Experimental report

29/07/2021

Proposal: 8-03-986 **Council:** 4/2019

Title: Structural characterization of theself assembly of biological Nanowires.

Research area: Biology

This proposal is a new proposal

Main proposer: **Thomas CLARKE**

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Samuel PIPER

Local contacts: Anne MARTEL

Samples: 20-heme porin cytochrome complex

Instrument	Requested days	Allocated days	From	To
D22	2	2	21/01/2020	23/01/2020

Abstract:

Recently SANS at the ILL was used to generate the molecular envelope of a transmembrane 20 heme cytochrome complex. We have generated new forms of this complex by recombining the component proteins and added new ligands close to potential heme sites. We now wish to use small angle neutron scattering 2 to generate scattering curves of the new complexes in Fos-choline at the deuterium match point of 15 %. These scattering curves will help to determine how the complexes assembled in vitro differ from the native form.

Small-Angle-Neutron Scattering (SANS) to Determine the Assembly of a Labelled Nanowire.

Instrument :D22
Users: Tom Clarke, Julea Butt, Samuel Piper, Marcus Edwards
Local contact: Anne Martel.

Background:
The process of mineral respiration has important environmental impact in controlling the availability of biologically essential elements such as iron and manganese, as well as toxic elements such as uranium and arsenic. Under anaerobic conditions mineral respiring organism of the genus Shewanella transfer electrons to a ~ 195 kDa outer membrane porin-cytochrome complex known as the Mtr complex. This consists of a 37 kDa periplasmic cytochrome MtrA, an 85 kDa transmembrane porin MtrB and a 75 kDa extracellular decahaem cytochrome MtrC that is associated with the outer membrane.
We aim to understand how the Mtr complex is assembled, and whether it is possible to assemble the complex *in vitro* by combining MtrAB and soluble MtrC separately. This would allow chemical modification and assembly of MtrC prior to incorporation into the complex. For example, site directed mutagenesis of MtrC has been used to attach the photosensitiser RuII(2,2'-bipyridine)3 (Ru-Me) close to the heme termini of MtrC and when irradiated, electrons can flow from a sacrificial electron donor into the Mtr complex,but it is not known whether this reassembled complex contains a electron transfer pathway similar to the isolated one.In this proposal we intended to use SANS to generate scattering curves of Mtr complex *in vitro* and compare with scattering curves obtained for the Mtr complex assembled *in vivo*. complexes.

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 Mtr complex. SANS measurements were made on samples of wild type Mtr complex (WT MtrCAB), as well as Mtr complexes assembled *in vitro* using soluble MtrC (MtrC-SII: MtrAB) and Ru-Me conjugated MtrC (RuMe-MtrC:MtrAB). All complexes were purified using gel filtration prior to analysis. Solutions of each of these complexes were prepared at 6.3 and 3.1 mg/mL in HNF Buffer with 13% D_2O. Previous experiments had established that 13% D_2O was the match point for Fos-choline 12 micelles. Scattering curves for the samples were obtained on the D22 instrument at Institut Laue-Langevin (ILL) at three detector distances. These profiles were found to scale linearly with protein concentration indicating that concentration dependant aggregation was not occurring. The background signal from the buffer was subtracted and the profiles were merged to give scattering curves covering Q = 0.006 to Q = 0.3 (figure 1A).

Data analysis
Scattering curves from the three Mtr complexes were compared to the MtrCAB and MtrAB complexes obtained previously.
The scattering curves shown in fig 1A were then loaded into ATSAS 3.0.1 software where Guinier plots were generated by plotting the natural log of the scattering intensity against Q^2 (Fig 1B). Scattering curves in this form should be linear at low values of Q^2 ($\leq$0.002 $Å^{-2}$). The slope of these lines can be used to approximate the radius of gyration (R_g) of the species which is the root-mean-square distance to the centre of mass for all atoms in the species. Continuing in ATSAS, Kratky plots were produced for the five complexes (fig 1C). Plotting the curves in this way allows the global flexibility of the protein structures to be measured. Globular proteins with minimal flexibility show a bell-shaped peak in a Kratky plot and the curve will smoothly converge to the x-axis. This is seen for MtrAB and the previously studied MtrCAB. The protein complexes used in this study converge to the x-axis at higher Q angles, indicative of structures with multiple protein domains. The reason for this difference is unclear and may

be a result of slight differences in buffer subtraction. Crucially the Kratky plots of all five protein complexes converge to the x-axis.

