

Supporting Information for

Ti(IV) and the Siderophore Desferrioxamine B: A Tight Complex Has Biological and Environmental Implications

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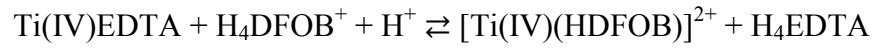
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Table S1. Protonation constants and stability constants used in calculations for fitting spectropotentiometric data and competition data with EDTA¹⁻³

Ligand	Equilibria	Constants
DFOB	$H^+ + [DFOB]^{3-} \rightleftharpoons [HDFOB]^{2-}$	$\log \beta_1 = 10.84$
	$2H^+ + [DFOB]^{3-} \rightleftharpoons [H_2DFOB]^-$	$\log \beta_2 = 20.39$
	$3H^+ + [DFOB]^{3-} \rightleftharpoons [H_3DFOB]$	$\log \beta_3 = 29.37$
	$4H^+ + [DFOB]^{3-} \rightleftharpoons [H_4DFOB]^+$	$\log \beta_4 = 37.69$
EDTA	$H^+ + [EDTA]^{4-} \rightleftharpoons [HEDTA]^{3-}$	$\log \beta_1 = 9.52$
	$2H^+ + [EDTA]^{4-} \rightleftharpoons [H_2EDTA]^{2-}$	$\log \beta_2 = 15.65$
	$3H^+ + [EDTA]^{4-} \rightleftharpoons [H_3EDTA]^-$	$\log \beta_3 = 18.34$
	$4H^+ + [EDTA]^{4-} \rightleftharpoons [H_4EDTA]$	$\log \beta_4 = 20.34$
Ti(IV) hydrolysis	$Ti^{4+} + H_2O \rightleftharpoons [Ti(OH)]^{3+} + H^+$	$\log K_1 = 0.3$
	$Ti^{4+} + 2H_2O \rightleftharpoons [Ti(OH)_2]^{2+} + 2H^+$	$\log K_2 = 1.38$
	$Ti^{4+} + 3H_2O \rightleftharpoons [Ti(OH)_3]^+ + 3H^+$	$\log K_3 = -0.72$
	$Ti^{4+} + 4H_2O \rightleftharpoons [Ti(OH)_4] + 4H^+$	$\log K_4 = -4.42$
	$Ti^{4+} + 5H_2O \rightleftharpoons [Ti(OH)_5] + 5H^+$	$\log K_5 = -14.52$
M-L conditions	$Ti(IV) + [EDTA]^{4-} \rightleftharpoons [TiEDTA]$	$\log \beta = 19.4$
	$Ti(IV) + H^+ + [DFOB]^{3-} \rightleftharpoons [TiHDFOB]^{2+}$	$\log \beta_{111} = 41.7$
	$Ti(IV) + [DFOB]^{3-} \rightleftharpoons [TiDFOB]^+$	$\log \beta_{110} = 38.1$
	$Ti(IV) + H_2O + [DFOB]^{3-} \rightleftharpoons [Ti(IV)(OH)DFOB] + H^+$	$\log \beta_{11-1} = 30.1$
	$Ti(IV) + [HDFOB]^{2-} \rightleftharpoons [TiHDFOB]^{2+}$	$\log K_f = 30.9$

Calculation of Binding Constants

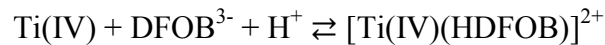
To determine the stability constant for $[\text{Ti(IV)(HDFOB)}]^{2+}$ by using ITC, a complex of a moderate ligand (EDTA) bound to Ti(IV) is titrated into a stronger ligand (DFOB) to obtain an apparent equilibrium constant (K_{ITC}). The overall reaction is:



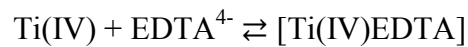
$$K_{\text{ITC}} = \frac{[\text{Ti(IV)(HDFOB)}]^{2+}[\text{H}_4\text{EDTA}]}{[\text{Ti(IV)EDTA}][\text{H}_4\text{DFOB}^+][\text{H}^+]}$$

The ITC fit returns the apparent dissociation constant ($K_{\text{D,app}} = 2.41 \times 10^{-6}$); this value corresponds to a K_{ITC} for the reaction above of $K_{\text{ITC}} = 1/K_{\text{D,app}} = 4.15 \times 10^5$.

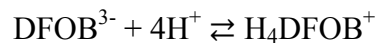
Relevant individual equilibria include:



$$\beta_{111} = \frac{[\text{Ti(IV)(HDFOB)}]^{2+}}{[\text{H}^+][\text{Ti(IV)}][\text{DFOB}^{3-}]}$$



$$\beta_{\text{Ti-EDTA}} = \frac{[\text{Ti(IV)EDTA}]}{[\text{Ti(IV)}][\text{EDTA}^{4-}]} = 10^{19.4}$$



$$\beta_{\text{DFOB-4H}} = \frac{[\text{H}_4\text{DFOB}^+]}{[\text{DFOB}^{3-}][\text{H}^+]^4} = 10^{37.69}$$



