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A label-free colorimetric isothermal cascade amplification for the detection of disease-related nucleic acids based on double-hairpin molecular beacon

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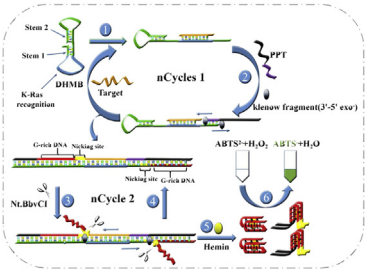
HIGHLIGHT

- A new colorimetric assay for K-Ras gene detection was developed based on double-hairpin molecular beacon (DHMB).
- Utilizing this colorimetric scheme, target DNA detection and screening of point mutations were accomplished by the naked eye.
- The colorimetric cascade amplification platform also holds the potential for common non-oligonucleotide target detection.
- The DHMB-based colorimetric strategy is a promising candidate platform for diagnostic applications.

GRAPHICAL ABSTRACT



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ABSTRACT

K-Ras mutations at codon 12 play an important role in an early step of carcinogenesis. Here, a label-free colorimetric isothermal cascade amplification for ultrasensitive and specific detection of K-Ras point mutation is developed based on a double-hairpin molecular beacon (DHMB). The biosensor consists of DHMB probe and a primer-incorporated polymerization template (PPT) designed partly complementary to DHMB. In the presence of polymerase, target DNA is designed to trigger strand displacement amplification (SDA) via promote the hybridization of PPT with DHMB and subsequently initiates cascade amplification process with the help of the nicking endonuclease. During the hybridization and enzymatic reaction, G-quadruplex/hemin DNAzymes are generated, catalyzing the oxidation of $ABTS^{2-}$ by H_2O_2 in the presence of hemin. Utilizing the proposed facile colorimetric scheme, the target DNA can be quantified down to 4 pM with the dynamic response range of 5 orders of magnitude, indicating the substantially improved detection capability. Even more strikingly, point mutation in K-ras gene can be readily observed by the naked eye without the need for the labeling or expensive equipment. Given the high-performance for K-Ras analysis, the enhanced signal transduction capability associated with double-hairpin structure of DHMB provides a novel rout to screen biomarkers, and the descripted colorimetric biosensor seems to hold great promise for diagnostic applications of genetic diseases.

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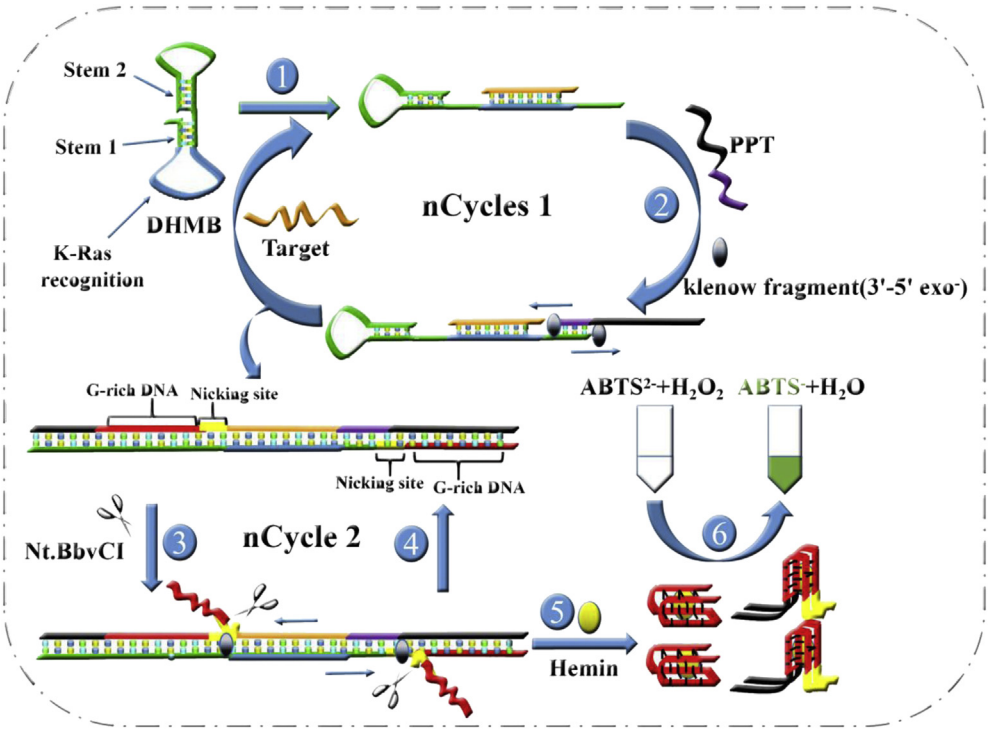
1. Introduction

