Electronic Supplementary Material (ESI) for Analyst.


# Supporting Information

## Ultra-thin silica shell-guarded nanoflares for high-fidelity live cell miRNA-21 imaging by fully avoiding interference from biothiols

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## 1. Materials and chemicals

All DNA strands were synthesized and purified by Sangon Biotech (Shanghai, China). Hydrogen tetrachloroaurate (III) ($HAuCl_4 \cdot 4H_2O$, 99.99%) was purchased from RHAWN. (3-Aminopropyl) trimethoxysilane (APTES), PBS, ethanol, dimethyl sulfoxide (DMSO), sodium citrate, glutathione (GSH), N-ethylmaleimide (NEM), and sodium citrate were obtained from Aladdin Industrial Co., Ltd. Sodium silicate, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), and N-hydroxy succinimide (NHS) were purchased from Sigma-Aldrich. Fetal bovine serum (FBS), dulbecco's modification of eagle's medium (DMEM), trypsin and penicillin-streptomycin were purchased from Gibco.

## 2. Characterization

Transmission electron microscopy (TEM) images of AuNPs and $Au@SiO_2$ were obtained by JEOL JEM 2100. Energy dispersive X-ray spectroscopy (EDS) of material was measured by JSM-5610. All pH values were measured by the pH meter (YOKE INSTRUMENT, China). The dynamic light scattering (DLS) and zeta potentiometer were measured with Zetasizer Nano ZS (Malvern, UK). UV−vis absorption spectra were measured by a UV-2550 spectrophotometer. The CCK8 assays were performed by a microplate reader (Multiskan FC, ThermoFisher Science). The fluorescence spectra were collected on an LS55 luminescence spectrometer (Perkin Elmer, America). Fourier transform infrared spectroscopy (FT-IR) spectra were measured by Nicolet 380. Human cervical carcinoma cells (HeLa) and Human embryonic kidney cells 293 (HEK293) with fluorescent labeling were observed by confocal laser scanning microscopy (CLSM) (Nikon, Japan).

## 3. Experiments

### Preparation of the AuNPs

The AuNPs were synthesized by the classical sodium citrate reduction method.[1] Firstly, 100 mL $HAuCl_4 \cdot 4H_2O$ (0.01% wt) was heated to boiling with vigorous stirring, and 2.5 mL of trisodium citrate solution (1% wt) was rapidly added under stirring. The color of the solution changed from pale yellow to colorless and finally to red wine. The solution was kept boiling for 15 min, and then cooled down to room temperature under stirring. Afterward, the AuNPs were washed 2-3 times with $H_2O$, the solution stored at 4 °C for further use.

### Preparation of $Au@SiO_2$

Firstly, freshly prepared solution of APTES (0.25 mL, 1 mM) was added to AuNPs (50 mL, 1 nM) solution under vigorous magnetic stirring. The mixture of APTES and AuNPs was allowed to stand for 15 min to ensure complete complexation of the amine groups with the AuNPs surface. The active silica solution was prepared by gradually adding 5% HCl to reduce the pH value to 10. Then 2 mL of active silica was added to 50 mL of surface modified AuNPs under intense magnetic agitation. Under the condition of 80°C water bath, silica gel layers with thicknesses of 2, 5, and 8 nm can be obtained by strictly controlling the time of 10, 20, and 40 min. The mixture was centrifuged three times at 11, 000 rpm for 30 min and then redispersed in water.

### Preparation of amino-modified $Au@SiO_2$ ($Au@SiO_2$-$NH_2$)

APTES (1 mL, 10 mM) and $Au@SiO_2$ (9 mL, 1 nM) are stirred vigorously at 60 °C and reacted in ethanol solvent for 6 h. The solution was centrifuged , and then washed 2-3 times with ethanol and redisperse it into water.

