 2H NMR experiments reported in the literature (DPPC,[3] POPC,[4] POPC:POPS (5:1),[14] POPC:POPG mixtures with 0.1M NaCl[12] and POPC:POPG (1:1) without NaCl[11]). As expected, the decrease of order parameters with the added $CaCl_2$ is more pronounced for systems with larger fraction of negatively charged lipids, indicating larger amount amount of bound cations.

S3 Calibration of PC headgroup order parameter response to the bound cations

Before using the headgroup order parameters to compare ion binding affinity between simulations and experiments, it is important to quantify the response of the order parameters to the bound charge in simulations.[15,16] Comparison of the headgroup order parameter response to the cationic surfactants in POPC bilayer to the experiments[6] shows that the both order parameters are too sensitive to the bound charge in the Lipid14 model, while CHARMM36 gives better agreement for the α carbon (Fig. S4). The ratio $\Delta S_{\mathrm{CH}}^{\beta}/\Delta S_{\mathrm{CH}}^{\alpha}$ from Lipid14 model was in good agreement with experiments in the previous study[15] because both order parameters are equally too sensitive to the bound cations (Fig. S4). The ratio was, however, overestimated for the CHARMM36 model because the β order parameter relatively more sensitive than the α order parameter. These have to be taken into account when analysing the ion binding affinities using headgroup order parameters in simulations.
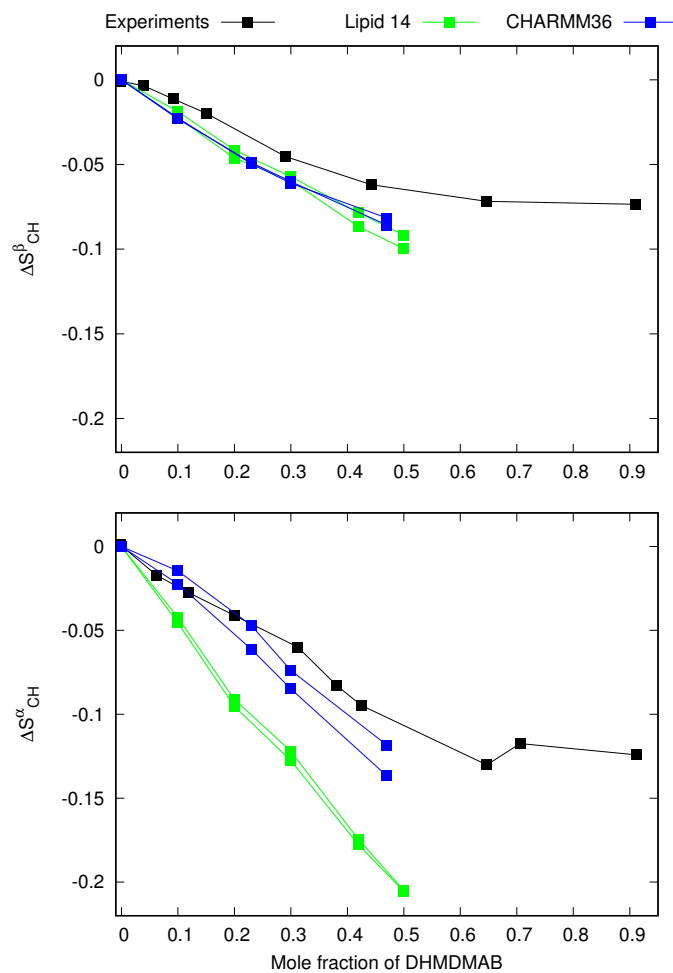
Figure S4: The response of headgroup order parameters to the fixed amount of cationic surfactants in POPC bilayer is compared between simulations and experiments.[6]

