	Pc	CBP	AcoP	AcNir	Nitrosocyanin
Copper ligands	$Hx_N.....Cx_2Hx_4M$	$Hx_N.....Cx_4Hx_4M$	$Hx_N.....Cx_6Hx_4M$	$Hx_N.....Cx_8Hx_4M$	$Ex_N.....Cx_2Hx_4H$
Geometry	classic blue T1 Tetrahedral	perturbed blue Distorted tetrahedral	T?	green T1.5 Distorted tetragonal	red T2 Tetragonal
PDB #	PDB: **1PLC** Guss JM et al. Acta Crystallogr B (1992) 48, p. 790-811	PDB: **2CBP** Guss JM et al. J Mol Biol (1996) 262, p. 686-705	PDB: **7Z3B** This study	PDB: **2BW4** Antonyuk SV et al. Proc Natl Acad Sci U S A (2005) 102, p. 12041-6	PDB: **1IBY** Lieberman RL et al. Biochemistry (2001) 40, p. 5674-81
Cu to ligand bond length	Cu-N(His37) = 1.91 Å Cu-S(Cys84) = 2.07 Å Cu-N(His87) = 2.06 Å Cu-S(Met92) = 2.82 Å	Cu-N(His39) = 1.93 Å Cu-S(Cys79) = 2.15 Å Cu-N(His84) = 1.95 Å Cu-S(Met89) = 2.61 Å	Cu-N(His85) = 2.00 Å Cu-S(Cys139) = 2.23 Å Cu-N(His166) = 2.10 Å Cu-S(Met171) = 2.91 Å	Cu-N(His95) = 2.05 Å Cu-S(Cys136) = 2.23 Å Cu-N(His145) = 2.04 Å Cu-S(Met150) = 2.49 Å	Cu-O(Glu60) = 2.10 Å Cu-S(Cys95) = 2.23 Å Cu-N(His98) = 2.01 Å Cu-N(His103) = 1.98 Å Cu-O(H_2O) = 2.06 Å
UV-Vis ABS spectrum	Adapted from Solomon EI et al. Chemical Reviews (2004) 104, p. 419-58	Adapted from Solomon EI et al. Chemical Reviews (2004) 104, p. 419-58	Adapted from Roger M et al. PLoS One (2014) 9, p. e98941	Adapted from Solomon EI et al. Chemical Reviews (2004) 104, p. 419-58	Adapted from Basumalick L et al. J Am Chem Soc (2005) 127, p. 3531-44
Em (vs. SHE) (mV)	+ 350	+ 317	+ 570*	+ 240	+ 85

* Determined at pH 5.

Figure S1: Some spectral and geometrical properties of T1 / T1.5 / T2 cupredoxins and AcoP.
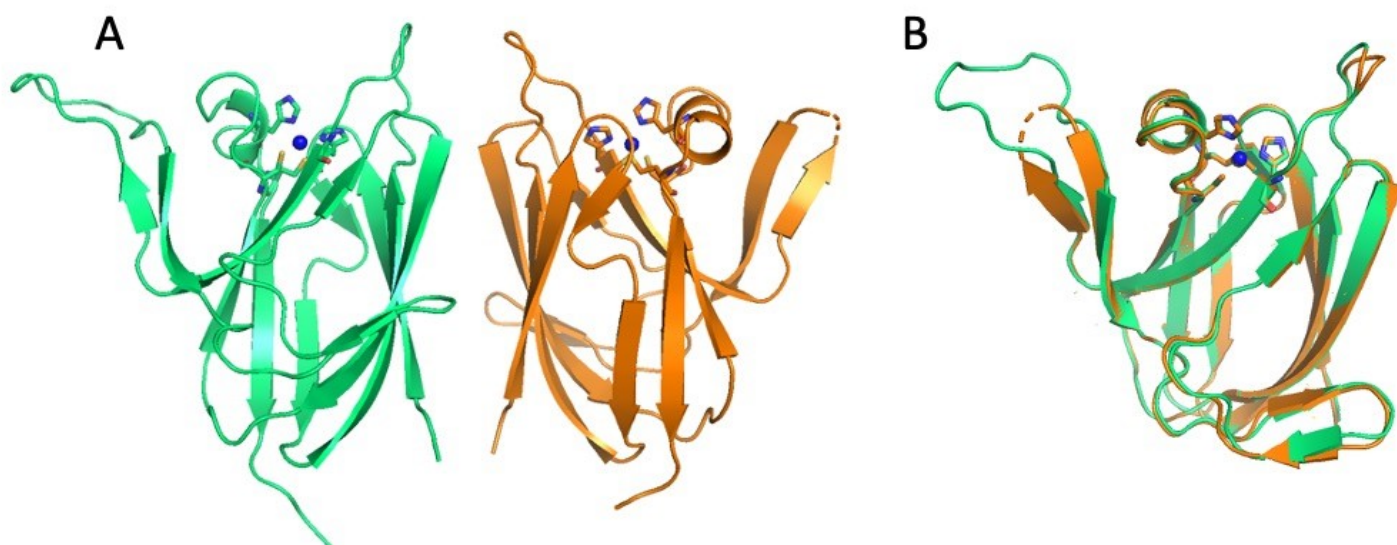
Figure S2: Structural comparison between molecule A and B of wild type AcoP. A) The two molecules present in the asymmetric unit, and B) their superposition are displayed (molecule A in green, molecule B in orange).

