Supporting Information

Incorporating Luminescence-Concentrating Upconversion Nanoparticles and DNA Walkers into Optical Tweezers Assisted Imaging: A Highly Stable and Ultrasensitive Bead Supported Assay

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Experimental Section

Materials

Oleic acid (OA, 90%), 1-octadecene (ODE, 90%), polyacrylic acid (PAA, average MW ~ 1.8 KDa), 1-ethyl-3-(3-dimethyllamino-propyl)carbodiimide hydrochloride (EDC·HCl, 99.5%), 2-(N-morpholino)ethanesulfonic acid (MES, 99.5%), tris(hydroxymethyl)aminomethane (Tris·HCl, 99.5%), and bovine serum albumin (BSA, 99.5%) were purchased from Sigma-Aldrich Co. 3 μm monodispersed amino-modified silica beads (ASBs, coefficient variation ~ 2%, concentration ~ 25 mg/mL), $YCl_3 \cdot 6H_2O$ (99.99%), $YbCl_3 \cdot 6H_2O$ (99.99%), $ErCl_3 \cdot 6H_2O$ (99.5%), diethylene glycol (DEG, 99.9%), and NH_4F (99.99%) were supplied by Aladdin Industrial Inc. (China). Nicking endonucleases were obtained from Thermo Fisher Scientific. Alpha fetal protein (AFP, > 95%) and prostate specific antigen (PSA, > 95%) were provided by Zhengzhou Biocell Biotechol. Co. Ltd. (China). Other chemical solvents including methanol, ethanol, hexane, chloroform, and acetone bought from Sinopharm Chemical Reagent Co. (China) were of analytical grades. All reagents were used as received and buffers were prepared throughout from ultrapure water (Resistivity ≥ 18.25 $M\Omega \cdot cm^{-1}$). DNA and RNA sequences listed in **Table S1** were synthesized and purified with HPLC by Shanghai Sangon Biotech. (China).

Instrumentations

Transmission electron microscopy (TEM) images were obtained on a JEM-2100

transmission electron microscope (Japan) operating at 100 kV, under which high-resolution imaging and energy dispersive spectrometer determination (EDS) were simultaneously implemented. X-ray diffraction (XRD) patterns ranging from 10° to 80° were token on an X-ray diffractometer (PANalytical, Netherlands) under Cu-Kα radiation (λ = 1.5406 Å). Fourier transform infrared (FT-IR) spectra were acquired on a FT-IR spectrometer (NICOLET 5700, USA) by depositing the solid samples on KBr tablets. Zeta potential distributions and hydrodynamic diameters were measured on a dynamic light scatting (DLS, Zetasizer Nano ZS90, Malvern Instruments) system. Scanning electron microscopy (SEM) images were captured on a field emission scanning electron microscope (Zeiss SIGMA, UK) operating at 20 kV. UV spectra were detected on a Shimadzu UV-2550 spectrophotometer (Japan) by setting the slits at 2 nm. Upconversion luminescence spectra were recorded on a Hitachi F-4700 spectrophotometer (Japan) equipped with an external 980 nm continuous laser (Changchun New Industries Optoelectronics Technology Co., China) by setting the λ_{em} at 10 nm. Native PAGE electrophoresis experiments were conducted on a voltage type electrophoresis imaging apparatus (JY04S-3C, Beijing Junyi Co., China).

Synthesis of hydrophobic core UCNPs ($NaYF_4$) capped with OA

Initially, 1 mmol of $YCl_3 \cdot 6H_2O$ was added into a 100 mL three-neck round-bottom flask containing 21 mL of OA/ODE (v/v = 2:5) mixture. Then, the flask was processed with vacuum treatment for 40 min under gentle stirring. After heating to 150 °C and mainating the Ar flow for at least 60 min, the slurry became into a clear

solution. On cooling to room temperature, the system was injected dropwise with 10 mL of freshly prepared methanol dissolving with NH_4F/NaOH (4 mmol/2.5 mmol) and continued to vigorous stirring for 30 min until form the white turbidity oleate intermediates, followed by degrassing at 100 °C for 20 min to remove residual oxgen and other low boiling impurities. Thereafter, the flask temperature was rapidly increased to 310 °C in 25 min and kept Ar flow for 60 min. Subsequently, excess ethanol was fully mixed together with the cooled golden yellow solution to precipitate out the products. Next, the precipitates were collected by centrifugation, and orderly washed several times with water/ethanol mixture (v/v = 1:1) and hexane/ ethanol mixture (v/v = 1:6) to reomve the unreacted oraginc solvents and inorganic salts. Finally, the as-purified nanoparticles were performed with ultrasonic for 5 min in 8 mL of chloroform to obtain a homogeous solution for later use (stored at 4 °C, concentration ~ 10 mg/mL).

Synthesis of hydrophobic core-shell UCNPs ($NaYF_4$@$NaYF_4$, Yb, Er) capped with OA

0.5 mmol of lanthanide chloride mixture with a specific ratio (Y/Yb/Er =0.8: 0.18: 0.02) were first stirred together with 10.5 mL of OA/ODE (v/v = 1:6) mixture in a 50 mL three-neck round-bottom flask and then processed with vacuum treatment for 40 min. Upon forming a clear pale yellow solution at 150 °C under Ar flow, the ealier retrieved OA capped core UCNPs were added dropwise into the flask. Next, it was allowed to degass at 100 °C for 20 min to remove the needless chloroform and inject

slowly with 10 mL of freshly prepared methanol containing NH_4F/NaOH (2 mmol/1.25 mmol). By keeping vigorous stirring for 30 min, the white turbidity oleate intermediates were further degassed at 100 °C for 20 min, followed by quickly heating to 310 °C and maintaining Ar flow for 75 min. After precipitating out the products with ethanol, the resultant nanoparticles were washed, purified, and finally dispersed into 4 mL of chloroform for later use (stored at 4 °C, concentration ~ 20 mg/mL).

Synthesis of hydrophobic SUCNPs ($NaYF_4$@$NaYF_4$, Yb, Er@$NaYF_4$) capped with OA

First, 0.5 mmol of $YCl_3 \cdot 6H_2O$ was mixed together with 10.5 mL of OA/ODE (v/v = 1:6) mixture in a 50 mL three-neck round-bottom flask. Subsequently, the slurry was handled with vacuum treatment for 40 min. After heating to 150 °C and keeping gentle stirring to form a clear solution, the ealier retrieved OA capped core-shell UCNPs were added dropwise into the flask. Then, it was degassed at 100 °C for 20 min and injected slowly with 10 mL of freshly prepared methanol containing NH_4F /NaOH (2 mmol/1.25 mmol), followed by vigorous stirring for 30 min to obtain the white turbidity oleate intermediates. Upon further degassing at 100 °C for 20 min, the system temparature was allowed to raise to 310 °C as quickly as possible and maintain Ar flow for 75 min. By precipitating, collecting, washing and purifying the products, the final nanoparticles were performed with ultrasonic in 4 mL of chloroform for at least 10 min for later use (stored at 4 °C, concentration ~ 20

mg/mL). When introducing different ratios of OA/ODE, the controllable tunning of the tickness of outer shell can be realized in this synthetic pathway.

