ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Graphene-coated copper-doped ZnO quantum dots for sensitive photoelectrochemical bioanalysis of thrombin triggered by DNA nanoflowers

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EXPERIMENTAL SECTION

Chemical and reagent

T4 DNA ligase, exonuclease I (Exo I), Glucose oxidase (GOx), Horseradish Peroxidase (HRP), hydrogen peroxide (H_2O_2), phi29 DNA polymerase, and deoxynucleotides (dNTPs) were purchased from Sangon Biotechnology. Zinc acetate dehydrate[$Zn(CH_3COO)_2 \cdot 2H_2O$], N,N-Dimethylformamide, ethanol (DMF), Copper(II) acetate monohydrate [$Cu(CH_3COO)2 \cdot H2O$], H_2SO_4 (>95 %), HNO_3 (>95 %), Sodium nitrate (>99 %), 30 % (w/w) solution of H_2O_2, 37 % reagent grade HCl and $KMnO_4$ (99 %), Graphite powder, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Ultrapure water obtained from a Millipore water purification system was used in all runs (18.2 MΩ cm, Milli-Q). All oligonucleotide sequences and magnetic beads (MBs) were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). The human serum samples were collected from normal human blood donated by Maidao Community Health Service Station (Qingdao, China). A conventional three-electrode system consisting of a graphene-coated copper doped Zinc oxide-graphene oxide quantum dots-modified FTO working electrode (WE), a Pt-wire counter electrode (CE) and a saturated calomel electrode (SCE) as the reference electrode (RE). All determinations were measured at least three times.

These sequences are listed as follows (5'-3'):

Padlock probe: phosphate-AATATTATTCCAGCTGGCAGTCACCCCAACCTGCC
CTACCACGGACTGACTGCACCTTGAACGCTTATTATGATT

Primer: CTGGAATAATATT AATCATAATAAGC

Apt15: GGT TGG TGT GGT TGG-biotin

Preparation of GO

GO was prepared by the modified Hummers' method. In a typical reaction, 1.0 g graphite, 1.0 g sodium nitrate ($NaNO_3$) and 46 mL Sulphuric acid were mixed in a flask. The mixture was stirred for 4 h in an ice bath. Subsequently, 6.0 g $KMnO_4$ was added to the solution drop by drop with stirring, and then stirred in the ice bath for 2 h. 92 mL ultrapure water was added to the reaction flask, and

then stirred at 35 °C for 1 h. The solution maintained at 98 °C for 2 h before cooling naturally to room temperature. After adding 200 mL water and stirring for 1 h, 20 mL of 30% (w/w) H_2O_2 was added to the solution stirred for 1 h. The golden-brown solution was centrifuged and washed once with 5 mL HCl (36.5-38%) and then with water until the pH of the solution reached 6. The resulting product was dried in an oven overnight at 50 °C.

Preparation of circular template DNA

The circular template was prepared using a linear padlock probe, a primer and T4 DNA ligase. Briefly, 10 μL of 5′-phosphorylated padlock probe (100 μM) was hybridized with 20 μL of primer (100 μM) in 1× T4 ligase reaction buffer (40 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM DTT, 0.5 mM ATP, pH 7.8), followed by heating at 95 °C for 5 min and slowly cooling to 25 °C for 3 h. Subsequently, 10 μL of T4 DNA ligase was added and the mixture was incubated at 16 °C overnight. The ligase was inactivated by heating at 65 °C for 10 min. Finally, the circular DNA template was obtained by treating with 5 μL of exonuclease I (Exo I, 20 U/μL) in 1× Exo I buffer (67 mM glycine-KOH, 6.7 mM MgCl2, 1.0 mM DTT, pH 9.5) at 37 °C for 1.5 h to degrade the uncircularized padlock probes and excess primers, followed by heating at 80 °C for 15 min to inactivate Exo I.

