Supporting Information:

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

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Contents

S1 R-PDLF and SDROSS experiments

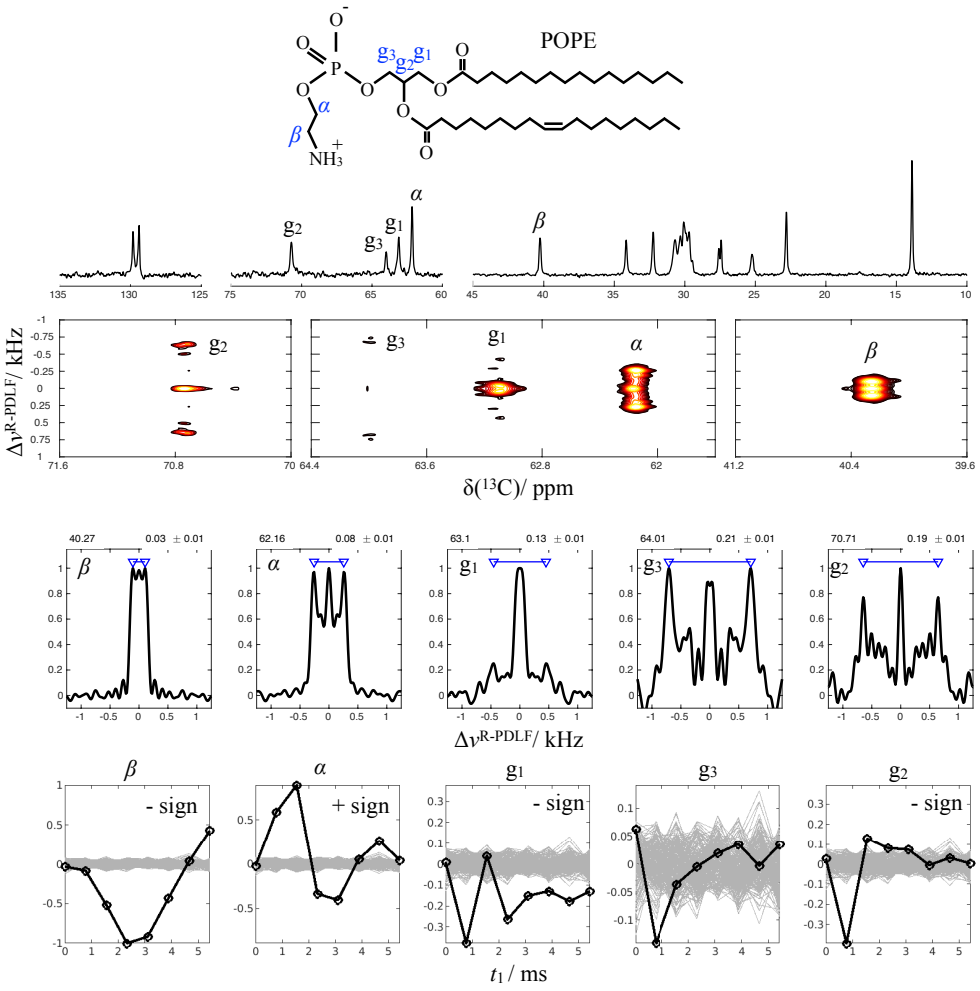

Figure S1: (A) Chemical structure of POPE with the labeling of headgroup and glycerol backbone carbons. (B) INEPT spectra from POPE sample with the headgroup and glycerol backbone peaks labeled. (C) 2D R-PDLF spectra (D) Dipolar sliced from the 2D R-PDLF spectra with the resulting order parameters on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

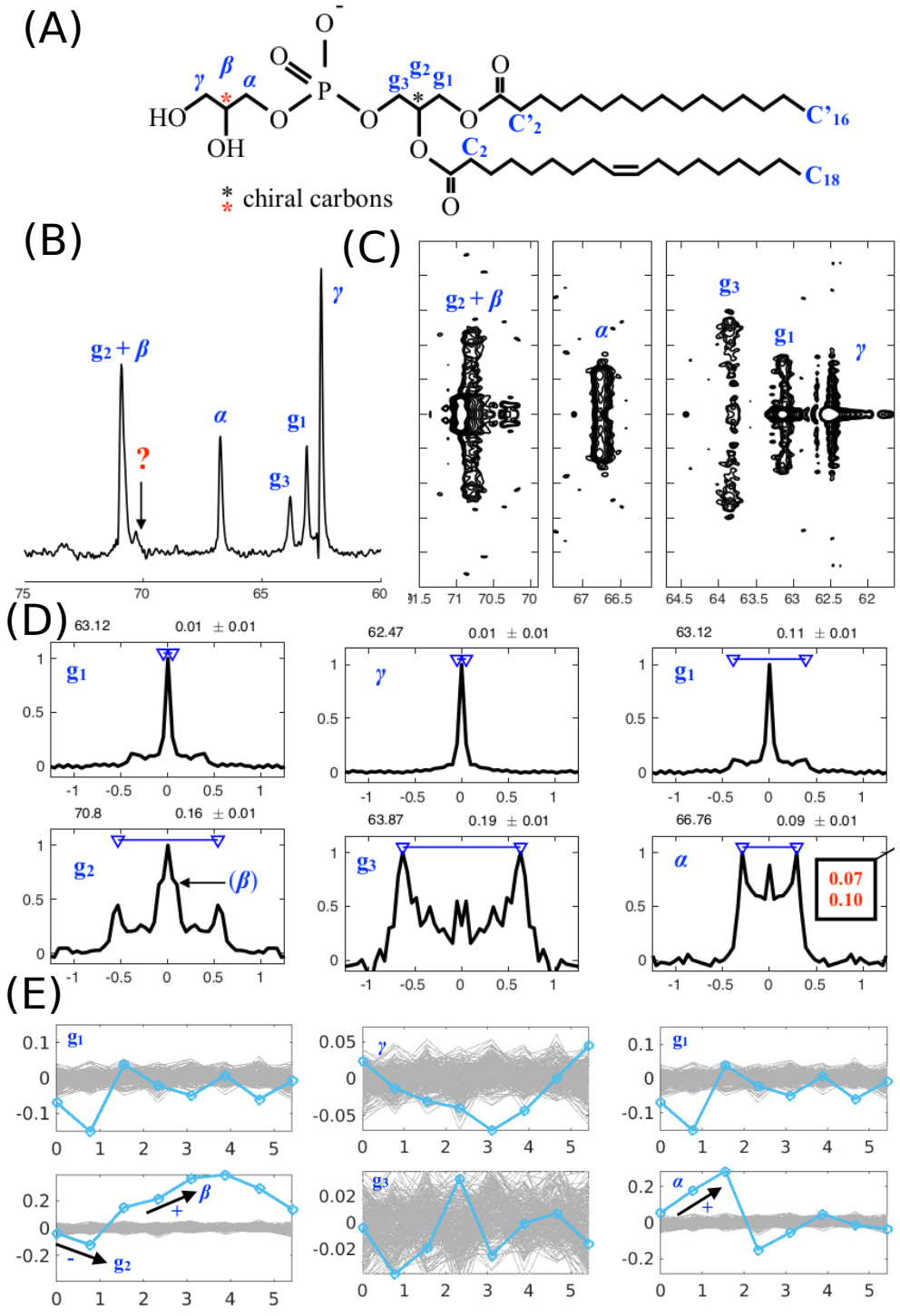

Figure S2: (A) Chemical structure of POPG with the labeling of headgroup and glycerol backbone carbons. (B) INEPT spectra from POPG sample with the headgroup and glycerol backbone peaks labeled. (C) 2D R-PDLF spectra (D) Dipolar sliced from the 2D R-PDLF spectra with the resulting order parameters on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

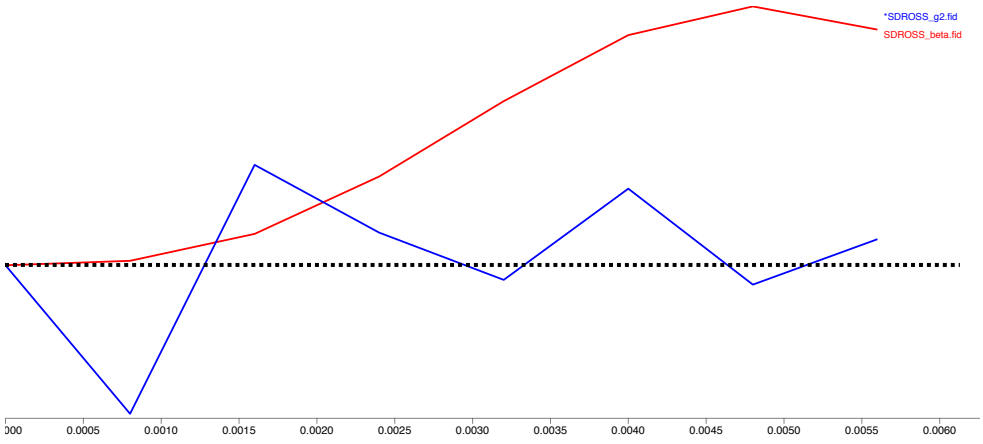

Figure S3: Simpson simulaton of S-DROSS curve of β-carbon of POPG.

