Supplementary Information

Target-assisted self-cleavage DNAzyme electrochemical biosensor for MicroRNA detection with signal amplification

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Materials and Reagents. All oligonucleotides, TE buffer (1×, low EDTA, PH 8.0), 5×TBE buffer (250 mM Tris, 10 mM EDTA, 250 mM H_3BO_3, pH 8.0), and agarose (conventional) were purchased by Sangon Biotech Co., Ltd. (Shanghai, China). Chloroauric acid ($HAuCl_4 \cdot 3H_2O$, AR), magnesium chloride ($MgCl_2$, AR), potassium chloride (KCl, AR), hexamercaptan (HT, 96%), disodium hydrogen phosphate (Na_2HPO_4, AR), potassium dihydrogen phosphate (KH_2PO_4, AR), potassium ferricyanide ($K_3[Fe(CN)_6$, AR), potassium ferrocyanide ($K_4[Fe(CN)_6$, AR). Alumina polishing powder was purchased from Tianjin Aida Hengsheng Technology Development Co., Ltd. (Tianjin, China). A 5.0 mM $[Fe(CN)_6]^{3-/4-}$ solution (5.0 mM $K_3[Fe(CN)_6]$, 5.0 mM $K_4[Fe(CN)_6]$, 0.1 M KCl, pH 7.4) was used as the supporting electrolyte for cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) detection. Prepare square wave voltammetry (SWV) detection buffer with PBS. The aqueous solution was prepared from ultrapure water (Shanghai Hetai Instrument Co., Ltd.) obtained by the Hitech-Sciencetool water purification system. Oligonucleotide sequences were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China), and the sequences were listed in Table S1.

Apparatus. In this work, all electrochemical measurements were performed at the CHI 660E electrochemical workstation (Shanghai Chenghua Instrument Co., Ltd.) using a conventional three-electrode system, in which the glassy carbon electrode (GCE, Φ = 4 mm) was the working electrode, the Ag/AgCl electrode (saturated KCl) was the reference electrode, and the platinum wire was the auxiliary electrode. The electrodes used in the experiment were purchased from Tianjin Aida Hengsheng Technology Development Co., Ltd. (Tianjin, China). 4% agarose gel electrophoresis results were obtained in JY300E.

Construction of Biosensor. First, the Φ = 4 mm glassy carbon electrode (GCE) was polished by aluminum oxide powder (0.3 μM and 0.05 μM) and ultrasonicated in ethanol and water for 1 min, respectively. Then, in order to modify the electrode, the pretreatment GCE electrodeposited -0.2V for 30 s in 1 % $HAuCl_4$ solution for the formation of Au nanoparticles (dep-Au) to obtain Au nanoparticles modified layer (named GCE/dep-Au). In sequence, with the help of Au-S bond, probe DNAzyme (10

μL, 3 μM) was successfully incubated on the dried electrode overnight at 4 °C. Separating excess substances with distilled water, then the resultant electrode was incubated with HT (10 μL, 5 mM) for 30 min for blocking the nonspecific sites. At last, the assembled electrode was washed with distilled water to remove unbound substances for further use.

Table S1. The sequences are listed below as text sequences annotated with segment names.

Names	Sequence (5′ to 3′)
DNAzyme	GGGGTATTTAACTrAGGTCTTTTTTTTTGACTCCGAGCCGGACGA AGTTCAAACTGACATTTTTTTTTT
Dual-labeled DNAzyme	Fc-GGGGTATTTAACTrAGGTCTTTTTTTTTGACTCCGAGCCGGACGA AGTTCAAACTGACATTTTTTTTTT-SH
miRNA-223	TGTCAGTTTGTCAAATACCCCA
miRNA-122	TGGAGTGTGACAATGGTGTTTG
miRNA-21	TAGCTTATCAGACTGATGTTGA
miRNA-155	TTAATGCTAATCGTGATAGGGGT
miRNA-16	TAGCAGCACGTAAATATTGGCG

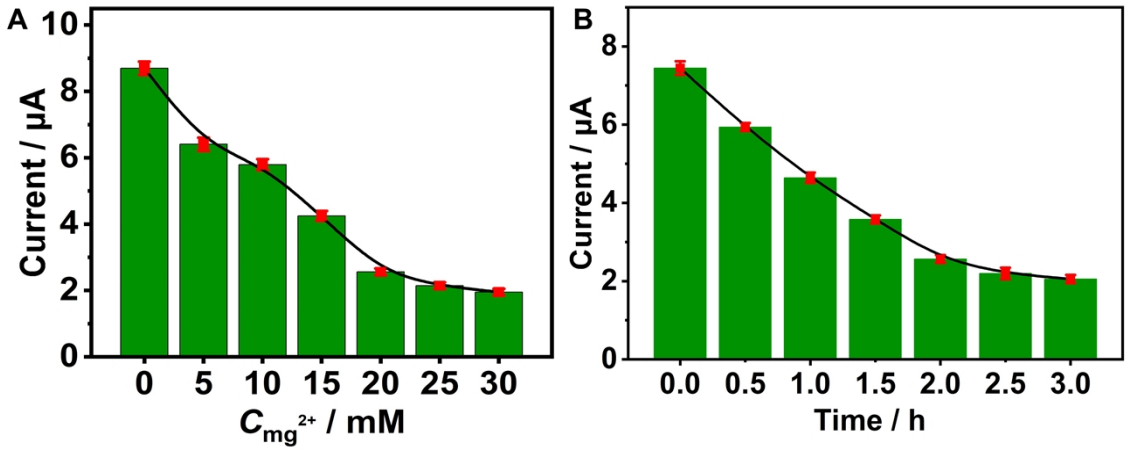

Figure S1. Effects of (A) Mg^{2+} concentration and (B) reaction time on the electrochemical responses. The SWV signals were monitored with 50 nM miRNA-223. Error bars: SD, n = 3.

