

Supporting Information

New Nanoplatforms Based on Upconversion Nanoparticle and Single-Walled Carbon Nanohorns for Sensitive Detection of Acute Promyelocytic Leukemia

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Materials and Chemicals

All the chemicals were used without further purification: $\text{YCl}_3 \cdot 6\text{H}_2\text{O}$ (99.99%), $\text{YbCl}_3 \cdot 6\text{H}_2\text{O}$ (99.99%) and $\text{ErCl}_3 \cdot 6\text{H}_2\text{O}$ (99.99%) were purchased from Sigma Aldrich. Oleic acid (90%, technical grade), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, 98%), N-Hydroxysuccinimide (NHS, 98%), trisodium citrate (anhydrous, 99%) were purchased from Alfa Aesar. 1-octadecene (>90%, gas chromatography), methanol (99.5%), diethylene glycol (>98%, gas chromatography), ammonium fluoride (guaranteed reagent, 98%), sodium hydroxide (guaranteed reagent, 97%, flakes) were purchased from Aladin Company. Acetone (99.5%, analytical reagent), cyclohexane (99.5%, analytical reagent), anhydrous ethanol (99.7%, analytical reagent), toluene (99.5%, analytical reagent), chloroform (analytical reagent), were obtained from Sinopharm Chemical Reagent Co., Ltd. Single-walled carbon nanohorns (JCSCH-95-90-15n, 95%, external diameter: 80~100 nm, length: 10~20 nm), graphene oxide (JCGO-99-1-2, >99%) were purchased from

Nanjing Jenano Tech Co., Ltd. Probe DNA (5'-NH₂-TCT CAA TGG CTG CCT CCC-3'), target DNA or PML/RAR α fusion gene (5'-GGG AGG CAG CCA TTG AGA-3'), non-complementary DNA (5'-AGT TCA TCC TGC GCT CTT-3'), single-base mismatch DNA (5'-GGG AAG CAG CCA TTGAGA-3') were purchased from Sangon Biotech (Shanghai) Co., Ltd.

Instrumentation and Characterization

The size and morphology of nanoparticles were measured by using a JEM-200CX transmission electron microscope operated at 120 kV and 200 kV using a JEM-2010F high-resolution transmission electron microscope. The crystal phase of UCNPs was identified by X-ray diffraction (XRD) measurements carried out on a D\max-2200 X-ray diffractometer using Cu K α ($\lambda = 1.54 \text{ \AA}$) radiation with 2θ range from 10° to 90° at a scanning rate of 8°/min. Fourier transform infrared spectroscopy (FT-IR) spectra were acquired in the spectral range from 4000 to 400 cm⁻¹ with an Avatar 370 by using pressed KBr pellet technique. The upconversion luminescence spectra were obtained on an Edinburgh LFS 920 luminescence spectrometer equipped with an external 0–800 mW 980 nm adjustable CW laser. The Z-potential and dynamic light scattering measurements of the materials were performed at 25°C using a ZETASIZER 3000HS instrument (Malvern Instruments, U.K.). Nitrogen (N₂) adsorption/desorption isotherms were measured using an ASAP2020M+C analyzer with nitrogen.

Synthesis of NaYF₄:20%Yb,2%Er (UCNPs) and NaYF₄:20%Yb, 2%Er@NaYF₄ (CS-UCNPs)

The hydrophobic NaYF₄: 20%Yb, 2%Er nanoparticles were synthesized via a solvent-thermal method.¹ 1.56 mL of YCl₃ (1.0 M), 0.40 mL of YbCl₃ (1.0 M) and 0.40 mL of ErCl₃ (0.1M) were added to a 100 mL two-necked flask and the mixture was heated to 110 °C. Then, 12 mL of oleic acid and 30 mL of 1-octadecene were added into the mixture following heated to 150 °C. After cooling down to room temperature, 20 mL of methanol solution including NH₄F (0.3 g, 8 mmol) and NaOH (0.2 g, 5 mmol) were added and the solution was stirred at 110 °C to removing methanol, then the reaction solution was heated to 300 °C and kept for 1 hour under Ar atmosphere. After

cooling down to room temperature naturally, the obtained suspension was separated by centrifugation and washed with cyclohexane and acetone. At last, the obtained sample was dispersed in 20 mL of cyclohexane. The synthesis procedure of NaYF₄:Yb,Er@NaYF₄ nanoparticles was similar to that of NaYF₄:Yb,Er except that 0.80 mL of YbCl₃ (1.0 M) was replaced by 5.0 mL of the prepared NaYF₄:Yb,Er.

Synthesis of citrate-capped UCNPs (Cit-UCNPs)

To impart UCNPs hydrophilicity, the water-soluble UCNPs were synthesized according to the ligand-free methods reported previously.²⁻⁴ Sodium citrate (2 mmol) and diethylene glycol (DEG) (15.0 mL) were added to a 50mL three-necked flask and the mixture was heated to 110 °C for 30 min under argon atmosphere. After the solution was cooled naturally, 10 mg of CS-UCNPs dispersed in chloroform and toluene solution (5 mL, v/v = 3:2) were injected into the above mixed solution slowly and the mixture was heated to 130 °C to removing chloroform and toluene. Finally, the solution was heated to 180 °C for another 2 h until the solution became yellow. After the solution was cooled down to room temperature, the precipitates were collected by centrifugation (10,000 rpm, 15 min) and washed two times with ethanol and deionized water(v/v = 1:1), and then dispersed in deionized water.

