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

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S1 R-PDLF and SDROSS experiments

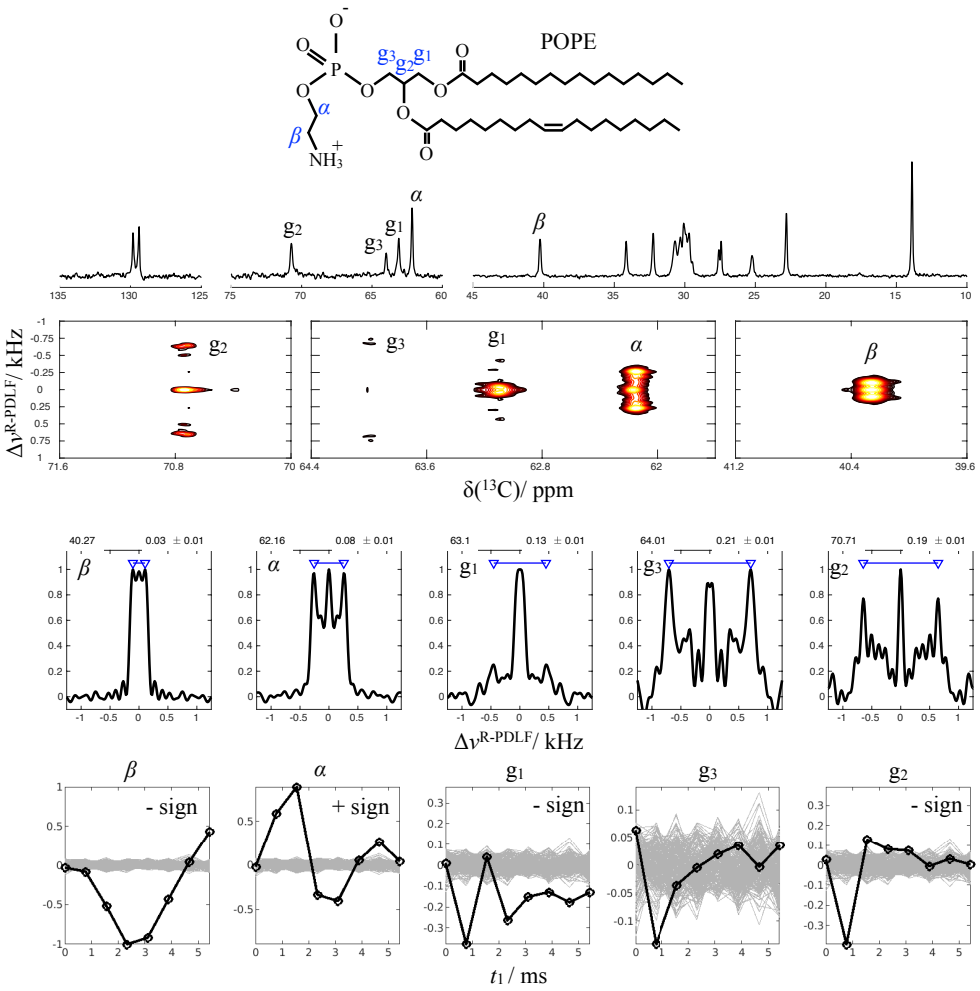

Figure S1: (A) Chemical structure of POPE with the labeling of headgroup and glycerol backbone carbons. (B) INEPT spectra from POPE sample with the headgroup and glycerol backbone peaks labeled. (C) 2D R-PDLF spectra (D) Dipolar sliced from the 2D R-PDLF spectra with the resulting order parameters on top of figures. (E) Experimetal S-DROSS curves giving signs of the order parameters.

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

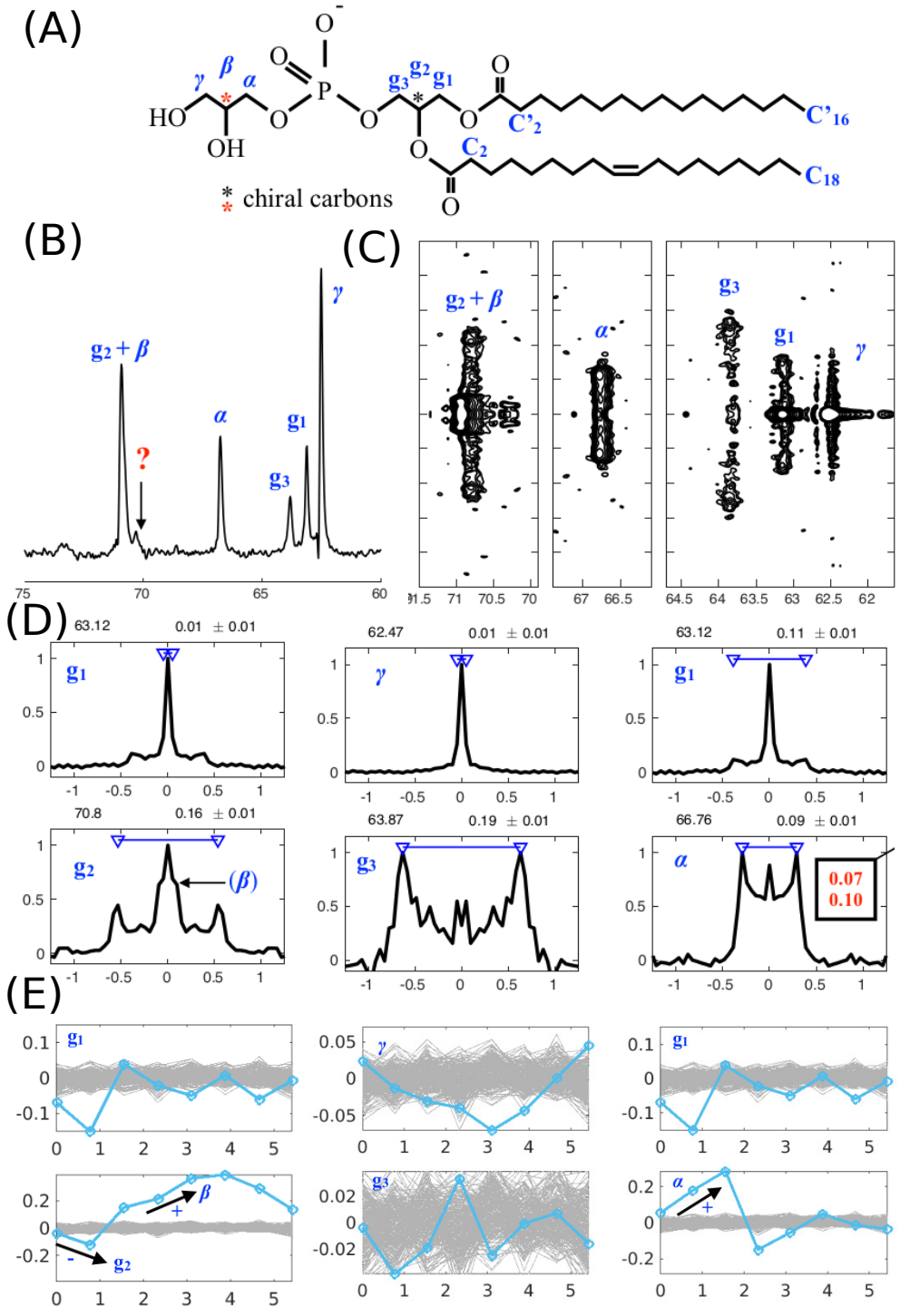

Figure S2: (A) Chemical structure of POPG with the labeling of headgroup and glycerol backbone carbons. (B) INEPT spectra from POPG sample with the headgroup and glycerol backbone peaks labeled. (C) 2D R-PDLF spectra (D) Dipolar sliced from the 2D R-PDLF spectra with the resulting order parameters on top of figures. (E) Experimetal S-DROSS curves giving signs of the order parameters.

