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Electronic Supplementary Information (ESI)

Label-free, regenerative and sensitive surface plasmon resonance and electrochemical aptasensors based on graphene

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Experimental Section

Reagents. GO was synthesized from graphite (spectral pure, Sinopharm Chemical Reagent Co., Ltd., China) by Hummers method with little modification.[1] Well dispersed GN was prepared by the chemical reduction of GO with hydrazine.[2] 3-mercaptopropionic acid (MPA) and Poly(diallydimethylammonium chloride) (PDDA, average Mw <100,000), were purchased from Alfa Aesar. Oligonucleotide containing specific sequences (TBA, 5′ GGT-TGG-TGT-GGT-TGG 3′) and the mismatch aptamer sequence (MAS, 5′ GGT-TCG-TGT-GCT-TGG 3′) were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). DNA oligonucleotide stock solutions were prepared with Tris-HCl buffer (25 mM Tris-HCl, 300 mM NaCl, pH=8.2) and kept frozen. α-thrombin, lysozyme and BSA, purchased from Sigma, were prepared in the Tris-HCl buffer (20 mM Tris-HCl, 140 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, pH=7.4). All other chemicals not mentioned here were of analytical reagent grade and used as received. Double distilled water was used throughout.

Instrumentation. SPR measurements were carried out for characterizing the fabrication of the sensing interface using an Autolab SPR instrument (Eco Chemie BV, The Netherlands). The measured Δθ values corresponded to the amount of adsorbed material with a mass sensitivity factor of 120 mDeg per 100 ng/cm^2. Cyclic voltammetry (CV) and EIS were performed on an Autolab PGSTAT30 (Utrecht, The Netherlands). A conventional three-film system, with a Au electrode (3 mm in diameter) as working film, a Ag/AgCl reference film, and a platinum wire as counter film was used. The cell was housed in a homemade Faraday cage to reduce stray electrical noise. All the measurements with the

Autolab were carried out at room temperature. EIS was performed under an oscillation potential of 5 mV over the frequency range of 10 kHz to 0.1 Hz. The amplitude of the alternate voltage was 5 mV. All the measurements (EIS, CV) were performed in the solution of 5 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ (in 67 mM PBS buffer, pH = 7.0). Atomic force microscopy (AFM) was performed on a SPI3800N microscope instrument (Seiko Instruments, Inc., Japan) in tapping-mode in air at ambient temperature.

Modification Process to Fabricate the SPR Sensing Surface. a) Prepare p-Au film. SPR Au film was first immersed in MPA (0.01M in ethanol) for 2 h to form negatively charged surface. After washing and N_2 drying, the substrate was immersed in 1% PDDA solution (1 h). The successfully preparation of p-Au film was investigated by SPR technique (Fig. S2). b) Prepare TBA/GN/p-Au sensing interface. P-Au film was immersed in 0.25 mg/mL GN solution for 1 h to assembly GN on p-Au film due to electrostatic interaction between PDDA and GN, followed by rinsing with water. 10 μM TBA (25 mM Tris-HCl, 300 mM NaCl, pH=8.2) was then added for 1 h to fabricate the TBA/GN/p-Au sensing interface.

SPR Detection of α-Thrombin and Regeneration of the TBA/GN/p-Au Sensing Interface. The as-prepared sensing interface was immersed in a series of α-thrombin solutions with different concentrations for 1 h. Control experiments were also carried out. The sensing interface was immersed in 40 nM BSA, and 40 nM lysozyme for 1 h respectively. A random oligonucleotide sequence containing two bases mismatch in α-thrombin aptamer (MAS) was also used for the control experiment. All the assembly processes were the same as before except using MAS instead of TBA, then the sensing interface was dipped into 40 nM α-thrombin solution. After each of detection, the used sensing interface could be regenerated easily by adding 10 μM TBA solution for 1 h to fabricate the TBA/GN/p-Au sensing interface.

Application of the Aptasensor in Biological Assay. Pretreated human plasma was used to confirm the applicability of the aptasensor. Healthy human plasma was pretreated with salt solution to avoid the formation of fibrin and the rapid sample clotting according to the previous articles.[3] Different concentrations of standard solutions of α-thrombin were spiked into the diluted pretreatment of 1%

plasma to test the performance of the aptasensor. All experimental conditions were the same as the foregoing detection.

