# Supporting Information

## Multifunctional electrochemiluminescence and photoelectrochemical biosensor based on quantum dots ion-exchange reaction for two-channel detection of thrombin

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**Table of Contents**



**Experimental section**

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*Chemicals and Reagents*

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS), 3-aminopropyltriethoxysilane and Exo III exonuclease were purchased from Shanghai Aladdin Reagent Co., Ltd. Silver nitrate and potassium persulfate were purchased from Sinopharm Group Chemical Reagent Co., Ltd. $CdCl_2 \cdot 2.5H_2O$ (98.0%) was purchased from Shanghai Runjie Chemical Reagent Co., Ltd. Sodium borohydride (>99.0%), tellurium powder (99.9%), sodium nitrate and 3-n-morino-propyl sulfonic acid were purchased from Qingdao Zhengye Reagent and Instrument Co., Ltd. $SiO_2$ microspheres were purchased from Baseline Chromatographic Technology Development Center of Tianjin. Mercaptopropionic acid (98.0%, MPA) was purchased from Beijing Bailingwei Technology Co., Ltd. All reagents were of analytical grade and used as received. Ultrapure water from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used throughout the experiment.

All the DNA sequences were synthesized and purified by Sangon Biotech (Shanghai) Co., Ltd. The DNA sequences used in this work are listed in Table S1.

**Table S1**. Sequences of the DNA

| Name | From（5'→ 3'） |
|---|---|
| **Apt** | **GTG GTT GGT GTG GTT GG** |
| **HP** | **NH2-TTT TTT TGG GGG GGA ATT GAG TGA AAC CCC ACG GGG GGA TTC CCC CCC A** |
| **P** | **TTT TTG GGG GGG AAT CCC CCC GTG GGG TTT CAC ACC AAC CAC** |

Apt is a TG-rich DNA sequence screened by the Sugimoto group to form the G-quadruplex necessary for thrombin binding, with a binding constant Ka of $5.9\times10^7\ M^{-1}$.[S1]

*Apparatus*

Transmission electron micrograph (TEM) image was acquired using a JEM-2100 instrument (JEOL, Japan). Scanning electron microscopy (SEM) image was acquired using a S-4800 instrument (Hitachi, Japan) with the voltage of 20 kV. All of the photoelectrochemical (PEC) signals were recorded on a PEAC 200A PEC analyzer (Aida Hengyi Technology Development Co., Ltd., Tianjin, China). The reference electrode was an Ag/AgCl electrode, the working

electrode was Indium tin oxide (ITO) glass (0.4 cm×4 cm), and a Pt wire was the counter electrode. UV-vis absorption spectra were measured with Lambda 35 UV Visible Spectrometer (Japan). Polyacrylamide gel electrophoresis (PAGE) analysis was performed on the Beijing JUNYI electrophoresis analyzer and imaged on the Biorad ChemDoc XRS (USA). X-ray photoelectron spectroscopy (XPS) was obtained on Multifunctional imaging electron spectrometer (Thermo ESCALAB 250Xi). X-ray diffraction (XRD) analysis was performed with Cu Kα radiation on a D/Max 2500V/PC Rigaku diffractometer.

**Synthesis of CdTe QDs by solvothermal method**

Quantum dots were synthesized on the basis of the literature method.[S2] 0.6 mmol $CdCl_2$ and 1.02 mmol MPA were mixed in 120 mL solution in a three-neck flask, and 1.0 M NaOH was added under agitation to adjust the solution pH to 11.8. After 30 min of deoxygenation of high-purity $N_2$, 120 mg $NaBH_4$ and 0.06 mmol $Na_2TeO_3$ were added sequentially. The molar ratio of $Cd^{2+}$: $Te^{2-}$: MPA was 1:0.1:1.7. The mixed solution was heated to 100 °C and refluxed under $N_2$ for 6 h. Finally, the heating was stopped, and the reaction products were collected and stored at 4 °C for later use.

**Pretreatment of DNA**

The newly ordered dry powder DNA was centrifuged at 4000 rpm for 30-60 s, and the dry powder DNA was collected to the bottom of the tube. Then the cap of the centrifuge tube was slowly opened, and a certain volume of TE buffer solution was added to the centrifuge tube to dissolve the DNA. The mixed DNA solution was prepared with a concentration of 100 μM and stored at -20 ℃ for later use.

