DNA Computing

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A Reversible DNA Logic Gate Platform Operated by One- and Two-Photon Excitations

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Abstract: *We demonstrate the use of two different wavelength ranges of excitation light as inputs to remotely trigger the responses of the self-assembled DNA devices (D-OR). As an important feature of this device, the dependence of the readout fluorescent signals on the two external inputs, UV excitation for 1 min and/or near infrared irradiation (NIR) at 800 nm fs laser pulses, can mimic function of signal communication in OR logic gates. Their operations could be reset easily to its initial state. Furthermore, these DNA devices exhibit efficient cellular uptake, low cytotoxicity, and high bio-stability in different cell lines. They are considered as the first example of a photo-responsive DNA logic gate system, as well as a biocompatible, multi-wavelength excited system in response to UV and NIR. This is an important step to explore the concept of photo-responsive DNA-based systems as versatile tools in DNA computing, display devices, optical communication, and biology.*

Molecular logic gates have attracted significant interests across widespread scientific researches because they hold great potential applications in nanotechnology, nanomedicine, and computation.[1] Various logic gates based on molecular assemblies[2] and inorganic/organic materials[3] have been developed. Indeed, DNA has been considered as an ideal candidate in designing logic gates because of its outstanding features, such as information processing and data storage capacity, ease of chemical synthesis, predictable hybridization ability, and biocompatibility.[4] Many reported DNA devices could be controlled by several stimuli, such as pH,[5] metal ions,[6] aptamer,[7] small molecules,[8] or sequence-specific oligonucleotides[9] to perform logic gate operations. However, the complicated handling procedures in terms of added external signal inputs limits their application to a certain extent.[10]

To address these problems, light would be an ideal signal input for exogenous control of the performance of DNA logic gates because it is a clean, non-invasive, and non-contact source.[11] No waste is accumulated to interfere with the working efficiency. Importantly, optical inputs can be remotely controlled while detectable signal outputs can be monitored instantaneously, even on the sub-femtosecond time scale. On the other hand, as the systems are sensitive to multiple wavelength ranges of excitation light, multi-wavelength excited systems could be developed based on DNA nanotechnology for potential applications in display devices, optical information storages, and camouflage technology. Among various reported DNA logic gates,[12] there are no demonstrated uses of multiple wavelength ranges of excitation light as inputs to trigger their operations. Thus, designing a photo-responsive DNA-based system which mimics function of signal communication in logic gates is highly significant and emerging, but still remains a challenge.

Herein, we develop a DNA device (D-OR) where the fluorescence signal output(s) can be regulated based on fine differences in the wavelengths of excitation light. Under two-photon excitation irradiation (Input A) and/or one-photon excitation (Input B), photo-cleavage of 4-nitro-4′-phenoxy-1,1′-biphenyl (4-NB) and/or 2-nitrophenyl (2-NP) molecule(s) results in selective release of piece(s) of DNA and detectable fluorescence signal(s) at 564 nm and/or 520 nm (Output) respectively. Importantly, this logic gate system could be easily reset to its original state and performed repeatedly. Furthermore, D-OR exhibited efficient cellular uptake, low cytotoxicity, high bio-stability, and biocompatibility.

Initial efforts concentrated on preparing a single-stranded and cyclic DNA template (T), according to our reported method.[13] Cyclic T consisting of three distinct segments, QQ′, S1′, and S2′, was generated from chemical ligation reaction (Supporting Information, Figures S1–S2).[14] Once we had this T in hand, three complementary DNAs were designed. The S1 strand, which was purchased from IDT, is labeled with a fluorophore Cy3 at the 5′ terminus and a 2-NP molecule as non-nucleosidic molecule at position 13. The successful labelling of 2-NP and Cy3 fluorophore on S1 was confirmed by MALDI-TOF, UV, and fluorescence studies (Figures S3–S4). In contrast, the S2 strand was designed and chemically synthesized to consist of a fluorophore FAM at the 3′ terminus and a 4-NB at position 9 by standard cyanoethyl-phosphoramidite chemistry. Synthesis and characterization of 2-cyanoethyl((4-nitro-4′-(trityloxy)-[1,1′-biphenyl]-3-yl)methyl)diisopropylphosphoramidite (TPM-P) is detailed in the

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Supporting Information (Figures S5–S8). The formation of S2 was confirmed by HRMS, UV/Vis, and fluorescence studies (Figures S9–S10).

