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A novel ratiometric SERS biosensor with one Raman probe for ultrasensitive microRNA detection based on DNA hydrogel amplification

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Materials and reagents

3-Mercaptophenylboronic acid (3-MPBA), 3-hydrocythiophenol (3-HTP), β-glucose and glucose oxidase (GOx) were purchased from TCI Chemical Industry Co., Ltd. (Shanghai, China). HPLC-purified microRNA was prepared from Takara Biotechnology Company Ltd. (Dalian, China). The DNA oligonucleotide probes were synthesized from Sangon, Inc. (Shanghai, China). And the corresponding sequences are listed in Table S1. Duplex-specific nuclease (DSN) was obtained from Newbornco Co., Ltd (Shenzhen, China). N, N, N', N'-tetramethylethylenediamine (TEMED), Ammonium persulfate ($(NH_4)_2S_2O_8$), ethylene diamine tetraacetic acid (EDTA), acrylamide, sodium chloride (NaCl), magnesium chloride ($MgCl_2$) were purchased from ChengDu Kelong Chemical Reagent Company (Chengdu, China). Gold chloride ($HAuCl_4$) and L-ascorbic acid (L-AA) were obtained from Sigma Chemical Co. (St.Louis, MO). The solutions used in this work were prepared using ultrapure water (specific resistance of 18 MΩ·cm).

Table S1. Sequence information for the nucleic acids used in this study.

Name	Sequence (5'-3')
miRNA 122 (target)	UGG AGU GUG ACA AUG GUG UUU G
ssDNA (S)	CAA ACA CCA TTG TCA CAC TCC A
Link DNA (L)	TCG CAG GAA AAA CTC GAT AGT CAG CAC TCT TCA AAC ACC AGC AAT GT
Released DNA (R)	TGG AGT ACA TTG CTG GTG TTT GAA GAG TGC TGA CTA TCG AGT TTT TCC TGC GA
Acrydite-modified DNA (SA)	ACA TTG CTG GTG TTT GAA GA
Acrydite-modified DNA (SB)	GTG CTG ACT ATC GAG TTT TT

Apparatus

The morphologies of Au nanoflowers were tested by a Tecnai G2 F20 S-Twin 200 KV microscope (TEM, FEI, USA). The SERS spectra used in this work were tested by a Raman spectrometer (Renishaw Invia Raman spectrometer, Invia, UK).

Preparation of DNA hydrogel

Firstly, two acrydite-modified short DNA strands SA and SB were separately prepared at 10 μM with stock solution (10 mM Tris, 1 mM EDTA (pH = 8.0), 200 mM NaCl, 4% acrylamide, 10 μM SA or SB). Then, the above two solutions were mixed together to form solution A at room temperature. Subsequently, nitrogen was bubbled through the mixed solution A for 5 min to remove the oxygen. Afterwards, 2.8 μL mixture B (containing 0.05 g $(NH_4)_2S_2O_8$, 25 μL TEMED and 0.5 mL H_2O) was dropped into solution A with additional 5 min nitrogen added into the mixture. At the same time, 2 mg mL^{-1} glucose oxidase (GOx) was added into the above solution with gentle blending to ensure the GOx would distribute evenly among the solution. Finally, in order to obtain GOx-captured DNA hydrogel, 10 μM Link DNA was added and incubated at 25 ^oC for 10 min.

Preparation of Au nanoflower

The Au nanoflowers (AuNFs) were obtained according to the reference.[1] 20 mL 19.8 mM L-AA and 80 μL 1% $HAuCl_4$ were rapidly mixed with an ice temperature. And the mixtures turned colorless to faint blue rapidly. The obtained AuNFs were collected after centrifugal washing with ultrapure water three times for further use.

SERS strategy assay process

5 μL 100 μM ssDNA (S) was firstly partly hybridize with 5 μL 100 μM Released DNA (R) in 10 μL phosphate buffer solution (PBS) (pH = 7.4) containing 0.25 mM $MgCl_2$ for 1 h with 37 ^oC. Afterwards, target miRNA with different concentrations were respectively added in the above solution for 40 min. By this way, S DNA can completely complement with target miRNA to release R DNA. Meanwhile, 5 μL 0.03 U DSN were put into the solution to cleave S DNA releasing miRNA. After the cleave reaction, 3 μL DSN stop solution was added into the amplification products for denaturation of DSN enzyme. As a result, lots of R DNA can be obtained through this DSN-induced cycle amplification.

