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ARTICLE

Universal and Label-Free Photosensitization Colorimetric Assays Enabled by Target-Induced Termini Transformation of dsDNA Resistant to Exo III Digestion

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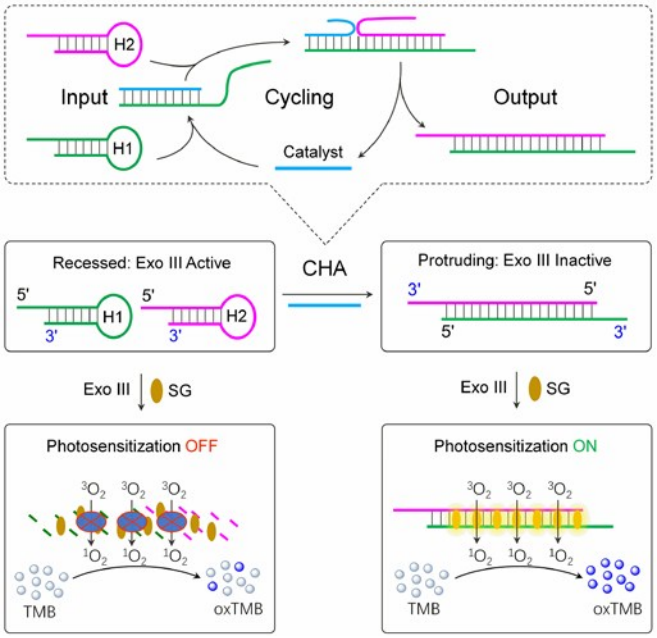
A label-free and universal photosensitization colorimetric assay based on target-induced dsDNA termini switch has been developed for analysis of nucleic acids, proteins, small molecules and metal ions. This strategy is highly sensitive and versatile, the principle of which will be desirable for various biosensor development and applications.

Binding-induced conformation changes of DNA has been explored as an interesting and effective strategy for biosensor development.[1] In these assays, chemically labelling of DNA and proteins with various signal tags (such as fluorescence[2] and electrochemistry[3]) are required for generating detection signals. For example, in the well-known molecular beacons with dual labels of fluorophore and quencher, the structure interconversion between hairpin and double stand DNA (dsDNA) results in efficiency change of fluorescence resonance energy transfer.[4] Although widely used and highly effective, chemical labelling can often be tedious involving complicated synthesis/purification steps and inevitably increase the cost. Therefore, label-free strategies capable of generating sensitive detection signals in response to conformational changes will be more desirable.[5]

Herein, we propose a conformation change-based DNA bioassay, in which the presence of target induces the termini switch of dsDNA from recessed 3'-termini to protruding 3'-termini (Scheme 1). Such conformational changes allow target-specific DNA degradation with exonuclease III (Exo III). A colorimetric assay combining the target-induced DNA hairpin assembly, selective DNA degradation, and DNA-mediated photosensitization,[6] was developed. As we make use of double-stranded DNA (dsDNA) and intercalating dyes for generating detection signals, highly sensitive colorimetric signal can be generated without the need for chemical labelling.[7] We also demonstrate that our strategy is highly universal,[8] where the same assay can be expanded to multiple classes of targets, including nucleic acids, proteins, small molecules, and metal ions, without the need for significantly altering the design of the signal-report sequences (such as G-quadruplex).

The principle of our dsDNA termini transformation assay was illustrated in Scheme 1, which is a combination three strategies: target-induced catalytic hairpin assembly (CHA) for dsDNA termini switch, structure-specific Exo III digestion, and photosensitized oxidation of TMB into colorimetric products. Two DNA hairpins (H1 and H2), both bearing recessed 3'-termini were designed as the precursors for the termini switch. In the presence of the target, the 3'-end recessed H1 and H2 were catalysed to produce a dsDNA with protruding 3'-termini via



Scheme 1 Schematic illustration of the target-induced recessed-to-protruding termini transformation for photosensitization colorimetric assay.

