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Supporting Information

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Biomimetic Surface Engineering of Lanthanide-Doped Upconversion Nanoparticles as Versatile Bioprobes**

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Experimental Section

Chemicals: All of the chemicals used were of analytical grade and were used without further purification. Rare earth chlorides $YCl_3 \cdot 6H_2O$, $YbCl_3 \cdot 6H_2O$, $ErCl_3 \cdot 6H_2O$, Gd $Cl_3 \cdot 6H_2O$, oleic acid, 1-octadecenewas were purchased from Sigma-Aldrich. Tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were from Thermo Scientific. Phospholipids with different functional headgroups (Scheme S1) including 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG-COOH), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG-Maleimide), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[folate(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG-Folate), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) (PE-Rhod) were all purchased from Avanti Polar Lipids. The thiolated DNA were synthesized and purified by Integrated DNA Technologies, Inc. (IDT, Coralville, IA). The sequences are as follows: 5'-CCA ACC ACA GTG TTT TTT TTT-thiol-3' (DNA to modify Lipo-UCNPs and also act as noncomplementary DNA to modify AuNPs), 5'-CAC TGT GGT TGG TTT TTT TTT-thiol-3′ (complementary DNA to modify AuNPs). AuNPs (5 nm diameter) were purchased from Ted Pella, Inc. (Redding, CA).

Synthesis of oleic acid capped UCNPs: β-$NaYF_4$:Yb,Er and β-$NaGdF_4$ nanoparticles were synthesized according to the literature methods using rare earth chlorides as precursor and oleic acid as stabilizing agents.[1] These UCNPs were purified by centrifugation after the addition of ethanol as the flocculent, washed extensively with ethanol/water (1:1), and redispersed in non-polar solvents for further experiments.

Synthesis of Phospholipids coated UCNPs (Lipo-UCNPs): The oleic acid capped UCNPs (0.05 mmol) in 0.5 mL of chloroform was mixed with a chloroform solution (2 mL) containing 12.5 mg designed phospholipids (one kind or a mixture of two different kinds of designed phospholipids) in a screw-neck glass bottle (5mL). Leave the glass bottle open in a fume hood for two days at room temperature to evaporate the chloroform slowly. The obtained mixed film was heated at 75 °C for 5 min to completely remove chloroform. Then the film was hydrated with MilliQ water (5 mL), and the UCNPs became soluble after vigorously sonication, which can be further stirred vigorously at 75 °C for 10 min. The solution was transferred to a microtube and centrifuged lightly, the sediment was discarded to remove possible large aggregates. Excess lipids were purified from Lipo-UCNPs by ultracentrifugation (15000 rpm, 6 min) and washing. For the preparation of DSPE-PEG-Maleimide coated Lipo-UCNPs, there is a little bit modification of the procedure: the chloroform was completely removed by pumping at room temperature for one hour but not heating at 75 °C, and the clear solution need not to be stirred at 75 °C after vigorously sonication.

DNA conjugation: DNA was conjugated to the maleimide groups of the DSPE-PEG-Maleimide coated Lipo-UCNPs by sulfhydryl-maleimide coupling. Briefly, 300 nmol disulfide-modified DNA was freshly cleaved by three equivalent of TCEP for 2 h under pH 5.2 at room temperature to obtain free sulfhydryl groups. The cleaved DNA were purified using NAP-10 columns. Then, the activated DNA was mixed with 5 mg Lipo-UCNPs with maleimide on the surface in the HEPES buffer (20 mM, 100 mM NaCl, pH 7.2) and shaken at room temperature

for 24 h. The particle was recovered by centrifugation and washing with the buffer and water.

Synthesis of DNA functionalized 5 nm AuNPs: Thiol-modified DNA were chemically attached to the AuNPs (5 nm) according to our previously reported method.[2] Thiol-modified DNA molecules were first activated by 1.5 equiv of TCEP for 1 h under pH 5.2 at room temperature before use. Glass vials (20-mL volume) were soaked in 12 M NaOH for 1 h and rinsed with DI water before use. AuNPs were loaded into the NaOH-treated glass vials, and thiol-modified DNA was added to a final concentration of 3 μM. The vials were capped and kept at room temperature for ∼16 h. Tris acetate buffer (pH 8.2) was then added to the nanoparticles to a final concentration of 5 mM, and NaCl was added to a final concentration of 100 mM. The functionalized 5 nm AuNPs solutions were incubated for another day before usage. To purify the product, a microcon (Ultracel YM-100, MWCO = 100K; Microcon) was used by following the instructions from the manufacturer.

Synthesis of bioinspired Lipo-UCNP-AuNPs nanoassemblies directed by DNA: The DNA conjugated Lipo-UCNPs was incubated with cDNA or noncomplementary DNA functionalized 5 nm AuNPs in Tris-HCl buffer (20 mM, 100 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, pH7.4) over night. The nanoparticles were then centrifuged and washed to remove unassembled AuNPs.

***Flow chamber and in vitro Lipo-UCNP samples*:** A glass slide (Fisher Scientific) with two holes was drilled at 1.5 – 2 cm separation. A glass coverslip (Fisher Scientific) with two strips of double-stick tape was added to form a flow chamber, followed by 5 Minute Epoxy (ITW Devcon) to seal the side of the flow chamber. 100 μL 1 mg/mL BSA-biotin in pH 7.0 Phosphate Buffered Saline (PBS) buffer was flowed through the chamber and allowed to sit for 10 min at room temperature (RT), and then washed with 60 μL PBS buffer. 100 μL 0.5 mg/mL Neutraavidin (Invitrogen) in PBS buffer was flowed through the chamber and allowed to sit for 10 min at RT, and then washed with 60 μL ddH_2O. 100 μL 1 mg/mL biotinylated Lipo-UCNPs in ddH_2O was flowed through the chamber and incubated at RT for 10 min. Excessive nanoparticles were then washed away with 60 μL ddH_2O. The single molecule Lipo-UCNP sample was subsequently imaged on an Olympus IX71 microscope with widefield and scan excitations.

