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 Box C/D snoRNPs and Reductase domain of TomAB Non-ribosomal peptide synthase	**Experiment number**: MX1974
Beamline:	**Date of experiment**: from: 2nd Dec 2017 to: 3rd Dec 2017	**Date of report**: 01.03.2018
Shifts:	**Local contact(s)**: Dr. GIACHIN Gabriele	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

1) Neha Dhimole*: Laboratory Leibniz Hannover University BMWZ Schneiderberg 38 DE - 30167 HANNOVER
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3) Simone Hoefler*: Laboratory Leibniz Hannover University BMWZ Schneiderberg 38 DE – 30167 HANNOVER

Report: Samples from the proposal number MX-1974 were measured on 2nd and 3rd of December 2017. There were no technical problems with the beamline during the measurements. **System 1** of proposal was concerned with the Box C/D snoRNP system from eukaryots, which is responsible for transferring the 2'-O-Methylations on ribosomal RNAs. This protein-RNA complex is composed of four different proteins (Snu13p, Nop1p, Nop56p and Nop58p) and one Box C/D guide snoRNA, which transfers the target specificity. Two chimeric complexes of different composition could be assembled for SAXS measurements. The first one consisted of the eukaryotic components Snu13p, Nop1p and a selected guide snoRNA and the chimeric Nop556 protein, which contains parts of the Nop5 protein, from the well studied archaeal Box C/D snoRNP complex, and the part of the eukaryotic Nop56p protein that interacts with the Nop1p protein.

The second complex consisted of the eukaryotic Snu13p and the same selected guide snoRNA and the Nop5 and Fibrillarin proteins from the archaeal Box C/D system. For both complexes only the selected guide snoRNA assembled into complexes good enough to perform meaningful SAXS measurements. Addition of targets was not performed because the complexes showed the formation of an non-homogeneous higher oligomeric state upon target RNA addition, on gel filtration during the sample preparation. For both complexes different concentrations (ranging from 0.5 -5.5mg/mL) were measured in batch mode. The obtained Rg values were found to increase with increasing concentration, signifying a concentration dependent change in the oligomeric state of the complexes, even though the best possible complexes were chosen. Despite the good indication on the oligomeric state of the complexes obtained from SAXS data further studies will be required to confirm the final oligomeric of state of this snoRNP.

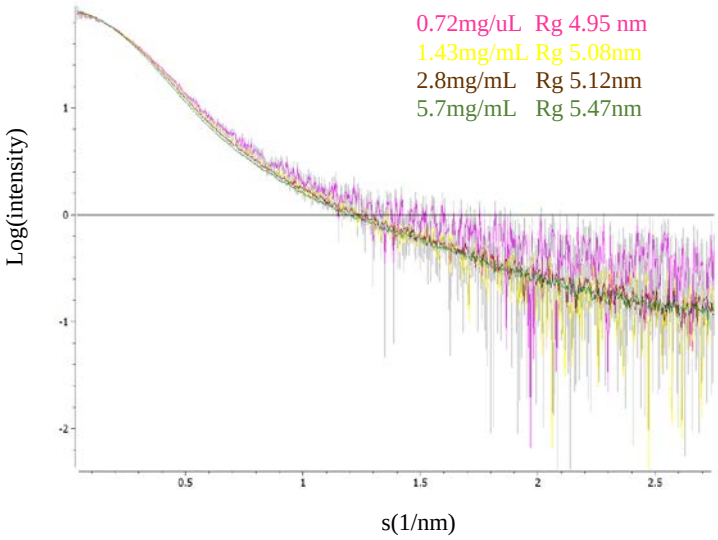

Figure 1: Shown here are the scattering curves of the chimeric Box C/D complex consisting of Snu13p, Nop1p, eukaryotic guideRNA and the Nop556 hybrid protein

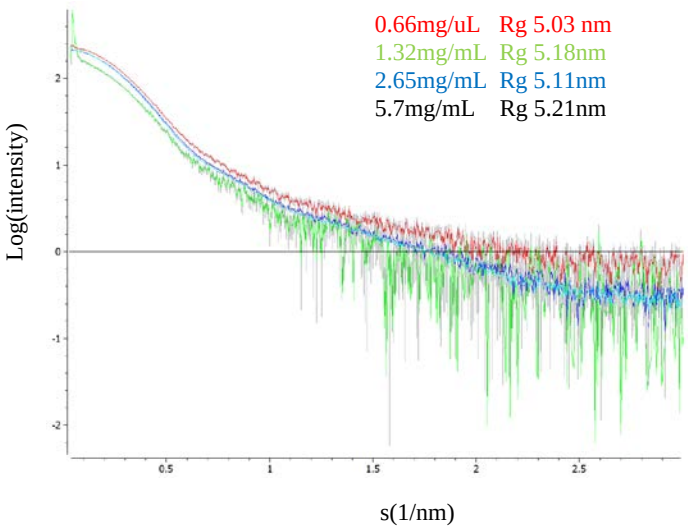

Figure 2: Shown here are the scattering curves of the chimeric Box C/D complex consisting of Snu13p, eukaryotic guideRNA and Nop5 and Fibrillain with the archael system

System 2 of the proposal was concerned with the the reductase domain of the TomAB non-ribosomal peptide synthase. Reductase domain of the Non–ribosomal peptide synthases (NRPS) is a unique domain that catalyzes both product release and reduction as the last step in the biosynthesis of the peptides, using the cofactor NADPH.

