**Supporting Information for**

# Photosensitiser functionalised luminescent upconverting nanoparticles for efficient photodynamic therapy of breast cancer cells

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# 1. Detailed experimental methods

## 1.1. Synthesis of UCNPs

*Synthesis of* β-*$NaYF_4$:Yb,Er,Gd core nanoparticles*

The synthesis of $Ln^{3+}$ doped $NaYF_4$ (20% Yb, 20% Gd, 2% Er) upconversion nanoparticles is based on a reported procedure.[1] The following description is for a 5 mmol approach of total lanthanide and rare earth chlorides. The synthesis was performed in a 250 mL three-neck round bottom flask under nitrogen flow. 387.5 mg $YbCl_3 \cdot 6H_2O$ (1 mmol), 371.7 mg $GdCl_3 \cdot 6H_2O$ (1 mmol), and 38.2 mg $ErCl_3 \cdot 6H_2O$ (0.1 mmol) were dissolved in 20 mL of methanol together with 879.7 mg $YCl_3 \cdot 6H_2O$ (2.9 mmol). 40 mL of oleic acid and 75 mL of octadecene were added and the cloudy solution was heated to 160 °C using a heating mantle with temperature control. The reaction mixture was stirred for 30 min *in vacuo* at 160 °C to obtain a clear solution of the oleate precursors and then was cooled to room temperature. Afterwards, a solution of 0.740 g $NH_4F$ (20.0 mmol) and 0.5 g of NaOH (12.5 mmol) in 20 mL of methanol was added. The white suspension was heated to 120 °C for 30 min to remove the methanol. The clear solution was heated to reflux (*ca.* 320 °C) to induce the growth of the nanoparticles. As soon as the temperature reached 300 °C, a timer was started and the occurring luminescence of the upconversion nanoparticles was controlled with a 980 nm laser module (200 mW, cw). After luminescence was visible (*ca.* 10 min), the nanoparticles were stirred for another 10 min for growth and self-focusing (Ostwald ripening). The dispersion was cooled down to room temperature and the particles were precipitated by addition of ethanol (*ca.* 200 mL). The white particles were collected by centrifugation (1,000 xg, 5 min). The precipitate was dispersed twice in 20 mL chloroform and precipitated again with ethanol (*ca.* 200 mL). This washing step was repeated with cyclohexane and acetone three times until a clear dispersion of the nanoparticles in cyclohexane was obtained. In a last step the particles were dissolved in 20 mL cyclohexane and salt/aggregates were separated by centrifugation (1,000 xg, 3 min). The supernatant containing the β-$NaYF_4$:Yb,Er,Gd core nanoparticles was stored at 4 °C for further usage.

*Synthesis of α-$NaYF_4$ nanoparticles (shell precursor)*

The synthesis was performed in a three-neck round bottom flask under a nitrogen flow. 1516.8 mg $YCl_3 \cdot 6H_2O$ (5 mmol) was dissolved in 10 mL methanol. 40 mL of oleic acid and 75 mL of octadecene were added and the cloudy solution was heated to 160 °C by a heating mantle with temperature control. The reaction mixture was stirred for 30 min *in vacuo* at 160 °C in order to obtain a clear solution of the oleate-capped nanoparticles. Afterwards, a solution of 0.740 g $NH_4F$ (20.0 mmol) and 0.5 g of NaOH (12.5 mmol) in 20 mL methanol was added at room temperature. The white suspension was heated to 120 °C for 30 min to remove the methanol. The solution was heated to 240 °C to induce the growth of the nanoparticles. After 30 min the dispersion was cooled to room temperature and the nanoparticles were purified as described for the β-$NaYF_4$:Yb,Er,Gd core nanoparticles.

*Synthesis of $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell (oleate-capped) nanoparticles*

The synthesis of the $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell (oleate-capped) nanoparticles was performed in two three-neck round bottom flasks under a nitrogen flow. In the first flask, 2 mmol of the β-$NaYF_4$:Yb,Er,Gd core particles were heated together with 5 mL oleic acid and 5 mL octadecene to 120 °C. In the second flask, 0.5 mmol of the α-$NaYF_4$ shell-material dispersed in cyclohexane was heated with 2.5 mL oleic acid and 2.5 mL octadecene to 120 °C. Both dispersions were stirred for 30 min *in vacuo* at 160 °C to remove the cyclohexane. While the mixture of the β-$NaYF_4$:Yb,Er,Gd core particles was heated to 325 °C under reflux, the shell material, α-$NaYF_4$ nanoparticles, was kept at 120 °C. The shell material was injected stepwise (0.5, 1, 1.5 and 2 mL) through a septum to the core particles. The time interval between each injection was 10 min. During the injection of the shell material the temperature should be above 300 °C to obtain a homogeneous shell growth. After the last injection, the $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell nanoparticles were stirred for another 10 min at reflux and then cooled to room temperature. The core-shell nanoparticles were purified as described for the β-$NaYF_4$:Yb,Er,Gd core nanoparticles.

