Sensing of biologically relevant d-metal ions using Eu(III)-cyclen based luminescent displacement assay in aqueous pH 7.4 buffered solution

Oxana Kotova,* Steve Comby and Thorfinnur Gunnlaugsson*

Electronic Supplementary Information

Experimental Section

Materials and Methods. All solvents and chemicals were purchased from commercial sources and used without further purification. Water was purified using a Millipore Milli-Q water purification system. **BPS** (4,7-Diphenyl-1,10-phenanthroline-disulfonic acid disodium salt trihydrate, puriss. p.a., ≥99.0 %) was purchased from Fluka and $MCl_2 \cdot xH_2O$ (M = Cd(II), Ca(II), Mg(II), Cu(II), Co(II), Ni(II) and Fe(II) with $x = 0, 2, 4$ or 6), $MCl_3 \cdot 6H_2O$ (M = Fe(III), Cr(III)) and $Zn(ClO_4)_2 \cdot 6H_2O$ were purchased either from Sigma or Aldrich, as well as BDH Ltd, Poole, England for the Co(II) chloride. Sodium hydrogen carbonate, sodium L-lactate, sodium citrate tribasic dihydrate, sodium phosphate dibasic were purchased from Fischer Scientific, Aldrich, Sigma-Aldrich and Sigma, respectively. The Hepes buffer (titration, ≥99.5 % from Sigma) solution was prepared by dissolving 4-(2 hydroxyethyl)piperazine-1-ethanesulfonic acid (Hepes, 0.1 M) and NaCl (ionic strength, 0.1 M) in Millipore water before the pH was adjusted to 7.4 with NaOH.

The cyclen complex, **1·Eu**, was synthesised and characterised according to Massue *et al.*[1,2]

Photophysical measurements. Otherwise stated, all measurements were performed at 298 K in Hepes buffer at pH 7.4, while the ionic strength was kept constant by the addition of NaCl (0.1 M). UV-visible absorption spectra were measured in 1-cm quartz cuvettes on a Varian Cary 50 spectrophotometer. Baseline correction was applied for all spectra. Emission (fluorescence, phosphorescence and excitation) spectra and lifetimes were recorded on a Varian Cary Eclipse Fluorimeter. Quartz cells with a 1 cm path length from Hellma were used for these measurements. The temperature was kept constant throughout the measurements at 298 K by using a thermostated unit block. Phosphorescence lifetimes of the Eu($^5D_0$) excited state were measured in both water and deuterated water in time-resolved mode at 298 K. They are averages of three independent measurements, which were made by monitoring the emission decay at 616 nm, which corresponds to the maxima of the Eu(III) $^5D_0 \rightarrow ^7F_2$ transition, enforcing a 0.1 ms delay, and were analyzed using Origin 7.5®. The number of coordinated water molecules (q) for the ternary complex **1·Eu·BPS** was calculated from the equation $q = A\left(\left(\tau_{H2O}^{-1} - \tau_{D2O}^{-1}\right) - k_{corr}\right)$, where τ_{H2O} and τ_{D2O} are the lifetimes in H_2O and D_2O, respectively and with A = 1.11, k_{corr} = 0.31 ms^{-1} [3] or with A = 1.2, k_{corr} = 0.25 ms^{-1}.[4]

Spectrophotometric titrations and binding constants. The formation of the luminescent 1:1 ternary complex, **1·Eu·BPS**, was ascertained by both UV-visible and luminescence titrations of a solution of **1·Eu** (10^{-5} M) with **BPS** (0→8 equivalents). The data were fitted using the non-linear regression analysis program, SPECFIT®.[5,6] The binding constant determined were confirmed by linear fitting using the following equation:[7]

$$\log\left(\frac{F - F_{\min}}{F_{\max} - F}\right) = \log K_a + n \cdot \log[BPS]$$

, where F is the emission intensity, $F_{\min}$, the minimum emission intensity at zero [**BPS**] and $F_{\max}$, the maximum emission intensity at saturating [**BPS**], *i.e.* when each **1·Eu** formed a ternary complex with **BPS**.

The **1·Eu·BPS** system was then titrated with alkaline earth and transition metals (0→10 equivalents). In a typical experiment, one equivalent of **BPS** was added prior titration to a 2.7 mL Hepes-buffered solution of **1·Eu** 10^{-5} M. After each metal addition, UV-visible, fluorescence, phosphorescence and excitation spectra were recorded at 298 K.

FIGURES

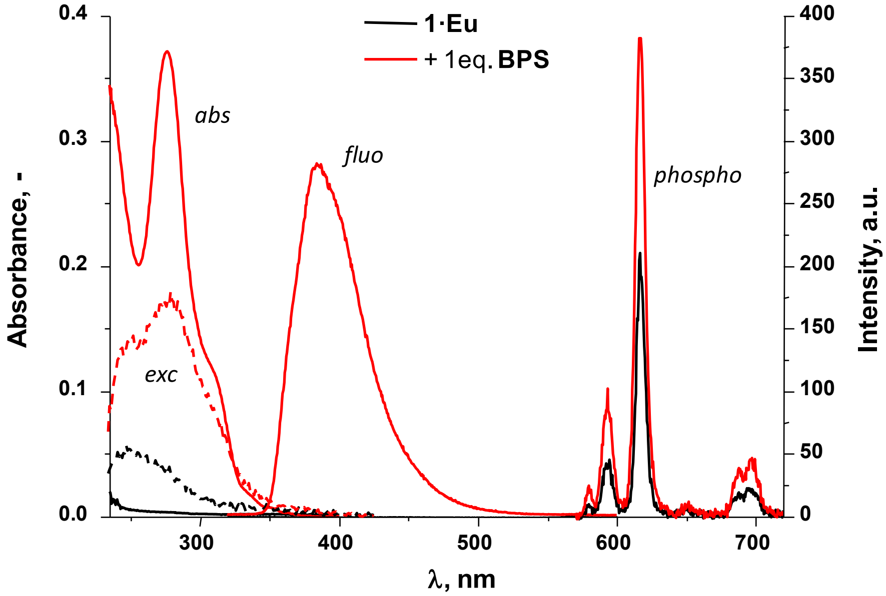

Figure S1. Absorption, emission (λ_{ex} = 278 nm) and excitation (λ_{an} = 616 nm) spectra of **1·Eu** in Hepes-buffered solution in the absence (black curves) and presence (red curves) of 1 equivalent of **BPS**.

