

## Supporting Information

### **Intracellular Low-abundance MicroRNA Imaging by NIR-Assisted Entropy-Driven DNA System**

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## **Experimental Procedures**

**Materials and Reagents.** Copper(II) chloride ( $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ), Poly(vinylpyrrolidone) (PVP-K30), sodium sulfide ( $\text{Na}_2\text{S}$ ) hydrazine anhydrous were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Tris(2-carboxyethyl)phosphine (TCEP) and ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma-Aldrich (St. Louis, MO). Phosphate buffer saline (PBS, pH 7.4) was purchased from Life Technologies Corporation (Los Angeles, CA, USA).

All the DNA and miRNA sequences were synthesized by Sangon Biological Engineering Technology & Co., Ltd (Shanghai, China) and Gene-Pharma Co., Ltd. (Shanghai, China). The ultrapure water ( $\geq 18 \text{ M}\Omega$ ) was used in all the experiments.

**Characterization.** Transmission electron microscopy (TEM; JEM-2010F from JEOL, 200 kV) were performed to exam the morphologies of HCuSNPs. The UV-vis absorption spectra were recorded with a UV-1800 spectrometer (Shimadzu, Kyoto, Japan). All the fluorescence measurements were measured on a F-4500 fluorescence spectrometer (Hitachi, Tokyo, Japan). The Dynamic light scattering (DLS) and zeta potential analysis was performed on a Zetasizer Nano S system (Malvern Instruments, Malvern, U.K.). All the fluorescence images were recorded by using a confocal laser scanning fluorescence microscope (CLSM, FV1200, Olympus, Japan)

**Native Polyacrylamide Gel Electrophoresis.** Firstly, 4.17 mL 30% acrylamide/bis-acrylamide gel solution (29:1), 1 mL  $10 \times$  TAE buffer (pH 8.3), 70  $\mu\text{L}$  10% Ammonium persulfate (APS), 3.5  $\mu\text{L}$  N,N,N',N'-tetramethylethylenediamine (TEMED) and 4.93 mL deionized water were mixed in a conical flask and reacted for 5 h at room temperature to prepare the 12.5% native polyacrylamide gel. The PAGE was performed in ice bath at 100 V for about 60 min and stained with  $3 \times$  Gel-Red TM Nucleic Acid Gel Stain solution (Biotium, USA) for 15 min, the gel was imaged using an Alliance Ld2 (Uvitec, Cambridge, U.K.).

### **Preparation of DNA-HCuSNPs.**

The HCuSNPs were synthesized and modified according to the previously reported.<sup>1,2</sup> Firstly,  $\text{CuCl}_2$  solution (200  $\mu\text{L}$ , 0.5 mol/L) was added to a solution containing

Polyvinylpyrrolidone (PVP-K40, 0.48 g) of deionized water (50 mL) under stirring at room temperature, followed by adding NaOH solution (50 mL, pH = 9.0) and reacted for 2 min. The  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$  (12.8  $\mu\text{L}$ ) was added to the mixture. 400  $\mu\text{L}$   $\text{Na}_2\text{S}$  (320 mg/mL) was added to the solution and kept the flask at 75 °C for 2 h. Afterwards, the HCuS NPs was collected by centrifugation and washed with deionized water several times.

DNA-HCuSNPs conjugates were prepared as follows. First, an 20-fold equivalent of TCEP solution was added a 1.5 mL centrifuge tube to activate the thiol -modified DNA1 (100  $\mu\text{M}$ , 10  $\mu\text{L}$ ) at room temperature for 1 h. Afterward, the activated thiol-modified DNA was added in 300  $\mu\text{L}$  HCuSNPs solution (300  $\mu\text{g}/\text{mL}$ ) at room temperature for 24 h with gentle stirring. The resultant mixture solution was washed by centrifugation at 6000 rpm for 20 min with deionized water for twice. Then, Fuel DNA (100  $\mu\text{M}$ , 10  $\mu\text{L}$ ) was added and kept at 37 °C for 2 h. The fuel probes modified HCuSNPs solution was washed by washed for twice at 6000 rpm for 20 min. The substrate peobes was modified on HCuSNPs using same measures. Finally, the DNA-HCuSNPs solution stored in 10 mM PBS (pH 7.4, 137 mM NaCl) buffer at 4 °C for the next step.

