EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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 flexibility and conformational basis of the macromolecular complexes involving STAM2 and Tri-ubiquitin**	**Experiment number**: MX-1954
Beamline: BM29	**Date of experiment**: from: 29/06/17 to:29/06/17	**Date of report**: 27/09/17
Shifts: 1	**Local contact(s)**: Petra PERNOT	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

H. KIM*[1], M. NGUYEN*[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

STAM2 and *Tri-ubiquitin* are flexible multidomain proteins and their interactions are known to play a key role in the regulation of their target proteins during endocytosis. We have obtained our *SAXS* measurements in June 2017. During the course of our beamtime we have managed to measure different protein constructs and complexes (*STAM2 UIM-SH3 & VHS-UIM-SH3*; *Tri-Ubiquitin*; *Tri-Ubiquitin:STAM2* complexes at different molar ratios; *Mono-ubiquitin* & *mono-ubiquitin:STAM2* controls; *AMSH & STAM2:ubiquitin:AMSH di/trimeric complexes*, etc.) (*Fig. 1*) at various concentration ranges. 82 samples were measured in total. In terms of sample quality, *STAM2*, *Tri-Ubiquitin (Ub3)* and their dimeric complexes at various molar ratios showed curves with good signal / noise within the *q* range between 0.007 and 0.490 $Å^{-1}$ at concentrations ranging between 0.75 and 8 mg / ml. Strong interparticle efects were not evident. In the case where there was a slight concentration-dependent interparticle effect, the final scattering curve was obtained by merging the curves from the lower (for the low angle) and the higher (for the higher angle data) concentrations which was then used for further analysis. *STAM2* and *Ub3* data analyses, modeling and interpretation focus primarily on the flexible nature of the proteins using a combination of methods including *monte-carlo* conformational sampling, *Crysol*, *EOM* and *MultiFoXS* (*Fig. 2*). *STAM2:Ub3* complex data were analysed and quasi-atomic models are being proposed through optimisation of the rigid-body docking using methods such as *FoXS Dock* and *pyDockSAXS* (*Fig. 2*). Given the interesting preliminary results of the complex *SAXS* data, it awaits further experimental proofs and computational refinements from *RDC*, *MD*, *SEC-SAXS*, etc. On the other hand, albeit our continued effors to ensure monodispersity and homogeneity for all our samples, *AMSH* was severely aggregated due to its inherent instability even at a concentration as low as 0.1 mg / ml. *Mono-Ubiquitin* requires further adjustment in its sample quality which will be incorporated in our next available beamtime.

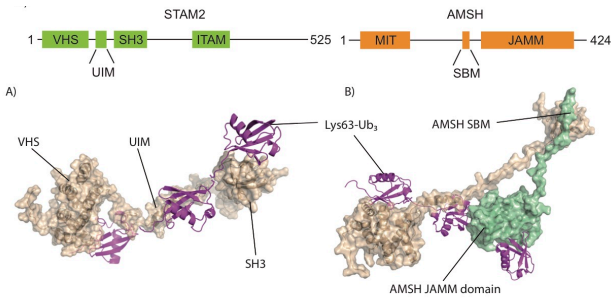

Figure 1. (top) Schematic showing the boundaries of STAM2 and AMSH constructs. (bottom) Model of the potential structural organisation by AMSH (green) - STAM2 (brown) - Ubiquitin (purple) complex (Hologne et al., 2016).

Preliminary Results & upcoming plans

Figure 2 shows the processed *SAXS* curves for tri-ubiquitin (*Ub3*) in blue, *VHS-UIM-SH3 (VUS)* in purple and *Ub3:VUS* complex at 1:1 molar ratio in red, which were fit against each of the theoretical scattering

curves represented as solid lines.

