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

Supporting Information

for

NIR excitation of upconversion nanohybrids containing a surface grafted Bodipy induces oxygen-mediated cancer cell death

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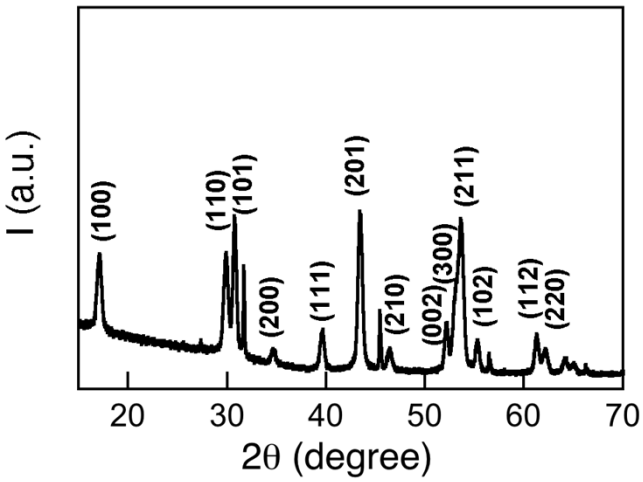


Fig. S1. X-ray power diffraction (XRD) spectrum of $NaYF_4$:Yb^{3+}, Er^{3+} hexagonal nanoprisms (JCPDS standard card no. 28-1192).[1]

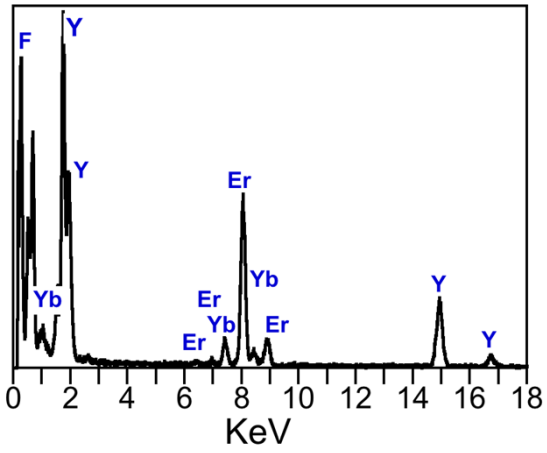


Fig. S2. EDX spectrum of spectrum of $NaYF_4$ (81%):Yb^{3+}(16%),Er^{3+}(3%).

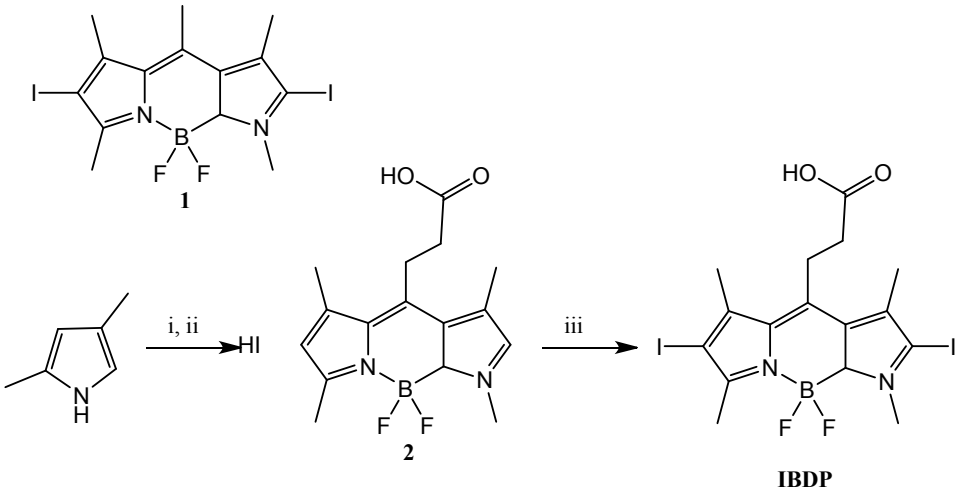
Methods. TLC was performed on silica gel precoated aluminum foils, Merck 60F 254, 0.25 mm. Flash column chromatography was carried out on silica gel from Merck, 230-400 mesh. Yields are referred to isolated pure compounds. ^1H and ^{13}C NMR spectra were registered at room temperature in $CDCl_3$ solution in Varian INOVA-300 and -400 spectrometers. Chemical shifts are reported in parts per million (ppm) using as internal reference the peak of the trace of undeuterated solvent (δ 7.26) or the carbon signal of the deuterated solvent (δ 77.0). The following abbreviations are used to describe: s (singlet), d (doublet), t (triplet), q (quartet), m (complex multiplet). Assignments were based on HSQC and HMBC experiments. IR spectra were recorded in a Perkin Elmer FT 681 spectrophotometer. Low-resolution mass spectra were registered in an Agilent HP 1100 LC/MSD spectrometer using ESI or APCI sources. High resolution mass spectra (HRMS) were recorded in an Agilent 6520 Q-TOF instrument with a ESI source, or in a QTOF QSTAR model (Applied Biosystems) by electro spray ionization in the positive mode (ESI^+) using as phase acetonitrile plus 0.1% formic acid, or in

