Experimental report

10/09/2018

Proposal: **INTER-382** **Council:** 4/2018

Title: SEC-SANS 2 col. option test

Research area:

This proposal is a new proposal

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Samples: Proteines recombinantes purifiées et lipides et détergents

Instrument	Requested days	Allocated days	From	To
D22	6	6	08/03/2018	12/03/2018

Abstract:

Experimental report

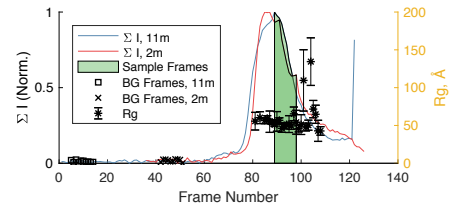
Proposal:	INTER-382
Instrument:	D22
Local Contacts:	Anne Martel, Lionel Porcar
Date:	8/3 – 9/3, 2018
Crew:	Nicolai Tidemand Johansen, Søren Roi Midtgaard

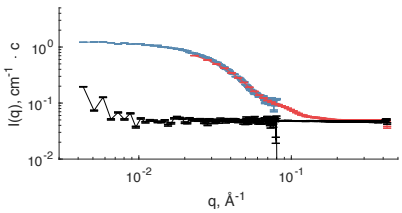
Aim

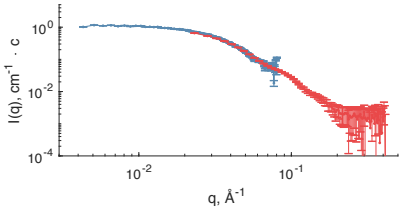
The two aims of this experiment were to: 1) test a new two-column option for the SEC-SANS setup at D22 and 2) obtain high quality SANS data on nanodisc and membrane protein samples.

Scientific motivation

High quality SANS data of completely monodisperse samples have been difficult to obtain compared to when performing highly complementary SAXS experiments. With SEC-SANS, this issue has been effectively solved, as we earlier demonstrated on a series of nanodisc samples (proposal 8-03-885). The SEC-SANS setup is still relatively new and undergoing continuous upgrading that will allow for more efficient data collection. One such upgrade is the introduction of extra valves to run two columns in parallel, whereby column equilibration can be done simultaneously on one column while eluting sample and collecting SANS data from the other column. Here, we set out to test the setup on a number of nanodisc and membrane protein samples, but we ran into unforeseen issues with hardware that meant that only a few complete data sets were obtained.





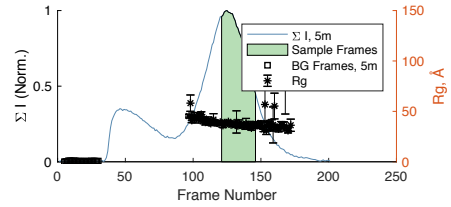


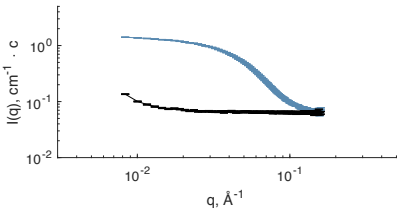
Experiment

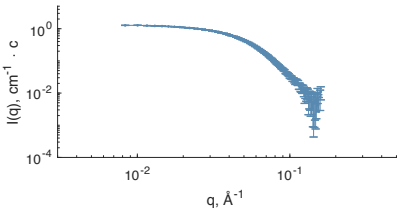
We have previously been measured the magnesium transporter CorA in match-out deuterated with static SANS and obtained data that indicated presence of dimers or other potential oligomeric states. This was not only surprising to us, but also hampered data analysis. Here, we measured CorA again with SEC-SANS and obtained promising data that showed that frames of monomeric CorA could be extracted (figure 1), which will make data analysis much simpler. Unfortunately, we experienced problems with high pressure on the HPLC during the night, which we were not able to solve, and we could not obtain data on CorA in other structural states. Thus, the data set remains incomplete for now.

Next, a few nanodisc samples were measured after fixing the pressure problem in the morning. Here, we wished to follow the radius of gyration (Rg) as a function of retention volume. The obtained data (figure 2) is shows a clear trend that the Rg is decreasing over the nanodisc peak. Further nanodisc samples were measured to compare the effect between different types of nanodiscs. As for CorA, a representative data set is

shown for the nanodisc sample, indicating no presence of aggregation as determined by the very nice Guinier-region. The data was complemented by SEC-SAXS data collected at BM29 at ESRF. Other types nanodisc samples will be studied in future SEC-SAS experiments to understand the relationship between structural dispersion and nanodisc size.







Overall, we are still impressed by the ability to obtain fully non-aggregated SANS data with good data statistics. It was unfortunate that we experienced trouble with the system pressure on the HPLC, which ultimately meant that we could not measure all of the proposed samples. Furthermore, it made it difficult to truly test the two-column system. One experience that we can share here is that, unlike in SEC-SAXS, where the two columns can be run at the same flow rate, the variable flow rates in SEC-SANS makes it difficult to overlap sample runs on the two columns. As such, we recommend users to use the two columns only for equilibration of one column into a new buffer while measuring SANS on the other column.

Further points for upgrades have been discussed on site with the D22 team. With more experience and having the pressure problem solved, we are looking forward to using the setup again.