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Supporting Information

Homogeneous Photoelectrochemical Biosensing *via* Synergy of G-quadruplex/Hemin Catalysed Reaction and Inner Filter Effect

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Experimental Section

Reagents and Materials

The 5′-phosphorylated hairpin DNA probe (HP) and microRNAs were purchased from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China), with their sequences listed in Table S1. KCl, $MgCl_2$, NaCl, $Cd(NO_3)_2 \cdot 4H_2O$, $Zn(NO_3)_2 \cdot 6H_2O$, Tris(hydroxymethyl)aminomethane (Tris), thioacetamide (TAA), ammonia solution (25%~28% in weight), acetic acid, absolute ethanol and H_2O_2 were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Dopamine hydrochloride (DA) (purity > 98%) and GelRed were purchased from Solebo Biotechnology Co., Ltd. (Beijing, China). λ-Exo and 10 × reaction buffer (pH 8.0, containing 50 mM NaCl, 25 mM Tris-HCl, 0.1 mM EDTA, 1mM dithiothreitol and 50% glycerol) were purchased from New England Biolabs, Ltd. (Beijing, China). Diethyl pyrocarbonate (DEPC), hemin and dimethyl sulfoxide (DMSO) were purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China). The reagents were of analytical grade and used without further purification. HP and microRNA stock solutions were prepared by diluting with Tris-HCl buffer (pH 7.4, 10 mM Tris, 100 mM NaCl, and 10 mM $MgCl_2$). Before use, the HP stock solution was heated up to 95 ℃, maintained at this temperature for 10 min, and then slowly cooled down to room temperature. The hemin stock solution (10 mM) was prepared in DMSO and stored in the dark at -20 ℃. Indium tin oxide (ITO) glass (sheet resistance < 10 Ω/square) was purchased from Shenzhen Nanbo Display Technology Co. Ltd. (Shenzhen, China). The ultrapure water (resistivity of 18.2 MΩ cm at 25 ℃) was provided by a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA), and DEPC-treated ultrapure water was used for all experiments involving microRNAs.

Apparatus

The PEC measurements were performed with a Zahner PEC measurement system (Zahner-Elektrik GmbH & Co. KG, Germany), using LED light sources with different wavelengths. All PEC experiments were carried out at room temperature using a conventional two-electrode system: an ITO/ZnO or ITO/ZnO/CdS electrode with an active area of 0.25 cm^2 (*i.e.* 0.5 cm × 0.5 cm) was used as the working electrode, and a platinum wire was employed as the counter electrode. Electrochemical impedance spectroscopy (EIS) was performed with an Autolab PGSTAT302N electrochemical workstation (Metrohm, the Netherlands) using a three-electrode system (a bare or modified ITO electrode as the working electrode, Ag/AgCl as the reference electrode, and a platinum wire as the counter electrode) at an alternating voltage of 5 mV over a frequency range of 0.01 to 100 kHz and controlled by Nova 1.1 in KCl solution (0.1 M) containing $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (5.0 mM, 1:1) mixture as the redox probe. The UV-visible absorption spectra were obtained on a U-3900 spectrophotometer (Hitachi, Japan). The polyacrylamide gel electrophoresis (PAGE) was performed on a Bio-Rad electrophoresis system (Bio-Rad Laboratories, Inc., U.S.A.), and the images of the obtained gel were scanned by a Gel Doc XR+ Imaging System (Bio-Rad Laboratories, Inc., U.S.A.). The scanning electron microscopy (SEM) (operating at 2 kV) and the energy dispersive spectroscopy (EDS) were performed on a JSM-7500F field emission scanning electron microscope (JEOL, Japan).

Preparation of ITO/ZnO/CdS Electrodes

The ITO glass was cut into 0.5 cm × 2.8 cm slices, which were cleaned by ultrasonic treatment in 1 M NaOH of water/ethanol mixture (1:1, v/v) for three times, 30 min each, then washed with ultrapure water and dried at 80 ℃ for 2 h. The zinc oxide (ZnO) nanorod (NR)

