Supporting information for

Cu(I) Controls Conformational States in Human Atox1 Metallochaperone: An EPR and Multiscale Simulation Study

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Table S1. Free energy profiles for proton transfer between Cys15 and Lys60.

	$\Delta G^{\ddagger}$	ΔG_r (kcal/mol)
PMF-indirect proton transfer	21.5	19.5
PMF-direct proton transfer	28.4	27.0

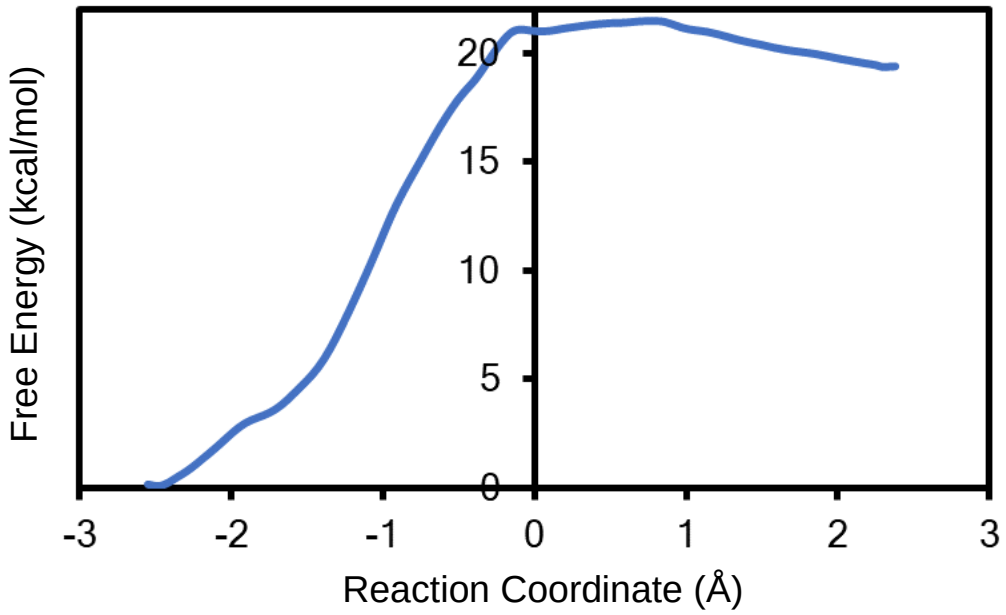

Figure S1. Free energy profiles for an indirect proton transfer between Lys60 and Cys15 via a bridging water molecule.