Analysis of cellular uptake HeLa cells were grown in chamber slides in 1 mL Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 units/ml aqueous penicillin G, 100 μg/mL streptomycin, 0.4 mM L-Glutamine and 10% FBS in 37°C CO_2 incubator at concentrations to allow 70% confluence in 24 h (i.e., 40,000 cells per cm^2). On the day of experiments, the medium was removed and cells were then incubated in 1 mL prewarmed fresh aforementioned cell medium with 100 μg/mL Lipo-UCNPs added, in 37°C CO_2 incubator for 1 h. Medium was then removed and treated HeLa cells were washed with prewarmed PBS (3 × 1 mL). The cell samples were subsequently imaged on an Olympus IX71 microscope with widefield or scan excitations.

Instrumentation. Transmission electron microscopy (TEM) images were taken on the JEOL 2100 Cryo transmission electron microscope with an accelerating voltage of 200 kV. Dynamic light scattering (DLS) experiments were carried out on a Malvern Zetasizer 3000 (Malvern Instruments). Fluorescent spectra were recorded on a FluoroMax-P fluorimeter (HORIBA Jobin Yvon Inc., Edison, NJ) equipped with a commercial CW IR laser (980 nm) (Thorlabs, Inc.). UV/Vis spectra were recorded on a Hewlett–Packard 8453 spectrometer. The

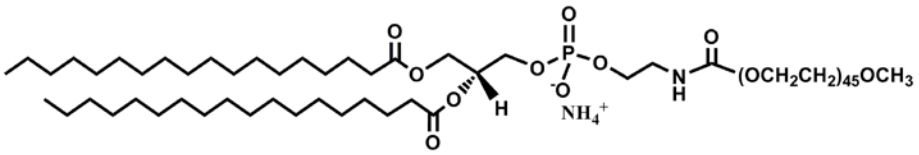
upconversion luminescence images of *in vitro* immobilized individual biotinylated Lipo-UCNPs and folate conjugated Lipo-UCNPs internalized in HeLa cells were acquired with an Andor iXon+ EMCCD camera through HQ535/50m (Chroma) + FF01-720/SP (Semrock) emission filters on an Olympus IX71 microscope, excited by a Spectra-Physics Mai Tai HP pulsed laser at 980 nm (widefield) or 785 nm (multipoint matrix scan). For 3D matrix scan imaging, the z-sections were taken at every 100 nm, equal to the x-y image pixel size.

Reference

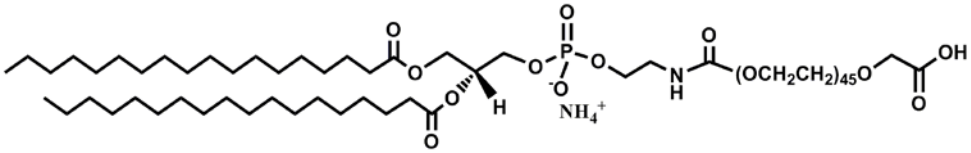
[1] (a) Z. Q.; Li, Y. Zhang, *Nanotechnology* **2008**, *19*, 345606. (b) Shi, F.; Wang, J.; Zhang, D.; Qin, G.; Qin, W. *J. Mater. Chem.* **2011**, *21*, 13413–13421. (c) Johnson, N. J. J.; Oakden, W.; Stanisz, G. J.; Prosser, R. S.; van Veggel, F. C. J. M. *Chem. Mater.* **2011**, *23*, 3714–3722.
[2] Liu, J.; Lu, Y. *Nat. Protoc*. **2006**, *1*, 246–252.

The complete reference for Ref. 8c and Ref. 9 in the Main Text:

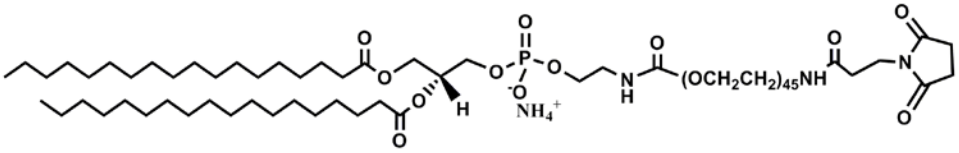
[8c] Y. Namiki, T. Namiki, H. Yoshida, Y. Ishii, A. Tsubota, S. Koido, K. Nariai, M. Mitsunaga, S. Yanagisawa, H. Kashiwagi, Y. Mabashi, Y. Yumoto, S. Hoshina, K. Fujise, N. Tada, *Nat. Nanotechnol*. **2009**, *4*, 598–606.
[9] C. E. Ashley, E. C. Carnes, G. K. Phillips, D. Padilla, P. N. Durfee, P. A. Brown, T. N. Hanna, J. Liu, B. Phillips, M. B. Carter, N. J. Carroll, X. Jiang, D. R. Dunphy, C. L. Willman, D. N. Petsev, D. G. Evans, A. N. Parikh, B. Chackerian, W. Wharton, D. S. Peabody, C. J. Brinker, *Nat. Mater.* **2011**, *10*, 389–397.



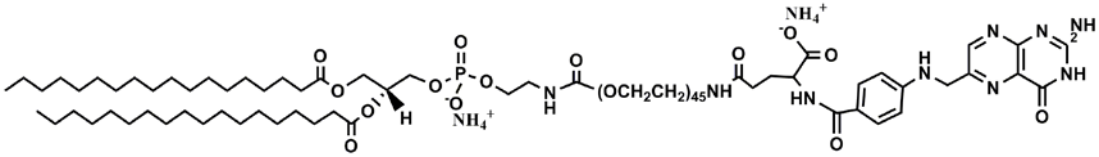
DSPE-PEG



DSPE-PEG-COOH



DSPE-PEG-Maleimide



DSPE-PEG-Folate

Scheme S1. The selectively used phospholipids with different functional headgroups.

