The Functionality of the three-sited Ferroxidase center of E. coli Bacterial Ferritin (EcFtnA)

SUPPORTING INFORMATION

The Functionality of the Three-Sited Ferroxidase Center of *E. Coli* Bacterial Ferritin (EcFtnA) [*]

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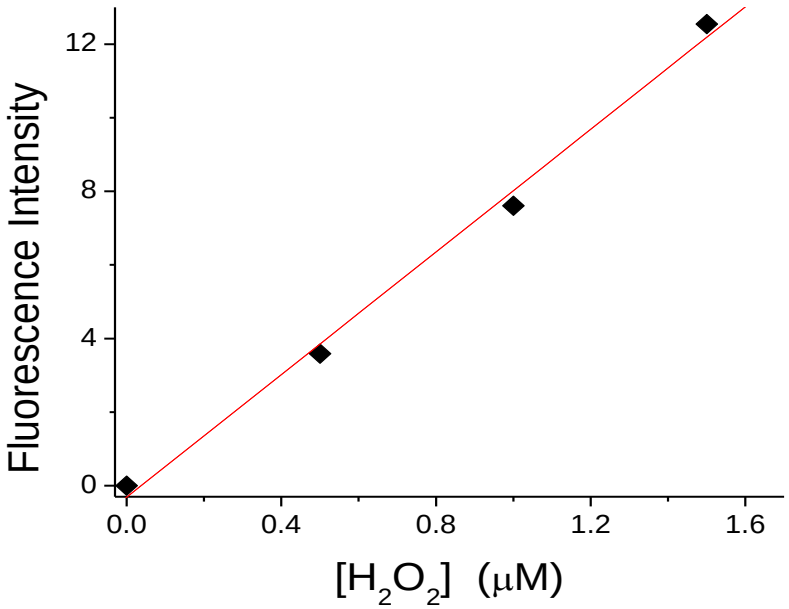


Figure S1: Standard curve for Amplex Red assay.

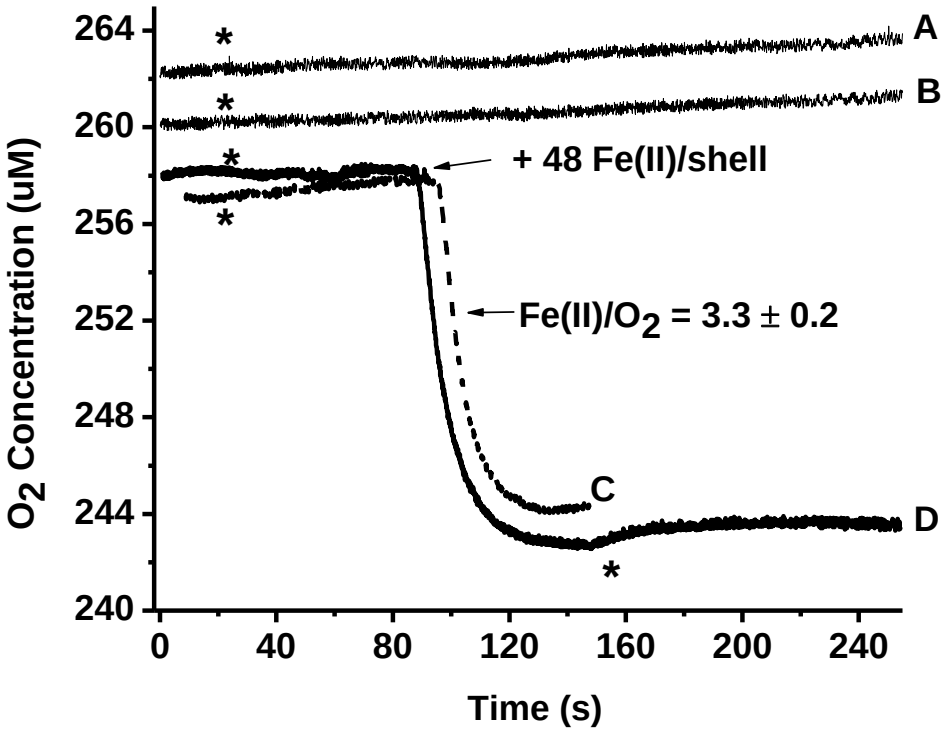


Figure S2: Oxygen consumption curves for (A) ApoEcFtnA (1 uM) in 0.1 M Mops, 50 mM NaCl, pH 7.0 + 1 ul catalase (1300 units); (B) buffer alone + 1 ul catalase; (C) EcFtnA (1 uM) in 0.1 M Mops, 50 mM NaCl, pH 7.0 + 1 ul catalase followed by 48 Fe(II)/shell; (D) EcFtnA (1 uM) in 0.1 M Mops, 50 mM NaCl, pH 7.0 + 48 Fe(II)/shell followed the addition of 1 ul catalase at the end of the oxidation reaction (the star denotes the addition of catalase).

