Supplementary information for

Engineering the Metal-binding Loop of a Blue Copper Protein by Circular Permutation

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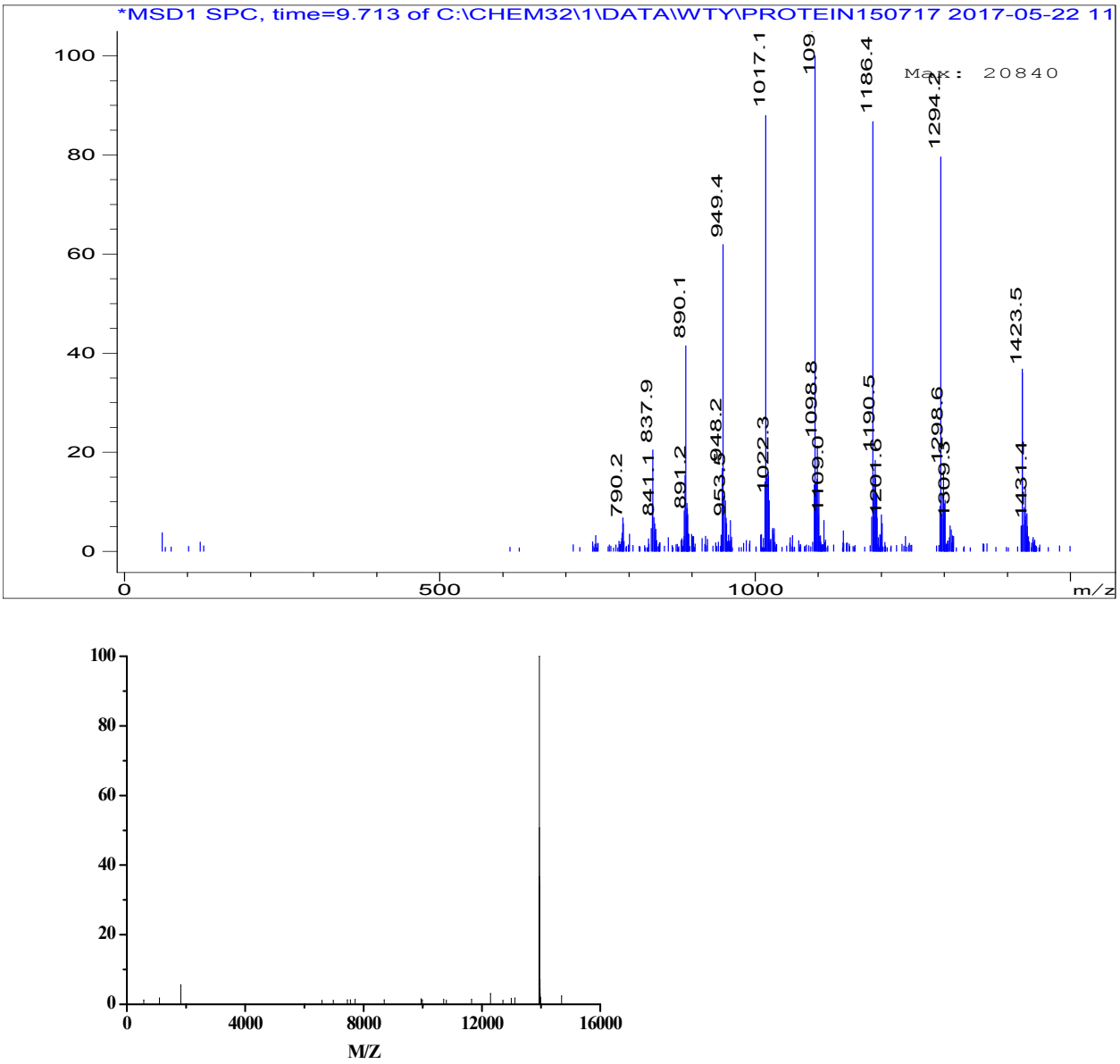

Figure S1. ESI Mass spec of Az-loop, the lower panel showed the deconvoluted spectrum. Az-loop, calculated: 14230, observed: 14226.

