Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

A fluorescent assay for microRNA let-7a by double-stranded DNA modified gold nanoparticles nanoprobe combined with graphene oxide

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Experimental section

Materials and Reagents

GO and AuNPs with 15 nm diameter were provided by XFNANO Materials Tech Co., Ltd. (Nanjing, China). Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) was purchased from Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Na_2HPO_4, NaH_2PO_4, NaCl, and KCl were obtained from Sinopharm Chemical Reagents, Co., Ltd. (Shanghai, China). Human serum samples were supplied by the First Affiliated Hospital of Hainan Medical University.

All oligonucleotides used in this experiment were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China) and the purification level is HPLC. The nucleic acid sequences are listed in Table S1.

Table S1. Nucleic acid sequences used in this study.

Name	Sequences (5′ to 3′)
Recognition DNA	(SH)-AAAAAAAAAAACTATACAACCTACTACCTCATAGGTAC
HP DNA	ACAACCTATGAGGTAGTAGGTTGT
Fuel DNA	(FAM)-G*T*A*CCTATGAGGTAGTAGGT*T*G*
MicroRNA let-7a	UGAGGUAGUAGGUUGUAUAGUU
Non-recognition sequences	(HS)-AAAAAAAAACCGATCACAACGTACTACCTCAAAGGTTG
One base mismatch microRNA	UGAGGUAGUAGGUUGUCUAGUU
MicroRNA inhibitor	mAmAmCmUmAmUmAmCmAmAmCmCmUmAmCmUmAmCmCmUmCmA

(FAM: 6-carboxyfluorescein. *: phosphorothioate bonds. C: mismatched base in the sequence. m: 2′-O-methyl.)

Apparatus

Fluorescence spectra were recorded on FL-8500 fluorescence spectrophotometer (PerkinElmer, Shelton, USA). Ultraviolet-visible (UV-vis) absorption spectra were recorded using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA).

Preparation of GO solution

In brief, 10 mg of GO powder was added to 10 mL of deionized water. The mixture was immediately treated by ultrasonic processing in an ice bath for 3 hours. A homogeneous brownish yellow dispersion was ultimately collected and kept at room temperature for further use.

Preparation of dsDNA-AuNPs

Recognition DNA (thiolated oligonucleotide) was reduced for 1h by TCEP at a molar ratio of 1:100 to prevent the formation of disulfide bonds. Recognition DNA and HP DNA were mixed in phosphate-buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4) at a molar ratio of 1:1.2. The mixture was heated to 75°C for 10 min and allowed to natrually cool to room temperature for rapid annealing. Subsequently, the mixture solution was added to AuNP solution (20 nM) and kept at room temperature for 12 h. Then, the solution was centrifuged at 13,000 rpm for 20 min and the supernatant was decanted to remove unbound DNA. Finally, the resulting solution was washed two times and stored in PBS solution in the dark at 4°C.

MicroRNA let-7a fluorescence detection

The fluorescence assay of microRNA let-7a was performed as follows. 20 nM fuel DNA solution was added to the GO solution. The dsDNA-AuNPs solution was added to the mixture. Different concentrations of target microRNA let-7a (0 pM-1 nM) were added and incubated at 37 °C for 30 min. The fluorescence of the mixture was recorded by fluorescence spectra (excitation wavelength: 488 nm, emission wavelength: 520 nm).

Additional Figures

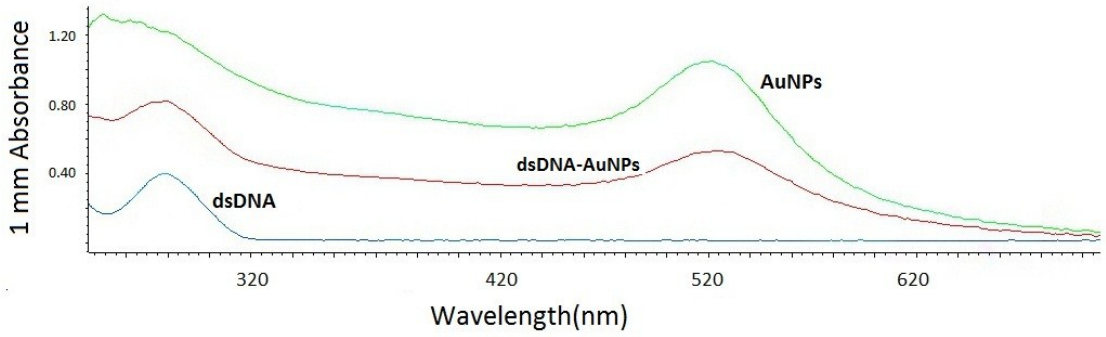

Figure S1. UV-vis characterizations of AuNPs, dsDNA-AuNPs and dsDNA.

