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Supporting Information

DNA Cycle Amplification Device on Magnetic Microbeads for Determination of Thrombin Based on Graphene Oxide Enhancing Signal-On Electrochemiluminescence

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Materials. Natural graphite powder was purchased from Sinopharm Chemical Reagent Co. Ltd. and used without further purification. Oligonucleotides were synthesized by SBS Genetech Inc. Sequences of the oligonucleotides are listed in Table 1. The endonuclease was purchased from Fermentas. Ramos cells were obtained from Chinese Academy of Medical Sciences. 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), bovine serum albumin (BSA) and poly(dimethyldiallylammonium chloride) (PDDA) were purchased from Sigma. 2-sulfanylethane sulfonic acid, sodium bis-(2-ethylhexyl)sulfosuccinate, n-hexane and cystamine were purchased from Alfa Aesar. AFP antibody was purchased from Biocell Bioeng. Co. (Zhengzhou, China). Purified thrombin (lyophilized powder) was purchased from dingguo biological Technology Corporation (Beijing, China). Mercaptoethylamine (MCH) was obtained from Fluka (USA). Superoxide Dismutase (SOD) was obtained from Worthington Biochemical Corporation. Magnetic microbeads modified with carboxyl groups (MB) was obtained from BaseLine Chrom Tech Research Centre (Tianjin, China). All of the other reagents were purchased from Sinopharm Chemical Reagent Co. Ltd. and used without further purification. Deionized water was used throughout all of the experiments.

Table S1. Oligonucleotides Sequences

DNA	**Sequences (5′to 3′)**
Aptamer	AGTCCGTGGTAGGGCAGGTTGGGGTGACT
oligonucleotides **1**	HS-TTTTGACT CCTAAGCCGATTTT-NH_2
oligonucleotides **2**	TTTATCG GCTTAGG AGTCACCCCAACCTCCGATC
oligonucleotides **3**	HS-TTTTT
oligonucleotides **4**	HS-TTTTGACTCC

Apparatus. ECL measurements were carried with a MPI-A ECL analyzer (Xi'An Remax Electronic Science & Technology, Xi'An, China) using a three-electrode system. The Au electrode (diameter 4 mm) or GOE acted as a working electrode. An Ag/AgCl electrode (saturated KCl) acted as a reference electrode. A Pt wire acted as a counter electrode. Cyclic voltammetry (CV)

and electrochemical impedance spectroscopy (EIS) were performed on a CHI 660C electrochemical workstation (Shanghai CH Instruments, China), using the same three-electrode system as for ECL detection. Transmission electron microscopy (TEM) images were obtained using a JEOL JSM-6700F instrument (Hitachi, Japan). Scanning electron microscopy (SEM) was carried out on a JEOL JSM-6340 F instrument (Hitachi, Japan). Fourier transform infrared transmission spectra were recorded using a Nicolet 510P FT-IR spectrophotometer. UV-visible spectra were carried out on a Cary 50 UV-Vis-NIR spectrophotometer (Varian).

Synthesis of CdS nanoparticles (CdS NPs). CdS NPs were prepared according to the literature [1, 2] with a slight modification. In brief, 14.0 g of sodium bis-(2-ethylhexyl)sulfosuccinate was dissolved in a mixture of water-n-hexane (4 mL/200 mL). The resulting mixture was separated into 120 mL and 80 mL subvolumes. 0.48 mL of 1.0 M $Cd(NO_3)_2$ solution was added to the 120 mL subvolume, while a 0.32 mL of 1.0 M Na_2S solution was added to the 80 mL subvolume. The two subvolumes were stirred for 1 h, and then mixed and stirred under N_2 for 1 h. 0.34 mL of 0.32 M cystamine solution and 0.66 mL of 0.32 M 2-sulfanylethane sulfonic acid were added stirring for 24 h under N_2. After vacuum evaporation under vacuum and the produce was successively washed with pyridine, n-heptane and methanol to yield the water-soluble CdS NPs.

