Structure, Volume *24*

Supplemental Information

Structural/Functional Properties of Human NFU1, an Intermediate [4Fe-4S] Carrier in Human Mitochondrial Iron-Sulfur Cluster Biogenesis

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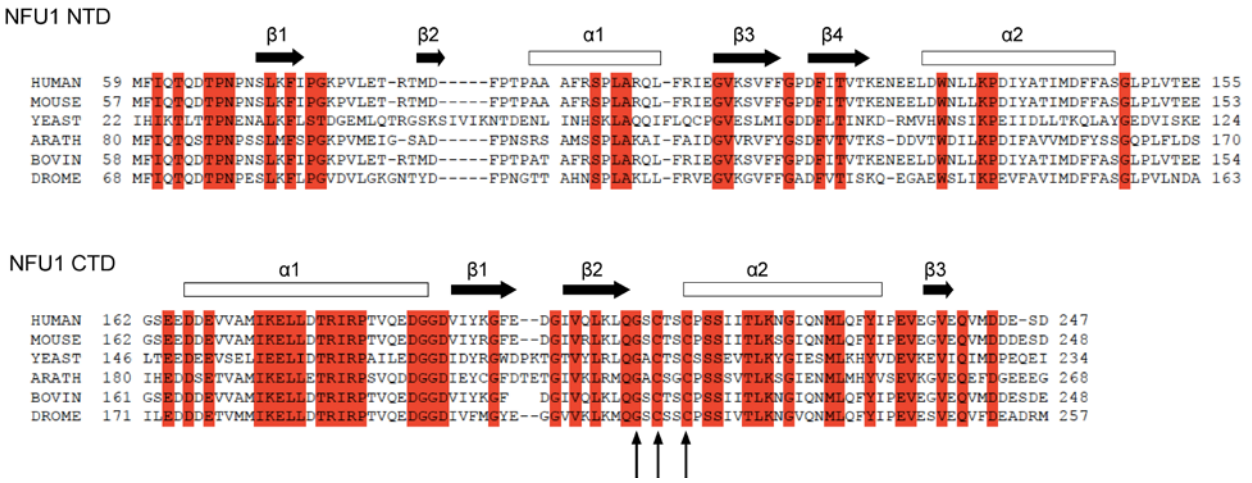

Figure S1, related to Figure 1. Sequence alignments and secondary structural features of the N-terminal domain (NTD) and C-terminal domain (CTD) of NFU1 whose structures are shown in Figure 1.

The residues highlighted in red are identically conserved in the sequences compared here. The arrows indicate the cysteine residues located in the conserved 'CXXC' motif. These residues from two subunits ligate the [4Fe-4S] cluster. The third arrow shows the position of the identically conserved residue (G193 in human NFU1). Abbreviations used are: HUMAN, *Homo sapiens*; MOUSE, *Mus musculus*; YEAST, *Saccharomyces cerevisiae* (strain ATCC 204508 / S288c); ARATH, *Arabidopsis thaliana* mitochondrial; BOVIN, *Bos tarus*; and DROME, *Drosophila melanogaster*.

