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Reusable molecular sensor based on photonic activation control of DNA probes

Takahiro Nishimura,* Yusuke Ogura, and Jun Tanida

Graduate School of Information Science and Technology, Osaka University, 1-5 Yamadaoka, Suita, Osaka, 5650871, Japan

**t-nishimura@ist.osaka-u.ac.jp*

Abstract: We propose a photoactivatable and resettable molecular sensor using DNA probes. The functionality is achieved by reversible change of DNA structure induced via photonic signals. Based on the mechanism, the sensor can detect target molecules at a desired instant and can be returned to its initial state after detection. The experimental results demonstrate that the concentrations of the target molecules are detected correctly and repeatedly according to the light signal.

OCIS codes: (280.1415) Biological sensing and sensors; (350.5130) Photochemistry; (170.2655) Functional monitoring and imaging.

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1. Introduction

Observation of spatial distribution of biomolecules and their temporal variations is important for the analysis of the behavior and exploring function of biological systems. The capability of DNA in molecular recognition is useful in the detection of specific biomolecules such as DNA, RNA and proteins. From this viewpoint, DNA probes have been widely used for biomolecular sensing to achieve, for example, live cell imaging and quantitative analyses [1, 2]. Continuous improvement of the DNA probe technology contributes to the development of a new class of analysis techniques.

A DNA probe can detect a target molecule based on the capability in selective binding to a specific molecule. The binding process is based on the autonomous nature of interactions between DNA and the target molecule, so that DNA probes are useful for sensing of a variety of biomolecules. However, autonomous behavior prevents us from reusing the DNA probes. An approach to overcome the problem is to control the binding between a DNA probe and a target molecule using external signals. The control of the binding offers detection of the target molecule at a desired instant. In addition, such DNA probes can release the target molecule to be returned to the initial state. The capability is important to reuse the DNA probes, and repeated sensing with periodic external signals enables us to observe time variations of target molecules. Furthermore, controlling the instants of activation and inactivation of the DNA probes is effective for shortening the time of binding to the target molecule for the detection. This is expected to reduce undesired influence on the native functions of the target molecule by binding for detection.

Light is the most effective carrier for non-invasive transfer of signals from outside the molecular environment. Light signals can be spatially and temporally modulated using appropriate optical methods, and a photothermal or photochemical effect can be induced locally. To date, the photoregulation of DNA hybridization has been achieved by modifying DNA using photochromic molecules [3–5] and gold nanoparticles [6]. Using photoregulation, the DNA structures can also be controlled remotely and repeatedly [7–11]. Photoregulation of DNA structures enables molecular function regulation including manipulation of enzyme inhibition [12] and switch of RNA digestion [13]. These features are attractive for controlling the binding between a DNA probe and a target molecule. This control is achieved without significantly affecting the native molecular environment. A DNA probe with a photoactivation functionality, which is called a caged molecular beacon, has been demonstrated by Wang *et al.* [14]. Although this method allows fast, remote and spatiotemporal control, the target molecules that are bound to the controlled DNA probe cannot be released and the repeated operation is impossible.

In this paper, we propose a photoactivatable molecular sensor unit with reuse capability. The sensor unit can be switched between activated and inactivated states by photoinduced structural change of DNA. Based on the functionality, the sensor unit can detect target molecules at a desired instant according to light signals. The molecular sensor unit releases the target molecule and returns to the initial state after detection, so that it is possible to reduce effects caused by the binding for the detection.

2. Photoactivatable molecular sensor unit with reuse capability

Activation of the molecular sensor unit is achieved with a photoinduced structural change of a hairpin DNA. As shown in Fig. 1(A), the hairpin DNA consists of two functional regions:

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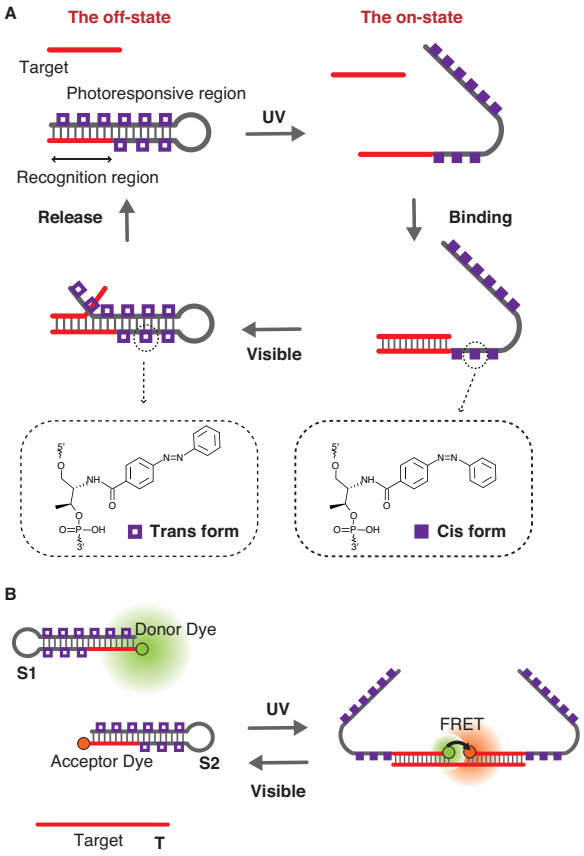

