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SUPPORTING INFORMATION

The Kinetics of Dimethylhydroxypyridinone Interactions wth Iron(III) and the Catalysis of Iron(III) Ligand Exchange Reactions: Implications for Bacterial Iron Transport and Combination Chelation Therapies

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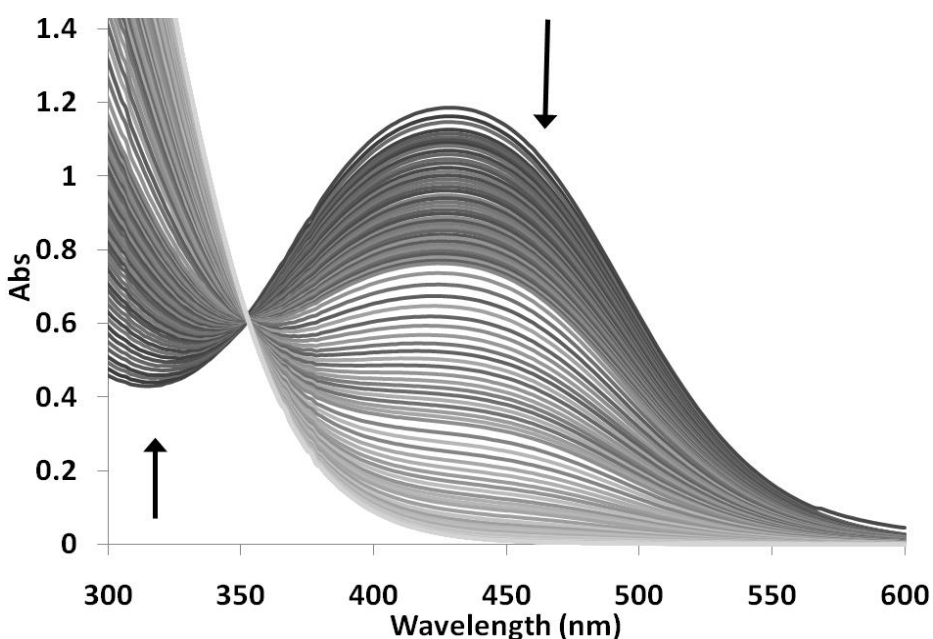[1] RTI International, Research Triangle Park, NC, United States

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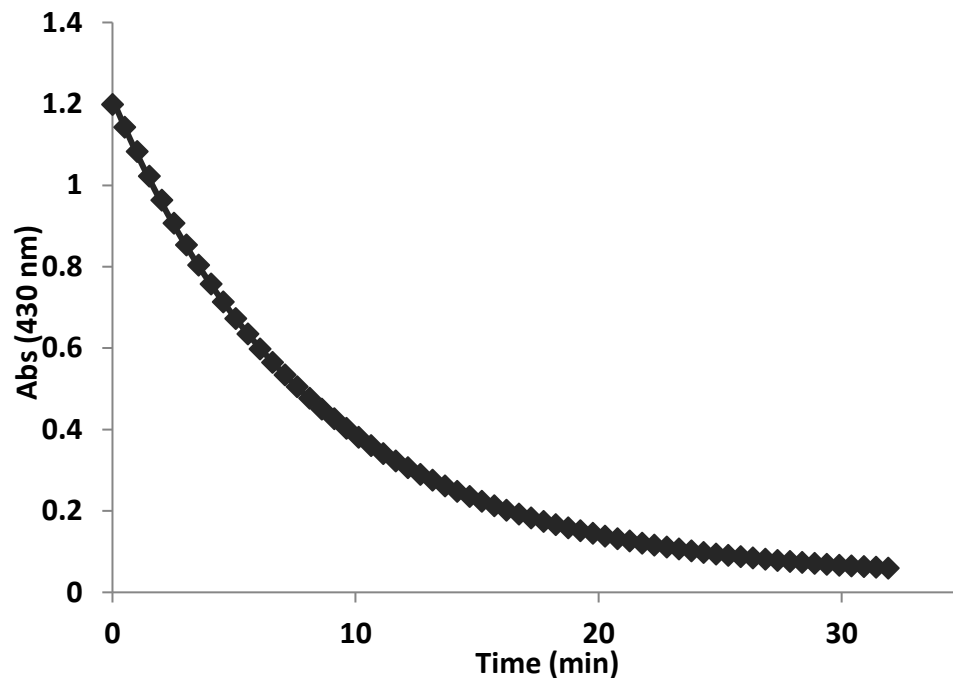CONTENTS

<DMHP catalysis Supp_Info Jan 26 2018.docx>

Figure S1. Representative time resolved spectra measured during the iron(III) exchange

reaction between ferrioxamine B and EDTA in the absence of H(DMHP) (Reaction (2);

[HDMHP] = 0). The arrows show the direction of spectral change with time over 250 minutes.

