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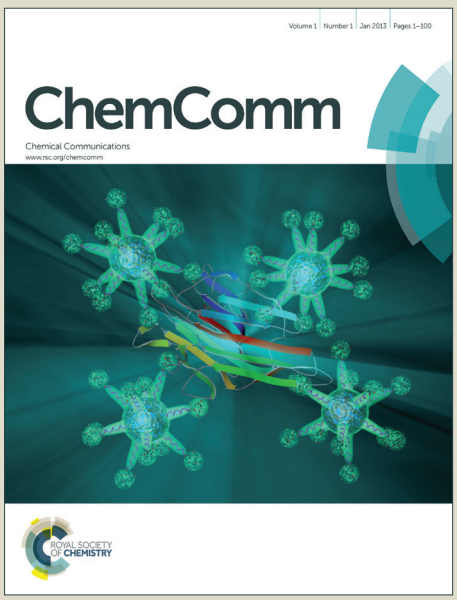

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Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

Photocatalytic Oxidation of TMB with the Double Strand DNA-SYBR Green I Complex for Label-Free and Universal Colorimetric Bioassay

Xinfeng Zhang,[a] Chengpeng Huang,[a] Shuxia Xu,[a] Junbo Chen,[b] Ying Zeng,[a] Peng Wu,[b,*] and Xiandeng Hou[b,*]

Received (in XXX, XXX) XthXXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

We report here a newly discovered photocatalytic activity from dsDNA-SYBR Green I (SG) complex, which can catalyze the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) under light irradiation corresponding to the excitation of dsDNA-SG complex. The most appealing feature of the photocatalytic system here is that it can be obtained with random DNA sequences that can form duplex. Taking the universality of the photooxidase, a label-free and universal platform was proposed for highly sensitive visual bioassays.

Besides natural hybridization, currently there is intensive interest in using DNA for various functions, such as apamter for specific recognition,[1] catalysis,[2] logic computation,[3] and DNA nanotechnology[4]. Basing on these functions, there are numerous emerging sensing and biomedical applications of DNA. For example, single-stranded DNA (ssDNA) or RNA can bind to their complementary strands with high specificity and is useful for nucleic acid detection.[5] Use of G-quadruplex-based DNAzyme as catalytic labels has boosted the development of a variety of chemo/bio-sensors.[6] However, most of the above applications are based on the intrinsic nature of DNA, i.e., hybridization and folding. Such biological functions are truly obsessing. In contrast, the non-biological functions of DNA are less concerned.[7]

Apart from hybridization and folding, DNA is also capable of binding a large number of organic dyes and transition metalcomplexes.[8] One of the most famous intercalating agents is SYBR Green I (SG), which has been commonly used as a fluorescent tag (nucleic acid stain) in molecular biology laboratories for techniques such as agarose gel electrophoresis. After binding with double-strand DNA (dsDNA), the fluorescence of SG will be intensified greatly,[9] resulting in visible orange fluorescence for identifying the stripes of DNA in gel. In analytical chemistry, the significantly intensified fluorescence of SG after binding with dsDNA is also harvested for developing of various label-free fluorescent sensing schemes.[10] However, few other applications about such dsDNA-SG complex have been reported.

Herein, we reported a new discovery that the dsDNA-SG complex possessed photocatalytic activity, which could catalyze the oxidation of oxidase substrates (3,3',5,5'-tetramethylbenzidine, TMB) with dissolved oxygen under photo-irradiation (Fig. 1A). Compared with G-quadruplex-based DNAzyme exhibiting similar catalytic activity,[6b, 11] such photocatalytic activity is truly versatile since it can be obtained from random dsDNA sequences. Taking advantage of the universality of the proposed dsDNA-SG system, we proposed a label-free general platform for visual bioassays (for DNA, proteins, small molecules, and metal ions) based on formation or de-formation of the DNA-dye complex.

