SUPPORTING INFORMATION

One-Pot Synthesis of Theranostic Nanocapsules with Lanthanide Doped Nanoparticles

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Experimental Procedures

Synthesis of $NaGdF_4$:Er^{3+} (2%), Yb^{3+} (20%) UCNPs

Gadolinium oxide (Gd_2O_3, 99.99%), erbium oxide (Er_2O_3, 99.99%), ytterbium oxide (Yb_2O_3, 99.99%), triflouroacetic acid (CF_3COOH, 99%), sodium trifluoroacetate (CF_3COONa, 98%), oleic acid (technical grade, 90%), and 1-octadecene (technical grade, 90%) were all purchased from Sigma-Aldrich and were used without further purification.

$NaGdF_4$:Er^{3+} (2%), Yb^{3+} (20%) UCNPs were synthesized *via* the hot-injection thermolysis approach[1-3], which allows to directly decompose the metal trifluoroacetate precursors. Briefly, the obtained $(CF_3COO)_3Ln$ (Ln = Gd, Yb, Er) precursors and CF_3COONa were first dispersed in 7.5 mL each of 1-octadecene and oleic acid (OA) and degassed with stirring at 125 °C. Then, the precursor solution was injected into the degassed and preheated (315 °C) mixture of 12.5 mL each of 1-octadecene and oleic acid at a rate of 1.5 mL min^{-1} (Harvard Apparatus Pump 11 Elite). The mixed solution was then left to stir vigorously under an inert argon atmosphere for 1 h. After the reaction was completed, the solution was cooled down to room temperature and the synthesized UCNPs were precipitated using absolute ethanol and centrifuged at 6500 rpm for 15 min. The solids were then washed with a mixture of hexane/ethanol (1:4) twice and finally re-dispersed in hexane.

Polymeric micelle/silica encapsulation of UCNPs and ZnPc

Pluronic® F127 ((ethylene oxide)$_{106}$(propylene oxide)$_{70}$(ethylene oxide)$_{106}$, PEO-PPO-PEO, MW = 12,600 g mol^{-1}), tetramethoxysilane (TMOS, 98%), tetrahydrofuran (THF, ≥99.9%) and zinc phthalocyanine (ZnPc, 97%) were purchased from Sigma-Aldrich. ZnPc was dissolved in THF at a concentration of 2 mg mL^{-1} (stock solution). The synthesized $NaGdF_4$:Er^{3+} (2%), Yb^{3+} (20%) UCNPs dispersed in hexane (31.8 mg mL^{-1}) were used as stock solution.

Polymeric micelle/silica nanocapsules with various payloads were prepared by modifying the interfacial templating condensation approach.[4] We have made nanocapsules with ZnPc (ZnPc@NCs), nanocapsules with various number (n) of UCNPs, namely S-UCNPs@NCs (n < 3) and M-UCNPs@NCs (n > 3), and nanocapsules with both UCNPs and ZnPc (UCNPs-ZnPc@NCs).

To prepare ZnPc@NCs, F127 (37.5 mg) was dissolved in THF (750 µL) and mixed with ZnPc (150 µL). After stirring at room temperature for 3 h, TMOS (55 µL) was added to the solution and sonicated for 1 min. The solution was injected into stirred deionized (DI) water (10 mL) at a rate of 60-90 µL min^{-1}. Stirring was continued for another 4 days for THF evaporation as well as TMOS hydrolysis and condensation. Afterwards, the solution was dialyzed against DI water for 24 h using a dialysis membrane (MWCO of 10,000 g mol^{-1}, Spectra/Por® regenerated cellulose). Finally, the solution was centrifuged for 10 min at 10,000 rpm, the supernatant was further passed through a 0.2 µm filter to remove large aggregates.

