

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

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Electronic Supplementary Information

10 pages

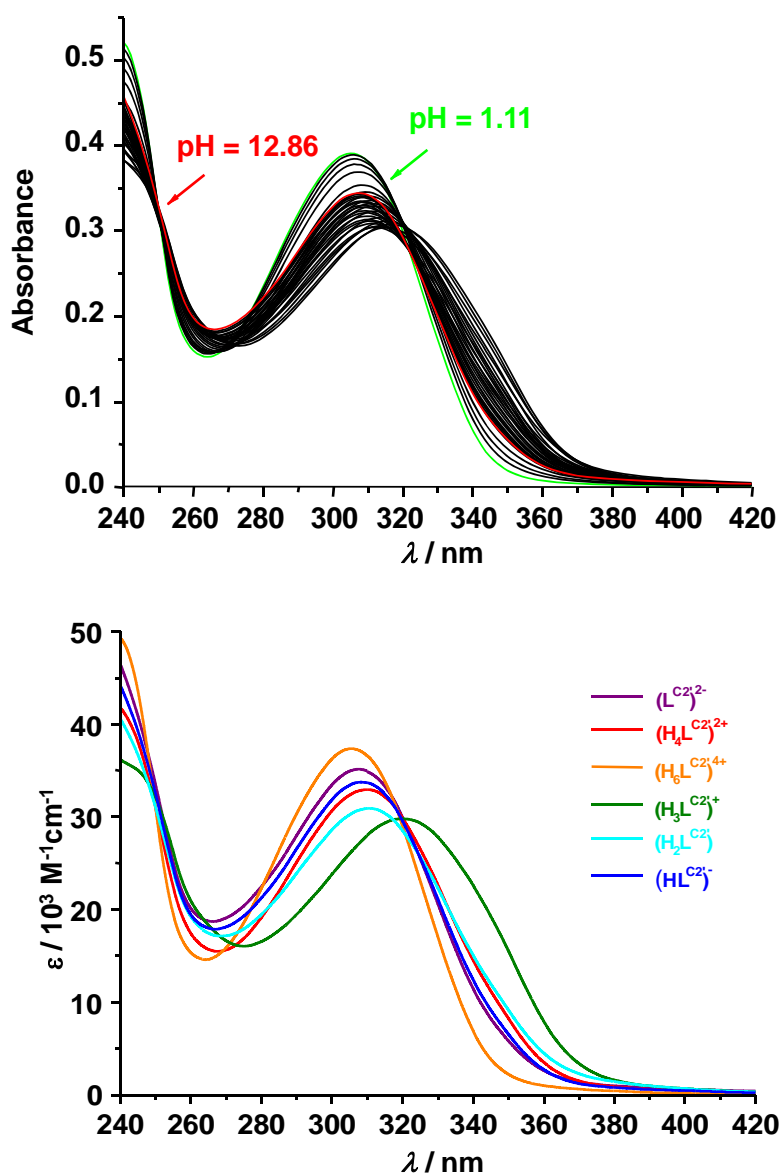


Figure S1. (Top) UV-vis spectra of $\text{H}_2\text{L}^{\text{C}2'}$ 1.06×10^{-5} M versus pH, $T = 298$ K, $\mu = 0.1$ M (KCl). (Bottom). Recalculated absorption spectra of the various species evidenced during titration with NaOH.