Immobilization of Cit-UCNPs with capture probe DNA (Cit-UCNPs–ssDNA)

2 mg of EDC and 3 mg of NHS were firstly added to 1 mL of water containing 1 mg of Cit-UCNPs and the mixture stirred for 2 h at 4 °C to activate the surface carboxylic acid group. The precipitates were collected by centrifugation (10,000 rpm, 15 min) and washed three times with deionized water to remove the excess EDC and NHS. And then 1 mL of H₂O containing 20μL of probe ssDNA (100 μM) was added and the solution was stirred for 10 h at 4 °C. After centrifugation, the precipitates were washed three times with water. Finally, Cit-UCNPs–ssDNA were obtained and stored in water at 4 °C for further use.

Procedures for upconversion luminescence quenching reaction

For the luminescence quenching experiments, the single-walled carbon nanohorns stock solution (0.1 mg/mL) was individually added to Cit-UCNPs–ssDNA solution (0.11 mg/mL) by using a micropipette and incubated for 60 min at 4 °C, and then

upconversion luminescence measurements were performed with an external 980 nm laser as the excitation source, and the emission intensity at 545 nm was taken for quantification. The experiment of graphene oxide (0.1 mg/mL) with Cit-UCNPs–ssDNA was performed following the similar procedures.

Procedures for PML/RAR α fusion gene (Target DNA) detection

In a typical LRET assay process, the SWCNHs were added to Cit-UCNPs–ssDNA solution (0.11 mg/mL) with an ultimate concentration of 4.65 μ g/mL followed by incubation for 60 min. The target DNA was individually added into the above solutions (Cit-UCNPs–ssDNA-SWCNHs) with an ultimate concentration of 43.5 nM followed by incubation for another 60 min. Subsequently, the upconversion luminescence measurements were carried out with the excitation of 980 nm. To examine the specificity of this UCNPs-LRET nanoplatfrom for PML/RAR α fusion gene, some other ssDNA including non-complementary DNA, single-base mismatch DNA were added individually into the Cit-UCNPs–ssDNA-SWCNHs system in place of PML/RAR α fusion gene under the same experimental conditions. The experiment of GO with Cit-UCNPs–ssDNA was performed following the similar procedures.

Table S1. Probe DNA , target DNA or PML/RAR α fusion gene , non-complementary DNA and single-base mismatch DNA Sequences. The single-base mismatch positions are highlighted as underlined.

Name	Sequence 5' \rightarrow 3'
Probe DNA	NH ₂ -TCT CAA TGG CTG CCT CCC
target DNA	GGG AGG CAG CCA TTG AGA
non-complementary DNA	AGT TCA TCC TGC GCT CTT
single-base mismatch DNA	GGG A <u>A</u> G CAG CCA TTGAGA

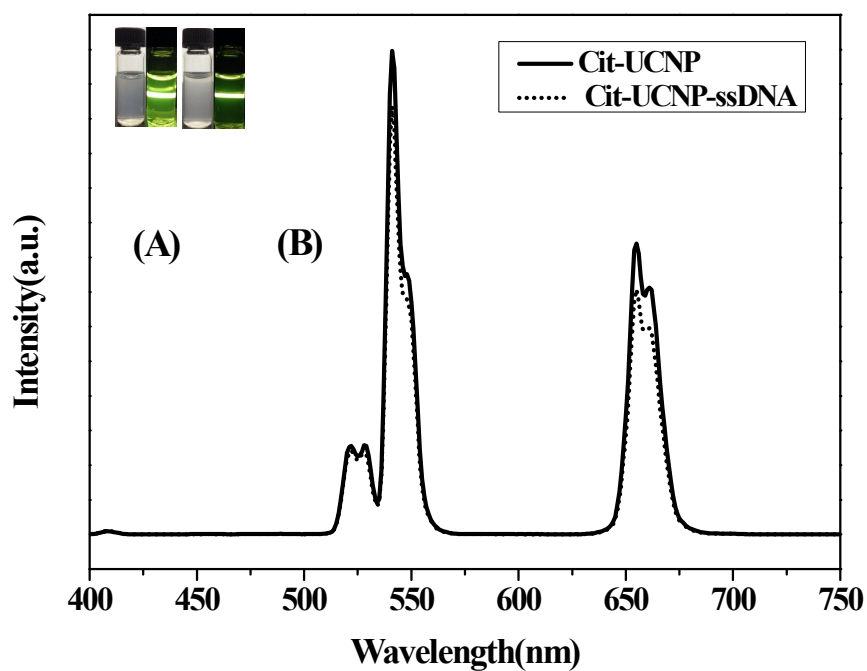


Fig. S1. Upconversion luminescence spectra of Cit-UCNPs (the solid line) and Cit-UCNPs-ssDNA (the short dot line). The inset shows the photograph of (A) bright-field images and luminescence images of Cit-UCNPs (B) bright-field images and luminescence images of Cit-UCNPs-ssDNA. All the solutions are excited with an external 980 nm laser. The concentration of Cit-UCNPs and Cit-UCNPs-ssDNA are 6.5 mg/mL.