**Determination the amount of DNA probes on HCuSNPs.** The amounts of DNA probes were evaluated as follows: HCuSNPs coated with FAM-DNA1 were prepared and purified according to the above protocol. Then, 1000  $\mu\text{L}$  FAM-DNA1 HCuSNPs (100  $\mu\text{g}/\text{mL}$ ) was mixed with 60  $\mu\text{L}$  DL-Dithiothreitol (DTT, 10 mM) at 60 °C for 1 h. Finally, the solution was centrifuged at 6000 rpm for 20 min and the supernatant was collected for fluorescence measurement to quantify the amount of the DNA probe modified on the HCuSNPs surface.

#### **Optimization of experimental conditions.**

The released temperature of the fuel probe was first investigated using a FAM-labeled fuel probe. 200  $\mu\text{L}$  HCuSNP-Fuel probes hybridization systems were prepared at a concentration of 140 nM and kept at different temperature (30 °C, 34 °C, 38 °C, 42 °C,

46 °C, 50 °C) for 10 minutes, respectively. The supernatant was collected by centrifugation, and the fluorescence intensity was measured.

To optimize the molar ratio of fuel probes and substrate probes, 0.2  $\mu\text{L}$ , 20  $\mu\text{M}$  miRNAs were added into 100  $\mu\text{L}$  of HCuSNP-DNA solution (70 nM) with different molar ratios of substrate probes and fuel probes (4: 1, 2: 1, 1: 1, 1: 2, 1: 3, 1: 4, and 1: 5) and kept at 37 °C for 2 h. After irradiating with a 1064 nm laser (1.0W/cm<sup>2</sup>) for 5 min, the fluorescence intensity was measured.

### **miRNA-155 Fluorescence Detection**

For the miRNA-155 detection, HCuSNP-DNA solution (100  $\mu\text{g}/\text{mL}$ , 70 nM substrate probes and 140 nM fuel probes) was incubated with different concentrations of miRNA-155 (7 fM -140 nM) at 37 °C. After 4 h, all the groups were irradiated with a 1064 nm laser (1.0W/cm<sup>2</sup>) for 5 min and kept at 37 °C for another 6 h. The fluorescence intensity was measured on a F-4500 fluorescence spectrometer.

### **Specific test**

We selected the complete miRNA-155 target (CM, 400 pM), the single base mismatched target fragment (SM, 400 pM), the triple base mismatched target fragment (TM, 400 pM) and miRNA-21 (400 pM), miRNA-205 (400 pM) for detection, the experimental reaction conditions and procedures consistent with the above operation.

### **Photothermal performance in vitro**

The photothermal performance was measured as in our previous reports.<sup>3</sup> In brief, the quartz cuvette containing 1 mL of HCuSNPs-DNA solution (0, 25, 50, 100 and 200  $\mu\text{g}/\text{mL}$ ) was irradiated with a 1064 nm NIR laser at a power intensity of 1.0 W/cm<sup>2</sup> for different duration, the temperature was record by an OMEGA 4-channel datalogger thermometer.

### **Cell Viability Assay**

The cells were cultured as the previously reported.<sup>4, 5</sup> NHDF cells, MCF-7 cells and A549 cells were added into 96-well plates for 24 h and then transfected HCuSNPs-DNA with different concentrations. Replacing of the transfection media with fresh

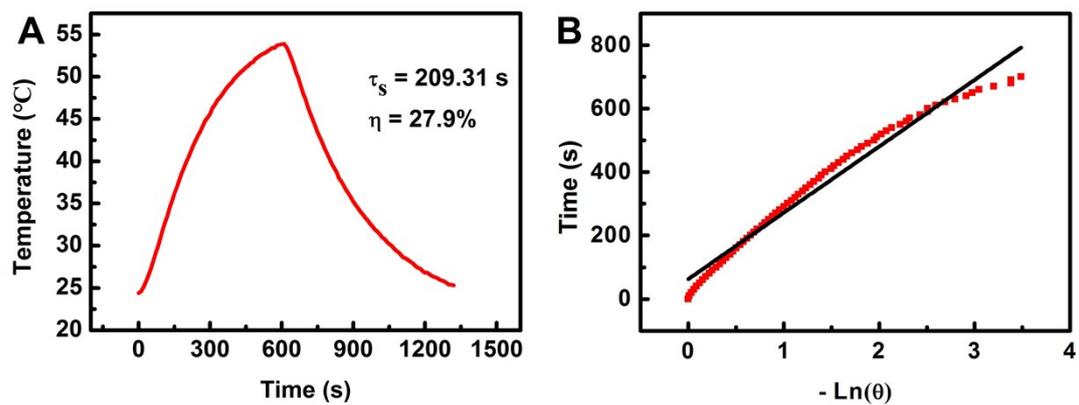
media 4 h later and cultivated for another 12 h. 20  $\mu$ L MTT (5 mg/mL) was added to each well. After 4 h, the media was removed and 100  $\mu$ L DMSO was added. The absorbance at 492 nm was measured by a microplate reader.