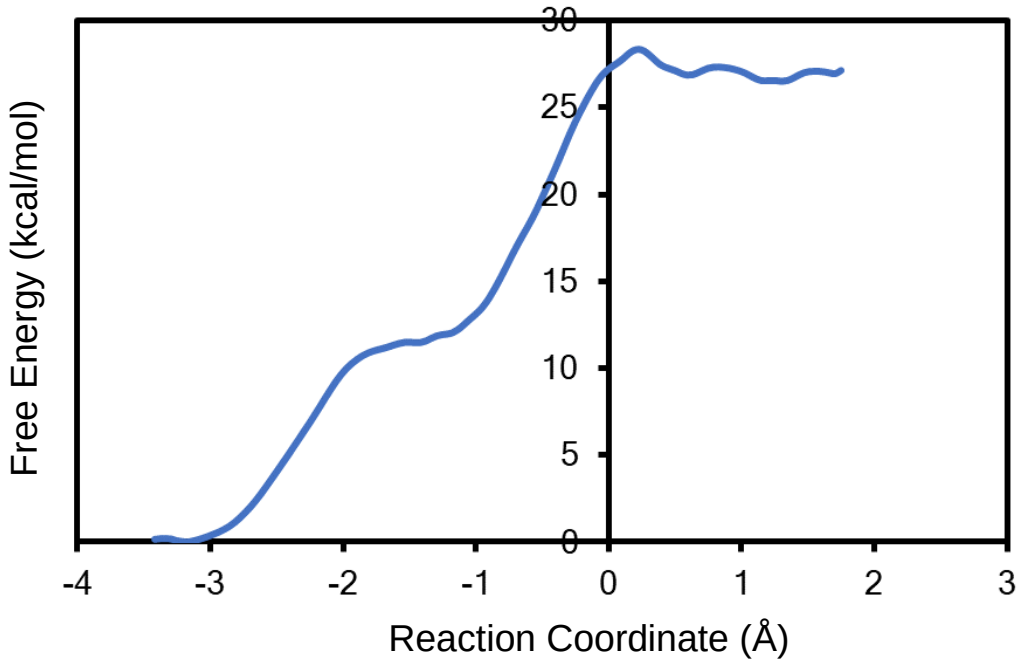

Figure S2. Free energy profile for a direct proton transfer between Lys60 and Cys15.

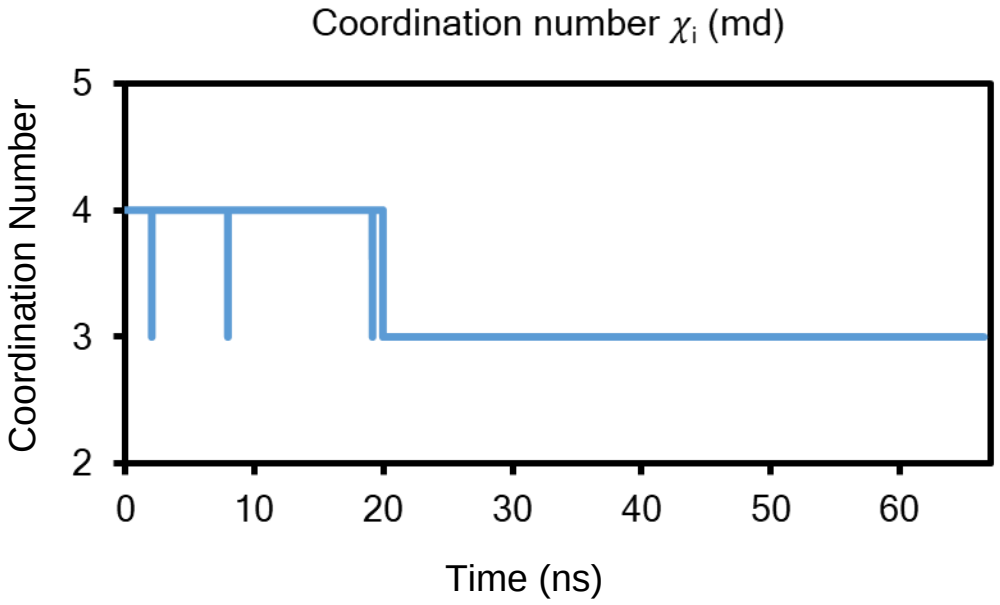

Figure S3. Atox1-Cu(I) coordination states during MD simulation (first replica).

