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**A novel host-guest recognition-assisted electrochemical
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ABSTRACT

In this work, an elegantly designed host-guest recognition-assisted electrochemical release was established and applied in a reusable electrochemical biosensor for the detection of microRNA-182-5p (miRNA-182-5p), a prostate cancer biomarker in prostate cancer, based on the DNA cross configuration-fueled target cycling and strand displacement reaction (SDR) amplification. With such design, the single target miRNA input could be converted to large numbers of single-stranded DNA (S1-Trp and S2-Trp) output which could be trapped by cucurbit[8]uril-methyl viologen (CB-8-MV²⁺) based on the host-guest recognition, significantly enhancing the sensitivity for miRNA detection. Moreover, the nucleic acids products obtained from the process of cycling amplification could be utilized sufficiently, avoiding the waste and saving the experiment cost. Impressively, by resetting a settled voltage, the proposed biosensor could release S1-Trp and S2-Trp from the electrode surface, attributing that the guest ion-methyl viologen (MV²⁺) was reduced to MV^{•+} under this settled voltage and formed more stable CB-8-MV^{•+}-MV^{•+} complex. Once O₂ was introduced in this system, MV^{•+} could be oxidized to MV²⁺, generating the complex of CB-8-MV²⁺ for capturing S1-Trp and S2-Trp again in only 5 min. As a result, the simple and fast regeneration of biosensor for target detection was realized on the base of electrochemical redox-driven assembly and release, overcoming the challenges of time-consuming, burdensome operations and expensive experimental cost in traditional reusable biosensors, and updating the construction method for reusable biosensor. Furthermore, the biosensor could be reused for more than ten times with the

regeneration rate of 93.20 % to 102.24 %. After all, the conception of this work provides a novel thought for the construction of effective reusable biosensor to detect miRNA and other biomarkers and has great potential application in the area requiring the release of nucleic acids or proteins.

Keywords: microRNA, electrochemical biosensor, cucurbit[8]uril, host-guest recognition, reusable detection

INTRODUCTION

In recent years, cancers have developed the major cause of mortality in the whole world, thus exploring new method for the prevention and treatment of cancers has become extremely urgent.¹ It is well known that the specific expression of DNA or microRNA (miRNA) is interrelated with the occurrence, progression and tumorigenesis of cancer.² Therefore, ultrasensitive and selective detection of various nucleic acids related cancer has an important significance in biological studies, forensic evidence and early screening of cancers.³⁻⁶ Unfortunately, the DNA or miRNA sequences which are worth to be researched may be presented in very small amounts, thus it is essential to exploit various amplification strategies that could determine trace levels of specific sequences.^{7,8} Recently, the target cycling amplification strategy combined with diverse DNA structures, for example, Hairpin DNA,⁹ Y junction DNA,^{10,11} DNA tweezer,¹² and so on, is especially intriguing in biosensor for improving the sensitivity for trace level of target detection.¹³⁻¹⁶ Although the sensitivity has been developed to some extent, large numbers of DNA strands generated from these cycling procedures are wasted,¹⁷⁻¹⁹ leading to low-effective and

high-cost for the detection of target. Therefore, it should be noted that designing rational DNA structure to achieve target cycling amplification as well as avoid the waste for enhancing analytical performances is still in high demand to satisfy the development demands of biological research and clinic diagnostics.

To the best of our knowledge, regenerated strategies which possess the advantages of being re-utilized for many times, saving material resources and experiment cost are widely applied in the areas of biomaterial, chemical absorption, medicine, etc.^{20,21} Therefore, the regeneration researches in electrochemical biosensors for miRNA detection have attracted increasing attentions in recent years.²² However, the regeneration in traditional biosensors is commonly time-consuming and needs to introduce additions, for example, functionalized nanomaterials or another new DNA,^{23,24} resulting in the construction of reusable electrochemical sensors being burdensome operations and expensive experimental cost. Therefore, how to inaugurate a way to overcome these difficulties for excavating simple and fast regeneration method is still a challenge. As it is well-known that electrochemical regulating and controlling could adhere or release small molecules on electrode surfaces to alter sensing platform, which could change the electrochemical behaviors and achieve the regeneration of electrode surface properties.²⁵⁻²⁸ Moreover, it possesses the advantages of simple, high sensitive and selective, fast response, inexpensive instrumentation as well as low fabrication cost.^{29,30} Therefore, electrochemistry regulating and controlling of regeneration sensing platform would be not only an extremely attractive method for the construction of reusable biosensor, but

also open up a new-style strategy for miRNA detection. However, this kind of reusable biosensor for miRNA or even other biomarkers detection has not yet appeared in previous articles.

