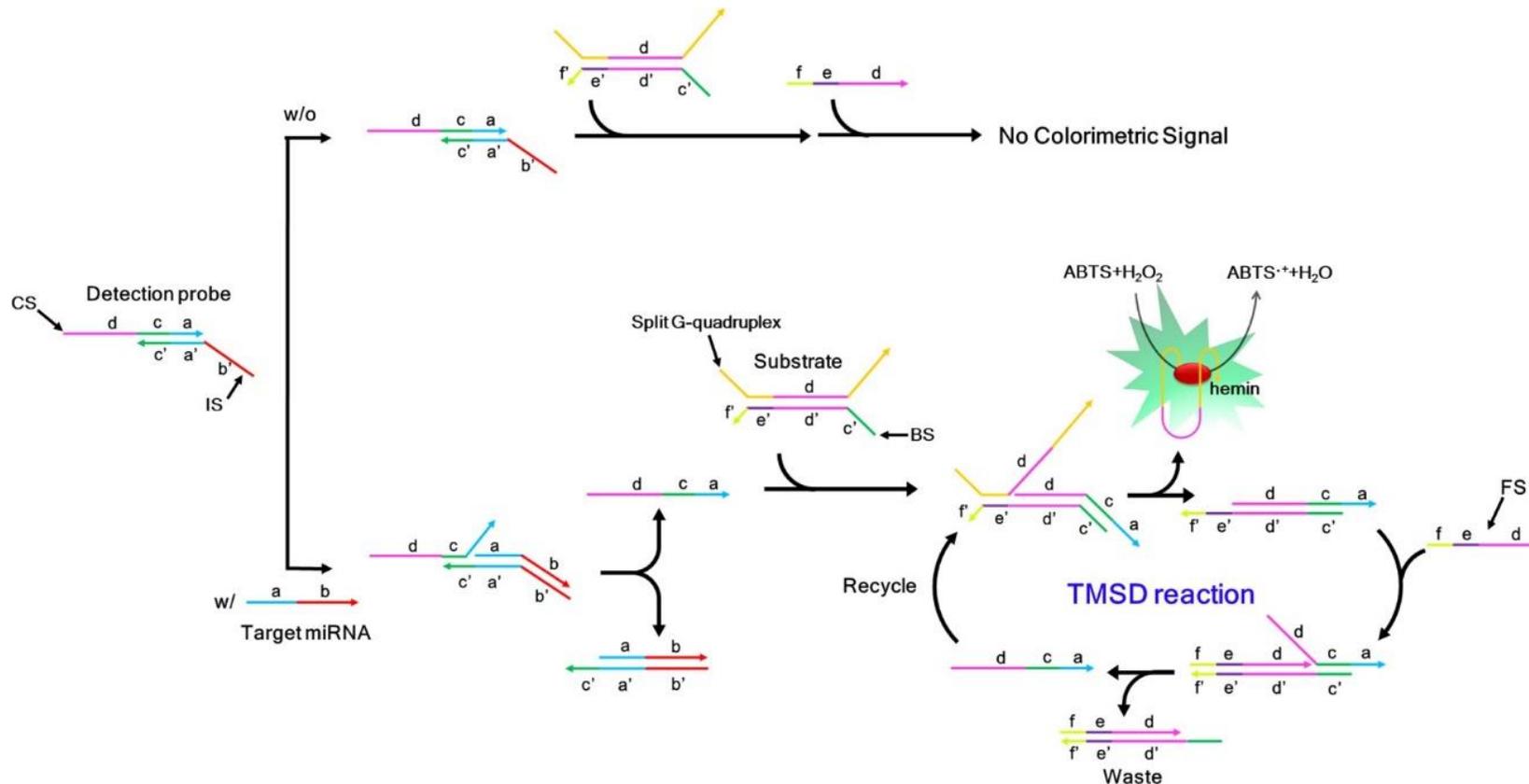


Universal miRNA detection based on target induced toe-hold-mediated strand displacement reactions

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1. Introduction

1) miRNA:

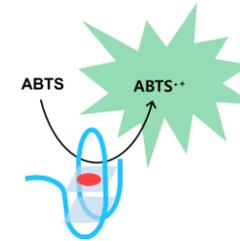
- MicroRNAs (miRNAs) are important diagnostic and prognostic biomarkers as their abnormal expressions are closely associated with diseases, including cancers, neuronal diseases, and diabetes.

