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DNA Computation: A Photochemically Controlled AND Gate

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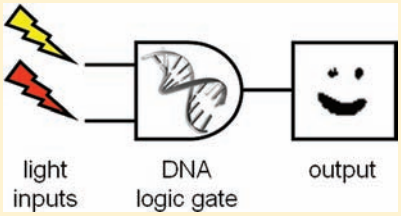
Supporting Information

ABSTRACT: DNA computation is an emerging field that enables the assembly of complex circuits based on defined DNA logic gates. DNA-based logic gates have previously been operated through purely chemical means, controlling logic operations through DNA strands or other biomolecules. Although gates can operate through this manner, it limits temporal and spatial control of DNA-based logic operations. A photochemically controlled AND gate was developed through the incorporation of caged thymidine nucleotides into a DNA-based logic gate. By using light as the logic inputs, both spatial control and temporal control were achieved. In addition, design rules for light-regulated DNA logic gates were derived. A step-response, which can be found in a controller, was demonstrated. Photochemical inputs close the gap between DNA computation and silicon-based electrical circuitry, since light waves can be directly converted into electrical output signals and vice versa. This connection is important for the further development of an interface between DNA logic gates and electronic devices, enabling the connection of biological systems with electrical circuits.



INTRODUCTION

Since Adleman showed a solution to the Hamiltonian path problem in 1994 through DNA hybridization,[1] demonstrating that an algorithm can be encoded in DNA and used to perform computation operations, there have been many developments in the field of DNA computation. A variety of DNA based chemical circuits have been engineered to facilitate DNA and ligand inputs that produce output signals that can be used as molecular computation devices. DNA logic gates have been designed as synthetic chemical circuits based on nucleic acid base hybridization,[1] deoxyribozyme function,[2] aptamer ligand binding,[3] molecular beacon probes,[4] and toe-hold mediated strand displacement.[5,6] DNA logic gates are a powerful computational device because the outputs are chemically equivalent to the inputs, such that the output of one gate can act as the input for a following gate. The advances in DNA logic gate engineering have enabled serial connections of gates, thus generating signaling cascades that can be assembled into complex molecular circuits.[7,8]

Though there are many hurdles in producing DNA computation devices that would rival silicon based computation, there are distinct advantages in developing biologically relevant computation systems. DNA computation devices have the ability to interact with biological and chemical environments, which is an important step toward developing in vivo cellular computation.[9] Interactions between biological systems and DNA computation devices may allow a cell to be programmed to process chemical and biological inputs and give a defined output response. Cells have been programmed to recognize DNA hybridization for cellular self-assembly pathways,[10] showing the potential for linking DNA computation with biological systems. Since DNA logic gates can be constructed to accept a variety of inputs, the ability to interface with biological systems is a strong driving force to further develop DNA computing devices that recognize specific biological changes. These attributes are paramount toward the eventual goal of developing modular cellular circuitry and molecular computation devices.

Light represents a powerful input with a wide range of advantages over chemical or biological inputs.[11] The use of chemical inputs introduces variables, such as cellular uptake, processing, and diffusion that reduce the reliability of a logic gate to be controlled in a biological environment. A system in which the logic gate machinery is preassembled and later activated with light provides enhanced control and specificity. Caged nucleic acids allow for light-activation of DNA hybridization in a precise manner that other research tools cannot accommodate. Previous examples have shown that photocaged oligonucleotides can be used for recombinant DNA manipulation,[12−14] DNA aptamer activation,[15] ribozyme and deoxyribozyme regulation,[16−18] and control of gene expression through antisense technology[19−23] as well as RNA interference mechanisms.[24−26] However, photocaged nucleic acids have not been used in cellular computation or the development of DNA based logic gates. Here, we are demonstrating that photochemical control of logic gate function can be achieved by employing caging groups on DNA strands responsible for toe-hold displacement. The photochemical triggering of a functional logic gate allows for spatial and temporal activation which can be used to enhance control over signaling cascades of complex DNA computation circuits.[7]

RESULTS AND DISCUSSION

A light-triggered AND gate was designed on the basis of the concept of toe-hold mediated strand displacement, which forms the basis for many DNA computational elements.[8] A toe-hold is a short sequence of single stranded DNA (approximately 6 nt long) that binds to a complementary sequence on another

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strand.[5,27] The purpose of a toe-hold is to facilitate strand displacement and hybridization by bringing two strands within close proximity. In the absence of a toe-hold, strand displacement is kinetically slow and offers little or no thermodynamic benefit.

