Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Ultrasensitive electrochemical detection of miRNA based on DNA strand displacement polymerization and Ca^{2+}-dependent DNAzyme cleavage

Dawei Yang, Wenbo Cheng, Xifeng Chen, Yuguo Tang, and Peng Miao*

Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences, Suzhou 215163, P. R. China

* Corresponding author. E-mail: miaopeng@sibet.ac.cn

Experimental

Materials and reagents

Mercaptohexanol (MCH), diethypyrocarbonate (DEPC), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), hexaammineruthenium(III) chloride ($[Ru(NH_3)_6]^{3+}$) and ethylenediaminetet-raacetic acid (EDTA) were obtained from Sigma (USA). $CaCl_2$, NaCl, KCl, and $MgCl_2$ were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Fetal bovine serum was from Hangzhou Sijiqing Biological Engineering Material Co., Ltd. (Hangzhou, China). All other reagents were of analytical grade and were used as received. qRT-PCR kit was purchased from Life Technologies (USA). Klenow fragment polymerase, dNTP mixture, and Nt.BbvCI nicking endonuclease were purchased from New England Biolabs Ltd. (Beijing, China). Water was purified by a Millipore system with a resistivity higher than 18 MΩ•cm, which was further treated with DEPC (0.1%) before use. All oligonucleotides were synthesized and purified by Takara Biotechnology Co., Ltd. (Dalian, China). The sequences and modifications were shown in Table S1.

Gold electrode modification

The substrate gold electrode (2 mm) was firstly cleaned by piranha solution (98% H_2SO_4 : 30% H_2O_2 = 3 : 1) for 5 min (*Caution: highly corrosive*). After carefully rinsed with double-distilled water, the electrode was polished to a mirror-like surface using P5000 silicon carbide paper and then 1, 0.3, 0.05 μm alumina slurry, respectively. Next, it was sonicated in ethanol and then double-distilled water for 5 min. Subsequently, it was incubated with 50% HNO_3 for 30 min and then electrochemically cleaned using 0.5

M H_2SO_4. After the electrode was dried by nitrogen, it was incubated with DNA probe c (0.5 μM, 10 mM Tris-HCl, 10 mM TCEP, 1 mM EDTA, 0.1 M NaCl, pH 7.4) for 9 h. Then, the electrode was rinsed with double-distilled water and immersed in 0.1 M MCH for 30 min.

miRNA-trigged strand displacement reaction

miRNA standard solutions with various concentrations were firstly prepared. Then, Klenow fragment catalyzed polymerization and Nt.BbvCI catalyzed cleaving reaction solution was prepared with 0.2 μL Klenow fragment, 0.3 μL Nt.BbvCI, 2.5 μL dNTPs (2.5 mM), 1.0 μL DNA probe a (5.0 μM), 5 μL miRNA, 1.0 μL 10×NEB buffer 2.1. The final volume of the reaction solution was 10 μL. After incubating at 37°C for 90 min, the reactions were terminated by heating to 80°C for 20 min.

DNAzyme formation and cleavage reaction

The above solution was dipped on DNA probe c modified electrode for 1 h. Afterward, the electrode was rinsed with double-distilled water and then immersed in DNAzyme reaction solution (1.2 mM Ca^{2+}, 20 mM LiCl, 10 mM HEPES, pH 7.6) for 1 h for DNAzyme-catalyzed cleavage.

Preparation of cell lysate

Human bladder cancer T24 cells were cultured in 1640 culture medium with the addition of 10% fetal bovine serum (v/v) at 37 °C in 5% CO_2 atmosphere. After the cells reached a confluence of more than 80%, the cells were washed by phosphate buffer (PBS). Cell lysates were prepared by applying SingleShotTM

Cell Lysis Kit according to the manufacturer's procedure, which were then analyzed by the proposed electrochemical biosensor and qRT-PCR method.

Electrochemical measurement

All electrochemical experiments were carried out with a CHI 660D electrochemical workstation (CH instruments, China). A traditional three electrode system was applied, which was consisted of a saturated calomel reference electrode, a platinum wire auxiliary electrode and the modified gold electrode as the working electrode. Chronocoulometry (CC) experiments were performed in 10 mM Tris-HCl buffer solution containing 50 μM $[Ru(NH_3)_6]^{3+}$. The pulse period was set to be 250 ms. Electrochemical impedance spectroscopy (EIS) experiments were performed using 5 mM $[Fe(CN)_6]^{3-/4-}$ containing 1 M KCl with the parameters of 0.205 V bias potential, 5 mV amplitude, and 0.1 to 100 000 Hz frequency range. Square wave voltammetry (SWV) experiments were performed in 20 mM Tris-HCl (pH 7.4) containing 140 mM NaCl and 5 mM $MgCl_2$. The potential scan range was from 0 to -0.7 V. The other parameters included 25 mV modulation amplitude, 80 Hz frequency and 4 mV step potential.

