Supplementary Information: A Critical Comparison of Biomembrane Force Fields: Structure and Dynamics of Model DMPC, POPC, and POPE Bilayers

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Table S1: Volumes of individual water molecule (in nm^3) simulated using force field specific parameter file (mdp files in GROMACS) calculated out of 4 ns simulations containing 14 572 water molecules.

Force field	**water model**	**320 K**	**300 K**	**308 K**
CHARMM36	TIPS3P [1]	0.03020	0.02966	0.02988
Slipids	TIP3P [2]	0.03097	0.03038	0.03061
Lipid14	TIP3P [2]	0.03097	0.03039	0.03061
GROMOS54a7	SPC [3]	0.03125	0.03074	0.03093

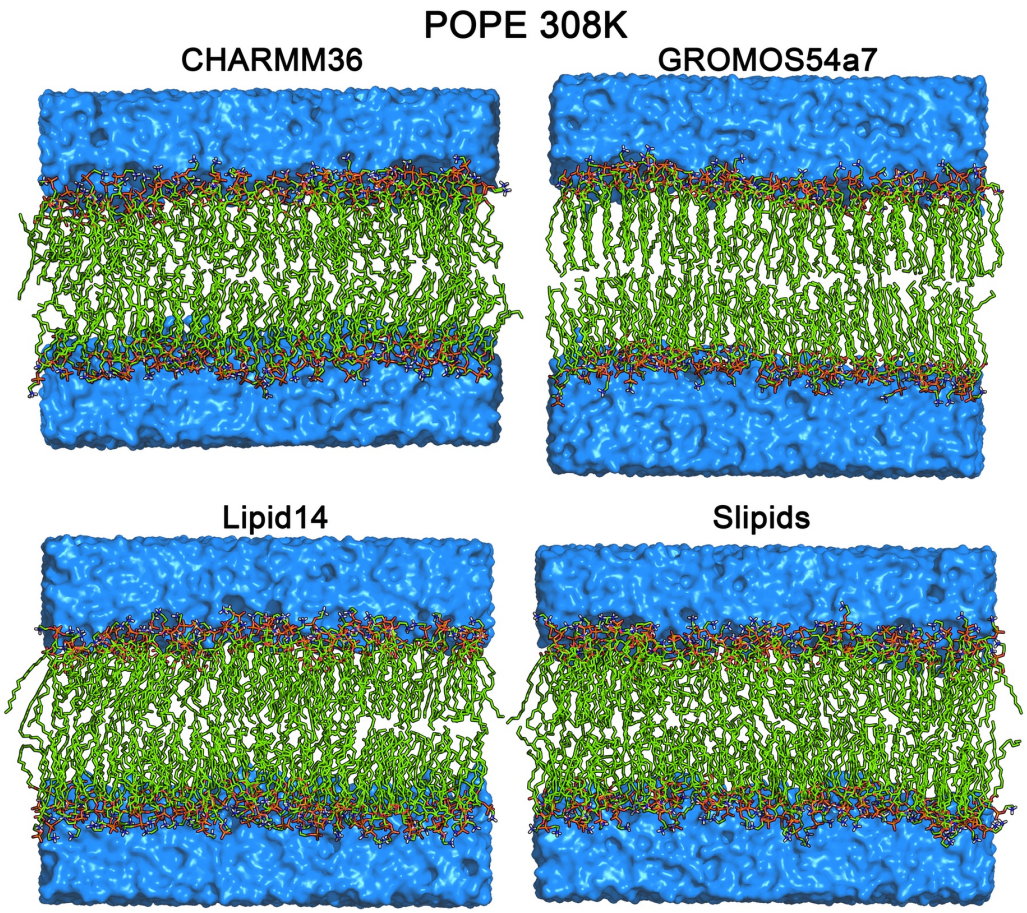

Figure S1: POPE simulation systems after 200 ns simulation time at 300 K. Only GROMOS54a7 which is partially in a gel phase doesn't exhibit a proper fluid phase. Hydrogen atoms were omitted for clarity. Water is shown as blue surface, DMPC as atom-color-coded sticks (carbon in green, oxygen in red, nitrogen in blue, and phosphorus in orange).

