Electronic Supplementary Information

An ATP-Fueled Nucleic Acid Signal Amplification Strategy for Highly Sensitive MicroRNAs Detection

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Table of Contents

Experimental Section

Chemicals and Materials. HCl, NaCl and $MgSO_4$ were obtained from Sinopharm Chemical Reagents, Co., Ltd. (Shanghai, China). Tris was purchased from Sigma (St. Louis, MO, USA). TE buffer (pH = 8.0) were prepared by 100 mM Tris, 100 mM HCl, 50 mM NaCl and 50 mM $MgSO_4$ standard stock solutions. Adenosine triphosphate (ATP), cytidine triphosphate (CTP), uridine triphosphate (UTP) and guanosine triphosphate (GTP) were purchased from Worthington Biochemicals (Lakewood, NJ, USA). Phosphate buffer saline (PBS), Dulbecco's modified eagle's medium (DMEM) and other cell culture products were purchased from Dingguo Biological Technology Co., Ltd. (Chongqing, China). The cervical cancer Hela cells and human breast cancer MCF-7 cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). All the other chemicals were of analytical grade and used without further purification. Ultrapure water with a resistivity of 18.2 MΩ/cm was used throughout this study.

All oligonucleotides were custom-synthesized by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). The sequences information was listed as Table S1. Before using, the hairpin nucleotides (H_1, H_2, H_3 and H_4) were heated to 95 °C for 2 min and then cooled to room temperature to form stem-loop structure.

Table S1. Oligonucleotide sequences.

Names	Sequences (5'-3')
MicroRNA-21	UAG CUU AUC AGA CUG AUG UUG A
aptamer	TAG CTT ATC CAA GCT ACT TAC CTG GGG GAG TAT TGC GGA

	GGA AGG TTC AAC ATC AGT CTG ATA AGC TA
assist-1	TAG CTT ATC AGA CTG CAC TCG TCC CT
assist-2	AGT CTG ATA CCT TCC TCC GCA ATA CTC CCC
H_1	AGG GAC GAG TGC AGT CTG ATA AGC TAG GGG AGT ATT GCG GAG GAA AGT ATC AGA CTG CAC TCG TCC CTA ACA TCT CAA GC
H_2	GCT TGA GAT GTT AGG GAC GAG TGC GCA CTC CAC AAG GCA CTC GTC CCT-FAM-AAC ATC CAC TGA GTG C
H_3	AGG GAC GAG TGC CTT GTG GAG TGC AAC ATC TCA AGC GCA CTC CAC AAG GCA CTC AGT GGA TGT T-TAMRA
H_4	CTT GTG GAG TGC GCT TGA GAT GTT GCA CTC GTC CCT AAC ATC TCA AGC GCA CTC AGT GGA TGT T-TAMRA
MicroRNA-141	UAA CAC UGU CUG GUA AAG AUG G
MicroRNA-155	UUA AUG CUA AUC GUG AUA GGG GU

Apparatus. Fluorescence was recorded on a F-7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). Gel Doc XR$^+$ System (Bio-Rad, California, USA) was used to take images of polyacrylamide gels.

Fluorescence Experiments. To study the relationship between target concentrations and corresponding signal intensities, fluorescence experiments were carried out on a F-7000 fluorescence spectrophotometer. All DNA probes used in this work were dissolved to final concentration of 100 mM by TE buffer, and stored at -20 °C for later use. Following, 200 nM DNA probe (aptamer, assist-1, assist-2) and 800 nM hairpin probe (H_1, H_2, H_3, H_4) were reacted with different concentrations of the targets for 4 h at 37 °C. After the reaction, the fluorescence was monitored at appropriate excitation wavelengths, the excitation and emission wavelength are 496 nm and 515 to 595 nm, respectively. In all fluorescence experiments, the slit widths of the both the excitation and emission were set as 5 nm, and the PMT voltage was set as 950 V.

Native polyacrylamide gel electrophoresis (PAGE). Firstly, 10 μL of each sample

were mixed with 2 μL of 6 × loading buffer. And then, 10 μL of mixture was transferred into the gel electrophoresis system, respectively. Electrophoresis was performed in 1 × TBE (pH 8.0) at a 120 V constant voltage for 180 min. The gels were then stained with ethidium bromide for 20 min, followed by photographing with the Gel Doc XR^+ System.

Cell Culture. The cervical cancer Hela cells and human breast cancer MCF-7 cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Hela and MCF-7 cells were grown in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum, 1% non-essential amino acids and 10 mg mL^{-1} insulin. All of the cells maintained in a humidified atmosphere of 5% CO_2 at 37 °C.

Optimization of ATP Concentration

In order to quantitative determination of miRNA-21 *in vitro*, the concentration of ATP used as fuel in the proposed fluorescent assay should be optimized. Figure S1 revealed that when the concentration of miRNA-21 was constant, the FRET ratio was finely controlled by varying the concentration of ATP. Specifically, the FRET ratio was efficiently enhanced with the ATP concentration in the range from 0.01 mM to 0.25 mM. However, the FRET ratio was decreased obviously with 0.5 mM ATP as fuel. Thus, considering the effects of ATP concentration, 0.25 mM ATP was used as fuel in the detection system to achieve optimized condition for miRNA-21 quantitative determination.