S2 Lipid ligand names in PDB used in the analysis of conformations of protein-bound lipids

PC: PLC, PX4, 6PL, LIO, HGX, PC7, PC8, P1O, 6O8, XP5, EGY, PLD, SBM, HXG, and PCW

PE: 8PE, PTY, 3PE, PEH, PEF, 6OE, 6O9, 9PE, PEV, 46E, SBJ, L9Q, PEK, EPH, ZPE, 9TL, 9Y0, 6OU, LOP, and PEE

PG: PGT, PGK, LHG, 44G, PGV, OZ2, D3D, PGW, DR9, P6L, PG8, H3T, and GOT

PS: PSF, PS6, Q3G, P5S, D39, PS2, 17F, and 8SP.

S3 Evaluation of simulations against NMR experiments

S3.1 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. Most importantly for this work, the CHARMM36 captures the distinct headgroup order parameters for PG and PS lipids observed in NMR experiments (Figs. 1 and 2 in the main text).

It should be noted that 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]

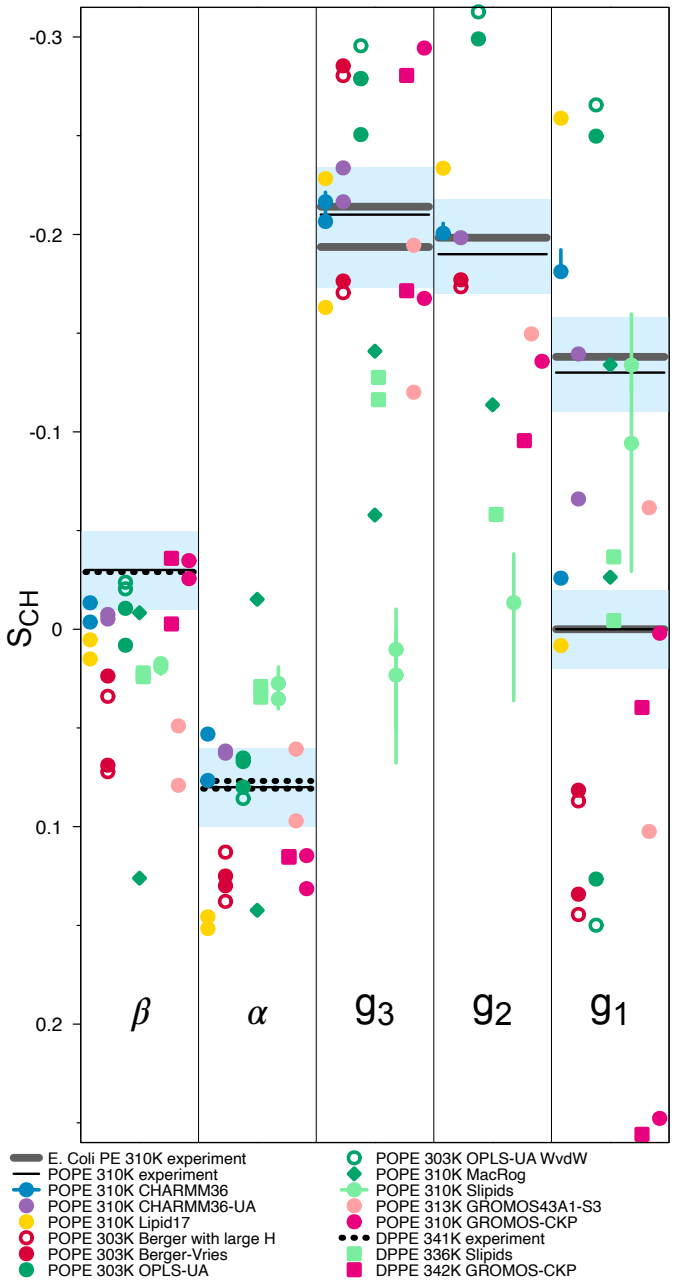

Figure S4: The headgroup and glycerol backbone order parameters of PE lipids from experiments (POPE and signs this work, DPPE from Ref. 4 and E.coliPE from Ref. 5) and simulations with different force fields.

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.

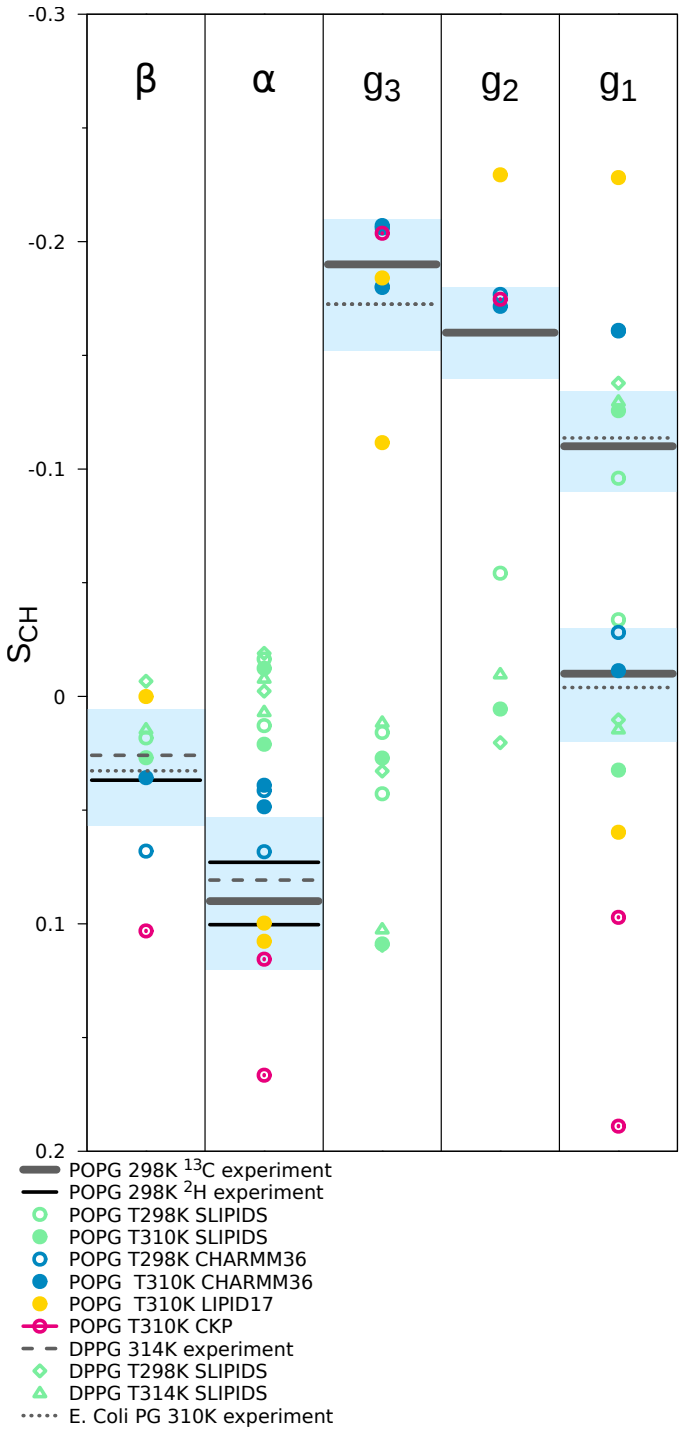

Figure S5: The headgroup and glycerol backbone order parameters of PG lipids from experiments (POPG and signs from this work and from Ref. 6, DPPG with 100mM NaCl from Ref. 3,and E.Coli PG results from Ref. 5) and simulations with different force fields.