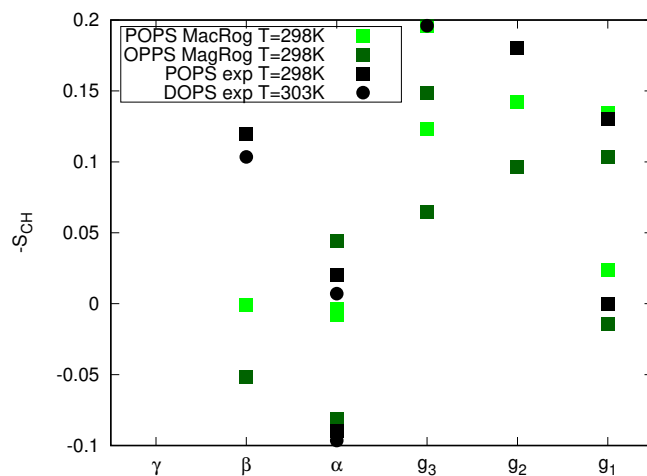# S4   Difference between POPC and OPPS in MacRog model

Figure S5: Headgroup order parameters from POPS and OPPS simulations with MacRog model.

S5 Dihedrals

The experimental results show essentially no changes in the order parameters as a function of added NaCl, while significant changes are observed in simulations. However, the minimum buffer concentration of NaCl in the experimental was 100mM.[13] Therefore, we cannot exclude the possibility that the NaCl induced changes were already saturated with 100mM NaCl concentration, which was the case for $CaCl_2$ in Fig. **??**.
