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ADVANCED MATERIALS

Supporting Information

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Propeller-Like Nanorod-Upconversion Nanoparticle Assemblies with Intense Chiroptical Activity and Luminescence Enhancement in Aqueous Phase

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Experimental Section

Preparation of gold nanorods. NRs with different longitudinal plasmon absorption bands were synthesized using the modified Au seed-mediated growth method [1]. Typically, 0.6 mL of ice-cold sodium borohydride solution ($NaBH_4$, 10 mM) was quickly added to a 10 mL mixture of 0.25 mM tetrachloroaurate trihydrate ($HAuCl_4$) and 0.1 M hexadecyltrimethylammonium bromide (CTAB) solution. After vigorous stirring for 5 min, the seed solution was obtained and left at 25°C before use. The NR growth solution was obtained by adding in the following order: CTAB (0.1 M, 4 mL), $HAuCl_4$ (0.01 M, 2 mL), silver nitrate ($AgNO_3$, 0.4 mL of 0.01 M), and hydrochloric acid (HCl, 0.8 mL of 1 M) to 40 mL of 0.1 M CTAB. Following the subsequent addition of ascorbic acid (0.1 M, 0.32 mL), the solution turned colorless, 0.1 mL of pre-synthesized seed solution was added and left undisturbed at 30°C for 12 h. The prepared NR solution was then centrifuged at 8500 rpm for 15 min, and re-dispersed in 0.1 M CTAB. To obtain expected NRs with varied longitudinal plasmon absorption (NR_{700}, NR_{750}, NR_{800}, NR_{850}), additional $HAuCl_4$ solution (0.01 M) was added at 30°C for 2 h. The additional $HAuCl_4$ consisted of 0.07, 0.08, 0.09, and 0.1 mL, for NR_{850}, NR_{800}, NR_{750} and NR_{700}, respectively. For NR_{900}, the preparation procedure was exactly the same as that for the pre-synthesized NR except that 2 mL CTAB solution was added.

Preparation of $NaGdF_4$:Yb,Er nanoparticles (UCNPs). $NaGdF_4$:Yb,Er NPs (20 and 35 nm) were prepared according to previous studies with small modifications[1b, 2]. Briefly, $GdCl_3$ (0.80 mmol), $YbCl_3$ (0.18 mmol), and $ErCl_3$ (0.02 mmol) were dissolved in ultrapure water (2 mL). The resulting solution was then added into mixed solvents containing oleic acid (OA, 5 mL) and octadecene (ODE, 15 mL). A homogeneous solution was formed under nitrogen protection, when the above mixture was heated to 150°C. When the solution had cooled to room temperature, 5 mL methanol solution with NaOH (2.5 mmol) and NH_4F (4 mmol) was dropped into the solution with stirring for 2 h. Subsequently, the reaction solution was heated to 100°C for 30 min to remove the methanol. After heating to 300°C, the formed mixture was reacted for 1.5 h under reduced pressure, which was terminated by cooling to room temperature. The resultant UCNPs were washed with ethanol 3 times, and finally re-dispersed in cyclohexane.

Ligand Exchange of UCNPs. To exchange with the biocompatible ligand, the purified UCNPs

(10 mg) and mal-PEG-dp (120 mg) were added into tetrahydrofuran (6 mL), and then underwent the ligand exchange reaction for 12 h at 40°C[2]. After washing with cyclohexane three times, the PEGylated UCNPs were obtained and stored at room temperature.

Preparation of single-stranded DNA-modified NRs. Firstly, the NRs were centrifuged at 8500 rpm for 15 min to remove excess CTAB and re-dispersed in 5 mM CTAB solution. Next, 1 μL of helper DNA (DNA 13, Table S1) with a final molar ratio of 80 to NRs, was added to block the end sites of the NRs. After centrifugation at 8500 rpm for 15 min, the pellet was re-dispersed in 10 mM Tris–HCl buffer containing 5 mM CTAB solution. Then, 1 μL of thiolated single-stranded DNA at an equivalent molar ratio (1:1) to NR was added to the above solution, followed by incubation for 2 h at room temperature. Subsequently, thiolated-PEG was introduced into the solution at a ratio of 200 to NR-DNA. The DNA-modified NRs were finally obtained after being centrifuged twice.

Preparation of single-stranded DNA-modified UCNPs. The maleimide-PEGylated UCNPs were functionalized with corresponding complementary thiolated DNA sequences, according to the typical thiol-maleimide "click" reaction[2]. Approximately 1 μL of thiolated single-stranded DNA (30 μM, Table S1) was added to 100 μL Tris–HCl buffer (10 mM, pH 7.5) containing 100 nM maleimide-PEGylated UCNPs and stirred for 30 min. The molar ratio of single-stranded DNA to UCNPs was approximately 3. The resultant solution was then transferred to an ultrafiltration tube (10 kD), centrifuged three times at 10,000 rpm for 20 min, and finally re-suspended in 100 μL Tris–HCl buffer (10 mM, pH 7.5).

Assembly of NR-UCNP tetramers. In order to form the NR-UCNP tetramers, 5 μL DNA-modified UCNPs were mixed with 200 μL of DNA-modified NRs. The mixture was then incubated with gentle shaking for 2.5 h at room temperature. The solution was then centrifuged at 8000 rpm for 15 min and re-dispersed in 100 μL Tris–HCl buffer (10 mM, pH 7.5).

Cancer biomarker detection. The target DNA concentration was obtained by serial dilution method in Tris–HCl buffer (10 mM, pH 7.5), following a typical analytical chemistry procedure[3]. Then, a 1 μL sample of each solution was added to 100 μL of the tetramer assembly solution. Following incubation, CD and UCL spectra were measured for each sample. For all the CD measurements, Tris–HCl buffer (10 mM, pH 7.5) was used as the blank baseline, and the sample

collection rate is 1nm/0.2s.

Theoretical simulations. The FIFD method was employed to calculate the chiroptical properties and electromagnetic field intensities of the tetramers, which were carried out based on CST Microwave Studio®. The calculations of optical properties were performed using different elements of a standard scattering matrix. Surface electric field enhancement simulations were carried out using a linearly polarized beam with an electric field vector parallel and perpendicular to the longitudinal direction of the NRs. The excitation beam for *E*-field calculations was set at 980 nm.

Calculation of the anisotropy factor (g-factor). The anisotropy factor was calculated as follows: $g = \Delta A/A = \theta\ [mdeg] / (32980\times A)$, $\theta[mdeg]$ and A were the chiral intensity of samples and the corresponding absorption, respectively.

