Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Poly(3-hexylthiophene) nanoparticles for biophotonics: study of the mutual interaction with living cells

Elena Zucchetti,[a,b] † Mattia Zangoli,[c] † Ilaria Bargigia,[a] Caterina Bossio,[a] Francesca Di Maria,[c] Giovanna Barbarella,[c] Cosimo D'Andrea,[a,b] Guglielmo Lanzani[a,b*] Maria Rosa Antognazza[a,*]

[a]Center for Nano Science and Technology @Polimi, Istituto Italiano di Tecnologia, Via Pascoli 70/3, 20133 Milano, Italy

[b]Politecnico di Milano, Dip.to di Fisica, P.zza L. da Vinci 32, 20133 Milano, Italy

[c]Institute for Organic Synthesis and Photoreactivity, CNR-ISOF, Via Piero Gobetti 101, 40129 Bologna, Italy.

†These authors contributed equally to this work

Corresponding Authors

*E-mail: mariarosa.antognazza@iit.it; guglielmo.lanzani@iit.it

Synthesis of poly(3-hexylthiophene)

Commercial 3-hexylthiophene (1 g, 0.006 mol) was dissolved in 50 mL of distilled $CHCl_3$, using dry $FeCl_3$ (3.87 g, 0.024 mol) suspended in 100 mL of distilled $CHCl_3$ as described in reference 23. The reaction mixture was stirred overnight at reflux under N_2 atmosphere. The resulting crude product was extracted with a 2% aqueous HCl solution until complete disappearance of $FeCl_3$ in excess, monitoring the dissociated iron in aqueous phases with ammonium thiocyanate. The resulting polymer was filtered and further purified by removing the shorter fractions soluble in methanol and acetone. Molecular weight (MW, 73600), Molar mass (M_n, 41530) and polydispersity index (PDI, 1.77) were determined by gel permeation chromatography in THF solvent with a polystyrene standard. Absorption and photoluminescence spectra both in chloroform solution and in solid film are reported in **Figure S1**, together with the ^{1}H-NMR spectrum.

