Experimental report

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Proposal: 8-03-831 **Council:** 10/2014

Title: Structure of a biological trans-membrane electron conduit.

Research area: Biology

This proposal is a new proposal

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Samples: MtrCAB

Instrument	Requested days	Allocated days	From	To
D22	2	2	22/07/2015	24/07/2015

Abstract:

Many bacteria are able to respire using solid minerals and metals as electron acceptors in the nabsence of oxygen or other diffusible substrates. In Shewanella oneidensis this respiration requires a porin-cytochrome complex that assembles in the outer membrane. This complex is composed of a trans-membrane porin MtrB, a periplasmic deca-haem cytochrome MtrA and a surface exposed outer membrane cytochrome MtrC. We intend to obtain information on the shape and size of the MtrCAB complex, which will inform on the orientation of exposed MtrC as well as whether MtrA either fully or partially enters MtrB, thus providing evidence for MtrB functioning as a sheath for MtrA. We have already collected data on SANS2D at the UK ISIS facilty using MtrCAB in different concentrations of deutereated buffer and have prepared samples in deuterated detergents ready for analysis. By collecting a full set of scattering measurements we hope to be able to generate a molecular envelope of the full MtrCAB cytochrome.

Structure of a biological trans-membrane electron conduit.

Instrument :D22
Users: Tom Clarke, Marcus Edwards, Gaye White
Local contact: Anne Martel.

Background.

The MtrCAB protein complex is a transmembrane electron conduit that allows electron transfer across the bacterial outer membrane. This ability to transfer electrons to the cell surface allows bacteria such as *Shewanella oneidensis* to respire on solid extracellular substrates such as iron and manganese oxides.
The molecular structure of the complex is not known, but has been shown to consist of a transmembrane porin MtrB that controls access to two cytochromes MtrC and MtrA from opposite sides of the membrane.
Understanding the structure of this complex would help to inform a number of important questions, such as (1) how MtrC can interact with solid mineral surfaces. (2) What is the orientation of the elliptical MtrC on MtrB (3) To what extent does MtrA insert into MtrB?

Experimental procedure

In order to obtain structural information for the complex we utilized small angle neutron scattering on instrument D22 at ILL to study the MtrCAB complex. We isolated MtrCAB from two different strains of *Shewanella*, *Shewanella oneidensis* MR-1 and *Shewanella baltica OS185.* The complexes were purified to homogeneity and exchanged into a buffer containing either a 60:40 deuterated/non-deuterated fos-choline mix (*S. oneidensis* MtrCAB) or a solely non-deuterated fos-choline (*S. baltica* MtrCAB). In order to minimise the contribution of the detergent micelles to the scattering, the D2O match point was determined.

Match points for deuterated and non-deuterated fos-choline.

Solutions of h-fos-choline and *d/h*-fos-choline micelles (0.5%) were prepared in buffer containing increasing concentrations of D_2O and the scattering intensity measured. Data was fitted to linear curves in order to measure D_2O concentrations where the scattering from micelles would be minimal. These match points were measured as 15 % and 65 % for fos-choline and *d/h*-fos choline respectively (Figure 1).

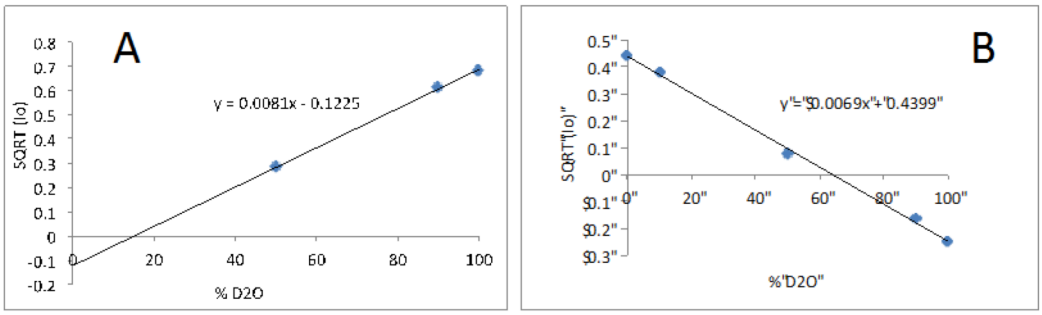

Figure 1: Change in scattering intensity with D2O concentration for (**A**) 0.5% Fos-choline (**B**) 0.5% Deuterated/Non-deuterated fos-choline mix.

SANS analysis of S. oneidensis MR-1 MtrCAB complex solublised in deuterated/non-deuterated fos-choline mix

Concentrations of 2.5, 5 and 10 mg mL^{-1} *S. oneidensis* MtrCAB solublised in 60:40 *D/H*-fos choline were dialysed into buffer containing 65 % D_2O. Data were collected for protein and buffer samples at three detector distances (2/5.6/17.6m) before being merged, reduced and buffer subtracted on site.
Analysis of reduced 1D scattering curves revealed that there was likely to be some precipitation or other aggregation in the sample, most likely caused by the stability of *S. oneidensis* MtrCAB in 65 % D_2O. Plotting ln(I) vs S^2 gave Guinier plots that showed non-linear variation at low S, consistent with aggregation (Figure 2). It was also observed during scaling that there was a large mismatch between protein and buffer samples, likely to be due to aggregation/precipitation preventing efficient dialysis. This meant the data could not be reliably baseline subtracted.