an AutoSpecEQ EI apparatus by electron impact (EI, 70 eV). High resolution TEM (HRTEM) and energy dispersive X-rays spectroscopy (EDX) were carried out by using a Field Emission Gun (FEG) TECNAI G2 F20 microscope operated at 200 kV.

X-ray power diffraction spectra were performed by using a PANalytical Empyrean X-ray powder diffractometer Cu radiation Oxford Cryostream with hybrid monochromator (Cu K alpha 1), a focussing mirror, and PIXcel detector XRPD for capillary measurements.

Synthesis of Bodipy compounds

2,6-Diiodo-1,3,5,7,8-pentamethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (**1**). was synthesized using the method described by Yogo *et al.*[2] Scheme 1 shows the synthetic pathway. 3-(2',6'-Diiodo-1',3',5',7'-tetramethyl-4',4'-difluoro-4'-bora-3'a,4'a-diaza-s-indacen-8'yl)propanoic acid (**IBDP**) was prepared by a modified protocol. Briefly, it was prepared by iodation with iodic acid and iodine of compound 2, which was synthesized from 2,3-dimethylpyrrole and succinyl chloride in the presence of triethylamine and boron trifluoride diethyl etherate. Our characterization data matches that previously reported.[3]



Scheme S1. i) $(CH_2COOH)_2$/DCM; ii) Et_3N/BF_3OEt_2; iii) I_2, EtOH, HIO_3, H_2O, 99%. Structure of IBDP dye[2] is also shown.

3-(1',3',5',7'-tetramethyl-4',4'-difluoro-4'-bora-3'a,4'a-diaza-s-indacen-8' yl)propanoic acid (**2**): A mixture of 2,4-dimethylpyrrol (1 g, 10 mmol) and succinyl chloride (0.58 mL, 5.2 mmol) in dichloromethane (50 mL) was refluxed under argon for 30 min. First, triethylamine (6 mL, 60 mmol) was added at room temperature and then, after 30 min, boron trifluoride

diethyl etherate (6.3 mL, 50 mmol). The mixture was refluxed for 2 h under argon and the reaction was quenched with 60 mL of 0.1 M HCl aqueous solution. Extraction was performed and the organic fractions were combined and washed three times with 40 mL of $NaHCO_3$ sat. aqueous solution. Then, the aqueous fractions were combined and acidulated with 0.1 M HCl aqueous solution, extracted with ethyl acetate and dried over magnesium sulphate. The orange residue was purified by flash column chromatography (silica gel, hexane-ethyl acetate 1:1 as eluent). Orange crystals, yield 0.5 g (30%). TLC (ethyl acetate): R_f = 0.15. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.44 (s, 6 H, C*H*$_3$-C1', C*H*$_3$-C7'), 2.52 (s, 6 H, C*H*$_3$-C3', C*H*$_3$-C5'), 2.66 (m, 2 H, C*H*$_2$-CO), 3.34 (m, 2 H, C*H*$_2$-C8'), 6.09 (s, 2H, *H*-C2', *H*-C6'), (OH not observed) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.48, 15.6 (*C*H$_3$-C3', *C*H$_3$-C5', *C*H$_3$-C1', *C*H$_3$-C7'), 23.5 (*C*H$_2$-C8'), 34.1 (*C*H$_2$-CO), 121.0 *C*2', *C*6'), 130.1 (C-7'a, C-8'a), 139.0 (C-1', C-7'), 141.7 (C-8'), 153.8 (C-3', C-5'), 176.2 (C=O) ppm. EIMS, m/z (%) = 277 [M^+-COO^-] (93), 254 (100).

