

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) Experimental 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 slices 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 S3: Simpson simlaton of S-DROSS curve of β -carbon of POPG.

S2 Evaluation of simulations against NMR experiments

S2.1 Conformational ensembles of headgroup and glycerol backbone in PE and PG lipids

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.

It should be noted that the PG headgroup is biologically abundant R enantiomer in all simulations, while our ¹³C NMR experiments has a racemic mixture. Nevertheless, previous ²H NMR experiments comparing results between different enantiomers concluded that the structural differences between these are minor.³

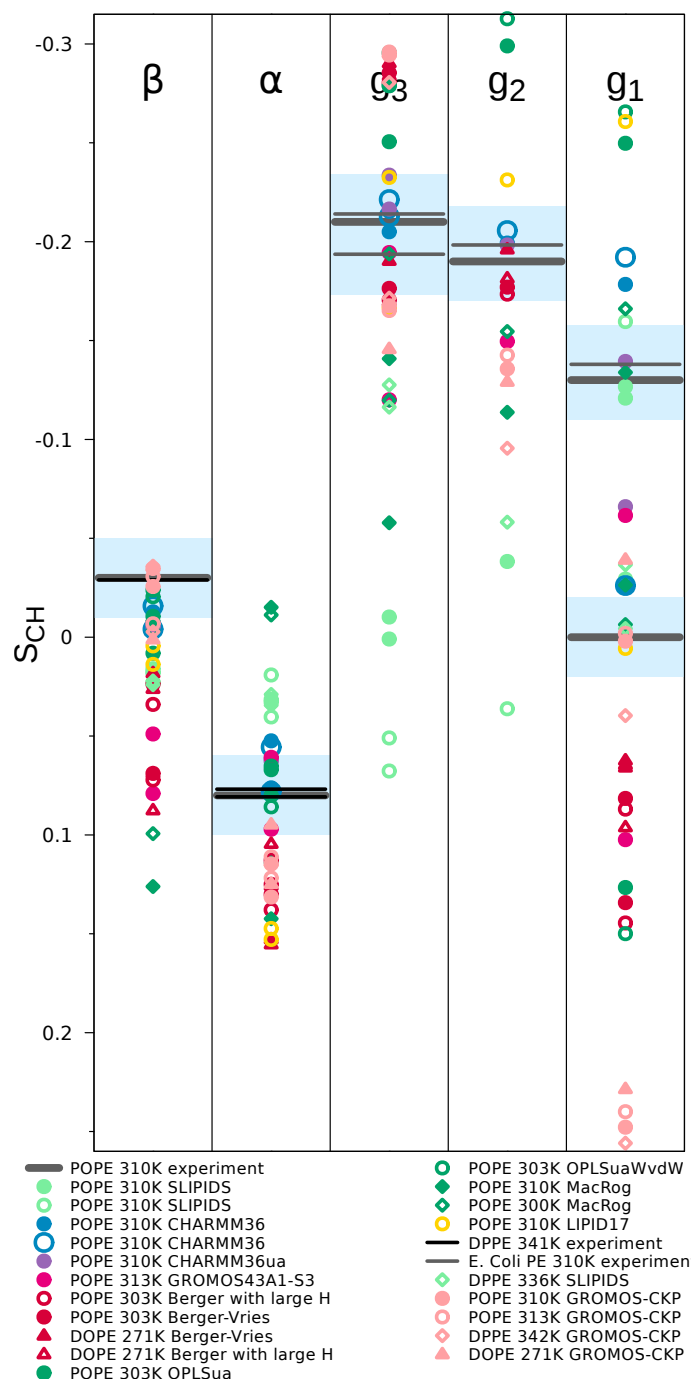


Figure S4: The headgroup and glycerol backbone order parameters of PE lipids from experiments (POPE and signs this work, DPPE from Ref. 4 and E.coliPE from Ref. 5) and simulations with different force fields.

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.

3.Lipid17 data should be updated to the one with correct dihedrals reported here

<https://github.com/NMRLipids/NMRLipidsIVPEandPG/issues/12#issuecomment-756641407>