The 2-NP motif is only active in one-photon excitation, while the 4-NB motif is not only active in one-photon excitation, but also in two-photon NIR excitation (Figure S11).[15] To explore the photocleavage of S1 and S2, their photolysis ability was investigated and confirmed by denaturing PAGE analysis and HRMS studies. Under UV irradiation at 200 W, the DNA band intensity of S1 decreased as a function of irradiation time while two broken pieces of 8-mer DNAs labeled with Cy3 (S1a) and 12-mer DNAs (S1b) appeared. One-photon photolysis of S1 was found to be completed after 1 min (Figure S12a). The two cleaved products S1a and S1b were confirmed with their corresponding MALDI-TOF *m/z* peaks at 6235.2642 $[M]^+$ (calcd *m/z* 6228.10) and 3772.3022 $[M]^+$ (calcd *m/z* 3772.87) (Figure S12b). To investigate the two-photon photo-cleavage ability of S2, we monitored the amount of S2 cleaved under NIR irradiation at 800 nm fs laser pulses as a function of irradiation time. Denaturing PAGE analysis showed the DNA band intensity of S2 also decreased, while two cleaved pieces of 8-mer DNA labeled with FAM (S2a) and 12-mer DNA (S2b) were observed (Figure S12c). The two-photon photolysis of S2 was completely done after 30 min. These two cleaved pieces were also confirmed with their corresponding MALDI-TOF *m/z* peaks at 6410.6322 $[M]^+$ (calcd *m/z* 6405.80) and 3539.6211 $[M]^+$ (calcd *m/z* 3539.70) (Figure S12d). The two-photon photolysis process of this 4-NB motif has been verified systemically, with a slope close to 2 from a quadratic dependency study.[13]

To form the photon-responsive DNA-based system, QQ, S1, and S2 hybridize to the corresponding segments QQ′, S1′, and S2′ on T respectively (Figure 1a). Their successful hybridization was verified by native polyacrylamide gel electrophoresis (PAGE) analysis in a quantitative yield. The fluorescent signals (Figure 1a, images ii and iii) confirmed the stepwise addition of S1 and S2 and coexistence of two fluorophores on a single D-OR. Subsequently, the fluorescence quenching effect by Iowa Black FQ was clearly revealed in lane 4 after adding QQ strand to T-S1-S2. This phenomenon was in good agreement with the fluorescence change of samples in solution phases (Figure 1b). The fully closed form of the D-OR device was also confirmed by TEM and FRET studies (Figures S13–S14).

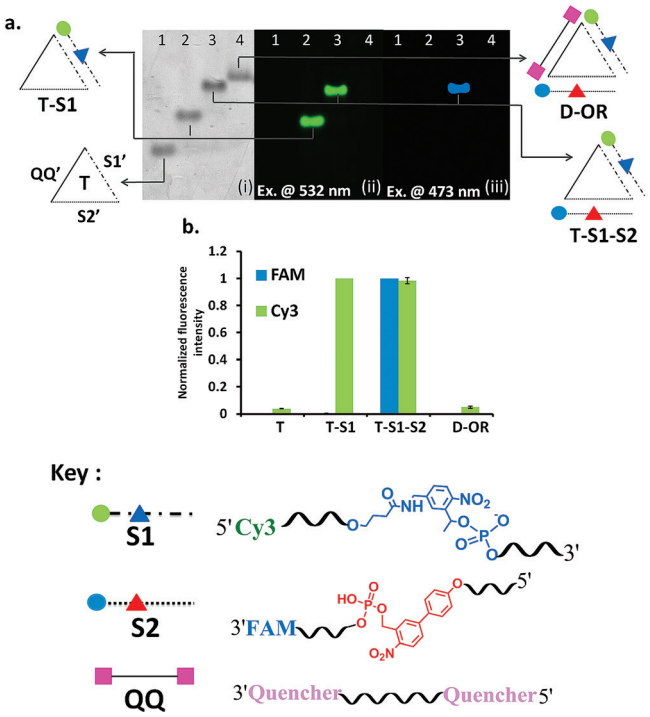
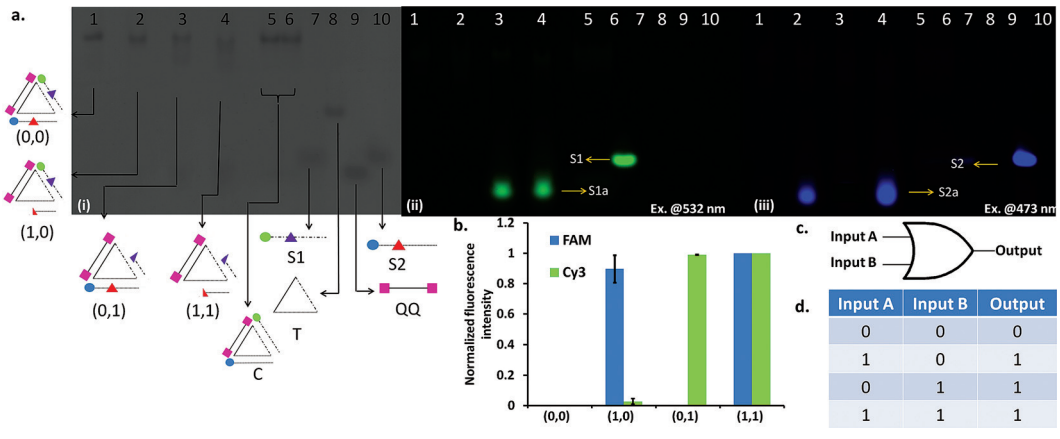


