Supporting Information

Triple-Emitting Dumbbell Fluorescent Nanoprobe for Multicolor Detection and Imaging Applications

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Potentiometric Studies on the binding constant of Eu:DTPA-AMC complex, Cu:DTPA-AMC complex, Tb:DTPA-cs124 complex and Cu:DTPA-cs124 complex.

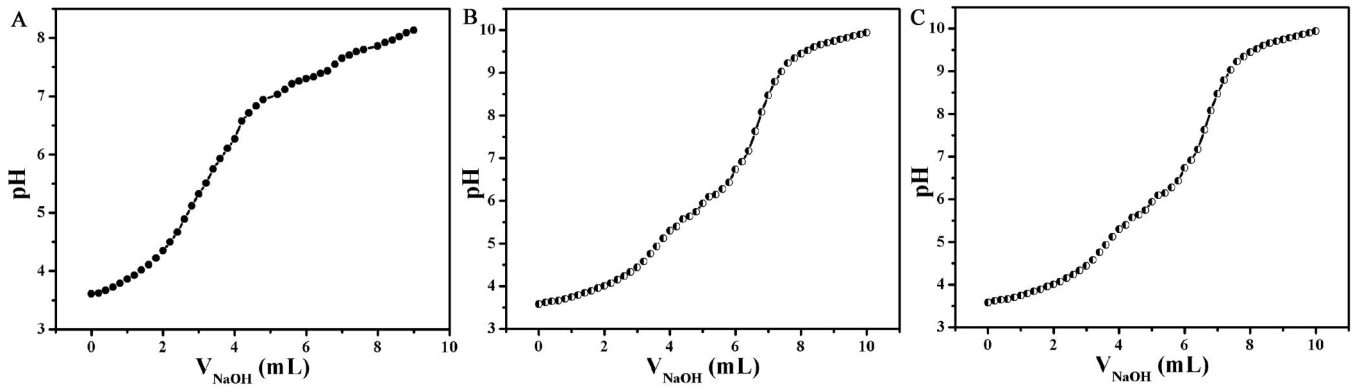

Figure S1. Potentiometric titration V-pH curves for the DTPA-cs124 (A), Tb:DTPA-cs124 complex (B) and Cu:DTPA-cs124 complex (C) system at 25 °C and I = 0.1 mol dm^{-3} NaCl.

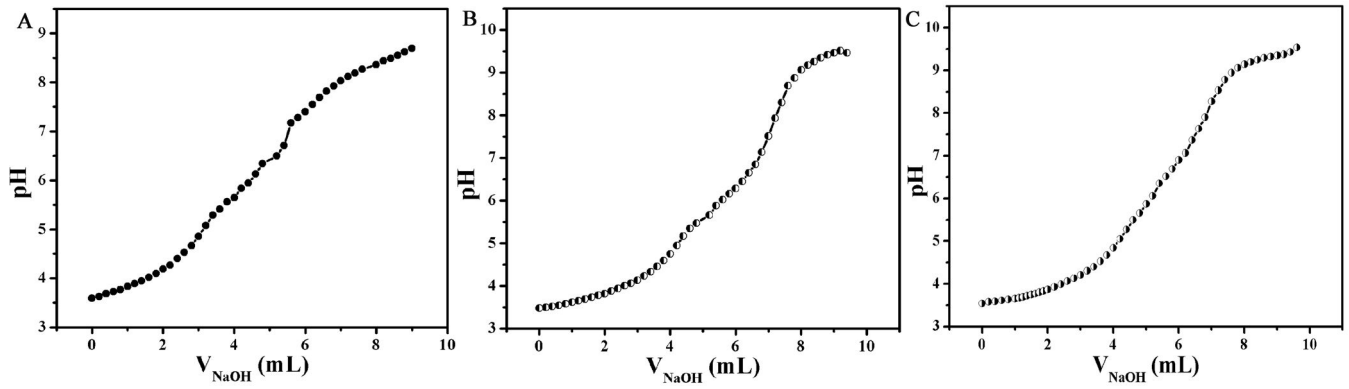

Figure S2. Potentiometric titration V-pH curves for the DTPA-AMC (A), Eu:DTPA-AMC complex (B) and Cu:DTPA-AMC complex (C) system at 25 °C and I = 0.1 mol dm^{-3} NaCl.

