Electronic Supplementary Material (ESI) for Metallomics.

Redox Sulfur Chemistry of the Copper Chaperone Atox1 as Catalyzed by the Enzyme Glutaredoxin 1 is Regulated by Both the Reduction Potential of the Glutathione Couple GSSG/2GSH and the Availability of Cu(I)

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Supporting Information

Determination of K_D for variants hGrx1-C23S and -C26S

In a buffer containing $[Cu^I(Bca)_2]^{3-}$ with p[Cu^+] ~ 13, both variants hGrx1-C23S and hGrx1-C26S removed Cu(I) from the probe complex but the protein products precipitated. In the same metal buffer with the free Cu_{aq}^+ buffered at a lower concentration (p[Cu^+] ~ 14), both variants competed for Cu(I) effectively and precipitation was slow. This allowed an estimation of their Cu(I) affinities via eqns 1 and 2 (Figure S1):-[1]

$$[Cu^IL_2]^{3-} + P \rightleftharpoons Cu^I\text{-}P + 2\,L^{2-} \qquad (L = Bca) \tag{1}$$

$$\frac{[P]_{total}}{[Cu]_{total}} = K_D\beta_2\left(\frac{[L]_{total}}{[Cu^IL_2]} - 2\right)^2[Cu^IL_2]\left(1 - \frac{[Cu^IL_2]}{[Cu]_{total}}\right) + 1 - \frac{[Cu^IL_2]}{[Cu]_{total}} \tag{2}$$

where the term $[Cu^IL_2]$ is the equilibrium concentration of probe complex $[Cu^IL_2]^{3-}$ in eqn 1 and may be determined directly from the solution absorbance at equilibrium under the condition that this complex is the only absorbing species. The other terms in eqn 2 are the known total concentrations of the relevant species. Curve fitting of the experimental data to eqn 2 based on the known β_2 for $[Cu^I(Bca)_2]^{3-}$ ($10^{17.2}$ M^{-2})^2 estimated $K_D = 10^{-13.5}$ M and $10^{-13.3}$ M for hGrx1-C23S and hGrx1-C26S, respectively (Figure S1). However, such weaker Cu(I) binding caused protein precipitation readily, likely due to considerable conformation change upon Cu(I) binding.

References

(1) Xiao, Z.; Gottschlich, L.; van der Meulen, R.; Udagedara, S. R.; Wedd, A. G. *Metallomics* **2013**, *5*, 501-13.

(2) Xiao, Z.; Brose, J.; Schimo, S.; Ackland, S. M.; La Fontaine, S.; Wedd, A. G. *J. Biol. Chem.* **2011**, *286*, 11047-55.

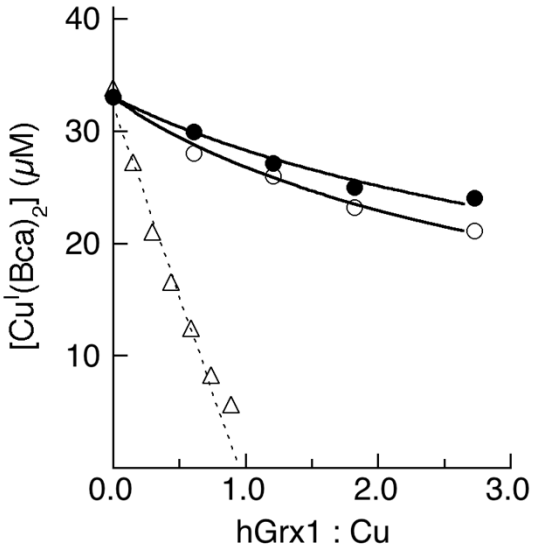


Figure S1. Determination of the Cu(I) affinities for hGrx1-C23S (empty circle) and hGrx1-C26S (solid circle). The probe complex anion $[Cu^{I}(Bca)_2]^{3-}$ was prepared with following compositions: $[Cu]_{tot}$ = 33 μM; $[Bca]_{tot}$ = 200 μM and $[NH_2OH]_{tot}$ = 1.0 mM in KPi buffer (50 mM, pH 7.0). The solid traces are the fitting curves of the experimental data points to eqn (2) which generate Cu(I) logK_D = -13.5 and -13.3 for hGrx1-C23S and hGrx1-C26S, respectively. Shown in empty triangles are the experimental data points with hGrex1-wt under the same conditions.

