Electronic Supplementary Information

Europium(III) Complex-Based Luminescent Sensing Probes for Multi-phosphate Anions:

Modulating Selectivity by Ligand Choice

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1. Experimental Section

1.1 Materials. The tetradentate β-diketone derivatives, **L^1**-**L^4** were synthesized by using a previous method.[S1] The compounds were confirmed by element analysis and ^{1}H NMR. 1.0×10^{-3} M stock solutions of the organic ligands were prepared in ethanol. Work solutions of the ligands were obtained by diluting the stock solution with Tris/HCl buffer (pH 7.2). EuCl$_3$ solution was prepared by dissolving Eu$_2$O$_3$ in 0.01 M HCl and diluting with water. Stock solutions of 0.05 M of sodium salts of all kinds of anions were prepared by dissolving the appropriate amount of each compound in water. The aqueous solutions of all surfactants were prepared from analytical-reagent grade. Except as specified, all measurements were performed at room temperature, all other reagents were of analytical-reagent grade, and all aqueous solutions were prepared with double deionized water.

1.2 Absorption Spectroscopy. The UV spectroscopic titrations of the ligands with Eu^{3+} or PPi in the CPB micellar aqueous solution were undertaken in 5 mL flasks at pH 7.2. Ligand concentration was fixed and the ratio of Eu^{3+} to ligand was varying, and the corresponding UV-vis absorption spectra were recorded on a Hitachi U-3010 UV-Vis spectrophotometer (Kyoto, Japan) using quartz cells (1.0×1.0 cm^2 cross section).

1.3 Steady-State Luminescence Spectroscopy and Binding Constants. Steady-state luminescence spectroscopy was recorded with a Hitachi F-4500 spectrofluorometer (Kyoto, Japan). Excitation and emission slits with a nominal band pass of 10 nm were used for all measurements. The titration of Eu^{3+} with the ligand was performed using 1.0 cm path length quartz cuvettes with fixed concentration of Eu^{3+} (1.0×10^{-6} M) in Tris/HCl buffer at pH 7.2 and varying the ligand amount. The luminescence emission spectra were obtained by exciting at the maximal absorption wavelength of the ligand as determined by UV spectroscopy. The association constant of the metal complex was obtained using a curve fitting procedure, operating in the Sigma plot software. Assuming a 2 : 1 of ligand (**L**)-to-Eu^{3+} binding stoichiometry, according to the derivation reported previously,[S2] a response function for the ligand concentration can be given as

$$[L]^2 = \frac{1}{K} \cdot \frac{1-\alpha}{\alpha} \qquad (S1)$$

where [L] denotes the ligand concentration. The α term is a response parameter that can be experimentally determined by measuring the Eu^{3+} luminescence intensity changes,

$$\alpha = \frac{F_{obs} - F_{init}}{F_{final} - F_{init}} \qquad (S2)$$

where F_{obs} is the Eu^{3+} luminescence intensity at 612 nm in the presence of different concentrations of ligand, and F_{init} and F_{final} are the limiting values of the Eu^{3+} luminescence intensity at zero ligand concentration and at final (plateau) concentration, respectively. Combining eqs of S1 and S2, the dependence of fluorescence intensity on the L concentration is taken, allowing determination of binding constant via curve fitting.

The anion spectrofluorimetric titrations were carried out in the buffer solution by adding different concentrations of anions to the buffer solution containing the Eu^{3+} complexes in CPB. After completely mixing the components, luminescence emission spectra were obtained by exciting at the maximal absorption wavelength of the ligand as determined by UV spectroscopy. The obtained data of the luminescent intensity of $\Delta J = 2$ manifold (centered at 612 nm) were analyzed for association constant, K, by curve fitting analysis. Assuming a $n : 1$ of anion (X)-to-Eu^{3+} binding stoichiometry, the over-all equilibrium between the Eu^{3+} ligation complex and X can be represented as following:

$$EuL_2 + nX \; \underset{}{\overset{K_x}{\rightleftharpoons}} \; EuX_n + 2L \qquad (S3)$$

According to the derivation reported,[S2] a response function for the anion concentration can be given as

$$[X]^n = \frac{4 \cdot C_T^2}{K_x} \cdot \frac{(1-\alpha)^3}{\alpha} \qquad (S4)$$

where [X] denotes the anion concentration, C_T is the initial concentration of EuL_2 (for simpleness, charge number of anion and Eu^{3+} complexes in eqs of S3 and S4 are omitted). The α term is a response parameter that can be experimentally determined by measuring the Eu^{3+} luminescence intensity changes,

$$\alpha = \frac{F_{obs} - F_{final}}{F_{init} - F_{final}} \qquad (S5)$$

where F_{obs} is the Eu^{3+} luminescence intensity at 612 nm in the presence of different concentrations of anion, and F_{init} and F_{final} are the limiting values of the Eu^{3+} luminescence intensity at zero anion concentration and at final (plateau) anion concentration, respectively. Combining eqs of S4 and S5 the dependence of luminescence intensity on the anion concentration is taken, allowing determination of stoichiometric ratio and binding constant via curve fitting (see below).

1.4 Determination of Luminescence Quantum Yield (Φ): For measurement of the quantum yields of the europium complexes, the complex solutions at pH 7.2 was adjusted to an absorbance of ~0.05. The emission spectra were recorded with the excitation wavelength of 335 nm, and the integrated areas of the

luminescence corrected spectra were measured. The quantum yields were then calculated by comparison with quinine sulfate as reference using the following equation,[S3]

$$\Phi = \frac{I}{I_R} \cdot \frac{A_R}{A} \cdot (\frac{n}{n_R})^2 \cdot \Phi_R \qquad (S6)$$

where Φ is the quantum yield, I is the integrated area under the luminescence spectra. A is the absorbance, n is the refractive index of the solvent, and R refers to the reference fluorophore, quinine sulfate. $\Phi_R = 0.56$ in 0.1M sulfuric acid was used as the reference quantum yield.[S4]

1.5 ^{31}P NMR. The ^{31}P NMR spectra were obtained with an Invoa-400 (Invoa 400, 400 MHz) spectrometer at 298 K. The samples were placed into a 4 mm broadband/1H dual-frequency magic-angle-spinning probehead and the spectra were acquired at 400 MHz. The chemical shifts were referenced relative to external H_3PO_4 85% (0 ppm).