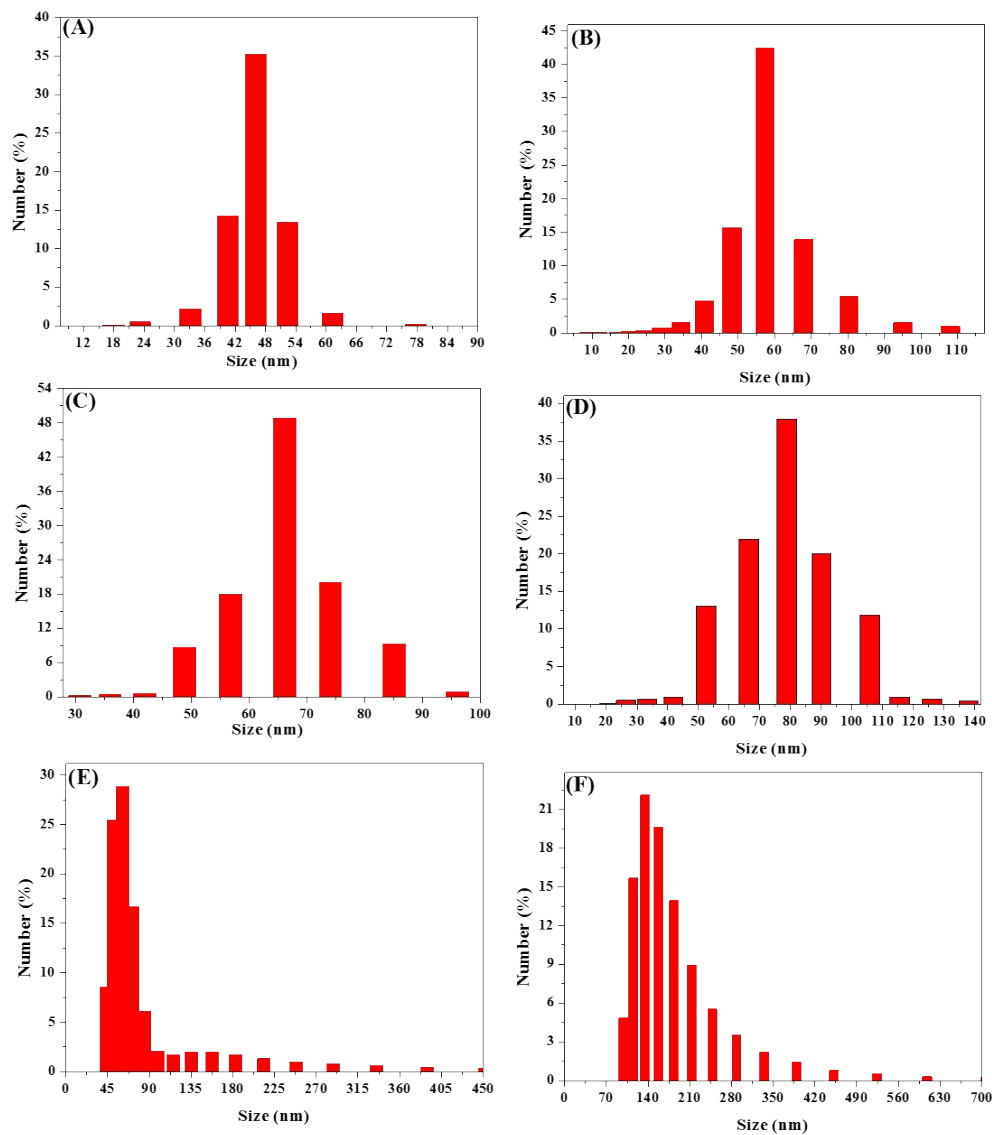


Fig. S2. Dynamic light scattering (DLS) analysis of (A) UCNPs and (B) CS-UCNPs in cyclohexane; (C) Cit-UCNPs, (D) Cit-UCNPs-ssDNA, (E) SWCNHs and (F) GO in water.

