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Supporting Information

Electrochemical sensing of attomolar miRNA combining cascade strand displacement polymerization and reductant-mediated amplification

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Experimental

Materials and Chemicals

Ethylenediaminetetraacetic acid (EDTA), tris(2-carboxyethyl) phosphine hydrochloride (TCEP), mercaptohexanol (MCH), diethypyrocarbonate (DEPC), trypsin, and hexaammineruthenium(III) chloride ($[Ru(NH_3)_6]^{3+}$) were purchased from Sigma (USA). The other reagents were of analytical grade and were used as received without further purification. Klenow fragment polymerase, dNTP mixture, Nb.BssSI, Nt.BstNBI, Nb.BsmI, Nb.BsrDI and Nb.BbvCI nicking endonuclease were purchased from New England Biolabs Ltd. (Beijing, China). Quant One Step qRT-PCR Kit was purchased from Tiangen Biotech Co., Ltd. (Beijing, China). Human cervical cancer cell line (HeLa), human pulmonary carcinoma cell line (A549), human renal cubularepithelial cell line (HK-2), human breast adenocarcinoma cell line (MCF-7) were provided by the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle medium (DMEM) was purchased from Gibco (Gaithersburg, USA). Fetal bovine serum was from Hangzhou Sijiqing Biological Engineering Material Co., Ltd. (Hangzhou, China). All oligonucleotides and 20 bp DNA Ladder were obtained from Takara Biotechnology Co., Ltd. (Dalian, China). The sequences of DNA and RNA were shown in Table S1. Double-distilled water was purified with a Millipore system under 18 MΩ cm resistivity, which was treated with DEPC before use.

Instruments

Electrochemical experiments were carried out on a CHI 660D electrochemical workstation (CH instruments, China). A three electrode system was applied, which included a saturated calomel reference electrode, a platinum wire counter electrode and a gold electrode as the working electrode.

Square wave voltammetry (SWV) measurements were performed with a step potential of 4 mV, a frequency of 70 Hz, and an amplitude of 25 mV by scanning the potential from 0.1 V to -0.6 V in working buffer solution (20 mM Tris-HCl, 160 μM TCEP, 100 mM NaCl, 50 mM $MgCl_2$, pH 7.4). Chronocoulometry (CC) was carried out 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) was conducted in 5 mM $[Fe(CN)_6]^{3-/4-}$ with 1 M KNO_3. The Bias potential was 0.215V, the amplitude was 5 mV and the frequency range was from 0.1 to 100000 Hz. All the experiments were run in triplicates. Polyacrylamide gel was photographed under UV light by Gel Doc^{TM} XR^{+} Imaging System (Bio-Rad, USA). qRT-PCR experiments were performed on an ABI 7500 Real-Time PCR System (ABI Life Technologies, USA).

Working Electrode Cleaning

The substrate gold electrode (2 mm) was pretreated according to a previous report.[1] Briefly, it was immersed in piranha solution (98% H_2SO_4 : 30% H_2O_2 = 3 : 1) for 5 min (*Caution: Piranha solution reacts violently with organic solvents and should be handled with great care!*). After rinsed with double-distilled water, it was polished to mirror-like surface using P5000 sand paper and 1, 0.3, 0.05 μm alumina slurry, respectively. Next, the gold electrode was soaked in ethanol and then double-distilled water with ultra-sonicating process. Subsequently, it was incubated with 50% HNO_3 for 30 min and then electrochemically cleaned with 0.5 M H_2SO_4 so as to remove any remaining impurities. It was then carefully rinsed and dried with nitrogen. The cleaned gold electrode was incubated with DNA probe d (1 μM, 10 mM Tris-HCl, 1 mM EDTA, 10 mM TCEP, 0.1 M NaCl, pH 7.4) for 8 h.[2] Subsequently, it was rinsed and treated with 0.1 M MCH for 30 min.[3]

MiRNA-Trigged Cascade Strand Displacement Polymerization

MiRNA standard solutions were firstly prepared with a series of concentrations. Isothermal polymerization and nicking reaction solution was prepared with 0.2 μL Klenow fragment, 0.3 μL Nb.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, which was dipped on the DNA probe d modified electrode surface at 37 ℃ for 2 h. After that, the enzymes are inactivated by heating to 80 ℃ for 20 min. The electrodes were carefully rinsed and then electrochemically measured with the amplification of TCEP.