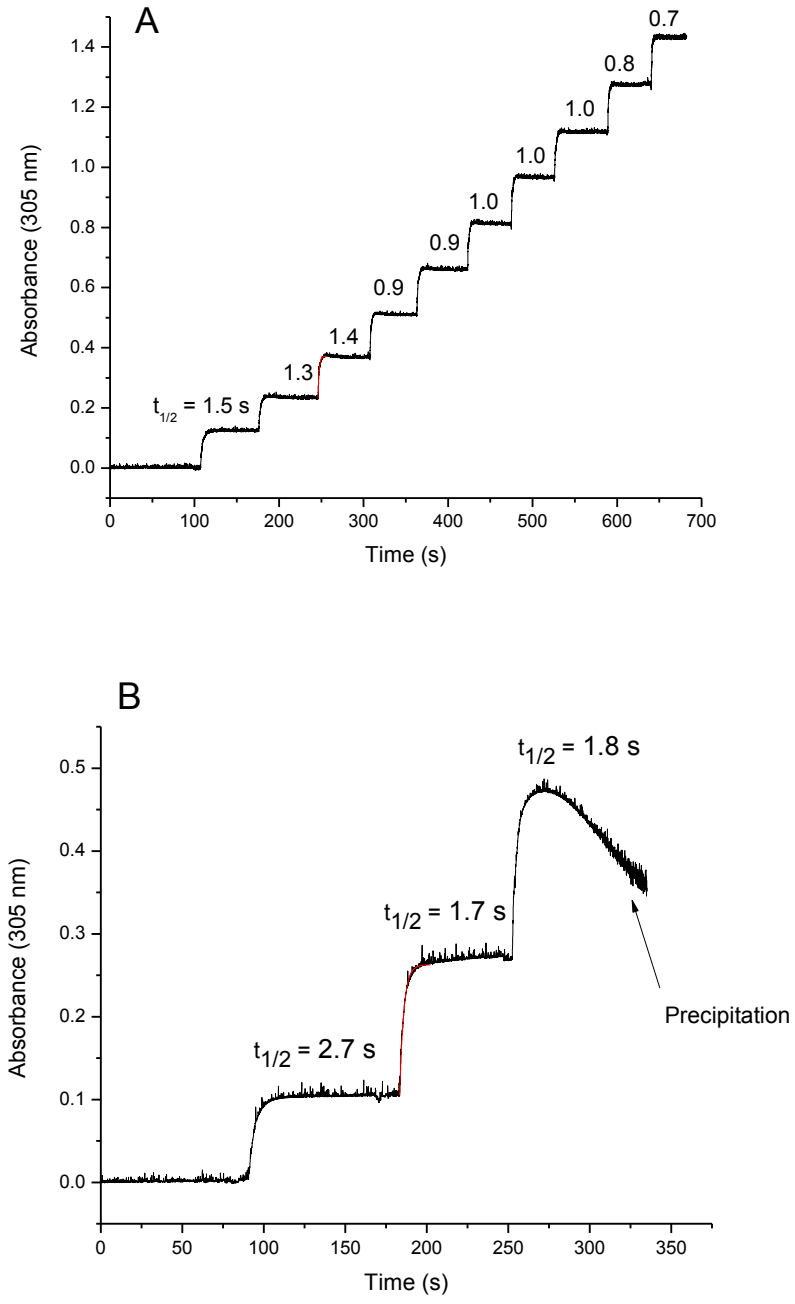
