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Instrument-free visual detection of tetracycline on an autocatalytic DNA machine using caged G-quadruplex as the signal reporter

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A instrument-free visual biosensor for the amplified detection of tetracycline has been successfully constructed using an autocatalytic DNA machine as the signal amplifier and a caged G-quadruplex as the signal reporter. The assay is ultrasensitive, enabling the visual detection of trace levels of tetracycline as low as 1 pM without instrumentation.

Tetracycline is a broad-spectrum antibiotic which has been extensively used in veterinary and human therapy.[1] The abuse of tetracycline has led to antibiotic residues in food products that could be a serious threat to human health.[2] Thus, it is of great significant to fabricate efficient detectors for tetracycline monitoring with high sensitivity and selectivity.[3-7] Conventional analytical methods, including high-performance liquid chromatography (HPLC), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and capillary electrophoresis (CE) are widely utilized for tetracycline analysis.[8,9] However, these approaches require expensive equipments and highly trained personnel, limiting the use of these techniques for on-site screening in remote settings. As an alternative, colorimetric biosensors have attracted remarkable attentions for target determination because of their advantages of low cost, simplicity, rapid response time, and on-site analysis.[10-14] Importantly, the output of the colorimetric detector can be recognized readily by the naked eye without requiring additional instrumentation.[15-18] However, the colorimetric assay is often confronted with unsatisfactory sensitivity. Thus, it is still highly desirable to develop a new biosensor for instrument-free visual detection of tetracycline with amplified colorimetric signal.

DNAzymes have attracted considerable interest as amplifying labels for biosensing events due to the high cleavage capacity, good stability, low nonspecific adsorption, and easy preparation.[19,20] DNAzymes are synthetic nucleic acids which show high catalytic cleavage activity toward specific substrates.[21,22] As biocatalytic amplifiers, the catalytic cleavage reaction of DNAzymes can be carried out at room temperature with multiple turnovers.[23,24] The unique advantages make DNAzymes ideal biocatalysts for amplified sensing applications. For example, the Mg^{2+}-dependent DNAzyme has been used to design enzyme-free amplified assay platforms for convenient and highly sensitive detection of DNA and other biomolecules.[25-28] It was reported that the Mg^{2+}-dependent DNAzyme can be split into two inactive components with no catalytic cleavage activity, which will be assembled to form activated conformation by target-induced cooperative binding of the subunits.[29,30] In this study, we developed an autocatalytic DNA machine for instrument-free visual detection of tetracycline with cascade signal amplification using split Mg^{2+}-dependent DNAzyme subunits as the sensing elements and caged G-quadruplex as the reporter unit. The combination of DNAzyme technology with G-quadruplex to give a simple colorimetric readout can be used in remote settings.

The design strategy for instrument-free visual detection of tetracycline on the basis of autocatalytic DNA machine and caged G-quadruplex is schematically demonstrated in Scheme 1. The two nucleic acids DNA1 and DNA2 include the domains I and II that correspond to the subunits of the Mg^{2+}-dependent DNAzyme, the domains b and c are complementary to the loop region of the hairpin substrate (H1), and domains a and a* that reveal base complementarities. In the absence of the target tetracycline, segment a of DNA1 is occluded by hybridization with the anti-tetracycline aptamer, which prevents the interaction between DNA1 and DNA2 to form an active DNAzyme. The introduction of tetracycline to the sensing system induces dehybridization of the partially complementary duplex between DNA1 and the aptamer. As a consequence, segment a is exposed and can bind with segment a*. Such binding generates an active DNAzyme. In the presence of Mg^{2+}, the synergistically-stabilized supramolecular DNAzyme structure leads to the cleavage of the substrate H1, resulting in the release of the caged G-rich sequence (domain d). The