Cell Culture and Lysis

HeLa, A549, HK-2, and MCF-7 cells were cultured in DMEM medium with the addition of 10% fetal bovine serum (v/v) in 5% CO_2 atmosphere. The culture temperature was set to be 37°C. After the cells reached a confluence of more than 80%, the four cells were detached by trypsin and washed with phosphate buffered saline. The collected cells were then seeded in 96-well plate. The concentrations were about 10000 cells per well. Cell lysates were obtained by applying SingleShot™ Cell Lysis Kit according to the manufacturer's procedure, which were then analyzed by the proposed electrochemical biosensor and qRT-PCR method.

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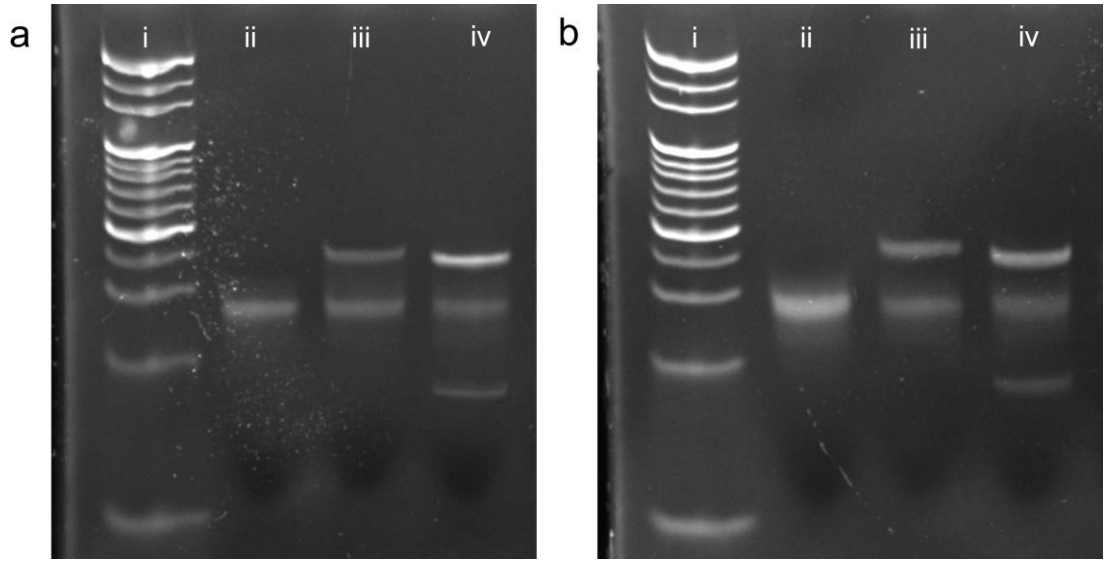

Fig. S1 Polyacrylamide gel electrophoresis analysis of oligonucleotides in this work. (a): (i) 20 bp DNA Ladder, (ii) DNA probe a, (iii) DNA duplex formed by DNA probe a and b, (iv) the previous DNA duplex in the presence of Klenow fragment and Nb.BbvCI. (b): (i) 20 bp DNA Ladder, (ii) DNA probe d, (iii) DNA duplex formed by DNA probe c and d, (iv) the previous DNA duplex in the presence of Klenow fragment and Nb.BbvCI.

