

Supporting Information

Lanthanide-Complex-Loaded Polymer Nanoparticles for Background-Free Single-Particle and Live-Cell Imaging

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Experimental Details

Microscopy

Steady-state imaging.

For ECP-NP detection, cells were imaged with an Olympus IX71 microscope and illuminated with an X-Cite 120Q mercury source (EXFO Photonic Solutions Inc.) at ~15 W through a 365 nm excitation band-pass filter (Hg01-365-25, Semrock Inc.) and 495 nm dichroic mirror (FF495-Di03, Semrock Inc.). The fluorescent signal was detected using a 60x objective (UPLSAPO, Olympus) and a 605 nm emission filter (FF01-605/15-25, Semrock Inc.). PL signal was recorded using a CMOS camera (pco.edge, PCO) with an acquisition time of 333 ms.

3D imaging.

Fluorescent images of HeLa cells were acquired on a wide-field inverted microscope Olympus IX83 using an X-Cite Exacte illumination source (Excelitas Technologies) and iXon Ultra 888 EMCCD camera (Andor). ECP-NPs were excited with a 100 W source power using 320 nm excitation filter (FF01-320/40-25, Semrock Inc.) and 355 nm dichroic mirror (Di01-R355, Semrock Inc.) and their emission collected using 605 nm filter (FF01-605/15-25, Semrock Inc.). DiD was excited with 10 W illumination power using 623 nm band-pass filter (FF01-623/24-25, Semrock Inc.), 649 nm dichroic mirror (FF649-Di01, Semrock Inc.) and the emission was collected through a 676 nm bandpass-filter (FF01-676/29-25, Semrock Inc.). Sample was imaged using UPLSAPO 60x objective (Olympus). Z-stack images were obtained by a displacement of a step of 0.25 μm . Images of ECP-NPs were acquired with x750 EM gain, 30 MHz readout speed, 1.1 μs vertical shift speed, and x2 preamplifier sensitivity. Images of plasma membrane stained with DiD were acquired with the same parameters except EM gain at x2. iXon camera and Olympus microscope were controlled by the Micromanager software.

Photon counting

Photon counting imaging was performed for PMMA-COOH 10% particles loaded with 40wt% [Eu(tta)₃phen] as a reference on a wide-field inverted microscope Olympus IX83 using X-Cite Exacte illumination source at 2 W (Excelitas Technologies) and iXon Ultra 888 EMCCD camera (Andor), 365 nm excitation filter (Hg01-365-25, Semrock Inc.) and 405 nm dichroic mirror (Di02-R405, Semrock Inc.). The excitation power density was measured to be 0.02 W/cm² at the sample plane and the illumination area was 0.005 cm².

The number of photons collected by the camera per pixel can be calculated from the equation:

$$Nb\ of\ collected\ photons = \frac{(signal-background) \times camera\ sensitivity}{analog\ gain \times EM\ gain \times QE} \quad (1)$$

Where the camera sensitivity (electrons to counts conversion) was 5.17, analog gain 2, EM gain 1000 and quantum efficiency (QE) 95 % at 605 nm. To obtain the total number of collected photons per particle, the measured numbers of photons per pixel were integrated over an area of typically 21 pixels (circular region of diameter 5 pixels, containing > 95 % of the detected photons) The number of emitted photons per particle can then be determined using:

$$Nb\ of\ emitted\ photons = \frac{\int Nb\ of\ collected\ photons}{\%photons\ into\ objective \times T\ obj \times T\ filter} \quad (2)$$

Objective transmittance ($T\ obj$) of UPLSAPO 60x in ECP-NPs emission wavelength range and FF01-605/15-25 emission filter transmittance ($T\ filter$) were 90% and 33%, respectively. The collection half-angle of the objective was 64.4° , corresponding to a solid angle of 3.6 sr. 28 % of the emitted photons are thus collected by the objective. The number of photons emitted per particle per second can be obtained using the following equation:

$$Nb\ of\ emitted\ photons/s = \frac{Nb\ of\ emitted\ photons}{t_{exposure}} \quad (3)$$

Exposure time ($t_{exposure}$) was fixed at 50 ms.

