Paper-Based Bipolar Electrode Electrochemiluminescence Platform for Detection of Multiple miRNAs

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Table of contents

S1. Reagent and Instrument

S1.1 Reagent

Cadmium Chloride ($CdCl_2$), 3-Mercaptopropionic acid (MPA), sodium tellurite (Na_2TeO_3), trisodium citrate dihydrate, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), N-Hydroxy succinimide (NHS), melamine, potassium persulfate ($K_2S_2O_8$) hydroxylammonium chloride and gold chloride ($HAuCl_4$), were obtained from Shanghai Aladdin biochemical technology co., LTD. (Shanghai, China). 6-Mercapto-1-hexanol (MCH) and monoethanolamine (MEA) were provided by Shanghai yi 'en chemical technology co., LTD. (Shanghai, China). H_2O_2 was purchased from Shanghai vok biotechnology co., LTD. (Shanghai, China). The used chemicals are not required to be repurified and can be used according to the original analytical grade purchased. 0.1 M phosphate buffer solution (PBS) was prepared by mixing Na_2HPO_4 and KH_2PO_4 stock solution in appropriate proportion. All solutions were freshly prepared using ultrapure water (resistivity ≥ 18 MΩ·cm) as a solvent. Whatman chromatography paper 1 was purchased from GE Healthcare Worldwide (Shanghai, China). The biological chains (DNA and RNA fragments) used in the study were supplied by Sangon (Shanghai, China). The sequences of the DNA probes and RNA targets used in this study were presented as follows:

Table S1

name	sequences (from 5′ to 3′)
H1	TAATCGTGATAGGGGTATGGACATGGAACCCCTATCACGATTAGCATTAAAGA-NH_2
H2	ATGGACATGGATAATCGTGATAGGGGTTCCATGTCCATACCCCTATGAAGGAGCG ACT-NH_2
S1	HS-CTCTGCCCTCCTTCCTAGCCGGATCGCGCTGGCCAGATGATATAAAG
S2	HN-CTTTATATCATCTGGCCAGCGCGATCCGGCTAGGAAGGAGGGCAGAAG
h1	GCATTTTGGTATTATTCTATCTGATTCTTAGTGTGTACCAAAAGTAATAATG-NH2
h2	GCATTTTGGTATTATTCTATCTGATTCTTAGTGTGTACCAAAAGTAATAATG
DNA1	NH2-TTTTGATGTGGCTGAGGATGTACCGTTTTTTTTGGG-SH
DNA2	SH-CCCAAAAAAACCATCCTCA
miRNA-126	CAU UAU UAC UUU UGG UAC

miRNA-155	UUAAUGCUAAUCGUGAUAGGGGU
miRNA-141	UAA CAC UGU CUG GUA AAG AUG G
miRNA-101	UACAGUACUGUGAUAACUGAA
miRNA-210	CU GUG CGU GUG ACA GCG GCU GA

S1.2 Instrument

The DC power supply (MS-305D) was provided by Dongguan Maihao Electronic Technology Co., Ltd. (Guangdong, China). CHI 760D Electrochemical Workstation was purchased from Shanghai Chenhua Instrument Co., Ltd., (Shanghai, China), which provide electrochemical impedance spectroscopy (EIS). ECL emission measurements were monitored with an MPI-A multifunctional ECL analytical system (Xi'an Remax Electronic Science & Technology Co. Ltd., Xi'an, China). The UV-visible (UV-vis) absorption spectra were tested on a UV-2550 ultraviolet-visible spectrophotometer (Shimadzu, Japan). Scanning electron microscopy (SEM) images, energy dispersive spectroscopy (EDS) and mapping were acquired with a scanning electron microscope (regulus 8100) (Hitachi hi-tech company, Japan). The transmission electron microscopy (TEM) images were obtained with a JEOL 4000 EX microscope. X-ray diffraction (XRD) were tested on a D8 advance diffractometer system equipped with Cu Ka radiation (Bruker Co., Germany). The two driving electrodes in the paper chip are made from screen-printed graphite.