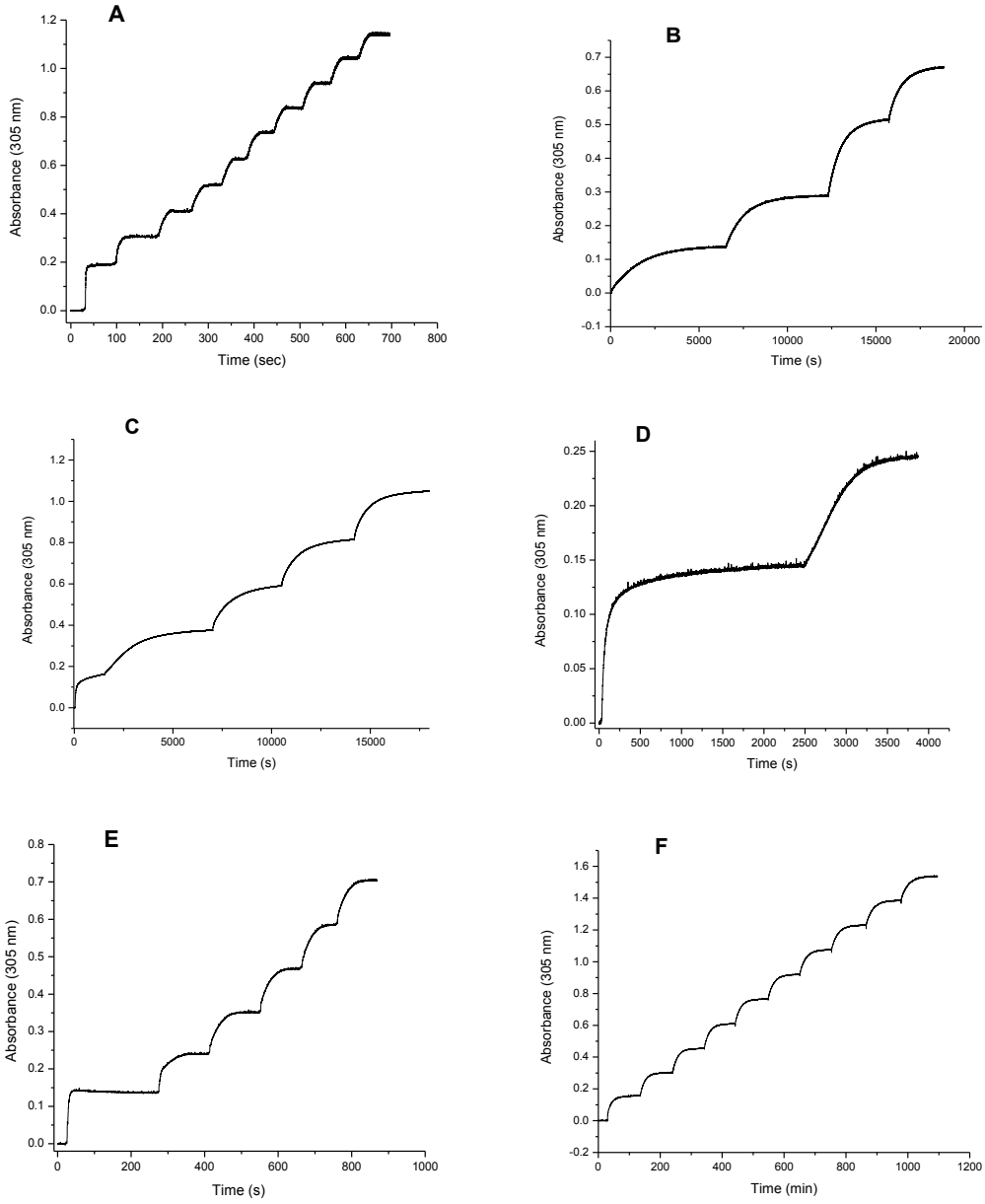