S3.2 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 is surprising here 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]

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

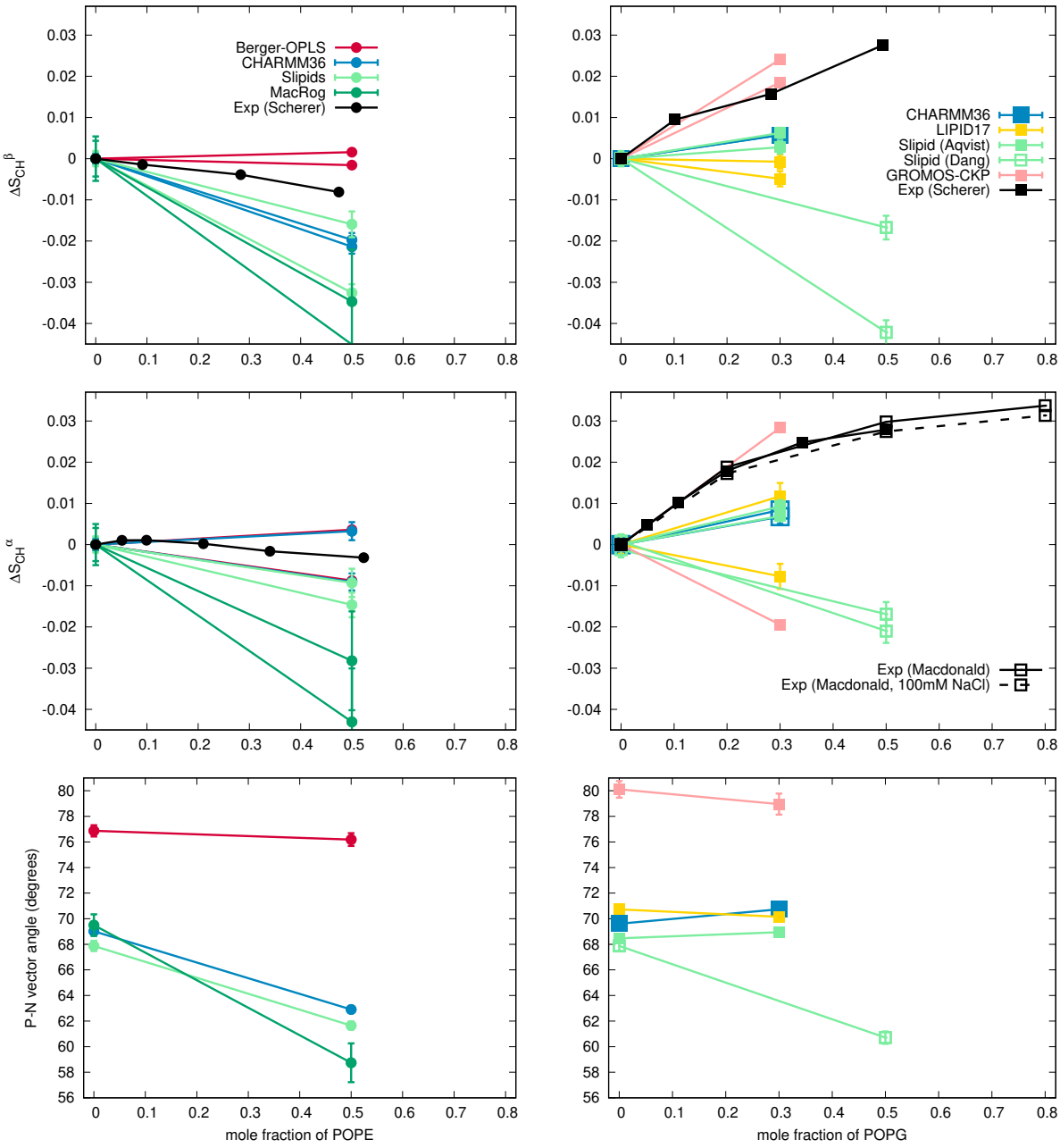

Figure S6: Modulation of POPC headgroup order parameters with increasing amount of POPE (left) and POPG (right) in bilayer from experiments at 298 K[7,8] and simulations with different force fields (temperatures listed in tables S3 and S4 are between 298-310 K). Signs are determined as discussed in Refs. 1,9.

S3.3 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 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.

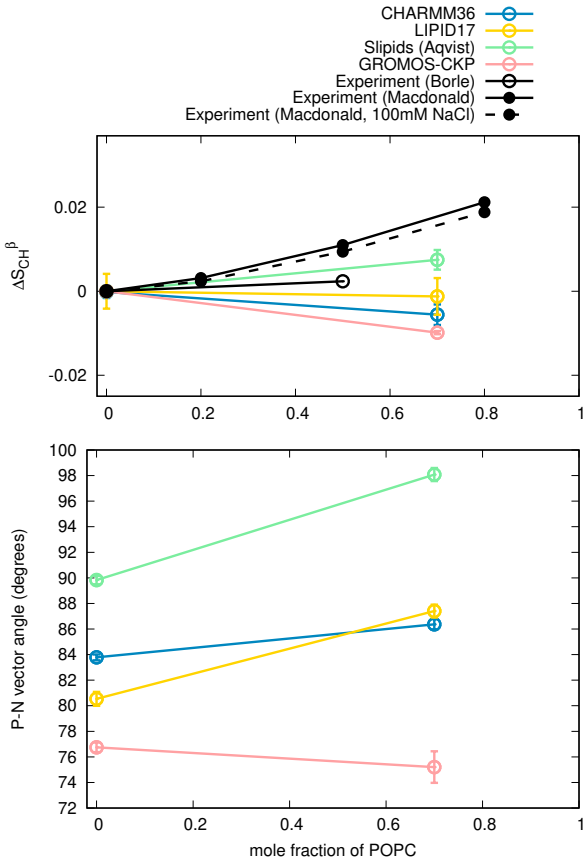

Figure S7: Modulation of PG lipid headgroup order parameters with the increasing amount of PC in lipid bilayer from experiments at 298 K[6,8] and simulations with different force fields at 310 K.

S3.4 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]