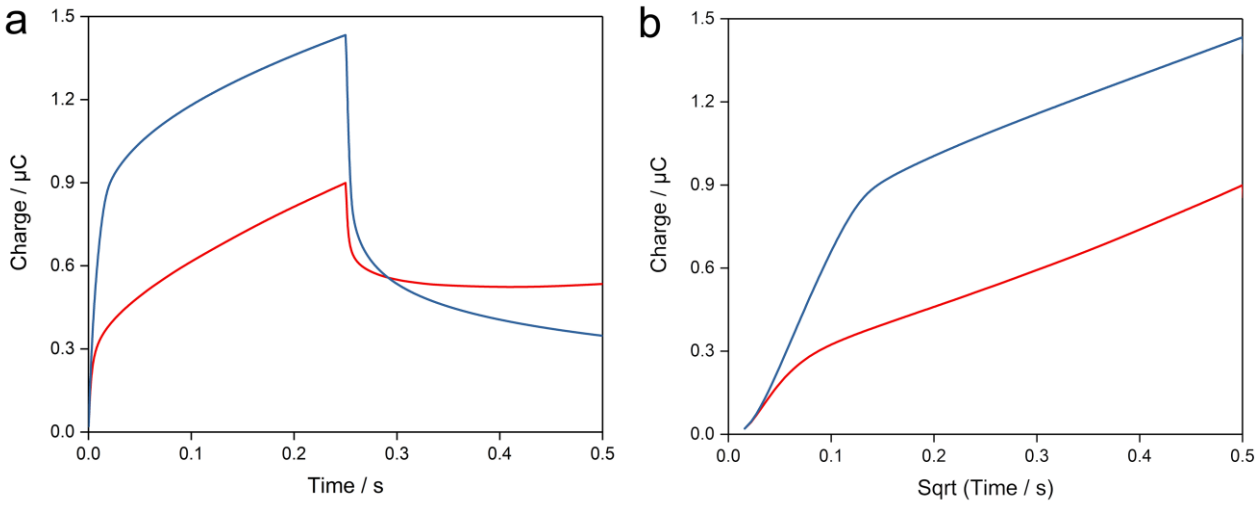

Fig. S2 (a) Chronocoulometry curves for the electrode modified with MCH (bottom) and DNA probe d before MCH (top). (b) Chronocoulometry curves of charge versus $t^{1/2}$.

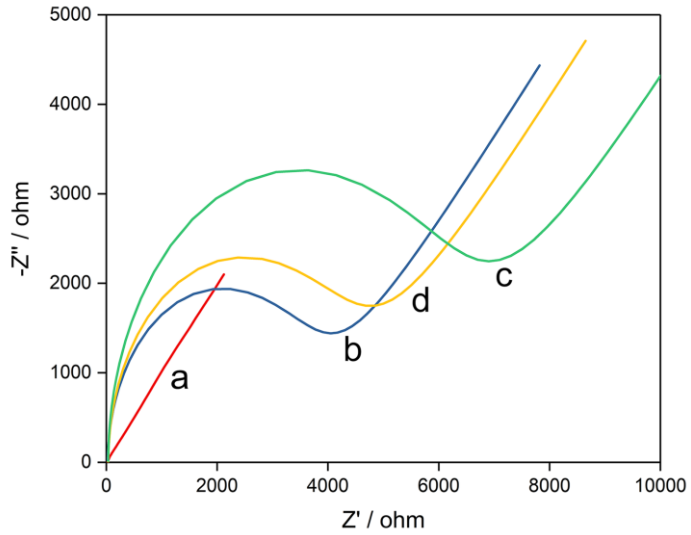

Fig. S3 Nyquist plots corresponding to (a) bare gold electrode, (b) DNA probe d modified electrode, (c) after miRNA-initiated cascade strand displacement polymerization in the presence of DNA probe a, (d) DNA probe d modified electrode after incubation with DNA probe c.