## 1.2. Inductively coupled plasma optical emission spectroscopy (ICP-OES) measurements

A known amount of β-$NaYF_4$:Yb,Er,Gd core nanoparticles or $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell nanoparticles (*ca.* 0.3 mg) were dried at 70 °C in the oven. The dry sample was dissolved in 417 µL of sulfuric acid (96%) supported by 15 minutes of sonication. The solution was first diluted with double distilled water to 7.5 mL and afterwards with 1 M nitric acid to 15 mL. The emission wavelengths for the lanthanide ions were 363.312 nm for yttrium, 342.247 nm for gadolinium, 349.910 nm for erbium and 401.225 nm for ytterbium.

## 1.3. Biological experiments

*Imaging medium*

The imaging medium based on Hank's balanced salt solution (HBSS) was prepared in water containing NaCl (120 mM), KCl (5 mM), $CaCl_2 \cdot 2H_2O$ (2 mM), $MgCl_2 \cdot 6H_2O$ (1 mM), $NaH_2PO_4 \cdot 2H_2O$ (1 mM), $NaHCO_3$ (1 mM) and 4-(2-hydroxyethyl)piperazine-4-ethanesulfonic acid (HEPES, 25 mM); and supplemented with bovine serum albumin (1 $mg \cdot mL^{-1}$) and glucose (11 mM). The pH of the imaging medium was adjusted to 7.2 using aqueous solutions of NaOH (1 M) and HCl (0.6 M). Prior to use, the imaging medium was sterilised by filtration through a 0.22 µm filter unit.

*Phosphate buffer saline (PBS)*

The phosphate buffered saline solution used for the cell-based experiments was prepared by dissolving 10 PBS tablets in water (1 L). The solution was sterilised by autoclaving at 110 °C for 10 min. The as-prepared PBS solution contained $Na_2HPO_4$ (8 mM), $KH_2PO_4$ (1 mM), NaCl (160 mM) and KCl (3 mM); and had a pH value of 7.3.

*Propidium iodide solution in PBS*

A solution of propidium iodide (1 $mg \cdot mL^{-1}$) was prepared in PBS. The PBS buffer (10 mM) used to dissolve the propidium iodide was prepared using $NaH_2PO_4 \cdot 2H_2O$ and $Na_2HPO_4$ stock

solutions (200 mM). The as-prepared PBS solution contained NaCl (150 mM) and calcium chloride dihydrate (100 µM). The pH of the PBS was adjusted to 7.4 using aqueous solutions of NaOH (1 M) and HCl (0.6 M). The propidium iodide solution was sterilised by filtration through a 0.22 µm filter unit.

*SK-BR-3 cell culture*

SK-BR-3 human breast adenocarcinoma cells were cultured in McCOY's 5A phenol red free medium supplemented with 10% foetal bovine serum. The cells were routinely cultured at 37 °C in a 5% $CO_2$ atmosphere in 75 $cm^3$ Nunc Easy tissue culture flasks with porous caps. Subcultures (1:4) were made every 5 days by washing the cells with PBS and dislodging the cells from the flask surface using trypsin 0.25% (1x) EDTA.

*Culture of SK-BR-3 cells onto coverslips*

The SK-BR-3 cells were cultured at 37 °C in a 5% $CO_2$ atmosphere in a 75 $cm^3$ tissue culture flask until they reached near confluence. Following incubation, the cells were washed with PBS and harvested from the flask using trypsin as described previously. A sterile 18 mm diameter glass coverslip was placed in each well of a 6-well Nunc multidish. An aliquot of SK-BR-3 cells (3 mL, $2x10^4$ cells·$mL^{-1}$) was added to each well covering the coverslip. The cells were incubated at 37 °C in a 5% $CO_2$ atmosphere for *ca.* 48 h.

