Supporting Information

Non-Enzymatic Synthesis of the P-Cluster in the Nitrogenase MoFe Protein: Evidence for the Involvement of All-ferrous $[Fe_4S_4]^0$ Intermediates

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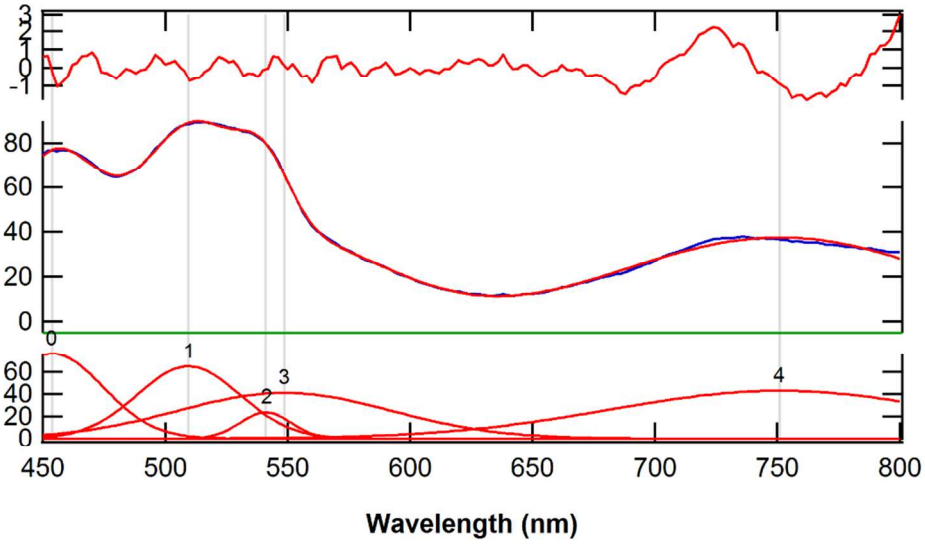
A



B

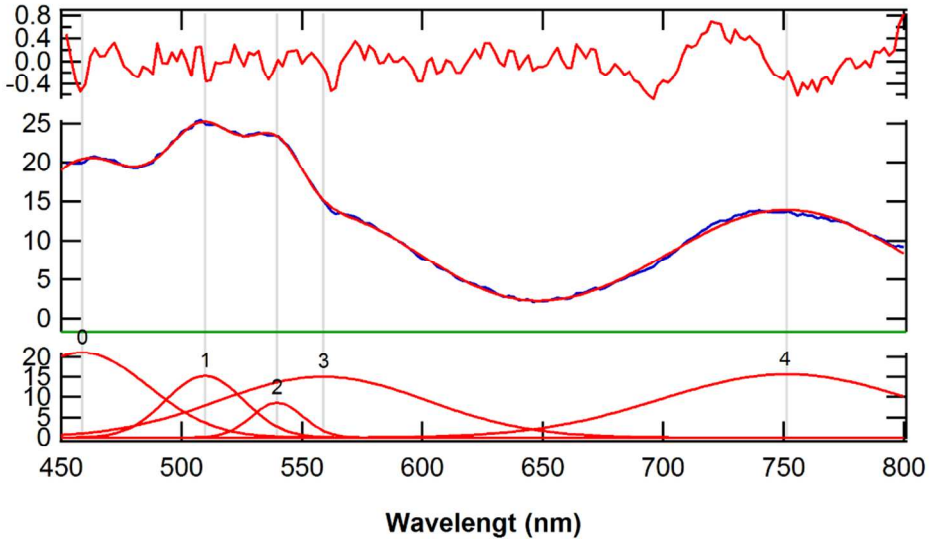


Figure S1. Gaussian Simulations of the MCD Spectra of Ti(III) Citrate Reduced Δ*nifH* NifDK (A) and NifH (B) at 1.6K and 6T. Top Curves in each figure: Intensity difference between simulation and spectrum. Middle Curves: Simulated (Red) and experimental (Blue) spectra. Bottom Curves: Gaussian simulations of the various individual transitions. The first simulation is the initial reference point and is labeled 0 with subsequent simulations labeled 1, 2, etc.

