Photo- and Thermoregulation of DNA Nanomachines

Keiichiro Takahashi[⋆], Satsuki Yaegashi[⋆], Hiroyuki Asanuma[‡] and Masami Hagiya[⋆†]

[⋆]NovusGene Inc., 2-3 Kuboyama-cho, Hachioji-shi, Tokyo 192-8512, Japan
Olympus Corporation Utsugi Technical Research Institute
takahashi-k@novusgene.co.jp

[⋆]Japan Science and Technology Corporation (JST-CREST)

[†]Department of Computer Science, Graduate School of Information Science and Technology, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
hagiya@is.s.u-tokyo.ac.jp

[‡]Department of Molecular Design and Engineering, Graduate School of Engineering, Nagoya University, Chikusa, Nagoya 464-01, Japan

Abstract

We have been investigating DNA state machines, especially those based on the opening of hairpin molecules in which state transitions are realized as hairpin loops are opened by molecules called openers. This paper introduces photo- and thermoregulation of such hairpin-based DNA machines, in which the openers become active by sensing external signals in the form of light or heat. We conducted fluorescence experiments and show that photo- and thermoregulation is possible. In the experiments, the openers become active when they are irradiated by UV light or when they receive heat as external input. For photoregulation, we use azobenzene-bearing oligonucleotides developed by the third author.

1 Introduction

Implementing controllable molecular nanomachines made of DNA is one of the goals of DNA computing and DNA nanotechnology, and a variety of implementations of DNA machines have been reported [1–5]. One of the most typical methods for controlling such DNA machines is to use DNA strands that hybridize with target machines and drive their state transition [1, 2, 7]. DNA strands can also be used as catalysts for the formation of double helices in such machines [6, 13]. As another approach to control DNA machines, Mao et al. showed that the B-Z transition of DNA owing to a change in solution condition can switch the conformation of their DNA motor [3].

In this paper, we show that signals in the form of light or heat can be used as another means to control DNA machines. We have been investigating DNA state machines that are based on the opening of hairpin molecules, in which state transitions are realized as hairpin loops are opened by molecules called openers [8–10]. Signals in the form of light or heat change the activity of the openers. The proposed reaction systems will be used as components of a larger molecular system, consisting of various sensors, computing elements, and actuators. We envision that such general-purpose molecular systems can be constructed from a network of DNA machines based on hairpins or other kinds of loop structure, as proposed by Seelig et al. [6] and the authors [12], the operations of which are driven by the formation of double helices and the dissociation of loops. The photo- and thermoregulation of hairpin-based machines introduced here can be incorporated into such systems and extend their range of application.

In this paper, we first briefly describe our previous experiments on hairpin dissociation using various kinds of opener. Then, we present experimental results of photo- and thermoregulation, and discuss our model of hairpin dissociation.

The figures are in colors. The pdf file of this paper, which shows the colors, is available at the following URL.

http://hagi.is.s.u-tokyo.ac.jp/pub/staff/hagiya/dna11/pt.pdf

2 Previous Work

Before describing the reaction systems operated by light or heat, let us briefly introduce our previous work [11], which constitutes the basis of those systems. In these preliminary experiments, we measured the efficiency of various kinds of opener molecules using fluorescence, and introduced some techniques to achieve robust hairpin dissociation, as seen in the work of Yurke's group [13,14].

The main reaction system in our study is depicted in Figure 1. We call the hybridization site of the hairpin or opener the *lead section*. The overhang of the hairpin is the hairpin's lead section, and this hybridizes with the lead section of the opener. The substrand of the opener that invades the hairpin and replaces one of the stem strands via branch migration is called an *invasion section* (green substrand).

In the study, we varied the length of the opener's lead section from 0 to 20 (20, 10, 7, and 0). The lead section with length 10 resulted in the most efficient kinetic rate (3.9×10^5 $M^{-1}s^{-1}$) in our experiment (0.05μM hairpin molecules and 0.05μM opener molecules in 1×SSC buffer at 25°C). Although a longer lead section causes a faster reaction in general [15], the kinetic rate for the 20-base lead section resulted in about 1.5×10^5 $M^{-1}s^{-1}$ because the opener strand folds into a conformation more stably than the openers with a lead section 10 or 7 bases long (see Fig. 2(a)).