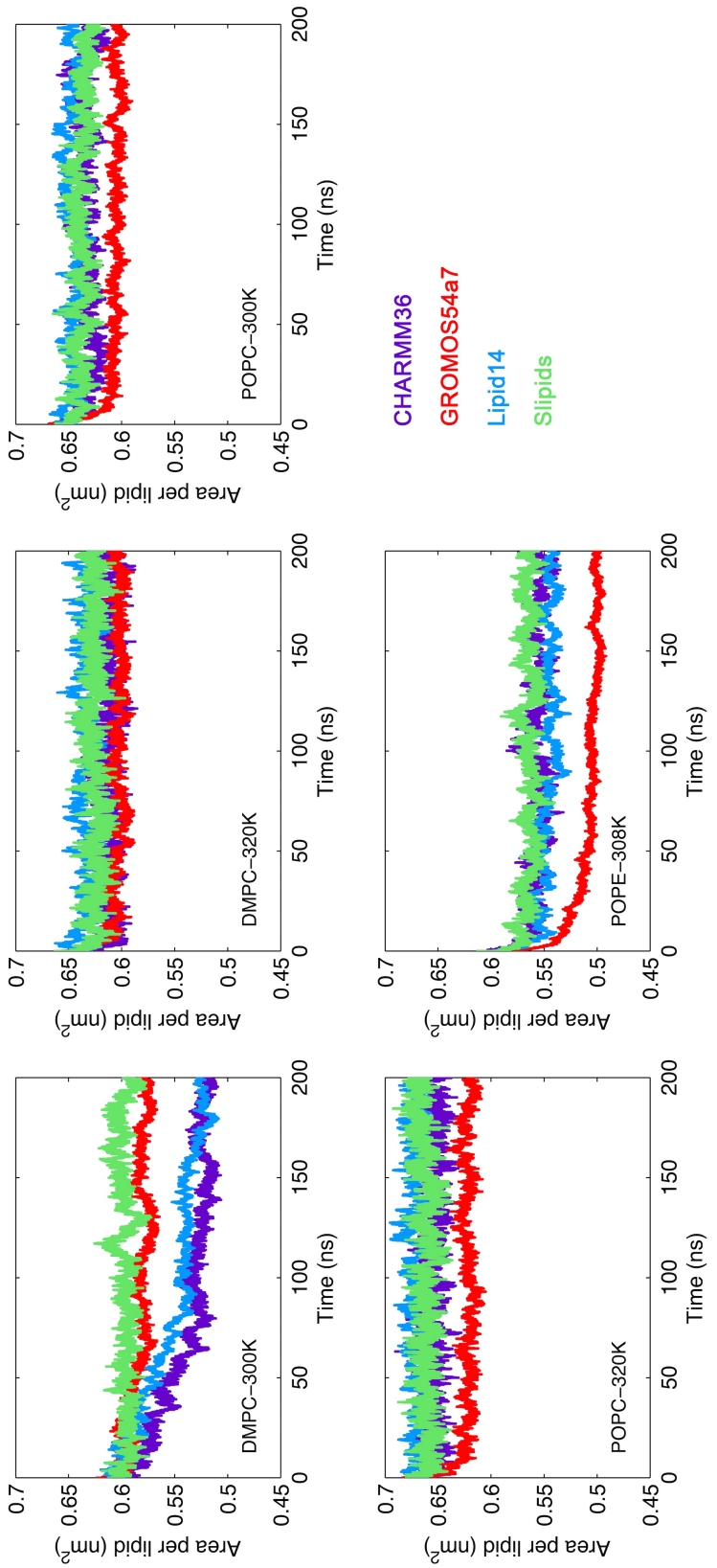

Figure S2: Evolution of the area per lipid over time.