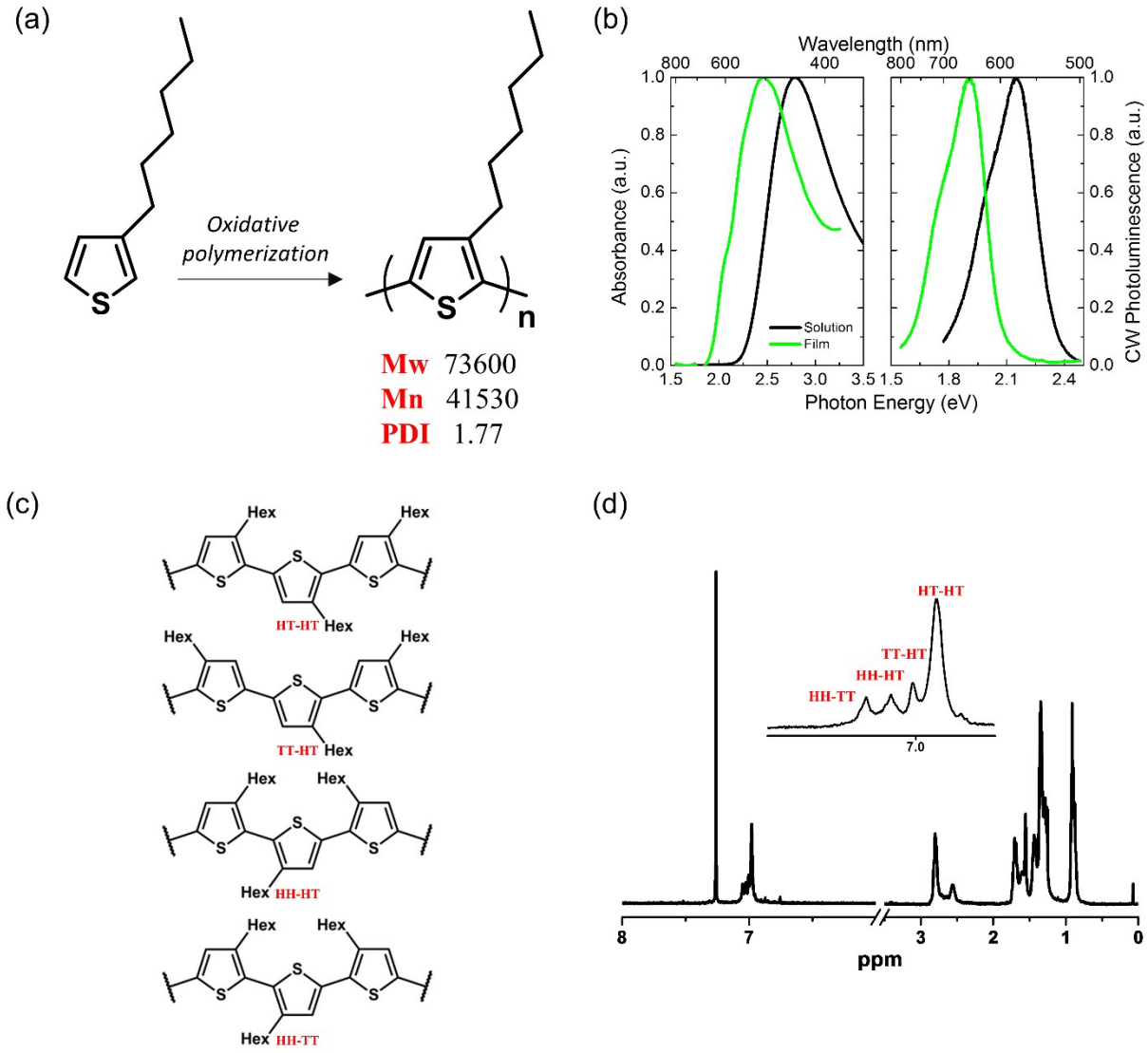


Figure S1. (a) Poly (3-hexylthiophene), P3HT, prepared by oxidative polymerization with $FeCl_3$. (b) Normalized photoluminescence and absorption spectra of P3HT in $CHCl_3$ (black trace) and cast film from $CHCl_3$ (green trace). (c) ^{1}H-NMR spectrum of P3HT in $CHCl_3$.

