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Supporting Information

Ultrasensitive Photoelectrochemical Biosensor for MiRNA-21 Assay Based on Target-Catalyzed Hairpin Assembly Coupled with Distance-Controllable Multiple Signal Amplification

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1. Materials and reagents

Perylene-3,4,9,10-tetracarboxylic dianhydride (PTCDA) was bought from Lian Gang Dyestuff Chemical Industry Co., Ltd. (Liaoning, China). Sodium hydroxide (NaOH), gold chloride tetrahydrate ($HAuCl_4 \cdot 4H_2O$), hexanethiol (HT), ascorbic acid (AA) and sodium chloride (NaCl) were supplied by Chengdu Kelong Co., Ltd (Chengdu, China). $K_4[Fe(CN)_6]$ and $K_3[Fe(CN)_6]$ were bought from Beijing Chemical Reagent Co. (Beijing, China). 0.1 M phosphate buffered solution (PBS, pH = 7.0) containing 0.1 M KCl, 0.1 M Na_2HPO_4 and 0.1 M KH_2PO_4 was served as the working buffer for photoelectrochemical (PEC) measurement. Tris-HCl (pH = 7.4) contains 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$ and 1 mM $MgCl_2$ was used to dilute the oligonucleotides. MicroRNA-21, microRNA-141, microRNA-155, microRNA-126 and all oligonucleotides in this work were bought from Sangon Biotech Co., Ltd. (Shanghai, China). All the nucleotide sequences are listed as follows (from 5′ to 3′):

Table S1. Information of Synthetic Nucleotide Sequences in This Study (from 5′ to 3′)

HP1	TCA ACA TCA GTC TGA TAA GCT ACC ATG AGA TAG CTT ATC AGA CTT TTT GGT AGT AGG TTG TAT AGA AAA C
HP2	TAA GCT ATC TAC ACA TGG TAG CTT ATC AGA CTC CAT GTG TAG ATT TTG GTA GTA GGT TGT ATA GAA AAC
SP-MB	MB-ACC CTC TGT AGG TTG TAT AGA AAA CAG AGG GT-$(CH_2)_6$-SH
TP-Fc	GTT TTC TAT ACA ACC TAC TAC CAA A
MiRNA-21	UAG CUU AUC AGA CUG AUG UUG A
MiRNA-141	UAA CAC UGU CUG GUA AAG AUG G
MiRNA-126	CAU UAU UAC UUU UGG UAC
MiRNA-122	UGGAGUGUGACAAUGGUGUUUG
MiRNA-55	UUAAUGCUAAUCGUGAUAGGGGU

2. Instrument

The PEC determination was performed on a PEC workstation (Ivium, Netherlands) with three-electrode system which including the working electrode of modified glassy carbon electrode (GCE, Φ = 4 mm), reference electrode of an $Hg/HgCl_2$ (saturated KCl) electrode and the counter electrode of platinum wire, respectively. Electrochemical impedance spectroscopy (EIS) was implemented on a CHI 760e electrochemistry workstation (Shanghai Chenhua Instrument, China). The morphology of prepared materials was characterized by the scanning electron microscopy (SEM, S-4800, Hitachi, Japan) with the voltage of 20 kV. The Molecular Imager Gel Doc XR+ and Image Lab software (Bio-Rad, California, USA) were used to photograph the image of gel electrophoresis.

3. Fabrication of PEC biosensor

The photoactive material PTCA from the hydrolysis of Perylene-3,4,9,10-tetracarboxylic dianhydride (PTCDA)[1] was firstly modified onto the cleaned GCE surface for drying at 37 °C. In the condition of -0.2 V constant potential, the Au nanoparticle (depAu) layer was obtained by immersing the modified electrode in 1% $HAuCl_4$ to electrodeposit for 15 s. Prior to incubate the dsDNA (SP-TP) onto the depAu/PTCA/GCE surface, the mixture of TP and SP was slowly heated to 85℃ for 5 minutes and cooled down to 25℃ for obtaining dsDNA. Then, the obtained dsDNA was dipped onto electrode to incubate at 4 ℃ for 12 h. Thereafter, 5 μL 1.0 mM HT was dropped onto modified surface with incubation of 40 minutes at room temperature to block nonspecific adsorption sites.

4. Target amplification procedure triggered by CHA.

Firstly, the HP1 and HP2 were slowly annealed at 95℃ for 5 minutes and cooled down to 25 ℃ to form the hairpin structure. After that, HP1 and HP2 were diluted into 2 μM with Tris-HCl (pH =

7.4), respectively. Then, the equal volumes of HP1 and HP2 were mixed with various concentrations of miRNA-21 (input) and maintained for 30 minutes at room temperature to obtain the (output) dsDNA (HP1-HP2). In this progress, the hairpin DNA HP1 was firstly opened when added target miRNA-21 and then exposed part complementary sequence for further hybridizing with hairpin DNA HP2 to displace the miRNA-21 and form into the dsDNA (HP1-HP2). Thus, the released miRNA-21 could be recycled to trigger the next CHA for achieving signal amplification.

