Multifunctional azo-BODIPY-functionalised upconversion nanoparticles as sensors of hypoxia in biological environments

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Electronic Supplementary Information

Section S1. Energy level scheme of UCNPs

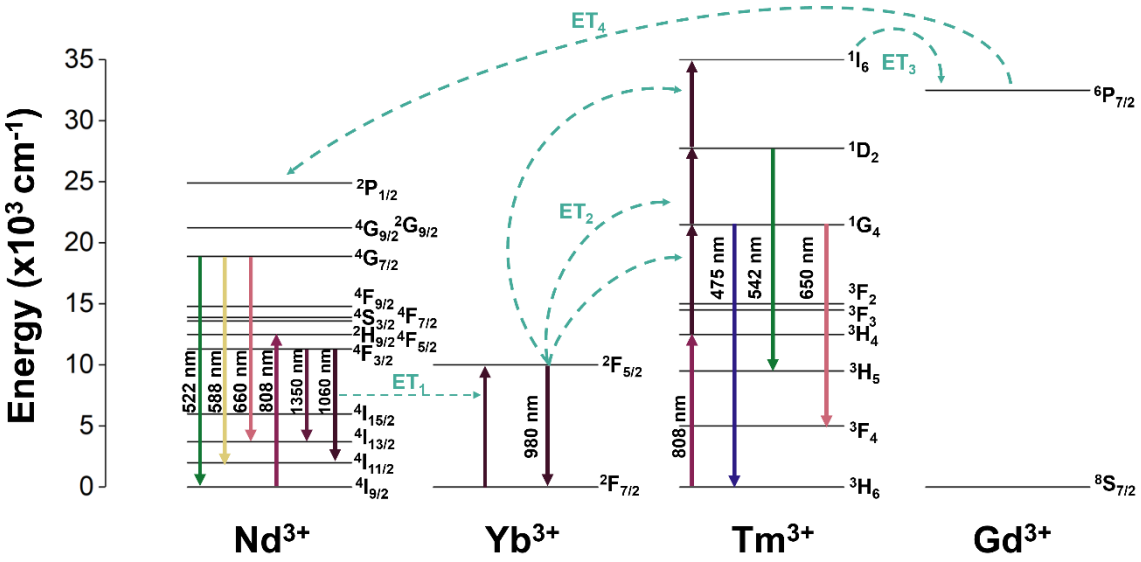


Figure S1. Energy level diagram for the $NaGdF_4$: 2% Yb^{3+}, 3% Nd^{3+}, 0.2% Tm^{3+} and $NaGdF_4$: 2% Yb^{3+}, 3% Nd^{3+}, 0.2% Tm^{3+}/$NaYF_4$ nanoparticles used in this work.

Section S2. Dynamic Light Scattering

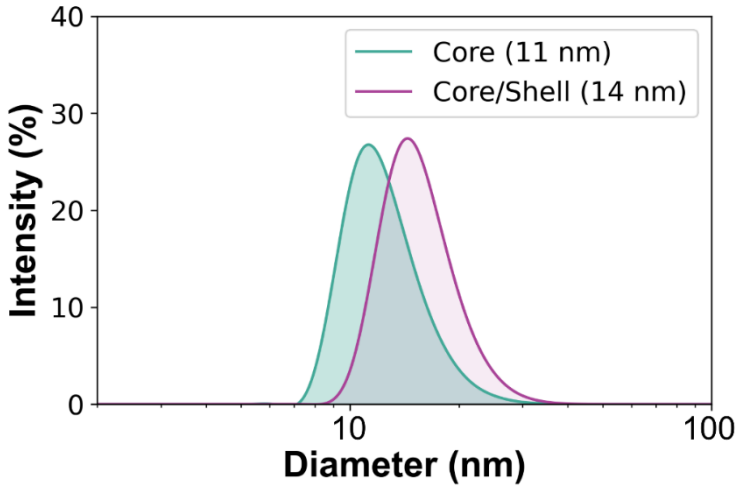
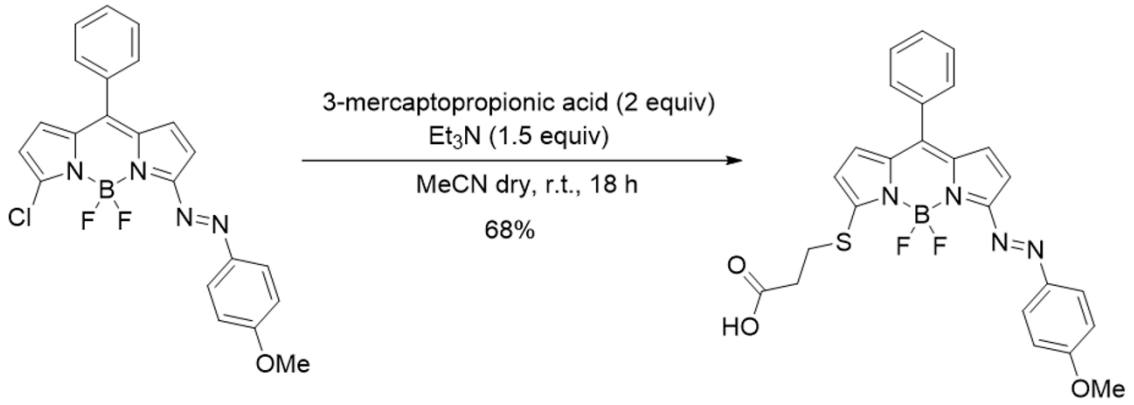