Time window: 10ns Linear fit: 2-5ns	Time window: 70ns Linear fit: 10-40ns	Time window: 140ns Linear fit: 10-50ns	Time window: 140ns Linear fit: 50-80ns

		COM bilayer removal Atoms	COM monolayer removal Atoms			COM monolayer removal COM lipid		FF literature	Experimental
CHARMM36	T (K)	D (10^{-8} cm^2/s)^A	D (10^{-8} cm^2/s)^B	D (10^{-8} cm^2/s)^C	D (10^{-8} cm^2/s)^D	D (10^{-8} cm^2/s)^E	D (10^{-8} cm^2/s)^F	D (10^{-8} cm^2/s)^G		
DMPC	300	3.3 (± 0.23)	3.1 (± 0.16)	1.03 (± 0.13)	0.89 (± 0.12)	0.60	1.38 (± 0.11)	0.62 (± 0.10)	-	5 (300K); (a) 10.01 (300K); (b)
	320	21.8 (± 0.65)	20.6 (± 0.57)	13.93 (± 0.92)	13.90 (± 1.60)	14.55	14.88 (± 0.58)	13.75 (± 1.55)	-	12 (320K); (a) 19.448 (320K); (b)
POPC	300	14.3 (± 0.51)	13.56 (± 0.35)	6.32 (± 0.46)	6.10 (± 0.01)	4.34	7.5 (± 0.35)	5.37 (± 0.003)	-	5.52 (300K); (b) 4.0 (303K); (c)
	320	22.0 (± 0.49)	20.9 (± 0.51)	12.70 (± 1.13)	12.25 (± 1.65)	12.70	14.01 (± 0.50)	11.95 (±1.65)	-	18.068 (320K); (b) 7.0 (323 K); (c)
POPE	308	11.6 (± 0.54)	10.41 (± 0.29)	6.00 (± 0.27)	6.34 (± 0.52)	5.37	5.97 (± 0.26)	5.81 (± 0.49)	-	5.2 (307 K); (d)

Figure S3: Diffusion coefficients calculated in different ways. In each simulation the first 60 ns were excluded for equilibration. The center of mass (COM) motion of the whole bilayer (A) or separately of each monolayer (B-G) was removed. The mean square displacement (MSD) was calculated for lipid atoms (A-E) or for the lipid center of masses (F-G). In green columns (A, B, F), the diffusion was calculated by splitting the simulations in 10 ns windows (linear fit on 2-5 ns). In blue columns (C), the simulations were split in 70 ns time windows (linear fitting from 10 to 40 ns). In red (D, G) and yellow (E) columns, the diffusion was calculated from the whole 60 – 200 ns simulations with linear fitting on 10 – 50 ns and 50 – 80 ns, respectively. The diffusion coefficient was calculated separately for each monolayer and averaged over all atoms/molecules and over time. Experimental findings were taken from (a) Almeida *et al.* (1992),[4] (b) Filippov *et al.* (2003),[5] (c) Köchy *et al.* (1993),[6] and (d) Jin *et al.* (1999; 78 mol % PE).[7] The difference between the diffusion coefficients, calculated with the aid of the lipid atoms, and a removal of the bilayer (A) and monolayer (B) COM motion, is rather small. The same holds true for the diffusion coefficients obtained by lipid atoms (D) and lipid COM (G) for the whole 140 ns interval. Overall, the diffusion coefficients calculated over larger time windows exhibit smaller values compared to time windows of small duration.

