Electronic Supplementary Material (ESI) for RSC Advances

SUPPORTING INFORMATION

A Novel Fluorescence Probe of dsDNA-Templated Copper Nanoclusters for Quantitative Detection of MicroRNAs

Xin-Ping Wang, Bin-Cheng Yin, and Bang-Ce Ye

Reagents and Materials. HPLC-purified miRNAs were purchased from Takara Biotechnology Co. Ltd. (Dalian, China). The oligonucleotide probes were synthesized and purified by Sangon Inc. (Shanghai, China). The sequences of these miRNAs and probes are listed in Table S1. The nicking endonuclease (*Nt.Bst.NBI*) and *VentR*® *(exo-)* DNA polymerase were purchased from New England Biolabs (Beverly, MA, USA). The L-Ascorbic acid was purchased from Sinopharm Chemical Reagent Co.,Ltd. (Shanghai, China). In order to create and maintain an RNase-free environment, the solutions were treated with 0.1% DEPC and autoclaved. The tips and tubes are RNase-free and do not require pretreatment to inactivate RNases. The buffers were prepared using distilled water purified by a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA) with an electrical resistance of 18.2 MΩ·cm.

Table S1. Sequences of miRNAs targets and probes used in this work.

Name	Sequences (5′-3′)*
T	CGCTCATACGTTCATTCTTGACTCTCATCATTACCAGGCAGTATTA TCTTGACTCTCATCATTACCAGGCAGTATTA-Pi
miR-200b	UAAUACUGCCUGGUAAUGAUGA
miR-141	UAACACUGUCUGGUAAAGAUGG
miR-200a	UAACACUGUCUGGUAACGAUGU
miR-200c	UAAUACUGCCGGGUAAUGAUGGA
miR-429	UAAUACUGUCUGGUAAAACCGU
DNA1	AGTTGCAAGAAGATGACAGAGAAGT
DNA2	ACTTCTCTGTCATCTTCTTGCAACT
DNA3	ATGAACGTATGAGCG
DNA4	CGCTCATACGTTCAT
DNA5	ATGAACGTATGAGC
DNA6	GCTCATACGTTCAT
DNA7	GTTCATCACG
DNA8	CGTGATGAAC

* The mutation bases are indicated in underlined portion.

Instrumentation. Photoluminescence spectroscopy was performed in a fluorescence microplate reader (Bio-Tek Instrument, Winooski, USA) using a black 384 well microplate (Fluotrac 200, Greiner, Germany). The isothermal amplification reaction was performed using a MJ Mini^{TM} personal Thermal Cycler (Bio-Rad, CA, USA).

Target-Triggered Isothermal Exponential Amplification Reaction. The target-triggered isothermal exponential amplification reaction (TIEAR) was carried out as follows. First, a mixture containing 3 μL AT (1 μM), 0.5 μL NEB buffer (500 mM Tris-HCl, pH 7.9, 1 M NaCl, 100 mM $MgCl_2$, 10 mM Dithiothreitol), 1 μL dNTPs mixture (2.5 mM), and 1 μL miRNA target was incubated at 88°C in the PCR amplifier for 10 min and then chilled on ice rapidly. Subsequently, 0.5 μL Thermopol buffer (200 mM Tris-HCl, pH 8.8, 100 mM KCl, 100 mM $NH_4(SO_4)_2$, 20 mM

$MgSO_4$, 1% Triton X-100), 3 μL ddH_2O, 0.5 μL *Nt.Bst.NBI* (10 U μL^{-1}) and 0.5 μL *Vent*$_R$®*(exo*$^-$*)* polymerase (2 U μL^{-1}) were added into the above mixture to yield a total volume of 10 μL. After incubating at 55°C for 40 min, the mixture was heated at 90°C for 10 min to inactivate the enzymes.

Preparation of DNA-Templated Copper Nanoclusters and Fluorescence Measurements. Following amplification reaction, the solution of TIEAR was added 10 μL **DNA4** solution (10 μM), 45 μL ddH_2O, and 20 μL hybridization buffer (20 mM MOPS, 300 mM $NaNO_3$, 2 mM $Mg(NO_3)_2$), and then the solution was heated to 90°C for 10 min and allowed to cool naturally to room temperature for 1.5 hours. After that, the solution were added 5 μL $CuSO_4$ solution (1 mM) and 10 μL freshly prepared L-Ascorbic acid (20 mM), and vigorous shaken for 3 min. The as-prepared solution was scanned with fluorescence microplate reader from 360 nm to 800 nm (excited at 340 nm).

