Supporting Information

Facile Phase-Transfer and Surface Biofunctionalization of Hydrophobic Nanoparticles Using Janus DNA Tetrahedron Nanostructures

Juan Li,[1,2,3,§] Cheng-Yi Hong,[1,§] Shu-Xian Wu,[1] Hong Liang,[1] Li-Ping Wang,[1] Guoming Huang,[1] Xian Chen,[1] Huang-Hao Yang,[1,*] Dihua Shangguan[2,4,*] and Weihong Tan[2,3,*]

[1] The Key Lab of Analysis and Detection Technology for Food Safety of the MOE and Fujian Province, State Key Laboratory of Photocatalysis on Energy and Environment, College of Chemistry, Fuzhou University, Fuzhou 350002, China

[2] Molecular Sciences and Biomedicine Laboratory, State Key Laboratory for Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering and College of Biology, Collaborative Innovation Center for Molecular Engineering and Theranostics, Hunan University, Changsha 410082, China

[3] Department of Chemistry and Department of Physiology and Functional Genomics, Center for Research at the Bio/Nano Interface, UF Health Cancer Center, University of Florida, Gainesville, FL 32611-7200, USA Fax: (+1) 352-846-2410

[4]Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P. R. China

Experimental Section

Materials and Apparatus

All oligonucleotides (see Table S1) were synthesized on an ABI 3400 DNA/RNA synthesizer (Applied Biosystems, Foster City, CA, USA). The DNAs were then purified by HPLC (ProStar, Varian, Walnut Creek, CA, USA). The high-quality IONPs were purchased from Ocean NanoTech (Springdale, AR, USA). $HAuCl_4$, trisodium citrate, tannic acid, potassium carbonate (K_2CO_3), bis(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (BSPP) and tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich. Trifluoroacetic acid (CF_3COOH, 99%) and Erbium oxide (Er_2O_3, 99.99%) was purchased from Aladdin. Yttrium oxide (Y_2O_3, 99.99%), sodium trifluoroacetate (CF_3COONa, 98%), oleic acid (OA), and cyclohexane were from Sinopharm Chemical Reagent Co. (China). All other chemicals were of analytical grade and were used as received from manufacturer. All solutions were prepared with ultrapure water obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA) with resistivity of 18.2 MΩ·cm.

Transmission electron microscopy (TEM) images were taken on the FEI Tecnai G20 transmission electron microscope with an accelerating voltage of 200 kV.

Ultraviolet-visible-near-infrared light (UV-Vis-NIR) absorption spectrum was recorded using a SH-1000 Lab microplate reader (Corona Electric, Hitachinaka, Japan) at room temperature. T_2-Weighted phantom images and relaxation times were measured on a 0.5 T MRI scanner (NMI20-015V, Niumag Corporation, Shanghai, PR China). A NIR laser (Shaanxi Kai Site Electronic Technology Co., Ltd, 980 nm, 5 W, China) was used as the excitation source for UCNPs. The fluorescence images of cells were taken on laser-scanning confocal microscopy system (Olympus, Fluoview FV1200, USA).

Preparation of DNA tetrahedron

The aptamer-pendant DNA tetrahedron nanostructure (Apt-tet) was prepared as the following process: strand A, B, C and D were mixed in equimolar ratio in TM buffer (10 mM Tris-HCl, 50 mM $MgCl_2$, pH 8.0) with a final concentration of 10 μM. The solution was heated to 95 °C and then cooled to 4 °C. The random sequence-pendant DNA tetrahedron nanostructure (Rdm-tet) was prepared under the same condition using strand A, B, C and E. Non-modified DNA tetrahedron nanostructure (Nmo-tet) was prepared under the same condition using strand D, F, G, and H. Three kinds of DNA tetrahedron were stored at 4 °C for further use.

Synthesis of DNA tetrahedron-functionalized IONPs

The oleic acid capped IONPs in 0.2 mL of chloroform was slowly added into 0.2 mL water solution containing 10 μM Apt-tet, and the solution was vigorously stirred for 12 h. Afterward, the IONPs were transferred into the upper water layer from the chloroform layer. Then the water solution was transferred to a microtube. Finally, the Apt-tet-IONPs were re-dispersed in the TM buffer and stored at 4 °C for further use.

