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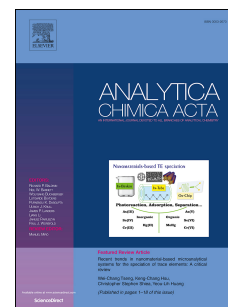
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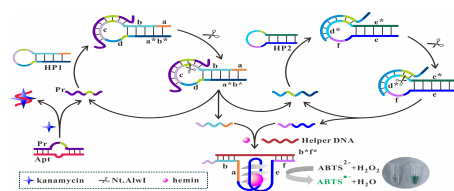
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Low-background and Visual Detection of Antibiotic Based on Target-activated Colorimetric Split Peroxidase DNAzyme Coupled with Dual Nicking Enzyme Signal Amplification

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Abstract

Herein, we have reported the development of a simple, rapid, and low cost colorimetric method for the detection of antibiotic based on target-activated split peroxidase DNAzyme coupled with dual nicking enzyme signal amplification (NESA). To lower background signal in G-quadruplex DNAzyme-based detection, the two split G-rich parts are caged into two different hairpin probes, respectively, preventing the two parts from assembling into the G-quadruplex structure. By the combination of restriction endonuclease-assisted cleavage reaction with the split G-quadruplex probes, target-modulated release of the two split G-rich parts is achieved, affording high specificity of antibiotic detection. Our strategy features with several aspects. First, the less background signal produced by the self-assembly of G-quadruplex in the absence of target is effectively eliminated owing to the pre-blocking of the two split G-rich parts. Second, dual NESA coupled G-quadruplex DNAzyme amplification strategy is integrated with colorimetric assay of antibiotic, which significantly improves the detection sensitivity. Third, peroxidase-mimicking DNAzyme is used as biocatalyst in our reaction system, which can catalyze the oxidation of 2,2' - azino - bis (3 - ethylbenzothiazoline - 6 - sulfonic acid) (ABTS^{2-}) mediated by H_2O_2 to generate the colored radical anion ($\text{ABTS}^{\cdot-}$), allowing to low cost and visual detection of antibiotic by the naked eye. Under optimized conditions, the results revealed the proposed biosensor exhibits excellent specificity and sensitivity toward kanamycin with a detection limit as low as 14.7 pM. Hence, the target-activated split G-quadruplex DNAzyme and dual NESA-based strategy provides a useful and practical platform for antibiotic

47 residues determination and other analytes detection in bio-analysis.

48 **Keywords:** split G-quadruplex probes; peroxidase DNAzyme; NESA; colorimetric;

49 antibiotic

50

1. Introduction

Antibiotics are mainly used to kill or inhibit microbes and bacteria, which are widely used in human and veterinary drugs [1]. Antibiotics can accumulate through the food chain in the human body, which may have a negative impact on human health even at low concentration, such as causing hearing impairment, toxic to organs [2]. In addition, their abuse causes humans to produce the super bacteria with tolerance to antibiotics, which will reduce the efficiency of disease treatment [3,4]. Antibiotic-resistant bacteria may spread to other microbial populations that pose a potential threat to human and animal health [5]. So, it is essential to control the intake of antibiotic and it is necessary to test whether the remaining antibiotics in the food contain more than the residue limit (MRL) before selling and distributing the food. [6] Therefore, the development of simple, rapid, high sensitive and specific assay methods for the determination of antibiotic residues in foodstuffs and environmental samples is in great need.

The conventional methods of detecting antibiotic residues mainly includes high-pressure liquid chromatography (HPLC) [7], gas chromatography (GC) [8], mass spectrometry (MS) [9], capillary electrophoresis (CE) [10], and liquid chromatography-tandem mass spectrometry (LC-MS) [8,11]. Despite their widespread applications, these methods suffer from some inherent shortcomings such as laborious pretreatments of samples, expensive instruments, and trained technical personnel. More recently, enzyme-linked immunosorbent assay (ELISA) [12] and electrochemistry-based methods have been reported for quantitative analysis of antibiotics [13,14].

73 These methods afford high sensitivity and demonstrate applicability in biological
74 samples. However, the procedures need tedious culture and wash steps, and careful
75 reagent handling, which is rather time-consuming and labor-intensive. Hence, it is
76 urgent for developing sensitive, robust, and ready-to-use strategy for antibiotic resi-
77 dues quantification.

