

Supporting Information:

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

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S1 Comparison of headgroup order parameters from different force fields to experiments

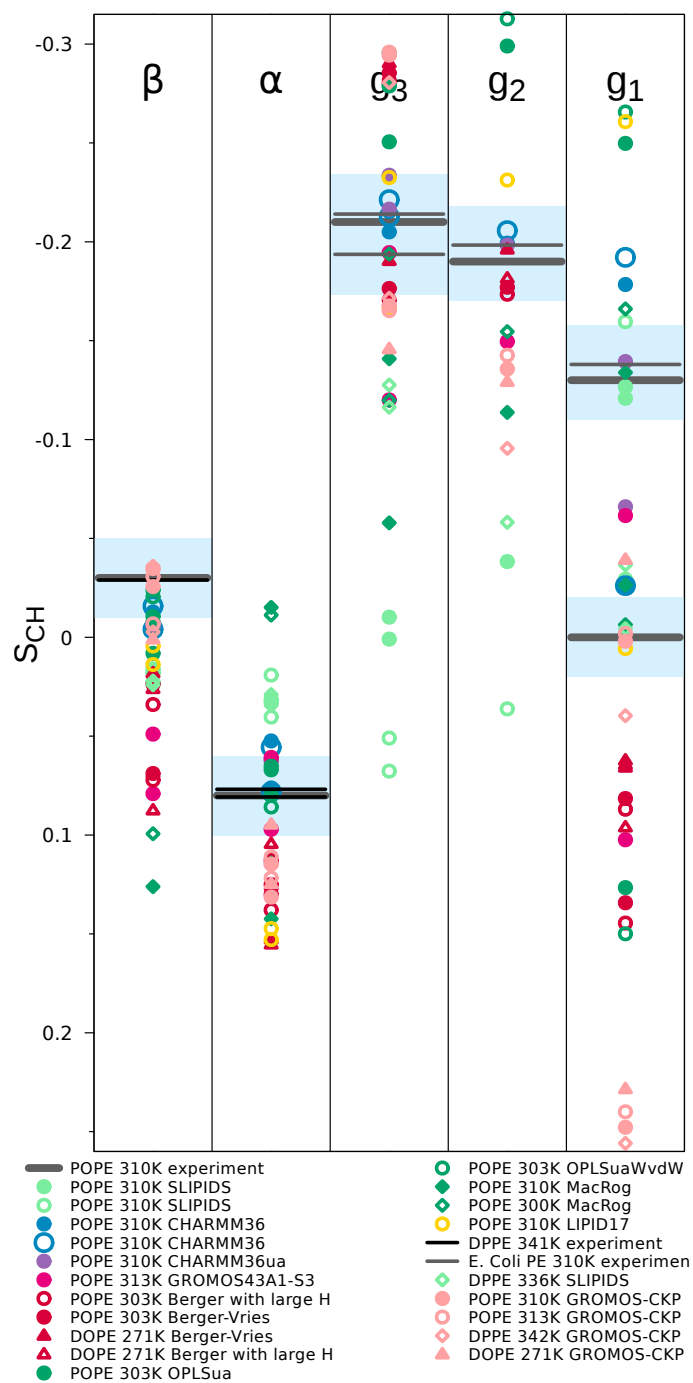


Figure S1: The headgroup and glycerol backbone order parameters of PE lipids from experiments (POPE and signs this work, DPPE from Ref. 1 and E.coliPE from Ref. 2) and simulations with different force fields.

1. This should be clarified as in NMRlipidsI and error bars should be added. Probably larger error bars for united atom models based on the report by Fuchs et al.

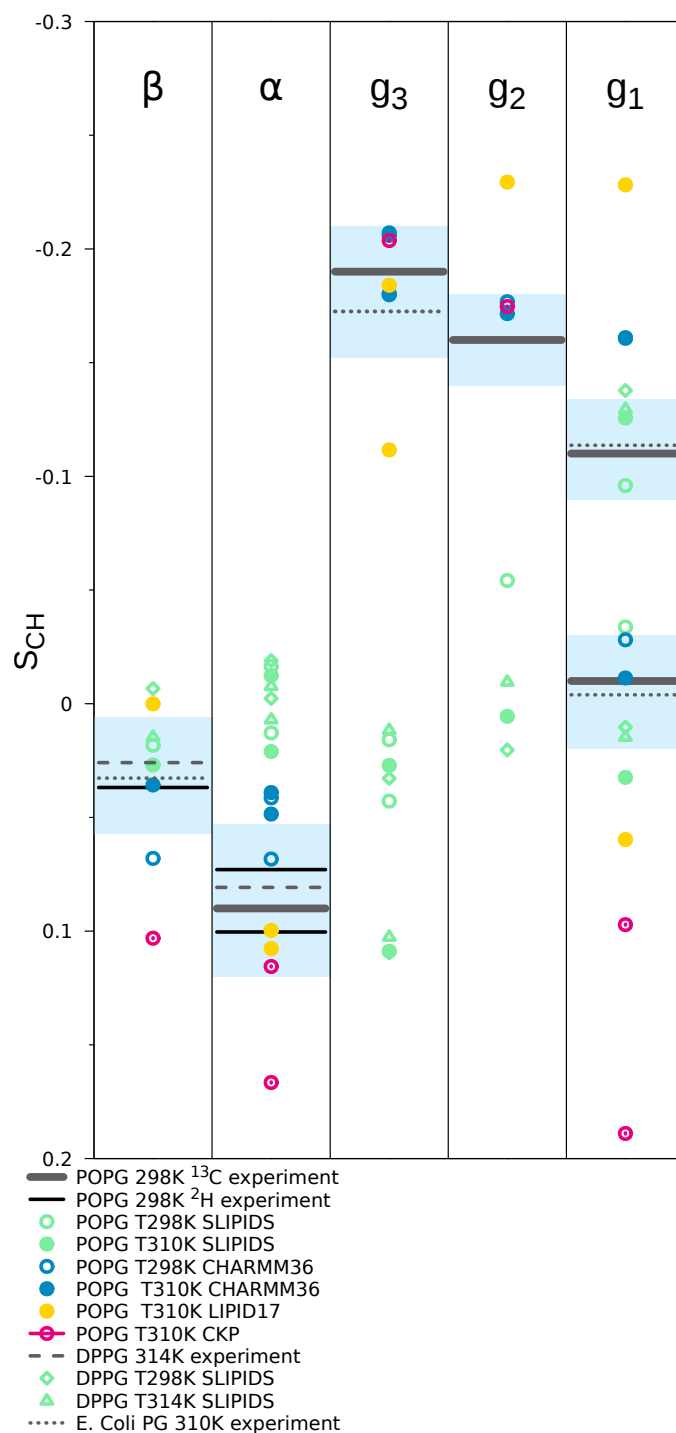


Figure S2: The headgroup and glycerol backbone order parameters of PG lipids from experiments (POPG and signs from this work and from Ref. 3, DPPG with 100mM NaCl from Ref. 4, and E. Coli PG results from Ref. 2). and simulations with different force fields.

