Electronic Supplementary Information

A Novel "Signal On" Photoelectrochemical Strategy Based on Dual Functional Hemin for MicroRNA Assay

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1. Reagents and Materials

Hemin, cadmium chloride ($CdCl_2$), ammonium chloride (NH_4Cl), sodium sulfide (Na_2S), gold chloride ($HAuCl_4$), 1-hexylthiol (HT), gel green, polyamidoamine dendrimer with arboxyl terminated (PAMAM, fifth generation), 3-aminopropyltriethoxysilane (APTMS), ethylsilicate (TEOS), Nhydroxysuccinimide (NHS), N-(3-(dimethylamino)propyl)-N′-ethylcarbodiimidehydrochloride (EDC) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Ethanol (C_2H_5OH) and H_2O_2 were from Kelong Chemical Industry (Chengdu, China). T7 exonuclease (T7 Exo) was supplied by New England Biotechnology Co., Ltd. (Beijing, China). MicroRNA-141 was obtained from Takara Biotechnology Company Ltd. (Dalian, China). $TiSO_4$ and other sequences used in this work were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The sequences were listed in Table S1.

Table S1. Sequences Information for the Nucleic Acids Used in This Work

Name	Sequence (5′- 3′)
S1	CCA TCT TTA CCA GAC AGT GTT AAA
MicroRNA-141	UAA CAC UGU CUG GUA AAG AUG G
MicroRNA-21	UAG CUU AUC AGA CUG AUG UUG A
MicroRNA-182-5p	UUU GGC AAU GGU AGA ACU CAC ACU
MicroRNA-155	UUA AUG CUA AUC GUG AUA GGG GU
H1	TGG TGA CTG TCT GGT AAA TTT TTT TTT TTT TTT TTT-NH_2
H2	H_2N-TTT TTT ACC AGA CAG TCA CCA TCT GGT AAA

2. Apparatus

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were monitored by a CHI 660e electrochemistry workstation (Shanghai Chenhua

Instruments, China). A traditional three electrode electrochemical system with a modified glassy carbon electrode (GCE, Φ = 4 mm) as the working electrode, a platinum wire as the auxiliary electrode and an Ag/AgCl electrode as the reference electrode. PEC signal was measured by a PEC workstation from Ivium (The Netherlands). Gel Doc XR^+ System (Bio-Rad, California, USA) was used to take images of gels. The morphologies of the synthesized materials were characterized using a scanning electron microscopy (SEM, S-4800, Hitachi, Japan) and a high-resolution transmission electron microscopy (HRTEM, JEM-2100, JEOL, Japan).

3. Preparation of CdS QDs

The preparation process of CdS QDs was reported by Xu's group. In detail, 0.1256 g of $CdCl_2 \cdot 2.5H_2O$ was dissolved in 30 mL distilled water and the solution was heated to 70 ℃ with stirring. Then, 30 mL of Na_2S solution (19.69 mg/mL) was slowly added into the prepared $CdCl_2$ solution and the mixture was orange. Next, the mixture was continuously refluxed at 70 ℃ for about 3 h. The product was obtained after centrifugation and washing with ethanol for 2 times and water for 3 times of the precipitate. Lastly, the product was dispersed into distilled water and stored at 4 ℃.

4. Preparation of $MTiO_2$-Hemin

In a typical preparation process of $MTiO_2$, NH_4Cl (0.134 g) and $TiSO_4$ (0.5 g) were dissolved into 40 mL of distilled water in the high pressure reactor. And then the reactor was placed in an oven at 200 ℃ for 12 h. Next, the precipitate was collected and rinsed with ethanol for 3 times and distilled water for 3 times, respectively. Finally, the $MTiO_2$ particles were obtained after drying in an oven at 80 ℃.

