Supplemental Data

Evidence that His349 acts as a pH-inducible switch to accelerate receptor-mediated iron release from the C-lobe of human transferrin

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Equation for A→B→C where $k_1 \neq k_2$

The time dependence of the fluorescence intensity *F(t)* is given by the following equation:

$$F(t) = F_A[A]_o e^{-k_1 t} + F_B \frac{k_1[A]_o}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) + F_C[A]_o \left[1 + \frac{1}{k_1 - k_2}(k_2 e^{-k_1 t} - k_1 e^{-k_2 t})\right]$$

Here F_A, F_B and F_C are the molar fluorescence intensity constants of species A, B and C. This equation is derived in the Supplemental Data section of Byrne, S. L., Chasteen, N. D., Steere, A. N., and Mason, A. B. (2010) The unique kinetics of iron-release from transferrin: The role of receptor, lobe-lobe interactions and salt at endosomal pH, *J Mol Biol 396*, 130-140.

Origin formula:

y=FA*exp(-k1*x)+ ((FB*k1)/(k2-k1))*(exp(-k1*x)-exp(-k2*x))+(FC*(1+ (((k1*exp(-k2*x))-(k2*exp(-k1*x)))/(k2-k1)))) + yo

When using the above equation, F_A is taken as zero because only differences in fluorescence intensity are important for kinetic analysis. In the situation where $k_1 \sim k_2$, the numerator and denominators of the second and third terms of the above equation both tend toward zero and the equation becomes indeterminate, resulting in large fitting errors. In this situation, the equation for the special case where $k_1 = k_2$ must be used.

Derivation of model for special case of A→B→C where $k_1 = k_2$

The solution for the case where $k_1 = k_2$ can be obtained by applying L'Hopital's rule in the limit $k_1 \rightarrow k_2$ to the above equation for the case where $k_1 \neq k_2$. Alternatively, the appropriate differential equations can be solved as detailed below. Approaches to solving the following differential equations can be found in M. R. Spiegel, *Applied Differential Equations*, Prentice-Hall, Inc., Engelwood Cliffs, NJ, 1958, pp 41-42).

$$A \xrightarrow{k} B \xrightarrow{k} C$$

Differential rate laws:

$$-\frac{d[A]}{dt} = k[A] \tag{1}$$

$$\frac{d[B]}{dt} = k[A] - k[B] \tag{2}$$

$$\frac{d[C]}{dt} = k[B] \tag{3}$$

Integrated rate law for *[A]*:

$$[A] = [A]_o e^{-kt} \tag{4}$$

Integrated rate law for *[B]*:

Substitution of equation 4 into equation 2 gives:

$$\frac{d[B]}{dt} + k[B] = [A]_o k e^{-kt} \tag{5}$$

Equation 5 is a first-order linear differential equation of the form $\frac{dy}{dx} + P(x)y = Q(x)$ with an integrating factor of $e^{\int P(x)dx} = e^{\int kdt} = e^{kt}$

Multiplication of equation (5) by e^{kt} gives equation 6.

$$\frac{d[B]}{dt} e^{kt} + k[B]e^{kt} = [A]_o k \tag{6}$$

In differential form eq (6) becomes eq (7).

$$e^{kt} d[B] + k[B]e^{kt} dt = [A]_o k dt \tag{7}$$

The left side of eq (7) is equivalent to $d[B]e^{kt}$ and thus:

$$d[B]e^{kt} = [A]_o k dt \tag{8}$$

Integration of equation (8) gives $[B]e^{kt} = [A]_o kt + c$ where *c* is a constant of integration. Rearrangement results in eq (9).

$$[B] = [A]_o kte^{-kt} + ce^{-kt} \quad (9)$$

The solution is subject to the boundary conditions *[B]* = 0 when $t = 0$ and $t = \infty$ which requires that $c = 0$. The final expression for *[B]* is therefore:

$$[B] = [A]_o kte^{-kt} \quad (10)$$

Integrated rate law for *[C]*.

