

# The structure of ions and zwitterionic lipids regulates the charge of dipolar membranes

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## **Supporting information**

### *Ion adsorption to PC membranes*

The binding constants of several ions to PC membranes are summarized in Tables S1-S6.

Table S1: Binding constants of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) with several ions:

Ref. number	T [ $^{\circ}\text{C}$ ]	method	$\text{Ca}^{2+}$ [ $\text{M}^{-1}$ ]	$\text{Ca}^{2+}$ [ $\text{M}^{-1}$ ] (in the presence of NaCl)	$\text{Mg}^{2+}$ [ $\text{M}^{-1}$ ]	$\text{Na}^{+}$ [ $\text{M}^{-1}$ ]	$\text{Cl}^{-}$ [ $\text{M}^{-1}$ ]	$\text{La}^{3+}$ [ $\text{M}^{-1}$ ]
(1)	25	SAXS, osmotic stress	1 - 300	10 (100 mM)				
(2)	5	DLVO theory	$21 \pm 9$		$2.5 \pm 0.7$			
(3)	22	Measurement of forces between mica supported bilayers using a surface force apparatus	120		20			
(4)	25	Zeta potential measurements by particle electrophoresis	37			0.25	0.28	
(5)	40-43 (below $T_m$ )	Zeta potential measurements by particle electrophoresis	$441.3 \pm 40$					
	43-46.5 (above $T_m$ )		$190 \pm 15$					
(6)	59	$^2\text{H}$ -NMR	19					120
(7)	30	Theory	100					
(8)	50	Molecular Dynamics (MD) simulations				0.15 - 0.61	0.16	

Table S2: Binding constants of dimyristoyl-sn-glycero-3-phosphocholine (DMPC) with several ions:

Ref. number	T [ $^{\circ}\text{C}$ ]	method	$\text{Ca}^{2+}$ [ $\text{M}^{-1}$ ]	$\text{Ca}^{2+}$ [ $\text{M}^{-1}$ ] (in the presence of NaCl)	$\text{Mg}^{2+}$ [ $\text{M}^{-1}$ ]	$\text{NO}_3^-$ [ $\text{M}^{-1}$ ]	$\text{Br}^-$ [ $\text{M}^{-1}$ ]	$\text{SCN}^-$ [ $\text{M}^{-1}$ ]	$\text{I}^-$ [ $\text{M}^{-1}$ ]	$\text{ClO}_4^-$ [ $\text{M}^{-1}$ ]
(3)	22	Measurement of forces between mica supported bilayers using a surface force apparatus	46		8.6					
(5)	22-25 (below $T_m$ )	Zeta potential measurements by particle electrophoresis	$391.7 \pm 30$							
	25-28 (above $T_m$ )		$256.4 \pm 25$							
(9)	37	Solid state $^2\text{H}$ -NMR and calcium electrode concentration measurements		37.7 (10 mM)						
(10)		Electrophoretic mobility measurements				$2.1 \pm 0.2$	$3.6 \pm 0.3$	$10.0 \pm 0.7$	$40 \pm 6$	$220 \pm 10$

Table S3: Binding constants of 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) with different ions:

Ref. number	T [°C]	method	Ca <sup>2+</sup> [M <sup>-1</sup> ]	Mg <sup>2+</sup> [M <sup>-1</sup> ]	Br <sup>-</sup> [M <sup>-1</sup> ]	Cl <sup>-</sup> [M <sup>-1</sup> ]
(3)	22	Measurement of forces between mica supported bilayers using a surface force apparatus	15	10		
(11)	25	Small Angle X-ray Scattering (SAXS), osmotic stress			0.22	negligible
This work	25	Atomic absorption	10.8 ± 0.9			
		Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES)	15 ± 2			

Table S4: Binding constants of natural EggPC with divalent and trivalent cations:

