Spectroscopic Analysis of Ligand Binding to Lanthanide-Macrocycle Platforms

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Supporting Information

Materials. MES monohydrate (*2*-(N-morpholino)ethanesulfonic acid monohydrate), MOPS (*3*-(N-morpholino)-propanesulfonic acid), CHES (N-cyclohexyl-*2*-aminoethanesulfonic acid) and CAPS (N-cyclohexyl-*3*-aminopropanesulfonic acid) buffers were purchased from Alfa Aesar. TAPS (N-tris(hydroxymethyl)methyl-*3*-aminopropanesulfonic acid) buffer was purchased from TCI America. All buffers were greater than 98% pure.

Methods. Unless otherwise specified, all samples were prepared to a final volume of 4.00 mL in disposable acrylate cuvettes (Spectrocell®), 1 cm pathlength, and were allowed to equilibrate for at least 4 days before analysis using a Fluorolog-3 Fluorescence Spectrometer (Horiba Jobin-Yvon).

Jobs Method of Continuous Variations. From 4.00 mM stock solutions of Eu^{3+}, DPA and DO2A, samples were prepared in triplicate with the concentrations of Eu^{3+} and DPA varied inversely from 0 to 12.0 μM in 1.0 μM increments, with 100 μM DO2A in 100 mM buffer. Five buffers were used: MES ($pK_a = 6.1$), MOPS ($pK_a = 7.2$), TAPS ($pK_a = 8.4$), CHES ($pK_a = 9.3$)

and CAPS (pK_a = 10.4), with pHs adjusted to within 0.1 of the pK_a value using 50% NaOH added dropwise.

pH Dependence Study. From 4.00 mM stock solutions of Eu^{3+}, DPA and DO2A, samples were prepared in triplicate to contain 10.0 μM Eu(DO2A)(DPA)⁻ in 100 mM buffer. Five buffers were used: MES (pK_a = 6.1), MOPS (pK_a = 7.2), TAPS (pK_a = 8.4), CHES (pK_a = 9.3) and CAPS (pK_a = 10.4), with pHs adjusted to within 0.1 of the pK_a value using 50% NaOH added dropwise.

Calculation of K_a. The titrimetric method developed by Jones and Vullev[12] was employed to calculate K_a, the binding affinity of Eu^{3+} for DPA^{2-}. Samples were prepared in triplicate in 200 mM sodium acetate, pH 7.4, such that the concentration of Eu^{3+} varied from 10.0 nM to 40.0 μM while the concentration of DPA^{2-} was maintained at 10.0 nM. The normalized integrated luminescence intensity (600-630 nm) was fit according to the following model:

$$\log\left(\frac{R}{C_{Tb}}\right) = \log(1-R) + \log K_a$$
$$R = \frac{[EuDPA]_{eq}}{[EuDPA]_{eq} + [DPA]_{eq}} \tag{S1}$$

A linear fit was performed for each trial and subsequently averaged to produce the final slope and y-intercept.

Results

In order to verify binding stoichiometries for the various complexes over our pH range of interest, a method of continuous variations was employed, where the concentrations of Eu^{3+} and DPA^{2-} were varied inversely to obtain a range of ratios. In the presence of the DO2A ligand, we see formation of the ternary complex in all cases where $[Eu^{3+}] \geq [DPA^{2-}]$, and when the pH is above 7.2, the ternary complex is formed preferentially even when $[Eu^{3+}] << [DPA^{2-}]$ (see Figure S1). These results are corroborated by a pH dependence study (Figure S2), indicating that the ternary complex is stable throughout the pH range of 6.1 to 10.3, while the free Eu^{3+} ion without DO2A precipitates above pH 8.4 as $Eu(OH)_3$.

To obtain the analogous binding constant K_a necessary to calculate the association constant K_a' from the competition equilibrium constant K_c, a titration of DPA^{-2} with Eu^{3+} was performed and fit to a model developed previously for the $Tb(DPA)^+$ system (see Figure S3).[12] The slope of equation S1, which corresponds to the number of DPA molecules bound per Eu^{3+} ion, was calculated to be 0.971, and the y-intercept produced a value of log $K_a = 7.40 \pm 0.03$.