Synthesis of hydrophilic SUCNPs modified with PAA

1600 mg of PAA was first added into a 50 mL three-neck round-bottom flask and then dissolving into 20 mL of DEG under gentle strirring for 20 min. After vacuum treatment, the clear solution was heated to 120 °C under Ar flow, followed by injecting dropwise with the ealier retrieved OA capped SUCNPs. Next, the system was allowed to degass for 10 min to remove the needless chloroform. Subsequently, the flask temperature was raised to 240 °C in 15 min and kept gentle stirring under Ar flow for 4 h. Upcon mixing togther with 20 mL of acetone, the products were precipitated out from the golden yellow solution and collected by high-speed centrifugation at 18000 rpm for 15 min. By further removing the unreacted ploymers with ethanol for five times, the final nanoparticles were treated with ultrasonic in 2 mL of ultrapure water for long-term storage (stored at 4 °C, concentration ~ 20 mg/mL).

Construction of home-built optical tweezers device

An optical fiber coupled 980 nm CW laser (M^2 ~ 1.1, output power < 950 mW, Connet, China) equipped with a collimator is operated as both trapping and exciation sources. The collimated beam (~ 3 mm) first passes through an adjusting frame to actualize axial height regulation and then is guided into a expander system (1:3),

leading to a spot size (~ 9 mm) capable of properly overfilling the pupil of oil immersion objective (100×, NA 1.30, Olympus). After proceeding with a telescope system (1:1) to accurately make the trapping plane consistent with the imaging plane, the larger-size beam is imported into an inverted microscope system holding an infinite optical path (Olympus IX70), followed by reflecting upward by its internal dichroic mirror (850 nm). Upon tightly focusing the adjusted Guassian beam on the mobilizable sample stage, a diffraction limited spot (~ 1 μm) is generated into the specially-made specimen chamber to perform the expectative events. Before collecting the upconversion luminescence ranging from 510 to 550 nm with a cooled EMCCD (Evolve 512 Delta, Photometrics, Canada), it is necessary to eliminate the interference of residual 980 nm laser by a blocking filter (760 nm). Beyond that, the bright-field illumination realized by a converged LED lamp is recorded in real time by a digital camera. Owing to the optical loss of the above components, about 45% of the output power can finally reach the focus area.

Assembling ASBs with SUCNPs-PAA

Approximately 125 μg of ASBs were first collected by centrifuging at 3500 rpm for 5 min and then washed twice times with ultrapure water. Next, the as-washed ASBs were suspended into 50 μL of MES buffer solution (10 mM, pH 6.8) and treated with ultrasonic for 5 min in a 1.5 mL tube. Thereafter, 1 μL of SUCNPs-PAA and 5 μL of freshly prepared 10 mg/mL EDC solution were orderly added into the tube. After gentle shaking at room temperature for 60 min, the mixture was additionally

introduced with 100 μg of EDC to facilitate the activation process and continued to incubate for 2 h. By removing the unreacted SUCNPs-PAA with centrifugation, the final assembled beads were washed three times with MES buffer containing 0.5% Tween 20 for further use (stored at 4 °C).

Co-conjugating DNA substrates and DNA walkers onto SUCNPs-PAA assembled beads

Initially, the as-prepared SUCNPs-PAA assembled beads were treated with ultrasonic for 3 min. Then, a total of 0.1 nmol of DNA walkers and DNA substrates (1:9) were added into the suspension solution and further mixed together *via* vortex for 2 min. After that, the mixture were gently shaken with 5 μL of freshly prepared 10 mg/mL EDC solution for 30 min at room temperature, followed by providing with 1 μL of 100 mg/mL EDC solution. Upon incubating for another 2 h, the conjugated beads were collected by centrifugation at 8000 rpm for 6 min to remove the residual DNAs and washed three times with 100 μL of Tris buffer (20 mM, 10 mM $MgCl_2$, pH 8.0) containing 0.5% Tween 20 for further use (stored at 4 °C).

Linking protecting DNA onto beads

The as-conjugated beads were first mixed together with 0.2 nmol of protecting DNAs *via* vortex and then gently incubated at 35 °C for 2 h. During the hybridization process, ultrasonic treatment was required every 30 min to prevent the beads from depositing to the bottom. After centrifugating to remove the unreacted protecting

DNAs and washing, the as-obtained beads were re-suspended into 100 μL of Tris buffer for further use (stored at 4 °C).

Fabrication of specimen chamber

First, a polydimethylsiloxane (PDMS) film was bound with a cover glass (22 × 50 × 0.17 mm) under a high vacuum and dust-free environment. Then, the PDMS was punched with a hollow hole (2 mm in diameter). To prevent the beads from absorbing onto the bottom cover glass, the specimen chamber should be further soaked in 1% BSA solution for blocking treatment.

Calibration of optical tweezers setup

A certain amount of ASBs were first loaded into the home-made sample chamber and then diluted 10-fold with ultrapure water to ensure that only a few beads were presented in the view field. Afterwards, the sample chamber should be further sealed with another cover glass (22 × 22 × 0.17 mm) to avoid the medium evaporation produced by the laser heating effect. The horizontal manipulation (X-Y directions) of a trapped ASB was realized through a passive approach performed by slowly moving the sample chamber. The axial manipulation (Z direction) was achieved through an active approach conducted by gradually adjusting the working distance of the objective.

Quantitative analysis of standard miRNA-21 sequences in buffer

The protecting DNAs linked beads were first divided into twelve RNA enzyme-away tubes and then mixed together with various amounts of standard miRNA-21 sequences (0, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 200, 300 amol). Subsequently, these tubes were supplied with centerin amounts of Tris buffer to hold their final total volumes at 100 μL and further gently incubated at 37 °C for 2 h. Notably, the as-incbuated beads were also treated with ultrasonic every 30 min during the hybridization process. Subsequently, each tube was introduced with 1 μL of 5 U/mL nicking endonucleases and continued to gentle shaking at 37 °C for 60 min. After collecting and washing, the as-prepared beads were diluted appropriately and transferred to perform the optical trapping based imaging detection. For each test group, 100 beads were optically trapped and their upconversion luminescence gray levels were simultaneously measured with the exposure time setting at 300 ms.

Specificity Examination

Slightly unlike the standard sequences analysis, the enriching targets were changed to 300 amol of single-base to three-base mismatched sequences, random sequences and three proteins (BSA, AFP and PSA).

Cell culture

Three cancer cell lines (MCF-7 and HepG2 cells in RPMI-1640 media, and Jurkat T cells in DMEM media) were cultured with an initial seeding density of 2×10^4

cells/cm^2 in a 40 mm petri dish placed under an environment of 37 °C and 5% CO_2. All the media were supplied with 1% penicillin/ streptomycin to prevent bacteria infection and 10% fetal bovine serum to provide additional nutrients.

