Experimental Report

Proposal: **8-03-771** **Council:** 4/2012

Title: Stealth Nanodiscs: Development of contrast optimized carrier systems for membrane proteins

This proposal is resubmission of: 8-03-751

Researh Area:

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Samples: D2O Tris Buffer
H2O Tris Buffer
protonated membrane protein
Deuterated protein

Instrument	Req. Days	All. Days	From	To
D11	1	1	07/05/2013	08/05/2013

Abstract:

In the last few years it has been demonstrated that membrane proteins can be successfully reconstituted into small membrane-like devices termed Nanodiscs. Consisting of a 10-14 nm sized phospholipid bilayer stabilized by an amphipatic protein belt, MSP, the Nanodisc stabilizes the membrane protein by providing a native-like environment. Considerable insight into the structural organization of the Nanodisc has been obtained by our group in previous experiments at ILL and ESRF through a combined SANS/SAXS approach. These data have enabled us to start optimizing the Nanodisc-carrier to be used as a platform for structural studies of membrane proteins in solution. The latest development of the system is the construction of a selectively deuterated and contrast minimized Nanodisc carrier, done in close collaboration with the D-LAB, Grenoble. This “stealth nanodisc”-carrier will then, relatively, enhance the neutron signal from the incorporated membrane protein, which is the system in focus. The aim of this proposed experiment is to establish the proof-of-concept of this “stealth Nanodiscs” approach to investigate membrane proteins.

Background:

This beamtime was applied for as part of our continuous effort of using so-called nanodiscs and similar particles for structural elucidation of integral membrane proteins. Membrane proteins are inherently difficult to study due to their amphipathic nature. Due to this and the complication of low availability and low stability, structural knowledge regarding membrane proteins is highly under-represented in the deposited structures available in the protein data bank (PDB). Membrane proteins are though vital to study due to their biological role, and multiple nobel prizes have been awarded based on structural elucidation of integral membrane proteins, latest in 2012 to Brian K. Kobilka and Robert Lefkowich for the investigating of G-protein coupled receptors. This means that further development of techniques for studying membrane protein structure is in demand and highly relevant. We are focusing on using small-angle scattering together with the soluble nanodisc based technology for low resolution information of membrane proteins.

Nanodiscs are lipid-protein particles consisting of a small central lipid bilayer core encircled by two amphipathic proteins (so-called membrane scaffold proteins (MSP)) on the perimeter of the discoidal particle. They have proven to be a good soluble self-assembling "sampleholder" for membrane proteins, being relatively monodisperse and providing high membrane protein stability compared to traditional detergent micelles. We have recently shown that it is indeed possible to extract information of a nanodisc incorporated membrane protein using a combination of small-angle neutron scattering (SANS) and small-angle X-ray scattering (SAXS) together with a rigorous modelling approach[1,2]. We have furthermore showed the possibility of matchout deuterating the nanodiscs to ease the data analysis of the SANS data[3] and the use of small synthetic peptides to form similar particles[4].

Experimental report:

The key focus of the experiment was to collect data on our contrast matched out nanodisc system[3] with incorporated membrane proteins. While the membrane protein systems and match-out deuterated lipids were ready for the experiment, we unfortunately ran into experimental problems during the final purification and preparation stages of the match out deuterated protein belts for the nano disc carriers. So while our (less sample volume demanding) SAXS data analysis showed that we indeed were able to produce nanodiscs from the stealth systems (see fig 1a), we unfortunately ended up not having enough sample to get good SANS data (See fig 1b). The work will be pursued in upcoming beam times.

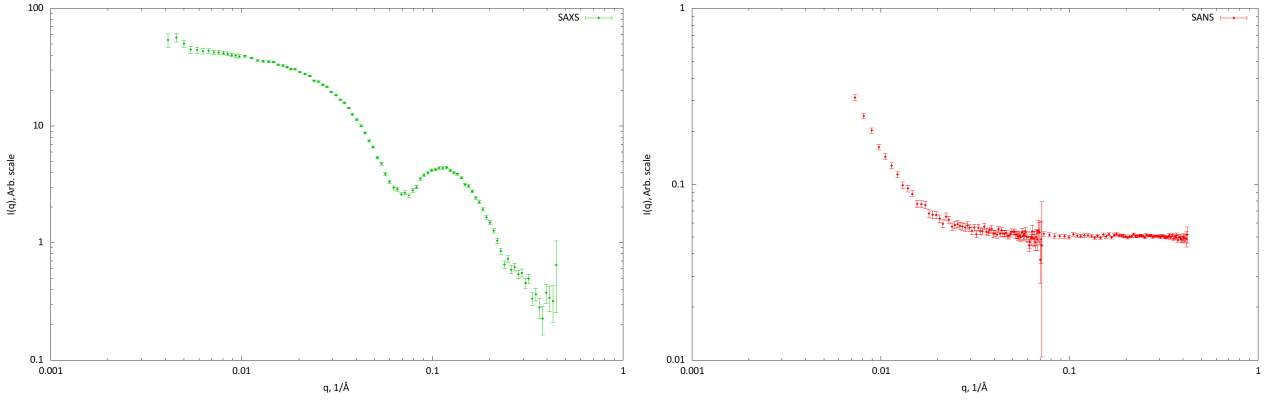

Figure 1: Showing SAXS (left) and SANS data (right) respectively for the same sample of the matched out nanodiscs with a membrane protein incorporated.

