Supporting Information for

Fluorescence resonance energy transfer using DNA-templated copper nanoparticles for ratiometric detection of microRNA

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Materials and methods

Materials

The oligonucleotides used in this study (shown in Table S1) were purchased from Integrated DNA Technologies (Skokie, IL, USA) and Bionics (Seoul, Korea). Sodium chloride (NaCl) and sodium ascorbate were purchased from Samchun Chemical (Seoul, Korea). Copper sulfate ($CuSO_4$) and 3-(N-morpholino) propanesulfonic acid (MOPS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Moloney murine leukemia virus (M-MLV) reverse transcriptase and deoxynucleoside triphosphates (dNTPs) were purchased from Enzynomics (Daejeon, Korea). Taq DNA polymerase was purchased from iNtRON Biotechnology (Seongnam, Korea). Greenstar, deionized water, and diethylpyrocarbonate-treated water were purchased from Bioneer (Daejeon, Korea). All chemicals of analytical grade were used without further purification.

Detection procedure using CuNP-based FRET

The reaction solutions composed of 200 nM CuNP probe, 200 nM Cy5 probe, and target (MIR-21, 5–20 nucleotide probes, or various types of miRNA; Table S1) at different concentrations in MOPS buffer (20 mM MOPS, 160 mM NaCl, pH 8.0) were first incubated for 5 min at room temperature. MIR-21 is a single stranded DNA oligonucleotide, whose sequence is identical to that of the miRNA molecule, miR-21, except that uracil is substituted with thymine. Next, 2 mM sodium ascorbate and 460 μM $CuSO_4$ were added sequentially. The fluorescence signals of the prepared samples were measured at an excitation wavelength of 340 nm using a microplate reader (Spectramax iD5; Molecular Devices, Sunnyvale, CA, USA).

FRET efficiency (E) and FRET ratio (R) were calculated according to the following equation.

$$E(\%) = \left(1 - \frac{emission\ of\ CuNP\ after\ FRET}{emission\ of\ CuNP\ before\ FRET}\right) \times 100\%$$

$$R = \frac{emission\ of\ Cy5\ at\ 660\ nm}{emission\ of\ CuNP\ at\ 620\ nm}$$

Reverse transcription and asymmetric PCR

First, cDNA was synthesized by incubating miR-21 at various concentrations in the reverse transcription reaction solutions (10 μL) composed of 250 μM of each of the four dNTPs, 4 U/μL M-MLV reverse transcriptase, 0.4 U/μL RNAse inhibitor, 100 nM hairpin primer, 50 mM Tris-HCl (pH 8.3), 3 mM $MgCl_2$, 10 mM dithiotreitol, and 75 mM KCl at 16 °C for 30 min, 42 °C for 60 min, and 85 °C for 5 min. Next, asymmetric PCR was performed. The asymmetric PCR solutions (20 μL) composed of 250 μM each of dNTPs, 0.025 U/μL M-MLV Taq DNA polymerase, 1 μM forward primer, 50 nM reverse primer, 2 μL cDNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 2 mM $MgCl_2$ were incubated at 95 °C for 30 s and then subjected to 37 temperature cycles (95 °C for 10 s, 54 °C for 15 s, and 72 °C for 20 s). The resulting PCR products (10 μL) were analyzed in a similar manner to that described in the previous section, except that the concentration of sodium ascorbate used was 4 mM.

Quantitative reverse transcription PCR

cDNA was prepared in the same manner as described in the previous section. Next, 1 μL cDNA was similarly subjected to qRT-PCR, except that 1X SYBR green I was additionally used. The PCR was programmed for incubation at 95 °C for 30 s and subsequently subjected to 37 temperature cycles (95 °C for 10 s, 54 °C for 15 s, and 72 °C for 20 s). The fluorescence signal of SYBR green during PCR amplification was measured on a real-time PCR detection system (CFX Connect, Bio-Rad, Hercules, CA, USA).

Table S1. Sequences of DNA/RNA oligonucleotides. DNA sequences are written in uppercase letters, and RNA sequences are written in lowercase letters.