References

[S1] J. Yuan, K. Matsumoto, H. Kimura, *Anal. Chem.*, 1998, **70**, 596-601; F. B. Wu, C. Zhang, *Anal. Biochem.*, 2002, **311**, 57-67.

[S2] Y. Zhang, R. H. Yang, F. Liu, K. A. Li, A. *Anal. Chem.*, 2004, **76**, 7336-7345.

[S3] S. Aromori, M. L. Bell, C. S. Oh, K. A. Frimat, T. D. James, *J. Chem. Soc., Perin, Trans. 1*, 2002, 803-808; M. Onoda, S. Uchiyama, T. Santa, K. Imai, *Anal. Chem.*, 2002, **74**, 4089-4096.

[S4] W. H. Melhuish, *J. Sci. Technol. B,* 1955, **37**, 142-149.

## 2. **The Molecular Model of the Luminescent Recognition of PPi**
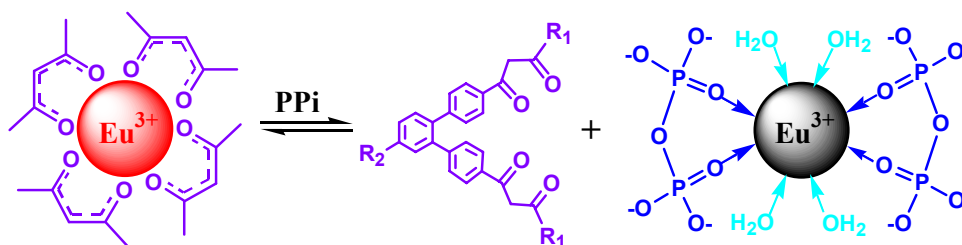
3. Spectroscopic Data

3.1 UV absorption spectra of 5.0×10^{-6} M $\mathbf{L^1}$ in 5.0×10^{-5} M CPB micellar solutions at pH 7.2: (a) $\mathbf{L^1}$ alone, (b) $\mathbf{L^1}$ + Eu^{3+}, (c) $\mathbf{L^1}$ + Eu^{3+} + PPi. [Eu^{3+}] = 2.5×10^{-6} M and [PPi] = 5.0×10^{-6} M.
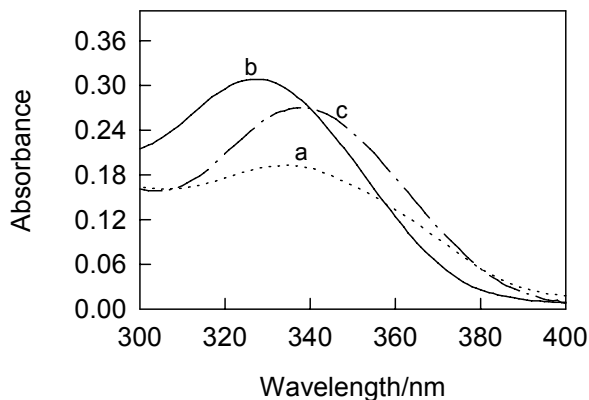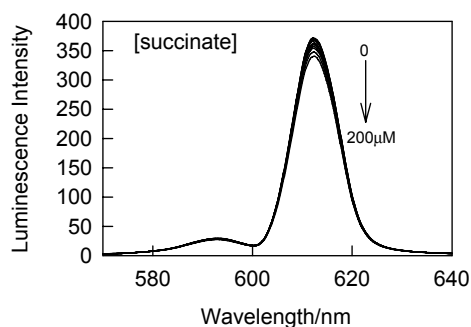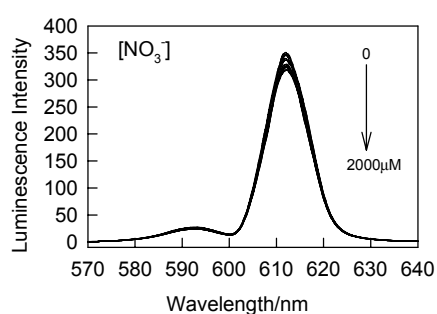

3.2 Luminescence emission spectra of 1.0×10^{-6} M Eu^{3+} (λ_{ex}=335 nm) in 5.0×10^{-5} M CPB in the presence of different concentrations of $\mathbf{L^1}$-$\mathbf{L^4}$.
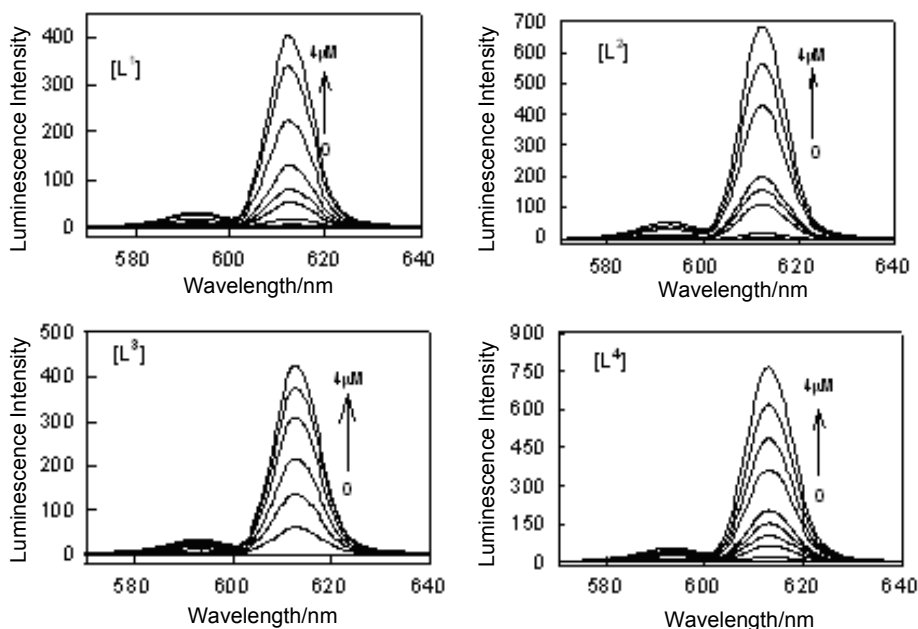
3. 3 Luminescence responses of $\mathbf{L^1}$-Eu^{3+} in 5.0×10^{-5} M CPB to selected substrates. [Eu^{3+}] = 1.0×10^{-6} M, [$\mathbf{L^1}$] = 2.0×10^{-6} M. The arrows indicate the signal changes as increasing in the anion concentration.

