# Bacterial Spore Detection by [$Tb^{3+}$(macrocycle)(dipicolinate)] Luminescence

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## Supporting Information

**Experimental Section.**

**Materials.** DPA (dipicolinic acid) was purchased from Sigma-Aldrich and used as received. $TbCl_3$ was purchased from Alfa Aesar and used as received. DO2A (*1*,*4*,*7*,*10*-tetraazacyclododecane-*1*,*7*-diacetate) was obtained after hydrolysis (see reference 11) of DO2A-*t*-Bu-ester (*1*,*4*,*7*,*10*-tetraazacyclododecane-*1*,*7*-di(*t*-butyl acetate)), purchased from Macrocyclics. DO2A•2.80HCl•1.00$H_2O$. Anal. Calcd (found) for $C_{12}H_{24}N_4O_4$•2.80HCl•1.00$H_2O$ (fw = 408.45): C, 35.29 (35.36); H, 6.42 (6.89); N, 13.72 (13.80); Cl, 24.30 (24.00).

**Synthesis of [Tb(DO2A)(DPA)]$^-$.** 0.1898 g (0.51 mmol) $TbCl_3$•6$H_2O$ and 0.2074 g (0.51 mmol) DO2A•2.80HCl•1.00$H_2O$ were dissolved in distilled deionized water. 1 molar equivalent of DPA was added to this solution, and the pH was adjusted to 8.5 with 10.63 mL (3.22 mmol) of 10% tetrabutylammonium hydroxide (TBAOH) in isopropanol. The orange mixture was filtered, freeze-dried, and resuspended in 20.0 mL of filtered acetone. The mixture was then centrifuged at 8000 rpm (25°C) for 20 min, and the transparent orange solution was decanted and filtered through a fine frit. Colorless crystals were obtained after slow evaporation overnight. Suitable crystals were collected for the X-ray diffraction study.

**Elemental Analysis of [Tb(DO2A)(DPA)]$^-$.** 10.30 mg of crystals, dried over $P_2O_5$ under vacuum for 3 days, were sent to Desert Analytics, Inc. for elemental analysis in duplicate. Anal. Calcd (found) for $Tb_1C_{35}H_{61}N_6O_8$•1.00$C_3H_6O$ •4.00$H_2O$ (fw = 982.97): C, 46.43 (46.63); H, 7.69 (8.17); N, 8.55 (8.71); Tb, 16.17 (15.65).

**X-ray crystallographic characterization of [Tb(DO2A)(DPA)]$^-$.** A clear colorless crystal of TBA•Tb(DO2A)(DPA) (0.35 x 0.31 x 0.11 $mm^3$) was secured on a glass fiber with Dow-Corning

grease. Data were collected at 100 ± 2 K on a Bruker SMART 1000 CCD area detector diffractometer equipped with graphite monochromated MoKα radiation (λα = 0.710 73 Å). The structure was solved by direct methods and refined by full-matrix least-squares calculations on $F^2$ (Bruker XS v6.12). Non-hydrogen atoms were refined anisotropically. The hydrogen atoms were introduced in calculated positions. Crystallographic data have been deposited at the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK and copies can be obtained on request, free of charge, by quoting the publication citation and the deposition number 629354.

**Quantum yield measurements.** The experiments were performed on a Jobin Yvon Fluorolog fluorescence spectrometer at 298 K. Measurements of Tb samples in aqueous solution were carried out at pH 5.5 and $\lambda_{ex}$ = 270 nm. An aqueous solution of $Ru(bpy)_3Cl_2$ ($\lambda_{ex}$ = 452 nm; $\Phi_{ref}$ = 0.042) was used as a reference. All emission signals were recorded in the S/R mode and the absorption intensities of all samples and standards at their corresponding $\lambda_{ex}$ were within the same range of 0.02. The measured spectroscopic information was used to calculate the emission quantum yield (with a 15% error) based on the following expression:

$$\Phi_{em} = \frac{\Phi_{ref} \times \eta^2 \times \int F(\nu)d\nu \times A_{ref}}{\eta_{ref}^2 \times A \times \int F_{ref}(\nu)d\nu}$$

where $\eta$ = refractive index of solvent

$\int F(\nu)d\nu$ = integrated fluorescence intensity ($cm^{-1}$)

