

Supplementary material for:

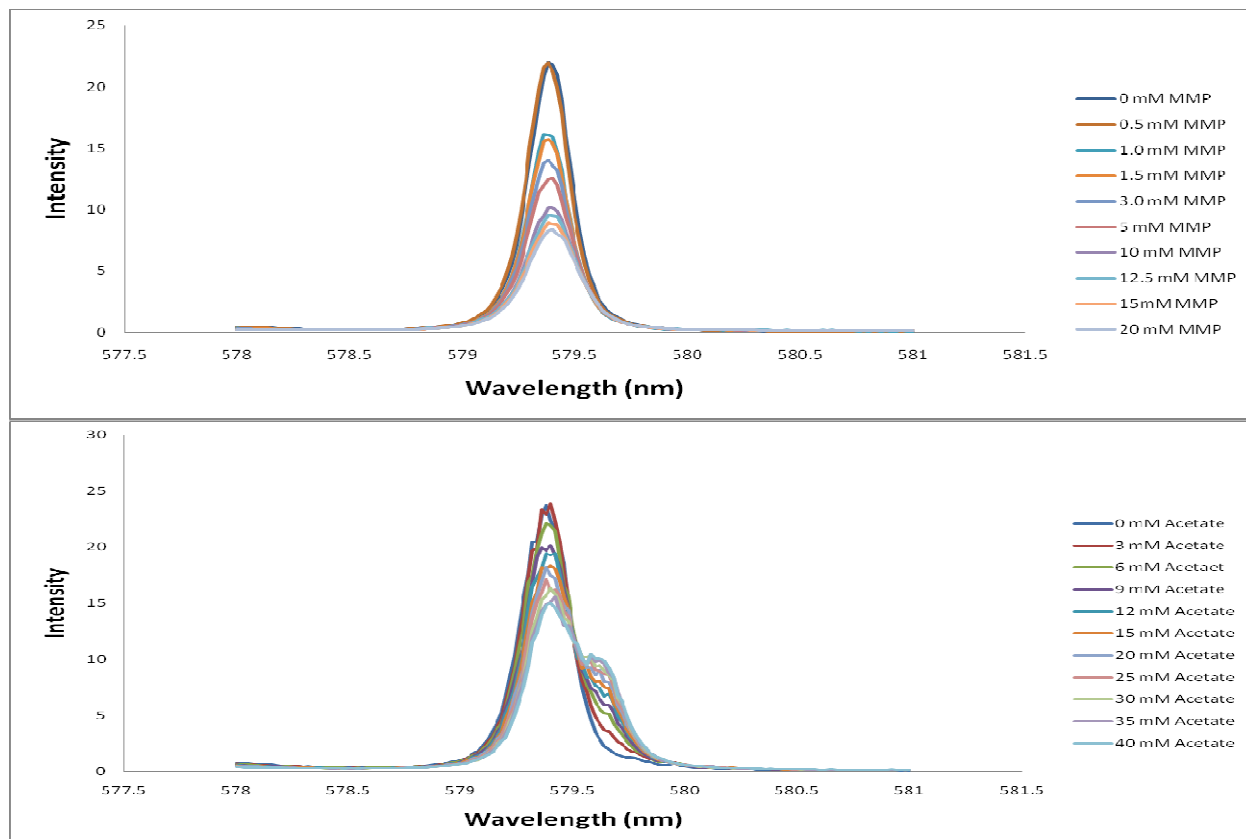
**Eu(III) complexes as Anion-responsive Luminescent Sensors and PARACEST**

**Agents**

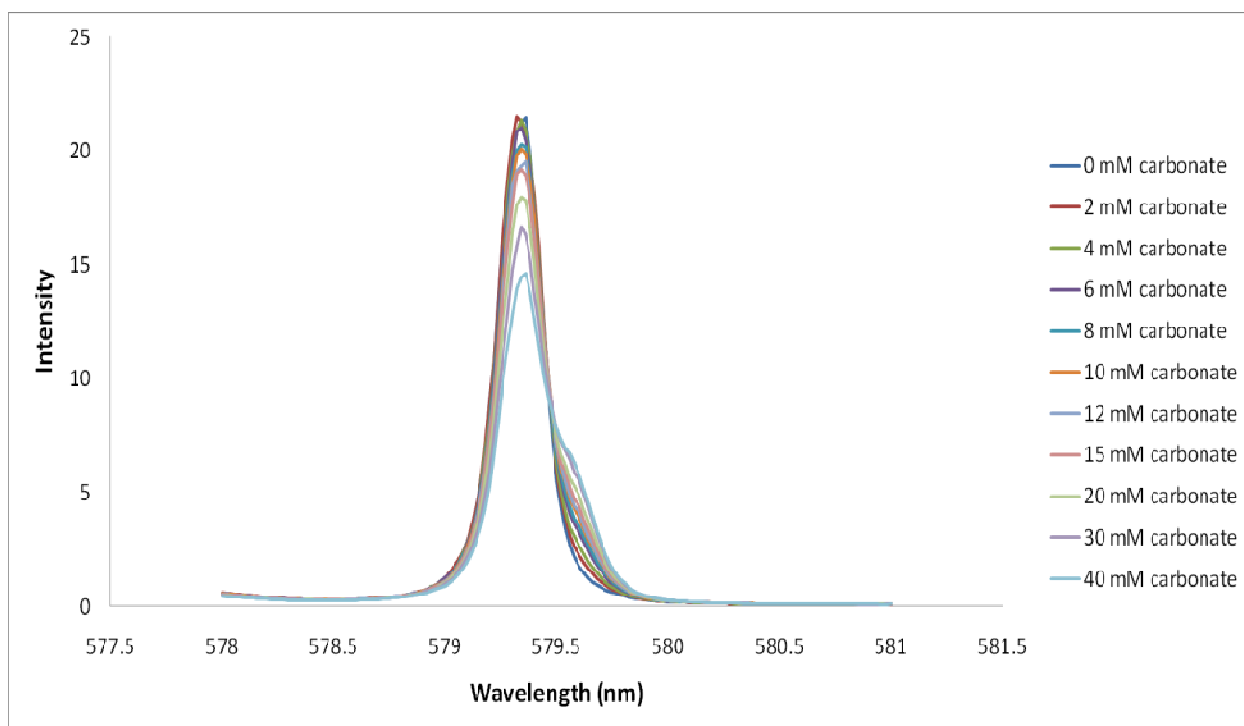
Jacob Hammell, Leandro Buttarazzi, Ching-Hui Huang and Janet R. Morrow\*

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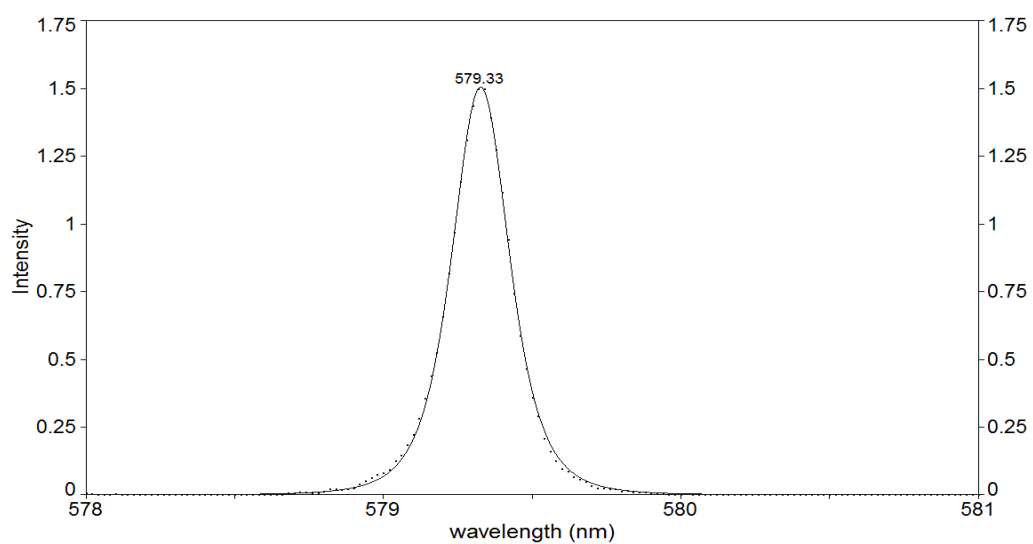
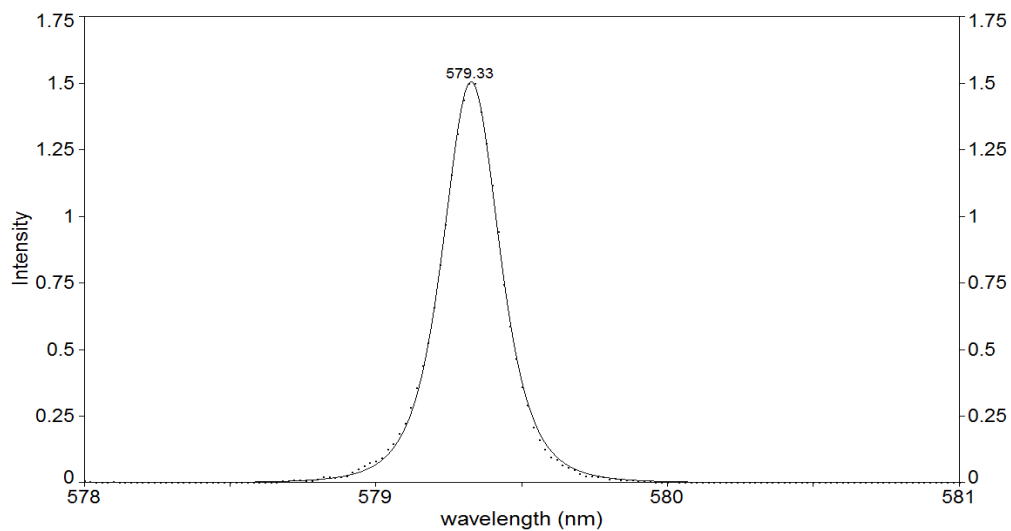
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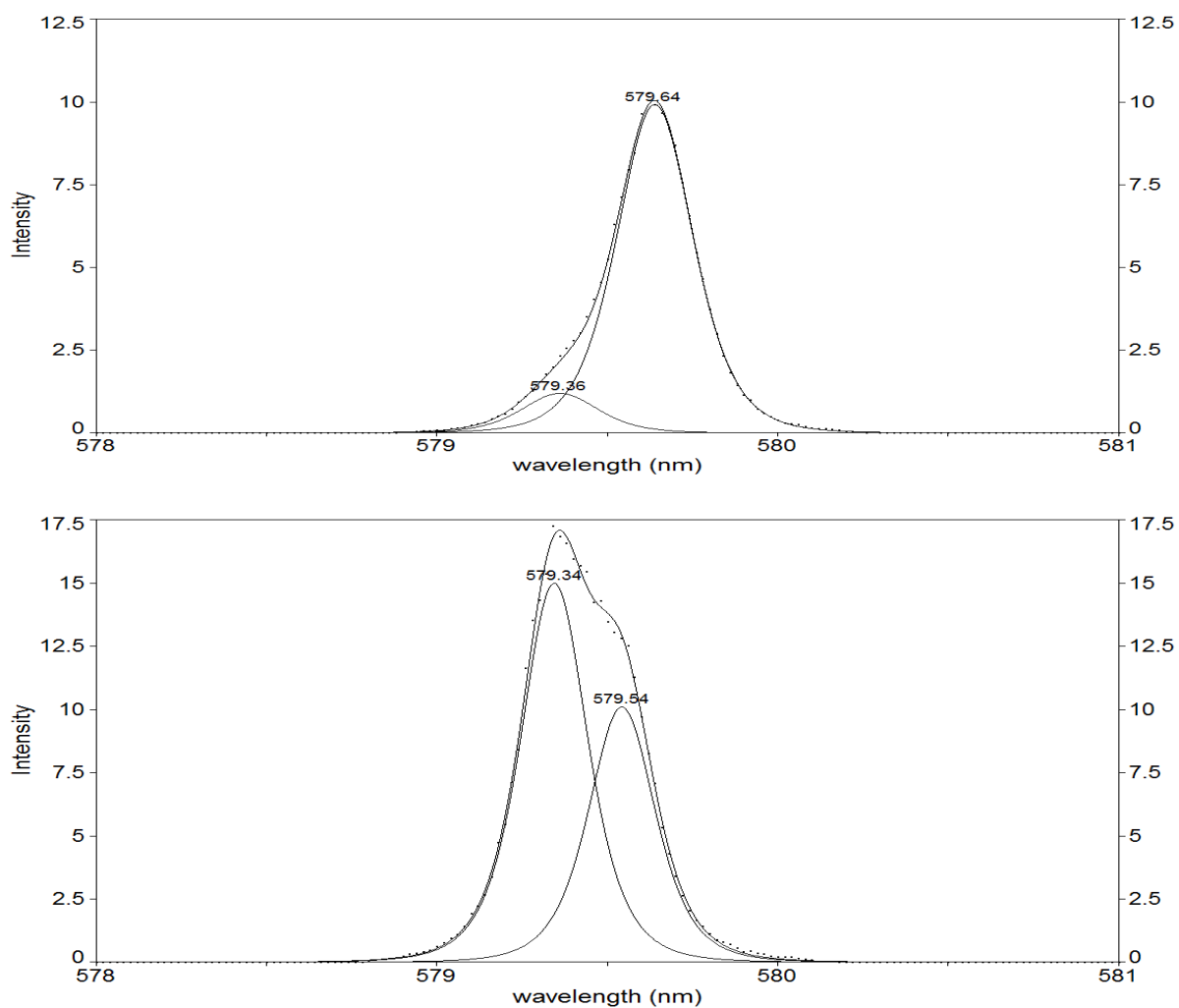
**Figure S1a:** Direct excitation  ${}^7F_0 \rightarrow {}^5D_0$  spectra with emission at  $628 \pm 27$  nm of 1.00 mM  $\text{Eu}(\text{S-THP})^{+3}$  with 20 mM HEPES, 100 mM NaCl and the addition of (a) MMP at pH 7.2 and (b) acetate at pH 7.2.



