Supporting Information

Reactivities of [4Fe-4S]-containing and cluster-free forms of *Streptomyces* WhiD

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Table S1. [4Fe-4S] WhiD cluster reactivities.

	Cluster			
	time = 0 min (μM)	**t = 115 min (μM) [a]**	**% Remaining**	**k_{obs} x10^{-3} (min^{-1})**
O_2	7.01	2.58	38	12.26 ± 0.52
O_2 [b]	7.07	2.90	41	30.38 ± 2.57
H_2O_2 [c]	7.06	2.88	40	15.47 ± 1.29
H_2O_2 [c, d]	6.23	4.11	66	48.80 ± 9.14
KO_2 [e]	7.07	Not determined [i]	Not determined	748.95 ± 35.57
KO_2 [d, e]	7.60	Not determined	Not determined	720.38 ± 36.03
KO_2 [f]	7.07	Not determined	Not determined	96.35 ± 27.65
Methyl-mycothiol	7.00	3.72	53	15.33 ± 1.10
Glutathione	6.97	3.57	51	20.30 ± 0.61
Trx [g]	7.05	4.48	63	28.49 ± 2.23
L-Cys	6.98	4.63	66	35.98 ± 1.10
Oxidised DTT [h]	7.03	2.88	41	13.38 ± 0.18
Glutathiol [h]	7.07	2.85	40	17.13 ± 0.49

(a) Estimated from remaining A406 nm
(b) Contained *in vitro* refolded/reconstituted [4Fe-4S] WhiD
(c) Anaerobically prepared (see *Experimental procedures*)
(d) Contained 2 mM glutathione.
(e) Contained catalase (*see Experimental procedures*)
(f) Contained superoxide dismutase (*see Experimental procedures*)
(g) Contained thioredoxin (see *Experimental procedures*)
(h) Control reactions carried out under anaerobic conditions confirmed that [4Fe-4S] WhiD was stable in the presence of the low molecular weight compounds used in this study, at least over the same time frame as aerobic experiments.
(i) The reaction with KO_2 was complete well before 115 min (see Figure 6C). Measurement of the UV-visible spectrum of [4Fe-4S] WhiD at 25 min after addition of KO_2 revealed that no cluster remained (see *Supplemental* Figure S2).

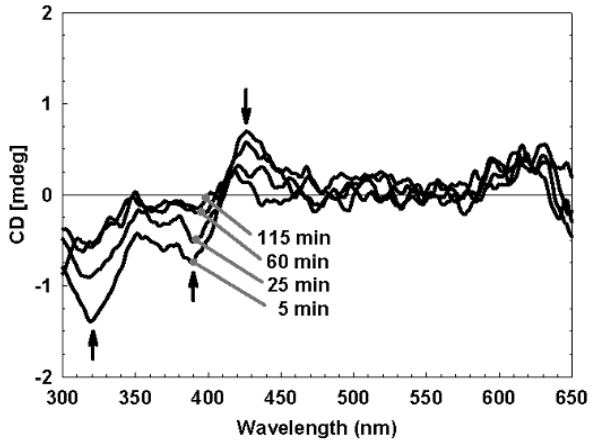

Figure S1. Reactivity of the [4Fe-4S] cluster monitored by CD spectroscopy. Near UV-visible CD spectra of native-WhiD (~7 μM [4Fe-4S]) following addition to an aerobic buffer; spectra were recorded at intervals, as indicated. Arrows indicate the direction of movement of spectral features. The buffer (20 mM Tris HCl, 20 mM Mes, 20 mM BisTrisPropane, 100 mM NaCl 5% Glycerol (v/v), pH 8.0) contained ~234 μM dissolved O_2.

