Double-Stranded DNA Matrix for Photosensitization Switching

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Photosensitization, originated from the activation of triplet states, is the basis of many photodynamic applications, but often competes with a series of nonradiative processes. Herein, we communicate a new function of double-stranded DNA (dsDNA) for label-free photosensitization switching. Up to ~70-fold singlet oxygen generation boosting was observed for SYBR Green I (SG) upon binding with dsDNA. Detailed photophysical and theoretical studies have revealed the role of dsDNA as a matrix, which could efficiently suppress the nonradiative transitions of SG. Such photosensitization modulation is universal for a series of dsDNA-binding photosensitizers, including both base intercalators and minor groove binders. In conjunction with photochemical oxidation of chromogenic substrates, a simple and low-cost photosensitization-based colorimetric detection protocol has been developed, with sensitivity comparable to that of fluorescence detection. Through loop-mediated isothermal amplification (LAMP), colorimetric detection of hepatitis B virus (HBV) was achieved with a limit of detection (LOD) down to 1 aM, which is comparable with that of the standard quantitative polymerase chain reaction (PCR). To facilitate point-of-care testing, a simple and low-cost paper strip has been developed for distance-based detection of LAMP amplicons with a LOD of 100 aM for HBV DNA.

Introduction
Photosensitized singlet oxygen (¹O₂) generation has attracted significant research attention due to its intriguing applications in a myriad of photodynamic fields, such as photodynamic therapy (PDT),¹–³ sunlight-activated insecticides,⁴ chromophore-assisted light inactivation of proteins,⁵ and analytical applications such as luminescent oxygen channeling immunoassay (LOCI)⁶ and in situ RNA localization assay.⁷,⁸ In a typical photosensitization process (Scheme 1a), a photosensitizer (PS) is first excited to its excited singlet state upon light irradiation (absorption). The excited-state energy can be either relaxed through emissions of prompt fluorescence or migrated to the triplet state (T₁) via intersystem crossing (ISC). Subsequently, triplet-state PS may decay to the ground

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state (S0) to emit phosphorescence (anaerobic) or interact with dissolved oxygen to induce the generation of \(^{1}\text{O}_2\) (photosensitization). Therefore, modulating the excited state (S1) of PSs is an efficient strategy for photosensitization regulation,\(^9\)\(^{-12}\) which complements common strategies such as heavy atom effect.\(^13\),\(^14\)

As shown in Scheme 1a, photosensitization efficiency (\(\Phi_{\Delta}\) generation efficiency, \(\Phi_{\Delta}\)) as well as prompt fluorescence, both compete with a series of nonradiative processes, such as intramolecular motions and intermolecular collisions.\(^15\) Therefore, suppression of nonradiative transitions represents an efficient strategy for photosensitization modulation. Previously, we reported an interesting phenomenon that double-stranded DNA (dsDNA) could modulate the photosensitization of SYBR Green I (SG, a typical dsDNA-staining dye),\(^16\),\(^17\) but the mechanism was unclear. Herein, we have performed detailed photophysical and theoretical studies to reveal the role of dsDNA as a matrix in fixing PS SG, allowing activation of \(T_1\) by suppressing nonradiative transitions (Scheme 1b). For the first time, \(O_2\)-free aqueous room-temperature phosphorescence (RTP) of SG was observed, together with up to a ~74-fold increase of \(^{1}\text{O}_2\) generation efficiency. The fixing effect of dsDNA originates from the double-helix structure (Scheme 1b), which allows distinct host–guest chemistry over single-strand DNA (ssDNA) for dye inclusion.\(^5\),\(^8\) Upon binding with dsDNA, SG can be isolated from its surrounding environment by the rigid double helix to exclude the intermolecular (collisions) and intramolecular (rotations) nonradiative processes. We also confirmed that the matrix effect of dsDNA is universal for several PSs, including both base intercalators and minor groove binders.

