# Experimental report

01/08/2019

**Proposal:** 8-02-852 **Council:** 10/2018

**Title:** Novel high-pressure/high-temperature phases in archaeal membranes

**Research area:** Biology

**This proposal is a continuation of 8-02-818**


**Main proposer:** Marta SALVADOR CASTELL

**Experimental team:** Judith PETERS
Marta SALVADOR CASTELL
Philippe OGER

**Local contacts:** Bruno DEME


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

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| D16 | 8 | 8 | 08/07/2019 | 17/07/2019 |


## 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 is due to the presence, in the midplane of the bilayer, of apolar hydrocarbons. Our previous experiments (ILL report 8-02-762 and 8-02-809) have demonstrated that the apolar molecule are located in the midplane of the lipid bilayer.
Using different approaches (FTIR,SAXS,DSC), we have demonstrated the impact of the presence of squalane inside the membrane on the physicochemical properties of the bilayer, and especially the predicted increase in stability/rigidity. We observed a dependence of these variations with the squalane/polar lipid ratio.
Noticeably, we observe novel and yet uncharacterized structuration of the lamellar phases. We have recently demonstrated the possibility to analyze these membranes under controlled and combined pressure and temperature. Here, we would like to examine how the concentration of squalane affects the behavior of the DoPhPC:DoPhPE lipid bilayer as a function of P and T , with an ememphasis on these novel phases.

# Report on D16 experiment #8-02-852

## Novel high-pressure/high-temperature phases in archaeal membranes

Organisms living at deep-sea hydrothermal vents have to thrive with harsh and changing conditions of temperature and pressure. To avoid cell membranes' damages, many extremophiles contain bipolar tetraether lipids, which conform a more rigid and resistant lipid monolayer instead of the usual lipid bilayer. Nevertheless, piezophilic and hyperthermophilic Archaea also synthesize monopolar, bilayer forming, diether lipids [1]. This apparent contradiction was recently explained by the presence of apolar isoprenoids lipids, as squalane, in the lipid bilayer that could modify physicochemical membrane properties [1].

### Project

In a previous ILL experiment (#8-02-818, manuscript submitted for publication), we have demonstrated that squalane at 1 mol% is placed in the midplane of a lipid bilayer composed by synthetic archaeal lipids as DoPhPC (1,2-di-O-phytanyl-*sn*-glycero-3-phosphocholine) and DoPhPE (1,2-di-O-phytanyl-*sn*-glycero-3-phosphoethanolamine) at the temperature range of 298K to 343K and the pressure range of 20 bar to 1000 bar. Moreover, this previous experiment allowed us to detect a new lipid phase induced by the presence of squalane.

Similarly, during this beam time, we have studied three different membranes (DoPhPC:DoPhPE (9:1), DoPhPC:DoPhPE (9:1) + h-squalane and DoPhPC:DoPhPE (9:1) + d62-squalane) with different squalane concentrations (2.5 mol%, 5 mol% and 10 mol%) under wide temperature (298K to 358K) and pressure ranges (20 bar to 1000 bar). Based on the experience of the last experiment, here, we could rapidly obtain results with enough resolution to show that squalane is placed in the lipid bilayer mid-plane under all these extreme conditions of temperature and pressure.

### Results

We have achieved to obtain a temperature and pressure scan for each scheduled sample. Four pressure points (0 bar, 250 bar, 500 bar and 1000 bar) were studied at five different temperatures (298K, 313K, 328K, 343K, 358K and back to 298K) which made a total of 24-28 hours of scans. Despite the usual loss of diffraction signal at high temperatures, we have obtained enough quality data to locate squalane in the lipid bilayer at temperatures up to 343K and pressures up to 1000 bar. The extracted preliminary neutron scattering length densities reveal significant differences on the bilayer regions that correspond to the bilayer midplane (d = 0 Å) between the sample containing h-squalane and the one with d62-squalane (Figure 1). This indicates that squalane, independently of its concentration, is placed in the bilayer midplane, perpendicular to lipids, even at high temperatures and high hydrostatic pressures.

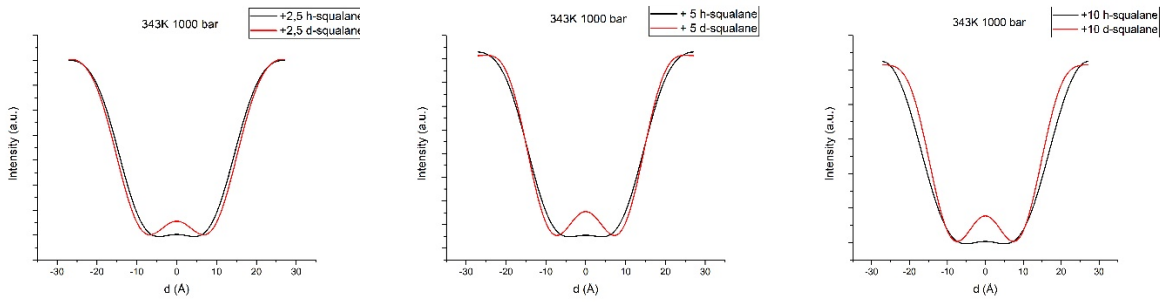


*Figure 1. Neutron scattering length densities of DoPhPC:DoPhPE (9:1) in presence of h-squalane (black) and d62-squalane (red) in different proportions (2.5 mol%, 5 mol%, 10 mol%) at 343K and 1000 bar.*

