

## Supporting Information

### **Electrophoresis separation assisted G-quadruplex DNAzyme-based chemiluminescence signal amplification strategy on the microchip platform for highly sensitive detection of microRNA**

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## Experimental section

**Materials and reagents:** All DNA and RNA oligonucleotides used in this work were obtained from Takara Biotech Co., Ltd. (Dalian, China). The sequences of these miRNAs and DNA probe are given in Table S1. The oligonucleotides were used as provided and diluted in pH 7.4, 20 mM Tris-HCl buffer solution (containing 100 mM NaCl, 20 mM KCl, and 2 mM MgCl<sub>2</sub>) to give stock solutions of 10  $\mu$ M. And each oligonucleotide was heated to 95°C for 10 min, and slowly cooled down to room temperature before use. Streptavidin (SA), 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol), hemin, ethidium bromide (EB) and Triton X-100 were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and sodium hydrogen carbonate (NaHCO<sub>3</sub>) were obtained from Taopu Chemicals (Shanghai, China). The nicking enzyme Nb.BbvCI and 10 $\times$ NEB buffer 2 were purchased from the New England Biolabs, Inc. (Ipswich, MA, USA). Agarose G-10 was obtained from Biowest. All other reagents were of analytical grade and were used without further purification. The water used in MCE-CL assay was purified by employing a Milli-Q plus 185 equip from Millipore (Bedford, MA, USA).

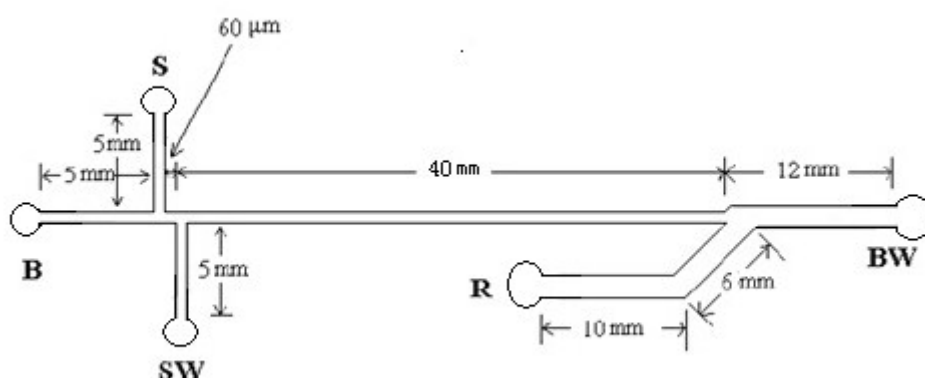
**Table S1.** Sequences of DNA probe and micorRNAs used in this work

Name	Sequences (5'to3')	Description
H- probe	CACACTGCTGAGTGTAGGATGTTTACAAGCCTCAGCAGTGTG	
Bio-G4	Bio— AAAAAAGGTGACTGCTGAGGCTAAATTAAAGGGGTAGGGCGG	
miR-30b	GTTGGG	
miR-30a	UGUAAACAUCUACACUCAGCU	
miR-30c	UGUAAACAUCUUGACUGGAAG	
miR-30d	UGUAAACAUCCTACACUCUCAGC	
miR-30e	UGUAAACAUCUCCGACUGGAAG	
miR-21	UGUAAACAUCUUGACUGGAAG	
Let-7d	UAGCUUAUCAGACUGAUGUUGA	

**Apparatus:** The MCE–CL detection was performed using a laboratory built system described previously.<sup>1</sup> A home-made glass/PDMS microchip was used in this work, and its schematic layout is illustrated in Figure S0. The procedure of microchip fabrication was as described previously.<sup>1</sup> The channel between reservoir S and SW was used for sampling, the channel between B and BW was used for the separation and the channel between R and BW was used for the oxidizer introduction. The width of microchannels is 70  $\mu\text{m}$  (except oxidizer introduction channel is 250  $\mu\text{m}$ ). The depth of all microchannels is 25  $\mu\text{m}$ , and the double “T” size is 60  $\mu\text{m}$ .

## References

[1] S. Zhao, Y. Huang, M. Shi, Y. M. Liu, *J. Chromatogr. A* **2009**, 1216, 5515-5519.



