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A novel bi-directional DNA walking machine and its application in enzyme-free electrochemiluminescence biosensor for sensitive detection of microRNAs

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ABSTRACT: Herein, a dual microRNA (miRNA)-powered bi-directional DNA walking machine with precise control was developed to fabricate an enzyme-free biosensor on the basic of distance-based electrochemiluminescence (ECL) energy transfer for multiple detection of miRNAs. By using miRNA-21 as the driving force, the DNA walker could move forth along the track and generated quenching of ECL response due to the proximity between Au nanoparticles (AuNPs) and Mn^{2+} doped CdS nanocrystals (CdS:Mn NCs) film as the ECL emitters, realizing ultrasensitive determination of miRNA-21. Impressively, once miRNA-155 was introduced as the driving force, the walker could move back along the track automatically, and surface plasmon resonance (SPR) occurred owing to the appropriate large separation between AuNPs and CdS:Mn NCs, achieving an ECL enhancement and realizing ultrasensitive detection of miRNA-155. The bi-directional movement of the DNA walker on the track led to continuous distance-based energy transfer from CdS:Mn NCs film by AuNPs, which resulted in significant ECL signal variation of CdS:Mn NCs for multiple detection of miRNA-21 and miRNA-155 down to 1.51 fM and 1.67 fM respectively. Amazingly, the elaborated biosensor provided a new chance for constructing controllable molecular nanomachines in biosensing, disease diagnosis and clinical analysis.

KEYWORDS: DNA walking machine, biosensor, electrochemiluminescent, microRNA.

INTRODUCTION

MicroRNAs (miRNAs) with approximate 19-23 nucleotides are single-stranded, short, endogenous and non-protein-coding RNAs, which play important roles in modulating over 30% mammalian genes in time of development, apoptosis,

metabolism and environmental response.[1-4] Scientists have paid increasing attention to sensitively analyze them with developing different methods in recent years, because miRNAs can serve as desirable biomarkers for the onset and progression of cancers and various genetic disorders.[5] Hence, the developments of reliable, universal and sensitive methods for miRNA analysis are critical for cancer diagnosis.[6]

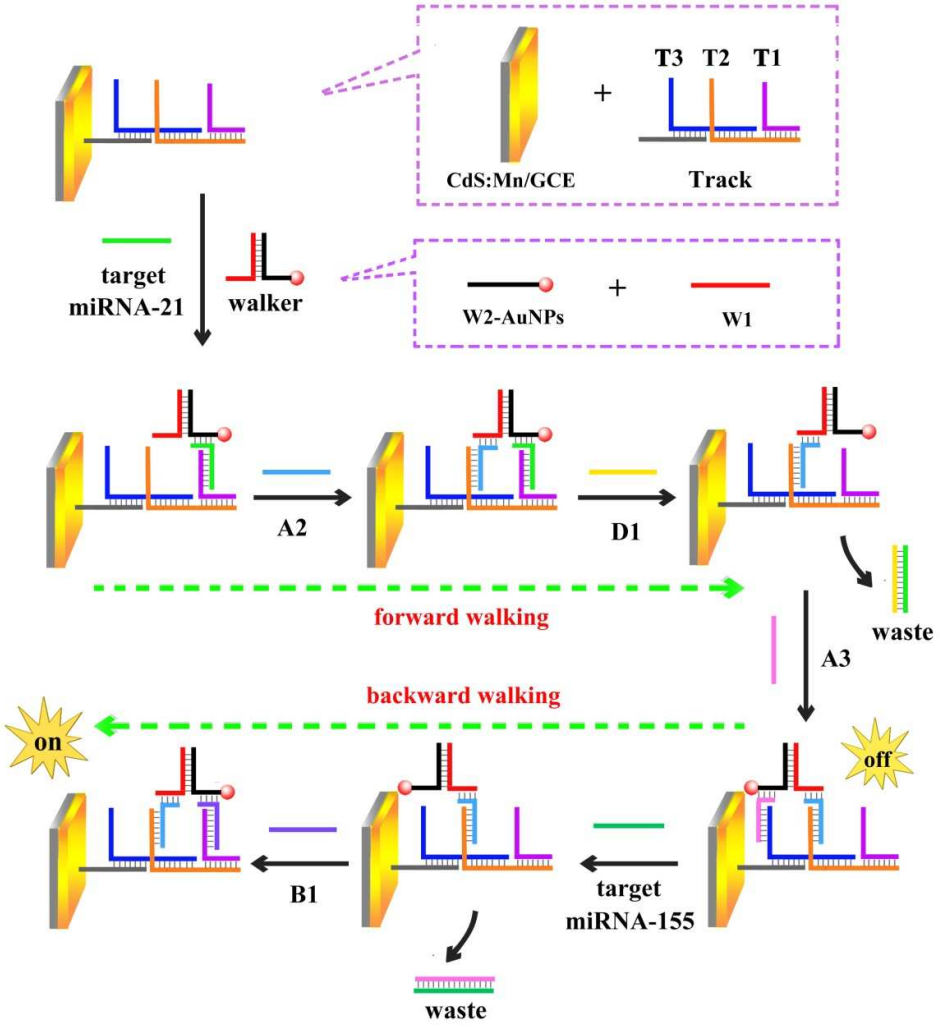
Until now, a variety of analytical methods, such as electrochemical,[7-10] fluorescent[11-13] and electrochemiluminescence (ECL),[14, 15] have been widely used for the sensitive detection of miRNA. Among diverse protocols, ECL is a particularly excellent detection technology because of its simplified operation and high sensitivity.[16, 17] In ECL system, energy transfer served as a possibly promising amplification strategies in biosensing and nanotechnology with wide applications.[18, 19] For example, Xu's group proposed a sensitive distance-based ECL system that the ECL quenching of Mn^{2+} doped CdS nanocrystals (CdS:Mn NCs) was obtained when Au nanoparticles (AuNPs) were closely contacted because of the ECL energy transfer between CdS:Mn NCs and AuNPs, and an ECL enhancement occurred when AuNPs were separated from CdS:Mn NCs due to surface plasmon resonance (SPR).[20] Although the distance-based ECL system was sensitive to target detection, only one target can be analyzed due to the traditional fixed hairpin structure, which limited the application of the sensitive ECL system. Therefore, seeking for a kind of novel DNA structure with more regulation and elasticity to expand the ECL application is a realistic challenge.

