Near-Infrared Activation of an Anticancer Pt^{IV} Complex by Tm-Doped UpconversionNanoparticles

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Supporting Information

Experimental details

Materials

All chemical reagents, yttrium(III) acetatehydrate (99.9%), ytterbium(III) acetate tetrahydrate (99.9%), thulium(III) acetate hydrate (99.9%), 1-octadecene (technical grade, 90%), oleic acid (technical grade, 90%), sodium hydroxide (\geq 97%), ammonium fluoride (98%), potassium iodide (99%), potassium hydroxide (90%), silver nitrate (\geq 99%), sodium chloride (99%), succinic anhydride (\geq 99%), hydrogen peroxide solution (30 wt. % in H₂O), N,N'-dicyclohexyl carbodiimide (99%), N,N-Dimethylpyridin-4-amine (\geq 99%), and solvents were purchased from Sigma-Aldrich. The amphiphilic polymer DSPE-PEG(2000) amine, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethyleneglycol)-2000] (ammonium salt), was purchased from Avanti Polar Lipids, Inc. The precursor of the metal complex potassium tetrachloroplatinate(II) (99%) was purchased from Precious Metals Online PMO Pty Ltd. Nanopure water was obtained using the Barnstead Nanopure Ultrapure Water Purification System.

Synthesis of core-NaYF₄:Yb³⁺/Tm³⁺

The *core*-NaYF₄:Yb³⁺/Tm³⁺ (rare earth element ratio 69.5/30/0.5 mol%) synthesis was carried out following a slightly modified literature procedure (E. Ruggiero *et al.*, *Chem. Commum.* **2014**, *50*, 1715–1718).

In a typical synthesis (total rare earth amount, 3 mmol),yttrium(III) acetate hydrate (555 mg, 2.1 mmol), ytterbium(III) acetate tetrahydrate (380 mg, 0.90 mmol) and thulium(III) acetate hydrate (5.2 mg, 0.015 mmol) were dissolved in 1-octadecene (25 mL) and oleic acid (15 mL) in a 100 mL three-neck round-bottom flask with coil condenser. The suspension was heated up to 120 °C with a slow temperature ramp of 3.2 °C/min under stirring and vacuum. Once reaction mixture reached such temperature, it was kept in these conditions for 30 min in order to form a clear solution and eliminate residual water and oxygen. The system was then allowed to cool to 50 °C under a flow of nitrogen gas.

A solution of sodium hydroxide (300 mg, 7.5 mmol) and ammonium fluoride (444 mg, 12.0 mmol) in methanol (8 mL) was added to the reaction flask drop wisein 10 min. The subsequent cloudy solution was stirred for 30 min at 50°C and for 30 min at 70°C under nitrogen to evaporate the entire amount of methanol from the solution. Successively, the system was heated up to 300°C with a fast temperature ramp of 13.5 °C/min under stirring and nitrogen and maintained in such conditions for 90 min. The reaction mixture progressively changed from turbid to yellow and transparent.

Next, the flask was left cooling to room temperature, and nanoparticles were purified by centrifugation (4500 rpm at 20 °C for 15 min) to remove any excess of reagents and solvents. The white pellet was washed once with of ethanol (40 mL) and once with THF/ethanol (5/35 mL) and recollected by centrifugation. Upconversion nanoparticles were dried at room

temperature overnight. Typically, ca. 600 mg of $NaYF_4$: Yb^{3+}/Tm^{3+} nanoparticles are obtained employing the reaction conditions described.

Synthesis of core-shell NaYF₄:Yb³⁺/Tm³⁺@NaYF₄

Synthesis of core-shell NaYF₄:Yb³⁺/Tm³⁺@NaYF₄ nanoparticles was realized by employing a slightly modified version of the procedure published by J-C. Boyer *et al.* (*J. Am. Chem. Soc.* **2010**, *132*, 15766–15772). The previously-obtained NaYF₄:Yb³⁺/Tm³⁺nanoparticles were used as seeds to grow aNaYF₄ shell.