78 Very recently, the colorimetric assay has drawn more and more concerns owing
79 to its superior performances of rapid feedback, cost-effectiveness, and visual detection
80 toward target analytes [17,18,19,20]. Until now, various efforts have been devoted to
81 the design of colorimetric strategies to meet the requirement for trace determination of
82 antibiotics residues in foodstuffs and environmental samples [21]. For example, Shen
83 et al. reported the development of a rapid colorimetric sensing of tetracycline antibi-
84 otics based on in situ growth of gold nanoparticles [22]. Duyen et al. developed a
85 simple colorimetric paper-based biosensor based on a novel principle for the detection
86 of antibiotics inhibiting bacterial protein synthesis [23]. Although the methods com-
87 bine some appealing features such as rapidness and simplicity, it is not difficult to
88 find their deficiency of non-ideal sensitivity. Therefore, the development of high sen-
89 sitive and easy-to-use colorimetric strategy for antibiotic residues detection is highly
90 desirable.

91 G-rich probes, which can reconstitute into a special G-quadruplex structure
92 [24,25] become active peroxidase-mimicking DNAzymes when bound with hemin in
93 the presence of K^+ [26,27,28]. G-quadruplex DNAzyme has gained increasing popu-
94 larity because it allows a simple detection via colorimetric means without the need of

additional labile reagents such as horseradish peroxidases conjugates. It has been reported that it is possible for G-rich probe to split the full-length single strand into two parts owing to the unique structure of the four GGG repeats [29]. The split strategy has been widely for detecting nucleic acid, in which the two G-rich parts can easily assemble to form an active anti-hemin aptamer in the presence of target DNA. However, it can't be ignored that less background signal produces because the two split parts have the tendency to assemble into active aptamer in the absence of target.

To lower background signal in G-quadruplex DNzyme-based detection, it is desirable to reduce or eliminate the possibility of forming active anti-hemin aptamer in the absence of target. One effective approach is pre-blocking the G-rich probe, in which the formation of G-rich structure is modulated by target analyte. For example, the frequently used signal-on sensing mode is generally achieved by target-triggered assembly of split DNzyme. However, these methods undoubtedly suffer from less background signal generated by free G-rich parts.

To resolve the problem of high background signal, we attempt to cage the two split G-rich sequence into two different hairpin structures, preventing the two G-rich parts from assembling into the G-quadruplex structure. Meanwhile, to achieve target-controlled liberation of the two split G-rich probes, restriction endonuclease-assisted cleavage reaction is used to cut off the hairpin probe. More importantly, dual nicking enzyme signal amplification (NESA) is integrated with the split G-quadruplex DNzyme to improve the sensitivity of colorimetric assay of analyte. The dual NESA consists of two cycles of NESA reaction, in which the upstream

products generated by the first cycle of NESAs reaction can act as the “DNA trigger” of the downstream cycle (the second cycle of NESAs reaction).

Herein, a simple, rapid, and low cost colorimetric method for the detection of antibiotic based on target-activated split peroxidase DNase coupled with dual NESAs has been developed. It is noteworthy that, besides the low background and high sensitive detection of analyte, our strategy also includes additional significant aspects. First, the nicking endonuclease *Nt.AlwI* can accurately and strongly identify the specific recognition sequence. By the specific design of the two hairpin probes, the two split G-rich parts producing after target-aptamer binding induced dual NESAs reaction, affording high specificity of target detection. Moreover, the proposed amplification system is based on the use of sole endonuclease, which saves the assay cost and decreases the risk of false-positive signal output. Second, we use peroxidase-mimicking DNase as biocatalyst in our reaction system, which can catalyze the oxidation of 2,2' - azino - bis (3 - ethylbenzothiazoline - 6 - sulfonic acid) (ABTS²⁻) mediated by H₂O₂ to generate the colored radical anion (ABTS^{•-}), therefore visual detection by the naked eye can be achieved [30,31]. Third, by redesigning the corresponding sequence for target recognition, our proposed target-activated split peroxidase DNase coupled with dual NESAs-based strategy can further extended for the detection toward a wide spectrum of analytes including nucleic acid, protein, and small molecules. Additionally, the split peroxidase DNase-based strategy can be also associated with other analytical techniques such as chemiluminescence. Thus, it might create a simple and highly efficient biosensing platform for antibiotic residues determination and oth-

er analytes detection in bio-analysis.