## 2. Characterisation of UCNPs

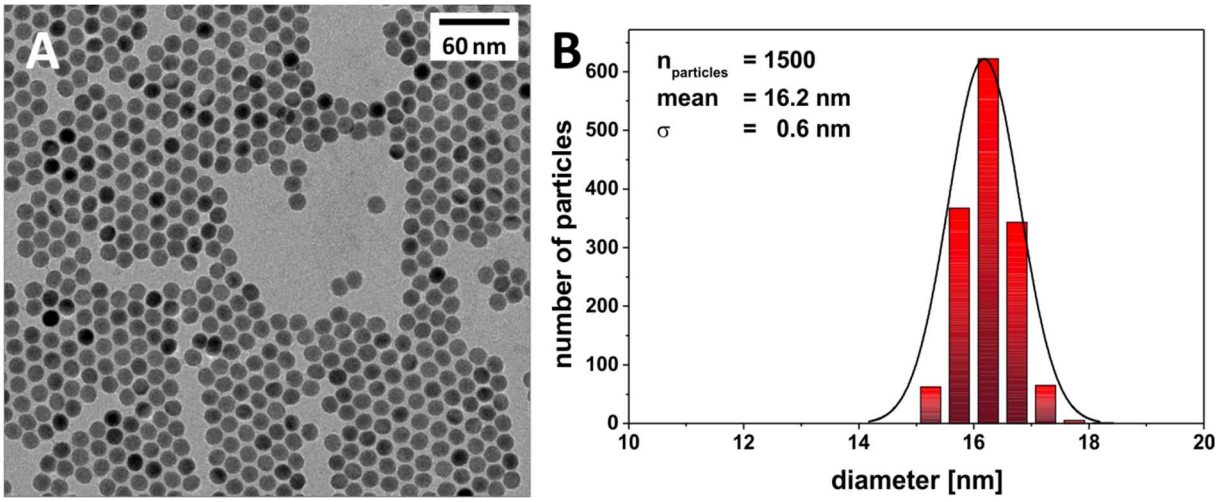


**Fig. S1.** (**A**) Transmission electron micrograph of a sample of β-$NaYF_4$:Yb,Er,Gd core upconverting nanoparticles (scale bar represents 60 nm); and (**B**) corresponding histogram of the size distribution of the β-$NaYF_4$:Yb,Er,Gd core upconverting nanoparticles showing a Gaussian fit. The average size of the β-$NaYF_4$:Yb,Er,Gd core upconverting nanoparticles was determined to be 16.2 ± 0.6 nm (n = 1500 nanoparticles).

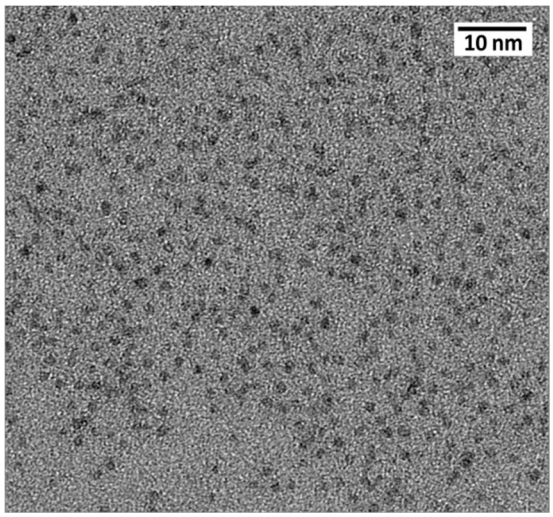


**Fig. S2.** Transmission electron micrograph of a sample of α-$NaYF_4$ upconverting nanoparticles dispersed in cyclohexane (scale bar represents 10 nm). According to the TEM images, the size of the α-$NaYF_4$ nanoparticles was estimated to be 2 – 3 nm.

