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

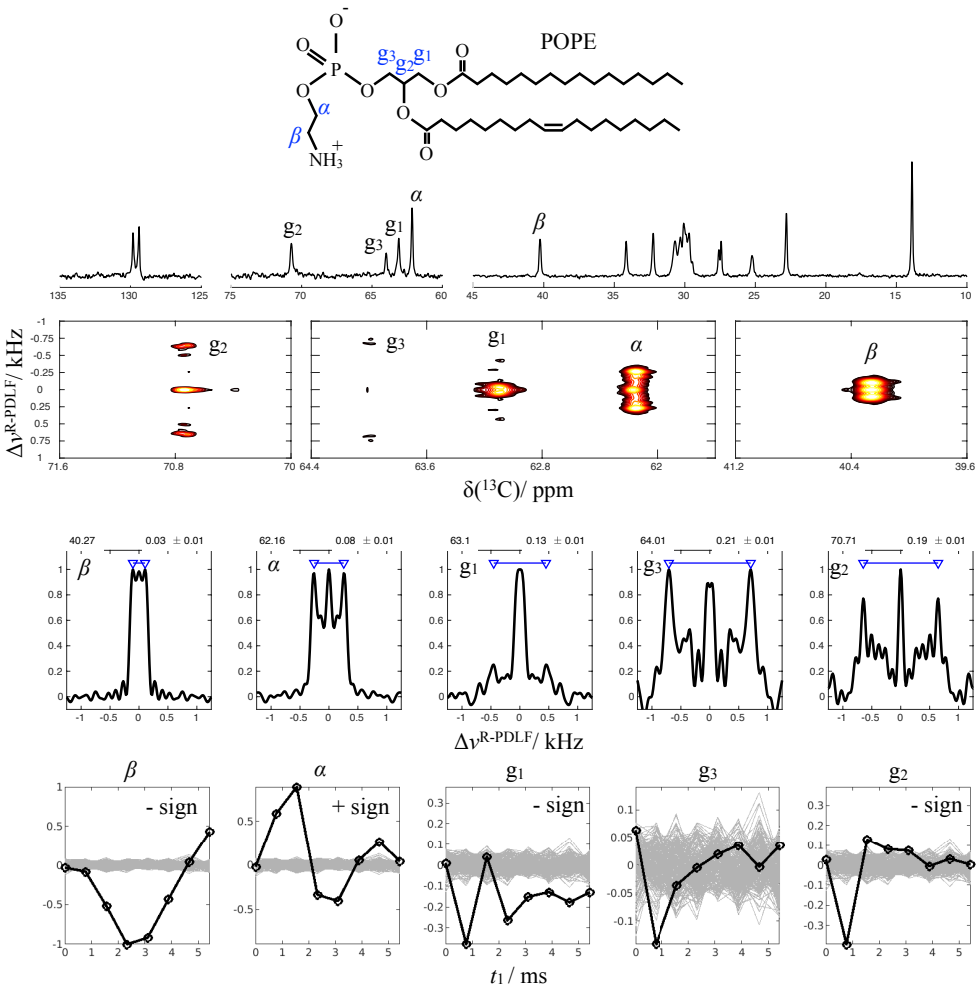

Figure S1: (A) Chemical structure of POPE with the labeling of headgroup and glycerol backbone carbons. (B) INEPT spectra from POPE sample with the headgroup and glycerol backbone peaks labeled. (C) 2D R-PDLF spectra (D) Dipolar sliced from the 2D R-PDLF spectra with the resulting order parameters on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