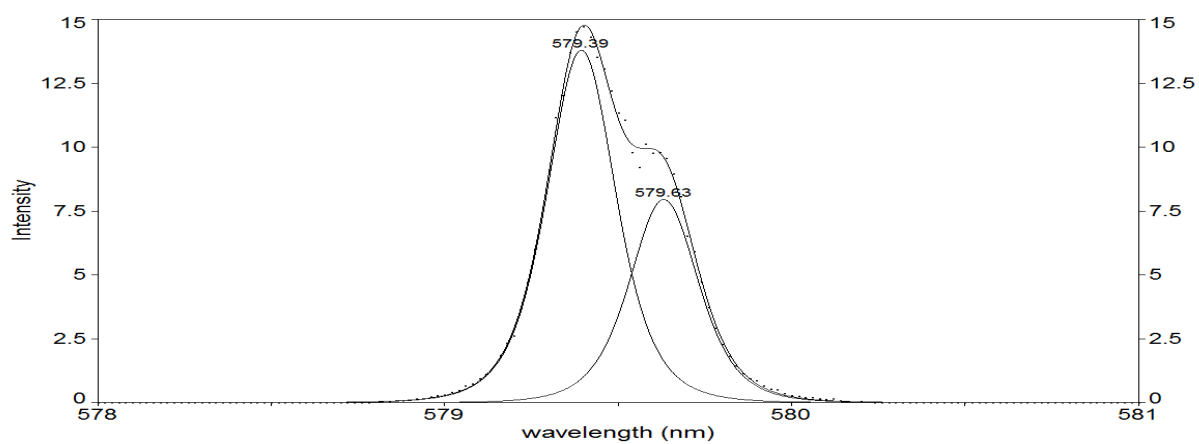
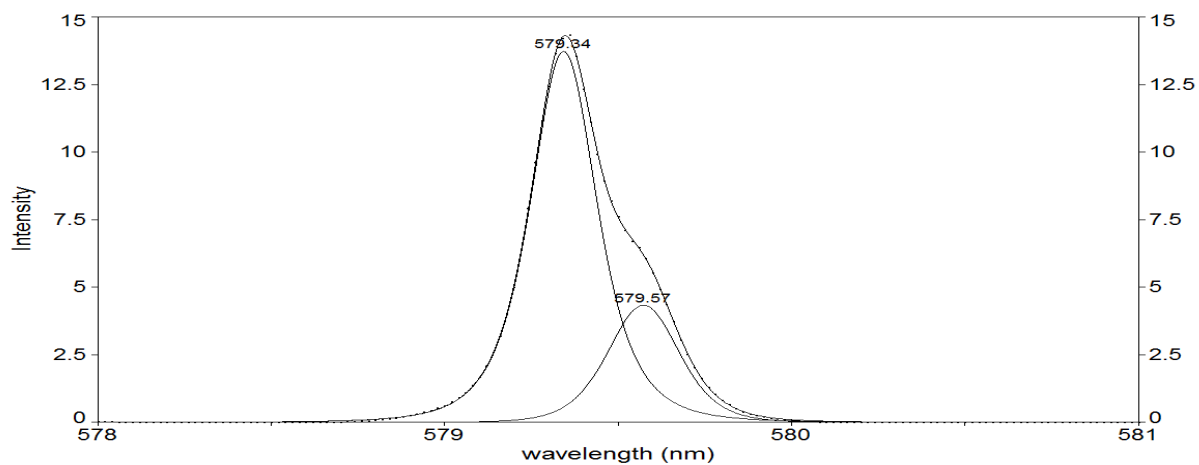
**Figure S1b:** Direct excitation  ${}^7F_0 \rightarrow {}^5D_0$  spectra with emission at  $628 \pm 27$  nm of 1.00 mM Eu (S-THP) $^{+3}$  with 20 mM HEPES at pH 7.2, 100 mM NaCl and the addition of carbonate.



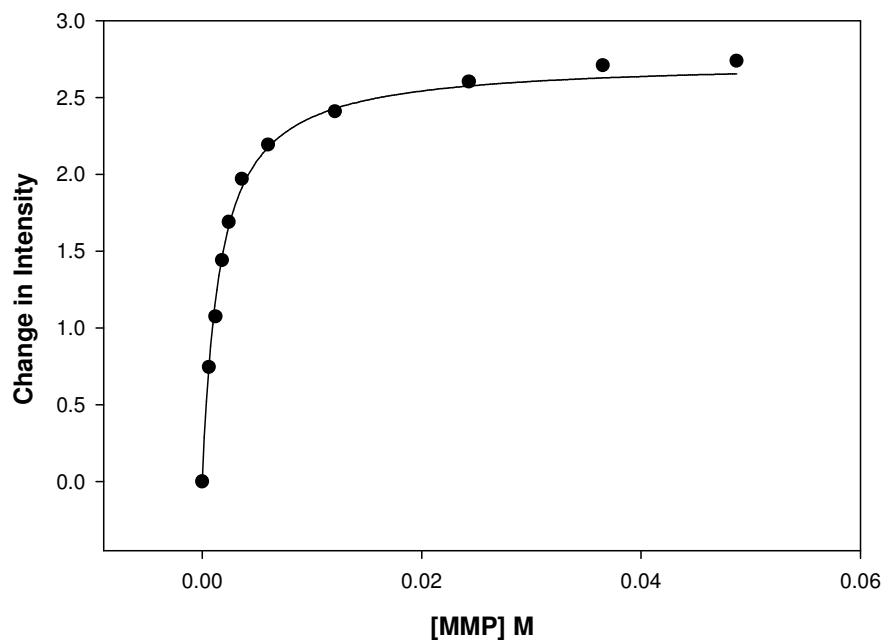
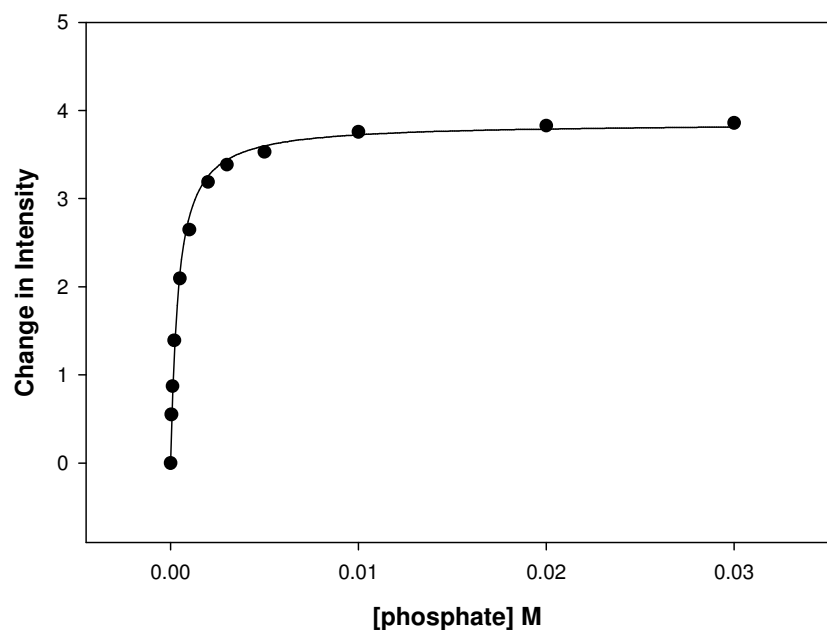
**Figure S2a:** Peak fitting analysis of 1.00 mM Eu (**S-THP**) in 20 mM HEPES and 100 mM NaCl, 20 mM **methylphosphate** (Top) and **phosphate** (bottom) at pH 7.2.



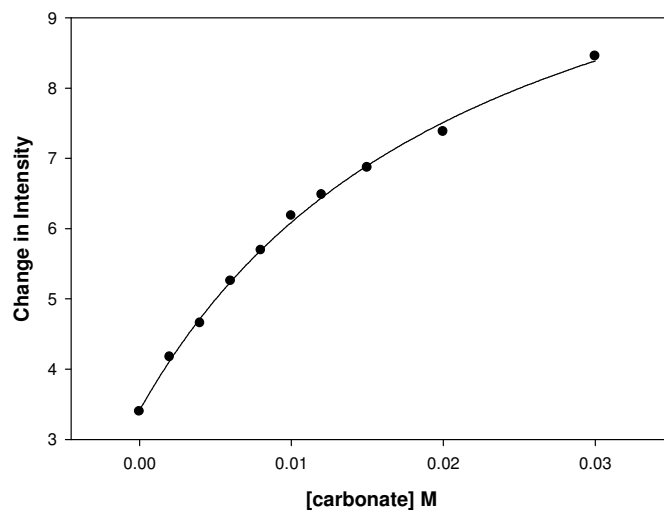
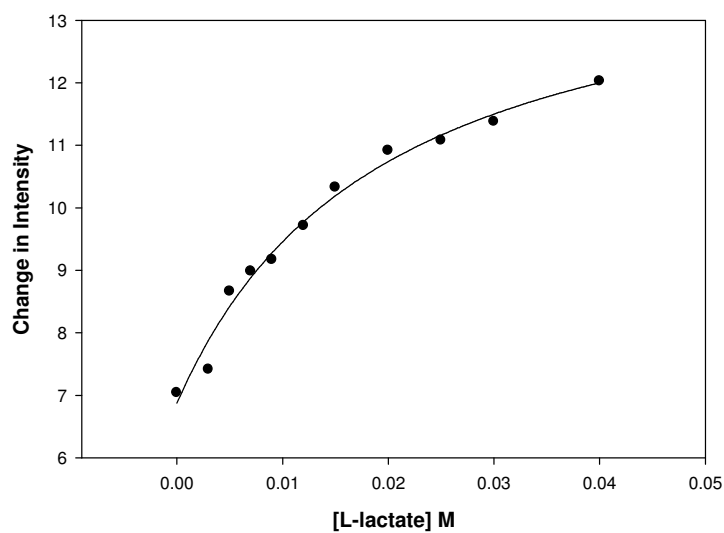
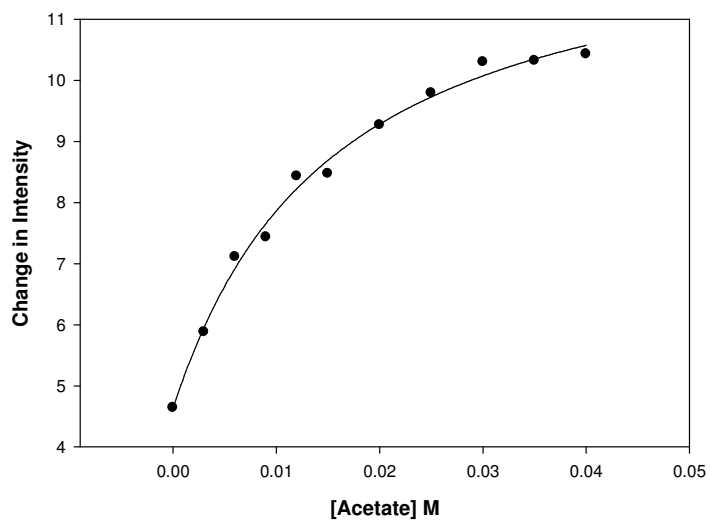
**Figure S2b.** Peak fitting analysis of 25  $\mu$ M Eu(S-THP) in 20 mM HEPES and 100 mM NaCl with 1.00 mM **citrate** (top) and **lactate** (bottom) at pH 7.2.



**Figure S2c.** The peak fitting analysis of 1.00 mM Eu(**S-THP**) in 20 mM HEPES and 100 mM NaCl with 40 mM **carbonate** (top) and **acetate** (bottom) at pH 7.2.

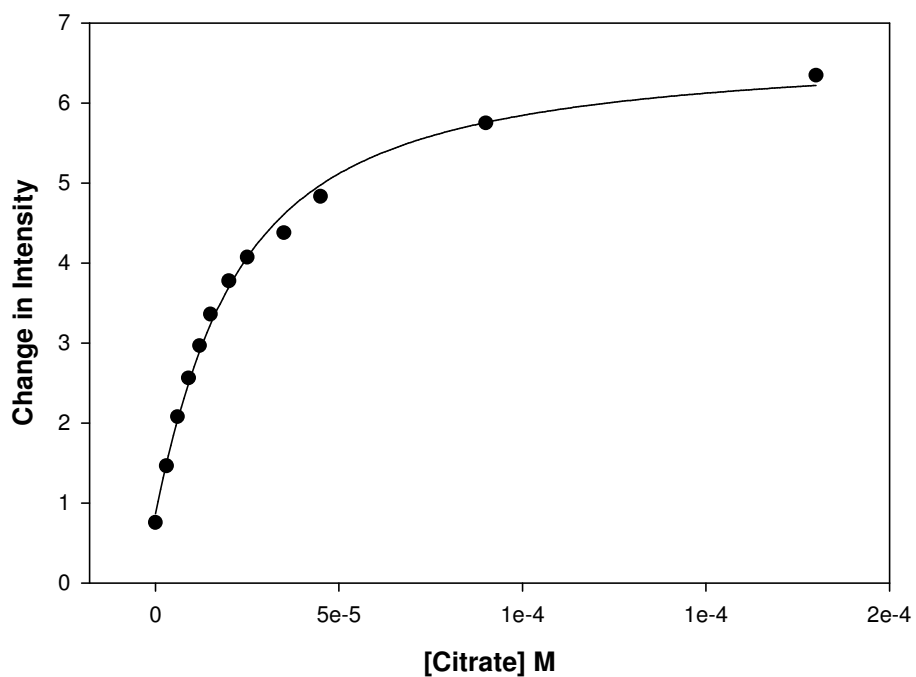


**Figure S3a.** Binding isotherms from a plot of the change in the  ${}^7F_0 \rightarrow {}^5D_0$  excitation peak intensity as a function of **phosphate** (top) or **methylphosphate** (MMP) bottom. Samples contained 0.100 mM Eu(**S-THP**), 0.1 M NaCl and 20 mM HEPES at pH 7.2. Data are fit to eq. 1 to give  $K_d$ 's of 0.3 and 1.8 mM respectively.

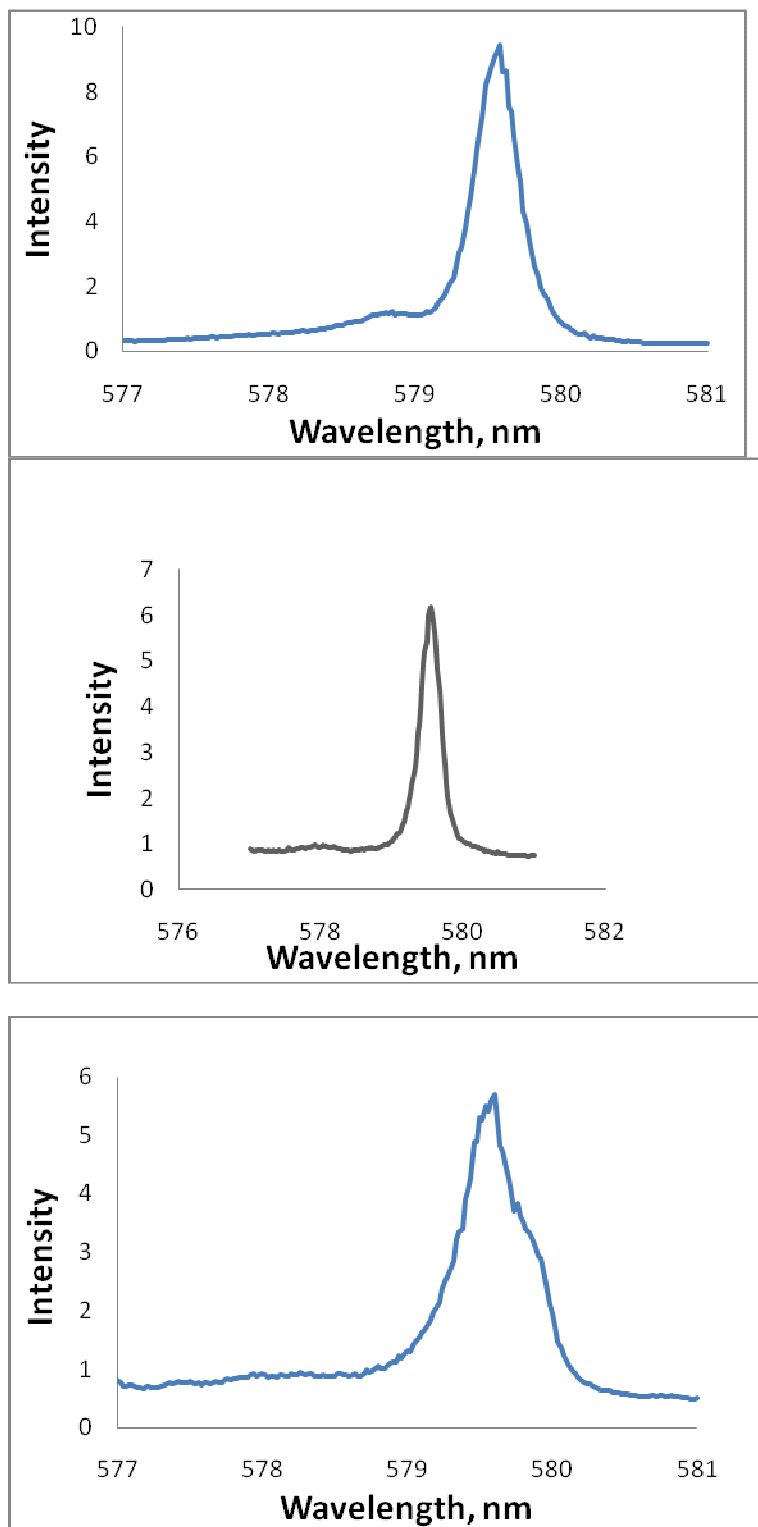


**Figure S3b.** Binding isotherms from a plot of the change in intensity of the  ${}^7F_0 \rightarrow {}^5D_0$  excitation peak as a function of anion concentration. Samples contain 0.100 mM Eu(S-THP), 0.100 M NaCl and 20 mM HEPES at pH 7.2 The  $K_d$ 's obtained, using equation 1, are 38, 33, and 19 mM respectively.