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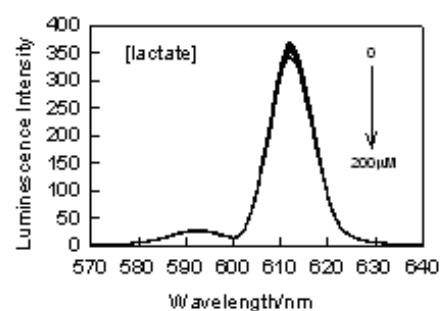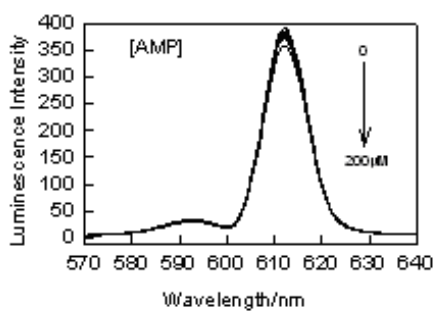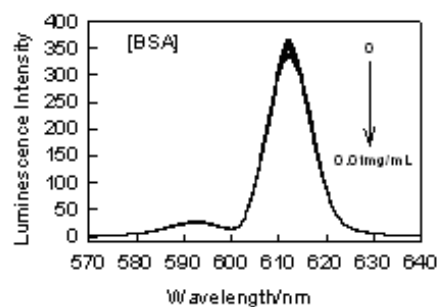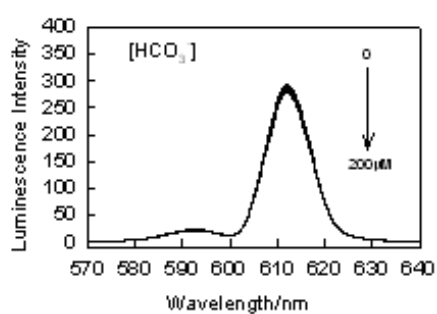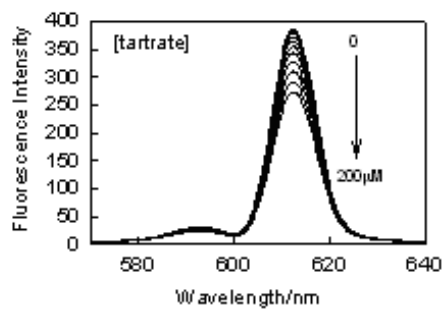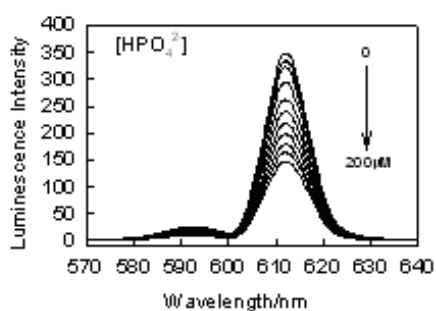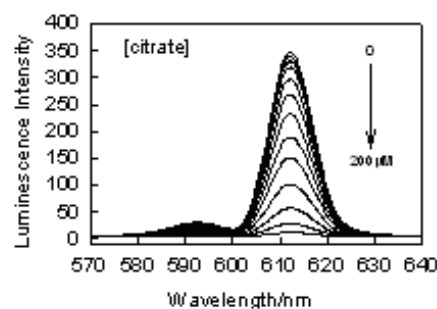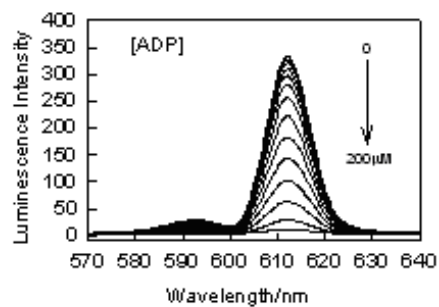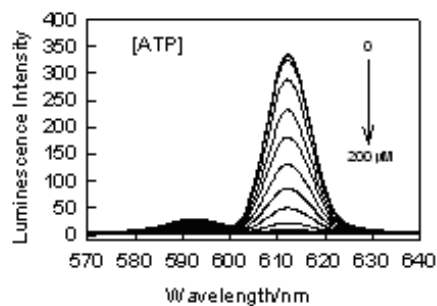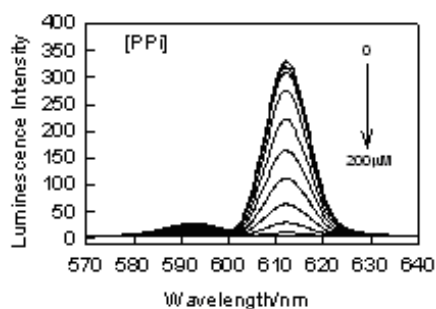
3.4 Luminescence responses of $\mathbf{L^2}$-Eu^{3+} in 5.0×10^{-5} M CPB to selected substrates. $[Eu^{3+}] = 1.0 \times 10^{-6}$ M, $[\mathbf{L^2}]$ $= 2.0 \times 10^{-6}$ M. The arrows indicate the signal changes as increasing in the anion concentration.
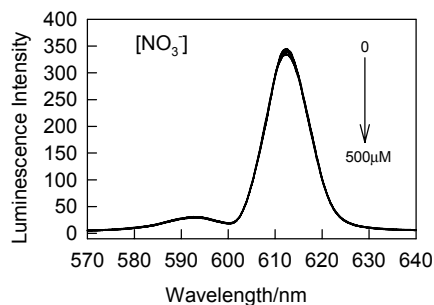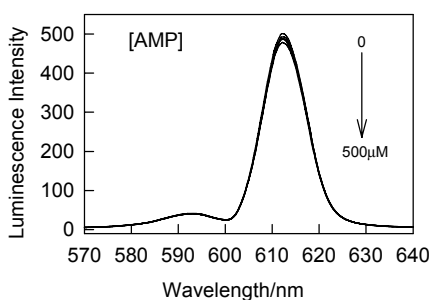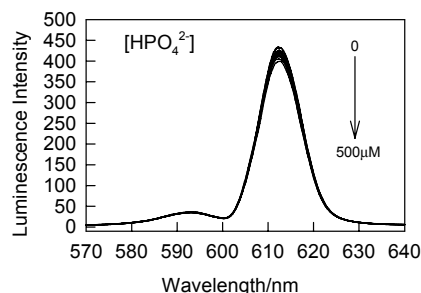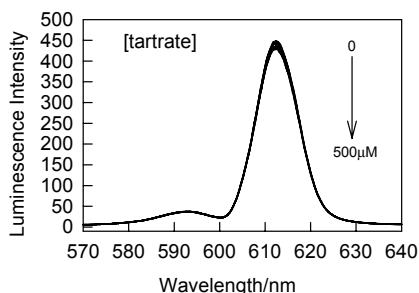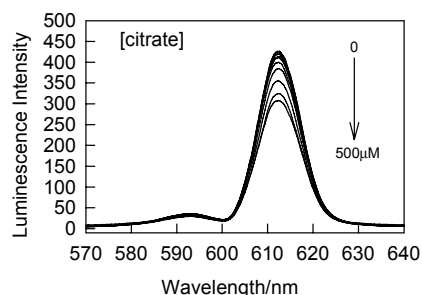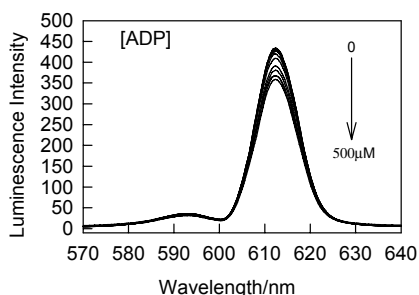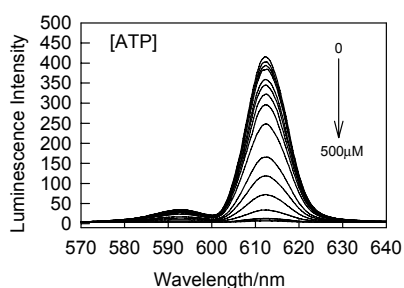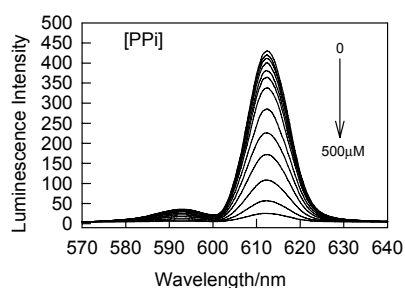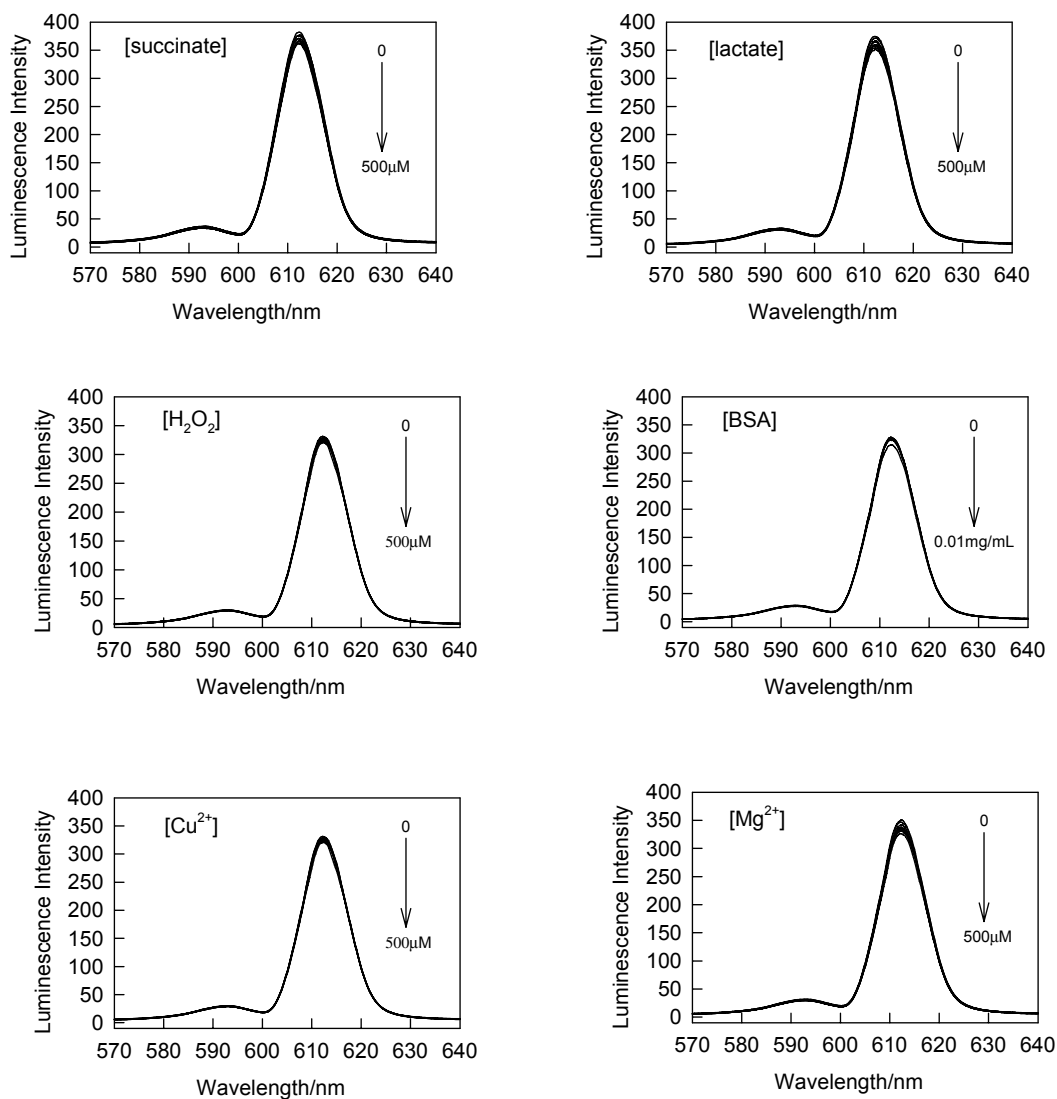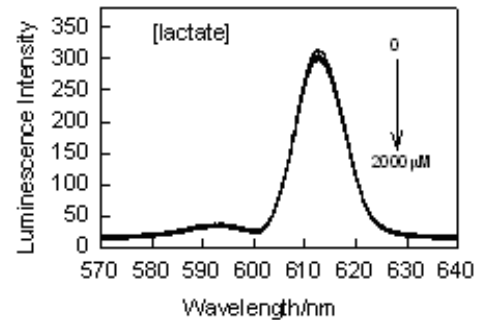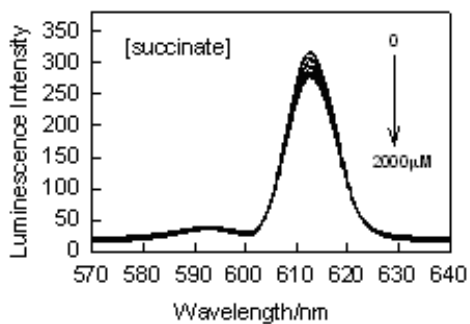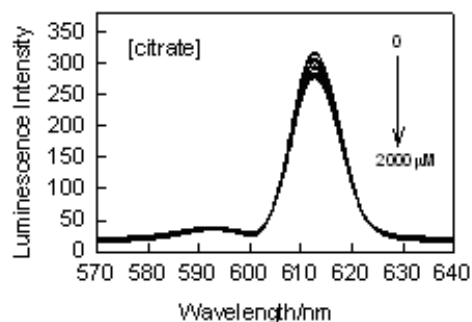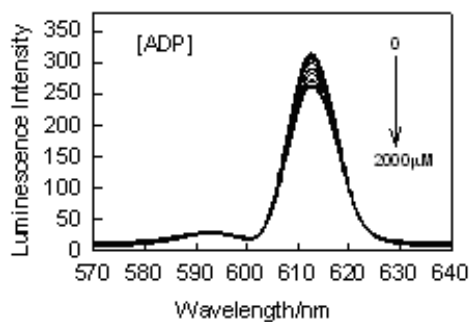