Cucurbit[8]uril (CB-8) is an excellent host for the formation of charge-transfer complexes by combining an aromatic electron-deficient molecule (π acceptor) with a partner containing an aromatic electron-rich residue (π donor).³¹⁻³³ It has been demonstrated that CB-8-methyl viologen (CB-8-MV²⁺) could not only bind tryptophane (Trp), glutamate, dopamine and peptide to form 1:1:1 heteroternary complexes spontaneously, but also realize controlled-release of these hosts from guests with electrochemistry strategies under a settled voltage.³⁴⁻³⁷ The crucial point for these phenomena were on account of redox-driven assembly or release, which were simple and fast process. Inspired by these demonstrations, we believe that this kind of stimuli-responsive composite materials based on CB-8-MV²⁺ could be easily modulated on the electrode surface and benefit to construct reusable biosensor for the detection of miRNA. Consequently, in this work, we firstly introduced Trp to label DNA strands which participate in target cycling and strand displacement reaction (SDR) amplification, and the cycling productions could be trapped on the CB-8-MV²⁺ modified electrode surface based on host-guest recognition. Then the electrochemical redox-driven assembly or release could be conducted under a settled voltage, finally the reusable electrochemical biosensor for miRNA detection was successfully constructed.

This article aimed at designing reusable electrochemical protocol for the

detection of miRNA-182-5p reported as a new molecular biomarker of prostate cancer^{38,39} in highly controllable and accurate based on the DNA cross configuration-fueled target cycling and SDR amplification, and host-guest recognition-assisted electrochemical release. Firstly, a DNA cross configuration was designed, which contained two hairpin DNA (H1, H2) labeled with Trp (H1-Trp, H2-Trp) at 3'-end respectively. When the target was introduced, it could be complementary with the part of H1-Trp to form target-H1-Trp, meanwhile the part of H2-Trp was exposed. Once the primer2 DNA was adopted in this system, it could be completely complementary with H2-Trp for the formation of double-stranded DNA2 (dsDNA2) with the help of Klenow Fragment and dNTPs. Finally the obtained dsDNA2 was cleaved by the Nt.BbvCI, and S2-Trp was released with the polymerization action of Klenow Fragment. Simultaneously, the exposed section in the formed target-H1-Trp could be complementary with the primer1 DNA forming dsDNA1 cleaved by Nt.BbvCI, and then S1-Trp as well as target miRNA were released. The released target miRNA could take part in the next cycling with the same way, and the released single strand DNA (ssDNA) including S1-Trp and S2-Trp could be immobilized on the electrode modified with CB-8-MV²⁺/PAMAM-CNTs-Pt/Nano-Au based on host-guest recognition. Subsequently, DNA1 (A1) and DNA2 (A2) could complement with S1-Trp and S2-Trp respectively and was hunted on the functionalized electrode to trigger the hybridization chain reaction (HCR) for capturing larger amounts of electroactives to produce strong electrochemical signal, realizing the sensitive detection of miRNA. More importantly, S1-Trp and S2-Trp

[illegible]

EXPERIMENTAL SECTION

The analytical apparatus, measurements and oligonucleotide sequences applied in this study, AFM characterization of electrode modification, polyacrylamide gel electrophoresis analysis and application were shown in the Supporting Information.

Preparation of PAMAM-CNTs-Pt Nanomaterials. MWCNTs modified with carboxyl groups (-COOH) was performed by the following procedure.⁴⁰ 10 mg MWCNTs was put into 10 mL a mixture ($\text{H}_2\text{SO}_4\text{:HNO}_3$) in a volume ratio 3:1 and vigorously sonicated for 2 h. After that, the obtained MWCNTs-COOH was centrifuged and washed several times with ultrapure water until the solution reached neutral in order to eliminate acid residues. Afterward, 1 mg functionalized MWCNTs and 100 μL 0.01 M amination PAMAM were added in 2 mL ultrapure water and sonicated to obtain uniform black solution (PAMAM-CNTs). Subsequently, 400 μL H_4PtCl_4 (1 %) was mixed into the obtained solution and then 300 μL new prepared NaBH_4 (0.06 mM) was added into the above solution dropwise with violently stirring. Afterwards, the mixture was centrifuged and washed with ultrapure water for three times to obtain PAMAM-CNTs-Pt nanomaterials which was stored in 4 $^\circ\text{C}$ refrigerator for further use.