Because only A is present at the start of the reaction, we can write $[C] = [A]_0 - [A] - [B]$ or that

$$[C] = [A]_0(1 - e^{-kt} - kte^{-kt}) \quad (11)$$

Fluorescence Intensity

The fluorescence intensity F(t) as a function of time is given by $F(t) = F_A[A] + F_B[B] + F_C[C]$ where F_A, F_B and F_C are the molar fluorescence intensity constants. After substitution of equations 4, 10 and 11 for the concentrations, we obtain the final expression for *F(t)*:

$$F(t) = F_A[A]_o e^{-kt} + F_B[A]_o kte^{-kt} + F_C[A]_0(1 - e^{-kt} - kte^{-kt}) \quad (12)$$

Origin formula:

y=FA*exp(-k*x)+FB*k*x*exp(-k*x)+FC*(1-exp(-k*x)-k*x*exp(-k*x)) + yo

Fitting parameters: k, FA, FB, FC, yo (offset term added)

Figure S1.

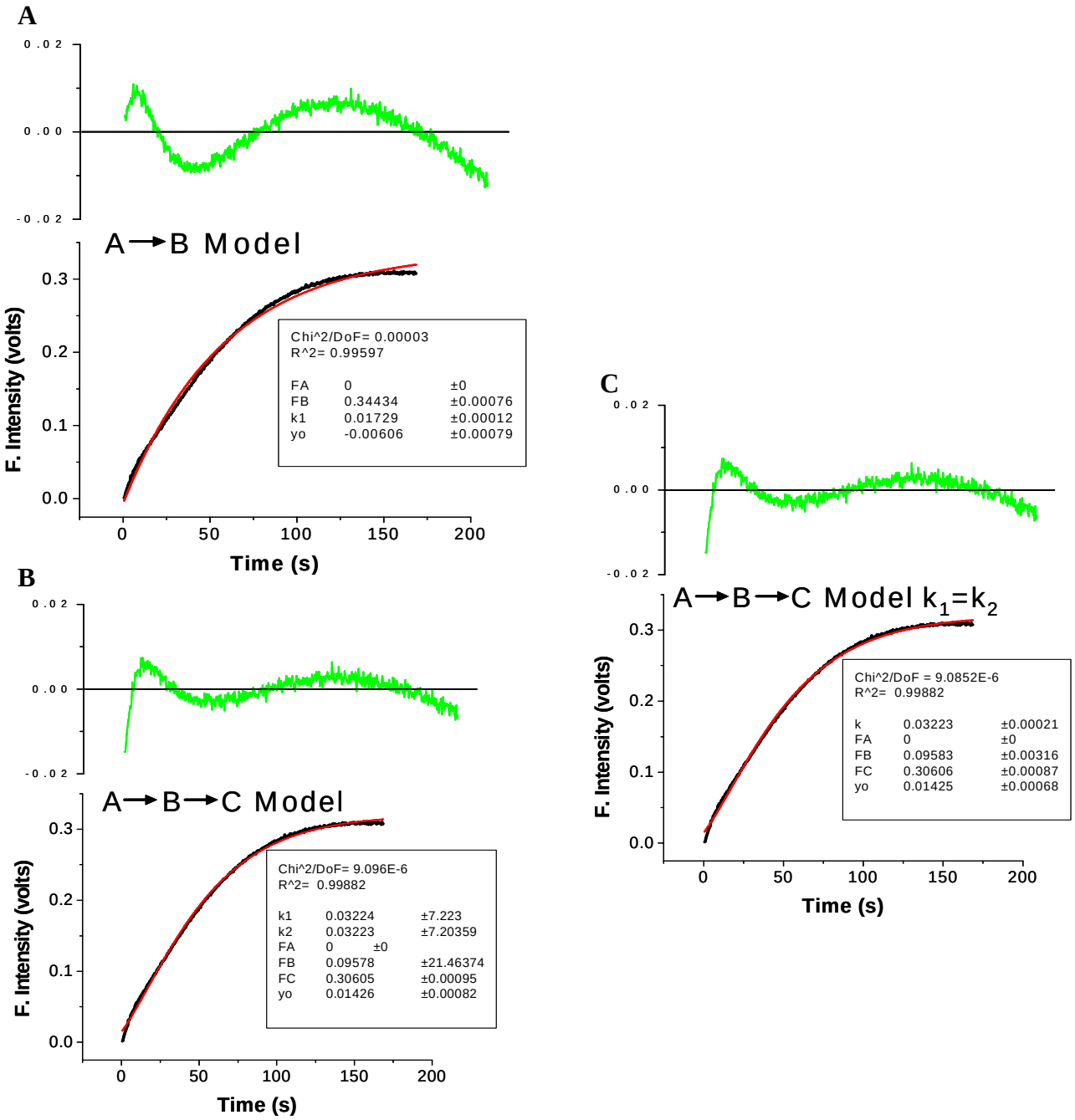

Figure S1. Comparison of curve fits for H349K Fe_ChTF + sTFR to the A→B, A→B→C, and A→B→C where k_1=k_2 models. (A) A simple A→B model (red) is insufficient to describe the data as evidenced by the residuals (green). **(B)** A two step A→B→C model (red) provides a better fit to the data (as shown by the residuals and reduced chi-square value), however the two rates are equivalent (k_1=k_2) and have large associated error (± 7.2). **(C)** The data are best fit to a two step model in which k_1=k_2 (red). It is clear that this model produces the most reasonable fit to the data given the reduced chi-square value and the elimination of the large parameter errors.

Figure S2.

