

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 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 S3: Simpson simlaton 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.

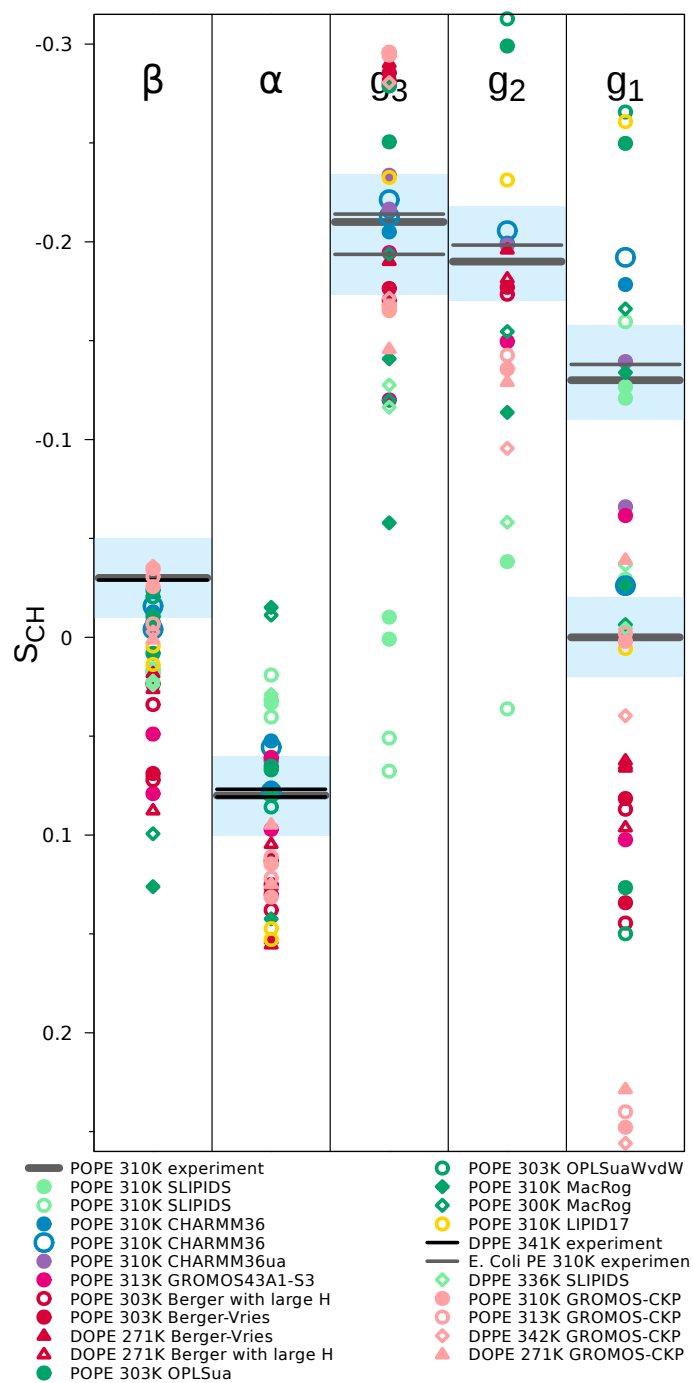


Figure S4: The headgroup and glycerol backbone order parameters of PE lipids from experiments (POPE and signs this work, DPPE from Ref. 3 and E.coliPE from Ref. 4) and simulations with different force fields.

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.