As shown in Figure 1, we designed a light-triggered AND gate based on a gate complex[5] as well as a caged (A_4) and a

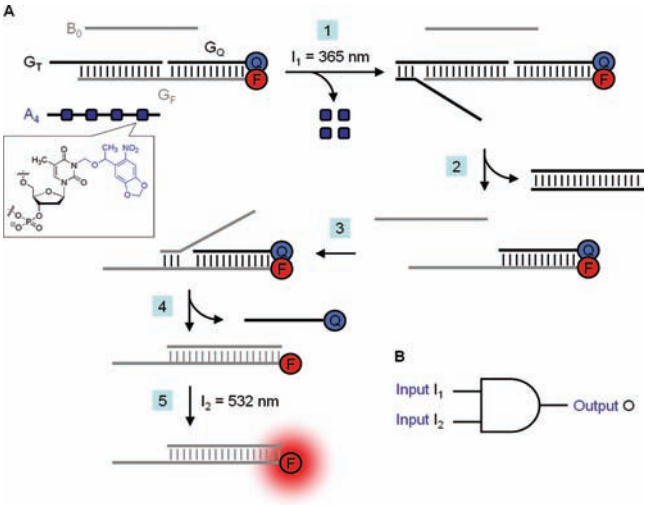

Figure 1. (A) Light-triggered DNA-based AND gate using irradiation at 365 and 532 nm as input signals I_1 and I_2, respectively, and fluorescence as the output signal. The NPOM (6-nitropiperonyloxymethylene) caging group installed on thymidine nucleotides is represented by a blue square.[28] Quencher Q = Iowa Black RQ. Fluorophore F = tetramethylrhodamine (TAMRA). (B) AND gate electrical symbol defining inputs I_1 and I_2 and the output O.

noncaged (B_0) DNA strand. The AND logic gate will only deliver an output signal if both photochemical input signals of different wavelengths (I_1 and I_2) are present. The gate complex is composed of three ssDNA oligomers: a fluorophore strand G_F, a quencher strand G_Q, and a toe-hold containing strand G_T. The fluorophore and quencher moieties are in close proximity preventing fluorescence. In order to activate the gate, A_4 and B_0 need to induce a toe-hold displacement cascade resulting in the removal of G_Q from G_F. The A_4 strand binds to the toe-hold of G_T separating the gate complex, allowing B_0 to bind to the toe-hold exposed on G_F. This event releases G_Q, permitting emission of the excited fluorophore. We hypothesized that caging groups installed on select thymidine bases of the A_4 strand will prevent hybridization and thus prevent strand exchange. Therefore, without the proper light inputs for decaging (input I_1 = 365 nm) and excitation (input I_2 = 532 nm) no output signal will be observed. Thus, step 1 involves UV irradiation at 365 nm for decaging of the nucleotides. After caging group removal, complementary regions are exposed, enabling DNA:DNA hybridization. In step 2, A_4 will dislodge G_T via a toe-hold mediated strand displacement mechanism.[8] Following step 2, the gate complex consists of only fluorophore and quencher strands. Step 3 occurs spontaneously because a second toe-hold region is exposed on the gate complex after the G_T strand was expelled by the A_4 strand. During step 4, quencher and fluorophore strands are separated by a second toe-hold mediated exchange with the strand B_0. In step 5, irradiation at 532 nm now leads to excitation of the fluorophore and observation of fluorescence emission as the output signal.

