The Structure of Membrane Proteins from Small Angle Neutron Scattering: The Purple Bacterial Reaction Centre

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Introduction.

Most small angle scattering (SAS) studies are carried out on soluble proteins using Synchrotron X-rays. However the requirement of membrane proteins (MPs) for a lipid environment, usually a lipid vesicle, leads to the vesicle dominating the scattering over that of any inserted protein. This is therefore a natural case where the contrast variation made possible by neutron scattering provides significant advantages. By using deuterated solvent and lipids it is possible to substantially remove the vesicle component from the scattering pattern so that the protein structure can be resolved. The reconstruction tools that have been applied with such success to proteins in solution can then be applied to generate a low resolution structure of the protein. A number of groups have described experiments in which the membrane (or surfactant) component of a protein:lipid complex have been matched out (Hunt et al., J Mol Biol, 273:1004, Bu et al, J Mol Biol, 332:23, Zimmer et al, Biophys J, 90:1752) . However these experiments remain challenging and the optimal experimental approach is not clear.

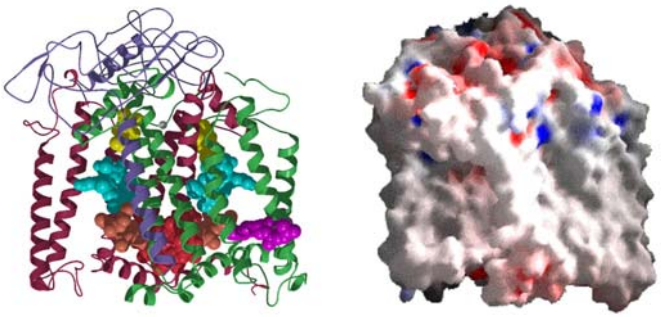

Figure 1. The reaction centre from Rb. sphaeroides. (a) X-ray crystal structure and (b) surface potential.

Our ultimate target is to develop and optimise both sample preparation and data analysis procedures so as to develop a generic methodology that can be applied to as wide a range of MPs as possible. Our first goal is to demonstrate proof of principle and optimise the contrast matching protocol to reduce the vesicle scattering as far as is possible. Therefore, we have selected as a model system one of the few MPs that has had its structure determined by crystallography, the photosynthetic reaction centre from the purple bacterium *Rhodobacter sphaeroides* (pbRC, Figure 1). The prBC has many advantages as a model system. It can be readily produced on a (reasonably) large scale, it can be reconstituted into a range of lipid environments, a range of structural variants can be prepared, and it shows a high degree of structural stability particularly when in a lipid environment . Most importantly there are significant structural questions remaining which have the potential to be tackled by SANS, including whether the pbRC dimerises when incorporated into an artificial bilayer.

Experimental Strategy

There are a wide range of experimental parameters to be explored both in terms of sample preparation and data collection.

Contrast Matching: The key first step is to identify the optimal lipid and solvent composition to reduce scattering from the lipid vesicles to the minimum possible while maximising the scattering signal from the embedded protein. Our first experiment (scheduled for April/May) will investigate a range of lipid compositions based on fully deuterated, tail-D, headgroup-D and fully hydrogenated lipid for optimally matching out vesicle scattering. An alternate approach is to use fully (or predominantly) H-lipids matched in ~15% D_2O. This reduces the contrast between protein and solvent but makes a wider range of lipid compositions available. This approach could also be appropriate if deuterated protein were available. We will investigate the full range of options for vesicle and solvent compositions to identify the optimal approach.

Data Collection: An important question for both data treatment and analysis is the importance of explicitly accounting for vesicle scattering through subtraction of an experimental or fitted vesicle scattering 'background'. It may for instance be more effective to allow sufficient residual scattering from the vesicle to allow the fitting of a vesicle model which can then be subtracted from the experimental data. Accordingly we will investigate the contribution of the vesicle scattering to the overall scattering curve by obtaining 'off-match' data over a wider angular range (i.e. requiring at least two detector positions – the other experiments can be carried out at a single sample to detector distance). The use of the pbRC is ideal here as its good thermal stability means that the samples will stable enough to allow measurements at multiple detector positions, and also we will be able to use DPPC vesicles (gel transition temperature 42° C) which are significantly more stable than DMPC vesicles.

Interference effects: Hunt et al. (see above) have described a protein:lipid ratio-dependant artefact at low Q which they ascribe to protein-dependent interparticle interactions. The effect can be controlled by obtaining data at a range of protein:lipid ratios and using only data in the Q-range which does not show this effect. We will investigate whether the same effect is seen for the pbRC and if so whether its cause can be understood and modelled. In this context the question of whether pbRC behaves as a monomer or dimer in this system will also be investigated.

Data Analysis

The ultimate aim of this experiment is to demonstrate the use of the ATSAS package to correctly reconstruct a low resolution structure of the pbRC. The key to this is the successful subtraction of any residual vesicle scatter from the experimental data. We will investigate simple subtraction of the scattering from protein-free vesicles from the data for the proteoliposomes as well as extrapolation of the scattering pattern from 'off match' data (e.g. 90% and 100% D_2O) to the exact match point and subtraction of a fitted vesicle scattering profile.

The need for neutrons and D22: This experiment is absolutely dependent on the potential for contrast variation provided by neutron scattering. The combination of high flux and wide simultaneous Q-range on D22 makes it ideally suited for obtaining the high quality data required for experiments of this type.