array were synthesized *via* hydrothermal reaction on ITO electrode according to literature [S1]. Briefly, 1.36 g of $Zn(NO_3)_2 \cdot 6H_2O$ was dissolved in 100 mL of ultrapure water to make the stock solution, 20 mL of which was then transferred into a 50 mL Teflon-lined stainless-steel autoclave, followed by adding 1 mL of concentrated ammonia solution. Several clean ITO electrodes were immersed in this solution with the conductive sides facing down, and heated at 95 ℃ for 4 hours. After slowly cooling down to room temperature, the ZnO NRs modified ITO electrodes, denoted as ITO/ZnO, were rinsed subsequently with ethanol and ultrapure water for 3 times each, and dried in air. Afterwards, the *in-situ* growth of CdS nanoparticles on the surface of ZnO NRs was carried out according to literature [S2]: the as-prepared ITO/ZnO electrode was soaked in an aqueous solution containing 10 mM $Cd(NO_3)_2$ and 10 mM TAA at room temperature for a certain period of time, and the as-obtained ITO/ZnO/CdS electrodes were then washed with ultrapure water, dried in air and stored for later use.

PEC Detection of MicroRNA

First, 10 μL of HP (10 μM) and 20 μL of target microRNA with different concentrations were added to 58 μL of Tris-HCl buffer (10 mM Tris, 100 mM NaCl, 100 mM KCl, and 10 mM $MgCl_2$, pH 7.4), and the mixture solution was incubated at 37 ℃ for 2 h. With 2 μL of λ-Exo (10 U μL^{-1}) and 10 μL of 10 × reaction buffer (pH 8.0, 50 mM NaCl, 25 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol and 50 % glycerol) being added, the above reaction solution was then incubated at 37 ℃ for another 1 h. Next, 20 μL of hemin stock solution (5 μM) was added, and the reaction solution was incubated at 37 ℃ for 30 min. Finally, 20 μL of dopamine hydrochloride solution (10 mM), 20 μL of H_2O_2 solution (100 mM) and 40 μL of Tris-HCl buffer (pH 7.4) were added into the reaction solution to bring the total volume to 200 μL, and then the

resulting solution was incubated at 37 ℃ for 15 min. Finally, the PEC measurement was carried out.

Nondenaturing Polyacrylamide Gel Electrophoresis (PAGE)

Different reaction solutions were mixed with 6× loading buffer and loaded on the 12% nondenaturing polyacrylamide gel. Then gel electrophoresis was carried out in 1× TBE buffer (pH 7.9, 9 mM TrisHCl, 9 mM boric acid, 0.2 mM EDTA) at room temperature for 55 min, with the voltage set to 110 V. After being stained by GelRed solution for 30 min, the gel was imagined using a Gel Doc XR+ Imaging System.

Table S1 Sequence information of the oligonucleotides used in this work[a]

Name	Sequence
HP (5′-phosphorylated hairpin DNA)	5′-(PO_4)-**TAC AGT GCT TCA TCT CA**T TTT TCC CTA AGG GTT AGG GTT AGG GTT AGG GAA AAA-3′
miRNA-143 (target microRNA)	5′-**UGA GAU GAA GCA CUG UA**G CUC A-3′
miRNA-141	5′-UAA CAC UGU CUG GUA AAG AUG G-3′
miRNA-155	5′-UUA AUG CUA AUC GUG AUA GGG GU-3′
miRNA-199a	5′-ACA GUA GUC UGC ACA UUG GUU A-3′
miRNA-21	5′-UAG CUU AUC AGA CUG AUG UUG A-3′

[a] The underlined letters in HP represent the sequences in the stem of the DNA hairpin probe, and the bold letters in HP and miRNA-143 stand for the sequences complementary to each other.

Table S2 Comparison of the analytical performance for microRNA assay between our strategy and those reported in literature.

Detection Method	Linear Detection Range	Detection Limit	Ref.
Electrochemiluminescence	0.1 pM ~10 nM	0.1 pM	S3
Electrochemistry	10 fM ~1 nM	10 fM	S4
electrochemistry	1 pM ~ 10 nM	0.26 pM	S5
Fluorescence	100 fM ~ 10 nM	58 fM	S6
Fluorescence	1 nM ~ 16 nM	47 pM	S7
Colorimetry	0.01 nM ~ 2.0 nM 0.01 nM ~ 2.5 nM	4.2 pM 3.6 pM	S8
Photoelectrochemistry	50 fM ~100 pM	34 fM	S9
Photoelectrochemistry	350 fM ~ 5 nM	153 fM	S10
Homogenous photoelectrochemistry	0.2 pM ~ 25 nM	65 fM	This work

Table S3 Determination of miRNA-143 spiked in human serum samples.