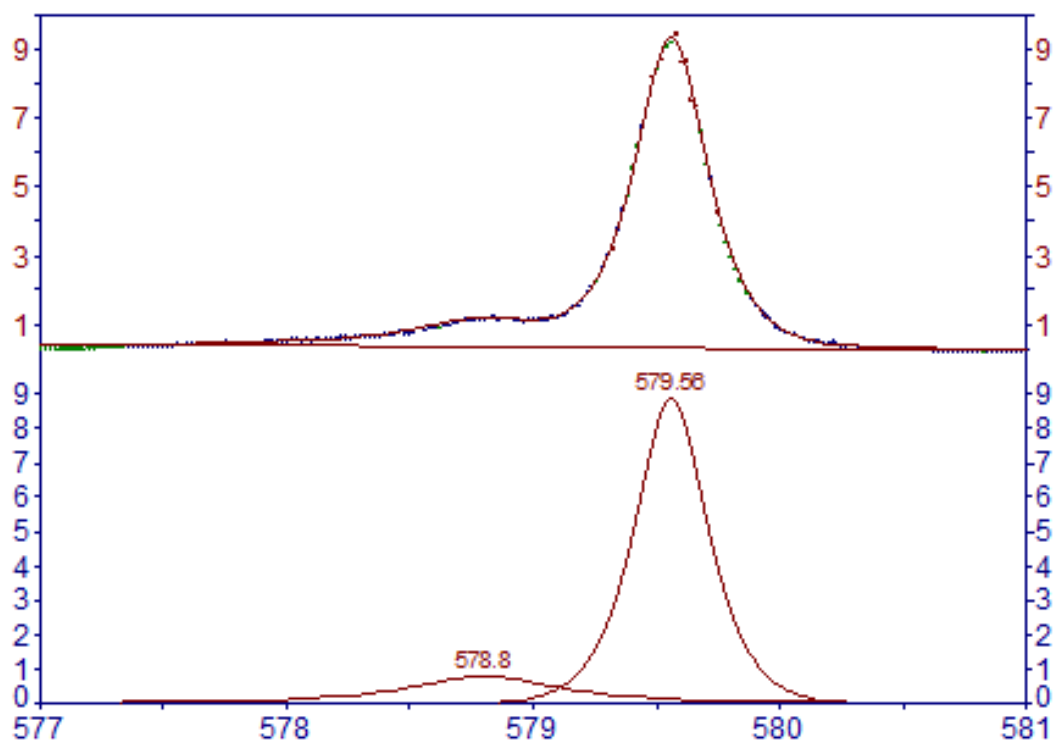




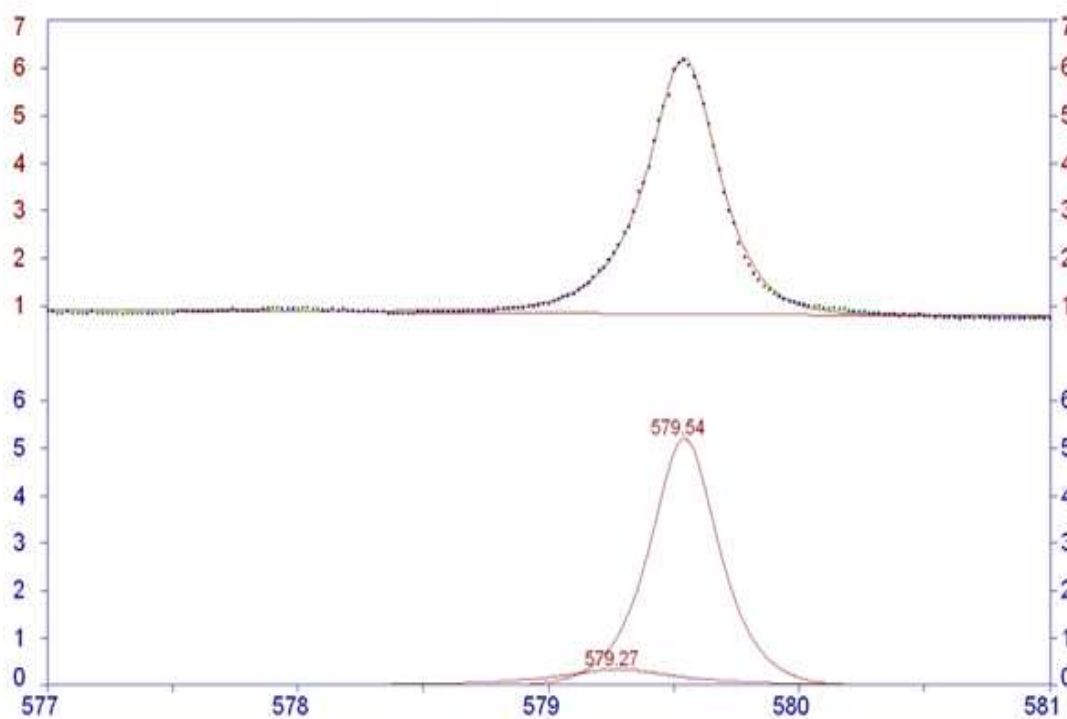
**Figure S3c.** Binding isotherms from a plot of the change in intensity of the  ${}^7F_0 \rightarrow {}^5D_0$  excitation peak as a function of **citrate** concentration. The sample contains 25  $\mu\text{M}$  Eu (**S-THP**), 0.1 M NaCl, and 20 mM HEPES at pH 7.2. The  $K_d$  obtained using equation 1 is 17  $\mu\text{M}$ .



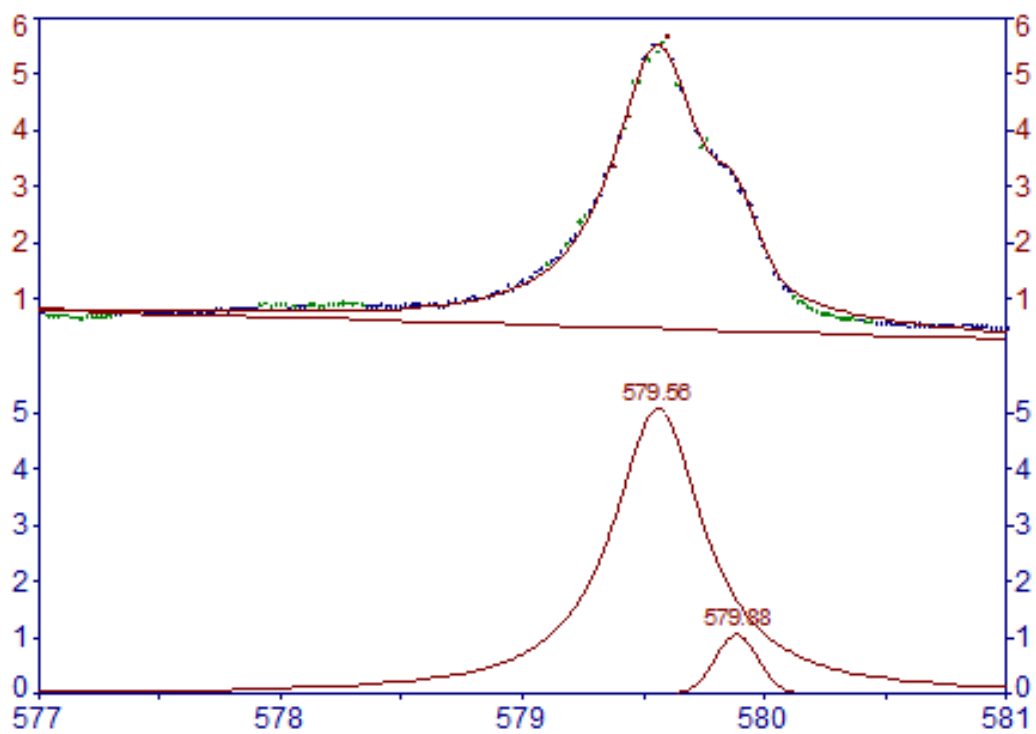
**Figure S4a:** Direct excitation  ${}^7F_0 \rightarrow {}^5D_0$  spectra with emission at  $628 \pm 27$  nm of bottom: 25  $\mu\text{M}$  Eu (THPC) with 20 mM HEPES, pH 7.2, 100 mM NaCl and 0.70 mM citrate, middle: complex alone, top: 10 mM phosphate.



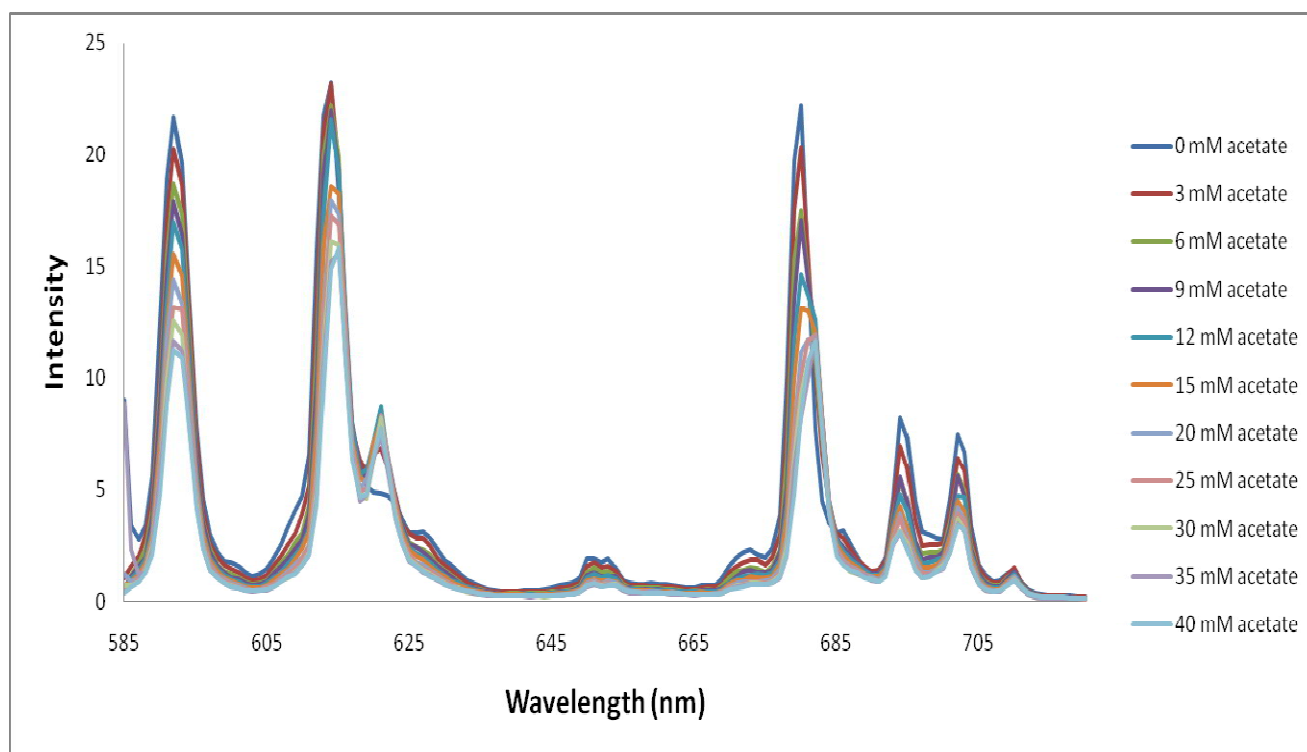
**Figure S4b.** Peak fitting analysis of the  ${}^7F_0 \rightarrow {}^5D_0$  excitation spectrum of 25  $\mu\text{M}$  Eu(THPC) in 20 mM HEPES at pH 7.2. Lower panel shows the peaks used for fit and top shows superposition of the data. The peak at 578.8 nm is attributed to Eu(III) aquo ion.



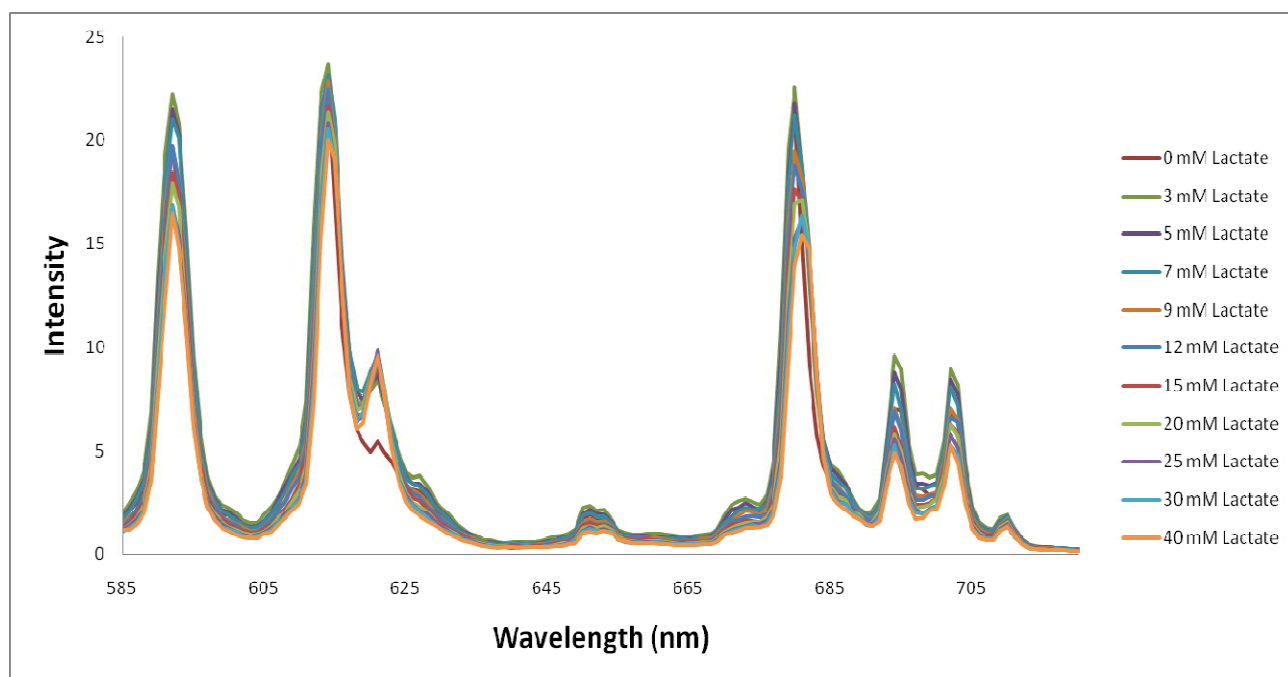
**Figure S4c.** Peak fitting analysis of the  ${}^7F_0 \rightarrow {}^5D_0$  excitation spectrum of 25  $\mu\text{M}$  Eu(THPC) in 20 mM HEPES and 100 mM NaCl and phosphate at pH 7.2. Lower panel shows the peaks used for fit and top shows superposition of the data.



