Electronic Supplementary Information (ESI) for

In Situ Chemical Redox and Functionalization of Graphene Oxide: Toward New Cathodic Photoelectrochemical Bioanalysis

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This material includes:

Experimental section

Chemicals and Apparatus. Alkaline phosphate (ALP, EC 3.1.3.1) and tyrosinase (TYR, EC 1.14.18.1) were purchased from Sigma-Aldrich (St. Louis, USA). Graphite was purchased from Qingdao JinRi Lai Graphite Co., Ltd. (Qingdao, China). Catechol, $NaNO_3$, $KMnO_4$, 30% H_2O_2, cysteine (Cys), glutathione (GSH), bovine serum albumin (BSA), phosphorus pentoxide, diethyl ether, anhydrous sodium sulfate, dichoromethane, methanol, and tyrosine were all obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Strepavidin-alkaline phosphatase conjugates (SA-ALPs) were purchased from Beyotime Biotechnology (Shanghai, China). Human α-fetoprotein (AFP) antigen, anti-human α-fetoprotein monoclonal antibody (capture antibody, Ab_1, and detection antibody, Ab_2), carcinoembryonic antigen (CEA), and prostate-specific antigen (PSA) were obtained from Jorferin Biotechnology Co., Ltd. (Beijing, China). All oligonucleotides used in the immunoassay were synthesized by Sangon Biotech. Co., Ltd. (Shanghai, China). Each oligonucleotide was heated at 90℃ for 10 min, and slowly cooled down to room temperature before use. The base sequences of the used oligonucleotides are listed as follows.

Capture probe (C_{DNA}): 5'-SH-$(CH_2)_6$-AAA AAA GAA GGA GGG GCG ACT-3';

Biotin-H1 (Biotinylated hairpin probe 1): 5'-biotin-$(CH_2)_6$-GGG GCG ACT TGA AAC AGT CGC CCC TCC TTC-3';

Biotin-H2 (Biotinylated hairpin probe 2): 5'-biotin-$(CH_2)_6$-GTT TCA AGT CGC CCC GAA GGA GGG GCG ACT-3'.

The X-ray powder diffraction (XRD) was obtained with an X'Pert Philips Materials Research Diffractometer (Brook AXS, Germany). Raman spectra were gained on a confocal micro-Raman spectrometer (Renishaw, Britain) with 532 nm laser excitation. Fourier transform–infrared spectroscopy (FT–IR) spectra were acquired on a FTLA 2000-104 spectrometer (ABB Bomem, Canada). The X-ray photoelectron spectra (XPS) were gained by an AXIS Ultra^{DLD} X-ray Photoelectron Spectroscopy (Shimadzu-Kratos, Japan). UV-Vis absorption spectroscopic measurements were completed through a TU-1901 spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., China).

Fabrication of the GO modified ITO electrode. GO was synthesized by a modified Hummer's method.[1] Briefly, 3.0 g of $KMnO_4$ was slowly added into the mixture of 0.5 g of graphite and 16.5 mL of cold 98% H_2SO_4 under stirring at 0 ºC in an ice bath. About 15 min later, 0.5 g of $NaNO_3$ was added followed by stirring for 1.0 h. Then, the mixture was heated around 35 to 40 ºC under stirring for 1.5 h before the addition of distilled water. Next, the reaction temperature was increased to 95 ºC and maintained at this temperature for 40 min. Afterwards, 1 mL of 30% H_2O_2 were added into the mixture to stop the reaction. Finally, the product was collected by filter flask and rinsed repeatedly with 5% HCl aqueous solution, followed by drying at 50 ºC in a vacuum drying oven. After ultrasonication, the obtained GO powder can be redispersed in water easily for future use.

The as synthesized GO was used to modify the pretreated ITO electrode (treated according to our previous report[2]). As shown in Fig. S1 (a), the sheet-like GO synthesized by a modified Hummer's method[1] was negatively charged (with the Zeta potential of -29 mV) and can be readily adsorbed onto the positively charged polyelectrolyte poly(diallyldimethylammonium chloride) (PDDA) modified tin indium oxide (ITO) electrode. The cleaned ITO slices were firstly dipped into a solution of 2% PDDA containing 0.5 M NaCl and for 10 min, and then carefully washed with ultrapure water. Then, the as obtained GO solution (with a concentration of 0.5 mg/mL) was dropped onto the PDDA deposited ITO electrode and dried in air to obtain GO modified ITO (ITO/GO) electrode.