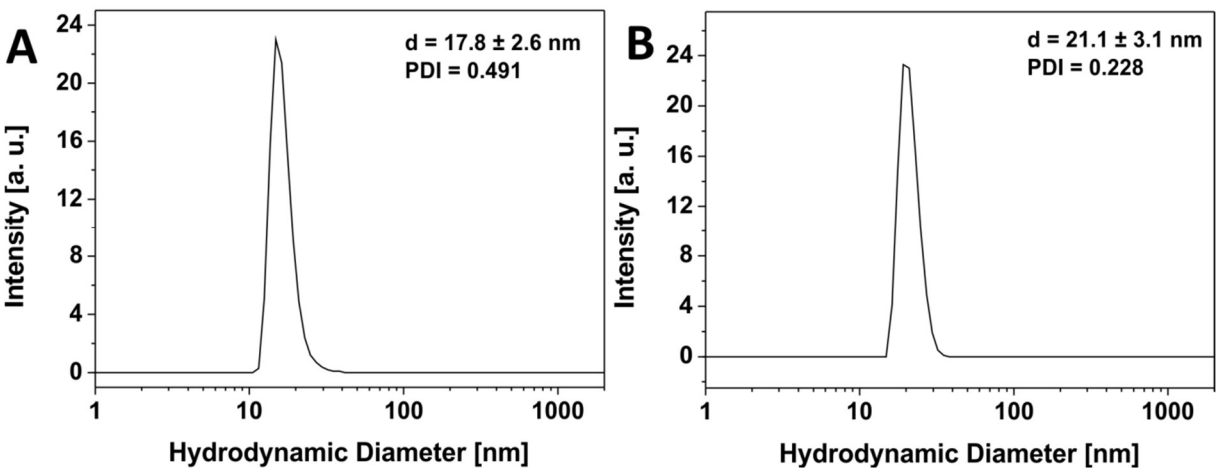


**Fig. S3.** DLS measurements of the β-$NaYF_4$:Yb,Er,Gd core nanoparticles (**A**) and the oleate-capped β-$NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell nanoparticles (**B**) in cyclohexane. The curve shows the DLS signal weighted for number with a solvodynamic diameter of 17.8 ± 2.6 nm (**A**) and 21.1 ± 3.1 nm (**B**). The polydispersity index (PDI) was 0.491 for the core particles and 0.228 for the core-shell nanoparticles.

**Table S1.** Composition (%) of the β-$NaYF_4$:Yb,Er,Gd core (16.2 nm) and of the oleate-capped $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell (17.2 nm) upconverting nanoparticles determined by ICP-OES measurements. (n = 3).

| Composition [%] | $Y^{3+}$ | $Gd^{3+}$ | $Er^{3+}$ | $Yb^{3+}$ |
|---|---|---|---|---|
| Core particles | 59.0 | 20.3 | 1.0 | 19.7 |
| Core-Shell particles | 68.1 | 16.4 | 0.9 | 14.4 |

