EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

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:** Unraveling of PICK1 oligomerization in solution using SEC-SAXS	**Experiment number**: MX 1818
Beamline: BM29	**Date of experiment**: from: 9:00 to: 01:00	**Date of report**:
Shifts: 2	**Local contact(s)**: Julien Perard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Søren Roi Midtgaard, The Niels Bohr Institute, University of Copenhagen
*Viktor Holm, The Niels Bohr Institute, University of Copenhagen
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*Kenneth Madsen, Faculty of Health, University of Copenhagen
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*Nicolaj Riis Christensen, Faculty of Health, University of Copenhagen
-Lise Arleth, The Niels Bohr Institute, University of Copenhagen

Report:

Background:
This beamtime was applied for to probe two different protein systems. Common for the two probed systems was the tendency to aggregate that have hindered multiple previous attempts to obtain good, non-aggregated, SAXS data.

1) The PICK1 system has previously been probed extensively using SAXS by the proposers of this experiment[1]. This system has a high tendency to aggregate and previous SAXS data has consisted of multiple oligomeric species. In this experiment, the use of SEC-SAXS was used in order to obtain data of PICK1 specific stabilized oligomers. This was highly successful, and an illustrative dataset is seen in figure 1 where it can be seen that the aggregation has been removed from the column.

[1] Karlsen, M. L.; Thorsen, T. S.; Johner, N.; Ammendrup-Johnsen, I.; Erlendsson, S.; Tian, X.; Simonsen, J. B.; Høiberg-Nielsen, R.; Christensen, N. M.; Khelashvili, G.; et al. Structure of Dimeric and Tetrameric Complexes of the BAR Domain Protein PICK1 Determined by Small-Angle X-Ray Scattering. *Structure* **2015**, *23*, 1258–1270.

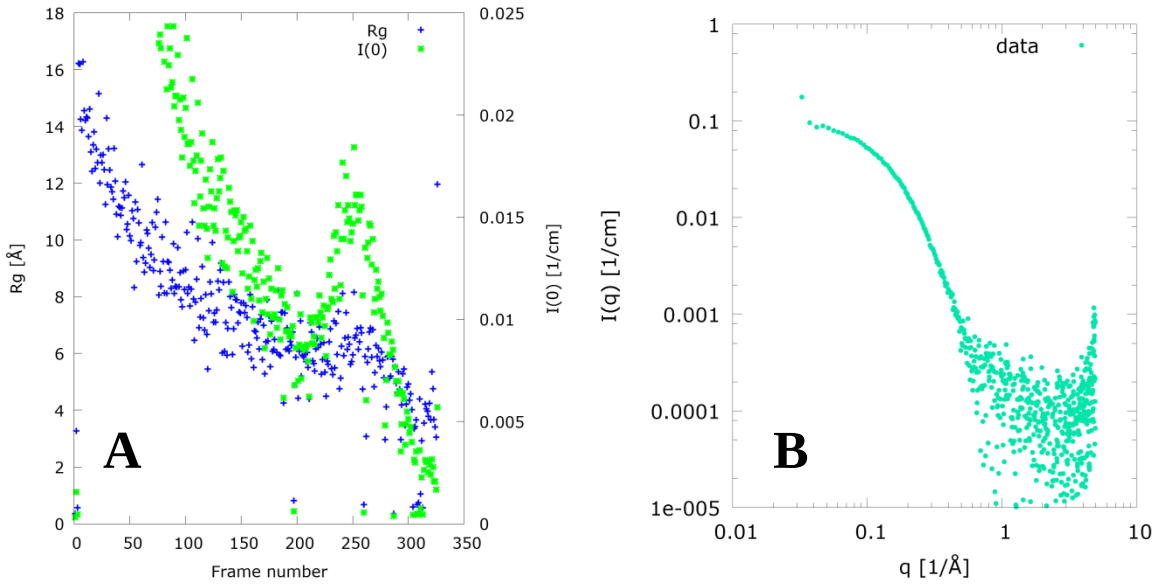

Figure 1: A) Showing Rg (blue) and I(0) (green) as a function of the SAXS frames recorded during the SEC run of a PICK1 sample. B) SAXS data from the I(0) peak, showing a non-aggregated sample.

The data collected on the PICK1 system will become the basis for a publication, and further studies for stabilizing PICK1 will also be based on the SEC-SAXS approach..

2) Photosystem 1 (PS1) from Barley is a very large membrane protein. This was used in the present study where multiple different platforms were tested for stabilizing PS1 in solution. Previous attempts have not been successful due to the tendency for this system to aggregate. In figure 2A, is the Rg and I(0) over the acquired frames over the SEC run shown. It is clear that that multiple species has been separated. Comparing the SAXS data from the peak of the I(0) with previous data (Figure 2, panel B), using the SEC-SAXS combination has helped gaining the needed data required for this system.

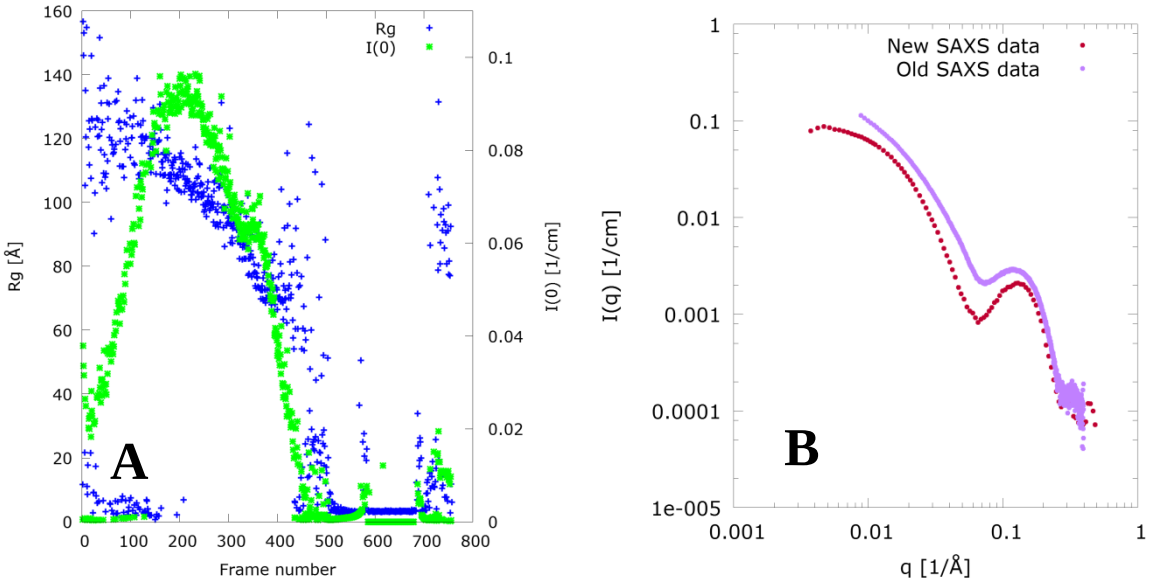

Figure 2: A) Showing Rg (blue) and I(0) (green) as a function of the SAXS frames recorded during the SEC run of a PS1 sample. B) SAXS data from the peak (red), showing a non-aggregated sample and an older dataset (purple) showing the aggregation.

The data collected on the different PS1 stabilizing systems will be the basis of a publication together with activity data on the effects of different solubilization systems on activity of photosystem 1 protein.