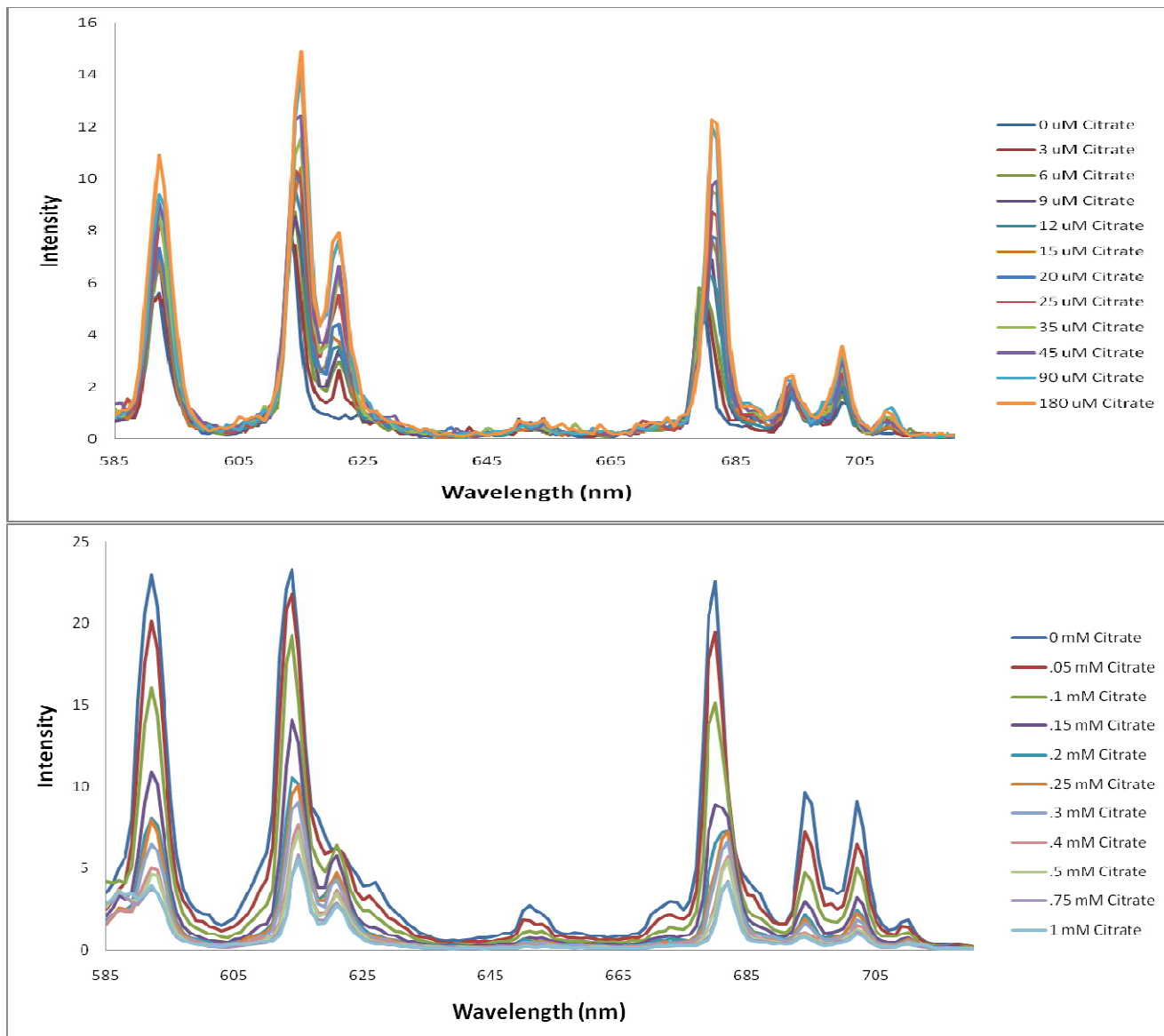
**Figure S4d.** Peak fit analysis of direct excitation spectrum ( ${}^7F_0 \rightarrow {}^5D_0$ , emission at  $628 \pm 27$  nm) of  $25\mu\text{M}$  Eu(**S-THPC**) with 20 mM HEPES, 100 mM NaCl at pH = 7.3 and 0.70 mM citrate.



**Figure S5.** Emission spectra (excitation at 579.36 nm) of 1.00 mM Eu (**S-THP**) in 20 mM HEPES, 100 mM NaCl upon addition of acetate at pH 7.2.

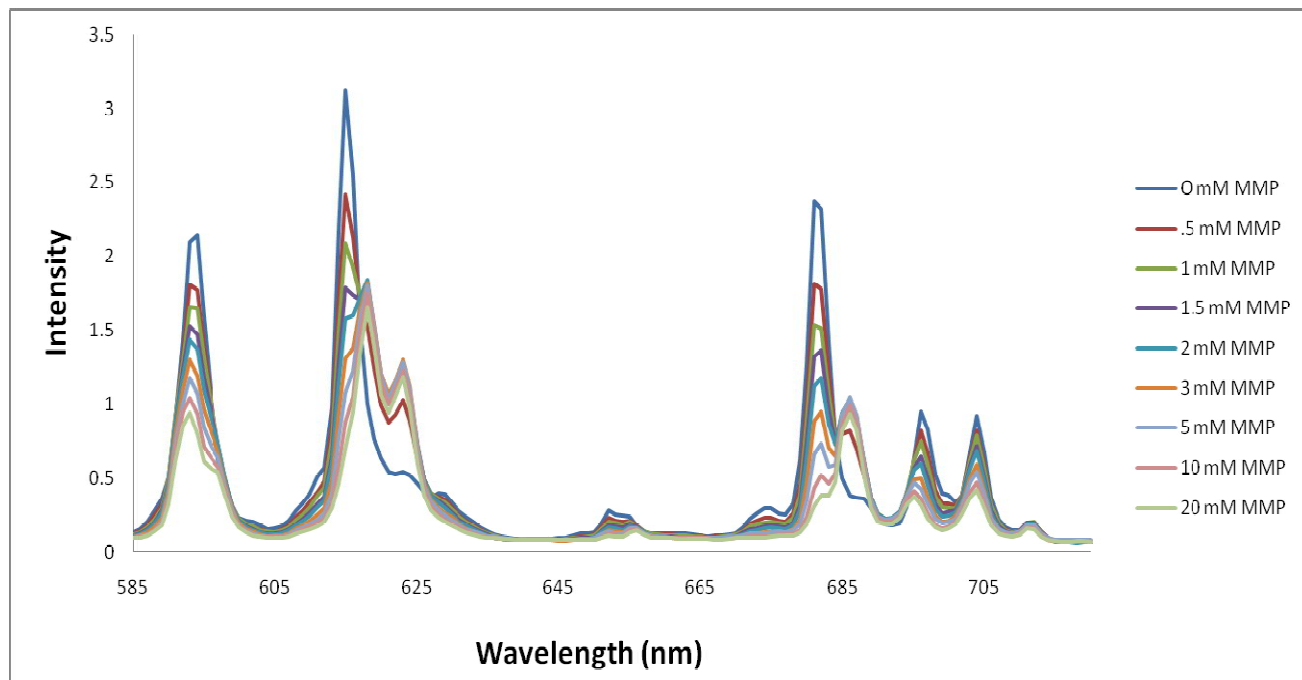


**Figure S6:** Emission spectra (excitation at 579.36 nm) of 1.00 mM Eu (**S-THP**) in 20 mM HEPES, 100 mM NaCl upon addition of **lactate**, at pH 7.2.

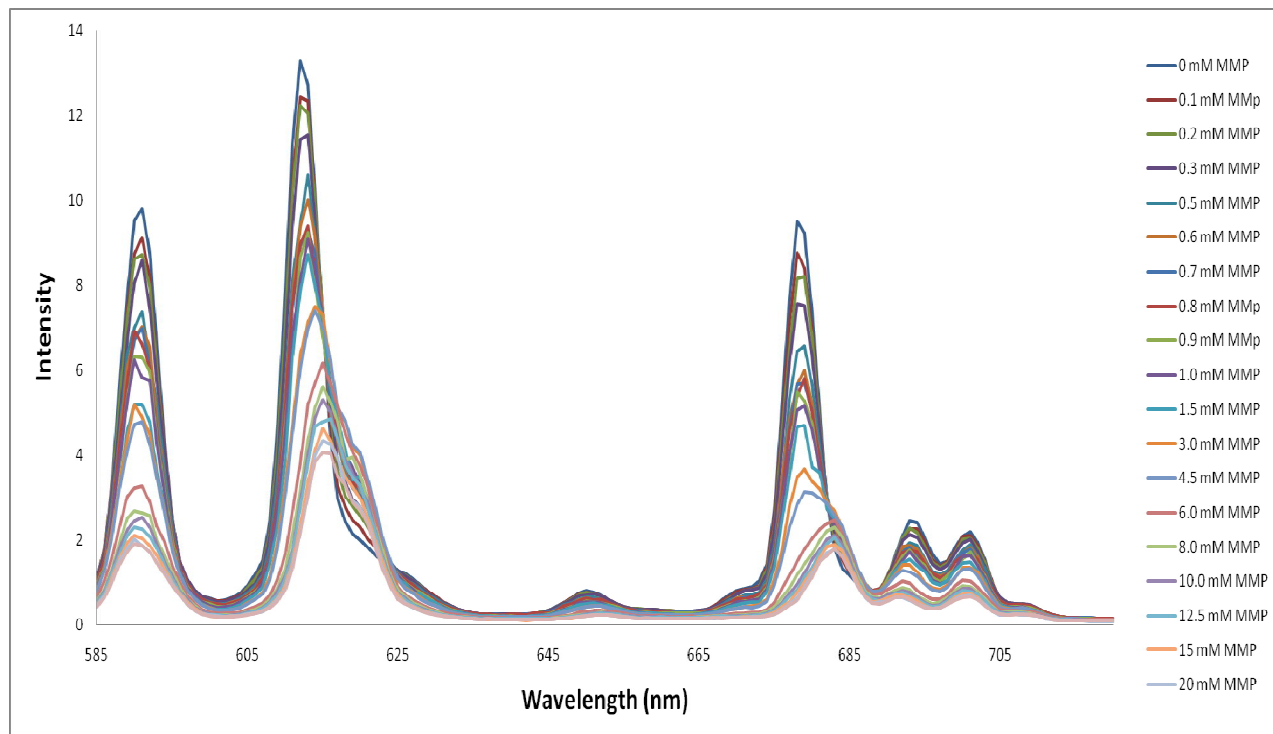


**Figure S7:** Emission spectra with excitation at 579.36 nm (top) or excitation at 579.58 nm (bottom) of 25  $\mu\text{M}$  Eu(S-THP) in 20 mM HEPES, 100 mM NaCl upon addition of citrate at pH 7.2.

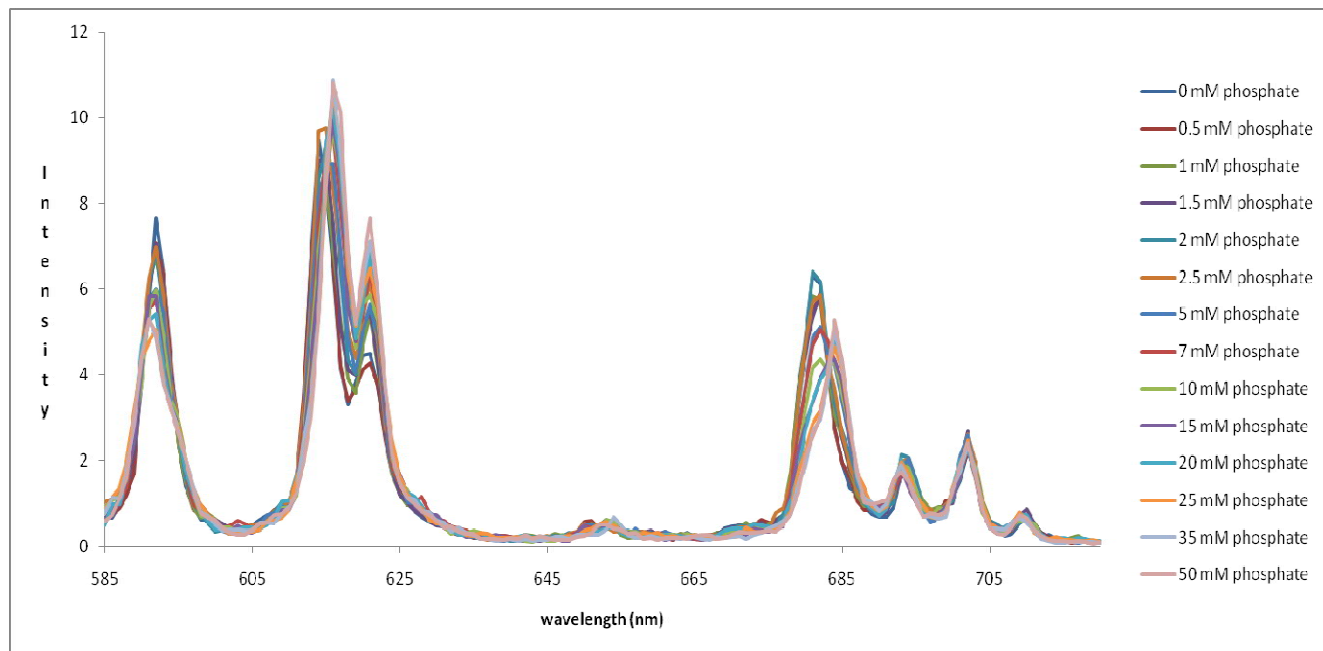




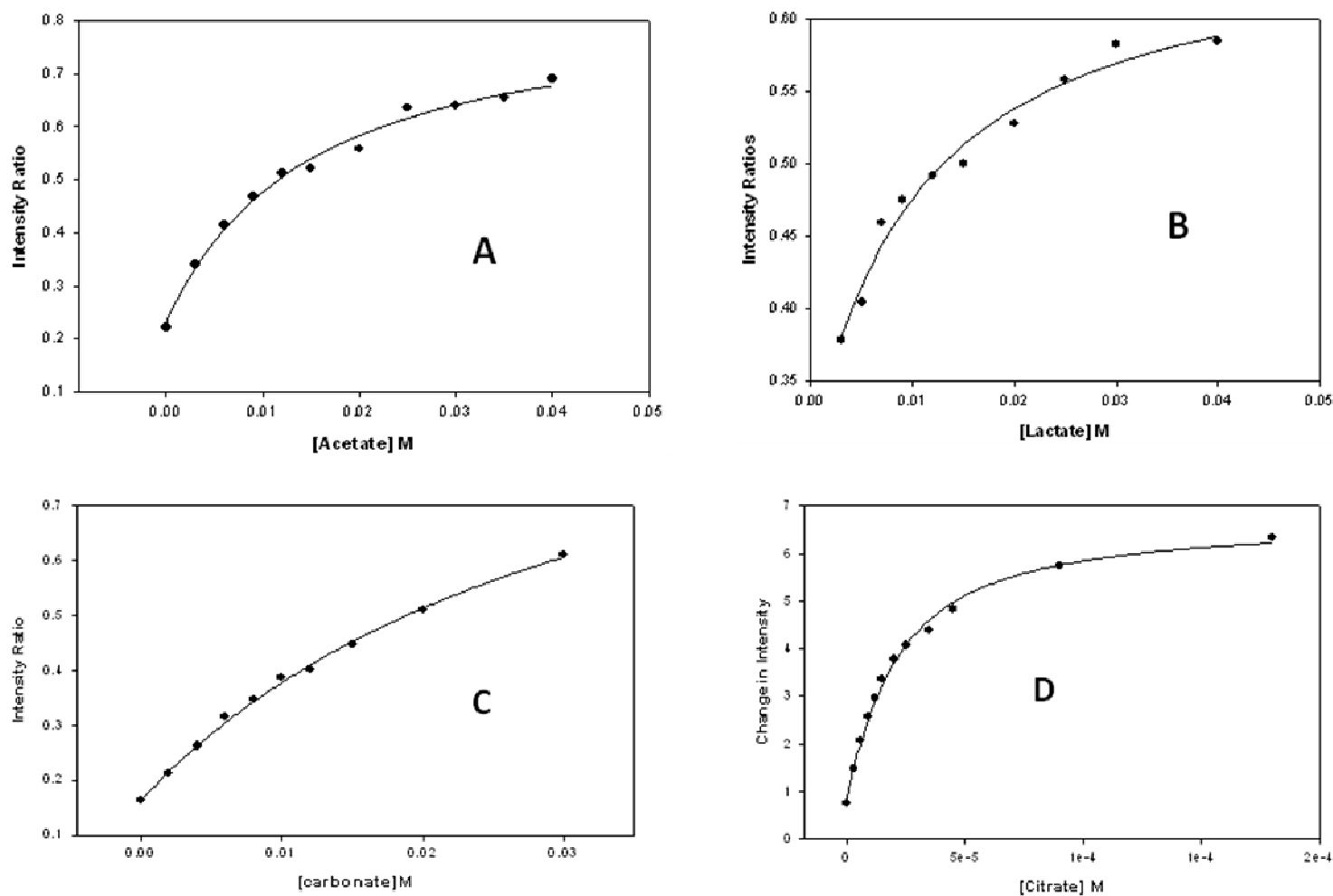
**Figure S8.** Emission spectra (excitation at 579.36 nm) of 1.00 mM Eu (**S-THP**) in 20 mM HEPES, 100 mM NaCl and the addition of methylphosphate (MMP), at pH 7.2.



