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Supporting Information

Highly sensitive and selective miRNA detection based on closed ring probe and multiple signal amplification

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Experimental Section

Materials. All DNA was purchased from Sangon Biotech Shanghai Co. Ltd. All RNA was purchased from Shanghai GenePharma Co. Ltd. The reaction solution of miRNA was prepared by DEPC-treated water. Their concentrations were determined by measuring the absorbance at 260 nm in a 1 mL quartz cuvette with a Cary 60 UV-Vis Spectrophotometer on the basis of manufacturer's instructions (Agilent Technologies, USA). 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was obtained from Sangon Biotech Shanghai Co. Ltd. PFP was were kindly given by Prof. Shu Wang (Institute of Chemistry, Chinese Academy of Sciences, Beijing, China). Human breast cancer cells (MCF-7), human umbilical vein endothelial cells (HUVEC) and Human lung adenocarcinoma epithelial cells (A549) were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All other chemicals were of analytical grade and were used as received from manufacturer.

Cell culture. A549, MCF-7 cells and HUVEC were cultured in Dulbecco's modified Eagle's medium (DMEM, high glucose) containing 10% heat-inactivated fetal bovine serum (FBS, HyClone, Logan, UT, USA) with 1% penicillin/streptomycin. All cells were maintained under standard culture conditions of 37 °C and 5% CO_2. Cells were passed every 2-4 days.

Instruments. Fluorescence measurements were carried out in a 3 mL quartz cuvette at room

temperature using a Hitachi F-7000 fluorometer equipped with a xenon lamp excitation source. The spectra were measured in HEPES buffer solution (200 mM, pH 8.0) including 200 mM NaCl. Other fluorescence data were monitored using the TriStar LB 941 multimode microplate reader (Berthold, Germany). Water was purified using a Millipore filtration system (Millipore CoCP., Bedford, MA). A DYY-6C electrophores is power supply and a DYCZ-24D polyacrylamide gel electrophoresis cell (Beijing Liuyi Instrument Factory) were used in polyacrylamide gel electrophoresis (PAGE) analysis. The images were recorded by the Gel Doc EZ system (Bio-Rad, USA).

Preparation of sealed circular DNA (CD). CD was prepared according to our previously reported method, and the synthetic route of CD is shown in Figure S1. Briefly, molecular beacon like DNA with amine at both ends (AM) was first annealed at 80°C for 20 min and slowly cooled to room temperature to get the hairpin structure. AM in distilled water was reacted with para nitrophenol activated cyclooctyne (60 equiv.) dissolved in dimethyl sulphoxide (DMSO). After overnight incubation at room temperature, the residual cyclooctyne and DMSO were eliminated through dialysis in deionized water, and then the product (CMD) was purified by HPLC and confirmed by ESI mass spectrum (MW: 13284.8). CMD was mixed with 1, 3-diazidopropane in aqueous solution. After overnight incubation at 4 °C, the residual 1, 3-diazidopropane was removed through dialysis, and the successful preparation of CD was further confirmed by mass spectrum (MW: 13410.1).

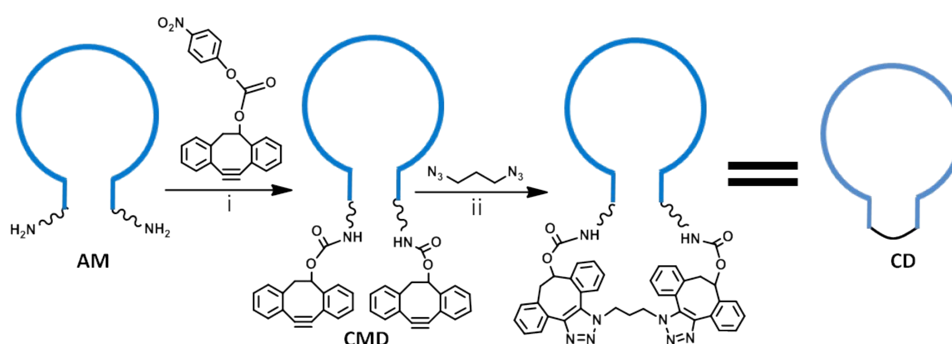

Figure S1. Synthetic routes of CD. (i) Et_3N, $DMSO/H_2O$, rt, 2h (ii) H_2O, rt, 1h

Detection of miRNA. let-7a was added to the mixture of CD-17ES and F-SS, after incubation with the presence of Pb2^{+}(50 μM) and Mg2^{+}(2 mM) at 37℃ for about 1.5 h, PFP was added, and the mixture was diluted with HEPES buffer solution (200 mM, pH 8.0, 200 mM NaCl) to a total volume of 1 mL (PFP=1 μM, CD-17ES=10 nM, F-SS=10 nM). The fluorescence spectra were measured with an excitation wavelength of 380 nm at room temperature. In the case of mismatched miRNA detection, the assays were performed under the same conditions as stated above.