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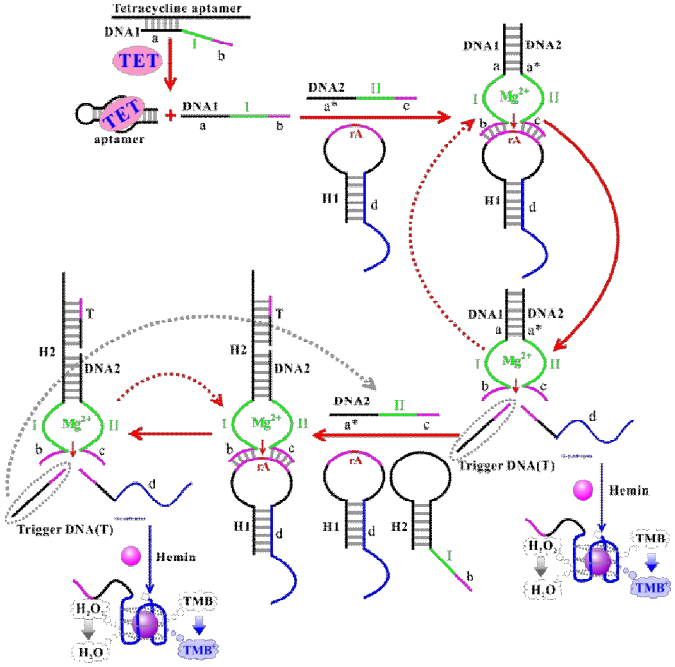
association of hemin to the G-rich sequence yields the G-quadruplex-hemin DNAzyme that catalyzes the H_2O_2-mediated oxidation of TMB to generate a colored signal readout, which can be easily recognized by the naked eye. In addition, the trigger DNA (T) is also liberated from the caged H1 to initiate the autocatalytic system.

The free trigger DNA opens the hairpin DNA H2, which contains the same sequence as DNA1. The complementary hybridization between the opened H2 and DNA2 bridges the DNAzyme subunits, thus generating another active DNAzyme structure that cooperatively binds the substrate H1. The cleavage of H1 leads to the release of the caged G-rich sequence (domain d) and the trigger DNA. The trigger DNA can be recycled to open many H2, resulting in the autocatalytic accumulation of the G-quadruplex, which gives rise to the enhanced colorimetric signal. Importantly, each target-induced activated Mg^{2+}-dependent DNAzyme can undergo many cycles to catalyze the cleavage of numerous H1 substrates, achieving an amplified output signal for tetracycline.



Scheme 1. Schematic illustration of the design strategy for instrument-free visual detection of tetracycline (TET) on an autocatalytic DNA machine using caged G-quadruplex as the signal reporter. The target-induced assembly of the active Mg^{2+}-dependent DNAzyme from the subunits (domians I and II) leads to the cleavage of the hairpin substrate H1 and to the release of the caged G-quadruplex for the formation of the color signal.

To verify the feasibility of the designed DNAzyme-mediated cascade signal amplification strategy for target detection, the UV-vis absorption spectra and color changes of the solution were investigated and compared under different conditions. As shown in Fig. 1, only a small background absorption was observed in the absence of target tetracycline or DNA2 (curves a and b), indicating that almost no active DNAzyme was formed and no cleavage reaction toward H1 could occur, and thus the G-quadruplex sequence was caged in H1. The further control experiment without H1 in the sensing system showed the negligible response (curve c). With the introduction of H1 into the mixture but no H2, the absorbance intensity at 650 nm increased obviously (curve d), which can be attributed to the fact that the catalytic cleavage of the substrate H1 by the formed active DNAzyme liberates the caged G-quadruplex sequence for giving the colorimetric output. Importantly, the absorption signal increased sharply (curve e) after the addition of H2 into the sensing system. This signal increase indicated that the autocatalytic DNA machine in the presence of H2 induces the autonomous cyclic formation of the active DNAzyme to catalyze the cleavage of H1 substrate, thereby generating numerous free G-quadruplex molecules to give an amplified signal. The corresponding color changes of the solution (inset in Fig. 1) were also recorded. These results strongly evidenced that our proposed sensing system can feasibly provide an amplified colorimetric signal for target detection.