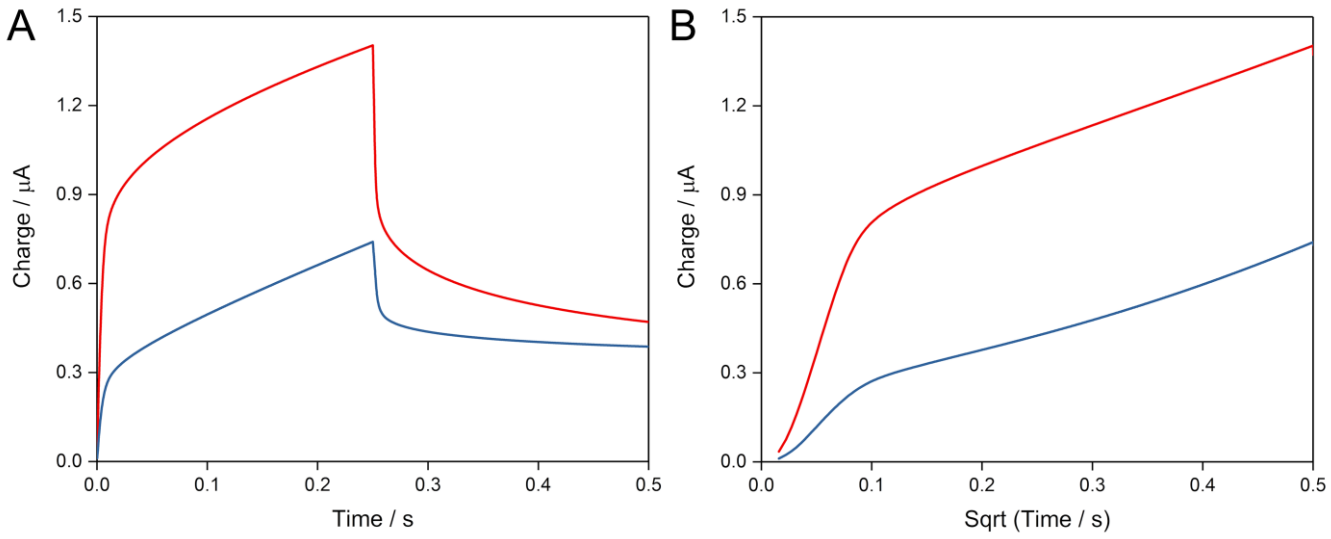

Fig. S1 (A) Chronocoulometry curves for the electrode modified with single MCH (bottom) and DNA probe c with MCH (top). (b) Chronocoulometry curves of charge versus $t^{1/2}$.

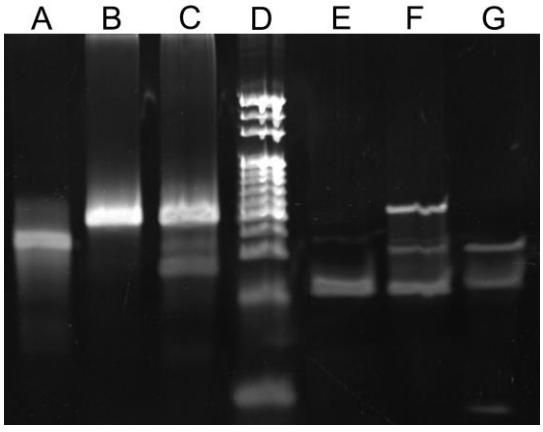

Fig. S2 Gel electrophoresis analysis of strand displacement polymerization and Ca^{2+}-dependent DNAzyme cleavage. Lane A is DNA probe a; lane B and C are the products of miRNA induced strand displacement reactions in the absence and presence of Nt.BbvCI; lane D is 20 bp DNA Ladder; lane E is DNA probe c; lane F and G are the mixture of DNA probe b and c in the absence and presence of Ca^{2+}.