Dividing the number of emitted photons by the number of incoming photons per second ($3.6 \times 10^{16} \text{ cm}^{-2}$) yields the fluorescence cross section σ .

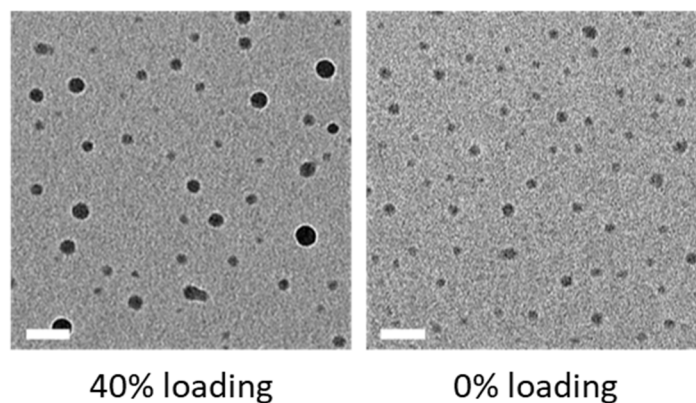


Figure S1. Comparison of TEM images of PMMA-COOH 10% ECP-NPs with (left) or without (right) [Eu(tta)₃phen] loading. Here no staining with uranyl acetate was applied. The scale bars correspond to 100 nm.