S1.1 PC headgroup interactions with PE and PG

In experiments, the PC headgroup order parameters increase with the addition of negatively charged PG or PS lipids, but are not affected by the addition of zwitterionic PE and SM

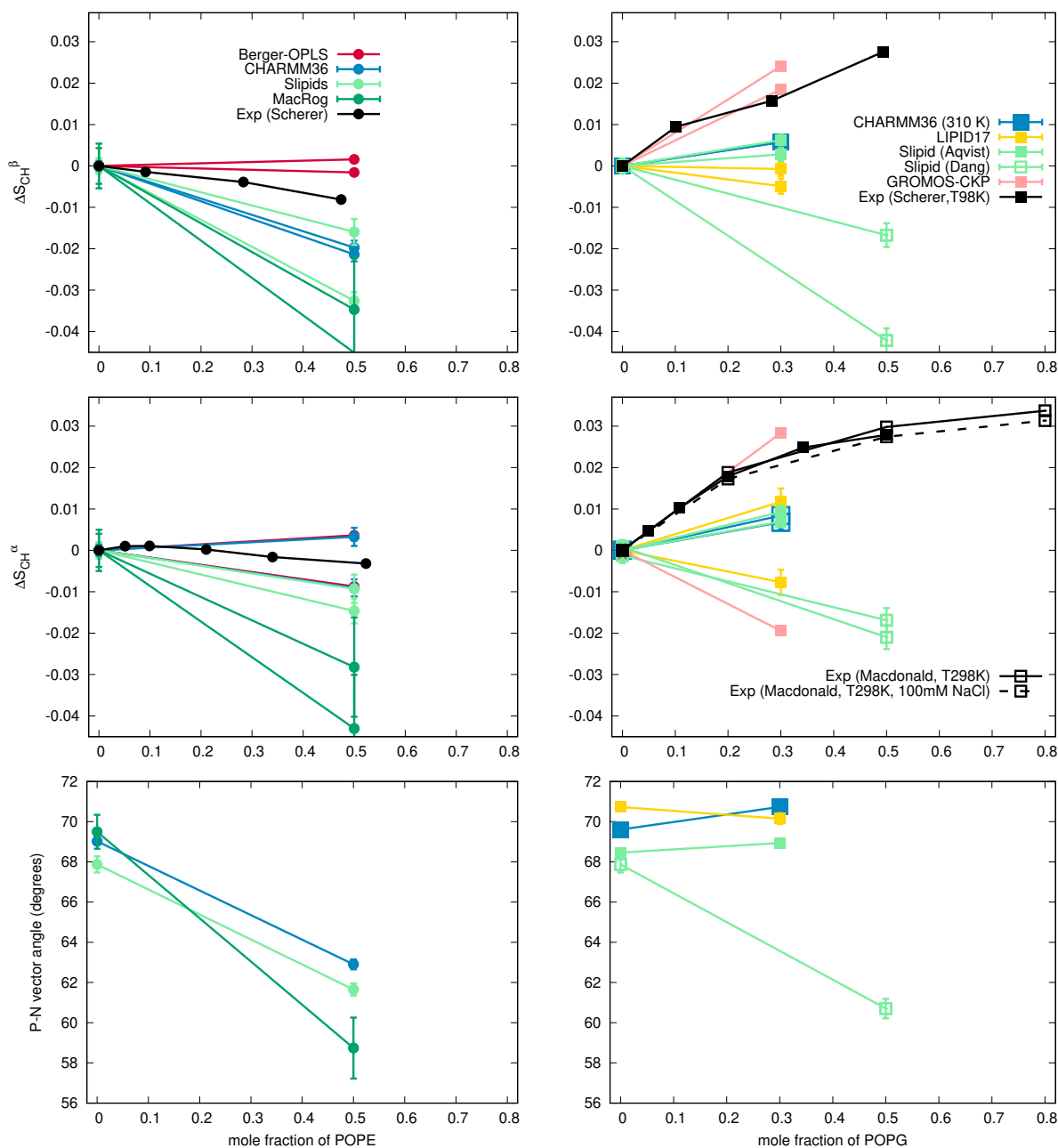


Figure S3: Modulation of POPC headgroup order parameters with increasing amount of POPE (left) and POPG (right) in bilayer from experiments^{5,6} and simulations with different force fields. Signs are determined as discussed in.^{7,8}

2.P-N vector angles from Berger-OPLS and GROMOS-CKP simulations are yet to be analyzed.

lipids or cholesterol (Fig. S3). This can be explained by the electrometer concept, which suggests that the headgroup dipole tilts more parallel to the membrane plane upon addition of negative charge to the membrane.^{5,9,10} The response of PC headgroup order parameters to PE by the tested CHARMM36 and Berger-OPLS force fields, although CHARMM36 slightly overestimates the changes (Fig. S3). The good performance of Berger-OPLS simulations is notable because the response of headgroup order parameters to cholesterol was significantly overestimated by the Berger/HÅültje force field in our previous work.⁷ **3.This is text by P. Fuchs, copied from the blog.**

Area results in nm², the error is <= 0.003 nm²

- pure POPC

CHARMM36: 0.624

Berger : 0.649

- POPC/POPE 50:50

CHARMM36 : POPC 0.609, POPE 0.557

Berger-hacked: POPC 0.637, POPE 0.632

One can see that CHARMM 36 predicts a drop in the area on going from pure POPC to POPC/POPE 50:50. This means that POPC pack tightly to POPE. In contrast, the values for Berger are not that changed. The POPE value predicted by CHARMM 36 (in the mixture POPC/POPE 50:50) is much smaller than that predicted by Berger.

The experimental acyl chain order parameters for POPE¹¹ seem larger than reported for POPC,¹² which supports the more condensed PE bilayer. This is interesting, but not exactly the core message of the manuscript. Maybe we should mention this very briefly? For example, we could just report the areas per lipid (without distinguishing PC and PE) and mention the difference between CHARMM36 and Berger. I have opened an issue for this: <https://github.com/NMRLipids/NMRLipidsIVPEandPG/issues/7>

None of the force fields fully reproduces the PC headgroup order parameter response to

the increasing amount of PG, which may be related to the counterion binding affinity (see also the next section).¹³ In all force fields except Slipids, the order parameters of different hydrogens attached to the α -carbon are responding differently when mixed with PE or PG lipids 4.Maybe we should figure out what is the reason for this?

Maybe we should analyze the P-N vector angle from different simulations?

<https://github.com/NMRLipids/NMRLipidsIVPEandPG/issues/10>.

For β -carbon order parameter in PG headgroup, experiments report mild increase⁶ or no change³ upon addition of PC lipids (Fig. S4). Simulations with all the tested force fields give only very small changes also for the α -carbon order parameter (Figs. S10 and S4). Therefore, the simulations are generally in line with experiments, suggesting that the interactions with PC do not essentially effect the PG headgroup structure. This suggests that the interactions between PG and PC headgroups are captured better in simulations than for PS headgroup, where all the force fieds significantly overestimated the structural response of PS headgroup to the interactions with PC lipids.¹⁰

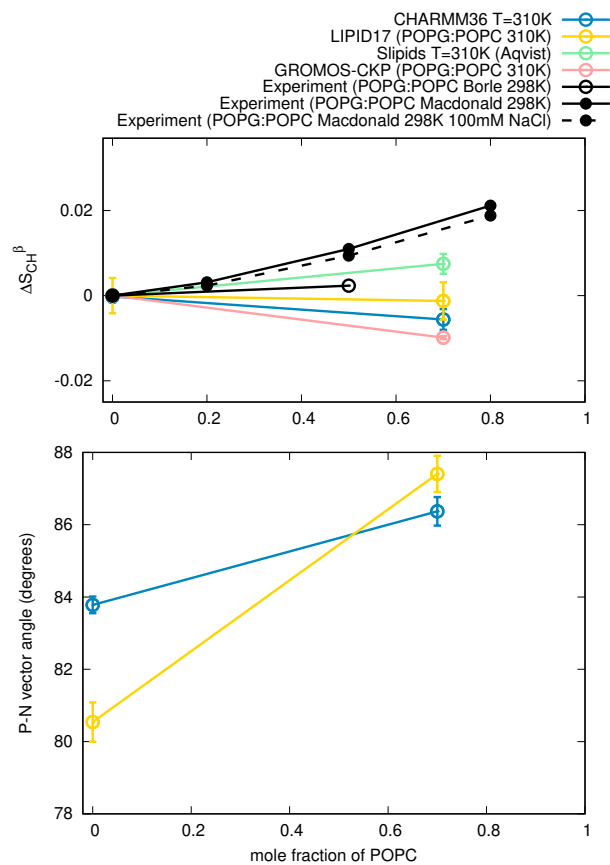


Figure S4: Modulation of PG lipid headgroup order parameters with the increasing amount of PC in lipid bilayer from experiments^{3,6} and simulations with different force fields.

5.P-N angles from Slipids and GROMOS-CKP yet to be calculated.