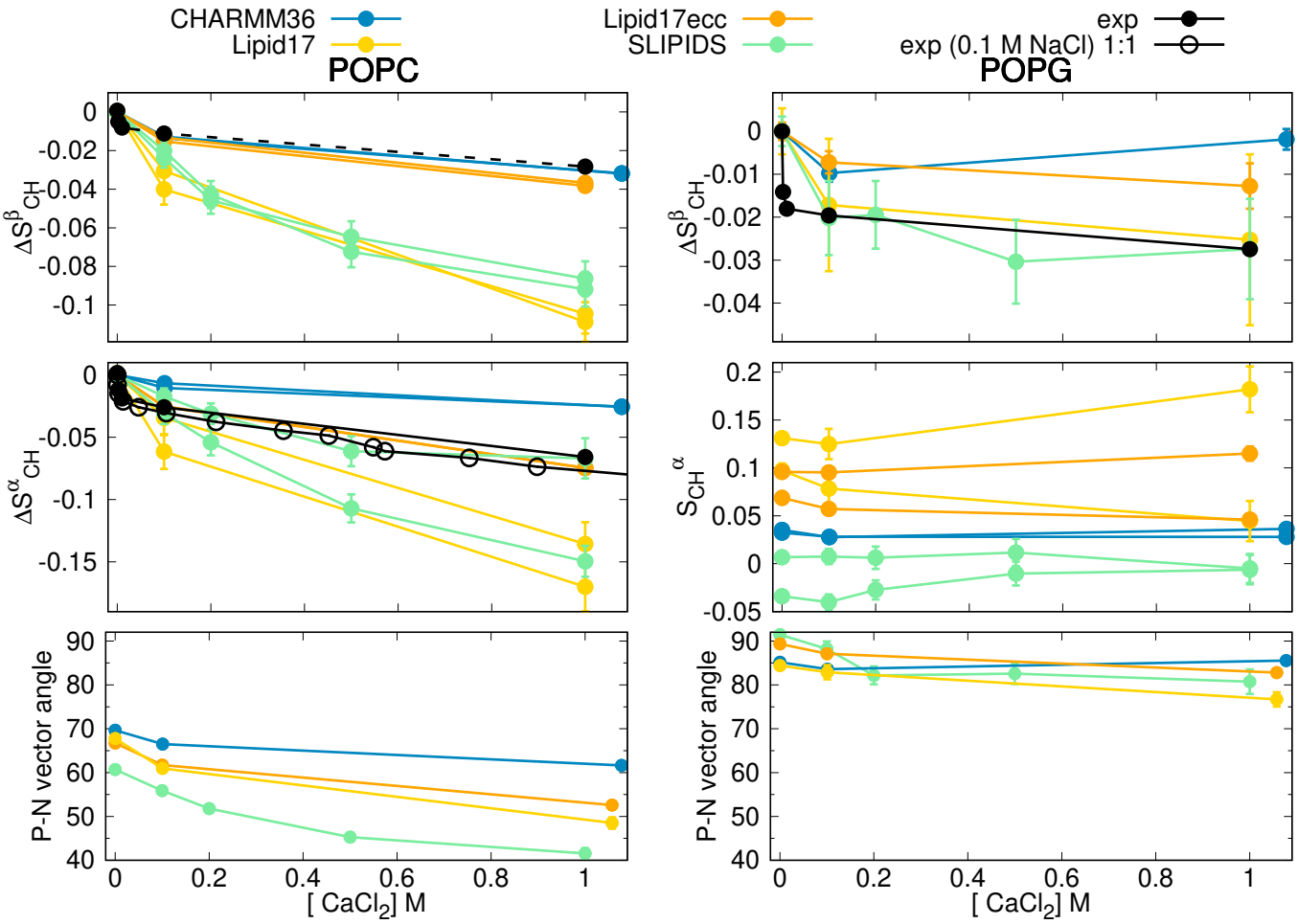

Figure S8: Modulation of headgroup order parameters of POPC (*left*) and POPG (*right*) in POPC:POPG (1:1) mixture upon addition of $CaCl_2$ in 298 K temperature from experiments[6,8] and simulations. The β-carbon order parameter of POPC (dashed line on top left) is not directly measured but calculated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

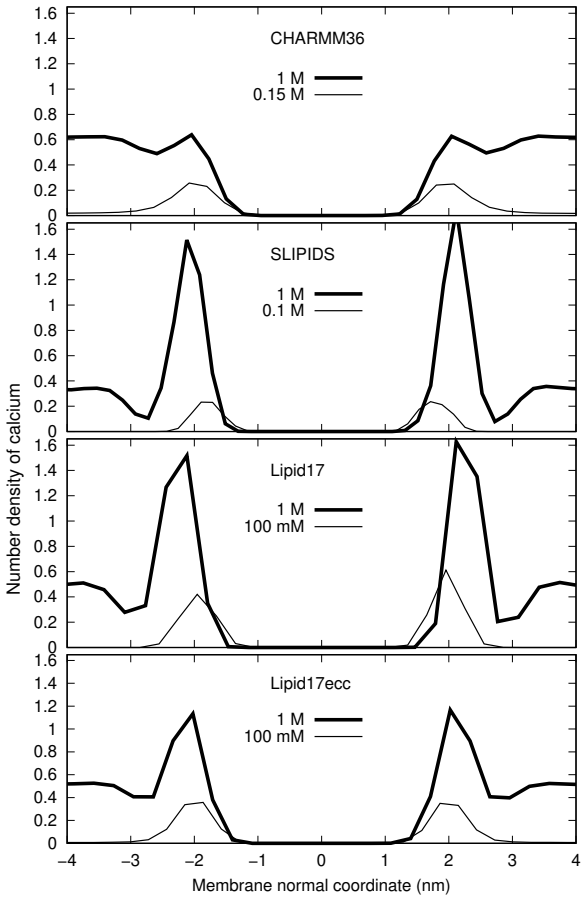

Figure S9: Calcium ion density profiles along membrane normal from simulations of POPC:POPG (1:1) mixtures with different force fields. The changes in the order parameters upon addition of $CaCl_2$ are compared with experiments in figure S8.

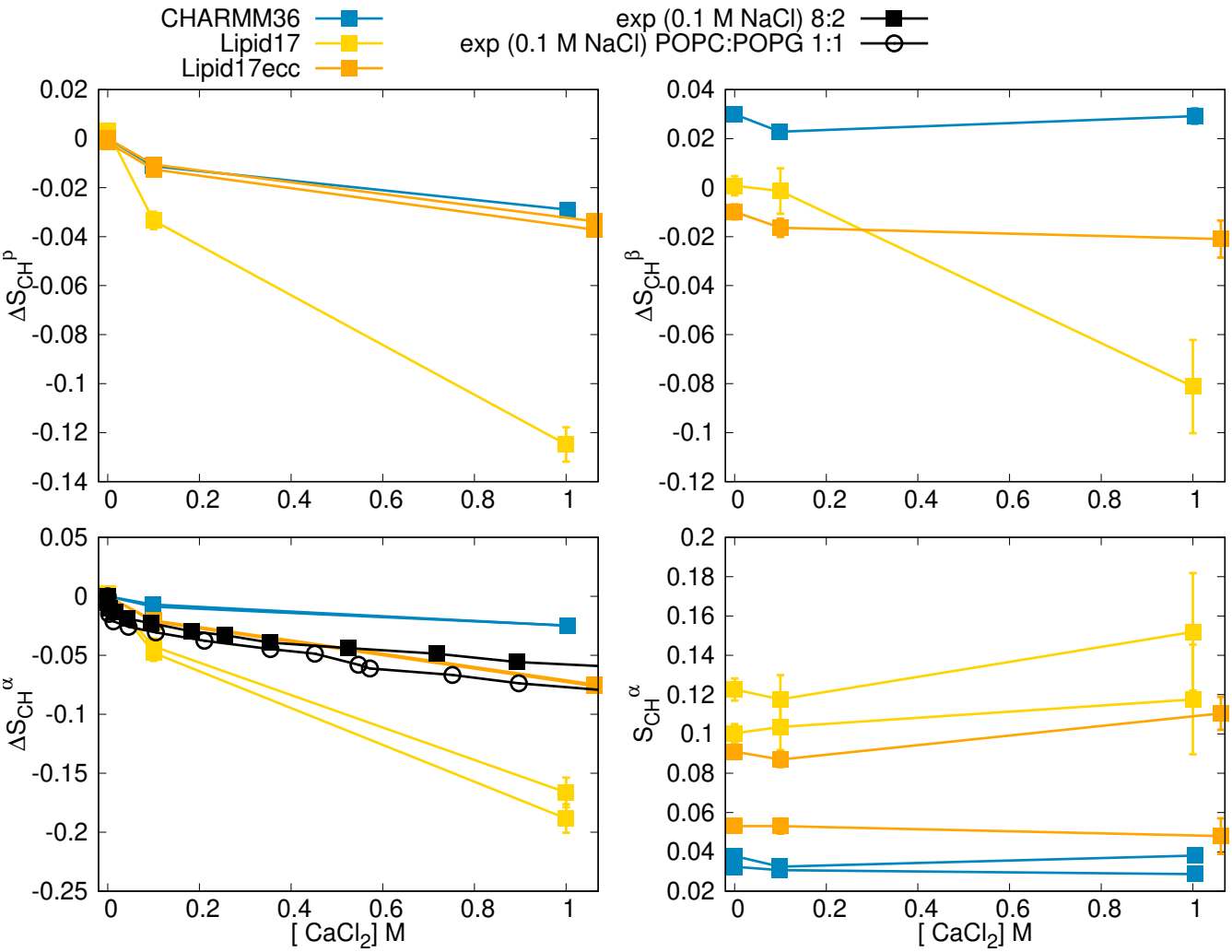

Figure S10: Modulation of headgroup order parameters of POPC (*left*) and POPG (*right*) in POPC:POPG (4:1) mixture upon addition of $CaCl_2$ in 298 K temperature from experiments[8] and simulations. The changes with respect to the systems without $CaCl_2$ are shown for other data than for the α-carbon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