Then, we verified that openers with a mismatching lead section cannot open the hairpin, and that if the hairpin's lead section is covered with its complementary strand, no proper openers can open the hairpin (see Fig. 2(b)).

We also tested openers with a lead section (seven bases) that was complementary to the hairpin loop and with an invasion section that might invade the hairpin stem from top to bottom (in the direction opposite to that of ordinary openers) (see Fig. 2(b)). Unlike the so-called molecular beacon [16], the small loop of the hairpin inhibits the invasion of the openers. An extra random coil attached to the lead section strengthens this inhibition, as reported by Yurke et al. [13]

3 Photoregulation

3.1 Photoregulation with Azobenzene

Using the isomerization of azobenzene residues in the side chain of an oligonucleotide (see Fig. 3(a)), hybridization between the oligomer and its complement can be photoregulated [17, 18]. When azobenzenes are isomerized from the *trans* form to the *cis* from by irradiating them with UV light ($300 \text{ nm} < \lambda < 400 \text{ nm}$), the melting temperature of the duplex is lowered considerably. Moreover, when the *cis*-form azobenzenes are irradiated with visible light ($\lambda > 400$ nm), the azobenzene residues are isomerized back to the *trans* form. Using these properties of azobenzenes, we show the feasibility of controlling hairpin opening with light.

Figure 3(b) depicts the reaction system used for photoregulation. Opener (B) has a 10-base lead section and openers (C-1) and (C-2) fold into a stem-loop in the *trans* condition. Even when opener (B) isomerized to the *cis* form is added to the solution containing hairpin molecules, the

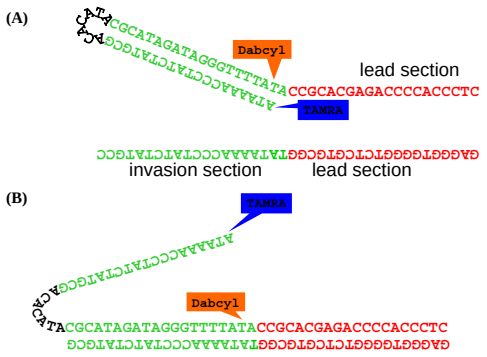


Figure 1: [Hairpin Structure and Detection Scheme]: The hairpin molecule is labeled at the 5′-end with TAMRA and at the end of the stem with Dabcyl. (A) If TAMRA and Dabcyl are in close proximity, Dabcyl quenches the fluorescence of TAMRA. (B) The fluorescence intensity increases in proportion to the opening of the hairpin structure.

hairpin structure is retained because the lead section of the *cis* opener has a much lower melting temperature. Once the solution of dissolved molecules of the hairpin and its *cis* opener is irradiated with visible light, the reaction between the hairpin and the opener is permitted. By contrast, openers (C-1) and (C-2) will open the hairpin structure (A) when the solution is irradiated with UV light, because the stem-loop of the openers is dissociated and the lead section is exposed.

3.2 Materials and Methods

The oligomers intercalating azobenzenes, shown in Table 1, were synthesized by the third author, while *H-TAM* was synthesized by Sigma-Aldrich Japan, Genosys Division. Each opener strand has four azobenzenes in its lead section; these are located every two bases. The difference between *Azo-type2-1* and *Azo-type2-2* is that in *Azo-type2-1*, part of the lead section is exposed in the loop, while in *Azo-type2-2*, it is completely concealed in the stem.

