Supporting Information:

Aptamer-based, multifunctional ligand-modified UCNPs for targeted PDT and bioimaging

Weijia Hou,[a] † Yuan Liu,[ab] † Ying Jiang,[ab] Yuan Wu,[ab] Cheng Cui,[a] Yanyue Wang,[a] Liqin Zhang,[a] I-Ting Teng,[a] and Weihong Tan*[ab]

a. Department of Chemistry and Physiology and Functional Genomics, Center for Research at the Bio/Nano Interface, Health Cancer Center, University of Florida, Gainesville, FL 32611-7200, USA
b. Molecular Science and Biomedicine Laboratory, State Key Laboratory of Chemo/Bio-Sensing and Chemometrics, College of Biology and College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, China
† These authors contributed equally to this work.

Experimental section

1 Materials

All reagents used were purchased from Sigma-Aldrich, unless otherwise stated. HS-PEG-FITC was purchased from Nanocs, Inc. All the DNA were synthesized by automated DNA synthesizer ABI4300 (Applied Biosystems, Foster City, CA). DNA synthesis reagents were acquired from Glen Research Corp. and ChemGenes Corp. All oligonucleotides were synthesized and purified in our group. The water used was purified on a Milli-Q Biocell System. All cells were obtained from ATCC (Manassas, VA).

2 Synthesis of oleic acid-capped $NaYF_4$ (Yb 30%, Er 2%) UCNPs

$Y(CH_3CO_2)_3$ (0.68 mmole), $Yb(CH_3COO)_3$ (0.3 mmole) and $Er(CH_3COO)_3$ (0.02 mmole) were added to a mixture of oleic acid (6 mL) and 1-octadecene (15 mL) in a 100 mL round-bottom flask. The solution was heated to 150 ℃ and vigorously stirred for 30 min. After cooling to 50 ℃, NH_4F (4 mmole) and NaOH (2.5 mmole) dissolved in 20 mL CH_3OH were injected into the flask drop-by-drop. The mixture was kept at 50 ℃ for 30 min before heating up to 145 ℃ and

application of argon flow to remove methanol. The reactor was further degassed for 15 min and then kept at 305 ℃ for 90 min. After cooling to room temperature, the product was washed three times with hexane and ethanol and redispersed in THF.

3 Synthesis of N-(2-[3,4-dihydroxyphenyl] ethyl) acrylamide (dopamine acrylamide)

Dopamine acrylamide was synthesized as previously reported. [1]

4 Acrylation of UCNP

Dopamine acrylamide (100 mg) was dissolved in 5 mL THF in a 25 mL three-necked flask. After the solution was heated to 40 ℃, UCNPs (30 mg) in 2 mL THF were added dropwise and stirred for 3 hours. After cooling to room temperature, the nanoparticles were washed three times with ethanol and THF and redispersed in THF.

5 Synthesis of thiol-T(NH_2)-sgc8-tmr

DNA was synthesized on an ABI 3400 DNA synthesizer (Applied Biosystems, Foster City, CA, USA) in a 1 μmol scale with a TAMRA-labeled controlled pore glass (CPG) and Amino-Modifier C6 dT, as well as Thiol-Modifier C6 S-S coupled with longer reaction time. After on-machine synthesis, the DNA was cut from the CPG and deprotected with 2.5 mL APA solution (ammonium hydroxide/propylamine/water 2:1:1) at 65 ℃ for 45 min. The supernatant was collected, and 6 mL cold ethanol and 250 μL 3.0M NaCl were added. The mixture was kept frozen at -20 ℃ overnight before DNA precipitation. The sequence was centrifuged, and the pellet was dissolved in 400 μL 0.2 M TEAA water for HPLC purification. HPLC purification was conducted on reversed-phase HPLC (ProStar, Varian, Walnut Creek, CA, USA) with a C18 column and acetonitrile, and 0.2 M TEAA water was used as elution buffer. The collected product was dried and detritylated with 200 μL 80% acetic acid for 45 min. After ethanol precipitation and drying, the DNA was reconstituted in 200 μL H_2O. The concentration was determined by 260 nm absorption (Shimadzu UV-1800 spectrophotometer).