Film Cleaning, Pretreatment, and Modification Process for the EIS Detection of α-Thrombin. Au electrode (3 mm in diameter) was polished with 1.0 μm, 0.3 μm α-Al_2O_3 and then washed ultrasonically with pure water for three times successively, followed by electrochemically cleaning in 0.1 M H_2SO_4 by potential scanning between -0.2 to 1.6 V until a reproducible cyclic voltammetry was obtained. Then it was rinsed with a copious amount of pure water and finally blown dry with nitrogen before assembly. The modification process to fabricate the sensing surface and the detection process were the same as before except using a Au elctrode (3 mm in diameter) instead of SPR Au film.

EIS Detection of α-Thrombin at TBA/GN/p-Au Sensing Interface. The as-prepared sensing interface was immersed in a series of α-thrombin solutions with different concentrations for 1 h. Control experiments were also carried out. The sensing interface was immersed in 20 nM BSA, and 20 nM lysozyme for 1 h respectively. MAS/GN/p-Au film was also used for the control experiment. The sensing interface was dipped into 20 nM α-thrombin solution.

SPR Detection of α-Thrombin at TBA/p-Au Sensing Interface. p-Au film was immersed in 10 μM TBA (25 mM Tris-HCl, 300 mM NaCl, pH=8.2) for 1 h to fabricate the TBA/ p-Au sensing interface. The as-prepared sensing interface was immersed in a series of α-thrombin solutions with different concentrations for 1 h. Control experiments were also carried out. The sensing interface was immersed in 40 nM BSA, and 40 nM lysozyme for 1 h respectively. The results were shown in Fig. S5.

EIS Detection of α-Thrombin at TBA/p-Au Sensing Interface. The modification process to fabricate the TBA/p-Au sensing surface and the detection process were the same as SPR detection of α-thrombin at TBA/p-Au sensing interface except using a Au elctrode (3 mm in diameter) instead of SPR Au film. The results were shown in Fig. S11.

SPR and EIS response of thrombin was performed at GN/p-Au. When the concentration of thrombin was less than 50 nM, there was no SPR angle shift. Moreover, there was no selectivity for the detection of thrombin at GN/p-Au at all. There was also no selectivity for the EIS detection of

thrombin at GN/p-Au.

Fig. S1 Tapping mode AFM images of GN on freshly cleaved mica substrate.

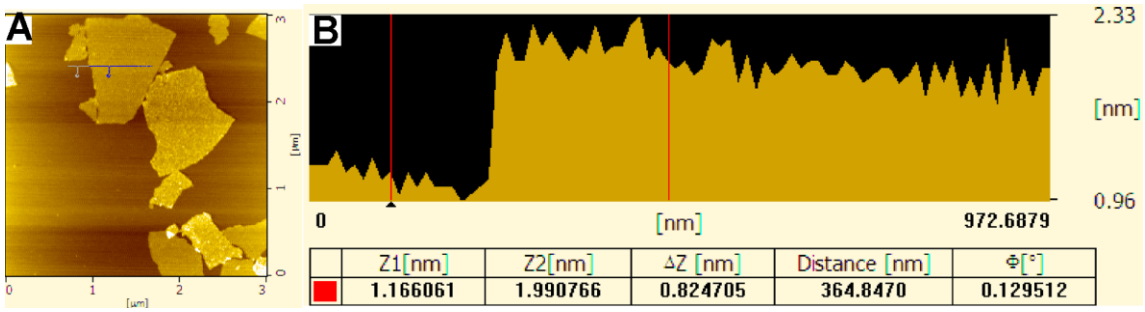
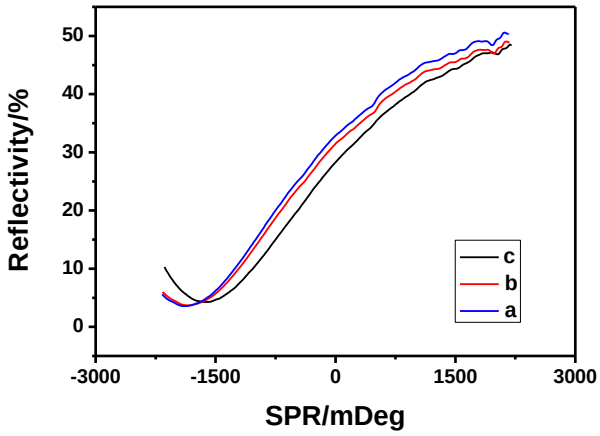


