Supporting Information:

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

Amélie Bacle,[†] Pavel Buslaev,[‡] Rebeca García Fandiño,[§] Fernando Favela-Rosales,[⊥] Tiago Ferreira,[#] Patrick F.J. Fuchs,[@,△] Ivan Gushchin,[¶] Matti Javanainen,[▽] Anne M. Kiirikki,[††] Jesper J. Madsen,[‡‡,¶¶] Josef Melcr,[▽,§§] Paula MilÃąn RodrÃŋguez,[‖‖] Markus S. Miettinen,[⊥⊥] O. H. Samuli Ollila,[*,††] Chris G. Papadopoulos,[##] Antonio Peón,[@@] Thomas J. Piggot,[△△] Ángel Piñeiro,[▽▽▽] and Salla I. Virtanen[††]

[†]*Laboratoire Coopératif "Lipotoxicity and Channelopathies âĂŞ ConicMeds", Université de Poitiers, 1 rue Georges Bonnet, 86000 Poitiers, France*

[‡]*Nanoscience Center and Department of Chemistry, University of JyvÃđskylÃđ, P.O. Box 35, 40014 JyvÃđskylÃđ, Finland*

[¶]*Research Center for Molecular Mechanisms of Aging and Age-related Diseases, Moscow Institute of Physics and Technology, 141701 Dolgoprudny, Russia*

[§]*Center for Research in Biological Chemistry and Molecular Materials (CiQUS), Universidade de Santiago de Compostela, E-15782 Santiago de Compostela, Spain*

[‖]*CIQUP, Centro de InvestigaÃğÃčo em QuÃŋmica, Departamento de QuÃŋmica e BioquÃŋmica, Faculdade de CiÃłncias, Universidade do Porto, Porto, Portugal*

[⊥]*Departamento de Ciencias Básicas, Tecnológico Nacional de México, ITS Zacatecas Occidente, México*

[#]*Halle, Germany*

[@]*Sorbonne Université, Ecole Normale Supérieure, PSL University, CNRS, Laboratoire des Biomolécules (LBM), 75005 Paris, France*

[△]*Université de Paris, UFR Sciences du Vivant, 75013, Paris, France*

[▽]*Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nám. 542/2, CZ-16610 Prague 6, Czech Republic*

[††]*Institute of Biotechnology, University of Helsinki*

[‡‡]*Department of Chemistry, The University of Chicago, Chicago, Illinois, United States of America*

[¶¶]*Global and Planetary Health, College of Public Health, University of South Florida, Tampa, Florida, United States of America*

[§§]*Groningen Biomolecular Sciences and Biotechnology Institute and The Zernike Institute for Advanced Materials, University of Groningen, 9747 AG Groningen, The Netherlands*

[‖‖]*Sorbonne UniversitÃĩ, Ecole Normale SupÃĩrieure, PSL University, CNRS, Laboratoire des BiomolÃĩcules (LBM), 75005 Paris, France*

[⊥⊥]*Department of Theory and Bio-Systems, Max Planck Institute of Colloids and Interfaces, 14424 Potsdam, Germany*

[##]*UniversitÃĩ Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France*

[@@]*Spain*

[△△]*Chemistry, University of Southampton, Highfield, Southampton SO17 1BJ, United Kingdom*

[▽▽▽]*Departamento de Física Aplicada, Facultade de Física, Universidade de Santiago de Compostela, E-15782 Santiago de Compostela, Spain*

E-mail: samuli.ollila@helsinki.fi

Contents

S1 R-PDLF and SDROSS experiments

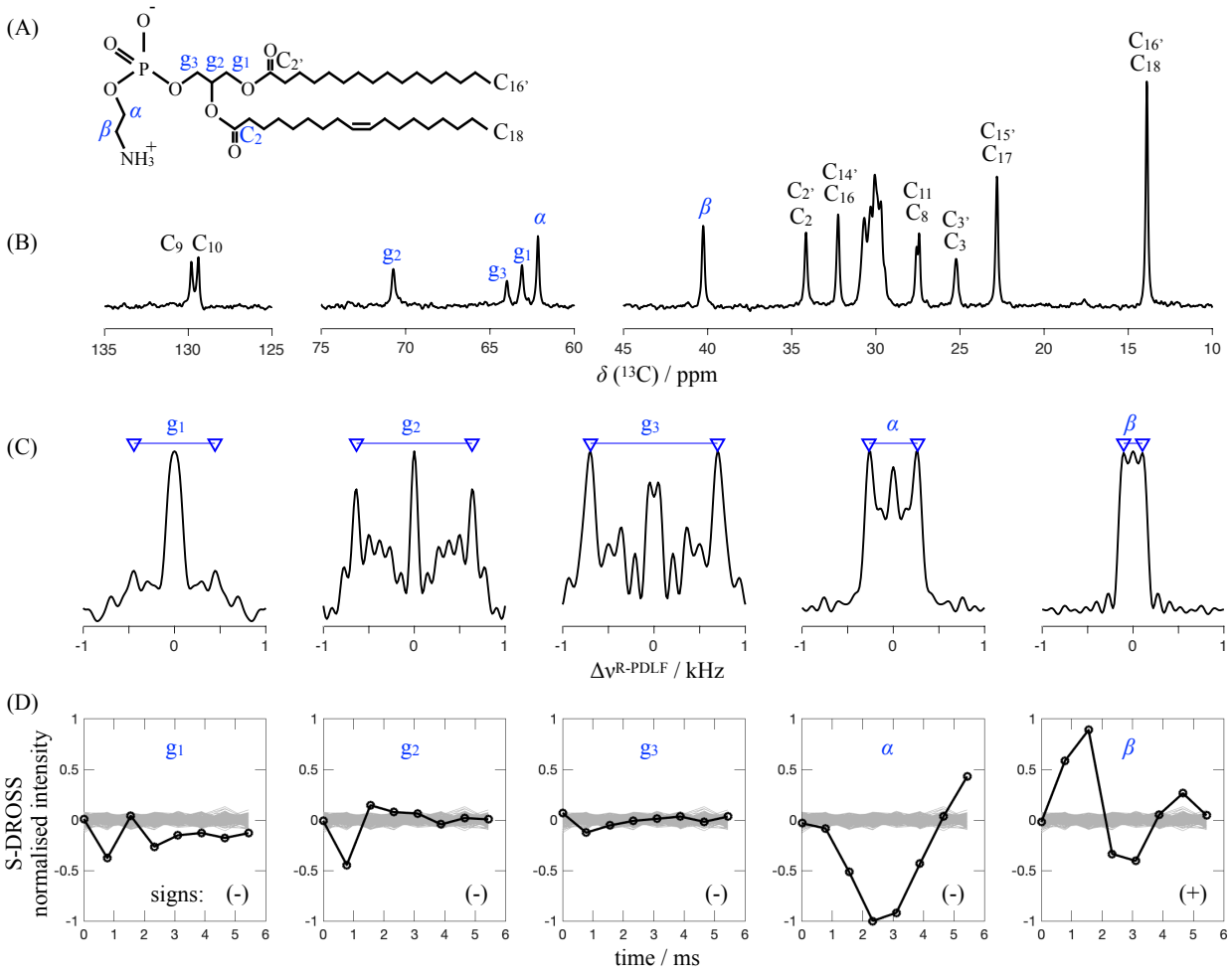

Figure S1: (A) Chemical structure of POPE with carbon labels. (B) Refocused INEPT ^{13}C spectrum of POPE MLVs. The peak assignment was based on previous work. (C) Headgroup and glycerol backbone R-PDLF dipolar slices. The arrows indicate the splittings used to determine the C–H bond order parameters by using $|S_{CH}| = \Delta\nu/0.315/d_{CH}$. The rigid coupling d_{CH} used was 22 kHz. (D) Experimental S-DROSS curves giving signs of the order parameters measured.

1.NMR figures in SI to be updated.

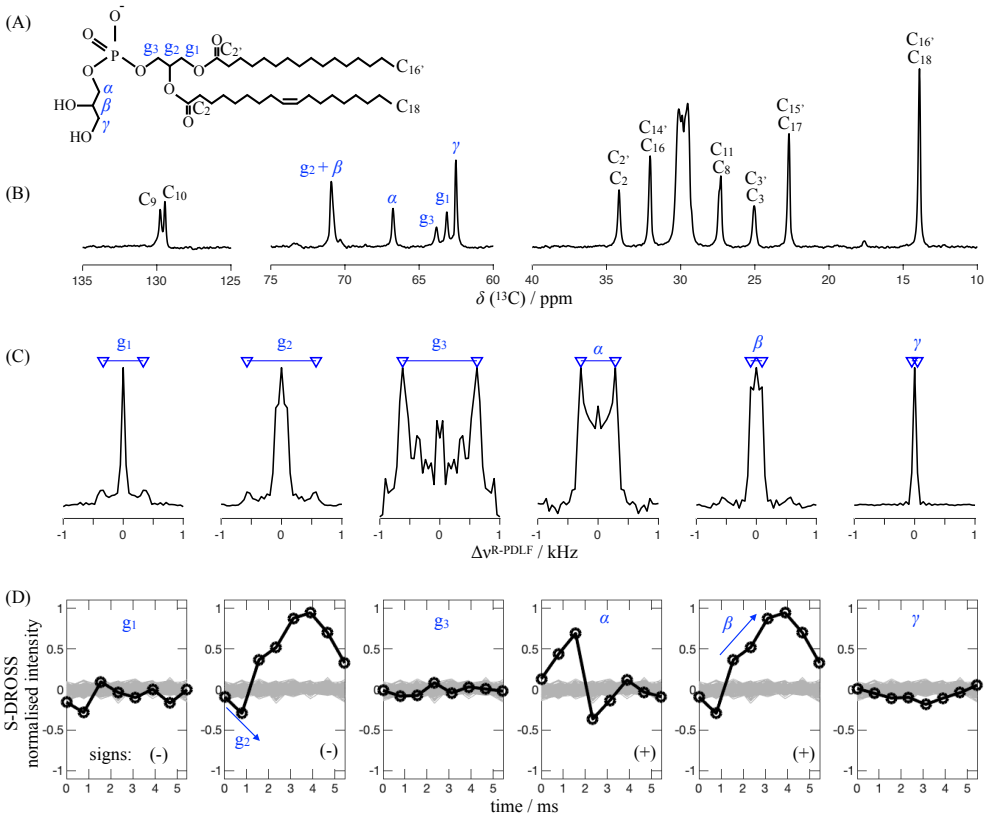

Figure S2: (A) Chemical structure of POPG with the labeling of headgroup and glycerol backbone carbons. (B) INEPT spectra from POPG sample with the headgroup and glycerol backbone peaks labeled. (C) Dipolar sliced from the 2D R-PDLF spectra with the resulting order parameters on top of figures. (D) Experimetal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

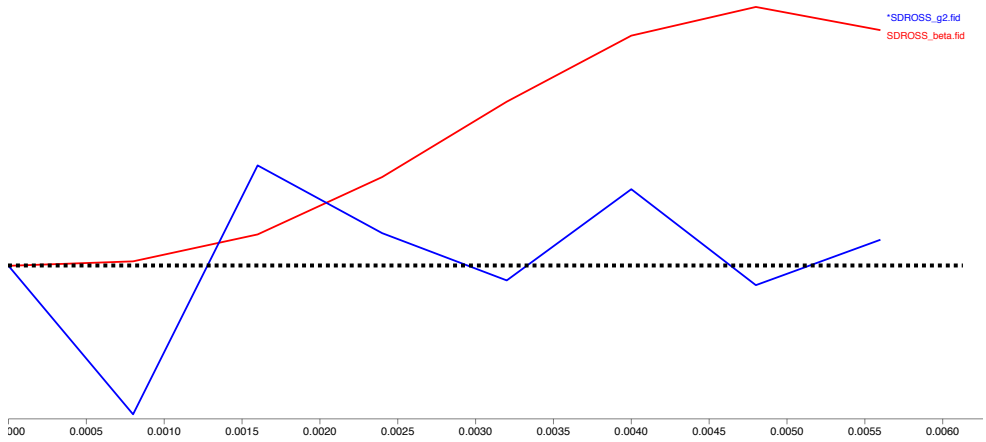

Figure S3: Simpson simulaton of S-DROSS curve of β-carbon of POPG.

