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Photo-pH Dually Modulated Fluorescence Switch Based on DNA Spatial Nanodevice

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In this paper, we describe our strategy on the design, construction, and characterization of a novel molecular device. By integrating a photoregulated fluorescent switch and a DNA-based nanomachine, the distance-dependent FRET process between fluorescein and photochromic moieties can be further modulated by the spatial output of a unique proton-driven DNA manomachine. This device could be reversibly switched in a reliable manner, and the fluorescence variation behavior, exhibited by this dually modulated fluorescence switch, can also mimic the function of a Boolean logic operation.

Introduction

Toward the ambitions to miniaturize and integrate electronics, photoelectronics, and mechanical devices for their widespread applications, development of molecular functional systems has been growing at a tremendous pace recently.[1–3] By making using of the features of photochromic compounds, a number of interesting molecular switches are reported;[4] for example, a spiropyran-based energy donor−acceptor (D−A) system has been demonstrated to be able to associate with fluorescein to make a photoregulated fluorescence switch through the fluorescene resonance energy transfer (FRET) process.[5] Meanwhile, the progressive insight into the structural and functional features of biomacromolecules has provided new inspirations to design molecular machinery devices such as DNA-based nanomachines.[6–11] However, the integration of different types of functional molecular systems is far behind the development of each single aspect. In this paper, we will show our attempt at integrating a photoregulated fluorescence switch and a DNA-based nanomachine, where the distance-dependent FRET process between fluorescein and the photochromic compound spiropyran can be further modulated by the spatial output of a unique proton-driven DNA manomachine. This device could be reversibly switched in a reliable manner, and the fluorescence variation behavior, exhibited by this dually modulated fluorescence switch, can also mimic the function of a Boolean logic operation.[12,13]

In our strategy, an established photoregulated fluorescence switch, fluorescein (Flu) and the light-induced structural transformation of spiropyran (SP)/merocyanine (MC) dyad (chemical structures are shown in Figure 1a; for detailed synthesis and characterization please see Supporting Information),[5] was modified with appropriate functional groups to connect with the pH-driven DNA motor. According to fluorescent spectroscopy studies (see Supporting Information), Flu molecule (the esterified form of fluorescein rather than the standard acid form is used in this work to eliminate its pH dependence; see its chemical structure in Figure 1a) shows broad absorption in the range of 400−500 nm, and strong emission around 520 nm when excited by 430 nm light; although the photochromic molecule SP has no obvious absorption at the wavelength larger than 400 nm,[14] its isomer MC (merocyanine) has large overlap with Flu's emission spectrum. The transformation between SP and MC could be manipulated by light irradiations;[15] thus a photoinduced fluorescence switch could be realized based on an intramolecular FRET process. Since the FRET intensity is proportional to the inverse sixth power of the D−A distance,[16] changing the spatial relation of donor and acceptor could be another route to modulate such a molecular switch.

Recently, kinds of molecular motors based on DNA have been reported which can produce nanometer spatial changes driven by different mechanisms. Among them, a DNA motor based on the "i-motif" structure, a four-strand DNA structure formed by an oligonucleotide containing tracts of cytosines at mild acid pH,[17] could undergo a clean, swift, and reversible extend−shrink movement on a pH change.[11a] This process has been demonstrated strong enough to move a functional group attached to its ends for ∼5 nm[18] or even drive a microcantilever move.[19] This system and well-established DNA modification technology enable us to incorporate a spatial control into the above-mentioned photomodulated FRET switch and acquire much advanced functional molecular devices.

Experimental Section

DNA sequence X (5′ HS-CCCTAACCCTAACCCTAACCC-NH_2 3′) was purchased from Takara Bio. (Dalian), with thiol modification at the 5′ end and amino modification at the 3′ end. DNA sequence Y (5′ GTTAGTGTTAGTGTTAG 3′) was purchased from SBS Genetech (Beijing).

Circular dichroism (CD) spectra were collected on a JASCO J-810 circular dichroism spectrometer. Ultraviolet−visible (UV−vis) studies were performed on a Varian Cary 100 spectrometer. Fluorescence spectra were recorded on a Hitachi F-4500 spectrophotometer using an excitation wavelength at 430 nm.