Due to the impact of toe-holds on strand displacement kinetics, we initially selected toe-holds as the primary targets for the installation of nucleotide caging groups. If needed, additional caging groups were added as evenly as possible throughout the remainder of the oligonucleotide. Thus, a set of caged and noncaged DNA oligonucleotides consisting of input strands or the gate itself were synthesized on the basis of previous reports[5,6] using standard oligonucleotide polymerization chemistry (see Experimental Section), in order to develop and investigate a light-triggered AND gate (Table 1).

In order to determine the effect of caging groups for the photochemical control of an AND gate, caging groups were initially added to the A_0 strand. A set of four oligonucleotides, A_1–A_4, bearing 1–4 caging groups was synthesized and individually tested for function to study the design requirements for suppression of strand displacement. As the number of caging groups was increased, the fluorescence output of the gate linearly decreased in the absence of UV irradiation with I_1 = 365 nm (Figure 2). Optimal suppression of the output signal was observed with A_4, which contained four NPOM caging groups evenly distributed throughout the DNA strand and displayed no activity. Thus, the presence of only one or two caging groups in the six nucleotide toe-hold region was not sufficient to prevent initiation of the strand displacement reaction and subsequent gate function.

An optimization of the UV irradiation time for decaging was conducted, and a time course was performed with the A_4 strand. Maximum fluorescence was observed after 15 min of UV irradiation at I_1 = 365 nm followed by a brief excitation at I_2 = 532 nm (Figure 3). Longer I_1 irradiation times lead to a decrease in fluorescence, possibly due to photobleaching of the

Table 1. Sequences of Caged and Noncaged Oligomers Used in the Light-Triggered AND Gate[a]

strand	sequence (5′ → 3′)
G_Q	Q-GTTAGATGTTAGTTTCACGAAGACAATGAT
G_F	TGTTTATGTGTTCCCTGATCTTTAGCCTTAATCATTGTCTTCGTGAAACTAACATCTAAC-F
B_0	GTTAGATGTTAGTTTCACGAAGACAATGATTAAGGC
B_4	GTTAGAT*GTTAGTTT*CACGAAGACAAT*GATT*AAGGC
G_T	TAAGGCTAAAGATCAGGGAACACATAAACAACCATA
G_{T1}	TAAGGCTAAAGATCAGGGAACACATAAACAACCAT*A
A_0	TATGGTTGTTTATGTGTTCCCTGATCTTTAGCCTTA
A_1	TAT*GGTTGTTTATGTGTTCCCTGATCTTTAGCCTTA
A_2	TAT*GGT*TGTTTATGTGTTCCCTGATCTTTAGCCTTA
A_3	TAT*GGT*TGTTTATGT*GTTCCCTGATCTTTAGCCTTA
A_4	TATGGT*TGTTTATGT*GTTCCCT*GATCTTT*AGCCTTA

[a]Toehold regions are underlined and NPOM-caged thymidines are highlighted as T*. Q = Iowa Black RQ quencher. F = tetramethylrhodamine (TAMRA) fluorophore.

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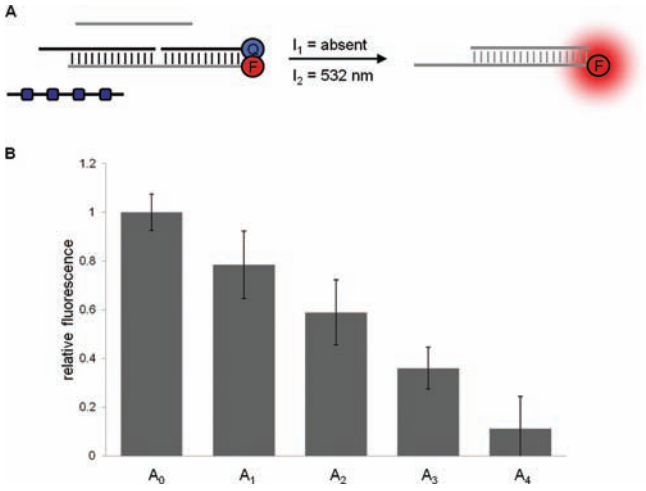