		AcoP 7Z3B red	AcoP 7Z3F ox	AcoP 7Z3G H166A	AcoP 7Z3I M171A	AcoP ox - red	Pn PC ox - red	Se PC ox - red	Acv PC ox - red
DISTANCES									
Cu-N(H85) (Å) (equatorial)	mol A	2.00	2.03	1.89	2.01	0.03			
	mol B	1.99	2.05	1.96	2.04	0.06			
	< AB >	**2.00**	**2.04**	**1.93**	**2.03**	**+ 0.04**	**- 0.06**	**- 0.12**	**- 0.02**
	mol B-A	-0.01	0.02	0.07	0.03	0.03			
Cu-S(C159) (Å) (equatorial)	mol A	2.23	2.34	2.14	2.27	0.11			
	mol B	2.24	2.21	2.17	2.23	-0.03			
	< AB >	**2.24**	**2.28**	**2.16**	**2.25**	**+ 0.04**	**- 0.08**	**- 0.04**	**+ 0.05**
	mol B-A	0.01	-0.13	0.03	-0.04	-0.14			
Cu-N(H166) (Å) (equatorial)	mol A	2.10	2.17	NR	2.06	0.07			
	mol B	2.11	2.19	NR	2.05	0.08			
	< AB >	**2.10**	**2.18**		**2.06**	**+ 0.08**	**- 0.06**	**- 0.36**	**- 0.03**
	mol B-A	0.01	0.02		-0.01	0.01			
Cu-S(M171) (Å) (axial)	mol A	2.85	2.74	2.79	NR	-0.11			
	mol B	2.98	2.80	2.82	NR	-0.18			
	< AB >	**2.92**	**2.77**	**2.81**		**- 0.15**	**NR**	**+ 0.14**	**+ 0.01**
	mol B-A	0.13	0.06	0.03		-0.07			
ANGLES									
I - (M171)S-Cu-N(H85) (°) (axial-bond to equatorial-bond angle)	mol A	80.5	81.2	81.9	NR	0.6			
	mol B	76.8	81.9	81.1	NR	5.0			
	< AB >	**78.7**	**81.5**	**81.5**		**+ 2.8**	**- 1.0**	**- 5.8**	**+ 0.4**
	mol B-A	-3.7	0.7	-0.8		4.4			
II - (M171)S-Cu-N(166) (°) (axial-bond to equatorial-bond angle)	mol A	107.8	113.3	NR	NR	5.5			
	mol B	104.4	111.5	NR	NR	7.1			
	< AB >	**106.1**	**112.4**			**+ 6.3**	**- 1.0**	**- 1.1**	**+ 1.6**
	mol B-A	-3.4	-1.8			1.6			
III - M171)S-Cu-S(C159) (°) (axial-bond to equatorial-bond angle)	mol A	109.1	113.7	115.7	NR	4.6			
	mol B	109.2	109.8	120.7	NR	0.6			
	< AB >	**109.1**	**111.8**	**118.2**		**+ 2.6**	**- 3.0**	**- 7.8**	**- 1.6**
	mol B-A	0.1	-3.9	5.0		- 4.0			
α - (H85)N-Cu-C(C159) (°) (inter equatorial-bond angle)	mol A	146.0	144.6	159.9	152.1	- 1.4			
	mol B	147.4	147.7	157.6	149.9	0.3			
	< AB >	**146.7**	**146.2**	**158.7**	**151.0**	**- 0.5**	**+ 2.0**	**- 9.2**	**- 4.0**
	mol B-A	1.4	3.1	- 2.3	- 2.2	1.8			
β - (C159)S-Cu-N(166) (°) (inter equatorial-bond angle)	mol A	109.2	104.4	NR	103.0	- 4.8			
	mol B	108.9	106.3	NR	104.2	- 2.6			
	< AB >	**109.1**	**105.4**		**103.6**	**- 3.7**	**+ 4.0**	**+ 12.9**	**+ 1.0**
	mol B-A	- 0.3	2.0		1.2	2.2			
γ - (H166)N-Cu-N(85) (°) (inter equatorial-bond angle)	mol A	97.8	97.7	NR	95.0	- 0.1			
	mol B	100.0	96.2	NR	94.9	- 3.8			
	< AB >	**98.9**	**96.9**		**94.9**	**- 2.0**	**+ 1.0**	**+ 8.1**	**+ 2.7**
	mol B-A	2.1	- 1.5		- 0.2	- 3.7			