A = absorbance

**Detail of X-ray crystallographic analysis**

**Table S1**. Summary of Important Crystal Parameters for [TBA][Tb(DO2A)(DPA)]

| | |
|---|---|
| Chemical formula | $[C_{19}H_{25}N_5O_8Tb]^-[C_{16}H_{36}N]^+ \bullet 0.47(C_3H_8O)$ $0.53(C_3H_6O)$ $3(H_2O)$ |
| Formula weight | 964.94 |
| Temperature | 100(2) K |
| Wavelength | 0.71073 Å MoKα |
| Crystal system | Monoclinic |
| Space group | $P2_1/c$ |
| Unit cell dimensions | $a$ = 13.1047(5) Å |
| | $b$ = 13.3397(5) Å  β = 90.0130(10)° |
| | $c$ = 26.0901(9) Å |
| Volume | 4560.9(3) $Å^3$ |
| $Z$ | 4 |
| Density (calculated) | 1.405 $Mg/cm^3$ |
| Absorption coefficient | 1.613 $mm^{-1}$ |
| Crystal size | 0.35 x 0.31 x 0.11 $mm^3$ |
| Reflections collected | 78773 |
| Independent reflections | 20316 [$R_{int}$ = 0.0879] |
| Data/restraints/parameters | 20316 / 0 / 577 |
| Goodness-of-fit on $F^2$ | 1.077 |
| Observed reflections [$I > 4\sigma(I)$] | 11252 |
| Final $R$ indices [$I > 4\sigma(I)$] | R1 = 0.0384, wR2[a] = 0.0639 |
| $R$ indices (all data) | R1 = 0.0900, wR2[a] = 0.0717 |

[a] $R1 = \Sigma[(F_o - F_c)]/ \Sigma (F_o)$. $wR2 = \{ \Sigma [w(F_o^2 - F_c^2)^2/ \Sigma [w(F_o^2)^2]\}^{1/2}$. $w = 1/[\sigma^2(F_o^2) + (0.0990P)^2]$, where $P = (F_o^2 + 2F_c^2)/3$.

**Table S2**. Selected interatomic distances (Å) for $[Tb(DO2A)(DPA)]^-$

| | |
|---|---|
| Tb-N1 | 2.4918(19) |
| Tb-N2 | 2.6437(17) |
| Tb-N3 | 2.5657(18) |
| Tb-N4 | 2.6550(18) |
| Tb-N5 | 2.5564(18) |
| Tb-O1 | 2.3879(15) |
| Tb-O3 | 2.3987(15) |
| Tb-O5 | 2.3573(15) |
| Tb-O7 | 2.3467(14) |

**Table S3**. Selected interatomic angles (º) for $[Tb(DO2A)(DPA)]^-$

| | |
|---|---|
| N1-Tb-O1 | 64.54(5) |
| N1-Tb-O3 | 64.78(6) |
| N1-Tb-O5 | 71.66(5) |
| N1-Tb-O7 | 72.84(5) |
| N1-Tb-N2 | 124.89(5) |
| N1-Tb-N3 | 129.99(6) |
| N1-Tb-N4 | 126.78(5) |
| N1-Tb-N5 | 129.68(6) |