2. Materials and methods

2.1. Chemicals and reagents

Kanamycin, penicillin G, penicillin, tetracycline, aztrconam, streptomycin, L-tyrosine, tylosin, tilmicomisin, spiramycin, Chloromycetin, ampicillin were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). *Nt.A/wI* (10000 U/mL) and 10× reaction buffer (500 mM NaCl, 100 mM Tris-HCl, 100 mM MgCl₂, and 10 mM dithiothreitol, pH 7.9) were purchased from the New England Biolabs Ltd. (Beijing, China). Disodium 2,2' - azinobis (3 - ethylbenzothiazoline - 6 - sulfonic), Hydrogen peroxide and hemin were purchased from Fuchen Chemical Reagents. (Tianjin, China). All the chemicals were of analytical grade and the solutions were prepared using ultrapure water with an electric resistance > 18.25 MΩ, which was obtained through a Millipore Milli-Q water purification system (Billerica, MA, USA). Oligonucleotides used in this work were synthesized and purified by Sangon Biotechnology Co. Ltd. (Shanghai, China), and the sequences were listed in Table S1.

2.2. Antibiotic detection

The solution consisted of 2 μL 5 μM antibiotic aptamer (Apt) and its complementary oligonucleotide (Primer) were previously hybridized in equal proportions were incubated at 37 °C for 1 hour. Then 4 μL of this solution was added to 2 μL of kanamycin solution with different concentrations (ranging from 0 to 500 nM) and incubated at 37 °C for 1 h.

The DNA amplification analysis was performed by mixing the above DNA pri-

mer solution (6 μ L), 2 μ L 5 μ M HP1 solution, 2 μ L 5 μ M HP2 solution, NEB buffer (10 $\times$, 2 μ L) and 2 μ L 1000 mM KCl were incubated at 37 $^{\circ}$ C for 1h. Then, 10 U *Nt.AlwI* and 2 μ L 50 μ M helper DNA were added and allowed to incubate for 2 h at 37 $^{\circ}$ C. Subsequently, the final concentration of 0.6 μ M hemin was added and incubated for 1 h at 37 $^{\circ}$ C to form hemin/G-quadruplex structure. Finally, we added the final concentration of 2 mM ABTS and H₂O₂ respectively. The color change was observed and absorbance spectrum was obtained by UV-vis spectrometer (Shimadzu UV-2600, Japan). Real time spectral scanning was performed in the wavelength range from 400 to 500 nm at a fixed time interval of 5 min.

2.3. Real sample analysis

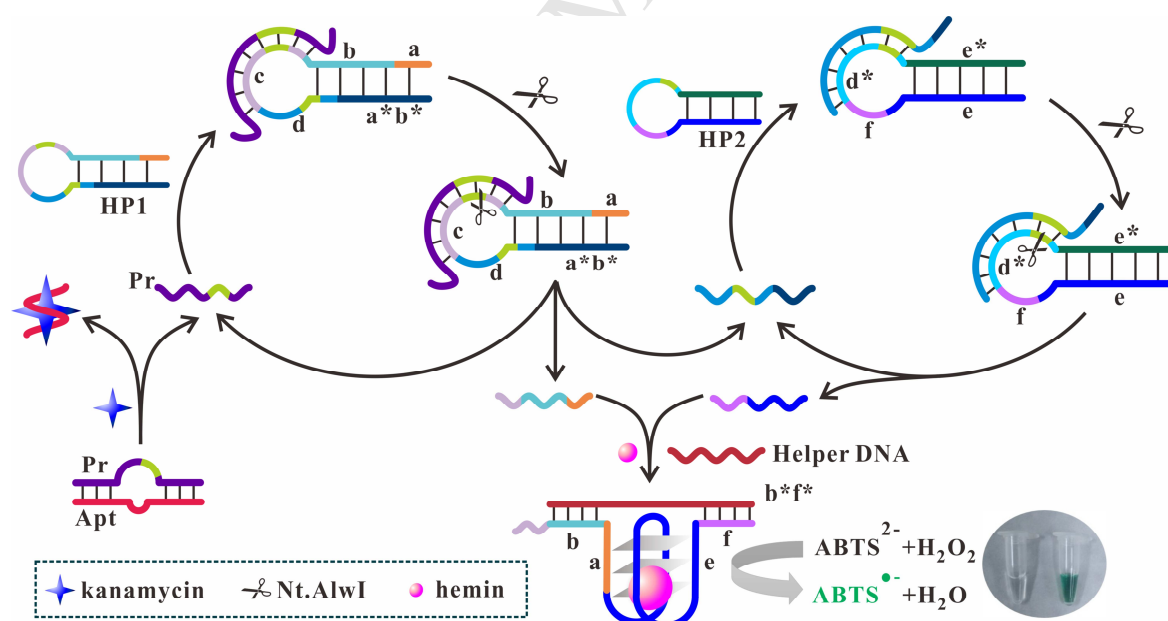
Milk was bought from a local supermarket. 2 mL of the milk was added to 8 mL of PBS (10 mM, pH 7.4) and completely mixed for 10 min. Then different concentrations of kanamycin were spiked into the diluted milk samples and detected directly without any pretreatments. The spiked samples were further quantified by a classic ELISA method and compared with results obtained with our method.

