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Supporting Information

A Versatile Ditopic Ligand System for Sensitizing the Luminescence of Bimetallic Lanthanide Bio-imaging Probes

Anne-Sophie Chauvin*, Steve Comby, Bo Song, Caroline D. B. Vandevyver*, and Jean-Claude G. Bünzli*[a]

[a] Laboratory of Lanthanide Supramolecular Chemistry, École Polytechnique Fédérale de Lausanne, , BCH 1401, CH-1015 Lausanne (Switzerland)

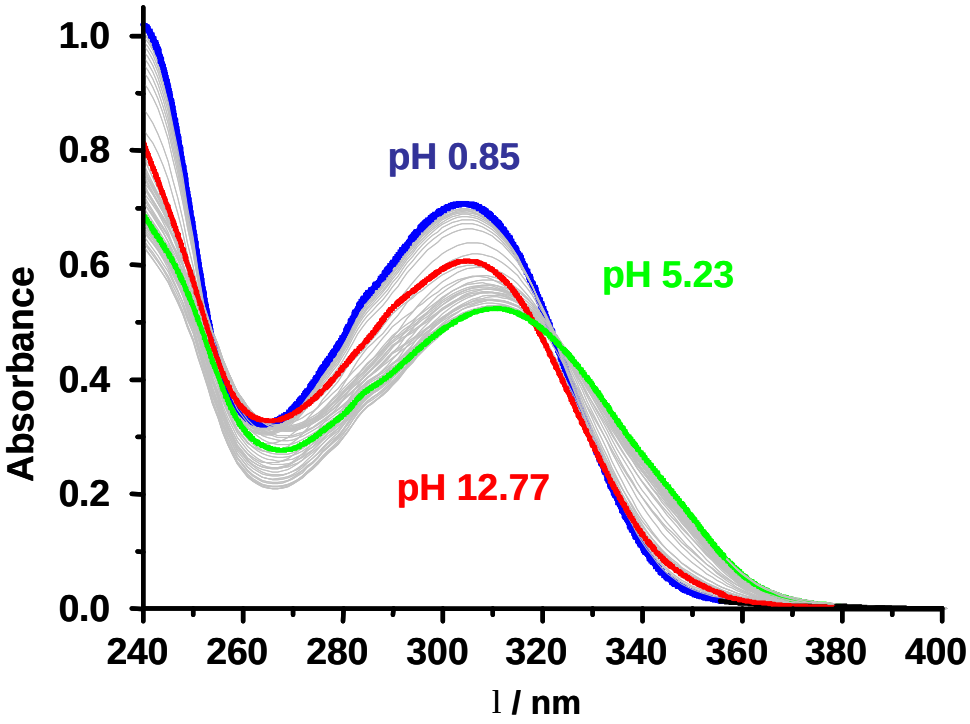

Figure S1. UV-vis titration of H_2L^{C2} 2.25×10^{-5} M by NaOH in the pH range 0.85-12.77, μ = 0.1 M (KCl), at 298 K.

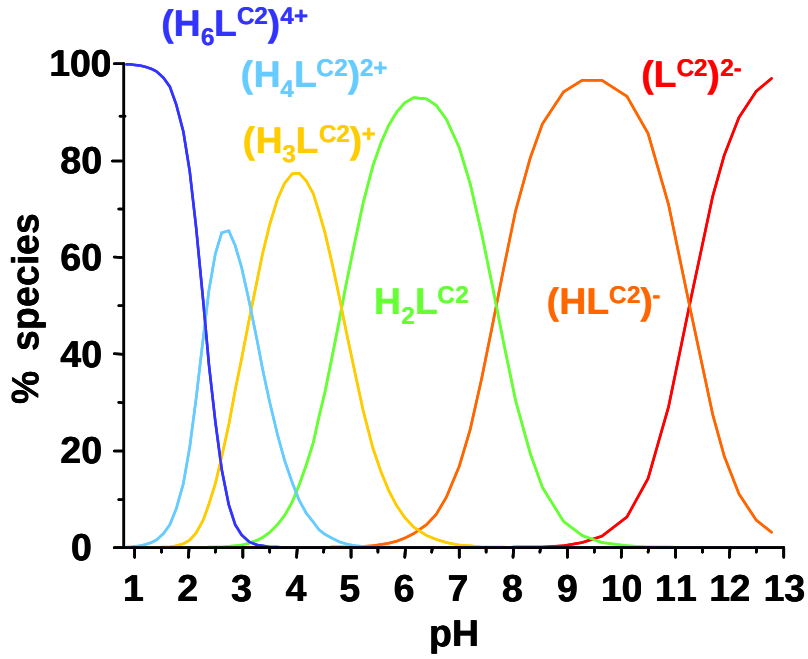

Figure S2. Distribution diagram for H_2L^{C2} 2.25×10^{-5} M at μ = 0.1 M (KCl) and 298 K.