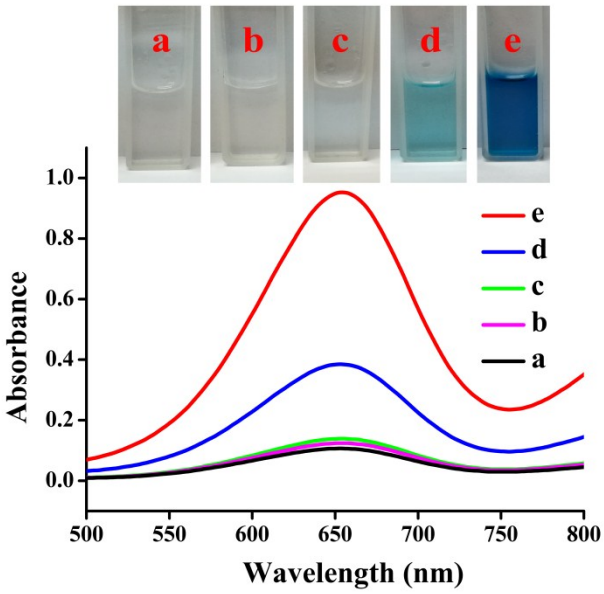


Fig. 1 UV-vis absorbance spectra of the test solution at different experimental conditions: (a) no tetracycline, (b) 10 nM tetracycline, no DNA2, (c) 10 nM tetracycline, no H1, (d) 10 nM tetracycline with H1, (e) 10 nM tetracycline, with H1 and H2. Experimental results were obtained in the TMB–H_2O_2 reaction solution. Insets: the corresponding photographs of the naked-eye color difference.

To evaluate the utility of the designed autocatalytic DNA machine for target detection, different concentrations of tetracycline were measured under the optimized conditions (Fig. S1-S4, ESI†). As shown in Fig. 2A, with the increase of tetracycline concentration, the color of the solution gradually intensified, indicating that more free G-quadruplex are generated by the cleavage of the caged substrate. Fig. 2B depicts the corresponding UV-vis absorbance spectra of the solution. As higher concentrations of tetracycline were added, the colorimetric responses increased. The absorption intensity at 650 nm were also recorded in Fig. 2C. The resulting calibration curve shows that a linear dependence between the absorption intensity and the logarithm of tetracycline

concentration was obtained in the range from 1 pM to 100 nM with a correlation coefficient (R^2) of 0.997 (Fig. 2C, inset). The linear regression equation is y = 0.179 x + 0.21 (y and x represent the absorption peak intensity and the logarithm of tetracycline concentration, respectively). Importantly, even 1 pM tetracycline can be easily distinguished by the naked eye according to the remarkable color change between the sample and the blank solution. Such a low visual detection limit is two orders of magnitude higher than the aptamer-based unamplified colorimetric sensors for tetracycline detection.[3,7] The calculated limit of detection (LOD) is 0.2 pM. The calculated LOD is defined by $3S_0/S$,[31,32] where 3 is the factor at the 99% confidence level, S_0 is the standard deviation of the blank measurements (n = 12), and S is the slope of the calibration curve. Such impressive sensitivity can be attributed to the fact that the autocatalytic DNA machine can stimulate the formation of numerous G-quadruplex-hemin molecules even at a low concentration of target tetracycline. As the European Union (EU) has set maximum residue limits (MRL) of tetracycline in milk to be 225 nM,[1,2] our proposed instrument-free visual detection strategy holds great promise for on-site monitoring of tetracycline residue in milk samples with the high sensitivity.

