Electronic Supplementary Information

Direct visualizing sub-femtomolar circulating microRNAs in serum based on duplex-specific nuclease-amplified oriented assembly of gold nanoparticle dimers

Longhua Guo*, Yi Lin, Chen Chen, Bin Qiu, Zhenyu Lin*, and Guonan Chen

Institute of Nanomedicine and Nanobiosensing; The Key Lab of Analysis and Detection Technology for Food Safety of the MOE and Fujian Province; College of Chemistry, Fuzhou University, Fuzhou, 350116, China.

Experimental details:

Materials and Apparatus

All oligonucleotides and modifications were conducted by Sangon Biotech Co., Ltd. (Shanghai). Detail sequences and modifications of the oligonucleotides were shown in Table 1. Sodium citrate, poly(ethylene glycol) (average M_n 8,000), poly(ethylene glycol) 2-thioethyl ether acetic acid (thiol-PEG-acid, average M_n 1,000), and gold(III) chloride trihydrate (99.9%) were obtained from Sigma-Aldrich. Tris(hydroxymethyl)aminomethane (Tris) and Ethylenediaminetetraacetic acid disodium salt (EDTA) were purchased from Aladdin (Shanghai, China). GeneFinder™ was purchased from Bio-V biotechnology (Xiamen, China). AmpliTaq

Gold polymerase, including reaction buffer and $MgCl_2$, were purchased from Perkin-Elmer (Madrid, Spain). Brilliant III Ultra-Fast QRT-PCR Master Mix was purchased from Stratagene Products Division, Agilent Technologies (La Jolla, CA). The serum samples of healthy donor, patients with breast cancer, colorectal cancer and lung cancer were obtained from Fujian Provincial Cancer Hospital (FPCH). All other reagents were of analytical purity and used as received. Ultrapure water used in all experiments was obtained from a Milli-Q water purification system (≥18MΩ).

Oligonucleotides stock solutions were prepared in TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA) and diluted to the requested concentrations with ultrapure water shortly before use. The hybridization buffer was a 20 mM Tris buffer solution (pH 7.4) containing 15% formamide, 10% dextran sulfate, 0.3 M NaCl and 3.75 mM $MgCl_2$. DSN buffer (1×) was consisted of 50 mM Tris-HCl, pH 8.0; 5 mM $MgCl_2$, and 1 mM DTT.

Scanning electron microscopy (SEM) images were obtained using an FE-SEM (Nova NanoSEM 230, FEI, USA). The samples were prepared by dropping the AuNP solution on silicon wafers and were observed under FE-SEM without coating. All absorbance spectra were measured with a portable Maya2000 Pro UV-Vis spectrometer (Ocean Optics, Dunedin, Florida, USA). The spectra were taken in a transmission mode using a pair of optical fiber bundles. The concentration of AuNPs was calculated by the absorbance at 543 nm and 450 nm, respectively, with a method reported previously.[1] The real-time PCR reactions were carried out on an Mx3000P™ QPCR System (Stratagene).

The preparation of DNA probes asymmetrically functionalized AuNPs

DNA probe 1 and probe 2 asymmetrically modified AuNPs were prepared with a method reported previously.[2] Briefly, AuNPs with an average diameter of 48 nm were synthesized via the sodium citrate reduction of $HAuCl_4$ as previously described.[3] Procedures for fabricating the asymmetrically functionalized AuNPs were divided into the following 5 steps:

1) A glass cover slip was first activated by immersing it in a "piranha" solution (30% H_2O_2 mixed in a 1:4 ratio with concentrated H_2SO_4) for 15 min. The cover slip was then washed thoroughly with distilled water and stored in ethanol prior to use.

2) The activated cover slip was immersed in a solution containing 0.05 M CTAB for 30 min and then washed three times with distilled water.

3) The CTAB-modified glass cover slip was then immersed in the citrate-stabilized 43-nm AuNP (which is negatively charged) solution for 5 min. The glass substrate was then removed and washed with water three times to remove any loosely bound AuNPs.