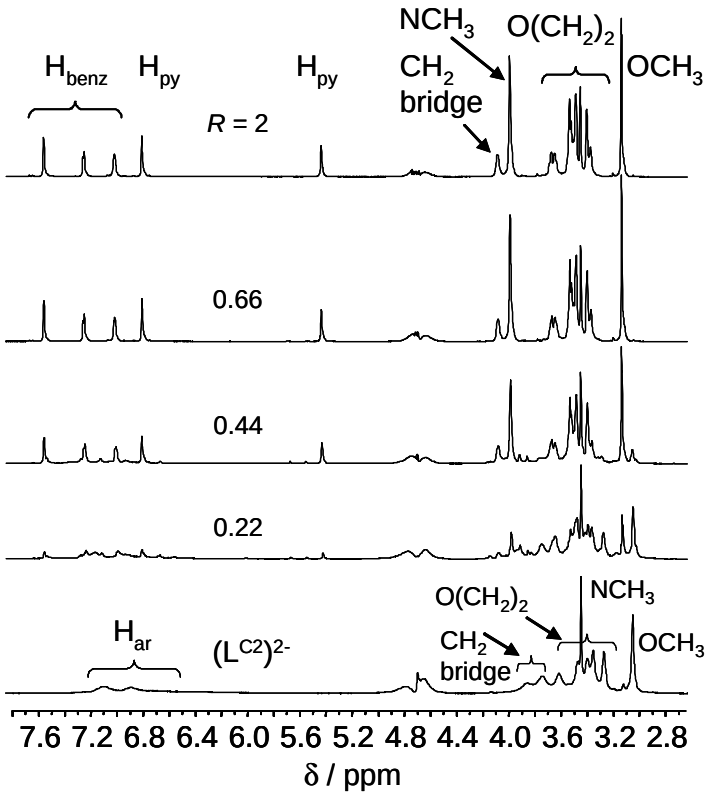

Figure S3. ^{1}H-NMR titration of H_2L^{C2} 10^{-3} M by $Lu(ClO_4)_3$ in Tris-d_{11}-DCl (0.1 M, pD = 7.8); R = $[Lu^{III}]_t/[H_2L^{C2}]_t$

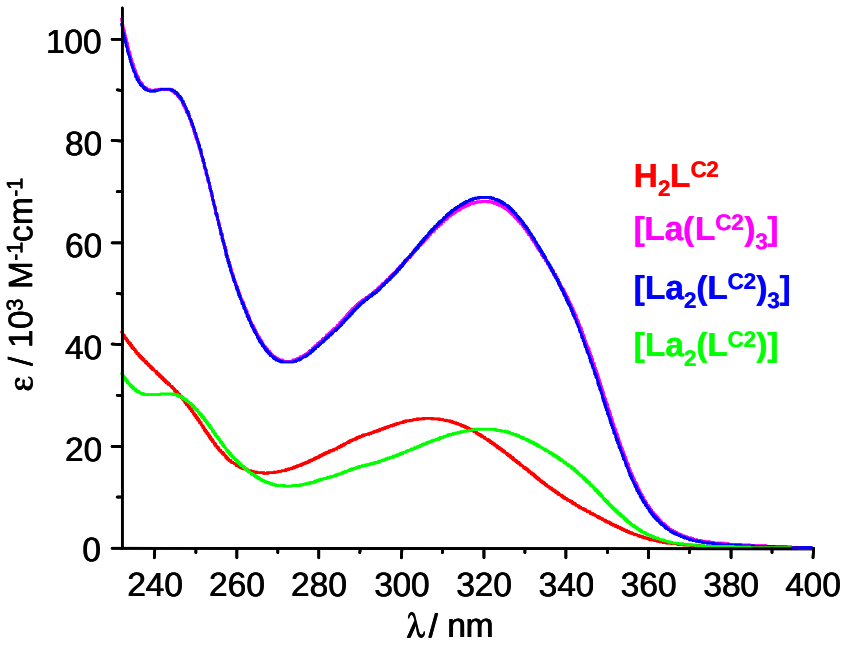

Figure S4. Recalculated spectra from the titration of H_2L^{C2} 10^{-5} M by La^{III}; μ = 0.1 M (KCl) and 298 K.

