Supporting Information

Integrating Optical Tweezers with Up-converting Luminescence: A Non-amplification Analytical Platform for Quantitative Detection of MicroRNA-21 Sequences

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Construction of the setup

The laser beam is first collimated by a fiber-port and regulated its longitudinal height to match the inverted microscope platform (Olympus IX70, Japan) through an adjusting frame. Then the collimating beam is expanded 3-fold by an expander system, resulting in a final diameter of approximately 8 mm to slightly overfill the back aperture of the objective (100×, NA = 1.30, Olympus, Japan). After passing through a telescope system which is used to keep consistency between the imaging plane and the trapping plane, the laser beam is directed into the microscope and reflected upward by a dichroic mirror (DM, 850 nm, Edmund). Finally, the beam is tightly focused with the high numerical objective to reach the home-made sample chamber, forming a diffraction limited spot of about 0.92 μm to realize optical trapping. The up-converting luminescence images are captured by an EMCCD (Photometrics, Canada) after blocking the NIR laser by a short-pass filter (SPF, FF01-760/SP-25, Semrock) inserted into the filter cube and a converged white-light LED source can be acted as the bright-field illumination to achieve the real-time records for the trapping events with a digital camera (DC). The actual laser power on sample chamber is ~40% of the output power measured by a NIR power meter (Coherent, USA).

Materials

Ytterbium chloride hexahydrate ($YbCl_3$ $6H_2O$, 99.9%), yttrium chloride hexahydrate (YCl_3 $6H_2O$, 99.9%), erbium chloride hexahydrate ($ErCl_3$ $6H_2O$, 99.5%), and diethylene glycol (DEG, 99%) were purchased from Aladdin Industrial Inc. (China). Oleic acid (OA, 90%), 1-octadecene (ODE, 95%), polyacrylic acid (PAA, average

MW ~1800), 1-ethyl-3-(3-dimethyllamino-propyl)carbodiimide hydrochloride (EDC·HCl, 99%), and bovine serum albumin (BSA) were obtained from Sigma-Aldrich Co. (USA). Carboxyl coated polystyrene microspheres (CPSs, 50 mg/ml) with uniform sizes of 3 μm were provided by Shanghai Huge Biotechnol. Co. Ltd. (China). Other reagents with analytical grades were supplied by Sinopharm Chemical Reagent Co. (China). All the nucleic acid sequences purified with HPLC were received from Shanghai Sangon Biotech. (China) as shown below:

Amino-modified capture DNA: 5′-NH_2-$(CH_2)_{12}$-GGTCAACATCAGT-3′

Amino-modified probe DNA: 5′-CTGATAAGCTAGG-$(CH_2)_6$-NH_2-3′

MicroRNA-21 (T): 5′-UAGCUUAUCAGACUGAUGUUGA-3′

Single-base mismatched RNA (MT1): 5′-UAGCUUAGCAGACUGAUGUUGA-3′

Two-base mismatched RNA (MT2): 5′-UAGCUUAGUAGACUGAUGUUGA-3′

Random RNA: 5′-AACUUCGGUCCCUGCAGUUCG-3′

Instrumentations

TEM images of the prepared UCNPs were recorded by a JEM-2100 transmission electron microscope (JEOL, Japan) operating at 100 KV, under which energy dispersive X-ray (EDX) analyses were simultaneously performed. Zeta potential measurements were carried out on a dynamic light scattering system (Zetasizer Nano ZS90). XRD spectra ranging from 10° to 80° were acquired on an X-ray diffractometer (PANalytical, Netherlands) with a Cu-Kα radiation (λ = 1.5406 Å). IR spectra were measured with a FT-IR spectrometer (NICOLET 5700, USA) with the KBr pellet technique. UV-vis absorption spectra were acquired on a UV-2550

spectrophotometer (Shimadzu, Japan) with the slits set at 2 nm. Up-converting luminescence spectra were detected with a fluorescence spectrometer (Hitachi F–4600, Japan), which was equipped with a 980 nm CW diode laser (Beijing Hi-Tech Optoelectronic Co., Ltd.) as the excitation light source. Reverse transcription PCR (RT-PCR) were performed on a PCR amplification instrument (Bio-Rad, USA). Agarose gel electrophoresis was implemented on a voltage steady electrophoresis apparatus with an imaging system (DYY-6C, Beijing Liuyi Co., China).

Synthesis of high-quality hydrophobic UCNPs capped with OA

Rare earth chloride mixtures (1 mmol) containing 78% $YbCl_3$ $6H_2O$, 20% YCl_3 $6H_2O$, and 2% $ErCl_3$ $6H_2O$ were added into a 100 mL three-necked flask and then mixed with 15 mL of ODE and 6 mL of OA under argon protection. Subsequently, the temperature of this system was increased to 150 ℃ to accelerate the dissolution rate. After obtaining a pale yellow homogenous solution, it was allowed to cool to room temperature. Rare earth oleate precursors were gained by slowly injecting 10 mL of methanol solution dissolving with 4 mmol of NH_4F and 2.5 mmol of NaOH into the flask and stirring for 30 min to form a white turbid liquid. Afterwards, the solution was kept at 110 ℃ under vacuum for 15 min to remove methanol, residual water and oxygen. Then the reaction mixture was heated to 320 ℃ at a rate of 10 ℃/min in argon atmosphere and maintained for 70 min with vigorous stirring to mediate the nanocrystals. Finally, 20 mL of ethanol was added into the above golden yellow solution to precipitate the nanoparticles and purified them by separately washing two times with ethanol/water (v/v = 1:1) and hexane/ethanol (v/v = 1:4) to remove the

unreacted precursors and organic reagents. The as-prepared hydrophobic UCNPs were dispersed in 8 mL of chloroform to keep the final concentration to 10 mg/mL for later use.

Synthesis of high-quality hydrophilic UCNPs modified with PAA

4 mL of the as-obtained OA capped UCNPs were first dissolved into 15 mL of DEG solution containing 600 mg of PAA to form a transparent solution and then transferred into a 100 mL three-necked flask. Afterwards, the solution was heated to 110 ℃ and kept for 10 min to remove chloroform under vacuum, and then maintained at 240 ℃ for 3 h to accomplish the ligand exchange process with vigorous stirring. The final hydrophilic UCNPs were washed three times with ethanol to remove the residual OA and dispersed in 4 mL of ultrapure water for long-term storage.