**Figure S0.** The schematic layout of glass/PDMS microchip used in this work. S: sample reservoir; B: buffer reservoir; SW: sample waste reservoir; BW: buffer waste reservoir; R: the oxidizer reagent reservoir.

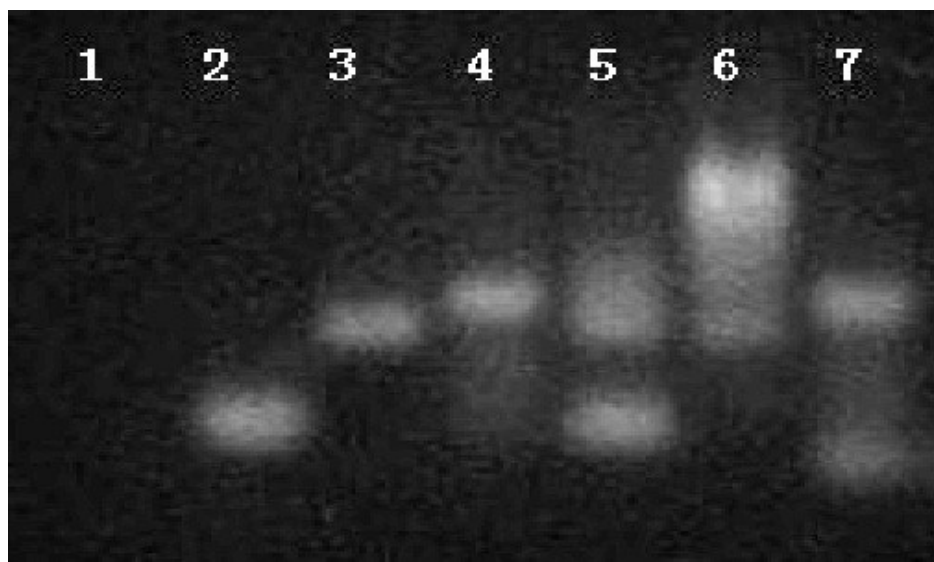
**Cell culture and sample preparation:** Human liver cancer cell lines (HepG2) were cultured in RPMI 1640 medium (Thermo Scientific Hyclone) supplemented with 10% fetal bovine serum, 10 U/mL penicillin and 10  $\mu\text{g/mL}$  streptomycin. These cells were cultured at 37  $^{\circ}\text{C}$  in a humidified atmosphere containing 5%  $\text{CO}_2$  for 24 h. Cells ( $1 \times 10^6$ ) were dispensed in an RNase-free 1.5 mL centrifuge tube, washed twice with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4) and centrifuged at

2000 rpm for 3 min, and then suspended in 10 mM Tris-HCl with pH 7.4. The suspended cells were sonicated with an ultrasonicator for 10 min on ice, and then centrifuged at 12000 rpm for 20 min at 4 °C. The extract was used immediately for miRNA assay or stored at - 80 °C.

**Procedure for signal mplification reaction:** H-probe (4  $\mu$ L, 1.25  $\mu$ M) was mixed with different concentrations of target miRNA (2  $\mu$ L) at 37 °C for 1 h. After that Bio-G4 (2  $\mu$ L 5 $\mu$ M) was added, and the signal mplification reaction was performed by incubating the resulting mixture with 10 unit of Nt.BbvCI (1  $\mu$ L) at 37 °C for another 2 h in NEB buffer 2 (1  $\mu$ L, 10 mM Tris-Cl, 50 mM NaCl, 10 mM MgCl<sub>2</sub>, and 1 mM DTT, pH 7.9). Then SA solution (1  $\mu$ L 10  $\mu$ M) was added, and incubating at 37 °C for 30 min. After incubation, the solution was diluted with HEPES buffer solution (88  $\mu$ L, pH 8.0, 25 mM HEPES, 0.05% (w/v) Triton X-100, 1% (v/v) DMSO, 20 mM KCl, 200 mM NaCl). Subsequently, hemin (1  $\mu$ L, 50  $\mu$ M) was added to obtain the mixture with final volume of 100  $\mu$ L, and incubated for 1 h at room temperature to form G-quadruplex DNAzyme. The resulting solution was analyzed by MCE–CL.

