EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

# Experiment Report Form

**The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF**.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

*http://193.49.43.2:8080/smis/servlet/UserUtils?start*

***Reports supporting requests for additional beam time***

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

***Reports on experiments relating to long term projects***

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

***Published papers***

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

## Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

## Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

| ESRF | **Experiment title:** Homeostasis in deep sea barophilic sea barophilic bacteria | **Experiment number**: SC-2799 |
|---|---|---|
| **Beamline**: ID02 | **Date of experiment**: from: 4/11/2009 to: 6/11/2009 | **Date of report**: 27/8/2010 |
| **Shifts:** 6 | **Local contact(s)**: Michael Sztucki | *Received at ESRF:* |


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## *Report*

Deep sea piezophilic organisms (organisms which grow well at high pressure) have to regulate and maintain the fundamental properties of their membranes in the face of extreme pressures. This homeostasis is achieved by altering the lipid fatty acid chain composition of their bilayer membranes in response to the external environment[1]. This process is vital to preserve both the basic integrity of the membrane and its micromechanics which are highly important for maintaining mobility and activity of the proteins, peptides and other bio-molecules which are embedded in the membrane[2]. It has been shown that mono- and poly-unsaturated fatty acids are crucial for modulating membrane fluidity, however the regulatory mechanisms which underlie this composition control remain poorly understood.

We are currently building a library of pressure-temperature phase behaviour data for lipid extracts from SS9, a piezophilic organism. This will allow us to further understand the regulatory factors which lead to lipid modulation in the membranes of these organisms. Further data is required to clarify this, however once complete, will represent the first study of the membrane properties which form a feedback mechanism for lipid composition in these organisms.

During this beamtime we have also taken a ‘bottom up’ approach by investigating the pressure dependent fluidising effect of modulating hydrophobic chain composition in model membrane systems. We have studied the phase behaviour of fatty alcohol and phosphotidycholine lipid systems as a function of pressure and temperature. The phase behaviour of 2:1 1-tetradeconal, di-myristoyl-phosphatidylcholine (DMPC) in excess water was particularly interesting and was characterised from 40-70$^{o}$C and 0-700 bar. Pure DMPC forms a lamellar gel phase over these temperature and pressure regimes and hence any change in its phase behaviour on addition of other components can be attributed to these additives. Results show the formation of several liquid crystalline phases; an inverse hexagonal phase coexisting with two different, swollen, biocontinuous cubic phases ($Q_{II}^{P}$ and $Q_{II}^{D}$) is formed at relatively low temperatures and pressures. The formation of these structured with inverse curvature indicates that addition of tetradecanol increases the lateral stress in the chain region but additionally fluidises the model membrane, transforming the system from a gel to a liquid crystal structure.

Interestingly, whilst carrying out these experiments we observed formation of partially aligned inverse bicontinucubic phases, indicated by non-uniform distribution of intensity around diffraction rings. This has allowed us to index distinct diffraction spots and we have been able to find, for the first time, experimental evidence for an epitaxial relationships between the observed $Q_{II}^{P}$ and $Q_{II}^{D}$ inverse bicontinuous cubic phases. In addition, time resolved studies of the lamellar to inverse hexagonal transition in tetradecanol/DMPC has given important information about the $Q_{II}^{P}$ is orientation. Analysis is ongoing to extract further results from these information rich data.

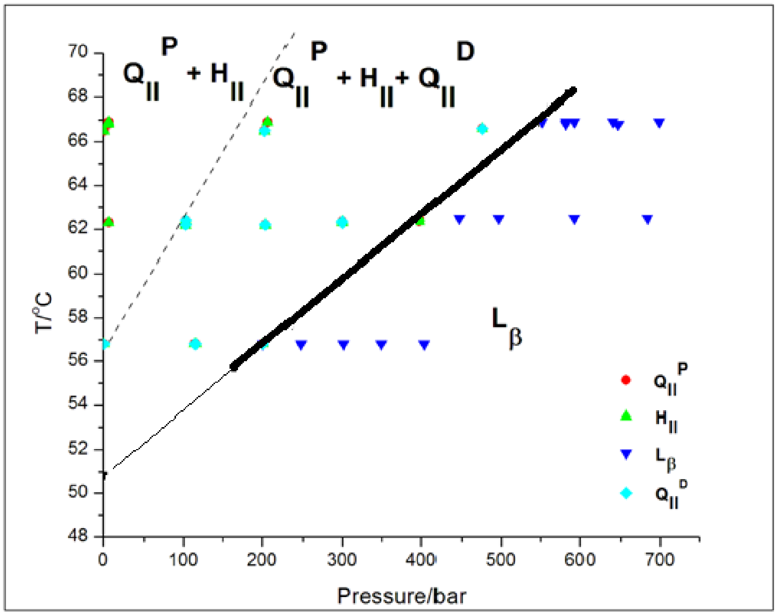


**Figure 1** Pressure-Temperature phase diagram of 2:1 1-tetradeconal:DMPC

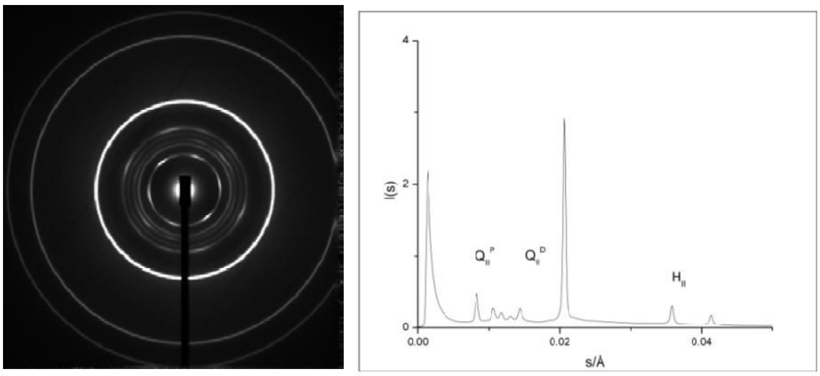


**Figure 2** SAXS diffraction pattern showing phase coexistence of $Q_{II}^{P}$,$Q_{II}^{D}$ and $H_{II}$ phases in 2:1 tetradeconal/DMPC in excess water at 67°C and 200 bar where the inverse bicontinuous phase share a 111-111 epitaxial relationship

## *References*


1. Cossins, A.R. and A.G. MacDonald, *Homeoviscous theory under pressure: II. The molecular order of membranes from deep-sea fish.* Biochimica et Biophysica Acta (BBA) - Biomembranes, 1984. **776**, 1, 144-150

2. Cantor, R.S., *Lateral Pressures in Cell Membranes: A Mechanism for Modulation of Protein Function.* The Journal of Physical Chemistry B, 1997. **101**, 10, 1723-1725