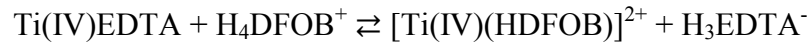
$$\beta_{\text{EDTA-4H}} = \frac{[\text{H}_4\text{EDTA}]}{[\text{EDTA}^{4-}][\text{H}^+]^4} = 10^{20.34}$$

so that $K_{ITC} = \frac{[[\text{Ti(IV)HDFOB}]^{2+}][\text{H}_4\text{EDTA}]}{[\text{Ti(IV)EDTA}][\text{H}_4\text{DFOB}^+][\text{H}^+]} = \beta_{111} \cdot (1/\beta_{\text{Ti-EDTA}}) \cdot (1/\beta_{\text{DFOB-4H}}) \cdot \beta_{\text{EDTA-4H}}$

$$4.15 \times 10^5 = \beta_{111} \cdot (1/10^{19.4}) \cdot (1/10^{37.69}) \cdot (10^{20.34})$$

$$\beta_{111} = 42.4$$

We note that this analysis considers H_4EDTA as the predominant form of EDTA, which it would be under the reaction conditions (Figure S9). If however, the form of released EDTA were H_3EDTA^- , then the relevant equilibria would be:



$$K_{ITC} = \frac{[[\text{Ti(IV)HDFOB}]^{2+}][\text{H}_3\text{EDTA}^-]}{[\text{Ti(IV)EDTA}][\text{H}_4\text{DFOB}^+]}$$



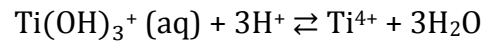
$$\beta_{\text{EDTA-3H}} = \frac{[\text{H}_3\text{EDTA}^-]}{[\text{EDTA}^{4-}][\text{H}^+]^3} = 10^{18.34}$$

with the corresponding $\beta_{111} = 44.4$.

Determining K_f using the paradigm of Whisenhunt *et al.*:⁴

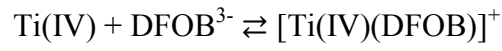
$$K_f = \frac{(K_{ML})(K_{MLH})(K_{comp})}{(K_{Ti(OH)_3})(K_2^H)} \quad \text{where } K_{ML} = 10^8 \text{ and } K_{MLH} = 10^{3.8}$$

and



$$K_{Ti(OH)_3} = \frac{[Ti^{4+}]}{[Ti(OH)_3^+][H^+]^3} = 10^{0.72}$$

and



$$K_{comp} = \frac{[[Ti(IV)(DFOB)]^+]}{[Ti(OH)_3][DFOB]^{3-}} = 10^{38.1}$$

yielding a value of $\log K_f = 39.4$ as defined above at $pH > 3.5$.

