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

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Here, with the enzyme-free catalytic hairpin assembly (CHA) target recycling generated dsDNA (HP1-HP2) as regulator for synchronously controlling the departure of quencher ferrocene (Fc) and approach of sensitizer methylene blue (MB) toward photoactive perylene-3,4,9,10-tetracarboxylic acid (PTCA), a distance-controllable multiple signal amplification based photoelectrochemical biosensor was proposed for ultrasensitive MiRNA-21 assay.

MiRNA-21, a small noncoding RNA molecule with the short length of 22 nucleotides, have abnormally high expression in various cancer cells lines, such as pancreatic cancer, cervical cancer, breast cancer and lung cancer.[1-3] Accordingly, the accurate and simple detection of miRNA-21 is of great significance for the diagnosis and treatment of diseases. In the respect, various effective analytical techniques have been proposed for its detection, such as electrochemiluminescence (ECL),[4,5] fluorimetry (FL),[6-8] electrochemistry (EC)[9,10] and chemiluminescence (CL)[11] and photoelectrochemistry (PEC)[12,13]. Among them, PEC as a novel and valuable analytical technique with simple equipment, low background and high sensitivity, has promptly become a subject of hot research interest[14-16]. It is reported that the photocurrent signal amplification is one of the important ways to improve sensitivity of PEC assay, and thus ongoing efforts have been devoted to exploit simple and effective amplifying strategies.

DNA recycling amplification strategies, such as enzyme-assisted target recycling amplification[17-20], metal-ion dependent DNAzyme recycling amplification[21,22] and non-enzymatic target recycling amplification[23-25], have showed desiring application prospect in signal enhancement owing to the generation of massive duplicate DNA units induced by even slight plenty of targets. Meanwhile, the sensitizer can facilitate photoelectric conversion efficiency of photoactive material by reducing the recombination of electron-hole pairs and increasing the absorption in visible region,[26-28] thereby also enhancing PEC signals to improve the detection sensitivity of biosensors. In this respect, supposing the DNA recycling products served as distance regulator to adjust the distance between sensitizer and photoactive material, the amplification capability would be significantly and simply enhanced in PEC assay systems, not only inhering the merits of DNA recycling amplification, but also avoiding the usage of extra fussy operation. More importantly, this approach endows the further combination of quencher for decreasing background signal and amplifying the signal intensity depended on distance control for improving the sensitivity in advance. However, to the best of our knowledge, there are few works reported on this.

To make this suppose into reality, we chose the 3,4,9,10-perylene tetracarboxylic acid (PTCA) as the photoelectric material for PEC sensing system. PTCA is a conjugated organic dye containing five-benzene cores, which possesses a narrow band gap energy (2.2 eV), remarkably optical and electrical properties,[29-31] thereby providing an ideal photoelectric material for PEC sensing system. Moreover, the methylene blue (MB) and ferrocene (Fc) could treat as the sensitizer and quencher toward PTCA under the same wavelength, respectively, which provided an opportunity to achieve the distance-controllable multiple signal amplification for further improving detection sensitivity. Thus, we innovatively coupled the enzyme-free catalytic hairpin assembly (CHA) target recycling[32-37] with the distance-controllable multiple signal amplification obtained by dsDNA (HP1-HP2) synchronously triggering the departure of quencher Fc and the approach of sensitizer MB toward PTCA for effectively improving detection sensitivity, which was illustrated in Scheme 1. Firstly, the photoactive material PTCA that directly dripped on the electrode surface provided a high PEC signal with the assistance of dep Au. Then, the hybrid complexes of SP labeled with MB and TP labeled with Fc were immobilized on the