Table S1 : Detailed copper centre distances and angles and comparison between redox states. Left pane: distance and angle values for AcoP copper-to-ligand bonds, for each AcoP monomer (A and B) in the asymmetric unit, average value (< AB >: bold values, same as in Table 2) and discrepancy in bond length and angles between monomer A and B (mol B-A: grey values). Angles (I, II, III, α, β, γ) are named according to what shown in Supp Figure 3. Right pane: differences for the same bond lengths and angles are shown for the red-to-ox transition (operation: ox – red) in AcoP (based on values on the left). Positive values (increased length or angle) are in green, negative ones (decreased length or angle) in red. Length and angle variations smaller than 0.04 Å and 0.5° respectively were considered as not relevant (grey text, no color). Variations in each monomer (A and B) are reported too, and colored accordingly (light green and light red boxes). The same variations (ox – red) are reported for: *Populus nigra* plastocyanin (Pn PC), *Synechococcus elongatus* (Se PC) and *Adiantum capillus-veneris* (Acv PC). Values for Pn PC were based on Solomon (2006)[8]; values from Se and Acv PC were obtained by comparing respectively pdb 1BXU (ox) and 1BXY (red), as well as 1KDJ (ox) and 1KDI (red).
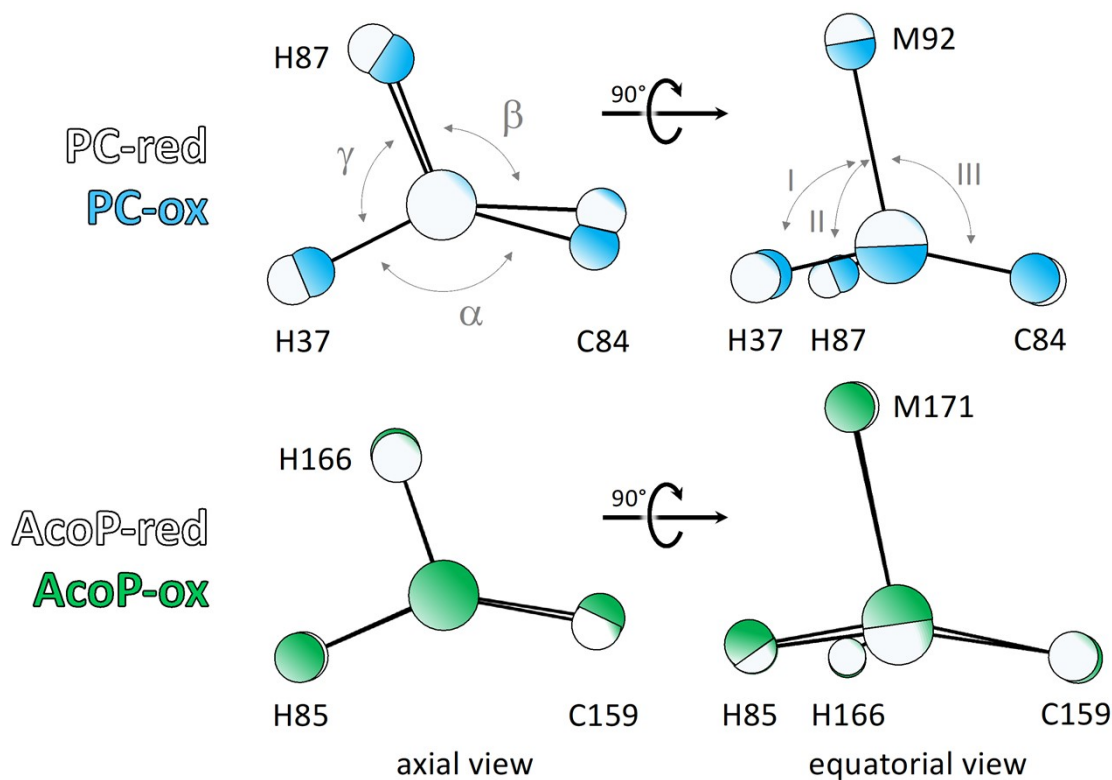