Analytical calculations for LOD. The LOD was determined according to the analytical methods, as follows:

$$y = \mathrm{a} + \mathrm{b}x \quad (1)$$

$$y = \mathrm{C_{blank}} - 3\mathrm{SD} \quad (2)$$

$$LOD = 10^{\frac{(C_{blank}-3SD)-a}{b}} \quad (3)$$

The formula (1) was the plotted calibration curve, where a and b were the variable obtained at x nM of DNA concentration and y mdeg of CD signal.

SD was the standard deviation, and $\mathrm{C_{blank}}$ was the CD signal of the blank sample without DNA.

Instrumentation. TEM micrographs were obtained using a JEOL-2010 microscope operated at 200 kV. The 3D reconstruction of cryo-electron tomography was collected by a Tecnai Spirit 120 kV TEM. The UV–vis spectra were obtained in a quartz cell using an ultraviolet–visible spectrometer (200–1000 nm). The CD spectra were measured using a Bio-Logic MOS-450 CD spectrometer. DLS data and zeta potentials were obtained using a Malvern Zetasizer ZS instrument with a 632.8 nm laser source. The upconversion luminescent spectra were acquired via a QuantaMaster TM40 spectrofluorometer with a 250 mW 980 nm NIR laser diode.

Supplementary Table

Table S1. DNA Sequences used in the tetramer assemblies and DNA detection (DNA strands 1-12 bear a hexyl-thiol linker at 5’ end, while DNA strands 14-15 bear a hexyl-thiol linker at 3’ end).

Types	Sequence (5’-3’)
DNA 1	GCT GCT GCT TTT TTT TTT TTT TTT TTT TTT
DNA 2	TCG TCG TCG AAA AAA AAA AAA AAA AAA AAA
DNA 3	TTT TTT TTT TT
DNA 4	AAA AAA AAA AA
DNA 5	GCT GCT TTT TTT TTT TTT
DNA 6	TCG TCG AAA AAA AAA AAA
DNA 7	GCT GCT GCT TTT TTT TTT TTT TTT
DNA 8	TCG TCG TCG AAA AAA AAA AAA AAA
DNA 9	GCT GCT GCT TTT TTT TTT TTT TTT TTT TTT TTT TTT
DNA 10	TCG TCG TCG AAA AAA AAA AAA AAA AAA AAA AAA AAA
DNA 11	GCT GCT GCT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT
DNA 12	TCG TCG TCG AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA
DNA 13	CCC CCC CCC CCC
DNA 14	TTA GAG TTG CTT TTT ATG GAT GAT GTG GTT T
DNA 15	ACC ACA TCA TCC ATA TAA AGA GGA GTT AAT CCA TGC AAC TCT AAA A
Target DNA	TTA GAG TTG CAT GGA TTA ACT CCT C
Negative D1	AGA AGA TAT TTG GAA TAA CAT GAC C
Negative D2	GGA GTA AAT GTT GGA GAA CAG TAT C
Negative D3	TTG GCT TTC AGT TAT ATG GAT GAT G
Negative D4	AGT TGT AAC GGA AGA TGC AAT AGT A
Negative D5	GAG GGA TTA TTG TTA AAT ATT GTA A

Table S2. Practical analysis of Hepatitis A virus Vall7 polyprotein gene (HVA) in serum samples.

Human serum sample [a]	Spiked concentration [b] (fM)	Detected concentration [c] (fM)	Detected concentration [d] (fM)	Recovery [e] (%)	Recovery [f] (%)
1	0.05	0.06 ± 0.02	0.04 ± 0.02	120 ± 9.7	80 ± 10.2
2	0.5	0.47 ± 0.03	0.46 ± 0.05	94 ± 3.8	92 ± 4.7
3	5	4.56 ± 0.15	5.33 ± 0.24	91.2 ± 6.3	106.6 ± 8.9

[a] Human serum samples are sampling from healthy donor at the Second Hospital in Wuxi, China. And they were negative samples, confirmed by the standard reverse transcription PCR (RT-PCR) procedures. The experiment was approved by the Ethics Committee of the Second Hosptial.

[b] Different concentrations of target DNA were added to the serum samples, thorough mixed and then stood for at least 3 h.

[c] The targets were detected through CD spectra; [d] The targets were detected through UCL spectra. SD was calculated based on five parallel experiments for each sample.

[e] Recovery for chiroplasmonic method; [f] Recovery for UCL method.

Supplementary Figures

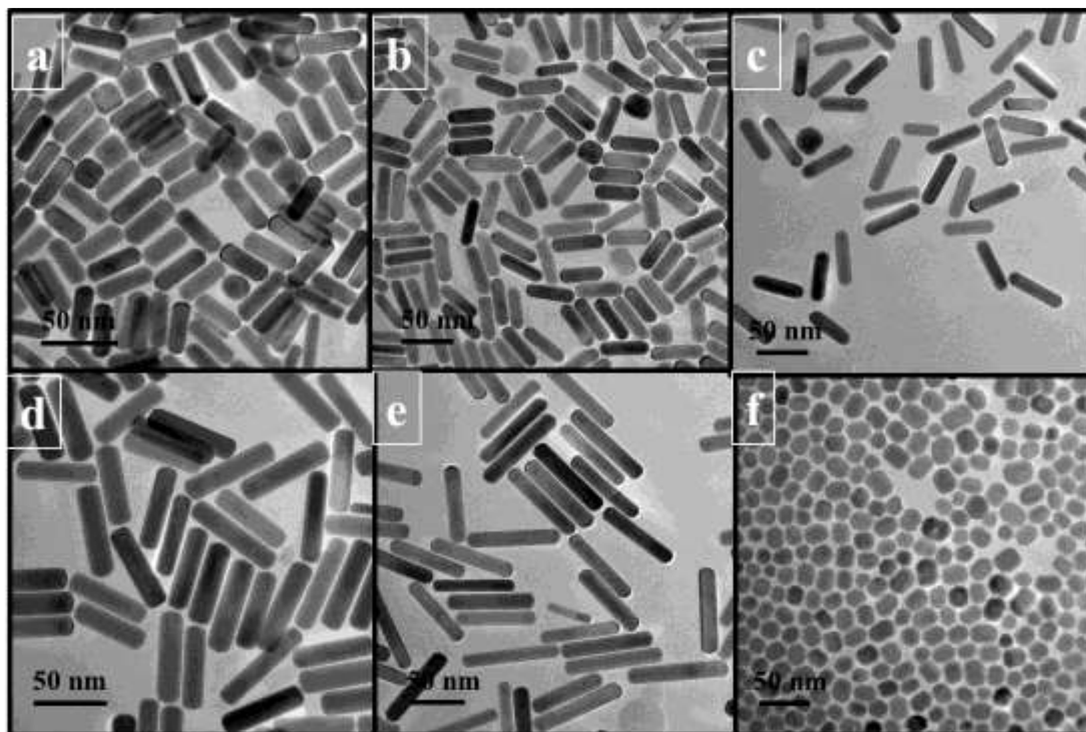

Figure S1. Representative TEM images of NR (a-e) and UCNP (f). The longitudinal absorption peak of NR was 700 nm (a), 750 nm (b), 800 nm (c), 850 nm (d), and 900 nm (e), respectively.

