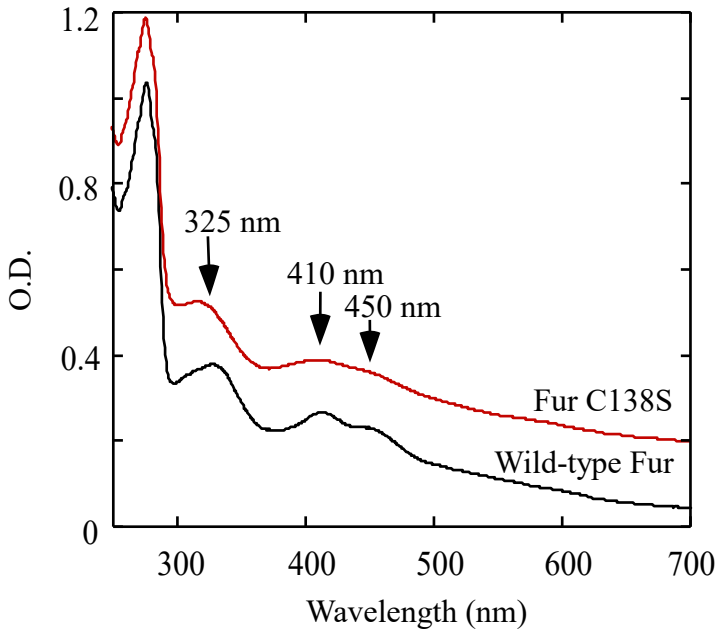
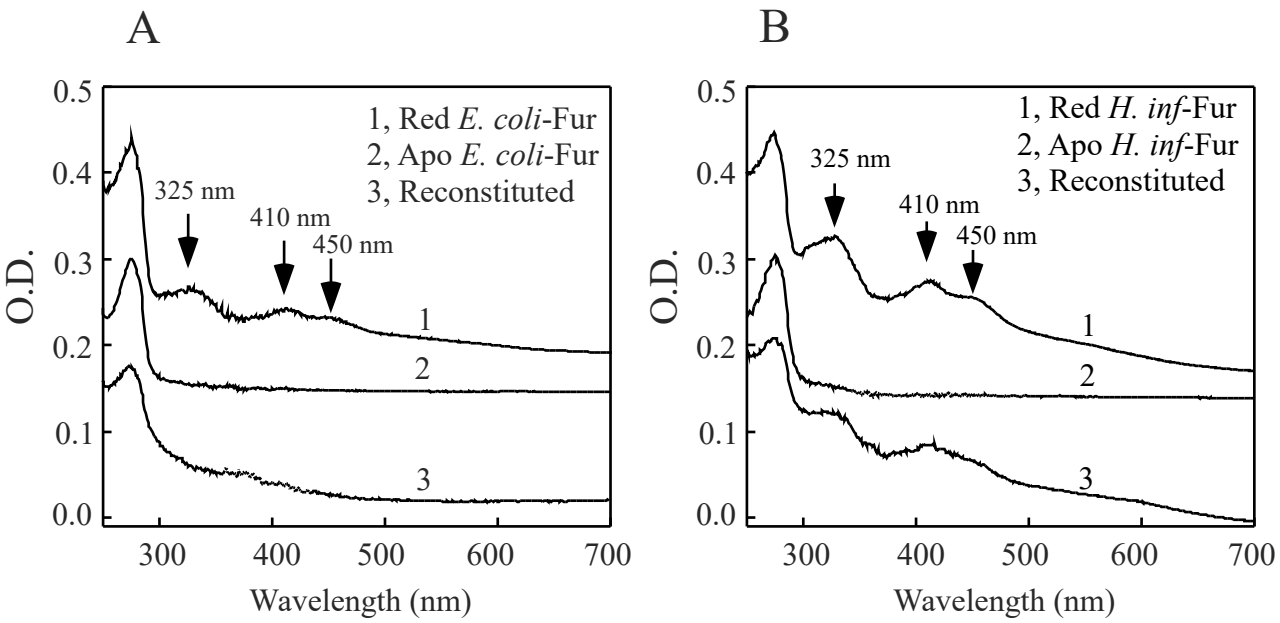
Supplementary Figure 1



Supplementary Figure 1. UV-Visible absorption spectrum of the *E. coli* Fur mutant C138S. Wild-type *E. coli* Fur and the Fur mutant C138S were purified from the *E. coli iscA/sufA* mutant cells grown in LB medium. The protein concentration was about 100 μM. The spectrum of the Fur mutant C138S was offset by O.D. of 0.2.