To explore the practical applicability of dsDNA-switched photosensitization, loop-mediated isothermal amplification (LAMP) was introduced to construct a nucleic acid testing (NAT) platform. NAT is the benchmark technology for disease diagnosis and monitoring,\(^19\),\(^20\) but is mostly performed in centralized laboratories with high-end instrumentation and highly skilled personnel,\(^21\),\(^22\) that is, not suitable for point-of-care testing (POCT). Therefore, NAT methods with simple operation and low-cost instrumentation are highly desired. Here, through exploration of a simple light-emitting diode (LED)-assisted photochemical oxidation protocol (\(O_2\)-induced chromogenic reaction of 3,3',5,5'-tetramethylbenzidine (TMB)), a colorimetric LAMP method was developed for the analysis of the hepatitis B virus (HBV) gene. Since photosensitization-mediated colorimetric signals can accumulate through prolonged light irradiation, the newly developed colorimetric LAMP assay features comparable analytical performances as that of a SG fluorescence assay. To facilitate POCT, we also developed a simple and low-cost paper strip for distance-based detection of LAMP amplicons with a limit of detection (LOD) of 100 aM for HBV DNA.

**Experimental Section**

**Materials**

SG was primarily studied in this work, and several other dsDNA-binding dyes were also included. Detailed information about the materials and oligonucleotides is given in Supporting Information Tables S1 and S2, respectively. The concentration of SG (10,000x) was calculated to be 3.95 mM according to Vitzthum’s work.\(^23\) Oligonucleotides were provided by Shanghai Sangon Biotech Co., Ltd. (Shanghai, China).

**Study on the photosensitization performance of SG**

The detailed instrumental information are given in Supporting Information Table S3. To study the photosensitization performance of SG, oligonucleotides and SG were mixed and diluted to 1 mL with citrate-phosphate buffer (pH 4.5, 10 mM MgCl\(_2\)). The final concentrations of dsDNA and SG were 100 nM and 0.8 μM (2x), respectively. The mixture was incubated for 10 min, followed by the addition of TMB (0.2 mg/mL), and then irradiated with a cyan LED (495 nm, 3 V, 3 W) for another 2 min. The \(^{1}\text{O}_2\) quantum yields (\(\Phi_{\Delta}\)) of SG were evaluated...
using Ru(bpy)$_2^{2+}$ as the standard (see Section 3 for details in Supporting Information Figures S8–S12).

To confirm the matrix effect of DNA, radiative ($k_r$) and nonradiative transitions ($k_{nr}$) were calculated through the following equations:

$$\Phi = \frac{k_r}{k_r + k_{nr}}$$  \hspace{1cm} (1)

$$\tau = \frac{1}{k_r + k_{nr}}$$  \hspace{1cm} (2)

$$k_{nr} = \frac{1 - \Phi}{\tau}$$  \hspace{1cm} (3)

where $\Phi$ and $\tau$ are the quantum yield and lifetime of fluorescence and phosphorescence, respectively. Specifically, SG and dsDNA were mixed and diluted to 2 mL with phosphate-buffered saline (PBS) buffer (pH 7.4, 10 mM). The final concentrations of dsDNA and SG were 1.0 $\mu$M and 0.8 $\mu$M (2x), respectively. Detailed measurements of $\Phi_{FL}$, $\Phi_{PHOS}$, $\tau_{FL}$, and $\tau_{PHOS}$ are given in the Supporting Information.

**Theoretical calculations**

For density functional theory (DFT) and time-dependent DFT (TD-DFT) calculations, Gaussian 09 was applied. The ground- and excited-state geometries were optimized at the B3LYP/6-31g (d) level. The Solvent Model based on Density (SMD) was applied for all calculations, and water was used as the solvent here. A potential energy scan of the $S_0$ was based on a ground-state optimized molecular structure, and the $S_1$ potential energy surface calculation was based on an excited-state optimized molecular structure. The potential energy surface calculation was carried out using relaxed potential energy surface scanning at the B3LYP/6-31g (d) level. On the basis of optimized geometries in the $S_1$, the excitation energies were both calculated using TD/B3LYP/def-TZVP for the electronically excited $\text{O}_2$ and $\text{T}_1$. At the same level, the spin–orbit coupling (SOC) matrix elements between the singlet and $\text{T}_1$ were given by ORCA 4.1.1 (free access from the Max-Planck-Institute).

**Molecular docking**

Molecular docking was performed with Surfex-Dock available in Sybyl-X 2.0 program (Tripos Inc., St Louis, USA). A dsDNA structure (sequence: 5’-ACA GAC ACC T-3’ and its complementary strand) was built in the bio-polymer builder module, and all the hydrogen atoms were added to define the correct configuration and tautomeric states. After adding the charge with the Gasteiger–Marsili charges, the model structure was energy minimized using the Powell energy minimization algorithm with a MMFF94 force field to obtain the DNA molecule for next docking analysis. The SG structure was designed in the Sybyl program and energy minimized to reach a reasonable three-dimensional (3D) conformation utilizing the Tripos force field with distance-dependent dielectric and Powell energy minimization algorithm. The maximum number of iterations performed in the minimization was set as 1000, and other parameters were set as default.