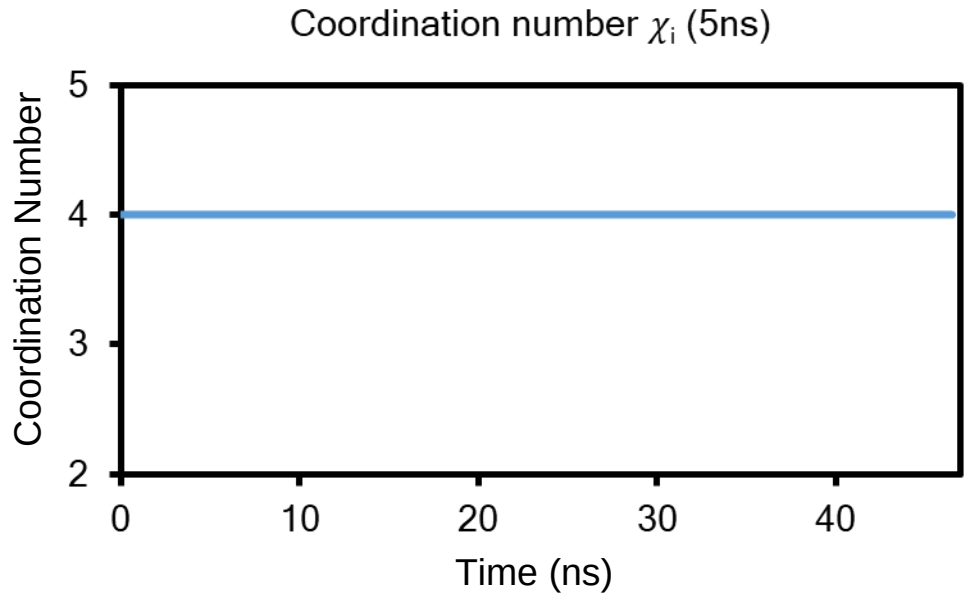

Figure S4. Atox1-Cu(I) coordination states during MD simulation (second replica).

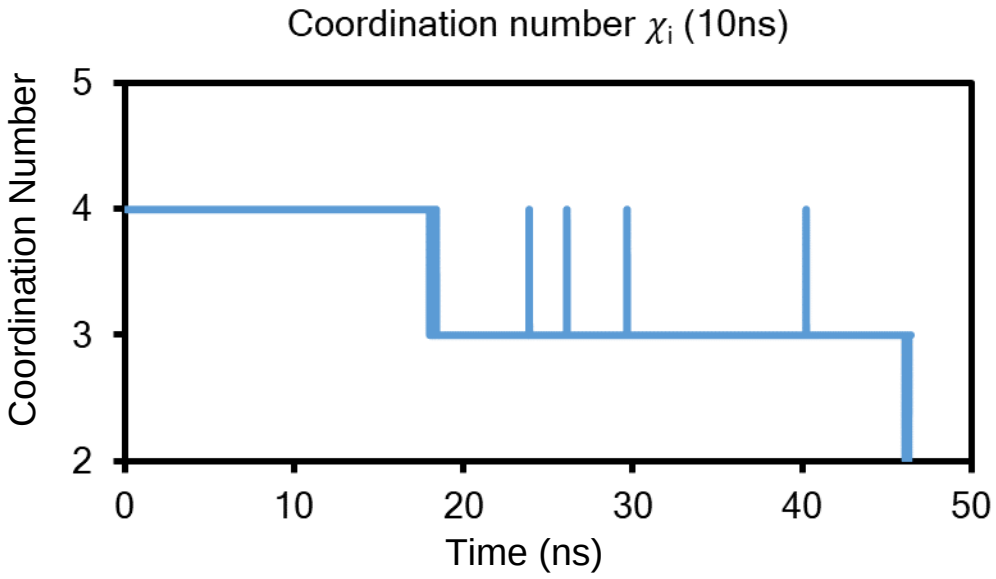

Figure S5. Atox1-Cu(I) coordination states during MD simulation (third replica).