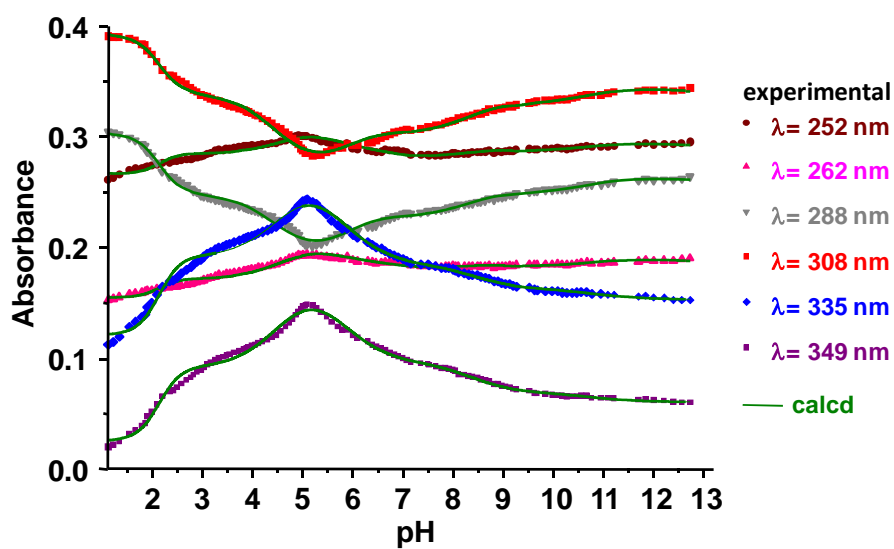


Figure S2. Comparison of the absorbance at six different wavelengths versus pH with re-calculated absorbance during the titration of H_2L^{C2-} with NaOH.

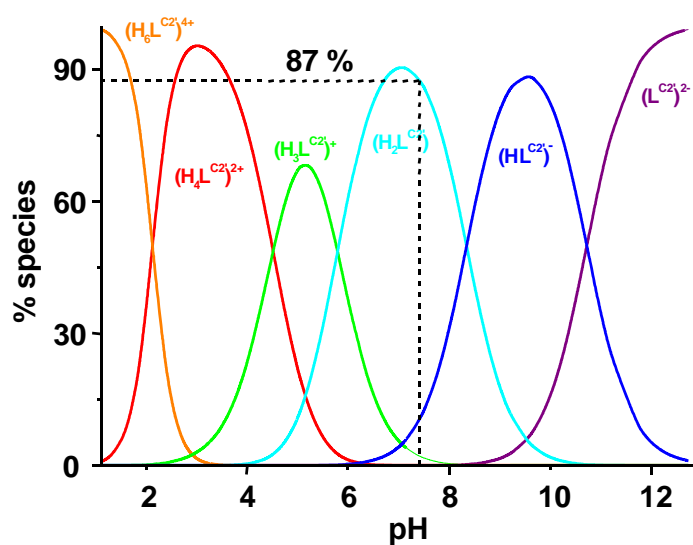


Figure S3. Distribution diagram for H_2L^{C2-} as computed from the pK_a s reported in the text.

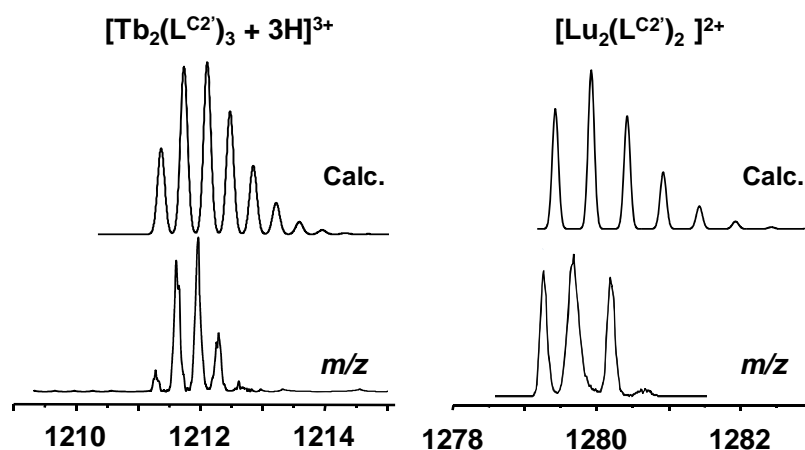


Figure S4. Measured and calculated isotopic distributions for a 2:3 and a 2:2 complex species.

