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 Ln³⁺ is any lanthanide, and which has the corresponding equilibrium expression written in [2].

\[
\text{Ln}^{3+} + \text{DPA}^{2-} \rightleftharpoons \text{Ln}^{3+}(\text{DPA})^{+} \tag{1}
\]

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

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

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

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

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

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

\[
[\text{DPA}^{2-}]_{\text{eq}} = C_{DPA} - [\text{Ln(DPA)}^{+}]_{\text{eq}} \tag{6}
\]
Substituting equations [5] and [6] into equation [2], we have equation [7].

\[ K_a = \frac{[\text{LN(DPA)}^+]_{eq}}{(C_{Ln} - [\text{LN(DPA)}^+]_{eq})(C_{DPA} - [\text{LN(DPA)}^+]_{eq})} \]  

[7]

Rearranging, we have equation [8].

\[ K_a = \frac{[\text{LN(DPA)}^+]_{eq}}{C_{Ln}C_{DPA} - [\text{LN(DPA)}^+]_{eq}C_{DPA} - [\text{LN(DPA)}^+]_{eq}C_{Ln} + ([\text{LN(DPA)}^+]_{eq})^2} \]  

[8]

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

\[ R = \frac{[\text{LN(DPA)}^+]_{eq}}{[\text{LN(DPA)}^+]_{eq} + ([DPA])^2_{eq}} \]  

[9]

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

\[ R = \frac{[\text{LN(DPA)}^+]_{eq}}{C_{DPA}} \]  

[10]

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

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

[11]

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

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

[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(CTb – RCDPA) vs log (R/(1 – R)) with slope set to unity and y-intercept corresponding to log Ka. 10.0 nM DPA titrated with TbCl3 in 0.2 M sodium acetate, pH 7.4, 24.5°C (λex = 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: $\lambda_{\text{Sm}} = 600$ nm, $\lambda_{\text{Eu}} = 615$ nm, $\lambda_{\text{Tb}} = 544$ nm, $\lambda_{\text{Dy}} = 574$ 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].

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

where

- $\Phi$ = Quantum yield
- $E$ = Fluorescence emission intensity
- $A$ = Absorbance at the excitation wavelength $\lambda$
- $I$ = Intensity of excitation light at wavelength $\lambda$
- $\eta$ = 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].

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

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

$$\Phi_X = \Phi_{ST} \cdot \frac{Grad_X}{Grad_{ST}} \cdot \frac{I_{ST}(\lambda_{ST})}{I_X(\lambda_X)} \cdot \frac{\eta_X^2}{\eta_{ST}^2}$$ \[3\]

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 ($\varepsilon_{Exp} = 5277.4 \text{ M}^{-1}\text{cm}^{-1}$, $\varepsilon_{Theor} = 5502 \text{ M}^{-1}\text{cm}^{-1}$).\[29\]
Molar extinction coefficients were also calculated for all four ternary complexes and the dipicolinate anion by plotting absorbance against concentration.

Table S1. Molar extinction coefficients of the Ln(DO2A)(DPA)- complexes (Ln = Sm, Eu, Tb, Dy) and the DPA2- anion.

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

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.

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

<table>
<thead>
<tr>
<th>Eq. Time</th>
<th>pH</th>
<th>log $K_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 days</td>
<td>7.5</td>
<td>9.25 ± 0.13$^§$</td>
</tr>
<tr>
<td>5 months</td>
<td>7.4</td>
<td>9.30 ± 0.19</td>
</tr>
<tr>
<td>11 months</td>
<td>7.5</td>
<td>9.12 ± 0.24</td>
</tr>
</tbody>
</table>

$^§$ Previous work.$^{27}$
Figure S6. Emission spectra of various terbium complexes, 10.0 μM in 0.2 M sodium acetate, pH 7.4 ($\lambda_{ex} = 278$ 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 ($\lambda_{ex} = 278$ nm).
Figure S8. Emission spectra of samarium complexes, 10.0 μM in 0.2 M sodium acetate, pH 7.4 ($\lambda_{ex} = 278$ 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; $\lambda_{ex} = 278$ 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; $\lambda_{ex} = 278$ 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; \( \lambda_{\text{ex}} = 278 \) 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; \( \lambda_{\text{ex}} = 278 \) 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:

$\Delta H_{\text{Tb}} = -1960$ J, $\Delta S_{\text{Tb}} = 108$ J·K$^{-1}$. $\Delta H_{\text{Eu}} = -2480$ J, $\Delta S_{\text{Eu}} = 76$ J·K$^{-1}$. 

$y = 2356.3x + 12.948$  
$R^2 = 0.5793$

$y = 2984x + 9.1694$  
$R^2 = 0.9221$
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 ($\lambda_{em} = 544$ nm). Signal amplitude was calculated by subtracting the maximum observed intensity in the range of 530 – 560 nm from the minimum observed intensity.

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:

$$\frac{S}{N} = \frac{\text{Max}_{\text{Sample}} - \text{Min}_{\text{Sample}}}{\text{Max}_{\text{Control}} - \text{Min}_{\text{Control}}}$$

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

<table>
<thead>
<tr>
<th>Trial</th>
<th>Tb(^{3+})</th>
<th>Tb(DO2A)(^{+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.8</td>
<td>41.1</td>
</tr>
<tr>
<td>2</td>
<td>14.2</td>
<td>38.8</td>
</tr>
<tr>
<td>3</td>
<td>11.6</td>
<td>57.8</td>
</tr>
<tr>
<td>4</td>
<td>15.8</td>
<td>37.1</td>
</tr>
<tr>
<td>5</td>
<td>15.9</td>
<td>51.2</td>
</tr>
</tbody>
</table>

Avg 14.1 ± 1.9 45.2 ± 8.9