

## Supporting Information for

### Regiospecific Hetero-Assembly of DNA-Functionalized Plasmonic Upconversion Superstructures

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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  $\text{YCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{YbCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{ErCl}_3 \cdot 6\text{H}_2\text{O}$ , oleic acid (90%), 1-octadecene ( $\geq 95\%$ ) were purchased from Sigma-Aldrich. 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG) was purchased from Avanti Polar Lipids. Tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were from Thermo Scientific. AuNPs with different sizes were purchased from Ted Pella, Inc. (Redding, CA). All DNA molecules were synthesized and purified by Integrated DNA Technologies, Inc. (IDT, Coralville, IA):

Sequence of DNA used for the assembly (from left to right: 5' to 3').

A27: HS-AAA AAA AAA AAA AAA AAA AAA AAA AAA

T27: HS-TTT TTT TTT TTT TTT TTT TTT TTT TTT

C27: HS-CCC CCC CCC CCC CCC CCC CCC CCC CCC

G20: HS-GGG GGG GGG GGG GGG GGG GG

Aptamer AS1411: HS-TTT TTT TTT GGT GGT GGT GGT TGT GGT GGT GGT GGT.

**Synthesis of UCNPs:**  $\beta\text{-NaYF}_4\text{:Yb,Er}$  nanoparticles were synthesized according to the literature methods.<sup>[1]</sup> Typically, 0.80 mmol of  $\text{YCl}_3 \cdot 6\text{H}_2\text{O}$ , 0.18 mmol of  $\text{YbCl}_3 \cdot 6\text{H}_2\text{O}$  and 0.02 mmol of  $\text{ErCl}_3 \cdot 6\text{H}_2\text{O}$  dissolved in an aqueous solution (2 mL) was added to a 50-mL three-neck flask charged with oleic acid (5 mL) and 1-octadecene (15 mL). The resulting mixture solution was heated to 160 °C for 45 min and then cooled down to room temperature. A methanol solution (10 mL) of  $\text{NH}_4\text{F}$  (4 mmol) and NaOH (2.5 mmol) was added and stirred at 50 °C for 30 min, followed by heating to 100 °C for 45 min to remove the methanol. Then the solution was heated to 315 °C under an argon atmosphere for 1.5 h and then cooled down to room temperature. The UCNPs were purified by centrifugation after the addition of ethanol

as the flocculent, washed extensively with ethanol/water (1:1), and re-dispersed in 10 mL of cyclohexane for further experiments.

***Synthesis of Phospholipids coated UCNPs (Lipo-UCNPs):*** Phospholipids coated UCNPs were prepared according to our previously reported method.<sup>[2]</sup> The oleic acid capped UCNPs in 1 mL of chloroform (100 nM) was mixed with 1 mL chloroform solution containing 15 mg DSPE-PEG in a screw-neck glass bottle. The glass bottle was left open in a fume hood for two days to evaporate the chloroform. The obtained mixed film was heated at 75 °C for 5 min to completely remove chloroform and then hydrated with MilliQ water (3 mL), and the UCNPs became soluble after vigorously sonication, which can be further stirred vigorously at 75 °C for 5 min. After cooled down to room temperature, the Lipo-UCNPs were purified by centrifugation to remove excess lipids, washed with MilliQ water, and re-dispersed in 3 mL MilliQ water for further experiments.

***Synthesis of DNA functionalized AuNPs (DNA-AuNPs):*** Polyvalent DNA-AuNP conjugates, regardless of DNA sequence, were prepared according to our previously reported method.<sup>[3]</sup> Thiol-modified DNA molecules were first activated by 1.5 equiv of TCEP under pH 5.2 for 1 h at room temperature before usage. A small volume (11 µL) of activated DNA (9 nmol) was added into 3 mL of AuNPs (10 nM, molar concentration) in a glass vials. Then, the vials were capped and kept at room temperature for about 16 h. Tris acetate buffer (500 mM, pH 8.2) was then added to the DNA/AuNPs solution to a final concentration of 5 mM, and NaCl (1M) was added to a final concentration of 100 mM. The DNA-functionalized AuNPs solution 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.

***Calculation of molar ratio of NPs:*** AuNPs of known molar concentration were purchased from Ted Pella, Inc. (Redding, CA). For UCNPs, the volume of a single hexagonal prism UCNPs was calculated from the TEM result. The single NP mass of UCNPs was estimated through multiply this volume by the density of NaYF<sub>4</sub> (4.21 g/cm<sup>3</sup>)<sup>[4]</sup>. Then the molar concentrations of UCNPs were calculated from their mass concentrations. Finally, molar ratio of AuNPs to UCNPs could be calculated through used molar concentrations and solution volume, respectively.

***Hetero-assembly of DNA-AuNPs/UCNP superstructures:*** In a typical hetero-assembly process, a small volume (i.e., 10 - 100  $\mu$ L) of concentrated DNA-AuNPs (A27 modified AuNPs) solution was added into Tris-HCl buffer solution (20 mM, 100 mM NaCl, 2 mM KCl, 1 mM MgCl<sub>2</sub>, pH 7.4) of Lipo-UCNPs (1.25 nM, molar concentration) with different NPs ratio (i.e., DNA-AuNPs : Lipo-UCNPs = 10:1, 26:1, 60:1, 200:1), which was then kept at room temperature overnight with shaking. The resulted DNA-AuNPs/UCNP assemblies are collected by centrifugation and washed with MilliQ water to remove unassembled DNA-AuNPs. The resulted assemblies were re-dispersed in MilliQ water after sonication for further usage.

For the preparation of Top assemblies, DNA molecules (A27, 5  $\mu$ M) were first incubated with Lipo-UCNPs (1.25 nM) in Tris-HCl buffer (20 mM, 100 mM NaCl, 2 mM KCl, 1 mM MgCl<sub>2</sub>, pH7.4) with shaking for 2 h. The nanoparticles were recovered by centrifugation and re-dispersed in buffer solution. A small volume of concentrated DNA-AuNPs (A27-modified AuNPs) solution was then added into the above Lipo-UCNPs solution with the NPs ratio of 30:1, which was then kept at room temperature for 3 h with shaking. The Top assemblies were purified by centrifugation to remove unassembled DNA-AuNPs, washed with MilliQ water, and re-dispersed in 1 mL MilliQ water for further usage.

