Electronic Supplementary Material (ESI) for ChemComm.
This journal is © The Royal Society of Chemistry 2014

Electronic Supplementary Information

Rapid detection of microRNA based on p19-enhanced fluorescence polarization

Yuan-Chen He, Bin-Cheng Yin,* Lihua Jiang, and Bang-Ce Ye*

1. Experiment section

Chemicals and materials: All chemicals used in this work were purchased from commercial sources and directly used without additional purification. The p19 protein was purchased from New England BioLabs, Inc. (Nebraska, America). RNase Inhibitor was purchased from Thermo Fisher Scientific Co., Ltd. HPLC-purified miRNAs, RNA probes, DNA probe and diethylpyrocarbonate (DEPC)-treated water were purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China). All solutions were made using Milli-Q water and filtered through a 0.22 μm filter. 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 miRNA sequences were selected from the Sanger Center miRBase (http://microrna.sanger.ac.uk/sequences). The buffers used in cellular extracts were prepared as follows. Buffer A was 10 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 10 mM KCl and 0.5 mM DTT. Buffer B was 20 mM HEPES (pH 7.9), 25% (v/v) glycerol, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and 0.5 mM DTT. Buffer C was 20 mM HEPES (pH 7.9), 20% (v/v) glycerol, 0.1 M KCl, 0.2 mM EDTA, 0.5 mM PMSF, and 0.5 mM DTT.

Table S1. The sequence information of miRNAs, RNA probes, and DNA probe used in this work.

Name	Sequence (5′-3′)
Probe-1	FITC-ACACCAUUGUCACACUCCAUAU
Probe-2	FITC-ACACCATTGTCACACTCCATAT
Probe-3	FITC-AACACCAUUGUCACACUCCAUA
Probe-4	FITC-CAAACACCAUUGUCACACUCCA
miR-122	UGGAGUGUGACAAUGGUGUUUG
DNA-122	TGGAGTGTGACAATGGTGTTTG
miR-221	ACCUGGCAUACAAUGUAGAUUU
miR-223	UGUCAGUUUGUCAAAUACCCCA
miR-21	UAGCUUAUCAGACUGAUGUUGA
let-7a	UGAGGUAGUAGGUUGUAUAGUU
let-7b	UGAGGUAGUAGGUUGUGUGGUU
let-7c	UGAGGUAGUAGGUUGUAUGGUU
let-7d	AGAGGUAGUAGGUUGCAUAGUU
let-7e	UGAGGUAGGAGGUUGUAUAGU
let-7f	UGAGGUAGUAGAUUGUAUAGUU
let-7g	UGAGGUAGUAGUUUGUACAGU

let-7i	UGAGGUAGUAGUUUGUGCUGUU
miR-141	UAACACUGUCUGGUAAAGAUGG
miR-429	UAAUACUGUCUGGUAAAACCGU
miR-200a	UAACACUGUCUGGUAACGAUGU
miR-200b	UAAUACUGCCUGGUAAUGAUGA
miR-200c	UAAUACUGCCGGGUAAUGAUGGA

Fluorescence polarization measurement: The reaction mixture was prepared by adding dye-labeled RNA probe, miRNA target, p19 protein, and 0.8 U/μL RNase Inhibitor together in a reaction buffer with a volume of 80 μL. Then the mixture was kept at 25°C for 5 min and transferred to a black 96 well half area microplate (Fluotrac 200, Greiner, Germany). Readout of fluorescence polarization was carried out by a fluorescence microplate reader (Bio-Tek Instrument, Winooski, USA) with an excitation wavelength at 485 nm and an emission wavelength at 528 nm. Unless noted otherwise, all experiments in this work were repeated three times.

Cell culture. Human hepatocellular carcinoma cell lines (HepG2) was obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). HepG2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 100 U/mL penicillin-streptomycin at 37ºC in a humidified 5% CO_2 incubator, respectively. The cells were grown with fresh medium at 50 mm glass-bottom dishes. Then the cells were harvested by trypsinization and washed with fresh medium for three times and suspended in fresh medium for following studies.