Electrophoresis analysis of DNAzyme cleavage. The mixtures of CD-17ES and F-SS before and after adding let-7a in the presence of Pb^{2+} (50 μM) and Mg^{2+} (2 mM) were respectively loaded onto 16% polyacrylamide gel, and a photograph was taken with gel imaging system after electrophoresis at 190V for 1h. The direct cleavage product by 17ES and F-SS was used a positive control.

Extraction of total RNA from A549, MCF-7 and HUVEC Cells. Total RNA of HUVEC, A549, and MCF-7 were extracted using cell total RNA extraction kit (GenePharma, Shanghai) according to the manufacturer's protocol. The extracted total RNA was quantitated by measuring its optical density (OD) at 260 nm using a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, USA).

Detection of let-7a in cell lyastes. Total RNA (1 μg) of HUVEC, A549 and MCF-7 was respectively added to the mixture of CD-17ES and F-SS in the presence of Pb^{2+}(50 μM) and Mg^{2+}(2 mM) in a total volume of 10 μL, after incubation at 37°C for about 1.5h, the mixture was diluted 10 times, and 3 μL of this diluted solution was further diluted with HEPES buffer (200 mM, 200 mM NaCl) to a final volume of 100 μL containing PFP (0.1 μM), the fluorescence intensity of the three mixtures was then measured using the microplate reader separately.

Quantification of let-7a in Cells by Quantitative Real-Time PCR (qRT-PCR). The content of let-7a in HUVEC, A549 and MCF-7 was quantified by qRT-PCR. The miRNA expression level of let-7a was

measured using threshold cycle (Ct) which is the cycle number at which the fluorescence generated within a reaction crosses the threshold. The Ct values were then converted to absolute amount using a standard curve (Figure S3).

Fluorescence resonance energy transfer (FRET) spectra

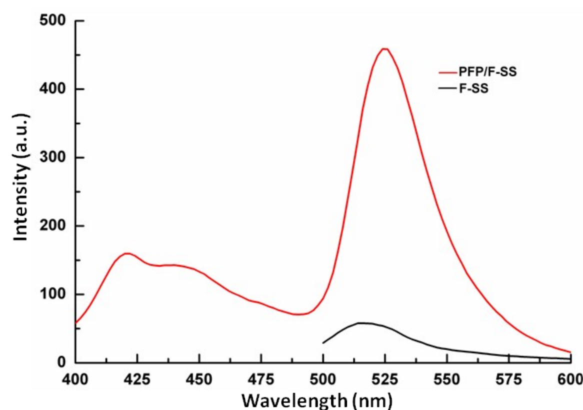

Figure S2. Emission spectra of F-SS before and after adding PFP in HEPES buffer solution (200 mM, pH = 8). [PFP] = 1.0 x 10^{-6} M in RUs, [DNA] = 1.0 x 10^{-8} M.

Probe and template sequences

Probe (**AM**): 5'-NH_2-GCGAGT TCAACATCAGTC TGATAAGCTATTTCGCAACTCGC-NH_2-3'
Enzyme strand (**17ES**): 5'-CATCTCTTCTCCGAGCCG GTCGAAATAGCTTATCAGACT-3'
Substrate strand (**F-SS**): 5'-FAM-CTCAT rAGGAAGAGATG-3'
Non complementary miRNA (**N-miRNA**): 5'- UAGCUUAUCAGACUGAUGUUGA-3'

Standard curve of let-7a measured by qRT-PCR.

(a)

Cell Type	Ct		
HUVEC	17.855	17.995	17.965
A549	18.613	18.571	18.274
MCF-7	18.505	18.319	18.219

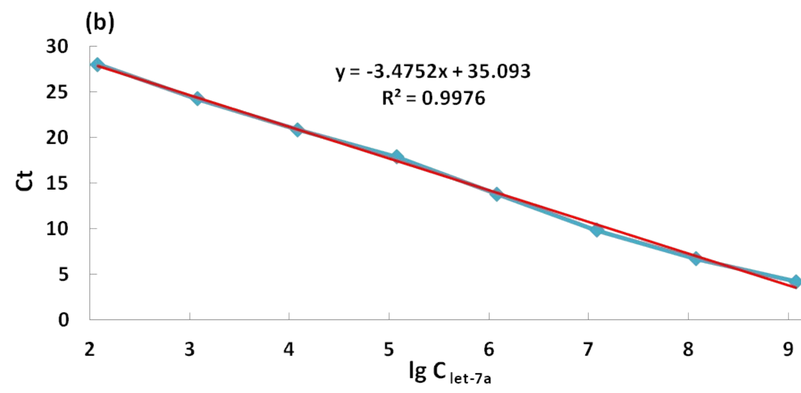

Figure S3. (a) Ct value of HUVEC, A549 and MCF-7 cells. (b) Correlation of let-7a with the threshold cycle values was determined by qRT-PCR assay.