Electronic Supplementary Information (ESI)

Label-free fluorescence detection of microRNA based on target induced adenosine$_2$-coralyne-adenosine$_2$ formation

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Experimental Section

Materials and chemicals

The DNA oligonucleotides used in this study (sequences shown in Table S1) were synthesized and purified through HPLC by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). RNase-free H_2O and RNase inhibitor were obtained from Takara Biotechnology Co., Ltd. (Dalian, China). Coralyne chloride hydrate (coralyne) was obtained from Sigma-Aldrich (St. Louis, MO). SYBR Green I (10,000× stock solution in dimethyl sulfoxide) was obtained from Life Technologies Corporation (USA). Streptavidin magnetic beads (SA-MB) and poly(A) polymerase were purchased from the New England Biolabs Ltd. (Beijing, China). ATP and SSC buffer (20×, 3 M NaCl, 300 mM Sodium citrate, pH 7.0) were obtained from Sangon Biotech (Shanghai, China) Co., Ltd. (Shanghai, China). All solutions used in the assay were prepared using RNase-free H_2O.

Procedures of miRNAs detection

The assay procedures can be briefly described as follows. Firstly, reaction mixture containing different concentrations of target miRNA, together with 1 U/μL of RNase inhibitor, 0.05 U/μL of poly(A) polymerase, 1 mM ATP, and 1× poly(A) buffer (50 mM Tris-HCl, 250 mM NaCl, 10 mM $MgCl_2$, pH 7.9) was incubated at 37 ℃ for 2 h. Following the addition of 2 μL of 2 μM biotin-DNA together with SYBR Green I and coralyne, 20 μL 10× SSC and 5 μL 4 mg/mL SA-MB (streptavidin magnetic beads) was added and incubated at room temperature for 30 min. After rinsed with 1× SSC for three times, the beads were directly subjected to for fluorescence assay.

Table S1. Sequences of Oligonucleotides

Name	Sequence (5′-3′)
DNA probe	TCAACATCAGTCTGATAAGCTATTTTTTTTTT-biotin
MiRNA-21	UAGC UUAU CAGA CUGA UGUU GA
Let-7a	UGA GGU AGU AGG UUG UAU AGU U
MiRNA-141	UAA CAC UGU CUG GUA AAG AUG G
MiRNA-143	UGA GAU GAA GCA CUG UAG CUC A

Table S2 Comparison of this method and other assays

Methods	Detection limit	Analytical Time	Specificity	Complexity level	Ref.
Label-free SERS	28 nM	overnight	Not reported	High complexity including synthesis and careful treatment of silver nanorod	1
Silver nanocluster DNA Probe	20 nM	~1.5 h	High specificity toward detecting specific miRNA sequences is possible	High complexity including synthesis of silver nanocluster	2
Multiplexed detection using cationic polythiophene	1.26 nM	~4.5 h	Good specificity for the use of inherently encoded nanorods	High complexity including synthesis of metallic striped nanorods	3
Colorimetric detection	16 pM	~4 h	Good selectivity due to the high specificity of the duplex-specific nuclease to perfectly matched duplexes	High complexity including preparation of oligonucleotide-functionalized AuNPs	4
label-free assay based on fluorescence quenching of AuNPs	3.8 pM	~7.5 h	High specificity of discriminating single-nucleotide differences	High complexity including preparation of AuNPs and immobilization of probe DNA	5
simple molecular beacon	3.8 pM	~2 h	Good specificity for the intrinsic property of duplex-specific nuclease	High complexity including dual labeling and high cost	6
microRNA-activated molecular machine	80 fM	~3.5 h	High selectivity for the base-mismatch discrimination capability of the toehold strand displacement reaction	High complexity including dual labeling and the design of three-strand DNA complex probes	7
Direct detection by using the p19 protein-functionalized magnetic beads (PFMBs)	1 fM	~9 h	High selectivity due to the low nonspecific adsorption of PFMBs	High complexity including the design of multiple probes	8
Electrochemiluminescent biosensor	0.3 fM	~5.5 h	High specificity induced by the dual signal amplification	High complexity including electrode modification and Rolling Circle Amplifications reaction	9
Enzymeless electrochemical signal amplification	0.1 fM	~0.5 h	Good selectivity resulting from the distinct thermodynamic properties in the triple-stem structure	High complexity including preparation of the electrode and DNA monolayer assembly	10
Label-free fluorescence detection	0.5 nM	~2.5 h	Improved specificity because of poly(A) polymerase reaction	Low complexity with simple design of probe, label-free detection and easy operation, low cost	This study

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Fig. S1. Fluorescence responses of the system at different concentrations of poly(A) polymerase. F and F_0 were fluorescence intensity in the presence or absence of target miRNA, respectively.