S2. The AI design picture and actual photos of the closed parallel bipolar electrode paper chip

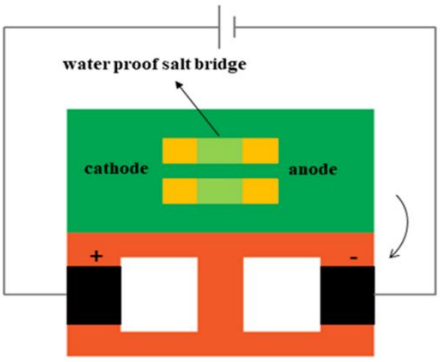

Figure S1. Fabrication of the paper-based closed-bipolar electrode device for miRNA-

155 and miRNA-155 assay.

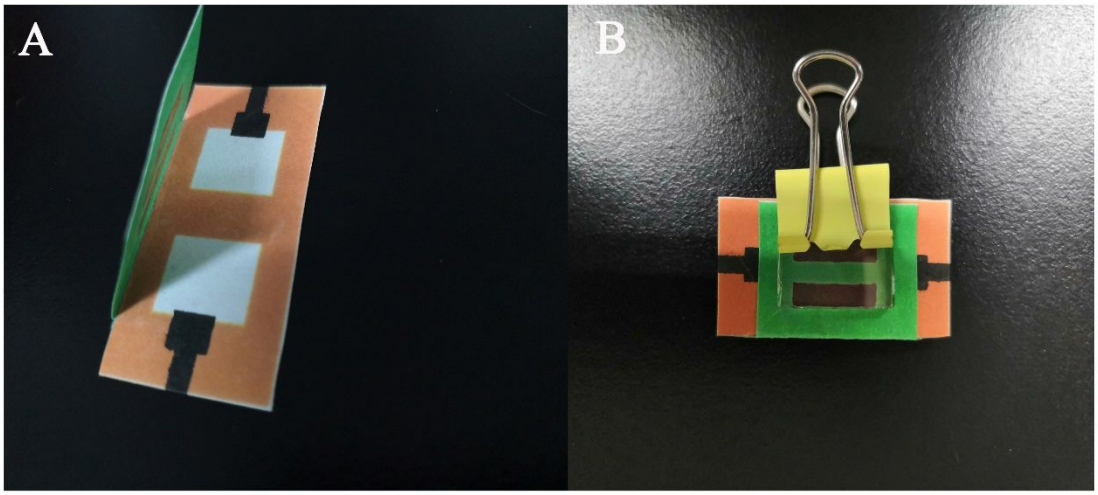

Figure S2. The real sample's figure of paper-based closed bipolar electrode

S3. Synthesis of materials.

S3.1 Synthesis of CdTe QDs-H2.

Firstly, EDC (15 μL, 0.2 M) and NHS (15 μL, 0.05 M) were mixed with H2 (50 μL, 5 μM) to activate the amino groups of H2. Then, 10 μL of CdTe QDs (0.05 M) were added to the above mixture solution and reacted at room temperature for 12 h. Finally, the CdTe QDs-H2 signal probe labeled with luminescent reagent was synthesized.

S3.2 Synthesis of Fe3O4-H1-S2 Magnetic Nanomaterial.

The aptamer oligonucleotides were first denatured at 95 °C for 5 min to form a hairpin structure before use. First of all, prepare the purchased carboxylated Fe_3O_4 magnetic nanoparticles. H1 (200 μL, 2 μM) and S2 (100 μL, 2 μM) were added into the 500 μL of Fe_3O_4 solution, and then placed the above mixture on a shaker and reacted at 25 °C for 12 h. In order to make H1, S1 and Fe_3O_4 magnetic nanoparticles combine fully through the interaction between amino and carboxyl groups loaded by themselves. The next, 1 mM MCH was used to block the nonspecific binding sites at room temperature for 2 h. Fe_3O_4-S2-H1 magnetic nanomaterials were separated from the solvent for three times by magnetic separation to remove unreacted reagents. Eventually, the magnetic nanocomposites were dispersed in ultra-pure water for further

use.

