Electronic Supplementary Information

Ultrasensitive Photoelectrochemical MicroRNA Biosensor Based on Doxorubicin Sensitized Graphitic Carbon Nitride Assisted by Target-activated Enzyme-free DNA Walker

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1. Materials and Reagents

Urea and 30% hydrogen peroxide (H_2O_2) were supplied by Kelong Chemical Inc. (Chengdu, China). Hexanethiol (HT), and gold chloride tetrahydrate ($HAuCl_4 \cdot 4H_2O$) were purchased from Sigma Chemical Co. (St. Louis. MO, USA). Doxorubicin (Dox) was bought from Aladdin Industrial Corporation (Shanghai, China). TBE buffer (5×; 250 mM Tris, 250 mM H_3BO_3, 10 mM EDTA; pH 8.0) was applied for the polyacrylamide gel electrophoresis (PAGE) experiments. Phosphate buffered solution (PBS) was composed of 0.1 M KCl, 0.1 M NaOH, 0.1 M KH_2PO_4 and 0.1 M Na_2HPO_4. 5.0 mM ferricyanide/ferrocyanide mixed solution ($[Fe(CN)_6]^{3-/4-}$) was made by dissolving potassium ferricyanide and potassium ferrocyanide in PBS solution. The microRNA-141 (miRNA-141) was synthesized by TaKaRa (Dalian, China). All DNA oligonucleotides were bought from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). The sequences used in the experiment were listed as the following (Table S1).

Table S1. Sequences used in the experiment

Name	Sequence (5′→ 3′)	Function
H1	NH2-AAA AAT CTT TAC CAG ACA GTG TTA GC CCC TGC TTC CTG GTA AAG	capture probe
H2	NH_2-AAA AAT CCC CCC TGC TTC CTT TAC CAG GAA GCA GGG GCG ACT	capture probe
H3	GAA GCA GGG GCG ACT G TGC TTC CTG GTA AAG GAA GCA GGG GGG	trigger probe

H4	GGG GCG ACT TGA AAC AGT CGC CCC TGC TTC	helper probe
H5	GTT TCA AGT CGC CCC GAA GGA GGG GCG ACT	helper probe
miRNA-141	UAA CAC UGU CUG GUA AAG AUG G	interferent
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A	interferent
miRNA-126	CAU UAU UAC UUU UGG UAC	interferent
miRNA-122	UGG AGU GUG ACA AUG GUG UUU G	interferent

2. Apparatus

The PEC measurements were performed in a PEC workstation (Ivium, Netherlands). A three-electrode system was used for photocurrent measurement, in which a glassy carbon electrode (GCE, Φ = 4 mm), an Ag/AgCl (saturated KCl) and a platinum wire electrodes were served as the working electrode，the reference and the counter electrode, respectively. The measurements of cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed on a CHI 760E electrochemistry workstation (Shanghai Chenhua Instrument, China). Gel Doc XR$^+$ System (Bio-Rad, California, USA) was used to take images of gels. The characterizations of the used materials were carried out on scanning electron microscopy (SEM, S-4800, Hitachi, Japan) and UV-2450 ultraviolet-visible (UV-vis) absorption spectrum (Shimadzu, Tokyo, Japan).

3. Polyacrylamide gel electrophoresis (PAGE)

The freshly 16% polyacrylamide gel was prepared by using 1 × TBE buffer and the electrophoresis was conducted at 120 V for 120 min. The electrophoresis image was observed under UV light after the gel was dyed by gel green for 20 min.

4. Synthesis of g-C_3N_4

The g-C_3N_4 was synthesized based on the previous reports with a minor modifacation.[1] 10 g of urea in a crucible without a cover in a vacuum drying oven was dried at 80 °C for 24 h, and then the crucible with a cover was put into a muffle furnace. After being heated at 550 °C for 4 h with a heating rate of 10 °C/min, the yellow production produced by calcination was washed by deionized water and ethanol with several times, respectively. Finally drying at 80 °C for several hours, the obtained yellow production was collected for further use.