Preparation of capture microspheres

5 μL of the original concentration of CPSs were first washed two times with 100 μL of MES buffer solution (10 mM, pH 5.5) and then mixed with 0.3 nmol of amino-modified capture DNA in a 2 mL centrifuge tube. Subsequently, 3.6 μL of 10 mg/mL EDC was added into the tube and incubated for 1 h at room temperature with gentle shaking. Thereafter, 1 μL of 100 mg/mL EDC was needed to add into the reaction mixture to ensure the conjugating efficiency and gently shook it for 10 h at room temperature. The final capture microspheres were obtained by washing three times with 100 μL of Tris-HCl buffer solution (50 mM, 200 mM NaCl, pH 8.0) to remove the unreacted reagents and stored at 4 ℃ for further use.

Preparation of UCNP probes

0.6 mg of PAA modified UCNPs were first mixed with 0.2 nmol of amino-modified probe DNA in a 2 mL centrifuge tube and then 5 μl of 10 mg/mL EDC was added into the solution. Afterwards, 100 μL of MES buffer solution was added into the tube and maintained the reaction mixture for 10 h at room temperature with gentle shaking. Finally, the DNA modified UCNPs were washed three times with 50 μL of Tris-HCl buffer solution to remove the unreacted reagents and stored at 4 ℃ for further use.

Preparation of complex microspheres

A facile one-step nucleic acid hybridization process, which was realized by mixing the capture microspheres, the UCNP probes and the microRNA-21 sequences, was adopted to get the complex microspheres enriched with various amount of targets. In a typical procedure, 4 fmol of targets, 2.5 μg of capture microspheres and 6 μg of UCNP probes were incubated at 30 ℃ for 4 h. The final microspheres were recovered by washing three times with Tris-HCl buffer solution to remove the unreacted capture microspheres and UCNP probes, and stored at 4 ℃ for later use.

Three-dimensional optical trapping and manipulation of a single complex microsphere

Two cover glasses were first blocked with 1% BSA to prevent the complex microspheres from absorbing onto their surfaces due to physical interaction. Then a home-made sample chamber based on these cover glasses was sealed by vacuum silicone with thickness ~0.84 mm to avoid the evaporation of medium too fast because of laser thermal effect. The as-prepared complex microspheres were first sonicated for about 3 min and then loaded into the sample chamber by diluting

ten-fold to reduce the interference from surrounding microspheres during the experiment. A passive manipulation method, which was performed by slowly moving the sample stage in the transverse direction, was employed to realize the horizontal manipulation of a trapped complex microsphere. An active manipulation method, which was implemented by gradually regulating the objective in axial direction, was applied to achieve the longitudinal movement of a trapped complex microsphere.

Quantitative detection of standard miRNA-21 sequences

To acquire the best signal-to-background data and avoid the local signals from the trapped complex microspheres exceed the upper limit of EMCCD response (65535 gray-level per pixel), the exposure time was chosen as 100 ms. For each concentration group, 60 complex microspheres were trapped and detected within the time interval of 3 s for two consecutive measurements and their mean gray-level is used to represent the luminescence intensity.

Selectivity investigation

The experimental procedures were similar as mentioned above except that the targets were changed to same amount (4 fmol) of single-base mismatched sequences, two-base mismatched sequences and random sequences.

Cell culture

MCF-7 cells and Jurkat T cells were grown following the same procedure by using RPMI-1640 medium containing with 1% bovine insulin as the culture medium. The cells in chamber slides were supplemented with 1% penicillin/ streptomycin and 10% FBS, and then cultured at 37 ℃ in an incubator supplied with 5% CO_2.

Total RNA extraction

Total RNA from two kinds of cells were extracted according to a mature experimental protocol provided by a RNA extraction kit (EZ-10 DNAaway RNA mini-prepes kit, Shanghai Sangon Biotech.). (1) Sample preparation: 10^4 cells were first centrifuged at 300 × g for 2 min to remove the supernatant and then immediately mixed with 350 ul of buffer lysis-DR in a 1.5 mL RNase-free centrifuge tube by vortex. (2) RNA purification: DNA was first removed by centrifuging the lysate in a DNA eliminator column at 9000 × g for 1 min and then 250 μL of ethanol was added into the flow-through followed by centrifuging at 9000 × g for 1 min in a RNA column. Then the as-obtained total RNA was separately washed with 500 μL of GT solution and 500 μL of NT solution. After completely evaporating the residual ethanol, the extracted RNA was dissolved in 50 μL of RNase-free water and kept at -80 ℃ for long-term storage.

Quantitative detection of miRNA-21 sequences in cells

The experimental procedures were similar to the detection of standard miRNA-21 sequences except that the targets were changed to the total RNA extracted from two kinds of cells, namely MCF-7 cells and Jurkat T cells. The original total RNA from 10^4 cells was step-wise diluted when investigating the sensitivity for cell detection.

RT-PCR detection

RT-PCR relating to absolute quantification was conducted according to the instructions provided by manufacturer. cDNA was synthesized by reverse transcription of the extracted total RNA from 10^6 cells using a designed specific

primer(5′-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAACATC-3′) and then performed PCR amplification to get the desired gene (Thermo Scientific™ EP0733). The detailed conditions were as follows: (1) Denaturation temperature was 94 ℃ and kept for 30 s; (2) Annealing temperature was 57 ℃ and kept for 30 s; (3) Extension temperature was 72 ℃ and kept for 30 s; (4) All objects were cycled with 35 times.