With the remarkable locomotion and controllability, DNA walking machine has

provided an avenue for the wide development of DNA nanotechnology.[21] The synthetic DNA walking machine is comprised of four parts: a DNA track with overhanging single-stranded branches as footholds, a bipedal DNA walker with two respective single-stranded "legs", attachment strands and detachment strands.[22, 23] The attachment strands connect the legs and the footholds, and then the detachment strands which are perfectly complementary with attachment strands are used to release the legs. With the fundamental characteristics of processivity, directionality, repetitive operation, progressive operation and autonomous operation,[24-26] DNA walking machine which duplicates "machine-like" function has been paid considerable attentions. In our previous work, a restriction enzyme (Nt.AlwI) triggered the walker to move along the DNA track whose four footholds labeled with the ECL emitters to propose an ECL biosensor for the high sensitive detection of target DNA.[27] Furthermore,on the basis of a DNA walking machine and target transduction, a ferrocene-switched ECL ''off-on'' biosensor was constructed for sensitively detecting cardiac troponin I (cTnI).[28] More recently, a DNA walker biosensor for the sensitive detection of miRNA was fabricated by Li's group based on the rolling circle amplification reaction and the enzymatic recycling cleavage strategy.[29] Although, the reported DNA walking machine studies realized sensitive detection of target, these studies can only detect one target for that the walker can only move along in a single direction. Thus, designing a bi-directional walking machine which enables multiple targets detection is of great importance in the development and application of DNA nanomachine.

Herein, a novel bi-directional DNA walking machine was constructed by designing a DNA walker which could migrate along the track and then move back along the previous steps to return the original position. The bi-directional DNA walking machine induced by two miRNAs was applied in constructing an ECL biosensor with use of CdS:Mn NCs as the ECL emitters for the sensitive detection of dual miRNAs on the basis of energy transfer. As expressed in Scheme 1, firstly, the DNA track, which had three protruding footholds (T1, T2, T3), was immobilized on the CdS:Mn NCs modified sensing surface. Next, the DNA walker containing W1 and AuNPs labeled-W2 (W2-AuNPs) was introduced on the sensing surface. Then, after target miRNA-21 hybridized with W2-AuNPs and T1, an enhanced ECL signal was obtained because of surface plasmon resonance (SPR) between AuNPs and CdS:Mn NCs film with appropriate large separation. Next, an attachment strand (A2) hybridized with W1 andT2. After that, a detachment strand (D1) fully hybridized with miRNA-21 to release the part of W2-AuNPs that could hybridize with an attachment strand (A3), and then AuNPs were proximally closed to CdS:Mn NCs, resulting in the ECL quenching owing to energy transfer between AuNPs and CdS:Mn NCs. It was worth pointing out that the quenching of ECL could reduce the background signal and improved the sensitivity of the proposed biosensor. Impressively, by introducing target miRNA-155, the walker began to move back along the track by the hybridization of A3 and miRNA-155 to produce duplex waste and release the part of W2-AuNPs. At last, W2-AuNPs and T1 hybridized with an attachment strand (B1), which prompted the walker back to the original position successfully. Through the