In a typical synthesis (total rare earth amount, 0.9 mmol), yttrium (III) acetate hydrate (240 mg, 0.9 mmol) was dissolved in 1-octadecene (15 mL) and oleic acid (6 mL) in a 100 mL three-neck round-bottom flask with coil condenser.

As for core nanoparticles, the suspension was heated up to 120 °C with a slow temperature ramp of 3.2 °C/min under constant stirring and vacuum, and maintained in such conditions for 30 min. Afterwards, the solution was cooled down to 80 °C and placed under a flow of nitrogen gas.

In the same time, a suspension of NaYF₄:Yb³⁺/Tm³⁺ (260 mg) in hexane (10 mL) was prepared via sonication and added to the reaction flask drop by drop (10 min). The resulting turbid solution was heated up to 110 °C (3.2 °C/min) under vacuum to eliminate the hexane. After 30 min the solution was again cooled down to 50 °C and a methanolic (5 mL) solution of sodium hydroxide (88 mg, 2.2 mmol) and ammonium fluoride (130 mg, 3.5 mmol) was added to the system drop wise over 5 min. The flask was maintained for 30 min at 50 °C and for 30 min at 70 °C under nitrogen to evaporate the entire amount of methanol from the reaction mixture. Afterwards, the reaction flask was heated up at 300 °C with a fast temperature ramp of 13.5 °C/min and heating was kept for 90 min. During the nucleation process the solution switches from cloudy to slightly yellow and transparent.

The solution was then cooled down to room temperature, and formation of a white precipitated is observed on the bottom of the flask. The so-obtained core-shell nanoparticles were purified by centrifugation (4500 rpm at 20 °C for 15 min). The pellet was washed with ethanol and THF and dried overnight. Typically, ca.500 mg of core-shell NaYF₄:Yb³⁺/Tm³⁺@NaYF₄ NPs are obtained.

Synthesis of cis, cis, trans-[Pt(NH₃)₂(Cl)₂(O₂CCH₂CH₂CO₂H)₂] (1)

The Pt prodrug was synthesized by complying the procedure described by M. Reithofer *et al.* (*Eur. J. Inorg. Chem.* **2006**, 2612–2617).

Synthesis of DSPE-PEG-Pt^{IV}(2)

The amphiphilic polymer 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethyleneglycol)-2000] (ammonium salt, DSPE-PEG(2000) amine) was functionalized with **1** using the protocol reported by J. Hernández-Gil, J. C. Mareque-Rivas *et al.* (Submitted).

Briefly, $cis, cis, trans - [Pt(NH_3)_2(Cl)_2(O_2CCH_2CH_2CO_2H)_2]$ (1) (9.6 mg, 18.0 µmol), dicyclohexyl carbodiimide (3.7 mg, 18.0 µmol) and 4-(dimethylamino)pyridine (1.0 mg, 7.2 umol) were dissolved in DMSO (130 uL). After 10 minutes, the solution containing the activated Pt^{IV} complex was added to a DMSO solution of DSPE-PEG(2000)-NH₂ (10 mg, 3.6 µmol, 170 µL). The resulting mixture was allowed to react at room temperature for 72 h under continuous stirring. Water was then added to the reaction mixture. The solution was centrifuged and the supernatant was passed through a 0.45 µm filter to remove insoluble traces. Then, the supernatant was dialyzed in a Slide-A-Lyzer Dialysis Cassete (Thermo Scientific) (MW cutoff of 2,000) against water (3 x 500 mL). The dyalizate, containing the pure product was lyophilized and the residue dried in vacuo over P₂O₅ (10.86 mg, 90%). ¹H NMR (500 MHz, CDCl₃) for **2**: δ 0.88 (t, CH₃, 6H), 1.25 (s, CH₂, 56H), 1.61 (m, CH₂CH₂CO, 4H), 2.32 (m, CH₂CH₂CO, 4H), 2.60 (m, CH₂-succinic, 8H), 3.44 (m, CH₂CH₂N, 2H), 3.64 (s, PEG ~ 180 H), 3.96 (m, CH₂CH₂NH, 2H), 4.22 (m, trans-PO₄CH₂CH, 1H), 4.32 (m, COOCH₂COH, 2H), 4.40 (m, cis-PO₄CH₂CH, 1H), 5.26 (m, PO₄CH₂CH, 1H), 6.25 (bs, NH₃, 6H), 6.68 (bs, NHCOCH₂), 6.82 (bs, NHCOOCH₂). UPLC/MS $\tau_r = 11.38$ min; m/z for $[C1_{40}H_{289}N_7O_{61}PPtCl_2]^{3+}$ expected = 1114.28, found = 1114.17 (ESI+).

