Supporting Information

Detection of Bacterial Spores with Lanthanide-Macrocycle Binary Complexes

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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].

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

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

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

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

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

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

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

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

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].

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

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

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

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

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

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

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

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

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

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.

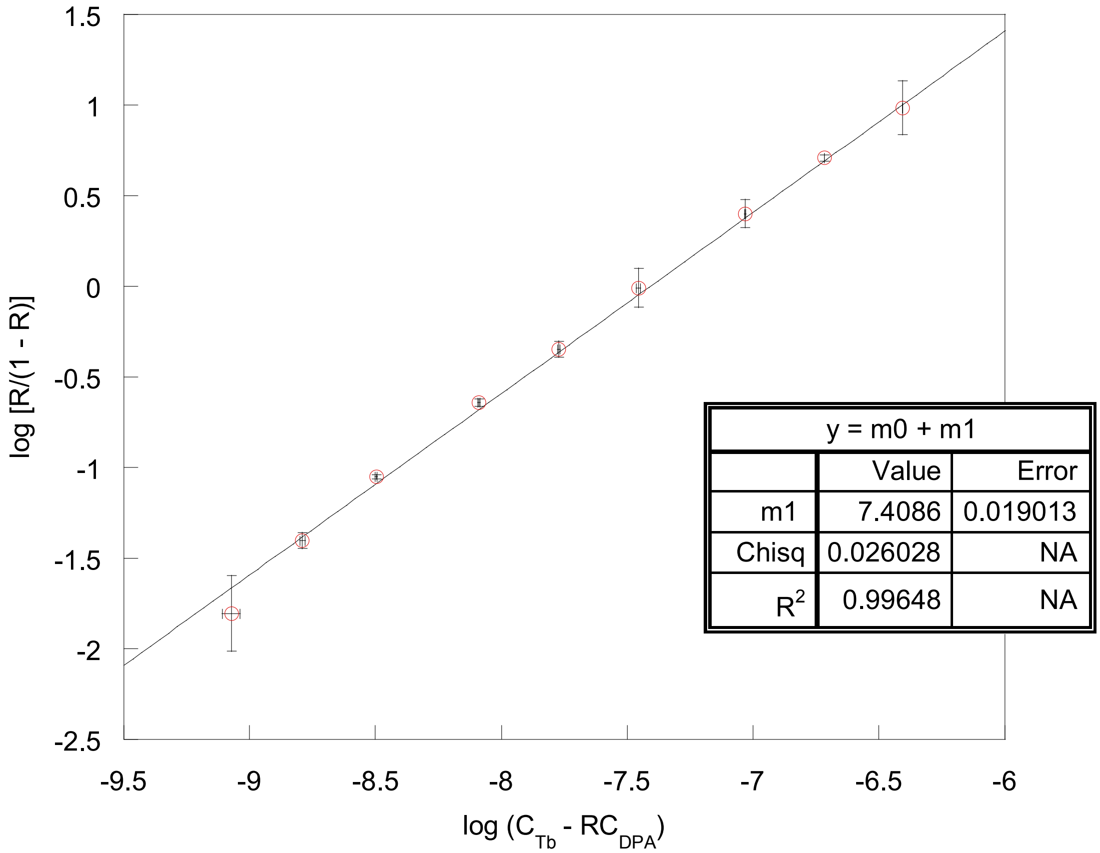

Figure S1. Linear fit of $\log(C_{Tb} - RC_{DPA})$ vs $\log(R/(1 - R))$ with slope set to unity and y-intercept corresponding to $\log K_a$. 10.0 nM DPA titrated with $TbCl_3$ in 0.2 M sodium acetate, pH 7.4, 24.5°C (λ_{ex} = 278 nm).

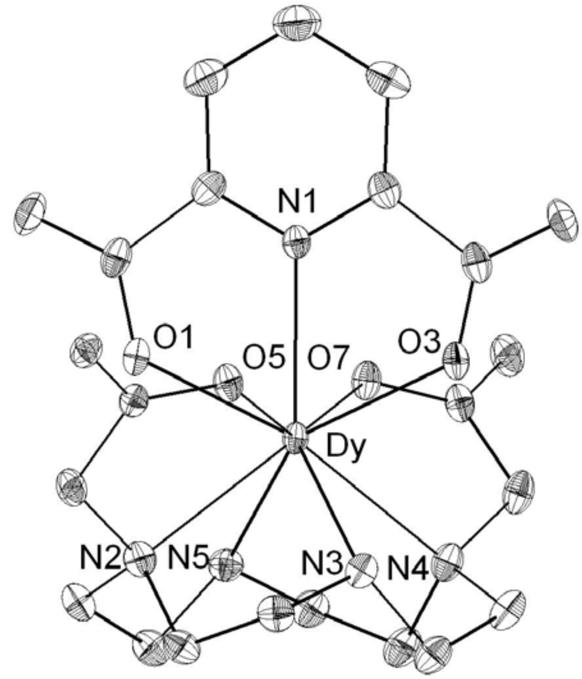

Figure S2. Thermal ellipsoid plot of the Dy(DO2A)(DPA)$^{-}$ ternary complex with 50% probability. Hydrogens omitted for clarity.

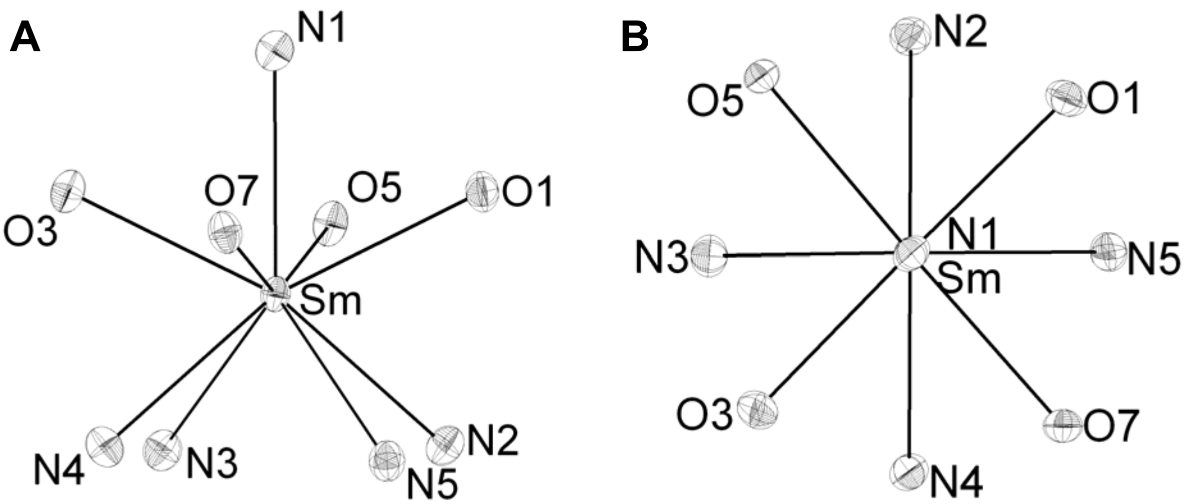

Figure S3. Thermal ellipsoid plots of the Sm coordination geometry in the Sm(DO2A)(DPA)$^{-}$ ternary complex with 50% probability. (A) Looking across the complex, with DO2A below and DPA above the Sm^{3+} central ion. (B) Looking down the DPA ligand (the N1 of the DPA is obstructing the view of the Sm).

