

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://www.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.


Experiment title:

SAXS studies on eukaryotic snoRNP complexes and piRNA biogenesis factor Qin using BM29 beamline.

Experiment number:

MX 1922

Beamline:

BM29

Date of experiment:

from: 23rd July 2017 to: 24th July 2017

Date of report:

10 Sep 2017

Shifts: 3
Local contact(s):

Dr. GIACHIN Gabriele

Received at ESRF:

Names and affiliations of applicants (* indicates experimentalists):

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Report: Samples from the proposal number MX-1922 were measured on 23rd-24th of July 2017. There were no technical problems with the beamline during the measurements.

The Eukaryotic Box C/D system that catalyse the methylation of 2'-OH groups in ribosomal RNAs was the **system1** of the proposal. It is composed of proteins Snu13p, Nop1, Nop56 and Nop58 and snoRNAs for specificity. Only sub-complex of Box C/D snoRNA complex could be assembled. The complete complex assembly required proteins from the well studied archeal Box C/D snoRNA, resulting in a chimeric complex with only Snu13p and snoRNA from eukaryotic system. Of the several snoRNA probed by SAXS only few resulted in the assembly of the complex. These complexes were unstable and dissociated on substrate ribosomal RNA addition, moreover the oligomeric state of the complex was also found to be concentration dependent. This was apparent from the batch mode measurement of the complex which gave different Rg values as a function of protein complex concentration as shown in the figure below:

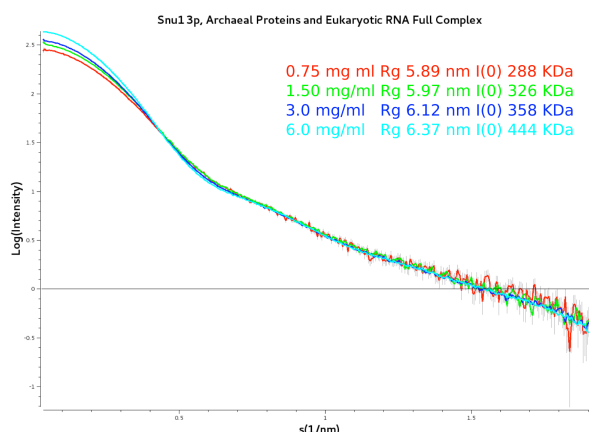


Figure 1: Shown here is the scattering curves for the Apo complex of chimeric eukaryotic Box C/D snoRNA complex.

System 2 was composed Qin protein that contains 5 tudor domains and is involved in piRNA biogenesis. Several constructs of Qin protein that contained tandem tudor domains were measured in batch, along with few measurement of single tudor domain of Qin. The SAXS measurement of the tandem tudor domains showed a strong concentration dependent inter-particle effect making the data analysis challenging. Only one of the constructs containing domain 3 and 4 showed slightly better results, shown in figure 2 with a zero extrapolation curve. Still the concentration dependent inter-particle effect are obvious in the curves, which was evident as the change of R_g and $I(0)$ with change in concentration. Despite this we were able to model the flexible linker between the tudor domain 3 and 4 using BUNCH.

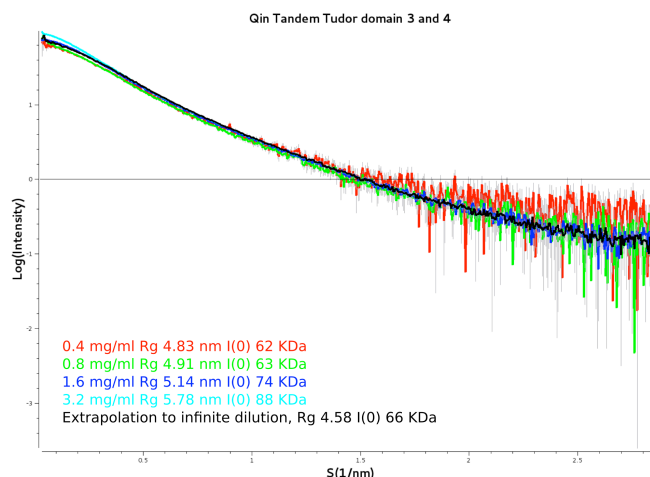


Figure 2: Scattering curves for various concentrations of tandem tudor domain 3 and 4 of Qin are shown. The concentration dependent inter-particle effect and zero-extrapolation is also shown along with the R_g and $I(0)$ values at different concentrations.