To study the effect of salt concentration and pH on the assembly, the NPs assembly was conducted in Tris-HCl buffer (pH 7.4, 20 mM) containing varying concentrations of NaCl or designed buffer solutions of different pH value (acetate 5 and Tris for pH 7.4 and 8.6, 20 mM each). To investigate whether this method can be generally applied to DNA strands with different sequences, thiolated T27, C27, and G20 were used to modify the AuNPs and repeat the above assembly procedures with a DNA-AuNPs to Lipo-UCNPs ratio of 26:1. Oleic acid free UCNPs were prepared according to the literature methods,<sup>[5]</sup> and used for the NPs assembly with a DNA-AuNPs to UCNPs ratio of 26:1.

***Analysis of cellular uptake:*** HeLa cells were grown in chamber slides in 2 mL Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 units/ml aqueous penicillin G, 100  $\mu$ g/mL streptomycin, 0.4 mM L-Glutamine and 10% FBS in 37°C CO<sub>2</sub> incubator at concentrations to allow 70% confluence in 24 h. Chambers are rinsed once with fresh medium,

before they are refilled with pre-warmed fresh aforementioned cell medium containing DNA-AuNPs/UCNP nanoparticles (1.25 nM). After incubation in 37°C CO<sub>2</sub> incubator for 12 h, medium was then removed and treated HeLa cells were washed with prewarmed PBS (3 × 2 mL). Thereafter, fluorescence images are immediately taken on a Zeiss LSM 710 Scanning Confocal Microscope. Nucleuses were stained by DAPI.

**Targeted dual-modality imaging of cancer cells:** 4T1 cells were grown in chamber slides in RPMI-1640 medium supplemented with 100 units/ml aqueous penicillin G, 100 µg/mL streptomycin, and 10% FBS at concentrations to allow 70% confluence in 48 h. On the day of experiments, chambers are rinsed once with fresh medium, before they are refilled with pre-warmed Opti-MEM medium containing aptamer or control DNA modified nano-assemblies (100 µg/ml). After incubation for 2 h, 4T1 cells were washed with prewarmed PBS (3 × 200 µL) and fixed with 4% (wt/wt) paraformaldehyde. Dual-modality imaging was taken on an FV1000 multiphoton confocal microscopy. Upconverted luminescence cellular imaging was excited in a 150µm × 150µm area by a 980 nm laser with a power of 2.4 mW at 20 µsec/pixel.

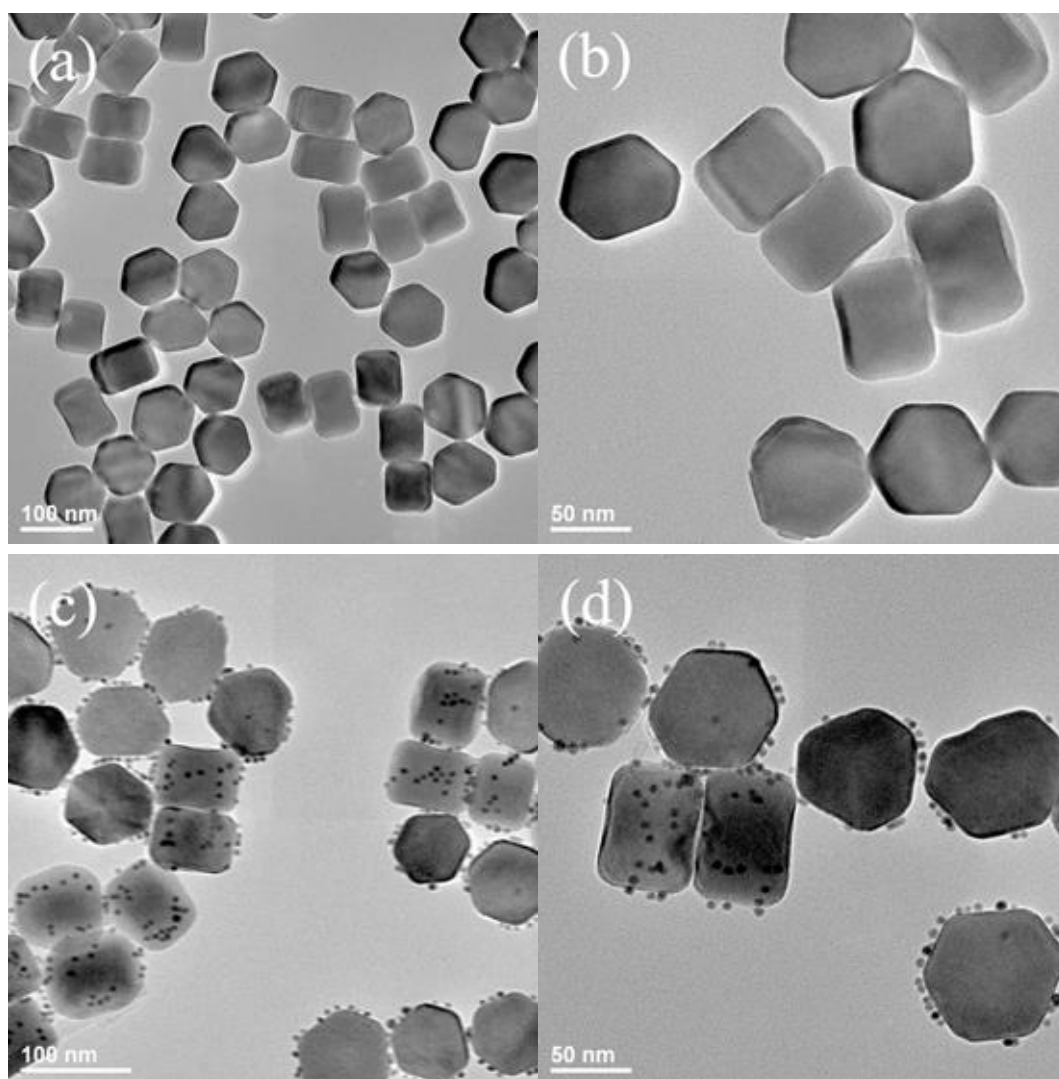
**Instrumentation.** Transmission electron microscopy (TEM) images were taken on the JEOL 2100 Cryo transmission electron microscope with an accelerating voltage of 200 kV. UV/Vis spectra were recorded on a Hewlett–Packard 8453 spectrometer. Fluorescent spectra were recorded on a FluoroMax-P fluorimeter (HORIBA Jobin Yvon Inc., Edison, NJ) coupled with a commercial CW IR laser (980 nm) (Thorlabs, Inc.). Confocal microscopy images were taken by Zeiss LSM 710 Laser Scanning Confocal Microscope equipped with a Spectra-Physics Mai Tai HP pulsed laser.

