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Supplemental Information

Methanol Accelerates DMPC Flip-Flop and Transfer: A SANS Study on Lipid Dynamics

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Materials & Methods

1,2-dimyristoyl-sn-glycero-3-phosphocholine (14:0/14:0 PC, h-DMPC), 1,2-dimyristoyl-d54-sn-glycero-3-phosphocholine (14:0(d27)/14:0(d27), d-DMPC) 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol (14:0/14:0 PG, DMPG) were purchased from Avanti Polar Lipids (Alabaster, AL). Lipids were received and used without further purification. Lipids in powdered form were dissolved in HPLC-grade chloroform prior to sample preparation. Deuterated methanol (d-methanol) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA).

h-DMPC/d-DMPC Large Unilamellar Vesicle Sample Preparation

Exact quantities of lipids were mixed then placed under a constant stream of nitrogen and then dried in a vacuum oven at 50 °C to ensure organic solvent evaporation. Each sample included 5% DMPG to promote unilamellarity. The resulting lipid films were then hydrated in the pertinent D_2O mixture solvent to produce samples of ca. 17 mg/mL. Subsequently, h-DMPC and d-DMPC samples were extruded separately through 100 nm pores in polycarbonate filters at 35 °C, above their phase transition temperature. Prior to any measurements, samples with d-methanol added were let stand for 1 hour to ensure equilibrium was reached before heating and measurements. Using a Wyatt DynaPro NanoStar, the mean particle diameter was determined to be ~140 nm, before methanol addition and after methanol incubation in small angle neutron scattering (SANS) banjo cells. Sizing measurements were taken at 30 °C.

Determining Bilayer Structure

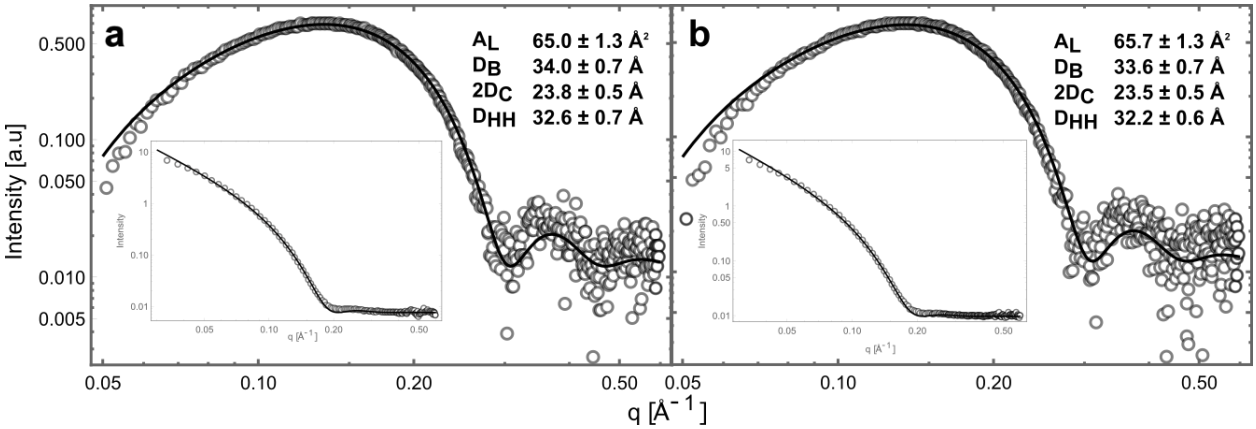

Figure S1. Small angle X-ray scattering (SAXS, open circles) and SANS (open circle inset) curves of pure h-DMPC LUVs in 100% D_2O (a) and in 3% d-methanol, D_2O solvent (b). Bold continuous lines represent fits using a 5-slab model which jointly analyzed both SANS and SAXS curves to robustly determine relevant bilayer parameters (*2*), as shown in the tables within the figure. All measurements shown here were conducted at 37 °C. Parameter uncertainty is estimated to be 2% according to Eicher et al. (*2*).

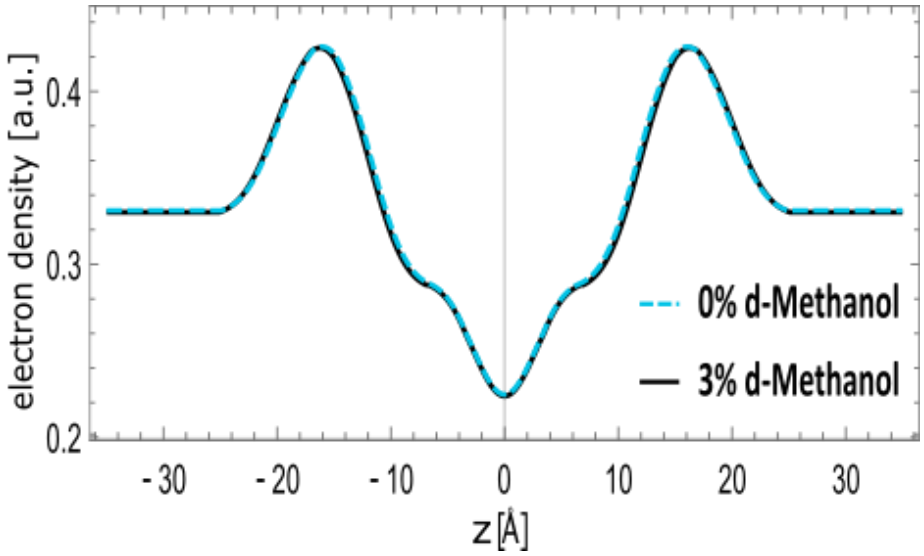

Figure S2. Electron density profiles (EDP) of DMPC in pure D_2O (black line) and 3% v/v d-methanol (teal dashes). EDPs were derived from the joint refinement of SANS and SAXS curves found in Fig. S1. The EDPs are essentially identical, highlighting the unchanged DMPC bilayer structure after d-methanol treatment.