Figure S3: Change of cupredoxin metal centres upon oxidation. Metal centre rearrangements upon Cu oxidation in *Populus nigra* (poplar) plastocyanin (PC) (top view; adapted from Solomon (2006)[8]) and AcoP (bottom view: atomic coordinates from pdb 7Z3F and 7Z3B, for the oxidized and reduced states respectively. Atoms corresponding to the oxidized states are colored in blue and green for PC and AcoP respectively. Atoms corresponding to the reduced states are colored in white. Left panes: view of the equatorial ligands (from the axial ligand point of view). Right panes: 90° away view, from aside the equatorial plane. Inter equatorial-bond angles (α, β, γ) and axial-bond to equatorial-bond angles (I, II, III) are named according to Supp Table 1.
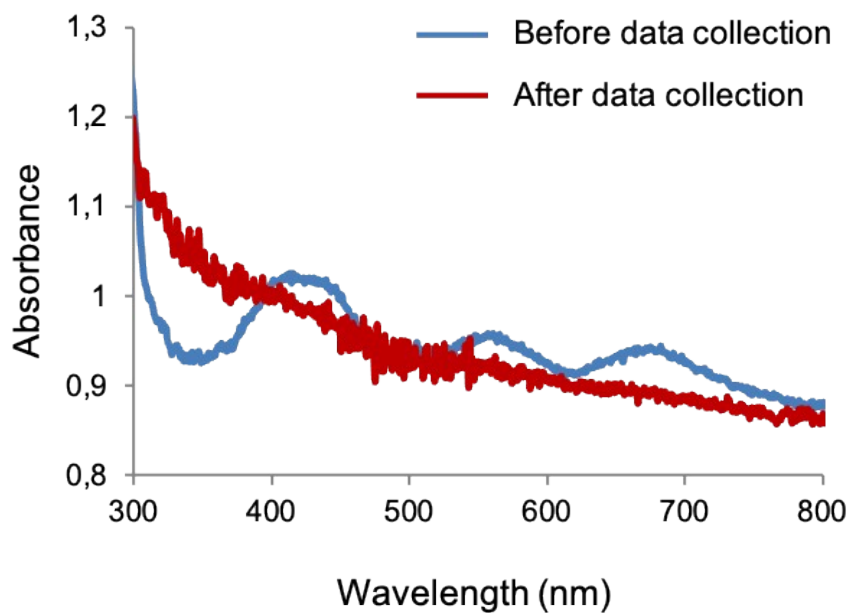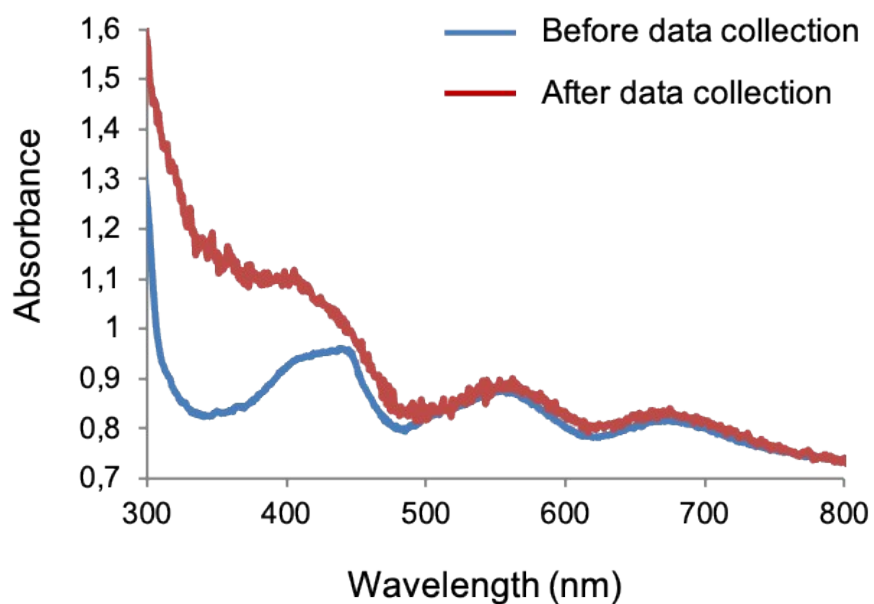
Figure S4 : Online microspectrophotometry of AcoP oxidized crystal. A) Spectra of an AcoP oxidized crystal before data collection (blue) and after classical data collection, irradiating the whole crystal (red), showing complete photoreduction. B) Spectra of the crystal from which the oxidized AcoP structure has been derived (PDB: 7Z3F) before data collection (blue) and after helical data collection (red). The noisy aspect and the increase in absorbance at shorter wavelengths, also visible in panel A, is a common feature in the spectra of X-ray-exposed protein crystals, due to photogenerated radicals.