## Reference

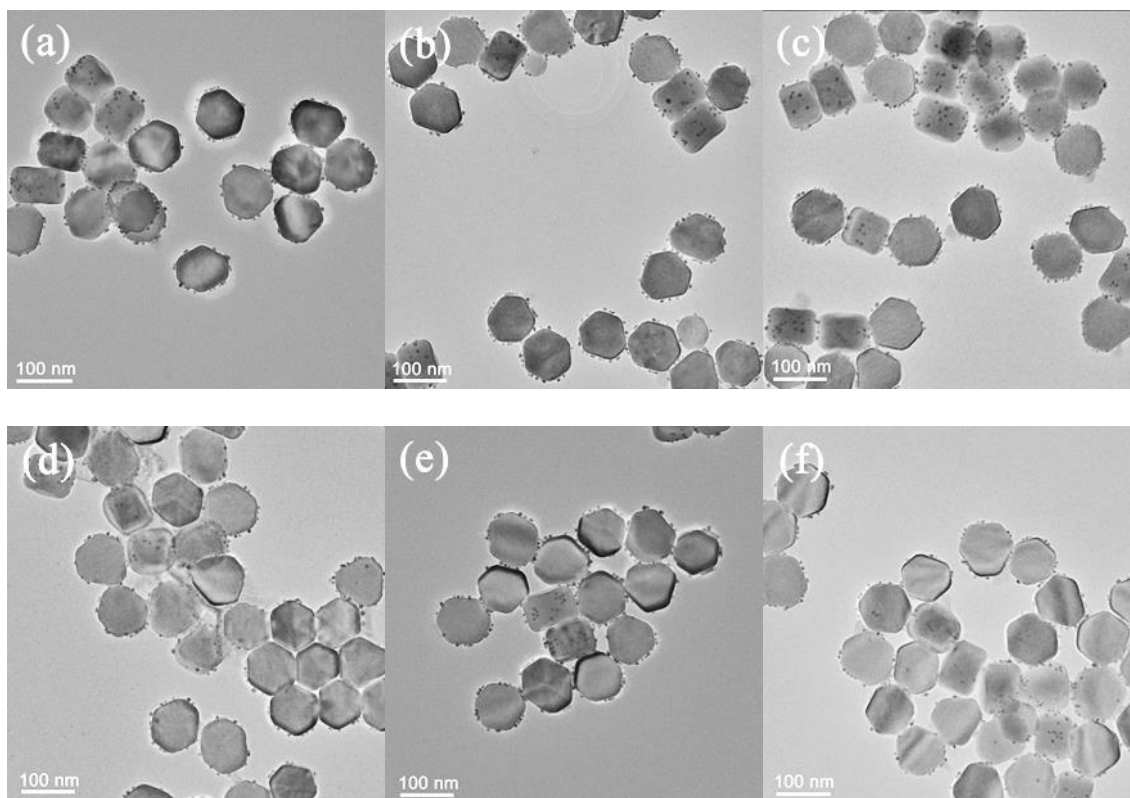
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- [2] Li, L.-L.; Zhang, R.; Yin, L.; Zheng, K.; Qin, W.; Selvin, P. R.; Lu, Y. *Angew. Chem., Int. Ed.* **2012**, *51*, 6121.
- [3] Liu, J.; Lu, Y. *Nat. Protoc.* **2006**, *1*, 246.

[4] (a) Pyatenko, Y. A.; Voronkov, A. A. *J. Struct. Chem.* **1962**, *3*, 696; (b) Cheng, L.; Yang, K.; Zhang, S.; Shao, M.; Lee, S.; Liu, Z. *Nano Res.* **2010**, *3*, 722.

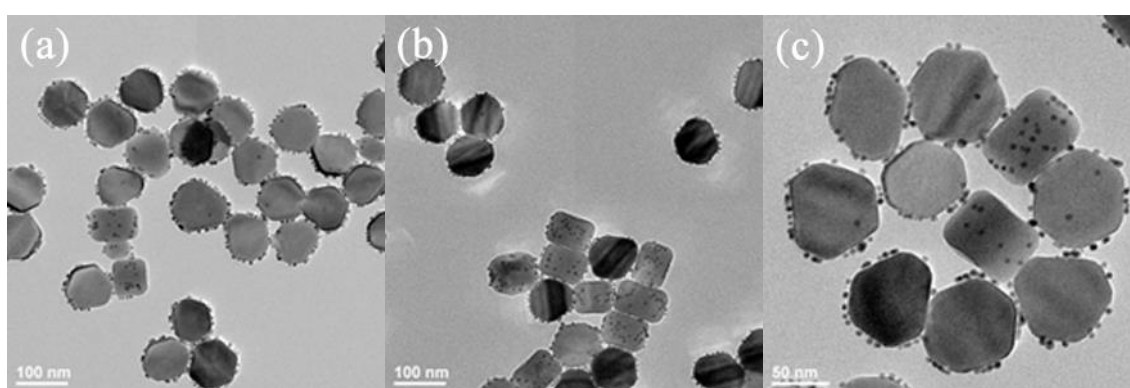
[5] Dong, A.; Ye, X.; Chen, J.; Kang, Y.; Gordon, T.; Kikkawa, J. M.; Murray, C. B. *J. Am. Chem. Soc.* **2011**, *133*, 998.