PEC detection of versatile targets. (i) The PEC probing the activity of ALP was conducted as follows: o–Phosphonoxyphenol (OPP), the substrate of ALP, was firstly synthesized by our previously reported method.[3] That is, catechol (1.24 g, 11.25 mmol) was warmed at a temperature slightly higher than its melting point (110 ℃), and then diphosphorus pentaoxide (1.06 g, 7.5 mmol) was slowly added under vigorous stirring within 2 h. As the reaction proceeded, the mixture became a brownish viscous liquid. After cooling to room temperature, the mixture was dissolved in dichloromethane (300 mL) containing a few drop of methanol, followed by concentration. Finally, the precipitate was collected by filtration and dried in vacuum to give OPP as a white power.

For the detection of ALP, 30 μL of OPP (0.01 M), 20 μL of different concentrations of ALP and 250 μL of tris–HCl solution (pH 8.0) were mixed for an incubation time of 50 min in 96 well plates. Following that, the ITO/GO electrodes were immersed in the above reaction mixture for 5 min followed by careful washing with tris–HCl buffer solution (pH 8.0), and finally immersed in 0.1 M tris–HCl (pH 8.0) for photocurrent measurements at 0 V (vs Ag/AgCl). The PEC measurements were performed with a homemade PEC system including a 500 W Xe lamp equipped with an ultraviolet cutoff filter ($\lambda \geq 400$ nm) as the irradiation source and a CHI 800C electrochemical workstation for photocurrent record. A saturated Ag/AgCl, a Pt wire and a modified ITO electrode was employed as the reference, counter and working electrode, respectively.

(ii) PEC immunoassay of AFP was implemented using ALP as the catalytic label. In this assay, the anti-AFP antibody (detection antibody, Ab_2) and capture DNA (C_{DNA}) co–immobilized Au NPs (Ab_2-Au NPs-C_{DNA}) were used to signal the immunoassay. To fabricate the Ab_2-Au NPs-C_{DNA} bioconjugates, the Au NPs were firstly prepared according to the previous protocol.[4] Specifically, 50 mL of 0.01% $HAuCl_4$ solution was heated to boiling under vigorous stirring, followed by the quick addition of 1.25 mL of 1% trisodium citrate solution to obtain Au NPs with dark red color. Subsequently, 1 mL of Au NPs' solution was adjusted to pH 7.6 using 0.1 M Na_2CO_3 aqueous solution. Next, 20 μL of 0.1 mg/mL Ab_2 was added to the solution, followed by incubation at 4℃ for 2 h with slow stirring. The resulting Ab_2-attached Au NPs was then reacted with 20 μL of 100 μM capture DNA (C_{DNA}) that was activated by the addition of TCEP for 12 h. Afterwards, 200 μL of 1% BSA blocking solution was dropped into the solution and incubated for another 30 min. The bioconjugates of the

Ab_2-Au NPs-C_{DNA} were centrifuged and washed for three times with ultrapure water before their final redispersion in tris–HCl buffer solution.