Blood samples

All the blood samples including 20 breast cancer patients and 10 healthy donors, were supplied by Hubei Cancer Hospital (China). The study of human blood and cancer cell lines was approved by the Ethical Committee of Hubei Cancer Hospital.

Total RNAs extraction from cancer cell lines

A custom EZ-10 DNAaway RNA mini-prepes kit (Shanghai Sangon Biotech.) was used to extract total RNAs by following the two main procedures. (1) Sample preparation: 5×10^4 of cells were first eluted with 1.5 mL of trypsin and then incubated immediately with 500 μL of lysis buffer *via* vortex. After that, the lysates were collected by centrifugation. (2) RNA purification: the needless DNA in the lysates was first removed by processing two times with a DNA eliminator column. Then, the collected DNA-away lysates were treated with 250 μL of ethanol to precipitate out and remove the needless proteins. After using a RNA column to further purify the supernatants, the flow-through was successively washed three times with NT and GT buffer solutions. Finally, the as-obtained pure RNAs were stored at -80 °C for long-term storage.

RT-PCR detection

A specific DNA primer (5′-CTCAACTGGTGTCGTGGAGTCGGCAA TTCAGTTGAGTCAACATC-3′) designed by Primer Premier 5.0 software was employed to conduct PCR analysis. All samples were cycled with 45 times by successively following the pre-denaturation (95 °C, 3 min), denaturation (94 °C, 30 s), annealing (57 °C, 30 s), extension (72 °C, 30 s), and repair extension (72 °C, 8 min) processes.

Quantitative analysis of miRNA-21 sequences in cancer cell lysates

The experimental procedures were similar to that of standard miRNA-21 sequences except that the enriching targets were changed to the total RNAs extracted from the three cancer cell lines.

Quantitative analysis of miRNA-21 sequences in human serums

Apart from changing the enriching targets to the human serums extracted from 20 breast cancer patients and 10 healthy donors, the procedures were similar to that of standard miRNA-21 sequences.

Table S1. Nucleic acid sequences used in this work

Type	Sequences (5' to 3')
DNA walker	5′-NH_2-(T)40-CAGACTGATGTTGACCTCAGC-3′
DNA substrate	5′-NH_2-CCGC*TGAGGAT-BHQ-1-3′ (*cleavage site)
Locked DNA	5′-AGGTCAACATCAGTCTGATAAGCTA-3'
MiRNA-21	5′-UAGCUUAUCAGACUGAUGUUGA-3′
M1	5′-UAGCUUAGCAGACUGAUGUUGA-3′
M2	5′-UAGCUUAGUAGACUGAUGUUGA-3′
M3	5′-UAGCUUAGUCGACUGAUGUUGA-3′
R	5′-AACUUCGGUCCCGUGCAGUUCG-3′

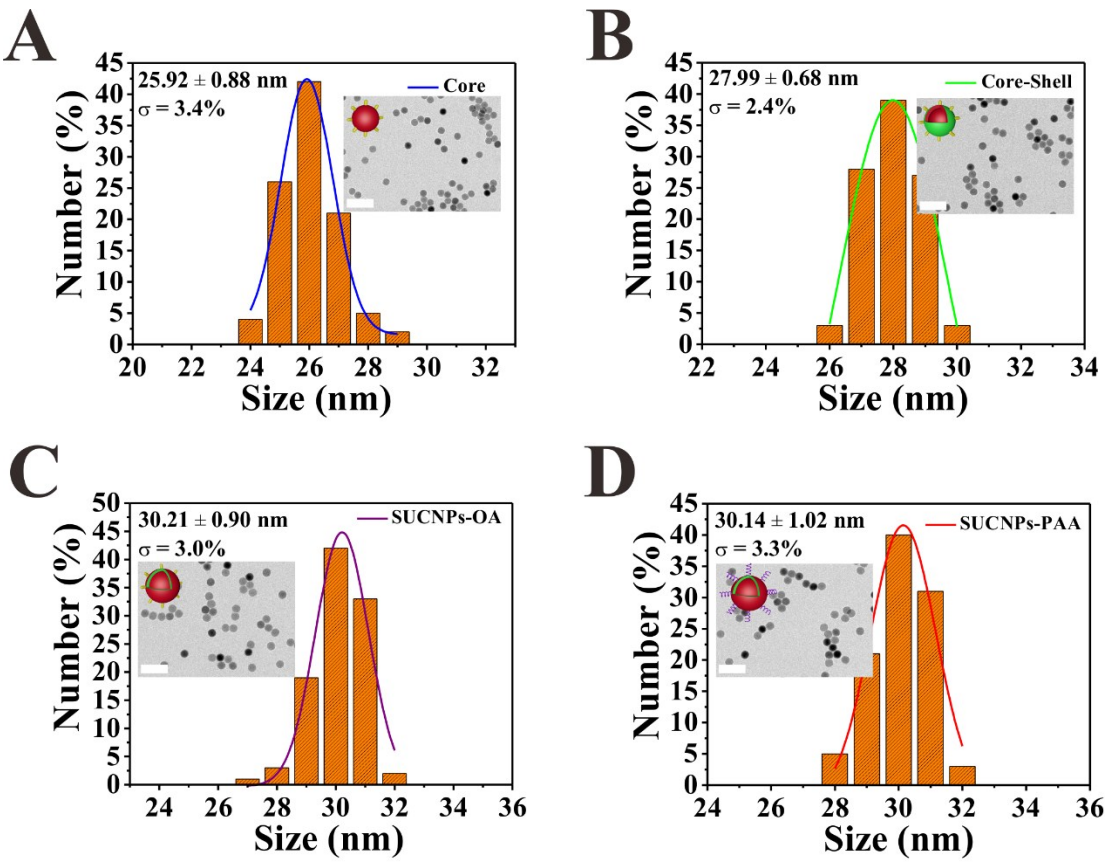

Fig. S1. Size diagrams of core (A), core-shell (B), SUCNPs-OA (C) and SUCNPs-PAA (D). Inset: corresponding TEM images (scale bars are 100 nm for all cases)