Nucleic acid analysis plays an important role in gene therapy, molecular diagnostics, and biomedical research [1–3]. As we know, mutated Ras is related to cancers [4]. The mutations in K-Ras found in codons 12 and 13 appear early during tumorigenesis, and thus can be used as an important marker for the early diagnosis of cancer [5–7]. In spite of the considerable importance, screening K-Ras point mutations is challenging because the large excess of wild-type DNA and other DNAs usually found in clinical samples [8,9]. The general strategy used to overcome this difficulty is often based on the enrichment of the mutation DNA before target hybridization detection [10]. However, multiple procedural manipulations involved makes this-type of method time-consuming and cost-ineffective. As the classical target amplification method, polymerase chain reaction (PCR) has been used for single-molecule analysis [11]. Unfortunately, PCR requires precise control of temperature cycling and primer design and is also prone to generate false positives [12]. In recent years, some alternative signal amplification strategies to PCR have been exploited for target nucleic acid detection, including the hybridization chain reaction (HCR) [13,14], strand displacement amplification [15–17], exonuclease III-assisted target recycling amplification [18–20], and the rolling circle amplification (RCA) technique [21,22]. Nevertheless, each of these methods has its own strengths and limitations. For instance, RCA sometimes is time-consuming and labor-intensive to generate the circular padlock and to form a long complementary repeated DNA product and in some cases inefficient.

It is well known that, for optical biosensors, the target detection is easy to implement, and high sensitivity, good stability and fast response can be achieved [8]. Moreover, as a simple and convenient method, colorimetric analysis is especially suited for rapid and effective point-of-care diagnostics because the experimental results are able to be monitored with the naked eye. For example, hemin/G-quadruplex horseradish peroxidase (HRP)-mimicking DNAzyme was introduced to develop optical biosensors for detection of DNA [23]. This catalytic nucleic acids were also employed to design the MBzyme for the ultrasensitive colorimetric amplification analysis of DNA hybridization via the direct visualization of the color change [17], offering an opportunity to propose new-type of colorimetric technique for visual readouts of K-Ras point mutations.

In the present study, we described a label-free colorimetric method for isothermal cascade amplification detection of disease-related nucleic acids K-Ras based on double-hairpin molecular beacon (DHMB) and strand displacement amplification (SDA). The signaling strategy is depicted in Scheme 1, where the nicking-mediated SDA is assigned to promote the continuous accumulation of HRP-mimicking DNAzyme. Specifically, in the presence of target K-Ras, one loop of DHMB is opened, making PPT bind to the DHMB/target duplex to initiate bidirectional polymerization. This not only can form double-stranded DNA (dsDNA) containing two binding sites for nicking enzyme but also peels off hybridized target. The displaced target DNA can initiate the next round reactions. With the help of Nt.BbvCI endonuclease, nicking/SDA reaction occurs repeatedly on two regions of the resulting dsDNA, dramatically generating the large amount of G-quadruplex-contained DNAzyme. This DNA species can bind to hemin and catalyze the oxidation of $ABTS^{2-}$ by H_2O_2, producing the highly sensitive colorimetric signal. Herein, the signaling principle of DHMB-based colorimetric amplification detection system was elucidated, the working conditions were optimized and the assay performance was evaluated by comparison with conventional MB. Strikingly, the point mutations occurring in K-Ras can be visualized by the naked



Scheme 1. Schematic illustration of colorimetric biosensor based on double hairpin molecular beacons (DHMB) for amplification detection of K-Ras DNA. Step ①: hybridization between the loop region of DHMB and target K-Ras to make DHMB stem 2 open; Step ②: PPT binding to the exposed stem to allow bidirectional polymerization by KF polymerase, which displaces the target DNA that can bind to another DHMB to initiate a new cycle; Step ③: Nt.BbvCI specifically recognizes the nicking site to cleave the extended DNA strand, and polymerization occurs from the nick, releasing the G-rich oligonucleotide; Step ④: Reforming a complete duplex; Step ⑤: Forming the DNAzyme upon addition of hemin, which can act as peroxidase mimics to catalyze the $ABTS^{2-}$- H_2O_2 system.

eye. Seemingly, the proposed biosensor may become a potential tool for diagnostic applications.

2. Experimental section

2.1. Chemicals and materials

Oligonucleotides used in this study were synthesized by Invitrogen Bio Inc. (Guangzhou, China). Their sequences are listed in Table 1. All oligonucleotides were dissolved and diluted with TE buffer (10 mM Tris, 1 mM EDTA, pH = 7.6) to a concentration of 10 μM followed by storage at 4 °C prior to use.

