EUROPEAN SYNCHROTRON RADIATION FACILITY

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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:** Stealth Nanodiscs: Development of contrast optimized carrier systems for membrane proteins	**Experiment number**: MX-1518
Beamline:	**Date of experiment**: from: May 5, 2013 to: May 6, 2013	**Date of report**: September 17, 2013
Shifts: 2	**Local contact(s)**: Petra Pernot	*Received at ESRF:*

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Report:

Membrane proteins remain very difficult to work with and therefore, the structural information on this important type of proteins is sparse. This has lead to a huge demand for new methods to investigate the structure of this class of proteins. Solution based techniques such as small-angle scattering seem to be a promising strategy as an alternative to x-ray crystallography; however, these still rely on successful reconstitution of membrane proteins into bilayer-mimicking carriers. One such carrier is the so-called nanodiscs, a 10-14 nm sized phospholipid bilayer stabilized by two amphipathic membrane scaffolding proteins (MSP).

This nanodisc system is very well structurally characterized and has shown a great potential as a carrier for structural studies of membrane proteins using a combined SAXS/SANS approach together with computational modeling techniques developed in our group (1, 2).

The present report shows the latest step in our development of this carrier system and its use in reconstitution of membrane proteins. We wish to structurally characterize membrane proteins using a new modeling technique that has been developed in the group to solve low resolution structures (3). Furthermore, we also employ the so-called stealth nanodiscs, which have been developed by selective deuteration to minimize the scattering intensity of neutrons (4). In the May 2013 experiment, we performed measurements on various nanodiscs systems, among all a variation series with cholesterol content and substitution of MSP for the native Apo A-1. Different membrane proteins in nanodiscs were studied including OmpF, proteorhodopsin, sensory rhodopsin and CorA. The results from two of these systems are described in further detail in the following.

The 7 TM membrane protein sensory rhodopsin was reconstituted into stealth nanodiscs using both selectively deuterated MSP and PC-lipids. The collected data implied successful reconstitution, unfortunately, the sample quality was not sufficiently good for further structural analysis and SANS data collection.

CorA, a large 2 TM pentameric magnesium transporter, was reconstituted into nanodiscs in its magnesium free state using POPC lipids. Both SAXS and SANS data was with great success collected from this assembly, shown in figure 1.

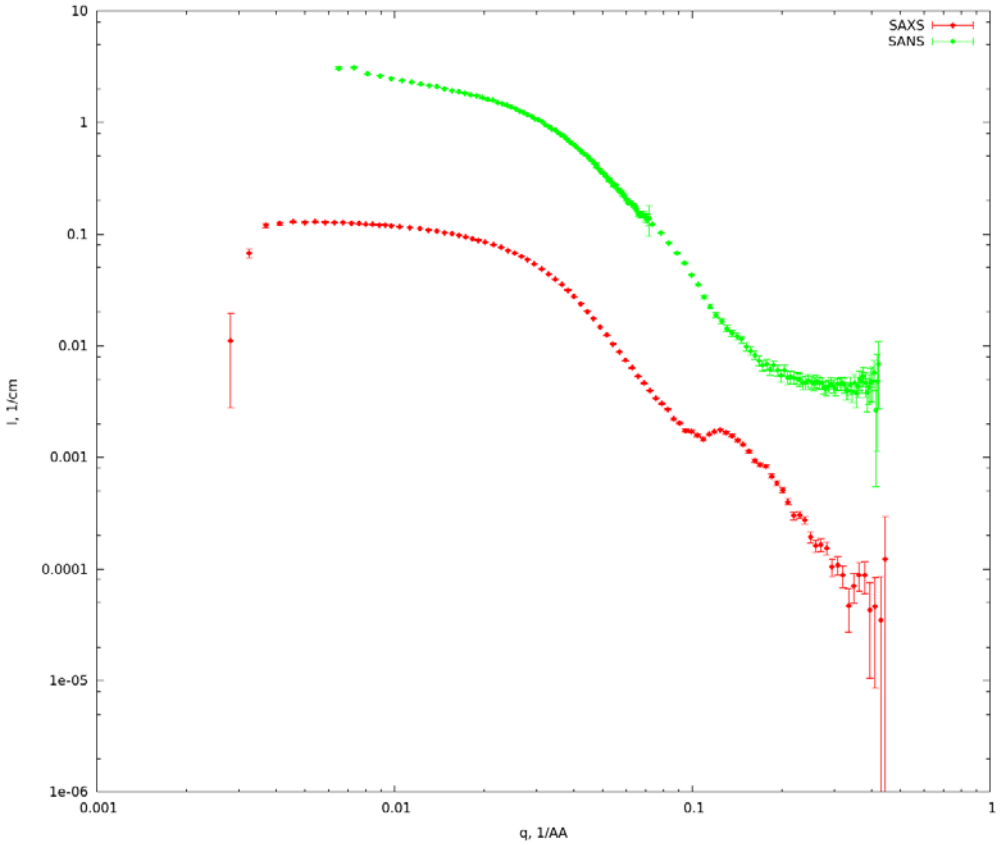

Figure 1: Scattering data of CorA reconstituted in nanodiscs. SANS data shown in green, SAXS data in red. SANS data was collected at beamline D11, Institute Laue-Langevin and SAXS data at beamline BM29, European Synchrotron Radiation Facility.

The data show CorA was successfully reconstituted into nanodiscs and very promising for further determination of the structure of CorA. The data analysis and structural characterization of CorA are still in progress. Furthermore, we wish to study CorA in its magnesium-bound state, as no crystal structure is available.

References

1. Skar-Gislinge N, *et al.* (2010) Elliptical structure of phospholipid bilayer nanodiscs encapsulated by scaffold proteins: casting the roles of the lipids and the protein. *Journal of the American Chemical Society* 132(39):13713-13722.
2. Skar-Gislinge N & Arleth L (2011) Small-angle scattering from phospholipid nanodiscs: derivation and refinement of a molecular constrained analytical model form factor. *Physical chemistry chemical physics : PCCP* 13(8):3161-3170.
3. Kynde S, *et al.* (2013) Small-angle scattering gives direct structural information about membrane protein inside lipid environment, *Submitted*
4. Maric S, *et al.* (2013) Stealth carriers for low resolution structural determination of membrane proteins in solution. *Submitted*