Figure S2. Dynamic light scattering measurements of the Core and Core/Shell nanoparticles directly after synthesis dispersed in hexane, showing a mean hydrodynamic diameter of 11 nm and 14 nm, respectively.

Section S3. Synthesis of (BDP-azo) **(2):**

3-Mercaptopropionicacid (14.6 mg, 0.138 mmol, 12.0 µL) was dissolved in dry acetonitrile (5 mL). Then triethylamine (10.3 mg, 0.102 mmol, 14.0 µL) was added and stirred. A solution of azo-BDP **1** (30.0 mg, 0.068 mmol) in dry acetonitrile (5 mL) was added slowly to this solution. The reaction mixture was stirred for overnight at room temperature. Organic layer was washed with 1N aqueous HCl and brine solution and then dried over anhydrous $Na_2S_2O_4$. The crude product was purified by silica gel column chromatography using dichloromethane/methanol (95:5). Yield: 23.4 mg (68%).

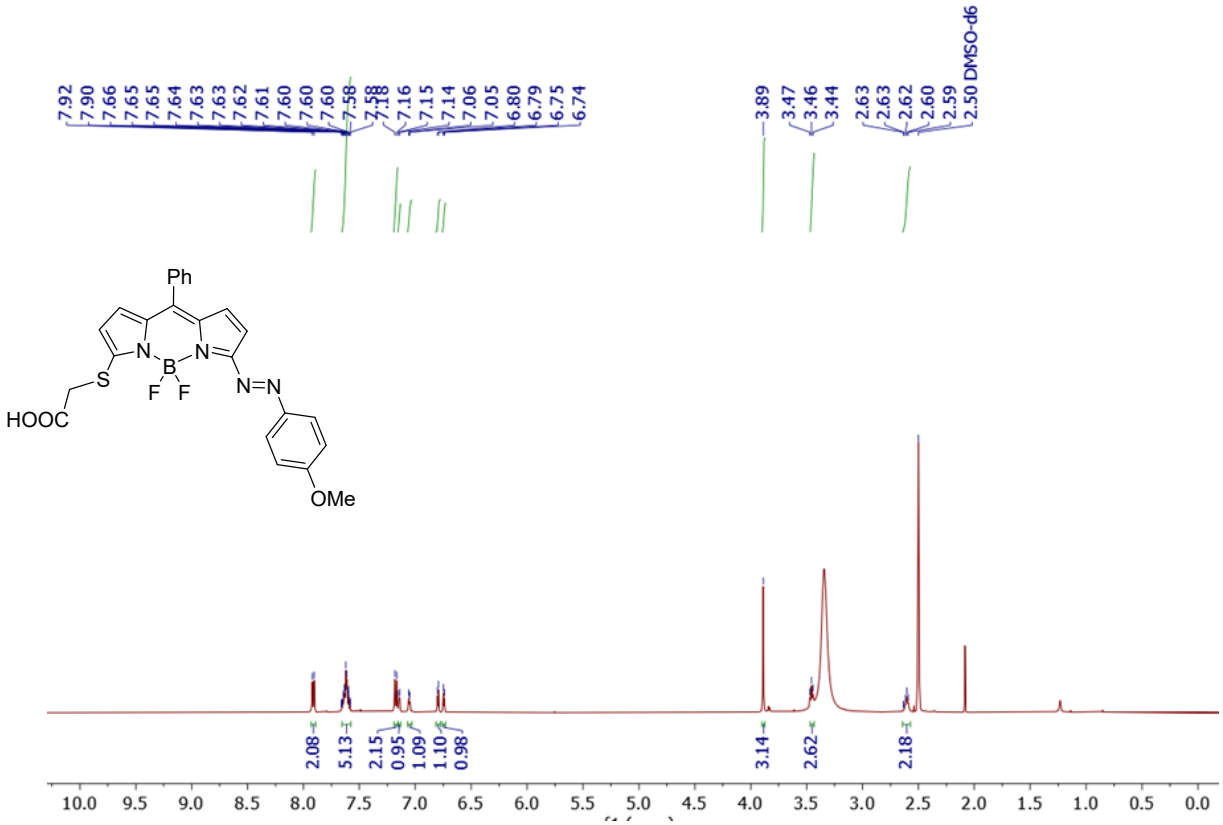


BDP-azo, 1 **BDP-azo, 2**

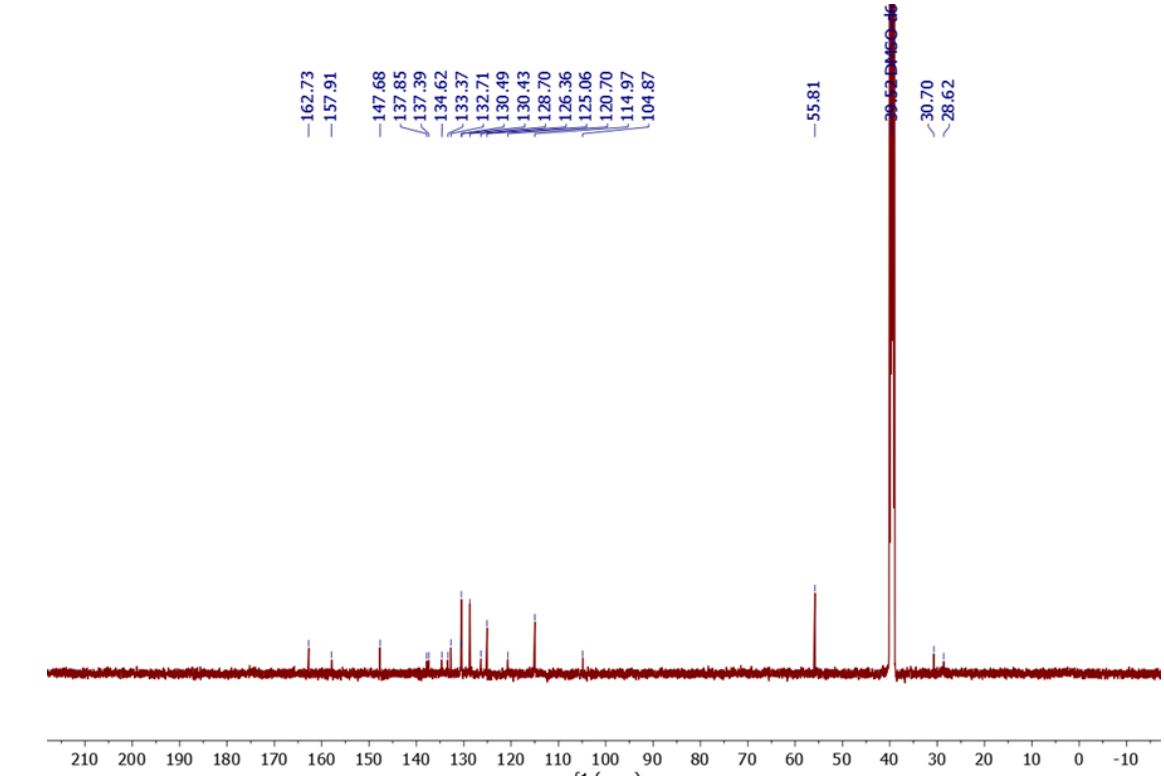
Figure S3. Schematic of the process followed to synthesise the BDP-azo **2**.