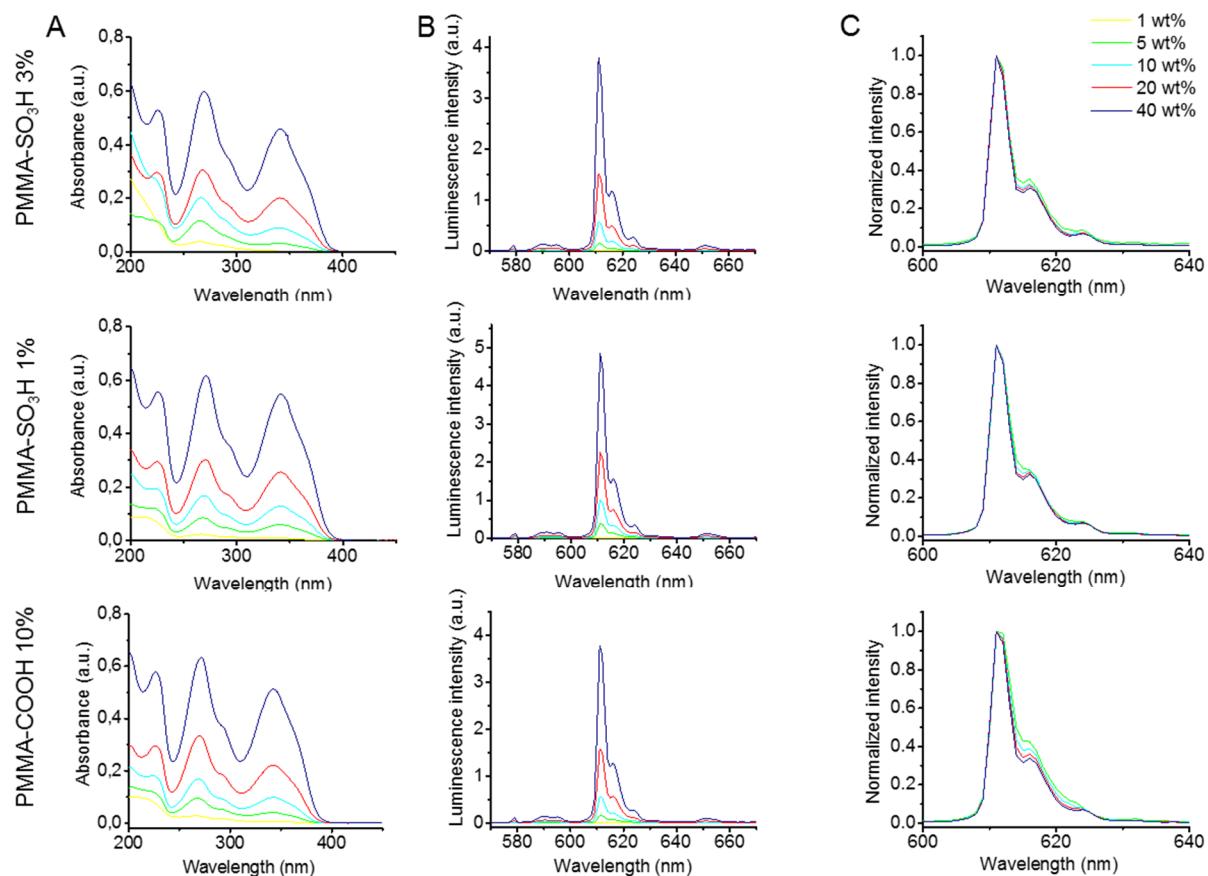


Figure S2. Absorbance (A), emission (B), and normalized emission (C) spectra of PMMA-SO₃H 1% and 3% and PMMA-COOH 10% NPs loaded with 1, 5, 10, 20, and 40 wt% [Eu(tta)₃phen] (λ_{exc} = 350 nm).

Table S1. PL quantum yields (ϕ) of solutions of [Eu(tta)₃phen] in acetonitrile and of ECP-NPs in water (r.t., $\lambda_{exc} = 350$ nm). The Eu^{III}-centered QY ($\phi_{Eu}^{Eu} = 0.50$) was calculated according to Equation 1,¹ from which a sensitization efficiency $\eta_{sens} = 0.56$ was calculated using Equation 5.

Sample	ϕ_{Eu}^L (%)	ϕ_{Eu}^{Eu} (%) ^{a)}
[Eu(tta) ₃ phen]	0.30	0.50
PMMA-COOH 10% - 40% [Eu(tta) ₃ phen]	0.26	0.52
PMMA-COOH 10% - 20% [Eu(tta) ₃ phen]	0.21	0.54
PMMA-COOH 10% - 10% [Eu(tta) ₃ phen]	0.16	0.59

^{a)} according to Equation 4. Estimated errors: $\pm 15\%$.

$$\phi_{Eu}^{Eu} = \tau_{H_2O} / \tau_R, \text{ with } \tau_R = (1/A_{MD,0}n^3)(I_{MD}/I_{tot}) \quad (4)$$

where $A_{MD,0} = 14.65 \text{ s}^{-1}$ arises for the spontaneous emission probability for the $^5D_0 \rightarrow ^7F_1$ transition in vacuum, n is the refractive index of the solution ($n = 1.341$ at 25°C for acetonitrile), I_{MD} and I_{tot} are the area of emission of the $^5D_0 \rightarrow ^7F_1$ transition and of all the transitions respectively.