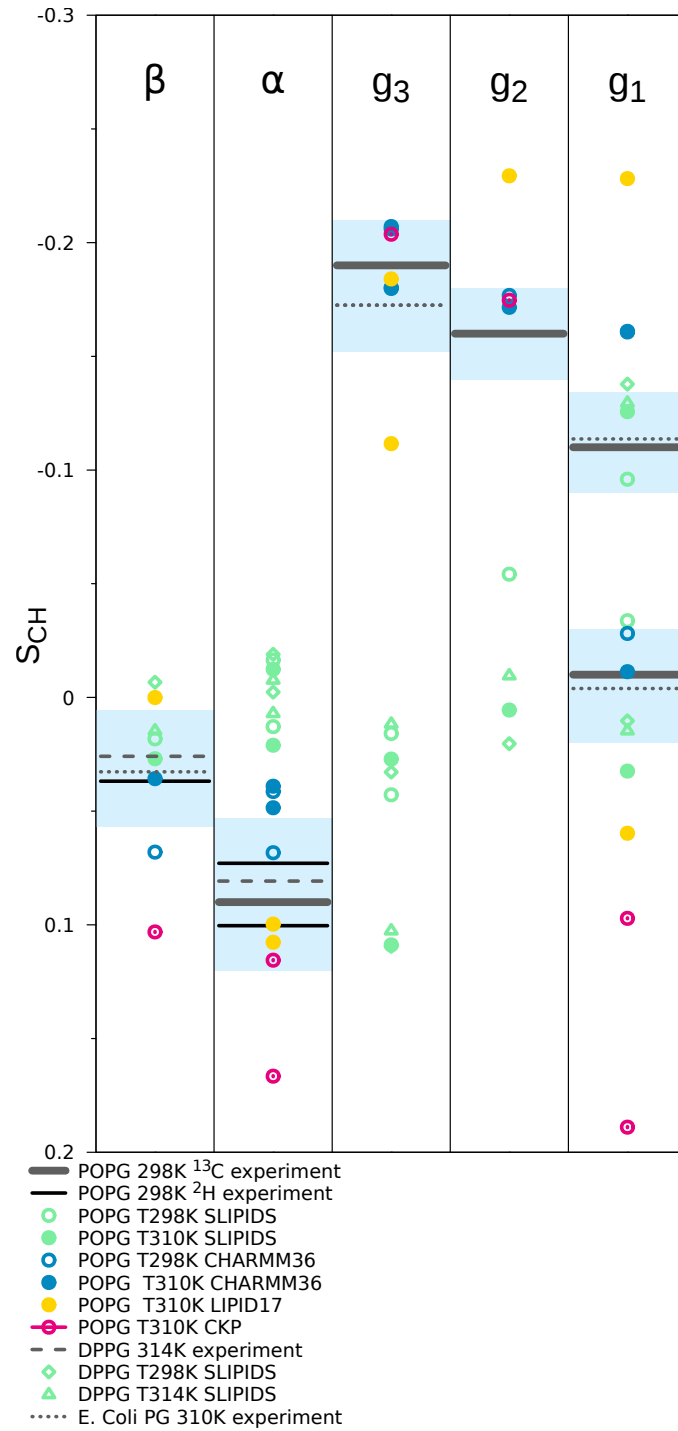


Figure S5: The headgroup and glycerol backbone order parameters of PG lipids from experiments (POPG and signs from this work and from Ref. 5, DPPG with 100mM NaCl from Ref. 6, and E. Coli PG results from Ref. 4). and simulations with different force fields.

S3 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

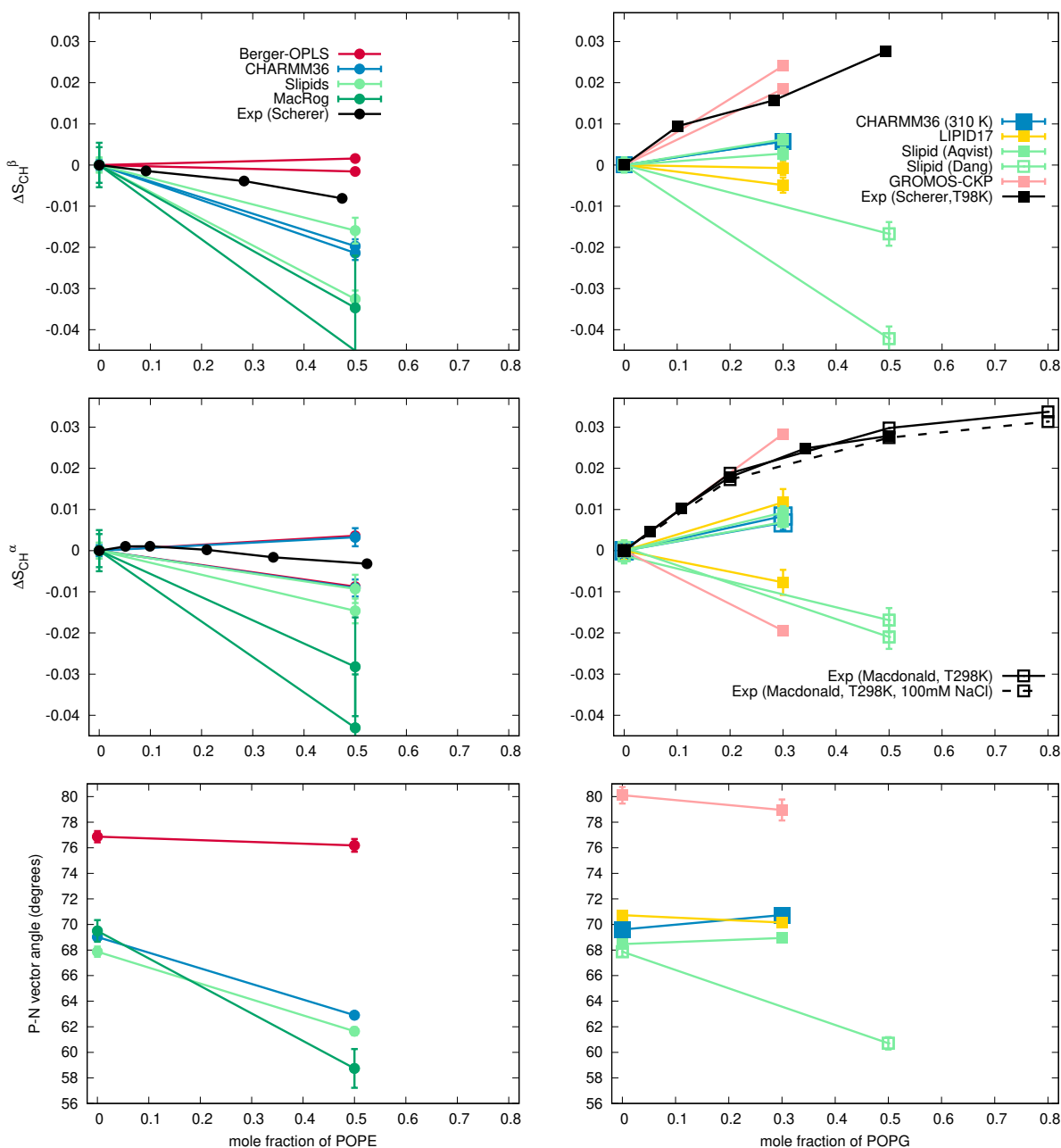


Figure S6: Modulation of POPC headgroup order parameters with increasing amount of POPE (left) and POPG (right) in bilayer from experiments^{7,8} and simulations with different force fields. Signs are determined as discussed in.^{1,9}

S4 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 unchanged⁵ 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.

