Electronic Supplementary Information (ESI) for Chemical Communications

Cross-triggered and cascaded recycling amplification system for electrochemical detection of circulating microRNA in human serum

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Experimental Section

Materials: Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), $HAuCl_4 \cdot 4H_2O$ and 6-mercapto-1-hexanol (MCH) were bought from Sigma-Aldrich (St. Louis, MO, USA). High performance liquid chromatography-purified RNA and DNA sequences listed in Table S1 were provided by Sangon Biotechnology Co., Ltd. (Shanghai, China). Choline chloride (Ch) was purchased from Shanghai Chemical Co. Ltd. (Shanghai, China). All chemical reagents were of analytical grade, and aqueous solutions were prepared or diluted with diethyprocarbonated-treated water for RNA assays.

Pretreatment of the sensing interface: A gold nanoparticles-modified glassy carbon electrode (AuNPs/GCE, Φ = 3 mm) was prepared as follows. Briefly, the GCE was polished with alumina oxide slurry, and sonicated with distilled water and ethanol for 5 min, respectively. Then, the GCE was scanned from −1.7 V to 1.8 V for six cycles in Ch

(1.5 mM) containing 0.1 M KCl at a scan rate of 20 mV s^{-1}, and then rinsed with water to remove residual Ch. Finally, the Au nanoparticles were deposited on Ch/GCE by cyclic voltammetry (CV) scans in 0.1 M Na_2SO_4 containing 0.30 mg mL^{-1} $HAuCl_4$ with a potential range from 0.2 V to −1.0 V for 10 cycles at 25 mV s^{-1}.

Operation of the sensing system: Prior to probe immobilization, the SH-SP (0.3 μM) and Sub (0.3 μM) were hybridized for 60 min in 20 mM Tris-HCl buffer (140 mM NaCl, 5 mM KCl, pH 7.5) to yield DSP, followed by reduction of the disulfide bond of SH-SP by incubation with TCEP (10 mM) for 60 min. Then, the AuNPs/GCE was incubated with the DSP complex (10 μL) overnight. Next, MCH (1 mM) was incubated with the DSP/AuNPs/GCE interface for 2 h to block the electrode, and washed with 10 mM phosphate-buffered saline (PBS, 150 mM NaCl, 10 mM $MgCl_2$, pH 7.5) to yield MCH/DSP/AuNPs/GCE. The MCH/DSP/AuNPs/GCE was subsequently incubated in solutions containing AP1 (0.3 μM) and AP2 (0.3 μM) with different concentrations of miRNA 122 for 80 min in 10 mM PBS. After rinsing with PBS, the sensing interface was subjected to electrochemical measurements.

Apparatus: Scanning electron microscope (SEM) photographs of the AuNPs were obtained using a SU8010 scanning electron microscopy (Hitachi, Japan). CV and square wave voltammetry (SWV) were performed using a CHI 660E electrochemical workstation (CH Instruments, Shanghai, China). The electrochemical cell consisted of a modified working electrode, a platinum wire counter electrode, and an Ag/AgCl reference electrode was applied for all electrochemical experiments. CVs were performed in $[Fe(CN)_6]^{3-/4-}$ solution (1 mM, 0.1 M KCl) from 0.6 V to −0.1 V at 50 mV s^{-1}. SWV measurements were carried out from −0.5 V to 0 V with an amplitude of 25 mV, a frequency of 25 Hz, and a step potential of 4 mV in 10 mM PBS.

miRNA extraction from human serum samples: Circulating miRNAs containing miRNA 122 were extracted from healthy human serum and liver injury patient serum samples using miRNeasy RNA isolation kits according to the Qiagen's instructions. In brief, serum sample (200 μL) was firstly added into Qiazol solution (1.0 mL), followed by incubation under stirring at room temperature for 5 min to dissociate nucleoprotein complexes. Then, chloroform (200 μL) was further added into the mixture and vigorously vortexed for 30 s. After centrifugation at 12 000 × g for 15 min at 4 °C, purification of miRNA in upper phase was completed, and the precipitation was performed based on the recommended protocol.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) experiments: Extracted RNA samples were converted to cDNA using the Revert Aid Premium Reverse Transcriptase Reverse Transcription Kit (Thermo) based on the manufacturer's instructions. SMA4000 spectrophotometer (Merinton Instrument, Inc.) was used to detect the concentration of extracted total RNA. The reaction conditions were listed as follows: 10 min at 20 °C, 30 min at 50 °C for reverse transcription, and 85 °C for 5 min to stop the reaction. Subsequently, the produced cDNA samples were further analyzed by qRT-PCR experiments, which were carried out on a Step One Plus real-time PCR instrument using the Fast qPCR Master Mix kit. The reaction system was incubation at 95 °C for 180 s, followed by 45 cycles of 95 °C for 5 s and 60 °C for 30 s.

Live subject statement: The human serum related experiments were performed in accordance with the WHO guidelines on blood drawing (WHO Publication ISBN-13: 978-92-4-159922-1, 2010) and were approved by the Ethics Committee of the affiliated hospital of Xuzhou Medical University. All of human subjects signed an informed consent form before detection.