S3.3 Synthesis of Au@g-C3N4 NSs -DNA1

By slightly improving the experimental method of traditional preparation of g-C_3N_4 NSs[1-3], small-sized g-C_3N_4 NSs (Figure S3) was obtained. First, $HAuCl_4$ (20 μL, 0.01 M) solution is added to the prepared g-C_3N_4 NSs (4 mL) suspension. The mixed solution was treated with ultrasonic for 10 min followed by stirring 100 min at room temperature and repeated for 3 times. After that, the freshly prepared $NaBH_4$ (50 μL, 0.01 M) was quickly added to the above suspension to stir 30 min. Then, 10 μL of 0.01 M sodium citrate solution was added to the suspension and stirred for 30 min. Au@g-g-C_3N_4 NSs solution was obtained by 3~4 times centrifugal washing and dispersed in 2ml ultra-pure water. Finally, 10 μL Au@g-C_3N_4 NSs solution was mixed with DNA1 (50 μL, 2 μM) at room temperature for 2 h. The Au@g-C_3N_4 NSs-DNA1 signal probe labeled with luminescent reagent was synthesized.

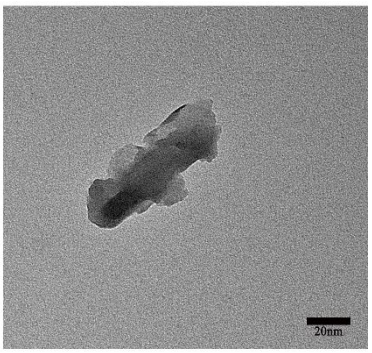

Figure S3. The TEM of g-C_3N_4 NSs.

S4. Condition optimization and electrochemical characterization of the potential-resolved bipolar electrode electrochemiluminescence biosensor.

In the typical BPE biosensor system, the ECL intensity of the luminescence system CdTe QDs-H2/$S_2O_8^{2-}$ ECL and Au@g-C_3N_4 NSs-DNA1/$S_2O_8^{2-}$ is closely related to the electric field intensity between the driving electrodes. As shown in Figure S4A, when the driving voltage was 9V, the ECL intensity of the CdTe QDs-H2/$S_2O_8^{2-}$system reached the maximum intensity. As shown in Figure S4B, when the driving voltage was 12V, the ECL intensity of the Au@g-C_3N_4 NSs-DNA1/$S_2O_8^{2-}$ system reached the

maximum intensity.

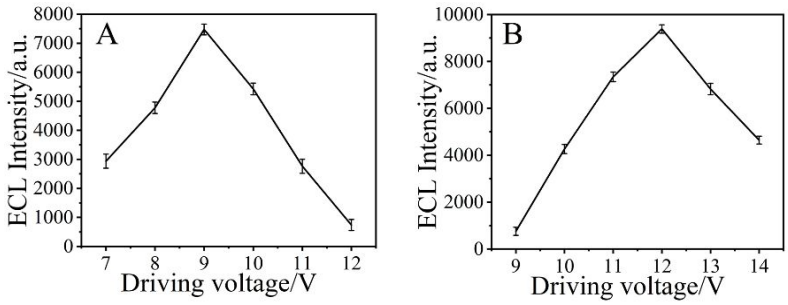

Figure S4. Optimization of experimental conditions for the proposed biosensor (A) The influence of drive voltage on CdTe QDs-H2/ $S_2O_8^{2-}$ ECL and (B) Au@g-C_3N_4 NSs-DNA1/ $S_2O_8^{2-}$ ECL

The sensitivity of the sensor to detect the target is greatly influenced by the material of the bipolar electrode. As shown in Figure S5A, it was obvious that the complete Au-BPE was the best choice for this BPE-ECL biosensor. Figure S5B shows that the EIS curve of the gradual modification of the detection in the process of detecting miRNA-155, in which the BPE cathode surface using $[Fe(CN)_6]^{3-/4-}$ as an electroactive probe consists of a semicircle in a higher frequency range and a straight line in a lower frequency range. In the high frequency region, the bare paper BPE is semicircular (Figure S5B, curve b), AuNPs is the improved BPE conductivity greatly increased, charge transfer resistance (R_{et}) decreased significantly (Figure S5B, curve a). The diameter of the semicircle increases gradually, and the electron transfer resistance R_{et} increases step by step (Figure S5B, curve c, d, e), which proves the successful modification of S1, MCH and Fe_3O_4-S2-H1-H2-CdTe QDs respectively.