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]

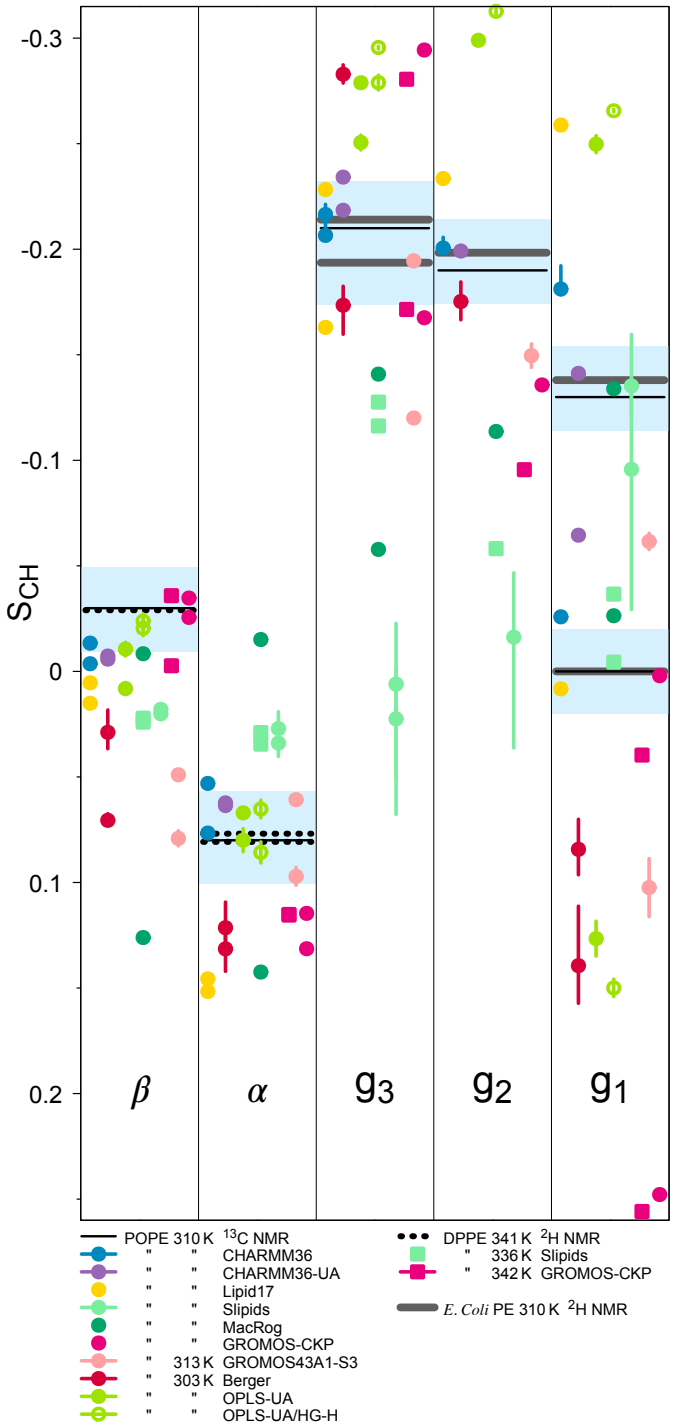

Figure S4: C–H bond order parameters, S_{CH}, of the PE headgroup (β and α) and glycerol backbone (g_3, g_2, g_1) carbons from NMR experiments (horizontal lines; POPE and signs this work, DPPE from Ref. 4, *Escherichia coli* PE from Ref. 5) and MD simulations with different force fields (symbols). The light blue areas span 0.04 units around the average of the extremal experimental values, in accordance with the expected quantitative accuracy of experiments.[6] The vertical bars shown for most simulation values are not error bars, but demonstrate that for these systems we had at least two data sets; the ends of the bars mark the extreme values from the sets, the symbol marks the measurement-time-weighted average.

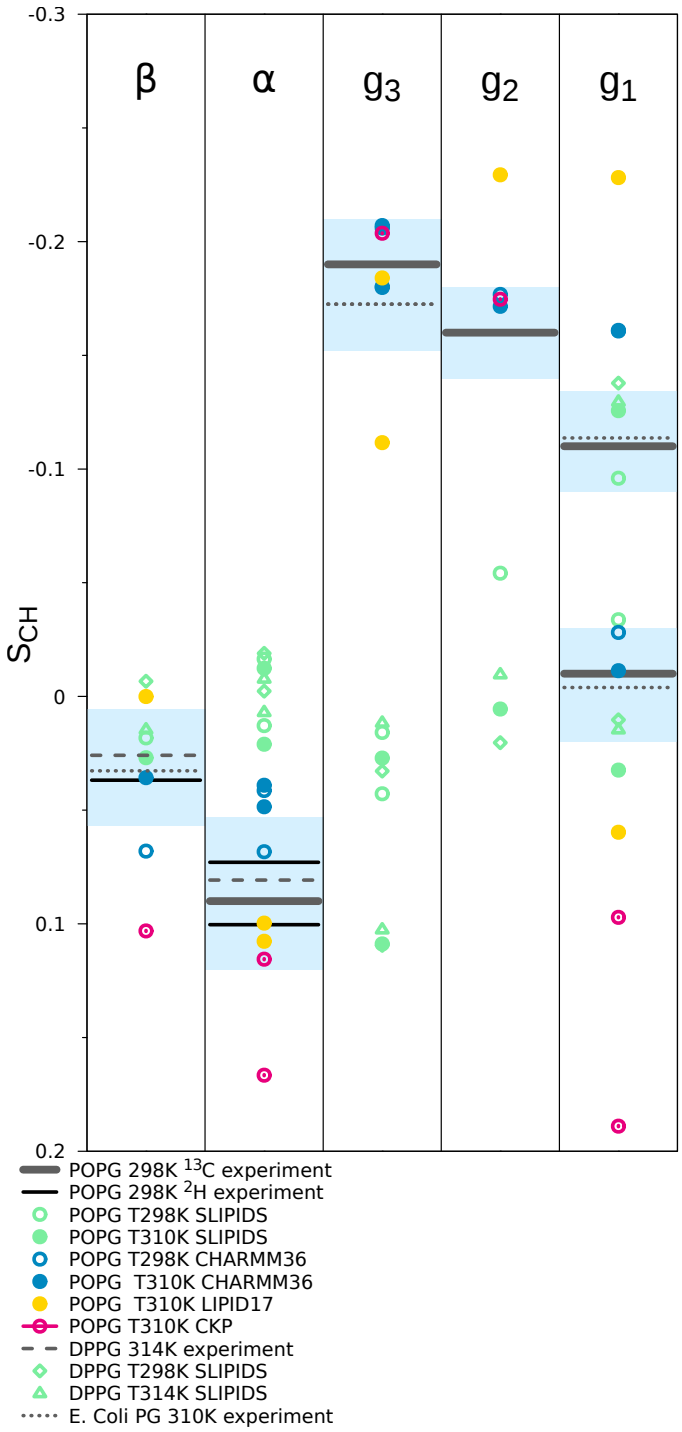

Figure S5: The headgroup and glycerol backbone order parameters of PG lipids from experiments (POPG and signs from this work and from Ref. 7, DPPG with 100mM NaCl from Ref. 3,and E.Coli PG results from Ref. 5) and simulations with different force fields.

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.[8,10,11] 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.[8] The good performance of Berger-OPLS simulations is surprising here 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.

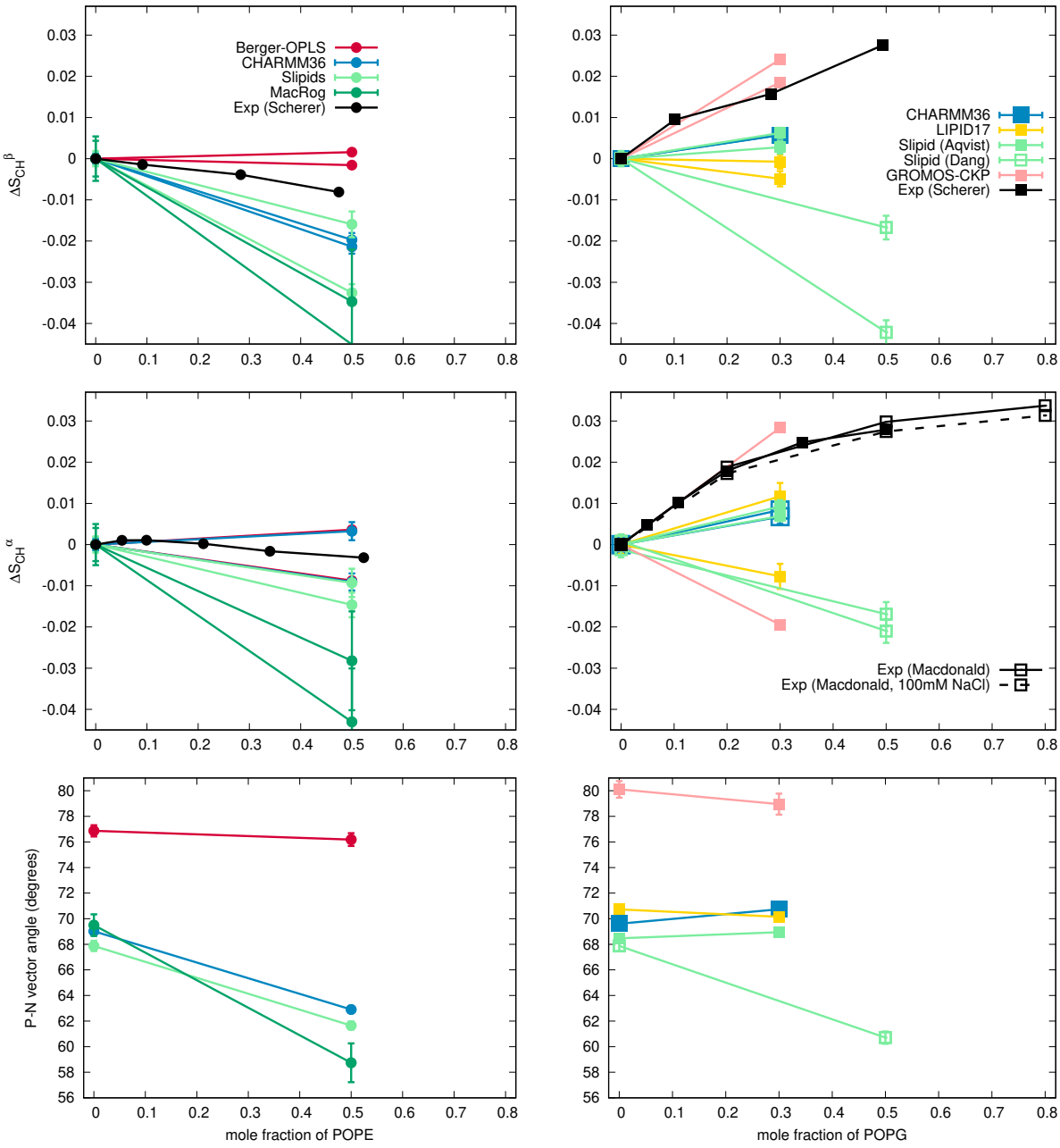

Figure S6: Modulation of POPC headgroup order parameters with increasing amount of POPE (left) and POPG (right) in bilayer from experiments at 298 K[8,9] and simulations with different force fields (temperatures listed in tables S3 and S4 are between 298-310 K). Signs are determined as discussed in Refs. 1,6.

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[9] or is unchanged[7] 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.

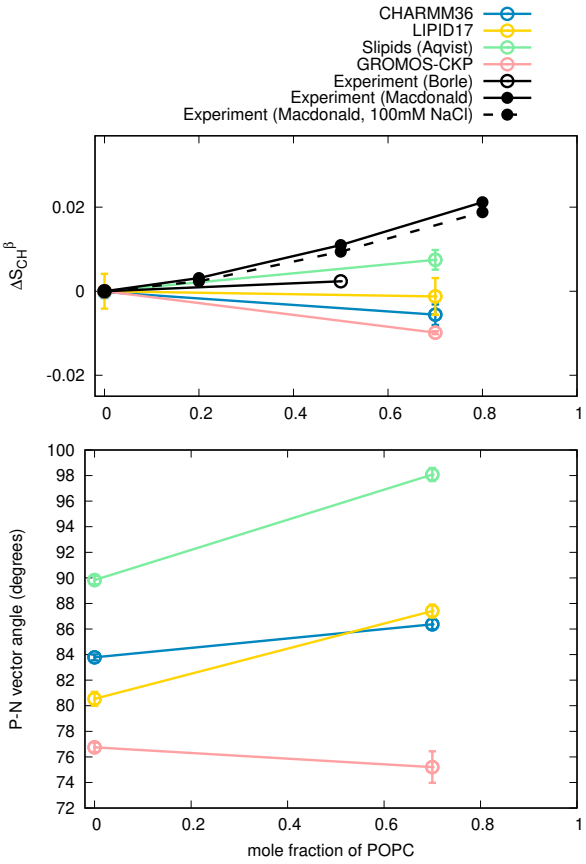

Figure S7: Modulation of PG lipid headgroup order parameters with the increasing amount of PC in lipid bilayer from experiments at 298 K[7,9] and simulations with different force fields at 310 K.