DNA Labeled with Tryptophan. 10 μL H1 was added into the mixture of 50 μL EDC (4 mM) and 50 μL NHS (10 mM), and then 100 μL tryptophan (Trp, 100 μM) solution was also added into the mixture reacted with vibration for 2 h for obtaining H1 labeled with tryptophan (H1-Trp). Following this, 210 μL ethyl alcohol was mixed with the as-prepared mixture in the CR-3 tube for 3-5 min in order to adsorb redundant or unreacted molecule and ion. Then, the solution was centrifuged and

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2
3
4 washed under 12000 rpm for 2 min to obtain the H1 labeled with Trp (H1-Trp).
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6 Subsequently, 210 μ L buffer RN was respectively added into the obtained H1-Trp and
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8 stood for 3 min, and then was centrifuged and washed for three times under 12000
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10 rpm. Finally, 100 μ L DEPC was used as solvent for H1-Trp and stored at 4°C for
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12 further usage. H2-Trp was obtained with the same method.
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16 **Target Cycling and SDR Amplification.** First of all, H1-Trp and H2-Trp dealt with
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18 annealing together and then reacted 2 h for the formation of DNA cross configuration.
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20 In the presence of target miRNA, H1-Trp was complementary with it forming
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22 target-H1-Trp, thus the part of H2-Trp was exposed and complementary with the
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24 primer2. With the help of dNTPs and Klenow Fragment, the primer2 could be
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26 extended along with H2-Trp until totally complementary with it forming dsDNA2,
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28 and then the resulted dsDNA2 was cleaved by Nt.BbvCI cleavage enzyme, thus the
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30 cleaved DNA sequence could take place polymerization action from 5' to 3' in the
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32 presence of dNTPs and Klenow Fragment, which resulted in the release of S2-Trp. At
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34 this time, the part of target-H1-Trp separated from the DNA cross configuration could
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36 be also complementary with the primer1, and then in the presence of dNTPs and
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38 Klenow Fragment, the primer1 could be completely complementary with H1-Trp for
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40 the formation of dsDNA1, thus the target miRNA was released and took part in the
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42 next cycling. Meanwhile, Nt.BbvCI cleavage enzyme could cleave the formed H1-Trp
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44 and the cleaved DNA could also polymerize from 5' to 3' depended on dNTPs and
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46 Klenow Fragment, so the S1-Trp was also released. With such cycling, the input
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48 single target miRNA could be converted to large numbers of output ssDNA
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immobilized on the modified electrode surface, significantly enhancing the sensitivity for miRNA detection.

The Fabrication of the Modified Electrodes. The proposed reusable biosensor for miRNA detection was performed as follows: First, 5 μL PAMAM-CNTs-Pt nanomaterials was dropped onto the electrode surface and dried under room temperature. Next, 20 μL of CB-8-MV²⁺ was introduced onto the prepared functionalized electrode for overnight, and then another 20 μL cycling amplification products (S1-Trp and S2-Trp) were added onto the surface of the modified electrode for 100 min. Afterward, 20 μL the mixture of A1, A2 and $[\text{Ru}(\text{NH}_3)_6]^{3+}$ was dropped onto the as-prepared electrode for 120 min. After being washed with ultrapure water, the finished electrode was prepared for electrochemical measurements.

The Release and Regeneration of the Prepared Electrochemical Biosensors. After measured with square-wave voltammetry (SWV), the release of the prepared electrode was under a settled voltage applied at -0.7 V for 5 min to ensure the complete single-electron reduction of MV²⁺ in 0.1 M PBS (pH=7) solution for obtaining more stable complex CB-8-MV⁺-MV⁺. Afterwards, the electrode was measured with CV. Subsequently, the electrode dealt with electrochemical release was immersed into the beaker containing CB-8 and MV²⁺ under O₂ flow for 5 min to oxidize MV⁺ to MV²⁺ for the formation of CB-8-MV²⁺ again. Next, the as-prepared electrode was modified with 20 μL the mixture of S1-Trp and S2-Trp for 100 min. After that, A1, A2 and $[\text{Ru}(\text{NH}_3)_6]^{3+}$ were dropped onto the handled electrode for 120 min and then washed with ultrapure water. Finally, the finished electrode was

measured by SWV in 2 mL of 0.1 M PBS, scanning from -0.5 to 0 V with a frequency of 25 Hz.