Preparation of GOx-DFs

The GOx-DFs were synthesized *via* RCA. A typical RCA reaction was carried out in 100 μL of solution containing a circular DNA template (0.36 μM), phi29 DNA polymerase (0.5 U/μL), dNTPs (1.0 mM), and GOx (24 μM) in buffer (33 mM Tris-acetate, 10 mM $Mg(CH_3COO)_2$, 66 mM CH_3COOK, 1% Tween 20, 1 mM DTT, pH 7.9) at 30 °C for 30 h. The RCA reaction was terminated by heating at 65 °C for 10 min. The RCA products were washed with ultrapure water three times by centrifugation at 8000 rpm for 10 min. The resulting HRP-DFs were re-dispersed in ultrapure water and stored at 4 °C before use.

Preparation of Apt15-immobilized MMPs

Magnetic microparticles (MMPs) (10 μL, 10 mg/mL) were washed three times with washing buffer and diluted to 1 mg/mL. biotin-modified thrombin aptamer Apt15 (10 μL, 100 μM) was added to the diluted MMPs solution and mixed for 120 min at room temperature, followed by magnetic separation

of the capture-labeled MMPs. The obtained capture-MMPs complexes were washed twice with a washing solution.

PARTIAL RESULTS AND DISCUSSION

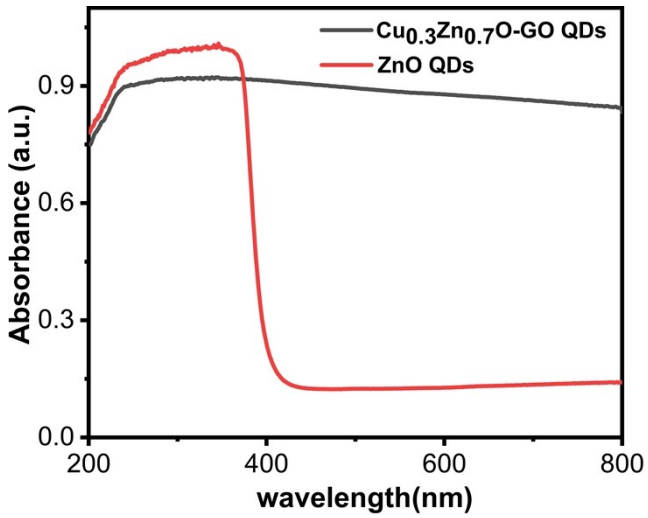

Fig. S1 UV-vis diffuse reflectance spectra of ZnO QDs and $Cu_{0.3}Zn_{0.7}O$–GO QD

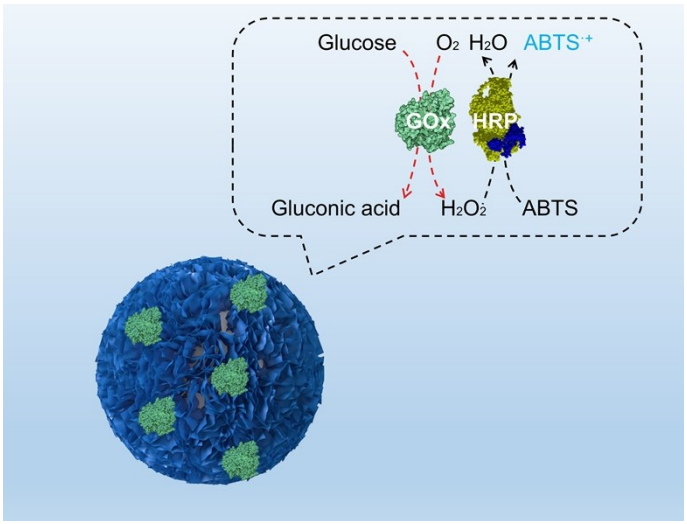

Fig. S2 Schematic illustration of enzyme catalytic reaction in the GOx-DFs. GOx catalyzes the oxidation of glucose to form gluconic acid and in situ generates H_2O_2. The generated H_2O_2 can oxide ABTS to $ABTS^{\cdot+}$ with HRP as the catalyst. The generated $ABTS^{\cdot+}$ can be detectable at 415 nm by using a UV-vis spectrophotometer. Because the amount of ABTS conversion and glucose consumption are positively correlated with the change of absorbance, the detected absorbance can be used to represent the catalytic performance of this enzyme system.