3. Results and discussion

3.1 The design of the hairpin probes

Our system involves of two hairpin DNA probes, an aptamer-primer (Ap-Pr) hybrid duplex probe, a helper DNA, and a nicking endonuclease *Nt.AlwI*. Two hairpin DNA probes (HP) are ingenious designed to be used for producing numerous unequal split of G-rich probes (one GGG repeat-inserted split probe and three GGG repeats-inserted split probe) with the aid of nicking endonuclease. The hairpin DNA probe 1 (HP1) consists of five regions: one GGG repeat-inserted split probe segment

(denoted in a in Scheme 1), the helper DNA-annealing segment (denoted in b), Pr-annealing segment containing the recognition sequence of nicking endonuclease *Nt.AlwI* (denoted in c in Scheme 1), the hairpin DNA probe 2 (HP2)-annealing segment (denoted in d), and split probe-blocking segment (denoted in a*b* in Scheme 1). The HP2 includes three GGG repeats-inserted split probe segment (denoted in e in Scheme 1), the helper DNA-annealing segment (denoted in f), HP1-annealing segment (denoted in d* in Scheme 1), and split probe-blocking segment (denoted in e* in Scheme 1). A helper DNA (denoted in b*f* in Scheme 1) is used for complementary with two split probes, respectively, thus generating assembly of G-quadruplex structure. The sequences and related descriptions of the used oligonucleotides were listed in table S1 in the supporting information.



Scheme 1 Schematic illustration of split peroxidase DNAzyme coupled with dual NESA-based colorimetric assay for antibiotic detection.

3.2 Principle of the colorimetric split peroxidase DNAzyme coupled with dual NESA strategy

Scheme 1 shows the working principle of the proposed strategy. The Ap-Pr hybrid duplex is pre-prepared by hybridizing anti-kanamycin aptamer with its complementary DNA (primer, Pr). In the presence of target kanamycin, the specific binding between anti-kanamycin aptamer and target induces the release of primer, which hybridizes with the loop region of HP1, thus forming double-stranded structures with specific recognition sites for *Nt.AlwI*. Immediately, HP1 is nicked and divided into two single-stranded DNA probes with the help of *Nt.AlwI*, which includes free one GGG repeat-inserted split G-quadruplex probes and the dissociated DNA probes used for complementary with the loop region of HP2. Meanwhile, the primer is dissociated from HP1 and again complementary to another HP1, initiating a new cycle of "hybridization-cleavage-dissociation" reaction. So, a great many of one GGG repeat-inserted split G-quadruplex probes are formed after target-activated NESA reaction. Then the dissociated DNA probes anneal with HP2, producing new DNA duplex containing the recognition sites for *Nt.AlwI*, thus HP2 is cleaved into two pieces with the aid of *Nt.AlwI*. Similarly, the falling DNA probes again anneal with other un-nicked HP2, which trigger dual NESA reaction and result in the formation of massive three GGG repeats-inserted split G-quadruplex probes. Subsequently, the produced two numerous split G-quadruplex probes are complementary to two termini of the helper DNA, respectively, resulting the overhung G-rich segments to assemble into G-quadruplex structure. After the addition of hemin, it binds to hemin and becomes G-quadruplex DNAzyme that possesses peroxidase-like activities. Thus, the produced DNAzyme catalyzes H_2O_2 -mediated ABTS^{2-} to generate the colored prod-

uct ABTS^{•-}, which enables the amplified colorimetric readout of the target concentration [32]. By the combination of target-controlled release of split G-quadruplex probes and dual NESA strategy, the current strategy is hopeful for offering a simple, rapid, and low cost colorimetric method for detecting antibiotics with low background and high sensitivity.