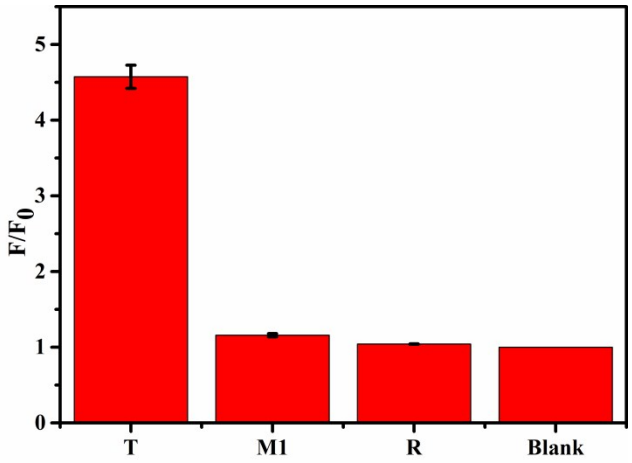

Figure S2. Selection of microRNA assay.

Table S2. Comparison between reported studies and the present study for microRNA detection.

Methodology	Analyte	Linear Range	LOD	Refs
Rolling circle amplification and lateral flow strip	microRNA let-7a	20 pM-200 nM	20.0 pM	1
G quadruplex-based signal quenching	microRNA 21	10 nM-1 μM	4.50 nM	2
Triple isothermal cascade amplification	microRNA 21	1 μM-100 μM	0.50 μM	3
DNA- gold nanoparticle coated silica nanorods	microRNA	10 pM-10 nM	10.0 pM	4
DNA nanoprobe based on dsDNA-AuNPs and GO	microRNA let-7a	5 pM-1 nM	3.9 pM	this study

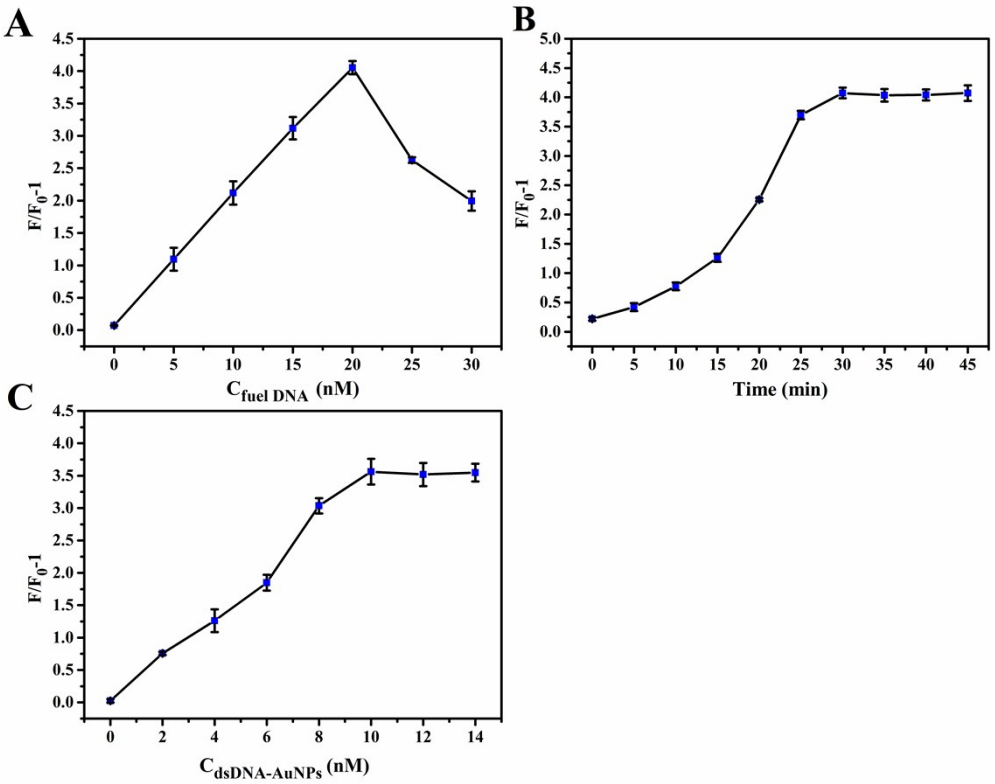

Figure S3. Effects of (A) fuel DNA concentrations, (B) the reaction time of two TSDRs and (C) dsDNA-AuNPs concentrations on the F/F_0−1 value of sensor system. Conditions: microRNA let-7a, 1 nM.

Supplementary references

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