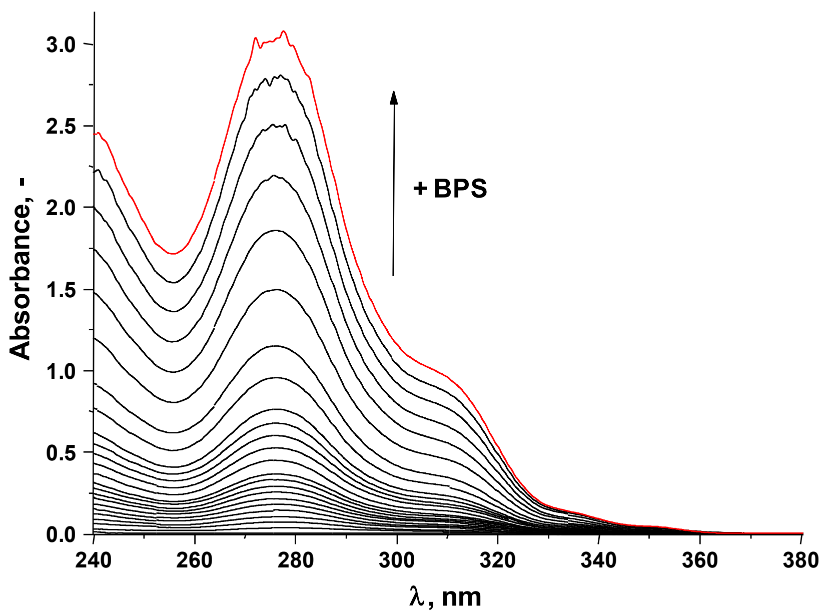

Figure S2. The evolution of the absorption spectrum of **1·Eu** (10^{-5} M) in Hepes-buffered solution (pH 7.4) upon addition of **BPS** (0→8 equivalents).

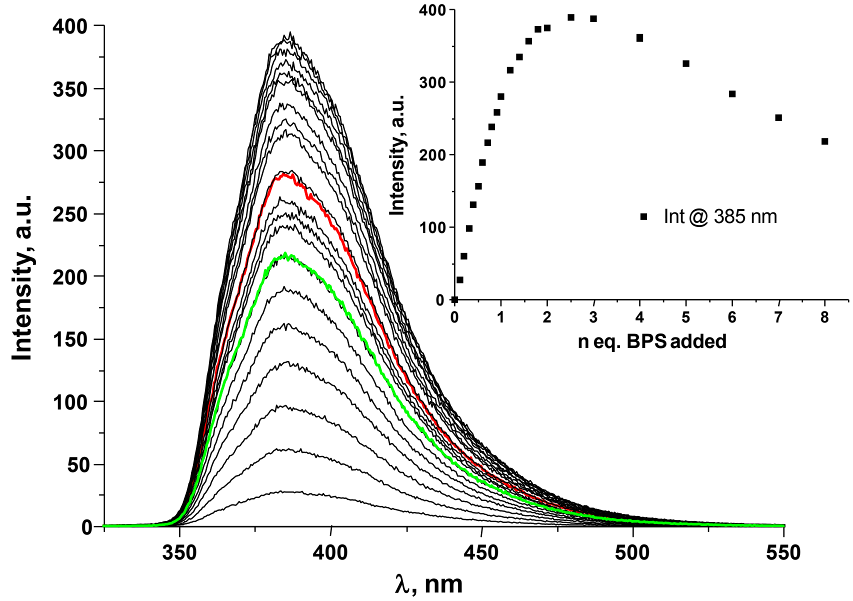

Figure S3. The evolution of the fluorescence emission of **1·Eu** (10^{-5} M) in Hepes-buffered solution (pH 7.4) upon addition of **BPS** (0→8 equivalents). Inset: The changes in the fluorescence intensity at 385 nm *vs.* equivalents of **BPS** added.

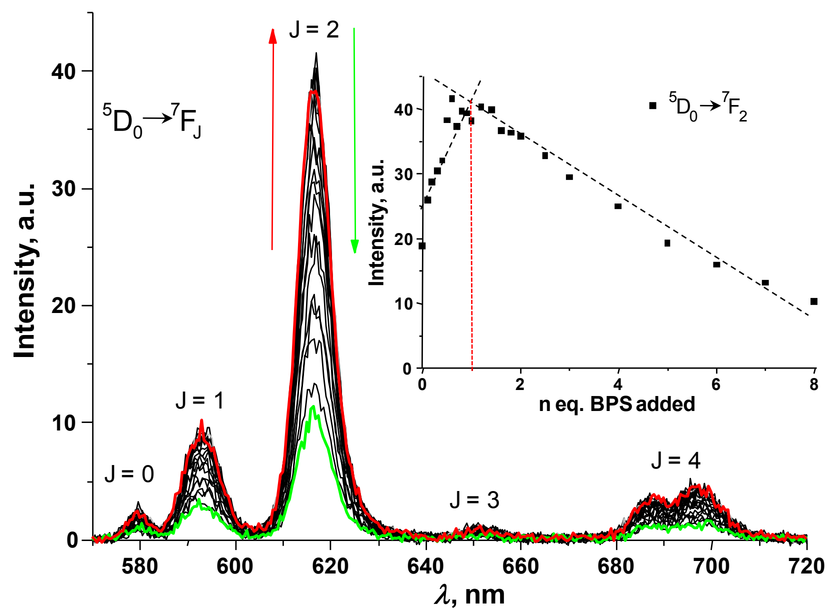

Figure S4. The evolution of the Eu(III)-centred emission of **1·Eu** (10^{-5} M) in Hepes-buffered solution (pH 7.4) upon addition of **BPS** (0→8 equivalents). Inset: The changes in the emission intensity of the Eu(III) $^5D_0 \rightarrow ^7F_2$ transition (at 616 nm) *vs.* equivalents of **BPS** added.

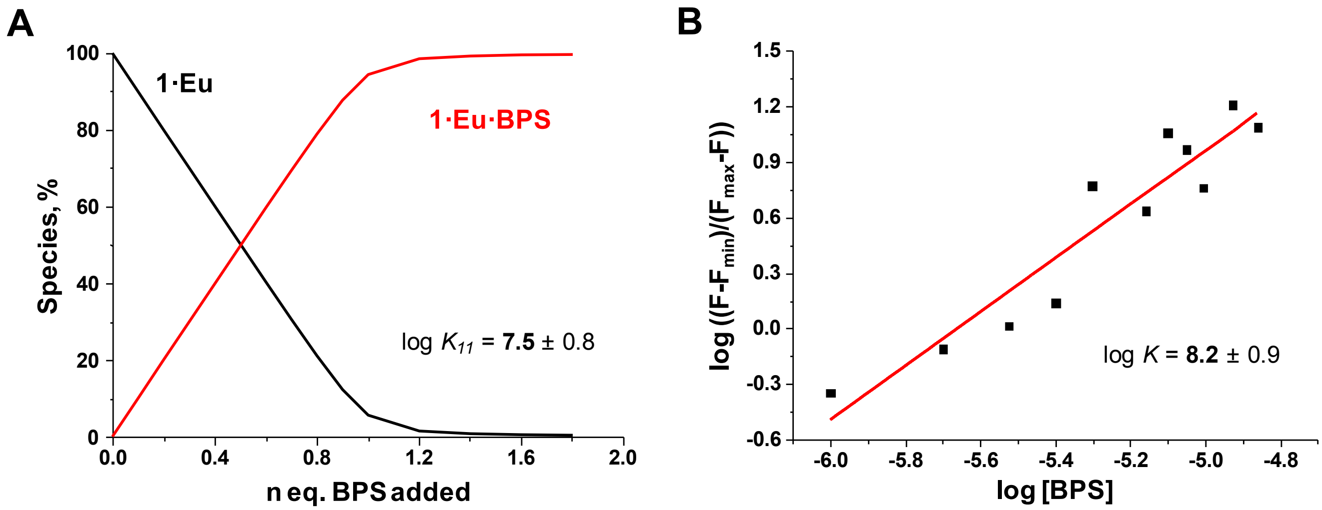

Figure S5. **A)** Speciation-distribution diagram obtained using SPECFIT® for the system **1·Eu·BPS**. **B)** The double logarithm plot of the Eu(III) emission in the presence of increasing amounts of **BPS** and the corresponding linear fitting (see Experimental Section).

