Data Collection Strategies for the Radiation Sensitive, Ferric Iron Binding Protein, FutA.

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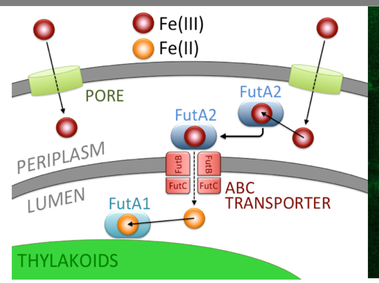
Background

FutA proteins are iron binding proteins that are typically found as two homologues in cyanobacterium; FutA2 is a periplasmic ferric iron binding protein associated with the Fut ABC transporter which mediates iron uptake, whilst FutA1 is a cytoplasmic ferrous iron binding protein suggested to protect photosystem II against oxidative stress [1].

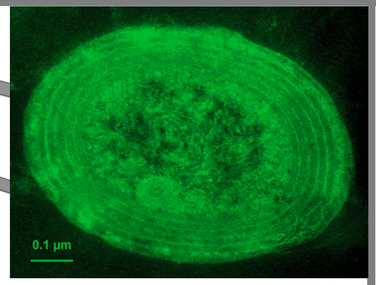
In order to survive in low nutrient waters many cyanobacteria have undergone extensive adaptions including large reductions in their genome size [2]. Interestingly, one such cyanobacterium *Trichodesmium* contains a single homologue of FutA that may have the capability to bind both ferric and ferrous iron depending on FutA localisation in the cytosol (reducing) or periplasm (oxidising) [3].

Prochlorococcus bacteria are the smallest photosynthetic organisms on earth and fix 4 gigatons of carbon every year, roughly equivalent to global agriculture [4]. Similarly to *Trichodesmium*, *Prochlorococcus* bacteria are able to thrive in low nutrient waters and contain only a single homologue of FutA.

Metalloproteins are particularly sensitive to specific radiation damage as metal ions are rapidly reduced by photoelectrons [5]. We study FutA from the cyanobacterium *Prochlorococcus* MED4 and utilise XFEL data collection, electron paramagnetic resonance (EPR), and neutron crystallography to probe ground state FutA, free from radiation damage. Finally, we characterise the sensitivity of FutA to specific radiation damage and exploit photoreduction of FutA to investigate it's physiological function.



Schematic diagram showing the Fut system.



Transmission electron micrograph (TEM) of *Prochlorococcus marinus* [6]. (Artificially coloured).

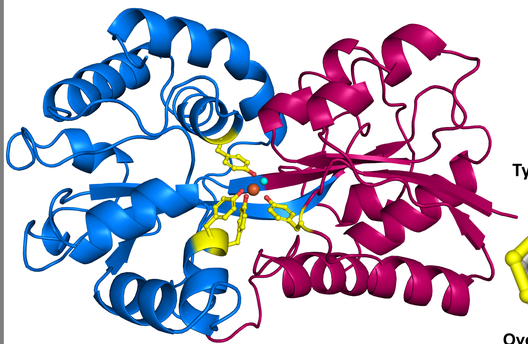
Aims

The aims for this project were:

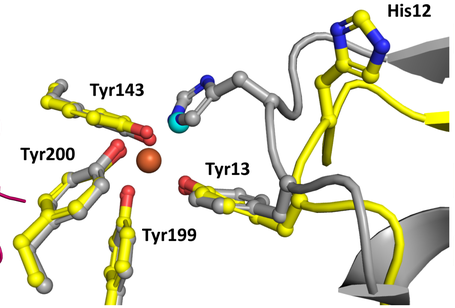
1. Obtain a radiation damage free ground state structure of FutA using serial femtosecond crystallography (SFX).
2. Investigate the iron redox state using electron paramagnetic resonance (EPR) spectroscopy and neutron crystallography.
3. Characterise the sensitivity of FutA to specific radiation damage and capture a ferrous state of FutA.

Results 1: Zero dose XFEL data collection reveals radiation damage free FutA.

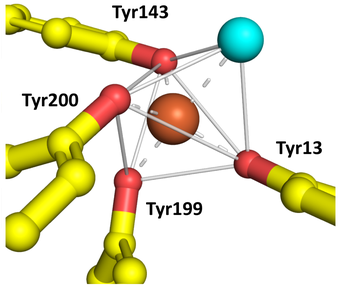
Data were collected at SACLA, Japan using silicon nitride fixed target chips for sample delivery [7]. In total 3 chips were collected equating to 76800 images, of which 24380 were scaled and merged to 1.6 Å.



Overall fold of *Prochlorococcus* MED4 FutA. The N-terminal domain is shown in magenta and the C-terminal domain in blue. The iron binding site sits within a cleft between both domains. Residues involved in iron binding are shown in yellow.

