Experimental report

06/09/2016

Proposal: 8-02-732 **Council:** 10/2014

Title: Combined SANS & NMR studies of seven transmembrane helical proteins in native-like membrane mimetic environment using isotope labelling

Research area: Biology

This proposal is a new proposal

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Experimental team: Juan Francisco BADA

Local contacts: Ralf SCHWEINS

Samples: Bacteriorhodopsin lipodisqs

Instrument	Requested days	Allocated days	From	To
D11	1	1	24/06/2015	25/06/2015

Abstract:

Structure determination of membrane proteins is challenging, accounting for <1% of the unique protein structures deposited in the PDB. In addition, most of these structures were not obtained in a native-like environment, adding doubt to their physiological relevance. Recently, a novel membrane mimetic, the lipodisq, has been developed that could enable the study of integral membrane proteins in a native-like bilayer environment by both NMR and SANS methods. We propose to insert a seven transmembrane helical receptor, bacteriorhodopsin, into lipodisqs and acquire high-resolution solution NMR spectra and low resolution SANS measurements of bacteriorhodopsin while in a 'stealth' carrier. If this is successful it will pave the way for many more structures of membrane proteins in native-like environments to be solved. Here we request beamtime to conduct SANS contrast variation experiments at ILL on the lipodisq-protein complex, which could provide novel and complementary data on the structure and oligomeric state of bacteriorhodopsin while in the novel lipodisqs.

Experimental Report
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Feasibility study using a combined NMR and SANS approach to study seven transmembrane helical proteins in a novel native-like membrane mimetic environment

Aims:

1) To express mg quantities of isotopically labeled (^{2}H, ^{13}C, ^{15}N) bacteriorhodopsin protein (bR) in *Halobacterium salinarum* (strain S9) cells (native source) for Nuclear Magnetic Resonance (NMR) spectroscopy and Small Angle Neutron Scattering (SANS) experiments (to be carried out in Deuteration Laboratory at ILL)
2) To acquire high-resolution solid state NMR spectra (enhanced by Dynamic Nuclear Polarisation) of isotopically labeled bR.
3) To reconstitute labeled bR into lipid nanoparticles for SANS in the presence of both deuterated and hydrogenated lipid, to determine particle structure.

Results:

Figure 1 shows the growth of *H. salinarum* cells expressing labeled bR in different growth media.

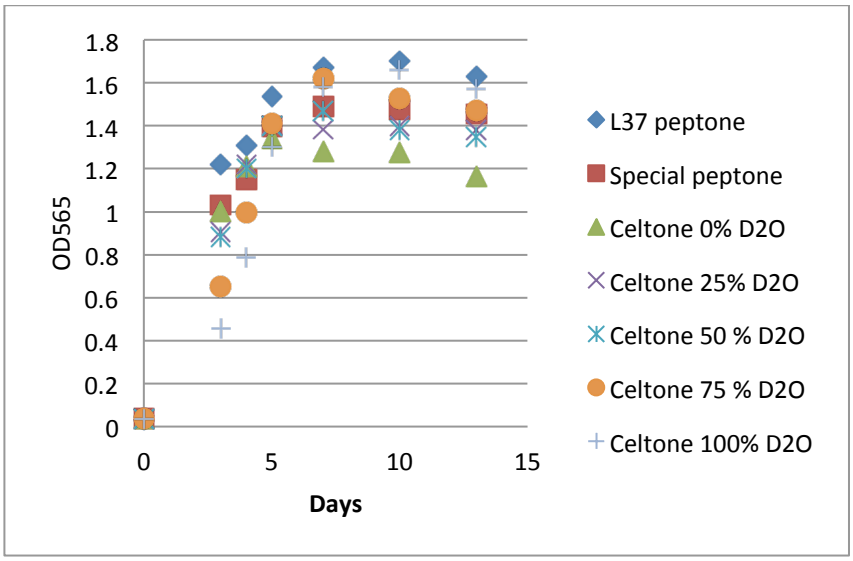

Figure 1: Growth of *H. salinarum* cells in different media and deuteration conditions as followed by optical density measurements at 565 nm.

Figure 2 shows an SDS PAGE electrophoresis gel of the various stages of bR purification, showing the purification of bR, as indicated by a band in the gel at 30 kDa (expected molecular weight for the non-labeled (natural abundance) protein is 27 kDa).

Cross-polarisation Magic Angle Spinning ssNMR spectra of labeled bR were successfully acquired (Figure 3) at a magnetic field strength of 6.6 T. The sample was prepared with 15mM AMUPOL (a stable radical species) and the NMR signal intensity was enhanced by Dynamic Nuclear Polarisation with a 187 GHz microwave source.

SANS experiments were performed on the D11 beamline at ILL. Samples subjected to SANS included bR reconstituted into lipid nanoparticles, prepared using either hydrogenated of chain-deuterated dimiyristyolphoshatidylcholine (DMPC). Figure 4 shows typical SANS data, modeled using SASview software.