No.	Added (pM)	Detected (pM) (n=5)	Mean recovery[a] (%)	RSD[b] (%)
1	0.00	Not detected	/	/
2	2.00	1.93 ±0.07	96.5	3.63
3	10.00	10.08 ±0.29	100.8	2.88
4	50.00	50.63 ±0.97	101.2	1.92

[a] recovery = ($C_{detected}$ / C_{added}) × 100%, [b] RSD: relative standard deviation

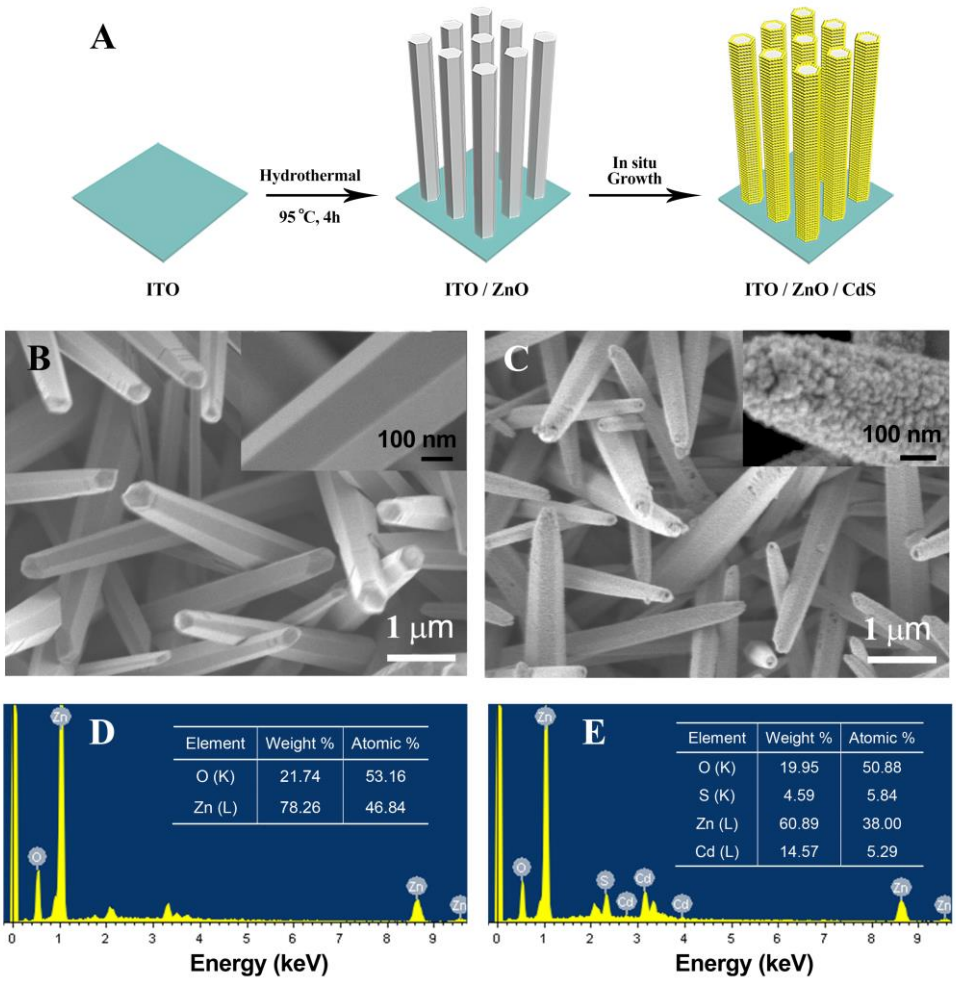

Fig. S1 (A) Fabrication procedure of ITO/ZnO/CdS electrode. Top-view SEM images of (B) ITO/ZnO electrode and (C) ITO/ZnO/CdS electrode, and the insets are the high-magnification SEM images. EDS spectra and elemental percentages of (D) ITO/ZnO and (E) ITO/ZnO/CdS electrodes.