Klenow fragment (3′-5′ exo$^-$) (5 U/μL) and Nt.BbvCI (10 U/μL) were purchased from New England Biolabs (USA) Ltd, while the deoxynucleotide solution mixture (dNTPs) was obtained from Dingguo Changsheng Biotechnology Co., Ltd (Beijing, China). Hemin and 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{2-}$) were purchased from Sigma-Aldrich Co. LLC, and hydrogen peroxide (H_2O_2) was obtained from Xilong Chemical Co., Ltd. All other reagents were of analytical grade. Hemin solution was prepared by dimethyl sulfoxide (DMSO) and stored in the dark at −20 °C, while all other solutions were prepared with double-distilled water (Kerton lab MINI water purification system, 18.25 MΩ cm).

2.2. Apparatus

A UV-2700 spectrophotometer (Shimadzu, Japan) was used to monitor the absorption spectra in the wavelength range from 350 to 500 nm at room temperature, and the absorption peak at 415 nm was chosen as signal readout to evaluate the proposed method. The concentration of oligonucleotides was measured using Q5000 UV/Vis spectrometer (Quawell Technology, Inc., USA) at 260 nm, and mobile phone (Huawei Honor 7) was used to take all photographs.

2.3. Isothermal cascade amplification detection of K-Ras based on double-hairpin molecular beacon

Before the experiment, DHMB was heated to 95 °C for 5 min, followed by gradually cooling down to room temperature to make the probe perfectly fold into a hairpin structure [24]. The detailed procedure for isothermal cascade amplification detection of K-Ras based on DHMB was as follows. First, 1 μL of 400 nM DHMB, 1 μL of K-Ras with specific concentration, and 1 μL of 400 nM PPT were added to tris-buffer (18 μL) (100 mM NaCl, 50 mM KAc, 10 mM $MgAc_2$ and 1 mM DTT, pH 8.2). After incubation at room temperature for 20 min, 1 μL of 10 mM dNTP, 0.3 μL of 5 U/μL Klenow fragment (3′-5′ exo$^-$) and 0.5 μL of 10 U/μL Nt.BbvCI were introduced to initiate strand displacement amplification (SDA), and the

Table 1
Oligonucleotides used in the experiments.[a]

Note	Sequence order (5'-3')
Double Hairpin Molecular Beacon (DHMB)	GTCAGCGCCCAACCCGCCCTACCCCGCTGACCAGGCT GAGGTGCC TAC GCC ACC AGC TCCCAG CCT GTC
Single Hairpin Molecular Beacon (SHMB)	CAATCCTCCCAACCCGCCCTACCCCGCTGACCAGGCTG AGGTGCC TAC GCC ACC AGC TCCCAG CCT GTC
PPT 1 (Primer-contained polymerization template1)	CCCAACCCGCCCTACCCGCTGAGGACAGG
PPT 2	CCCAACCCGCCCTACCCGCTGAGGACAGGC
PPT 3	CCCAACCCGCCCTACCCGCTGAGGACAGGCT
PPT 4	CCCAACCCGCCCTACCCGCTGAGGACAGGCTGG
PPT 5	CCCAACCCGCCCTACCCGCTGAGGACAGGCTGGGA
Target DNA (K-Ras gene)	TTGTGGTAGTTGGAGCTGGTGGCGTAGGCAAGAGTG
Mutant target DNA 1 (MT1)	TTGTGGTAGTTGGAGCTGcTGGCGTAGGCAAGAGTG
Mutant target DNA 2 (MT2)	TTGTGGTAGTTGGAGCTGcTGGCcTAGGCAAGAGTG
Mutant target DNA 3 (MT3)	TTGTGGTAGTTGGAcCTGcTGGCcTAGGCAAGAGTG

[a]For DHMB, the two underlined (stem 1) portions and two boxed portions (stem 2) are complementary to each other and can form two stems by self-hybridization, respectively. The shaded characters in DHMB (loop 2) indicate the K-Ras recognition sequences, which are complementary to the K-Ras target DNA. The double underline bases in PPT sequences represent the regions complementary to the part in double underline close to the 3' terminus of DHMB. Green letters represent the C-rich oligonucleotide. The letters in red bold were designed a half of the recognition sites of the nicking enzyme Nt.BbvCI; MT1, MT2, and MT3 have the same sequences as K-Ras but with one, two, and three points mutation in red lower case letter, respectively.

enzymatic reactions are designated to proceed at 37 °C for 2 h. Then, the mixture was heated to 80 °C for 20 min to terminate the activity of the polymerase and nicking endonuclease. Next, 2 μL of 50 μM hemin was added, and the mixture was incubated at 37 °C for another 2 h to generate a large number of DNAzymes. The total volume of the resulting solution was 25 μL.