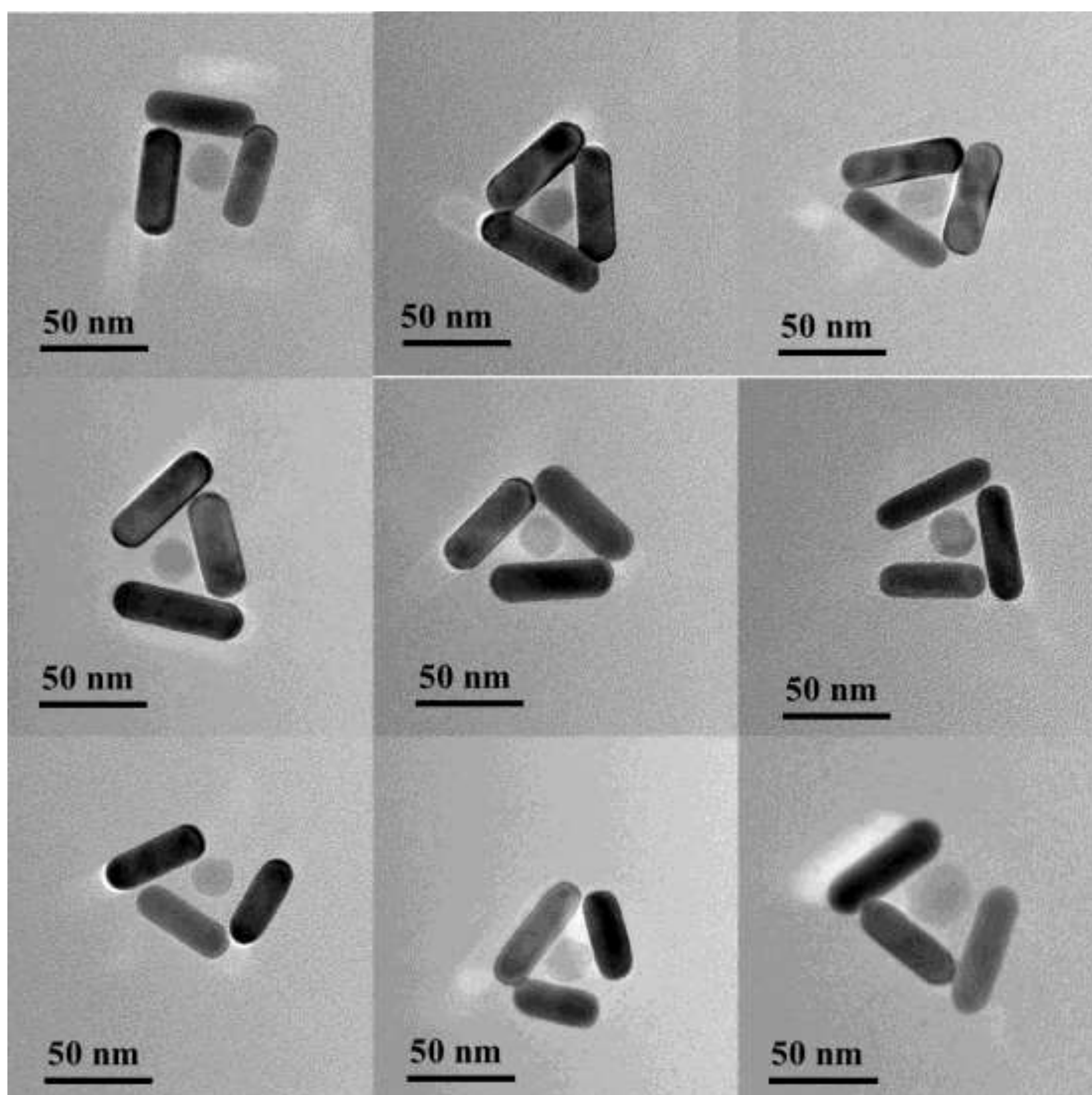

Figure S2. Representative TEM images of NR-UCNP tetramer assembly, where the longitudinal absorption peak of NR was 750 nm.

