Supporting Information

Glutathione Complexed Fe-S Centers

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Experimental Methods

Synthesis – Ferric chloride (20 mM) and sodium sulfide (20 mM) were added to 10 mL 40 mM glutathione solution, pH 8.6. A volume (40 mL) of ethanol was added to the mixture and mixed well by vortexing. The precipitate was collected by centrifugation at 13,000 rpm for 10 min, washed twice with ethanol and dried under vacuum.

Electronic spectroscopy and electrochemistry – UV-Vis measurements were obtained on a Varian Cary 50 UV-Vis Spectrophotometer, and cyclic voltammetric experiments conducted on Potentiostat/Galvonostat Model 263 (EG&G Princeton Applied Research) with 10 mM GSH-Fe-S complex in 50 mM Hepes, 100 mM NaCl, pH 7.5.

Enzymatic reconstitution of GSH-Fe-S – GSH was dissolved in water to a concentration of 1 mM and the pH adjusted to 8.6 by addition of a concentrated NaOH solution. The resulting solution was argon purged following addition of *Tm* Nifs to a concentration of 5 μM, and then $FeCl_3$ and cysteine were anaerobically added from concentrated stock solutions to a final concentration of 1 mM each. UV spectra were recorded every 10 min after the addition of cysteine.

Iron Quantitation.[1] A solution of $[Fe_2S_2](GS^-)_4$ (0.05 mM, 200 μL) in H_2O was acidified by concentrated HCl (60 μL) and heated to 100 ºC for 15 min. The resulting suspension was

centrifuged at 14,000 rpm for 2 min and the supernatant (100 μL) was diluted with Tris-HCl (0.5 M, 1.3 mL, pH 8.5). Solutions of sodium ascorbate (0.1 mL, 5%) and bathophenanthroline-disulfonate (0.4 mL, 0.1%) were sequentially added to the neutralized reaction solution with mixing between each addition. The solution was incubated at 25 ºC for 1 h and iron was quantitated by measuring the absorbance at 535nm on a UV-Vis spectrometer and calculated from a calibration curve made with 0.01-0.30 mM $FeCl_3$ standard solutions.

Sulfide Qunatitation.[2] All reagents and solvents were degassed and purged with argon gas prior to use. A solution of $[Fe_2S_2](GS\text{-})_4$ (0.04 mM, 1 mL) in H_2O was added with 0.1 mL DPD reagent (0.02 M *N,N*-dimethyl-*p*-phenylenediamine sulfate in 7.2 *N* HCl) and stirred to homogeneity. A solution of 0.03 M $FeCl_3$ in 1.2 *N* HCl (0.1 mL) was added to the reaction vial and incubated for 20 min. Sulfide was quantitated by measuring the absorbance at 650 nm on a UV-Vis spectrometer and calculated from a calibration curve made with 0.02 to 0.10 mM Na_2S standard solutions.

NMR. All NMR samples were dissolved in 100% D_2O in 5 mm NMR tubes (Wilmad-Labglass). Solution samples of glutathione and the [2Fe-2S] glutathione cluster complex were freshly prepared at concentrations of 4 mM (pH 8.6) and 1 mM, respectively. All NMR experiments were recorded at 300.1 K, except for the variable temperature experiments. Proton (^1H) spectra for all samples were recorded on a Bruker DRX 500 MHz spectrometer equipped with a 5 mm TXI cryo-probe and processed with XWIN-NMR v3.5 software. The T_1 and T_2 relaxation data were recorded on a Bruker DMX 600 MHz spectrometer equipped with a 5 mm triple-resonance probe with *x,y,z*-axes gradients and processed with XWIN-NMR v1.1 software. Variable temperature studies were recorded on a Bruker DPX 400 MHz equipped with a 5 mm BBI probe with *z*-axis gradient and processed with Topspin v1.3 software.

Mössbauer. A 10 mg sample of ^{57}Fe metal was dissolved in 250 μL of a 1:1 mixture of concentrated HCl and HNO_3. The suspension was stirred for ~ 10 min until all solids had

dissolved and gas evolution ceased. A solution of 5 M NaOH was then slowly added in aliquots of 20 μL and the pH of the solution checked after each addition. Additions continued until a pH ~ 7.4 was obtained; typically ~ 300-340 μL 5 M NaOH total. The color of the mixture turned light yellow, and then dark orange. This ^{57}Fe stock salt solution was subsequently used to synthesize ^{57}Fe-labeled clusters. The solution was centrifuged to discard any precipitate, and then 0.077 g GSH in 4 mL H_2O, pH 8.6 was added. A 500 μL solution of 200 mM Na_2S was added and reaction continued for 10 min prior to precipitation with a ten-fold volume excess of ethanol with stirring. The resulting solution was centrifuged at 14,000 rpm and the supernatant removed by decanting. The solid was resuspended two additional times in ethanol with stirring and was collected by centrifugation prior to final drying in a speedvac for a period of up to 4 h to obtain the ^{57}Fe-S glutathione cluster complex. . For the preparation of the Mössbauer absorber, a solution of this complex (9.3 mM, 600 μL) in GSH 10 mM (pH 8.6) was transferred into a 2 cm-diameter holder.

