Supporting information

MicroRNA-21 expression in single living cells revealed by fluorescence and SERS dual-response microfluidic droplet platform

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1. The statistic particle sizes of AuNPs

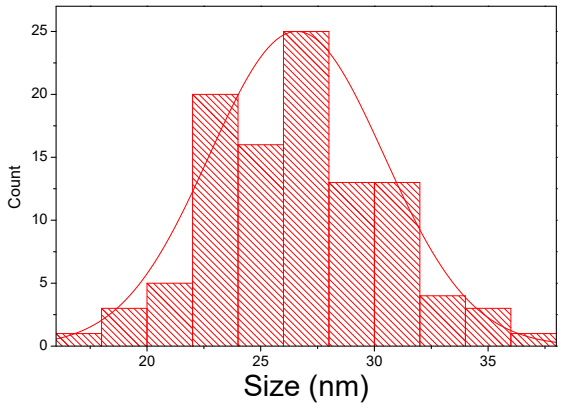

Fig. S1 The statistic particle sizes of the prepared AuNPs.

2. Optimization of the reaction conditions between the target miR-21 and the nanoprobe.

Condition optimization for quantifying miR-21 level was carried out. The incubation time for SERS intensity of nanoprobe at 1504 cm^{-1} in the presence of miR-21 (10 nM) was assessed in Fig. S2a. It is noted that SERS intensity at 1504 cm^{-1} is sharply increased within the first 80 min. When the reaction time is 100 min, the SERS intensity of 1504 cm^{-1} reaches the maximum value, and then the value remains almost unchanged with time (Fig. S2b). Thus, 100 min is chosen as the optimum incubation time.

Furthermore, the temperature and pH of the reaction solutions strongly influence DNA hybridization and are the two most important factors for optimization. Therefore, the intensity of SERS signal of nanoprobe was investigated under different temperature and pH conditions. The influence of temperature values ranging from 20 to 45℃ on the SERS signal intensity produced by 1.0×10^{-8} M miR-21 is shown in Fig. S2c and the SERS intensity at 1504 cm^{-1} reached a maximum at 35℃. Therefore, we selected 35℃ as the optimum temperature. As shown in Fig. S2e, the SERS intensity increased as the pH value increased from 5.5 to 8.5, reached a maximum at 7.5, and decreased gradually as the pH value was increased further. Thus, 7.4 was chosen as the optimum pH value.