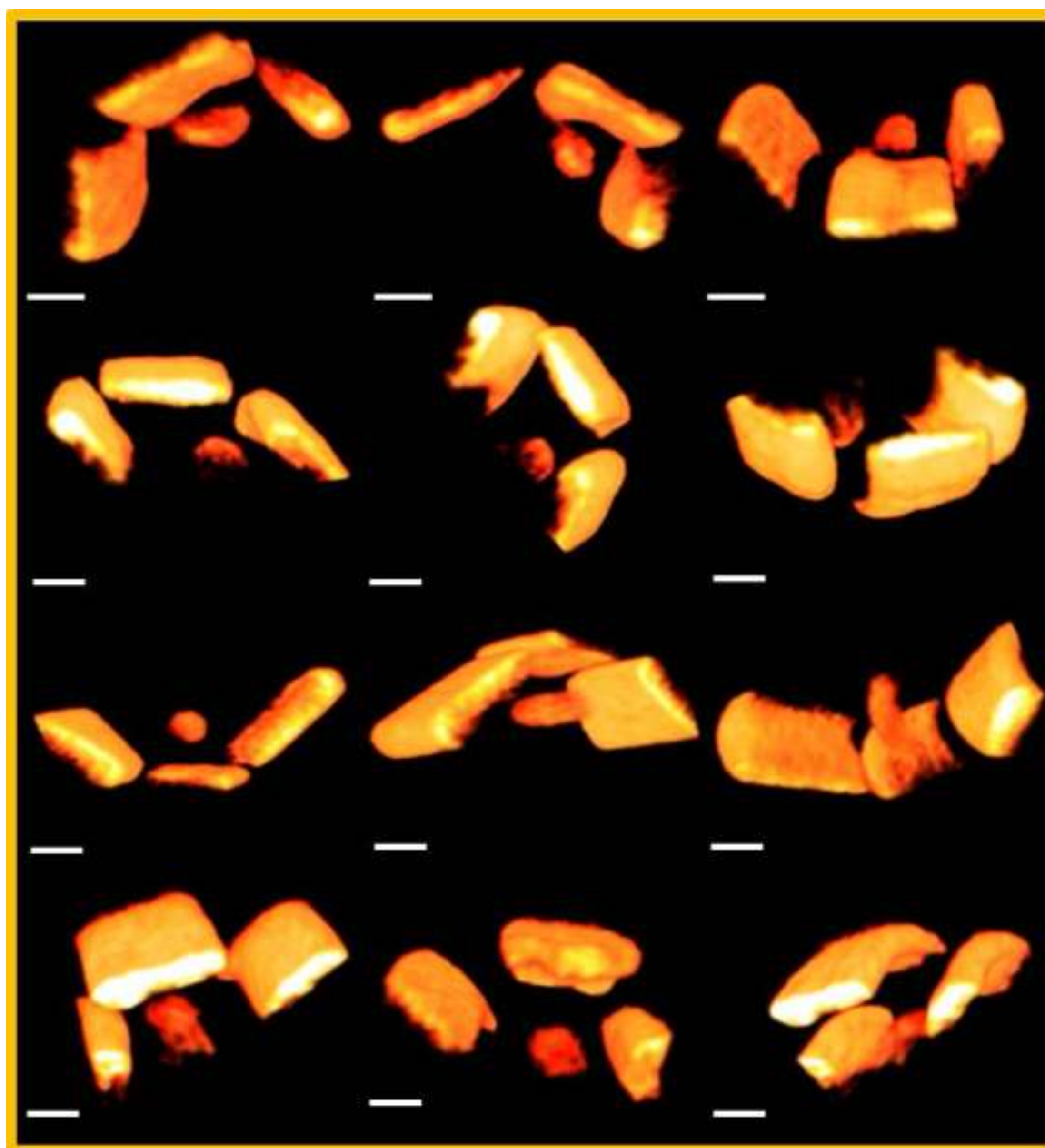

Figure S3. Representative 3D cryo-TEM tomography images of NR-UCNP tetramer assembly, where the longitudinal absorption peak of NR was 750 nm; scale bar, 20 nm.

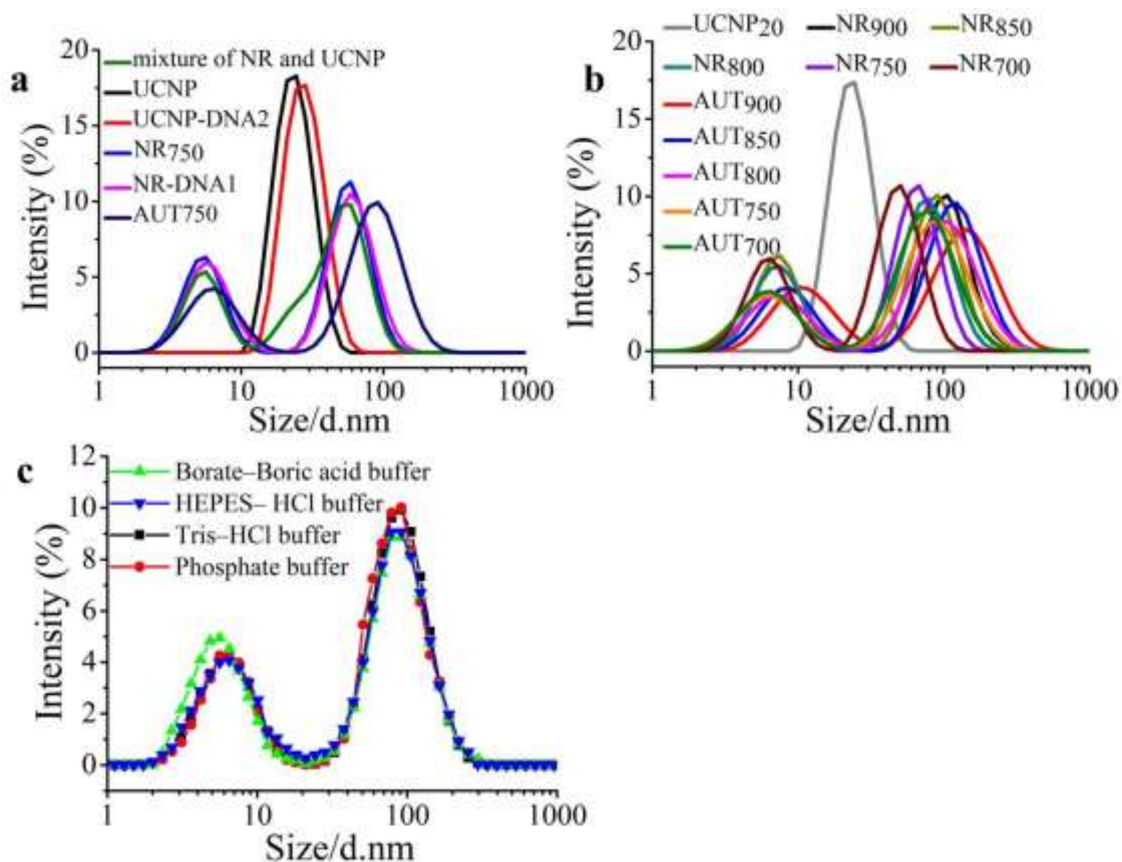

Figure S4. The dynamic light scattering (DLS) characterization for the NPs and their assemblies. (a) The DLS curves of NR-UCNP tetramer assembly (AUT), and their controls. (b) The DLS curves of NR-UCNP tetramer assemblied with different NRs. (c) The DLS curves of NR-UCNP tetramers assemblied under different buffer solutions. For (a) and (c), the tetramers were assembled with the longitudinal absorption peak of NR of 750 nm, 30 bp of DNA sequence, and 20 nm of UCNP. All the buffer solutions were 10 mM with pH of 7.5.