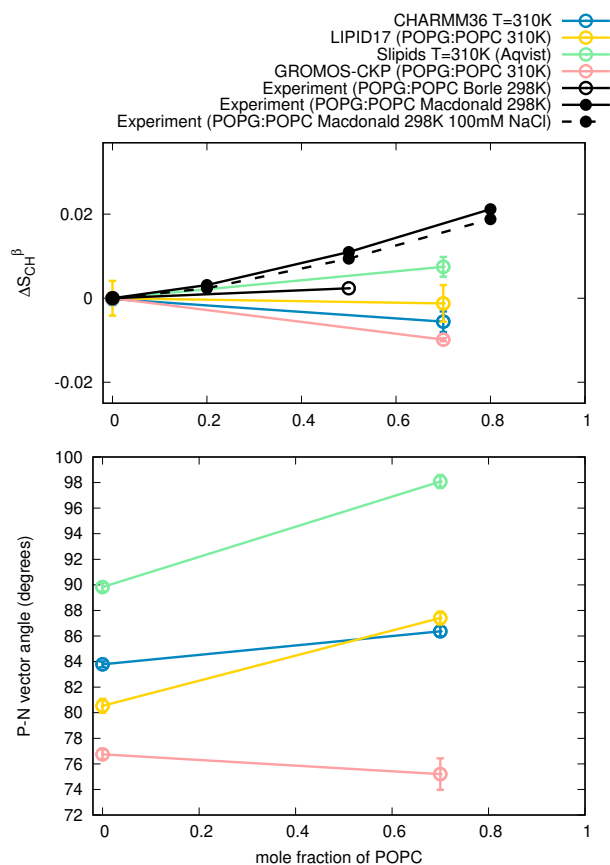


Figure S7: Modulation of PG lipid headgroup order parameters with the increasing amount of PC in lipid bilayer from experiments^{5,8} and simulations with different force fields.