Docking pockets were generated based on “Automatic mode”, and the binding site was determined by a protocol probe. An additional starting conformation was set as 10 with a minimum of 0.05 Root Mean Square Deviation (RMSD) between poses. The spin-alignment method was used with moderate accuracy (density of search, 6.0) and 12 spin per alignment, and other parameters were set as defaults. Docking results were ranked based on Surfex-Dock’s scoring function (an empirical scoring function), in terms of hydrophobic, polar, repulsive, entropic, and solvation. The lowest binding energy conformation was searched out of 20 different conformations and used for further analysis. We selected the top two structures in terms of score. All binding models were visualized in Pymol (version 2.4.0, Schrödinger, NY, USA).

**Fluorescence versus photosensitization**

To compare the photosensitization-based colorimetry and fluorescence, oligonucleotides of different lengths (10–130 bps) and PSs were mixed and diluted to 1 mL with citrate-phosphate buffer (pH 4.5, 10 mM, MgCl$_2$) for photosensitization and PBS buffer (pH 7.4, 10 mM) for fluorescence, respectively. The concentrations of dsDNAs of different lengths were 10 nM, and SG was 0.8 $\mu$M (2x). To obtain the LOD of the photosensitization-based colorimetric assay and fluorescence for DNA quantification, concentrations of the probe ssDNA were fixed (30–60 bps, 100 nM; 70–130 bps, 50 nM), while the final concentration of SG was 0.8 $\mu$M (2x). The mixture was incubated for 10 min, followed by the addition of TMB, and then irradiated with a cyan LED for another 2 min.

**LAMP design of paper strips for photosensitization-based colorimetric assays**

The designed patterns onto cellulose chromatographic paper used a XEROX ColorQube 8580 solid ink printer and then heated on a hot plate at 150 °C for 40 s. The strip was then fabricated by stacking the patterned paper and a layer of paraffin film on a microscope glass slide. This sandwiched device was then bonded by heating on the hot plate at 110 °C for 30 s. Finally, TMB was coated onto the test zone of the strip. To do so, TMB was first dissolved in acetonitrile and then deposited onto cellulose paper upon rapid solvent evaporation.

**Results and Discussion**

**Confirmation of the dsDNA-switched photosensitization switching of SG**

First, $^{1}\text{O}_2$ generation from the dsDNA-SG complex was confirmed with standard electron paramagnetic...
resonance (EPR) characterization. Upon mixing of SG with dsDNA (dsDNA-1; Figure 1a, Supporting Information Figures S1 and S2 and Table S2), a distinct EPR signal of $^1\text{O}_2$ from photosensitization was observed from the typical 1:1:1 triplet peaks of 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl (4-hydroxy-TEMPO; Figure 1b). In addition, a very weak 1275 nm characteristic peak of $^1\text{O}_2$ was also collected (Supporting Information Figure S3). However, for SG alone or the mixture of ssDNA and SG, no appreciable EPR signal was observed. Therefore, only binding of SG with dsDNA resulted in a type-II photosensitization process ($^1\text{O}_2$, Supporting Information Figures S4–S6). Quanti- tatively, the quantum yields ($\Phi_{\Delta}$) of SG for producing $^1\text{O}_2$ were evaluated using Ru(bpy)$_3^{2+}$ as a standard (see Section 3 in Supporting Information). As shown in Figure 1c, $\Phi_{\Delta}$ of free SG was extremely low ($\sim 0.3\%$), but increased $\sim 74$-fold after binding with dsDNA ($\sim 22.2\%$). Treating the dsDNA-SG complex with NaN$_3$ (a specific $^1\text{O}_2$ scavenger) further resulted in a sharp decrease of $\Phi_{\Delta}$ (Figure 1c). Besides dsDNA-1, it was found that other synthetic and natural dsDNAs could also switch the $^1\text{O}_2$ generation from SG (Supporting Information Figure S7), indicating that the double helix is responsible for regulating SG photosensitization (Scheme 1b).