RESULTS AND DISCUSSION

Characterization of PAMAM-CNTs-Pt Nanomaterials. As shown in the Figure 1A and 1B, the well-dispersed tubular structure represented the typical SEM images for the PAMAM-CNTs. And the bright spots and clusters appeared in both of the two images demonstrating that the Pt nanoparticles were displayed on the surface of PAMAM-CNTs *via* Pt-N bonding. These results proved the successful preparation of PAMAM-CNTs-Pt nanomaterials.

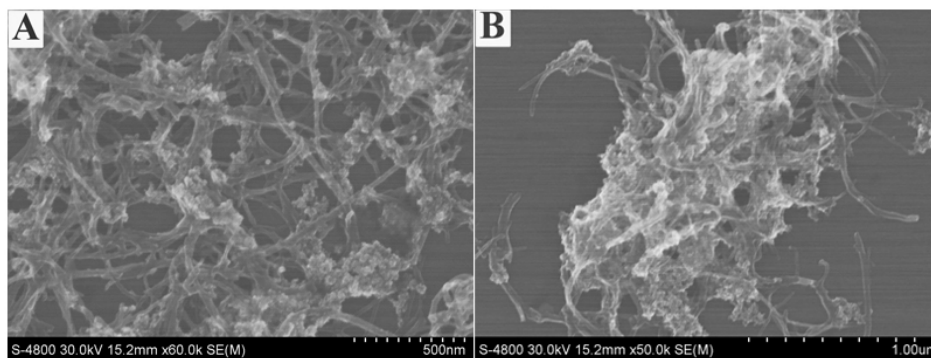


Figure 1 The SEM characterization of PAMAM-CNTs-Pt nanomaterials with 500 nm (A) and 1 μm (B).

The Optimization of the Experimental Conditions. The incubation time of host-guest recognition between CB-8-MV²⁺ and S1-Trp, S2-Trp would influence the performance of the electrochemical biosensor. Figure 2A showed the effect of the incubation time of CB-8-MV²⁺ and S1-Trp, S2-Trp on the electrode response for 10 pM miRNA-182-5p. The current signal increased gradually with the augment of incubation time and then reached to a plateau after 100 min, suggesting that 100 min

was enough for host-guest recognition. Thus, 100 min was employed as the incubation time of host-guest recognition between CB-8-MV²⁺ and S1-Trp, S2-Trp. Further, the incubation time of A1 and A2 would effect the HCR triggering, which may influence the electrochemical signal. Thus, the incubation time of A1 and A2 was investigated and the results obtained from Figure 2B showed that with the increase of incubation time, the current increased and then without change at 120 min. Therefore, 120 min was selected as the best incubation time of A1 and A2.

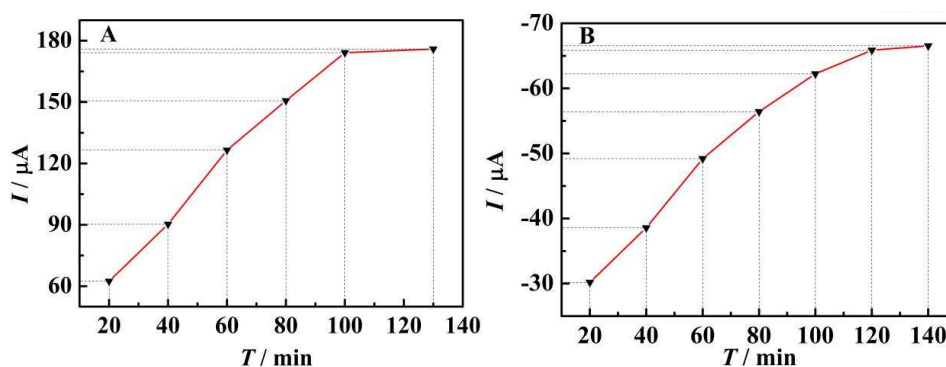


Figure 2 (A) Optimum time of host-guest recognition between CB-8-MV²⁺ and S1-Trp, S2-Trp; (B) Optimum incubation time of A1 and A2 for triggering HCR. The electrochemical signals were obtained with 10.0 pM miRNA-182-5p in 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 0.1 M PBS respectively.

