Electronic Supplementary Information (ESI)

Phosphomolybdic acid anion probe-based label-free, stable and simple electrochemical biosensing platform

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1. Experimental Methods.

1.1 Reagents and materials. Thrombin, chitosan, bovine serum albumin (BSA), hemoglobin (Hb) and human serum albumin (HSA) were acquired from Sigma-Aldrich. Graphite powder and phosphomolybdic acid was purchased from Sinopharm Chemical Reagent Co., Ltd. Other chemicals regents were analytical reagents and used without further purification. All solutions were prepared with double-distilled water, unless indicated otherwise.

Capture single-stranded DNA (DNA1) sequence:

5'-TTA CCG GGC TCT GCC ATC TT-3'

Wild type target (DNA2) sequence:

5'-AAG A**T**G GCA G**A**G CCC GGT AA-3'

Random sequence (DNA3):

5’-CCT GAT TGT GTT CCA TTG CC-3’

Single-base mismatched target (DNA4) sequence:

5’-AAG A**G**G GCA GAG CCC GGT AA-3’

Mutant type target (DNA5) sequence:

5’-AAG ATG GCA G**G**G CCC GGT AA-3’

Thrombin aptamer sequence:

5’-GGT TGG TGT GGT TGG-3’

1.2 Apparatus. Electrochemical experiments, including cyclic voltammetry (CV) and square wave pulse voltammetry (SWV) were performed with an electrochemical analyzer CHI 660D (CH Instruments, USA). The morphology of the modified films were sputter-coated with platinum and were characterized by a JSM-7600F field emission scanning electron microscopy (SEM) (JEOL, Japan) at an accelerating voltage of 10 kV. The electrochemical impedance spectroscopy (EIS) was carried out with an Autolab potentiostat/galvanostat PGSTAT302N (Eco chemie, BV, The Netherlands) and controlled by Nova 1.8 software with a three-electrode system under open circuit conditions. All experiments were conducted at ambient temperature. The voltage frequencies ranged from 10^5 Hz to 0.1 Hz and the AC voltage amplitude was 5 mV. The supporting electrolyte was 10 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl.

1.3 Fabrication of the biosensor. Graphite oxide was synthesized from graphite powder by the Hummers method,[S1] then dispersed in H_2O with sonication for 2 h to obtain graphene oxide (GO). Before modification, the glassy carbon electrode (GCE) was polished to a mirror finish with alumina slurry (0.3 and 0.05 μm, respectively), followed by sonication for 30 s to remove the alumina particles, and then rinsed with water, drying under nitrogen gas. 5 μL of chitosan solution (0.5 wt% in 1 % HAc solution) was dropped on the GCE

surface and allowed to air dry at room temperature for 1.5 h. Then 7 μL GO suspension (0.25 mg mL^{-1}) was dropped to coat the chitosan film and dried at room temperature. After thoroughly washing with double-distilled water, the electrode was immediately followed by incubation with 10 μL capture single-stranded DNA (DNA1) (1 μM) or aptamer (1 μM) for 1 h at room temperature. Finally, the electrode was washed with phosphate buffered saline (PBS) (8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.2~7.4) before use.

1.4 Measurement procedures. First, the GCE/chitosan/GO/DNA1 electrode was incubated with 10 μL of different concentrations of DNA2 for 2 h at 37 °C, followed by washing with PBS. Then the electrode (GCE/chitosan/GO/DNA1/DNA2) was incubated with 10 μL of phosphomolybdic acid solution (50 μM) for 50 min at room temperature and washed for the later determination. Thrombin was prepared in the Tris-HCl buffer (20 mM Tris-HCl, 140 mM NaCl, 5 mM KCl, pH 7.4). 10 μL of different concentrations of thrombin solution was accumulated onto the aptamer-modified electrode for 2 h at 37 °C, followed by washing with PBS. Subsequently, the electrode (GCE/chitosan/GO/aptamer/thrombin) was incubated with 10 μL of phosphomolybdic acid solution (50 μM) for 50 min at room temperature and washed for the later determination. The electrochemical detection was performed in 0.5 M H_2SO_4 solution containing 0.1 M NaCl. Square wave voltammetry paremeters: Init E (V): 0.52, Final E (V): 0.08, Incr E (V): 0.004, Amplitude (V): 0.025, Frequency (Hz): 15, Sensitivity (A/V): 1.e-006.