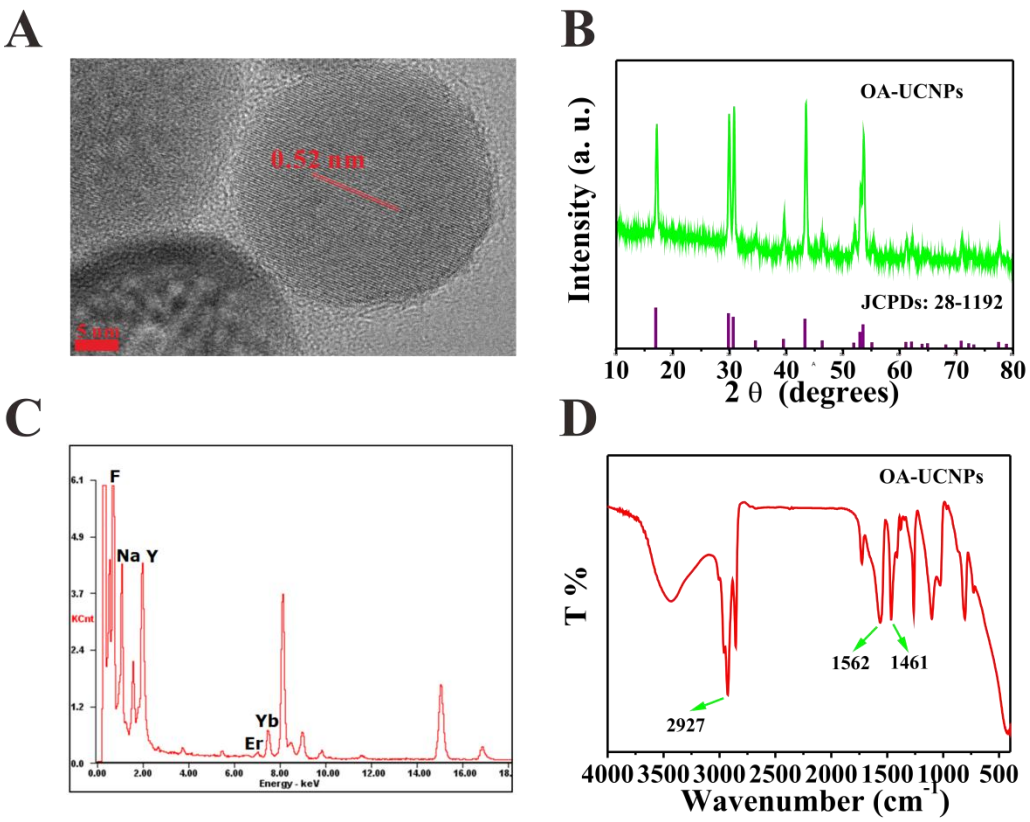


Fig. S1. (A) HRTEM image of OA-UCNPs. The scale bar is 5 nm. (B) XRD spectrum of OA-UCNPs. (C) EDX analysis of OA-UCNPs. (D) FT-IR spectrum of OA-UCNPs. All these characterizations were performed in solid state.

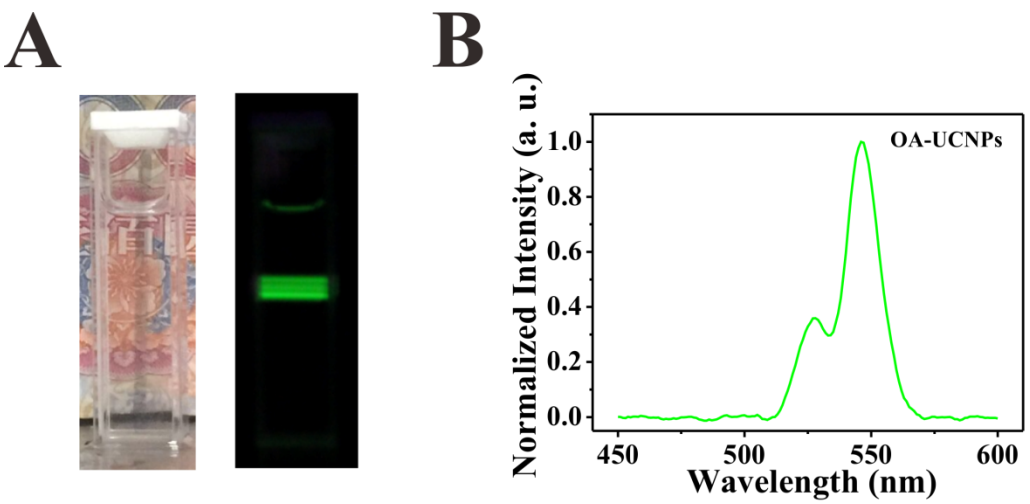
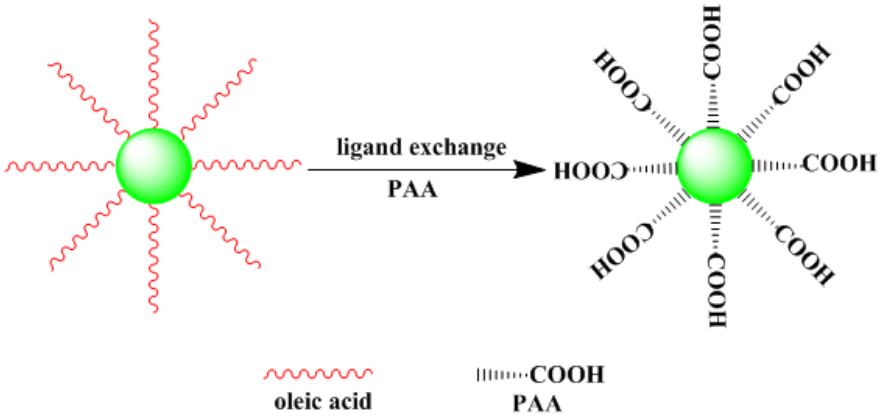


Fig. S2. (A) Digital images of the OA-UCNPs formed transparent solution in chloroform without laser irradiation (left) and under 980 nm laser irradiation with 500 mW power (right). (B) Up-converting luminescence spectrum of OA-UCNPs under 980 nm laser excitation with 500 mW power. The concentration of nanoparticles for these measurements is 10 mg/mL.



Scheme S1. Principle diagram of the ligand exchange process for the preparation of PAA-UCNPs from OA-UCNPs.