To conduct the immunoassay, a 20 μL of 0.1 mg/mL anti-AFP antibody (capture antibody, Ab_1) was added to the 96 well plates at 4 ℃ overnight followed by washing three times with the washing buffer (20 mM tris-HCl buffer solution (pH=7.4) containing 0.05% Tween-20). Then, the wells were blocked by BSA blocking solutions for 30 min. Next, 20 μL of the AFP antigen (Ag) solution with different concentrations were added to the wells and incubated for 60 min at 37℃ followed by washing. After the immunoreaction between Ab_1 and Ag, the Ab_2-Au NPs-C_{DNA} bioconjugates were injected to the wells and incubated for another 60 min. After removing the uncombined bioconjugates of Ab_2-Au NPs-C_{DNA} and washing completely, 40 μL of hybridization buffer (50 mM tris–HCl, pH=7.4, containing 1.0 M NaCl, 3.0 μM biotinylated hairpin probe H_1 and 3.0 μM biotinylated hairpin probe H2) were added and incubated for 60 min to trigger the HCR. In the HCR reaction, the captured DNA (C_{DNA}) conjugated on Au nanoparticles (NPs) was initially fixed on the 96 well plates through the sandwich immune reaction, and then it was opened by the hairpin probe 1 (H1) and the newly exposed sticky end of the H1 opened the hairpin probe 2 (H2) to expose a newly sticky end on H2 for extended hybridization. Since the two hairpins, H1 and H2, were labeled with biotin, the streptavidin labelled ALP (SA-ALPs) that catalyzed hydrolysis of o–Phosphonoxyphenol (OPP) to catechol for promoting the cathodic photocurrent generation was then captured on Au NPs through the high affinity between streptavidin and biotin. After rinsed thoroughly, 20 μL of 0.1 mg/mL streptavidin labeled alkaline phosphatase conjugates (SA–ALPs) were added and incubated for 60 min and again rinsed thoroughly. Finally, the plates were allowed for incubating in 0.1 M tris–HCl buffer solution (pH 8.0) containing 100 μL of 20 mM OPP for 50 min. Following that, the ITO/GO electrodes were immersed in the above mixture, carefully washed and finally used for photocurrent measurements as described above.

(iii) The detection of TYR was similar to the procedure for the detection of ALP except that 20 μL of tyrosine (0.01 M), 20 μL of different concentrations of TYR and 160 μL of tris–HCl solution (pH 8.0) replaced the reaction mixture of OPP and ALP. In the presence of TYR and dissolved O_2, tyrosine was converted to dopaquinone. Then, the formed dopaquinone was reduced to DOPA by reacting with 1.0 mM citric acid for 15 min.[5] Following that, the ITO/GO electrodes were used for photocurrent measurements as described above.

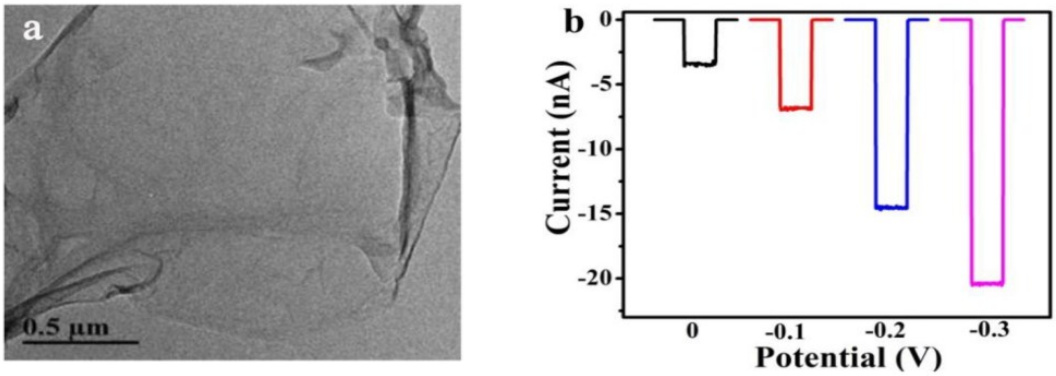


Fig. S1 (a) TEM image of the as synthesized GO and (b) effects of applied cathodic potential on photocurrent responses of the ITO/GO electrode under visible-light irradiation (λ≥400 nm) in 0.1 M tris–HCl solution (pH 8.0).

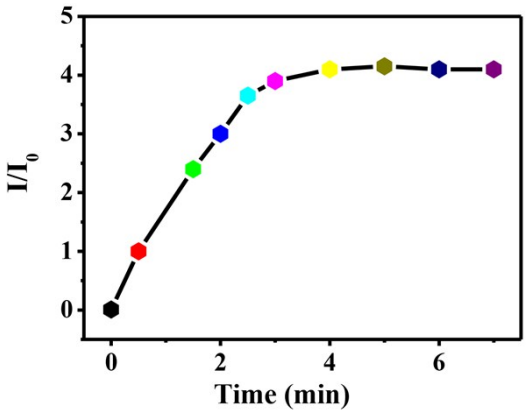


Fig. S2 Dependence of the photocurrent responses of the GO modified ITO electrode with 0.1 μM catechol on reaction time.

