Supporting Information for:

Dual-channel signal for intracellular mRNA detection via a PRET nanosensor

Tao Xie, Meng Li and Yi-Tao Long*

Key Laboratory for Advanced Materials & School of Chemistry and Molecular Engineering, East China University of Science and Technology, Shanghai, 200237, P. R. China.

* To whom correspondence should be addressed. E-mail: ytlong@ecust.edu.cn; Tel/Fax: +86-21-6425 2339.

Contents:

1. **Experimental Section……....S2**
2. **Supplementary Figures…… ..……..S6**

   Fig.S1 TEM image of AuNPs…….….S6

   Fig.S2 UV-Vis spectra of AuNP before and after modification…S7

   Fig.S3 Proof of nanoflares assembled onAuNP…….S7

   Fig.S4 Evaluation of amounts of nanoflares on each AuNP…….S8

   Fig.S5 DFM images of AuNP before and after modification……S8

   Fig.S6 Scattering spectra changes of AuNP before and after modification…….…..S9

   Fig. S7 TK1 mRNA in MCF-7 and HeLa cells treated with tamoxifen……S9

   Fig. S8 Fluorescence images of TAMRA in MCF-7 and HeLa cells by PRET-based nanosensors……..…S10

3. **The Calculation of Nanoflares Number on Each AuNP.....S10**

1. Experimental Section

Materials and Reagents. All the reagents, which were of analytical grade, were obtained from Sigma Aldrich Chemicals Co. (St. Louis, MO, USA). Aqueous solutions were prepared using ultra-pure water (18.2 MΩ cm) obtained from Milli-Q apparatus (China). The indium-tin oxide (ITO) slides were purchased from Geao Co. Ltd. (China). HeLa, MCF-7 and HaCaT cells were purchased from HaoChen Biotech. Co. Ltd. (Shanghai, China). Phosphate buffer saline (PBS, pH 7.4) contained 136.7 mM NaCl, 2.72 mM KCl, 8.72 mM Na_2HPO_4, and 1.41 mM KH_2PO_4. All oligonucleotides were synthesized and HPLC purified by Sangon Biotechnology Co., Ltd (China). The sequences of the involved oligonucleotides are listed as below:

Recognition sequence: 5'-SH-TTT TTT TTT TTG TAG CGA GTG TCT TTG GCA TAC TTG ATC A-3'

Dye-labelled consensus nanoflare: 5'-TAT GCC AAA GAC ACT CGC TAC ATT TGG CAT A-TAMRA-3'

Target DNA: 5'-TGA TCA AGT ATG CCA AAG ACA CTC GCT ACA-3'

One-based mismatched target: 5'-TGA TAA AGT ATG CCA AAG ACA CTC GCT ACA-3'

Synthesis and Characterization of Gold Nanoparticles. Gold nanoseeds were prepared by a traditional seeds growth method, which

was based on the citrate-mediated reduction of $HAuCl_4$. $HAuCl_4$ (0.01 wt%; 50 mL) was heated to reflux with vigorous stirring, and then sodium citrate (38.8 mM; 5 mL) was added quickly to the solution. The mixed solution was continued to heat for 15 minutes, then stopped heating and kept stirring for another 10 minutes. Preformed gold nanoseeds (1 mL) was mixed with ultra-pure water (25 mL) and $NH_2OH{\cdot}HCl$ (0.2 M; 100 μL), and then stirred vigorously at room temperature. $HAuCl_4{\cdot}3H_2O$ (0.1 wt%; 3.0 mL) was added dropwise. The resulting solution of 60 nm gold nanoparticle was characterized by UV-Vis and TEM (Figure 1).

Preparation of ITO Slides. Indium-tin oxide (ITO) slides were cleaned in an ultrasonic bath by using ethanol, acetone and ultra-pure water, respectively. Each sonication step was performed for 60 min. After sonication, the slides were rinsed with ultra-pure water and then dried under a stream of nitrogen prior to immobilization of the AuNPs. AuNPs were immobilized on a cleaned ITO slide via the immersion of a dilution 20 times of aqueous gold nanoparticle (60 nm) solution for 1 min.

Immobilization of PRET-based Nanosensor on the Surface of AuNP. DNA recognition sequences (1.0 μM) were immobilized on the freshly prepared AuNPs in PBS. Immobilization was carried out at 37 °C under constant mixing in a shaker for 24 h. Then the AuNPs modified ITO glass was rinsed with ultra-pure water and subsequent incubation

with 1.0 μM dye-labelled nanoflares (PBS, pH 7.4). The mixture was heated to 75 °C, then slowly cooled down to the room temperature, and shook for 24 h to form PRET-based nanosensors. Then the scattering spectra of PRET-based nanosensors were recorded by DFM.