$$\phi_{Eu}^L = \eta_{sens} \times \phi_{Eu}^{Eu} \quad (5)$$

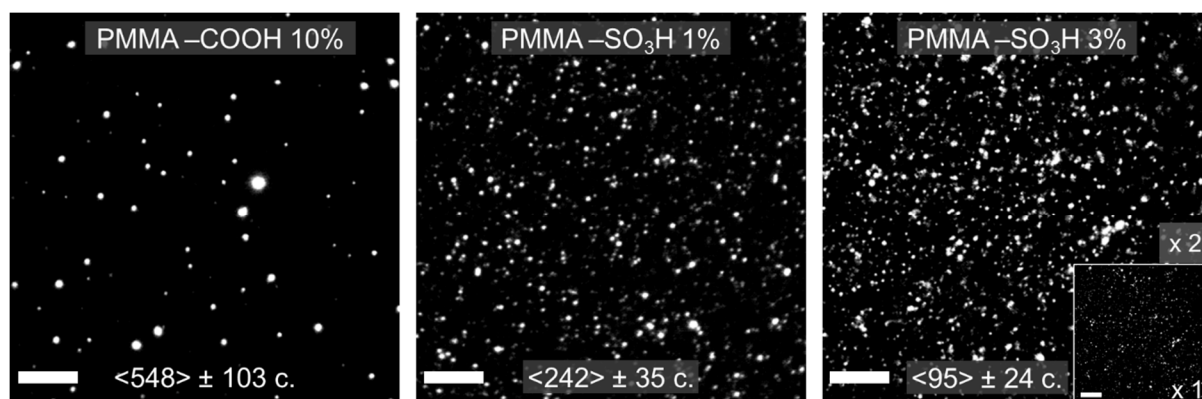


Figure S3. TG single-particle images of PMMA-COOH 10 %, PMMA-SO₃H 1 %, and PMMA-SO₃H 3 % at 40% [Eu(tta)₃phen] loading. Values at the bottom of the images are average PL intensities (in photon counts: c. \pm SD). For better visibility the intensity was increased twofold for PMMA- SO₃H 3% (original in inset). Scale bars: 10 μ m.

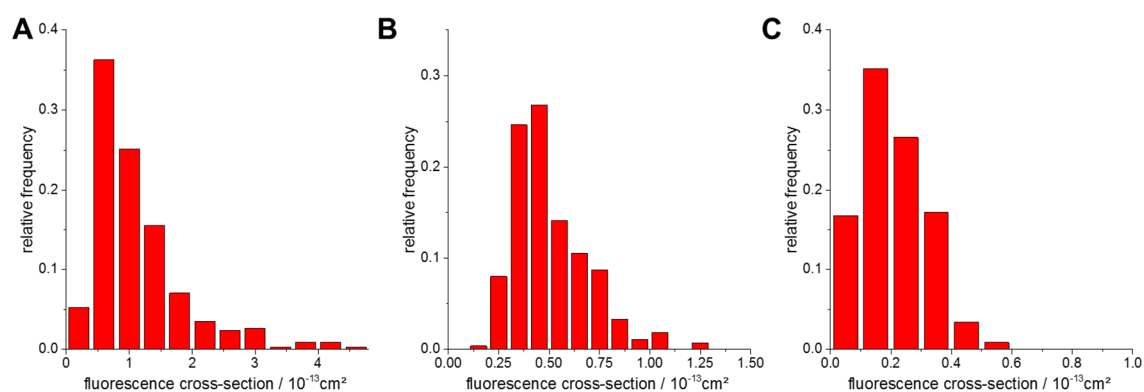


Figure S4. Histograms of single particle brightnesses of (A) PMMA-COOH 10 % NPs, (B) PMMA-SO₃H 1%, (C) PMMA-SO₃H 3%, all loaded with 40% [Eu(tta)₃phen]. At least 250 particles have been analyzed per condition.