3-(2',6'-Diiodo-1',3',5',7'-tetramethyl-4',4'-difluoro-4'-bora-3'a,4'a-diaza-s-indacen-8'-yl)propanoic acid (**IBDP**). A solution of iodic acid (0.325 g, 1.9 mmol) in water (5 mL) was added dropwise to a mixture of the former dye (300 mg, 0.93 mmol), iodine (0.354 g, 1.4 mmol) and ethanol (100 mL). The mixture was heated 20 min at 60 ºC. The workup yielded pure **2** as a pink solid, 0.5 mg (99%) and was used without further purification. TLC (ethyl acetate): R_f = 0.15. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.51 (s, 6 H, C*H*$_3$-C1', C*H*$_3$-C7'), 2.6 (s, 6 H, C*H*$_3$-C3', C*H*$_3$-C5'), 2.6 (m, 2 H, C*H*$_2$-CO), 3.40 (m, 2 H, C*H*$_2$-C8'), (OH not observed) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, δ): 15.6, 20.04 (*C*H$_3$-C3', *C*H$_3$-C5', *C*H$_3$-C1', *C*H$_3$-C7'), 23.3 (*C*H$_2$-C8'), 33.7 (*C*H$_2$-CO), 98.5 (C-2', C-6'), 130.1 (C-8'), 141.3 (C-1', C-7'), 141.6 (C-7'a, C-8'a), 153.3 (C-3', C-5'), 173.8 (C=O) ppm. MS (ESI^-), m/z (%) = 570.9 [M (nominal mass)-H^+]. IR (KBr): ν = 3391 (COO-H st), 1735 (C=O st), 1545 (COO- st as), 1200, 997 cm^{-1}.

Fig. S3. Photo showing a dispersion of 0.5 mg/mL of UCNP-IBDP@PEG in PBS.

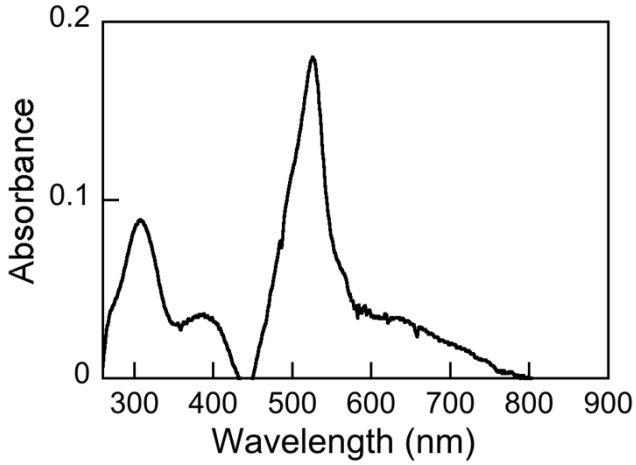


Fig. S4. Absorption spectrum of a dispersion of 1mg/1mL of UCNP-IBDP@PEG in PBS.

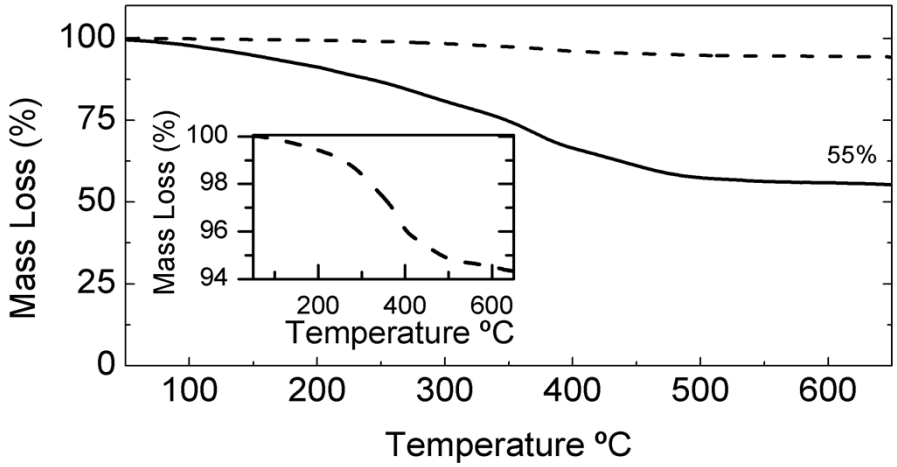


Fig. S5. TGA of UCNP@oleate (dashed line) and UCNP-IBDP@PEG (black line).