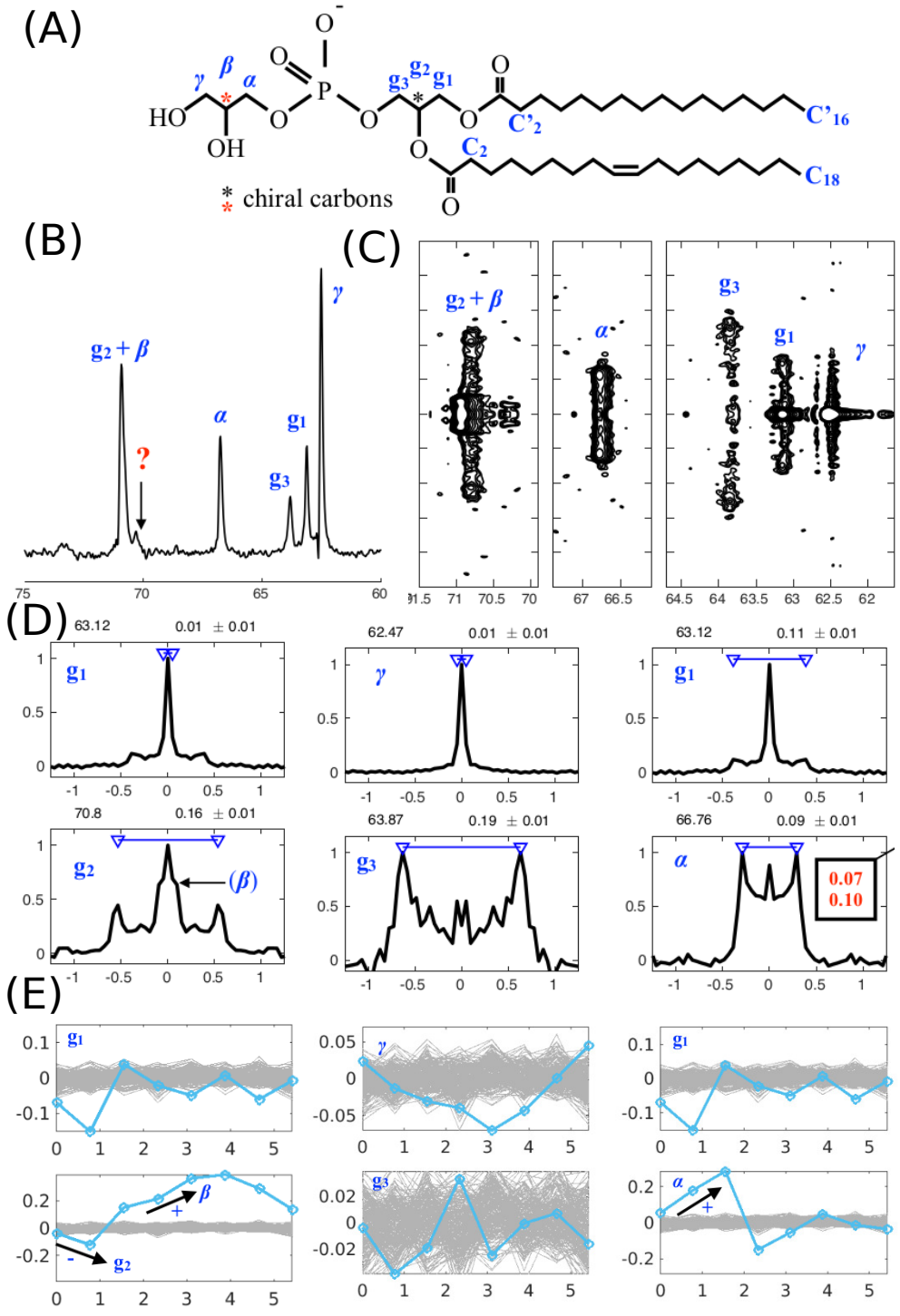

Figure S2: (A) Chemical structure of POPG with the labeling of headgroup and glycerol backbone carbons. (B) INEPT spectra from POPG sample with the headgroup and glycerol backbone peaks labeled. (C) 2D R-PDLF spectra (D) Dipolar sliced from the 2D R-PDLF spectra with the resulting order parameters on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

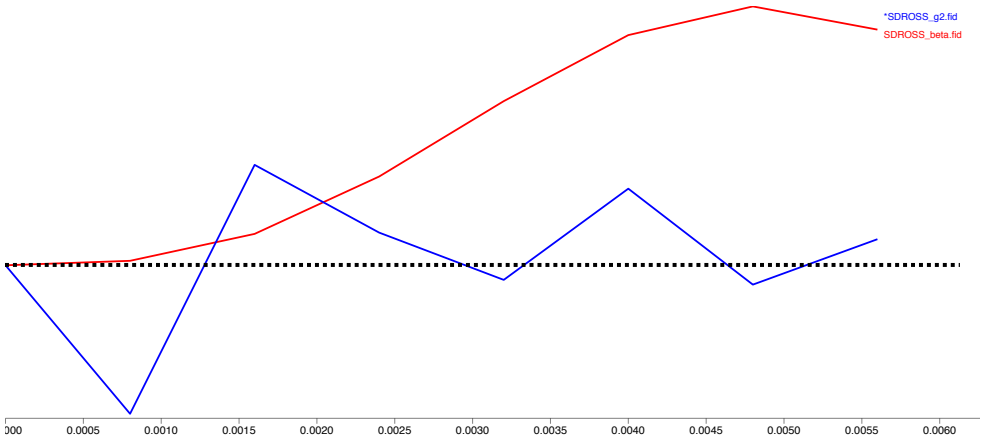

Figure S3: Simpson simulaton of S-DROSS curve of β-carbon of POPG.

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.

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

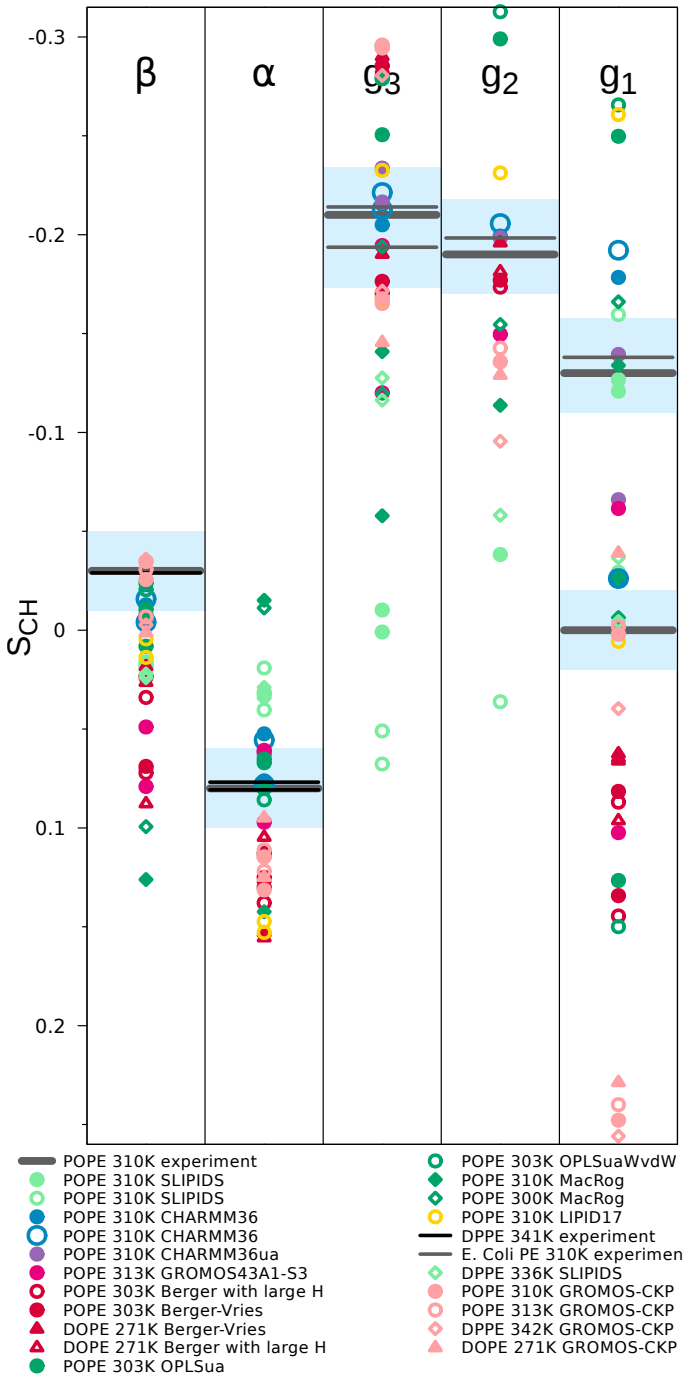

Figure S4: The headgroup and glycerol backbone order parameters of PE lipids from experiments (POPE and signs this work, DPPE from Ref. 4 and E.coliPE from Ref. 5) and simulations with different force fields.