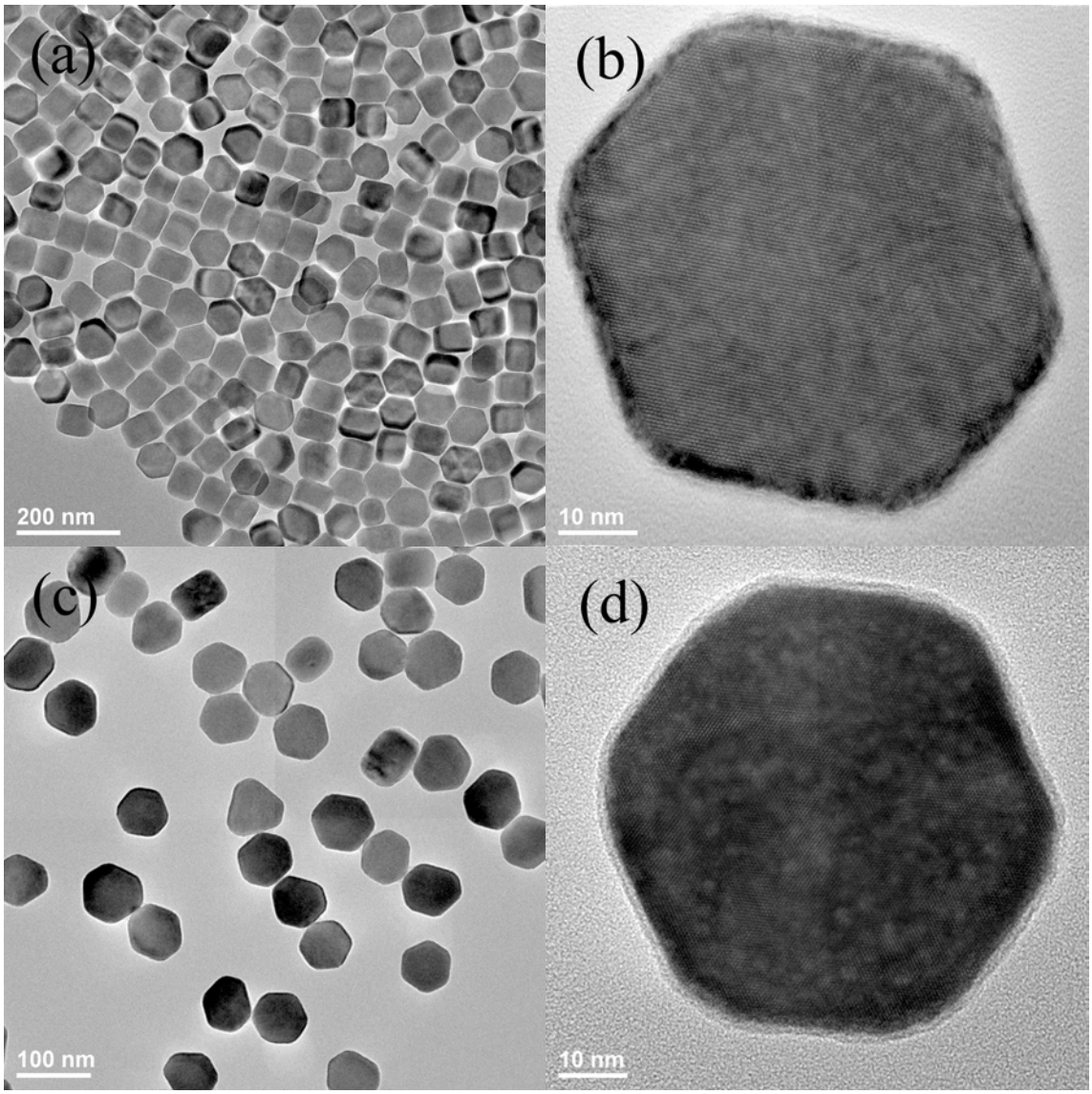


Figure S1. TEM images of the (a,b) as-prepared and (c,d) phospholipids coated Lipo-UCNPs deposited on TEM grid from a drop of UCNPs cyclohexane solution and Lipo-UCNPs water solution, respectively.

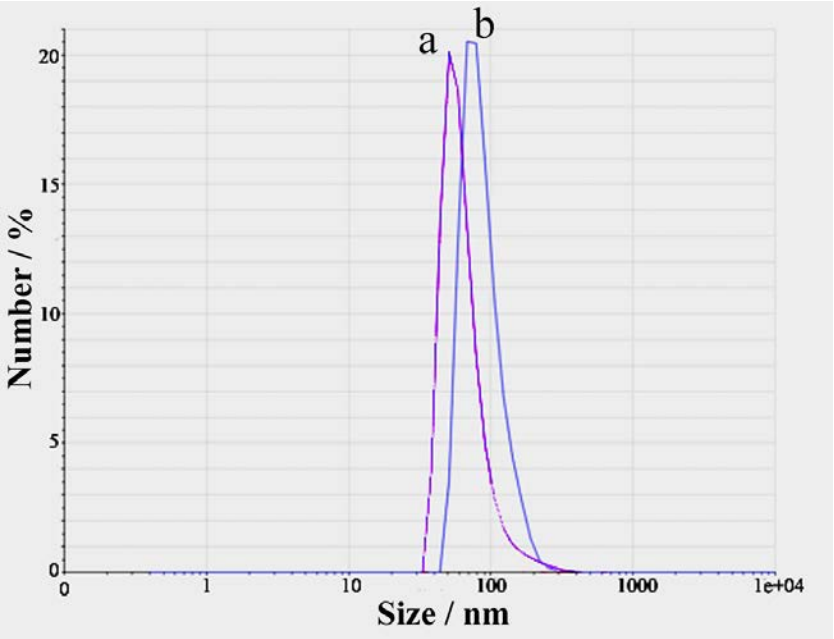


Figure S2. Hydrodynamic size distribution of (a) UCNPs in cyclohexane and (b) Lipo-UCNPs in water, determined by dynamic light scattering.