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[7,9] 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]

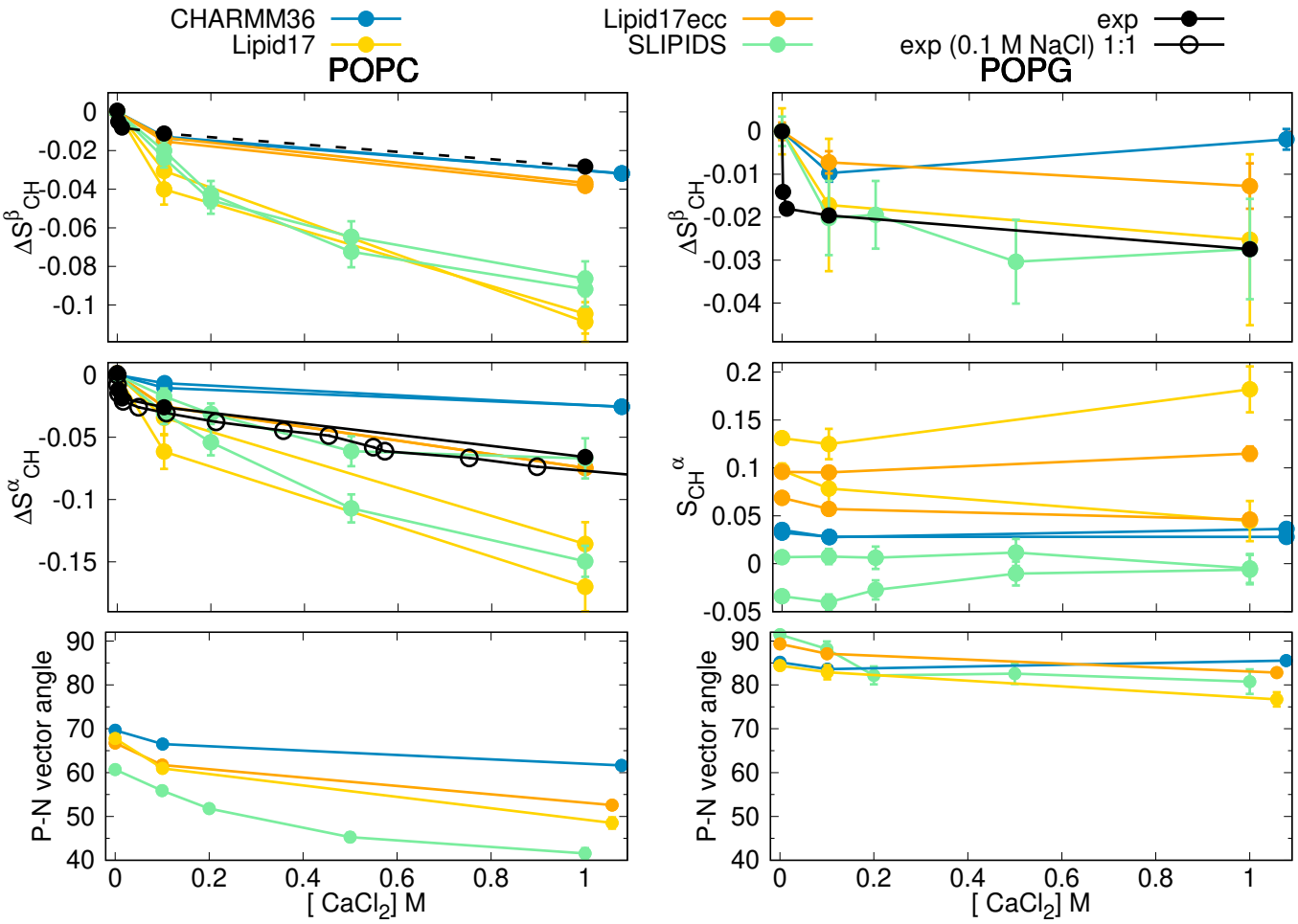

Figure S8: Modulation of headgroup order parameters of POPC (*left*) and POPG (*right*) in POPC:POPG (1:1) mixture upon addition of $CaCl_2$ in 298 K temperature from experiments[7,9] and simulations. The β-carbon order parameter of POPC (dashed line on top left) is not directly measured but calculated 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.

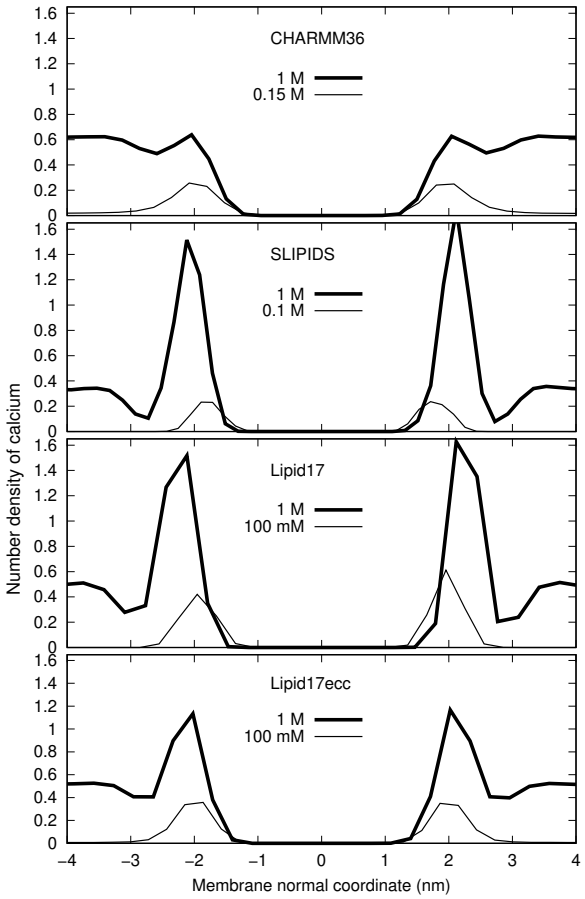

Figure S9: Calcium ion density profiles along membrane normal from simulations of POPC:POPG (1:1) mixtures with different force fields. The changes in the order parameters upon addition of $CaCl_2$ are compared with experiments in figure S8.

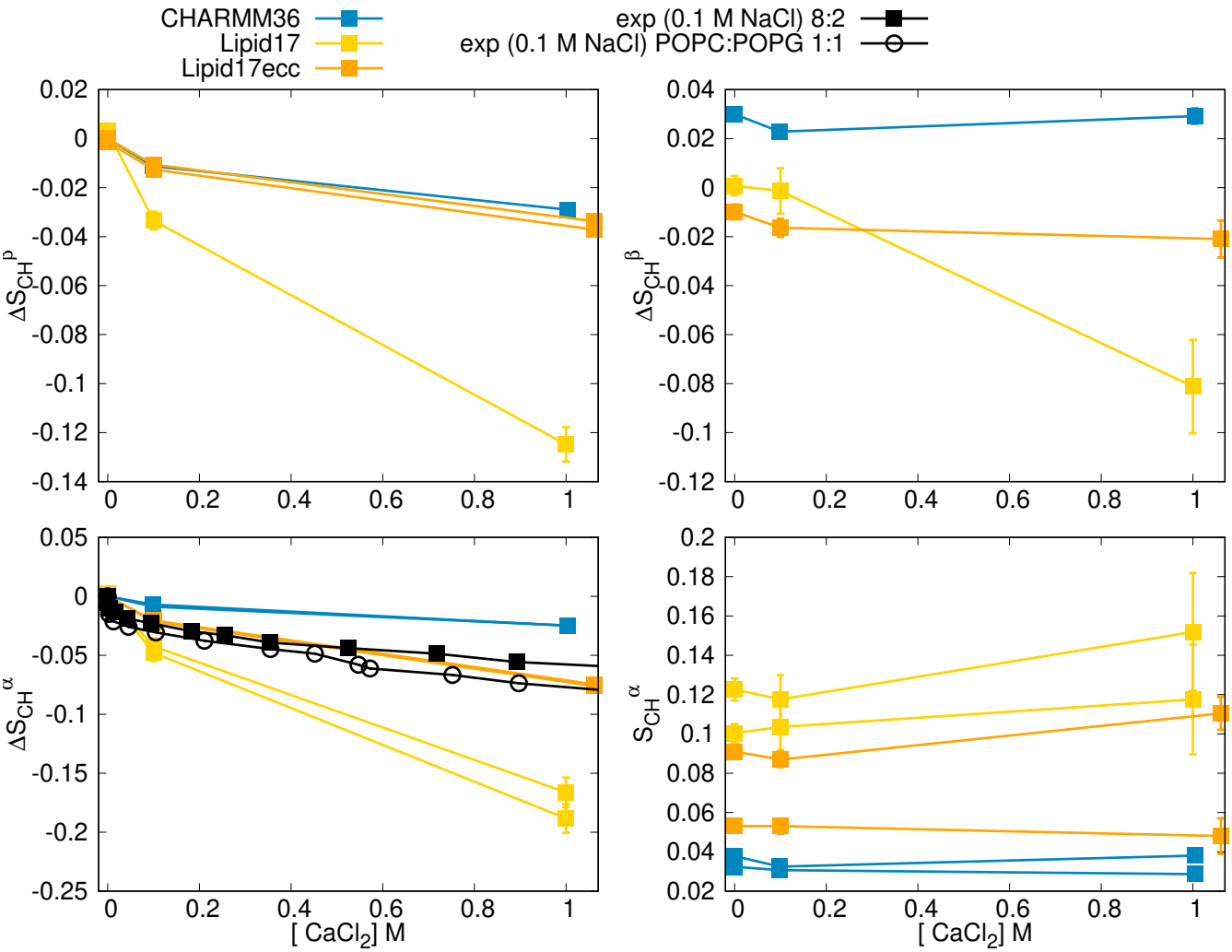

Figure S10: Modulation of headgroup order parameters of POPC (*left*) and POPG (*right*) in POPC:POPG (4:1) mixture upon addition of $CaCl_2$ in 298 K temperature from experiments[9] and simulations. The changes with respect to the systems without $CaCl_2$ are shown for other data than for the α-carbon of POPG for which experimental order parameter is not available.

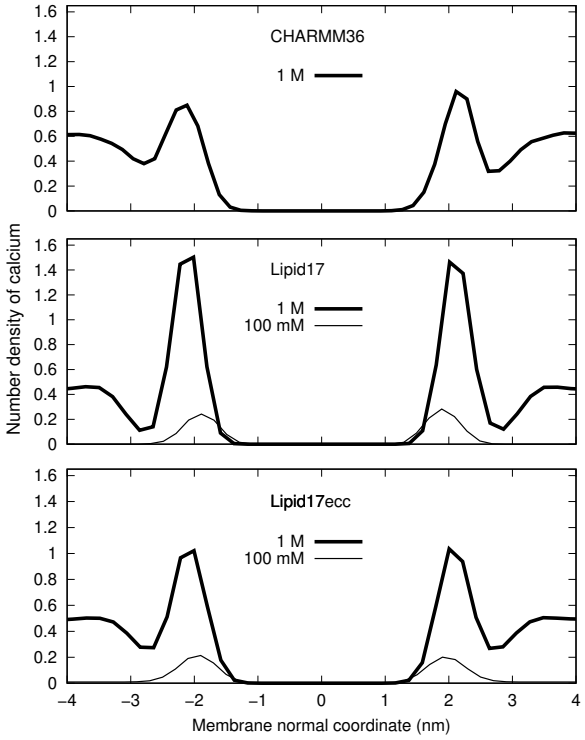

Figure S11: Calcium ion density profiles along membrane normal from simulations of POPC:POPG (4:1) mixtures with different force fields.

S4 Dihedral angle distributions and the analysis of relative energies

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

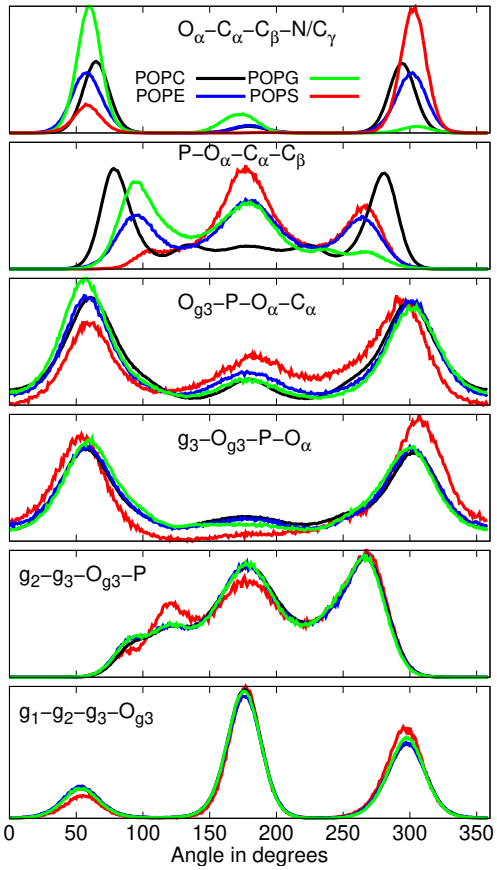

Figure S12: Heavy atom dihedral angle distributions from CHARMM36 simulations that correctly capture the order parameter differences between the force fields.