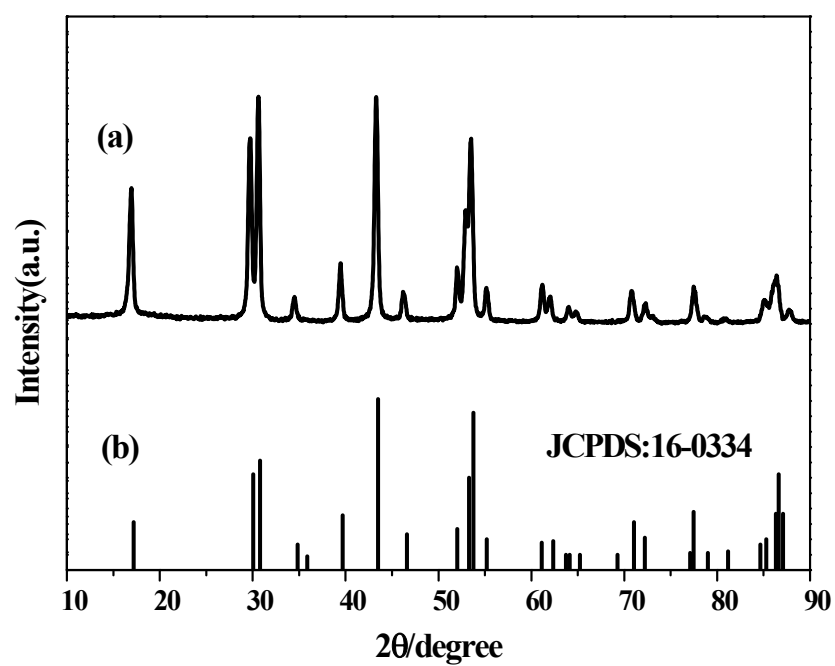


Fig. S3. XRD pattern of NaYF₄: 20%Yb, 2%Er nanocrystals (a) and (b) the standard card of hexagonal phase NaYF₄ (JCPDS file No.16-0334).

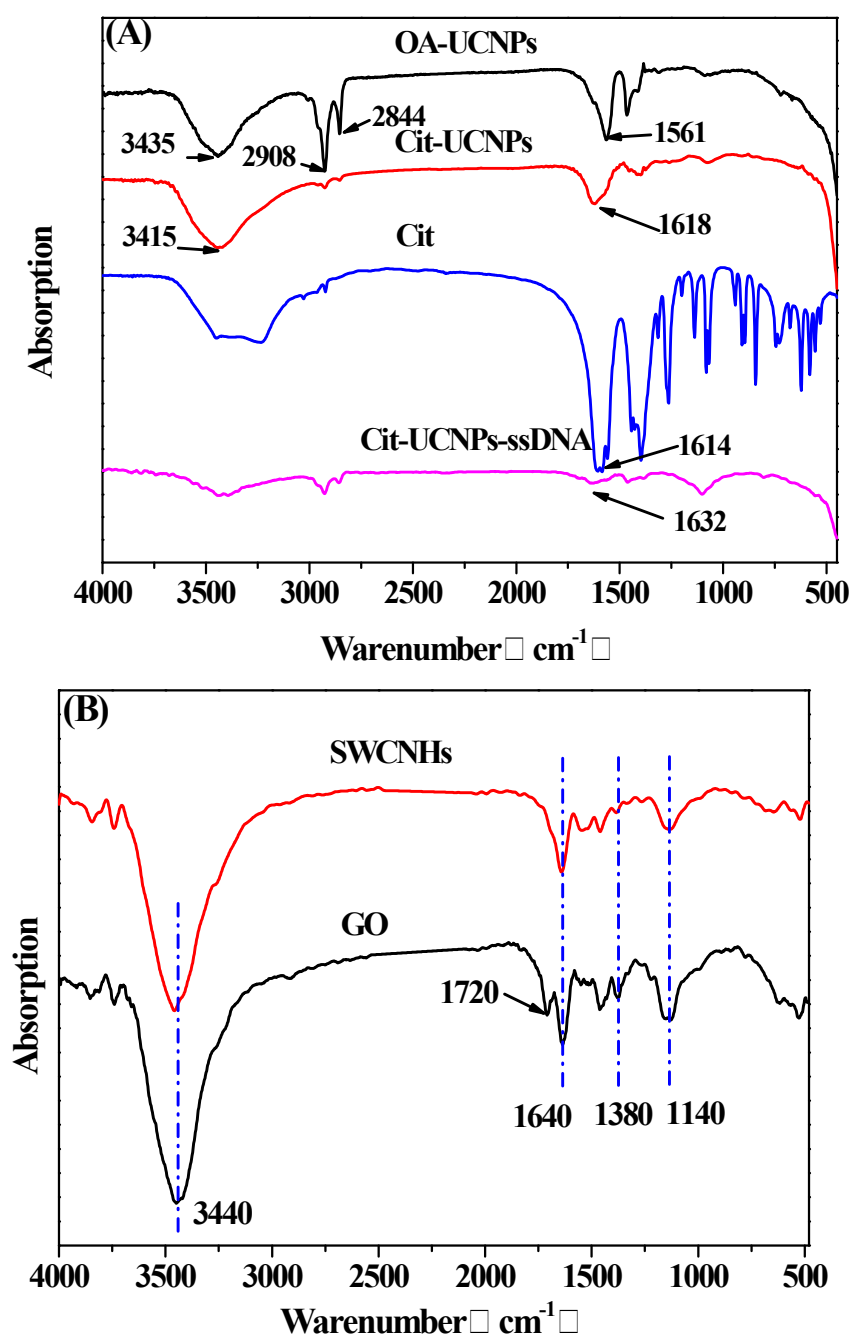


Fig. S4. FT-IR spectra of (A) OA-UCNPs, Cit-UCNPs, sodium citrate (Cit) and Cit-UCNPs-ssDNA; (B) single-walled carbon nanohorns (SWCNHs) and graphene oxide (GO).

