Supporting Information

Dual-responses for electrochemical and electrochemiluminescent detection based on a bifunctional probe

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EXPERIMENTAL SECTION

Reagents and materials

Perylene-3, 4, 9, 10-tetracarboxylic dianhydride ($C_{24}H_8O_6$, PTCDA) was purchased from Lian Gang Dyestuff Chemical Industry Co. Ltd (Liaoning, China). Toluidine blue (Tb), thrombin, hexanethiol (96%, HT) and gold chloride ($HAuCl_4$) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). $K_2S_2O_8$ was purchased from shanghai chemical Reagent company (Shanghai, China). Bovine serum albumin (BSA, 96-99 %), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimidehydrochloride (EDC) and *N*-hydroxy succinimide (NHS) were purchased from Shanghai Medpep Co. Ltd. (Shanghai, China). All other chemicals were of reagent grade and used as received. Thrombin binding aptamers (TBA) were purchased from TaKaRa (Dalian, China), and the sequences of the oligonucleotides were as follows:

TBA: 5'-SH-$(CH_2)_6$-GGT TGG TGT GGT TGG-3'

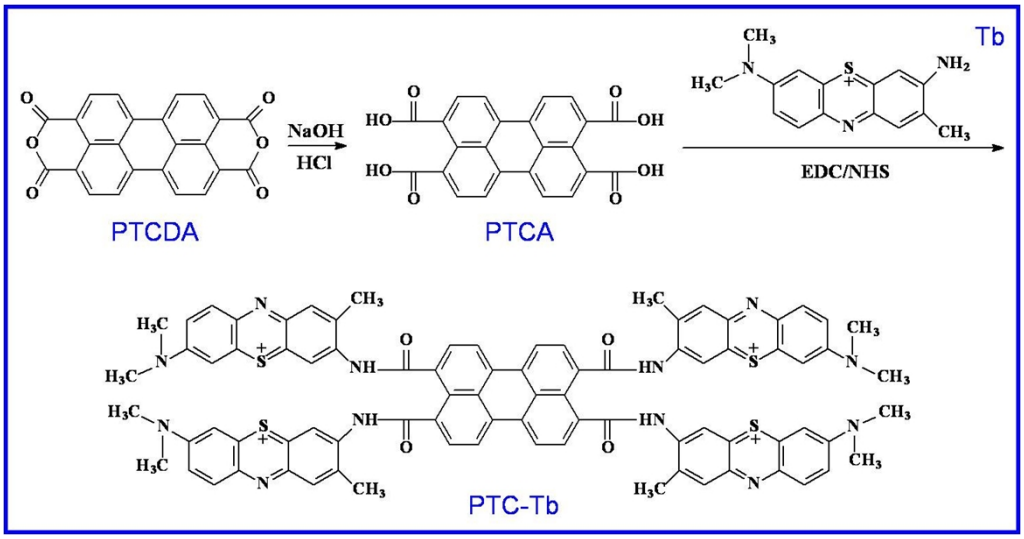
Doubly distilled water was used throughout this study. 0.1 M HAc-NaAc (pH 5.5) was employed to investigate the performance of electrodes. 20 mM Tris-HCl buffer (pH 7.4) containing 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$ and 1 mM $MgCl_2$ was used to prepare aptamer solutions.

Apparatus

The ECL emission was monitored with a model MPI-A electrochemiluminescence analyzer (Xi'an Remax Electronic Science & Technology Co., Ltd., Xi'an, China). Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were carried out with a CHI 660D electrochemical workstation (Shanghai CH Instruments, China). A conventional three-electrode system was used consisting of a modified gold electrode (AuE, Φ = 4 mm) as working electrode, an Ag/AgCl (sat. KCl) as reference electrode and a platinum wire as counter electrode, respectively. The scanning electron micrographs were taken with a scanning electron microscope (SEM, S-4800, Hitachi, Tokyo, Japan). X-ray photoelectron spectroscopy (XPS) measurement was carried out on a VG Scientific ESCALAB 250 spectrometer, using Al Ka X-ray (1486.6 eV) as the light source. UV-vis absorption spectra were recorded with a UV-2450 spectrophotometer (Shimadzu, Japan) at room temperature using a 300 μL black-body quartz curette with 1 cm path length. The Fourier transform infrared (FTIR) spectra were performed by using a Spectrum GX FTIR spectroscopy system (Perkin-Elmer, USA).