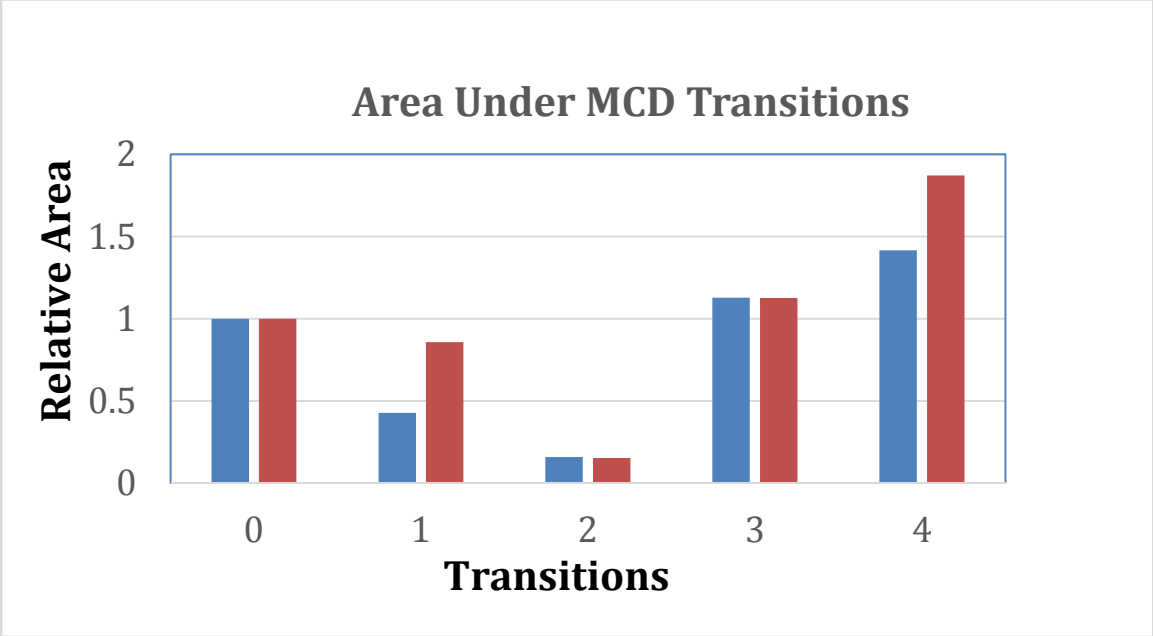


Table T1. Comparison of MCD Spectral Peaks and Areas of Gaussian Fits to Ti(III) Citrate Reduced NifH (Blue) and Δ*nifH* NifDK (Red). Plot of the integrated area under each of the five simulated major MCD spectral transitions (see S1) relative to the area of transitions 0 (normalized to 1). The five transitions for the reduced NifH are 0: 459 nm, 1: 510 nm, 2: 540 nm, 3: 559 nm and 4: 751 nm. The five transitions for the reduced Δ*nifH* NifDK are 0: 454 nm, 1: 510 nm, 2: 541 nm, 3: 549 nm and 4: 751 nm.