Conventional Mössbauer effect (ME) experiments at constant acceleration at 212 K were carried out in transmission geometry using a nominal 50 mCi ^{57}Co source in a Rh matrix. Instrumentation consists of ORTEC pulse processing modules (142pc, 572A, 551-TSCA), a LND-4045 proportional counter and CMTE MA-250 linear velocity transducer with MR-350 drive. Recently developed equipment was used in the acquisition of spectra and for velocity reference wave generation.[3] Isomer shifts are referred to α-Fe at room temperature. Mössbauer spectrum was fit to one Lorentzian doublet taking into account the effective thickness in the Mössbauer transmission integral.[4] The relevant fitted parameters were isomer shift and quadrupolar splitting being these $\delta = 0.393(1)$ mm/s and $\Delta E_Q = 0.676(2)$ mm/s respectively. This component can be associated with Fe(III) consistent with the existence of fully oxidized $[2Fe\text{-}2S]^{2+}$ clusters in the sample.[5]

Purification of Hs ISU and Tm Nifs. Protein expression and purification was performed as previously reported.[6-8] As noted in the text, the D46A ISU derivative was typically used since this is less prone to background cluster hydrolysis. Relative to earlier reports on this

derivative,[7,9] the residue numbering now reflects the full available NCBI sequence, gi|24307953.

Cluster Transfer. The absorbances at 330 nm and 415 nm correspond to cluster charge transfer bands that can be used to monitor the cluster formation or cluster transfer from ISU to GSH complex. The 330 nm absorbance is stronger. In the case of formation of the GSH-bound cluster, the absorbance change is large, and so we chose to monitor at the weaker 415 nm wavelength to keep the absorbance on-scale in the concentration range used (Figure 1). In the case of cluster transfer (Figure 3), the absorbance change is relatively small, and so we chose to monitor at 330nm which has a higher extinction coefficient and thereby minimizes errors.

A 25 μM solution of holo D46A ISU in 100 μL 50 mM Tris, 100 mM NaCl, pH = 8.6 was mixed with 10 mM GSH and the UV/Vis spectrum recorded at 2 min intervals for the first 20 min and every 5 min for an additional 70 min. The measured absorbance at 330 nm was plotted against time. Control experiment was performed without added GSH, and the experiment repeated under similar conditions, with the exception that the pH was held at 7.5. For cluster transfer in the reverse direction, a solution of 15 μM apo native ISU in 100 μL 50 mM Tris, 100 mM NaCl, pH=7.5 was mixed with 100 μM GSH-Fe-S complex and 1 mM DTT, and the absorbance at 330 nm was monitored every minute for 40 min.

Analysis of cluster transfer. The D46A derivative hISU (75 μM) in Tris-HCl (50 mM, pH 7.5, 100 μL) was incubated at room temperature with GSH buffer (10 mM, pH 8.6, 400uL) and applied to 3K Amicon™ Ultra Centrifugal Filters (Millipore) and centrifuged at 14,000 x *g* for 10 min. The retentate was recovered and the addition of GSH buffer was repeated four more times. Solution passed through the filter, containing GSH cluster complex and other small molecules, and the retained solution, containing ISU protein, were applied to ESI-MS, respectively.

Tables

GSH		GSSG		GSH + Fe^{3+}		$[Fe_2S_2](GS)_4$ Cluster					
	T_1 (ms)	T_2 (ms)	T_1 (ms)	T_2 (ms)	T_1 (ms)	T_2 (ms)	T_1 (ms)	T_2 (ms)	R_{1M}	R_{2M}	R_{1M}/R_{2M}
C_α	2725	278.0	2941	1669	2912	14.0	-	-	-	-	-
G_α	1286	1006	1017	547.0	1292	16.0	593.7	117.4	2.166	8.569	0.253
E_α	2346	445.2	3504	977.0	2225	14.0	-	-	-	-	-
$C_{\beta1}$	596.9	331.5	524.0	286.5	575.0	15.0	267.9	47.5	2.228	6.979	0.319
$C_{\beta2}$	601.2	303.6	524.0	286.0	590.0	14.0	226.3	49.9	2.657	6.084	0.437
E_γ	784.0	464.0	713.8	407.6	764.0	15.0	104.5	34.9	7.502	13.295	0.564
E_β	713.2	371.9	645.1	438.0	722.0	15.0	62.4	28.3	11.429	13.141	0.870

