Supporting information

Water-Dispersible Dye-Sensitized Upconversion Nanocomposite Modified with Phosphatidylcholine for Lymphatic Imaging

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Experimental Procedures

Matetials. All reagents and chemicals were purchased from commercial sources and used as received. Oleic acid (OA 90%), 1-octadecane (ODE 90%), 2,3,3-trimethylindolenine, 1,3-propane sultone and phosphatidylcholine (PC) were obtained from Sigma-Aldrich. Rare-earth oxides RE_2O_3 (99.999%) ($RE^{3+}=Yb^{3+}$, Y^{3+} , Er^{3+} , and Nd^{3+}) were purchased from Shanghai Yuelong New materials Co. Ltd. Methanol, ethanol, toluene and diethyl ether were obtained from Alfa Aesar Ltd. RECl₃ were prepared by dissolving the corresponding oxides in hydrochloric acid at elevated temperature then removed the solvent.

Characterization. ¹H NMR spectra were recorded on a Brucker spectrometer at 400 MHz. All chemical shifts are reported in the standard δ notation of parts per million. Electrospray ionization mass spectra (ESI–MS) were measured on a Micromass LCTTM system. The size and morphology of UCNPs were determined at 200 KV using a JEOL JEM-2010F low to high resolution transmission electron microscope (TEM). The as-prepared samples were dispersed in cyclohexane and dropped on the surface of a copper grid. Powder X-ray diffraction (XRD) measurements were performed on a Bruker D4 diffractometer at a scanning rate of 1°/min in the 20 range from 10 to 90° (Cu K α radiation, λ =1.54056 Å). FT-IR spectra were measured using an IR Prestige-21 spectrometer (Shimadzu) from samples in KBr pellets. UV-vis absorption spectra were recorded on a Shimadzu 3000 spectrophotometer. UCL emission spectra were measured on an Edinburgh FL-900 luminescence spectrometer with an external 0-1.5 W adjustable CW semiconductor laser (Shanghai Hi-Tech Optoelectronic Co., China).

Synthetic procedure to NIR dye Cy7



Scheme S1. Synthetic route of the NIR dye Cy7

Synthesis of compound 1. 2,3,3-trimethylindolenine (6.3 g, 62 mmol) and 1,3-propane sultone (8.2 mL, 94 mmol) were dissolved in toluene (50 mL), and the solution was heated under reflux for 18h. The reaction mixture was allowed to cool to room temperature and the resulting pink crystals were filtered and washed with acetone. The filtered product was recrystallized from a solution of MeOH and Et₂O. The crystals were collected and dried under vacuum.

Compound 2. A solution of POCl₃ (37 mL, 397 mmol) in DCM (35 mL) was slowly added to an ice-cooled solution of DMF (40 mL, 516 mmol) in DCM (40 mL). After the addition was finished, cyclohexanone (10 g, 100 mmol) was added in via syringe. The resulted reaction mixture was refluxed for 2 h. The mixture was then cooled in ice. Water (200 mL), pre-cooled to 0 °C was added slowly while the mixture was stirred. Then the mixture was stirred for 30 min. DCM layer was collected and the water layer was extracted with additional DCM. The DCM solutions were combined, passed through the $MgSO_4$ columm, concentrated on a rotavapor and treated with pentane (200 mL) to give compound 2 as yellow crystalline solid to reserve in the cool temperature.

Compound Cy7. Into a flask attached with Dean-Stark trap and a condenser were added compound 1 (7.9 g, 20 mmol), freshly prepared compound 2 (1.7 g, 10 mmol), n-butanol (200 mL) and benzene (20 mL). The mixture was heated to 120 oC for 24 h, resulting in a green solution. Solvents were removed on a rotavapor. ¹H NMR (300 MHz, MeOD) δ 8.44 (d, J = 14.1 Hz, 2H), 7.52 (d, J = 7.4 Hz, 2H), 7.48 – 7.39 (m, 4H), 7.33 – 7.23 (m, 2H), 6.46 (d, J = 14.1 Hz, 2H), 4.49 – 4.31 (m, 4H), 2.98 (t, J = 6.8 Hz, 4H), 2.77 (t, J = 5.9 Hz 4H), 2.34 – 2.19 (m, 8H), 1.93 (dd, J = 11.0, 4.5 Hz 2H), 1.73 (s, 12H). MS-ESI (M)⁺ m/z calcd for C₃₆H₄₂ClN₂O₆S₂: 699.3, found 699.3.