		COM bilayer removal Atoms	COM monolayer removal Atoms				COM monolayer removal COM lipid			
GROMOS54a7	T (K)	D (10^{-8} cm^2/s)^A	D (10^{-8} cm^2/s)^B	D (10^{-8} cm^2/s)^C	D (10^{-8} cm^2/s)^D	D (10^{-8} cm^2/s)^E	D (10^{-8} cm^2/s)^F	D (10^{-8} cm^2/s)^G	FF literature	Experimental
DMPC	300	3.43 (± 0.14)	3.21 (± 0.08)	0.77 (± 0.08)	0.72 (± 0.04)	0.49	1.12 (± 0.05)	0.36 (± 0.02)		5 (300K); (a) 10.01 (300K); (b)
	320	6.28 (± 0.37)	5.10 (± 0.14)	1.90 (± 0.15)	1.80 (± 0.12)	1.20	2.28 (± 0.10)	1.25 (± 0.01)	DPPC: 2.6 (323 K)	12 (320K); (a) 19.448 (320K); (b)
DMPC-PME	320	-	-	-	-	-	3.16 (+ 0.12)	2.18 (± 0.23)		
POPC	300	5.97 (± 0.33)	4.62 (± 0.10)	1.04 (± 0.03)	1.00 (± 0.07)	0.67	1.49 (± 0.06)	0.5 (± 0.04)	-	5.52 (300K); (b) 4.0 (303K); (c)
	320	7.72 (± 0.20)	6.64 (± 0.17)	1.86 (± 0.17)	1.95 (± 0.02)	1.82	2.67 (± 0.13)	1.23 (± 0.01)	-	18.068 (320K); (b) 7.0 (323 K); (c)
POPE	308	2.56 (± 0.28)	1.60 (± 0.07)	0.37 (± 0.04)	0.35 (± 0.008)	0.24	0.51 (± 0.04)	0.15 (± 0.0002)	-	5.2 (307 K); (d)

		COM bilayer removal Atoms	COM monolayer removal Atoms				COM monolayer removal COM lipid			
Lipid14	T (K)	D (10^{-8} cm^2/s)^A	D (10^{-8} cm^2/s)^B	D (10^{-8} cm^2/s)^C	D (10^{-8} cm^2/s)^D	D (10^{-8} cm^2/s)^E	D (10^{-8} cm^2/s)^F	D (10^{-8} cm^2/s)^G	FF literature	Experimental
DMPC	300	4.63 (± 0.21)	4.48 (± 0.18)	2.10 (± 0.32)	1.89 (± 0.40)	1.34	2.04 (± 0.11)	1.4 (± 0.35)	5.05 (303K)	5 (300K); (a) 10.01 (300K); (b)
	320	19.13 (± 0.49)	18.34 (± 0.47)	11.67 (± 1.08)	10.80 (± 0.50)	8.03	12.34 (± 0.45)	10.41 (± 0.49)	-	12 (320K); (a) 19.448 (320K); (b)
POPC	300	11.66 (± 0.18)	11.31 (± 0.22)	5.04 (± 0.27)	4.76 (± 0.005)	3.54	5.51 (± 0.19)	3.92 (± 0.07)	5.74 (303K)	5.52 (300K); (b) 4.0 (303K); (c)
	320	20.28 (± 0.46)	19.47 (± 0.46)	11.28 (± 0.75)	11.50 (± 0.70)	10.45	12.04 (± 0.44)	10.95 (± 0.65)	-	18.068 (320K); (b) 7.0 (323 K); (c)
POPE	308	9.39 (± 0.41)	8.17 (± 0.25)	3.30 (± 0.31)	3.71 (± 0.71)	3.76	4.3 (± 0.22)	3.13 (± 0.73)	4.67 (310K)	5.2 (307 K); (d)

		COM bilayer removal Atoms	COM monolayer removal Atoms				COM monolayer removal COM lipid			
Slipids	T (K)	D (10^{-8} cm^2/s)^A	D (10^{-8} cm^2/s)^B	D (10^{-8} cm^2/s)^C	D (10^{-8} cm^2/s)^D	D (10^{-8} cm^2/s)^E	D (10^{-8} cm^2/s)^F	D (10^{-8} cm^2/s)^G	FF literature	Experimental
DMPC	300	10.8 (± 0.4)	10.04 (± 0.27)	4.72 (± 0.46)	4.75 (± 0.07)	4.65	5.37 (± 0.23)	4.16 (± 0.10)	5.22 ± 0.49 (303K)	5 (300K); (a) 10.01 (300K); (b)
	320	20.64 (± 0.44)	19.75 (± 0.44)	12.48 (± 1.30)	13.25 (± 1.95)	15.50	13.94 (± 0.43)	12.9 (±2.00)	11.8 ±2.10 (323K)	12 (320K); (a) 19.448 (320K); (b)
POPC	300	12.44 (± 0.31)	11.92 (± 0.23)	5.26 (± 0.46)	5.08 (± 0.18)	4.65	5.96 (± 0.20)	4.26 (± 0.22)	4.71 ± 0.2 (303K)	5.52 (300K); (b) 4.0 (303K); (c)
	320	20.38 (± 0.67)	19.58 (± 0.54)	11.63 (± 0.66)	11.30 (± 0.2)	11.85	12.51 (± 0.51)	10.85 (± 0.25)	15.9 ± 0.8 (323K)	18.068 (320K); (b) 7.0 (323 K); (c)
POPE	308	10.41 (± 0.37)	8.55 (± 0.27)	4.36 (± 0.51)	4.48 (± 0.38)	5.09	4.52 (± 0.26)	3.86 (± 0.35)	2.12 ± 0.4 (303K)	5.2 (307 K); (d)