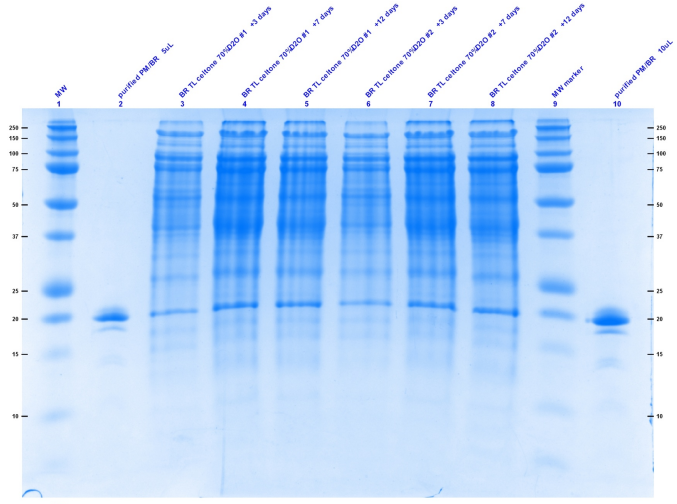

Figure 2: SDS PAGE electrophoresis gel, showing the various stages of bR purification. The intense band at 30 kDa corresponds to isotopically labeled bR.

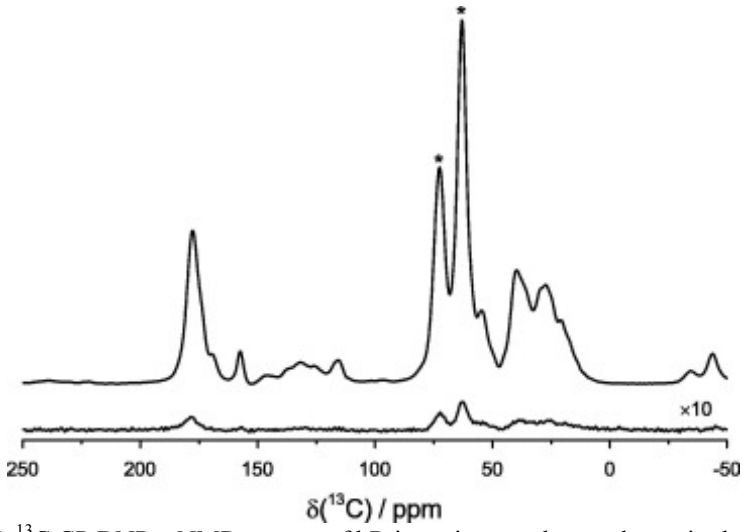

Figure 3: $^{1}H–^{13}C$ CP DNP ssNMR spectra of bR in native purple membrane in the presence of AMUPOL radical at 90 K. The unenhanced spectrum (lower) has been multiplied by 10 to allow better comparison. The two stars (*) denote glycerol peaks from the buffer. Both spectra were acquired with 256 co-added transients and a 3 s recycle delay. The enhancement achieved is approximately 120-fold. The sample temperature was 9 K. (Figure reproduced from Kemp *et al.* (2016).)

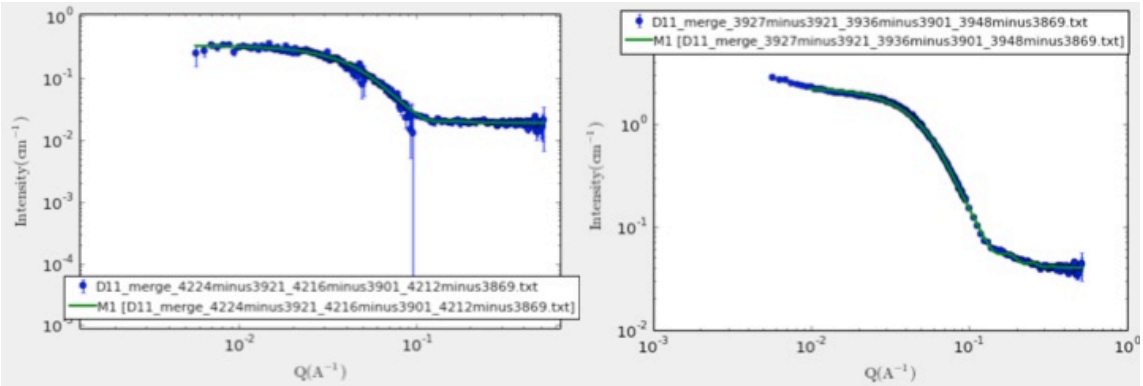

Figure 4: SANS scattering curves for SMA-1H-DMPC LQ in H_2O (left) and SMA-1H-DMPC-2H-bR(PM) (right). Data points are shown in blue (with error bars) and fitting curves are shown in green. ($\chi 2$ values for fits are 0.77 and 3.88 for A and B respectively.)

Current status:

We are in the final stages of analyzing the SANS data and are preparing a publication which includes this data. ssNMR data have already been published (*Journal of Magnetic Resonance*) and protein expression and purification data have been presented in a poster at the European Conference on Neutron Scattering.

Publications:

The work funded by this proposal has generated data included in the following publication:

Kemp TF, Dannatt HRW, Barrow NS, Watts A, Brown SP, Newton ME, Dupree R (2016) Dynamic Nuclear Polarization enhanced NMR at 187 GHz/284 MHz using an Extended Interaction Klystron Amplifier *J. Magnetic Resonance* 265 77–82

A further publication is currently in preparation.

Poster:

Data generated in this proposal was presented in the following poster at the European Conference on Neutron Scattering conference in Zaragoza, Spain (September 2015).

Bada JF, Taylor GF, Dannatt HRW, Judge PJ, Devos J, Schweins R, Haertlein M, Forsyth VT, Watts A (2015) Combined NMR and SANS solution studies of membrane proteins in a native-like membrane mimetic environment using multiple isotope labeling.