Supporting Information

Autocatalytic Amplified Detection of DNA Based on CdSe Quantum Dots/Folic Acid Electrochemiluminescence Energy Transfer System

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Table S1. The Sequences of the DNA in Scheme 1

DNA	Sequence
Target DNA1:	5’- GGA CAG AGTCTC-3’
DNA2：	5’- GAT ATC AGC GAT GAA GAC TC-3’
DNA3:	5’- GAGA CTC TGT CCG AGT CTT CCA CCC ATG TTA CTC T -3’
DNA4:	5’- NH_2-GCT GGA CAG AGT ATrA GGA TAT CAA TTT TTT TTT TTT AGT CCA GC-SH- 3’
single-base mismatched	
DNA1:	5’- CGA CAG AGT-3’
noncomplementary DNA1:	5’- CCT GTC TCA-3’

2. Experimental section

2.1. Materials and apparatus

2.1.1. Materials

$CdCl_2 \cdot 2.5H_2O$ (98%), selenium (99.9%, powder), $NaBH_4$ (GR, 99.8%), mercaptoacetic acid (AR, 99.8%), folic acid (AR, 97%), $MgCl_2$ (AR, 98%), Tris (AR, 99%), $K_2S_2O_8$ (AR, 99.5%), KCl (AR, 99.98%) and EDTA（AR, 99.9%）were purchased from Aladdin Industrial Corporation, Multi-walled carbon nanotubes (CNTs, CVD method, purity >95%, diameter 30–60 nm, length 0.5–15 μm) were purchased from Nanoport. Co. Ltd. (Shenzhen, China). Fifth-generation PAMAM dendrimers (ethylenediamine core, generation 5) were purchased as methanol solutions from Sigma-Aldrich (Shanghai) Trading Co., Ltd. All other reagents were of analytical grade. Double distilled water was used for all experiments. Phosphate buffer solutions (PBS, 0.1 M) at various pH values were prepared by mixing different amounts of stock solutions of NaH_2PO_4 and Na_2HPO_4. 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), and imidazole were obtained from Sigma. All of synthetic oligonucleotides were purchased from

Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (China). Sequences of the oligonucleotides are listed in Table S1.

2.1.2. Apparatus

Electrochemical and ECL measurements were carried out on a MPI-A ECL analyzer (Xi'An Remax Electronic Science & Technology, Xi'An, China) using a three-electrode system. The electrodes were an Au disk working electrode, a saturated calomel reference electrode, and a Pt counterelectrode. The spectral width of the photomultiplier tube (PMT) was 200–800 nm and the voltage of the PMT was 500–800V in the detection process. Transmission electron microscopy (TEM) images were recorded using a JEOL JSM-6700F instrument (Hitachi). Photoluminescence (PL) spectra were obtained on an RF-540 spectrophotometer (Shimadzu). Field-emission scanning electron microscopy (FE-SEM) was carried out on a JEOL JSM-6700F instrument.

2.2. Preparation of Water-Soluble Quantum Dots

Briefly, 0.05 g of selenium powder and 0.037 g of sodium borohydride were added to a small flask, then 4 mL of ultrapure water was added. The solution was degassed, refilled with nitrogen and heated to 80 ℃. After the selenium powder disappeared completely, the resulting clear NaHSe of 0.1 M was obtained.

Colloidal CdSe-COOH nanocrystals were prepared as described with a slight modification. Briefly, freshly prepared 0.1 M NaHSe solution was added to 1.25 mM N_2-saturated $CdCl_2$ solution, and the pH was adjusted to 11, then 200 μL of mercaptoacetic acid as the stabilizing agent was added. The molar ratio of Cd^{2+}/ TGA /NaHSe was fixed at 1:2.4:0.5. After the mixture was vigorously stirred for 10 min, it was refluxed for 3 h to control the growth of the CdSe nanocrystals. The TGA-capped QDs were extracted by centrifugation at 6000 rpm, purified twice with ethanol, and finally dissolved into 10 mL water of pH 8. The water-soluble QDs were obtained.

2.3. DNAzyme-mediated Amplified detection of DNA based on ECL quenching of CdSe QDs by folic acid

Preparation of the hairpin DNA4- folic acid quencher probe. Folic acid (4.4 mg, 10^{-5} mol) was dissolved in 10 mL of ultrapure water, and diluted to 10^{-5} mol·L^{-1}. Then EDC (0.1 mol/L, 50

μL) and NHS (0.025 mol/L, 50 μL) were added to 500 μL of the folic acid solution and incubated for 30 min. To form the quencher probe, 50 μL of 1.0×10^{-5} M amine-modified hairpin DNA4 was added to 60 μL of the activated folic acid solution, and reacted for 12 h at room temperature with gentle shaking.