5. Fabrication of the PEC Biosensor

Firstly, under the thoroughly polished with alumina slurry and sonicated with ethanol and ultrapure water in turn, a completely clean GCE surface could be obtained. Subsequently, 5 μL of g-C_3N_4 was dropped onto the prepared electrode and then dried at 37 °C to get thin films. Next, the modified electrode was immersed in 1% $HAuCl_4$ aqueous solution to obtain the gold nanoparticles layer (Dep Au) under a constant potential of -0.2 V for 15 s. After that, H1 (10 μL, 1 μM) and H2 (10 μL, 2 μM) were immobilized on the electrode at 4 °C for 12 h through Au-N bonds. To reduce nonspecific binding sites, HT (10 μL, 1 mM) was dropped onto the as-prepared electrode for 40 min.

6. Target-activated Enzyme-free DNA Walker Procedure

When 10 μL of target miRNA-141 was attached to the electrode surface at 37 °C for 2 h, H1 was first opened to form a DNA walker and the exposed tail of H1 was used as the swing arm of the DNA walker to further capture hairpin DNA H2 to generate a H1-target-H2 complex. With the addition of H3 (10 μL, 2 μM) on the

fabricated electrode, H3 could hybridize with H2 through a catalytic hairpin assembly (CHA) process, resulting in the release of swing arm and triggering the movement of DNA walker. After N cycles, a large number of H2-H3 complexes were formed on the electrode and the exposed tail of H3 could act as the initiator of the hybridization chain reaction (HCR). After the mixture solution of H4 (10 μL, 1 μM) and H5 (10 μL, 1 μM) were incubated on the electrode at 37 °C for 2 h, lots of long dsDNA copolymers could be generated. Finally, Dox (10 μL, 0.16 mM) was dropped on the modified electrode for 1 h at room temperature, resulting in an enhanced photocurrent signal. For removing residual reagents, the modified electrode was completely washed with distilled water after each fabricated step.

7. PEC Measurement procedure

The PEC measurements were carried out in 5 mL PBS (0.1 M, pH 7.0) containing 50 μL of H_2O_2 (30%) as electron donor. The light-emitting diode lamp was used as the excitation light source (λ = 365 nm) and switched "off-on-off" for 10-20-10 s under 0.0 V potential.

8. Electrochemical Characterizations of the PEC Biosensor

The fabricated process of the PEC biosensor was investigated by measuring the CV changes of the modified electrode in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ solution. As depicted in Figure S1A, a couple of well-defined peak current could be observed on the pretreated bare GCE (curve a). Because g-C_3N_4 could hinder electronic transmission, the peak current decreased (curve b) compared to the bare GCE. After Dep Au modified on the electrode surface, the peak current increased (curve c) due to the preeminent conductivity of Dep Au. When H1 and H2 were immobilized on the modified electrode

simultaneously, the peak current declined (curve d), which was mainly ascribed to the electronic repulsion between negatively charged DNA and redox probe ($[Fe(CN)_6]^{3-/4-}$). Then the peak current further reduced (curve e) after HT was dropped on the electrode, for the reason of the inhibition of electron transfer from HT. Results from CV measurements indicated that the sequential modification process of the biosensor was fabricated successfully.

EIS was also an useful assay to confirm the construction process of the biosensor by monitoring the impedance changes. As shown in Figure S1B, a small resistance (R_{et}) value (curve a) could be observed from the bare GCE. When g-C_3N_4 was assembled on the bare GCE, the R_{et} value (curve b) obviously increased because the g-C_3N_4 could hinder the electron transfer. Afterwards, the R_{et} value (curve c) clearly decreased with the modification of Dep Au on the electrode surface because Dep Au could promote the electron transfer. After the immobilization of H1 and H2, the R_{et} value (curve d) increased, which could be attributed to the electronic repulsion of negatively charged DNA. After blocking with HT, R_{et} value (curve e) further increased as HT could inhibit the electron transfer. These results showed that the biosensor was successfully constructed.