distance-based ECL response, the liner ranges for miRNA-21 and miRNA-155 detection were both from 5.0 fM to 500 pM, and the biosensor achieved the low detection limits of 1.51 fM and 1.67 fM respectively. Moreover, the walking system could serve as an ideal sensing candidate for analysis of other biomarkers by changing the sequence of target and relating DNA. Above all, the construction of the bi-directional DNA walker machine provided a new potential to explore various artificial movements of DNA nanomachines with precision control which were applied for detecting various biomarkers containing proteins and nucleonic acid.



Scheme 1. Schematic illustration of the miRNAs induced bi-directional DNA walking machine for sensitive detection of miRNAs

EXPERIMENTAL SECTION

Reagents and materials. Cadmium nitrate trtrahydrate ($Cd(NO_3)_2 \cdot 4H_2O$),Sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$), Sodium borohydride ($NaBH_4$), Sodium persulfate ($Na_2S_2O_8$) and Manganese acetate ($Mn(Ac)_2 \cdot 4H_2O$) was provided by Kelong Chemical Inc (Chengdu, China). 3-mercaptopropionic acid (MPA) was bought from Shanghai Medpep Co. Ltd. (Shanghai, China). N-hydroxy sulfosuccinimide sodium salt (NHS), 6-mercapto-1-hexanol (MCH) and N-(3-dimethylamminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) was obtained from Sigma Aldrich (St. Louis, MO, U.S.).The diethyl pyrocarbonate (DEPC) and the DNA oligonucleotides were supplied by Sangon, Inc. (Shanghai, China). The miRNA-21 and miRNA-155was produced by TaKaRa (Dalian, China). All the DNA and miRNA chains were stored at -20 °C and diluted by DNA hybridization buffer (HB1) and miRNA hybridization buffer (HB2),[30] respectively. The sequences of oligonucleotides are depicted in Table S1.

Instrumentation. The ECL measurements were conducted with a MPI-A ECL analyzer (Xi'an Remax Electronic Science & Technology Co. Ltd., Xi'an, China) with 800 V of the photomultiplier tube and the potential scan from -1.6 to 0.2 V. Cyclic voltammetry (CV) was accomplished with a CHI 660C electrochemistry workstation (Shanghai CH Instruments, Shanghai, China), and all experiments were carried out with a conventional three-electrode system which contained a modified glass carbon

electrode (GCE, Φ = 4 mm) as working electrode, a platinum wire as counter electrode and a Ag/AgCl (saturated KCl) as reference electrode. The surface appearances of CdS:Mn NCs and AuNPs were characterized by Transmission Electron Microscopy (TEM, Tecnai G2 F20, FEI, U.S.).

Synthesis of AuNPs. AuNPs were synthesized according to a well-established method with slight modifications.[19] Typically, 0.6 mL ice cold $NaBH_4$ (0.1 M) was added to 20 mL 1% $HAuCl_4$ containing sodium citrate (2.5×10^{-4} M) under stirring. Then, the color of solution changed from colorless to wine red suggesting the successful formation of AuNPs. The obtained AuNPs for further use was stored at 4 °C.

Preparation of CdS:Mn NCs. Hydrothermal method was applied in this study for the synthesis of CdS:Mn NCs.[19] Firstly, $Cd(NO_3)_2\cdot4H_2O$ (0.1683 g) was mixed with $Mn(CH_3COO)_2\cdot4H_2O$ (0.0134 g) in 30 mL water and heated to 70°C under stirring. Next, the obtained solution was introduced into $Na_2S\cdot9H_2O$ (0.6125 g) solution which was freshly prepared in 30 mL ultrapure water. The above mixture turned orange-yellow instantly, indicating the successful formation of the precursor. The reaction was kept continuously refluxing under 70°C for 3 h. Finally, the resulted of CdS:Mn NCs was centrifuged for several times by using absolute ethanol and stored at 4 °C.TEM characterizations of CdS:Mn NCs and AuNPs were shown in Supporting Information (Figure S1).

Fabrication of the biosensor with CdS:Mn NCs film and DNA track. Scheme 1 displayed the stepwise assembly process for the proposed biosensor. The bare GCE

was first coated with 30 μL CdS:Mn NCs solution and dried at room temperature. Next, the prepared electrode was orderly steeped in 3 mM MPA (1.0 mL) for 5 h. Then the electrode was immersed in cross-linking agent (10 mg NHS and 40 mg EDC) at 4°C for 2 h, achieving CdS:Mn NCs film with active carboxyl on the GCE.

