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

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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 written by Piggot and Ollila Simulations with excess sodium were taken directly from Ref. 1 and simulations with calcium directly from 2. Simulation of POPC at 310 K was taken directly from Ref. 3.

S1.5 GROMOS-CKP

To be written by Piggot

S1.6 Lipid17

To be written by Kav, Miettinen and Meclr.

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[4–7] (Fig. S1). Therefore, the headgroup order parameters can be used to measure the ion binding affinity to lipid bilayers containing PC lipids.[4–6,8–10] Changes of the headgroup order parameters of other lipids with bound charge, like negatively charged PS and PG lipids, are also systematic, but less well characterized.[8–11] Therefore, the ion binding affinity to negatively charged bilayers can be better characterized measuring the PC headgroup order parameters from mixed bilayers.[8–12]

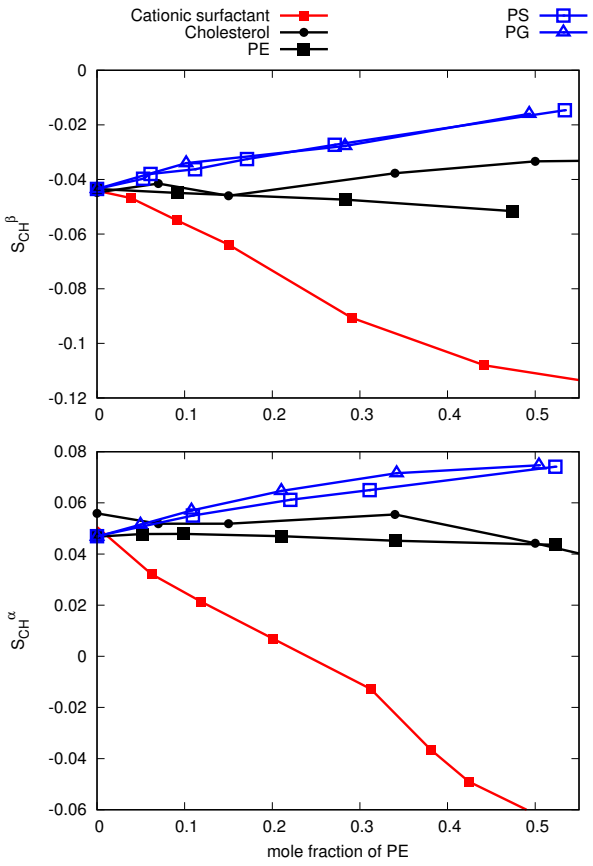

Figure S1: Headgroup order parameters of POPC measured from mixtures with PS (bovine brain), POPG, POPE, cholesterol and cationic surfactant.[7,13,14] Signs are taken from separate experiments.[15,16]

When comparing ion binding measured from the PC headgroup order parameters between neutral and anionic membranes, it is important to note that the order parameters increase due to the addition of negative charged lipids according to the electrometer concept[6,13] (Fig. S1). Therefore, the headgroup order parameters of POPC in mixtures with anionic lipids are larger than in pure POPC lipid bilayer, which is also seen in the ion binding experiments from different mixtures in the absence of added calcium (Fig. S2). Upon addition of $CaCl_2$, the order parameters 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). Around these concentrations the positive charge of bound Ca^{2+} cancels the negative charge lipids, resulting to a neutral membrane. Above such concentrations, the specific binding of calcium leads to the overcharging of the membrane.

Because the POPC headgroup order parameters in mixtures with different amounts of anionic lipids but without additional salt are not equal, the binding affinity of calcium can be better compared by plotting the changes of order parameters as a function of added calcium. As expected, such a plot reveals more pronounced order parameter decrease in systems with larger fractions of negatively charged lipids (Fig. S3), indicating an increase in the calcium binding affinity with the increasing amount of negatively charged lipids in membranes. In conclusion, the presented empirical comparison of headgroup order parameter changes from various mixtures of POPC and anionic lipids with added calcium gives physically consistent results, suggesting that the electrometer concept can be used to determine the cation binding affinity also to the lipid bilayers containing mixtures of PC and anionic lipids.

