"Tuning membrane thickness fluctuations in model lipid bilayers"

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S1. Characterization of the membrane phase transitions

Shifts in the transition temperatures are expected in lipids with deuterium labeling, depending on the deuteration level and the amount of the deuterated lipids in the sample. Previous temperature-dependent density measurements on single component DMPC and DSPC lipid bilayers showed significant shifts in the melting temperatures of tail-deuterated (dt) DMPC and DSPC lipids relative to their hydrogenated analogues (1). The reported temperatures for the different lipids are: T_m (DSPC) = 55 °C and T_m (DMPC) = 23 °C for the hydrogenated samples, and T_m (dtDMPC) = 20.5 ˚C and T_m (dtDSPC) = 50.5 ˚C. For the DMPC/DSPC mixtures in this study, the deuterium content in the samples depends on the mixing ratio of the two lipids and the deuteration requirements of the neutron scattering experiments. In this work, we investigate two samples of equimolar DMPC/DSPC bilayers with different neutron scattering contrasts: 1) fully hydrogenated bilayers in D_2O and 2) bilayers with the tail region contrast matched to D_2O. To account for deuteration effects, we use temperature-dependent densitometry to determine the phase boundaries of the two samples. The detailed composition of the two samples and their transition temperatures are reported in Table S1.

Table S1: Compositional (mass) description of the fully-hydrogenated and tail-contrast-matched vesicles of equimolar DMPC/DSPC mixtures used in this study, along with their lower and upper transition temperatures, T_l and T_u respectively, as obtained from density measurements. The mixtures were suspended in the required amount of D_2O at a lipid mass fraction of 10 mg/mL and 100 mg/mL for SANS and NSE measurements, respectively.

	dtDMPC	hDMPC	dtDSPC	hDSPC	T_l	T_u
Fully-hydrogenated	0 mg	9.240 mg	0 mg	10.765 mg	30.2 °C	43.6 °C
Tail-contrast-matched	8.301 mg	0.899 mg	9.677 mg	1.121 mg	27.5 °C	41 °C

S2. Neutron Spin Echo (NSE)

The NSE technique accesses the normalized intermediate scattering function $I(q,t)/I(q,0)$, which for the present system can be fitted to a stretched exponential of the form $\exp[-(\Gamma t)^{2/3}]$ where Γ is the decay rate

and t is the Fourier time (2). An example of the fits to the data collected on the tail-contrast-matched vesicles is shown in Fig. S1 for T = 65 °C. The decay rates are adequately described by a sum of the Zilman-Granek model (2) of bending fluctuations, including the refinement by Watson and Brown (3) and Lee *et al*. (4), and a thickness fluctuation term proposed by Nagao (1, 5, 6). The full expression used for modeling the membrane fluctuations is given by eq. 1 in the main text.

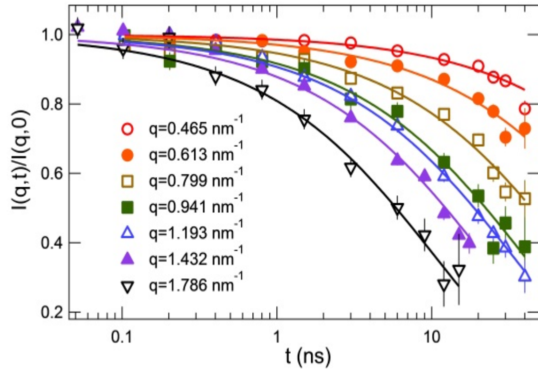

Fig. S1. Normalized intermediate scattering function, $I(q,t)/I(q,0)$, obtained from NSE measurements on the tail-contrast-matched sample at T = 65 °C. The solid lines are fits to the stretched exponential model.

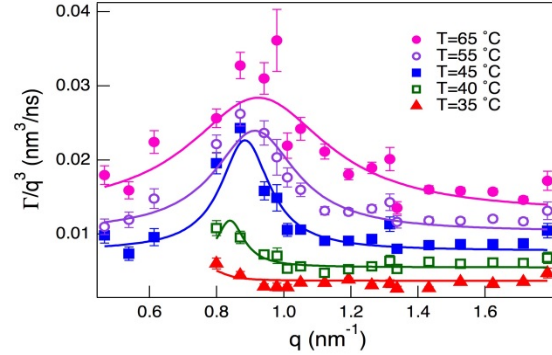

Fig. S2. Temperature variation of the q dependence of Γ/q^3 from the tail-contrast-matched sample. The lines are best fits to the data using eq. 1 in the main text with q_0 values obtained from the dip position in the corresponding SANS data. The shift of the peak position toward lower q with decreasing temperature indicates an increase in the membrane thickness as the mixture transitions into the coexistence phase.