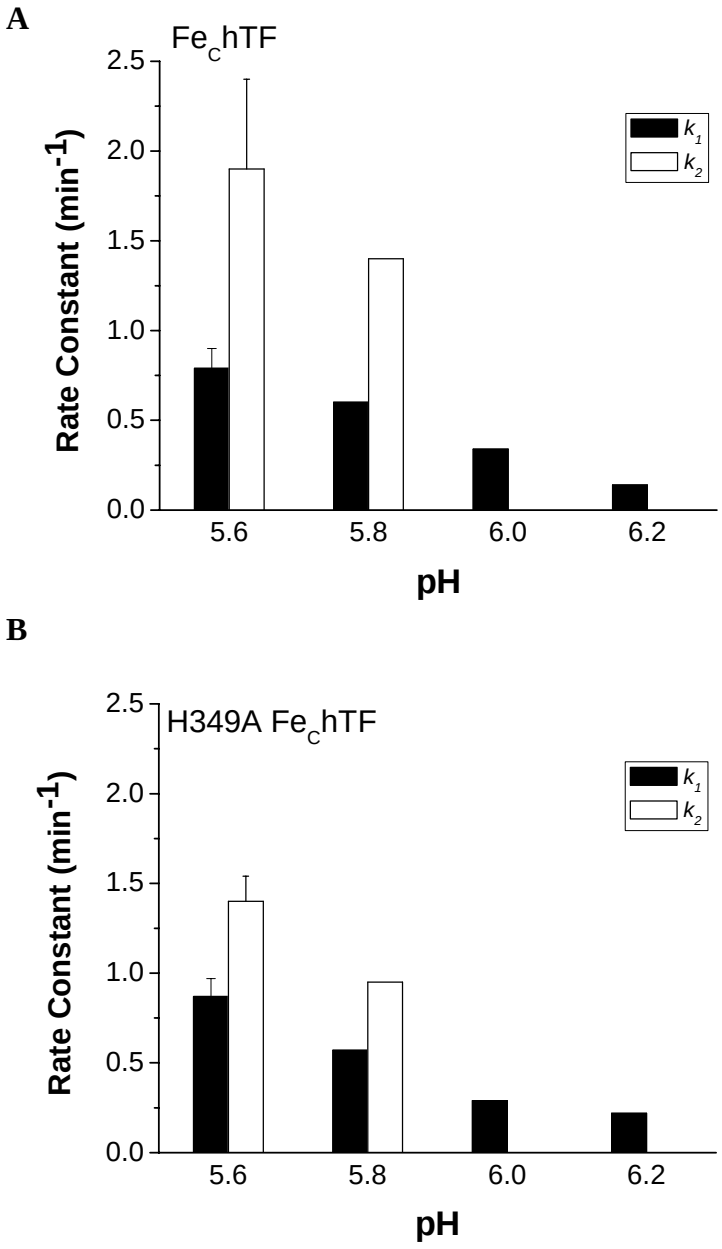

Figure S2. Effect of pH on rate constants from Fe$_C$hTF (A) and H349A Fe$_C$hTF (B) in the absence of the sTFR. Rate constants (k_1 (black) and k_2 (white)) as a function of pH are shown for the Fe$_C$hTF control **(A)** and the H349A Fe$_C$hTF mutant **(B)** in the absence of the sTFR. Except for the pH, the conditions are exactly as indicated in the legend to Fig. 3. Note that the rate constants are plotted on the same scale to provide a direct comparison between the H349A Fe$_C$hTF mutant and the Fe$_C$hTF control.

Figure S3.