Gold nanoparticle synthesis

AuNPs (5 nm diameter) were synthesized according to the literature.[1] Briefly, 1mL of $HAuCl_4$ (1%w/v) was added to 80 mL of water in a 250 ml erlenmeyer flaks and brought to 60 ℃. A reducing solution containing 16 mL H_2O, 4 mL trisodium citrate (1%), 1 mL tannic acid (1%), and 1mL potassium carbonate (3.46 mg/mL) was prepared and warmed to 60 °C in a water bath for 50 mins. Under vigorous stirring, the reducing solution was swiftly injected into the erlenmeyer flask and the reaction was kept at 60 °C 30 minutes and then at 90 °C for 10 mins. The heat was then turned off for the flask to cool to room temperature. 1 mL of 80 mg/mL bis(p-sulfonatophenyl) phenylphosphine dihydrate dipotassium salt (BSPP) was added to the nanoparticle solutions and stirred overnight to improve particle stability.

Synthesis of DNA-AuNPs and its assembly with DNA-IONPs

Synthesized of 5 nm gold nanoparticles were washed twice and concentrated in PBST (10 mM sodium phosphate buffer, 0.01% Tween 20, pH 7.2) by ultracentrifugation. Oligonucleotide

functionalization of gold nanoparticles was performed by incubating 200 nM gold nanoparticles with 10 μM thiolated DNA (cDNA, ncDNA) in PBST. Particles were aged in this mixture for 16 h. A solution of 3.43 M NaCl in PBST was added in 5 aliquots to this particle mixture to bring the final salt concentration in the solution to 0.5 M. The Apt-tet-IONPs were incubated with complementary DNA functionalized AuNPs (cDNA-AuNPs) or noncomplementary DNA functionalized AuNPs (ncDNA-AuNPs) for 2 h. The conjunct nanoparticles were centrifuged and washed to remove unassembled DNA-AuNPs.

Various pH solubility tests of DNA tetrahedron-functionalized IONPs

30 μL of the Apt-tet-IONPs was dissolved in 120 μL of water solution. The pH value of solution was changed between 2.0 and 11.0 by adding 0.1 M HCl or 0.5 M NaOH.

Phase-transfer ability of different DNA molecules

The oleic acid capped IONPs (100 μg/mL) in 0.2 mL of chloroform was slowly added into 0.2 mL water solution containing 10 μM Apt-tet, 0.2 mL water solution containing 10 μM Nmo-tet, and 0.2 mL water solution containing 50 μM strand I, respectively. Three solution mixtures were vigorously stirred for 12 h under the same condition.

In vitro magnetic resonance imaging

A series of Apt-tet-IONPs aqueous solutions containing different iron concentrations (0, 0.025, 0.05, 0.1, 0.2, and 0.4 mM) were prepared. T_2-weighted phantom images and relaxation times were measured on a 0.5 T MRI scanner under the following parameters: TR/TE = 2000/60 ms, 256 × 256 matrices, thickness= 1 mm.

Magnetic Resonance Imaging of Cells

MCF-7 cells and L02 cells seeded in 6-well plates (5×10^5 cells/well) were incubated with Apt-tet-IONPs and Rdm-tet-IONPs at different iron concentrations (0, 0.5, 1, and 2 mM) for 2 h, respectively. The cells were then washed with PBS and harvested before MRI.

Synthesis of $M(CF_3COO)_2$:$Y/Yb^{3+}/Er^{3+}$ UCNPs

Hexagonal phase $M(CF_3COO)_2$: 78% Y, 20% Yb^{3+}, 2% Er^{3+} nanoparticles were synthesized according to the literature.[2] 100 mL three-necked flask were added 0.3338g $Y(CF_3COO)_3$, 0.1024g $Yb(CF_3COO)_3$, 0.0101g $Er(CF_3COO)_3$, 0.2720g CF_3COONa, 5.65g oleic acid and 5.05g 1-octadecene (ODE). The mixture was first vigorous stirring under vacuum at room temperature for 30 min, and then heated to 120-140 °C for 30 min with vigorous stirring under vacuum. The resulting solution was heated to 320 °C to react for 40 min. After cooling to room temperature, 10 mL ethanol was added into the obtained nanoparticles. The mixed was centrifugation and then washed three times with ethanol and cyclohexane (v/v, 1:1) and recovered by centrifugation.