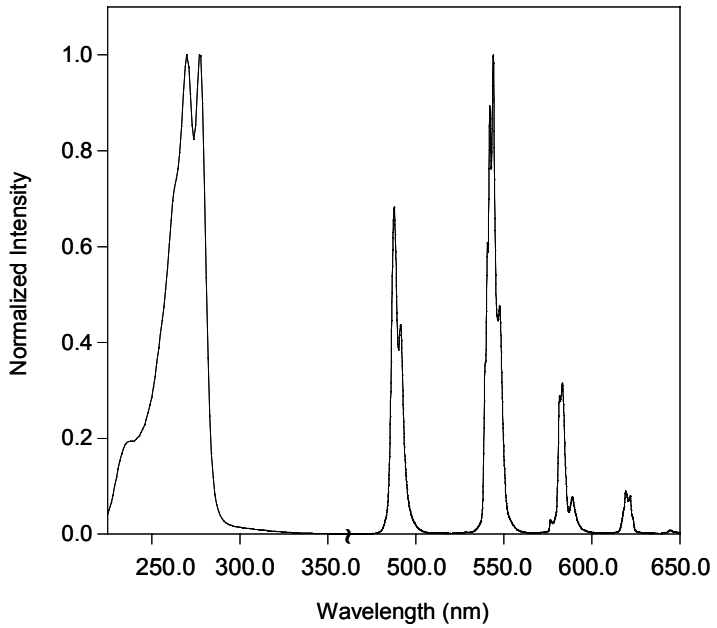


**Figure S1**. Excitation and emission spectra of the $[Tb(DO2A)(DPA)]^-$ ternary complex at 100 μM, pH 10. Excitation wavelength: 278 nm; emission wavelength: 544 nm.

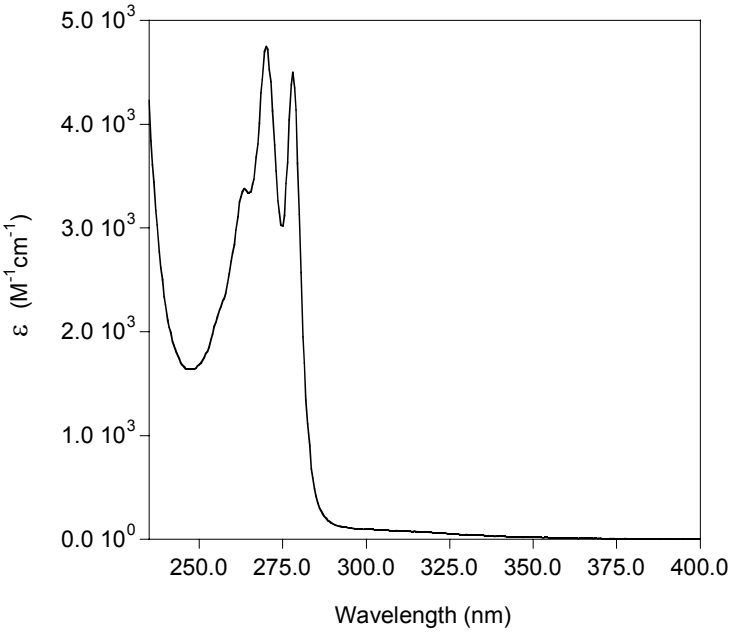


**Figure S2**. UV absorbance spectrum of the $[Tb(DO2A)(DPA)]^-$ ternary complex at 100 μM, pH 10.

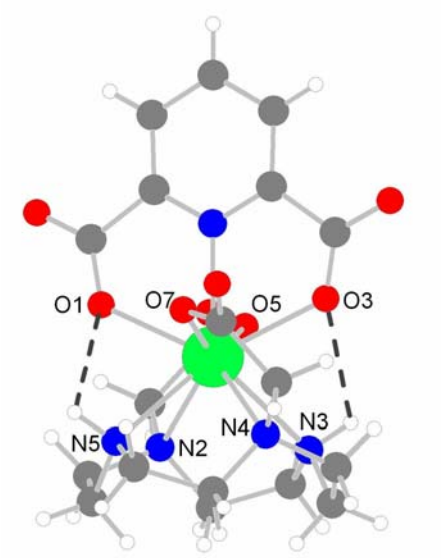


**Figure S3**. Ball and stick model of the $[Tb(DO2A)(DPA)]^-$ ternary complex. Dashed lines indicate the two putative interligand hydrogen bonds with bond lengths of 2.75 Å (left) and 2.76 Å (right).

