Supplementary Material

Analysis of copper-ligand bond lengths in X-ray structures of the different types of copper sites in proteins

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Table S1. Examples of copper-ligand bond lengths derived from EXAFS data for the types of copper sites studied here.

	Scys	ND/Ehis	Smet	Others	References
CuA (oxidized)					
Nitrous oxide reductase	2.24	1.92	2.93	Obb: 2.05	Charnock *et al.*, *Eur. J. Biochem.* 2000
COXII's CuA site (*T. thermophilus*)	2.25-2.29	1.94-1.96	2.55		Blackburn *et al.*, *JACS* 1997 and *Biochemistry* 1999
COXII's CuA site (*B. subtilis*)	2.3	1.95			Blackburn *et al.*, *JACS* 1997 and *Biochemistry* 1999
Type 1					
Rusticyanin (ox)	2.16	1.93-1.98	2.836		Holt *et al.*, *FEBS Letters* 1990 and Barret *et al.*, *Biochemistry* 2006
Rusticyanin (red)	2.21	2.02-2.07	2.795		Holt *et al.*, *FEBS Letters* 1990 and Barret *et al.*, *Biochemistry* 2006
Amicyanin (ox)	2.11-2.13	1.95-2.01			Lommen *et al.*, *Biochim. Biophys. Acta* 1991
Amicyanin (red)	2.19	2.18	2.41		Lommen *et al.*, *Biochim. Biophys. Acta* 1991
Stellacyanin (ox)	2.17-2.18	1.95-1.96			DeBeer *et al.*, *J. Phys. Chem. B* 2000
Azurin (ox.)	2.14-2.18	1.92-1.95			Berry *et al.*, *JACS* 2003 and *Clark et al.*, *JACS 2010*
Azurin (red.)	2.21	2.00			Berry *et al.*, *JACS* 2003
Type 2					
Cu,Zn superoxide dismutase		1.98			Blackburn *et al.*, *Biochem. J.* 1983
Galactose oxidase		1.97			Clark *et al.*, *Biochemistry* 1994
Homeostatic					
DR1885		2.00	2.30		Banci *et al.*, *PNAS* 2005
Sco1 from B. Subtilis (several forms)	2.251 – 2.264	1.967 – 2.026			Nittis *et al.*, *J. Biol. Chem.* 2001
Type 3 (oxidized)					
Fet3P		2.04		Bridging O: 1.94	Blackburn *et al.*, *Biochemistry* 2000

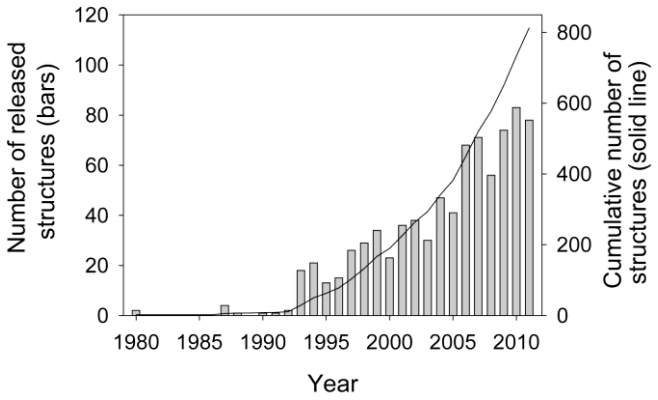

Figure S1. Number of structures of copper-containing proteins released by the Protein Data Bank between 1980 and 2011.