^{1}H-NMR (500 MHz DMSO-d_6): δ 7.92-7.90 (AA', 2H, anisole), 7.71 – 7.54 (m, 5H), 7.18-7.16 (BB', 2H, anisole), 7.14 (d, *J* = 4.7 Hz, 1H), 7.05 (d, *J* = 4.8 Hz, 1H), 6.80 (d, *J* = 4.5 Hz, 1H), 6.74 (d, *J* = 4.5 Hz, 1H), 3.89 (s, 3H), 3.46 (t, *J* = 6.8 Hz, 2H), 2.60 (t, *J* = 6.9 Hz, 2H). **^{13}C-NMR (126 MHz DMSO-d_6)**: δ 162.7, 157.9, 147.7, 137.8, 137.4, 134.6, 133.3, 132.7, 130.5, 130.4, 128.7, 126.3, 125.0, 120.7, 114.9, 104.8, 55.8, 30.7, 28.6. **^{11}B-NMR (160 MHz, DMSO-d_6)**: δ 0.98 (t, J = 29.4 Hz). **^{19}F-NMR (471 MHz, DMSO-d_6)**: δ -142.27 (dd, J = 58.8, 28.8 Hz). **MS (ESI)**: Calc for $C_{25}H_{20}BF_2N4O_3S$ $[M]^+$ 505.1395 found 505.1320.

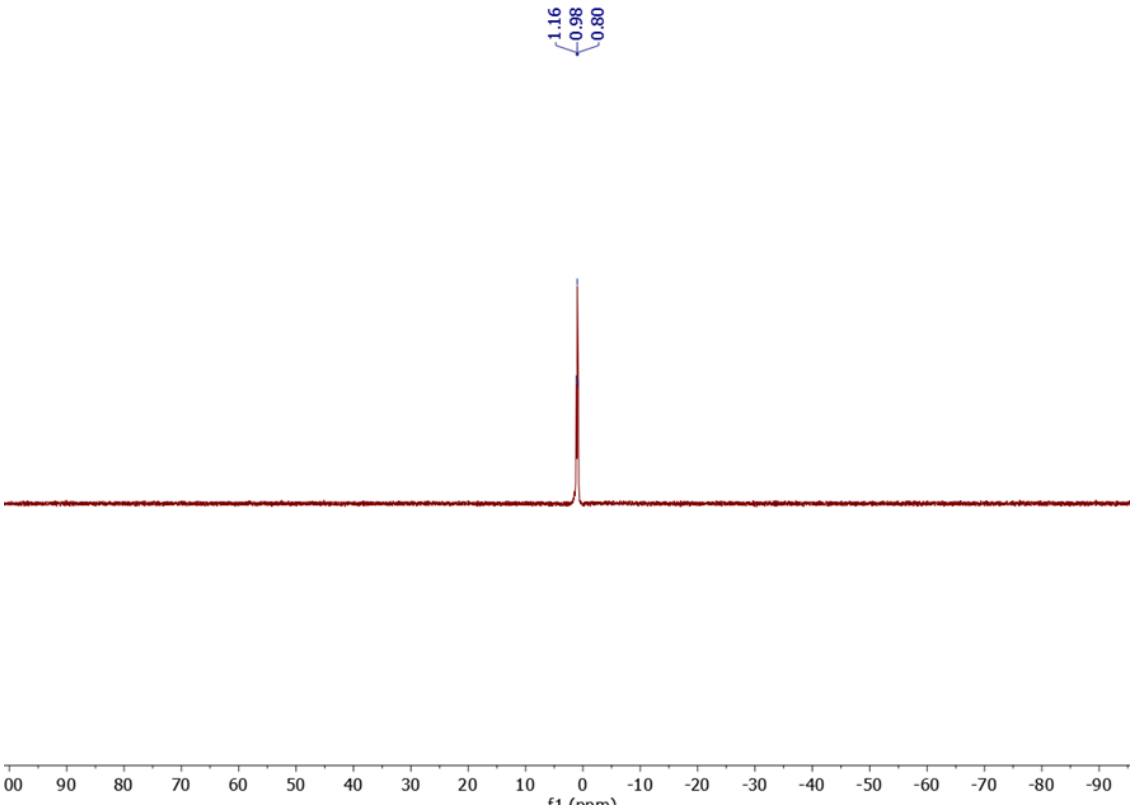
^{1}H-NMR (500 MHz DMSO-d_6) 2:



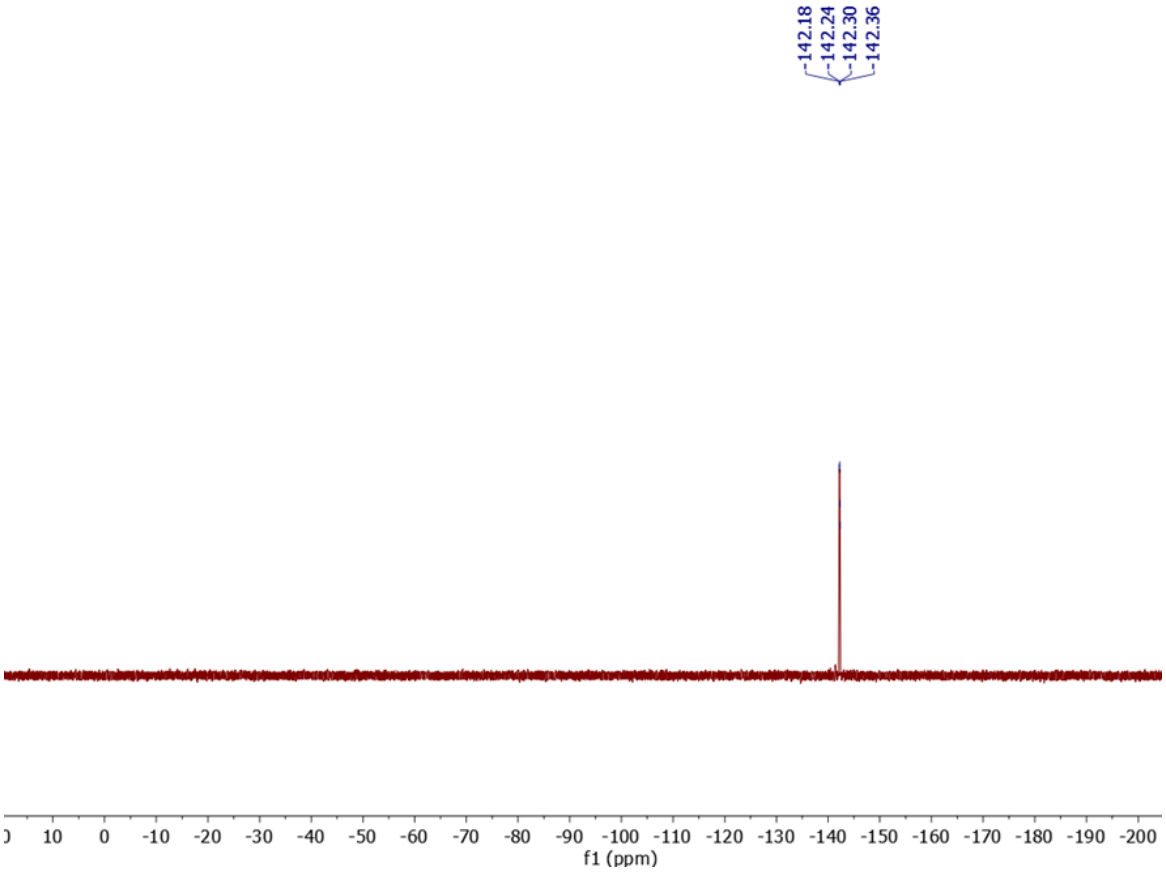
^{13}C-NMR (126 MHz DMSO-d_6) 2:



^{11}B-NMR (160 MHz, DMSO-d_6) 2:



^{19}F-NMR (471 MHz, DMSO-d_6) 2:



Section S4. Cleavage of azo-bond and Synthesis of BDP-NH_2 (3):

To a suspension of the corresponding BDP-azo **(2)** (10 mg, zinc dust (10 equiv) in CH_2Cl_2 (0.1 M) and ammonium formate (20 equiv), dissolved in the minimum amount of methanol, was added and the mixture was stirred at room temperature. After the completion of the reaction (monitored by TLC) the reaction mixture was filtered through a pad of Celite, the solvent was evaporated in vacuo, and the residue was purified by flash column chromatography (eluent CH_2Cl_2/MeOH, 95:5) to obtain **3** as an orange solid (7.5 mg, 0.019 mmol, 98%).