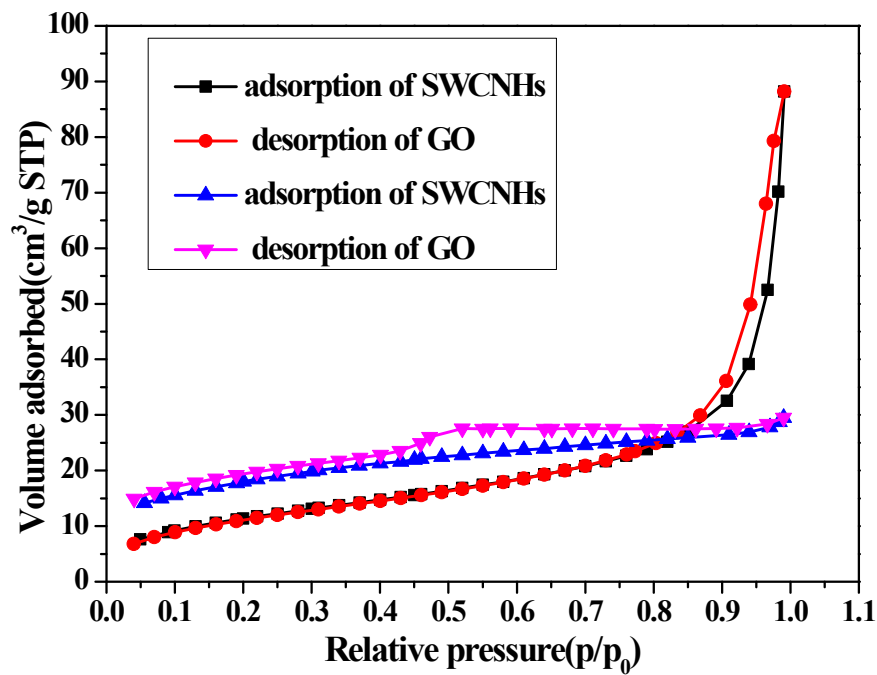


Fig. S5. N₂ adsorption-desorption isotherm of SWCNHs and GO;

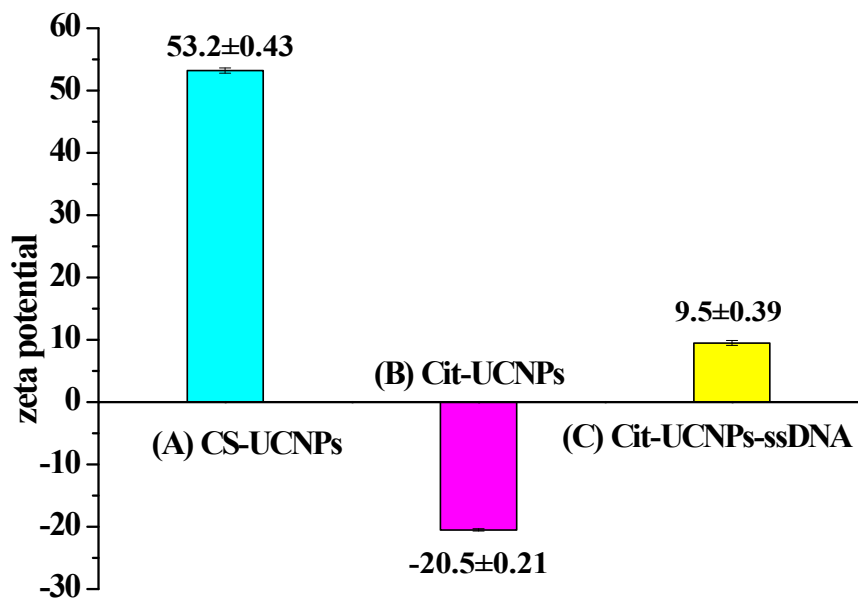


Fig. S6. Zeta potential distribution of CS-UCNPs in cyclohexane (A), and Cit-UCNPs (B), Cit-UCNPs-ssDNA (C) in deionized water.