In a typical procedure, DNA X was dissolved in 0.1 M potassium phosphate buffer (pH 7.0) to a final concentration of 100 μM. Maleimide-modified SP (compound **5**; see Supporting Information) and Flu (compound **8**; see Supporting Information) were dissolved in dry DMSO to final concentrations of 20 mM. To 100 μL of X solution was added 10 μL of 20 mM SP

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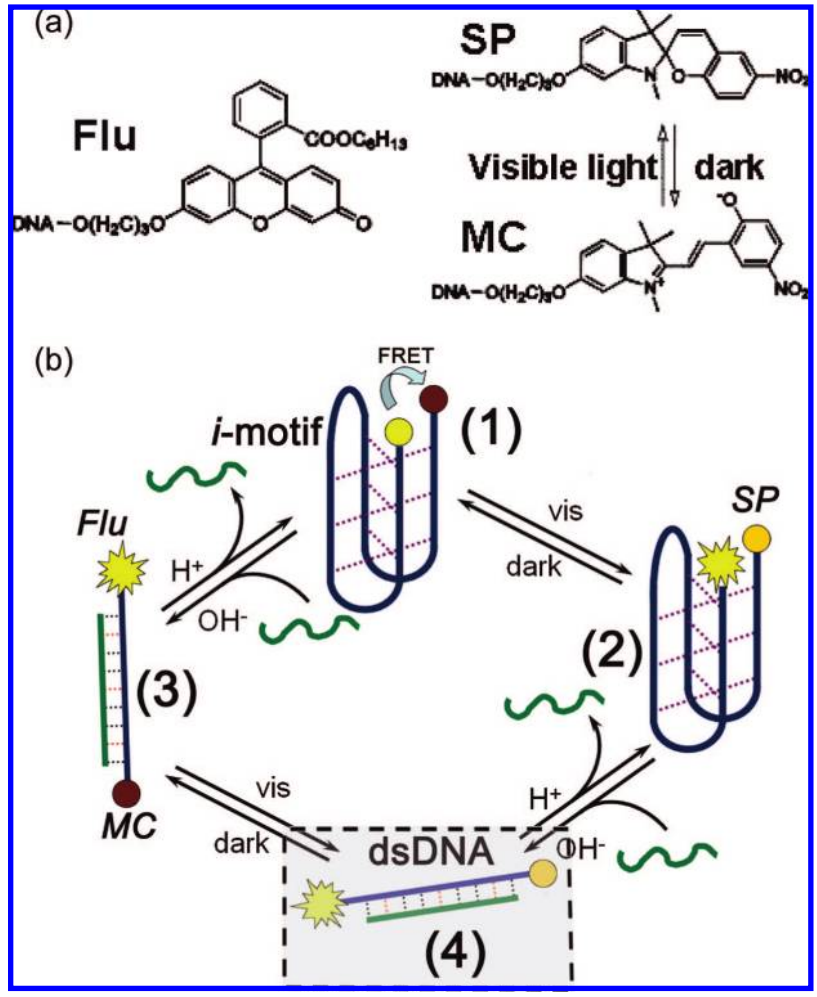

Figure 1. Working principle of photo-pH dually modulated fluorescence switch based on DNA spatial nanodevice. (a) Chemical structures of the dyes Flu (esterified form of fluorescein rather than standard acid form is used in this work to eliminate its pH dependence) and SP/MC. (b) Four stages of the switch constructed by oligonucleotide strands X (blue) and Y (green).

solution. Then it was kept at room temperature for 4 h; unreacted SP molecule was extracted by ethyl acetate. SP−DNA conjugate with attached SP at the 5′ end was purified through HPLC and redissolved in 0.1 M potassium borate buffer (pH 8.0) to a final concentration of 100 μM. To 100 μL of SP−DNA conjugate solution was added 10 μL of 20 mM dithiobis(propionic acid *N*-hydroxysuccinimide ester) (DTSP, from Sigma, dissolved in DMSO) solution. It was kept at room temperature for 24 h, and then it was treated with stoichiometric tris(2-carboxyethyl)phosphine hydrochloride (TCEP, from Shanghai Sangon Bio., dissolved in borate buffer) to cleave the disulfide bonds and leave the thiol group in the 3′ end of DNA. The above solution was reacted with 20 μL of 20 mM Flu solution for another 4 h, and unreacted Flu molecule was extracted by ethyl acetate. The final product of SP and Flu double-modified DNA, with SP at the 5′ end and Flu at the 3′ end, was purified by HPLC and redissolved in specific buffer solution for further tests.