3.5 Luminescence responses of L^3-Eu^{3+} in 5.0×10^{-5} M CPB to selected substrates. $[Eu^{3+}] = 1.0 \times 10^{-6}$ M, $[L^3]$ $= 2.0 \times 10^{-6}$ M. The arrows indicate the signal changes as increasing in the anion concentration.
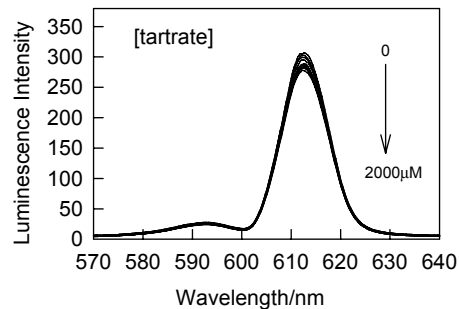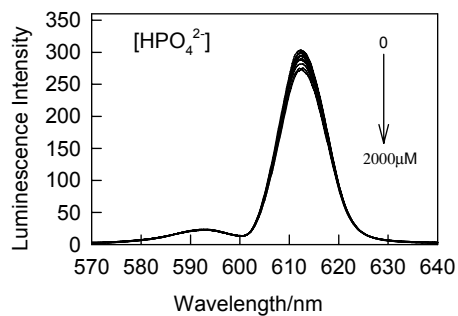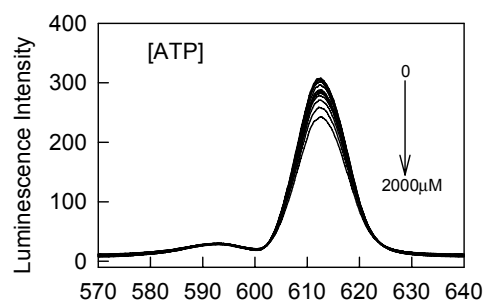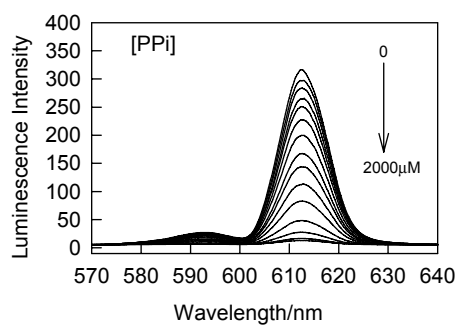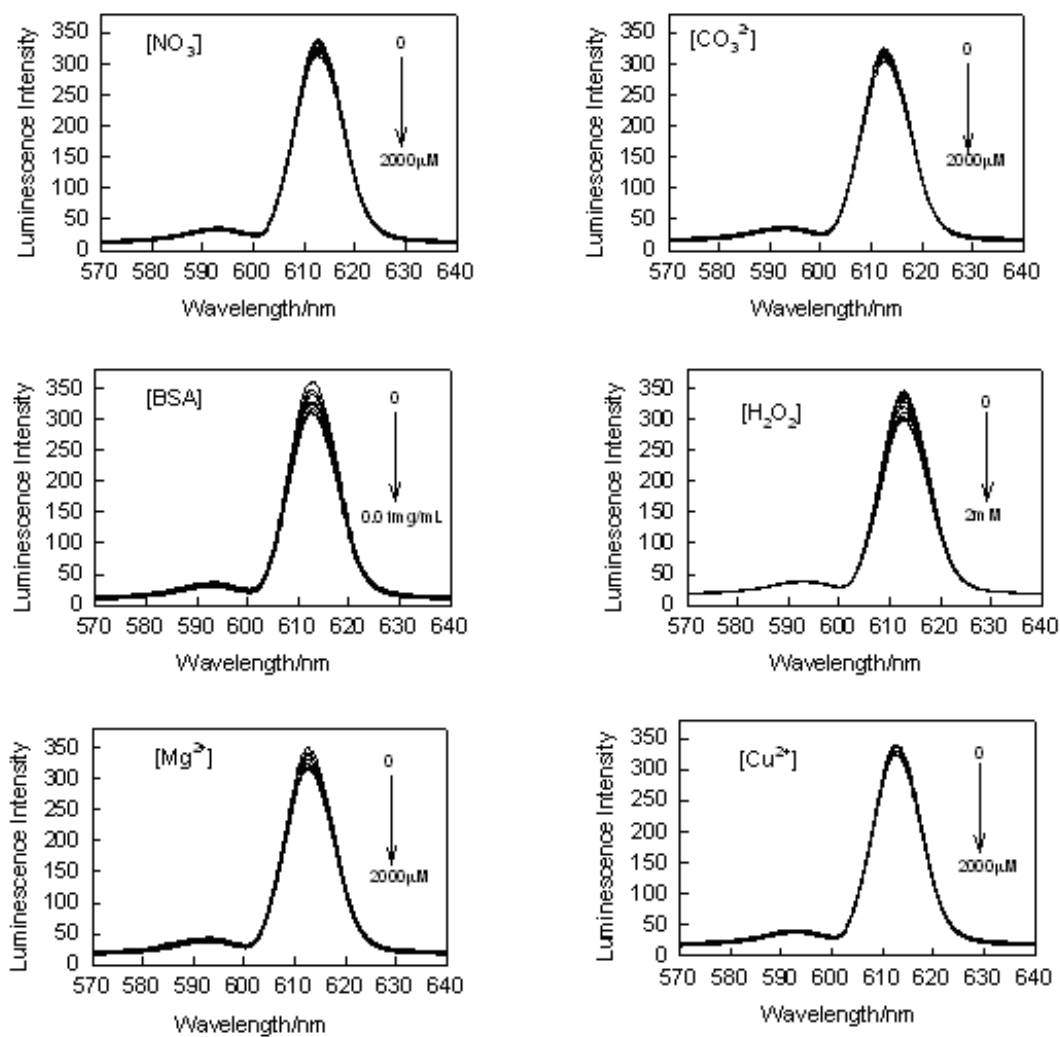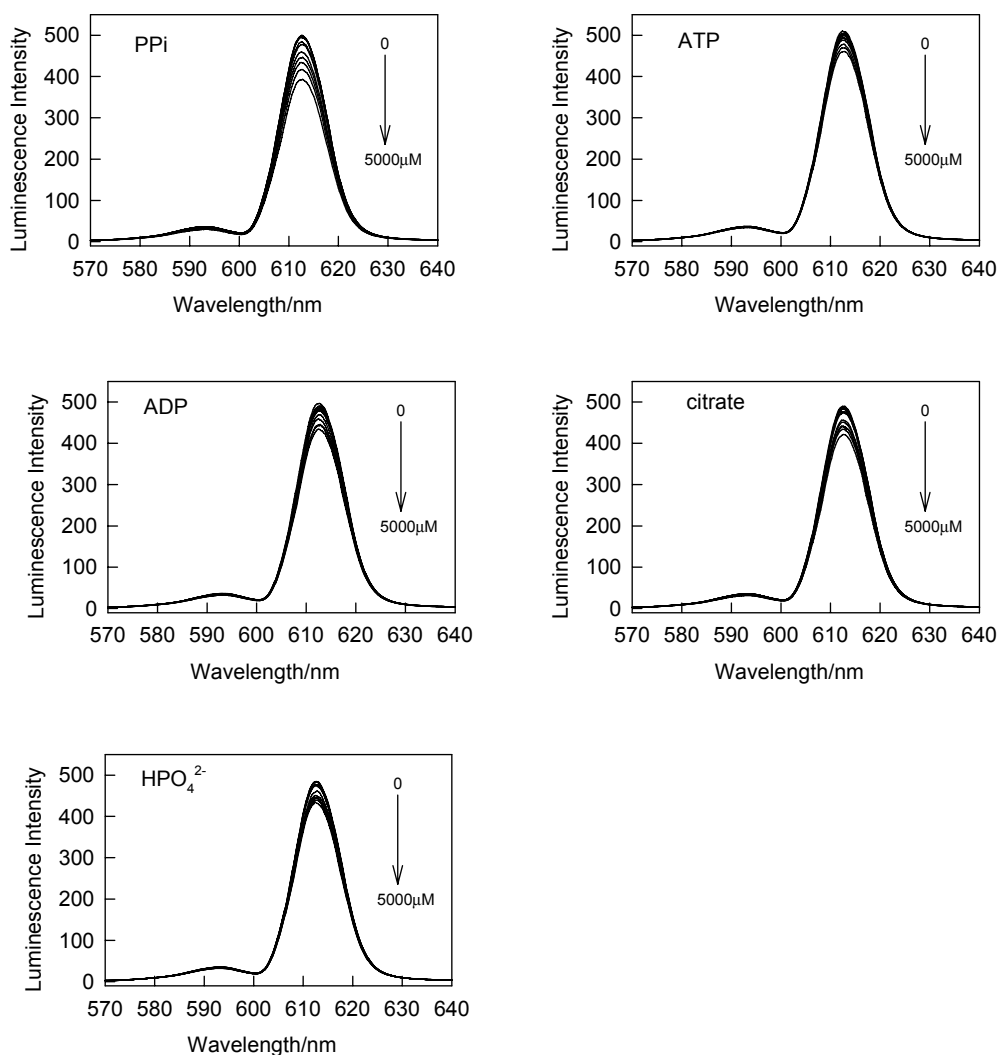
3. 6 Luminescence responses of L^4-Eu^{3+} in 5.0×10^{-5} M CPB to selected substrates. $[Eu^{3+}] = 1.0 \times 10^{-6}$ M, $[L^4] = 2.0 \times 10^{-6}$ M. The arrows indicate the signal changes as increasing in the anion concentration.
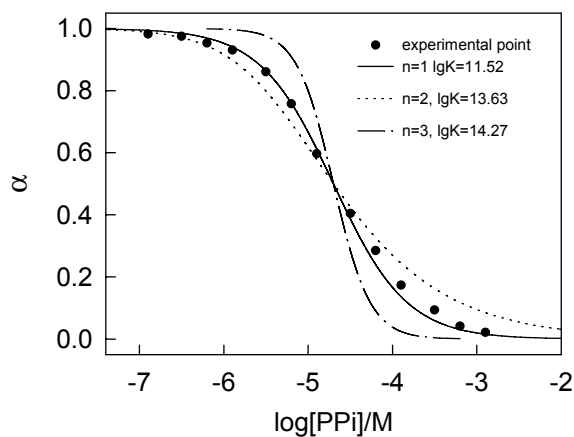
3.7 Response parameter values (α) as a function of the logarithm of PPi concentrations at pH 7.2. Theoretical response of PPi was predicated by eqs. (S4) and (S5). The experimental data ($\bullet$) were fitted to the equation with the different complex ratios and binding constants. The curve referring to $n = 2$ and log K=13.63 is the best one fitted to the experimental data.