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

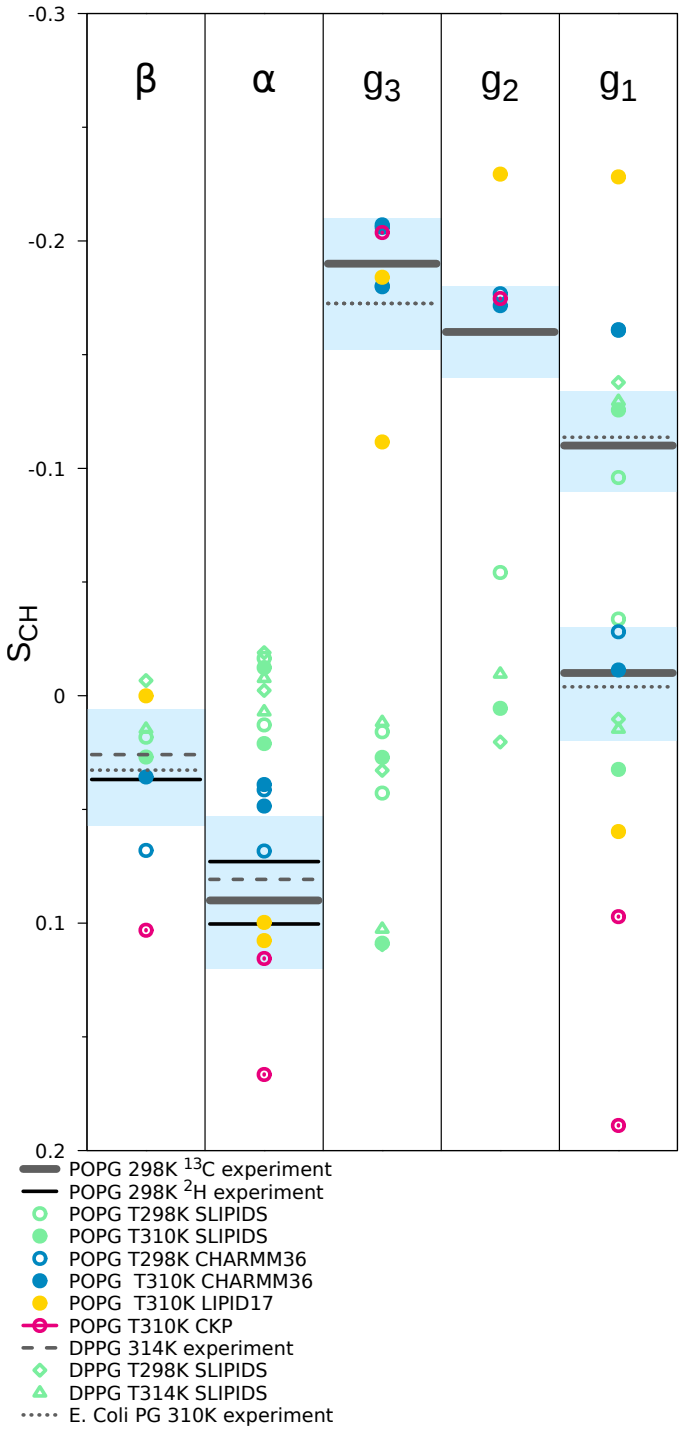

Figure S5: The headgroup and glycerol backbone order parameters of PG lipids from experiments (POPG and signs from this work and from Ref. 6, DPPG with 100mM NaCl from Ref. 3,and E.Coli PG results from Ref. 5) and simulations with different force fields.

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

https://github.com/NMRLipids/NMRlipidsIVPEandPG/issues/12#issuecomment-756641407

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 neturalizes 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 enemble 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]

