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Supporting Information

Target-induced structure switching of DNA for label-free and ultrasensitive electrochemiluminescent detection of protein

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Experimental Section

Materials and reagents: Dichlorotris (1,10-phenanthroline) ruthenium hydrate ($Ru(phen)_3Cl_2 \cdot H_2O$), 6-mercapto-1-hexanol (MCH), tripropylamine (TPrA), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), trypsin, bovine serum albumin (BSA) and lysozyme were purchased from Sigma-Aldrich (St. Louis, MO). Exonuclease III (Exo III) with 10×NEBuffer 1 was purchased from New England Biolabs (Beijing, China). The HPLC-purified oligonucleotide sequences were ordered from Shanghai Sangon Biotechnology (Shanghai, China) and the sequences were listed as follows:

Thiolated capture DNA (S1):

5'-AACCTGCCCTACCACGGACTGCTCTCAGAATGTATTTTTTT-$(CH_2)_6$-SH-3';

Assistant DNA (S2):

5'-*AGTCACCCC*ATACATTCTGAGAGC-3' (the italic sequence indicates S2*);

Thrombin binding aptamer (TBA):

5’-AGTCCGTGGTAGGGCAGGTTGGGGTGACT-3’;

All chemicals were of analytical grade and all solutions were prepared with ultrapure water (resistivity of 18.2 MΩ cm).

Pretreatment of the gold electrode (AuE): Prior to use, the AuE (3 mm in diameter, CH Instruments Inc., Shanghai, China) was immersed in a freshly prepared piranha solution (a 3:1 v/v mixture of concentrated sulfuric acid and 30% hydrogen peroxide solution) for 30 min. Subsequently, the electrode was polished with 0.3 and 0.05 μm aluminum slurry, followed by sonicating in ethanol and pure water for 5 minutes, respectively, to remove the residual alumina powder. Then, the well-polished electrode was subjected to cyclic voltammetric treatment in 0.5 M H_2SO_4 solution with a potential scan from 0.2 to 1.6 V until a stable characteristic voltammetric peak was obtained.[1]

Label-free and amplified electrochemiluminescent thrombin detection: Firstly, the mixture of S1 (2 μM) and S2 (2 μM) in 10 mM phosphate buffer solution (PBS, 0.1 M NaCl, pH 7.4) was heated to 85 °C for 10 minutes and allowed to cool to room temperature for at least 1 h to form the partially complementary dsDNA strands. Then, TCEP was added to the above mixture to a final concentration of 10 mM and incubated for 1 h to reduce the water-soluble alkyl disulfides. Next, 10 μL of the above solution was coated on the AuE overnight. After being rinsed with PBS, 1 mM freshly prepared MCH was incubated with the dsDNA-modified AuE for 2 h to block the surface. Followed by washing with PBS, 10 μL of TBA (2.4 μM) was incubated with the modified AuE for 1 h to form the sensing surface. Subsequently, the modified sensing AuE was soaked in 10 μL solution containing various concentrations of thrombin and 10 U Exo III at 37 °C for 2 h. After that, the sensor was rinsed with PBS and incubated with 10 μL of $Ru(phen)_3^{2+}$

(20 mM) for 7 h, followed by washing with PBS. Finally, the sensor was transferred into 0.1 M phosphate buffer (PB, pH 7.5) containing 20 mM TPrA for ECL measurements. ECL measurements were carried out with a conventional three-electrode system with the modified AuE, an Ag/AgCl (3 M KCl) reference electrode, and a platinum wire counter electrode. The ECL signals were recorded on a MPI-A electrochemiluminescence analyzer (Xi'an Remax Electronic Science & Technology Co. Ltd., Xi'an, China) by scanning the potential from 0 to 1.2 V (versus Ag/AgCl). The voltage of the photomultiplier tube (PMT) was set at 800 V and the scan rate was 50 mV s^{-1}.

Supplementary Figures:

1. The oxidation of TPrA on the modified electrode.

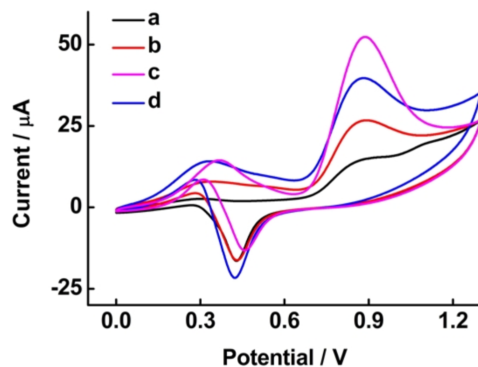

Fig. S1 Typical cyclic voltammograms recorded in 0.1 M PB (pH 7.5) containing 20 mM TPrA solution on different modified AuE: (a) bare AuE, (b) S1/S2/AuE, (c) S1/S2-TBA /AuE, (d) S1/S2-TBA/AuE after incubation with 100 pM target and 10 U Exo III. Scan rate: 50 mV s^{-1}.