		AcoP				AcoP	AcoP ox *vs* red *vs* mutants			
pdb :		7Z3B	7Z3F	7Z3G	7Z3I		M171A	ox	red	M171A
state :		red	ox	H166A	M171A	ox - red	- H166A	- H166A	- H166A	- red
DISTANCES										
Cu-N(H85) (Å)	mol A	2.00	2.03	1.89	2.01	0.03	0.12	0.14	0.11	0.01
(equatorial)	mol B	1.99	2.05	1.96	2.04	0.06	0.08	0.09	0.03	0.05
	< AB >	**2.00**	**2.04**	**1.93**	**2.03**	**+ 0.04**	**+ 0.10**	**+ 0.12**	**+ 0.08**	**+ 0.02**
	mol B-A	-0.01	0.02	0.07	0.03	0.03	-0.04	-0.05	-0.08	0.04
Cu-S(C159) (Å)	mol A	2.23	2.34	2.14	2.27	0.11	0.13	0.20	0.09	0.04
(equatorial)	mol B	2.24	2.21	2.17	2.23	-0.03	0.06	0.04	0.07	-0.01
	< AB >	**2.24**	**2.28**	**2.16**	**2.25**	**+ 0.04**	**+ 0.09**	**+ 0.12**	**+ 0.08**	**+ 0.01**
	mol B-A	0.01	-0.13	0.03	-0.04	-0.14	-0.07	-0.16	-0.02	-0.05
Cu-N(H166) (Å)	mol A	2.10	2.17	NR	2.06	0.07				-0.04
(equatorial)	mol B	2.11	2.19	NR	2.05	0.08				-0.06
	< AB >	**2.10**	**2.18**		**2.06**	**+ 0.08**				**- 0.05**
	mol B-A	0.01	0.02		-0.01	0.01				-0.02
Cu-S(M171) (Å)	mol A	2.85	2.74	2.79	NR	-0.11		-0.05	0.06	
(axial)	mol B	2.98	2.80	2.82	NR	-0.18		-0.02	0.16	
	< AB >	**2.92**	**2.77**	**2.81**		**- 0.15**		**- 0.03**	**+ 0.11**	
	mol B-A	0.13	0.06	0.03		-0.07		0.03	0.10	
ANGLES										
I - (M171)S-Cu-N(H85) (°)	mol A	80.5	81.2	81.9	NR	0.6		- 0.7	- 1.4	
(axial-bond to equa-	mol B	76.8	81.9	81.1	NR	5.0		0.8	- 4.3	
torial-bond angle)	< AB >	**78.7**	**81.5**	**81.5**		**+ 2.8**		**0.0**	**- 2.8**	
	mol B-A	-3.7	0.7	-0.8		4.4		1.5	- 2.9	
II - (M171)S-Cu-N(166) (°)	mol A	107.8	113.3	NR	NR	5.5				
(axial-bond to equa-	mol B	104.4	111.5	NR	NR	7.1				
torial-bond angle)	< AB >	**106.1**	**112.4**			**+ 6.3**				
	mol B-A	-3.4	-1.8			1.6				
III - M171)S-Cu-S(C159) (°)	mol A	109.1	113.7	115.7	NR	4.6		- 2.0	- 6.6	
(axial-bond to equa-	mol B	109.2	109.8	120.7	NR	0.6		- 10.8	- 11.5	
torial-bond angle)	< AB >	**109.1**	**111.8**	**118.2**		**+ 2.6**		**- 6.4**	**- 9.0**	
	mol B-A	0.1	-3.9	5.0		- 4.0		- 8.9	- 4.9	
α - (H85)N-Cu-C(C159) (°)	mol A	146.0	144.6	159.9	152.1	- 1.4	- 7.8	- 15.3	- 13.8	6.0
(inter equato-	mol B	147.4	147.7	157.6	149.9	0.3	- 7.8	- 9.9	- 10.2	2.4
rial-bond angle)	< AB >	**146.7**	**146.2**	**158.7**	**151.0**	**- 0.5**	**- 7.8**	**- 12.6**	**- 12.0**	**+ 4.2**
	mol B-A	1.4	3.1	- 2.3	- 2.2	1.8	0.1	5.4	3.6	- 3.6
β - (C159)S-Cu-N(166) (°)	mol A	109.2	104.4	NR	103.0	- 4.8				- 6.2
(inter equato-	mol B	108.9	106.3	NR	104.2	- 2.6				- 4.7
rial-bond angle)	< AB >	**109.1**	**105.4**		**103.6**	**- 3.7**				**- 5.5**
	mol B-A	- 0.3	2.0		1.2	2.2				1.5
γ - (H166)N-Cu-N(85) (°)	mol A	97.8	97.7	NR	95.0	- 0.1				- 2.8
(inter equato-	mol B	100.0	96.2	NR	94.9	- 3.8				- 5.1
rial-bond angle)	< AB >	**98.9**	**96.9**		**94.9**	**- 2.0**				**- 3.9**
	mol B-A	2.1	- 1.5		- 0.2	- 3.7				- 2.3

