# Supporting Information

## Human Ferredoxin-2 Displays a Unique Conformational Change

**Wenbin Qi [a], Jingwei Li [b] and J. A. Cowan[a,b,*]**

[a] *Ohio State Biochemistry Program, the Ohio State University, Columbus, OH 43210*

[b] *Department of Chemistry and Biochemistry, the Ohio State University, Columbus, OH 43210*

[*] *Corresponding author; email: cowan@chemistry.ohio-state.edu*

## Experimental Methods

### Cloning and Expression of human ferredoxin-2

A 100 ng sample of DNA, 100 ng of each primer, 2.5 units of cloned *Pfu* Turbo DNA polymerase, *Pfu*Turbo buffer, and 0.2 mM of each dNTP were used to amplify *Hs* Ferredoxin-2 (Q6P4F2 residues 53-183) via PCR. Primers were designed as follows: 5'-ttatcatatggctggagaggaggacgcg-3' and 5'-taagctcgagtcagtggggcttggggac-3', where the underlined regions denote the NdeI and XhoI restriction sites, respectively. The thermocycle used was identical to that described in the *Pfu*Turbo DNA polymerase manual (Stratagene). PCR products were digested with NdeI and XhoI and ligated to double-digested vector pET28-b(+) by T4 DNA ligase. Cloning into pET28-b(+) resulted in the production of the gene for N-terminal His-tagged *Hs* Fd2. Cloning results were confirmed by nucleotide sequencing, and E. *coli* BL21(DE3) was used for protein expression. A 10 ml Luria-Bertani (LB) culture with 30 mg/L kanamycin was grown

overnight as a starter culture, which was then used to inoculate 1 L LB medium and grown to an $OD_{600}$ of ~ 0.6 prior to induction with 0.3 mM isopropyl-1-thio-α-D-galactopyranoside (IPTG). Cells were pelleted 5 hours after induction and stored at –80 °C for future use.

**Purification of hFd2**

Cell pellets were resuspended in 20 mM Tris, 500 mM NaCl, 5 mM imidazole, pH = 8.0 and lysed by sonication. Cell debris was removed by centrifugation at 4 C at 15,000 rpm for 30 min. The supernatant was then loaded onto a Ni-NTA column and eluted by standard methods, and further purified by gel filtration chromatography using a Superose12 column equilibrated with 50 mM Hepes, 100 mM NaCl, pH = 7.5. Eluted proteins were analyzed by SDS-PAGE and concentrated by Amicon ultrafiltration.

**Purification of hFd1**

The expression vector for *Hs* Fd1 was kindly provided by J. Markley and protein was expressed and purified according to literature procedures (Xia, B., Cheng, H., Bandarian, V., Reed, G. H., and Markley, J. L. (1996) *Biochemistry 35*, 9488–9495).

**hFd1 and hFd2 cluster absorbance change monitored by UV/Vis spectroscopy**

A UV/Vis spectrum of 100 µL 80 µM hF1 or hFd2 was taken using a Cary-50 UV/Vis Spectrometer (Varian). In the heating cycle experiments, the sample temperature was raised to 60 C and cooled to 25 C at a rate of 1 C/minute. UV/Vis spectra were taken at 5 degree intervals. In the melting experiments the sample temperature was raised to 75 C at a rate of 1 C/minute. In the kinetics experiments the sample temperature was raised to 40, 45, 47.5, 50, 52.5, 55, 57.5, and 60 C, respectively, in 2 min and then the spectrum taken every 2 min. Absorbance measurements at 414 nm were plotted against time and fitted to a first order exponential equation to obtain the rate constant k (Table S1).

**hFd2 cluster CD signal change at 50** C

An 80 μM solution of hFd2 in 50 mM Hepes, 100 mM NaCl, pH=7.5 was heated and maintained at 50 C. CD spectra in the range of 300 nm to 600 nm were taken at 5 min intervals using a JASCO J-815 Circular Dichroism (CD) Spectrometer. The same experiment was performed with and without the addition of 1 mM TCEP in the buffer.

