Experimental report

15/09/2016

Proposal: 8-05-426 **Council:** 4/2016

Title: Dirty SANS: A feasibility study ofmembrane protein structure determination using cell-free protein synthesis: a new approach

Research area: Biology

This proposal is a new proposal

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Samples: Deuterated SDF1
Deuterated VDAC proteoliposome
Deuterated SDF1 with liposome

Instrument	Requested days	Allocated days	From	To
D22	2	1	02/09/2016	03/09/2016

Abstract:

We propose to carry out proof-of-principle measurements on instrument D22 in which we test the feasibility of analysing protein structures using SANS data recorded directly from the cell-free synthesis media in which they are produced. The advantage of being able to do this is the fact that it will avoid the need to purify - a major stumbling block afflicting this field. If successful, the novel deuteration approach involved will have wide application for membrane protein structure determination - an important area where structural information is in short supply.

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The samples measured during the beam-time on D22 were as follows:

1. **Partially purified deuterated SDF1 expressed by cell-free method**

   Samples used for experiment: i. Buffer at 42% D2O,
   ii. Partially purified SDF1 in 42% D2O

   Using the cell-free expression system, we have managed to express deuterated SDF1. The cell-free system offers an alternative to classical *in vivo* system whereby we can express *de novo* deuterated protein by supplementing the reaction with deuterated amino acids. We were able to obtain a signal for the partially purified protein, thereby matching out the residual contaminating hydrogenated protein. The signal was however quite low due to the low concentration of SDF1 present and its small size (10kDa). Despite being quite low, we could nevertheless confirm the signal that corresponds to the computationally estimated SAXS profile determined from FoXs. (http://modbase.compbio.ucsf.edu/foxs/about.html) (http://modbase.compbio.ucsf.edu/foxs/runs/1a15_32_16_9_2_8_116/).

2. **Partially purified Deuterated SDF1 added to Lysate**

   Samples used for experiment: i. Buffer at 42% D2O
   ii. Partially purified Deuterated SDF1 in 42% D2O
   iii. Partially purified D-SDF1 + 1% Lysate at 42% D2O
   iv. Partially purified D-SDF1 + 5% Lysate at 42% D2O
   v. Partially purified D-SDF1 + 10% Lysate at 42% D2O
   vi. Partially purified D-SDF1 + 20% Lysate at 42% D2O

   In order to simulate the in-situ expression of deuterated SDF1 from the cell-free reaction, we added deuterated SDF1 to increasing amounts of lysate. The samples comprised of SDF1 at the same concentration as in the purified SDF1 in (1), with lysate contamination at 1, 5, 10 and 20%. The samples had been dialysed extensively into 42% D2O to match out the hydrogenated contaminating protein. However, with the low signal obtained from (1), we could not however get a signal over the background even after long measurement times.

3. **Unpurified deuterated SDF1 produced in the cell-free system**

   Samples used for experiment: i. Buffer at 42% D2O
   ii. Control Lysate at 42% D2O
   iii. Unpurified D-SDF1 at 42% D2O
   iv. Unpurified D-SDF1 at 42% D2O, nuclease treated

   v. Buffer at 0% D2O
   vi. Control Lysate at 0% D2O
   vii. Unpurified D-SDF1 at 0% D2O
   viii. Unpurified D-SDF1 at 0% D2O, nuclease treated

ix. Buffer at 65% D2O
x. Control Lysate at 65% D2O
xi. Unpurified D-SDF1 at 65% D2O
xii. Unpurified D-SDF1 at 65% D2O, nuclease treated

xiii. Unpurified H-SDF1 at 42% D2O
xiv. Unpurified H-SDF1 at 42% D2O, nuclease treated

Deuterated SDF1 was produced in the cell-free system by the addition of deuterated amino acids in an overnight reaction. The samples were then correspondingly treated with nuclease to remove nucleic acid. The samples were then extensively dialysed against the corresponding buffer.

a. Verify the difference in signal obtained at different D2O concentrations

Apart from the *de novo* deuterated SDF1, the cell-free reaction however represents a large mix contaminating not only hydrogenated protein, but also nucleic acids and residual lipids. We therefore wished to verify whether there was any significant alteration to the signal at the different D2O concentrations which might arise due to these.

Overall, we could not see any difference in the signal. At 0%, 42% (approx. hydrogenated protein match-point) and 65% (approx. nucleic acid match-point) samples produced similar profiles and we could not differentiate the signal due to the contribution of protein-nucleic acid-lipid components. Similar profiles were observed between control lysate (only protein present) and cell-free reactions (protein and nucleic acids present). To further reinforce our confidence, we did not see any difference in the signal between nuclease treated and nuclease treated samples.

It was deemed that the signal contribution due to the protein component was far too strong, rendering the signal contribution from nucleic acid and residual lipid insignificant. This experiment therefore allows us to confirm that for future SANS experiments, the influence of nucleic acid and lipids is negligible, and contaminating proteins are the significant contributor to observed profile. The experiment indicates that following cell-free reaction further nuclease treatment is required, and that SANS measurements can be taken directly. We therefore have a baseline for the next SANS experiment where we would accurately define the match-point of cell-free reaction mix.

b. Obtain a signal for the unpurified deuterated expressed SDF1 in the cell-free system

Due to the very high contaminating background, and relatively low concentration of SDF1, we could not obtain a signal over the noise. We were however were expecting this result.

4. **Determine match-out point of liposome**

Samples used: i. 100% D2O Buffer
ii. Liposome at 0% D2O
iii. Liposome at 10% D2O
iv. Liposome at 42% D2O
v. Liposome at 65% D2O

Our main objective of the SANS experiments, is to be able to obtain structural insights of the membrane protein produced during cell-free reaction directly into liposomes as proteoliposomes. The liposome used is an in-house made component consisting of various lipids, including cholesterol. The liposome therefore represents an artificial mimic for the membrane in which inserts membrane proteins. We therefore were able to accurately determine the match-point of the liposome (10.7%). This information will allow us to plan subsequent SANS experiment in which deuterated membrane protein expressed by cell-free would be embedded.

5. **Verify the contribution of signal contribution Liposome with contaminating lysate**

Samples used: i. Lysate + Liposome at 42% D2O (protein match-out)
ii. Lysate + Liposome at 10.7% D2O (Liposome match-out)

With little beam-time remaining, we briefly wished to verify the whether we could observe a signal contribution due to the liposomes in the presence of the lysate. Here, we still need to properly analyse the data, but we anticipate that the signal contribution of hydrogenated protein overwhelms that of the liposome. Confirming no differences, would therefore indicate that it would be possible to directly obtain a signal of a deuterated membrane protein embedded in the liposome expressed by the cell-free reaction without the need of purification.

Concluding Remarks

The SANS data provided us with critical information for future experimental setups, as well as new perspectives on the project. We therefore intend to put forward a subsequent SANS proposal that would be based on this first series of SANS experiments.