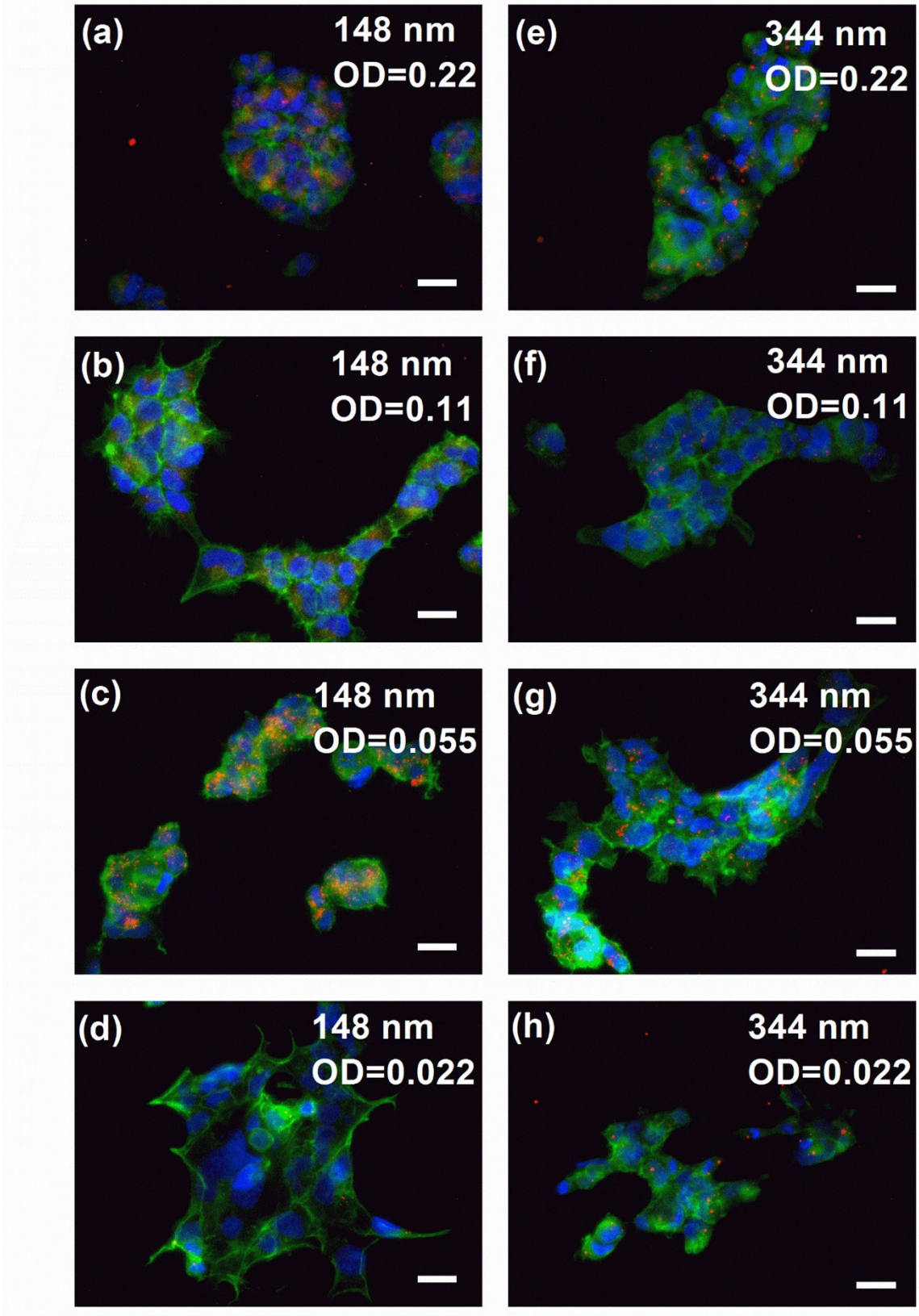