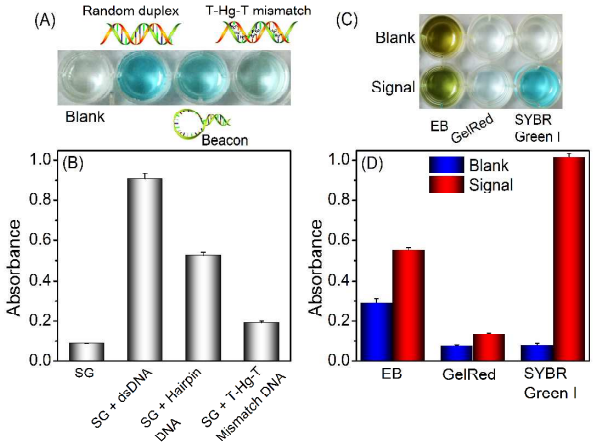


Fig. 1 dsDNA-SG complex shows photocatalytic activity for oxidation of TMB: (A) and (B), different dsDNA structures all exhibit the photocatalytic activity; and (C) and (D), dsDNA could also add photocatalytic activity to other intercalating dyes besides SG. Experimental conditions: dsDNA, 100 nM; SG, 3.92 μM; Gelred, 2×; EB,100 μM; and solution pH 4.5.

First, three random conformations of duplex DNAs, i.e., dsDNA, harpin, and T-Hg^{2+}-T mismatch were prepared with SG intercalated. Greatly intensified fluorescence of SG was observed after incubation of SG with the above duplex DNAs (Fig. S1, λ_{ex} = 525 nm), indicating sucessful intercalation of SG into the grooves of dsDNA. All these three duplex DNAs could catalyze the oxidation of the subtrate TMB under blue LED irradiation (Fig. 1A and Fig. 1B), giving rise to the characterized absorption centered at 650 nm. Control experiments showed that neither single strand DNA (ssDNA) nor dsDNA without SG intercalated exhibited similar photooxidase activity. The mixture of SG and ssDNA could only catalyze minimal TMB oxidation, i.e., very low photooxidase activity. The above experimental results

indicated that the formation of dsDNA- SG complex is the only prerequisite for the photooxidase activity.

Besides SG, there are also a large number of dyes that can be intercalated into dsDNA.[8, 10] Interestingly, we found dsDNA can also add similar photocatalytic activity to other dyes such as ethidium bromide (EB) and gel red (GR) (Fig. 1C and Fig. 1D). These phenomena verified that the proposed photocatalytic activity can be obtained from various dsDNA-dye complexes.

To further confirm the photocatalytic activity of the dsDNA-SG complex, four other commonly used colorimetric oxidase substrates, namely 3,3'-diaminobenzidine (DAB), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS), O-phenylenediamine (OPD), and a typical fluorecent oxidase substrate Amplex red were investigated in place of TMB. As shown in Fig. S2, catalytical oxidation of these substrates with dsDNA-SG complex under LED irradiation was all achieved, although the oxidation extent for OPD and ABTS were much lower than that of TMB.

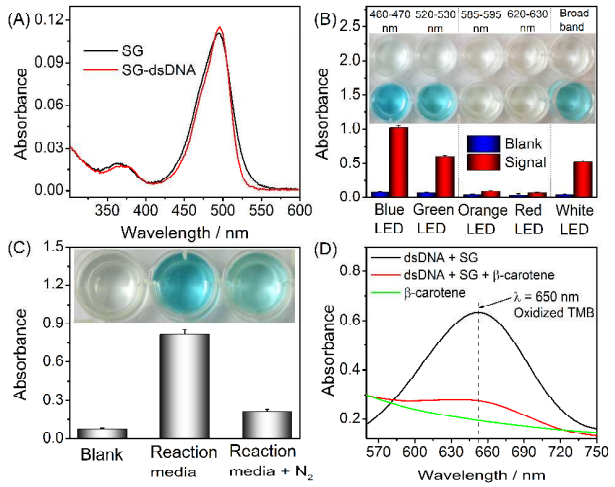


Fig. 2 Investigation of the mechanism of photocatalytic activity: (A) absorption spectra of SG and dsDNA-SG complex; (B) effect of LED wavelengths on photocatalytic activity of dsDNA-SG complex; (C) effect of dissolved oxygen on oxidation of TMB; and (D) β-carotene could alleviate the oxidation of TMB. Experimental conditions: DNA concentration, 100 nM; SG concentration, 3.92 μM; Incubation time, 15 min; solution pH, 4.5 (citrate buffer); and irradiation time, 15 min.