S5 Calcium binding to POPC:POPG mixtures

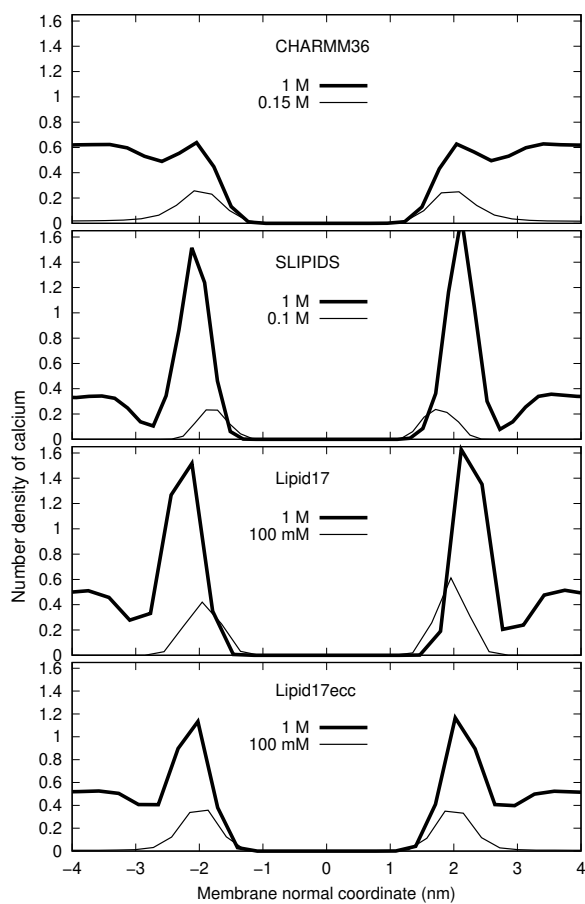
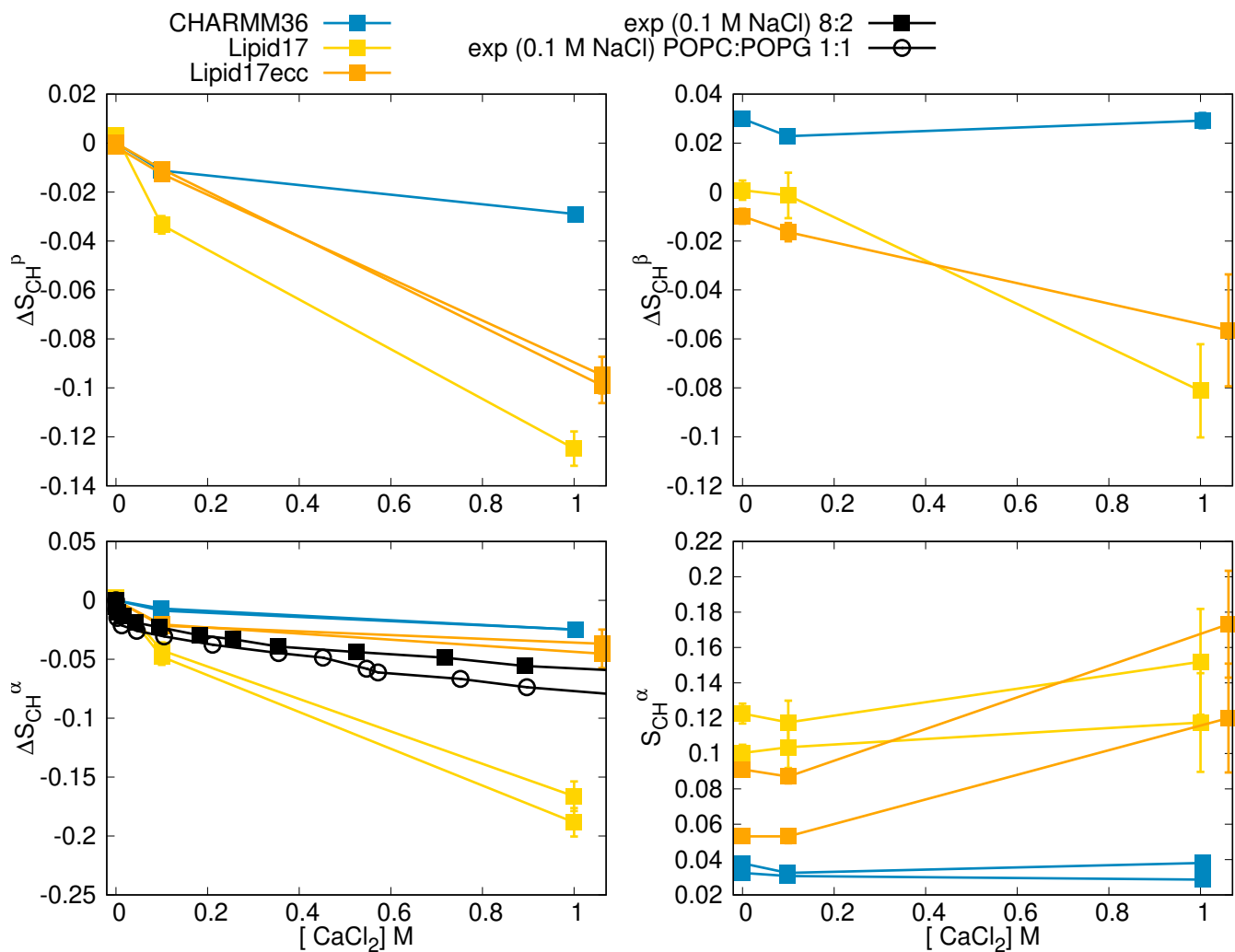


Figure S8: Calcium ion density profiles along membrane normal from simulations of POPC:POPG (1:1) mixtures with different force fields. The changes in the order parameters upon addition of CaCl₂ are compared with experiments in figure 10 in the main text.



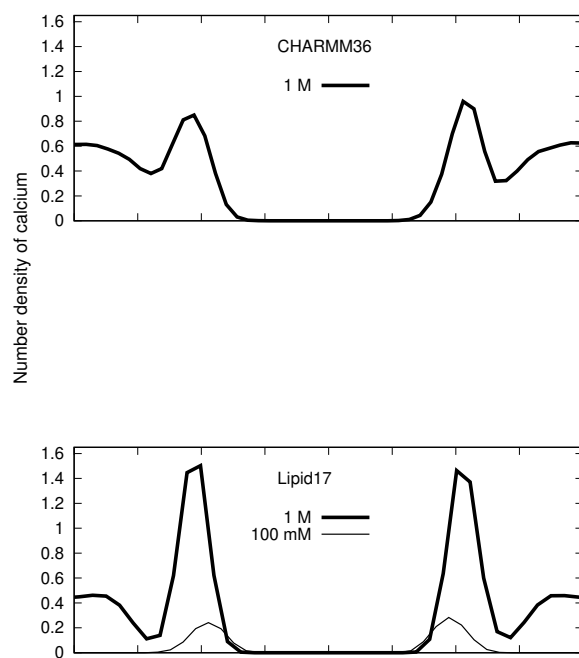


Figure S10: Calcium ion density profiles along membrane normal from simulations of POPC:POPG (4:1) mixtures with different force fields.