**MCE–CL assay:** The microchannels were rinsed sequentially with 0.1 M NaOH, water, and electrophoretic buffer for 5 min each. Prior to the MCE separation, the reservoirs B, S, SW, and BW were filled with the electrophoretic buffer, reservoir R was filled with the oxidizer solution, and vacuum was applied to the reservoir BW in order to fill the separation channel with the electrophoretic buffer. Then, the electrophoretic buffer in reservoir S was replaced by sample solution. For loading the sample solution, a set of electrical potentials was applied to five reservoirs: reservoir S at 600 V, reservoir B at 250 V, reservoir BW at 350 V, reservoir SW at grounded, and reservoir R floating. The sample solution was transported from reservoir S to SW in a pinched mode. After 20 s, potentials were switched to reservoir B, S, SW, and R at 2600, 1500, 1500, and 800 V, respectively, while reservoir BW was grounded for separation and detection.

### **The viability of proposed strategy:**

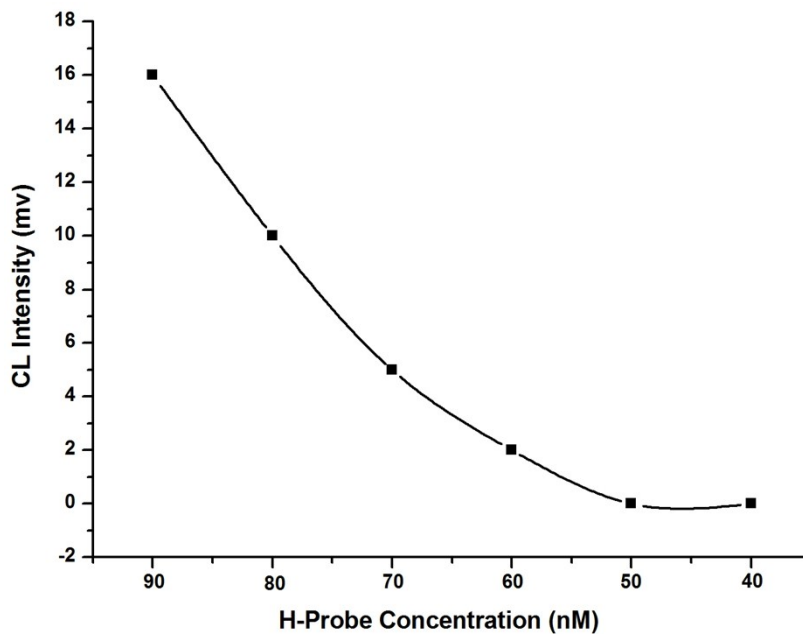


**Figure S1.** Agarose gel electrophoresis analysis: (1) miR-30b ; (2) H-probe ; (3) Bio-G4 ; (4) miR-30b/H-probe complex ; (5) mixture of H-robe and Bio-G4 ; (6) mixture of miR-30b, H-probe and Bio-G4 ; (7) mixture of miR-30b, H-robe, Bio-G4 and Nb.BbvCI.

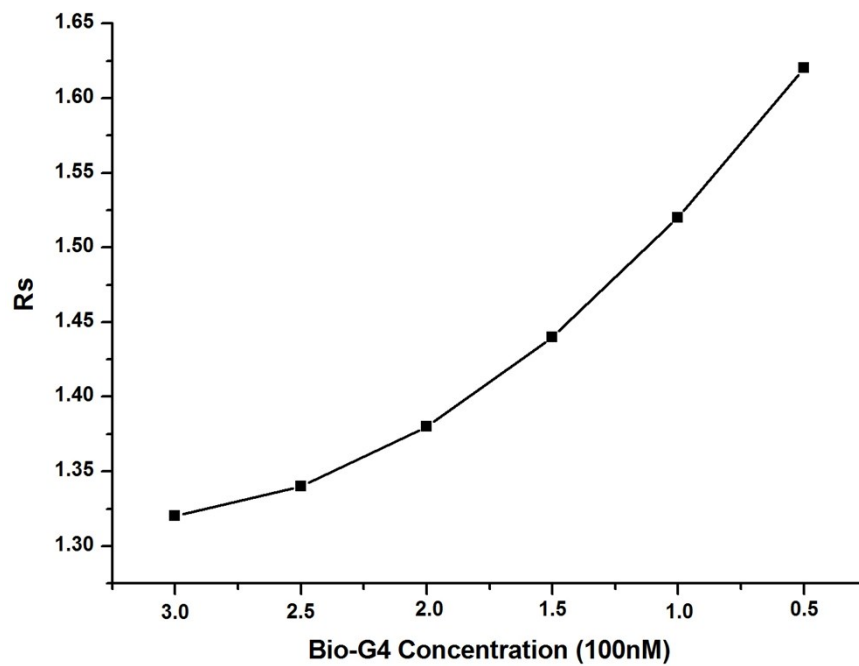
### **Optimization of the conditions for signal amplification and electrophoresis separation:**