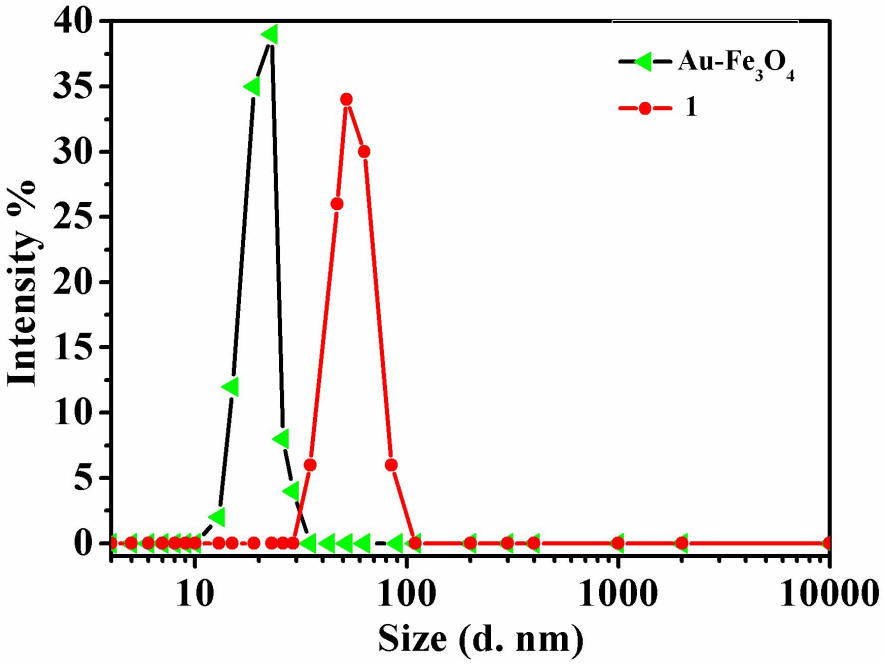

Figure S3. Hydrodynamic diameters measured by DLS for the as-synthesized Au-Fe_3O_4 NPs dispersed in hexane, and the **1** dispersed in H_2O.

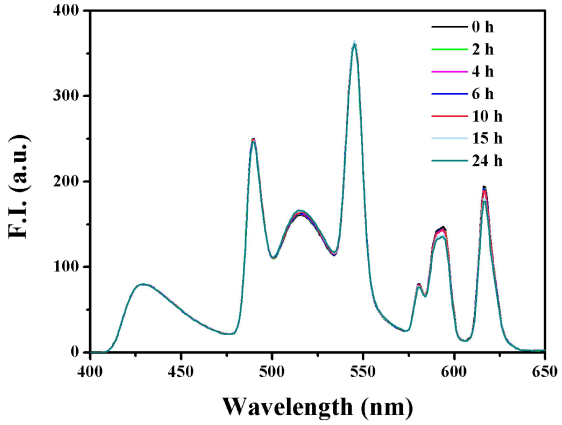

Figure S4. The fluorescence intensity of **1** in Tris-HCl buffer (50 mM, pH 7.20) at different time.

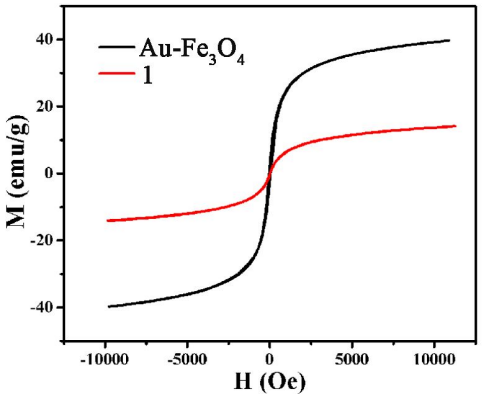

Figure S5. Magnetization measured at 300 K. The saturated magnetization (*M*) of as-synthesized Au-Fe_3O_4 NPs and **1**.

