Supporting Information

For

Modulation of dipalmitoylphosphatidylcholine monolayers by dimethyl sulphoxide

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Analysis of SNR data.

Analysis of the SNR data were performed using the optical matrix calculation in the RasCAL software.[1] The optical matrix models the surface as being composed of a series of discrete layers, each described by the thickness, scattering length density (SLD) and an interfacial roughness. The interfacial roughnesses were fitted to be the physically reasonable value of 3 Å, except for the interface of the head and subphase where the roughness was found to increase to 4 Å in the presence of DMSO. In the case of the current analysis, the DPPC monolayer was divided into two layers, namely a solvated lipid head group layer and an unsolvated lipid hydrocarbon chain layer. The SNR data obtained from the four isotopic contrasts were fitted simultaneously, linking parameters common to all contrasts, namely the thickness, hydration, and interfacial roughness.

The SLDs for each molecule or molecular moiety in the model ($SLD_{molecule}$) was calculated using:

$$SLD_{molecule} = \frac{\sum_{i=1}^{n} b_i}{vol_{molecule}} \quad \text{(Equation 1)}$$

where b_i is the scattering length of the i^{th} of n atoms in the molecule and $vol_{molecule}$ is the molecular volume of the molecule, assumed to be independent of deuteration. Supporting Information Table 1 shows the scattering lengths, molecular volumes and SLDs of the molecules used in this study. The molecular volume of the chains was taken as reported by Nagle and Wilkinson[2] for DPPC while the molecular volume of the heads was calculated as the sum of the molecular volumes of the phosphate, glycerol, choline and carbonyl moieties obtained by Armen et al.[3] For the pure solvents, the molecular volumes were calculated from the density data for water and DMSO.[4] In the case of mixed systems, such as the sub-phase containing both DMSO and water, the SLD was calculated based on the molar fractions of the components. The critical edge was fitted to determine the SLD of the sub-phase after addition of DMSO, allowing up to 5% uncertainty in the volume of DMSO injected into the trough. Note that as DMSO is not ideally miscible in water the slight differences in density[5] were taken into account in the calculation of sub-phase SLD.

Analysis of MD simulation trajectories.

The structure of the lipid monolayer has been characterised by determining the thickness and roughness of the monolayer. The thickness of the monolayer was determined by calculating the average difference between the maximum and minimum extent of a lipid molecule ($\Delta z_{H,T}$). Additionally, the average distance between the N atom in the choline group and the carbonyl O

(EO) atom of the ester group ($\Delta z_{N(CH3)3,COO}$) were calculated to characterise any changes in the thickness of the head group and the chain ($\Delta z_{H,T}$ - $\Delta z_{N(CH3)3,COO}$) regions as a result of the presence of DMSO. Whereas, the roughness of the monolayer/solvent interface was calculated by first calculating the mean z coordinates of the N atom in the choline group ($z_{N,avg}$) and the P atom in the phosphate group ($z_{P,avg}$). Then the roughness at the depth of the N atom in choline group of the lipid molecules was calculated as the average of the absolute value of the difference in z-coordinate of each N atom in the choline group of the various lipid molecules and $z_{N,avg}$ at each configuration, and finally averaged over all configurations. A similar calculation was done to measure the roughness at the depth of the P atom in the phosphate group of the lipid molecules.

The interaction of the solvent molecules and the PC head groups was characterised by calculating the number of solvating molecules as well as looking at the relative position of bound DMSO molecules around the choline group. The solvation of the PC head groups were described by calculating the number of water and DMSO molecules (bound, free and total = bound + free) around the various head group regions highlighted in Figure 1b (N = nitrogen atom in choline group, P = phosphorus atom in phosphate group and EO = double bonded oxygen atoms in the ester bonds). A solvent molecule is defined as bound if they are within a first neighbour distance as determined from radial distribution functions (not shown) measured from the simulation trajectories. A free solvent molecule is a solvent molecule whose oxygen atom has a z-coordinate within the range of z-coordinates spanned by the nearest lipid molecule's head group and is not bound.

Additionally, the positioning of the DMSO around the choline group within the PC head group has been determined. The position of DMSO around the nitrogen atom of the choline group was

calculated by finding the angle consisting of the vector between the N atom in the choline group and the oxygen atom of DMSO molecules bound to the choline group and the (0,0,1) vector, which is normal to the monolayer-solvent layer interface. The angle was defined such that if the angle is > 90°, then the oxygen atom of the DMSO molecule is located 'below' the choline group (in the solvent phase), if the angle is < 90°, then the oxygen atom of the DMSO molecule would be 'above' the choline group (more inserted into the monolayer), and if the angle is equal to 90° then it would be parallel to the nitrogen atom of the choline group (the cartoon in Figure 5a) demonstrates the relative above and below configurations).