Figure S3: (A) Multiple anaerobic additions of 48 Fe(II)/shell to apoEcFtnA (1 µM) in 0.1 M Mops, 0.1 M NaCl, pH 7.00 followed by 24 H_2O_2/shell. Half-lives in seconds from single exponential fits are indicated. Fitting errors are ± 0.02 s. (B) Two anaerobic additions of 500 Fe(II)/shell to apoEcFtnA (0.1 µM) in 0.1 M Mops, 0.1 M NaCl, pH 7.00 followed by 250 H_2O_2/shell. The third addition of 500 Fe(II)/shell led to protein precipitation. Half-lives from single exponential fits are indicated. Fitting errors are ± 0.03 s.


A
Absorbance (305 nm)
Time (sec)
B
Absorbance (305 nm)
Time (s)
C
Absorbance (305 nm)
Time (s)
D
Absorbance (305 nm)
Time (s)
E
Absorbance (305 nm)
Time (s)
F
Absorbance (305 nm)
Time (min)

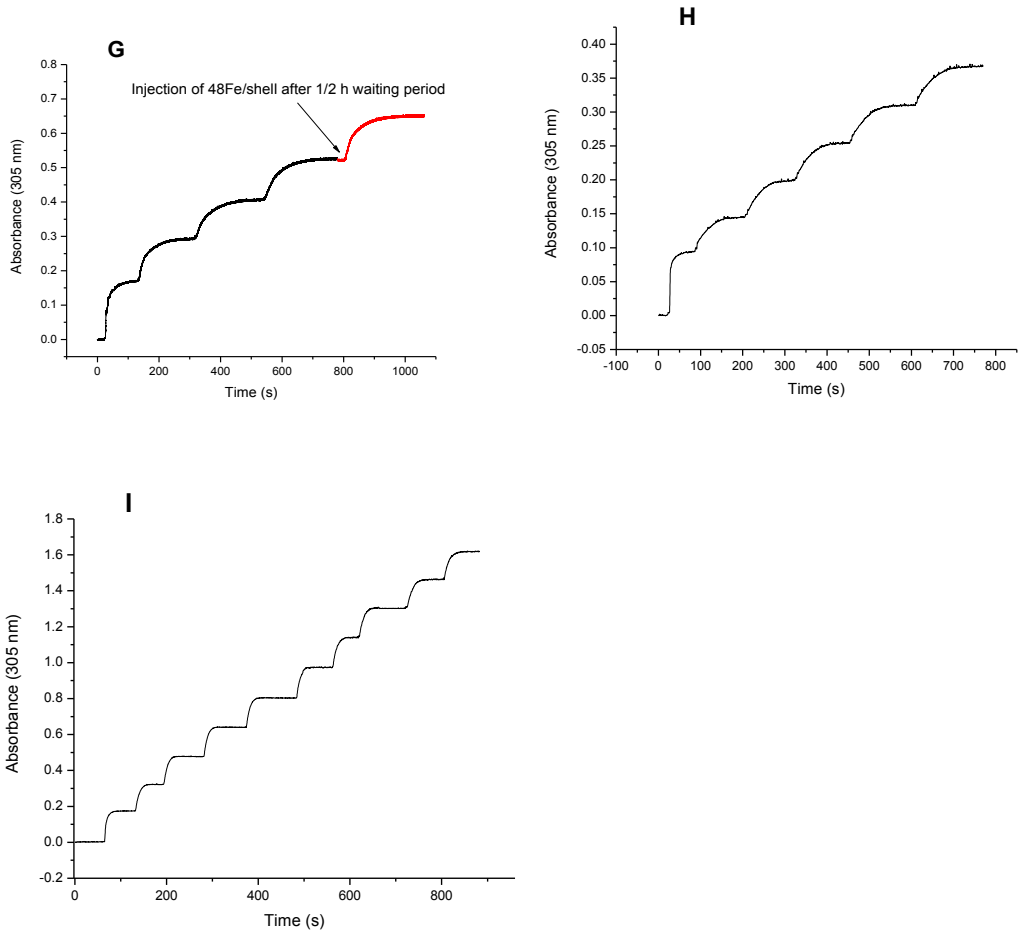


Figure S4: Absorbance-time curve for multiple aerobic additions of 48 Fe(II) per shell to 1 μM protein solutions (except Y24F at 0.5 uM) in 0.1 M Mops, 50 mM NaCl, pH 7.02. Derived half-lives for oxidation are given in Table 2. (A) Wt EcFTnA, (B) H53A, (C) E17A, (D) E94A, (E) E130A, (F) E 49A, (G) E126A, (H) Y24F, (I) HuHF