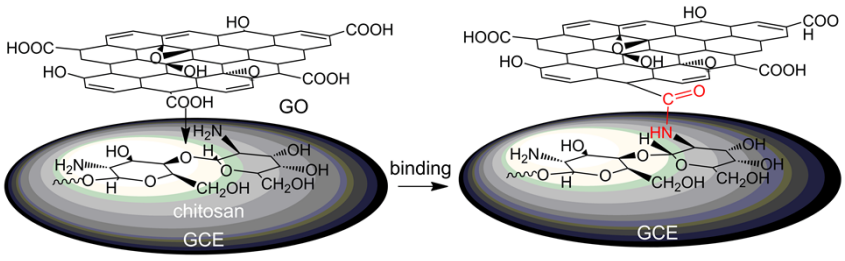

Fig. S1 Binding protocol of chitosan to GO at electrode surface.

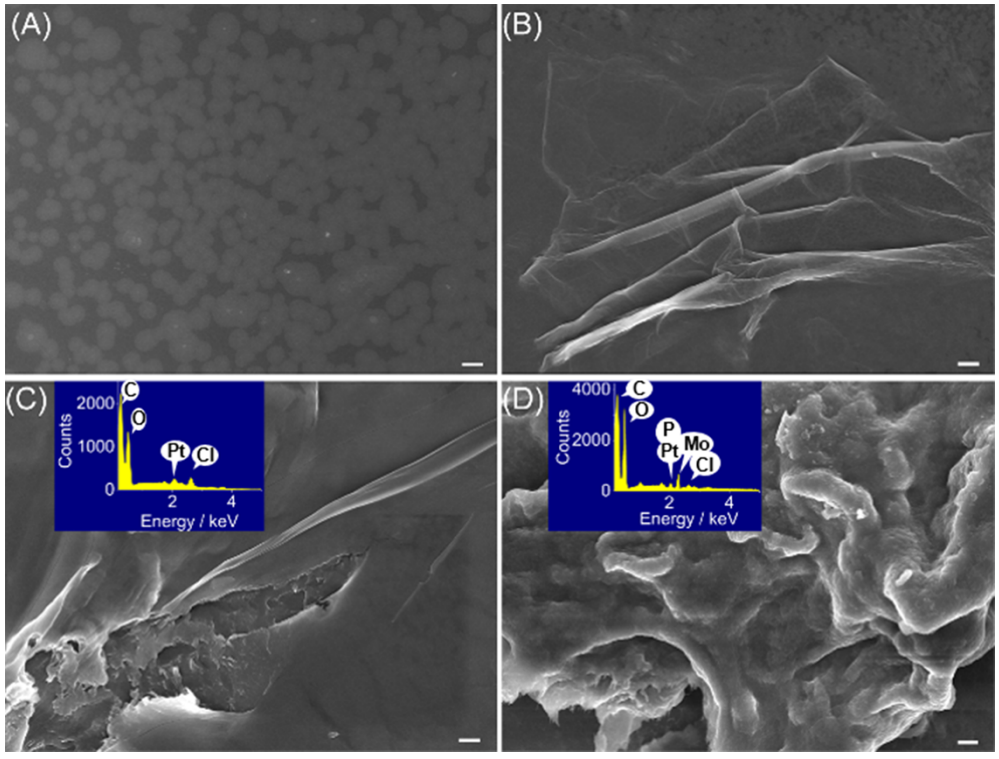

Fig. S2 SEM images of (A) chitosan, (B) chitosan/GO, (C) chitosan/GO/DNA1/PMo_{12}, (D) chitosan/GO/DNA1/DNA2/PMo_{12} films. Scale bar: 1 μm. Insets: EDS spectra.