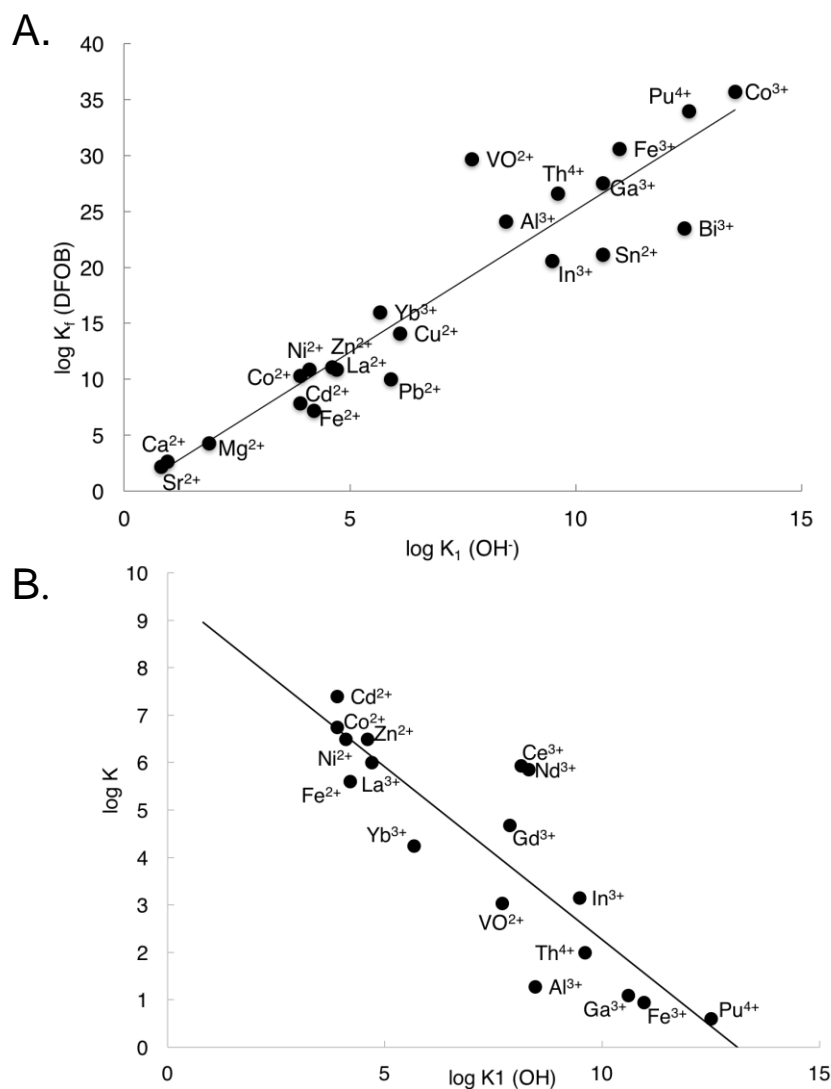


Figure S1. (A) Correlation for various metal ions between binding constant for HDFOB²⁻ ($\log K_f$ where $K_f = \frac{[M(\text{HDFOB})^{(n-2)+}]}{[M^{n+}][\text{HDFOB}^{2-}]}$) with binding constant for OH⁻ ($\log K_{1(\text{OH})}$ where $K_1 = \frac{[M(\text{OH})^{(n-1)+}]}{[M^{n+}][\text{OH}^-]}$),^{5,6} using published binding constants.^{1,4,5,7-10} According to this correlation, Ti(IV) ($\log K_{1(\text{OH})} = 14.3$)¹¹ would be predicted to have a $\log K_f$ (DFOB) of approximately 35. Smaller ions tend to bind more tightly to DFOB than this correlation suggests, whereas larger ones tend to bind less tightly, in some cases with deviations > 5 in the value of $\log K$. **(B)** Plot of $\log K$ where $K = \frac{[\text{MLH}_2]}{[\text{MLH}][\text{H}^+]}$ as a function of the metal hydrolysis constant. According to this trend, Ti(IV) would have a predicted protonation constant of -0.8, and therefore undetectable in our pH range. This correlation suggests that even at very low pH, Ti(IV) fully complexes with DFOB, deprotonating all the sites on DFOB, binding to $[\text{HDFOB}]^{2-}$.^{1,4,12-16} This chart was adapted from Hernlem *et al.* (1996)¹² and Boukhalfa *et al.* (2007)¹⁶.

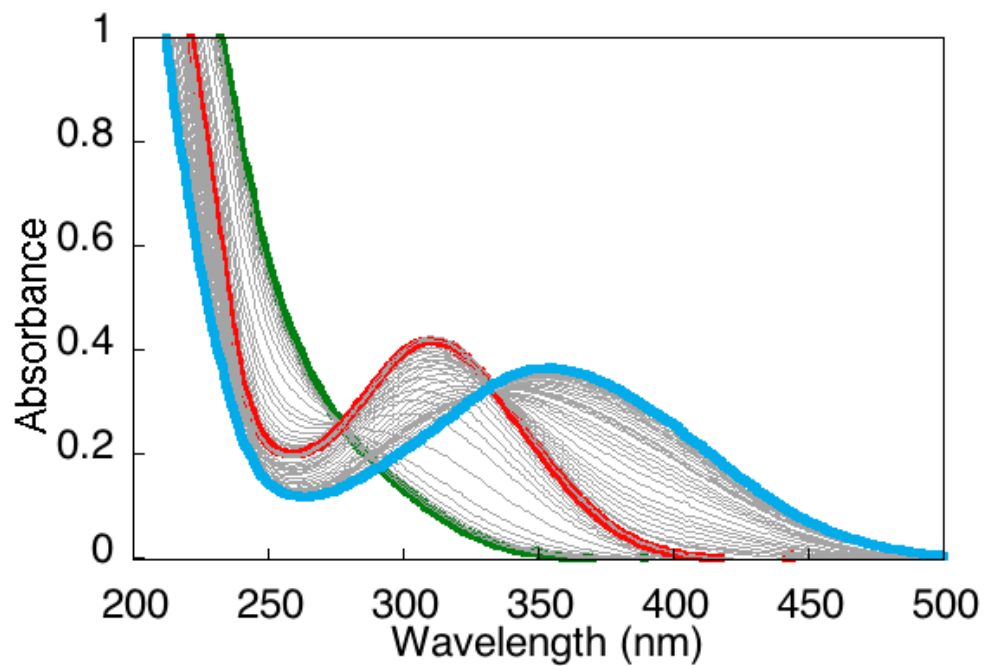


Figure S2. The 62 pH-dependent UV/vis spectra obtained during one spectrophotometric titration performed under Ar atmosphere of a 1:1, 0.4 mM DFOB/Ti(IV) system at 25 °C and set to $I = 0.1$ with KCl. The blue trace was obtained at pH 2.02, the red trace was obtained at pH 6.10, and the green trace was obtained at pH 10.09.