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

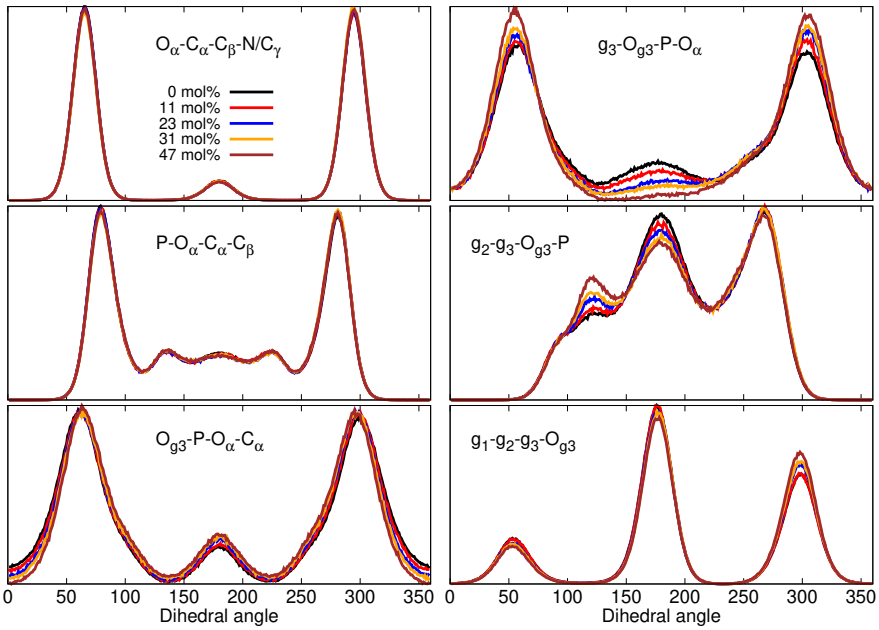

Figure S13: Changes in PC headgroup conformational ensembles upon increasing the amount of positive charge in bilayer, characterized by the heavy atom dihedral distributions, from CHARMM36 simulations.

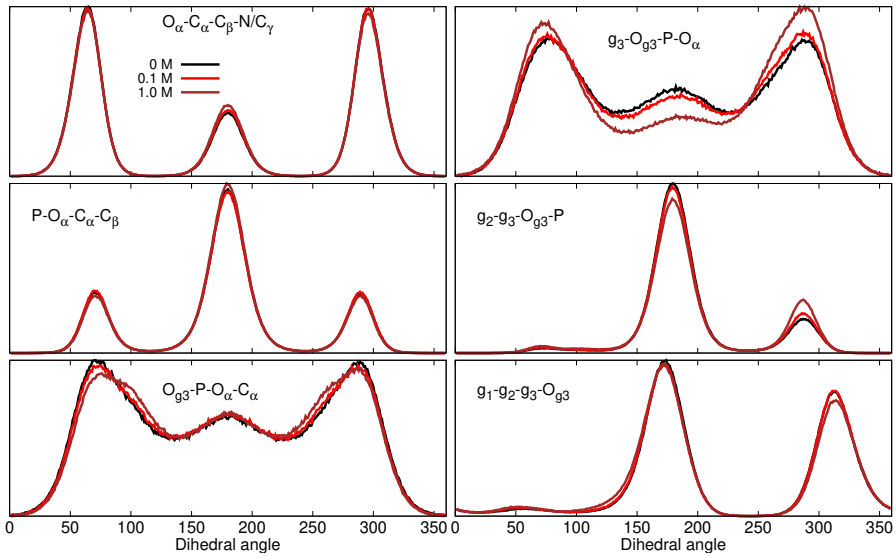

Figure S14: Changes in POPC lipid17ecc dihedrals with increasing amount of $CaCl_2$.

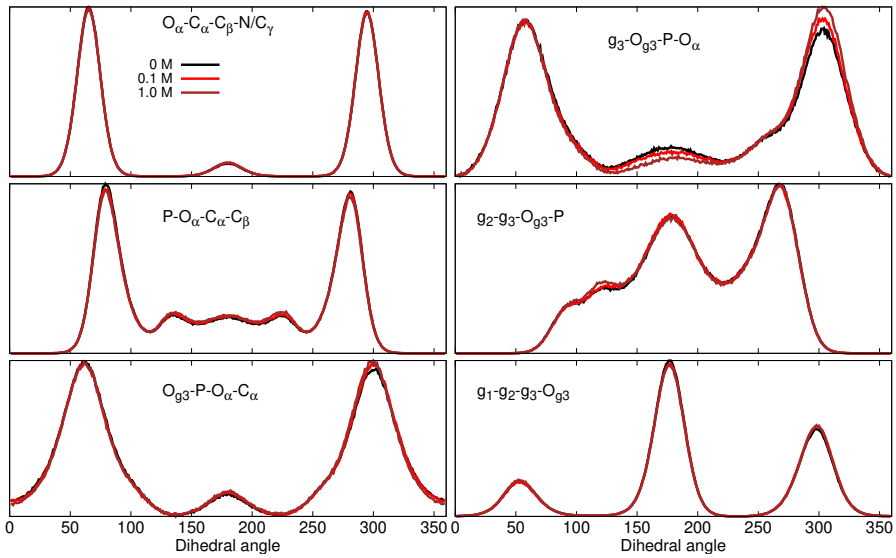

Figure S15: Changes in POPC CHARMM36 dihedrals with increasing amount of $CaCl_2$.

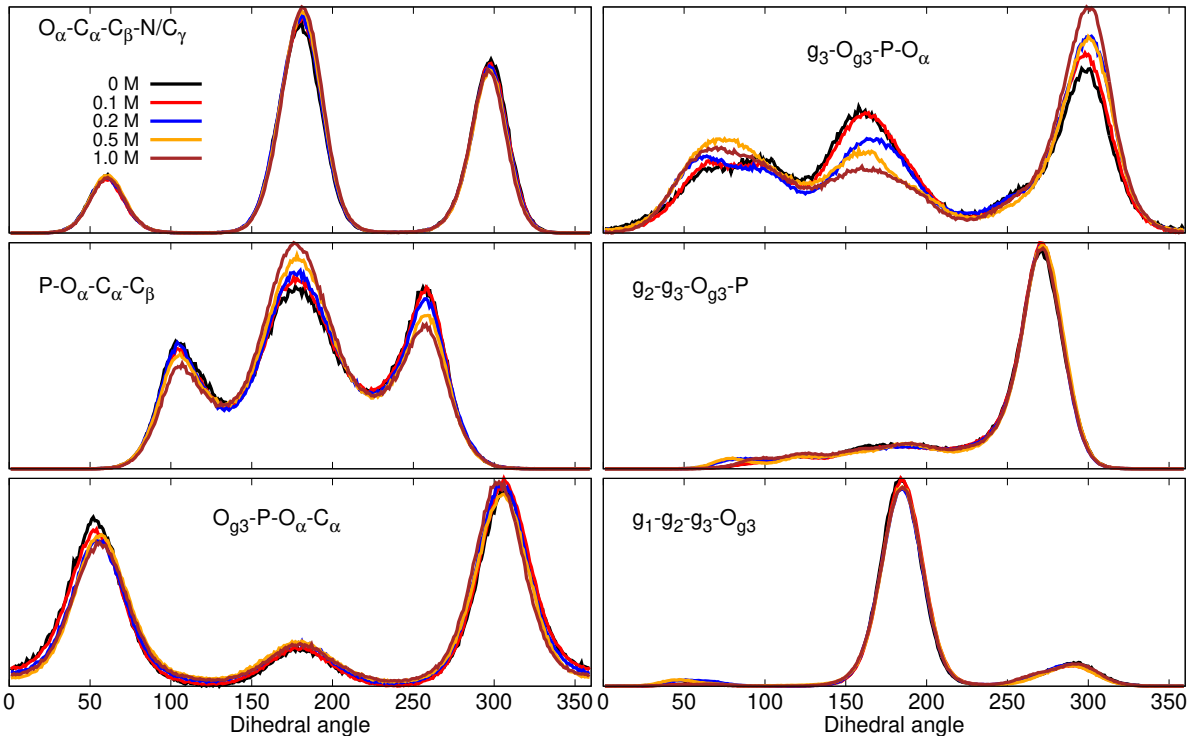

Figure S16: Changes in POPG Slipids dihedrals with increasing amount of $CaCl_2$.

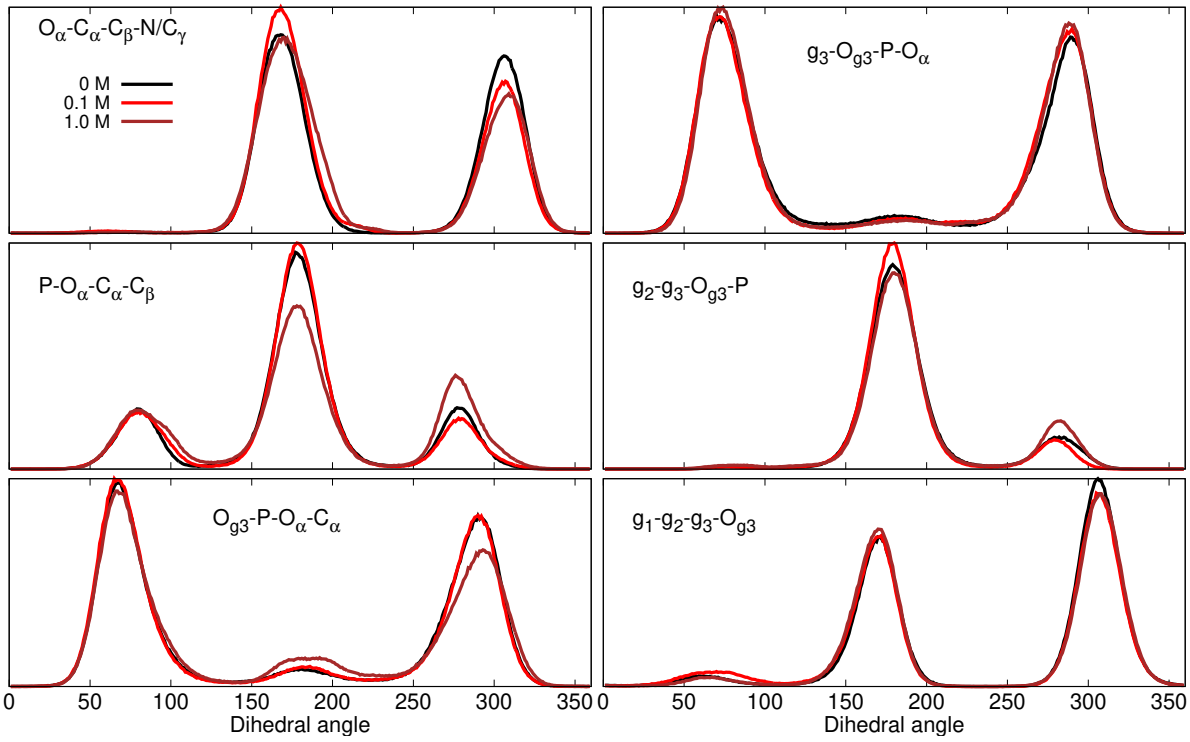

Figure S17: Changes in POPG lipid17 dihedrals with increasing amount of $CaCl_2$.