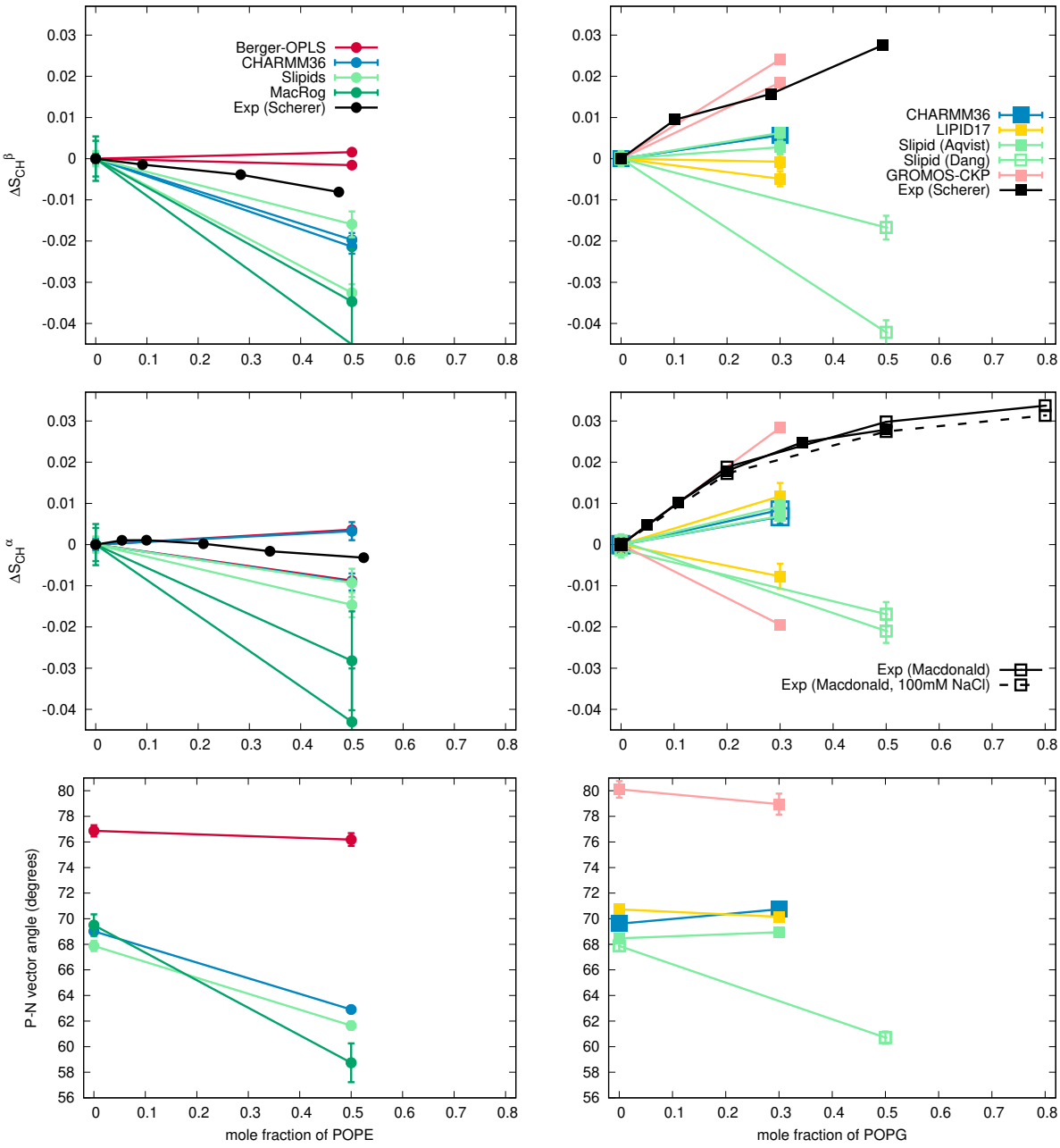

Figure S6: Modulation of POPC headgroup order parameters with increasing amount of POPE (left) and POPG (right) in bilayer from experiments at 298 K[7,8] and simulations with different force fields (temperatures listed in tables S3 and S4 are between 298-310 K). Signs are determined as discussed in Refs. 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 mildly[8] or is unchanged[6] 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.

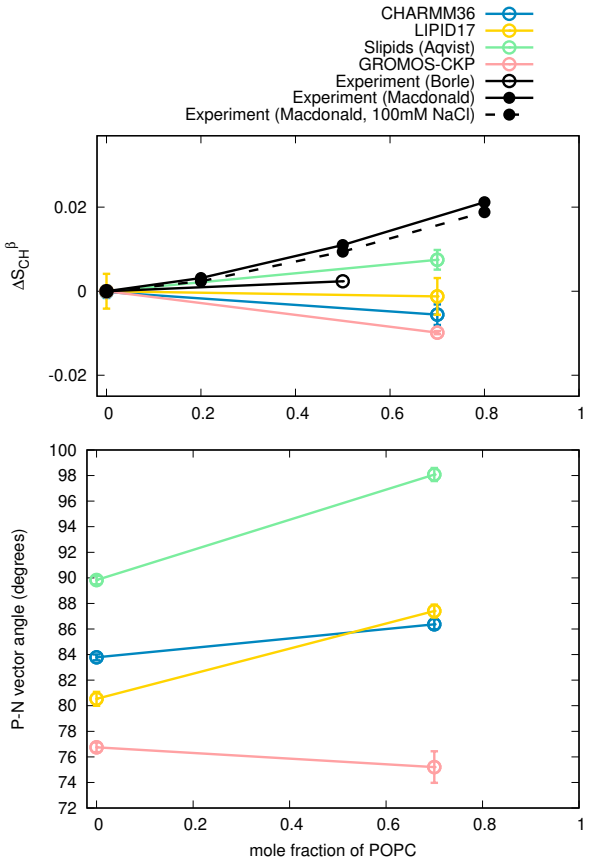

Figure S7: Modulation of PG lipid headgroup order parameters with the increasing amount of PC in lipid bilayer from experiments at 298 K[6,8] and simulations with different force fields at 310 K.

S5 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]