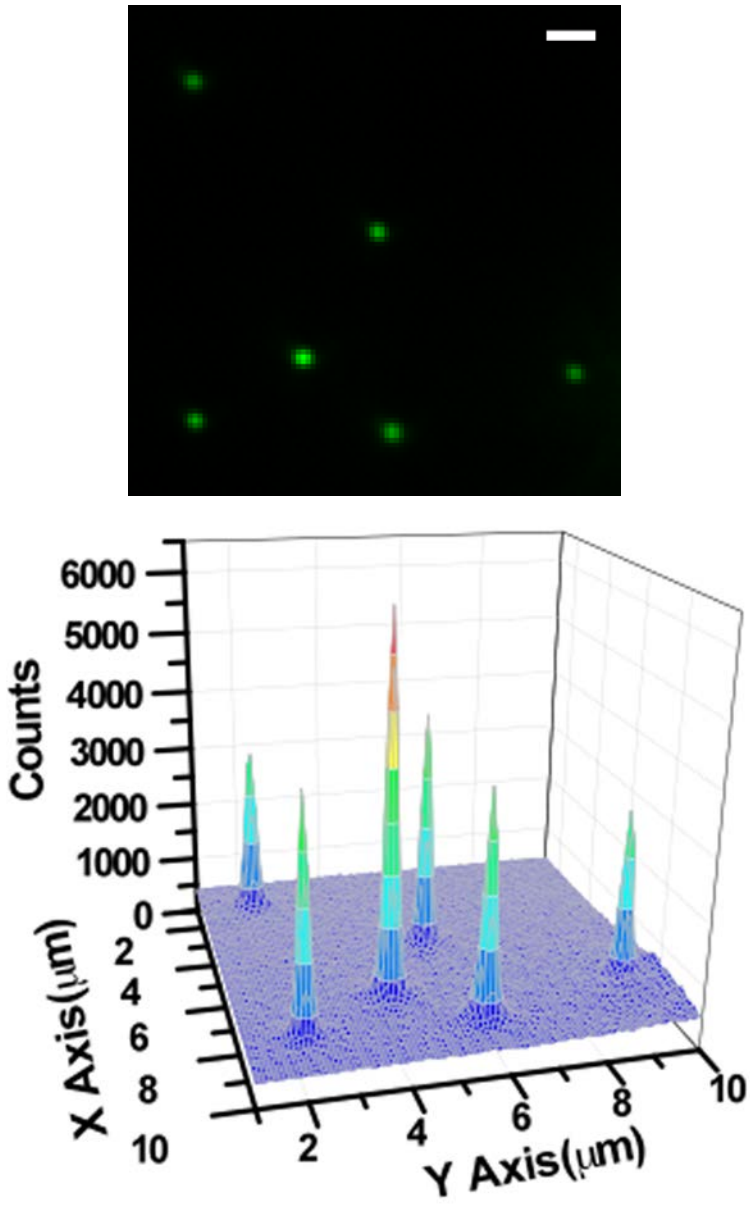


Figure S3. (Upper) Upconverted luminescent image of individual Lipo-UCNPs attached to a coverslip at the emission range of 510–560 nm under 980 nm pulsed laser widefield excitation and (Bottom) corresponding point spread functions (PSFs) of individual Lipo-UCNPs spots. Data taken at 30 ms exposure time.

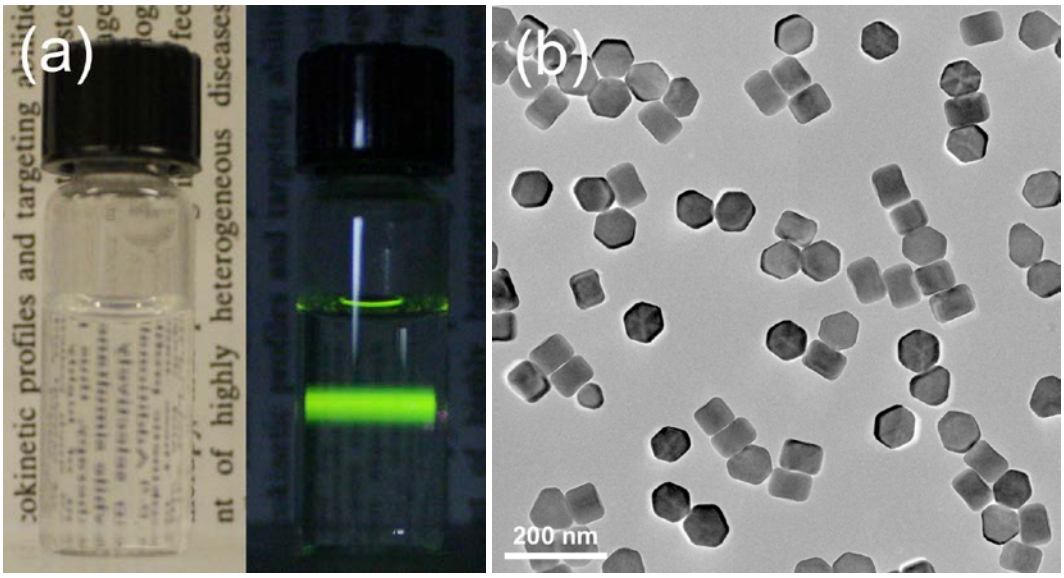


Figure S4. Lipo-UCNPs showed prominently long-term stability in water and resistance to aggregation over extended periods of time. (a) Photographs of the transparent water solution of Lipo-UCNPs stored in water at room temperature for three months without laser illumination (left) and the upconverted visible luminescence under continuous-wave 980 nm laser illumination (right). (b) TEM images of the Lipo-UCNPs sample stored in water at room temperature for three months, indicating they remain monodisperse in size without aggregation.