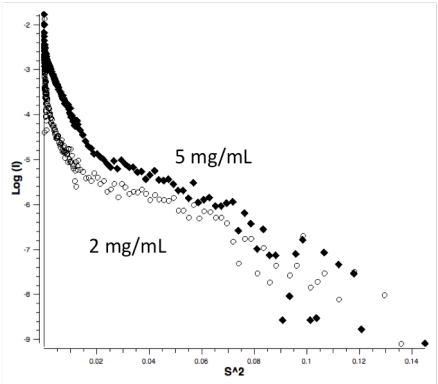

Figure 2: Guinier plot of *S. oneidensis MtrCAB* in 65 % D_2O shows evidence of aggregation at low Q.

SANS analysis of S. baltica MtrCAB solublised in fos-choline.

S. baltica MtrCAB in 2.5, 5 and 10 mg ml^{-1} in 0.1% fos-choline (non-deuterated) were dialysed into buffer containing 15 % D_2O. Data were collected for protein and buffer samples at three detector distances (2/5.6/17.6m) before being merged, reduced and buffer subtracted on site. (Fig 3A).

Unlike the MtrCAB from *S. oneidensis* the *S. baltica* MtrCAB did not show signs of aggregation over the range of 2.5 to 10 mg mL^{-1} tested (Figure 3B/C). A Guinier analysis gave R_g values of 55 -62 Å.

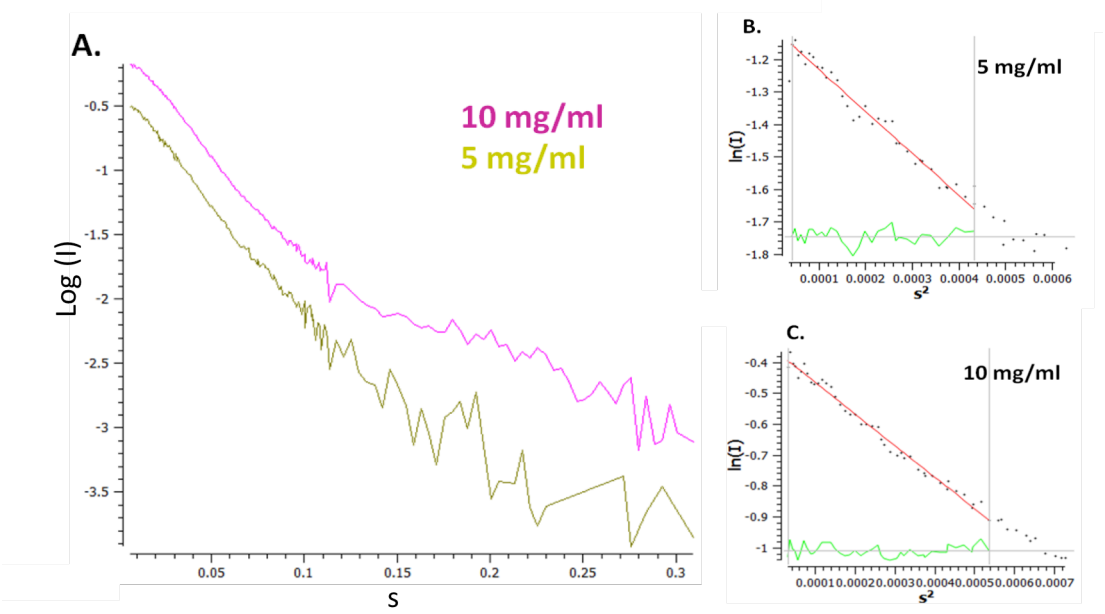

Figure 3: **A**. Scattering plots from 5/10 mg/ml samples of MtrCAB from *S.baltica* OS185. **B/C.** Guinier plot/analysis for 5 mg/ml (B) and 10 mg/ml samples

Generation of a transmembrane molecular envelope from *S. baltica MtrCAB.*

A P(r) plot was produced from the 10 mg/ml data with a Dmax of 220 Å (Fig 4 A). 24 models were produced using the modelling program GASBOR. These models were subsequently aligned and clustered and a representative model with a good fit to the experimental data was selected (Fig 4 B/C). The model was consistent with the expected dimensions and shape of the MtrCAB complex, and suggested that (1) MtrC was exposed on the surface of the complex, (2) MtrC was raised end-on on the MtrB complex and (3) that the MtrA cytochrome was buried deeply into the MtrB porin. Further experiments to differentiate the MtrB and MtrC components will be necessary before publication.

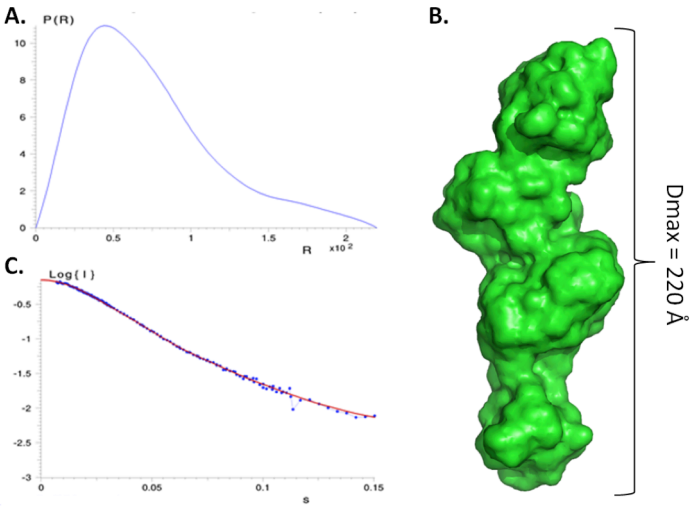

Figure 4. **A.** P(r) curve for the 10 mg/ml sample with a Dmax of 220 Å. **B.** Representative model produced using GASBOR. **C.** Fit of the model to the experimental data