Figure 2. Investigation of the number of caging groups on strand A_0 that are required to inhibit gate function. (A) Simplified schematic of the light-activation of the AND gate. (B) The logic gate was not irradiated with 365 nm light in order to keep all caging groups in place, but only with 532 nm light as I_2. A linear decrease in fluorescence was observed with increasing numbers of caging groups. Four caged thymidines on strand A_4 produced optimal suppression of fluorescent signal. An average of three independent experiments is shown, and error bars represent standard deviations. Time courses are shown in Supporting Information Figure S2.

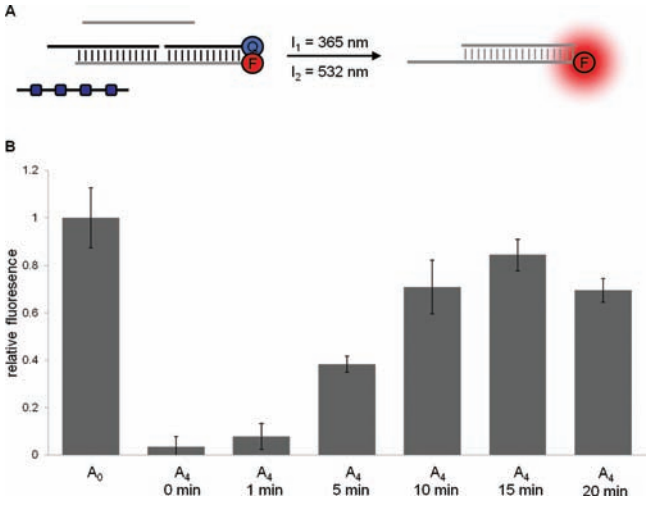

Figure 3. Time course of UV irradiation of the gate complex and the caged A_4 strand. (A) Simplified schematic of the light-activation of the AND gate. (B) A maximum fluorescence output signal is obtained with a 15 min irradiation at I_1 = 365 nm. An average of three independent experiments is shown, and error bars represent standard deviations. Time courses are shown in Supporting Information Figure S3.

fluorophore.[29] Activation of logic gates using noninvasive UV irradiation as an input signal shows that a DNA-based light switch can be generated, which holds promise for developing new applications of externally regulated DNA computation devices.

After the successful light-triggering of gate activity using the caged strand A_4, four NPOM-caged thymidine nucleotides were introduced into the B_0 strand (B_4) in order to test if the AND gate can also be photochemically controlled by caging B_0. Strands containing fewer than four caging groups were dismissed on the basis of the results from testing A_{1-4} (Figure 2). Here, the output signal was completely suppressed in the presence of B_4 and was only observed after irradiation with the input wavelengths of I_1 = 365 and I_2 = 532 nm (Figure 4). Thus, caged B_4 was also successful for photochemical control of the light-triggered AND gate.