Results and Discussion

Design of the Molecular Device. As illustrated in Figure 1b, Flu and SP/MC respectively have been attached to the ends of DNA motor X (5′ CCCTAACCCTAACCCTAACCC 3′) via established chemistry. At acid pH and in the dark, SP/MC is in its open state (MC) and keeps close to Flu by the close-state DNA motor which could lead to a highly efficient FRET process; then the fluorescence of Flu will be quenched (stage 1). MC could be transformed into the closed form (SP) by visible light irradiation, which will stop the FRET process and show strong fluorescent emission of Flu (stage 2). As a result, the fluorescent emission of Flu can be reversibly modulated by turning visible light on and off. Meanwhile, this process could also be implemented by the motion of the DNA nanomotor according to our design. Without switching the visible light, this molecular device could be changed into stage 3 through the addition of alkali, which could also give out strong fluorescent emission of Flu. According to the DNA motor's property, this process could be switched reversibly and swiftly. Moreover, even though they are based on different modulation mechanisms, stages 2 and 3 could also be switched via stage 4 without fluorescent output changes.

Switch among Four Stages. In order to prove the feasibility and validity of the design, we have employed circular dichroism (CD) and fluorescence spectroscopies to identify the existence of these four stages. At the beginning, DNA X with SP/MC and Flu modifications and its partially complementary strand Y were dissolved in 0.1 M potassium phosphate buffer (pH 5.5) with 0.1 M NaCl in the dark where the molecular device was set as stage 1. As shown in Figure 2, the CD spectrum showed a positive peak, a crossover, and a negative peak at around 285, 270, and 265 nm, respectively, demonstrating the formation of the i-motif structure;[20] the weak fluorescent signal in the fluorescent spectrum for Flu and the strong absorption peak of

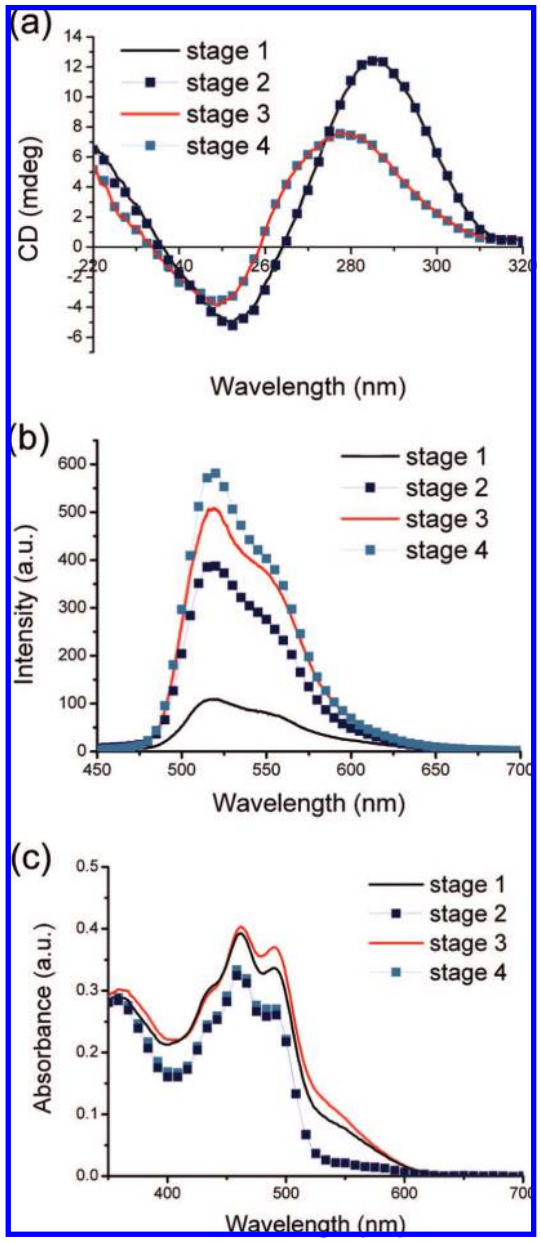

Figure 2. Spectroscopic characterizations of four stages of the switch. (a) CD spectra; (b) fluorescence spectra (excited wavelength is 430 nm); (c) UV−vis spectra. In these characterizations, 5 μM (for CD) or 15 μM (for fluorescence and UV−vis) DNA X with labeled dyes and Y were dissolved in 0.1 M NaCl and potassium phosphate buffer with pH 5.5/7.0. The time for visible light irradiation is 5 min, while that for standing in the dark is 24 h.