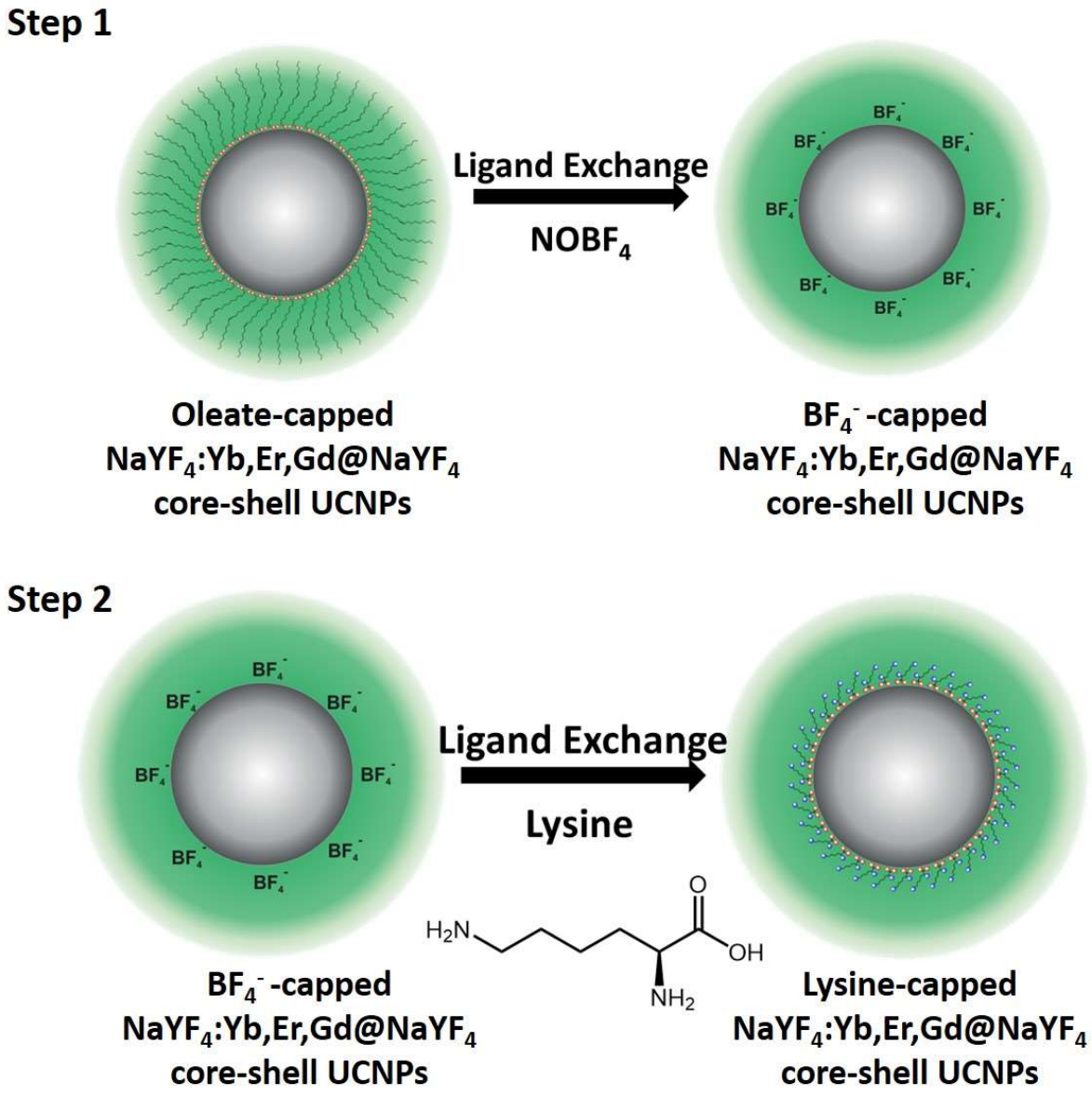


**Fig. S4.** Schematic representation of the two ligand exchange steps followed to obtain lysine-capped $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell UCNPs from the oleate-capped $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell UCNPs.

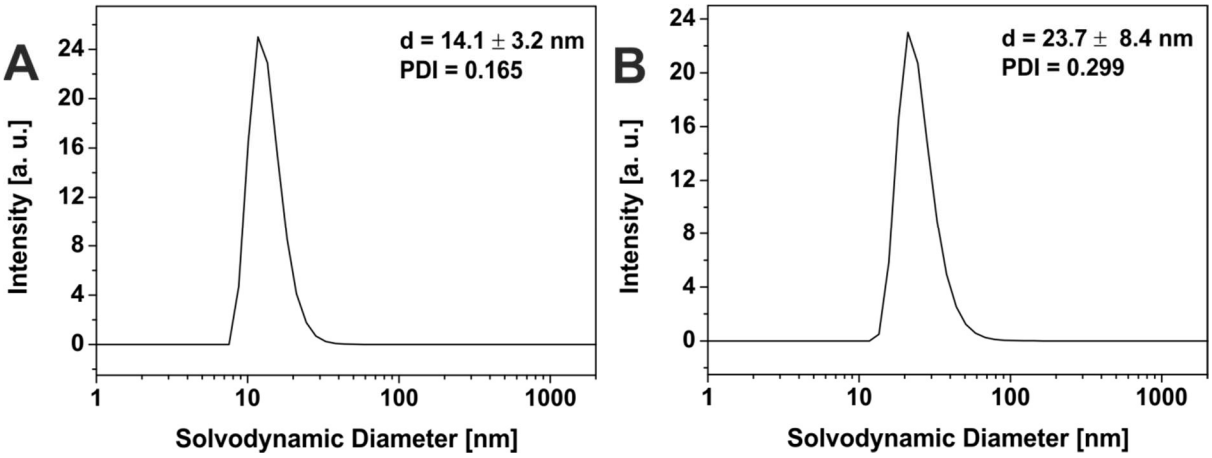


**Fig. S5.** DLS measurements of the $BF_4^-$-capped $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell UCNPs (**A**) and of the RB-lysine functionalised $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell nanoparticles (**B**) performed in DMF and DMSO, respectively. The curve shows the DLS signal weighted for number with a solvodynamic diameter of 14.1 ± 3.2 nm (**A**) and 23.7 ± 8.4 nm (**B**).