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	[a]N_l	[b]N_w	[c]T (K)	[d]t_{sim}(ns)	[e]t_{anal} (ns)	[f]files
POPE	CHARMM36[24]	144	5760	310	500	400	[25]
POPE	CHARMM36[24]	500	25000	310	500	100	[26]
POPE	CHARMM36-UA[?]	336	15254	310	2×200	2×100	[27]
DPPE	Slipids[28]	288	9386	336	200	100	[29]
POPE	Slipids[28]	336	?	310	2×200	2×100	[30]
POPE	Slipids[28]	500	25000	310	500	100	[31]
DPPE	GROMOS-CKP[?]	128	3655	342	2×500	2×400	[32]
POPE	GROMOS-CKP[?]	500	25000	310	500	100	[33]
POPE	GROMOS 43A1-S3[?]	128	3552	313	2×200	2×100	[34]
POPE	OPLS-UA/HG-H[?]	128	3328	303	2×200	2×100	[35]
POPE	OPLS-UA[?]	128	3328	303	2×200	2×100	[36]
POPE	OPLS-MacRog[37]	144	5760	310	500	350	[38]
POPE	Berger-POPE-2004[?]	128	3552	303	2×200	2×100	[39]
POPE	Berger-POPE-2018[?]	128	3552	303	2×200	2×100	[40]
POPE	Lipid17[41]	500	25000	310	500	100	[42]

[a]Number of lipid molecules
[b]Number of water molecules
[c]Simulation temperature
[d]Total simulation time
[e]Time used for analysis
[f]Reference for simulation files

2.Citation for CHARMM36ua?

3.Citation for GROMOS-CKP?

4.Citation for GROMOS 43A1-S3?

5.Citation for OPLS-UA models?

6.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[43]	0	118	4110	0	298	100	100	[44]
POPG	CHARMM36[43]	0	500	25000	0	310	500	100	[45]
POPG/Na^+	Slipids / Åqvist[46,47]	0	288	10664	0	298	250	100	[48]
DPPG/Na^+	Slipids / Åqvist[46,47]	0	288	11232	0	314	200	100	[49]
DPPG/Na^+	Slipids / Åqvist[46,47]	0	288	11232	0	298	400	100	[50]
POPG	Slipids / Åqvist[46,47]	0	500	25000	0	310	500	100	[51]
POPG	LIPID17 / Dang[41,52,53]	0	500	25000	0	310	500	100	[54]
POPG	GROMOS-CKP[?]	0	500	25000	0	310	500	100	[55]

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

7.Citation 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	[56]
POPC:POPG (7:3)	CHARMM36[24,43]	0	0	350	25000	0	310	500	100	[57]
POPC:POPG (1:1)	CHARMM36[24,43]	0	0	150:150	31500	0	298	500	400	[58]
POPC:POPG (1:1)	CHARMM36[24,43]	0	0.1	150:150	31329	57	298	400	300	[59]
POPC:POPG (1:1)	CHARMM36[24,43]	0	1.08	150:150	29766	578	298	500	400	[60]
POPC:POPG (4:1)	CHARMM36[24,43]	0	0	350:88	26280	0	298	500	400	[61]
POPC:POPG (4:1)	CHARMM36[24,43]	0	0.1	350:88	26280	47	298	500	400	[62]
POPC:POPG (4:1)	CHARMM36[24,43]	0	1.0	350:88	24927	451	298	500	400	[63]
POPC	CHARMM36[24]	0	0	256	8704	0	300	300	250	[64]
POPC:POPE (1:1)	CHARMM36[24,43]	0	0	128	8704	0	300	300	250	[65]
POPC	OPLS-MacRog[37]	0	0	128	5120	0	300	500	300	[66]
POPC:POPE (1:1)	OPLS-MacRog[37]	0	0	128	5120	0	300	500	300	[67]
POPC	Slipid[28]	0	0	512	23943	0	298	170	100	[68]
POPC:POPE (1:1)	Slipid[28]	0	0	128	5120	0	298	500	300	[69]
POPC	GROMOS-CKP / ??[? ?]	0	0	500	25000	0	310	500	100	[70]
POPC:POPG (7:3)	GROMOS-CKP / ??[? ?]	0	0	350:150	25000	0	310	500	100	[71]
POPC	Slipid[28]	0	0	500	25000	0	310	500	100	[72]
POPC:POPG (7:3)	Slipid / Åqvist[28,47]	0	0	350:150	25000	0	310	500	100	[73]
POPC:POPG (1:1)	Slipid / Dang[28,52,53,74]	0	0	128:128	12800	0	298	500	400	[75]
POPC:POPG (1:1)	Slipid / Dang[28,52,53,74]	0	0.1	128:128	12800	23	298	500	400	[75]
POPC:POPG (1:1)	Slipid / Dang[28,52,53,74]	0	0.2	128:128	12800	46	298	1500	500	[75]
POPC:POPG (1:1)	Slipid / Dang[28,52,53,74]	0	0.5	128:128	12800	115	298	1500	500	[75]
POPC:POPG (1:1)	Slipid / Dang[28,52,53,74]	0	1.0	128:128	12800	230	298	1500	500	[75]

[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

8.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[41,52,53]	0	0	350:88	26265	0	298	400	350	[76]
POPC:POPG (4:1)	Lipid17 / Dang[41,52,53]	0	0.1	350:88	26124	47	298	400	250	[77]
POPC:POPG (4:1)	Lipid17 / Dang[41,52,53]	0	1.0	350:88	24840	475	298	1200	200	[78]
POPC:POPG (1:1)	Lipid17 / Dang[41,52,53]	0	0	150:150	31572	0	298	320	200	[79]
POPC:POPG (1:1)	Lipid17 / Dang[41,52,53]	0	0.1	150:150	31401	57	298	718	198	[80]
POPC:POPG (1:1)	Lipid17 / Dang[41,52,53]	0	1.0	150:150	29865	569	298	720	200	[81]
POPC:POPG (4:1)	Lipid17ecc / ECC-ions[82–84]	0	0	350:88	26265	0	298	400	300	[85]
POPC:POPG (4:1)	Lipid17ecc / ECC-ions[82–84]	0	0.1	350:88	26124	47	298	400	300	[86]
POPC:POPG (4:1)	Lipid17ecc / ECC-ions[82–84]	0	1.0	350:88	24840	475	298	400	300	[87]
POPC:POPG (1:1)	Lipid17ecc / ECC-ions[82–84]	0	0	150:150	31572	0	298	347.8	333	[88]
POPC:POPG (1:1)	Lipid17ecc / ECC-ions[82–84]	0	0.1	150:150	29865	54	298	400	300	[89]
POPC:POPG (1:1)	Lipid17ecc / ECC-ions[82–84]	0	1.0	150:150	29865	569	298	600	400	[90]
POPC	Berger[?] 9.	0	0	256	10240	0	300	300	200	[91]
POPC:POPE (1:1)	Berger[?] 10.	0	0	128	11008	0	300	300	200	[92]

[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

11.Citation and description for "Berger" model?

S5.1 CHARMM36

POPE A lipid bilayer, consisting of a total of 144 POPE molecules, distributed equally between the two leaflets was set using CHARMM-GUI.[?] The bilayer was solvated by 5760 water molecules (40 per lipid). The random initial configuration and topologies were generated using the CHARMM-GUI web portal, which provides GROMACS-compatible simulation input files.[93] CHARMM36 lipid parameters[24] were used for POPE, whereas the CHARMM-specific TIP3P water model[94] was used for water. The bilayer was simulated for 500 ns using GROMACS 2018.6 at 310 K.

The recommended simulation parameters for CHARMM36 force field in GROMACS were used.[93] Namely, buffered Verlet lists were used to keep track of neighbouring atoms.[95] The Lennard-Jones potential was cut off at 1.2 nm with the forces switched to 0 between 1.0 nm and the cutoff value. The smooth PME algorithm[96,97] was used to account for long-range electrostatics. The temperatures of the lipid and the solvent were separately coupled to a Nosé-Hoover thermostat[98,99] with a time constant of 1 ps and a target temperature of 310 K. The system was coupled to a semi-isotropic (isotropic on the membrane plane) Parrinello–Rahman barostat[100] with a time constant of 5 ps, a reference pressure of 1 bar, and compressibility of 4.5×10^{-5} 1/bar. The bonds with hydrogen atoms were constrained using p-LINCS.[101,102]

The simulation files are available at Ref. 25.

POPG Lipid bilayer containing 118 POPG molecules, 4110 TIP3P water molecules, and 118 potassium ions was build using CHARMM-GUI.[93] The system was simulated 100 ns, coupled to 298 K using Nose-Hoover[98,99] thermostat and 1 bar with semi-isotropic Parrinello-Rahman[100] pressure coupling. The used default parameters and force field files from CHARMM-GUI were used. The used files are available from 44.

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

POPC:POPE mixtures Data is available at.[64,65] A pure POPC system and a 50:50 POPC:POPE mixture were built and equilibrated using CHARMM-GUI [https://doi.org/10.1021/acs.jctc.5b00935].

They contained 256 lipids (for the mixture 64 lipids per leaflet for each species) and 34 water molecules per lipid. No ions were added. The production simulations were run for 300 ns with a time step of 2 fs. The first 50 ns were discarded for the analysis. The simulations were run with the GROMACS 2016.4 [https://doi.org/10.1016/j.softx.2015.06.001] version. The v-rescale thermostat [http://dx.doi.org/10.1063/1.2408420] was used with a temperature of 300 K and a time constant of 1 ps; lipids and water were coupled separately to the heat bath. Pressure was kept constant at 1 bar using a semi-isotropic Parrinello–Rahman barostat [https://doi.org/10.1103/PhysRevLett.45.1196] with a time constant of 5.0 ps. A real space cut-off of 1.2 nm was employed for electrostatic interactions while the long-range part was evaluated using the PME method [https://doi.org/10.1063/1.464397, https://doi.org/10.1063/1.470117]. A force-based switching function was used to switch the Lennard-Jones forces to zero over a range of 1–1.2 nm. All bonds with hydrogen atoms were constrained with the LINCS algorithm [https://doi.org/10.1002/(SICI)1096-987X(199709)18:12Water molecules were kept rigid with the SETTLE algorithm [https://doi.org/10.1002/jcc. The simulation files are available from ref 64 [https://doi.org/10.5281/zenodo.1306800] (pure POPC) and 65 [https://doi.org/10.5281/zenodo.1306821] (POPC:POPE mixture).

POPC:POPG 1:1 and POPC:POPG 4:1 mixtures with additional calcium The initial structures were built with CHARMM-GUI Membrane Builder.[93] 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.[103] The Nose-Hoover thermostat[98,99] was used with temperature of 298 K and the time constant for temperature coupling was 1.0 ps. The semi-isotropic Parrinello-Rahman barostat[100] 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. 58–63.

POPC and POPC:POPG (7:3) mixture **13.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. 104–109. Default simulations parameters and force field files from CHARMM-GUI[93] 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[110] at the temperature of 313 K. All simulation files are available from Refs. 111,112.

S5.2 CHARMM36ua

POPE Data is available at.[27] **14.Simulation details by T. Piggot.**

S5.3 Slipids

POPE Data is available at.[30] **15.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.[113] The TIP3P[114] 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.[110] Timestep of 2 fs was used with the leapfrog integrator. The Nosé–Hoover thermostat[98,99] 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[100] with a time constant of 10.0 ps. Long-range electrostatic interactions were calculated using the PME method.[96,97] 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,[102] whereas water molecules were constrained using SETTLE.[115] 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.[113] The TIP3P[114] water model was used to solvate the system and Ions are described by the parameters derived by Åqvist.[47] 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.[113] The TIP3P[114] water model was used to solvate the system and Ions are described by the parameters derived by Åqvist.[47] 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:POPE mixture A POPC/POPE bilayer with its lipids distributed evenly among the leaflets was generated by from a pure POPC bilayer by removing and renaming atoms in the head group region. The bilayer contained a total of 100 POPC and 100 POPE lipids, and it was solvated by 45 water molecules per lipid (for a total of 9000 water molecules).

The Slipids force field[28,46,116] was used for lipids, and the TIP3P model[94] for water. A 300 ns-long simulation was performed using GROMACS 2019.4.[110] The simulation parameters were equal to those used for the POPC/POPG mixture with additional $CaCl_2$ (see below).

The simulation data are available at Ref. 69.