### Preparation of Au-S-ssDNA-Cy3 and $Au@SiO_2$-ssDNA-Cy3

A thiol and fluorophore (Cy3) dual-modified single stranded DNA (HS-ssDNA-Cy3) was attached to the AuNPs by the classic salt aging.[2] AuNPs (200 μL, 1 nM) was mixed with HS-ssDNA-Cy3 (4 μL, 50 μM) for 12 h. In addition, sodium chloride was continuously added to the solution, and the concentration of sodium chloride in the solution reached 0.2 M within 24 h, ensuring high-density DNA on AuNPs. $Au@SiO_2$-$NH_2$ and carboxyl DNA (HOOC-ssDNA-Cy3) are crosslinked by forming amide bonds via the EDC/NHS process. Firstly, EDC (10 μL, 10 mM) was mixed with HOOC-ssDNA-Cy3

(4 μL, 50 μM) for 4 h. After that, NHS (6 μL, 10 mM) was added for 2 h and mixed with the Au@$SiO_2$-$NH_2$ (200 μL, 1 nM) solution. Then, the mixture was centrifuged at 11, 000 rpm for 10 min. Lastly, the precipitates were washed 2-3 times with PBS buffer (125 mM NaCl, 10 mM phosphate, 2 mM $MgCl_2$, pH 7.2) to completely remove the free DNA. The final conjugates were re-dispersed in PBS buffer and stored at 4 °C for further use.

**GSH interference study *in vitro***

GSH (1 mM) was added into the Au-S-ssDNA-Cy3 and Au@$SiO_2$-ssDNA-Cy3 solutions (1 nM), respectively, and incubated at 37 °C for different times (0, 2, 4, 6 and 8 h). Then the fluorescence spectra of the solutions were measured at each time point. Moreover, the effect of different concentrations of GSH (0, 200, 400, 600, 800 and 1000 μM) on gold-sulfide and amide bonds were also investigated by incubating at 37 °C for 8 h. All experiments were repeated 3 times.

**Investigation on the thermal stability**

Au-S-ssDNA-Cy3 and Au@$SiO_2$-ssDNA-Cy3 (1 nM) were incubated at different temperatures (30, 40, 50, 60, 70 and 80 °C) for 12 h, then were gradually cooled down to room temperature. The fluorescence spectrum in each solution was then measured.

**Detection performance of Au@$SiO_2$ NFs in PBS buffer**

First, the effect of operating temperature on detection performance was investigated. Au@$SiO_2$ NFs solutions (1 nM) were incubated at different temperatures (30 to 50 °C) for 2 hours in the absence or presence of miRNA-21 (1 mM). And the fluorescence spectra were measured. Then Au@$SiO_2$ NFs was incubated with the target of different concentration for 2 h at 37 °C. Fluorescence spectra were measured after incubation with different concentrations of miRNA-21. Finally, the selectivity of Au@$SiO_2$ NFs was investigated. Fluorescence measurements of Au@$SiO_2$ NFs after 2 h incubation with different analytes. The concentration of each analyte was 1000 nM. All experiments were repeated three times.

**Cell culture**

HEK293 cells and HeLa cells were obtained from American Type Culture Collection. All cells were cultured in DMEM medium with 10% fetal bovine serum and 100 U/mL

1% antibiotics (penicillin/streptomycin) and maintained at 37°C in a 100% humidified atmosphere with 5% $CO_2$.

**Cytotoxicity test**

HeLa cells were first seeded in 96-well microplates, and after culturing for 24 h, and different concentrations of Au@$SiO_2$-ssDNA-Cy3 were added and incubated for another 12 h. Then CCK8 solution (0.5 mg/mL, 100 μL) was added to each well and treated for 4 h. Finally, the absorbances at 490 nm were recorded.