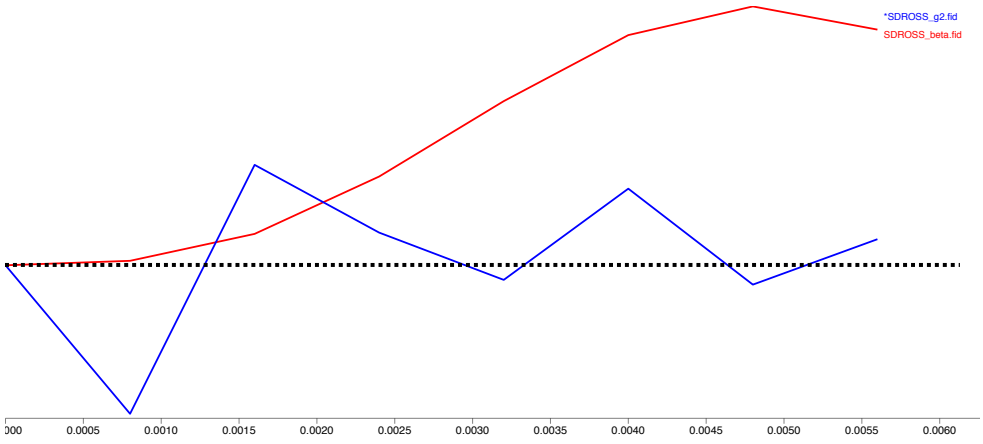

Figure S3: Simpson simulaton of S-DROSS curve of β-carbon of POPG.

S2 Comparison of headgroup order parameters from different force fields to experiments

The quality of PE and PG headgroup conformational ensembles in different simulations against NMR experiments is evaluated in figures S4 and S5 using C-H bond order parameters as in our previous studies for PC and PS lipids.[1,2] Conclusions are the same for all lipids: None of the force fields correctly captures the lipid headgroup conformational ensembles, but CHARMM36 gives results closest to experiments.

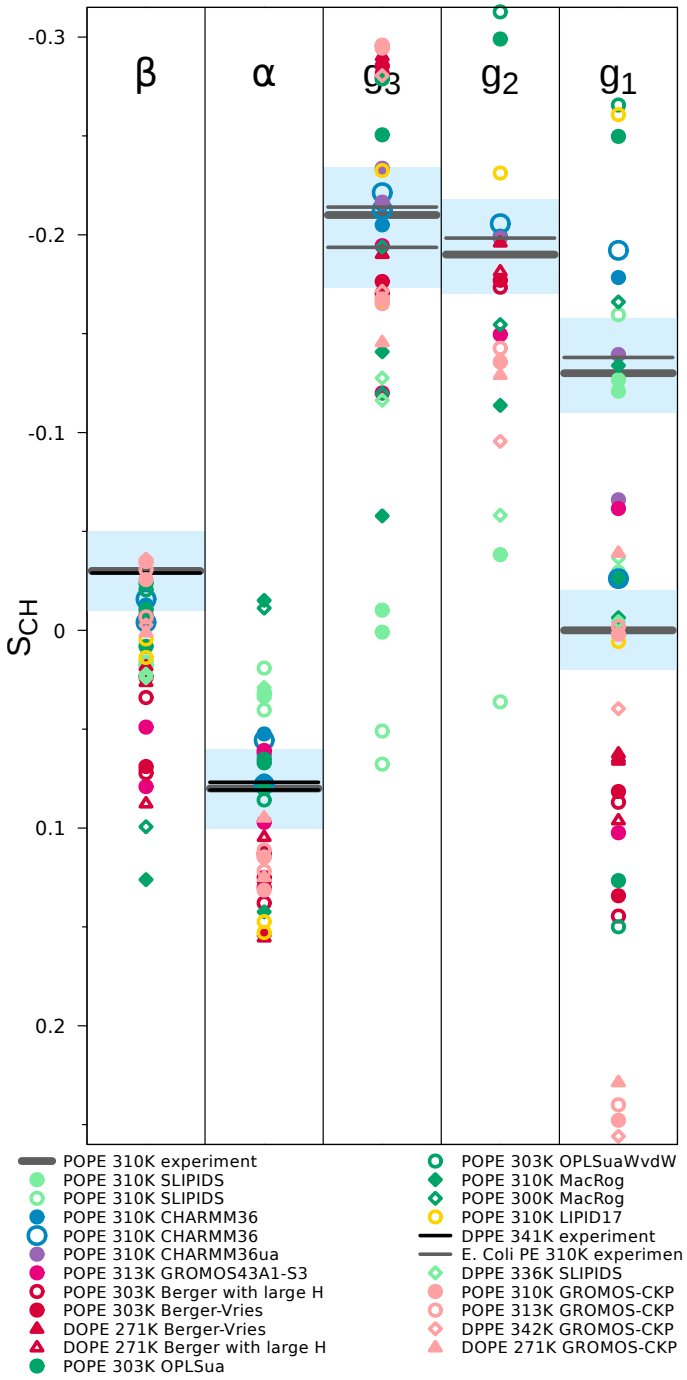

Figure S4: The headgroup and glycerol backbone order parameters of PE lipids from experiments (POPE and signs this work, DPPE from Ref. 3 and E.coliPE from Ref. 4) and simulations with different force fields.

2.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.

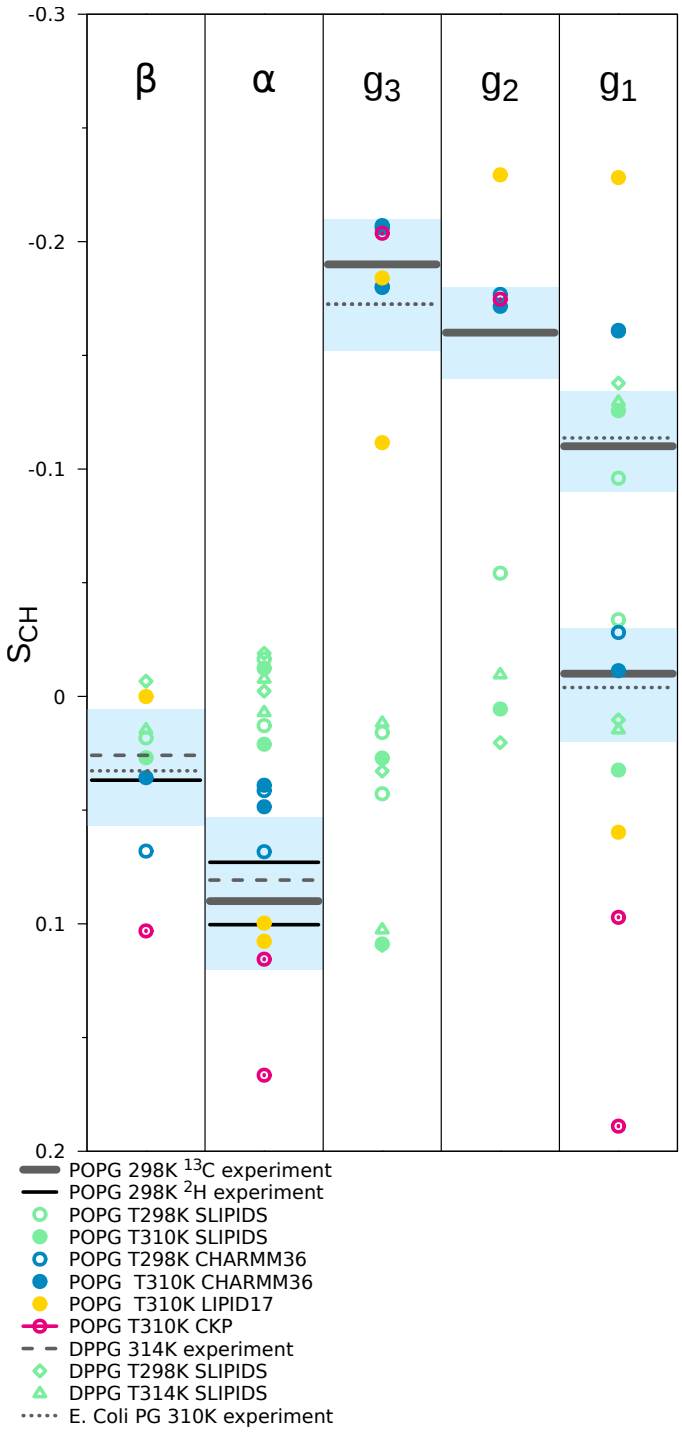

Figure S5: The headgroup and glycerol backbone order parameters of PG lipids from experiments (POPG and signs from this work and from Ref. 5, DPPG with 100mM NaCl from Ref. 6,and E.Coli PG results from Ref. 4). and simulations with different force fields.