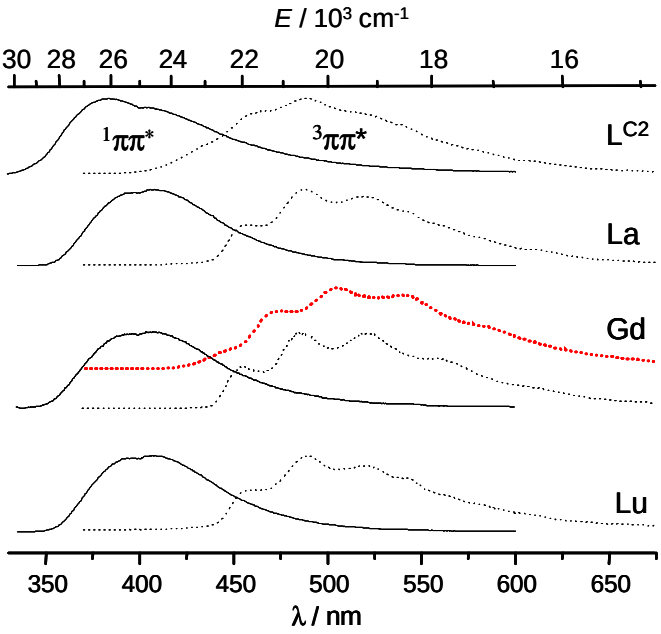

Figure S5. Ligand emission spectra at 295 K (plain lines) and 77 K (with time delay 0.05 ms; dotted lines) under excitation at 307-324 nm; $[H_2L^{C2}]$ = 4.5×10^{-5} M; $[Ln_2(L^{C2})_3]$ = 1.5×10^{-5} M; pH = 7.4 (Tris-HCl). The corresponding $^3\pi\pi^*$ emission from $[Gd_2(L^{C3})_3]$[1] is drawn in red for comparison purposes.

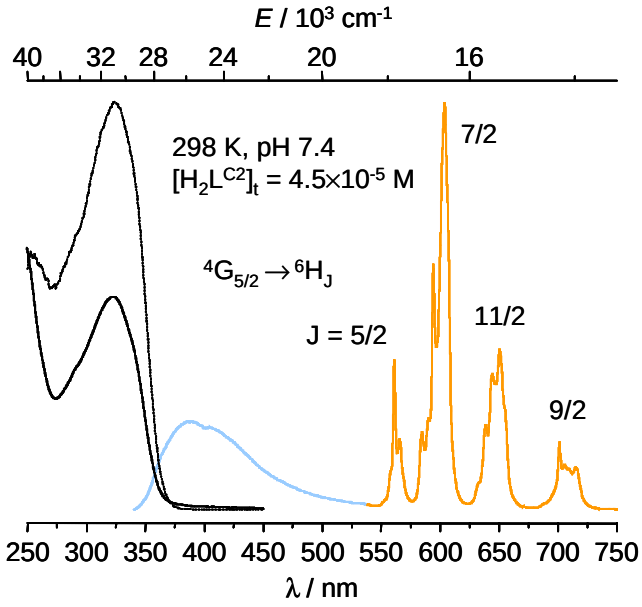

Figure S6. Left: absorption (solid line) and excitation (dotted line) spectra of $[Sm_2(L^{C2})_3]$. Right: corresponding emission spectrum. $[Sm^{III}]_t$ = 3.0×10^{-5} M, pH 7.4 (Tris-HCl 0.1 M), and T =298 K.