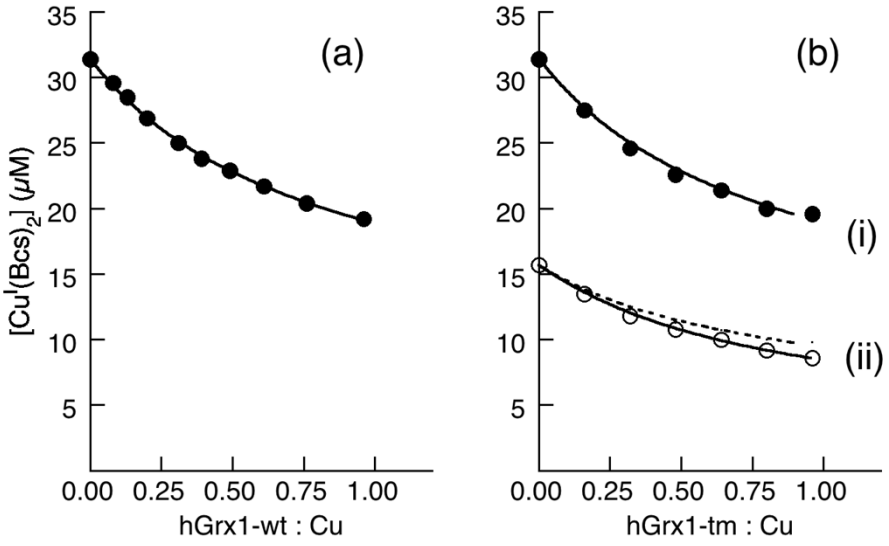


Figure S2. Determination of Cu(I) binding affinity in KPi buffer (25 mM, pH 7.0, 100 mM NaCl). Decrease in $[Cu^{I}(Bcs)_2]^{3-}$ concentration (total composition: $[Cu]_{tot}$ = 31.4 μM; $[Bcs]_{tot}$ = 80 μM; $[NH_2OH]_{tot}$ = 1.0 mM) with increasing concentrations of: (a) hGrx1-wt; (b) hGrx1-tm. The experimental data points shown in empty circles in (ii) were obtained from a 1:1 dilution of each solution in (i). The solid traces are the fitting curves of the experimental data points to eqn (2) and these curve-fittings derive a consistent Cu(I) $\log K_D$ = -15.6 ± 0.1 for both hGrx1-wt and hGrx1-tm proteins. The dash trace in (ii) is the simple 1:1 dilution curve of data set (i).