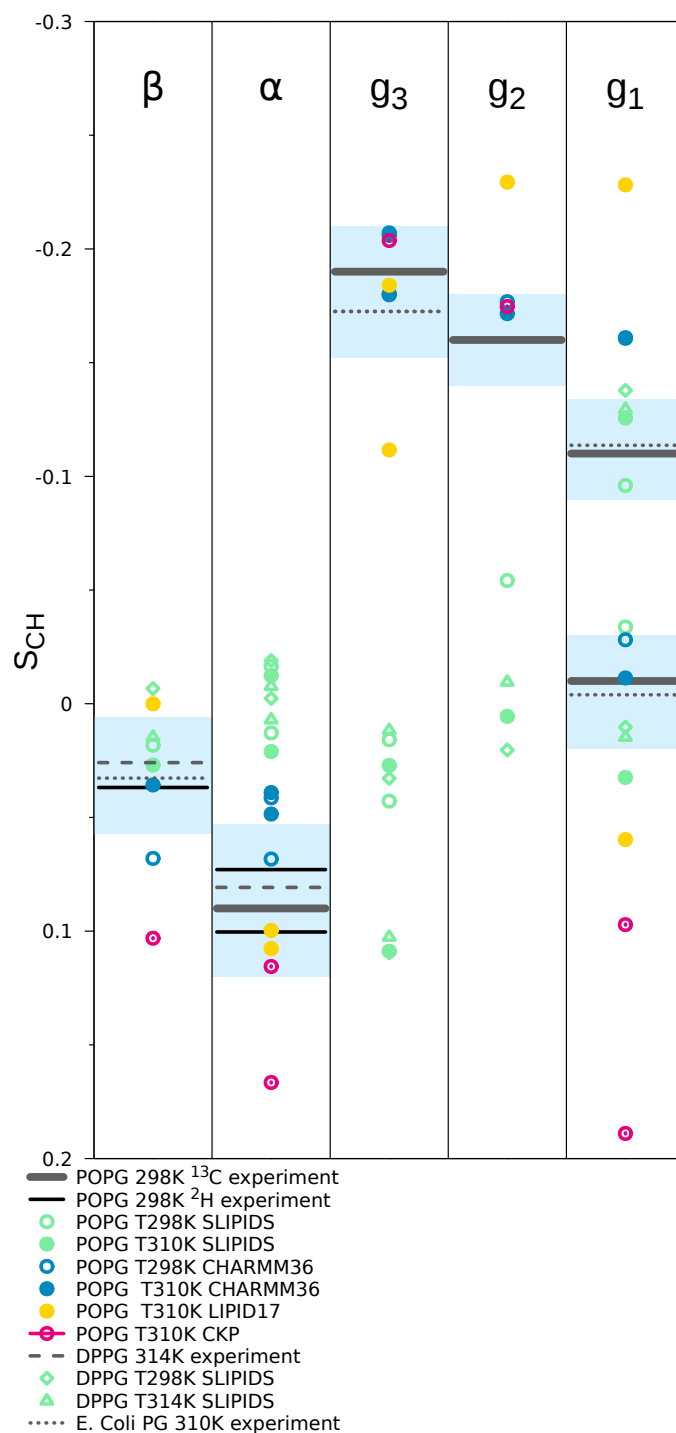


Figure S5: The headgroup and glycerol backbone order parameters of PG lipids from experiments (POPG and signs from this work and from Ref. 6, DPPG with 100mM NaCl from Ref. 3, and E. Coli PG results from Ref. 5) and simulations with different force fields.

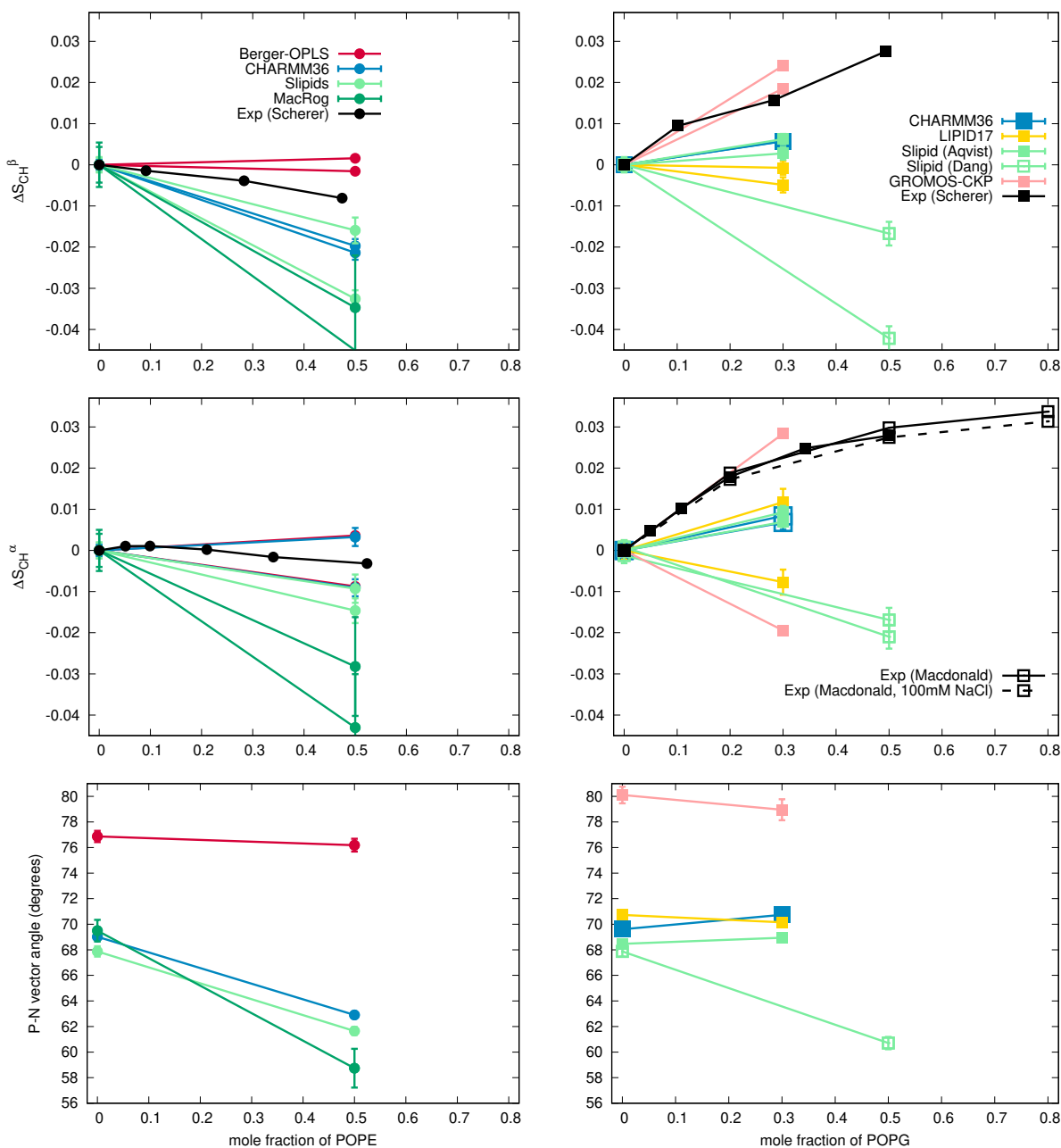
4. Lipid17 data should be updated to the one with correct dihedrals reported here

<https://github.com/NMRLipids/NMRLipidsIVPEandPG/issues/12#issuecomment-756641407>

S2.2 PC headgroup in mixtures with 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. None of the simulations reproduce neither the experimentally observed increase in PC headgroup order parameters with increasing amount of PG nor the related tilting of the headgroup more parallel with the membrane. Similar observations in our previous work for PS lipids were explained by the overestimated counterion binding affinity that neutralizes the effect of added negative charge.² All simulations except Berger-OPLS predict tilting of P-N headgroup outwards from the membrane and decrease of PC headgroup order parameters upon addition of PE lipids. These results are not in line with experiments where the PC headgroup order parameters are not affected by zwitterionic lipids.⁷ The good performance of Berger-OPLS simulations in here is surprising because headgroup conformational ensemble is not very close to experiments in this model and the response of headgroup order parameters to cholesterol was significantly overestimated by the Berger/Höltje force field in our previous work.¹

In conclusion, more accurate force fields are needed to correctly simulate the interactions between different headgroups.