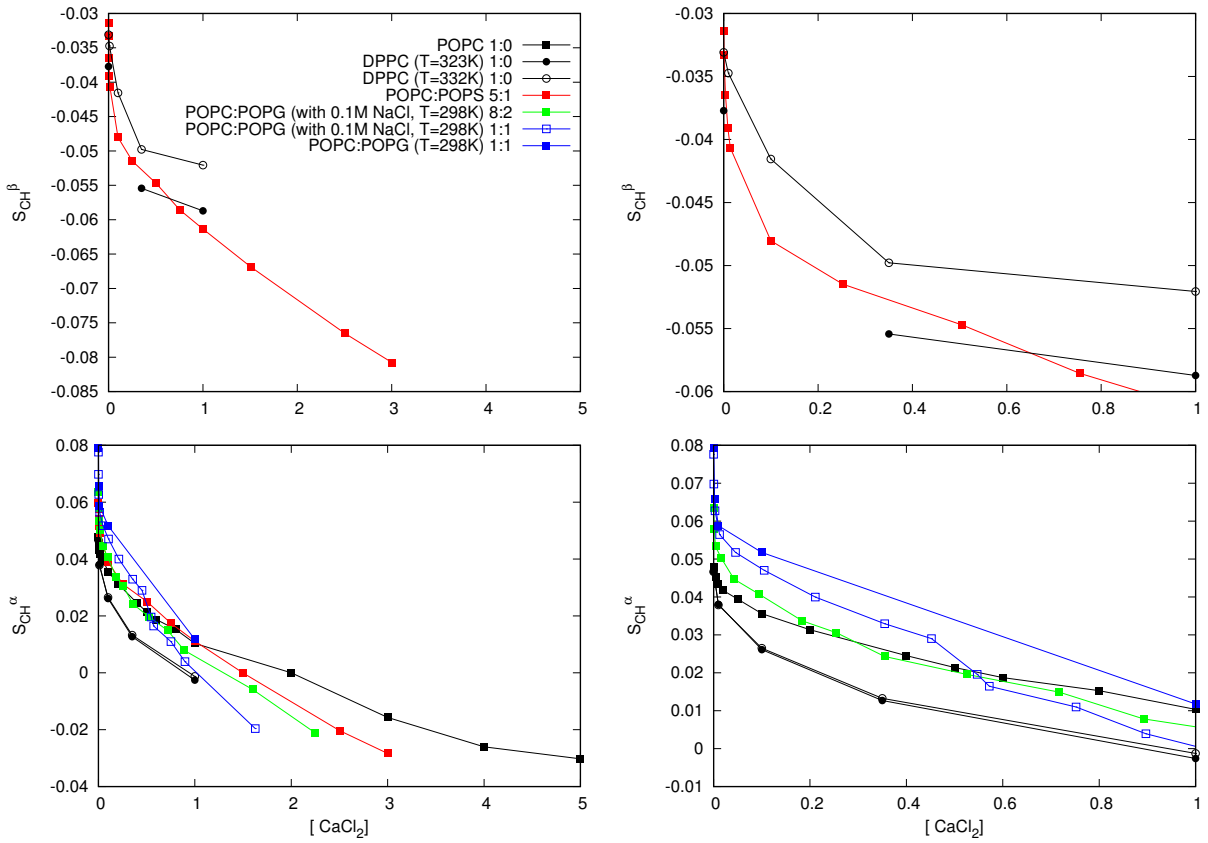

Figure S2: Headgroup order parameters of POPC as a function of CaCl concentration from experiments different mole fractions of negatively charged lipids (left column). Right column shows the same data zoomed to the concentrations below 1M. Data for Pure DPPC from,[4] for pure POPC from,[5] for POPC:POPS (5:1) mixture from,[10] for POPC:POPG (8:2,1:1) mixtures with 0.1M NaCl from[9] and for POPC:POPG (1:1) mixture data without NaCl from.[8]

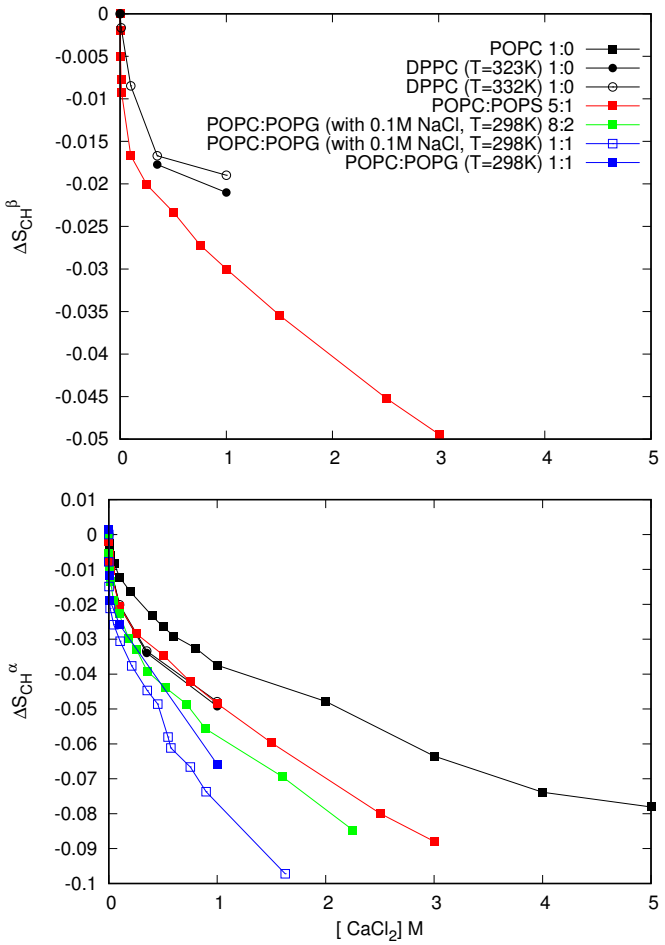

Figure S3: Changes of POPC headgroup order parameters as a function of $CaCl_2$ measured from mixed bilayers containing different amounts of anionic lipids. The original data is the same as in figure S2.