S3 Lipid headgroups in mixtures with PC and PE or PG lipids

Headgroup order parameters of PC lipids are unchanged upon addition of zwitterionic lipids or cholesterol in experiments, but increase upon addition of negatively charged PG or PS lipids because headgroup dipole tilts more parallel to the membrane plane after incorporation of negative charges into the membrane.[7,10,11] The response of PC headgroup order parameters to the addition of PE or PG lipids from different simulations is compared with experiments in figure S6. by the tested CHARMM36 and Berger-OPLS force fields, although CHARMM36 slightly overestimates the changes The good performance of Berger-OPLS simulations is notable because the response of headgroup order parameters to cholesterol was significantly overestimated by the Berger/HÃűltje force field in our previous work.[1]

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).[2] In all force fields except Slipids, the order parameters of different hydrogens attached to the α-carbon are responsing 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 increase[8] or no change[5] upon addition of PC lipids (Fig. S7). Simulations with all the tested force fields give only very small changes also for the α-carbon order parameter (Figs. S11 and S7). 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.[11]

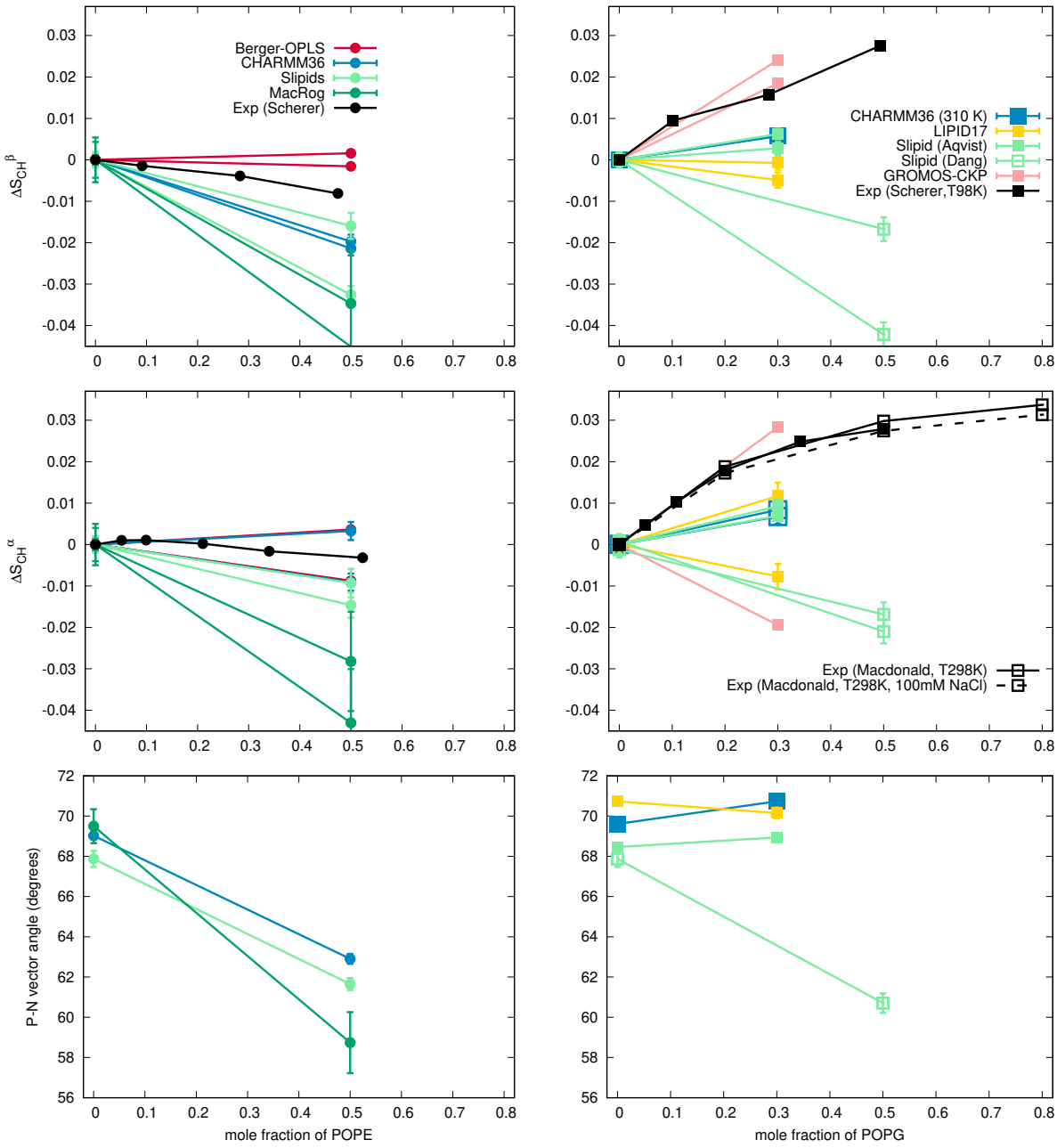

Figure S6: Modulation of POPC headgroup order parameters with increasing amount of POPE (left) and POPG (right) in bilayer from experiments[7,8] and simulations with different force fields. Signs are determined as discussed in.[1,9]

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

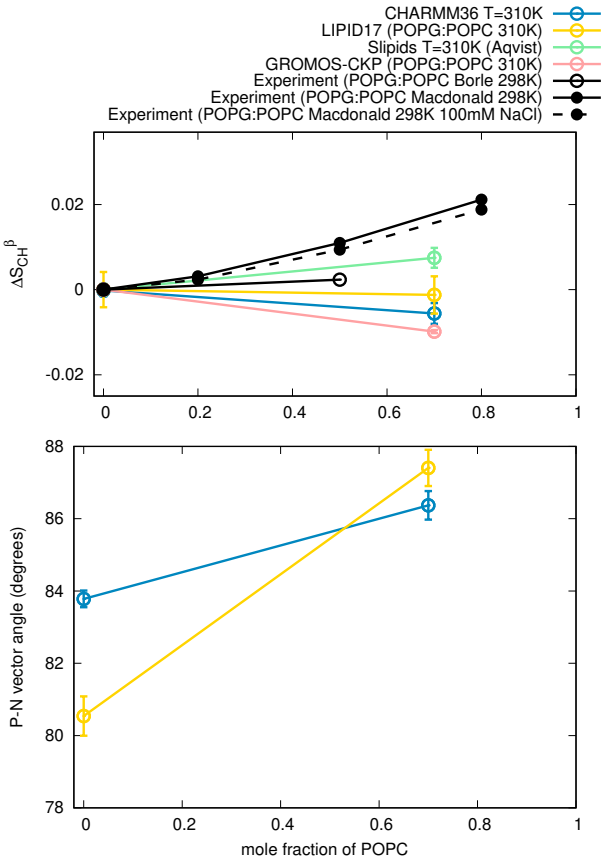

Figure S7: Modulation of PG lipid headgroup order parameters with the increasing amount of PC in lipid bilayer from experiments[5,8] and simulations with different force fields.