Table S2: Detailed results of lipid chain protrusion analysis. Analysis performed on 60 − 200 ns of simulations. Protrusions recorded at a single frame (10 ps) were excluded from the analysis. The value in paranthesis by median time denotes the interquartile range. Protrusion efficiency is a product of protrusion probability and the median time of a protrusion event.

Simulated System	layer	number of protrusions	probability per lipid [1/μs]	median time (IQR) [ns]	maximal time [ns]	protrusion efficiency
CHARMM36						
DMPC	lower	33	1.228	0.38 (1.68)	8.09	0.467
300K	upper	35	1.302	0.46 (3.23)	12.11	0.599
DMPC	lower	77	2.865	0.17 (0.50)	2.81	0.487
320K	upper	74	2.753	0.12 (0.37)	3.62	0.317
POPC	lower	44	1.637	0.41 (1.19)	3.48	0.663
300K	upper	46	1.711	0.34 (0.83)	2.94	0.582
POPC	lower	72	2.679	0.18 (0.43)	2.43	0.482
320K	upper	86	3.199	0.20 (0.41)	1.76	0.624
POPE	lower	7	0.260	0.04 (0.99)	2.53	0.010
308K	upper	12	0.446	0.13 (0.32)	2.61	0.056
Slipids						
DMPC	lower	65	2.418	0.27 (0.69)	4.86	0.653
300K	upper	59	2.195	0.29 (0.90)	5.62	0.637
DMPC	lower	146	5.432	0.24 (0.55)	3.14	1.304
320K	upper	121	4.501	0.24 (0.55)	2.28	1.080
POPC	lower	66	2.455	0.33 (0.83)	4.95	0.798
300K	upper	71	2.641	0.25 (1.11)	3.52	0.660
POPC	lower	162	6.027	0.17 (0.60)	3.32	0.994
320K	upper	152	5.655	0.13 (0.37)	2.3	0.707
POPE	lower	30	1.116	0.34 (0.82)	4.08	0.379
308K	upper	33	1.228	0.24 (0.75)	2.84	0.295
Lipid14						
DMPC	lower	326	12.128	0.40 (1.25)	20.85	4.791
300K	upper	209	7.775	0.57 (1.30)	22.43	4.432
DMPC	lower	362	13.467	0.16 (0.51)	3.09	2.155
320K	upper	367	13.653	0.18 (0.55)	3.83	2.458
POPC	lower	249	9.263	0.24 (0.63)	4.21	2.223
300K	upper	267	9.933	0.26 (0.70)	4.47	2.583
POPC	lower	426	15.848	0.17 (0.45)	4.55	2.694
320K	upper	424	15.774	0.19 (0.49)	3.58	2.997
POPE	lower	217	8.073	0.25 (0.78)	5.32	2.018
308K	upper	186	6.920	0.33 (0.72)	4.14	2.249
GROMOS54a7						
DMPC	lower	25	0.930	0.89 (1.74)	5.04	0.487
300K	upper	30	1.116	0.65 (1.51)	14.82	0.487
DMPC	lower	45	1.674	0.34 (1.28)	5.75	0.487
320K	upper	58	2.158	0.47 (1.53)	4.68	0.487
POPC	lower	22	0.818	0.50 (1.79)	6.48	0.487
300K	upper	28	1.042	0.68 (1.70)	3.96	0.487
POPC	lower	47	1.749	0.21 (1.15)	4.1	0.487
320K	upper	51	1.897	0.30 (1.36)	16.45	0.487
POPE	lower	1	0.037	1.33 (0.00)	1.33	0.049
308K	upper	1	0.037	0.02 (0.00)	0.02	0.001