**Confocal fluorescence imaging assays**

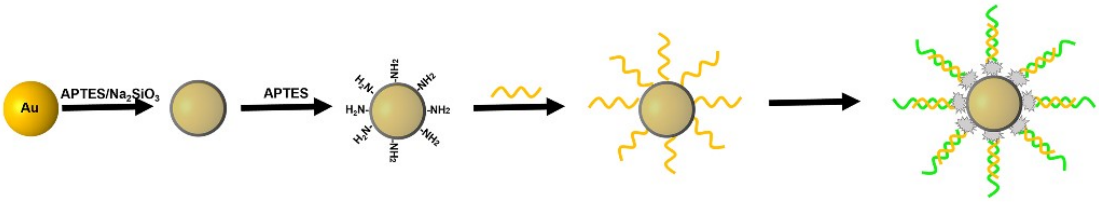
HeLa and HEK293 cells were incubated for 24 h in an incubator at 37 °C and 5% $CO_2$. The Au@$SiO_2$ NFs, Au-S NFs, Au@$SiO_2$-ssDNA-Cy3 and Au-S-ssDNA-Cy3 (1 nM) were separately added and incubated for another 2 h, and the resulting cells were washed 3 times with PBS buffer. Finally, the treated cells were observed with a confocal laser scanning microscopy (CLSM). The fluorescence intensity was analyzed by Image J software.

**References**

1 B. V. Enustun and J. Turkevich, *J. Am. Chem. Soc.*, 1963, **85**, 676–687.
2 J. Liu and Y. Lu, *Nat. Protoc.*, 2006, **1**, 246-252.

**Table. S1** Sequences of oligonucleotides used in this work.

| **Oligonucleotides** | **Sequences (from 5' to 3')** |
|---|---|
| HS-ssDNA-Cy3 | HS-AAACCCAGTCT-Cy3 |
| HOOC-ssDNA-Cy3 | HOOC-AAACCCAGTCT-Cy3 |
| Thiol modified anchor strand | HS-CCCTATAGCTTATCAGACT |
| Carboxyl modified anchor strand | HOOC-CCCTATAGCTTATCAGACT |
| Recognition strand | TCAACATCAGTCTGATAAGCTATAGGG-Cy3 |
| Target chain (miRNA-21) | TAGCTTATCAGACTGATGTTGA |
| Single-base mismatch | TAGCTTATCAGAGTGATGTTGA |
| Two-base mismatch | TAGCTTTTCAGAGTGATGTTGA |
| Three-base mismatch | TAGCTTTTCAGAGTGAAGTTGA |
| miRNA 12 analogue | GAGTGTGACAATGGTGTTTG |