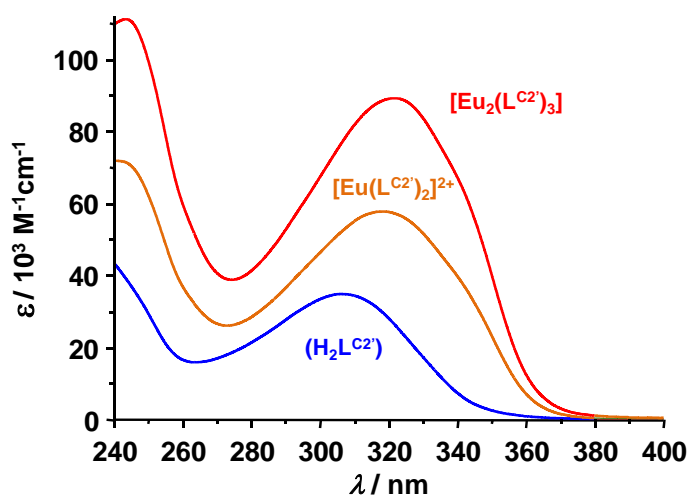


Figure S5. Re-calculated spectra from the titration of $\text{H}_2\text{L}^{\text{C}2'}$ by europium perchlorate.

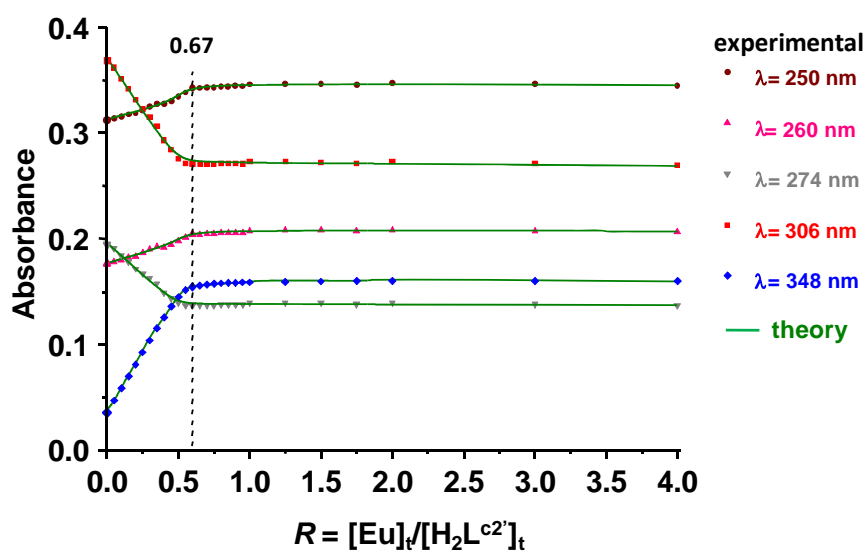


Figure S6. Absorbance values extracted at different wavelengths during the titration of $\text{H}_2\text{L}^{\text{C}2'}$ by europium perchlorate.

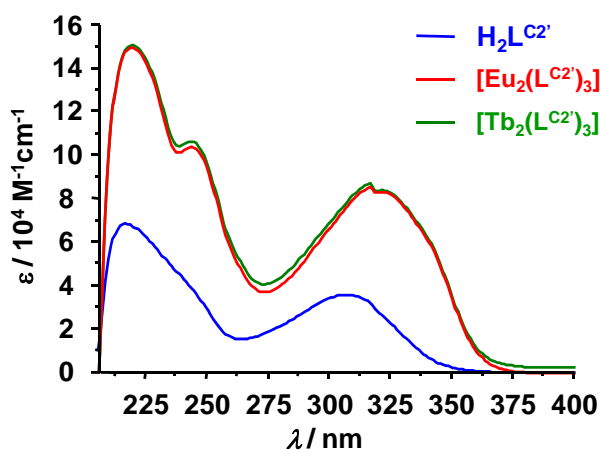


Figure S7. Molar absorption of $\text{H}_2\text{L}^{\text{C}2'}$ and of the Eu^{III} and Tb^{III} helicates at room temperature in Tris-HCl 0.1 M.