MC around 520 nm in the UV−vis spectrum also agreed with the expected FRET process. Just by exposing the molecular system to visible light, it could be switched to stage 2, where strong fluorescent emission could be observed at 520 nm with changes at 520−600 nm in UV−vis spectrum and no obvious CD spectrum change. Similarly, the molecular system could be switched from stage 1 to stage 3 by raising the pH value of the system to 7.0 with an appropriate amount of 1 M NaOH. As shown in Figure 2, tremendous changes in fluorescence spectra were observed, accompanying big changes in CD spectra but small changes in UV−vis spectra, which indicated the destroying of the i-motif structure and the formation of a designed double helix. Similarly, stage 4 could be obtained by switching stage 2 or stage 3 through pH change or light, respectively. It gave out strong fluorescent emission, low absorption at 520−600 nm in the UV−vis spectrum, and distinct double helix peaks in the CD spectrum.

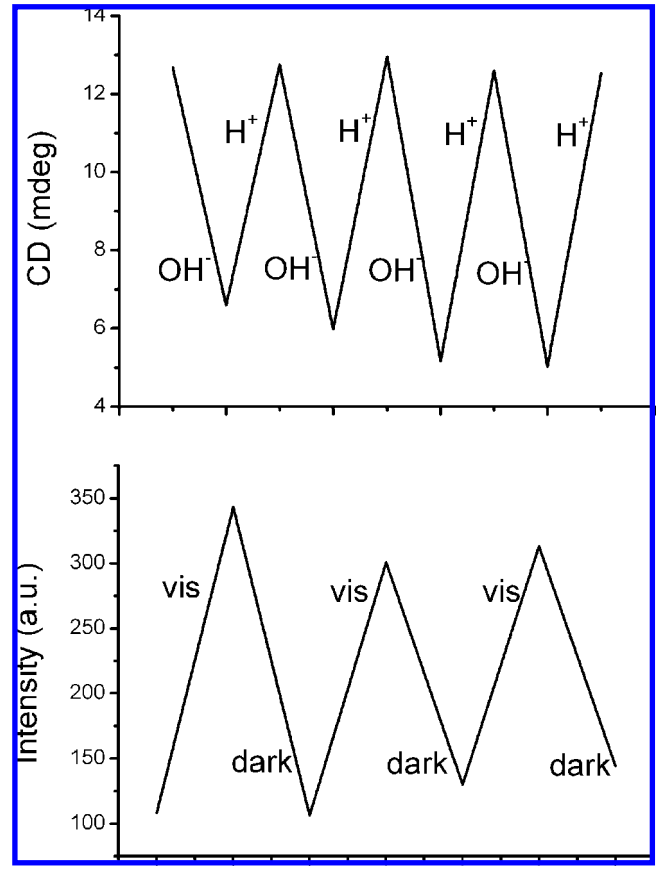

Figure 3. Reversible operations of the switch. (a, top) Switches between stages 1 and 3 monitored by CD spectra of wavelength at 285 nm. The alternative additions of acid and base will form and deform the i-motif structure. The time for each transition is 5 min. (b, bottom) Switches between stages 1 and 2 monitored by fluorescence spectra of emission wavelength at 520 nm that is excited by 430 nm light. The time for each transition in visible light is 5 min, while that for transition in the dark is 24 h.