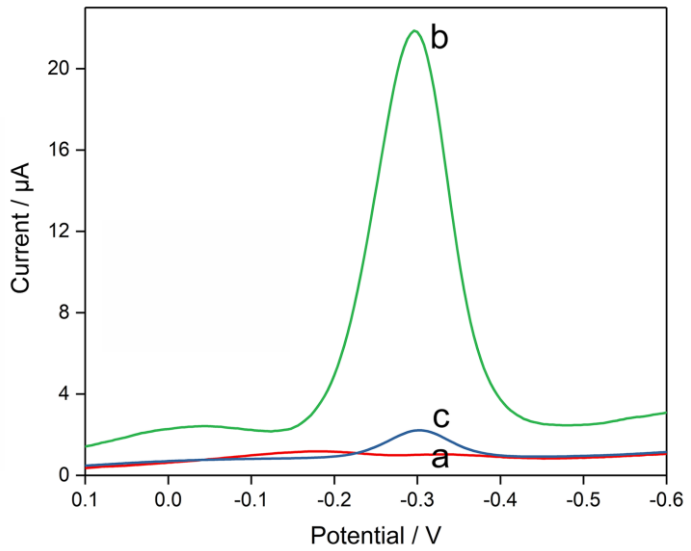

Fig. S4 Square wave voltammograms of (a) bare gold electrode, (b) DNA probe d modified electrode, (c) after miRNA-initiated cascade strand displacement polymerization (10^{-12} M).

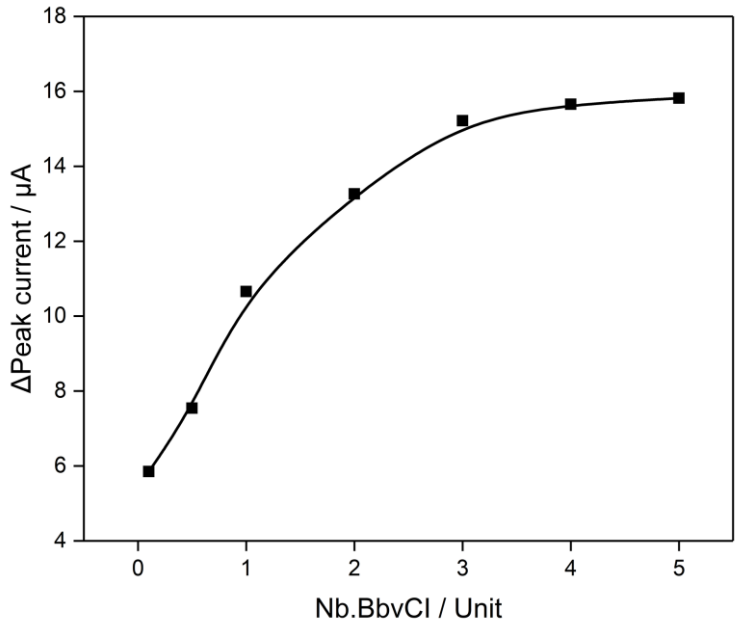

Fig. S5 Optimization of the amount of Nb.BbvCI used for DNA cleavage.

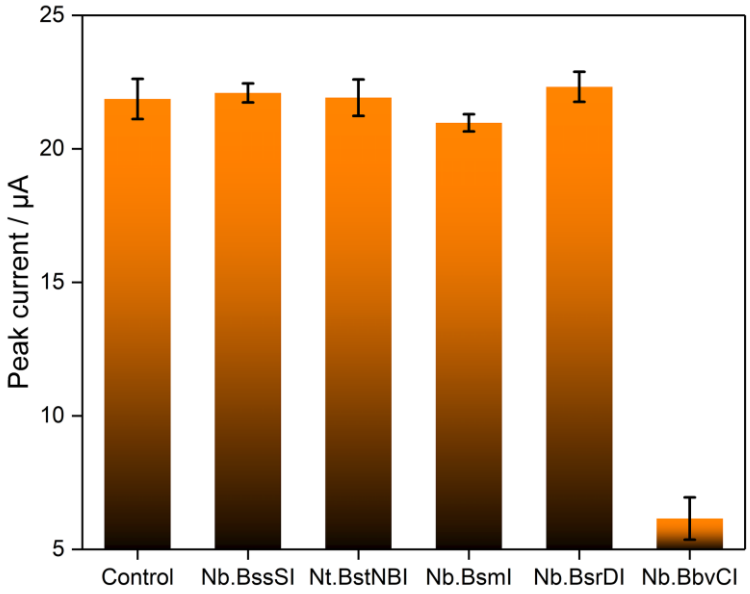

Fig. S6 Peak currents of the square wave voltammograms for the detection of 1 fM miRNA in the absence and presence of Nb.BssSI, Nt.BstNBI, Nb.BsmI, Nb.BsrDI and Nb.BbvCI nicking endonucleases, respectively.