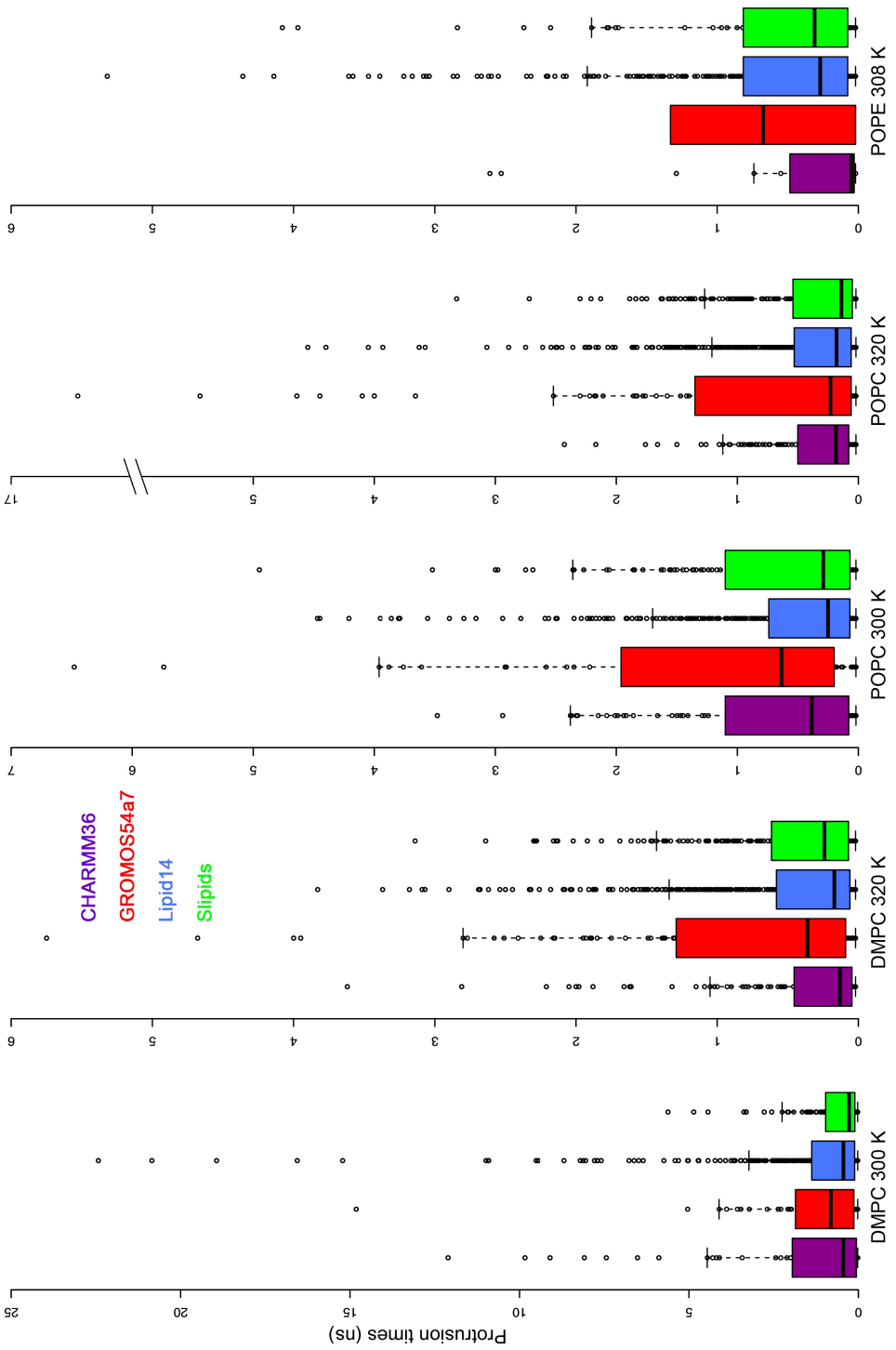

Figure S4: Box plots of lipid protrusion times for systems under study. CHARMM36 is colored in purple, GROMOS54a7 in red, Lipid14 in blue, and Slipids in green. Horizontal top and bottom lines of each box define the first and third quartiles, the thick line inside the box is the median. The whiskers (horizontal lines outside the box) determine the last data point that is no more away than 1.5 times the interquartile range (box length) from the box. Box plots were generated with RStudio.[8]