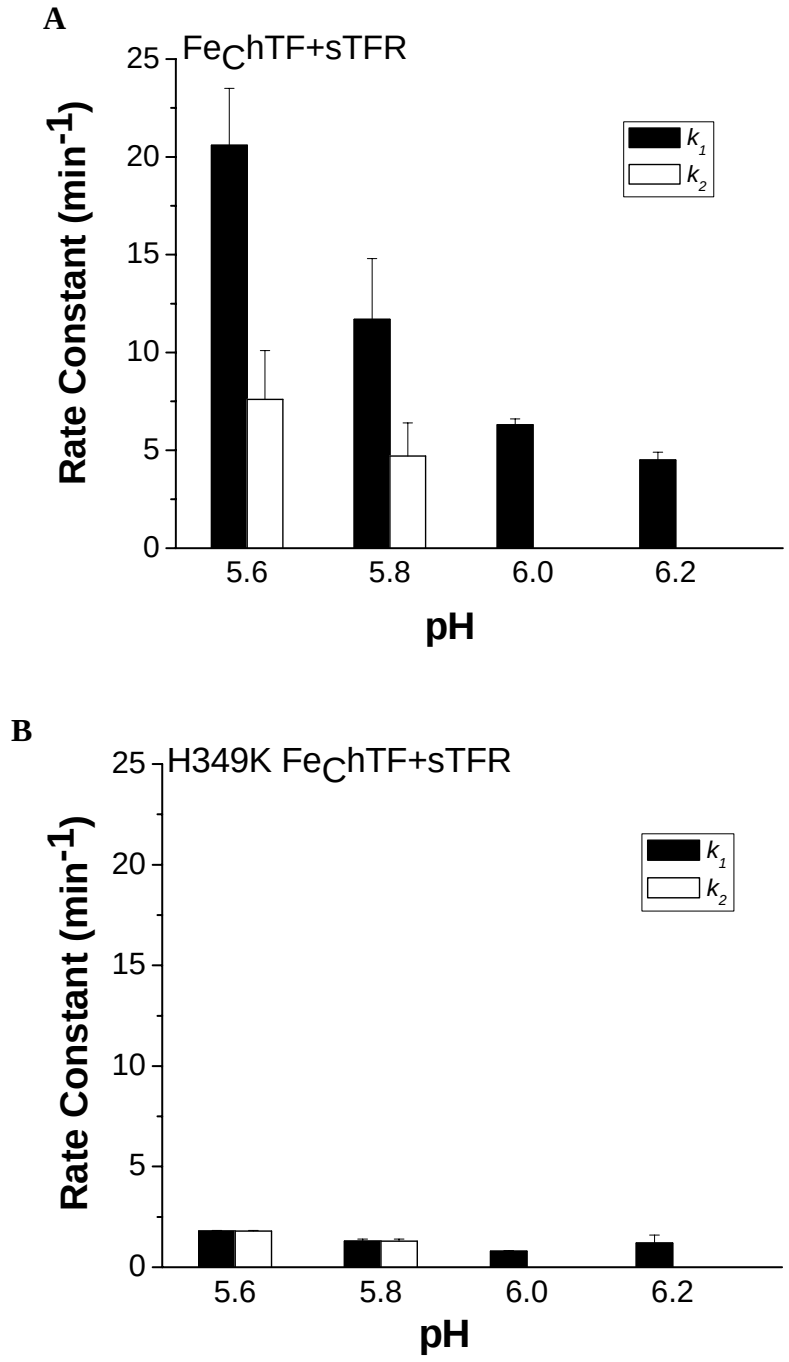

Figure S3. Effect of pH on rate constants from Fe$_C$hTF/sTFR (A) and H349K Fe$_C$hTF/sTFR (B) complexes. Rate constants (k_1 (black) and k_2 (white)) as a function of pH are shown for the Fe$_C$hTF control **(A)** and the H349K Fe$_C$hTF mutant **(B)** in the presence of the sTFR. Except for the pH, the conditions are exactly as indicated in the legend to Fig. 3. Note that the rate constants are plotted on the same scale to provide a direct comparison between the H349K Fe$_C$hTF/sTFR complex and the Fe$_C$hTF/sTFR control.

Figure S4.