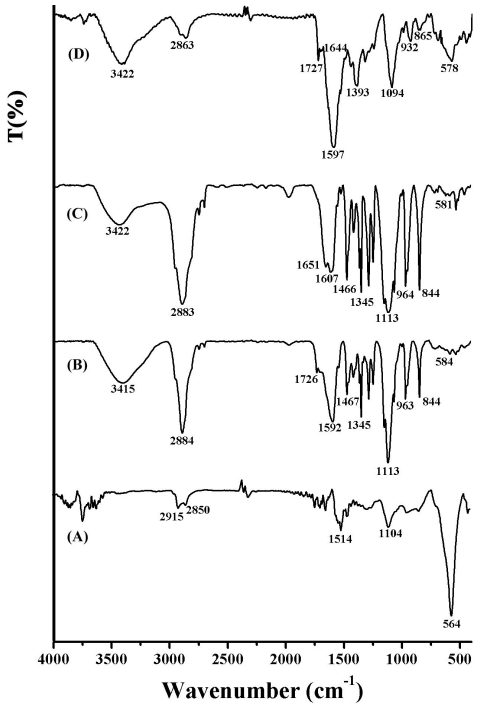

Figure S6. The FT-IR spectra of (A) as-sythesized Au-Fe_3O_4 NPs , (B) Eu:**1a**, (C) Tb:**1b,** (D) **1c.**

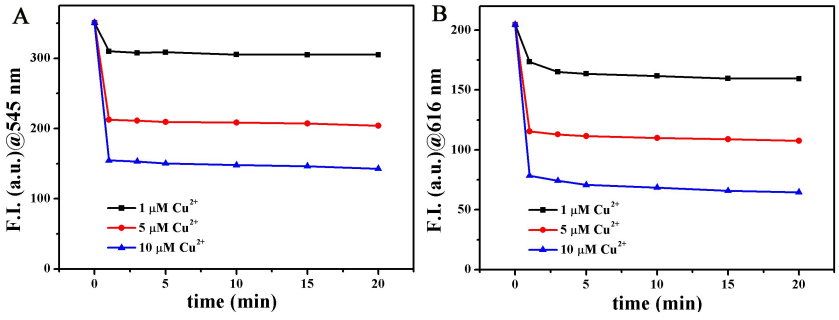

Figure S7. Reaction times on the emission intensity of **1** (3 μM Eu^{3+}, 0.3 μM Tb^{3+}) with 1 μM, 5 μM and 10 μM Cu^{2+} in Tris-HCl buffer (50 mM, pH 7.20) at 545 nm and 616 nm, respectively.

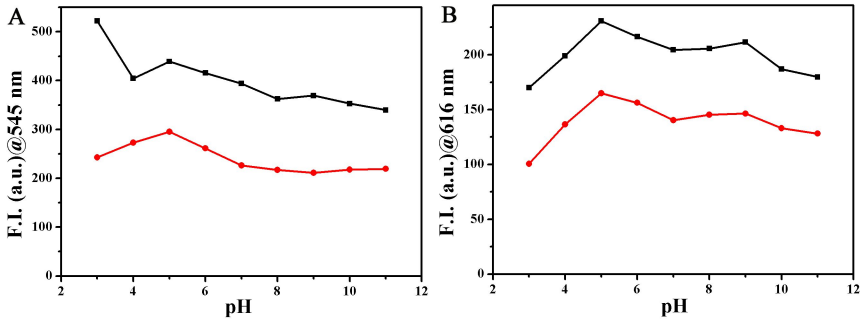

Figure S8. Effects of pH value on the emission intensity of **1** (3 μM Eu^{3+}, 0.3 μM Tb^{3+}) without Cu^{2+} (black) and with 5 μM Cu^{2+} (red) in Tris-HCl buffer (50 mM, pH 7.20) at 545 nm and 616 nm, respectively.

Table S1. The excited state $^5D_4$ lifetimes and the excited state $^5D_0$ lifetimes of nanocomposites **1** before and after addition of Cu^{2+}.