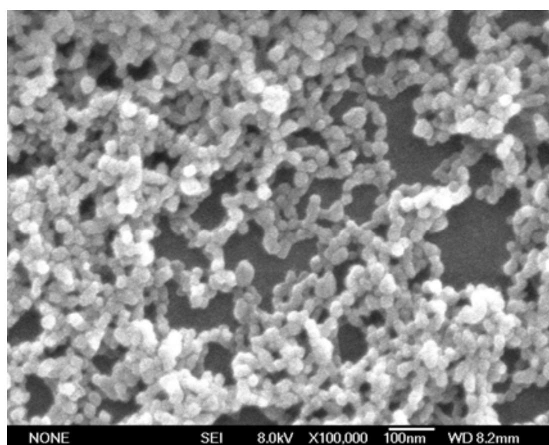

Fig. S1. SEM image of CdS NPs.

The morphological structures and sides of CdS NPs were studied by means of scanning electron microscopy (SEM), as shown Fig. S1. The particle size of above mentioned CdS NPs was about 15 nm.

Synthesis of Graphite Oxide. Graphite oxide (GO) were prepared from natural graphite powder according to the Hummers method [3], with a slight modification. The 3 g of graphite was added into 120 mL of H_2SO_4 (98%). The mixture was cooled to 0 ºC and added in 15 g of $KMnO_4$ under stirring. The temperature was raised to 35 ºC for 2 hours with stirring. The reaction mixture was diluted with 250 mL of deionized water in an ice bath. Then 20 mL of H_2O_2 (30 wt%) was added to the mixture. The mixture was washed with 5% HCl solution and then with deionized water. For purification, the product was dialyzed. Finally, GO dry powder was obtained after drying in the vacuum at room temperature.

The morphological structures and size of GO was obtained by means of transmission electron microscopy (TEM) (Fig. S2).

Fig. S2 TEM image of and GO.

FT-IR Spectrum of GO. GO was characterized by FT-IR (Fig. S3). The absorption peaks demonstrate many kinds of oxy-groups, such as C=O, C-OH and O-H were generated on the carbon surface after a oxidation procedure. It illustrated the successful oxidation of graphite.

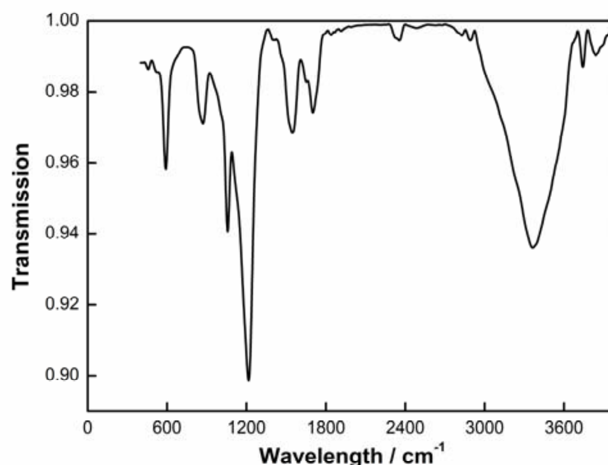

Fig. S3. FT-IR of GO.

Electrode Preparation and Modification. An Au electrode (4 mm diameter) was polished carefully with 1.0, 0.3, and 0.05 μm alumina powder sequentially and then washed ultrasonically with deionized water. The bare Au electrode was scanned in 0.5 M H_2SO_4 until a reproducible CV was obtained. The cleaned Au electrode was dried with nitrogen steam for the next modification. The GOE electrode was prepared by a two-step process. First, Au electrode was immersed in 1.0 mL of 1.0 mM mercaptoethylamine (MCH) for 16 h at 25 ºC. The MCH modified Au electrode was then thoroughly rinsed with water. For bonding the GO onto the MCH modified Au electrode, the MCH modified Au electrode was immersed into 1.0 mL GO dispersion containing 0.01 M EDC and incubated for 12 h with gently shaking at 25 ºC to produce a GO attached electrode (GOE). The product was washed with water then dried with pure N_2 before use.