H-probe concentration not only affects the signal intensity of the analytes, but also affects the intensit of the background signal. When the H-probe concentration is larger, the background value is larger. When the concentration is 50 nM, the background value was reduced to 0 (Fig. S2). The Bio-G4 concentration not only affects CL intensity, but also affects the CE separation. With the decrease of Bio-G4 concentration, the resolution ( $R_s$ ) of two kinds of G-quadruplex DNAzymes was gradually increased. When the concentration was 100 nM, a baseline separation ( $R_s >$

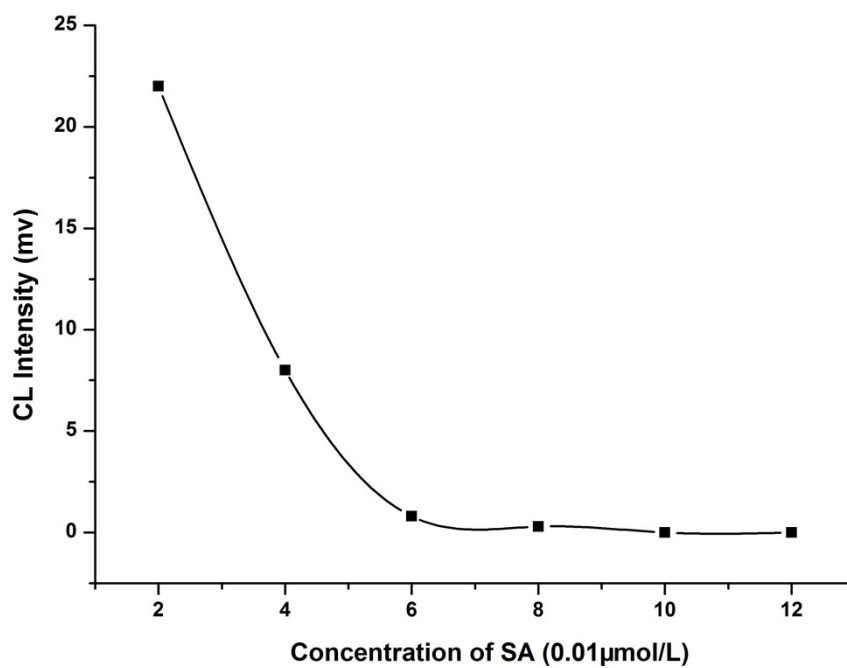
1.5) was achieved (Fig. S3,). SA concentration affects background value, when the concentration of SA was 100 nM, the background value was reduced to 0 (Fig. S4,).