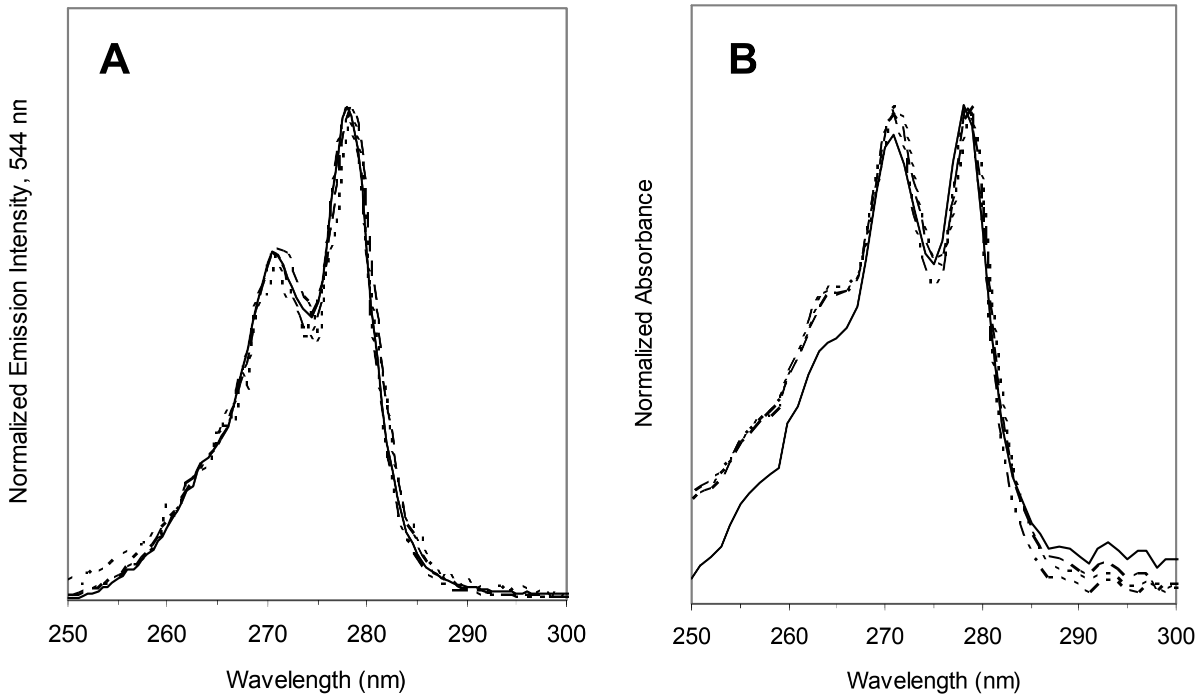

Figure S4. Normalized excitation (A) and absorption (B) spectra of $Ln(DO2A)(DPA)^-$ complexes, where Ln = Sm (dotted), Eu (dashed), Tb (solid) or Dy (dashed-dotted), at 10.0 µM in 0.1 M Tris, pH 7.9. Excitation wavelengths: λ_{Sm} = 600 nm, λ_{Eu} = 615 nm, λ_{Tb} = 544 nm, λ_{Dy} = 574 nm.

Calculation of Quantum Yields and Molar Extinction Coefficients for Ln(DO2A)(DPA)$^{-}$ Complexes.

We start with the standard equation for calculation of luminescence quantum yield in [1].

$$\frac{\Phi_X}{\Phi_{ST}} = \frac{E_X}{E_{ST}} \cdot \frac{A_{ST}(\lambda_{ST})}{A_X(\lambda_X)} \cdot \frac{I_{ST}(\lambda_{ST})}{I_X(\lambda_X)} \cdot \frac{\eta_X^2}{\eta_{ST}^2} \qquad [1]$$

where

Φ = Quantum yield
E = Fluorescence emission intensity
A = Absorbance at the excitation wavelength λ
I = Intensity of excitation light at wavelength λ
η = Refractive index of solvent
X = Sample
ST = Standard

To fit our data to a linear least-squares regression, we introduce a gradient relationship in [2].

$$\text{Grad} = \frac{E}{A} \qquad [2]$$

Substituting equation [2] into equation [1], we end with equation [3].

$$\Phi_X = \Phi_{ST} \cdot \frac{\text{Grad}_X}{\text{Grad}_{ST}} \cdot \frac{I_{ST}(\lambda_{ST})}{I_X(\lambda_X)} \cdot \frac{\eta_X^2}{\eta_{ST}^2} \qquad [3]$$

Data was plotted as emission intensity against absorbance, and the resulting values of Grad_X and Grad_{ST} were applied to equation [3] to obtain the quantum yield, with L-tryptophan as the standard ($\boldsymbol{\varepsilon}_{Exp}$ = 5277.4 $M^{-1}cm^{-1}$, $\boldsymbol{\varepsilon}_{Theor}$ = 5502 $M^{-1}cm^{-1}$).[29]

Molar extinction coefficients were also calculated for all four ternary complexes and the dipicolinate anion by plotting absorbance against concentration.