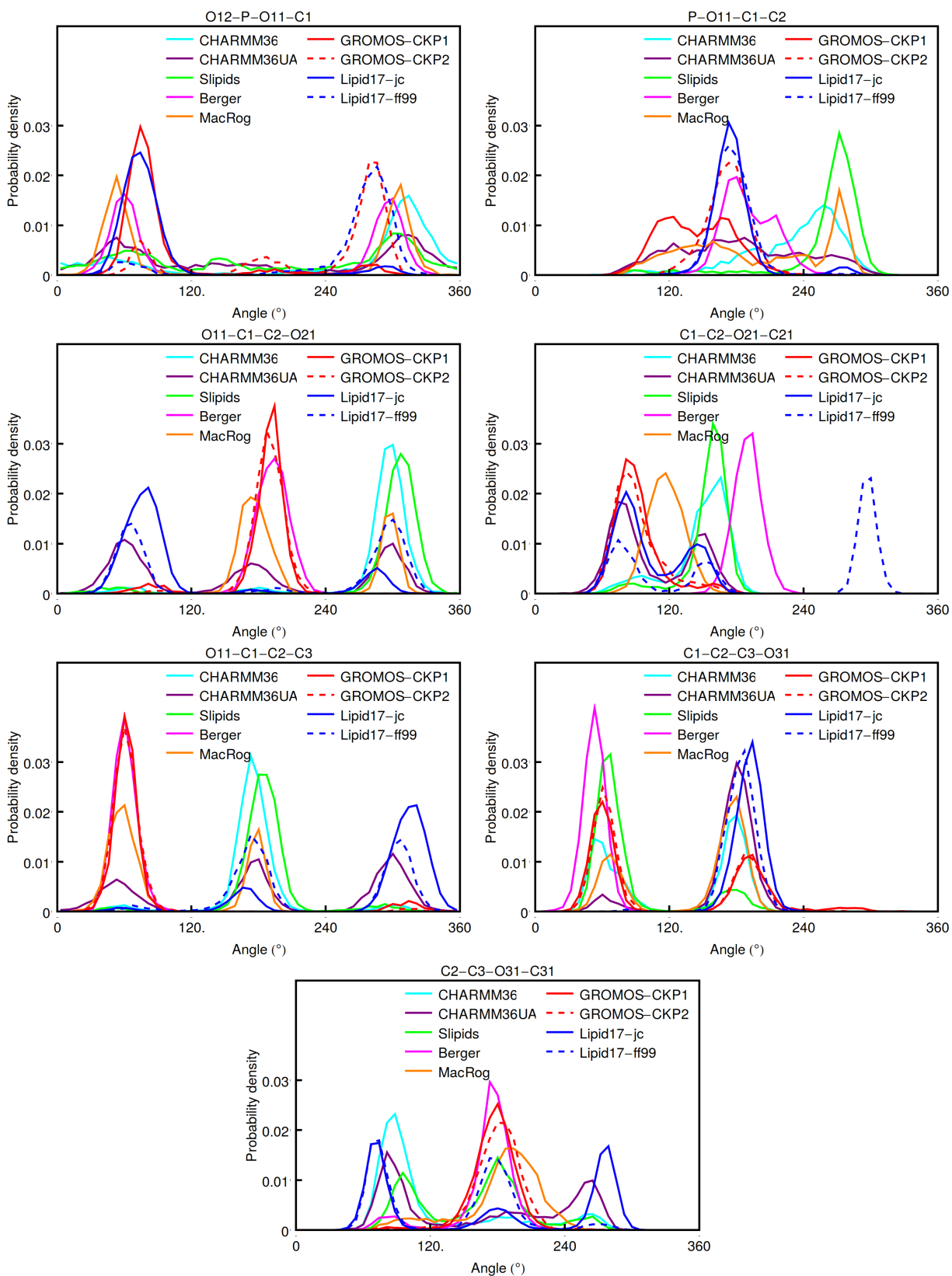
Figure S6: Dihedral angle distributions of bonds from phosphate to acyl chain carbonyls from different simulation models.
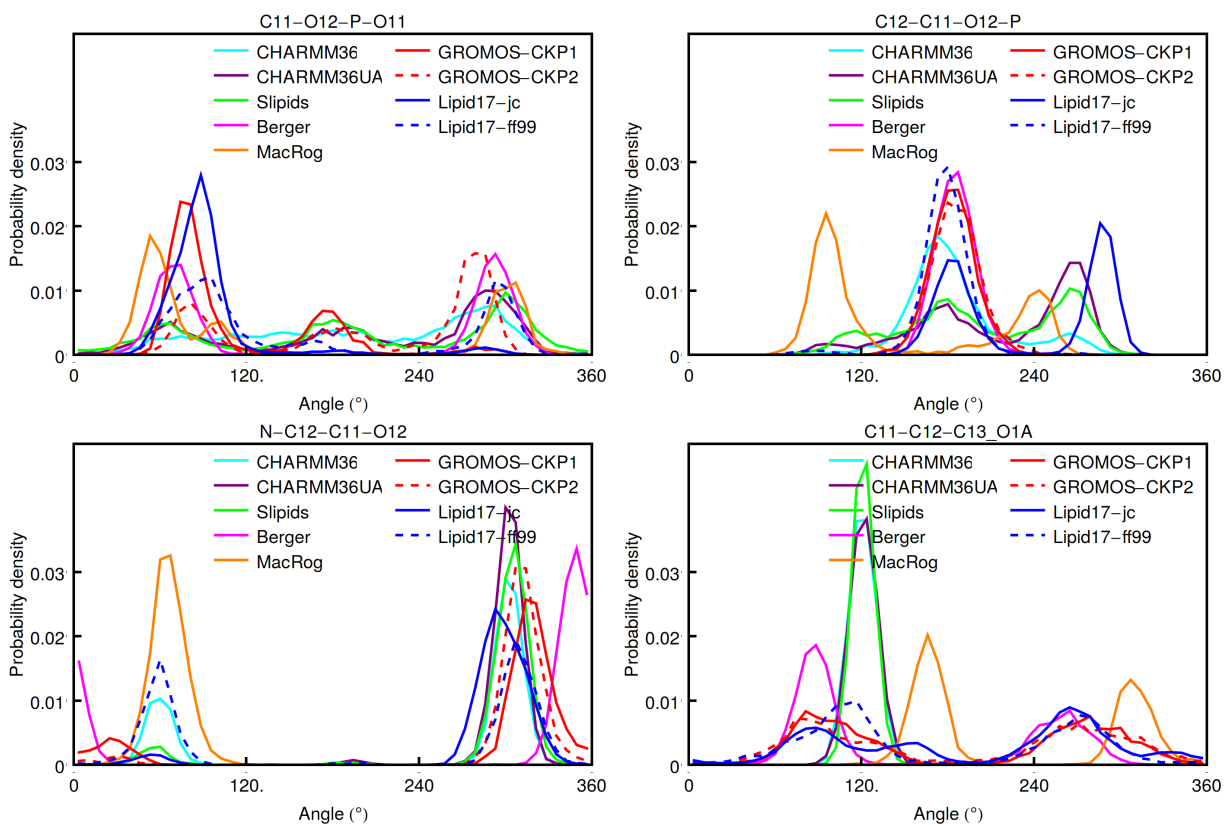
Figure S7: Dihedral angle distributions of bonds from phosphate to headgroup from different simulation models.
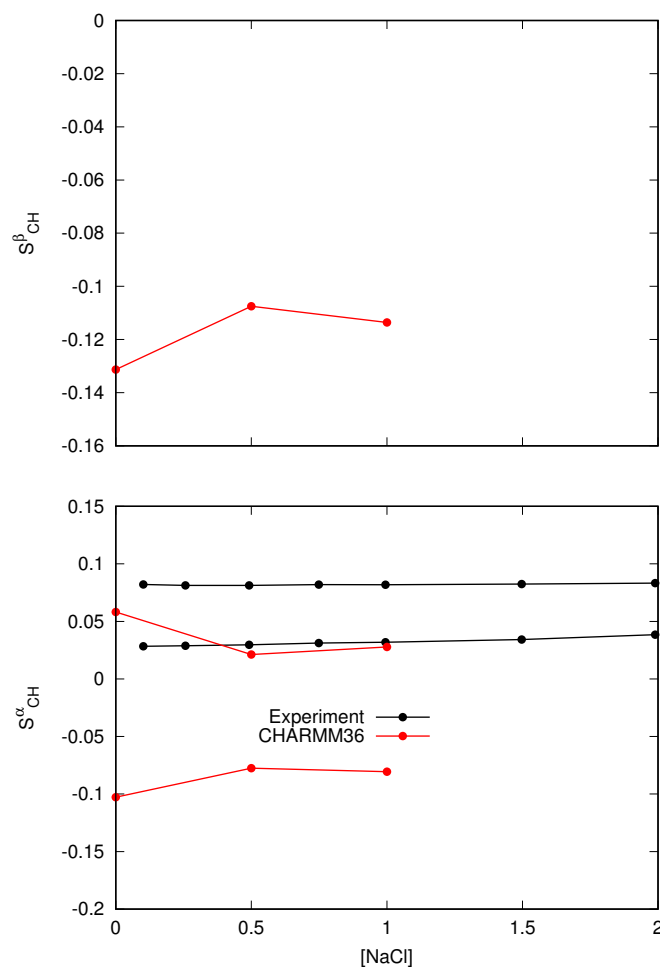
Figure S8: Order parameters of PS headgroup as a function of added NaCl measured from DMPC:DMPS (3:1) mixture.[13]