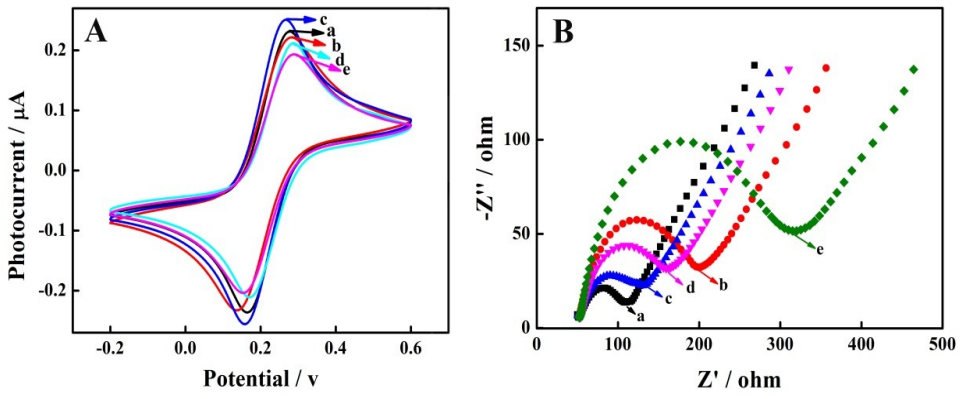

Figure S1. CV (A) and EIS (B) responses of the different modified electrodes: (a) bare GCE; (b) g-C_3N_4/GCE; (c) Dep Au/g-C_3N_4/GCE; (d) H1-H2/Dep Au/g-C_3N_4/GCE; (e) HT/H1-H2/Dep Au/g-C_3N_4 /GCE.

9. Optimal Conditions of the PEC Assay

To obtain a superior analysis performance of the PEC measurements, the concentrations of DNA probe H1 and H2, Dox, as well as the hybridization time for HCR, were optimized by comparing the photocurrent signals, respectively. As exhibited in Figure S2A, the photocurrent signals obviously increased with increasing the concentration of DNA probe H1 from 0.4 μM to 1 μM, and with the extension of the concentration from 1 μM to 1.5μM, the photocurrent signals was decreased. Thus, 1 μM was chosen as the optimal concentration for DNA probe H1. Figure S2B showed the optimal concentration of DNA probe H2, the photocurrent signals enhanced visibly along with the concentration of H2 increased from 0.5 μM to 2 μM, while it decreased when the concentration of H2 exceeded 2 μM. Therefore, 2 μM was selected as the optimal concentration for DNA probe H2. As illustrated in Figure S2C, a peak photocurrent was obtained when the concentration of Dox was 0.16 mM, then the photocurrent signals obviously decreased with the increasing concentration of Dox, which manifested that 0.16 mM was the appropriate concentration of Dox. At the same time, the hybridization time for HCR was investigated in this experiment. As shown in Figure S2D, the optimization of the hybridization time for HCR was selected in the range from 0.5 h to 3 h, the photocurrent signals gradually increased accompanying by the time reached to 2 h but decreased slightly after 2 h. Therefore, 2 h was chosen as the optimal time for the HCR.

