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:** SAXS studies of human tyrosine phosphatase SHP2 and T-Box riboswitches	**Experiment number**: MX-2058
Beamline: BM29	**Date of experiment**: from: 23rd June to: 24th June 2018	**Date of report**: 24th August 2018
Shifts: 3	**Local contact(s)**: Mark TULLY	*Received at ESRF:*

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Report:

System 1:

Samples from the proposal number MX-2058 were measured on 23rd and 24th of June 2018. There were no technical problems with the beamline during the measurements.

Protein tyrosine phosphatase is a 70-kDa enzyme composed of two regulatory SH2 domains (N-SH2 and C-SH2) connected to the catalytic PTP domain. There is evidence that SHP-2 exists in a closed, inactive conformation due to an interaction between N-SH2 and PTP; binding of phosphopeptides to the SH2 domain releases the inhibition by favoring an open conformation of the enzyme.

Two constructs of SHP-2 were measured: the first was the wild-type, catalytically competent enzyme, the second was an inactive form of SHP-2 in which the active-site C459 was mutated into a serine. In addition, each construct was measured in complex with the allosteric inhibitor SHP099, which is thought to prevent the opening of SHP-2 even in the presence of activating phosphopeptides. Five concentrations of SHP-2 were measured to allow proper treatment of interparticle effects: 3mg/ml, 1.5mg/ml, 0.75mg/ml, 0.37mg/ml, 0.18mg/ml, in 50mM HEPES, 150mM NaCl, 5mM, pH 7.6.

The SAXS curves of the catalytically competent SHP-2 reveal that the phosphatase does indeed adopt a compact fold in solution: the radius of gyration calculated from the extrapolated curves at infinite dilution (2.61 ± 0.01 nm) is consistent with the one determined from the crystal structure (2.6nm, pdb 2SHP). Furthermore, the distance distribution analysis revealed a compact fold, with a maximal end-to-end distance (Dmax) of 8.49 nm. Addition of SHP099 does not significantly affect the conformation of SHP-2 when it is not bound to activating phosphopeptides (Rg 2.61 ± 0.01nm, Dmax 8.39 nm). Therefore, it is likely that SHP099 acts by stabilizing the closed conformation of SHP-2 even in the presence of activators and preventing its opening.

On the other hand, the catalytically inactive form of SHP-2 (C459S) displayed a significantly different behavior, with a greater preference towards open states even without activators. This was demonstrated by a larger Rg value (2.86 ± 0.01nm) and a more skewed distance distribution curve. In this case, addition of

SHP099 caused a dramatic shift towards the compact state (Rg 2.6 ± 0.01 nm). We therefore speculate that the presence of the Serine residue in the catalytic site disrupts the physiological interaction between N-SH2 and PTP. Thus, a new inactive mutant of SHP-2 must be designed for structural studies.