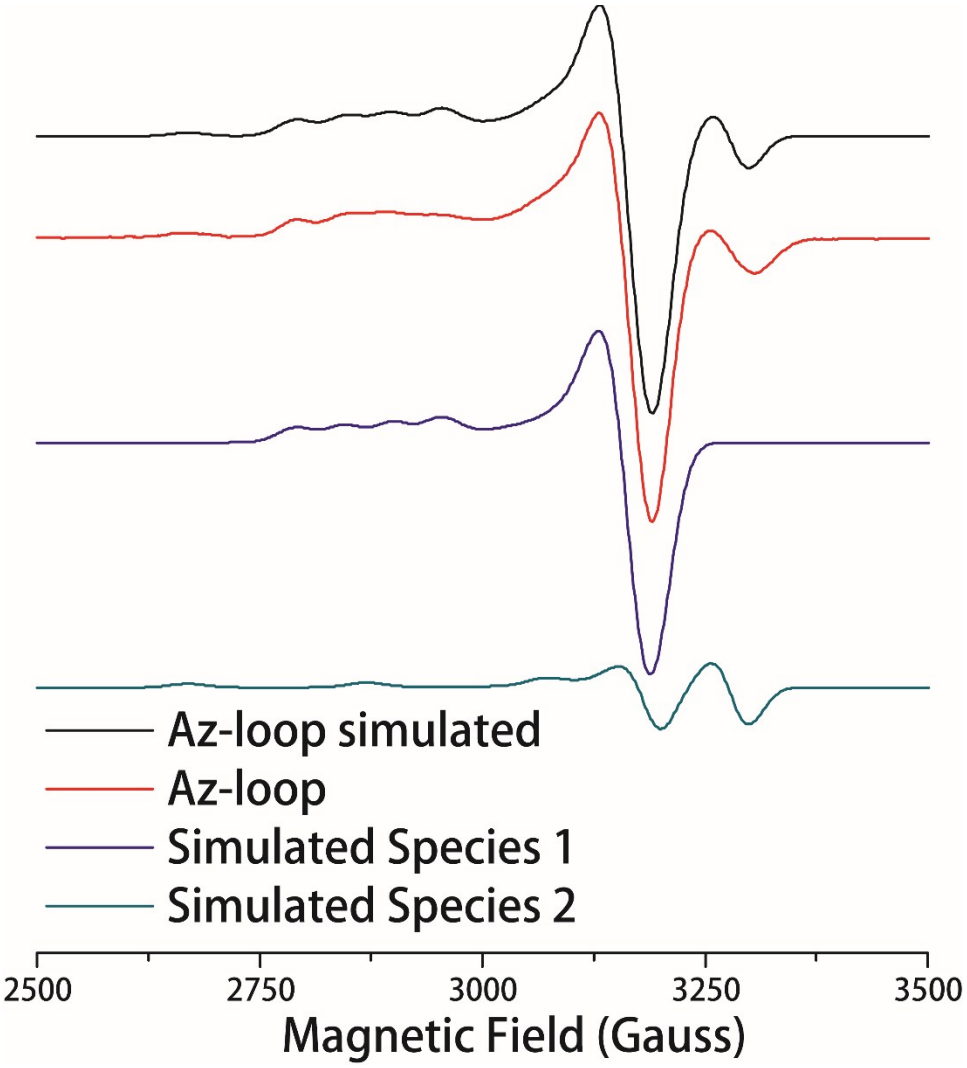

Figure S2. EPR spectrum Az-loop and simulation.

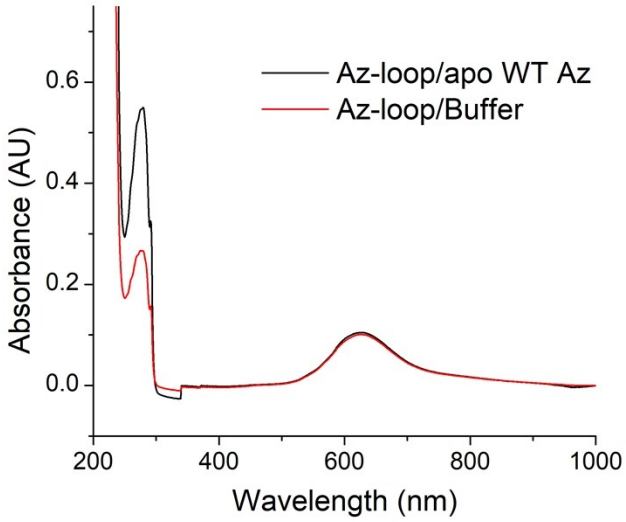

Figure S3. Absorption spectra of Cu(II) loaded Az-loop with equal volume, same concentration of WT Az (black) or buffer (red). Cu(II)-loaded Az-loop was incubated with apo WT Az for 0.5 h before the spectrum was taken.