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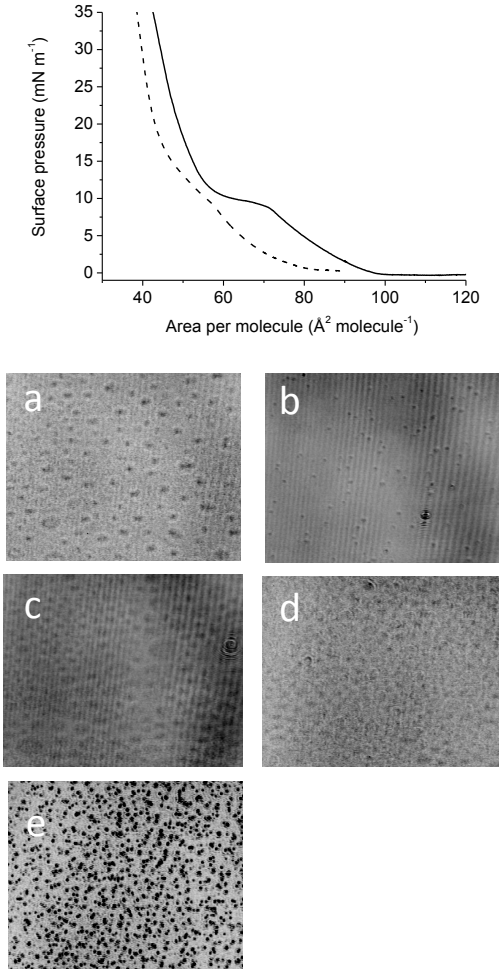
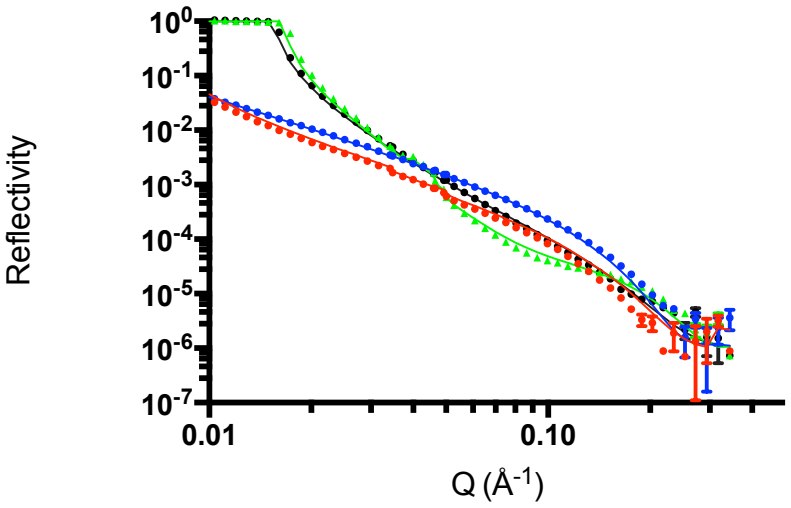


Supporting Information Figure 1. Surface pressure-area isotherm of DPPC deposited on a DPPC on a sub-phase of 0.1 mole fraction DMSO (broken line) compared to pure water sub-phase (solid line). BAM images taken of the DPPC monolayer on a sub-phase of 0.1 mole fraction DMSO at (a) 5, (b) 9, (c) 10, (d) 20, and (e) 30 mN m^{-1}. In contrast to DMSO injected underneath pre-formed DPPC monolayers (Figure 2) lateral hetergeneity in the form of large domains was observed. The size of each BAM image is 215 μm x 267 μm.



Supporting Information Figure 2. Neutron reflectometry profiles of a DPPC monolayer at 30 mN m^{-1} after addition of 0.05 mole fraction DMSO into the sub-phase. The contrasts shown are fully deuterated DPPC (d_{75}DPPC) on D_2O with hDMSO (green; contrast 2), chain-deuterated DPPC (d_{62}DPPC) on D_2O with d_6DMSO (black; contrast 1), d_{75}DPPC on ACMW with hDMSO (blue; contrast 3), and d_{62}DPPC on ACMW with d_6DMSO (red; constrast4). The line of best fit is shown as a solid line for each contrast, from the model fitted to the four contrasts simultaneously.

Supporting Information Table 1. Neutron scattering lengths, molecular volumes, and neutron scattering length densities of lipids and solvents used in this study.

Material	Molecular formula	Molecular volume ($Å^3$)	Scattering length (x 10^{-15} m)	Scattering length density (x 10^{-6} $Å^{-2}$)
d_{62}chains	$C_{30}D_{62}$	800	612.98	7.66
d_{13}PC	$C_{10}H_5NO_8PD_{13}$	344	195.39	5.68
hPC	$C_{10}H_{18}NO_8P$	344	60.04	1.75
d_{62}DPPC	$C_{38}H_{18}NO_8PD_{62}$	1144	673.02	5.88
d_{75}DPPC	$C_{38}H_5NO_8PD_{75}$	1144	808.38	7.07
hDMSO	$(CH_3)_2SO$	118	-0.50	-0.04
d_6DMSO	$(CD_3)_2SO$	118	61.97	5.26
Water	H_2O	30	-1.68	-0.56
Heavy water	D_2O	30	19.15	6.35

Supporting Information Table 2. The average number of solvent molecules in the head group region fitted from the specular neutron reflectivity (SNR) data at 25°C of a DPPC monolayer on a pure water or aqueous DMSO solution. Four isotopic contrasts were fitted with a two-layer optical matrix model. The total number of solvent molecules is the sum of the number of water molecules and DMSO obtained from the fitted scattering length density (SLD) of the head group region. The ratio is the number of DMSO molecules divided by the number of water molecules in the head group region.

DMSO (mole fraction)	Water molecules/lipid molecule	DMSO molecules/lipid molecule	Ratio Water: DMSO
0	3.0 ± 1	-	-
0.05	2.9 ± 0.1	0.1 ± 0.1	29:1
0.1	2.7 ± 0.1	0.3 ± 0.1	9:1