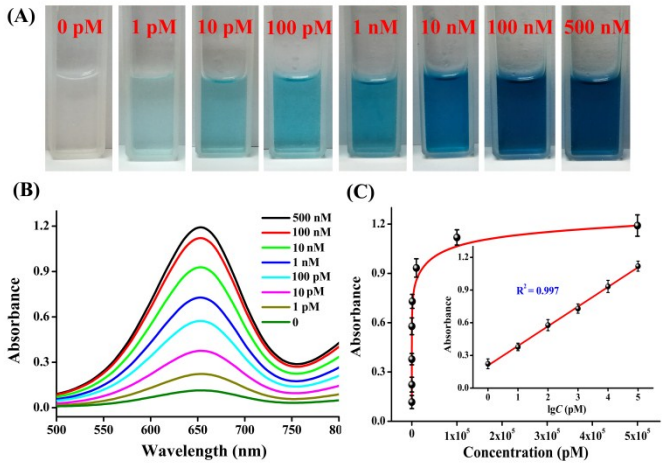


Fig. 2 (A) Photograph for visual tetracycline detection at different concentrations. (B) Their corresponding UV-vis absorption spectra. (C) Dependence of absorption intensity at 650 nm on target concentration. Inset depicts the linear relationship between the absorption peak intensity and the logarithm of tetracycline concentration in the range from 1 pM to 100 nM.

To investigate the selectivity of the fabricated biosensor, other antibiotics including clindamycin, amoxicilin, ciprofloxacin, chloramphenicol, kanamycin, ribostamycin, streptomycin, and penicillin were also analyzed by the present sensing system. As shown in Fig. 3, the addition of the target tetracycline (10 nM) could result in a bright blue color change of the probe solution, while all other control molecules (100 nM) did not lead to obvious variation of the solution color compared with the blank sample. These results clearly demonstrated the high specificity of our designed sensing system based on colorimetric assay. Such a high selectivity of the sensor can be related to the highly specific binding capability of the aptamer to the target analyte.

To validate the practical application of our described method in real food sample, recovery testing was carried out by spiking different concentrations of target tetracycline into the milk samples. The results were listed in Table S1 (ESI†). Values between 84% and 108% were obtained for the recovery experiments, which indicated that the possible interference from milk samples was negligible. Moreover, the concentration of tetracycline in spiked milk samples was further validated by comparing to conventional liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. The data revealed that no significant difference existed between the values measured using the proposed autocatalytic biosensor and LC-MS/MS method, confirming the accuracy of the developed sensor for detecting tetracycline. The above results suggested that the proposed instrument-free visual detection strategy for tetracycline could be used in complicated food samples.

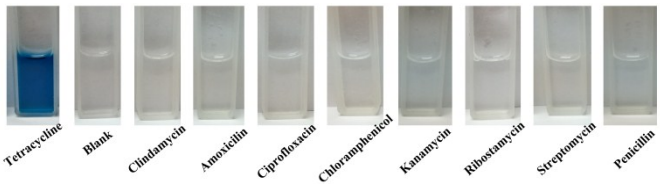


Fig. 3 Selectivity investigation of the fabricated biosensor or tetracycline against other antibiotics. The concentration is 10 nM for tetracycline and 100 nM for other antibiotics.

In summary, we have successfully developed a instrument-free biosensor for the visual detection of tetracycline on the basis of an autocatalytic DNA machine for cascade signal amplification. The binding of the aptamer to tetracycline triggers the cooperative assembly of the active Mg^{2+}-dependent DNAzyme from the subunits, leading to the cleavage of the hairpin-structured substrate and the release of the caged G-rich sequence. As a result, the liberated G-rich sequence associates with hemin to form the G-quadruplex-hemin complex that can catalyze the oxidation of colorless TMB solution to blue-colored product, which can be easily recognized by the naked eye. The autocatalytic DNAzyme recycling strategy generates significantly amplified colorimetric signals for tetracycline detection, with a visual detection limit of 1 pM can be achieved without instrumentation. The proposed sensing method also shows high selectivity toward the target tetracycline and can be applied to the determination of tetracycline in spiked milk samples. Moreover, the tetracycline biosensor has the distinct advantages of rapid naked-eye output, simplicity, cost-effectiveness, and instrument-free feature, making this sensing system particularly suitable for on-site monitoring of food samples in resource-constrained settings. Significantly, the developed autocatalytic DNA machine can be conveniently extended to fabricate versatile and robust sensing platforms for the detection of a variety of target analytes by selecting appropriate aptamers.

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