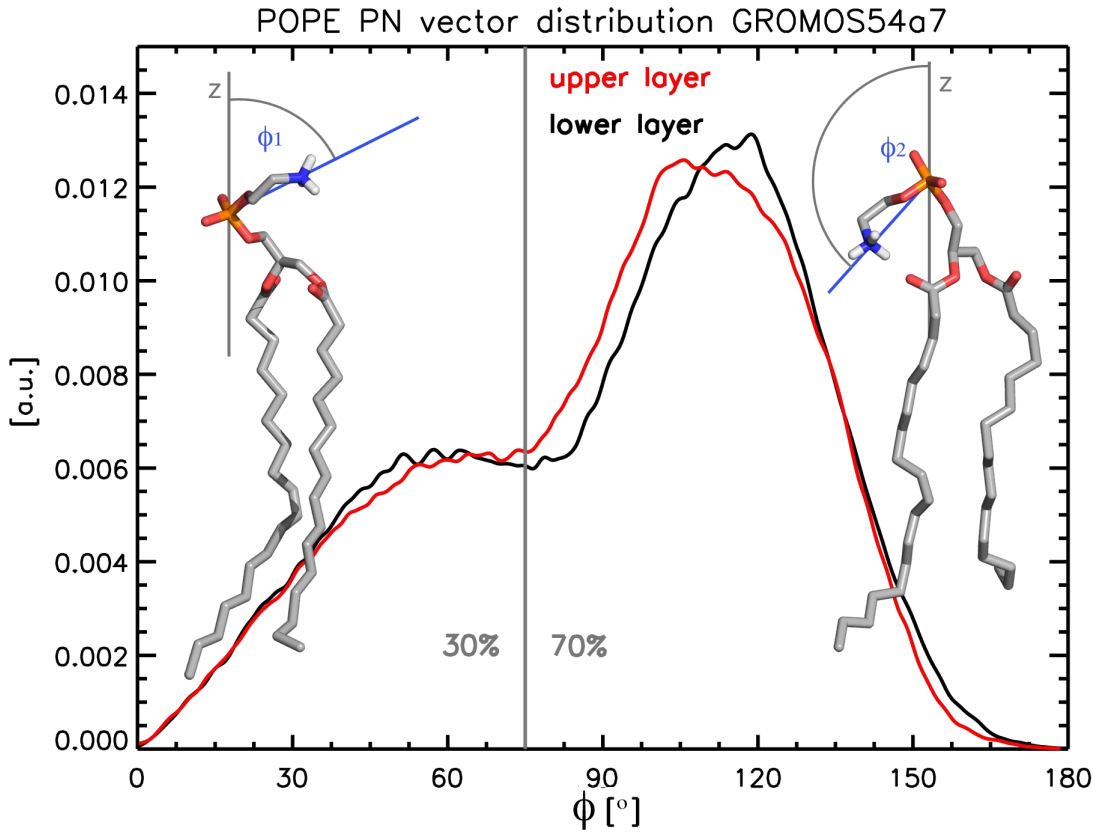

Figure S5: Distribution of PN vectors relative to the membrane normal for the POPE gel phase obtained by the GROMOS54a7 force field. The population of lipids with the PN vector smaller than 75° (an example lipid shown on the left) corresponds to 30%, the population of lipids with the PN vector larger than 75° (an example lipid shown on the right) makes up 70%.