S1.2 Sodium binding to PE and PG lipid bilayers

Sodium binding affinity to PE lipids has not been measured experimentally, but large differences to PC would be surprising. In simulations, the sodium binding affinity to POPE depends on the used force field (Fig. S5), but lesser extend than reported previously for PC.¹⁴

6.This will be finished once we have all the simulation details and Lipid17 simulations with correct dihedrals from issue <https://github.com/NMRLipids/NMRLipidsIVPEandPG/issues/12>, Because some simulation and ion parameters are not identical with the previous work,¹⁴ we compare POPE results to the POPC simulations ran with identical parameters (Fig. S11). In Lipid17 with the strongest sodium binding affinity to POPE, the binding affinity is approximately similar to POPC. Slipids and CHARMM36 exhibit slightly, and GROMOS-CKP substantially weaker binding to POPE than to POPC. Assuming that the binding to POPE would be similar than to POPC, the sodium binding affinity to POPE is potentially realistic in CHARMM36, Slipids, and GROMOS-CKP simulations here, but substantially overestimated in Lipid17 simulation.

Simulations with PG lipids give similar dependence on force field as observed in POPE simulations: Lipid 17 simulations with Dang ion parameters exhibits stronger counter-ion binding affinity to pure POPG bilayer than CHARMM36, Slipids, and GROMOS-CKP simulations, which are roughly similar (Fig. S6). Lipid17 also exhibits less increase in POPC headgroup order parameters upon addition of POPG than other simulations (Fig. S3), and lower area per molecule (59.5 \AA^2) than in experiments (66.1 \AA^2). In our previous study about PS lipids,¹⁰ such behaviour was related to the overestimated counterions binding and shielding the electrostatic repulsion between PG headgroups in bilayers. Even though the area per lipid in CHARMM36, Slipids, and GROMOS-CKP simulations is in good agreement with experiments (Fig. S6), the experimental increase in POPC headgroup order parameters upon addition of POPG are not fully reproduced (Fig. S3). Therefore we conclude that the counter-ion binding affinity is overestimated in Lipid17 simulations, while the other simulations are more realistic, but slight overbinding cannot be excluded.

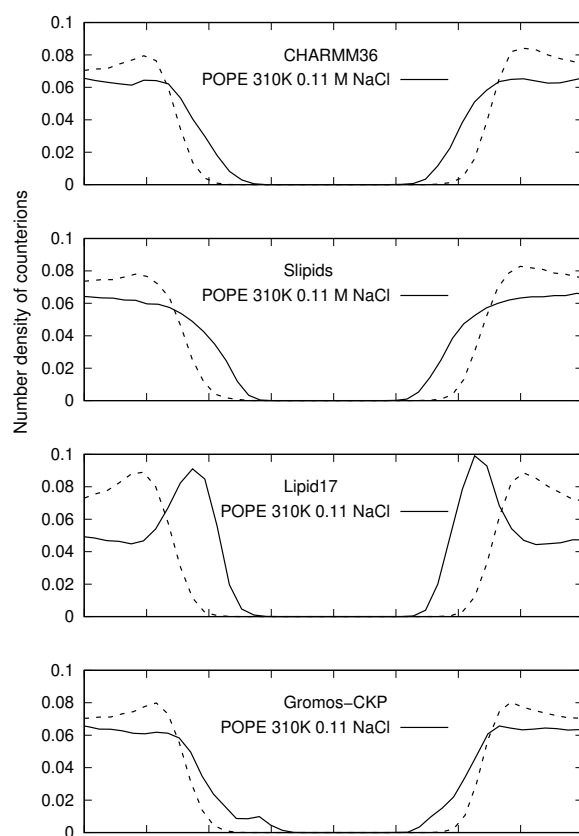


Figure S5: Sodium (solid line) and chloride ion density profiles along membrane normal from different simulations with PE lipids.

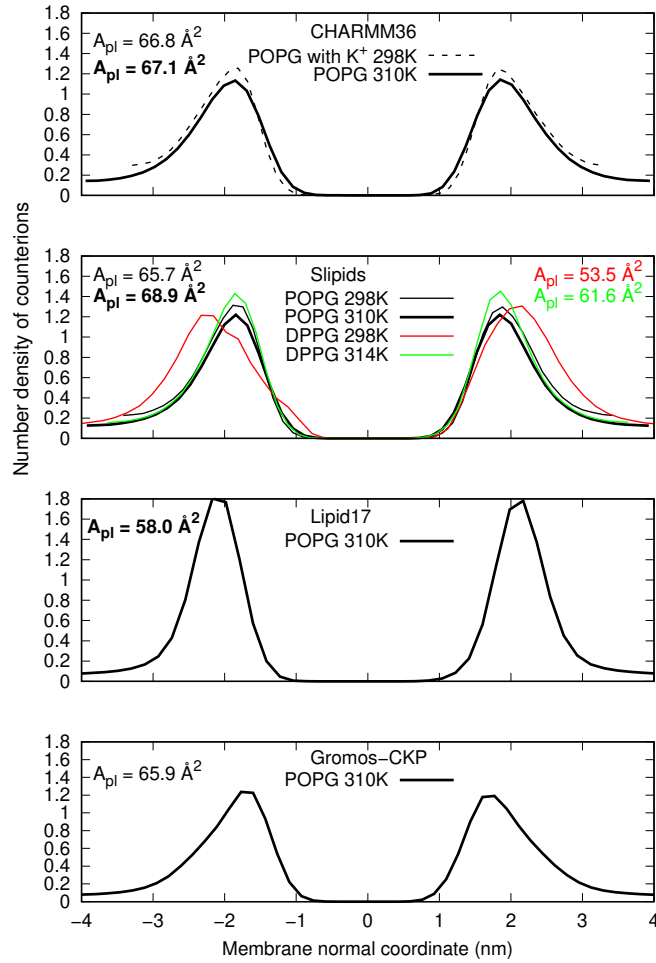


Figure S6: Counterion densities and area per lipids from simulations with PG lipids. Experimental area for POPG at 303 K is 66.1 \AA^2 and 67 \AA^2 for DPPC at 323 K.¹⁵

S2 Simulated systems

S2.1 CHARMM36

POPE [31.Simulation details by M. Javanainen.](#)

POPE with additional NaCl [32.Simulation details by A. Peon.](#)

POPG [33.Simulation details by Ollila.](#)

POPG with additional NaCl [34.Simulation details by A. Peon.](#)

POPC:POPE mixtures Data is available at.^{66,67} 300 K with v-rescale ($\tau=0.1$ ps), 1 bar with PR semiisotropic ($\tau=4$ ps, compressibility= $4.5\text{e-}5$ bar⁻¹), PME order 4 and space 0.12, rcoulomb and rvdw 1.0, 128 lipids per leaflet, no ion [35.Full simulation details by Fuchs et al.](#)

POPC:POPG mixture with additional calcium [36.Simulation details by J. Madsen.](#)

POPC:POPG mixture with additional NaCl [37.Simulation details by A. Peon.](#)

S2.2 CHARMM36ua

POPE Data is available at.¹⁹ [38.Simulation details by T. Piggot.](#)

S2.3 Slipids

POPE Data is available at.²² [39.Simulation details by T. Piggot.](#)

POPE with additional NaCl [40.Simulation details by A. Peon.](#) I have assumed that ion parameters are default Slipids, i.e., Åqvist, please correct if this is not true.

DPPE Data is available at.²¹ [41.Simulation details by F. Favela.](#)

POPG Data is available at.⁴⁸ [42.Simulation details by F. Favela.](#) I have assumed that ion parameters are default Slipids, i.e., Åqvist, please correct if this is not true.

POPG with additional NaCl [43.Simulation details by A. Peon.](#) I have assumed that ion parameters are default Slipids, i.e., Åqvist, please correct if this is not true.

DPPG Data in 298 K is available at⁵⁰ and in 314 K at.⁴⁹ [44.Simulation details by F. Favela.](#) I have assumed that ion parameters are default Slipids, i.e., Åqvist, please correct if this is not true.