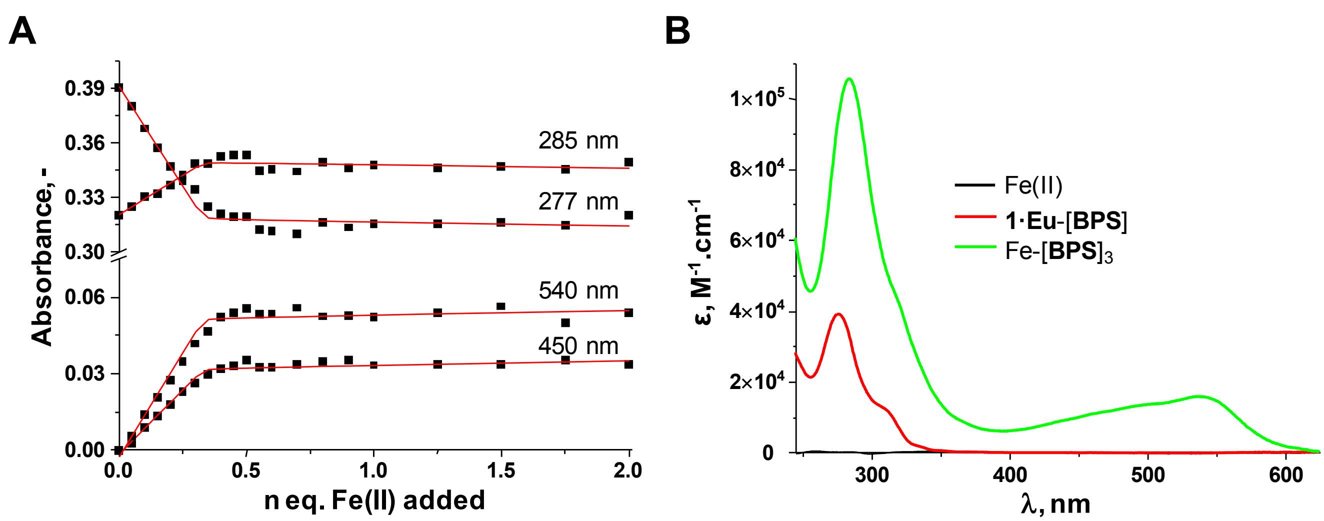

Figure S6. **A)** Experimental binding isotherms (····) for the UV-visible titration of **1·Eu·BPS** (10^{-5} M) with Fe(II) (0→4 equivalents) and their corresponding fit by means of SPECFIT® (—). **B)** Recalculated spectra for the three absorbing species in solution at pH 7.4, namely Fe(II), **1·Eu·BPS** and Fe:**BPS** in a 1:3 stoichiometric ratio.

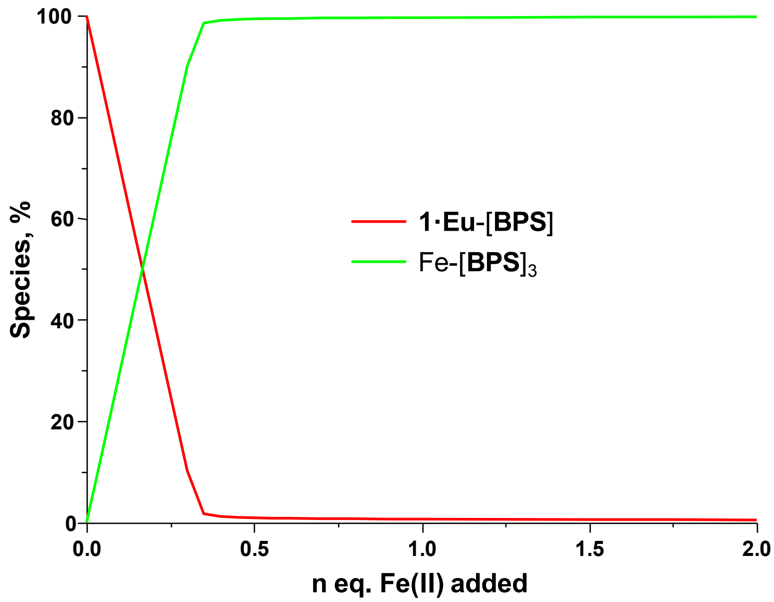

Figure S7. Speciation-distribution diagram obtained using SPECFIT® for the ternary complex **1·Eu·BPS** (10^{-5} M) in the presence of increasing amounts of Fe(II).

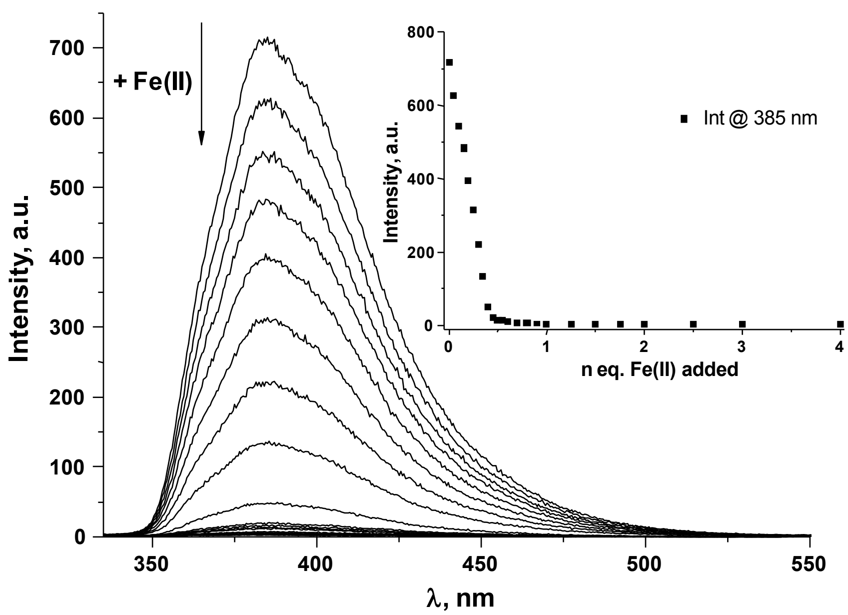

Figure S8. The evolution of the fluorescence emission of **1·Eu·BPS** (10^{-5} M) in Hepes-buffered solution (pH 7.4) upon addition of Fe(II) (0→4 equivalents). Inset: The changes in the fluorescence intensity at 385 nm *vs.* equivalents of Fe(II) added.