Table S2 : Detailed copper centre distances and angles and comparison between different AcoP forms. Left side: as in table S1, distance and angle values for for each AcoP monomer (A and B), average value (< AB >: bold values) and discrepancy in bond length and angles between monomer A and B (mol B-A: grey values). Angles (I, II, III, α, β, γ) are named according to what shown in Supp Figure 3. Right side: differences for the same bond lengths and angles are shown for different AcoP forms, assuming that AcoP mutants H166A (constantly reduced and non-oxidable) and M171A (constantly oxidized and non-reducible) might mimic, at least partially, the reduced and oxidized AcoP WT forms. Changes in lengths and angles are calculated as (ox – red) or (ox-like – red-like) operations. The color code is the same as in Table S1, and is reported also for monomer A and B separately (light green and light red boxes, respectively, for increase and decrease).
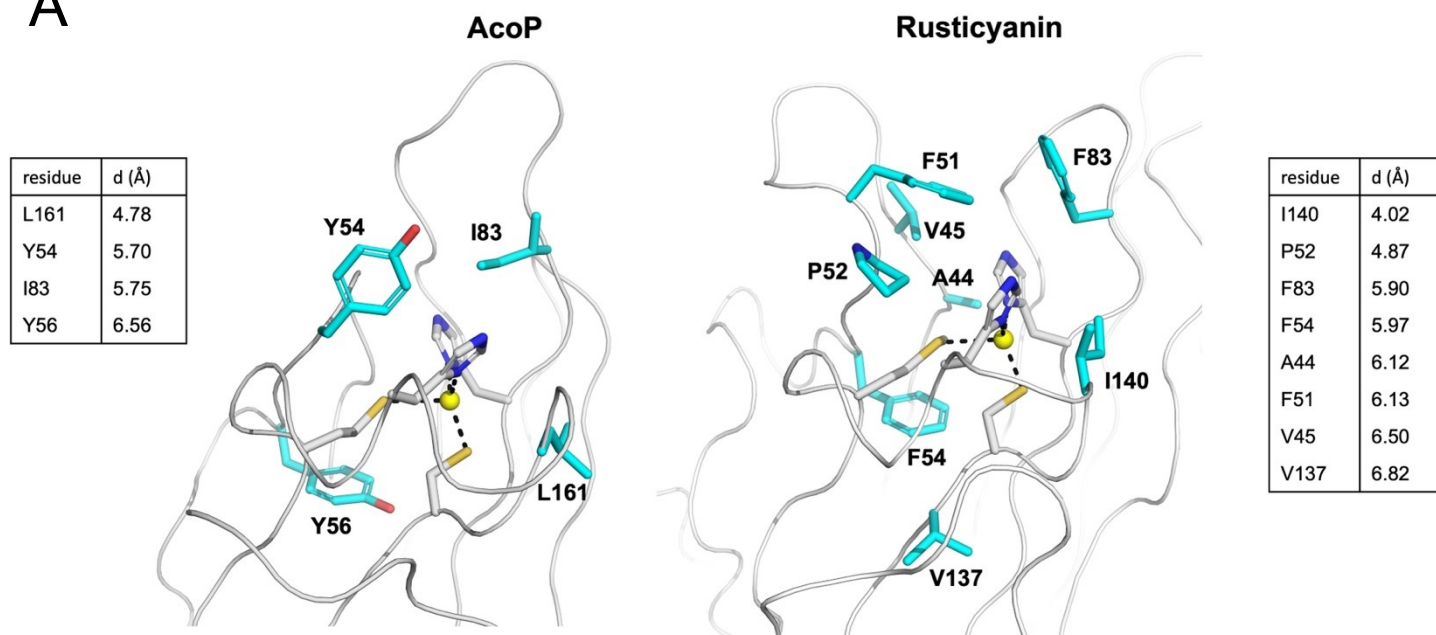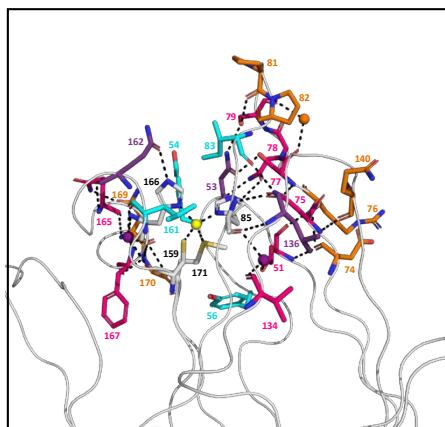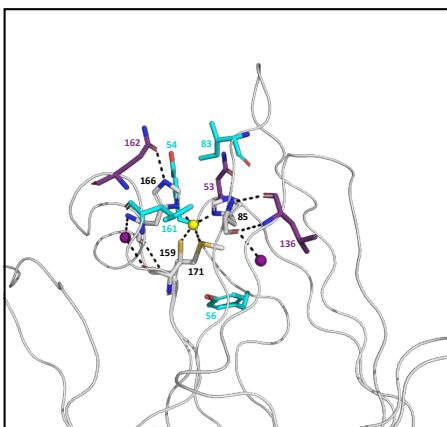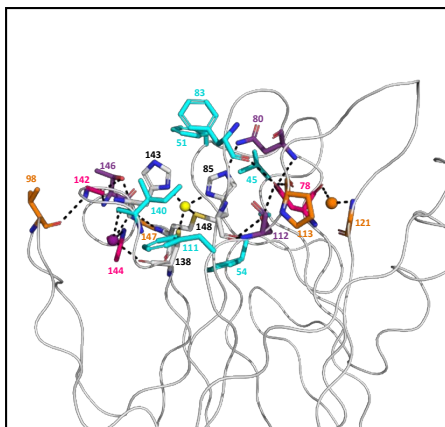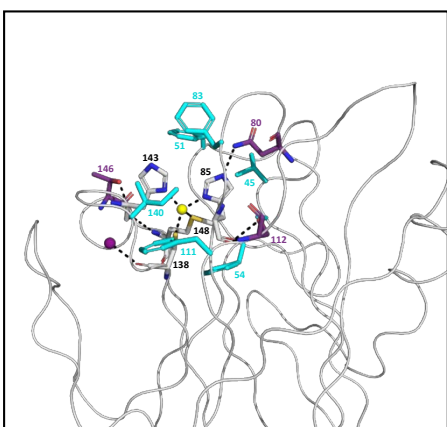