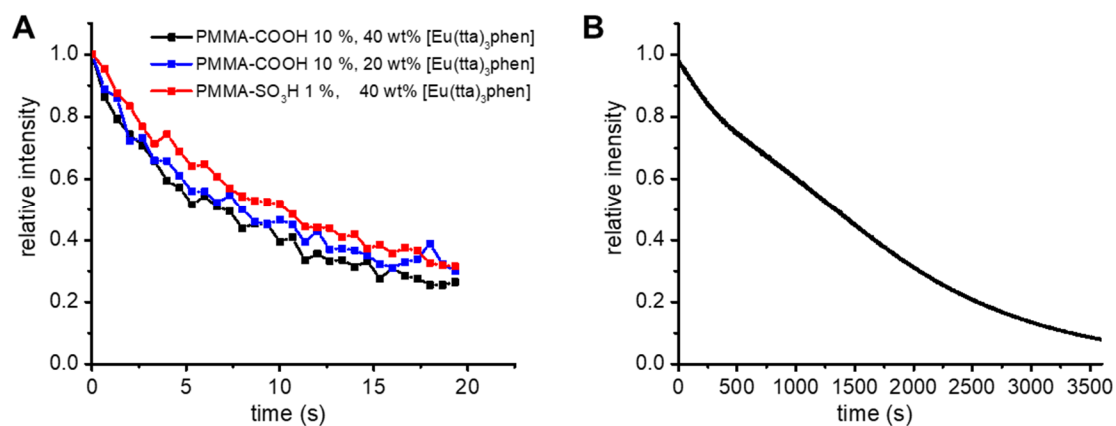


Figure S5. (A) Single particle photostability of Eu(tta)₃phen loaded NPs in TG imaging in water. Pulsed excitation with a Nd:YLF laser at 349 nm and 300 Hz was used. Acquisition was performed using a delay time of 10 μ s, a gate width of 0.6 ms, and 200 gates/exposure. NPs: PMMA-COOH 10% NPs loaded with 20 or 40 wt % Eu(tta)₃phen and PMMA-SO₃H 1% NPs loaded with 40 wt % Eu(tta)₃phen. (B) Ensemble photostability of Eu(tta)₃phen in acetonitrile solution as measured in a fluorometer ($\lambda_{\text{ex}} = 345$ nm, $\lambda_{\text{em}} = 612$ nm).