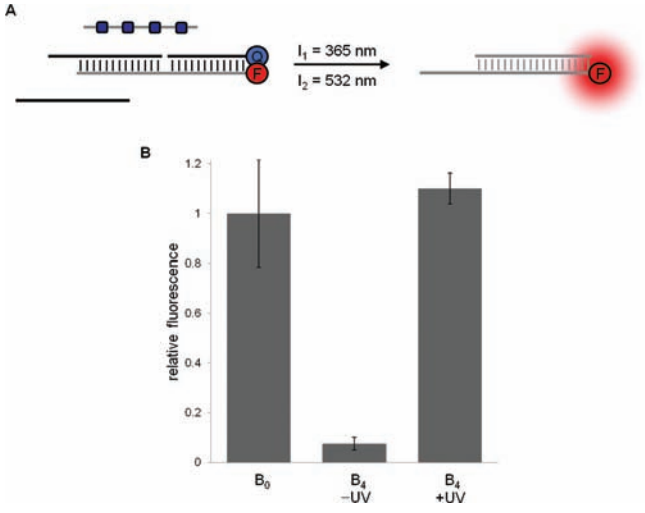

Figure 4. Gate activation through irradiation of a caged B_0 strand. Four caging groups on B_4 fully suppress AND gate function in the absence of UV irradiation, and decaging at I_1 = 365 nm (15 min) led to full restoration of DNA gate activity. (A) Simplified schematic of a light-triggered AND gate using the caged DNA strand B_4. (B) The logic gate was irradiated at I_2 = 532 nm with and without prior irradiation at I_1 = 365 nm. An average of three independent experiments is shown, and error bars represent standard deviations. Time courses are shown in Supporting Information Figure S4.

In addition, caging of a thymidine located in the toe-hold region of the gate strand (G_{T1}) was investigated in order to ascertain if caging of the initiator toe-hold of the AND gate would allow for photochemical control. When noncaged A_0 and B_0 were added to a caged gate complex containing G_{T1}, a signal comparable to the noncaged G_T strand was observed indicating full function of the gate despite the presence of the NPOM caging group (Figure 5). Thus, the output signal was not repressed through the addition of a single caging group to the gate complex toe-hold. The caged gate complex containing G_{T1} was also investigated with strands A_1 and A_2, which also contain caging groups in the toe-hold region of the complementary strand. Surprisingly, no complete suppression of logic gate function was observed by combining caging groups in the toe-holds of strands A_{1-2} with G_{T1}. Thus, in agreement with caged A_0 strands (Figure 2B), caging just the toe-hold regions of interacting strands is not sufficiently effective at suppressing the strand displacement reaction. In conclusion, caging groups need to be evenly distributed throughout the sequence, including toe-hold and body regions, in order to enable photochemical control of DNA logic gate operations.

To investigate whether the light-triggered AND gate could be controlled with temporal resolution using UV light as an input, three separate sets of experiments with A_4 were conducted and logic gates were irradiated at different time points (Figure 6A). The obtained fluorescent signal was only observed after UV irradiation at I_1 = 365 nm, but not before. Thus, temporal control over the light-triggered AND gate was achieved. Moreover, a step response of the gate was elicited through subsequent UV irradiations in two intervals (Figure 6B). The tunable nature of the step response displays a unique feature to control output intensity of a DNA-based AND gate using

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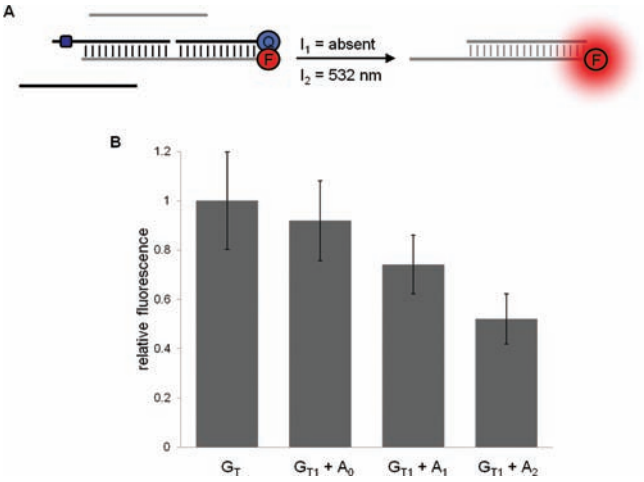

Figure 5. Caging of the toehold region of strands G_T. (A) Simple schematic of the caged AND gate containing caged G_{T1}. (B) The logic gate was not irradiated with 365 nm light, but only with 532 nm light. The addition of a caging group on strand G_T alone does not decrease fluorescence output. G_{T1} was analyzed with strands A_1 and A_2 to determine the effects of combining caged toe-hold regions on hybridizing strands. Full suppression of the gate output signal was not achieved, revealing an insufficient deactivation of gate function using caged toe-hold regions exclusively. An average of three independent experiments is shown, and error bars represent standard deviations. Time courses are shown in Supporting Information Figure S5.

subsequent input stimuli I_1. Achieving a tunable step-response allows light-triggered DNA logic gates to be used as molecular controllers that can be adapted to enhance circuit cascades. As discussed by Ellington,[9] a disadvantage of current DNA logic gate technology is a lack of real-time response to changes in the environment. Using photochemical activation to achieve temporal control allows for the advancement of DNA based computation by overcoming this hurdle and enhances time dependent computation applications. These factors demonstrate the improvements upon existing DNA logic gates through temporal activation with light input signals.