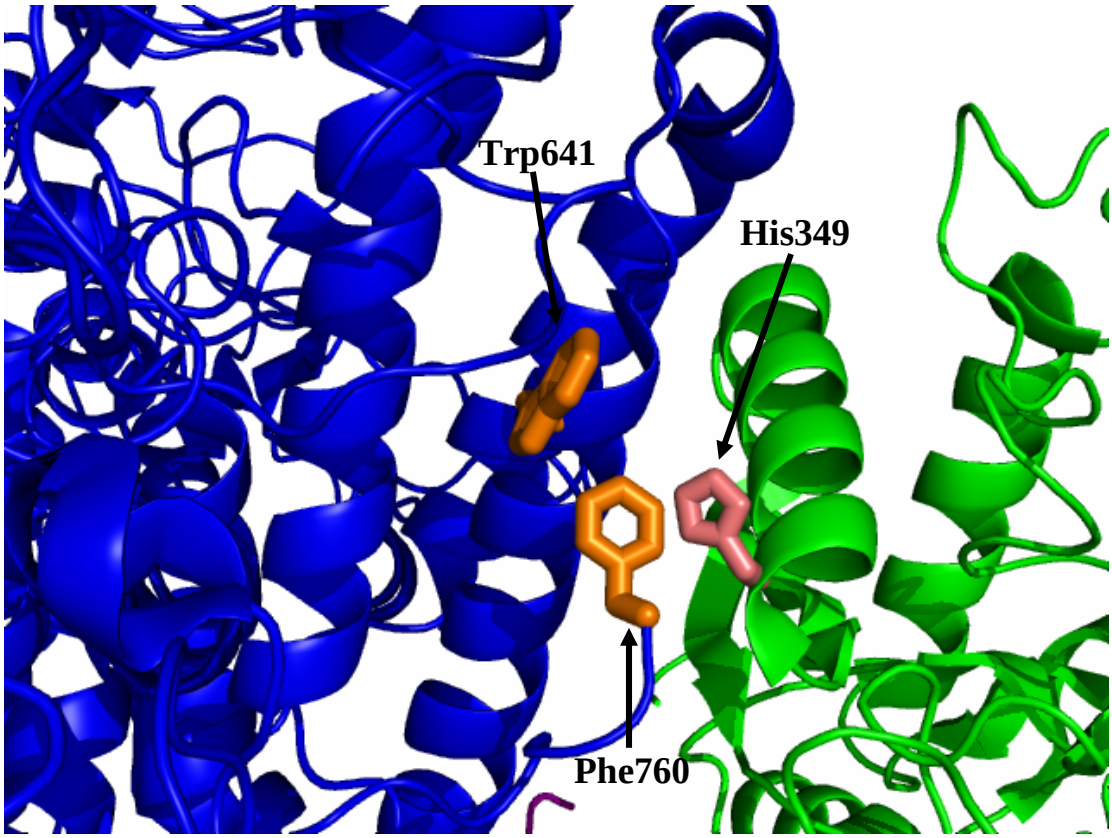

Figure S4. Close up view of proposed His349-Trp641/Phe760 interaction. TFR (blue), C-lobe of hTF (green). His349 in the CI subdomain of hTF (salmon) is proposed to interact with a hydrophobic patch on the TFR, comprised of Trp641/Phe760 (orange). Figure generated using PDB: 1SUV and Pymol (Cheng, Y., Zak, O., Aisen, P., Harrison, S. C. and Walz, T. (2004) Structure of the human transferrin receptor-transferrin