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

S3.1 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. S8), but lesser extend than reported previously for PC.[12] **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,[12] we compare POPE results to the POPC simulations ran with identical parameters (Fig. S12). 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 subtantially 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. S9). Lipid17 also exhibits less increase in POPC headgroup order parameters upon addition of POPG than other simulations (Fig. S6), and lower area per molecule (59.5 $Å^2$) than in experiments (66.1 $Å^2$). In our previous study about PS lipids,[11] 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. S9), the experimental increase in POPC headgroup order parameters upon addition of POPG are not fully reproduced (Fig. S6). 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.

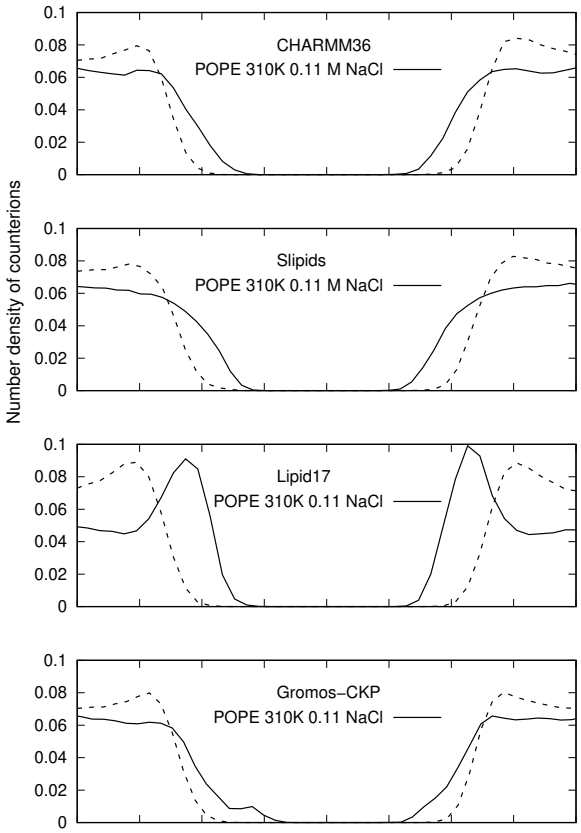

Figure S8: Sodium (solid line) and choride ion density profiles along membrane normal from different simulations with PE lipids.

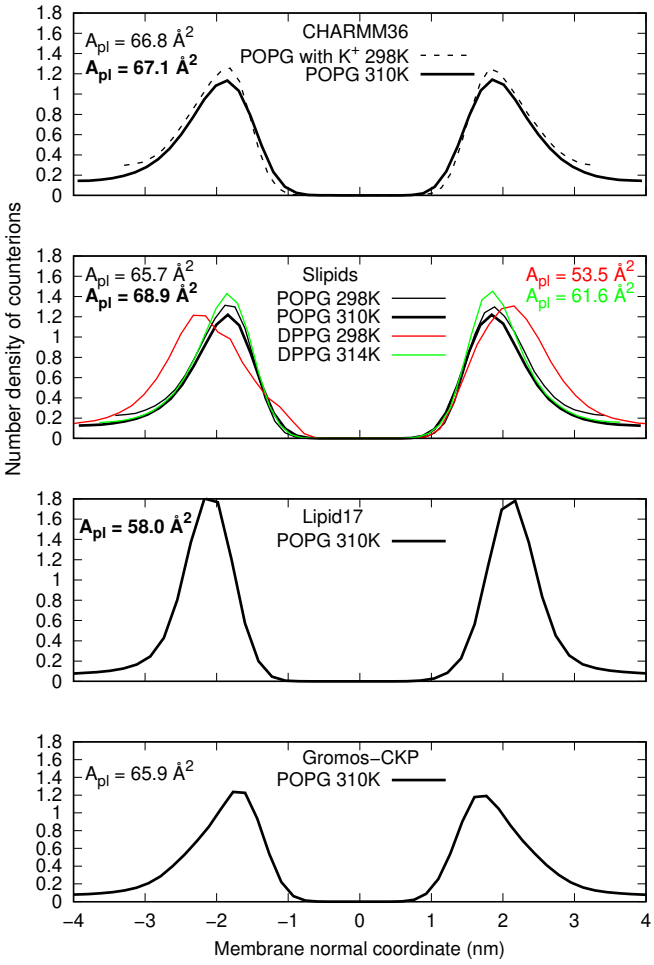

Figure S9: Counterion densities and area per lipids from simulations with PG lipids. Experimental area for POPG at 303 K is 66.1 Å^2 and 67 Å^2 for DPPC at 323 K.[13]

S4 Calcium density distributions

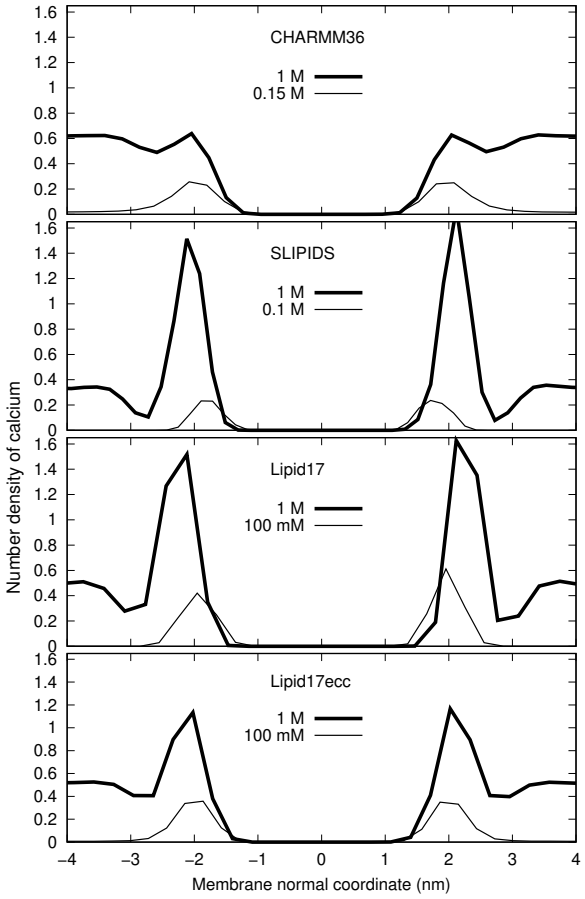

Figure S10: Calcium ion density profiles along membrane normal from simulations of POPC:POPG (1:1) mixtures with different force fields.

S5 Changes of PG headroup order parameters upon addition of PC

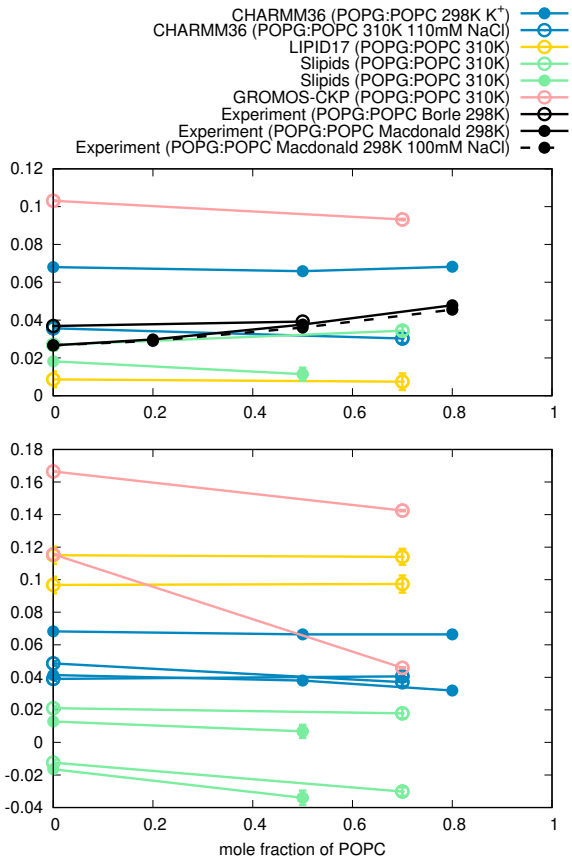

Figure S11: Modulation of PG lipid headgroup order parameters with the increasing amount of PC in lipid bilayer from experiments[5,8] and simulations with different force fields.