4) The AuNP-modified glass substrate was then immersed in a 10 mM Thiol-PEG-acid solution (0.1 M phosphate buffer, pH 8.0) and allowed to incubate for 24 hours. The substrate was then removed and washed thoroughly with water to remove any loosely bound PEG.

5) To remove the particles from the glass substrate after modification, the substrate was sonicated in 1.0 mL water for 1 min, resulting in a dispersion containing PEG asymmetrically functionalized AuNPs.

Prior to modify the oligonucleotide, the thiol groups were activated according to a method described in the literature.[4] The 5′-thiol oligonucleotides (0.1 mM) were deprotected with 0.l mM tris(2-carboxyethyl)phosphine (TCEP) in 20 mM tris buffer (pH 7.4) for 1 hour at room temperature. A 100 μL aliquot of the deprotected oligonucleotides (100 μM) was then mixed with a 1-mL PEG asymmetrically functionalized AuNP solution (20 mM Tris (pH 7.4), 100 pM AuNP, 0.01% SDS). The oligonucleotide/gold nanoparticle solution was incubated overnight at room temperature. Aqueous NaCl (2.0 M with 0.01% SDS) was then added to the solution to bring its total NaCl concentration to 0.1 M. The mixture was again incubated for 5 hours. The NaCl concentration was increased to 0.2 M using the same approach and incubated for another 5 hours. Then, the NaCl concentration was raised to 0.3 M. After additional 5-hour incubation, the gold nanoparticle solution was centrifuged and the supernatant removed leaving a pellet of gold nanoparticles at the bottom. The particles were then resuspended in 1 mL enzymatic reaction buffer. This washing process was repeated for three times.

DSNSA guided miRNA-cDNA reaction

A standard detection procedure for the preparation of the reaction system was as follow: 100 μL of miRNA target and 50 μL of cDNA stock solution (1.5 nM) were added to a volume of 150 μL reaction mixture (containing 1×DSN buffer, 0.1 U DSN, and 20 U RNase inhibitor). The mixture solution was then incubated in a thermal cycler at 60 °C for 30 min. Subsequently, the reaction mixture was added 200 μL 10 mM EDTA and incubated at 60 °C for 5 min to inactive DSN enzyme.

For direct profiling of circulating miR-21 in serum, each 100 μL of serum sample, obtained from Fujian Provincial Cancer Hospital (FPCH), was diluted with PBS to a final volume of 500 μL (1:5 dilution), heated at 95 °C for 5 min, and then cooled rapidly on ice for 5 min. Then, the heat-denatured serum lysates were centrifuged at 15000 g for 20 min at 4 °C. Finally, 100 μL of the supernatant (equal to 20 μL of serum) was used for DSNSA reaction.

Detection of cDNA based on oriented assembly of AuNP dimers

A typical procedure for the detection of cDNA was as follow: 300 μL cDNAs was combined with 300 μL of hybridization buffer and 400 μL of AuNP probes (AuNPs modified with P1 and P2 were mixed at a 1:1 ratio with a final AuNP concentration of 30 pM). The solution was heated to 68 °C for 1 min and incubated at room temperature for 60 min. The pictures of the solution were recorded by a digital camera (Canon EOS 600D with EF 100mm f/2.8L IS USM). Absorbance measurements were conducted with a portable Maya2000 Pro UV-Vis spectrometer (Ocean Optics, Dunedin, Florida, USA).

Quantitative RT-PCR detection of miRNA-21 in serum samples

The total RNA content of serum was extracted from human blood using the miRNeasy RNA isolation kit from Qiagen according to the manufacturer's instructions. To quantify the expression of mature miRNA-21, the following procedure was carried out: 1.5 μg of total RNA was reverse-transcribed to corresponding cDNA using AMV reverse transcriptase (TaKaRa, Dalian, China) and looped antisense primers. The resulted cDNA was then quantified by RT-PCR by an

