

Supporting information for: Folding catalysis by transient coordination of Zn^{2+} to the Cu ligands of the ALS-associated enzyme Cu/Zn superoxide dismutase 1

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Supporting information 1

Anisotropy of Cu^{2+} in the crystal structure of monomeric holoSOD1^{CuZn}

The present holoSOD1^{CuZn} structure is essentially identical to the atomic resolution structure of monomeric Cu/Zn SOD1 crystallised in the presence of Cd^{2+} (PDB entry 1MFM)¹. However in 1MFM the Cu-site is delocalised over two positions with approximately equal occupancies, separated by 1.6 Å, one of which contains a Cd^{2+} ion and the other Cu. This was interpreted as representing two binding sites, for Cu^{2+} and Cu^+ respectively. In contrast, in the present structure we observe only one Cu^{2+} ion, located very close to the Cd^{2+} ion in 1MFM. Even so, the holoSOD1^{CuZn} structure shows significant anisotropy for the Cu^{2+} ion. The anisotropy value (ratio of smallest to largest eigenvector in the anisotropic B-factors^{2,3}) is 0.20 for the Cu^{2+} ion and 0.68 for the Zn^{2+} ion. The major axis of this anisotropy, as determined from anisotropic atomic displacement parameters (ADP) from Refmac5, is approximately perpendicular to the line between the Cu- and Cd^{2+} -ions in the earlier 1MFM structure (Figure S1). This major anisotropic axis does not coincide with any of the coordination vectors from the NE2 atoms of any of the His

ligands, nor with the observed direction of disorder of Cu^{+2+} in previously reported structures^{1,4-7}. The Refmac5 anisotropy values were further confirmed with 20 cycles of anisotropic refinement using SHELXL-97².

Analysis of the anomalous scattering of the coordinated metal ions at different wavelengths confirms that the Cu-site is occupied by Cu alone. Anomalous scattering data was collected to 1.8 Å resolution at four wavelengths at the MAX-lab beam line I911-3: 1.23 Å (above the Zn and Cu absorption edges), 1.28 Å (at the Zn and above the Cu absorption edge), 1.33 Å (below the Zn and above the Cu absorption edge) and 1.38 Å (at the Cu and below the Zn absorption edge).

