Experimental report

13/04/2016

Proposal: 8-02-719 **Council:** 10/2014

Title: Molecular adaptation of plasmic membrane in archael piezophile

Research area: Biology

This proposal is a continuation of 8-04-691

Main proposer: Philippe OGER

Experimental team: Judith PETERS
Nicolas MARTINEZ
Philippe OGER

Local contacts: Bruno DEME

Samples: Lipids extracted from E. coli and T. barophilus

Instrument	Requested days	Allocated days	From	To
D16	3	3	18/09/2015	21/09/2015

Abstract:

In the past, neutron experiments proved to be successful in quantifying the extent to which macromolecular dynamics in bacterial cells is affected by adaptation to extreme temperatures and salinity (Tehei et al., 2004; Tehei et al., 2007). The results supported the hypothesis that the evolutionary selection of appropriate resilience in order to maintain macromolecular structure and flexibility within the narrow limits required by biological activity contributes to environmental adaptation. Archael piezophiles have the ability to tune the composition of their membranes in order to adapt to the external conditions (temperature and pressure). It has been shown that the composition of the membrane changes depending on the growth conditions, so that the membrane can maintain its fluidity. Preliminary data shows us that the phase transition temperatures depend greatly on the temperature and pressure at which the organism was cultivated.
The objective of our project is to map these phase transitions as a function of growth conditions by monitoring the d-spacing value of these membranes as function of temperature.

Report on D16 experiment 8-02-719

P. Oger, J. Peters, M. Golub, M. Salvador, B. Demé

Molecular adaptation of plasmic membrane in archael piezophile

Elastic incoherent neutron scattering experiments on IN13 have revealed important differences in the mean molecular dynamics between organisms adapted to high and low temperature habitats (Tehei et al., 2004). The mean flexibility and resilience of the macromolecules were obtained and gave valuable insights on how thermoadaptation in macromolecules is achieved. These experiments were performed on whole living cells thus allowing studying the dynamics of biomolecules in their cellular context. The signal obtained from elastic scattering experiments on whole cells is expected to be dominated by the contribution from the proteins (Tehei et al., 2004). However, the major macromolecular structure which is impacted by high hydrostatic pressure is the cell membrane, which may undergo a fluid-to-gel transition in the pressure domain known to harbor life. Thus, in order to characterize the behavior of pressure adapted microbes, and how these microbes adapt their membranes, a first approach would be to study natural membranes from deep sea procaryotic cells. A recent experiment on pure lipid model membranes under high pressure revealed not only a shift of the main phase transition temperature (Peters et al., 2012), but also a reduced mobility of the lipids under high pressure (Trapp et al., 2013). It is expected that microbes modify their lipid composition to maintain membrane fluidity, an adaptation coined the term "homeoviscous adaptation", and therefore their biological function, but this remains to be verified experimentally.

Project:

Recent solid state NMR experiments were undertaken at the platform TGIR-RMN in Bordeaux in collaboration with E. Dufourc and A. Grélard and data revealed that the phase diagram of *T. barophilus* (an obligate hyperthermophilic and piezophilic organism) varies greatly depending on the growth conditions. We investigated in fact lipids extracted from *E. coli*, and confirmed findings from the literature, and lipids extracted from *T. barophilus*, grown at native conditions (40 MPa and 85°C) and at ambient pressure and three different temperatures (75, 85 and 90°C). The RMN data permitted to calculate the spectral moment of the first order to visualize the phase transition as function of temperature. The transitions are rather broad, as the samples are composed by a mixture of lipids. At low and high temperature conditions, the transitions are closed, but vary considerably at the native growth conditions.

We recently got a financing from the CNRS (Défi Instrumentation aux limites) to develop a new cell for neutron scattering experiments which should withstand high pressure and high temperatures (up to 100°C) simultaneously. Such a cell was designed by the SANE group of the ILL using TiZr and Al for the different parts and was now ready for a test experiment with real samples. We therefore measured DMPC multi-lamellar vesicles (MLV) as reference system for which structural changes as function of temperature and pressure were known (Trapp et al., 2013) to validate the good performance of the cell. In addition we wanted to measure the phase transition temperature of lipids extracted from *T. barophilus*. Unfortunately, we had difficulties to solubilize the latter ones with different solvents and saw only the typical Bragg peaks of the Silicium support, but no signal from the lipids. We concluded that we were not able to produce a structured sample from these lipids. In contrary, the experiment went as expected with DMPC MLV (see figure 1) and we were able to extract a (p,T) diagram (figure 2) in agreement with values in the literature.

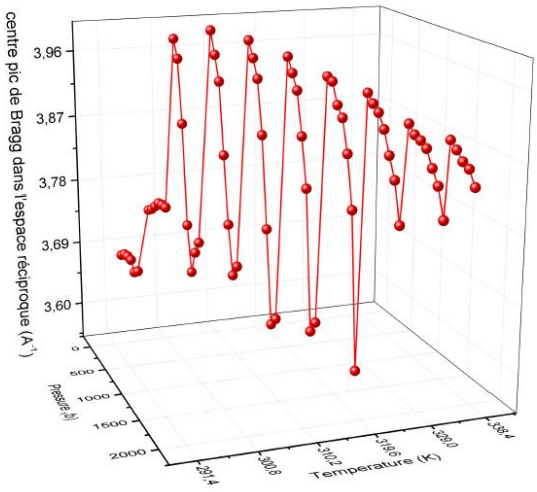

Figure 1: Center of the Bragg peaks of DMPC MLVs as function of temperature and pressure.

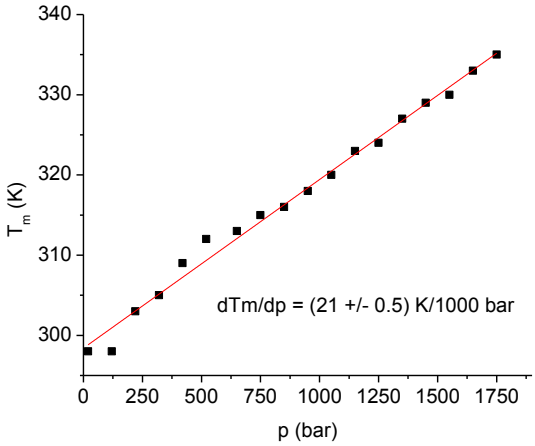

Figure 2: (p, T) phase diagram of DMPC MLVs in excess of D_2O.

Figure 1 presents the center of the Bragg peaks of DMPC MLVs as function of temperature and pressure in the reciprocal space. They permit to extract the repeat distances d through $d = 2\pi/Q$. It is known that they depend very sensitively on the lipid phase (Trapp et al., 2013). We extracted the phase transition temperature T_m corresponding to the main phase transition between the ripple and the liquid crystalline phase and plotted it as function of pressure (see figure 2). The slope was evaluated to $dT_m/dp = (21 \pm 0.5)$ K/1000 bar in very good agreement with values in the literature (Trapp et al., 2013, Winter and Pilgrim, 1989). The experiment permitted therefore to validate the good functioning of the high pressure equipment up to high temperatures.

References:

Tehei et al., (2004), EMBO Rep., **5**, 66-70.
Trapp et al. (2013), Phys.Chem. Chem. Phys., **15**, 20951.
Peters et al. (2012), High Pressure Research (2012) **32**, 97 – 102.
R. Winter and W.-C. Pilgrim, Ber. Bunsen-Ges., 1989, **93**, 708–717.