Electronic Supplementary Information (ESI)

Highly sensitive "signal-on" electrochemiluminescent biosensor for the detection of DNA based on dual quenching and strand displacement reaction

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1. Experimental section

1.1 Reagents and materials. Tris(hydroxymethyl) aminomethane (Tris) and Mercaptoacetic acid (MPA 97%) were purchased from Alfa Aesar Company. 6-mercapto-1-hexanol (MCH), N-hydroxysuccinimide (NHS, 98%), Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC) were purchased from Sigma-Aldrich Company. Cadmium chloride ($CdCl_2 \cdot 2.5H_2O$) was purchased from Shanghai Reagent Co., Ltd. (Shanghai, China). Selenium metal powder (≥99.95%) was obtained from Shanghai Meixing Chemical Co., Ltd. (Shanghai, China). Fetal bovine serum was purchased from Sunshine Biotechnology (Nanjing) Co., Ltd. All chemicals were of analytical reagent grade. Tris-HCl buffer (50mM, pH 7.4) containing 0.1 M $K_2S_2O_8$ and 0.1 M KCl was used as the test solution. All solutions were prepared with ultrapure water (18.2 MΩ·cm) from a Milli-Q purification system (Bedford, MA). DNA strands were purchased from Sangon Biological Engineering Technology & Company Ltd. (Shanghai, China) and purified with high-performance-liquid chromatography. Their base sequences were listed as below.

Probe DNA: 5′-NH_2-$(CH_2)_6$-AAAGAACGAGAGTCTTTCTC-$(CH_2)_3$-SH-3′

Helper DNA: 5′- TGGTTCTCGTTCTTT-Fc-3′

Target DNA: 5′-CAGAAAGACTCTCGTTCTTT-3′

M1: 5′-CAGAAAGACT***G***TCGTTCTTT-3′

M2: 5′-CAGAAAGACT***G***TCGT***A***CTTT-3′

M3: 5′-CAGAA***T***GACT***G***TCGT***A***CTTT-3′

The underlined bold letters represent the complementary sequences. The italic bold letters represent the mismatched bases

1.2 Apparatus. The ECL emission was detected with an MPI-A electrochemiluminescence analyzer (Xi′An Remax Electronic Science &Technology Co. Ltd., Xi′An, China). All experiments were carried out at room temperature using a conventional three-electrode system with a modified gold electrode (GE) (2 mm diameter) as working electrode, a platinum wire as the auxiliary electrode and an Ag/AgCl electrode as reference electrode. Ultraviolet-visible (UV-vis) absorption spectra were obtained on Cary 60 spectrophotometer (Agilent, USA). PL spectra were recorded on Fluoromax-4 Spectrofluorometer (USA). Electrochemical impedance spectroscopy (EIS) was carried out with an Autolab potentiostat/galvanostat PGSTAT302N (Eco chemie, BV, The Netherlands) in KCl solution (100 mM) containing a $K_3Fe(CN)_6/K_4Fe(CN)_6$ (5.0 mM, 1:1) mixture as a redox probe from 0.1 Hz-100KHz with a signal amplitude of 10 mV.

1.3 Synthesis of Water-soluble CdSe QDs. The CdSe QDs were synthesized according to the previous report[S1] with a slight modification. Briefly, after 50 mL deoxygen deionized water containing 2 mM $CdCl_2$ was mixed with 20 μL MPA, 1 M NaOH solution was added to adjust its pH value to 10.0. The mixture was bubbled with highly pure nitrogen for 30 min. Then freshly prepared NaHSe solution (0.7 mL, 70 mM) was injected into the mixture to obtain a clear light yellow MPA-CdSe QDs solution after refluxed at 100 ˚C for 4 h. After cooling under N_2 atmosphere, the obtained solution was stored at 4 ˚C.

1.4 Fabrication of ECL DNA Biosensor. The GE was polished carefully with 0.05 μm α-Al_2O_3 powders on chamois leathers and washed ultrasonically with water and ethanol, respectively. Then the electrode was electrochemically cleaned in 0.5 M H_2SO_4 solution with scanning from -0.3 and 1.6 V, followed by drying under nitrogen. 4 μL probe DNA (1 μM) was dropped on the GE surface and kept overnight at 4 ˚C, followed by washing to remove non-grafted DNA. Afterwards, the probe DNA modified GE was immersed into 1

mM MCH solution for 1 h to block the uncovered surface and reduce the nonspecific adsorption. Subsequently, 4 μL CdSe QDs solution and 10 μL EDC-NHS solution (5 mM EDC, 10 mM NHS in 10 mM Tris-HCl buffer, pH 7.4) were dropped onto the electrode surface sequentially. Followed by keeping at room temperature for 1 h, carboxyl groups on the surface of CdSe QDs were activated with EDC and NHS, and further implemented the attachment with amino groups at the terminal of probe DNA. Finally, 4 μL 1.5 μM helper DNA was dropped onto the electrode and incubated at 37 ˚C for 1 h. After rinsing with 0.01 M Tris-HCl buffer, the ECL DNA biosensor was obtained.

