Experimental report

15/09/2018

Proposal: 8-02-818 **Council:** 4/2018

Title: Constructing a comprehensive modelof the archaeal membrane

Research area: Biology

This proposal is a continuation of 8-02-762

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Samples: DoPhPC + DoPhPE
DoPhPC + DoPhPE + d-squalane
DoPhPC + DoPhPE + h-squalane

Instrument	Requested days	Allocated days	From	To
D16	8	7	05/09/2018	12/09/2018

Abstract:

We have recently proposed a novel membrane architecture model to explain the stability of lipid bilayers of archaeal cells at high temperatures (> 70°C) and high pressures (400 bar). In this architecture, the increase in membrane stability/rigidity is due to the presence, in the midplane of the bilayer, of apolar hydrocarbons, referred to as "apolar lipids". Our previous experiment (see ILL report 8-02-762) has demonstrated that the apolar lipid squalane can enter the membrane and is located in the midplane of the bilayer. We have started to characterize the impact of the presence of apolar lipids inside the membrane on the physicochemical properties of the bilayer using different approaches (FTIR, SAXS, DSC). We demonstrate the predicted increase in stability/rigidity, but also a concentration dependence of these variations with the apolar/polar lipid ratio. Up to now, no diffraction study has been done to determine the pressure effect on the model. Here, we would like to examine how the DoPhPC : DoPhPE lipid bilayer is affected by pressure and temperature and the physical effect of squalane under these conditions.

Report on D16 experiment 8-02-818

Constructing a comprehensive model of the archaeal membrane

Extremophiles have adapted all their cell components to live under harsh conditions. For example, they possess unique lipids to maintain the cell membrane functional but there still open questions about how their lipid bilayer can withstand extreme conditions. Our group have presented a novel model of membrane, in which apolar hydrocarbon chains are placed in the midplane of the bilayer [1]. In previous neutron experiments, we have demonstrated that the apolar isoprenoid molecule squalane is effectively placed in the midplane of an archaeal lipid bilayer, regardless of squalane concentration (from 1% to 10% molar) (8-02-762 and 8-02-809). Our previous neutron experiments were done at 25°C, so in this project, we wanted to investigate the effect of the temperature and the pressure on the squalane position to get closer to physiological conditions.

Project

We have used a mixture of DPhPC (1,2-di-O-phytanyl-sn-glycero-3-phosphatidyl-choline) and DPhPE (1,2-di-O-phytanyl-sn-glycero-3-phosphatidylethanolamine) in a 90:10 proportion as a model of the lipid bilayer of hyperthermophile and piezophile archaeon. We have incorporated hydrogenated or deuterated squalane at 1% molar concentration. The mixture was placed on ultra clean silicium wafers previously cut to fit the sample compartment of the high-pressure cell.

In this experiment, we wanted to control the temperature (up to 85°C) and the pressure (up to 1000 bars), which adds an important difficulty as lipids needs to be in contact with a transmitting pressure medium. At first, we have tried to use water around the wafer as transmitting medium but the water excess and the vertical position of the wafer made that our lipids flow and we lose the diffraction signal. After different trials, we have defined a protocol that allowed us to do the experiment controlling temperature and pressure conditions. For this, we have deposed 1.5 mg of lipid on two different wafers, and after they were completely hydrated, we have placed the wafers face to face and we have put them in the compartment of the high-pressure cell. We have added 5 µl of the D2O contrast and we let them rehydrate in the high-pressure cell, once lipids were completely hydrated, we have used fluorinert as transmitting medium inside the cell. We have controlled the level of hydration of lipids comparing the D-spacing obtained with the values of previous experiments.

Results

Despite the complexity of the experiment and after some initial problems, we have achieved to obtain a temperature and pressure scan for each sample (lipids without squalane and with hydrogenated and deuterated squalane). Four pressure points (0 bar, 250 bars, 500 bars and 1000 bars) were studied at five different temperatures (25°C, 40°C, 55°C, 70°C and 85°C) which made 20 hours of scan. For each studied sample, the diffraction patterns were measured with at least three orders of Bragg reflection, which will allow us to study the squalane position in further analysis as we have done before (see report #8-02-809).

According to a first inspection of the data, we can observe an important effect of pressure on our lipids. For example, figure 1 shows the 2D diffraction patterns obtained for DPhPC:DPhPE at 85°C and four different pressure values.

In this case, we recognize the apparition of a new lamellar phase at 85°C and ambient pressure which decreases when pressure is applied. Such new phase has a larger lamellar repeat distance (i.e. lower 2theta angle) than the original phase, 58 Å and 51.4 Å respectively. Moreover, this new lamellar phase that appears at 85°C and that is affected by pressure has another behavior when squalane is present. Detailed analysis will be done to clarify the effect of squalane.