The preparation process of $MTiO_2$-hemin was showed in Scheme 1A. 0.5 mg of hemin and 6.1 mg $MTiO_2$ particles were dissolved in 300 μL distilled water at 65 ℃. Then, the mixture solution was ultrasonicated for 30 min and set at room temperature for 24 h. Through such a proposal, Ti-O bonds were formed between $MTiO_2$ and hemin and $MTiO_2$-hemin compound was prepared after centrifugating the product under 8000 rpm for 5 min to remove extra hemin. At last, the prepared compound was washed with distilled water and dried in oven.[1]

5. T7 Exo-Initiated Target Cycle Amplification Process

The T7 Exo-initiated target cycle amplification process[2, 3] was shown in Scheme 1. The reaction system was consisted of H1, S1 and the target model (microRNA-141). Due to T7 Exo just cleaves the 5′ recessed DNA strand of the dsDNA, the duplex of H1/S1 will not be digested because of the 5′ protruding terminuses. However, with the addition of target, the strand displacement reaction (SDR) was triggered: H1 was displaced by microRNA-141 because the complementary sequences of duplex of H1/S1 were shorter than that of duplex of S1/microRNA-141. The duplex of microRNA-141/S1 was formed, which contains 5′ blunt terminus. Subsequently, the duplex of microRNA-141/S1 was cleaved by T7 Exo (from 5′ to 3′ direction), S1 was digested to nucleotides and the target was released, thereby realizing more cycles of SDR. As a result, a lot of H1 were retained on the electrode surface and hybridized with H2-CdS QDs to form H1/H2-CdS QDs hybridization on the electrode.

6. Fabrication of the PEC Biosensor

First of all, after polished by alumina powder (0.3 μm) and rinsed with distilled

water, the cleaned GCE was modified with 10 mL of $MTiO_2$-hemin (3.05 mg/mL) to obtain an initial PEC signal. When a film was formed, the resultant electrode was immersed in $HAuCl_4$ (1%) solution to deposit Au NPs layer under -0.2 V for 30 s. Then, H1/S1 duplex was attached onto the electrode by Au-N bond at 4 ℃ for 12 h. At last, for blocking the nonspecific binding sites, 10 mL of HT was introduced onto the electrode. The obtained electrode was washed with distilled water after each step.

7. PEC Measurement Condition

In the PEC detection process, 6 mL of PBS (0.1 M) buffer containing 50 μL of H_2O_2 (30%) was used as the test base solution. The LED lamp was used as the excitation light source (λ = 365 nm) and the test pattern was set as switched off-on-off for 10-20-10 s.

8. Polyacrylamide Gel Electrophoresis (PAGE)

The strand displacement reaction was investigated by PAGE. The prepared samples were analyzed by nondenaturing polyacrylamide gel, and electrophoresis was conducted at 120 V in 1×TBE buffer for 120 min. After dyeing by gel green, the electrophoresis image was observed under UV light.

9. PEC Characterization of the Proposed Biosensor

The prepared process of the PEC biosensor was characterized by PEC signal. As shown in Figure S1, the GCE showed a near-zero photocurrent signal (curve a). After the modification of hemin-merso-porous TiO_2 (hemin-$MTiO_2$) onto GCE, an increased photocurrent signal was observed due to the excellent photocurrent response of $MTiO_2$ and a quenching efficiency of 87% of hemin to $MTiO_2$. When the target was incubated

on the biosensor, a decreased photocurrent signal was obtained. It was noted that the photocurrent increased by the hybridization of H1 and H2-CdS QDs at the last step. Thus, the photocurrent characterization confirmed the successful construction of the biosensor.

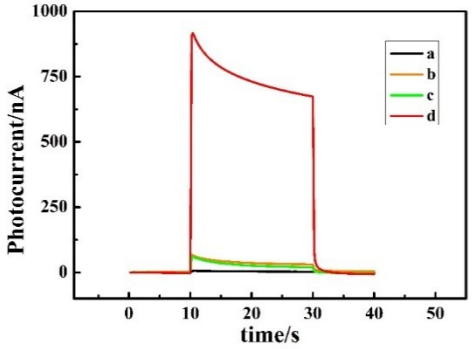

Figure S1. PEC responses of (a) bare GCE; (b) $MTiO_2$-hemin/bare GCE; (c) microRNA-141 /HT/H1/S1/Au NPs/$MTiO_2$-hemin/bare GCE; (d) H2-CdS QDs/T7 Exo/microRNA-141/HT/H1/S1/Au NPs/$MTiO_2$-hemin/bare GCE..