To synthesize the nanocapsules with UCNPs (S-UCNPs@NCs, M-UCNPs@NCs, and UCNPs-ZnPc@NCs), designated amounts of UCNP stock solution was firstly air dried to remove hexane. Then, the dried UCNPs were re-dispersed in THF and mixed with F127 or F127 and ZnPc, namely solution A1 (6.4 mg UCNPs, 15 mg F127, and 900 µL THF), solution A2 (9.5 mg UCNPs, 15 mg F127, and 900 µL THF), and solution A3 (6.4 mg UCNPs, 15 mg F127, 150 µL ZnPc, and 900 µL THF), respectively. After stirring at room temperature for 3 h and sonicating for 10 min, each of the solutions A(1-3) were individually injected into DI water (10 mL) at a rate of 60-90 µL min^{-1} while being stirred, to obtain respective solutions B(1-3). Stirring was continued for 2 more days to evaporate THF. Afterwards, 30 µL of TMOS was dissolved in THF (300 µL) and injected at a rate of 60-90 µL min^{-1} into each of the solutions B(1-3). It was stirred at room temperature for another 2 days for hydrolysis and condensation of TMOS. The solution was then subjected to centrifugation (6,000 rpm for 30 min), discarding the supernatant containing empty (i.e., UCNP-free) nanocapsules and re-dispersing the precipitates in DI water. The dispersion was further passed through a 0.2 µm filter to remove large aggregates.

Characterization

The crystalline phase of OA-capped UCNPs under investigation was analysed by X-ray diffraction (XRD) using a Bruker D8 Advanced Diffractometer, Cu Kα radiation (λ=1.5406 Å, power of the generator: 40 kV and 40 mA). Scan range was 20 – 80° 2θ, with a step size of 0.04° and a count time of 1s. The morphology and size distribution of the silica nanocapsules and UCNPs were determined by transmission electron microscopy (TEM) with a JEOL JEM-2010 microscope operating at 80 kV. The sample solution was prepared on a formvar/carbon film supported on a 300 mesh copper grid (3 mm in diameter). Inductively coupled plasma - atomic emission spectroscopy (ICP-AES) was used to determine the content of Na^+, Gd^{3+}, Yb^{3+}, and Er^{3+} in the UCNPs. The samples were digested in 70% HNO_3 with sonication at 65 °C, and then diluted to below 5% HNO_3 before conducting ICP-AES measurements. Dynamic light scattering (DLS) was performed with Malvern Zetasizer Nano-S using a HeNe laser (633 nm) to measure the hydrodynamic size and size distribution of different nanocapsules. All measurements were conducted using DI water as the dispersant in a glass cuvette. A Fourier-Transform Infrared (FTIR) spectrophotometer (Varian 3100 Excalibur) was used to characterize the chemical composition of the synthesised nanocapsules. For FTIR sample preparation, the aqueous suspensions of UCNPs-ZnPc@NCs were freeze-dried and dispersed in KBr pellets. Luminescent measurements were carried out under 980 nm excitation using a laser diode (BWT, China). The laser beam was focused on the sample using a lens to obtain a spot of a Gaussian intensity distribution with a 0.4 mm diameter. The emitted light was collected by a lens in a 90° configuration, and then transferred to a spectrophotometer (Avaspec-2048L-USB2) using an optical fiber. The UV-Visible (UV-VIS) absorption spectra were obtained using a Varian Cary 5000 spectrometer.

Evaluation of singlet oxygen ($^1O_2$) generation

$^1O_2$ production was detected by 9, 10-anthracenediyl-bis(methylene) dimalonic acid (ABDA) (Sigma-Aldrich).[5] In this method, the ABDA fluorescence emission is bleached due to reaction with the $^1O_2$. Briefly, ABDA was dissolved in dimethyl sulfoxide (DMSO) (Sigma Aldrich, USA) and diluted in water to a final concentration of 20 µM. UCNPs-ZnPc@NCs, UCNPs@NCs, or ZnPc@NCs alone were mixed with ABDA

and irradiated by a 980 nm CW laser (1.5 W cm^{-2}) for 0, 20, 40, and 60 min, respectively. Magnetic stirring and constant temperature (24 °C) were kept during the irradiation experiments. The fluorescence emission of ABDA at 431 nm (excited at 380 nm) was measured using a microplate fluorescence spectrophotometer (Cytation/5 imaging reader, BioTek).