**Rate of ferredoxin reduction by adrenodoxin reductase and cluster transfers monitored by the cytochrome c assay**

All solutions were purged by argon prior to use. For the measurement of enzymatic parameters, cytochrome c (80 μM), adrenodoxin reductase (200 nM) and NADPH (400 μM) were combined in 10 mM sodium phosphate buffer under anaerobic condition. Ferredoxin solutions at final concentrations of 0.1-8 μM were added and the absorbance at 550 nm was monitored over 5 min to define the initial slope of the kinetic profiles. For measurement of the holo-ISU to apo ferredoxin cluster transfer rate, a solution of 20 μL apo ferredoxin (200 μM) was added to 20 μL DTT (50 mM) and incubated at R.T. for 30 min. An aliquot of this solution (7 μL) was subsequently added to 35 μL of holo ISU (200 μM) and 6 μL of this mixture was used in the cytochrome c assay. For the cytochrome c assay, the total reaction volume used was 100 μL. This solution contained 80 μM cytochrome c, 200 μM adrenodoxin reductase, 400 μM NADPH, and 10 mM sodium phosphate at pH 7.4. The absorbance was measured at 550 nm for the first minute and the resulting initial slope was used for subsequent calculations. For details see previously published work from this laboratory.[16]

**Nuclear magnetic resonance spectroscopy**

All NMR samples (0.45 mM) were exchanged to 90% phosphate buffer (40 mM $Na_2HPO_4$, 100 mM NaCl, pH 7.4)/ 10% $D_2O$ with Illustra MicroSpin™ G-25 Column

(GE Healthcare) and data were acquired in 5 mm NMR tubes (Wilmad-Labglass). [$^{15}$N-$^{1}$H] Heteroquantum Single Quantum Coherence (HSQC) spectra for all samples were recorded on a Bruker DMX 600 MHz spectrometer (carrier frequency 600.13MHz) equipped with a 5 mm TXI ($^{13}$C/$^{15}$N) probe with x,y,z-axes gradients to a dimension of [1024 x 512] and processed with XWIN-NMR v1.1. Contour level was defined with a multiplier of 1.4 and a total of 8 positive layers. All NMR experiments were recorded at 301.1 K except for the variable temperature experiments, which were achieved with the spectrometer's built-in calibrated temperature controller.

# Figures

```
Fd1       ITVHFINRDGETLTTKGKVGDSLLDVVVENNLDIDGFGACEGTLACSTCHLIFEDHIYEK 60
Fd2       VNVVFVDRSGQRIPVSGRVGDNVLHLAQRHGVDLEG--ACEASLACSTCHVYVSEDHLDL 58
          :.* *::*.*: :...*:***.:*.:. .:.:*::*  ***.:*******: ..:.  :

Fd1       LDAITDEENDMLDLAYGLTDRSRLGCQICLTKSMDNMTVRVPETV 105
Fd2       LPPPEEREDDMLDMAPLLQENSRLGCQIVLTPELEGAEFTLPKIT 103
          * .  :.*:****:*  * :.******* ** .::.  . :*: .
```

Figure S1. Protein sequence alignment for hFd1 and hFd2. Cysteine residues are highlighted.

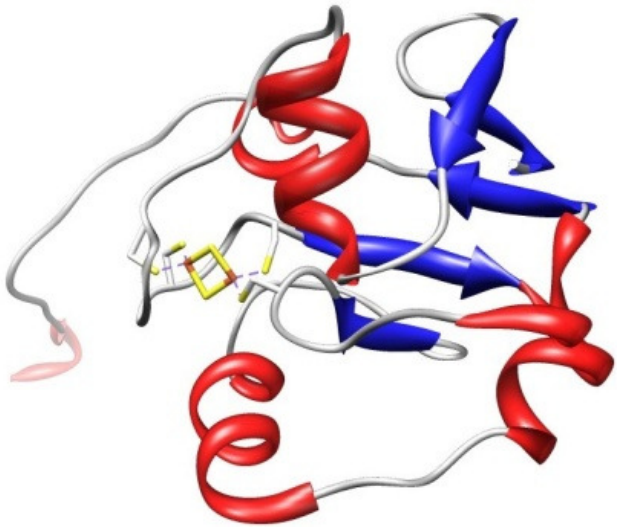

Figure S2. Crystal structure of human ferredoxin1 (PDB: 3P1M). The [2Fe-2S] cluster is ligated by four cysteine residues (shown in yellow).

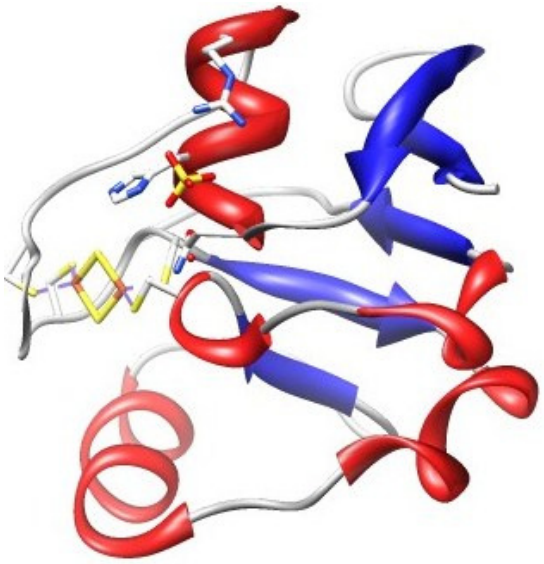

Figure S3. Crystal structure of human ferredoxin2 (PDB: 2Y5C). The [2Fe-2S] cluster is ligated by four cysteine residues (shown in yellow).