H-TAM	:	5’-GGCCTCACTC-CCAGTATATACCAGCACCTG(-TAMRA)
		-TTAGCCC-CAGGTGCTGGTATATACTGG-3’
Azo-type1	:	5’-(Dabcyl-)CAGGTGCTGGTATATACTGG-GAXGTXGAXGGXCC-3’
Azo-type2-1	:	5’-(Dabcyl-)CAGGTGCTGGTATATACTGGGAG
		-TGAGGCC-CTXCCXCAXGTXAT-3’
Azo-type2-2	:	5’-(Dabcyl-)CAGGTGCTGGTATATACTGG-GAGTGAGGCC
		-TAGTCAT-GGXCCXTCXACXTC-3’

Table 1: [Sequences of the Photo-Sensor Systems]: From top to bottom, these sequences correspond to structures (A), (B), (C-1), and (C-2) in Fig. 3(b). The sequence *H-TAM* folds into a hairpin stem with a sticky end. The base on the 5′-side of the closing pair is labeled with TAMRA. *Azo-type1, Azo-type2-1,* and *Azo-type2-2* are the hairpin openers intercalating azobenzenes, which are labeled with Dabcyl at the 5′-end. Each `X` represents an azobenzene residue.

In an actual application, we would use light as input to the entire molecular system. In our experiments, however, instead of irradiating the entire system, which consists of both the opener and the hairpin machine, we irradiated only the openers in advance, to facilitate quantitative measurement. These experiments were conducted at 40°C, which is roughly the melting temperature

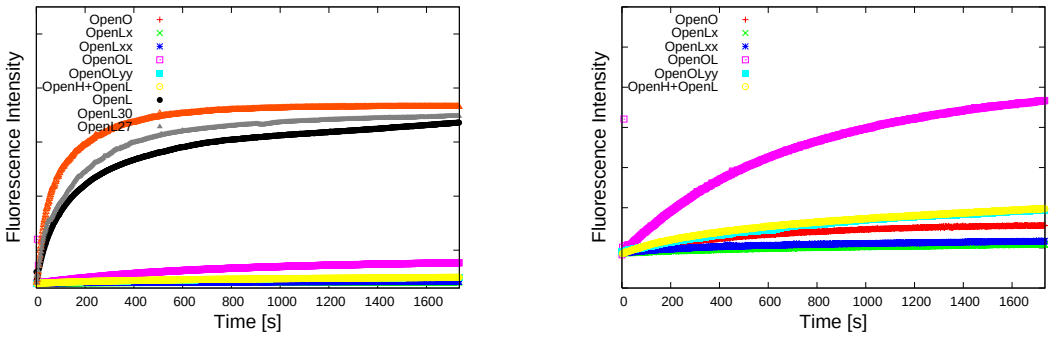


Figure 2: [Experimental Results of the Previous Study]: (a) From the top curve, the efficiencies of the openers that have 10-, 7- and 20-base lead sections are each depicted. And the other curves show the efficiencies of the suppressed openers. (b) The fluorescence intensity of the suppressed openers (scaled up). The efficiency of the opener consisting of the invasion section and the lead section complementary to the hairpin loop is drawn in the pink line (top), and the opener with a random coil reached down to the light blue line (under the yellow curve). And we observed that the opener without any lead section cannot open the hairpin (the red curve), that the openers with a mismatching lead section cannot open the hairpin (the green and the blue curves), and that if the hairpin's lead section is covered with its complementary strand, the hairpin cannot be opened by any proper opener (the yellow curve).