The step-by-step fabrication of the sensor was first characterized by cyclic voltammetry (CV) in 0.1 M PB (pH 7.5) containing 20 mM TPrA. As shown in Fig. S1, a small voltammetric peak at ~0.89 V is observed (curve a) on bare AuE. This peak can be

assigned to the oxidation of TPrA, which is in agreement with previous reports.[2] The self-assembly of the dsDNA strands (S1/S2) and subsequent hybridization with the TBA leads to further increase in the TPrA oxidation peak (curve b and c, respectively). Such increase is due to the fact that the surface-immobilized dsDNA strands can pre-concentrate TPrA via electrostatic interactions between $TPrAH^+$ (TPrA exists in the form of $TPrAH^+$ at pH 7.5) and the negatively charged phosphate backbones of the dsDNA.[3] Meanwhile, the formation of dsDNA strands can also improve the conductivity of the electrode, which favors TPrA oxidation. On the contrary, the incubation of the sensing surface with thrombin (100 pM) and Exo III (10 U) leads to apparent decrease in the oxidation peak of TPrA (curve d), ascribing to cyclic digestion of TBA and S2. These results reveal the successful preparation of the sensor.

2. Optimization of the assay conditions.

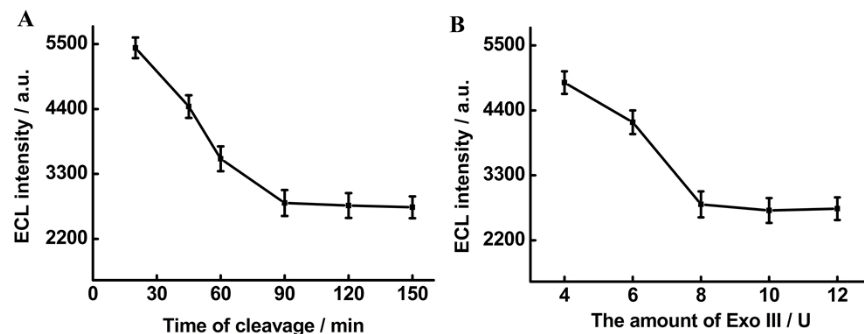

Fig. S2 Effects of the enzymatic reaction time (A) and the amount of Exo III (B) on the ECL response of the sensor for thrombin detection. Error bars, SD, n=3. ECL measurements were recorded in 0.1 M PB (pH 7.5) containing 20 mM TPrA. Scan rate: 50 mV s^{-1}.

After proof-of-concept demonstration of the signal amplification strategy, the assay conditions were optimized. The effect of enzymatic reaction time on the ECL intensity of the sensor with the presence of thrombin (20 pM) and Exo III (10 U) was first

investigated in the range from 25 min to 180 min. According to Fig. S2A, the ECL intensity decreases with prolonged enzymatic reaction time and levels off after 90 min, suggesting an optimal enzymatic reaction time of 90 min. The amount of Exo III was also optimized. From Fig. S2B, we can see that the ECL intensity decreases with increasing amount of Exo III up to 8 U in the investigated range from 4 to 12 U and remains stable thereafter. Therefore, the amount of 8 U Exo III was used in subsequent experiments.

3. Evaluation of thrombin in human blood serum.

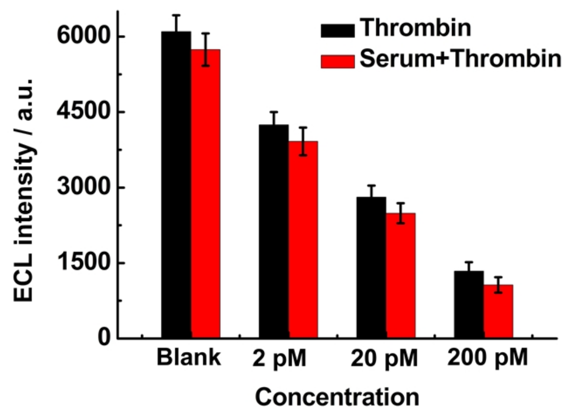

Fig. S3 ECL responses of the sensor for thrombin detection in PB buffer (black bars) and diluted human blood serum samples (red bars). Error bars, SD, n=3. The cleavage time of Exo III was 90 minutes and the amount of Exo III was 8 U.

To demonstrate the potential application of the sensing strategy for thrombin detection in complex biological environment, we employed the proposed method to detect thrombin in human blood serum samples (obtained from the 9th People's Hospital of Chongqing, China). The serum samples were collected by centrifugation of whole blood after coagulation. The chemical composition of serum is similar to that of plasma but without the presence of coagulation proteins such as thrombin or other coagulation factors.[4,5] Different concentrations of thrombin at 2 pM, 20 pM, 200 pM were separately spiked into the diluted serum (1:10 dilution ratio with NEB buffer) and the samples were

tested with the developed method. Results for the detection of thrombin in the serum samples and PB buffer are shown in Fig. S3. From Fig. S3, we can see that the ECL signals obtained in serum samples slightly decrease compared with those obtained in PB buffer. Such decrease is assembly due to the interferences of some other proteins and nucleases present in serum.[6-8] Despite these decreases, the presence of thrombin in serum can be detected with our developed method, indicating the applicability of this method for real samples.

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