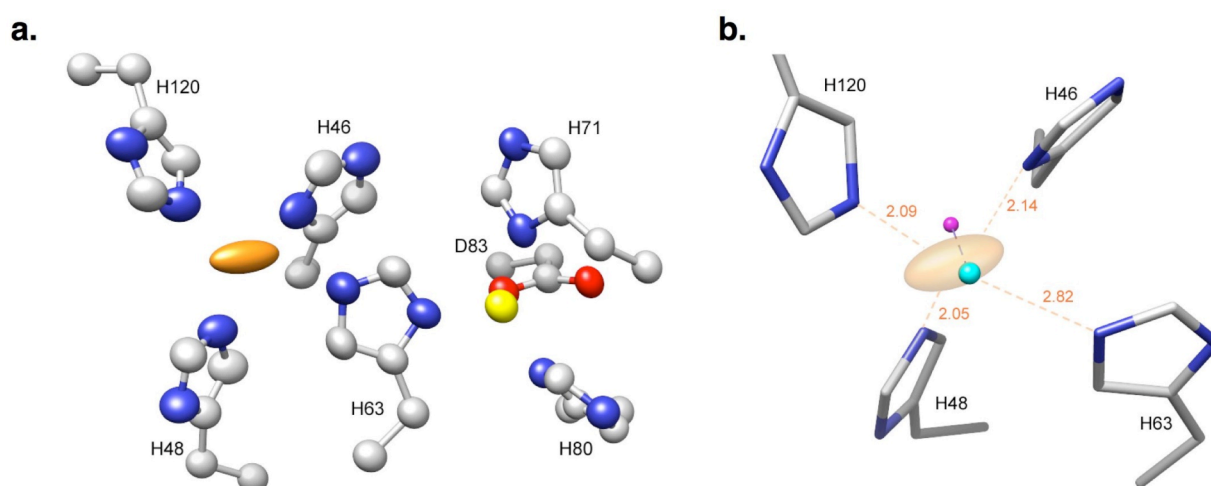


Figure S1. (a.) Thermal ellipsoid representation of the anisotropic B-factors in the active site of holoSOD1^{CuZn} at a 50% probability level as derived from ADPs. The Cu²⁺ ion is shown as an orange ellipsoid. Zn²⁺, N and O atoms are shown in yellow, blue and red respectively. **(b.)** The anisotropic disorder of Cu²⁺ in holoSOD1^{CuZn} (orange ellipsoid) is in a different orientation to the vector between the Cd²⁺ (cyan) and Cu⁺ (magenta) sites in the monomeric SOD1 structure 1MFM¹. The distances between Cu²⁺ and the side-chain ligands in holoSOD1^{CuZn} are shown in orange. The figures were drawn with Chimera³.

Supporting information 2

Alternate conformations of loop IV in the crystal structures of monomeric holoSOD1^{CuZn} and holoSOD1^{XZn}

Even though the dimer-breaking substitutions F50E /G51E do not alter the structure of the SOD1 β -barrel, they lead to structural heterogeneity of loop IV. The native topology of loop IV is normally supported by the dimer interface⁴, whereas in the crystal packing of the monomeric structures loop IV is completely exposed to solvent. Even so, the electron density for holoSOD1^{XZn} reveals a new, alternate conformation of loop IV (residues 52–56) that is closer to the β barrel (Figure S2) and also different from the conformation in the native dimer. As a result of this conformational heterogeneity, higher mean B-factors were observed for loop IV compared to the overall structure.

Accompanying the two conformations of loop IV we observe also two distinct conformations of the disulphide linkage between C57 and C146 (Figure S2). The conformation of the catalytically important R143^{11,12} is sensitive to the position of C57. As a result of the relatively high degree of order of C57 in the present structures (the C57 B-factors of holoSOD1^{CuZn} and holoSOD1^{XZn} structures are 40.0 and 27.7 Å², respectively, compared to 51.7 Å² in 1MFM¹), Arg143 is also well ordered in both structures and there is no deviation between them. R143 interacts with loop IV, hydrogen bonding to the carbonyl oxygens of C57 and T58. Although this superficially resembles the conformation in 1MFM, inspection of the electron density for 1MFM produced by the EDS server⁵ indicates that R143 in that model is highly disordered (side chain B-factors approaching 100 Å² at the tip) and is possibly incorrectly built.

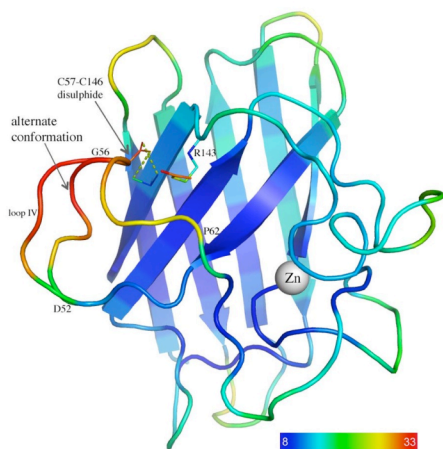
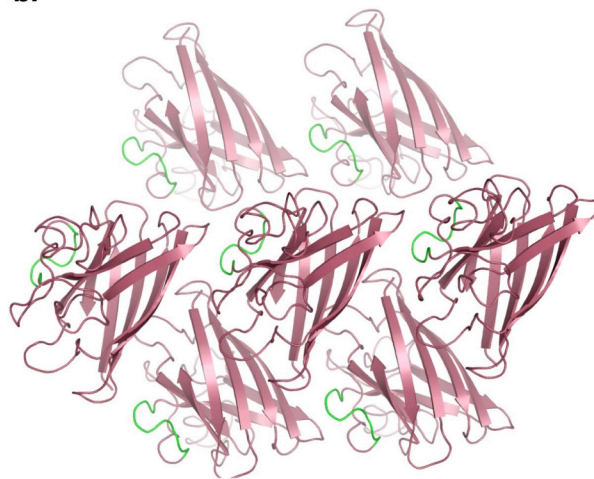
a.**b.**

Figure S2. **(a.)** The structure of holoSOD1^{XZn} showing alternate conformations of loop IV and the disulphide bond. The backbone is coloured according to the temperature factors of C α atoms, ranging from 8 Å² (blue) to 33 Å² (red). **(b.)** The packing of monomeric SOD1 molecules (holoSOD^{XZn}) in the crystal lattice is different from that of the native dimers. The part of loop IV that interacts with the dimer interface in the native structure is coloured in green.