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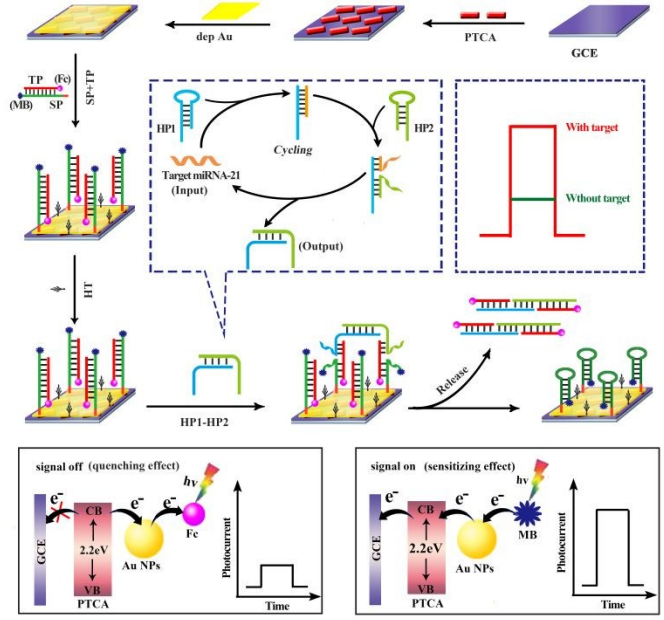
surface of modified electrode via Au-S bond for effectively reducing the background signal due to the proximate distance of quencher Fc to PTCA. Subsequently, the addition of output products (HP1-HP2) generated by CHA target recycling could displace TP to make Fc far away from PTCA as well as SP synchronously formed into hairpin structure to make MB approximate to PTCA, thereby achieving the multiple signal amplification to effectively improve detection sensitivity. Such proposed PEC biosensor not only reduced the background signal but also synchronously triggered the departure of quencher Fc and the approach of sensitizer MB to PTCA for achieving the ultrasensitive detection of miRNA-21. Importantly, this developed PEC biosensor held great potential to detect biomolecules in bioanalysis and disease diagnosis.



Scheme 1 Schematic of target-catalyzed hairpin assembly coupling with distance-controllable multiple signal amplification for the miRNA-21 detection.

The size and morphology of synthesized nanomaterials were characterized by SEM. As shown in Figure 2A, the SEM image of PTCA displayed the short and rod-like structure derived from π-π stacking, which was coincidence with previous reported litertures.[38] From the picture of Figure 2B, after the depAu were successfully deposited on the surface of PTCA, a typical Au nanoflowers morphology were appeared and exhibited a bright pattern due to the excellent conductivity of Au NPs. Moreover, as shown in Figure 2C, the PTCA exhibited two apparent absorption peaks at 456 nm and 553 nm compared with that of PTCDA. Both the SEM and UV-vis absorption spectroscopy results were demonstrated the PTCA was synthesized successfully.

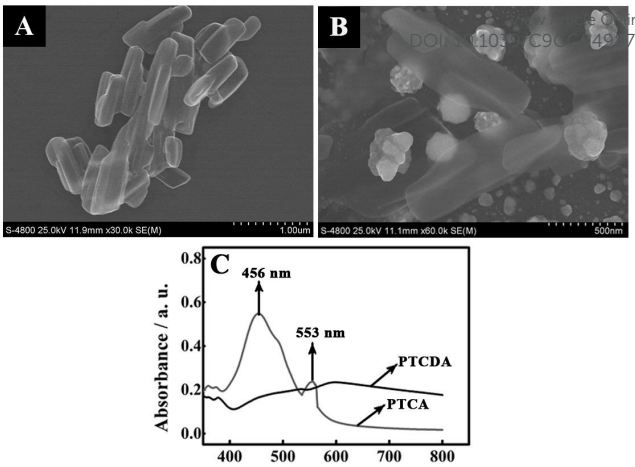


Figure 1 (A) SEM images of PTCA and (B) dep Au/PTCA (C) UV–vis absorption spectrum of PTCDA and PTCA.

The construction process of this PEC biosensor was characterized by PEC measurement. As depicted in Figure 2A, compared with the photocurrent of bare electrode (curve a), the PTCA modified electrode showed an obvious photocurrent (curve b) on account of the superior photoactivity of PTCA. After being modified with dep Au, an enhanced photocurrent was acquired (curve c) because the energetic electrons from the surface plasmons of the dep Au were injected into the LUMO orbit of the PTCA.[39] However, the photocurrent decreased significantly after incubating with dsDNA (SP-TP) (curve d), which could be interpreted that the quencher Fc was proximate to PTCA while the sensitizer MB was far away from PTCA. Sequentially, the modified electrode was further incubated with hexanethiol (HT), the photocurrent continually decreased (curve e). However, the photocurrent increased dramatically after the modified electrode incubating with the mixture of miRNA-21, HP1 and HP2 (curve f), which was ascribed to that the Fc proximate to electrode was removed from electrode via the hybridization between dsDNA (HP1-HP2) and TP while the SP concurrently turned into hairpin structure for making MB approach to PTCA.