Ref. number	T [°C]	method	Ca <sup>2+</sup> [M <sup>-1</sup> ]	Ca <sup>2+</sup> [M <sup>-1</sup> ] (in the presence of NaCl)	Mg <sup>2+</sup> [M <sup>-1</sup> ]	Mn <sup>2+</sup> [M <sup>-1</sup> ]	Co <sup>2+</sup> [M <sup>-1</sup> ]	Ni <sup>2+</sup> [M <sup>-1</sup> ]	Sr <sup>2+</sup> [M <sup>-1</sup> ]	Ba <sup>2+</sup> [M <sup>-1</sup> ]	Lanthnide [M <sup>-1</sup> ]	Eu <sup>3+</sup> [M <sup>-1</sup> ]	Tb <sup>3+</sup> [M <sup>-1</sup> ]	Ce <sup>3+</sup> [M <sup>-1</sup> ]
(5)	25	Zeta potential measurements by particle electrophoresis	42 ± 5	38 (100 mM)	30 ± 4				16 ± 2	10 ± 1				
(12)	30	<sup>31</sup> P-NMR	2.2										700	
(13)	30	<sup>31</sup> P-NMR									700			
(14)	25	NMR									29			
(15)	25	Electrophoretic mobility measurements	1		1	3.33	0.833	0.833	0.357	0.278				
(16)	25	Electrophoretic mobility measurements	2.7		1.8									
(17)	25	<sup>31</sup> P-NMR, competition titration	3									30		
(18)	35	gel filtration, in methanol	28.6		24.4									2500

Table S5: Binding constants of natural EggPC with monovalent cations ( $\text{Na}^+$ ) and anions:

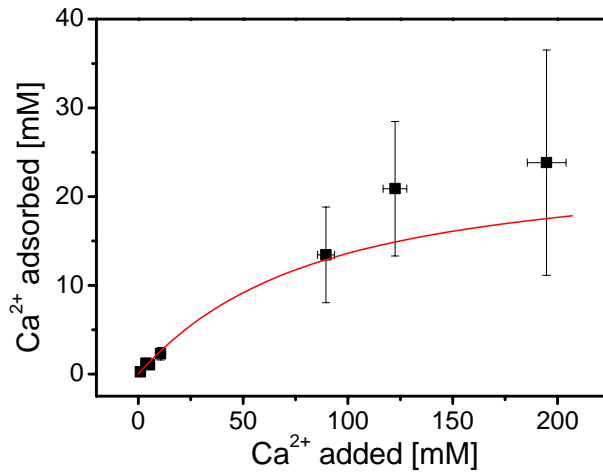
Ref. number	T [ $^{\circ}\text{C}$ ]	method	$\text{Na}^+$ [ $\text{M}^{-1}$ ]	$\text{Cl}^-$ [ $\text{M}^{-1}$ ]	$\text{Br}^-$ [ $\text{M}^{-1}$ ]	$\text{NO}_3^-$ [ $\text{M}^{-1}$ ]	$\text{ClO}_4^-$ [ $\text{M}^{-1}$ ]
(5)	25	Zeta potential measurements by particle electrophoresis	$0.15 \pm 0.1$	$0.2 \pm 0.1$	$2.0 \pm 1$	$2.8 \pm 1$	$70 \pm 10$
(12)	30	$^{31}\text{P}$ -NMR					
(13)	30	$^{31}\text{P}$ -NMR		0.9	2	8	
(15)	25	Electrophoretic mobility measurements		0.025			
(16)	25	Electrophoretic mobility measurements	0.6				

Table S6: Binding constants of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) with several ions:

Ref. number	T [°C]	method	Ca <sup>2+</sup> [M <sup>-1</sup> ]	Ca <sup>2+</sup> [M <sup>-1</sup> ] (in the presence of NaCl)	Na <sup>+</sup> [M <sup>-1</sup> ]	SCN <sup>-</sup> [M <sup>-1</sup> ]	I <sup>-</sup> [M <sup>-1</sup> ]	ClO <sub>4</sub> <sup>-</sup> [M <sup>-1</sup> ]	La <sup>3+</sup> [M <sup>-1</sup> ]
(19)	25	Atomic absorption and <sup>2</sup> H-NMR		13.8 (100 mM)					
	40		7						
(20)	25	Atomic absorption and <sup>2</sup> H-NMR	12.4		0.15				
(21)	27	Titration calorimetry							4100 ± 1100
(22)	22	Micropipette aspiration	7.3						
(23)		<sup>31</sup> P-NMR and <sup>2</sup> H-NMR				80	32	115	
(24)	25	<sup>2</sup> H-NMR				1.4			

### *The association constant of $\text{CaCl}_2$ to DLPC*

Measurements performed by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) are plotted in Figure S1.



