Effect of the length of polyoxyethylene substituents on luminescent bimetallic lanthanide bioprobes

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Electronic Supplementary Information

10 pages
Figure S1. (Top) UV-vis spectra of H$_2$L$^{C2'}$ 1.06×10$^{-5}$ M versus pH, $T = 298$ K, $\mu = 0.1$ M (KCl). (Bottom). Recalculated absorption spectra of the various species evidenced during titration with NaOH.
Figure S2. Comparison of the absorbance at six different wavelengths versus pH with recalculated absorbance during the titration of H$_2$L$^{2+}$ with NaOH.

Figure S3. Distribution diagram for H$_2$L$^{2+}$ as computed from the pK$_a$s reported in the text.
Figure S4. Measured and calculated isotopic distributions for a 2:3 and a 2:2 complex species.

Figure S5. Re-calculated spectra from the titration of $\text{H}_2\text{L}^{\text{C}2^-}$ by europium perchlorate.
Figure S6. Absorbance values extracted at different wavelengths during the titration of H$_2$L$^{C2^-}$ by europium perchlorate.

Figure S7. Molar absorption of H$_2$L$^{C2^-}$ and of the Eu$^{III}$ and Tb$^{III}$ helicates at room temperature in Tris-HCl 0.1 M.

Figure S8. Normalized luminescence spectra of H$_2$L$^{C2^-}$ and its helicates with non-luminescent Ln$^{III}$ ions, measured at 77 K in buffered aqueous solution (pH 7.4, Tris-HCl 0.1 M) containing 10% of glycerol; dotted lines: without time delay; plain lines: with a 0.05 ms time delay; [H$_2$L$^{C2^-}$] = 4.1×10$^{-5}$ M, [Ln$_2$(L$^{C2^-}$)$_3$] = 1.4×10$^{-5}$ M.
Figure S9. High-resolution emission spectrum of a frozen solution of [Eu$_2$(L$^{C2'}$)$_3$] 2.5$x$10^{-4} M in Tris-HCl (pH 7.4) recorded at 10 K under excitation at 331 nm. Insert: detail of the $^5$D$_0$$\rightarrow$$^7$F$_0$ transition.

Figure S10. (Left) WST-1 proliferation test of HeLa cells in absence or presence of various concentrations of [Eu$_2$(L$^{C2'}$)$_3$]. Each point is the average of three nominally identical
measurements. (Right). HeLa cell viability (WST-1 test) after 24h incubation with different concentrations of [Eu$_2$(L$^{C2}$)$_3$].

Figure S11. Time course of the [Eu$_2$(L$^{C2}$)$_3$] complex loading into HeLa cells. The cells were incubated in the presence of 500 μM of the complex in cell culture medium at 37°C. The images were taken using a Zeiss luminescence microscope Axiovert S100 (Objective: Plan-Neofluar, 20x; $\lambda_{exc} = 330$ nm, emission filter = LP 585 filter, acquisition time 60 s).
Figure S12. HeLa cells were incubated in presence of 100 μM [Eu₂(L²')₃] in RPMI-1640 for 7 h at 4°C or 37°C. The images were taken using a Zeiss luminescence microscope Axiovert S100 (Objective: Plan-Neofluar, 20x ; λ_{exc} = 330 nm, emission filter = LP 585 filter, exposure time 60 s).

Figure S13. Quantitation of the intracellular concentration of [Eu₂(L²')₃] using the Delfia® method. Each point represents the average of four nominally identical measurements.
Figure S14. Loading concentration dependence of the emitted luminescence for [Eu$_2$(L$^{C2'}$)$_3$] (red curve) and [Eu$_2$(L$^{C2}$)$_3$] (green curve). HeLa cells were incubated 7h at 37 °C. The images were taken on a Zeiss luminescence microscope Axiovert S 100 (objective: Plan-Neofluor 20 x; $\lambda_{exc}$: 330 nm; emission filter: LP 585; exposure time: 60 s).
Figure S15. Time-course dependence of the emitted luminescence for [Eu$_2$(L$^{C2}$)$_3$] (red curve) and [Eu$_2$(L$^{C2}$)$_3$] (green curve). The cells were incubated at 37 °C in presence of 500 μM helicate. Same imaging conditions as for Figure S14.