**Scheme S1** Schematic diagram of the synthesis of Au@$SiO_2$ NFs.

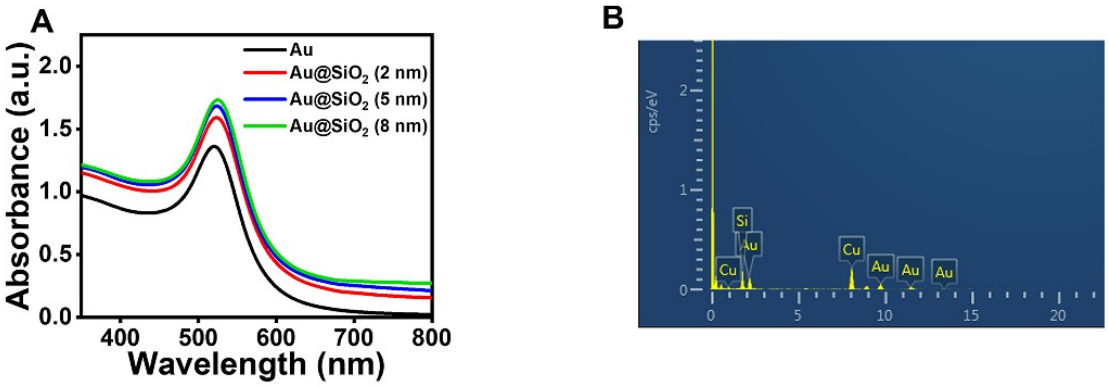



**Fig. S1** (A) UV−vis spectra of AuNPs and Au@$SiO_2$. (B) EDS of Au@$SiO_2$.

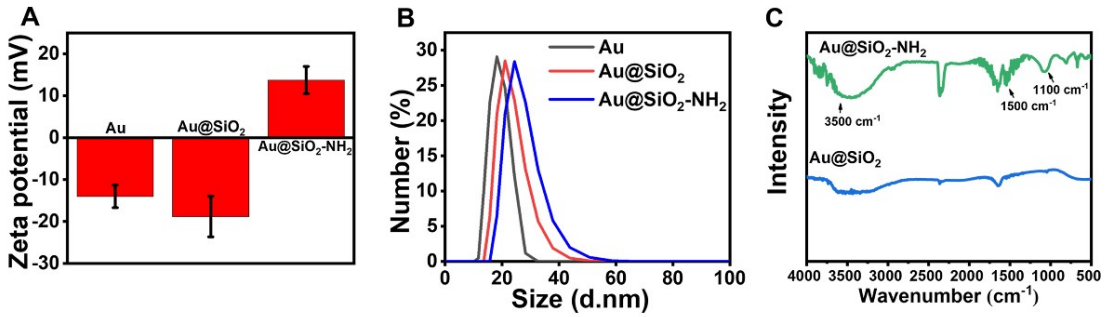



**Fig. S2** (A) Zeta-potentials of AuNPs, Au@$SiO_2$ and Au@$SiO_2$-$NH_2$. (B) Hydrodynamic size distribution of AuNPs, Au@$SiO_2$ and Au@$SiO_2$-$NH_2$. (C) FT-IR spectra of Au@$SiO_2$ and Au@$SiO_2$-$NH_2$.