Supplementary Tables and Figures

Table S1 Oligonucleotide sequences used in the proposed system

Oligonucleotide	Sequence (5′-3′)
Signal probe (SH-SP)	AAC ACC ATT TTT TTT T(SH) TTA GGT CTT GGA TTT CGA CC-MB
Substrate (Sub)	TGG AGT GTG ACA ATG GTG TTT GCA AT rA G ATG CAT TCC GAG CCG GTC GAA ATT GCA
Assistant probe 1 (AP1)	ATG CAT TCC GAG CTC ACA CTC CA
Assistant probe 2 (AP2)	CAA ACA CCA TTG CGG TCG AAA TTG CA
miRNA 122	UGG AGU GUG ACA AUG GUG UUU G
Single-base mismatched miRNA (sRNA)	UGG AGU GUU ACA AUG GUG UUU G
Non-complementary miRNA (nRNA)	UUC UCC GAA CGU GUC ACG UTT
miRNA 26a	UUC AAG UAA UCC AGG AUA GGC U
miRNA 141	CAU CUU CCA GUA CAG UGU UGG A

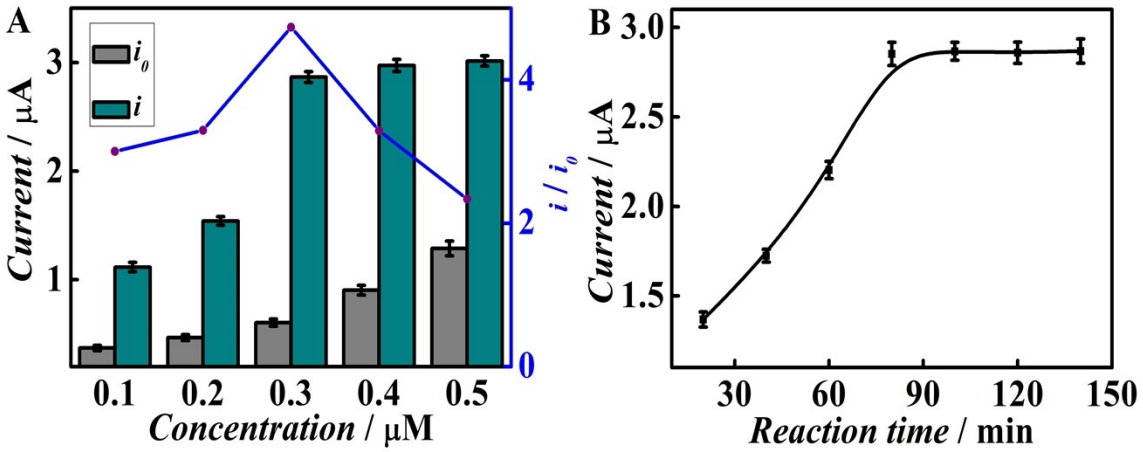

Fig. S1 (A) Effects of DSP concentration on the current response, background response, and signal-to-background ratio (i/i_0, blue curve, corresponding to the right Y axis). (B) Effect of reaction time on the current response to miRNA 122 (1 pM).

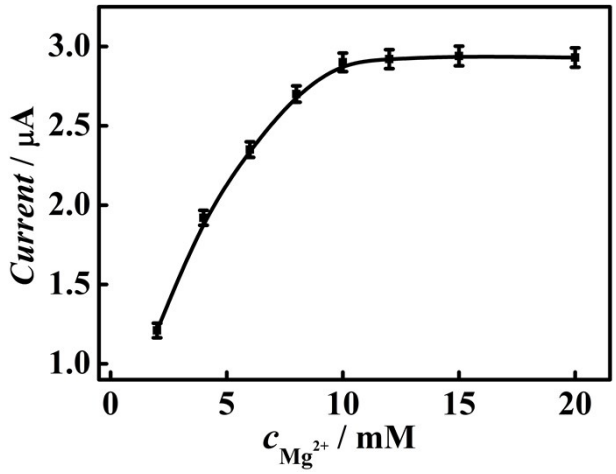

Fig. S2 The effect of Mg^{2+} concentration on the current response of 1 pM miRNA 122.

Table S2 Comparison of analytical parameters of this work with reference works for miRNA analysis

Detection technique	Linear range	Detection limit	Reference
Electrochemical	5 fM−100 pM	2.2 fM	S1
Electrochemical	10 fM–10 nM	1.1 fM	S2
Electrochemical	0.4 nM–140 nM	0.25 nM	S3
Electrochemical	0.1 pM–1 nM	30 fM	S4
Electrochemical	1.0 fM–2.0 pM	58 aM	S5
Electrochemical	10 fM–1000 fM	4.53 fM	S6
Electrochemical	10 fM–2 pM	5 fM	S7
Electrochemical	1.0 pM–10.0 nM	0.26 pM	S8
Electrochemical	100 fM–1 nM	78 fM	S9
Electrochemical	10.0 fM–0.1 nM	2.3 fM	S10
Electrochemical	1 fM–100 pM	0.34 fM	This work

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