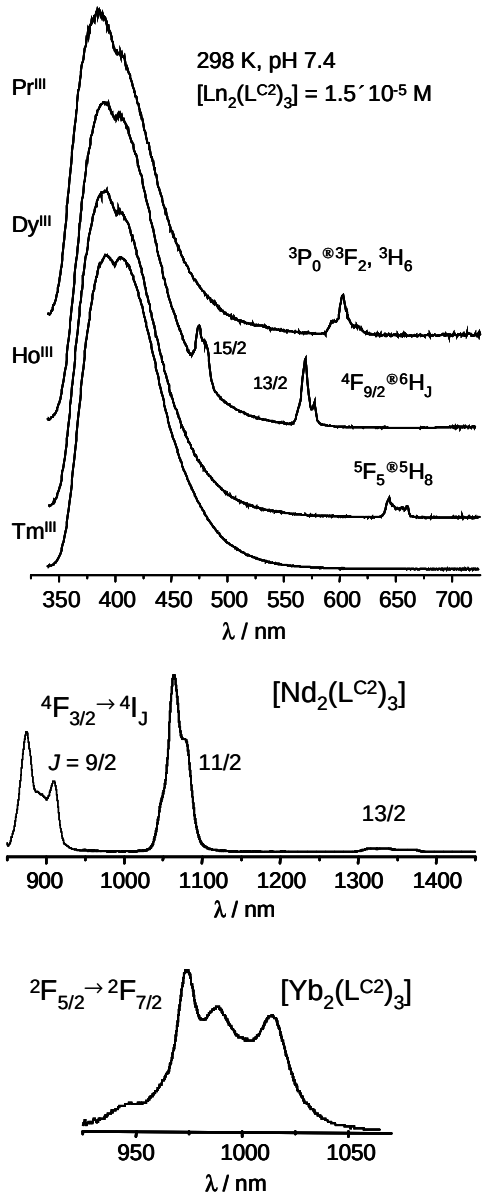

Figure S7. Emission spectra of $[Ln_2(L^{C2})_3]$ 1.5×10^{-5} M at pH 7.4 (Tris-HCl) and 298 K under ligand excitation (323 nm). (Top) Ln = Pr, Dy, Ho, and Tm. (Bottom) Ln = Nd, Yb.

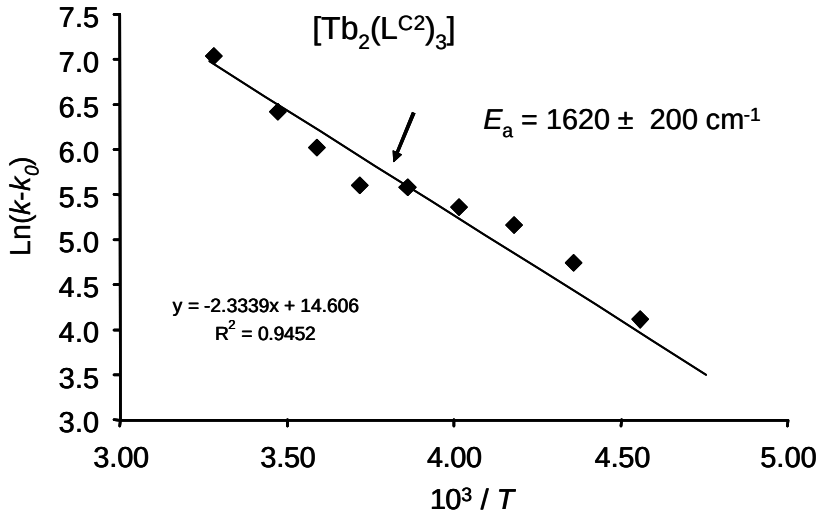

Figure S8. Analysis of the lifetime versus temperature data in term of activation energy for $[Tb_2(L^{C2})_3]$ in the range 10-295 K, recorded upon heating. The arrow points to the probable phase transition

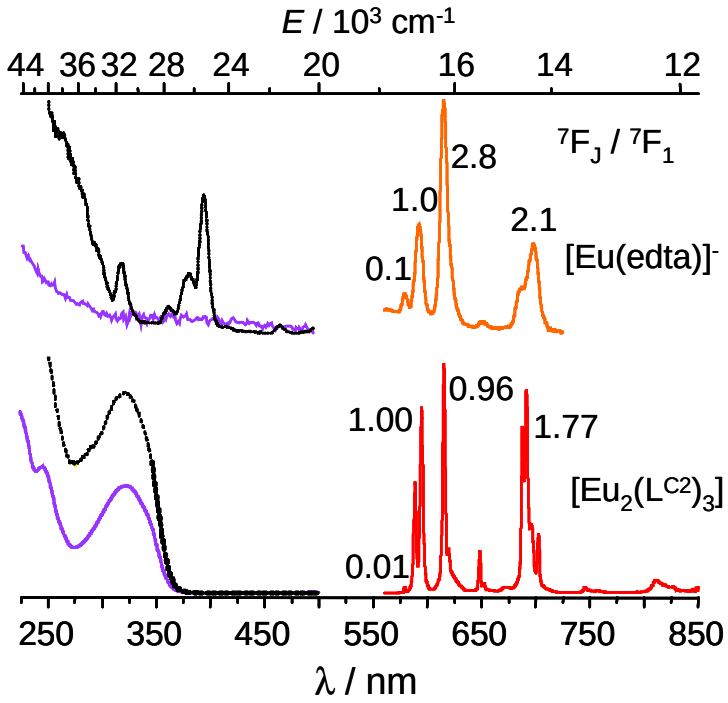

Figure S9. Left: absorption (solid violet line) and excitation spectra (black dotted line) of $[Eu(edta)(H_2O)_n]^-$(top) and $[Eu_2(L^{C2})_3]$ (bottom). Right: corresponding emission spectra (λ_{ex} = 323 nm) with the values of the intensity ratios $I(^5D_0 \rightarrow {}^7F_J)/I(^5D_0 \rightarrow {}^7F_1)$. For both complexes, $[Eu^{III}]_t$ = 3.0×10^{-5} M, pH = 7.4 (Tris-HCl 0.1 M), and T =298 K.