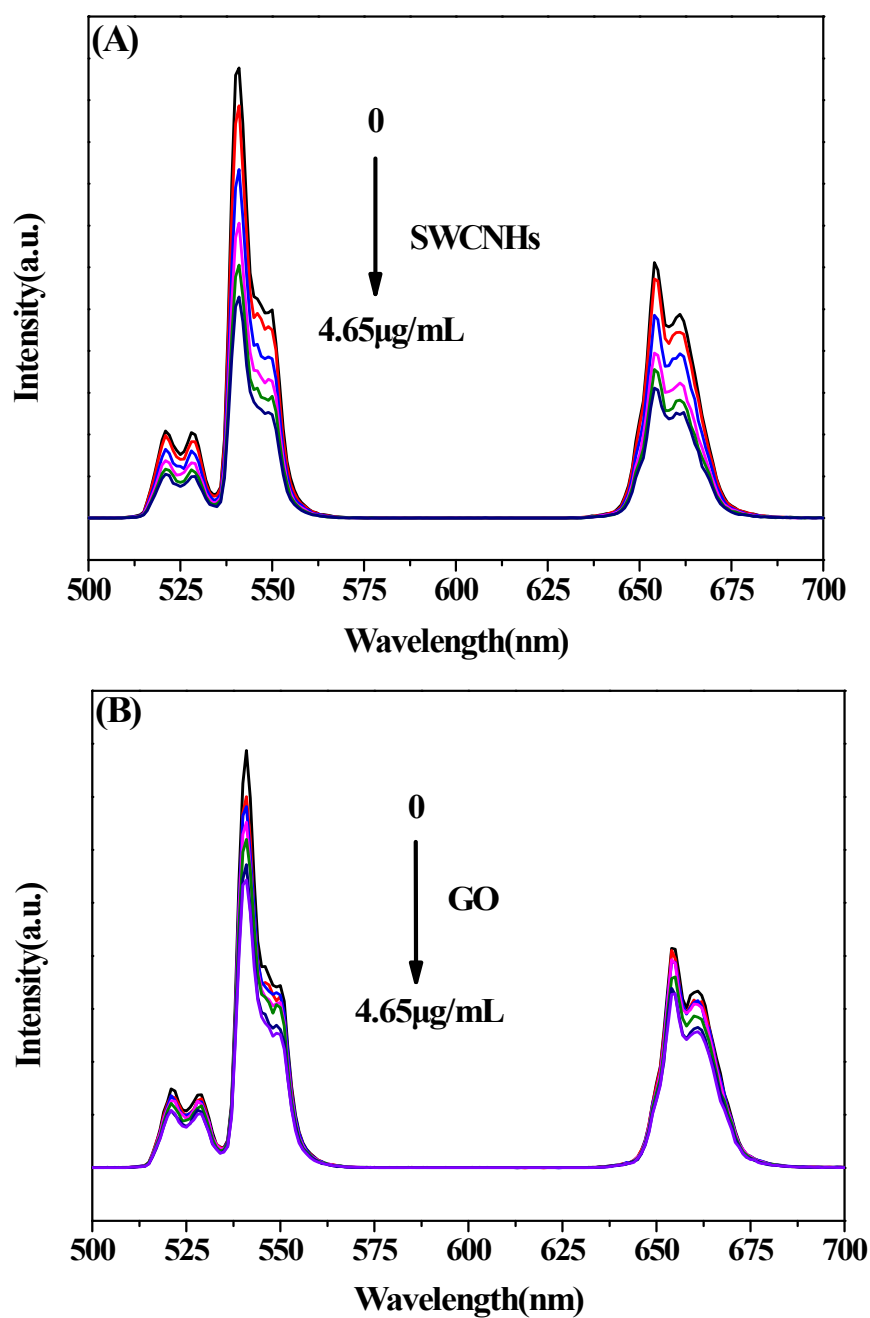


Fig. S7. Luminescence spectra of Cit-UCNPs-ssDNA after incubation with various concentrations of single-walled carbon nanohorns (A) and graphene oxide (B). The concentration of Cit-UCNPs-ssDNA is 0.11 mg/mL, the concentrations of SWCNHs and GO are ranged from 0 to 4.65 $\mu\text{g/mL}$, $\lambda_{\text{ex}} = 980 \text{ nm}$. Experiments were performed in deionized water.