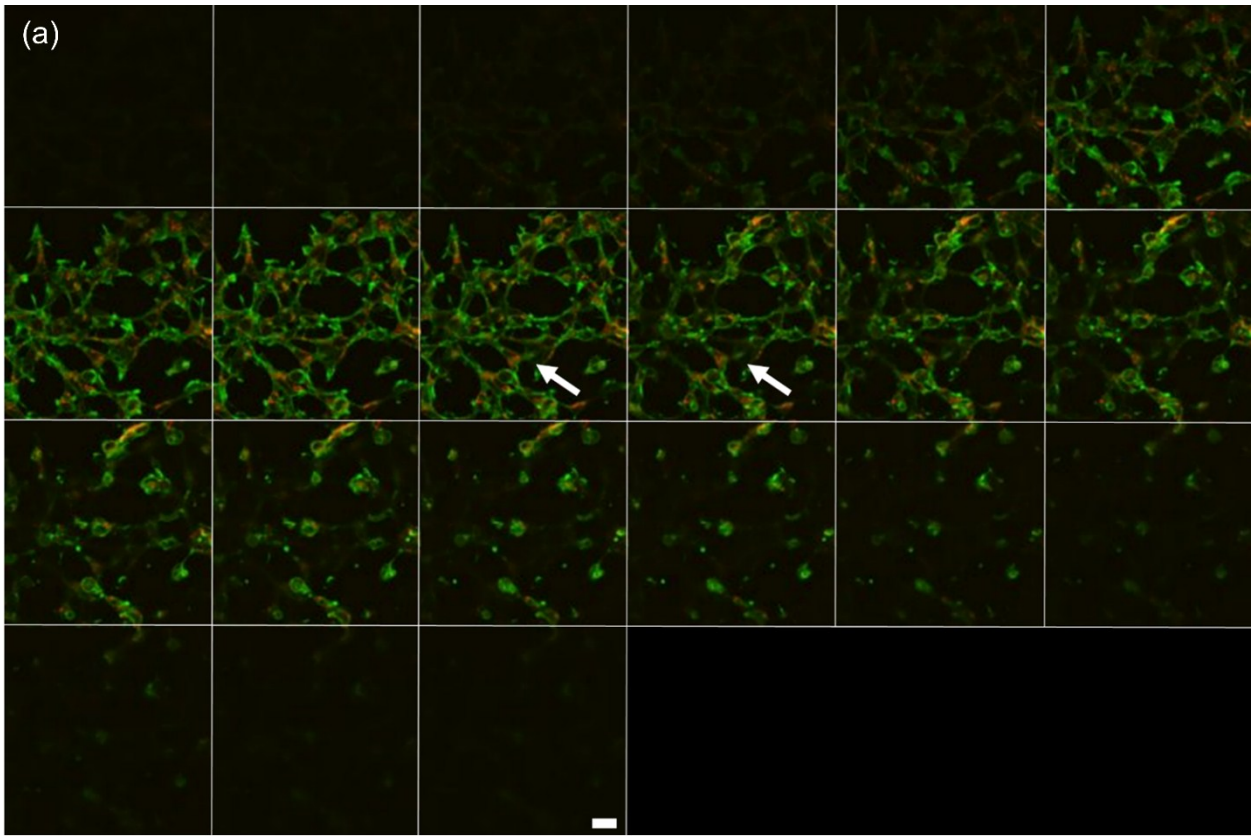