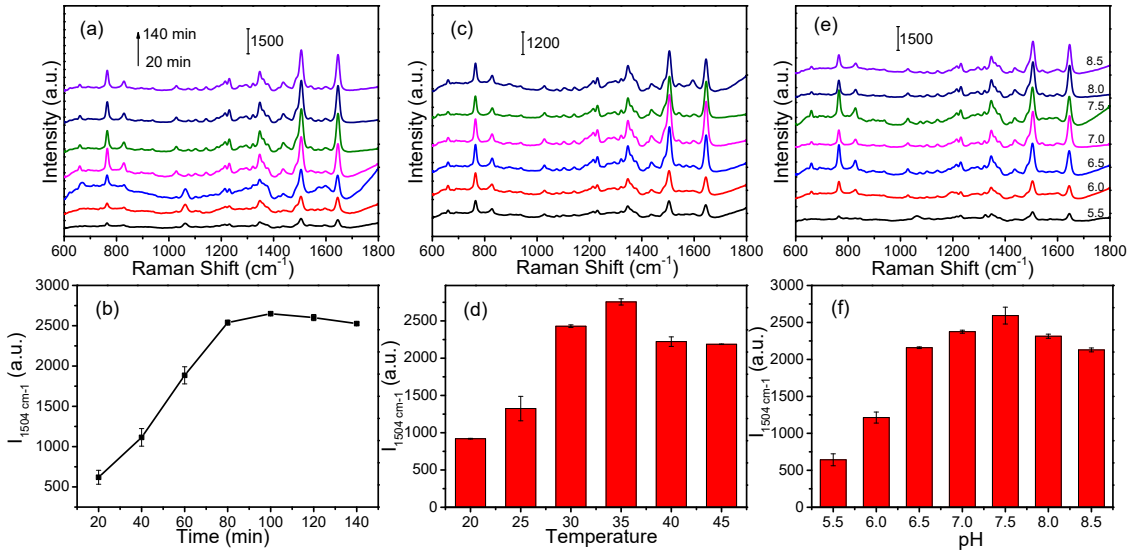

Fig. S2 (a) SERS spectra of nanoprobe at different reaction time (from bottom to top are 20, 40, 60, 80, 100, 120, and 140 min, respectively) (b) A plot of the SERS intensity of nanoprobe at 1504 cm^{-1} along with reaction time. (c) SERS spectra of nnaoprobe at different temperature (from bottom to top are 20, 25, 30, 35, 40, 45℃), while the reaction time kept at 100 min. (d) Plot of SERS intensity at 1504 cm^{-1} with the temperature. (e) and (f) The optimization of pH of the sensing reaction.

3. The fluorescence spectra of ROX dye

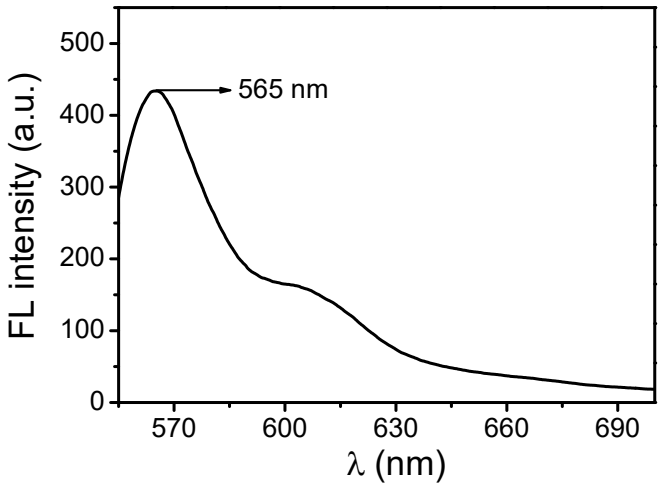

Fig. S3 The fluorescence spectra of Rox dye and the excitation wavelength for Rox was 514 nm.

4. miR-21 analysis in cell lysate

The miR-21 analysis in cell lysate was conducted as follows: miR-21 samples with different concentrations were added to the nanoprobe solution (the nanoprobes were dispersed into 30 μL cell lysate). SERS detections were carried out after incubation for 100 min. Three spectra were acquired from different sites of each sample and averaged to represent the SERS results. Error bars show the standard deviation of the three experiments.