On the other hand, silicon wafer was firstly immersed into ethanol and ultrapure water alternately and ultrasound 5 min to remove the impurities. Next, 10 μL AuNFs were dropped on the clean silicon wafer and dried. Then, 10 μL 1.5 mM 3-MPBA

was dropped on the Si@AuNFs for 2 h to immobilize 3-MPBA on Si@AuNFs by Au-S bond. And excess 3-MPBA can be washed by ultrapure water with a slight shaking of Si@AuNFs@3-MPBA. Later, 5 μL 10 mM glucose, 3 μL GOx captured DNA hydrogel and 3 μL products from DSN induced cycle amplification were added on the SERS substrates at 40 ^{o}C for 20 min. After washed the unreacted reagent, the SERS substrates were tested by 785 nm laser to obtain SERS spectrum.

Figure S1

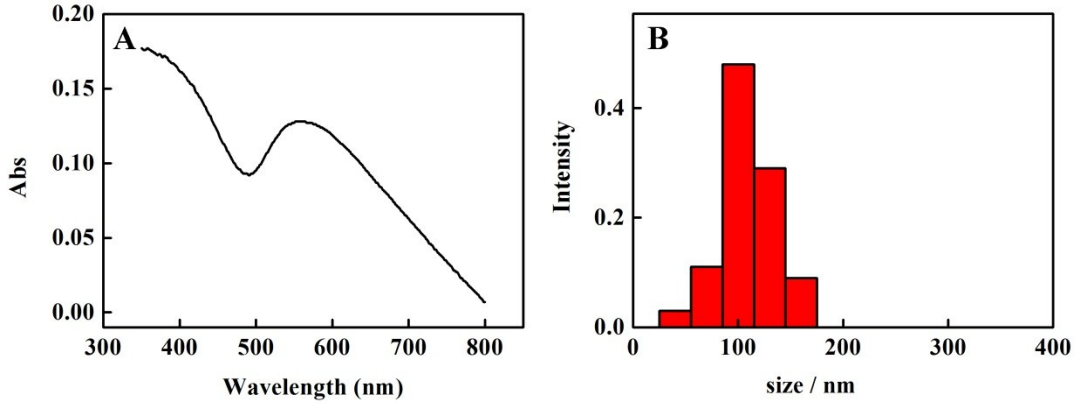

Figure S1. (A) The UV-Vis of Au NFs; (B) Size distribution of Au NFs.

Figure S2

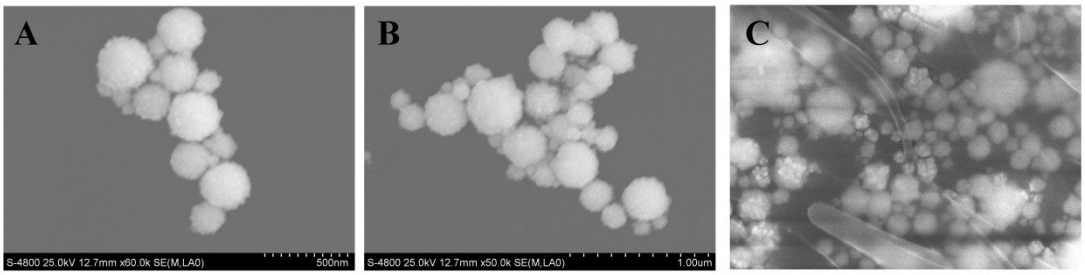

Figure S2. (A) SEM of Au NFs; (B) SEM of Au NFs@3-MBPA; (C) SEM of Au NFs@3-MBPA after treatment of DNA hydrogel.

Table S2. The sequences of miRNAs and DNAs.

Name	Sequence (5'-3')
miRNA 155	UUA AUG CUA AUC GUG AUA GGG GU
miRNA 21	UAG CUU AUC AGA CUG AUG UUG A
miRNA 141	UAA CAC UGU CUG GUA AAG AUG G
one base mismatch	TGG AGU GTG ATA ATG GTG TTT G

three base mismatch	TGG AGA GTG ATA ATG CTG TTT G
miRNA 122	UGG AGU GUG ACA AUG GUG UUU G

Reference

1 S. Boca, D. Rugina, A. Pintea, L. Barbu-Tudoran and S. Astilean, *Nanotechnology*, 2011, **22**, 055702.