All switch processes discussed above could be reversibly operated. Cycles between stages 1 and 3 were monitored by detecting DNA structural transitions with the CD method (Figure 3a). Like our previous work using fluorescence change as an indication, by alternating additions of HCl and NaOH, the switch of the system was able to complete in seconds in dual directions. Different from the above switching principle, transitions between stages 1 and 2 showed alternative FRET procedures on and off, while keeping the i-motif structure. From Figure 3b, the fluorescence intensities of Flu were cycled between high and low values synchronously with turning on and off the visible light. However, we note that the time needed for this photochromic reaction, especially for the dark process, was much longer than the neutralization between stages 1 and 3. Moreover, the photochromic switches between stages 3 and 4 could be detected by UV−vis spectra (data not shown). Another transition process between stages 2 and 4 with no photochromism could also be monitored by the CD method (data not shown) like that between stages 1 and 3. Overall, the switches among these four stages have been proven to be easily operated on the one hand. The waste products, on the other hand, were H_2O and NaCl in this case that would not interfere much with the working efficiency.

It is of great importance to mention that this is still a long-term goal for practical nucleic acids based devices. As a step

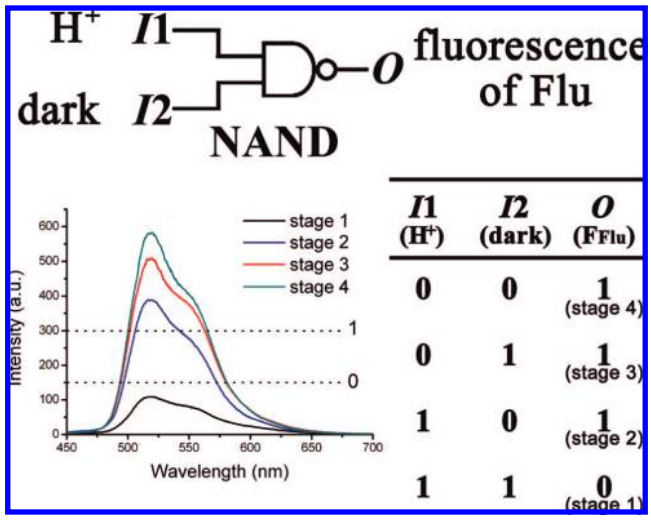

Figure 4. Illustrations of the design of NAND gate with acid and the dark as inputs and fluorescent signal as output.

beyond our two-state proton-driven DNA motor, the introduction of a photochromic D−A system makes this switch responsive to light besides pH. Providing a controlled external environment, each stage of this switch will display special DNA conformation, fluorescent signal, and absorption bands. The above features, with easy operations and less waste, make our switch a prototype for a multifunctional device.

Mimic NAND Logic. As an important feature of this switch, the dependence of the fluorescent intensities on the external stimulations, acid/base and visible light/dark, can mimic the function of signal communication in logic gates. As an example, Figure 4 illustrates a NAND gate based on our molecular device with the fluorescent signal of Flu as output. As far as we know, NAND logic is assembled connecting the output terminal of an AND gate to the input terminal of a NOT gate.[2] By defining H^+ and the dark as two inputs, the FRET process (Flu signal is low) will only happen in the presence of both of them. In other words, the output is "1" for all combinations of binary inputs except the "1,1" situation where the output becomes "0". Notably, the reversibility of our switch makes the logic gates feasible to realize system reset, whereas the hardly removable input chemicals or the degradation of the system interferes with most reported nucleic acids based logic gates seriously.[21–26]

Conclusions

In summary, three individual mechanisms—photochromism, FRET process, and pH-controlled DNA motor—have been combined in our present molecular fluorescence switch. In view of the designed principles, this device has the inherent advantages including easy operations, less waste, multifunction, and reversibility. Especially the driving forces for the switch, from reactions such as acid−base neutralization and photochromism, are hopefully to be extensively applied in the next generation of DNA/RNA devices. The application of this switch for logic gates achieves easy reversibility of the system. We also notice that the big and unequal time spans of the used photochromic reaction in dual directions are not well satisfied for logic operations in this step. However, the selected modified dyes could be changed to realize fast photochromism and complex functions in principle. In addition, the asymmetry in time spans makes us envisage other applications such as digital memory.[27] Anyway, our strategy provides inspirations for combing nucleic acid based devices with other chemical/physical processes and will shed light on the exploration toward practicability, multifunction, cleanness, and reversibility in nanoscale.

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Supporting Information Available: Synthesis of Flu and SP/MC, HPLC patterns, and UV−vis and fluorescence spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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