Synthesis of NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@2

Nanoparticle surface functionalization with DSPE-PEG-Pt^{IV}(2) was obtained following previous work by some of us with few adjustments (Gomez Blanco et al., Chem. Commum. **2012**, 48, 4211–4213). NaYF₄:Yb³⁺/Tm³⁺@NaYF₄ nanocrystals (12 mg) and **2** (4 mg) were solubilised in a glass flask containing 6 ml of CHCl₃. The solvent was left to evaporate overnight at room temperature under stirring. The flask was placed into an water bath at 80 °C for 30 s, then the nanoparticles were solubilised with 1.5 mL of H₂O obtaining a pearly solution. The solution was purified from the excess of polymer by centrifugation (8000 rpm at room temperature for 5 min), and the resulting white nanoparticle pellet was resuspended with 1.5 mL of H₂O. Later. the product was freeze-dried overnight and 8 mg of NaYF₄:Yb³⁺/Tm³⁺(a)NaYF₄(a)**2** NPs were collected.

Synthesis of NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@DSPE-PEG(2000)-NH₂

The same method illustrated above was used to prepareNaYF₄:Yb³⁺/Tm³⁺@NaYF₄@DSPE-PEG(2000)-NH₂ nanomaterials.

Instrumentation

Transmission Electron Microscopy (TEM)

TEM was performed on a JEOL JEM-1400 PLUS-HC microscope operating at 120 kV. The TEM samples were prepared by dropping 2 μ L sample solutions (0.1-1 mg/mL in THF or in water) onto a 400-mesh carbon coated copper grid (3 mm in diameter) followed by the evaporation of the solvent under vacuum. The nanoparticle sizes were estimated from over 150 nanoparticles.

Fourier Transform Infrared (FTIR)

FTIR spectra of oleic acid, $NaYF_4:Yb^{3+}/Tm^{3+}$, $NaYF_4:Yb^{3+}/Tm^{3+}@NaYF_4$, $NaYF_4:Yb^{3+}/Tm^{3+}@NaYF_4@2$ and 2 were recorded on a Nicolet FTIR 6700 spectrometer as KBr pellet.

X-Ray Photoelectron Spectroscopy (XPS)

XPS experiments were performed in a SPECS Sage HR 100 spectrometer with a nonmonochromatic X-ray source Magnesium K α line of 1253.6eV energy and a power applied of 250 W and calibrated using the 3d_{5/2} line of Ag with a full width at half maximum (FWHM) of 1.1 eV. All measurements were made in an ultra high vacuum (UHV) chamber at a pressure below $8 \cdot 10^{-8}$ mbar. Samples were measured on titanium surfaces.

Nuclear Magnetic Resonance (NMR)

¹H NMR and ¹H-¹H COSY 2D NMR spectra of the various samples were recorded in PBS/D₂O buffer solution (10 mM, pH = 7.3) on an AVANCE III Bruker 500 NMR spectrometer. Chemical shifts were reported in parts-per-million (δ , ppm) and referenced to the residual solvent peak.

UV-Vis absorption spectroscopy (UV-vis)

UV-visible-NIR absorption spectra of **1** and NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@**2** were acquired in aqueous solution using a Varian Cary 5000 spectrophotometer.