Fig. S1A shows an AFM image of the flat GN on mica. A height profile (Fig. S1B, ESI†) along the line shows the thickness of the nanosheets is about 0.82 nm. This value matches well with the reported apparent thickness of GN, [2,4,5] suggesting the single-sheet nature of GN obtained in this work.

Fig. S2 The angle-resolved SPR curves during the different steps (SPR Au film (a), MPA (b) and PDDA (c)) to fabricate the p-Au film.



SPR Au film was chosen to bind MPA through Au-S bond, and then PDDA was assembled on the film by electrostatic interaction between PDDA and MPA. As indicated in Fig. S2, the assembly of MPA and PDDA on SPR Au film showed angle shift of about 40, and 254 mDeg, respectively. The value of angle shift was mainly dependent on the molecular weight and surface coverage of the molecule adsorbed on the SPR Au film.

Fig. S3 Stepwise SPR response during the different steps (p-Au film (a), GN (b), TBA (c) and treatment with 1 nM α-thrombin (d)) of the sensor immobilization. DNA buffer was Tris-HCl buffer (25 mM Tris-HCl, 300 mM NaCl, pH=8.2). Thrombin buffer was Tris-HCl buffer (20 mM Tris-HCl, 140 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, pH=7.4).

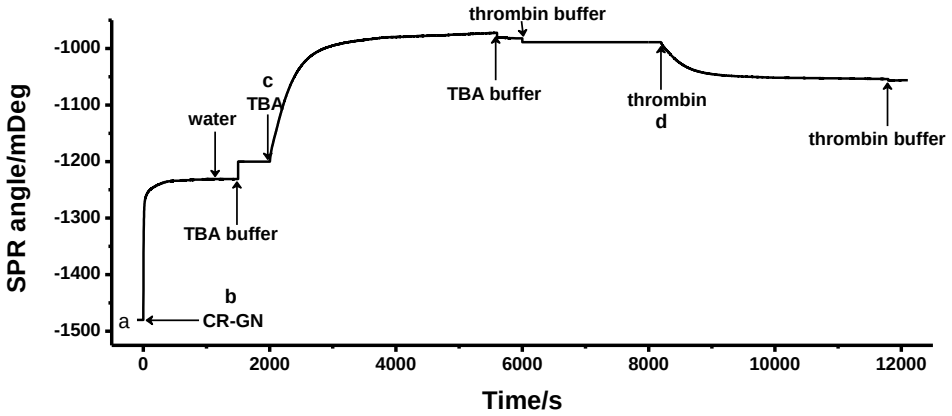


Fig. S4 The relationship between the SPR response and the concentration of α-thrombin. The average of the RSD was 0.0293. The corresponding regression coefficient of the linear part was 0.996.

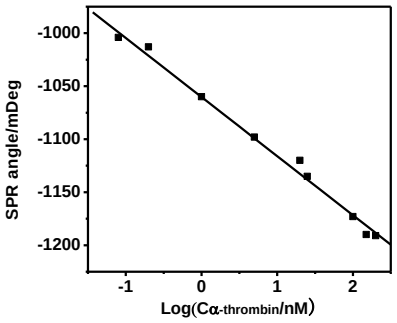
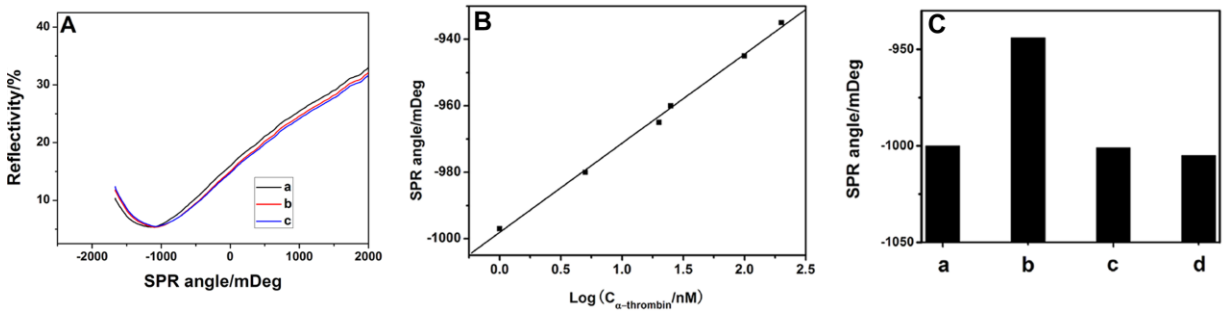