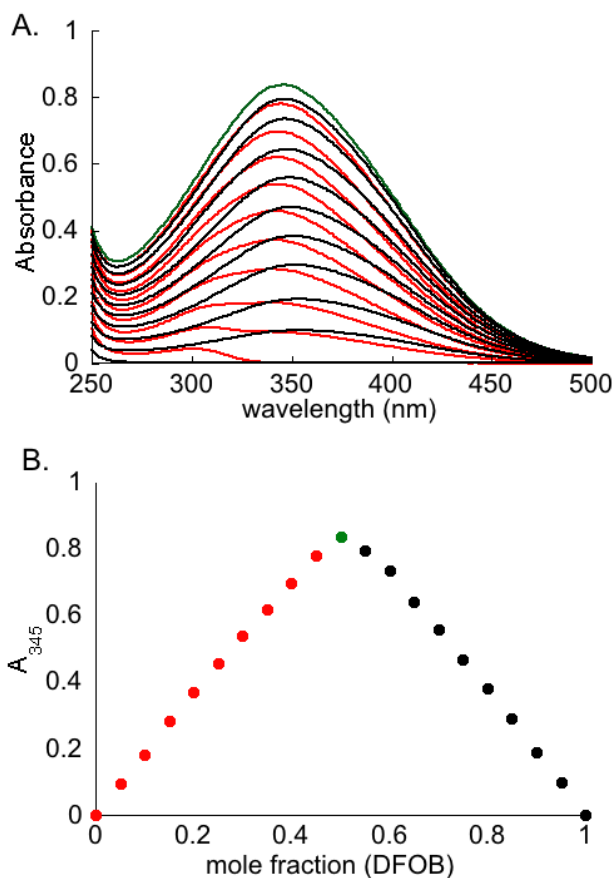


Figure S3. Job's Method of Continuous Variation. A) The UV/Vis spectra obtained of solutions at pH 2.1 and ionic strength set to 0.1 with KCl with varying DFOB/Ti(IV) ratios with the total concentrations of DFOB and Ti(IV) kept constant. The maximum absorbance was observed when the DFOB/Ti(IV)=1:1 (200 μ M/200 μ M) (green trace). The black traces were obtained when DFOB was in excess and the red traces were obtained when Ti(IV) was in excess. A shift to higher energy was observed when Ti(IV) was in excess (red traces). B) The plot of the absorbance at 345 nm versus the mole fraction of DFOB. The maximum absorption at 345 nm was observed when DFOB/Ti(IV) = 1:1.

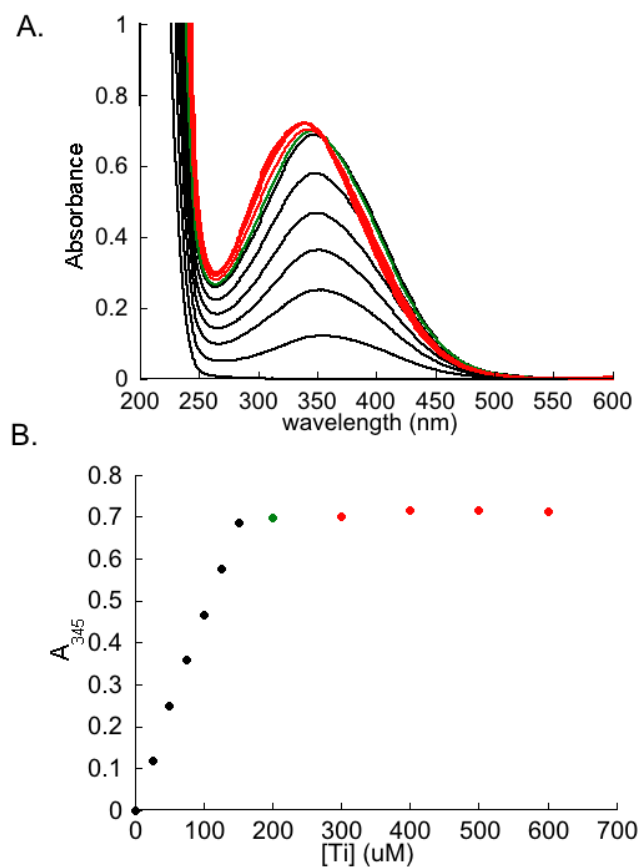


Figure S4. A.) The titration of Ti(IV) into 200 μM DFOB. UV/Vis spectra of samples with constant [DFOB] and increasing [Ti(IV)] at pH 2.1 and ionic strength set to 0.1 with KCl. The absorbance at 345 nm increased as Ti(IV)/DFOB increased to 1:1 (green trace). There was a clear shift of the spectra to high energy (red traces) when Ti(IV) was in excess. B) A plot of A_{345} of the UV/Vis spectra versus [Ti] (μM). The black circles correspond to the black traces in A, the green circles correspond to 1:1 Ti/DFOB in A, and the red circles correspond to the red traces in A.

