Supporting Information

A Dual-Signal Twinkling Probe for Fluorescence-SERS Dual Spectrum Imaging and Detection of miRNA in Single Living Cell via Absolute Value Coupling of Reciprocal Signals

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Experimental Section

Synthesis of Gold Nanoparticles (AuNPs). AuNPs were synthesized by the reduction of tetrachloroauric acid ($HAuCl_4$) with trisodium citrate [1]. After boiling 100 mL of 0.01% (w/w) $HAuCl_4$ in a flask under vigorous stirring, 1.5 mL of 1% (w/w) trisidium citrate was added to the flask rapidly, the color of the solution changing from black purple to orange red, and stirring and heating sustainably for 30 min. With continuous stirring, the solution was cooled naturally to the room temperature, and we could obtain clarified gold colloidal solutions. The prepared AuNPs were characterized by TEM (Figure S1) and stored in brown glass at 4 °C.

Cell Culture. MCF-7 cells were employed in this experiment. The cells were grown in serum medium at a density of 6×10^5 cells/dish in 25-cm^2 cell culture flasks, and cultured in medium (89%) with heat-inactivated bovine serum (10%), penicillin and streptomycin (1%) at 37 °C in a humidified atmosphere (5% CO_2 and 95% air).

Comparison Experiment. The concentration of intracellular miR-203 can be caught through the quantification measurement of MCF-7 cells. The homogeneous solution with the same concentration of miRNA with the cells was prepared and stored at 4 °C. The prepared cell lysates and homogeneous solution were added to the freshly prepared DSTPs respectively and incubated for 2 h. SERS measurements were taken and eleven parallel Raman spectra were measured from different sites of each sample and averaged to represent the final results, and the experiments were taken in triplicate.

Polyacrylamide Gel Electrophoresis. Firstly, moderate amount of the DNA solution were added to 10.0 μL of PBS solution, annealed at 95 °C for 5 min and cooled in ice bath before used. Secondly, 100.0 mL of 50 × TAE was prepared, including deionized water, glacial acetic acid, Tris and $Na_2EDTA\bullet 2H_2O$. Then, 17.5% polyacrylamide gel was prepared, including 40% PAGE Pre-Solution, TEMED, 10% APS, 50 × TAE and deionized water. Later, the gel was mingled adequately and added into the glass until the glass was filled with the gel. Besides, the electrophoretic comb was then inserted to the gel to form the lane and the gel was frozen for 2 h. Next, the electrophoretic comb was pulled out, and the 1 × TAE was poured into the electrophoretic pool and 110 V voltage was inputted for 10 min previously. And 10.0 μL DNA and 1.5 μL 6 × loading buffer were mingled and 10.0 μL mixture was drawn to the lane, 180 V voltage was inputted for 3 min and 125 V voltage was inputted for 90 min until the bright bands were reached at the 3/4 of the gel. Finally, the gel was stained with GelRed for 1h and imaged through UV.

Results and Discussion

Characterization of AuNPs. The synthesized AuNPs were characterized by the transmission electron microscopy (TEM) (Figure S1).

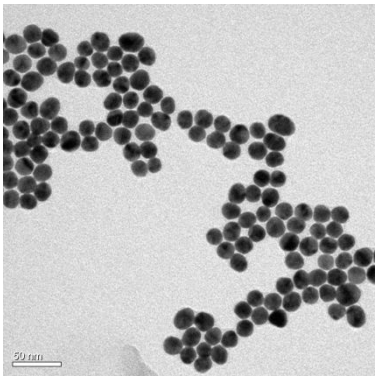

Figure S1. TEM image of AuNPs (the average diameter, 18 nm).

UV-visible Spectra of the DSTPs. The UV-visible spectra of AuNPs, the signal probes (5.0 μM S1: Rox-DNA) and the DSTPs (S1 and 5.0 μM S2: Cy3-DNA) were measured through a Cary 50 UV/Vis-NIR spectrophotometer. As is described in Figure S2A, curve a and b exhibit the characteristic absorption peak of AuNPs (~520 nm) and S1 (Rox-DNA, ~260 nm and a characteristic absorbance at 500~600 nm). And curve c, which exhibited the characteristic absorbance of both Rox-DNA and AuNPs, indicated that the Rox-DNA had successfully conjugated with the AuNPs. While Figure S2B shows that Rox-DNA and Cy3-DNA was also conjugated with AuNPs and the DSTP was successfully prepared.

