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Supporting Material

Molecular dynamics simulations of mixed acidic/zwitterionic phospholipid bilayers

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Supplementary Material

Appendix to Methods section

Data Analysis

The surface tension can be derived from thermodynamics using $\gamma=\delta F/\delta A$. Applying the pressure profile implementation by Gullingsrud et al. (1) we retrieved the lateral and normal pressure to calculate the surface tension (2).

The order parameters S_{CD} were calculated from the average value of the angle θ along the last 100 ns of each simulation of the C-D bond considered with the bilayer normal (3); defined by:

$$S_{CD}=\langle(\frac{3}{2}cos^2\theta-\frac{1}{2})\rangle \qquad (1)$$

Na^+ binding & lipid clustering

We used a radius of 3.3 Å to calculate the coordination number; 3.3 Å is the radius of the first solvation shell of Na^+ ions (4). The coordination number was calculated for the phosphate, lipid carbonyl and lipid headgroup oxygens. The number of Na^+ bound to the membrane per frame was calculated with CHARMM (5), applying a cutoff distance of 2.9 Å. A Na^+ cluster is defined as a Na^+ ion bound to at least two lipid molecules.

Hydrogen bonding

A hydrogen bond is considered to exist if the acceptor (A) to hydrogen distance is equal or below 2.4 Å, if the angle D-H-A (D: hydrogen bond donor) is equal or greater than 130° and if these criteria are met for at least 1 ps. For the PC headgroups we calculated the number of hydrogen bonds of the choline headgroup hydrogens with the headgroup oxygens rather than the charge pairs (6). Likewise, we calculated Na^+-bridges applying a cutoff distance of 2.9 Å and a minimum lifetime of 1 ps.

Surface Charge Density

The calculations of surface charge density were done on conformations stored every ps during the last 100 ns of each simulation. For each leaflet, we projected all charges (lipids headgroups, carbonyl and phosphate groups, bound sodium atoms) on a plane perpendicular to the z axis. The z coordinate is defined as the average z of the phosphorus atoms of the leaflet. The density is then obtained by dividing the total charge by the surface area of the corresponding conformation. Surface densities are then averaged over the last 100 ns of the simulations.

Figure S1: Number of ions bound to the mixed bilayers along NPγT MD simulations (γ = 17 dyn/cm): DMPC/DMPG (black line), DMPC/DMPS (blue line), DMPC/DMPA (green line).

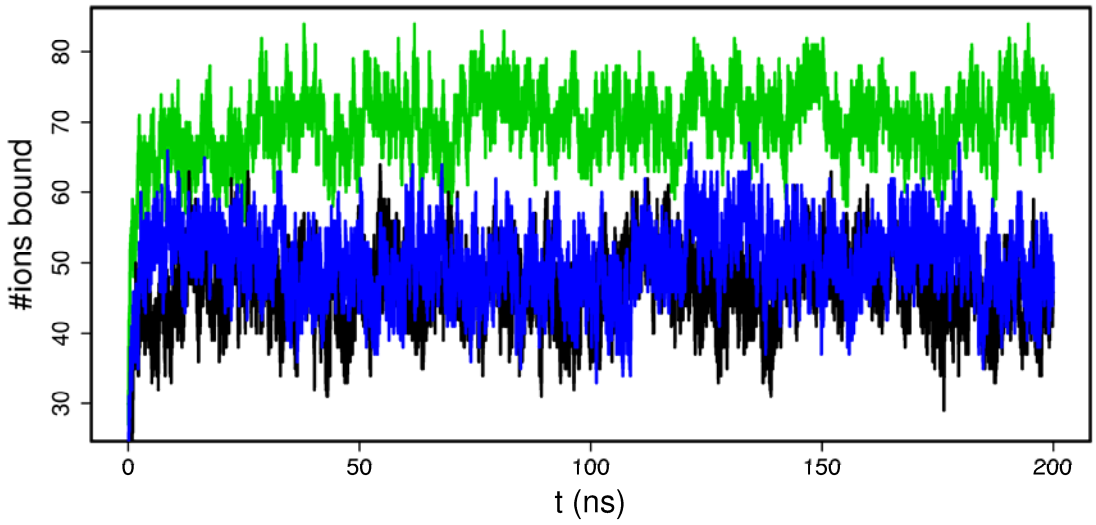
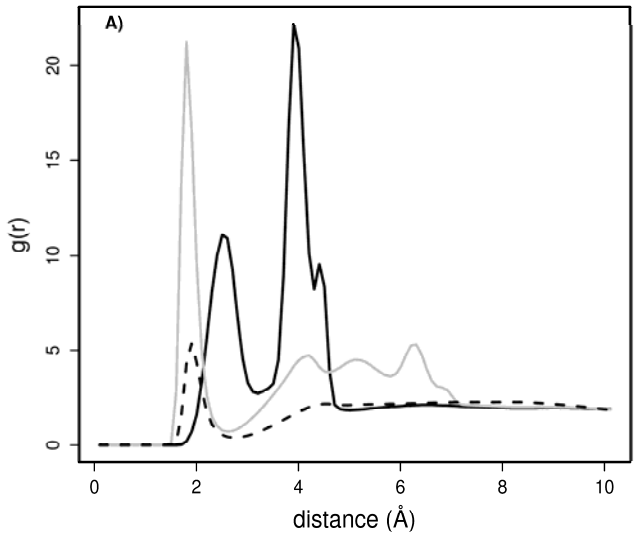