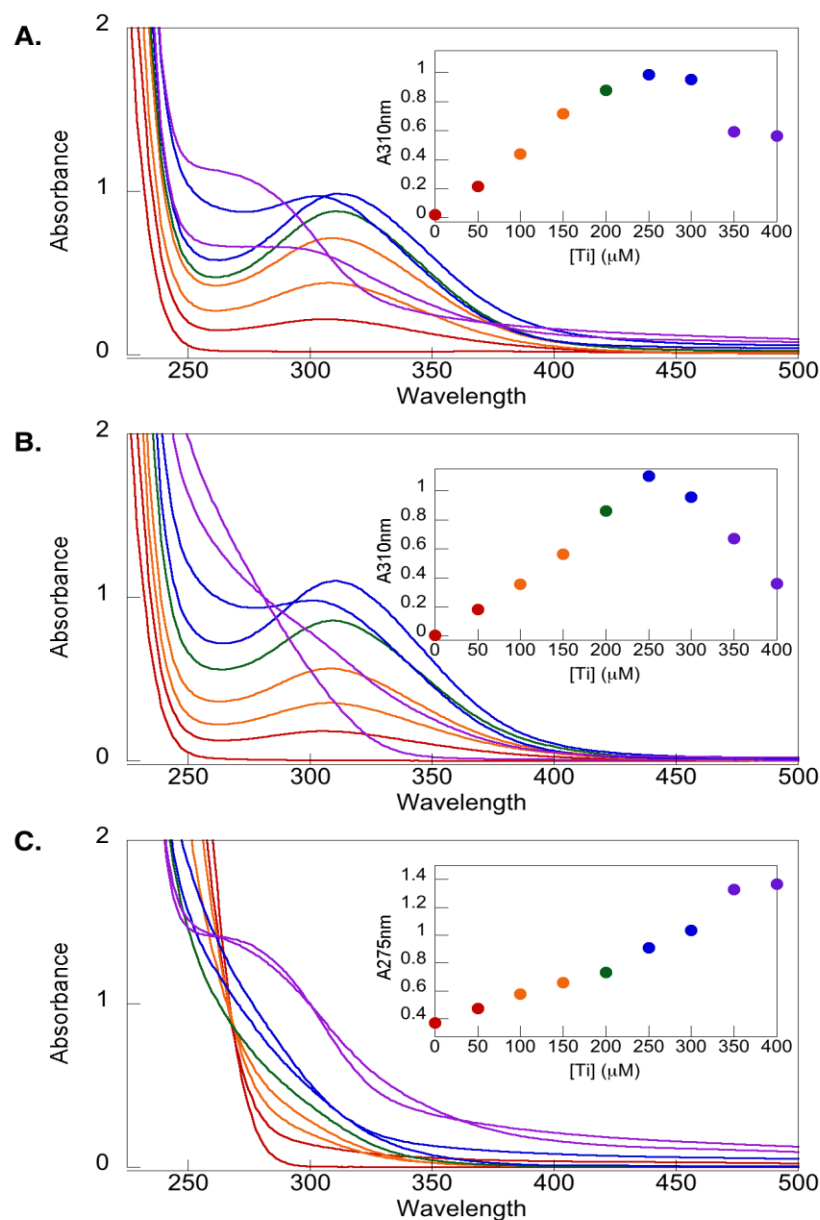


Figure S5. Job's Method of Continuous Variation. A) The UV/Vis spectra obtained of solutions at pH 6 and ionic strength set to 0.1 with KCl with varying DFOB/Ti(IV) ratios with the total concentrations of DFOB and Ti(IV) kept constant. The maximum absorbance was observed when the Ti(IV) was in slight excess to DFOB (250 μM /150 μM). B) The UV/Vis spectra obtained of solutions at pH 6 and ionic strength of 0.1 with KCl using Ti(IV)-citrate as the titanium source. The maximum absorbance was observed when Ti(IV)-citrate was in slight excess to DFOB (250 μM /150 μM). C) The UV/Vis spectra obtained of solutions pH 10 and ionic strength of 0.1 with KCl. No trend was observed due to the hydrolysis of Ti(IV). The insets on each graph show the plot of the maximum absorbance versus the concentration of Ti(IV) (μM).

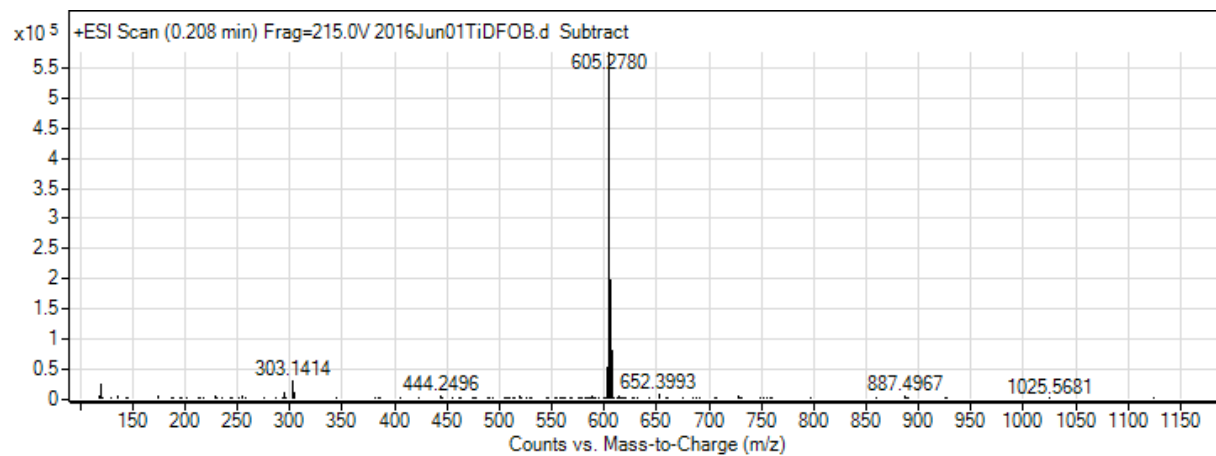


Figure S6. The ESI-QTOF mass spectrum of an aqueous solution with 10 μM Ti(IV)-DFOB = 1:1 at pH 2, I = 0.1 M KCl shows evidence for the fully complexed species $[\text{TiC}_{25}\text{H}_{46}\text{N}_6\text{O}_8]^{2+}$ ($[\text{Ti(IV)(HDFOB)}]^{2+}$) with $m/z = 303.1$ and $[\text{TiC}_{25}\text{H}_{45}\text{N}_6\text{O}_8]^+$ ($[\text{Ti(IV)(DFOB)}]^+$) with $m/z = 605.3$.

