Supplementary Information

for

"Absorption spectroscopy and binding constants for first-row transition metal complexes of a DOPA-containing peptide"

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Fitting Titration Data: Base Titration of $Fe(NO_3)_3$ and AdopaTP Peptide

Binding constants for metal-ligand complexes presented in this paper were determined by simultaneous fitting of ultraviolet-visible absorption spectra and pH data. The fit quality was judged by how well the spectra of calculated species matched the observed experimental UV-vis spectra both visually and mathematically. Here we describe the example of $Fe(NO_3)_3$ and AdopaTP in detail. All other titrations were handled in a similar manner.

Figure S1a shows UV-vis spectra obtained for base (NaOH at 0.1125M) titration into a solution of $Fe(NO_3)_3$ (0.3 mM) and AdopaTP peptide (0.9 mM). The titration had 41 points, thereby yielding 41 spectra. For clarity, all 41 spectra are not shown here. Interpretation of these spectra based upon maximizing of spectral features and then the number of base equivalents added suggested the presence of three iron-peptide complexes. The spectra for each complex at maximum formation are shown in Figure S1b.

When attempting to fit titration and pH data, the model used included three colored AdopaTP–Fe species (the 1:1, 2:1, and 3:1 peptide:metal complexes). Additionally, we imported and fixed into the model the aqueous $Fe(NO_3)_3$ spectrum. The resulting fit yielded binding affinities for the 1:1, 2:1, and 3:1 peptide:metal species of 18.19 ± 0.04, 32.75 ± 0.06, and 41.11 ± 0.07, respectively. In determining these binding constants, the SPECFIT program calculated expected spectra for each metal complex, shown in Figure S1c. As can be seen easily, the observed and calculated spectra match well, indicating a good fit to the data. The corresponding speciation diagram produced by SPECFIT with this model is presented in Figure S2. The experimentally observed spectrum of aqueous $Fe(NO_3)_3$ is shown in Figure S1c.

Alternate models were fit to the data, but with less success. For example, we used a model of three colored AdopaTP-Fe complexes, but did not include a defined spectrum for $Fe(NO_3)_3$, alone (Figure S3b). Although the calculated spectra for the three Fe-AdopaTP complexes were similar to those observed, the calculated spectrum of aqueous $Fe(NO_3)_3$ did not match the observed spectrum. Likewise, a model with only two Fe-AdopaTP peptide complexes could not account for all of the spectra obtained in the full titration (Figure S3c). A model containing four colored Fe-AdopaTP complexes was examined, however only three spectra and binding constants were found by SPECFIT.

In addition to visual inspection of spectra obtained with each model, binding constant error values and fitting parameters were examined. Tables S1-4 show calculated binding constants with error values and sigma Y values for different fits. Models including two, three, and four Fe-AdopaTP species were attempted. The model with only two species gave higher error and sigma Y values than the "best fit" model of three species (Table S1). Alternately, the model with four species could fit only three species (Table S1). Table S2 shows the values obtained from a model in which the spectrum of aqueous iron was not fixed. Lower errors were found with the fixed spectrum included. For titrations in which final species formation was maximized prior to addition of 10 base equivalents, the spectra showing only simple dilution of the final species were removed from the data set used. The presence or absence of these dilution spectra showed no significant effect on the binding constants obtained or the fit quality, as can be seen in Table S3. Table S4 shows that erroneous binding constant values and poor fits resulted only when initial guess values were extremely overestimated (*e.g.*, error on β_1 of ± 6.54 versus typical error of ± 0.06).

