Supplementary Information

The Effect of Divalent Cation Removal on the Structure of Gram-negative Bacterial Outer Membrane Models

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Surface Pressure vs. area isotherms obtained from RaLPS monolayers

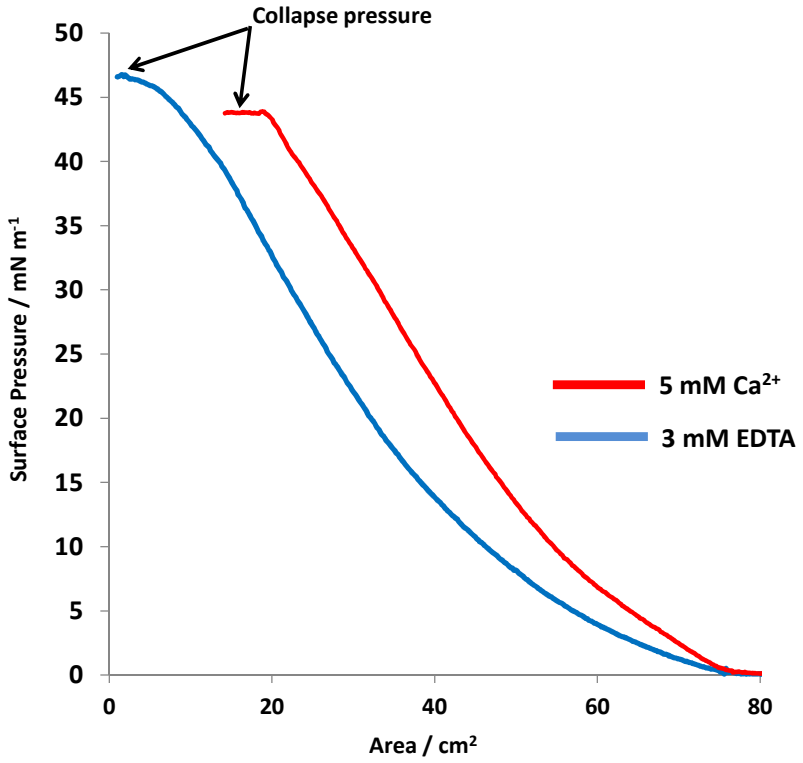

Figure S1, A comparison of the surface pressure vs area isotherms obtained from RaLPS monolayers deposited on liquid surfaces buffered with 20 mM HEPES at pH 7.2 containing either 5mM CaCl2 (red line) or 3 mM EDTA (blue line). The isotherm areas have been normalised so that the area where the initial increase in surface pressure is the same for both samples for ease of comparison.

Parameters obtained from model data fitting of reflectivity profiles obtained from asymmetric DPPC/RaLPS bilayers at the silicon/water interface

Table S1, Summary of known scattering length densities of the lipid components and the solution subphases.

Lipid / Solvent	**Neutron scattering length density (ρ) (10^{-6} Å^{-2})**
D_2O	6.35
Silicon matched water	2.07
H_2O	-0.56
Silicon	2.07
Silicon oxide (SiO_2)	3.41
DPPC head group	1.98
h-DPPC tails	-0.39
d-DPPC tails	7.45

Table S2, Fitting parameters obtained for six isotopic contrast fitting of an asymmetrical 1,2 dipalmitoylhposphatidylcholine (inner leaflet) Ra-LPS (outer leaflet) bilayer in the presence of a solution of 20 mM HEPES pH/D 7.2 5 mM $CaCl_2$.

