EUROPEAN SYNCHROTRON RADIATION FACILITY

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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 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: Investigation into the structural organization of the macromolecular complex between AMSH and STAM2 during protein de/ubiquitination: A combined SAXS / NMR approach**	**Experiment number**: MX-1881
Beamline: BM29	**Date of experiment**: from: 16/11/16 to:17/11/16	**Date of report**: 14/02/17
Shifts: 2	**Local contact(s)**: Petra PERNOT	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

H. KIM*[1], M. MARTIN*[1], M. HOLOGNE[1], F. GABEL[2], and O. WALKER[1]

[1]Institut des Sciences Analytiques, Villeurbanne, Fr.

[2]Institut de Biologie Structurale, Grenoble, Fr.

Report:

Experimental Summary

With the aim of understanding a complex structural arrangement involving STAM2-AMSH-Ubiquitin, which play key roles during lysosomal degradation pathway, we have obtained our first SAXS measurements in Nov. 2016. During the course of our beamtime we have managed to measure different protein constructs and complexes (STAM2 UIM-SH3 & VHS-UIM-SH3; AMSH; STAM2-AMSH) (Fig. 1) at various concentration ranges under different buffer and additive conditions. 30 samples were measured in total. In terms of sample quality, STAM2 proteins (UIM-SH3 in particular) showed curves with good signal / noise within the q range between 0.007 and 0.494 $Å^{-1}$ at concentrations ranging between 1 and 12 mg / ml. Strong interparticle efects were not evident and the final scattering curve was obtained by merging the curves from the lowest and the highest concentrations which was then used for further analysis. At the conclusion of our initial data analysis for STAM2, the SAXS data complements our recently published NMR data (Hologne et al., 2016) and moreover it opens up new prospects for our next beamtime which we are planning for in the upcomming months. On the other hand, despite our effors to ensure monodispersity and homogeneity for all our samples, one of our samples (AMSH) was severely aggregated even at a concentration as low as 0.1 mg / ml. This is expected of such protein with flexibility at its extremity. New efforts have been made to express, purify and optimise a full-length AMSH (FL-AMSH), evidence of which will be implemented in our next proposal.

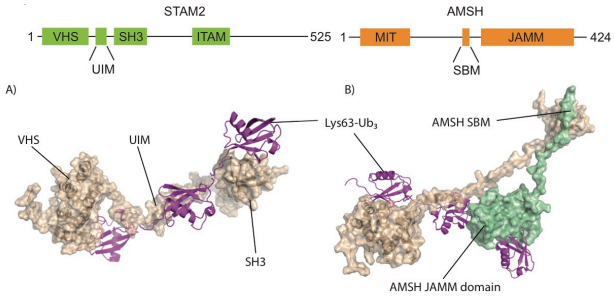

Figure 1. (top) Schematic showing the boundaries of STAM2 and AMSH constructs. (bottom) Model of the potential structural organisation by AMSH (green) - STAM2 (brown) - Ubiquitin (purple) complex (Hologne et al., 2016).

Preliminary Results & upcoming plans

Figure 2 shows the processed SAXS curves for UIM-SH3 in gray and VHS-UIM-SH3 in red, which were fit against each of the theoretical scattering curves in orange and black solid lines for UIM-SH3 and VHS-UIM-

SH3 respectively.