Figure 1. a) Native 8% PAGE gel showing the formation of DNA logic gates under (i) staining with StainAll, (ii) excited by 532 nm, and (iii) excited by 473 nm. b) The fluorescence intensities of DNA samples measured in solution. Excitation at 495 and 550 nm and emission collected at 520 nm and 565 nm for FAM and Cy3, respectively. Three independent experiments were averaged and the errors bars represent the standard deviation.

Native PAGE analysis clearly demonstrated the photon response of D-OR (Figure 2a). At the initial state of no input, the intact D-OR migrates as a single band with attenuated fluorescence emission from Cy3 and FMA owing to their close proximity to fluorescence quenchers (Figure 2a, lane 1). With light input A (NIR irradiation), a slightly lower DNA



Input A	Input B	Output
0	0	0
1	0	1
0	1	1
1	1	1

Figure 2. a) Native 8% PAGE gel analysis of DNA OR gate operation. Lane 1: D-OR; lane 2: D-OR + Input A; lane 3: D-OR + Input B; lane 4: D-OR + Input A + Input B; lane 5: Control (C); lane 6: C + input A + input B; lane 7: S1; lane 8: T; lane 9: QQ; lane 10: S2. Input A is UV excitation while input B is NIR at 800 nm fs laser pulses. b) The fluorescence intensities of DNA samples measured in solution. Excitation at 495 and 550 nm and emission collected at 520 nm and 565 nm for FAM and Cy3, respectively. Three independent experiments were averaged and the errors bars represent the standard deviation. c) Logic circuit and d) the truth table of D-OR.

band and a new band with higher mobility were observed (Figure 2ai, i and iii, lane 2). It is worth noting that the 4-NB molecule was cleaved selectively and the 8-base S2a was unhybridized to D-OR simultaneously at rt (T_m of S2a ≈17°C), at which point fluorescence of FAM measured at 520 nm (output) upon excitation at 473 nm is restored. In contrast, when light input B (UV irradiation) is applied for 1 min, a slightly lower DNA band and a new band of broken Cy3-labeled DNA fragment were also observed (Figure 2a, i and ii, lane 3. This is because UV light excitation activated the D-OR by selective cleaving of the 2-NP and then causing a spatial separation of the 8-base S1a at rt (T_m of S1a ≈13.5°C) from the quenchers, resulting in a restored fluorescence emission of Cy3 at 564 nm (output) upon excitation at 532 nm. Subsequently, in the presence of input A and B, both 4-NB and 2-NP molecules were cleaved correspondingly, at which point fluorescence signals of FAM and Cy3 are restored (Figure 2a, ii and iii, lane 4). Similar fluorescence changes were also observed in solution (Figure 2b). As an important feature of this device, the dependence of the readout fluorescent signals on the two external stimuli, UV excitation and/or NIR at 800 nm fs laser pulses, can mimic function of signal communication in OR logic gates. The initial quenched state of D-OR is given a false operating output signal of 0, and the fluorescent stated induced by the light inputs is allocated a true operating output signal of 1. Consistent with the truth table for the circuit, high fluorescence values are observed in the presence of either one or both inputs (Figure 2c–d). No such DNA cleavage and fluorescence enhancement were observed when control samples (C) were excited by these two light inputs (Figure 2a, lane 5–6). This system is different from the work done by Liu et al. where a DNA switch could mimic the function of signal communication in logic gates with the external stimulations including both acid/base and visible light/dark.[16] Compared with other reported DNA logic gates,[17] indeed, our DNA logic gate has the advantages of only using non-contact/non-invasive sources of light as signal inputs, remote control of signal inputs, and selective control of detectable signal outputs.