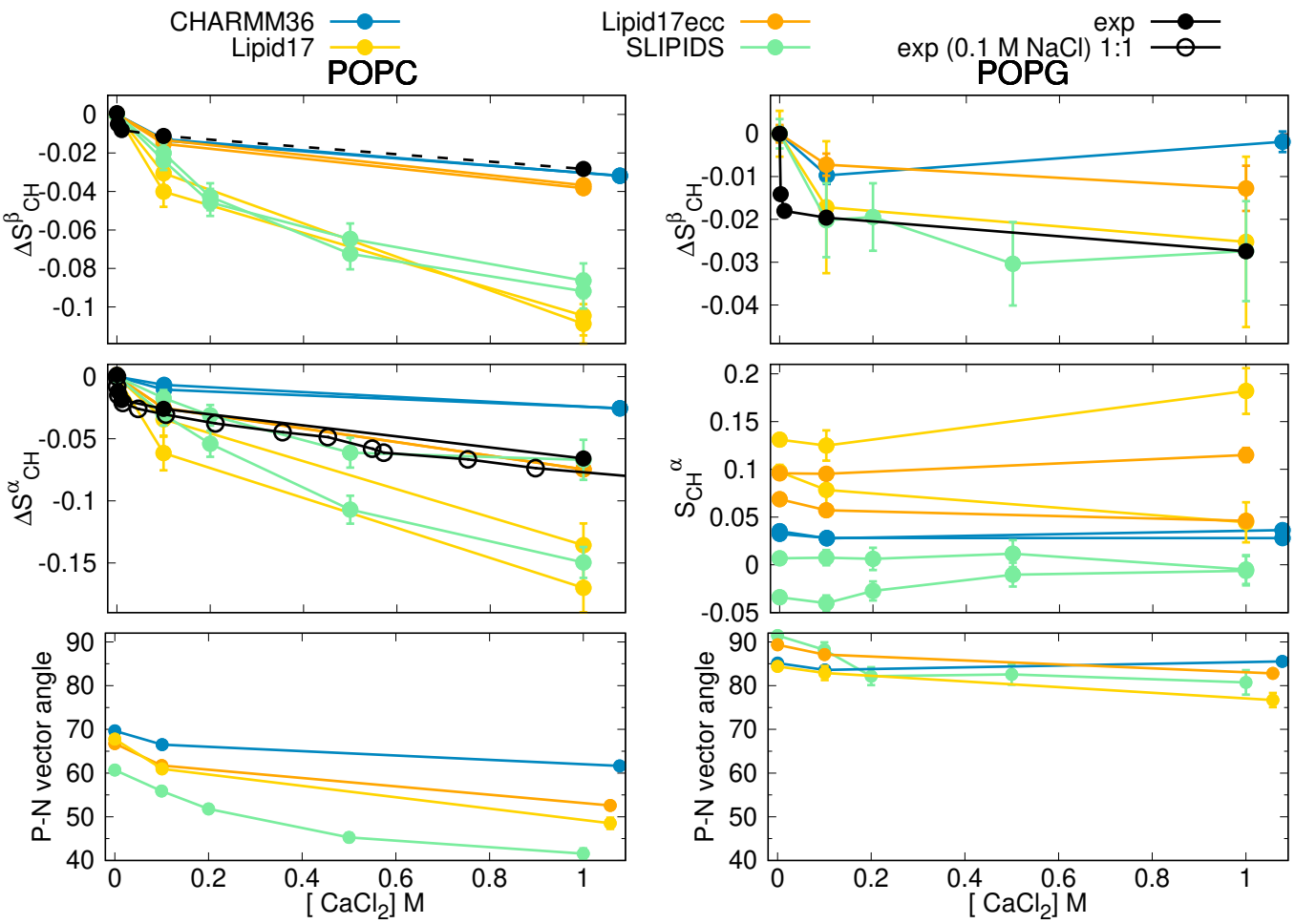

Figure S8: Modulation of headgroup order parameters of POPC (*left*) and POPG (*right*) in POPC:POPG (1:1) mixture upon addition of $CaCl_2$ in 298 K temperature from experiments[6,8] and simulations. The β-carbon order parameter of POPC (dashed line on top left) is not directly measured but calculated 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. Calsium density distributions are shown in figure S9.

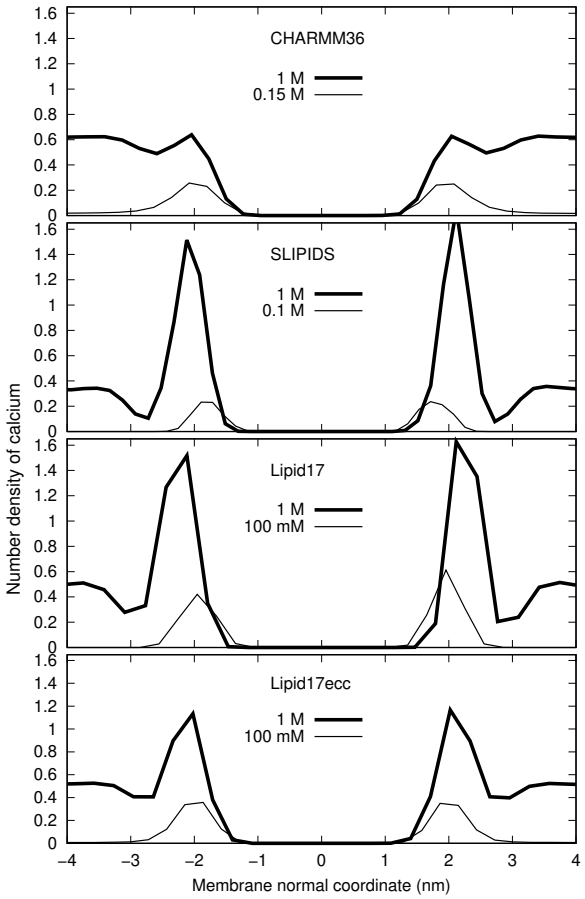

Figure S9: Calcium ion density profiles along membrane normal from simulations of POPC:POPG (1:1) mixtures with different force fields. The changes in the order parameters upon addition of $CaCl_2$ are compared with experiments in figure S8 in the main text.