Samples	τ_1 (μs)	τ_2 (μs)	$<\tau>$ (μs)	λ_{em} (nm)
1	83.13 [2.83%]	478.5 [97.17%]	467.31	545
1 + Cu^{2+}	0.33 [84.89%]	40.57 [15.11%]	6.41	545
1	337 [24.2%]	851.82 [75.8%]	727.23	616
1 + Cu^{2+}	55.42 [32.62%]	144.15 [67.38%]	115.21	616

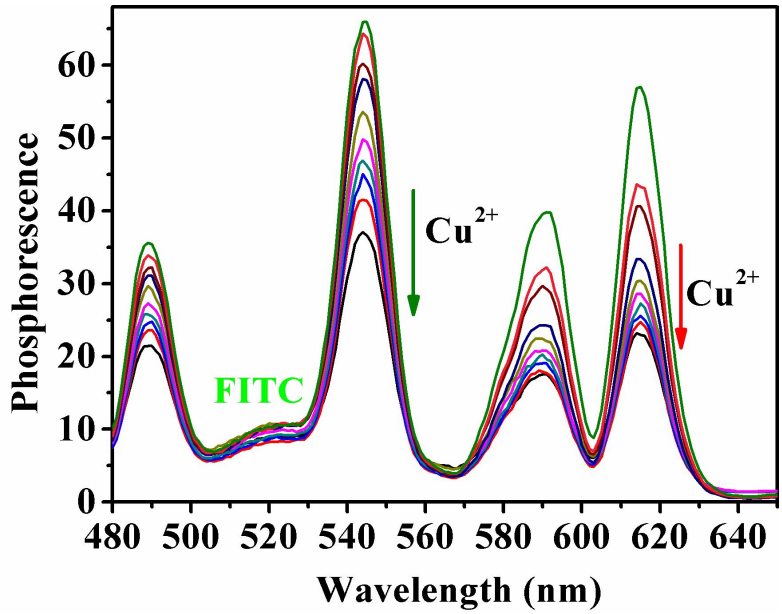

Figure S9. Phosphorescence spectra of **1** (3 μM Eu^{3+}, 0.3 μM Tb^{3+}) upon addition of Cu^{2+} from 0 μM to 10 μM in Tris-HCl buffer (50 mM, pH 7.20).

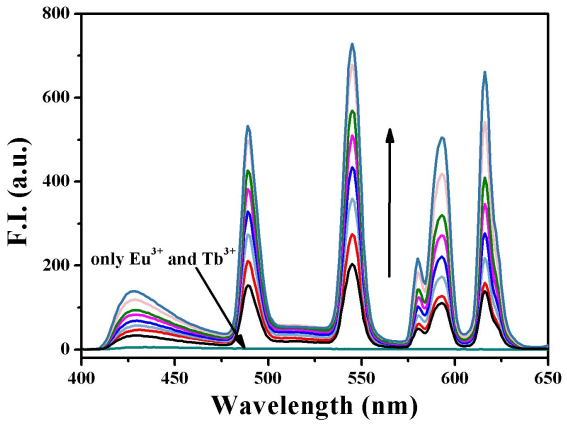

Figure S10. The fluorescent spectra of 50 μM Eu^{3+} and 5 μM Tb^{3+} in the presence of different concentration of cs124-DTPA-NH-PEG-DBA-Fe_3O_4-Au-PEG-NH-FITC (0, 0.2 mg/L, 0.4 mg/L, 0.6 mg/L, 0.8 mg/L, 1 mg/L, 1.2 mg/L, 2 mg/L, 3 mg/L).