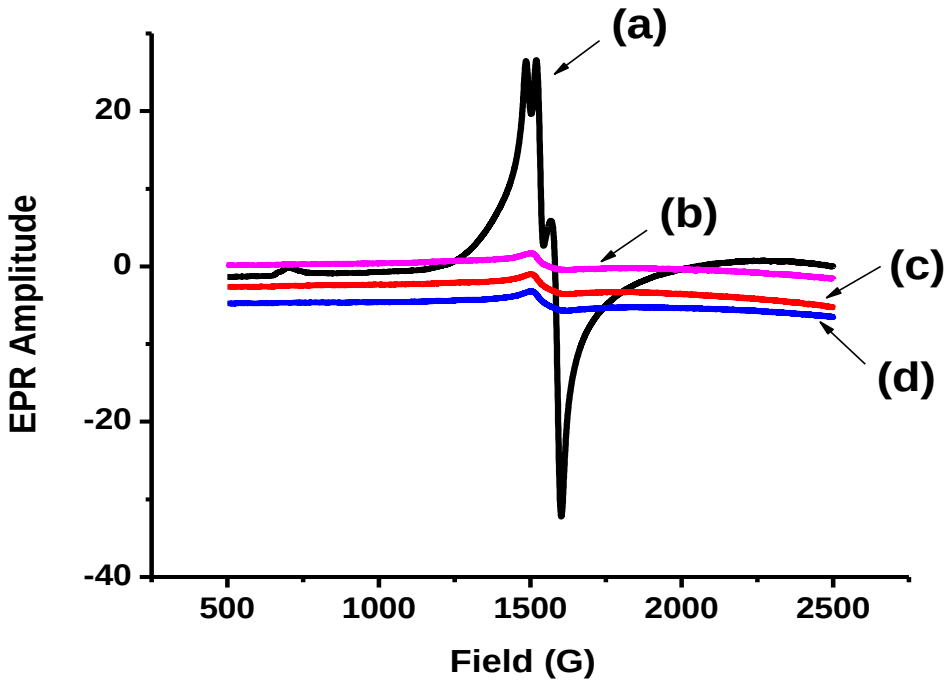


Figure S5: EPR spectra of frozen solutions of (A) Fe^{3+}–human transferrin (0.427 mM monoferric C-lobe transferrin) in 20 mM $NaHCO_3$, 50 mM Mops, pH 7.0, (B) EcFtnA + 48 Fe(II) per shell (0.427 mM Fe^{3+}), (C) sample B + another 24 Fe(II)/shell to give a total of 72 Fe(II)/shell (0.64 mM Fe^{3+}), (D) EcFtnA + 72 Fe(II) per shell (0.64 mM Fe^{3+}) added in one shot. Conditions: [EcFtnA] = 8.9 uM in 0.1 M Mops and 50 mM NaCl, pH 7.0. Sample B was frozen 2 minutes after adding Fe^{2+}; Sample C was frozen 2-3 minutes after thawing sample A and adding 24 more Fe/shell; Sample D was frozen 5 minutes following the one shot addition of 72 Fe(II)/shell.

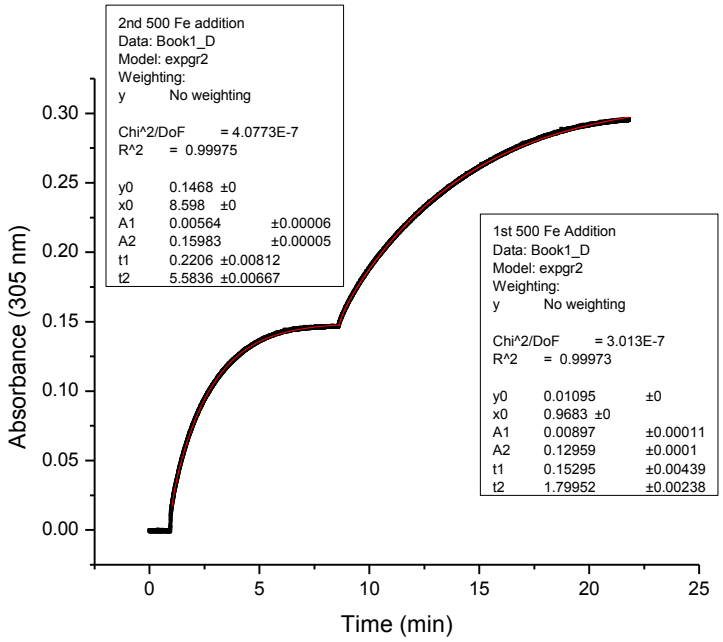


Figure S6: Iron uptake curves for two additions of 500 Fe(II) additions to apoEcFtnA (0.1 μM) in 0.1 M Mops, 0.1 M NaCl, pH 7.00. Red lines are fitted curves with fitting parameters given in the boxes. Fitting function