Characterization of the miRNA Biosensor. The fabrication process of the miRNA biosensor was characterized by CV measurement in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution with a scan rate of 100 mV/s. As is shown in Figure 3, the well-defined redox peaks of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ were obtained from a bare GCE (curve a). When PAMAM-CNTs-Pt was coated on the electrode, the current of the modified electrode increased obviously (curve b), proving that PAMAM-CNTs-Pt could accelerate the electron transfer

efficiently. The introduction of nano-Au resulted that the electrochemical signal was amplified because of the excellent conductivity of nano-Au (curve c). Once the CB-8-MV²⁺ was captured onto the modified electrode surface, the current decreased (curve d) owing to its hindrance to electron transfer. After S1-Trp and S2-Trp were assembled with CB-8-MV²⁺ by host-guest action, the peak current further decreased as no surprise (curve e), due to the diffusion block of DNA. When the modified electrode was measured with SWV, no obvious signal appeared (Figure 3B, curve a), due to lack of electroactive substances. Once A1, A2 and [Ru(NH₃)₆]³⁺ were introduced onto the prepared electrode, the obvious electrochemical signal was presented (Figure 3B, curve b), contributing that S1-Trp and S2-Trp could be complementary with A1 and A2 respectively, further trigger HCR for the immobilization of larger amounts [Ru(NH₃)₆]³⁺.

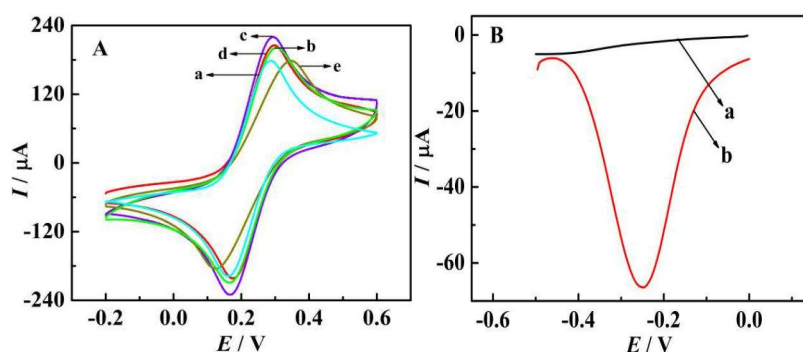


Figure 3 (A) Electrochemical characterization of different modified electrodes: (a) bare GCE; (b) PAMAM-CNTs-Pt/GCE; (c) nano-Au/PAMAM-CNTs-Pt/GCE; (d) CB-8-MV²⁺/nano-Au/PAMAM-CNTs-Pt/GCE; (e) S1-Trp + S2-Trp/CB-8-MV²⁺/nano-Au/PAMAM-CNTs-Pt/GCE after the biosensor was measured in 5 mM [Fe(CN)₆]^{3-/4-} solution. (B) SWVs of the modified electrodes: (a) S1-Trp + S2-Trp/CB-8-MV²⁺/nano-Au/PAMAM-CNTs-Pt/GCE; (b) [Ru(NH₃)₆]³⁺ + A1 + A2/

S1-Trp + S2-Trp/CB-8-MV²⁺/nano-Au/PAMAM-CNTs-Pt/GCE.

The Electrochemical Release and Regeneration of the Electrochemical Biosensor.

After the modified electrode was performed by a constant potential (-0.7 V) over 5 min to ensure the complete single-electron reduction of MV²⁺ in the solution of 0.1 M PBS (pH 7), the handled electrode was measured with CV. As shown in the Figure 4A, once the modified electrode undergo electrochemistry release (b), the electrochemical signal was higher than that of the electrode without electrochemistry release (a), modified with CB-8-MV²⁺/nano-Au/PAMAM-CNTs-Pt/GCE (d) and S1-Trp + S2-Trp/CB-8-MV²⁺/nano-Au/PAMAM-CNTs-Pt/GCE (e), owing that the complete single-electron reduction of MV²⁺ occurred and more stable complex CB-8-MV^{•+}-MV^{•+} was obtained, then a part of MV²⁺, S1-Trp, S2-Trp as well as a mount of A1 and A2 were away from the electrode surface. At the same time, these results also indicated the electrochemistry release was realized successfully. Once the electrode dealt with electrochemical release was immersed into the beaker that contained CB-8 and MV²⁺ under O₂ flow for 5min, the electrochemical signal increased, contributing that MV^{•+} was oxidized to MV²⁺ that was captured on the electrode surface by CB-8 based on host-guest recognition again. Not only that, the obtained electrochemical signal was equivalent to that of the electrode modified with CB-8-MV²⁺/nano-Au/PAMAM-CNTs-Pt, and the efficiency of electrode regeneration was 102.0%. Meanwhile, the regeneration of the miRNA biosensor was monitored by SWV. As shown in the Figure 4B, when the as-prepared electrode was measured with SWV, the obvious electrochemical signal was presented (a). After the