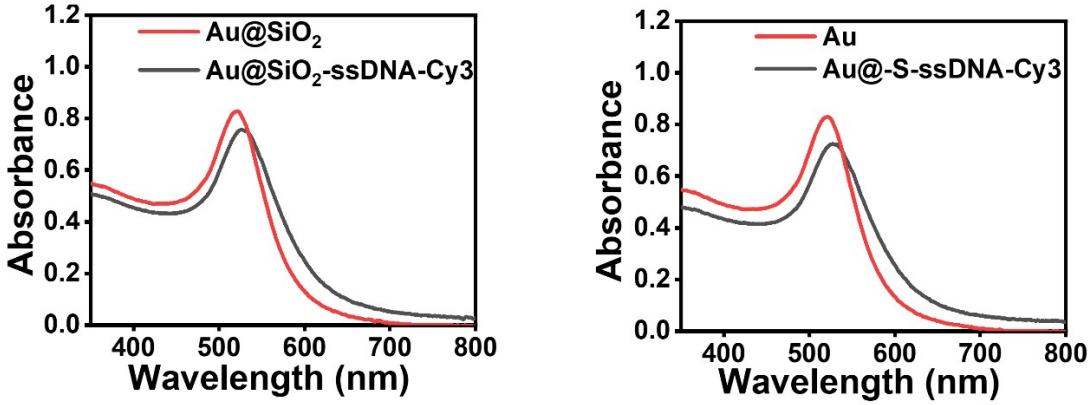



**Fig. S3** UV-vis spectra of AuNPs and $Au@SiO_2$ before and after the modification of HS-ssDNA-Cy3 and HOOC-ssDNA-Cy3.

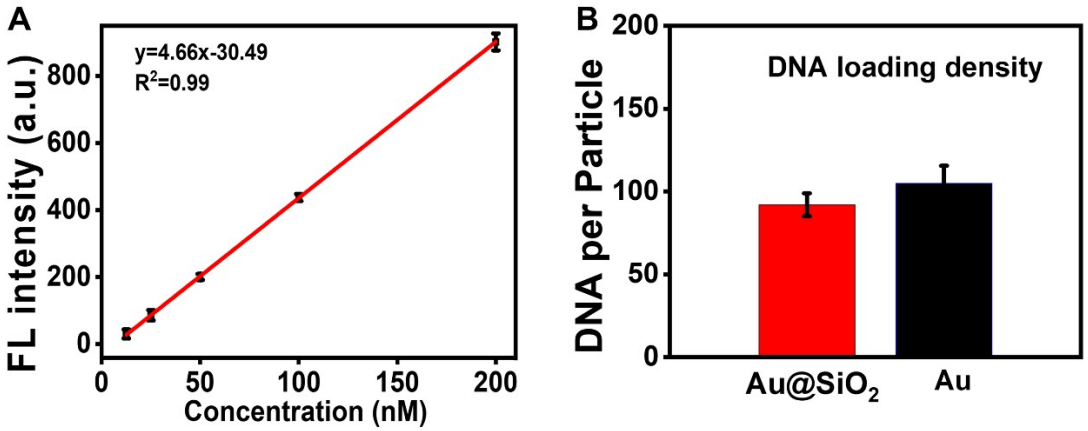



**Fig. S4** (A) Standard curve for quantifying the number of Cy3-labelled ssDNA. (B) The loading amount of DNA on AuNPs and $Au@SiO_2$, respectively.

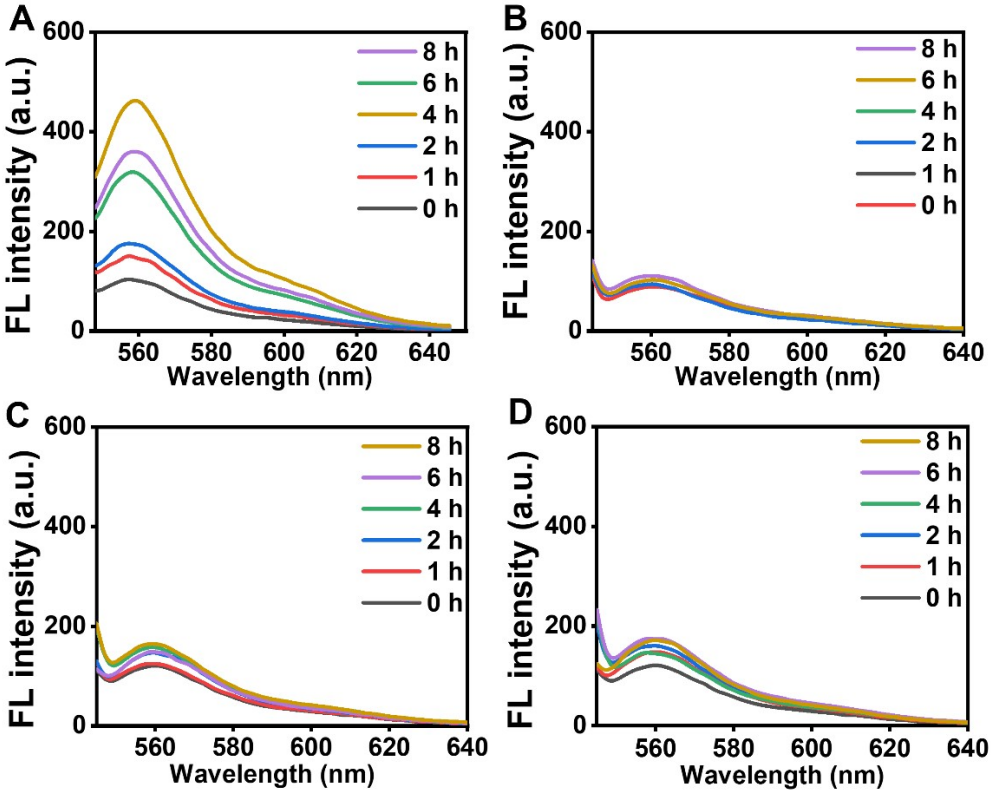



**Fig. S5** (A, B) Time-dependent fluorescence spectra of Au-S-ssDNA-Cy3 in the presence (A) and absence (B) of GSH (1 mM) at 37 °C. (C, D) Fluorescence spectra of Au@$SiO_2$-ssDNA-Cy3 in PBS buffer with (C) and without (D) GSH (1 mM).

