Supplementary Materials

Mapping Cellular Fe-S Cluster Uptake and Exchange Reactions - Divergent Pathways for Iron-Sulfur Cluster Delivery to Human Ferredoxins

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[†] **Author contributions:** IF and CW contributed equally to this project.

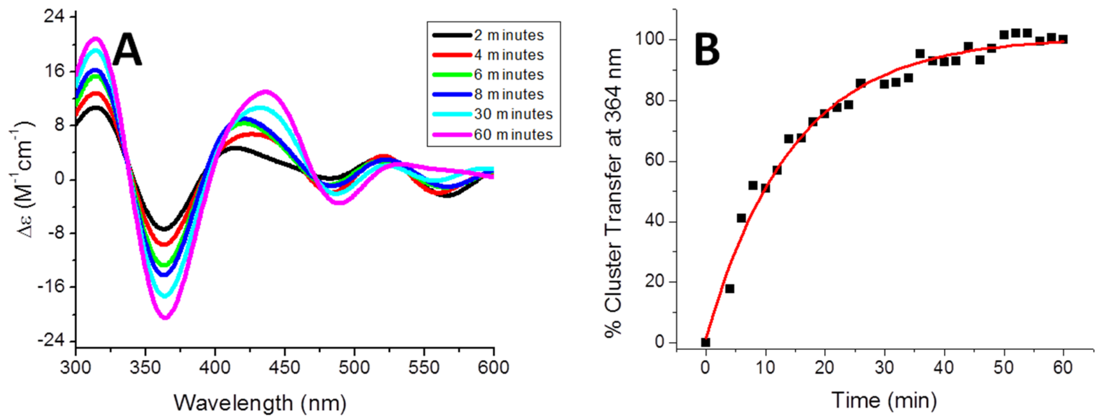

FIGURE S1. (A) Time course measurement for cluster transfer from $[2Fe\text{-}2S]^{2+}$ holo human Nfu to apo *Hs* Grx2 (50 µM each) was monitored by UV-visible CD spectroscopy under anaerobic conditions in semi-micro 1 cm cuvettes at room temperature. UV-visible CD spectra were recorded every 2 min for 60 min after addition of the cluster bound form of Nfu to apo Grx2. **(B)** The kinetics of appearance of the holo Grx2 CD signal following the cluster transfer from holo Nfu to apo Grx2 was also monitored. The cluster transfer reactions were carried out with a 1:1 donor:acceptor cluster stoichiometry in the presence of 3 mM GSH. The change in extinction values are based on the initial $[2Fe\text{-}2S]^{2+}$ cluster concentration. An apparent second order rate constant of $2000 \pm 150\ M^{-1}\ min^{-1}$ was obtained.