S6 Changes in headgroup conformations upon addition of CaCl_2

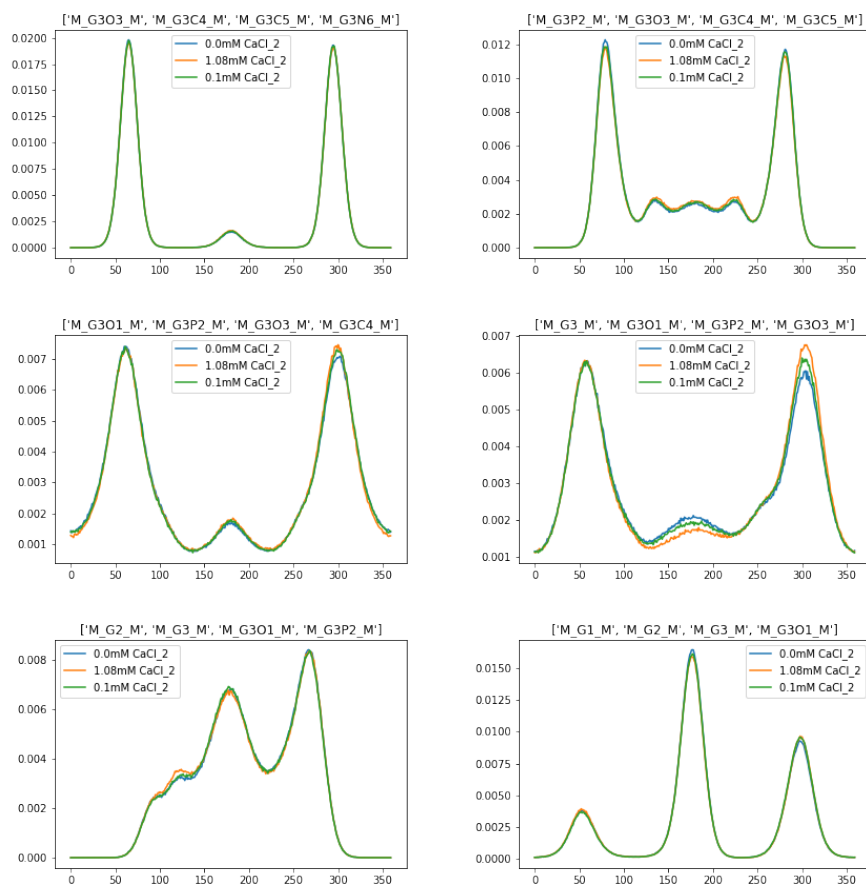


Figure S11: Changes in POPC CHARMM36 dihedrals with increasing amount of CaCl_2 .

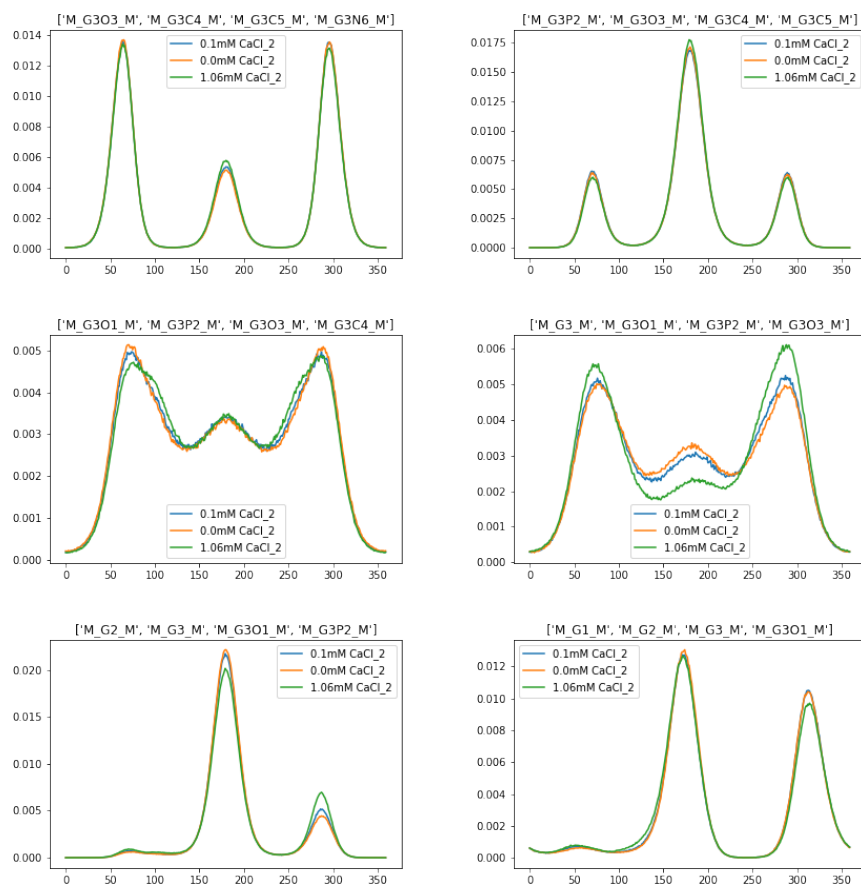


Figure S12: Changes in POPC lipid17ecc dihedrals with increasing amount of CaCl_2 .

