Experimental report

10/09/2019

Proposal: 8-03-959 **Council:** 10/2018

Title: SEC-SANS for investigation of three challenging and biologically relevant membrane proteins

Research area: Biology

This proposal is a new proposal

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Samples: MSP1E3D1
Tissue Factor
Matchout deuterated DDM
NHE1
Growth hormone receptor
Growth hormone

Instrument	Requested days	Allocated days	From	To
D22	2	2	19/06/2019	21/06/2019

Abstract:

We propose to utilize the SEC-SANS setup at D22 to investigate the three challenging and biologically relevant membrane proteins; growth hormone receptor (GHR), sodium/hydrogen exchanger1 (NHE1) and tissue factor (TF). All three proteins are expressed and purified in our lab, and solubilised in relevant car-rier systems. NHE1 will be investigated in match-out deuterated DDM, which will enable modeling of the protein structure without signal from the detergent micelle. GHR will be investigated in nanodiscs since the long intrinsically disordered intracellular domain of GHR might show a non-native interaction with its extracellular domain of when in a micelle. The nanodisc will also facilitate the investigation of the dimeri-zation and the interaction with growth hormone of the GHR. TF and the interaction with factor VIIa and factor Xa is highly dependent on the presence of a lipid bilayer and will hence also be studied in nano-discs. The SEC-SANS setup at D22 is a key factor in this approach, since the systems under investigation are highly delicate, and thus prone to form aggregates.

Experimental report

Proposal:	8-03-959
Instrument:	D22
Local Contact:	Anne Martel
Date:	19/6– 21/6, 2019

Overview

We investigated the structural states of three membrane proteins in matched-out carrier systems. Moreover, we investigated the exchange rate of lipids inside different nanodiscs at varying temperatures and concentrations. The SEC-SANS setup was further optimised in close collaboration with Anne Martel and Lionel Porcar.

Part one: structural states of membrane proteins

We had proposed to study the solution structures including conformations of highly disordered intracellular domains of the human growth hormone receptor (GHR), the sodium/proton exchanger 1 (NHE1), and Tissue Factor (TF) in complex with Factor VIIa, Factor Xa and the nematode anticoagulant protein c2 (NAPc2). Unfortunately, production of GHR and NHE1 samples, which is otherwise routine, was of low yield, meaning that samples of adequate quality for SANS could not be obtained. Instead, two other membrane proteins were investigated together with the TF complex; Spinach Aquaporin (SoPIP) and Gleobacter ligand-gated ion channel (GLIC).

SoPIP is a H_2O transporting aquaporin and was measured to examine its solution structure (Figure 1A). SoPIP was solubilised in matched-out deuterated DDM (dDDM) [1]. GLIC is a bacterial cation-selective ion channel. An open and a closed state had been proposed in the literature. We investigated a wild type (WT) construct at pH 7.5 which was expected to be in the closed form, and a mutant at pH 3.0, which was expected to be in an open form (Figure 1B), both solubilised in dDDM. The last protein was the TF complex involved in blood coagulation. This protein was solubilised in matched-out deuterated circularised and solubility-enhanced nanodiscs, developed in our group [2,3] (d-csND), made from biologically synthesised deuterated membrane scaffold protein produced at the D-lab at ILL and chemically synthesised deuterated POPC lipids produced at the National Deuteration Facility at ANSTO.

All proteins were measured in 100% D_2O (match-out conditions for dDDm and d-csND). TF and SoPIP were measured in a single 5.6m/5.6m setting (with detector offset), and GLIC was measured in 2m/2.8m (centered detector) as well as 11.2m/11.2m (detector offset) settings.

All proteins were measured first in SEC-SANS conditions, and peak fractions were collected and later measured in cuvette SANS mode to get better statistics at high-q and to measure the sample absorption independently to estimate the sample concentration better (data not included in this report). Examples of SEC-SANS data is shown in Figure 1.