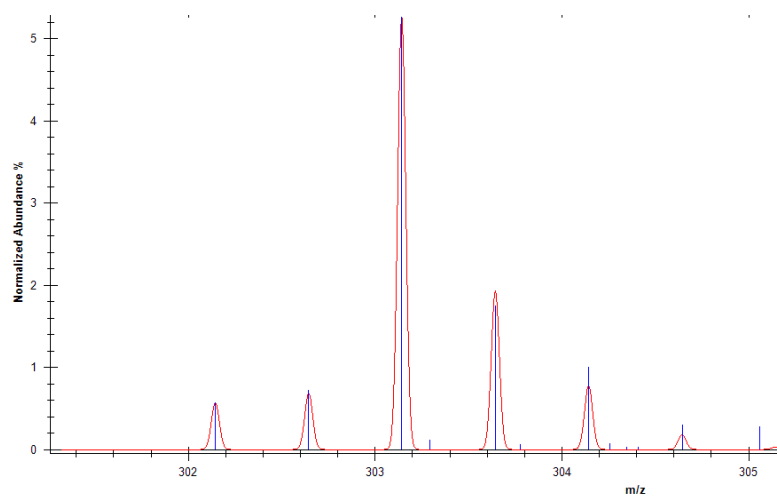
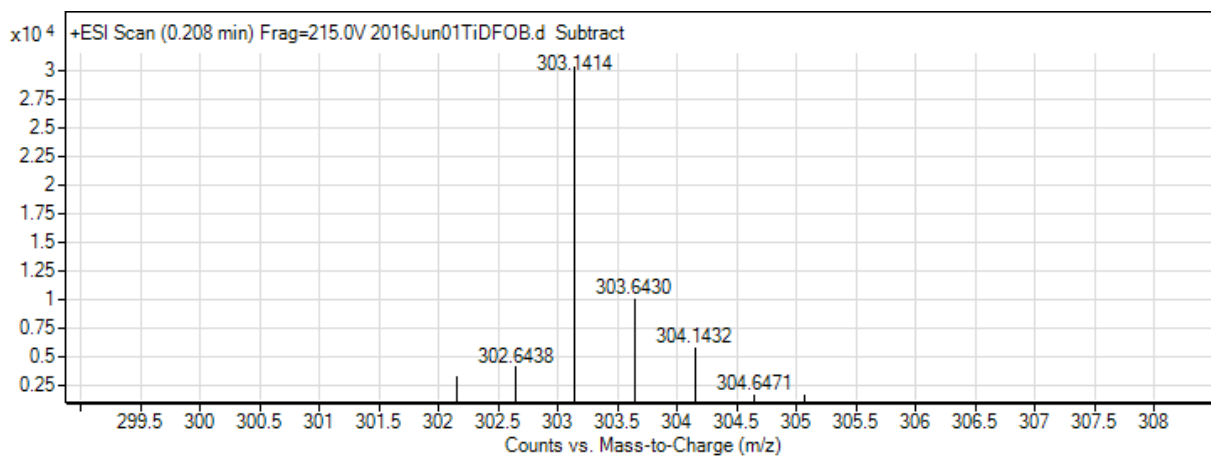


Figure S7. Modeling the peak at $m/z = 303.1$ in the ESI-QTOF. (top) the isotope pattern detected in Figure S6. (bottom) the isotope model for $[\text{TiC}_{25}\text{H}_{46}\text{N}_6\text{O}_8]^{2+}$ ($[\text{Ti}(\text{IV})(\text{HDFOB})]^{2+}$) which indicates that the peak at $m/z = 303.1$ is the protonated Ti-DFOB complex

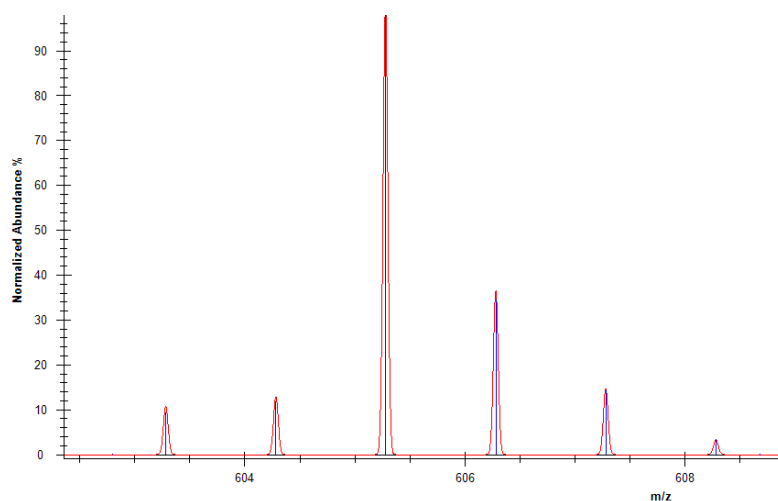
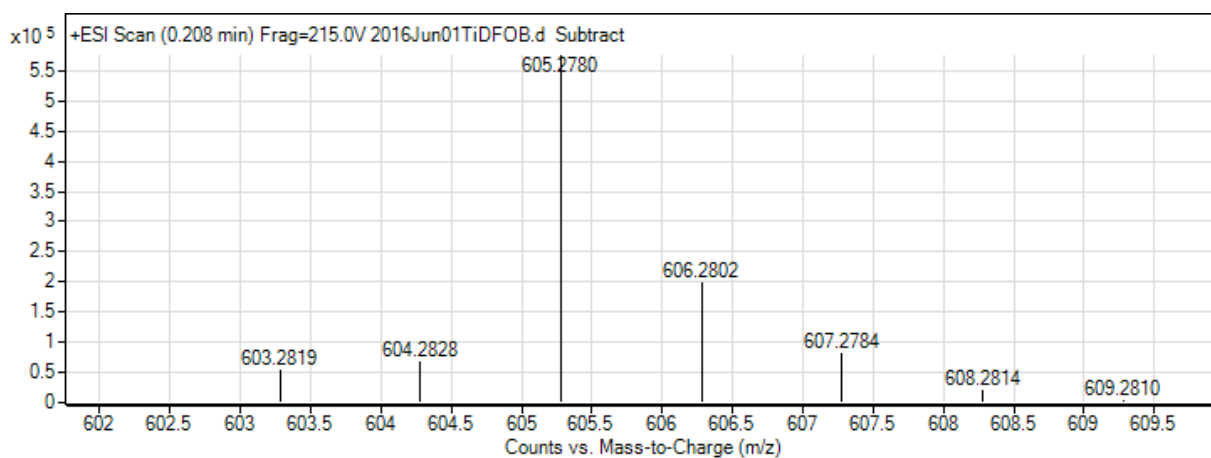