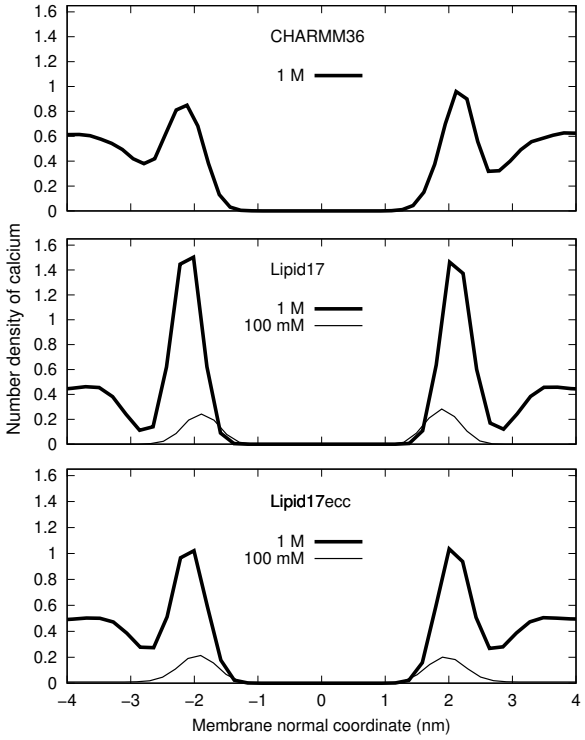

Figure S11: Calcium ion density profiles along membrane normal from simulations of POPC:POPG (4:1) mixtures with different force fields.

S4 Dihedral angle distributions and the analysis of relative energies

S4.1 Dihedral angles and relative energies of PC, PE, PG and PS headgroups

We estimated the relative energies of each dihedral angle value with respect to the most probable value (lowest energy) from the inverse Boltzmann formula $\Delta E(\theta) = -kT\left[\ln\left[p(\theta)\right] - \ln\left[p(\theta_0)\right]\right]$, where $p(\theta)$ is the dihedral angle distribution and θ_0 is the most probable angle from MD simulation. The dihedral angle distributions are shown in Fig. S12 and relative energies in Fig. 2 in the main text.

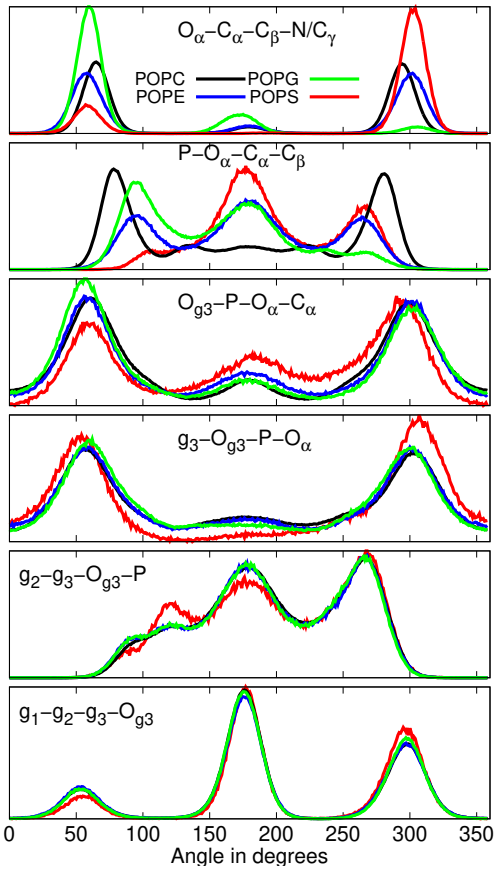

Figure S12: Heavy atom dihedral angle distributions from CHARMM36 simulations that correctly capture the order parameter differences between the force fields.

S4.2 Changes in headgroup conformations upon addition of charged surfactants or $CaCl_2$

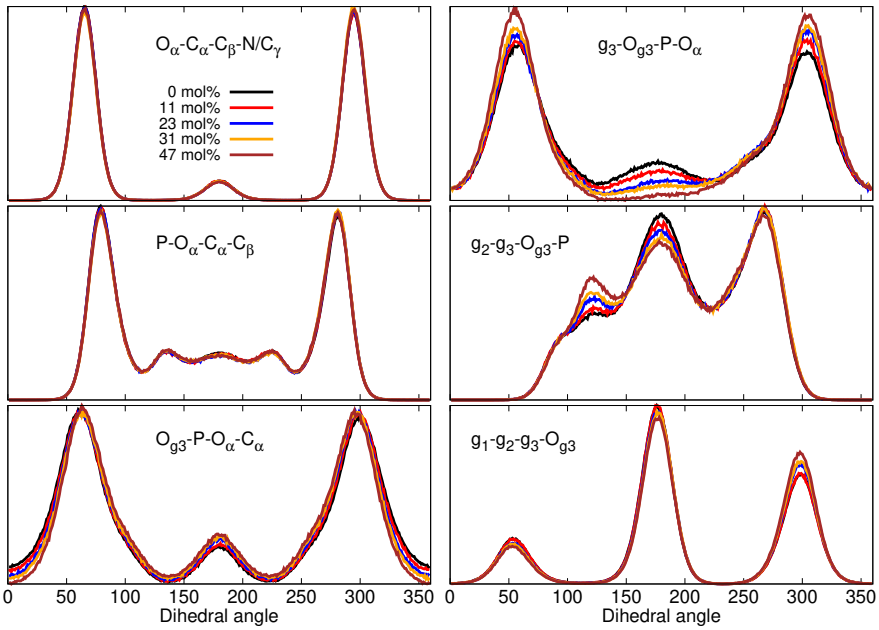

Figure S13: Changes in PC headgroup conformational ensembles upon increasing the amount of positive charge in bilayer, characterized by the heavy atom dihedral distributions, from CHARMM36 simulations.

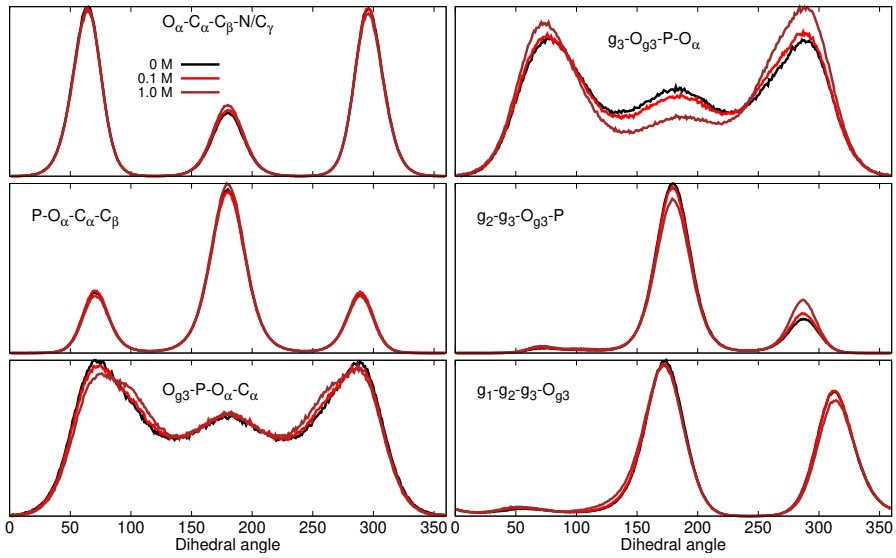

Figure S14: Changes in POPC lipid17ecc dihedrals with increasing amount of $CaCl_2$.