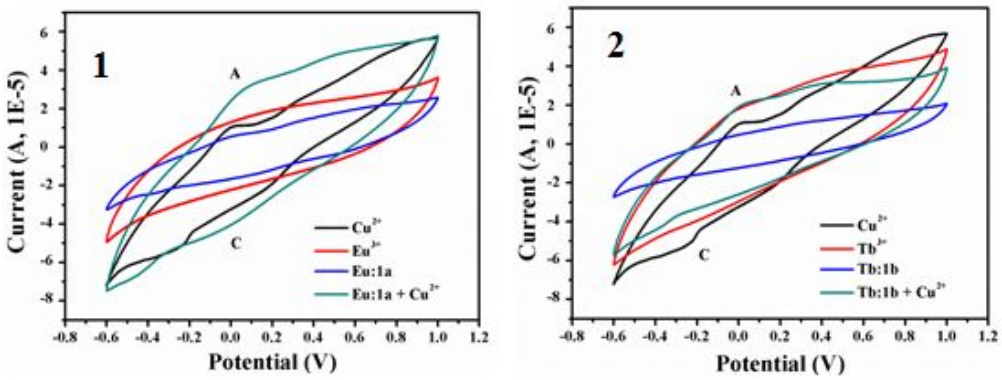

Figure S11. (**1**) Cyclic voltammograms of Cu^{2+}, Eu^{3+}, Eu:**1a** and Eu:**1a** reacting with Cu^{2+} in the electrode solution. (**2**) Cyclic voltammograms of Cu^{2+}, Tb^{3+}, Tb:**1b** and Tb:**1b** reacting with Cu^{2+} in the electrode solution.

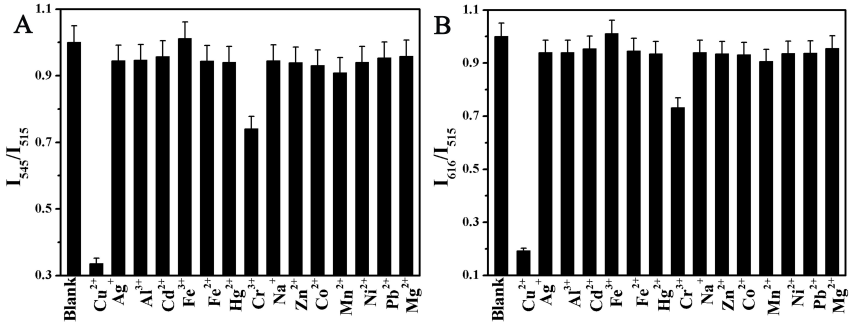

Figure S12. (A) Normalized fluorescence responses of **1** (10 μM, Eu^{3+}) to various metal ions (100 μM). Bars represent the final integrated ratiometric fluorescence response 545 nm over 515 nm. Excitation occurred at 330 nm. (B) Normalized fluorescence responses of **1** (10 μM, Eu^{3+}) to various metal ions. Bars represent the final integrated ratiometric fluorescence response of 616 nm over 515 nm.

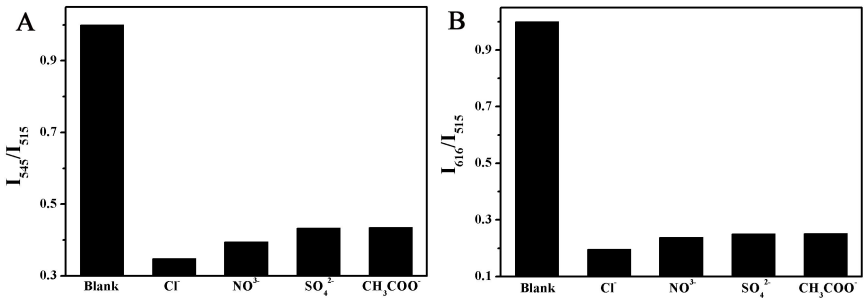

Figure S13. Normalized fluorescent response of **1** (10 μM Eu^{3+}, 1 μM Tb^{3+}) to $CuCl_2$, $Cu(NO_3)_2$, $CuSO_4$, $Cu(CH_3COO)_2$, (100 μM) with ratiometric fluorescence (A) 545 nm over 515 nm and (B) 616 nm over 515 nm in Tris-HCl buffer (50 mM, pH 7.20).