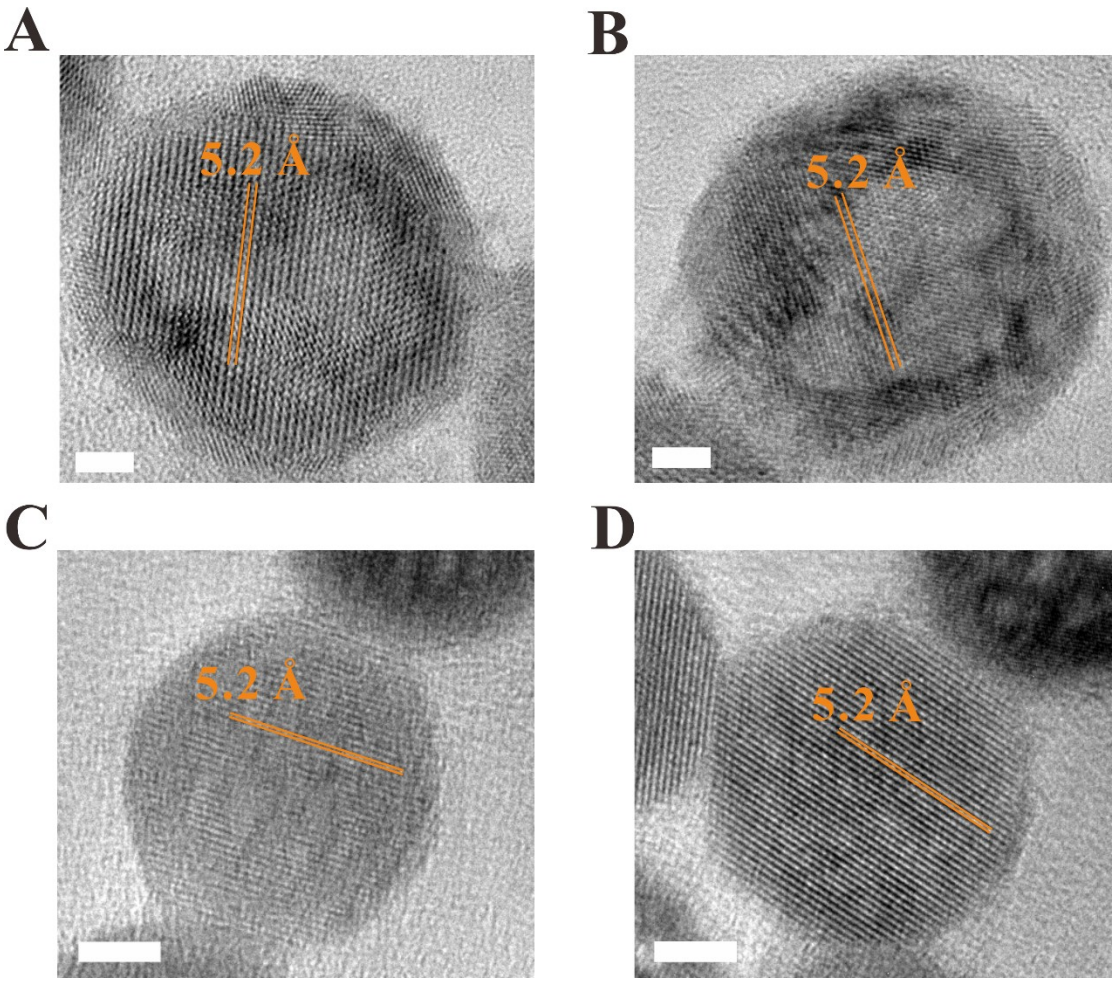

Fig. S2. High-resolution TEM images of core (A), core-shell (B), SUCNPs-OA (C) and SUCNPs-PAA (D). Scale bars are 5 nm for (A) and (B), and 10 nm for (C) and (D).

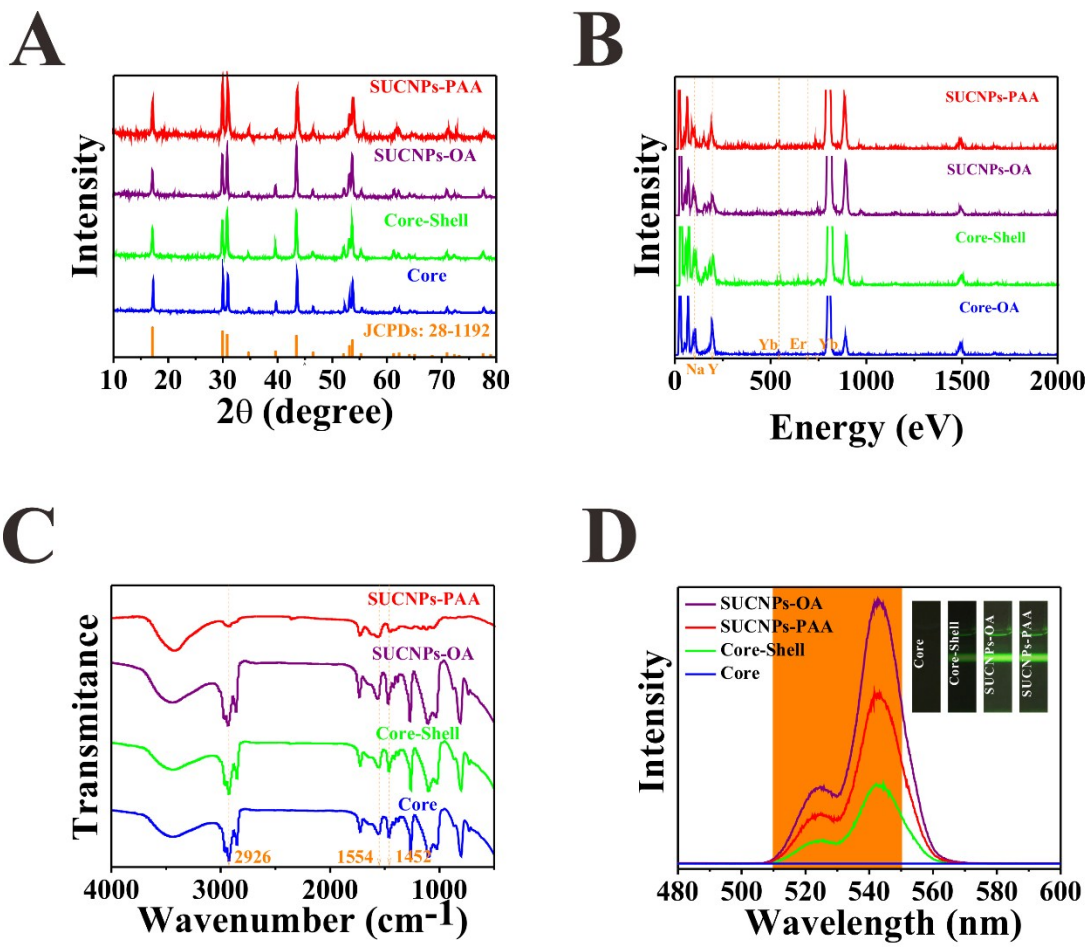

Fig. S3. (A)-(C) XRD patterns, EDS lines, FT-IR spectra of core, core-shell, SUCNPs-OA and SUCNPs-PAA. (D) Upconverion luminescence spectra of the nanoparticles dispersed in corresponding solvents (. Inset: digital images under the irradiation of 980 nm lasers (laser power is ~ 500 mW). Orange region indicates the signal acquisition channel (510-550 nm) for optical tweezers based imaging.