To investigate the origin of the photocatalytic activity, a dsDNA (30 bps)-SG system was employed since it gave the best catalytic oxidation performance for TMB. The absorption profiles of SG in the absence and presence of dsDNA both peaked at 490 nm (Fig. 2A). Interestingly, when irradiating the dsDNA-SG-TMB mixed solution, blue, green, or white LEDs could induce the oxidation of TMB (Fig. 2B), which well matches the absorption of the dsDNA-SG system. For orange- and red-emitting LEDs, the oxidation extent of TMB were quite low. Therefore, the wavelength of the LED used to trigger the oxidation of TMB should agree with the excitation of the dsDNA-SG complex.

Dissolved oxygen played a decicive role in dsDNA-SG-catalyzed photooxidation of TMB. As shown in Fig. 2C, deoxygenization of the dsDNA-SG-TMB via N_2 bubbling largely deactivated the photocatalyst for TMB oxidation. Apparently, without light irradiation, no TMB oxidation was observed.

It is well-known that when excited, dyes are prone to take part in photosensitized oxidation, leading to the generation of active singlet oxygen ($^1O_2$) by energy transfer from dyes to dissolved oxygen.[12] Therefore, we selected two typical $^1O_2$-generating dyes, namely phloxine[13] and riboflavin,[14] for interaction with TMB upon exitation. As shown in Fig. 3, TMB did be photocatalytically oxidized with phloxine and riboflavin. The oxidation was also largely attenuated after deoxygenization as in the case of dsDNA-SG. Therefore, singlet oxygen as the oxidant for TMB oxidation in these systems could be verified. In fact, many dyes have been explored for photodynamic therapy due to the generation of singlet oxygen upon excitation via a type II sensitization process.[15] It was further confirmed that several other dyes could also catalyze the oxidation of TMB when irradiated with light corresponding to their excitation (Fig. S3).

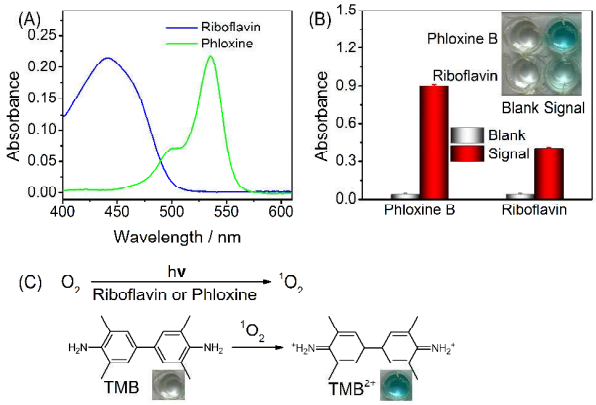
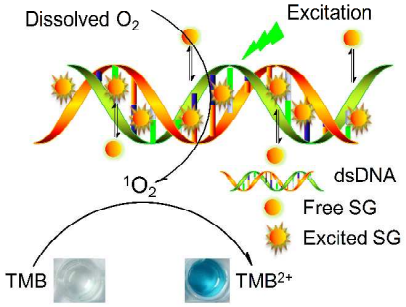


Fig. 3 Oxidation of TMB by singlet oxygen: (A) the absorption spectra of phloxine and riboflavin, with maximum absorption corresponding to green and blue light, respectively; (B) photooxidation of TMB catalyzed by phloxine (green LED) and riboflavin (blue LED); and (C) possible oxidation schemes. The concentrations of phloxine and riboflavin were both 100 nM and the concentration of TMB was 200 mg/L.



Scheme 1 Schematic illustration of the photosensitization of dsDNA-SG for TMB oxidation.

Accordingly, we speculated that singlet oxygen may also be generated from dsDNA-SG system when irradiated with green LED. To verify such a speculation, we used a well-known singlet oxygen quencher *β*-carotene,[16] to eliminate possible singlet oxygen generated from the dsDNA-SG system. As shown in Fig. 2D, upon incubating *β*-carotene with the dsDNA-SG system and irradiating with a blue LED for 10 min, the oxidation extent of TMB was largely attenuated over 75%. Accordingly, the observed photocatalytic activity of dsDNA-SG complex could be

ascribed to singlet oxygen generated upon blue light photosensitization (Scheme 1). For free SG, the generation efficiency of singlet oxygen is very low due to its low fluorescence efficiency. After intercalated into the grooves of duplex DNA, the fluorescence of the complex increased greatly, accompanied by the largely increased formation of singlet oxygen, which is responsible for the oxidation of TMB.

