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Letter

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Target-Responsive, DNA Nanostructure-Based E-DNA Sensor for microRNA Analysis

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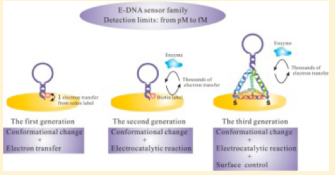
Supporting Information

ABSTRACT: Because of the short size and low abundance of microRNAs, it is challenging to develop fast, inexpensive, and simple biosensors to detect them. In this work, we have demonstrated a new generation (the third generation) of E-DNA sensor for the sensitive and specific detection of microRNAs. Our third generation of E-DNA sensor can sensitively detect microRNA target (microRNA-141) as low as 1 fM. The excellent specificity has been demonstrated by its differential ability to the highly similar microRNA analogues. In our design, the use of DNA tetrahedron ensures the stem-loop structure in well controlled density with improved reactivity. The regulation of the thermodynamic stability of the stem-loop structure decreases the background signal and increases the specificity as well. The enzymes attached bring the electrocatalytic signal to amplify the detection. The combination of these effects improves the sensitivity of the E-DNA sensor and makes it suitable to the microRNA detection. Finally, our third generation of E-DNA sensor is generalizable to the detection of other micro RNA targets (for example, microRNA-21).



Over the past decade, microRNAs have become progressively more important because of their functions in regulating many cellular processes.[1−3] Importantly, even small changes in the expression levels of microRNAs have significant influence to the target genes. Recently, accumulating evidence demonstrated that microRNAs are playing a very important role in the development of various cancers, which broaden the applications of microRNAs in diagnostics and therapy.[4−6] So the investigations on microRNAs and the determination of expression pattern of known microRNAs have profound meanings in biology and clinics. These all require an accurate and inexpensive detection platform for microRNAs.[7−11] In turn, the development of the detection platform will help the discovery of unknown biological functions of microRNAs.

However, the accurate and inexpensive detection of microRNAs is challenging.[7,8,12,13] The relatively short size and high similarity between microRNAs hinder the progress in the development of the detection platform. As a result, the pace of developing sensitive, accurate, and inexpensive detection methods is slower than that of the research on microRNA regulation and function. For example, the most classic method, polymerase chain reaction (PCR) suffers from the low efficiency and false positive because of the short primers in the amplification process of microRNAs. Northern blotting is time-consuming and less sensitive. Microarrays have the advantage of high-throughput; however, the cross-hybridization is a serious problem.

The E-DNA sensor was first invented in 2003,[14,15] and thereafter, the family members of E-DNA sensor have demonstrated great potential in the detection of DNA not only in pure solutions but also in many complex matrixes.[15−27] The E-DNA sensor represents a significant improvement in the DNA detection area with the advantages of fast response, easy to use, low cost and direct use in complicated samples. The prototype of E-DNA sensor employs a stem loop structure dually labeled with a thiol (for probe immobilization) and a ferrocene (for electrochemical signal output).[14] At the initial state, the probe adapts a stem-loop structure that make the ferrocene close to the electrode surface. The electrons can transfer to or from the electrode efficiently at this state. After hybridization, the conformational change forces the ferrocene away from the electrode that blocks the electron transfer. The detection sensitivity of the prototype E-DNA sensor is ∼30 pM with excellent specificity and selectivity. To improve the sensitivity, a second generation of E-DNA sensor has been developed by replacing the ferrocene with an enzyme.[28] The enzymatic catalytic reaction amplifies the electrochemical signal effectively, and the DNA target in the concentration in the femtomolar range can be sensitively detected.