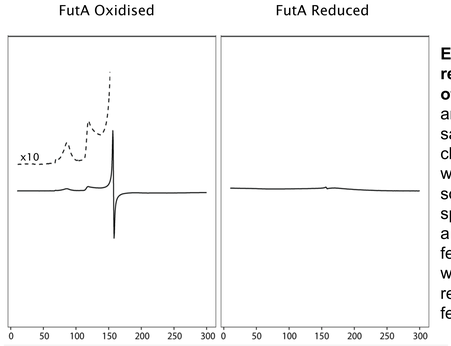


Overlay of *Prochlorococcus* MED4 FutA (yellow) and *Synechocystis* 6803 FutA2 (grey) [8]. *Prochlorococcus* MED4 FutA is crystallised in an open conformation where conserved His12 is unable to complete iron site closure and iron coordination is instead fulfilled by a water.

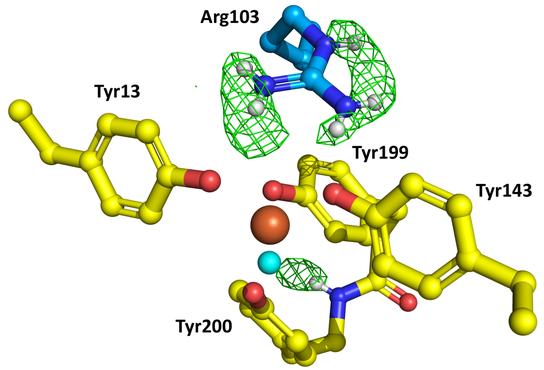


Ligand coordination by four tyrosines and a water shows the iron in a trigonal bipyramidal geometry. Red: oxygen, cyan: water, orange: ferric-ion, grey dashes: geometry.

Results 2: Does FutA bind ferric or ferrous iron?

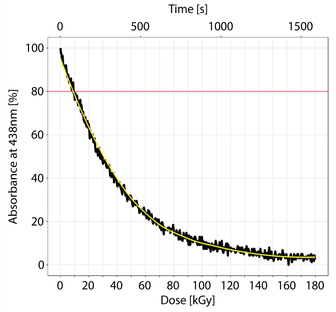


Electron paramagnetic resonance (EPR) spectra of FutA iron complex (left) and the chemically reduced sample (right). For chemical reduction FutA was incubated with x10 sodium dithionite. The EPR spectra (left) is indicative of a five-coordinate, high-spin ferric ion bound to FutA, which disappears after reduction to EPR-inactive ferrous iron (right).

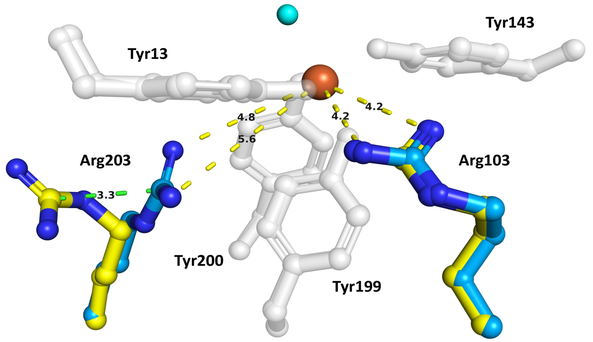


Neutron structure of FutA. The *Fo–Fc* neutron map is shown as meshes contoured at 3σ. Positive density (green) indicates protonation sites. Arg103 is fully protonated, providing a +1 charge, whilst the tyrosine hydroxyl groups are unprotonated, providing a total -4 charge. Overall, with an Fe (III) redox state of the bound iron, the net charge within the iron site is 0.

Results 3: The extreme radiation sensitivity of FutA provides insights into it's biological function.



UV-VIS spectroscopy of FutA crystals measured at 438 nm during X-ray irradiation. 438 nm corresponds to the absorbance maximum of the ferric iron which is lost due to X-ray induced photo-reduction. Data were collected at 100K. Iron in FutA is exquisitely sensitive to specific radiation damage, with 20% of the iron peak lost within the first 10 kGy of X-ray irradiation!



Room temperature single crystal structure of FutA (blue), overlaid with the SFX structure (yellow). The damaged state of FutA (blue) with an average incident dose of 50 kGy reveals a 3.3 Å movement of Arg203 into the iron binding site. The +1 charge provided by Arg203 would stabilise the shift in charge from Fe (III) to Fe (II) and may represent a mechanism by which *Prochlorococcus* FutA can bind ferrous iron. This ability to stabilise a ferrous charge supports the hypothesis that *Prochlorococcus* FutA may have both a periplasmic ferric iron binding function and a cytoplasmic ferrous iron binding function.

Conclusion

The radiation damage free structure of *Prochlorococcus* MED4 FutA was elucidated using serial femtosecond crystallography and the redox state of the iron was confirmed using both EPR spectroscopy and neutron crystallography. The acute sensitivity of FutA to specific radiation damage illustrates the requirement for dose limiting data collection regimes for metal binding substrate binding proteins (SBPs). Yet, it is the sensitivity of FutA to specific radiation damage which allowed a potentially biologically relevant ferrous state of FutA to be captured.

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References

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