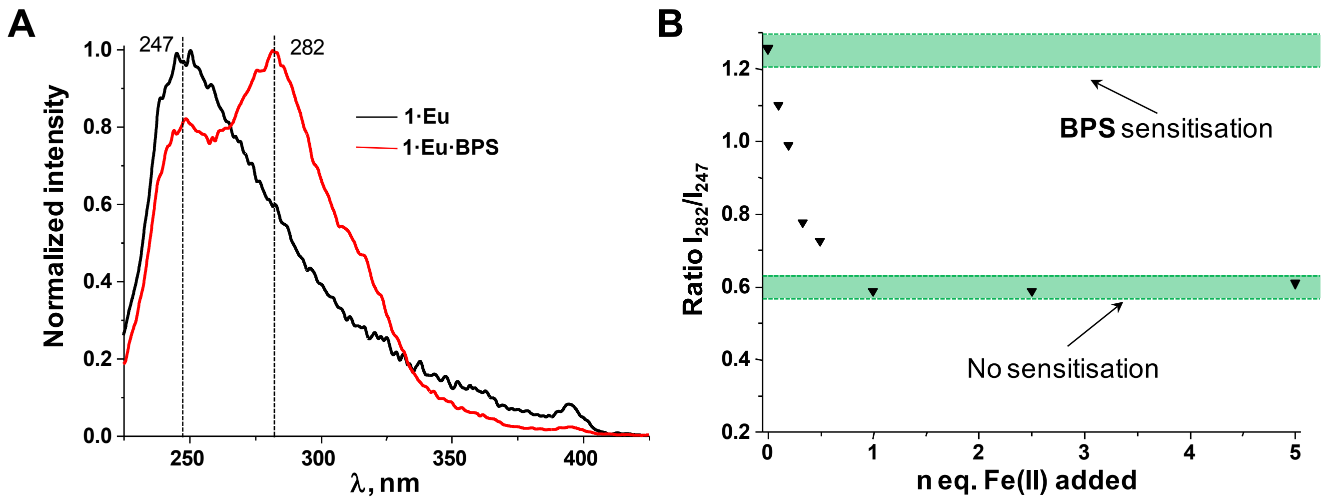

Figure S9. **A)** Normalized excitation spectra of **1·Eu** and **1·Eu·BPS** (λ_{an} = 616 nm). **B)** Ratio of the intensities monitored at 282 nm and 247 nm upon addition of Fe(II) (0→4 equivalents), with the green bands representing the range of values for which no sensitization (**1·Eu**) or sensitization through the **BPS** is observed.

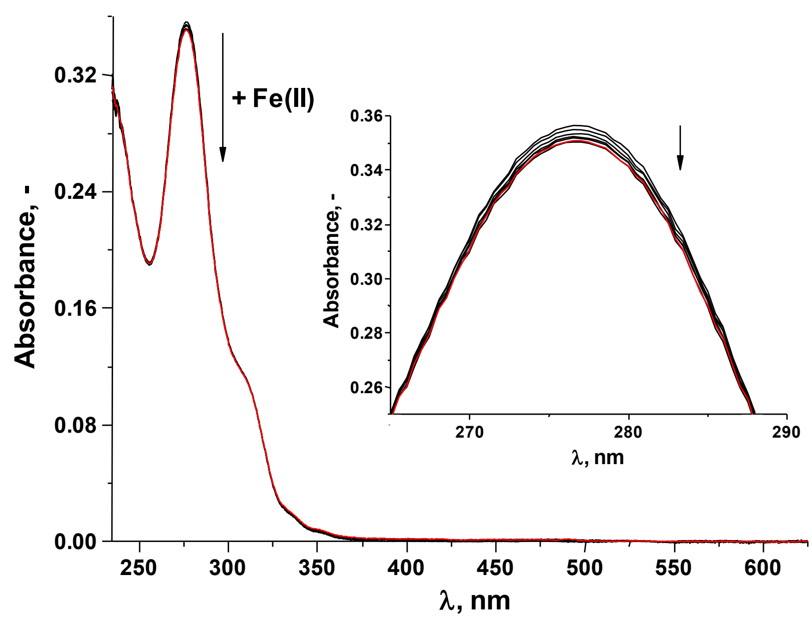

Figure S10. The evolution of the UV-visible absorption spectrum of **1·Eu·BPS** (10^{-5} M) in Hepes buffer (pH 7.4) upon titration with Fe(II) (0→6×10^{-6} equivalent).

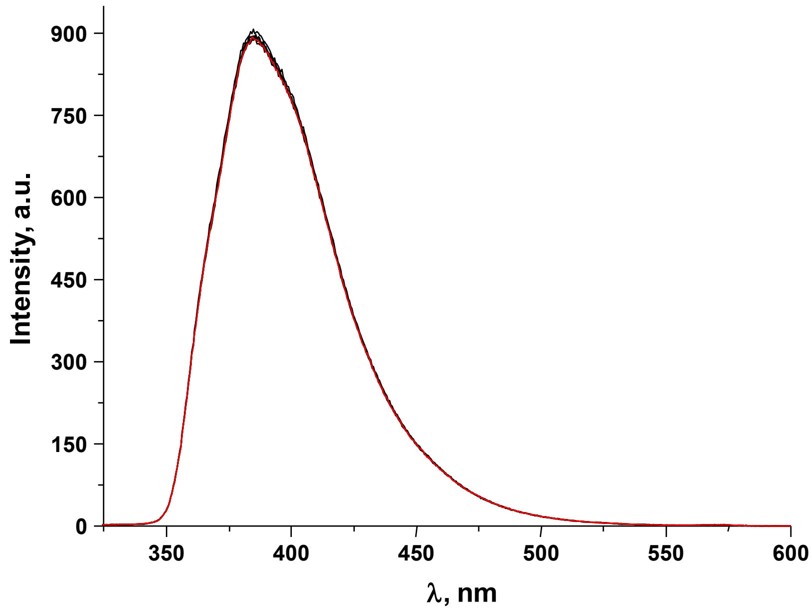

Figure S11. The evolution of the fluorescence spectrum of **1·Eu·BPS** (10^{-5} M) in Hepes buffer (pH 7.4) upon titration with Fe(II) ($0 \rightarrow 6\times10^{-6}$ equivalent).