Figure S5 : Second coordination sphere features in AcoP and other cupredoxins. A) Hydrophobic residues in the vicinity of AcoP and Rusticyanin (PDB: 1RCY) metal centres, whose distances to the copper ion are shorter than 7Å, are displayed in cyan. These residues, as well as the minimum distance (d) between their side chains and the copper, are listed in the enclosed tables. B) (next page) For four cupredoxins, hydrophobic residues within the same range are represented, surrounded by the hydrogen-bond networks described in Figure 5 of the main text: the first shell only (left panel) and the three shells (right panel) of residues connected to copper ligands by hydrogen bonds are depicted. As it can be easily seen, the biggest differences between the four analyzed cupredoxins are due to residues that are two or three hydrogen bonds away from copper ligands.
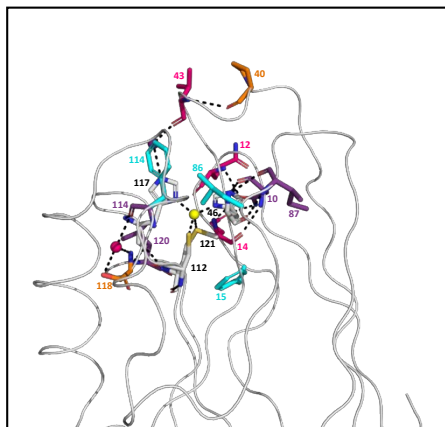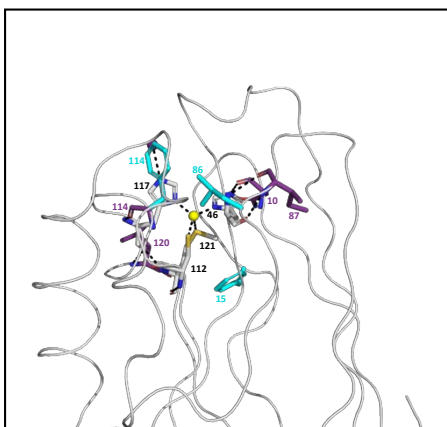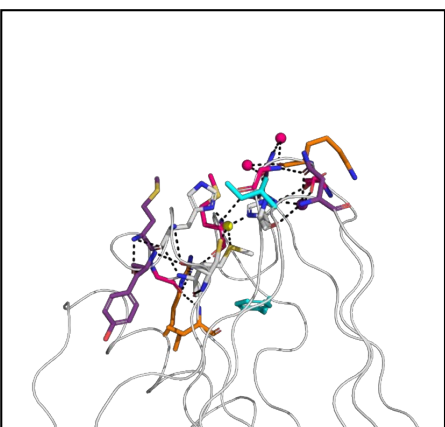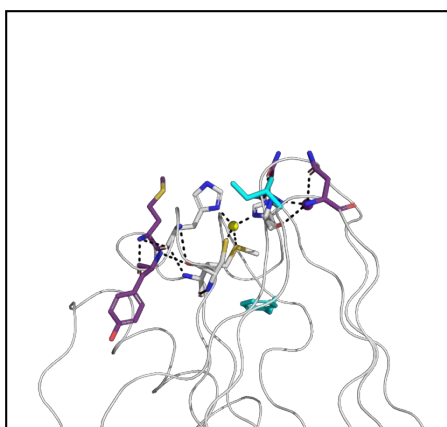
AcoP