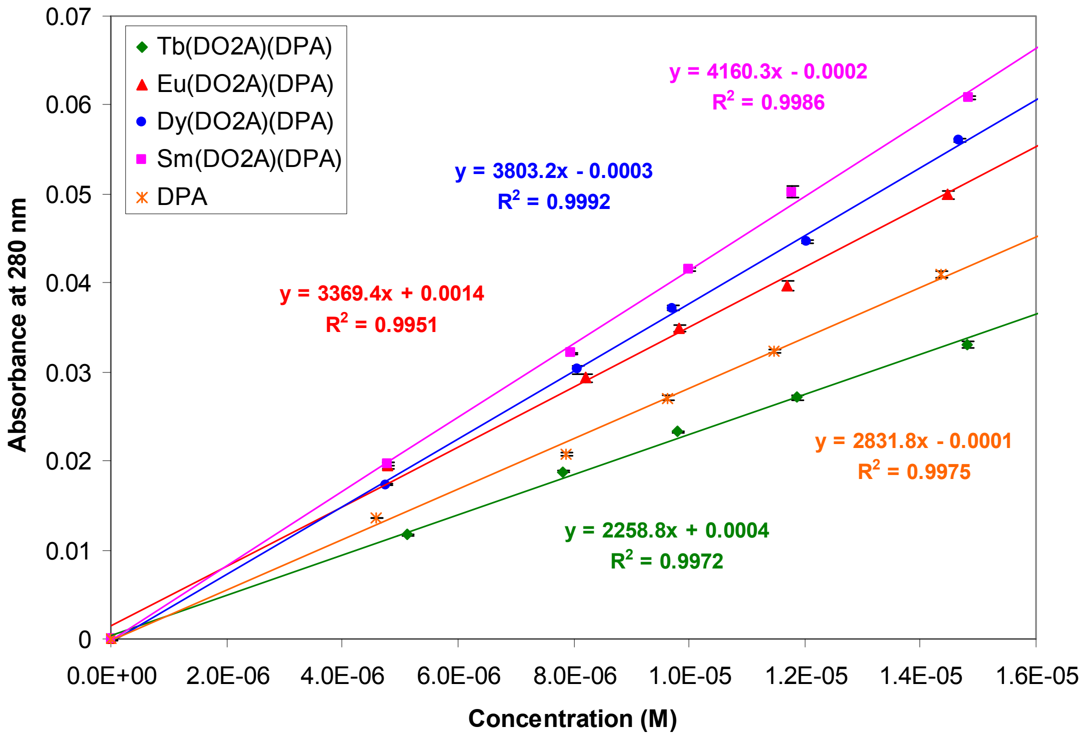

Figure S5. Linear fit of absorbance (λ_{abs} = 280 nm) versus concentration for the $Ln(DO2A)(DPA)^-$ complexes (Ln = Sm, Eu, Tb, Dy) in 0.1 M Tris buffer, pH 7.5.

Table S1. Molar extinction coefficients of the $Ln(DO2A)(DPA)^-$ complexes (Ln = Sm, Eu, Tb, Dy) and the DPA^{2-} anion.

Complex	Buffer	λ_{abs} (nm)	Temp (°C)	pH	ε_{Exp} ($M^{-1}cm^{-1}$)
$Sm(DO2A)(DPA)^-$	0.1 M Tris	280	22.0	7.49	4160 ± 10
$Eu(DO2A)(DPA)^-$			22.1	7.46	3369 ± 24
$Tb(DO2A)(DPA)^-$			22.0	7.43	2259 ± 10
$Dy(DO2A)(DPA)^-$			22.1	7.49	3803 ± 2
DPA^{2-}			22.3	7.50	2832 ± 21

Molar extinction coefficients are all in the same range of 10^3 $M^{-1}cm^{-1}$, which is expected as all contain the same amount of dipicolinate, the only strongly absorbing species.

Table S2. Stability of the $Tb(DO2A)(DPA)^-$ complex over time.

Eq. Time	pH	log K_a'
8 days	7.5	9.25 ± 0.13[§]
5 months	7.4	9.30 ± 0.19
11 months	7.5	9.12 ± 0.24

[§] Previous work.[27]

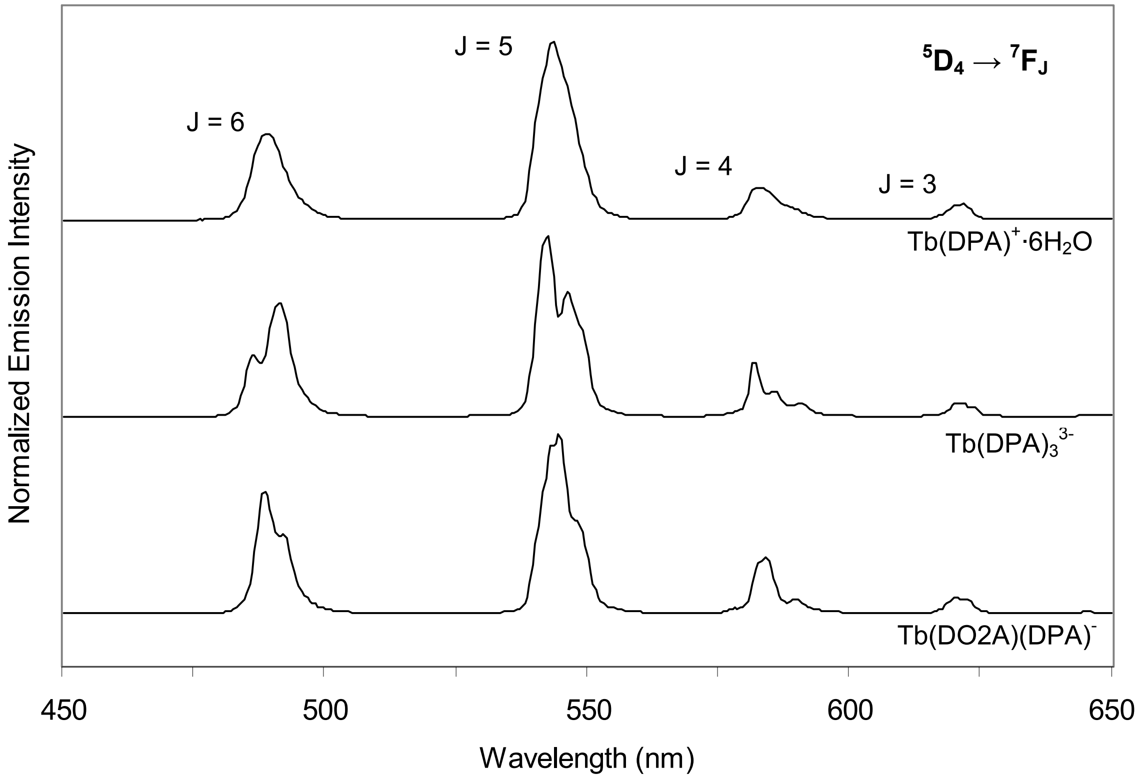

Figure S6. Emission spectra of various terbium complexes, 10.0 μM in 0.2 M sodium acetate, pH 7.4 (λ_{ex} = 278 nm), showing characteristic splitting as a result of changes in the symmetry of the Tb^{3+} coordination sphere.