The initial spectrum is consistent with iron(III) sequestered by desferrioxamine B as the

Fe(HDFB)$^+$ complex (λ_{max} = 430 nm), while the final spectrum is consistent with the conversion

to Fe(EDTA)$^-$.[1,2] Conditions: [FeHDFB$^+$] = 0.4 mM, [EDTA] = 10 mM, μ = 0.10 M (NaClO$_4$),

pH = 4.35 (100 mM NaOAc buffer), T = 25 °C.
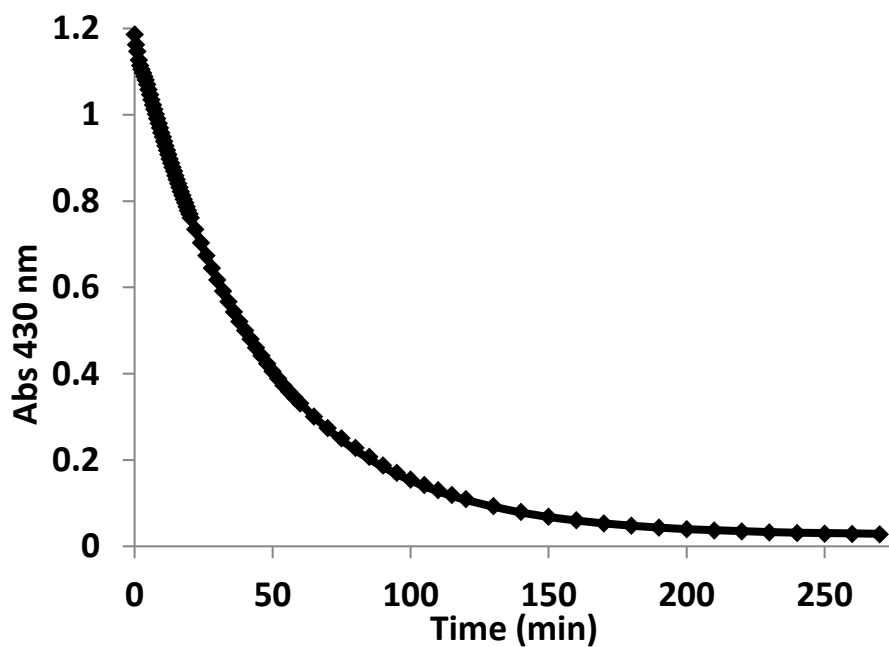
Figure S2. Representative plot of absorbance at 430 nm as a function of time for the iron(III) exchange reaction of ferrioxamine B with EDTA in the absence of H(DMHP) (Reaction (2)). The points represent experimental data, while the line represents the single order exponential decay fit of the data according to Eq. (1). Conditions are as in Fig. S1.

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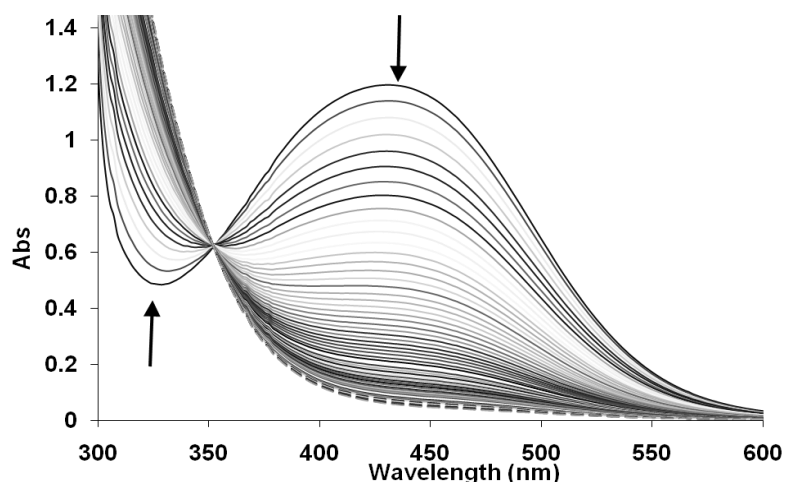
Figure S3. Representative time resolved spectra measured during the iron(III) exchange reaction between ferrioxamine B and EDTA in the presence of H(DMHP) (Reaction (2)). Arrows show the direction of spectral change with time over 900 secs. The initial spectrum is consistent with iron(III) being bound by desferrioxamine B as the FeHDFB$^+$ complex (λ_{max} = 430 nm), while the final spectrum is consistent with the Fe(EDTA)$^-$ complex.[1,2] Conditions: [FeHDFB$^+$] = 0.4 mM, [EDTA] = 10 mM, [DMHP] = 1.5 mM, μ = 0.10 M (NaClO$_4$), pH = 4.35 (50 mM NaOAc buffer), T = 25 °C.

<DMHP catalysis Supp_Info Jan 26 2018.docx>

Figure S4. Representative plot of absorbance at 430 nm as a function of time for the iron(III)

exchange reaction of ferrioxamine B with EDTA in the presence of H(DMHP) (Reaction (2)).

The points represent experimental data, while the line represents the single order exponential

decay fit of the data according to Eq. (1). Conditions: $[FeHDFB^+] = 0.4$ mM, $[EDTA] = 10$ mM,

$[H(DMHP)] = 1.5$ mM, $\mu = 0.10$ M $(NaClO_4)$, pH = 4.35 (50 mM NaOAc buffer), T = 25 °C.