The track was synthesized by T1 strand (1 μM), T2 strand (1 μM), T3 strand (1 μM) and ssDNA strand (1 μM) in HB1. Then, the track was incubated about 5 min under 95 °C and cooled to 37 °C for 3 h slowly.[28] Finally, 10 μL of the prepared track was casted onto the surface of the CdS:Mn NCs film. At last, the prepared electrode was incubated overnight at 37 °C and washed thoroughly with 1×PBS. The buffers which involved in this work were shown in Supporting Information.

Assembling of the DNA walking machine. Firstly, 10 μM of W2 was mixed with 500 μL of AuNPs to obtain the W2-AuNPs composites. Afterwards, the above composites were blocking with 5 μL of MCH (0.73 M). Subsequently, the obtained solution was centrifuged and washed with PBS twice. Then, the DNA walker was formed by mixing W1 and W2-AuNPs strands in equal molar ratio. At last, the solution was cooled slowly from 95 to 37 °C over 3 h.[31]

10 μL of walker and 10 μL of miRNA-21 was introduced onto the resulting electrode and then incubated at room temperature for 2 h. Then the modified electrode was washed again with 1×PBS. Next, 10 μL A2 (1 μM) was dropped on the electrode for 1 h at room temperature. After that, the electrode was dropped with 10 μL D1 (1 μM), and then W2 could react with 10 μL A3 (1 μM) for the next step. The proposed biosensor was detected in 0.05 M $K_2S_2O_8$ (pH 8.0) which served as coreactant of QDs.

Finally, miRNA-155 and B1 with equimolar amounts were successively incubated on the assembled biosensor, enabling the walker to move back.

Native polyacrylamide gel electrophoresis (PAGE). The synthesis of DNA walking machine was analyzed by PAGE. The first sample was achieved by the assembled track (2 μM) and the other samples were pre-incubated with the walker and different strands (2 μM) subsequently. The samples were loaded in 16% native denaturing gel and the gel electrophoresis was performed in 1×TBE buffer at 50V for 2.5 h. Finally, the gel was dyed with ethidium bromide (EB), and a digital camera was used for the electrophoresis image under UV light.

RESULTS AND DISCUSSION

Investigation of the bi-directional DNA walking machine. The operation of the DNA walker was confirmed by PAGE. Each complex migration exhibited a clear sharp band under native conditions. The walker moved along the track with strand displacement reaction by adding attachment strands (miRNA-21, A2, A3 and B1) and detachment strands (D1, miRNA-155) subsequently. As shown in Figure 1, a major band in lane 1 demonstrated that W2 and T1 hybridized with the target miRNA-21 successfully. The gel exhibited a band (lane 2) that W1 and T2 hybridized with A2 and showed slow mobility due to its high molecular weight. When D1 hybridized with miRNA-21, a band moved fast because the sample had low molecular weight (lane 3). When A3 was introduced, the band got less mobile (Lane 4), indicating that W2 was hybridized with A3 and both legs of the walker were consistent with the track. Moreover, miRNA-155 hybridized with A3 to release W2, which exhibited a fast

mobile band (Lane 5). Lane 6 showed that the PAGE was attributed to hybridization of the track with B1, which moved less slow. The above result demonstrated the successful movement of the designed DNA walking machine.

