Experimental report

18/06/2018

Proposal: 8-02-809 **Council:** 4/2017

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: squalane
,2-di-O-phytanyl-sn-glycero-3-phosphocholine
1,2-di-O-phytanyl-sn-glycero-3-phosphatidylethanolamine

Instrument	Requested days	Allocated days	From	To
D16	10	8	09/04/2018	18/04/2018

Abstract:

To explain the stability of bilayer membranes at temperature >110°C, we proposed a membrane ultrastructure in which apolar lipids insert in the bilayer, in order to increase of membrane rigidity and decrease membrane permeability. The results of ILL experiment 8-02-762 allow us to affirm that apolar lipids are located in the inner plane of the bilayer membrane formed by the synthetic archaeal lipids DPhPC and DPhPE and the apolar isoprenoid lipid squalane. The insertion of squalane inside the membrane bears resemblance to that of glycerol in eukaryal membranes, raising questions about the influence of the proportion of apolar lipids on the behavior and ultrastructure of the archaeal membrane.
This project aims investigating the dependence on the apolar lipid concentration and hydration the self-organization of archaeal membranes. Squalane will thus be incorporated at a relative concentration between 0 and 50% into membranes reconstructed from a mixture of synthetic or natural archaeal polar lipids. Membranes will be studied under varied hydration and temperature to determine membranes parameters under conditions reproducing the physiological conditions of the archaeal cells.

Report on D16 experiment 8-02-809

Investigating the ultrastructure of an archaeal membrane containing apolar structural lipids

Cell membranes have a fundamental role in the structure and cellular functions and they are adaptable to environmental changes (e.g. temperature, hydration…). Recently, a new model of membrane ultrastructure has been proposed for the membrane of hyperthermophilic archaea. The novel model suggests that the inner plane of the archaeal bilayer membrane is populated by apolar lipids [Cario 2015, Oger 2013], which would extend the stability of the membrane to higher temperatures [Haines 2001]. In a previous D16 experiment (8-02-762, article in preparation), we have demonstrated using 10% molar of the apolar lipid squalane that these apolar lipids are, indeed, situated in the midplane of an archaeal bilayer.

Project

Our original project was to investigate the dependence on the squalane concentration (between 0 and 50%) and its localization as it was not possible so far to determine precisely the content in natural membranes. Before conducting this neutron experiment, we realized that the content in cells was between 1% and 2.5% squalane and that just small concentrations of squalane, as 1% molar, can modify the physicochemical behavior of the lipid bilayer. In consequence, we decided to change the original squalane concentration and we used 0, 1%, 2.5% and 5% squalane instead of 0, 5%, 20% and 50% as it was originally stipulated. Squalane or deuterated squalane was incorporated at these different relative concentrations to determine squalane position in a lipid bilayer. Artificial multilayered membranes were used to mimic the membranes of hyperthermophilic archaeon using 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.

In addition to determining the position of different concentrations of squalane, we have characterized the different membranes varying the temperature (25°C, 40°C, 55°C, 70°C and 85°C). All those measurements were done changing the D_2O hydration contrast (8%, 50% and 100%) to be able to determine the phases for each sample.

Results

Results confirm that the position of apolar lipids inside the bilayer does not change in function of squalane concentration, at least up to 10% squalane. Our preliminary results show that at synthetic archaeal lipids with 1%, 2.5% and 5% squalane, apolar lipids are situated in the midplane of the bilayer (figure 1). This is an important finding as in natural cells membranes, the concentration of apolar lipids should vary in function of the requirements of the cell and if

apolar lipids position would have changed, the physicochemistry of the cell membrane would completely change.