Furthermore, EIS characterization was also used to describe the construction process of PEC biosensor. As illustrated in Figure 2B, the bare electrode showed a small R_{et} value (curve a). When the bare electrode modified with PTCA, the R_{et} value significantly increased (curve b). Nevertheless, after modifying with Au NPs (curve c), the electrode showed a small R_{et} value due to the fast electron transfer. When dsDNA (SP-TP) were decorated on the modified electrode (curve d), the R_{et} value was enhanced on account of the increased negative charges brought by DNA. After being immobilized with insert HT, the R_{et} value of the electrode was further enhanced (curve e). When the mixture of HP1, HP2 and miRNA-21 was dropped onto the surface of electrode (curve f), the R_{et} value increased due to the release of Fc labeled TP. The characterization results of EIS were consistent with PEC characterization, which indicated the feasibility of stepwise assembly of electrodes.

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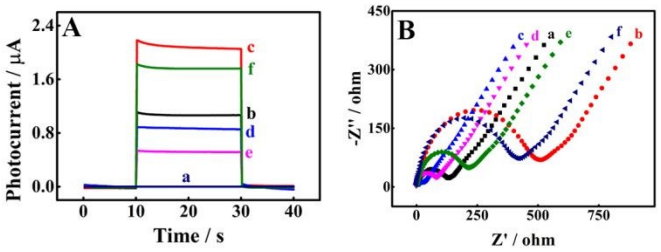

Figure 2 (A) PEC and (B) EIS profiles of (a) bare GCE, (b) PTCA/GCE, (c) depAu/PTCA/GCE, (d) SP-TP/depAu/PTCA/GCE, (e) HT/SP-TP/depAu/PTCA/GCE, (f) HT/SP-TP/depAu/PTCA/GCE treated with mixture of HP1, HP2 and miRNA-21. PEC was accomplished in 4 mL PBS (pH = 7.0) containing 0.1M AA at 0.0 V potential. EIS was performed in PBS (pH = 7.0) containing 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and 0.1 M KCl in the frequency range between 0.1 Hz and 100 kHz at the amplitude of 5 mV.

The analytical performance of the proposed PEC biosensor for detecting various concentrations of miRNA-21 was investigated. As shown in Figure 3A, the PEC responses continuously enhanced with the incremental concentrations of miRNA-21 in the range from 10 aM to 100 pM. As displayed in Figure 3B, the calibration curve between photocurrent and the logarithm (lg) miRNA-21 concentrations exhibited a good linear relationship. The corresponding liner regression equation was I = 0.1792 lg$c_{miRNA\text{-}21}$ + 1.080 (R^2 = 0.9974) with the detection limit of 3.3 aM (S/N = 3). Besides, the comparison between the previously reported strategies and the proposed PEC strategy for miRNA-21 detection was shown in Table S2. These results showed that this PEC biosensor constructed by coupling CHA target recycling with the departure of quencher Fc and the approach of sensitizer MB toward PTCA signal amplification strategy possessed a superior performance for miRNA-21 detection.

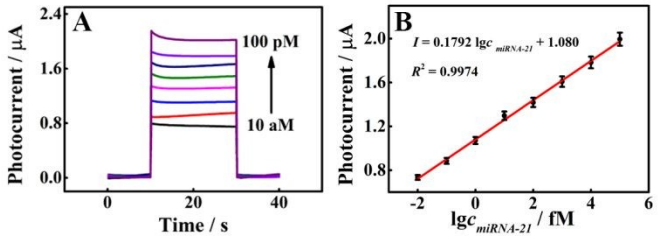

Figure 3 (A) PEC response of proposed biosensor to different concentrations of miRNA-21 in 0.1 M PBS containing 0.1M AA. The concentration of miRNA-21 were 10 aM, 100 aM, 1.0 fM, 10 fM, 100 fM, 1.0 pM, 10 pM and 100 pM. (B) The calibration curve for the different concentrations of miRNA-21 detection. Error bars: standard deviation (SD), n = 3.

