EUROPEAN SYNCHROTRON RADIATION FACILITY

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ESRF

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.

<table>
<tr><td> </td><td>Experiment title:
Annexin II binding to solid supported phospholipid membranes</td><td>Experiment number:
SC-2027</td></tr>
<tr><td>Beamline:</td><td>Date of experiment:
from: 06.12.06 to: 11.12.06</td><td rowspan="2">Date of report:

Received at ESRF:</td></tr>
<tr><td>Shifts:</td><td>Local contact(s):
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<tr><td colspan="3">Names and affiliations of applicants (* indicates experimentalists):
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Report:

We study the interaction of proteins with phospholipid membranes. A suitable and well-established model system for surface sensitive studies are substrate supported membranes [1]. The protein that was investigated in this study, Annexin II, is a periphere membrane protein that binds to negatively charged lipids in a calcium dependant manner [2, 3]. It is a dimer protein that can form tetramers by connecting to a second Annexin II by another protein p11. This feature enables the protein to assemble adjacent membranes, a task that is important in endo- and exocytosis. There are two possible conformations the protein may bind to a lipid membrane that differ in the height of the protein layer. The primary goal of this work was to reveal the actual conformation. Reflectivity measurements were conducted at the beamline ID01. We used a microfluidic chamber that allows for the control of the quality of the membrane by fluorescence microscopy and x-ray measurements at the same sample. We used an energy of 20 keV to provide transmission through the chamber and to minimize beam damage of our biological samples in aqueous environment [4]. Our substrates consist of 20x15 mm² silicon wafers with oxide layers of 20nm and 100nm, to minimize quenching of fluorescence dyes during fluorescence microscopy.

The membranes consisted of a 3:1 1-Palmitoyl-2-Oleoyl-*sn*-Glycero-3-Phosphocholine (POPC): 1-Palmitoyl-2-Oleoyl-*sn*-Glycero-3-[Phospho-L-Serine] (Sodium Salt) (POPS) where the POPS is negatively charged. As fluorescent probe 0.5 mol% of Texas Red DHPE lipid was added to this mixture.

To gain fluid bilayers of such a mixture on Silicon vesicle spreading with osmotic pressure was used. Figure 1 shows the microscopy picture of such a membrane after 50min of continous bleaching. The clearly visible white rim reveals an intact and fluid membrane [5].

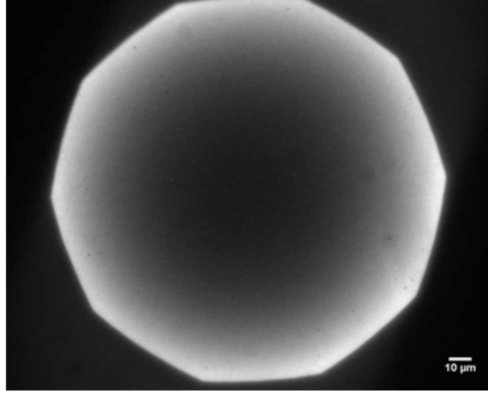

Figure 1. Fluorescence microscopy picture of a 3:1 POPC:POPS membrane after vesicle spreading with osmotic pressure. The picture was taken after 50min of continous bleaching. The white rim indicates a high mobility of the membrane lipids and thus an intact membrane.

After the control with fluorescence microscopy the samples were measured with x-ray reflectivity at ID01. Figure 2 shows the reflectivity data of a 3:1 POPC:POPS membrane on a 20nm silicon oxide layer (blue squares), the reflectivity data after incubation with Annexin II tetramer (red squares), and after rinsing the sample with an EGTA containing buffer (green squares).

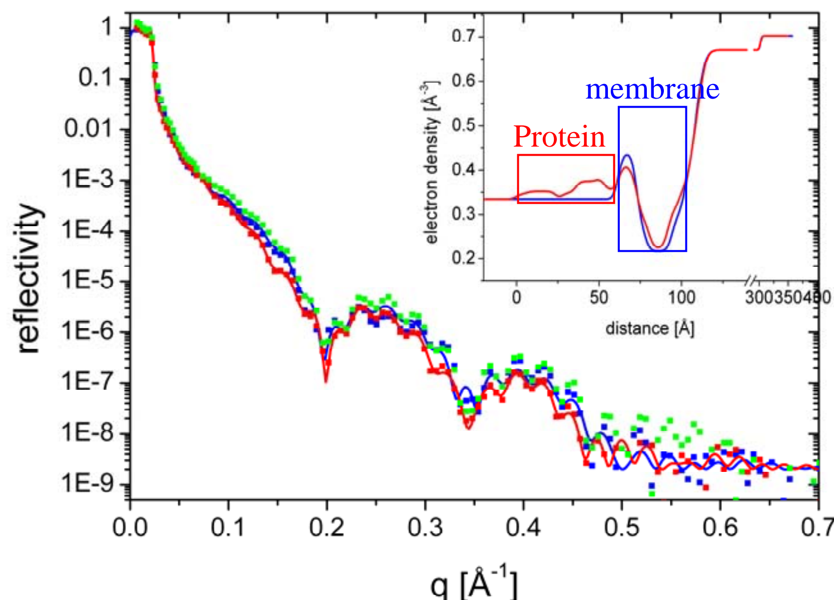

Figure 2: Reflectivity measurement of a 3:1 POPC:POPS membrane (blue squares), after incubation with Annexin II tetramer (red squares) and after rinsing with EGTA containing buffer (green squares). Inset: preliminary data analysis using the PARRAT algorythm.

The typical signal of a phospholipids membrane was investigated (fig.2) [4,6]. The inlet in figure 2 shows the electron density profile of the membrane as fitted with a 5 box model using the parrat algorythm. After adding Annexin II a change in signal could be observed (fig.2). The red line in figure 2 shows the electron density profile of membrane with bound Annexin II. In this case the electron density profile of membrane and protein was fittet using 5 boxes for the membrane and 4 boxes for the protein layer. To ensure the change in signal being due to the proteins specific binding, the membrane was rinsed with EGTA containing TRIS buffer and again measured. A change in direction to the original membrane signal could be observed (fig.2).

References

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