Figure S9: The response of headgroup order parameters to the fixed amount of cationic surfactants in POPC bilayer is compared between simulations and experiments.[6]
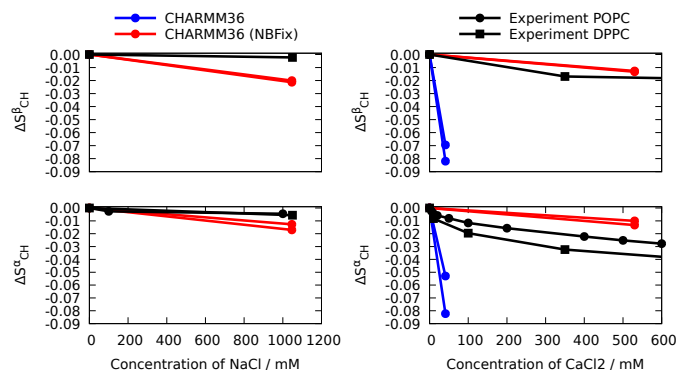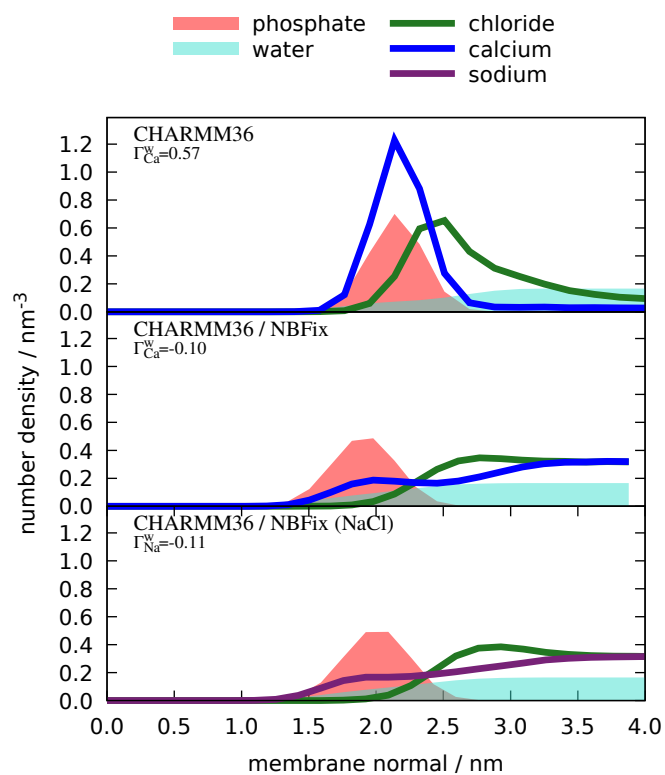
Figure S10: The response of headgroup order parameters to the fixed amount of cationic surfactants in POPC bilayer is compared between simulations and experiments.[6]