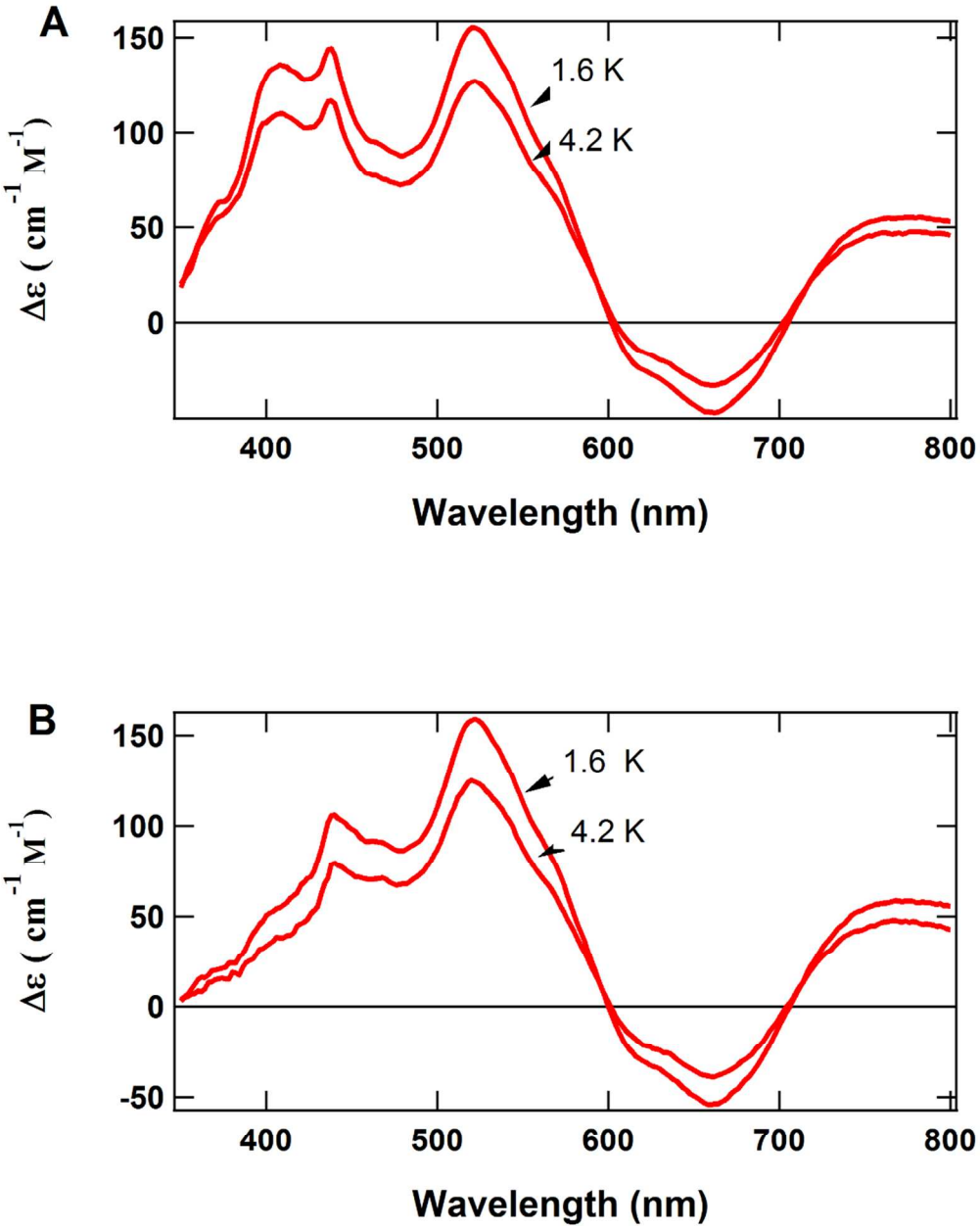


Figure S2. MCD Spectra of Δ*nifH* NifDK (A) in DTN Following Ti(III) Citrate Reduction and Subsequent IDS Oxidation and (B) Direct Equilibration in DTN Following Ti(III) Citrate Reduction Recorded at 1.6 K and 4.2 K. The uniform decrease in intensity with increased temperature is consistent with both spectra corresponding to only a single paramagnetic species. Note that both spectra have the same intensity, which represents about 30-35% that of the as-isolated protein.