Fig. S5 (A) The angle-resolved SPR curves during the different steps (p-Au film (a), TBA (b) and treatment with 1 nM α-thrombin (c)) of the sensor immobilization. (B) The relationship between the SPR response at TBA/p-Au film and the concentration of α-thrombin. The average of the RSD was 0.0239. The corresponding regression coefficient of the linear part was 0.995. (C) Control experiments for α -thrombin: (a) TBA/p-Au film; (b) 40 nM α-thrombin at TBA/p-Au film; (c) 40 nM lysozyme at TBA/p-Au film; (d) 40 nM BSA at TBA/p-Au film.



In the absence of GN, the amount of TBA assembled on the p-Au film was much less than that of GN/p-Au (please see Fig. 2A in the manuscript and Fig. S5 in ESI†). As shown in Fig. S5, we obtained a linear relationship between the SPR angle and the logarithm of α-thrombin concentrations over a range of 1 – 200 nM with the lowest detection limit down to 1 nM.

Fig. S6 SPR response of the sensing interface to α-thrombin at different concentrations in the 1% pretreated biological human plasma. The corresponding regression coefficient of the linear part was 0.989.

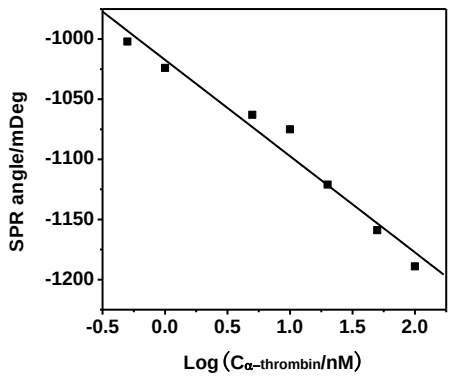
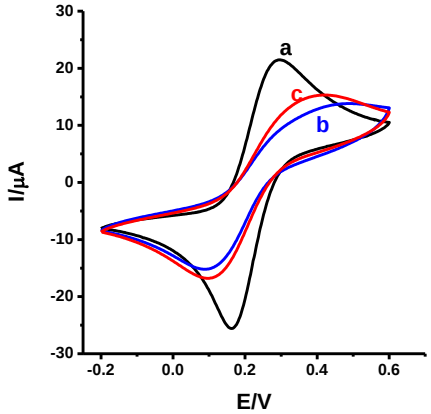
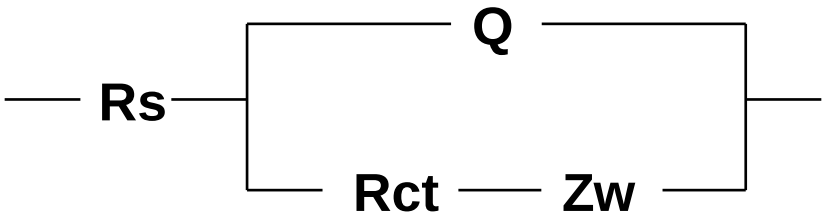


Fig. S7 CV response during the different steps (GN/p-Au film (a), TBA/GN/p-Au film (b) and treatment TBA/GN/p-Au film with 0.2 nM α-thrombin (c)) of the sensor immobilization.



As shown in Fig. S7, the CV response of electrochemical probe ($[Fe(CN)_6]^{4-/3-}$ anions) evidently decreases after TBA adsorption on GN/p-Au film. This illustrates that the TBA self-assembly layer is compact for blocking the redox probes from effective charge-transfer at the sensing interface. After treating the TBA/GN/p-Au film by α-thrombin solution, the CV response is enhanced due to the desorption of TBA from the sensing interface (Fig. S7 (c)).