Mx3000P™ QPCR System (Stratagene). The reaction conditions were as follows: 95 °C for 5 min, followed by 40 cycles with a 15 s interval at 95 °C and a 1 min interval at 60 °C. All reactions were performed in triplicate. The primers used for these assays were directly adopted for a previous literature[5] and listed as follows: miR-21 forward primer: 5'-ACA CTC CAG CTG GGT AGC TTA TCA GAC TGA-3'; miR-21 reverse primer: 5'-CTC AAC TGG TGT CGT GGA GTC GGC AAT TCA GTT GAG TCA ACA TC-3'; U6 forward primer: 5'-CTC GCT TCG GCA GCA CA-3'; miR-21 reverse primer: 5'-AAC GCT TCA CGA ATT TGC GT-3'.

Live subject statement

All experiments were performed in compliance with the relevant laws and institutional guidelines, and Fujian Provincial Cancer Hospital (FPCH) has approved the experiments. The informed consent was obtained for any experimentation with human subjects.

Table S1

Table S1 Sequences and modifications of oligonucleotides used in this work

Name	Sequence (5′-3′)
miR-21	UAGCUUAUCAGACUGAUGUUGA
cDNA	TCAACATCAGTCTGATAAGCTA
miR-16	UAGCAGCACGUAAAUAUUGGCG
miR-126	CAUUAUUACUUUUGGUACGCG
miR-141	UAACACUGUCUGGUAAAGAUGG
miR-122	UGGAGUGUGACAAUGGUGUUUG
miR-200b	UAAUACUGCCUGGUAAUGAUGA
let-7d	AGAGGUAGUAGGUUGCAUAGUU
DNA P1	SH-CTGTTACTGACTGATGTTGA
DNA P2	TAGCTTATCAGCAGTAACAG-SH

Figure S1

Herein, we showed three typical SEM images to demonstrate the dependence between concentration of cDNA and the production of AuNP dimers. It can be clearly seen that in the absence of the cDNA, AuNPs are distributed on the silicon wafer without aggregation (Figure S1 a); in the presence of a relatively low concentration of cDNA, some AuNPs are aggregated into dimers (Figure S1 b). However, most of the AuNPs are still in the monomer forms; in the presence of a relatively high concentration of cDNA, most of the AuNPs are formed into dimers (Figure S1 c).