Aptamer ligand sequence: disulfide-TTTTT(NH_2)TTT TTA TCT AAC TGC TGC GCC GCC GGG AAA ATA CTG TAC GGT TAG A-TAMRA

6 Ce6 coupling to thiol-T(NH_2)-sgc8-tmr

Five μmole Ce6 was mixed with 25 μmole 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 32.5 μmole N-Hydroxysuccinimide (NHS) and dissolved in 1250 μL N,N-dimethylformamide (DMF) for activation reaction with 30 min shaking. The solution was then added with 1250 μL DNA (250nmole) in 0.1M pH 7.5 $NaHCO_3$ solution and shaken overnight at room temperature before ethanol precipitation. Free Ce6 was removed by precipitating DNA in ethanol twice. The DNA-Ce6 conjugated product was dissolved in TEAA H_2O and purified with HPLC.

7 Thiol-Ce6-sgc8-tmr conjugation to UCNP

The disulfide bond was first reduced to a thiol group by dithiothreitol (DTT). Ten μmole DTT was added to DNA (40 nmole) in 250 μL H_2O and shaken for 20 min before purification with a NAP-5 desalting column (GE Healthcare) to eliminate free DTT. The obtained HS-DNA was dried to 120 μL and dropwise added to a glass vial containing UCNP in 800 μL THF (0.5 mg/mL). TEA (5 mg/mL, 200 μL) was added to the mixture as catalyst to promote the reaction. The reaction mixture was shaken overnight at room temperature before washing twice with water. Particles in 1mL H_2O were then reacted with thiol-PEG_{1000} in 200 μL H_2O (20 mg/mL) overnight at room temperature. Particles were washed three times with 1mL H_2O and then redispersed in H_2O at a concentration of 2 mg/mL.

8 Target binding test with flow cytometry

To test the target binding ability of the nanoplatform toward a specific cell line, fluorescence measurements were obtained with a FACSAria™ IIu cytometer (Becton Dickinson, San Jose, CA, USA), using a 555 nm laser as excitation source. After washing three times, 2×10^5 CEM and Ramos cells were incubated with 10 μg/mL tmr-sgc8-UCNP in 200 μg $binding\ buffer$ at 4 ℃ for 30 min. Then the cells were washed with PBS buffer three times and redispersed in binding buffer for flow cytometry analysis by counting 10,000 events.

9 Internalization study

To study the specific internalization of the tmr-sgc8-UCNP, 2×10^5 CEM and Ramos cells were incubated with 100 μg/mL tmr-sgc8-Ce6-UCNP in 200 μL RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO) supplemented with 100 U/mL aqueous penicillin G, 100 μg/mL streptomycin, 0.4 mM L-Glutamine and 10% Fetal Bovine Serum (Life Technologies, Carlsbad, CA) at 37 ℃ for 12h with 5% CO_2 atmosphere. Cells were then washed three times with PBS and resuspended in 200 μL PBS. Confocal images were obtained with a Leica TCS SP5 confocal microscope.

10 Cytotoxicity study

For both CEM and Ramos cells, 1.5×10^4 cells were washed and seeded in a 96-well plate in 100 μL RMPI-1640 complete medium. tmr-sgc8-Ce6-UCNP at different concentrations was added to each well and incubated with cells for 12 h at 37 °C in a 5% CO_2 atmosphere. Supernatant was removed after centrifugation, and cells were washed once and resuspended in 100 μL medium. Laser light of 980 nm was applied to each well for 10 min, and then cells were further incubated at 37 °C in a 5% CO_2 atmosphere for 48 h before MTS assay. The absorbance at 490 nm for each well was obtained by microplate reader.