Layer	Contrast	Thickness/Å	ρ / $\times 10^{-6}$ Å^{-2}	% Hydration	Roughness/Å
Layer 1 Silicon Oxide	N/A	14.6±1.2	3.41[nf]	13.0±2.0	3.0±0.2
Layer 2 Inner head-group	d-PC/Ra-LPS in D_2O	13.0±0.9	4.1±0.3	N/A	5.5±1.0
	d-PC/ Ra-LPS in SMW		2.1±0.3		
	d-PC/ Ra-LPS in H_2O		0.6±1.0		
	h-PC/ Ra-LPS in D_2O		4.1±0.3		
	h-PC/ Ra-LPS in SMW		2.1±0.3		
	h-PC/ Ra-LPS in H_2O		0.6±1.0		
Layer 3 Inner tails	d-PC/ Ra-LPS	17.0±0.2	6.0[A]±0.3	4.0±4.0	As above
	h-PC/ Ra-LPS		-0.39[nf]		
Layer 3 Outer tails	d-PC/ Ra-LPS	14.5±0.7	0.96[A]±0.2	4.0±4.0	As above
	h-PC/ Ra-LPS		-0.39[nf]		
Layer 4 Outer head-group	d-PC/ Ra-LPS in D_2O	31.0±1.0	4.9±0.5	N/A	As above
	d-PC/ Ra-LPS in SMW		2.7±0.1		
	d-PC/ Ra-LPS in H_2O		1.0±0.1		
	h-PC/ Ra-LPS in D_2O		4.9±0.5		
	h-PC/ Ra-LPS in SMW		2.1±0.1		
	h-PC/ Ra-LPS in H_2O		1.0±0.1		

A) Value refers to (ρ-($\varphi_{water} \times \rho_{water}$) which is fitted simultaneous for H_2O, SMW and D_2O solvent isotopic contrasts. Nf refers to a non-fitted parameter.

Table S3, Fitting parameters obtained for six isotopic contrast fitting of an asymmetrical 1,2 dipalmitoylhposphatidylcholine (inner leaflet) Ra-LPS (outer leaflet) bilayer in the presence of a solution of 20 mM HEPES pH/D 7.2 3mM EDTA.

Layer	Contrast	Thickness/Å	ρ / $\times 10^{-6}$ Å^{-2}	% Hydration	Roughness/Å
Layer 1 Silicon Oxide	N/A	13.9±5.0	3.41[nf]	12.0±2.0	2.9±0.2
Layer 2 Inner head-group	d-PC/Ra-LPS in D_2O	15.4±4.0	3.6±0.8	N/A	8.4±1.2
	d-PC/ Ra-LPS in SMW		2.7±0.4		
	d-PC/ Ra-LPS in H_2O		1.9±0.5		
	h-PC/ Ra-LPS in D_2O		3.6±0.8		
	h-PC/ Ra-LPS in SMW		2.7±0.4		
	h-PC/ Ra-LPS in H_2O		1.9±0.5		
Layer 3 Inner tails	d-PC/ Ra-LPS	15.9±1.0	4.5[A]±0.1	4±0.4	As above
	h-PC/ Ra-LPS		-0.39[nf]		
Layer 3 Outer tails	d-PC/ Ra-LPS	11.0±5.0	2.3[A]±0.3	4±4	As above
	h-PC/ Ra-LPS		-0.39[nf]		
Layer 4 Outer head-group	d-PC/ Ra-LPS in D_2O	28.4±1.2	5.2±0.6	N/A	As above
	d-PC/ Ra-LPS in SMW		2.4±0.07		
	d-PC/ Ra-LPS in H_2O		0.9±0.1		
	h-PC/ Ra-LPS in D_2O		5.2±0.6		
	h-PC/ Ra-LPS in SMW		2.4±0.1		
	h-PC/ Ra-LPS in H_2O		0.9±0.1		

A) Value refers to (ρ-($\varphi_{water} \times \rho_{water}$) which is fitted simultaneous for H_2O, SMW and D_2O solvent isotopic contrasts. Nf refers to a non-fitted parameter.

Mixing control measurements.