2.4. Colorimetric measurement

An 87.5-μL droplet of 2 × HEPES buffer (50 mM HEPES, 20 mM KCl, 0.4 M NaCl, 2% DMSO, 0.1% Triton X-100, pH = 5.2) was added to the above-mentioned enzymatic reaction solution. Then, 5 μL of 36 mM $ABTS^{2-}$ and 2.5 μL of 4 mM H_2O_2 were successively added to reach the final concentration of 1.5 mM and 83.3 μM, respectively, followed by incubation for 30 min. The UV–vis absorbance measurements of the resulting were performed on UV-2700 spectrophotometer.

3. Results and discussion

3.1. Working principle

The working principle of isothermal cascade amplification detection of K-Ras based on double-hairpin molecular beacon probe is shown in Scheme 1. In the probe design, double-hairpin molecular beacon (DHMB) probe consists of four domains: a K-Ras recognition domain, half recognition sequence of Nt.BbvCI, primer-containing fragment complementary to polymerization template (PPT), and the C-rich fragment, whose complementary sequence can fold into G-quadruplex structure responsible for the formation of HRP-mimicking DNAzyme. In the absence of K-Ras, the DHMB is in its initially locked configuration by self-hybridizing of stem 2. In other words, the formation of DNAzymes or the initiation of the polymerization by Klenow fragment (3′-5′ exo$^-$) is prohibited. In contrast, in the presence of K-Ras, the stem 2 of DHMB can be opened by hybridization with target gene. Then, the released single stranded domain at the 3′ end of DHMB is able to hybridize with PPT and initiates bi-directional polymerization induced by Klenow fragment (3′-5′ exo$^-$), generating double-stranded DNA (dsDNA) with two recognition sites for the nicking endonuclease Nt.BbvCI. Meanwhile, the hybridized target DNA is displaced and re-hybridizes to another DHMB, activating a new cycle. This constitutes the nCycles I after cycle-after-cycle of hybridization, polymerization and displacement. Furthermore, with the help of nicking endonuclease Nt.BbvCI, the cleavage of recognition site of dsDNA occurs, contributing to the generating a large number G-quadruplex through repetitive cycle of polymerization, nicking and displacement (called nCycles II). In this case, hemin can bind to G-quadruplex nanostructure, forming the HRP-mimicking DNAzyme that may catalyze the oxidation reaction of $ABTS^{2-}$-H_2O_2 system. As a result, not only can the amplified signal be achieved but also the level of K-Ras in sample can be detected according to the color change.

3.2. Feasibility of colorimetric assay

For proof-of-concept demonstration of the proposed colorimetric sensing method, K-Ras was used as the model target DNA and examined by UV–vis absorption spectroscopy. The experimental results are shown in Fig. 1. As a control experiment, the mixture containing hemin and $ABTS^{2-}$-H_2O_2 without target species was investigated, and a weak absorbance is observed at 415 nm (line a), indicating that mixture itself hardly generates a catalytic efficiency toward $ABTS^{2-}$-H_2O_2 without DNAzyme. When DHMB was introduced into the mixture, the absorption spectroscopy