S2.3 PG headgroup in mixtures with PC lipids

Changes in other than PC lipid headgroup with changing membrane composition are less extensively characterized in the literature. The β -carbon order parameter in PG headgroup increases mildly⁸ or is unchanged⁶ upon increasing amount of PC lipids (Fig. S7), but experimental data from α -carbon is not available. Also the tested force fields predict very small changes for the β -carbon order parameter, while the P-N vector tilt and its response to the increased amount of PC varies significantly between force fields in figure S7. Therefore, more experimental data and more accurate force fields are still required to resolve the PG conformational ensembles in mixtures with other lipids.

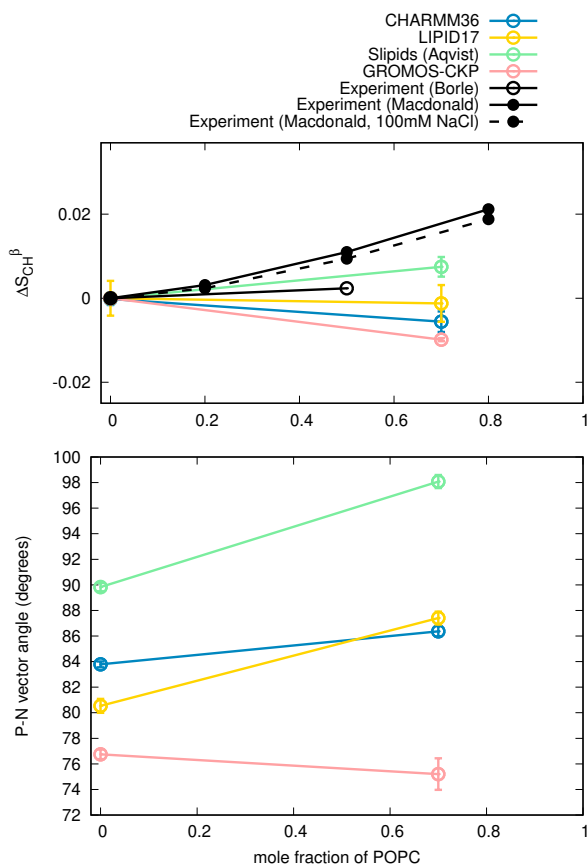


Figure S7: Modulation of PG lipid headgroup order parameters with the increasing amount of PC in lipid bilayer from experiments at 298 K^{6,8} and simulations with different force fields at 310 K.

S2.4 Calcium binding to POPC:POPG mixtures

The changes of headgroup order parameters in POPC:POPG mixtures upon addition of CaCl_2 between different simulations and experiments^{6,8} are compared in figures S8 (molar ratio 1:1) and S10 (molar ratio 4:1). The results are in line with our previous studies: most force fields overestimate the calcium binding,^{2,12} but CHARMM36 with the NBfix correction underestimates the binding affinity,² and the implicit inclusion of electronic polarizability using the electronic continuum correction (ECC) improves the results.^{13,14}

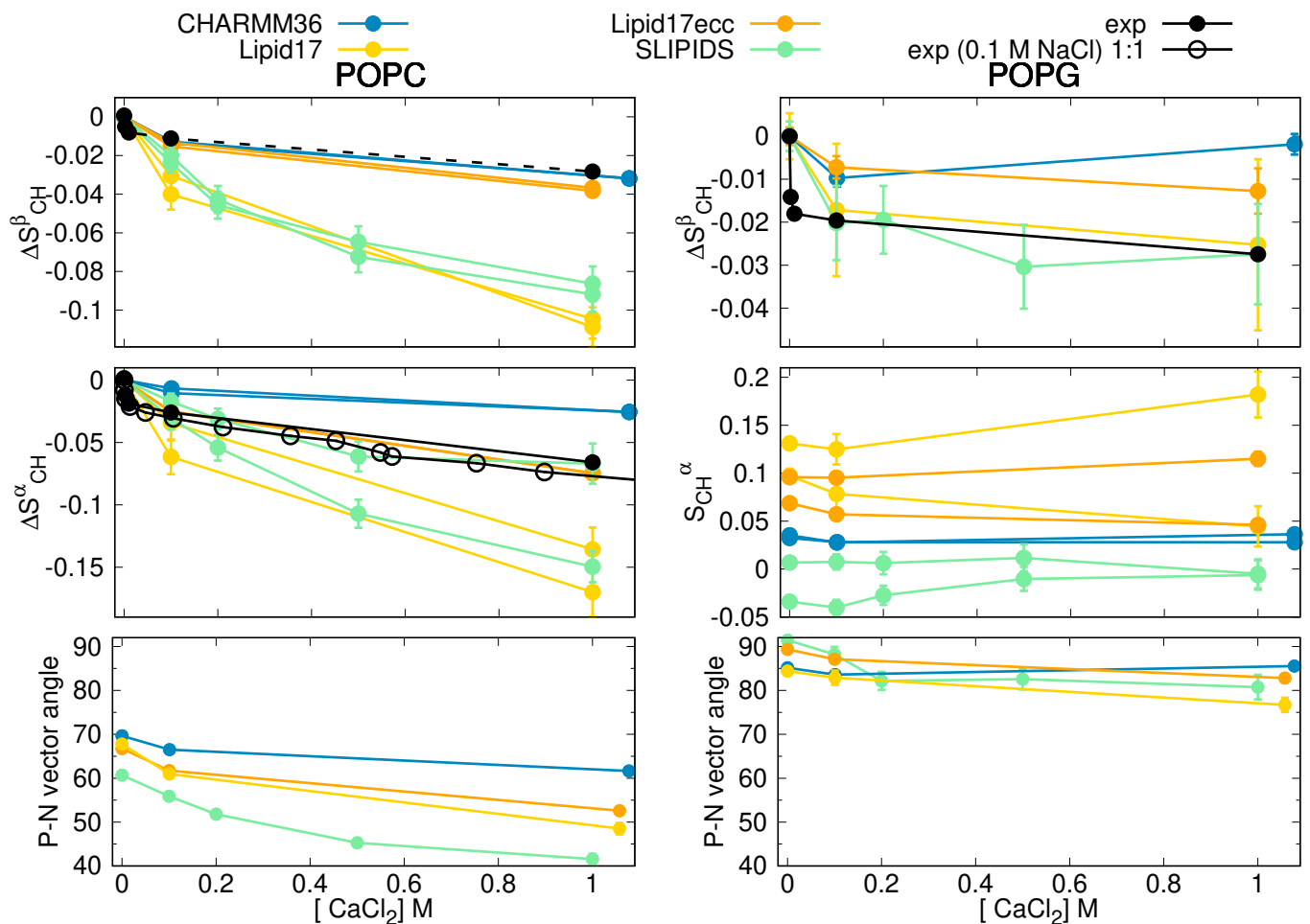


Figure S8: Modulation of headgroup order parameters of POPC (*left*) and POPG (*right*) in POPC:POPG (1:1) mixture upon addition of CaCl_2 in 298 K temperature from experiments^{6,8} and simulations. The β -carbon order parameter of POPC (dashed line on top left) is not directly measured but calculated from empirical relation $\Delta S_\beta = 0.43\Delta S_\alpha$.¹⁵ 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. Calcium density distributions are shown in figure S9.

