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 characterise the structure of these proteins, but also to identify the oxidation state of the co-factors involved. One approach for this 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 [1]. While being easy and quick to conduct, data collection for SpReAD refinement can expose crystals to a relatively high total dose, which can result in radiation damage, including reduction of metals.

Here, we have conducted experiments for SpReAD analysis on HZB-MX beamline 14.1, using *S. tokodaii* sulerythrin, a ruberythrin-like protein with a binuclear metal center as a model [2]. We show that data for SpReAD analysis can be collected in under 90 minutes and can reveal differences in oxidation states between individual metal atoms. We further analysed the effect of the total absorbed on the experiments by collecting data for SpReAD analysis at different flux rates. This shows 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 analysis.

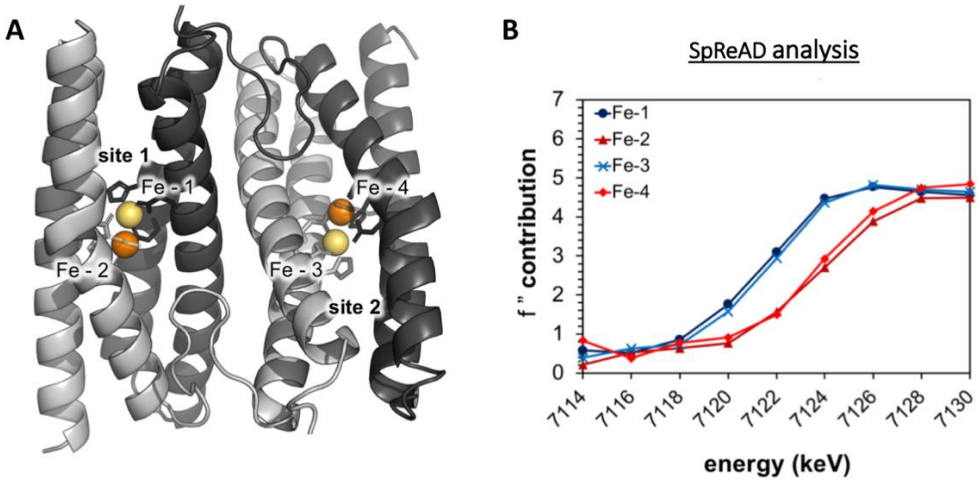

Figure 1: (A) Structure of *S. tokodaii* sulerythrin reconstituted with iron, showing individual iron atoms as spheres. (B) SpReAD analysis of data collected on sulerythrin crystals at low total dose (0.26 MGy), with SpReAD profiles for the iron atoms, colored by oxidation state (blue = more reduced,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

[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 Multiple X-ray Wavelengths.* J. Am. Chem. Soc. **129**, 2210–2211.

[2] 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.