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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:** **The effect of interfaces on protein-protein interaction as revealed from grazing incidence diffraction experiments**	**Experiment number**: SI-1995
Beamline: ID10b	**Date of experiment**: from: 12-APR-06 to: 18-APR-06	**Date of report**: 30-AUG-06
Shifts: 18	**Local contact(s)**: Dr. Leide Cavalcanti	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

M. Tolan, R. Winter, M. Paulus*, L Wiegert*, K. Shokuie*, M. Sulc*

Report:

We report an in-situ grazing incidence diffraction (GID) experiment and reflectivity study to investigate the protein solution/gas and the protein solution/lipid layer interface. Aim of the experiment was to reveal their effects on the intermolecular interaction and aggregation at conditions also including natural cell environment. The surface of aqueous lysozyme solutions were examined using different lysozyme concentrations between 5 and 15 weight percent. Additionally the lysozyme solutions were covered by a monolayer of DPPA (1,2-Dipalmitoyl-sn-Glycero-3-Phosphate) and DPPC (1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine) to study the influence of lipid membranes on lysozyme aggregation. The interfaces were studied by grazing incidence diffraction (GID) measurements at different

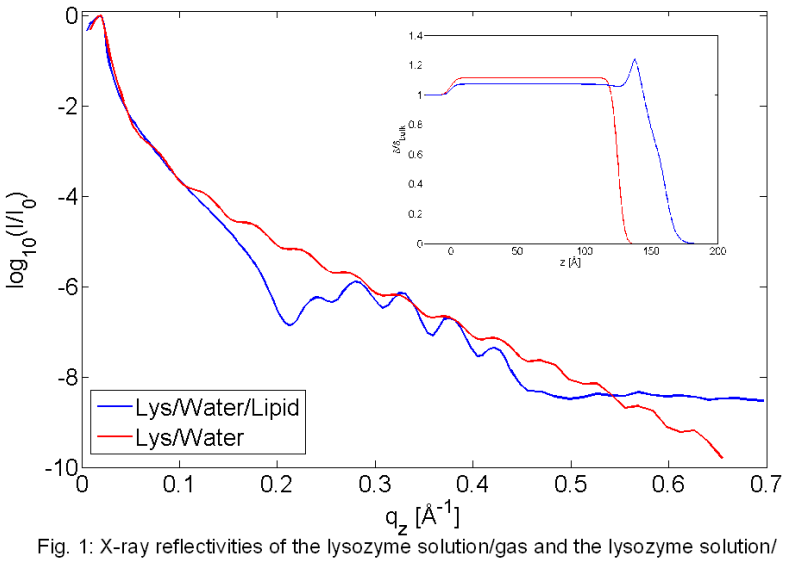

Fig. 1: X-ray reflectivities of the lysozyme solution/gas and the lysozyme solution/ lipid/gas interface. The inset shows the refined dispersion profiles.

angles of incidence below and above the critical angle of total reflection. For the GID measurements a position sensitive detector in combination with a sollar slit system was used, leading to minimum wave vector transfer parallel to the samples surface of $q_{||}$=0.14 Å^{-1}. Additionally x-ray reflectivity measurements of these interfaces were accomplished to study the vertical electron density profile of the interfaces. For these measurements a NaI detector was used. The incident photon energy was 8keV.

All lysozyme solutions excluding the 5 weight percent show the aggregation of lysozyme layers at the solutions surfaces. All layers have a thickness around 110 Å which points to aggregation of three or four layers of lysozyme molecules. The introduction time for aggregation varied between 4 and 9 hours, depending on concentration, and shows a rise in the layers electron density but not in its thickness. Two sample reflectivities and the refined electron density profiles are shown in figure 1. It is apparent that the lysozyme layers have nearly the same thickness and are sharply separated for the bulk phase as well as for the lipid layer.

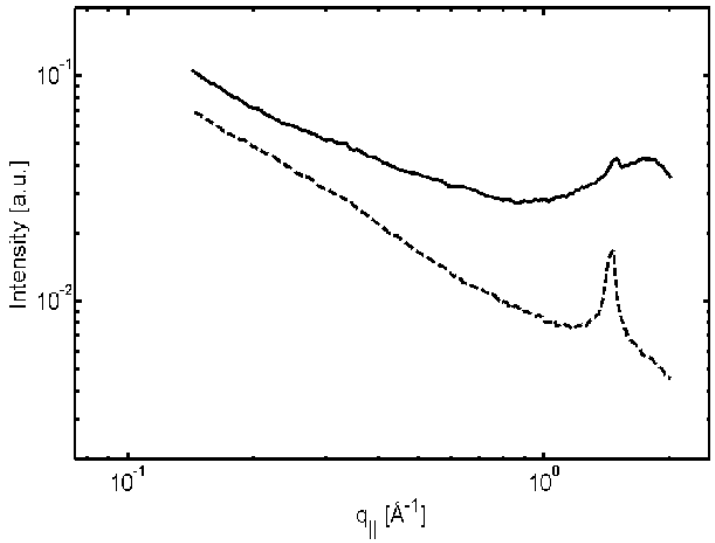

Fig. 2: GID scan of aqueous solution of lysozyme covered by DPPC. Dashed line: incitent angle of 0.1°, solid line: incident angle of 0.16°.

The GID measurements were used to study the lateral structure of the lysozyme film. In figure 2, for example, two different GID scans of lysozyme solution covered by a DPPC lipid layer are shown. The different scans were taken at an incidence angle of incidence of 0.1° and 0.16° which is below, slightly above the critical angle of total reflection. The first one shows the well ordered crystalline phase of the DPPC film covering the lysozyme solutions surface which implies that the lysozyme does not penetrate the lipid monolayer. The second scan includes scattering intensity of both, the lipid (the Bragg peak is still observable) and the protein. The intense broad peak at a wave vector transfer parallel to the samples surface $q_{||}$ ~1.5Å^{-1} originates from an overlap of water and protein structure peak. Thus the state of unfolding of the lysozyme molecules should be accessible via a complete analysis of peak position and width. A more detailed analysis of the GID identifies the lysozyme structure factor oscillation in the scattering intensity at low wave vector transfer (not shown in fig 2). Because of the use of the sollar slit, which was essential for high wave vector transfers the low q region below 0.15Å^{-1} was not experimentally accessible. Thus a second beamtime is reasonable to resolve lateral structures which are in the lateral dimension of the lysozyme molecules size and above. This would give access to the aggregated molecules shape and the ordering of molecules within the aggregation layer. The data analysis of these GID measurements is still in progress.