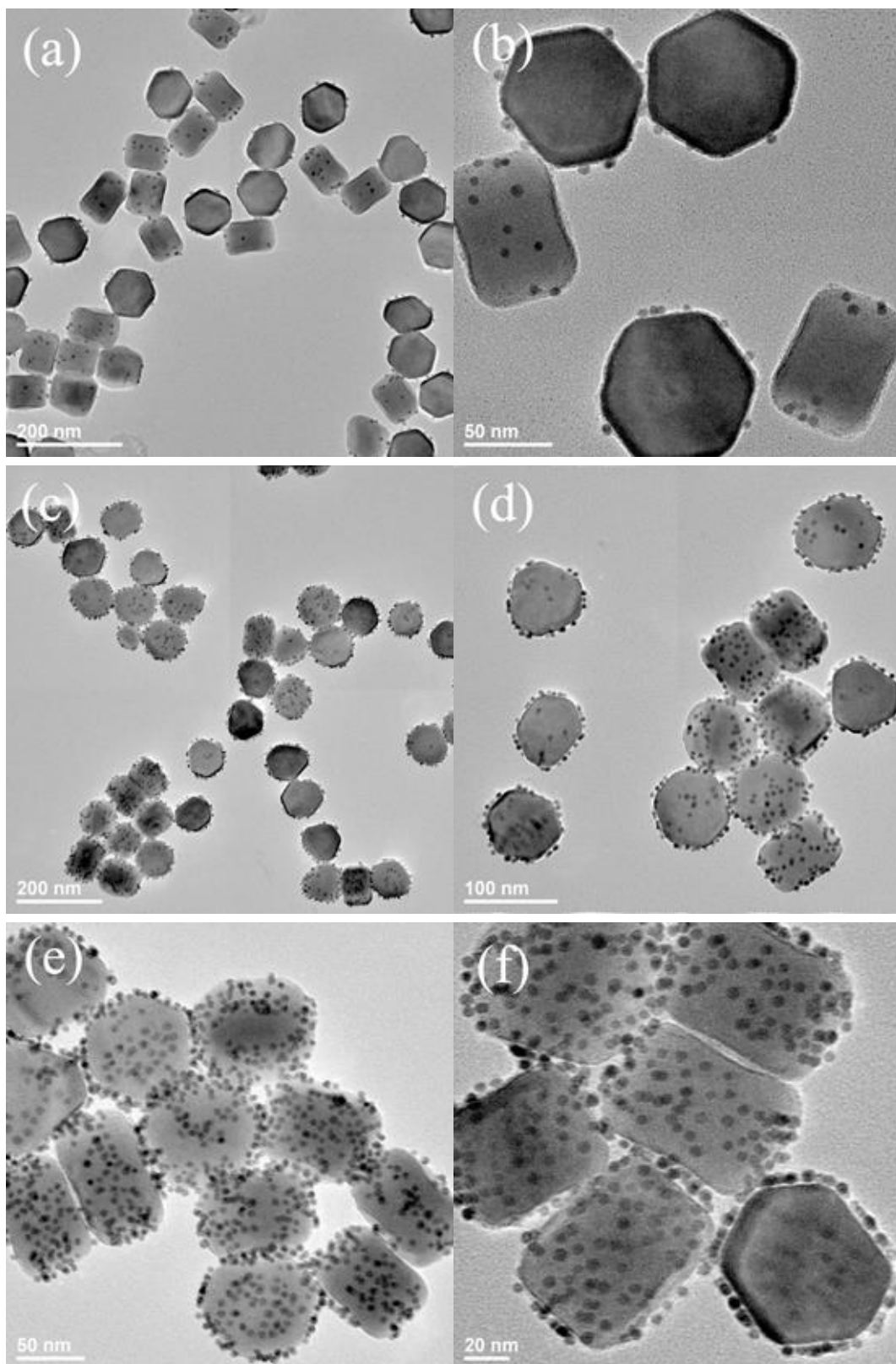
**Figure S1.** TEM images of the (a,b) as-prepared and (c,d) DNA-AuNPs/UCNP Side assemblies, which stand on the TEM grids on either the bottom face or the side face.



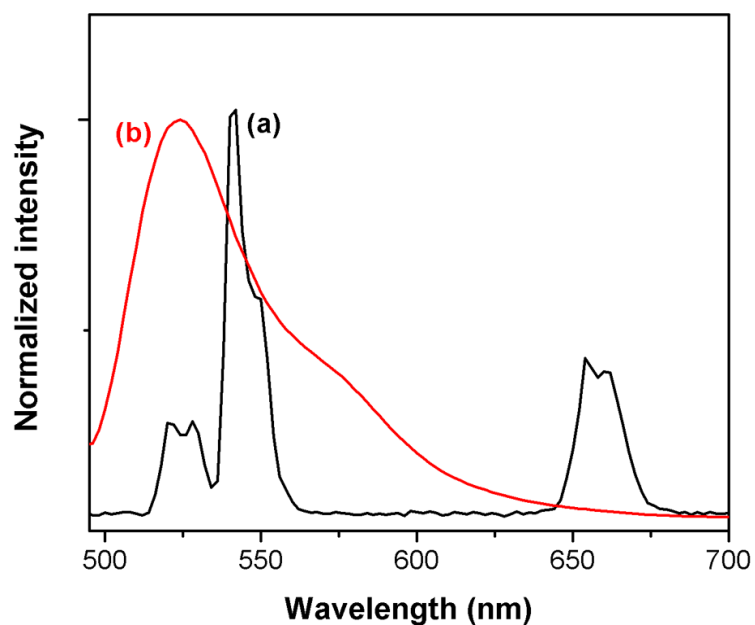
**Figure S2.** TEM images of the DNA-AuNPs/UCNP Side assemblies prepared in Tris-HCl buffer (pH 7.4, 20 mM) containing varying concentrations of NaCl: (a) 0 mM, (b) 50mM, (c) 150 mM; TEM images of the DNA-AuNPs/UCNP Side assemblies prepared in designed buffer solutions (20 mM) of different pH value: (d) acetate for pH 5, (e) Tris for pH 7.4 (f) Tris for pH 8.6.