C

S1: 5' **FAM**-ATCTGAACTAACGCTxTTxAAxGATCTCTxCTxTAxAAxGCxGTxTAxGTxTCA 3'

S2: 5' CACxGTxACxATxGCTxTTxAAxGATCTCTxCTxTAxAAxGCATGTACGTGCTA-**TAMRA** 3'

T: 5' CGTTAGTTCAGATATCATAGCACGTACATG 3'

CT: 5' CATGTACGTGCTATGATATCTGAACTAACG 3'

Fig. 1. (A) Schematic illustration of photonic activation of recognition function of the DNA using azobenzene-tethered hairpin DNA. (B) Schematic illustration of a photonically controlled molecular sensor. Purple squares represent the azobenzene positions. (C) Sequence and modification of the DNA strands used in this study. Here symbol x refers to the position of the azobenzene.

a recognition region and a photoresponsive region. The recognition region has a specific sequence that can bind to the target molecule. Recognition is activated by exposure of the recognition region which is covered with a part of the photoresponsive region that is modified by azobenzene [3]. Azobenzene changes its structure to the the *cis*-form upon UV irradiation and to the *trans*-form upon visible light irradiation. DNA binding strength is considered to depend on the polarity and the structure of the azobenzene [4]. Because the *trans*-form azobenzene is nonpolar and planar, it stacks between the DNA bases well, and the duplex with the trans-form azobenzene is stabilized. In contrast, the *cis*-form azobenzene is polar and nonplanar, and it makes the duplex less stable. The structure of the azobenzene moiety is shown in Fig. 1(A). For the *trans*-azobenzene, the hairpin DNA takes the closed structure and the recognition region

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is covered. In this case, the recognition function is inhibited, and thus the sensor unit is in the off-state. The hairpin structure is opened by ultraviolet-light irradiation to change the azobenzene to the *cis*-form, and the recognition region is then exposed to sense the target molecule. In other words, the sensor unit is in the on-state. When the functional DNAs are in the on-state, they can bind with the target molecule. To return the molecular sensor unit to the initial state after detection, visible light is irradiated to change the hairpin DNAs to the closed structure which causes the release of the target caused by branch-migration reaction. The hairpin DNA contributes to the reversible switching of the sensor unit between the activated and inactivated states by light irradiation on a single molecule. As a result, no additional molecules are required for the reaction control and no byproduct is produced by the operation. To report the presence of the target molecule, two DNA probes are designed to form a pair of fluorescent donor and acceptor chromophores as shown in Fig. 1(B). Fluorescence resonance energy transfer (FRET) occurs only when both the DNA probes are bound to the target molecule.

3. Experiments and discussions

The sequences and modifications used in this study are shown in Fig. 1(C). In this study, we designed a molecular sensor unit that is capable of detecting a 30-nt single-stranded DNA, T , as a target molecule. CT is the complementary DNA of T and it was used to decrease the concentration of the target molecules with single-stranded form. The sensor unit consists of two 43-nt hairpin DNAs, S1 and S2, incorporated with 11 azobenzene molecules. A carboxyfluorescein (FAM) was attached to the 5'-end of one hairpin DNA, S1, as the donor dye and a carboxytetramethylrhodamine (TAMRA) was attached to the 3'-end of the other hairpin DNA, S2, as the acceptor dye. The DNA strands, S1, S2, T, and CT were all purchased from Tsukuba Oligo Service (Japan).

S1, S2 and T were mixed in a phosphate buffer (1M NaCl, 1mM $MgCl_2$), and the solution was incubated at 95 °C, rapidly cooled to 4 °C, and again incubated at 4 °C for 20 min. The volume of the solution was 50 μL and the concentrations were controlled in each experiment. To irradiate the sample with ultraviolet (wavelength: 340 nm) and visible light (wavelength: 440 nm) and to measure a fluorescence emission spectrum (excitation wavewlength: 494nm), a spectrofluorometer (FP-6200, JASCO, Japan) equipped with a temperature-contorller (ETC-272T, JASCO, Japan) was employed. The initial state was measured after incubating the sample at 25 °C for 10 min. The time schedule of temperature for one cycle is shown in Fig. 2. One cycle was 22 min. The sample was irradiated with either UV or visible light for 2 min at 80 °C and incubated at 25 °C for 5 min and the spectrum was measured at 25 °C. The efficiency of the isomerization of azobenzenes from the *trans* to the *cis* form depends on the temperature during the UV irradiation [10]. We irradiated UV and visible light at 80 °C to obtain high control efficiency.