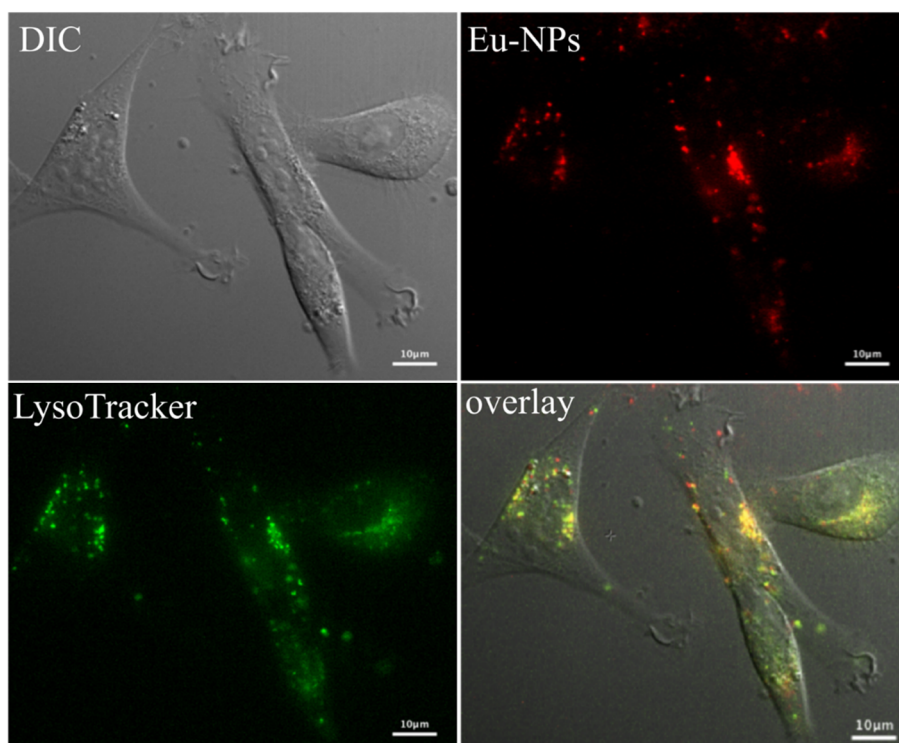


Figure S6. Live-cell images of HeLa cells costained with ECP-NPs (PMMA-COOH 10%, 40% [Eu(tta)₃phen]) and green LysoTracker. PL of ECP-NPs (red) and fluorescence of LysoTracker (green) were measured with steady-state detection. Cells were incubated for 3h in OptiMEM medium at 37°C followed by rinsing with PBS. After transferring cells into LabTek wells and letting them adhere overnight, they were incubated with green LysoTracker in complete DMEM at the final concentration of 50 nM for 30 minutes at 37°C. Images were acquired using a wide-field inverted Olympus IX71 with CMOS camera (500 ms) and illumination with a mercury source (15W).

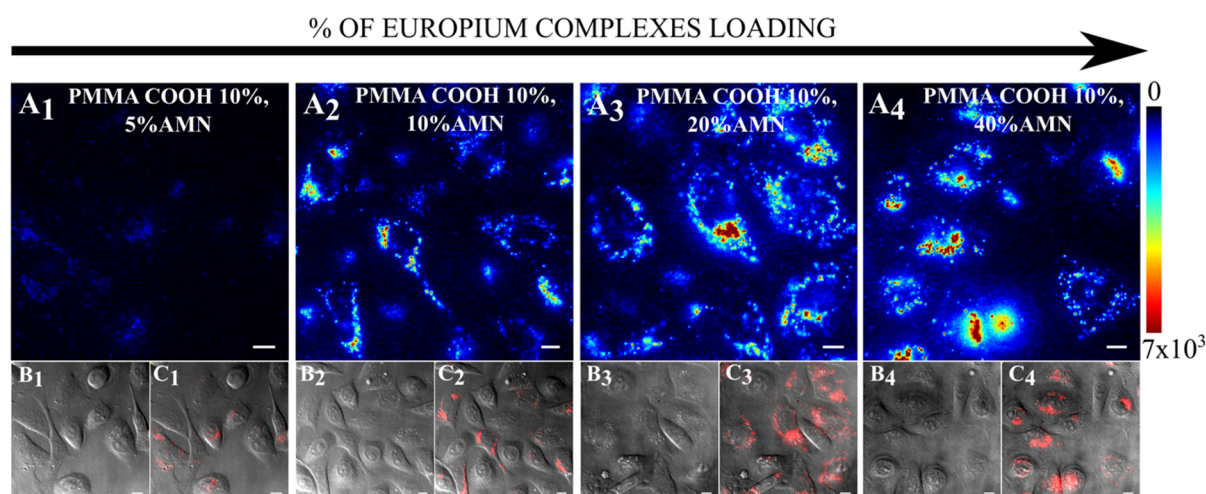


Figure S7. Live-cell images of HeLa cells incubated with ECP-NPs. (A) Time-gated PL signal from PMMA-COOH 10% NPs at 5, 10, 20 and 40% [Eu(tta)₃phen] complex loading. (B) Differential interference contrast (DIC) images. (C) Overlay: DIC overlaid with PL (ECP-NPs in red). The TG images were acquired on a wide-field inverted Olympus IX71 microscope with iCCD camera (100 gates/exposure, 0.01 ms delay time 0.6 ms gate time and 100V gain) and pulsed laser excitation at 349 nm and 300 Hz. Scale bars: 10 μm.