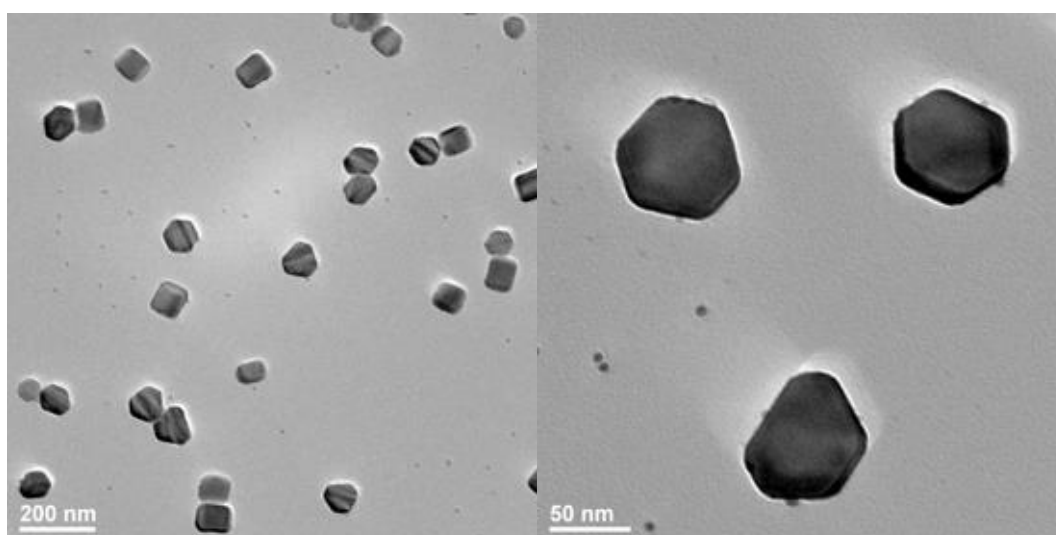
**Figure S3.** TEM images of the DNA-AuNPs/UCNP Side assemblies prepared from (a) T27, (b) C27, and (c) G20. It showed that DNA of other sequences could also be used by the same method for the hetero-assembly.



**Figure S4.** TEM images of the DNA-AuNPs/UCNP assemblies produced from the NPs ratio of Lipo-UCNPs to DNA-AuNPs of (a,b) 1:10, (c,d) 1:60 and (e,f) 1:200.

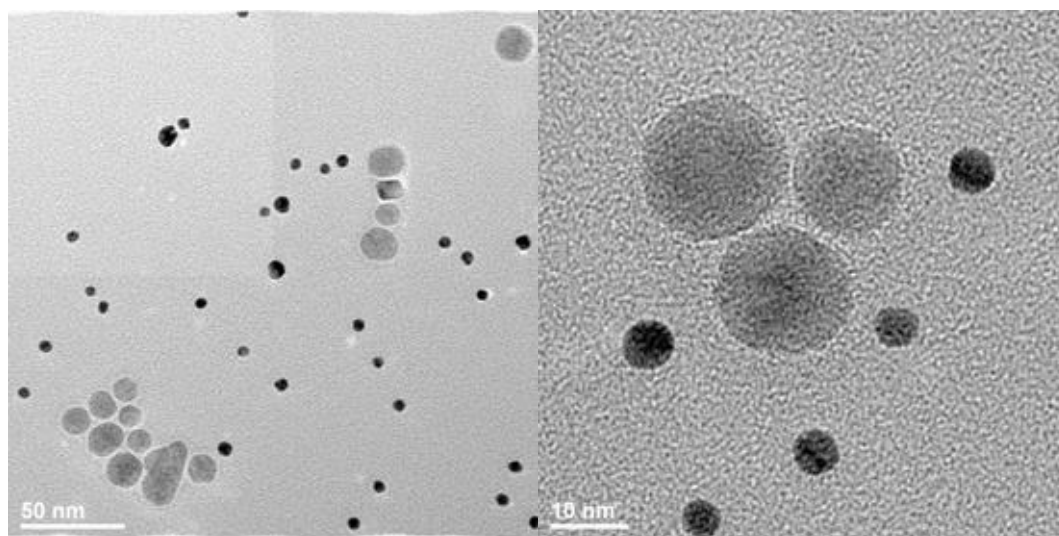


**Figure S5.** Room-temperature fluorescence spectra of Lipo-UCNPs after incubation with FAM dye-labeled DNA upon excitation with (a) 980 nm and (b) 488 nm, which was attributed to UCNPs and FAM dye respectively.

