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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:** SAXS analysis of the HAUSP/Usp7 C-terminal activating domain	**Experiment number**: 22856 MX-988
Beamline:	**Date of experiment**: from: 28/09/2009 to:	**Date of report**: 16/11/2009
Shifts:	**Local contact(s)**: **Dr Adam ROUND**	*Received at ESRF:*

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Report:

Aims of the experiment and scientific background

Deubiquitinating enzymes or DUBS are essential for regulating critical ubiquitin pathways (Nijman et al, 2005). Usp7/HAUSP is a ubiquitin specific protease that regulates P53 levels in the cell, by cleaving ubiquitin off P53 and Mdm2. It is important for decision making between proliferation, apoptosis and senescence. For these reasons it is pursued a drug target for cancer therapy.

The C-terminal domain of Usp7 is critical for its full catalytic activity. We have expressed this domain from the human Usp7/HAUSP and crystallized it. Our recent, yet unpublished X-ray structure revealed a modular arrangement. We would like to confirm their relative arrangement in solution using SAXS in order to understand if this arrangement is due to crystal contacts or present in solution. In addition we would like to understand how this domain activates the catalytic domain.

Crystal structures of the Usp7 catalytic domain are available (Hu et al, 2002) and we would like to study the complex of the catalytic domain and the C-terminal domain in solution in order to understand it activating effect.

Drosophila GMP synthase can active Usp7 against P53 and histone H2b (van der Knaap et al, 2005). The groups of Peter Verrijzer and ourselves have confirmed this interaction for the human GMP synthase and shown that it maps to the C-terminal domain. A crystal structure for human GMP synthase is available in the PDB. We have expressed human GMP synthase in insect cells and we would like to study the complex between the Usp7 C-terminal domain. and GMPS using SAXS.

Experimental method

Usp7/HAUSP C-terminal domain is expressed easily and can be purified very well. It is a single peak on a gelfiltration column with a monomer size. Constructs expressing the catalytic domain are also well behaved and amenable to SAXS experiments and this is also true for the insect cell expressed GMP synthase. In addition we measured samples of the Catalytic+C-terminal domain of Usp7 and truncations thereof.

Results obtained

We measured 15 samples from 5 constructs. Our measurements showed well behaved protein, with some aggregation and inter-particle effects at the highest concentrations. For the construct for which a crystal structure is available, the C-terminal domain, we performed fitting to zero. By comparing the SAXS curve with the theoretical scattering curve from our crystal structure, we could confirm the structure is different in solution (Fig 1) The curves did not fit and the differences exceeded Chi-values of 20. The crystal structure already suggested flexible hinges, and might therefore not be in one conformation. Sofar attempts to determine the SAXS structure using *ab initio* and rigid body modeling were not succesful, as the modeling did not converge to a single conformation.

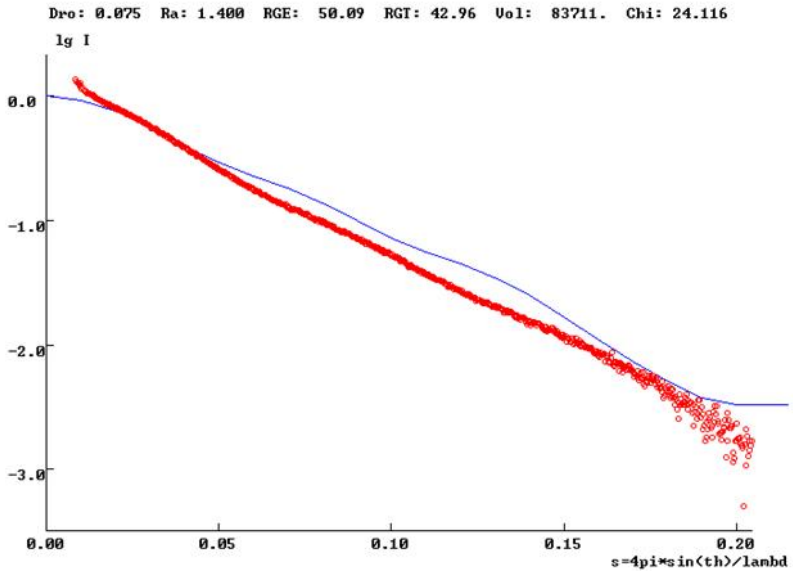

Fig 1: SAXS data of Usp7/HAUSP C-terminal domain was measured at three different concencentrations (1, 2 and 5 mg/ml), and thereafter scaled and extrapolated to zero concentration to correct for interparticle effects. When comparing to the theoretical scattering curve from our crystal structure, we observed large differences between SAXS data and crystal structure.

For some of the other constructs we did not have sufficient data to obtain the optimal experimental curve. Meanwhile we have also made additional constructs that we would like to test, in order to analyze the activating properties of this domain and the relative orientation with respect to the catalytic domain.