2. Electrochemical performance of GCE/chitosan/GO/PMo_{12}.

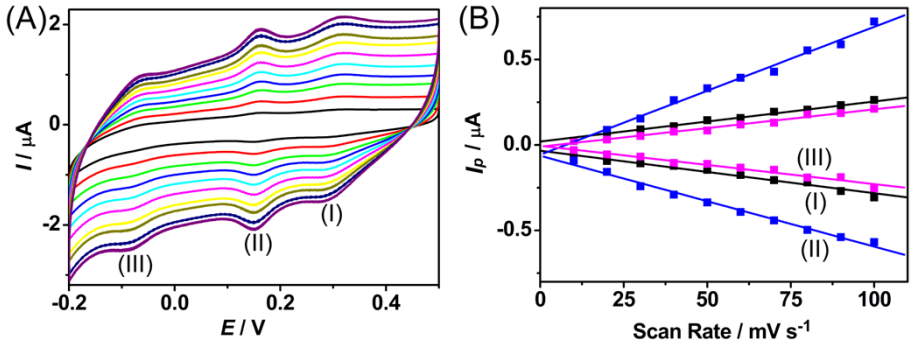

Fig. S3 (A) CVs and (B) peak current intensity variations of GCE/chitosan/GO/PMo_{12} at different scan rates (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mV s^{-1}) in 0.5 M H_2SO_4 containing 0.1 M NaCl medium. The reference electrode was a saturated calomel electrode.

3. Optimization of Detection Conditions.

Herein, we explored four factors, which would affect the SWV responses of the biosensor, including the concentration of DNA1 coated on the surface of GCE/chitosan/GO (Fig. S4A), DNA hybridization time (Fig. S4B), the incubation time of PMo_{12} (Fig. S4C) and the concentration of PMo_{12} (Fig. S4D). The concentration of DNA1 coated on the surface of GCE/chitosan/GO had a great influence on electrochemical responses. As shown in Fig. S4A, with increasing the concentration of DNA1 from 0 to 1 μM, the SWV peak currents obviously decreased to a much small signal and then the peak currents had little change from 1 to 3 μM DNA1, which might own to the fact that 1 μM DNA1 was enough to fully cover the surface of GO, which would make the similar charged PMo_{12} could not attach to the GO surface. Hence, 1 μM DNA1 was chosen as the optimized concentration. The DNA hybridization time was another important parameter. Fig. S4B showed that the SWV peak currents increased with increasing DNA hybridization time and then tended to level off after 2 h, thus a hybridization time of 2 h was adopted in the experiment. Fig. S4C showed that

the SWV peak currents increased with increasing incubation time of PMo_{12} and reached to a maximum value at 50 min, thus 50 min was selected as the incubation time of PMo_{12}. The electrochemical response was also related to the concentration of PMo_{12}. As revealed in Fig. S4D, the currents of the biosensor increased from 10 μM to 50 μM PMo_{12} with an appropriate rate. When further increasing the PMo_{12} concentration, the increasing rate of I_p obtained from the peak II of PMo_{12} advanced its speed, which might attribute to that PMo_{12} would compete with single-stranded DNA to bind with GO after reaching a certain amount, sequentially push aside the single-stranded DNA from the GO surface and occupy the vacancy on the GO surface. Therefore, 50 μM PMo_{12} was chosen as the optimized concentration to avoid the possible competitive reaction.