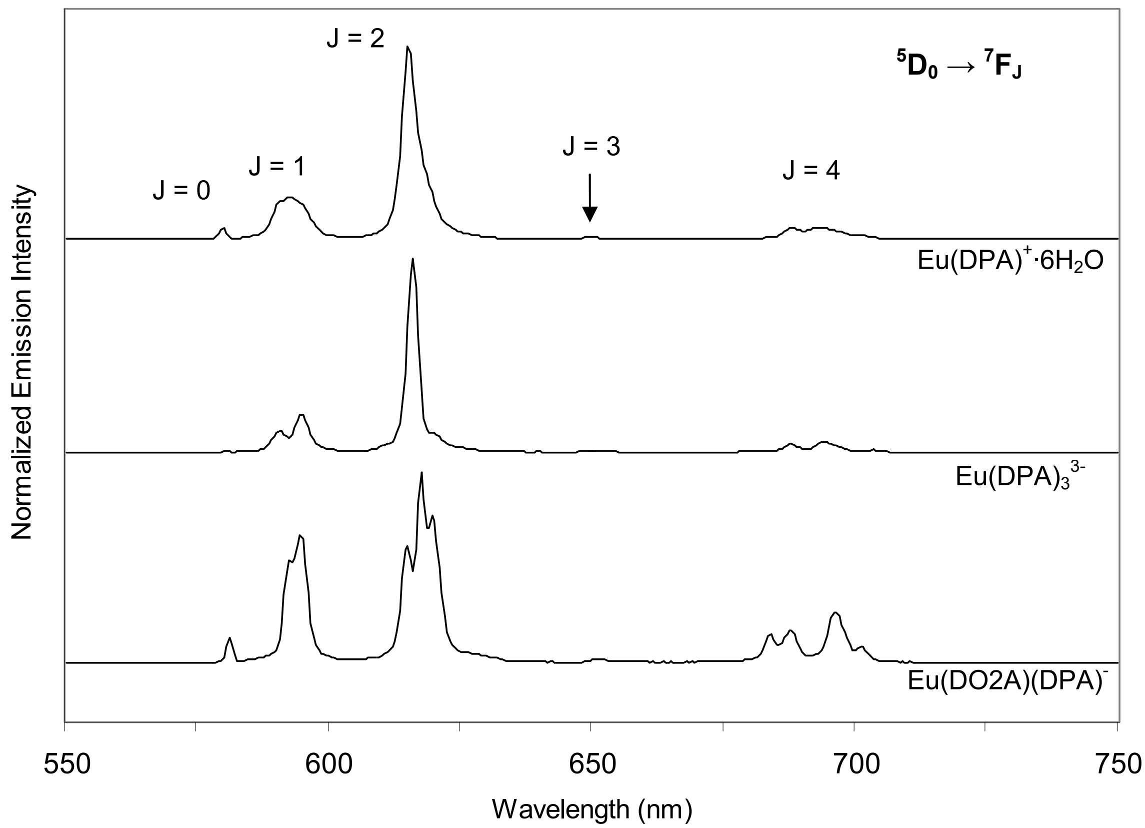

Figure S7. Emission spectra of europium complexes, 10.0 μM in 0.2 M sodium acetate, pH 7.4 (λ_{ex} = 278 nm).

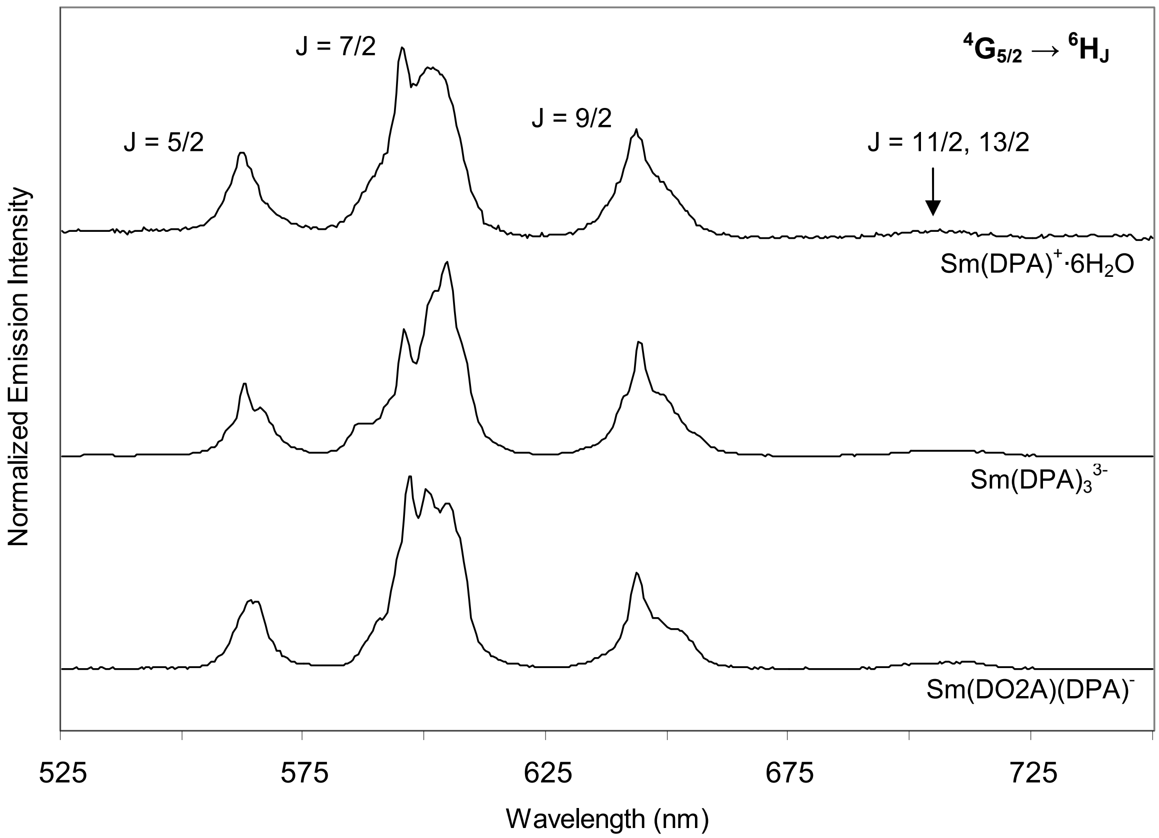

Figure S8. Emission spectra of samarium complexes, 10.0 μM in 0.2 M sodium acetate, pH 7.4 (λ_{ex} = 278 nm).