Figures

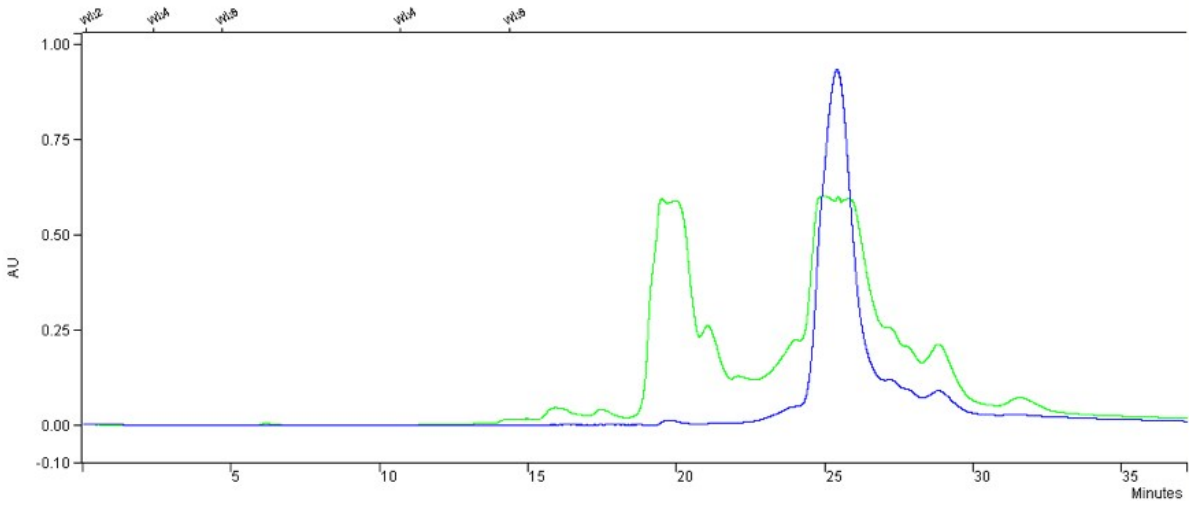

Fig. S1 HPLC chromatogram of the product after Ce6 and DNA conjugation. Green: 260 nm channel for DNA absorption; Blue: 404 nm channel for Ce6.

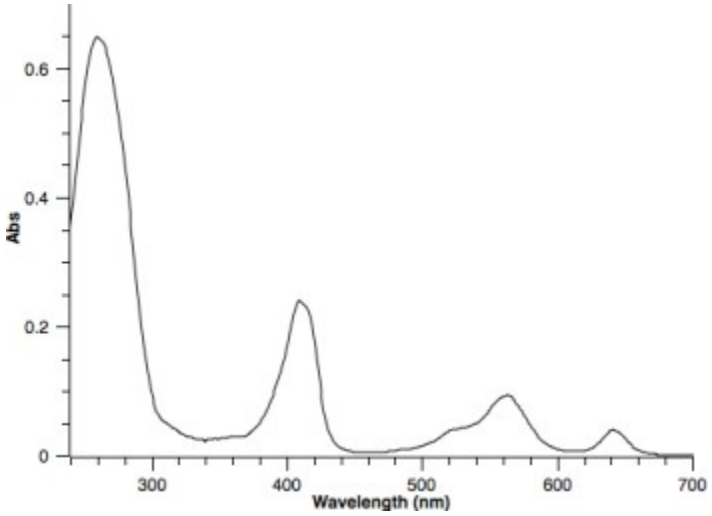

Fig. S2 UV/Vis absorption of Tmr-Ce6-sgc8 ligand.

