Experimental report

26/01/2018

Proposal: 1-20-46 **Council:** 10/2016

Title: Development of a method for matchout labelling of DNA for SANS stuctural studies of DNA-protein complexes

Research area: Biology

This proposal is a new proposal

Main proposer: Benjamin BROCCO

Experimental team: Trevor FORSYTH
Juliette DEVOS

Local contacts: Anne MARTEL

Samples: Protein
DNA

Instrument	Requested days	Allocated days	From	To
D11	0	0		
D22	1	1	24/02/2017	25/02/2017
D33	0	0		

Abstract:

Here we propose to study DNA extracted from a range of deuterated cultures to identify culture conditions that result in matchout labelled DNA. Such information has been developed for proteins and is now very widely used. Matchout labelled DNA would be of major importance for the study of protein/nucleic acid complexes and will further broaden the application of SANS methods for the study of biological systems.

Proposal 1-20-46: Development of a method for matchout labelling of DNA for SANS structural studies of DNA-protein complexes

B.Brocco, T.forsyth, J.Devos, M.Haertlein

Local contact: Anne Martel

The success of neutron studies involving composite biomolecules, such as membrane proteins or nucleic acid protein complexes, can be limited by insufficient contrast between the components. Indeed, because of their difference in chemical composition, proteins, lipids and nucleic won't have the same scattering length density and therefore a natural contrast will exist. However, if one wants to further improve the contrast to maximize the signal and optimize the phase differentiation during modelling, the deuteration of one of the component might be required. However, for nucleic acid bound proteins, and especially membrane proteins, the reconstitution of the complex is not necessarily possible. We therefore propose a method that allows for the selective labelling of only one of the biomolecules *in vivo* while keeping the other unlabeled.

A first step to achieve selective deuteration is to have a full understanding of the relation between culture conditions and deuteration levels. Previous studies have established this in proteins and lipids, using Small Angle Neutron Scattering. We further showed it is possible to produce, simultaneously and *in vivo*, lipids that are fully deuterated together with protein only deuterated at 22%. This selective deuteration labeling strategy therefore allow to increase the contrast between biomolecules and could serve as a new tool as a new tool for producing optimized samples for Small Angle Neutron Scattering.

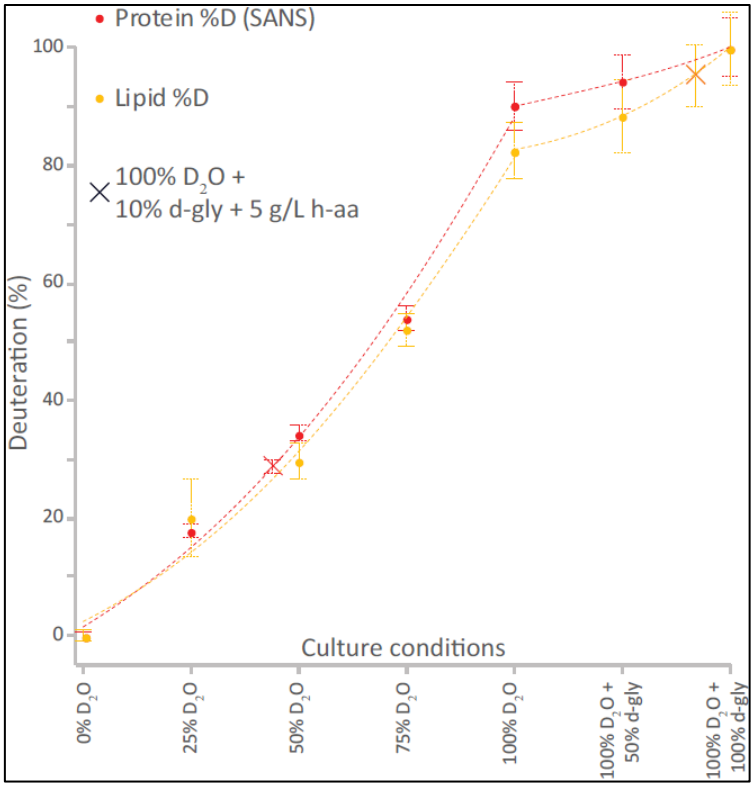

Figure 1 : Relation between culture condition and deuteration level of proteins and lipids. The crosses represent the condition with fully deuterated medium supplemented in hydrogenated amino acids. The supplementation with unlabeled amino acid greatly reduces the deuteration level of proteins while barely affecting lipids

The aim of this experiment is to extend the analysis to the third class of biomolecule : nucleic acids.

We cultivated *E.coli* in different deuteration regimes :

- 0% D_2O
- 25% D_2O
- 50% D_2O
- 75% D_2O
- 100% D_2O
- 100% D_2O + 50% d-glycerol
- 100% D_2O + 100% d-glycerol
- 100% D_2O + 100% d-glycerol + 5g/L of hydrogenated amino

For each condition, the nucleic acids were prepared by phenol chloroform extraction and resuspended in 0, 20, 40, 60, 80, 100% deuterated buffer for contrast variation experiment. The solubilization was assisted by sonication and heating. The sample, together with the buffers, where measured at 6 Å, with a detector distance of 2 and 8 meters.

Unfortunately, regardless of the sample, solvent and detector distance, the single stranded nucleic acid did not give any signal.

The main reason is probably the low concentration achieved, sub 1mg/ml, due to the preparation protocol which involves extensive drying step so no contamination by hydrogenated solvent could bias the results. Incidentally, the dried sample was very hard to resolubilize (despite the use of sonication and heating) and the sample had to be diluted to be homogenized. The contrast variation series were prepared by dilution from a stock solution (to ensure identical concentration), further reducing the concentration of the sample.

Another factor could be that, because of the elongated single stranded nature of the sample, the form factor is relatively low. Incidentally, interference is less likely to happen as compared to a globular protein or lipid vesicle, therefore leading to lower signal.

Improving the preparation protocol will be necessary before repeating the experiment. The main improvement would have to be done on the sample concentration and it is therefore required to improve the solubilization protocol. Extended solubilization times (several days at 4°C), together with optimizations of buffer's pH and salt concentration, could help increasing the solubility of the nucleic acid and therefore achieve higher final sample concentration. As for the form factor, the only option is to keep the nucleic acid in double stranded form to increase the likelihood of interference event.