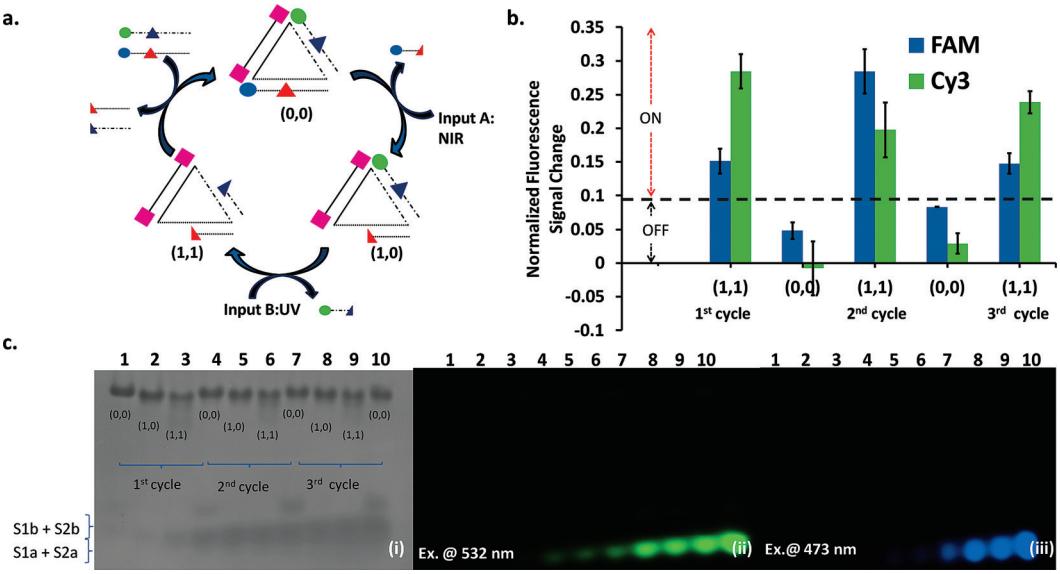


Figure 3. A reversible molecular DNA OR logic gate. a) Representation of the operational design of the OR gate with the cycle transformation between different states. b) Normalized fluorescence signal change (NfSC) of D-OR device in each cycle. NfSC at $(1,1)_n = Nf_n(1,1) - Nf_n(0,0)$ representing the ON state while $(0,0)_n = Nf_n(0,0) - Nf_n\text{-}1(1,1)$ representing the OFF state, where Nf stands for normalized fluorescence and *n* stands for number of cycle. c) Native 8% PAGE gel analysis of three cycles operation between state (0,0) and (1,1).

Reversibility is another key characteristic in DNA logic systems.[18] In our design, the final readout logic gate (1,1) can be easily reset to its original state (0,0) by a strand displacement strategy (Figure 3a). Native PAGE analysis was used to explore this recycle mechanism, showing reproducible performance of the DNA OR gate up to three cycles (Figure 3c). Addition of a corresponding amount of S1 and S2 to the readout logic gate system (1,1) would displace the two shorter pieces of S1b and S2b, resetting the DNA logic gates to the original state (0,0). Upon sequential addition of two light inputs repeatedly, the band intensities of displaced S1b and S2b were gradually increased to enhanced fluorescence signals of fluorophore-labeled S1a and S1b. A fingerprint DNA band pattern of the DNA components (S1a and S2a) could be tracked across each cycle. Fluorescence band intensities of FMA and Cy3 were obtained with ImageJ and further subtracted from the fluorescence from previous cycle. Quantitative analysis of native PAGE image confirmed the fluorescence switch-Off and On phenomenon of D-OR. We defined the normalized fluorescent intensity above the threshold of 0.1 as ON in the logic gate system (Figure 3b). To our knowledge, this is the first example of a reversible DNA logic gate utilizing different wavelengths of lights as inputs.