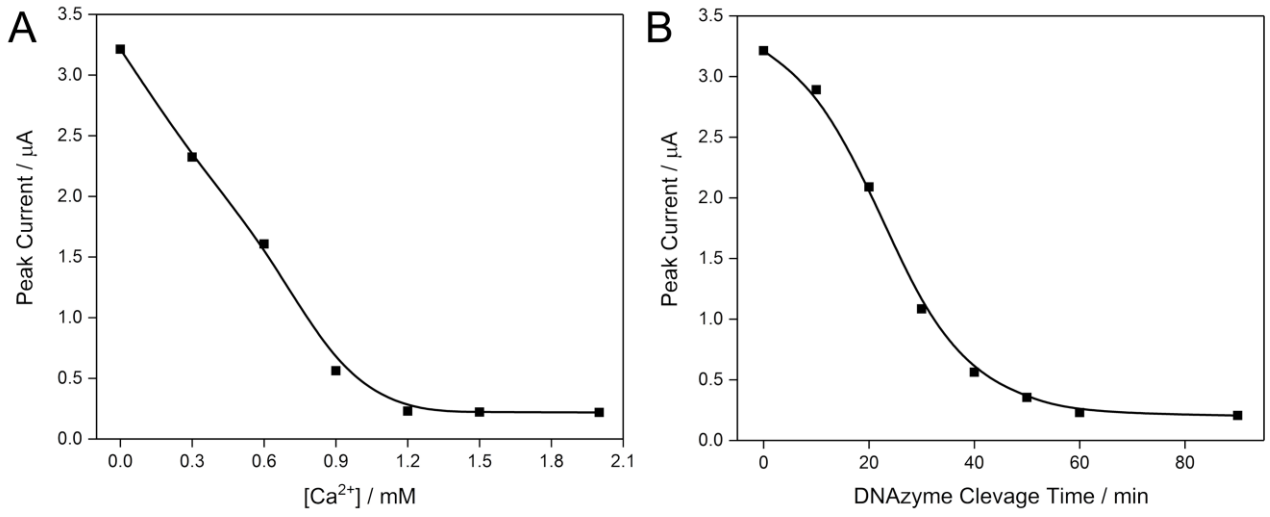

Fig. S3 Optimization of (A) Ca^{2+} concentration and (B) DNAzyme cleavage time. The concentration of used miRNA is 10^{-13} M.

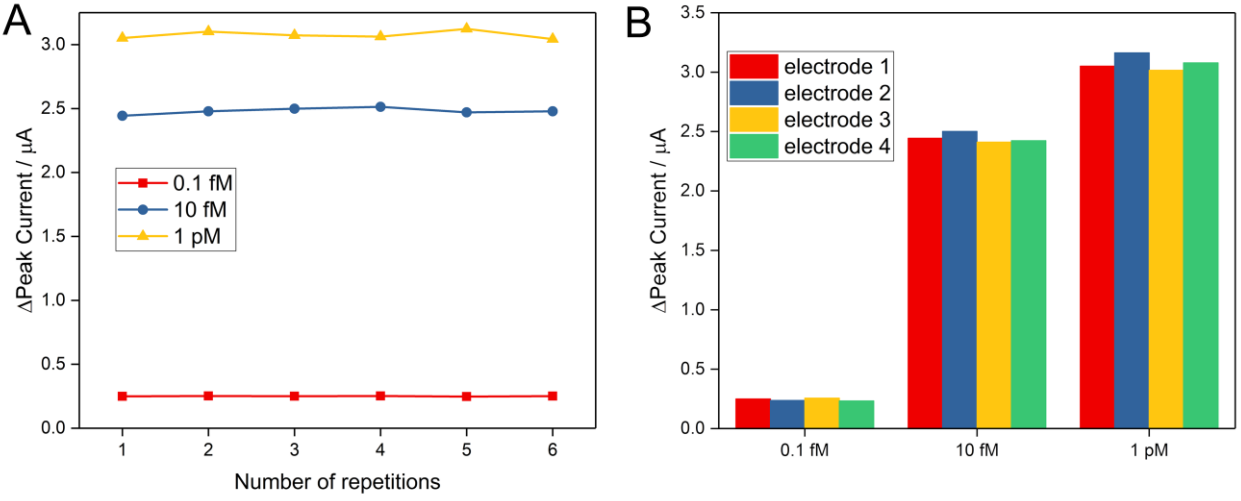

Fig. S4 Study of (A) run-to-run and (B) electrode-to-electrode reproducibility.

Table S1 DNA and RNA sequences used in this work.

Name	Sequence (from 5' to 3')
DNA probe a	GTCACGAGTCACTAACAACCTTAGCGAGTACGCTGTGAGAAAAGATGGCGAAA GCTGAGG**CCATCTTTACCAGACAGTGTTA**
DNA probe b	TCAGCTTTCGCCATCTTTTCTCACAGCGTACTCGCTAAGGTTGTTAGTGACTCGT GAC
DNA probe c	MB-*AAGTCACGAGTCACTA*TrAGGAAGATGGCGAAATAGCAAC*GACTCGTGACTT* TTTT-$(CH_2)_6$-SH
miR-141	UAACACUGUCUGGUAAAGAUGG
miR-141-1a	UAACACUGUCUGGUAAAGAUGA
miR-141-1b	UAACACUGUCUGGUACAGAUGG
miR-141-1c	UAACACUGUCAGGUAAAGAUGG
miR-141-2a	UAACACUGUAUGGCAAAGAUGG
miR-141-2b	UAACACGGUCUGGUACAGAUGG

The bold sequence of DNA probe a is complementary with miR-141. The yellow sequence is the recognition site of Nt.BbvCI. The green and blue sequences of DNA probe b and c are complementary with each other. The italic parts inside DNA probe c are complementary sequences. The underlined sequences in miRNAs are mismatched sites compared with miR-141.