The photocatalytic activity of dsDNA-SG is dependent on pH, SG concentration, incubation time of dsDNA and SG, and dsDNA concentration (Fig. 4). Similar to G-quadruplex-based DNAzyme and horseradish peroxidase (HRP),[17] the optimal pH of the dsDNA-SG was 4.5 (Fig. 4A). The SG concentration required for optimal photocatalytic activity (Fig. 4B, 15.68 μM) was larger than that required for intercalation into grooves of dsDNA (100 nM dsDNA, 30 bps). Probably, an equilibrium exists between SG and intercalated SG, thus requiring more SG than the stoichimetric amount. The optimal incubation time for dsDNA and SG was 15 min to form the dsDNA-EB complex (Fig. 4C). The double helix concentration was investigated with the same DNA concentration but different lengths of duplex (from 10 to 50 base pairs). As shown in Fig. 4D, the photocatalytic activity incrased linearly as the lengths of dsDNA elonged, because of increased dsDNA-SG complex.

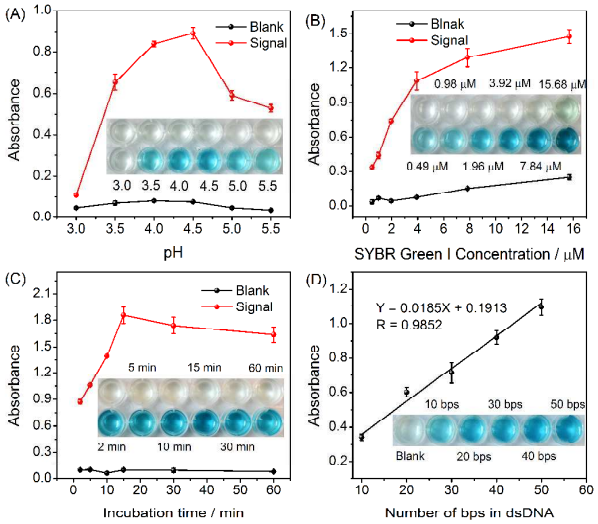


Fig. 4 Factors that influence the photocatalytic activity (evaluated through the absorbance change before and after dsDNA) of dsDNA-SG complex: (A) pH; (B) SG concentration; (C) incubation time of dsDNA and SG; and (D) dsDNA concentration (by varying the number of base pairs from 10 to 50). Experiments were carried out using 100 nM dsDNA and 3.92 μM SG in 0.1 M citrate buffer (pH 4.5) unless otherwise stated.

Considering the analyte recognition of various functional DNAs, such newly discovered photocatalytic activity of dsDNA-SG system can be easily engineered into a facile label-free and universal platform[18] for visual detection of a broad range of trace analytes, such as DNA, metal ions, proteins, or small molecules. To test the universality of the dsDNA-SG system for visual detection of a broad range of target analytes, we first demonstrated the detection of DNA (BRCA 1 gene). A DNA strand complementary to the target DNA was used as the probe. The presence of target DNA triggered the formation of dsDNA and thus activated its photocatalytic activity with SG, which could catalyze the oxidation of TMB to give color readout (Fig. 4A). Under the optimized conditions, the resultant color change allowed visual detection of DNA as low as 0.1 nM (observable detection limit, Fig. 4B). Using spectrophotometry, Fig. S5A showed the absorbance of the reaction solution at 650 nm increased linearly with the logarithm of DNA concentration in the range of 0.05-1000 nM, and the limit of detection was 0.024 nM (3σ). For one- and three-base mismatched DNA, the oxidation extent of TMB was decreased greatly, and the non-complementary DNA led to extremely low signal (Fig. S5B).