Cell culture and cytotoxicity assay

The breast cancer cells BT474 (American Type Culture Collection) were cultured in Dulbecco's modified Eagle medium (DMEM) (high glucose [4.5 g L^{-1}], with sodium pyruvate and L-glutamine), containing 10% fatal bovine serum (Invitrogen), 1% antibiotic with 100 UI ml^{-1} penicillin, and 100 mg ml^{-1} streptomycin (Invitrogen). Cell incubation was maintained at 37 °C, and 5% CO_2. The culture medium was changed two to three times a week and cells were passaged serially using 0.25% trypsin/EDTA (Invitrogen).

The cytotoxicity of S-UCNPs@NCs, ZnPc@NCs, and UCNPs-ZnPc@NCs was evaluated by determining the viability of BT474 breast cancer cells using Cell Counting Kit-8 (CCK-8) (Sigma-Aldrich),[6] of which highly water-soluble tetrazolium salt is reduced by dehydrogenases in cells to give an orange colour product (formazan). The amount of the formazan dye generated is directly proportional to the number of living cells. Briefly, BT474 cells were seeded into 96 well plates at a density of 10,000 cells per well and incubated for 24 h. Three groups of the nanocapsules with the designated concentrations, containing UCNPs (30, 100, 300 μM) or ZnPc (1, 3.5, 10 μM), were added to the cultured cells. After incubation with nanocapsules for 72 h, CCK-8 solution was added into cultured cells and incubated for another 2 h. The absorbance at 450 nm of the mixture was measured using a Benchmark Plus microplate spectrophotometer (Bio-Rad). The absorbance values obtained were recalculated as percentage values of viability,

Viability (%) = 100 x $(A_T-A_B)/(A_C-A_B)$

where A_T, is the absorption values of wells containing nanocapsules, A_C is the absorption values of control wells, and A_B is the absorption values of a blank solution. Data were expressed as mean ± standard deviation (SD). Statistical significance of differences observed between groups was calculated using a two-tail paired Student's t-Test at the 95% confidence level. Significance was represented as p-value < 0.05.

In vitro therapeutic efficacy of UC-PDT

To determine the therapeutic effect of UCNPs-ZnPc@NCs, we evaluated the growth inhibition and apoptosis of breast cancer BT474 cells after 980 nm irradiation (1.5 W cm^{-2}). The sequential irradiation was done as follows: 40 min of irradiation, 40 min rest, 40 min of irradiation. The total irradiation period was 80 min. Cell growth inhibition by UCNPs-ZnPc@NCs, UCNPs@NCs, and ZnPc@NCs was tested using CCK-8 cell proliferation assay as described in the previous section. The apoptosis induction was evaluated using Caspase-3/7 green detection reagent (Invitrogen), a fluorescence probe that presents green emission upon 488 nm excitation if reacted with the Caspase-3/7, released by the apoptotic cells. Briefly, 30,000 cells were seeded in eight-chambered cover glass plate (LAB-TEK, Chambered Cover glass System), and incubated with the above 3 groups of nanocapsules containing 100 μM UCNPs and / or 3.5 μM ZnPc for 24 h before the sequential irradiation treatment. After another 24 h of incubation, the Caspase-3/7 green detection reagent was added and incubated with the cells for 6 h to allow the accumulation of the reagent into the cells. Live cell imaging was performed using a confocal laser scanning microscope (Nikon). 20×/0.5 NA dry objective (Nikon) was selected for imaging acquisition. The fluorescence image was acquired at λ_{exc} = 488 nm and λ_{exc} = 530 nm.