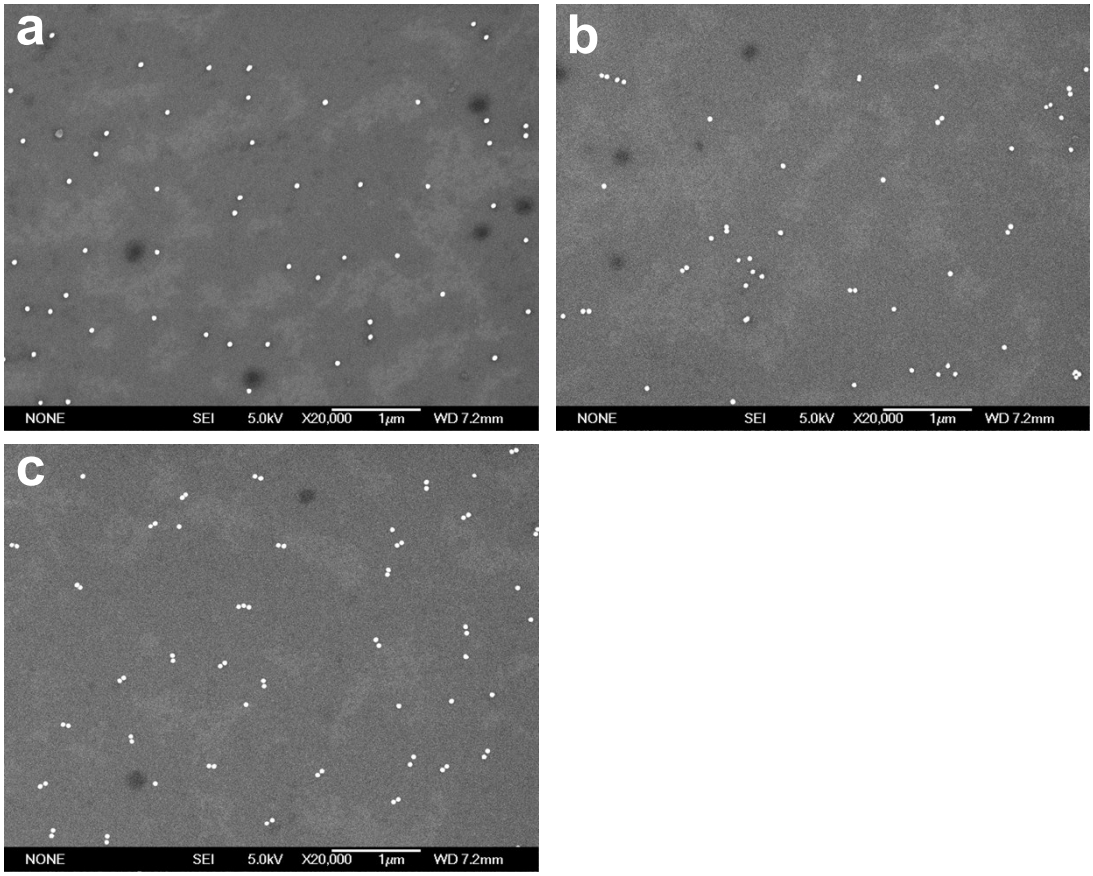


Figure S1 SEM images of the sensing system in the presence of different concentration of cDNA: a. 0 M; b. 64 pM; c. 1024 pM.

Figure S2

In our work, a calibration curve based on the signals obtained from a series of miRNA-21 standard solutions was established for the detection of miRNA-21 in unknown samples. Figure S2 shows the performances of using different data processing approaches to establish the calibration curve. Linear responses are observed for both calibration curves established based on the logarithm of concentration versus A_{600} (c) and the logarithm of concentration versus A_{600}/A_{540} (d). However, the adjusted R-squared of the calibration curve in (c) is 0.98446, while it is 0.96217 for the calibration curve in (d). Therefore, we concluded that calibration curve established based on the logarithm of concentration versus A_{600} is the most appropriate one for our work.