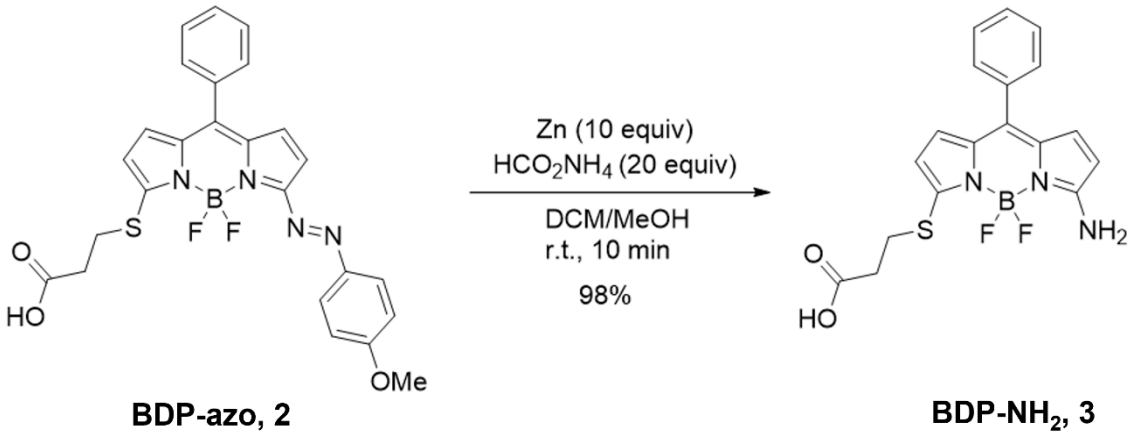


Figure S4. Schematic of the chemical Reduction of BDP-azo into BDP-NH_2 **3**.

Section S5. Absorbance and Emission of BDP-azo 2 and BDP-NH_2 3:

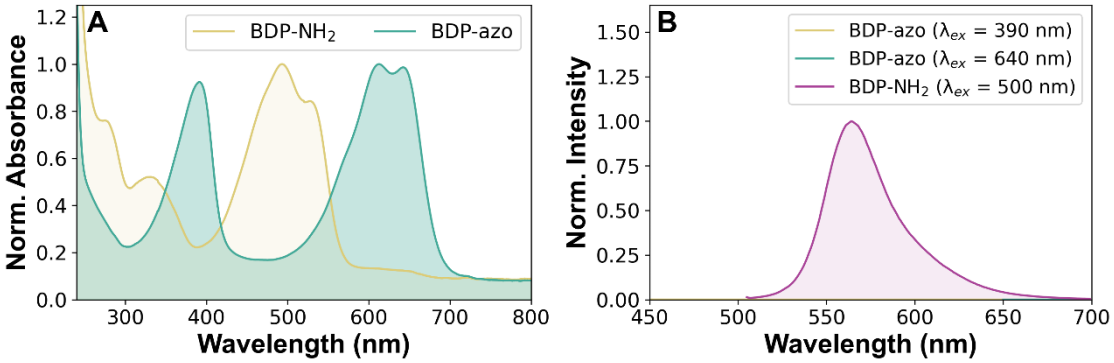


Figure S5. Normalised absorption (**A**) and emission (**B**) of the BDP-azo and BDP-NH_2 compounds in dichloromethane.

Section S6. NIR Imaging underneath phantom tissue

Powdered agar (200 mg) and 20% intralipid emulsion (200 µl) (both obtained from Sigma Aldrich) were added to de-ionized water (10 mL) at 90 ºC under heavy stirring. The resulting mix was stirred for another 15 min at this temperature and then cast forming 5 mm thick phantom tissue employing a 3 cm x 5 cm mold. After cooling down the gel was employed in the imaging experiment. NIR-imaging was performed in the PhotonSWIR Imager from BioSpace Lab (Nesles-la-Vallée, France, which combines an InGaAs CCD camera (WiDy SenS 640V-ST, New Imaging Technologies, Paris, France) with a long-pass filter at 850 nm (Thorlabs FEL850, Newton, NJ, USA) and a fiber-coupled 808 nm laser diode (LIMO) into one system. A cuvette containing a dispersion of UCNP-BDP-NH_2 (1 mg/mL) was illuminated with 50 W/cm^2 covered by the phantom tissue. The images were recorded with an exposure time of 0.1 s.