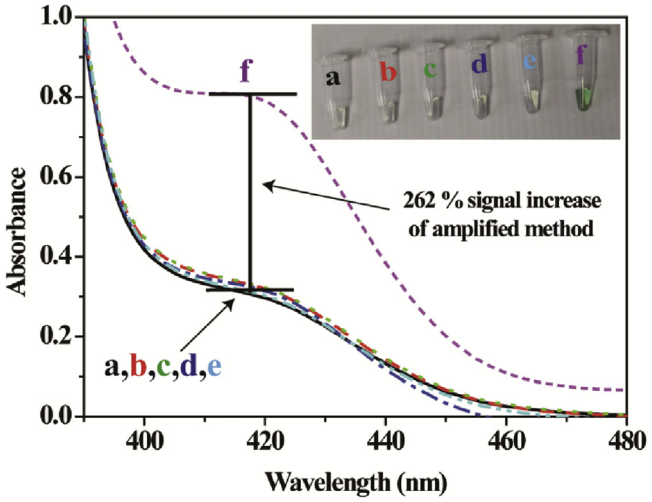


Fig. 1. UV–vis absorption spectra of $ABTS^{2-}$ under different conditions: (a) 8 μM hemin+1.5 mM $ABTS^{2-}$+83.33 μM H_2O_2, (b) 0.4 μM DHMB+8 μM hemin+1.5 mM $ABTS^{2-}$+83.33 μM H_2O_2, (c) 0.4 μM DHMB+0.4 μM PPT4+ 2.5 U KF+5 U Nt.BbvCI+8 μM hemin+1.5 mM $ABTS^{2-}$+83.33 μM H_2O_2, (d) 0.4 μM DHMB+0.4 μM target+0.4 μM PPT4+5 U Nt.BbvCI+8 μM hemin+1.5 mM $ABTS^{2-}$+83.33 μM H_2O_2, (e) 0.4 μM DHMB+0.4 μM target+0.4 μM PPT4+2.5 U KF+8 μM hemin+1.5 mM $ABTS^{2-}$+83.33 μM H_2O_2, (f) 0.4 μM DHMB+0.4 μM target+0.4 μM PPT4+2.5 U KF+5 U Nt.BbvCI+8 μM hemin+1.5 mM $ABTS^{2-}$+83.33 μM H_2O_2. The inset shows the photographic images of corresponding reaction tubes.

remains substantially unchanged (line b) relative to line a. Even if further introduction of PPT, Klenow fragment (3′-5′ exo$^-$) and Nt.BbvCI, the absorbance value (line c) still shows almost no change, suggesting generation of no hemin/G-quadruplex DNAzyme in the blank. More control experiments in the presence of K-Ras, but without one of two enzymes (namely, in the presence of Nt.BbvCI but no Klenow fragment for line d; in the presence of Klenow fragment but no Nt.BbvCI for line e) were explored, and no substantial change in absorbance at 415 nm is detected. However, a remarkable increase in absorbance intensity can be achieved when the coexistence of K-Ras, PPT, Klenow fragment (3′-5′ exo$^-$) and Nt.BbvCI (line f). These measured data indicate that the introduction of target DNA can significantly trigger polymerization, nicking and displacement in the presence of DHMB, PPT, Klenow fragment

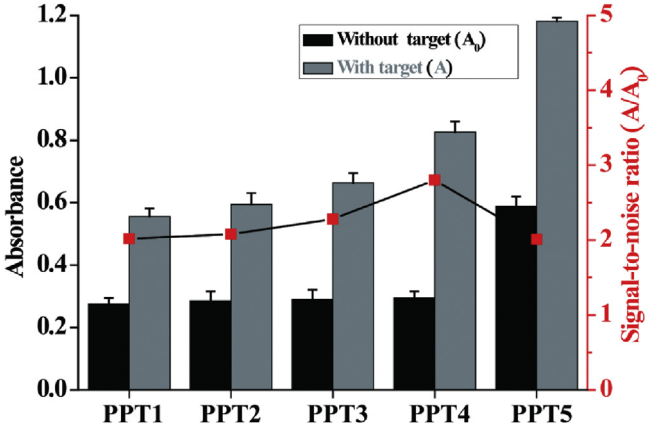


Fig. 2. Optimization of PPT sequence to improve the colorimetric analysis capability. A and A_0 are the UV–vis absorbance value at 415 nm in the presence and absence of target DNA, respectively. The heights of the bars represent the standard deviation calculated from three independent experiments (n = 3). The enzymatic reaction time was 90 min [DHMB] = 400 nM; [PPT] = 400 nM; [K-Ras] = 200 nM; [hemin] = 4 μM; [H_2O_2] = 83.33 μM; [$ABTS^{2-}$] = 1.5 mM.

and Nt.BbvCI, producing DNAzymes that accomplish the detection of K-Ras. In fact, the experimental results can be also observed by the naked eye as shown in the Inset. The enzymatic reactions were further validated by gel electrophoresis analyses, and the experimental results are shown in Fig. S1.

3.3. Optimization of PPT sequence

In the proposed signaling scheme, the bidirectional polymerization is dependent on the efficient hybridization of PPT with DHMB/target duplex, but PPT is required to be unable to the locked DHMB in the absence of target DNA. Thus, the PPT sequence has a direct effect on the amplification efficiency and is an important issue for colorimetric sensing performance. To optimize the PPT sequence, we designed different PPTs and investigated the target DNA-induced signal intensity. UV–vis absorbance peaks and the signal-to-noise ratios are presented in Fig. 2. With increasing the base number of double underlined fragment complementary to DHMB, both signal and noise increase (from PPT1 to PPT5), but PPT4 can generate the highest signal-to-noise ratio. This is because the interaction between PPT4 and DHMB/target DNA duplex is stable enough to initiate the bidirectional polymerization, and the background absorbance is substantially inhibited in the absence of target DNA. Thus, PPT4 was considered to be the optimal sequence for the colorimetric detection.