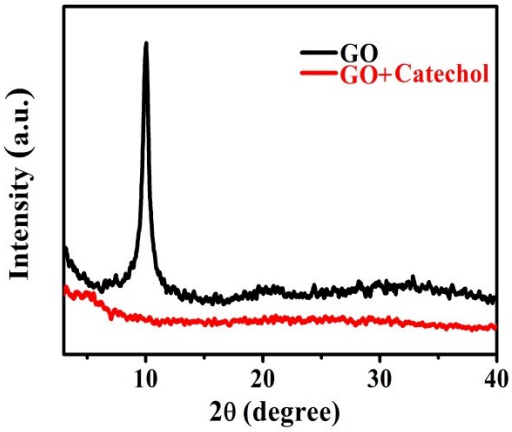


Fig. S3 XRD pattern of GO before and after reaction with catechol.

As shown in Fig. S4 (a), the CB potential of the as-generated rGO was determined by linear sweep voltammetry[6] as -0.93 V vs saturated Ag/AgCl reference electrode. Besides, the in situ formed poly(catechol)[7] can simultaneously act as an efficient electron acceptor because of its abundant benzoquinone (BQ) groups, which reduced the charge recombination of rGO for promoting photocurrent output. Cyclic voltammetry disclosed the reduction potential of -0.10 V for poly(catechol) as shown in Fig. S4 (b).

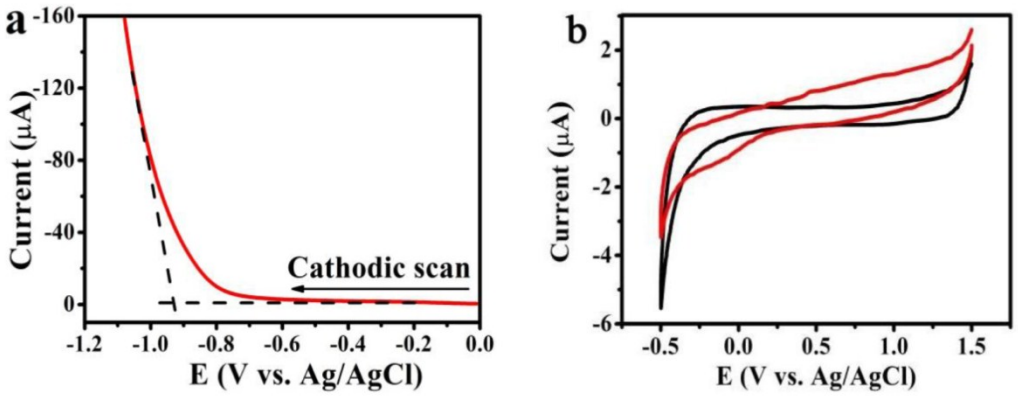


Fig. S4 (a) Cathodic linear potential scan for determining the CB edge of the rGO on the ITO electrode (the ITO/rGO electrode was obtained through the reaction of the ITO/GO electrode with 20 μM catechol and then the formed poly(catechol) was eliminated by treatment with 1.0 M NaOH[8]) specimens. The experiment was conducted in the deoxygenated 0.2 M Na_2SO_4 solution. (b) Cyclic voltammograms of the GO modified ITO electrode before (black line) and after (red line) reaction with 20 μM catechol.