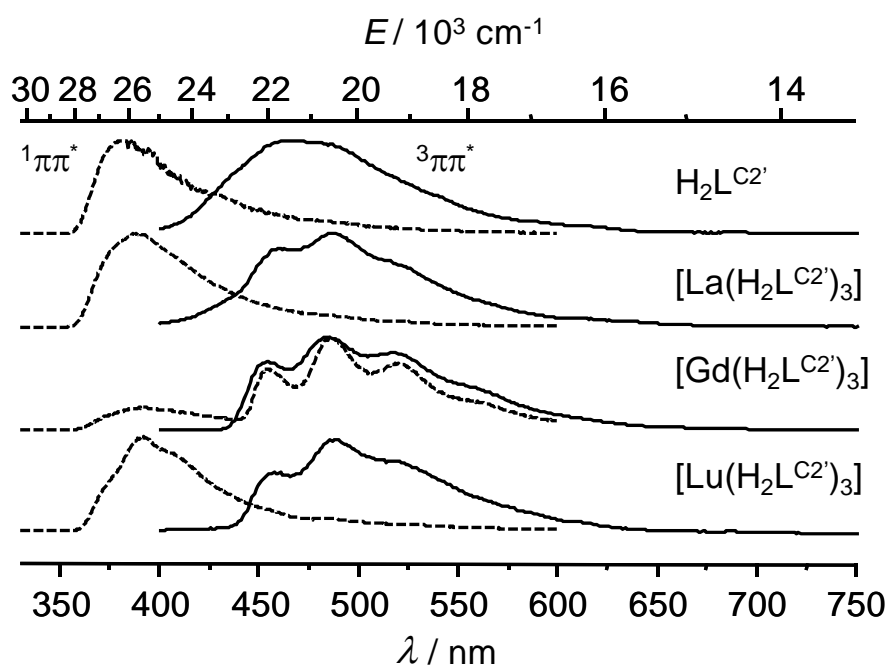


Figure S8. Normalized luminescence spectra of $\text{H}_2\text{L}^{\text{C}2'}$ and its helicates with non-luminescent Ln^{III} ions, measured at 77 K in buffered aqueous solution (pH 7.4, Tris-HCl 0.1 M) containing 10 % of glycerol; dotted lines: without time delay; plain lines: with a 0.05 ms time delay; $[\text{H}_2\text{L}^{\text{C}2'}] = 4.1 \times 10^{-5}$ M, $[\text{Ln}_2(\text{L}^{\text{C}2'})_3] = 1.4 \times 10^{-5}$ M.

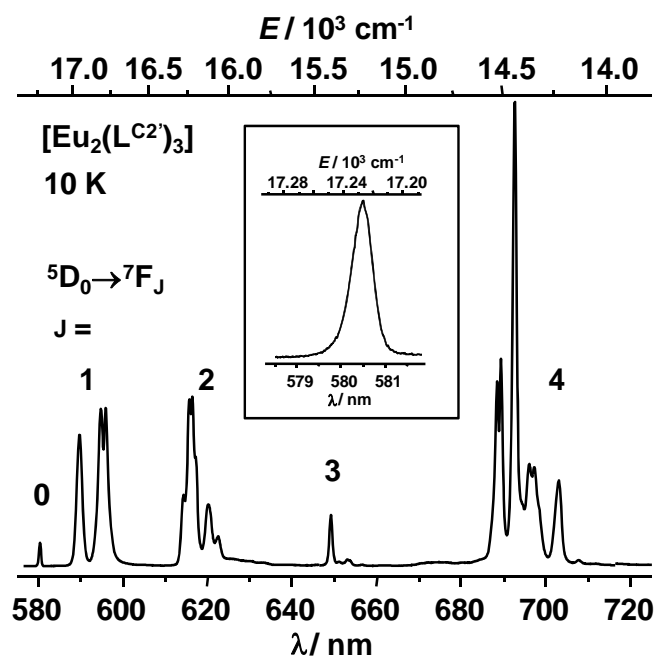


Figure S9. High-resolution emission spectrum of a frozen solution of $[\text{Eu}_2(\text{L}^{\text{C}2'})_3]$ 2.5×10^{-4} M in Tris-HCl (pH 7.4) recorded at 10 K under excitation at 331 nm. Insert: detail of the ${}^5\text{D}_0 \rightarrow {}^7\text{F}_0$ transition.