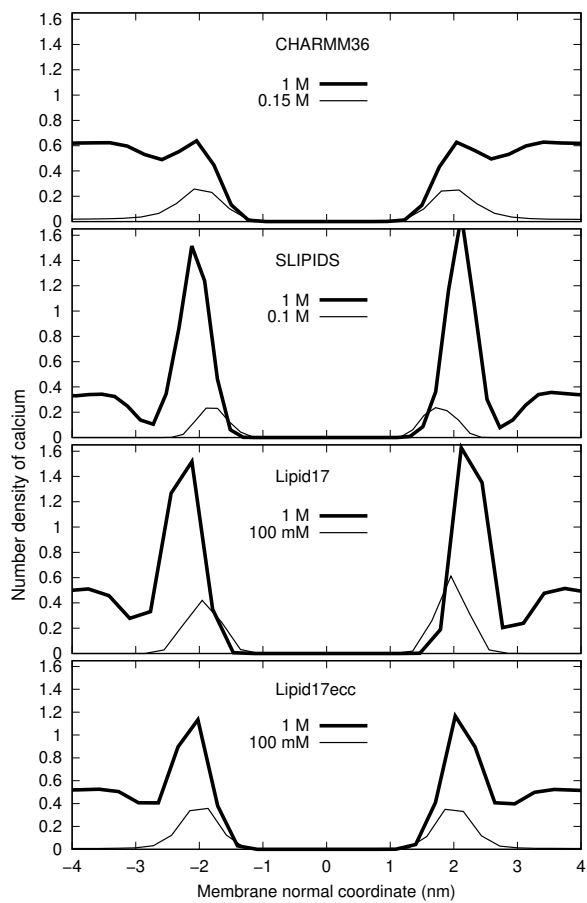
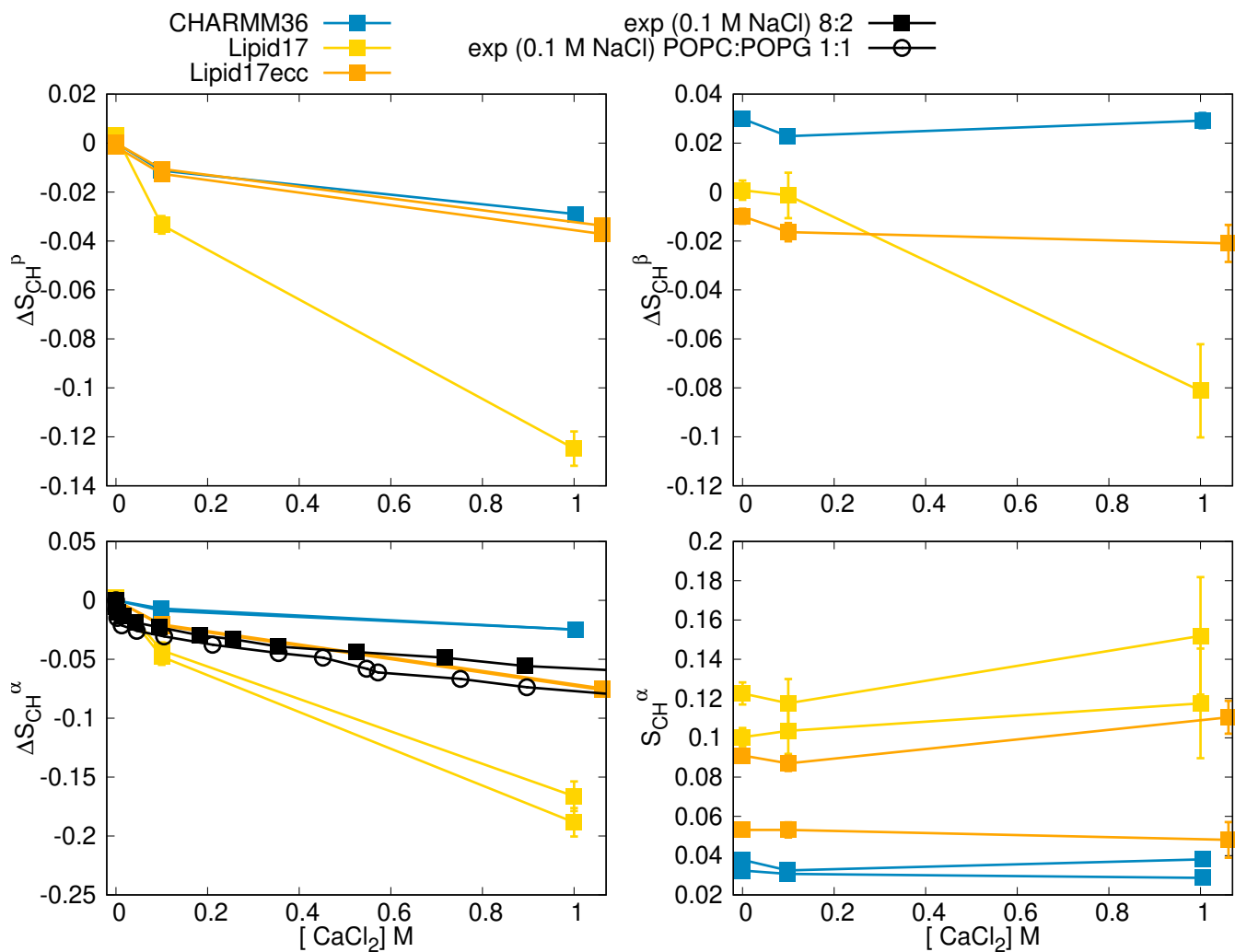


Figure S9: Calcium ion density profiles along membrane normal from simulations of POPC:POPG (1:1) mixtures with different force fields. The changes in the order parameters upon addition of CaCl_2 are compared with experiments in figure S8.