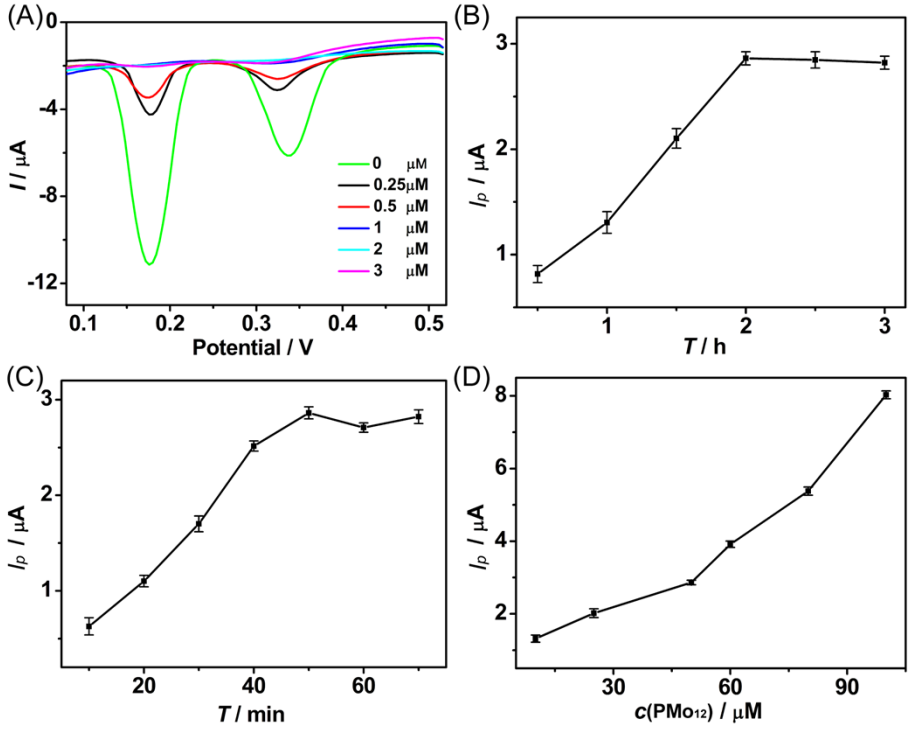

Fig. S4 Effects of (A) the concentration of DNA1 coated on the surface of GCE/chitosan/GO, (B) DNA hybridization time, (C) the incubation time of PMo_{12} and (D) the concentration of PMo_{12} in the detection solution on the SWV responses of the biosensor toward 100 pM DNA2.

4. Electrochemical performance of the proposed biosensor.

Table S1. Comparison of the sensitivity for different modified electrodes for DNA assay.

different modified electrodes	linear range	detection limit	reference
Exo III/MB-DNA/target DNA	20-300 pM	20 pM	S2
PGE/AuNPs/ssDNA/target DNA	10 nM-1 μM	6.9 nM	S3
probe 1/probe 2/target DNA/Exo III/$Os[(bpy)_2(dppz)]^{2+}$	2.5-100 nM	2.5 nM	S4
capture DNA/target DNA/detection DNA-Pt NPs	/	10 pM	S5
Co-S1-S2/AuNPs-MP	0.11-40 nM	72 pM	S6
chitosan/GO/DNA1/target DNA/PMo_{12}	0.5 pM-10 nM	0.2 pM	this work

Table S2. Comparison of the sensitivity for different modified electrodes for thrombin detection.

different modified electrodes	**linear range**	**detection limit**	**reference**
SiNCs/aptamer-FcSH/thrombin	0.1-5 nM	0.06 nM	S7
MWCNTs/aptamer/thrombin	0.39-1.95 nM	105 pM	S8
AuNPs/ Apt I/thrombin/multi-labeled PtNPs-redox probes-rGS	0.02-45 nM	11 pM	S9
aptamer/DNA2/thrombin/MB	6-60 nM	3 nM	S10
Au NPs/aptamer/thrombin/hemin/Exo I	/	0.1 nM	S11
chitosan/GO/aptamer/thrombin/PMo_{12}	10 pM-25 nM	5.8 pM	this work