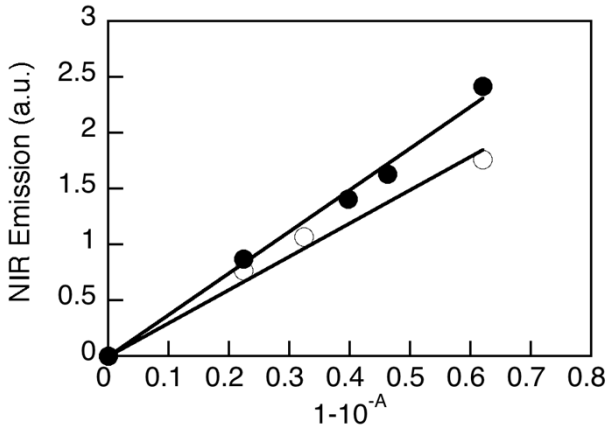


Fig. S6. Plot for singlet oxygen generation quantum yields of IBDP solutions (ᘓ) using RB (●) in methanol as standard.

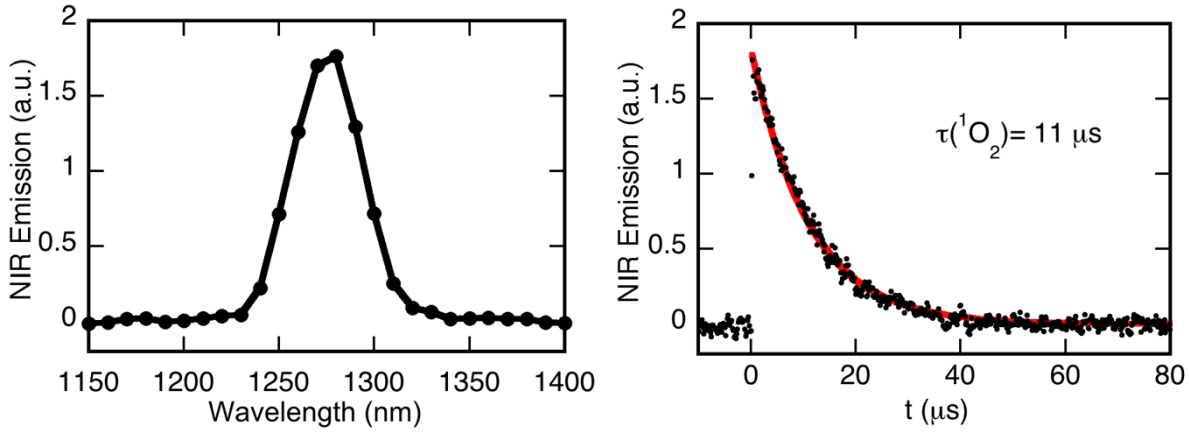


Fig. S7. A characteristic emission spectrum of singlet oxygen recorded upon excitation of an aerated methanol solution of IBDP (left). Decay of the singlet oxygen phosphorescence monitored at 1270 nm following laser excitation of IBDP at 532 nm (right).

Studies of optimisation of the IBDP loading

The nanohybrids were prepared as follows: a solution of UCNP@PEG (300 µL, 10 mg/mL) in TEA (40 mM, pH 7.5) was sonicated for 15 minutes and poured into a solution of IBDP (xmg IBDP per mg UCNP@PEG, m_{added}) in TEA (0.5 mg/mL). After stirring at RT for 24 h, the mixture was purified by centrifugation/sonication in acetonitrile (5 cycles of centrifugation for 10 minutes at 10000 rpm; sonication for 15 min). The nanohybrids (UCNP-IBDP@PEG) were re-suspended in 3 mL of milliQ water or TEA and stored at +4ºC.