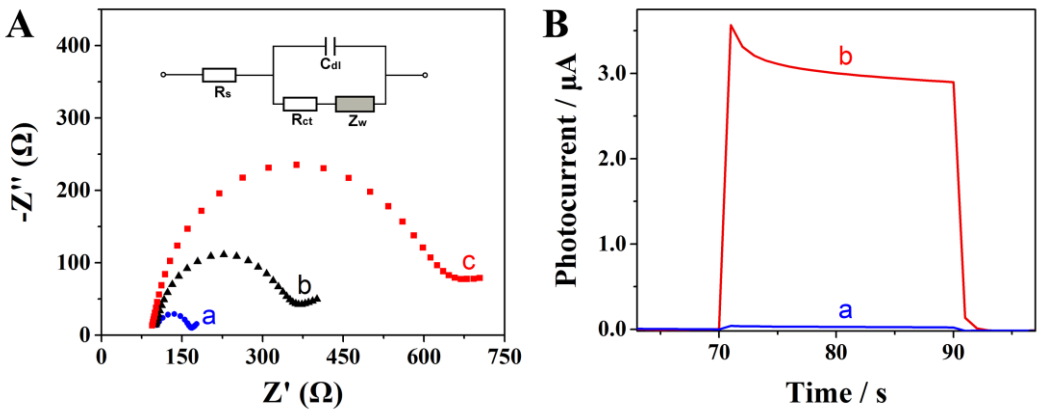

Fig. S2 (A) EIS Nyquist plots of ITO electrodes at different modification stages: (a) bare ITO, (b) ITO/ZnO, and (c) ITO/ZnO/CdS; Inset: equivalent circuit. (B) Photocurrent response of (a) ITO/ZnO, and (b) ITO/ZnO/CdS electrodes in Tris-HCl buffer (pH 7.4) containing 10 mM H_2O_2 and 1 mM dopamine.

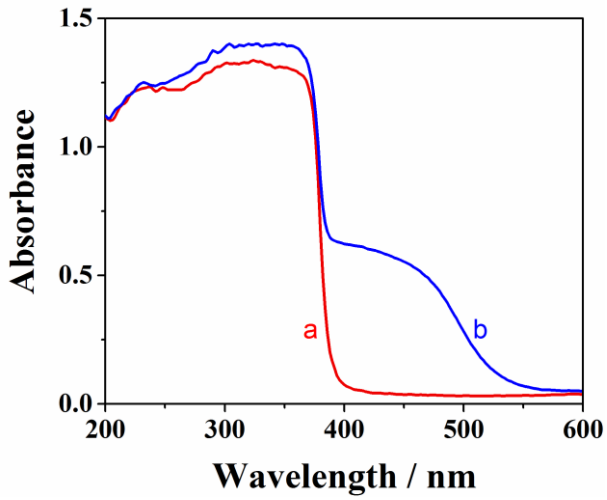

Fig. S3 UV-vis absorption spectra of (a) ZnO and (b) ZnO/CdS heterostructure.

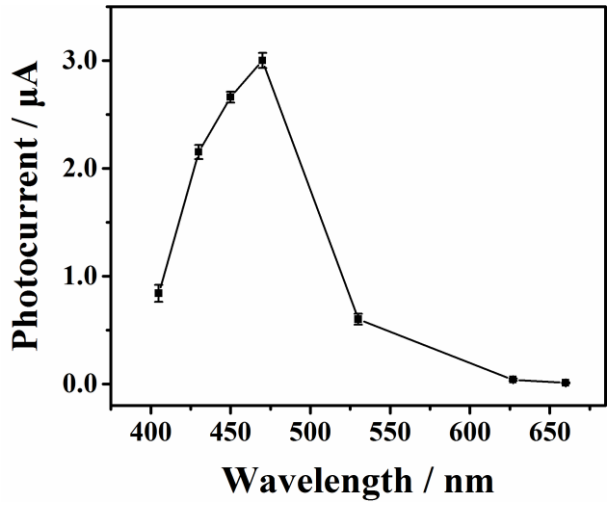

Fig. S4 The photocurrent response of ITO/ZnO/CdS electrode in Tris-HCl buffer (pH 7.4) containing 10 mM H_2O_2 and 1 mM dopamine using visible light source with different wavelengths: 405, 430, 450, 470, 530, 627 and 660 nm.