Preparation of Bifunctional Probe (PTC-Tb)

The synthesis was performed in the following manner. The first step: PTCA was made by hydrolyzing PTCDA with 1 M NaOH until the colour of the solution becoming yellow-green. After that the mixture solution was treated with HCl to neutralize the excess NaOH, red precipitate appeared and the pH of the solution maintained at slightly acidic. Subsequently, the product of PTCA was collected by centrifuged and then dried under vacuum at 60 °C. The second step: 1 mg of the above prepared PTCA was firstly dissolved in 5 mL an aqueous solution containing a proper amount of EDC and NHS (4:1) and stirred for 6 h at ambient condition. Afterwards, 1 mL of 5 mg mL^{-1} Tb aqueous solution was added dropwise into the above solution and the mixture was allowed to react overnight at 70-80 °C under continuous stirring. The synthesized product (PTC-Tb) was centrifuged and then washed with doubly distilled water. The process involved in fabricating the PTC-Tb is shown schematically in SFig. 1.



SFig. 1. Schematic diagram of the procedure used to prepare bifunctional PTC-Tb probe.

Preparation of nano-Au

Nano-Au was prepared according to the previous protocol [1]: In brief, 50 mL of

0.01% $HAuCl_4$ solution was heated to boiling with vigorous stirring, and then 1.25 mL 1% trisodium citrate solution was quickly added to the boiling solution. When the color of the solution turned deep red, the heating source was removed and the resulting solution was stirred for an additional 15 min to cool down at room temperature.

Pretreated gold electrode

To obtain mirror-like surface, gold electrode (AuE, Φ = 4 mm) was firstly polished successively with 0.3 and 0.05 μm alumina powder to remove adsorbed organic substances. Then it was electrochemically cleaned in 0.1 M H_2SO_4 *via* potential scanning between −0.2 and +1.6 V until a reproducible CV was obtained. Before modification, the AuE was dried with nitrogen at room temperature.

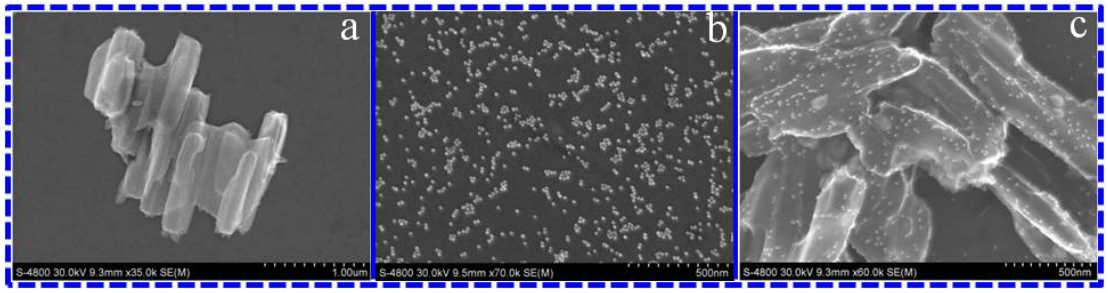
Detection Measurements

The EC detection in 0.1 M HAc-NaAc buffer solution (pH 5.5) was employed to investigate the performance of electrodes in the process of detection. DPV parameters applied were: 20 mV pulse amplitude, 50 ms pulse width, 0.2 s pulse period and voltage range from -0.6 to 0.1 V. CV parameters applied were: the potential range from -0.6 to 0.2 V at 50 mV s^{-1}. The ECL detection was performed at room temperature in a 10 mL homemade quartz cell. A high voltage of the photomultiplier tube was set at 800 V in the process of detection. The potential scan from -2.0 to 0 V and the scan rate of 0.1 V s^{-1} was applied to obtain ECL signal in 0.1 M HAc-NaAc (pH 5.5) containing 5 mM $K_2S_2O_8$.

RESULTS AND DISCUSSION

Characterizations of the Nanomaterials by SEM

The morphology and microstructure of the as-prepared nanomaterials were investigated by SEM (SFig. 2) at an acceleration voltage of 30.0 kV. SFig. 2a shows the SEM image of PTC-Tb which displays irregular quadrateshaped islands with the large specific surface area. SFig. 2b shows the typical SEM image of nano-Au. After nano-Au was adsorbed onto PTC-Tb film, many bright dots can be found (SFig. 2c), implying the successful assembling of the nano-Au on the PTC-Tb surface through electrostatic interaction.

