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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:** Development of a “stealth” carrier system for SANS studies of membrane proteins	**Experiment number**: 1455
Beamline: BM29	**Date of experiment**: from: 10th Sep 2012 to: 11th Sep 2012	**Date of report**: 12th April 2013
Shifts: 2	**Local contact(s)**: Petra Pernot	*Received at ESRF:*

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Report:

Membrane proteins remain extremely hard to crystallize leaving us with very limited structural information for some of the most important proteins of the cell. 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 nanodisc, a 10-14 nm sized phospholipid bilayer stabilized by two amphipathic protein belts. Structurally well-defined, this nanodisc system 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 the development of the nanodisc system where we have constructed a selectively deuterated and contrast minimized stealth version of the discs in close collaboration with the Deuteration Laboratory at the Institut Laue-Langevin in Grenoble. Our long term goal is to use these stealth discs as neutron-undetectable carriers (Fig. 1) which can function as a platform for low-resolution structural studies of membrane proteins in solution using SANS together with already available data analysis tools (3).

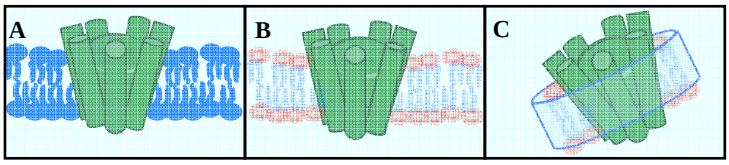

Fig 1 - (A) Membrane protein (green) and phospholipid bilayer (blue) in the standard contrast situation. Both bilayer and membrane protein are clearly visible. (B) Membrane protein in contrast-matched phospholipid bilayer. Only the membrane protein is visible, but it is confined to a 2D membrane geometry. (C) Membrane protein in contrast-matched Nanodisc. Only the membrane protein is visible and it is floating free in solution.

The stealth nanodiscs were assembled using deuterated amphipathic belts (MSP1D1) together with selectively deuterated PC-lipids, developed in collaboration with the D-Lab, ILL, Grenoble. The theoretical deuteration levels needed for full "invisibility" of the discs, which vary for the different parts of the nanodisc complex and even differ for the different parts of the phospholipid bilayer alone, were achieved through biosynthesis in a genetically modified *E-coli* strain grown in deuterated media. The deuteration levels and localization on the lipid molecule were regulated during lipid synthesis through careful design of the bacterial growth conditions.

The assembly of specifically deuterated stealth nanodiscs and their behaviour in both H_2O and D_2O, were investigated using SAXS as the main focus of the present experiment. In addition, several membrane proteins reconstituted into regular hydrogenated discs were investigated to determine their characteristics prior to further incorporation into the stealth discs. The obtained SAXS data of the stealth nanodiscs in H_2O showed a very distinct curve characteristic of the nanodisc system (Fig. 2A) (4). The resulting *p(r)* function indicated a maximum size of approximately 120 Å for the discs (Fig. 2B) consistent what has previously been observed for similarly prepared hydrogenated nanodiscs (1, 2). A mathematical model for the nanodisc system derived previously in our group was also fitted to the experimental data (Fig. 2A black) (4) and confirmed that the SAXS data were fully consistent with nanodiscs having the same structure as has previously been observed (1, 2).