5. PEC and EIS measurement.

The PEC determination was implemented in 4 mL PBS (pH = 7.0) containing 0.1 M electron donor AA. The recording of PEC signals was accomplished under an applied potential of 0.0 V and a 460 nm excitation light source according to switch off-on-off for 10-20-10 s. EIS was performed on a CHI 760e electrochemistry workstation with the working buffer of PBS (pH = 7.0) containing 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) and 0.1 M KCl. The frequency was ranged from 0.1 Hz to 100 kHz at the amplitude of 5 mV.

6. Gel electrophoresis.

Firstly, 2 μL loading buffer solution was added into 10 μL eight DNA samples, respectively. Then, the mixtures were subjected to eight lanes of freshly prepared polyacrylamide gel (16%). Afterwards, the electrophoresis was carried out in 1 × TBE buffer at a constant voltage of 120 V for 80 minutes. Subsequently, the gel was stained by ethidium bromide (EB) solution for 20 minutes and the Molecular Imaging Gel Doc XR system with Image Lab software was used to photograph the image of gel electrophoresis for obtaining electrophoresis results.

7. *The mechanisms of quenching and sensitizing effect.*

When Fc was trapped on PTCA surface, the light-generated electrons would transfer into the empty orbit of Fc rather than the electrode so as to the photocurrent signal was significantly reduced, which was termed as quenching effect. Instead, under the exciting light, the electrons generated from MB would be transferred into the conduction band (CB) of PTCA so as to obviously improve the photoelectric conversion efficiency to enhance the photocurrent, which was termed as sensitizing effect.

8. Characterization of proposed PEC strategy by PAGE.

The PAGE was used for proving this the feasibility of proposed PEC strategy. As illustrated in Figure S1, we could see two bright bands in lane 1 and lane 2, which represented ssDNA SP and TP, respectively. The band of lane 3 represented the hybridization product of SP and TP with slow migration speed. After adding miRNA-21 into hairpin DNA HP1 (lane 4), a dual band could be seen in lane 5. The bright band represented remaining HP1 and the blurred band in the top represented the hybridization product of HP1 and miRNA-21. After adding hairpin DNA HP2 (lane 6) into the mixture of HP1 and miRNA-21, five bands were appeared in Lane 7, which represented the hybridization product of HP1 and HP2, hybridization product of HP1 and miRNA-21, the excessive HP1, HP2 and miRNA-21 from top to bottom, respectively, suggesting that the target catalyzed hairpin assembly (CHA) was successfully achieved by the cycling of miRNA-21. Finally, six bands of lane 8 could be seen clearly, the uppermost band represented the hybridization product of HP1, HP2 and TP, which demonstrated that the dsDNA HP1-HP2 successfully hybridized with TP. The five remaining bands represented the hybridization product of HP1 and HP2, hybridization product of HP1 and miRNA-21, the excessive HP2, SP and miRNA-21.

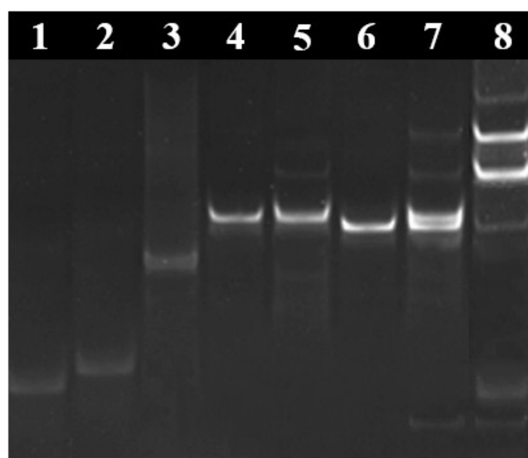

Figure S1. PAGE characterization of proposed PEC strategy: lane 1, SP; lane 2, TP; lane 3, SP+TP; lane 4, HP1; lane 5, HP1+miRNA-21; lane 6, HP2; lane 7, HP2+HP1+miRNA-21; lane 8, SP+TP+HP2+HP1+miRNA-21.