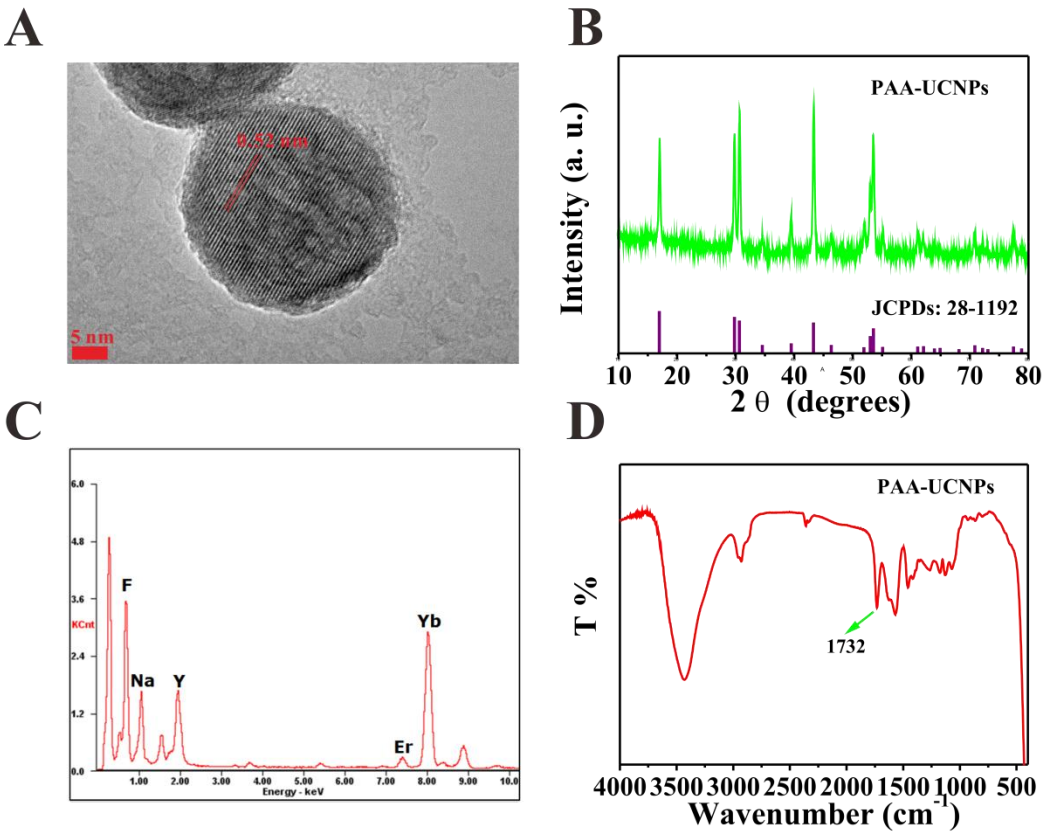


Fig. S3. (A) HRTEM image of PAA-UCNPs. The scale bar is 5 nm. (B) XRD spectrum of PAA-UCNPs. (C) EDX analysis of PAA-UCNPs. (D) FT-IR spectrum of PAA-UCNPs. All these characterizations were performed in solid state.

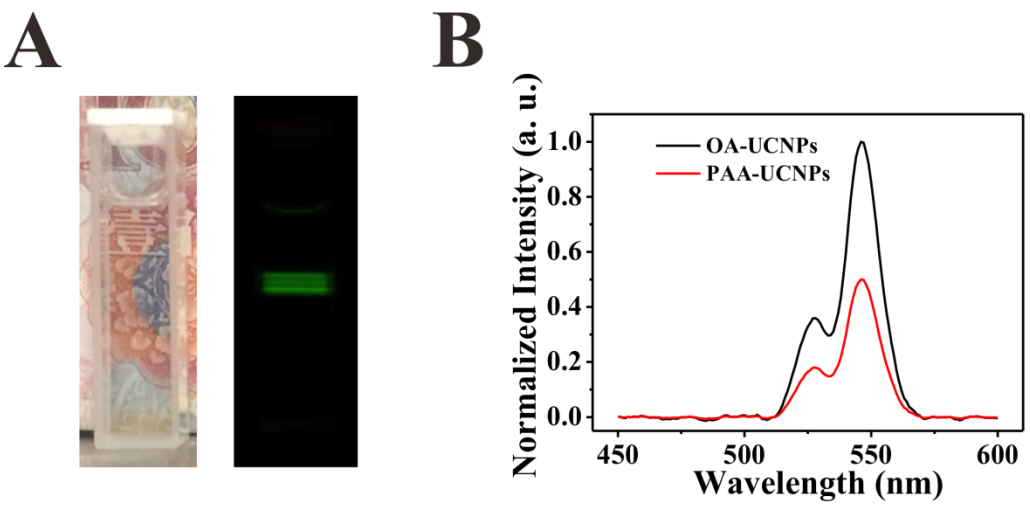


Fig. S4. (A) Digital images of the PAA-UCNPs formed transparent solution in water without laser irradiation (left) and under 980 nm laser irradiation with 500 mW power (right). (B) Up-converting luminescence spectra of OA-UCNPs and PAA-UCNPs under 980 nm laser excitation with 500 mW power. The concentration of nanoparticles for these measurements is 10 mg/mL.