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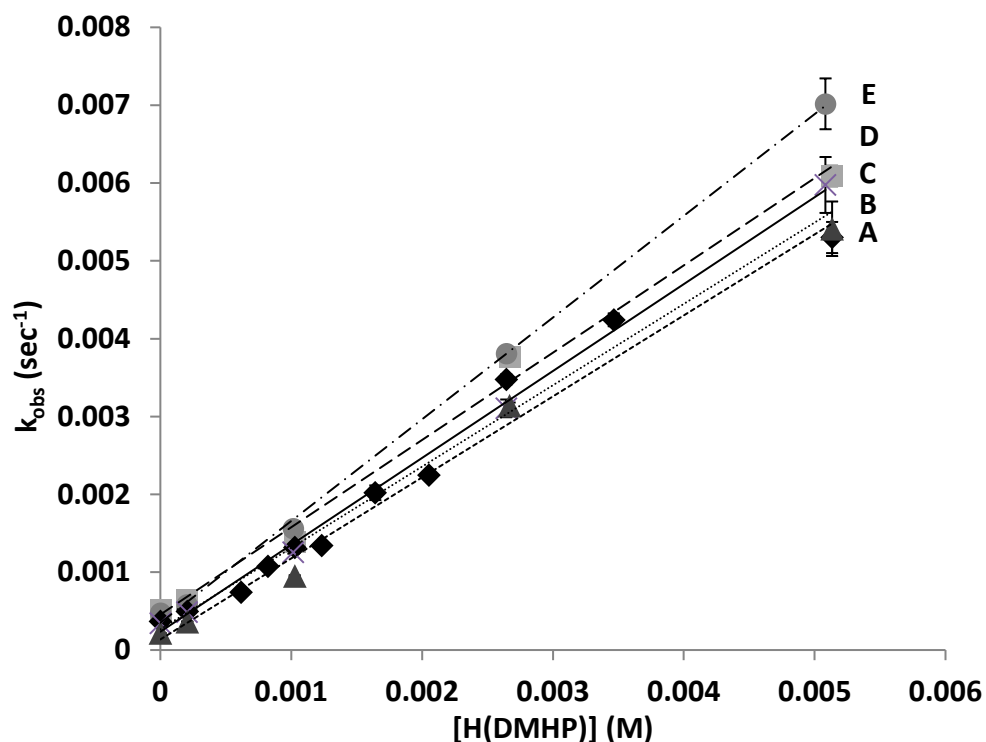
Figure S5. Plot of pseudo-first order rate constant, k_{obs} , as a function of [H(DMHP)]

concentration for Reaction (2) at five different EDTA concentrations. All reactions were

performed at [FeHDFB$^+$] = 0.4 mM, [DMHP] = 0-5 mM, μ = 0.10 M (NaClO$_4$), pH = 4.35 (50

mM NaOAc buffer), and T = 25 °C. Legend: A - [EDTA] = 5.0 mM, B - [EDTA] = 8 mM, C -

[EDTA] = 10 mM, D - [EDTA] = 12 mM, E - [EDTA] = 15 mM.

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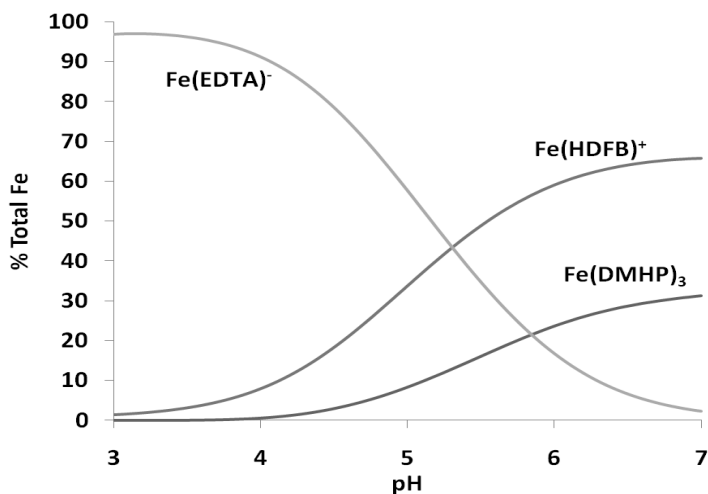
Figure S6. Speciation plot of the iron(III)-desferrioxamine B-H(DMHP)-EDTA system over a range of pH values and constant concentrations of H(DMHP), desferrioxamine B, and EDTA, calculated using the program Hyss and literature values for the relevant stability constants.[1-5] Conditions: $[Fe]_{tot} = 0.40$ mM, $[HDFB]_{tot} = 0.40$ mM, $[H(DMHP)]_{tot} = 5$ mM, $[EDTA]_{tot} = 10$ mM, T = 25 °C, $\mu = 0.10$.

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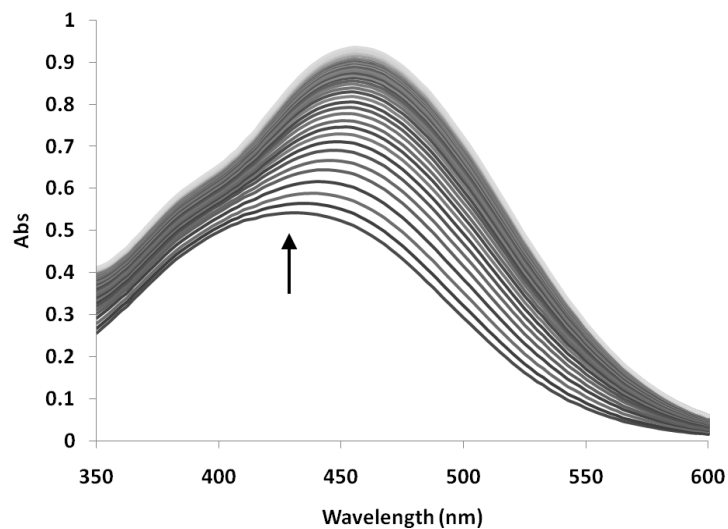
Figure S7. Representative spectra measured during the iron(III) exchange reaction from ferrioxamine B to H(DMHP) (Reaction (3)) over the course of 20 minutes. Arrow shows direction of spectral change with time. Conditions: $[FeHDFB^+] = 0.20$ mM, $[H(DMHP)] = 7$ mM, $\mu = 0.10$ M (NaClO$_4$), pH $= 4.35$ (100 mM NaOAc buffer), T $= 25$ °C.