The Hybridization between PRET-based Nanosensor and DNA Target. For mRNA detection, the PRET-based nanosensors were incubated in PBS and treated with increasing concentrations of the DNA targets (final DNA targets concentration: 0, 10, 25, 50, 75, 100, 150 and 200 nM). After incubation for 0.5 h at 37 °C, the AuNPs modified ITO glass were cleaned with ultra-pure water and gently dried in a weak stream of nitrogen.

The Detection of False Positive Signals. For DNase I effects, the PRET-based nanosensors were incubated in PBS at 37 °C. After allowing the samples to 10 min equilibrate, 10 μL DNase I (10 U/mL) was added into PBS solution. The scattering spectra of AuNPs were recorded for 50 min, and each spectrum was recorded after the slides were rinsed with ultra-pure water and gently dried in a weak stream of nitrogen. For GSH effects, the PRET-based nanosensors were incubated in PBS solution at 37 °C. After allowing the samples to 10 min equilibrate, 10 μL GSH (10 mM) was added in PBS solution. The incubation was last for 4 h and the scattering spectra of AuNPs were recorded at 0, 0.5, 1, 2, 4 h during this

period. Each spectrum was recorded after the slides were rinsed with ultra-pure water and gently dried in a weak stream of nitrogen.

Cell Culture for DFM Imaging and Detection. MCF-7, HaCaT and HeLa cells were cultured in Dulbecco's modified Eagles medium (DMEM, GIBCO, Sigma-Aldrich Inc., USA), which were supplemented with 10 % fetal bovine serum (FBS, Sigma) and antibiotics penicillin/streptomycin (100 μg/mL) at 37 °C in a 100% humidified atmosphere containing 5% CO_2. Then the cells were seeded in dishes (6 cm) and incubated with AuNPs in PBS for 1 h. After incubation, the dishes were washed by ultra-pure water and immersed in PBS for DFM imaging.

Scattering Spectroscopy by DFM. An inverted microscope (Eclipse Ti-U, Nikon, Japan) was used to observe the AuNPs in the dark-field, which was equipped with a dark-field condenser ($0.8 < NA < 0.95$) and 40× objective lens (NA = 0.6). The AuNPs were excited by a white light source (100 W, halogen lamp) and then the plasmon resonance scattering light of AuNPs was generated and captured through the objective lens, and split by a monochromator (SCT320, PI). The monochromator was equipped with a grating (grating density = 150 lines/mm) and recorded by a spectrometer CCD (ProEM 1600+, Princeton Instruments, USA) to obtain the scattering spectra of nanoparticles. Part of scattered light was collected by a true-color digital camera (Nikon DS-fi1c, Japan) to obtain

the dark-field color images. The other fraction of light was collected by a spectrometer to obtain single particle scattering spectra.[34] The scattering spectra were obtained after integrating over 10 s and calculated by the subtraction between the original response curve spectra and the background spectra of adjacent regions.

2. Supplementary Figures

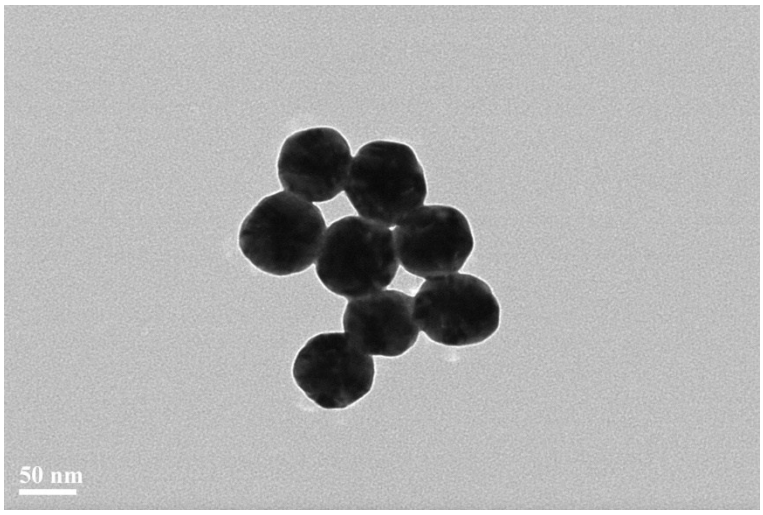

Fig. S1 TEM image of AuNPs.