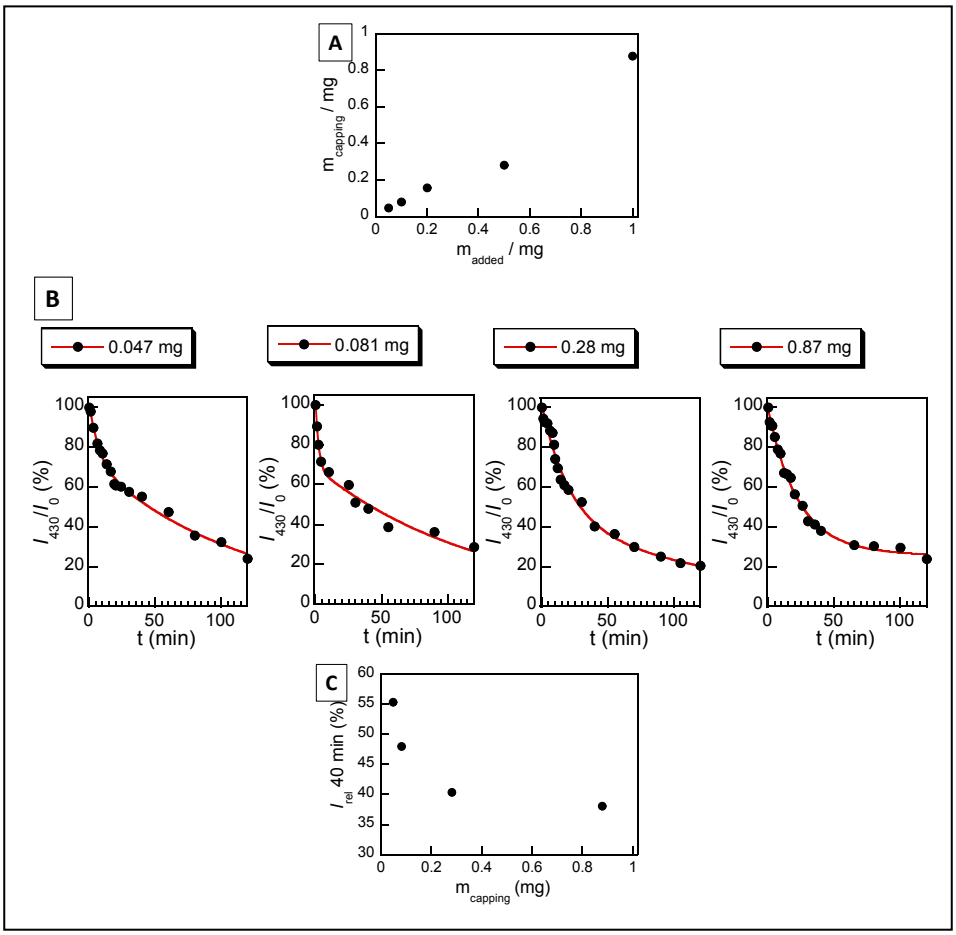


Figure S8. Figure S8A shows the IBDP attached to the nanoparticle vs the IBDP added per mg of UCNP@PEG ($m_{capping}$ vs m_{added}). Figure S8B compares the efficiency of the UCNP-IBDP@PEG nanohybrids in the photodegradation of ABDA by showing the decrease of ABDA fluorescence with time. Figure S8C compares the decrease of ABDA fluorescence after 40 minutes of irradiation with the IBDP loading. The UCNP-IBDP@PEG nanohybrids with $m_{capping}$ of 0.28 mg was chosen for the studies reported in the main text.

Stability of UCNP-IBDP@PEG in a cell culture medium

UCNP-IBDP@PEG was dispersed in phenol red free DMEM/F-12 medium (from Life Technologies, Carlsbad, CA) at 37 ºC. The mixture was centrifuged and the UV-Vis spectrum of the supernatant was recorded in order to check if any of the IBDP had detached from the UCNP surface.

Cell culture and uptake of UCNP-IBDP@PEG

SH-SY5Y cells (ATCC CRL-2266) were cultured in DMEM/F-12 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 1X non-essential aminoacids solution, and 1 mM sodium pyruvate (all from Life Technologies, Carlsbad, CA) at 37 ºC in a humidified atmosphere containing 5% CO_2. For nanostructure uptake assessment, cells in culture were seeded onto 60 mm culture dishes 24 h before imaging. The following day, the cells were incubated with UCNP-IBDP@PEG (10 µg/mL diluted in culture medium) for 2 h at 37 ºC and 5% CO_2. Cells were then washed with PBS buffer, stained with 4-6-diamidino-2-phenylindole (DAPI; Life Technologies) and rinsed with fresh complete medium. Photomicrographs were acquired by using an Olympus FV1000MPE confocal vertical microscope (Olympus, Münster, Germany) coupled with a Mai-Tai HP DeepSee laser (Spectra Physics, Irvine, CA) set at 975 nm. Fluorescent emission was detected using four filters: DAPI, 420-500 nm; UCNP green emission, 515-580 nm; UCNP red emission, 590-650 nm and 660-740 nm. All images were background-subtracted and analyzed using Fiji software.

