Electronic Supplementary Material (ESI) for Analyst.

Enzyme-free quantification of exosomal microRNA by the target-triggered assembly of polymer DNAzyme nanostructure

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EXPERIMENTAL SECTION

Materials

All oligonucleotides were purchased from Sangon Biotechnology Inc. (Shanghai, China). All buffers were prepared with ultra-pure MilliQ water (resistance >18.2 MΩ cm^{-1}). Other chemicals were used as received without further purification. Human cervical carcinoma cell line (HeLa) was obtained by the Cell Center of Xiangya Medical School. Normal human hepatocyte cell line (L02) was purchased from the Shanghai Cell Bank, Chinese Academy of Medical Sciences. The serum samples from cervical cancer patient and healthy human have been provided by the First Affiliated Hospital of Changsha Medical University (Hunan, China).

Hybridization assay of probe and target

MiRNA-21 was chosen as the model target molecule. Its DNA analogue was used for the following response experiments. Sequences of oligonucleotides are listed in Table S1. Each functional hairpin DNA (1 μM) was heated to 95 °C for 5 min and then allowed to cool down to room temperature (25 °C) for at least 2 h before use. Then, target sequence with variable concentration was added to 10 mM HEPES buffer containing 500 mM NaCl, 20 mM $MgCl_2$ and mixture hairpin DNA (100 nM H0, 500 nM H1, 500 nM H2 and 500 nM S). The time-dependent fluorescence changes upon adding different concentrations of target sequence were monitored at 37 °C using a fluorescence spectrofluorometer with excitation at 488 nm.

For AFM characterization of the formed wire-shaped DNA nanostructures that were produced by a HCR process, the concentrations of hairpins H0, H1 and H2 were 500 nM, and the target concentration was 10 nM. For polyacrylamide gel electrophoresis, the concentration of target was the same as those of other hairpins (200 nM) to obtain reasonably good quality of image.

Cell culture

HeLa cells and L02 cells were cultured in RMPI 1640 media supplemented with 10%

(v/v) exosomes-free fetal bovine serum (FBS) and 1% (v/v) penicillin and streptomycin. To obtain the exosomes-free FBS, the commercially available FBS was centrifuged at 4 °C at 100,000 g for 10 h using a TLA-100.3 fixed angle rotor in an ultracentrifuge (Optima TL-100, Beckman Coulter, U.S.) to remove the exosomes. The supernatant was then filtered using a 0.22 μm syringe-filter and stored at 4 °C. All cell lines were maintained in a humidified atmosphere of 5% CO_2 at 37 °C and incubated in media containing exosomes-free FBS for two days for the generation of exosomes from the cells.

Exosome isolation

Exosomes were isolated by a precipitation method using the ExoQuick kit (System Biosciences) from the supernatant media of HeLa and L02 cells after cell culture. In brief, the conditioned media were first centrifuged at 3000 rpm for 15 min, and the supernatant was incubated overnight with ExoQuick reagent solution according to the manufacturer's instruction. The next day, the mixture was centrifuged at 3000 rpm for 30 min, and the pellets were re-suspended in phosphate-buffered saline (PBS).

Subsequently, the isolated exosomes were absorbed onto a formvar carbon-coated grid for 10 min. After washing the grid with PBS, exosomes were fixed in 2% paraformaldehyde for 1 min. Then, the grids were negatively stained with 2% phosphotungstic acid for 2 min and washed twice with PBS. Samples were dried for 15 min and visualized using a JEM-3010 electron microscope (JEOL, Japan) operated at 60 kV.

Exosome quantification

To quantify the amount of exosome particles released, we conducted ELISA using an Exosome ELISA kit (System Biosciences). Briefly, the isolated exosomes were incubated overnight in the micro-titer plate provided in the kit. Standard exosomes with known concentration supplied in the kit also were run on ELISA. Then, exosome-specific anti-CD63 (System Biosciences) was added and incubated for 1 h. After washing, the plate was incubated with HRP-conjugated anti-rabbit antibody

(System Biosciences) for 1 h. After the final washing, the reaction was developed with TMB substrate followed by blocking with a stop buffer, and optical densities were recorded at 450 nm.