Preparation of cellular extracts. The cellular extracts were prepared according to the reported method with minor modification.[1] Briefly, 10^7 cells were washed once with phosphate-buffered saline and twice with Buffer A. The cell pellet was suspended in Buffer B/0.1% Nonidet P-40 (20 μL per 10^7 cells). After incubating for 15 min on ice, the lyzed cellular suspension was briefly mixed on a vortex and centrifugated for 10 min at 4 ºC. Then supernatant was diluted with 80 μL per 10^7 cells of Buffer C and stored at -80 ºC.

2. Optimization of the reaction buffer for p19-dsRNA binding

The influence of reaction buffer on p19-dsRNA binding were studied by testing four reported buffers including buffer 1 (20 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 1 mM TCEP, 0.02% Tween-20, pH 7.0), buffer 2 (137 mM NaCl, 2.6 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 10 mM DTT, pH 7.4), buffer 3 (137 mM NaCl, 2.6 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4), buffer 4 (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). As shown in Fig. S1, buffer 1 and buffer 3 provided the best performances with similar ΔFP values. Taking the cost of ingredients into account, we chose buffer 3 as reaction buffer in this work.

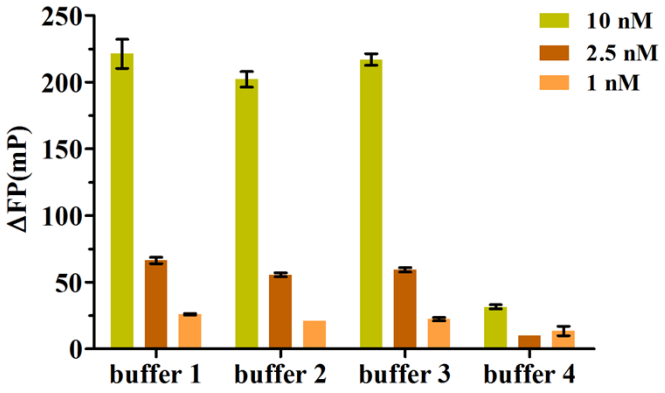

Fig. S1. Comparison of ΔFP values among the four tested reaction buffers. The reaction was performed with miR-122 at three concentrations (10 nM, 2.5 nM, and 1 nM), 10 nM Probe-1, 0.125 U/μL p19 protein, and 0.8 U/μL RNase Inhibitor in the reaction buffers, respectively.

3. Optimization of the amount of Probe-1

As shown in Fig. S2A, FITC-labeled Probe-1 with an increase concentration from 5 to 80 nM had a significant effect on the fluorescence intensity, but had a negligible effect on the fluorescence polarization. Fig. S2B showed that when the concentration of Probe-1 was 10 nM, it had the best performance for quantitative miR-122 analysis. Thus, we chose 10 nM Probe-1 in the following experiments.