3.3 Feasibility Study

Fig. 1 depicts the typical absorption spectral responses of the proposed strategy in the assay of kanamycin. As shown in Fig. 1, for the mixture of HP1, HP2, and the helper DNA probe, it was observed that the absorption peak was neglected and the absorption intensity at 418 nm of was only 0.103, which was attributed that hemin displayed a very low catalytic activity toward the H₂O₂-mediated oxidation of ABTS²⁻ (curve a). This signified the split G-rich probes couldn't assemble into G-quadruplex structure due to the ingeniously HP design that pre-blocking the two split G-rich sequence. For the blank sample, there was a similar absorption spectrum to the mixture of HP1, HP2, and the helper DNA probe (curve b). However, in the presence of 500 nM kanamycin, a green color for ABTS^{•-} and 657 % of signal increase in the absorption intensity at 418 nm were observed, demonstrating the formation of G-quadruplex/hemin complex (curve c). This revealed the specific binding of target and aptamer triggered *Nt.AlwI*-catalyzed cyclic nicking reaction, releasing the massive split G-rich probes, thus the G-quadruplex DNAzyme is formed in the presence of the helper DNA and hemin. Additionally, the absorbance almost approximate to that of the blank sample was obtained in the absence of *Nt.AlwI*, suggesting the ab-

sorbance enhancement was attributed to *Nt.AlwI*-assisted release of split G-rich probes (curve d). When kanamycin was replaced by non-target Ampicillin, there was very low absorption intensity at 418 nm (curve e). Furthermore, time-dependent absorption changes upon the above samples were investigated, and the data were shown in Fig. S1. The obtained results were consistent with that of the absorption spectral measurements. This implied the change of absorbance signal was induced by the specific binding of target and aptamer. Therefore, the proposed strategy could be used for amplified detection of kanamycin.

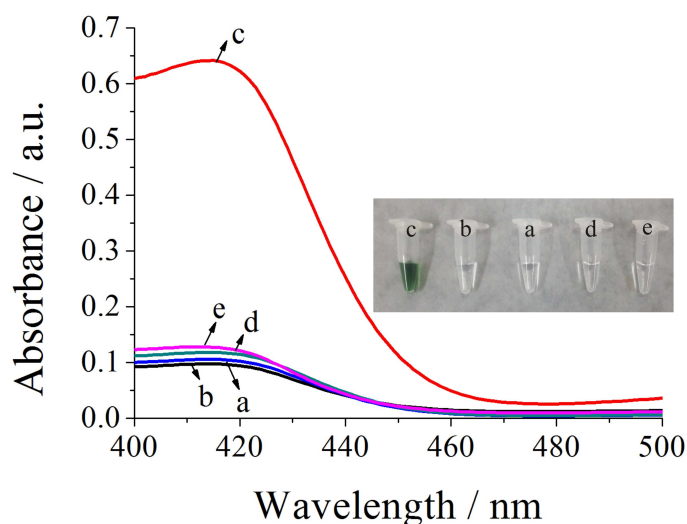


Fig. 1 Typical absorption spectral responses for colorimetric assay of kanamycin. 5 μ M HP1, 5 μ M HP2, and 5 μ M helper DNA (a), blank sample (b), positive sample (500 nM kanamycin) (c), 500 nM kanamycin without *Nt.AlwI* (d), 50 μ M Ampicillin in place of 500 nM kanamycin (e). Inset is the photograph.

3.4 Optimization of Experimental Conditions

In our assay, *Nt.AlwI*-assisted cleavage reaction is crucial for the formation of G-quadruplex DNAzyme. It is of great importance for the cleavage reaction time and

the concentration of *Nt.AlwI* to the yield of split G-rich probes. Different assay conditions were investigated in our studies, and the results were shown in Fig. 2. A long cleavage reaction time is expected to achieve enhanced signal amplification. The cleavage reaction time of *Nt.AlwI* was studied to probe the absorbance signal toward 500 nM kanamycin detection. As shown in Fig. 2A, it was found that the absorbance intensity for the positive sample increased continuously with the increase of the cleavage reaction time and kept constant when the reaction time reached 120 min, while there was no obvious change for that of the blank sample. Thus, the cleavage reaction time of 120 min was selected for the following experiments. The amount of employed *Nt.AlwI* had an effect on the efficiency of cleavage reaction as well. The concentration of *Nt.AlwI* also optimized on the basis of the absorbance signal toward the detection of 500 nM kanamycin, and the results were shown in Fig. 2B. In Fig. 2B, the positive sample, the absorbance intensity was intensified with the increase of the concentration of *Nt.AlwI* and experienced almost no change when the concentration reached 10 units; nevertheless, the absorbance signal was nearly unchanged for the blank sample. So, a concentration of 10 units was chosen as the optimized amount of *Nt.AlwI* for the cleavage reaction. Additionally, the amplification efficiency of this strategy may be influenced by the concentration of the helper DNA. So, the absorption responses to different concentrations of the helper DNA were investigated, and the results were shown in Fig. S2 in the supporting information. It was found that the concentration of 5 μ M gave the highest signal to noise ratio. Thus, the concentration of 5 μ M was used for the subsequent experiments.