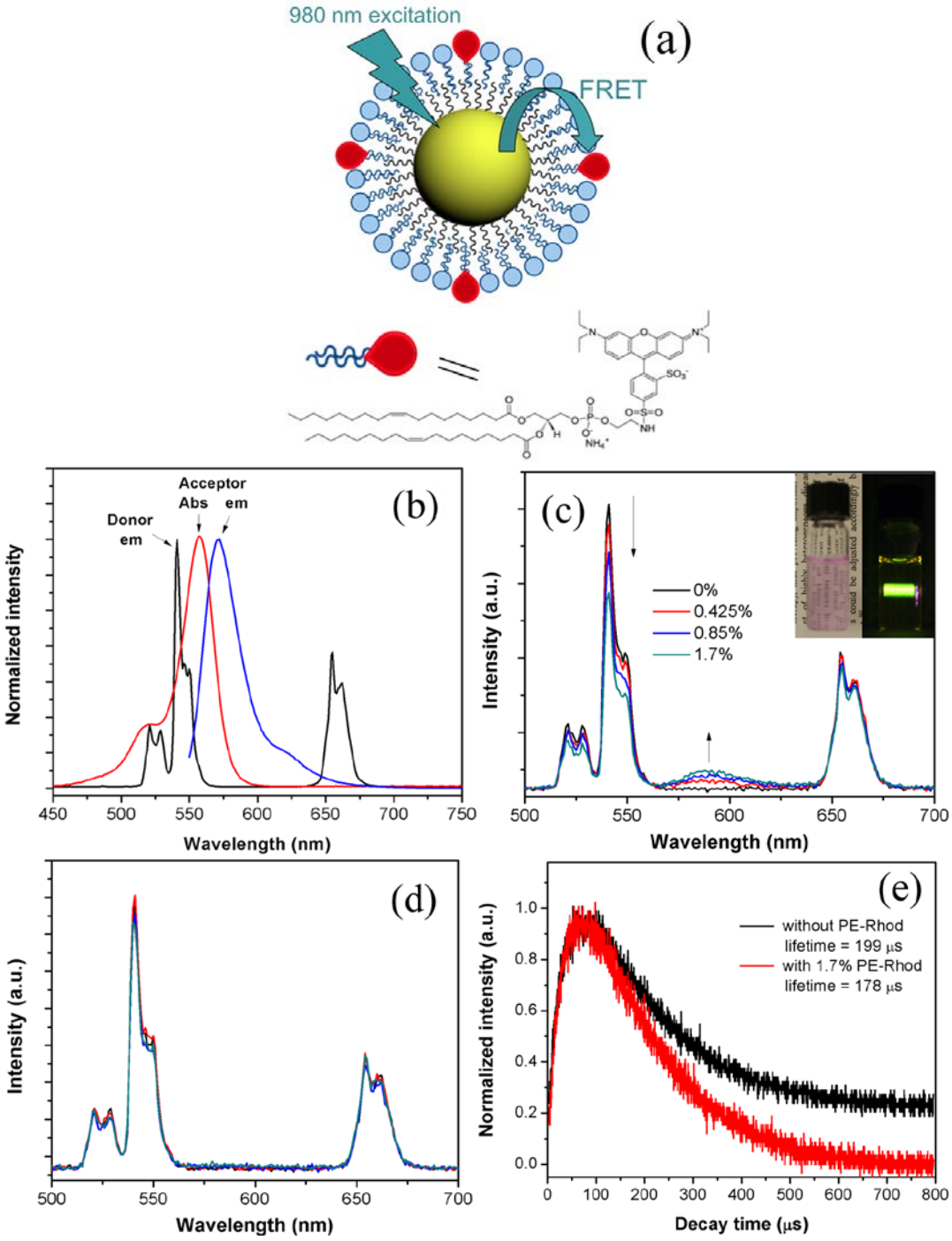


Figure S5. (a) Schematic illustration of the FRET nanoprobes based on organic dye (rhodamine B) attached Lipo-UCNPs, which was synthesized through one-step assembly by using DSPE-PEG and a rhodamine B labeled phospholipid (PE-Rhod). A series of Lipo-UCNPs with varying amounts of PE-Rhod were synthesized by changing the ratio of PE-Rhod and DSPE-PEG in their lipid coating. (b) Absorbance spectra (red line) and emission spectra (blue line) of PE-Rhod in chloroform, and upconversion luminescence spectra (black line) of Lipo-UCNPs in water. The luminescence emission peak (540 nm) of Lipo-UCNPs significantly overlap with the absorbance peak of rhodamine B, which is a prerequisite for FRET. (c) Fluorescence spectra of Lipo-UCNPs coated with varying amounts of PE-Rhod under 980 nm excitation (Inset: Photographs of the water solution of PE-Rhod coated (1.7%) Lipo-UCNPs without laser illumination (left) and the upconverted visible luminescence 980 nm laser illumination (right)). When exposed to 980 nm NIR laser, the characteristic emission peak of Lipo-UCNPs at 540 nm were reduced gradually with an increasing amount of PE-Rhod, while this decrease was accompanied by an increase in

the intensity of the emission peak of rhodamine B at 590 nm, suggesting the occurrence of FRET from UCNPs to the organic dye molecules. (d) Fluorescence spectra for the control experiments by mixing as-prepared UCNPs with the same amounts of PE-Rhod in cyclohexane under otherwise identical conditions, where neither the decrease of upconversion luminescence nor the appearance of the emission of the organic dye were observed, indicating that UCNPs and PE-Rhod are far apart in solution and hence no FRET occurs. This further confirms the FRET from UCNPs to the organic dyes for Lipo-UCNPs is related to the close coating of PE-Rhod lipids. (e) Luminescence decay curves of Lipo-UCNPs coated with varying amounts of PE-Rhod (0% and 1.7%). λ_{ex} = 980 nm, λ_{em} = 540 nm (represent the $^4S_{3/2}$ to $^4I_{15/2}$ transitions for Er^{3+}). It is well known that FRET would cause a decrease of the luminescence lifetimes of the donor. To confirm this point, we measured the luminescence lifetimes of the UCNPs emission for two different samples (with or without PE-Rhod). In Figure S5e the fluorescence decay curves portray an obvious reduction in the UCNPs excited state lifetime with the coating of PE-Rhod. The decreased luminescence lifetimes of the UCNPs observed further confirm the FRET from UCNPs donor to the dye molecule acceptor.

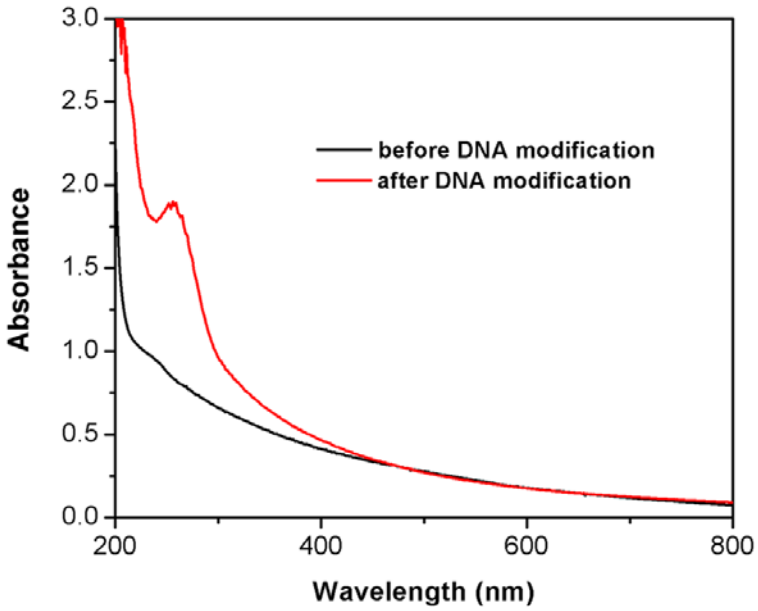


Figure S6. The UV/Vis spectrum of the maleimide-functionalized Lipo-UCNPs before and after the DNA-conjugation reaction.