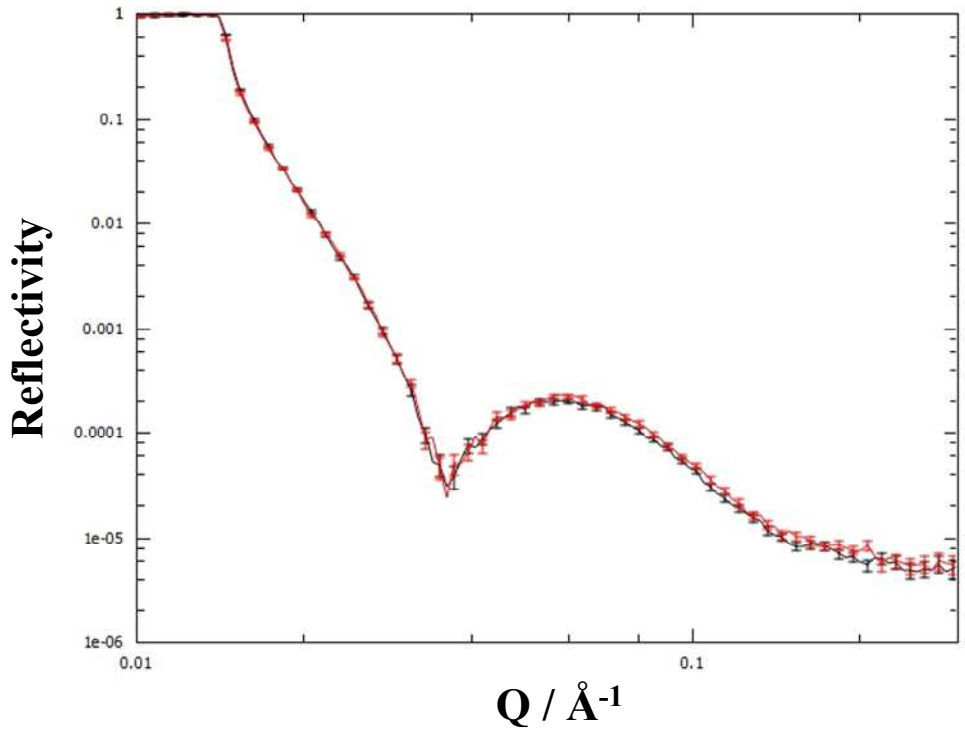

Figure S2, Bilayer mixing control measurements. The experimental neutron reflectometry profiles obtained from an asymmetrically deposited d-DPPC/Ra-LPS bilayer in 20 mM HEPES D_2O buffer at a pD of 7.2 containing 5 mM $CaCl_2$ after 22.5 ml (red) and 90 ml (black) of solution was passed through the sample cell.

Calculation of RaLPS : Ca^{2+} stoichiometry from X-ray reflectivity data.

Upon addition of EDTA to the Ca^{2+} containing sub-phase, the SLD of the inner headgroups is reduced from 14 x 10^{-6} to 13 x 10^{-6} $Å^{-2}$ with the change attributed to the removal of calcium ions. One can convert the SLD values to electron density [Eqn S1] and therefore calculate the number of calcium ions removed from per RaLPS molecule.

$$\rho_e = SLD / r_e \qquad \text{S1}$$

where r_e is the classical electron radius, 2.82 x 10^{-5} m, and ρ_e is the electron density, $e/Å^{-3}$. The change in SLD therefore corresponds to a $\Delta\rho_e$ of 0.0355 $e/Å^3$. An area per RaLPS of 128 $Å^2$, combined with a layer thickness of 23.5 Å (Table 1) results in a volume of 3008 $Å^3$ per headgroup. A change in ρ_e of 0.036 over this volume corresponds to a reduction of 106.8 electrons per headgroup. Given the atomic number of calcium, 20, this implies that on average there were 5.3 Ca^{2+} ions per headgroup. Using an ionic radius of 118 pm for Ca^{2+}, the ionic volume is 2.19 $Å^3$ compared to a volume of 30 $Å^3$ for a single water molecule. We have therefore neglected the impact that the slight increase in the number of water molecules would have on the electron density.