In order to assess the selectivity of this PEC biosensor, the miRNA-122 (100 pM), miRNA-155 (100 pM), miRNA-141 (100 pM) and miRNA-126 (100 pM) were used as interferences for comparing with 10 pM miRNA-21. As shown in Figure 4A, the PEC responses of interferences were relatively small in comparison with the PEC response of 10 pM miRNA-21, which demonstrated a satisfactory selectivity of this proposed PEC biosensor for miRNA-21 detection. Moreover, the stability of this PEC biosensor for 10 pM miRNA-21 was also investigated under continuous off-on-off light for 8 cycles. As shown in Figure 4B, the relative standard deviation (RSD) of this PEC photocurrent was 1.64%, which exhibited a desirable stability of this PEC biosensor. Furthermore, we also assessed the reproducibility of this PEC biosensor by comparing the PEC signal of the intra-assay and inter-assay to 10 pM miRNA-21 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 10 pM miRNA-21. As depicted in Figure 4C, we could see that the PEC signal of intra-assay variation was relatively small and the calculated RSD was 1.02%. Meanwhile, Figure 4D also showed small PEC signal of inter-assay variation and the calculated RSD was 1.59%. Those obtained results demonstrated that the proposed PEC strategy reflected an admirable intra-assay and inter-assay.

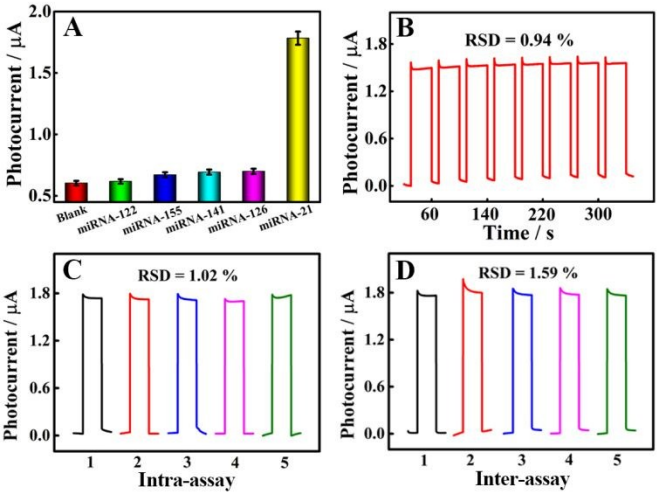

Figure 4 (A) Selectivity assessment of this biosensor toward 100 pM of miRNA-122, miRNA-155, miRNA-141 and miRNA-126, respectively. Error bars: standard deviation (SD), n = 3. (B) Stability of this biosensor in 10 pM miRNA-21with continuous off-on- off light for 8 cycles. (C) The PEC signal of intra-assay incubating with 10 pM miRNA-21. (D) The PEC signal of inter-assay incubating with 10 pM miRNA-21.

In summary, we successfully proposed an ultrasensitive PEC biosensor for miRNA-21 detection by coupling CHA target recycling with the signal amplification strategy of simulatously the departing of quencher Fc and the approaching of sensitizer MB toward PTCA. First, the quencher Fc that proximate to PTCA could effectively reduce background signal for improving sensitivity. Second, the distance-controllable synergetic signal amplification strategy of quencher departure and sensitizer approach toward the same photoactive material significantly enhanced the PEC signal for improving the detection sensitivity. Third, the utilization of CHA was enzyme-free to avoid the drawbacks of expensive reagents, complex modification processes, vulnerable to contamination, susceptible to external environment influence as well as achieved the recycle of target, thereby realizing the signal amplification to further improve the detection sensitivity. Moreover, this proposed PEC biosensor provided a versatile method to sensitively analysis diverse targets, which possessed great significance in bioanalysis and disease diagnosis.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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