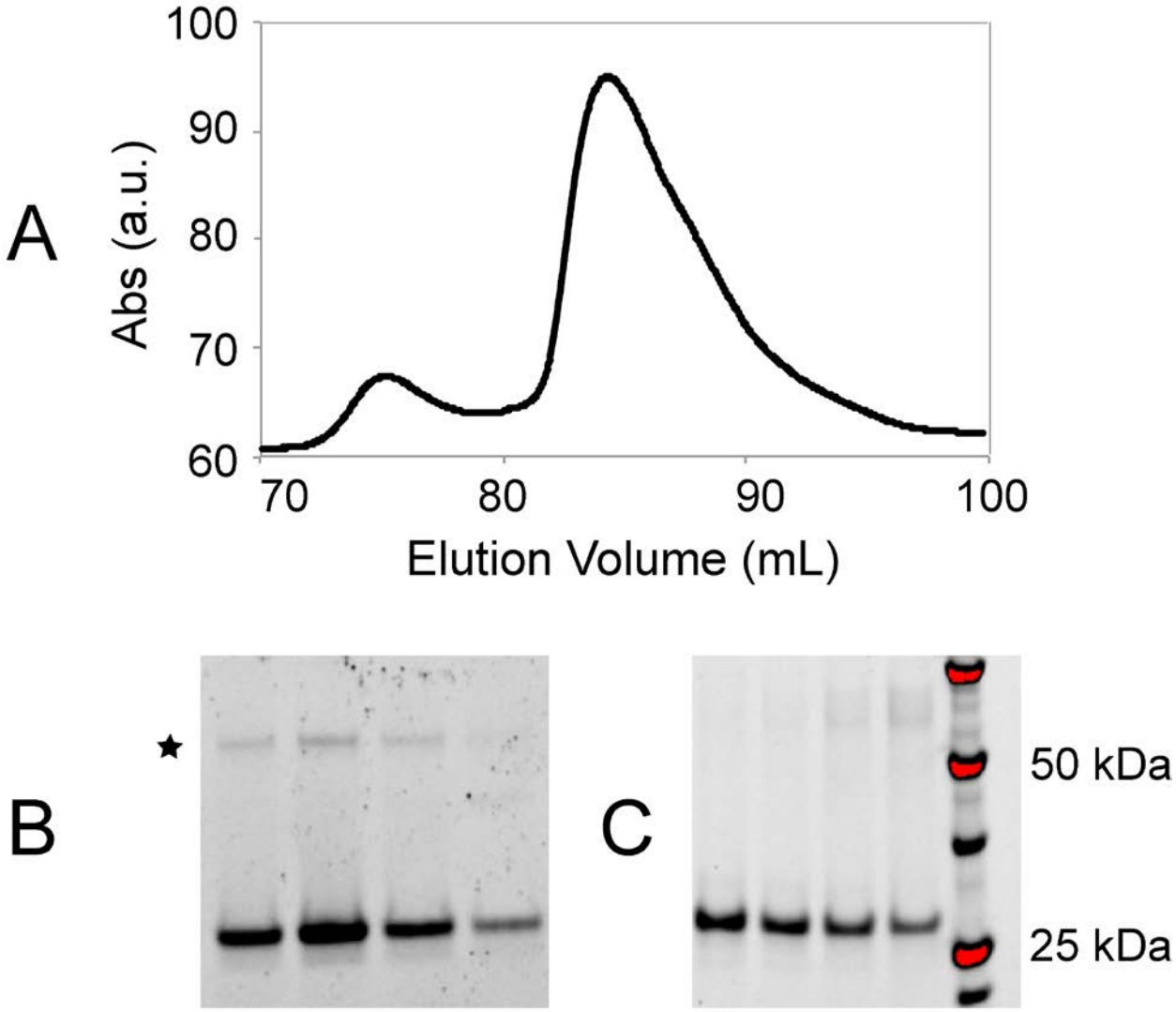

Figure S2, related to Figure 2. Evidence for the tendency of apo-NFU1 to dimerize through an intermolecular disulfide bond as discussed in the results of size exclusion chromatography (SEC) studies.

(*A*) Size exclusion chromatography (SEC) elution profile of NFU1. The peaks at ~75 mL and 86 mL correspond to NFU1 dimer and monomer, respectively.

(*B*) Non-reducing SDS-PAGE of the elution fractions collected from 74 to 80 mL.

(*C*) Reducing (50 mM DTT) SDS-PAGE of the elution fractions collected from 74 to 80 mL.