T_2 relaxivity measurements and T_2-weighted images

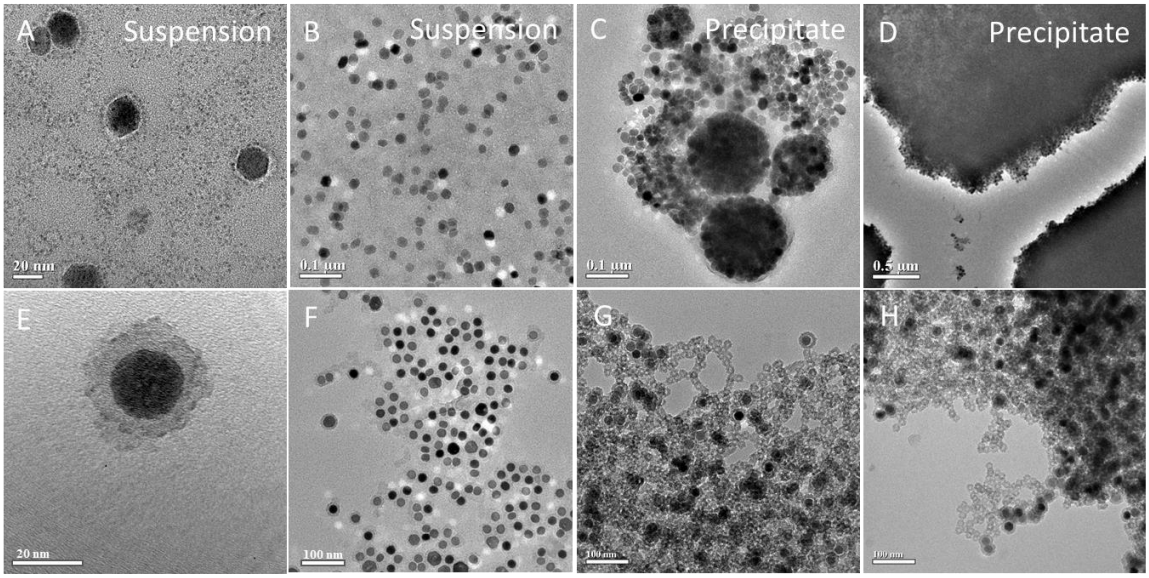
T_2 relaxation measurements and phantom images were obtained by using a 7-Tesla Bruker Clinscan MRI system. To determine the effect of polymeric micelle/silica encapsulation on T_2 relaxivity of UCNPs, the water dispersible ligand-free UCNPs were used as a non-encapsulation group. Ligand-free UCNPs were prepared by removing the capped OA ligands using hydrochloric acid solution (pH = 4).[7] Prior to imaging, a phantom was prepared by series dilution of ligand-free UCNPs, S-UCNPs@NCs and M-UCNPs@NCs at concentrations of 0.125, 0.06, 0.03, 0.015 mM. T_2 relaxation times were determined from a multi-echo spin-echo sequence: TR = 4000 ms; TE: 6.7–250.6 ms. The transverse (r_2) relaxivities were calculated from the slope of $1/T_2$ versus molar [Gd^{3+}] concentration plots. The relevant acquisition parameters were optimized to generate T_2-weighted images, being TR/TE = 3000/61 ms.

BT474 cells were seeded in a 6-well plate (30,000 cells per well) and incubated for 24 h, UCNPs-ZnPc@NCs containing 100 μM of Gd^{3+} were then added. After 24 h incubation, the cells were detached by 0.25% tripsin-EDTA (Invitrogen), and washed with 1x PBS 3 times to remove the non-uptaken UCNPs-ZnPc@NCs. Prior to imaging, a phantom was prepared by mixing the collected cells into a 0.5% agarose solution. T_2 -weighted images were obtained using the same acquisition parameters described above.