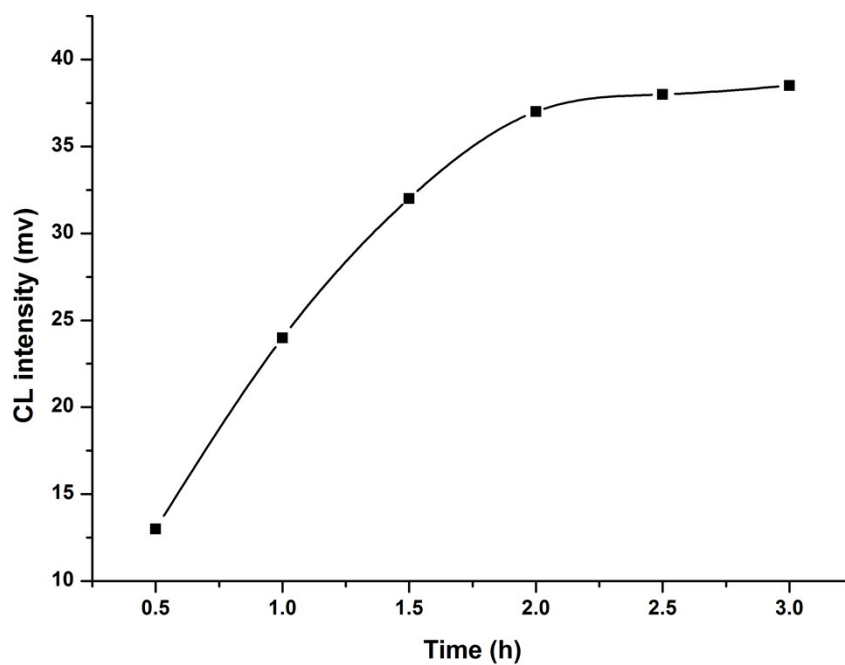
**Figure S2:** Effect of H-probe concentration on the background signal.



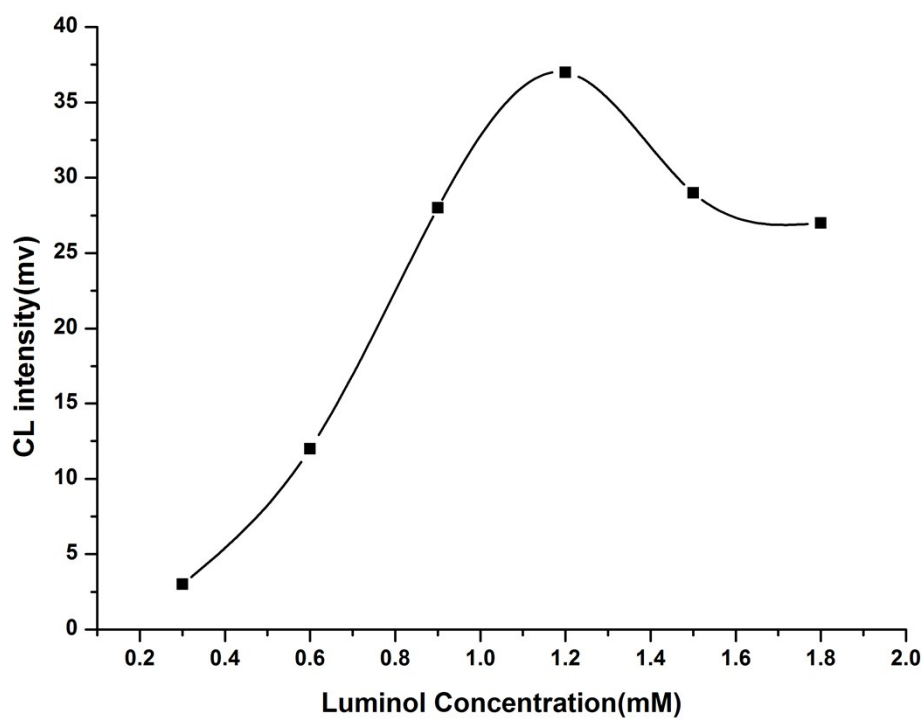
**Figure S3:** Effect of Bio-G4 concentration on the CE separation.