10. Electrochemical Characterization of the Proposed Biosensor

To characterize the preparation process of the biosensor, cyclic voltammetry (CV) measurements were employed step by step in 0.1 M PBS solution with 5 mM $[Fe(CN)_6]^{3-/4-}$ at the potential ranging from -0.2 to 0.6 V with a scan rate of 50 mV/s. As shown in Figure S2A, a pair of definite redox peaks of $[Fe(CN)_6]^{3-/4-}$ could be seen for the bare GCE (curve a). When $MTiO_2$-hemin was modified on GCE, the redox peak currents reduced significantly caused by the poor conductivity of $MTiO_2$-hemin (curve b). After the deposition of Au NPs, the redox peak currents were increased obviously due to the good conductivity and electron transfer speed of Au NPs (curve c). When duplex of H1/S1 was incubated onto the resultant electrode surface via Au-N bond between H1 and Au NPs, the redox currents decreased clearly attributed to the repulsion effect between $[Fe(CN)_6]^{3-/4-}$ and the negatively charged phosphate backbone of the DNA (curve d). A further decrease of peak current was obtained by immobilizing HT for blocking the nonspecific sites on the surface (curve e). The reason was that HT on

the electrode would retard the electron transfer because of the increased steric hindrance effect. Figure S2B showed the electrochemical impedance spectroscopy (EIS) measurements of the fabrication process of the biosensor. When $MTiO_2$-hemin was modified on GCE, a much larger R_{et} (curve b) than bare GCE (curve a) could be obtained. When Au NPs were deposited on the modified electrode, a decrease of R_{et} was presented in curve c. After the introducing of H1/S1, an increase of R_{et} was obtained due to the negatively charged H1/S1 (curve d). A further increase of R_{et} was founded by adding HT. Here, the electrochemical characterizations confirmed the successful construction of the biosensor.

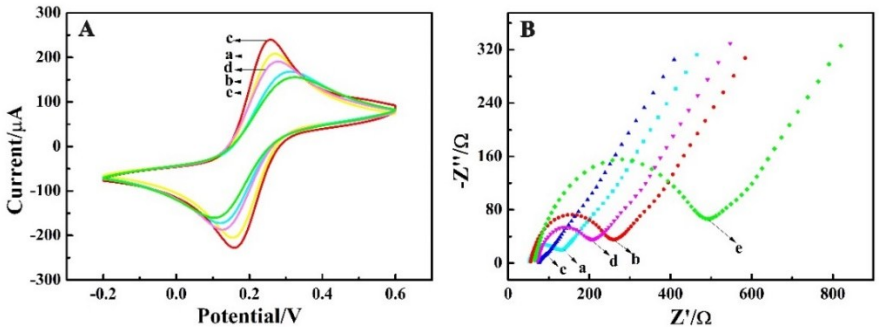

Figure S2. (A) CV profiles and (B) EIS measurement in 0.1 M PBS containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ toward (a) bare GCE; (b) $MTiO_2$/GCE; (c) Au NPs/$MTiO_2$/GCE; (d) H1/S1/Au NPs/$MTiO_2$/GCE; (e) HT/H1/S1/Au NPs/$MTiO_2$/GCE.

11. **Optimization of the Concentration of Substrate**

For the purpose of obtaining stable and continuous PEC signal, the concentration of matrix ($MTiO_2$-hemin) was a key factor for the performance of the proposed biosensor. Thus, the incubation concentration of $MTiO_2$-hemin was optimized in the range of 1.22 mg/mL to 6.10 mg/mL. As shown in Figure S3, the PEC signal enhanced with the increase of incubation concentration of $MTiO_2$ until 3.05 mg/mL, even it exceeded 3.05 mg/mL, a reduced PEC signal was found because of the matrix peeling off the electrode surface. Therefore, 3.05 mg/mL was chosen as the optimal

concentration of matrix.