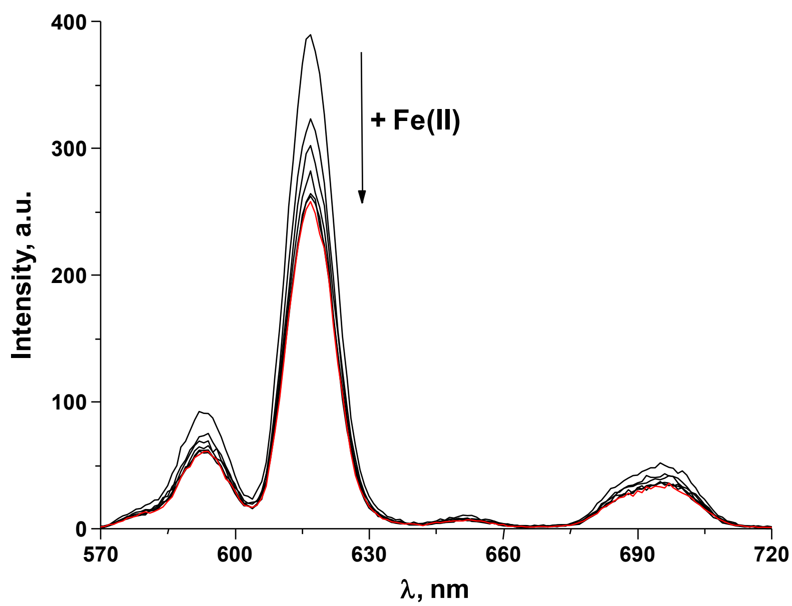

Figure S12. The evolution of the Eu(III) metal-centred emission of **1·Eu·BPS** (10^{-5} M) in Hepes buffer (pH 7.4) upon titration with Fe(II) ($0 \rightarrow 6\times10^{-6}$ equivalent).

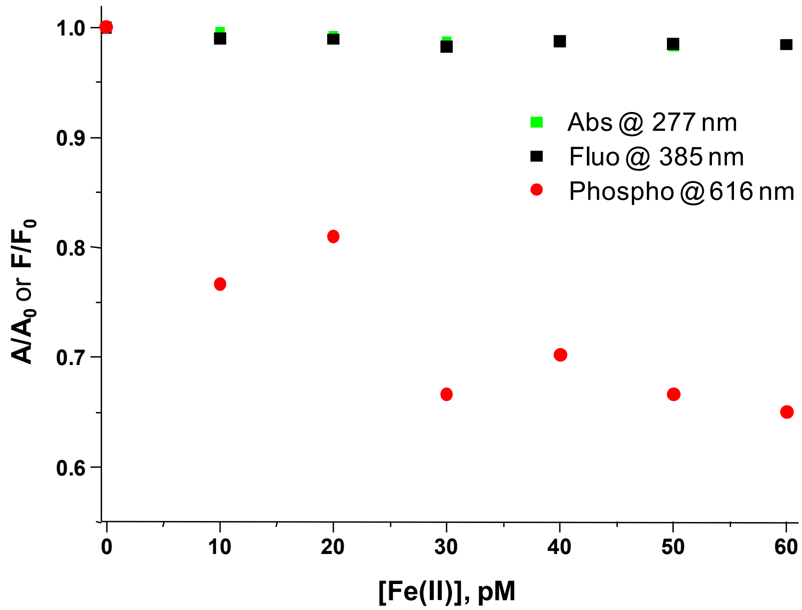

Figure S13. Experimental binding isotherms for the UV-visible (at 277 nm, green squares), fluorescence (at 385 nm, black squares) and phosphorescence (at 616 nm, red circles) spectra of **1·Eu·BPS** (10^{-5} M) *vs.* concentration of Fe(II) (0→60 pM).

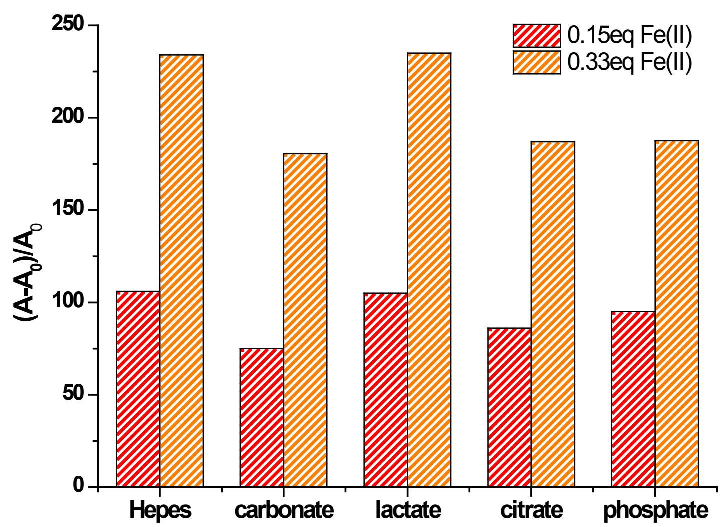

Figure S14. Bar chart diagram showing the absorbance response (at 535 nm) of **1·Eu·BPS** to 0.15 and 0.33 equivalents of Fe(II) in the presence of various biologically relevant anions; [carbonate] = 30 mM, [lactate] = 2.3 mM, [citrate] = 0.13 mM, [phosphate] = 0.9 mM.

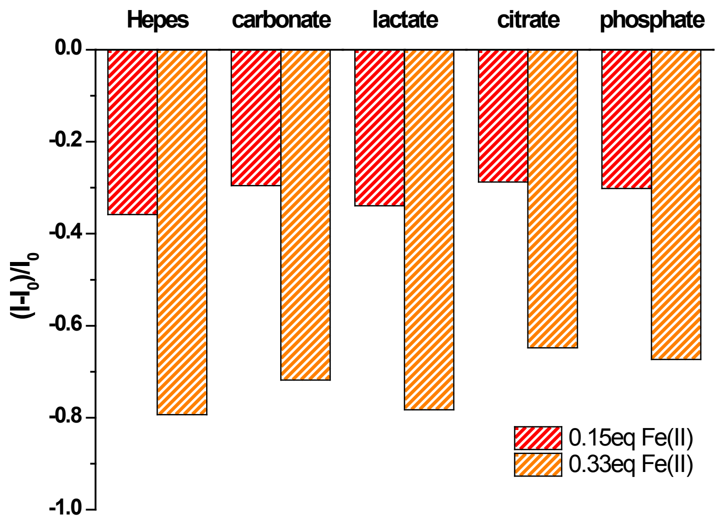

Figure S15. Bar chart diagram showing the fluorescence response of **1·Eu·BPS** to 0.15 and 0.33 equivalents of Fe(II) in the presence of various biologically relevant anions; [carbonate] = 30 mM, [lactate] = 2.3 mM, [citrate] = 0.13 mM, [phosphate] = 0.9 mM.