S6 Sodium binding to POPC simulations

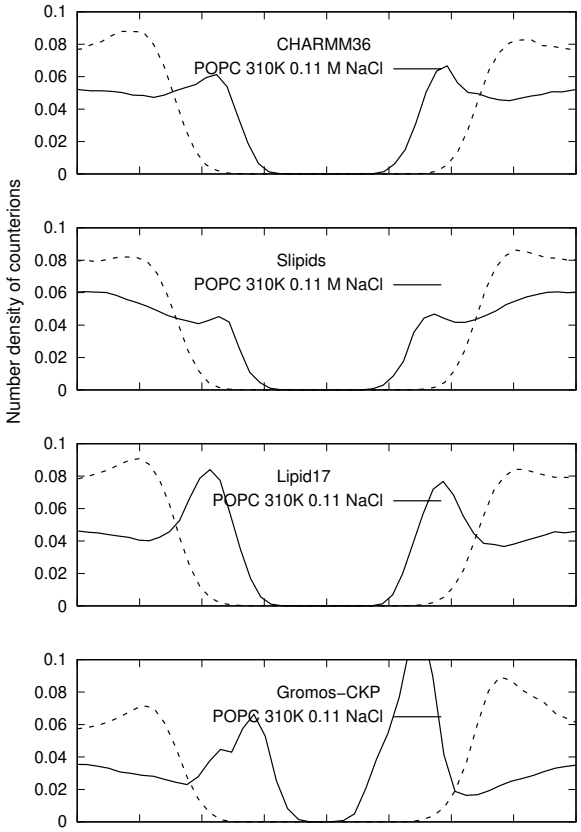

Figure S12: Sodium (solid line) and choride ion density profiles along membrane normal from different simulations with PC lipids.

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

S7 Calcium binding to POPC:POPG (4:1) mixtures

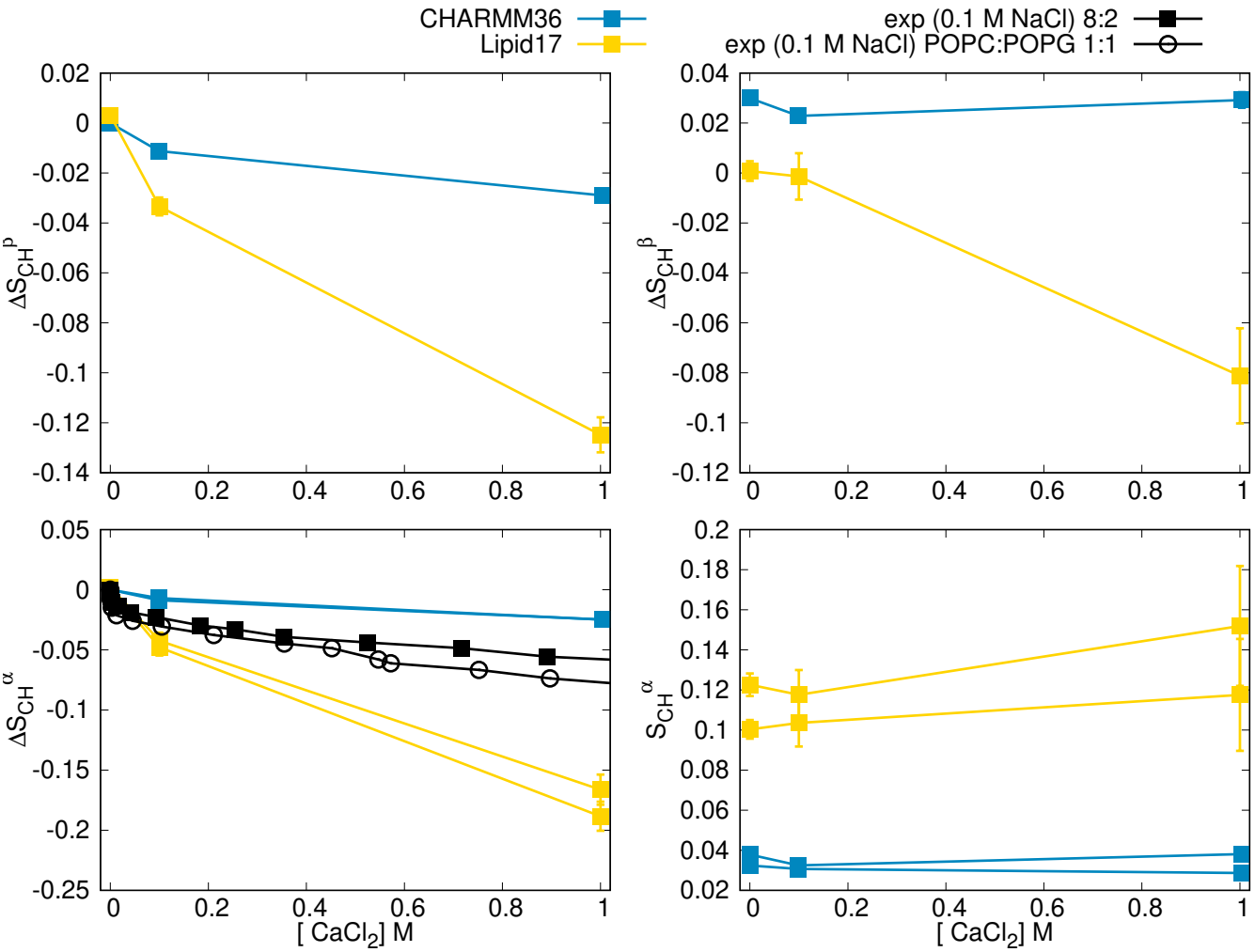

Figure S13: Modulation of headgroup order parameters of POPC (*left*) and POPG (*right*) in POPC:POPG (4:1) mixture upon addition of $CaCl_2$ in 298 K temperature from experiments[8] and simulations. The changes with respect to the systems without $CaCl_2$ are shown for other data than for the α-carbon of POPG for which experimental order parameter is not available.

8.Lipid17ecc data to be analyzed and added.

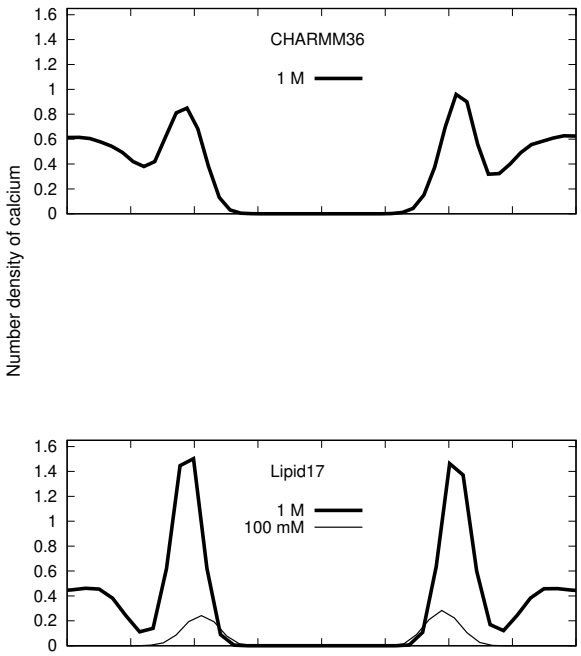

Figure S14: Calcium ion density profiles along membrane normal from simulations of POPC:POPG (4:1) mixtures with different force fields.