Table S1: List of MD simulations with PE lipids.

lipid/counter-ions	force field for lipids / ions	NaCl (M)	^a N _l	^b N _w	^c N _c	^d T (K)	^e t _{sim} (ns)	^f t _{anal} (ns)	^g files
POPE	CHARMM36 [?]	0	144	5760	0	310	500	400	16
POPE	CHARMM36 [?]	0	500	25000	0	310	500	100	17
POPE	CHARMM36 [?]	0.11	500	25000	50	310	500	100	18
POPE	CHARMM36ua [?]	0	336	15254	0	310	2×200	2×100	19
DPPE	Slipids ²⁰	0	288	9386	0	336	200	100	21
POPE	Slipids ²⁰	0	336	?	0	310	2×200	2×100	22
POPE	Slipids ²⁰	0	500	25000	0	310	500	100	23
POPE	Slipids / Åqvist ^{20,24}	0.11	500	25000	50	310	500	100	25
DPPE	GROMOS-CKP [?]	0	128	3655	0	342	2×500	2×400	26
POPE	GROMOS-CKP [?]	0	128	3552	0	313	2×500	2×400	27
POPE	GROMOS-CKP [?]	0	500	25000	0	310	500	100	28
POPE	GROMOS-CKP [?]	0.11	500	25000	50	310	500	100	29
DOPE	GROMOS-CKP [?]	0	128	4789	0	271	2×500	2×400	30
POPE	GROMOS 43A1-S3 [?]	0	128	3552	0	313	2×200	2×100	31
POPE	OPLS-UA vdW on H [?]	0	128	3328	0	303	2×200	2×100	32
POPE	OPLS-UA [?]	0	128	3328	0	303	2×200	2×100	33
POPE	OPLS-MacRog ³⁴	0	144	5760	0	310	500	350	35
POPE	OPLS-MacRog ³⁴	0	128	5120	0	300	500	300	36
POPE	Berger-Vries [?]	0	128	3552	0	303	2×200	2×100	37
POPE	Berger-largeH [?]	0	128	3552	0	303	2×200	2×100	38
DOPE	Berger-Vries [?]	0	128	4789	0	271	2×200	2×100	39
DOPE	Berger-largeH [?]	0	128	4789	0	271	2×300	2×100	40
POPE	LIPID17 ⁴¹	0	500	25000	50	310	500	100	42
POPE	LIPID17 ⁴¹	0.11	500	25000	50	310	500	100	43

^aNumber of lipid molecules with largest mole fraction

^bNumber of water molecules

^cNumber of additional cations

^dSimulation temperature

^eTotal simulation time

^fTime used for analysis

^gReference for simulation files

7.Citation for CHARMM36 PE?

8.Which ion model is used in ¹⁸?

9.Citation for GROMOS-CKP?

10.Citation for GROMOS 43A1-S3?

11.Citation for OPLS-UA models?

12.Citations for Berger-* simulations?

13.LIPID17 simulations with correct dihedrals still coming

Table S2: List of MD simulations with PG lipids.

lipid/counter-ions	force field for lipids / ions	NaCl (M)	^a N _l	^b N _w	^c N _c	^d T (K)	^e t _{sim} (ns)	^f t _{anal} (ns)	^g files
POPG/K ⁺	CHARMM36 [?] 14.	0	118	4110	0	298	100	100	⁴⁴
POPG	CHARMM36 [?]	0.11	500	25000	49	310	500	100	⁴⁵
POPG	CHARMM36 [?]	0	500	25000	0	310	500	100	⁴⁶
POPG/Na ⁺	Slipids / Åqvist ^{24,47}	0	288	10664	0	298	250	100	⁴⁸
DPPG/Na ⁺	Slipids / Åqvist ^{24,47}	0	288	11232	0	314	200	100	⁴⁹
DPPG/Na ⁺	Slipids / Åqvist ^{24,47}	0	288	11232	0	298	400	100	⁵⁰
POPG	Slipids / Åqvist ^{24,47}	0	500	25000	0	310	500	100	⁵¹
POPG	Slipids / Åqvist ^{24,47}	0.11	500	25000	49	310	500	100	⁵²
POPG	LIPID17 / Dang ^{41,53}	0	500	25000	0	310	500	100	⁵⁴
POPG	LIPID17 [?]	0.11	500	25000	49	310	500	100	⁵⁵
POPG	GROMOS-CKP [?]	0	500	25000	0	310	500	100	⁵⁶
POPG	GROMOS-CKP [?]	0.11	500	25000	49	310	500	100	⁵⁷

^aNumber of lipid molecules with largest mole fraction

^bNumber of water molecules

^cNumber of additional cations

^dSimulation temperature

^eTotal simulation time

^fTime used for analysis

^gReference for simulation files

15. Citations and ion model for CHARMM36?

16. Lipid17 simulation with correct dihedral potentials still coming.

17. Citation and ion model for GROMOS-CKP?

Table S3: List of MD simulations with PE and PG lipids mixed with PC.

lipid/counter-ions	force field for lipids / ions	NaCl (M)	CaCl ₂ (M)	^a N _l	^b N _w	^c N _c	^d T (K)	^e t _{sim} (ns)	^f t _{anal} (ns)	^g files
POPC	CHARMM36 [?]	0	0	500	25000	0	310	500	100	58
POPC:POPG (7:3)	CHARMM36 [?]	0	0	350	25000	0	310	500	100	59
POPC:POPG (1:1)	CHARMM36 [?]	0	0	150:150	31500	0	298	500	400	60
POPC:POPG (1:1)	CHARMM36 [?]	0	0.1	150:150	31329	57	298	400	300	61
POPC:POPG (1:1)	CHARMM36 [?]	0	1.08	150:150	29766	578	298	500	400	62
POPC:POPG (4:1)	CHARMM36 [?]	0	0	350:88	26280	0	298	500	400	63
POPC:POPG (4:1)	CHARMM36 [?]	0	0.1	350:88	26280	47	298	500	400	64
POPC:POPG (4:1)	CHARMM36 [?]	0	1.0	350:88	24927	451	298	500	400	65
POPC	CHARMM36 [?]	0	0	256	8704	0	300	300	250	66
POPC:POPE (1:1)	CHARMM36 [?]	0	0	128	8704	0	300	300	250	67
POPC	OPLS-MacRog ³⁴	0	0	128	5120	0	300	500	300	68
POPC:POPE (1:1)	OPLS-MacRog ³⁴	0	0	128	5120	0	300	500	300	69
POPC	Slipid ²⁰	0	0	512	23943	0	298	170	100	70
POPC:POPE (1:1)	Slipid ²⁰	0	0	128	5120	0	298	500	300	71
POPC	GROMOS-CKP / ?? ? ?	0	0	500	25000	0	310	500	100	72
POPC:POPG (7:3)	GROMOS-CKP / ?? ? ?	0	0	350:150	25000	0	310	500	100	73
POPC	Slipid ²⁰	0	0	500	25000	0	310	500	100	74
POPC:POPG (7:3)	Slipid / Åqvist ^{20,24}	0	0	350:150	25000	0	310	500	100	75
POPC:POPG (1:1)	Slipid / Dang ^{20,53,76,77}	0	0	128:128	12800	0	298	500	400	78
POPC:POPG (1:1)	Slipid / Dang ^{20,53,76,77}	0	0.1	128:128	12800	23	298	500	400	78
POPC:POPG (1:1)	Slipid / Dang ^{20,53,76,77}	0	0.2	128:128	12800	46	298	1500	500	78
POPC:POPG (1:1)	Slipid / Dang ^{20,53,76,77}	0	0.5	128:128	12800	115	298	1500	500	78
POPC:POPG (1:1)	Slipid / Dang ^{20,53,76,77}	0	1.0	128:128	12800	230	298	1500	500	78

^aNumber of lipid molecules with largest mole fraction

^bNumber of water molecules

^cNumber of additional cations

^dSimulation temperature

^eTotal simulation time

^fTime used for analysis

^gReference for simulation files

18. Citation and ion model for GROMOS-CKP?

19. Citation and description for "Berger" model?

20. Lipid17 POPC and POPC:POPG mixtures (<https://doi.org/10.5281/zenodo.3241242> and <https://doi.org/10.5281/zenodo.3237656>) should be added after simulated with corrected dihedrals.