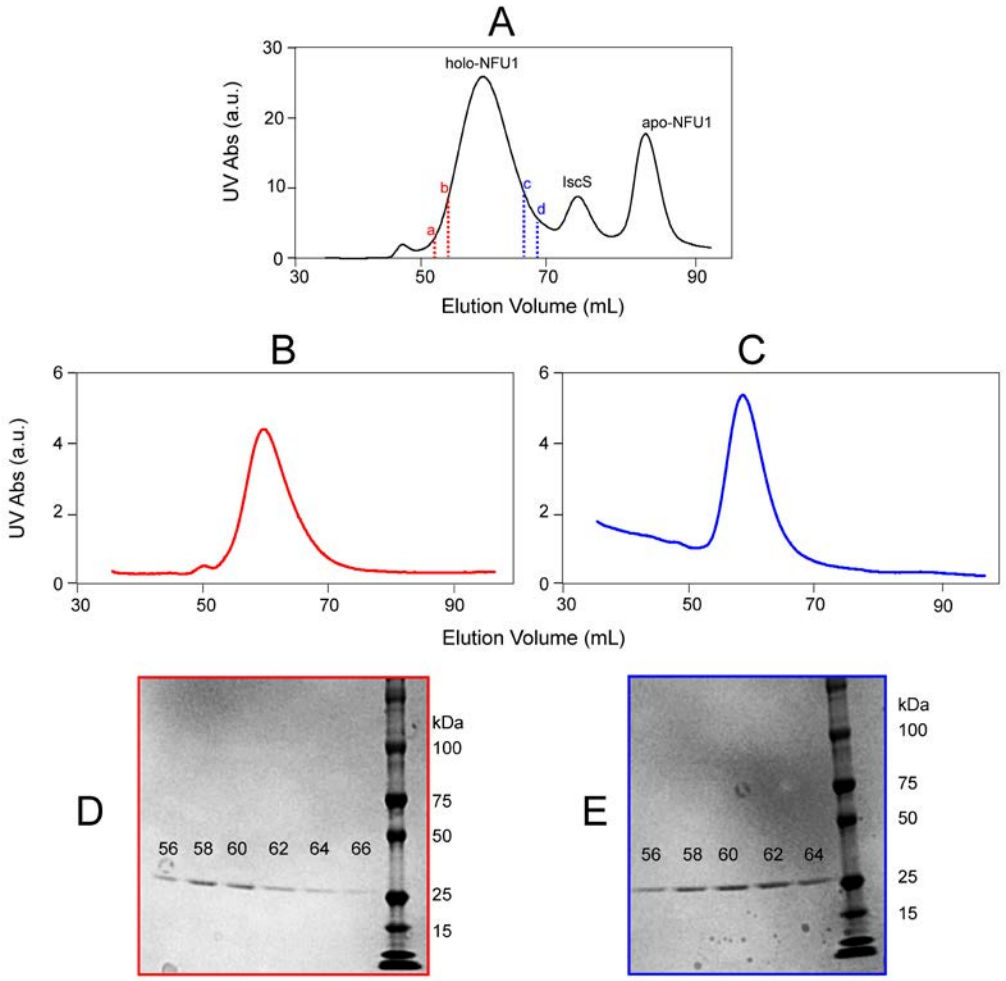

Figure S3, related to Figure 3. Results of rechromatographing the leading and trailing edges of the size exchange chromatography (SEC) peak assigned to holo-NFU1 in Figure 3*D* to determine that it represents a single species.

(*A*) SEC of the product of the cluster assembly reaction indicating the volume fractions used for re-chromatography: a-b, leading edge, c-d, trailing edge. The identities of the peaks are indicated.

(*B*) SEC of the leading edge fraction. The SEC profile shows a single peak with the same elution volume as that assigned to holo-NFU1 in panel A.

(*C*) SEC of the trailing edge fraction. The SEC profile shows a single peak with the same elution volume as that assigned to holo-NFU1 in panel A.

(*D*) SDS-PAGE of the fractions collected for the major peak in panel B. The numbers indicate the elution volume of each fraction.

(*E*) SDS-PAGE of the fractions collected for the major peak in panel C. The numbers indicate the elution volume of each fraction.