Dynamic Light Scattering

The hydrodynamic radius of NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@**2** was obtained measuring an aqueous solution containing the nanoparticles (0.5 mg/mL) with 173° scattering angle at 25 °C using a NanoSizer Malvern Nano-Zs.

Emission spectroscopy

Upconverted emission spectra of $NaYF_4:Yb^{3+}/Tm^{3+}$, $NaYF_4:Yb^{3+}/Tm^{3+}@NaYF_4$, $NaYF_4:Yb^{3+}/Tm^{3+}@NaYF_4@DSPE-PEG(2000)-NH_2$ and $NaYF_4:Yb^{3+}/Tm^{3+}@NaYF_4@2$ were

collected in THF or aqueous solution on a Fluorometer Fluorolog-TSPC (Horiba-Jovine Ivone) coupled with a 980 nm laser diode (CNI, MDL-N-980).

Photolysis experiments

Inside a 1 mL glass vial PBS/D₂O buffer solutions (10 mM, pH = 7.3) of **1** and **2** were irradiated with UVA (λ_{exc} = 385 nm) and NIR light (λ_{exc} = 980 nm) in the presence or absence of UCNPs. A Prizmatix LED Multi-Wavelength MWLLS-11 and a BWT diode laser DS3-11312-110 were employed for UVA and NIR irradiation experiments respectively.

At 385 nm, solutions of **1** (150 μ M) and **2** (150 μ M) were irradiated with a power density of 40 mW cm⁻² and formation of photoproducts was monitored by ¹H NMR.

In the case of UCNP-mediated photolysis, a power density of 4.9 and 7.3 W cm⁻² was employed for 980-nm excitation of **1** (150 μ M) with NaYF₄:Yb³⁺/Tm³⁺@NaYF₄ (5 mg), and for NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@**2** (5 mg) respectively. After irradiation, the nanoparticle solutions were centrifuged (5000 rpm, 5 min) and the supernatants were analysed by ¹H NMR. After each measurement, supernatants were combined again with the pellet, obtaining the initial solution used in the photoreaction. Such procedure was employed to improve the quality of NMR spectra. Control experiments consisted of exciting directly (no UCNPs) at 980 nm **1** (150 μ M) and **2** (150 μ M) in buffer using a power density of 6.5 and 8.8 W cm⁻² respectively.

Results



Figure S1 Size distribution histograms of (A) $NaYF_4:Yb^{3+}/Tm^{3+}$ and (B) $NaYF_4:Yb^{3+}/Tm^{3+}@NaYF_4$ nanoparticles. Average diameter of $NaYF_4:Yb^{3+}/Tm^{3+}$ is 30 ± 2 nm. Average diameter of $NaYF_4:Yb^{3+}/Tm^{3+}@NaYF_4$ is 37 ± 3 nm.



Figure S2 FT-IR spectra of (black) pure oleic acid, (light blue) $NaYF_4:Yb^{3+}/Tm^{3+}$, (blue) $NaYF_4:Yb^{3+}/Tm^{3+}@NaYF_4$, (green) $NaYF_4:Yb^{3+}/Tm^{3+}@NaYF_4@2$, (red) 2.



Figure S3 XPS spectra of (black) $NaYF_4:Yb^{3+}/Tm^{3+}and$ (red) $NaYF_4:Yb^{3+}/Tm^{3+}@NaYF_4$ nanoparticles confirming the presence of C, O, F, Na, Y, Tm and Yb as assigned by core level and Auger electrons.



Figure S4 Upconversion luminescence spectrum of NaYF₄:Yb³⁺/Tm³⁺ (0.5 mg·mL⁻¹ in THF) upon 980 nm excitation.



Figure S5 Upconversion luminescence spectra of (top) NaYF₄:Yb³⁺/Tm³⁺@NaYF₄ (0.5 mg·mL⁻¹ in THF) and (bottom) its magnification upon 980 nm excitation at different powers (3.4–20.6 W cm⁻²).