21. Upcoming Lipid17ecc with POPC:POPS (4:1) mixture simulations to be added.

Table S4: List of MD simulations with PE and PG lipids mixed with PC.

lipid/counter-ions	force field for lipids / ions	NaCl (M)	CaCl ₂ (M)	^a N _l	^b N _w	^c N _c	^d T (K)	^e t _{sim} (ns)	^f t _{anal} (ns)	^g files
POPC:POPG (4:1)	Lipid17 / Dang ^{41,53,77}	0	0	350:88	26265	0	298	400	350	79
POPC:POPG (4:1)	Lipid17 / Dang ^{41,53,77}	0	0.1	350:88	26124	47	298	400	250	80
POPC:POPG (4:1)	Lipid17 / Dang ^{41,53,77}	0	1.0	350:88	24840	475	298	1200	200	81
POPC:POPG (1:1)	Lipid17 / Dang ^{41,53,77}	0	0	150:150	31572	0	298	320	200	82
POPC:POPG (1:1)	Lipid17 / Dang ^{41,53,77}	0	0.1	150:150	31401	57	298	718	198	83
POPC:POPG (1:1)	Lipid17 / Dang ^{41,53,77}	0	1.0	150:150	29865	569	298	720	200	84
POPC:POPG (1:1)	Lipid17ecc / ECC-ions ⁸⁵⁻⁸⁷	0	0	150:150	31572	0	298	347.8	333	88
POPC:POPG (1:1)	Lipid17ecc / ECC-ions ⁸⁵⁻⁸⁷	0	0.1	150:150	29865	54	298	400	300	89
POPC:POPG (1:1)	Lipid17ecc / ECC-ions ⁸⁵⁻⁸⁷	0	1.0	150:150	29865	569	298	600	400	90
POPC	Berger [?] 22.	0	0	256	10240	0	300	300	200	91
POPC:POPE (1:1)	Berger [?] 23.	0	0	128	11008	0	300	300	200	92
POPC:DOPE (1:1)	Berger [?] 24.	0	0	128	10240	0	300	300	200	93
DOPC	Berger [?] 25.	0	0	256	11008	0	300	300	200	94
DOPC:DOPE (1:1)	Berger [?] 26.	0	0	128	11008	0	300	300	200	95

^aNumber of lipid molecules with largest mole fraction

^bNumber of water molecules

^cNumber of additional cations

^dSimulation temperature

^eTotal simulation time

^fTime used for analysis

^gReference for simulation files

27. Citation and ion model for GROMOS-CKP?

28. Citation and description for "Berger" model?

29. Lipid17 POPC and POPC:POPG mixtures (<https://doi.org/10.5281/zenodo.3241242> and <https://doi.org/10.5281/zenodo.3237656>) should be added after simulated with corrected dihedrals.

30. Upcoming Lipid17ecc with POPC:POPS (4:1) mixture simulations to be added.

POPC:POPG mixture with additional NaCl [45.Simulation details by A. Peon.](#) I have assumed that ion parameters are default Slipids, i.e., Åqvist, please correct if this is not true.

S2.4 Berger

POPE Data is available at. ^{37,38} [46.Simulation details by T. Piggot.](#)

DOPE Data is available at. ^{39,40} [47.Simulation details by T. Piggot.](#)

POPC:POPE, POPC:DOPE and DOPC:DOPE mixtures Data is available at. ^{91,92} 300 K with v-rescale (tau=0.1 ps), 1 bar with PR semiisotropic (tau=4 ps, compressibility=4.5e-5 bar⁻¹), PME order 4 and space 0.12, rcoulomb and rvdw 1.0, 128 lipids per leaflet, no ion [48.Simulation details by Fuchs et al.](#)

S2.5 GROMOS 43A1-S3

POPE Data is available at. ³¹ [49.Simulation details by T. Piggot.](#)

S2.6 OPLS-UA

POPE Data is available at. ³³ [50.Simulation details by T. Piggot.](#)

POPE with vdW interaction in H Data is available at. ³² [51.Simulation details by T. Piggot.](#)

S2.7 GROMOS-CKP and GROMOS-CKPM

POPE Data is available at. ²⁷ [52.Simulation details by T. Piggot.](#)

DOPE Data is available at. ³⁰ [53.Simulation details by T. Piggot.](#)

DPPE Data is available at. ²⁶ [54.Simulation details by T. Piggot.](#)

S2.8 Lipid17

S2.9 ECC-LIPID POPG

In ECC-lipid models, electronic continuum correction (ECC) is applied to implicitly include the missing electronic polarizability into the force field description.^{96,97} In practise, this is implemented by scaling the charges and Lennard-Jones σ s of headgroup, glycerol backbone, and carbonyl regions of Amber Lipid14/17 models are scaled by constant factors. Here, we follow the approach that previously improved ion binding to bilayers containing negatively charged PS lipids:⁹⁷ ECC-POPC parameters (scaling factors $f_q=0.8$ and $f_\sigma=0.89$ applied to Lipid14 POPC parameters)⁹⁶ were used for POPC and scaling factors of $f_q=0.75$ and $f_\sigma=0.89$ were applied to the charges and Lennard-Jones σ s of headgroup, glycerol backbone, and carbonyl regions of Amber Lipid17 POPG parameters. The Lipid17 parameters (described above) and initial configurations were taken from Ref.⁵⁴ with the correct dihedral type, and the resulting parameters are available from Ref. ? . ECC-ion parameters with the scaled charges,⁸⁵⁻⁸⁷ downloaded from bitbucket.org/hseara/ions/src/master/, were used in these simulations.