Preparation of the soluble PAMAM-CNTs. Carbon nanotubes (CNTs) were chemically shortened by ultrasonic agitation in a mixture of sulfuric acid and nitric acid (3:1) for about 3 h. The resulting CNTs were separated and washed repeatedly with distilled water by centrifugation until pH was 7. 1 mL of PAMAM dendrimers aqueous solution (0.1 % wt) was firstly prepared, and then 0.5 $mg \cdot mL^{-1}$ CNTs was dispersed in the PAMAM solution in the volume ratio of 1:1 , the resulting dispersion was sonicated for 30 min to give a homogeneous black suspension. Superfluous PAMAM was removed by centrifugation, the obtained PAMAM-CNTs was redispersed in water under gentle sonication.

Preparation of the ECL Biosensor for detection of DNA. The fabrication principle and procedure of the DNAzyme-mediated Amplified detection of DNA based on ECL quenching of CdSe QDs by folic acid are illustrated in Scheme 1. Briefly, gold disk electrodes (4 mm in diameter) were polished carefully with 1.0-, 0.3- and 0.05-μm α-Al_2O_3 powder on fine abrasive paper and washed ultrasonically with water. Before modification, the bare electrodes were scanned in 0.5 M H_2SO_4 between –0.2 and 1.5 V until a reproducible cyclic voltammogram was obtained. After the electrodes were thoroughly rinsed with deionized water and dried, 8 μL of PAMAM-CNTs was dropped on the electrodes and dried. After the CdSe-COOH QDs were activated by EDC（0.1 M）and NHS（0.025 M）for 30 min, the electrodes were immersed in the QDs solution and reacted for 12 h at room temperature. Then the electrodes were rinsed with 10 mM PBS buffer and performed the ECL measurement. Subsequently, 8 μL of the hairpin DNA4-folic acid quencher probe was dropped on the electrode and reacted for 12 h at room temperature. As a result, the DNA4-folic acid quencher probe with thiol groups on the other end of DNA was conjugated to the CdSe-COOH QDs on the electrode via S-Cd bond. After the electrodes were rinsed with 0.01 M PBS buffer to remove nonspecifically adsorbed quencher probe and performed the ECL measurement, the electrodes were immersed in the mixture with

analyte DNA1, DNA2 (10^{-5} mol·L^{-1}) and DNA3 (10^{-5} mol·L^{-1}) in TE buffer (10 mmol·L^{-1} Tris-HCl, 1 mmol·L^{-1} EDTA and 12.5 mmol·L^{-1} $MgCl_2$), which was incubated at 37 ºC for 2 h, and then washed twice for ECL measurements

ECL Measurement. The modified electrodes above were in contact with 0.1 M PBS (pH 7.4) containing 0.1 M $K_2S_2O_8$ and 0.1 M KCl and scanned from 0 to −1.5 V. ECL signals related to the DNA concentrations were measured.

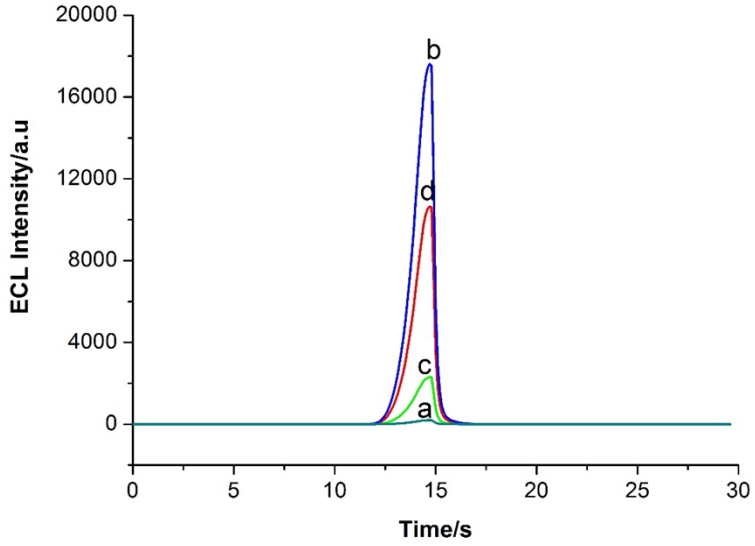

Figure S1 ECL-time curves for (a) bare electrode, (b) the PAMAM-CNT-QDs modified electrode, (c) ECL quenching by FA, (d) amplified detection of DNA in the presence of target DNA (10^{-6} mol·L^{-1}) based on Mg^{2+}-dependent DNAzyme autocatalytic system, 0.1 M PBS (pH =7.4) containing 0.1 mol·L^{-1} KCl and 0.05 mol·L^{-1} $K_2S_2O_8$.