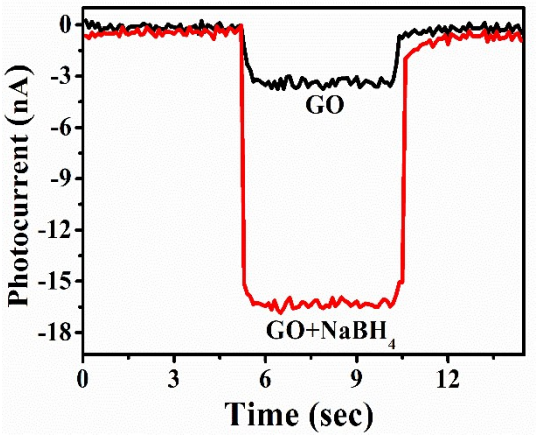


Fig. S5 Photocurrent responses of GO and rGO (obtained by reducing GO with $NaBH_4$ for 30 min) in Tris–HCl solution (pH 8.0) under visible-light irradiation (λ≥400 nm).

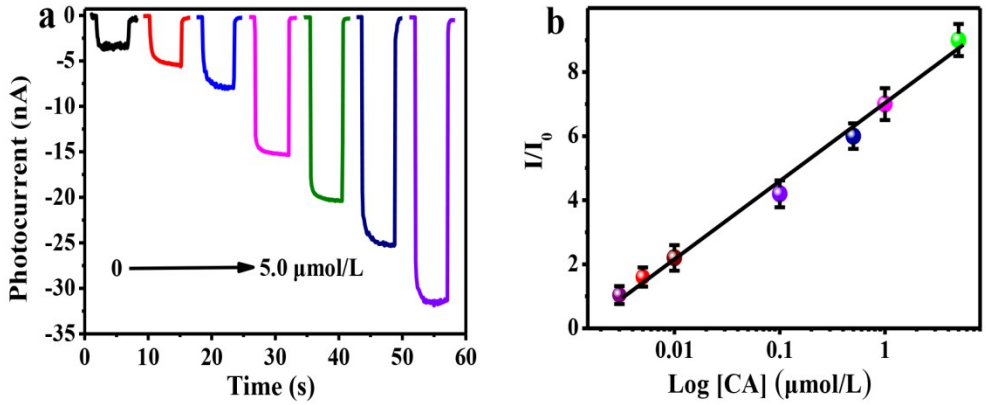
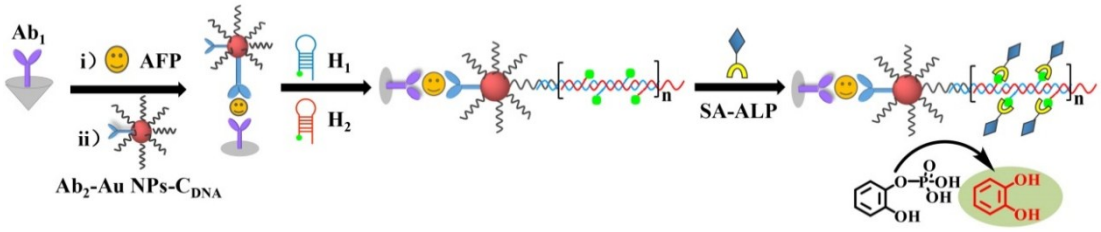


Fig. S6 (a) Photocurrent responses of the ITO/GO electrode to different concentrations of catechol: 0, 0.005, 0.01, 0.1, 0.5, 1.0, and 5.0 μmol/L in 0.1 M tris–HCl solution (pH 8.0) at 0 V (vs Ag/AgCl) under visible light (λ≥400 nm) irradiation. (b) Calibration curve relative to photocurrent enhancement change (I/I_0) and catechol concentration in logarithmic scale.

Table S1. Comparison with different methods for the detection of ALP

Method	Material	Linear range (U/L)	LOD (U/L)	Reference
Colorimetry	G_{20}–Cu(II)	20.0–2.0×10^2	0.8	9
Colorimetry	Au NPs	32.0–2.0×10^2	32.0	10
Fluorometry	CQDs	16.7–7.8×10^2	1.1	11
Fluorometry	CQDs	4.6–3.8×10^2	1.4	12
Fluorometry	K(FITC)FFYp	0−2.8×10^3	60.0	13
Raman scattering	Ag/PATP@SiO_2 NPs	15.0−1.5×10^5	15.0	14
Photoelectrochemistry	CdS NPs	unkown–50.0	0.7	15
Photoelectrochemistry	GO	5.0×10^{-3}–1.0×10^2	1.0×10^{-3}	This work



Scheme S1 Illustration for the immunoreaction of AFP in the microwell plates.