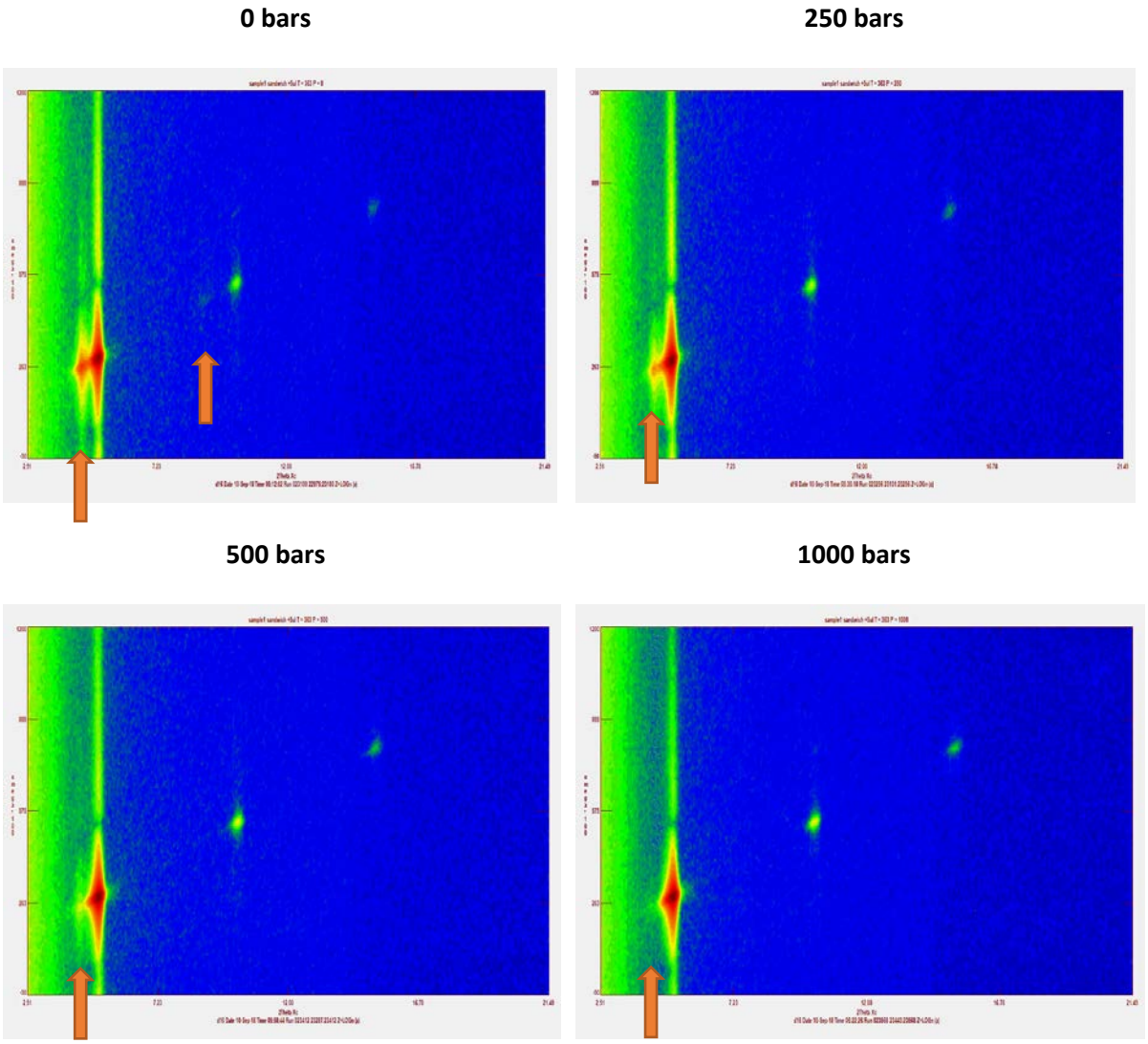

*Figure 1. Diffraction patterns of DPhPC : DPhPE (9 :1) at 85°C and 0, 250, 500 and 1000 bars of applied pressure measured at 50% D_2O. X-axis are 2theta values and Y-axis are omega*100. The orange arrow indicates a new lamellar and pressure-dependent phase.*

[1] Cario, A., Grossi, V., Schaeffer, P., and Oger, P. (2015). Front Microbiol **6**.doi: 10.3389/fmicb.2015.011152