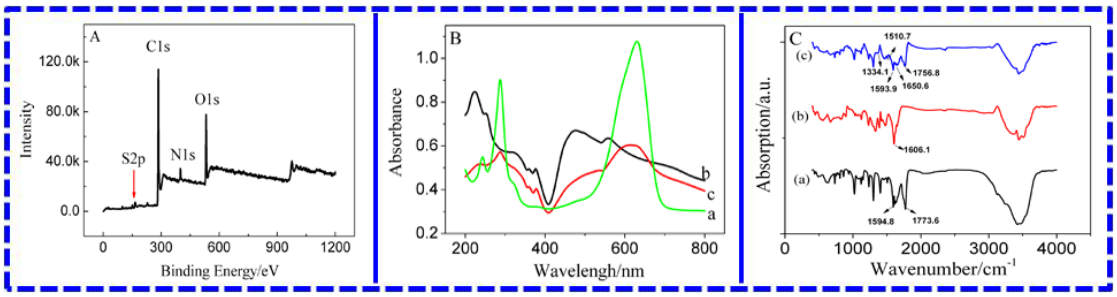


SFig.2 SEM images of (a) PTC-Tb and (b) nano-Au and (c) nano-Au/PTC-Tb.

Characterizations of the Nanomaterials by XPS, UV–vis and FTIR spectra

XPS analysis provides effective information on the chemical composition of the as-prepared nanocomposite. The full XPS pattern of PTC-Tb is shown in SFig. 3A. The photoelectron peaks of C, N and O are clearly distinguishable, corresponding to the element of PTCA. The new peak at about 164.1 eV is observed, which is the positions of S2p, indicating the successfully prepared PTC-Tb. Furthermore, in order to further verify the interaction between PTCA and Tb, the UV–*vis* absorption spectra of Tb, PTCA and PTC-Tb are also studied. PTCA contains the wide absorption bands at 557 and 476 nm as well as a series of weak maxima at 370 and 376 nm (SFig. 3B,

curve a). From SFig 3B, Tb shows two characteristic peaks at 630 and 287 nm (SFig. 3B, curve b). A new absorption band appear at 615 nm with the disappearance of maxima at 557 and 630 nm after the formation of PTC-Tb, (SFig. 3B, curve c), elucidating the successful synthesis of PTC-Tb. To confirm the successfully covalent binding between PTCA and Tb, the combination was characterized by FTIR spectroscopy. The peak at 1773.6 cm^{-1} (SFig. 3C, curve a) represents the characteristic stretching vibration of C=O bond related to the carboxylic acid groups of PTCA. The FTIR spectrum of the Tb (SFig. 3C, curve b) exhibits a sharp peak at 1606.1 cm^{-1} corresponding to the N–H bending vibration. Then the PTC-Tb (SFig. 3C, curve c) produces the absorption feature at 1334.1 cm^{-1} (–C–N– stretching of acylamino), implying the formation of PTC-Tb.



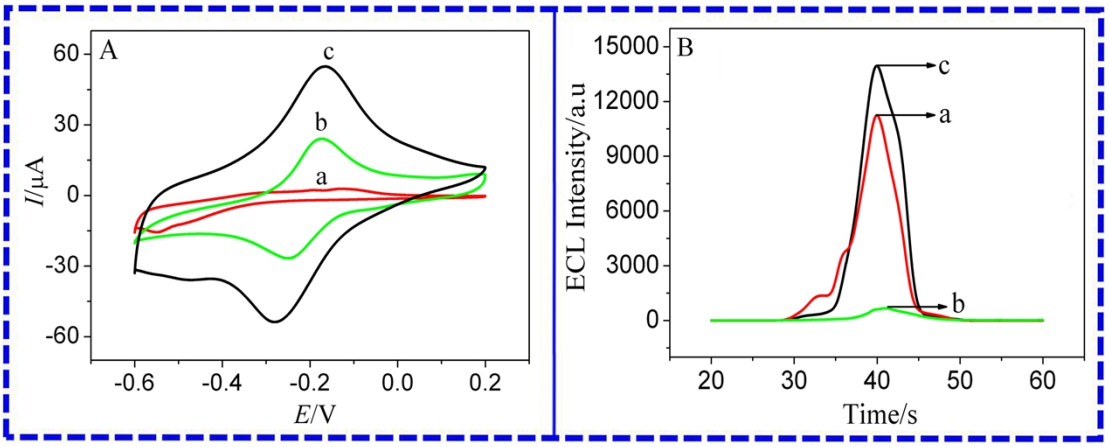
SFig.3 (A): XPS of PTC-Tb. (B) UV-*vis* and (C) FTIR spectra of PTCA (a); Tb (b) and PTC-Tb (c), respectivily.