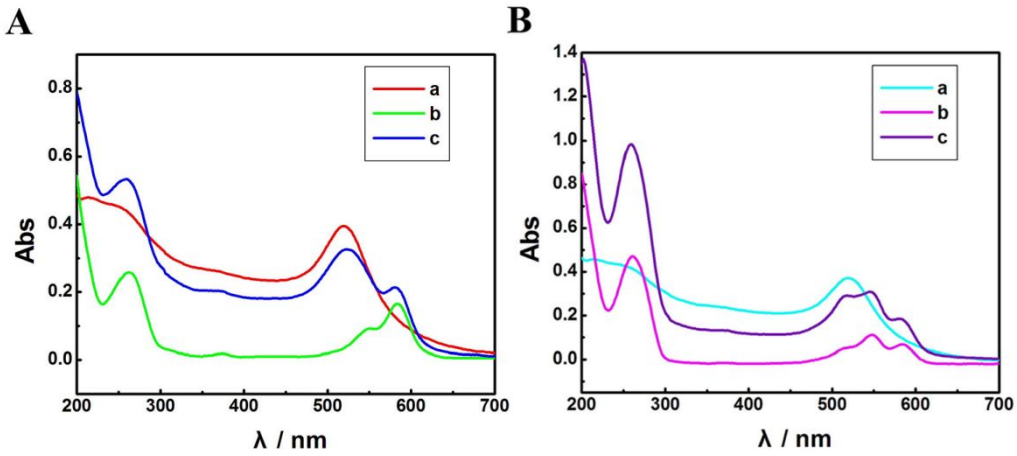

Figure S2. (A) UV spectra of the Au colloids (a), Rox-DNA (b), and Au-DNA-Rox (c). (B) UV spectra of the Au colloids(a), the complex of Rox-DNA-Cy3-DNA (b), DSTP(c).

Optimization of the Reaction Temperature and pH. The temperature and pH of the reaction solutions strongly influence DNA hybridization and are the two most important parameters for optimization. Therefore, we investigated the intensity of the Raman signal under different temperature and pH conditions. As shown in Figure S3A, the normalized Raman absolute value coupling of reciprocal signal increased as the temperature increased from 25 °C to 45 °C, reached a maximum at 37 °C, and decreased gradually as the temperature was increased further. Thus, 37 °C was chosen as the optimum temperature.

The influence of pH values ranging from 6.0 to 8.0 on the Raman signal intensity produced by 10 pM miR-203 is shown in Figure S3B; the normalized Raman absolute value coupling of reciprocal signal reached a maximum at pH 7.4. Therefore, we selected pH 7.4 as the optimum pH value.

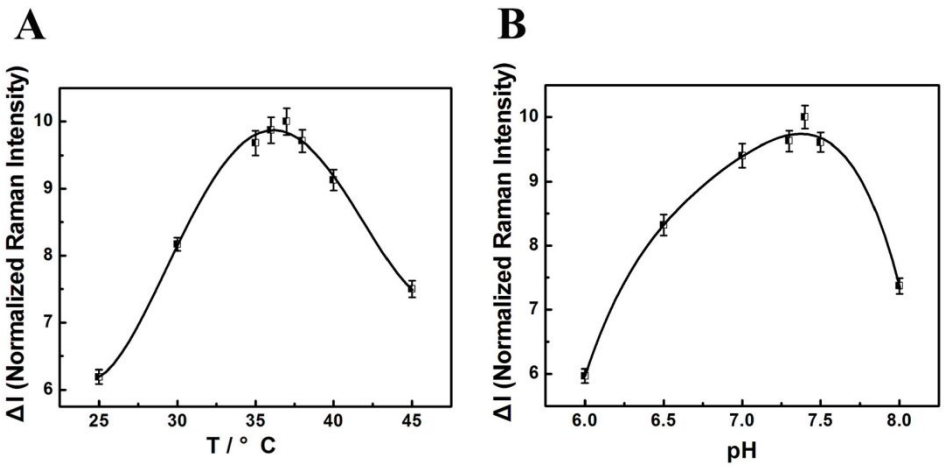

Figure S3. The influence of temperature (A) and pH (B) of the reaction on the intensity ratios of the signal in response to 10 pM miR-203.

Optimization of the Reaction Time of the System. The reaction time was investigated. Figure S4 shows the changes in the Raman signals generated by performing the experiments at different time intervals. The results revealed that the Raman intensity ratio increased rapidly as the incubation time increased and reached a plateau after 90 min. Therefore, we deduced that 60 min was the best incubation time for the assay.