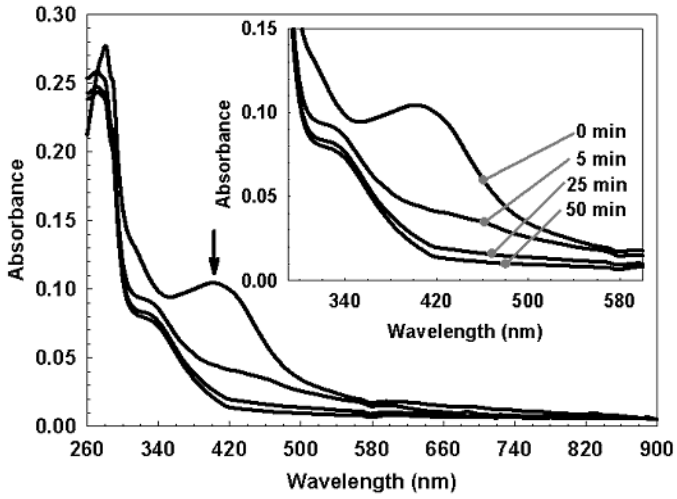

Figure S2. Reactivity of the [4Fe-4S] cluster with superoxide.
Absorption spectra of native-WhiD (~7 μM [4Fe-4S]) following addition of KO_2; spectra were recorded 0, 5, 25 and 50 min after exposure to KO_2, as indicated. *Inset*, Changes in the 300 – 600nm region in more detail. Arrows indicate the direction of movement of spectral features. The buffer (20 mM Tris HCl, 20 mM Mes, 20 mM BisTrisPropane, 100 mM NaCl 5% Glycerol (v/v), pH 8.0), contained ~43 μM dissolved superoxide ion.

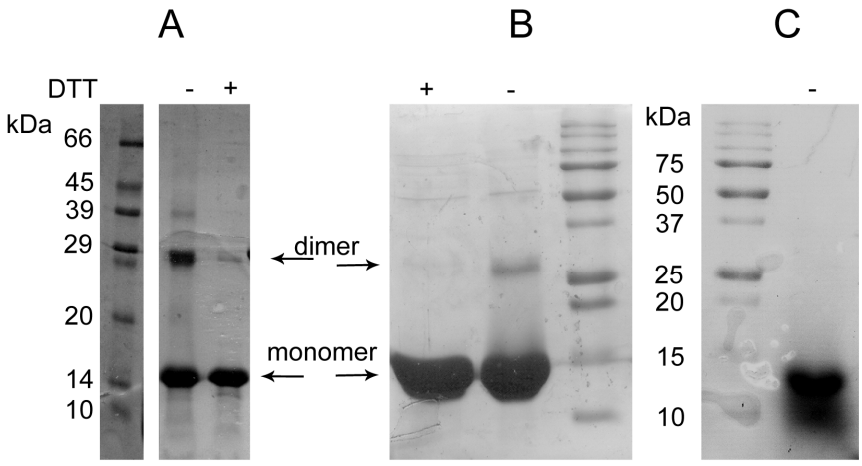

Figure S3. Analysis of WhiD by SDS-PAGE. A WhiD dimer that could be resolved on addition of DTT was observed by SDS-PAGE implying it contains an intra-molecular disulfide bond. **A)** SDS-PAGE of apo-WhiD in the presence (+) and absence (-) of 250 mM DTT. Coomassie stained bands corresponding to monomeric (~15 kDa) and dimeric WhiD (~30 kDa) are indicated. **B)** SDS-PAGE of as isolated WhiD (with 70% cluster occupancy) in the presence and absence of 250 mM DTT and coomassie stained. **C)** SDS-PAGE of as isolated WhiD run in the absence of 250 mM DTT and stained for iron according to the method of Chung (*1*), using 2-mercaptoethanol in place of thioglycolic acid. Iron is clearly associated with the monomeric form of WhiD but not with the dimeric form of WhiD, indicating that dimerization of WhiD occurs only in the absence of the cluster. The molecular weight markers were pre-stained (BioRad). SDS-PAGE was carried out using the Laemmli system, except that DTT was omitted as required (*2*).