**Figure S1:** Calcium adsorption isotherm produced by ICP-OES measurements, for  $15 \frac{\text{mg}}{\text{ml of salt solution}}$  DLPC membranes (solid symbols). Solid line: calculated fit to the experimental data, using Eq. (6) with  $K_a = 15 \pm 2 \text{ M}^{-1}$ .

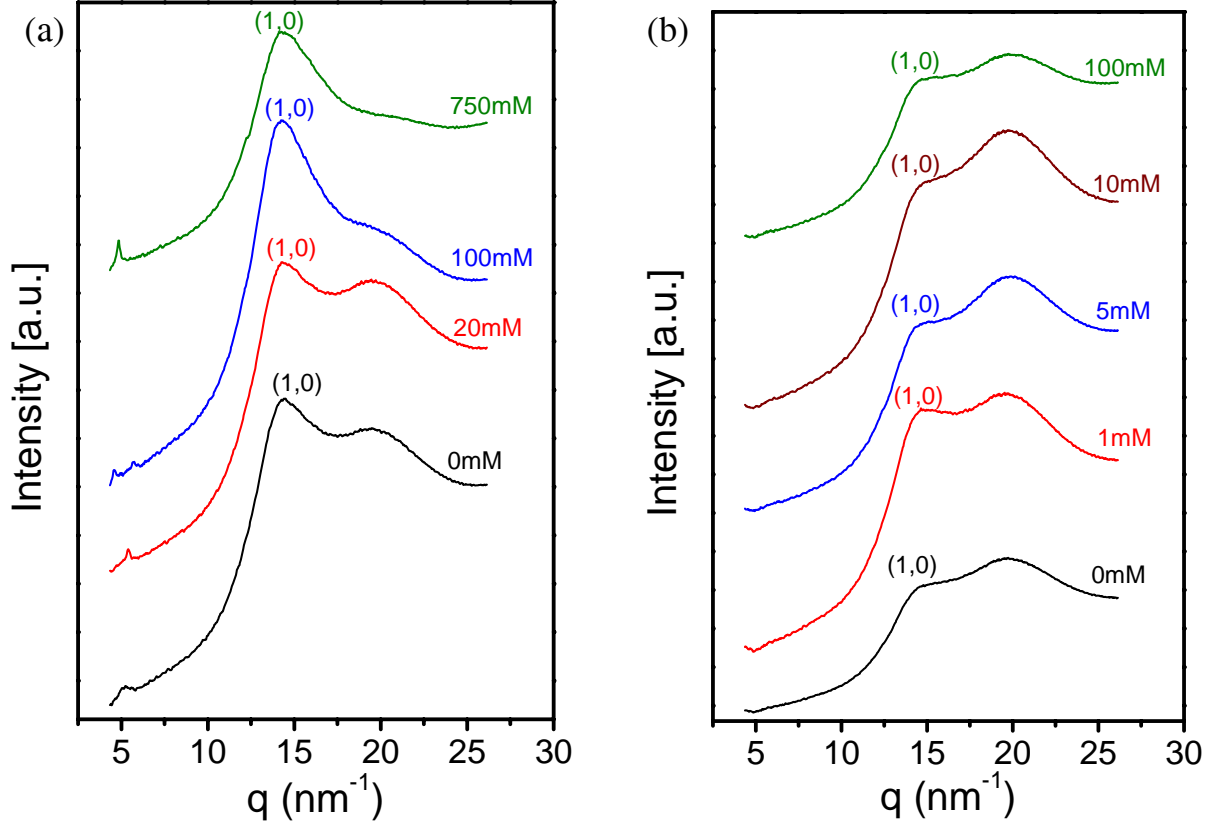
The binding constant extracted from fitting the data to Eq. (6) is  $K_a = 15 \pm 2 \text{ M}^{-1}$ .