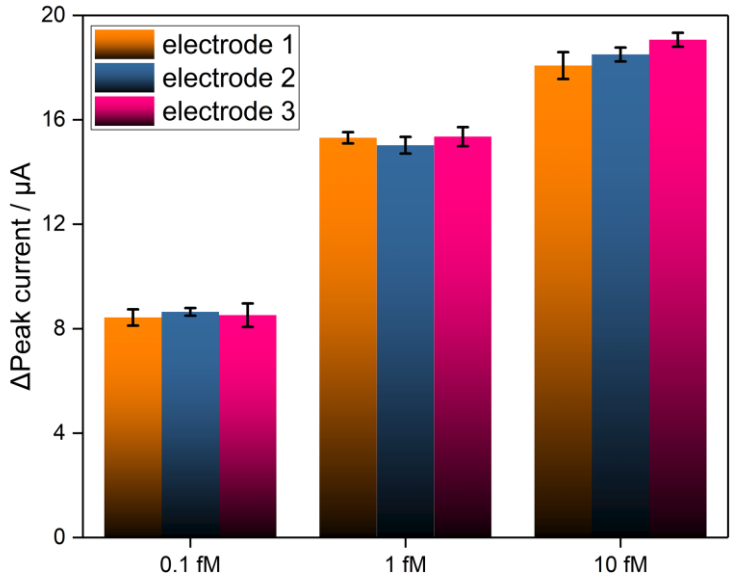

Fig. S7 Reproducibility of the proposed electrochemical biosensor in different concentrations.

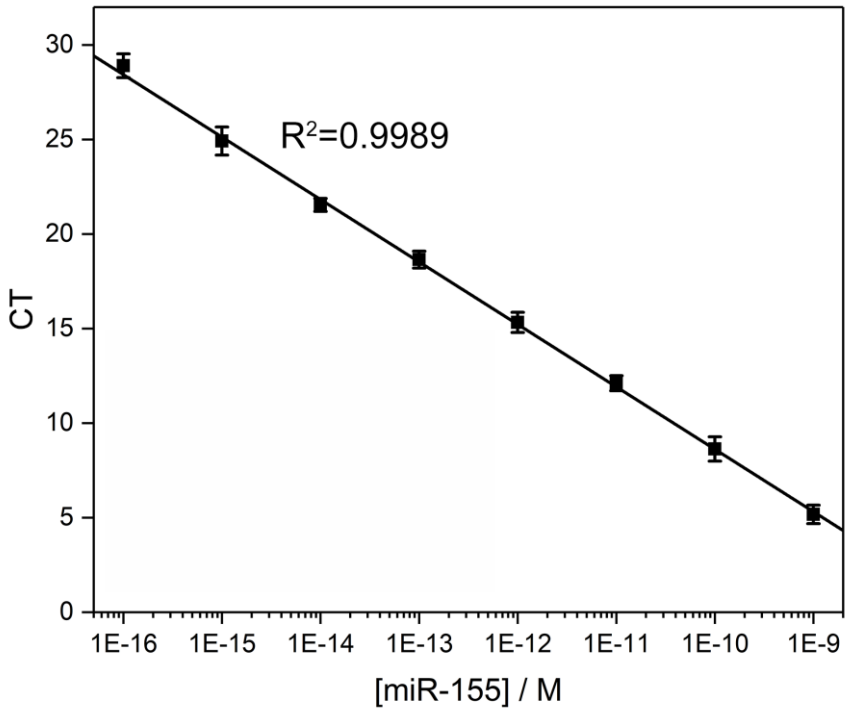

Fig. S8 Standard curve of qRT-PCR for 10-fold dilution series ranging from 10^{-16} to 10^{-9} M of synthetic miR-155. Standard deviations are obtained from three independent measurements.