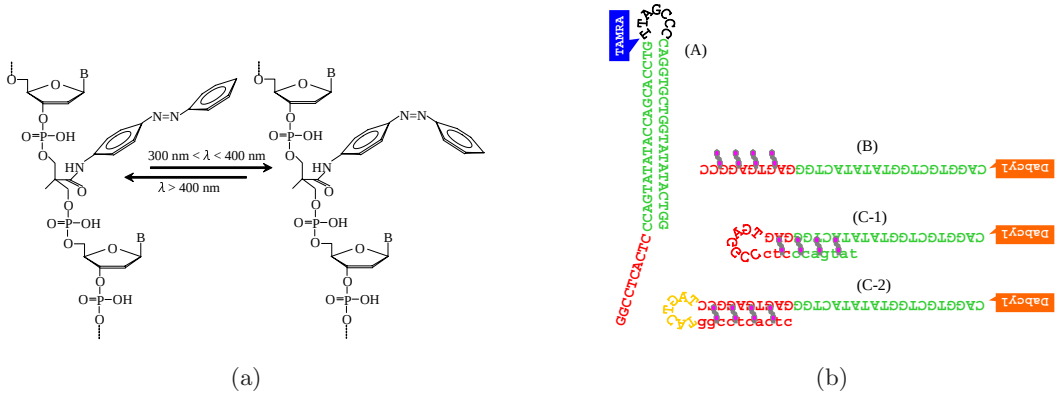
of *trans* [18]. The openers *Azo-type1*, *Azo-type2-1,* and *Azo-type2-2* were irradiated for isomerization before being added to the solution. The temperature of the sample cell fixed in a HITACHI F-2500 spectrophotometer was maintained with a LAUDA RC6 thermostatic bath. In each measurement, the opener and hairpin machine were diluted to 0.05μM and 0.0225μM in 1×SSC buffer, respectively. The concentration of the hairpin molecules was planned to be half that of the openers (0.025μM), but an approximately 9% difference occurred after quantitative adjustments. We preprocessed the sample tube to dissolve the opener by heating it at 60 °C before irradiating it because we can attain a higher rate of azobenzene isomerization at a higher temperature. Subsequently, we exposed the tube to UV light through UV-D36C glass (a filter from Asahi Techno Glass that transmits UV and absorbs visible light) with a UVP B-100AP 100-W lamp (the original light filter was removed) for five minutes in order to effectively isomerize the azobenzenes from *trans* to *cis.* This treatment resulted in an isomerization rate of about 80% (roughly three of the four azobenzenes were isomerized), as observed using BECKMAN DU 650 spectroscopy (data now shown).

For the experiments with the *trans*-form openers, we irradiated the openers through an L-39 filter (a UV blocking filter from Asahi Techno Glass) with the 100-W lamp, because some *cis* azobenzenes might exist in the normal condition.

As the "half life" of *cis*-form azobenzenes is about twelve hours at 37°C and about one day at room temperature, we dropped each opener into the solution containing the *H-TAM* molecules immediately after irradiating it with UV light. Therefore, although our experiments were conducted at 40°C for thirty minutes, the temperature would not have affected the isomerization back to the *trans* form.

3.3 Experimental Results

Figure 4 shows the experimental result for the reaction between the hairpin machine *H-TAM* and the opener *Azo-type1.* The fluorescence intensity decreases as the hairpin machines are opened, since the TAMRA is quenched by the Dabcyl on the opener. Although we tethered fluorescent



(a) (b)

Figure 3: [Photoregulation using Azobenzene]: (a) Isomerization scheme of an azobenzene in the side chain upon irradiation. (b) (A) A hairpin structure labeled at the top of the stem with TAMRA. (B) An opener for the hairpin structure labeled at the 5′-end with Dabcyl, which contains azobenzenes in its lead section. (C-1) and (C-2) Other openers with lead sections that form a stem region with their counterparts. These openers have supplementary bases allowing them to fold into a loop structure. The fluorescence intensity decreases as the reaction between the hairpin molecule and each opener molecule proceeds.

dyes at the bottom of the stem in the previous study while dyes are put at the top of the stem in this experiment, it seems that their position does not affect fluorescent properties judging from the kinetic rates (data will be shown later). The *trans* opener was expected to be far more efficient than the *cis* one for opening the hairpin structure. As the figure shows, however, in this experiment our expectation was rarely met. Assuming the reactions follow the second-order kinetics of ordinary duplex formation, we estimated that the rate constants are around 1.2×10^5 $M^{-1}s^{-1}$ for the *trans* openers and 8.1×10^4 $M^{-1}s^{-1}$ for the *cis* openers from fitting the fluorescence data. Therefore, even if the opener *Azo-type1* has a *cis* lead section, branch migration can proceed via partial hybridization between the lead sections.