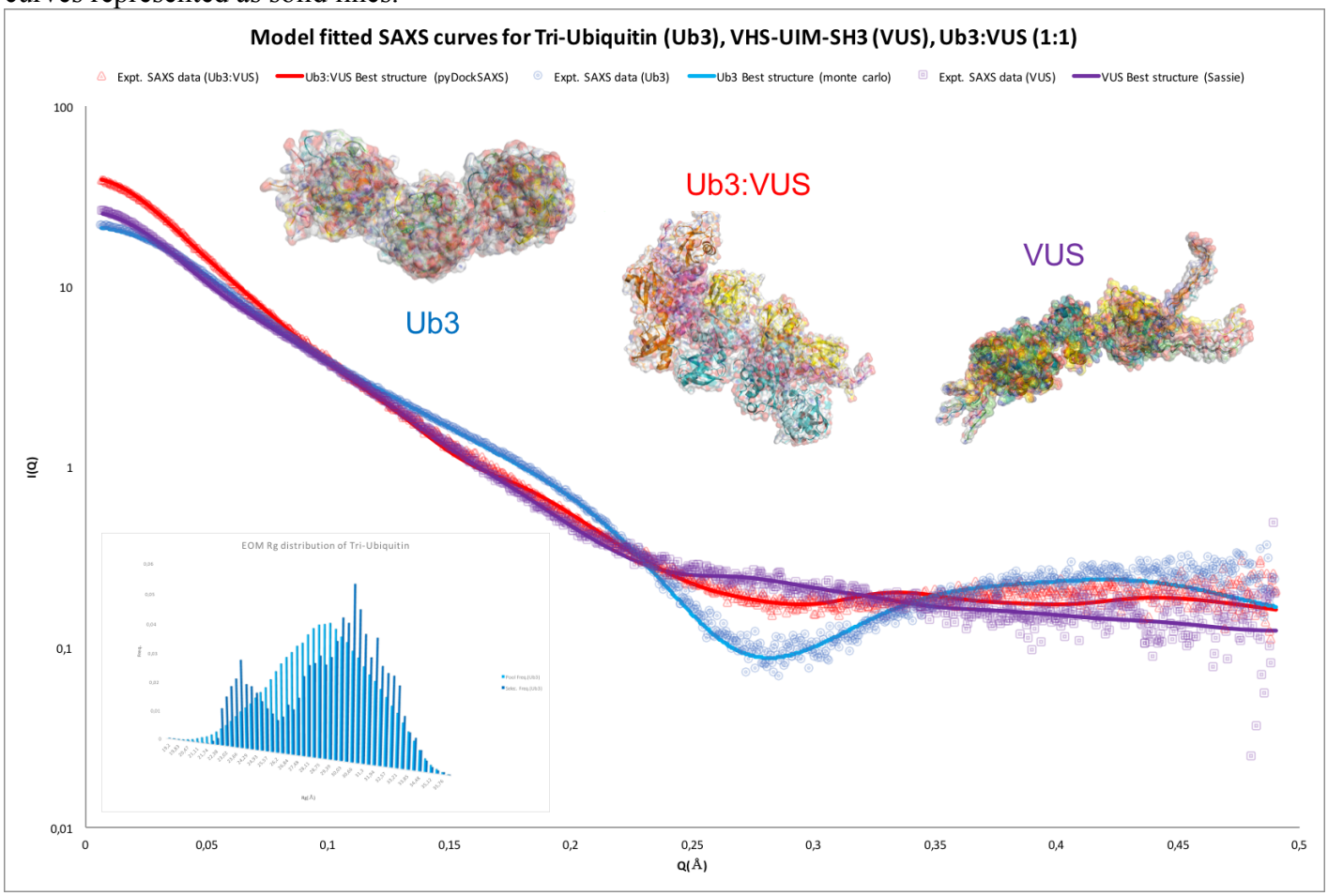

Figure 2. Experimental SAXS curves for Ub3 (blue), VUS (purple) and Ub3:VUS (1:1)(red) fitted against each of their best theoretical scattering curves. 10,000 conformers each were initially generated by mc conformational sampling for the Ub3 and VUS, and by rigid-body docking (pyDockSAXS) for the Ub3:VUS complex. The respective theoretical scattering curves were calculated from them, fitted against their experimental profiles, and scored according to χ^2 by Crysol. (above right) Graphical representation of each of the top structures. (below left) R_G distribution of Ub3 by EOM.

Taking the flexible nature of *STAM2* and *Ub3* into account, *EOM & MultiFoXS* were used in parallel to obtain the best ensemble structures from which the average theoretical curves were calculated. R_G distribution of both proteins indicates a presence of multiple states (2 and 3 for *Ub3* and *VUS* respectively) with the strong tendancy towards more extended conformations (*Fig. 2*). Overall, the quality of our experimental *SAXS* data, linearity of the Guinier plot, and model fiting by χ^2 minimisation were of good standard as summerised in *Table 1*. As we progress into the later phase of this project, we are further exploring the flexibility or 'unstructuredness' of STAM2. A series of truncated mutants of STAM2 have been designed, engineered, over-expressed and purified in line with our next beamtime details of which will be described in the next proposal.

Table 1. Experimental SAXS parameters and model fitting of Ub3, VUS and Ub3:VUS

	Tri-Ub. (Ub3)	**VHS-UIM-SH3 (VUS)**	**Ub3:VUS (1:1)**
Molecular mass (kDa) (theoretical)	25.7	29.9	55.6
Molecular mass (kDa) (SAXS experimental estimation)	17.2 – 22.9	27.0 – 36.0	36.5-48.7
R_G (Guinier fit, Experimental), Å	28.9 ± 0.1	36.4 ± 0.1	37.5 ± 0.2
I_0 (Guinier fit)	21.60 ± 0.02	31.75 ± 0.07	38.40 ± 0.12
Porod Vol. (nm^3)	35.61	53.83	75.92
χ^2	1.76	1.34	2.04
HS (%)	5.99	17.37	0