S3 R-PDLF and SDROSS experiments

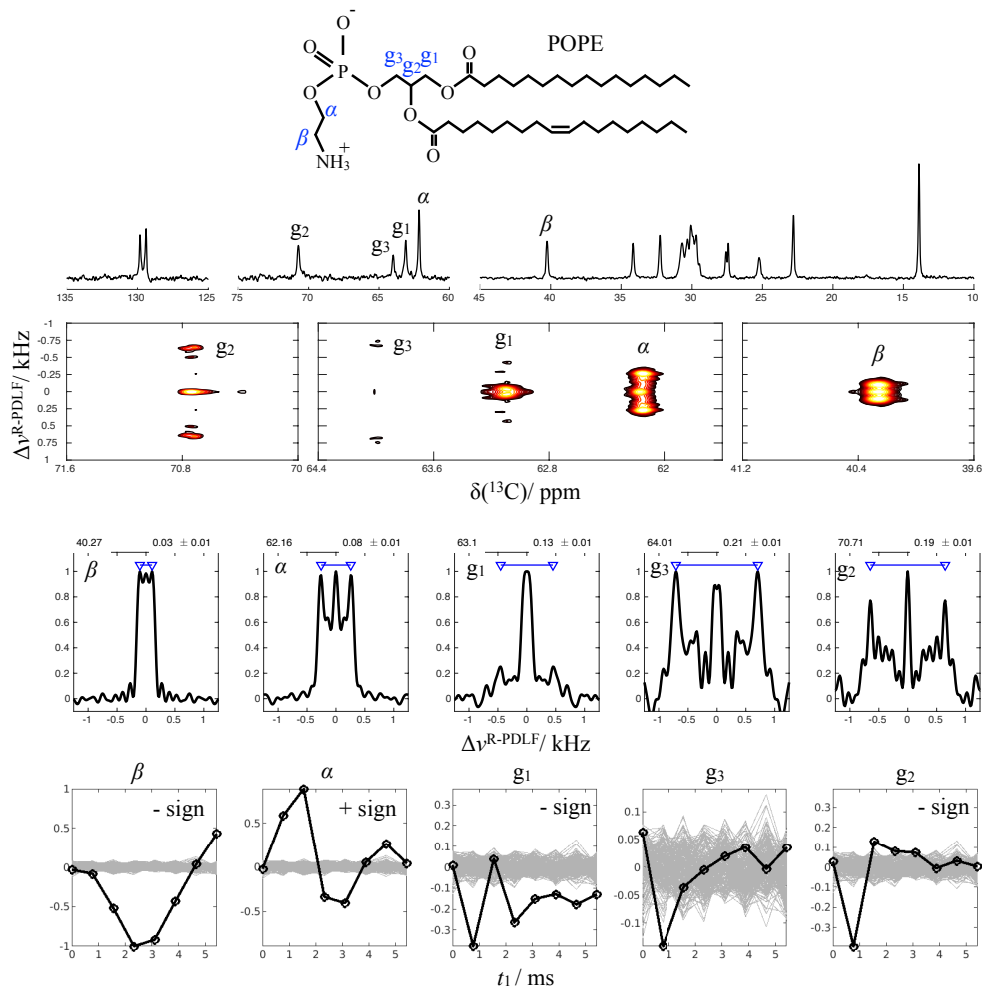


Figure S7: (A) Chemical structure of POPE with the labeling of headgroup and glycerol backbone carbons. (B) INEPT spectra from POPE sample with the headgroup and glycerol backbone peaks labeled. (C) 2D R-PDLF spectra (D) Dipolar sliced from the 2D R-PDLF spectra with the resulting order parameters on top of figures. (E) Experimental S-DROSS curves giving signs of the order parameters.

55.A, B etc. labels to be put in the figure.

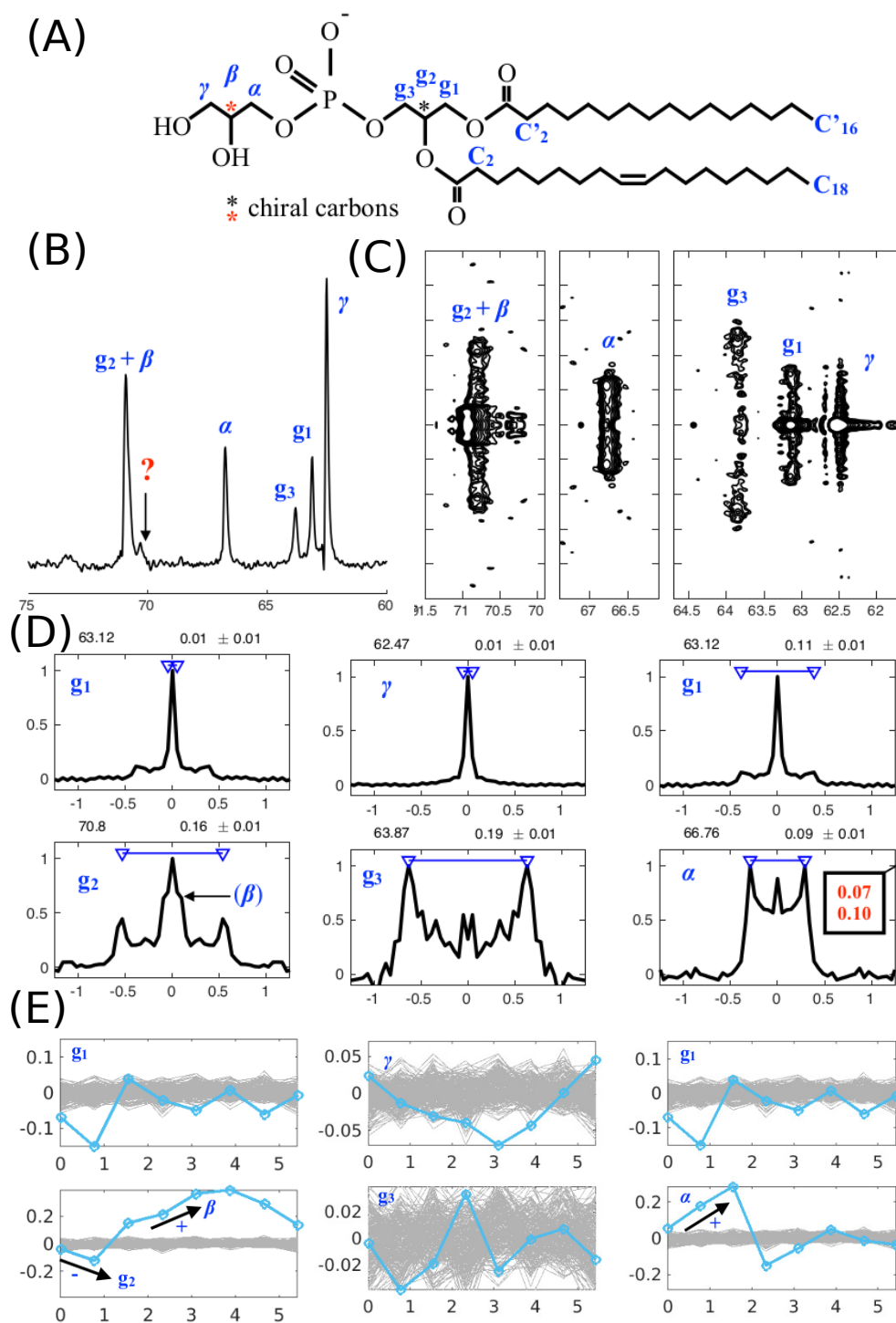


Figure S8: (A) Chemical structure of POPG with the labeling of headgroup and glycerol backbone carbons. (B) INEPT spectra from POPG sample with the headgroup and glycerol backbone peaks labeled. (C) 2D R-PDLF spectra (D) Dipolar sliced from the 2D R-PDLF spectra with the resulting order parameters on top of figures. (E) Experimental S-DROSS curves giving signs of the order parameters.

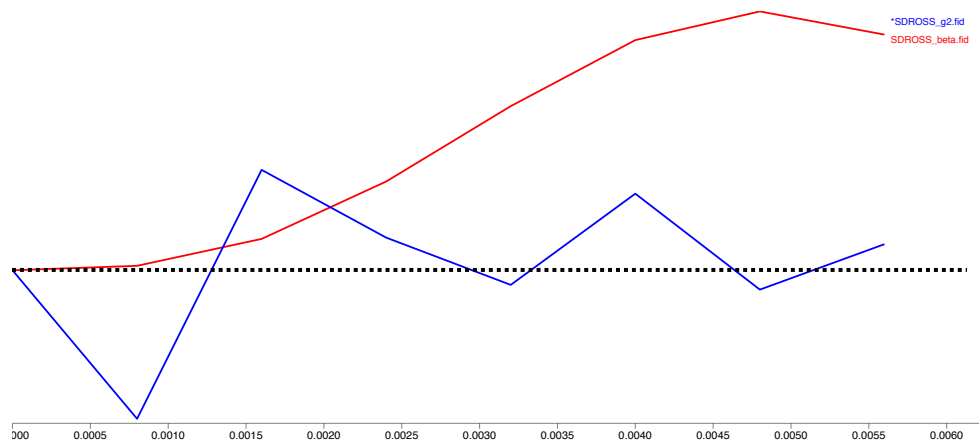


Figure S9: Simpson simlaton of S-DROSS curve of β -carbon of POPG.

S4 Changes of PG headroup order parameters upon addition of PC

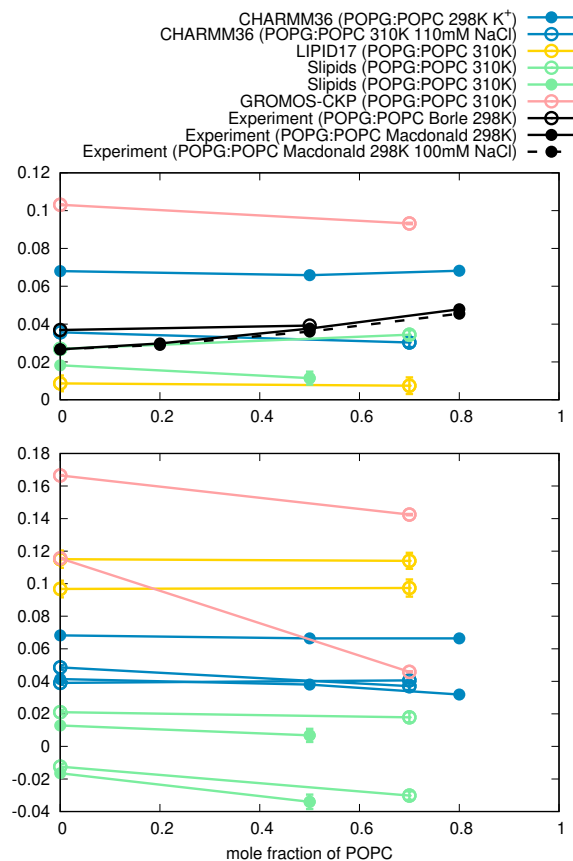


Figure S10: Modulation of PG lipid headgroup order parameters with the increasing amount of PC in lipid bilayer from experiments^{3,6} and simulations with different force fields.