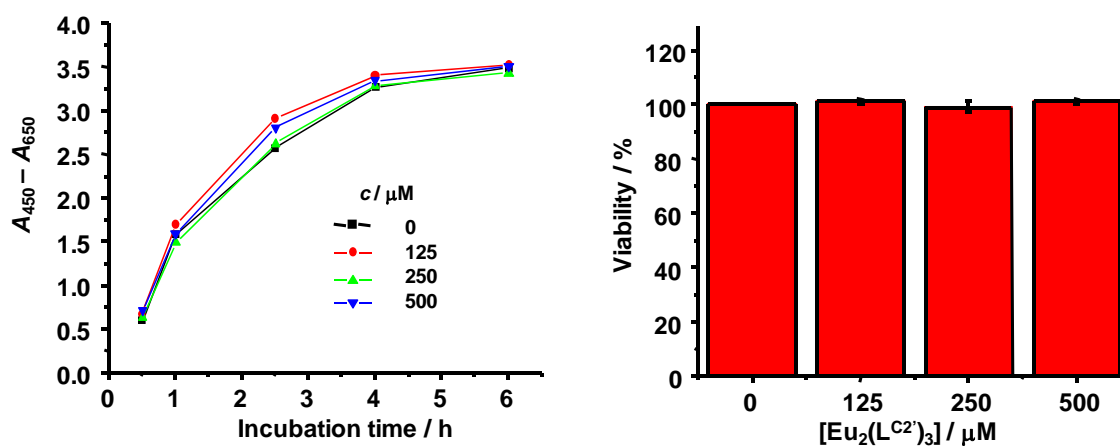


Figure S10. (Left) WST-1 proliferation test of HeLa cells in absence or presence of various concentrations of $[\text{Eu}_2(\text{L}^{\text{C}2'})_3]$. Each point is the average of three nominally identical

measurements. (Right). HeLa cell viability (WST-1 test) after 24h incubation with different concentrations of $[\text{Eu}_2(\text{L}^{\text{C}2'})_3]$.

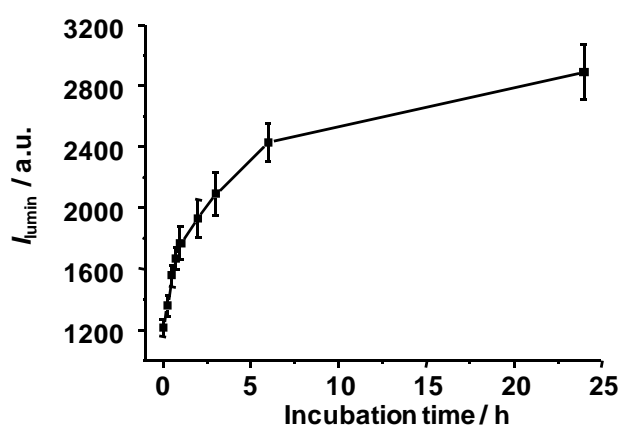
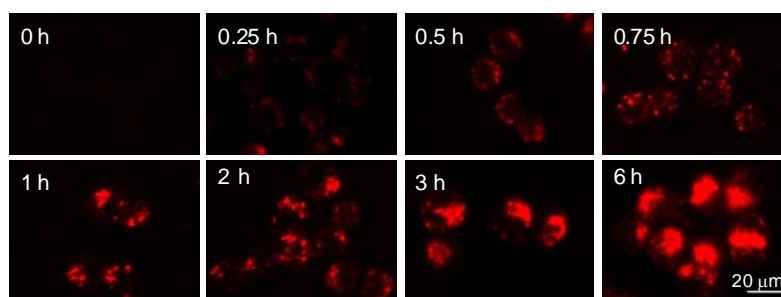


Figure S11. Time course of the $[\text{Eu}_2(\text{L}^{\text{C}2'})_3]$ complex loading into HeLa cells. The cells were incubated in the presence of $500 \mu\text{M}$ of the complex in cell culture medium at 37°C . The images were taken using a Zeiss luminescence microscope Axiovert S100 (Objective: Plan-Neofluar, $20\times$; $\lambda_{\text{exc}} = 330 \text{ nm}$, emission filter = LP 585 filter, acquisition time 60 s).