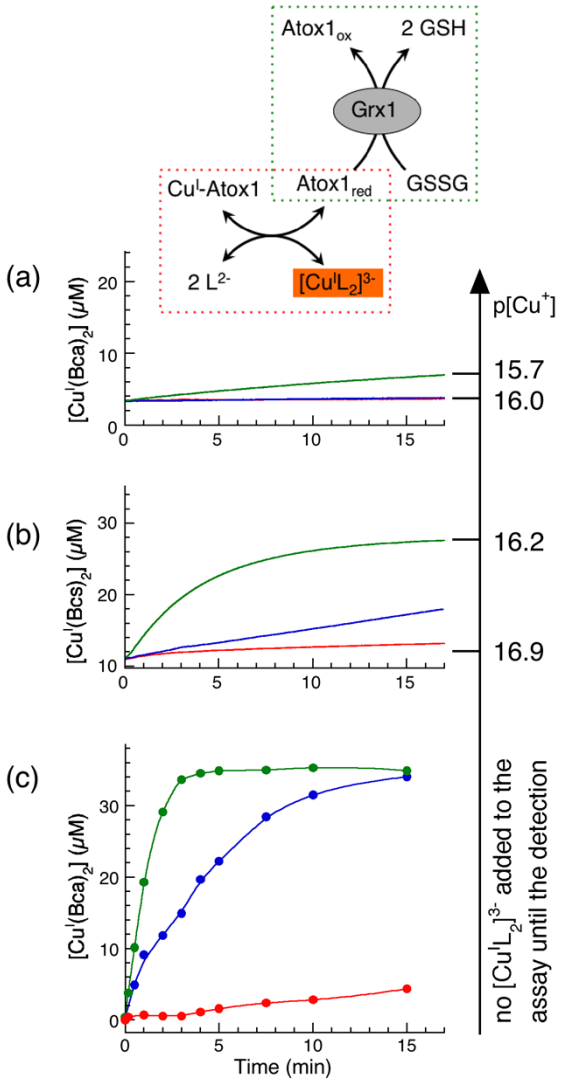


Figure S3. Comparison of oxidation rate of Atox1 (35 μM) by GSSG (400 μM) with catalyst (each 2.0 μM) of hGrx1-wt (shown in blue trace), hGrx1-C26S (shown in green trace) or no catalyst (shown in red trace) in KPi buffer (20 mM; pH 7.0; NH_2OH, 1.0 mM) with free [Cu_{aq}^{+}] buffered to: (a) ≥ 10^{-16} M by Cu_{aq}^{+}-buffer of $[Cu(I)]_{tot}$ = 35 μM and $[Bca]_{tot}$ = 500 μM; (b) ≥ 10^{-17} M by Cu_{aq}^{+}-buffer of $[Cu(I)]_{tot}$ = 35 μM and $[Bcs]_{tot}$ = 140 μM; (c) no Cu_{aq}^{+}-buffer. The rates in (a,b) were followed in real time by increase in concentration of the Cu(I) probe $[Cu^{I}(Bca)_2]^{3-}$ whereas the rate in (c) by quenching the oxidation at various reaction time points with the same probe $[Cu^{I}(Bca)_2]^{3-}$ but less $[Bca]_{tot}$ = 100 μM (see Experimental section for details). Inset: scheme for the assay that consists of the catalysis (framed in green dots) and the associated Cu_{aq}^{+}-buffering and Cu(I)-transferring detection (framed in red dots).

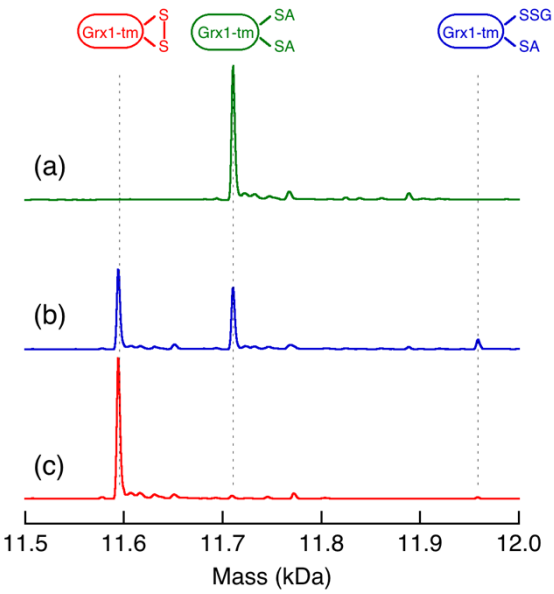


Figure S4. ESI-MS analysis of the oxidized forms of hGrx1 (10 µM) upon oxidation by GSSG in the following redox buffer (potential *E*): (a) GSSG (0.06 mM)/GSH (3.88 mM) (-222 mV); (b) GSSG (0.68 mM)/GSH (0.64 mM) (-145 mV); (c) GSSG (0.39 mM)/GSH (0.03 mM) (-69 mV). Each reaction mixture was incubated overnight under anaerobic condition in KPi (50 mM, pH 7.0), followed by alkylation with iodoacetamide (~10 mM) and ESI-MS analysis.