1.5 Detection of Target DNA. 5 μL different concentrations of target DNA were applied to the as-prepared GE and incubated at 37 ˚C for 75 min. Subsequently, the modified GE was swilled thoroughly to remove unbounded target DNA and transferred into Tris-HCl buffer saline (0.05 M, pH 7.4) containing 0.1 M $K_2S_2O_8$ and 0.1 M KCl to record the ECL response between 0 and -1.5 V at a 100 mV s^{-1}. The emission window was placed in front of the photomultiplier tube, which was biased at 800 V.

2. ECL behavior

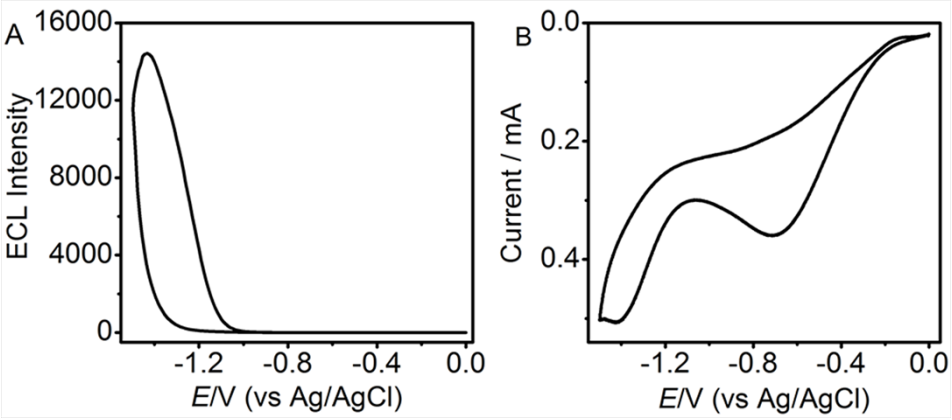

Fig. S1. (A) ECL-potential curve and (B) CV curve of CdSe modified GE in 50 mM Tris-HCl buffer containing 0.1 M $K_2S_2O_8$ and 0.1 M KCl.

3. Optimization of the detection conditions

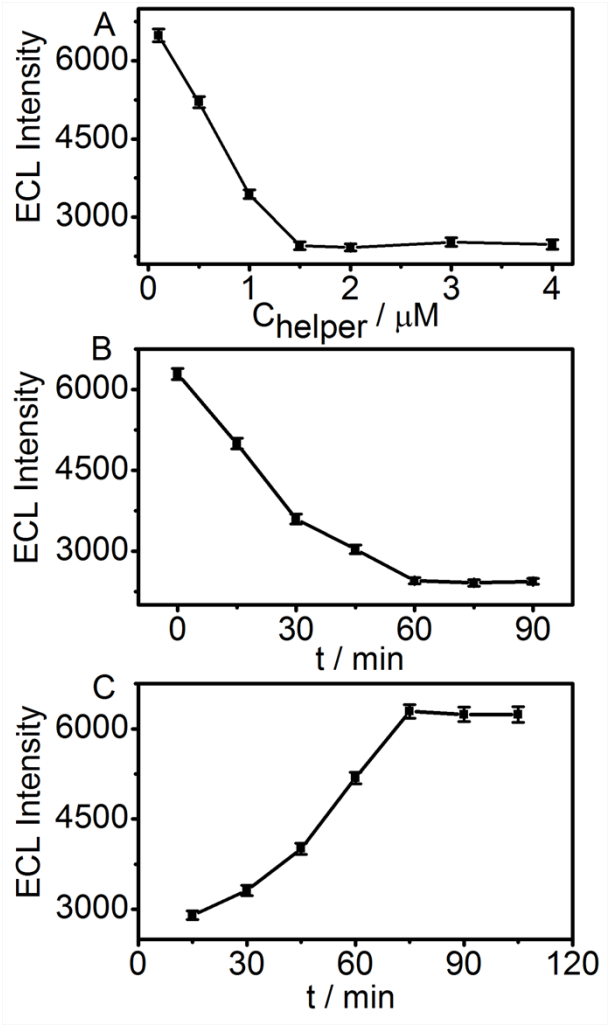

Fig. S2. Effects of (A) the concentration of helper DNA, (B) incubation time of helper DNA and (C) incubation time of target DNA on the ECL response of the DNA biosensor in 50 mM Tris-HCl buffer containing 0.1 M $K_2S_2O_8$ and 0.1 M KCl. The concentration of target DNA was 10 fM.

References

S1 X. X. Zeng, J. C. Bao, M. Han, W. W. Tu and Z. H. Dai, *Biosens. Bioelectron.* 2014, **54**, 331.