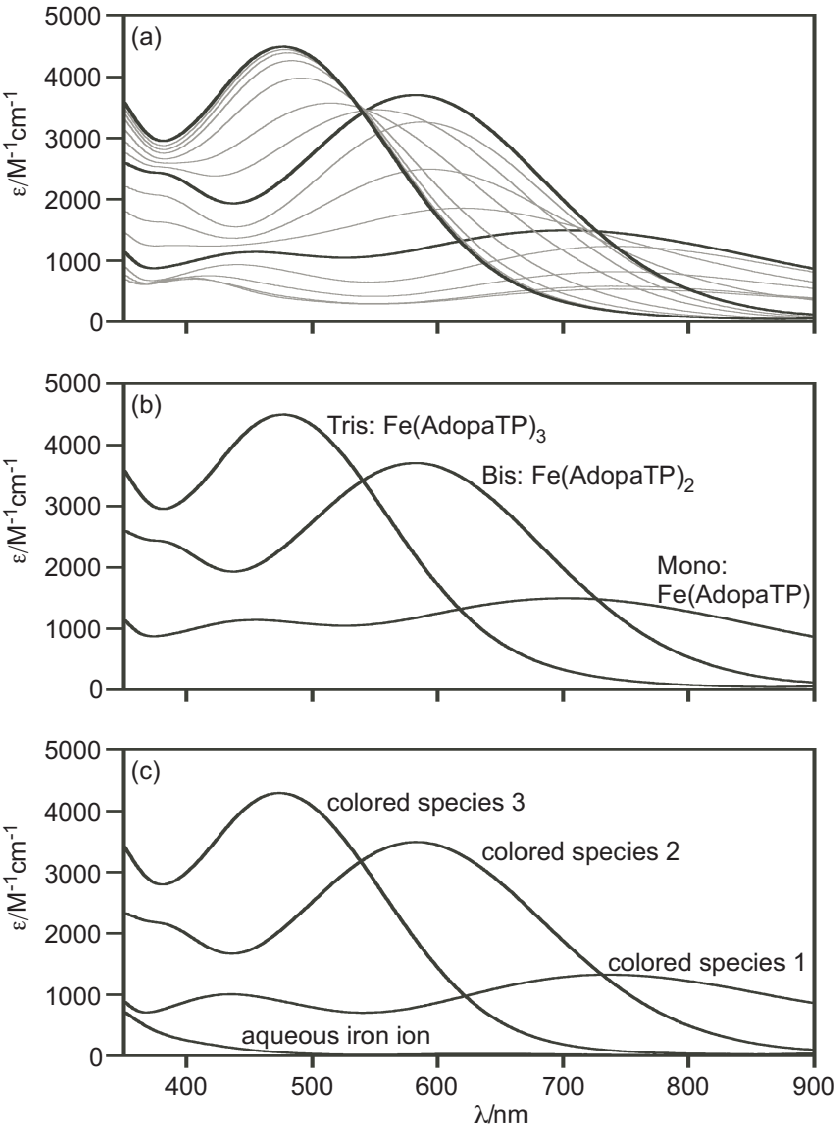

Figure S1. UV-vis spectra for complexes formed by base titration of $Fe(NO_3)_3$ and AdopaTP peptide: (a) Titration data adjusted for dilution and plotted versus extinction coefficient values. Three spectra are highlighted in black to show where spectral changes maximized, (b) The spectra of 1:1, 2:1, and 3:1 AdopaTP-iron species determined from titration data, (c) AdopaTP-iron species spectra calculated by SPECFIT for a model containing three colored metal-ligand species and a fixed spectrum for aqueous $Fe(NO_3)_3$, determined experimentally.

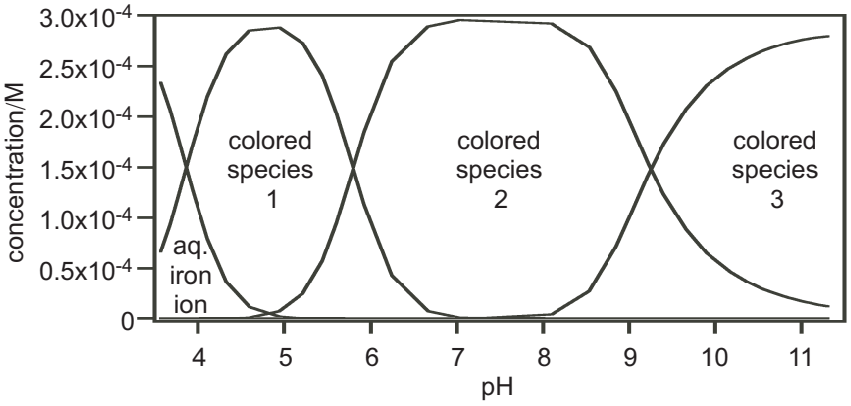

Figure S2. Speciation diagram for base titration of $Fe(NO_3)_3$ and AdopaTP calculated by SPECFIT for the "best fit" model containing three colored metal-ligand species and a fixed spectrum for aqueous $Fe(NO_3)_3$.