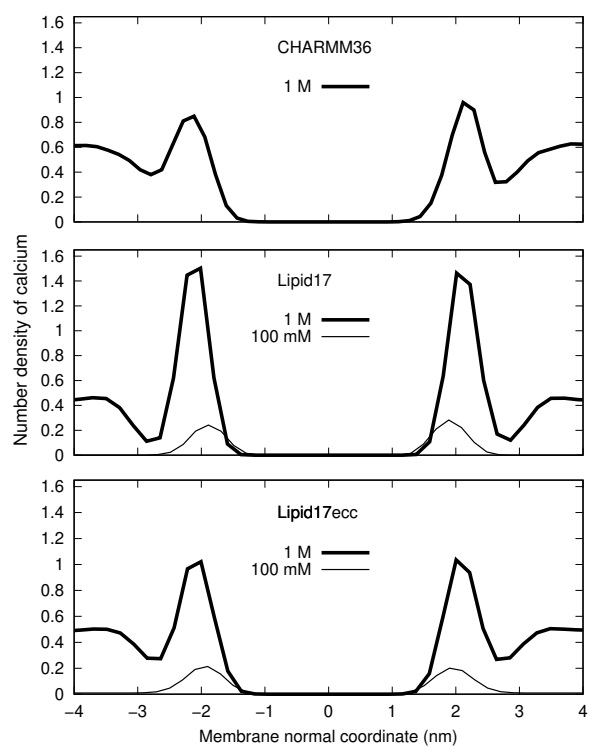


Figure S11: Calcium ion density profiles along membrane normal from simulations of POPC:POPG (4:1) mixtures with different force fields.

S3 Changes in headgroup conformations upon addition of CaCl_2

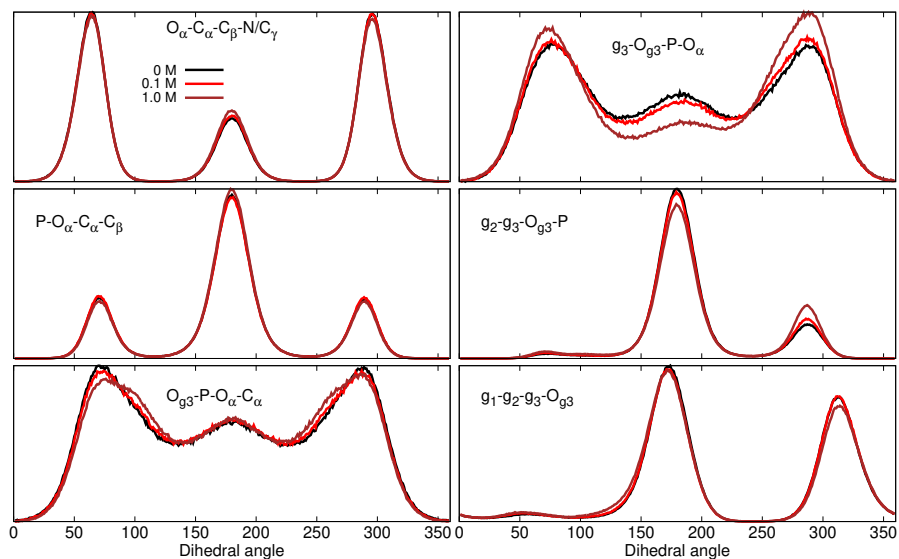


Figure S12: Changes in POPC lipid17ecc dihedrals with increasing amount of CaCl_2 .

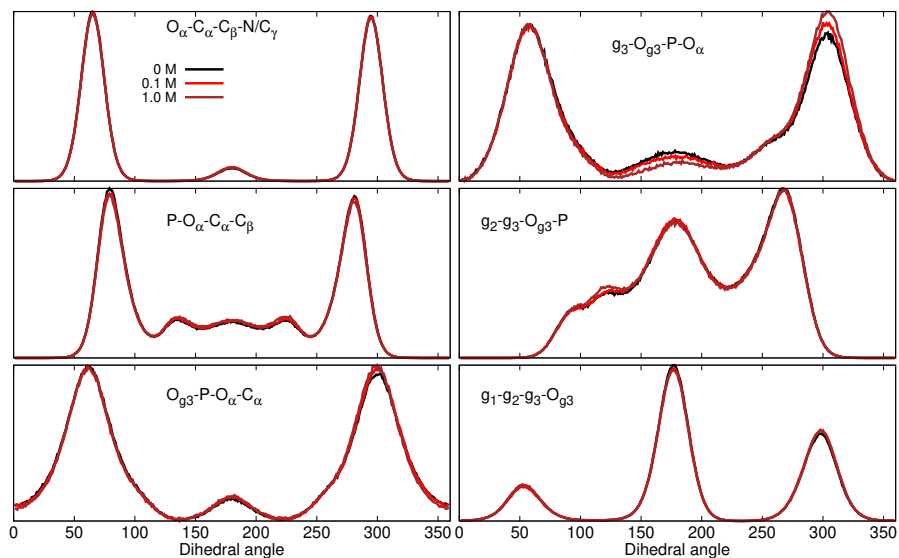


Figure S13: Changes in POPC CHARMM36 dihedrals with increasing amount of CaCl_2 .