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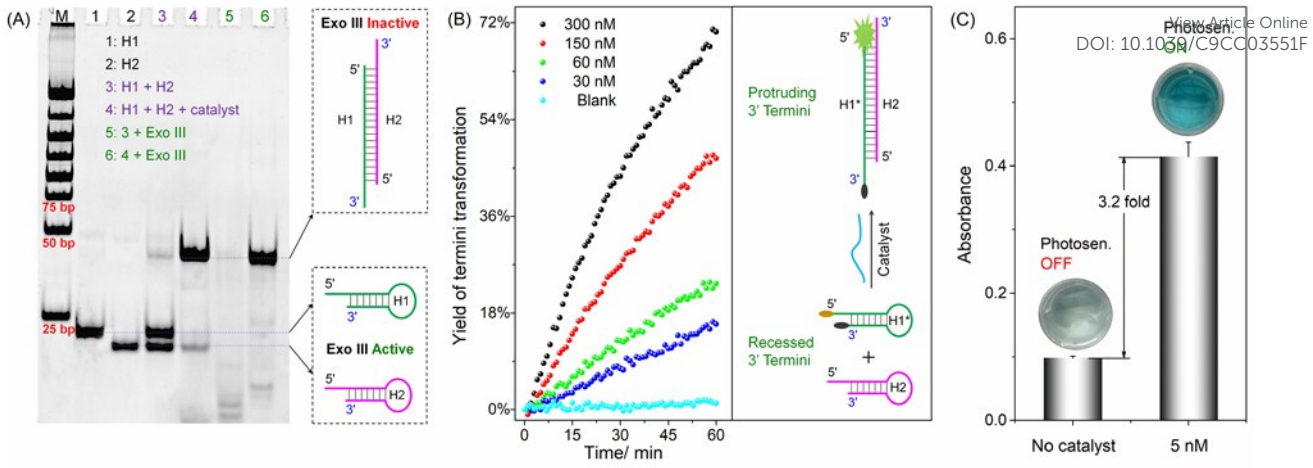

Fig. 1 (A) the relationship of the termini transformation yields with reaction time and catalyst concentrations; (B) conforming the Exo III activity for input hairpins and output dsDNA; (C) colorimetric results comparison between photosensitization off assay and photosensitization on assay.

catalytic hairpin assembly (CHA).[9] As both H1 and H2 bearing recessed 3'-termini, they can be rapidly hydrolysed by Exo III. However, upon the formation of dsDNA in response to the target, the product bearing protruding 3'-termini (5-7 bps) at both ends and thus becomes resistant to Exo III cleavage. As a result, the target-induced transformation allows the quantitative amplification and preservation of dsDNA as a final product in the solution. By adding SYBR Green I (SG) as a photosensitizer and TMB as a chromogenic substrate, we are able to quantify the CHA product in a label-free and colorimetric manner.[6]

To verify the feasibility of the proposed target-induced dsDNA termini switch, two hairpins featuring recessed 3'-termini (H1 and H2, see ESI† for detailed sequences) were designed (Scheme 1). The CHA-assisted assembly between the two hairpins and the target was confirmed with native polyacrylamide gel electrophoresis (PAGE). As shown in Fig. 1A, the mixture of H1 and H2 exhibited two separated bands (line 3). In the presence of the target, it catalysed the hybridization of H1 and H2 through CHA, evidenced by the new band emerged at a region of higher molecular weight (line 4).

For better monitoring the termini switch, hairpin H1 was labelled with a fluorophore (FAM) and a quencher (BHQ1) at the 5'- and 3'-end, respectively (H1*). The yield of termini switch (R) was calculated and quantified based on the following equation:

$$R = \frac{F - F_0}{F_A - F_0} \times 100\%$$

where F, F_0, and F_A denote the fluorescence in real time assembly, the fluorescence before CHA reaction, and the fluorescence after CHA reaction (Fig. S2), respectively. As shown in Fig. 1B, increasing the concentration of CHA catalyst and the assembly time can both increase the yield of output dsDNA. Therefore, the DNA termini switch could be successfully triggered by the target (CHA catalyst).

Next, Exo III-assisted signal generation through dsDNA termini switch was also confirmed. As shown in Fig. 1A, the mixture of H1 and H2 was successfully digested by Exo III (lane 5), but the product with switched termini could not (line 6). Such differential response can be ascribed to the selective recognition of 3'-recessed termini by Exo III.

When monitoring the Exo III-assisted DNA digestion using the photosensitization colorimetric assay (Fig. 1C, see details in ESI†), only weak TMB absorbance at 652 nm was observed in the absence of target, due to heavy digestion of hairpins with recessed 3'-termini by Exo III (photosensitization OFF). In the presence of target, strong TMB oxidation was harvested because of the reserved output dsDNA containing protruding 3'-termini (photosensitization OFF). Quantitatively, 5 nM target generated 3.2-fold higher TMB absorbance over that without target.