Fabrication for MB-stators. The MB-stators were prepared as follow. 40 μL of carboxylated magnetic microbeads (MB) was placed in a 1.5 mL Eppendorf tube (EP tube), washed three times with of 0.1 M imidazol-HCl buffer (pH 7.0). 100 μL of 0.2 M EDC was added to the EP tube, and

the mixture was incubated at 37 ºC for 1 h to activate the carboxylate groups on the MB. After washed with 0.01 M PBS buffer (pH 7.4), the MB-stators were synthesized by incubating the activated MB with 200 μL of 1.0×10^{-6} M 3'-amino group modified oligonucleotides 1 at 25 ºC for 12 h. Finally, the product (MB-stators) was washed with 0.01 M PBS buffer (pH 7.4), dispersed in 40 μL of 0.01 M PBS buffer (pH 7.4) and stored at 4 ºC until used.

Fabrication for MB-TStators. The stators were tagged according the reference with a slight modification. [1] The stators were tagged by adding 500 μL of CdS NPs and 200 μL of 1.0×10^{-7} M 5'-thiolated oligonucleotides 3 to 40 μL of MB-stators and shaking gently for 12 h at 37 ºC. Oligonucleotides 1 and 3-functionalized CdS NPs were formed. Oligonucleotides 3 played a placeholder part, which could avoid cross-reaction and introduce CdS NPs labeling on oligonucleotides 1 as much as possible to increase assay sensitivity. Finally the product (MB-TStators) was isolated by magnetic separation, washed with 0.01 M PBS buffer (pH 7.4) to remove excess CdS NPs and oligonucleotides, and resuspended in 40 μL of 0.01 M PBS buffer (pH 7.4) at 4 ºC until used.

Operation of the DNA cycle device. Aptamers are in vitro selected short oligonucleotides or peptide to bind to various targets [4] such as ions, proteins, and even cells with antibody-like specificity and affinity. Aptamer (10 nM) and its complementary oligonucleotides 2 (10 nM) were previously hybridized in 200 μL of 0.01 M PBS buffer (pH 7.4) at 37 ºC for 1 h to form the duplex structure. 50 μL of thrombin of different concentrations was interacted with the duplex structure at 25 ºC for 1 h. 40 μL of MB-TStators was added to capture the released oligonucleotides 2 by incubation at 25 ºC for 1 h. The hybridization results were separated from the incubation solution and washed three times with 0.01 M PBS buffer (pH 7.4) buffer, the next step to incubated in a 20

μL of Fermentas REase buffer R containing 0.5 U/μL nicking endonuclease (Nt. Bpu10 I). Nt. Bpu 10 I is a site and strand specific nicking endonuclease that cleaves only one strand of the DNA within its recognition sequence on double stranded DNA substrate. The operation of the oligonucleotides 2 was stopped by heating to 95 ºC for 5 min. After a magnetic separation, the solution 1 was stored at 4 ºC until used.

ECL Measurements. Rinsing the GOE surface with 0.01 M PBS buffer containing 0.5% SDS was very important to remove interferents. The solution 1 was dripped on GOE at room tempreture and then the electrode was rinsed with 0.01 M PBS buffer containing 0.5% SDS before ECL measurement. The modified electrode was in contact with 0.01 M PBS containing 0.1 M $K_2S_2O_8$ and 0.1M KCl and scanned from 0 V to -1.5 V.

Optimization of ECL Performance. The experimental parameters, the amounts of GO and the pH of PBS buffer solution, were investigated in order to achieve the high sensitivity ECL response for the detection of the analyte. All the investigations were performed with 1.0×10^{-14} M thrombin.

The pH value of detection solution could influence the π-π stacking interaction between the nucleobases and the GO. Therefore, the ECL intensity is undoubtedly influenced by the pH value of PBS buffer solution. The effect of pH was studied in the range of 5.5–7.8. As shown in Fig. S4A, the highest intensity occurred in pH 7.0 solution.