electrochemistry release took place, no signal of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ appeared (b), while the regeneration of the biosensor was carried out, the electrochemical signal of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ reappeared (c), demonstrating the successful regeneration of the constructed biosensor for the detection of miRNA. In addition, the regeneration of the proposed biosensor in ten times was investigated, and the regeneration rate was at the range of 92.99 % to 102.32 %, which further supported the well regeneration of the biosensor and proved the biosensor could be reused in many times.

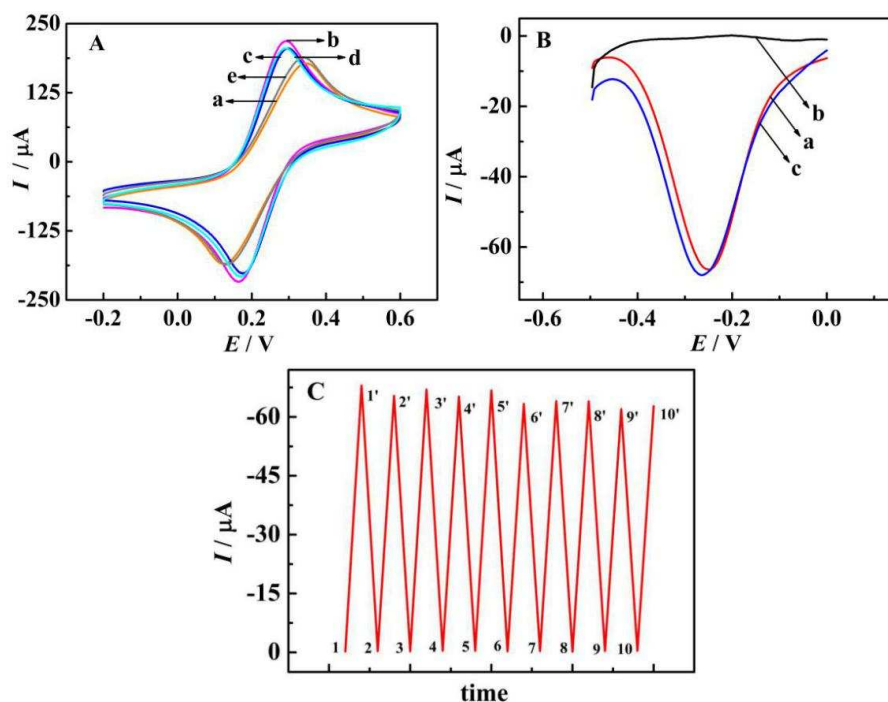


Figure 4 (A) CVs of (a) A1 + A2/S1-Trp + S2-Trp/CB-8-MV²⁺/nano-Au/PAMAM-CNTs-Pt/GCE; (b) the modified electrode was endured with electrochemistry release; (c) the released electrode was immersed into the beaker containing CB-8 and MV²⁺ under O₂ flow for 5 min; (d) CB-8-MV²⁺/nano-Au/PAMAM-CNTs-Pt/GCE; (e) S1-Trp + S2-Trp/CB-8-MV²⁺/nano-Au/PAMAM-CNTs-Pt/GCE; (B) SWVs of (a) before and (b) after the modified electrode undergo electrochemistry release; (c) the

regeneration of the modified electrode in one time. (C) The regeneration of the proposed biosensor for miRNA detection in ten times under the same condition.

