Experimental report

30/05/2019

Proposal: 9-13-636 **Council:** 4/2016

Title: Influence of charge on thickness fluctuations in lipid membranes

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Elizabeth KELLEY

Experimental team: Paul BUTLER
Michihiro NAGAO

Local contacts: Lionel PORCAR

Samples: DMPG lipid in D2O

Instrument	Requested days	Allocated days	From	To
IN15	9	0		
D22	1	1	04/06/2018	05/06/2018

Abstract:

Lipid membrane elastic properties play an important role in the membrane deformations necessary for cell function, motivating significant research efforts to understand the physics underlying biomembrane dynamics. The majority of research to date has focused on model membrane systems composed of zwitterionic lipids; however, nearly all biomembranes are negatively charged at physiological conditions. Accordingly, here we propose to expand the use of neutron spin echo to study thickness fluctuation dynamics in negatively-charged model membranes. Neutron spin echo is the only technique capable of probing the appropriate length scales and time scales to study thickness fluctuation dynamics, and the unique access to the long Fourier times and high flux available on the IN15 instrument is crucial for the proposed experiments. The experiments outlined below will provide new and necessary insights into charge effects on lipid membrane dynamics and elastic properties.

Experimental Report for ILL proposal 9-13-636

Influence of charge on thickness fluctuations in lipid membranes

Elizabeth Kelley, Paul Butler, Michihiro Nagao, Lionel Porcar, Bela Farago

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.

SANS data were collected on both the protiated and deuterated DMPG lipid vesicles to complement NSE experiments to measure the corresponding lipid dynamics (data not shown). The high q SANS data for 20 mg/mL protiated DMPG in D2O buffer (10 mM HEPES, 2 mM NaCl, pD 7.4) are shown in Fig. 1a. The first minima in the bilayer form factor ($q \approx 2$ nm^{-1}) shifted to lower q with decreasing temperature, suggesting an increase in bilayer thickness as the vesicles are cooled from the fluid phase (red curves), through the melting transition (purple curves) and into the gel phase (blue curves).

The changes in bilayer thickness were quantified by fitting the data with a bilayer form factor and the resulting values are plotted in Fig. 1b. The bilayer thickness increased ≈ 1 nm from the fluid to gel phase as reported for other phospholipids. Interestingly, there was no anomalous change in the bilayer thickness in the intermediate phase unlike the dynamics measured with NSE (data not shown). Instead, the bilayer thickness monotonically increased through the melting transition and followed the lipid volume measured with densitometry.

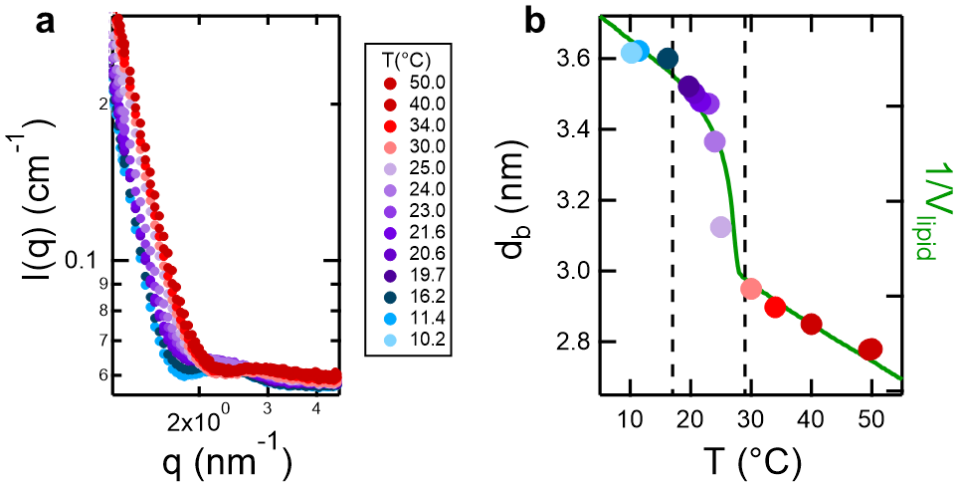

Fig. 1 *Temperature dependence of (a) the high q SANS data and (b) corresponding bilayer thicknesses (d_b) determined by fitting the data with a vesicle form factor. The green line in (b) shows that the changes in db directly follow the changes in lipid volume measured with densitometry. The red, purple, and blue symbols correspond to temperatures in the fluid phase, melting transition, and gel phase, respectively.*