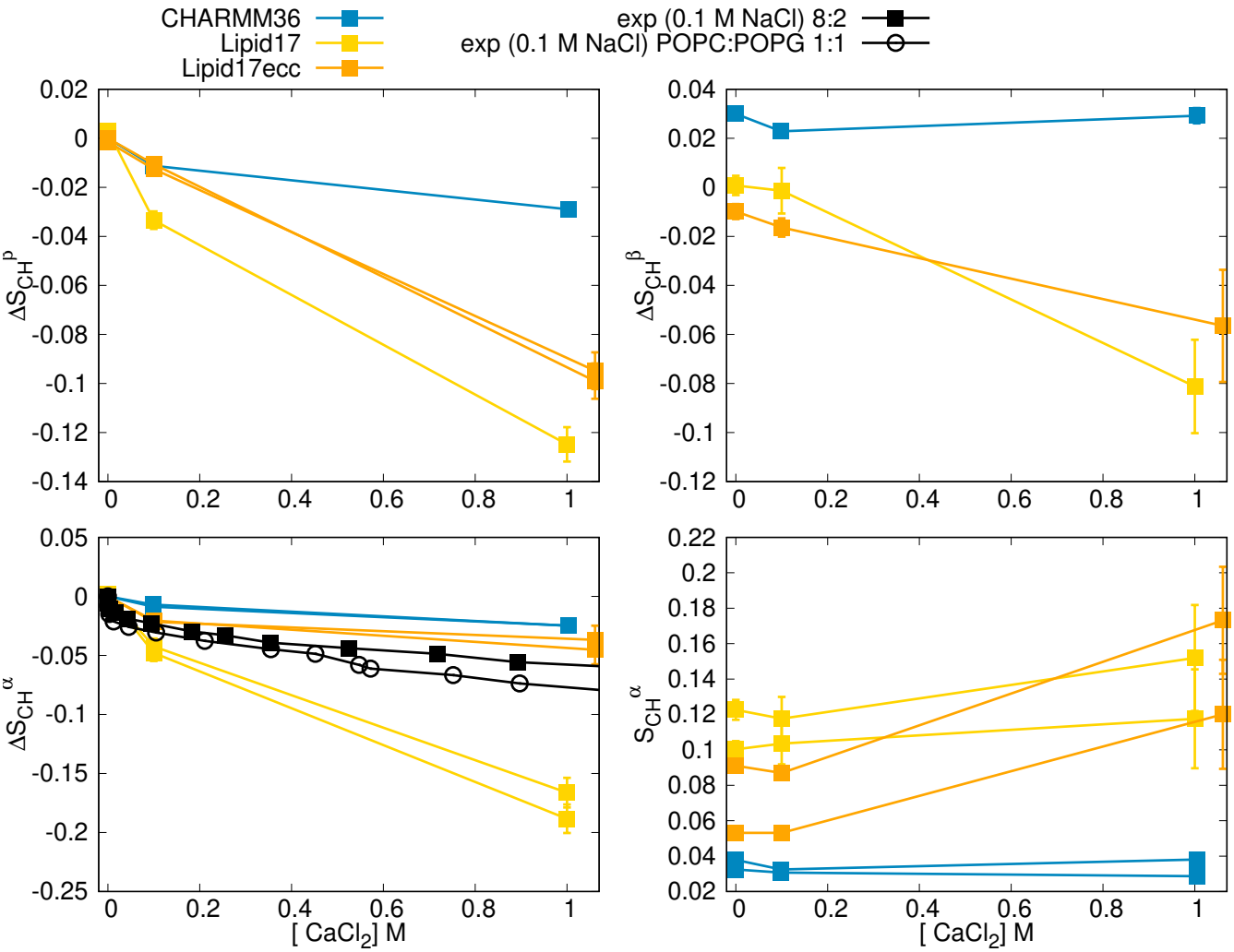

Figure S10: Modulation of headgroup order parameters of POPC (*left*) and POPG (*right*) in POPC:POPG (4:1) mixture upon addition of $CaCl_2$ in 298 K temperature from experiments[8] and simulations. The changes with respect to the systems without $CaCl_2$ are shown for other data than for the α-carbon of POPG for which experimental order parameter is not available.

5.There is something wrong the Lipid17ecc data at 1M, a new simulation is coming.

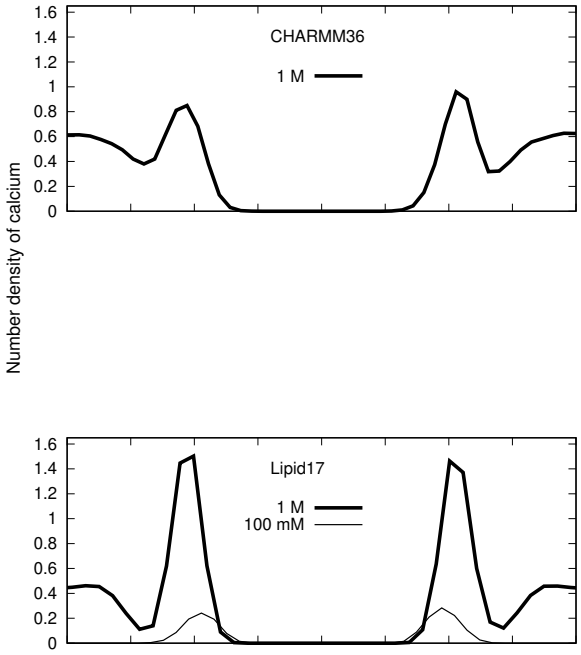

Figure S11: Calcium ion density profiles along membrane normal from simulations of POPC:POPG (4:1) mixtures with different force fields.

6.Density profiles from Lipid17ecc data will be added when we have the new simulations at 1M.

S6 Changes in headgroup conformations upon addition of $CaCl_2$

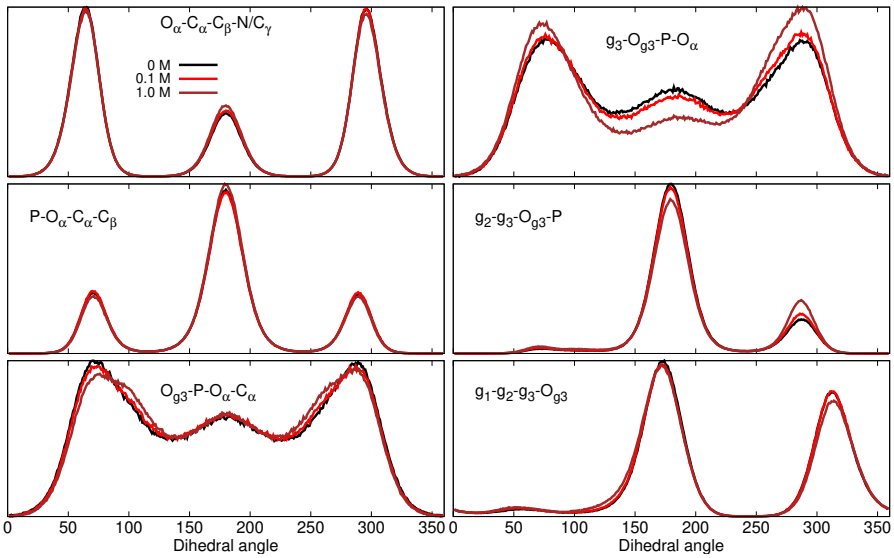

Figure S12: Changes in POPC lipid17ecc dihedrals with increasing amount of $CaCl_2$.