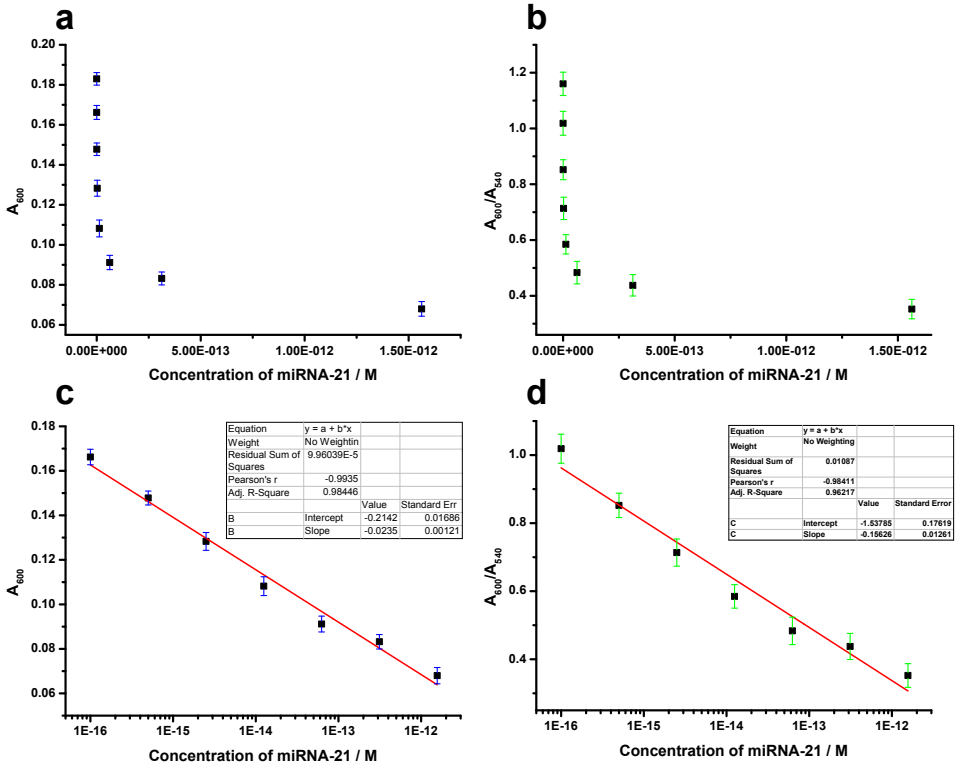


Figure S2 Calibration curves for the detection of miRNA-21 based on different data processing approaches. a. Concentration versus absorbance intensity at the wavelength of 600 nm (A_{600}); b. Concentration versus the ratio of absorbance intensity at the wavelength of 600 nm and 540 nm (A_{600}/A_{540}), respectively; c. The logarithm of concentration versus the absorbance intensity at the wavelength of 600 nm; d. The logarithm of concentration versus the ratio of absorbance intensity at the wavelength of 600 nm and 540 nm, respectively.

Figure S3

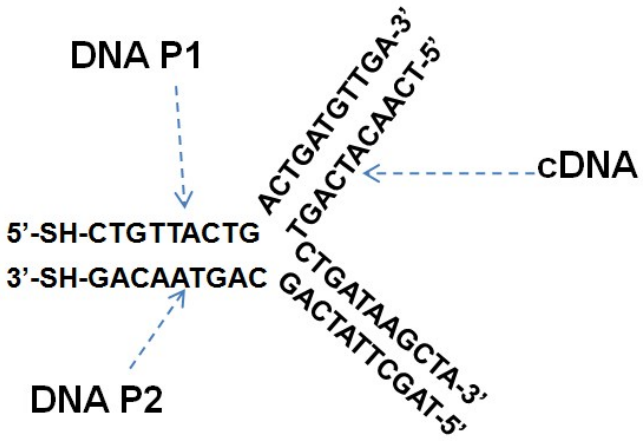


Figure S3 The Y-shaped DNA duplex structure formed when mixing DNA probe 1, DNA probe 2 and the cDNA oligonucleotides.

Figure S4

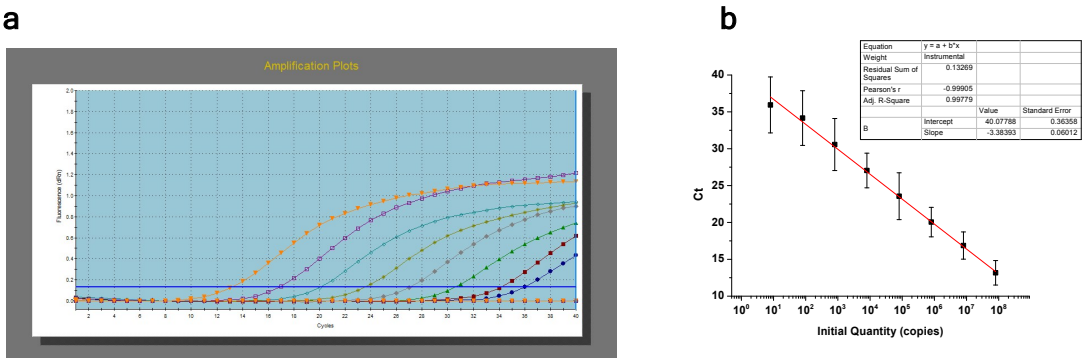


Figure S4 Typical amplification plots and corresponding standard curve for qRT-PCR analysis of miRNA-21.