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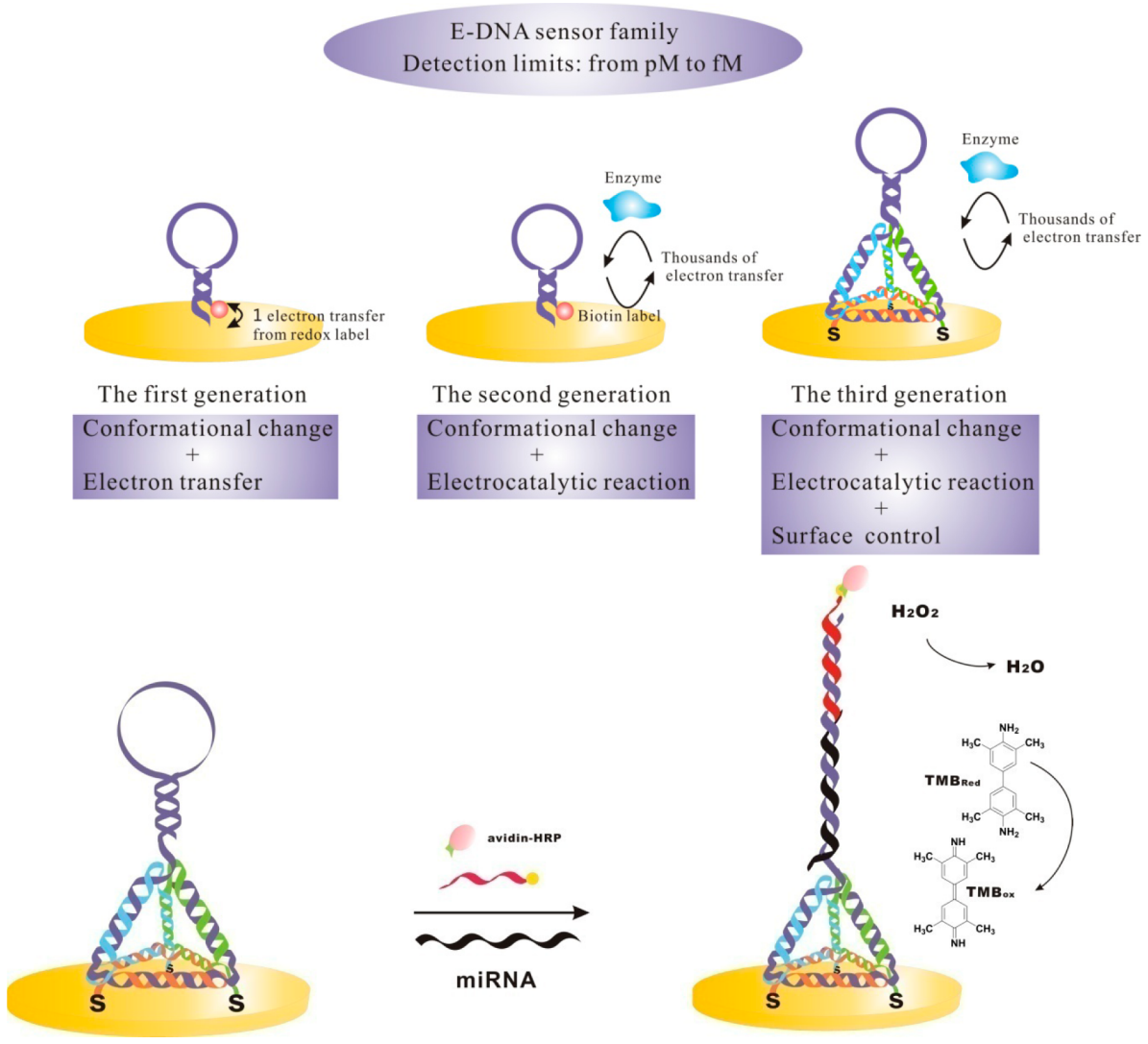

Figure 1. First generation of the E-DNA sensor is based on the electron transfer change and conformational change upon target binding. The fast response, simple operation, and electronic readout of the E-DNA sensor represent a significant step toward DNA analysis. To further improve the sensitivity of the E-DNA sensor, our group developed the second generation of the E-DNA sensor by combining the E-DNA sensor to enzyme catalytic amplification. Here, to fulfill the critical requirements of microRNAs detection, we demonstrated the third generation of the E-DNA sensor, by controlling the surface immobilization of the stem-loop structure through the DNA tetrahedron. Thus, the sensitive microRNA detection can be achieved with the detection limit of 1 fM.

To fulfill the critical requirements (the low abundance and small size of microRNAs) of microRNA detection, here, we have demonstrated the third generation of E-DNA sensor, which is based on a DNA tetrahedral probe[29] and enzymatic amplification. The tetrahedral structure ensures the DNA probes with well controlled density and orientation. Importantly, because of the small size of microRNA, our design is that the target microRNA completely hybridizes one part of the stem-loop probe by opening the stem-loop structure and then the signaling probe with biotin label hybridized to another part of it. With this design, we can easily regulate the stability of the stem-loop structure and then optimize the performance of the E-DNA sensor. Through the rational design and optimization, the third generation of the E-DNA sensor can detect microRNA as low as 1 fM with great differentiation ability in microRNA family members with high similarity (Figure 1).

