Experimental report

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Proposal: 9-13-705 **Council:** 4/2017

Title: Determining the effects of headgroup charge on the elastic and viscous properties of model lipid membranes

Research area: Soft condensed matter

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Samples: DMPG lipid in D2O buffer

Instrument	Requested days	Allocated days	From	To
D22	1	0		
IN15	9	5	06/06/2018	11/06/2018

Abstract:

Biological membranes are highly flexible self-assembled structures that experience dynamic motions ranging from individual lipid diffusion to undulations of micron-sized patches of the membrane. At intermediate length scales, lipid membranes undergo collective bending and thickness fluctuations that involve tens to hundreds of lipids. Importantly, these mesoscale dynamics are governed by the same elastic properties that determine the energy required for large scale membrane deformations necessary for cell functions such as growth and division. Here we propose to measure the effects of headgroup charge on the mesoscale thickness fluctuation dynamics and membrane elastic properties in model lipid bilayers using neutron spin echo (NSE). NSE is the only technique capable of accessing the necessary length scales and time scales to probe these dynamics and thereby provide important insights into the functional significance of charged lipid headgroups in tuning the dynamics of biological membranes.

Experimental Report for ILL proposal 9-13-705

Determining the effects of headgroup charge on the elastic and viscous properties of model lipid membranes

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Charged phosphatidylglycerols (PG) are anionic phospholipids found in mitochondrial membranes as a precursor to cardiolipin and are a major component of the cytoplasmic membrane of bacteria. The phase behavior of PG lipids not only depends on hydrocarbon tail length like other phospholipids, but also is highly sensitive to the pH and ionic strength of the surrounding solution. In particular, the phase behavior of dimyristoylphosphatidylglycerol (DMPG) with saturated 14 carbon tails is highly unusual. The calorimetric profile shows several broad peaks than span 20 °C and sharpen with increasing buffer ionic strength. The wide melting transition is associated with changes in the lipid membrane structure as well as the bulk solution properties including the viscosity and ionic conductivity.(1) The aim of this experiment was to better understand the collective membrane dynamics on the nanoscale during the melting transition and link these dynamics to the changes in membrane structure and bulk solution properties.

Solutions containing 2 mg/mL protiated DMPG lipid were prepared in a D_2O buffer containing 10 mM HEPES-d18 and 2mM NaCl, pD 7.4. Relatively monodisperse lipid vesicles were prepared by extrusion. SANS data (not shown) indicated that the final vesicles were ≈ 60 nm in diameter.

Fig. 1a shows differential scanning calorimetry data measured at the PSCM prior to beginning the NSE measurements. As expected, the data contain several broad features between ≈ 35 °C and ≈ 15 °C. NSE data were collected at 45 °C and 23 °C corresponding to the fluid phase and melting transition, respectively. The NSE data were fit with a stretched exponential

$$I(q,t)/I(q,0) = \exp\left[-(\Gamma t)^{2/3}\right]$$

predicted by Zilman and Granek (ZG) for single membrane bending fluctuations in which Γ, is the decay rate.(2)The fits are plotted as the solid lines in the Fig.1b and 1c. The data are well described by a stretch exponential in the fluid phase and at long time in the melting transition; however, the data markedly deviate from the fits at 23°C for t < 10 ns at the highest q-values.

Recent work by Gupta et al. suggests that the deviations from the ZG model at short times are due to the short range motions of the individual lipids within the membrane.(3) Our results in Fig.1 indicate that the dynamics of the individual lipids are enhanced during the melting transition and that additional contributions beyond the ZG model will need to be included to correctly fit the data.