3.8 Luminescence excitation spectra (λ_{em} = 612 nm) and emission spectra (λ_{ex} = 335 nm) of Tris/HCl buffer solutions containing $\mathbf{L^1}$-Eu^{3+} ([Eu^{3+}] = 1.0 × 10^{-6} M, [$\mathbf{L^1}$] = 2.0 × 10^{-6} M) in the absence and presence of 5.0 ×10^{-5} M CPB.
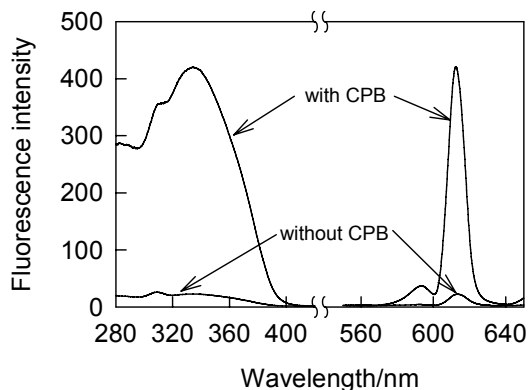
3. 9 Luminescence response of $\mathbf{L^1}$-Eu^{3+} ([Eu^{3+}] = 1.0 × 10^{-6} M, [$\mathbf{L^1}$] = 2.0 × 10^{-6} M) to different concentrations of PPi in Tris/HCl buffer solutions at pH 7.2 in the absence of surfactant.
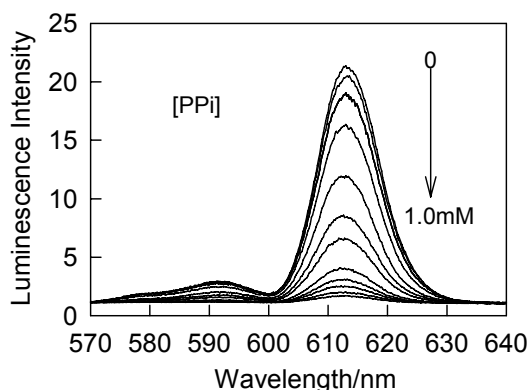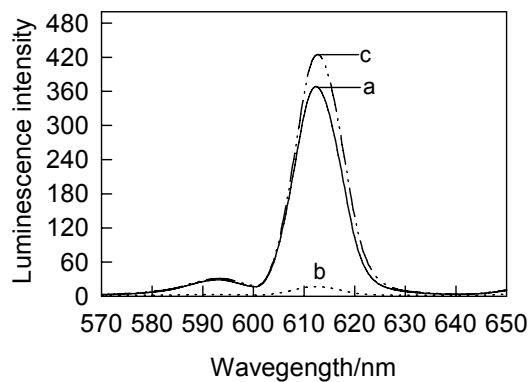