Figure 5 depicts the fluorescence intensities of the reactions between the hairpin machine and the hairpin openers *Azo-type2-1* and *Azo-type2-2*. As the opener *Azo-type2-1* has a partially exposed lead section in its hairpin loop, this opener is more likely to open the hairpin machine in the *trans* condition as compared to *Azo-type2-2* (see Fig. 6(b)). In other words, the length of the lead section on the hairpin structure *H-TAM* is slightly shorter to avoid the interference between the lead sections [11]. By contrast, *Azo-type2-2*, which carries the *trans*-form azobenzenes, is more robust, as the lead section of the opener is completely concealed in the stem region. However, in exchange for this advantage, *Azo-type2-2* is less capable of breaking the hairpin machine (see Fig. 6(a)) because it is more stable. On the assumption that the reaction of the *Azo-type2* openers takes the second-order kinetics, we also tried to fit the curves and estimated the rates; 1.2×10^5 $M^{-1}s^{-1}$ for the *cis* form *Azo-type2-1* and 8.3×10^4 $M^{-1}s^{-1}$ for the *cis* form *Azo-type2-2*. As for the *trans* form openers, we could not fit the curves well. If we borrow the coefficients obtained for the *cis* form other than the reaction rate and use the fluorescent intensity at 1800s, the reaction rate for the *trans* form is approximated by 5.1×10^4 $M^{-1}s^{-1}$ and 5.3×10^4 $M^{-1}s^{-1}$ for *Azo-type2-1* and *Azo-type2-2,* respectively.

Figure 6(c) compares the *switched-on* openers. *Azo-type1* with *trans* azobenzenes and *Azo-type2-1* with *cis* azobenzenes have equal ability, while the *cis* opener *Azo-type2-2* is slightly less efficient. Fig. 6(d) compares the *switched-off* openers. Unfortunately, *Azo-type1* works as if it were an *always switched-on* opener in contrast to other suppressed openers.

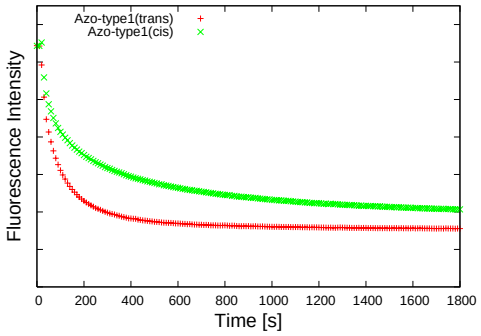
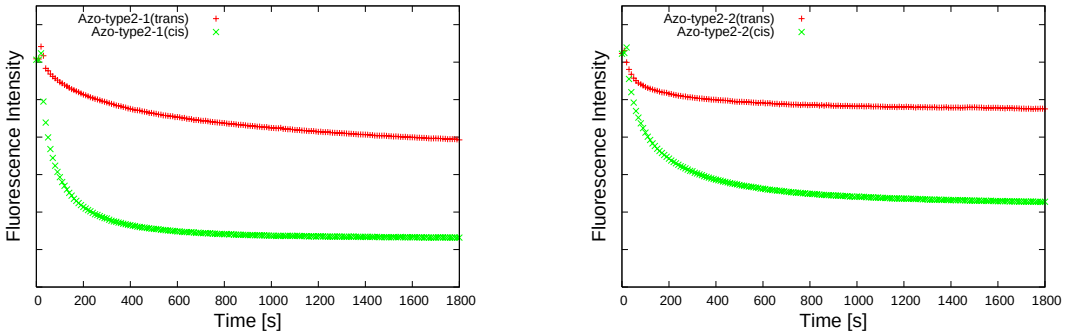


Figure 4: [Comparison of the *trans* and *cis* forms of *Azo-type1*]: The upper line is the change in fluorescence intensity as the reaction between the *trans* opener *Azo-type1* and the hairpin structure *H-TAM* proceeds. The lower line is for the reaction between the *cis* opener *Azo-type1* and the hairpin structure.



(a) (b)

Figure 5: [The Efficiencies of *Azo-type2-1* and *Azo-type2-2*]: (a) This figure shows the difference in the efficiency of *Azo-type2-1* between the *trans* and *cis* conditions. (b) This figure shows the difference in the efficiency of *Azo-type2-2* between the *trans* and *cis* conditions.

4 Thermoregulation

As another possible means of sensing environmental changes, this section introduces thermoregulation of hairpin opening.

4.1 Materials and Methods

We verified the dissociation of a hairpin machine as a thermo-sensor using the two openers *Th8* and *Th6* listed in Table 2. *Hairpin* is the same molecule that we used in our preliminary experiment. These oligomers were also synthesized by Sigma-Aldrich Japan, Genosys Division. The secondary structure of each sequence is shown in Fig. 8, where the hairpin loops of the openers might be closed by wobble pairs of (T, G).