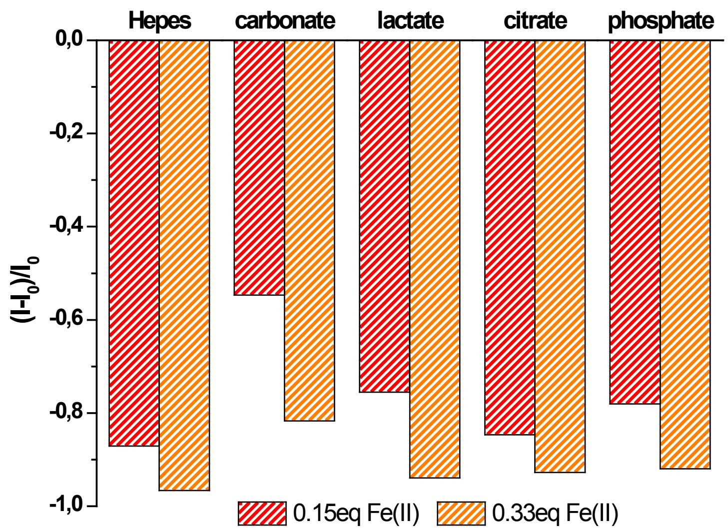

Figure S16. Bar chart diagram showing the Eu(III) emission response of **1·Eu·BPS** to 0.15 and 0.33 equivalents of Fe(II) in the presence of various biologically relevant anions; [carbonate] = 30 mM, [lactate] = 2.3 mM, [citrate] = 0.13 mM, [phosphate] = 0.9 mM.

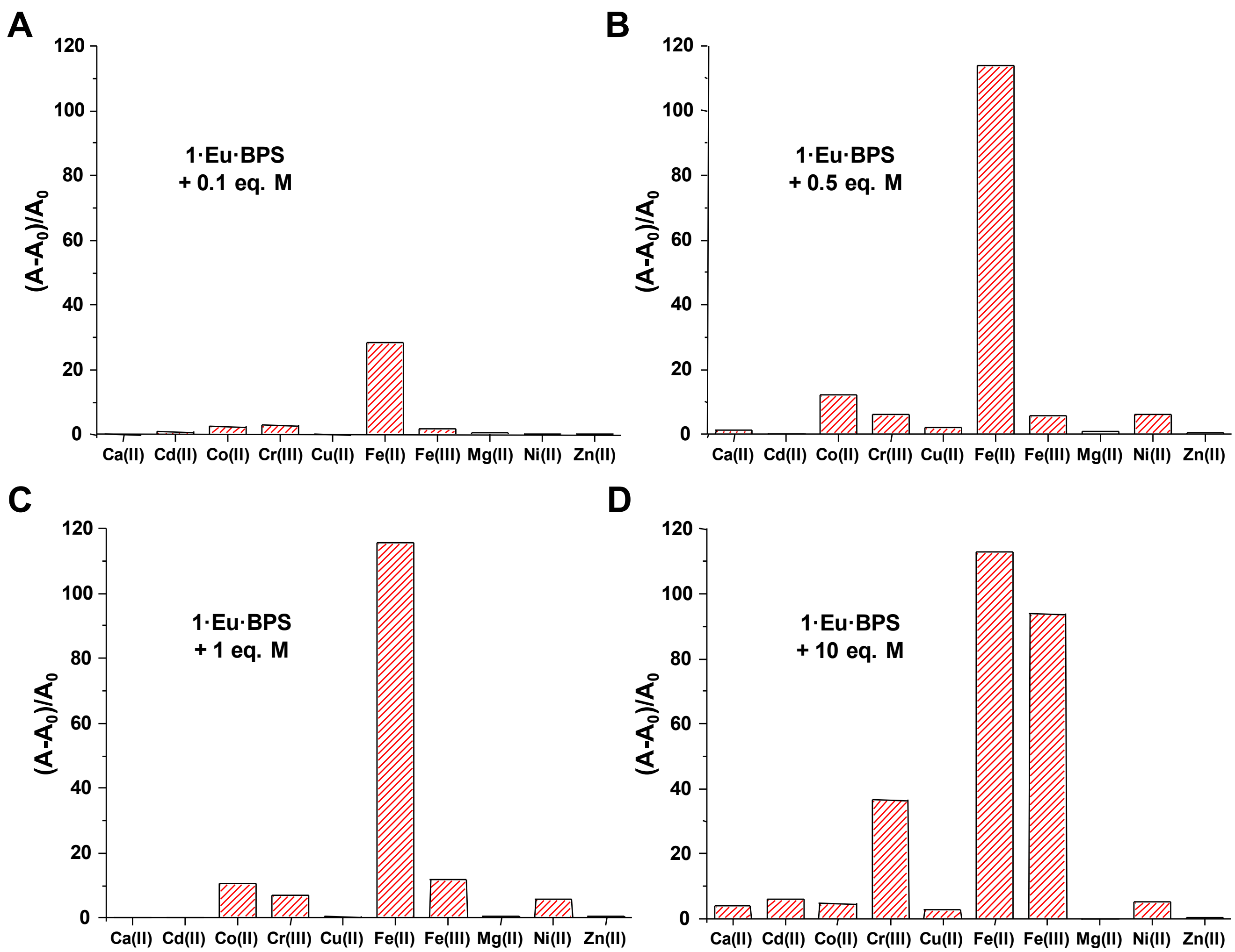

Figure S17. Bar chart diagrams showing the absorbance responses of **1·Eu·BPS** (10^{-5} M) at 535 nm to the presence of 0.1 (**A**), 0.5 (**B**), 1 (**C**) and 10 (**D**) equivalents of different cations.