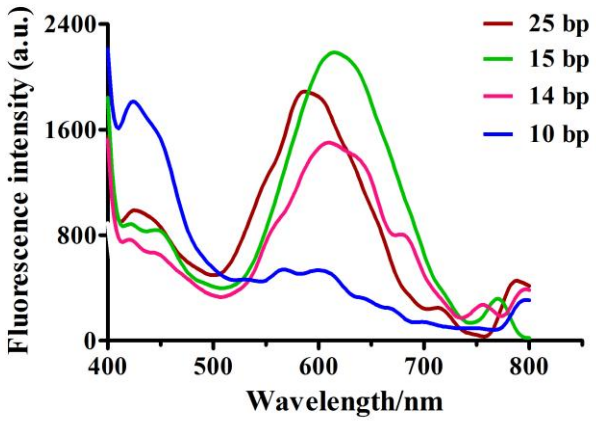

Figure S1. Fluorescence emission spectra of the synthesized dsDNA/CuNCs using four different pairs of DNA duplex with different lengths (25-bp **DNA1**/**DNA2**, 15-bp **DNA3**/**DNA4**, 14-bp **DNA5**/**DNA6**, 10-bp **DNA7**/**DNA8**).

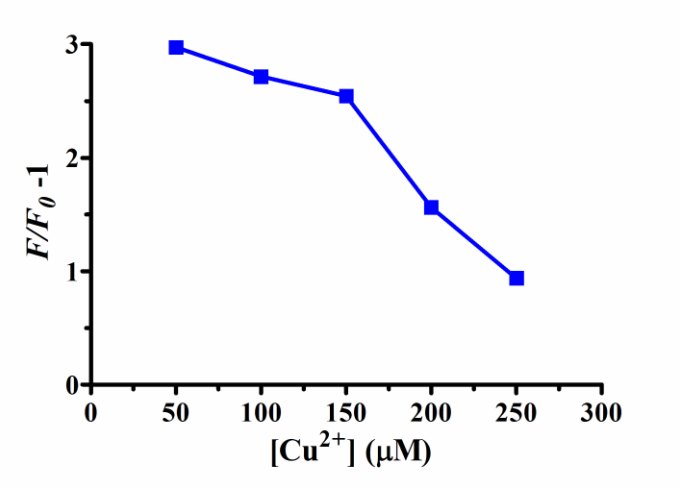

Figure S2. The influence of copper ion concentration. Five copper concentrations were tested: 50 μM, 100 μM, 150 μM, 200 μM, and 250 μM.

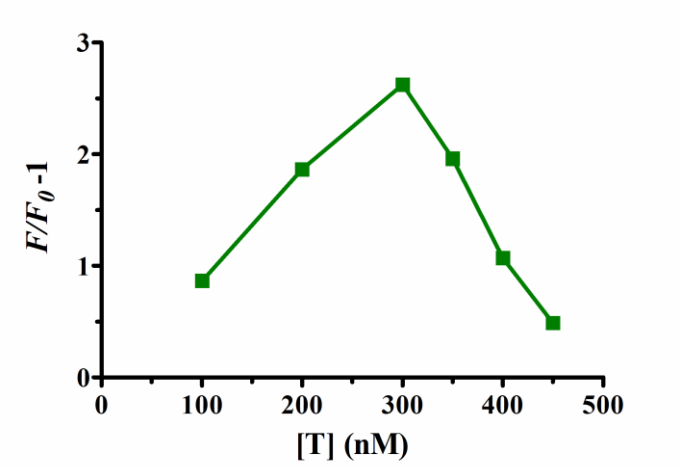

Figure S3. The influence of amplification template concentration. Six concentrations were tested: 100 nM, 200 nM, 300 nM, 350 nM, 400 nM, and 450 nM.

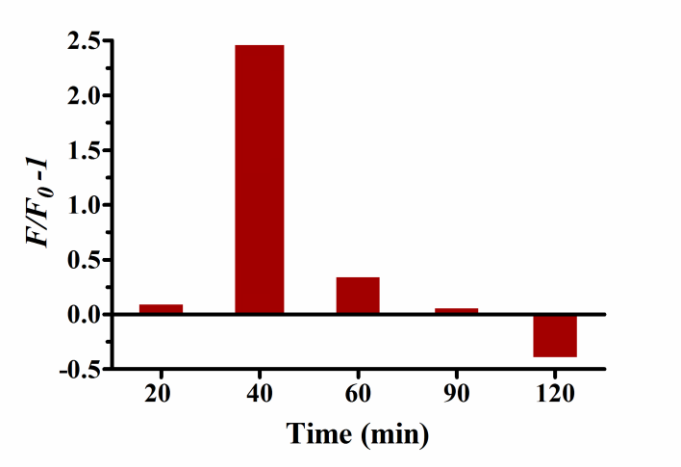

Figure S4. The influence of the time of isothermal amplification reaction. Five times were tested: 20 min, 40 min, 60 min, 90 min, and 120 min.