S6 Details of the rough subjective force field ranking (Fig. ??)

The assessment was based fully on the Fig. **??**. First, for each carbon (the columns in Fig. **??**) 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. **??**).

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 β and g_2, which have only one hydrogen. For the α carbon, for which a considerable forking of 0.105 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. **??**) was $0.08 < D < 0.13$ units for all the calculated OPs (that is, for all different lipids).

1 (ꜰ): $(0.06 < D < 0.08)$ OR $(0.13 < D < 0.15)$.

2 (F): $(0.04 < D < 0.06)$ OR $(0.15 < D < 0.17)$.

3 (F): $(0.02 < D < 0.04)$ OR $(0.17 < D < 0.19)$.

4 (F): $(D < 0.02)$ OR $(0.19 < D)$.

For the g_3 carbon, for which no forking is indicated by experiments, the following 5-step scale was used:

0 ( ): $D < 0.02$.

1 (F): $0.02 < D < 0.04$.

2 (F): $0.04 < D < 0.06$.

3 (F): $0.06 < D < 0.08$.

4 (F): $0.08 < D$.

For the g_1 carbon, for which a considerable forking of 0.13 is experimentally seen, the following 5-step scale was used:

0 ( ): $0.11 < D < 0.15$.

1 (F): $(0.09 < D < 0.11)$ OR $(0.15 < D < 0.17)$.

2 (F): $(0.07 < D < 0.09)$ OR $(0.17 < D < 0.19)$.

3 (F): $(0.05 < D < 0.07)$ OR $(0.19 < D < 0.21)$.

4 (F): $(D < 0.05)$ OR $(0.21 < D)$.

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. 4. (Note that for β and 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. **??**, was then used to (roughly and subjectively, as should be clear from the above description) rank the force fields.

References

(1) Melcrová, A.; Pokorna, S.; Pullanchery, S.; Kohagen, M.; Jurkiewicz, P.; Hof, M.; Jungwirth, P.; Cremer, P. S.; Cwiklik, L. The complex nature of calcium cation interactions with phospholipid bilayers. *Sci. Reports* **2016**, *6*, 38035.

(2) Ollila, S.; Hyvönen, M. T.; Vattulainen, I. Polyunsaturation in Lipid Membranes: Dynamic Properties and Lateral Pressure Profiles. *J. Phys. Chem. B* **2007**, *111*, 3139–3150.