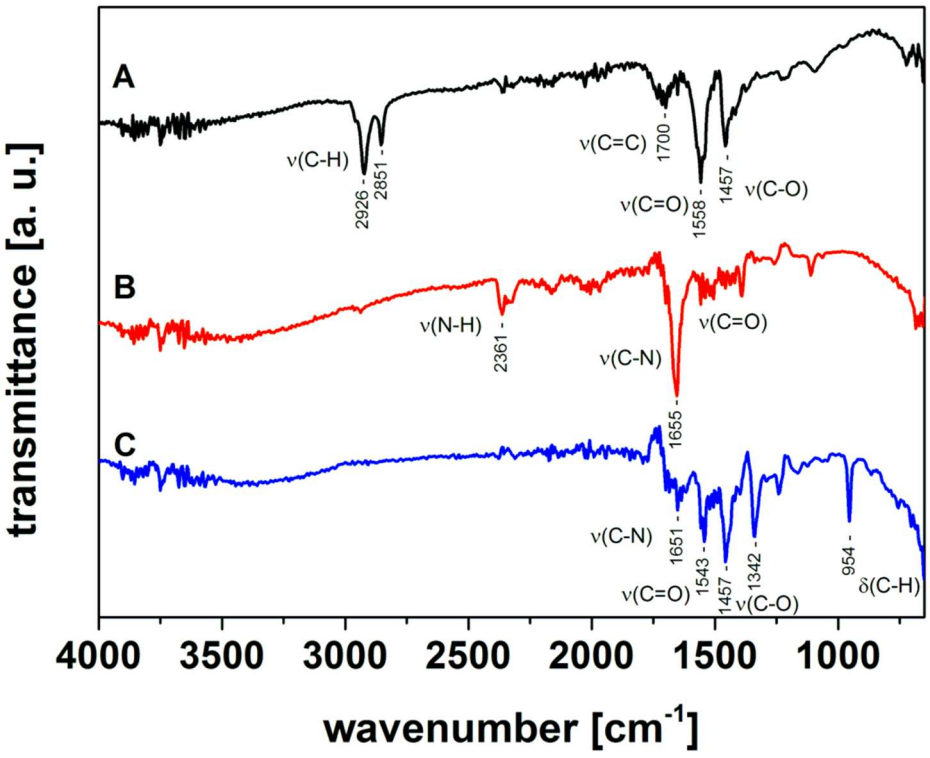


**Fig. S6.** FTIR spectra of: **A**) oleate-capped $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell UCNPs, **B**) l-lysine-capped $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell UCNPs and **C**) RB-lysine functionalised $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell UCNPs.

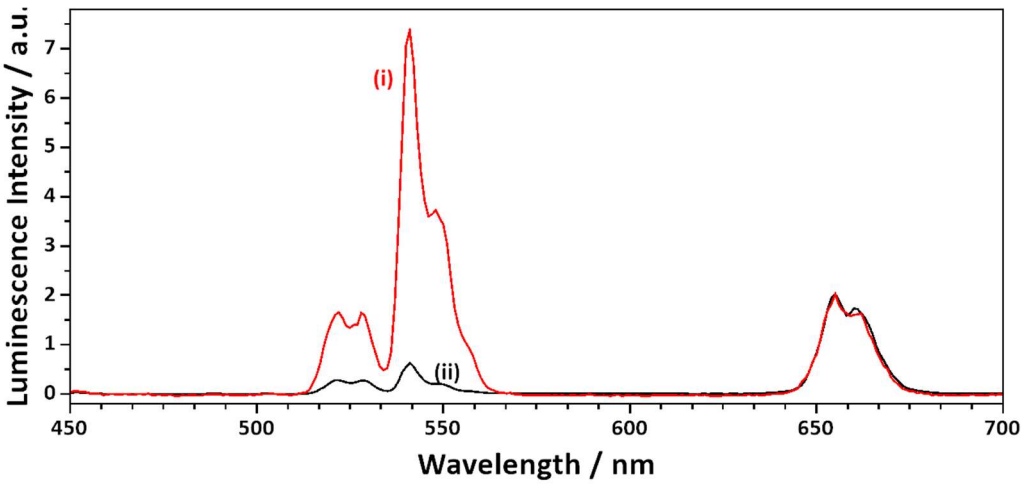


**Fig. S7.** Luminescence spectra of the **(i)** lysine functionalised $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell nanoparticles and **(ii)** RB-lysine functionalised $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell nanoparticles recorded in water following excitation at 980 nm (cw 200 mW). The emission of the photosensitiser RB is no longer visible in **(ii)** as compared to **Fig. 3b (i)**. The enlarged luminescent lifetime of RB after the energy transfer makes the excited state of the dye more susceptible to water quenching.