Detection of exosomal miR-21

The certain amounts of exosomes derived from HeLa or L02 cells were obtained. Then the obtained exosomes were lysed to release all miRNAs into the solutions. The target miR-21 in the extracted solutions of exosomes derived from HeLa (HeLa-Exo) and/or L02 (L02-Exo) cells was detected by the developed method. The concentration of exosomes was 3 nM. Subsequently, in order to study the sensitivity of the detection method, the as-synthesized miR-21 was spiked in the extracted solution of HeLa-Exo (0.03 nM) to prepare the samples with various concentrations (10 fM-10 nM) of miR-21. The samples were then added into 0.5 mL of HEPES buffer (10 mM, pH 7.4) containing 500 mM NaCl, 20 mM $MgCl_2$ and four functional probes (H0, H1, H2 and S). Fluorescence intensities at the wavelength of 526 nm were obtained by the F-7000 spectrofluorometer with excitation at 488 nm. Moreover, the miR-21 concentration calculated by the above-mentioned method was compared with that determined by traditional PCR analysis.

Moreover, the content of exosomal miR-21 in HeLa-Exo without added miR-21 was carefully analyzed. The extracted solutions of HeLa-Exo with different concentrations (0-2.4 nM) were first prepared. The obtained sample solutions were added into 0.5 mL of HEPES buffer (10 mM, pH 7.4) containing 500 mM NaCl, 20 mM $MgCl_2$ and four functional probes (H0, H1, H2 and S), and then allowed to hybridize at 37 °C for 2 h. The mixture solution was immediately subjected to fluorescence measurements. Fluorescence spectra were recorded by the F-7000 spectrofluorometer with excitation at 488 nm.

For the detection of exosomal miR-21 in serum samples (0.5 mL) from cervical cancer patient and heathy human, exosomes was respectively isolated from their serum samples by using the commercially available ExoQuick reagent (System Biosciences). Then the total miRNAs were extracted from the exosomes. The

resulting extracted solutions were added into 0.5 mL of HEPES buffer (10 mM, pH 7.4) containing 500 mM NaCl, 20 mM $MgCl_2$ and four functional probes (H0, H1, H2 and S), and allowed to hybridize at 37 °C for 2 h. The mixture solution was immediately subjected to fluorescence measurements.

Table S1 DNA sequences used to construct the detection platform

Name	Sequences (5'-3')
miR-21	UAG CUU AUC AGA CUG AUG UUG A
DNA analogue of miR-21	TAG CTT ATC AGA CTG ATG TTG A
D1	GAT ATC AGC GAT CTT CTG ATA AGC TA
D2	TCA ACA TCA GT AAG CAC CCA TGT TAC TCT
Substrate (S)	BHQ1- AGA GTA T rA G GAT ATC -FAM
H0	TCA ATT TGA AGT CAA CAT CAG TCT GAT AAG CTA CAT AAG ACT TCT AAT TGA
Control DNA	AAT CAA CTG GGA GAA TGT AAC T
H1	GAT ATC AGC GAT CTT CTA ATT GAA AGT TAT TAA TCA ATT AGA AGT CTT ATG AAG CAC CCA TGT TAC TCT
H2	GAT ATC AGC GAT CTT TTA ATA ACT TTC AAT TAG CAT AAG ACT TCT AAT TGA AAG CAC CCA TGT TAC TCT
1-Mismatched DNA	TAG CTA ATC AGA CTG ATG TTG A
Deleted DNA	TAG CTT ATC GAC TGA TGT TGA
Inserted DNA	TAG CTT ATC ATG ACT GAT GTT GA
Random DNA	GCT AGA GAT TTA TCC ACA CTG A