Table S1. Comparison of the proton longitudinal relaxation rate (T_1), the transverse relaxation rate (T_2) and the enhancement R_{1M} and R_{2M}, respectively between free, oxidized, GSH/Fe^{3+} control and cluster-bound glutathione. R_{1M}/R_{2M} values demonstrate that contact relaxation is more dominant over dipolar relaxation for the $C_{\beta1}$ and $C_{\beta2}$ protons relative to the E_β and E_γ protons.

Figures

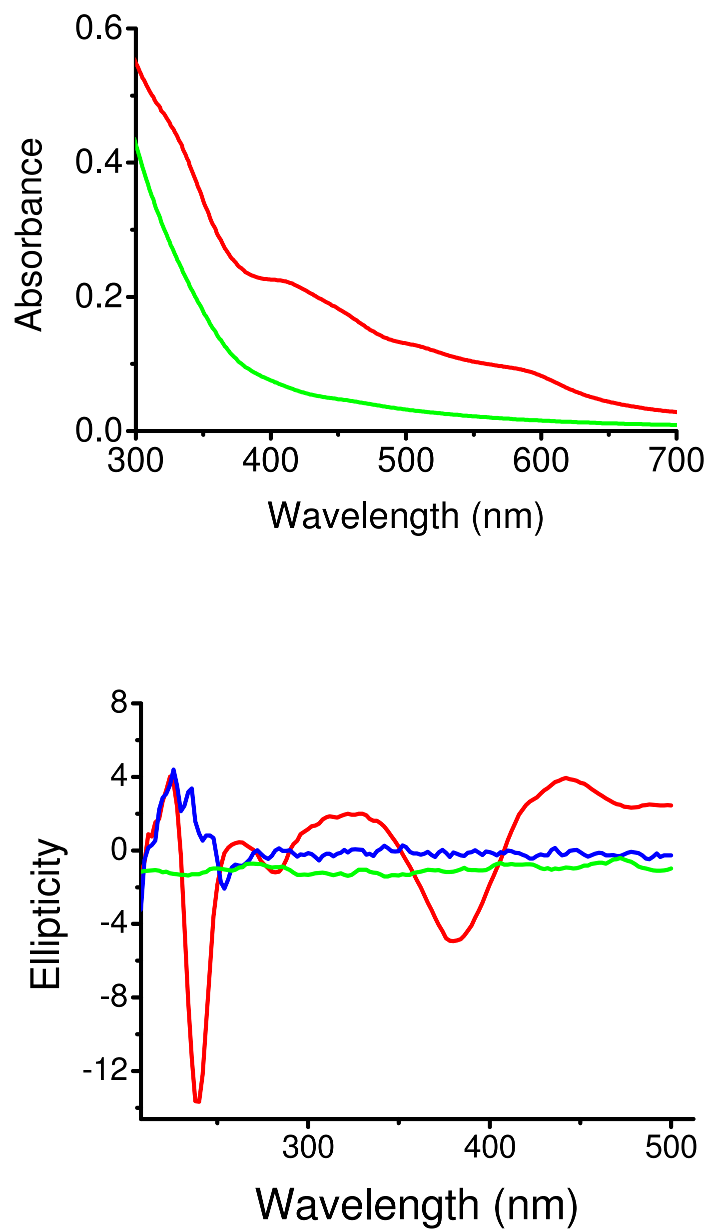

Figure S1. (top) The UV-Vis spectrum of $[Fe_2S_2](GS)_4$ contains characteristic iron-sulfur cluster peaks (red). A control following addition of ferric ion to GSH is also shown (green). (bottom) CD spectrum of holo ISU (red), GSH (blue) and GS-Fe-S complex (green).

