Identifying Metal Redox States Through Low Dose Measurements for Spatially Resolved Anomalous Dispersion Refinement

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Protein-mediated redox reactions play a critical role in a numerous biological processes and are often catalysed at centres that contain elemental co-factors, such as transition metals. To understand the exact mechanisms behind these reactions it is important to not only characterize the structure of these proteins, but also to identify the oxidation state of the individual co-factors involved. This normally requires at least two sets of experiments, collecting the X-ray diffraction data for structure determination, and separate experiments using methods such as EXAFS or XANES, which are integrative and do not allow assignment of oxidation states to individual metal sites. A method that overcomes these issues is spatially resolved anomalous dispersion (SpReAD) refinement, which is based on collecting several X-ray diffraction datasets across the X-ray absorption edge of the metal in question. SpReAD refinement allows assignment of oxidation states to individual metals, but has previously only been used on large, iron-sulfur-cluster containing proteins [1].

Here, we have tested the feasibility of this approach for small, non-iron–sulfur redox centres using S. tokodaii sulerythrin [2], a ruberythrin-like protein with a binuclear metal center reconstituted with iron. We show that data collection for SpReAD refinement is not only fast (< 90 minutes total data collection time), but that it also reveals a mixed-valence state previously not observed for sulerythrin, highlighting the usefulness of the SpReAD method to identify such intermediate reaction states. Since the collection of several full data sets exposes the crystal to a relatively high radiation dose, we further analysed the effect of the total absorbed dose on the experiments and tested whether data collected at low total dose are suitable for SpReAD analysis. We demonstrate that while data collection at high total doses leads a partial photoreduction of individual metal atoms, data collected at low total dose do not suffer from this effect and are indeed highly suitable to identify metal oxidation states by SpReAD refinement.

{1} Einsle, O., Andrade, S. L. A., Dobbek, H., Meyer, J. & Rees, D. C. (2007). Assignment of Individual Metal Redox States in a Metalloprotein by Crystallographic Refinement at

{2} Multiple X-ray Wavelengths. J. Am. Chem. Soc. 129, 2210–2211.

Fushinobu, S., Shoun, H. & Wakagi, T. (2003). Crystal Structure of Sulerythrin, a Rubrerythrin-Like Protein from a Strictly Aerobic Archaeon, Sulfolobus tokodaii Strain 7, Shows Unexpected Domain Swapping. Biochemistry. 42, 11707–11715.

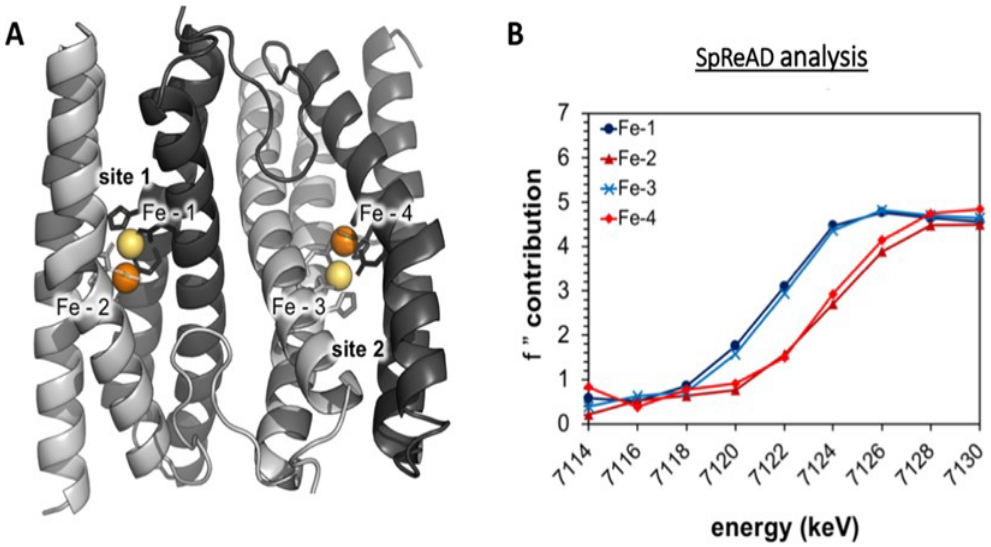

Figure 1: (A) Structure of *S. tokodaii* sulerythrin reconstituted with iron, showing the individual iron atoms as spheres. (B) SpReAD analysis of data collected on sulerythrin crystals at low total dose (0.26 MGy), showing the SpReAD profiles for the iron atoms, colored by oxidation state (blue = more reduced, red = more oxidised).