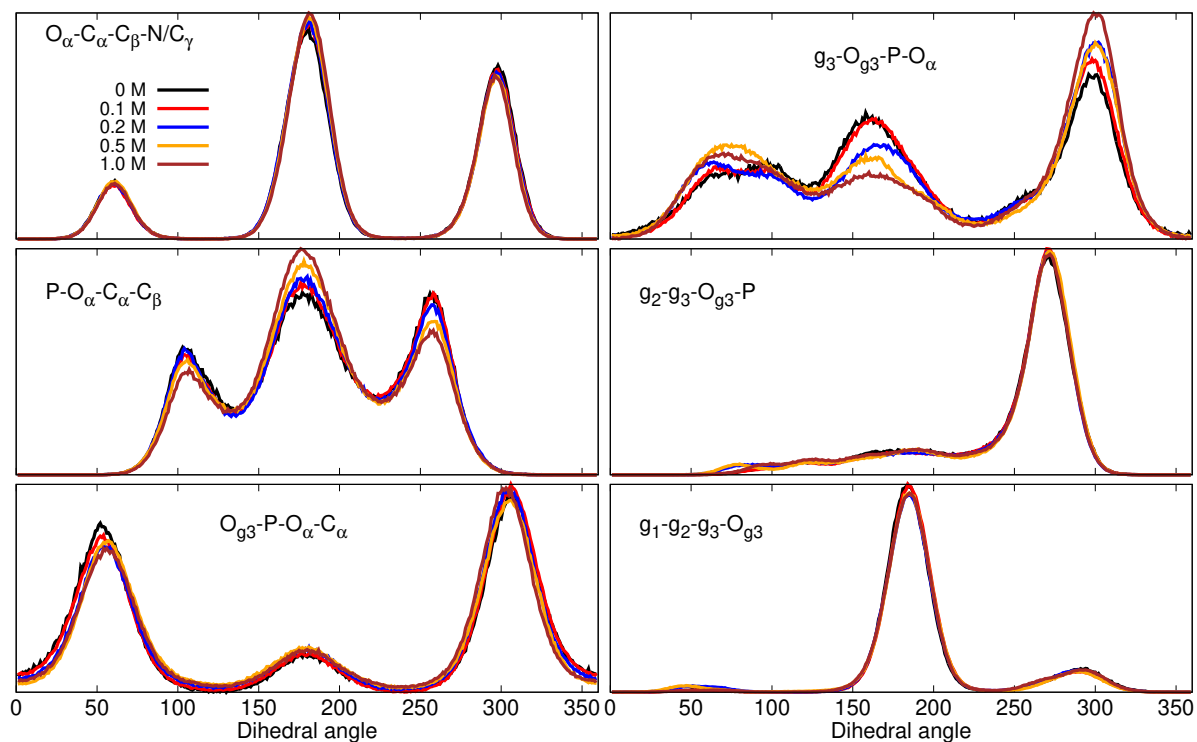


Figure S14: Changes in POPG Slipids dihedrals with increasing amount of CaCl_2 .

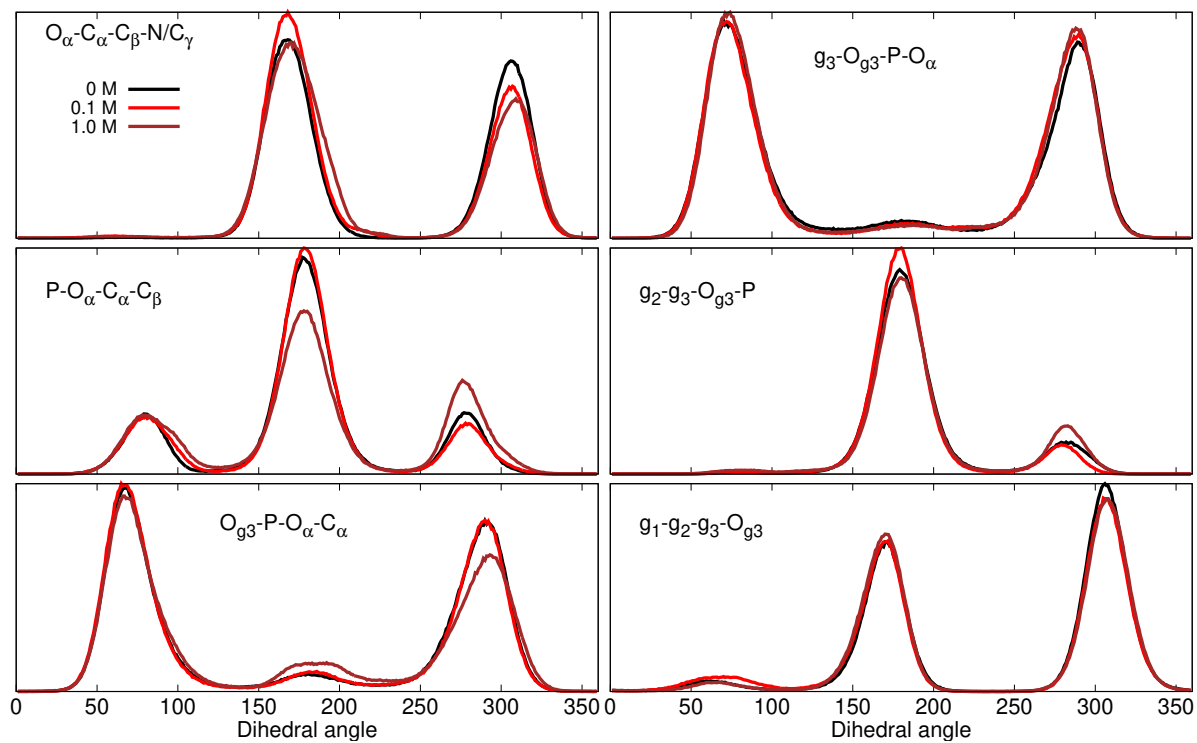


Figure S15: Changes in POPG lipid17 dihedrals with increasing amount of CaCl_2 .

S4 Simulated systems

The simulated systems of pure PE and PG bilayers without additional ions are listed in Tables S1 and S2, and lipid mixtures with additional ions in Tables S3 and S4. the simulation data are indexed in a searchable database available at www.nmrlipids.fi, and in the NMRLipids/MATCH repository (github.com/NMRLipids/MATCH). The large set of MD simulation data was analysed using the development version of NMRLipids databank. Unique naming convention for lipid atoms in each force field was defined using the mapping files and analysis for all simulations indexed in NMRLipids databank manner were performed using Python codes.