$$y = y_0 + A_1[1-\exp((x_0 - x)/t_1)] + A_2[1-\exp((x_0 - x)/t_2)]$$

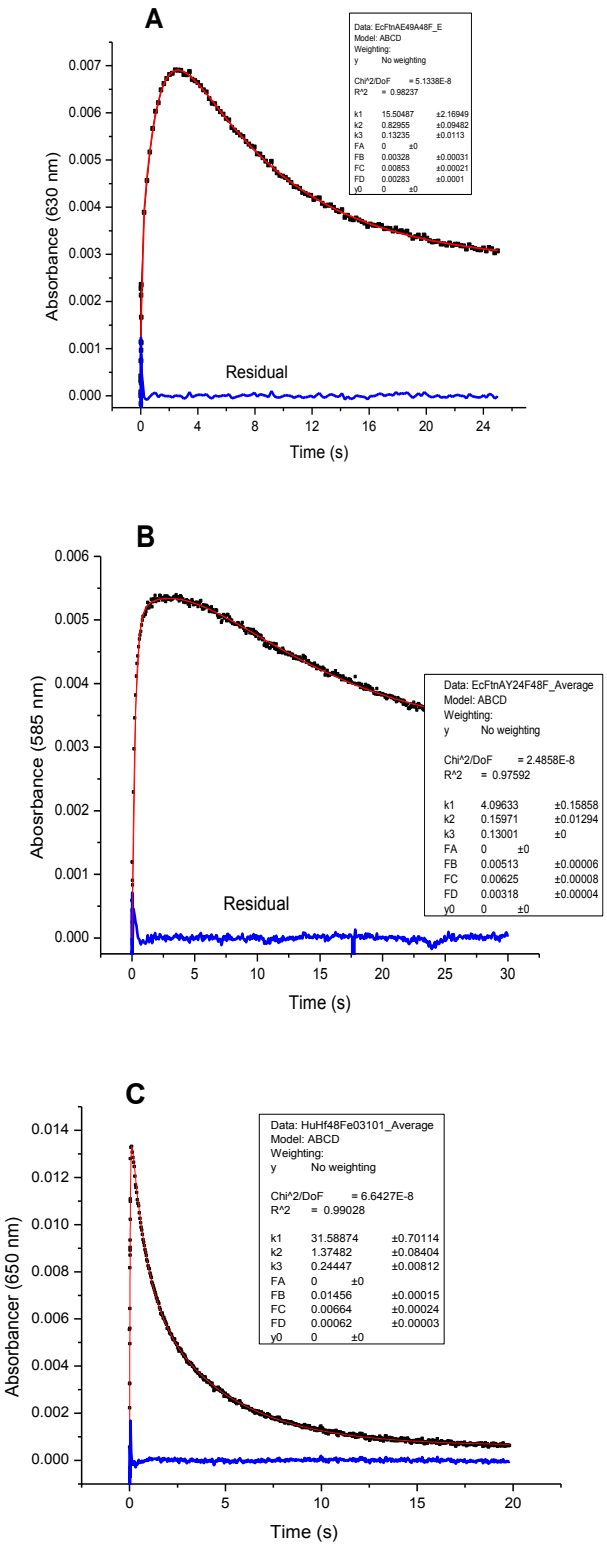


Figure S7: Stopped-flow kinetics curve for peroxo complex formation and decay in (A) E49A, (B) Y24F and (C) HuHF.

