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 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:** High energy study of protein aggregation at the solid liquid interface	**Experiment number**: SC-2360
Beamline: ID15 A	**Date of experiment**: from: 12-SEP-07 to: 18-SEP-07	**Date of report**:
Shifts: 18	**Local contact(s)**: Federica VENTURINI	*Received at ESRF:*

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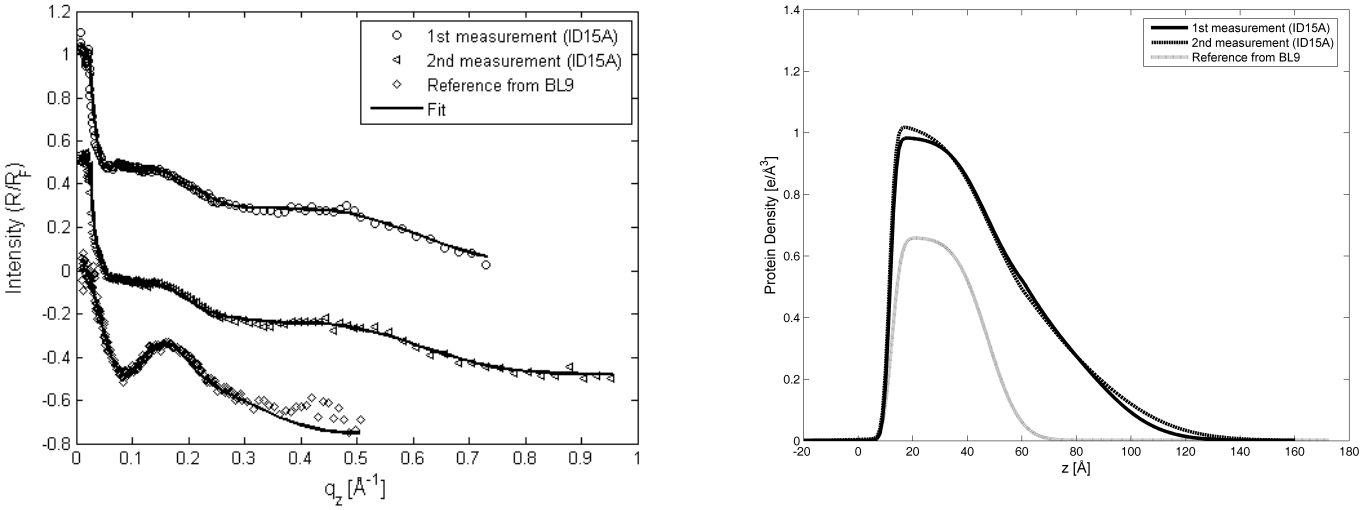
Report:

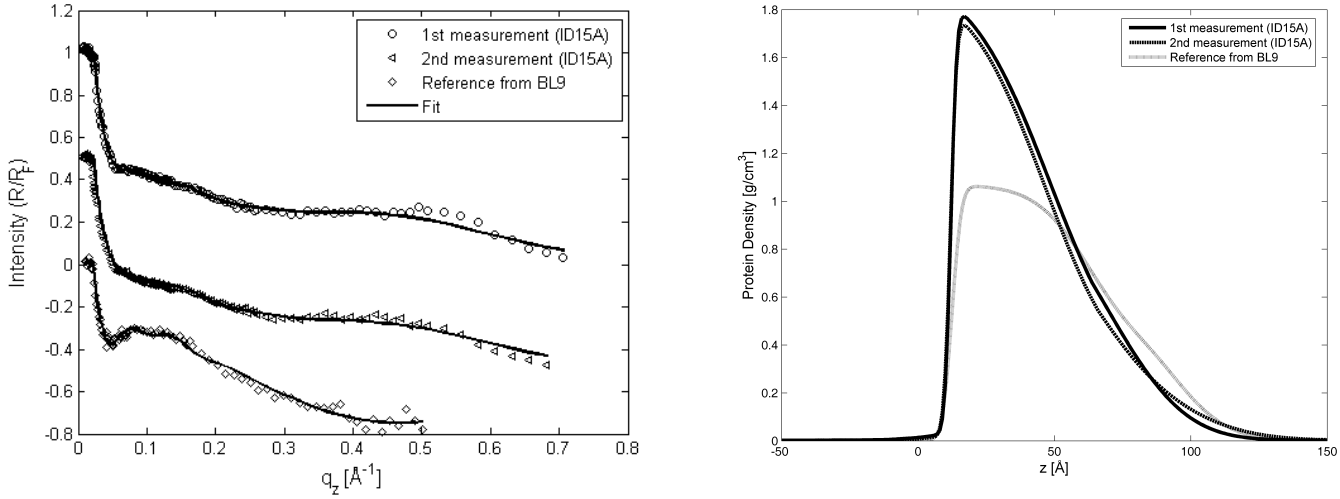
Protein adsorption is a process which plays a major roll whenever proteins come in contact with an interface. Of particular interest is the process of protein adsorption when it takes place at a solid/liquid interface. Processes like fouling of contact lenses and thrombosis on implant materials are only two of much more examples which suggest that studies of protein adsorption are significant for fundamental research and the development of new biotechnological materials. In order to probe the protein adsorption techniques are desired which allows an in situ investigation. One powerful technique is X-ray reflectometry at high energy which enables the investigation of the vertical density profile of the adsorbed protein layer. The high incident energy is required to decrease the absorption of the X-ray-beam while penetrating the liquid phase of the sample. Using synchrotron radiation with high brilliance makes it also possible to probe the protein layer structure with unprecedented accuracy.

Using synchrotron light with high flux density on biological samples raises the question of beam damages on the sample. Measurements at beamline ID15A demonstrate that high flux densities can cause beam damages or at least can influence the structure adsorbed protein layer. In order to have a comparison to X-ray-reflectivities of the same sample system with lower flux densities additional measurements were performed at BL9 of the Dortmunder Elektronenspeicherringanlage (DELTA). The proteins used for this purpose were lysozyme from hen-egg-white and hemoglobin from bovine blood. The substrate used for the measurements was silicon with a natively grown silicon-dioxide layer.

The reflectivitiy data and the refinements of calculated model reflectivities are shown in the left part of the figures 1 and 2. The calculation of the reflectivities was carried out by applying the recursive Parratt-formula on a stack model for the different layers.



Picture 1: Left: X-ray-reflectivities and refinements of the calculated reflectivities. Right: density profiles of the adsorbed lysozyme layers. In all cases the Protein concentration was 0.1 mg/ml at pH 11.



Picture2: Left: X-ray-reflectivities and refinements of the calculated model reflectivities. For clarity the intensities were normalized to the Fresnel reflectivity and shifted on the y-axis. Right: density profiles of the adsorbed Hemoglobin layers. The concentration of Hemoglobin was 0.1 mg/ml at pH 7 for the two measurements at the ID15A and 0.03 mg/ml at pH 7 for the measurements at BL 9.

In both cases the density profiles of measurements at the ID15A with differently prepared samples were conform. Hence artifacts which can be attributed to misalignment during measurement or impureness of the sample can be ruled out.

In the case of lysozyme both the thickness and the maximum density are higher than those measured at the BL9 (here we observed the adsorption of one lysozyme mono layer) though the same sample system with identical pH and concentration was applied (pH 11, c=0.1 mg/ml). One possible explanation of the difference between these two measurements could be radiation damage. In comparison to BL9, the flux density at ID15A is approx. 10^5 times higher.

Another hint for the X-ray damage is the density profile of the adsorbed layer which can be explained by the assumption of densely packed fragments of lysozyme whose density decays exponentially with the hight by the transition to the aqueous solution.

In the case of hemoglobin the thickness of the layer seems to be slightly less than that measured at the BL9. But the maximum density of the layer is even much higher than the van-der-Waals-density of a single hemoglobin molecule (M_{Hem}/v_{Hem}=1.24 g/cm^3). This could be even an indication for the loss of the tetrameric structure of hemoglobin molecules due to X-ray damage. In summary we could observe a drastic effect on the adsorption behavior of proteines caused by the high flux density at ID15A.