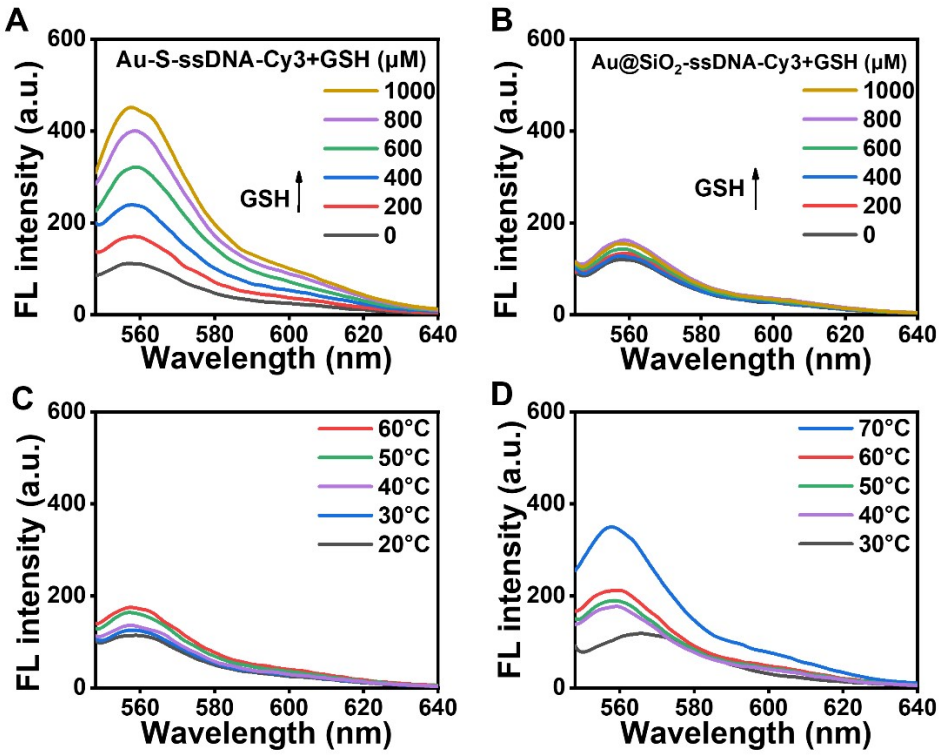



**Fig. S6** (A, B) Fluorescence spectra of Au-S-ssDNA-Cy3 (A) and Au@$SiO_2$-ssDNA-Cy3 (B) in PBS buffer treating with different concentrations of GSH for 12 h. (C, D) Fluorescence spectra of Au@$SiO_2$-ssDNA-Cy3 (C) and Au-S-ssDNA-Cy3 (D) after being treated at different temperatures for 12 h.