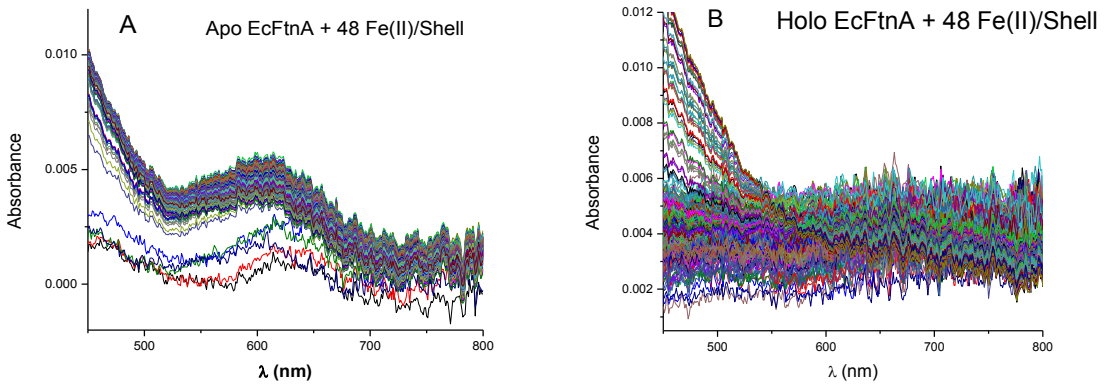


Figure S8: Multiwavelength stopped-flow spectra of (A) ApoEcFtnA (1 μM) in 0.1 M + 48 Fe(II)/shell and (B) Holoferritin (1.5 μM) containing 72 Fe(III)/shell + 48 Fe(II)/shell, both in 0.1 M Mops, 0.1 M NaCl, pH 7.0. Spectra scaled to 1 μM protein concentration.

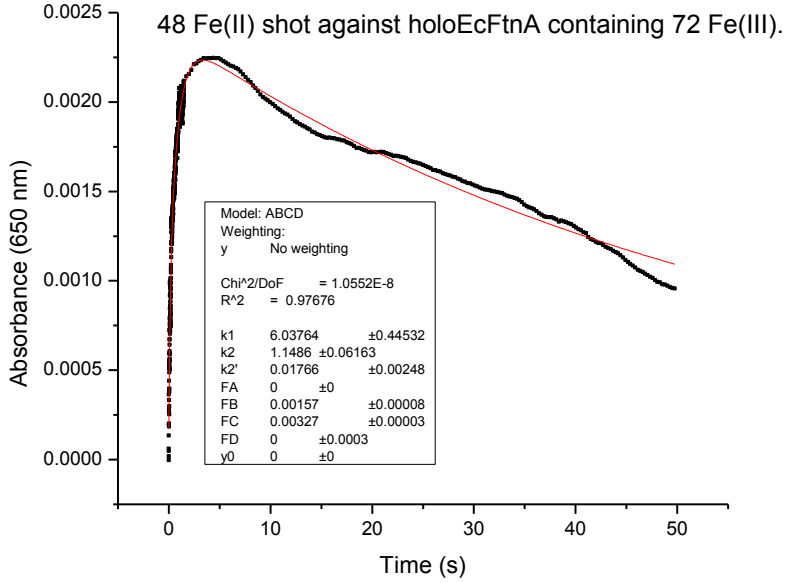


Figure S9: Stopped-flow kinetic curve at 650 nm for weak absorbance in Fig. S7B. Fit is only approximated by a $A \xrightarrow{k_1} B \xrightarrow{k_2} B' \xrightarrow{k_2'} C$ model.