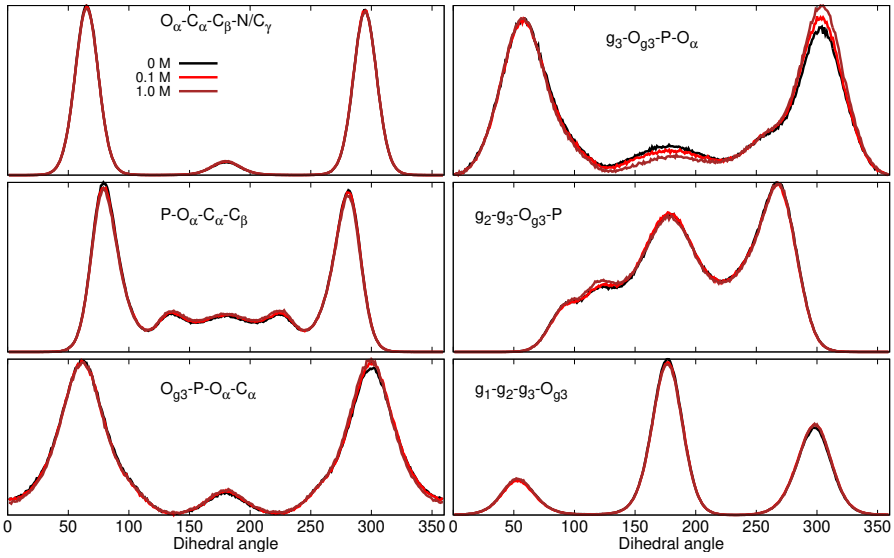

Figure S13: Changes in POPC CHARMM36 dihedrals with increasing amount of $CaCl_2$.

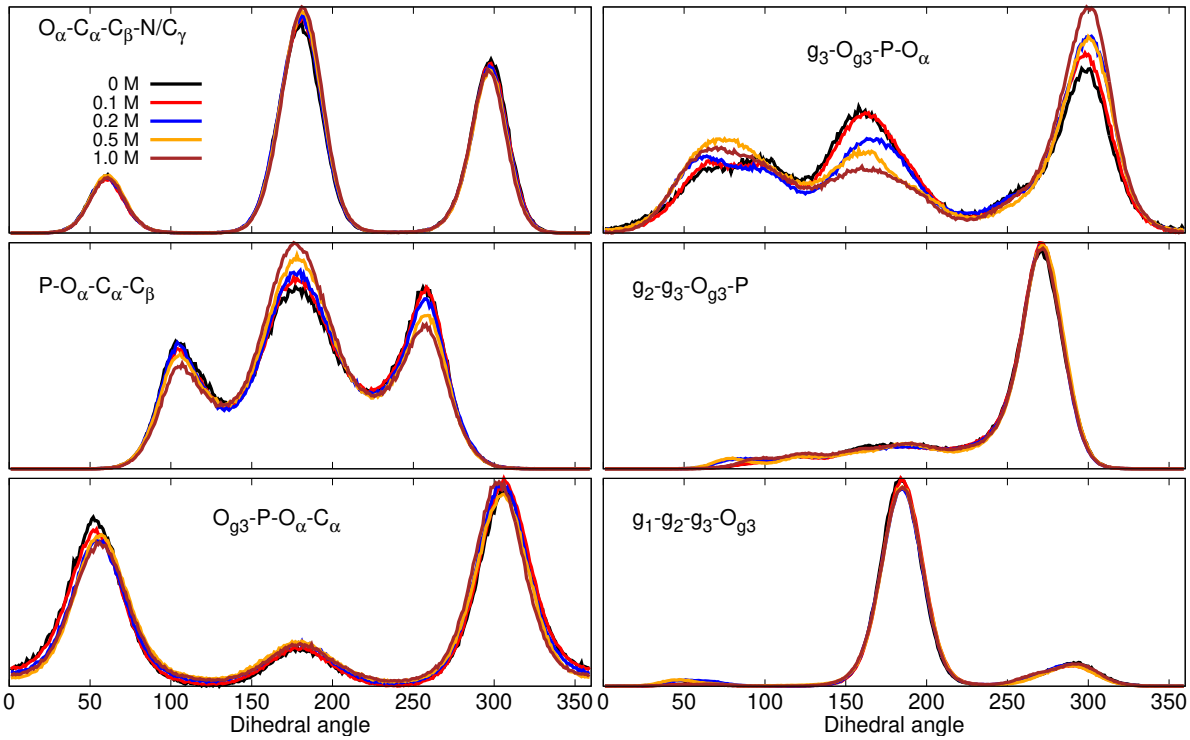

Figure S14: Changes in POPG Slipids dihedrals with increasing amount of $CaCl_2$.