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, the response of the order parameters to the bound charge in simulations should be quantified against experiments.[17,18] In our previous work,[17] the ratio $\Delta S^{\beta}_{\mathrm{CH}}/\Delta S^{\alpha}_{\mathrm{CH}}$ was in good agreement with the experiments[4] in the Lipid14 model, but was underestimated by other models. In the more recent study,[18] the headgroup order parameter responses were compared more carefully with the experiments of cationic surfactants in POPC bilayer.[7] The comparison reveals that the both headgroup order parameters in the Lipid14 model are too sensitive to the bound charge, while CHARMM36 gives better agreement for the α carbon (Fig. S4). Therefore, the headgroup order parameter response to the bound charge is actually more realistic in CHARMM36 model than in the Lipid14. In the latter, both order parameters are equally too sensitive to the bound cations giving a good result for the $\Delta S^{\beta}_{\mathrm{CH}}/\Delta S^{\alpha}_{\mathrm{CH}}$ ratio. The ratio was overestimated for the CHARMM36 model because the β-carbon order parameter is relatively more sensitive than the α-carbon order parameter. These results have to be taken into account when analysing the ion binding affinities using headgroup order parameters in simulations. However, we conclude that the discrepancies arising from the sentivity of lipid headgroup to bound charge are typically smaller than then discrepancies arising from ion binding affinity.

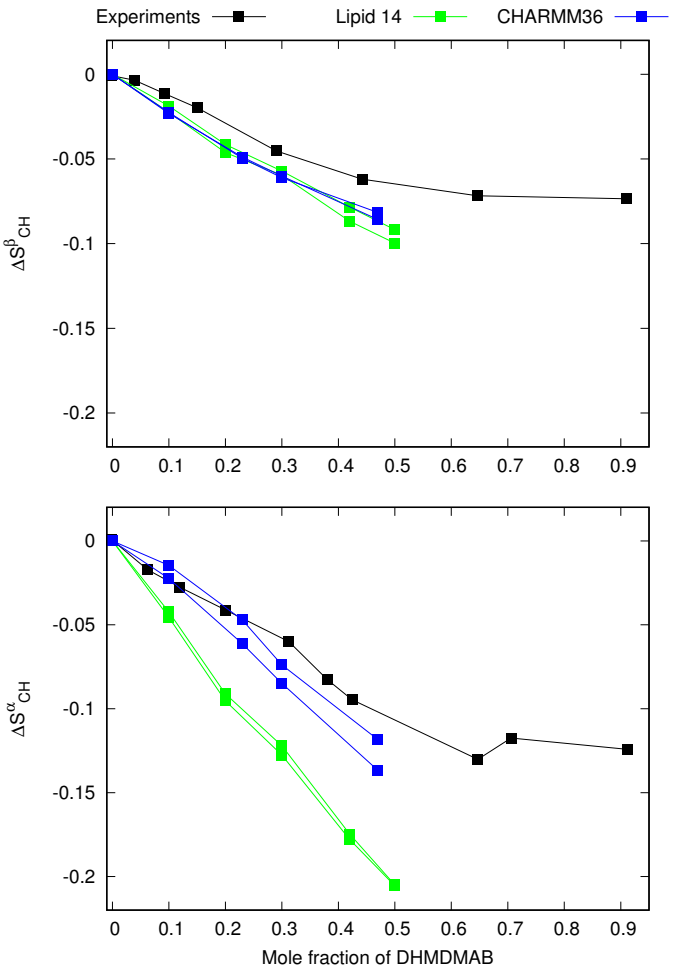

Figure S4: Responses of headgroup order parameters to the fixed amount of cationic surfactants in POPC bilayer from simulations and experiments.[7] The simulation results for Lipid14 are directly from Ref. 18. The CHARMM36 simulation data and details are available from Ref. 19.

S4 Effect of the definition of concentration on the headgroup order parameters as a function of ion concentration.