Comparison of Differently Modified Electrodes

To demonstrate the EC property of proposed modified electrode, PTCA (a), Tb (b) and PTC-Tb (c) were dropped on the different AuE surfaces and subjected to CV analysis in HAc-NaAc (pH 5.5) at a scan rate of 50 mV s^{-1}. As illustrated in SFig 4A, curve (a) exhibits redox-activity owing to the electronic property of PTCA

but there were miscellaneous redox peaks in the potential region from -0.6 to 0.2 V, which led to potential signal interference in target quantitative detection. Curve (b) shows a pair of well-defined redox peaks, corresponding to the reversible redox reaction of Tb. Interestingly, when PTC-Tb was employed to modify electrode (SFig 4A, curve c), a pair of well-defined redox peaks could be obtained, which not only conciliates the miscellaneous redox peaks of PTCA but also can effectively promote the electron transfer of Tb modified electrode.

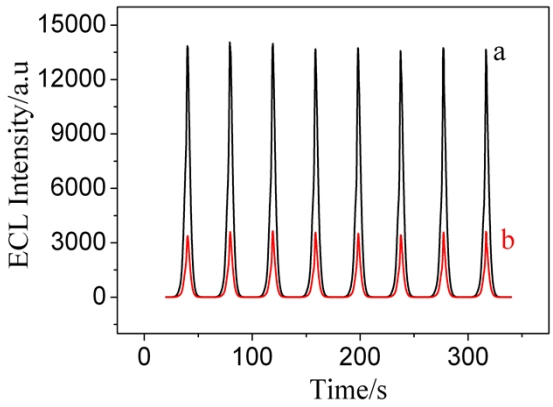
To investigate the ECL efficiency of PTC-Tb, the contrast experiment to compare their ECL responses of different modified electrodes under the same conditions were investigated and the results were shown in SFig. 4B. When PTCA was coated on the electrode (SFig. 4B, curve a), a noticeable ECL signal of 11242 a.u. was obtained. Tb modified electrode (SFig. 4B, curve b) shows a weak ECL signal only about 665 a.u.. After PTC-Tb was employed to modify electrode, the ECL intensity dramatically raised to 13952 a.u. (SFig. 4B, curve c). The enhancement can be attributed to synergistic effect of PTCA and Tb. On the basis of EC and ECL results, we can make a conclusion that PTC-Tb is not only a well-defined EC redox molecule but also a highly efficient co-reactant of ECL $O_2/S_2O_8^{2-}$ system.



SFig. 4 (A) CV responses of different electrodes in 0.1 M HAc-NaAc buffer (pH 5.5) at a scan rate of 50 mV s^{-1} and (B) ECL intensities of different electrodes in 0.1 M HAc-NaAc buffer containing 5 mM $K_2S_2O_8$ (the voltage of the photomultiplier tube was set at 800 V) at a scan rate of 100 mV s^{-1}: (a) PTCA/AuE; (b) Tb/AuE; (c) PTC-Tb/AuE.

Possible Luminescence Mechanism of PTC-Tb

SFig. 5 shows the ECL mechanism of the $O_2/S_2O_8^{2-}$ system by performing ECL measurements on the PTC-Tb modified AuE in air-saturated and N_2-saturated conditions. In the air-saturated HAc-NaAc buffer (pH 5.5) containing 5 mM $K_2S_2O_8$, PTC-Tb/AuE exhibits a strong and stable ECL response during the potential scan (SFig. 5 a). Nevertheless, after purging the above detection buffer with high purity N_2 for 30 min (N_2-saturated solution), the ECL signal is sharply decreased (SFig. 5 b *vs*. SFig. 5 a).



SFig. 5 ECL intensity-time curves on the PTC-Tb/AuE (a) in O_2-saturated and (b) N_2-saturated 0.1 M HAc-NaAc buffer (pH 5.5) containing 5 mM $K_2S_2O_8$.