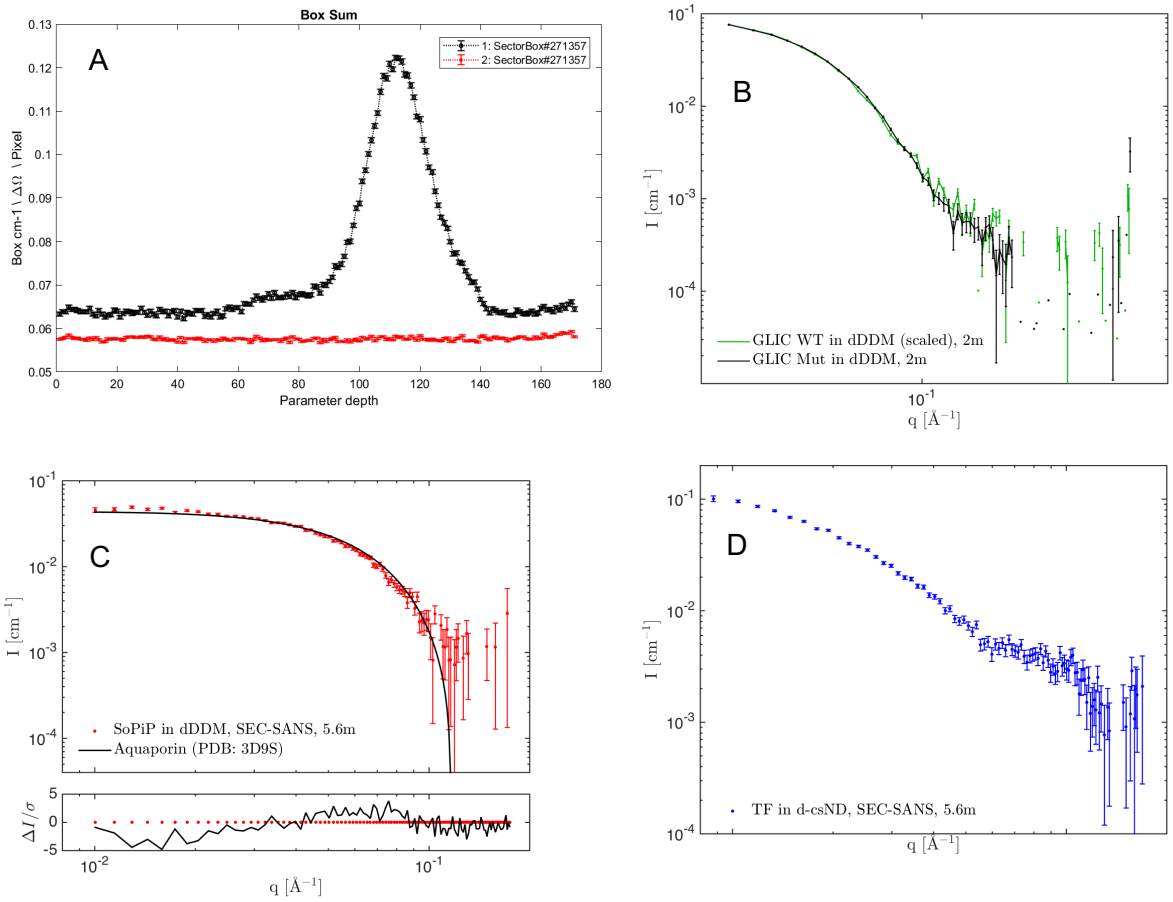

Figure 1. SEC-SANS data. (A) SANS scattergram, i.e. intensity integrated over a low-q range (black) and high-q range (red) for frames of GLIC WT in dDDM at 11m. (B) GLIC WT and GLIC Mut, both in dDDM. (C) SoPIP fitted with a model of aquaporin (PDB 3D9S). (D) TF in d-csND.

Part 2: lipid exchange in nanodiscs

We investigated the exchange of lipids between nanodiscs of different composition and sizes, at temperatures between 30 and 37 °C along with different concentrations at 37 °C. The experiment was performed by mixing nanodiscs with deuterated lipids (tail deuterated DMPC: d54-DMPC) and nanodisc with hydrogenated lipids (DMPC) at time 0. At full exchange, the mixed nanodiscs are contrast matched with the buffer (50% D2O). The low-q intensity is monitored over time to measure exchange rate (example showed in Figure 2).

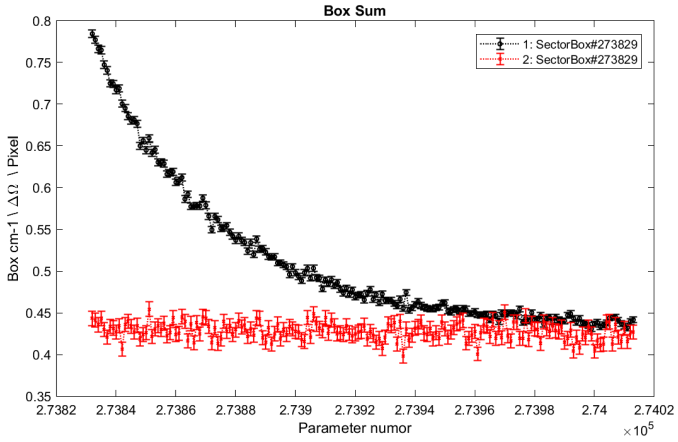

Figure 2. Lipid exchange experiment. Here for a nanodiscs with circularized solubility enhanced MSP1E3D1 and DMPC lipids measured at 37 C. The black data points are the low-q intensity and the red data points is high-q intensity. The data was visualised with GRASP during the experiment.

Notes on SEC-SANS

We discussed point of improvements with Anne Martel and Lionel Porcar. We highly appreciate the SEC-SANS setup and encourage time and effort to be put in improvement of both sample environment and collection software. It is a pleasure to see the developments on the hardware site, especially with the air-cooled cabinet around the HPLC. The HPLC is still very sensitive to small errors, such as tiny air bubbles or slight unintentional movements of parts in the autosampler. If the equipment continues to cause trouble for users, it should be considered to go for more typical FPLC systems used for protein purification, such as Äkta, Biorad etc.

Provisional conclusions

The SEC-SANS setup was used successfully to measure three challenging membrane protein systems in order to assess the solution structure. Moreover, the static SANS setup was used to measure the kinetics of lipids exchanging between nanodiscs. Static SANS was also used as supplement to the SEC-SANS measurements to enhance the statistics, in particular at high-q, for finer structural details. The SoPIP and TF data will be used to complement current structural modeling of these proteins inside nanodiscs. These projects are on a mature state with manuscripts in preparation. The GLIC data will serve a basis for MD simulations on the switch between open/closed conformations in this protein and serve to guide for which samples and conditions will be further measured at a SANS beamtime at D22 later this year. The nanodisc lipid exchange data obtained here was of superior quality compared to data obtained on similar samples at other neutron facilities. While this is of course very positive, we would also like to remeasure samples that have been measured elsewhere than D22. Thus, we will apply for 12 hrs of beamtime at D22 to conclude the nanodisc lipid exchange study.

References

[1] Midtgaard et al, FEBS J. 2018, 285, 357-371.
[2] Maric et al, Acta Cryst D, 70, 2014, 317-328.
[3] Johansen et al, FEBS J., 2019, 286, 1734-1751.