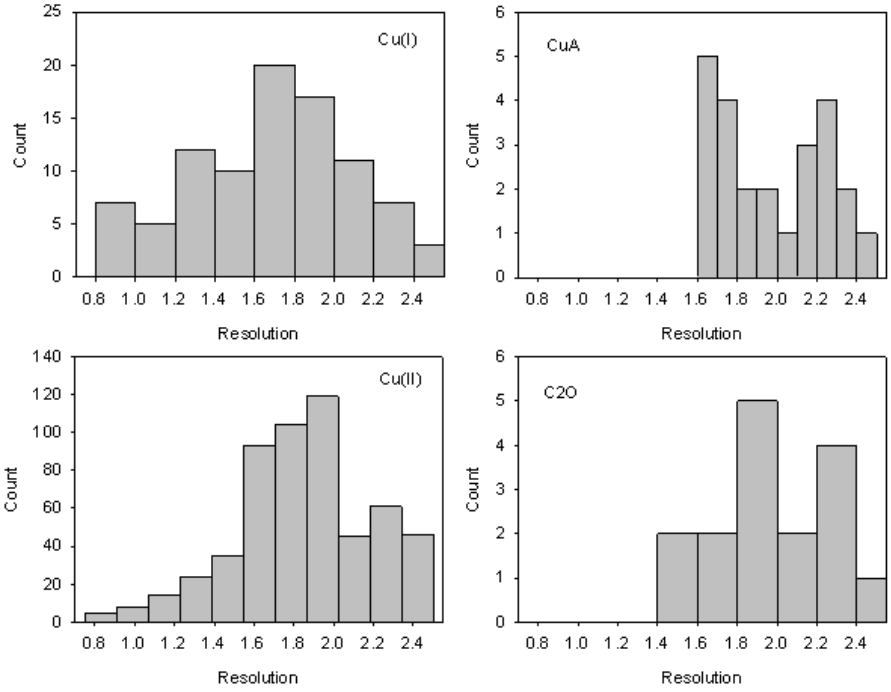

Figure S2. Distribution of structure resolutions within each group of copper sites studied. Only structures with resolution better than 2 Å were analyzed in the case of mononuclear sites (except for NDhis and NEhis bond lengths where all data was used for the plots in figure S3). All structures were analyzed for CuA and Type 3 (C2O) proteins.

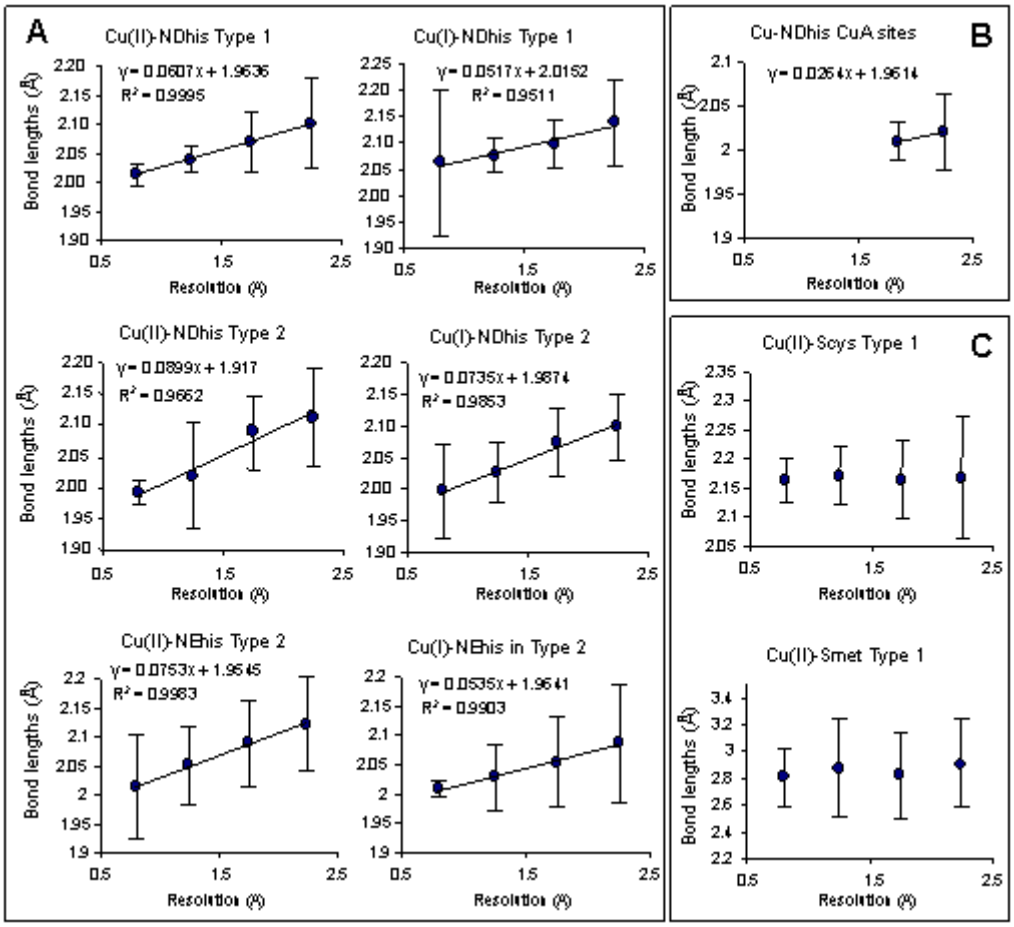

Figure S3. Sample plots showing the dependence of Cu-ligand bond lengths on structure resolution. Copper-atom bond lengths were computed for subsets of proteins grouped by resolution, and the averages were plotted against the mid resolution of the bins. In all cases, the uncertainties of the intercepts (± values in Table 2 of the main text) were calculated through Monte Carlo simulations considering the standard deviation of each averaged bin. Panel (A) shows plots for 6 bond lengths between Cu(II) and histidine ND and NE atoms in Type 1 and Type 2 sites and their fits to straight lines. Panel (B) shows a plot for the ND atom of CuA centers and a straight line that goes through these two points. Panel (C) shows sample plots in which no dependence was observed (but as detailed in the text, their whole averages match well with EXAFS data, which was not the case for histidine N atoms).