Unfortunately, the different constructs of reductase domain of the TomAB system severely aggregated at concentrations required for the dilution series in the SAXS measurements and hence were not amenable for further studies. Moreover, the cofactor NADPH was found to be highly unstable in the solution conditions required for SAXS measurements. However, another domain of the TomAB non-ribosomal system involved in catalysis of peptide bond formation (BC), which also purportedly interacts with the reductase domain, was used for the experiments. The constructs of the protein with varying lengths of the N-terminus were probed by SAXS to check for the effect of the N-terminal residues on the folding and conformational properties of the protein. A dilution series was performed on both the constructs: BC and BCdelN. Figure 1 shows the logarithmic plot of logIq vs s for BC. The linear behavior in the Guinier region indicates that there are no interparticle effects even at higher concentration of the protein indicating that this protein remains monodisperse and monomeric.

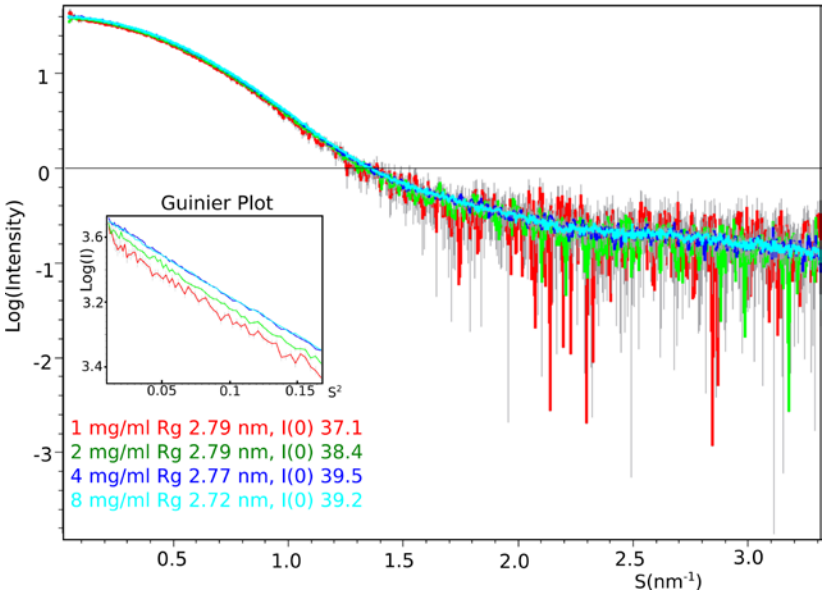

Figure 1: Scattering curves for various concentration of BC of TomAB NRPS system. The inlet shows the linear guinier region of the curves alongwith their respective Rg and I(0).

Figure 2 shows the scattering curve for the truncated BCdelN.
In case of BCdelN, different structural models predicted by homology modeling for this protein were scored against the observed SAXS data. Figure 2 shows the scattering curve of BCdelN alongwith the predicted curve for one of the models. The observed and back-calculated curves match and so do the R(g)s. This shows that we could discriminate against current available structures that vary in conformational flexibility. We will use the best-fit models for further computational and structural studies.

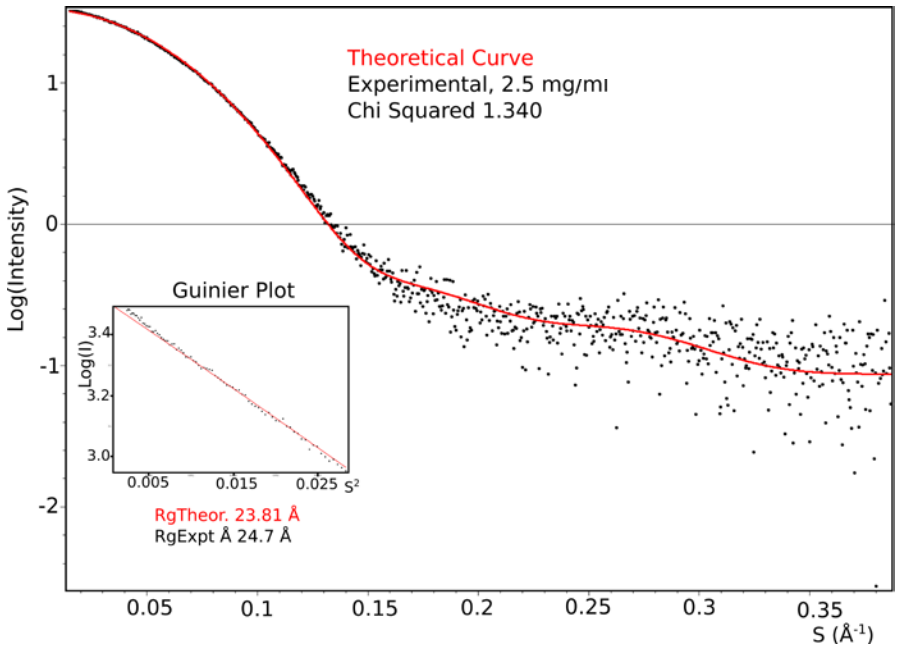

Figure 2: Scattering curve for the N-terminal truncated form of BC. The theoretical curve was calculated using Crysol.