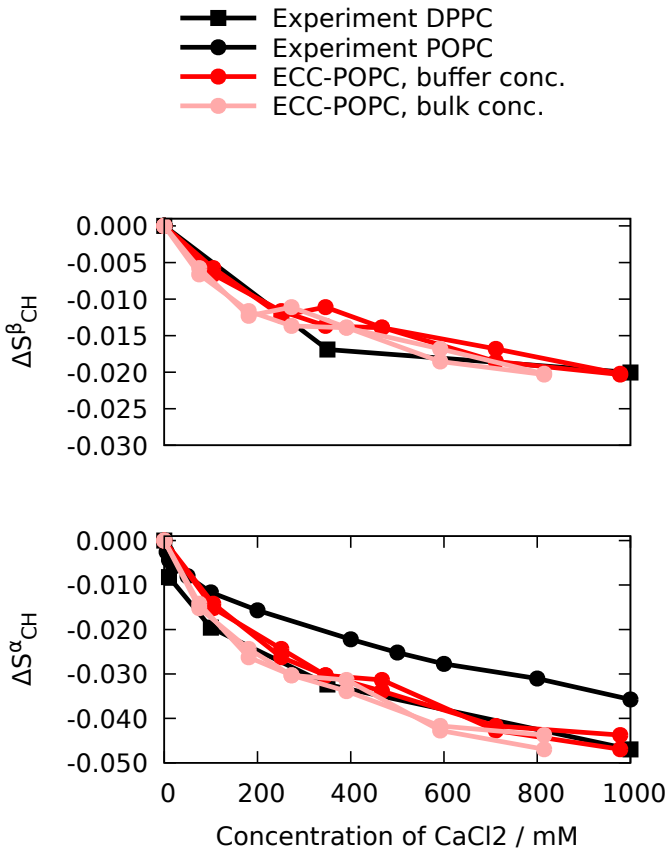

Figure S5: Difference between order parameter changes as a function of $CaCl_2$ concentration demonstrated for the two possible definitions of ion concentration, concentration in buffer before solvating the lipids (buffer concentration) and concenrtration in bulk after solvating the lipids (bulk concentration).[18]

S5 Dipolar slices of the R-PDFL experiment

Slices of the R-PDFL spectra (Fig. S6) show a single splitting for the β-carbon with the order parameter value of 0.12, and a superposition of a large and a very small splitting for the α-carbon. The larger splitting gives a order parameter value of 0.09, while the numerical value from the small splitting cannot resolved with the available resolution.

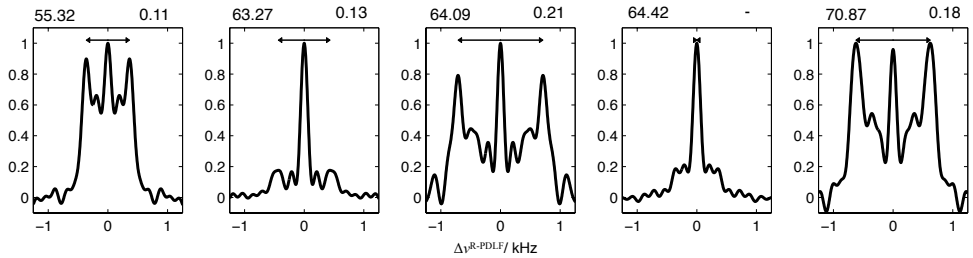

Figure S6: Dipolar coupling slices from the R-PDFL experiment.
Labeling of carbons and explanations of numbers (chemical shift and order parameter) would be good. Also, the size of the figure in the file may not be optimal.

S6 Dihedral angle distributions of the headgroup and glycerol backbone regions of PS lipids from different simulation models