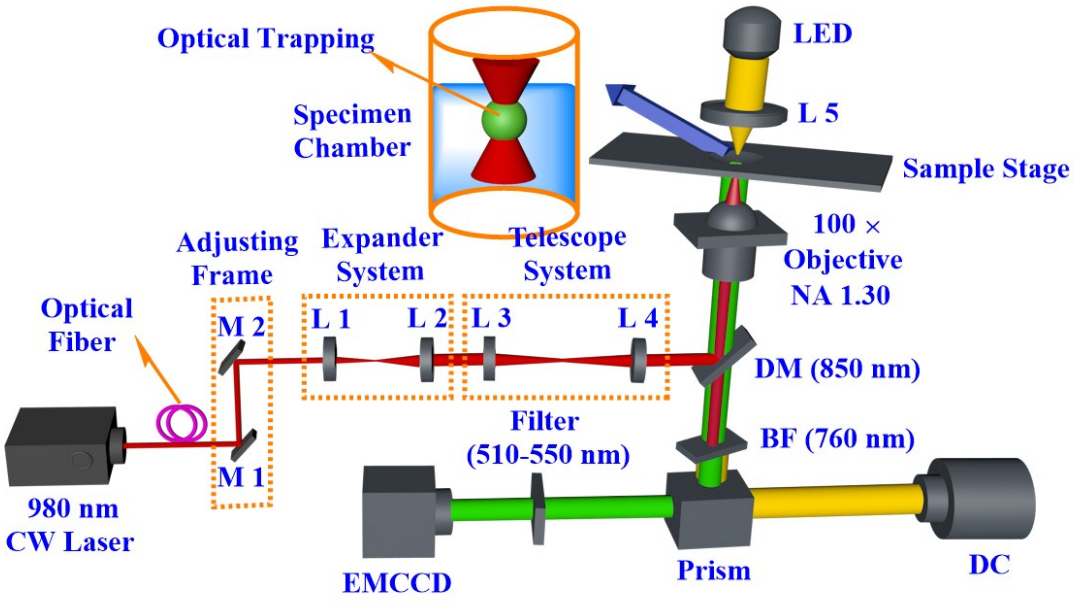

Fig. S4. Schematic diagram (not to real scale) of the self-constructed optical tweezers device. Abbreviations used: M = mirror, L = lens, DM = dichroic mirror, DC = digital camera. Inset: the specially-made specimen chamber. Red line: optical path of 980 nm laser beam. Yellow line: illumination path of converged LED lamp. Green line: upconversion luminescence path.

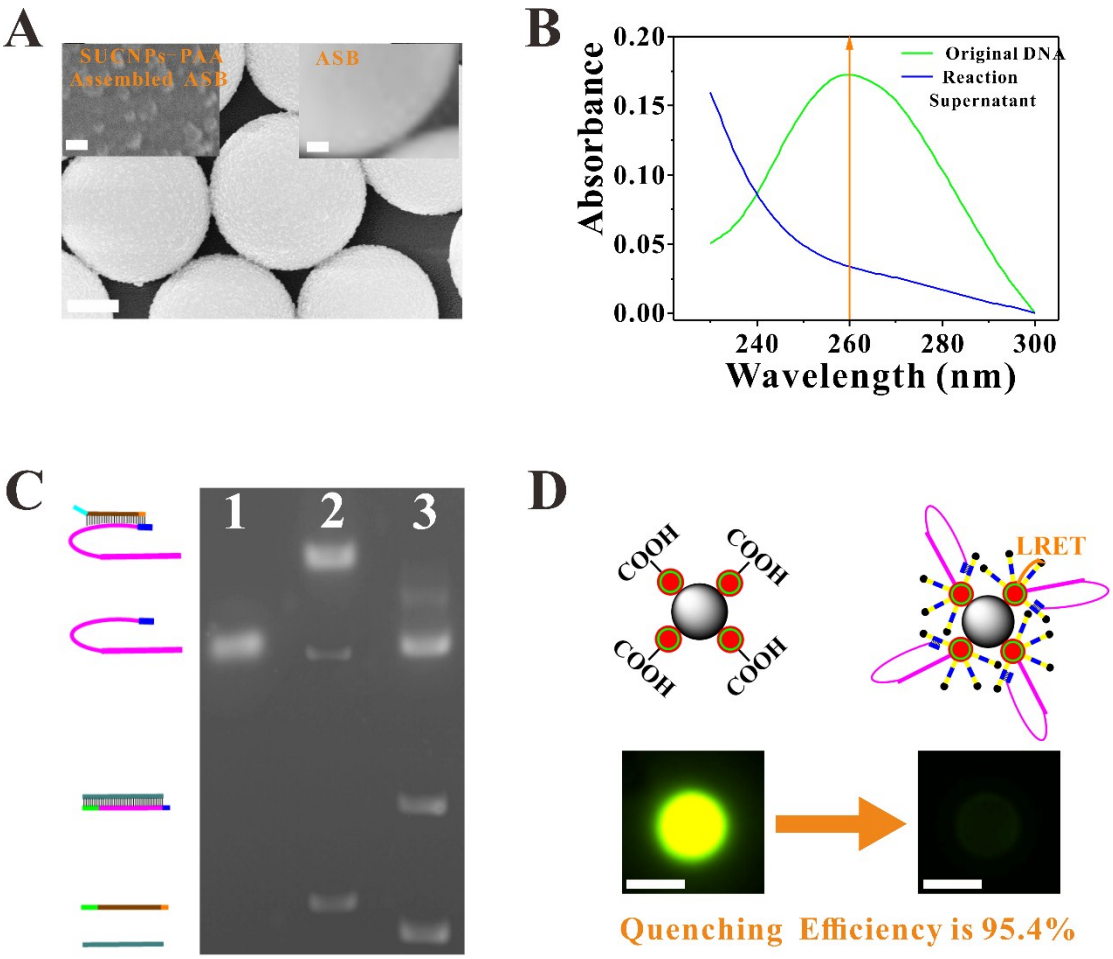

Fig. S5. (A) SEM image of SUCNPs-PAA assembled ASBs. Scale bar is 3 μm. Inset: the magnified area of a single assembled bead (left) and pure ASB (right). Scale bars are 100 nm. (B) UV spectra of the original DNA and the reaction supernatant. (C) 12.5% native PAGE images of the DNA walkers' release process in the presence of targets (1 μM). Lane 1: DNA walkers, lane 2: DNA walkers + locked DNA, lane 3: DNA walkers + locked DNA + miRNA-21. (D) Corresponding upconversion luminescence images before and after conjugating with the specifically combined DNA (walker : substrate = 1 : 9).