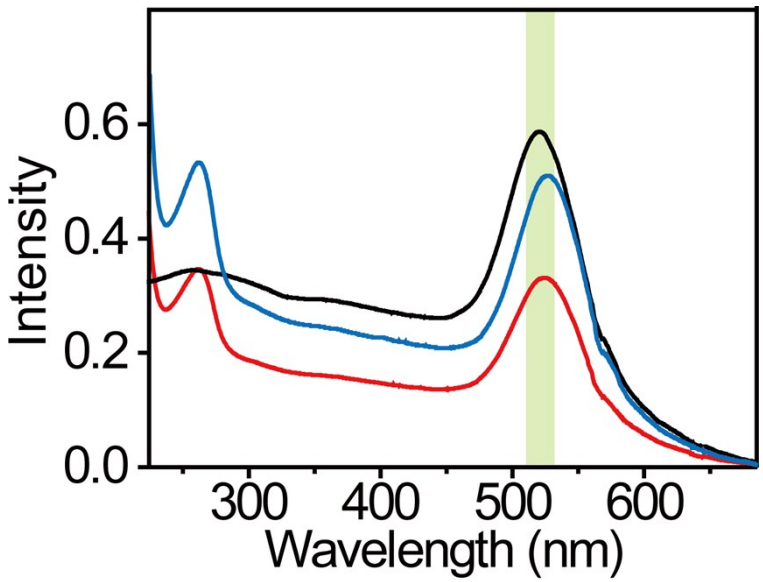

Fig. S2 UV-Vis spectra of AuNP (black), recognition sequences/AuNP (blue), and recognition sequences/nanoflares/AuNP (red).

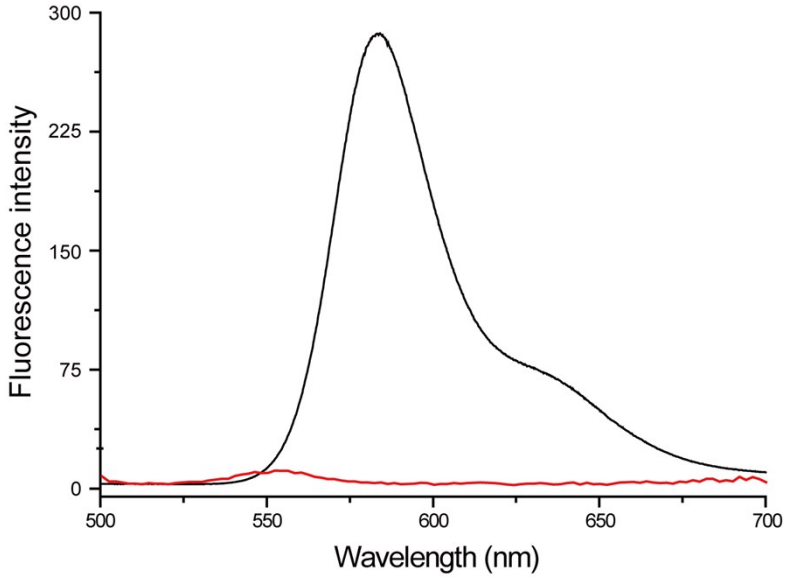

Fig. S3 Fluorescence spectra of the supernatant with (black line) and without (red line) adding 0.78 mg mercaptoethanol (10.0 mM) into 1.0 mL nanosensors solution. The fluorescence peak at 573 nm indicated that TAMRA-labelled flares were replaced by mercaptoethanol and released into the supernatant.

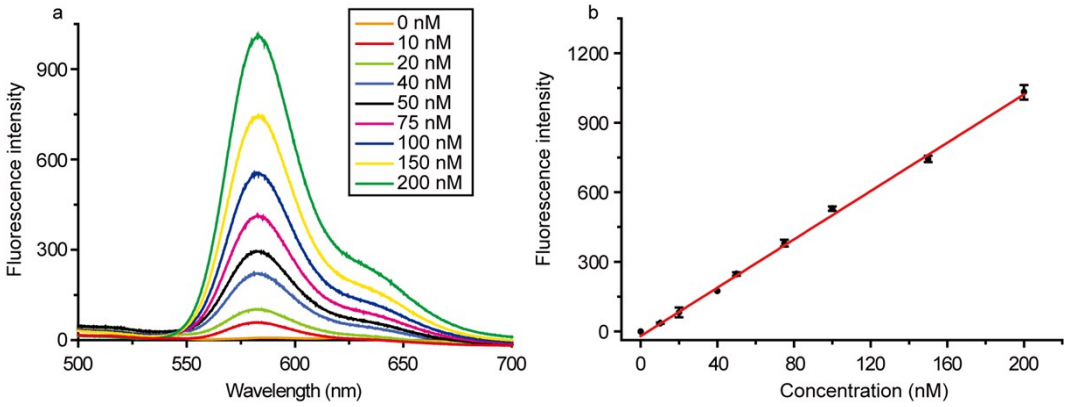

Fig. S4 Standard linear calibration curve of fluorescence intensity with the TAMRA-labeled consensus nanoflares (final concentration: 0, 10, 20, 40, 50, 75, 100, 150, 200 nM). The excitation wavelength was 480 nm, and the emission wavelength was 573 nm. The error bars represent the standard deviations.