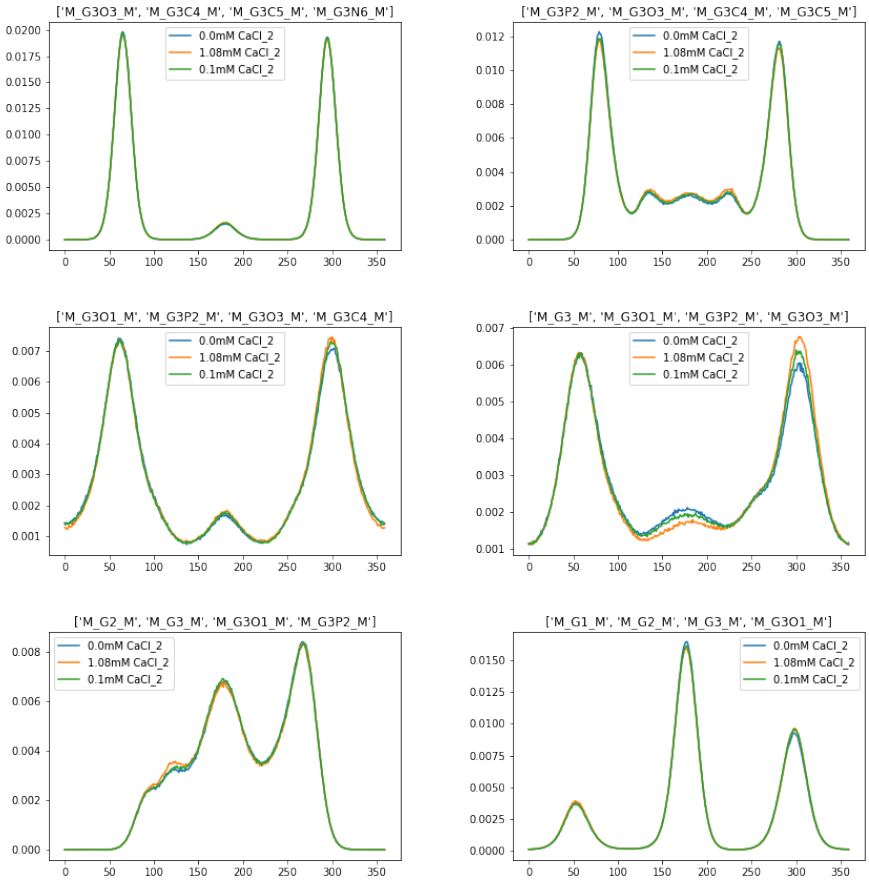

Figure S15: Changes in POPC CHARMM36 dihedrals with increasing amount of $CaCl_2$.

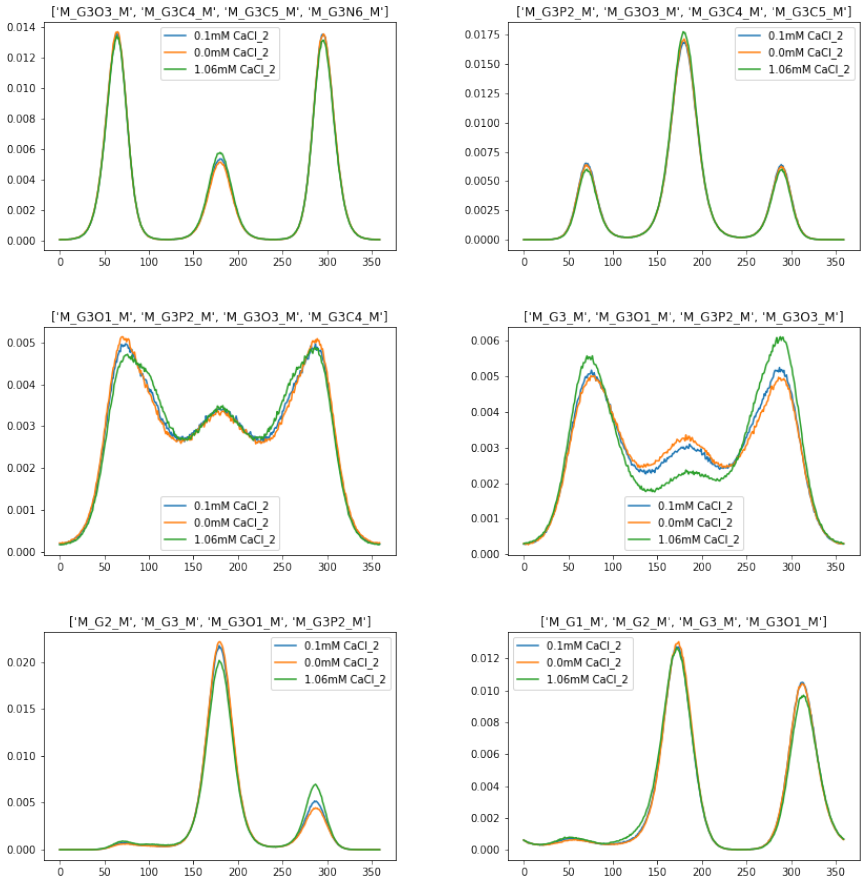

Figure S16: Changes in POPC lipid17ecc dihedrals with increasing amount of $CaCl_2$.

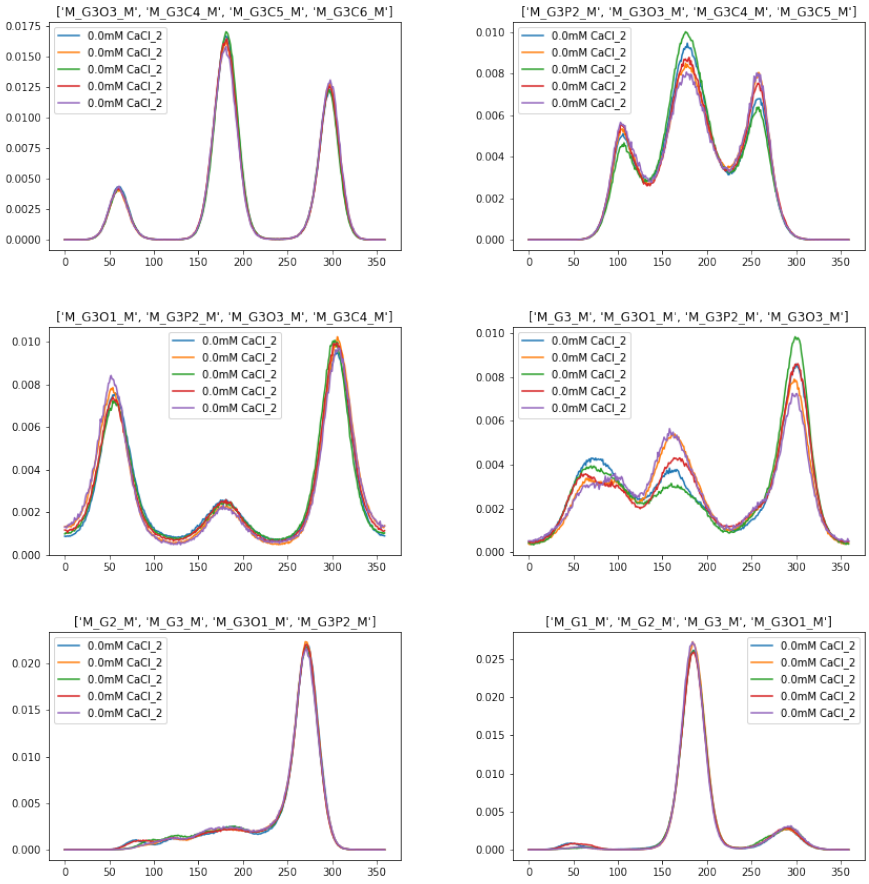

Figure S17: Changes in POPG Slipids dihedrals with increasing amount of $CaCl_2$.