Rusticyanin

Azurin

Pseudoazurin

B

Figure S6 : DPI-derived and other errors on Cu-S(Cys) and Cu-S(Met) distances. (A) The errors shown are those associated with the set of cupredoxin metal centre distances used to trace Figure 6 (main text). The drawn errors are derived from the DPI positional errors of Cu, S and N atoms, and correspond to the square root of $DPI_{Cu}^2 + DPI_{S,N}^2$ for a distance between Cu and S or N atoms. The DPI approach redistributes per atom the average positional error of a pdb structure as a function of the atomic B-factors: as such, atoms with the same B-factors are attributed an identical DPI error. (B) Distances and errors in structures with redundant asymmetric units. The precision of crystallographically-derived distances might be even higher than what suggested by panel A, especially for lower resolution structures. A very good example is the structure of Ami-TtCuA solved at 2.3 Å resolution in the space group P 2_1 2_1 2_1. In fact, in this case, the eight molecules present in the crystallographic asymmetric unit represent eight independent measurements, yet derived from one experimental dataset only (so, highly comparable in terms of quality and statistics). As it can be easily seen, the absolute uncertainty and the corrected standard deviation calculated on the average Cu-ligand distances in the eight molecules represented about 20% and 13%, respectively, of the DPI-derived error. Similar trends can be derived from the pdb structures of two more cupredoxins, not included in our CSC plot because of their poor resolution: auracyanin B (1OV8, 4 subunits per asymmetric unit) and spinach plantacyanin (1F56, 3 subunits per asymmetric unit), solved at 1.9 and 2.1 Å resolution respectively. Absolute uncertainty was calculated using the formula (max-min)/2, where max and min are the longer and the shorter measurements for a set of distances, and corrected standard deviation was calculated using the square root of $\Sigma^N (x - <x>)^2 / (N - 1)$, where N is the number of measurements. The DPI assumption is that atoms with same B-factors have same positional errors. If this is possibly true for C, N and O atoms, in the case of Cu, 28 electrons, instead of 7 in average, more strongly contribute to the crystallographic structure factors, implying that the error could be, for same B-factors, much smaller for Cu atoms than what predicted by DPI analysis, at least for structures at ~2.0 Å resolution.

Panel B tables:

distances in 5U7N (9 subunits ; 2.3 Å)

molecule	Cu-Met	Cu-Cys
A	2,33	2,38
B	2,43	2,5
C	2,37	2,4
D	2,32	2,44
E	2,29	2,27
F	2,27	2,44
G	2,36	2,46
H	2,37	2,37

errors in 5U7N :	Cu-Met err	Cu-Cys err	% of DPI err	% of DPI err
DPI-derived	0,47	0,47	100,0	100,0
absolute uncertainty	0,080	0,115	17,0	24,2
corrected standard deviation	0,0509	0,0703	10,8	14,8

distances in 1OV8 (5 subunits ; 1.9 Å)

molecule	Cu-Met	Cu-Cys
A	2,896	2,067
B	2,876	2,189
C	2,917	2,013
D	2,913	2,183

errors in 1OV8 :	Cu-Met err	Cu-Cys err	% of DPI err	% of DPI err
DPI-derived	0,19	0,19	100,0	100,0
absolute uncertainty	0,021	0,088	10,9	46,3
corrected standard deviation	0,0187	0,0872	10,0	45,9

distances in 1F56 (3 subunits ; 2.05 Å)

molecule	Cu-Met	Cu-Cys
A	2,717	2,173
B	2,776	2,194
C	2,603	2,183

errors in 1F56 :	Cu-Met err	Cu-Cys err	% of DPI err	% of DPI err
DPI-derived	0,29	0,27	100,0	100,0
absolute uncertainty	0,086	0,011	30,2	3,9
corrected standard deviation	0,0879	0,0105	30,7	3,9