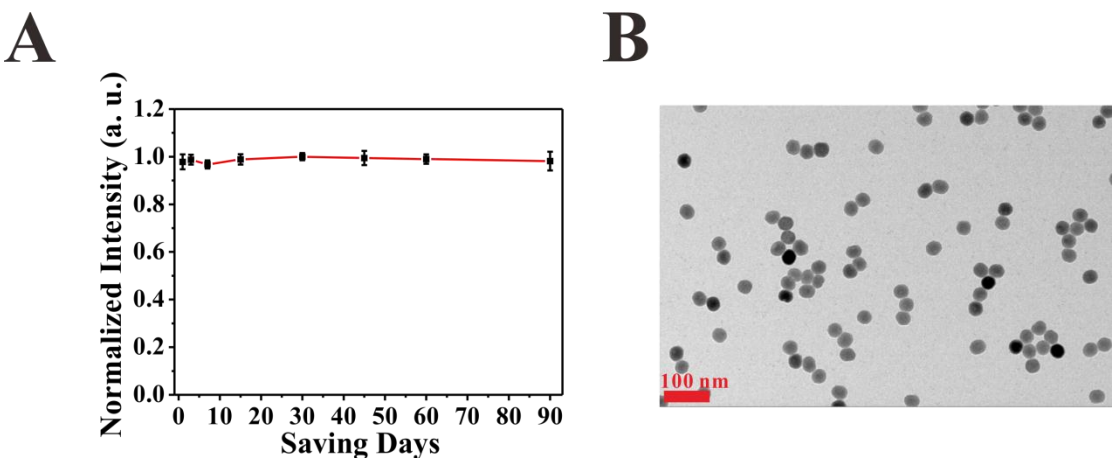
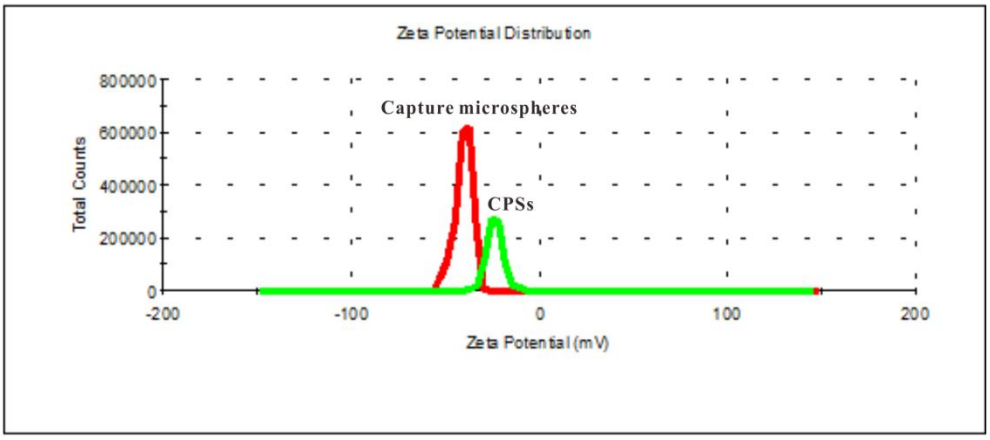


Fig. S5. (A) Optical stability of PAA-UCNPs with time evolution. (B) TEM image of PAA-UCNPs saved for three months.

A



B

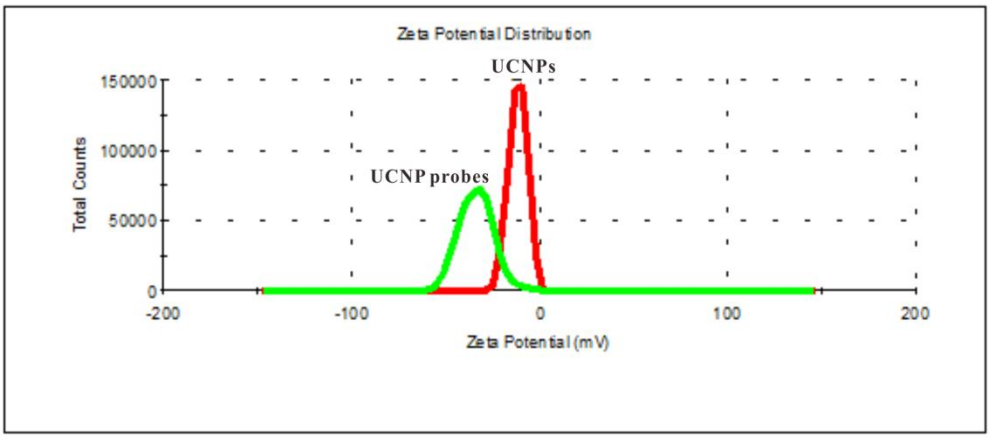


Fig. S6. (A) Zeta potential distributions of CPSs before and after binding with capture DNA. (B) Zeta potential distributions of PAA-UCNPs before and after binding with probe DNA.

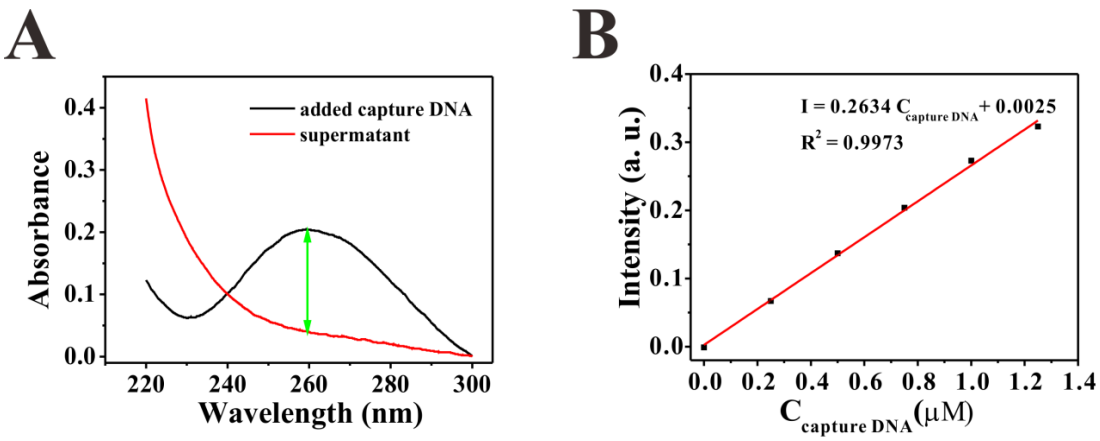


Fig. S7. (A) UV-vis absorption spectra of added capture DNA and supernatant from the reaction system for the preparation of capture microspheres. (B) Standard curve of various concentrations of capture DNA (0, 0.25, 0.75, 1, 1.25 μM).