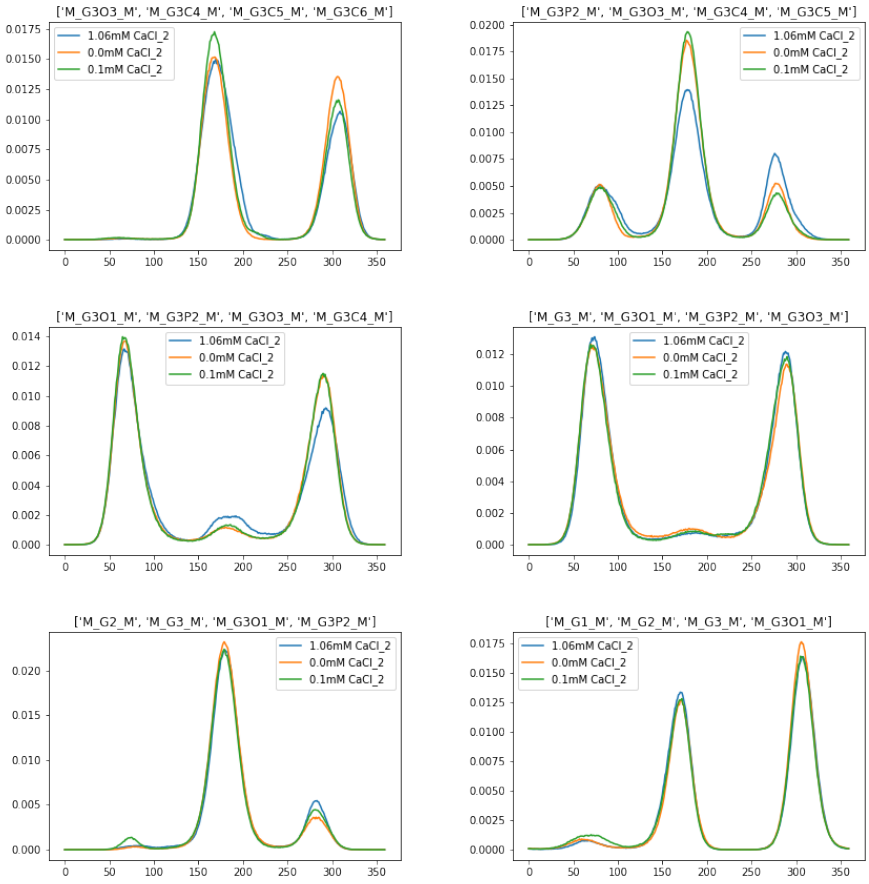

Figure S18: Changes in POPG lipid17 dihedrals with increasing amount of $CaCl_2$.

S8 Changes in headgroup conformations upon addition of $CaCl_2$

S9 Simulated systems

S9.1 CHARMM36

POPE **33.Simulation details by M. Javanainen.**

POPE with additional NaCl **34.Simulation details by A. Peon.**

POPG **35.Simulation details by Ollila.**

POPG with additional NaCl **36.Simulation details by A. Peon.**

POPC:POPE mixtures Data is available at.[64,65] 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 **37.Full simulation details by Fuchs et al.**

POPC:POPG mixture with additional calcium **38.Simulation details by J. Madsen.**

POPC:POPG mixture with additional NaCl **39.Simulation details by A. Peon.**

S9.2 CHARMM36ua

POPE Data is available at.[17] **40.Simulation details by T. Piggot.**

S9.3 Slipids

POPE Data is available at.[20] **41.Simulation details by T. Piggot.**

POPE with additional NaCl **42.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.[19] **43.Simulation details by F. Favela.**

POPG Data is available at.[46] **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	[14]
POPE	CHARMM36[?]	0	500	25000	0	310	500	100	[15]
POPE	CHARMM36[?]	0.11	500	25000	50	310	500	100	[16]
POPE	CHARMM36ua[?]	0	336	15254	0	310	2×200	2×100	[17]
DPPE	Slipids[18]	0	288	9386	0	336	200	100	[19]
POPE	Slipids[18]	0	336	?	0	310	2×200	2×100	[20]
POPE	Slipids[18]	0	500	25000	0	310	500	100	[21]
POPE	Slipids / Åqvist[18,22]	0.11	500	25000	50	310	500	100	[23]
DPPE	GROMOS-CKP[?]	0	128	3655	0	342	2×500	2×400	[24]
POPE	GROMOS-CKP[?]	0	128	3552	0	313	2×500	2×400	[25]
POPE	GROMOS-CKP[?]	0	500	25000	0	310	500	100	[26]
POPE	GROMOS-CKP[?]	0.11	500	25000	50	310	500	100	[27]
DOPE	GROMOS-CKP[?]	0	128	4789	0	271	2×500	2×400	[28]
POPE	GROMOS 43A1-S3[?]	0	128	3552	0	313	2×200	2×100	[29]
POPE	OPLS-UA vdW on H[?]	0	128	3328	0	303	2×200	2×100	[30]
POPE	OPLS-UA[?]	0	128	3328	0	303	2×200	2×100	[31]
POPE	OPLS-MacRog[32]	0	144	5760	0	310	500	350	[33]
POPE	OPLS-MacRog[32]	0	128	5120	0	300	500	300	[34]
POPE	Berger-Vries[?]	0	128	3552	0	303	2×200	2×100	[35]
POPE	Berger-largeH[?]	0	128	3552	0	303	2×200	2×100	[36]
DOPE	Berger-Vries[?]	0	128	4789	0	271	2×200	2×100	[37]
DOPE	Berger-largeH[?]	0	128	4789	0	271	2×300	2×100	[38]
POPE	LIPID17[39]	0	500	25000	50	310	500	100	[40]
POPE	LIPID17[39]	0.11	500	25000	50	310	500	100	[41]

[a]Number of lipid molecules with largest mole fraction
[b]Number of water molecules
[c]Number of additional cations
[d]Simulation temperature
[e]Total simulation time
[f]Time used for analysis
[g]Reference for simulation files

9.Citation for CHARMM36 PE?

10.Which ion model is used in[16]?

11.Citation for GROMOS-CKP?

12.Citation for GROMOS 43A1-S3?

13.Citation for OPLS-UA models?

14.Citations for Berger-* simulations?

15.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? 16.	0	118	4110	0	298	100	100	[42]
POPG	CHARMM36?	0.11	500	25000	49	310	500	100	[43]
POPG	CHARMM36?	0	500	25000	0	310	500	100	[44]
POPG/Na^+	Slipids / Åqvist[22,45]	0	288	10664	0	298	250	100	[46]
DPPG/Na^+	Slipids / Åqvist[22,45]	0	288	11232	0	314	200	100	[47]
DPPG/Na^+	Slipids / Åqvist[22,45]	0	288	11232	0	298	400	100	[48]
POPG	Slipids / Åqvist[22,45]	0	500	25000	0	310	500	100	[49]
POPG	Slipids / Åqvist[22,45]	0.11	500	25000	49	310	500	100	[50]
POPG	LIPID17 / Dang[39,51]	0	500	25000	0	310	500	100	[52]
POPG	LIPID17?	0.11	500	25000	49	310	500	100	[53]
POPG	GROMOS-CKP?	0	500	25000	0	310	500	100	[54]
POPG	GROMOS-CKP?	0.11	500	25000	49	310	500	100	[55]

[a]Number of lipid molecules with largest mole fraction
[b]Number of water molecules
[c]Number of additional cations
[d]Simulation temperature
[e]Total simulation time
[f]Time used for analysis
[g]Reference for simulation files

17.Citations and ion model for CHARMM36?