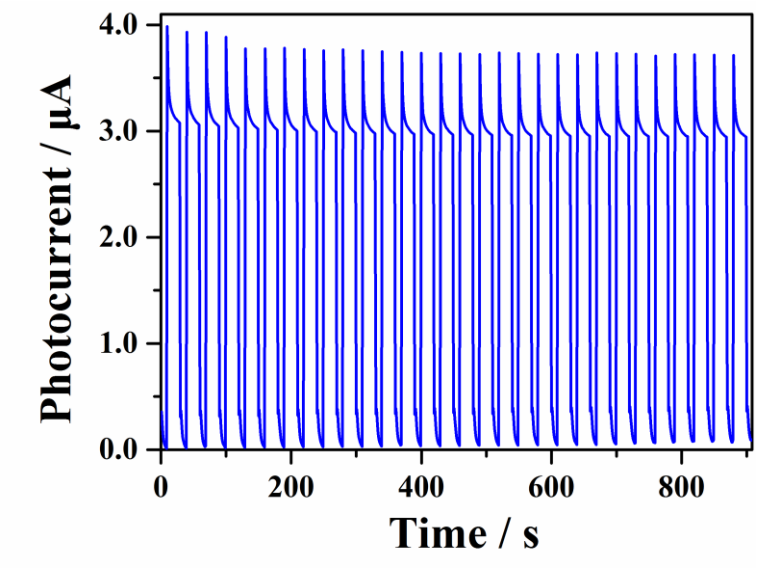

Fig. S5 Photostability study: PEC response of ITO/ZnO/CdS electrode during 30 repeated on/off illumination cycles in the presence of 10 mM H_2O_2 and 1 mM dopamine.

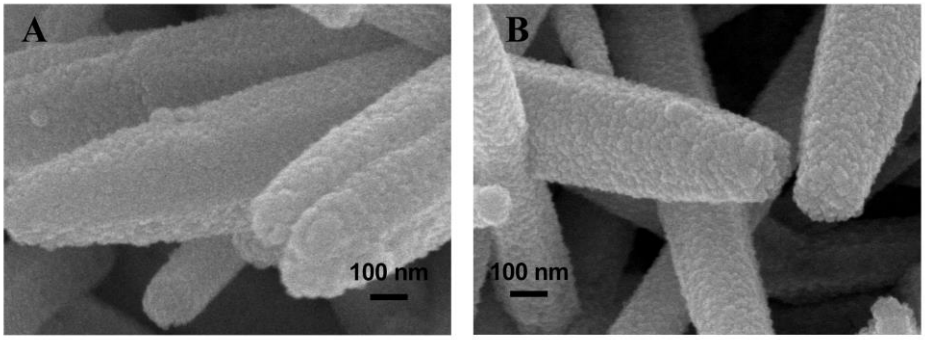

Fig. S6 Top-view SEM images of ITO/ZnO/CdS electrode (A) before and (B) after photocurrent measurements.

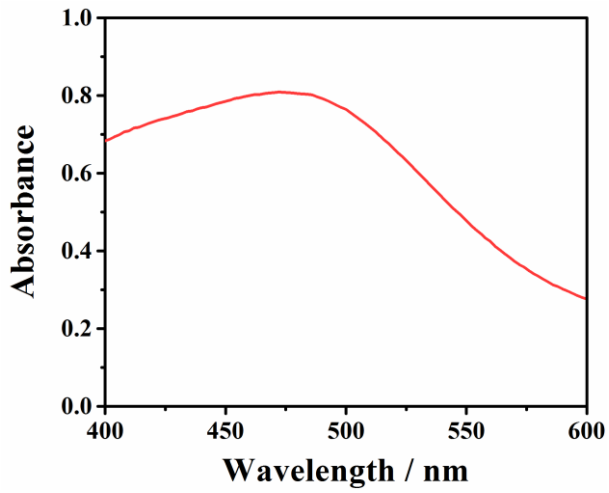

Fig. S7 UV-vis absorption spectrum of aminochrome.