3.10 Luminescence intensity-pH profiles for titrations of the buffered-CPB solution containing 1.0 × 10^{-6} M Eu^{3+} and 2.0 × 10^{-6} M $\mathbf{L^3}$ in the absence (a) and the presence (b) of 1.0 ×10^{-5} M PPi. The excitation was at 334 nm, and emission was recorded at 612 nm.
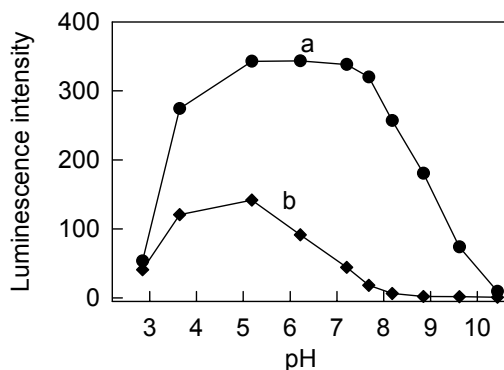
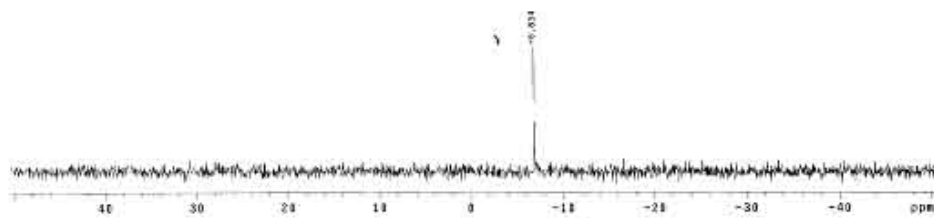**3. 11** Luminescence emission spectra of $\mathbf{L^1}$-Eu^{3+} in: (a) CPB micelles, (b) (a) + 1.0 ×10^{-5} M PPi, and (c) (b)