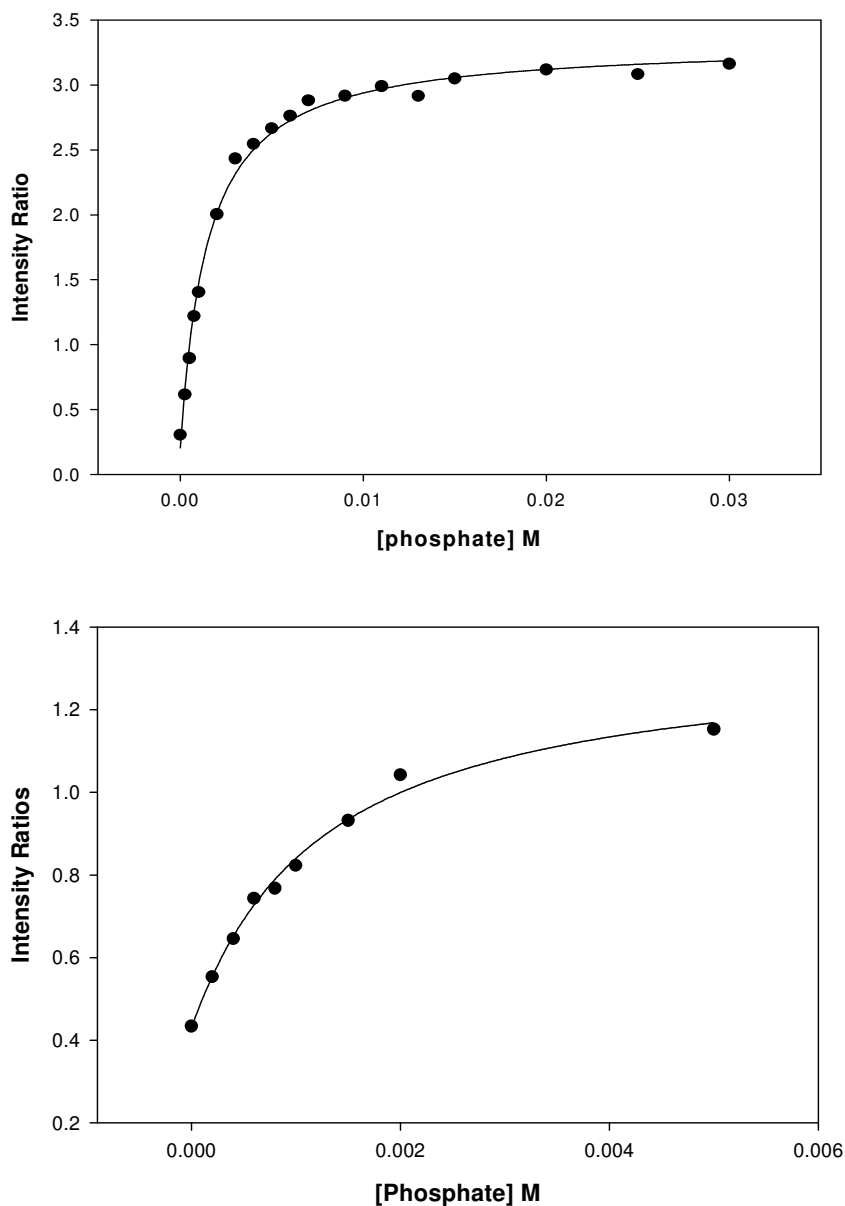
**Figure S9.** Emission spectra of 0.100 mM Eu(S-THP), 20 mM HEPES, 100 mM NaCl, pH 7.2 upon addition of methylphosphate (MMP) showing bimodal changes in intensity for two distinct binding events.



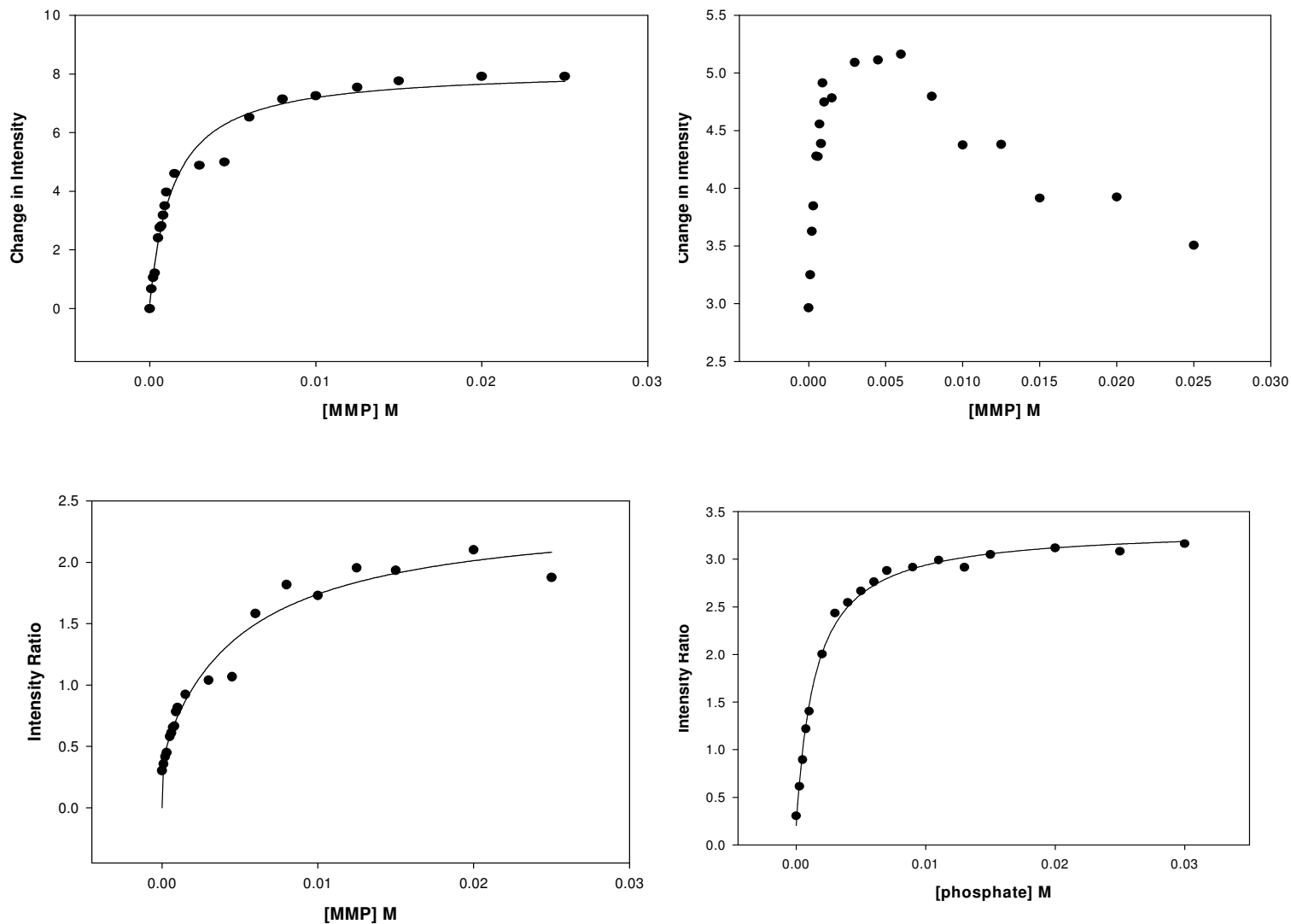
**Figure S10.** Emission spectra (excitation at 579.36 nm) of 0.100 mM Eu(S-THP) in 20 mM HEPES, 100 mM NaCl, 20 mM sodium carbonate, 2.6 mM sodium L-lactate, and 0.100 mM sodium citrate, at pH 7.2.



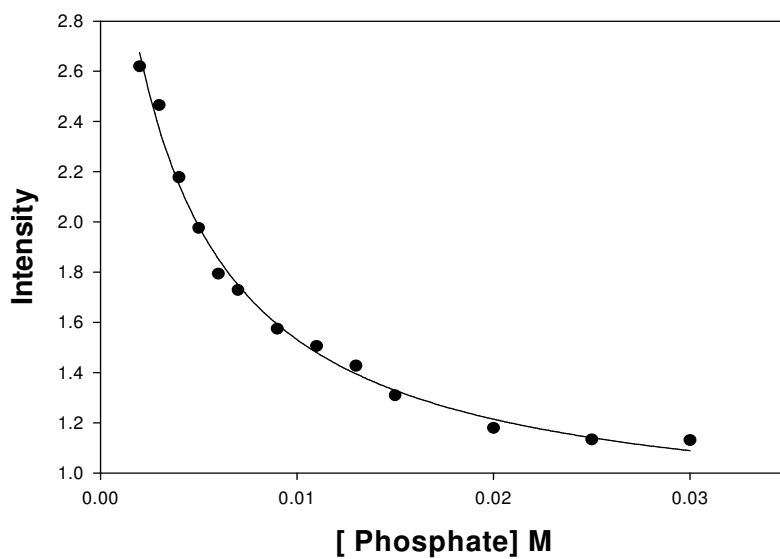
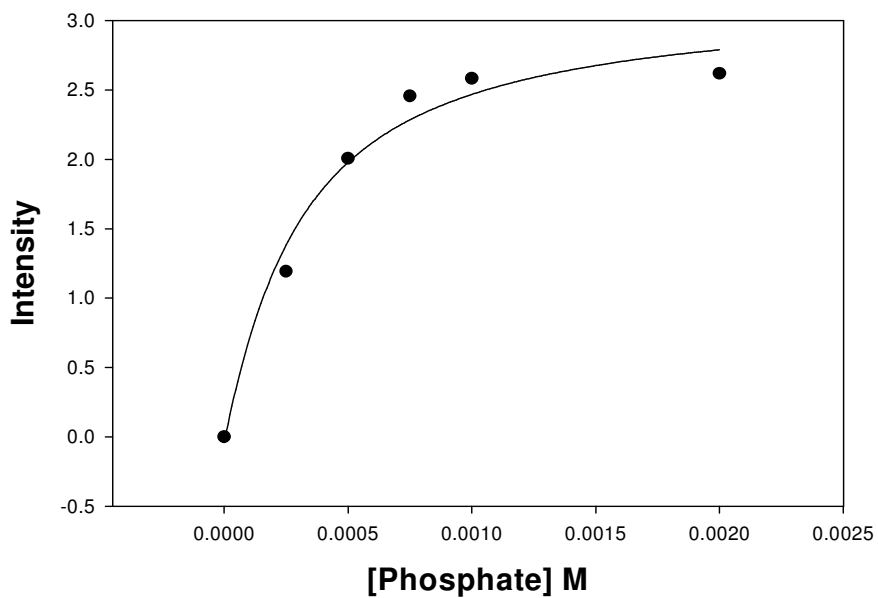
**Figure S11a.** Binding isotherms (excitation at 579.36 nm) from plots of the emission intensity at 622/593 nm of 1.00 mM Eu(S-THP) in 20 mM HEPES, pH 7.2, 100 mM NaCl, with the addition of (A) acetate, (B) lactate, and (C) carbonate (D) binding isotherm from plot of intensity at 622 nm ( $\lambda_{ex} = 579.54$  nm) as a function of citrate (25  $\mu$ M Eu(S-THP)). The  $K_d$  values from fitting of the data to eq. 1 are 14 mM, 13 mM, 33 mM, 17  $\mu$ M respectively.