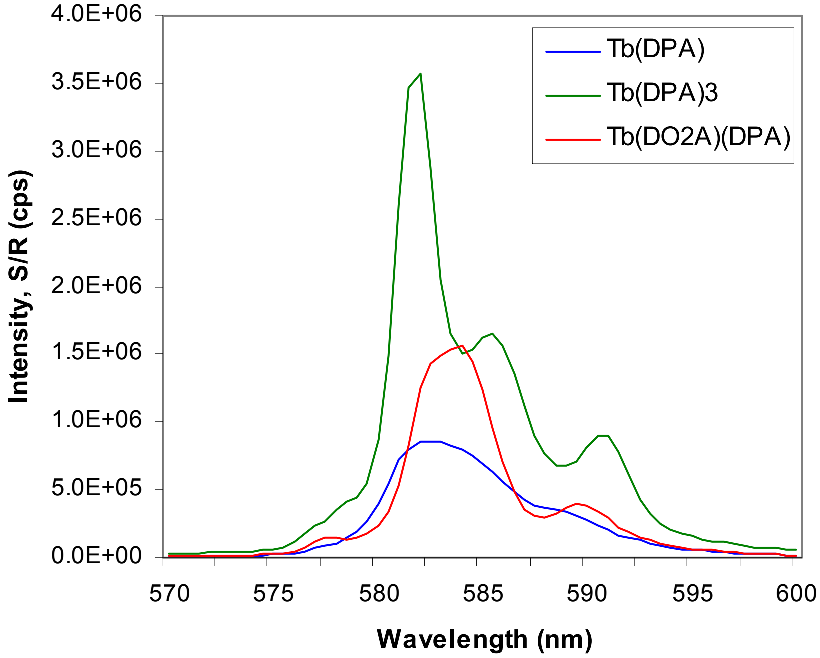

Figure S9. Emission spectra of the three terbium dipicolinate complexes, all 10.0 μM in 0.1 M MOPS buffer, pH 7.4.

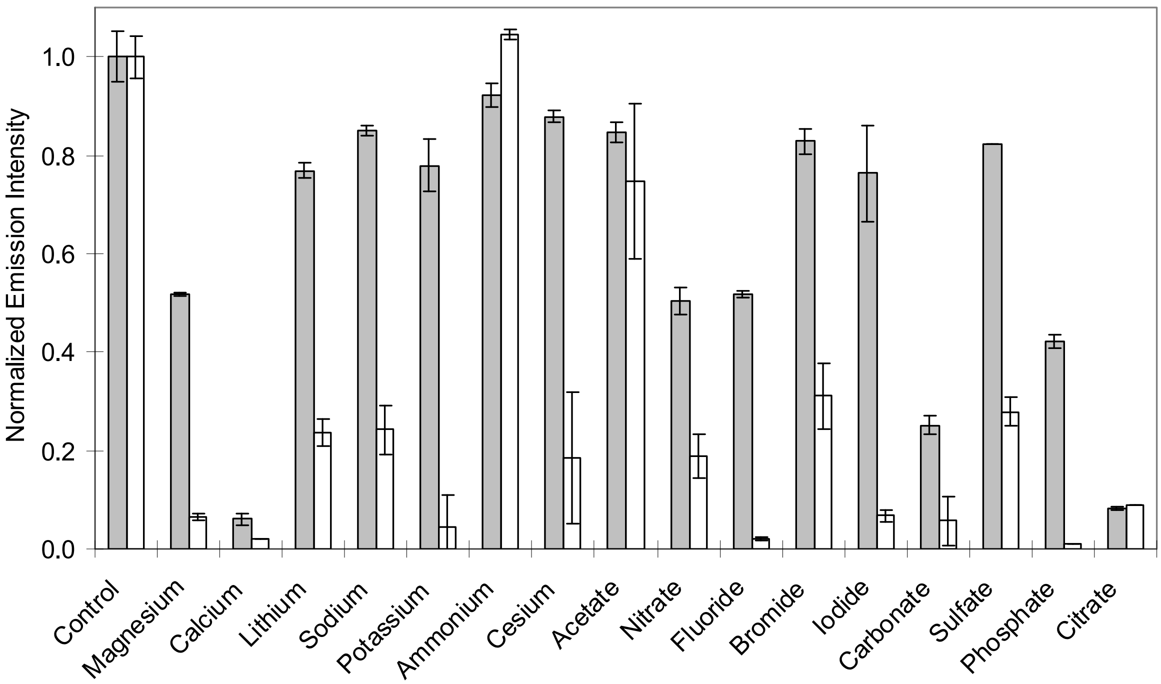

Figure S10. Emission intensity variation of 0.1 μM Tb(DO2A)(DPA)$^{-}$ complex (gray bars) or Tb(DPA)$^{+}$ complex (white bars) with the addition of 0.1 M ion, pH 6.6. Normalized integrated emission intensity, 530 – 560 nm; λ_{ex} = 278 nm.

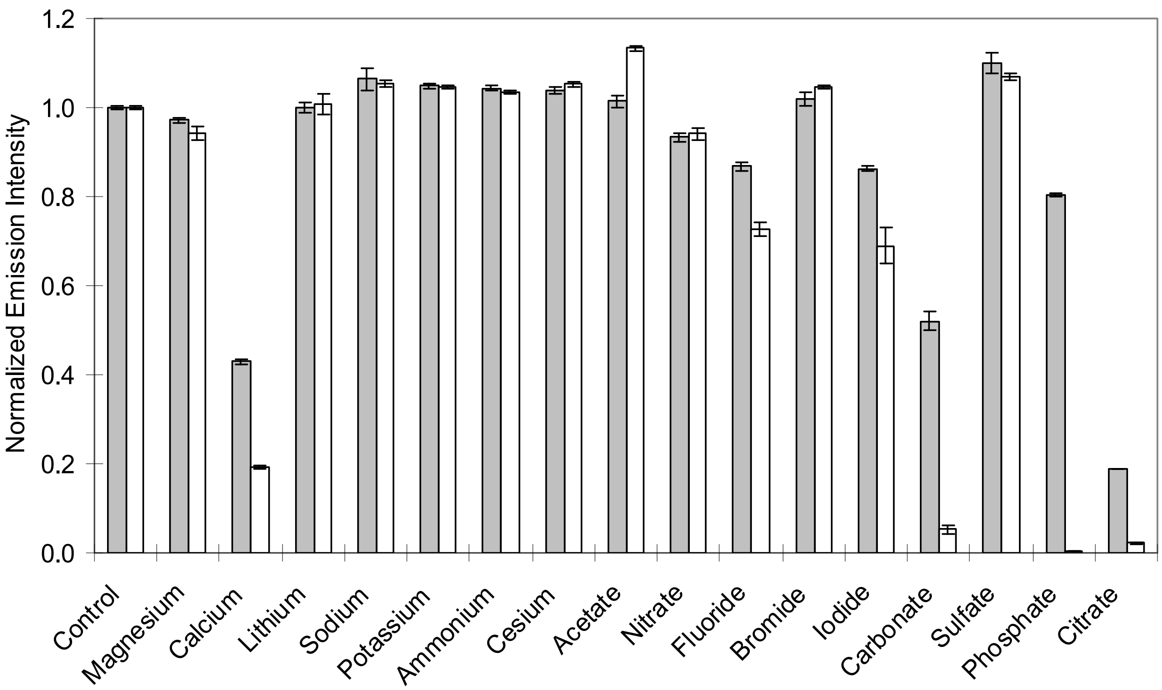

Figure S11. Emission intensity variation of 0.1 μM Tb(DO2A)(DPA)$^{-}$ complex (gray bars) or Tb(DPA)$^{+}$ complex (white bars) with the addition of 0.01 M ion, pH 5.6. Normalized integrated emission intensity, 530 – 560 nm; λ_{ex} = 278 nm.