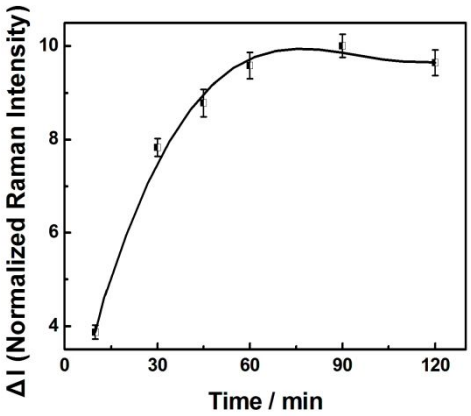

Figure S4. Effect of reaction time on the intensity ratios of the signal in response to 10 pM miR-203.

MTT Experiment. The cytotoxicity of the DSTPs in MCF-7 cells was tested by the MTT experiment. The cells were incubated with 0.5 mL of culture medium containing the DSTPs (5.0 nM) for different time (6, 12, 24, 48 h). Next, the medium was replaced by new medium with MTT (50.0 μL, 1 mg mL^{-1}), and incubated for 12 h at 37 °C, after which the medium was removed. 100.0 μL of dimethyl sulfoxide (DMSO) was to each dish and vibrated for 15 min to dissolve the crystals in the living cells; the absorbance at 490 nm was determined on a RT 6000 microplate reader to obtain the relative cell viability (%), and the results are shown in Figure S5.

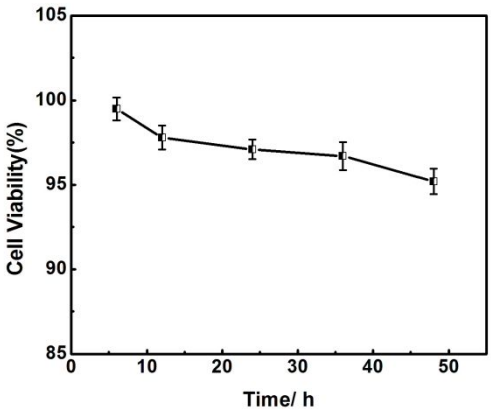

Figure S5. MTT experiment of MCF-7 cells.

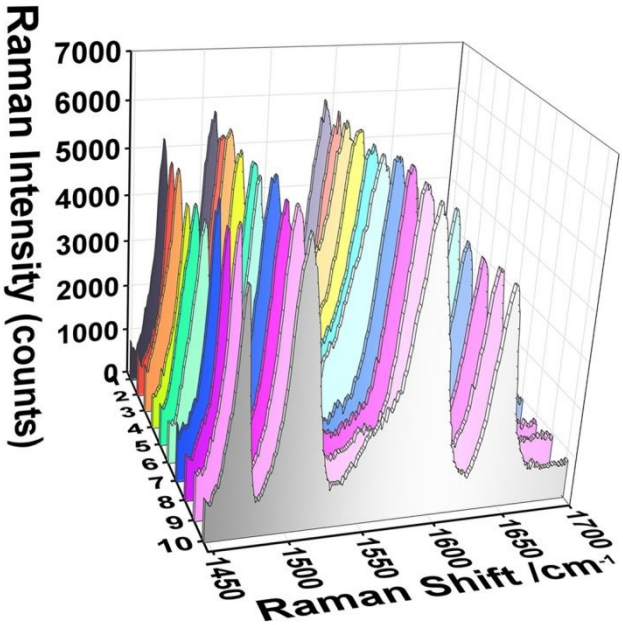

Figure S6. SERS spectra of the DSTP probe for the detecting of miR-203 in 10 different MCF-7 cells, incubating time is 40 min.

Table S1. Oligonucleotide sequences used in our experiments.

Oligonucleotide name	Sequences (5' to 3')
S1	Rox-CACTAGAAAGAAAGAATCTAGTGGGGG-SH
S2	Cy3-TTTCTTTCTTTCTAGTGGTCCTAAACATTTCTTTCTTTCTT
miR-203	GUGAAAUGUUUAGGACCACUAG
miR-21	UAGCUUAUCAGACUGAUGUUGA
miR-141	UAACACUGUCUGGUAAAGAUGG
miR-155	UUAAUGCUAAUCGUGAUAGGGGU

Reference:

[1] NATURE PHYSICAL SCIENCE, 1973, VOL.241, 20-22