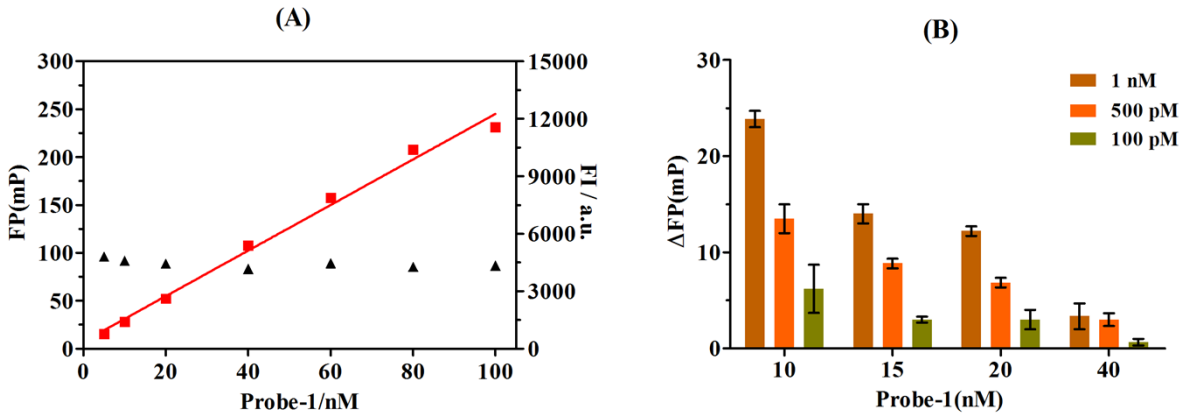

Fig. S2. (A) Investigation the effect of the amount of Probe-1 on the signals of fluorescence polarization (black triangle) and fluorescence intensity (red square). The reactions were performed with Probe-1 (5 nM, 10 nM, 20 nM, 40 nM, 60 nM, 80 nM) and 0.8 U/μL RNase Inhibitor in buffer 3, respectively. (B) The effect of the amount of the Probe-1 on performance of quantitative miR-122 analysis. The reactions were performed with Probe-1 (10 nM, 15 nM, 20 nM, 40 nM), miR-122 (1 nM, 500 pM, 100 pM), 0.125 U/μL p19 protein, and 0.8 U/μL RNase Inhibitor in buffer 3, respectively.

4. Optimization of the amount of p19 protein

The amount of p19 protein was investigated from 0.0625 U/μL to 0.3125 U/μL. As shown in Fig. S3, the tested amounts of p19 protein were all sufficient for binding its substrate of 10 nM Probe and 10 nM miRNA target. Thus, we chose an amount of 0.1250 U/μL p19 protein in the following experiments.

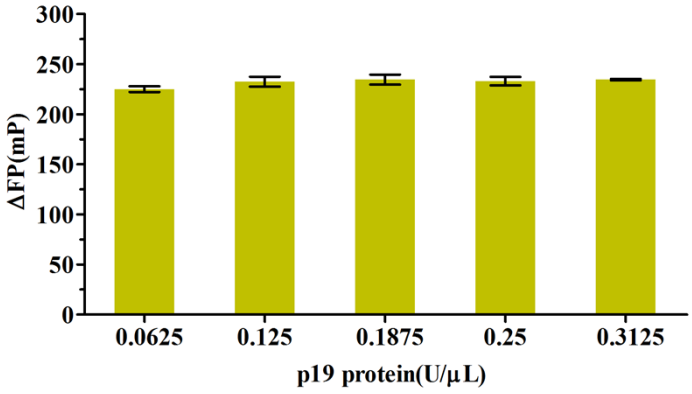

Fig. S3. Optimization of the amount of p19 protein. The reaction were performed with 10 nM Probe-1, 10 nM miR-122, p19 protein (0.0625 U/μL, 0.125 U/μL, 0.1875 U/μL, 0.25 U/μL, 0.3125 U/μL), and 0.8 U/μL RNase Inhibitor in buffer 3.

5. Determination of spiked miR-122 in cell lysis buffer

The spiked samples at four concentrations of 1000, 800, 600 and 400 pM, were prepared in cell lysis buffer (20 mM HEPES, 25% glycerol(v/v), 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM PMSF, 0.5 mM DTT, pH 7.9) and quantitatively detected by our method. The experiments were repeated five times. The result is listed in Table S2.

Table S2. Detection of spiked miR-122 in cell lysis buffer

Spiked amount (pM)	Detected amount (pM)	Recoveries (%)	CV (%)
1000	944.70	94.47	5.43
800	799.40	99.93	6.12
600	615.51	102.59	12.45
400	412.20	103.05	7.78

6. Determination of spiked miR-122 in cell lysate

The spiked samples at two concentrations of 1000 and 500 pM, were prepared in HepG2 cell lysate and quantitatively detected by our method. The experiments were repeated five times. The result is listed in Table S3.

Table S3. Detection of spiked miR-122 in HepG2 cell lysate

Spiked amount (pM)	Detected amount (pM)	Recoveries (%)	CV (%)
1000	896.14	89.51	5.33
500	427.46	85.50	10.08

Reference

1 L. Osborn, S. Kunkel, G. J. Nabel, *Proc. Natl. Acad. Sci. U S A* 1989, **86**, 2336.