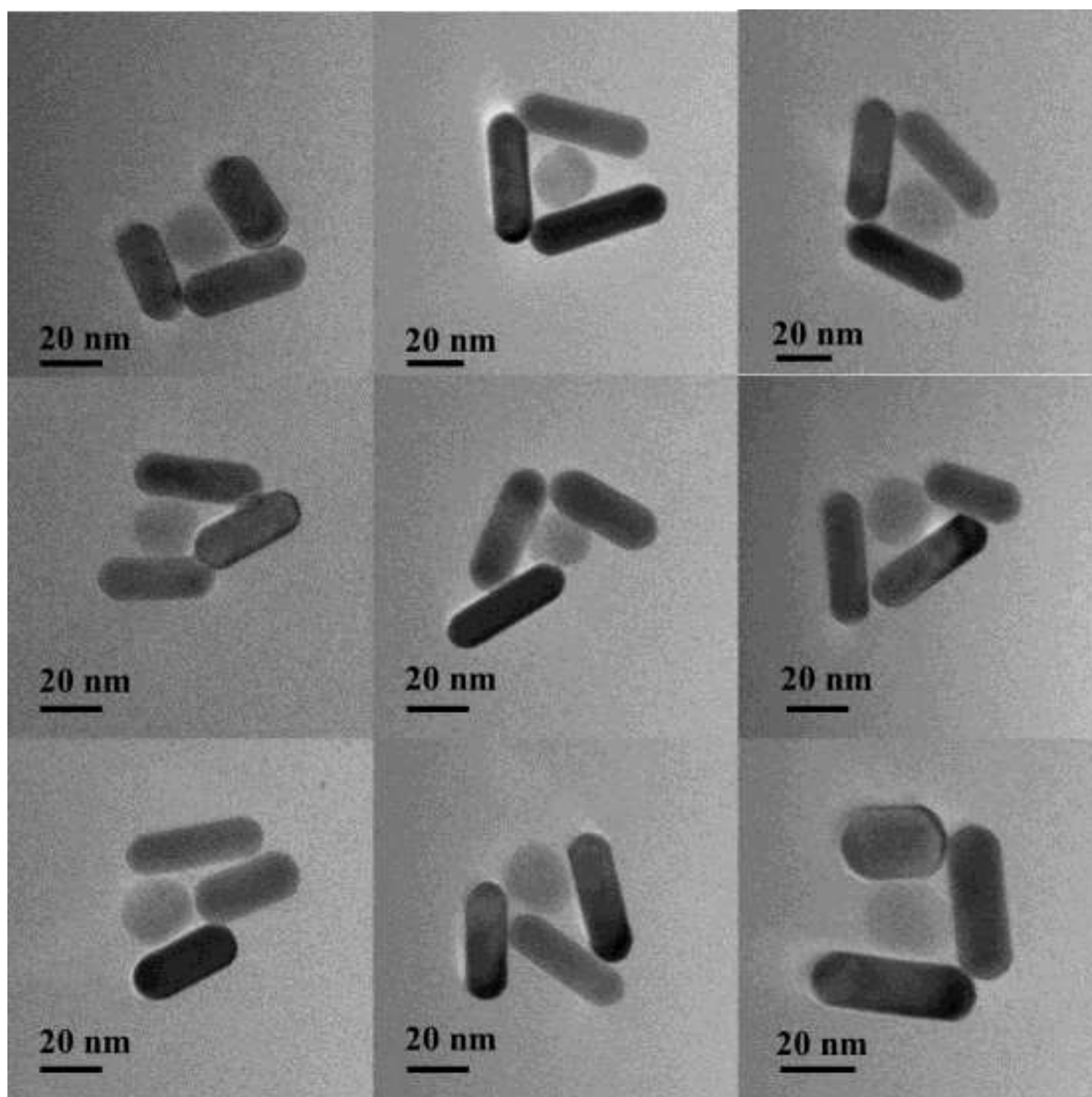

Figure S5. Representative TEM images of NR-UCNP tetramer assembly, where the longitudinal absorption peak of NR was 700 nm.

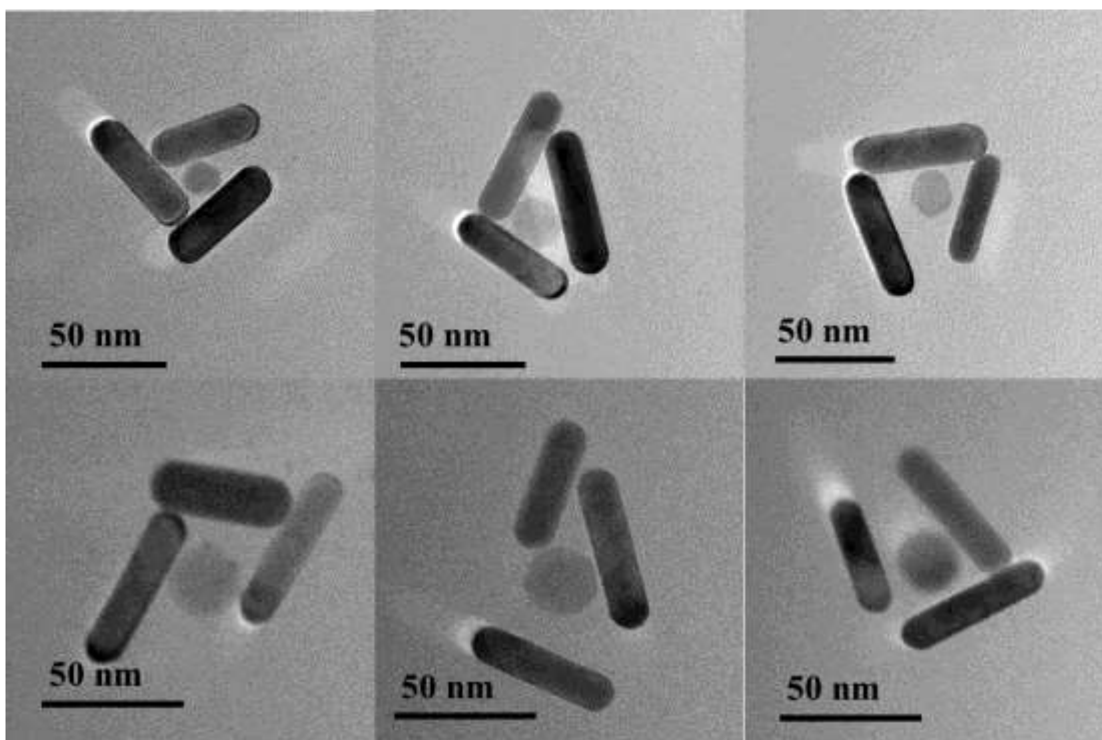

Figure S6. Representative TEM images of NR-UCNP tetramer assembly, where the longitudinal absorption peak of NR was 800 nm.