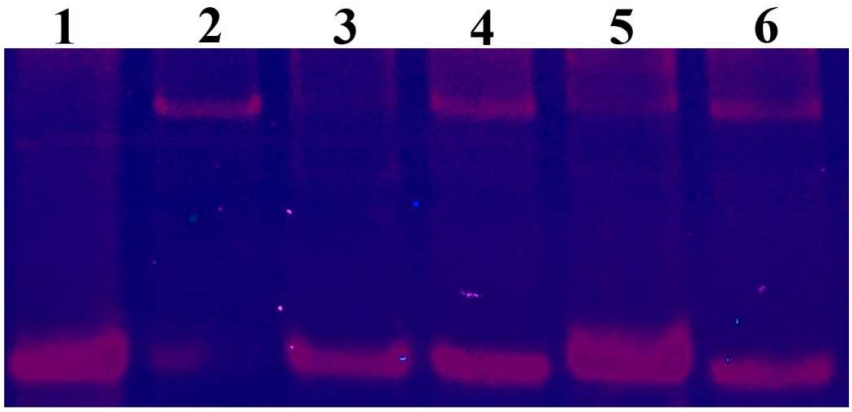


Figure1. PAGE characterization of miRNAs induced bi-directional DNA walker machine. Lane 1: mixture of Track (T), Walker (W) and miRNA-21; Lane 2: mixture of T, W, miRNA-21 and A2; Lane 3: mixture of T, W, miRNA-21, A2 and D1; Lane 4: mixture of T, W, miRNA-21, A2, D1 and A3; Lane 5: mixture of T, W, miRNA-21, A2, D1, A3 and miRNA-155; and Lane 6: mixture of T, W, miRNA-21, A2, D1, A3, miRNA-155 and B1. The mixtures were incubated at room temperature for 1 h.

Electrochemical characterization of the biosensor. The fabrication process of the biosensor was characterized by CV in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ from -0.2 to 0.6 V at a scan rate of 0.1 V s^{-1}. The walker moved forth and back along the track is shown in Figure 2. The bare GCE exhibited a couple of well-defined redox peaks (curve a), while the CV signal decreased (curve b) after modification of CdS:Mn NCs film owing to the hindrance of CdS:Mn NCs for electron transmission. Then, the CV curve decreased (curve c) when the DNA track was incubated on the electrode due to the

nonelectroactive character. After the electrode was incubated with miRNA-21 and walker, the CV response remained decline (curve d) ascribe to the electron transfer obstruction of oligonucleotides. Subsequently, a continued decreased CV response was obtained (curve e), testifying that A2 was immobilized onto the electrode. Followed by immobilizing D1 onto the electrode, the peak current slightly decreased (curve f). However, after incubating with A3, the current response enhanced (curve g) because the AuNPs-labeled walker was proximally closed to the surface of the electrode, which accelerated the electron transmission of AuNPs. Subsequently, the current response greatly reduced (curve h) after the electrode was incubated with miRNA-155, because miRNA-155 displaced A3 for making negative effect of electron transmission. Finally, the peak current (curve i) declined continuously after B1 was immobilized onto the electrode, demonstrating that the walker leaved away from the surface of electrode to reduce electron transfer.

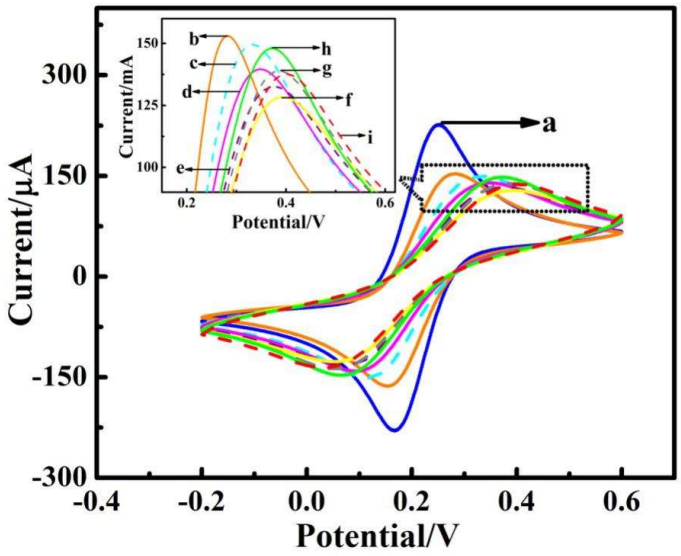


Figure2. Electrochemical characterization of the assembled process of the bi-directional DNA walking machine: (a) bare GCE, (b) CdS:Mn NCs/a, (c) track/b, (d) miRNA-21/c, (e) A2/d, (f) D1/e, (g) A3/f, (h) miRNA-155/g, (i) B1/h. The CVs of the proposed biosensor were carried out from -0.2 V to 0.6 V in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution.

ECL performance of the bi-directional DNA walking machine. ECL performance had been interpreted to reflect the assembled process of the bi-directional DNA walking machine. The corresponding ECL detection was employed in 2 mL PBS (pH 8.0) with 0.05 M $K_2S_2O_8$, which was cycled (scanned positively from 0 V) in the range from -1.6 to 0.2 V. As displayed in Figure 3, there was almost no ECL response with the bare GCE (curve a). After the immobilization of CdS:Mn NCs film on the GCE, the ECL signal enhanced obviously (curve b) because of the introduction of ECL luminphore. After the DNA track was modified on

electrode, the decline was achieved because oligonucleotides could block the electron transfer (curve c). When walker and miRNA-21 were assembled on the electrode, the ECL intensity increased obviously, testifying that AuNPs could amplify the ECL signal of CdS:Mn NCs through energy transfer (curve d). Subsequently, after assembling A2, D1 and A3 on the sensor, the walker was activated to move along the track, which led the ECL signal (curve e) down due to energy transfer between CdS:Mn NCs and AuNPs. Finally, the ECL intensity increased when miRNA-155 and B1 were assembled on the proposed biosensor, because the energy transfer occurred with the long-distance of AuNPs and CdS:Mn NCs. According to the experimental result, it can be confirmed that the bi-directional DNA walking biosensor with distance-dependent energy transfer strategy was fabricated successfully.