Fig. S8 Circuit for the EIS.



In the circuit, Rs corresponds to the resistance of the solution; Rct represents the resistance to charge transfer between the solution and the electrode surface; Zw is the Warburg impedance due to the contribution of diffusion; and Q is the constant phase element.

Fig. S9 The relationship between the EIS response and the concentration of α-thrombin. The average of the RSD was 0.0282. The corresponding regression coefficient of the linear part was 0.998.

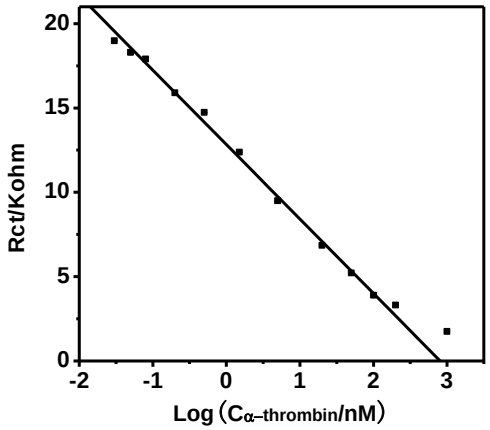
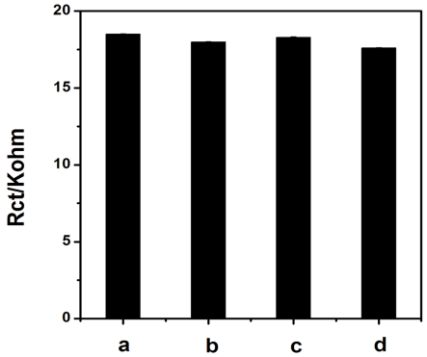


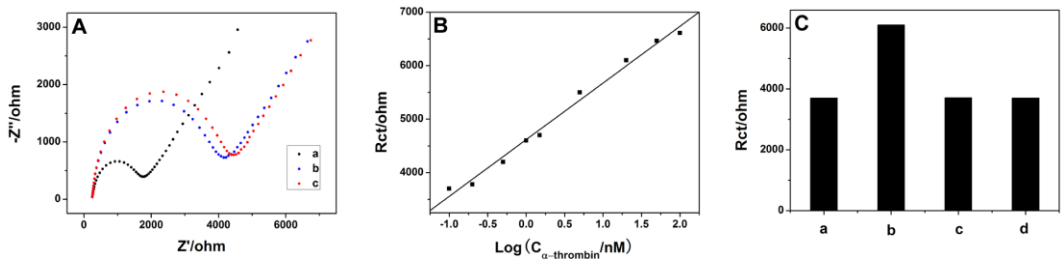
Fig. S10 Control experiments for α-thrombin: (a) TBA/GN/p-Au film; (b) 20 nM BSA at TBA/GN/p-Au film; (c) 20 nM lysozyme at TBA/GN/p-Au film; (d) 20 nM α-thrombin at MAS/GN/p-Au film.



After 1 h immersion in 20 nM BSA (Fig. S10 (b)) and 20 nM lysozyme (Fig. S10 (c)) respectively, the TBA/GN/p-Au electrode shows almost no change of EIS signal. MAS/GN/p-Au electrode is also

used for the control experiment. The sensing interface is dipped into 20 nM α-thrombin solution. There is also almost no change of Rct value at the MAS/GN/p-Au electrode (Fig. S10 (d)).

Fig. S11 (A) The EIS response during the different steps (p-Au film (a), TBA (b) and treatment with 1 nM α-thrombin (c)) of the sensor immobilization. (B) The relationship between the EIS response at TBA/p-Au film and the concentration of α-thrombin. The average of the RSD was 0.0297. The corresponding regression coefficient of the linear part was 0.991. (C) Control experiments for α-thrombin: (a) TBA/p-Au film; (b) 20 nM α-thrombin at TBA/p-Au film; (c) 20 nM lysozyme at TBA/p-Au film; (d) 20 nM BSA at TBA/p-Au film.



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