(3) Akutsu, H.; Seelig, J. Interaction of metal ions with phosphatidylcholine bilayer membranes. *Biochemistry* **1981**, *20*, 7366–7373.

(4) Altenbach, C.; Seelig, J. Calcium binding to phosphatidylcholine bilayers as studied by deuterium magnetic resonance. Evidence for the formation of a calcium complex with two phospholipid molecules. *Biochemistry* **1984**, *23*, 3913–3920.

(5) Seelig, J.; MacDonald, P. M.; Scherer, P. G. Phospholipid head groups as sensors of electric charge in membranes. *Biochemistry* **1987**, *26*, 7535–7541.

(6) Scherer, P. G.; Seelig, J. Electric charge effects on phospholipid headgroups. Phosphatidylcholine in mixtures with cationic and anionic amphiphiles. *Biochemistry* **1989**, *28*, 7720–7728.

(7) Scherer, P.; Seelig, J. Structure and dynamics of the phosphatidylcholine and the phosphatidylethanolamine head group in L-M fibroblasts as studied by deuterium nuclear magnetic resonance. *EMBO J.* **1987**, *6*.

(8) Ferreira, T. M.; Coreta-Gomes, F.; Ollila, O. H. S.; Moreno, M. J.; Vaz, W. L. C.; Topgaard, D. Cholesterol and POPC segmental order parameters in lipid membranes: solid state ^{1}H-^{13}C NMR and MD simulation studies. *Phys. Chem. Chem. Phys.* **2013**, *15*, 1976–1989.

(9) Botan, A.; Favela-Rosales, F.; Fuchs, P. F. J.; Javanainen, M.; Kanduč, M.; Kulig, W.; Lamberg, A.; Loison, C.; Lyubartsev, A.; Miettinen, M. S. et al. Toward Atomistic Resolution Structure of Phosphatidylcholine Headgroup and Glycerol Backbone at Different Ambient Conditions. *J. Phys. Chem. B* **2015**, *119*, 15075–15088.

(10) Ollila, O. S.; Pabst, G. Atomistic resolution structure and dynamics of lipid bilayers in simulations and experiments. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2016**, *1858*, 2512 – 2528.

(11) Borle, F.; Seelig, J. Ca2+ binding to phosphatidylglycerol bilayers as studied by differential scanning calorimetry and 2H- and 31P-nuclear magnetic resonance. *Chemistry and Physics of Lipids* **1985**, *36*, 263 – 283.

(12) Macdonald, P. M.; Seelig, J. Calcium binding to mixed phosphatidylglycerol-phosphatidylcholine bilayers as studied by deuterium nuclear magnetic resonance. *Biochemistry* **1987**, *26*, 1231–1240.

(13) Roux, M.; Neumann, J.-M. Deuterium NMR study of head-group deuterated phosphatidylserine in pure and binary phospholipid bilayers. *FEBS Letters* **1986**, *199*, 33–38.

(14) Roux, M.; Bloom, M. Calcium, magnesium, lithium, sodium, and potassium distributions in the headgroup region of binary membranes of phosphatidylcholine and phosphatidylserine as seen by deuterium NMR. *Biochemistry* **1990**, *29*, 7077–7089.

(15) Catte, A.; Girych, M.; Javanainen, M.; Loison, C.; Melcr, J.; Miettinen, M. S.; Monticelli, L.; Maatta, J.; Oganesyan, V. S.; Ollila, O. H. S. et al. Molecular electrometer and binding of cations to phospholipid bilayers. *Phys. Chem. Chem. Phys.* **2016**, *18*, 32560–32569.

(16) Melcr, J.; Martinez-Seara, H.; Nencini, R.; Kolafa, J.; Jungwirth, P.; Ollila, O. H. S.

Accurate Binding of Sodium and Calcium to a POPC Bilayer by Effective Inclusion of Electronic Polarization. *The Journal of Physical Chemistry B* **2018**, *122*, 4546–4557.