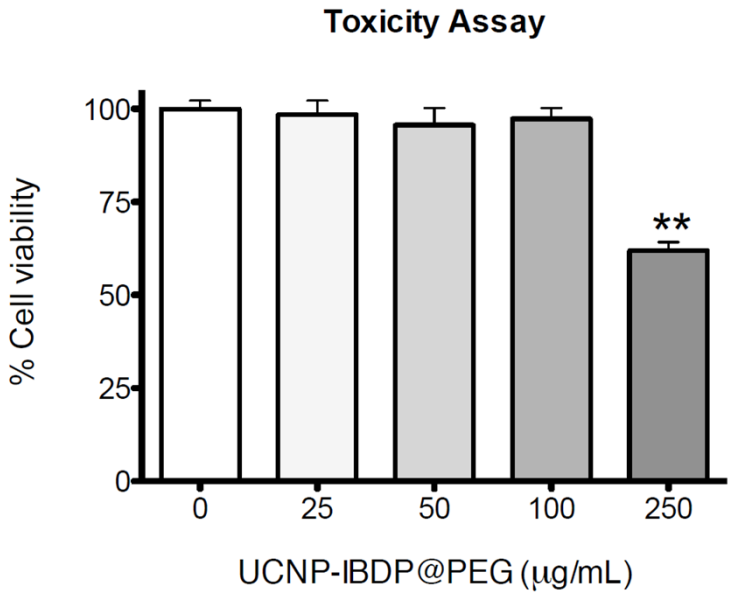


Fig. S9. *In vitro* cell viability of SH-SY5Y cells incubated at different concentrations of UCNP-IBDP@PEG for 24 h. Cells proliferated without significant changes under 0, 25, 50 and 100 μg/mL of the nanohybrid, but the highest 250 μg/mL concentration led to a drastic drop in cell viability.

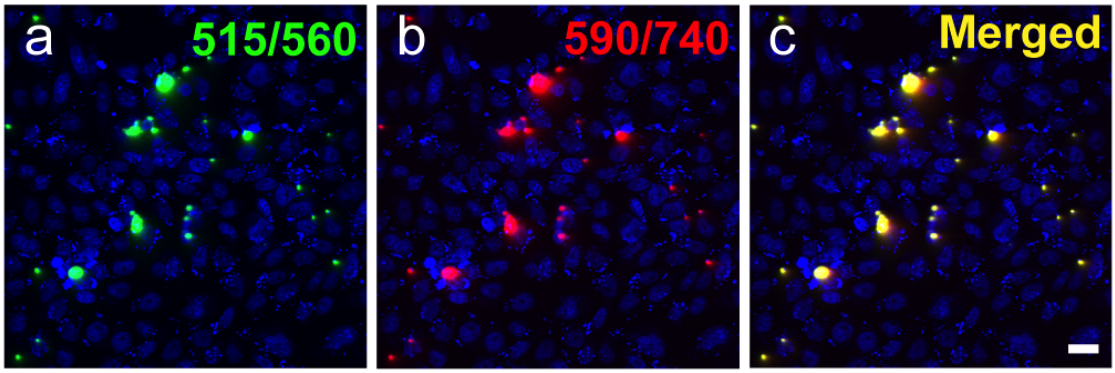


Fig. S10. UCNP-IBDP@PEG uptake by SH-SY5Y cells. When excited by 975 nm tissue-penetrating infrared light, engulfed nanoparticles emit in the green and the red visible spectra (a and b respectively). High co-localization corroborates emission by upconversion nanoparticles (c). Cell nuclei are labelled with DAPI. Scale bar = 20 μm.

