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

Specific radiation damage is a pervasive problem in X-ray crystallography, often occurring before a complete dataset can be collected, at doses as low as tens of kGy [1,2]. Metalloproteins are particularly susceptible to specific radiation damage as the metal ions are rapidly reduced by solvated electrons [3].

The FutA-proteins found in cyanobacterium are characterised as both, ferric Fe(III) and ferrous Fe(II) iron binders. 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 [4].

We currently study FutA from the cyanobacterium *Prochlorococcus MED4*, which contains only a single homologue of FutA known to bind ferric iron. The sensitivity of this FutA protein to radiation damage is demonstrated by the complete reduction of the ferric iron to ferrous iron after a dose of 200 kGy at 100 K, as measured by absorbance spectroscopy.

Using *Prochlorococcus MED4* FutA, various room temperature data collection strategies were employed to study the iron binding site of FutA. Single crystal rotation data revealed the damaged state of FutA, which may relate to a biologically relevant ferrous iron binding state. Furthermore, serial synchrotron and serial femtosecond crystallography revealed the progression of radiation damage within the protein and the undamaged state of FutA.

Overall, this work aims for the successful characterisation of the FutA iron binding site and to offer insight into the advantages and disadvantages of different room temperature data collection techniques.

References

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