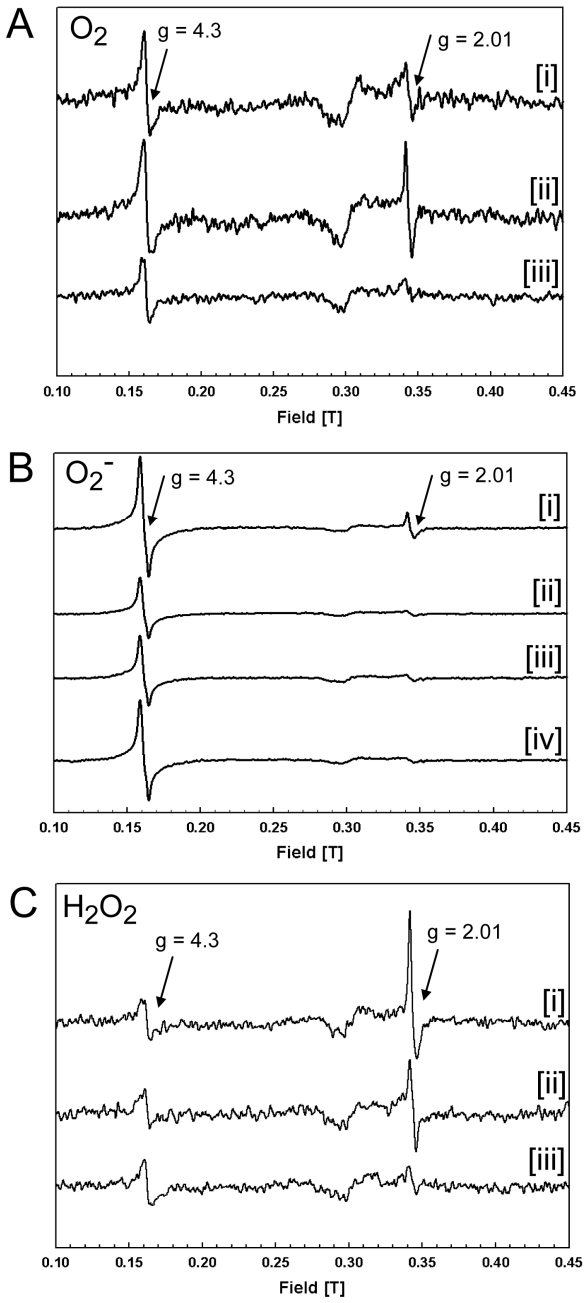

Figure S4. Oxidation of [4Fe-4S] WhiD followed by EPR spectroscopy. EPR spectra of [4Fe-4S] WhiD. **A)** Following exposure to ~234 μM O_2 for [i] 55 sec, [ii] 4 min, and [iii] 18 min. The g = 2.01 signal displayed the temperature and power characteristics of a S= ½ $[3Fe\text{-}4S]^{+}$ species. Spin quantitation of the g = 2.01 $[3Fe\text{-}4S]^{1+}$ signal after 4 min gave ~0.16 μM, accounting for a <1% of the original cluster concentration. **B)** Following exposure to ~563 μM H_2O_2 for [i] 2 min, [ii] 50.7 μM H_2O_2 for 2 min, and [iii] 10 min. Spin quantitation of the g = 2.01 signal after 2 min exposure to ~563 μM H_2O_2 gave ~0.9 μM, accounting for ~4% of the original cluster, whilst a 2 min exposure to ~50.7 μM H_2O_2 gave ~0.5 μM (~2% of the cluster). **C)** Following exposure to ~43 μM KO_2 in the presence of 1267 units of catalase, for [i] 55 sec, [ii] 3 min, [iii] 6 min and [iv] 12 min. Spin quantitation of the g = 2.01 signal after 55 sec gave ~0.5 μM (~2% original cluster). EPR spectra were recorded at 15 K, with microwave frequency of 9.68 GHz and power of 2 mW normalised to the same receiver gain; modulation amplitude 1 mT and frequency 100 kHz. The dip at 0.30 T was present in the empty cavity. WhiD (20 μM in [4Fe-4S]) was in 20 mM MES, 20 mM Tris, 20 mM BisTrisProrpane, 100 mM NaCl, 5% glycerol, pH 8.0.

References

1. Chung, M. C. (1985) A specific iron stain for iron-binding proteins in polyacrylamide gels: application to transferrin and lactoferrin. *Anal Biochem. 148*, 498-502
2. Laemmli, U. K. (1970) Cleavage of structural proteins during assembly of head of bacteriophage T4. *Nature 227*, 680-685.