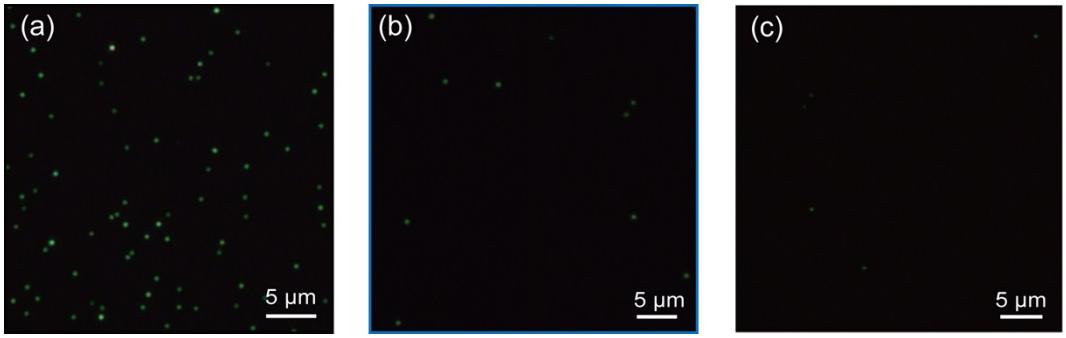

Fig. S5 The DFM images of (a) AuNPs, (b) recognition sequences/AuNP, and (c) recognition sequences/nanoflares/AuNP.

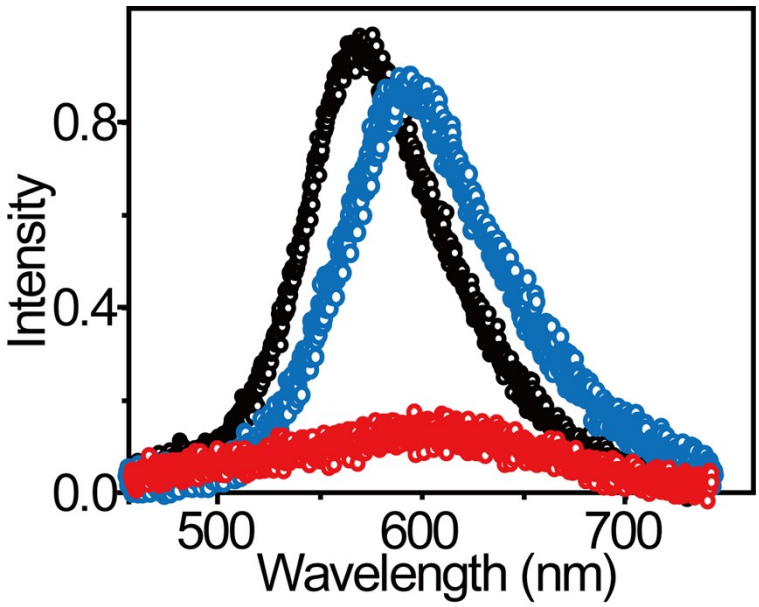

Fig. S6 The scattering spectra of AuNP (black), recognition sequences/ AuNP (blue), and recognition sequences/nanoflares/AuNP (red).

