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

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

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.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number 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:** Measuring Intramolecular Distance Distributions in Proteins by Anomalous Small-Angle X-ray Scattering	**Experiment number**: LS-2945
Beamline:	**Date of experiment**: from: 12 Oct 2020 to: 14 Oct 2020	**Date of report**: 09 Mar 2021
Shifts:	**Local contact(s)**: Michael Sztucki, Narayanan Theyencheri	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Prof. Dr. Jan Lipfert, LMU Munich, Chair of Biophysics and Applied Materials
* Anna Baptist, LMU Munich, Chair of Biophysics and Applied Materials
* Samuel Stubhan, LMU Munich, Chair of Biophysics and Applied Materials

Report:

The beamtime allocated to proposal LS-2945 was used to measure ASAXS on Maltose-binding-protein (MalE) labeled with two gold-nanoparticles. In the MalE mutants used, two amino-acids are replaced by cysteines gold nanoparticles are coupled via the thiol bond. Two different mutants were measured: MalE with cysteines at site 36 and 352 (MalE 36-352) and MalE with cysteines at site 31 and 212 (MalE 31-212). As a control we measured both mutants without nanoparticles. All measurements were performed in KCl-TRIS buffer at pH = 8.2.

The double-labeled proteins were measured at 9 different energies around the the gold L-III absorption edge from 300 eV below to 50 eV above (all energies shown in the inset in Fig 1 a). The scattering curves were normalized and a correction for a residual fraction of single-labeled proteins was applied. We used a matrix inversion approach to isolate the gold-gold interaction term G_{Au} (Fig. 1b) from the other scattering contributions. The distance distributions (Fig. 1c) are calculated from the gold-gold term G_{Au} by regularized Fourier transformation using a maximum entropy algorithm.

Although the purification of proteins labeled with gold is challenging and high concentrations were hard to obtain, we managed to experimentally determine label-label distance distributions for both proteins. The distance distrbution feature main peaks that match the expected distances from the known structure. For MalE 31-212 the main peak is at 6.2 nm (expected 6.1 nm) and for MalE 36-352 the main peak is at 5.8 nm (expected 5.7 nm). The distributions also feature two smaller peaks, one at around 1.5 nm, which resembles the size of the gold labels, and one at ~3.5 nm, which may arise from single labeled proteins or from protein aggregates connected by gold labels.
The results from run LS-2945 demonstrate -for the first time- that it is possible to determine label-label distances in proteins by ASAXS. We want to follow up on these highly encouraging results by measuring the same proteins with improved purification protocols; in addition, we want to perform measurements in the absence and presence of ligands to monitor conformational changes. We hope to again measure at beamtime ID02, which is uniquely suited for these demanding measurements.

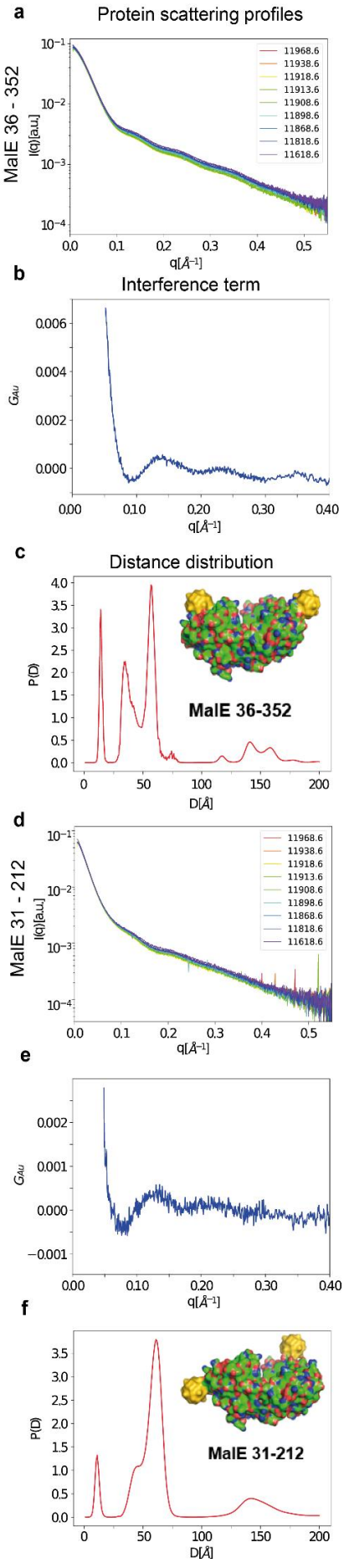

Figure 1: Scattering Intensities at different energies, Gold-gold interference terms and distance distributions for MalE 36 – 352 (a-c) and MalE 31-252 (d-f). Insets in c) and f) show pdb-models of the MalE mutants.