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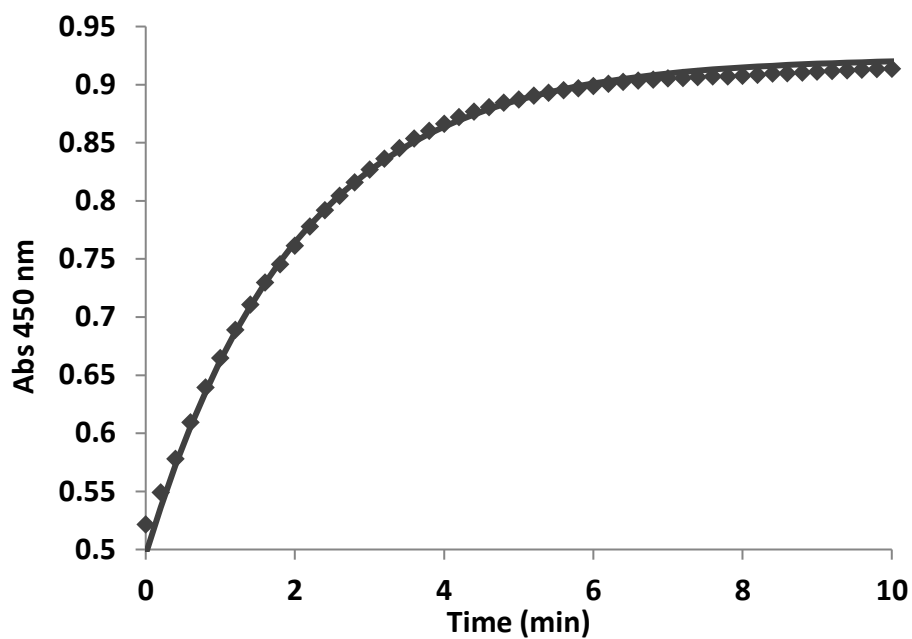
Figure S8. Representative plot of absorbance at 450 nm as a function of time for the iron(III) exchange reaction between ferrioxamine B and H(DMHP) (Reaction (3)). The line represents the single exponential decay fit of Eq. (1) to the data. Conditions: $[FeHDFB^+]$ = 0.20 mM, $[H(DMHP)]$ = 7 mM, μ = 0.10 M ($NaClO_4$), pH = 4.35 (100 mM NaOAc buffer), T = 25 °C.

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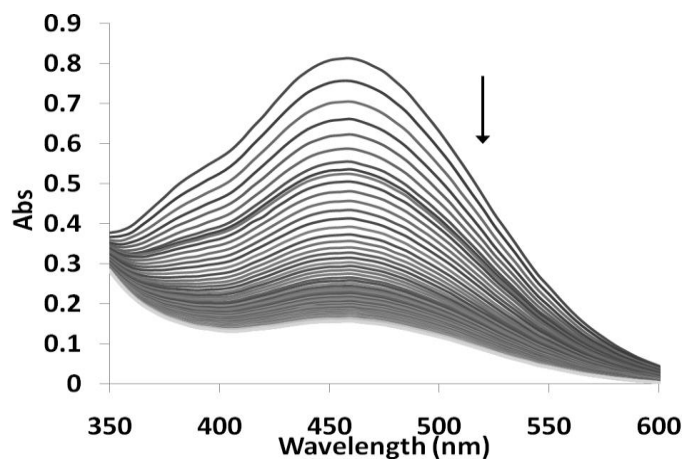
Figure S9. Spectra measured during the iron(III) exchange reaction of the $Fe(DMHP)_3$ complex with EDTA (Reaction (4)). Arrow shows the direction of spectral change with time over 120 secs. The initial and final spectra are indicative of reactant and products as shown in Reaction (4), taking into account the protonation states of the ligands at pH 4.35. Conditions: $[Fe^{3+}] =$ 0.20 mM, $[H(DMHP)] = 2.0$ mM, $[EDTA] = 5.0$ mM, $\mu = 0.10$ M ($NaClO_4$), pH = 4.35 (50 mM NaOAc buffer), T = 25 °C.