Synthesis of oleic acid coated NaYF₄:Yb,Nd,Er@NaYF₄:Nd (denoted as OA-CS:Nd). NaYF₄:Yb,Nd,Er core nanoparticles were prepared with a solvothermal method.^[1] Typically, 1 mmol RECl₃ (RE³⁺= Y³⁺, Yb³⁺, Nd³⁺, and Er³⁺) with the molar ratio of 68.5:30:1:0.5 was mixed with 6 mL oleic acid (OA) and 15 mL octadecene (ODE). The mixture was heated to 140 °C for 30 min to obtain a pellucid solution and then cooled down to room temperature. Subsequently, 8 mL methanol solution containing NaOH (2.5 mmol) and NH₄F (4 mmol) was slowly dropped into the flask. After being stirred at 100 °C for 30 min to remove methanol, the solution heated to 300 °C and maintained for 1 h under Ar protection. When reaction was completed, excess ethanol was added and the products were collected by centrifugation. OA-CS:Nd core-shell nanoparticles were synthesized through epitaxial growth. YCl₃ (0.80 mmol), NdCl₃ (0.20 mmol) were added into 6 mL OA and 15 mL ODE and heated to 140 °C to obtain a clear solution, and then cooled down to room temperature. 5 mL cyclohexane solution of the assynthesized NaYF₄:Yb,Nd,Er core nanoparticles was added dropwise into the solution along with a 8 mL methanol solution of NaOH (2.5 mmol) and NH₄F (4 mmol). After being stirred at 100 °C for 20 min to remove methanol, then the solution was heated to 300 °C under Ar protection for 1 h. After being cooled to room temperature, nanoparticles were precipitated from the solution with ethanol, collected by centrifugation, washed with ethanol and cyclohexane several times. Finally, redispersed in cyclohexane.

Assembly of OA-CS:Nd, PC and Cy7 (denoted as CS:Nd-Cy7@PC). The OA-CS:Nd (10 mg) was dispersed in the 5 mL CH₂Cl₂ by ultrasonication, and then the NIR dye was added, the mixture was stirred at room temperature to obtain a homogeneous phase. Furthermore, the amphiphilic phosphatidylcholine (10 mg) was added, and then the mixture was stirred overnight at room temperature. The mixture was centrifugated, the collected solid was repeatedly washed with water. The precipitate could be redispersed in deionized water.

Cell Culture. Human cervical carcinoma HeLa cells were provided by the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences (CAS). The HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % Fetal Bovine Serum (FBS) and 1% antibiotic/antimycotic solution (penicillin and streptomycin, Invitrogen). All cells were cultured at 37 °C under 5% CO₂.

Cytotocicity of CS:Nd-Cy7@PC.^[2] *In vitro* cytotoxicity was measured by performing methyl thiazolyl tetrazolium (MTT) assay. HeLa cells were cultivated in 96-well plates containing DMEM at 37 °C and 5% CO_2 for 12 h. Subsequently, the medium was next replace by fresh medium containing different mass concentrations of CS:Nd-Cy7@PC (0, 100, 200, 300,

400, 600 and 800 μ g/mL). After 24 h, MTT (10 μ L; 5 mg/mL) was added to each well and the plate was incubated for an additional 4 h at 37 °C under 5% CO₂. After the addition of 100 μ L DMSO, the assay plate was allowed to stand at room temperature for 2 h. A Tecan Infinite M200 monochromator-based multifunction microplate reader was used to measure the optical density OD 570 value (Abs) of each well with background subtraction at 690 nm. The following formula was used to calculate the inhibition of cell growth:

cell viability(%) = (mean Abs value of treatment group/mean Abs value of control) $\times 100\%$

LSUCLM imaging. HeLa cells were washed with PBS buffer for three times, and then the cells were incubated with 300 µg/mL CS:Nd-Cy7@PC and CS:Nd@PC in PBS for 2 h at 37 °C, respectively. Cell imaging was then carried out after washing the cells with PBS. LSUCLM imaging was performed with an OLYMPUS FV1000 scanning unit. Cells loaded with CS:Nd-Cy7@PC and CS:Nd@PC were excited by a CW laser at 808 nm (Connet Fiber Optics, China). UCL emission was collected at 500-560 nm.

Upconversion luminescence imaging *in vivo*.^[3] Animal procedures were in agreement with the guidelines of the institutional Animal Care and Use Committee. *In vivo* UCL imaging was performed with a modified upconversion luminescence imaging system designed by our group. External 0-3.5 W adjustable CW 808 nm semiconductor laser. A cooled electron-multiplying charge-coupled device (EMCCD, Andor DU897) was used as the signal collector. Imaged of luminescence signals were analyzed with Kodak Molecular Imaging Software. UCL signals were collected at 540 nm \pm 12 nm.



Figure S1. TEM (a) and HR-TEM (b) images of NaYF4:30%Yb,1%Nd,0.5%Er (core). TEM (c) and HR-TEM (d) images of NaYF4:30%Yb,1%Nd,0.5%Er@ NaYF4:20%Nd (CS:Nd), and (inset) EDS line-scan profile of a single particle. (e) X-ray diffraction pattern of core and CS:Nd. The standard pattern of β -NaYF4 (JCPDS no. 16-0334) is also shown. (f). UCL emission spectra of core and CS:Nd under 808 nm irradiation.



Figure S2 The EDXA of NaYF₄:Yb,Nd,Er (a) and NaYF₄:Yb,Nd,Er@ NaYF₄:Nd (b).