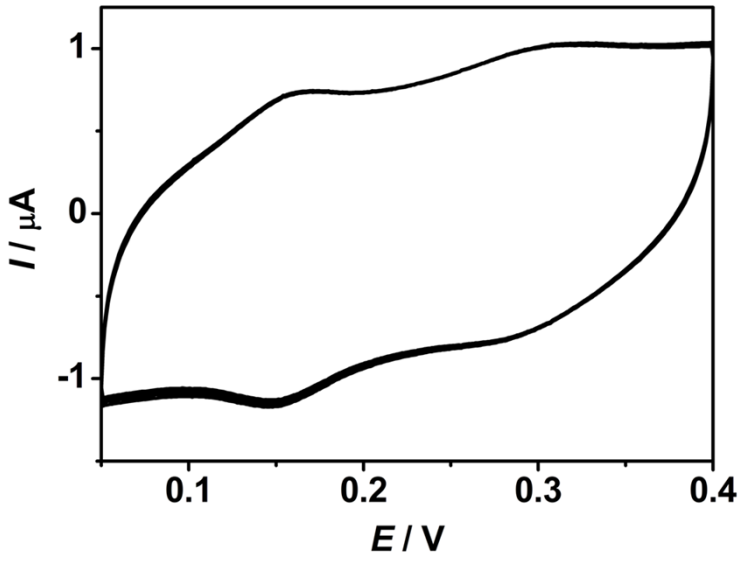

Fig. S5 CVs of GCE/chitosan/GO/DNA1/DNA2/PMo_{12} in 0.5 M H_2SO_4 + 0.1 M NaCl medium for 20 cycles.

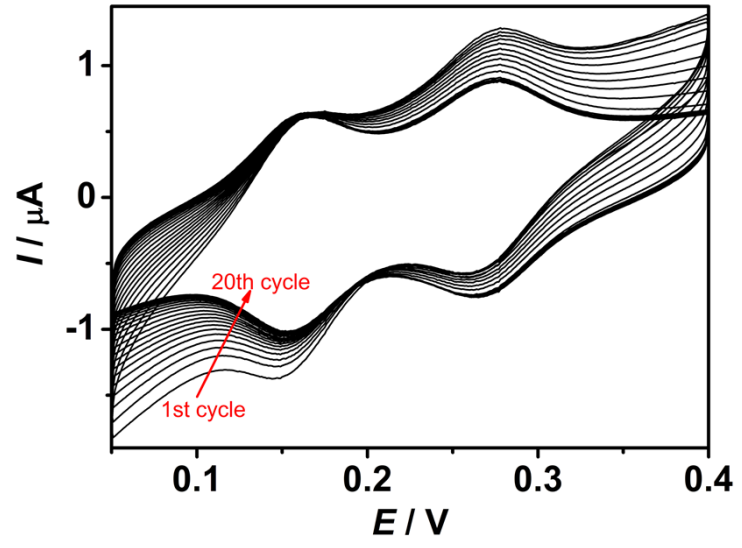

Fig. S6 CVs of GCE/GO/DNA1/DNA2/PMo_{12} in 0.5 M H_2SO_4 + 0.1 M NaCl medium for 20 cycles.