Other Cu-atom bond lengths

There is limited information for NEgln and Otyr, so the reported values are only indicative and should not be utilized for modeling and refinement. In particular, the NEgln atom, which acts as axial ligand in some Type 1 copper centers, can be misidentified with its corresponding OEgln. Assuming no such errors in the X-ray structures, the computed standard deviation is relatively small considering the low number of averaged values. Tyrosine's phenolic O atom is found as equatorial or axial ligand in some Type 2 centers with a large dispersion of bond lengths (Figure 3B).

Other ligands found within 3 Å spheres of Cu(II) ions in Type 2 sites were OGser, NZlys, NEarg and NH2arg atoms. These atoms were not observed for Type 1, Type 3 sites, Cu_A or homeostatic sites, whose coordination spheres are much more conserved than those of Type 2 sites. A particular, unique case in the group of proteins involved in copper homeostasis is that of CusF, where the aromatic ring of a tryptophan residue makes a π-cation bond with the copper ion (structure depicted in Figure 1) (Xue *et al.*, 2008).

Comments on the observed values for Cu-atom bond lengths in different types of copper sites

Cu-Nhis bond lengths are similar between all oxidized copper sites and between all reduced sites, being slightly but consistently longer in the later, in agreement with EXAFS data. However, Cu-Scys bond lengths do vary between sites with the same oxidation state. The shortened Cu-Scys bond distance in Type 1 sites relative to homeostatic sites arises from the strain imposed by the protein matrix in an entatic state that ensures low reorganization energy values to help maximize electron transfer rates (Gray *et al.*, 2000; Paraskevopoulos *et al.*, 2006; Cheung *et al.*, 2000) . In Cu_A sites the average Cu-Scys bond length is again similar to that of homeostatic sites, consistently with EXAFS data, reflecting the bridging nature of cysteine S atoms in these sites, where the strain is probably utilized to bring the two copper ions together and force it into a σ_u^* ground state (Gorelsky *et al.*, 2006; Abriata *et al.*, 2009). Another difference between Type 1 and Cu_A sites is on the Smet and Obb axial ligands. Although they both show larger dispersions than Scys distances (Figures 3A and 3B for Scys and Smet, and 4A for Obackbone), the distribution is much broader in Type 1 sites. It has been shown that in both types of sites the nature and bonding length of the axial ligands can tune the reduction potential (Garner *et al.*, 2006; Ledesma *et al.*, 2007); thus the broader distributions in Type 1 sites are consistent with their wider range of potentials. Also, the lower variability in bonding distances

for Smet in Cu_A sites is in line with an increased stiffness that confers lowered reorganization energies compared to Type 1 sites (Gray *et al.*, 2000; Hay & Lu, 2000; Gray & Winkler, 1996).

Backbone O atoms are also common ligands in homeostatic and Type 2 sites, for which the distributions are broader than in Cu_A but sharper than in Type 1 sites, at least sharp enough to disclose a population of first-shell Obb atoms and another of second-shell atoms (Figure 4A). The other copper-binding atom of the protein backbone is the peptide N, almost exclusive to Type 2 sites with a marked preference for oxidized over reduced copper (Figure 4B).

Single and bridging O atoms from H_2O, OH^-, etc. are mainly found in oxidized Type 2 and Type 3 sites. The distribution of bond lengths for oxidized Type 2 sites (Figure 4B) is very disperse, with a flat maximum from 1.8 to 2.8 Å that goes down and then rises by the contribution of outer-sphere atoms. In line with the lower affinity of reduced copper for these molecules, there are few counts at small bond lengths while the distribution rises at longer distances due to outer-shell water molecules. Thus, as expected from the affinities of Cu(I) and Cu(II), water can be a first-shell ligand in oxidized Type 2 sites, but only a second-shell ligand in reduced sites, similarly to the case of backbone N atoms. In Type 3 sites the peak is sharper and is located at a shorter average bond length, reflecting stronger activation by the two copper ions.

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