The surprising results are shown in Fig. 2 where the low q SANS data showed a marked change at temperatures corresponding to the lipid melting transition. The I(q → 0) dropped as the temperature decreased and then was recovered upon further cooling in the gel phase. Moreover, the original scattering curve was completely recovered after reheating the sample back to the fluid phase, suggesting the changes in low q were not due to a change in the vesicle structure.

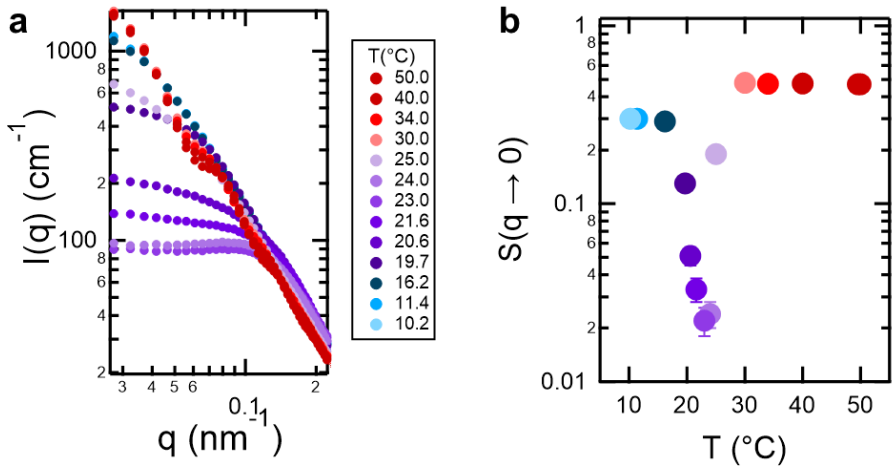

Fig. 2 *Temperature dependence of (a) low q SANS data and (b) extrapolated S(q → 0). Values for S(q → 0) were determined by diving the measured SANS data by the calculated vesicle form factor. The red, purple and blue symbols correspond to temperatures is the fluid phase, melting transition and gel phase, respectively.*

Instead we speculate that the changes in low q are due to an increase in repulsive interactions in the intermediate phase and associated changes in the structure factor. S(q→0) values calculated by dividing the measured intensity by the calculated vesicle form factor are shown in Fig 2b. S(q→0) drops by as much as an order of magnitude during the melting transition. This significant increase in repulsions may be do the enhanced dynamics measured with NSE (data not shown) and associated increase in the undulation repulsion forces between vesicles.(2)

Together the structural and dynamic data collected on D22 and IN15 show interesting and potentially complementary trends at temperatures corresponding to the intermediate phase. We are currently working to better analyze the SANS data and correlate the structural results with the dynamics data. So far, the data suggest that the bilayer structure does not significantly change during the melting transition and instead point to a change in the effective interactions between vesicles potentially caused by an increase in the bilayer undulations. The enhancement may be 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.(3)

References:

1. Lamy-Freund MT & Riske KA (2003) The peculiar thermo-structural behavior of the anionic lipid DMPG. *Chem. Phys. Lipids* 122(1–2):19-32.
2. Helfrich W (1978) STERIC INTERACTION OF FLUID MEMBRANES IN MULTILAYER SYSTEMS. *Zeitschrift Fur Naturforschung Section a-a Journal of Physical Sciences* 33(3):305-315.
3. Kelley EG, Butler PD, & Nagao M (2019) Softening of DMPG Lipid Membranes along the Anomlous Gel-Fluid Transition. *Biophys. J.* 116(3):364a.