Calculation of order parameters of unsaturated carbons

Using the GROMACS's tool *g_order* to calculate the order parameters around unsaturated carbons succeeds by following following workflow. The test case is a lipid containing one saturated chain (palmitoyl carbons named C2A - C2P) and one monounsaturated (oleoyl, carbons C1A-C1R) chain.

Step 1. Calculation of standard saturated order parameters for all carbons.

First index files have to be created for each chain individually containing only carbons for which order parameters should be calculated. Please note, that using *g_order* no order parameter value can be calculated for the first and the last carbon in the chain.

```
make_ndx -f backmapped.gro -o sn1.ndx << EOF
del 0-20
a C2A
a C2B
a C2C
a C2D
a C2E
a C2F
a C2G
a C2H
a C2I
a C2J
a C2K
a C2L
a C2M
a C2N
a C2O
a C2P
q
EOF

make_ndx -f backmapped.gro -o sn2.ndx << EOF
del 0-20
a C1A
a C1B
a C1C
a C1D
a C1E
a C1F
a C1G
a C1H
```

```
a C1I
a C1J
a C1K
a C1L
a C1M
a C1N
a C1O
a C1P
a C1Q
a C1R
q
EOF
```

Next *g_order* is used to calculate the order parameters for each chain.

```
g_order -s topol.tpr -f traj.xtc -n sn1.ndx -d z -od deuter_sn1.xvg

g_order -s topol.tpr -f traj.xtc -n sn2.ndx -d z -od deuter_sn2.xvg
```

Step 2. Calculation of order parameters for unsaturated carbons

The double bond is found between atoms C1I and C1J. Calculation of the order parameter for atom C1J (- C1I = C1J – C1K -) succeeds following

```
make_ndx -f backmapped.gro -o sn2-C1J.ndx << EOF
del 0-20
a C1I
a C1J
a C1K
q
EOF

g_order -unsat -f traj.xtc -s topol.tpr -n sn2-C1J.ndx -d z
-od deuter_C1J.xvg
```

The order parameter for atom C1I is calculated following the opposite direction (- C1J = C1I – C1H -).

```
make_ndx -f backmapped.gro -o sn2-C1I.ndx << EOF
del 0-20
a C1J
a C1I
a C1H
q
EOF
```

```
g_order -unsat -f traj.xtc -s topol.tpr -n sn2-C1I.ndx -d z
-od deuter_C1I.xvg
```

Step 3. Combining results

In the last step the order parameters in the file deuter_sn2.xvg of atoms C1J and C1I are replaced by results obtained by calculation using the option `-unsat` and proper atom order.

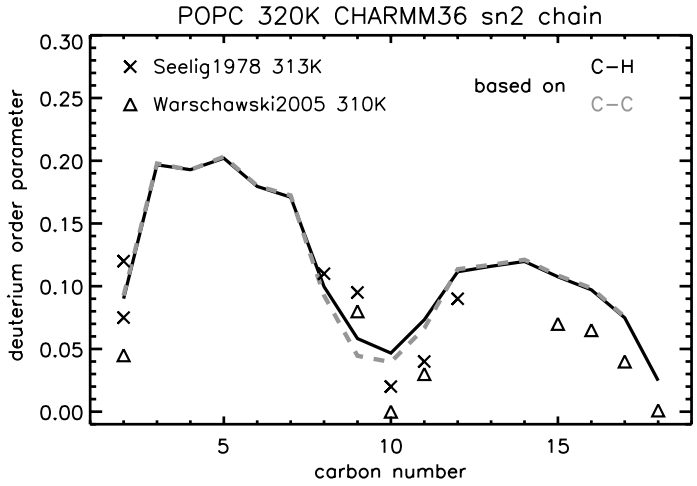

Figure S6: Deuterium order parameters of the unsaturated sn2 chain of POPC simulated by CHARMM36 at 320 K calculated based on C-H orientation (black) or C-C orientation (grey). The latter method is applied in GROMACS' tool g_order. The spread of the experimental data (crosses and triangles) is larger than the difference between the two theoretical methods.

References

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