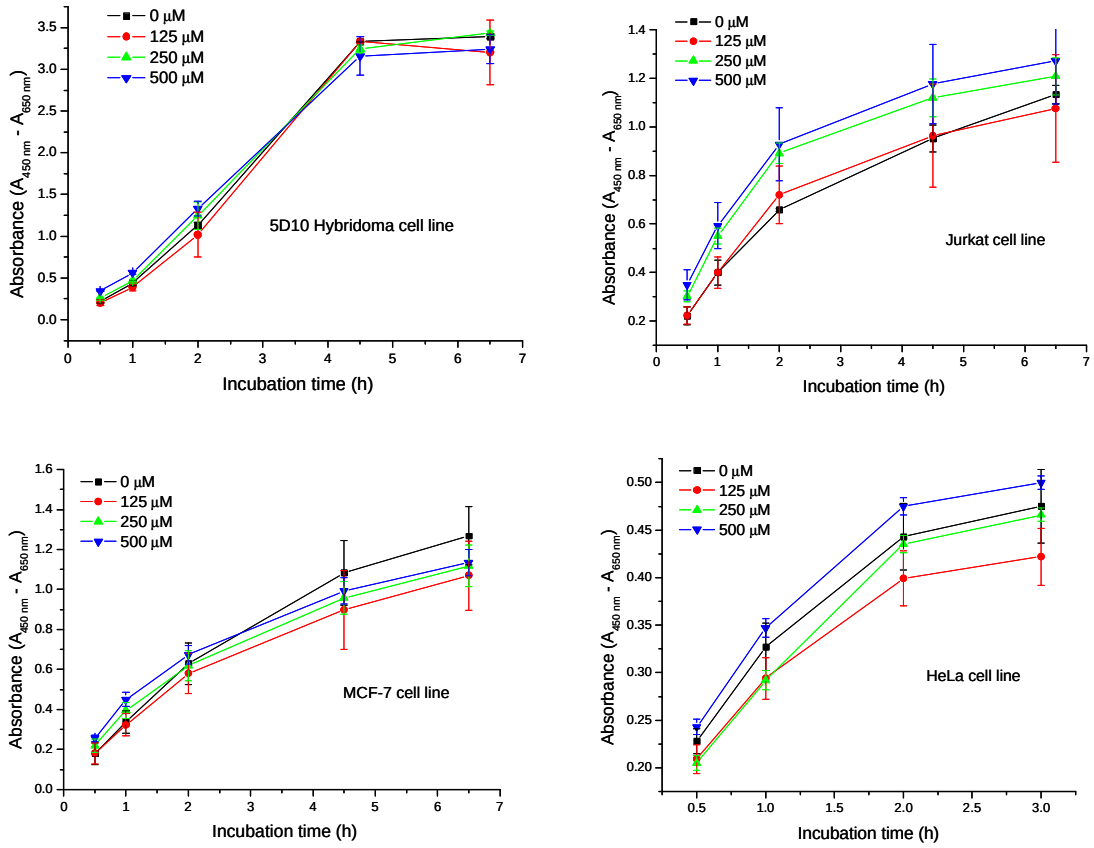

Figure S10. WST-1 proliferation test of different cell lines in absence or presence of different concentrations of $[Eu_2(L^{C2})_3]$. Each point represents the average over three nominally identical measurements.