Apart from the position of squalane, we were also interested to study new lipid phases that were induced by the presence of the apolar molecule. According to Bragg peaks' intensities, such new phase is proportional to squalane concentration and it is more present at high temperatures and low hydrostatic pressures. Although we could not determine the position of squalane at the highest temperature (358K), measurements at such temperature allowed us to clearly see the total disappearance of the initial lipid phase and the opposite effect of pressure on the lipid bilayer structure, as the Bragg's peaks corresponding to the initial phase present higher intensities at high pressures (Figure 2).

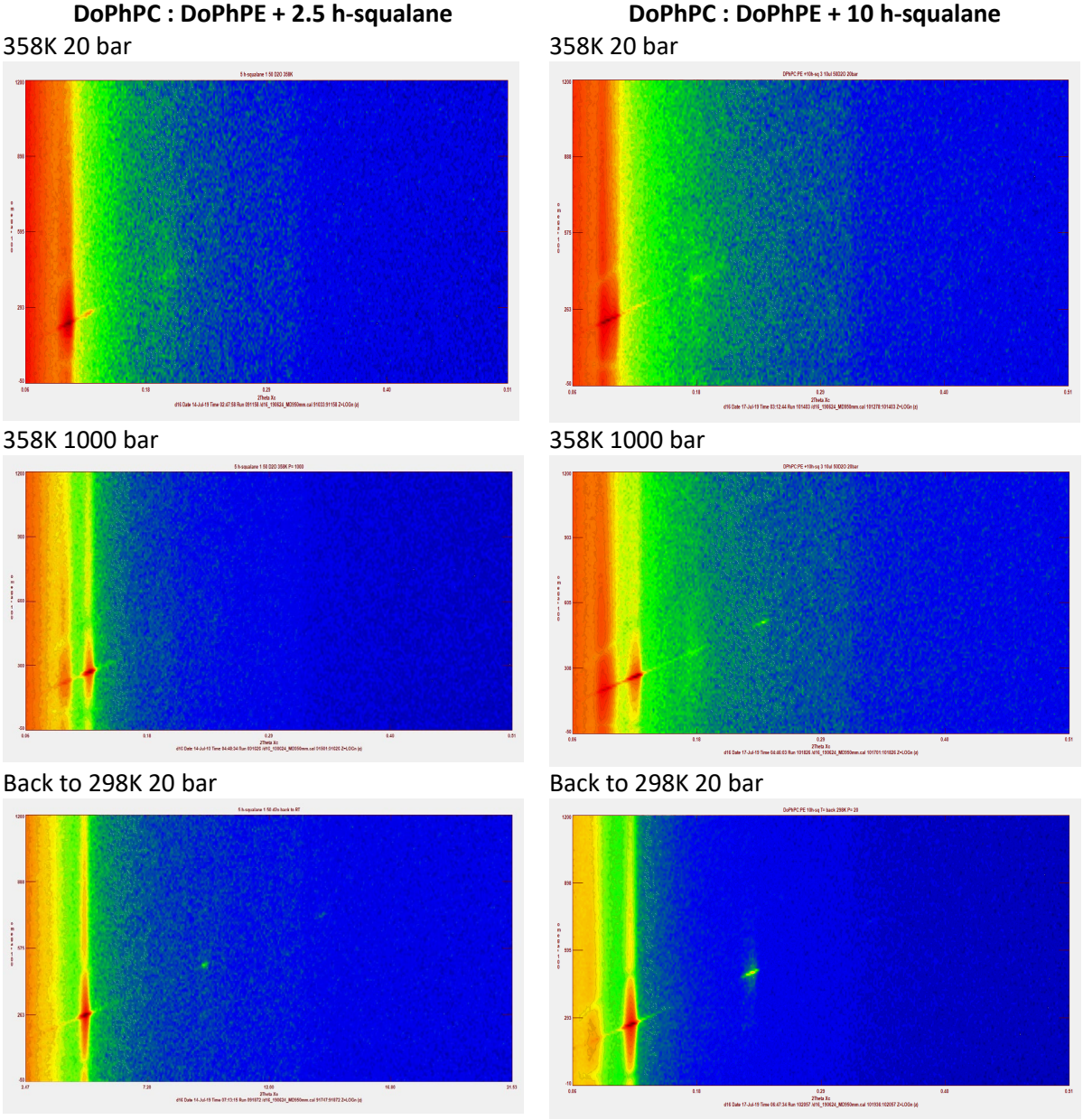


*Figure 2. 2D diffractograms obtained for DoPhPC:DoPhPE (9:1) + 2.5 mol% h-squalane (left column) and DoPhPC:DoPhPE (9:1) + 10 mol% h-squalane (right column) at 358K 20 bar (top), 358K 1000 bar (middle) and after coming back to 298K 20 bar (bottom). Y-axes are omega *100 and X-axes are q.*

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