**The process of gel electrophoresis**

The results of polyacrylamide gel electrophoresis (PAGE) imaging were used to determine the feasibility of DNA hybridization. First, we prepared 50×TAE buffer (tris 24.2 g, EDTA 3.72 g, glacial acetic acid 5.71 mL, add water to 100 mL). 10 mL was taken from the centrifuge tube, then water (4256 μL), 40% polyacrylamide gel (3500 μL), 50×TAE buffer (160 μL), APS (80 μL), TEMED (4 μL) were added, and mixed. The newly prepared gel was added to the gel box and insert the comb into the gel. Then it reacted at room temperature for 2 hours. Next, the gel box was transferred to the electrophoresis tank and a 1×TAE buffer was added to two-thirds of the tank. The comb was removed from the gel and the sample was added to the gel hole. The

electrophoresis was performed with 1× triacetic acid EDTA (pH=8.0) at 180 V constant pressure for 3 min and 135 V constant pressure for 1.5 h. After EB staining, the gel was scanned with a gel imaging analyzer.

**ECL measurement**

The detection method was carried out in phosphate buffer solution (pH 7.4, 0.05 M) with 0.05 M $K_2S_2O_8$, using a three-electrode system: ITO was the working electrode, Pt wire was the counter electrode, and Ag/AgCl electrode was the reference electrode. The photomultiplier tube (PMT) voltage was set at -750 V, the potential scan was set from 0 V to -1.5 V, and the scan rate was set at 100 $mV \cdot s^{-1}$.

**PEC measurement**

The PEC biosensor was constructed in the same way as ECL. For PEC assays, photocurrent measurements were performed in PBS (pH 7.4, 0.1 M) containing 0.1 M ascorbic acid (AA), and blue light was used as the excitation light source.

**1. Results and discussion**

**Table S2.** Comparison of different methods for detecting Thrombin

| Methond | Linear range | LOD | Ref |
|---|---|---|---|
| Fluorescence | 40 pM-100 nM | 40 pM | S3 |
| Direct Electrochemistry | 120 fM-15 nM | 120 fM | S4 |
| Electrochemical | 100 fM-20 nM | 38 fM | S5 |
| Colorimetric detection | 50 pM-5 nM | 20 pM | S6 |
| Fluorescence | 0.01 nM-25 nM | 3.5 pM | S7 |
| Electrochemiluminescence | 0.01 nM-10 nM | 0.2 pM | S8 |
| Photoelectrochemical | 20 fM-10 pM | 9.6 fM | S9 |
| Electrochemiluminescence | 10 fM- 10 nM | 2.5 fM | This work |
| Photoelectrochemical | 10 fM- 10 nM | 5.0 fM | This work |

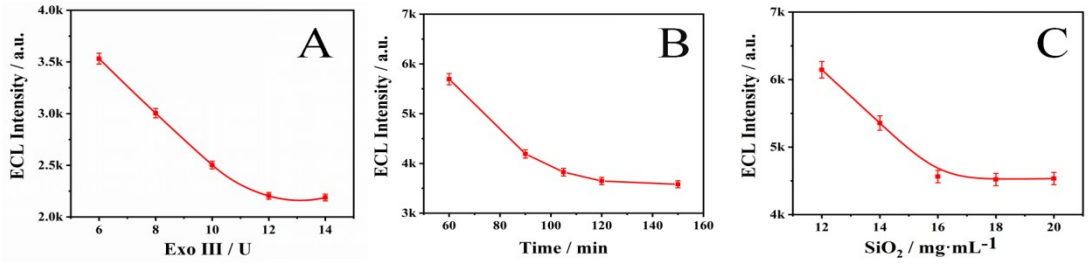


**Fig. S1**. Optimization conditions: (A) the concentration of Exo III, (B) Ratio of CPDNA to DNA1 dosage, (C) the concentration of Exo III, (D) the time of target-circulation amplification reaction.

**Table S3**. Determination of Thrombin in real human serum samples

| Sample | Added/pM | Obtain/pM | Recovery/% | RSD/% |
|---|---|---|---|---|
| 1 | 0 | 0.46 | | 3.72 |
| 2 | 3 | 3.41 | 98.3 | 4.84 |
| 3 | 5 | 5.32 | 97.2 | 2.85 |
| 4 | 10 | 10.95 | 104.9 | 4.87 |
| 5 | 0 | 0.42 | | 3.25 |
| 6 | 3 | 3.35 | 97.7 | 3.98 |
| 7 | 5 | 5.43 | 100.2 | 4.45 |
| 8 | 10 | 10.78 | 103.6 | 3.51 |

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