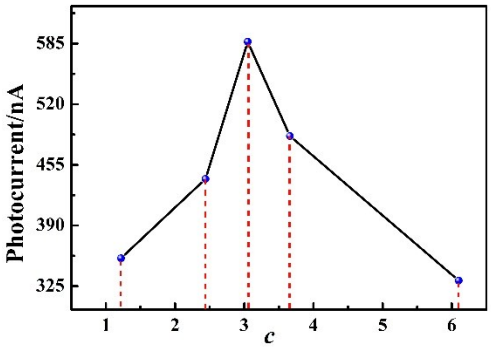

Figure S3. Optimization of the concentration of matrix.

12. PAGE Analysis

The feasibility of T7 Exo-assisted target-recycle amplification technology was confirmed with 16% PAGE. As shown in Figure S4, lane 1, 2, 4 and 7 represented S1, H1, microRNA-141 and H2 respectively, presenting four bands of single strip. Lane 3 was the PAGE result of S1/H1 composite and exhibited a slower mobility compared with lane 1 and 2, which demonstrated the successful hybridization of S1 and H1. Subsequently, lane 5 and 6 were the PAGE results of the target recycle amplification process in the absence or presence of T7 Exo after adding microRNA-141 into H1/S1. Lane 5 showed two distinct bands of S1/microRNA-141 hybridization and H1/S1 hybridization respectively, suggesting that although S1 could hybridize with microRNA-141, a low hybridized efficiency was obtained in the absence of T7 Exo. As expect, lane 6 had two bands, indicating the target recycle amplification reaction occurred. Lastly, lane 8 displayed three distinct bands, representing the hybridization of H1/H2, the resultant microRNA-141 and H2, respectively. All the above results verified the successful construction of the biosensor.

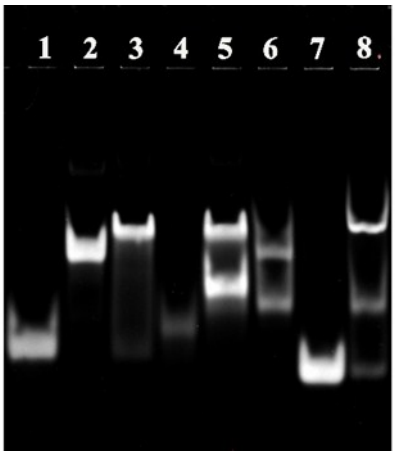

Figure S4. PAGE image of the nucleic acid reaction products: lane 1, single-stranded DNA S1; lane 2, hairpin DNA H1; lane 3, the mixture of S1 and H1 without target; lane 4, single-stranded RNA microRNA-141; lane 5, the mixture of S1, H1 and microRNA-141 in the absence of T7 Exo; lane 6, the mixture of S1, H1 and microRNA-141 in the presence of T7 Exo; lane 7, hairpin DNA H2; lane 8, the mixture of H2 and the product of the T7 Exo-initiated target-recycle amplification reaction.

Table S2 Comparison of the Reported Works with Our Proposal Strategy for Detection of MicroRNA.

analytical method	detection range	limit of detection	references
fluorescent	3.8 pM ~ 10 nM	3.8 pM	4
fluorescent	0.1 nM ~ 8 μM	33.3 pM	5
fluorescent	10 fM ~ 500 fM	3 fM	6
EIS	2.0 fM ~ 2.0 pM	1.0 fM	7
chemiluminescence	10 fM ~ 50 pM	3.02 fM	8
PEC	1 fM ~ 1 pM	0.23 fM	9
PEC	0.25 fM ~ 2.5 nM	139 aM	***Our work***

Abbreviation: Electrochemical Impedance Spectroscopy (EIS).

Reference

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