In order to demonstrate spatial control of DNA computation via locally restricted light irradiation, the AND gate complex and the caged strand A_4 were embedded into a low-melt agarose gel. To ensure that the AND gate still functioned correctly in an agarose gel, a truth table was first completed (Figure 7A). The gel was either kept in the dark or spot irradiated with I_1 = 365 nm UV light, followed by imaging of the gel via excitation at I_2 = 532 nm. A distinct signal was obtained, and no fluorescence was observed in the absence of I_1 or I_2, demonstrating the ability to apply the developed light-triggered AND gate in spatially controlled DNA computation. DNA computation in a spatially restricted fashion was achieved through patterned UV irradiation using two different masks (Figure 7B). A fluorescent output was only observed in irradiated areas that performed an AND logic operation (I_1 = 365 nm, I_2 = 532 nm) but not in areas where one input was missing (I_1 = absent, I_2 = 532 nm). This demonstrates that logic gate operation can be performed in semisolid structures and is not limited to solution based applications. Electronic systems depend on solid structures and spatially separated devices. Identification and recognition of spatially separated signals allows organization of objects and circuits and creates an important link between nonelectronic and electronic computational systems.

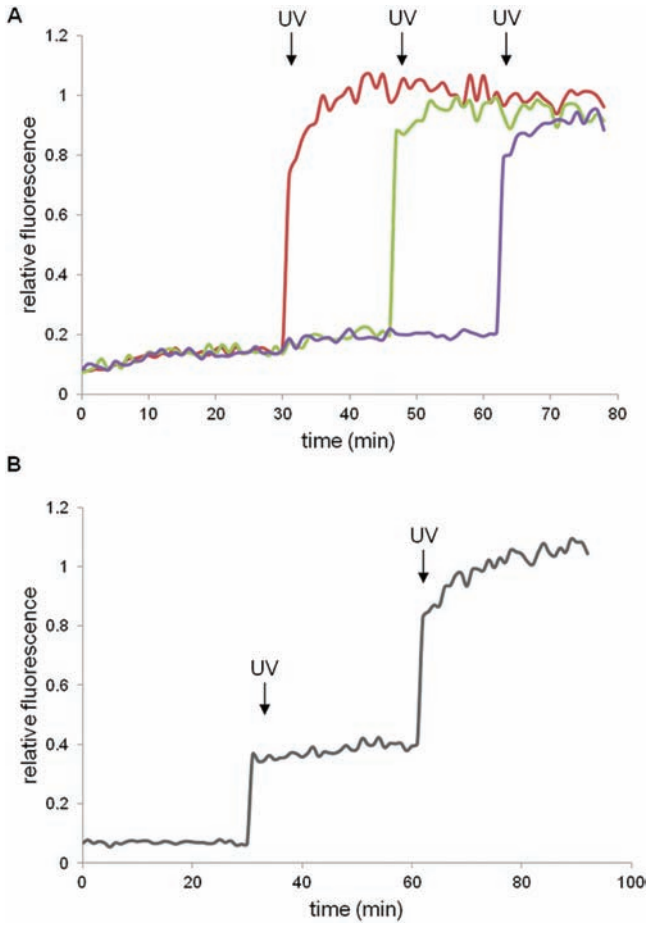

Figure 6. UV irradiation of the gate complex containing caged A_4 at different time points in order to demonstrate temporal control over DNA computation. (A) Baseline fluorescence was measured for 30 min, and three individual gates were irradiated with I_1 = 365 nm light at 30 (red), 45 (green), and 60 min (purple). (B) A single logic gate was irradiated for two intervals resulting in a steplike response. The output signal of the caged AND gate is dependent upon the irradiation interval and increases with additional UV exposure. Graphs represent an average of three independent experiments.