During the procedure, the nicking endonuclease activity is very important to the cleave-substrate efficiency which is related to ECL intensity. The temperature is one of the key elements which determine the nicking endonuclease activity. The nicking endonuclease activity would increase at higher temperature within a specific range. However, at too high temperature, the nicking endonuclease activity would decline, which induced less efficiency. The temperature

was examined from 25 ºC to 50 ºC (Fig. S4B). The temperature of the maximum ECL intensity was at 37 ºC

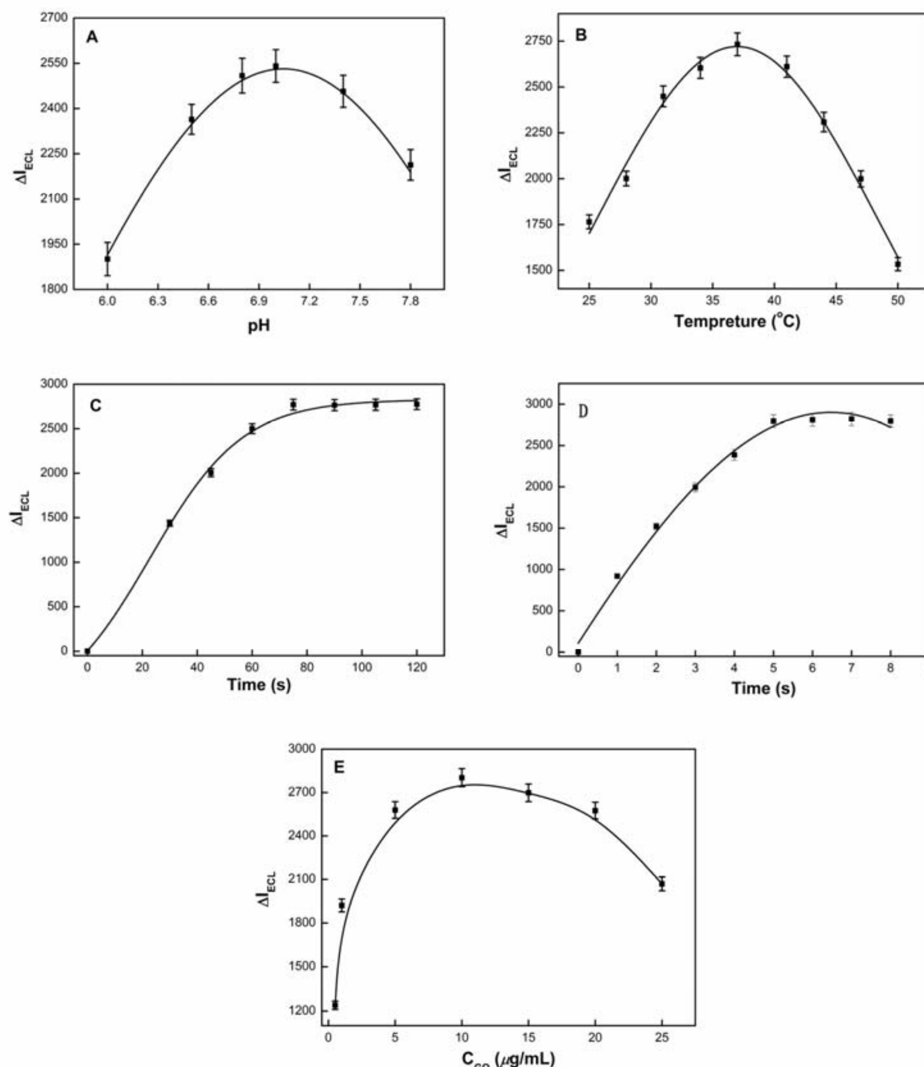

Fig. S4 Optimization of relative parameters (A) the pH value of detection solution, (B) the nicking endonuclease reaction temperature, (C) the nicking endonuclease reaction time, (D) the GOE capture time (E) the concentration of GO. All other factors are at their optimal values during optimizing one parameter. All the ECL signals were detected in the presence of 1.0×10^{-14} M thrombin.

In addition, to ensure the method time-saving, the effect of nicking endonuclease incubation time was investigated. Fig. S4C shows the influence of the incubation time of nicking endonuclease used in the DNA device. The ECL intensity increased as time increased from 30 min to 75 min and changed very little at the time beyond 75 min. Therefore, the time of 75 min was

chosen for nicking endonuclease.

Also, the time was spend capturing signal DNA by GOE was investigated. As shown in Fig. S4D, the ECL intensity increased as time increased from 0 min to 5 min and changed very little at the time beyond 5 min. The time seems quite short while capturing.