Supporting Information 3

Deviation from first-order unfolding kinetics of holoSOD1^{XZn} at low concentrations of solvent Zn²⁺.

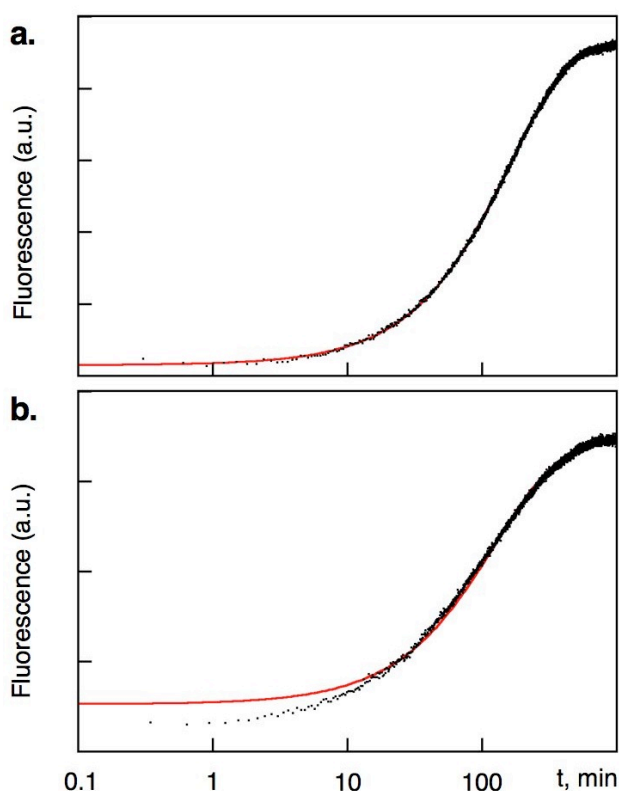


Figure S3. Deviation from first-order unfolding kinetics of holoSOD1^{XZn} at low concentrations of solvent Zn²⁺. (a.) Unfolding of holoSOD1^{XZn} displays pseudo-first order kinetics in the presence of 500 μ M of ZnCl₂ (8.7 M urea). The fit shows a single exponential function. (b.) Unfolding of ‘as purified’ holoSOD1^{XZn} in the absence of ZnCl₂ (7.4 M urea) deviates from exponential behavior. The reason for this deviation is that the concentration of solvent Zn²⁺ increases from zero to 5 μ M during the course on the unfolding reaction, c.f. Scheme 1. The fit shows a single exponential function.

Supporting Information 4

Crystal structure refinement

During model building, pronounced difference electron densities for Zn^{2+} ions were observed at the crystal contacts in both the holoSOD1^{CuZn} and holoSOD1^{XZn} structures. These Zn^{2+} positions correspond to the Cd^{2+} positions observed in the atomic resolution structure of monomeric holo SOD1¹. The presence of Zn^{2+} ions at the crystal contacts emphasizes our earlier observation that divalent metal ions can reproducibly crystallize many variants of this engineered monomeric SOD1⁶. Furthermore, in the holoSOD1^{XZn} structure, pieces of difference electron density found near the crystal contacts were modelled as polyethylene glycol, sodium and acetate ions, consistent with their presence in the crystallization drops. Water molecules were added where stereo-chemically plausible and where the $2|F_o| - |F_c|$ and $|F_o| - |F_c|$ maps revealed densities of more than 1.0 and 3.5σ respectively.

In order to produce the total B-factors, the TLS contribution was added to the anisotropic B-factor in the final round of refinement in Refmac5. The anisotropic displacement parameters were analyzed using the program ANISOANL⁷, implemented in the CCP4 suite.

Supporting Information 5

Complete Reference (25):

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