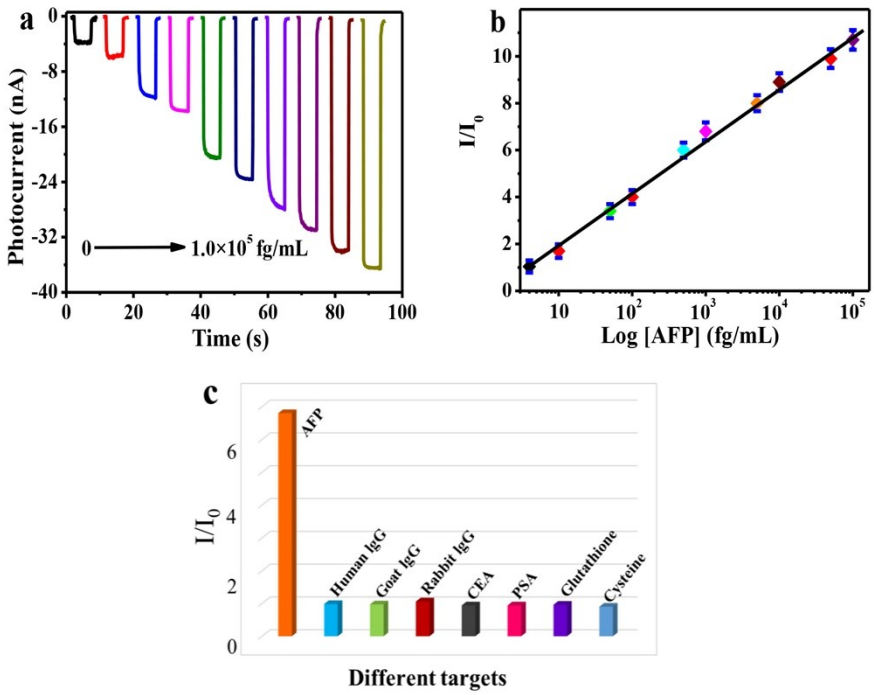


Fig. S7 (a) Photocurrent responses of the detection system for valuated concentrations of AFP: 0, 4.0, 10, 100, 500, 1.0×10^3, 5.0×10^3, 1.0×10^4, 5.0×10^4, and 1.0×10^5 fg/mL. (b) Calibration curve relative to photocurrent enhancement change (I/I_0) and AFP concentration in logarithmic scale. (c) Selectivity of the immunoassay to AFP (0.1 pg/mL) compared with some other interferents: carcinoembryonic antigen (CEA), prostate specific antigen (PSA), human IgG, goat IgG and rabbit IgG were at 10.0 pg/mL level; glutathione and cysteine were at 5.0×10^{-6} mol/L level.

Table S2. Comparison with different methods for the detection of AFP

Method	**Material**	**Linear range (pg/mL)**	**LOD (pg/mL)**	**Reference**
Fluorometry	Magnetic beads	5.0–1.0×10^3	1.6	16
Electrochemistry	IrO_x-CS	50.0–1.5×10^5	20.0	17
Electrochemistry	CuNPs/AgNPs	4.0×10^2–1.0×10^6	10.0	18
Electrochemiluminescence	PET membrane	2.0×10^4–2.0×10^5	1.0×10^4	19
Electrochemiluminescence	CdTe/CdSe NCs	5.0×10^{-2}–1.0×10^2	1.0×10^{-2}	20
Electrochemiluminescence	Au NPs	0–1.0×10^3	5.6×10^{-2}	21
Photoelectrochemistry	ZnO	1.0×10^2–5.0×10^5	10.0	22
Photoelectrochemistry	GO	4.0×10^{-3} –1.0×10^2	1.5×10^{-3}	This work

Table S3. Determination results for AFP in serum samples using the proposed method and the reference ELISA method

Sample	Proposed method (pg/mL)	Reference method (ng/mL)	Relative error (%)
1	20.0*	2.12	-5.7
2	17.4*	1.93	-9.8
3	53.5*	5.61	-4.6
4	34.4*	3.30	+4.2

*The serum samples were diluted by 100 times (to ensure that the concentrations are within the linear range of the method) before using the proposed method for detection.