Figure S3. ESI-MS spectra of Cy7



Figure S4 ¹H NMR spectra of Cy7 in the MeoD



Figure S5. (a) Absorption spectra of pure Cy7 dye and Cy7-attached CS:Nd core-shell upconverting nanoparticles. (b) A comparison of luminescence spectra of Cy7 in a free pure dye solution and in a dye-attached CS:Nd upconverting nanoparticle solution under laser excitation at 785 nm.



Figure S6. (a) Absorption spectra of NaYF₄:30%Yb,1%Nd,0.5%Er@ NaYF₄:20%Nd with varied concentration of Cy7 dye for sensitization and the absorption at 790 nm as a function of Cy7 dye concentration (inset). (b) Fluorescence emission spectra of NaYF₄:30%Yb,1%Nd,0.5%Er@ NaYF₄:20%Nd with varied concentration of Cy7 dye for sensitization and fluorescence emission at 800 nm as a function of Cy7 dye concentration (inset)



Figure S7. Fluorescence decay of the Cy7 dye in the absence and the presence of an CS:Nd. The lifetime of Cy7 fluorescence was shortened from 1.07 ns to 0.41 ns.



Figure S8. The relative up-conversion quantum yield of CS:Nd nanoparticles with and without Cy7 dye versus the excitation power density.



Figure S9. (a, b) Absorption and photoluminescence of Cy7 in EtOH, DMSO, DMF, and H2O (Concentrations are all 5 μ M). UCL spectra of (e) Cy7-attached CS:Nd and (f) free CS:Nd dispersed in EtOH with varied volume of water and the intensities of the band at 540 nm as a function of H₂O/EtOH ratio.

Table S1. The relative quantum efficiency^[a] of Cy7 in different solvents and of Cy7@PC in Water

Sample		C	Cy7@PC	ICG (Ref)		
Solvent	EtOH	DMSO	DMF	H ₂ O	H ₂ O	DMSO
QY	15.2%	13.4%	16.5%	4.2%	8.0%	13%

[a] Fluorescence standard: indocyanine green in dimethylsulfoxide (DMSO); Φ_{fl} =10.6% ^[4]



Figure S10. Absorption spectra and fluorescence emission of free Cy7 dye (a, b) and Cy7 dye associated with CS:Nd. (c, d) in 2mL EtOH upon gradual addition of water. (e, f) Absorption intensities at 790 nm and fluorescence emission intensities band at 800 nm versus the volume of water.



Figure S11. (a) Absorption spectra of dye in the supernatant of Cy7-attached CS:Nd core-shell upconverting nanoparticles after centrifuging when introducing varied volumes of water into the Cy7-attached CS:Nd dispersed in 2 mL EtOH.



Figure S12. TEM and HR-TEM images of CS:Nd-Cy7@PC



Figure S13. FTIR spectra of OA-CS:Nd, PC, Cy7, and CS:Nd-Cy7@PC.



Figure S14. (a) The absorption and (b) fluorescence spectrum of Cy7 and Cy7@PC in aqueous phase.

Samples	UCNPs	РС	Dye	Loaded Dye	Load rate
	(mg)	(mg)	(10 ⁻⁶ mmol)	(10 ⁻⁶ mmol)	
a	10	10	20	18	90%
b	10	10	40	37	92%
c	10	10	60	51	85%
d	10	10	80	70	87%
e	10	10	100	79	79%
f	10	10	120	95	79%
g	10	10	150	115	76%
h	10	10	200	125	63%

Table S2. The amount of loaded Cy7 in different samples



Figure S15 The concentration of Cy7 loaded in the CS:Nd-Cy7@PC was calculated according to the unloaded dye measured by the absorption spectroscopy technique. (a) Absorption spectra of the Cy7 with different concentrations of $0.1-5.0 \mu$ M in EtOH/H₂O (v/v, 1:1, dash line) and the absorption of unloaded dye in different samples after diluted 16 times (solid line). (b) the absorbance at 785 nm as a function of Cy7 concentration.



Figure S16. Upconversion emission spectra of CS:Nd@PC and CS:Nd-Cy7@PC in aqueous phase under irradiation at 785 nm (a) and 760 nm (b), respectively.



Figure S17 Evaluation of the stabilities of CS:Nd-Cy7@PC by changes in absorption (a) and UCL intensities (b) for different time.



Figure S18. In vitro cell viability of Hela cells incubated with CS:Nd-Cy7@PC and CS:Nd@PC at different concentration for 24 h, respectively.



Figure S19. Luminescence lymphatic imaging at 30 min post injection of (a) CS:Nd-Cy7@PC under irradiation at 785 nm; (b) CS:Nd @PC excited at 785 nm; (c) CS:Nd-Cy7@PC under irradiation at 760 nm; (d) CS:Nd @PC excited at 760 nm. (λ_{UCL} = 540 ± 12 nm).

References

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