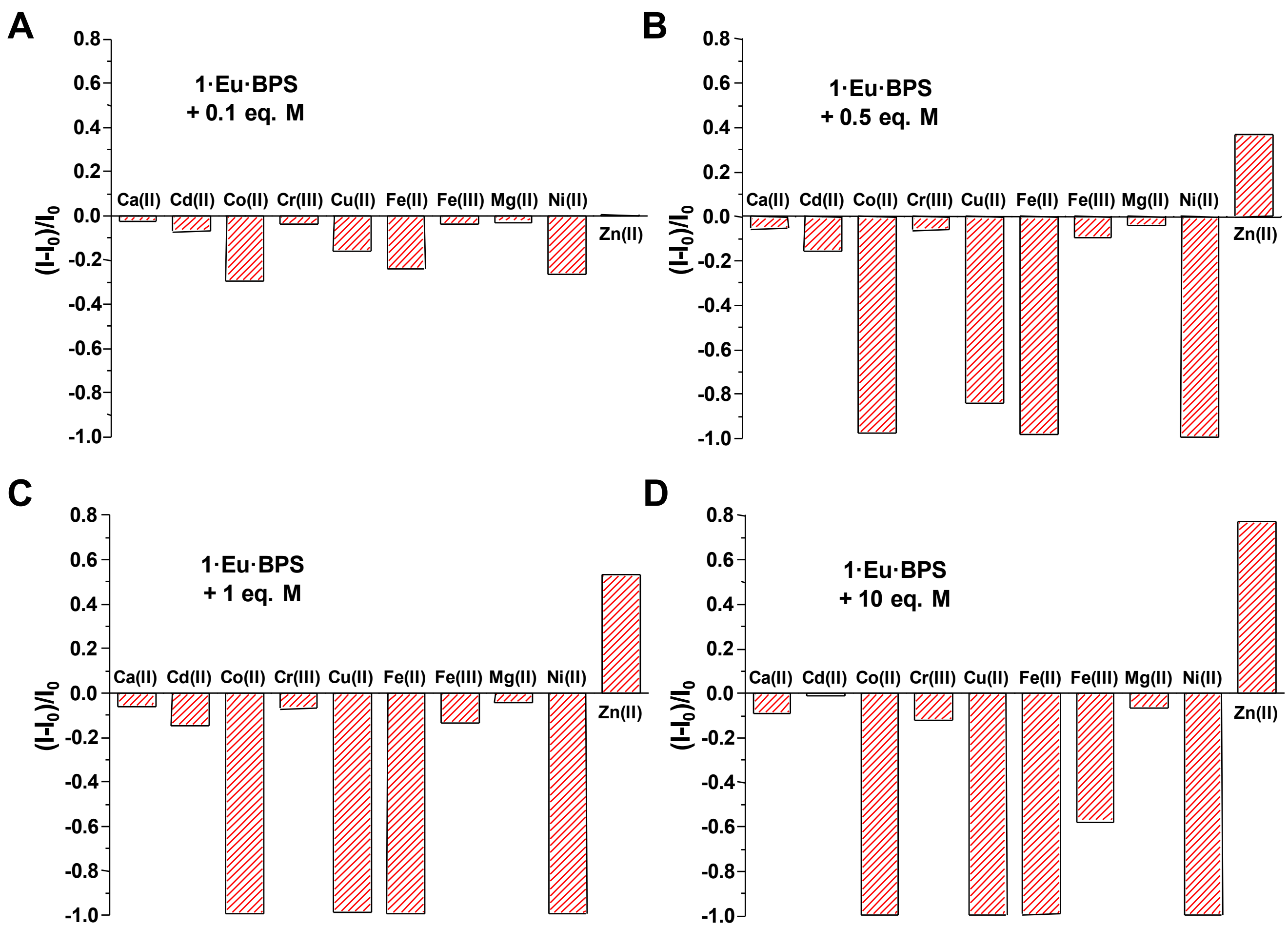

Figure S18. Bar chart diagrams showing the fluorescence responses (integrals) of **1·Eu·BPS** (10^{-5} M) to the presence of 0.1 (**A**), 0.5 (**B**), 1 (**C**) and 10 (**D**) equivalents of different cations.

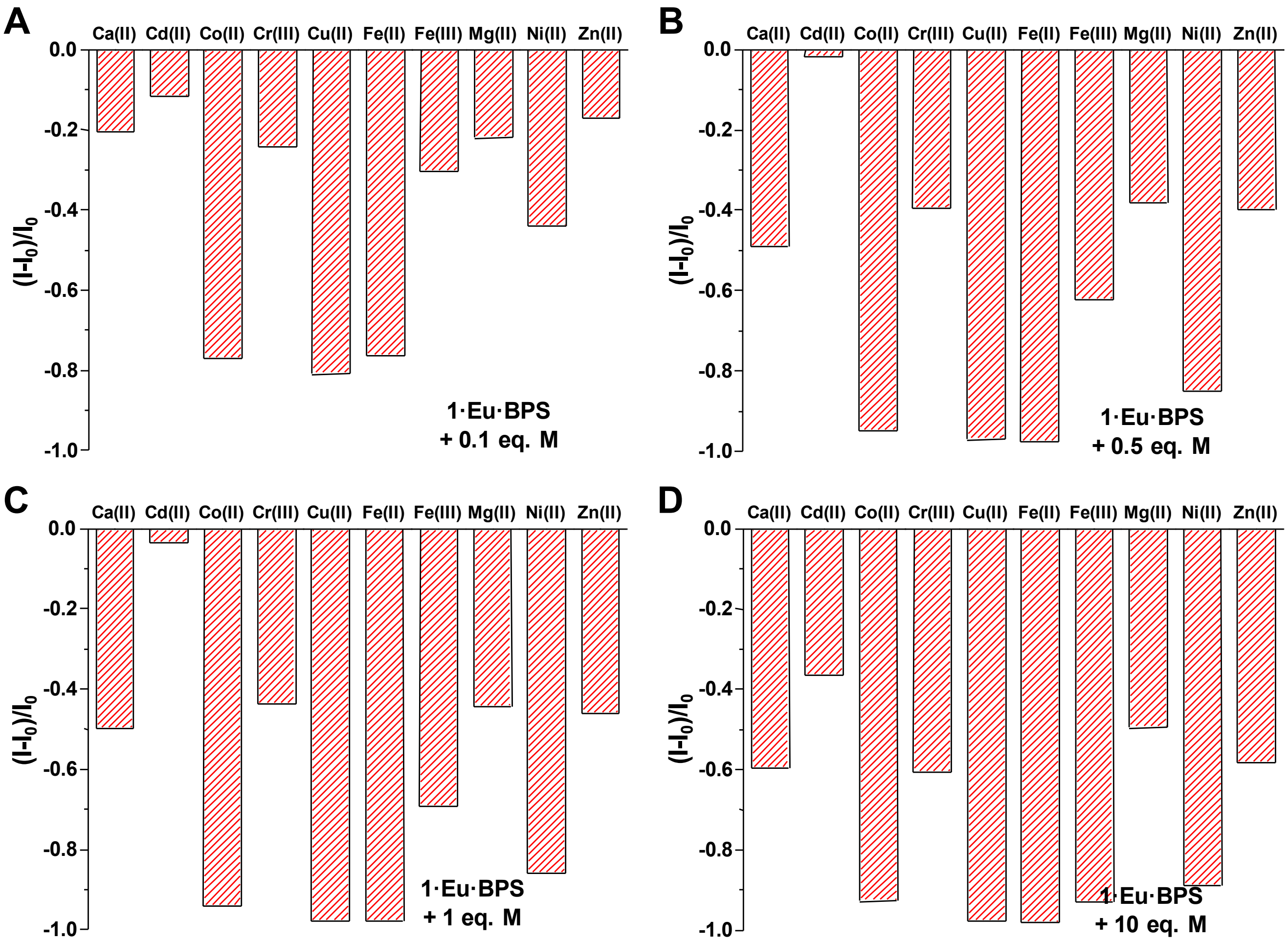

Figure S19. Bar chart diagrams showing the phosphorescence responses (integrals) of **1·Eu·BPS** (10^{-5} M) to the presence of 0.1 (**A**), 0.5 (**B**), 1 (**C**) and 10 (**D**) equivalents of different cations.