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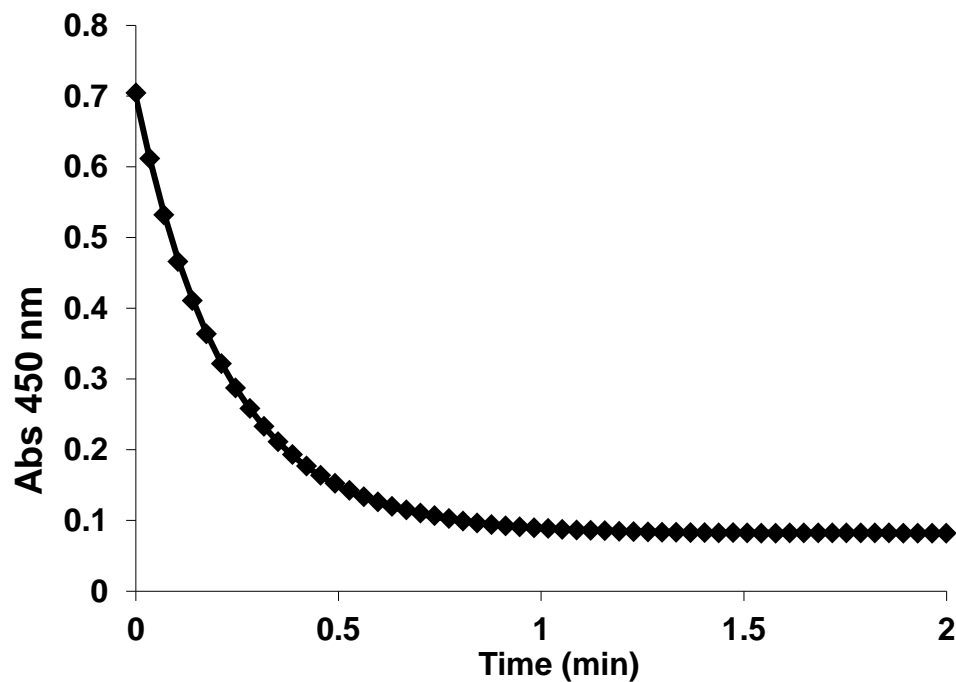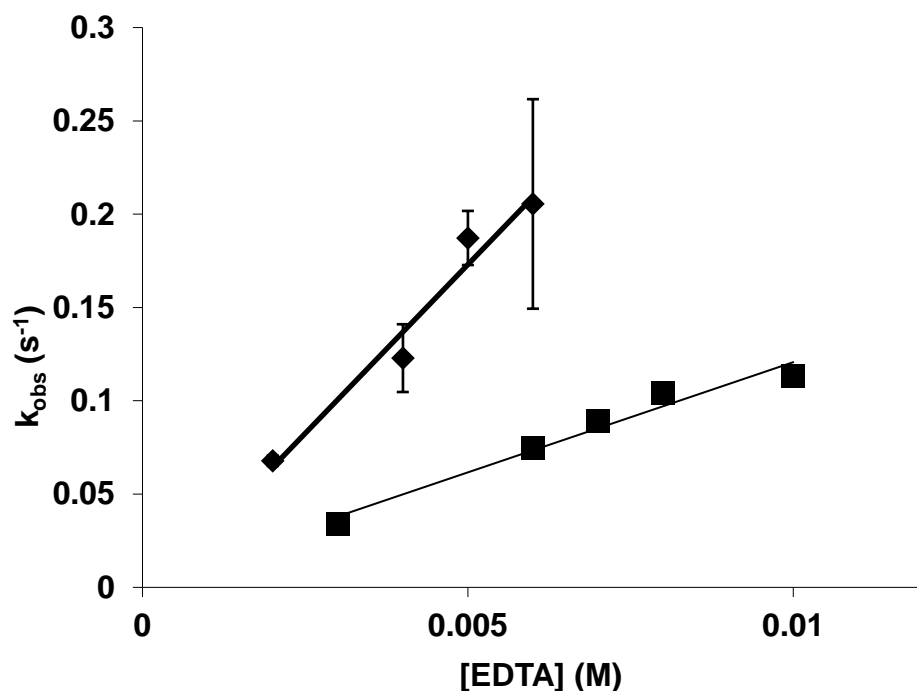

Figure S10. Representative plot of absorbance at 450 nm as a function of time for the iron(III) exchange reaction between Fe(DMHP)$_3$ and EDTA (Reaction (4)). The line represents the single exponential decay fit of Eq. (1) to the data. Conditions: $[Fe^{3+}] = 0.20$ mM, $[H(DMHP)] = 5.0$ mM, $[EDTA] = 6.0$ mM, $\mu = 0.10$ M (NaClO$_4$), pH = 4.35 (50 mM NaOAc buffer), T = 25 °C.

<DMHP catalysis Supp_Info Jan 26 2018.docx>

Figure S11. Plot of observed pseudo-first order rate constant for the iron(III) exchange reaction between DMHP and EDTA (Reaction (4)) as a function of EDTA concentration at two different excess H(DMHP) concentrations. The upper plot (diamonds) represents the reaction performed at 2 mM H(DMHP), while the lower plot (squares) represents the reaction performed at 5 mM H(DMHP). Conditions: $[Fe^{3+}] = 0.20$ mM, $\mu = 0.10$ M (NaClO$_4$), pH $= 4.35$ (50 mM NaOAc buffer), and T $= 25$ °C. Error bars on the plot at 5 mM H(DMHP) concentration represent the standard deviation of the average observed rate constant measured at four discrete wavelengths in a single experiment. Error bars on the plot at 2 mM H(DMHP) concentration represent the standard deviation of the average observed rate constant measured at two discrete wavelengths, with two replicates performed at each wavelength (four total replicates). If error bars are not visible (as in the squares), they are smaller than the data points.