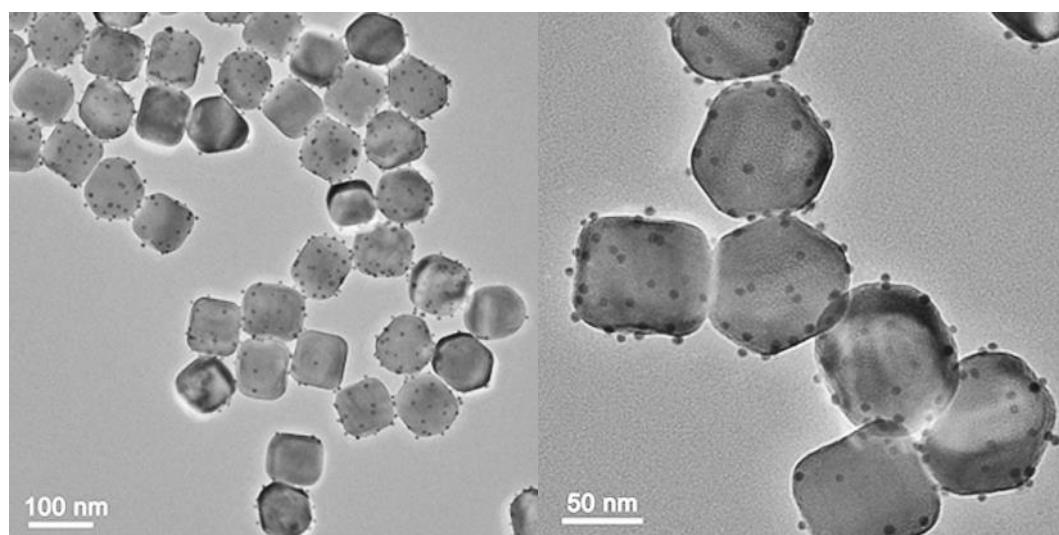


**Figure S6.** TEM images of the Lipo-UCNPs after incubation with PEG-modified AuNPs.

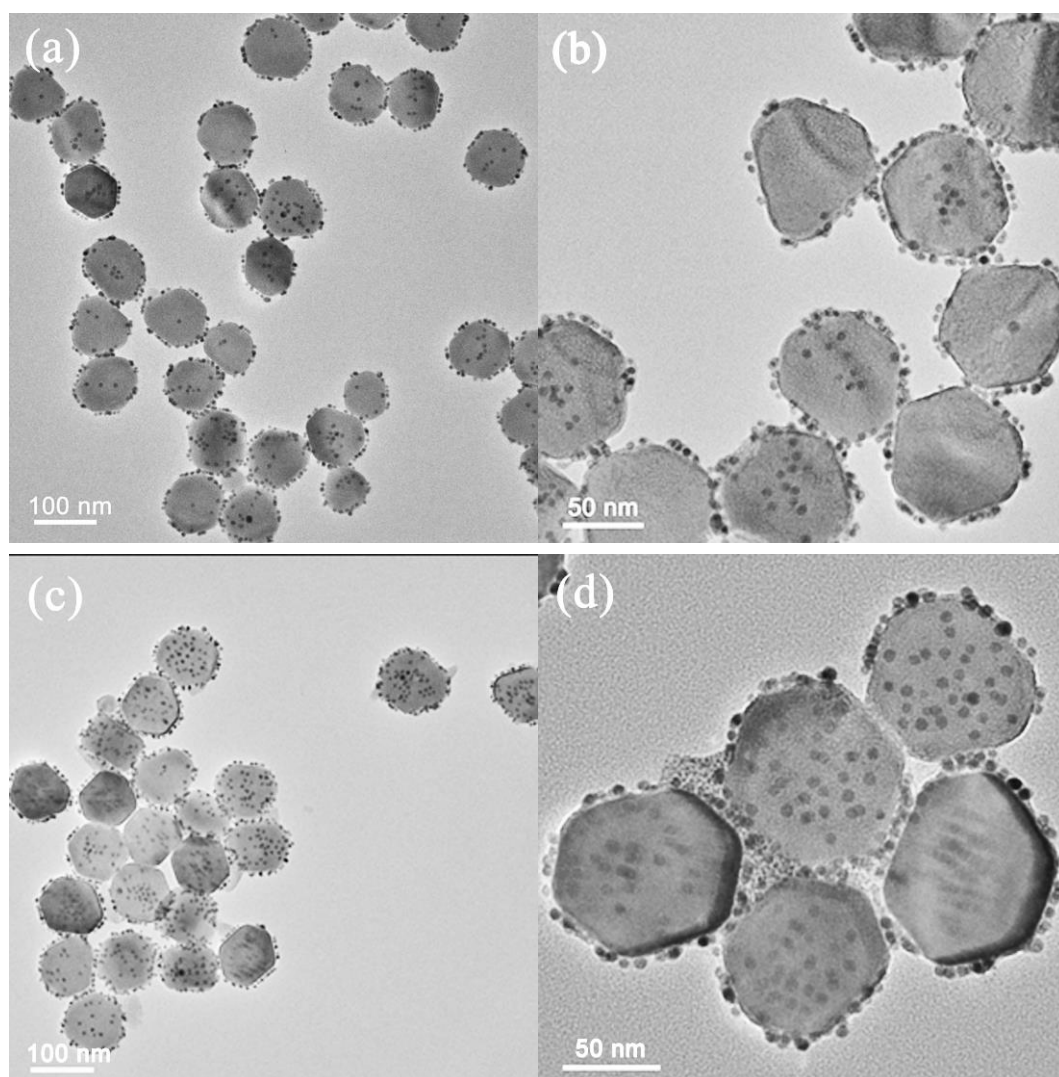




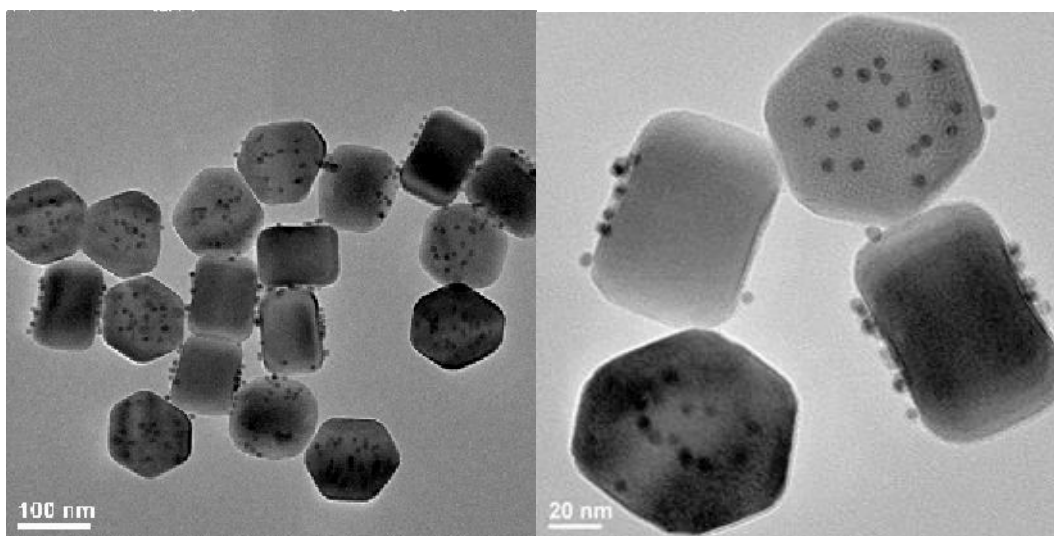
**Figure S7.** TEM images of the DSPE-PEG coated iron oxide nanoparticles (about 20 nm) after incubation with DNA-AuNPs.