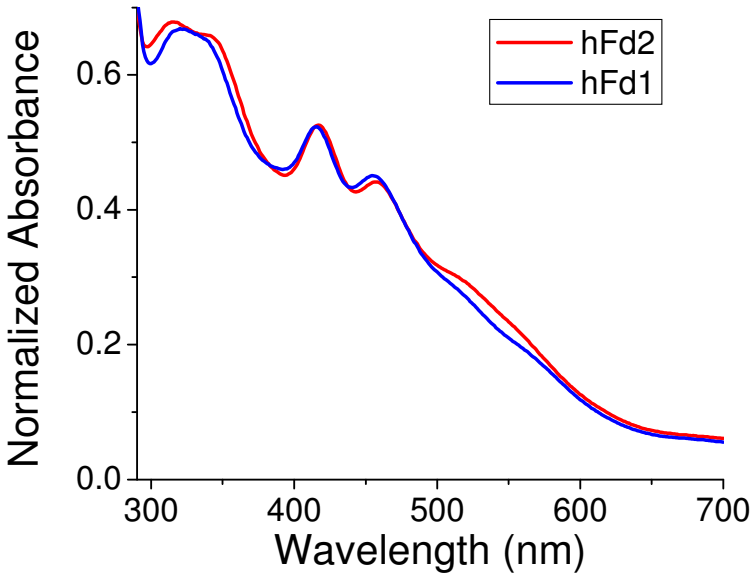


Figure S4. Comparison of UV/vis spectra for holo hFd1 and holo hFd2.

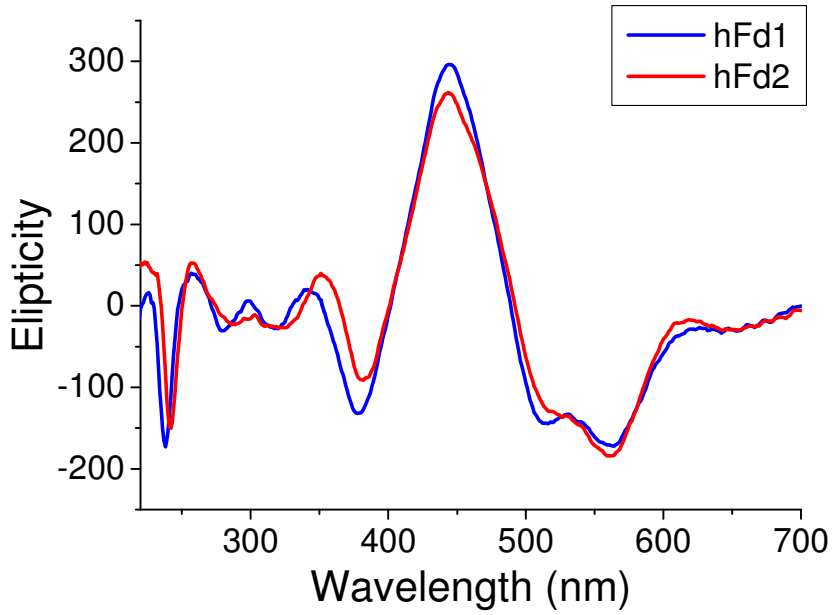


Figure S5. Comparison of CD spectra for holo hFd1 and holo hFd2.

