Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information for

Kinetically Stable Sub-50nm Fluorescent Block Copolymer Nanoparticles via Photomediated RAFT Dispersion Polymerization for Cellular Imaging

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I. Calculation of colloidal data

I.1 Number of particles (N_p)

$$N_p = \frac{6\tau}{\pi \rho_{PS} D_p^3} \qquad (1)$$

Where

ρ_{PS}: density of polystyrene = 1.047 [g cm^3]

D_p: diameter of particles determined by TEM images using ImageJ software [nm]

τ: mass concentration of copolymer (2) [g L^{-1}]

I.2 Mass concentration of copolymer calculations (τ)

$$\tau = \frac{Conv. \cdot m_{monomer} + m_{macro-CTA}}{\frac{m_{solvent}}{\rho_{solvent}} + \frac{m_{H_2O}}{\rho_{H_2O}}} \qquad (2)$$

Where

$Conv.$: Conversion of styrene at current time []

$m_{monomer}$: initial mass of monomer [g]

$m_{macro-CTA}$: initial mass of macro-CTA [g]

$m_{solvent}$: initial mass of solvent [g]

$\rho_{solvent}$: volumetric mass density of solvent [g L^{-1}]

m_{H_2O}: initial mass of water [g]

ρ_{H_2O}: volumetric mass of water [g L^{-1}]

I.3 Polydispersity index calculations (PDI)

$$PDI = \frac{D_w}{D_n} \qquad (3)$$

Where

D_w: weight-average diameter [nm]

D_n: number-average diameter [nm]

Weight-average diameter calculations (D_w) :

$$D_w = \frac{\sum {D_p}^4}{\sum {D_p}^3} \quad (4)$$

Where

D_p: diameter of particles determined by TEM [nm]

Number-average diameter calculations (D_n) :

$$D_n = \frac{\sum D_p}{n} \quad (5)$$

Where

D_p: diameter of particles determined by TEM [nm]

n : number of particles used in manual treating of TEM images with ImageJ software [-]

II. Additional tables

Table S1. Synthesis of POEGA macro-CTAs with different chain lengths

macro-CTA	***Time*** **(h)**	M_n **(g/mol)**	M_w/M_n
POEGA$_{18}$-CTA	21	8240	1.15
POEGA$_{27}$-CTA	21	11675	1.17
POEGA$_{35}$-CTA	21	14501	1.19

Time – time of irradiation. M_n – molecular weight determined by SEC in DMF. M_w/M_n – dispersity determined by SEC in DMF

Table S2. Number of NR molecules per NP (N_{dye}) and theoretical brightness (B_p) in POEGA$_{27}$-b-PBzA$_{172}$ NP. Theoretical brightness per NP (B_p) was also determined. B_p is defined as product of extinction of fluorophore at maximum absorption (ε), quantum yield yield (Φ_{fluo}) and number of dye par particle (N_{dye}).[1] For POEGA$_{27}$-b-PBzA$_{172}$ NPs containing the lowest content in fluorophore (0.15 w/w% of polymer), the theoretical brightness was $\varepsilon \times \Phi_{fluo} \times N_{dye} = 3.8\ 10^4 \times 0.55 \times 52 = 1.09 \times 10^6$ M cm^{-1}. The value is one order of magnitude smaller than that found in another study describing NR encapsulated PS nanoparticles.[2] In this example, the number of dyes per particle was higher through an encapsulation in the apolar core. As expected, increasing the dye content (0.30 %w to polymer) enables to produce brighter particles ($B_p = 1.91 \times 10^6$ M cm^{-1}) through a higher number of dye molecules per particle

System	N_{dye}	B_p (10^6 M^{-1} cm^{-1})
POEGA$_{27}$-b-PBzA$_{172}$ ω(Nile red) = 0.15 w%	52	1.09
POEGA$_{27}$-b-PBzA$_{172}$ ω (Nile red) = 0.30 w%	100	1.91

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[1] A. Reisch and A. S. Klymchenko, Small, 2016, 12, 1968–1992.

[2] T. Behnke, C. Würth, E.-M. Laux, K. Hoffmann and U. Resch-Genger, Dyes Pigments, 2012, 94, 247–257.