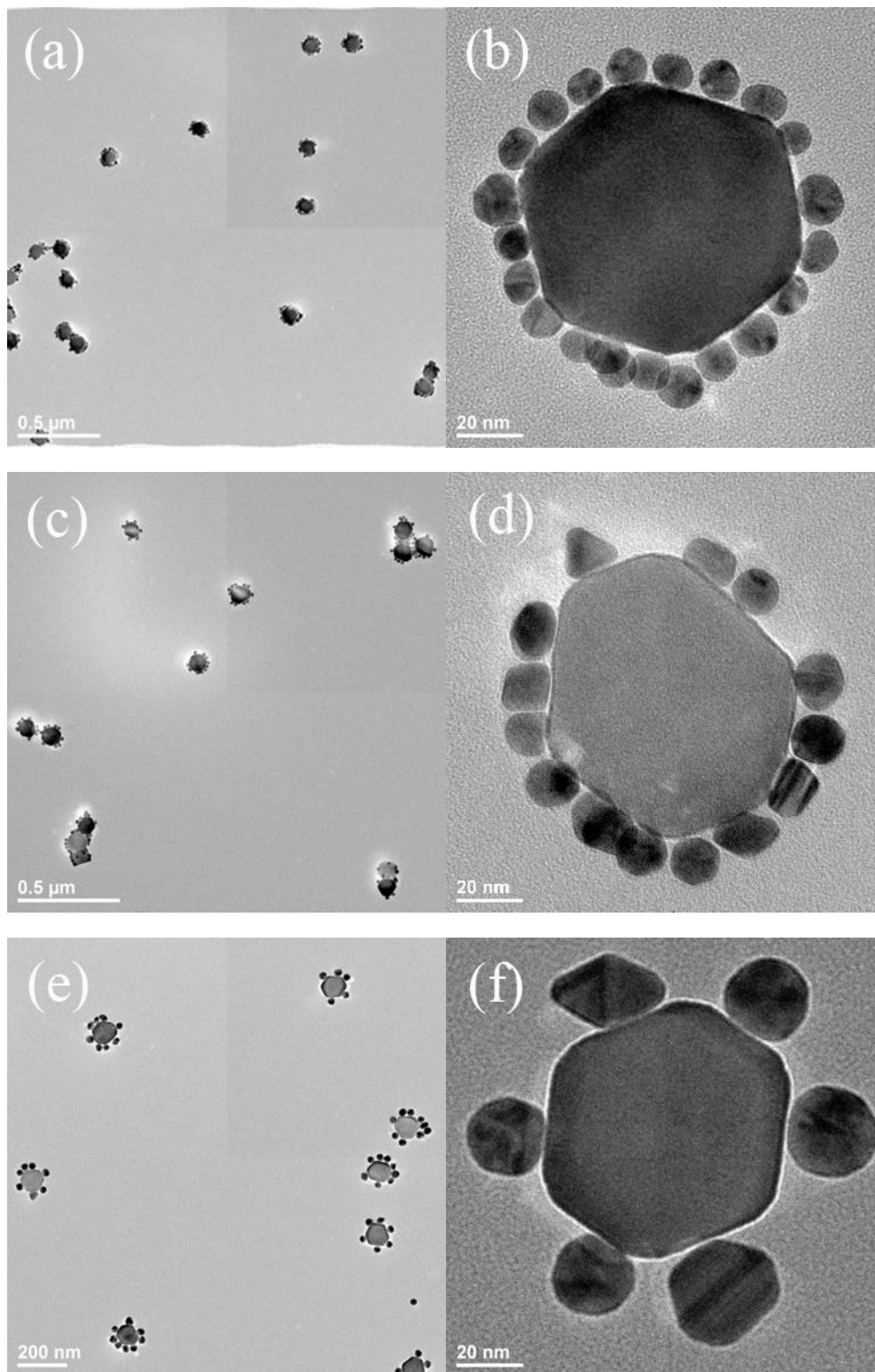
**Figure S8.** TEM images of the superstructures assembled from oleic acid free UCNPs and DNA-AuNPs with a NP ratio of 1:26.



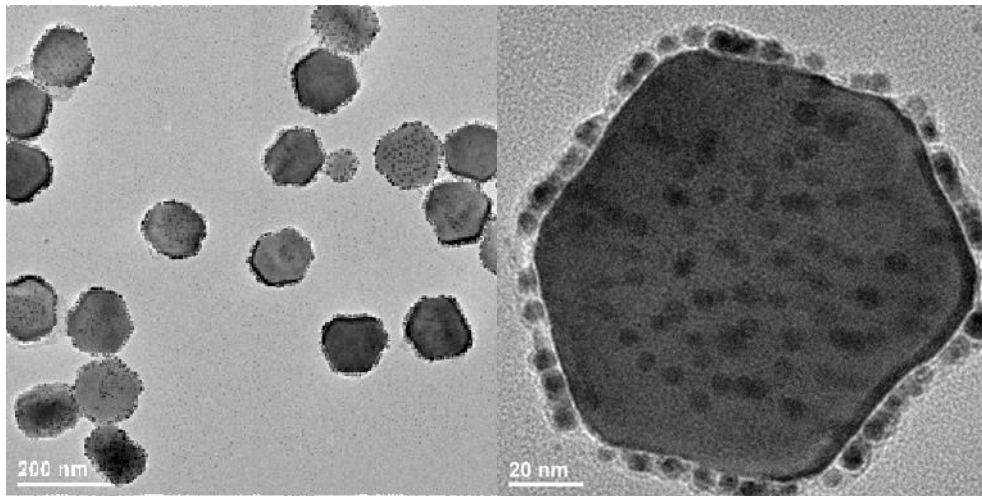
**Figure S9.** TEM images of the superstructure (assembled from DNA-AuNPs and Lipo-UCNPs with a NP ratio of 60:1) (a,b) before and (c,d) after incubation with DNA-AuNPs with a NP ratio of 1:26.



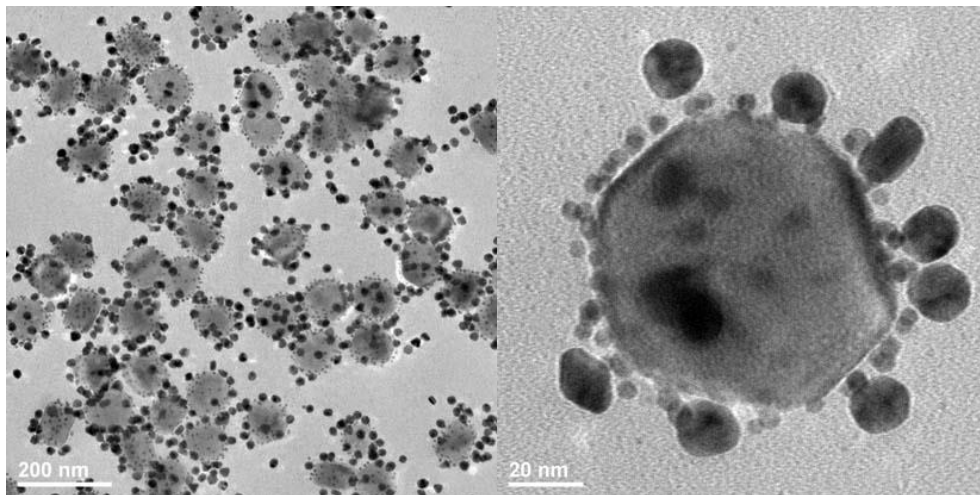
**Figure S10.** TEM images of DNA-AuNPs/UCNP Top assemblies.