It is apparent from Figure S1 that the binding isotherm reaches saturation at high  $\text{CaCl}_2$  concentrations, where about 20 mM of  $\text{Ca}^{2+}$  ions are adsorbed to the membrane surface, indicating a binding stoichiometry of 1:1 (bound  $\text{Ca}^{2+}$  : lipid).

### *Wide Angle X-ray Scattering (WAXS) measurements*

WAXS measurements provided additional structural information. The liquid disordered lamellar phase,  $L_\alpha$ , is characterized by a wide correlation scattering peak at the wide angle region that corresponds to the ordering of the tails in a “hexagonal-like” lattice<sup>25-32</sup>. DOPC and DLPC membranes have a low melting temperature and are therefore in the liquid disordered phase (at ambient room temperature), as can be seen in Figure S2. This phase is therefore much less ordered than a hexagonal phase in the gel-phase. Nevertheless, the correlation peak suggests that the tails still maintain spatial correlations and there is a characteristic spacing between them, from which the area per lipid can be estimated.

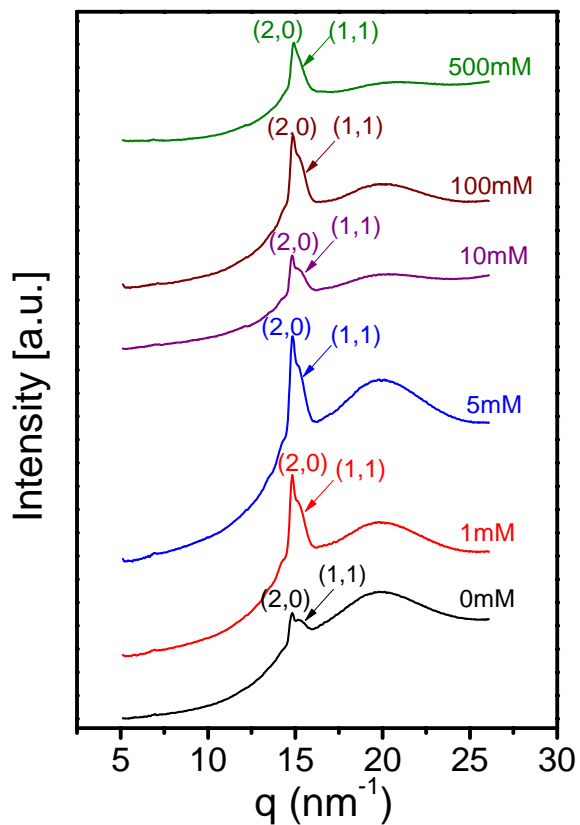




**Figure S2:** Wide angle X-ray scattering (WAXS) curves as a function of the momentum transfer,  $q$ , for (a) DOPC membranes and (b) DLPC membranes in different  $\text{CaCl}_2$  concentrations, as shown in the figure. In all the samples the final total lipid concentration was  $15 \frac{\text{mg}}{\text{ml of salt solution}}$ . The (1,0) peak corresponds to the tail-tail correlations, assuming a “hexagonal-like” lattice, typical for the  $L_\alpha$  liquid phase<sup>25</sup>. This phase is fluid and much less ordered than a hexagonal phase in the gel-phase, yet the tails still maintain spatial correlations.

