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Supporting Information

Label-free and sensitive thrombin sensing on molecularly grafted aptamer on graphene

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Chemicals. 15-mer thrombin-binding aptamer (TBA), with sequence of 5'-NH₂-GGT TGG TGT GGT TGG-3', was purchased from Bioneer Trade Co., Ltd. (Shanghai, China).

Human α -thrombin was supplied by Sigma (St. Louis, MO, USA). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (Spain). Bovine serum albumin (BSA) and trypsin (TYP) were obtained from RED Chemical Co., Ltd. (Shanghai, China). Graphene oxide (GO) was prepared according to the literature [1]. Healthy human serum was provided by Nanjing Maternity and Child Health Care Hospital. All other chemicals were of analytical grade. Double-distilled water (DDW) was used in making all aqueous solutions.

Apparatus. Cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) were conducted using CHI660B electrochemical analyzer (CH Instruments, Chenhua Inc., Shanghai, China). A three-electrode system was employed consisting of a modified glassy carbon working electrode, a platinum wire counter electrode and a Ag/AgCl (saturated with KCl) reference electrode. Fourier transform infrared (ATR-FTIR) spectrum was recorded on a Cary 5000 spectrophotometer (Varian Co., USA). Raman spectrum was recorded on a Labram HR800 (Jobin Yvon Co., France). All experiments were conducted at ambient temperature.

Preparation of graphene (GR) modified electrode. The glassy carbon electrode (GCE) was sequentially polished to a mirror finish with 0.3 μm and 0.5 μm alumina slurry and washed with absolute alcohol followed by rinsing thoroughly with DDW. Finally, the polished electrode was allowed to dry at room temperature after ultrasonic cleaning for 5 min in DDW. 2 μL of chitosan/graphene oxide (CS/GO) was coated on the cleaned GCE surface and dried for 6 h in air. The CS/GO coated electrode was immersed in 1 mL hydrazine and heated for 6 h at 60 °C. After reduction reaction, the electrode was rinsed thoroughly with 0.1 M phosphate buffer solution (PBS, pH 7.4) and DDW and a graphene modified electrode (GR-GCE) was thus obtained.

Fabrication of GR-TBA modified electrode. GR-GCE was electrochemically pretreated by applying an oxidative potential of 1.5 V for 5 min, after which the electrode was activated with 0.5 mL of 0.1 M PBS containing 10 mM EDC and 10 mM NHS for 16 h. Subsequently, the electrode was immersed in 1 mL of 1 μM TBA containing 0.1 M NaCl and 4 M EDTA for at least 24 h at ambient temperature. Finally, the electrode was rinsed with copious DDW to remove unfixed aptamer molecules.

Electrochemical measurements. The electrochemical performances of the modified electrode were investigated by CV and EIS. CV was conducted with potential scanning range of -0.1 to 0.6 V and a scan rate of 50 mV/s. EIS was performed in 0.1 M PBS (pH=7.4) containing 10 mM K₃Fe(CN)₆/K₄Fe(CN)₆ and 0.1 M KCl,^[2-3] over a wide range of frequencies from 1 to 10⁵ Hz with an initial potential of 0.28 V.

Detection of thrombin was carried out by immersing the sensing interface into 0.1 M PBS containing a certain concentration of target thrombin for 20 min. The resulting change of peak current upon the binding of aptamer with thrombin was monitored using DPV. DPV was performed by potential scanning between -0.1 V and 0.6 V with amplitude of 0.05 V.

In order to investigate the conductivity of the electrodes, CVs were conducted in 0.10 M PBS. Figure S1 shows the CV curve of bare electrode, GR modified electrode and GR-TBA modified electrode. As it is commonly known, the bare GCE electrode displays pure capacitive feature and has no electrochemical redox reaction in blank PBS. After modification with GR, the electrode still represents strong capacitive characteristics but possesses high ability to accommodate charges in comparison with bare GCE. Interestingly, the

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functionalized electrode displays a couple of well-defined redox peaks with a peak-to-peak separation (ΔE_p) of 53 mV, implying good conductivity of the biofilm-modified electrode.

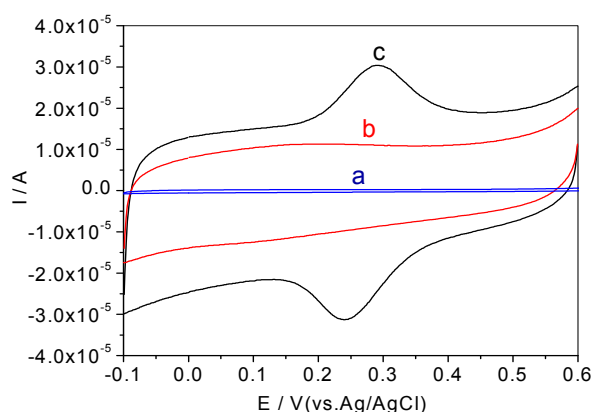


Figure S1. CVs of bare GCE (a), GR-GCE (b), GR-TBA-GCE (c) in 0.10 M PBS. Scan rate 50 mVs⁻¹.

To evaluate the binding specificity to target protein of the aptasensor, control experiments were performed. Bovine serum albumin (BSA) and trypsin (TYP) co-existing with thrombin in blood were used in our study and the results were shown in Figure S2. The presence of these proteins in a mixture without thrombin has a negligible effect upon the current, whereas substantial drop in current was observed after the aptasensor was immersed in a mixed protein solution containing thrombin. The current in the presence of mixed solution with 50 fM thrombin is much lower than that with 5 fM thrombin. The phenomena imply that the thus prepared sensor has good specific binding between aptamer and thrombin.

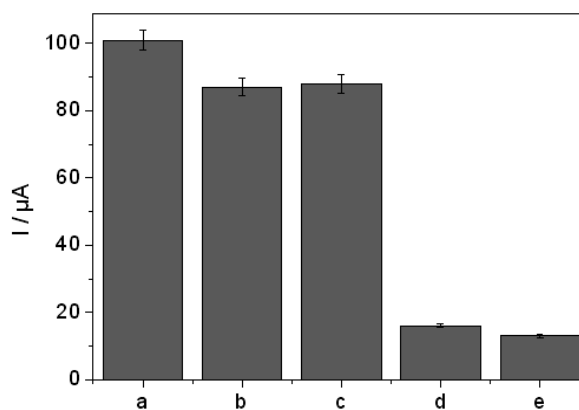


Figure S2. Specificity analysis of aptasensor tested in blank (a) and in the presence of 25 nM BSA (b); 25 nM TYP (c); 25 nM BSA, 25 nM TYP and 5 fM thrombin (d) and 25 nM BSA, 25 nM TYP and 50 fM thrombin (e).

References:

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