Experimental Report

Proposal: **9-13-454** **Council:** 4/2012

Title: Confinement induced phase transitions in lipid bi-layers.

This proposal is a new proposal

Researh Area: Soft condensed matter

Main proposer: **DE VOS Wiebe**

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Samples: 1,2-palmitoyl-sn-glycero-3-phosphocholine (DPPC)
1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC)

Instrument	Req. Days	All. Days	From	To
D17	4	3	30/10/2012	02/11/2012

Abstract:

A recent ILL experiment showed the unique result that confinement can induce a liquid to gel phase transition in lipid bi-layers. However, the exact mechanism for this transition needs to be elucidated and to achieve this we need to study it in much greater detail. We propose to determine how much the transition temperature changes under different strengths of confinement for the lipids DPPC and DMPC. Key to this investigation is a unique and recently developed surface force style apparatus that allows direct measurements of the structure of thin layers under a confining pressure using neutron radiation. We will use this to measure the inter-layer thickness and overall thickness of a confined stack of bi-layers for a range of temperatures, the phase transition will show up as a large increase in layer thickness with decreasing temperature. Furthermore, DPPC is known to have a pre-transition to a ripple phase a few degrees below its transition temperature, our experiments will also determine if such a pre-transition still exists under confinement.

Title: Confinement induced phase transitions in lipid bilayers.

Experiment: 9-13-454 Dates: 29/10/2012 - 01/11/2012 Instrument: D17
Team: L.L.E. Mears, R. Barker, S.W. Prescott, S.B. Abbott, W.M. de Vos, R.M. Richardson.
Local contact: R. Barker

Abstract

A previous ILL experiment, our first on the confinement of lipid bilayer stacks, showed an interesting difference in the behaviour of the lipid DPPC under confinement in the gel and fluid phases. In the gel phase the interlayer spacing decreased whereas in the fluid phase it increased indicative of the DPPC undergoing a confinement induced phase transition. This experiment looked deeper into the temperature dependence of the structure of the lipid DMPC while under confinement. It indicated that once confined the interlayer spacing for DMPC was independent of temperature.

Introduction

The phase behaviour of lipid bilayers is very important for their biological functions in cell membranes and it is influenced by environmental conditions, such as temperature, pressure and hydration. In a previous experiment using the D17 reflectometer [1] our novel surface force type apparatus [2] was used to probe the effect confinement has on spin coated lipid bilayer stacks. In those experiments hydrogenous phosphatidylcholine lipids, of different tail lengths, were hydrated with D_2O vapour prior to confinement. The data suggested that under confinement some of this D_2O is removed from the stack, a consistent feature for all the lipids in both the fluid (L_α) and gel (L_β) phases. For lipids in the gel phase the shift in Bragg peak position indicated an associated thinning of the layers. However for DPPC, the lipid with the longest tail length, which we looked at in the fluid phase during that experiment, there was a surprising increase in the repeat distance. A subsequent experiment [3] has confirmed that this is also the case for the slightly longer chained DSPC. This appeared to be a confinement induced phase transition and therefore the following experiment was proposed to look further into how the phase transition temperature shifts for phosphatidylcholine lipids under confinement.

Experimental results

Samples were prepared by spin coating from chloroform onto 3” Si blocks, to form ~8 bilayers (40-50nm) of hydrogenous 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC). D_2O was used to fully hydrate the samples, using a few drops on the surface prior to confinement, in order to enhance the contrast. During the experiment the temperature of the sample was varied using an external circulating water bath connected to a heat exchanger on the sample environment.

At lower temperatures used, 8 and 14°C in the gel phase, we saw some interesting kinetic behaviour with the interlayer spacing increasing, as if entering the ripple phase, before it

thinned to show the same overall trend as the other lipids. At 8°C the sample took a very long time to equilibrate but this was quicker when 3 bar was applied. One sample was heated under confinement having started at a temperature in the gel phase. Another was cooled after initial confinement in the fluid phase at 39°C. The data from these two samples are shown in the figure alongside some reflectivity data. Although the interlayer spacing is slightly different between the heating and cooling runs it remains almost constant after confinement and is significantly different from the interlayer spacing without confinement. We believe that it had not fully equilibrated at 8°C before the heating cycle due to the very slow kinetics at this temperature. Further analysis to understand more about the distribution of water in the layer, using model fitting of the reflectivity data from this and other experiments, is ongoing.