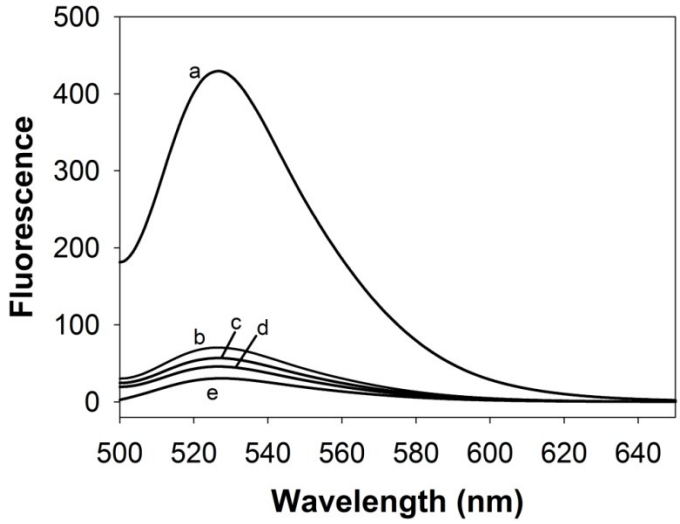

Fig. S1 Fluorescence spectra under different conditions: (a) H0, H1, H2, S, target sequence and Mg^{2+}; (b) H0, H1, H2, S, control sequence and Mg^{2+}; (c) H1, H2, S, target sequence and Mg^{2+}; (d) H0, H1, H2, S and target sequence; (e) H0, H1, H2, S and Mg^{2+}.

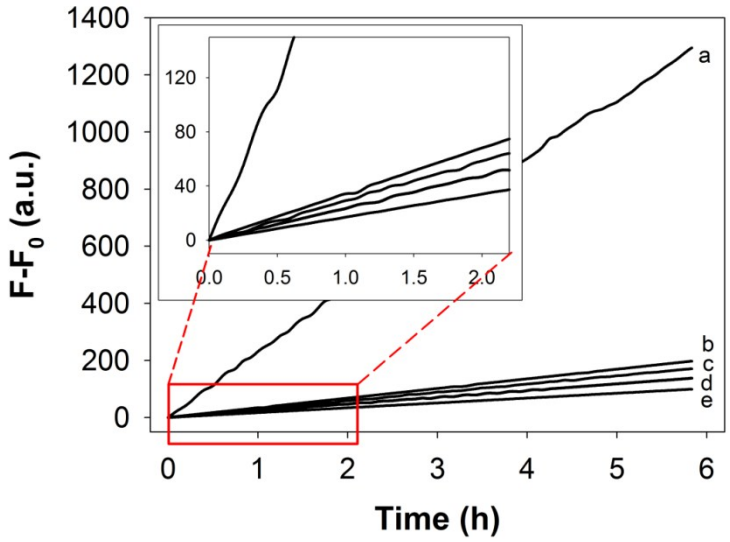

Fig. S2 Time-dependent fluorescence changes under different conditions: (a) H0, H1, H2, S, target sequence and Mg^{2+}; (b) H0, H1, H2, S, control sequence and Mg^{2+}; (c) H1, H2, S, target sequence and Mg^{2+}; (d) H0, H1, H2, S and target sequence; (e) H0, H1, H2, S and Mg^{2+}. F_0 is a background fluorescence signal.

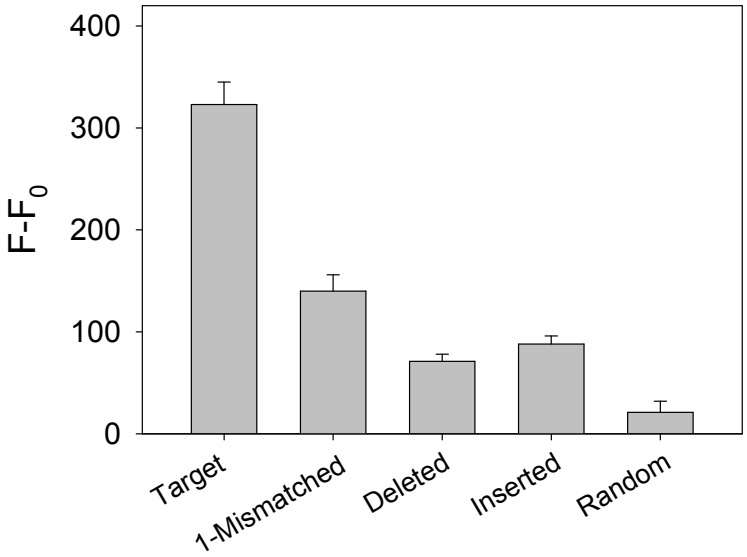

Fig. S3 Relative fluorescence intensity of FAM in the presence and absence of different analytes: target DNA, one-base mismatched (1-mismatched) DNA, deleted DNA, inserted DNA, and random DNA. The concentration of all analytes was 5 nM. F_0 is a background fluorescence signal in the absence of target and control sequences.