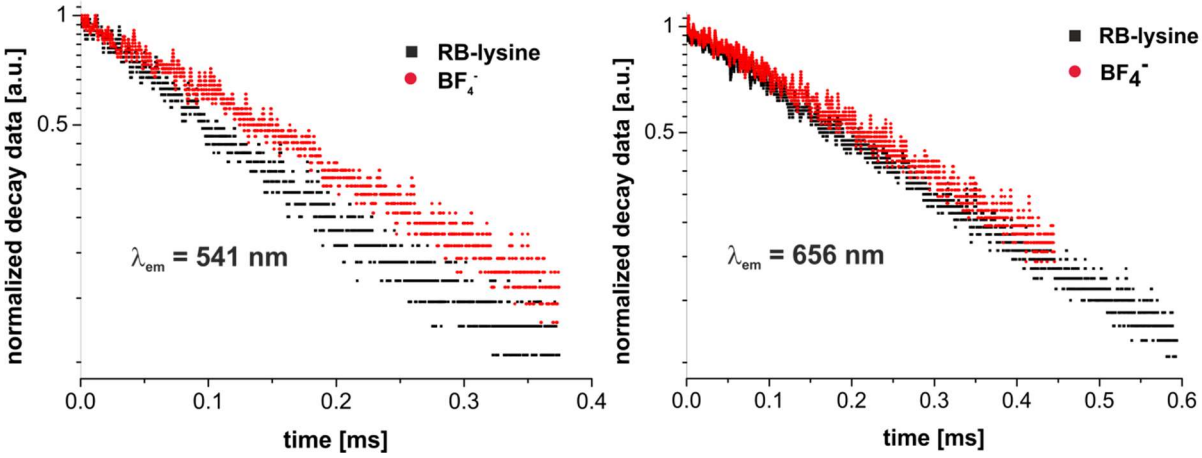


**Fig. S8.** Normalised decay data of UCNPs before ($BF_4^-$-capped $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell UCNPs) and after (RB-lysine functionalised $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell UCNPs) attachment of Rose Bengal (RB). The nanoparticles, dispersed in DMSO, were excited at 980 nm and the luminescence decay of the green (541 nm – graph on the left) and red (656 nm – graph on the right) upconversion emission was recorded. A reduction of the green upconversion luminescence lifetime was observed following binding the photosensitiser RB to the surface of the nanoparticles.

**Table S2.** Luminescence lifetimes of the green and red UCNP emission bands were measured before ($BF_4^-$-capped $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell UCNPs) and after (RB-lysine functionalised $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell UCNPs) the attachment of RB. The particles were dispersed in DMSO. (n = 3)

| | RB-lysine - UCNPs | $BF_4^-$ - UCNPS |
|---|---|---|
| $\tau_{Green}$ [µs] | 161 ± 5 | 215 ± 6 |
| $\tau_{Red}$ [µs] | 266 ± 3 | 342 ± 20 |

Calculation of FRET efficiency: $E = 1 - \frac{\tau_{DA}}{\tau_D}$ (1)

$\tau_{DA}$: lifetime of the donor in presence of the photosensitizer; $\tau_D$: lifetime of the donor without acceptor.

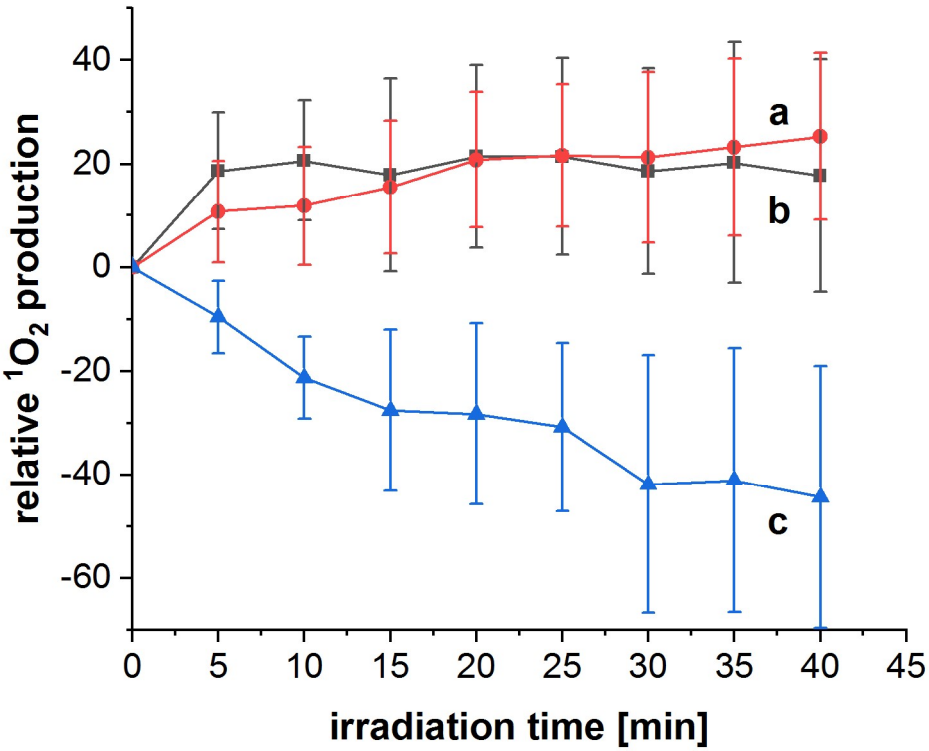


**Fig. S9.** Singlet oxygen ($^1O_2$) production by three different systems related to RB-lysine functionalised $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell UCNPs in water. The relative fluorescence intensity of ABMA at 409 nm following NIR light irradiation was calculated for: lysine capped $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell UCNPs with a) RB non-covalently linked to the nanoparticles in DMSO b) RB covalently linked to the nanoparticles in DMSO and c) RB non-covalently linked to the nanoparticles in water. As reference ($I_{ref}$) the systems with the lysine capped $NaYF_4$:Yb,Er,Gd@$NaYF_4$ core-shell UCNPs with RB covalently linked to the nanoparticles in water was used. The relative $^1O_2$ production was calculated with: $(I_{ref}-I)/(I_{ref})$. In all of the samples the amount of Rose Bengal molecules in solution or covalently attached to the particles was identical as verified by absorption measurements. (Particle concentration $\beta$ = 0.5 mg·mL$^{-1}$, N = 3.)