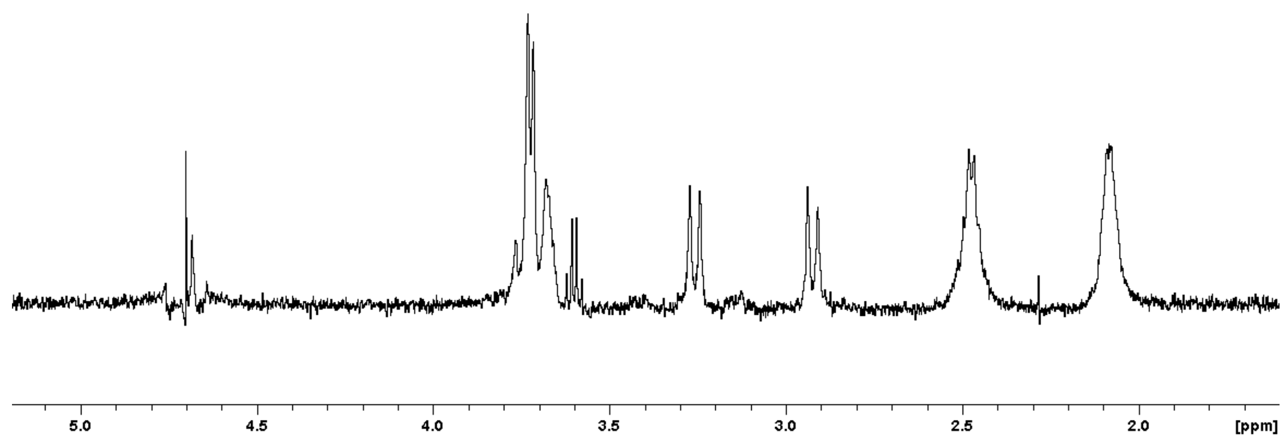

Figure S2. Results of a proton homonuclear decoupling experiment. Saturation of the resonances ~ 4.70 ppm yielded doublets for the $C_{\beta 1}$ and $C_{\beta 2}$ protons, supporting the conclusion that the C_{α} proton had shifted to the position of the water peak following cluster formation (see Figure 2 for labeling).