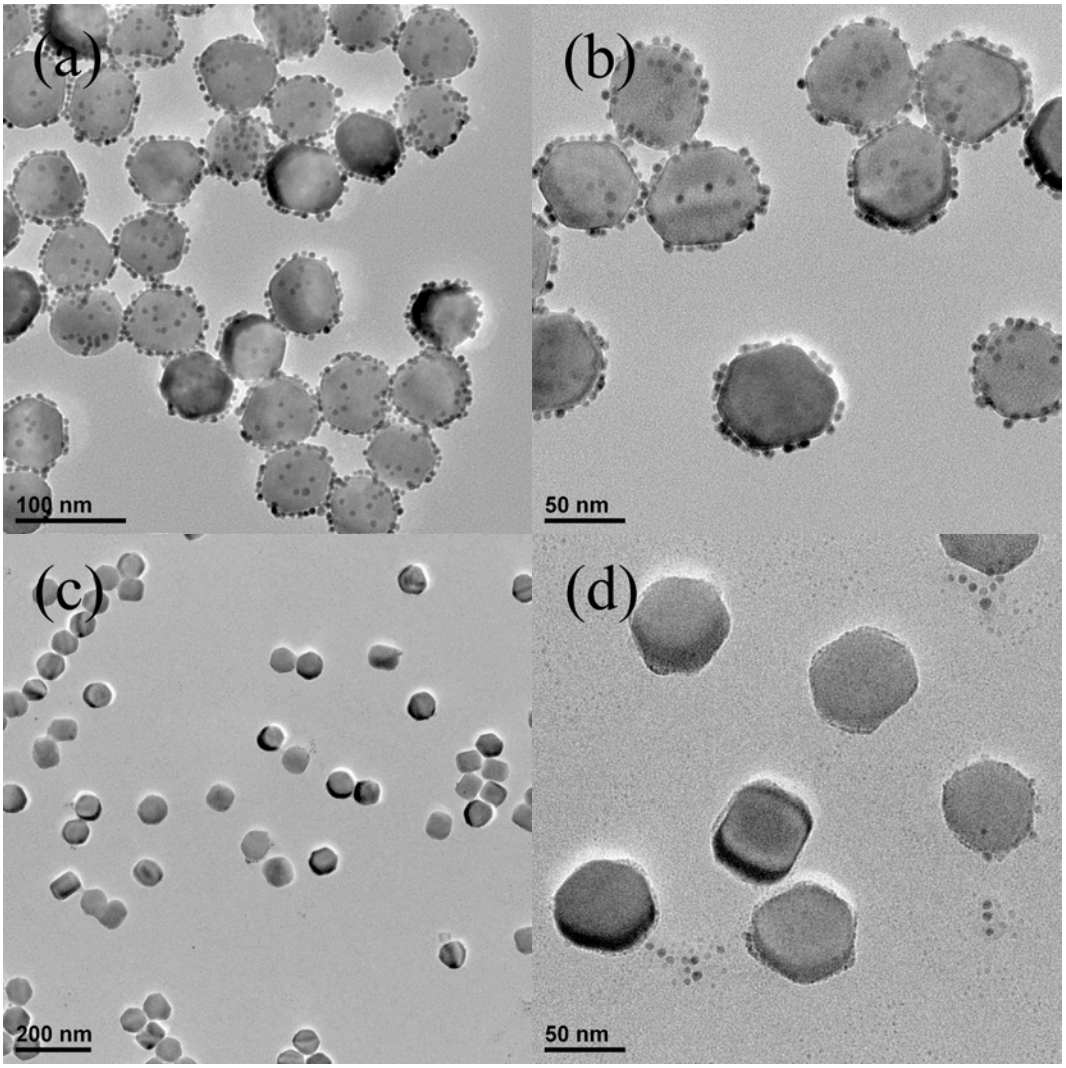


Figure S7. TEM images of bioinspired Lipo-UCNP-AuNPs nanoassemblies directed by (a,b) complementary DNA and (c,d) noncomplementary DNA.

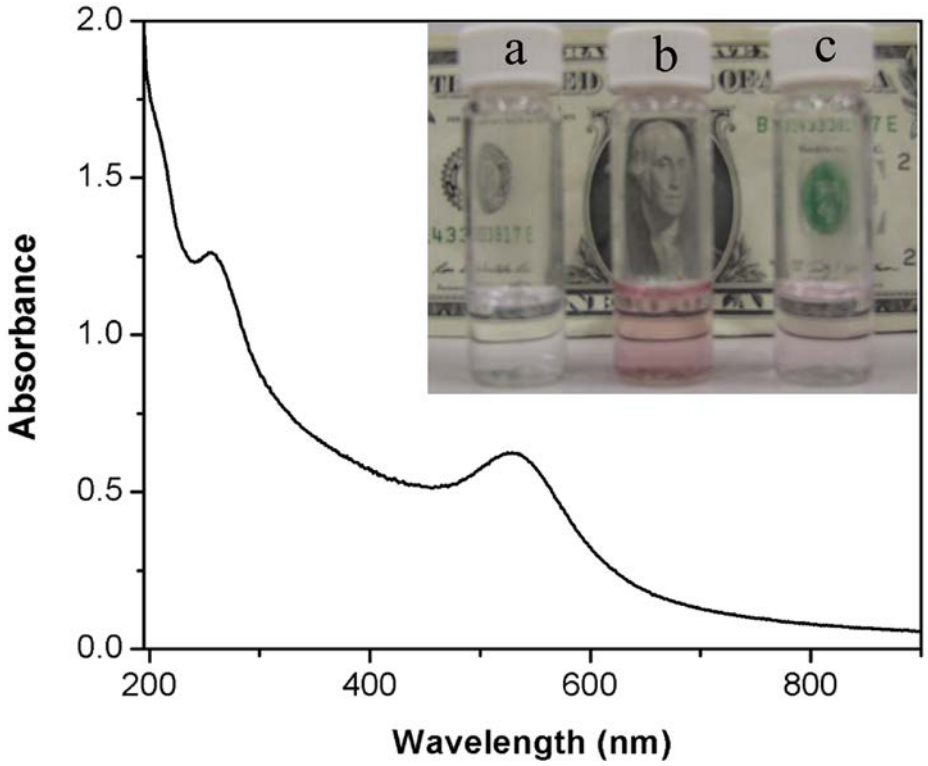


Figure S8. The UV/Vis spectrum of the bioinspired Lipo-UCNP-AuNPs nanoassemblies directed by complementary DNA. The bands at ca. 260 and 520 nm were assigned to DNA and attached AuNPs, respectively. Inset: Photographs of the water solution of (a) the DNA conjugated Lipo-UCNP, (b) DNA conjugated Lipo-UCNP after assembly with AuNPs that was modified with complementary DNA, (c) DNA conjugated Lipo-UCNP after assembly with AuNPs that was modified with noncomplementary DNA.