RESULTS AND DISCUSSION

In our third generation of E-DNA sensor, we designed a pyramidal like probe by using DNA tetrahedron as a scaffold with a pendant stem-loop structure (Figure 1). Our previous results demonstrated that the DNA tetrahedral probes can be easily immobilized on the gold surface by labeling three vertices of tetrahedron with thiols.[29] Importantly, the relatively rigid DNA nanostructure of tetrahedron supports the DNA recognition probe in the upright orientation. The distance between the stem-loop structures can be well controlled. As such, the entanglement between stem-loop structures on the gold surface can be eliminated. This point is especially important when using stem-loop structures as recognition probes because the intramolecular interactions are more serious in this case.

Our stem-loop structure has two regions, one of which hybridizes to the microRNA target and the other hybridizes to a signaling probe. At last, the avidin-HRP (avidin modified horse

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radish peroxidase) is attached to produce enzyme catalytic signal (Figure 1). Compared to the traditional sandwich assay, our design is suitable to detect the short microRNA targets. In the traditional sandwich assay, the DNA recognition probe hybridizes to a part of microRNA which is short (~10 base pairs) resulting in low hybridization efficiency and low stability. As a contrast, in our design, the whole sequence of microRNA target is used to open the stem-loop structure, The resulting hybridized helix has the free energy of −26.3 kcal/mol. Compared to some traditional design of the sandwich assay (half sequence of microRNA target is used to hybridize the recognition probe with the free energy of −12.2 kcal/mol), the hybridized helix in our design is much more stable in thermodynamics.

Then, we used the third generation of the E-DNA sensor to detect the cancer related biomarker-microRNA 141. Without the target, we observed two pairs of well-defined redox peaks in the cyclic voltammetry that originated from the electron mediator-TMB (3,3′,5,5′-tetramethylbenzidine) (Figure S1 in the Supporting Information). After hybridization with microRNA (1 nM) (Figure S1 in the Supporting Information), the obvious electrocatalytic phenomenon occurs and the catalytic current increases. When we decrease the concentration of microRNA to 1 fM, the catalytic current decreased too. These results demonstrated that our E-DNA sensor works well.

Then, interestingly, we found that the background signal can be optimized by regulating the reaction temperature (Figure 2, top). At 25 °C, the background signal is relatively high because of the undesired hybridization between the signaling probe and the stem-loop structure, which increases the nonspecific signal without the target. When we increased the temperature to 37 °C, the undesirable hybridization is unfavorable because of the thermodynamics (Figure 2, top). The predicted melting temperatures (via mfold) for the signaling probe and the target are 48.9 and 64.9 °C, respectively. Increasing the temperature may avoid undesirable binding of the signaling probe and the recognition probe.

Next, we realized that the thermodynamic stability of the stem-loop structure is critical to decrease the background signal. We designed a series of stem-loop structures with different lengths of the stem region (Figure 2, bottom, Table S1 in the Supporting Information).When we employed the stem-loop with six-base pairs in the stem region, we obtained a high background signal (~750 nA), which indicated that this stem-loop structure was thermodynamically unstable. As a result, the signaling probe can easily hybridize to the unstable stem-loop structure to produce a background signal in the absence of target microRNA. The high background signal leads to a low signal to background ratio. Along with the length increase in the stem region, the thermodynamic stability increased. Then, the background signal was decreased. Finally, we used a stem-loop structure with eight base pairs in the stem region to obtain a satisfactory background signal.

Then, we investigated the detection sensitivity of the third generation E-DNA sensor. Impressively, we can detect as low as 1 fM target microRNA 141 (Figure 3, top) with a broad dynamic range. The sensitivity was better than the prototype of