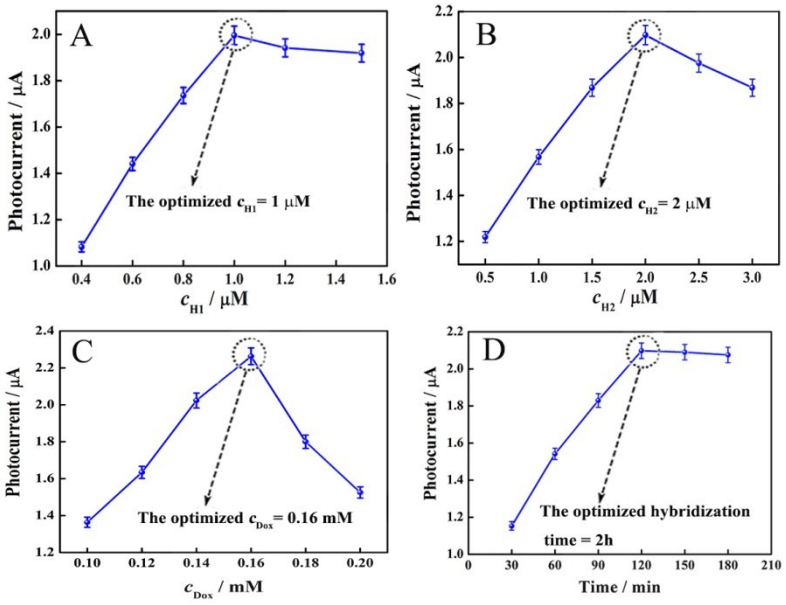

Figure S2. The conditions optimization of (A) the concentration of DNA probe H1; (B) the concentration of DNA probe H2; (C) the concentration of Dox and (D) the incubation time for HCR.

10. The Energy Band Gap (*Eg*) Computational Process for Dox

According to the previous literature: $Eg = 1240/\lambda_{onset}$, where λ_{onset} is the onset absorption wavelength.[2] As a result, the *Eg* of Dox was calculated as 2.14 eV owing to the λ_{onset} of Dox was about 580 nm from the UV-vis absorption spectrum (Figure 1D).

11. PAGE Analysis

To investigate the feasibility, PAGE was performed in this experiment. All the hairpin DNA probes were heated to 95 °C for 2 min and then allowed to cool to room temperature for 1 h before use. As illustrated in Figure S3, lanes 1, 2, 3 exhibited a distinct single band for H1, H2 and target, respectively. When target was reacted with H1, a new band (lane 4) could be observed, corresponding to the DNA duplex of H1-target, indicating the successful hybridization of target and H1. Lane 5 had two bands, a higher position band could be noticed, corresponding to the complex of H1-target-H2,

demonstrating the successful hybridization of H2 and H1. Lane 6 represented the H3. Lane 7 displayed four distinct bands, representing the residual H1-target-H2, the DNA duplex of H2-H3, the residual H1-target and H2 from top to bottom, manifesting the successful assembly of the CHA reaction. Lastly, lane 8 showed the HCR products mainly posed the highest position, which confirmed the validity of proposed HCR strategy. Lane 9 and 10 represented the H4 and H5, respectively. All the above results verified the successful construction of the biosensor as expected.

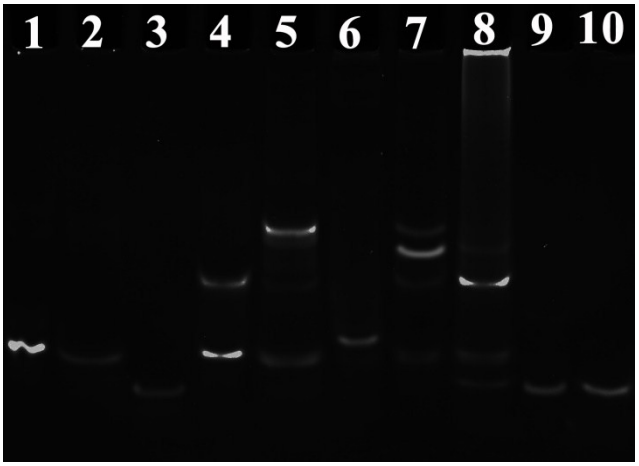

Figure S3. Gel electrophoresis analysis of different samples. Lane 1: H1; Lane 2: H2; Lane 3: target; Lane 4: H1-target; Lane 5: H1-target-H2; Lane 6: H3; Lane 7: the CHA reaction; Lane 8: the HCR reaction; Lane 9: H4; Lane 10: H5.