2) G quadruplex DNzyme:

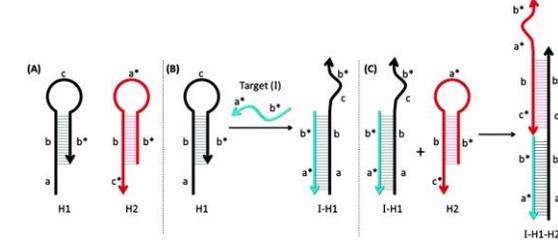
- G rich DNA sequence with horseradish peroxidase activity

3) Non enzymatic amplification:

- Methods such as hybridization chain reaction (HCR) and catalytic hairpin assembly (CHA) that attain high amplification efficiencies without the need for enzymes



G quadruplex DNzyme



Hairpin Chain Reaction (HCR)

Herein, we developed an enzyme-free strategy for the colorimetric detection of miRNA, based on target induced toehold-mediated strand displacement (TMSD) reactions. A detection duplex probe is designed to recognize target miRNA and release a catalyst strand (CS) intended to trigger subsequent TMSD reactions. The TMSD reactions result in the formation of active G-quadruplex DNzymes. Distinct colorimetric signal is generated by the ABTS oxidation promoted by the G-quadruplex DNzymes. Based on this strategy, we successfully detected miR-141, a biomarker for human prostate cancer, down to 0.48 nM with high selectivity.

2. Feasibility

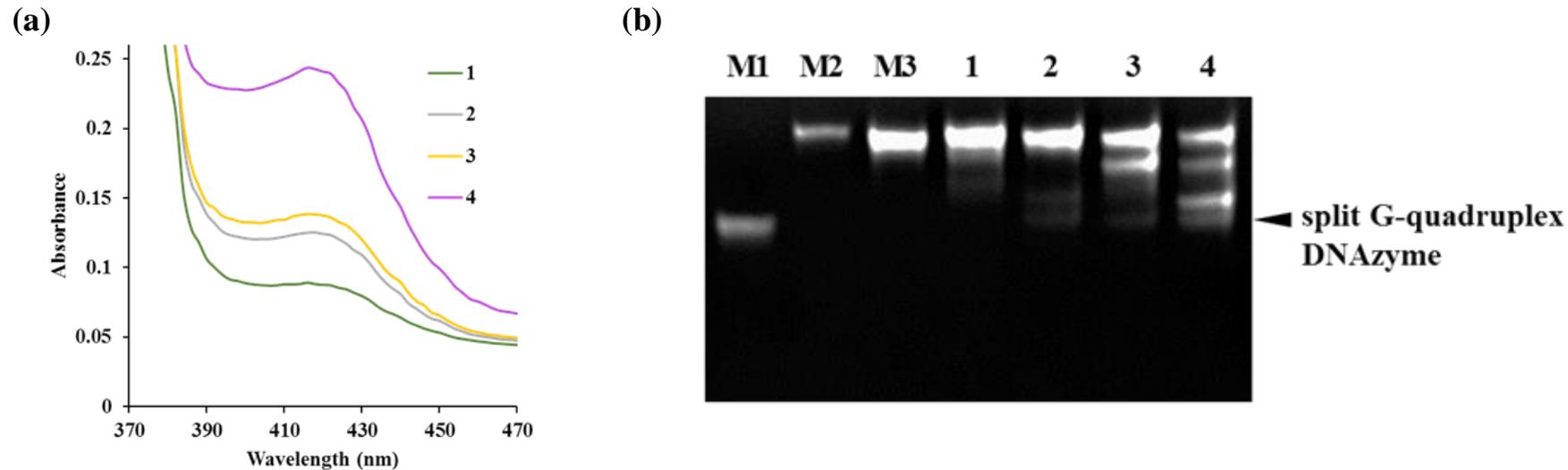


Figure 1. Feasibility of the miRNA detection strategy. **(a)** Absorbance spectra resulting from the ABTS oxidation promoted by the released G-quadruplex DNzymes under various conditions. **(b)** Polyacrylamide gel electrophoresis (PAGE) image for the products obtained after target catalyzed TMSD reaction (1: blank, 2: FS, 3: miR-141, 4: miR-141 + FS). Lanes M1, M2, and M3 show the markers for G-quadruplex DNzyme, detection probe, and substrate, respectively.