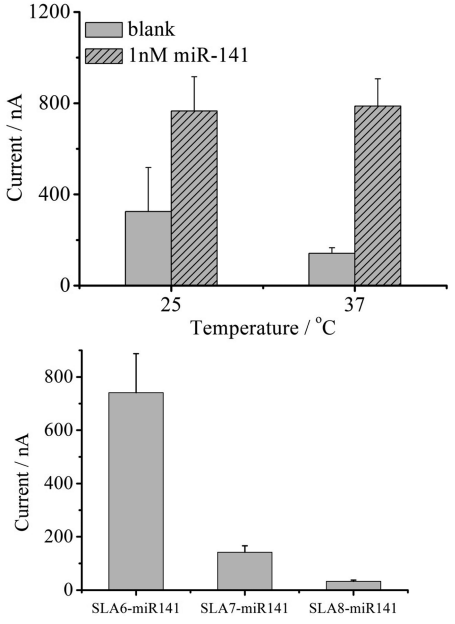

Figure 2. (top) We regulated the temperature in our detection and found out that the background signal can be decreased efficiently by increasing the temperature from 25 to 37 °C. The target concentration was 1 nM. The electrochemical signal was collected through the *I*−*t* analysis (see detailed information in the Experimental Section in the Supporting Information). (bottom) The thermodynamic stability of the stem-loop structure was critical to decrease the background. We investigated different lengths in the stem region and concluded that eight base pairs in the stem-loop region had the lower background than that of six and seven base pairs in the stem regions.

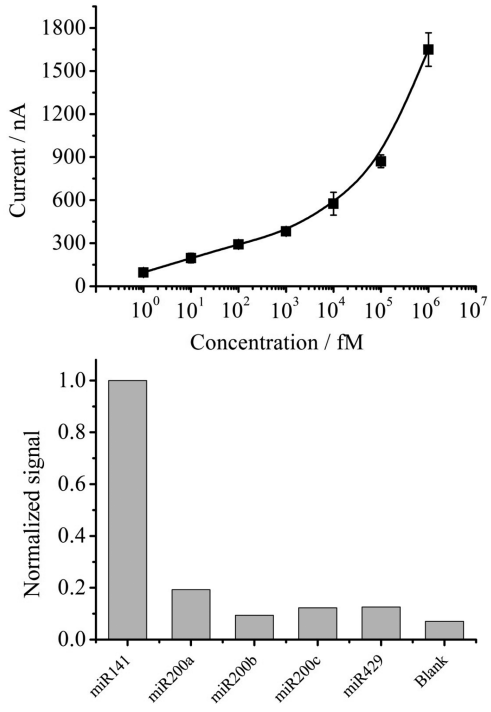

Figure 3. (top) Our third generation of E-DNA sensor is sensitive to detect as low as 1 fM microRNA 141. The electrochemical signal from a target of 1 fM is still larger than the mean blank value (32.5 nA) plus 3 standard deviations (>3SD, standard deviation is 5.2 nA). The dynamic range is broad which spans 6-orders of magnitude. (bottom) We detected the microRNAs with high similarity to microRNA 141, which include microRNA 200a, microRNA 200b, microRNA 200c, and microRNA 429. All these microRNAs brought signals similar to the background signal, while, the high signal from microRNA 141 was obtained.

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E-DNA sensor and the second generation of the E-DNA sensor.[14,15,28] In the design of prototype of E-DNA sensor, a redox label (ferrocene) was used to generate an electrochemical signal, which means a single hybridization event can only bring the signal from one electron. That is the reason that limits the sensitivity. To improve this, in the second generation of E-DNA sensor, an enzyme was used to replace the redox label, which can amplify the signal through the catalytic reaction. Thus, a single hybridization event can bring the signal from thousands of electrons. In this work, we designed the third generation of E-DNA sensor to further improve the sensitivity. By using the DNA nanotechnology, the probe density and orientation can be well controlled in our new generation of E-DNA sensor. The surface states of probes do affect the hybridization efficiency as previous studies.[30] Since our electrode is modified with DNA nanostructures with recognition probes situated on them, the sensing surface in our system can be considered as a nanostructured surface. As stated before, the hybridization efficiency can be improved greatly by using a nanostructured electrode.[30−32]

At last, we challenged our sensor with microRNAs that are highly similar to our target microRNA-141 (Figure 3, bottom). The results indicated that our E-DNA sensor had excellent differentiability in microRNA family members. Our E-DNA sensor can be inherently generalizable to the detection of other microRNA targets. We designed another stem-loop structure for the detection of microRNA 21 and obtained the signal of ∼1700 nA (with 1 nM of microRNA 21), while the background signal was only ∼25 nA (Figure S2 in the Supporting Information). The high signal-to-noise ratio of our third generation of E-DNA sensor is really impressive.

■ ASSOCIATED CONTENT

Supporting Information

Supporting tables and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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