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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 H₂O₂/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 H₂O₂/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.
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 μM) 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-lobef 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.
Power Saturation Curve of tyrosyl radical at 77 K. Data fitted to the saturation function
\[ Y = \frac{K \cdot P^{1/2}}{1 + P/P_{1/2}^{1/2}} \]

Data: Data1_A
Model: PowSat
Weighting: y = No weighting

\[ \text{Chi}^2/\text{DoF} = 310.32361 \]
\[ R^2 = 0.99924 \]

\[ K = 621 \pm 0 \]
\[ P_{1/2} = 0.71057 \pm 0.04782 \]
\[ b = 0.183 \pm 0 \]

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