Experimental report

06/11/2018

Proposal:	**9-13-783**	**Council:**	4/2018
Title:	Measuring the interbilayer pressure in glucolipids lamellar phases using a humidity chamber		
Research area:	Soft condensed matter		

This proposal is a new proposal

Main proposer:	**Niki BACCILE**
Experimental team:	Niki BACCILE
Local contacts:	Viviana CRISTIGLIO

Samples: C24H46O8

Instrument	Requested days	Allocated days	From	To
D16	4	4	11/10/2018	15/10/2018

Abstract:

In a recent set of experiments, we have measured the variation of the interbilayer distance with temperature on a lamellar phase composed by a natural glucolipid. At room temperature, the interbilayer distance is about 18 nm, but below -20°C, ice forms and interbilayer distances between 4.7 and 4.2 nm are measured, where the bilayer thickness is about 3 nm. In order to associate these values to the pressure exerted by ice during freezing and that for cryo-preservation applications, we want to measure the pressure corresponding to water thickness values ranging from 0.1 nm to 1.5 nm using a humidity chamber, because unfortunately the freezing device commonly used required acquisition times that are not compatible with neutron scattering. The sample is constituted by a glucolipid lamellar phase which has been largely characterized in previous SAXS experiments and on which we have collected temperature-dependent diffraction data. At the bottomlime, we plan to draw a pressure-interbilayer distance map which can be used to estimate the pressure below the water freezing point, using the interbilayer distance as common standard.

Standard Project

Experimental Report template

Proposal title: Measuring the interbilayer pressure in glucolipids lamellar phases using a humidity chamber		**Proposal number:** **9-13-783**
Beamline: D16	**Date(s) of experiment:** from: 11/10/2018 to: 15/10/2018	**Date of report:** 06/11/2018
Days: 4	**Local contact(s):** V. Cristiglio	*Date of submission: 06/11/2018*

Background:
Life under extreme conditions such as strong dehydration or temperatures much below zero is an extremely passionate, but also highly complex phenomenon to study. The way living cells survive under high pressures is a matter of strong interest not only for fundamental questioning about the origin of life but also for more contemporary problems like cryo-preservation. [1] In a recent set of experiments we have shown that living cells can be encapsulated in hybrid organic/inorganic matrices under controlled temperature gradients. [2] In the literature cell viability has been ascribed to the formation of intracellular ice. Beyond this assumption, other questions are still pending. Our hypothesis is that the strong pressures generated by the liquid-solid phase transition during freezing, and due to dehydration, is responsible for cell death. However, the actual pressure felt by the cell under standard cryopreservation conditions is not known. The goal of this proposal was to collect a set of pressure-distance data to evaluate the pressure of exerted by hydration/dehydration on a bilayer, composed of a new microbial glycolipid, GC18:0, forming a lamellar phase.

Results and the conclusions of the study (main part):

Two 1 wt% GC18:0 D_2O solutions at pH 6.5 and at [Na] respectively of 16 mM and 100 mM are prepared and dispersed on two separate 5 cm x 2 cm silicon wafers by simple drop cast (volume dropped: 500 µL). To enhance homogeneous spreading of the solution onto the substrate, we have used a horizontal suport levelled with a 2D spirit level. The silicon substrates were let drying in an oven at 40°C until a homogeneous coating was obtained. The samples were then introduced within a Pressure chamber, provided at the beamline, and set under vacuum at T= 25°C. The temperature of the D_2O water bath below the sample was modified to set chamber at the desired RH% value. Sample at 16mM was let equilibrate at 98 RH% before studying, where relative umidity was lowered. The 100 mM sample was let equilibrating at 10 RH% and humidity was then increased.
The experiments have been done with a wavelength of 4.5 Ångstrom; an equilibration time ranging between 60 min and 120 min was employed after each humidity change (value of omega is 2°). Equilibration time was considered to be long enough when the peak position reached a plateau. After equilibration, a full omega scan between -1° and 8° performed every 0.05° is recorded.
Figure 1a shows the evolution of the (100) Bragg diffraction peak of the GC18:0 (pH 6.5, [Na] 16 mM) supported lamellar phase with decreasing RH%, while Figure 1b shows the corresponding pressure-distance curve for 16 mM and 100 mM [Na]. As expected, the pressure increases for small interbilayer distances (low RH%) while it drops for large distances (high RH%). These data will be used to determine the repulsive hydration contribution to the total osmotic pressure. We

also observe no major variation of the pressure below about 4.5 nm, indicating that salt has no specific influence in the short-range distance regime of the osmotic pressure, as expected. We recall that the initial equilibrium interbilayer distances before drop casting for the 16 mM and 100 mM solutions are respectively 26.35 nm and 21.05 nm (measured by solution SANS). As a consequence, the salt concentration values in the high pressure regime (low RH%) increase by a factor 6.5 and 5.2 for, respectively, the 16 mM and 100 mM initial solutions, of which the end [Na] becomes 104.1 mM and 519.8 mM.

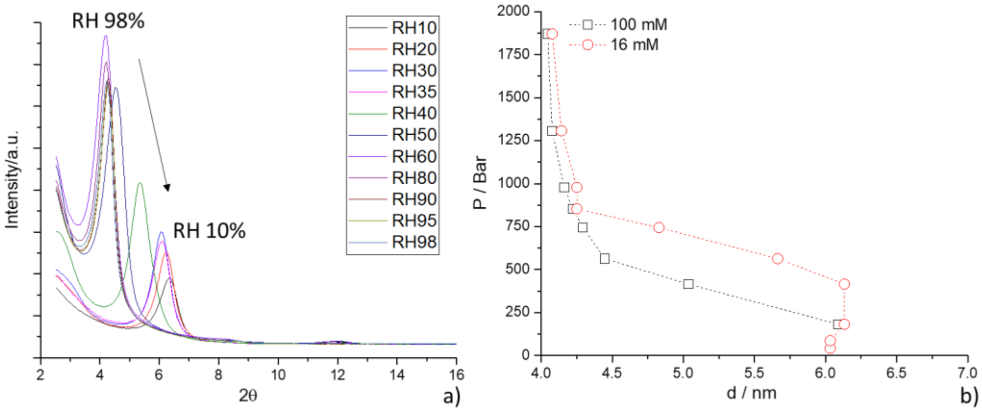

Figure 1 – a) Evolution of the (100) reflection of a GC18:0 (16 mM [Na]) supported lamellar phase as a function of relative humidity; b) Evolution of the osmotic pressure as a function of the interbilayer periodicity for two samples prepared at 16 mM and 100 mM [Na].

Data treatment

All data have been treated using the LAMP software. The classical I vs 2θ profile for each RH% is obtained by summarizing each integrated 2D image measured at a given value of omega. The value of the peak position at a given RH% is obtained by fitting the peak with a gaussian profile.

Justification and comments about the use of beam time:

The D16 beamline is the only one equipped with a pressure chamber to precisely explore the high-pressure (low RH%) regime in the pressure-distance curve measured on a supported lamellar phase. This piece of information was not known on the family of pH-responsive glycolipids used in this study and it will contribute to both better understand this class of molecules and use them as dehydration preservatives in living cellular systems.

Problems during beamtime:

We did not experience any trouble during the beamtime. However, there was a beam shutdown on the October 14th at about 8 pm, which caused a loss of 11 h of experiment.