Supplementary Material

To determine the relationship between loss of Cu and electrophoretic migration on native gels lacking SDS, which are identical to the system used to study tissues from mice (see Fig. 4), we treated preparations of wild-type human SOD1 isolated from red blood cells (Sigma-Aldrich, St. Louis, MO) with metal chelators and reducing agents (Supplementary Figure 2). SOD1 in these preparations migrates as a doublet in native gels possibly due to oxidative modification of Cys 111 (1). Removal of metal cofactors and reduction of the disulfide bond in each subunit of SOD1 have been shown to dissociate SOD1 into monomeric subunits (2;3). Incubation of SOD1 in 10 mM EDTA alone did not lead to alterations in electrophoretic mobility. Treatment of the enzyme with 20 mM EDTA and heat to 80ºC for 5 minutes, produced faster migrating species of SOD1, but the addition of DTT to 100 mM with EDTA (10 and 20 mM) shifted the migration of almost all protein to a single faster migrating species. These data demonstrate that the complete removal of metal cofactors and complete reduction of disulfide bonds are required to produce a single faster migrating species of SOD1 as seen in other preparations of monomeric SOD1 protein examined by native gel electrophoresis (4;5).

Reference List

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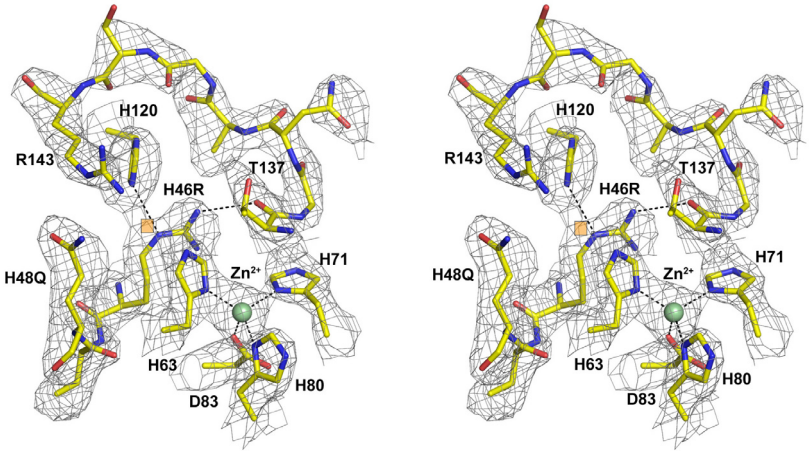
Supplementary Figure Legends

Fig. S1. Divergent stereo view of the metal-binding site in a subunit of the H46R/H48Q double copper-site SOD1 mutant that most closely resembles wild-type SOD1. The active site is superimposed on 2.4 Å electron density with coefficients $2mF_o$-DF_c contoured at 1.2 σ. Arg 143 and residues corresponding to metal ligands in the wild type enzyme (46, 48, 63, 71, 80, 83, and 120) are labeled. The orange square represents the position copper would occupy in the wild-type enzyme. Arg 46 donates a hydrogen bond to the carbonyl oxygen of Thr 137 (see text).

Fig. S2. Removal of metals by chelation and reduction of disulfide alters the electrophoretic mobility of wild-type SOD1 in native polyacrylamide gels. To examine the electrophoretic mobility of human SOD1 (hSOD1) in native gel after removal of metals and reduction of disulfide bonds, hSOD1 from human erythrocytes was examined (Sigma Aldrich). Aliquots containing 2 μg of hSOD1 were suspended in 40 μl of Tris-glycine buffer (25 mM Tris-Cl , 192 mM glycine) containing .002% bromophenol blue and 10% glycerol with the following additions; no treatment (lane 1), 10mM EDTA (2nd lane), 20 mM EDTA + 80ºC for 5 minutes (3rd lane), 10mM DTT + 10 mM EDTA (4th lane), 100 mM DTT + 10 mM EDTA (5th lane), 100 mM DTT + 20 mM EDTA (6th lane). Samples were electrophoresed on 10% Tris-HCl gels (no SDS) (Bio-Rad Laboratories, #345-0023) for 2.5 hours at 125 v and then a further 1 hour at 150 v. The gel was rinsed with water 3 times 5 min each and then stained in GelCode Blue (Pierce Biotechnology Inc., #245900) for ~1 hour and then enhanced in water for 1 hour before capturing an image.

Supplemental Figure 1



Supplemental Figure 2