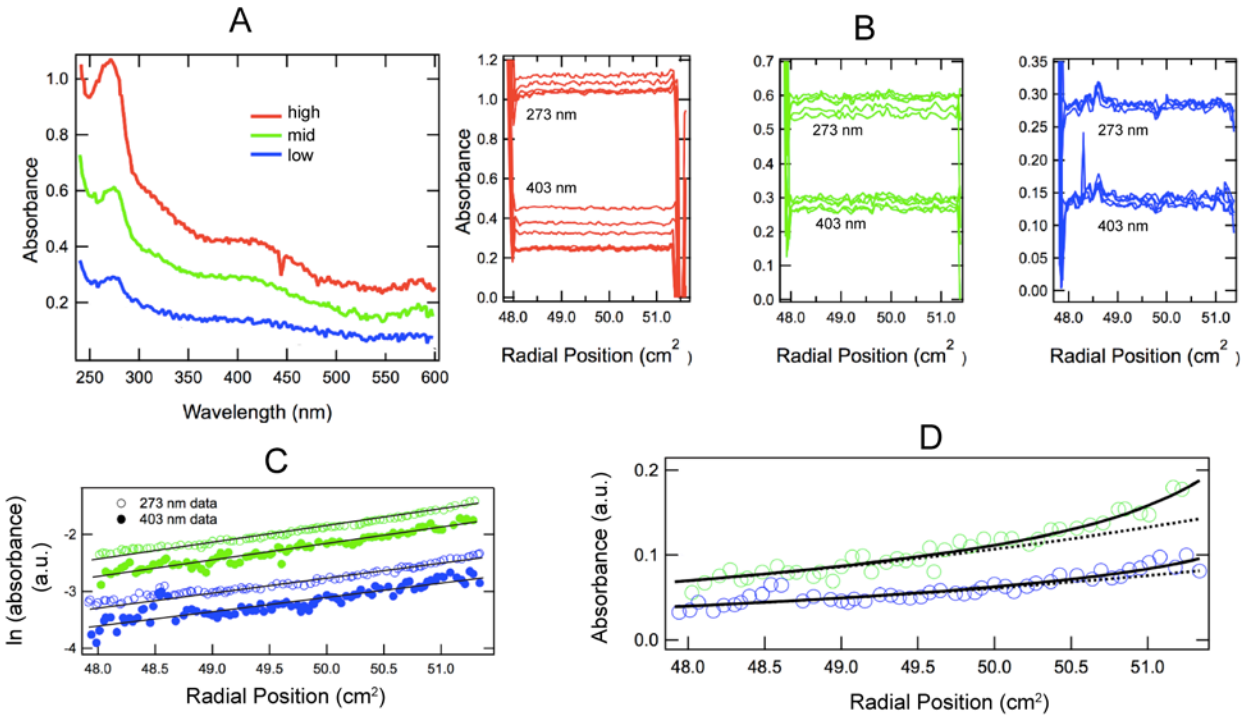

Figure S4, related to Figure 3. Sedimentation equilibrium results with the holo-NFU1 fraction shown in Figure 3*D*. Three different concentrations of holo-NFU1 were used as color coded (red) 4 mg/mL (high), (green) 2 mg/mL (mid), and (blue) 1 mg/mL (low). The solutions contained 20 mM HEPES buffer, pH 7.6, 150 mM NaCl, and 3 mM tris(2-carboxyethyl)phosphine (TCEP) as the reducing agent. All the equilibrium data were collected at 4800 rpm. The Fe-S clusters on holo-NFU1 are susceptible to oxidative damage. Although the experiment was carried out under vacuum, some cluster loss was observed during the long run, especially for the 4 mg/mL sample.

(*A*) Initial uv-vis spectra of the three concentrations of holo-NFU1 used.

(*B*) Radial scans as a function of time of absorbance at 273 nm and 403. The large decrease in absorbance at 403 nm for the most concentrated sample (4 mg/mL) indicated cluster loss. The other two samples (2 mg/mL and 1 mg/mL) exhibited much lower decrease in absorbance, indicating that their clusters remained reasonably intact.

(*C*) Plot of ln(absorbance) as a function of radial position. The open circles denote data collected at 273 nm and solid dots denote data collected at 403 nm. The parallel straight lines for the 2 mg/ml and 1 mg/ml samples indicate the presence of a single species. Results from the 4 mg/ml protein sample are not shown because of cluster loss.