Synthesis of DNA tetrahedron-functionalized UCNPs

The oleic acid capped UCNPs in 0.2 mL of cyclohexane was slowly added into 0.2 mL water solution containing 10 μM Apt-tet, and the solution was vigorously stirred for 12 h. Afterward, the UCNPs were transferred into the water layer from the cyclohexane layer. Then the water solution was transferred to a microtube. The excess Apt-tet was removed from Apt-tet-UCNPs by centrifugation and washing. Finally, the Apt-tet-UCNPs were re-dispersed in the TM buffer and stored at 4 °C for further use.

Targeting cancer cells imaging

MCF-7 and L02 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) media with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 mg/mL) at 37 °C in a 5% CO_2 incubator. The cells were plated at 60% - 70% confluency for 24 h before imaging experiments in 35-mm culture dishes. Cultured cells were washed with PBS (pH 7.4) two times prior to imaging. After incubation with Apt-tet-UCNPs for 1 h, medium was removed. The treated MCF-7 cells or L02 cells were washed with PBS (pH 7.4). Thereafter, fluorescence images were immediately taken using laser-scanning confocal microscopy system (Olympus, Fluoview FV1200, USA), modified to allow illumination with a NIR 980 nm laser.

Table S1. The detailed information of DNA sequences

Name	Detailed sequence information (from 5' to 3')
Strand A	COOH-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAG GGTCCAATAC
Strand B	COOH-TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATG GCGGCTCTTC
Strand C	COOH-TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGA CCCTCGCAT
Strand D	GGTGGTGGTGGTTGTGGTGGTGGTGGTTTTTTACATTCCTAAGTCTGAAA CATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
Strand E	GAGTGGTTTCACATAAAATGTACCAATTTTTTACATTCCTAAGTCTGAAAC ATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
Strand F	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCC AATAC
Strand G	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGC TCTTC
Strand H	TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTC GCAT
Strand I	COOH-TTTTTTTTTTTTTTTTTTTTTTTTT
cDNA	CCACCACCACCACAACCACCACCACC-$(CH_2)_6$-SH

ncDNA	GGTTTGGTTAGGTTAGCTTAGGTTAG-$(CH_2)_6$-SH

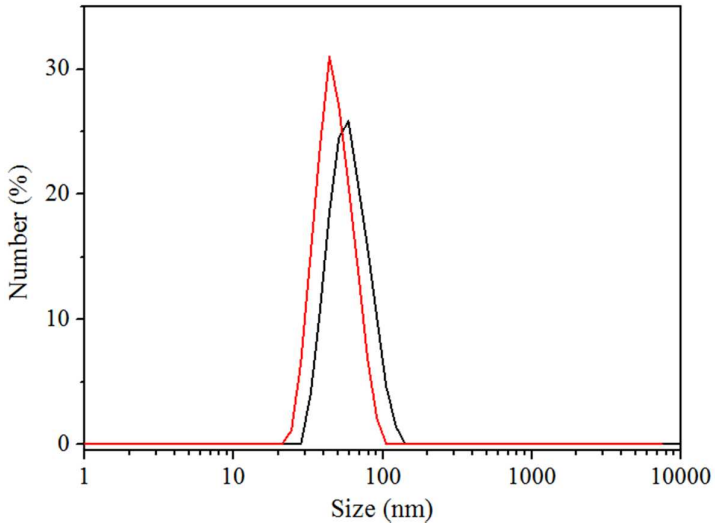

Figure S1. Dynamic light scattering (DLS) data of IONPs in chloroform (red) and Apt-tet-IONPs in water (black).