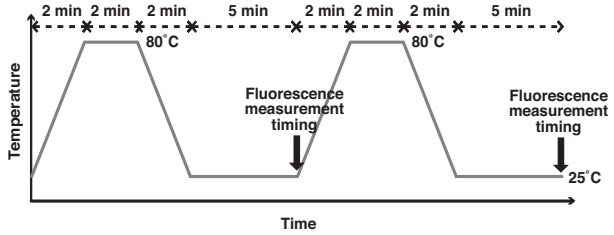

Fig. 2. Time course of temperature control for one cycle. Light irradiation can be carried out at 80 °C for 2 min.

Figure 3(A) shows the fluorescence emission spectra measured at each state during one cycle

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of the On/Off operation. After UV light was irradiated to turn on the sensor unit, the fluorescence intensity of FAM at the maximum emission wavelength (517 nm) decreased and the fluorescence intensity of TAMRA (580 nm) increased when compared with those of the initial state. This indicates that FRET occurs because of the binding of the target molecule to the DNA probes. After visible light irradiation to turn off the sensor unit, the fluorescence emission spectrum was similar to the initial state. This indicates that the target molecule was released from the sensor unit by switching the hairpin structure into the initial state.

To confirm that the sensor unit is controlled by light irradiation, not by the temperature control, the FRET ratio was measured with/without light irradiation under the temperature control. The FRET ratio R is defined as $R = 1 - F/F_0$, where F and F_0 are the fluorescence intensities of FAM at the maximum emission in the individual operations and at the initial state, respectively. Figure 3(B) shows the FRET ratio obtained with/without light irradiation in one cycle of the temperature control. The shown values are averaged ones for three times of measurements. The sensor unit was in the on-state at 11 min owing to UV light irradiation and in the off-state at 22 min owing to visible light irradiation. As can be seen in Fig. 3(B), the FRET ratio without light irradiation was approximately zero during the operation. In contrast, the FRET ratio with light irradiation changed depending on the state of the sensor unit. This result indicates that the sensor unit can be regulated by light irradiation but not just by temperature change with high reproducibility. The FRET ratio at the on-state was 0.24, which was lower than the estimation by considering the length between FAM and TAMRA. This is because the efficiency for photoregulating the hairpin-DNA structure from the closed state to the open state is not high. The efficiency is estimated as about 0.4 [15]. In the proposed sensor unit, FRET occurs only when both of two hairpin-DNAs bind with a single target molecule, so that the FRET ratio decreases to less than 0.4. To improve the FRET ratio, increasing efficiency for photoregulation is required.

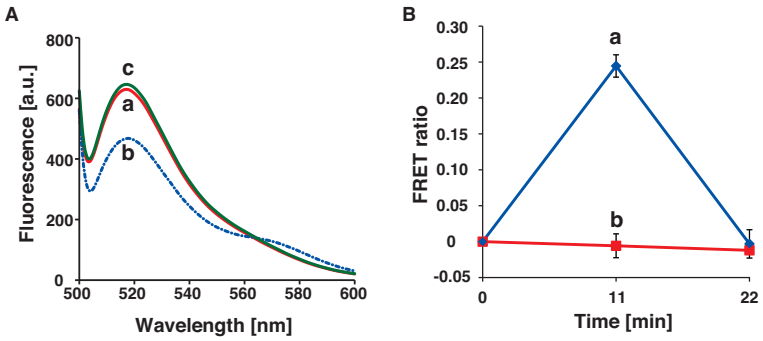

Fig. 3. (A) Fluorescence emission spectra measured at the initial state (a), the on-state (b) and the off-state (c). (B) FRET ratio in light irradiation (a) and no light irradiation (b) under the temperature control. The sample concentrations of the sensor and the target were 1 μM in a phosphte buffer (NaCl 1 M, $MgCl_2$ 1 mM).

Figure 4(A) shows the static response of the FRET ratio to concentrations of the target molecule. The FRET ratio increased in response to the concentration. This shows that the sensor unit can detect the concentration of the target molecule. To confirm the repeated regulation of the sensor unit, three operating cycles were carried out. Figure 4(B) shows the FRET ratio during the repetitive operation. The ratios at the on-state had almost the same value, and the ratios at the off-state were nearly zero during the repeated operation. The sensor unit maintained an approximately uniform performance during the repeated operations, and this is suitable for realizing a reusable molecular sensor.