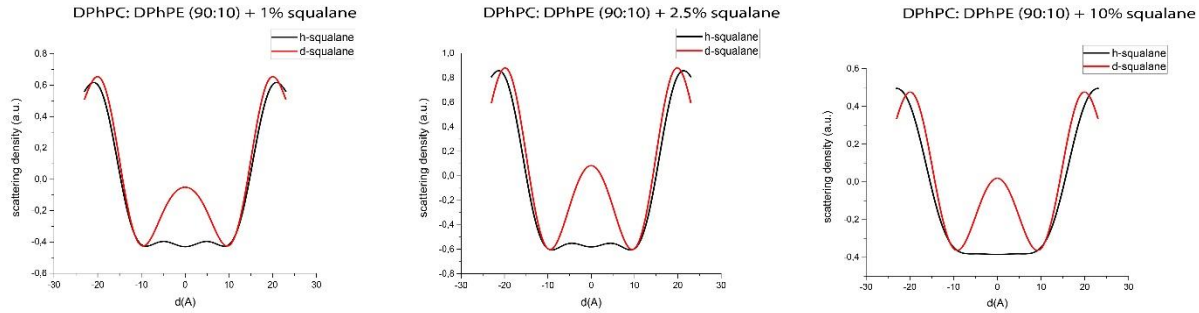

Figure 1. Scattering density function of the DPhPC:DPhPE (90:10 mol%) membrane containing diferent percentates of protonated squalane (black line) or deuterated squalane (red line), 8% D2O contrast.

The 2D diffraction patterns of temperature scans have revealed different information. At 25°C, fourth order of Bragg diffractions were present for all samples (figure 2a), by adding squalane we have seen the promotion of a new phase (figure 2b) placed at lower 2theta values, this phase is highly present at high temperatures (figure 2c and 2d). Moreover, a new kind of reflection appears when squalane is added, this reflection could be linked to a new period in the plane, which could indicate that there are domains at the lipid bilayer. Nevertheless, it is necessary a deeper analysis to characterize the new lipid phase and to connect all the new Bragg peaks.

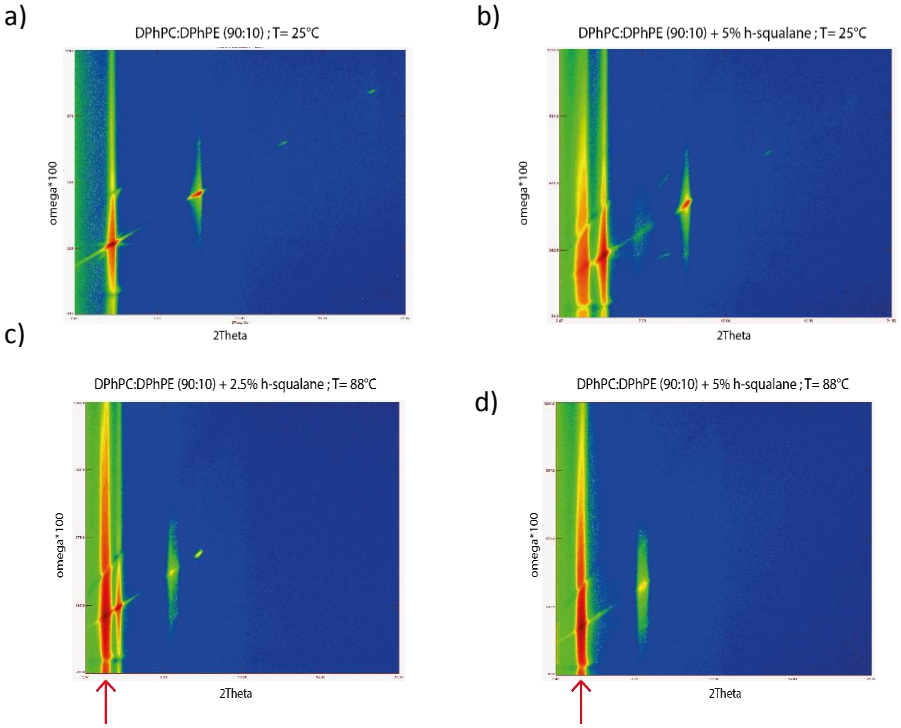

Figure 2. Diffraction patterns of

a) DPhPC:DPhPE (90:10 mol%) at 25°C;

b) DPhPC:DPhPE + 5% h-squalane at 25°C;

c) DPhPC:DPhPE +2.5% h-squalane at 88°C;

d) DPhPC:DPhPE + 5% h-squalane at 88°C.

100% humidity. 100% D2O contrast. The red arrows indicates the position of new phase.

References

Cario, A., Grossi, V., Schaeffer, P., and Oger, P. (2015). Front Microbiol **6**.doi: 10.3389/fmicb.2015.011152
Haines, T.H. (2001). Prog Lipid Res **40**: 299-324.
Oger, P., and Cario, A. (2013). Biophys Chem **15**: 42-56.