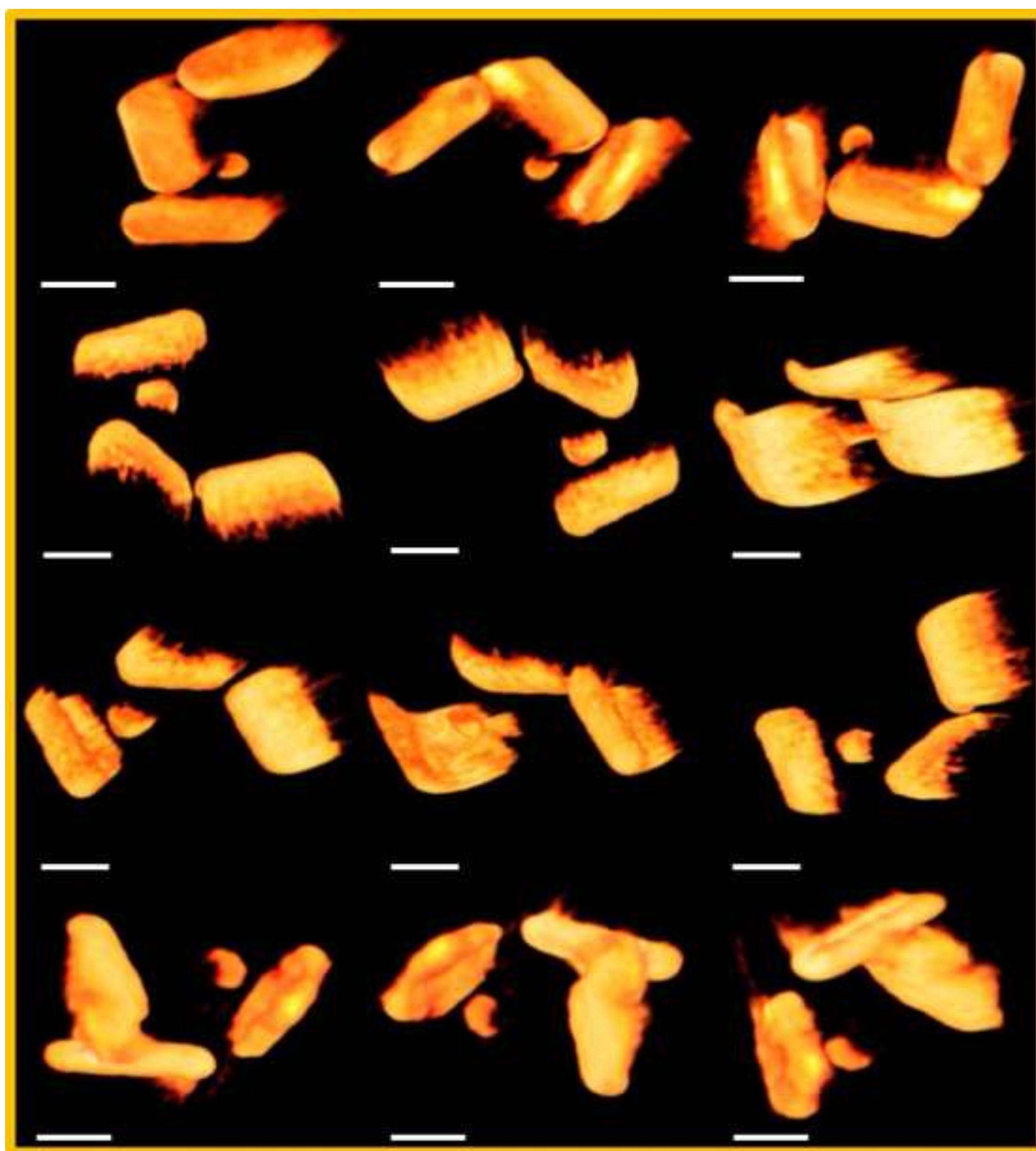

Figure S7. Representative 3D cryo-TEM tomography images of NR-UCNP tetramer assembly, where the longitudinal absorption peak of NR was 700 nm; scale bar, 20 nm.

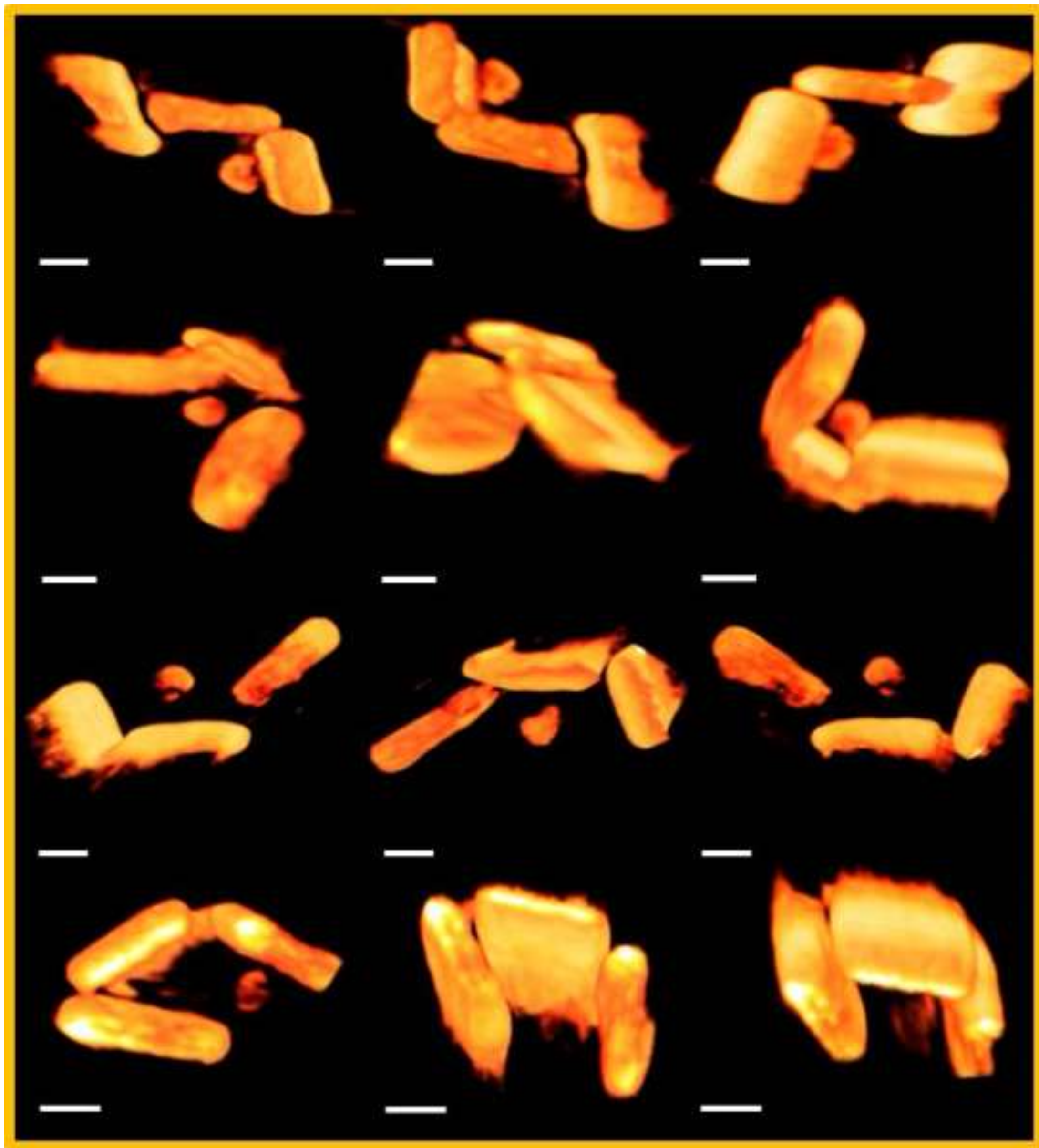

Figure S8. Representative 3D cryo-TEM tomography images of NR-UCNP tetramer assembly, where the longitudinal absorption peak of NR was 800 nm; scale bar, 20 nm.