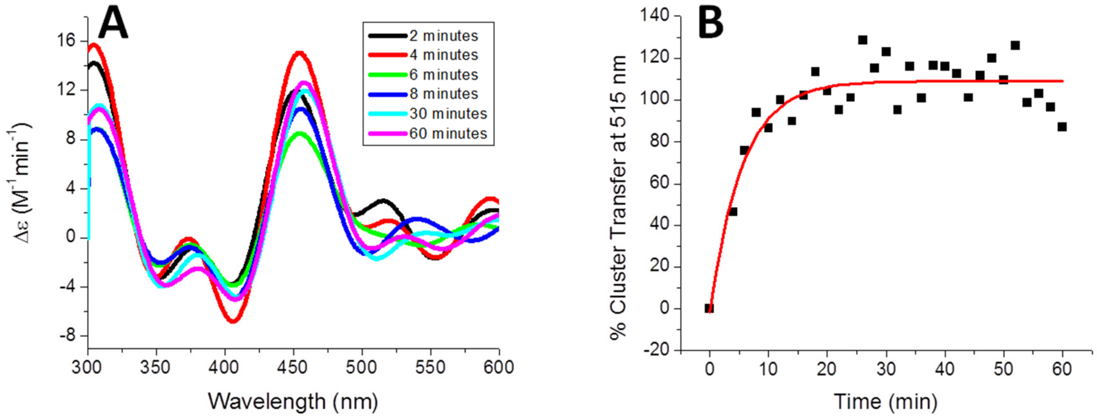

FIGURE S2. **(A)** Time course measurement for cluster transfer from $[2Fe\text{-}2S]^{2+}$ holo *S. pombe* Isa1 to apo *S. cerevisiae* Grx3 (120 µM each) was monitored by UV-visible CD spectroscopy under anaerobic conditions in semi-micro 1 cm cuvettes at room temperature. UV-visible CD spectra were recorded every 2 min for 60 min after addition of the cluster bound form of Isa1 to apo Grx3. **(B)** The kinetics of appearance of the holo Isa1 CD signal following cluster transfer from holo Isa1 to apo Grx3 was also monitored. The cluster transfer reactions were carried out with a 1:1 donor:acceptor cluster stoichiometry in the presence of 3 mM GSH. The change in extinction values are based on the initial $[2Fe\text{-}2S]^{2+}$ cluster concentration. An apparent second order rate constant of $6200 \pm 1900\ M^{-1}\ min^{-1}$ was obtained.

TABLE S1: Second order rate constants for all the transfer reactions demonstrated in this work and previous work from our laboratory.

Transfer Reaction	Rate (M^{-1} min^{-1})
Holo *Hs* IscU to apo *Hs* Fdx1	8700 ± 590
Holo *Hs* IscU to apo *Hs* Fdx2	2400 ± 1400
Holo *S. pombe* Isa1 to apo *Hs* Fdx1	6500 ± 1970
Holo *S. pombe* Isa1 to apo *Hs* Fdx2	8500 ± 2380
Holo *S. cerevisiae* Grx3 to apo *Hs* Fdx1	2100 ± 500
Holo *S. cerevisiae* Grx3 to apo *Hs* Fdx2	695 ± 138
Holo *Hs* Grx2 to apo *Hs* Fdx1	1160 ± 200
Holo *S. pombe* Isa1 to apo *Hs* Grx2	3500 ± 1300
Holo *S. pombe* Isa1 to apo *Hs* IscU	2900 ± 600
Holo *S. pombe* Isa1 to apo *Hs* Nfu	6700 ± 1560
Holo *S. pombe* Isa1 to apo *S. cerevisiae* Grx3	6200 ± 1900
Holo *Hs* Nfu to apo *Hs* Fdx1	4695 ± 823[1]
Holo *Hs* Nfu to apo *Hs* Fdx2	3849 ± 1242[1]
Holo *Hs* Nfu to apo *Hs* Grx2	2000 ± 150
[2Fe-2S]$(GS)_4$ to apo *Hs* Nfu	1930 ± 212[1]
Holo *Hs* Nfu to form [2Fe-2S]$(GS)_4$ via GSH extraction	130 ± 22[1]
[2Fe-2S]$(GS)_4$ to apo *Hs* IscU	4100 ± 1500[2]
Holo *Hs* IscU to form [2Fe-2S]$(GS)_4$ via GSH extraction	40 ± 3.4[2]
[2Fe-2S]$(GS)_4$ to apo *S. cerevisiae* Grx3	1360 ± 110[2]
Holo *S. cerevisiae* Grx3 to form [2Fe-2S]$(GS)_4$ via GSH extraction	130 ± 27[2]
[2Fe-2S]$(GS)_4$ to apo *S. pombe* Isa1	7400 ± 1500[2]
Holo *S. pombe* Isa1 to form [2Fe-2S]$(GS)_4$ via GSH extraction	51 ± 8.5[2]

References

1. C. Wachnowsky, I. Fidai and J. A. Cowan, Iron-sulfur cluster exchange reactions mediated by the human Nfu protein, *J. Biol. Inorg. Chem*, 2016, **21**, 825-836.
2. I. Fidai, C. Wachnowsky and J. A. Cowan, Glutathione-complexed [2Fe-2S] clusters function in Fe-S cluster storage and trafficking, *J. Biol. Inorg. Chem*, 2016, **21**, 887-901.