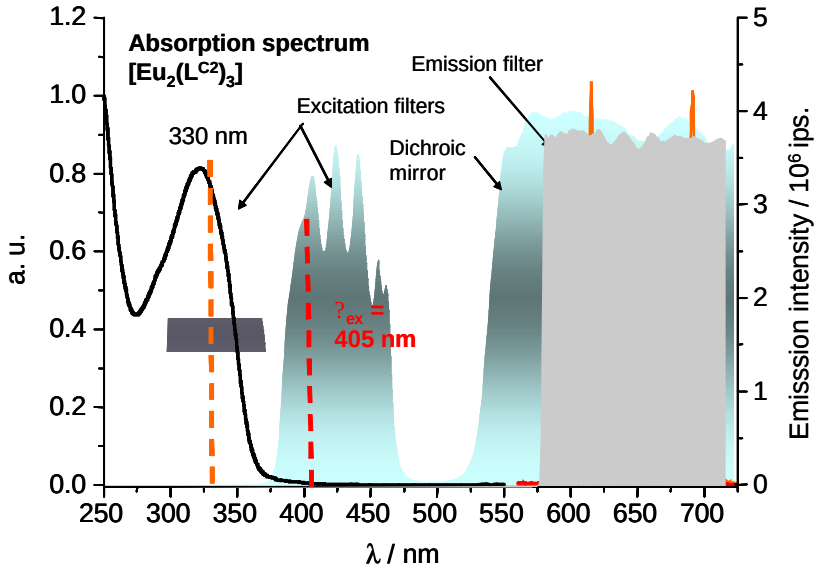

Figure S11. Experimental conditions used for luminescence microscopy.

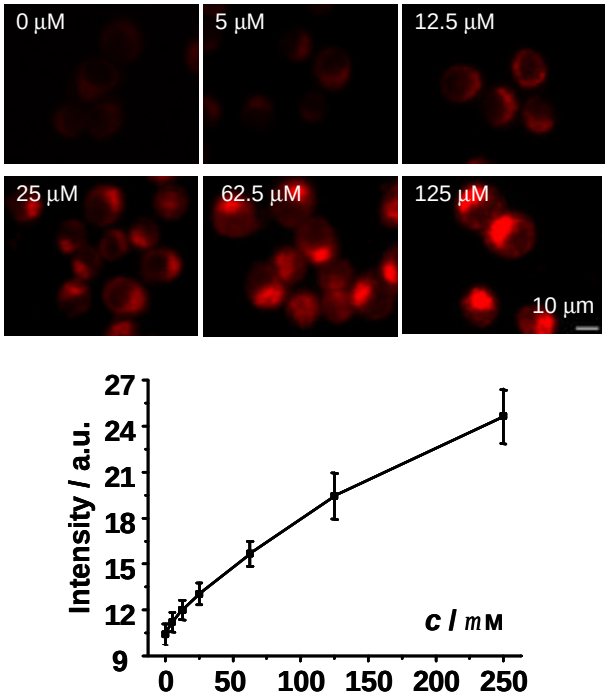

Figure S12. (Top) Luminescence images of HeLa cells loaded with different concentrations of the $[Eu_2(L^{C2})_3]$ in RPMI-1640 for 7 h at 37°C. The images were taken using a Zeiss fluorescence microscope Axiovert S100 (Objective: Plan-Neofluar, 20x ; λ_{exc} = 330 nm (BP 80 nm) , emission filter = LP 585, exposure time 60 s). (Bottom) Corresponding graph showing the luminescence intensity versus concentration of the helicate.

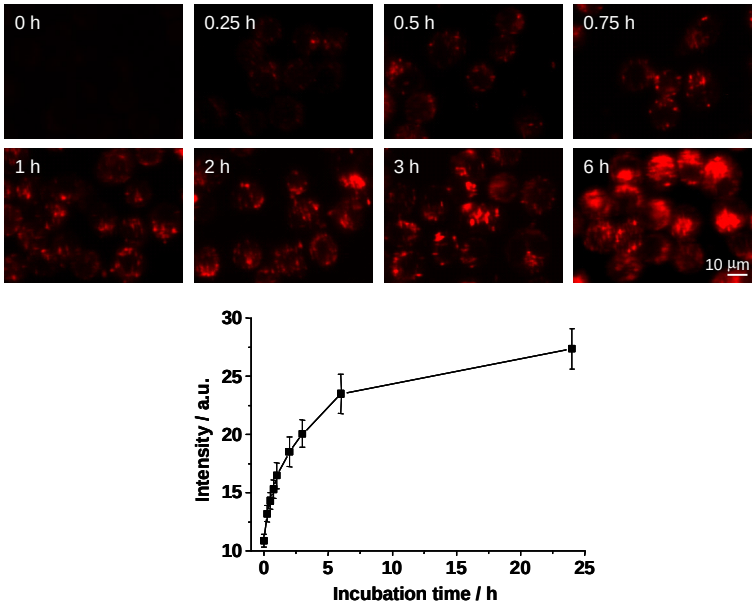

Figure S13. Time course of $[Eu_2(L^{C2})_3]$ loading into HeLa cells. The cells were incubated in the presence of 250 µM of the complex in cell culture medium at 37°C. The images were taken using a Zeiss fluorescence microscope Axiovert S100 (Objective: Plan-Neofluar, 20x ; λ_{exc} = 330 nm (BP 80 nm) , emission filter = LP 585 nm, exposure time 60 s).