Due to the ubiquity of the method, we performed this BAC assay in four different buffers to determine the dependence of our competition association constant (K_c) on pH (Figure S4). Over the pH range analyzed (6.1 − 8.0), we found that the affinity of the $Eu(DO2A)^+$ binary complex for the DPA^{2-} analyte increases with increasing bascicity by nearly an order of magnitude (Table S1). This result is consistent with the expected increase in stability of the $Eu(DO2A)^+$ binary complex, and is also advantageous in terms of preventing Eu^{3+} precipitation as $Eu(OH)_3$ if the DO2A ligand were not present. In fact, we would have liked to conduct further experiments at higher pH levels to determine the optimal K_c for this system, which according to our pH study may be around 9.0, but Eu^{3+} precipitation prevented experimentation

over a pH of 8.0. Regardless, the association constants calculated using the lanthanide

competition experiment indicate both that the Eu(DO2A)$^+$ binary complex is an effective

receptor of DPA^{2-} at physiological pH, and that the use of the DO2A ligand increases the pH

range significantly over which bacterial spore detection assays may be used.
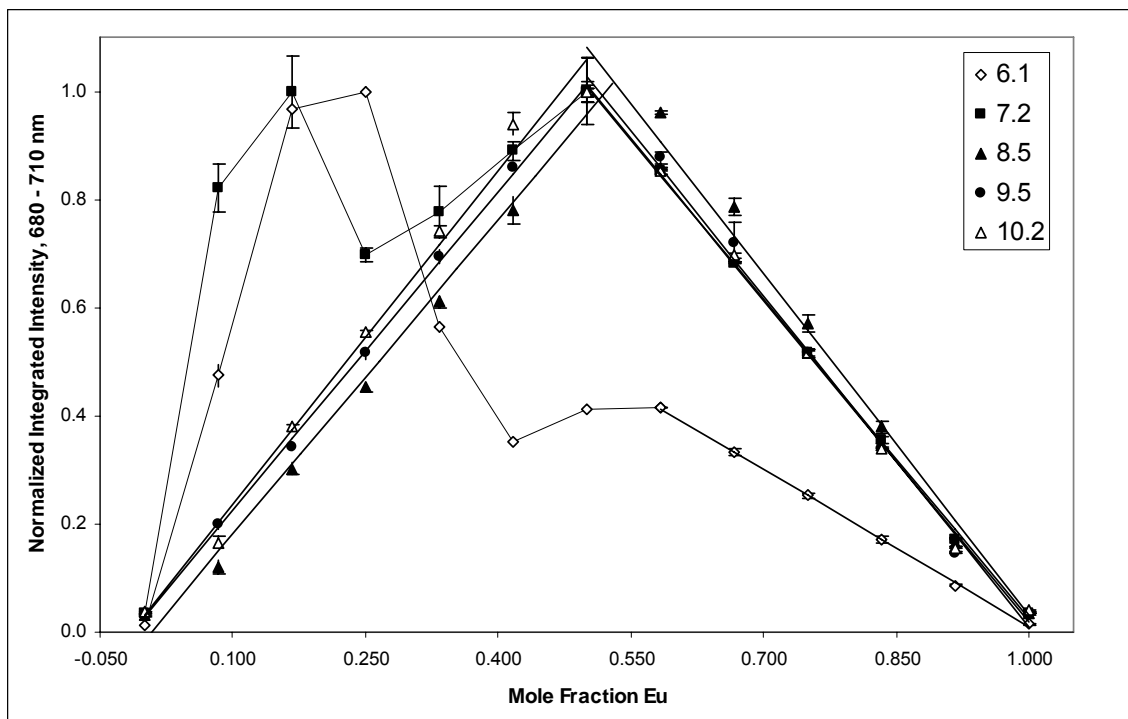