To overcome this problem few of the constructs were run on the gel filtration column to make use of in-line SAXS measurements. Unfortunately the presence of dirt and contaminants from either the capillary or the column resulted in aggregates and unstable baseline. The analysis of such data has not been straight forward. One such run for tudor domain 4 of Qin is shown in figure 3 and for tandem tudor domain 1 and 2 in figure 4.

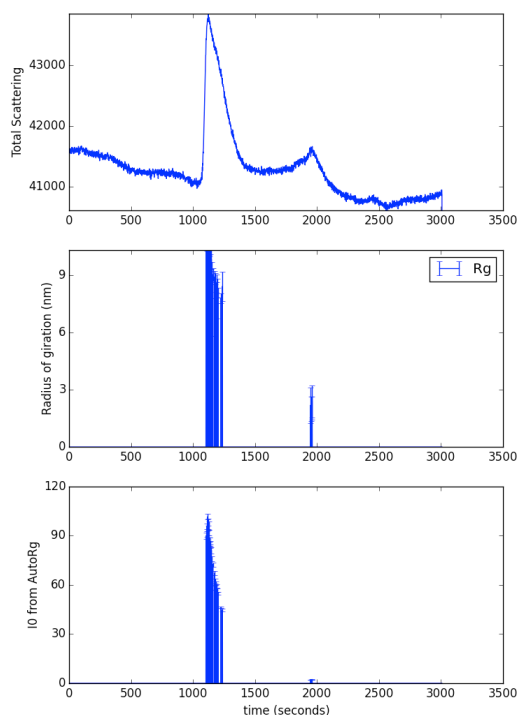


Figure 3: Scattering curves for gel filtration run of tudor domain 4 is shown in the top panel while the R_g and $I(0)$ are in the bottom panel. The uneven baseline is apparent.

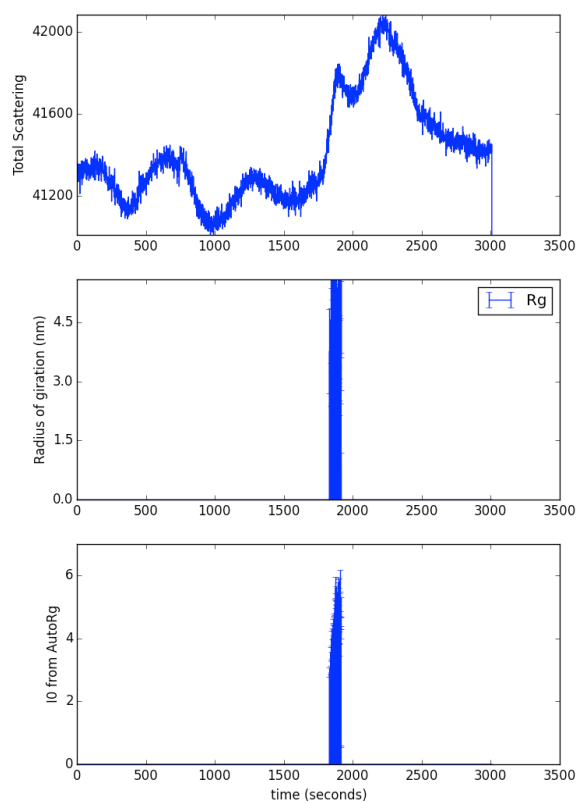


Figure 4: Similar to figure 3, the scattering curves for gel filtration run of tandem tudor domain 1 and 2 is shown in the top panel with R_g and $I(0)$ in the bottom panel.