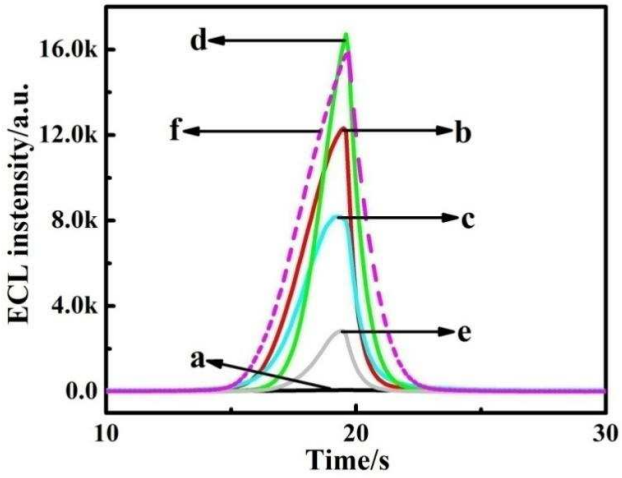


Figure 3. ECL-time curves of the stepwise-modified electrode: (a) bare GCE, (b) CdS:Mn NCs / GCE, (c) track / CdS:Mn NCs / GCE, (d) miRNA-21 / walker / track / CdS:Mn NCs / GCE, (e) A2 / D1 / A3 / miRNA-21 / walker / track / CdS:Mn NCs /

GCE, (f) B1 / miRNA-155 / A2 / D1 / A3 / miRNA-21 / walker / track / CdS:Mn NCs / GCE. The biosensor was analyzed in PBS solution (pH = 8.0) containing 0.05 M $S_2O_8^{2-}$; Scan rate, 100 mV s^{-1}.

ECL detection and stability of the bi-directional DNA walking biosensor. Under optimal experimental conditions (Figure S2 in the Supporting Information), the bi-directional DNA walking biosensor was investigated to sensitively quantify miRNA-21 and miRNA-155 respectively. As displayed in Figure 4A, the ECL peak decreased with elevating concentration of miRNA-21 from 5.0 fM to 500 pM with a detection limit of 1.51 fM. As shown in Figure 4B, the linear regression equation was y = 6675.5 - 1882.7 lgc_1, where y was the ECL intensity and $c_1$was the concentration of the miRNA-21. The detection limit is estimated according to $3S_B/m$, where S_B is the standard deviation of the blank measures and m is the slope of the linear regression equation. According to Figure 5A, under the constant concentration of miRNA-21 (500 fM), it can be seen that the ECL intensity increased accordingly upon increasing concentration of miRNA-155 in the range from 5 fM to 500 pM. The linear relationship could be represented as y = 10150.6 + 1927.6 lgc_2, where y was the ECL intensity and c_2 was the concentration of miRNA-155 (Figure 5B). The proposed biosensor for miRNA-155 detection achieved high sensitivity with a low detection limit of 1.67 fM.

Moreover, Figure. 4A and Figure. 5A showed excellent stability of the as-prepared biosensor with 3 cycles at different concentrations of miRNA-21 and miRNA-155 accordingly. Significantly, compared to other biosensors, as can be seen

in Table 1, the proposed biosensor had an attractive wider linear range and relative lower detection limit. The proposed biosensor for the highly sensitive detection of miRNA-21 and miRNA-155 might have potential application in clinical detection.