As shown in Fig. S4E, the ECL intensity was influenced by the amount of GO on GOE. The ECL intensity was achieved optimum effects, when the concentration of GO was 10.0 μg/mL.

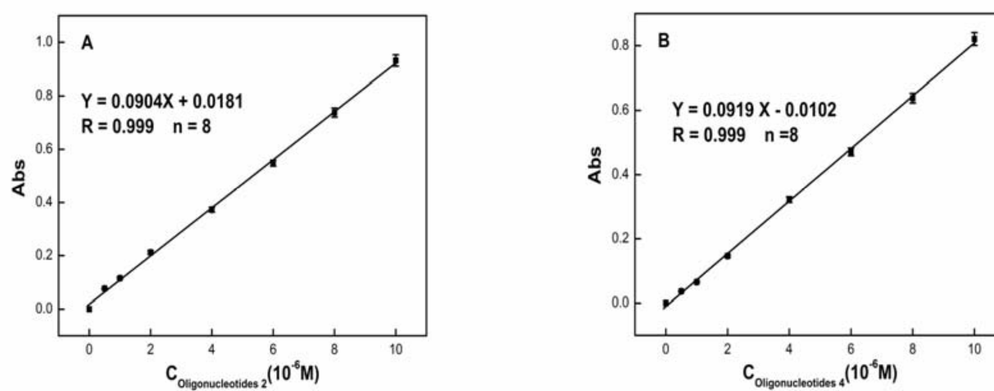

Fig. S5 (A) UV-vis absorbance calibration curve of oligonucleotides **2**. The regression equations could be expressed as Y = 0.0904 X + 0.0181 (X was the concentration of oligonucleotides **2**, 10^{-6} M; Y was the absorbance of UV-vis, n = 8, R = 0.999). (B) UV-vis absorbance calibration curve of oligonucleotides **4**. The regression equations could be expressed as Y = 0.0919 X - 0.0102 (X was the concentration of oligonucleotides **4**, 10^{-6} M; Y was the absorbance of UV-vis, n = 8, R = 0.999).

Estimation the cycle steps. One step was defined as a oligonucleotides 2 moving from one stator to another and generating one signal DNA. To estimate the cycle steps in the DNA device, a program was designed with the following process. 3.0 mL of the prepared MB-stators was isolated by magnetic separation to remove the PBS buffer, added into 2.0 mL of 5.0×10^{-6} M oligonucleotides 2 with incubation at 25 ºC for 1 h. The supernatant solution was separated and detected by UV-vis spectrophotometer. The absorbance of the supernatant was measured at 260 nm to obtain the amount of the nonhybridization oligonucleotides 2 according Fig. S5A. The number of oligonucleotides 2 molecules hybridized on the MB (N_1) can be quantitatively

calculated from the absorbance difference at 260 nm between the oligonucleotides 2 solution before immobilization and the supernatant after hybridization. The hybridization results the next step to incubated in a 1.0 mL of Fermentas REase buffer R containing 0.5 U/μL nicking endonuclease at 37 ºC for 75 min. After a magnetic separation, the signal DNA was collected by centrifugation and then detected by UV-vis spectrophotometer. The absorbance of the signal DNA was measured at 260 nm to obtain the amount of the signal DNA (N_2) according Fig. S5B. The ratio of N_2 and N_1 was the cycle steps. From the calculation, the cycle step was 463.

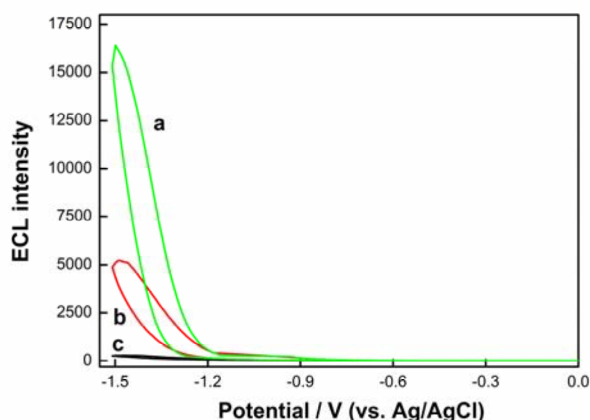

Fig. S6 ECL-potential curves of (a) CdS NPs modified GOE in 0.01 M PBS buffer containing 0.1 M $K_2S_2O_8$ (b) CdS NPs modified GOE in 0.01 M PBS buffer containing 0.1 M $K_2S_2O_8$ and 100 µg/mL SOD (c) CdS NPs modified GOE in 0.01 M PBS buffer without $K_2S_2O_8$. The voltage of the PMT was -600 V.