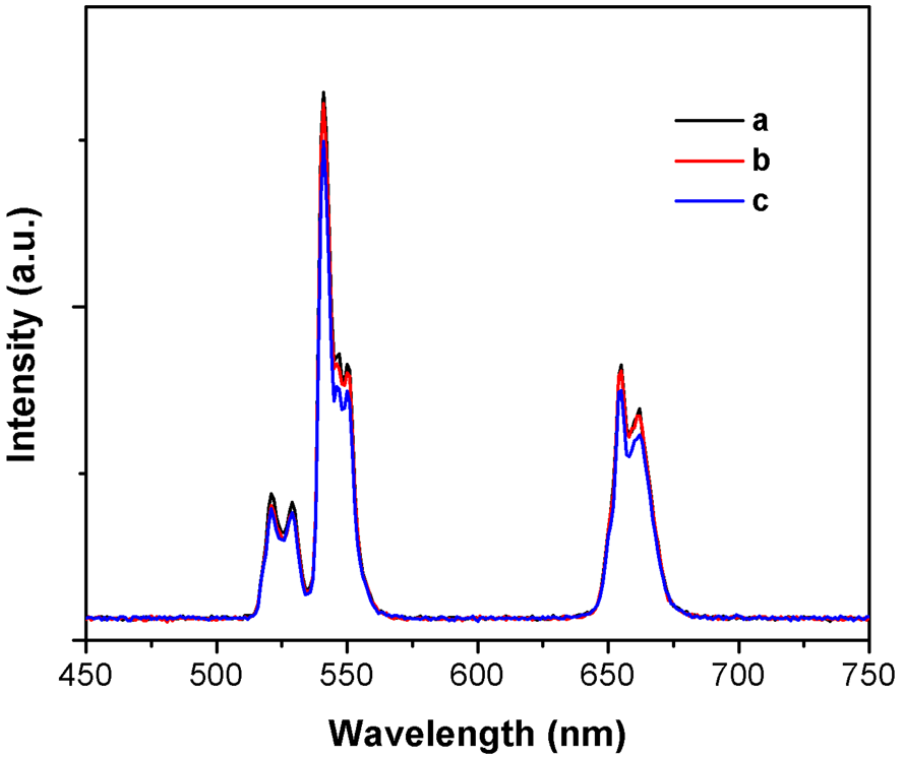


Figure S9. Room-temperature upconversion luminescence spectra of (a) the DNA conjugated Lipo-UCNP, (b) DNA conjugated Lipo-UCNP after assembly with AuNPs that was modified with noncomplementary DNA, (c) DNA conjugated Lipo-UCNP after assembly with AuNPs that was modified with complementary DNA.

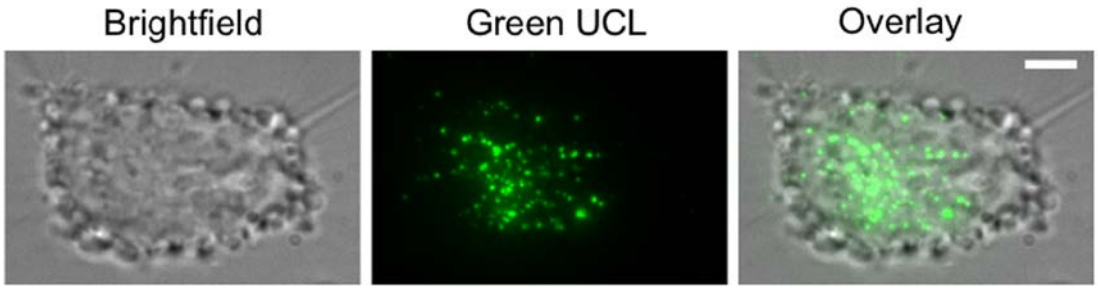


Figure S10. Images of HeLa cells treated with Lipo-UCNP-FA. The luminescent UCNP image is the z projection of a stack of 3D scan images of the cell, and the emission was collected in the range of λ_{em} = 510–560 nm. Scale bar = 5 μm. For 3D representation, see Movie S1.