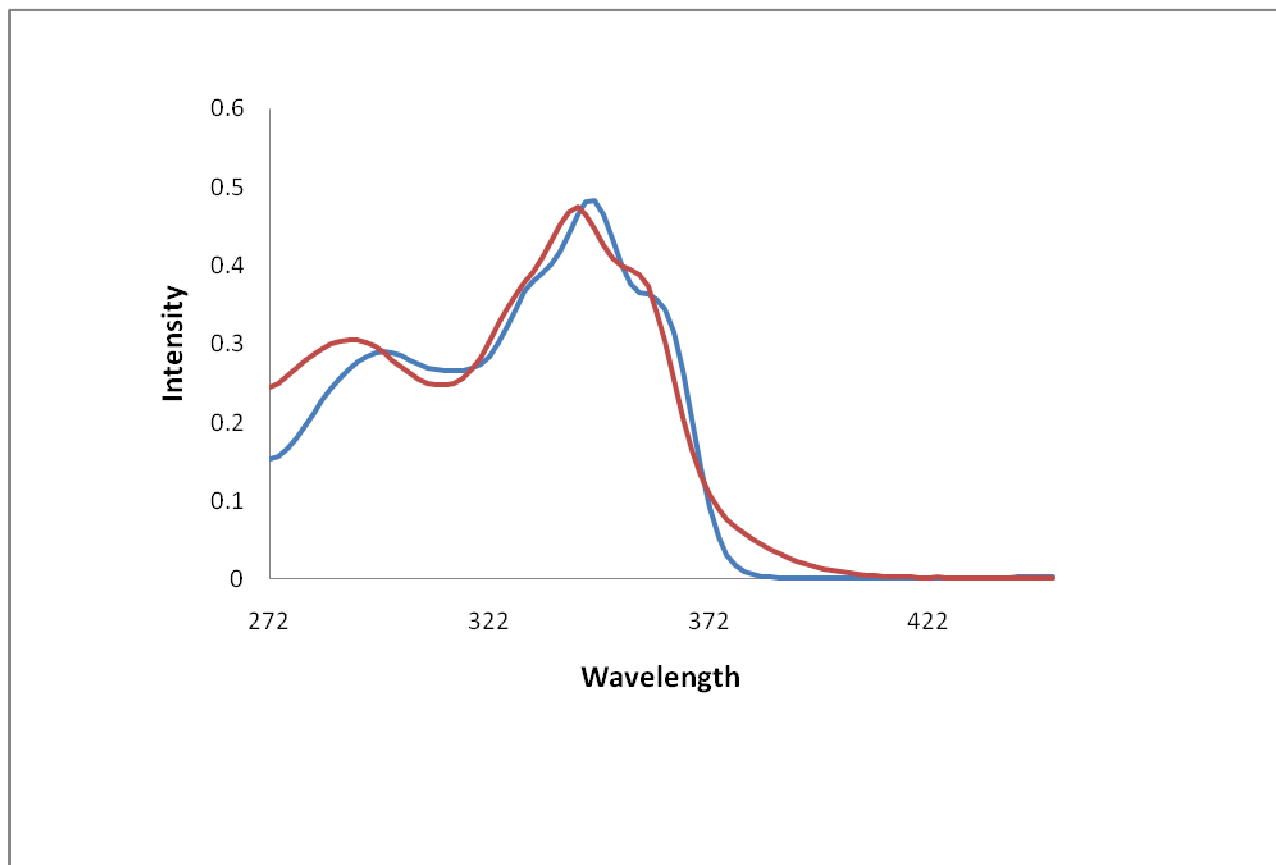
**Figure S11b.** Binding isotherms (excitation at 579.36 nm) from plots of the emission intensity at 622/593 nm of 25  $\mu\text{M}$  Eu(**S-THP**) in 20 mM HEPES, pH 7.2, 100 mM NaCl, with the addition of phosphate (top) and plot of the ratio of 618/593nm from the addition of phosphate to a solution containing 1.00 mM Eu(**S-THP**), 20 mM sodium carbonate, 2.60 mM sodium L-lactate, 0.100 mM sodium citrate, 100 mM NaCl, and 20 mM HEPES at pH 7.2 (bottom). The  $K_d$  values from fitting of the data to eq. 1 are 0.70 mM and 14 mM, respectively, for top and bottom graph.



**Figure S12a.** Binding isotherms from a plot of the luminescence intensity of Eu(S-THP) as a function of anion: top left: MMP addition, 593 nm emission peak, top right: intensity of the 622 nm emission peak, bottom left: the ratio of the two emission peaks, bottom right: ratio of emission intensities at 622/593 nm upon addition of phosphate. Data at bottom right is fit to equation 1 and remaining are fit to equation 2. All solutions contain 0.100 mM Eu(S-THP), 100 mM NaCl, and 20 mM HEPES at pH 7.2

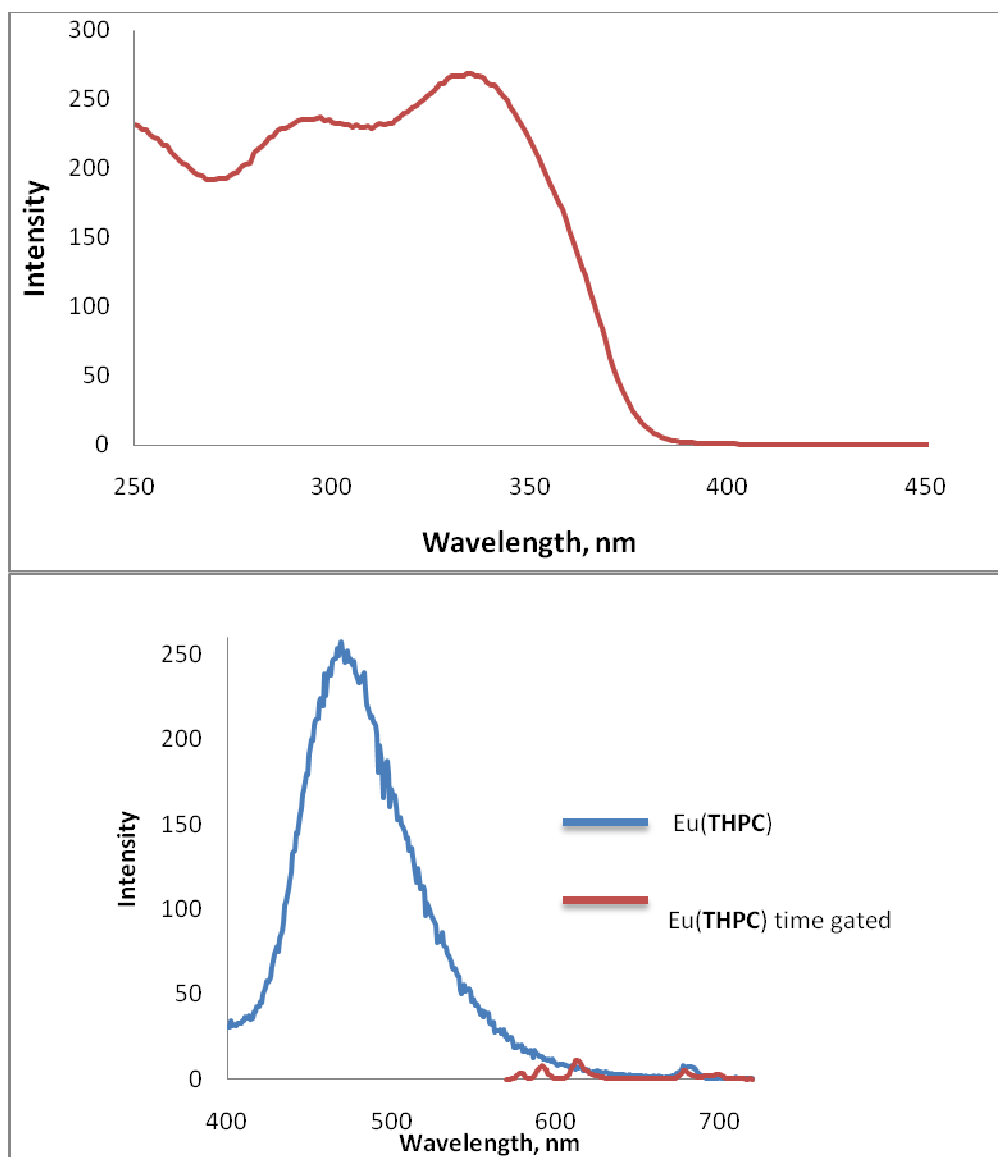


**Figure S12b:** Binding isotherms from a plot of the luminescence intensity of the 618 nm emission peak from 0.0 to 2.0 mM phosphate (top) and 2.0 to 30 mM phosphate (bottom) for a solution containing 0.100 mM Eu(**S-THP**), 100 mM NaCl, and 20 mM HEPES at pH 7.2. Dissociation constants are 0.30 and 3.1 mM respectively.

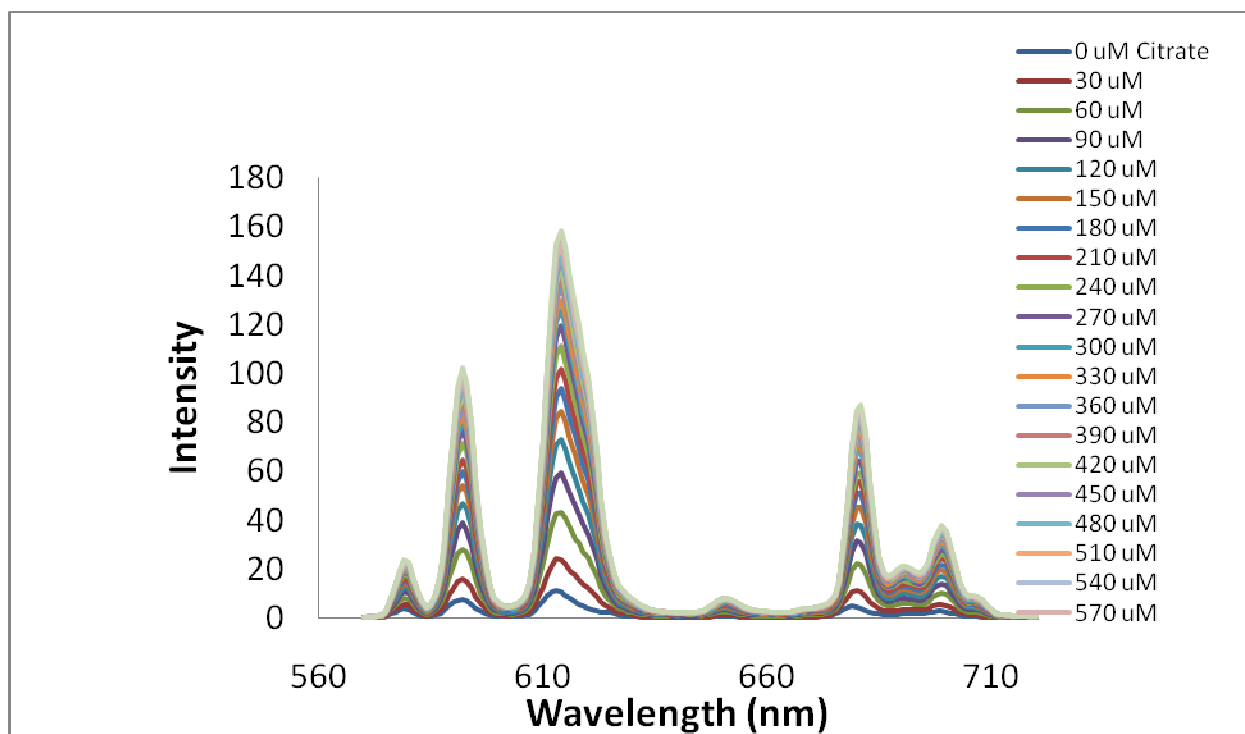


**Figure S13.** Absorbance spectra of **THPC** ligand (blue) and **Eu(THPC)** complex (red) at 21  $\mu\text{M}$ , pH 7.2, 100 mM NaCl, HEPES buffer.

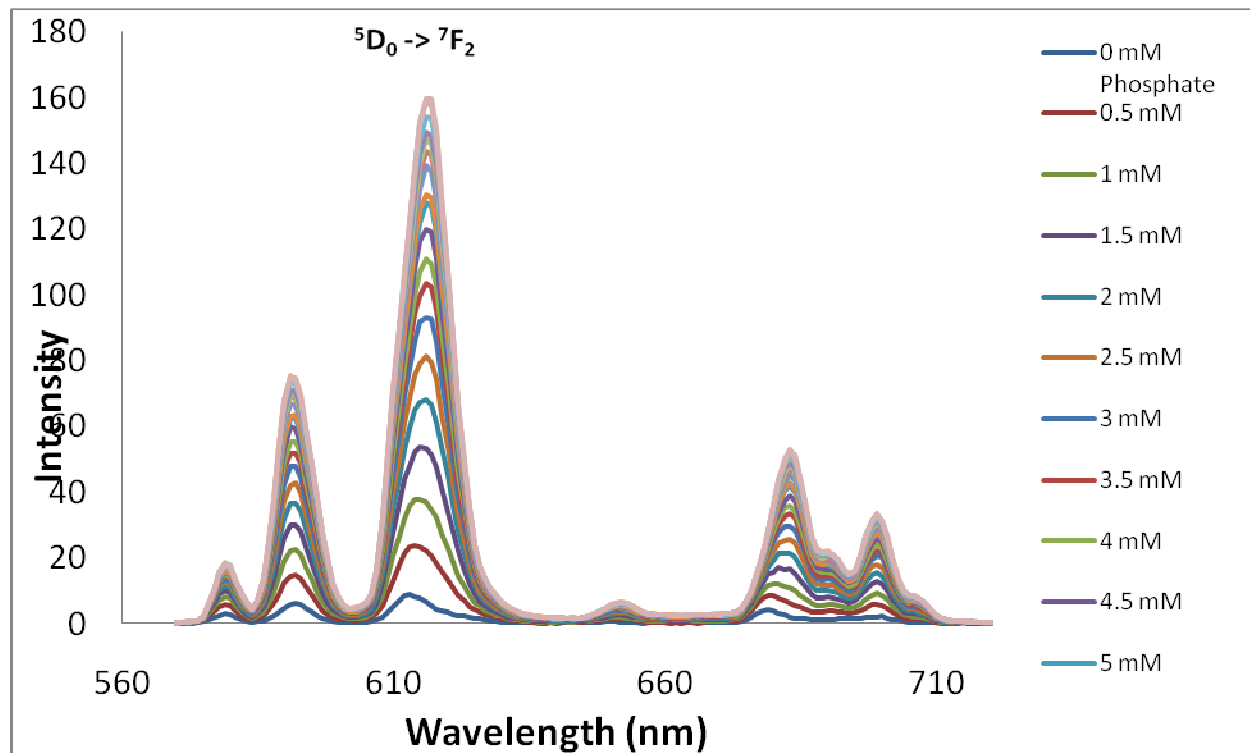




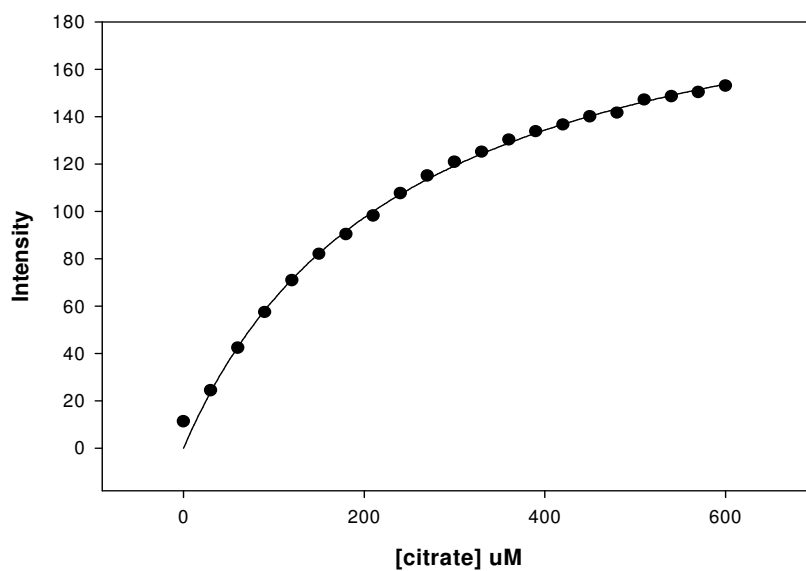
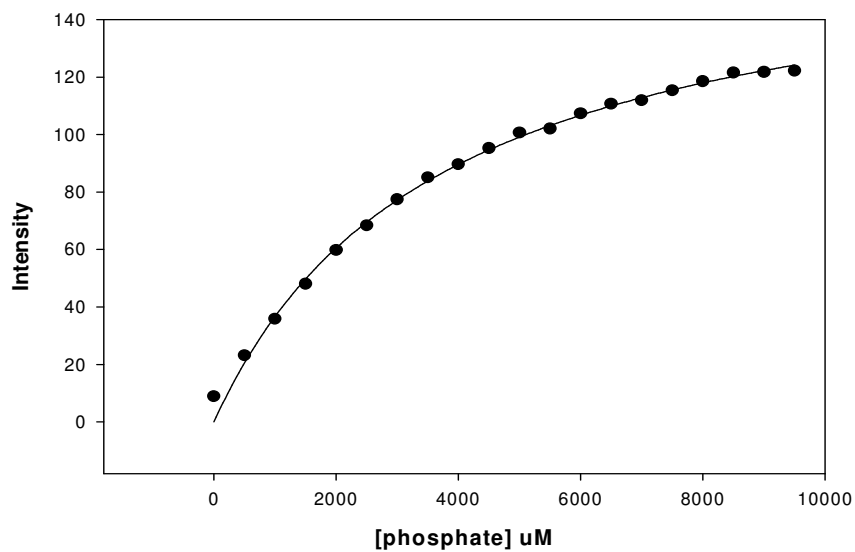
**Figure S14.** Excitation (top) and emission(bottom) spectra of Eu(THPC) at 100  $\mu$ M complex, 100 mM NaCl, 20 mM Hepes buffer at pH 7.2 with (red) or without (blue) a time gate of 51 us. Emission monitored at 615 nm, excitation at 340 nm.