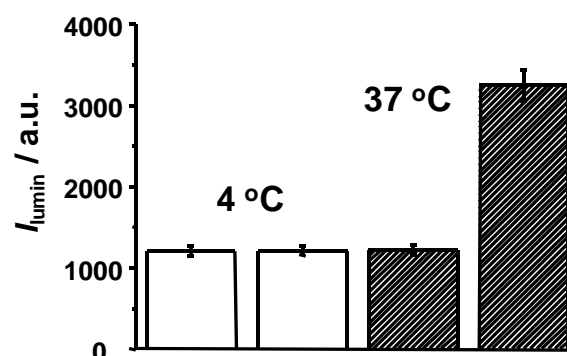
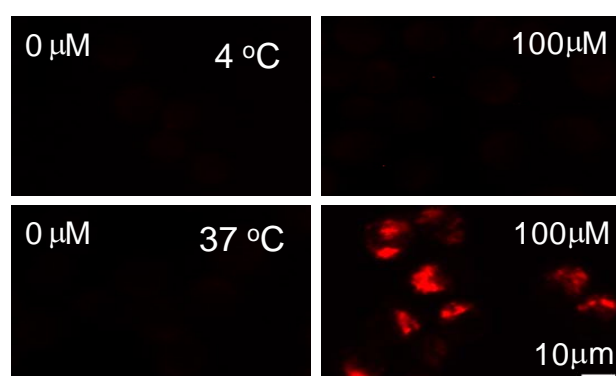


Figure S12. HeLa cells were incubated in presence of $100 \mu\text{M}$ $[\text{Eu}_2(\text{L}^{\text{C}2'})_3]$ in RPMI-1640 for 7 h at 4°C or 37°C . The images were taken using a Zeiss luminescence microscope Axiovert S100 (Objective: Plan-Neofluar, 20x ; $\lambda_{\text{exc}} = 330 \text{ nm}$, emission filter = LP 585 filter, exposure time 60 s).

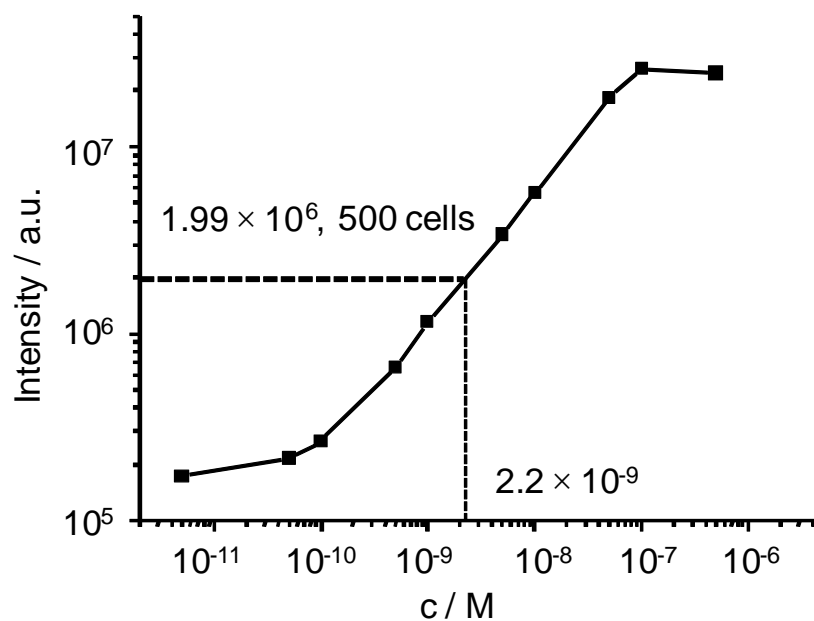


Figure S13. Quantitation of the intracellular concentration of $[\text{Eu}_2(\text{L}^{\text{C}2'})_3]$ using the Delfia[®] method. Each point represents the average of four nominally identical measurements.