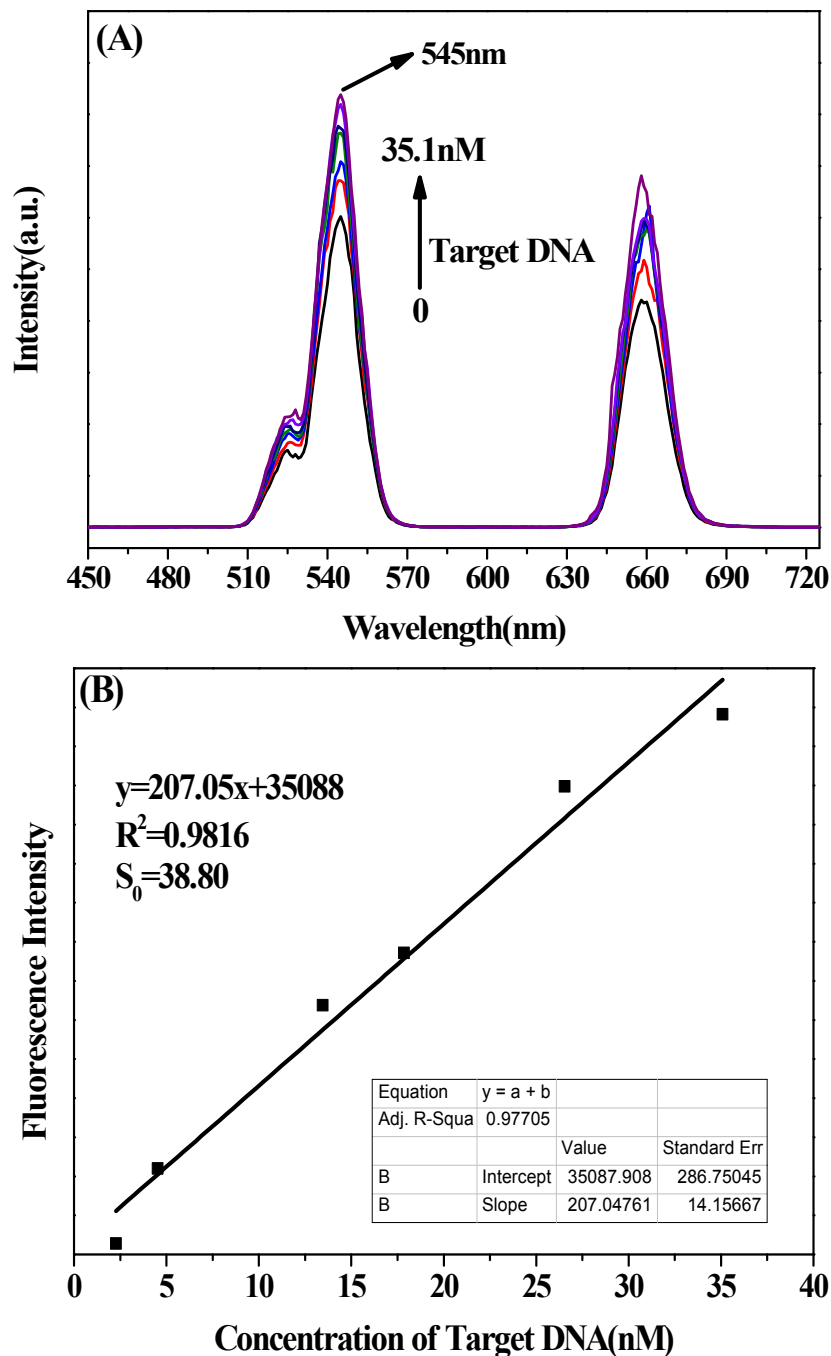


Fig. S8. (A) The luminescence spectra of multiplexed Cit-UCNPs-ssDNA-GO nanoplatform with various concentrations of target DNA (PML/RAR α fusion gene). (B) Linear relationship between upconversion luminescence intensity recorded at 545 nm versus target DNA concentrations. Target DNA concentration is in the range of 0 nM to 35.1 nM; the final concentration of Cit-UCNPs-ssDNA used is 0.11 mg/mL; GO: 4.65 μ g/mL; λ_{ex} =980 nm. Experiments were performed in deionized water.