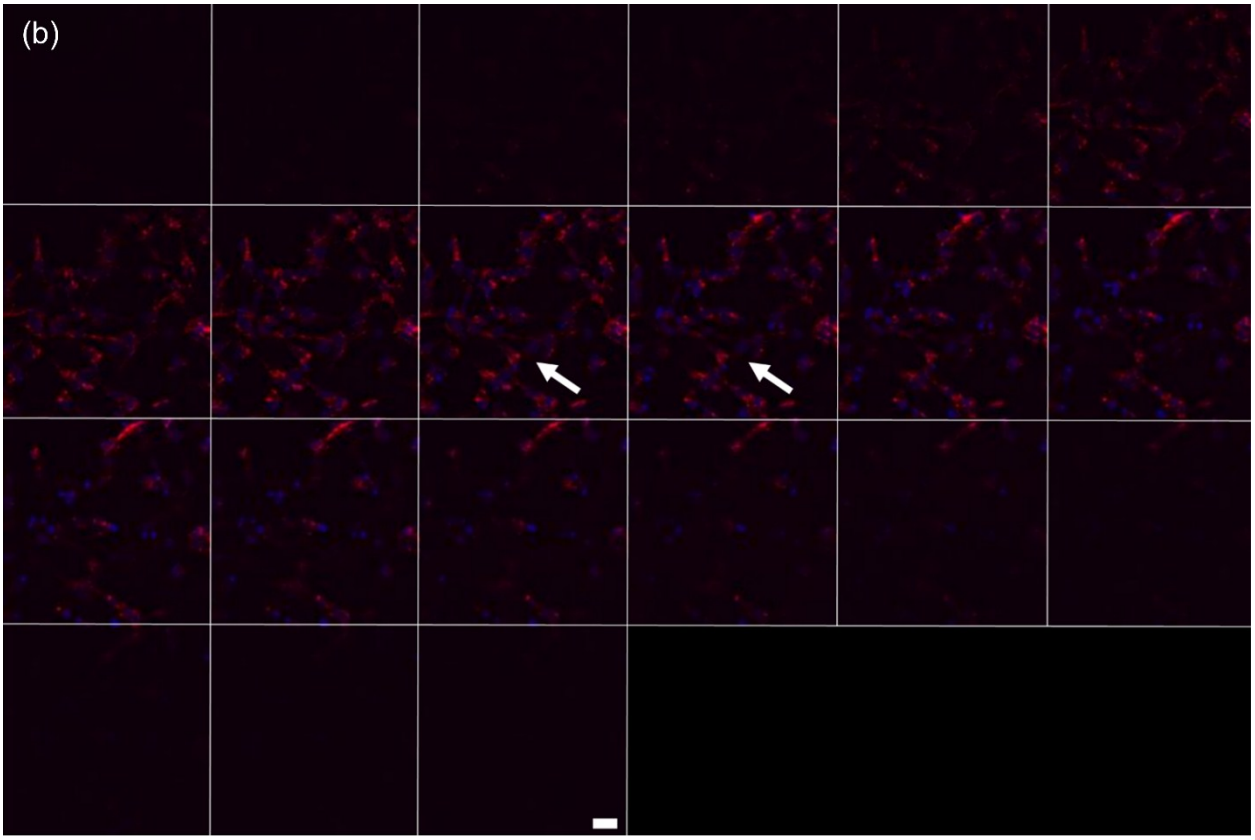