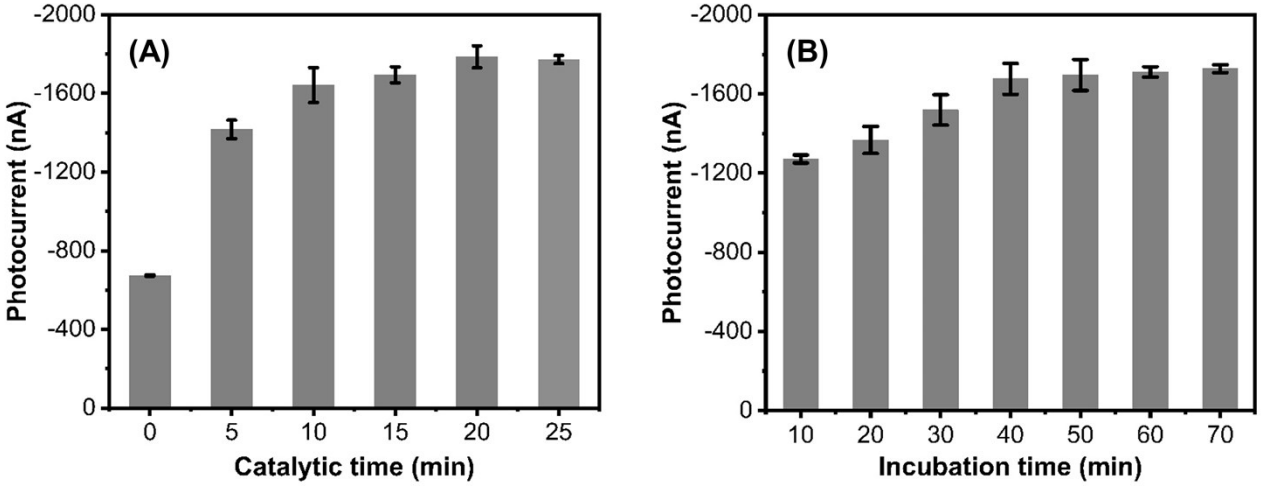

Fig. S3 Effects of (A) the catalytic time of GOx-DFs, and (B) incubation time on the photocurrent of the $Cu_{0.3}Zn_{0.7}O$-GO QDs electrode in PBS (3000 fM thrombin used in this case).

Table S1 Determination of thrombin added in human serum with the PEC biosensor.

Serum samples	Added thrombin (fM)	Found thrombin (fM , *n*=3)	Relative standard deviation (RSD; %)	Recovery (%)
1	50.00	51.68	2.2	103.4
2	100.00	100.35	2.6	100.4
3	500.00	499.93	0.4	100.0
4	1000.00	1035.34	3.3	103.5
5	10000.00	9977.76	5.6	99.8

Table S2. Comparison of different thrombin detection schemes on analytical properties.

Transduced signal	Dynamic range	LOD	Ref.
Metal organic framework-based electrochemiluminescence biosensor	1 - 10000 fM	0.02 aM	1
GOx/HRP DNA nanoflowers-based electrochemical biosensor	15 – 10^8 fM	12.77 fM	2
Aptamer hydrogel-functionalized metamaterial-based molecule-specific terahertz biosensors	0.5 – 500 pM	0.40 pM	3
Dual-recognition colorimetric aptasensor	108.1 - 2.7 × 10^7 pM	27.8 pM	4
Smart nanostructure-based self-powered biosensor	0.6 - 142.9 pM	0.2 pM	5
Photo-induced enhanced Raman spectroscopy-based detection platform	50 - 1× 10^6 pM	50 pM	6
PEC biosensing strategy	50-1000 fM	29 fM	This work

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