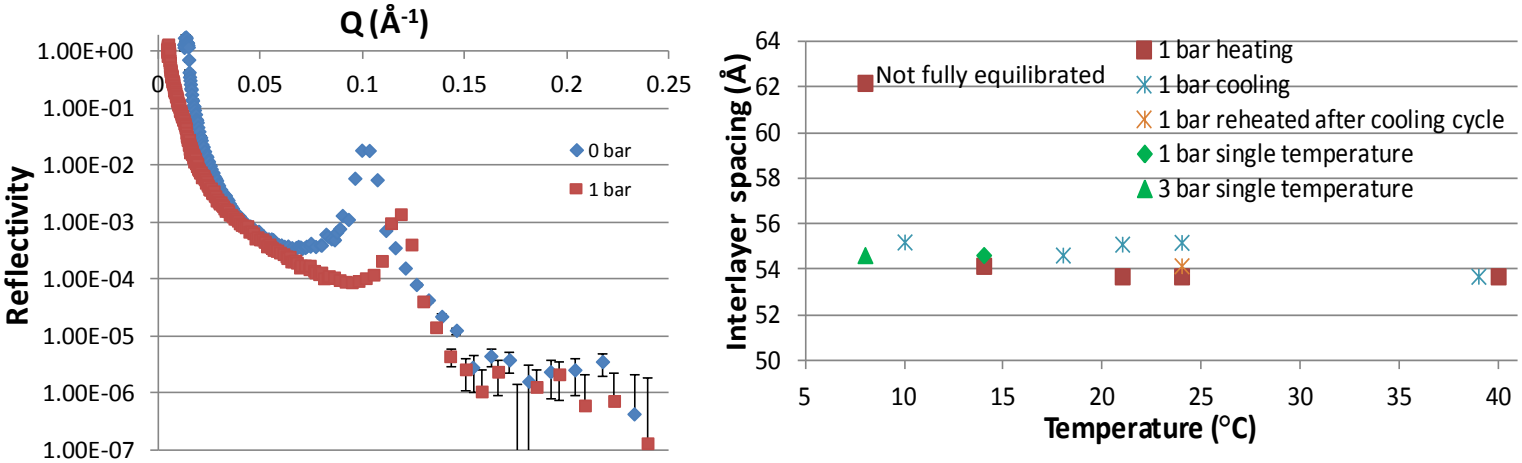

Figure 1 Left: Reflectivity data for DMPC lipid bilayer stacks fully hydrated with D_2O at 39°C both without and with 1 bar of confinement applied, the shift of the Bragg peak to a higher value of Q (smaller interlayer spacing) is clear. Right: The change in interlayer spacing as a function of temperature for a heating cycle, a cooling cycle including a reheat to 24°C, and application of 1 and 3 bar at two different temperatures to indicate that a similar spacing could be achieved by confinement without a subsequent temperature change.

Conclusions

The experiment has investigated the temperature dependence of the internal structure of DMPC bilayers under confinement. Although there are small differences between the heating and cooling cycles in the data, the interlayer spacing does not show the significant change expected across the ripple phase for a sample under ambient conditions. The data from this experiment and from the other neutron experiments referred to will be analysed further in order to fully understand the phase behaviour of lipids under confinement.

References

1. W.M. de Vos, L. L. E. Mears, S. W. Prescott, R. M. Richardson, T. Cosgrove, and R. Barker, *Exp. report ILL, D17*, 9-13-375 (2011).
2. W.M. de Vos, L.L.E. Mears, R.M. Richardson, T. Cosgrove, R.M. Dalgliesh, S.W. Prescott, *Rev. Sci. Instrum.*, 83, 113903 (2012).
3. L. L. E. Mears, W.M. de Vos, S. B. Abbott, S. W. Prescott, T. Cosgrove, R. M. Richardson, and R. Barker, *Exp. report ILL, D17*, 9-13-496 (2013).
4. L. L. E. Mears, S. B. Abbott, W.M. de Vos, S. W. Prescott, T. Cosgrove, R. M. Richardson, and R. Barker, *Exp. report ILL, D17*, 9-13-518 (2013).