SG binding with dsDNA not only switched on the photosensitization of SG, but also the fluorescence and phosphorescence. As shown in Figure 1d, the SG fluorescence increased over 1000-fold, accompanied by a largely increased lifetime (Figure 1e). Meanwhile, distinct O$_2$-free RTP emerged (Figure 1f) with a lifetime of $\sim 3.07$ ms (Figure 1g). Compared with fluorescence, SG phosphorescence shows another Stokes shift of $\sim 100$ nm but the same excitation (Supporting Information Figure S13). For free SG, no significant phosphorescence was observed. This is the first report of RTP collection from pure organic PSs upon interaction with dsDNA in a label-free manner. Therefore, SG binding with dsDNA could activate the T$_1$ of SG, thereby switching on the photosensitization.

To study the mechanism of fluorescence and phosphorescence modulation, the radiative ($k_r$) and nonradiative ($k_{nr}$) rate constants of SG before and after binding with dsDNA were derived with eqs 1–3 (see Experimental Section). As shown in Table 1, although the radiative rate constants of SG only doubled for fluorescence and tripled for phosphorescence, the corresponding nonradiative rate constants decreased by $\sim 391$-fold (fluorescence) and $\sim 5850$-fold (phosphorescence), respectively. Therefore, the dsDNA-induced luminescence lighting up and photosensitization switching of SG can be ascribed to dsDNA matrix-restricted nonradiative transitions (Scheme 1b). Herein, the inhibition of the nonradiative transition of the T$_1$ is much more significant, probably because the long-lived phosphorescence is prone to deactivation.

**Origin of the nonradiative process of SG**

Next, the origin of the nonradiative transitions of SG was investigated. Structurally, SG is similar to the typical molecular motor thioflavin T (ThT), which contains electron acceptor (benzothiazole) and electron donor (1,4-dihydroquinoline) moieties. Thus, DFT and TD-DFT calculations were employed to confirm the above prediction. Results of the coordinate-driving potential surface
scanning indicates that when excited, a relaxation process with a barrierless rotation of SG starts until the dihedral angle (θ) between the 1,4-dihydroquinoline and benzothiazole moieties reaches 90° (S′₁, the most stable (S₁)) (Figure 2a). In such a process, a nonradiative twisted intramolecular charge-transfer (TICT) process will occur, with extremely low oscillator strength (f) of almost zero (Figure 2b). Such relaxation is fastest due to the small energy gap (S′₁ → S′₀, Figure 2c). Therefore, the radiative processes (fluorescence and phosphorescence) and photosensitization are expected to be off at this stage.

To verify the above transitions, temperature-dependent fluorescence and phosphorescence of SG were collected. As shown in Figures 2d and 2e, the fluorescence and phosphorescence of SG were extremely weak at room temperature [298 K, 2Me-tetrahydrofuran (THF) as solvent], which agrees well with the results in the absence of dsDNA (Figures 1d and 1f). With decreasing temperature, appreciable fluorescence (∼525 nm) and phosphorescence (∼625 nm, delay time of 2 ms) were observed only after freezing (136 K, the ice point of the solvent 2Me-THF), with spectral features similar to those in the presence of dsDNA (Figures 1d and 1f). Further temperature decreases from 136 K to 77 K resulted in increased emission intensity and lifetime, but was not as significant as freezing (from 298 K to 136 K).

Table 1 | Photophysical Parameters of SG before and after Binding with dsDNA

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SG (1)</th>
<th>SG + dsDNA (2)</th>
<th>(2)/(1)</th>
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<tr>
<td>Φ₅₅ (%)</td>
<td>0.6</td>
<td>66.5</td>
<td>110.8</td>
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<tr>
<td>τ₅₅ (ns)</td>
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<td>k₉ (FL, 10⁸ s⁻¹)</td>
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<td>1.5</td>
<td>2</td>
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<tr>
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<td>1/391</td>
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<tr>
<td>Φ₄₅ (%)</td>
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<td>0.057</td>
<td>~60</td>
</tr>
<tr>
<td>τ₄₅ (ms)</td>
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<td>3.07</td>
<td>600</td>
</tr>
<tr>
<td>k₉ (PHOS, s⁻¹)</td>
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<td>0.18</td>
<td>3</td>
</tr>
<tr>
<td>τ₄₅ (PHOS, 10⁴ s⁻¹)</td>
<td>193</td>
<td>0.033</td>
<td>1/5850</td>
</tr>
</tbody>
</table>