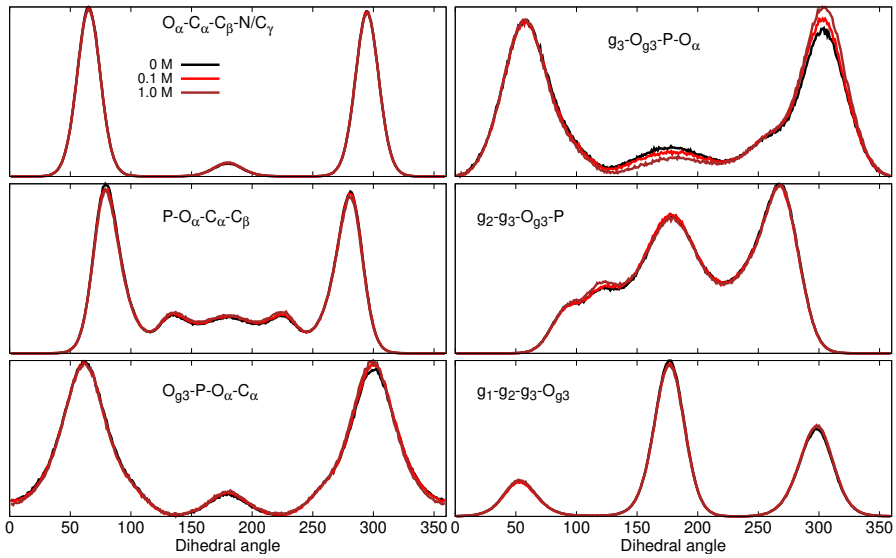

Figure S15: Changes in POPC CHARMM36 dihedrals with increasing amount of $CaCl_2$.

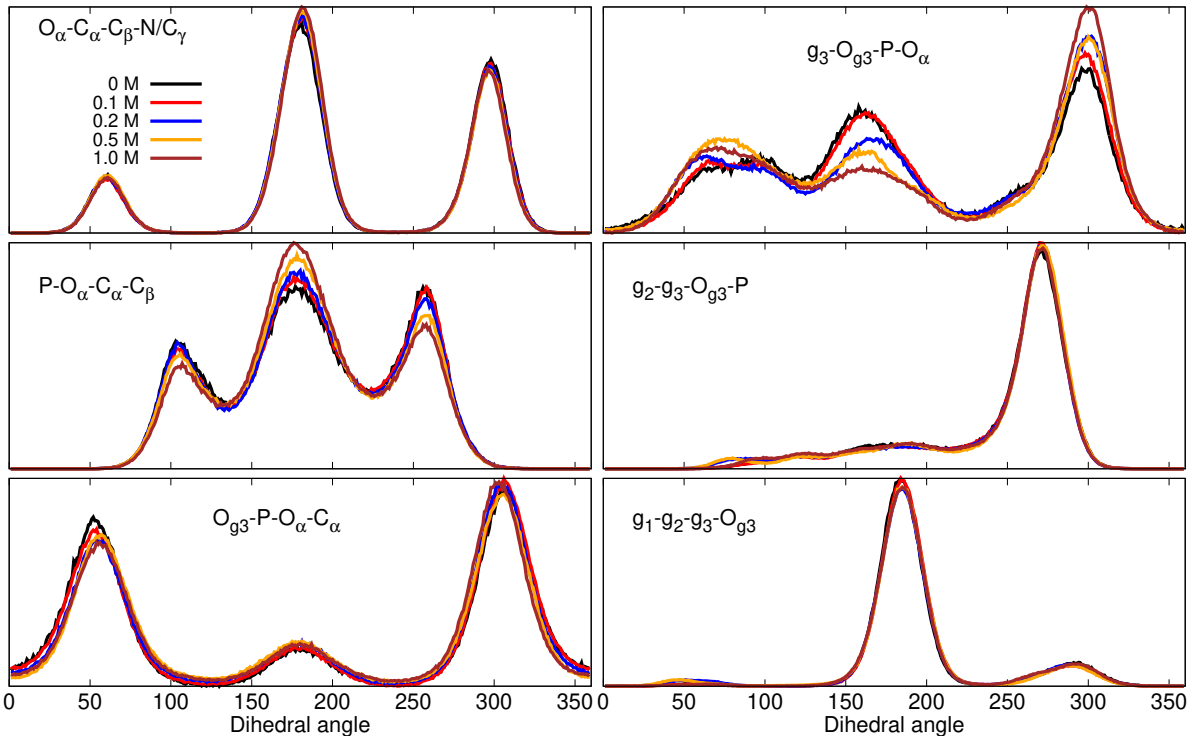

Figure S16: Changes in POPG Slipids dihedrals with increasing amount of $CaCl_2$.

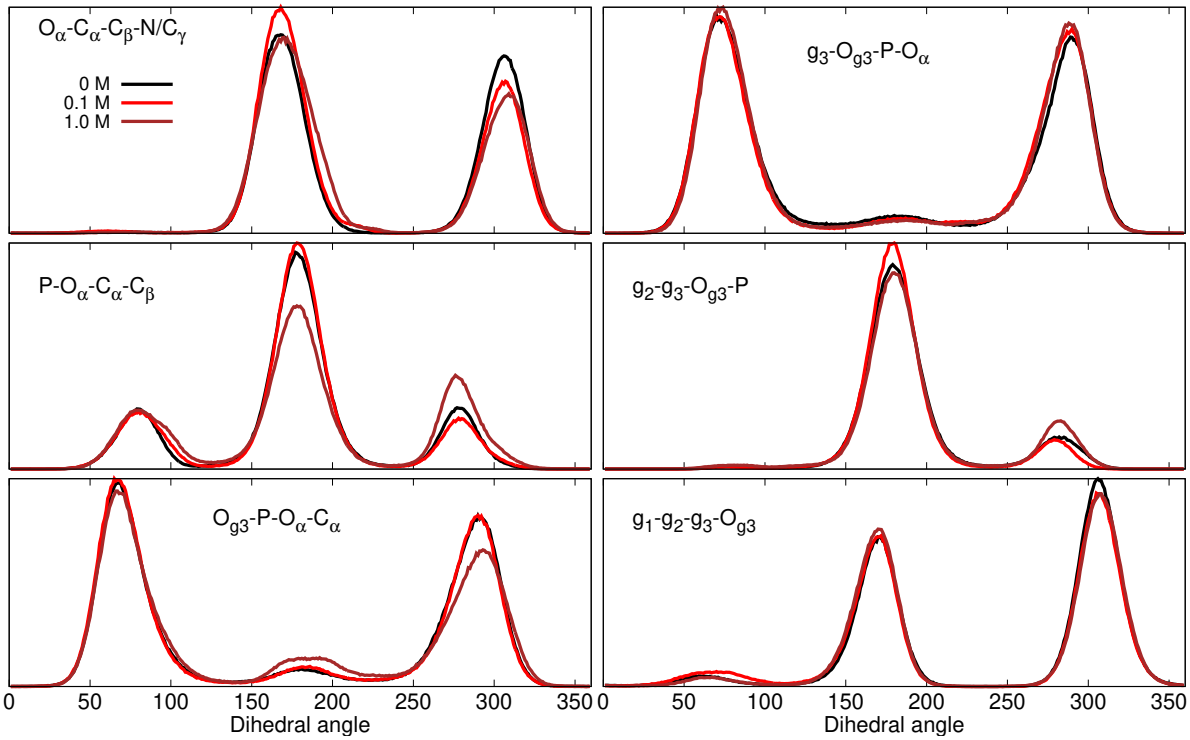

Figure S17: Changes in POPG lipid17 dihedrals with increasing amount of $CaCl_2$.