Measurement of brightness. The brightness of each particle can be expressed as $B_P = \phi_f \times \mathrm{N}_{dye} \times \varepsilon$, where ϕ_f is the fluorescence quantum yield; N_{dye} is the number of NR molecules per particle; ε the molar absorption coefficient of NR at λ = 600 nm (ε = 38000 mol L^{-1} cm^{-1}). N_{dye} was calculated according to the following equation:

$$\mathrm{N}_{dye} = C(NR) \times N_{agg} / C(copolymer)$$

where $C(NR)$ is the concentration of Nile Red encapsulated in NPs; N_{agg} is the number of copolymer chains per particle and $C(copolymer)$ is the concentration of copolymer determined by gravimetry

III. Additional figures

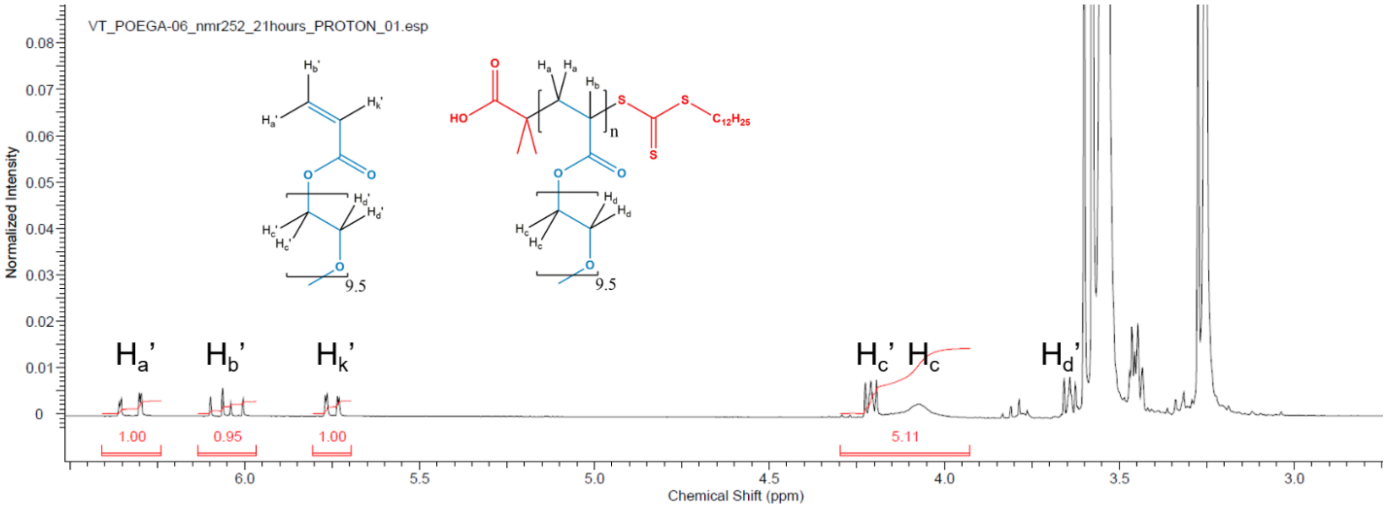

Fig. S1. ^{1}H NMR spectrum of POEGA$_{27}$-CTA. *Conv.* = $100 \times \left(1 - \frac{2\cdot\left(I(H_a')+I(H_b')+I(H_k')\right)}{3\cdot\left(I(H_c')+I(H_c\ )\right)}\right) \cdot = 61\%$. Experimental conditions: OEGA : CTA = 44 : 1; [OEGA]$_0$ = 13.410 mM in ethanol, blue LED (0.8 mW·cm^{-2}, 455 nm, 30 °C, 21 h).

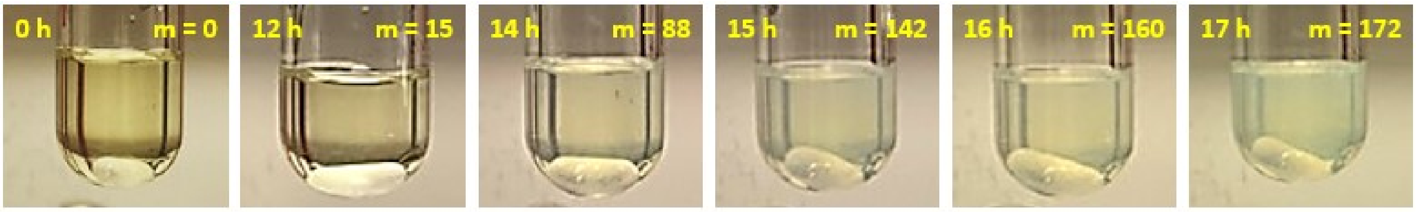

Fig. S2. Series of photographs showing the evolution of the reaction medium during the synthesis of POEGA$_{27}$-b-PBzA$_{172}$ NP by photomediated RAFT dispersion polymerization of benzyl acrylate.