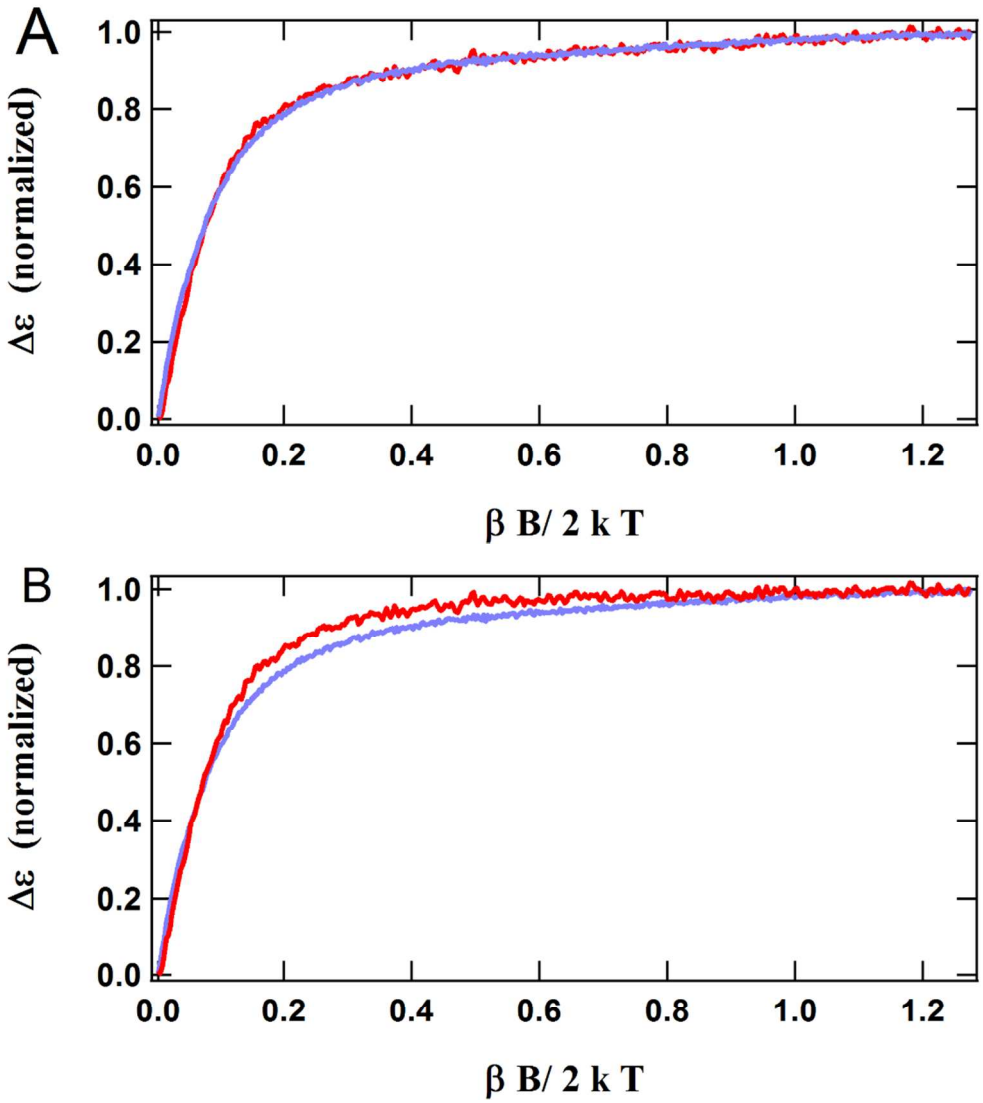


Figure S3. Simulation (Blue) of the Magnetization Curve of OAR at 1.6 K and 790 nm using $S = 1$ (A) and $S = 2$ (B) for X compared to the known (Red) magnetization curve for P^{2+}. The magnetization curve of X at 1.6 K was approximated by assuming that the composite experimental curve (i.e., X + P^{2+}) at 790 nm is due to 75% P^{2+} and 25% X, as deduced from the spectral simulation (see text). Because X is EPR silent in the IDS oxidized state, it is assumed to be an integer state as is P^{2+}. The parameters used for the best-fit simulations are (A) $D = -4.9$ cm^{-1}, $E/D = 0.296$ using Polarizations $M_{xy} = 0.5$, $M_{xz} = 0.8$ and $M_{yz} = 1.0$ and (B) $D = -5.05$ cm^{-1}, $E/D = 0.151$ using Polarizations $M_{xy} = 1.1$, $M_{xz} = 0.8$ and $M_{yz} = 0.9$.