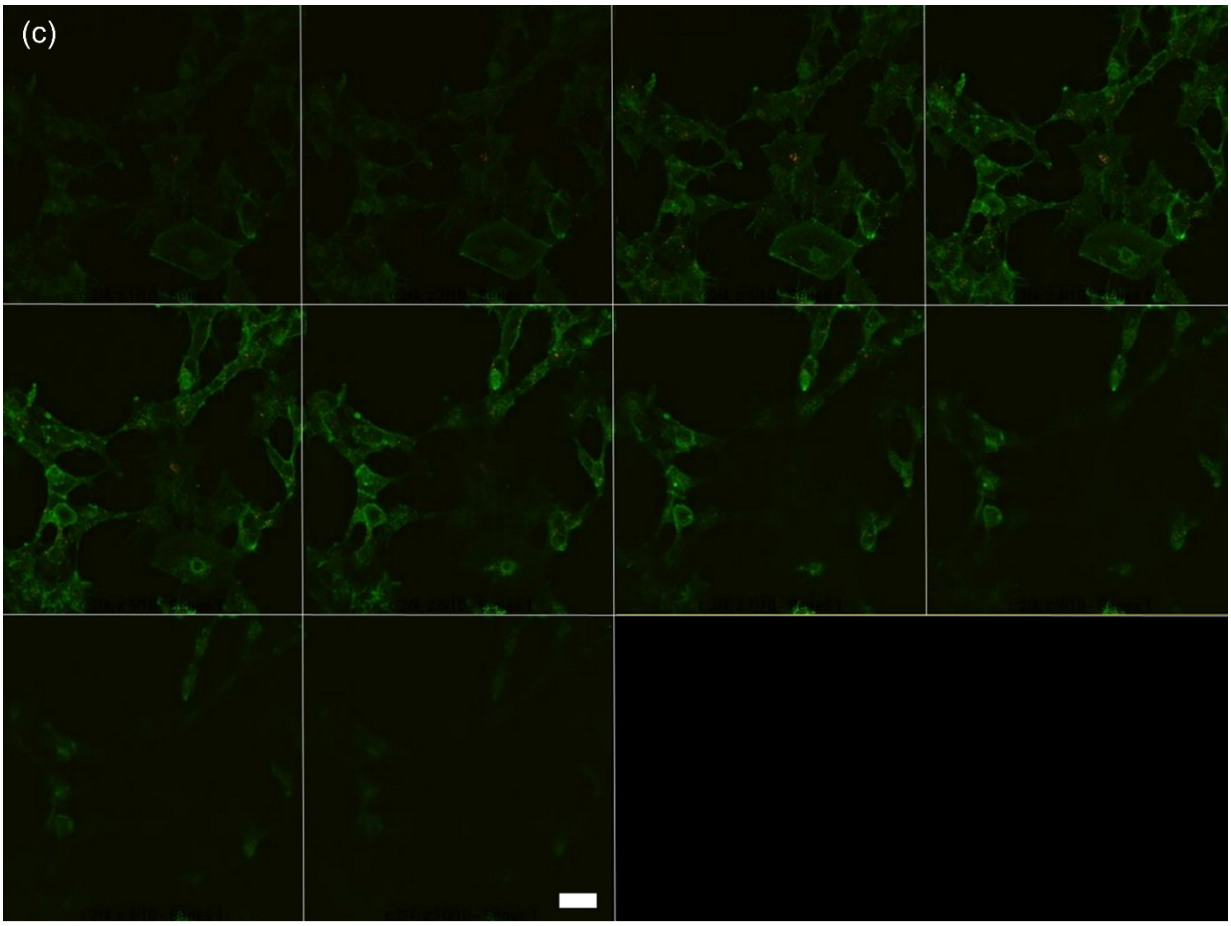

