Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

New Photoelectrochemical Aptasensor for the Thrombin Detection Based on Functionalized Graphene and CdSe Nanoparticles Multilayers

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1. Reagents and Apparatus

Reagents. Graphite powder, NaOH, KMnO4, $K_2S_2O_8$, P_2O_5, H_2SO_4, triethylamine, N-methylpyrrolidone, 30% H_2O_2 aqueous solutions and hydrazine monohydrate were all analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd.. PAA (Mw=2000), pyrenylmethylamine hydrohloride, 1,3-dicyclohexyl-carbodiimide (DCC), 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC), PDDA (20%, w/w in water, molecular weight 200 000~350 000) and 2-aminothanethiol (AET) were purchased from ACROS Organics (Japan) and used as received. Bovine plasma albumin (BSA) and thrombin were obtained from Sigma. The oligonucleotide aptamer used in this study was purchased from SBS Genetech Co.,Ltd. (China) with the following sequences: 5'-PO_3H -TTTTTTGGTTGGTGTGGTTGG-3'

Apparatus. The photocurrent was measured on an electrochemical workstation (Zahner Zennium, Germany). A three-electrode system was employed with Pt wire as an auxiliary electrode, Ag/AgCl as a reference electrode and ITO conductive glass supplied by Weiguang Corp. (Shenzhen, People's Republic of China, ITO coating 180±25 nm, sheet resistance ≤10 Ω/square) as a working electrode. Scanning electron microscopy (SEM) (JSM-6700F, JEOL, Japan) was used to examine the morphology of modified electrodes.

2. Preparation of Graphene Oxide (GO)

Graphene oxide was synthesized from natural graphite by a modified Hummers method.[1,2] Graphite powder (10 g), $K_2S_2O_8$ (5 g), and P_2O_5 (5 g) were added into solution of concentrated H_2SO_4 (15 mL). The mixture was kept at 80 °C for 6 h. Then cooled down to room temperature and diluted with 1 L of DDW. The diluted mixture was filtered and washed to remove the residual acid until the pH value of rinse water became neutral. The product was dried under ambient

condition for 3 days. This oxidized graphite powder was then subjected to oxidation using Hummers' method. The preoxidized graphite powder (2 g) was put into concentrated H_2SO_4 (46 mL) at 8 °C, $KMnO_4$ (6 g) was added gradually with stirring and ice-bath cooling, then the mixture was stirred at 35 °C for 2 h and water (95 mL) was added. In 15 min, the reaction was terminated by adding a large amount of water (280 mL) and 30% H_2O_2 solution (5 mL). The mixture was filtered and washed with 1:10 HCl solution in order to remove metal ions. The GO product was diluted in water forming 2% GO dispersion, and then the dispersion was filtered and washed until the water reached a neutral pH. Finally, the product was vacuum-dried.

3. Poly(aclic acid) labelled with 1-pyrenylmethylamine (PAA-Pyr)[3]

A mixture of PAA (l.0 g) in 1-methylpyrrolidone (50 mL) was heated to 60°C for 2 h under nitrogen atmosphere to allow the polymer to dissolve. A solution of 1-pyrenylmethylamine hydrochloride (0.0956 g) in l-methylpyrrolidone (5 mL) was added quickly to the resulting solution, followed by triethylamine (65 μL) and a solution of DCC (0.0947 g) in l-methylpyrrolidone (5 mL). The reaction mixture was stirred at 60°C for 24 h in the dark, cooled in an ice-bath and neutralized by dropwise addition of a concentrated sodium hydroxide solution. The resulting precipitation was separated by filtration and washed three times with hot (60°C) 1-methylpyrrolidone (20 mL) and three times with cold methanol (20 mL). The resulting solid was dried in vacuum. It was converted to the acid form by elution through a cation-exchange column. Freeze-drying yielded 1-pyrenylmethylamine labeled PAA as a fluffy white solid.

4. Preparation of PAA-Modified Graphene Sheets.[4]

Graphene oxide obtained above was sonicated for half an hour in water to form a stable solution. For the chemical reduction of graphene oxide, 25 mL of 0.5 mg/mL graphene oxide solution was mixed with 25 mL of 0.5 mg/mL PAA-Pyr, followed by the addition of 10 mg of NaOH and 1 mL of hydrazine monohydrate. The solution was kept in a water bath at 80 °C for 24 h. The as-prepared solution was denoted as PAA-G.

5. Synthesis of CdSe-NH_2 NPs

Colloidal CdSe NPs were prepared as described with a slight modification.[5] Briefly, freshly prepared 0.1 M NaHSe solution was added to 1.25 mM N_2-saturated $CdCl_2$ solution at pH 5.6-5.9 in the presence of AET as a stabilizing agent. The molar ratio of Cd^{2+}/AET/HSe^- was fixed at 1:2.4:0.5. After vigorously stirring the mixture for 10 min, it was refluxed for 3 h to control the growth of the CdSe NPs.