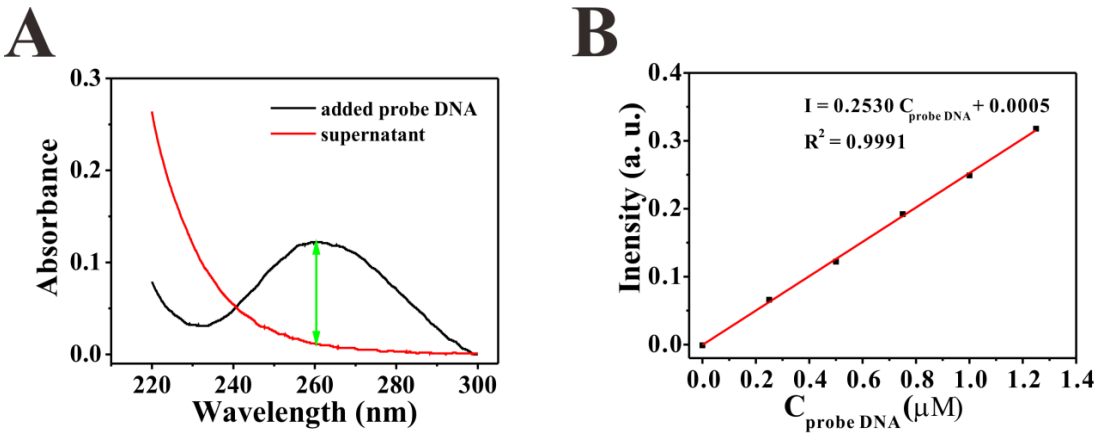


Fig. S8. (A) UV-vis absorption spectra of added probe DNA and supernatant from the reaction system for the preparation of UCNP probes. (B) Standard curve of various concentrations of probe DNA (0, 0.25, 0.75, 1, 1.25 μM).

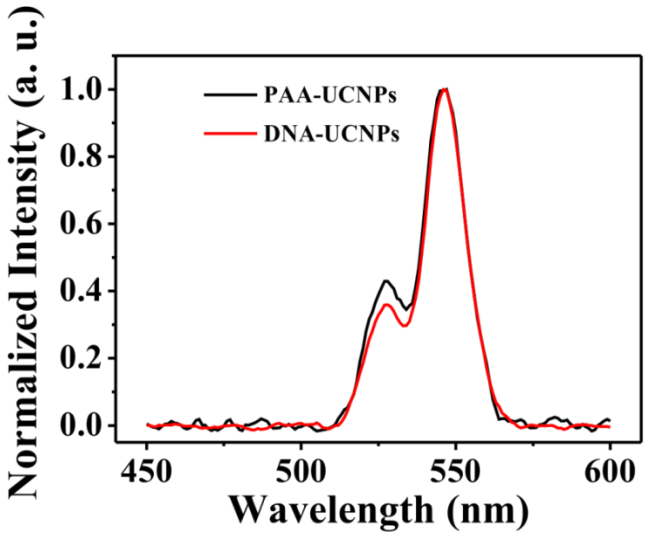


Fig. S9. Up-converting luminescence spectra of PAA-UCNPs before and after conjugating with probe DNA.

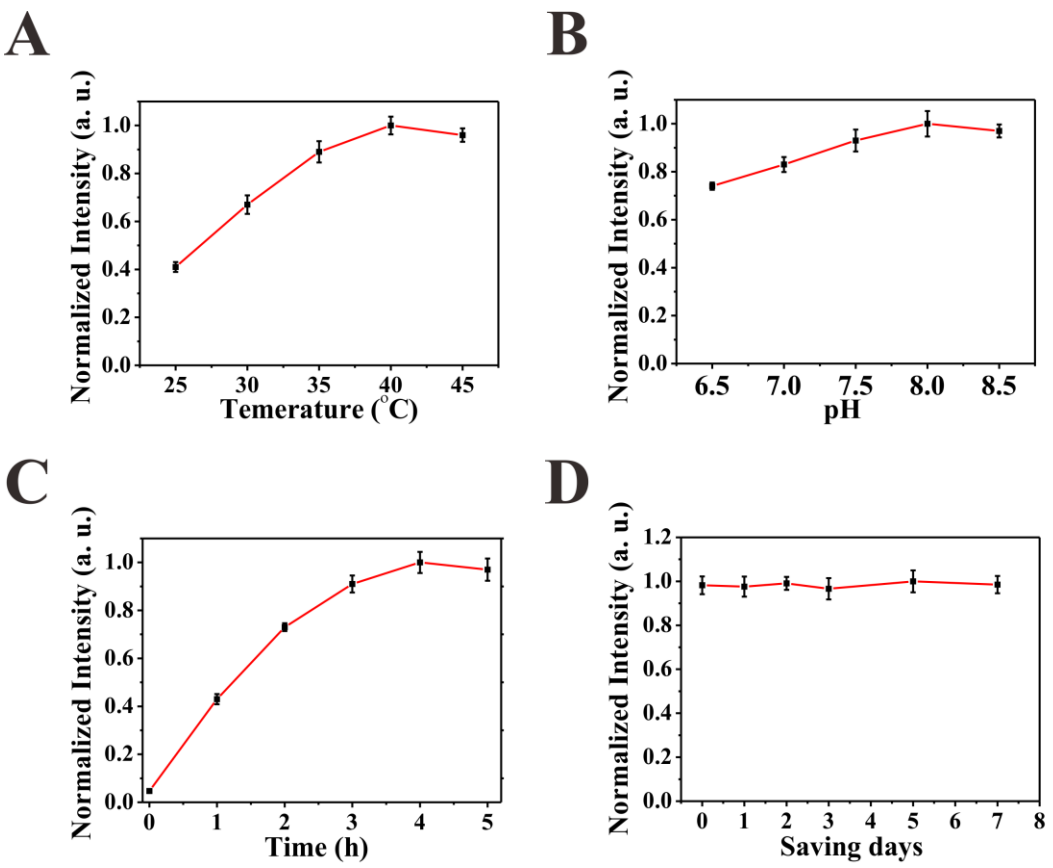


Fig. S10. (A) Luminescent intensity versus reaction temperature. (B) Luminescent intensity versus reaction pH. (C) Luminescence intensity versus reaction time. (D) Luminescence stability of the prepared complex microspheres.