3.4. Optimization of colorimetric detection conditions

In order to achieve the analytical performance, the corresponding experimental conditions including the concentration of hemin and H_2O_2, the amount of Klenow fragment (3′-5′ exo$^-$) and Nt.BbvCI, and the reaction time and temperature were investigated. The signal-to-noise ratio (SNR) was used to evaluate the sensor performance. As shown in Fig. 3A, the SNR data indicates that the incubation of 90 min almost makes the signal reach a plateau. Therefore, 90 min was adopted as the optimal reaction time. Hemin can externally stack on the G-quadruplex structure owing to strong interaction [25]. As shown in Fig. 3B, the SNR increases with the increase of hemin concentration from 1 to 4 μM, followed by the

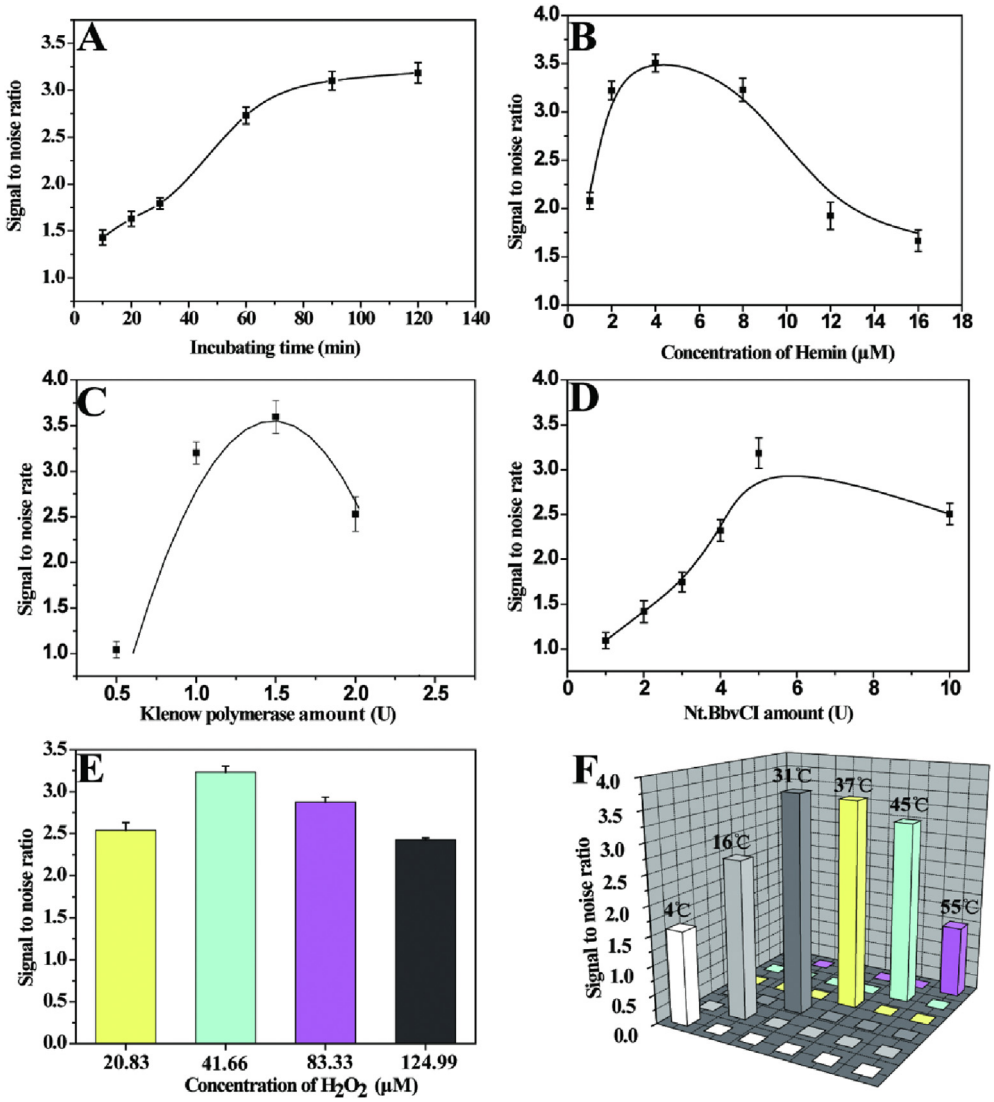


Fig. 3. The dependence of colorimetric amplification assay (estimated from the signal-to-noise ratio) on the reaction time **(A)**, concentration of hemin **(B)**, amount of Klenow exo$^-$ DNA polymerase **(C)**, amount of Nt.BbvCI nicking endonuclease **(D)**, H_2O_2 concentration **(E)** and incubating temperature **(F)**. The error bars represent the standard deviation of three measurements. The used DNA concentrations are described as follows: [DHMB] = 400 nM; [PPT4] = 400 nM; [K-Ras] = 200 nM; [ABTS] = 1.5 mM.