In principle, our self-assembled DNA devices can be activated and switched ON if the corresponding wavelengths of excitation light are dominant under an electromagnetic spectrum. As light is a useful information carrier, our DNA device could be used as a multi-information carrier for potential applications in display devices, optical information storage, and camouflage technology, as well as DNA comput-

ing. In biology, light is considered as a favorable source of energy and a detrimental source of cellular damage.[19] Some proteins or biomolecules could be systematically activated by different wavelengths of light, and show promise for use in regulating a number of downstream functions/biological mechanisms in living organisms.[20] Therefore, we believe that this single DNA device would also become a versatile tool for monitoring physiological or photochemical processes directly related to light sensing.

Before performing any operation inside cells, it is necessary to explore the bio-stability and biocompatibility of D-OR. To test the sensitivity of D-OR towards nucleases, a fetal bovine serum (FBS) digestion test is performed. The denaturing PAGE showed that D-OR exhibited an enhanced resistance to nucleases as compared to duplex DNA, with a mean half-life of 18.7 h (Figure S15 and Table S1). We strongly believe that these photonic D-OR were significantly protected from enzyme degradation, suggesting that delivery of the device to cells is feasible.

Colocalized confocal fluorescence images of D-OR in several common cell lines, including HeLa, MCF-7, and A549, confirmed their efficient cellular uptake and localization in lysosomes (Figure 4a and S16). These results indicated that

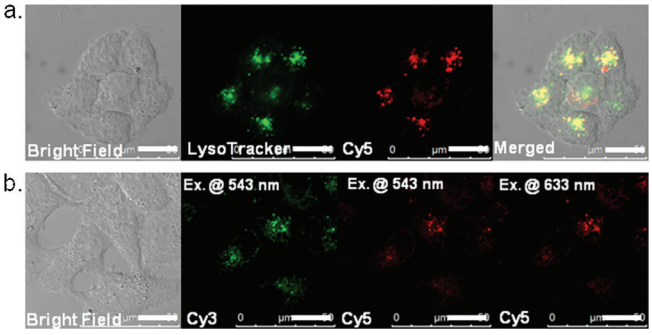


Figure 4. a) Intracellular localization of Cy-5 labeled DNA OR logic device in lysosomes in MCF-7 cells. b) In vitro FRET studies of doubly Cy-labeled DNA OR gate in MCF-7 cells. Scale bar = 20 μm.

delivery platforms, such as commercially available transfection agents or microinjection, are not needed for efficient cellular internalization.[21] Förster resonance energy transfer (FRET) experiments were also conducted to confirm the structural integrity of doubly Cy-labeled DNA logic gates in a cellular environment. This D-OR is cell permeable and enters cell as a cohesive and intact structure rather than broken pieces of its individual components (Figure 4b). Among the numerous reports of DNA-based logic gates, there are very few examples of intracellularly stable DNA logic gates.[22]

MTT and live/dead assays were also performed to evaluate the cytotoxicity of D-OR. Most of the cells (> 99 %) are viable after taking up of different concentrations of D-OR for 24 h (Figures S17–S18). Consequently, these results highly suggest that these photo-responsive DNA devices could function as logic gates but, more importantly, also are suitable as smart materials for biology applications. The logic gate operation involved inside the cells is currently under exploration.

In summary, we have successfully demonstrated the use of two different wavelength ranges of excitation light as inputs to remotely trigger the responses of the self-assembled DNA devices. As an important feature of this device, the dependence of the readout fluorescent intensities on the two external stimuli, UV excitation, and/or NIR at 800 nm fs laser pulses, can mimic function of signal communication in OR logic gates, and can be considered as an example of a photo-responsive DNA logic gate system. Our strategy does not rely on toehold-mediated strand displacement, therefore no waste is accumulated to harm its operational efficiency. Remarkably, this simple but versatile DNA logic gate system can be reversibly operated, which allows the ON-OFF function to be achieved. Furthermore, a biocompatible, multi-wavelength excited system is also developed based on DNA nanotechnology. This work is a step towards employing photo-responsive DNA devices as versatile materials in biology, DNA computing, and optical communication.

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