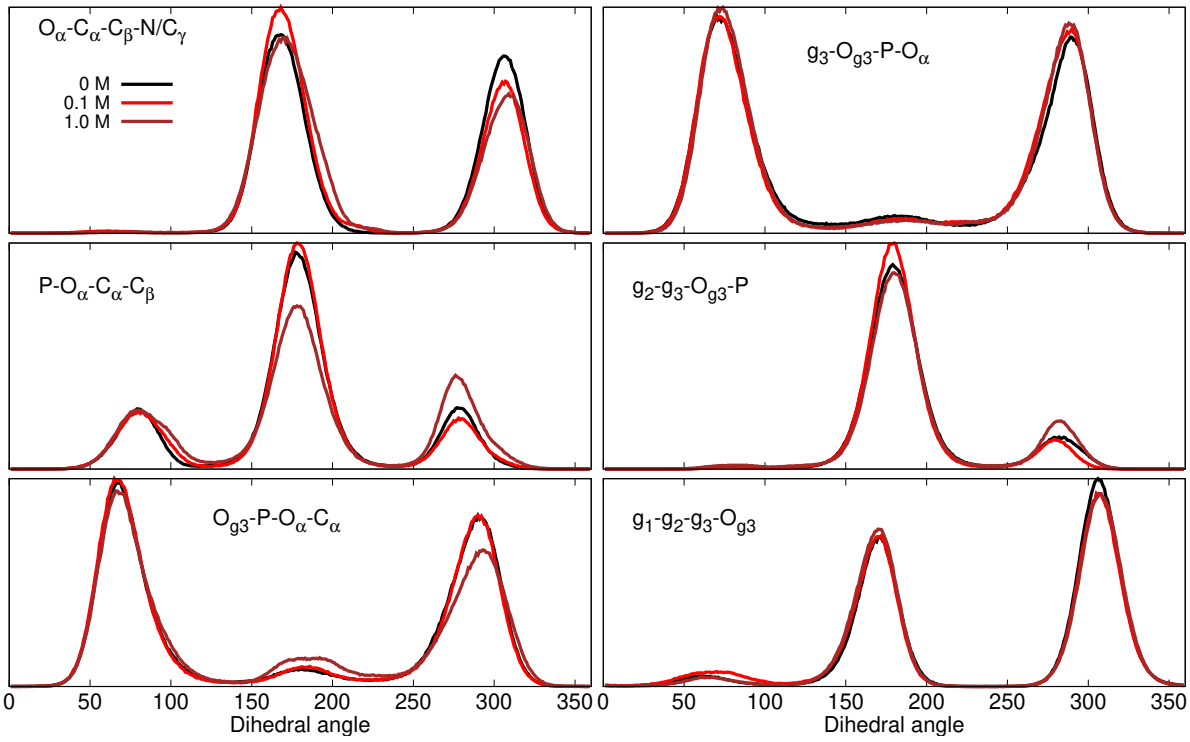

Figure S15: Changes in POPG lipid17 dihedrals with increasing amount of $CaCl_2$.

S7 Simulated systems

S7.1 CHARMM36

POPE **29.Simulation details by M. Javanainen.**

POPE with additional NaCl **30.Simulation details by A. Peon.**

POPG **31.Simulation details by Ollila.**

POPG with additional NaCl **32.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=4.5e-5 bar^{-1}), PME order 4 and space 0.12, rcoulomb and rvdw 1.0, 128 lipids per leaflet, no ion **33.Full simulation details by Fuchs et al.**

POPC:POPG mixture with additional calcium **34.Simulation details by A. Kiirikki.**

POPC and POPC:POPG (7:3) mixture **35.Simulation details by A. Peon.**

S7.2 CHARMM36ua

POPE Data is available at.[19] **36.Simulation details by T. Piggot.**

S7.3 Slipids

POPE Data is available at.[22] **37.Simulation details by T. Piggot.**

POPE with additional NaCl **38.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] **39.Simulation details by F. Favela.**

POPG Data is available at.[48] **40.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 **41.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[50] and in 314 K at.[49] **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.**

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]

[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

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

8.Citation for CHARMM36 PE?

9.Which ion model is used in[18]?

10.Citation for GROMOS-CKP?

11.Citation for GROMOS 43A1-S3?

12.Citation for OPLS-UA models?

13.Citations for Berger-* simulations?

14.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? 15.	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]

[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

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

17.Citations and ion model for CHARMM36?

18.Lipid17 simulation with ions 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	[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]

[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.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 (4:1)	Lipid17ecc / ECC-ions[85–87]	0	0	350:88	26265	0	298	400	300	[88]
POPC:POPG (4:1)	Lipid17ecc / ECC-ions[85–87]	0	0.1	350:88	26124	47	298	400	300	[89]
POPC:POPG (4:1)	Lipid17ecc / ECC-ions[85–87]	0	1.0	350:88	24840	475	298	400	300	[90]
POPC:POPG (1:1)	Lipid17ecc / ECC-ions[85–87]	0	0	150:150	31572	0	298	347.8	333	[91]
POPC:POPG (1:1)	Lipid17ecc / ECC-ions[85–87]	0	0.1	150:150	29865	54	298	400	300	[92]
POPC:POPG (1:1)	Lipid17ecc / ECC-ions[85–87]	0	1.0	150:150	29865	569	298	600	400	[93]
POPC	Berger[?] **22.**	0	0	256	10240	0	300	300	200	[94]
POPC:POPE (1:1)	Berger[?] **23.**	0	0	128	11008	0	300	300	200	[95]
POPC:DOPE (1:1)	Berger[?] **24.**	0	0	128	10240	0	300	300	200	[96]
DOPC	Berger[?] **25.**	0	0	256	11008	0	300	300	200	[97]
DOPC:DOPE (1:1)	Berger[?] **26.**	0	0	128	11008	0	300	300	200	[98]

[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

27.Citation and description for "Berger" model?

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

POPC:POPG mixture 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.**

POPC:POPG mixture with additional CaCl **44.Simulation details by M. Javanainen.**

S7.4 Berger

POPE Data is available at.[37,38] **45.Simulation details by T. Piggot.**

DOPE Data is available at.[39,40] **46.Simulation details by T. Piggot.**

POPC:POPE, POPC:DOPE and DOPC:DOPE mixtures Data is available at.[94,95] 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 **47.Simulation details by Fuchs et al.**

S7.5 GROMOS 43A1-S3

POPE Data is available at.[31] **48.Simulation details by T. Piggot.**

S7.6 OPLS-UA

POPE Data is available at.[33] **49.Simulation details by T. Piggot.**

POPE with vdW interaction in H Data is available at.[32] **50.Simulation details by T. Piggot.**

S7.7 GROMOS-CKP and GROMOS-CKPM

POPE Data is available at.[27] **51.Simulation details by T. Piggot.**

DOPE Data is available at.[30] **52.Simulation details by T. Piggot.**

DPPE Data is available at.[26] **53.Simulation details by T. Piggot.**

POPG **54.Simulation details by A. Peon.**

POPC:POPG mixture **55.Simulation details by A. Peon.**

S7.8 OPLS-MacRog

POPE **56.Simulation details by M. Javanainen and P. Fuchs.**

POPC:POPE mixtures **57.Simulation details by P. Fuchs.**

S7.9 Lipid17

POPE **58.Simulation details by A. Peon.**

POPG **59.Simulation details by A. Peon.**

POPC:POPG **60.Simulation details by S. Virtanen or O. H. S. Ollila.**

S7.10 Lipid17ecc

61.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 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:[?] ECC-POPC parameters (scaling factors f_q=0.8 and f_σ=0.89 applied to Lipid14 POPC parameters)[13] 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.[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.

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