Analytical Property of the Reusable Biosensor for miRNA Detection. To investigate the potential quantitative application and the sensitivity of the proposed platform, various concentrations of miRNA were detected by the prepared biosensor under the optimal conditions. Figure 5A depicted the strong correlation between the electrochemical intensity and the logarithm (lg) of miRNA concentration. The calibration plot was illustrated in Figure 5B, the electrochemical response was proportional to the logarithm (lg) of miRNA concentration from 0.001 pM to 500 pM with a regression equation expressed as $I = -57.433 - 10.813 \lg c_{\text{target}}$, and the correlation coefficient was 0.992, where c_{target} represents the different concentrations of target miRNA-182-5p, and the physical quantity is M (mol/L). I corresponds to the current value of biosensors with different concentrations of target miRNA-182-5p, and the physical quantity is μA . The limit of detection (LOD) for target detection can be estimated as 0.5 fM, according to the formula of $\text{LOD} = 3S_b/m$, where m is the slope of the corresponding calibration curve and S_b is the standard deviation of the blank signals.⁴¹⁻⁴³ The analytical performance and regeneration of the proposal method were compared with other reported methods for miRNA detection (Table 1). As shown, the proposed biosensor exhibited excellent analytical performance, which should be attributed to the DNA cross configuration inducing target cycling strategy for signal amplification and host-guest recognition-assisted HCR. More importantly, the proposed biosensor could release and regenerate with simple operation, which

could provide a new avenue for the construction of reusable biosensor for miRNA detection.

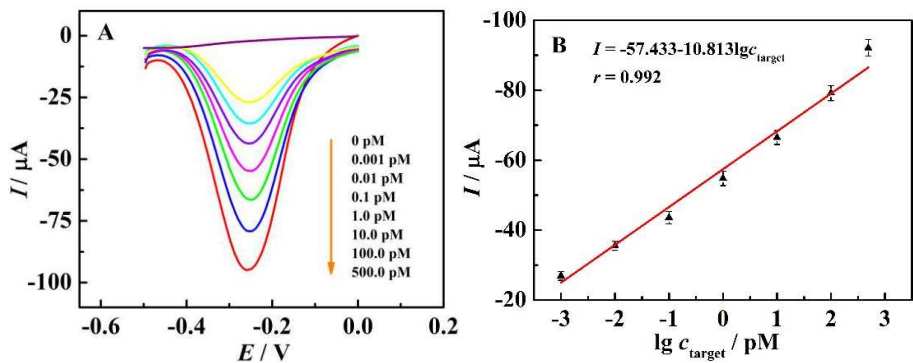


Figure 5 (A) Electrochemical intensity of the biosensors incubated with different concentrations of miRNA-182-5p: 0 pM, 0.001 pM, 0.01 pM, 0.1 pM, 1.0 pM, 10.0 pM, 100.0 pM and 500.0 pM in 2 mL PBS (pH 7.0). (B) Calibration plot of signal intensity vs the logarithm (lg) of miRNA concentration.

Table 1 Comparison of the analytical performance and regeneration of the proposed method with other reports for miRNA detection

Analytical method	target	Linear range	Detection limit	regeneration	Ref.
Square-wave voltammetry	miRNA-21	1.0 pM~25.0 nM	0.6 pM	No	44
Chronocoulometry	miRNA-21	2.0 fM~1.0 nM	30.0 fM	No	45
Square-wave voltammetry	miRNA-155	0.01 nM~1.0 μM	5.2 pM	No	46
Differential Pulse Voltammetry	miRNA-let-7	1.0 pM~100.0 nM	1.2 fM	No	47
Differential Pulse Voltammetry	miRNA-21	0.5 fM~5.0 pM	1.92 fM	No	48
Square-wave voltammetry	miR-29b-1	1.0 fM~1.0 nM	5.0 fM	No	49
Square-wave voltammetry	miRNA-182-5p	0.001 pM~500.0 pM	0.5 fM	Yes	The work

Comparison of Different Probes. In this work, the HCR was employed as effective carriers for binding electroactive substances $[\text{Ru}(\text{NH}_3)_6]^{3+}$. For comparing and demonstrating the advantages of the biosensor with HCR, the electrochemical signal intensity of the miRNA biosensor with or without HCR was investigated in the same working buffer. The same batch of biosensors were constructed for the detection of 10 pM miRNA, and then incubated with A1 + $[\text{Ru}(\text{NH}_3)_6]^{3+}$ and A1 + A2 + $[\text{Ru}(\text{NH}_3)_6]^{3+}$ respectively. The changed electrochemical signal values (ΔI) of the biosensors with or without HCR were displayed in the Figure 6. Compared with the blank experiment (blank cave), ΔI_1 of the biosensor without HCR was 13.97 μA (red cave). Once HCR was triggered, a remarkable increased electrochemical signal intensity of the biosensor was observed and ΔI_2 was 64.49 μA (blue cave), indicating that the immobilized amount of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ was obviously improved by HCR and the sensitivity of the biosensor was also improved.