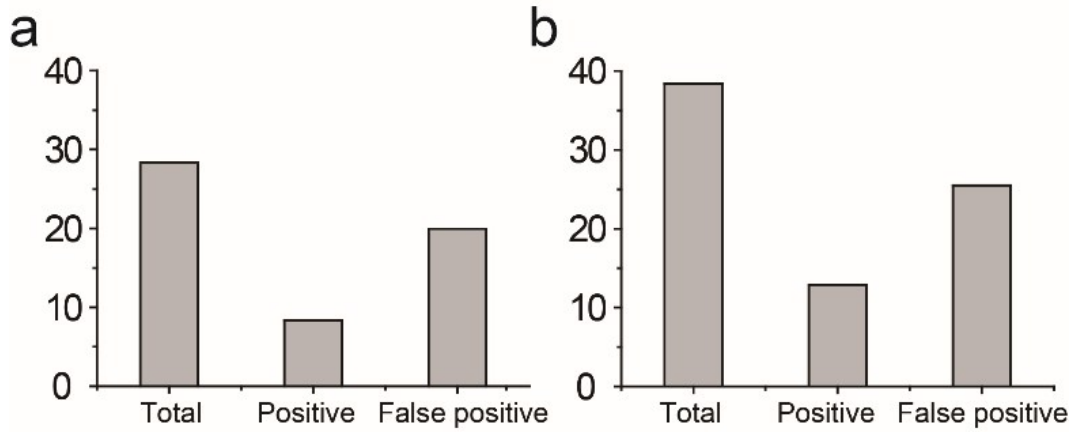

Fig. S7 Histogram of the positive and false positive signals of TK1 mRNA in (a) MCF-7 and (b) HeLa cells treated with tamoxifen by nanoflares-functionalized AuNPs.

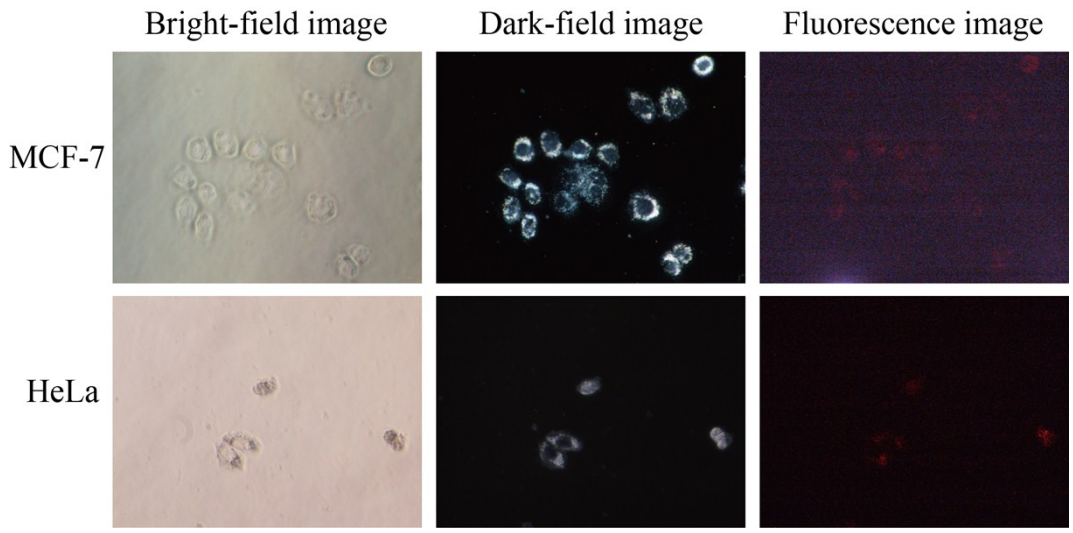

Fig. S8 Fluorescence images of TAMRA in MCF-7 and HeLa cells by PRET-based nanoflares.

3. The Calculation of Nanoflares Number on Each AuNP

The concentration of AuNPs was calculated according to Beer-Lambert Law:

$$A = Kbc$$

Here, ***A*** was the absorption intensity, ***K*** was the molar extinction coefficient, ***b*** was the thickness of absorbed layer and ***c*** was the concentration. According to the absorption spectra of AuNPs in Fig. S2, we obtained the molar extinction coefficient of AuNPs (where ***K***=1.57×10^{10}) from Table S3 in the previous research.[1] Then we put the absorption intensity of AuNPs (***A*** =0.58) and the thickness of cuvette (***b*** =0.1 cm) into the Beer-Lambert Law, and we calculated that the concentration of prepared AuNPs solution was 0.4 nM.

In order to calculate the number of nanoflares modified on each AuNP, we supposed the number of dye-labelled nanoflares in the supernatant was equal to the number of dye-labeled nanoflares released from the surface of AuNPs after adding mercaptoethanol. Based on the standard linear calibration curve of fluorescence intensity against the concentration of TAMRA (Fig. S4b), the dye-labelled nanoflare modified on AuNPs was 51.7 nM because the fluorescence intensity of the supernatant was 275 (Fig. S3). By controlling the AuNPs solution used for modification was kept at the same concentration, there were 51.7 nM nanoflares modified on the surface of 0.4 nM AuNPs, which equaled to approximately 130 nanoflares existed on the surface of each AuNP.

1 W. Haiss, N. T. Thanh, J. Aveyard and D. G. Fernig, *Anal. Chem.*, 2007, **79**, 4215-4221.