POPC:POPG mixture with additional $CaCl_2$ A lipid bilayer consisting of a total of 256 lipids

(128 POPC + 128 POPG) spread equally between the two leaflets was generated using CHARMM-GUI.[93] The membrane was solvated by 50 water molecules per lipid (a total of 12800), and 128 Na^+ counter ions for the POPG charges. The initial random configuration was equilibrated using the CHARMM-GUI protocol and using the CHARMM36 force field.[24] Next, additional ions were added to obtain initial $CaCl_2$ concentrations of 0, 100, 200, 500, or 1000 mM (0/0, 23/46, 46/92, 115/230, 230/460 Ca^{2+}/Cl^- ions) The systems were energy-minimized and equilibrated using the Slipids force field[28,46,116] before production simulations at 298 K. The ion parameters by Dang et al. were used.[53]

The neighbour lists with a cutoff of 1.0 nm were updated every 10 simulation steps. The smooth PME algorithm was used to calculate long-range electrostatics.[96,97] The Lennard-Jones potential was cut off at 1.0 nm, and the dispersion corrections[117] were applied to energy and pressure. The stochastic velocity rescaling thermostat[118] with a time constant of 0.5 ps and a target temperature of 298 K was applied separately to lipids and the solvent. A constant pressure of 1 bar was maintained by a Parrinello–Rahman barostat[100] with a time constant of 10 ps and compressibility of 4.5×10^{-5} 1/bar. The pressure coupling was performed semi-isotropically with the two simulation box vectors aligned along the membrane plane considered isotropic. All bonds were constrained using the p-LINCS algorithm.[101,102]

The systems simulated for 1500 ns ($CaCl_2$-containing systems) or 500 ns (systems without $CaCl_2$). The simulations were performed using GROMACS 2019.4,[110] and the simulation files are available at Ref. 75.

S5.4 Berger

Following the earlier convention in the NMRlipids Project,[1] for the Berger-based models we use the following naming convention: Berger - {*molecule name*} - {*year when model published first time*} {*citation*}.

POPE Data are available at Ref. 39 for Berger-POPE-2004[?] and at Ref. 40 for Berger-POPE-2018.[?] **16.Simulation details by T. Piggot.**

POPC:POPE mixtures Data is available at.[91,92] Two systems were simulated using the Berger force field [https://doi.org/10.1016/S0006-3495(97)78845-3], pure POPC and a mixture 50:50 POPC:POPE. For POPE, we additionnally used the de Vries modification implementing a repulsion of the hydrogen atoms located on the amino group of ethanolamine [https://doi.org/10.1021/jp0366926]. Starting from a PDB file of a pure POPC system with Berger atom names, a 50:50 POPC:POPE mixture was built by mutating randomly methyl groups to hydrogens (POPC -> POPE). Each system contained 256 lipids (for the mixture 64 lipids per leaflet for each species) and about 40 water molecules per lipid. No ions were added. Both systems were minimized and equilibrated. The production simulations were run for 300 ns with a time step of 2 fs. The first 100 ns were discarded for the analysis (@Samuli: this sentence may be removed if the analysis was done on a different time window). The simulations were run with GROMACS 4.5.3 [https://doi.org/10.1093/bioinformatics/btt055] version. The v-rescale thermostat [http://dx.doi.org/10.1063/1.2408420] was used with a temperature of 300 K and a time constant of 0.1 ps; lipids and water were coupled separately to the heat bath. Pressure was kept constant at 1 bar using a semiâĂŞisotropic ParrinelloâĂŞRahman barostat [https://doi.org/10.1103/PhysRevLett.45.1196] with a time constant of 4.0 ps. A real space cut-off of 1.0 nm was employed for van der Waals and electrostatic interactions. The long-range part of electrostatic interactions was evaluated using the PME method [https://doi.org/10.1063/1.464397, https://doi.org/10.1063/1.470117] with a grid spacing of 0.12 nm and an interpolation order of 4. All bonds with hydrogen atoms were constrained with the LINCS algorithm [https://doi.org/10.1002/(SICI)1096-987X(199709)18:12

S5.5 GROMOS 43A1-S3

POPE Data is available at.[34] **17.Simulation details by T. Piggot.**

S5.6 OPLS-UA

POPE Data is available at.[36] **18.Simulation details by T. Piggot.**

POPE with vdW interaction in H (OPLS-UA/HG-H) Data is available at.[35] **19.Simulation details by T. Piggot.**

S5.7 GROMOS-CKP and GROMOS-CKPM

POPE Data is available at.[33] **20.Simulation details by A. Peon.**

DPPE Data is available at.[32] **21.Simulation details by T. Piggot.**

POPG **22.Simulation details by A. Peon.**

POPC:POPG mixture **23.Simulation details by A. Peon.**

S5.8 OPLS-MacRog

POPE

A bilayer patch with a total of 144 POPE molecules, distributed evenly between two leaflets, was created by reordering atoms in a final structure of a POPE simulation performed using the CHARMM36 force field. The bilayer was hydrated by 40 water molecules per lipid for a total of 5760 water molecules. The OPLS-based MacRog force field was used[119] for lipids and the TIP3P model[94] for water.

Buffered Verlet lists[95] were used to keep track of neighbours. The Lennard-Jones potential was cut off at 1.0 nm, and dispersion corrections were applied to energy and pressure.[117] Smooth PME algorithm was used to calculate long-range electrostatics.[96,97] The Nosé–Hoover thermostat was used to keep the temperatures of the lipids and water at 310 K. These groups were coupled separately, and a time constat of 0.4 ps was used. The Parrinello–Rahman barostat was used to keep the pressures in the membrane plane as well as normal to it constant at 1 bar. For the barostat, a time constant of 10 ps was used, and the membrane compressibility was set to 4.5×10^{-5} 1/bar. All bonds were constrained using p-LINCS.[101,102]

The system was simulated for 500 ns using GROMACS 2019.2,[110] and the simulation data are available at Ref. 38.

POPC:POPE mixtures

Force field details Simulations of POPC:POPE mixtures using the OPLS-MacRog force field [https://doi.org/10.1021/jp5016627] were performed. The initial force field parameter files in GROMACS format were taken from ref [https://doi.org/10.1016/j.dib.2016.03.067]. However, a number of errors were detected in the published files so we fixed them in the following way. For POPE, the two aliphatic chains sn-1 and sn-2 were switched (in the file the lipid was in fact OPPE); one atom (named C27) was not connected to the previous atom in the aliphatic chain so bonds, angles, dihedrals, pairs were included to create the connection; two atoms were called C27 thus one of them was renamed C28. For POPC, some impropers were missing leading to non planar systems for double bonds (in particular the carbonyls of sn-1 and sn-2 as well as the double bond of the oleoyl chain), we thus added back those impropers. The resulting fixed files can be found on Zenodo (https://zenodo.org/record/3741793/files/popc_fixed.itp, https://zenodo.org/record/3725637/files/pope_fi
To check our fix, one simulation of pure POPC was also run and the order parameter compared to previous published results of ref [https://doi.org/10.1021/acs.jpcb.5b04878]. We found very similar values. All the details of the procedure described here are described in https://www.dsimb.inserm.fr/ fuchs/project_Samuli/POPC_POPE/report_results_comparison.pdf.

The data for pure POPC are available from [https://zenodo.org/record/3741793], for the POPC:POPE mixture from [https://zenodo.org/record/3725637].

Simulation details Two systems of pure POPC and 50:50 POPC:POPE mixture were built using CHARMM-GUI [https://doi.org/10.1021/acs.jctc.5b00935]. They contained 128 lipids and 5120 TIP3 water molecules. The initial PDB file was modified to match OPLS-MacRog nomenclature and atom order. No ions were added. The production simulations were run for 500 ns with a time step of 2 fs. The first 200 ns were discarded for the analysis (@Samuli: this sentence may be removed if the analysis was done on a different time window). The simulations were run withthe GROMACS 2018.5 [https://doi.org/10.1016/j.softx.2015.06.001] version. The v-rescale thermostat [http://dx.doi.org/10.1063/1.2408420] was used with a temperature of 300 K and a time constant of 0.1 ps; lipids and water were coupled

separately to the heat bath. Pressure was kept constant at 1 bar using a semiâĂŞisotropic ParrinelloâĂŞRahman barostat [https://doi.org/10.1103/PhysRevLett.45.1196] with a time constant of 4.0 ps. Long-range electrostatic interactions were calculated using the PME method [https://doi.org/10.1063/1.464397, https://doi.org/10.1063/1.470117]. A real space cut-off of 1.0 nm was employed with a grid spacing of 0.1 nm in the reciprocal space. Lennard-Jones potentials were cut off at 1.0 nm. All covalent bonds in lipids were constrained using the LINCS algorithm [https://doi.org/10.1002/(SICI)1096-987X(199709)18:12

S5.9 Lipid17

POPE **24.Simulation details by A. Peon.**

POPG **25.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. 120. Force field parameters from the same reference were used **26.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[103] with the time step of 2 fs. The non-bonded interactions were calculated directly within 1.0 nm cutoff; the Verlet scheme was used;[95] and the long-range electrostatic forces were calculated using particle mesh Ewald.[97] The bond lengths of hydrogen atoms were constrained using LINCS.[102] Temperature was coupled to the velocity rescaling thermostat[118] at 298 K with a coupling constant of 1 ps. Pressure was coupled to the Parrinello–Rahman barostat[100] at 1 bar with a coupling constant of 10 ps. For simulations with $CaCl_2$, appropriate amount of ions with Dang[52,53] parameters were added into the solvent. The simulation files are available from Refs. 76–81

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.[121] 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. 120 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[82–84] from `bitbucket.org/hseara/ions/src/master/`, and SPC/E water model[122] were used in these simulations. Rest of the simulation parameters and initial configurations were taken from Lipid17 simulations.[76–81] Simulation files of Lipid17ecc simulations are available from Refs. 85–90.

S6 Author contributions

Amélie Bacle Amélie Bacle set up, performed and analysed POPC and POPC:POPE (1:1) simulations with the Berger Force Field.

Pavel Buslaev Performed the analysis of dihedrals and isomers of lipids. Analysed lipid structures from Protein Data Bank. Prepared panels for figures 2 and 4. Participated in discussions.

Rebeca García Fandiño

Fernando Favela-Rosales set up and performed DPPE, POPG and DPPG simulations with the Slipids Force Field.

Tiago M. Ferreira

Patrick F.J. Fuchs supervised Paula Milán Rodríguez, Amélie Bacle and Chris Papadopoulos, created the buildH software, contributed to many discussions.

Ivan Gushchin supervised Pavel Buslaev and contributed to the analysis of lipid structures in the Protein Data Bank.

Matti Javanainen set up and performed simulations using CHARMM36, Slipids, and MacRog lipid models. He contributed to the organization of the manuscript.

Anne M. Kiirikki

Jesper J. Madsen set up, performed, and analysed several of the CHARMM36 simulations. Provided comments on the manuscript.

Josef Melcr

Paula Milán Rodríguez set up, performed and analysed POPC and POPC:POPE (1:1) simulations with the MacRog Force Field. She also fixed the initial force field (itp) files prior to the simulations.

Markus S. Miettinen

O. H. Samuli Ollila designed the project and managed the work. Ran and analysed several simulations. Wrote the manuscript.

Chris G. Papadopoulos set up, performed and analysed POPC and POPC:POPE (1:1)

simulations with the CHARMM36 Force Field.

Antonio Peón

Thomas J. Piggot

Ángel Piñeiro

Salla I. Virtanen

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.

(2) Antila, H. S.; Buslaev, P.; Favela-Rosales, F.; Mendes Ferreira, T.; Gushchin, I.; Javanainen, M.; Kav, B.; Madsen, J. J.; Melcr, J.; Miettinen, M. S. et al. Headgroup Structure and Cation Binding in Phosphatidylserine Lipid Bilayers. *The Journal of Physical Chemistry B* **2019**, acs.jpcb.9b06091.

(3) Wohlgemuth, R.; Waespe-Sarcevic, N.; Seelig, J. Bilayers of phosphatidylglycerol. A deuterium and phosphorus nuclear magnetic resonance study of the head-group region. *Biochemistry* **1980**, *19*, 3315–3321.

(4) Seelig, J.; Gally, H. U. Investigation of phosphatidylethanolamine bilayers by deuterium and phosphorus-31 nuclear magnetic resonance. *Biochemistry* **1976**, *15*, 5199–5204.

(5) Gally, H. U.; Pluschke, G.; Overath, P.; Seelig, J. Structure of Escherichia coli membranes. Glycerol auxotrophs as a tool for the analysis of the phospholipid head-group region by deuterium magnetic resonance. *Biochemistry* **1981**, *20*, 1826–1831.

(6) Ollila, O. S.; Pabst, G. Atomistic resolution structure and dynamics of lipid bilayers in

simulations and experiments. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2016**, *1858*, 2512 – 2528.

(7) Borle, F.; Seelig, J. Ca2+ binding to phosphatidylglycerol bilayers as studied by differential scanning calorimetry and 2H- and 31P-nuclear magnetic resonance. *Chemistry and Physics of Lipids* **1985**, *36*, 263 – 283.

(8) Scherer, P.; Seelig, J. Structure and dynamics of the phosphatidylcholine and the phosphatidylethanolamine head group in L-M fibroblasts as studied by deuterium nuclear magnetic resonance. *EMBO J.* **1987**, *6*.

(9) Macdonald, P. M.; Seelig, J. Calcium binding to mixed phosphatidylglycerol-phosphatidylcholine bilayers as studied by deuterium nuclear magnetic resonance. *Biochemistry* **1987**, *26*, 1231–1240.