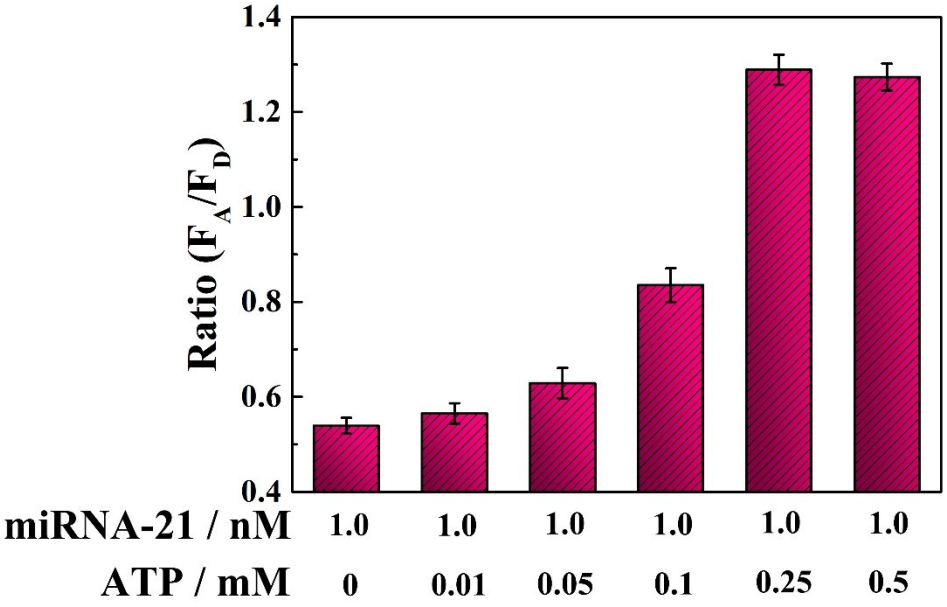

Figure S1. Effects of the ATP concentration on the FRET ratio. F_A and F_D was fluorescence intensity of acceptor and donor, respectively.

The qRT-PCR of miRNA expression

The concentration of miRNA-21 in MCF-7 lysates calculated by with RT-PCR method and this method, and the results was shown in Figure. R3. The results received using the our method were in good agreement with the qRT-PCR method. These results demonstrate that the proposed method provided an efficient and promising alternative tool for determining miRNA-21 in clinic analysis.

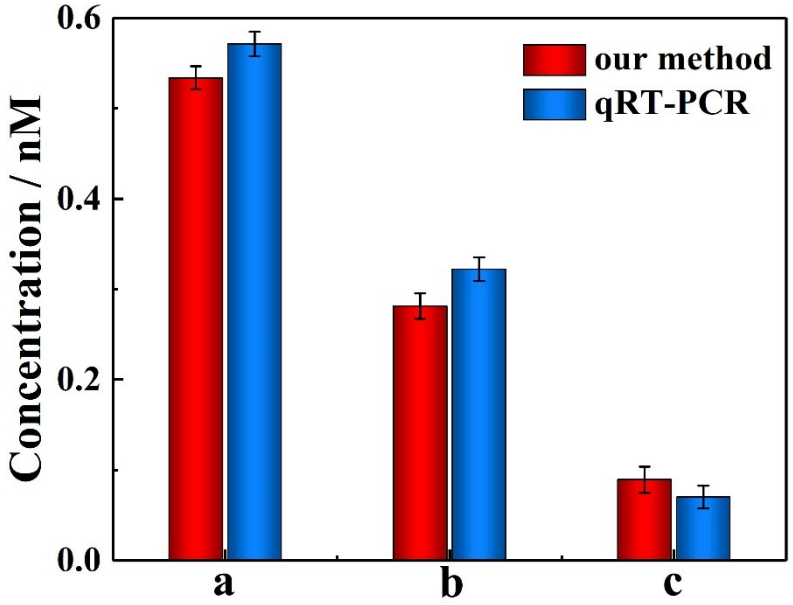

Figure S2. The comparison of the concentration of miR-21 ((a) 100×10^4 cells, (b) 50×10^4 cells, (c) 20×10^4 cells) in MCF-7 lysates calculated by qRT-PCR method and our method.

Table S2. Comparison of the proposed fluorescent assay with other detection methods.

Target	Detection method	Limit of Detection	Linear range	Ref.
miRNA-21	Electrochemistry	0.04 nM	0.14-10 nM	1
miRNA-21	Fluorescence	0.3 nM	1-5000 nM	2
miRNA-21	Fluorescence	3 nM	10-100 nM	3
miRNA-21	Fluorescence	0.68 nM	2-60 nM	4
miRNA-21	Fluorescence	47 pM	0.1-16 nM	5
miRNA-21	Fluorescence	18pM	0.05-10 nM	This work

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