Figure S1

Figure S2: RDFs. Phosphate ester oxygen O12 (black line), phosphate non-ester oxygens (gray line) and ester carbonyl oxygens (black dashed line) relative to A) the secondary hydroxyl group (HG2) and B) the terminal hydroxyl group (HG3).



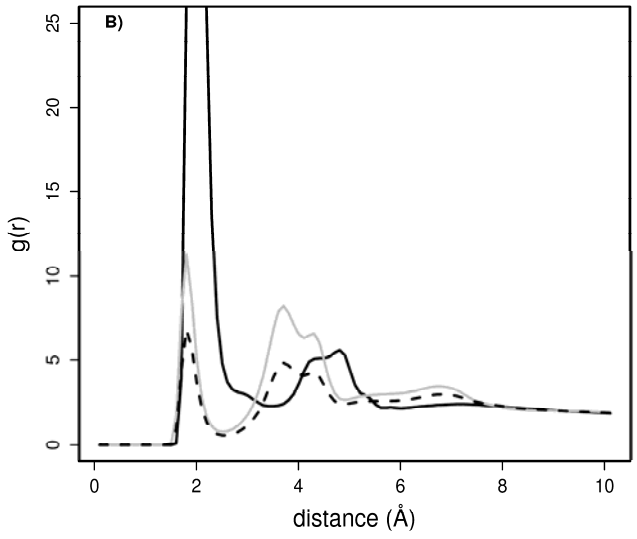


Figure S2

Figure S3: Distribution of the angle of the DMPC P-N vectors (phosphorus to ammonium nitrogen) with the bilayer normal in equimolar mixtures with DMPG, DMPS and DMPA. P-N vector distribution: DMPC (black solid line), DMPC/DMPG (dashed black line), DMPC/DMPS (dashed blue line) and DMPC/DMPA (dotted green line).
To characterize the PC headgroup orientation in the different mixtures we calculated the angle of the P-N vector with the bilayer normal and found 79.1±1.6° for DMPC/DMPG, 75.9±1.5° for DMPC/DMPS and 78.6±1.5° for DMPC/DMPA, close to the value of 74.1±0.8° in pure DMPC. The average angle of the P-N vector (phosphorus to ammonium nitrogen) of DMPS lipids with the bilayer normal is with 72.9 ±1.9° very similar to the P-N vector orientation of the DMPC lipids (74.1±0.8°). The ammonium group is not more solvent exposed than the choline group, as it facilitates strong intermolecular headgroup-headgroup hydrogen bonds. The carboxylate oxygens are slightly above the membrane plane (59.6±2.3°); the carboxylate group seems more solvent exposed than the ammonium group. The P-C13 vector (phosphorus to the terminal C-atom) of DMPG lipids has an average angle of 82.4±1.5° in the DMPC/DMPG mixture. As in the pure DMPG bilayer (83.5±1.6°) the glycerol group is lying parallel to the lipid-water interface.