- To verify the feasibility, absorbance spectra resulting from the ABTS oxidation promoted by the released G-quadruplex DNzymes were measured under varying conditions. As anticipated, negligible absorbance signal was produced in the absence of both miR-141 and FS (curve 1, figure 1(a)). When either miR-141 or FS was present, a slightly enhanced, but still weak absorbance signal was observed, implying that CS or FS alone cannot effectively promote the TMSD reaction (curve 2 and 3, figure 1(a)). Most importantly, obvious enhancement of absorbance signal was observed when both miR-141 and FS were applied (curve 4, figure 1(a)), indicating that both the target-induced release of CS and the FS-mediated recycling of CS are critical for the proposed strategy to sensitively detect target miRNA.
- PAGE analysis of products obtained after target-catalyzed TMSD reaction was also performed to support the absorbance results.

3. Sensitivity

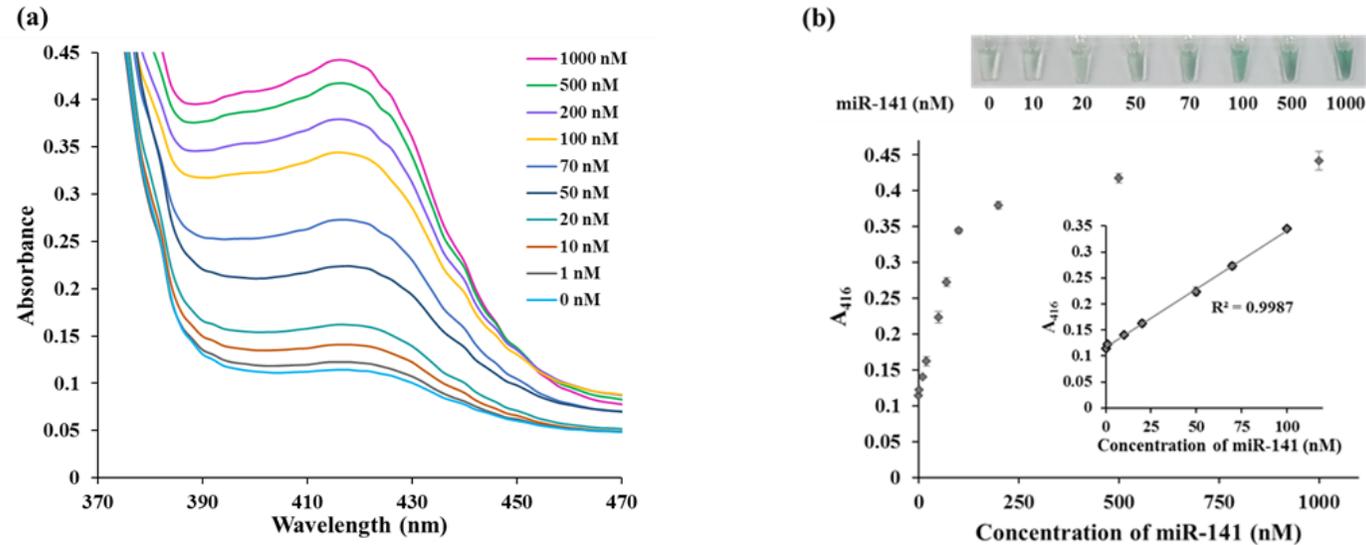


Figure 2. Sensitivity of the miRNA detection system. (a) Absorbance spectra and (b) absorbance intensity at 416 nm (A_{416}) resulting from the ABTS oxidation promoted by the released G-quadruplex DNazymes in the presence of miR-141 at varying concentrations. Inset: linear relationship between A_{416} and miR-141 concentration (0–100 nM). Photograph: images obtained from the sample solutions in the presence of miR-141 at varying concentrations.

Under the optimized conditions, the detection sensitivity was determined by measuring the absorbance intensities at 416 nm, the maximum wavelength of oxidized ABTS, as a function of miR-141 concentration. The results show that the absorbance intensity increased with increasing concentrations of miR-141 up to 500 nM, and then reached a plateau at concentrations over 500 nM (figure 2). An excellent linear relationship ($R_2 = 0.9987$) exists in the range of 0–100 nM and the limit of detection (LOD) ($3\sigma/\text{slope}$) is ca. 0.48 nM, a value that is comparable or better than those from previous colorimetric miRNA detection systems (figure 2(b))