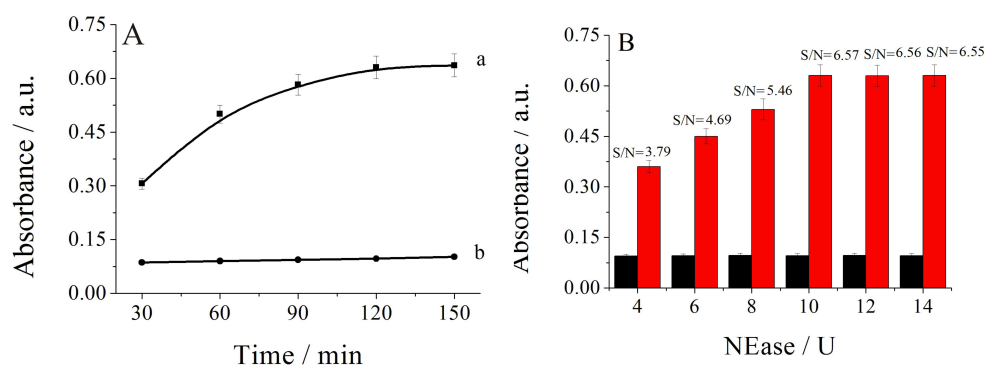


Fig. 2 (A) Effects of the incubation time of *Nt.AlwI* on the absorption signal of biosensor in the presence (curve a) and absence (curve b) of 500 nM kanamycin. (B) Effects of the concentration of *Nt.AlwI* on the absorption signal of biosensor in the presence (the red bar) and absence (the blank bar) of 500 nM kanamycin.

3.5 Analytical performance for kanamycin detection

Under the optimized experimental conditions, the analytical performance of constructed colorimetric assay method was investigated towards the detection of kanamycin with different concentrations. Fig. 3 displayed absorption spectral responses of the proposed method in the assays under the optimized conditions. It was found that in Fig. 3A the green color of the product $ABTS^{\cdot-}$ were gradually deepened with increasing concentration of kanamycin. Fig. 3B depicted time-dependent absorbance changes upon analyzing different concentrations of kanamycin. The absorbance values increased accordingly with the increasing concentration of kanamycin from 0 pM to 500 nM, which was consistent with that of the visual observations. This suggested more active G-quadruplex / hemin DNAzyme were formed with the increasing concentrations of kanamycin. This also verified the assembly of split G-quadruplex DNAzyme was initiated by the specific binding of target and aptamer. Additionally,

the absorption spectral in the range of 400-500 nm in the assays of kanamycin with different concentrations was inspected, and the results were shown in Fig. S2. It was observed that the absorption intensity at 418 nm increased accordingly in the increasing of kanamycin concentration. Fig. 3C displayed a good linear correlation of the absorption intensity of ABTS^{•+} at 418 nm to the logarithm of the concentrations of kanamycin ranging from 50 pM to 500 nM. The regression equation was $Y = 1.47 + 0.12 \lg C$ with a correlation coefficient of 0.992, where Y and C represented the absorbance intensity and kanamycin concentration, respectively. The detection limit was calculated to be 14.7 pM by evaluating the average response of blank sample plus 3 times standard deviation, indicating superior detection sensitivity compared with those previous reported methods. The detailed comparison of our proposed strategy with other methods was illustrated in Table S2 in the supporting information. The high sensitivity could be ascribed to extremely low background and high amplification efficiency of the present target-activated split G-quadruplex DNzyme and dual NESA strategy.