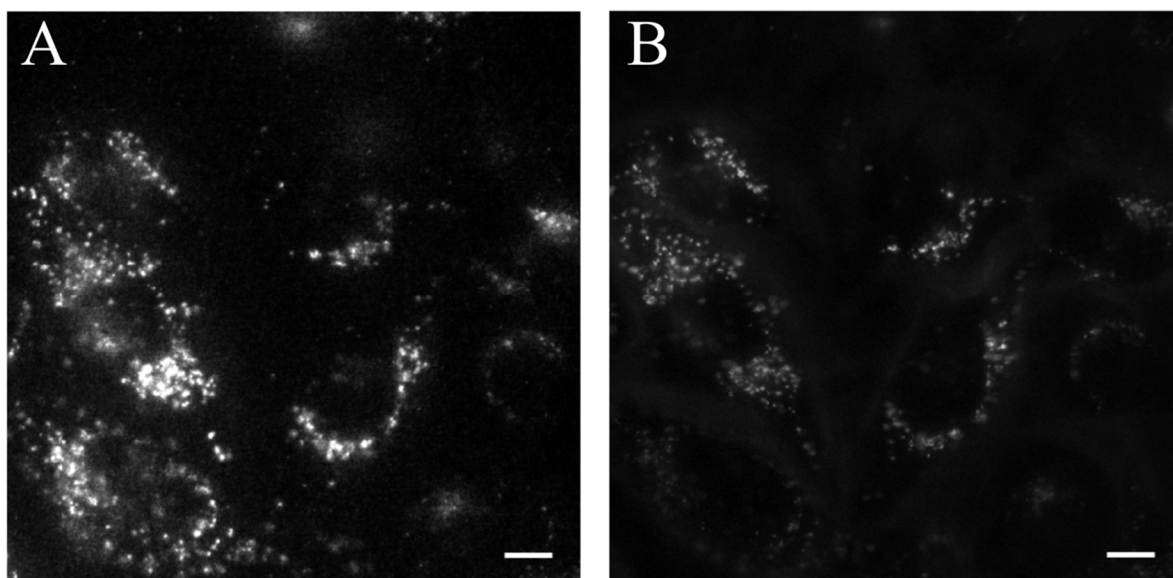


Figure S8. Live-cell images of HeLa cells incubated for 3h with PMMA-COOH 10%, 20% [Eu(tta)₃phen] NPs(A) TG PL intensity recorded on a wide-field inverted Olympus IX71 microscope with iCCD camera (100 gates/exposure, 0.01 ms delay time 0.6 ms gate time and 100 V gain) and pulsed laser excitation at 349 nm and 300 Hz. (B) Steady-state image acquired on the same microscope with CMOS camera (333 ms) and illuminated with mercury source (10 W). Intensity scale was adjusted from 0 to 5000 counts. Scale bars: 10 μ m.

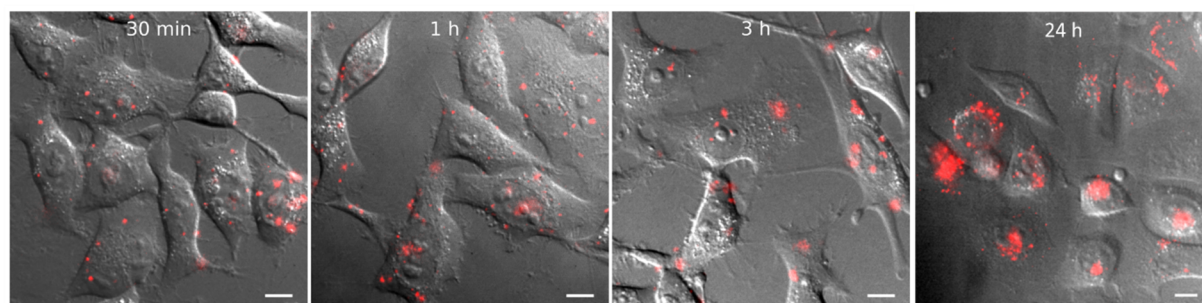


Figure S9. Time-lapse of Eu-NPs PMMA-SO₃H 1% at 40% [Eu(tta)₃phen] localization in live HeLa cells incubated with Eu-NPs The image after 24h shows the cells after trypsination and transfer to a new well. Merge of DIC image with TG-PL (Eu-NPs are in red). Images were acquired on a wide-field inverted Olympus IX71 microscope with iCCD camera (100 gates/exposure, 0.01 ms delay time 0.6 ms gate time and 100V gain) and pulsed laser excitation at 349 nm and 300 Hz. The NPs intensity range was adapted to each case in order to appreciate the localization and not the intensity of PL signal Scale bars: 10 μ m.