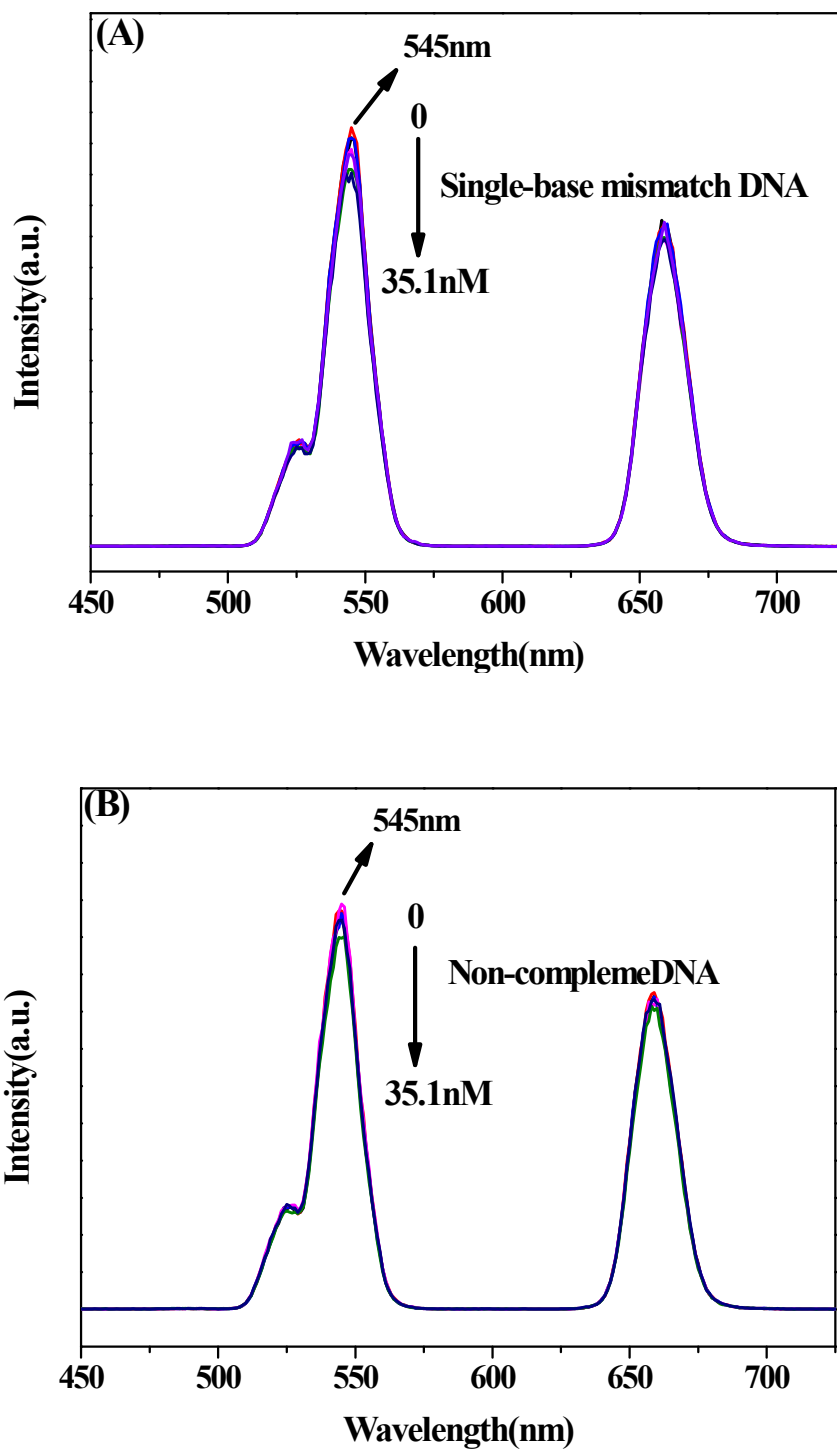


Fig. S9. Upconversion luminescence spectra of the Cit-UCNPs-ssDNA-SWCNHs system in the presence of single-base mismatches DNA (A) and non-complementary DNA (B). All of the ssDNA concentration are ranged from 0 to 35.1 nM; the final concentration of Cit-UCNPs-ssDNA is 0.11 mg/mL; SWCNHs: 4.65 $\mu\text{g/mL}$; λ_{ex} =980 nm. Experiments are performed in deionized water.

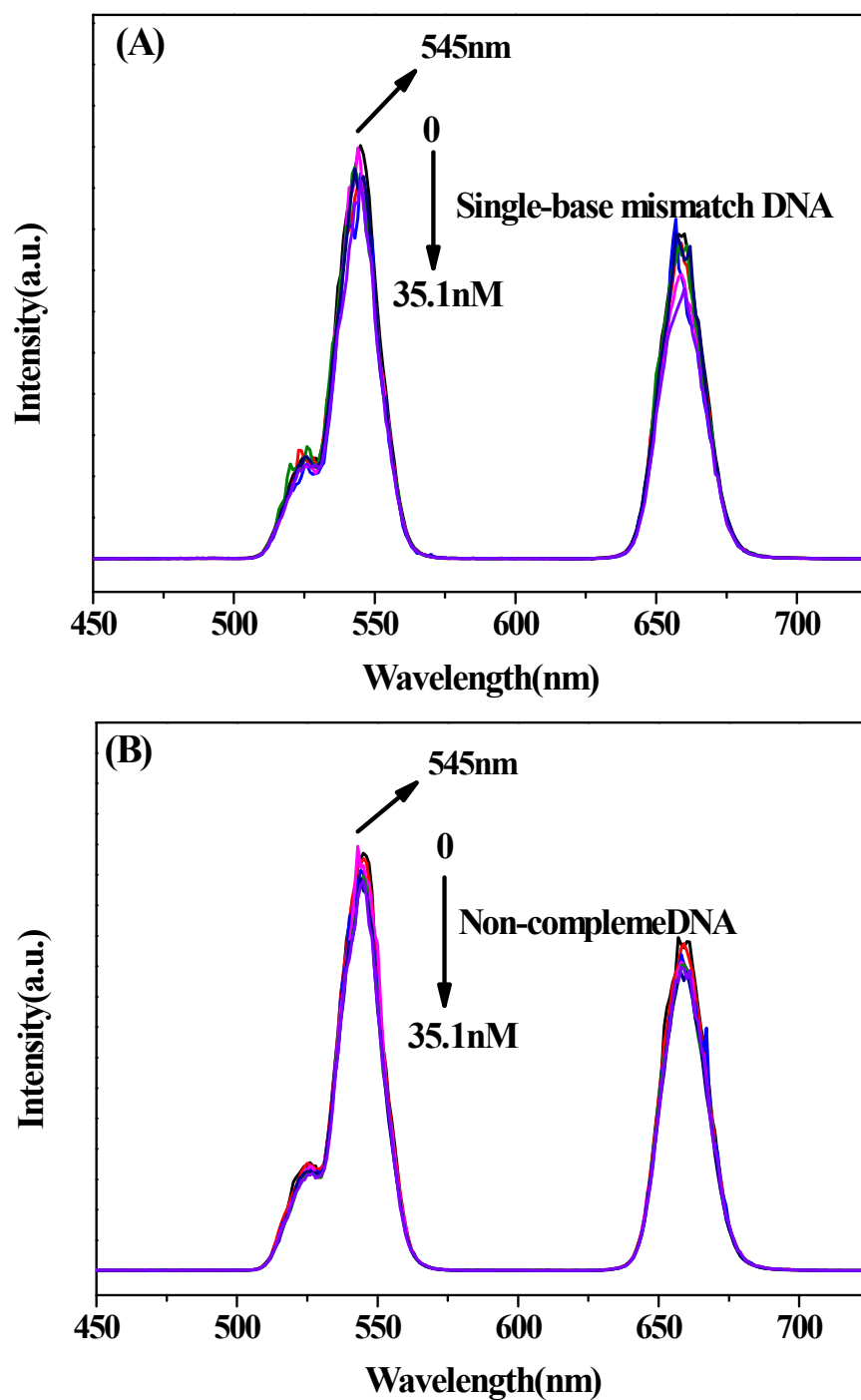


Fig. S10. Luminescence spectra of the Cit-UCNPs-ssDNA-GO system in the presence of single-base mismatch DNA (A) and non-complementary DNA (B). All of the ssDNA concentration are ranged from 0 to 35.1 nM; the final concentration of Cit-UCNPs-ssDNA and GO are 0.11 mg/mL and 4.65 μ g/mL, respectively. Experiments are performed in deionized water (λ_{ex} =980 nm).

Reference

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