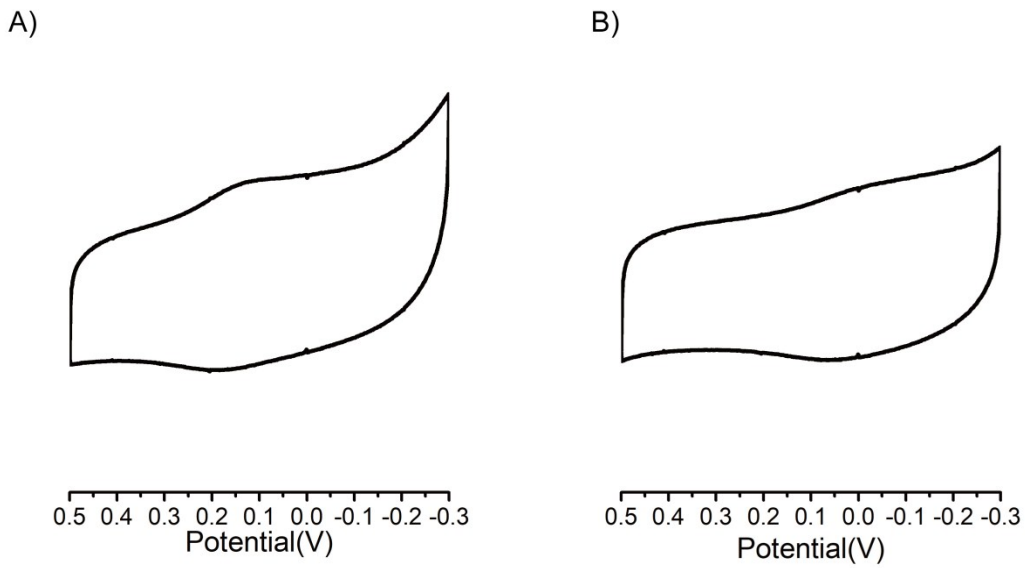

Figure S4. Cyclic voltammogram of Az-loop at pH 4 (left) and pH 7 (right).

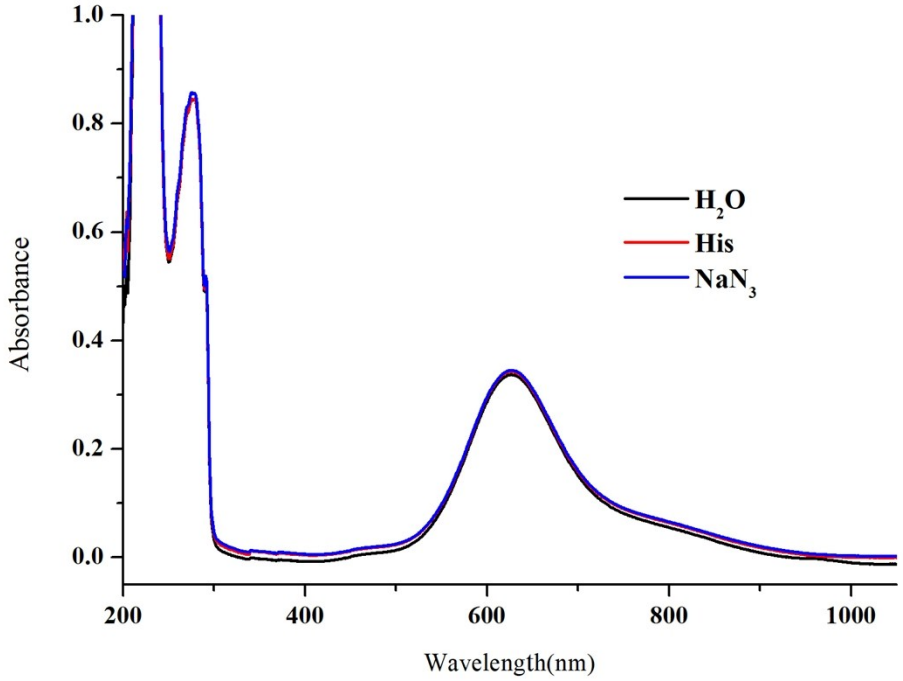

Figure S5. Electronic absorption spectra of Az-loop (0.1 mM) upon addition of water, histidine (3 mM) and NaN_3 (3 mM).

