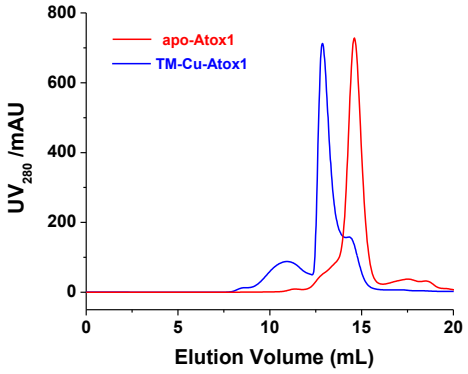
Electronic Supplementary Material (ESI) for Metallomics.

Supporting Information

for

Tetrathiomolybdate Inhibits the Reaction of Cisplatin with Human Copper Chaperone Atox1

A



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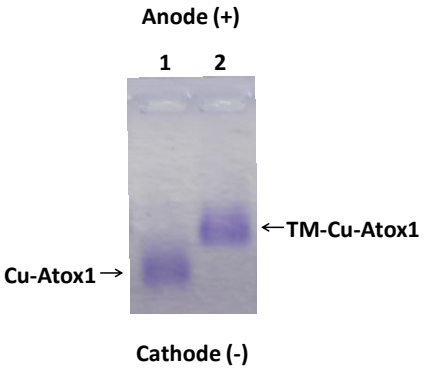

Fig. S1 Formation of the trimeric TM-Cu-Atox1 complex. (A) Analytical size exclusion chromatography analysis of apo-Atox1 (red) and TM-Cu-Atox1 complex (blue). The TM-Cu-Atox1 complex was prepared by incubation of 800 µM Cu-Atox1 with 320 µM TM for 2 hours. The main peak of TM-Cu-Atox1 appears at the position corresponding to trimeric species. In comparison, the apo-Atox1 appears as a monomeric protein. (B) Electrophoresis analysis on native agarose gel. 1: Cu-Atox1; 2: Cu-Atox1+ 0.4 TM. A 0.8% agarose gel was used in 25 mM Tris-Cl buffer (pH 8.5, 19.2 mM glycine).

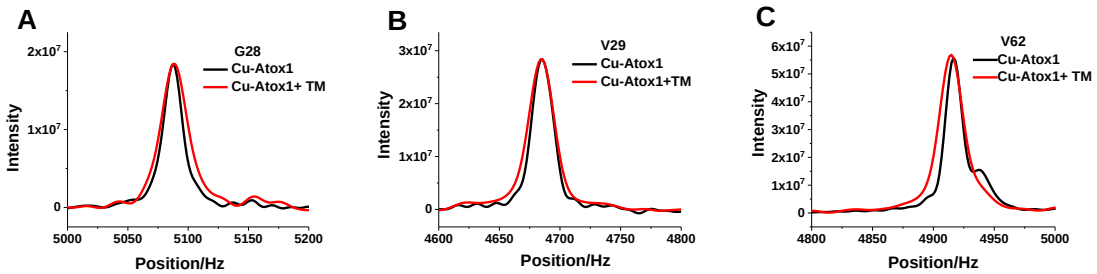

Fig. S2 Lineshape of representative NMR signals. The slices of ^{1}H dimension were taken from ^{1}H ^{15}N HSQC spectra in Fig. 2B. (A) G28; (B) V29; (C) V62. The color of lines denotes the signal of Cu-Atox1 in the absence (black) or in the presence (red) of TM.

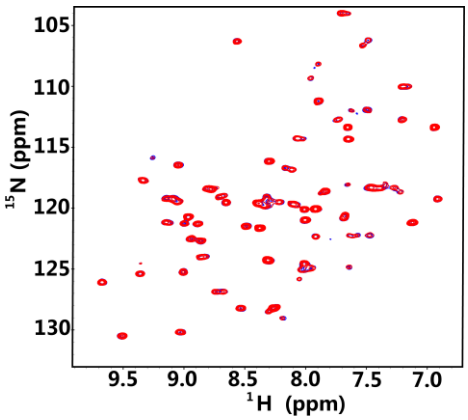

Fig. S3 ^{1}H-^{15}N HSQC NMR spectra of the TM-Cu-Atox1 complex incubated with 1.3 eq. of cisplatin for 2 h (blue) and 8 h (red). The two sets of peaks are well overlapped. This result shows that no reaction occurs between TM-Cu-Atox1 and cisplatin even after 8 h incubation.