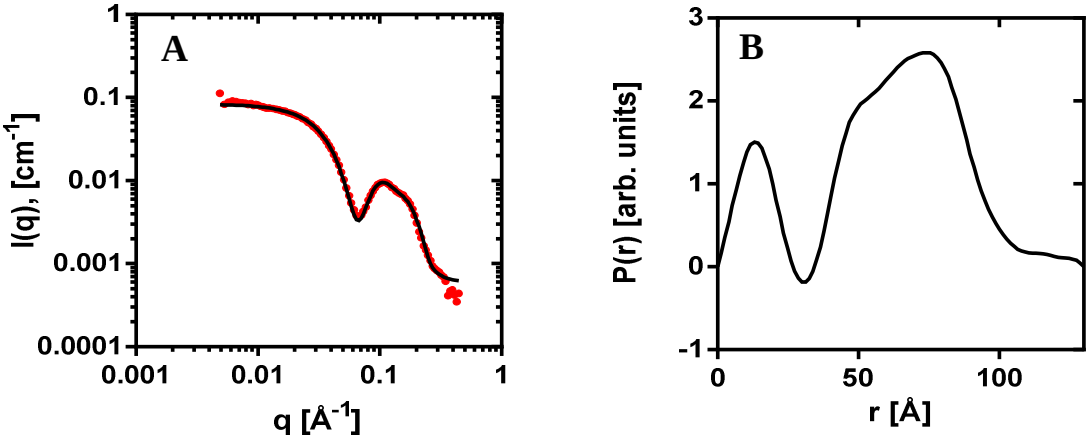

Fig. 2 - Stealth Nanodisc assembly. (A) small-angle x-ray scattering data from nanodiscs in H_2O (red) shown together with the fitted structural model (black). (B) shows the pair distance distribution function for the stealth discs with information about the disc size.

The SAXS analysis was followed by a SANS contrast variation study in aqueous buffer to determine the match point and internal homogeneity of the scattering length density within the carriers. The SANS data, collected at the FRMII in Munich showed a decrease in scattering intensity with increasing level of D_2O in solution (Fig. 3A) (4). For each contrast measurement the forward scattering was determined by the indirect Fourier transform method and the scattering data normalized by sample concentration (4). The match point was then determined by fitting a second order polynomial to the forward scattering plotted as a function of volume fraction of D_2O in the buffer solution (Fig. 3B) (4).

A minimum in the overall I(q) can be seen giving a match point at 100% D_2O, however absolute zero is not reached. This can be due to inhomogeneities in the nanodisc components arising from a small distribution in deuteration of the phospholipid bilayer and possible inhomogeneity arising from the deuteration in membrane scaffold protein. However the intensity decreases to a level comparable to that of the experimental background making the signal negligible when seen in combination with a possible incorporated protein (4). We therefore see this as a very promising new development for structural investigation of membrane proteins and plan to follow up with studies on several membrane proteins incorporated into the aforementioned contrast-matched stealth carriers.

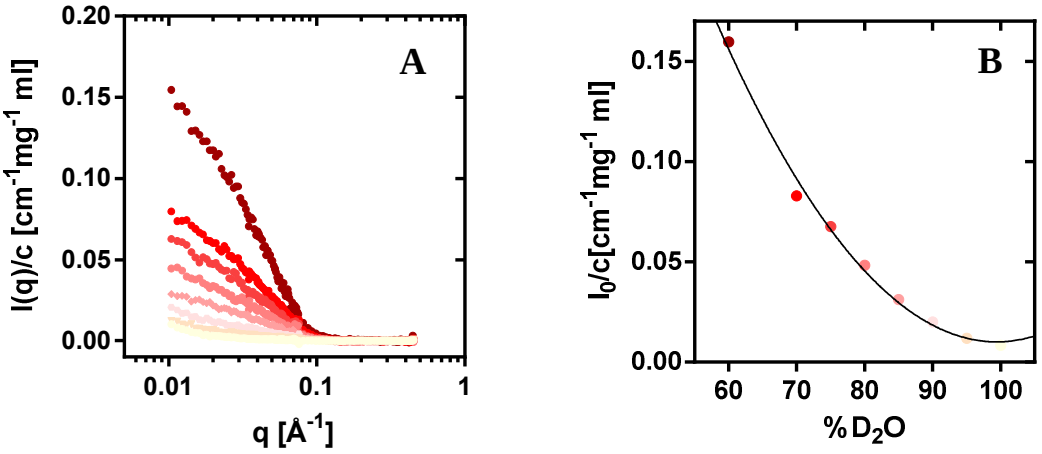

Fig. 3 - Stealth Nanodisc SANS contrast variation. (A) SANS data showing the decrease in scattering intensity from the stealth nanodiscs with increasing buffer contents of D_2O in solution. (B) SANS forward scattering as a function of the D_2O contents of the buffer.

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. Petoukhov MV & Svergun DI (2012) Applications of small-angle X-ray scattering to biomacromolecular solutions. *The international journal of biochemistry & cell biology* 45(2):429-437.
4. Maric S, *et al.* (2013) Stealth carriers for low resolution structural determination of membrane proteins in solution. *Submitted to PNAS.*