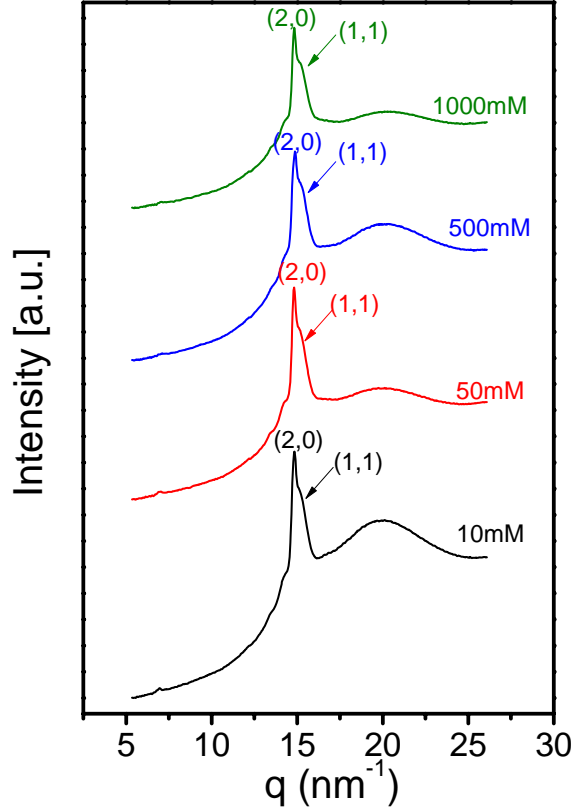
When the  $\text{CaCl}_2$  concentration increases to 100 mM or higher, the tail-tail correlation peak in DOPC sharpens, indicating an increase in the domain size of lipids that are in lateral positional correlation within the bilayers.

The gel-phase lamellar structure,  $L_\beta$ , is characterized by a sharp peak in the wide angle region, followed by a diffuse scattering<sup>28-30, 33-36</sup>. The DPPC membranes, at ambient room temperature, are in the  $L_\beta$  phase, which has similar characteristics to the  $L_\beta$  phase (Figures S3 and S4) in the wide angle region, but in the  $L_\beta$  phase the chains are tilted with respect to the bilayer normal<sup>30, 33</sup>.



**Figure S3:** Radially integrated WAXS scans for  $15 \frac{\text{mg}}{\text{ml of salt solution}}$  DPPC membranes in various  $\text{CaCl}_2$  solutions, as indicated in the figure. The (2,0) and (1,1) peaks correspond to the tail-tail correlations, forming a rectangular lattice, typical for the  $L_{\beta'}$  gel phase<sup>37</sup>.

The results show that  $\text{CaCl}_2$  (Figure S3) or  $\text{NaCl}$  (Figure S4) have immeasurable effect on the packing of the  $L_{\beta'}$  gel phase of the DPPC membranes, in the range of salt concentrations used in our experiments.



**Figure S4:** Radially integrated WAXS scans for  $15 \frac{\text{mg}}{\text{ml of salt solution}}$  DPPC membranes in the presence of NaCl solutions, as indicated in the figure. The (2,0) and (1,1) peaks correspond to the tail-tail correlations, forming a rectangular lattice, typical for the  $L_{\beta}$  gel phase<sup>37</sup>.

The peaks found by WAXS were fitted to a two dimensional lattice of the hydrocarbon chains, and the lattice parameters are summarized in Table 7S. Even though the DOPC and DLPC hydrocarbon chain are in the fluid-phase, their arrangement in the membrane is treated as a 2D hexagonal lattice (Figure S5a), and the area per head-group is calculated by<sup>38, 39</sup>:

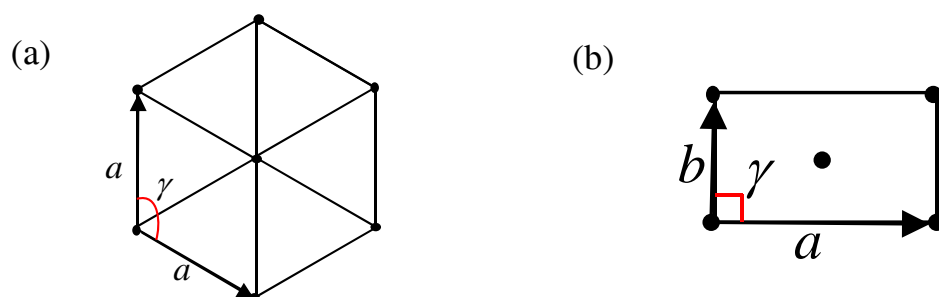
$$(S1) \quad A \cong 2 \cdot 1.32 \cdot \left( \frac{9\pi}{4q_0} \right)^2,$$

where  $q_0$  is the position of the wide-angle scattering peak (in  $\text{nm}^{-1}$ ).