(*D*) Absorbance at 403 nm as a function of radial position for two samples (2 mg/mL and 1 mg/mL). The solid line represents the fit to the data, and the dotted line represents a fit to six times the molecular weight of a single NFU1 chain (neglecting the [4Fe-4S] cluster). The data are consistent with a hexamer as the dominant species.

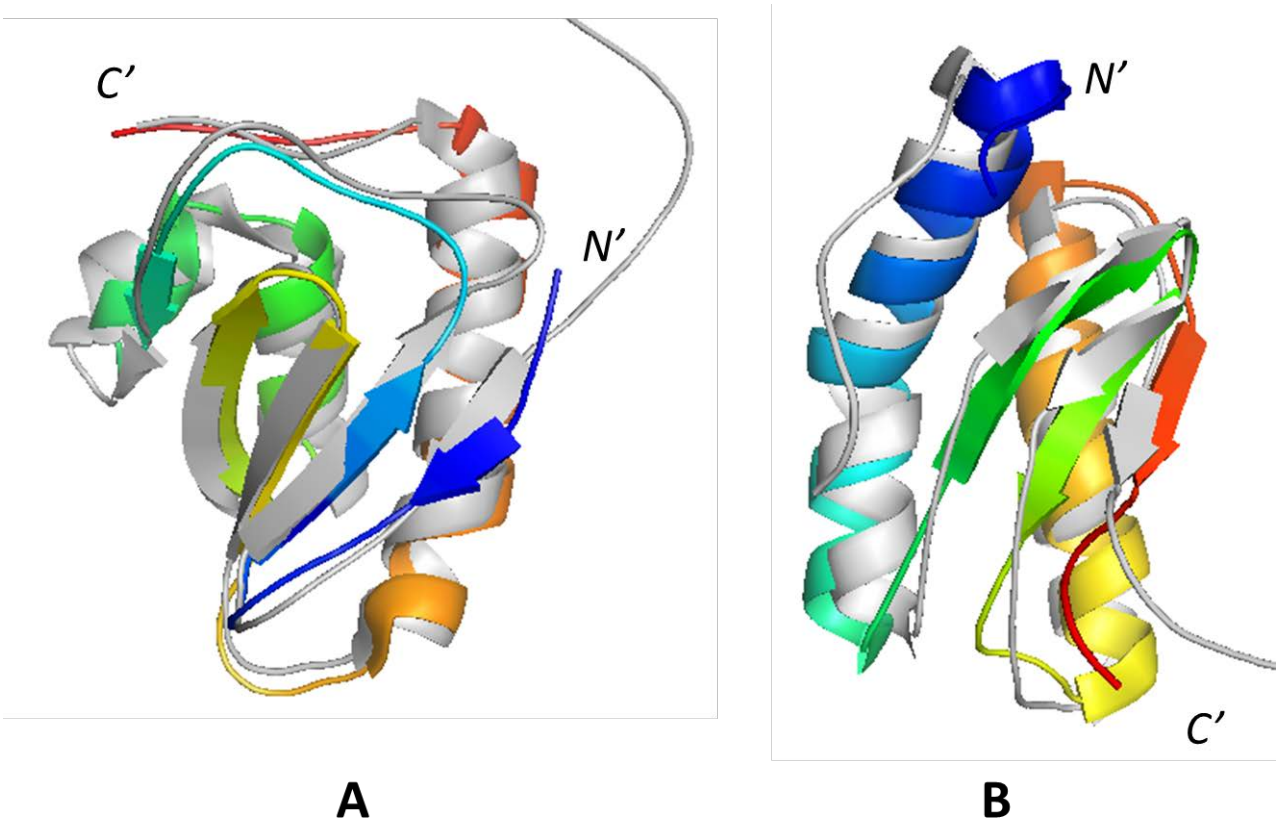

Figure S5, related to Figure 1. Comparison of the solution NMR structures of the N-terminal and C-terminal domains of NFU1 from Figure 1 with structures of Nfu1 domains from different organisms.