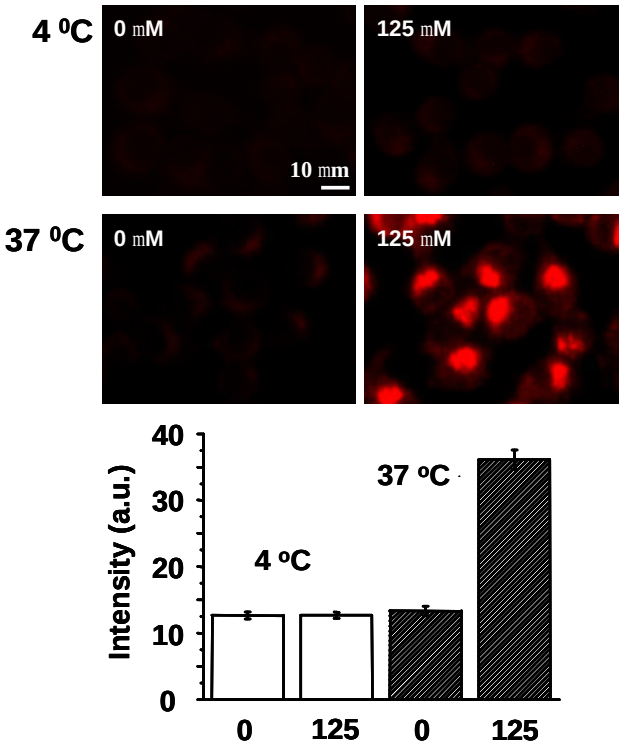

Figure S14. HeLa cells were incubated in presence of 125 µM $[Eu_2(L^{C2})_3]$ in RPMI-1640 for 7 h at 4 °C or 37 °C. The images were taken using a Zeiss luminescence microscope Axiovert S 100 (Objective: Plan-Neofluar 20x, $?_{exc}$ = 330 nm (BP 80 nm) , emission filter = LP 585 nm, exposure time 60 s).

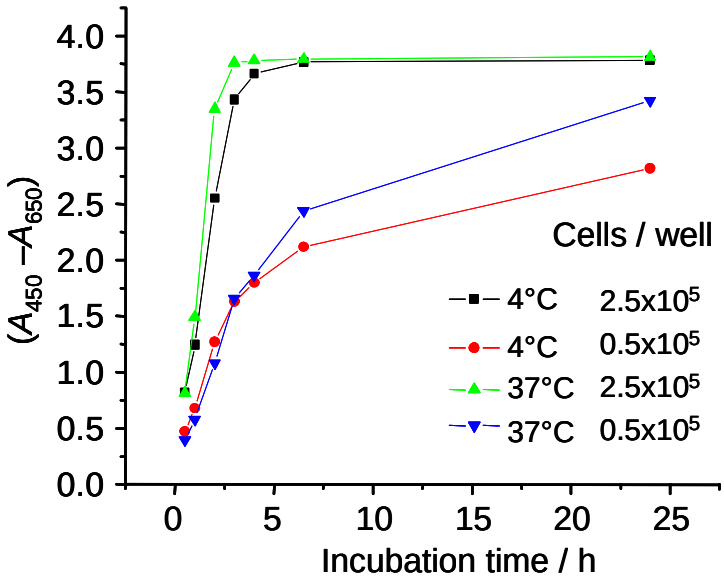

Figure S15. WST-1 proliferation test of HeLa cells after incubation overnight at 4°C or 37°C. Each point represents the average over six nominally identical measurements.

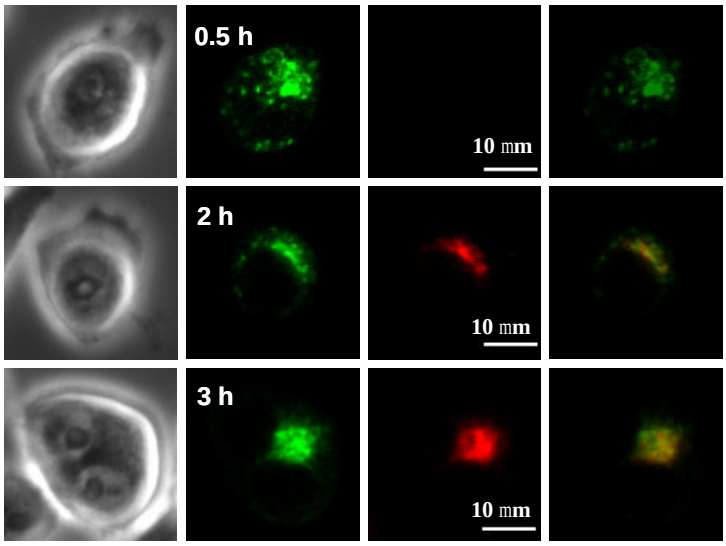

Figure S16. Time course of the co-localization experiments with labeled transferrin. Same conditions as for Figure 8. First column: bright field images; 2nd column, transferrin; 3rd column Eu^{III} luminescence; 4th column: merged images.