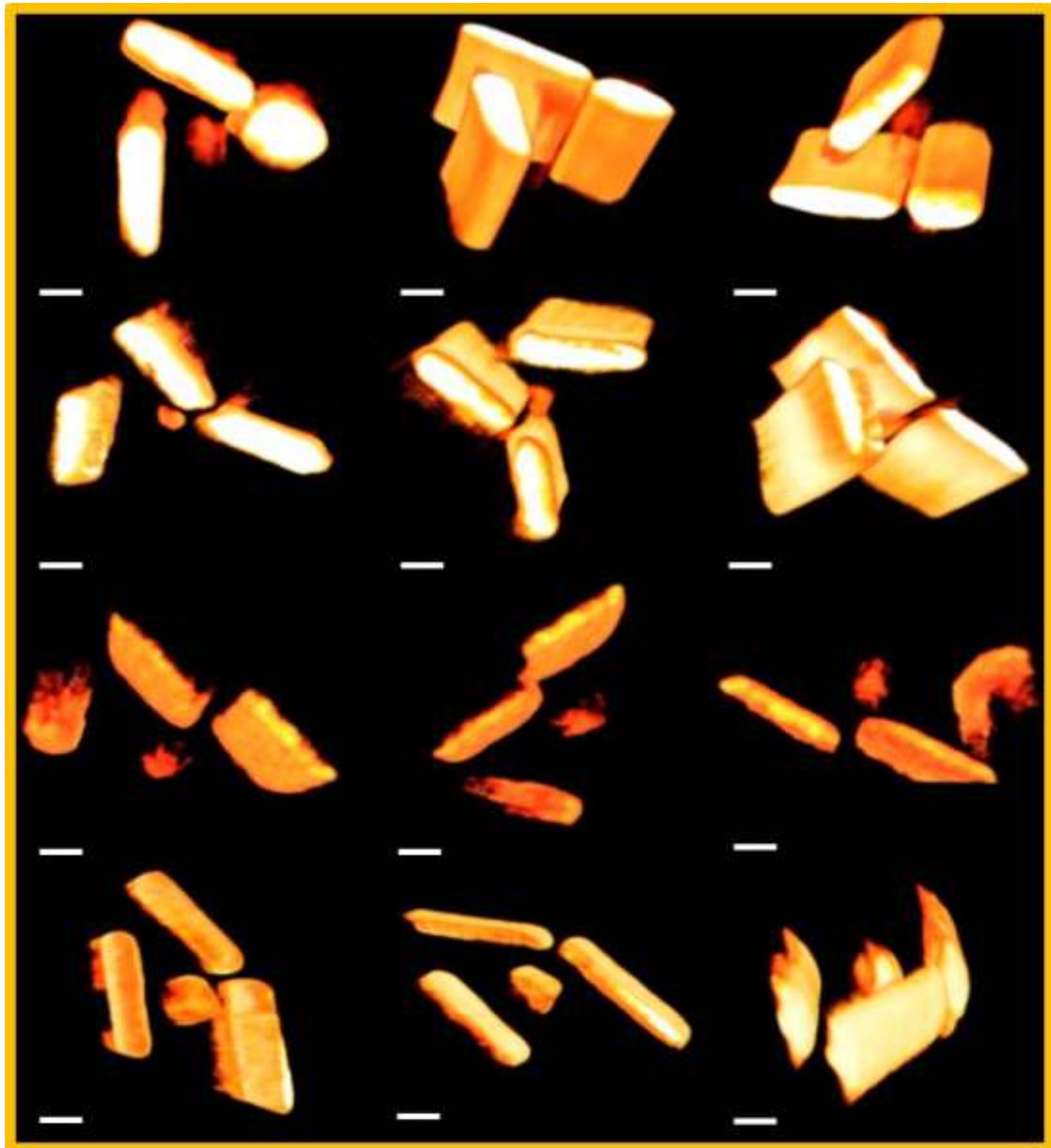

Figure S9. Representative 3D cryo-TEM tomography images of NR-UCNP tetramer assembly, where the longitudinal absorption peak of NR was 850 nm; scale bar, 20 nm.