Figure S8. Modeling the peak at $m/z = 605.3$ in the ESI-QTOF mass spectrum (top) the isotope pattern detected in Figure S6. (bottom) the isotope model for $[\text{TiC}_{25}\text{H}_{45}\text{N}_6\text{O}_8]^+$ ($[\text{Ti(IV)(DFOB)}]^+$) which indicates that the peak at $m/z = 605.3$ is the deprotonated Ti-bound DFOB species

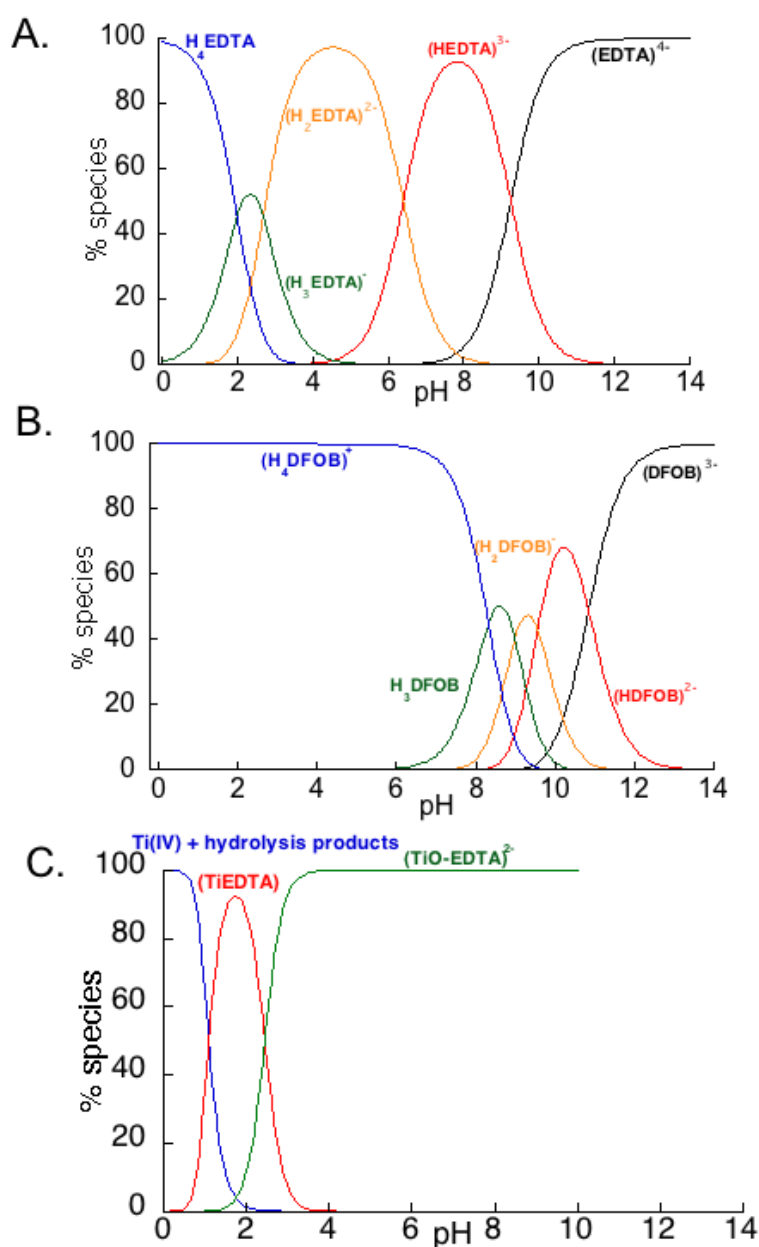


Figure S9. pH-dependent speciation of species relevant to the DFOB/EDTA competition titrations. All species are at 1 mM. Beta values are from the NIST database¹ unless otherwise noted. (A) EDTA alone, (B) DFOB alone, (C) Ti(IV) and EDTA, using values of $\beta_{11-2} = 14.5$ (as $[(Ti(IV)O)(EDTA)]^{2-}$) and $\beta_{110} = 19.4$ ($Ti(IV)EDTA$).^{2,3} Hydrolysis constants were not invoked in (C) because hydrolysis of Ti(IV) overcame the speciation of Ti-EDTA species; however, the species is kinetically stable to this hydrolysis and hydrolytic precipitation was not observed at pH 2.