The openers have a lead section of length 10 enclosed in their hairpin loop. These openers exist as hairpins until they receive external input in the form of heat, i.e., until the temperature is raised (to 50°C in this system) so that their hairpin structure is dissociated. Therefore, the thermo-sensing system consisting of the hairpin machine and opener should measure the difference in temperature between 25°C (the room temperature) and 50°C. The hairpin machine and its opener were each diluted to 0.05μM in 1×SSC buffer, and each experiment was measured using a

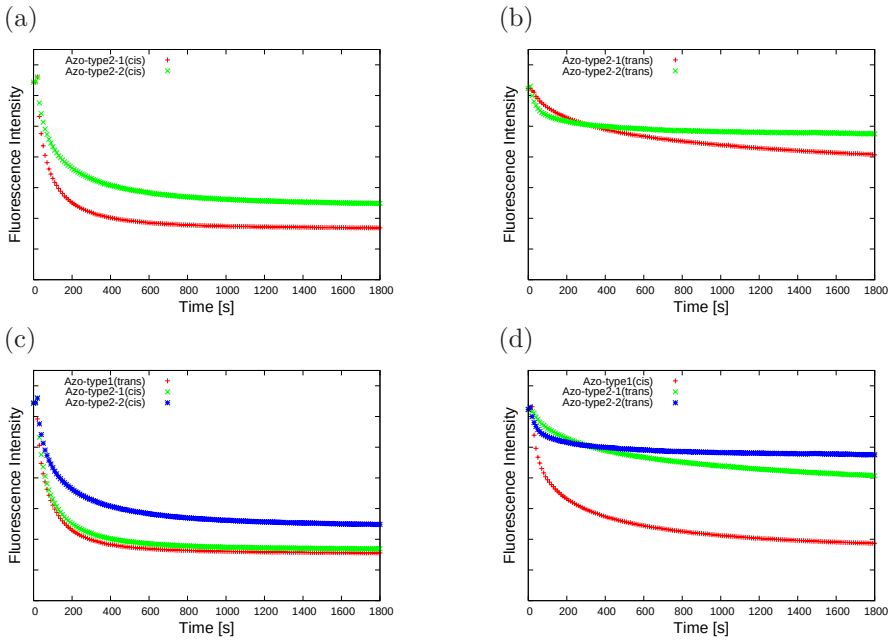


Figure 6: [Some Comparisons of the Photoregulation]

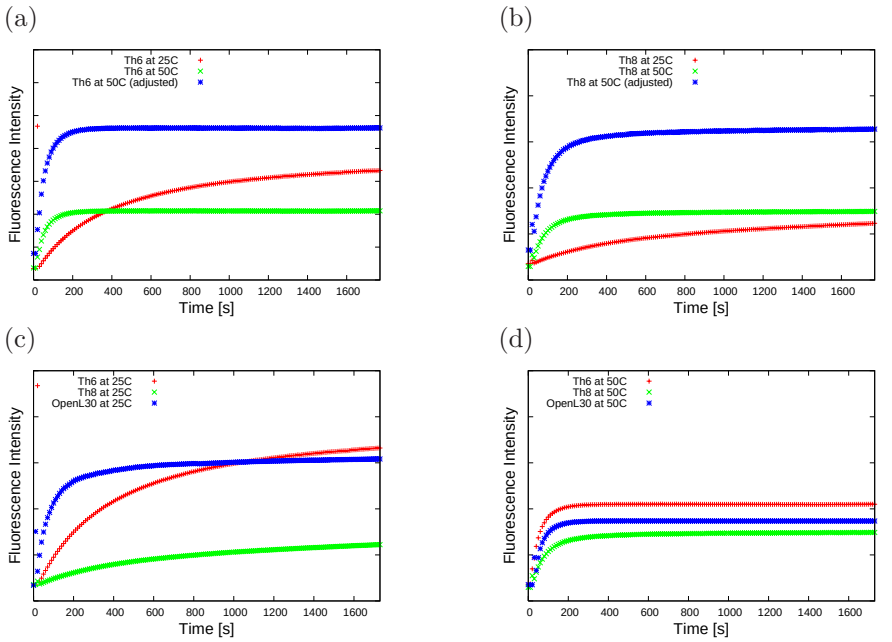


Figure 7: [Experimental Results of Thermoregulation]