Figure 2 | Study on the photophysical properties of free SG: (a) potential energy (eV) versus dihedral angle (θ) at the ground and the S₁; (b) oscillator strength versus dihedral angle (θ); (c) scheme of the rotational mobility of SG between dihedral angle of 90° and <90°, with Jablonski diagram of the plausible mechanism showing inset; (d) temperature-dependent fluorescence and phosphorescence (delay time: 2 ms) emission spectra; and (e) temperature-dependent fluorescence and phosphorescence lifetime. The temperature-dependent investigations were carried out in 2Me-THF media and the concentration of SG was 0.8 μM.
Solution viscosity-dependent investigations further verified the above deactivation process of SG (Supporting Information Figure S14). These phenomena confirmed the TICT nature of SG, which causes significant nonradiative transitions. Upon freezing or viscosity increasing, the bond rotation at the S1 was restricted ($\theta < 90^\circ$), together with significantly enhanced $f$, making the relaxation pathway ($S_1 \rightarrow S'_1 \rightarrow S'_0$) less probable. Therefore, fluorescence ($S_1 \rightarrow S_0$) and phosphorescence ($S_1 \rightarrow T_1 \rightarrow S_0$) would be switched ON (Figure 2c). On the basis of the above spectral features, it is expected that SG binding with dsDNA would result in the restriction of TICT in the S1, leading to the activation of the excited T1 of SG and, in turn, photosensitization (Scheme 1).

### Influence of dsDNA binding on the ISC process of SG

Besides nonradiative competition, ISC also contributes to the activation of the excited T1. In recent studies, it was proposed that PSs with intramolecular charge-transfer (ICT) state may be benefit from the separated donor and acceptor structure in generating activated excited T1.\(^{30-32}\) Therefore, the dihedral angle change of SG after binding with dsDNA may contribute to the ISC of SG. The binding conformation of SG with dsDNA was first simulated with molecular docking (Sybyl-X 2.0).\(^{33,34}\) As shown in Figure 3a, groove binding of SG with the double helix can be identified, which is consistent with the previous research.\(^{35}\) In such a restricted microenvironment, the most probable dihedral angles of SG were in the range of 60–70° (inset in Figure 3a).

The probability of ISC ($k_{ISC}$) is mainly determined by the energy difference between $S_1$ and $T_1$ ($\Delta E_{ST}$) and the SOC $T[H_{soc}]S$:\(^{36}\)

$$k_{ISC} \propto \frac{(T[H_{soc}]S)^2}{\Delta E_{ST}^2}$$  \hspace{1cm} (4)

The $S_1$ molecular orbitals and electronic structures of SG under various dihedral angles were further calculated with TD-DFT.\(^{37}\) As shown in Figure 3b, Supporting Information Figures S15 and S16, increasing the dihedral angle (0–90°) results in increases of the SOC constants ($\zeta$) and decrease of the singlet–triplet energy gap ($\Delta E_{ST}$), which is consistent with previous works.\(^{30-32}\) However, the oscillator strength of SG at 90° is nearly zero, which is not feasible for the whole transition. Therefore, D–A separation may be a design principle for highly efficient PSs (different from the quasi-plane structure requirement of fluorescence), but the oscillator strength should also be taken into consideration (e.g., TICT-based PSs\(^{38,39}\)). Here, the activated $S_0$ of SG after binding with dsDNA may be a compromise between ISC and oscillator strength, again confirming the unique role of the dsDNA matrix in the photosensitization activation of SG (Scheme 1).