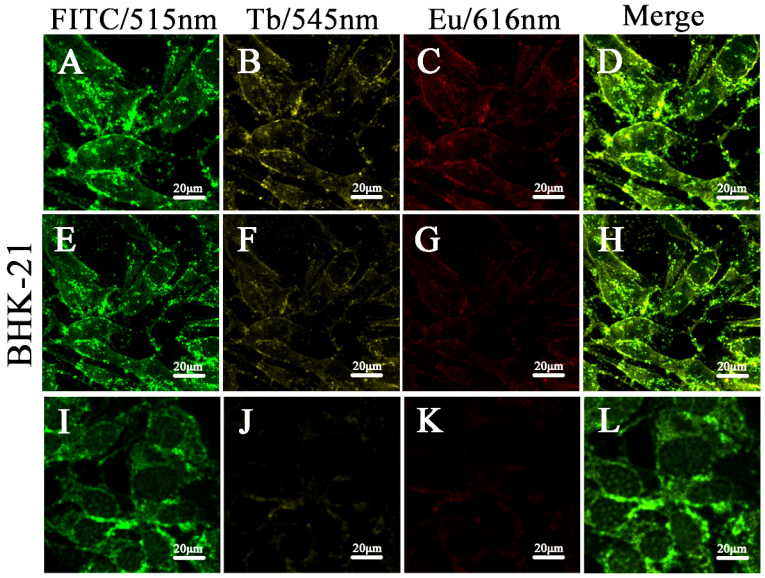

Figure S14. Confocal fluorescence images of BHK-21 cells (A-D) after incubation with **1** and (E-H) after addition 10 μM Cu^{2+} to the **1** treated cells and (I-L) after addition 25 μM Cu^{2+} to the **1** treated cells for 1 h. Excitation at 335 ± 20 nm and Emission at 515, 545 and 616 nm. (A, E and I) Fluorescence image of FITC (green); (B, F and J) fluorescence image of Tb(III) complex (yellow); (C, G and K) fluorescence image of Eu(III) complex (red); (D) overlay of (A), (B) and (C); (H) overlay of (E), (F) and (G); (L) overlay of (I), (J) and (K).

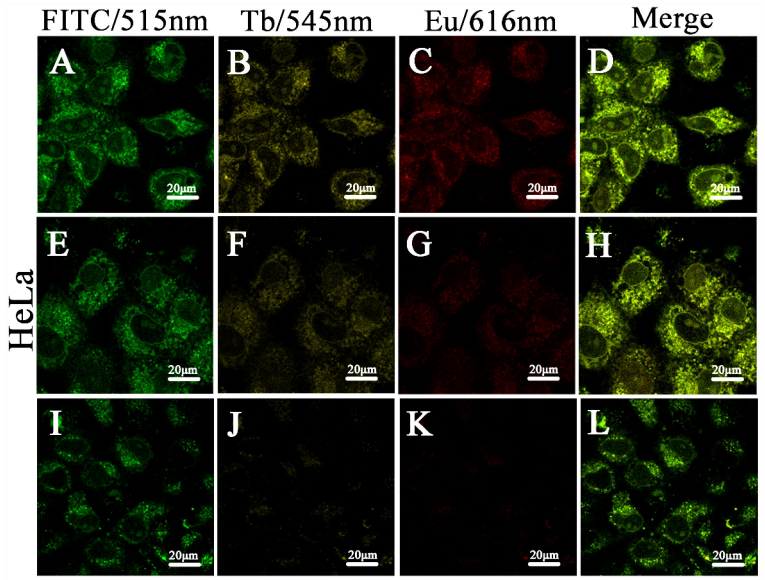

Figure S15. Confocal fluorescence images of HeLa cells (A-D) after incubation with **1** and (E-H) after addition 10 μM Cu^{2+} to the **1** treated cells and (I-L) after addition 25 μM Cu^{2+} to the **1** treated cells for 1 h. Excitation at 335 ± 20 nm and Emission at 515, 545 and 616 nm. (A, E and I) Fluorescence image of FITC (green); (B, F and J) fluorescence image of Tb(III) complex (yellow); (C, G and K) fluorescence image of Eu(III) complex (red); (D) overlay of (A), (B) and (C); (H) overlay of (E), (F) and (G); (L) overlay of (I), (J) and (K).