The C–H bond order parameters were calculated directly from the carbon and hydrogen positions using the definition

$$S_{\text{CH}} = \frac{1}{2} \langle 3 \cos^2 \theta - 1 \rangle, \quad (1)$$

where θ is the angle between the C–H bond and the membrane normal (taken to align with z , with bilayer periodicity in the xy -plane). Angular brackets denote average over all sampled configurations. The order parameters were first calculated averaging over time separately for each lipid in the system. The average and the standard error of the mean were then calculated over different lipids. Python programs that use the MDAnalysis library^{16,17} used for all atom simulations are available in Ref. 18 (`scripts/calcOrderParameters.py`). For united atom simulations, the trajectories with hydrogens having ideal geometry were constructed first using either `buildH` program¹⁹ or (`scratch/opAAUA_prod.py`) in Ref. 18, and the order parameters were then calculated from these trajectories. This approach has been tested against trajectories with explicit hydrogens and the deviations in order parameters are small.^{19,20}

The number density profiles of ions were calculated using the `gmx density` tool of the GROMACS software package.²¹

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	22
POPE	CHARMM36 [?]	0	500	25000	0	310	500	100	23
POPE	CHARMM36 [?]	0.11	500	25000	50	310	500	100	24
POPE	CHARMM36ua [?]	0	336	15254	0	310	2×200	2×100	25
DPPE	Slipids ²⁶	0	288	9386	0	336	200	100	27
POPE	Slipids ²⁶	0	336	?	0	310	2×200	2×100	28
POPE	Slipids ²⁶	0	500	25000	0	310	500	100	29
POPE	Slipids / Åqvist ^{26,30}	0.11	500	25000	50	310	500	100	31
DPPE	GROMOS-CKP [?]	0	128	3655	0	342	2×500	2×400	32
POPE	GROMOS-CKP [?]	0	128	3552	0	313	2×500	2×400	33
POPE	GROMOS-CKP [?]	0	500	25000	0	310	500	100	34
POPE	GROMOS-CKP [?]	0.11	500	25000	50	310	500	100	35
DOPE	GROMOS-CKP [?]	0	128	4789	0	271	2×500	2×400	36
POPE	GROMOS 43A1-S3 [?]	0	128	3552	0	313	2×200	2×100	37
POPE	OPLS-UA vdW on H [?]	0	128	3328	0	303	2×200	2×100	38
POPE	OPLS-UA [?]	0	128	3328	0	303	2×200	2×100	39
POPE	OPLS-MacRog ⁴⁰	0	144	5760	0	310	500	350	41
POPE	OPLS-MacRog ⁴⁰	0	128	5120	0	300	500	300	42
POPE	Berger-Vries [?]	0	128	3552	0	303	2×200	2×100	43
POPE	Berger-largeH [?]	0	128	3552	0	303	2×200	2×100	44
DOPE	Berger-Vries [?]	0	128	4789	0	271	2×200	2×100	45
DOPE	Berger-largeH [?]	0	128	4789	0	271	2×300	2×100	46
POPE	LIPID17 ⁴⁷	0	500	25000	50	310	500	100	48
POPE	LIPID17 ⁴⁷	0.11	500	25000	50	310	500	100	49

^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

5. Simulations with added NaCl are not currently used here, maybe should be removed from the table?

6. Citation for CHARMM36 PE?

7. Which ion model is used in ²⁴?

8. Citation for GROMOS-CKP?

9. Citation for GROMOS 43A1-S3?

10. Citation for OPLS-UA models?

11. Citations for Berger-* simulations?

12. 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 [?] 13.	0	118	4110	0	298	100	100	50
POPG	CHARMM36 [?]	0.11	500	25000	49	310	500	100	51
POPG	CHARMM36 [?]	0	500	25000	0	310	500	100	52
POPG/Na ⁺	Slipids / Åqvist ^{30,53}	0	288	10664	0	298	250	100	54
DPPG/Na ⁺	Slipids / Åqvist ^{30,53}	0	288	11232	0	314	200	100	55
DPPG/Na ⁺	Slipids / Åqvist ^{30,53}	0	288	11232	0	298	400	100	56
POPG	Slipids / Åqvist ^{30,53}	0	500	25000	0	310	500	100	57
POPG	Slipids / Åqvist ^{30,53}	0.11	500	25000	49	310	500	100	58
POPG	LIPID17 / Dang ^{47,59}	0	500	25000	0	310	500	100	60
POPG	LIPID17 [?]	0.11	500	25000	49	310	500	100	61
POPG	GROMOS-CKP [?]	0	500	25000	0	310	500	100	62
POPG	GROMOS-CKP [?]	0.11	500	25000	49	310	500	100	63

^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

14.Simulations with added NaCl are not currently used here, maybe should be removed from the table?

15.Citations and ion model for CHARMM36?

16.Lipid17 simulation with ions 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	64
POPC:POPG (7:3)	CHARMM36 [?]	0	0	350	25000	0	310	500	100	65
POPC:POPG (1:1)	CHARMM36 [?]	0	0	150:150	31500	0	298	500	400	66
POPC:POPG (1:1)	CHARMM36 [?]	0	0.1	150:150	31329	57	298	400	300	67
POPC:POPG (1:1)	CHARMM36 [?]	0	1.08	150:150	29766	578	298	500	400	68
POPC:POPG (4:1)	CHARMM36 [?]	0	0	350:88	26280	0	298	500	400	69
POPC:POPG (4:1)	CHARMM36 [?]	0	0.1	350:88	26280	47	298	500	400	70
POPC:POPG (4:1)	CHARMM36 [?]	0	1.0	350:88	24927	451	298	500	400	71
POPC	CHARMM36 [?]	0	0	256	8704	0	300	300	250	72
POPC:POPE (1:1)	CHARMM36 [?]	0	0	128	8704	0	300	300	250	73
POPC	OPLS-MacRog ⁴⁰	0	0	128	5120	0	300	500	300	74
POPC:POPE (1:1)	OPLS-MacRog ⁴⁰	0	0	128	5120	0	300	500	300	75
POPC	Slipid ²⁶	0	0	512	23943	0	298	170	100	76
POPC:POPE (1:1)	Slipid ²⁶	0	0	128	5120	0	298	500	300	77
POPC	GROMOS-CKP / ?? [?] ?	0	0	500	25000	0	310	500	100	78
POPC:POPG (7:3)	GROMOS-CKP / ?? [?] ?	0	0	350:150	25000	0	310	500	100	79
POPC	Slipid ²⁶	0	0	500	25000	0	310	500	100	80
POPC:POPG (7:3)	Slipid / Åqvist ^{26,30}	0	0	350:150	25000	0	310	500	100	81
POPC:POPG (1:1)	Slipid / Dang ^{26,59,82,83}	0	0	128:128	12800	0	298	500	400	84
POPC:POPG (1:1)	Slipid / Dang ^{26,59,82,83}	0	0.1	128:128	12800	23	298	500	400	84
POPC:POPG (1:1)	Slipid / Dang ^{26,59,82,83}	0	0.2	128:128	12800	46	298	1500	500	84
POPC:POPG (1:1)	Slipid / Dang ^{26,59,82,83}	0	0.5	128:128	12800	115	298	1500	500	84
POPC:POPG (1:1)	Slipid / Dang ^{26,59,82,83}	0	1.0	128:128	12800	230	298	1500	500	84

^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?