After demonstration of the working principle, the proposed assay was explored first for the detection of nucleic acids. Before such attempts, the liner relationship between absorbance of the oxidized TMB and the concentration of dsDNA was confirmed (Fig. S3). Here, both DNA and miRNA were detected, and sequences for these assays were given in Table S1 (ESI†). As illustrated in Fig. 2A, the TMB absorbance @ 652 nm was gradually intensified upon increasing the concentration of the target DNA, with linear response in the concentration range from 0.025 nM to 5 nM. The limit of detection (LOD) was determined to be 9.8 pM. For miRNA detection, miRNA-141 was used as the target. Similar to DNA target (Fig. S4), miRNA-141 also resulted in CHA assembly and enhanced TMB oxidation, with linear response in the concentration range from 0.01 nM to 2.5

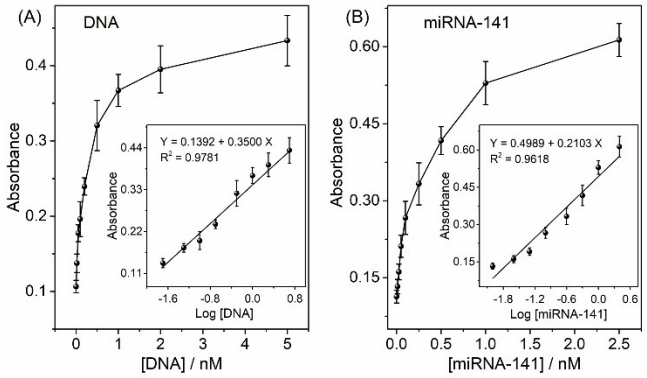

Fig. 2 (A) Plot of absorbance for an increasing concentration of target DNA. Inset: linear correlation between absorbance intensity and logarithm of DNA concentration from 0.025 nM to 5 nM; (B) Plot of absorbance for an increasing concentration of miRNA 141. Inset: linear correlation between absorbance intensity and logarithm of miRNA 141 concentration from 0.01 nM to 2.5 nM.

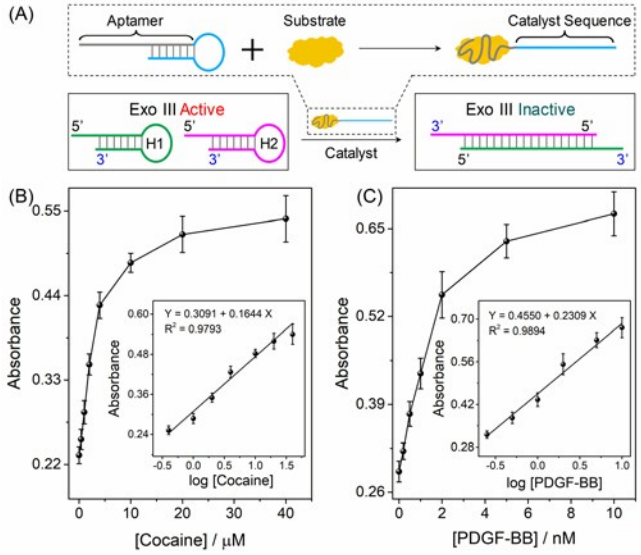

Fig. 3 (A) Analysis of aptamer substrates through aptamer-contained hairpins; (B) plot of absorbance versus varying concentration of cocaine. Inset: linear correlation between absorbance and logarithm of cocaine concentration from 0.4 μM to 40 μM; (C) plot of absorbance versus varying concentration of PDGF-BB. Inset: linear correlation between absorbance and logarithm of PDGF-BB concentration from 0.2 nM to 10 nM.

nM and LOD of 4.2 pM (Fig. 2B). Therefore, this assay offers high sensitivity for nucleic acid analysis.

To demonstrate that our assay is a universal detection platform, we engineered the DNA probes for non-nucleic-acid targets through the uses of aptamers. Here, cocaine and platelet-derived growth factor (PDGF) were used as representative targets for small molecules and proteins, respectively. Specifically, the catalyst for the CHA assembly was designed to contain the aptamer sequences (grey part in Fig. 3A). In the absence of the targets, the catalyst was blocked through partial complementary base pairing (Fig. 3A). Upon binding with the corresponding substrates, the catalyst was released for activation of the subsequent CHA assembly and termini switch (Fig. S5 and S6). Afterwards, the product was detected with the Exo III-assisted photosensitization colorimetric assay. The performance for cocaine was shown in Fig. 3B, with linear response in the concentration range from 0.4 μM to 40 μM and LOD of 0.27 μM. For PDGF, the linear response range was found to be from 0.2 nM to 10 nM (Fig. 3B), with LOD of 89 pM. Therefore, through proper designing of the catalyst sequences, the termini switch assay can be extended for detection of small molecule and protein.

To conform the applicability of this sensing method in practical sample analysis, human serum was selected as a real sample matrix. We compared the sensing performance in reaction buffer and diluted human serum (diluted by reaction buffer). Both of the analytical results for miRNA-141 (Fig. 4A) and PDGF-BB (Fig. 4B) showed minimal difference in reaction. Hence, the system has potential application in analysis of serum molecules.