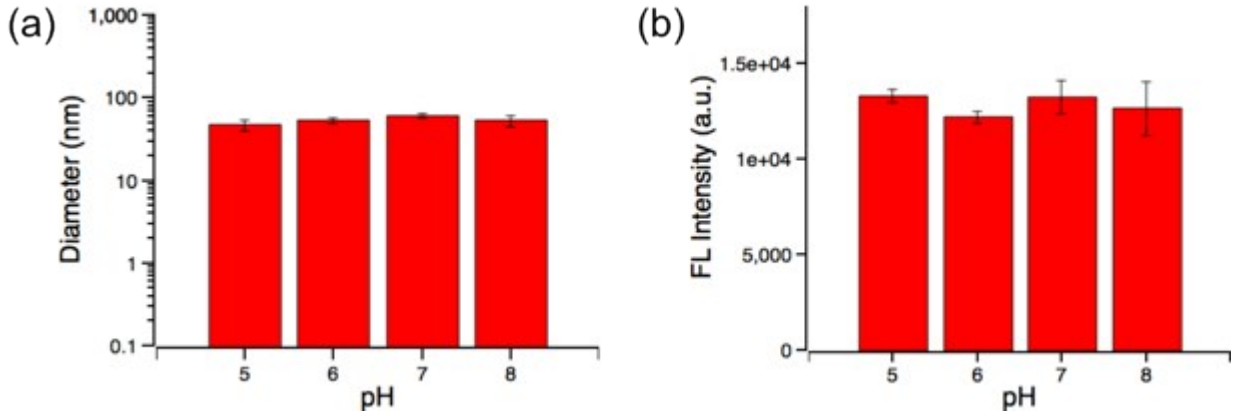

Fig. S3 Hydrodynamic diameter (a) and fluorescence intensity (b) over a pH range of physiological condition in PBS buffer.

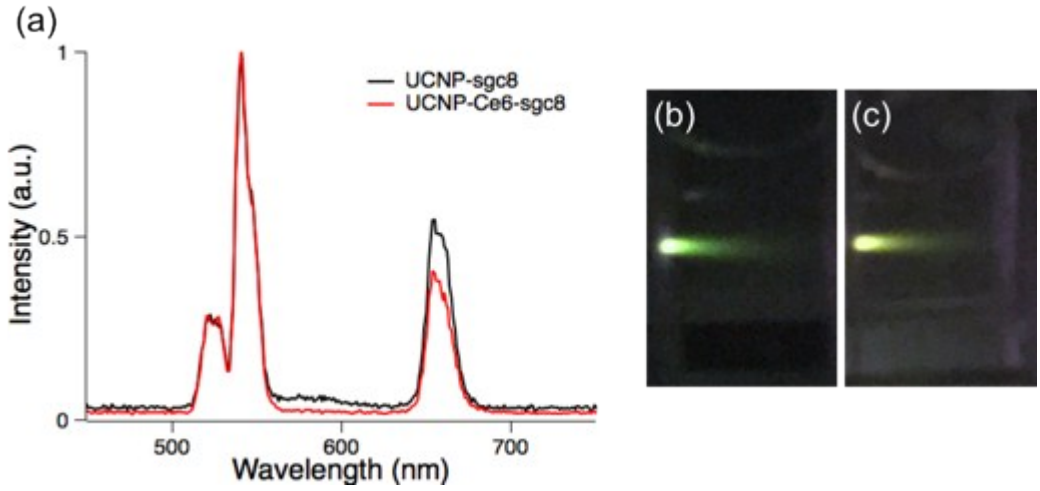

Fig. S4 (a) Emission spectrum of UCNP-sgc8 and UCNP-Ce6-sgc8. Luminescence upon excitation with 980 nm laser of (b) sgc8-Ce6-UCNP and (c) sgc8-UCNP with no Ce6 loading.

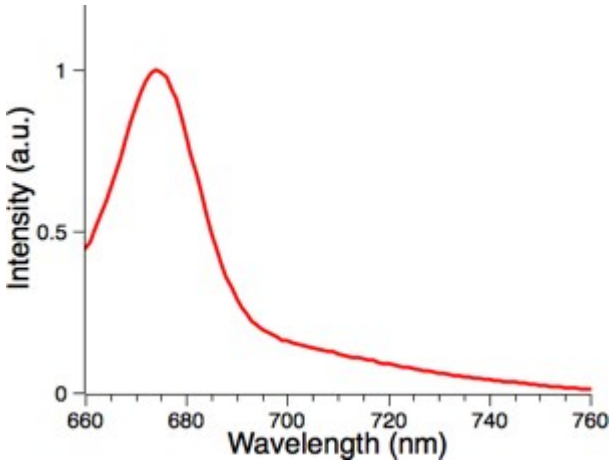

Fig. S5 Emission spectrum of Ce6 in ethanol.

Reference

1. Y. Liu, W. Hou, H. Sun, C. Cui, L. Zhang, Y. Jiang, Y. Wu, Y. Wang, J. Li and B. S. Sumerlin, *Chemical science*, 2017, **8**, 6182-6187.