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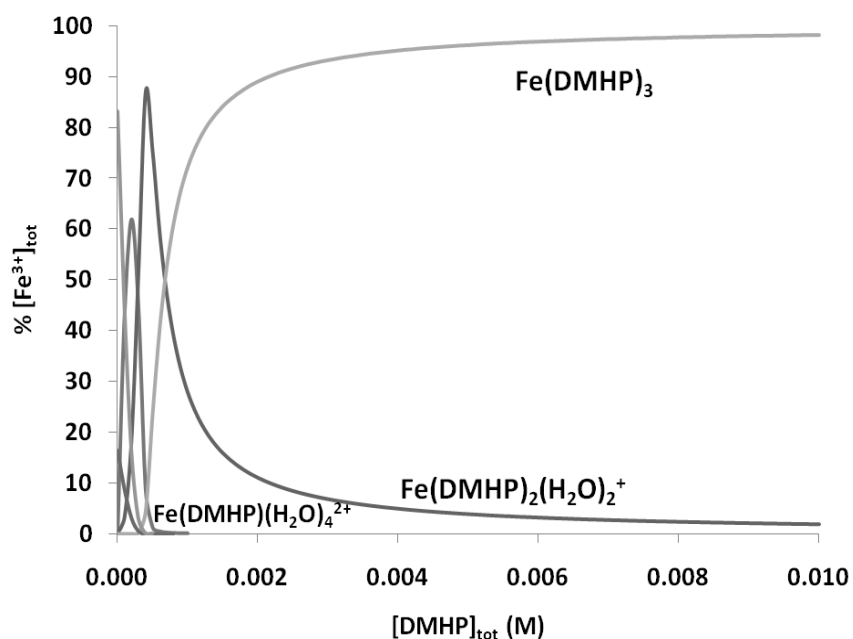
Figure S12. Speciation diagram for the Fe-DMHP system with varying DMHP concentration. The concentrations were calculated using the literature values of the complex formation constants obtained from reference [18]. Conditions: $[Fe^{3+}]_{tot} = 0.20$ mM, pH $= 4.35$, T $= 25$ °C, $\mu = 0.10$ M, and [DMHP] $= 0 - 10$ mM.

Derivation of Eq. (7):

$$\text{Rate} = k_{obs}[\text{FeHDFB}^+] = \{ k_2[\text{DMHP}] + k_3[\text{EDTA}]\}[\text{FeHDFB}^+] \qquad (7)$$

The rate law for the iron(III) exchange Reaction (2) from ferrioxamine B to EDTA in the presence of H(DMHP) that is based on the plot of k_{obs} as a function of [H(DMHP)] at five different excess EDTA concentrations (Fig. S5) is:

$$\text{Rate} = k_{obs}[\text{FeHDFB}^+] \qquad\qquad\qquad (S1)$$

$$k_{obs} = k_2[\text{DMHP}] + C_1 \qquad\qquad\qquad (S2)$$

where k_{obs} represents the observed pseudo first-order rate constant, k_2 represents the second-order rate constant for the reaction with H(DMHP) at a fixed constant EDTA concentration obtained from the slopes of the linear plots shown in Fig. S5, and C_1 represents a constant component to the observed rate constant obtained from the intercepts of the plots in Fig. S5.

A series of kinetic experiments was also performed for Reaction (2) at five different fixed excess H(DMHP) concentrations over a range of EDTA concentrations. The observed rate constants for the reaction exhibited a linear dependence on the concentration of EDTA (Fig. 1), suggesting first-order dependence of the reaction on EDTA concentration. The linear plot of the observed pseudo first-order rate constant as a function of EDTA concentration at a constant H(DMHP) concentration takes the form

$$k_{obs} = k_3[\text{EDTA}] \qquad + C_2 \qquad\qquad\qquad (S3)$$

where k_3 is the slope of the plot in Fig. 1 and represents the second order rate constant for Reaction (2) with respect to EDTA at a fixed H(DMHP) concentration, and C_2 represents an

EDTA independent component that varies with H(DMHP) concentration and was obtained from the intercepts of the plots in Fig. 1.

The value for k_2 determined from the linear plots in Fig. S5 is 1.12 ± 0.09 M^{-1} s^{-1} (Table 1). The value for k_3 determined from the linear plots in Fig. 1 is 0.06 ± 0.02 M^{-1} s^{-1} (Table 1).

The inter-relatedness of the slopes and intercepts of the linear plots of the kinetic data in the presence of variable excess concentrations of EDTA and H(DMHP) for the iron(III) exchange Reaction (2) in Figs. 1 and S5 (and consequently the internal consistency of our kinetic data) can be demonstrated by comparing the slopes of the plots obtained at one fixed component concentration (H(DMHP) or EDTA) to the y-intercepts of the plots obtained when the other component concentration is fixed. It can be shown that there is a relationship between the constants, C_1 (Eq. (S2)) and C_2 (Eq. (S3)) and the rate constants, k_3 and k_2, as shown in Eqs. (S4) and (S5).

$$C_1 = k_3[\text{EDTA}] \qquad\qquad\qquad (S4)$$

$$C_2 = k_2[\text{H(DMHP)}] \qquad\qquad\qquad (S5)$$

By dividing the y-intercept of the linear plots in Fig. S5 by the EDTA concentration of for each line, the obtained second order rate constant (k_3) is calculated as 0.028 ± 0.005 M^{-1} s^{-1}, which is equivalent within experimental error to the second order rate constant from the slopes of the linear plots in Fig. 2 corresponding to k_3 (0.06 ± 0.02 M^{-1} s^{-1}; Table 1). It is also important to note that the values of the rate constant k_3 that are determined both from Fig. 1 and Fig. S5 resemble the value of the rate constant for the iron(III) exchange reaction performed without H(DMHP) within experimental error, 0.030 ± 0.08 M^{-1} s^{-1} (Table 1). The same observation can be made for the y-intercepts of the plots of k_{obs} as a function of EDTA concentration in Fig. 1, where the second order rate constant, k_2, calculated from the y-intercepts through division by the

<DMHP catalysis Supp_Info Jan 26 2018.docx>

H(DMHP) concentration for each line (Eq. (S5)) is 1.0 ($\pm$ 0.1) M^{-1} s^{-1}, the same value within experimental error as that observed from the slope of the plots of k_{obs} against H(DMHP) concentration (Eq. (S2)) in Fig. S5 (1.12 $\pm$ 0.09 M^{-1} s^{-1}; Table 1).

