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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:** Pressure jump Time-resolved Studies of Transitions in Sphingomyelin / Cholesterol Membranes	**Experiment number**: SC 2196
Beamline:	**Date of experiment**: from:15 Jun 2007 to: 18 June 2007	**Date of report**: 31 August 2009
Shifts:	**Local contact(s)**: Dr Pierre Panine	*Received at ESRF:*

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Report:

Sphingomyelins (SM) are a group of phospholipids based on a sphingosine backbone with a hydrocarbon chain attached via an amide linkage. They play a highly important role in the plasma membrane of many mammalian cells and while being structurally similar to phosphatidylcholine (PC) head group glycerophospholipids e.g. dipalmitoylphosphatidylcholine (DPPC), SM shows some key differences. The most important of these are: 1) the interfacial region contains an amide linkage in place of one of the ester linkage found in glycerol based lipids 2) while phosphatidylcholines can only act as hydrogen bond acceptors, the amide of sphingomyelin allows it to both accept and donate hydrogen bonds 3) it has two long, often mismatched hydrocarbon chains, 4) it has a high gel-to-fluid transition temperature (chain melting temperature, Tm), typically around 37°C. Sphingomyelin concentrations typically reach 15% of the total phospholipid content in the outer leaflet of mammalian cell plasma membranes, however this can rise above 50% in the myelin sheath which surrounds nerves. As well as forming part of the membrane structural matrix, sphingomyelin and its metabolic products have also been proposed as important signalling molecules in programmed cell death and cellular apoptosis. There is also evidence that SM is linked to other biomedical processes including ageing and neural development and raised concentrations have been found in the brain tissue of Alzheimer's patients.

During this experiment, we investigated the pressure-temperature phase behaviour and kinetics of phase transitions in three different sphingomyelin natural extracts from egg yolk (EYSM), bovine brain (BBSM) and milk (MSM).

The equilibrium phase behaviour of each extract was studied from 5 – 65 °C and 0 – 3000 bar. The most striking result from this section of the experiment was the existance of a rippled gel phase in both EYSM and BBSM. At atmospheric pressure, the diffraction pattern from this ripple phase is poorly resolved, however, the use of high pressure has allowed us to resolve the diffraction pattern and extensively index it by inducing a barotropic transition at high temperature, see figure 1 below. In both extracts, the ripple of the gel phase can be flattened out by applying increased pressure to form a flat lamellar gel. Interestingly, adding cholesterol to the rippled gel phase at atmospheric pressure was also found to cause an 'un-rippling' to form a flat lamellar gel. While MSM also showed a lamellar gel phase, this was not rippled in its gel phase; however the SAXS data suggest that this is a tilted gel which undergoes an un-tilting prior to melting with increasing temperature or decreasing pressure.