Renewing for the GOE. GO could bind ssDNA through π-stacking interactions between the nucleobases and GO surface. SDS with π-electrons can be used to renew GOE with sonication. [5] It was sonicated in PBS solution of pH 7.4 containing 0.5% SDS for 3min and then rinsed with water carefully. ECL experiments displayed that CdS NPs/ssDNA could be removed from the GOE. One prepared GOE was used to blind CdS NPs/ssDNA, and then renewed over and over again. After the 50th detection, the variation of ECL intensity was no more than 8%. (Fig. S7). The CV analysis is commonly complicated by surface fouling. No such response is observed at the renewed GOE surface. No evidence of electrode fouling is apparent at the renewed surface,

indicating that the reusability of the GOE was satisfactory. All the investigations were performed with 1.0×10^{-14} M thrombin.

To indentify the effect of GO immobilization using MCH and EDC, a control experiment was performed. GO was assembled by poly(diallylammonium chloride) (PDDA) on bare Au electrode. Other steps were mentioned as above. After the 10th detection, the variation of ECL intensity was more than 20%. It indicated an unsatisfactory reusability. From the point of view, the immobilization using MCH and EDC was necessary.

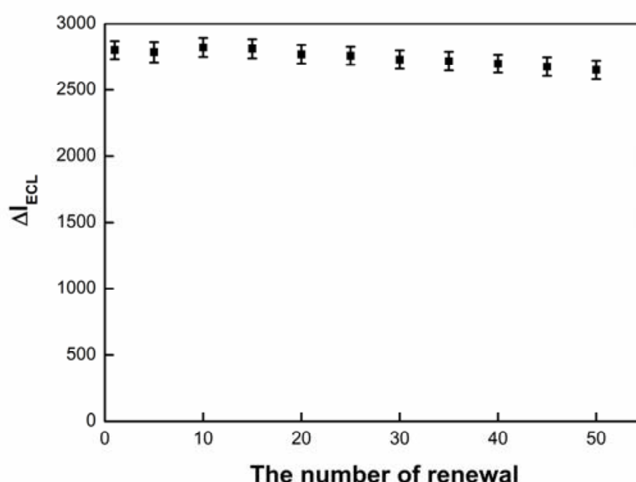

Fig. S7 Typical records of the renewed GOE

Interference Effect. In order to assess the possibility of interference, Bovine Serum Albumin (BSA), immunoglobin G (IgG), alpha fetoprotein (AFP), horseradish peroxidase (HRP) and lysozyme were selected to study the specificity. 8.0 $\times10^{-15}$ M thrombin standard solutions were detected, containing 1.0 $\times10^{-8}$ M BSA, 1.0 $\times10^{-8}$ M IgG, 1.0 $\times10^{-8}$ M AFP, 1.0 $\times10^{-12}$ M BSA, 1.0 $\times10^{-12}$ M HRP and 1.0 $\times10^{-12}$ M lysozyme respectively. It could be seen that the average degree of interference from interfering substances was no more than 7.6%. Thus, the response of the present method was very selective for thrombin.

Detection of thrombin in complex samples. Thrombin is a coagulation protein in the blood

stream. However, healthy human serum sample does not contain thrombin. [6] To investigate the proposed strategy could detect thrombin in a complex sample matrix, thrombin solutions were spiked into human serum samples or Romas cellular extract sample to test the performance.