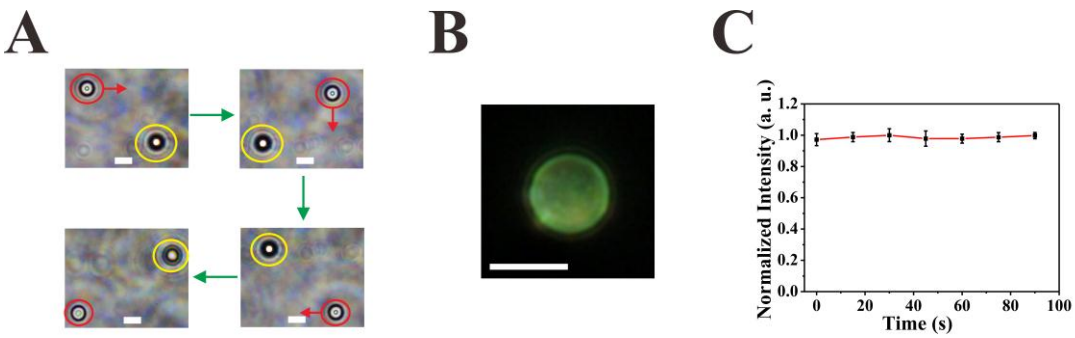


Fig. S11. (A) Bright-field images of three-dimensional optical trapping and manipulation of a single complex microsphere enriched with 4 pM miRNA-21 sequences along a square trajectory under the tightly focused 980 nm laser beam recorded by a digital camera (DC). The red circle and the yellow circle represent the trapped and a stationary microsphere, respectively, and the scale bars are 3 μm for all cases. (B) DC captured color image of the trapped complex microsphere as that of Figure S11A when turning off the LED lamp (exposure time 1 s). The scale bar is 3 μm. (C) Optical stability of the same trapped complex microsphere with time evolution.

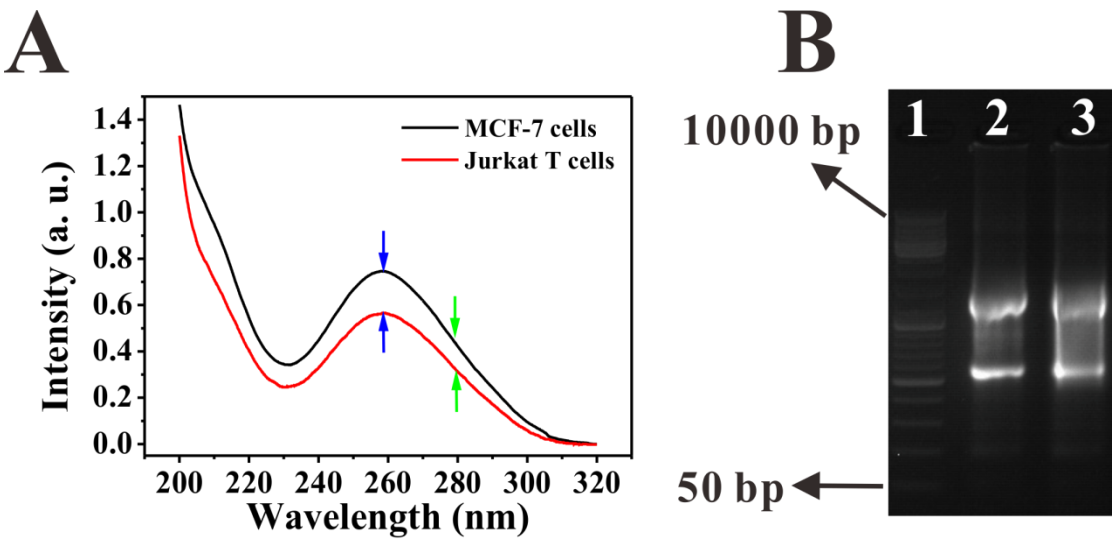


Fig. S12. (A) UV-vis absorption spectra of the extractive from 10^4 MCF-7 cells and 10^4 Jurkat T cells. (B) Agarose gel electrophoresis images of the extractive, where band 2 and band 3 represent MCF-7 cells and Jurkat T cells, respectively, and band 1 represents the markers. This experiment was performed by loading the extractive into 1.5% agarose and then run under the voltage of 120 V for 30 min.