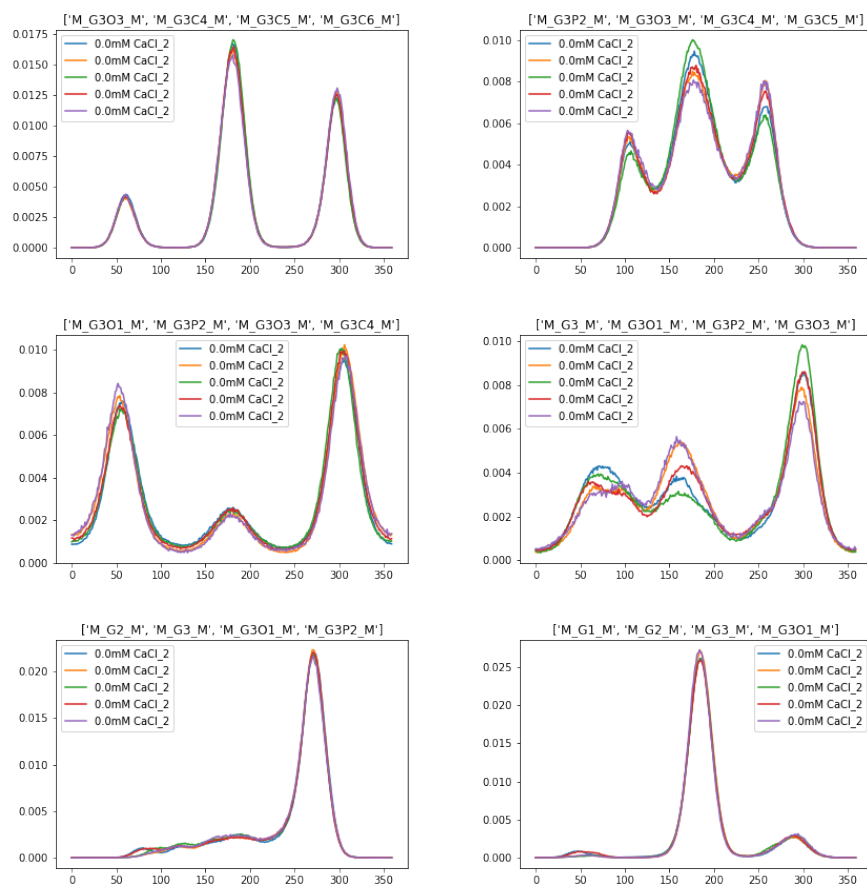


Figure S13: Changes in POPG lipids dihedrals with increasing amount of CaCl₂.

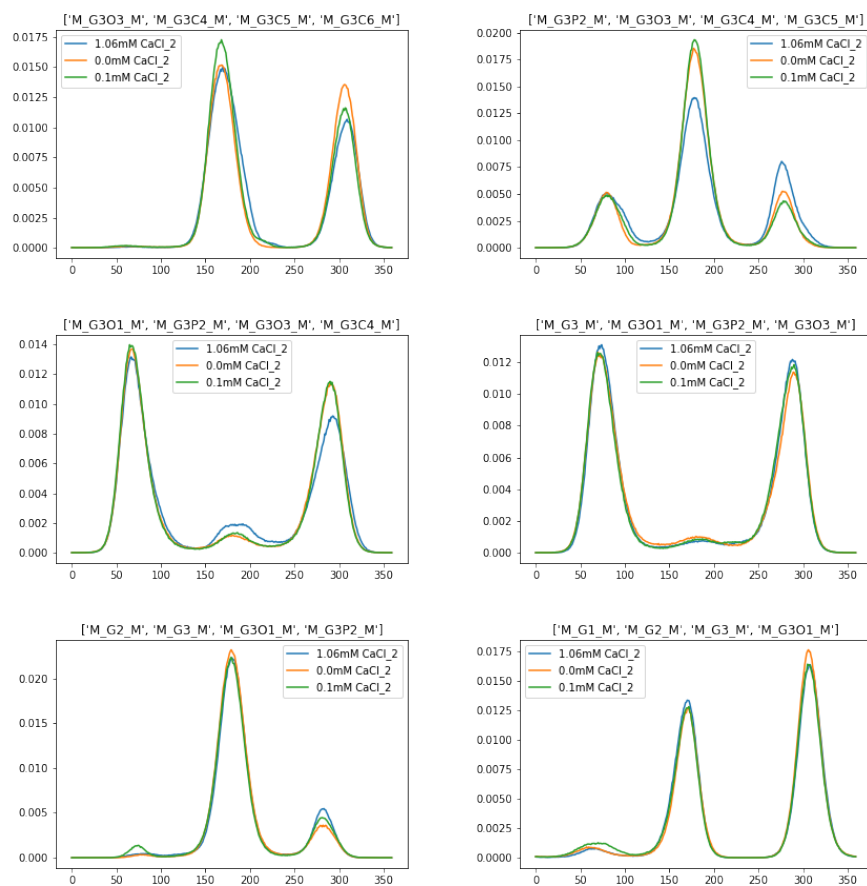


Figure S14: Changes in POPG lipid17 dihedrals with increasing amount of CaCl₂.

S7 Simulated systems

S7.1 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.^{62,63} 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 [29.Full simulation details by Fuchs et al.](#)

POPC:POPG mixture with additional calcium [30.Simulation details by J. Madsen.](#)

POPC:POPG mixture with additional NaCl [31.Simulation details by A. Peon.](#)

S7.2 CHARMM36ua

POPE Data is available at.¹⁵ [32.Simulation details by T. Piggot.](#)

S7.3 Slipids

POPE Data is available at.¹⁸ [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.¹⁷ [35.Simulation details by F. Favela.](#)

POPG Data is available at.⁴⁴ [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 at⁴⁶ and in 314 K at.⁴⁵ [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.

