EUROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY

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:** Static and time-resolved pressure jump studies of membrane protein-lipid systems	**Experiment number**: SC-2532
Beamline: ID02	**Date of experiment**: from: 14 Nov 2008 to: 17 Nov 2009	**Date of report**: 25 Aug 2009
Shifts: 9	**Local contact(s)**: Michael Sztucki	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):
Prof. Richard Templer, Imperial College London
Prof. John Seddon, Imperial College London *
Dr. Oscar Ces, Imperial College London *
Prof. Roland Winter, University of Dortmund

Report:

In-cubo crystallization of membrane proteins has attracted a huge amount of interest recently and is driving the structure determination of many proteins which have proved difficult or impossible to crystallize by other techniques, however the mechanism of this process has remained poorly understood. While it is accepted that key to the crystallization process is mixing solubilized protein with a lipidic lyotropic cubic phase, it has been suggested in the literature that it is in fact a lamellar lipid phase that mediates crystallization (Caffrey 2003; Qutub, Reviakine et al. 2004; Cherezov and Caffrey 2007).

In this experiment, we have explored the role of lipid lamellar phases in *in-cubo* crystallization by *in-situ* studies of crystallization. Samples of the lipid monoolein, which exhibits wide areas of bicontinuous cubic phase in its binary phase diagram, were prepared with various concentrations of bacteriorhodopsin (bR) added. In all mixtures, we have observed clear cubic phase diffraction patterns but also see coexisting additional diffraction signatures corresponding to a lamellar phase. Furthermore, by varying the concentration of bR in the mixture, the study revealed that increasing levels of bacteriorhodopsin (bR) in a monoolein crystallization matrix promoted the formation of a lamellar phase at the expense of the cubic phase, note: this was observed both before and after significant crystallization had occurred. This is a highly significant result and demonstrates that the protein component is driving a phase transition in the lipid system, not the other way round. This observation, coupled with the fact that at all times the majority of the lipid adopts cubic symmetry indicates that the lamellar phase is a side effect or marker for the crystallization process and not a driving force as previously suggested. In mixtures where bR crystals have been allowed to form (all samples older than 5 days), lamellar phases are promoted close to the crystal edges where the

concentration of bR in the lipid matrix is highest. Moving away from the edge-front of a bR crystal, the bR concentration falls and the lipid adopts its equilibrium cubic phase.

Experimental details:

SAXS patterns were gathered at the ID02 beamline, ESRF with an X-ray energy of 17 keV (λ=0.07514nm).

Independent samples were prepared in 3 mm diameter capillary tubes every day for 15 days prior to the experiment commencing to maximize output from the beamtime. This sequential sample preparation allowed us to effectively study the effect of crystallization time. Diffraction patterns were recorded at several different points in the sample by moving the capillary sample holder between diffraction pattern acquisitions.

High hydrostatic pressure and pressure jumps were used to evaluate the relative stability of the cubic and lamellar phases (analysis ongoing).

This work forms the basis of a manuscript which is currently in preparation.

References:

M. Caffrey, *J. Structural Bio.*, 2003, **142**, 108, “Membrane protein crystallization”

Y. Qutub, I. Reviakine, C. Maxwell, J. Navarro, E. M. Landau, P. G. Vekilov, *J. Molec. Bio.*, 2004, **343**, 1243, “Crystallization of transmembrane proteins in cubo: Mechanisms of crystal growth and defect formation”

V. Cherezov, M. Caffrey, *Faraday Discussions*, 2007, **136**, 195, “Membrane protein crystallization in lipidic mesophases. A mechanism study using X-ray microdiffraction”