Finally, we extended our method to the analysis of toxic metal ion, such as Hg^{2+}. Hg2+ is an emerging pollutant that is harmful to both humans and the environment.[10] To be responsive

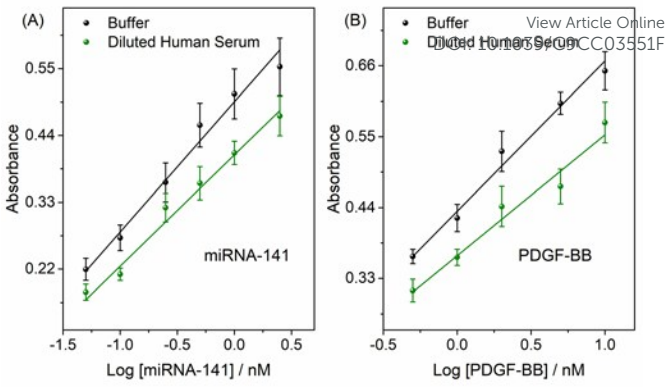

Fig. 4 (A) The linear relationship between absorbance and logarithm of different concentration miRNA-141 in buffer and diluted human serum; (B) the linear relationship between absorbance and logarithm of different concentration PDGF-BB in buffer and diluted human serum.

to Hg^{2+}, the well-known T-Hg-T mismatch[11] was explored. As shown in Fig 5A, to facilitate the catalyst effect of Hg^{2+} for CHA, an engineered H1 containing several T sites was designed. In the presence of Hg^{2+}, allosteric switch of the engineered H1 resulted in liberation of the pre-blocked 3'-end, which subsequently triggered the assembly with H2. Upon further strand displacement, the output dsDNA was obtained, which can be quantified through the Exo III-assisted photosensitization colorimetric assay. Meanwhile, the captured Hg^{2+} was released for next cycle of CHA. The whole assembly process was verified with native PAGE (Fig. S7). The performance for Hg^{2+} was shown in Fig. 5B, with linear response in the concentration range from 10 nM to 500 nM and LOD of 5.6 nM. Considering the existing C-Ag-C[12] and also T-melamine-T[13] structures, the proposed design can be easily extended to Ag^{+} and melamine analysis.

In summary, this study has demonstrated a new conformation change-based DNA bioassay, in which the presence of target induced the termini switch of dsDNA from recessed 3'-termini to protruding 3'-termini. Such termini switch can be easily read out with Exo III-assisted photosensitization colorimetric assay in a label-free format. In combination with aptamer for probe design, the proposed assay could be

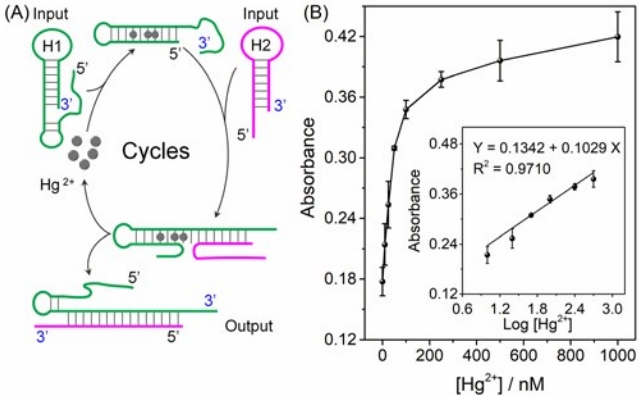

Fig. 5 (A) Analysis of Hg^{2+} through T-Hg-T base pair; (B) plot of absorbance versus varying concentration of PDGF-BB. Inset: linear correlation between absorbance and logarithm of Hg^{2+} concentration from 10 nM to 500 nM.

engineered for a universal bioassay platform, i.e., nucleic acids (DNA, LOD of 9.8 pM; miRNA, LOD of 4.2 pM), small molecules (cocaine, LOD of 0.27 μM), proteins (PDGF, LOD of 89 pM), and metal ions (Hg^{2+}, LOD of 5.6 nM). Since the photosensitization colorimetric assay only requires DNA and SG for signal generation, no specific DNA structures (e.g., G-quadruplex[14] or i-motif[15]) are thus required, which further simplify the assay design (Table S2). On the basis of the label-free, facile design and H_2O_2-free signalling, we believe that this platform will provide a new way to construct biosensors and have potential applications, such as point-of-care testing (POCT).[16]

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Conflicts of interest

There are no conflicts to declare.

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