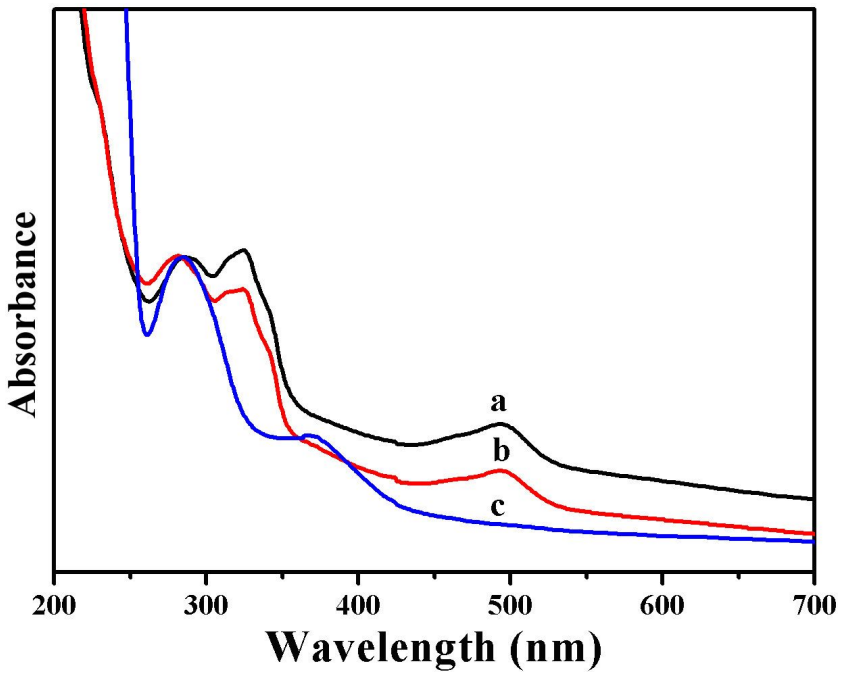

Figure S16. UV-vis spectra of **1** (a)**, 3** (b) and **1f** (c) in aqueous solution. The absorption peaks at 283 nm and 370 nm belong to folic acid (FA), 323 to cs124 and AMC, and 493 nm to fluorescein isothiocyanate (FITC).

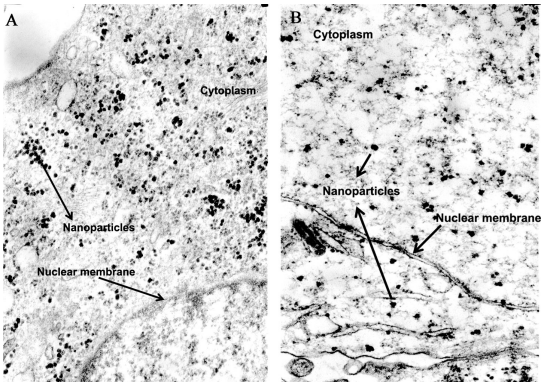

Figure S17. TEM images of HeLa cells interacted with (A) nanocomposites **1** and (B) nanocomposites **3**.

Calculation of Eu and Tb complexes number per Fe_3O_4 NPs[1]: Each Fe_3O_4 NP has fcc structure with a=0.485 nm. Each periodic lattice has 4 atoms. Each Fe_3O_4 NP is a sphere with diameter 12 nm.

Volume of Fe_3O_4 NPs: $V_1 = \frac{4}{3}\pi r^3 = \frac{4\times 3.14\times(6\times10^{-9})^3}{3} = 904\times10^{-27}\,m^3$

Volume of lattice is: $V_2 = a^3 = (0.485\times10^{-9})^3 = 0.582\times10^{-27}\,m^3$

Thus the number of atom of per NPs is $n = \frac{V_1}{V_2}\times 4 = 6213$

Weight percentage of Eu/Fe is 63.3% and Tb/Fe is 6.33%, thus atom ratio of Eu/Fe is 63.3:100 and Tb/Fe is 6.33:100. Take into the number of complexes per NPs, Eu/Fe_3O_4 NPs is 3933 and Tb//Fe_3O_4 NPs is 393.

Reference

1. Xu, C. J.; Wang, B. D.; Sun, S. H. *J. Am. Chem. Soc.* **2009**, *131*, 4216–4217.