# Supporting Information

# Photo-Driven Regeneration of G-quadruplex Aptasensor for Sensitively Detecting Thrombin

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Contents

| | | Page |
|---|---|---|
| **1.** | **General** | **S-2** |
| **2.** | **Ethics Statement** | **S-2** |
| **3.** | **Blood Sample Preparation** | **S-2** |
| **4.** | **Supplementary Figures** | **S-3** |
| | **Figure S1** | **S-3** |
| | **Figure S2** | **S-4** |
| | **Figure S3** | **S-4** |
| | **Figure S4** | **S-5** |
| **5.** | **References** | **S-6** |

## 1. General

Thrombin, haemoglobin, fibrinogen, bovine serum albumin, immunoglobulin G, lysozyme, and cytochrome C were purchased from Sangon Biotech (Shanghai, China) Co., Ltd. Alkaline phosphatase and T4 polynucleotide kinase were provided by New England Biolabs Inc. (NEB, UK). DNAs were synthesized by Sangon Biotech (Shanghai, China). All the solutions were prepared with ultrapure water obtained from a Millipore water purification system (>18.2 MΩ • cm). All other chemicals were of analytical grade.

Electrochemical experiments were performed on electrochemical workstation (model CHI 660E, CHI Instruments, Shanghai, China). The three-electrode system were gold electrode modified with Fc-labeled thrombin-binding aptamer modified gold electrode was employed as the working electrode, Ag/AgCl (KCl-sat.) electrode and platinum wire were used as the reference electrode and the counter electrode respectively. 20 mM Tris, contained 140 mM NaCl (pH 7.6) was used as the electrolyte. Differential pulse voltammetry experimental conditions were: pulse height, 50 mV; step potential, 7 mV; scan rate, 0.03 $V \cdot s^{-1}$; rest time 2 seconds. Electrochemical impedance spectroscopy (EIS) was performed in 20 mM Tris buffer containing 140 mM NaCl and 20 mM ~~$Na_3[Fe(CN)_6]$~~ within a frequency range from $2\times10^4$ to 0.1 Hz. The electrode was scanned in a 0.5 M sulfuric acid solution at a sweep rate of 0.5 $V \cdot s^{-1}$ across the potential range of -0.20 V and +1.60 V until a typical clean gold electrode characteristic cyclic voltammogram was obtained. After rinse the electrode with ultrapure water, 3 $\mu$M thrombin-aptamer was dropped onto the surface of the cleaned electrode and allowed to react for 2 hours. When the self-assembly reaction was completed, the electrode was immersed into 20 mM Tris buffer containing 0.10 M 3-mercaptopropionic acid for 10 minutes to further form a submonolayer at the unoccupied Au surface. The regeneration schematic illustration of the regeneration progress was shown in Figure S3.

The $K_d$ value of thrombin and aptamers were determined in real time using the Biacore T200 system. After loading CM5, equal volume of SDS and NaOH were injected at a flow rate of 30 $\mu L \cdot min^{-1}$ respectively to clean the CM5 sensor chip (GE Healthcare) for an injection time of 120 seconds. The cleaning process was repeated three times. 100 mM of EDC and 25 mM NHS mixture was circulated through the surface of the chip for 6 minutes at a rate of 5 $\mu L \cdot min^{-1}$ to activate the carboxyl groups on the surface of the dextran chip. The thrombin was diluted to 50 $\mu$M with sodium acetate (pH 4.0), continuously injected for 2 minutes, and then ethanolamine (pH 7.0) was injected to block the unreacted carboxymethyl site. Finally, 4532 RU of thrombin was coupled to the sensor surface. The oligonucleotide was dissolved in PBS buffer. Then the DNA was diluted and injected into the sensor surface at a rate of 30 $\mu L \cdot min^{-1}$ for 6 minutes, and the dissociation was realized in 1M NaCl solution at 30 $\mu L \cdot min^{-1}$ for 5 minutes. Analyze experimental data by curve fitting were calculated using Biacore evaluation software (version 2.0).

## 2. Ethics Statement

All experimental procedures and sample collection were according to the Regulations for the Administration of Affairs Concerning Experimental Animals (Ministry of Science and Technology, China) and approved by the Institutional Animal Care and Use Committee of Anhui Agricultural University, Hefei, China[1-4].

## 3. Blood Sample Preparation

Three 25 weeks-old healthy female Shelducks were provided by Anhui Jiuming Poultry Co., Ltd. China. The ducks' weights were 1049g, 1243g, and 1207g respectively. The ducks were reared on floor litter and artificial nest and exposed to natural lighting and temperature and had free access to food and water. Ducks were bled by using a sterile needle and syringe. Total 5 mL of fresh blood was drawn from the vein under the wing of each duck. The ducks were euthanized by exsanguination under anesthesia (intravenous injection of phenobarbitone, 100 mg/kg). The whole blood samples were detected by the aptasensor immediately. The serum samples were prepared after been centrifuged at 1,000 rpm for 10 minutes in a refrigerated centrifuge.

## 4. Supplementary Figures

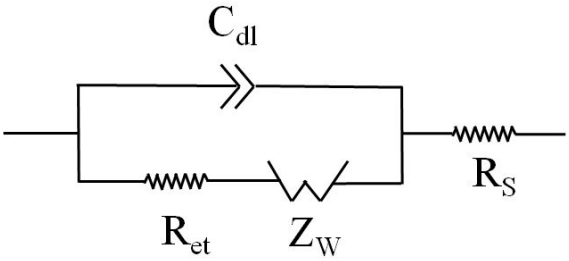


**Figure S1** Equivalent circuit model for the impedance plots. $R_s$ refers to the circuit includes the ohmic resistance of the electrolyte solution; $Z_W$ means Warburg impedance of ions' diffusion from the bulk of the electrolyte to the interface; $C_{dl}$ is double-layer capacitance; $R_{et}$ is the electron-transfer resistance.