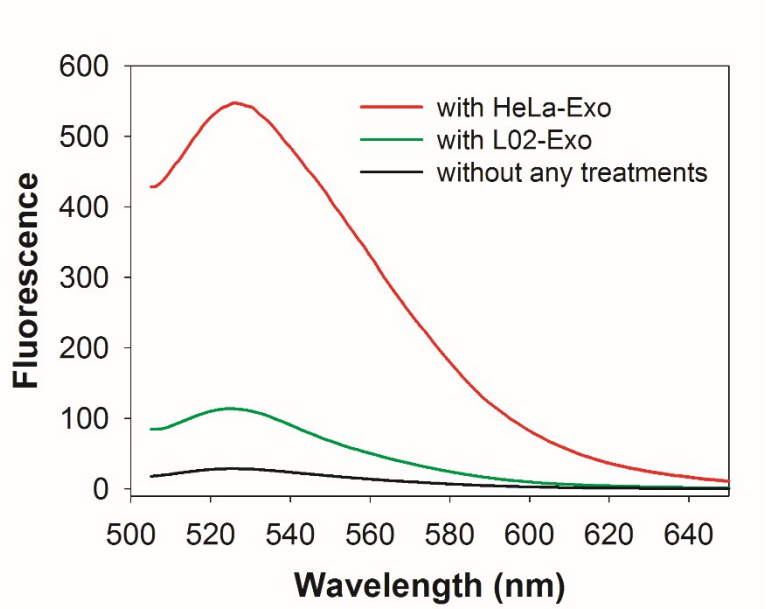

Fig. S4 Fluorescence spectra of the probes before and after adding the extracted solutions of HeLa-Exo or L02-Exo. The concentration of exosomes used is 10 nM.

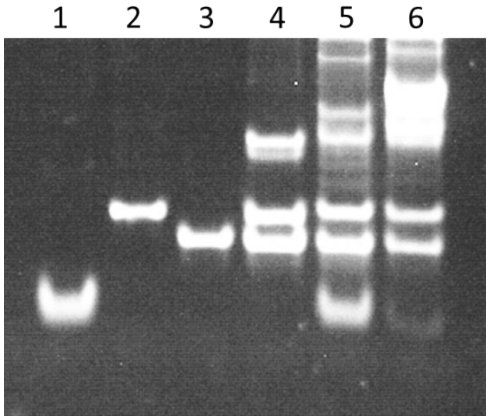

Fig. S5 Gel analysis: H0 (lane 1); H1 (lane 2); H2 (lane 3); H1, H2 with extracted solution of HeLa-Exo (lane 4); H0, H1, H2 with extracted solution from L02-Exo (lane 5); H0, H1, H2 with extracted solution of HeLa-Exo (lane 6).

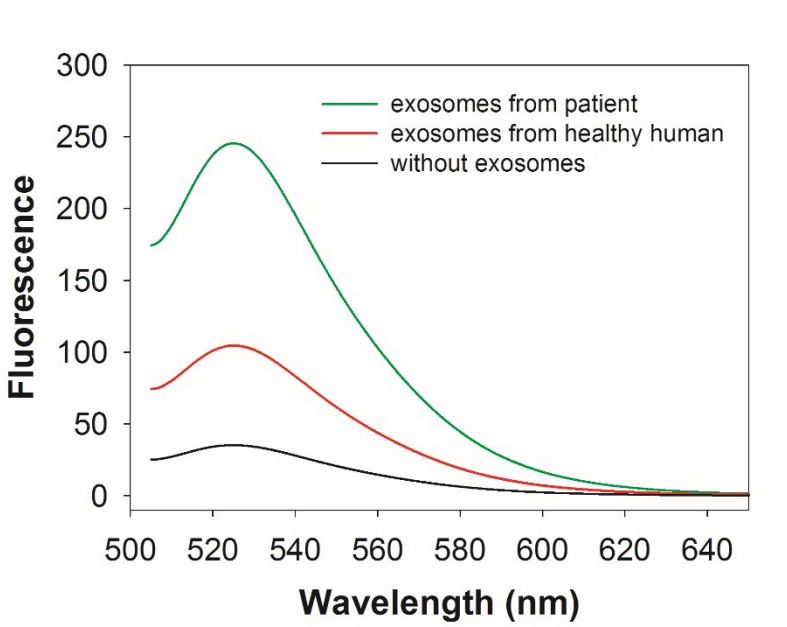

Fig. S6 Fluorescence spectra of the probes before and after adding the exosomes-extracted solution of a cervical cancer patient or a healthy donor.