(10) Seelig, J.; MacDonald, P. M.; Scherer, P. G. Phospholipid head groups as sensors of electric charge in membranes. *Biochemistry* **1987**, *26*, 7535–7541.

(11) Antila, H. S.; Buslaev, P.; Favela-Rosales, F.; Mendes Ferreira, T.; Gushchin, I.; Javanainen, M.; Kav, B.; Madsen, J. J.; Melcr, J.; Miettinen, M. S. et al. Headgroup Structure and Cation Binding in Phosphatidylserine Lipid Bilayers. *The Journal of Physical Chemistry B* **0**, *0*, null.

(12) Catte, A.; Girych, M.; Javanainen, M.; Loison, C.; Melcr, J.; Miettinen, M. S.; Monticelli, L.; Maatta, J.; Oganesyan, V. S.; Ollila, O. H. S. et al. Molecular electrometer and binding of cations to phospholipid bilayers. *Phys. Chem. Chem. Phys.* **2016**, *18*, 32560–32569.

(13) Melcr, J.; Martinez-Seara, H.; Nencini, R.; Kolafa, J.; Jungwirth, P.; Ollila, O. H. S. Accurate Binding of Sodium and Calcium to a POPC Bilayer by Effective Inclusion of Electronic Polarization. *The Journal of Physical Chemistry B* **2018**, *122*, 4546–4557.

(14) Melcr, J.; Ferreira, T. M.; Jungwirth, P.; Ollila, O. H. S. Improved Cation Binding to Lipid Bilayers with Negatively Charged POPS by Effective Inclusion of Electronic Polarization. *Journal of Chemical Theory and Computation* **2020**, *16*, 738–748.

(15) Akutsu, H.; Seelig, J. Interaction of metal ions with phosphatidylcholine bilayer membranes. *Biochemistry* **1981**, *20*, 7366–7373.

(16) Michaud-Agrawal, N.; Denning, E. J.; Woolf, T. B.; Beckstein, O. MDAnalysis: A toolkit for the analysis of molecular dynamics simulations. *Journal of Computational Chemistry* **2011**, *32*, 2319–2327.

(17) Richard J. Gowers,; Max Linke,; Jonathan Barnoud,; Tyler J. E. Reddy,; Manuel N. Melo,; Sean L. Seyler,; Jan DomaÅĎski,; David L. Dotson,; SÃĺbastien Buchoux,; Ian M. Kenney, et al. MDAnalysis: A Python Package for the Rapid Analysis of Molecular Dynamics Simulations. Proceedings of the 15th Python in Science Conference. 2016; pp 98 – 105.

(18) McGibbon, R. T.; Beauchamp, K. A.; Harrigan, M. P.; Klein, C.; Swails, J. M.; Hernández, C. X.; Schwantes, C. R.; Wang, L.-P.; Lane, T. J.; Pande, V. S. MDTraj: A Modern Open Library for the Analysis of Molecular Dynamics Trajectories. *Biophysical Journal* **2015**, *109*, 1528 – 1532.

(19) Abraham, M.; van der Spoel, D.; Lindahl, E.; Hess, B.; the GROMACS development team, GROMACS user manual version 5.0.7. 2015.

(20) project, N. NMRLipidsIVb GitHub repository. `https://github.com/NMRLipids/NMRlipidsIVPEandPG`.

(21) ohsOllila,; et al., MATCH GitHub repository. `https://github.com/NMRLipids/MATCH`.

(22) Fuchs, P.; et al., BuildH GitHub repository. https://github.com/patrickfuchs/buildH.

(23) Piggot, T. J.; Allison, J. R.; Sessions, R. B.; Essex, J. W. On the Calculation of Acyl Chain Order Parameters from Lipid Simulations. *J. Chem. Theory Comput.* **2017**, *13*, 5683–5696.

(24) Klauda, J. B.; Venable, R. M.; Freites, J. A.; O'Connor, J. W.; Tobias, D. J.; Mondragon-Ramirez, C.; Vorobyov, I.; MacKerell Jr, A. D.; Pastor, R. W. Update of the CHARMM All-Atom Additive Force Field for Lipids: Validation on Six Lipid Types. *J. Phys. Chem. B* **2010**, *114*, 7830–7843.

(25) Javanainen, M. Simulation of a POPE bilayer at 310K with the CHARMM36 force field. 2019; https://doi.org/10.5281/zenodo.2641987.

(26) PEON, CHARMM36 POPE Bilayer Simulation (Last 100 ns, 310 K). 2019; https://doi.org/10.5281/zenodo.3237461.

(27) Piggot, T. CHARMM36-UA POPE Simulations (versions 1 and 2) 310 K (NOTE: hexagonal membrane and POPE is called PEUA). 2018; https://doi.org/10.5281/zenodo.1293774.

(28) Jämbeck, J. P. M.; Lyubartsev, A. P. An Extension and Further Validation of an All-Atomistic Force Field for Biological Membranes. *J. Chem. Theory Comput.* **2012**, *8*, 2938–2948.

(29) Favela-Rosales, F. MD simulation trajectory of a fully hydrated DPPE bilayer: SLIPIDS, Gromacs 5.0.4. 2017. 2017; https://doi.org/10.5281/zenodo.495247.

(30) Piggot, T. Slipids POPE Simulations (versions 1 and 2) 310 K (NOTE: hexagonal membrane). 2018; https://doi.org/10.5281/zenodo.1293813.

(31) Peon, A. SLIPID POPE Bilayer Simulation (Last 100 ns, 310 K). 2019; https://doi.org/10.5281/zenodo.3231342.

(32) Piggot, T. GROMOS-CKP DPPE Simulations (versions 1 and 2) 342 K. 2018; https://doi.org/10.5281/zenodo.1293957.

(33) PEON, A. GROMOS POPE Bilayer Simulation (Last 100 ns, 310 K). 2019; https://doi.org/10.5281/zenodo.3237754.

(34) Piggot, T. GROMOS 43A1-S3 POPE Simulations (versions 1 and 2) 313 K (NOTE: anisotropic pressure coupling). 2018; https://doi.org/10.5281/zenodo.1293762.

(35) Piggot, T. OPLS-UA POPE Simulations (versions 1 and 2) 303 K with vdW on H atoms. 2018; https://doi.org/10.5281/zenodo.1293853.

(36) Piggot, T. OPLS-UA POPE Simulations (versions 1 and 2) 303 K. 2018; https://doi.org/10.5281/zenodo.1293855.

(37) RÃşg, T.; OrÅĆowski, A.; Llorente, A.; Skotland, T.; SylvÃďnne, T.; Kauhanen, D.; Ekroos, K.; Sandvig, K.; Vattulainen, I. Data including GROMACS input files for atomistic molecular dynamics simulations of mixed, asymmetric bilayers including molecular topologies, equilibrated structures, and force field for lipids compatible with OPLS-AA parameters. *Data in Brief* **2016**, *7*, 1171 – 1174.

(38) Javanainen, M. Simulation of a POPE bilayer, lipid model based on OPLS-aa by Rog et al. 2019; https://doi.org/10.5281/zenodo.3571071.

(39) Piggot, T. Berger POPE Simulations (versions 1 and 2) 303 K - de Vries repulsive H. 2018; https://doi.org/10.5281/zenodo.1293889.

(40) Piggot, T. Berger POPE Simulations (versions 1 and 2) 303 K - larger repulsive H. 2018; https://doi.org/10.5281/zenodo.1293891.

(41) Gould, I.; Skjevik, A.; Dickson, C.; Madej, B.; Walker, R. Lipid17: A Comprehensive AMBER Force Field for the Simulation of Zwitterionic and Anionic Lipids. 2018; In preparation.

(42) PEON, A. LIPID17 POPE Bilayer Simulation (Last 100 ns, 310 K) using dihedral 9. 2019; https://doi.org/10.5281/zenodo.4424292.

(43) Venable, R. M.; Luo, Y.; Gawrisch, K.; Roux, B.; Pastor, R. W. Simulations of Anionic Lipid Membranes: Development of Interaction-Specific Ion Parameters and Validation Using NMR Data. *The Journal of Physical Chemistry B* **2013**, *117*, 10183–10192.

(44) Ollila, O. H. S. POPG lipid bilayer simulation at T298K ran with MODEL_CHARMM_GUI force field and Gromacs. 2017; https://doi.org/10.5281/zenodo.1011096.

(45) ANTONIO, CHARMM36 POPG Bilayer Simulation (Last 100 ns, 310 K). 2019; https://doi.org/10.5281/zenodo.3237463.

(46) Jämbeck, J. P. M.; Lyubartsev, A. P. Implicit inclusion of atomic polarization in modeling of partitioning between water and lipid bilayers. *Phys. Chem. Chem. Phys.* **2013**, *15*, 4677–4686.

(47) Åqvist, J. Ion-water interaction potentials derived from free energy perturbation simulations. *J. Phys. Chem.* **1990**, *94*, 8021–8024.

(48) Favela-Rosales, F. MD simulation trajectory of a fully hydrated POPG bilayer: SLIPIDS, Gromacs 5.0.4. 2017. 2017; https://doi.org/10.5281/zenodo.546133.

(49) Favela-Rosales, F. MD simulation trajectory of a fully hydrated DPPG bilayer @314K: SLIPIDS, Gromacs 5.0.4. 2017. 2017; https://doi.org/10.5281/zenodo.546136.

(50) Favela-Rosales, F. MD simulation trajectory of a fully hydrated DPPG bilayer @298K: SLIPIDS, Gromacs 5.0.4. 2017. 2017; https://doi.org/10.5281/zenodo.546135.

(51) PeÃşn, A. LIPID17 POPG Bilayer Simulation (Last 100 ns, 310 K) using dihedral type 9. 2019; https://doi.org/10.5281/zenodo.3832274.

(52) Smith, D. E.; Dang, L. X. Computer simulations of NaCl association in polarizable water. *J. Chem. Phys* **1994**, *100*, 3757–3766.

(53) Dang, L. X.; Schenter, G. K.; Glezakou, V.-A.; Fulton, J. L. Molecular simulation analysis and X-ray absorption measurement of Ca2+, K+ and Cl- ions in solution. *J. Phys. Chem. B* **2006**, *110*, 23644–54.

(54) PeÃşn, A. LIPID17 POPG Bilayer Simulation (Last 100ns, 310 K) using dihedral type 9 instead of type 1. 2020; https://doi.org/10.5281/zenodo.3832219.

(55) PEON, A. GROMOS POPG Bilayer Simulation (Last 100 ns, 310 K). 2019; https://doi.org/10.5281/zenodo.3266166.

(56) PEON, A. CHARMM36 POPC Bilayer Simulation (Last 100 ns, 310 K). 2019; https://doi.org/10.5281/zenodo.3247813.

(57) PEON, A. CHARMM36 POPC-POPG 7:3 Bilayer Simulation (Last 100 ns, 310 K). 2019; https://doi.org/10.5281/zenodo.3248689.

(58) Kiirikki, A. M.; Ollila, O. H. S. POPC:POPG 1:1 MD simulation with CHARMM36 in water and Na+ counter ions. 2020; https://doi.org/10.5281/zenodo.3997116.

(59) Kiirikki, A. M.; Ollila, O. H. S. POPC:POPG 1:1 MD simulation with CHARMM36 in 0.1 M CaCL solution and Na+ counter ions. 2020; https://doi.org/10.5281/zenodo.4005515.

(60) Kiirikki, A. M.; Ollila, O. H. S. POPC:POPG 1:1 MD simulation with CHARMM36 in 1 M CaCL solution and Na+ counter ions. 2020; https://doi.org/10.5281/zenodo.3997135.

(61) Kiirikki, A. M.; Ollila, O. H. S. POPC:POPG 4:1 MD simulation with CHARMM36 in water with Na+ counter ions. 2020; https://doi.org/10.5281/zenodo.3996952.

(62) Kiirikki, A. M.; Ollila, O. H. S. POPC:POPG 4:1 MD simulation with CHARMM36 in 0.1 M CaCL2 solution with Na+ counter ions. 2020; https://doi.org/10.5281/zenodo.3997019.

(63) Kiirikki, A. M.; Ollila, O. H. S. POPC:POPG 4:1 MD simulation with CHARMM36 in 1 M CaCL2 solution with Na+ counterions. 2020; https://doi.org/10.5281/zenodo.3997037.

(64) Papadopoulos, C.; Fuchs, P. F. CHARMM36 pure POPC MD simulation (300 K - 300ns - 1 bar). 2018; https://doi.org/10.5281/zenodo.1306800.