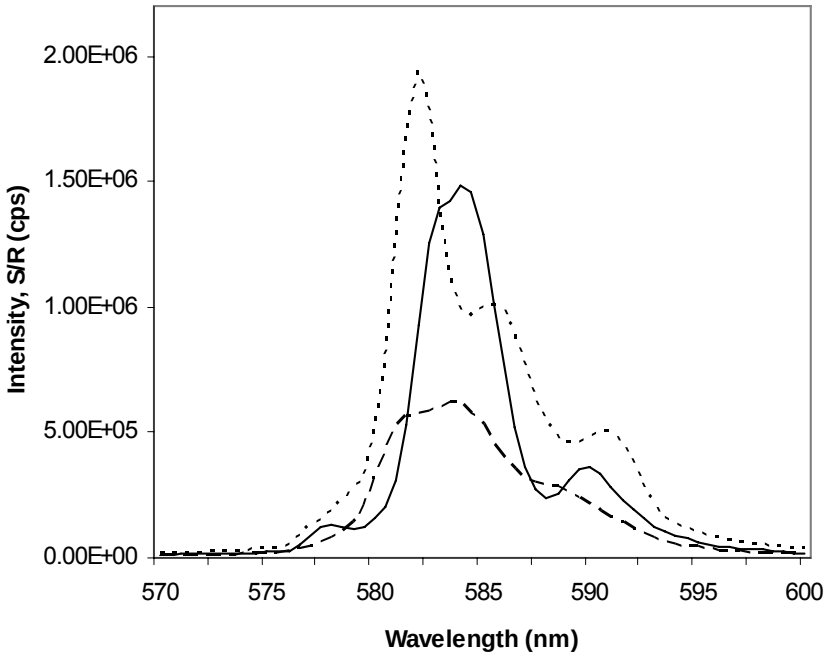


**Figure S4**. Emission spectra of the $^5D_4 \rightarrow {}^7F_4$ transition spectroscopic handle (583 nm) for the $[Tb(DO2A)(DPA)]^-$ (solid line), $[Tb(DPA)\cdot 6H_2O]^+$ (dashed line) and $[Tb(DPA)_3]^{3-}$ (dotted line) complexes at 10 μM in $H_2O$, with the $[Tb(DO2A)(DPA)]^-$ in 1 mM NaOH (pH 10).

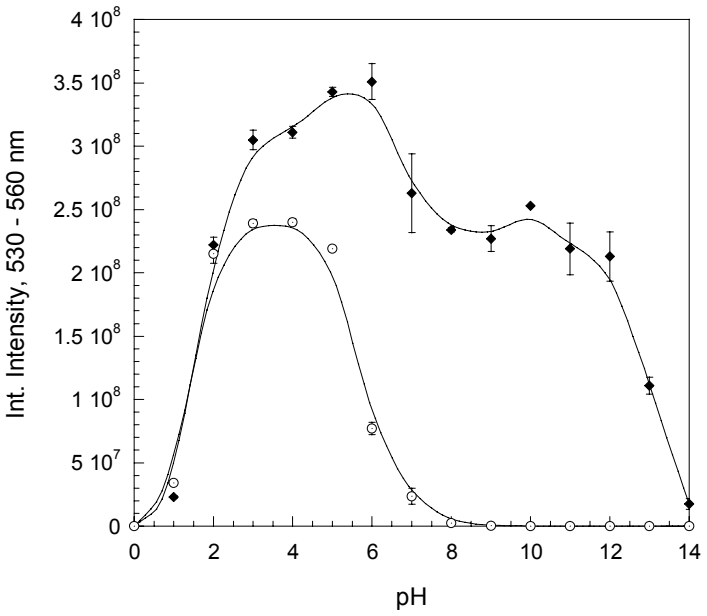


Figure S5. Dependence of luminescence intensity on pH for the $[Tb(DO2A)(DPA)]^-$ (♦) and $[Tb(DPA)\cdot 6H_2O]^+$ (○) complexes at 10 μM. The pH was adjusted with HCl or NaOH.