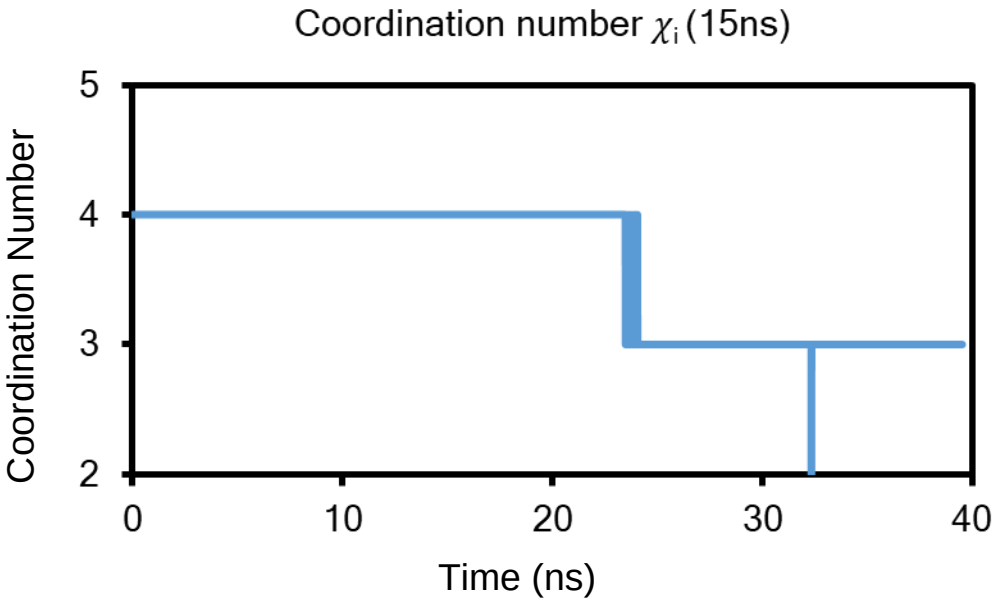

Figure S6. Atox1-Cu(I) coordination states during MD simulation (fourth replica).

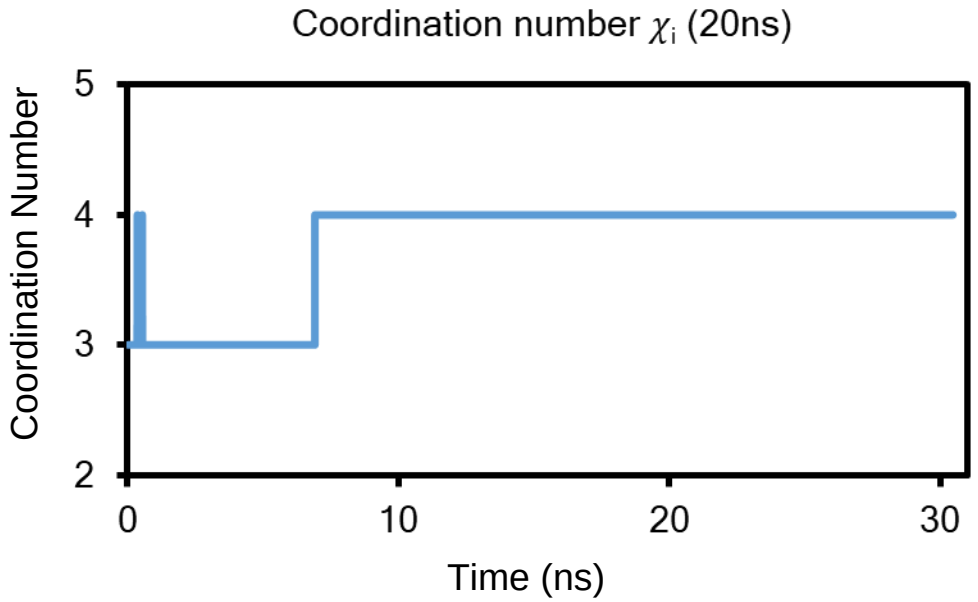

Figure S7. Atox1-Cu(I) coordination states during MD simulation (fifth replica).

Mathematical definition of coordination state in Atox1 (χ_i):

$$\chi_i = 4 - \sum_{i=1}^{4} \delta_i \qquad \delta_i = \begin{cases} 0 & if\ (r_i < 3.0) \\ 1 & if\ (r_i \geq 3.0) \end{cases}$$

Table S2. Total coordination abundance (based on five MD replicas).

Coordination #	**Abundance (%)**
3	42.5
4	57. 5

Table S3. Total coordination swaps (based on five MD replicas).

Coordination change	**Occurrence**
3 to 4	9
4 to 3	11
3 to 2	2
2 to 3	2

Table S4. Averaged distances (Å) for both coordination states (based on five MD replicas).[a]

	4	**3**
S(Cys12A)-Cu	2.17±0.08	2.12±0.07
S(Cys15A)-Cu	2.2±0.1	3±1
S(Cys12B)-Cu	2.16±0.08	2.13±0.08
S(Cys15B)-Cu	2.2±0.1	2.6±0.8
S(Cys15A)-N(Lys60A)	5.1±0.3	5.3±0.8
S(Cys15B)-N(Lys60B)	5.2±0.4	4.9±0.7
O(Thr11A)-S(Cys12B)	4±1	4±1
O(Thr11B)-S(Cys12A)	4±1	5.2±0.9
N(Lys60A)-O(Gly14B)	5±1	8±1
N(Lys60B)-O(Gly14A)	5±1	8±1
N(Lys60A)-O(Gly14A)	5±1	7±1
N(Lys60B)-O(Gly14B)	5±1	6.2±0.5
N(Lys60A)-N(Lys60B)	3.8±0.4	3.8±0.4

[a] The MD trajectories were divided into two groups based on the main Cu coordination states, 3 and 4 using the definition appearing in conjunction with Table S2.