Name	Sequence (5′ → 3′)	Length
CuNP probe	TCAACATCAGTATATATATATATATATATTTTTTATA TATATATATATATAT	52
Cy5 probe	Cy5-CTGATAAGCTA	11
MIR-21	TAGCTTATCAGACTGATGTTGA	22
5 nt probe*	TAGCTTATCAG*CCCCC*ACTGATGTTGA	27
10 nt probe*	TAGCTTATCAG*CCCCCCCCCC*ACTGATGTTGA	32
15 nt probe*	TAGCTTATCAG*CCCCCCCCCCCCCCC*ACTGATGTTGA	37
20 nt probe*	TAGCTTATCAG*CCCCCCCCCCCCCCCCCCCC*ACTGAT GTTGA	42
Hairpin primer	GTCGTATCCACACGTCCCAGGCTCCAATTCCGTGTG GATACGACTCAACA	50
Forward primer	TCGCCTAGCTTATCAGACTGA	21
Reverse primer	CACGTCCCAGGCTCCA	16
miR-21	uagcuuaucagacugauguuga	22
miR-122	uggagugugacaaugguguuug	22
miR-141	caucuuccaguacaguguugga	22
miR-155	uuaaugcuaaucgugauagggguu	24
miR-200a	caucuuaccggacagugcugga	22
miR-200b	caucuuacugggcagcauugga	22
miR-200c	cgucuuacccagcaguguuugg	22
miR-375	gcgacgagccccucgcacaaacc	23
miR-429	uaauacugucugguaaaaccgu	22

*nt represents nucleotides. Italicized letters show the part of the probe that is variable in length, which alters the distance between CuNP and Cy5 when they are hybridized to the target.

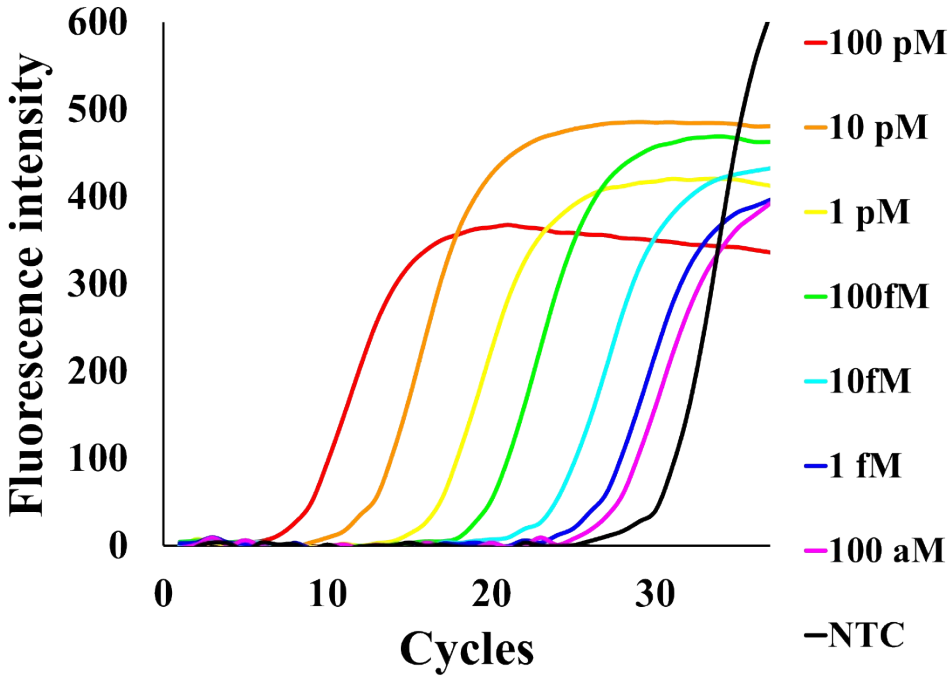

Figure S1. Amplification plot of qRT-PCR at different concentrations of miR-21.

Table S2. Difference between CuNP and quantum dots.

	CuNP probe	**Quantum dot probe**
Toxic chemicals	Copper (Guideline value = 2.0 mg/L) [S1]	Cadmium (Guideline value = 0.003 mg/L) [S1] Lead (Guideline value = 0.01 mg/L) [S1]
Synthesis condition	At room temperature and atmospheric pressure	At high temperature or high pressure[S2]
Synthesis time	Short	Long[S2]
Functional group on DNA to make probe	No need	Need DNA modification[S3]
Functional group on nanomaterial to make probe	No need	Need functionalization of quantum dot[S3]
Additional purification process	No need	Electrophoresis, ultracentrifugation or chromatographic separation[S3]

S1. World Health Organization, Guidelines for Drinking-water Quality, World Health Organization, Geneva, 4th edn, 2011

S2. O. Carion, B. Mahler, T. Pons and B. Dubertret, *Nat. Protoc.*, 2007, **2**, 2383-2390.

S3. A. Banerjee, T. Pons, N. Lequeux and B. Dubertret, *Interface Focus*, 2016, **6**, 20160064.