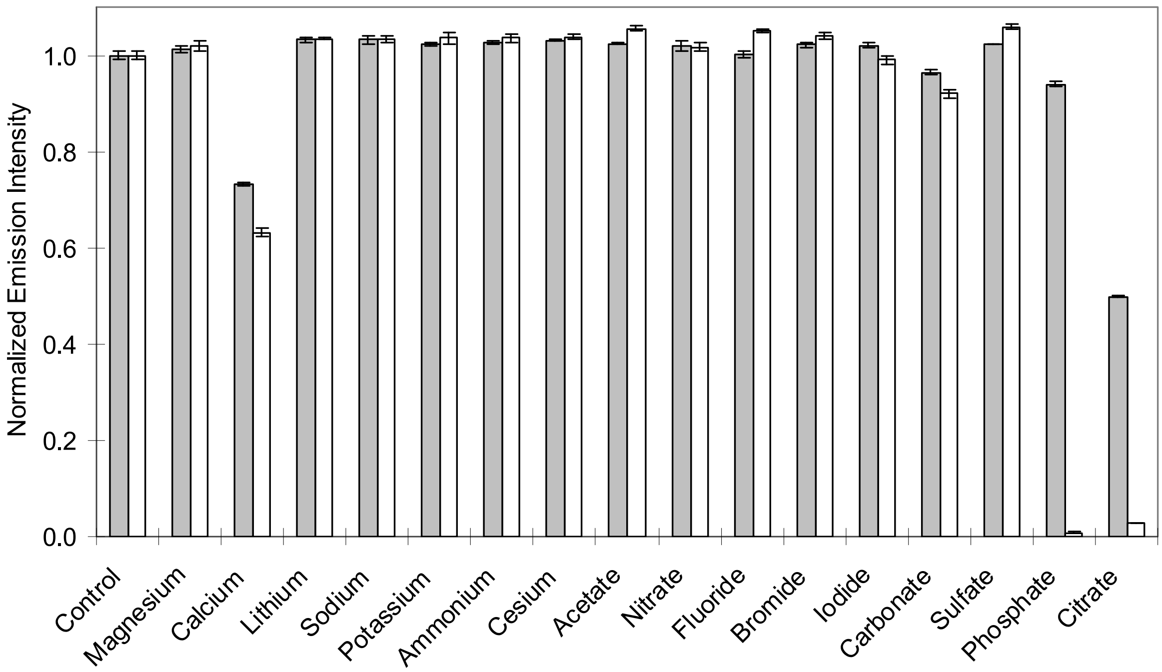

Figure S12. Emission intensity variation of 0.1 μM Tb(DO2A)(DPA)$^-$ complex (gray bars) or Tb(DPA)$^+$ complex (white bars) with the addition of 1.0 mM ion, pH 5.3. Normalized integrated emission intensity, 530 – 560 nm; λ_{ex} = 278 nm.

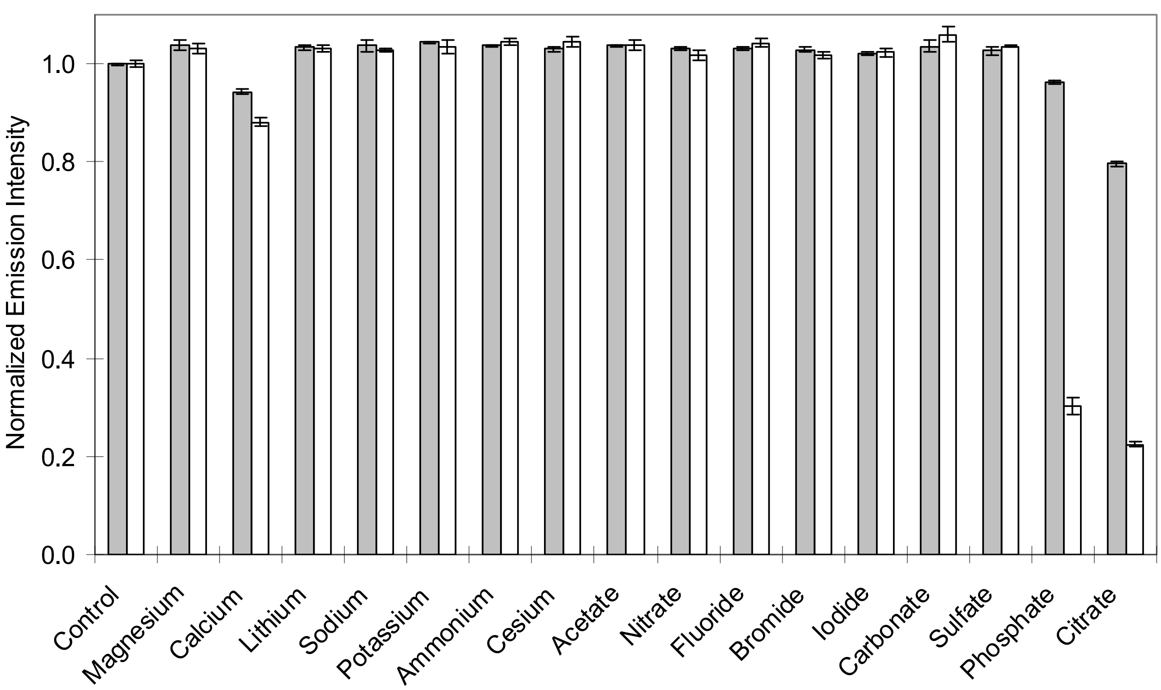

Figure S13. Emission intensity variation of 0.1 μM Tb(DO2A)(DPA)$^-$ complex (gray bars) or Tb(DPA)$^+$ complex (white bars) with the addition of 0.1 mM ion, pH 5.0. Normalized integrated emission intensity, 530 – 560 nm; λ_{ex} = 278 nm.

