EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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 via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can 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:** **The influence of hydrostatic pressure and cosolvents on proteins at the solid-liquid interface**	**Experiment number**: SC-3717
Beamline: ID15A	**Date of experiment**: from: 22/01/14 to: 28/01/14	**Date of report**: 29/08/2014
Shifts: 18	**Local contact(s)**: Dr. Veijo Honkimäki	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Florian Wirkert*, Julia Nase*, Michael Paulus, Holger Göhring*, Christopher Weis*, Metin Tolan

TU Dortmund, Physik / DELTA, Otto-Hahn-Str.4, 44227 Dortmund

Report:

The behaviour of proteins under different environmental conditions is of great interest, because the functionality of proteins is important for many biological, biochemical and biophysical reactions. It is of special interest how the adsorption of proteins at a certain interface can be triggered and how the presence of an interface affects proteins, respectively. Since the application of high hydrostatic pressure (HHP) has proven to be a strong tool for the study of biosystems, we used HHP to examine the behaviour of the model protein lysozyme at the solid-liquid interface, and so broaden the knowledge of protein behaviour at interfaces. The experimental technique was chosen to be x-ray reflectometry (XRR) because of its possibility to reliably resolve the structure of thin layer systems and small changes in such systems.

Our experiments were carried out at beamline ID15A. The incident energy was set to 70 keV, the beam height was 5 µm at the sample. Si-wafers of the size 8 x 8 mm², coated with a monolayer of octadecyl-trichlorosilane (OTS), were used. With our custom made sample cell pressures up to 5 kbar can be applied. A two cell design allows the separation of the sample liquid from the pressure transmitting liquid via a flexible membrane. The outer cell can also be flushed with a tempered liquid to keep the temperature stable during the measurements. The experimental procedure was to record reflectivity curves of the solid – liquid interface surface each at a different pressure beginning at near ambient pressure going up to 5 kbar and back to near ambient pressure again. In order to avoid beam damage, each reflectivity was measured at a different spot on the samples surface.

We found that a monolayer of lysozyme adsorbs at a solid surface at ambient pressure and that the adsorption of lysozyme cannot be increased with the application of HHP in the regime up to 5 kbar alone (data not shown). Thus, we added destabilizing cosolvents to the lysozyme solution. In order to exclude the possibility of measuring only the adsorption of the cosolvent, the fist series of measurements was performed using a pure cosolvent solution. The well known chaotropic cosolvents urea and guanidinium chloride were used. For each

the reflectivity curves at different pressures are plotted in figure 1. The experimental data is shown normalized to the Fresnel reflectivity of a perfectly smooth silicon surface. Both series show a slight pressure induced shift of the minima, which seems to be completely reversible.