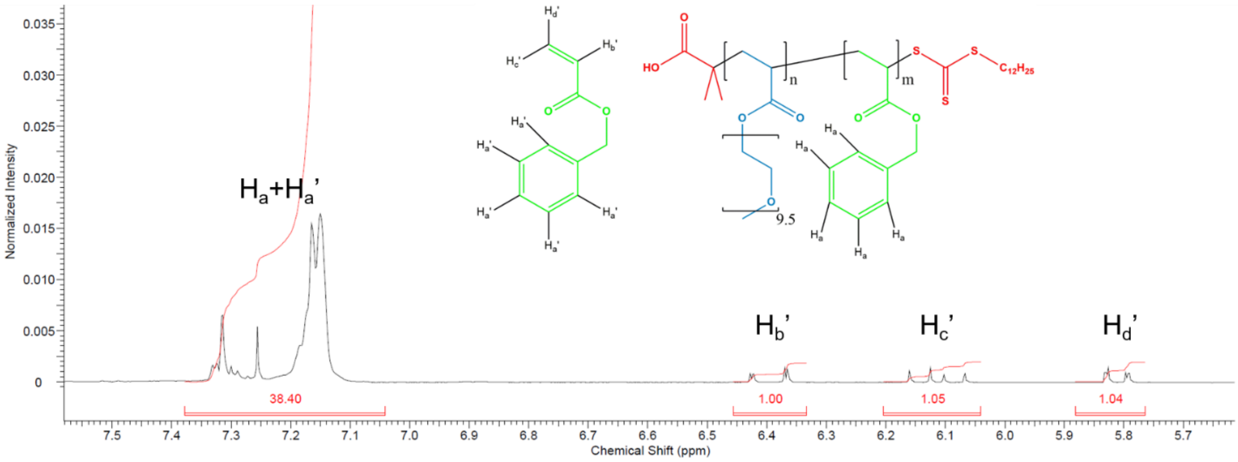

Fig. S3 Example of ^{1}H NMR spectrum of POEGA$_{27}$-b-PBzA$_{172}$. *Conv.* = $100 \times (1 - \frac{5\left(I(H_b') + I(H_c') + I(H_d')\right)}{\left(I(H_a') + I(H_a\ )\right)3}) = 86\%$. Experimental conditions: POEGA-CTA : BzA =1 : 200; [POEGA-CTA]$_0$ = 0.067 mM in methanol/water mixture (70/30 w/w %), blue LED (0.8 mW·cm^{-2}, 455 nm, 30 °C, 17 h).

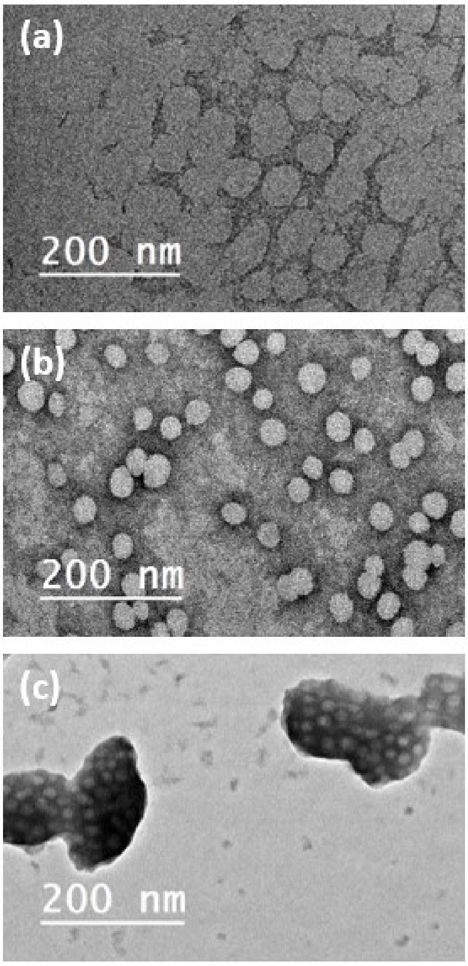

Fig. S4. Negative-stain TEM images of (**a**) POEGA$_{18}$-b-PBzA$_{186}$, (b) POEGA$_{27}$-b-PBzA$_{172}$ and (c) POEGA$_{35}$-*b*-PBzA$_{180}$ samples.