S5 Sodium binding to POPC simulations

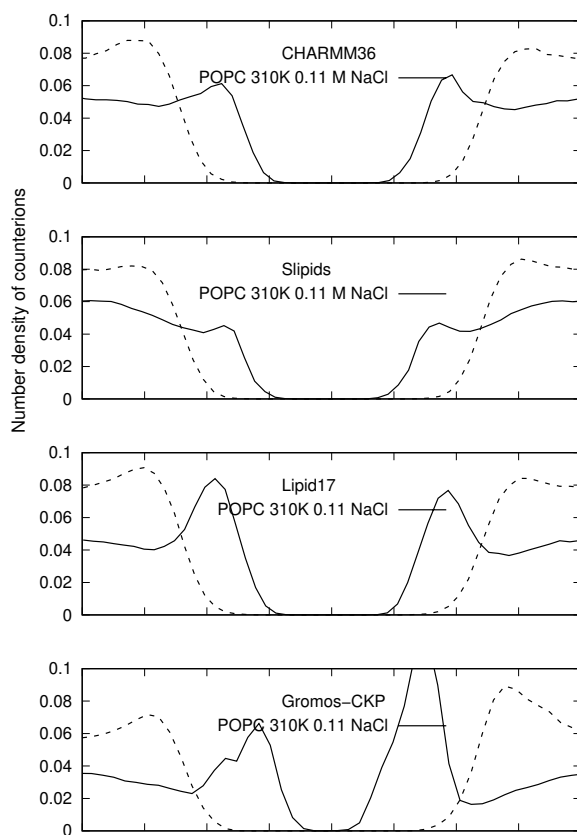


Figure S11: Sodium (solid line) and chloride ion density profiles along membrane normal from different simulations with PC lipids.

56.Discussion about differences to the NMRlipids II to be discussed once we have the details on ions models.

S6 Calcium binding to POPC:POPG (4:1) mixtures

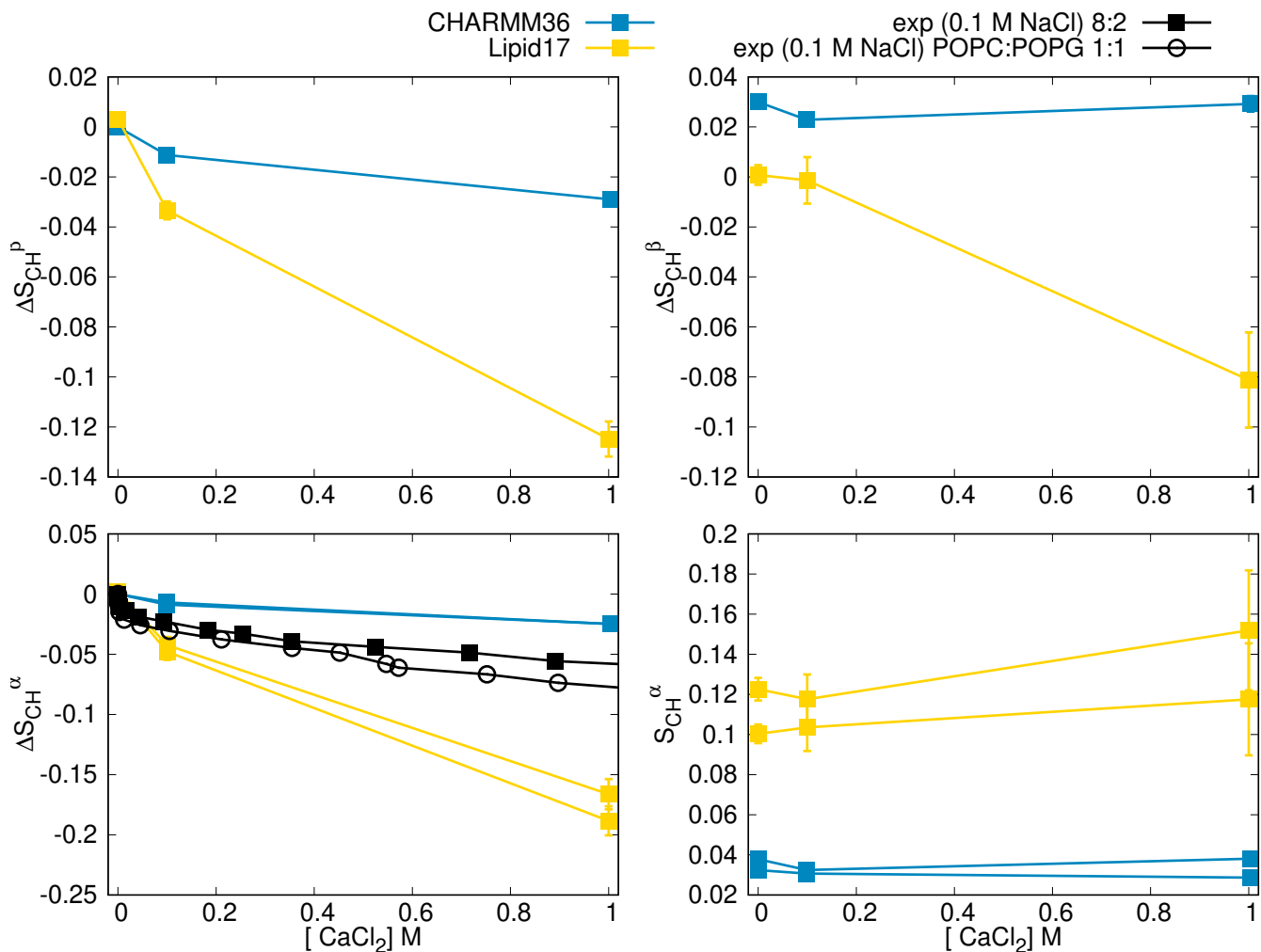


Figure S12: Modulation of headgroup order parameters of POPC (*left*) and POPG (*right*) in POPC:POPG (4:1) mixture upon addition of CaCl_2 in 298 K temperature from experiments⁶ and simulations. The changes with respect to the systems without CaCl_2 are shown for other data than for the α -carbon of POPG for which experimental order parameter is not available.

57.Lipid17ecc data to be analyzed and added.

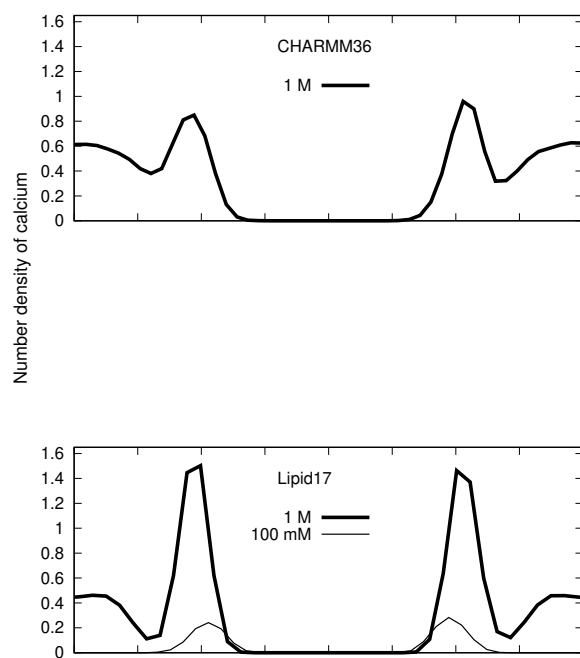


Figure S13: Calcium ion density profiles along membrane normal from simulations of POPC:POPG (4:1) mixtures with different force fields.