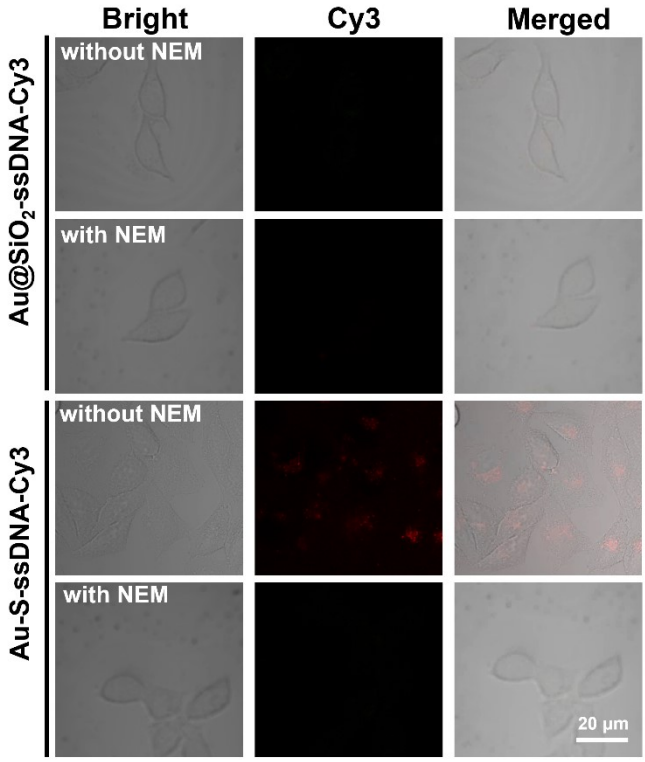



**Fig. S7** CLSM images of HeLa cells. Scale bar: 20 μm.

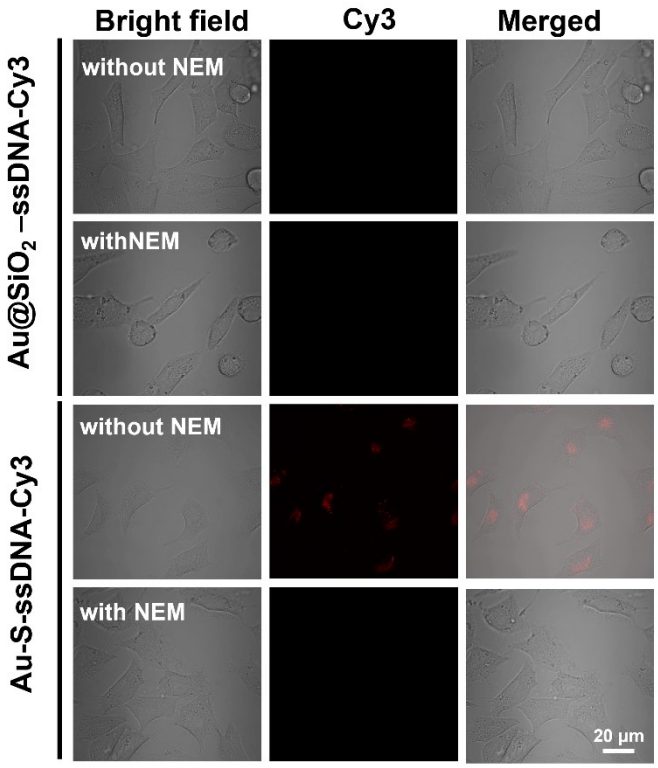



**Fig. S8** CLSM images of HEK293 cells. Scale bar: 20 μm.

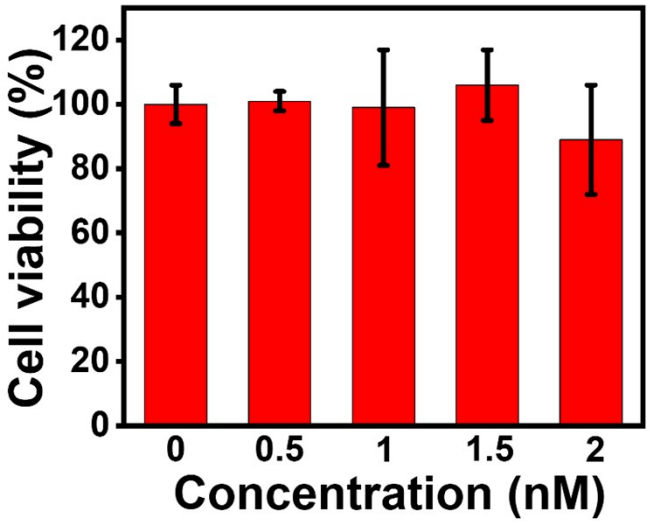



**Fig. S9** Viabilities of Hela cells after treatment with different concentrations of Au@$SiO_2$–ssDNA-Cy3. The error bars were the standard deviations from four independent measurement.