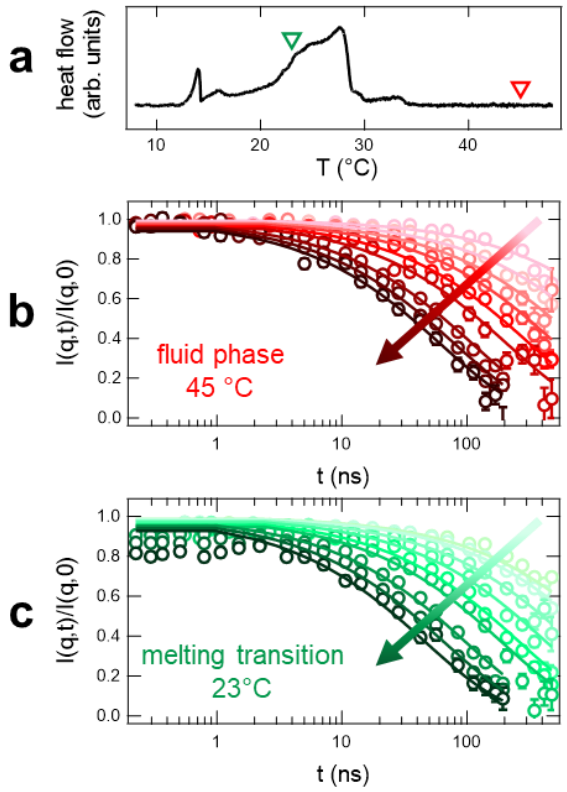

Fig. 1 *(a) DSC data for protiated DMPG and NSE data corresponding to temperatures in the lipid (b) fluid phase and (c) melting transition. The colored symbols in (a) correspond to the temperatures of the NSE experiments in (b) and (c). The arrows in (b) and (c) show the trend in NSE data with increasing q.*

Comparing the averaged q-dependence of Γ determined from the ZG fits to the data at long Fourier times (Table 1) also suggests that the dynamics are faster in the melting transition. The increase in $\langle \Gamma/q^3 \rangle$ is associated with a decrease in the membrane bending modulus and a softer membrane. (2)

Table 1. *Average q-dependence of decay rates determined from fits to the NSE data in Fig. 1*

T (°C)	$\langle \Gamma/q^3 \rangle$ (Å^3/ns^{-1})
45	16.5 ± 0.3
23	23.4 ± 0.2

A decrease in the membrane bending modulus should also be associated with a decrease in the membrane area compressibility modulus (K_A). We recently showed that K_A can be extracted from NSE measurements of the membrane thickness fluctuations.(4) Therefore, we also prepared a 20 mg/mL sample of DMPG-d54 in the same deuterated buffer to measure the temperature dependence of the thickness fluctuations and K_A. The deuterated lipids were used to contrast match the lipid tails to the solvent and emphasize the collective dynamics of the lipid headgroups.

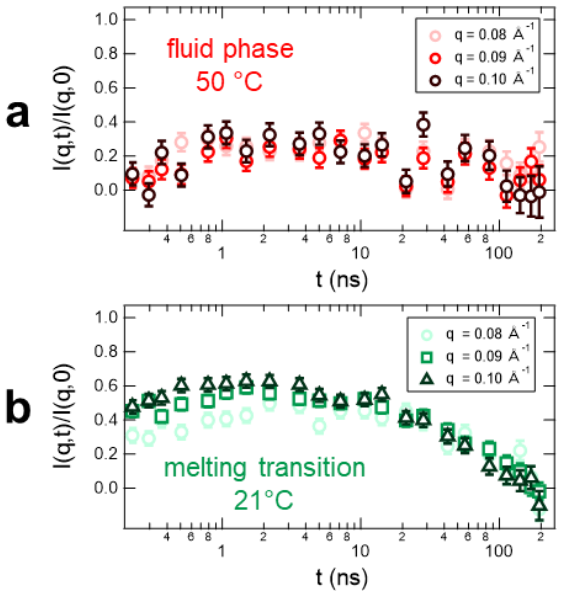

Fig. 2 *NSE data for DMPG-d54 corresponding to temperatures in the lipid (a) fluid phase and (b) melting transition.*

The NSE data for DMPG-d54 did not show the trends reported for other lipids. As seen in Fig. 2a, there was no measurable signal above background in the fluid phase and we were therefore unable to determine any information related to the thickness fluctuation dynamics. There was a measurable signal in the intermediate phase. However, the intermediate scattering function did not start at 1.0 suggesting the presence of the much faster process. Also, the decay at longer Fourier times did not have a noticeable q-dependence as expected for thickness fluctuation data. One possibility is that there was hydrogen-deuterium exchange between the hydroxyl groups on the lipid headgroups and the solvent that was affecting the results. Future measurements would require a different contrast scheme to isolate the membrane dynamics of interest.

While we are unable to reliably extract information on the thickness fluctuation dynamics from our measurements, the bending results are an interesting and novel result. We are currently working to better analyze the data in Fig. 1 and correlate the dynamic results with structural data. So far, the data suggest that both the individual lipid dynamics and collective membrane dynamics are faster in the melting transition. This enhancement is consistent with the increase in density fluctuations predicted at the melting transition and may help explain results in literature that suggest DMPG bilayers become porous during the melting transition.(5)

References:

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4. Nagao M, Kelley EG, Ashkar R, Bradbury R, & Butler PD (2017) Probing Elastic and Viscous Properties of Phospholipid Bilayers Using Neutron Spin Echo Spectroscopy. *The Journal of Physical Chemistry Letters* 8(19):4679-4684.
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