Blood sample contains dissolved proteins including fibrinogen. Thrombin can convert soluble fibrinogen into fibrin, as well as catalyzing many other coagulation-related reactions. To avoid this phenomenon, 2.0 M ammonium sulfate and 0.1 M sodium chloride was used to pretreated blood samples before the addition of thrombin. [7] After mixed for 5 min, the mixture was centrifuged at 10 000 rpm for 10 min at 4 ºC. The supernatant was serum. Serum remained from whole blood minus both the cells and the clotting factors. It does not contain coagulation factors, so the addition of thrombin did not affect phase transformations. Thrombin solutions with 5.0×10^{-15} M, 2.0×10^{-13} M, 9.0×10^{-11} M were spiked into the serum. Thrombin was measured after appropriate dilution and was calculated from the measured concentrations after correction for dilution. Fig. S8 shows the results for the thrombin in the serum sample. Five replicates for each concentration were determined by ECL.

A suspension of 2.94×10^{5} cells for Romas cells dispersed in RPMI cell media buffer, was centrifuged at 1000 rpm for 5 min at 4 ºC, washed with phosphate-buffered saline and resuspended in 0.2 mL of deionized water. Then, the cells were disrupted by sonication for 20 min at 0 ºC. To remove the homogenate of cell debris, the lysate was centrifuged at 16 000 rpm for 30 min at 4 ºC. Finally thrombin solutions with 5.0×10^{-15} M, 2.0×10^{-13} M, 9.0×10^{-11} M were spiked into the cellular extract. Thrombin was measured after appropriate dilution and was calculated from the measured concentrations after correction for dilution. Fig. S8 shows the results for the thrombin in the cellular extract. Five replicates for each concentration were determined by ECL.

The statistical data for the quantization of thrombin were shown in Fig. S8, which indicated the proposed method developed in this study is content to determine thrombin in complex samples.

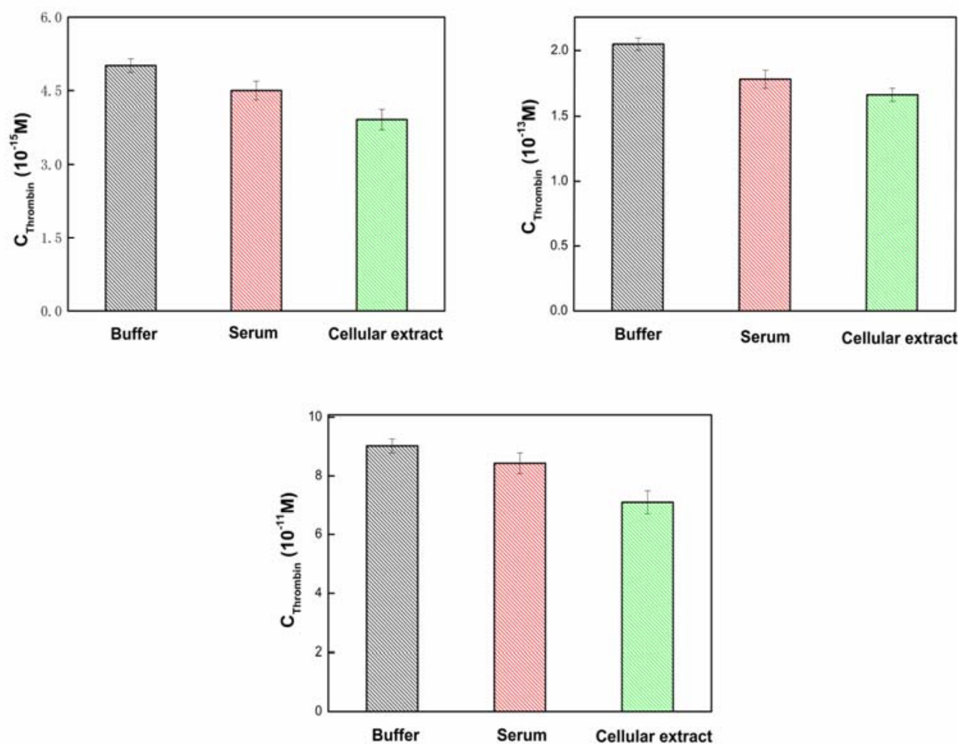

Fig. S8 The columns of different concentrations of thrombin in buffer, human serum and Ramos cellular extract .

Cells culture. Romas cell line was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 IU mL^{-1} penicillin-streptomycin. Then the cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2. The cell density was counted using a hemocytometer.

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