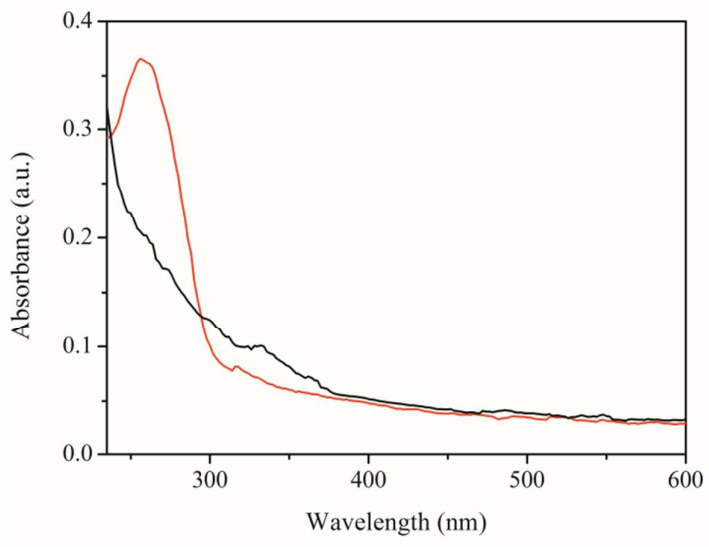

Figure S2. UV-Vis spectra of Apt-tet-IONPs in water (red) and unmodified IONPs in chloroform (black). The UV-vis spectrum of Apt-tet-IONPs showed a characteristic peak of DNA at 260 nm after DNA tetrahedron functionalization (red), while that of IONPs exhibited no peak (black).

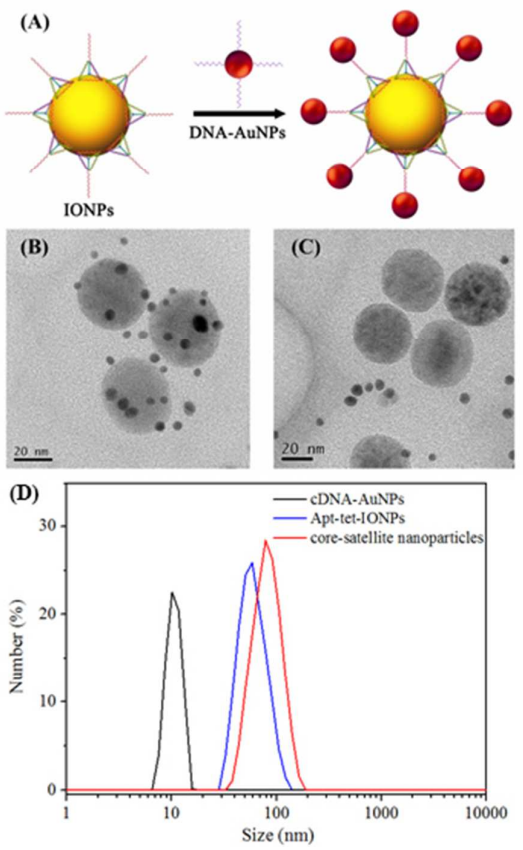

Figure S3. (A) The assembly of DNA tetrahedron-functionalized IONPs (Apt-tet-IONPs) and DNA-AuNPs. TEM images of Apt-tet-IONPs assembled with AuNPs that was modified with (B) complementary DNA (cDNA-AuNPs) and (C) noncomplementary DNA (ncDNA-AuNPs). (D) Dynamic light scattering (DLS) data of cDNA-AuNPs (black), Apt-tet-IONPs (blue) and core-satellite nanoparticles assembled with Apt-tet-IONPs and cDNA-AuNPs (red).

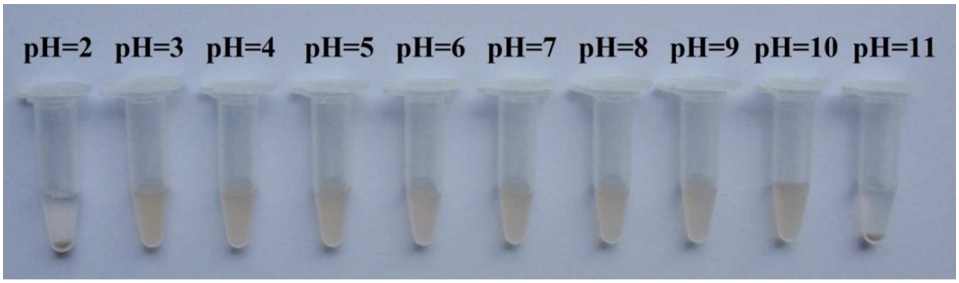

Figure S4. The Apt-tet-IONPs in the buffer solutions with different pH values after 48 hours.