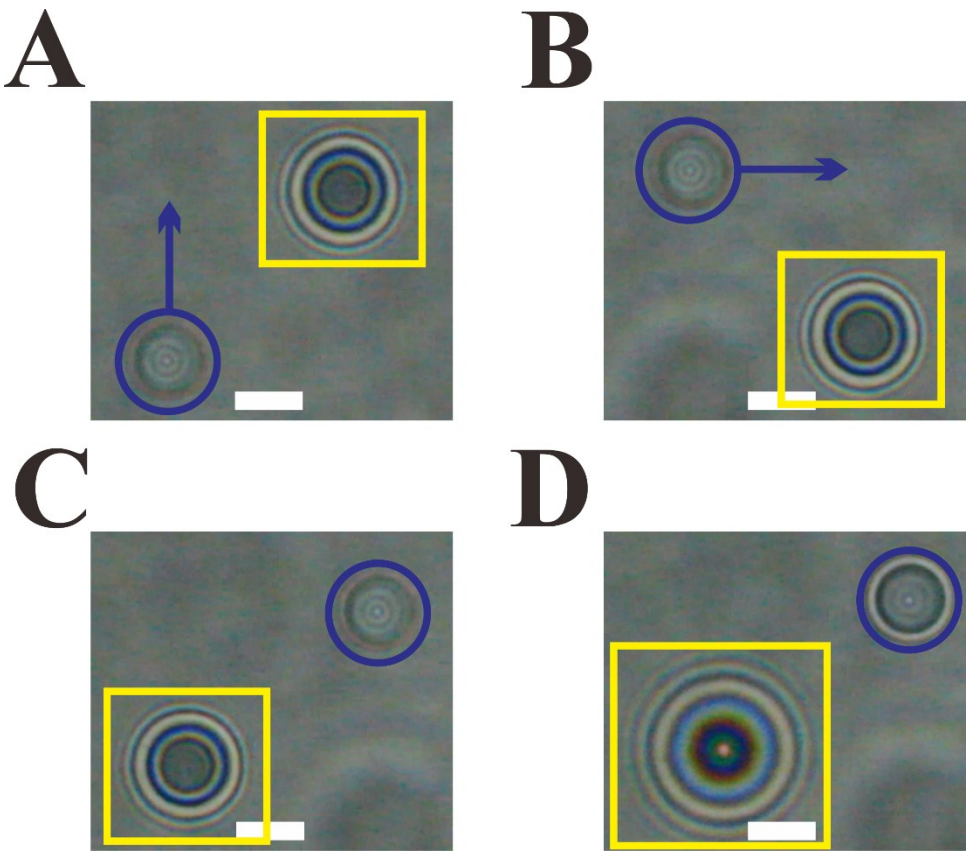

Fig. S6. (A)-(C) Optical trapping and manipulation of a single SUCNPs-PAA assembled ASB in X-Y directions *via* a passive approach. (D) Optical manipulation of this trapped bead in Z direction *via* an active approach. Blue circle and yellow box indicate the trapped bead and the referential bead, respectively, and blue arrow represents the manipulation pathway. Scale bars are 3 μm for all cases.

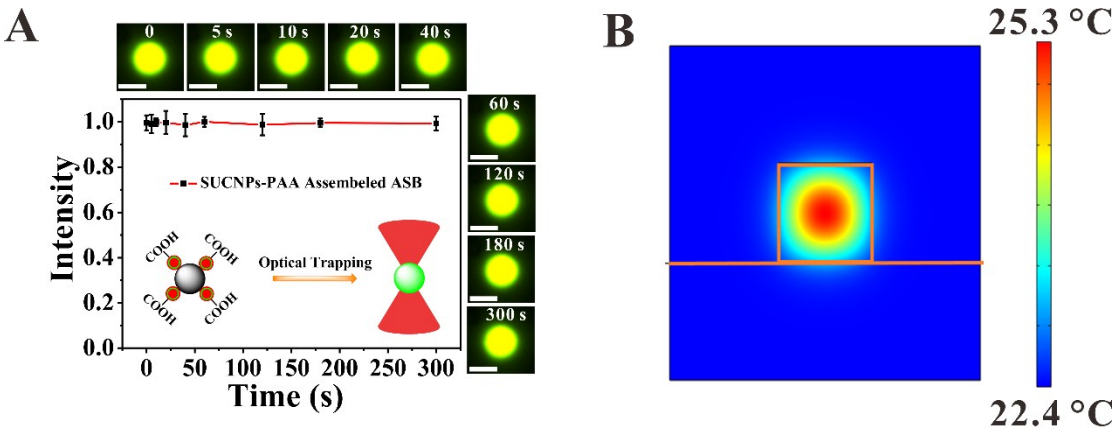

Fig. S7. (A) Luminescence maintainance of the trapped bead. (B) Finite elements simulation of the 2D temperature profile around the focal point calculated with COMSOL Multiphysics software.

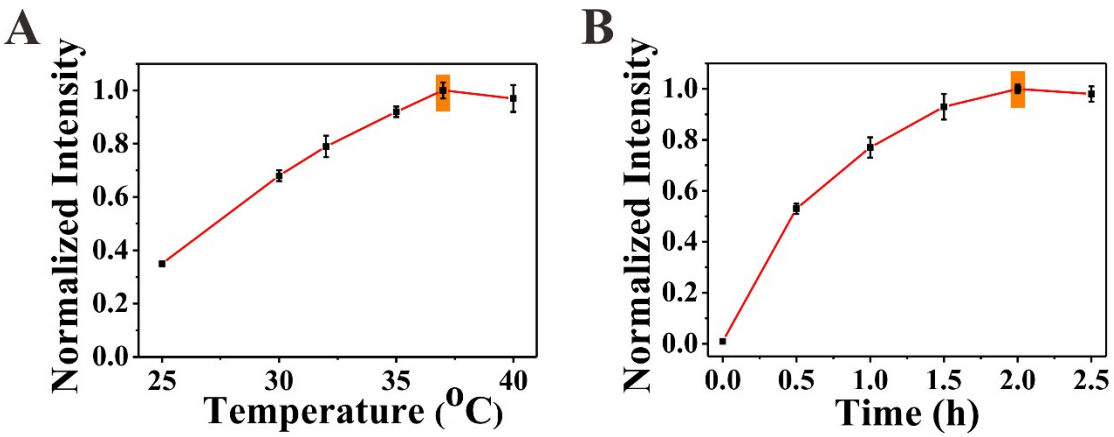

Fig. S8. (A)-(B) Reaction temperature and time optimization of the target specific identification.

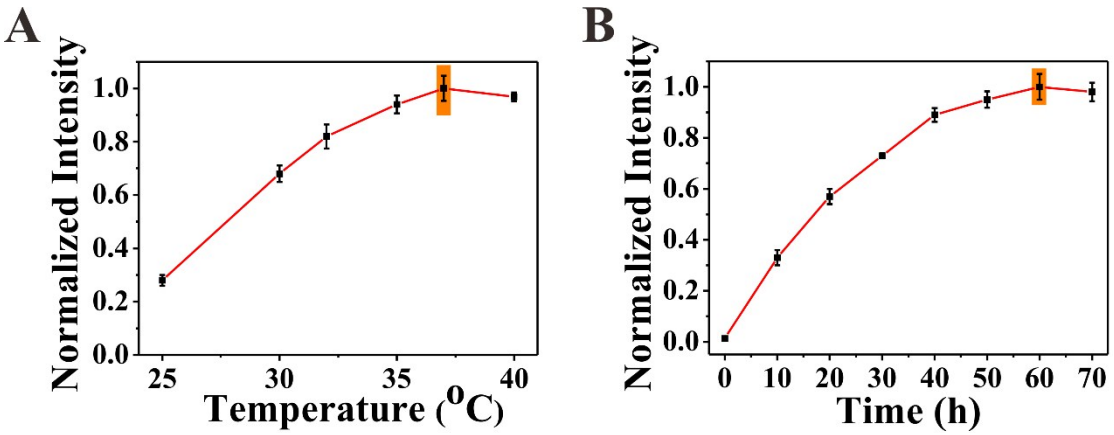

Fig. S9. (A)-(B) Reaction temperature and time optimization of the endonuclease catalytic.