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	12
POPE	CHARMM36 [?]	0	500	25000	0	310	500	100	13
POPE	CHARMM36 [?]	0.11	500	25000	50	310	500	100	14
POPE	CHARMM36ua [?]	0	336	15254	0	310	2×200	2×100	15
DPPE	Slipids ¹⁶	0	288	9386	0	336	200	100	17
POPE	Slipids ¹⁶	0	336	?	0	310	2×200	2×100	18
POPE	Slipids ¹⁶	0	500	25000	0	310	500	100	19
POPE	Slipids / Åqvist ^{16,20}	0.11	500	25000	50	310	500	100	21
DPPE	GROMOS-CKP [?]	0	128	3655	0	342	2×500	2×400	22
POPE	GROMOS-CKP [?]	0	128	3552	0	313	2×500	2×400	23
POPE	GROMOS-CKP [?]	0	500	25000	0	310	500	100	24
POPE	GROMOS-CKP [?]	0.11	500	25000	50	310	500	100	25
DOPE	GROMOS-CKP [?]	0	128	4789	0	271	2×500	2×400	26
POPE	GROMOS 43A1-S3 [?]	0	128	3552	0	313	2×200	2×100	27
POPE	OPLS-UA vdW on H [?]	0	128	3328	0	303	2×200	2×100	28
POPE	OPLS-UA [?]	0	128	3328	0	303	2×200	2×100	29
POPE	OPLS-MacRog ³⁰	0	144	5760	0	310	500	350	31
POPE	OPLS-MacRog ³⁰	0	128	5120	0	300	500	300	32
POPE	Berger-Vries [?]	0	128	3552	0	303	2×200	2×100	33
POPE	Berger-largeH [?]	0	128	3552	0	303	2×200	2×100	34
DOPE	Berger-Vries [?]	0	128	4789	0	271	2×200	2×100	35
DOPE	Berger-largeH [?]	0	128	4789	0	271	2×300	2×100	36
POPE	LIPID17 ³⁷	0	500	25000	50	310	500	100	38
POPE	LIPID17 ³⁷	0.11	500	25000	50	310	500	100	39

^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

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

4. Citation for CHARMM36 PE?

5. Which ion model is used in ¹⁴?

6. Citation for GROMOS-CKP?

7. Citation for GROMOS 43A1-S3?

8. Citation for OPLS-UA models?

9. Citations for Berger-* simulations?

10. 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 [?] 11.	0	118	4110	0	298	100	100	40
POPG	CHARMM36 [?]	0.11	500	25000	49	310	500	100	41
POPG	CHARMM36 [?]	0	500	25000	0	310	500	100	42
POPG/Na ⁺	Slipids / Åqvist ^{20,43}	0	288	10664	0	298	250	100	44
DPPG/Na ⁺	Slipids / Åqvist ^{20,43}	0	288	11232	0	314	200	100	45
DPPG/Na ⁺	Slipids / Åqvist ^{20,43}	0	288	11232	0	298	400	100	46
POPG	Slipids / Åqvist ^{20,43}	0	500	25000	0	310	500	100	47
POPG	Slipids / Åqvist ^{20,43}	0.11	500	25000	49	310	500	100	48
POPG	LIPID17 / Dang ^{37,49}	0	500	25000	0	310	500	100	50
POPG	LIPID17 [?]	0.11	500	25000	49	310	500	100	51
POPG	GROMOS-CKP [?]	0	500	25000	0	310	500	100	52
POPG	GROMOS-CKP [?]	0.11	500	25000	49	310	500	100	53

^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

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

13.Citations and ion model for CHARMM36?

14.Lipid17 simulation with ions with correct dihedral potentials still coming?

15.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	54
POPC:POPG (7:3)	CHARMM36 [?]	0	0	350	25000	0	310	500	100	55
POPC:POPG (1:1)	CHARMM36 [?]	0	0	150:150	31500	0	298	500	400	56
POPC:POPG (1:1)	CHARMM36 [?]	0	0.1	150:150	31329	57	298	400	300	57
POPC:POPG (1:1)	CHARMM36 [?]	0	1.08	150:150	29766	578	298	500	400	58
POPC:POPG (4:1)	CHARMM36 [?]	0	0	350:88	26280	0	298	500	400	59
POPC:POPG (4:1)	CHARMM36 [?]	0	0.1	350:88	26280	47	298	500	400	60
POPC:POPG (4:1)	CHARMM36 [?]	0	1.0	350:88	24927	451	298	500	400	61
POPC	CHARMM36 [?]	0	0	256	8704	0	300	300	250	62
POPC:POPE (1:1)	CHARMM36 [?]	0	0	128	8704	0	300	300	250	63
POPC	OPLS-MacRog ³⁰	0	0	128	5120	0	300	500	300	64
POPC:POPE (1:1)	OPLS-MacRog ³⁰	0	0	128	5120	0	300	500	300	65
POPC	Slipid ¹⁶	0	0	512	23943	0	298	170	100	66
POPC:POPE (1:1)	Slipid ¹⁶	0	0	128	5120	0	298	500	300	67
POPC	GROMOS-CKP / ?? [?] ?	0	0	500	25000	0	310	500	100	68
POPC:POPG (7:3)	GROMOS-CKP / ?? [?] ?	0	0	350:150	25000	0	310	500	100	69
POPC	Slipid ¹⁶	0	0	500	25000	0	310	500	100	70
POPC:POPG (7:3)	Slipid / Åqvist ^{16,20}	0	0	350:150	25000	0	310	500	100	71
POPC:POPG (1:1)	Slipid / Dang ^{16,49,72,73}	0	0	128:128	12800	0	298	500	400	74
POPC:POPG (1:1)	Slipid / Dang ^{16,49,72,73}	0	0.1	128:128	12800	23	298	500	400	74
POPC:POPG (1:1)	Slipid / Dang ^{16,49,72,73}	0	0.2	128:128	12800	46	298	1500	500	74
POPC:POPG (1:1)	Slipid / Dang ^{16,49,72,73}	0	0.5	128:128	12800	115	298	1500	500	74
POPC:POPG (1:1)	Slipid / Dang ^{16,49,72,73}	0	1.0	128:128	12800	230	298	1500	500	74