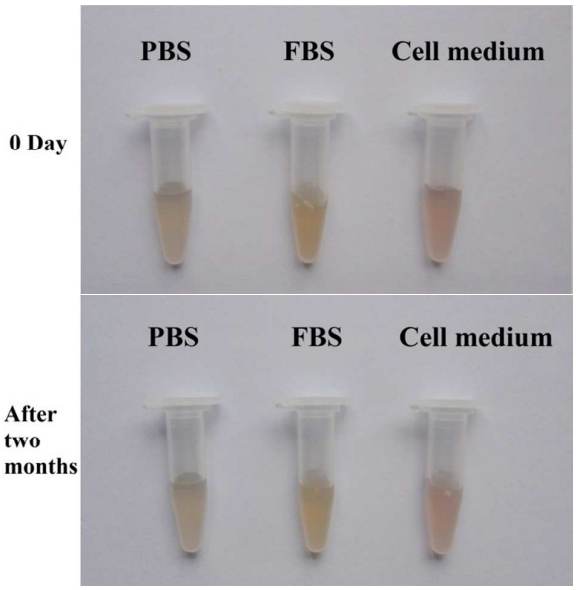

Figure S5. Photos of Apt-tet-IONPs in different solutions including PBS, FBS and RPMI-1640 with 10% fetal bovine serum (FBS) cell culture medium.

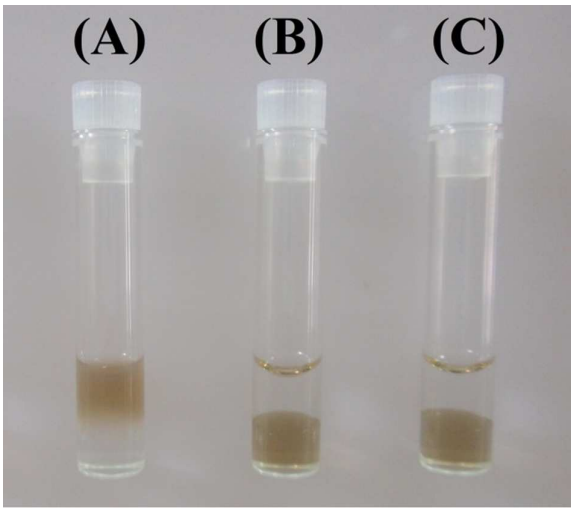

Figure S6. Solvent dispersity of IONPs using difference DNA molecules functionalization. (A) carboxyl group-modified DNA tetrahedron, (B) non-modified DNA tetrahedron, (C) carboxyl-terminated single-stranded (ss-) DNA.

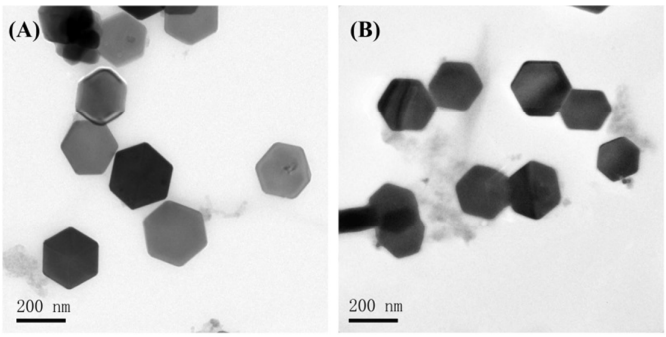

Figure S7. TEM images of UCNPs before DNA tetrahedron functionalization in cyclohexane (A) and after DNA tetrahedron functionalization in water (B).