Supplementary Experimental Data

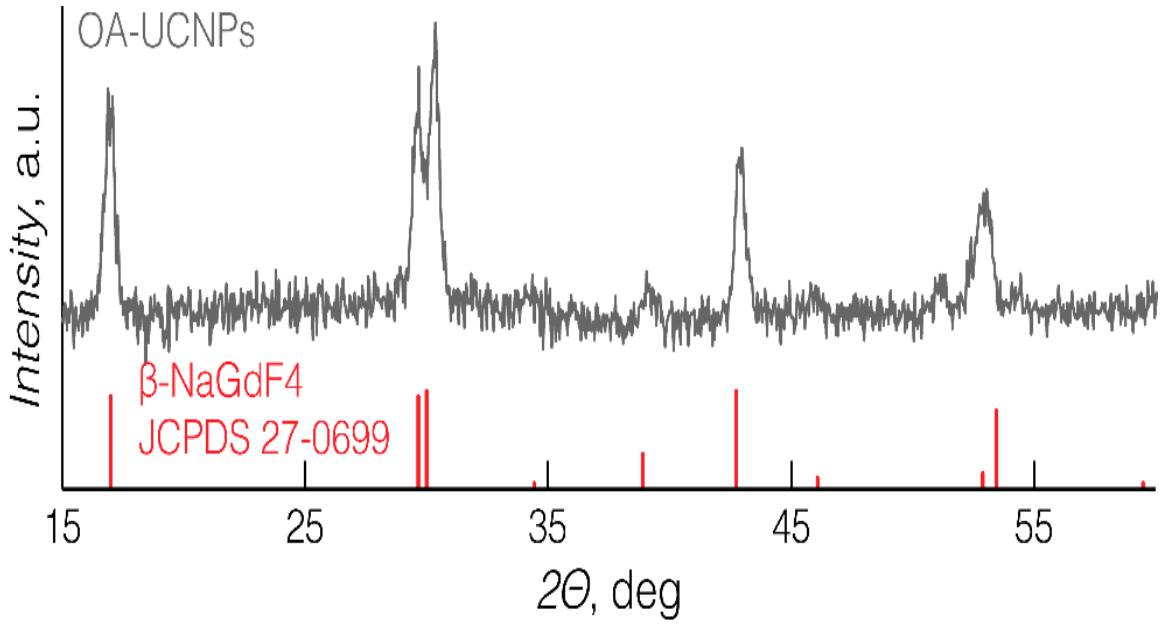
TEM images demonstrating the influence of TMOS and ZnPc on the encapsulation

Figure S1. (A-D) TEM images of nanocapsules from the failed encapsulation when simultaneously injecting the mixture of TMOS, F127 and UCNPs in water. Nanocapsules with UCNPs are demonstrated in the suspension of the resultant solution and aggregated UCNPs are observed in the precipitate of the resultant solution. (E-H) TEM images demonstrating the co-encapsulation of UCNPs and ZnPc at various loading amounts of ZnPc: morphology of the monodispersed UCNPs-ZnPc@NCs with a single UCNP at the loading dose of 0.3 mg (E and F), failed encapsulations at the loading dose of 0.6 (G) and 1.2 mg (H), respectively.



XRD analysis of OA-UCNPs

Figure S2. (A) The diffraction peaks of XRD indicated a pure β-phase, in agreement with the reference data (JCPDS 27-699).

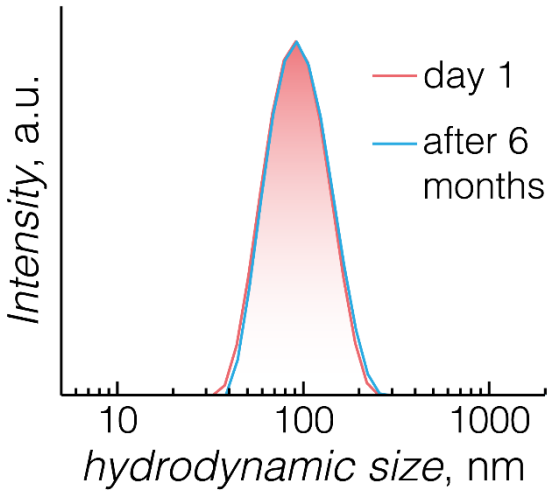


DLS analysis of the NCs studied

Table S1. The hydrodynamic sizes and polydispersity indexes (PDI) of nanocapsules analysed by DLS.

Nanocapsules (NCs)	Z-ave Size (nm)	Main Peak (nm)	PDI
S-UCNPs@NCs	92.8	110	0.199
M-UCNPs@NCs	145.3	180	0.217
ZnPc@NCs	43.04	71.92	0.392
UCNPs-ZnPc@NCs	85.6	97.2	0.113
UCNPs-ZnPc@NCs_6 Months	90.1	99.6	0.154

Figure S3. The hydrodynamic size of UCNPs-ZnPc@NCs analysed after 6 months storage at room temperature.