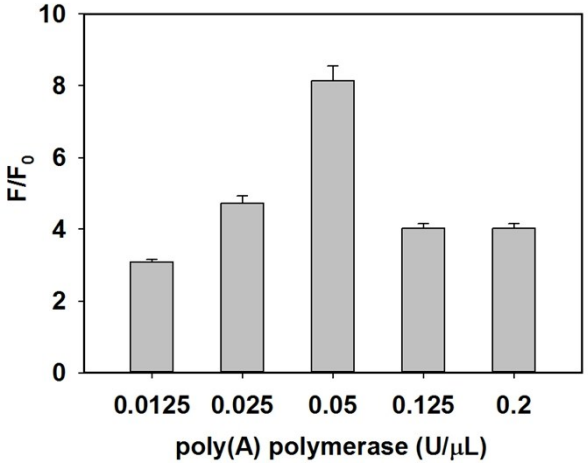

Fig. S2. Fluorescence responses of the system at different concentrations of coralyne. F and F_0 were fluorescence intensity in the presence or absence of target miRNA, respectively.

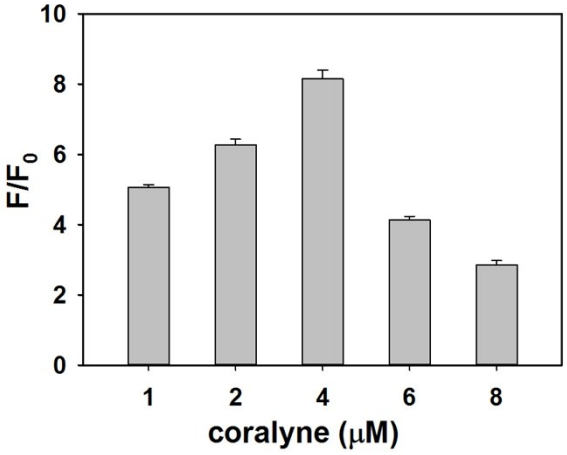

Fig. S3 Fluorescence responses of the system at different concentrations of ATP. F and F_0 were fluorescence intensity in the presence or absence of target miRNA, respectively.

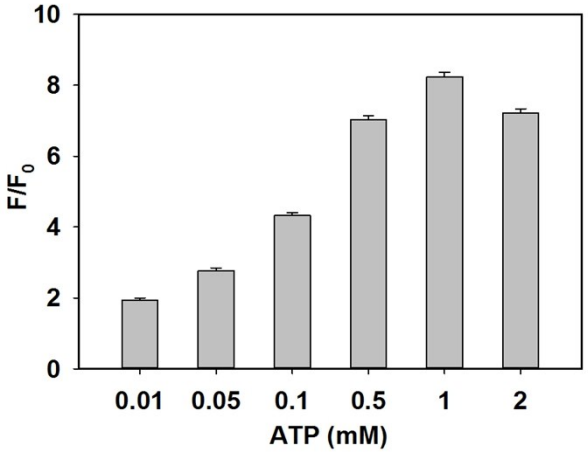

Fig. S4 Fluorescence responses of the system at different concentrations of biotin-DNA probe. F and F_0 were fluorescence intensity in the presence or absence of target miRNA, respectively.

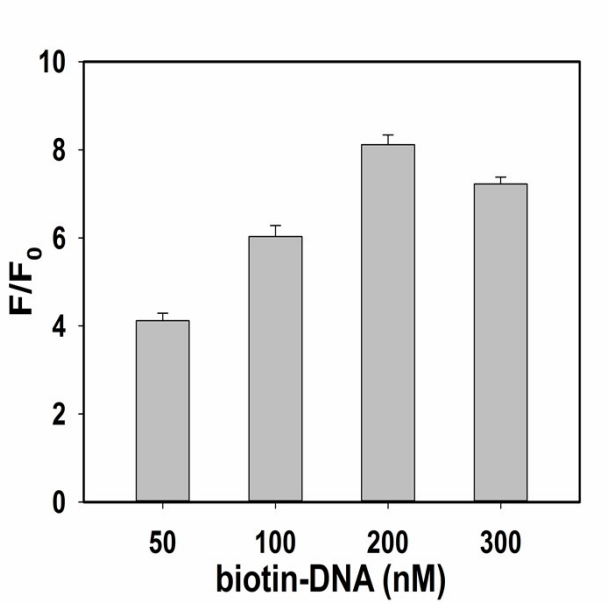

Fig. S5 Fluorescence responses of the system at different concentrations of SA-MB. F and F_0 were fluorescence intensity in the presence or absence of target miRNA, respectively.