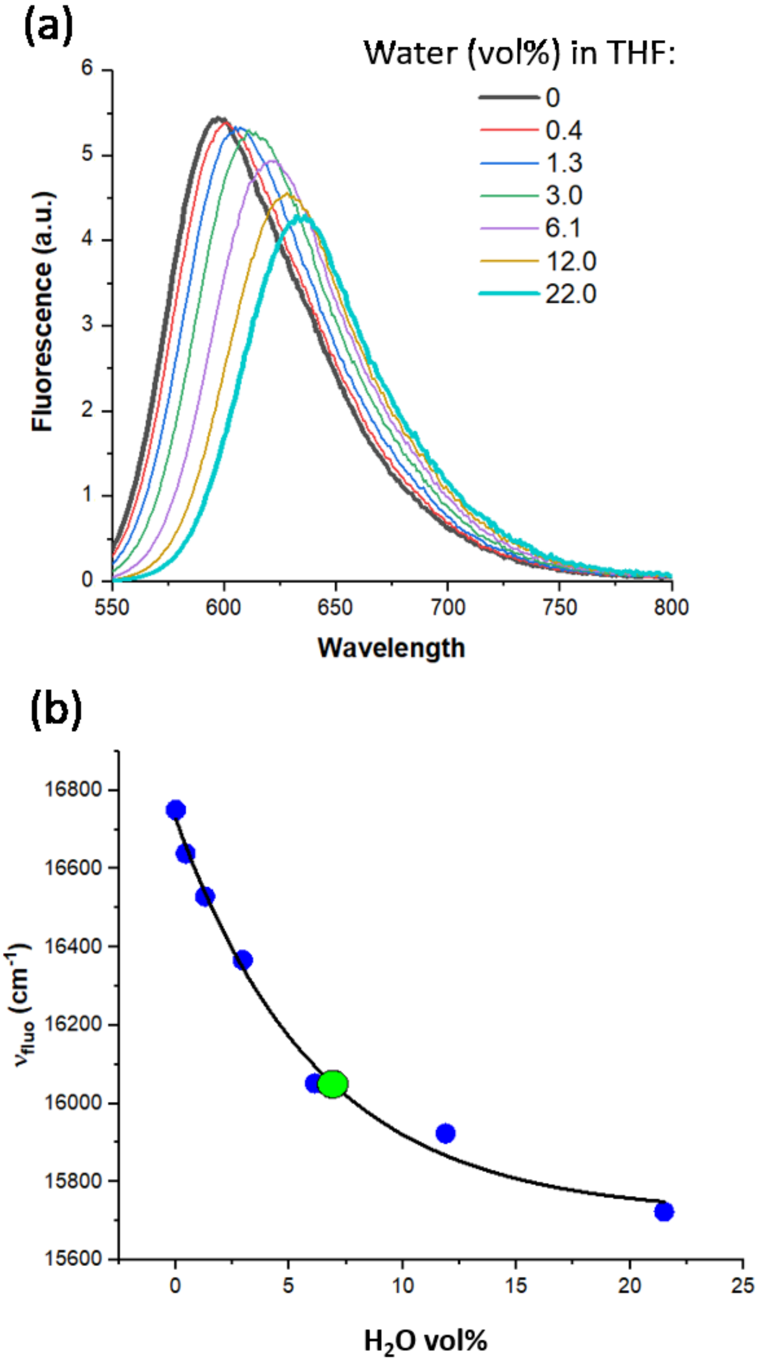

Fig. S5. (**a**) Fluorescence emission spectra of NR in THF-water mixture. (**b**) NR in THF-water mixture (blue dots) and POEGA$_{27}$-b-PBzA$_{172}$ (ω(NR) = 0.15 w/w% of polymer, green dot). Plots **a** and **b** show respectively the fluorescence band red shift of NR in THF upon gradual addition of water and the corresponding maximum emission wavenumbers as function of the volume fraction of water. It is clearly evidenced that very few amount of water strongly influences the spectral position of the NR emission band. For instance, the addition of ~ 6 v% of water into THF solution of NR leads to a ν_{fluo}^{max} which is similar that recorded for NPs (see green dot**)**. Therefore, we have the confirmation that NR is preferentially localized in the POEGA shell.