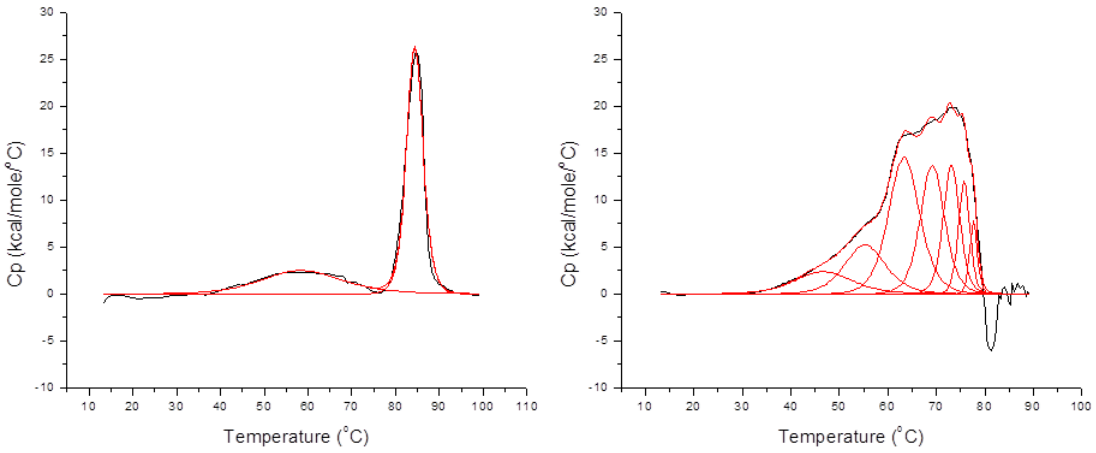


Figure S6. Differential scanning calorimetry experiments of hFd2 (left) and hFd1 (right).

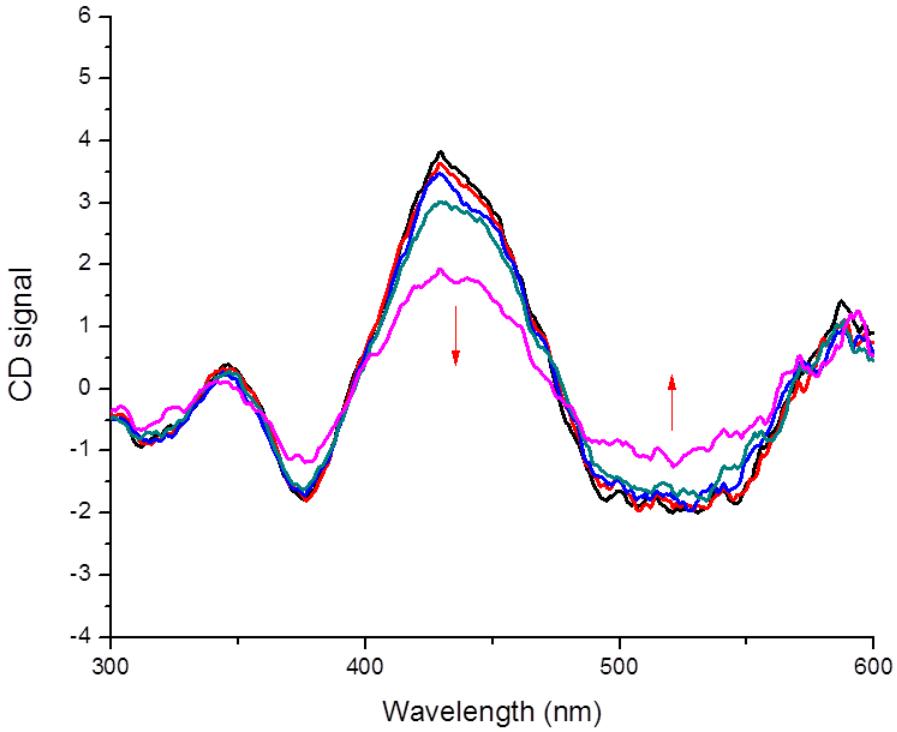


Figure S7. Change in the CD spectrum for holo hFd2 (from 300 to 600 nm) following incubation from 25 C (black) to 65 C (pink).