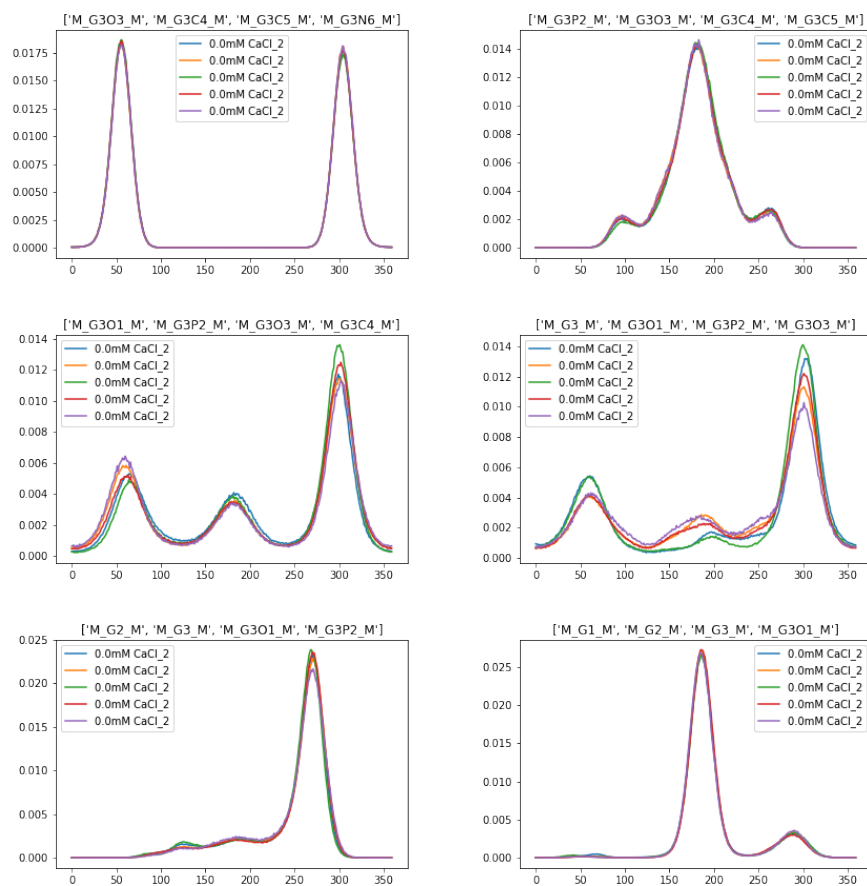


Figure S14: Changes in Slipids dihedrals with increasing amount of CaCl_2 .

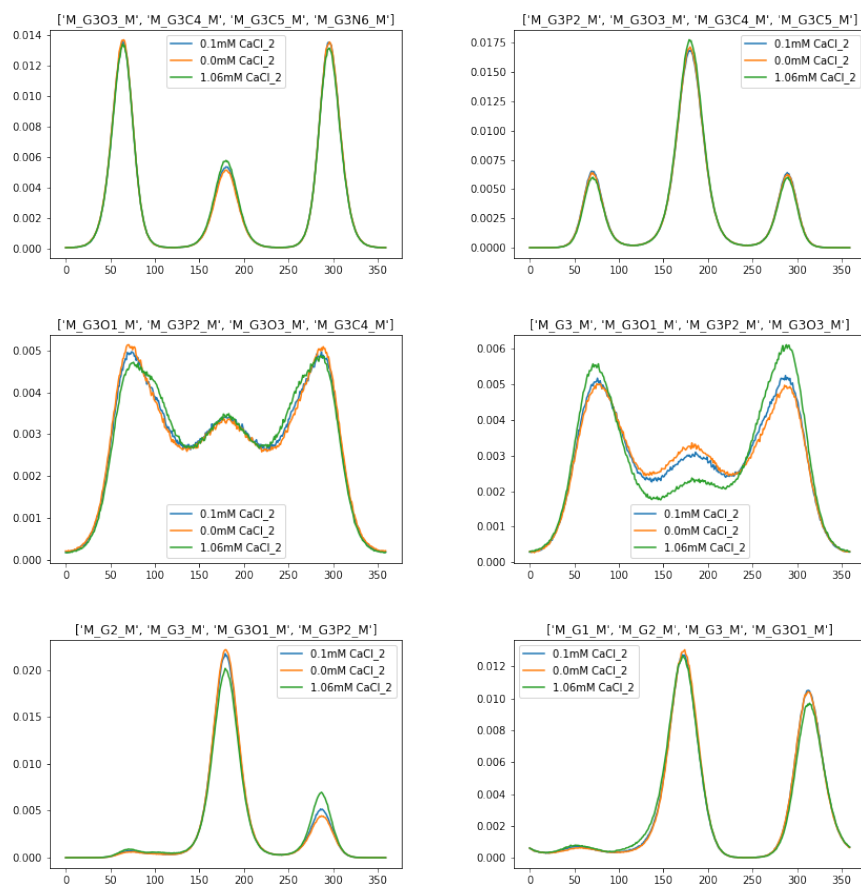


Figure S15: Changes in lipid17ecc dihedrals with increasing amount of CaCl₂.

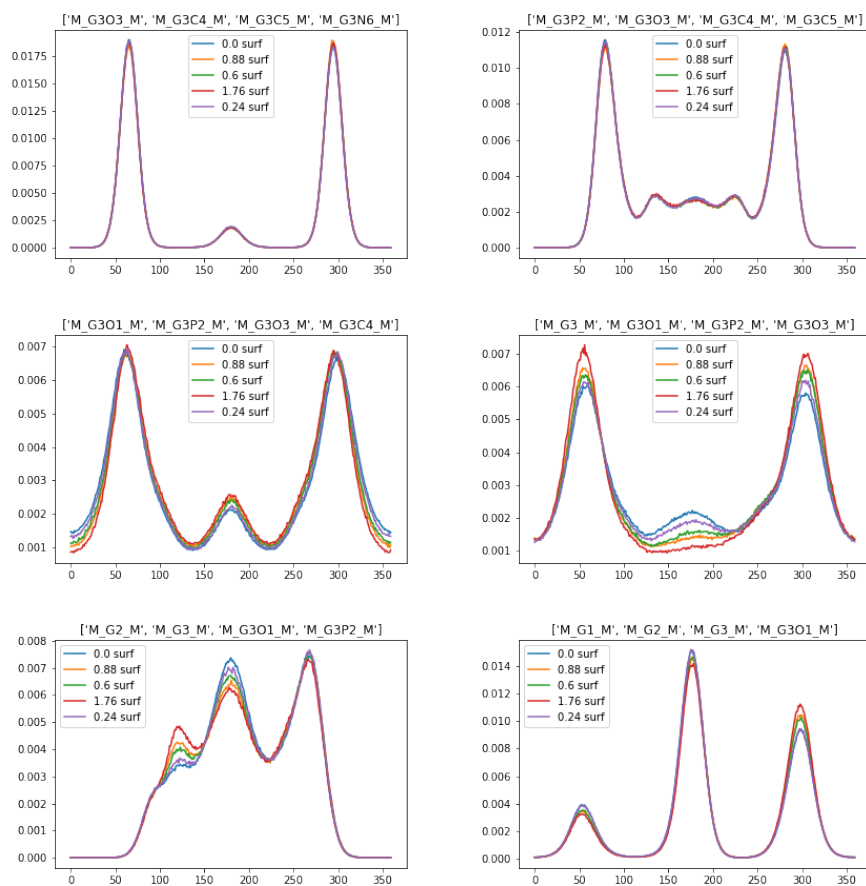


Figure S16: Changes in CHARMM36 dihedrals with increasing amount of cationic surfactant.

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