(65) Papadopoulos, C.; Fuchs, P. F. CHARMM36 POPC/POPE (50%-50%) MD simulation (300 K - 300ns - 1 bar). 2018; https://doi.org/10.5281/zenodo.1306821.

(66) Milan Rodriguez, P.; Fuchs, P. F. MacRog pure POPC MD simulation (300 K - 500ns - 1 bar). 2020; https://doi.org/10.5281/zenodo.3741793.

(67) Milan Rodriguez, P.; Fuchs, P. F. MacRog POPC/POPE 1:1 MD simulation (300 K - 500ns - 1 bar). 2020; https://doi.org/10.5281/zenodo.3725637.

(68) Favela-Rosales, F. MD simulation trajectory of a lipid bilayer: Pure POPC in water. SLIPIDS, Gromacs 4.6.3. 2016. 2016; https://doi.org/10.5281/zenodo.166034.

(69) Javanainen, M. Simulation of POPC:POPE 1:1 membrane with the Slipids force field. 2020; https://doi.org/10.5281/zenodo.3605386.

(70) PEON, A. GROMOS-CKP POPC Bilayer Simulation (Last 100 ns, 310 K). 2019; https://doi.org/10.5281/zenodo.3247435.

(71) PEON, A. GROMOS-CKP POPC-POPG 7:3 Bilayer Simulation (Last 100 ns, 310 K). 2019; https://doi.org/10.5281/zenodo.3266240.

(72) PEON, A. SLIPID POPC Bilayer Simulation (Last 100 ns, 310 K). 2019; https://doi.org/10.5281/zenodo.3235552.

(73) PeON, A. SLIPID POPC-POPG 7:3 Bilayer Simulation (Last 100 ns, 310 K). 2019; https://doi.org/10.5281/zenodo.3240156.

(74) Jämbeck, J. P.; Lyubartsev, A. P. Another piece of the membrane puzzle: extending slipids further. *Journal of chemical theory and computation* **2012**, *9*, 774–784.

(75) Javanainen, M. Simulations of POPC:POPG 1:1 membranes with varying levels of CaCl_2 using the Slipids force field. 2020; https://doi.org/10.5281/zenodo.3613573.

(76) Virtanen, S.; Ollila, O. H. S. LIPID17 POPC-POPG 80:20 MD simulation, Na+ counterions, 298K. 2019; https://doi.org/10.5281/zenodo.3693681.

(77) Virtanen, S.; Ollila, O. H. S. LIPID17 POPC-POPG 80:20 MD simulation, Na+ counterions and 100mM CaCl2, 298K. 2019; https://doi.org/10.5281/zenodo.3833725.

(78) Virtanen, S.; Ollila, O. H. S. LIPID17 POPC-POPG 80:20 MD simulation, Na+ counterions and 1000mM CaCl2, 298K. 2019; https://doi.org/10.5281/zenodo.3874378.

(79) Virtanen, S.; Ollila, O. H. S. LIPID17 POPC-POPG 50:50 MD simulation, Na+ counterions, 298K. 2019; https://doi.org/10.5281/zenodo.3857816.

(80) Virtanen, S.; Ollila, O. H. S. LIPID17 POPC-POPG 50:50 MD simulation, Na+ counterions and 100mM CaCl2, 298K. 2019; https://doi.org/10.5281/zenodo.3871590.

(81) Virtanen, S.; Ollila, O. H. S. LIPID17 POPC-POPG 50:50 MD simulation, Na+ counterions and 1000mM CaCl2, 298K. 2019; https://doi.org/10.5281/zenodo.3864993.

(82) Pluhařová, E.; Fischer, H. E.; Mason, P. E.; Jungwirth, P. Hydration of the chloride ion in concentrated aqueous solutions using neutron scattering and molecular dynamics. *Mol. Phys.* **2014**, *112*, 1230–1240.

(83) Kohagen, M.; Mason, P. E.; Jungwirth, P. Accounting for Electronic Polarization Effects in Aqueous Sodium Chloride via Molecular Dynamics Aided by Neutron Scattering. *J. Phys. Chem. B* **2016**, *120*, 1454–1460.

(84) Martínek, T.; Duboué-Dijon, E.; Timr, Š.; Mason, P. E.; Baxová, K.; Fischer, H. E.; Schmidt, B.; Pluhařová, E.; Jungwirth, P. Calcium ions in aqueous solutions: Accurate force field description aided by ab initio molecular dynamics and neutron scattering. *J. Chem. Phys.* **2018**, *148*, 222813.

(85) Kiirikki, A. M.; Ollila, O. H. S. Lipid17ecc POPC:POPG 4:1 MD simulation in water with Na+ counter ions. 2020; `https://doi.org/10.5281/zenodo.3997154`.

(86) Kiirikki, A. M.; Ollila, O. H. S. Lipid17ecc POPC:POPG 4:1 bilayer simulation in 0.1 M CaCl2 solution and Na+ counter ions. 2020; `https://doi.org/10.5281/zenodo.3997176`.

(87) Kiirikki, A. M.; Ollila, O. H. S. Lipid17ecc POPC:POPG 4:1 bilayer simulation in 1 M CaCl2 solution and Na+ counter ions. 2020; `https://doi.org/10.5281/zenodo.4492639`.

(88) Ollila, O. H. S.; Virtanen, I. S. ECC-LIPID17 POPC-POPG 50:50 MD simulation, Na+ counterions, 298K. 2020; `https://doi.org/10.5281/zenodo.3859339`.

(89) Ollila, O. H. S.; Virtanen, I. S. ECC-LIPID17 POPC-POPG 50:50 MD simulation, Na+ counterions and 100mM CaCl2, 298K. 2020; `https://doi.org/10.5281/zenodo.3855729`.

(90) Ollila, O. H. S.; Virtanen, I. S. ECC-LIPID17 POPC-POPG 50:50 MD simulation, Na+ counterions and 1000mM CaCl2, 298K. 2020; https://doi.org/10.5281/zenodo.3862036.

(91) AmÃľlie, B.; F.J., F. P. Berger pure POPC MD simulation (300 K - 300ns - 1 bar). 2018; https://doi.org/10.5281/zenodo.1402417.

(92) AmÃľlie, B.; F.J., F. P. Berger POPC/POPE (50:50 ratio) MD simulation (300 K - 400ns - 1 bar). 2018; https://doi.org/10.5281/zenodo.1402449.

(93) Lee, J.; Cheng, X.; Swails, J. M.; Yeom, M. S.; Eastman, P. K.; Lemkul, J. A.; Wei, S.; Buckner, J.; Jeong, J. C.; Qi, Y. et al. CHARMM-GUI Input Generator for NAMD, GROMACS, AMBER, OpenMM, and CHARMM/OpenMM Simulations Using the CHARMM36 Additive Force Field. *Journal of Chemical Theory and Computation* **2016**, *12*, 405–413.

(94) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of simple potential functions for simulating liquid water. *The Journal of chemical physics* **1983**, *79*, 926–935.

(95) Páll, S.; Hess, B. A flexible algorithm for calculating pair interactions on {SIMD} architectures. *Comput. Phys. Commun.* **2013**, *184*, 2641 – 2650.

(96) Darden, T.; York, D.; Pedersen, L. Particle mesh Ewald: An N·log(N) method for Ewald sums in large systems. *J. Chem. Phys.* **1993**, *98*, 10089–10092.

(97) Essman, U. L.; Perera, M. L.; Berkowitz, M. L.; Larden, T.; Lee, H.; Pedersen, L. G. A smooth particle mesh ewald potential. *J. Chem. Phys.* **1995**, *103*, 8577–8592.

(98) Nose, S. A molecular dynamics method for simulations in the canonical ensemble. *Mol. Phys.* **1984**, *52*, 255–268.

(99) Hoover, W. G. Canonical dynamics: Equilibrium phase-space distributions. *Phys. Rev. A* **1985**, *31*, 1695–1697.

(100) Parrinello, M.; Rahman, A. Polymorphic transitions in single crystals: A new molecular dynamics method. *J. Appl. Phys.* **1981**, *52*, 7182–7190.

(101) Hess, B. P-LINCS: A Parallel Linear Constraint Solver for Molecular Simulation. *J. Chem. Theory Comput.* **2008**, *4*, 116–122.

(102) Hess, B.; Bekker, H.; Berendsen, H. J. C.; Fraaije, J. G. E. M. LINCS: a linear constraint solver for molecular dynamics simulations. *J. Comput. Chem.* **1997**, *18*, 1463–1472.

(103) PÃąll, S.; Zhmurov, A.; Bauer, P.; Abraham, M.; Lundborg, M.; Gray, A.; Hess, B.; Lindahl, E. Heterogeneous parallelization and acceleration of molecular dynamics simulations in GROMACS. *The Journal of Chemical Physics* **2020**, *153*, 134110.

(104) Ollila, O. H. S. POPC bilayer simulated at T313K with the Lipid14 model using Gromacs. 2017; `https://doi.org/10.5281/zenodo.1020709`.

(105) Ollila, O. H. S. POPC bilayer with 10% of dihexadecyldimethylammonium simulated at T313K with the Lipid14 model using Gromacs. 2017; `https://doi.org/10.5281/zenodo.1020240`.

(106) Ollila, O. H. S. POPC bilayer with 20% of dihexadecyldimethylammonium simulated at T313K with the Lipid14 model using Gromacs. 2017; `https://doi.org/10.5281/zenodo.1020593`.

(107) Ollila, O. H. S. POPC bilayer with 30% of dihexadecyldimethylammonium simulated at T313K with the Lipid14 model using Gromacs. 2017; `https://doi.org/10.5281/zenodo.1020623`.

(108) Ollila, O. H. S. POPC bilayer with 42% of dihexadecyldimethylammonium simulated

at T313K with the Lipid14 model using Gromacs. 2017; https://doi.org/10.5281/zenodo.1020671.

(109) Ollila, O. H. S. POPC bilayer with 50% of dihexadecyldimethylammonium simulated at T313K with the Lipid14 model using Gromacs. 2017; https://doi.org/10.5281/zenodo.1020689.

(110) Abraham, M. J.; Murtola, T.; Schulz, R.; Páll, S.; Smith, J. C.; Hess, B.; Lindahl, E. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* **2015**, *1*, 19–25.

(111) Ollila, POPC CHARMM36 T313K. 2020; https://doi.org/10.5281/zenodo.4040423.

(112) Ollila, O. H. S. CHARMM36 simulations of POPC mixed with cationic surfactant. 2018; https://doi.org/10.5281/zenodo.1288297.

(113) Ghahremanpour, M. M.; Arab, S. S.; Aghazadeh, S. B.; Zhang, J.; van der Spoel, D. MemBuilder: a web-based graphical interface to build heterogeneously mixed membrane bilayers for the GROMACS biomolecular simulation program. *Bioinformatics* **2013**, *30*, 439–441.

(114) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* **1983**, *79*, 926–935.

(115) Miyamoto, S.; Kollman, P. A. SETTLE: An analytical Version of the SHAKE and RATTLE Algorithm for Rigid Water Models. *J. Comput. Chem* **1992**, *13*, 952–962.

(116) Jämbeck, J. P. M.; Lyubartsev, A. P. Derivation and Systematic Validation of a Refined All-Atom Force Field for Phosphatidylcholine Lipids. *J. Phys. Chem. B* **2012**, *116*, 3164–3179.

(117) Shirts, M. R.; Mobley, D. L.; Chodera, J. D.; Pande, V. S. Accurate and Efficient Corrections for Missing Dispersion Interactions in Molecular Simulations. *The Journal of Physical Chemistry B* **2007**, *111*, 13052–13063.

(118) Bussi, G.; Donadio, D.; Parrinello, M. Canonical sampling through velocity rescaling. *J. Chem. Phys.* **2007**, *126*.

(119) Kulig, W.; Pasenkiewicz-Gierula, M.; Róg, T. Cis and trans unsaturated phosphatidylcholine bilayers: A molecular dynamics simulation study. *Chem. Phys. Lipids* **2016**, *195*, 12 – 20.

(120) PEÃŞN, A. LIPID17 POPC-POPG 7:3 Bilayer Simulation (Last 100 ns, 150 mM NaCl, 310 K). 2019; `https://doi.org/10.5281/zenodo.2585523`.

(121) DubouÃĬ-Dijon, E.; Javanainen, M.; Delcroix, P.; Jungwirth, P.; Martinez-Seara, H. A practical guide to biologically relevant molecular simulations with charge scaling for electronic polarization. *The Journal of Chemical Physics* **2020**, *153*, 050901.

(122) Berendsen, H. J. C.; Grigera, J. R.; Straatsma, T. P. The missing term in effective pair potentials. *The Journal of Physical Chemistry* **1987**, *91*, 6269–6271.