Experimental report

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Proposal: 8-03-940

Council: 4/2018

Title: SEC-SANS for probing the structureof membrane proteins in match-out deuterated carrier systems

Research area: Biology

This proposal is a new proposal

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Samples: Matchout deuterated MSP
CorA
SERCA
Proteorhodopsin
Matchout deuterated DDM
NHE1
Matchout deuterated POPC

Instrument	Requested days	Allocated days	From	To
D22	3	2	22/06/2018	24/06/2018

Abstract:

We propose to utilize the SEC-SANS setup at D22 to investigate the full potential of the match-out (MO) deuterated membrane protein carrier systems used in our group. Chemically synthesized deuterated de-tergents and lipids and in vivo deuterated membrane scaffolding proteins allow for obtaining scattering data on membrane proteins in both MO deuterated detergents and NDs. This both improves the infor-mation obtained on the membrane proteins and make the subsequent data analysis much easier. The newly developed SEC-SANS setup at D22 is a key factor in this approach, since the systems under investi-gation are highly delicate, and thus prone to form aggregates, which will be removed by the SEC-column. Furthermore, the SEC-SANS setup may provide valuable structural information about dimers or higher order oligomers which can only be separated by the SEC. In addition to a selection of model protein sys-tems, which serves to evaluate the method, the sodium-hydrogen antiporter, NHE1 will be measured. Structural information about NHE1 in solution is scarce, and high-quality SANS data on this protein in MO deuterated carriers would be of very high value to the field.

Experimental report

Proposal: 8-03-940
Instrument: D22
Local Contacts: Anne Martel, Lionel Porcar
Date: 22/6– 24/6, 2018

Scientific motivation

It is difficult to make samples of membrane proteins suited for SANS with the required concentration, homogeneity and stability. On two other occasions (proposals INTER-382 and 8-03-885), we have successfully utilized the new SEC-SANS setup at D22 to obtain excellent SANS data on such difficult samples. Aggregates were effectively separated from the desired protein, resulting in a monodisperse sample while SANS was measured. For the present experiment, we aimed for measuring a number of different membrane proteins in two different match-out deuterated carrier systems, DDM micelles (d-DDM) and phospholipid nanodiscs (d-ND), as well as some membrane proteins in standard nanodiscs (h-ND). The membrane proteins under investigation include a number of systems that have previously been measured with SANS and yielded poor quality data due to sample aggregation.

Experiment

Figure 1 shows example data sets obtained from SEC-SANS; three different membrane proteins in d-DDM (A), CorA in d-NDs (B), and two other membrane proteins in h-NDs (B). In general, samples were run with 0.3-0.5 ml/min until sample elution, upon where the flow rate was reduced to 0.05-0.1 ml/min. The data shown here are the average of frames selected from the peak of the elution profile.

For all samples, data with well-defined and flat Guinier-regions, indicating aggregation-free samples, could be obtained. This was even the case for the membrane protein SERCA in d-DDM, which precipitated within a few minutes after elution (data not shown). The good quality of the obtained data is here exemplified by CorA and AQP0, respectively, in d-DDM (Figure 1A, blue and red), as well as CorA in d-NDs and SoPip and TF-FVII, respectively, in h-NDs (Figure 1B). Even for the membrane protein GLIC, which had a low concentration before loaded on the column, the data is suited for Guinier analysis, which gives cause to continue studying this membrane protein and its structural rearrangements using SANS in future experiments (Figure 1A green). In general, the data statistics at low-q are very good, whereas at high-q, the data are useful up to around q = 0.2 (depending on the samples concentration). This could be a practical limit because of the columns that are typically used for sample purification (1 cm in diameter, 30 cm long), which have a maximum capacity and thereby limits the achievable sample concentration in the elution-peak.

It should be noted that, for the first time, we succeeded in obtaining SANS data on a membrane protein in a d-ND. The data of CorA in d-NDs (Figure 1B, blue) is of good quality, featuring a well-defined Guinier-region at low-q and several distinct features at medium-q. Data obtained with and without Mg^{2+} present will serve the basis for modeling the proposed structural rearrangements in CorA.

For nanodisc samples, a complementary SAXS contrast was obtained at BM29 at the ESRF after the beamtime at D22. One example is the human growth hormone receptor (GHR) that was measured both by SEC-SANS and SEC-SAXS. In both experiments, the elution profile featured a broad peak, possibly containing multiple species (data not shown). Here, it was extremely convenient to be able to take out frames from specific parts of the peak to yield good quality data. GHR in h-NDs is a very difficult sample that would not have yielded good quality data with standard cuvette/robot SANS/SAXS. The obtained SEC-SANS/SEC-SAXS data is currently being modelled to provide some of the first structural insights to the full-length GHR.

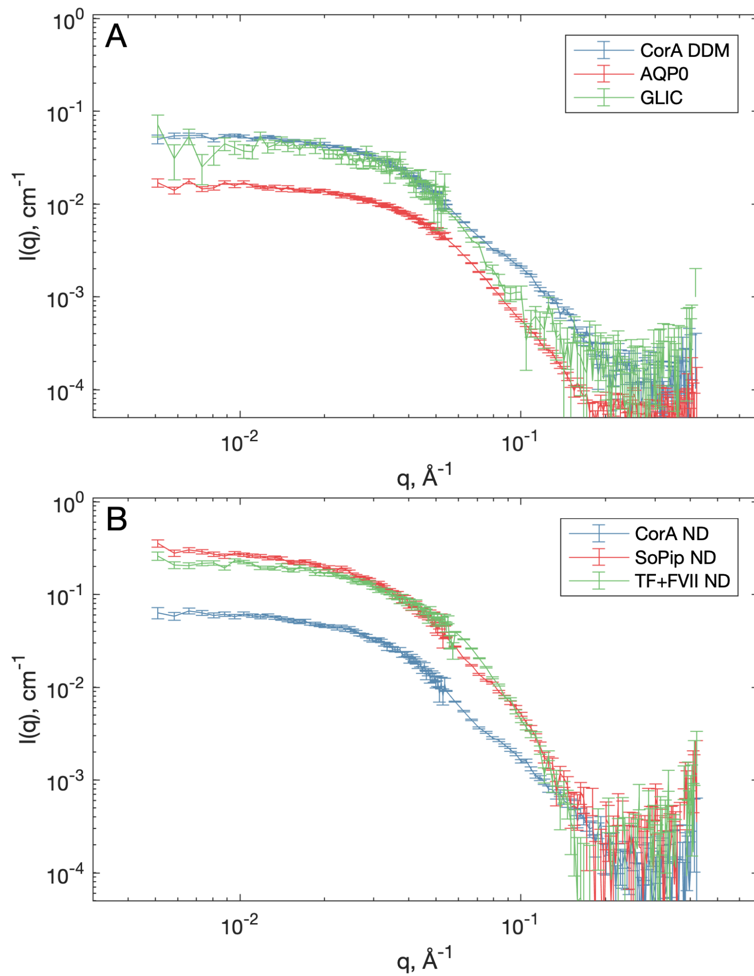

Figure 1: A: CorA, Aqp0 and GLIC in match-out deuterated DDM. B: CorA in match-out deuterated nanodisc. SoPip and TF (+FVIIa) in h-nanodiscs.

In conclusion, we had proposed a very optimistic sample list for this experiment but still ended up with mostly good data sets on all the investigated samples. This reflects that SEC-SANS is indeed advantageous for difficult samples. As the setup is still under development, we met a number of bugs that could be solved before future experiments. Some of these were discussed with the local contact during the beam time, and furthermore, we have had follow-up discussions since.