UV−Vis measurements were performed using a Carry 5000 spectrometer at room temperature. Measurements were carried out in a high-precision cell with a 10 mm optical path length. cell purchased from Hellma Analytics.

BCA (Bicinchoninic acid disodium salt hydrate; Sigma-Aldrich, St. Louis, MO, USA) was added to Atox1 samples and their UV-VIS spectra were recorded. The BCA concentration was 120 μM to ensure the absence of free Cu(I) from the solution. Spectra were recorded from 200 to 800 nm with a step size and bandwidth of 0.5 nm. The spectra were baselined according to the absorption value at 800 nm, which is zero.

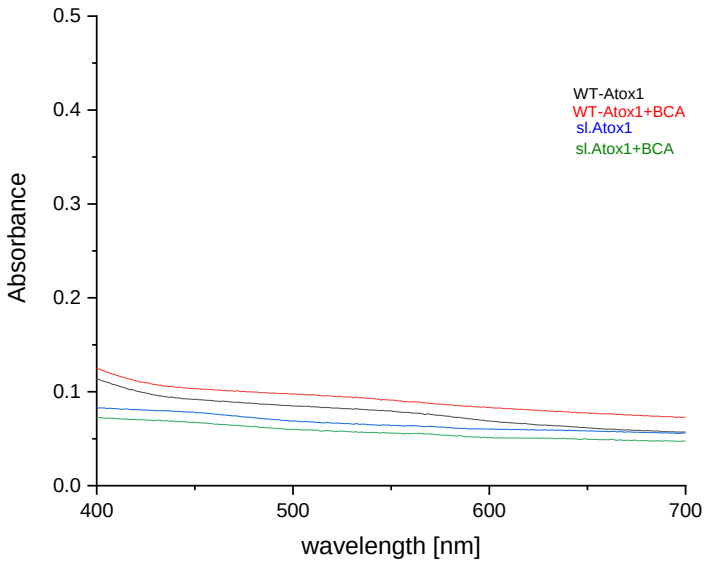

Figure S8. UV-Visible spectra of 60 μM WT-Atox1 and 60 μM spin-labeled (sl) Atox1 in the absence and in the presence of 120 μM BCA reagent. The absorption peak at 562 nm, which corresponds to Cu(I)-BCA_2 complex was not observed in UV-Vis measurements. These results confirm the absence of Cu(I) in the protein samples.

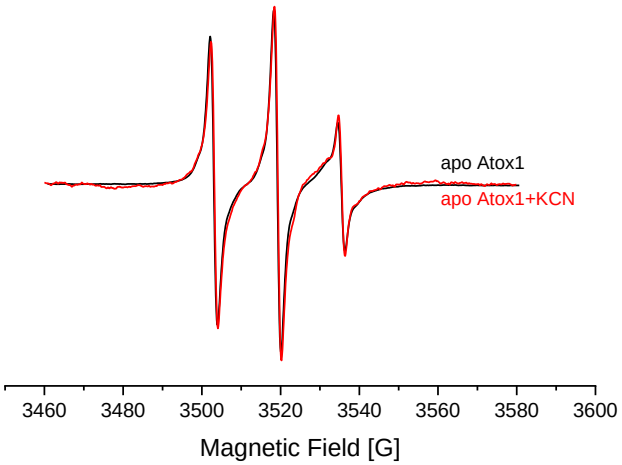

Figure S9. CW-EPR spectra of apo WT-Atox1 in the absence and presence of 0.1 mM KCN. The specra are identical confirms no free Cu(I) ions after dialysis in the solution.