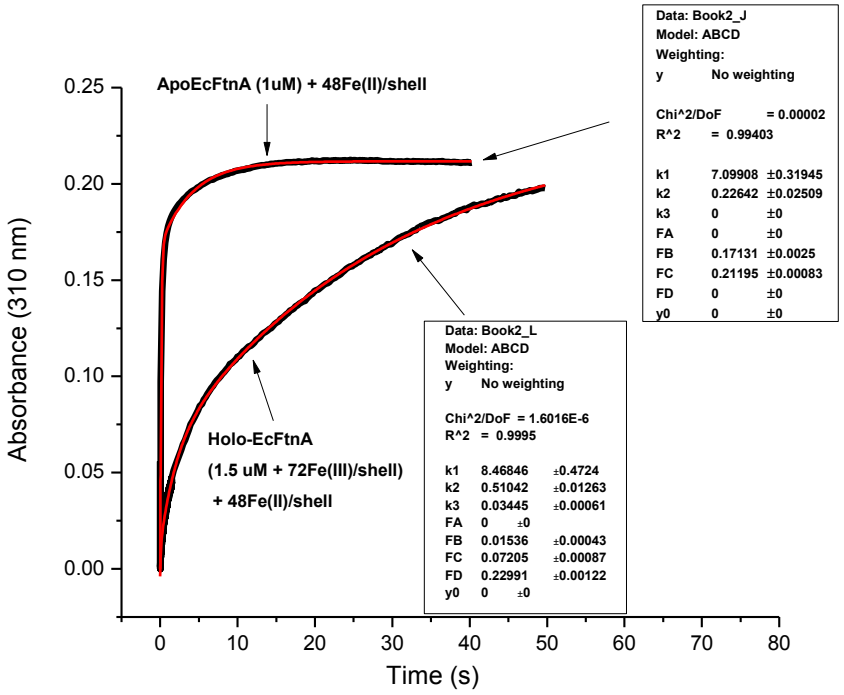


Figure S10: Stopped-flow absorbance-time curve at 310 nm for apoEcFtnA + 48 Fe(II)/shell and Holo EcFtnA + 48 Fe(II)/shell. Curve for HoloEcFtnA has been scaled to 1 μM protein concentration. Conditions: 0.1 M Mops, 0.1M NaCl, pH 7.

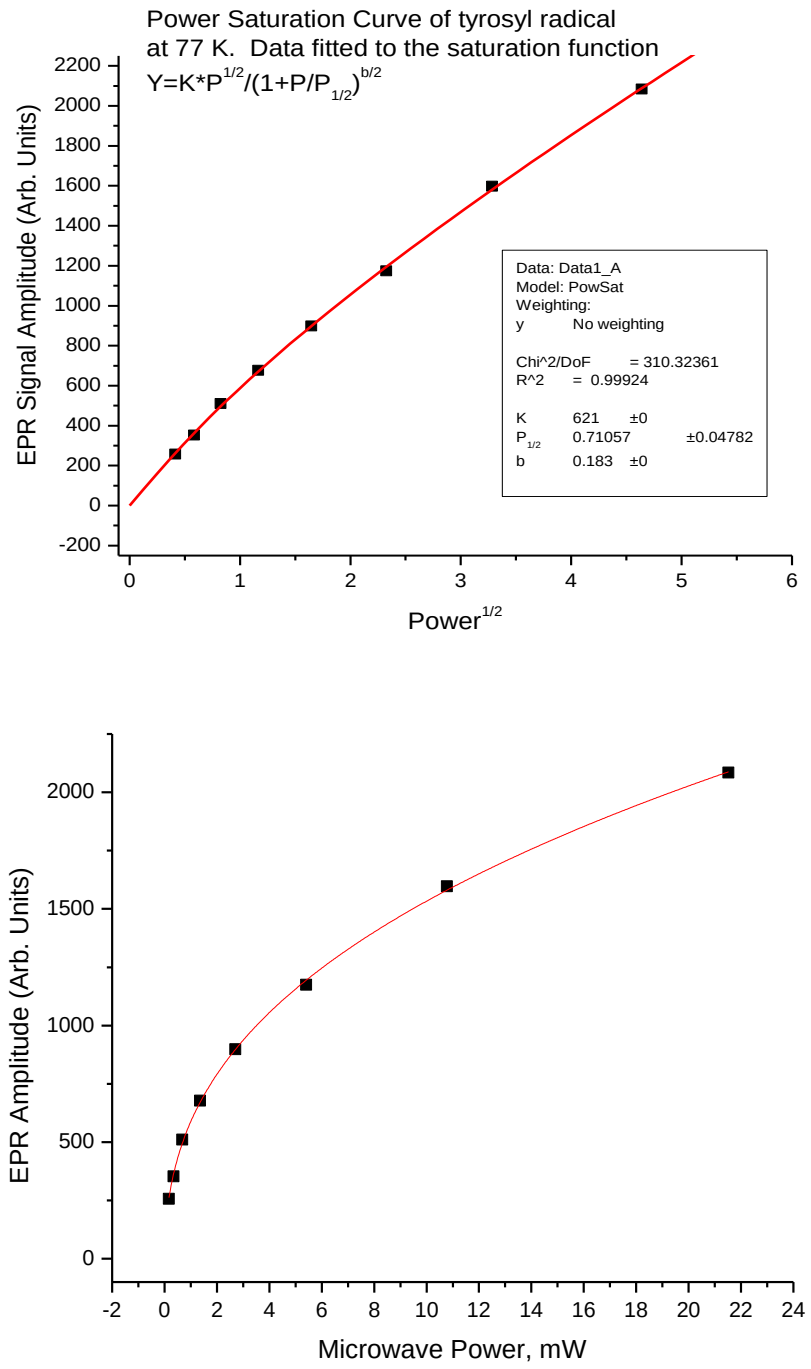


Figure S11: EPR power saturation curves of tyrosine radical signal.