Supplementary Figure 2



Supplementary Figure 2. ***In vitro*** **reconstitution of the [2Fe-2S] cluster in the *E. coli* Fur and the *H. influenzae* Fur. A),** recombinant *E. coli* Fur proteins were prepared from the *E. coli iscA/sufA* mutant cells grown in LB medium containing 0 (spectrum 1) or 200 μM (spectrum 2) of 2,2'-dipyridyl. Apo-form *E. coli* Fur protein (50 μM) was then incubated with 200 μM $Fe(NH_4)_2(SO_4)_2$, 1 mM L-cysteine, 1 μM *E. coli* cysteine desulfurase (IscS), and 4 mM dithiothreitol for 30 minutes, followed by passing through a High-Trap Desalting column to re-purify the protein. Re-purified Fur protein was subjected to the UV-Visible absorption measurement (Spectrum 3). The protein concentrations were about 20 μM. Spectra of the Fur proteins were offset for clarity. **B)**, as in A), except recombinant *H. influenzae* Fur proteins were prepared from the *E. coli iscA/sufA* mutant cells grown in LB medium containing 0 (spectrum 1) or 200 μM (spectrum 2) of 2,2'-dipyridyl. Spectrum 3, apo-form *H. influenzae* Fur protein after reconstitution as described in A).

Supplementary Table 1. Mössbauer Parameters for Selected Protein-Bound Fe-S clusters

			Experimental MB parameters at 4.2 K		
Cluster Type	Protein	Cluster spin	δ (mm/s)	ΔE_Q (mm/s)	Ref.
[Fe]-4Cys	*Rubredoxin (C. pasteurianum)*	5/2	0.24	0.5	(1)
[Fe]-4Cys	*Desulforedoxin (D. gigas)*	5/2	0.25	0.75	(2)
[Fe]-3Cys1Ser	*C42S Rubredoxin (C. pasteurianum)*	5/2	0.26	0.7	(1)
[Fe]-3Cys1Ser	*C9S Rubredoxin (C. pasteurianum)*	5/2	0.26	0.7	(1)
[2Fe-2S]-4Cys	*Ferredoxin (P. aeruginosa)*	0	0.27	0.60	(3)
[2Fe-2S]-3Cys1His	*Rieske protein (T. thermophilus)*	0	0.32; 0.24	0.91; 0.52	(4)
[2Fe-2S]-3Cys[(a)]	*Glutaredoxin (human)*	0	0.27	0.61	(5)
[2Fe-2S]-3Cys[(a)]	*Rubredoxin C42A (C. pasteurianum)*	0	0.30; 0.29	0.71; 0.58	(6)
[2Fe-2S]-3Cys1His	*mitoNEET (human)*	0	0.26; 0.30	0.47; 0.96	(7)
[2Fe-2S]-3Cys1His	*IscU (A. vinelandii)*	0	0.27; 0.32	0.66; 0.94	(8)
[3Fe-4S]-3Cys	*Ferredoxin (A. vinelandii)*	½	0.27	0.63	(9)
[4Fe-4S]-4Cys	*Ferredoxin (B.stearothermophilus)*	0	0.43; 0.42	1.50; 1.20; 1.10; 0.66	(10)
[4Fe-4S]-4Cys	*FNR anaerobic (E. coli)*	0	0.45	1.22	(11)
[2Fe-2S]-4Cys	*FNR exposed to O_2 (E. coli)*	0	0.28	0.58	(11)
[2Fe-2S]-3Cys	*Fur (E. coli)*	0	0.29	0.53	This work

Notes: (a) in these proteins only three ligands were identified as Cysteine. The respective studies indicated that the fourth ligand is unknown, but it is unlikely to be His.

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