4. Selectivity

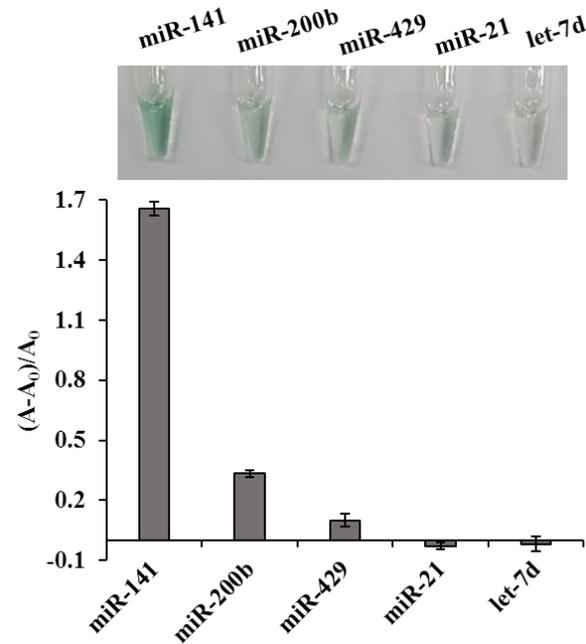


Figure 3. Selectivity of the miRNA detection system. The degree of absorbance enhancement at 416 nm in the presence of miR-141 (100 nM) and other miRNAs (100 nM). The degree of absorbance enhancement is defined as $(A-A_0)/A_0$, where A and A_0 are the absorbance at 416 nm resulting from the ABTS oxidation promoted by the released G-quadruplex DNazymes in the presence and absence of miRNA, respectively. Photograph: images obtained from the sample solutions in the presence of miR-141 and other miRNAs.

In order to assess the selectivity of the present detection method, other miRNAs were examined for their abilities to induce the enhancement of colorimetric signal, which were then compared with that of target miR-141. Family members of miR-141, miR-200b and miR-429 with similar sequences were included for comparison. As shown in figure 3, target miR-141 led to the significantly enhanced absorbance signal as evidenced by the intense green color, while negligible absorbance signals were observed in the presence of non-target miRNAs.

5. Real sample test

(a)

Added miR-141 ^a (nM)	Measured miR-141 ^b (nM)	SD ^c	CV ^d (%)	Recovery ^e (%)
40	43.0	2.62	6.08	108
60	59.2	3.77	6.36	98.7
90	89.9	2.04	2.27	99.9

(b)

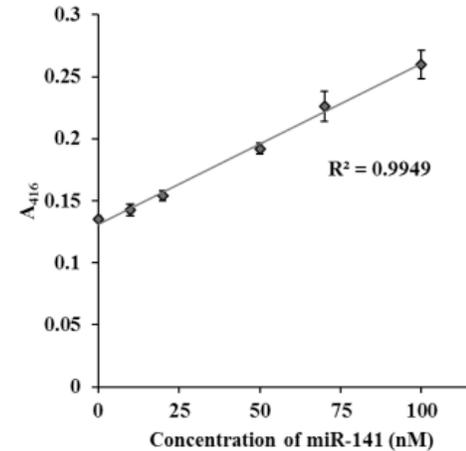


Figure 4. Determination of miR-141 in human serum. **(a)** ^aTo measure the concentration of miR-141, a calibration curve was first created by using standards containing a known concentration of miR-141 spiked in diluted human serum (1%). Based on this calibration curve, the absorbance at 416 nm from the unknown samples was used to determine the concentration of miR-141 present in human serum. ^b Mean of three measurements. ^c Standard deviation of three measurements. ^d Coefficient of variation = SD/mean × 100. ^e Measured value/added value × 100. **(b)** Absorbance intensity at 416 nm (A_{416}) resulting from the ABTS oxidation promoted by the released G-quadruplex DNAzymes in the presence of miR-141 at varying concentrations spiked in human serum.

The practical applicability of the proposed strategy was also demonstrated by determining miRNA in human serum sample. For this, mock clinical samples were prepared by spiking various concentrations of miR-141 into the diluted human serum and subjected to the detection procedures used in the above experiments. As shown in figure 4(b), good linear relationship ($R_2 = 0.9943$) was obtained in mock clinical samples and the concentrations of miR-141 were successfully determined with the coefficients of variation less than 6.36% and recovery rates between 98.7% and 108% (figure 4(a)), confirming the excellent reproducibility and precision of this method.