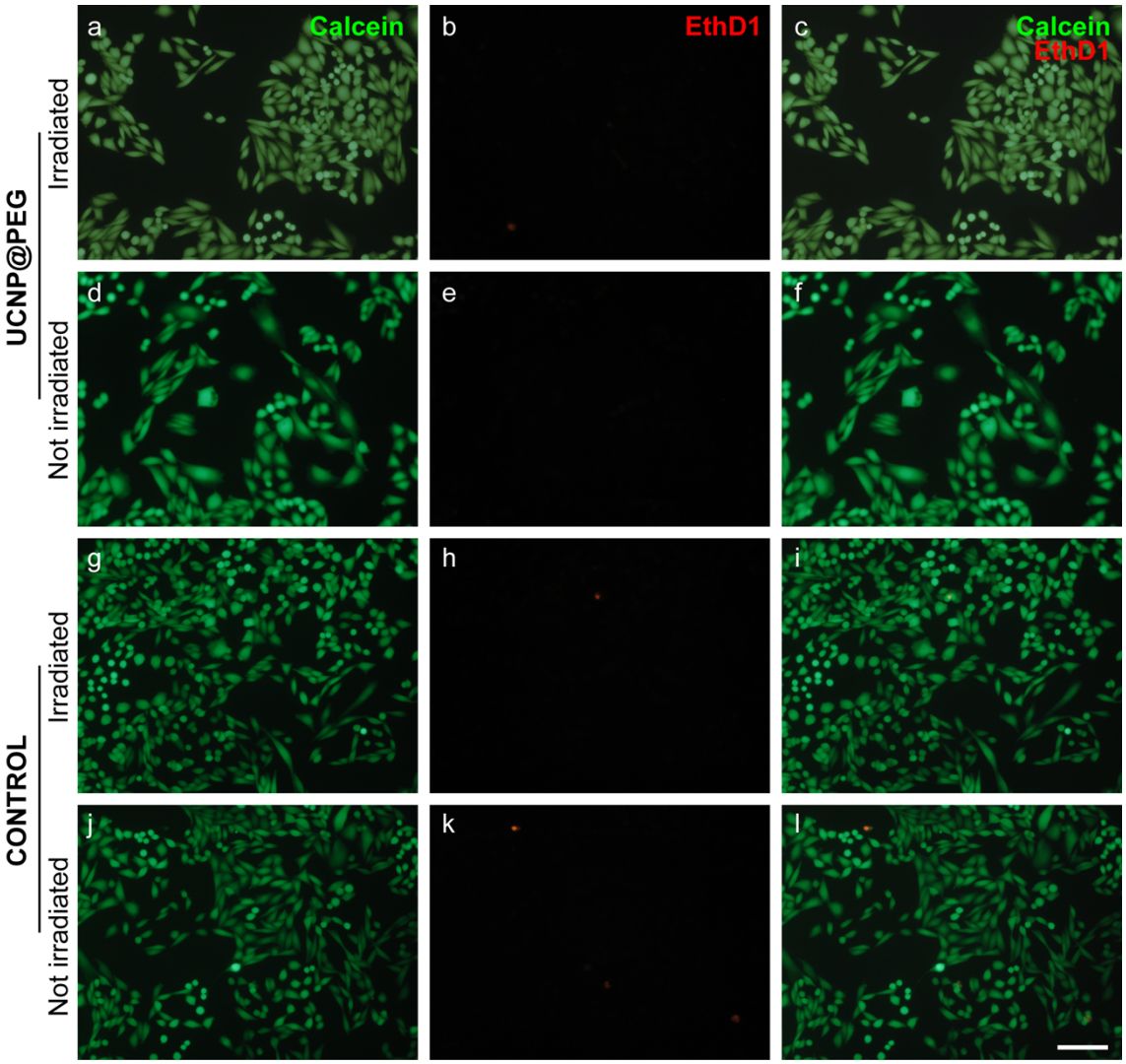


Fig. S10. SH-SY5Y cells incubated with UCNP@PEG (a-f) or control medium (g-l), were irradiated with a CW laser at 975 nm for 45 min. Cell viability was subsequently assayed with Life Technologies LIVE/DEAD® Kit. Cells with compromised membranes exhibit red-fluorescence from the live-cell impermeant nucleic acid stain ethidium homodimer-1. Cells with intact cell membranes are able to use nonspecific cytosolic esterases to convert nonfluorescent calcein AM into green-fluorescent calcein. Scale bar = 100 μm.

References:

1. Z. Li and Y. Zhang, *Nanotechnology*, 2008, **19**, 345606-345610.

2. T. Yogo, Y. Urano, Y. Ishitsuka, F. Maniwa and T. Nagano, *J. Am. Chem. Soc.*, 2005, **127**, 12162-12163.

3. S. Hui Lim, C. Thivierge, P. Nowak-Sliwinska, J. Han, H. van den Bergh, G. Wagnières, K. Burgess and H. B. Lee, *J. Med. Chem.*, 2010, **53**, 2865–2874.