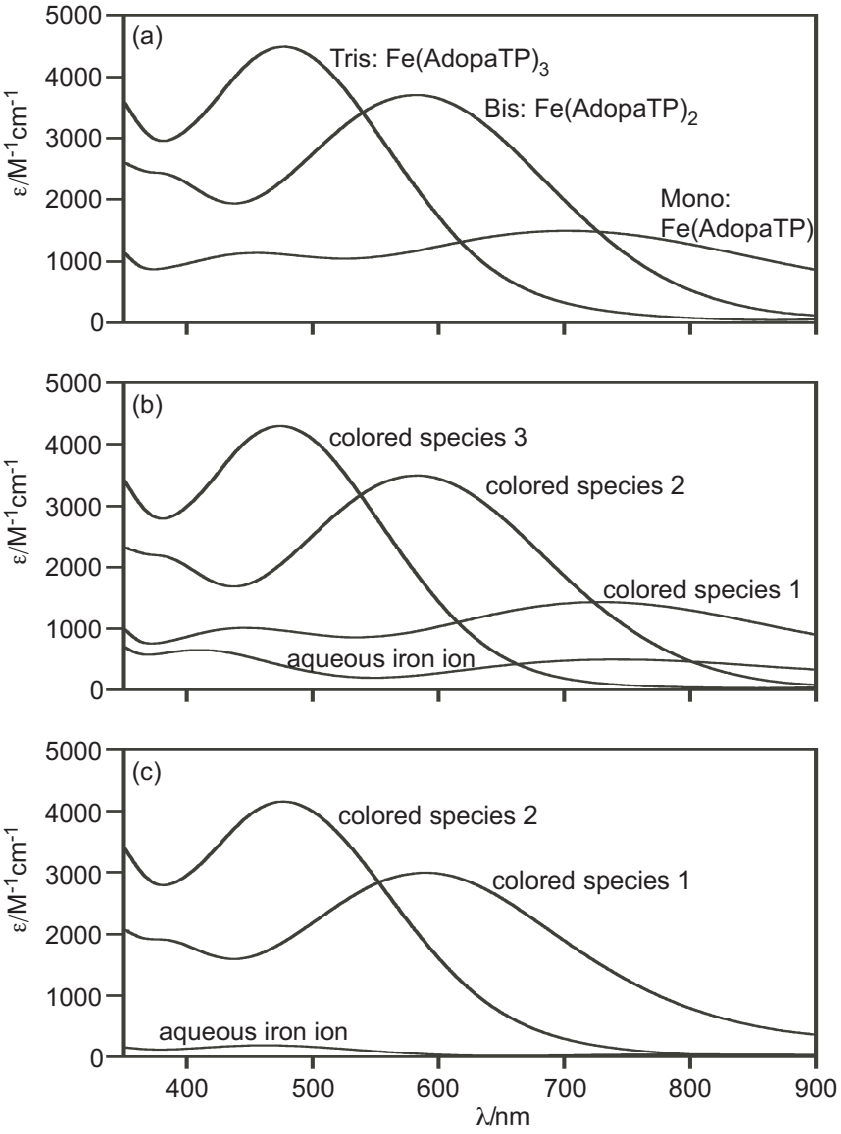

Figure S3. Observed and calculated Fe-AdopaTP species UV-vis spectra for the base titration of $Fe(NO_3)_3$ and AdopaTP peptide: (a) The spectra of 1:1, 2:1, and 3:1 AdopaTP-iron species determined from titration data, (b) Spectra of AdopaTP-iron species calculated by SPECFIT for a model containing three colored metal-ligand species, but without a fixed spectrum for aqueous $Fe(NO_3)_3$, (c) Calculated AdopaTP-iron spectra from SPECFIT for a model containing two colored metal-ligand species and a fixed spectrum for aqueous $Fe(NO_3)_3$.

Table S1. Effect of different number of complex species in model

# of species	Fe^{3+}_{aq} spectrum	# of spectra	Initial β_x estimates 1st	2nd	3rd	4th	mono β_1	bis β_2	tris β_3	β_4	sigma Y
2	yes	41	20	30	-	-	15.56 ± 0.11	23.58 ± 0.16	-	-	0.09302
3	yes	41	20	30	40	-	18.19 ± 0.04	32.75 ± 0.06	41.11 ± 0.07	-	0.02351
4	yes	41	20	30	40	50	18.20 ± 0.04	32.78 ± 0.05	41.17 ± 0.07	NA	NA

Table S2. Effect of including a fixed spectra of aqueous $Fe(NO_3)_3$

# of species	Fe^{3+}_{aq} spectrum	# of spectra	Initial β_x estimates 1st	2nd	3rd	4th	mono β_1	bis β_2	tris β_3	β_4	sigma Y
3	yes	41	20	30	40	-	18.19 ± 0.04	32.75 ± 0.06	41.11 ± 0.07	-	0.02351
3	no	41	20	30	40	-	17.32 ± 0.08	31.79 ± 0.09	40.14 ± 0.10	-	0.01916

Table S3. Effect of extra spectra on fit

# of species	Fe^{3+}_{aq} spectrum	# of spectra	Initial β_x estimates 1st	2nd	3rd	4th	mono β_1	bis β_2	tris β_3	β_4	sigma Y
3	yes	41	15	25	35	-	18.19 ± 0.04	32.75 ± 0.06	41.11 ± 0.07	-	0.02351
3	yes	31	15	25	35	-	18.20 ± 0.05	32.77 ± 0.06	41.14 ± 0.08	-	0.02435
3	yes	25	15	25	35	-	18.20 ± 0.05	32.78 ± 0.07	41.18 ± 0.09	-	0.02642

Table S4. Effect of initial β_x estimates on fit

# of species	Fe^{3+}_{aq} spectrum	# of spectra	Initial β_x estimates 1st	2nd	3rd	4th	mono β_1	bis β_2	tris β_3	β_4	sigma Y
3	yes	41	0	0	0	-	18.19 ± 0.04	32.75 ± 0.06	41.11 ± 0.07	-	0.02351
3	yes	41	15	25	35	-	18.19 ± 0.04	32.75 ± 0.06	41.11 ± 0.07	-	0.02351
3	yes	41	20	30	40	-	18.19 ± 0.04	32.75 ± 0.06	41.11 ± 0.07	-	0.02351
3	yes	41	30	40	50	-	36.28 ± 6.54	51.10 ± 0.07	59.48 ± 0.07	-	0.04193