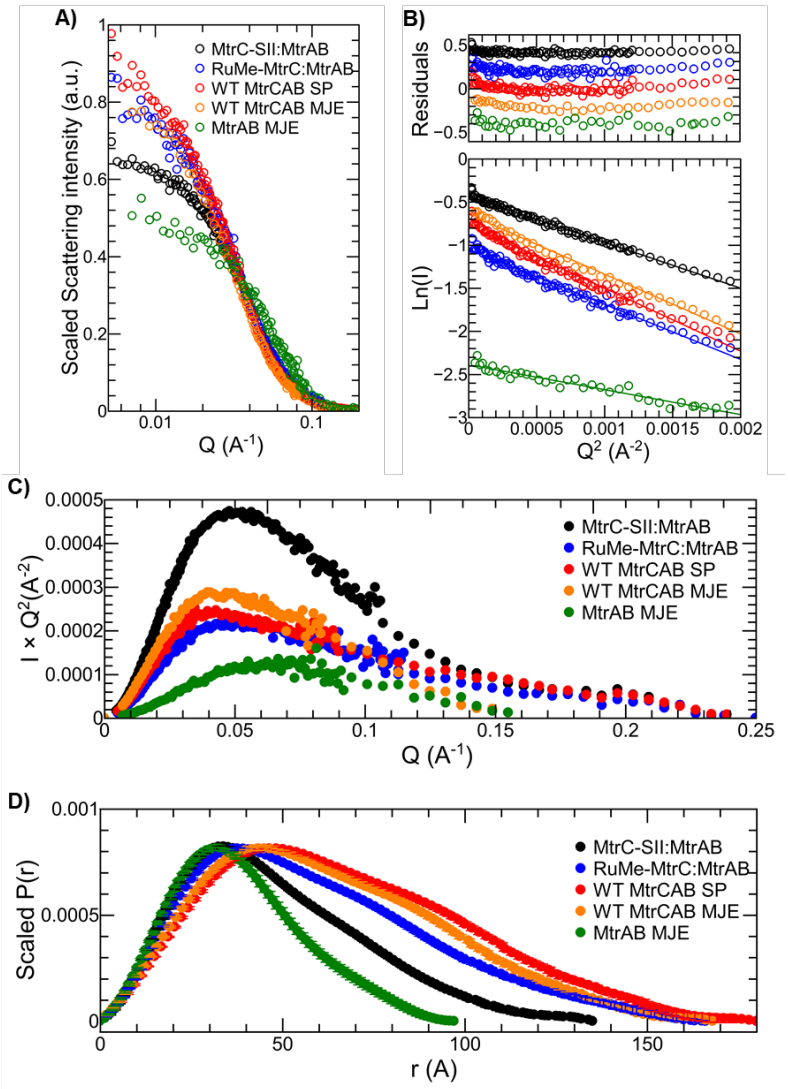

Figure 1 - SANS curves of Mtr protein complexes: MtrC-SII: MtrAB (black); RuMe-MtrC:MtrAB (blue); WT MtrCAB measured in this work (Red); WT MtrCAB (orange) and MtrAB (green) measured on a previous occasion and reported in Edwards et al. 2018. Scattering curves produced by merging profiles from different detector distances, data has been scaled to be equal at Q=0.03 A-1; B) Guinier region of the scattering curves are shown in the lower panel, lines are linear fits to the data and residuals are shown in the upper panel where they have been staggered by 0.2 to aid distinction; C) Kratky plots of the scattering curves; D) P(r) curves produced from the scattering curves, curves have been scaled to have equal maxima.

Scattering curves produced by merging profiles from different detector distances, data has been scaled to be equal at Q=0.03 A-1; B) Guinier region of the scattering curves are shown in the lower panel, lines are linear fits to the data and residuals are shown in the upper panel where they have been staggered by 0.2 to aid distinction; C) Kratky plots of the scattering curves; D) P(r) curves produced from the scattering curves, curves have been scaled to have equal maxima. Finally, P(r) curves were generated (Fig 1d). These curves represent the distribution of interatomic distances in the complexes. From these curves we can derive the maximum dimensions of the complex (D_{max}) and reobtain the R_g which should be similar to the approximated value. These parameters reveal that WT MtrCAB and RuMe-MtrC:MtrAB have similar overall dimensions whereas the MtrC-SII:MtrAB sample has a significantly lower D_{max}