Figure S6 Power dependence of the emission at 360 nm of $NaYF_4:Yb^{3+}/Tm^{3+}@NaYF_4$ upon NIR irradiation.



Figure S7 Power dependence of the emission at 450 nm of $NaYF_4:Yb^{3+}/Tm^{3+}@NaYF_4$ upon NIR irradiation.



Figure S8 Power dependence of the emission at 475 nm of $NaYF_4:Yb^{3+}/Tm^{3+}@NaYF_4$ upon NIR irradiation.



Figure S9 ¹H NMR spectra of **1** in PBS/D₂O buffer (10 mM, pH = 7.3) before (bottom) and after (top) irradiation at 980 nm light for 7 hours (6.5 W cm⁻²).



Figure S10 ¹H NMR spectra of **1** in PBS/D₂O buffer (10 mM, pH = 7.3) before (bottom) and after (top) it was exposed to 385 nm light for 15 min (40 mW cm⁻²).



Figure S11 Pt 4f XPS spectra of (A) **1** (150 μ M) without irradiation, (B) after 7h of irradiation at 980 nm (6.5 W cm⁻²), (C) after 15 min of irradiation at 385 nm (40 mW cm⁻²). Based on fit peak areas, the ratio Pt^{II}:Pt^{IV} in the spectra are (A) 0:100, (B) 0:100 and (C) 56:44.



Figure S12 Pt 4f XPS spectra for pellet of (A) **1** (150 μ M) with NaYF₄:Yb³⁺/Tm³⁺@NaYF₄ (5 mg) after 6 h NIR irradiation at 4.9 W cm⁻² and (B) NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@**2** (5 mg) after 3.5 h NIR irradiation at 7.3W cm⁻². Both spectra do not present Pt in the pellet.



Figure S13 TEM images of NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@2 nanoparticles (1 mg·mL⁻¹ in H₂O).



Figure S14 XPS spectrum of NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@**2** with assignment for C, O, F, Na, Y, Tm and Yb by core level and Auger electrons. Inset: zoomed spectrum in the 69–82 eV energy range confirming the presence of Pt^{IV} on the surface of NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@**2**.



Figure S15 UV-visible-NIR absorption spectrum of $NaYF_4$: Yb³⁺/Tm³⁺@NaYF₄@2 (0.5 mg·mL⁻¹ in H₂O).



Figure S16 Upconversion luminescence spectra of (top) $NaYF_4:Yb^{3+}/Tm^{3+}@NaYF_4@2$ and (bottom) $NaYF_4:Yb^{3+}/Tm^{3+}@NaYF_4@DSPE-PEG(2000)-NH_2$, (0.5 mg·mL⁻¹ in H₂O) upon 980 nm excitation at different powers (0.4–2.4 W cm⁻²).



Figure S17 Histogram illustrating hydrodynamic size distributions of NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@**2** (0.5 mg·mL⁻¹ in H₂O) measured by Z-sizer. The hydrodynamic radius was determined to be equal to 157 ± 32 nm.



Figure S18 ¹H NMR spectra of **2** in PBS/D₂O buffer (10 mM, pH = 7.3) before (bottom) and after (top) irradiation at 385 nm light for 10 min (40 mW cm⁻²).



Figure S19 ¹H-¹H COSY 2D NMR spectrum of **2** in PBS/D₂O buffer (10 mM, pH = 7.3) after irradiation at 385 nm light for 10 min (40 mW cm⁻²).



Figure S20 ¹H NMR spectra of **2** in PBS/D₂O buffer (10 mM, pH = 7.3) before (bottom) and after (top) after irradiation at 980 nm light for 5 h (8.8 W cm⁻²).



Figure S21 Pt 4f XPS spectra of **2** (150 μ M) (A) without irradiation, (B) after 5 h of irradiation at 980 nm (8.8 W cm⁻²), (C) after 10 min of irradiation at 385 nm (40 mW cm⁻²). Based on fit peak areas, the ratio Pt^{II}:Pt^{IV} in the spectra are (A) 0:100, (B) 0:100 and (C) 55:45.