The rate law derived for Reaction (2) from the experiments performed at fixed EDTA, H(DMHP), and H^+ concentrations feature second-order rate constants k_2 and k_3 that differ by over an order of magnitude (Table 1). The combined rate law is shown in the manuscript as Eq. (7).

$$\text{Rate} = k_{obs}[\text{FeHDFB}^+] = \{ k_2[\text{DMHP}] + k_3[\text{EDTA}]\}[\text{FeHDFB}^+] \qquad (7)$$

Derivation of Equation (16):

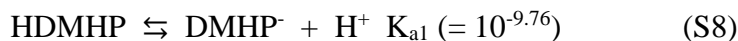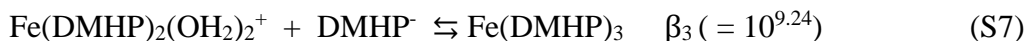$$\text{Rate} = k_7[\text{EDTA}][\text{Fe(H(DMHP))}_3][\text{H}^+] / (K_3[\text{H(DMHP)}]) \qquad (16)$$

Fig. S11 and Fig. 5 show that the exchange Reaction (4) is first order in [EDTA] and inverse first order in [H(DMHP)]. Our objective was to obtain a value for the second order rate constant for Reaction (4) and to identify the reactive iron complex species leading to Fe(EDTA)$^-$ product. The observed rate constants were fit to models representing all possible Fe(DMHP)$_x$ (x = 1, 2, 3) complexes to determine the reaction path for Reaction (4). The best fit to the data was found to represent the exchange of iron(III) from Fe(DMHP)$_2$(H$_2$O)$_2^+$ to EDTA. Inverse first order dependence of the rate on [DMHP] may therefore be accommodated by considering the following dissociation reaction to produce the reactive species Fe(DMHP)$_2$(OH$_2$)$_2^+$, followed by rate determining reaction with EDTA.

$$\text{Fe(DMHP)}_3 + \text{H}^+ \leftrightarrows \text{Fe(DMHP)}_2(\text{OH}_2)_2^+ + \text{H(DMHP)} \quad 1/K_3 \qquad (12)$$

$$1/K_3 = [\text{Fe(DMHP)}_2(\text{OH}_2)_2^+][\text{HDMHP}] / ([\text{Fe(DMHP)}_3][\text{H}^+]) = 1/(\beta_3 K_{a1}) \qquad (S6)$$

$$\text{Fe(DMHP)}_2(\text{OH}_2)_2^+ + \text{EDTA} \rightarrow \text{FeEDTA}^- + 2\,\text{H(DMHP)} \quad k_7 \qquad (13)$$

A numerical value for $1/K_3$ (= $10^{0.52}$) may be obtained from literature values for the equilibria in Reactions (S7) and (S8).[5]

$$\text{Fe(DMHP)}_2(\text{OH}_2)_2^+ + \text{DMHP}^- \leftrightarrows \text{Fe(DMHP)}_3 \quad \beta_3\,(=10^{9.24}) \qquad (S7)$$

$$\text{HDMHP} \leftrightarrows \text{DMHP}^- + \text{H}^+ \quad K_{a1}\,(=10^{-9.76}) \qquad (S8)$$

The rate expression for the two-step process, assuming that Reaction (12) is a rapidly established pre-equilibrium is shown in Eq. (14).

$$\text{Rate} = k_7[\text{EDTA}][\text{Fe(H(DMHP))}_2(\text{OH}_2)_2{}^+] \qquad (14)$$

Solving for $[\text{Fe(DMHP)}_2(\text{OH}_2)_2{}^+]$ using Eq. (S6),

$$[\text{Fe(DMHP)}_2(\text{OH}_2)_2{}^+] = [\text{Fe(DMHP)}_3][\text{H}^+] \ / \ K_3[\text{H(DMHP)}] \qquad (S9)$$

Substituting into Eq ~~(12)~~ (14) we have Eq ~~(14).~~ (16).

$$\text{Rate} = k_7[\text{H}^+][\text{EDTA}][\text{Fe(DMHP)}_3] \ / \ K_3[\text{HDMHP}] \qquad (16)$$

Consequently, for the experimentally observed pseudo first-order rate expression Eq (15), k_{obs} may be expressed as Eq. (S10).

$$\text{Rate} = k_{obs}[\text{Fe(DMHP)}_3] \qquad (15)$$

$$k_{obs} = k_7[\text{H}^+][\text{EDTA}] \ / \ K_3[\text{HDMHP}] \qquad (S10)$$

where k_7 is the second order rate constant for Reaction (13), K_3 is as defined above in Reaction (12) and [H(DMHP)] is the free equilibrium concentration of H(DMHP) calculated from Eq. (S11).