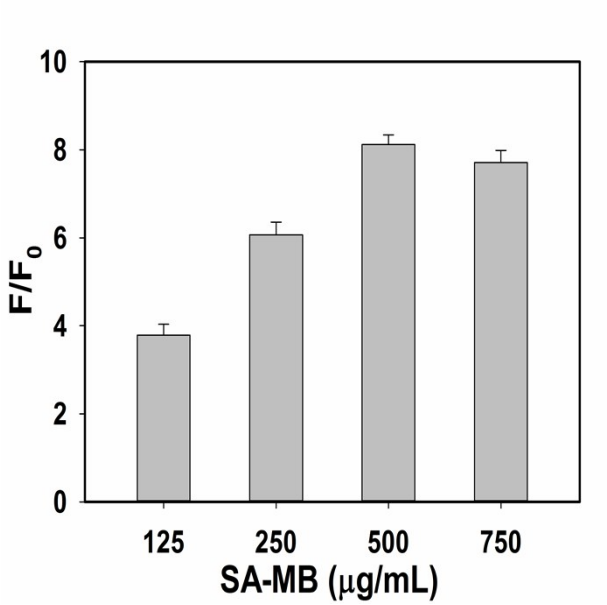

Fig. S6 Fluorescence responses of the system at different concentrations of SYBR Green I (SG). F and F_0 were fluorescence intensity in the presence or absence of target miRNA, respectively.

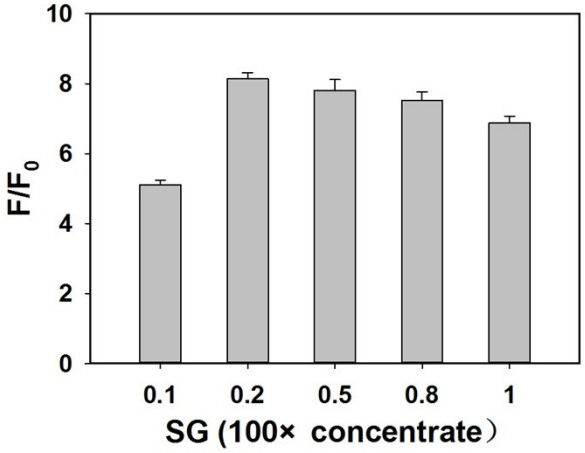

Fig. S7 Fluorescence responses of the system at different time. F and F_0 were fluorescence intensity in the presence or absence of target miRNA, respectively.

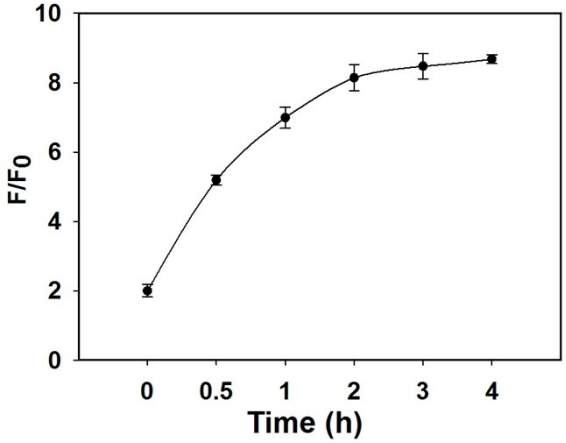

Table S3. Recovery experiments of miR-21 in 10% human sera samples

Sample	Added miR-21 (nM)	Detected miR-21 (nM)	Recovery (%)
human sera sample	0	0.0254 ± 0.0522	Not detectable
	20	18.827 ± 0.2397	94.0
	50	48.606 ± 0.9514	97.2
	100	93.700 ± 5.4329	93.7

Average of three determinations ± standard deviation