ICP data demonstrating the content of UCNPs and ZnPc encapsulated in the nanocapsules studied

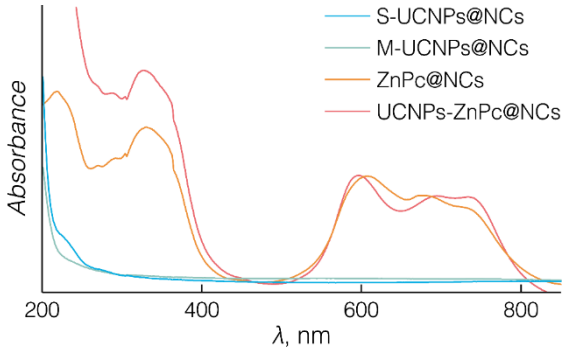
Table S2. Content of $NaGdF_4:Er^{3+}$ (2%), Yb^{3+} (20%) UCNPs and ZnPc encapsulated in the synthesized polymeric micelle/silica nanocapsules analysed by ICP

Nanocapsules (NCs)	mM (mmol l^{-1})			
	$[Gd^{3+}]$	$[Yb^{3+}]$	$[Er^{3+}]$	$[Zn^{2+}]$
S-UCNPs@NCs	0.789	0.191	0.018	x
M-UCNPs@NCs	0.820	0.202	0.018	x
UCNPs-ZnPc@NCs	1.386	0.341	0.036	0.031
ZnPc@NCs	x	x	x	0.046

X: not detected

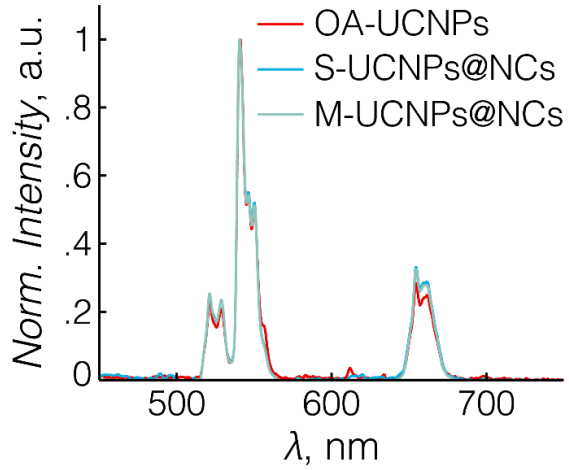
Light absorbance spectrum of ZnPc encapsulated in the nanocapsules studied

Figure S4. Absorbance spectrum of the synthesized nanocapsules demonstrating ZnPc molecules were encapsulated in UCNPs-ZnPc@NCs and ZnPc@NCs, respectively.



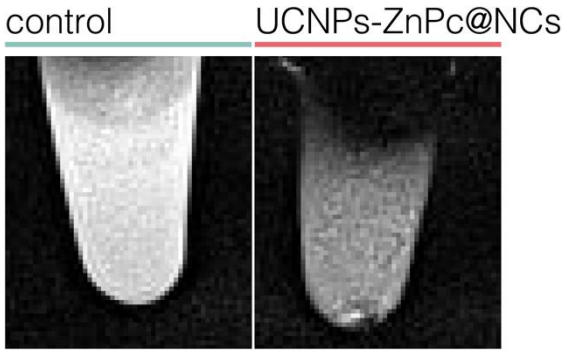
Comparison of upconversion spectrum between OA-UCNPs and encapsulated UCNPs

Figure S5. The upconversion spectra of OA-capped UCNPs in hexane and encapsulated UCNPs in water. Spectra are normalized to the maximum intensity.



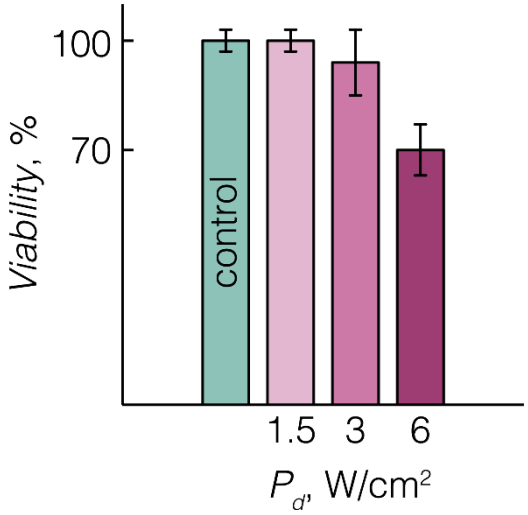
In vitro T_2 contrast enhancement of UCNPs-ZnPc@NCs by MRI

Figure S6. T_2-weighted image demonstrates the enhanced negative contrast in the BT474 cells incubated with UCNPs-ZnPc@NCs compared to the non-treated cells at 7 T magnetic field.