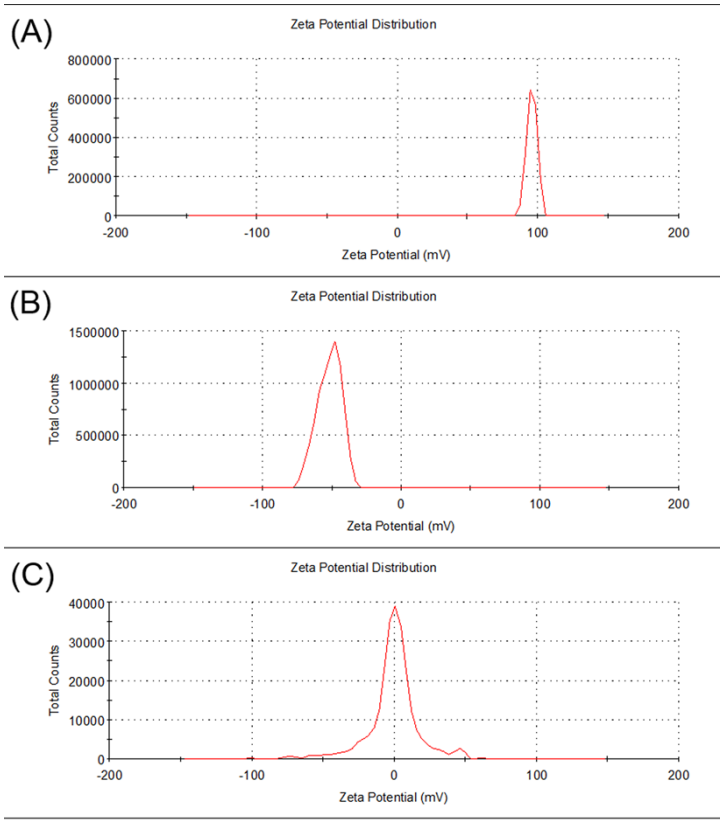

Fig. S7 ζ potential of (A) a solution of chitosan, (B) a suspension of GO, (C) a mixture of GO and chitosan.

Zeta potential measurements showed that while the GO suspension had a negative ζ potential centered at -51.9 mV (Fig. S7A), the solution of chitosan held a positive ζ potential at about +97 mV. (Fig. S7B). However, when the two were mixed, the ζ potential of the mixture was about -1.2 mV (Fig. S7C).

5. Thrombin analysis in real samples.

To evaluate the applicability of the biosensing platform, the proposed biosensor was used for detecting the concentration of thrombin in human serum samples. The proposed assay was tested using human serum as matrix, which was diluted 100-fold prior to assay. A series of thrombin human serum samples were used to test the accuracy of the electrochemical quantitative approach. Different amounts of thrombin were spiked into human serum samples. The recovery results were summarized in Table S3, indicating that the electrochemical biosensing method was reliable and accurate.

Table S3. Recovery results of thrombin assay for different concentrations spiked into human serum samples.

sample no.	spiked	found	recovery (%)	RSD (%)
1	100 pM	113 pM	113	6.09
2	1.00 nM	1.08 nM	108	3.12
3	5.00 nM	4.96 nM	99.3	4.86

*Each sample was repeated for three times and averaged to obtain the recovery and relative standard deviation (RSD) values.

References

S1 W. S. Hummers and R. E. Offeman, *J. Am. Chem. Soc.* 1958, **80**, 1339.

S2 F. Xuan, X. T. Luo and I. M. Hsing, *Anal. Chem.* 2012, **84**, 5216.

S3 N. Y. Wu, W. Gao, X. L. He, Z. Chang and M. T. Xu, *Biosens. Bioelectron.* 2013, **39**, 210.

S4 R. Miranda-Castro, D. Marchal, B. Limoges and F. Mavre. *Chem. Commun.* 2012, **48**, 8772.

S5 S. J. Kwon and A. J. Bard, *J. Am. Chem. Soc.* 2012, **134**, 10777.

S6 F. Li, Y. Feng, P. J. Dong, L. M. Yang and B. Tang, *Biosens. Bioelectron.* 2011, **26**, 1947.

S7 Y. H. Wang, X. X. He, K. M. Wang, X. Q. Ni, J. Su and Z. F. Chen, *Biosens. Bioelectron.* 2011, **26**, 3536.

S8 P. Kara, A. De La Escosura-Muniz, M. Maltez-Da Costa, M. Guix, M. Ozsoz and A. Merkoci, *Biosens. Bioelectron.* 2010, **26**, 1715.

S9 L. J. Bai, R. Yuan, Y. Q. Chai, Y. Zhuo, Y. L. Yuan and Y. Wang, *Biomaterials* 2012, **33**, 1090.

S10 F. F. Yan, F. Wang and Z. L. Chen, *Sens. Actuator B-Chem.* 2011, **160**, 1380.

S11 B. Y. Jiang, M. Wang, C. Li and J. Q. Xie, *Biosens. Bioelectron.* 2013, **43**, 289.