$$[\text{HDMHP}] = [\text{HDMHP}]_{Tot} - 3[\text{Fe(DMHP)}_3] - 2[\text{Fe(DMHP)}_2(\text{OH}_2)_2{}^+] - [\text{Fe(DMHP)}(\text{OH}_2)_4{}^{2+}]$$

$$(S11)$$

<DMHP catalysis Supp_Info Jan 26 2018.docx>

There are two methods for determining the value of k_7 in Eqs. (16) and (S10) from our data. In the first method the linear relationship in Fig. S11 is used as in Eq. (S12)

$$k_{obs} \; = \; m[EDTA] \; + \; b \qquad\qquad\qquad (S12)$$

where the intercept **b** is zero within experimental error and the slope m is represented as in Eq. (S13).

$$m \; = \; k_7[H^+] \, / \, (K_3[H(DMHP)]) \qquad\qquad (S13)$$

For the data set represented by the diamonds in Fig. S11 where $[H(DMHP)] = 2.0$ mM and $[H^+] = 10^{-4.35}$ M, the slope $m = 3.62 \times 10^1$ M^{-1}s^{-1} and k_7 is calculated as 3.42×10^2 M^{-1}s^{-1}. For the data set represented by the squares in Fig. S11 where $[H(DMHP)] = 5.0$ mM and $[H^+] = 10^{-4.35}$ M, the slope $m = 1.18 \times 10^1$ M^{-1}s^{-1} and k_7 is calculated as 3.51×10^2 M^{-1}s^{-1}. Thus the two data sets in Figure S11 give internally consistent values for k_7.

The second method uses the larger data set at a constant [EDTA] concentration shown in Fig. 5, where the best fit expression is shown in Eq. (S15)

$$k_{obs} \; = \; a \, / \, [H(DMHP)] \qquad\qquad\qquad (S14)$$

and **a** is an adjustable parameter, which in the context of Reaction (4) may be represented as Eq. (S15).

$$a \;=\; k_7[\text{EDTA}][\text{H}^+] \,/\, (K_3) \qquad\qquad (S15)$$

The best fit line (Eq. (S14)) of the experimental data shown in Fig. 5 yields $\mathbf{a} = 3.01 \text{ x } 10^{-4} \text{ Ms}^{-1}$,

which at the fixed [EDTA] and [H$^+$] concentrations yields k_7 as $3.39 \text{ x } 10^2 \text{ M}^{-1}\text{s}^{-1}$.

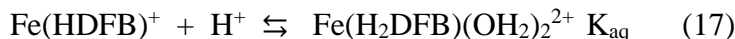The agreement obtained in the values calculated for k_7 using the three independent data

$$\text{Rate} = k_7[\text{EDTA}][\,\text{Fe(H(DMHP))}_2(\text{OH}_2)_2{}^+] \qquad\qquad (14)$$

sets at different variable concentration conditions described above (339, 342, and 351 $\text{M}^{-1}\text{s}^{-1}$)

confirms the internal consistency of our data and analysis methods.

Derivation of Equation (19):

Rate = $k_B K_{aq}[H^+][HDMHP][Fe(HDFB)^+]$ (19)

We propose the following mechanism for the formation of $Fe(H_2DFB)(DMHP)^+$ as a reactive intermediate in Reaction (2) and Reaction (3).

$Fe(HDFB)^+ + H^+ \leftrightarrows Fe(H_2DFB)(OH_2)_2^{2+}$ K_{aq} (17)

$Fe(H_2DFB)(OH_2)_2^{2+} + HDMHP \rightarrow Fe(H_2DFB)(DMHP)^+ + H^+$ k_B (18)

For Reaction (2) the intermediate $Fe(H_2DFB)(DMHP)^+$ proceeds to react with EDTA in a rapid step to form $Fe(EDTA)^-$ product

$Fe(H_2DFB)(DMHP)^+ + EDTA \rightarrow Fe(EDTA)^- + H_4DFB^+ + H(DMHP)$ fast   (S16)

and for Reaction (3) the intermediate $Fe(H_2DFB)(DMHP)^+$ proceeds to react with H(DMHP) in two rapid steps to form $Fe(DMHP)_3$ product.

$Fe(H_2DFB)(DMHP)^+ + 2\ HDMHP \rightarrow Fe(DMHP)_3 + H_4DFB^+$ fast   (S17)
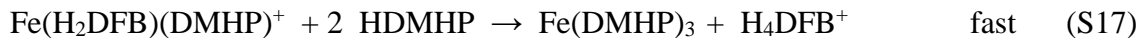
Assuming Reaction (17) is a rapidly established pre-equilibrium[6] followed by Reaction (18) as the rate determining step, we have the rate expression Eq. (S18) for the [H(DMHP)] dependent path in Reaction (2) and Reaction (3).

Rate = $k_B[FeH_2DFB)(OH_2)_2^{2+}]_{eq}[HDMHP]$ (S18)

where $[FeH_2DFB)(OH_2)_2^{2+}]_{eq}$ may be expressed as follows

$[FeH_2DFB)(OH_2)_2^{2+}]_{eq} = K_{aq}[FeHDFB^+]_{eq}[H^+]$ (S19)

Substituting in Eq (S18) and noting that at pH 4.35 and $K = 10^{0.94}$ [7] that $[FeHDFB^+]_{eq} \approx [FeHDFB^+]_{tot}$ we have Eq. (19) in the main body of the manuscript.

Rate = $k_B K_{aq}[H^+][HDMHP][FeHDFB^+]$ (19)

References

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