Figure S5

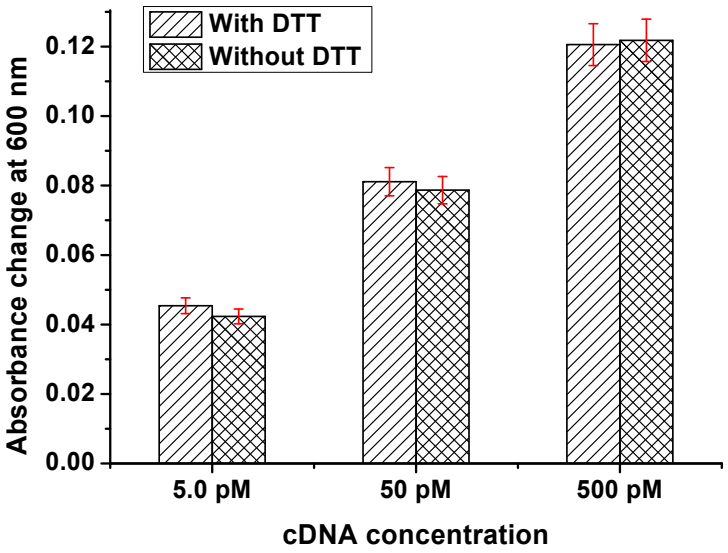


Figure S5 cDNA induced oriented assembly of AuNP dimers in the presence and absence of DTT. Procedures to conduct these experiments are the same as Figure 2 except for the addition of miRNAs.

Figure S6

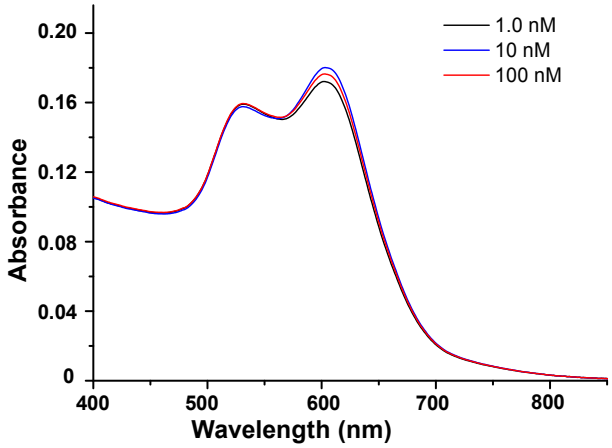
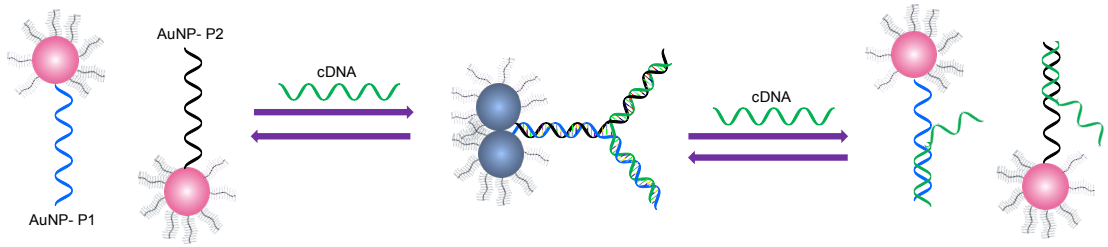


Figure S6 cDNA induced oriented assembly of AuNP dimers. Procedures to conduct these experiments are the same as Figure 1.

Figure S6 shows the absorption spectra of the sensor in the presence of high concentration of cDNAs (1-100 nM). It can be clearly seen that maximum A_{600} was achieved when a concentration of 10 nM of cDNA was presented. When the cDNA concentration was increased to 100 nM, slight absorbance decrease was observed at 600 nm, and the absorbance at 540 nm increased slightly at the same time. No significant absorbance change at the wavelength range of 620 nm to 900 nm, indicating that the presence of a high concentration of cDNA is not leading to random nanoparticle aggregates. Instead, the disassembly of AuNP dimers was happened.

Scheme S1

Scheme S1 Mechanism for the formation and disassembly of AuNP dimers in the presence of different amount of cDNAs



Therefore, if more excess of cDNA was added, it would neither form new AuNP dimers nor form random AuNP aggregates, but induce the disassembly of the AuNP dimers.

References:

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