## 3. Control experiments with SK-BR-3 cells

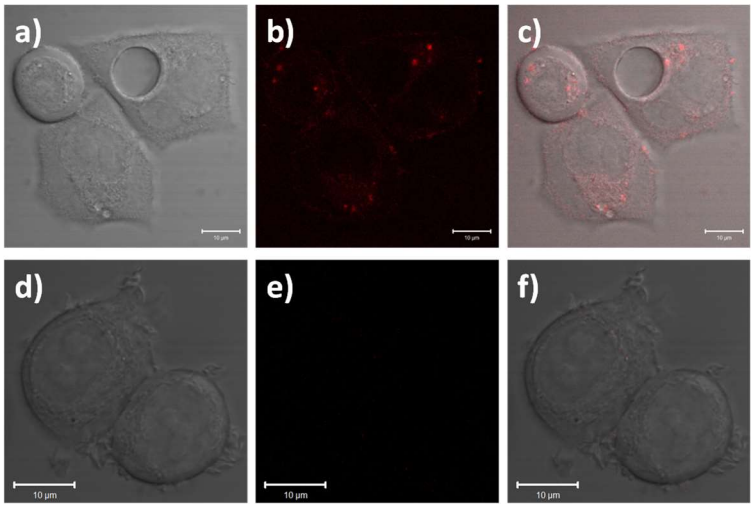


**Fig. S10.** Laser scanning confocal microscopy images of non-irradiated SK-BR-3 cells: **a** – **c**) incubated with the functionalised UCNPs (15 µg·mL$^{-1}$); and **d** – **f**) without functionalised UCNPs. The images were taken before treatment with propidium iodide and the fluorescence emission due to the RB on the UCNPs was collected in the red channel (560 – 615 nm; **b** and **e**) following excitation at 543 nm using a 100% laser power. DIC images were obtained using a 488 nm laser (**a** and **d**). Images c and f are composite images of the red and DIC channels. Scale bars are 10 µm.

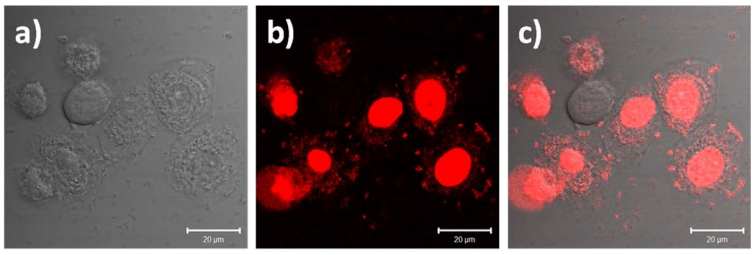


**Fig. S11.** Laser scanning confocal microscopy images of irradiated SK-BR-3 cells incubated with the functionalised UCNPs (15 µg·mL$^{-1}$) and treated with propidium iodide. **a)** DIC image obtained using a 488 nm laser; **b)** fluorescence emission due to the RB on the UCNPs and the propidium iodide (red channel, 560 – 615 nm) following excitation at 543 nm (laser power 4%); and **c)** composite image of the red and DIC channels. Scale bars are 20 µm.

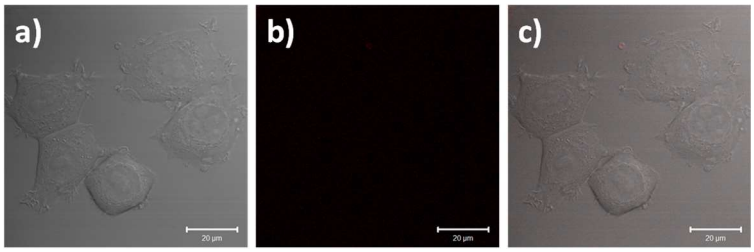


**Fig. S12.** Laser scanning confocal microscopy images of non-irradiated SK-BR-3 cells without functionalised UCNPs that had been treated with propidium iodide. **a)** DIC image obtained using a 488 nm laser; **b)** fluorescence emission (red channel, 560 – 615 nm) following excitation at 543 nm (laser power 4%) showing no fluorescence from neither the RB on the UCNPs nor the propidium iodide; and **c)** composite image of the red and DIC channels. Scale bars are 20 µm.

## 4. References

1. S. Wilhelm, M. Kaiser, C. Wurth, J. Heiland, C. Carrillo-Carrion, V. Muhr, O. S. Wolfbeis, W. J. Parak, U. Resch-Genger and T. Hirsch, Water dispersible upconverting nanoparticles: effects of surface modification on their luminescence and colloidal stability, *Nanoscale*, 2015, **7**, 1403-1410.