### **Confocal Fluorescence Imaging**

NHDF cells, MCF-7 cells and A549 cells were seeded into culture dishes for and HCuSNPs-DNA probe (100  $\mu$ g/mL, 70 nM substrate probes and 140 nM fuel probes) were transfected by using fresh Opti-MEM. After 4 h, removed the Opti-MEM and washed twice with PBS and cultivated for 8 h. Then, one group was irradiated with laser for 5 min, and all the groups were cultured for another 8 h. The fluorescence imagings were carried out by using a confocal laser scanning fluorescence microscope. The miRNA-155 inhibitor (300 nM) was transferred follow the according to the manufacturer's instructions.

### **Fluorescence Imaging in vivo**

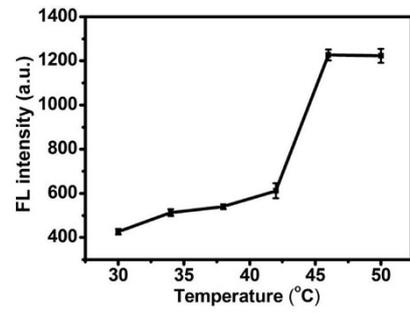
All animal experiments were approved by the Institutional Animal Care and Use Committee of the Beijing institute of Basic Medical Science (Beijing, China). The whole animal experiment was conducted in compliance with the National Regulation of China for Care and Use of Laboratory Animals. To perform the fluorescence imaging, female nude mice (6 weeks old) were acquired from and tumor xenografts as the previously reported.<sup>1, 6</sup> The tumor-bearing mice were injected 200  $\mu$ L of HCuSNPs-DNA solution (1 mg/mL). After 8 h of injection, the tumors areas were exposed to a 1064 nm laser (1.0 W/cm<sup>2</sup>) for 5 min. Fluorescence imaging was acquired using a IVIS Spectrum Imaging System (USA) at 8 h after laser irradiation.



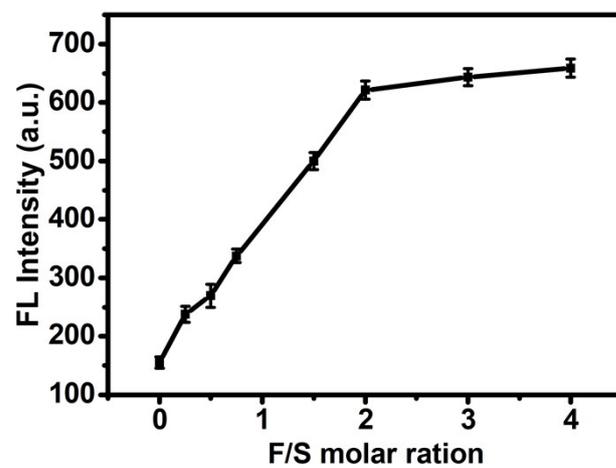
**Figure S1.** (A) Photothermal effect of the irradiation of the HCuSNPs-DNA solution (100  $\mu\text{g/mL}$ ) with a laser irradiation (1064 nm, 1.0  $\text{W/cm}^2$ ) and the laser was switched off after 10 min, the cooling rate was recorded. (B) Plot of time versus negative natural logarithm of the temperature increment for the cooling cycle (after 10 min in A).