+ 2.0×10^{-6} M $\mathbf{L^4}$. $[\mathbf{L^1}]$ = 2.0×10^{-6} M, $[Eu^{3+}]$ = 1.0×10^{-6} M. λ_{ex} = 335 nm.

3.12 ^{31}P NMR of PPi alone (a), PPi + Eu^{3+} (b), and $\mathbf{L^3}$ + Eu^{3+} + PPi (c)
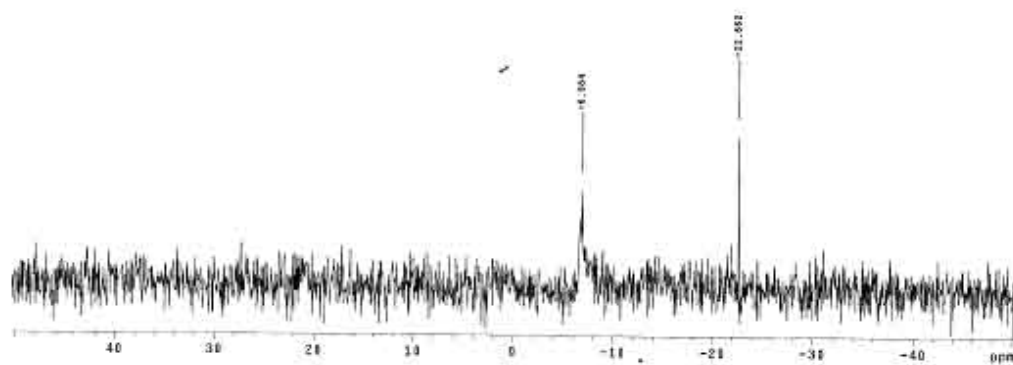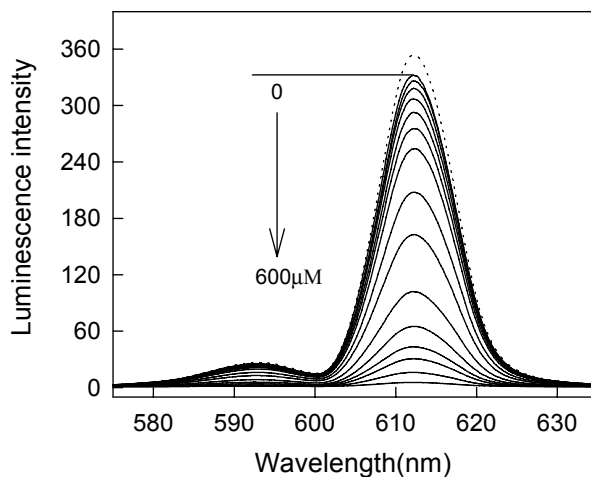
a)