### Versatility of the dsDNA matrix for photosensitization switching

The matrix effect of dsDNA for photosensitization switching was not only limited to SG, but also a
series of other PSs. As shown in Supporting Information Figures S17–S19, typical groove binders, Pico-Green PG) and Hoechst 33258 (H33258),40,41 and base intercalators, ethidium bromide (EB) and TOTO-1 (1,1'-((4,4,7,7-tetramethyl-4,7-diazaundecamethylene)bis-4-(3-methyl-2,3-dihydro(benzo-1,3-thiazole)-2-methylidene)quinoinium),42,43 were also found to receive photosensitization activation upon binding to dsDNA. Further photophysical studies indicate that nonradiative rate constants of these PSs greatly decrease after binding with dsDNA (Supporting Information Figures S20–S24 and Table S4). Therefore, dsDNA may be a universal matrix for harvesting photosensitization energy. On the other hand, based on the above data and also previous studies,44 there are three main factors permitting well-performed photosensitization of the dsDNA-binding PSs: (1) high affinity to dsDNA allowing efficient binding; (2) TICT structures for efficient quenching of photosensitization before binding with dsDNA; and (3) structures containing benzothiazole or related moieties to ensure intrinsic photosensitization capacity, which can be switched on after dsDNA binding. Therefore, among the studied PSs, SG, PG, and TOTO-1 generally exhibit better performances over EB and H33258.

Analytical performance comparison of colorimetric photosensitization and fluorescence detection

Previously, we determined that 1O2 generated from photosensitization could oxidize the chromogenic substrate TMB with high efficiency,45,46 yielding sensitive colorimetric signal (Figure 4a) for DNA analysis.16 Herein, we coupled the colorimetric signal from dsDNA-switched

Figure 4 | Comparison of the analytical performances of fluorescence and photosensitization for DNA sensing: (a) scheme of energy diagram of fluorescence and photosensitization colorimetry; (b) comparison of the irradiation time-dependent signal of fluorescence and photosensitization colorimetry; and (c) comparison of the signal-to-background ratio of fluorescence and photosensitization for detection of dsDNA (10 nM) of different lengths (10–130 bps). Due to the lower redox potential of TMB (+0.54 V)47 over the guanine base (+1.47 V),48 and electrostatic interaction of dsDNA backbone and TMB, 1O2 generated from photosensitization will first oxidize TMB rather than the guanine base (Supporting Information Figure S25). Optimizations of the assay sensitivity are presented in the Supporting Information. Experimental conditions: the concentration of SG, 0.8 μM (2x); media for fluorescence, pH 7.4 phosphate buffer; media for photosensitization, pH 4.5 phosphate-citrate buffer; irradiation time for cyan LED, 2 min. The sequences of the DNAs used here are given in Supporting Information Table S2.

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photosensitization with isothermal DNA amplification to develop a new and simple NAT platform. SG was chosen as the model PS for this purpose due to its excellent photosensitization performance over other PSs (Supporting Information Figure S17). As shown in Figure 4b, photosensitization-based colorimetric signals of the dsDNA–SG system sharply increased along the irradiation time, since the consumption of $^{1}\text{O}_2$ (TMB oxidation) could be supplemented through further photosensitization. Such photochemical transduction ($^{1}\text{O}_2 \rightarrow$ oxidized TMB) allowed efficient signal accumulation, which is impossible for fluorescence. Accordingly, the signal-to-background ratio of photosensitization-based colorimetric detection (lighting time: 2 min) was comparable with that of fluorescence (dsDNA: 10 nM; dsDNA lengths: 10–130 bps; Figure 4c). Moreover, when compared with phosphorescence (here, the dsDNA matrix could also induce $^{3}\text{O}_2$-free phosphorescence), the sensitivity of the proposed colorimetric detection was slightly higher (Supporting Information Figure S26 and Table S5). Since they share the same relaxation pathway, the accumulated color signal during photosensitization can be considered an enhancement of the weak phosphorescence signal. This unique assay principle is considerably different from more existing colorimetric methods with much lower sensitivities than fluorescence, leading to comparable LODs through photochemical signal transduction (Supporting Information Figures S27 and S28 and Tables S5 and S6).

### NAT through dsDNA-switched photosensitization coupled with LAMP

On the basis of the excellent DNA detection performance of the current photosensitization switching, we further combined it with LAMP for NAT applications. LAMP is an isothermal amplification protocol that possesses outstanding performance in DNA amplification for diverse applications.\(^\text{49,50}\) Besides, LAMP is also capable of generating dsDNA products after amplification, which can be readily taken as the matrix for photosensitization switching. As a proof-of-principle, a set of six primers were designed to selectively amplify a 192 bps HBV-S gene fragment at location 1081–1272 on the HBV vector (Figure 5a). To ease the operation of photosensitization-based colorimetric detection, a series of a simple LED arrays (96-plex, 48-plex, or 24-plex, based on the plate used) were designed for irradiation. As each LAMP reaction produced massive dsDNA amplicons in terms of both size and concentration, strong color transitions were observed immediately after LED irradiation. In addition to its high sensitivity, LAMP is also well known as a rugged amplification system and much less sensitive to interferences than polymerase chain reaction (PCR). Indeed, we found that LAMP photosensitization could be performed directly in undiluted human serum samples without additional extraction or purification steps. More interestingly, LAMP induced extensive precipitations in HBV-positive serum samples, which significantly