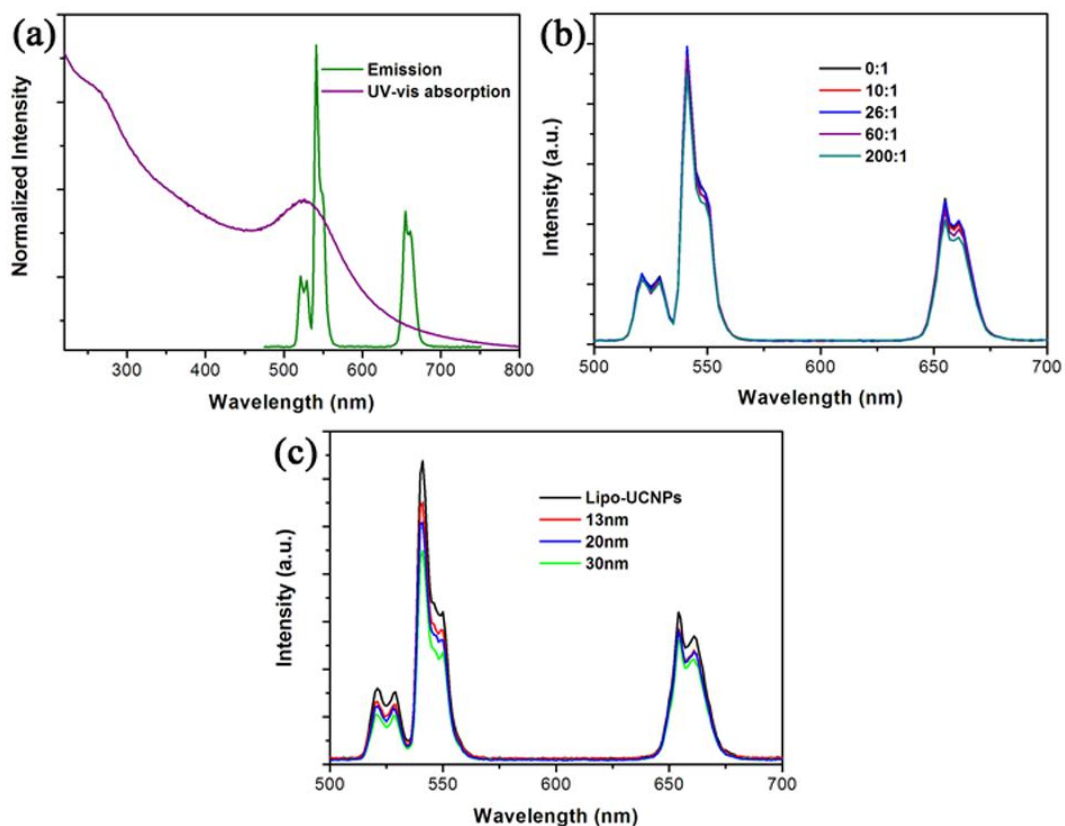
**Figure S11.** TEM images of DNA-AuNPs/UCNP superstructures assembled from Lipo-UCNPs and DNA-modified (a,b) 13 nm, (c,d) 20 nm, and (e,f) 30 nm AuNPs.



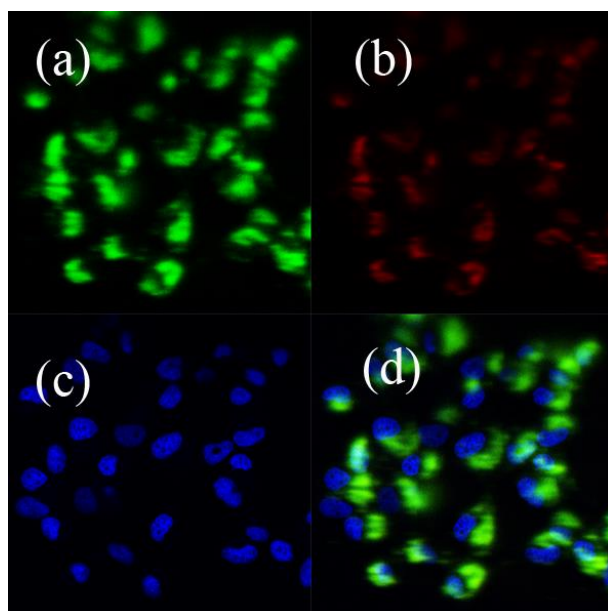
**Figure S12.** TEM images of DNA-AuNPs/UCNP Satellite superstructures assembled from 150 nm Lipo-UCNPs and DNA-modified 5 nm AuNPs.



**Figure S13.** TEM images of ternary NPs superstructures assembled from 20 nm AuNPs modified with cDNA and Satellite assemblies.



**Figure S14.** (a) UV-vis absorption spectrum and upconversion emission spectrum ( $\lambda_{\text{ex}} = 980$  nm) of DNA-AuNPs/UCNP Satellite superstructure. (b) Upconversion emission spectra of the DNA-AuNPs/UCNP superstructures assembled from different NPs ratio of DNA-AuNPs to Lipo-UCNP. (c) Upconversion emission spectra of DNA-AuNPs/UCNP superstructures assembled from Lipo-UCNPs and DNA-modified 13 nm, 20 nm, and 30 nm AuNPs. The distance between AuNP to UCNP was estimated as about 10 nm based on the length of DNA molecules (A27) used for the assembly. In these systems, minimal AuNPs-induced change of upconversion luminescence was observed with increasing loading of DNA-AuNPs on UCNP. However, with increasing the size of the assembled AuNPs, the luminescence quenching effect was observed probably due to the much higher extinction coefficients of larger AuNPs.



**Figure S15.** Confocal Microscopy images of HeLa cells treated with DNA-AuNPs/UCNP Satellite assemblies. For UCNP imaging channel,  $\lambda_{\text{ex}} = 980$  nm, and emission was collected in the range of (a)  $\lambda = 510\text{--}560$  nm and (b)  $630\text{--}680$  nm; (c) Fluorescence imaging of DAPI-stained nuclei; (d) The overlay of panels a–c.