APPENDIX A

Derivation of Model for Ln(DPA) Binding Affinity

We start with the equilibrium described in [1], where Ln^{3+} is any lanthanide, and which has the corresponding equilibrium expression written in [2].

$$Ln^{3+} + DPA^{2-} \overset{K_a}{\rightleftharpoons} Ln(DPA)^{+} \qquad [1]$$

$$K_a = \frac{[Ln(DPA)^+]_{eq}}{[Ln^{3+}]_{eq}[DPA^{2-}]_{eq}} \qquad [2]$$

We can write the total concentrations of lanthanide and DPA, or C_{Ln} and C_{DPA}, as follows in equations [3] and [4].

$$C_{Ln} = [Ln^{3+}]_{eq} + [Ln(DPA)^+]_{eq} \qquad [3]$$

$$C_{DPA} = [DPA^{2-}]_{eq} + [Ln(DPA)^+]_{eq} \qquad [4]$$

These can be rearranged to produce equations [5] and [6].

$$[Ln^{3+}]_{eq} = C_{Ln} - [Ln(DPA)^+]_{eq} \qquad [5]$$

$$[DPA^{2-}]_{eq} = C_{DPA} - [Ln(DPA)^+]_{eq} \qquad [6]$$

Substituting equations [5] and [6] into equation [2], we have equation [7].

$$K_a = \frac{[Ln(DPA)^+]_{eq}}{(C_{Ln} - [Ln(DPA)^+]_{eq})(C_{DPA} - [Ln(DPA)^+]_{eq})} \qquad [7]$$

Rearranging, we have equation [8].

$$K_a = \frac{[Ln(DPA)^+]_{eq}}{C_{Ln}C_{DPA} - [Ln(DPA)^+]_{eq}C_{DPA} - [Ln(DPA)^+]_{eq}C_{Ln} + ([Ln(DPA)^+]_{eq})^2} \quad [8]$$

Let us introduce a normalization factor, R, given in equation [9].

$$R = \frac{[Ln(DPA)^+]_{eq}}{[Ln(DPA)^+]_{eq} + [(DPA)^{2-}]_{eq}} \quad [9]$$

Substituting equation [6] into equation [9], we have equation [10].

$$R = \frac{[Ln(DPA)^+]_{eq}}{C_{DPA}} \quad [10]$$

Substituting equation [10] into equation [8] and simplifying, we have equation [11].

$$K_a = \frac{R}{C_{Ln} - RC_{DPA} - RC_{Ln} + R^2C_{DPA}} \quad [11]$$

Rearranging, we end with equation [12], which has a linear relationship between two components dependent on $[Ln(DPA)^+]_{eq}$, C_{Ln} and C_{DPA}.

$$\log\left(\frac{R}{1 - R}\right) = \log\left(C_{Ln} - RC_{DPA}\right) + \log\left(K_a\right) \quad [12]$$

Thus, a plot of $\log(C_{Ln} - RC_{DPA})$ vs $\log(R/(1 - R))$ will produce a linear fit with a slope of unity and a y-intercept equal to the logarithm of K_a.

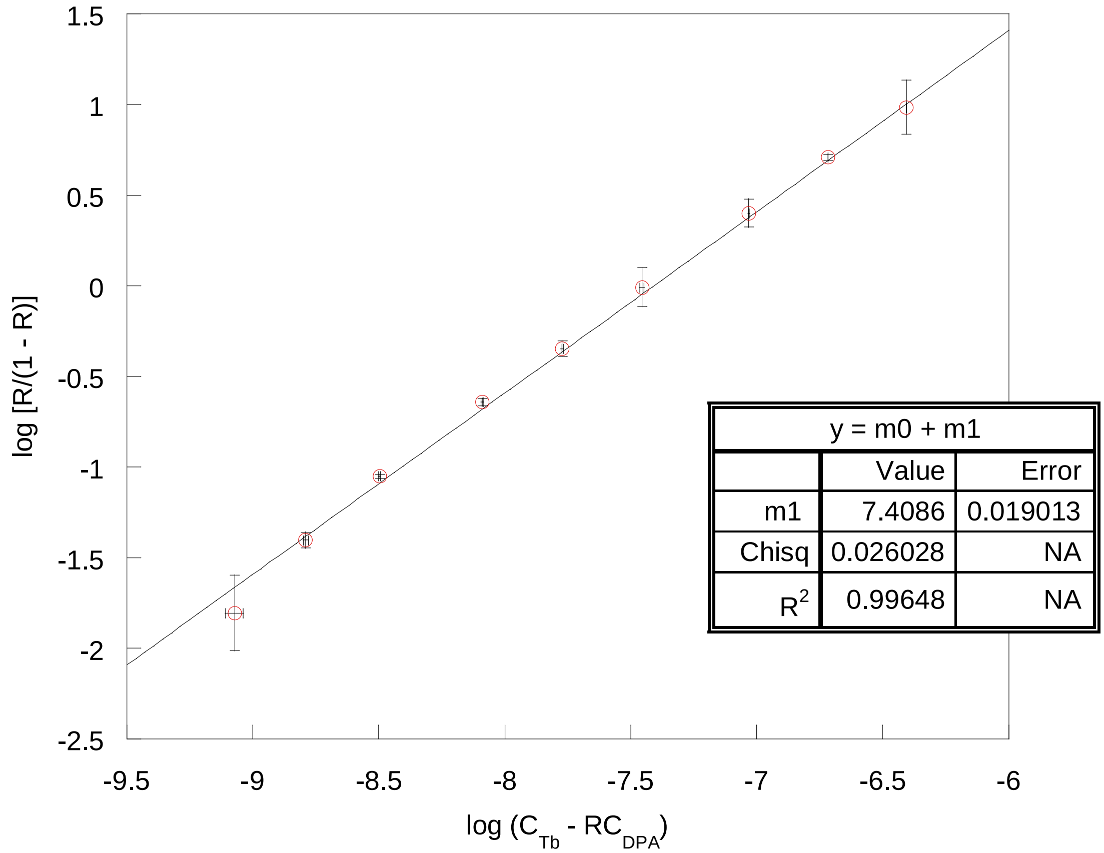

Figure A1. Linear fit of log(C_{Tb} – RC_{DPA}) vs log (R/(1 – R)) with slope set to unity and y-intercept corresponding to log K_a. 10.0 nM DPA titrated with $TbCl_3$ in 0.2 M sodium acetate, pH 7.4, 24.5℃ (λ_{ex} = 278 nm).