SANS and SAXS Structural Measurements

Elastic SANS curves were measured on the Extended Q-range SANS (EQ-SANS) instrument located in Oak Ridge National Laboratories (ORNL) (*3*). A sample-to-detector distance of 1.6 m was set, and a neutron beam with a wavelength band of 4 to 8 Å was used to produce a scattering vector, q, range of 0.02 $Å^{-1}$ to 0.8 $Å^{-1}$ where q represents the scattering vector, found by $q = 4\pi\sin\theta/\lambda$, where 2θ is the angle of scattered neutrons. The resulting scattered neutrons were detected via a 2D ^{3}He-based detector and radially averaged to produce a 1D *I(q)* scattering curve. Data processing was done using Mantid Software provided by ORNL where an absolute scale was established using a porous silica standard, as well as subsequent reductions accounting for sample transmission, pixel sensitivity, dark current and sample background. SANS curves can be found in Figure S1 as the red open circle plots in the foreground.

Complementary X-ray scattering data were measured using a Rigaku BioSAXS-2000 home source with a Pilatus 100 K detector. H-DMPC samples in pure D_2O or 3% d-methanol solutions were measured at a fixed sample-to-detector distance of 480 mm. As seen in Figure S1 (inset), resultant curves were averaged and subtracted via the relevant solvent backgrounds using ATSAS software (*4*).

In Fig. S1, several important bilayer parameters are shown: A_L, D_B, $2D_C$, and D_{HH}. AL represents the lateral area per lipid which is a good measure of lipid packing. D_B represents the bilayer (or Luzzati) thickness, as determined by the SANS portion of the fitting. $2D_C$ is the hydrocarbon thickness. D_{HH} is another measure of bilayer thickness but is determined by the SAXS data and is representative of the distance between phosphate-phosphate groups. The parameter values for methanol-treated and untreated DMPC liposomes are almost unchanged, revealing that the statistical average of the vesicle structure is unchanged as well.

Measuring Lipid Flip-Flop and Transfer Rates Using Small Angle Neutron Scattering

Dynamical lipid SANS measurements were conducted on the Very Small-Angle Neutron Scattering (VSANS) instrument located at the National Institute of Standards and Technology Center for Neutron Research (NIST-CNR). The white beam option was used on VSANS to maximize neutron count rates and minimize the required count times. A neutron wavelength (λ) of 5.3 Å with a wavelength spread $\Delta\lambda/\lambda$ of 40% was used with two detector carriages with sample-to-detector distances of 4 m and 19 m was used to accesses yielded a q-range of 0.003 $Å^{-1}$ to 0.12 $Å^{-1}$. Data were collected using 3 min. acquisition times. Lipid samples were mixed and immediately measured on SANS using 1 mm or 2 mm path length quartz banjo cells at 37 °C. Each sample was run until the intensity decayed to a minimum and therefore reached the fully scrambled equilibrium. The total intensity was calculated using the Igor Pro reduction software and VSANS macros provided by the NIST-CNR by subtracting contributions from the external background, sample transmission, empty cell scattering as well as by correcting for detector pixel sensitivity. This resulted in well populated intensity vs. q curves after stitching.

Subsequent analysis follows the scheme used by Nakano et al. (*1*). Each sample possessed numerous intensity scattering curves, each representing a measurement taken at a single time point. All curves were converted into a single contrast decay curve per sample after normalization using $\Delta\rho(t)/\Delta\rho(0) = (I(t)^{0.5}-I(\infty)^{0.5})/(I(0)^{0.5}-I(\infty)^{0.5})$, where $\Delta\rho(t)/\Delta\rho(0)$ represents the normalized total intensity decay, while I(t), I(∞), and I(0) are the scattering intensity at some time after mixing, scrambled infinity, and initial mixing time, respectively. Each sample was allowed to run until base-line, or full decay, was reached, signifying fully mixed vesicles and that our contrast match solvent was adequate. These final fully decayed curves provided an infinity point to use for normalization. The resultant decay curves were then fitted using

$$\frac{\Delta\rho(t)}{\Delta\rho(0)} = \left(0.5 - \frac{k_f}{X}\right)e^{\left(-\frac{k_e+2k_f+X}{2}t\right)} + (0.5 + \frac{k_f}{X})e^{\left(-\frac{k_e+2k_f-X}{2}t\right)}$$

where $X = (4k_f^2+k_e^2)^{0.5}$ to find DMPC flip-flop (k_f) and transfer rates (k_e), respectively. Half-times for these rates were calculated via $t_{1/2} = (\ln 2)/k$

Disclaimer

Certain commercial equipment, instruments, or materials are identified in this paper to foster understating. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, not does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Supporting References

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