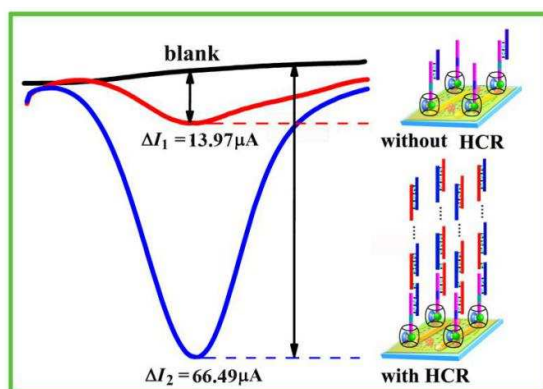


Figure 6 The contrast for electrochemical signal after the different electrodes modified with S1-Trp + S2-Trp/CB-8-MV²⁺/nano-Au/PAMAM-CNTs-Pt/GCE (blank cave); A1 + $[\text{Ru}(\text{NH}_3)_6]^{3+}$ /S1-Trp + S2-Trp/CB-8-MV²⁺/nano-Au/PAMAM-CNTs-Pt/GCE (red cave) and A1 + A2 + $[\text{Ru}(\text{NH}_3)_6]^{3+}$ /S1-Trp

+S2-Trp/CB-8-MV²⁺/nano-Au/PAMAM-CNTs-Pt/GCE (blue cave) respectively.

Reproducibility, Stability and Selectivity of the Reusable miRNA biosensor. To investigate the reproducibility, we studied the batch-to-batch precision of the biosensor toward three concentrations (low, middle, high), 0.1 pM, 10 pM and 500 pM miRNA-182-5p, and the relative standard deviations (RSD) were 4.9%, 6.3% and 5.6% ($n = 3$) respectively, suggesting acceptable accuracy and reproducibility of the electrochemical assay. The stability was monitored by the as-prepared fresh electrode, and the electrochemical signal of the prepared electrode was measured after 5d (97.45%), 10d (95.07%), 20d (93.39%), 25d (92.84%) and 30d (92.06%). The obtained results implied that the biosensor owed an excellent stability. To further explore the selectivity, miRNA-21, miRNA-141 and miRNA-155 were chosen as interfering agents and the blank sample was also carried out as the control experiment . According to Figure 7, no significant electrochemical responses of the miRNA-21(100.0 pM), miRNA-141(100.0 pM), miRNA-155 (100.0 pM) and blank sample were observed except that for target miRNA-182-5p (10.0 pM). These results indicated good selectivity of this strategy for miRNA detection.

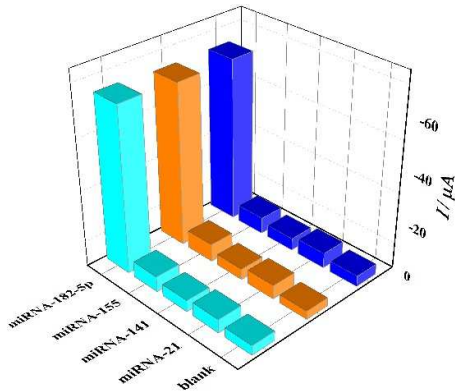


Figure 7 Specificity of the electrochemical biosensor (10.0 pM miRNA-182-5p,

100.0 pM miRNA-155, 100.0 pM miRNA-141, 100.0 pM miRNA-21 and blank sample were used in this case respectively).

CONCLUSIONS

In summary, this work has established a reusable electrochemical biosensor for the detection of miRNA based on DNA cross configuration-fueled target cycling and SDR amplification, and host-guest recognition-assisted electrochemical release. The designed DNA cross configuration could convert the single target miRNA input to large numbers of ssDNA (S1-Trp and S2-Trp) output after cycling amplification, which could improve the amplification efficiency, further enhance the sensitivity of the biosensor. Meaningfully, the electrochemical biosensor could release S1-Trp and S2-Trp from the electrode surface under a settled voltage and quickly regenerate in only 5 min based on electrochemical redox-driven assembly and release under a circumstance of CB-8, MV^{2+} and O_2 , and the regeneration rate was from 93.20 % to 102.24 % in ten times, which overcome the difficulties of burdensome operations, time-consuming and expensive experimental cost in traditional reusable electrochemical biosensors. After all, the present method proposes a new perspective for the construction of miRNA and other targets biosensors, and also opens up a new way to design reusable biosensor and release nucleic acids or proteins.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

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NOTES

The authors declare no competing financial interest.

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