# Assignment of Individual Metal Redox States in a Metalloprotein by Crystallographic Refinement at Multiple X-ray Wavelengths

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## Supporting Information

### Crystallization and Data Collection

Crystals of Fd4 were grown by sitting drop vapour diffusion from a reservoir solution containing 25 % (*w/v*) of polyethylene glycol 400, 0.05 M cadmium chloride and 0.1 M of sodium acetate/acetic acid buffer, pH 4.6, in a flexible glove box (Coy Laboratories, Grass Lake, MI) at less than 1 ppm of oxygen. 1 µl of protein solution at a concentration of 10 $mg \cdot ml^{-1}$ was mixed with 1 µl of this reservoir solution and incubated in sitting drop well plates at 293 K. Large single crystals of Fd4 grew within two to three days and were harvested into a solution containing 33 % (*w/v*) of polyethylene glycol 400, 0.05 M cadmium chloride and 0.1 M sodium acetate/acetic acid buffer, pH 4.6, and flash-cooled in liquid nitrogen. Preliminary tests were conducted at Stanford Synchrotron Radiation Laboratory, Palo Alto, and final data collection was carried out at the EMBL BW7A beam line at the DORIS storage ring, DESY, Hamburg, Germany. After mounting the crystal, a fluorescence scan of the K-edge region of iron (7070 - 7180 eV) was taken from which the wavelengths for the individual data sets were chosen. Data collection was carried out starting at the highest energy (strongest anomalous signal). At each energy, 180 images with 1° rotation angle per image were collected, covering the same angular range of the same crystal in each case and extending over the resolution range of 50.0 - 1.8 Å.

### Data Processing and Refinement

Data sets were integrated with the HKL suite of programs[1], fixing unit cell dimension and crystal orientation for each energy to the values obtained from refining the first data set (7140

eV). The crystal belonged to space group C2, with cell dimensions of **a** = 67.48 Å, **b** = 59.95 Å, **c** = 46.26 Å, and β = 109.09°. All data sets were converted to structure factor amplitudes and scaled together using programs of the CCP4 suite[2]. The model of Fd4 (PDB ID 1F37) was refined to an *R*-value of 0.182 at a resolution of 1.80 Å using REFMAC5[3] against the data set taken at 7140 eV. 325 water molecules and two cadmium ions were added to the model and the latter were also included into the edge refinement procedure, due to their significant anomalous signal in the iron K-edge region.

**Diffraction Data Statistics**

| **Energy [eV]** | **7100** | **7114** | **7116** | **7118** | **7120** | **7122** | **7124** | **7126** | **7140** |
|---|---|---|---|---|---|---|---|---|---|
| Unique reflections | 15 621 | 15 625 | 15 608 | 15 626 | 15 629 | 15 614 | 15 601 | 15 585 | 15 601 |
| Completeness [%] | 96.4 | 96.4 | 96.4 | 96.4 | 96.3 | 96.4 | 96.3 | 96.4 | 96.4 |
| $R_{sym}$* | 0.066 | 0.065 | 0.066 | 0.068 | 0.070 | 0.073 | 0.075 | 0.075 | 0.077 |
| $R_{sym}$ (last shell)** | 0.219 | 0.217 | 0.214 | 0.239 | 0.221 | 0.301 | 0.231 | 0.223 | 0.231 |
| I/σ(I) | 13.98 | 13.95 | 13.96 | 13.82 | 13.88 | 13.68 | 13.85 | 13.87 | 13.84 |
| I/σ(I) (last shell)** | 5.01 | 5.18 | 5.23 | 4.82 | 5.01 | 3.02 | 4.05 | 4.14 | 4.26 |
| Multiplicity | 3.7 | 3.7 | 3.7 | 3.7 | 3.7 | 3.7 | 3.7 | 3.7 | 3.7 |
| Scale factor | 1.013 | 1.013 | 1.013 | 1.014 | 1.013 | 1.013 | 1.012 | 1.013 | 1.000 |
| wt. *R* for scaling | 0.041 | 0.048 | 0.048 | 0.047 | 0.049 | 0.049 | 0.046 | 0.046 | 0.000 |
| $\lvert\Delta_{ano}\rvert$ / σ($\Delta_{ano}$) | 0.64 | 0.64 | 0.65 | 0.67 | 0.71 | 0.74 | 0.80 | 0.81 | 0.84 |

* $R_{sym} = \Sigma \mid I - \langle I \rangle \mid / \Sigma I$

** The last resolution shell comprised the range between 1.83 and 1.80 Å.

**Supplementary References**


1. Otwinowski, Z.; Minor, W., *Methods Enzymol.* **1996,** 276, 307-326.
2. Collaborative Computational Project No. 4, *Acta Cryst.* **1994,** D50, 760-763.
3. Murshudov, G. N.; Vagin, A. A.; Dodson, E. J., *Acta Cryst.* **1997,** D53, 240-255.