(a)
148 nm
OD=0.22
(e)
344 nm
OD=0.22
(b)
148 nm
OD=0.11
(f)
344 nm
OD=0.11
(c)
148 nm
OD=0.055
(g)
344 nm
OD=0.055
(d)
148 nm
OD=0.022
(h)
344 nm
OD=0.022

Figure S2. Optical microscope images of stained cell cultures showing nuclei (in blue), actin filaments (in green) and the emission of NPs (in red) for 148 nm and 344 nm NPs suspensions. Scale bar, 10 μm. Each NPs dispersion was diluted at different concentrations in ultrapure water, in order to obtain four different optical densities values, measured at 460 nm and ranging from 0.022 to 0.22. We aimed at finding a suitable compromise between maximization of NPs cellular uptake and appearance of detrimental effects on cell morphology. In the case of smaller NPs, the highest considered concentration (panel a) clearly lead to altered cellular shape and reduced cellular membrane polarization, thus possibly indicating the occurrence of apoptotic processes. We thus fixed the working optical density of the dilution at 0.11 (panel b). Conversely, bigger NPs could be used at the highest tested concentration (panel e), which however could not be further increased in order not to add to the extracellular solution a water excess larger than 10% in volume.


(a)


(b)


(c)

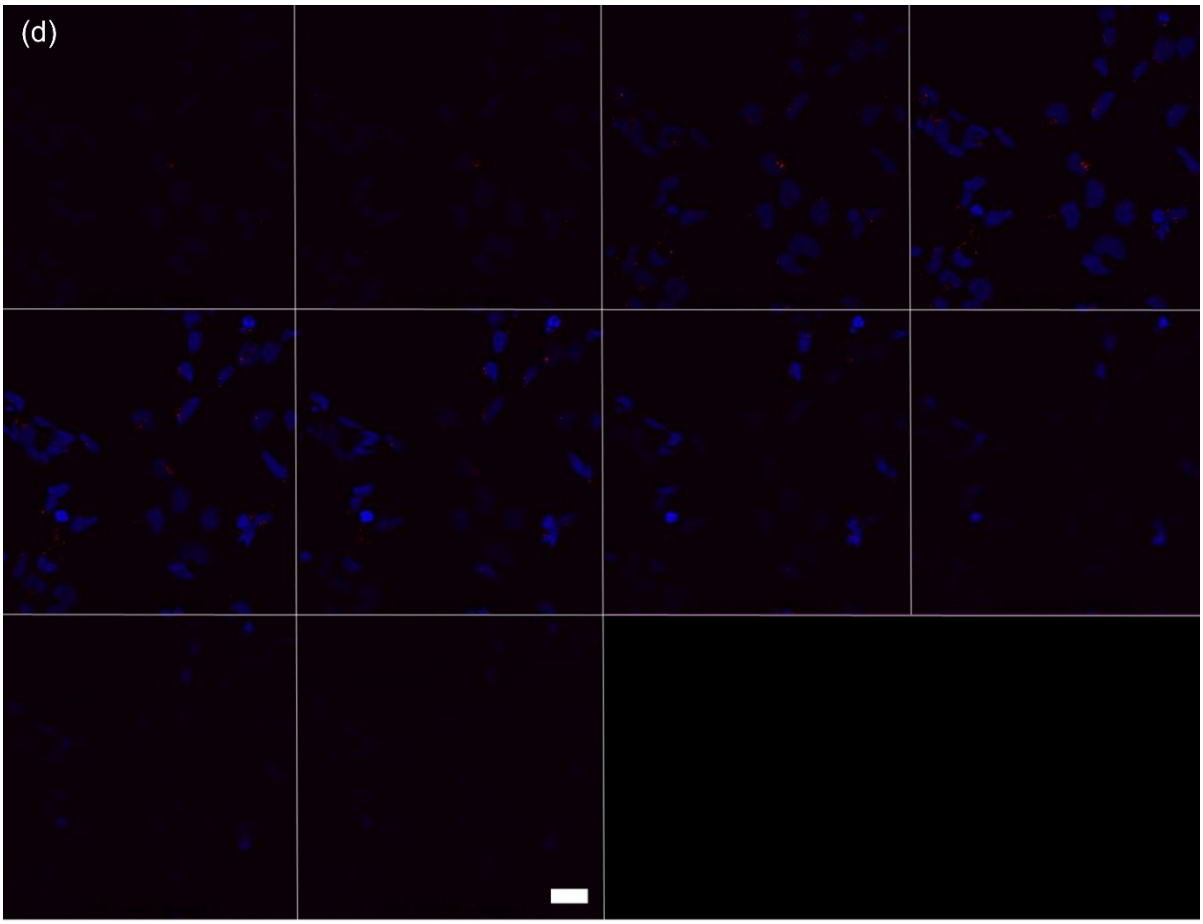


Figure S3. Confocal optical sections of cells loaded with 148 nm (panels (a) and (b)) and 344 nm (panels (c) and (d), stained with DAPI (blue, nuclei) and phalloidin (green, actin). Emission from NPs is visible in the red channel. By inspection of subsequent stacks, acquired from the bottom to the upper cell surface, it is possible to appreciate that NPs have been internalized within the cytosol, but do not cross the nuclear membrane. Scale bar, 30 μm.