For the gel phase lipid (DPPC), the hydrocarbon chain arrangement in the membrane is treated as a 2D rectangular lattice (Figure S5b)<sup>37</sup>, in which each unit cell contains two tails, and the area per lipid is the unit cell area. On the other hand, the WAXS data can also be analyzed as the DPPC forms a 2D hexagonal lattice<sup>30, 40</sup>, in which each unit cell contains only one tail, and the area per lipid is twice the area of the unit cell. The peak indexing in Figures S3 and S4 assumes a rectangular lattice, as in an earlier study of DPPC membranes in the  $L_{\beta}$  gel phase<sup>37</sup>.

**Table S7:** Structural parameters, obtained by WAXS.

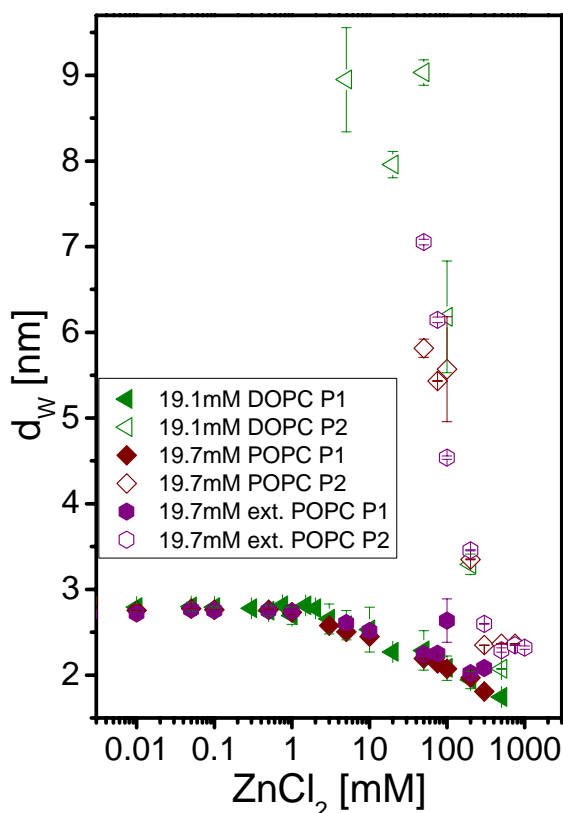
Lipid	Phase	a [ $\text{\AA}$ ]	b [ $\text{\AA}$ ]	$\gamma$	Area per head-group [ $\text{\AA}^2$ ]
DOPC	“Hexagonal-like”	$5.09 \pm 0.02$	$5.09 \pm 0.02$	$120^\circ$	$64.9 \pm 0.2$
DLPC	“Hexagonal-like”	$5.07 \pm 0.02$	$5.07 \pm 0.02$	$120^\circ$	$64.6 \pm 0.2$
DPPC	Rectangular	$8.47 \pm 0.01$	$4.69 \pm 0.01$	$90^\circ$	$39.7 \pm 0.2$
DPPC	Hexagonal	$4.90 \pm 0.02$	$4.90 \pm 0.02$	$120^\circ$	$41.5 \pm 0.1$



**Figure S5:** Illustrations of the tail unit cells in a hexagonal lattice (a) and a rectangular lattice (b). Each dot represents a lipid tail.

## The Interaction of $\text{ZnCl}_2$ with Unsaturated or Hybrid Lipids

Figure S6 shows that the interaction of DOPC or POPC with  $\text{Zn}^{2+}$  ions is similar to their interaction with  $\text{Ca}^{2+}$  ions.



**Figure S6:** Inter-lamellar spacing,  $d_w$ , as a function of  $\text{ZnCl}_2$  concentration, of lipid solutions containing the unsaturated DOPC lipid or the hybrid lipid, POPC that was prepared with or without extrusion, as indicated in the figure. In all the samples the final total lipid concentration was  $15 \frac{\text{mg}}{\text{ml of salt solution}}$  ( $\sim 1.5$  wt% lipid). The corresponding molar concentration of each lipid is indicated in the figure. The solid symbols represent the major phase that does not adsorb the ions. The open symbols represent the secondary phase (in minority) that adsorbs some of the ions.

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