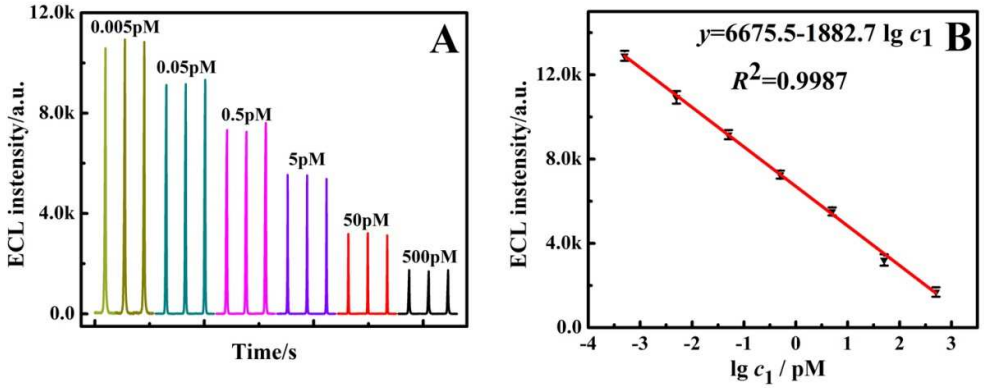


Figure 4. (A) ECL intensity-time curves of the miRNA-21 with different concentrations: 0.005 pM, 0.05 pM, 0.5 pM, 5 pM, 50 pM and 500 pM. (B) The corresponding calibration curve of miRNA-21 analysis.

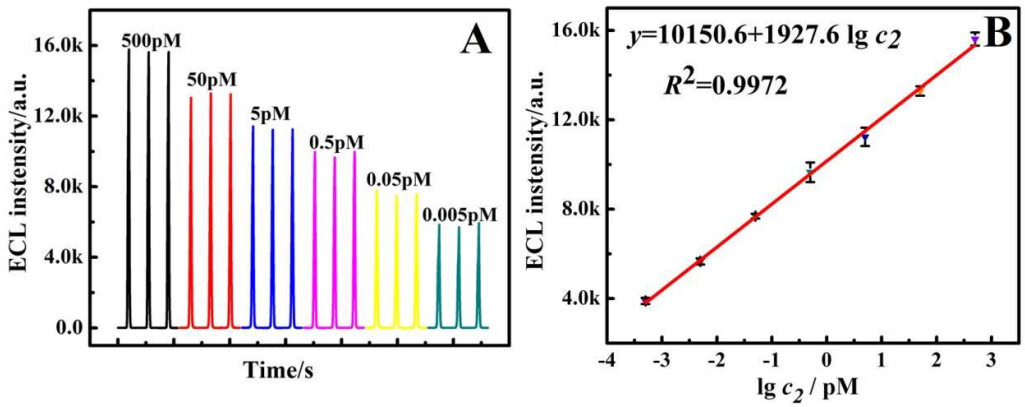


Figure 5. (A) ECL intensity-time curves of the miRNA-155 with different concentrations: 0.005 pM, 0.05 pM, 0.5 pM, 5 pM, 50 pM and 500 pM. (B) The corresponding calibration curve of miRNA-155 analysis.

Table 1. Comparison of the Proposed Biosensor with Other Detection Strategies

Analytical methods	Linear range	Detection limit	Ref.
Fluorescence	100 fM~10 nM	58 fM	29
Surface Enhanced Raman Scattering	0.5 nM~5 nM	485 pM	32
ECL	10 fM~1 pM	10 fM	33
Fluorescence	10 fM~1 nM	10 fM	34
Colorimetric	50 nM~1 μM	10 nM	35
Electrochemical	5 fM~5 pM	10fM	36
ECL	5 fM~500 pM	1.51 fM(miRNA-21) 1.67 fM(miRNA-155)	*This work*

Reproducibility and selectivity of the bi-directional DNA walking biosensor. The reproducibility of the designed biosensor was explored by analysis of different batches. As displayed in Figure S3, the relative standard deviation (RSD) values for the detection of miRNA-21and miRNA-155 at 500 pM were not more than 5%, which demonstrated that the biosensor had efficient reproducibility and accuracy.

To evaluate the selectivity of the developed biosensor, p53 gene, oral cancer (ORVOA 1) gene,miRNA-199, miRNA-182-5p were chosen as interfering agents. According to Figure 6A, the presence of the interference substances (5 nM) displayed strong ECL emissions. When the biosensor was incubated with miRNA-21 (500 fM)), the ECL signal decreased significantly. Besides, compared to the miRNA-21 solution, the mixture in the presence of miRNA-21 (500 fM) with interference substances displayed no remarkable change in the ECL response. The result suggested that the biosensor possessed a high selectivity toward miRNA-21. Furthermore, as can be seen from Figure 6B, the biosensor incubated with the interference substances (5 nM)

exhibited low ECL signals. The ECL intensity of miRNA-155 (500 fM) exhibited obvious increase compared with the interference substances. When the ECL response of the mixture containing 5 nM p53 gene, 5 nM ORVOA 1 gene, 5 nM miRNA-199, 5 nM miRNA-182-5p and 500 fM miRNA-155 was compared with that of the obtained solution from the 500 fM miRNA-155, no obvious difference was observed. The above result demonstrated that the designed protocol displayed good selectivity toward miRNA-155 against other control miRNAs. The comparison above illustrated the superior specificity of the bi-directional DNA walking biosensor.