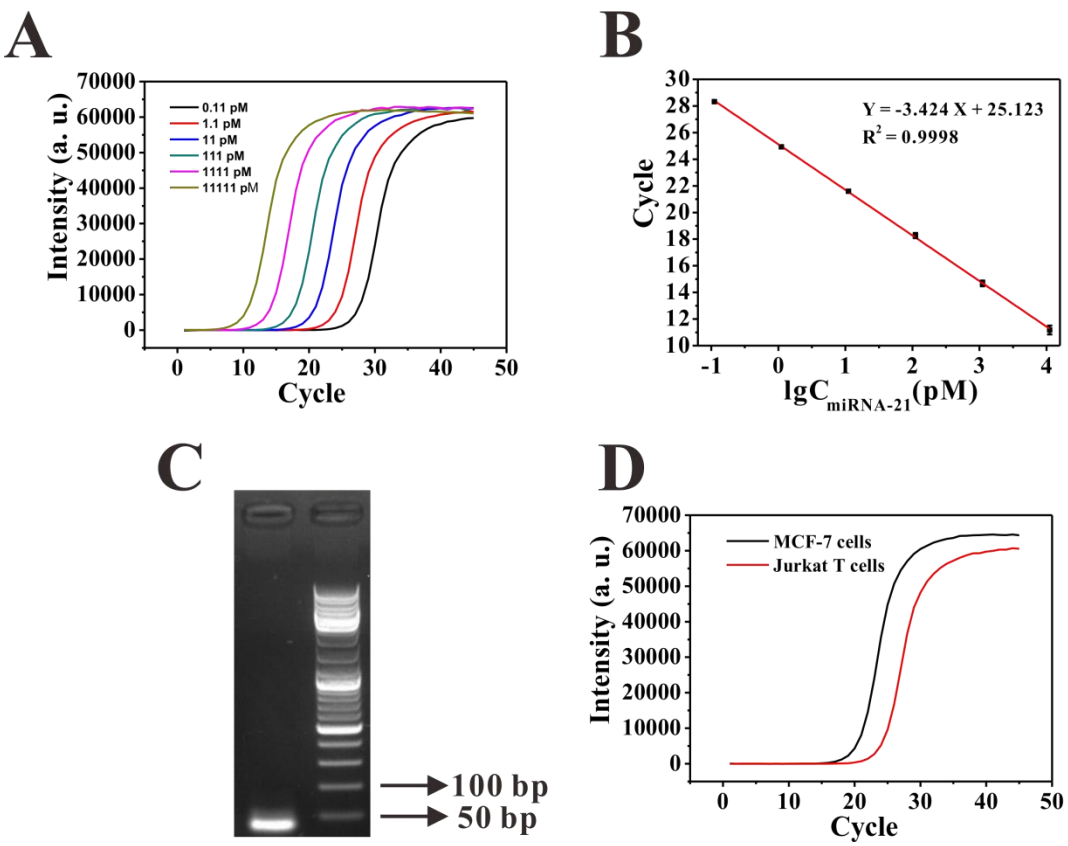


Fig. S13. Detection results of miRNA-21 by RT-PCR. (A) Real-time luminescence intensity at various concentrations of miRNA-21 sequences. (B) Liner relationship between cycle times and the logarithm of the concentration of miRNA-21 sequences. (C) Agarose gel electrophoresis image of the PCR amplification products of standard miRNA-21 sequences. (D) Cycle curves of the extractives from 10^6 MCF-7 cells and 10^6 Jurkat T cells.

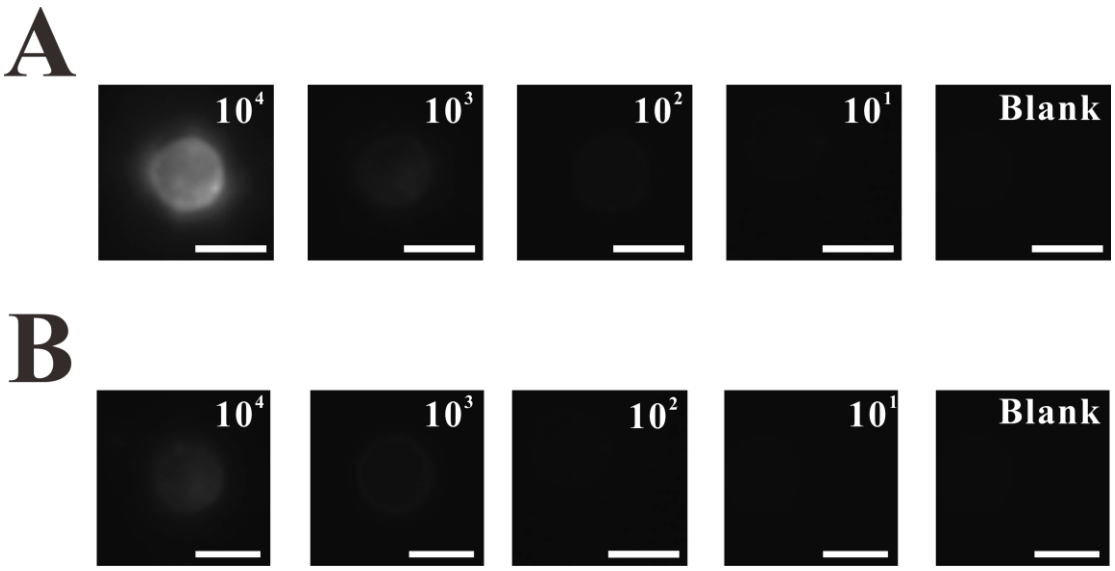


Fig. S14. (A) Representative luminescence images of the trapped complex microspheres enriched with miRNA-21 sequences from diluted MCF-7 cells. (B) Representative luminescence images of the trapped complex microspheres enriched with miRNA-21 sequences from diluted Jurkat T cells. The scale bars are 3 μm for all cases.

Table S1. Quantitative detection of standard miRNA-21 sequences

Concentration (pM)	Intensity (a. u.)	CV (n = 60, %)
4	11872	4.6
3	8478	4.1
2	5944	4.7
1	3674	3.5
0.5	2180	3.8
0.3	1573	4.9
0.2	1101	3.4
0.06	995	3.6
0.02	700	2.4
0	560	1.9

Table S2. Comparison with other luminescence-based approaches for miRNAs detection

method	LOD	amplification	cells
paper based biosensing platform using polymer dyes as luminescent reporters[1]	10 nM	no	no
using DNA templated silver nanocluster probes[2]	1 nM	no	no
enhanced luminescence on a photonic cystal[3]	0.5 nM	yes	no
double-strand displacement biosensor with a complementary luminescent reporter[4]	0.1 nM	yes	10^5
using photonic-crystal micropattern supported dual-modal upconversion bioprobes[5]	10 pM	yes	10^5
using templated Ru^{II}-catalyzed unmasking of a fluorophore[6]	5 pM	yes	10^5
FRET between graphene quantum dots and ssDNA-UCNP@SiO_2[7]	10 fM	yes	no
plasmon-enhanced hybridization chain reaction[8]	1 fM	yes	5
this method	12 fM	no	10^2

Table S3. Determination the number of miRNA-21 sequences in a single cell

	intensity (gray-level)[a]	molar level of miRNA-21 for 10^4 cells (amol)[b]	number of miRNA-21 in a single cell (copies)[c]
MCF-7 cell	9762	33.33	2007
Jurkat T cell	2325	5.997	361

[a] Intensity is the counted value of the mean gray-level from 60 complex microspheres enriched with miRNA-21 sequences extracted from 10^4 cells.

[b] Molar level of miRNA-21 for 10^4 cells is calculated according to the acquired linear equation between luminescence intensity (I) and concentration of miRNA-21 ($C_{miRNA-21}$) ($I = 2720\ C_{miRNA-21} + 693.5$), where the volume is 10 μL.

[c] Number of miRNA-21 in a single cell is calculated following the equation of $N= nN_A$, where n and N_A represents the molar level of miRNA-21 in a single cell and Avogadro constant, respectively.

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