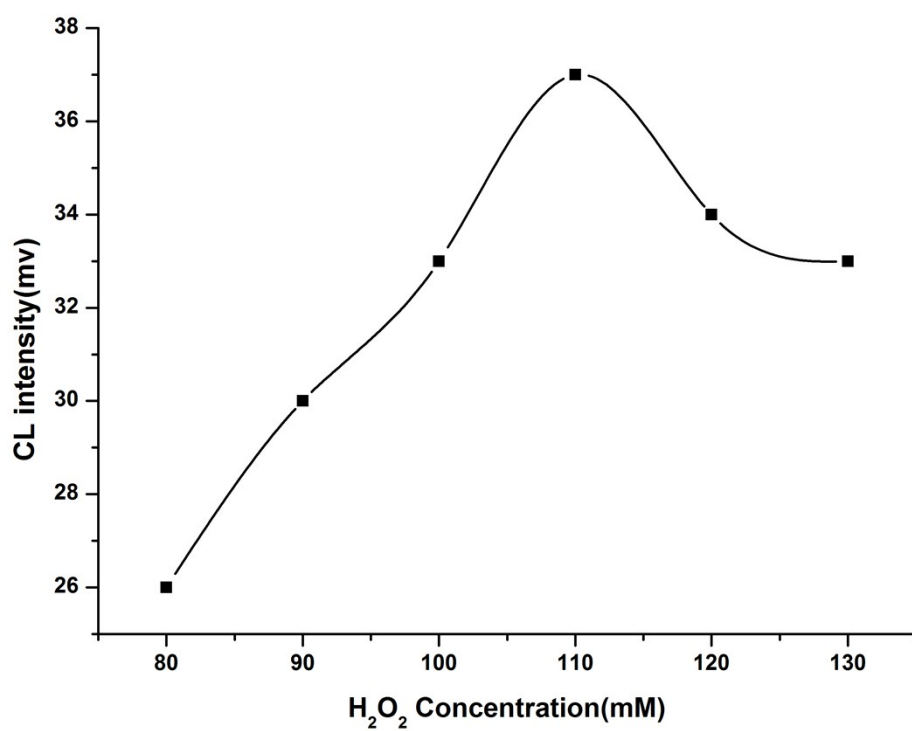
**Figure S4:** Effect of SA concentration on the CL background signal.