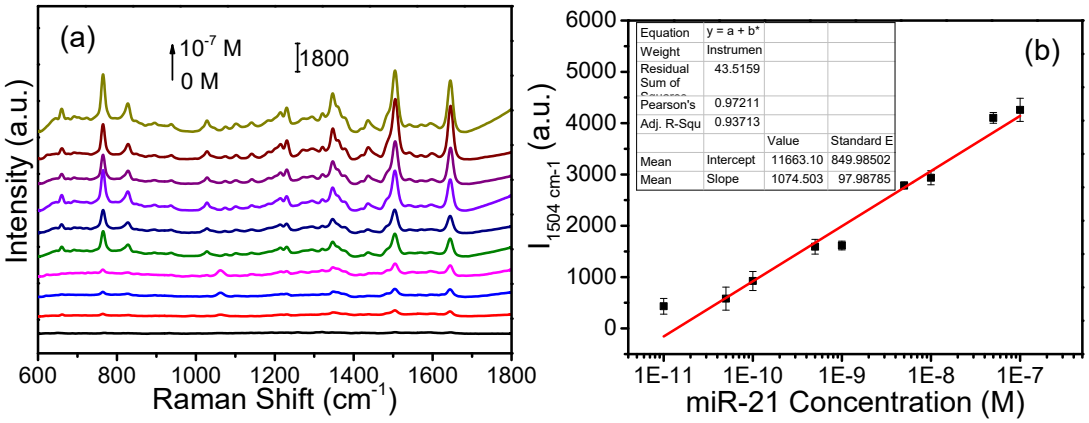

Fig. S4 (a) SERS spectra of the nanoprobe in the appearance of different concentrations of miR-21 (from bottom to top 0, 10^{-11}, 5.0×10^{-11}, 10^{-10}, 5.0×10^{-10}, 10^{-9}, 5.0×10^{-9}, 10^{-8}, 5.0×10^{-8}, 10^{-7} M) in cell Lysates. (b) A plot of the $I_{1504\ cm\text{-}1}$ of ROX with the concentration of miR-21.

5. The evaluation of selectivity and stability

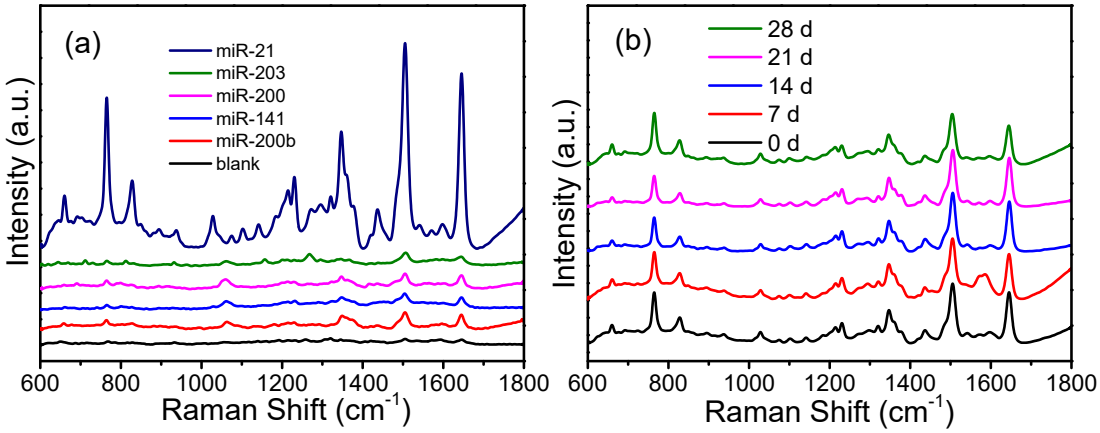

Fig. S5 (a) SERS spectra studies of selectivity of this method with miR-21 (10 nM)

and other miRNAs (100 nM). (b) The stability evaluation of the nanoprobe for detecting miR-21 (10 nM).