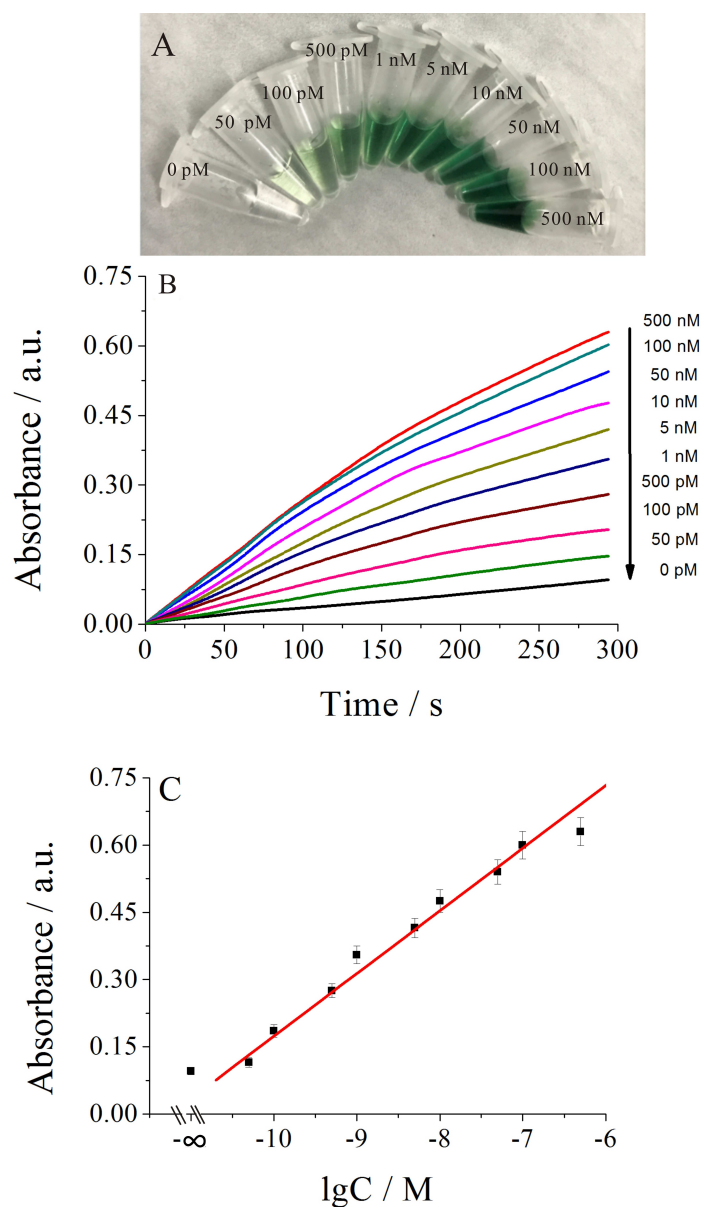


Fig. 3 (A) Pictures of the colorimetric method in the assay of kanamycin with varying concentrations. (B) Time-dependent absorbance changes upon analyzing kanamycin with varying concentrations. (C) The calibration curve of absorption peak intensity at 418 nm versus the logarithm of kanamycin with varying concentrations. Error bars are standard deviations across three repetitive measurements.

3.6 Detection specificity

The specificity of sensing strategy is highly desirable during practical applica-

tions. In order to evaluate the selectivity of the proposed method, we challenged the system with the target kanamycin and several non-target antibiotics such as ampicillin. The results were shown in Fig. 4. It was observed that the system only showed a remarkable absorbance signal in the presence of target kanamycin, while the absorption peak intensity was negligible in the presence of other non-target antibiotics. These results clearly demonstrated the absorbance signal was specifically triggered by target-activated split G-quadruplex DNzyme and dual NESA. This confirmed the high specificity of the proposed colorimetric strategy for kanamycin identification.

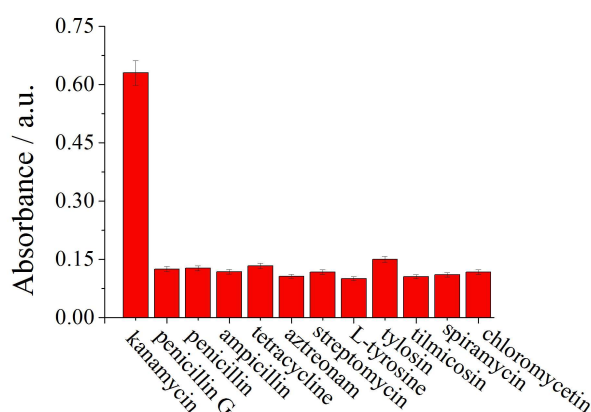


Fig. 4 Absorption peak intensities at 418 nm obtained in colorimetric assay in the presence of different antibiotics. The concentration of kanamycin is 500 nM. The concentration of non-target antibiotic is 50 μ M. Error bars are standard deviations across three repetitive experiments.