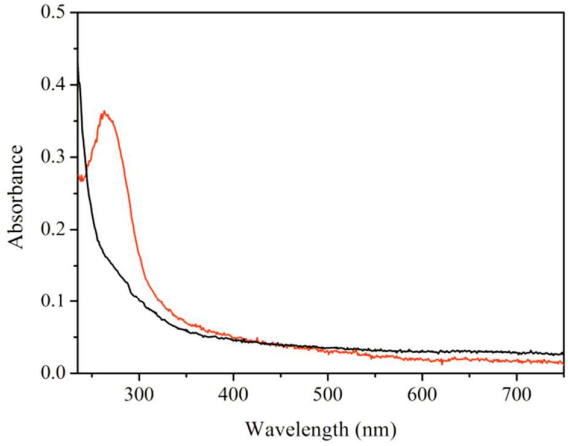

Figure S8. UV-Vis spectra of Apt-tet-UCNPs in water (red) and unmodified UCNPs in cyclohexane (black). The UV-vis spectrum of Apt-tet-UCNPs showed a characteristic peak of DNA at 260 nm after DNA tetrahedron functionalization (red), while that of UCNPs exhibited no peak (black).

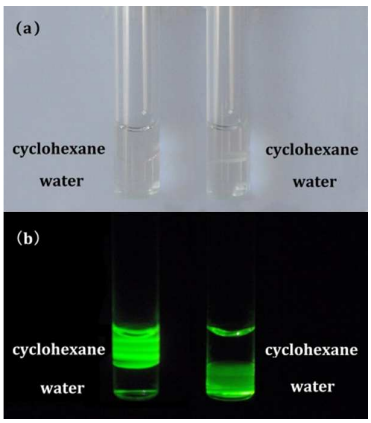

Figure S9. (a) Photograph of solvent dispersity of UCNPs before (left) and after (right) DNA tetrahedron functionalization taken under ambient light. (b) Photograph of solvent dispersity of UCNPs (left) and after (right) after DNA tetrahedron functionalization in the dark under excitation by a 980 nm laser.

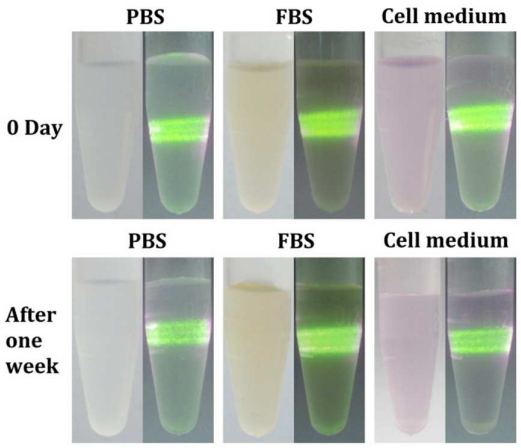

Figure S10. Photos of Apt-tet-UCNPs in different solutions including PBS, FBS and RPMI-1640 with 10% fetal bovine serum (FBS) cell culture medium. For each picture, photographs of Apt-tet-UCNPs were taken under ambient light (left) and corresponding luminescence photograph taken under excitation by a 980 nm laser (right).

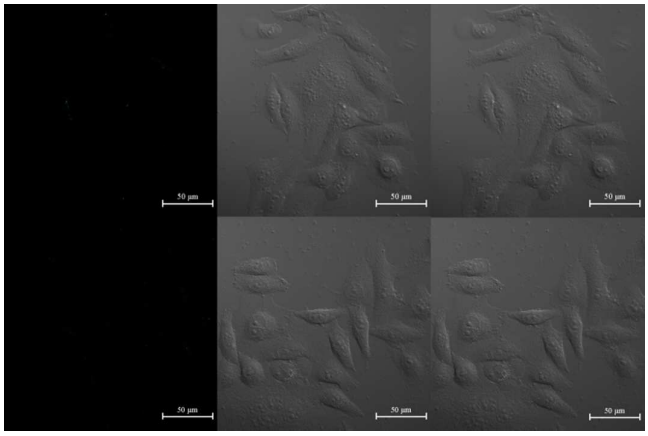

Figure S11. Confocal Microscopy images of the L02 cells treated with Apt-tet-UCNPs (upper) and Rdm-tet-UCNPs (lower) for 1 h. λ_{ex}= 980 nm, emission was collected in the range λ= 510-560 nm.

Reference

(1) Chou, L. Y. T.; Zagorovsky, K.; Chan, W. C. W. *Nat. Nanotechnol.* **2014**, *9*, 148-155.

(2) Wang, J.; Wei, T.; Li, X.; Zhang, B.; Wang, J.; Huang, C.; Yuan, Q. *Angew. Chem., Int. Ed.* **2014**, *53*,1616-1620.