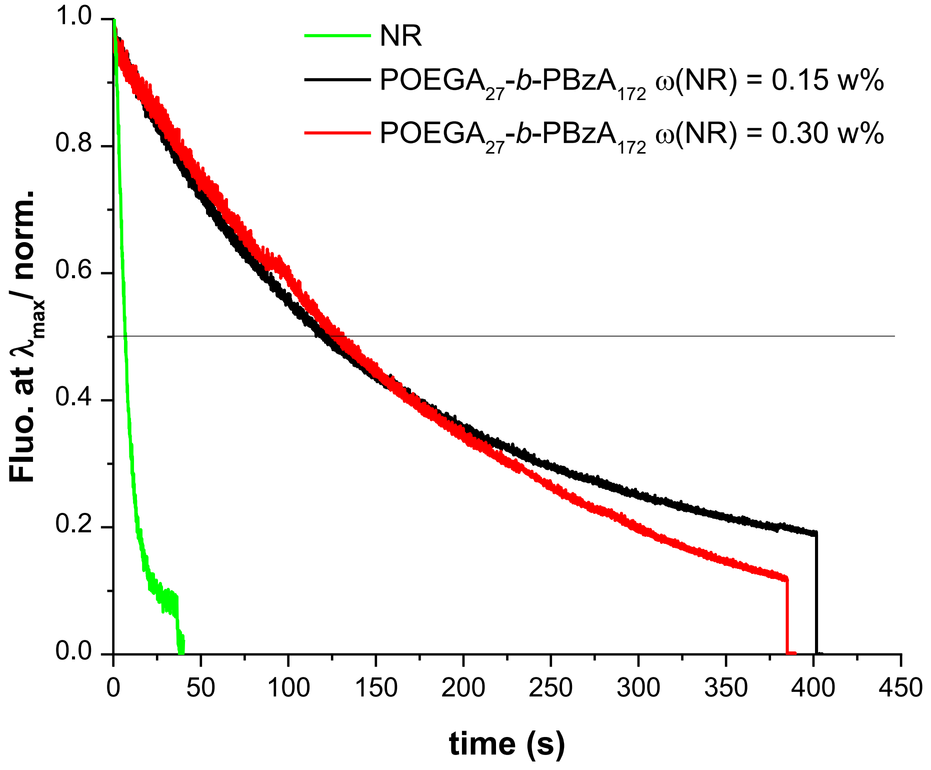

Fig. S6. Photostability study of dried NR-loaded POEGA$_{27}$-b-PBzA$_{174}$ NP, and comparison with an analogue prepared from a THF solution. Power of irradiation P_{irr} = 135 µW at 485 nm, Numerical Aperture = 0.65 and Irradiance = 76 kW cm^{-2}. The photostability of NPs was evaluated by monitoring their time-dependent fluorescence intensity decreases upon a high photon flux exposure at 485 nm and under air atmosphere. For this purpose, the dried NPs were irradiated using an epi-fluorescence microscope. The figure shows the normalized time-dependent photobleaching profiles of both NPs. For comparison, we have also studied a reference sample prepared similarly but from a THF solution of NR (10^{-8} M). Regardless of the NR content, the NPs specimens exhibit similar photobleaching profiles with a bleaching half-time ($t_{1/2}$ ~ 125 s) which is 10 times higher than that of the control experiment. The difference of photostability can be understood on the basis of the shielding effect of the NPs polymer matrix, which drastically reduces the atmospheric oxygen diffusion toward encapsulated dye.

Measurement of NP Photostability. The photostability of NPs was evaluated upon monitoring their time-dependent fluorescence intensity decrease upon irradiation at 485 nm using an Olympus IX73 inverted microscope equipped with a 75 W Xe lamp housing. The NPs were deposited on a glass coverslip upon slow evaporation of a solution containing the NPs then irradiated at 485 nm through a 40X, 0.65-NA objective using a fluorescence mirror unit (U-FBW from Olympus) associating a band pass filter centred at 485 nm (BP460-495), dichroic mirror (DM505) and long pass filter (BA510IF). The epifluorescence signal from the NPs is collected by the same objective, passes through the same fluorescence mirror unit and is either collected by a spectrometer (USB4000-UV-Vis Ocean Optics) Neutral filters were placed on the excitation optical path of the microscope to attenuate the irradiation power which was adjusted to a value of 135 µW.