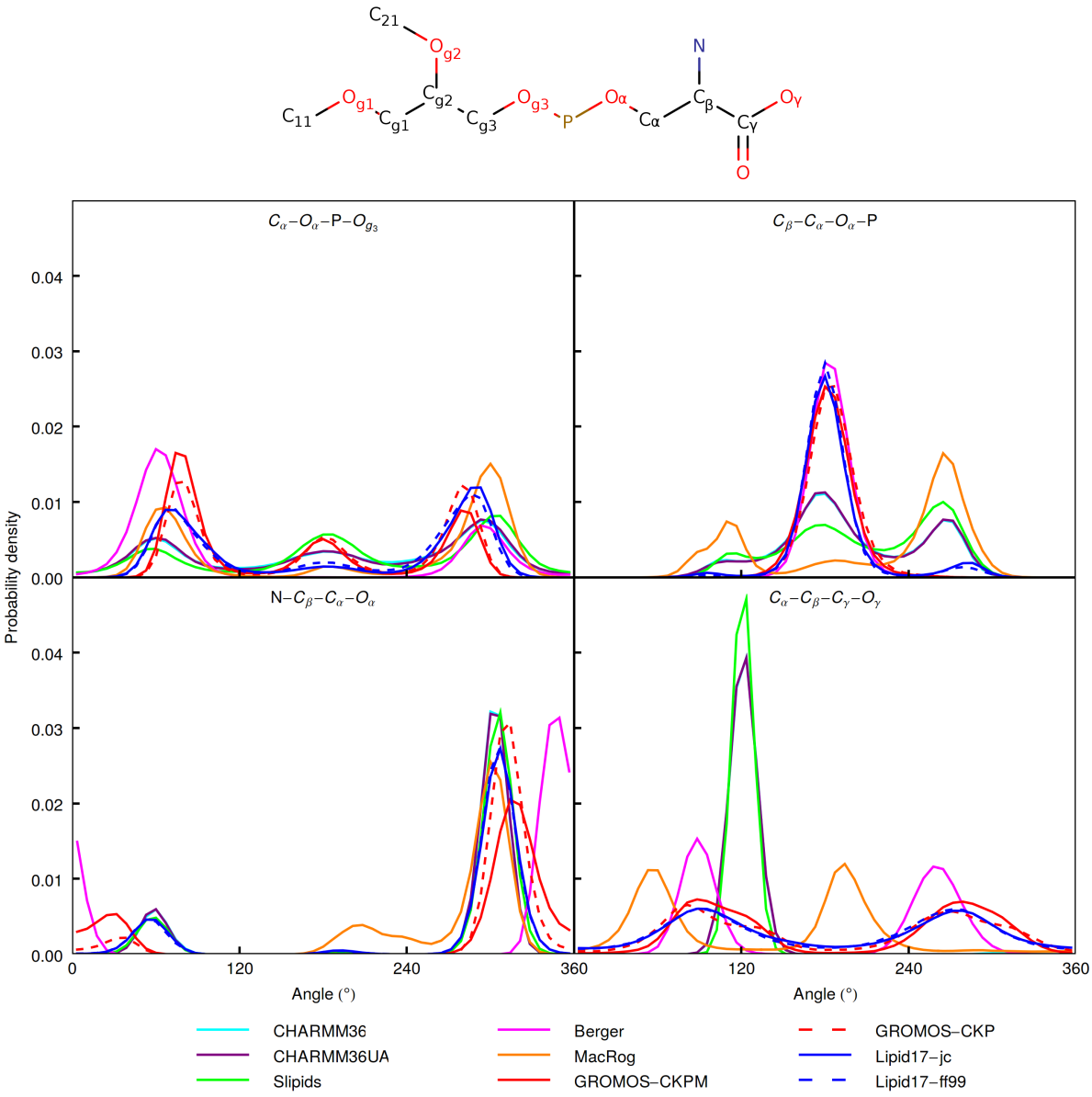

Figure S7: Dihedral angle distributions of headgroup region of PS lipids from different simulation models.

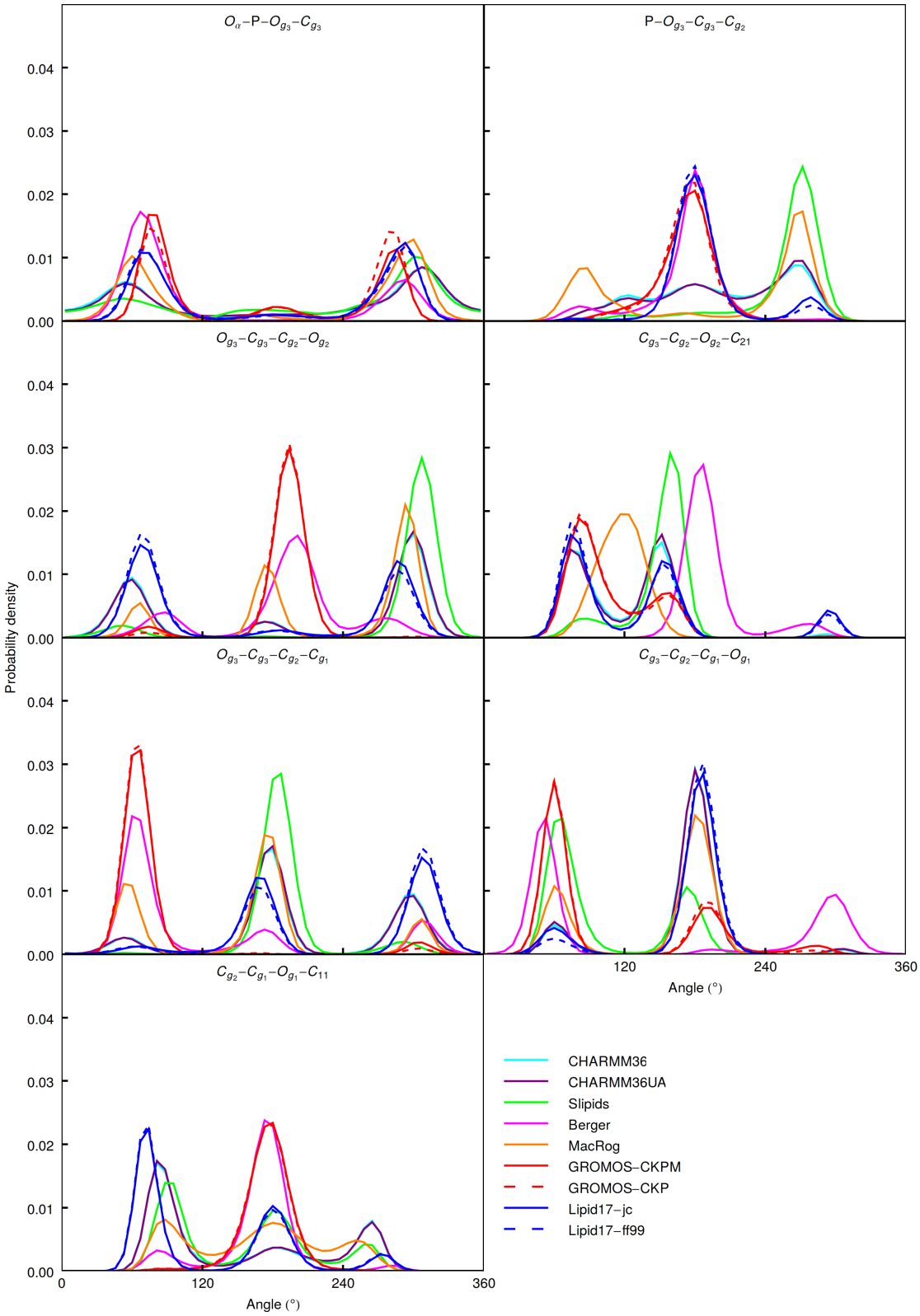

Figure S8: Dihedral angle distributions of glycerol backbone region of PS lipids from different simulation models.