S5 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 simulations were analyzed using preliminary versions of the NMRlipids databank (`www.nmrlipids.fi`, `github.com/NMRlipids/MATCH` and `https://github.com/NMRLipids/NMRlipidsIVPEandPG/tree/master/Data/Simulations`) and unique naming convention for lipid atoms (`http://nmrlipids.blogspot.com/2015/03/mapping-scheme-for-lipid-atom-names-for.html`), which enable automatic analysis of simulations with different force fields with varying atom naming conventions. The automatic analyses were implemented using MDAnalysis[16,17] and MDTraj[18] python libraries, and tools in the GROMACS sofware package.[19] All codes are available from the project's GitHub repository.[20]

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

$$S_{\mathrm{CH}} = \frac{1}{2}\left\langle 3\cos^2\theta - 1 \right\rangle, \tag{1}$$

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. Code for all atom simulations is available in Ref. 21 (`scripts/calcOrderParameters.py`). For united atom simulations, we first constructed trajectories including hydrogens with ideal geometry using either `buildH` program[22] or (`scratch/opAAUA_prod.py`) in Ref. 21, 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.[22,23]

Table S1: List of MD simulations with PE lipids.

lipid	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[24]	0	144	5760	0	310	500	400	[25]
POPE	CHARMM36[24]	0	500	25000	0	310	500	100	[26]
POPE	CHARMM36ua[?]	0	336	15254	0	310	2×200	2×100	[27]
DPPE	Slipids[28]	0	288	9386	0	336	200	100	[29]
POPE	Slipids[28]	0	336	?	0	310	2×200	2×100	[30]
POPE	Slipids[28]	0	500	25000	0	310	500	100	[31]
DPPE	GROMOS-CKP[?]	0	128	3655	0	342	2×500	2×400	[32]
POPE	GROMOS-CKP[?]	0	500	25000	0	310	500	100	[33]
POPE	GROMOS 43A1-S3[?]	0	128	3552	0	313	2×200	2×100	[34]
POPE	OPLS-UA vdW on H[?]	0	128	3328	0	303	2×200	2×100	[35]
POPE	OPLS-UA[?]	0	128	3328	0	303	2×200	2×100	[36]
POPE	OPLS-MacRog[37]	0	144	5760	0	310	500	350	[38]
POPE	Berger-Vries[?]	0	128	3552	0	303	2×200	2×100	[39]
POPE	Berger-largeH[?]	0	128	3552	0	303	2×200	2×100	[40]
POPE	LIPID17[41]	0	500	25000	50	310	500	100	[42]

[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

3.Citation for CHARMM36ua?

4.Which ion model is used in[43]?

5.Citation for GROMOS-CKP?

6.Citation for GROMOS 43A1-S3?

7.Citation for OPLS-UA models?

8.Citations for Berger-* simulations?

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[44]	0	118	4110	0	298	100	100	[45]
POPG	CHARMM36[44]	0	500	25000	0	310	500	100	[46]
POPG/Na^+	Slipids / Åqvist[47,48]	0	288	10664	0	298	250	100	[49]
DPPG/Na^+	Slipids / Åqvist[47,48]	0	288	11232	0	314	200	100	[50]
DPPG/Na^+	Slipids / Åqvist[47,48]	0	288	11232	0	298	400	100	[51]
POPG	Slipids / Åqvist[47,48]	0	500	25000	0	310	500	100	[52]
POPG	LIPID17 / Dang[41,53,54]	0	500	25000	0	310	500	100	[55]
POPG	GROMOS-CKP[?]	0	500	25000	0	310	500	100	[56]

[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 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[24]	0	0	500	25000	0	310	500	100	[57]
POPC:POPG (7:3)	CHARMM36[24,44]	0	0	350	25000	0	310	500	100	[58]
POPC:POPG (1:1)	CHARMM36[24,44]	0	0	150:150	31500	0	298	500	400	[59]
POPC:POPG (1:1)	CHARMM36[24,44]	0	0.1	150:150	31329	57	298	400	300	[60]
POPC:POPG (1:1)	CHARMM36[24,44]	0	1.08	150:150	29766	578	298	500	400	[61]
POPC:POPG (4:1)	CHARMM36[24,44]	0	0	350:88	26280	0	298	500	400	[62]
POPC:POPG (4:1)	CHARMM36[24,44]	0	0.1	350:88	26280	47	298	500	400	[63]
POPC:POPG (4:1)	CHARMM36[24,44]	0	1.0	350:88	24927	451	298	500	400	[64]
POPC	CHARMM36[24]	0	0	256	8704	0	300	300	250	[65]
POPC:POPE (1:1)	CHARMM36[24,44]	0	0	128	8704	0	300	300	250	[66]
POPC	OPLS-MacRog[37]	0	0	128	5120	0	300	500	300	[67]
POPC:POPE (1:1)	OPLS-MacRog[37]	0	0	128	5120	0	300	500	300	[68]
POPC	Slipid[28]	0	0	512	23943	0	298	170	100	[69]
POPC:POPE (1:1)	Slipid[28]	0	0	128	5120	0	298	500	300	[70]
POPC	GROMOS-CKP / ??[? ?]	0	0	500	25000	0	310	500	100	[71]
POPC:POPG (7:3)	GROMOS-CKP / ??[? ?]	0	0	350:150	25000	0	310	500	100	[72]
POPC	Slipid[28]	0	0	500	25000	0	310	500	100	[73]
POPC:POPG (7:3)	Slipid / Åqvist[28,48]	0	0	350:150	25000	0	310	500	100	[74]
POPC:POPG (1:1)	Slipid / Dang[28,53,54,75]	0	0	128:128	12800	0	298	500	400	[76]
POPC:POPG (1:1)	Slipid / Dang[28,53,54,75]	0	0.1	128:128	12800	23	298	500	400	[76]
POPC:POPG (1:1)	Slipid / Dang[28,53,54,75]	0	0.2	128:128	12800	46	298	1500	500	[76]
POPC:POPG (1:1)	Slipid / Dang[28,53,54,75]	0	0.5	128:128	12800	115	298	1500	500	[76]
POPC:POPG (1:1)	Slipid / Dang[28,53,54,75]	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

10.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[41,53,54]	0	0	350:88	26265	0	298	400	350	[77]
POPC:POPG (4:1)	Lipid17 / Dang[41,53,54]	0	0.1	350:88	26124	47	298	400	250	[78]
POPC:POPG (4:1)	Lipid17 / Dang[41,53,54]	0	1.0	350:88	24840	475	298	1200	200	[79]
POPC:POPG (1:1)	Lipid17 / Dang[41,53,54]	0	0	150:150	31572	0	298	320	200	[80]
POPC:POPG (1:1)	Lipid17 / Dang[41,53,54]	0	0.1	150:150	31401	57	298	718	198	[81]
POPC:POPG (1:1)	Lipid17 / Dang[41,53,54]	0	1.0	150:150	29865	569	298	720	200	[82]
POPC:POPG (4:1)	Lipid17ecc / ECC-ions[83–85]	0	0	350:88	26265	0	298	400	300	[86]
POPC:POPG (4:1)	Lipid17ecc / ECC-ions[83–85]	0	0.1	350:88	26124	47	298	400	300	[87]
POPC:POPG (4:1)	Lipid17ecc / ECC-ions[83–85]	0	1.0	350:88	24840	475	298	400	300	[88]
POPC:POPG (1:1)	Lipid17ecc / ECC-ions[83–85]	0	0	150:150	31572	0	298	347.8	333	[89]
POPC:POPG (1:1)	Lipid17ecc / ECC-ions[83–85]	0	0.1	150:150	29865	54	298	400	300	[90]
POPC:POPG (1:1)	Lipid17ecc / ECC-ions[83–85]	0	1.0	150:150	29865	569	298	600	400	[91]
POPC	Berger[?] **11.**	0	0	256	10240	0	300	300	200	[92]
POPC:POPE (1:1)	Berger[?] **12.**	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

13.Citation and description for "Berger" model?

S5.1 CHARMM36

POPE **14.Simulation details by M. Javanainen.**

POPG Lipid bilayer containing 118 POPG molecules, 4110 TIP3P water molecules, and 118 potassium ions was build using CHARMM-GUI.[94] The system was simulated 100 ns, coupled to 298 K using Nose-Hoover[95,96] thermostat and 1 bar with semi-isotropic Parrinello-Rahman[97] pressure coupling. The used default parameters and force field files from CHARMM-GUI were used. The used files are available from 45.

15.Simulation details for larger simulation by A. Peon.