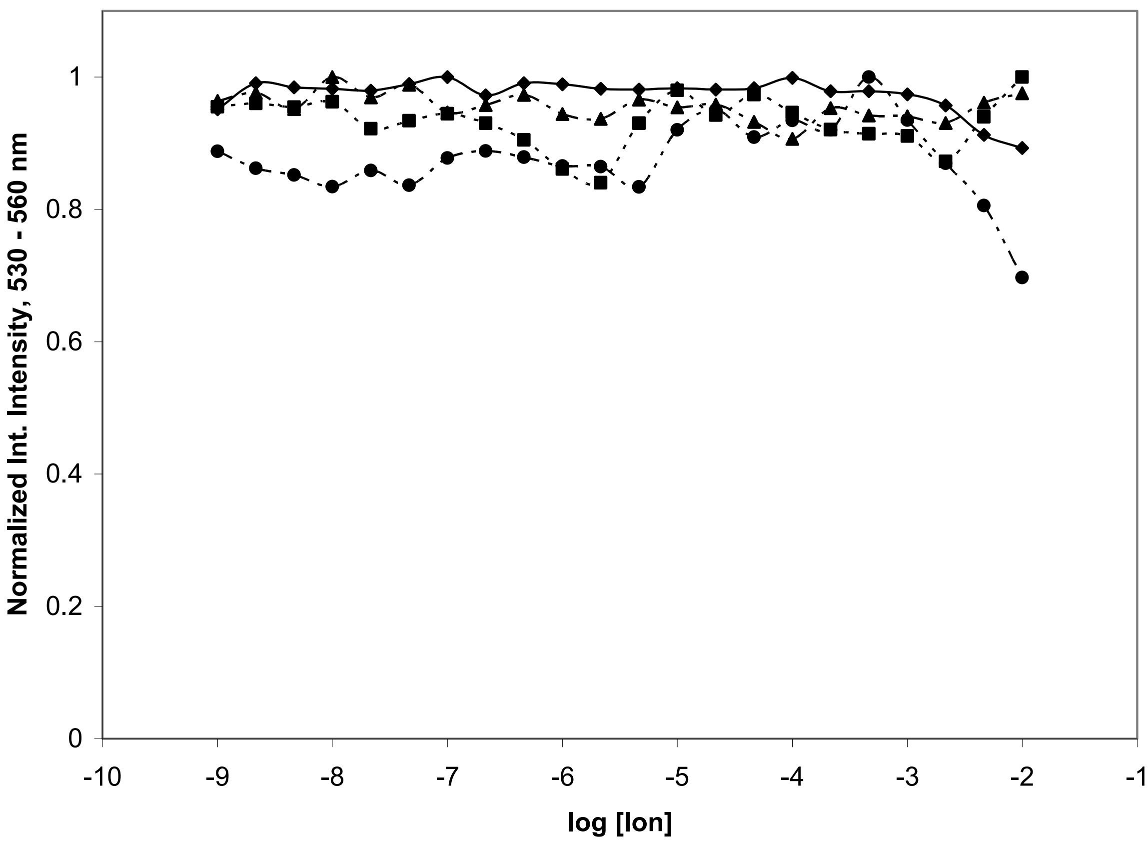

Figure S14. Ion competition experiment of 0.1 µM Tb(DO2A)(DPA)$^-$ titrated with phosphate (♦), sulfate (■), potassium (▲) or carbonate (●) over a concentration range from 1.0 nM to 100 mM, pH 7.5 (0.1 M MOPS). Carbonate appears to be the only ion that competes, and only at very high concentrations (1:10^5 [Tb(DO2A)(DPA)$^-$] : [CO_3^{2-}]).

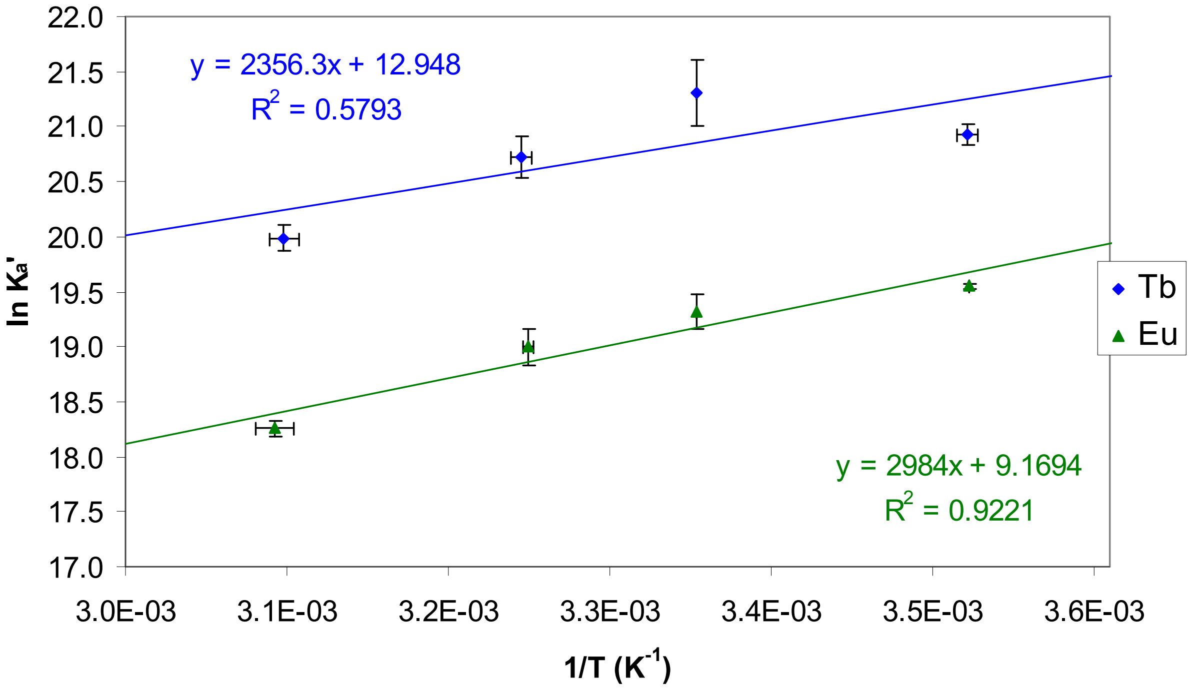

Figure S15. Plot of ln K_a' versus 1/T for $Tb(DO2A)(DPA)^-$ (blue) and $Eu(DO2A)(DPA)^-$ (green), 200mM NaAc, pH 7.4. Calculations of enthalpy and entropy for each complex give the following: ΔH_{Tb} = -1960 J, ΔS_{Tb} = 108 J·K^{-1}. ΔH_{Eu} = -2480 J, ΔS_{Eu} = 76 J·K^{-1}.