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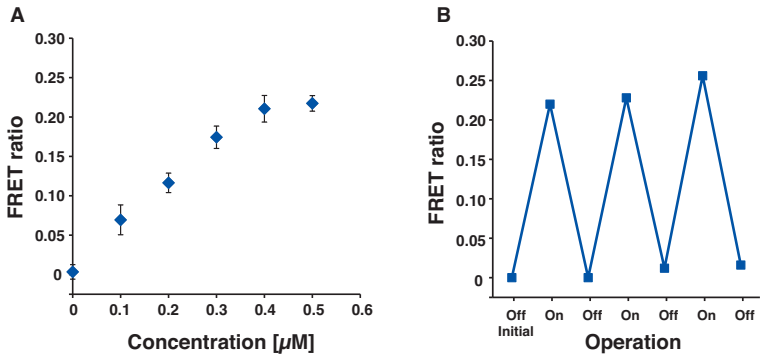

Fig. 4. (A) Measurement result for different concentrations of the target, 0, 0.1, 0.2, 0.3, 0.4, and 0.5 μM with 0.5 μM of the sensor. (B) FRET ratio during a repeated operation. The sample concentrations of the sensor and the target were 1 μM in a phosphate buffer (NaCl 1 M, $MgCl_2$ 1 mM).

The final experiment was aimed at confirming the capability of the sensor unit in tracking the target concentration at a desired instant. We added the target molecule or the complementary DNA of the target after one operating cycle of the operations to change the concentration of the target. The time-scale of the concentration change was expected to be much shorter compared to the sampling interval (22min). The concentration of the target molecule was increased from 0.1 μM to 0.3 μM, after which the second measurement was carried out. The FRET ratio increased from 0.07 to 0.14, as shown in Fig. 5(A). On the other hand, when the complementary DNA, CT, was added after the first measurement, the FRET ratio decreased from 0.17 to 0.12 as shown in Fig. 5(B). This is because the complementary DNA was bound to the target and the concentration of the target molecules in the single-strand form decreased. If all the complimentary DNA bound to the target, the concentration of the single-stranded target was estimated to decrease from 0.4 μM to 0.2 μM. As shown in Figs. 5(A) and 5(B), the FRET ratio reported from the sensor unit was in agreement with the concentration of the target in accordance with the activated timing. The result shows that the sensor unit can monitor the change of the target concentration.

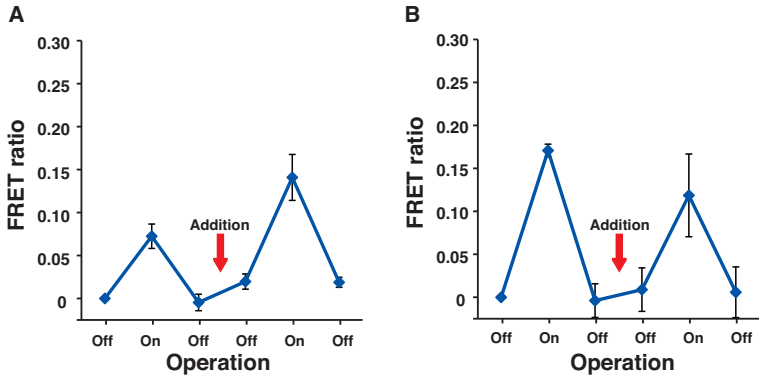

Fig. 5. Results of the tracking of concentration when the target molecule (A) increased from 0.1 μM to 0.3 μM and (B) decreased from 0.4 μM to 0.2 μM. The concentration of the sensor was 0.5 μM.

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The advantages of the proposed method are the capabilities of remote regulation, releasing the analyte after detection, and repeated operation. These features enable the detection of the target molecule at desired intervals and the continuous monitoring of biological events. For applying the method to living cells or any other biosystems, it is required to improve photoactivation efficiency, to relax the requirement on the temperature, and to shorten the operation cycle. Possible solutions are to optimize the sequences of the photoresponsive region and to incorporate more azobenzenes to the hairpin structure. The photoregulation efficiency of the DNA reaction with azobenzene depends on the position and the number of incorporated azobenzenes [3]. Another solution involves the use of metal nanoparticles to regulate DNA structures [6]. The photothermal effect induced by a pulse light can be used to control DNA reactions. This method contributes to the speeding-up of the photoregulation of the sensor unit.

4. Conclusion

In conclusion, we have demonstrated a photoactivatable molecular sensor with reuse capability. Based on photoinduced structural changes, the sensor unit switches between the on-state to detect a target molecule and the off-state to dissociate it. The experimental results show that the sensor unit correctly reports the concentration of the target molecule and that the target molecule is released after the detection according to the light signal. This photoactivation strategy of the molecular sensor unit is expected to provide a new methodology for biological analyses.

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