complex. Cell. **116**, 565-576; Delano, W. L. (2002) The PyMOL Molecular Graphics System.).

Figure S5.

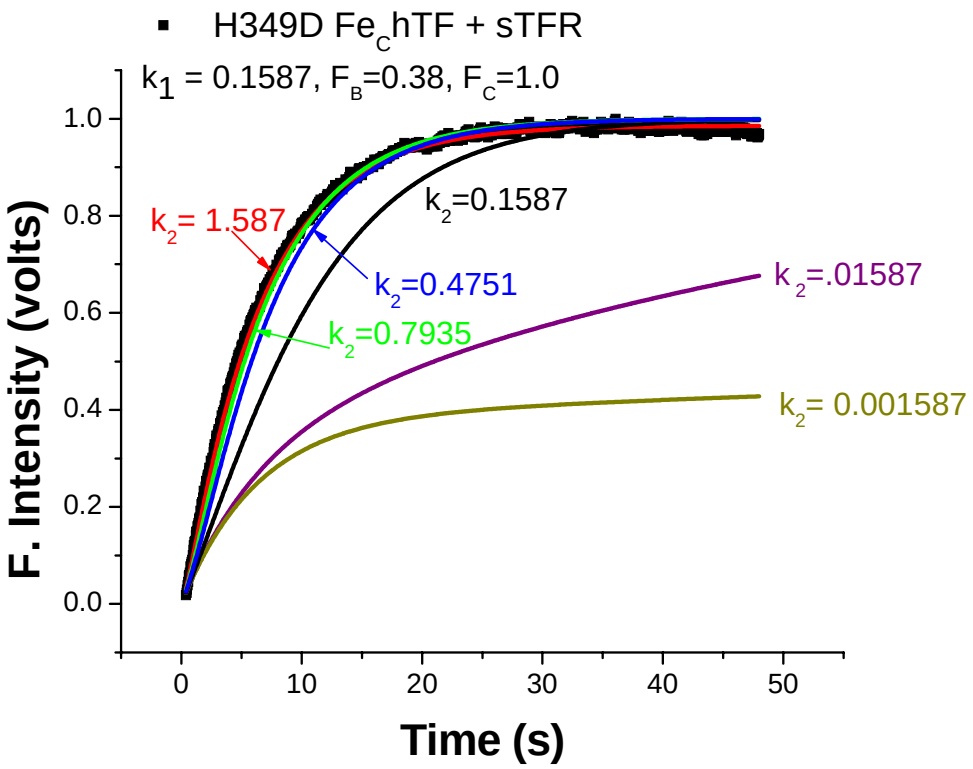

Figure S5. Simulations of the stopped-flow progress curve for H349D + sTFR. In this series of simulations, k_1 = 0.1587 s^{-1}, F_B = 0.38 and F_C = 1.0 where held fixed and k_2 was varied between 0.0001587 and 1.587 s^{-1}. A single exponential is obtained when $k_2 >> k_1$ with a rate constant corresponding to k_1 = 0.1587.