Experimental report

13/02/2017

Proposal: 8-03-885 **Council:** 4/2016

Title: SEC-SANS for probing the structureof membrane proteins in lipid-protein nanodiscs

Research area: Biology

This proposal is a new proposal

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Samples: Bacteriorhodopsin
MSP1D1
1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)
Tissue Factor
Factor 7a

Instrument	Requested days	Allocated days	From	To
D22	2	2	20/09/2016	22/09/2016

Abstract:

During the last five years our group has focused on developing platforms and approaches to enable combined SANS and SAXS based structural studies of membrane proteins under solution conditions. SANS holds a particularly large potential in this context due to the opportunity for H-D based contrast variation. Unfortunately, the 100% D2O based buffers which in theory provides optimal contrast and signal-to-noise, destabilize many proteins and promote aggregation in the experimental samples. These D2O induced aggregation effects have unfortunately been clearly reflected in many of our previous SANS experimental data and often we have had to discard the data. D22 at ILL has recently, as the first neutron user-facility internationally, implemented a so-called size-exclusion chromatography (SEC) setup in the SANS sample environment enabling SEC-SANS. Based on our experience with similar SEC-SAXS setups, we have a strong expectation that this setup will solve the above mentioned problems with D2O-induced aggregation and we here propose an ambitious experiment to investigate membrane proteins relevant for the understanding and treatment of hemophilia.

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Scientific motivation:

Membrane proteins are notoriously difficult to handle. Due to their hydrophobic membrane spanning segments, they are inherently insoluble in water and need stabilizing entities such as detergent micelles or lipid bilayers to keep them in solution. In our group, we have been successful in stabilizing membrane proteins with nanodiscs, which are disc shaped lipid bilayer mimicking particles[1]. The nanodisc is composed of a lipid bilayer with a diameter of ~10nm, whose hydrobobic rim is stabilized by two copies of an amphipathic alpha-helical protein (MSP1D1) derived from the high density lipoprotein constituent ApoA1[2]. Due to its lipid bilayer, nanodiscs are thought to provide a more native environment for membrane proteins compared to detergent micelles.

To obtain a more detailed description of membrane proteins in nanodiscs, our aim is to employ small-angle neutron scattering combined with selective deuteration to match out the different components of the nanodisc in D_2O. However, working with 100% D_2O has yielded problems with the stability of our protein samples, most often resulting in a higher propensity for aggregation. In our small-angle scattering data, this is seen as a distinct up-turn in the low-*q* region[1,2]. We applied for SEC-SANS beam time at D22, because gel filtration should effectively separate aggregates from monodisperse articles. First, we sought to obtain good data on "empty" nanodiscs with no membrane protein incorporated, and secondly we wanted to study nanodiscs with incorporated membrane proteins.

Experiment report:

The key focus of our study was originally to investigate the highly interesting Tissue Factor:Factor VII[3] complex in nanodiscs. Although the system had been tested and was well-established, Tissue Factor could not be purified for this experiment. After this beam time, we have localized the cause to a bad DNA construct and this has since then been fixed.

To benchmark the new SEC-SANS setup, we measured a number of samples that we had previously obtained small-angle scattering data on but with light aggregation present. These samples mainly included empty nanodiscs made from MSP1D1 and POPC[2]. With the new SEC-

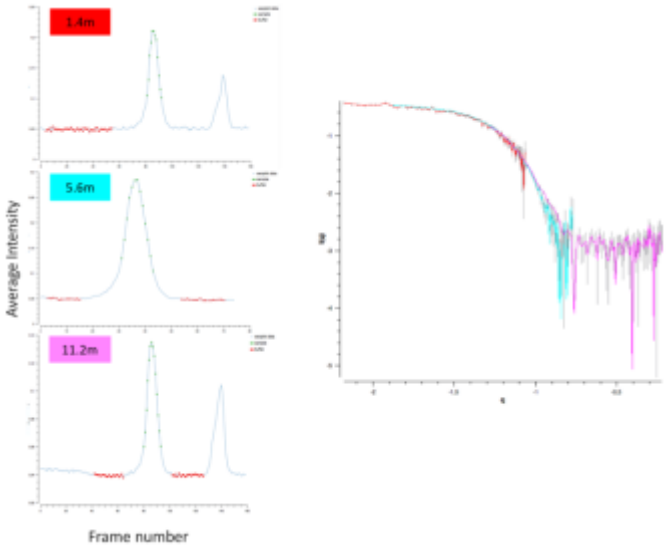

Figure 1 Nanodiscs composed of MSP1D1 and POPC lipids. Data processed in secplot (part of the ATSAS package[5]): On the chromatograms, selected data frames are marked with green squares and selected buffer frames with red squares. Each chromatogram is marked with a color code corresponding to the 1D SANS data shown in the plot on the right and the sample to detector distance is indicated. The 1D data was manually scaled to overlay.

