

Experimental Section

Reagents and Materials

DNA oligonucleotides were synthesized by Sangon Inc. (Shanghai, China). And their sequences are shown below:

Capture probe ssDNA(1) :



Detection probe ssDNA(2) :



Tris(2,2'-bipyridyl) ruthenium(II)-doped silica nanoparticles (Ru-SNPs) were prepared according to the previous literature [RS1] and labeled on the end of ssDNA(2) according the published procedure [RS2]. Briefly, the prepared Ru-SNPs were functionalized with amine at first, then DNA buffer solution was added to the functionalized Ru-SNPs solution and stirred for 120 min in 37 °C water bath, Ru-SNPs then labeled on the end of ssDNA(2) through covalent interaction of oligonucleotides, After this, the production was washed, centrifuged and resuspended in PBS and stored at 4 °C.

Human thrombin (TB), bovine serum albumin (BSA), bovine hemoglobin (BHb), immunoglobulin G (IgG), 2-(dibutylamino) ethanol (DBAE) and 6-mercaptohexanol (MCH) were purchased from Sigma (St. Louis, MO). Other chemicals employed were all of analytical grade.

Aqueous solutions were prepared with ultra-pure water (18.2-M Ω resistance). Buffers for electrochemiluminescence (ECL) detection were 10 mM Tris-HCl solution

(pH 7.4) with 100 mM NaCl. DNA buffer solution is 10 mM Tris-HCl buffer (pH=7.4) containing 4 mM ethylenediaminetetraacetic acid (EDTA), 10 mM tris(2-carboxyethyl) phosphinehydrochloride (TCEP) and 100 mM NaCl. Above choice of buffer solution, pH and salts was optimized for this experiment.

Apparatus

ECL detection system consisted of a BPCL ultra-weak luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China) and a CHI 660a electrochemical system (CH Instruments, Shanghai, China). Electrochemical signals were recorded by CHI 660 electrochemical system. ECL signals were captured by a personal computer with BPCL program (Institute of Biophysics, Chinese Academy of Science). A three-electrode quartz cell with an optically flat bottom was used. Gold electrodes (diameter: 3.0 mm, surface area: 7.07 mm², CH Instruments, Shanghai, China) were used as the working electrodes. Platinum wire and Ag/AgCl (saturated with KCl) were used as counter and reference electrodes, respectively. ECL buffer solutions were purged with highly purified nitrogen for 20 min before measurement and kept in the cell during determinations. The electrochemical cell was placed directly in front of a photomultiplier (PMT, operated at -800 V) and the PMT window was only opened to the working electrode to reduce the interference of ECL from the counter electrode.

Sensor preparation

Gold electrodes were cleaned following prior published procedure [RS3]. First, the gold electrodes were polished with 1.0, 0.3 and 0.05 μm $\alpha\text{-Al}_2\text{O}_3$ powders and

washed with deionized water and absolute alcohol for 3 min, respectively. The electrodes were then electrochemically cleaned in fresh 0.5 mol/L sulfuric solution until got a stable cyclic voltammetry (CV) curve. Finally the activated gold electrodes were rinsed with water and dried with nitrogen. ssDNA(1) buffer solution (capture probe) was dropped on the surface of gold electrodes to form self-assemble monolayer through interaction of thiol-Au. Then the modified electrodes were immersed in MCH solution for 1h to cover the nonspecific sites. The Ru-SNPs-labeled detection probe was mixed with different concentration of thrombin at 4 °C for 1 h, and then the above capture probe-modified electrode was incubated into the mix solution for 2 h at 4 °C. After these procedures, the electrodes were washed with washing buffer and dried in the nitrogen atmosphere.

ECL Measurements

The ECL measurements were carried out in a quartz cell at room temperature. The cell contains 2mL Tris-HCl solution and 5×10^{-3} mol/L DBAE (worked as coreaction reagent [RS4]). Cyclic voltammetry (CV)(ranged from 0.8V to 1.6V) was preformed to get the ECL signal. The intensity of ECL signal was recorded for quantitative detection.

References

- [RS1] Santra, S.; Zhang, P.; Wang, K. M.; Tapeç, R.; Tan, W. *Anal. Chem.* 2001, 73, 4988-4993.
- [RS2] Zhu, N. N.; Cai, H.; He, P.G.; Fang, Y. Z. *Anal. Chim. Acta.* 2003, 481,181-189.

[RS3] Zhang, J.; Song, S.; Wang, L.; Pan, D.; Fan, C.; Nat. Prot. 2007, 2, 2888-2895.

[RS4] Liu, X. Q.; Shi, L. H.; Niu, W. X.; Li, H. J.; Xu, G. B. Angew. Chem. Int. Ed. 2007, 46, 421-424.

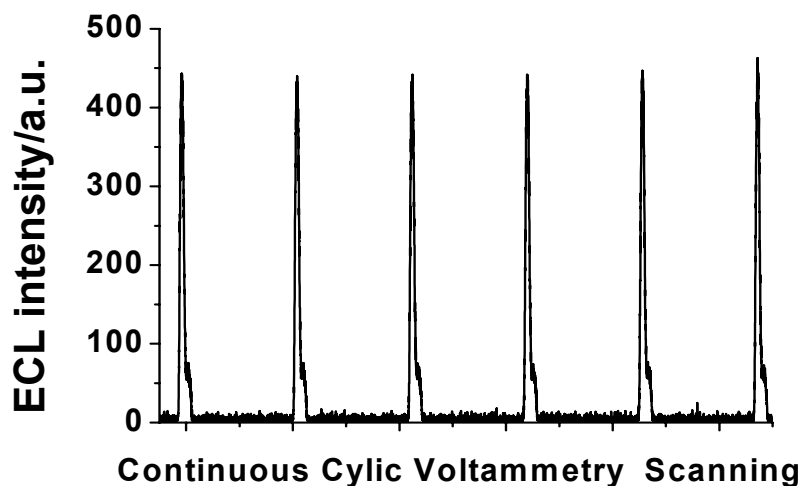


Fig. S1 Reproducibility of the aptamer-based ECL sensor after reaction with 3.3×10^{-11} mol/L thrombin

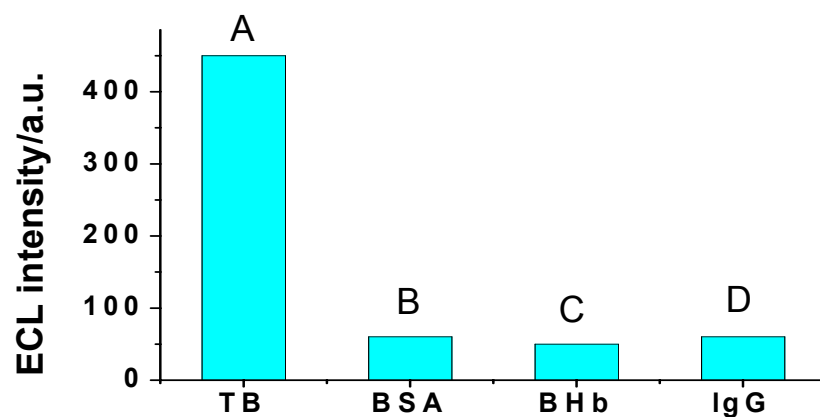


Fig. S2 ECL intensity on the modified gold electrodes after they are exposed to the solution with TB, BSA, BHb, IgG, respectively, the columns A to D are the intensity of ECL signal after reacting with TB, BSA, BHb, IgG, respectively. The concentration of TB is 3.3×10^{-11} mol/L, the others are 5.0×10^{-8} mol/L.