6. Fabrication of the biosensor and thrombin analysis

The graphene-CdSe NPs multilayer film was prepared by a LBL assembly of negatively charged PAA-modified graphene and positively charged CdSe NPs capped with AET. Briefly, the ITO slices were sonicated in acetone, NaOH (1 M) in 1:1 (v/v) ethanol/water and water, respectively, for about 15 min each. The multilayer film was grown firstly by casting of 2% PDDA containing 0.5 M NaCl (5 μL) on the cleaned ITO slices, and then the as-obtained PAA-modified graphene solution (5 μL) and CdSe NPs solution (5 μL) were alternately casted on PDDA modified ITO electrode for 15 min, respectively. Between each step, the surface was rinsed with DDW and blown dry under a nitrogen stream. This process was repeated to obtain a desired

number of $(PAA\text{-}G/CdSe)_n$ multilayer.

Conjugation of aptamers onto a CdSe nanoparticles modified electrode was achieved by using the classic EDC coupling reactions between-NH_2 groups on the surfaces of the AET-capped CdSe NPs and $–PO_3H$ groups of the aptamer. Briefly, 20 μL of 10^{-5}M phosphoryl-modified aptamer was activated in 40 μL of 0.1 M imidazole buffer (pH 6.8) containing 0.2 M EDC 40 μL. Then actived aptamer (10 μL) was casted onto the surface of $(PAA\text{-}G/CdSe)_n$ modified electrode and incubated at room temperature overnight. After incubation, the electrode was rinsed with the buffer solution. This electrode was then blocked with 10 μL of 2% BSA for 1 h at room temperature, washed with 0.05% Tween and buffer solution thoroughly. Finally, the modified electrode was immersed in 1 mL of thrombin solutions and incubated at 37 °C for 1 h followed by washing with the buffer solution three times.

Photoelectrochemical detection was carried out in 0.1 M PBS (pH=7.4) containing 0.1 M ascorbic acid (AA) which was served as a sacrificial electron donor during the photocurrent measurement. Light excitation of 430 nm was switched every 10 s. The applied potential was 10 mV. Radiation intensity is $48.22W\cdot m^{-2}$。

7. Preparation of multilayered CdSe NPs ($(PAA/CdSe)_n$)

$(PAA/CdSe)_n$ multilayers were obtained by casting 5 μL PAA (0.25 mg/mL) and 5 μL CdSe NPs solution obtained above on PDDA modified ITO electrode for 15 min, alternately. Between each step, the surface was rinsed with DDW and blown dry under a nitrogen stream. This process was repeated to obtain a desired number of $(PAA/CdSe)_n$ multilayers.

8. UV-*vis* spectra of graphene oxide (GO) and PAA modified graphene (PAA-G)

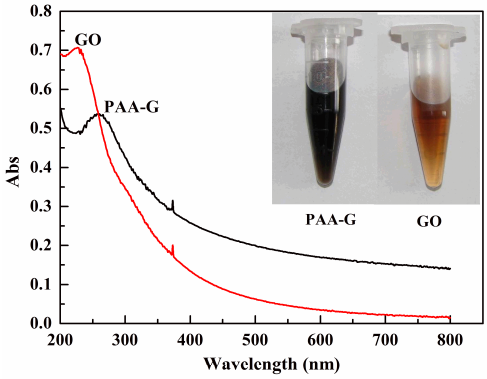

Fig. S1. UV-*vis* spectra of graphene oxide (GO) and PAA modified graphene (PAA-G). The inset: photo of GO and PAA-G aqueous solution.

8. Comparation of sensivivity with other methods.

The value of detection limit in this method (4.5× 10^{-13} M) was lower than that obtained from the amperometric detection using Pt nanoparticles as catalytic labels (1 nM),[6] electrochemical molecular beacon aptasensor (0.5 nM),[7] chemiluminescence detection amplified by the RCA and DNAzyme (6.6 pM)[8] and graphene fluorescence resonance energy transfer aptasensor (31.3 pM).[9]

9. Control experiments

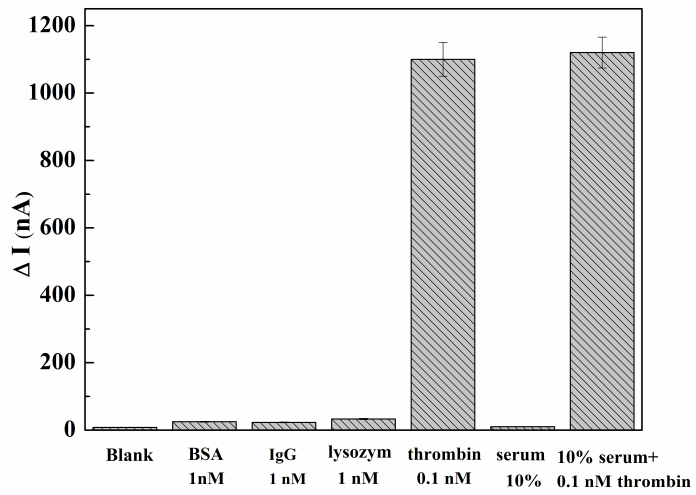

Fig. S2. Selectivity of thrombin toward different analytes.

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