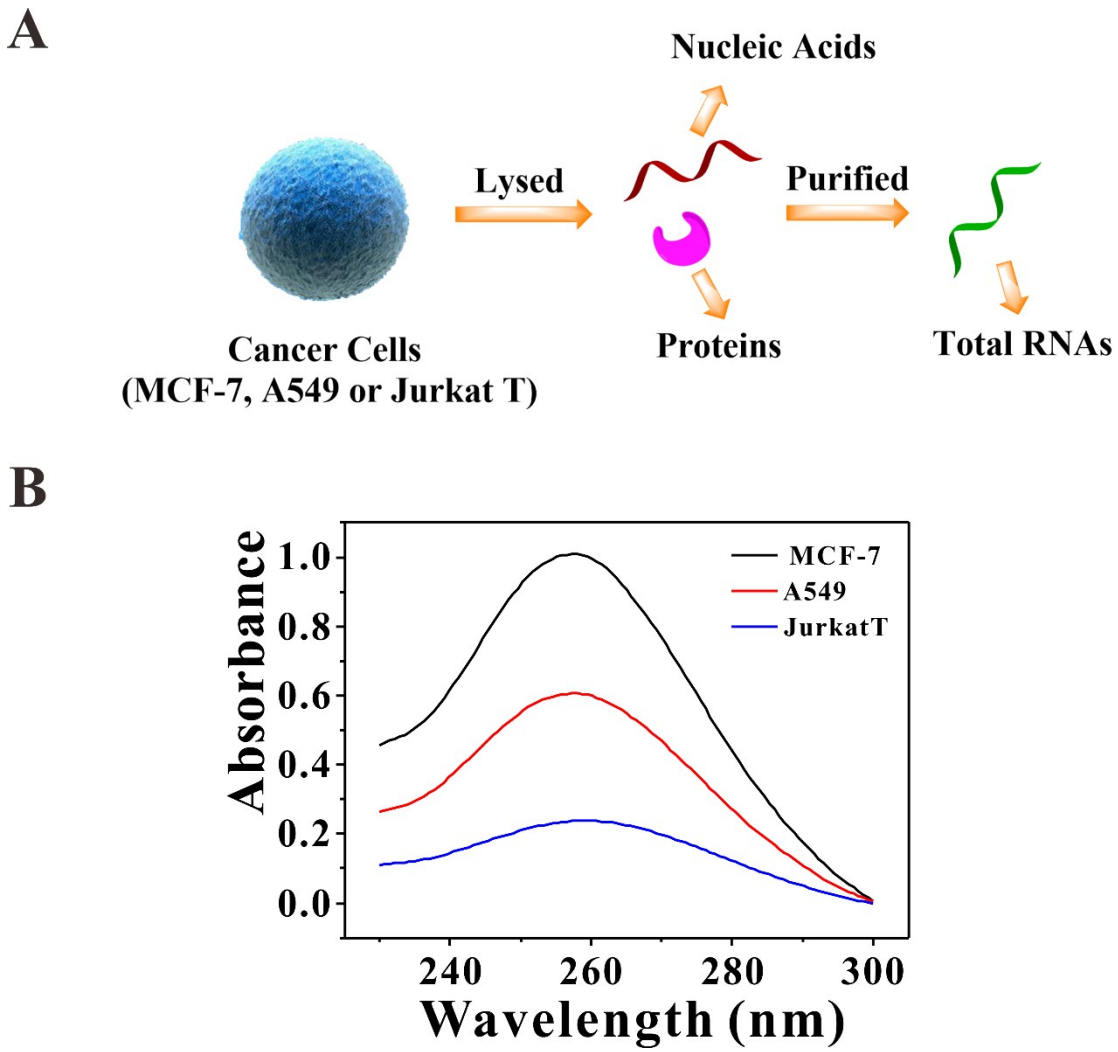

Fig. S10. (A) Total RNAs extraction procedures for three cancer cell lines including MCF-7, HepG2 and Jurkat T. (B) UV spectra of the total RNAs extracted from 5 × 10^4 of MCF-7, HepG2, Jurkat T cells.

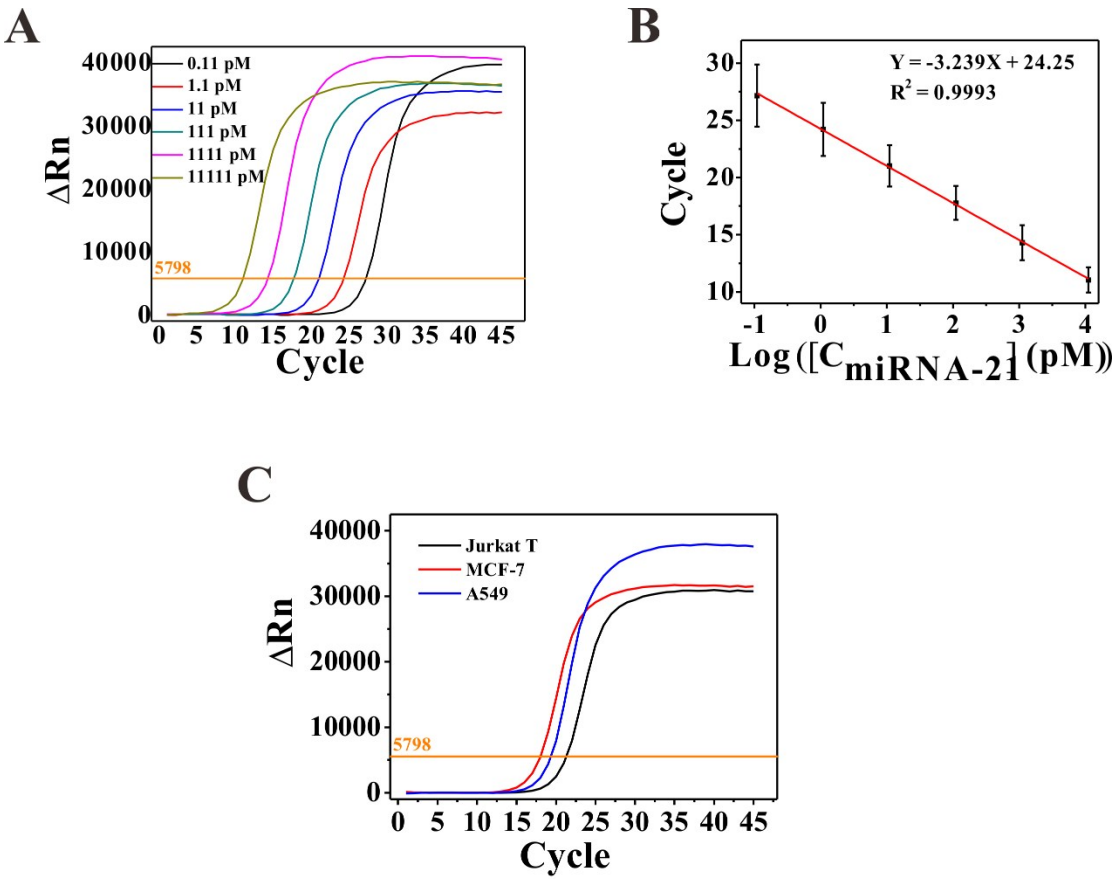

Fig. S11. A) Cycle curves toward various concentrations of standard miRNA-21 sequences. (B) Linear relationship between the cycle times and the logarithm concentration of standard miRNA-21 sequences. (D) Cycle curves toward the total RNAs extracted from 10^6 of MCF-7, HepG2 and Jurkat T cells.