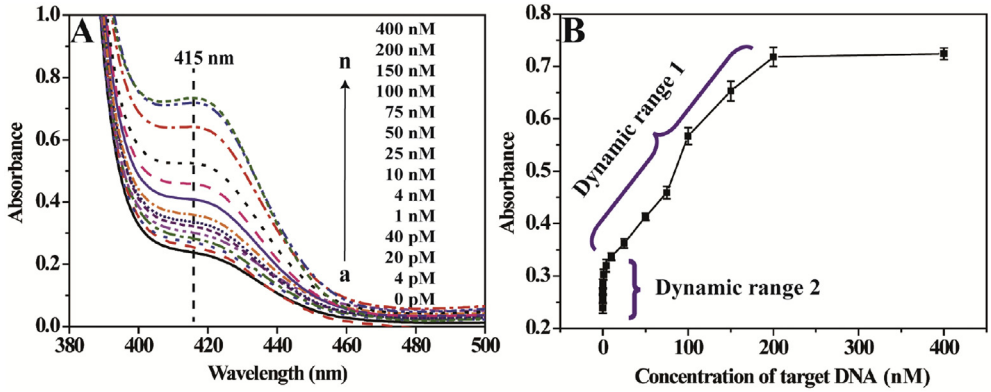


Fig. 4. Capability of the proposed colorimetric sensing method to detect K-Ras. **(A)** UV–vis absorption spectra in the presence of the different concentrations of K-Ras: (a), Control; (b–n), 4 pM to 400 nM. **(B)** The dynamic relationship between UV–vis absorption peak at 415 nm and target DNA concentration from 0 to 400 nM.

Table 2
Comparison of DHMB-based detection method with MB-based FRET assay and isothermal amplification methods.

Analysis method	Detection technique	Detection limit	Reference
Molecular beacon-based FRET assay	fluorescence	20 nM	[26]
The label-free luminescence switch-on method	luminescence	30 nM	[27]
nicking endonuclease signal amplification	colorimetry	1 pM	[28]
The gold nanoparticle-based colorimetric assay	colorimetry	10 nM	[29]
Based on amplifying fluorescent conjugated polymer	fluorescence	0.1 nM	[30]
Double-hairpin molecular beacon-based amplification	colorimetry	4 pM	This work

decrease beyond hemin concentration of 4 μM. Thereby, the 4 μM hemin was selected for the subsequent research. The amounts of Klenow fragment (3′-5′ exo$^-$) and Nt.BbvCI were also optimized. As presented in Fig. 3C and D, 1.5 U Klenow fragment and 5 U Nt.BbvCI were considered to be optimum amount used in this colorimetric system. This is because that the low concentration of enzymes cannot induce efficiently polymerization and nicking reaction to amplify the signal, while the very high concentration of enzymes would make the background increase, compromising the capability to detect the target DNA (namely, Signal-to-noise ratio). H_2O_2 plays a crucial role in the color change of the mixture. Therefore, the concentration of H_2O_2 was studied. As shown in Fig. 3E, 41.6 μM H_2O_2 was selected for further research. Because of the dependence of enzyme bioactivity strongly on the ambient temperature, the effect of enzymatic temperature on the amplification efficiency was studied from 4 to 55 °C. As shown in Fig. 3F, the SNR almost remains constant between 31 °C and 37 °C. Properly because higher incubating temperature affects the biological activity of enzymes, increasing further incubation temperature compromises the response signal. In the present study, 37 °C was chosen for the subsequent colorimetric assay.

3.5. Colorimetric assay of K-Ras gene

To evaluate the assay performance of the proposed colorimetric sensing strategy to quantify K-Ras, a series of samples containing different concentrations of K-Ras gene have been analyzed under optimized conditions. As described in Fig. 4A, the absorption peak increases with the increase of K-Ras concentration in the range of 0–400 nM. It is worth noting that 4 pM target DNA can be easily detected compared with the blank, and the target concentration is defined as the detection limit, which is comparable to or even lower