Figure S1. Binding stoichiometries of Eu^{3+} and DPA^{2-} in excess of DO2A at various pH values, using a method of continuous variations. The concentrations of Eu^{3+} and DPA^{2-} were varied inversely from 0 – 12.0 μM with 100 μM DO2A in 100 mM buffer.
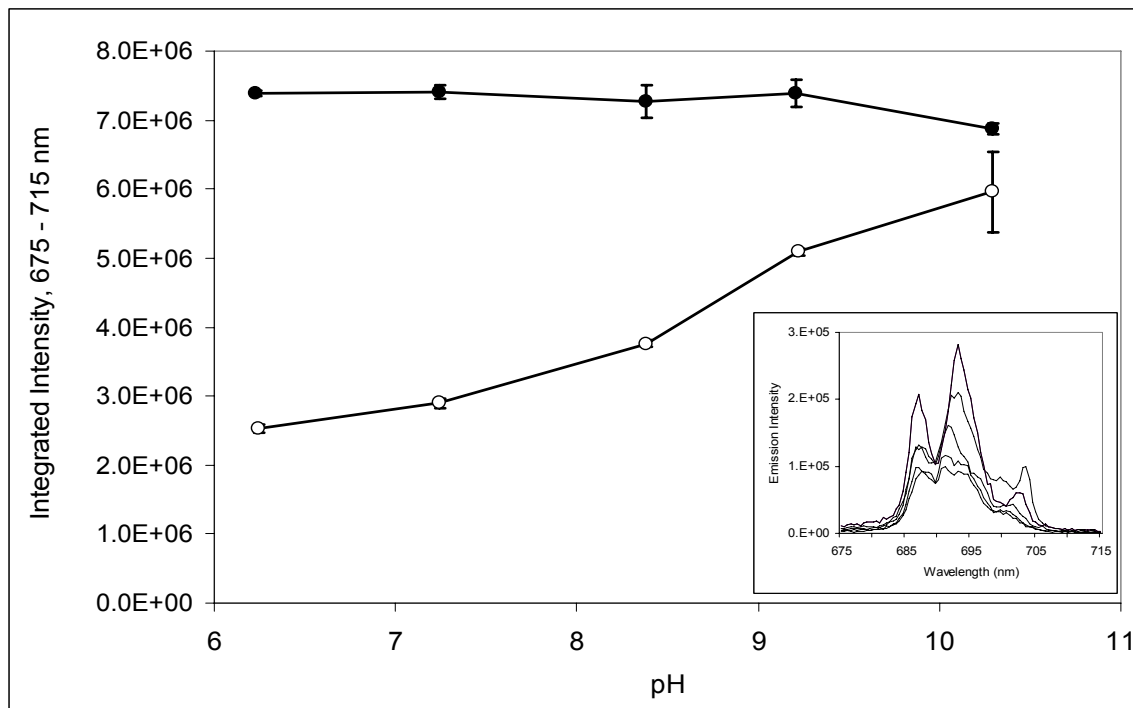
Figure S2. Relationship of emission intensity to pH of the Eu(DO2A)(DPA)⁻ ternary complex (●) and the mono-Eu(DPA)⁺ complex (○) at 10 µM in 100 mM buffer. Emission increase in the binary complex with pH is due to precipitation of Eu^{3+} as $Eu(OH)_3$ and formation of the more intense tris-$Eu(DPA)_3^{3-}$ species, as evidenced by the $^5D_0 \rightarrow {}^7F_3$ ligand field-sensitive peak (inset). Excitation wavelength: 278 nm; emission wavelength: 614 nm.
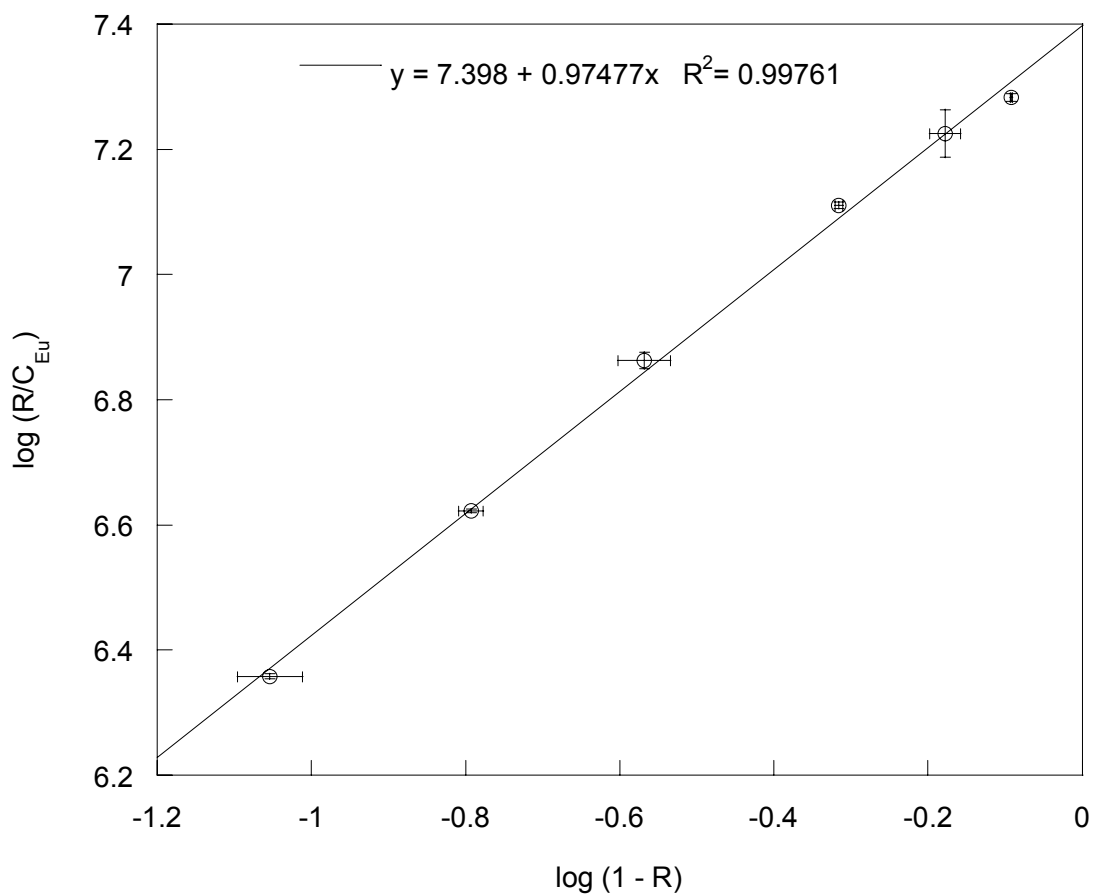
Figure S3. Calculation of K_a via titration of 10.0 nM DPA^{2-} with Eu^{3+} in 200 mM NaAc, pH 7.4. Slope corresponds to number of DPA molecules bound per Eu^{3+}; y-intercept corresponds to log K_a. Excitation wavelength: 278 nm.
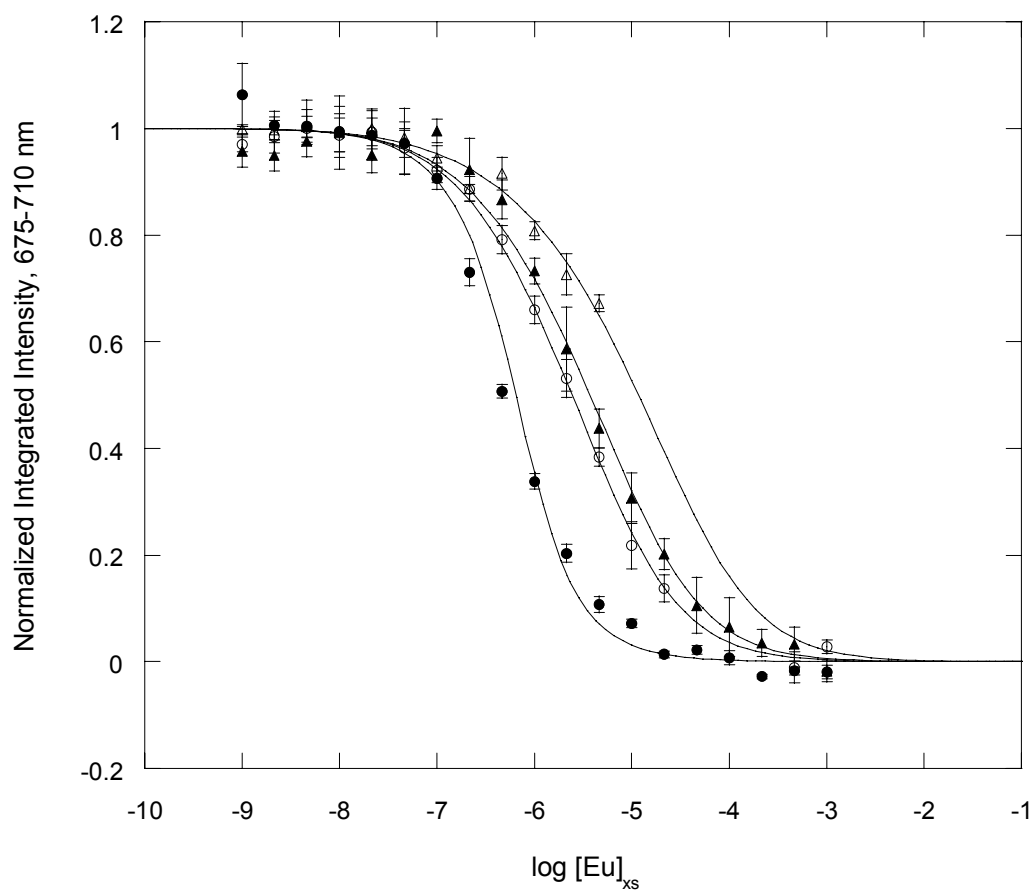
Figure S4. Lanthanide competition performed at pH 6.1 (●), 7.4 (○), 7.5 (▲) and 8.0 (△) to determine the dependence of the competition association constant (K_c) on pH. Initial concentration of Eu(DO2A)(DPA)$^-$ was 1.0 μM.