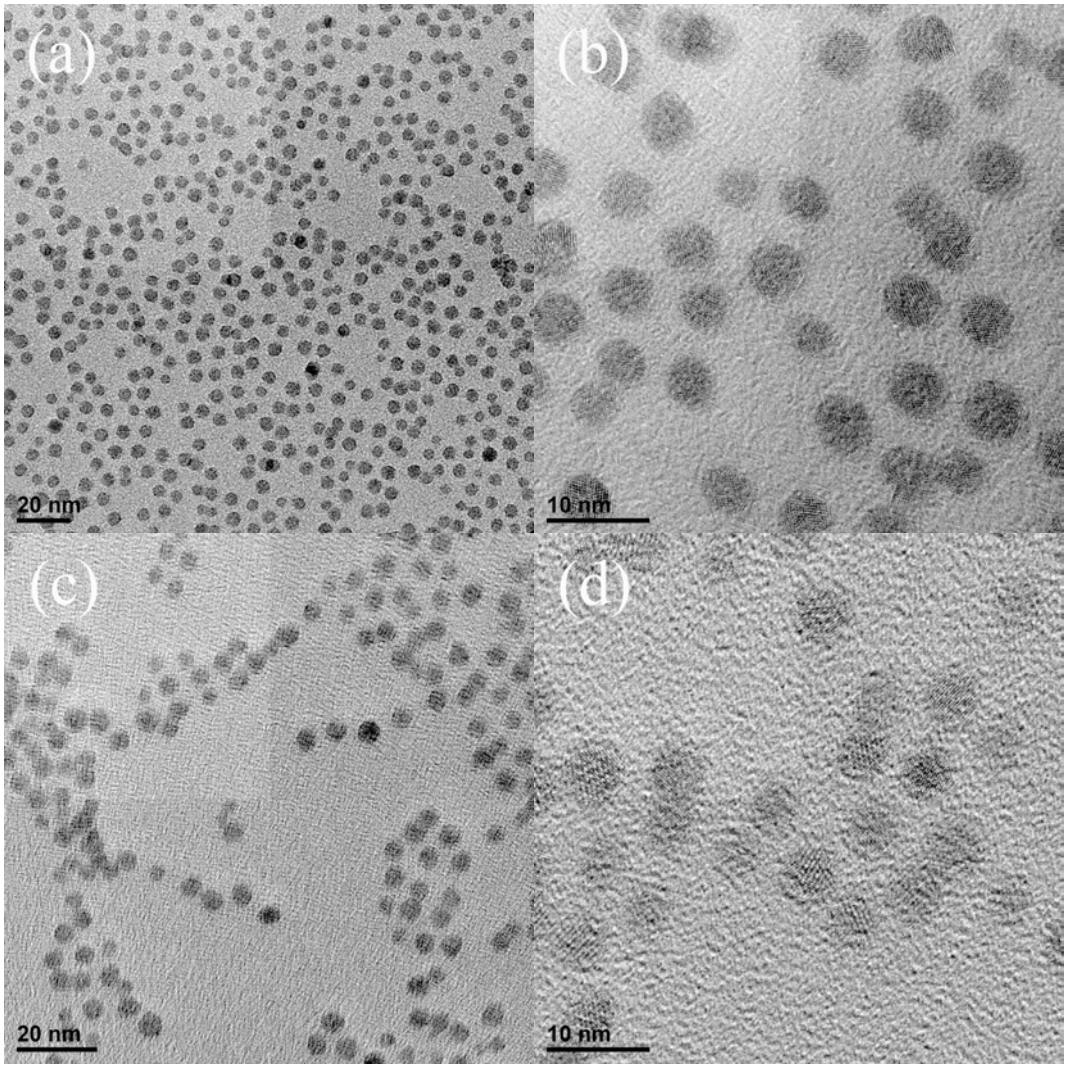


Figure S11. TEM images of the (a,b) as-prepared and (c,d) phospholipids DSPE-PEG coated ~6 nm $NaGdF_4$ nanoparticles deposited on TEM grid from a drop of $NaGdF_4$ cyclohexane solution and Lipo-$NaGdF_4$ water solution, respectively.

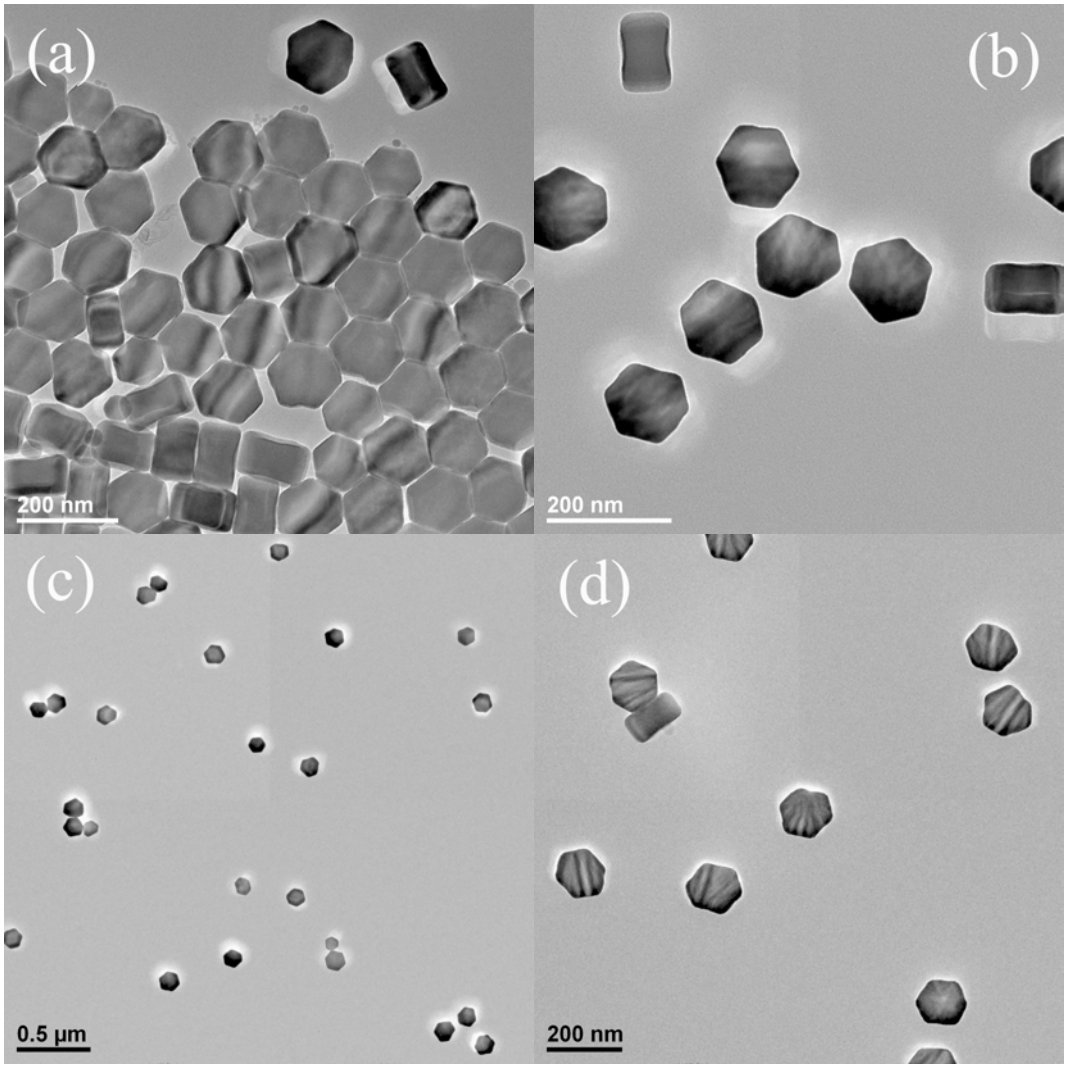


Figure S12. TEM images of the (a,b) as-prepared and (c,d) phospholipids DSPE-PEG coated ~130 nm $NaYF_4$ nanoparticles deposited on TEM grid from a drop of $NaYF_4$ cyclohexane solution and Lipo-$NaYF_4$ water solution, respectively.