In CV and DPV technologies, the Fc moieties fabricated on the DNA chain of the aptamer were used as the probe. In CV curve, the value of peak current ($I_p$) is linear with the amount of redox moiety near the electrode surface, $I_p = n^2F^2v\Gamma^0_{Fc}/RT$ ($n$ is the number of electrode transfer of each redox moiety; $F$ means Faraday constant; $v$ is the scan rate; $\Gamma^0_{Fc}$ refers to the amount of Fc group near the electrode surface; $R$ is gas constant; $T$ refers to the temperature). When the electrode irradiated by UV light before combined with thrombin, most of the Fc moieties were far away from the electrode and the value of $\Gamma^0_{Fc}$ is a small one. After incubation with thrombin, the DNA chain would fold into a G- quadruplex conformation and the amount of Fc groups which near the electrode would increase. The $I_p$ value would increase with the $\Gamma^0_{Fc}$ as a result of thrombin-binding. Difference with CV technology, DPV add pulse electric potentials in the process of scan. The pulse potential could reduce the charge current and increase the sensitivity of the sensor. In EIS technology, ~~$Na_4[Fe(CN)_6]$~~ was dissolved in the electrolyte as the probe. Before incubated with thrombin, the anion probe $Fe(CN)_6^{3-/4-}$ could easily diffuse to the electrode surface, and the impedance value ($R_{et}$) was small. After incubated with negative-charged thrombin, the aptamer on the electrode would binding with its target. Thrombin which binding with aptamer can blocks the electron transfer between $Fe(CN)_6^{3-/4-}$ and electrode surface, then the value of $R_{et}$ would increase[5-9].

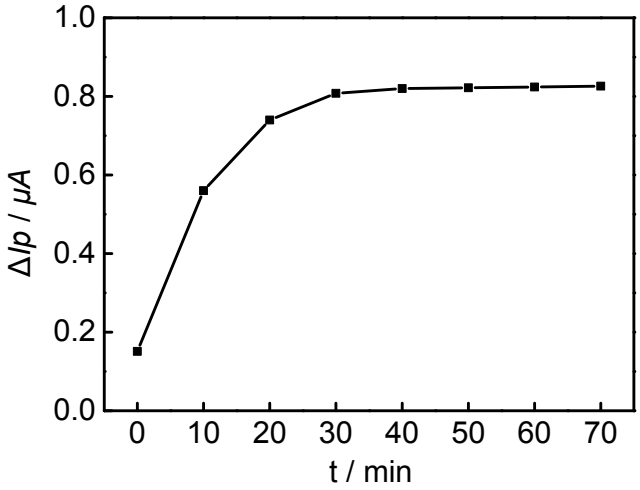


**Figure S2** Dynamic curve for the signals of aptamer 1 modified electrode to the time of incubation with 5 $\mu$M thrombin.

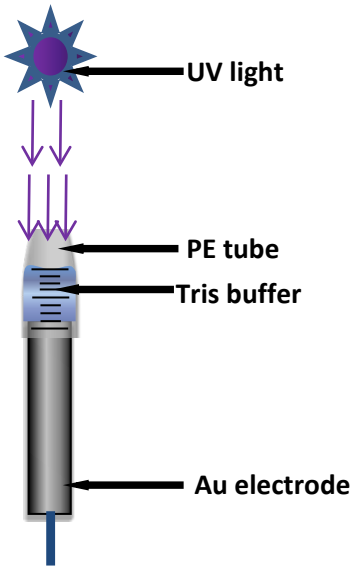


**Figure S3** Schematic illustration of the experimental setup for photoregeneration of the electrochemical aptasensor. To regenerate the aptasensor, the aptasensor previously being challenged was covered by a PE tube, in which 100 $\mu$L of Tris buffer (20 mM) was filled. The end of PE tube was cut off to permit irradiation of UV light. The UV lamp was placed above the electrode.

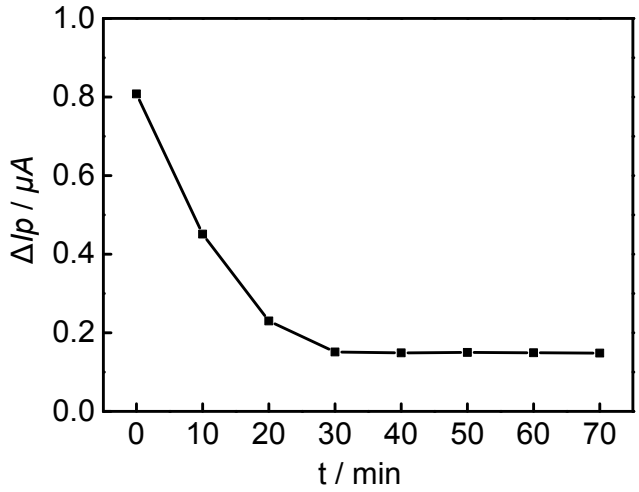


**Figure S4** Light-induced change of the regenerated signals on the aptamer 1 modified electrode as a function of UV-light irradiation time.

## 5. References


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