S7 Headgroup response to the additional counterions in POPC:POPS (5:1) mixtures

To evaluate counterion binding in different simulation models against experimental data,[10] we plot the headgroup order parameters measured from POPC:POPS 5:1 mixture as a function of different monovalent ions added to the buffer (Fig. S9). Experimental order parameter data for POPC headgroup in the mixture is available as a function of LiCl and KCl concentrations, while POPS headgroup order parameters are measured also as a function of NaCl. Lithium interacts more strongly with PS headgroups than other monovalent ions,[10,11,20–22] as also observed for PC headgroups.[23] This is evident also in the changes of PS headgroup order parameters, which decrease with the addition of lithium but increase with the addition of sodium or potassium (Fig. S9). POPC headgroup order parameters exhibit a clear decrease as a function of LiCl concentration but only modest changes as a function of KCl concentration, indicating singificant Li^+ binding but only weak Na^+ binding to the mixture when interpreted using the electrometer concept.[4–6] In simulations with the Berger model, the headgroup order parameter response of POPC to the added NaCl is similar to the experiments of LiCl, indicating overestimated binding affinity of sodium, in line with the results for PC bilayers.[17] Indeed, the sodium density profile shows a significant binding peak in the Berger model (Fig. S10). Potassium binding in the MacRog simulation is significantly weaker (Fig. S10) and the headgroup order parameter changes are also in better agreement with simulations (Fig. S9). Discussion about Lipid17 to be written when we have the density profiles. All the tested models overestimate the changes of POPS headgroup order parameters as a function of monovalent ions (Fig. S9), suggesting that model development is necessary to interpret the PS headgroup-ion interactions from MD simulations.