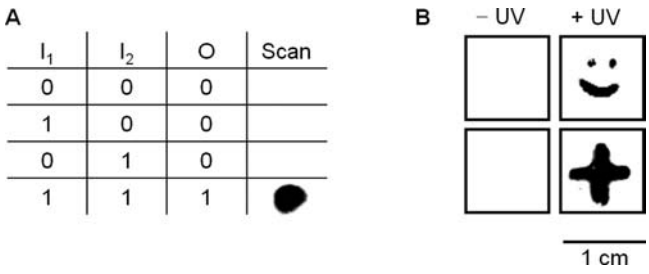

Figure 7. Spatial control of DNA logic gate function. (A) Truth table of the AND gate. Low-melt agarose containing the gate complex, the A_4 strand, and the B_0 strand was tested using all combinations of I_1 (365 nm) and I_2 (532 nm), providing the expected AND gate result. (B) Spatially restricted activation of the DNA logic gate through patterned UV irradiation using masks. Gel imaging revealed patterned fluorescence only in areas previously irradiated with I_1 = 365 nm light, followed by scanning of the entire gel with I_2 = 532 nm.

CONCLUSIONS

In conclusion, a photochemically controlled AND gate was developed through the incorporation of caged thymidine

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nucleotides in a DNA-based logic gate. Strands of DNA were synthesized using specialized phosphoramidites, which enabled the use of specific wavelengths of light as inputs for a DNA-based AND gate. Many DNA-based computation methods rely on toe-hold mediated strand displacement. Thus, the design of caged oligomers was primarily focused on controlling gate activity by caging toe-hold regions. However, our experiments showed that exclusive caging of the toe-hold regions and the introduction of fewer than four evenly spaced caged nucleotides per 36 bases is ineffective for the photochemical control of strand displacement and DNA computation. Temporal control over DNA computation was achieved through introducing four caging groups and activating separate gate complexes at different time points, displaying fundamental properties of a light-switch for molecular circuits. When a single gate complex was irradiated at two intervals, a steplike response in the output signal was observed, suggesting that the phototriggered AND gate can act as a tunable DNA-based circuit. Integration of a light-activated AND gate for purposes of a step response could allow the gate to function as a manual feedback controller. Within a cascade of gates, the light-triggered AND gate can operate as a switch or controller and will allow for more complex and better controlled circuit designs. Moreover, photochemical activation enabled DNA-based logic operations in a spatially localized fashion. This was demonstrated by light-triggered pattern formation in a semisolid substrate, where DNA computation events were only observed in areas that received irradiation with both input wavelengths. Design rules were established that enabled light-activation of the gate and will be applicable to further developments, e.g., the generation of other light-triggered logic gates. The use of light to control a DNA-based logic gate creates a new paradigm of inputs that will be beneficial when used in a biological context. Light allows for spatial and temporal control with high specificity, while overcoming the downfalls of chemical based inputs such as diffusion and delivery kinetics. Photochemical inputs also shorten the gap between DNA computation and silicon-based electrical circuitry, since light waves can be directly converted into electrical output signals and vice versa. This connection is supremely important for further developing the interface of DNA logic gates and electronic devices and, thus, the interface of biological systems with electrical circuits. Thus, the photochemical control demonstrated here lays the foundation for the programming of complex, DNA-based computation devices with unprecedented spatial and temporal resolution.