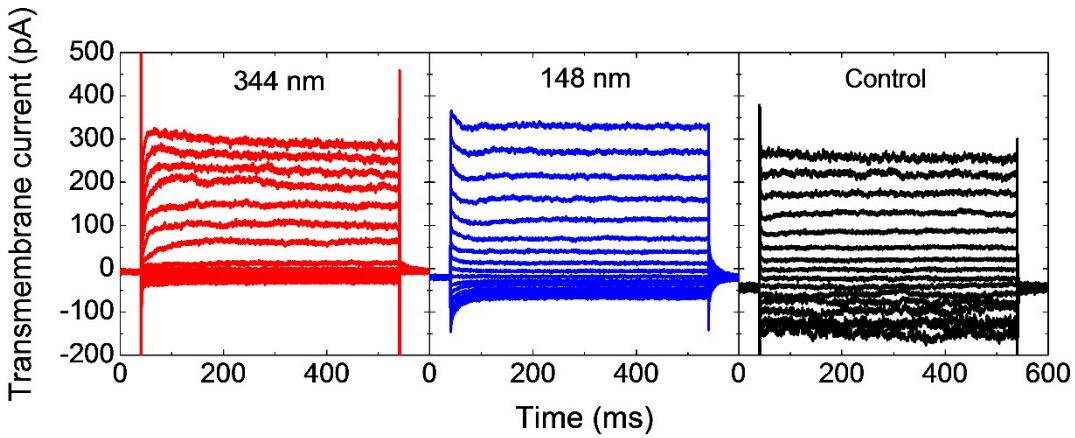


Figure S4. Representative trans-membrane current measured in whole-cell, voltage clamp configuration in samples treated with NPs (red and blue curves) and controls (black curves). 500-ms voltage steps have been subsequently applied (holding potential, -40 mV), ranging from -100 mV to 50 mV, in 10 mV increments.

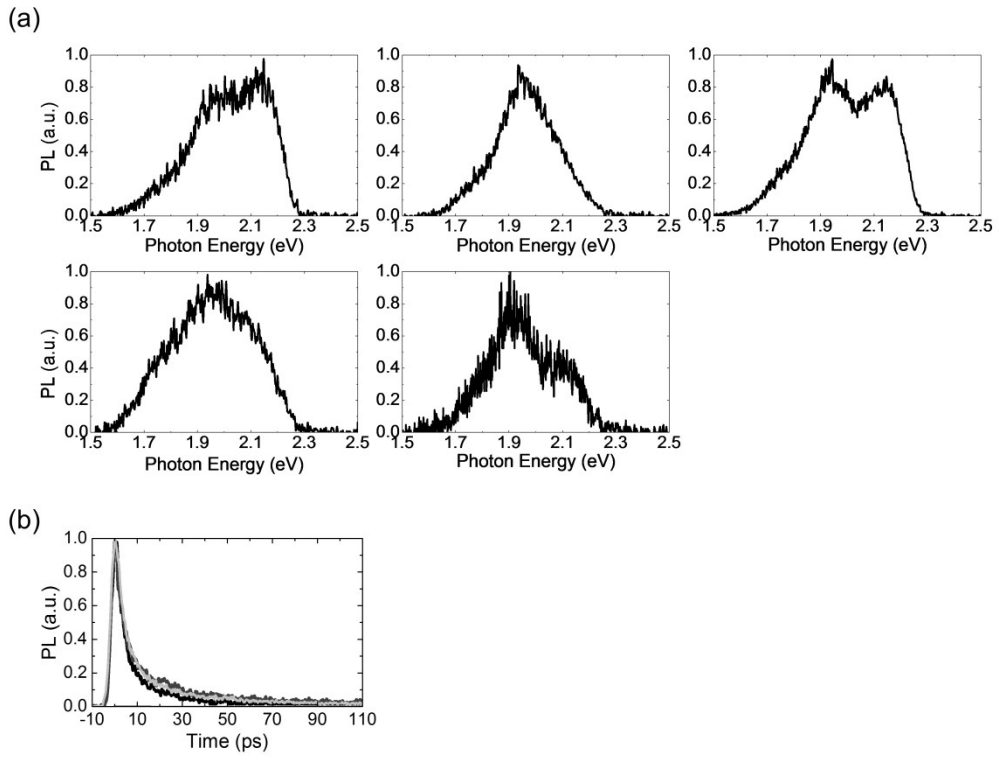


Figure S5. Examples of fluorescence spectra (a) integrated over the first 128 ps and representative kinetic traces (b) integrated between 1.82 and 2 eV obtained from measures of P3HT-NPs within living cells.

	A_1 (%)	τ_1 (ps)	A_2 (%)	τ_2 (ps)
Water	0.85	7	0.15	64
KRH	0.77	7	0.23	59
Cells	0.81±0.08	4±0.68	0.19±0.08	42±14

Table S1. Relative percentage weight of short- and long-lived components (A_1 and A_2, respectively) used to fit the fluorescence dynamics of NPs dispersed in water, KRH and internalized within the cytosol. Corresponding time constants are also reported. Data for living cells are reported as average values ± standard deviation, over a statistical sample of n = 16 cells.