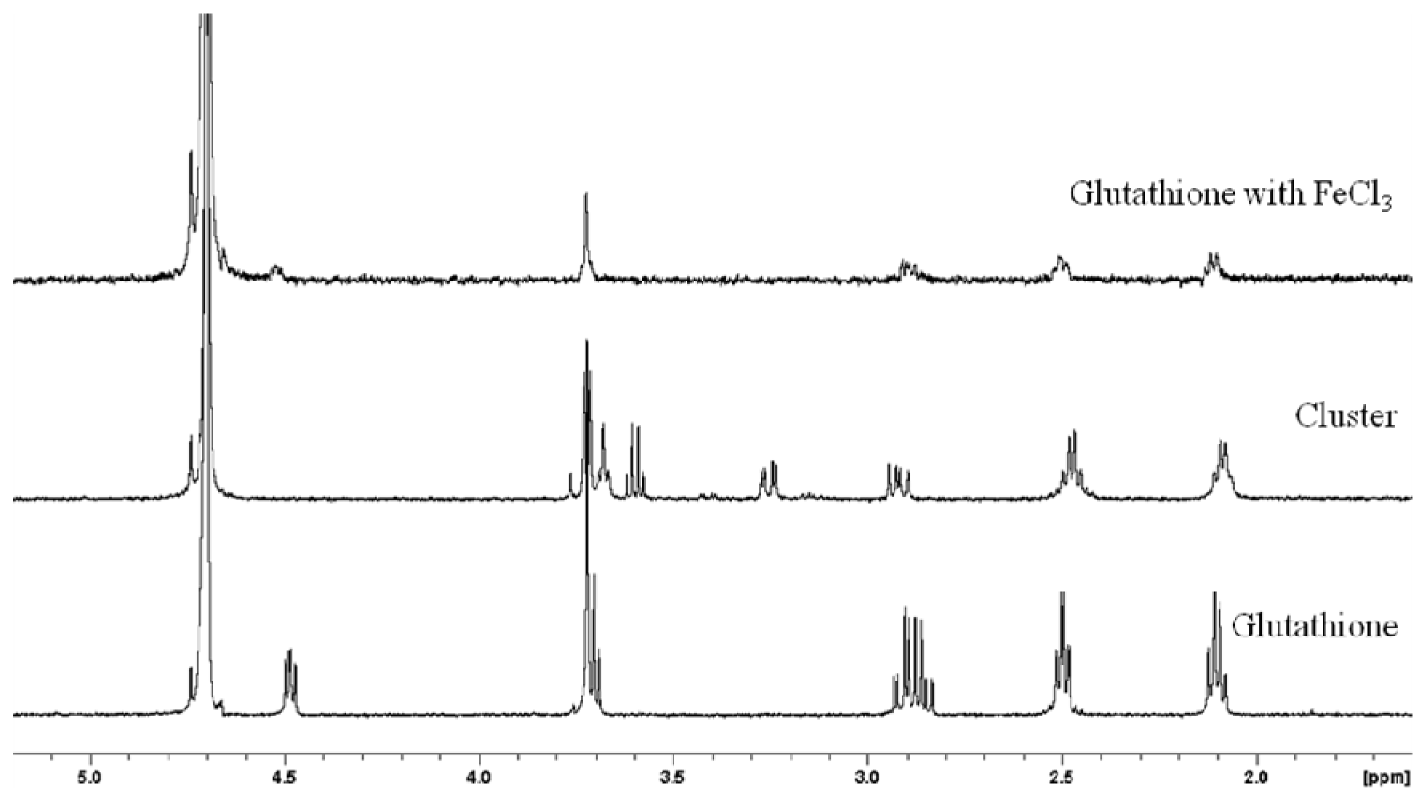

Figure S3. Addition of $FeCl_3$ to glutathione resulted in line broadening as a result of paramagnetic relaxation and a decrease in signal-to-noise ratio. The cluster-bound glutathione protons remained relatively sharp as a result of strong antiferromagnetic coupling between the Fe^{3+} ions.

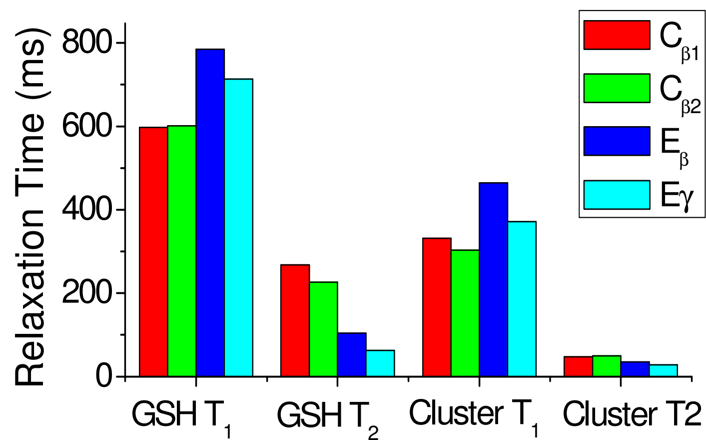

Figure S4. Comparisons of T_1 and T_2 relaxation times for $C_{\beta 1}$, $C_{\beta 2}$, E_β and E_γ protons for free and cluster-bound glutathione. Spin-spin (T_2) relaxation times show a significant cluster-induced change in relaxation, reducing the proton T_2's by almost 6-fold. Data were obtained from a 1 mM $[[Fe_2S_2]^{2+}(GS^-)_4]^{2-}$ solution in D_2O at 300.1 K, using a Bruker DRX 500 MHz spectrometer.

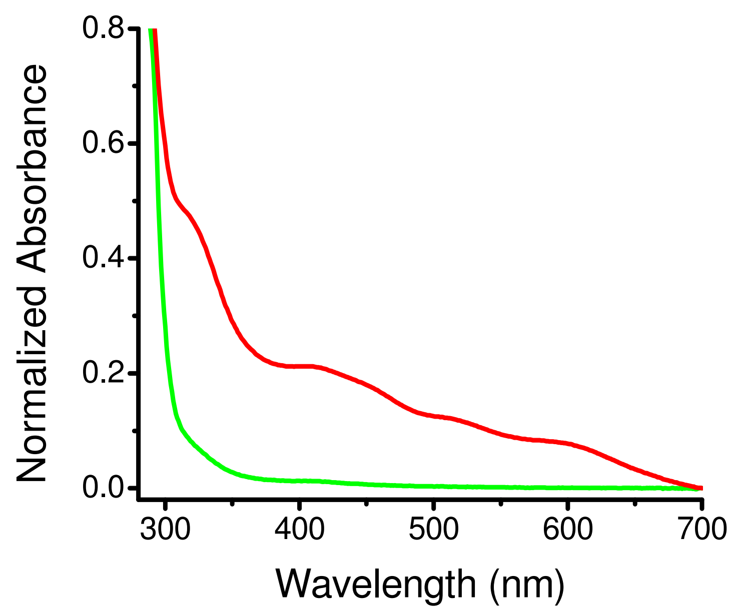

Figure S5. Comparison of the UV spectra for ISU before (green) and after (red) the cluster transfer reaction from GSH cluster complex to apo ISU. ISU was isolated from other reaction components using a Talon metal affinity resin.

References

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