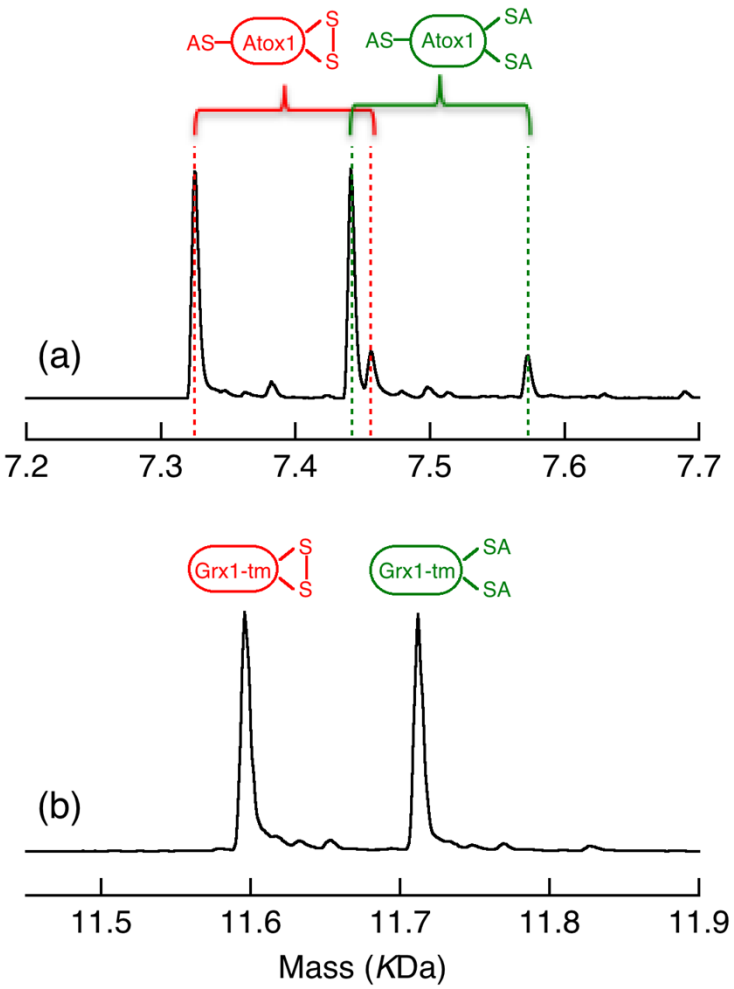


Figure S5. Control experiments for comparison of ESI-MS peak intensities from a solution mixture containing equal molar concentrations of reduced and oxidised forms (both pre-alkylated and quantified separately before mixing) of either Atox1 (a) or hGrx1-tm (b).

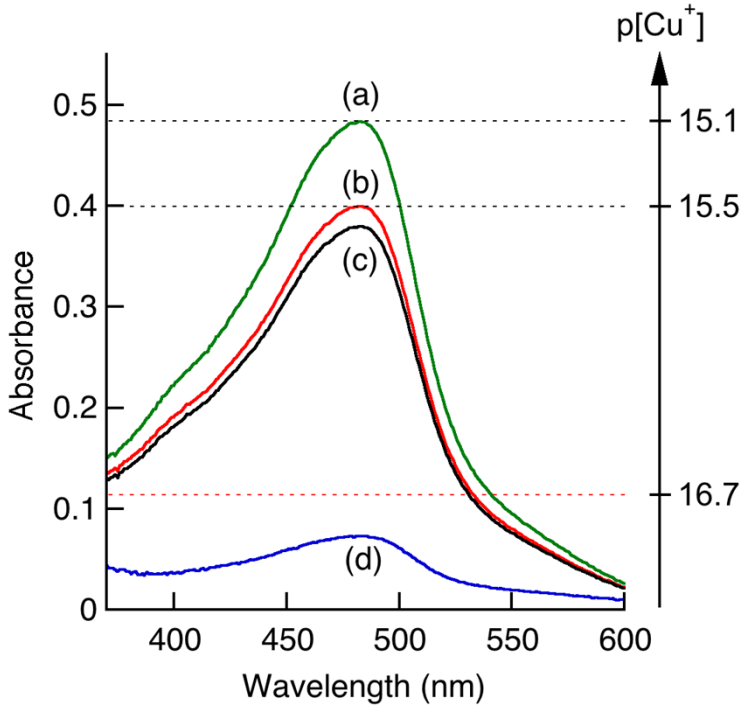


Figure S6. Solution spectra in KPi buffer (50 mM, pH 7.0; 100 µM NH_2OH) with following further composition:

(a) $[Cu^{I}(Bcs)_2]^{3-}$ generated from $[Cu]_{tot}$ = 37 µM and [Bcs] = 100 µM;

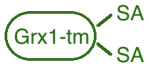
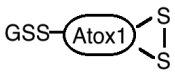
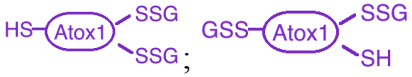
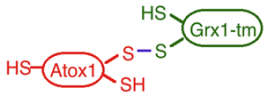
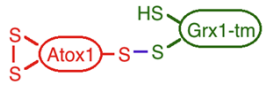
(b) as (a) but with extra GSH (800 µM) (no change with addition of extra 50 µM hGrx1-C26S);

(c) as (b) but with extra hGrx1-tm (50 µM)

(d) as (b) but with extra fully reduced Atox1 (50 µM).

The **red** dashed line indicated the solution absorbance when Atox1-(SS) (50 µM) reached a reduction equilibrium in solution (b).

Table S1. ESI-MS data of protein species [a]

Protein	Formula	MW (Da) (calc.)	MW (Da) (found)
hGrx1-wt	$C_{516}H_{845}N_{143}O_{152}S_5$	11,644.5	11,644.7
hGrx1-C23S	$C_{516}H_{845}N_{143}O_{153}S_4$	11,628.4	11,628.4
hGrx1-C26S	$C_{516}H_{845}N_{143}O_{153}S_4$	11,628.4	11,628.8
hGrx1-C23,26S (hGrx1-dm)	$C_{516}H_{845}N_{143}O_{154}S_3$	11612.4	11,612.2
hGrx1-C8,79,83S (hGrx1-tm)	$C_{516}H_{845}N_{143}O_{155}S_2$	11596.3	11,596.3
	$C_{516}H_{843}N_{143}O_{155}S_2$	11,594.3	11,594.2
	$C_{520}H_{851}N_{145}O_{157}S_2$	11,710.3	11,710.1
	$C_{528}H_{863}N_{147}O_{162}S_3$	11,958.6	11,958.7
Atox1	$C_{322}H_{531}N_{85}O_{101}S_6$	7,401.6	7,401.7
	$C_{317}H_{522}N_{84}O_{100}S_5$	7,270.4	7,270.5
	$C_{322}H_{529}N_{85}O_{101}S_6$	7,399.6	7,399.8
	$C_{317}H_{520}N_{84}O_{100}S_5$	7,268.4	7,268.6
	$C_{324}H_{532}N_{86}O_{102}S_6$	7,456.6	7,456.6
	$C_{319}H_{523}N_{85}O_{101}S_5$	7,325.4	7,325.5
	$C_{328}H_{540}N_{88}O_{104}S_6$	7,572.7	7,572.6
	$C_{323}H_{531}N_{87}O_{103}S_5$	7,441.5	7,441.3
	$C_{332}H_{544}N_{88}O_{107}S_7$	7,704.8	7,704.6
	$C_{327}H_{535}N_{87}O_{106}S_6$	7,573.6	7,573.7
	$C_{336}H_{552}N_{90}O_{109}S_7$	7,821.0	7,821.0
	$C_{331}H_{543}N_{89}O_{108}S_6$	7,689.8	7,689.9
	$C_{342}H_{561}N_{91}O_{113}S_8$	8,012.2	8,012.1
	$C_{337}H_{552}N_{90}O_{112}S_7$	7,881.0	7,881.0
	$C_{352}H_{576}N_{94}O_{119}S_9$	8,317.5	8,317.3
	$C_{347}H_{567}N_{93}O_{118}S_8$	8,186.3	8,186.2
	$C_{838}H_{1374}N_{228}O_{256}S_8$	18,995.7	18,995.5
	$C_{833}H_{1365}N_{227}O_{255}S_7$	18,864.5	18,864.3
	$C_{838}H_{1372}N_{228}O_{256}S_8$	18,993.7	ND
	$C_{833}H_{1363}N_{227}O_{255}S_7$	18,862.5	ND

[a] -SA: acetamidated cysteine thiol; -SSG: glutathionylated cysteine thiol; ND: not detected.