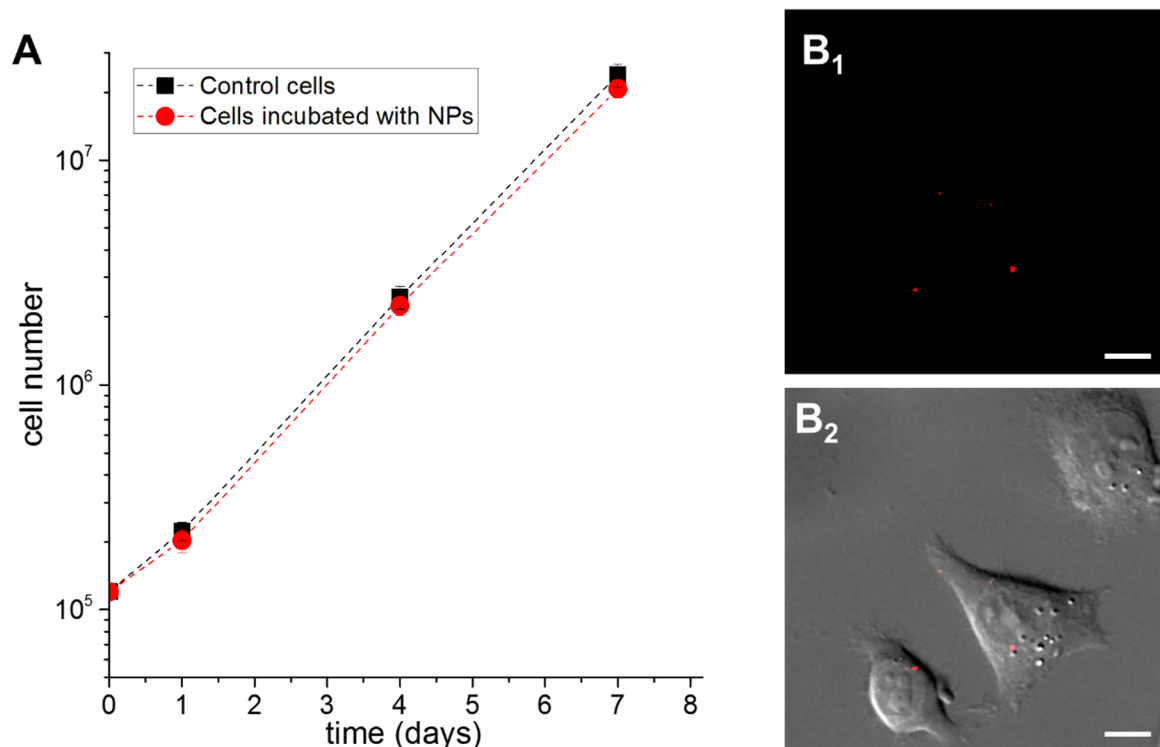


Figure S10. Cell growth of HeLa cells investigated on control cells (black squares) and cells incubated with NPs PMMA-SO₃H 3% (red circles) at 20% [Eu(tta)₃phen] loading (A). Error bars correspond to SD. No significant difference between these two conditions were observed (test T concluded no difference between mean cell number values within 95% confidence level at 1 and 4 days and 99% at 7days). (B) Live-cell images of HeLa cells incubated with NPs. TG PL images with NPs after 7 days (B₁) and overlay with DIC images (B₂). Scale bars correspond to 10 μm.

References

- (1) Werts, M. H. V.; Jukes, R. T. F.; Verhoeven, J. W. The Emission Spectrum and the Radiative Lifetime of Eu³⁺ in Luminescent Lanthanide Complexes. *Phys. Chem. Chem. Phys.* **2002**, *4* (9), 1542–1548. <https://doi.org/10.1039/B107770H>.