The thickness fluctuation term accounts for the enhancement of the decay rates caused by membrane thickness fluctuations as shown in Fig. S2. The maximum enhancement is observed at q-values that coincide with the dip position in the corresponding SANS scattering pattern (see Fig. S3). The dip position in the SANS signal corresponds to the membrane thickness and can be calculated from a lamellar form factor (7) as $q_0 = \pi/(d_m - d_h)$ where d_m and d_h are the total membrane thickness and the lipid headgroup thickness, respectively. On the other hand, similar form factor calculations on a fully hydrogenated bilayer (that lacks contrast between head and tail groups) show that the peak position, q_0, in this case occurs at $2\pi/d_m$; *i.e.* $q_0 \sim$ twice the value for the tail-contrast matched bilayers. This value is outside the measurement window of the NSE experiment. Consequently, thickness-fluctuation enhancement of the decay rates of the hydrogenated bilayers cannot be observed over the accessed q-range, as evident from the data shown in Fig. 2 in the main text.

S3. Structural characterization of the lipid bilayers

The response of the structural parameters of the vesicles to the phase transitions was studied by small-angle neutron and x-ray scattering (SANS and SAXS) over a wide temperature range. Fig. S4 shows SANS profiles of tail-contrast-matched unilamellar vesicles at three select temperatures in each of the regions in Fig. 1 in the main article. The data show a dip around q = 1 nm^{-1} which further shifts toward lower q with decreasing temperature, indicating an increase in the bilayer thickness as the lipid

components transition into their gel phase. The solid lines are least-mean square fits to the data using a scattering model for a three-shell vesicle (8). The model takes into account the contrast variations of the head and tail groups of the lipid molecules within the vesicle. Accordingly, the vesicle model is divided into three layers or shells such that the innermost and outermost shells account for the headgroup regions of the inner and outer leaflets, respectively, and the intermediate shell depicts the overall lipid-tail region in both leaflets. Each shell is described by a given thickness and scattering contrast, which are obtained from fits to the scattering data. The same model is used for fitting the SAXS data with careful substitution of the neutron scattering length density by the electron density of the corresponding shell. The membrane thickness, obtained from SANS and SAXS data at various temperatures, is shown in Fig. 6 in the main manuscript.

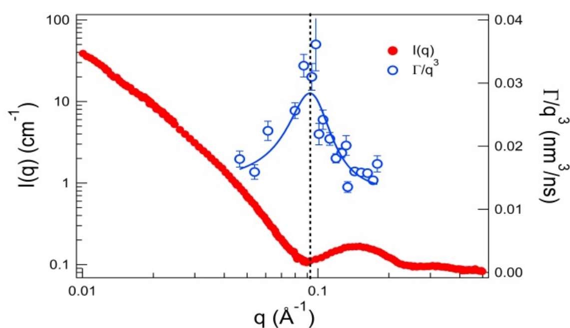

Fig. S3. Overplot of NSE and SANS data on the tail-contrast matched equimolar DMPC/DSPC vesicles at T = 65 °C. The thickness fluctuation enhancement in the NSE signal occurs at the same *q*-value as the dip signifying membrane thickness in the SANS signal.

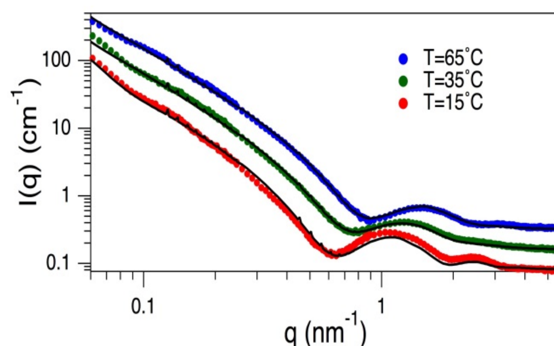

Fig. S4. SANS data from the tail-contrast-matched equimolar DMPC/DSPC unilamellar vesicles, shifted vertically for clarity. The solid lines are the fit results to a three-shell vesicle model.

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