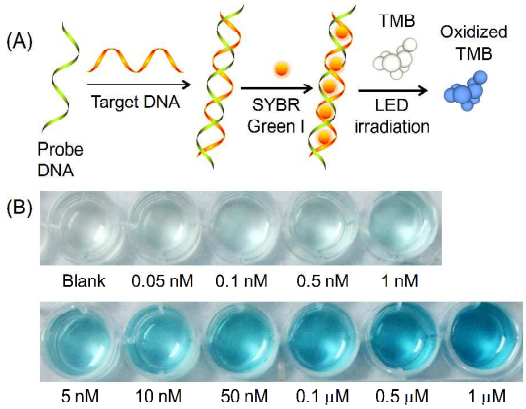


Fig. 5 Design and performance of the visual sensor based on photosensitization of dsDNA-SYBR green complex for DNA detection: (A) the scheme of the biosensor; and (B) photographs of the visual detection of 0.05 nM-1000nM of BRCA1 gene.

To demonstrate the universality of this assay, the photocatalytic activity of dsDNA-SG complex was also successfully expanded for protein (thrombin, Fig. S6 and Fig. S7), small molecule (cocaine, Fig. S8 and Fig. S9), or metal ion (Hg^{2+}, Fig. S10 and Fig. S11) detection. For thrombin detection, a DNA duplex containing thrombin aptamer and its complementary sequence was explored as the probe and SG was pre-intercalated into the duplex. The presence of thrombin departures the dsDNA-SG complex and inhibits of the photocatalytic activity, due to the formation of thrombin-aptamer complex that dissociates the DNA duplex. The resultant color change allows visual detection of thrombin down to 500 pM (Fig. S7). For cocaine and Hg^{2+} detection, anti-cocaine aptamer and Hg^{2+} aptamer recognize their targets respectively, and yielded dsDNA or dsDNA-containing structure that permitted SG to be embedded in. The resultant color change supported facile discrimination of cocaine and Hg^{2+} down to 20 μM and 0.25 μM, respectively.

Compared with G-quadruplex-based DNAzyme,[6b-d] peroxidase-mimicking nanozyme,[17, 19] and HRP also that also exhibit similar catalytic activity for TMB oxidation, the dsDNA-SG proposed here offers several distinct advantages for sensing applications (Table S4). First, dsDNA-SG complex is truly simple and versatile as it can be obtained with random dsDNAs, while G-quadruplex-based DNAzyme requires specific DNA sequences. Second, the random dsDNAs in dsDNA-SG complex permit label-free detection in contrast to HRP- or nanozyme-labeled bioassays. Third, application of dsDNA-SG complex for visual sensing is universal for inorganic ions, proteins, small molecules, and DNA with convenient biosensor designs. For

DNAzyme, the corresponding bioassays can be made universal, but the biosensor designs may be sophisticated since the G-quadruplex structure must be included. For nanozyme and HRP, the bioassays are generally not universal. Fourth, dsDNA-SG complex works with dissolved oxygen only and the sensing process is convenient, only requiring mixing several solutions and irradiation with compact LEDs. But for DNAzyme, nanozyme, and HRP, H_2O_2 is the oxidant (with a few exceptions for nanozyme[20]), which causes slight oxidation of TMB directly.

In summary, we reported here a new photocatalytic activity from dsDNA-SG complex, which was ascribed to the singlet oxygen generated upon excitation. The most appealing characteristics of the photocatalytic activity proposed here are its universality with random dsDNA sequences, leading to the development of a label-free universal platform for visual bioassay of metal ions, small molecules, DNA, or proteins. Due to the involvement of singlet oxygen, selective photo-dynamic therapy is also expected in the near future through activation of the photocatalytic activity with cancer cell aptamers and other intercalated dyes, it is also potentially useful in selective photo-dynamic therapy.

The authors gratefully acknowledge the financial support from the National Natural Science Foundation of China (No. 21475013) and Doctoral Fund of Ministry of Education of China (No. 20120181120070).

Notes and references

[a]*College of Materials and Chemistry & Chemical Engineering, Chengdu University of Technology, Chengdu 610059, China*

[b]*Analytical& Testing Center, Sichuan University, 29 Wangjiang Road, Chengdu 610064, China. E-mails: wupeng@scu.edu.cn; houxd@scu.edu.cn*

† Electronic Supplementary Information (ESI) available: Experimental details. See DOI: 10.1039/b000000x/

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