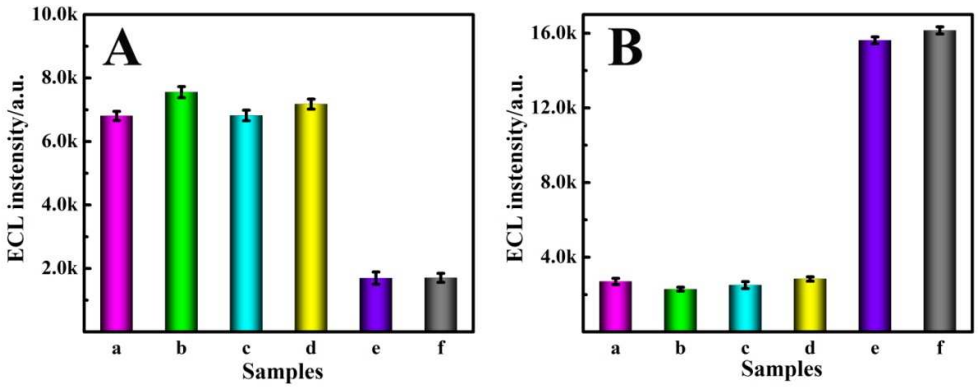


Figure 6. (A) Selectivity of the proposed biosensor when employed with (a) p53 gene; (b) ORVOA 1 gene; (c) miRNA-199a; (d) miRNA-182-5p; (e) miRNA-21; (f) mixture of p53 gene, ORVOA 1 gene, miRNA-199, miRNA-182-5p and miRNA-21. (B) Selectivity of the biosensor when analyzed with (a) p53 gene; (b) ORVOA 1 gene; (c) miRNA-199a; (d) miRNA-182-5p; (e) miRNA-155; (f) mixture of p53 gene, ORVOA 1 gene, miRNA-199, miRNA-182-5p and miRNA-155. Error bars, SD, $n = 3$.

Analytical recovery experiment of the elaborated biosensor. To testify the applicability, different concentrations of miRNA in PBS were monitored respectively

by the proposed biosensor. According to sample 1 to 4 in Table 2, the recovery experiment was evaluated by adding different concentrations of miRNA-21 into PBS. As a result, we found that the recovery was ranging from 94.3% to 103.7%. Similarly, sample 5 to 8 in Table 2, a series of samples were prepared by adding miRNA-155 of different concentrations into PBS and the recovery of the miRNA-155 analysis was ranging from 96.1% to 101.8%. The obtained result showed that the developed biosensor could be used to monitor different miRNAs with a desirable recovery and could serve as a potential platform in real application.

Table 2. Analytical Recovery Experiment of Dual miRNAs by Elaborated Biosensor

Sample	Added/pM	Found/pM	Recovery/%
1	0.005	0.00418	94.3%
2	0.05	0.0485	97.2%
3	0.5	0.519	103.7%
4	5	5.06	101.2%
5	0.005	0.00481	96.1%
6	0.05	0.0492	98.3%
7	0.5	0.509	101.8%
8	5	5.02	100.3%

CONCLUSION

In summary, an enzyme-free ECL biosensor was fabricated by bi-directional DNA walking machine for sensitive detection of two different miRNAs, and the proposed strategy has brought forth the following two novel points. First, with rational design, a novel bi-directional DNA walking machine enabled the walker to move forth and back on the DNA track, which was precisely controlled by strand

displacement reactions. The designed bi-directional DNA walking machine with systematicness and scientificness had great prospect for further application in drug discovery and cancer diagnostics. Second, the innovative combination of distance-dependent energy transfer and the bi-directional movement of DNA walking machine achieved excellent analytical performance for the detection of miRNA-21 and miRNA-155, respectively. The scheme proposed a substantial progress in the anticipated developing field of DNA-based machines. Moreover, based on the bi-directional DNA walking machine, the sensing strategy provided a promising way for constructing controllable DNA nanomachines in clinical diagnostics and biomedical research.

ASSOCIATED CONTENT

Supporting Information

Additional electronic information as pointed in the essay. This information is available free of charge via the Internet at http://pubs.acs.org

The oligonucleotides sequences used in this work, the buffers involved in this work, characterization of the proposed CdS:Mn NCs and Au NPs, optimum of the volume of CdS:Mn NCs and the concentration of the DNA track, reproducibility of the proposed biosensor.

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