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 ^{47,59,83}	0	0	350:88	26265	0	298	400	350	85
POPC:POPG (4:1)	Lipid17 / Dang ^{47,59,83}	0	0.1	350:88	26124	47	298	400	250	86
POPC:POPG (4:1)	Lipid17 / Dang ^{47,59,83}	0	1.0	350:88	24840	475	298	1200	200	87
POPC:POPG (1:1)	Lipid17 / Dang ^{47,59,83}	0	0	150:150	31572	0	298	320	200	88
POPC:POPG (1:1)	Lipid17 / Dang ^{47,59,83}	0	0.1	150:150	31401	57	298	718	198	89
POPC:POPG (1:1)	Lipid17 / Dang ^{47,59,83}	0	1.0	150:150	29865	569	298	720	200	90
POPC:POPG (4:1)	Lipid17ecc / ECC-ions ⁹¹⁻⁹³	0	0	350:88	26265	0	298	400	300	94
POPC:POPG (4:1)	Lipid17ecc / ECC-ions ⁹¹⁻⁹³	0	0.1	350:88	26124	47	298	400	300	95
POPC:POPG (4:1)	Lipid17ecc / ECC-ions ⁹¹⁻⁹³	0	1.0	350:88	24840	475	298	400	300	96
POPC:POPG (1:1)	Lipid17ecc / ECC-ions ⁹¹⁻⁹³	0	0	150:150	31572	0	298	347.8	333	97
POPC:POPG (1:1)	Lipid17ecc / ECC-ions ⁹¹⁻⁹³	0	0.1	150:150	29865	54	298	400	300	98
POPC:POPG (1:1)	Lipid17ecc / ECC-ions ⁹¹⁻⁹³	0	1.0	150:150	29865	569	298	600	400	99
POPC	Berger [?] 19.	0	0	256	10240	0	300	300	200	100
POPC:POPE (1:1)	Berger [?] 20.	0	0	128	11008	0	300	300	200	101
POPC:DOPE (1:1)	Berger [?] 21.	0	0	128	10240	0	300	300	200	102
DOPC	Berger [?] 22.	0	0	256	11008	0	300	300	200	103
DOPC:DOPE (1:1)	Berger [?] 23.	0	0	128	11008	0	300	300	200	104

^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

24. Citation and description for "Berger" model?

S4.1 CHARMM36

POPE ²⁵.Simulation details by M. Javanainen.

POPE with additional NaCl ²⁶.Simulation details by A. Peon.

POPG ²⁷.Simulation details by Ollila.

POPG with additional NaCl ²⁸.Simulation details by A. Peon.

POPC:POPE mixtures Data is available at.^{72,73} 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 ²⁹.Full simulation details by Fuchs et al.

POPC:POPG mixture with additional calcium ³⁰.Simulation details by A. Kiirikki.

POPC and POPC:POPG (7:3) mixture ³¹.Simulation details by A. Peon.

S4.2 CHARMM36ua

POPE Data is available at.²⁵ ³².Simulation details by T. Piggot.

S4.3 Slipids

POPE Data is available at.²⁸ ³³.Simulation details by T. Piggot.

POPE with additional NaCl ³⁴.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.²⁷ ³⁵.Simulation details by F. Favela.

POPG Data is available at.⁵⁴ ³⁶.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 ³⁷.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 at⁵⁶ and in 314 K at.⁵⁵ ³⁸.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 [39.Simulation details by A. Peon.](#) I have assumed that ion parameters are default Slipids, i.e., Åqvist, please correct if this is not true.

POPC:POPG mixture with additional CaCl [40.Simulation details by M. Javanainen.](#)

S4.4 Berger

POPE Data is available at. ^{43,44} [41.Simulation details by T. Piggot.](#)

DOPE Data is available at. ^{45,46} [42.Simulation details by T. Piggot.](#)

POPC:POPE, POPC:DOPE and DOPC:DOPE mixtures Data is available at. ^{100,101} 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 [43.Simulation details by Fuchs et al.](#)

S4.5 GROMOS 43A1-S3

POPE Data is available at. ³⁷ [44.Simulation details by T. Piggot.](#)

S4.6 OPLS-UA

POPE Data is available at. ³⁹ [45.Simulation details by T. Piggot.](#)

POPE with vdW interaction in H Data is available at. ³⁸ [46.Simulation details by T. Piggot.](#)

S4.7 GROMOS-CKP and GROMOS-CKPM

POPE Data is available at. ³³ [47.Simulation details by T. Piggot.](#)

DOPE Data is available at. ³⁶ [48.Simulation details by T. Piggot.](#)

DPPE Data is available at. ³² [49.Simulation details by T. Piggot.](#)

POPG [50.Simulation details by A. Peon.](#)

POPC:POPG mixture [51.Simulation details by A. Peon.](#)

S4.8 OPLS-MacRog

POPE 52.Simulation details by M. Javanainen and P. Fuchs.

POPC:POPE mixtures 53.Simulation details by P. Fuchs.

S4.9 Lipid17

POPE 54.Simulation details by A. Peon.

POPG 55.Simulation details by A. Peon.

POPC:POPG 56.Simulation details by S. Virtanen or O. H. S. Ollila.

S4.10 Lipid17ecc

57.This is to be finished and POPC:POPG mixtures to be described In ECC-lipid models, electronic continuum correction (ECC) is applied to implicitly include the missing electronic polarizability into the force field description.^{13?} 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:[?] ECC-POPC parameters (scaling factors $f_q=0.8$ and $f_\sigma=0.89$ applied to Lipid14 POPC parameters)¹³ 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.⁶⁰ with the correct dihedral type, and the resulting parameters are available from Ref. ? . ECC-ion parameters with the scaled charges,⁹¹⁻⁹³ downloaded from bitbucket.org/hseara/ions/src/master/, were used in these simulations.

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