Table S1. Competition association constants for the equilibria between [Eu(DO2A)]$^+$ and DPA^{2-} at various pH levels.

pH	K_c
6.1	3.302
7.4	0.154
7.5	0.257
8.0	0.044

X-ray crystallographic characterization of TBA·Eu(DO2A)(DPA). A clear colorless

crystal of TBA·Eu(DO2A)(DPA) (0.34 x 0.13 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 = 0.71073

Å). The structure was solved by direct methods and refined by full-matrix least-squares

calculations on F^2 (SHELXL-97). 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 634507.

Detail of X-ray crystallographic analysis

Table S2. Summary of Important Crystal Parameters for TBA·Eu(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	957.23
Temperature	100(2) K
Wavelength	0.71073 Å MoKα
Crystal system	Triclinic
Space group	P1
Unit cell dimensions	$a = 13.1473(4)$ Å
	$b = 13.2269(4)$ Å $\beta = 90.0540(10)°$
	$c = 26.2248(8)$ Å
Volume	4560.4(2) Å^3
Z	4
Density (calculated)	1.394 Mg/cm^3
Absorption coefficient	1.438 mm^{-1}
Crystal size	0.34 x 0.13 x 0.11 mm^3
Reflections collected	126185
Independent reflections	27668 [$R_{int} = 0.1140$]
Data/restraints/parameters	27668 / 0 / 560
Goodness-of-fit on F^2	1.004
Observed reflections [$I > 4\sigma(I)$]	12878
Final R indices [$I > 4\sigma(I)$]	R1 = 0.0437, wR2 = 0.0750
R indices (all data)	R1 = 0.1187, wR2 = 0.0854