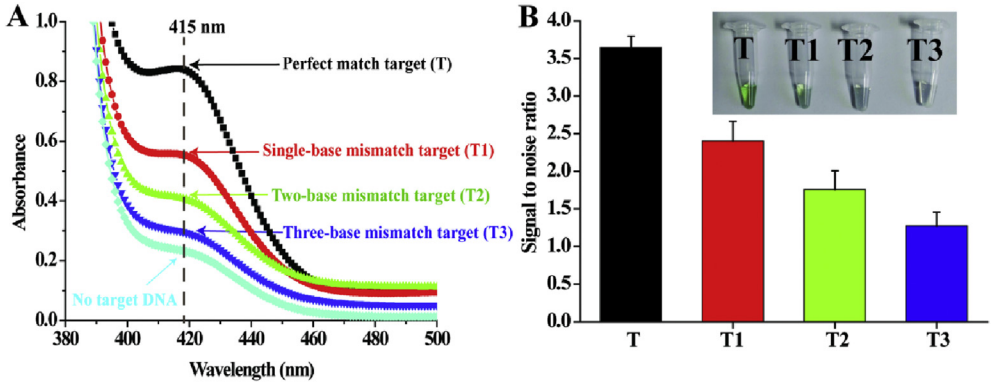


Fig. 5. Specificity of the proposed colorimetric assay. **(A)** UV–vis absorption spectra of the colorimetric biosensor exposed to different target DNAs: perfect matched target (T), single-base mismatched target (T1), two-base mismatched target (T2) and three-base mismatched target (T3). **(B)** The signal-to-noise ratio (A/A_0) values obtained from (A), where A and A_0 are the absorbance peak at 415 nm in the presence and absence of tested target, respectively. Inset: photographic images showing colorimetric responses to the different target DNAs. The concentration of perfect matched and mismatched targets is 200 nM. The concentrations of other species involved are seen in Fig. 2.

than the amplification methods as reporters [26–30] (Table 2). The absorbance peak at 415 nm was used to quantitatively scale the DNA concentration. As illustrated in Fig. 4B, we find that the plot of the response signal vs K-Ras concentration can be divided into two different dynamic response ranges. The absorbance peak exhibits a linear relationship in the concentration range from 0 pM to 4 nM with a correlation coefficient of 0.9889 (Fig. S2A). In the higher concentration range from 4 to 200 nM, a good linear relationship is also achieved with a correlation coefficient of 0.9924 (Fig. S2B). For comparison, we designed a single hairpin molecular beacon (SHMB), and the detection of target DNA was also conducted using the same protocol. As shown in Fig. S3, the linear range and detection limit were 1–100 nM and 1 nM, respectively. Namely, the screening capability of DHMB is approximately 3 orders of magnitude higher than SHMB, indicating the intriguing signal transduction efficacy of double-hairpin structure of molecular beacon.

3.6. Assay specificity

The detection specificity of the proposed DNA biosensor was investigated by measuring the absorption spectrum in the presence of four kinds of DNA sequences, the perfect matched target (T), single-base mismatched target (T1), two-base mismatched target (T2) and three-base mismatched target (T3). The capability to discriminate the perfect matched target from the mismatched ones is evaluated by calculating the signal-to-noise ratio (SNR). The measured results are described in Fig. 5. One can see that there is the obvious difference in absorption spectrum between perfect matched and mismatched target DNAs. Fig. 5B exhibits that the SNR values exhibit a step decrease from T to T3, clearly suggesting that the colorimetric sensing system displays the good detection specificity toward point mutations in K-Ras gene. More strikingly, as shown in Fig. 5B Inset, all mismatched target DNAs can be screened by the naked eye. It is known that the assay specificity of sensing system is associated with intermolecular hybridization [31] and probe structure [32]. Besides the high fidelity of DNA polymerase and the desirable specificity of Nt.BbvCI nicking endonuclease, the high detection specificity achieved in the newly-proposed system should be closed associated with the unique structure of DHMB. Double hairpin structure could easily screen the difference between perfect matched and mismatched target DNAs.

4. Conclusion

In this study, we have developed a label-free, isothermal, simple, rapid, highly selective and ultrasensitive colorimetric sensing platform for the detection of K-Ras gene based on DHMB, where polymerization/nicking reactions can repeatedly proceed and achieves the cascade amplification effect. The proposed method exhibits several advantages. First, the target DNA detection is very simple and convenient because only several mixing steps are involved during the signal transduction, and both hybridization, enzymatic reactions and oxidation of $ABTS^{2-}$ occur in an isothermal environment. Second, if two enzymes are not considered, the sensing system is significantly cost-effective and easy-to-use because the detection probes involved do not require any chemical modification and even the signal measurement can be accomplished without any expensive equipment [33]. Third, the proposed strategy exhibits remarkably high sensitivity and wide dynamic range. Fourth, a good assay specificity is achieved and the point mutations can be visualized by the naked eye. Furthermore, K-Ras recognition domain is expected to be replaced by aptamer to recognize other target species, and the colorimetric cascade amplification platform holds the potential for the common non-oligonucleotide target detection. The DHMB-based colorimetric strategy is a promising candidate platform for diagnostic applications.

Competing interests

The authors have declared that no competing interest exists.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.aca.2016.12.023.

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