9. Cyclic voltammetric (CV) characterizations of this proposed PEC biosensor

The related CV characterizations of this biosensor were also investigated to describe the interface properties of the modified electrode. As depicted in Figure S2, compared with the redox peaks of bare GCE (curve a), the PTCA modified electrode showed a decreased redox peaks (curve b). After depAu was electrodeposited onto the PTCA modified electrode, the redox peaks obviously increased due to the excellent electron transfer properties of depAu (curve c). When dsDNA (SP-MB and TP-Fc) was immobilized onto depAu/PTCA/GCE, the redox peaks decreased (curve d). Afterward, the obtained electrode was further incubated with HT, the redox peaks decreased again due to the inaccessibility created by HT (curve e). However, the redox peaks increased after incubating with the mixture of HP1, HP2 and miRNA-21 (curve f).

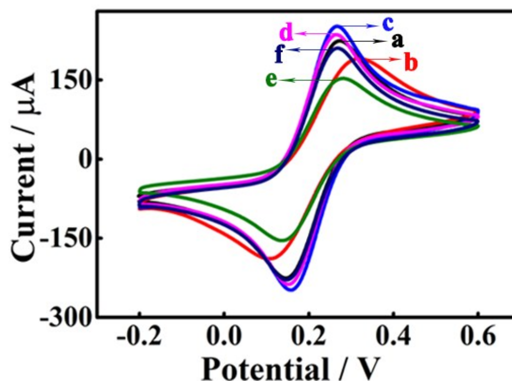

Figure S2. CV profiles of (a) bare GCE, (b) PTCA/GCE, (c) depAu/PTCA/GCE, (d) SP-TP/depAu/PTCA/GCE, (e) HT/SP-TP/depAu/PTCA/GCE, (f) HT/SP-TP/depAu/PTCA/GCE treated with mixture of HP1, HP2 and miRNA-21. CV was performed in PBS (pH = 7.0) containing 5.0 mM K3[Fe(CN)6]/K4[Fe(CN)6] and 0.1 M KCl with the scanning potential from -0.2 to 0.6 V (scan rate of 100 mV/s).

10. Optimization of experiment condition

In order to receive a superior performance of this PEC biosensor, the reaction time of catalyzed hairpin assembly triggered by target miRNA-21 and the concentration of electron donor AA were optimized. The optimization of reaction time was performed ranging from 20 minutes to 100 minutes. As shown in Figure S3 A, the PEC signal increased with the increment of reaction time and trended to level off after 60 minutes in the presence of 10 pM miRNA-21, indicating that 60 minutes was the optimal reaction time. Moreover, in order to optimize the concentration of AA, the PEC signal for various concentrations of AA was recorded in the presence of 10 pM miRNA-21. As illustrated in Figure S3 B, the PEC signal was constantly enhanced with increasing AA concentration from 0.025 M to 0.1 M, while the PEC signal decreased slightly after the AA concentration reaching 0.1 M. Therefore, 0.1 M AA was selected as the optimum concentration.

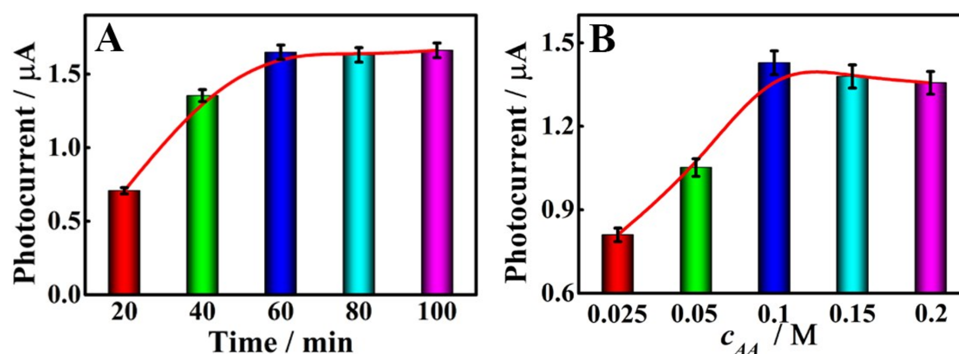

Figure S3. The experiment parameters were optimized by PEC measurement (A) the reaction time of catalyzed hairpin assembly triggered by target miRNA-21 and (B) the concentration of electron donor AA. Error bars: standard deviation (SD), $n = 3$.

Table S2. Comparison for the miRNA-21 Detection with Other Analytical Methods

Analitical method	Detection range	Detection limit	Ref.
chemiluminescence	10 fM-100 pM	3.5 fM	[2]
electrochemical	1.0 fM-1.0 nM	0.34 fM	[3]
fluorescence	1 fM-1 pM	0.35 fM	[4]
electrochemiluminescence	0.1 fM-10 pM	0.03 fM	[5]
photoelctrochemical	1 fM-100 pM	0.31 fM	[6]
photoelctrochemical	10 aM-100 pM	3.3 aM	This work

Notes and references

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