(A) Structure shown in colors of the N-terminal domain of *Homo sapiens* NFU1 (PDB ID: 2ltm) compared to the structure shown in gray of the N-terminal domain of *Saccharomyces cerevisiae* Nfu1 (PDB ID 2ltl).

(B) Structure shown in colors of the C-terminal domain of *Homo sapiens* NFU1 (PDB ID: 2m5o) compared to the structure shown in gray of the C-terminal domain of *Mus musculus* Nfu1 (PDB ID: 1veh). The residues in each domain from human NFU1 are colored from blue at the N-terminus to red at the the C-terminus, as in Figure 1. The N-, C- termini are labeled as N′ and C′, respectively.

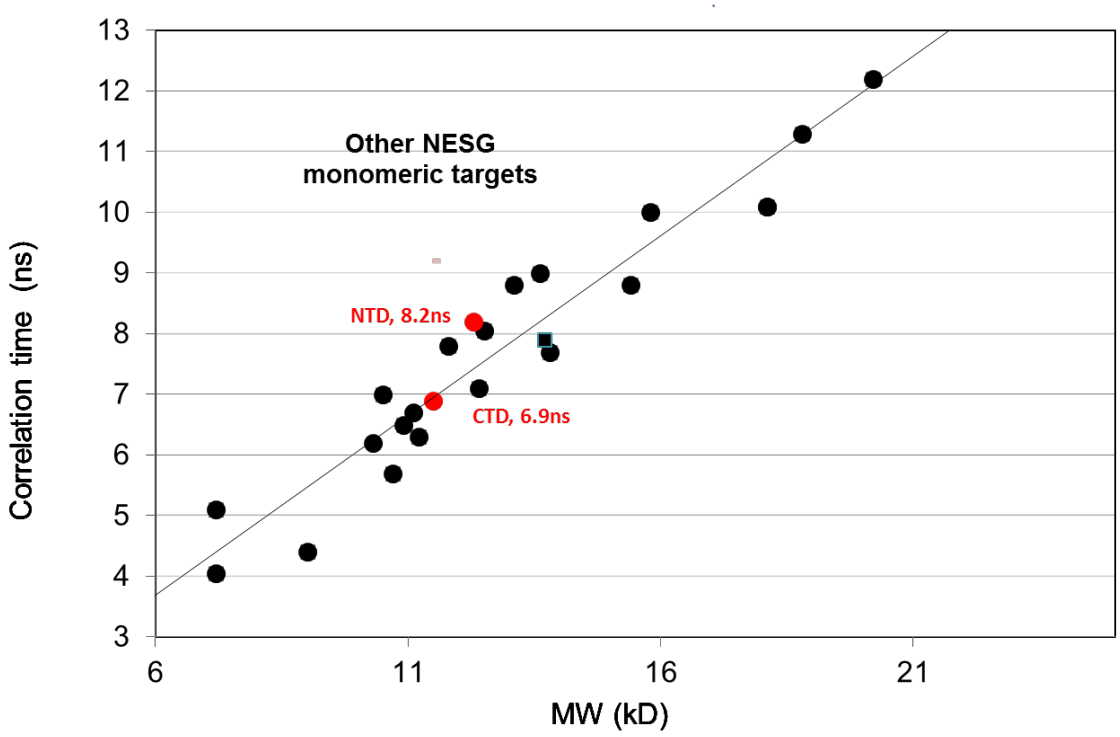

Figure S6, related to Figure 1. (*Red circles*) Experimental rotational correlation times, τ_c (ns), for NFU1 NTD and CTD determined at 25 ˚C under reducing conditions determined from 1D ^{15}N T_1/T_2 relaxation data plotted vs. protein construct molecular weight (kDa). These conditions are the same as those used for the NMR structure determinations of these domains shown in Figure 1. (*Black circles*) Data from an archive of monomeric proteins of known molecular weights and oligomerization states. These results demonstrate that both the NTD and CTD are monomeric under the conditions used for the NMR structure determination.