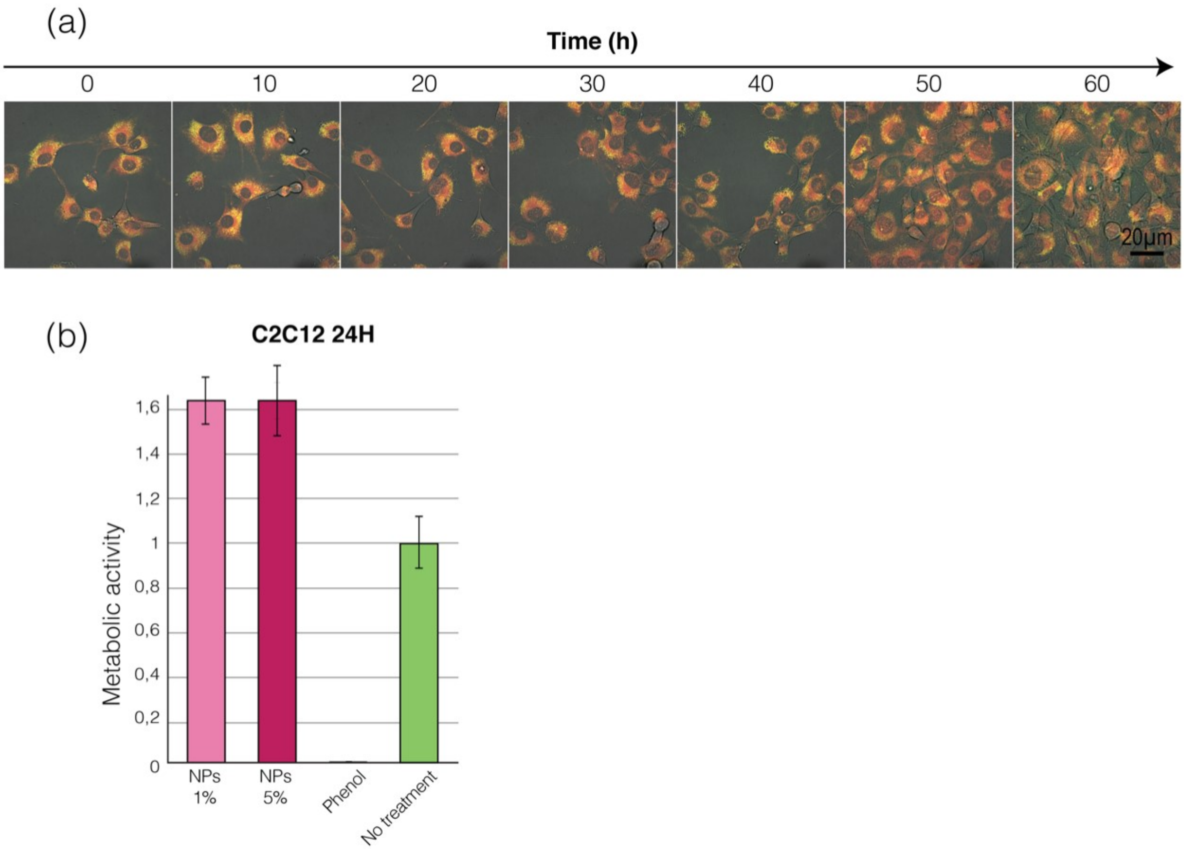

Fig. S7. (**a**) CFM images of C2C12 cells incubated with NR-labelled POEGA$_{27}$-b-PBzA$_{172}$ NPs (C_P = 80 µg mL^{-1}, *ω(NR)* = 0.30 w/w% of copolymer) versus time after the administration of NPs showing a proliferation of cells. (**b**) Cell viability assays (MTT) after 24h of the same NPs in C2C12 cells at C_P = 16 µg mL^{-1} (1%) and 80 µg mL^{-1} (5%) suggesting that NPs are not toxic for either