As a part of the experiment, a large pentameric membrane protein system, CorA (a magnesium transporter), was investigated with the ordinary hydrogenated nanodisc system. The CorA was obtained through

collaboration with Mikaela Rapp, Department of Medical Biochemistry and Biophysics, The Karolinska Institute at Stockholm University. This system provided very promising data, both from the SAXS and the SANS analysis (see fig 2). A detailed analysis, building on the experience gained through our previous work[1,5] (See also our previous experimental report) is presently in the process.

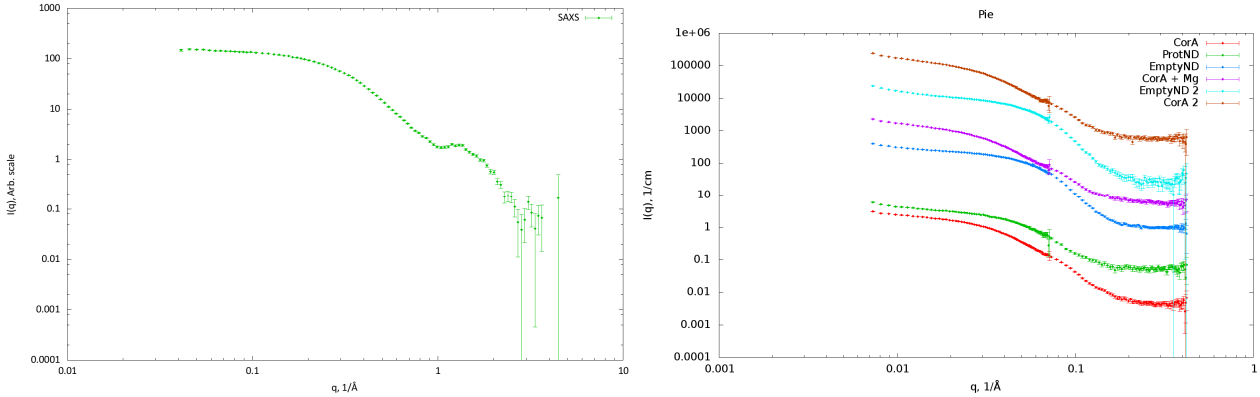

Figure 2: Showing SAXS (left) and SANS data (right) respectively for the CorA incorporated nanodiscs.

Finally, SANS experiments were performed on nanodiscs prepared from different dimers of the so-called 18A-system. This is a further development on peptide based nanodiscs previously investigated at ILL and recently published[4] in collaboration with Prof Knud J. Jensen from the Chemical biology and Nanobioscience group at University of Copenhagen. A publication based on the obtained data presented in figure 3 is presently being written.

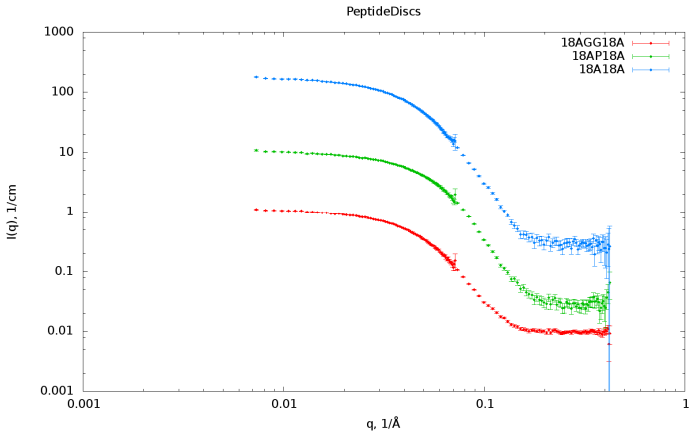

Figure 3: SANS data for the 3 different peptide based nanodiscs.

(1) Kynde, S. A. R.; Skar-Gislinge, N.; Pedersen, M. C.; Midtgaard, S. R.; Simonsen, J. B.; Schweins, R.; Mortensen, K.; Arleth, L. Small-Angle Scattering Gives Direct Structural Information about a Membrane Protein inside a Lipid Environment. *Acta Crystallogr. D. Biol. Crystallogr.* **2014**, *70*, 371–383.

(2) Pedersen, M. C.; Arleth, L.; Mortensen, K. WillItFit : A Framework for Fitting of Constrained Models to Small-Angle Scattering Data. *J. Appl. Crystallogr.* **2013**, *46*.

(3) Maric, S.; Skar-Gislinge, N.; Midtgaard, S.; Thygesen, M. B.; Schiller, J.; Frielinghaus, H.; Moulin, M.; Haertlein, M.; Forsyth, V. T.; Pomorski, T. G.; et al. Stealth Carriers for Low-Resolution Structure Determination of Membrane Proteins in Solution. *Acta Crystallogr. D. Biol. Crystallogr.* **2014**, *70*, 317–328.

(4) Midtgaard, S. R.; Pedersen, M. C.; Kirkensgaard, J. J. K.; Sørensen, K. K.; Mortensen, K.; Jensen, K. J.; Arleth, L. Self-Assembling Peptides Form Nanodiscs That Stabilize Membrane Proteins. *Soft Matter* **2014**, *10*, 738–752.

(5) Skar-Gislinge, N.; Simonsen, J. B.; Mortensen, K.; Feidenhans'l, R.; Sligar, S. G.; Lindberg Møller, B.; Bjørnholm, T.; Arleth, L. Elliptical Structure of Phospholipid Bilayer Nanodiscs Encapsulated by Scaffold Proteins: Casting the Roles of the Lipids and the Protein. *J. Am. Chem. Soc.* **2010**, *132*, 13713–13722.