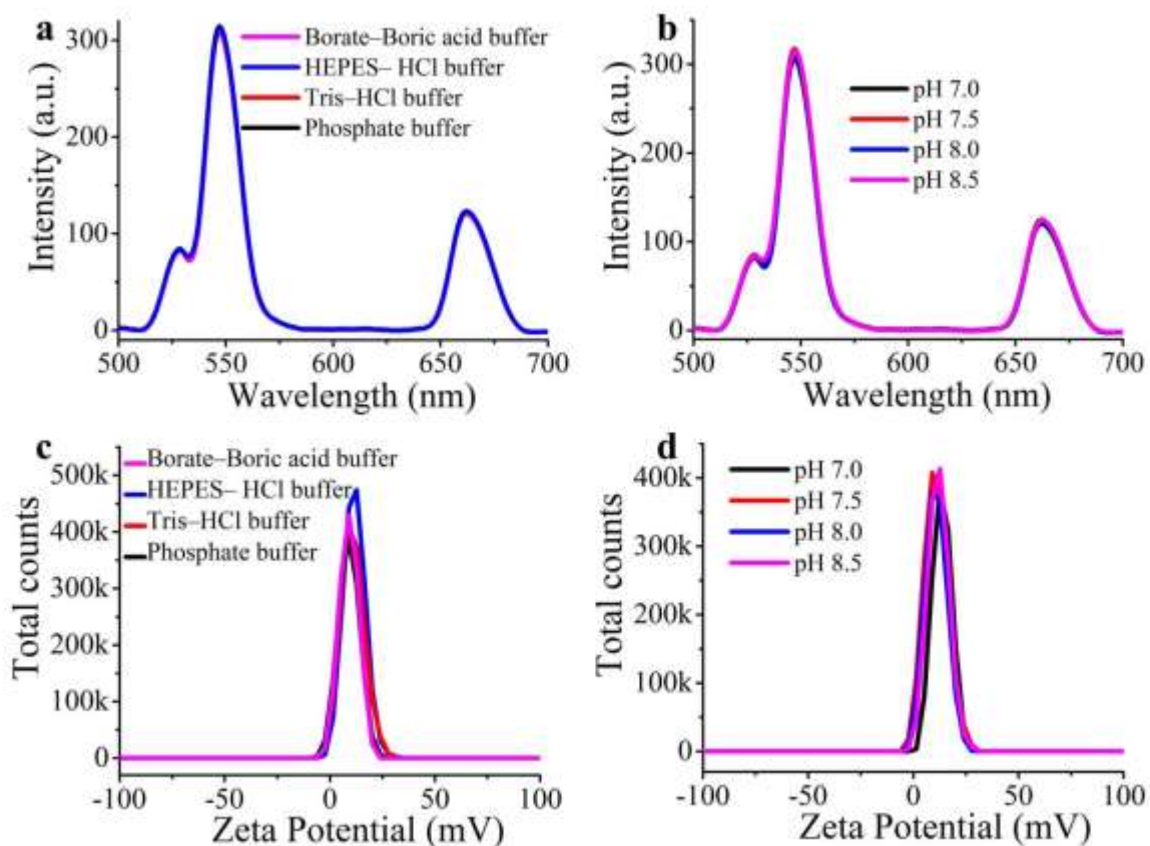

Figure S10. Surface charge properties of the NR-UCNP tetramer assembly. (a,b) Up-conversion luminescence spectra and (c,d) zeta potentials curves of NR-UCNP tetramer assembly under different buffer solutions (a,c) and different pH values (b,d), where the tetramers were assembled with the longitudinal absorption peak of NR of 750 nm, 30 bp of DNA sequence, and 20 nm of UCNP. All the buffer solutions in (a,c) were 10 mM with pH of 7.5. While for (b,d), the tetramers were in the Tris–HCl buffer of 10 mM.

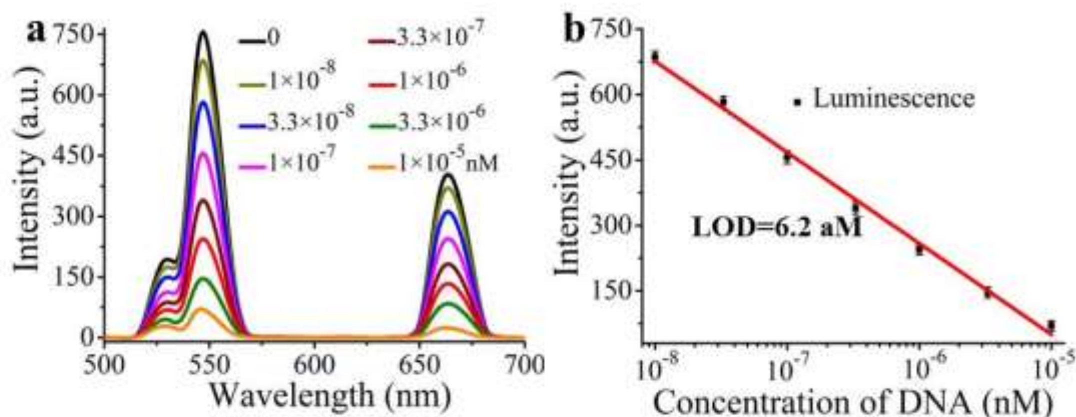

Figure S11. DNA detection by up-conversion luminescent and chiroplasmonic techniques with the NR-UCNP tetramer assembly. (a) The up-conversion luminescence spectra and (b) calibration curves for DNA detection. The tetramers were assembled with 20 nm of UCNP and the longitudinal absorption peak of NR using for assembly was 900 nm.

To compare this method with the chiroplasmonic method used in biomedical analysis, we also chose the UCL system with the maximum enhancement factor. The highest I/I_0 ratio was observed for assemblies with DNA bridges 30 bp long that corresponded to at a distance of approximately 10 nm, NRs with λ_L at 900 nm, and 20 nm UCNPs in the tested tetramers.

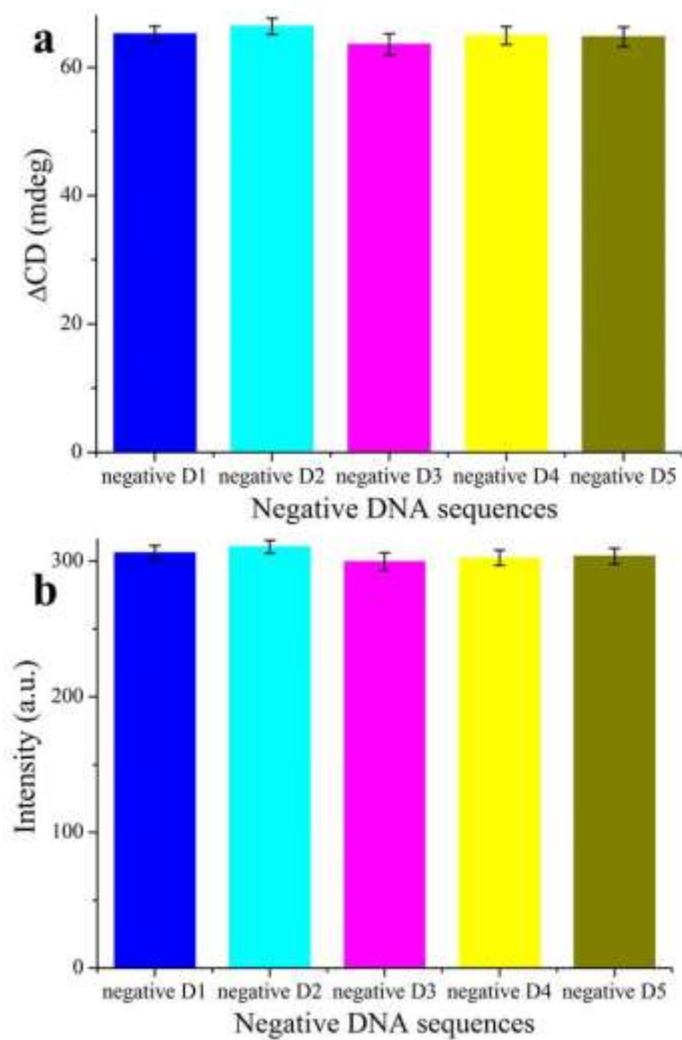

Figure S12. Negative controls tests through NR-UCNP tetramer system assembly for DNA detection by using (a) chiroplasmonic technique and (b) up-conversion luminescence method. The tetramers were assembled with the longitudinal absorption peak of NR of 750 nm and 20 nm of UCNP. The concentration of negative DNA sequences were all 5 nM.

Supplementary References

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