ESRF	**Experiment title:** SAXS studies of mRNP components from yeast	**Experiment number**: MX-775
Beamline:	**Date of experiment**: from: 12.12.2008 to: 12.12.2008	**Date of report**:
Shifts: 2	**Local contact(s)**: Petra Pernot	*Received at ESRF:*

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Report:

In our proposal, we suggested two projects to be analyzed by small angle X-ray scattering (SAXS) at ID14-3. ESRF granted us 2 shifts (8 hours each), which where both scheduled for Dec. 12. 2008. Since to date we had only very limited experience in SAXS measurements, our main goals of our visit to the ESRF were
(i) to get familiar with this new technology,
(ii) evaluate the state of the beamline development at ID14-3, and
(iii) to obtain first data for our two proposed projects.

(i) During the double-shift at ID14-3 we were able to achieve a basic routine during data collection, data quality assessment, and data processing. This was possible mainly through the superb support by the two beamline scientists, who were extremely supportive and helpful. At our home institute we are currently processing and analyzing our data in more detail.

(ii) Although the beamline was still in the early phase of public use and several things have not been established yet (like a GUI interface), the beamline was easy to handle. Among the number of planned improvements, we consider the commissioning of a sample changer in combination with the opportunity to perform remote data collection to be a highly interesting option. A graphical user interface would of course also be nice…

(iii) We had proposed to characterize two core proteins of a yeast mRNA transport complex by SAXS. In the first project, we aimed to characterize the oligomerization of the unusual RNA-binding protein She2p (Müller et al., 2007) in solution. In a high-resolution crystal structure, She2p forms a dimer (Niessing et al., 2004). In solution, however, larger complexes are formed (D.N. unpublished). Because no crystal structures could be obtained from these larger oligomers, we proposed to use SAXS instead. Our measurements of wild-type She2p at ID14-3 resulted in high-quality scattering curves that are consistent with a larger oligomer in solution. Comparative analyses with a mutant version of She2p that fails to form these oligomers have

been performed as well. From this comparison, we already see that the mutant has an altered shape and oligomeric state. Further data processing and modelling will be required before we understand what the exact differences between both species are. Because our measurements suggested that further measurements at higher protein concentrations and altered buffer conditions could further improve our data analyses, we plan to apply for additional SAXS beamtime in the near future.

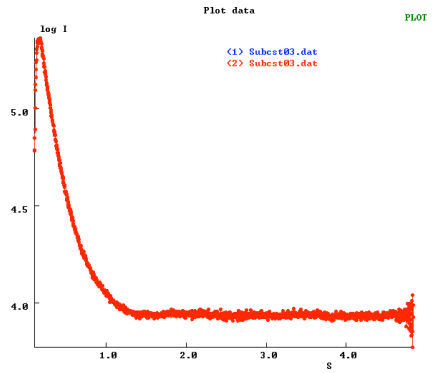

Fig. 1: single measurement curve of She2p
after buffer substraction.

The second project was less successful. We planned to obtain SAXS data from a myosin tail domain that interacts with the mRNA-cargo complex (Heuck et al., 2007; Müller et al., 2007). Unfortunately, different protein fragments and co-complexes of this myosin tail all showed unacceptable amounts of aggregation. After a number of measurements at ID14-3, we came to the conclusion that this protein either did not survive the transport to the ESRF well or that it is not suitable for SAXS measurements. Will will further investigate this problem by dynamic light scattering and then decide if we would like to test the protein again in SAXS measurements.

In summary, we have obtained high quality data for the first project, which we will continue to characterize and improve. Because SAXS has high potential in combination with X-ray crystallography, we hope to use this technique in future for other projects of our laboratory as well. We would consider it very convenient, if we could have the chance to combine the dates for our MX and SAXS measurements in future.

References:

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