Calculation of LOD

According to related references and the IUPAC recommendation[1-3], the limit of detection (LOD) was be estimated as LOD = kS_b/m, in which S_b was the standard deviation of the blank signals (n_b = 20), m was the analytical sensitivity which could be estimated as the slope of calibration curve at lower concentration ranges and k is the numerical factor chosen in accordance with the desired confidence level. As suggested by Long and Winefordner[4], the use of k = 3 allows a confidence level of 99.86% for a normal distribution of the blank signals. So, LOD was usually defined as LOD = 3S_b/m. Firstly, to calculate the LOD of the AND measuring automata, the trends of current change values with the concentration of miRNA-223 was showed. As shown in Figure S2, the insert presented that current change values (ΔI) were linearly related to the concentration of miRNA-223 (c / fM) at a low concentration range. The corresponding linear equation was ΔI = -0.1150 c + 6.8066 and the S_b of twenty times zero-dose was about 0.07351. Therefore, the LOD of the proposed measuring biosensor were 1.92 fM (LOD = 3 × 0.07351 ÷ 0.1150 = 1.92 fM).

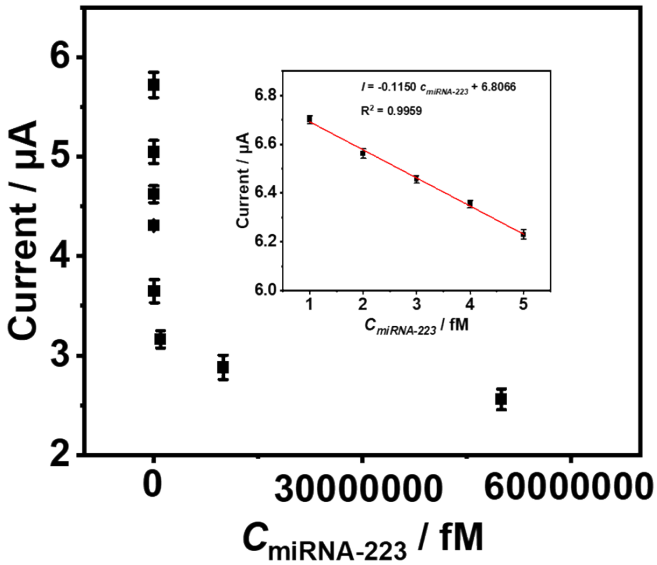

Figure S2. (A) The variation trend of electrochemical sensing system responses with miRNA-223 concentration. The inset shows the calibration curve of current change responses changing with the target in the lower concentration range.

Table S2. Comparison of different methods for miRNA detection

detection methods	detection range	detection limit	ref
Fluorescence	0.2 nM~10 nM	Not mentioned	5
Fluorescent	50 fM~30 nM	269 fM	6
Fluorescence	100 fM-100 nM	Not mentioned	7
surface-enhanced Raman scattering (SERS)	1 fM to 100 nM	374 aM	8
Fluorescence	2~25 nM	0.45 nM	9
ECL	10 fM~50 nM	1.92 fM	This work

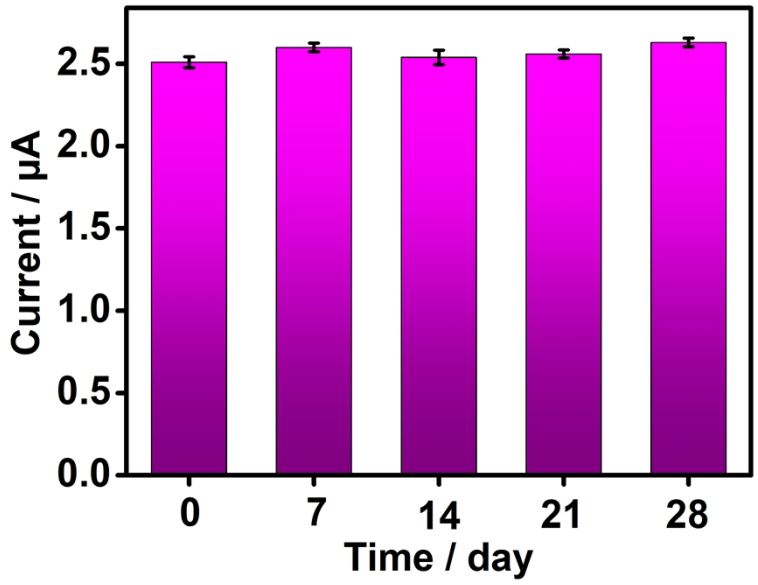

Figure S3. Stability of the designed strategy. Error bars: SD, n = 3.

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