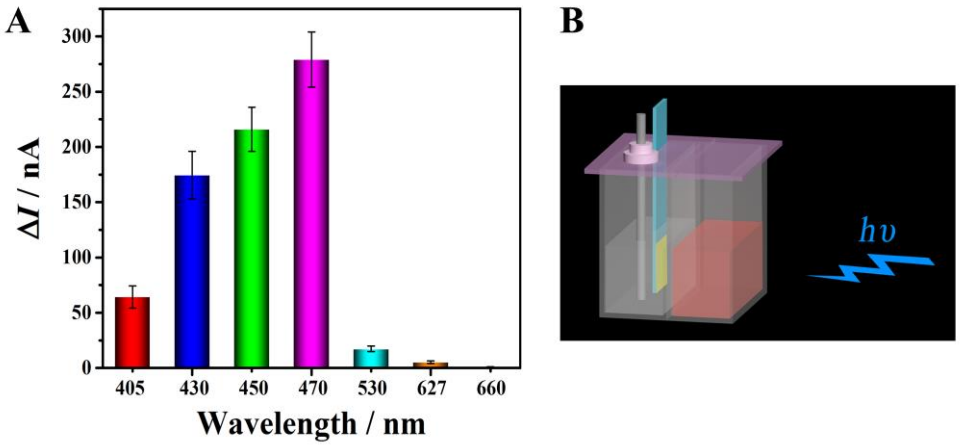

Fig. S8 (A) Comparison of the photocurrent change (ΔI) using excitation light source with different wavelengths (405, 430, 450, 470, 530, 627 and 660 nm). Here, $\Delta I = I_0 - I$, where I_0 is the photocurrent of Tris-HCl buffer (pH 7.4) containing 10 mM H_2O_2 and 1 mM dopamine using ITO/ZnO/CdS working electrode, and I is the photocurrent of the same solution with another quartz cuvette containing 200 μL aminochrome solution placed in front of the light source to absorb the light, whose setup is shown in (B). Herein, the aminochrome solution was the catalysed product resulted from the reaction system containing 10 nM miRNA-143, and the reaction conditions are the same as those mentioned in Experimental Section.

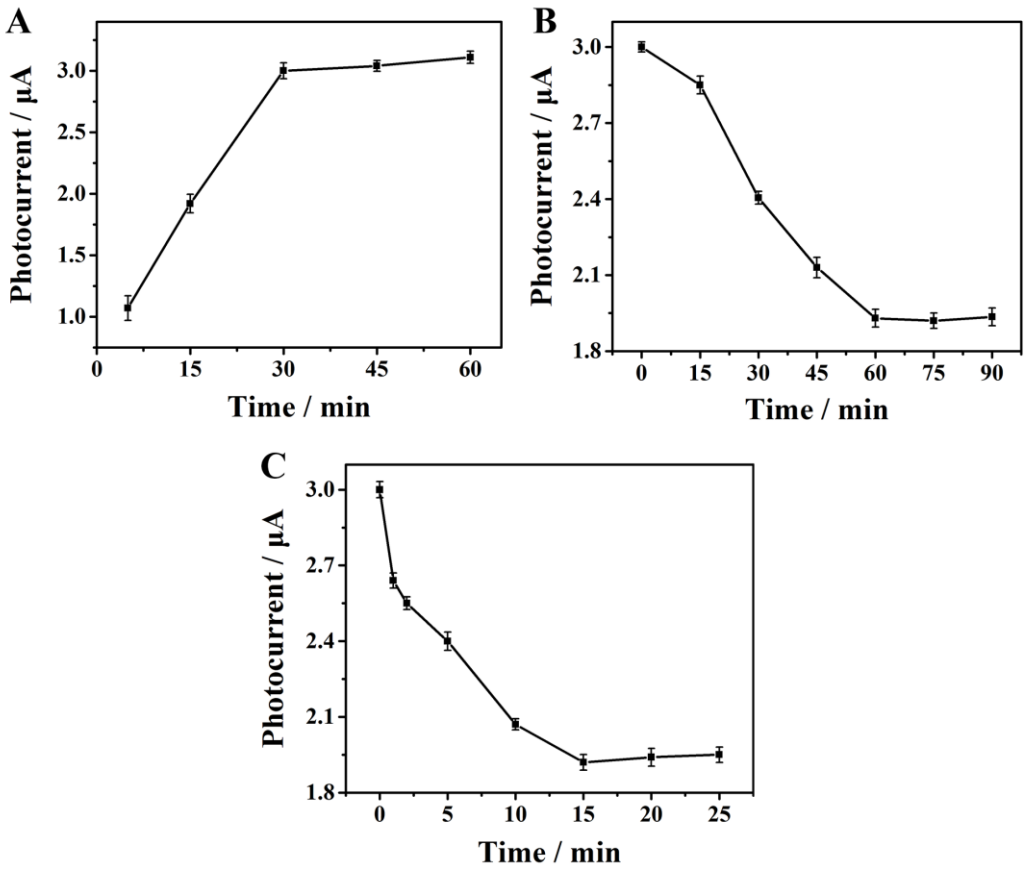

Fig. S9 Optimization of the experimental conditions. The photocurrent versus (A) the *in-situ* deposition time of CdS (5, 15, 30, 45 and 60 min), (B) the reaction time of λ-Exo catalyzed digestion (0, 15, 30, 45, 60, 75 and 90 min), and (C) the catalytic reaction time of G-quadruplex/hemin (0, 1, 2, 5, 10, 15, 20 and 25 min).

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