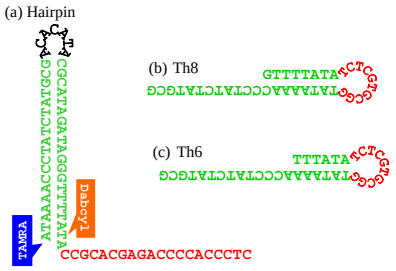


Figure 8: [Thermo-Sensor Systems] : (a) The sequence of the hairpin structure is the same as in our preliminary experiments. (b,c) The openers for the thermo-sensor machine. *Th8* has a stem of length 8 and *Th6* has a stem of length 6, and both have a lead section of length 10.

HITACHI spectrophotometer and a LAUDA thermostatic bath.

Hairpin	:	TAMRA-5'-TATAAAACCCTATCTATGCG-ACACATA
		-CGCATAGATAGGGTTTTAT(-Dabcyl-)A
		-CCGCACGAGACCCCACCCTC-3'
Th8	:	5'-GTTTTATA-TCTCGTGCGG-TATAAAACCCTATCTATGCG-3'
Th6	:	5'-TTTATA-TCTCGTGCGG-TATAAAACCCTATCTATGCG-3'

Table 2: [Sequences of the Thermo-Sensor Systems]

4.2 Experimental Results

Figure 7(a) plots the fluorescence intensity of TAMRA as a function of time during the reaction of *Hairpin* and *Th6*. The red line shows the efficiency of the hairpin-formed opener at 25 °C and the green line shows the efficiency of the single-stranded opener at 50 °C. As the fluorescence intensity of TAMRA is inversely proportional to the temperature, we adjusted the green curve to the fluorescence intensity at 25 °C and obtained the blue curve. In addition, the efficiency of opener *Th8* at 25 °C and 50 °C is shown in Figure 7(b).

Since these reactions do not follow the ordinal hybridization kinetics, we could not fit the curves. However, Figure 7(a) clearly shows that we cannot effectively control the dissociation of the haripin machine with *Th6,* while Figure 7(b) shows that *Th8* at 25°C does not have much ability to open the machine. Figure 7(c) and (d) depict the comparisons between the two openers at 25°C and 50°C, respectively. We also compare the two openers against the proper opener with the 10-base lead section used in our preliminary experiment (Fig. 2(a)). The adjustment of the fluorescence intensity in (a) and (b) is made according to the measurement of this opener. In exchange for the good controllability, *Th8* is totally inferior to *Th6* in terms of efficiency because it has a more stable stem.

5 Discussion

In this paper, we showed that photoregulation and thermoregulation of hairpin opening are possible in the same framework of hairpin opening. Note that there are a variety of methods for thermoreg-

ulation other than the method proposed in this paper, but the openers for thermoregulation in this study can be used in conjunction with other kinds of openers, including those for photoregulation and those containing aptamers as proposed by Dirks et al. [19].

In the photoregulation experiments, we succeeded in controlling the opening of the hairpin machine using UV light. However, control using visible light (as was expected for *Azo-type1*) remains a future goal. In the thermoregulation experiments, we succeeded in regulating the conformational change of the hairpin machine with openers that changed structure according to external input in the form of heat. These kinds of sensors will be used to regulate general-purpose molecular systems such as DNA logical circuits [12].

Compared with the functionally suppressed openers used in the preliminary experiments mentioned in the first section, we have not adequately inhibited the *switched-off* openers. In order to apply the current results effectively, we need to construct more robust sensor machines by suppressing the *switched-off* openers more strongly. As for the *switched-on* openers, on the other hand, we need to make hairpin opening more efficient. As suggested by an anonymous reviewer, in order to drive the equilibrium of the system towards the complete hairpin opening, we could add a few bases to the invading substrand, which complements the first few bases on the hairpin loop.

Acknowledgments

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