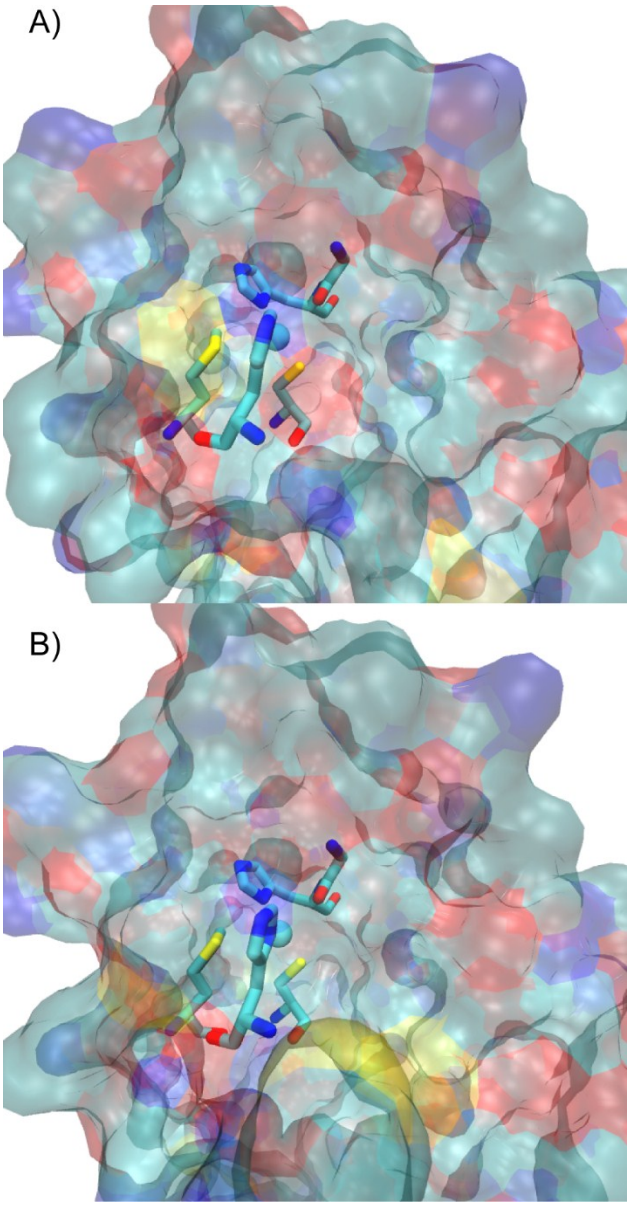

Figure S6. Solvent accessibility to copper site in WT Az (A) and Az-loop (B).

Table S1. EPR simulation parameters

		g_x	g_y	g_z	A_x /10^{-4} cm^{-1}	A_y /10^{-4} cm^{-1}	A_z /10^{-4} cm^{-1}	Ratio
WT Az		2.032	2.054	2.256	8.0	8.0	54.3	1
Az-loop	Species 1	2.032	2.054	2.256	8.0	8.0	54.3	0.84
	Species 2	2.036	2.043	2.182	8.0	8.0	200.0	0.16

The assignment of x vs. y is arbitrary.

Table S2. Statistics of data-collection and refinement

Diffraction data	
Space group	*P*2
Cell dimensions	
a, b, c (Å)	49.9, 88.0, 66.1
α, β, γ (°)	90, 110.2, 90
Resolution range (Å)	62.02-1.66 (1.69-1.66)
Number of unique reflections	62796
Data completeness (%)	99.5 (100)
Redundancy	7.4 (7.4)
$\langle I \rangle / \langle \sigma(I) \rangle$	48.4 (9.1)
R_{merge} [a]	0.113 (0.530)
Refinement	
R-factor / R_{free} [b]	0.162/0.217
Number of reflections used	59724
r.m.s.d. bond length (Å)	0.008
r.m.s.d. bond angles (°)	1.356
Mean *B* factor (Å^2)	
protein main-chain atoms	22.9
protein side-chain atoms	26.9
water molecules	33.8
Cu	18.5
No. atoms	
protein	4033
water molecules	496
Cu	4
Ramachandran plot statistics	
In preferred regions (%)	97.0

In allowed regions (%)	3.0
Outliers (%)	0

[a] $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $\langle I(hkl)\rangle$ is the main value of $I(hkl)$.

[b] R-factor $= \sum||F_{obs}| - |F_{calc}||/\sum|F_{obs}|$, where F_{obs} and F_{calc} are observed and calculated structure factors.

The free R factor was calculated using 5% of reflections omitted from the refinement.

Table S3 Distances between ligands and copper for Az-loop

Distance (Å)	Chain A	Chain B	Chain C	Chain D	WTAz
S_{Cys131}-Cu	2.179	2.186	2.140	2.181	2.24
N_{His65}-Cu	2.081	2.089	2.146	2.075	2.01
N_{His3}-Cu	2.089	2.088	2.071	2.093	2.08
S_{Met7}-Cu	3.263	3.357	3.323	3.220	3.15
O_{Gly64}-Cu	2.729	2.753	2.825	2.766	2.97
N_{Asn66}-S_{Cys131}	3.492	3.520	3.525	3.482	3.61
N_{Phe133}-S_{Cys72}	3.448	3.433	3.485	3.492	3.46
O^{γ}_{Asn66}-N_{Thr132}	2.928	2.894	2.853	2.923	2.89
N^{δ}_{Asn66}-O^{γ}_{Thr132}	2.987	2.962	2.877	2.956	2.91