Kinetics of Reaction. The kinetic process of the DNAzyme-mediated amplified ECL detection of DNA was studied by monitoring the ECL change in the presence of target DNA. Following the immobilization of QDs, conjugation of the DNA4-folic acid quencher probe on the electrode, DNAzyme-mediated circular amplification, an increase in ECL intensity was observed. As shown in Figure S2, at the beginning, slow increase in ECL intensity was observed because the concentration of target DNA and released quencher probe was low. Subsequently, an immediate rise in ECL intensity was observed, signifying the formation of DNAzyme-mediated circular amplification after quick cleavage of hairpin substrate in the presence of Mg^{2+} ions, and the release of quencher fragment as DNA triggers. After 60 min, the ECL intensity increased to a platform due to the depletion of the reaction components. These results further confirm that the

DNAzyme-mediated circular amplification occurred as expected and the detection of DNA can be accomplished within 2 h.

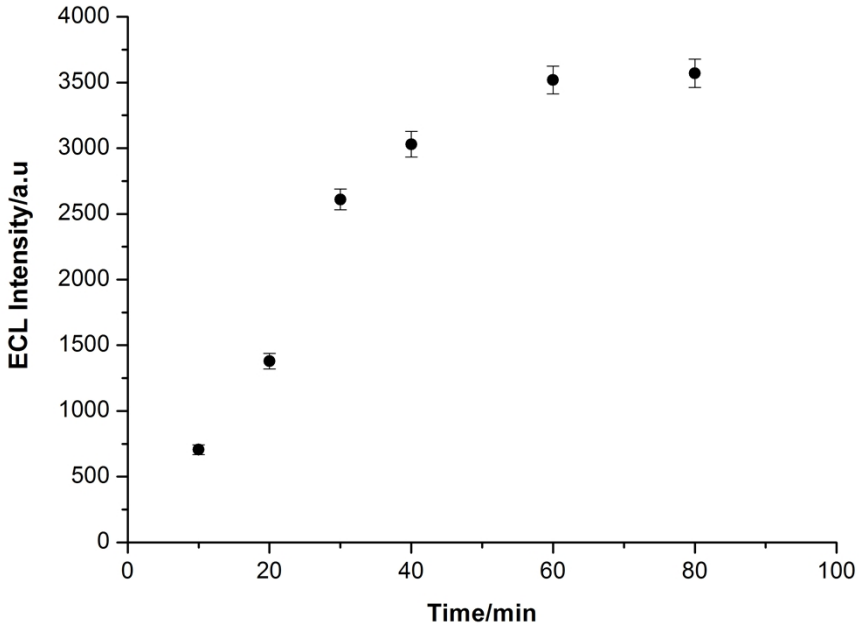

Figure S2. Kinetic response of the DNAzyme-mediated amplified ECL detection of DNA. Target DNA sequences (1.00 nmol·L^{-1}). The error bar represents the standard deviation of three measurements.

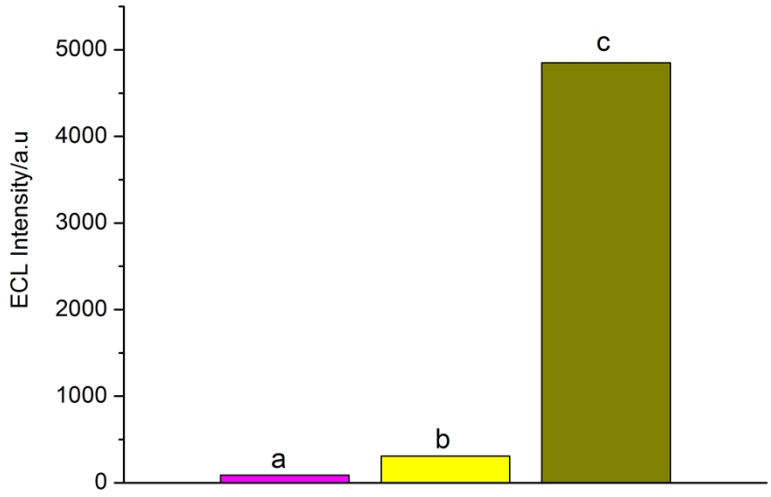

Figure S3 ECL responses for different target DNA sequences (10.0 nmol·L^{-1}). (a) completely mismatched DNA, (b) single-base mismatched DNA, (c) the complementary target DNA. The blank was deducted.