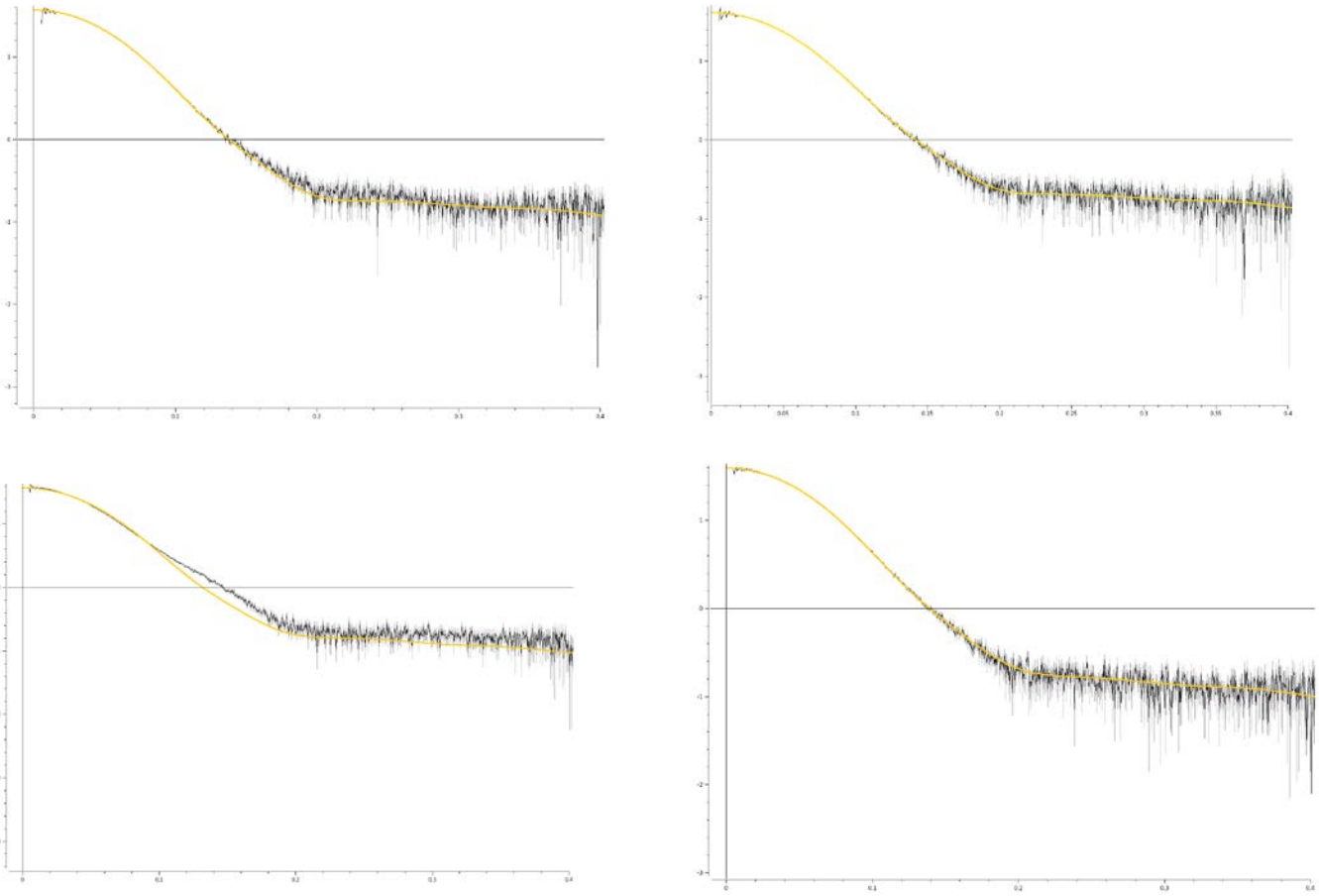

Figure 1: Shown here are the scattering curves of the two constructs of SHP-2 (extrapolated at infinite dilution and fitted against the theoretical curve generated from pdb 2SHP), in the absence and presence of allosteric inhibitor SHP099.
Top left: catalytically competent SHP-2, top right: catalytically competent SHP-2 with SHP099.
Bottom left: catalytically incompetent SHP-2, bottom right: catalytically incompetent SHP-2 with SHP099.

System 2:

Samples from proposal number MX-2058 were measured on 23rd June 2018. No technical errors were encountered during the experiments.

The proposal was related to the *met*-T-box riboswitch from *Staphylococcus aureus*, which is involved in regulation of amino acid metabolism in most gram-positive bacteria. T-box RNA sense and respond to aminoacylated state of tRNA as a measure of amino acid availability and then regulate the expression of downstream genes for biosynthesis of amino acids.

The predicted secondary structure of T-box-RNA suggests the existence of several folded domains (primarily stem-1, linker and terminator) which possibly interact during the tRNA recognition. Three constructs *i.e.* full length T-box RNA, and linker were measured at different concentration (0.6 to 4.4 mg/ml) so as the understand the overall fold.

The data for full length T-box RNA shows interparticle interaction at higher concentration. The curves were analysed to obtain R_g values of 5.15 nm. Similar experiment with stem-1 shows higher R_g values, which is in agreement with its predicted elongated shape as compared to the full length. The data from linker indicated towards comparatively compact fold. The data obatined in these experiments will be combined with other studies to understand the overall structure of the *met*-T-box RNA.