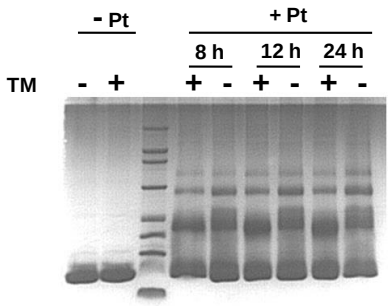

Fig. S4 Effect of TM on the aggregation of apo-Atox1 induced by cisplatin. The reactions were conducted using 300 µM apo-Atox1 or [TM + apo-Atox1] and 1.3 molar equivalents of cisplatin. The sample of [TM + apo-Atox1] was prepared by incubation of 300 µM apo-Atox1 with equimolar TM for 2 h. Samples were analyzed using Tricine-SDS-PAGE analyses after 8, 12 or 24 h incubation (reaction time is labeled). The results show that TM does not inhibit the aggregation of apo-Atox1.

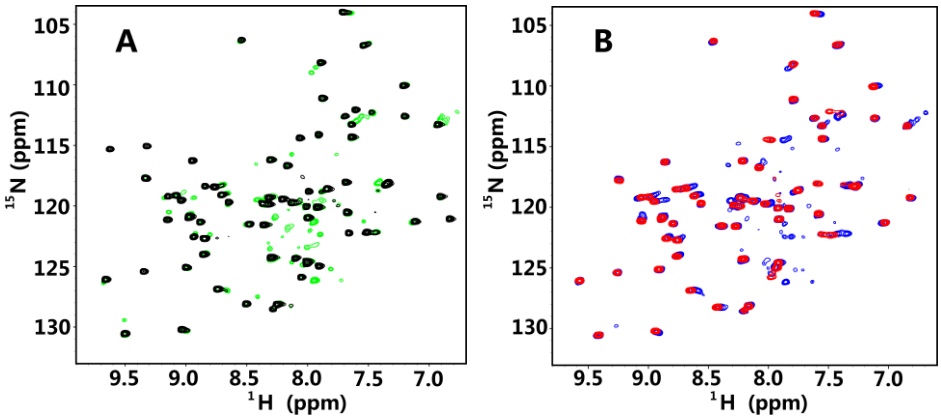

Fig. S5 Effect of TM on cisplatin binding to Ag-Atox1 monitored by ^{1}H-^{15}N HSQC spectroscopy. (A) Spectra of Ag-Atox1 before (black) and after (green) reaction with cisplatin (1.3 eq. for 8 h). (B) Spectra of the mixture of Ag-Atox1 and 0.4 eq. TM before (red) and after (blue) reaction with cisplatin (1.3 eq. for 8 h). A 0.5 mM concentration of ^{15}N-labeled protein was used in all these NMR experiments.

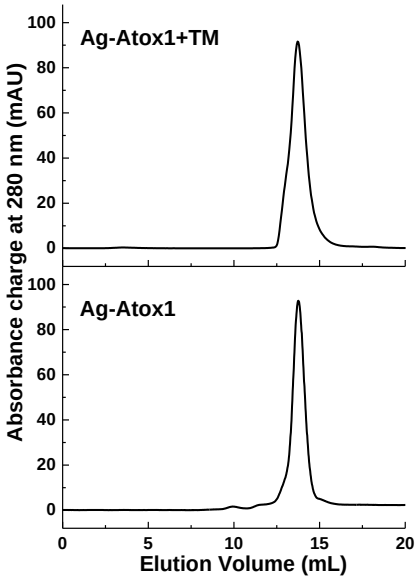

Fig. S6 Size exclusion chromatographic analysis of Ag-Atox1 in the absence and in the presence of TM. Ag-Atox1 was prepared by incubation of 200 µM apo-Atox1 (in 20 mM HEPES, 150 mM $NaNO_3$, pH 7.4) with 300 µM $AgNO_3$ in the presence of 1 mM DTT for 15 minutes. The [TM + Ag-Atox1] sample was prepared by incubation of 200 µM Ag-Atox1 (in 20 mM HEPES, 150 mM $NaNO_3$, pH 7.4) with 80 µM TM for 2 h.

Table S1. ICP-MS snalysis of metal concentrations in the TM-Cu-Atox1 complex

Species*	Monomer			Trimer			Oligomer		
	Atox1	Cu	Mo	Atox1	Cu	Mo	Atox1	Cu	Mo
Concentration (µM)	0.63	0.32	0.00	3.43	4.41	1.33	4.43	3.62	1.01
Relative ratio to Atox1	1.00	0.51	0.00	1.00	1.29	0.39	1.00	0.82	0.23

* The fractions corresponding to the three peaks in Fig. S1 were collected from the size exclusion chromatographic apparatus.

Table S2. ESI-MS spectra analyses of the platination adducts of Cu-Atox1

	Composition	Observed *m/z*	Calculated *m/z*
1	$[Atox1+6H]^{6+}$	1279.47	1279.90
2	$[Atox1+Cu+5H]^{6+}$	1290.41	1290.30
3	$[Atox1+Cu+2Na+3H]^{6+}$	1296.80	1297.63
4	$[Atox1+Pt(NH_3)_2+4H]^{6+}$	1317.64	1317.80
5	$[Atox1+Pt(NH_3)_2+H_2O+4H]^{6+}$	1321.47	1320.80