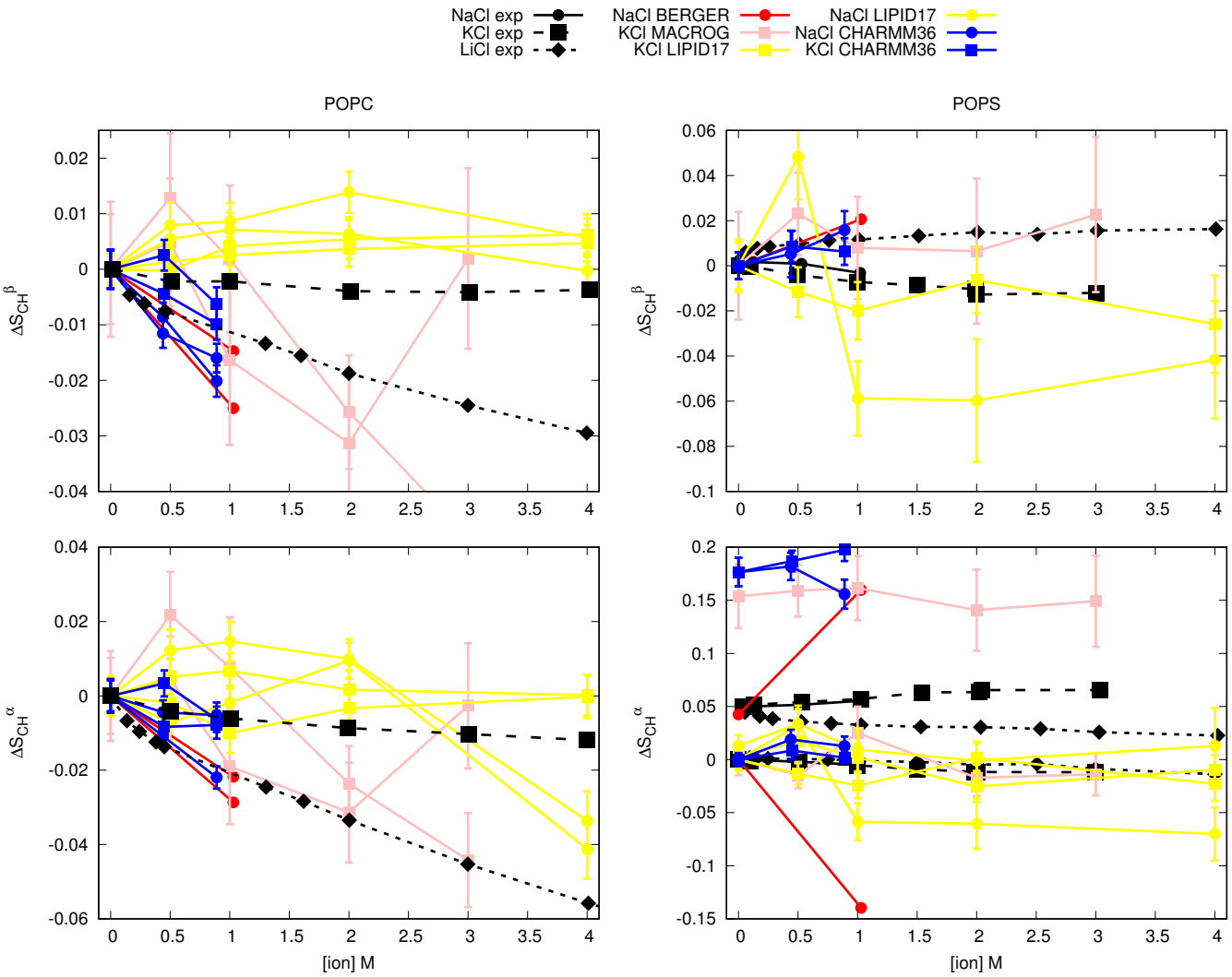

Figure S9: Changes of the PC (left) and PS (right) headgroup order parameters as a function of added NaCl, KCl and LiCl from POPC:POPS (5:1) mixture at 298 K (except Berger simulations are (4:1) mixture at 310 K). The experimental data is from Ref. 10. The values from counterion-only systems are set as a zero point of y-axis. To correctly illustrate the significant forking of the α-carbon order parameter in PS headgroup (bottom, right), the y-axis is tranferred with the same value for both order parameters such that the lower order parameter value is at zero.

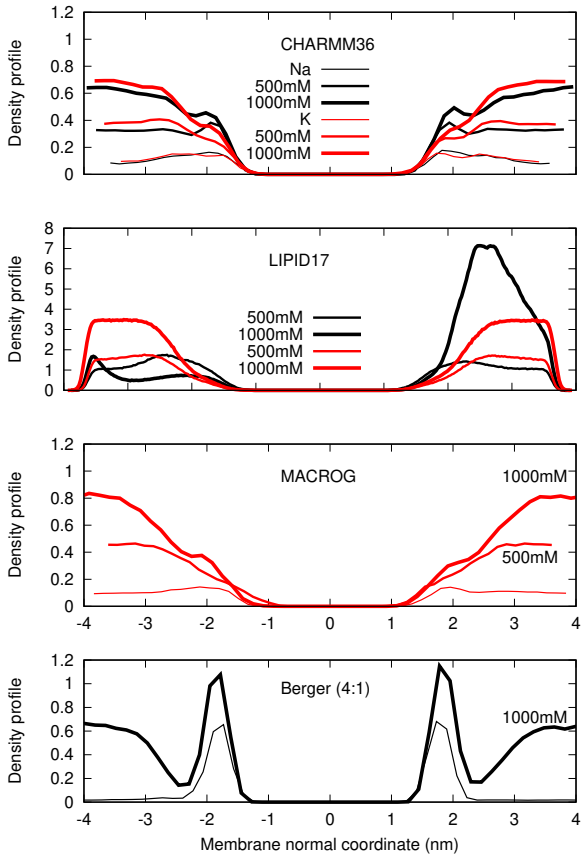

Figure S10: Counterion density distributions from PC:PS mixtures.
Lipid 17 is to be added when we have the density profiles. There is something wrong with the current plots in git: https://github.com/NMRLipids/MATCH/pull/40

S8 Calcium binding to POPC in CHARMM36 simulation with NBfix

The response of POPC headgroup order parameters to the $CaCl_2$ concentration are underestimated in simulations of POPC:POPS (5:1) mixture with CHARMM36 containing the NBfix for interactions between calicum and lipid oxygens[24] (Fig. 8 in the main text), indicating that the calcium binding to the bilayer is too weak with these parameters. Without the NBfix term, the calcium binding affinity to pure POPC lipid bilayers was overestimated in the CHARMM36 model.[17] However, after employing the NBfix term, the response of headgroup order parameters (Fig. S11) and the binding affinity (Fig. S12) of calcium also to a pure POPC bilayer are underestimated. Notably, CHARMM36 simulations with the NBfix terms[24,25] employed predict similar binding affinity for sodium and calcium. In conclusion, the special NBfix[24] for calcium, incorporated in the parameters give by the CHARMM-GUI at the time of running the simulations (January 2018), leads to the underestimation of calcium binding affinity to pure PC and mixed PC:PS bilayers.