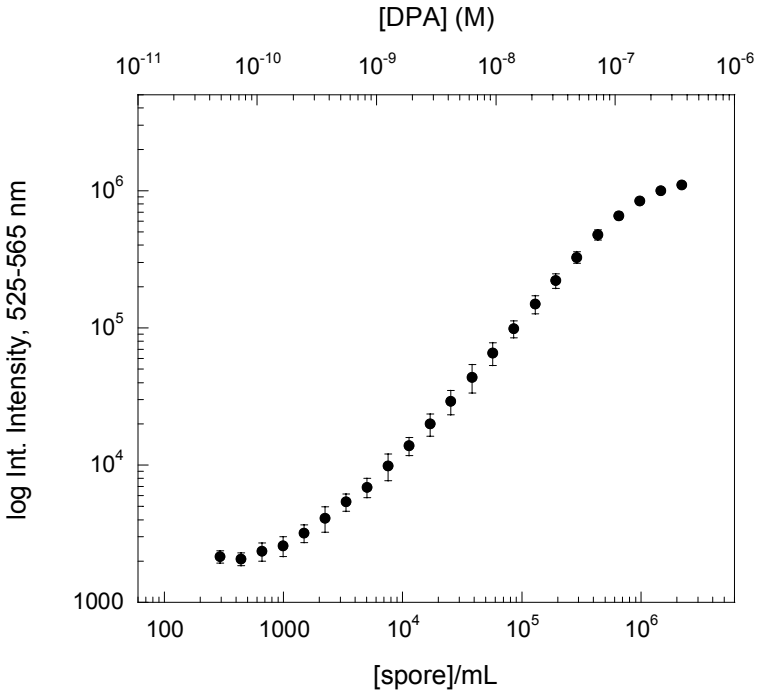


Figure S6. Serial dilution of 2.2 x $10^6$ spores/mL (370 nM DPA) from *Bacillus atrophaeus* (ATCC 9372) spores with 1 μM $[Tb(DO2A)^+]$ in 1 mM NaOH, pH 10. (Shafaat, H.; Cable, M. L.; Ponce, A., unpublished results).

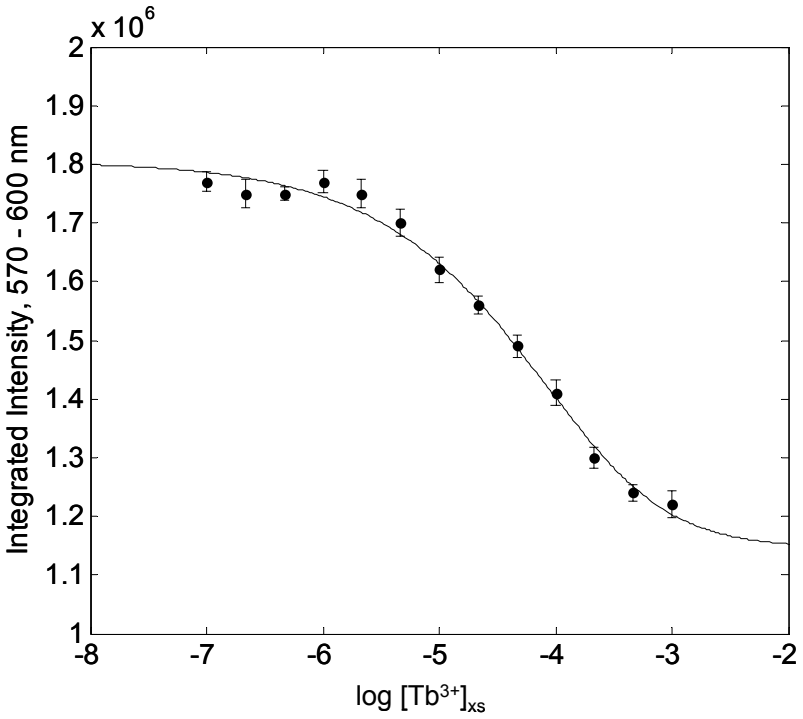


Figure S7. Titration of 1 μM $[Tb(DO2A)(DPA)]^-$ with excess $Tb^{3+}$, pH 7.4. Data was fit using the following equation:

$$I_{obs} = \left(1 - \frac{[Tb(DPA)]_{eq}}{[Tb(DO2A)(DPA)]_T}\right) I_{max} + \left(\frac{[Tb(DPA)]_{eq}}{[Tb(DO2A)(DPA)]_T}\right) I_{min}$$

From this fit, $[Tb^{3+}]_{xs}$ at the equivalence point was determined, and the binding constant, K, calculated from the following:

$$K = \frac{K_1 \{[Tb(DO2A)(DPA)^-]_{eq} ([Tb^{3+}]_T - [Tb(DO2A)(DPA)^-]_{eq} - 2[Tb(DPA)^+]_{eq})\}}{([Tb(DPA)^+]_{eq})^2}$$

where $K_1 = 10^{8.68}$ $M^{-1}$ (see reference 16)