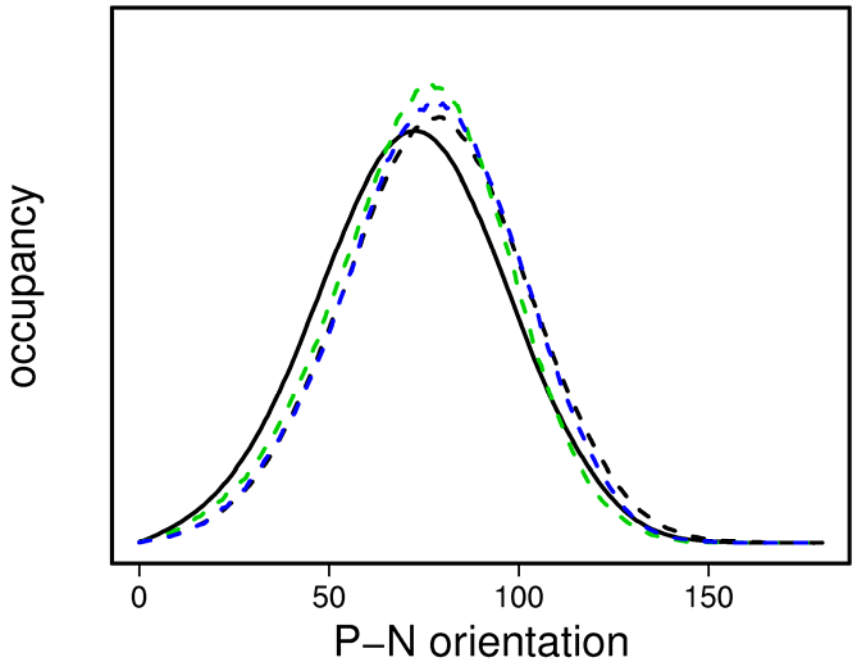


Figure S3

Table S1: Average number per frame of intermolecular hydrogen bonds formed by the hydrogens of choline groups of DMPC. The hydrogen bonds to the oxygens of the different lipids in the mixtures are compared. O_{head}: the headgroup oxygens (see Figure 1 for atom names).

	DMPC	DMPC/DMPG		DMPC/DMPS		DMPC/DMPA	
	DMPC	DMPG	DMPC	DMPS	DMPC	DMPA	DMPC
O13 & O14	136.2	35.2	30.8	40.9	32.2	34.2	30.0
O11	39.6	11.8	10.3	14.0	10.3	14.7	9.7
O12	32.9	5.1	5.6	4.9	6.7	12.4	6.2
O22	23.4	6.6	6.4	9.0	6.6	8.0	7.6
O32	26.3	8.5	6.6	9.0	5.8	8.7	6.1
O_{head}	-	11.6	-	7.6	-	-	-
Total	258.4	78.8	59.7 138.5	85.4	61.6 147.0	78.0	59.6 137.8

Table S2: Average number per frame of intermolecular hydrogen bonds formed in the equimolar DMPC/DMPG mixture; the average hydrogen bond lifetime is given in parenthesis. The hydrogen bonds of the hydrogens of terminal hydroxyl groups (HG3) and of the secondary hydroxyl groups (HG2) of DMPG formed with DMPC and DMPG lipids are compared (atom names see Figure 1).

	HG2		HG3	
	DMPG	DMPC	DMPG	DMPC
O13 & O14	14.2 (22)	13.7 (22)	6.1 (19)	7.6 (19)
O11	1.7 (7)	1.3 (7)	0.6 (7)	0.6 (7)
O12	0.3 (5)	0.5 (6)	0.2 (4.3)	0.4 (6)
O22	2.0 (10)	2.0 (11)	1.0 (10)	1.0 (9)
O32	1.8 (11)	2.5 (13)	0.7 (9)	0.8 (9)
OG2	1.7 (10)	-	0.9 (9)	-
OG3	2.1 (10)	-	0.7 (7)	-
Total	23.8	20.0	10.2	10.4
		43.8		20.6
				64.4

Table S3: Average number per frame of intermolecular hydrogen bonds formed by the choline groups of DMPC versus the number of hydrogen bonds formed by the ammonium group of DMPS. The hydrogen bonds to the oxygen atoms of DMPC and DMPS are compared (see Table S1).

	ammonium		choline	
	DMPS	DMPC	DMPS	DMPC
O13 & O14	47.4	53.7	40.9	32.2
O11	0.8	1.0	14.0	10.3
O12	0.5	1.0	4.9	6.7
O22	2.1	2.1	9.0	5.8
O32	2.7	1.8	9.0	6.6
O_{head}	50.6	-	7.6	-
Total	104.1	59.6	85.4	61.6
		163.7		147.0

References

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