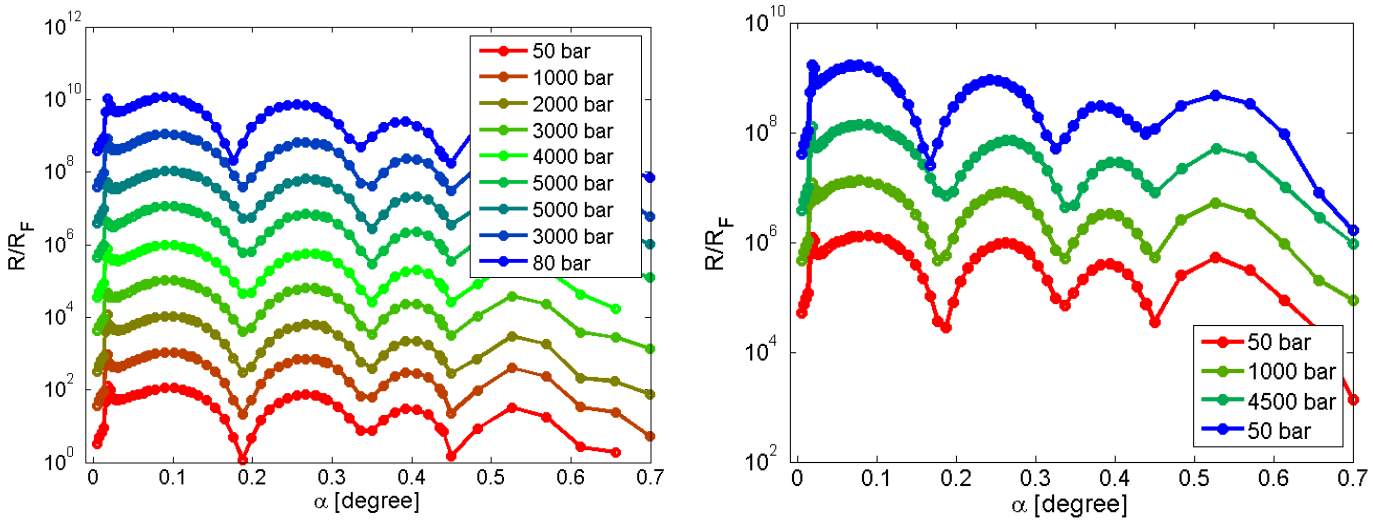

Figure 1: Reflectivity curves for pure cosolvent solutions. Small and reversible effects for both, urea (left) and guanidinium chloride (right) are visible.

In figure 2 the results of the measurements using protein-cosolvent solutions are shown. Applying urea, we see a more pronounced but still reversible effect compared to the pure cosolvent solution. No increased adsorption of lysozyme can be directly deduced from the reflectivity data. However, with guanidinium chloride we see strong changes in the reflectivity curve, which can be attributed to an increased adsorption of lysozyme with rising pressure. Returning to ambient pressure shows, that this additional adsorption is not completely reversible. Thus, although both cosolvents are known as strong denaturants we observe a different adsorption behavior, pointing to specific cosolvent – protein interactions. However, a detailed analysis of the reflectivity data is still in progress.

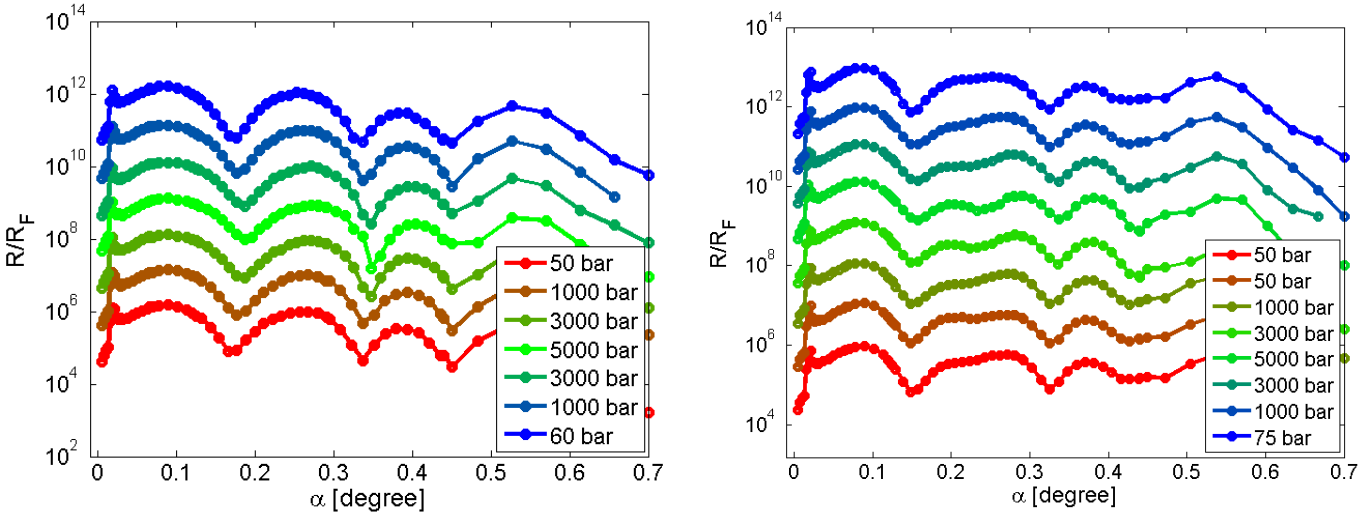

Figure 2: Reflectivity curves for protein-cosolvent solutions. With urea (left) the observed shift of oscillation minima is completely reversible, with guanidinium chloride an increased lysozyme adsorption was observed.