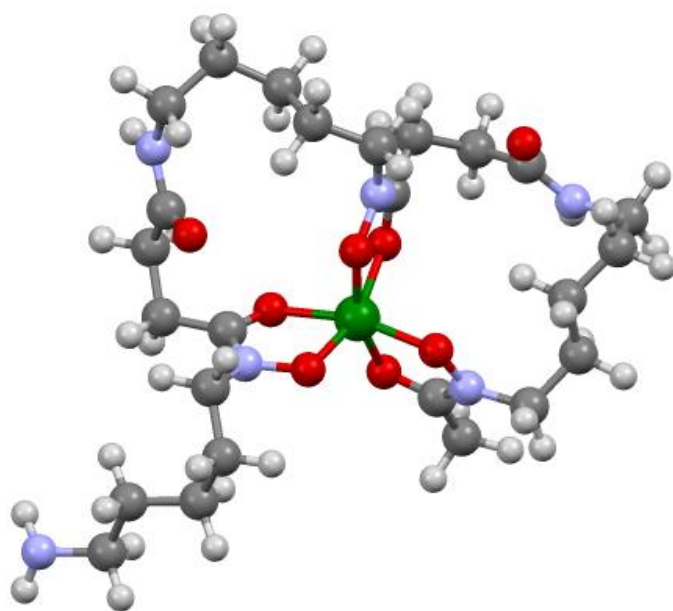


Figure S10. Optimized Ti(IV)-DFOB structure in its deprotonated (+1) with B3LYP 6-31G(d)

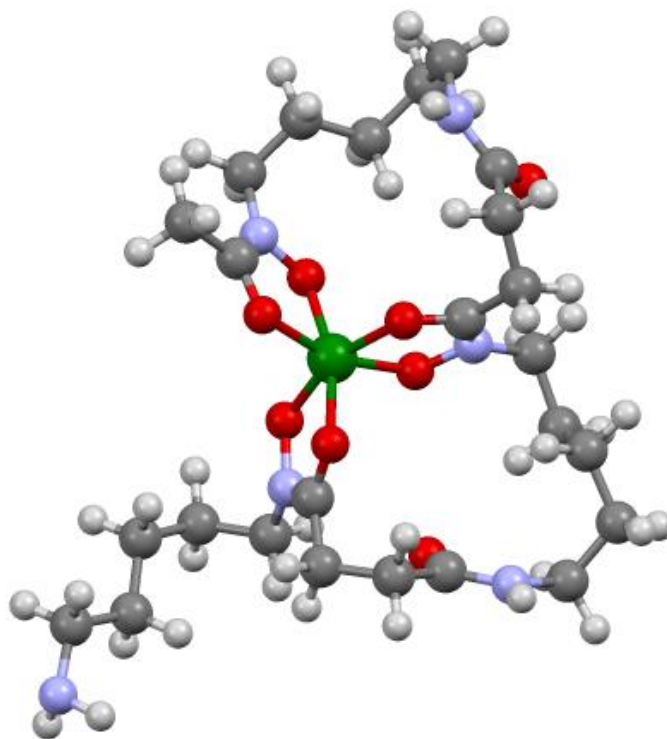


Figure S11. Optimized Ti(IV)-DFOB structure in its deprotonated (+1) using LANL2DZ-electron core potential on Ti(IV) and 6-31G(d) on the rest of the atoms.

O-Ti-O angle (degrees)	O2	O5	O16	O18	O29	O31
O2						
O5	76.14					
O16	92.59	100.2				
O18	159.09	88.1	76.56			
O29	97.52	162.8	96.01	101.31		
O31	107.01	89.99	159.66	86.33	76.44	
Ti-O length	1.907	2.06	1.899	2.025	1.906	2.029

Table S2. Calculated bond angles from O-Ti-O bonds of all 6 oxygens and Ti-O bond lengths for B3LYP-6-31G(d) structure of $[\text{Ti(IV)HDFOB}]^{2+}$

O -Ti- O angle (degrees)	O2	O5	O16	O18	O29	O31
O2						
O5	76.59					
O16	92.67	100.53				
O18	159.98	88.87	72.32			
O29	96.86	163.05	95.33	100.74		
O31	106.7	90.62	159.52	86.86	76.15	
Ti-O length (Å)	1.995	2.02	1.907	2.025	1.914	2.022

Table S3. Calculated bond angles from O-Ti-O bonds of all 6 oxygens and Ti-O bond lengths for LANLD2Z ECP structure of $[\text{Ti(IV)DFOB}]^+$

O-Ti-O angle (degrees)	O2	O5	O16	O18	O29	O31
O2						
O5	76.77					
O16	93.26	100.36				
O18	160.25	88.37	76.49			
O29	96.8	163.05	95.62	100.92		
O31	106.37	90.33	159.42	86.38	76.3	
Ti-O length (Å)	1.891	2.035	1.904	2.04	1.913	2.039

Table S4. Calculated bond angles from O-Ti-O bonds of all 6 oxygens and Ti-O bond lengths for B3LYP-6-31G(d) structure of $[\text{Ti(IV)DFOB}]^+$

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