Table S1 DNA and RNA sequences used in this work.[α]

Name	Sequence (5’ to 3’)
DNA probe a	ACTAGCTAAGGCATCATAGGTATCTAGCTAAAGCCCTCAGCACCCC TATCACGATTAGCATTAA
DNA probe b	TGAGGTTAATGCTAATCGTGATAGGGGT
DNA probe c	TGAGGGCTTTAGCTAGATACCTATGATGCCTTAGCTAGT
DNA probe d	MB-ACCCCTATCACGATTAGCATTAACCTCAGCACTAGCTAAGGCA TCATAGGTATCTAGCTAAAGCCCTCA-$(CH_2)_6$-SH
miR-155	UUAAUGCUAAUCGUGAUAGGGGU
mismatch 1	AUAAUGCUAAUCGUGAUAGGGGU
mismatch 2	ACAAUGCUAAUCGUGAUAGGGGU
mismatch 3	UUAAUGCUAAUCGUGAUAGGGGG
mismatch 4	UUAAUGCUAAUCGUGAUAGGGAG
mismatch 5	UUAAUGCUAAUGGUGAUAGGGGU
mismatch 6	UUAAUGCUAAUGCUGAUAGGGGU

[α] The blue and green colored sequences are the same or complementary sequences. The black colored sequences of DNA probes are parts of restriction sites of Nb.BbvCI nicking endonuclease. The underlined parts of mismatched miRNAs represent the mismatch sites which cover 5’ end, 3’ end and middle sections of the sequences

Table S2 Comparison of the analytical performances of recent miRNA assays.

Technique	Strategy	Detection range (M)	LOD (M)	Ref.
fluorescence	ratiometric probe with catalyzed hairpin assembly	5×10^{-10} to 5×10^{-8}	7.2×10^{-11}	4
electrochemistry	thionine and gold nanoparticles co-functionalized MoS_2 nanosheet	10^{-12} to 10^{-8}	2.6×10^{-13}	5
electrochemistry	Double-loop hairpin probe and doxorubicin-loaded gold nanoparticles	10^{-12} to 10^{-8}	1.7×10^{-13}	6
electrochemistry	gold-loaded nanoporous superparamagnetic nanocubes	10^{-13} to 10^{-6}	10^{-13}	7
fluorescence	target-fueled DNA walker	10^{-13} to 10^{-9}	5.8×10^{-14}	8
fluorescence	near-infrared Ag_2S quantum dots-based logic gate	10^{-16} to 10^{-11}	1.2×10^{-14}	9
fluorescence	helicase-assisted hybridization chain reaction	10^{-14} to 2×10^{-12}	4.2×10^{-15}	10
fluorescence	isothermal exponential amplification and graphene oxide	10^{-14} to 10^{-11}	3×10^{-15}	11
electrochemistry	enzyme-free target recycling signal amplification	5×10^{-15} to 5×10^{-10}	1.4×10^{-15}	12
colorimetry	gold nanoparticles and duplex specific nuclease	2×10^{-15} to 10^{-12}	10^{-15}	13
electrochemistry	gold nanoparticles@copper metal-organic frameworks	10^{-15} to 10^{-8}	3.5×10^{-16}	14
electrochemiluminescence	DNAzyme and rolling circle amplification	10^{-15} to 10^{-10}	3×10^{-16}	15
electrochemiluminescence	the assembly of the DNA tweezer	10^{-16} to 10^{-11}	3×10^{-17}	16
electrochemistry	cascade strand displacement polymerization	10^{-17} to 5×10^{-15}	3.2×10^{-18}	this work

Table S3 Electrochemical detection of miRNA added in human serum samples.

Sample	Added (fM)	Found (fM)	Recovery (%)	RSD (%)
1	10	10.15	101.5	4.12
2	50	49.21	98.42	3.55
3	100	104.51	104.51	3.24
4	500	510.26	102.05	4.73
5	1000	978.30	97.83	3.71