As supported by the experimental results in SFig. 5, the ECL mechanism of the PTC-Tb in $O_2/S_2O_8^{2-}$ system can be expressed as the following equations:

$$S_2O_8^{2-} + e^- \rightarrow SO_4^{\bullet -} + SO_4^{2-}$$

$$SO_4^{\bullet -} + H_2O \rightarrow HO^{\bullet} + HSO_4^-$$

$$HO^{\bullet} \rightarrow HOO^{\bullet} + H_2O$$

$$O_2 + H_2O + e^- \rightarrow HO^- + HOO^{\bullet}$$

$$SO_4^{\bullet -} + HOO^{\bullet} \rightarrow HSO_4^- + {}^1(O_2)_2{}^*$$

$${}^1(O_2)_2{}^* \rightarrow 2{}^3O_2 + h\nu$$

$$\text{PTC-Tb} - e^- \rightarrow \text{PTC-Tb}^{\bullet +}$$

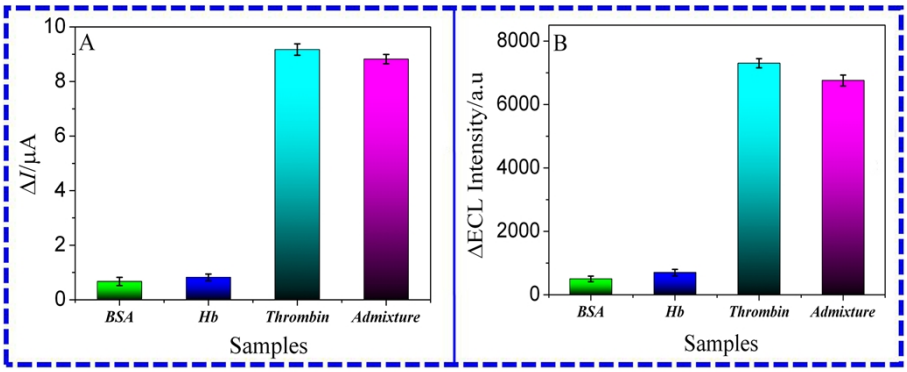
$$\text{PTC-Tb}^{\bullet +} \rightarrow \text{PTC-Tb}^{\bullet} + H^+$$

$$\text{PTC-Tb}^{\bullet} + HOO^{\bullet} \rightarrow \text{PTC-Tb} + {}^1(O_2)_2{}^*$$

$${}^1(O_2)_2{}^* \rightarrow 2{}^3O_2 + h\nu$$

Selectivity

To investigate the selectivity of the proposed aptasensor, the control experiments were performed by using bovine serum albumin (BSA, 100 nM) and hemoglobin (Hb, 100 nM) to replace thrombin (10 nM), respectively. The change of the EC (ΔI) and ECL (ΔECL) responses were used to evaluate the selectivity of the proposed aptasensor toward thrombin, which are given by $\Delta I = I - I_0$ and $\Delta\text{ECL} = |\text{ECL} - \text{ECL}_0|$, respectively (The background noises are recorded as I_0 and ECL_0 toward zero analyte, while the EC and ECL responses are recorded as I and ECL toward different interferents). As shown in SFig. 6, it is found that no remarkable signal was observed in comparison with that in the presence of thrombin. Further, the proposed aptasensor in a mixture solution (10 nM thrombin containing 100 nM BSA and 100 nM Hb) was also investigated. Although the high concentration of BSA and Hb are coexisted, the detection signals have no apparent difference. The experimental results implied acceptable selectivity of the proposed dual-responses aptasensor.



SFig. 6. (A) EC and (B) ECL selectivity investigation for thrombin (10 nM) detection against the interference proteins: BSA (100 nM), Hb (100 nM), Admixture (10 nM thrombin containing 100 nM BSA and 100 nM Hb). The error bars represent the standard deviations of three measurements.

Analytical application of the aptasensor

The analytical reliability and possible application of the proposed aptasensor was investigated by recovery experiments. A series of samples were prepared by adding thrombin of different concentrations into 10-fold-diluted healthy human serum samples (obtained from Ninth People's Hospital of Chongqing, China). As shown in STable 1, the EC and ECL recoveries are in the range of 94.0%–110.4% and 93.6%–104.0%, respectively. These results clearly demonstrated that the proposed dual-responses aptasensor provided a potential application in real biological samples.

STable 1. Detection of thrombin added in human serum (n = 3) with the proposed aptasensor.

Sample No.	Added thrombin (nM)	Found thrombin (nM)[a]		Recovery (%)	
		EC	ECL	EC	ECL
1	0.75	0.80	0.78	106.7	104.0
2	1.25	1.38	1.17	110.4	93.6
3	2.50	2.35	2.39	94.0	95.6
4	4.75	4.88	4.91	102.7	103.4
5	8.00	7.82	7.80	97.8	97.5

[a] The values shown here are the average values from three measurements.

References

[1]: A. Ambrosi, M. T. Castaneda, A. J. Killard, M. R. Smyth, S. Alegret and A. Merkoci, *Anal. Chem.* 2007, **79**, 5232.