**A**

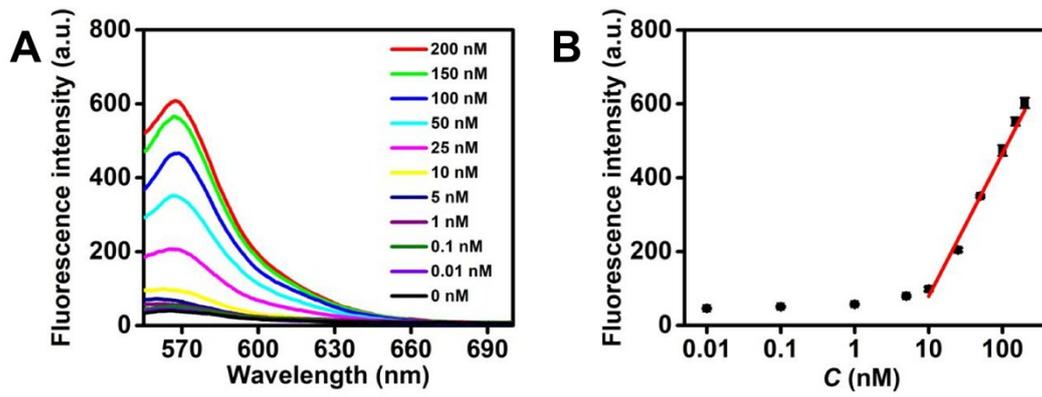
	T <sub>m</sub> (°C)
DNA1 and Fuel	48.87

**B**

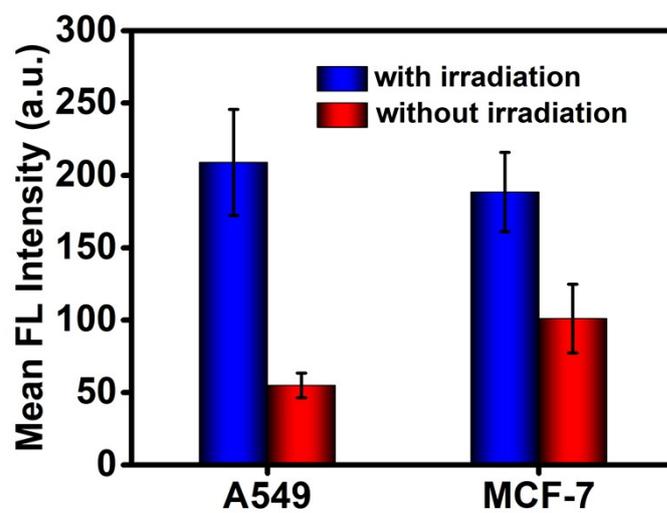
**Figure S2.** (A) The T<sub>m</sub> of DNA1 and Fuel calculated by *Oligonucleotide Properties Calculator*. (B) Optimization the released temperature of the fuel DNA.



**Figure S3.** Optimization of molar ratio of fuel probes (F) and substrate probes (S).



**Figure S4.** (A) Fluorescence intensity corresponding to MBs (100 nM) response to different concentrations of miR-155. (B) The linear correlation between the FL intensity and the logarithm of miRNA-155 concentration



**Figure S5.** The mean fluorescence intensity of A549 cells and MCF-7 cells with and without irradiation.

**Table S1. DNA and RNA sequences used in this work**

<b>Name</b>	<b>Oligonucleotide Sequence (5'–3')</b>
DNA1	/SH/(CH <sub>2</sub> ) <sub>6</sub> /CCGACGATTAGCATT
DNA2	/SH/(CH <sub>2</sub> ) <sub>6</sub> /CCAGGCATCCTTCAC/BHQ/
DNA3	/Cy3/CGGAACTTAATGCTAATCGT
Fuel DNA	ATCCTTCACCGGAACTTAATGCTAATCGT
DNA1-FAM	/SH/(CH <sub>2</sub> ) <sub>6</sub> /CCGACGATTAGCATT/FAM/
Fuel-FAM	ATCCTTCACCGGAACTTAATGCTAATCGT/FAM/
Linker	ACCCCTATCACGATTAGCATTAAAGTTCCGGTGAA GGATGCCTGG
SM-155	UUA AUGCUAAUCGUGAUAGGAGU
TM-155	UUCAUGCUAAUAGUGAUAGGAGU
miRNA-21	UAGCUUAUCAGACUGAUGUUGA
miRNA-155	UUA AUGCUAAUCGUGAUAGGGG
miRNA-205	UCCUUCAUCCACCGGAGUCUG

## Reference

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