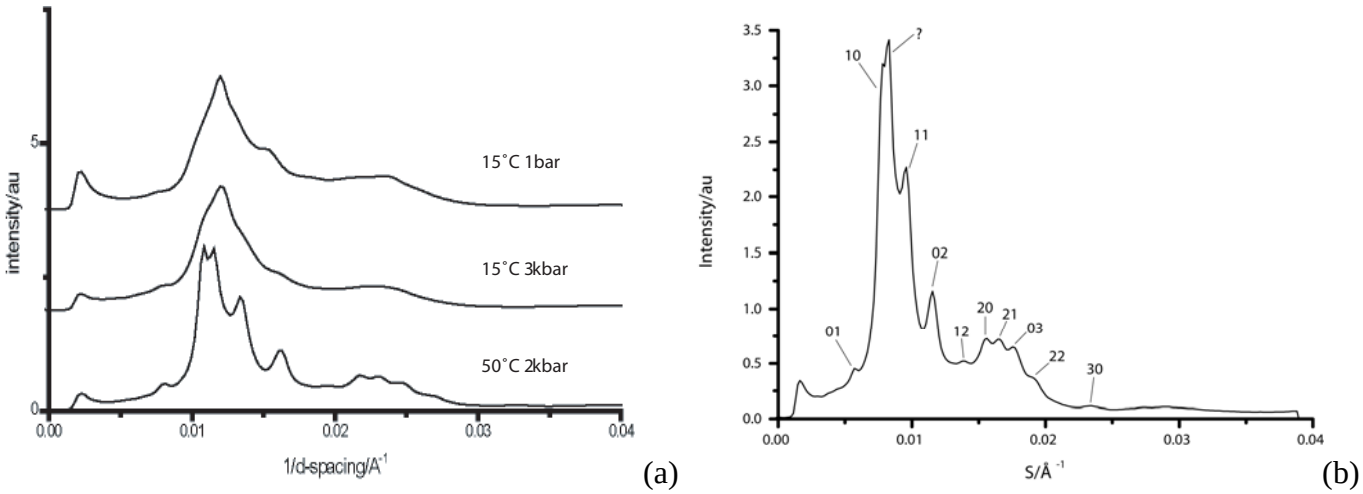

Figure 1. (a) SAXS diffraction patterns of BBSM at 15C, 1bar (top), 15C 3kbar (middle) and 50C 2kbar (bottom). The ripple diffraction pattern is resolved at high temperature and high pressure. (b) Resolved ripple gel diffraction pattern with peak indexing – note the peak marked ? originates from a co-existing flat lamellar gel

While a ripple gel phase has previously been reported in BBSM (Meyer, 1999), this was determined by freeze fracture microscopy and we believe that this is the first X-ray diffraction evidence for a ripple gel phase in any natural sphingomyelin extract. Additionally, this is the first time that the ripple period for the gel phase has been determined with sufficient accuracy to determine the gradual un-rippling mechanism for the ripple – flat gel transition as shown in figure 2 below.

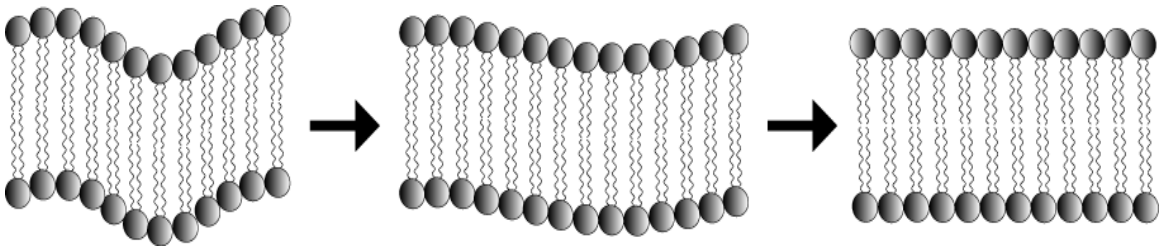

Figure 2. Diagram showing the gradual loss of the ripple to form a flat lamellar phase via a “stretching out” mechanism induced by increasing pressure or addition of cholesterol.

The second section of the experiment was an investigation of the kinetics of phase transitions between gel and fluid lamellar phases in the BBSM and MSM sphingomyelin extracts mentioned previously. We gathered a huge amount of data for this section so only a *very* brief summary is given here.

The kinetics of the lamellar transitions are dependent upon temperature, jump amplitude and the direction of the transition. The further beyond the phase boundary the jump ends, the faster the transition occurs however, the starting point of the jump does not affect the rate of the transition.

The fluid-gel transition proceeds more quickly at low temperatures however, the gel-fluid transition occurs more rapidly at high temperature.

In BBSM, there were clearly two separate processes involved during the fluid to gel transition, firstly the fluid chains freeze to form a flat gel then the gel ripples, this mechanism is echoed in reverse for the gel to fluid transition.

Again in the gel to fluid transition in MSM is actually made up to two separate transitions, a tilted gel - flat gel transition and the main melting transition to a fluid lamellar phase.

This data forms the basis of two manuscripts that are currently in preparation.