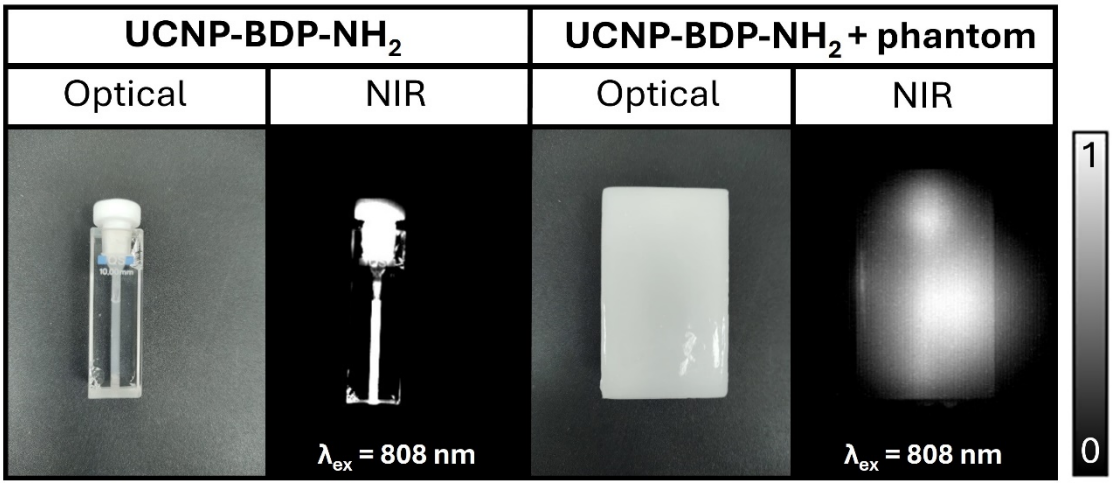


Figure S6. NIR imaging of UCNP-BDP-NH_2 under phantom tissue. The left two pictures show the cuvette filled with the nanoparticle dispersion taken with a normal camera and a NIR fluorescence image when exciting with the 808 nm laser. In the right two pictures the cuvette is placed underneath a phantom tissue (2 % agar, 2 % intralipids. Although diffuse, the signal from the dispersion in the cuvette is still recognizable.

Section S7. Dark Toxicity of UCNP and UCNP-BDP-azo.

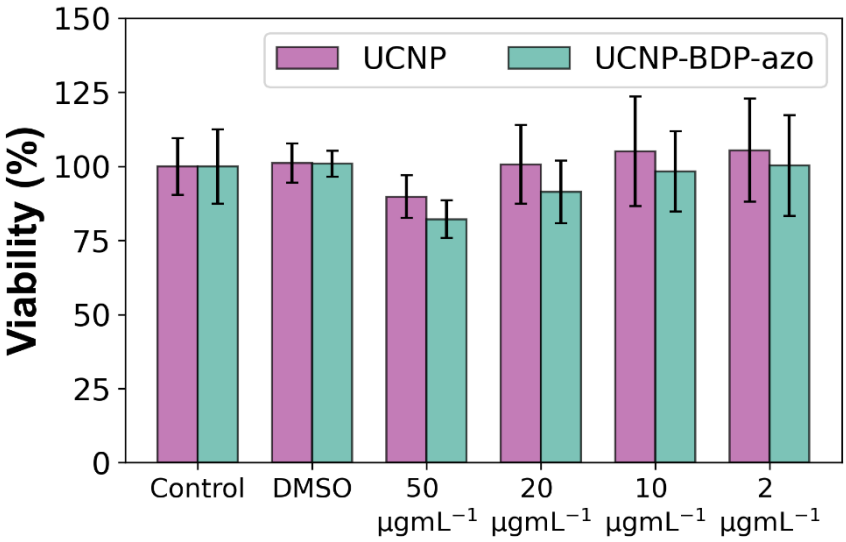


Figure S7. Dark toxicity experiments showing the cell survival percentage after incubation with four different concentrations of UCNP and UCNP-BDP-azo (pink and blue, respectively). A control for DMSO is also included in the graph.