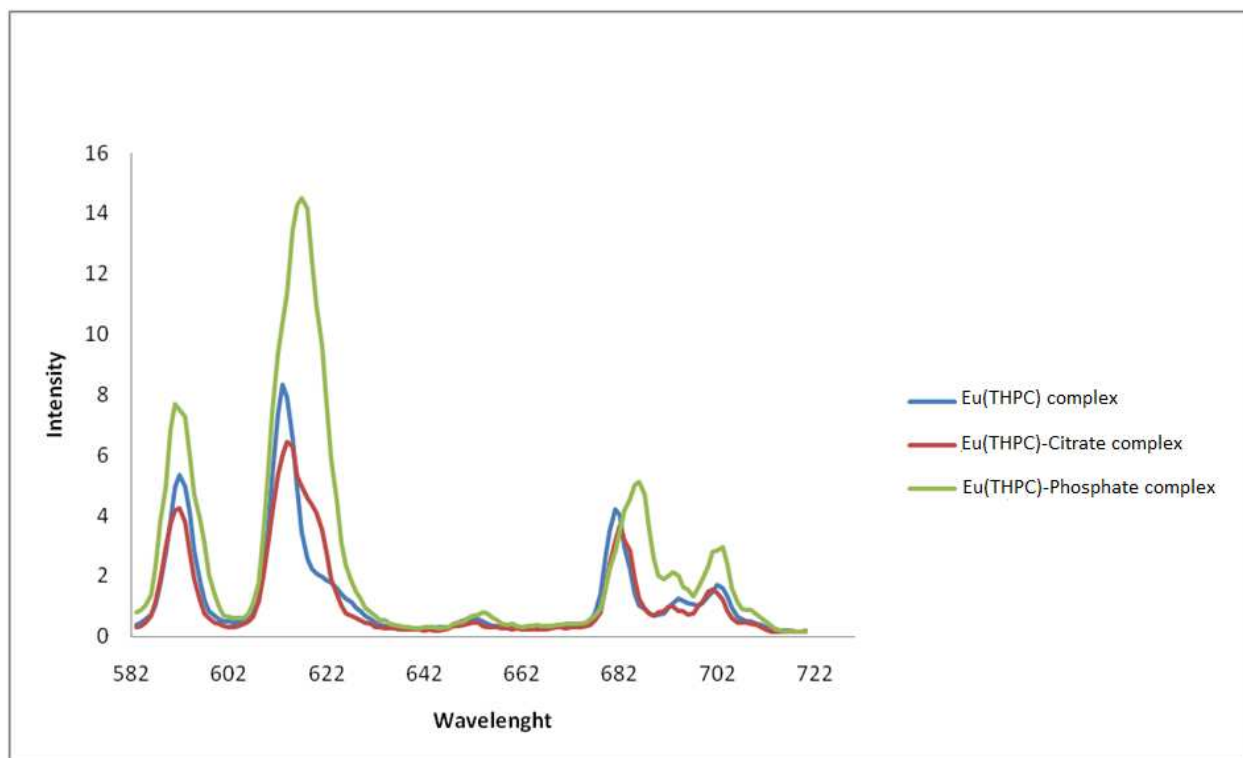
**Figure S15:** Emission spectra (excitation at 340 nm) of 100 μM Eu(THPC) in 20 mM HEPES, 100 mM NaCl upon addition of citrate at pH 7.2.



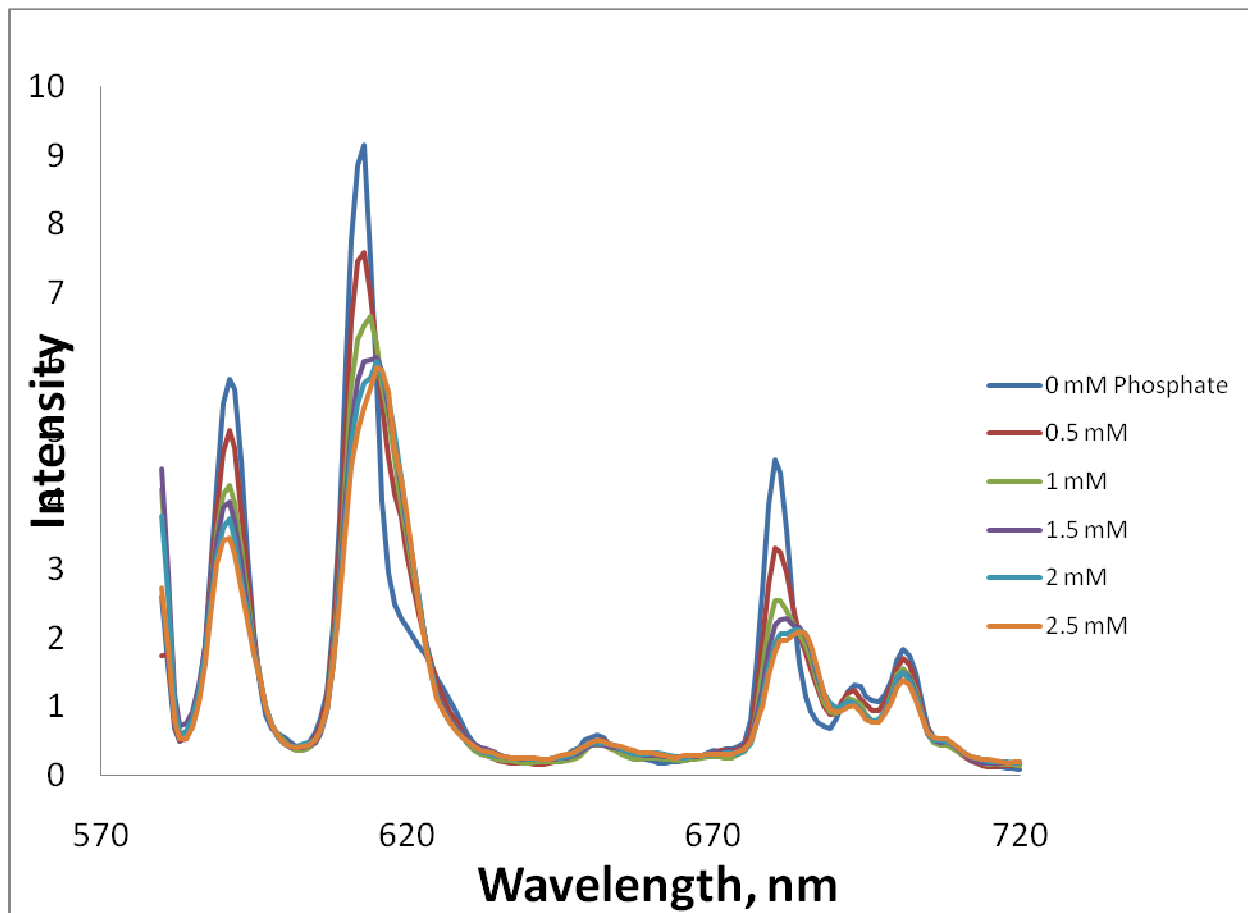
**Figure S16:** Emission spectra with excitation at 340 nm of 100  $\mu$ M Eu(THPC) in 20 mM HEPES, 100 mM NaCl upon addition of phosphate at pH 7.2.



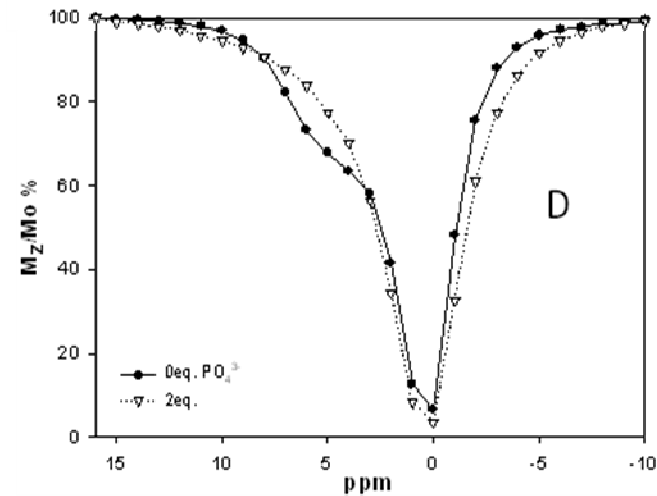
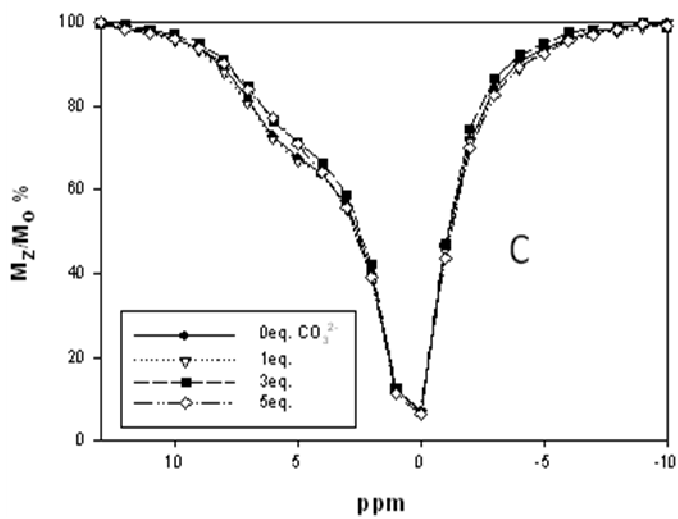
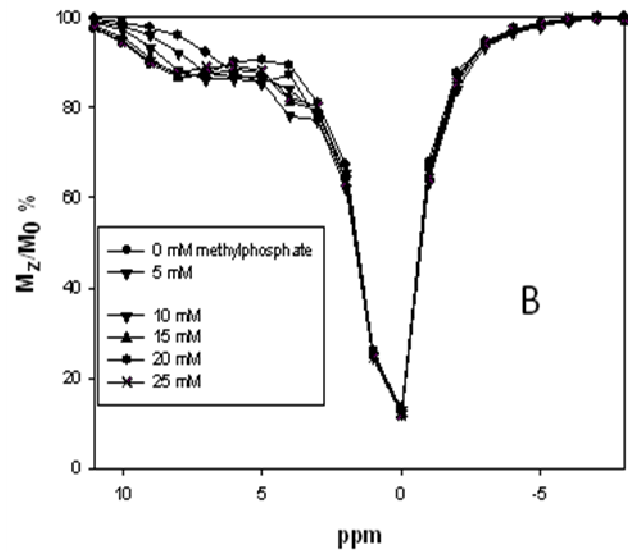
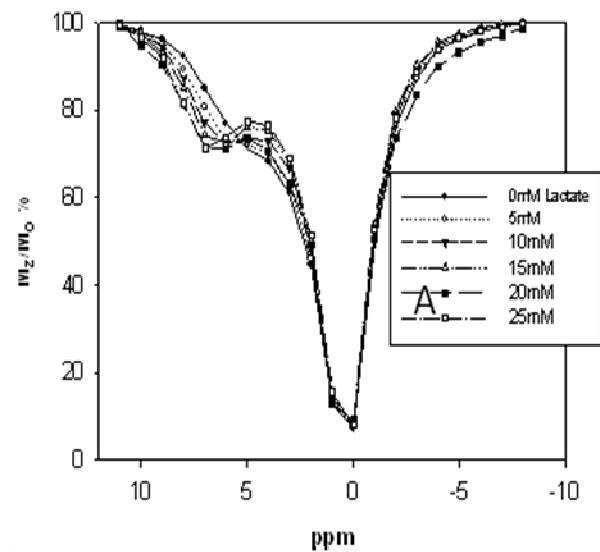
**Figure S17.** Binding isotherms from a plot of the luminescence intensity of : **A)** 614 nm emission peak upon addition of phosphate or, **B)** the 622 nm emission peak upon addition of citrate for a solution containing 0.100 mM Eu(**THPC**), 100 mM NaCl, and 20 mM HEPES at pH 7.2. Data is fit to a simple 1:1 binding isotherm. Excitation was at 340 nm.



**Figure S18.** Emission spectra of 25  $\mu\text{M}$  Eu(THPC), 20 mM HEPES, 100 mM NaCl, pH 7.2 upon addition of phosphate or citrate. Excitation at 579.54 nm.



**Figure S19.** Emission spectra of 25  $\mu\text{M}$  Eu(THPC), 20 mM HEPES, 100 mM NaCl, pH 7.2 upon addition of phosphate. Excitation ( ${}^7\text{F}_0 \rightarrow {}^5\text{D}_0$ ) at 579.54 nm.



**Figure S20.** CEST spectra of 5.00 mM Eu(S-THP), 100 mM NaCl, 20 mM HEPES, pH 6.5, 22 °C with added lactate (A), methylphosphate (B), carbonate (C) or phosphate (D).

$$F = [A]_{tot} + [M]_{tot} + K_d$$

$$[MA] = \frac{F - \sqrt{F^2 - 4[M]_{tot}[A]_{tot}}}{2} \quad \text{eq. S1-b}$$

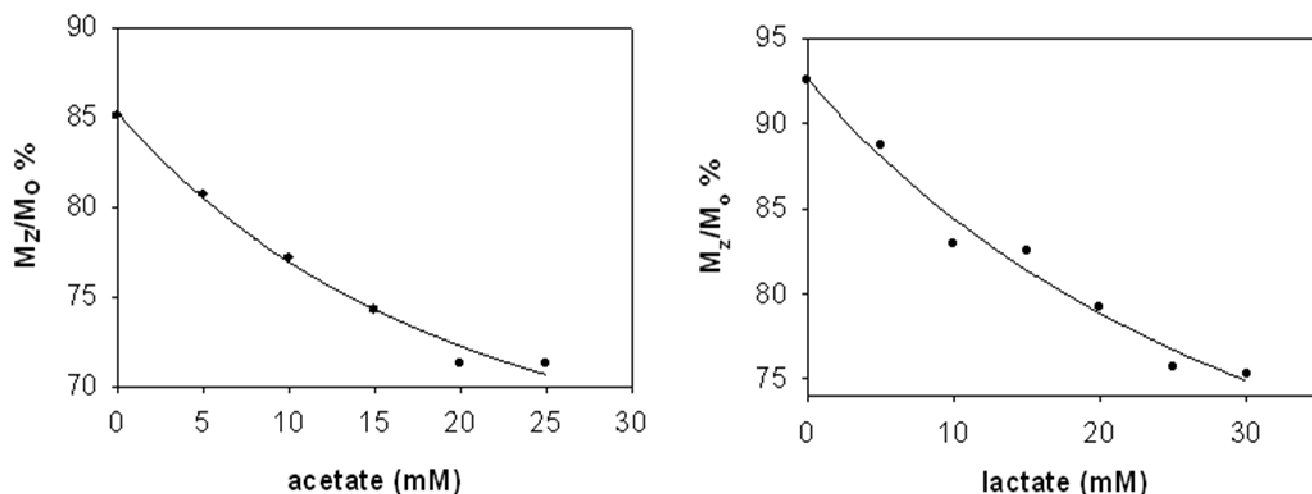
$$X_{MA} = [MA] / [M]_{tot} \quad \text{eq. S1-c}$$

$$X_M = 1 - X_{MA} \quad \text{eq. S1-d}$$

$$y = X_M k_M + X_{MA} k_{MA} \quad \text{eq. S1-e}$$

$$y = \frac{M_z}{M_0} \%$$

**M** = metal complex; **A** = anion;  $K_d$  = estimated binding constant



**Figure S21.** Binding isotherms from a fit of the CEST peak of Eu(S-THP) in solutions containing 100 mM NaCl, 20 mM HEPES, at pH 6.5 for binding of acetate (left) or lactate (right). Data is fit to eq. S1 to give dissociation constants of 20 mM or 34 mM for acetate and lactate respectively.