EXPERIMENTAL SECTION

Caged DNA Synthesis Protocol. DNA synthesis was performed using an Applied Biosystems (Foster City, CA) model 394 automated DNA/RNA synthesizer and standard β-cyanoethyl phosphoramidite chemistry. The caged oligonucleotides were synthesized on a 40 nmol scale, with solid-phase supports obtained from Glen Research (Sterling, VA). Reagents for automated DNA synthesis were also obtained from Glen Research. Standard synthesis cycles provided by Applied Biosystems were used for all normal bases with 25 s coupling times. The coupling time was increased to 2 min for incorporation of caged deoxythymidine modified phosphoramidite. The NPOM-caged deoxythymidine phosphoramidite was resuspended in anhydrous acetonitrile to a concentration of 0.1 M.

Preparation of the Logic Gate. Noncaged strands G_Q, G_T, A_0, and B_0 were purchased from Integrated DNA Technologies (IDT), and strand G_F was purchased from Alpha DNA. Logic gates were assembled and quantified according to the procedures by Winfree.[5]

Fluorescence Experiments. Fluorescence was measured on a BioTek Synergy 4 plate reader using an excitation wavelength of 532 nm and emission wavelength of 576 nm. A black walled and clear bottom 96-well plate designed for fluorescence contained all reaction samples. Reaction buffer (TAE/Mg^{2+} buffer) contained 0.04 M tris-acetate, 1 mM ethylenediaminetetraacetic acid (EDTA), and 12.5 mM magnesium acetate. Positive controls were tested for each experiment and contained a total sample volume of 100 μL reaction buffer with a gate concentration of 200 nM, strand A_{0-4} concentration of 800 nM, and strand $B_{0,4}$ concentration of 800 nM. Samples were irradiated at 365 nm with a hand-held UV lamp. Relative fluorescence represents fluorescence of each sample relative to the positive control (set as 1.0).

Investigation of Optimal Caging Group Number and Localization on Strand A. Samples were prepared in triplicate, and wells contained 100 μL total of 200 nM gate complex and 800 nM B_0 in TAE/Mg^{2+} buffer. Initial fluorescence (532 nm) was measured for 20 min. Caged oligomers A_1–A_4 were added (800 nM), and fluorescence was measured for 20 min.

Irradiation Time Course. Samples were prepared in triplicate for each experiment (0, 1, 5, 10, 15, and 20 min). Sample wells contained 100 μL of a total of 200 nM gate complex, 800 nM B_0, and 800 nM A_4 in TAE/Mg^{2+} buffer. Initial fluorescence (532 nm) was measured for 30 min. Wells were irradiated at 365 nm for the indicated time followed by fluorescence measurement.

Light-Activation of Strand B. Samples were prepared in triplicate, and wells contained 100 μL total of 200 nM gate complex and 800 nM A_0 in TAE/Mg^{2+} buffer. Initial fluorescence (532 nm) was measured for 30 min. Strand B_4 was added (800 nM), and fluorescence was measured for 30 min. The wells containing B_4 were irradiated at 365 nm for 15 min followed by fluorescence measurement for 30 min.

Investigation of Toe-Hold Caging. Samples were prepared in triplicate, and wells contained 100 μL total of 200 nM caged gate complex in TAE/Mg^{2+} buffer. Initial fluorescence (532 nm) was measured for 30 min. Strand B_0 (800 nM) and strand A_0, A_1, or A_2 (800 nM) were then added followed by fluorescence measurement for 30 min.

Spatial Control of Gate Function. A 100 μL 1.5% agarose solution in TAE/Mg^{2+} buffer was heated in a microwave until all agarose was dissolved. Before the agarose solidified, the gate complex (200 nM), A_4 (800 nM), and B_0 (800 nM) were added and mixed. The gel containing the gate and caged strand was spread on a glass slide and allowed to solidify for 20 min in the dark. The gel was imaged on a General Electric Typhoon FLA 7000 phosphorimager for background fluorescence with an excitation wavelength of 532 nm and a 580 nm emission filter. The gel was then irradiated on a UVP high performance UV transilluminator with 365 nm light passing through patterned aluminum foil. After UV irradiation, the gel was again imaged.

ASSOCIATED CONTENT

Supporting Information

Fluorescence time courses of gate activation experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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