6. Optimization of endocytosis time of nanoprobes.

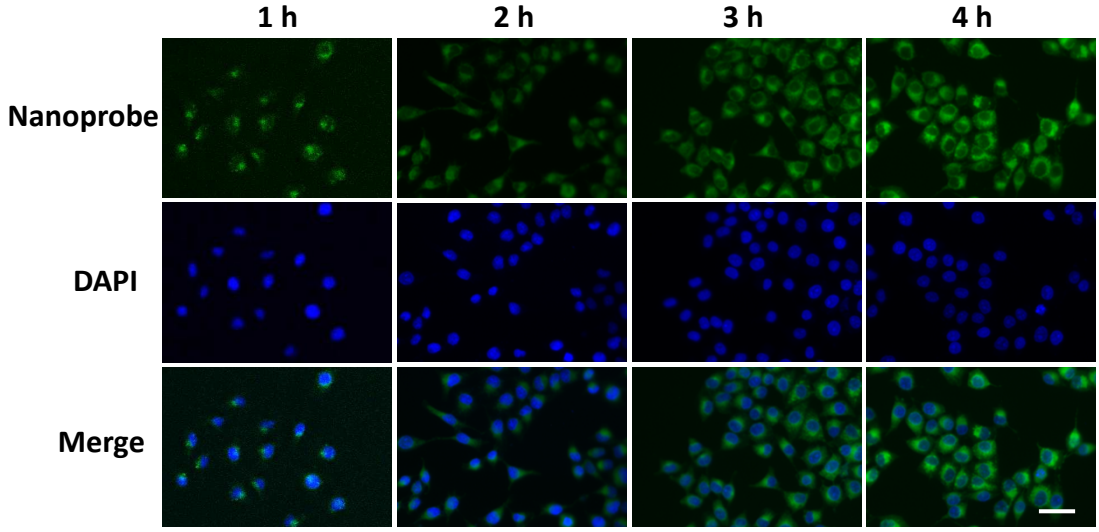

Fig. S6 Fluorescence images (from top to bottom: the images of the naoprobe, DAPI and the overlapping images). MCF-7 cells incubated with 0.4 nM nanoprobe for 1, 2, 3, and 4 h (scale bar = 50 μm).

7. The probability of droplet encapsulating single cell

The probability of produced droplets encapsulating individual cells was studied in Fig. S7, where orange arrows highlight the cell-bearing droplets. The Poisson distribution of cells encapsulated into droplets is given as following:

$$f(\lambda ;n) = (\lambda^n \ e^{-\lambda}) / n!$$

where n is the number of cells in the droplets and λ is the average value of cells encapsulated into per droplet. We have evaluated the distributions under the different densities of cells into every droplets. We have calculated the values of λ was 0.2, 0.3, and 0.5 respectively, which are typical values of interest for single cell experiments. It can ensure that very few droplets containing multiple cells. In our work, the value of λ used is about 0.3 which is in good agreement with those results calculated from Poisson statistics (Fig. S7) under the density of cells about 3.5×10^6 cells/mL. Therefore, we have obtained the probability of single cells encapsulated into one droplet was about～20% while ensuring that fewer than 6% have two or more cells. Although the number of single-cell-bearing droplets is rather low, it could not influence the detection of single cells in this work, because the high production and

screening rate can be achieved with microfluidic devices to obtain the single cells encapsulated into one droplet.

Fig. S7 (a) The probability of a droplet encapsulating a single cell. n is the number of cells in the drops and λ is the average number of cells per droplet. Dashed and solid lines show the predicted values from Poisson statistics and experimental results. (b) Single cell encapsulated in an individual droplet. The cell bearing drops are highlighted by the orange arrows. Scale bar represents 50 μm.

Table. S1 Oligonucleotide sequences used in our experiments

Oligonucleotides name	Sequences
probe DNA strand	5'-ROX—GCGAGCTAGCTTAT CAGACTGCTCGCTTTT TT-SH-3'
capture DNA strand	5'-TCAACATCAGTCTGATAAGCTA-3'
microRNA-21	5'-UAGCUUAUCAGACUGAUGUUGA-3'
miRNA-203	5'-GUGAAAUGUUUAGGACCACTU G-3'
miRNA-141	UAA CAC UGU CUG GUA AAG AUG G
miRNA-200	CUG UGC GUG UGA CAG CGG CUG A

Table. S2 The comparison of different methods for miR-21 detection

Method	The limit of detection	The linear range	Ref
Digital PCR	20 aM	20 aM to 200 fM	[1]
Isothermal amplification	0.5 nM	1 nM to 100 nM	[2]
Nanoplasmon-enhanced droplet screening platform	0.1 nM	0.1 nM to 1000 nM	[3]
Chemiluminescence	10 fM	40 fM to 1 nM	[4]
Fluorescence	18.5 pM	0.2–2 × 10^{-9} M	[5]
SERS-fluorescence	10 pM	1.0×10^{-11} - 1.0×10^{-7} M	Our woks

References

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