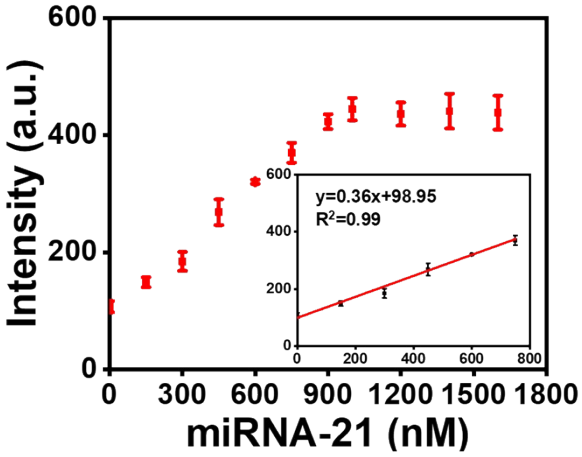



**Fig. S10** Fluorescence intensities of the Au-S NFs at different miRNA-21 concentrations. Inset: Linear calibration curve.

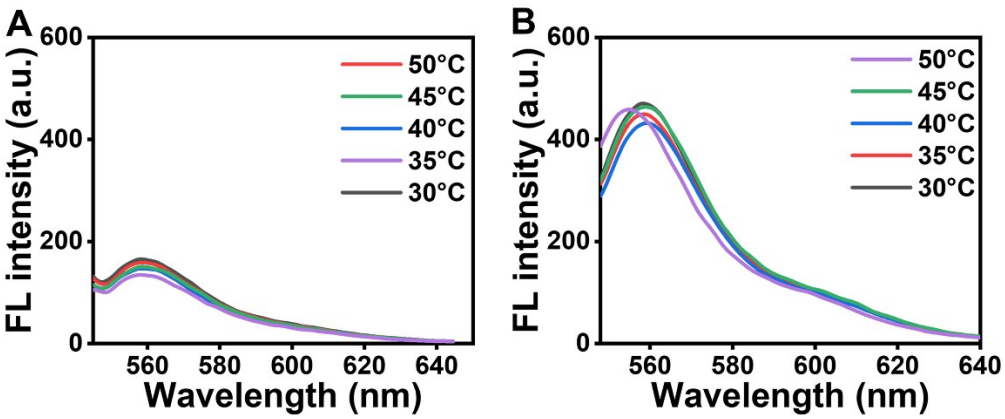



**Fig. S11** Fluorescence spectra of Au@$SiO_2$ NFs in the absence (A) or presence (B) of 1 mM miRNA-21 at different reaction temperatures.

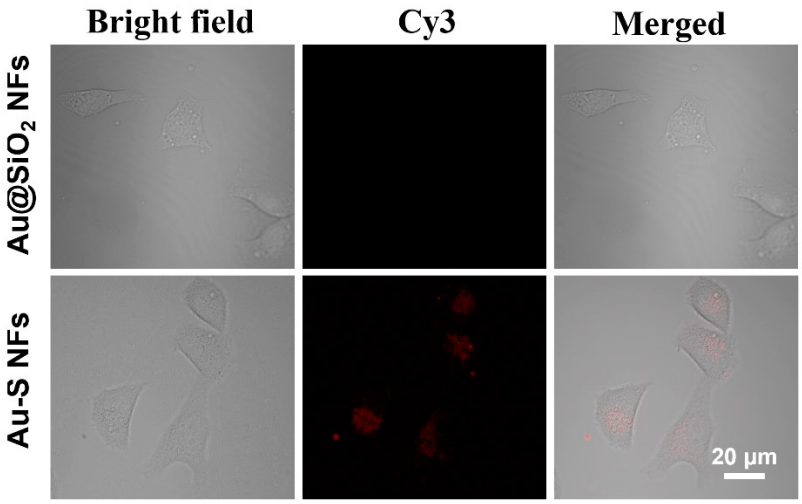



**Fig. S12** CLSM images of HEK293 cells incubated with Au@$SiO_2$ NFs and Au-S NFs.