![Figure 5](https://example.com/figure5.png)

**Figure 5** | Photosensitization-based colorimetry coupled with LAMP for NAT of HBV-S gene fragment: (a) schematic illustration of LAMP principle; (b) photosensitization solution colorimetry coupled with LAMP for direct analysis of HBV genomic DNA in undiluted human serum samples without the need for any sample preparation steps, HBV-positive serum samples could be examined visually (top images) or quantitatively using the absorbance detection (bottom); and (c) further simplifying the photosensitization-based colorimetric readout on paper strip for analysis of HBV genomic DNA. The target sequence for LAMP is given in Supporting Information Table S7. The detailed operation procedure of LAMP and fabrication of the paper strip are given in Supporting Information.
enhanced the colorimetric readout, whereas the HBV-negative serum remained clear (Supporting Information Figure S29).

To demonstrate the utility of photosensitization-based colorimetric detection for quantification of LAMP amplicons, we monitored the detection of LAMP-amplified HBV DNA in undiluted human serum samples for 30 min. With a 5-min color development, HBV-positive serum samples with concentrations ranging from 10 aM to 100 fM could be visually discriminated from the negative serum control (Figure S29). Real-time monitoring of this color development also enabled a quantifiable range from 10 aM to 1 fM.

Since the operation of photosensitization-based colorimetric detection can be simply performed with LEDs, and the color development can be directly read out with naked eye, we introduced a paper-based strip with the goal of further simplifying the assay readout (no instrumental need). This strip was fabricated using wax printing, where well-defined circular sample loading zones and linear testing zones were created by hydrophobic wax barriers. A thin layer of TMB was then homogeneously deposited to the testing zone for color development (Figure 5c). Upon SG deposition onto the sample loading zone, it was tightly retained and could only be eluted into the testing zone in the presence of dsDNA (here LAMP amplicons). Therefore, a quantitative relationship could be established between the migration distance (dM) of SG and concentration of dsDNA. Using TMB-coated paper strips, amplicons produced by LAMP could be visually quantified by measuring the dM as readout. Through distance development for 10 min followed by photosensitization color development for 1 min, the propagation of blue color was observed in the testing zone, providing a semiquantitative readout that clearly differentiated HBV DNA (100 aM, dM = ∼29 mm) from the blank (dM = ∼12 mm) without the need for an external reader (Figure 5c, Supporting Information Figure S30).

Conclusion

The role of dsDNA in photosensitization switching of SG was identified as a matrix, resulting in great inhibition of the nonradiative transitions (TICT) and up to a 70-fold 1O2 generation increase. Through photochemical signal transduction, a simple and sensitive colorimetric DNA sensing protocol was developed, yielding comparative analytical performances (signal-to-background ratio and LODs) with homologous fluorescent detection. Due to its simplicity and high sensitivity, such photosensitization switching is easy to be combined with various DNA amplification protocols, such as LAMP. When coupled with varying detection platforms, the analytical performances (LOD: <10 aM for the solution and <100 aM for the paper strip) were comparable with those of typical PCR technology. To further increase the analytical sensitivity, propelling energy from fluorescence to photosensitization via grafting halogen atoms to the structure of SG (heavy atom effect) can be expected.

The dsDNA in this work, namely the matrix for photosensitization switching, offers a new idea for chemical usage of DNA, which may contribute to the RTP area due to the intrinsic association between RTP and photosensitization. For example, the current commercial nucleic acid analysis is almost exclusively based on PCR with fluorescence detection, which may be problematic for POCT due to the high cost and bulky instrumentation of fluorescence. Here, the photosensitization switch (from RTP) shares the same energy origin as fluorescence but allows the development of much simpler and low-cost LED-aided colorimetric assay. Considering that PSs have already been extensively used for PCR, the developed colorimetric assay is appealing for further POCT diagnosis, especially for resource-limited areas.

Supporting Information

Supporting Information is available.

Conflict of Interest

The authors declare no conflict of interests.

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