^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

16. Citation and ion model for GROMOS-CKP?

17. 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 ^{37,49,73}	0	0	350:88	26265	0	298	400	350	75
POPC:POPG (4:1)	Lipid17 / Dang ^{37,49,73}	0	0.1	350:88	26124	47	298	400	250	76
POPC:POPG (4:1)	Lipid17 / Dang ^{37,49,73}	0	1.0	350:88	24840	475	298	1200	200	77
POPC:POPG (1:1)	Lipid17 / Dang ^{37,49,73}	0	0	150:150	31572	0	298	320	200	78
POPC:POPG (1:1)	Lipid17 / Dang ^{37,49,73}	0	0.1	150:150	31401	57	298	718	198	79
POPC:POPG (1:1)	Lipid17 / Dang ^{37,49,73}	0	1.0	150:150	29865	569	298	720	200	80
POPC:POPG (4:1)	Lipid17ecc / ECC-ions ⁸¹⁻⁸³	0	0	350:88	26265	0	298	400	300	84
POPC:POPG (4:1)	Lipid17ecc / ECC-ions ⁸¹⁻⁸³	0	0.1	350:88	26124	47	298	400	300	85
POPC:POPG (4:1)	Lipid17ecc / ECC-ions ⁸¹⁻⁸³	0	1.0	350:88	24840	475	298	400	300	86
POPC:POPG (1:1)	Lipid17ecc / ECC-ions ⁸¹⁻⁸³	0	0	150:150	31572	0	298	347.8	333	87
POPC:POPG (1:1)	Lipid17ecc / ECC-ions ⁸¹⁻⁸³	0	0.1	150:150	29865	54	298	400	300	88
POPC:POPG (1:1)	Lipid17ecc / ECC-ions ⁸¹⁻⁸³	0	1.0	150:150	29865	569	298	600	400	89
POPC	Berger [?] 18.	0	0	256	10240	0	300	300	200	90
POPC:POPE (1:1)	Berger [?] 19.	0	0	128	11008	0	300	300	200	91
POPC:DOPE (1:1)	Berger [?] 20.	0	0	128	10240	0	300	300	200	92
DOPC	Berger [?] 21.	0	0	256	11008	0	300	300	200	93
DOPC:DOPE (1:1)	Berger [?] 22.	0	0	128	11008	0	300	300	200	94

^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

23. Citation and description for "Berger" model?

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

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.

S7.4 Berger

POPE Data is available at. ^{33,34} [40.Simulation details by T. Piggot.](#)

DOPE Data is available at. ^{35,36} [41.Simulation details by T. Piggot.](#)

POPC:POPE, POPC:DOPE and DOPC:DOPE mixtures Data is available at. ^{90,91} 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 [42.Simulation details by Fuchs et al.](#)

S7.5 GROMOS 43A1-S3

POPE Data is available at. ²⁷ [43.Simulation details by T. Piggot.](#)

S7.6 OPLS-UA

POPE Data is available at. ²⁹ [44.Simulation details by T. Piggot.](#)

POPE with vdW interaction in H Data is available at. ²⁸ [45.Simulation details by T. Piggot.](#)

S7.7 GROMOS-CKP and GROMOS-CKPM

POPE Data is available at. ²³ [46.Simulation details by T. Piggot.](#)

DOPE Data is available at. ²⁶ [47.Simulation details by T. Piggot.](#)

DPPE Data is available at. ²² [48.Simulation details by T. Piggot.](#)

S7.8 Lipid17

S7.9 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.^{95,96} 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:⁹⁶ ECC-POPC parameters (scaling factors $f_q=0.8$ and $f_\sigma=0.89$ applied to Lipid14 POPC parameters)⁹⁵ 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.⁵⁰ with the correct dihedral type, and the resulting parameters are available from Ref. ? . ECC-ion parameters with the scaled charges,⁸¹⁻⁸³ downloaded from bitbucket.org/hseara/ions/src/master/, were used in these simulations.

References

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