**Figure S22.** CEST spectra of solutions containing anion and 5.00 mM Eu(S-THP), 100 mM NaCl, 20 mM HEPES, at pH 7.0 (carbonate), 7.0 (phosphate), 7.2 (citrate), 7.0 methylphosphate (MMP).

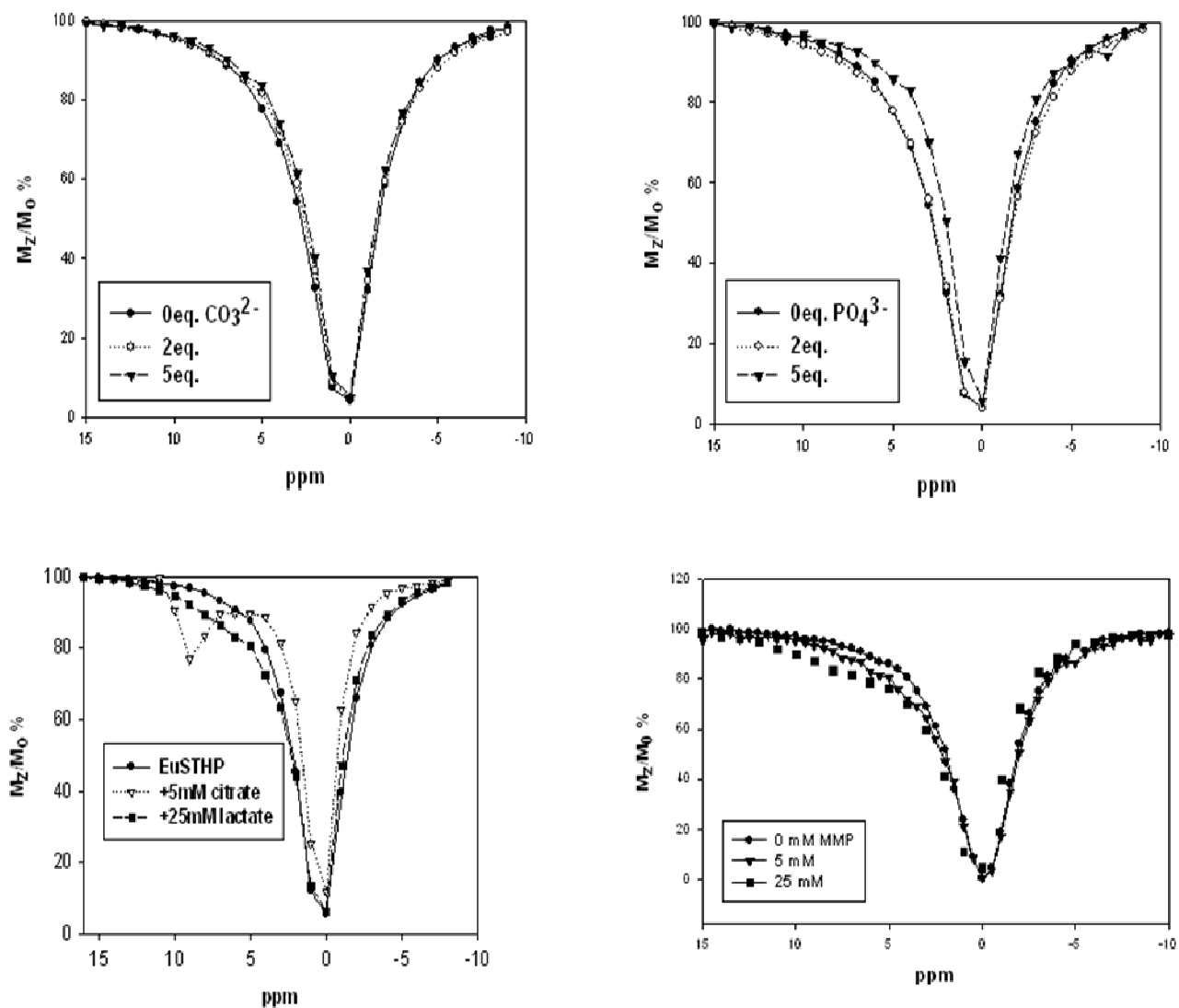
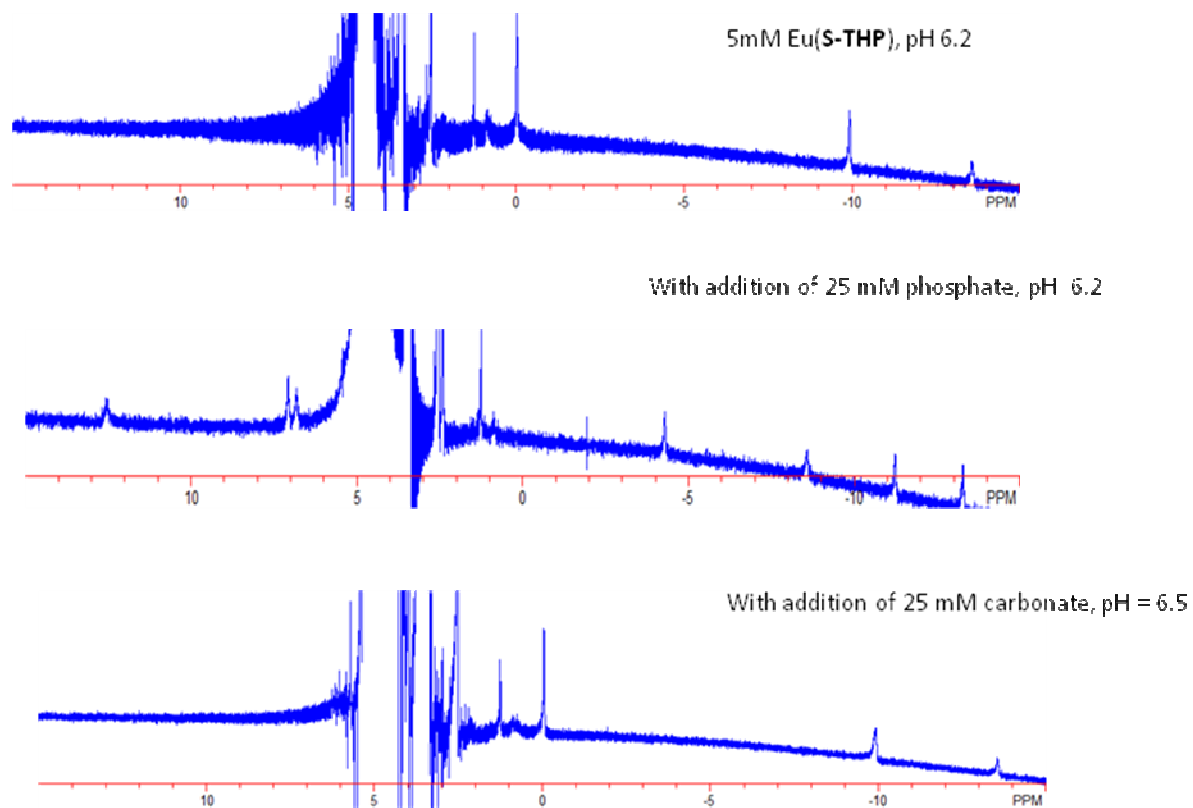
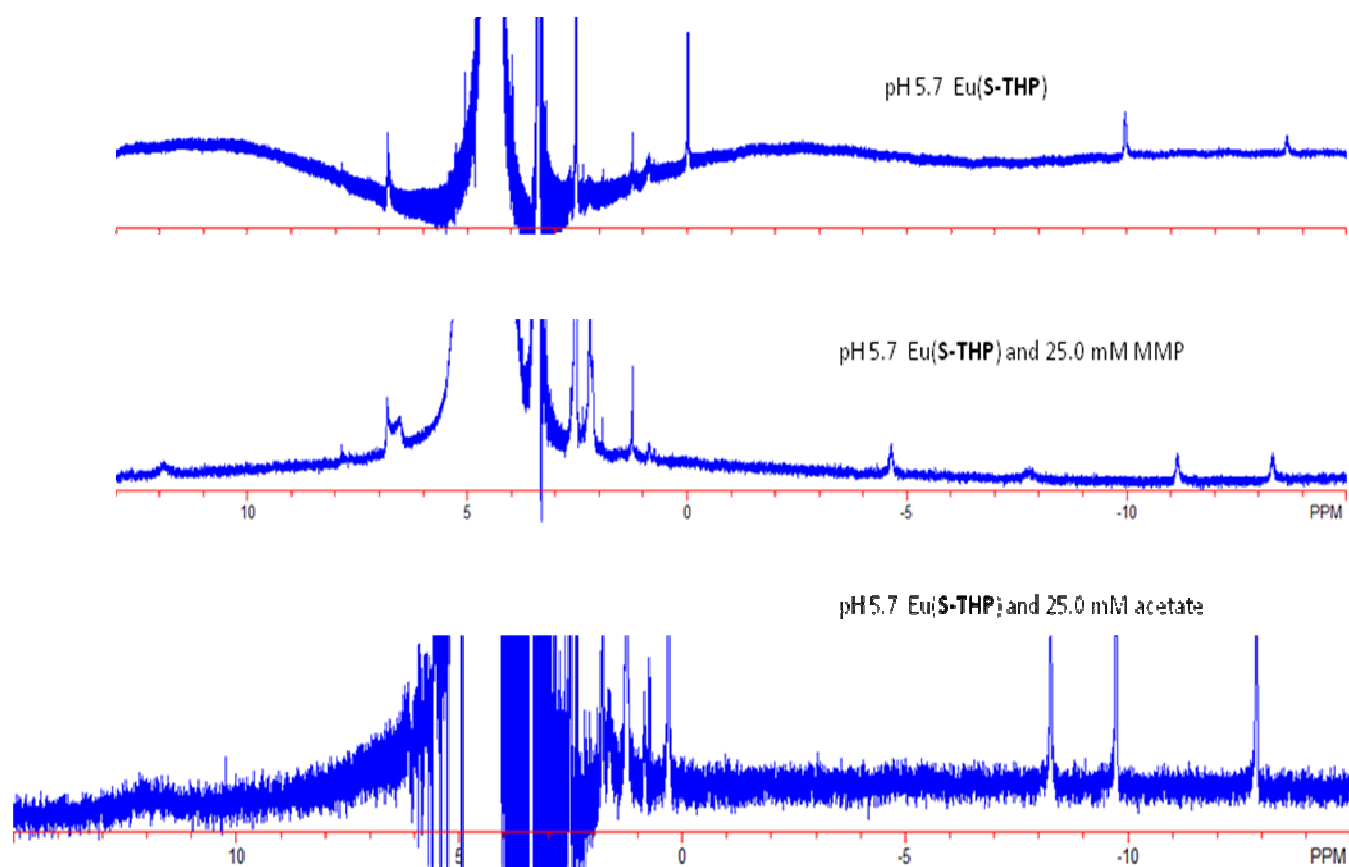
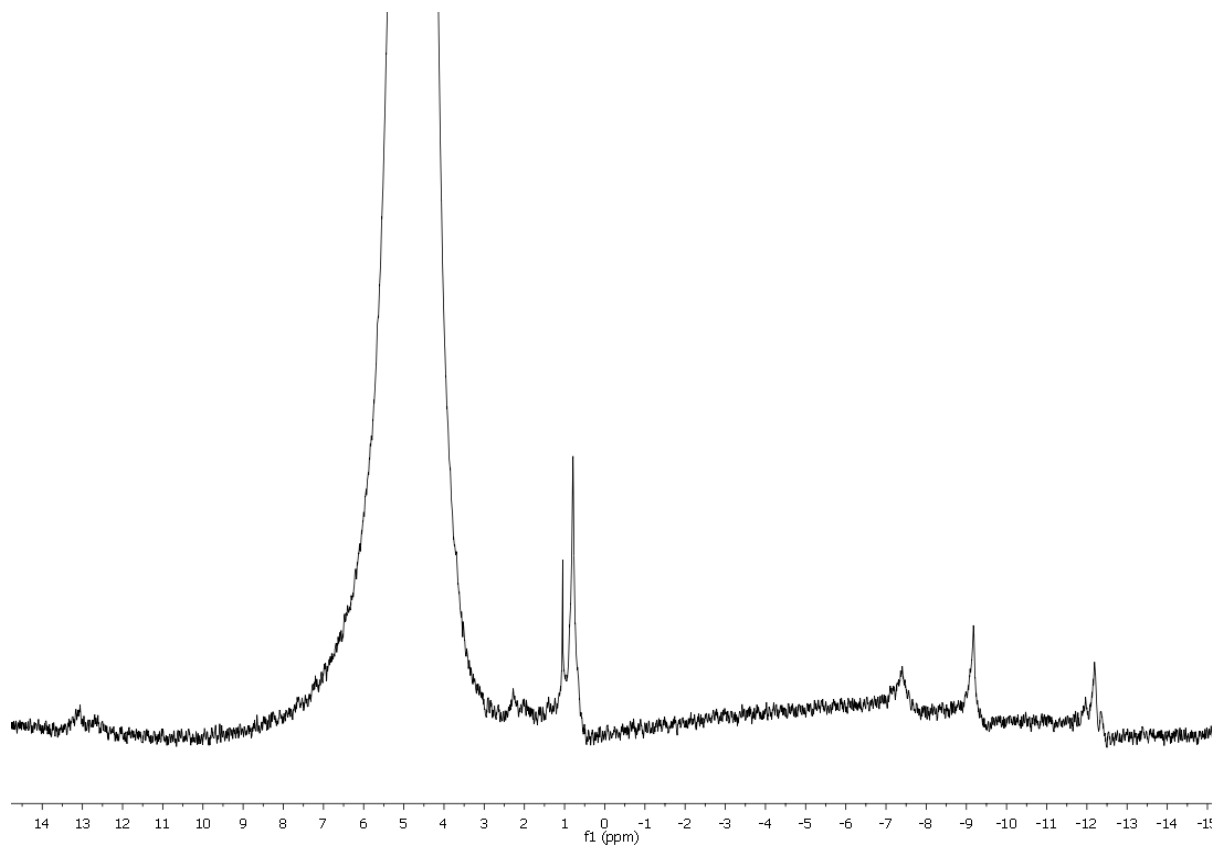


Figure S23.  $^1\text{H}$  NMR spectra of Eu(S-THP) with phosphate or carbonate in aqueous solution.



**Figure S24.**  $^1\text{H}$  NMR spectra of  $\text{Eu}(\text{S-THP})_3$  with methyl phosphate, acetate or citrate in aqueous solution.





**Figure S25.**  $^1\text{H}$  NMR spectra of 5 mM Eu(**S-THP**) with 5 mM citrate in aqueous solution, pH 5.5.