b)
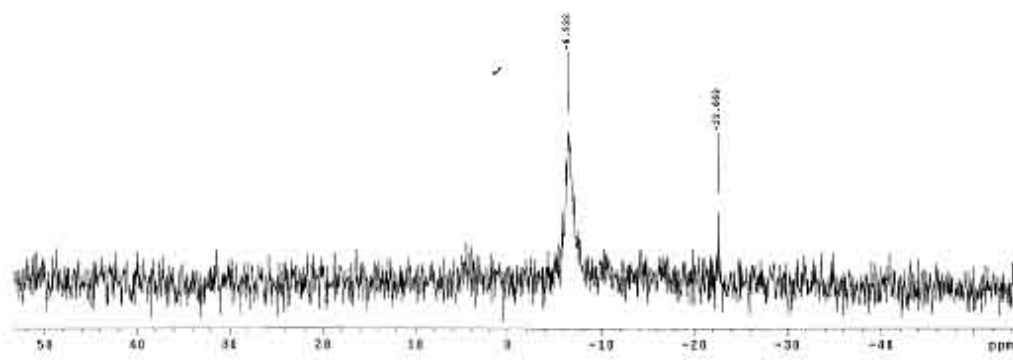
c)

3.13. Emission spectra of L^3-Eu^{3+} solution (dash line) and the solution containing fixed amounts of competitive substrates with increasing concentrations of PPi (0, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10, 20, 50, 100, 200, 400, and 600 µM). λ_{ex} = 334 nm.

3.14. Hydrolysis kinetic curves of PPi at different PPase concentrations using L^2-Eu^{3+} at pH 7.2: (a) 0.3 units; (b) 0.6 units; (c) 1.8 units; (d) 3.6 units; (e) 5.4 units; and (f) 7.2 units. λ_{ex} = 334 nm, λ_{em} = 612 nm.