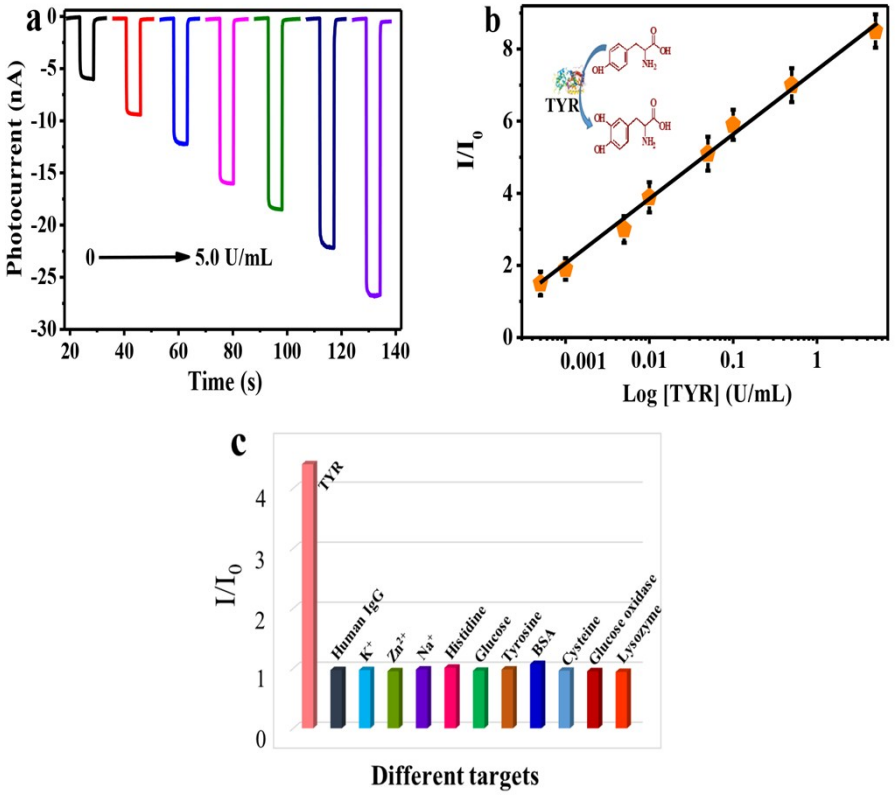


Fig. S8 (a) Photocurrent responses of the detection system for different concentrations of TYR: 0, 5.0×10^{-4}, 1.0×10^{-3}, 5.0×10^{-3}, 0.01, 0.05, 0.1, 0.5, 5.0 U/mL. (b) Calibration curve relative to photocurrent enhancement change (I/I_0) and TYR concentrations in logarithmic scale. (c) Selectivity of the method for TYR detection. The concentration of TYR was 0.01 U/mL; K^+, Zn^{2+}, Na^+, histidine, glucose, and tyrosine was 8.0×10^{-4} mol/L; cysteine was 5.0×10^{-6} mol/L; glucose oxidase and lysozyme were 5.0 μg/L; bovine serum albumin (BSA) and human IgG were 10.0 mg/L.

Table S4. Comparison with different methods for the detection of TYR

Method	Material	Linear range(U/L)	LOD (U/L)	Reference
Fluorometry	Dopa-CQDs	23.2−1.4×10^4	7.0	23
Fluorometry	HB-NP	5.0×10^2−6.0×10^4	5.0×10^2	24
Fluorometry	Au NCs	6.0−3.6×10^3	6.0	25
Fluorometry	Melanosome	5.0×10^2−6.0×10^4	7.0	26
Photoelectrochemistry	PbS QDs	5.0×10^{-3}−50.0	1.6×10^{-3}	27
Photoelectrochemistry	GO	0.1−5.0×10^3	2.0×10^{-2}	This work

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