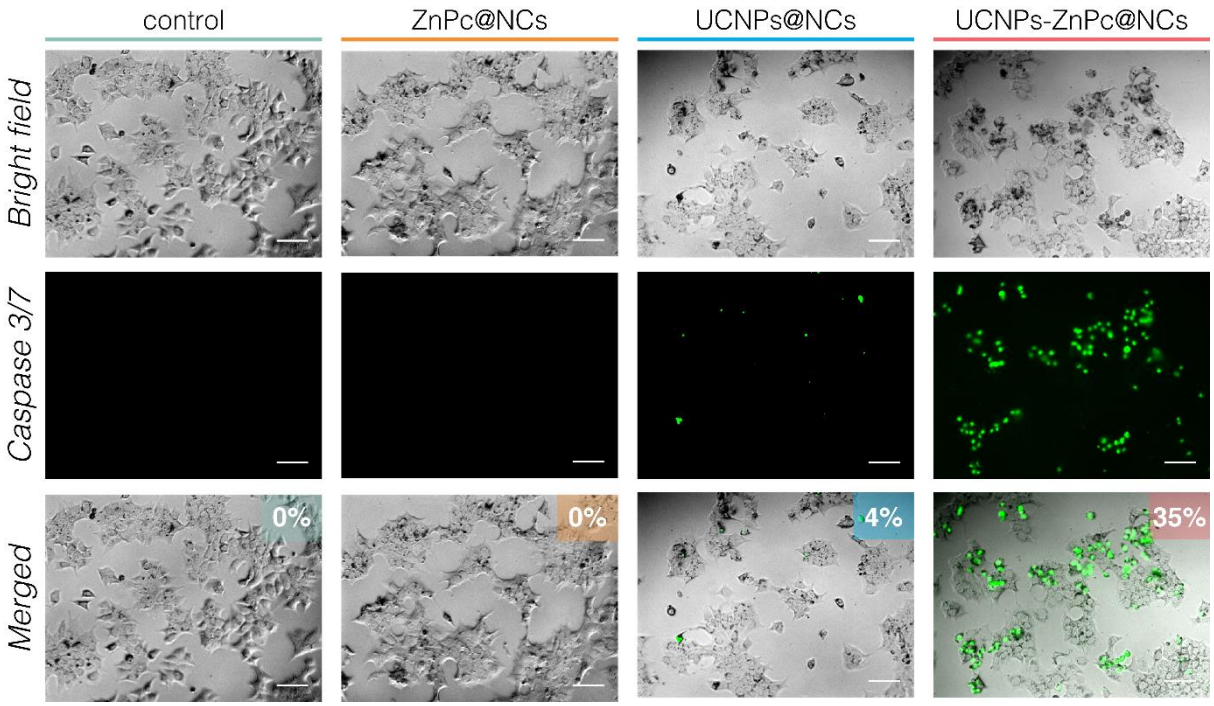
The viability of BT474 cells upon 980 nm irradiation

Figure S7. The viability (%) of BT474 cells upon 980 nm irradiation. BT474 cells were seeded into 96 well plates at a density of 10,000 cells per well and incubated for 24 h, then irradiated by 980 nm light for 40 min at various power densities (1.5, 3, and 6 W cm^{-2}). Non-irradiated wells were set as control. The cell viability was analysed by CCK-8. Data were expressed as mean ± standard deviation (SD) of triplicated experiments.



In vitro UC-PDT effect of UCNPs-ZnPc@NCs in BT474 breast cancer cells.

Figure S8. Fluorescence microscopy images of BT474 cells, incubated with the 4 groups of NCs for 24 h before 980 nm irradiation. Cell nuclei were stained with caspase 3/7 detection reagent (λ_{ex} = 488 nm, λ_{em} = 530 nm), green colour indicates the apoptotic cells. The % corresponds to the apoptosis induced by each group. The scale bars in all images stand for 200 µm.



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