and Rg. These initial results provided from simple data manipulations support the initial hypothesis that RuMe-MtrC:MtrAB resembles WT MtrCAB in its global structure. Given this, it is unusual that MtrC-SII:MtrAB appears to have different structural properties. MtrC-SII more closely resembles WT MtrC than RuMe-MtrC and would therefore be expected to form a complex that is more WT in nature. Further investigation revealed MtrC-SII:MtrAB to be contaminated with excess MtrAB.
For the remaining four samples, models of the possible protein structure were produced using DAMMIN software5 as part of the ATSAS online suite of tools. Such Models were generated for RuMe-MtrC:MtrAB, WT MtrCAB SP+MJE, and MtrAB MJE (Fig 2). The models produced were then aligned to the structure of WT MtrCAB or MtrAB, also provided by Dr. Edwards, using SUPCOMB software as part of the ATSAS package. The aligned structures (Fig 2) show that the DAMMIN models of RuMe-MtrC:MtrAB and WT MtrCAB fit well with the MtrCAB structure, having similar dimensions and good overlap.

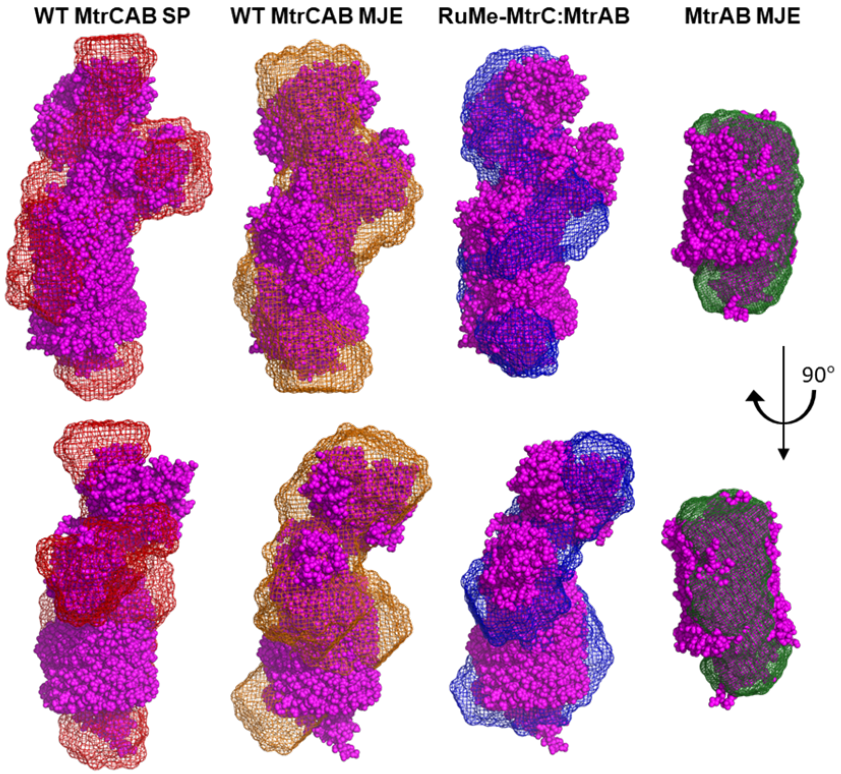

Figure 4.3.2. 4 DAMMIN envelopes shown as surface mesh and aligned to the structure of MtrCAB (PDB ID 6R2Q) or MtrAB using SUPCOMB. Mtr(C)AB is shown as purple spheres. DAMMIN models coloured as previously. The aligned structures are shown in two orientations as indicated by the arrow.

Conclusions.
Using SANS we were able to demonstrate that Ru-Me labelled MtrC is capable of associating to MtrAB in an position that closely resembles the in vivo assembled Mtr complex. This work has now been accepted for publication in the following journal.

Bespoke Biomolecular Wires for Transmembrane Electron Transfer: Spontaneous Assembly of a Functionalized Multiheme Electron Conduits (2021)
Samuel E. H. Piper, Marcus J.Edwards, Jessica H. van Wonderen1, Carla Casadevall, Anne Martel, Lars J. C.Jeuken, Erwin Reisner, Thomas A.Clarke and Julea N. Butt
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