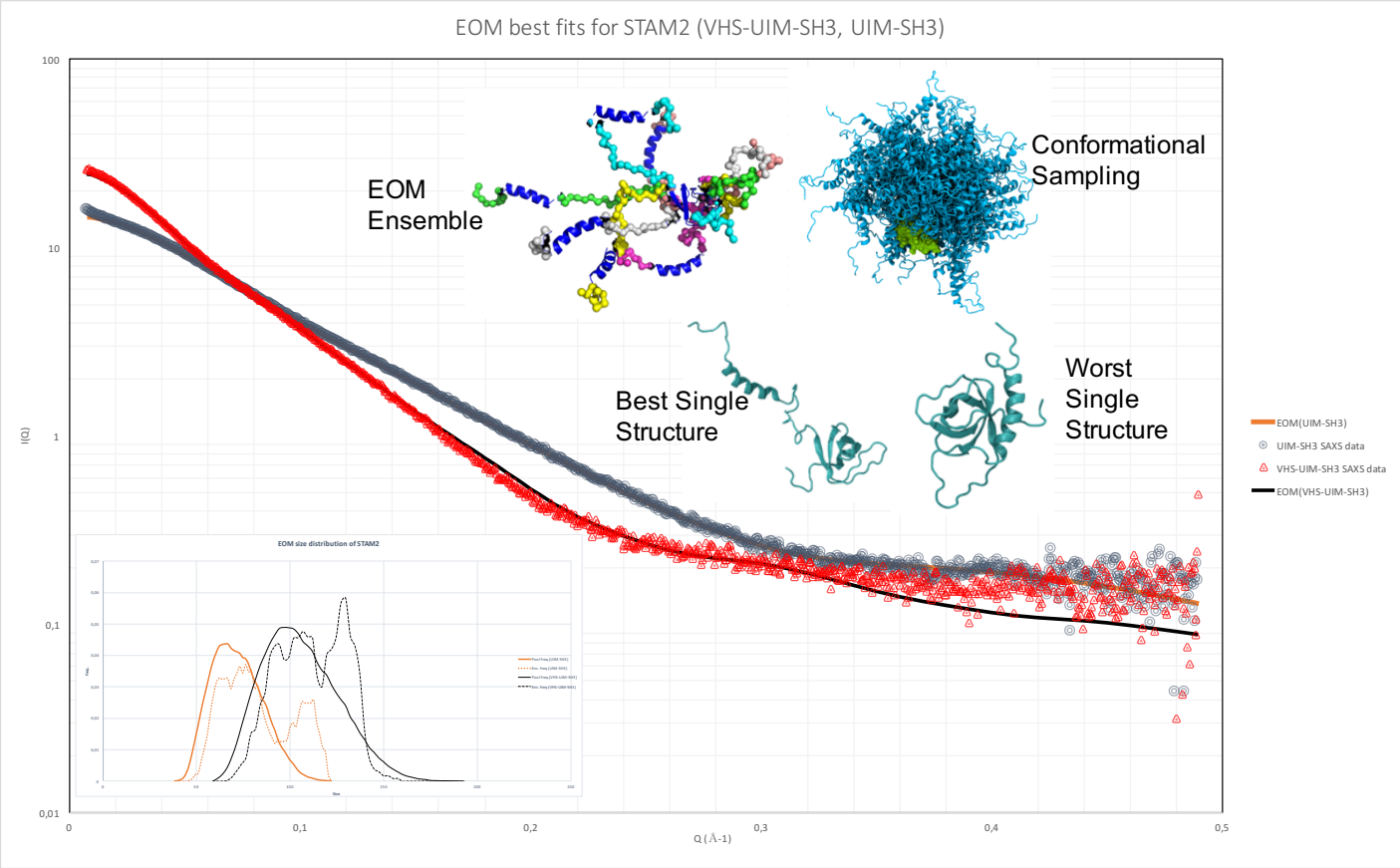

Figure 2. Experimental SAXS curves for UIM-SH3 (gray) and VHS-UIM-SH3 (red) fitted against each of the average theoretical scattering curves calculated from EOM. (above right) Graphical representation of the pool of random structures, best ensemble, as well as the best and worst single structure for UIM-SH3. (below left) Size distribution of UIM-SH3 (orange) and VHS-UIM-SH3 (black).

Given the flexibility of STAM2, EOM (Ensemble Optimisation Method) (Tria et al., 2015) was used to obtain the best ensemble structures from which the average theoretical curves were calculated. Quality of the experimental data, linearity of the Guinier plot, and model fiting by χ^2 minimisation were of good standard (Table 1). Nevertheless, further improvement in sample quality and model precision is being implemented for our next proposal. The data was analysed by SASSIE (http://www.smallangles.net/sassie/SASSIE_HOME.html) in parallel which also resulted in a similar preliminary outcome as the EOM. AMSH, known for its unstability showed strong signs of aggregation/oligomerisation during our last beamtime. Since then, fresh efforts have been made to improve this by expressing and purifying a new full length (FL) AMSH in its monodisperse form which we are planning to measure in our next available beamtime as well as the STAM2-AMSH FL complex. Ubiquitin in monomeric and/or trimeric forms are also in preparation which will be measured in complex with STAM2 (and AMSH). Conformational changes which may occur upon these interactions and whether the overall shape and size of the complex agree with (or not?) our proposed model system will be of our utmost interest in our next experiment at BM29.

Table 1. Experimental SAXS parameters and EOM analyses of two & three domain STAM2

	UIM-SH3	**VHS-UIM-SH3**
Molecular mass (kDa) (theoretical)	12.5	29.9
Molecular mass (kDa) (SAXS experimental estimation)	8.8 – 12.9	24 – 32
R_G (Guinier fit, Experimental), Å	24.1 ± 0.1	38.0 ± 0.1
R_G (Final ensemble, EOM), Å	26.01	35.93
χ^2	0.802	3.594

References

HOLOGNE et al. (2016) NMR reveals the interplay between the AMSH SH3 binding motif, STAM2 and Lys63-linked diubiquitin. ***J. Mol. Biol.*** 428 (22); 4544-4558

TRIA, G. et al. (2015) Advanced ensemble modelling of flexible macromolecules using X-ray solution scattering. ***IUCrJ*** 2, 207-217.