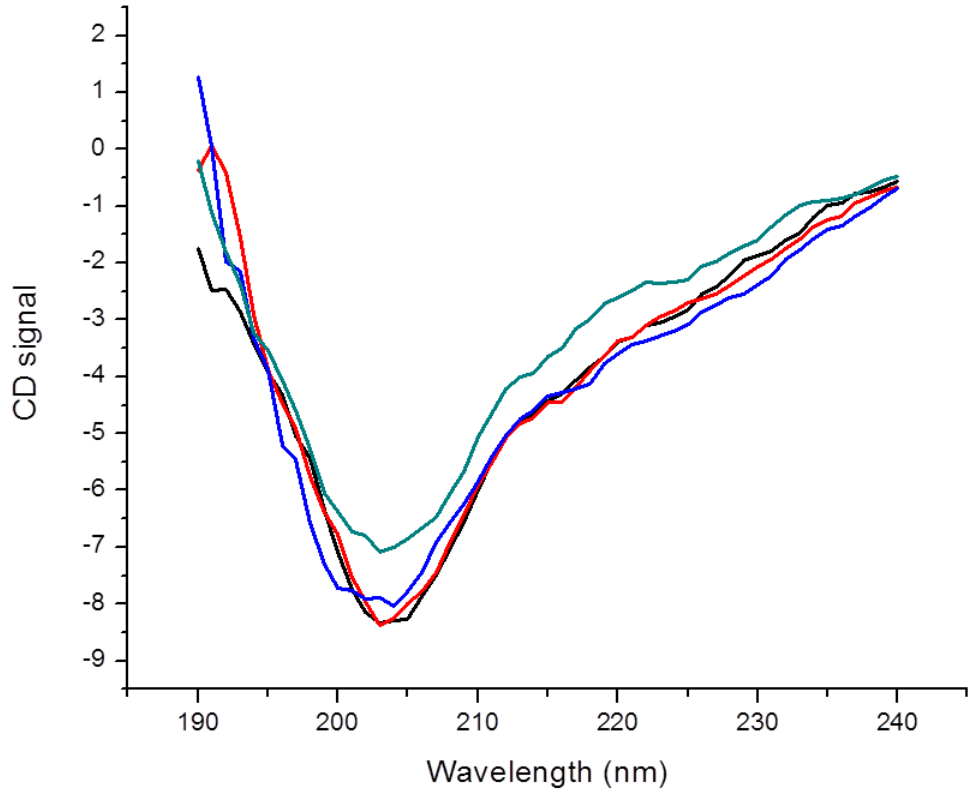


Figure S8. Far UV absorption spectra of hFd2 obtained at various temperatures: 35 ºC (black), 55 ºC (red), 75 ºC (blue) and 95 ºC (teal). Secondary structural analyses by K2D3 are summarized in Table S2.

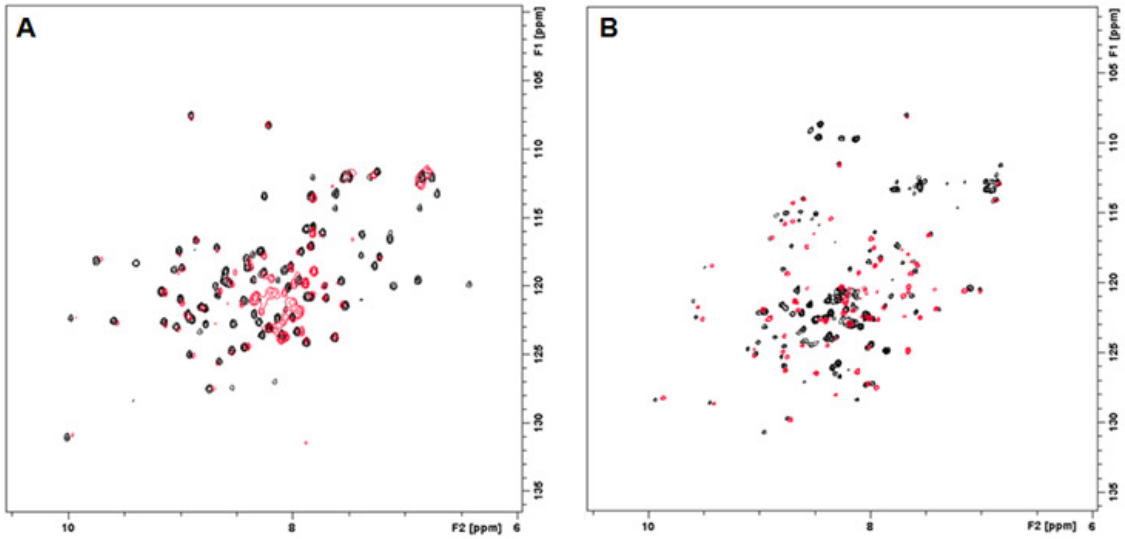


Figure S9. Overlay of variable temperature [$^{15}$N-$^{1}$H] HSQC spectra for hFd1 (A) and hFd2 (B) at 28 C (black) and 55 C (red).

Table S1. Dependence of rate constant on temperature.

| T (ºC) | 40 | 45 | 47.5 | 50 | 52.5 | 55 | 57.5 | 60 |
|---|---|---|---|---|---|---|---|---|
| k ($min^{-1}$) | 0.0099 | 0.0495 | 0.0676 | 0.135 | 0.204 | 0.278 | 0.455 | 0.909 |

Table S2. Temperature dependent secondary structural prediction and analysis for hFd2 by K2D3

| T (ºC) | Prediction | 35 | 45 | 55 | 65 | 75 | 85 | 95 |
|---|---|---|---|---|---|---|---|---|
| α-helix (%) | 17.5 | 16.4 | 19.2 | 17.3 | 18.2 | 17.4 | 9.7 | 9.7 |
| β-strand (%) | 21.2 | 22.6 | 21.6 | 22.6 | 22.3 | 22.3 | 26.8 | 26.9 |