3.7 Real sample analysis

To explore the applicability of the proposed method in complex matrixes, the quantitative assay of kanamycin in the spiked milk samples was conducted using our method. Different concentrations of kanamycin were spiked into the milk samples and

measured directly without any pretreatment except for dilution with a buffer solution. For comparison, ELISA method was also used for the analysis of the same sample. The results were listed in Table. 1. As can be seen, these data obtained by our method and ELISA method showed good agreement with each other, and the discrepancies between the two methods were all smaller than 11.5 %. Besides, the recovery of the proposed method was in the range of 93.4-104.7 %, indicating acceptable accuracy of the method for kanamycin determination in complex matrixes. This clearly revealed that the proposed approach might hold great potential for real sample analysis with great accuracy and reliability.

Table. 1 Application of the colorimetric assay for kanamycin determination in milk and comparison with ELISA detection.

Spiked amount (nM)	Measured (nM)		Recovery (%)	
	Our method	ELISA	Our method	ELISA
1.000×10^{-1}	$(1.048 \pm 0.042) \times 10^{-1}$	$(0.977 \pm 0.038) \times 10^{-1}$	104.7 $\pm$ 4.3	97.7 $\pm$ 3.7
1.000×10^0	$(0.936 \pm 0.036) \times 10^0$	$(1.038 \pm 0.024) \times 10^0$	93.4 $\pm$ 3.7	103.8 $\pm$ 2.5
1.000×10^1	$(1.028 \pm 0.015) \times 10^1$	$(1.017 \pm 0.022) \times 10^1$	102.9 $\pm$ 1.4	101.7 $\pm$ 2.2
1.000×10^2	$(1.017 \pm 0.009) \times 10^2$	$(0.988 \pm 0.016) \times 10^2$	101.7 $\pm$ 0.9	98.7 $\pm$ 1.7

Conclusion

In summary, we have constructed a simple, rapid, and low cost colorimetric assay method for low-background detection of antibiotics based on target-activated split G-quadruplex DNzyme and dual NESAs. In our assay, the two split G-rich probes were caged into two hairpin structures, respectively, eliminating the possibility of assembling into active peroxidase DNzyme in the absence of target, thus low-background detection of target could be achieved. In the presence of target, pe-

359 roxidase DNAzyme could be easily recovered via target-triggered release of split
360 G-quadruplex probes with the aid of restriction endonuclease. This work is to our
361 knowledge the first report that split G-quadruplex DNAzyme coupled with dual
362 NESA has been used for colorimetric assay of antibiotics. Based on the amplification
363 strategy, this proposed biosensor can detect kanamycin with the detection limit down
364 to 14.7 pM without any labeling procedures and expensive equipment. Furthermore,
365 by redesigning the corresponding sequence for target recognition, the target-activated
366 split peroxidase DNAzyme and dual NESA strategy can be applied for the detection
367 of various aptamer binding molecules including nucleic acid, protein, and small mol-
368 ecules. In addition, this constructed strategy can be also associated with other analyti-
369 cal techniques such as chemiluminiscence. Hence, this method provides a versatile
370 and practical platform for detecting analytes with trace amounts in bio-analysis.

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Highlights

1. This sensor is the first report based on the pre-blocking of the two split G-rich parts could effectively eliminate the background signal produced by G-quadruplex in the absence of target.
2. We designed the dual NESA coupled G-quadruplex DNzyme amplification strategy is significantly improves the detection sensitivity.
3. We used the peroxidase-mimicking DNzyme as biocatalyst in our reaction system, which can catalyze the oxidation of ABTS^{2-} mediated by H_2O_2 to generate the colored radical anion $\text{ABTS}^{\bullet-}$.
4. The proposed biosensor can detect kanamycin with excellent sensitivity, and the LOD can be as low as 14.7 pM.
5. This biosensor created a versatile platform in detecting molecules with trace amounts in bioanalysis and molecular diagnosis.

Vitae

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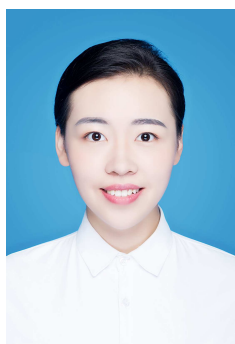
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