POPC:POPE mixtures Data is available at.[65,66] 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 **16.Full simulation details by Fuchs et al.**

POPC:POPG 1:1 and POPC:POPG 4:1 mixtures with additional calcium The initial structures were built with CHARMM-GUI Membrane Builder.[94] The TIP3P water model was used to solvate the systems. The simulations were run for 400 ns with timestep 2 fs and the first 100 ns were discarded as equilibration time. The simulations were run with GROMACS version 2020.2.[98] The Nose-Hoover thermostat[95,96] was used with temperature of 298 K and the time constant for temperature coupling was 1.0 ps. The semi-isotropic Parrinello-Rahman barostat[97] was used with reference pressure 1.0 bar and with a time constant of 5.0 ps with compressibility of 4.5e-5 bar^{-1}. Long range electrostatic interactions were calculated with the PME method. All bonds with hydrogen atoms were constrained with LINCS algorithm. The simulation files are available from Refs. 59–64.

POPC and POPC:POPG (7:3) mixture **17.Simulation details by A. Peon.**

POPC and cationic surfactant (dihexadecyldimethylammonium) mixture Intial structures were taken from similar previously published[13] simulations with Amber lipid14 force field, which are available from Ref. 99–104. Default simulations parameters and force field files from CHARMM-GUI[94] were used, except for dihexadecyldimethylammonium for which the atom types and partial charges of Amber lipid14 parameters from previous work[13] were

modified to correspond Charmm36 force field. Systems contained 50 POPC molecules, 3983 water molecules, and 12, 30, 44, or 88 dihexadecyldimethylammonium molecules. Chloride ions were used as counterions for dihexadecyldimethylammonium. Reference system without cationic surfactants contained 200 POPC and 9000 water molecules. Systems were simulated 200 ns (the first 20 ns was discarded as an equilibration period) using Gromacs 5[105] at the temperature of 313 K. All simulation files are available from Refs. 106,107.

S5.2 CHARMM36ua

POPE Data is available at.[27] **18.Simulation details by T. Piggot.**

S5.3 Slipids

POPE Data is available at.[30] **19.Simulation details by T. Piggot.**

DPPE with 288 lipids. The starting structure for simulation with 288 DPPE lipids and 9386 water molecules was constructed with the MEMBRANE BUILDER website.[108] The TIP3P[109] water model was used to solvate the system. Simulation was performed for 200 ns, and the last 100 ns were used for the analysis. Simulation was carried out within the NPT ensemble using the GROMACS 5.0.4 package.[105] Timestep of 2 fs was used with the leapfrog integrator. The Nosé–Hoover thermostat[95,96] was used with reference temperature of 336 K and a relaxation time constant of 0.5 ps; lipids and water were coupled separately to the heat bath. Pressure was kept constant at 1.013 bar using a semi–isotropic Parrinello–Rahman barostat[97] with a time constant of 10.0 ps. Long-range electrostatic interactions were calculated using the PME method.[110,111] A real space cut-off of 1.0 nm was employed with grid spacing of 0.12 nm in the reciprocal space. Lennard-Jones potentials were cut off at 1.4 nm, with a dispersion correction applied to both energy and pressure. All covalent bonds in lipids were constrained using the LINCS algorithm,[112] whereas water molecules were constrained using SETTLE.[113] Twin-range cutoffs, 1.0 nm and 1.6 nm, were used for the neighbor lists with the long-range neighbor list updated every 10 steps.

POPG with 288 lipids. The starting structure for simulation with 288 POPG lipids, 10664 water molecules and 288 Na ions was constructed with the MEMBRANE BUILDER website.[108] The TIP3P[109] water model was used to solvate the system and Ions are described by the parameters derived by Åqvist.[48] Simulation was performed for 250 ns, and the last 100 ns were used for the analysis. Same simulation conditions as DPPE with reference temperature of 298 K.

DPPG with 288 lipids. The starting structure for simulation with 288 DPPG lipids, 11232 water molecules and 288 Na ions was constructed with the MEMBRANE BUILDER website.[108] The TIP3P[109] water model was used to solvate the system and Ions are described by the parameters derived by Åqvist.[48] For the 298 K temperature, simulation was performed for 400 ns, and the last 100 ns were used for the analysis. For the 314 K temperature, simulation was performed for 200 ns, and the last 100 ns were used for the analysis. Same simulation conditions as DPPE for both temperatures.

POPC:POPG mixture with additional CaCl **20.Simulation details by M. Javanainen.**

S5.4 Berger

POPE Data is available at.[39,40] **21.Simulation details by T. Piggot.**

POPC:POPE mixtures Data is available at.[92,93] 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 **22.Simulation details by Fuchs et al.**

S5.5 GROMOS 43A1-S3

POPE Data is available at.[34] **23.Simulation details by T. Piggot.**

S5.6 OPLS-UA

POPE Data is available at.[36] **24.Simulation details by T. Piggot.**

POPE with vdW interaction in H Data is available at.[35] **25.Simulation details by T. Piggot.**

S5.7 GROMOS-CKP and GROMOS-CKPM

POPE Data is available at.[33] **26.Simulation details by A. Peon.**

DPPE Data is available at.[32] **27.Simulation details by T. Piggot.**

POPG **28.Simulation details by A. Peon.**

POPC:POPG mixture **29.Simulation details by A. Peon.**

S5.8 OPLS-MacRog

POPE **30.Simulation details by M. Javanainen**

POPC:POPE mixtures **31.Simulation details by P. Fuchs.**

S5.9 Lipid17

POPE **32.Simulation details by A. Peon.**

POPG **33.Simulation details by A. Peon.**

POPC:POPG 4:1 and POPC:POPG 1:1 mixtures with different $CaCl_2$ concentrations Initial structures were build by removing appropriate amount of lipids from POPC:POPG 7:3 mixture available from Ref. 114. Force field parameters from the same reference were used **34.We still need description from A. Peon how these were obtained**, except that incorrect dihedrals with type 1 were changed to type 9 (for details, see discussion in `https://github.com/NMRLipids/NMRlipidsIVPEandPG/issues/12`). Simulations were performed using the Gromacs simulation package[98] with the time step of 2 fs. The non-bonded interactions were calculated directly within 1.0 nm cutoff; the Verlet scheme was used;[115] and the long-range electrostatic forces were calculated using particle mesh Ewald.[111] The bond lengths of hydrogen atoms were constrained using LINCS.[112] Temperature was coupled to the velocity rescaling thermostat[116] at 298 K with a coupling constant of 1 ps. Pressure was

coupled to the Parrinello–Rahman barostat[97] at 1 bar with a coupling constant of 10 ps. For simulations with $CaCl_2$, appropriate amount of ions with Dang[53,54] parameters were added into the solvent. The simulation files are available from Refs. 77–82

S5.10 Lipid17ecc

POPC:POPG 4:1 and POPC:POPG 1:1 mixtures with different $CaCl_2$ concentrations Implicit inclusion of electronic polarizability by electronic continuum correction (ECC), implemented by scaling the partial charges in force fields, can be used to improve ion interactions with lipids and other biomolecules in classical MD simulations.[117] For Amber Lipid14/17 force fields, ECC has been previously implemented by scaling the charges and Lennard-Jones σs of headgroup, glycerol backbone, and carbonyl regions by constant factors.[13,14] Here, we apply similar ECC approach to Amber Lipid17 PG parameters as done previously for PS:[14] charges and Lennard-Jones σs of headgroup, glycerol backbone, and carbonyl regions of parameters POPG from Ref. 114 were scaled by factors of f_q=0.75 and f_σ=0.89, respectively (and the dihedral types were corrected to type 9 as in previous section). Previously introduced ECC-POPC parameters (scaling factors f_q=0.8 and f_σ=0.89 applied to Lipid14 POPC parameters) were used for POPC.[13] ECC-ion parameters with the scaled charges[83–85] from `bitbucket.org/hseara/ions/src/master/`, and SPC/E water model[118] were used in these simulations. Rest of the simulation parameters and initial configurations were taken from Lipid17 simulations.[77–82] Simulation files of Lipid17ecc simulations are available from Refs. 86–91.

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