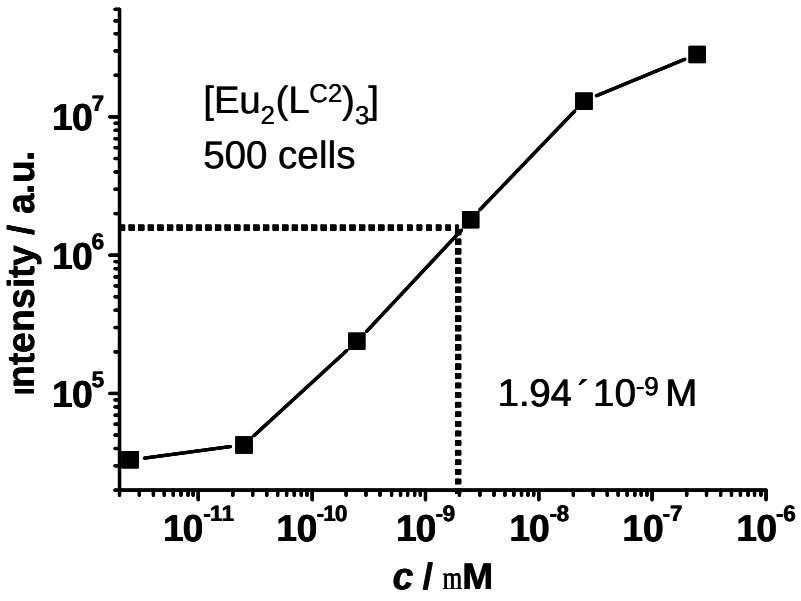

Figure S17. Determination of the helicate concentration: calibration curve of the luminescence intensity (*I*) versus the concentration (*c*) of the solution with which 500 HeLa cells were incubated (logarithmic scales).

Table S1. Corrected intensity ratios $I(^5D_0 \rightarrow {}^7F_J)/I(^5D_0 \rightarrow {}^7F_1)$ = $^7F_J/^7F_1$ versus time for $[Eu(edta)(H_2O)_n]^-$, $[Eu_2(L^{C2})_3]$, and a solution of the latter to which were added 15 equivalents edta (t = 0) and further 85 equivalents after 44 h.

Compound	t / h	$^7F_0/^7F_1$	$^7F_1/^7F_1$	$^7F_2/^7F_1$	$^7F_3/^7F_1$	$^7F_4/^7F_1$
$[Eu(edta)(H_2O)_n]^-$	0	0.12	1.00	2.83	0.10	2.10
$[Eu_2(L^{C2})_3]$	0	0.01	1.00	0.96	0.12	1.77
$[Eu_2(L^{C2})_3]$ + 15 eqs edta	44	0.01	1.00	0.96	0.11	1.74
$[Eu_2(L^{C2})_3]$ + 100 eqs edta	68	0.01	1.00	0.96	0.12	1.74
	236	0.01	1.00	0.96	0.12	1.73
	670	0.01	1.00	0.96	0.12	1.75

Table S2. Corrected intensity ratios $^7F_J/^7F_1$ (Eu^{III}) and $^7F_J/^7F_4$ (Tb^{III}) for $[Ln_2(L^{C2})_3]$ 10^{-4} M in water at pH 7.4 and after incubation (125 μM, 15 h, 37 °C). Excitation wavelength: 330 nm, emission bandpass 0.5 nm.

Exp. Cond.	Corrected intensity ratios				
Eu	0/1	1/1	2/1	3/1	4/1
Solution	0.01	1.00	1.00	0.09	1.77
In cellulo	0.01	1.00	0.96	0.08	1.35
Tb	6/4	5/4	4/4	3/4	2/4
Solution	0.54	1.00	0.43	0.18	0.02
In cellulo	0.56	1.00	0.44	0.18	0.02

Reference List

(1) Vandevyver, C. D. B.; Chauvin, A.-S.; Comby, S.; Bünzli, J.-C. G. *Chem. Commun.* **2007**, 1716.