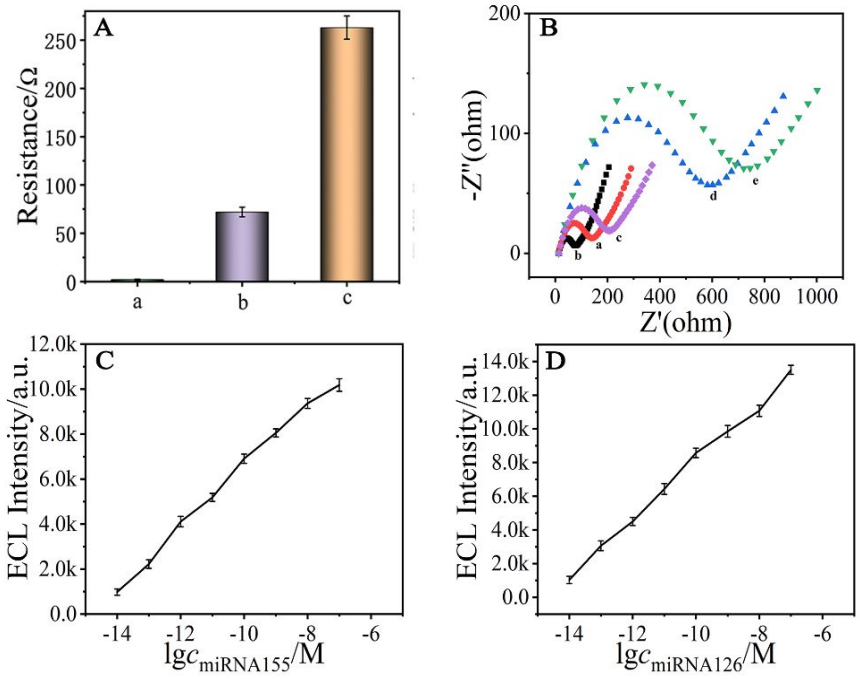

Figure S5. (A) Optimization of bipolar electrode materials (a) AuNPs were all grown in the BPE region, (b) the BPE area is screen-printed with graphite electrodes loaded with gold at both ends, (c) screen printed graphite electrode in BPE area. (B) EIS of electrodes with different modified in PBS (0.1 M pH 7.4) including 5.0 mM $[Fe(CN)_6]^{3-/4-}$ (a) bare BPE, (b) Au-BPE, (c) S1/Au-BPE, (d) MCH/ S1/Au-BPE, (e) Fe_3O_4-S2-H1-H2-CdTe QDs/ MCH/ S1/Au-BPE. (C)The logarithmic relationship between the concentration of the miRNA-155 and the electrochemiluminescence intensity. (D)The logarithmic relationship between the concentration of the miRNA-126 and the electrochemiluminescence signal.

Table S2

Performance of miRNA detection contrasted with other works.

analytical method	detection limit	linear range	Refs.
Fluorescence	2.3 pM	5 pM ~ 0.5 nM	[4]
Electrochemical	0.6 pM	1 pM ~ 25 nM	[5]
Fluorescence	0.41 nM	0.5 nM ~ 100 nM	[6]
photoelectrochemistry	0.31 pM	1 pM ~ 100 nM	[7]
Chronoamperometry	3 pM	10 fM ~ 5 pM	[8]
electrochemiluminescence	5.7 fM /4.2 fM	100 nM ~ 10 fM	this work

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