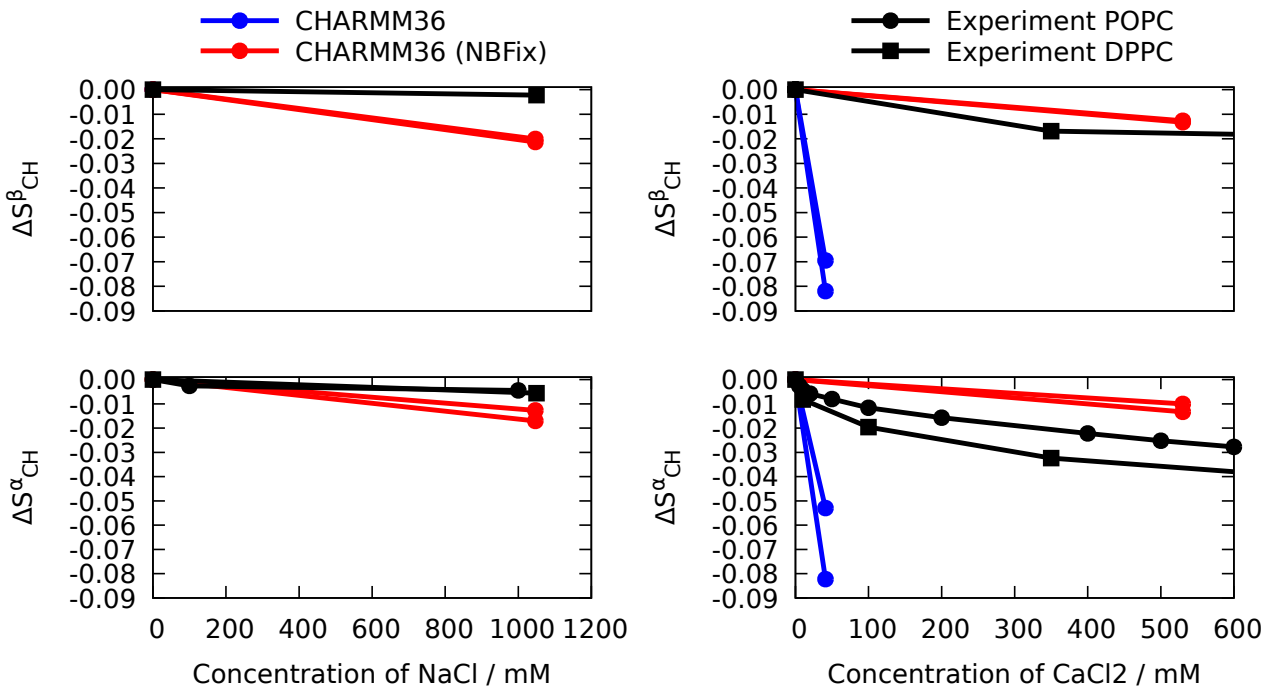

Figure S11: Headgroup order parameters from CHARMM36 simulations of POPC mixed with ions having the NBfix term employed for sodium[25] (*left*) and calcium[24] (*right*) compared with the experimental data.[4,5] Simulation files without ions are available at Ref. 26, with the NBfix term in sodium at Ref. 27, with the NBfix term in calcium at Ref. 27 and without the NBfix in calcium at Ref. 28.

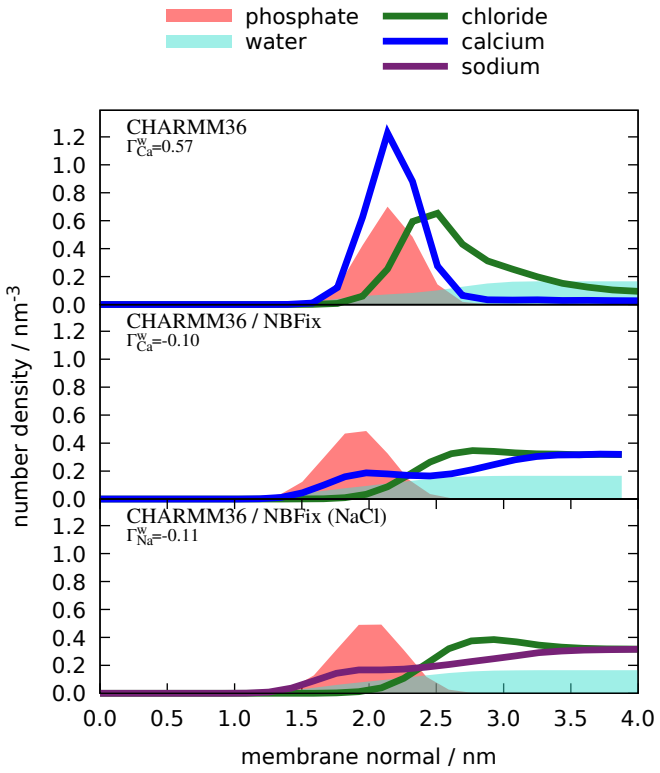

Figure S12: Density profiles along membrane normal from CHARMM36 simulations with (middle) and without (top) the NBfix term for calcium[24] compared to the simulation with the NBfix term for sodium[25] (bottom). The simulation data are the same as in figure S11.

S9 Details of the rough subjective force field ranking (Fig. 5)

The assessment was based fully on the Fig. 3. First, for each carbon (the columns in Fig. 3) 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. 3).

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. 3) 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. 3, was then used to (roughly and subjectively, as should be clear from the above description) rank the force fields.

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