Table S3. Selected interatomic distances (Å) for Eu(DO2A)(DPA)$^-$

Eu-N1	2.5146(18)
Eu -N2	2.6711(18)
Eu -N3	2.5947(18)
Eu -N4	2.6692(18)
Eu -N5	2.5848(18)
Eu -O1	2.4038(15)
Eu -O3	2.4140(15)
Eu -O5	2.3801(14)
Eu -O7	2.3740(15)

Table S4. Selected interatomic angles (°) for [Tb(DO2A)(DPA)]⁻

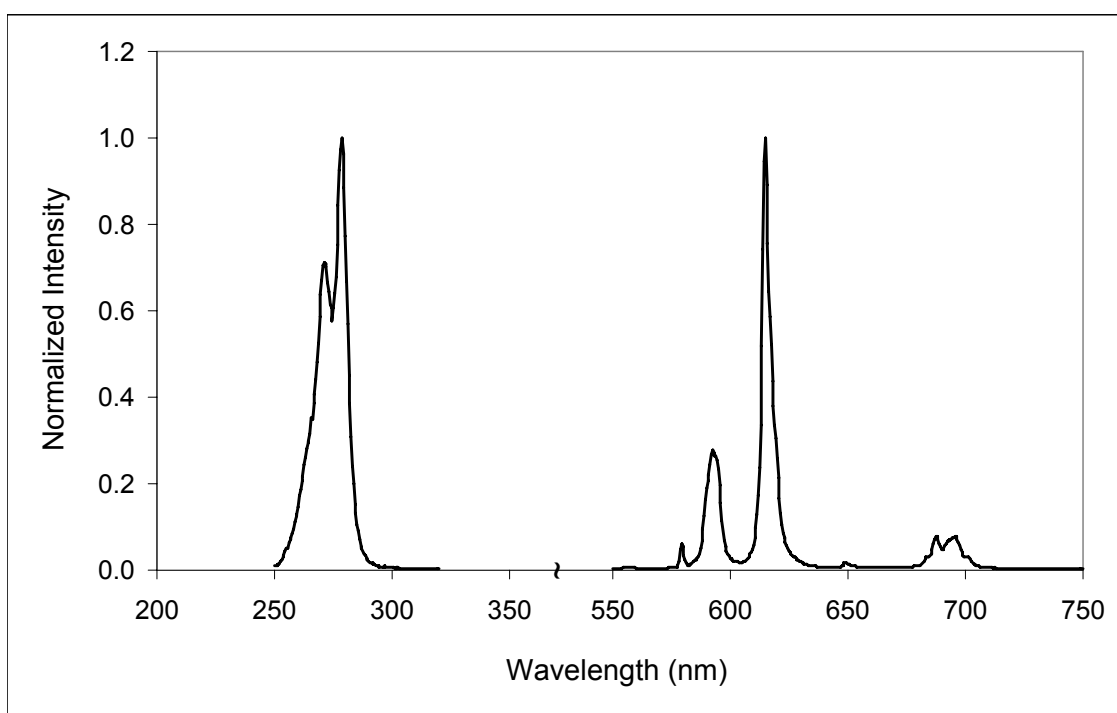|	
N1-Eu-O1	64.12(5)
N1-Eu-O3	64.18(5)
N1-Eu-O5	72.30(5)
N1-Eu-O7	73.56(5)
N1-Eu-N2	125.21(5)
N1-Eu-N3	130.00(6)
N1-Eu-N4	127.07(6)
N1-Eu-N5	130.32(6)

Figure S4. Excitation and emission spectra of the Eu(DO2A)(DPA)⁻ ternary complex at 10 µM, pH 7.4. Excitation wavelength: 278 nm; emission wavelength: 614 nm.