Section S8. Control without nanoparticles.

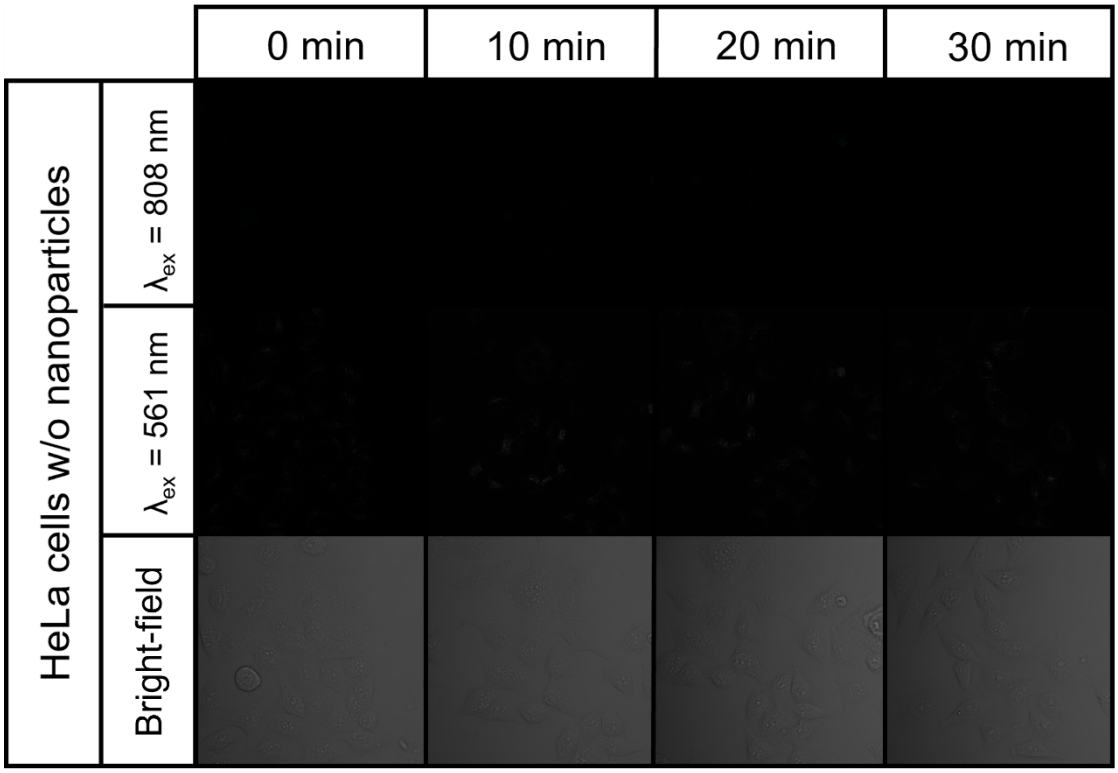


Figure S8. Confocal microscope control images of HeLa cells without any type of nanoparticle.

Section S9. Fluorescence microscopy with NIR excitation

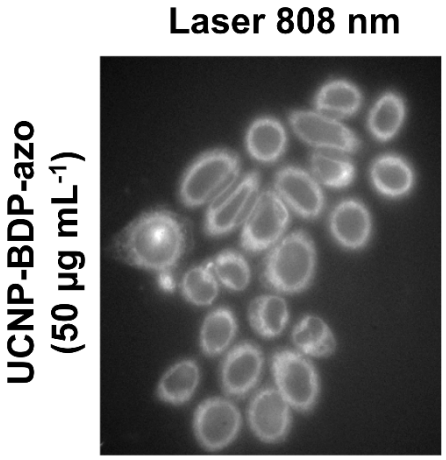


Figure S9. UCNP-BDP-azo (50 µg·mL^{-1}) were incubated with HeLa cells for 2 h and sealed to achieve anoxia**.** The integration of an 808 nm light source (diode laser, Lumics) with sufficient power density to induce upconversion into the standard Nikon Eclipse inverted fluorescence microscope was achieved via a collimator module together with a 10x IR objective that was attached to the top of the microscope, while the emission fluorescence was recollected through the normal visible pathway and employing a short-pass filter (750 nm FESH) below the probe stage and observed with a greyscale visible camera (Ixxon, Andor Technologies).