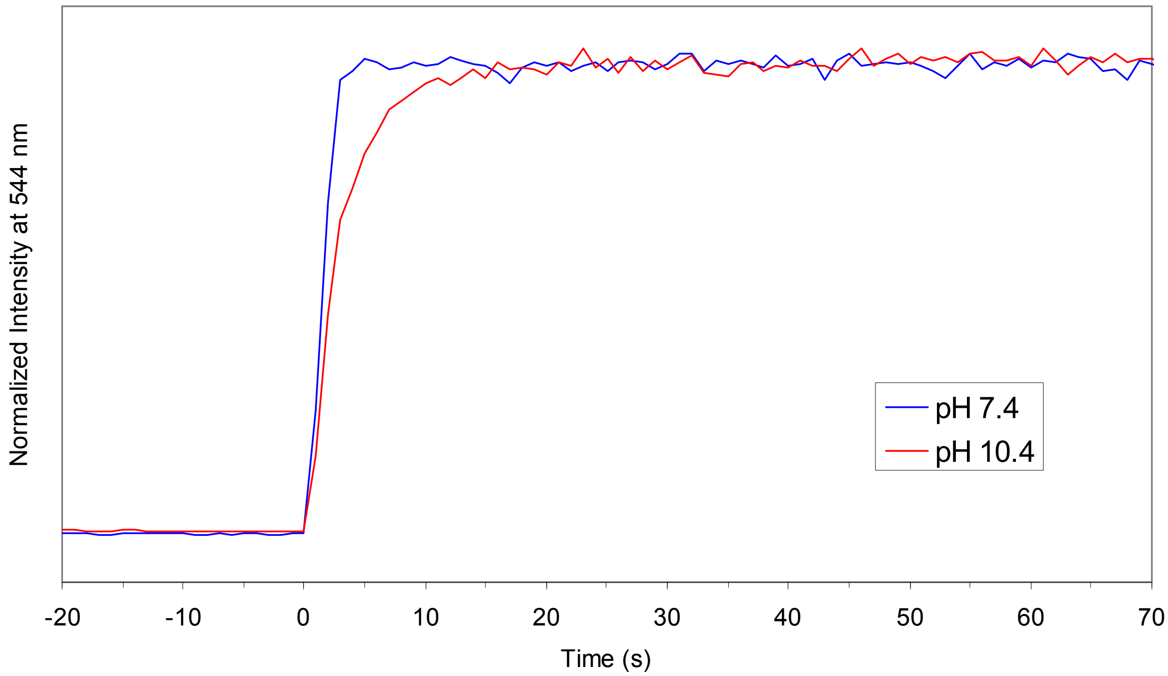

Figure S16. Time course of addition of 1.0 μM DPA to 1.0 μM $Tb(DO2A)^+$ in 0.1 M MOPS buffer (pH 7.4) and 0.1 M CAPS buffer (pH 10.4). Emission intensity was monitored at 544 nm (λ_{ex} = 278 nm) before, during and after DPA addition (T = 0). Complete $Tb(DO2A)(DPA)^-$ formation was observed after approx. 3 sec at pH 7.4, 15 sec at pH 10.4.

Calculation of Signal-to-Noise Ratio for Bacterial Spore Detection Study.

In spectroscopy, the signal-to-noise (S/N) ratio is defined as the ratio of the amplitude of the desired signal to the amplitude of noise signals. To calculate the S/N ratio for our bacterial spore detection experiment, we use the most intense peak of the Tb emission spectrum (λ_{em} = 544 nm). Signal amplitude was calculated by subtracting the maximum observed intensity in the range of 530 – 560 nm from the minimum observed intensity.

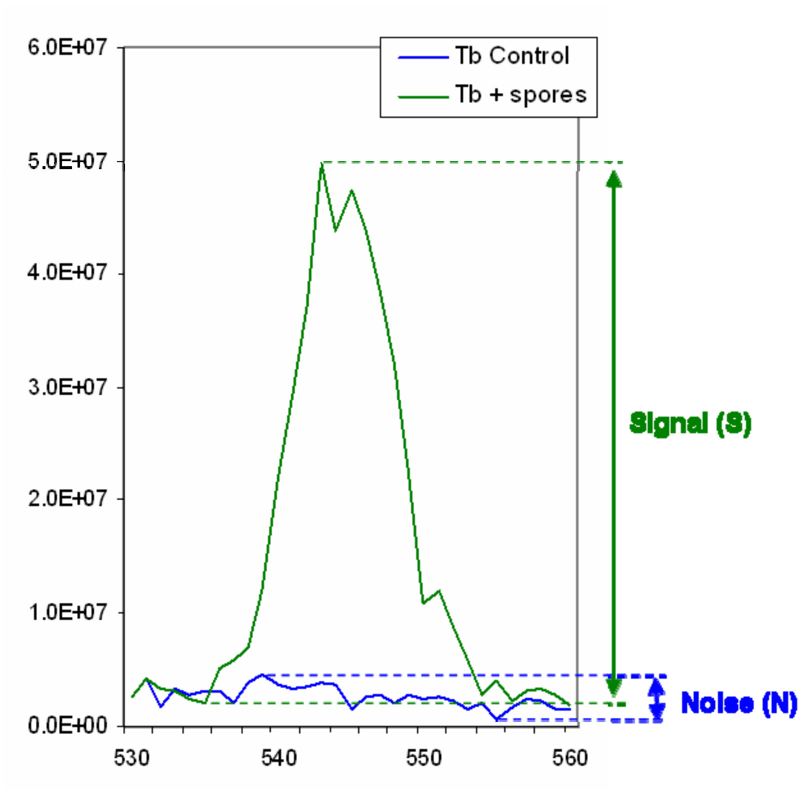

Figure S17. Example of signal and noise amplitude measurements from emission spectrum, 530 – 560 nm.

This was performed both for the sample, which contained the lysed bacterial spores and either the Tb^{3+} or $Tb(DO2A)^{+}$ complex, and for the analogous control, containing either Tb^{3+} or $Tb(DO2A)^{+}$ alone. The ratio of these two amplitudes produced the S/N ratio:

$$S/N = \frac{Max_{Sample} - Min_{Sample}}{Max_{Control} - Min_{Control}}$$

The calculated S/N ratios for each of the five trials were averaged to produce the final values for the Tb^{3+} and $Tb(DO2A)^{+}$ complexes (see Table S3).

Table S3. Calculated S/N ratios for the bacterial spore detection study

Trial	Tb^{3+}	$Tb(DO2A)^+$
1	12.8	41.1
2	14.2	38.8
3	11.6	57.8
4	15.8	37.1
5	15.9	51.2
Avg	14.1 ± 1.9	45.2 ± 8.9