SANS setup, we could effectively get rid of the distinct upturn in low-*q*, obtaining data with no trace of aggregation in the data (figure 1).

To test a model membrane protein incorporated into nanodiscs, we measured proteorhodopsin[4] (figure 2). A lot of our sample had aggregated while in storage, but although most of the sample eluted in the void volume, this test highlighted that SEC-SANS can be really useful to analyze unstable samples by separating the aggregates from the good sample, since some of the sample eluted later than the void volume. However, the elution profile did not match the expected for this protein. Even data sets from the small particle side of the eluted peak contained larger particles than expected for proteorhodopsin in nanodiscs.

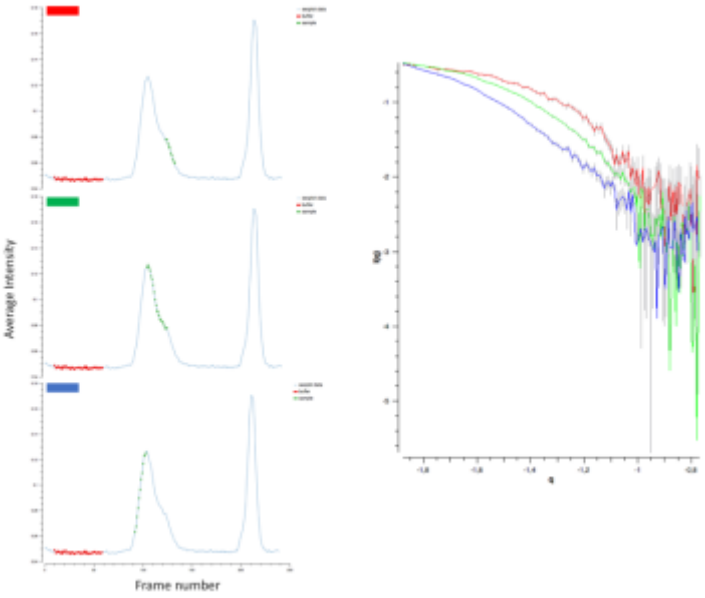

Figure 2 Proteorhodopsin incorporated in nanodiscs composed of MSP1D1 and POPC lipids. Data processed in "SECplot" (part of the ATSAS package[5]): On the chromatograms, selected data frames are marked with green squares and selected buffer frames with red squares. Each chromatogram is marked with a color code corresponding to the 1D SANS data shown in the plot on the right. The 1D data was manually scaled to overlay in *I*(0). The detector setting was 5.6m.

All in all, we were very impressed with the new SEC-SANS setup and the ability to obtain fully non-aggregated SANS data with useable data statistics. While we did not obtain a good dataset for the proteorhodopsin nanodisc due to a bad sample, we obtained the knowhow and hands-on experience needed to proceed with this and other membrane-protein related projects.

Due to the very recent implementation of the SEC-SANS setup, we found a number of points where we could see potential for further development and optimization of the system. These points have been delivered to the D22 team via a Skype meeting and will thus not be listed here.

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2. Skar-Gislinge, N. *et al.* Elliptical structure of phospholipid bilayer nanodiscs encapsulated by scaffold proteins: Casting the roles of the lipids and the protein. *J. Am. Chem. Soc.* **132,** 13713–13722 (2010).
3. Morrissey, J. H. *et al.* Blood clotting reactions on nanoscale phospholipid bilayers. *Thromb. Res.* **122,** 23–26 (2008).
4. Gourdon, P. *et al.* Optimized in vitro and in vivo expression of proteorhodopsin: A seven-transmembrane proton pump. *Protein Expr. Purif.* **58,** 103–113 (2007).
5. Petoukhov, M. V. *et al.* New developments in the ATSAS program package for small-angle scattering data analysis. *J. Appl. Crystallogr.* **45,** 342–350 (2012).