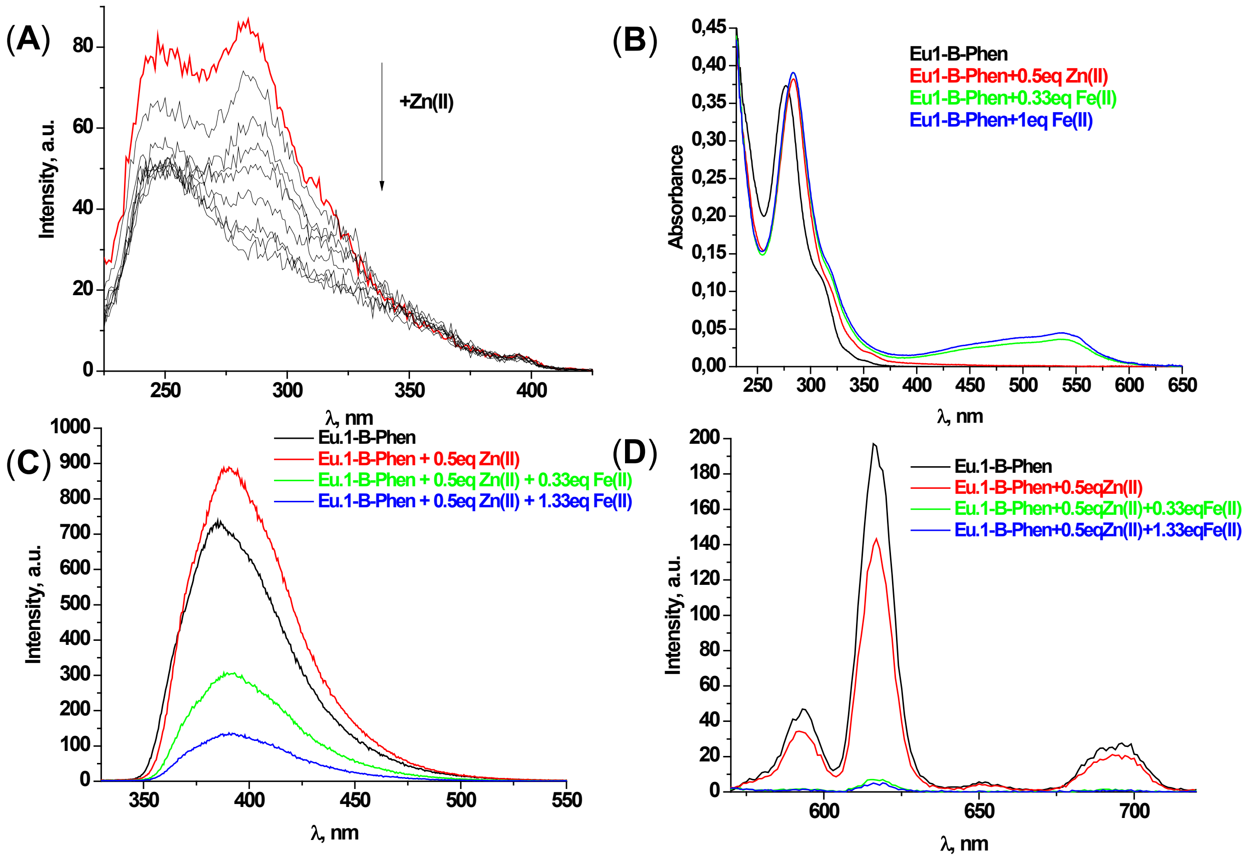

Figure S20. **A)** The excitation spectra of **1·Eu·BPS** upon addition of Zn(II) (0→10 equivalents), and **B)** the absorption **C)** fluorescence and **D)** phosphorescence spectra of **1·Eu·BPS** upon addition of Zn(II) (0.5 equivalents) with the following addition of Fe(II) (0.33 and 1.0 equivalents) in Hepes-buffered solution (pH 7.4).

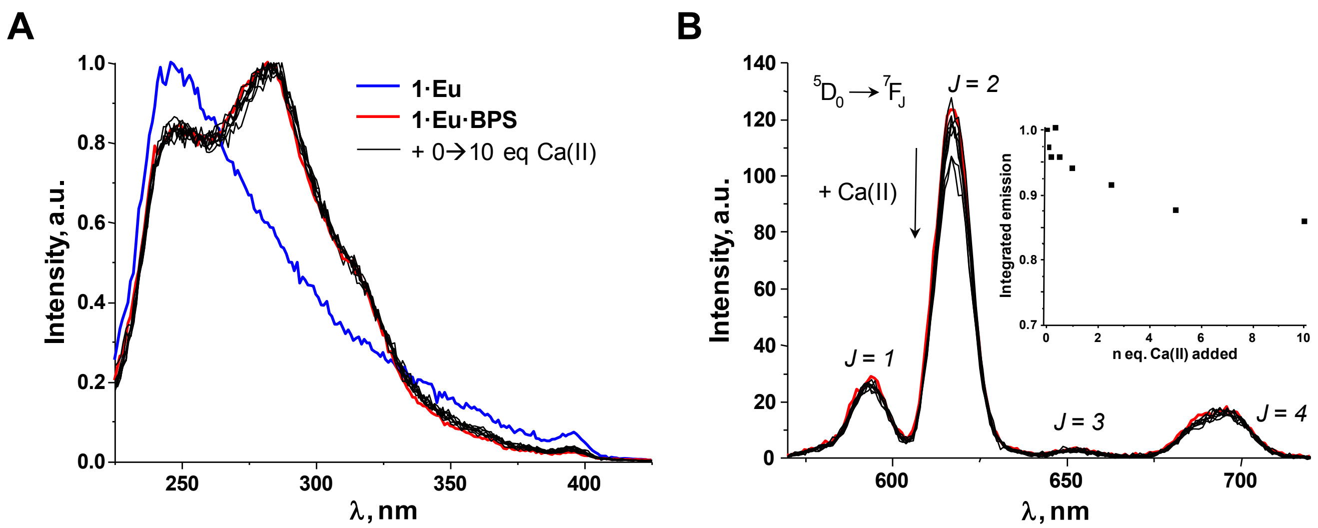

Figure S21. **A)** The normalized excitation spectra of **1·Eu** and **1·Eu·BPS** upon titration with Ca(II) (0→10 equivalents) in Hepes-buffered solution. **B)** The evolution of the Eu(III)-centred emission of **1·Eu** (10^{-5} M) in Hepes-buffered solution (pH 7.4) upon addition of Ca(II) (0→10 equivalents). Inset: The changes in the emission integrals of the Eu(III) $^5D_0 \rightarrow ^7F_J$ transitions (J = 0-4) *vs.* equivalents of Ca(II) added.

1. Massue, J.; Plush, S. E.; Bonnet, C. S.; Moore, D. A.; Gunnlaugsson, T. *Tetrahedron Lett.* **2007,** *48*, 8052-8055.
2. Massue, J.; Quinn, S. J.; Gunnlaugsson, T. *J. Am. Chem. Soc.* **2008,** *130*, 6900-6901.
3. Supkowski, R. M.; Horrocks, W. DeW., Jr. *Inorg. Chim. Acta* **2002**, *340*, 44.
4. Beeby, A.; Clarkson, I. M.; Dickins, R. S.; Faulkner, S.; Parker, D.; Royle, L.; de Sousa, A. S.; Williams, J. A. G.; Woods, M. *J. Chem. Soc., Perkin Trans. 2* **1999**, 493.
5. Gampp, H.; Maeder, M.; Meyer, C. J.; Zuberbühler, A. D. *Talanta* **1985,** *32*, 95-101.
6. Gampp, H.; Maeder, M.; Meyer, C. J.; Zuberbühler, A. D. *Talanta* **1986,** *33*, 943-951.
7. Kamiya, M.; Johnsson, K. *Anal. Chem.* **2010,** *82*, 6472-6479.