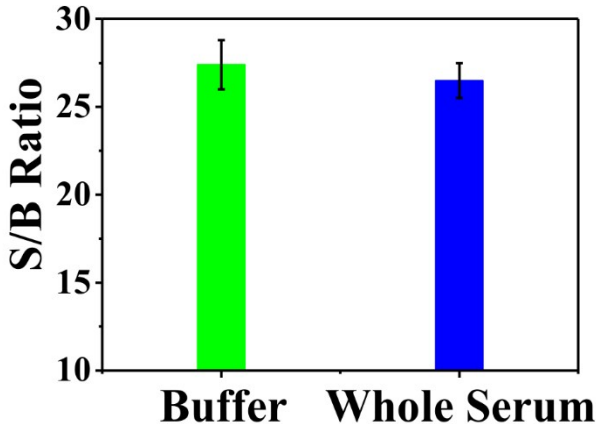

Fig. S12. S/B ratios for buffer system and human whole serum.

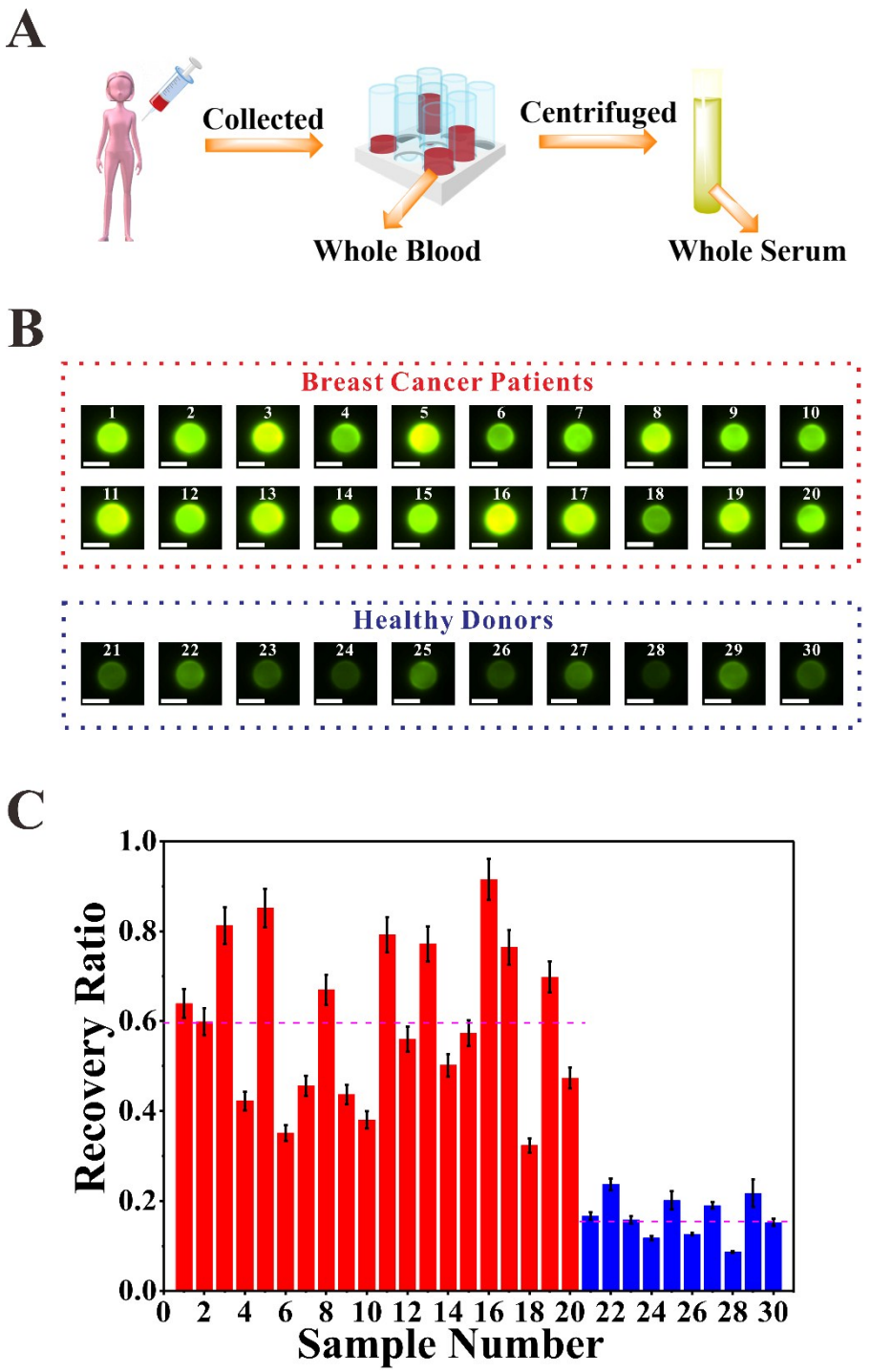

Fig. S13. (A) Human serum extraction procedures for 30 individual venous blood samples from 20 cases of breast cancer patients and 10 cases of healthy donors. (B) Analysis results towards the extracted whole serums. Scale bars and exposure time are 3 μm and 300 ms for all cases, respectively. (C) Corresponding recovery ratios obtained from (B) and dotted purple lines represent the average values.

Table S2. Detection of standard miRNA-21 sequences

Concentration	Recovery Ratio	CV (n = 100, %)
3 pM	0.9210	3.9
2 pM	0.9156	2.8
1 pM	0.8513	4.6
0.5 pM	0.7659	3.7
0.1 pM	0.6246	1.8
50 fM	0.5158	3.0
10 fM	0.3837	2.4
5 fM	0.3187	3.1
1 fM	0.2212	4.8
500 aM	0.1489	2.3
100 aM	0.0452	2.8

Table S3. Comparison with current optical methods toward miRNAs analysis

method	LOD	Ref.
double-strand displacement optical sensor	5 nM	1
RNA hybridization and ligation steps	0.9 nM	2
combination of isothermal amplification and SYBR green I fluorescence platform	1 pM	3
isothermal helicase-dependent amplification	12.8 fM	4
FRET between graphene quantum dots and ssDNA-UCNP@SiO_2	10 fM	5
duplex specific nuclease-assisted target recycling and pyrene excimer switching	580 aM	6
DNA-capped-Au assembled hydrogels for imaging	179 aM	7
this method	69 aM	/

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