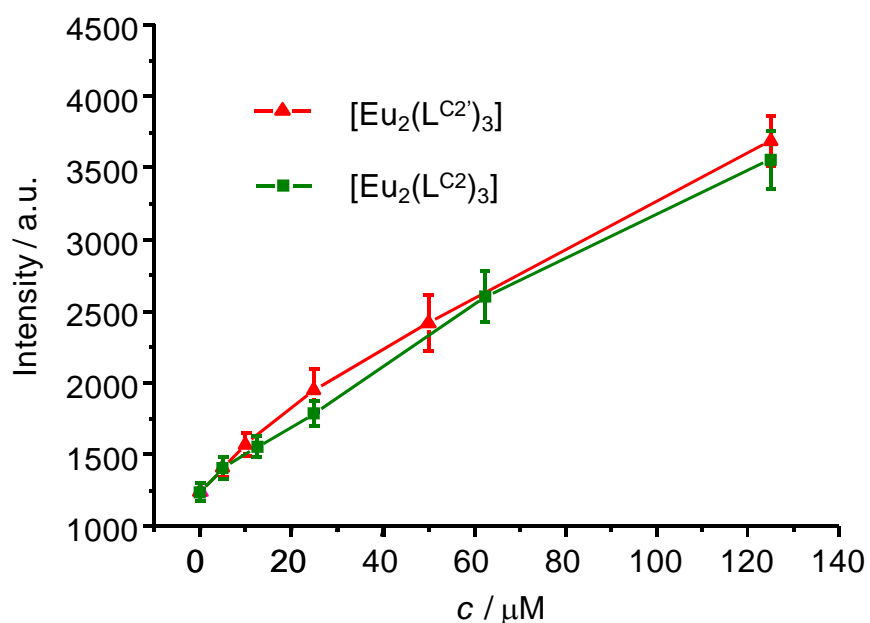


Figure S14. Loading concentration dependence of the emitted luminescence for [Eu₂(L^{C2'})₃] (red curve) and [Eu₂(L^{C2})₃] (green curve). HeLa cells were incubated 7h at 37 °C. The images were taken on a Zeiss luminescence microscope Axiovert S 100 (objective: Plan-Neofluor 20 x; λ_{exc}: 330 nm; emission filter: LP 585; exposure time: 60 s).

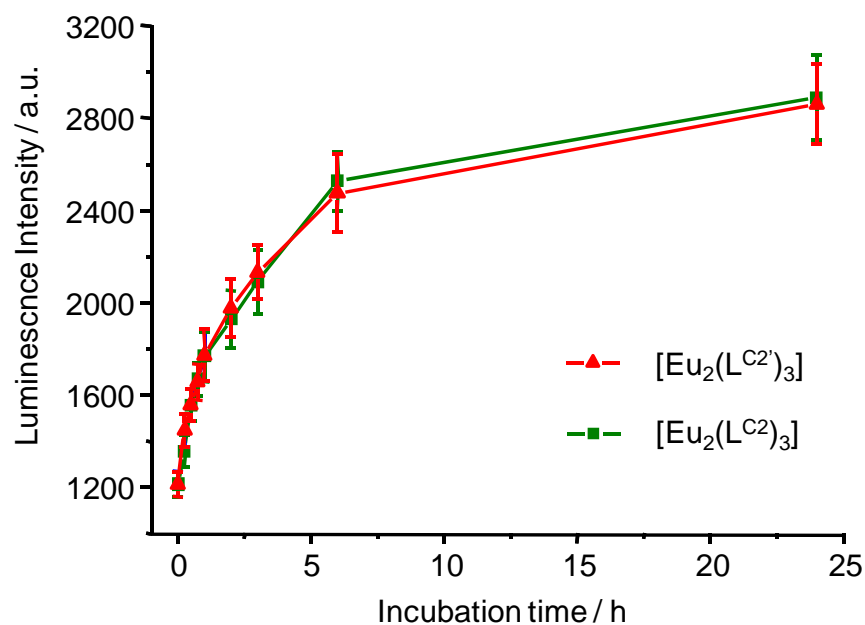


Figure S15. Time-course dependence of the emitted luminescence for $[\text{Eu}_2(\text{L}^{\text{C}2'})_3]$ (red curve) and $[\text{Eu}_2(\text{L}^{\text{C}2})_3]$ (green curve). The cells were incubated at 37 °C in presence of 500 μM helicate. Same imaging conditions as for Figure S14.