12. The Reproducibility of the Biosensor

The reproducibility of the PEC biosensor was assessed based on the relative standard deviation (RSD) by comparing the PEC signal of the inter-assay and intra-assay to 2.5 pM miRNA-141 under the same experiment conditions. The intra-assay was measured in the five same-batches of electrodes and inter-assay was carried out in five different batches of electrodes in the presence of 2.5 pM miRNA-141. As depicted in Figure S4A, the PEC signal of inter-assay showed small PEC variation and the

calculated RSD was 3.78%. Meanwhile, Figure S4B also showed the PEC signal of intra-assay and the calculated RSD was 2.4%. Those obtained results indicated a satisfactory reproducibility of this proposed biosensor.

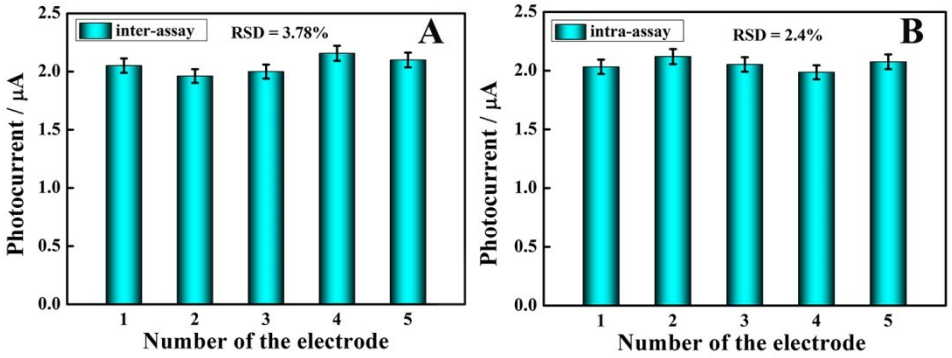

Figure S4. The reproducibility of inter-assay and intra-assay of the biosensor to 2.5 pM miRNA-141.

13. The Feasibility of the Developed Biosensor in Real Samples

To assess the feasibility of the developed biosensor for the detection of miRNA-141, the expression of miRNA was investigated in MB231 (human breast cancer cells) with high expression of miRNA-141 and HeLa (human cervical cancer cells) with low expression of miRNA-141. As exhibited in Figure S5, with increasing the cell numbers from 1×10^3 to 100×10^3, the photocurrent signals gradually increased. At the same time, MB231 showed significantly higher photocurrent signal than HeLa in the same cell number, which was consistent with the previous reports.[3] Thus, the proposed PEC biosensor could be generalized to the application in the early diagnosis of cancers.

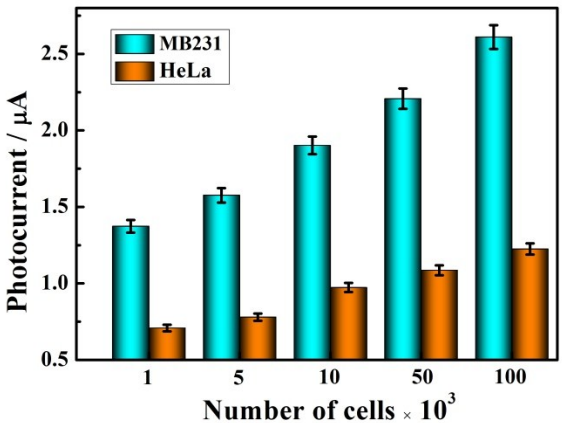

Figure S5. The application of PEC biosensor for sample analysis in MB231 and HeLa cells under different cell numbers.

Table S2. Comparison of this PEC biosensor with other reported detection strategies for miRNA-141

Analytical method	Linear range	Detection limit	References
Fluorescent	0.1 pM to 10 pM	80 fM	4
Electrochemical	5 fM to 50 pM	4.2 fM	5
Chemiluminescence	20 fM to 5 pM	10 fM	6
Photoelectrochemical	1 fM to 10 pM	0.5 fM	7
Photoelectrochemical	350 fM to 5 nM	153 fM	8
Photoelectrochemical	0.25 fM to 2.5 nM	83 aM	***Our work***

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