**Figure S5:** Effect of incubation time at room temperature on the CL signal amplification reaction.

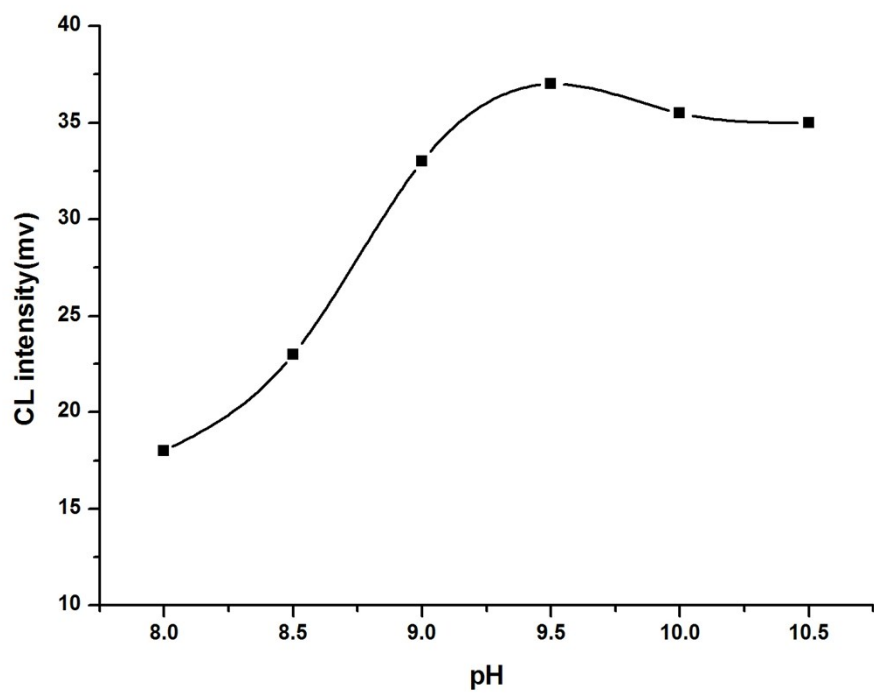


**Figure S6.** Effect of luminol concentration on in-line CL reaction.

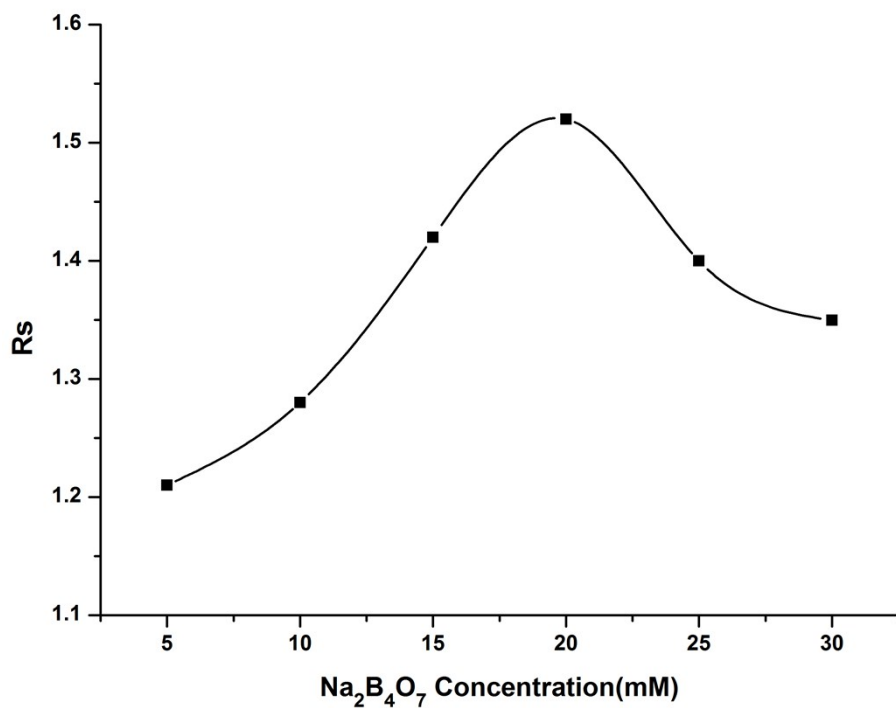


**Figure S7.** Effect of H<sub>2</sub>O<sub>2</sub> concentration on in-line CL reaction.

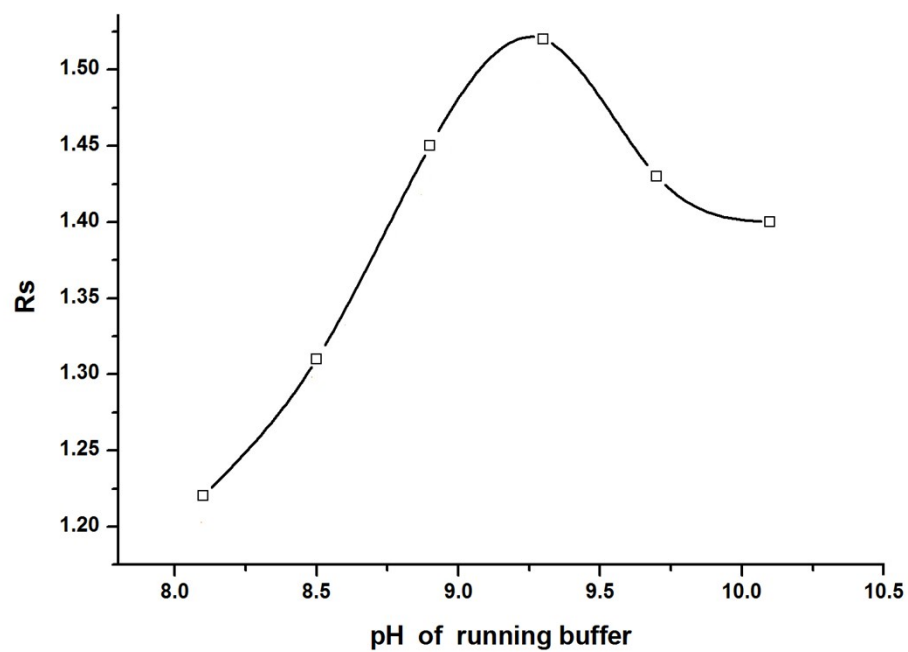




**Figure S8.** Effect of the pH value of reaction medium on in-line CL reaction.

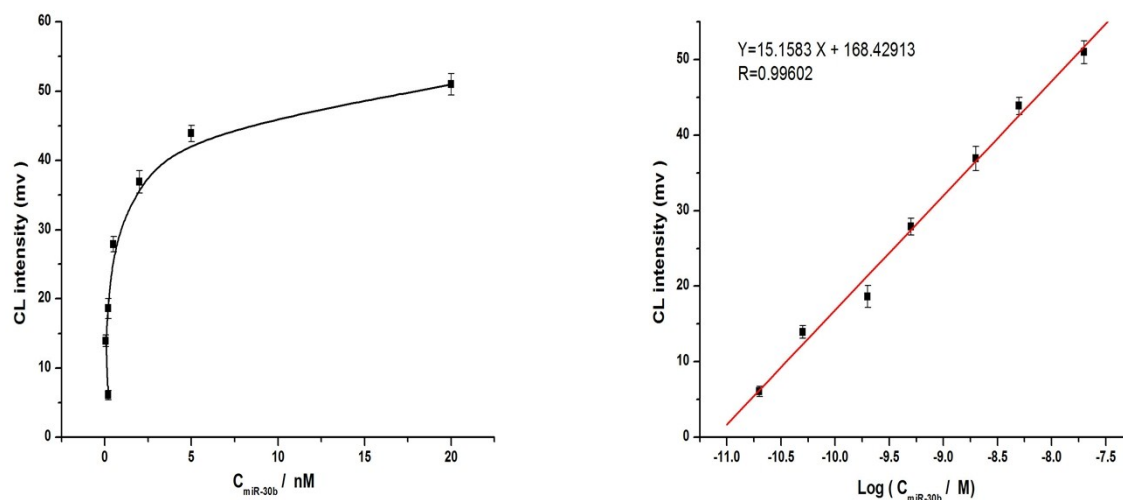


**Figure S9.** Effect of the concentration of borax buffer on the electrophoresis separation.

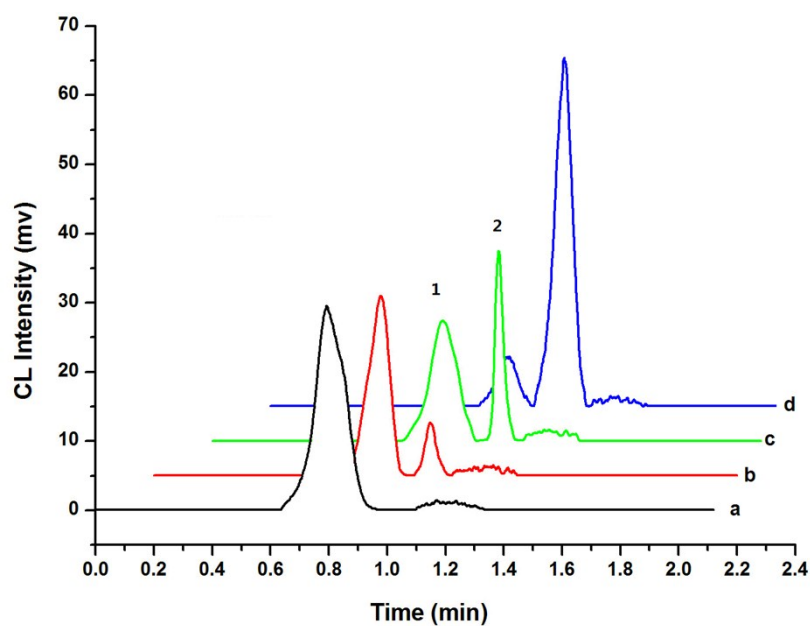


**Figure S10.** Effect of the pH value of borax buffer on the electrophoresis separation.

## Potential application:



**Figure S11.** The response linearity of the peak height (CL intensity) of G-quadruplex DNAzyme with the log miR-30b concentration.



**Figure S12.** The electropherograms obtained upon the analysis of the cell lysate samples. (a) HepG2 cell lysate; (b) HepG2 cell lysate+20 pM miR-30b; (c) HepG2 cell lysate+0.5 nM miR-30b; (d) HepG2 cell lysate+20 nM miR-30b. Peak identification: (1) SA-Bio-G4 complex; (2) G-rich DNA segment. The concentrations of H-probe, Bio-G4, SA and hemin are  $5 \times 10^{-8}$  M,  $1 \times 10^{-7}$  M,  $1 \times 10^{-7}$  M and  $5 \times 10^{-7}$  M, respectively.