18.Lipid17 simulation with correct dihedral potentials still coming.

19.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	[56]
POPC:POPG (7:3)	CHARMM36[?]	0	0	350	25000	0	310	500	100	[57]
POPC:POPG (1:1)	CHARMM36[?]	0	0	150:150	31500	0	298	500	400	[58]
POPC:POPG (1:1)	CHARMM36[?]	0	0.1	150:150	31329	57	298	400	300	[59]
POPC:POPG (1:1)	CHARMM36[?]	0	1.08	150:150	29766	578	298	500	400	[60]
POPC:POPG (4:1)	CHARMM36[?]	0	0	350:88	26280	0	298	500	400	[61]
POPC:POPG (4:1)	CHARMM36[?]	0	0.1	350:88	26280	47	298	500	400	[62]
POPC:POPG (4:1)	CHARMM36[?]	0	1.0	350:88	24927	451	298	500	400	[63]
POPC	CHARMM36[?]	0	0	256	8704	0	300	300	250	[64]
POPC:POPE (1:1)	CHARMM36[?]	0	0	128	8704	0	300	300	250	[65]
POPC	OPLS-MacRog[32]	0	0	128	5120	0	300	500	300	[66]
POPC:POPE (1:1)	OPLS-MacRog[32]	0	0	128	5120	0	300	500	300	[67]
POPC	Slipid[18]	0	0	512	23943	0	298	170	100	[68]
POPC:POPE (1:1)	Slipid[18]	0	0	128	5120	0	298	500	300	[69]
POPC	GROMOS-CKP / ??[? ?]	0	0	500	25000	0	310	500	100	[70]
POPC:POPG (7:3)	GROMOS-CKP / ??[? ?]	0	0	350:150	25000	0	310	500	100	[71]
POPC	Slipid[18]	0	0	500	25000	0	310	500	100	[72]
POPC:POPG (7:3)	Slipid / Åqvist[18,22]	0	0	350:150	25000	0	310	500	100	[73]
POPC:POPG (1:1)	Slipid / Dang[18,51,74,75]	0	0	128:128	12800	0	298	500	400	[76]
POPC:POPG (1:1)	Slipid / Dang[18,51,74,75]	0	0.1	128:128	12800	23	298	500	400	[76]
POPC:POPG (1:1)	Slipid / Dang[18,51,74,75]	0	0.2	128:128	12800	46	298	1500	500	[76]
POPC:POPG (1:1)	Slipid / Dang[18,51,74,75]	0	0.5	128:128	12800	115	298	1500	500	[76]
POPC:POPG (1:1)	Slipid / Dang[18,51,74,75]	0	1.0	128:128	12800	230	298	1500	500	[76]

[a]Number of lipid molecules with largest mole fraction
[b]Number of water molecules
[c]Number of additional cations
[d]Simulation temperature
[e]Total simulation time
[f]Time used for analysis
[g]Reference for simulation files

20.Citation and ion model for GROMOS-CKP?

21.Citation and description for "Berger" model?

22.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.

23.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[39,51,75]	0	0	350:88	26265	0	298	400	350	[77]
POPC:POPG (4:1)	Lipid17 / Dang[39,51,75]	0	0.1	350:88	26124	47	298	400	250	[78]
POPC:POPG (4:1)	Lipid17 / Dang[39,51,75]	0	1.0	350:88	24840	475	298	1200	200	[79]
POPC:POPG (1:1)	Lipid17 / Dang[39,51,75]	0	0	150:150	31572	0	298	320	200	[80]
POPC:POPG (1:1)	Lipid17 / Dang[39,51,75]	0	0.1	150:150	31401	57	298	718	198	[81]
POPC:POPG (1:1)	Lipid17 / Dang[39,51,75]	0	1.0	150:150	29865	569	298	720	200	[82]
POPC:POPG (1:1)	Lipid17ecc / ECC-ions[83–85]	0	0	150:150	31572	0	298	347.8	333	[86]
POPC:POPG (1:1)	Lipid17ecc / ECC-ions[83–85]	0	0.1	150:150	29865	54	298	400	300	[87]
POPC:POPG (1:1)	Lipid17ecc / ECC-ions[83–85]	0	1.0	150:150	29865	569	298	600	400	[88]
POPC	Berger[?] **24.**	0	0	256	10240	0	300	300	200	[89]
POPC:POPE (1:1)	Berger[?] **25.**	0	0	128	11008	0	300	300	200	[90]
POPC:DOPE (1:1)	Berger[?] **26.**	0	0	128	10240	0	300	300	200	[91]
DOPC	Berger[?] **27.**	0	0	256	11008	0	300	300	200	[92]
DOPC:DOPE (1:1)	Berger[?] **28.**	0	0	128	11008	0	300	300	200	[93]

[a]Number of lipid molecules with largest mole fraction
[b]Number of water molecules
[c]Number of additional cations
[d]Simulation temperature
[e]Total simulation time
[f]Time used for analysis
[g]Reference for simulation files

29.Citation and ion model for GROMOS-CKP?

30.Citation and description for "Berger" model?

31.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.

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

POPG 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.**

DPPG Data in 298 K is available at[48] and in 314 K at.[47] **46.Simulation details by F. Favela. I have assumed that ion parameters are default Slipids, i.e., Åqvist, please correct if this is not true.**

POPC:POPG mixture with additional NaCl **47.Simulation details by A. Peon. I have assumed that ion parameters are default Slipids, i.e., Åqvist, please correct if this is not true.**

S9.4 Berger

POPE Data is available at.[35,36] **48.Simulation details by T. Piggot.**

DOPE Data is available at.[37,38] **49.Simulation details by T. Piggot.**

POPC:POPE, POPC:DOPE and DOPC:DOPE mixtures Data is available at.[89,90] 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 **50.Simulation details by Fuchs et al.**

S9.5 GROMOS 43A1-S3

POPE Data is available at.[29] **51.Simulation details by T. Piggot.**

S9.6 OPLS-UA

POPE Data is available at.[31] **52.Simulation details by T. Piggot.**

POPE with vdW interaction in H Data is available at.[30] **53.Simulation details by T. Piggot.**

S9.7 GROMOS-CKP and GROMOS-CKPM

POPE Data is available at.[25] **54.Simulation details by T. Piggot.**

DOPE Data is available at.[28] **55.Simulation details by T. Piggot.**

DPPE Data is available at.[24] **56.Simulation details by T. Piggot.**

S9.8 Lipid17

S9.9 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.[94,95] In practise, this is implemented by scaling the charges and Lennard-Jones σs 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:[95] ECC-POPC parameters (scaling factors f_q=0.8 and f_σ=0.89 applied to Lipid14 POPC parameters)[94] were used for POPC and scaling factors of f_q=0.75 and f_σ=0.89 were applied to the charges and Lennard-Jones σs of headgroup, glycerol backbone, and carbonyl regions of Amber Lipid17 POPG parameters. The Lipid17 parameters (described above) and initial configurations were taken from Ref.[52] with the correct dihedral type, and the resulting parameters are available from Ref. **?** . ECC-ion parameters with the scaled charges,[83–85] downloaded from `bitbucket.org/hseara/ions/src/master/`, were used in these simulations.

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