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A DNA Nanomachine Powered by Light Irradiation

Xingguo Liang,[b] Hidenori Nishioka,[b] Nobutaka Takenaka,[b] and Hiroyuki Asanuma*[a]

Over the past decade, DNA has been widely used for the development of nanomaterials because it undergoes highly sequence-specific hybridization and forms a highly regular double-helical structure with suitable flexibility.[1–4] DNA is probably one of the most promising biomolecules for future applications in nanotechnology and materials science.[5] Many 2D and 3D nanostructures with determined shapes and geometries have been reported recently in which DNA is used as the building blocks and mortar.[3,6,7] More excitingly, several types of DNA nanomachines, fuelled with DNA oligonucleotides[8] or other molecules such as intercalators[9] and metal ions,[10] have been constructed.[4,5] During these 10 years of development, substantial progress has been made in the design of DNA-based devices such as tweezers, walkers, and gears, which can perform mechanical functions such as scission, directional motion, or rolling.[11–13] The prospects of this field are extraordinarily promising, and several valuable applications of DNA nanomachines as sensors, transporters, and drug-delivery systems have also been reported.[5]

For most of the DNA nanomachines constructed so far, oligonucleotides have been generally used as the fuel. In many of these systems, the mechanical motion was usually carried out by hybridization of one DNA fuel molecule to target sequences followed by its removal with another DNA sequence that is completely or partially complementary to the first.[5] Yurke et al. demonstrated the first DNA machine that functioned as "tweezers" fuelled by two strands of DNA with tailored complementarity.[8a] As the energy for operating these DNA nanomachines is produced by a strand-exchange strategy, a DNA duplex is produced as a waste product in every working cycle. Thus, the operating efficiency decreases gradually with the accumulation of "wastes". A new strategy is therefore required to overcome this problem for the further development of DNA nanotechnology.

Over the past decade, we have developed a series of photoresponsive DNAs by covalently tethering azobenzene moieties onto the DNA strand.[14–19] Hybridization of these photoresponsive DNAs to single-stranded DNA (to form duplexes), RNA (to form DNA–RNA hybrids), or double-stranded DNA (to form triplexes) can be efficiently switched "on" and "off" by simply irradiating with UV and visible light. This is based on the following mechanism: the planar *trans*-azobenzene intercalates between adjacent base pairs and stabilizes the duplex or triplex structure by stacking interactions, whereas the nonplanar *cis*-azobenzene destabilizes it by steric hindrance.[19] The successful photoregulation of primer elongation, transcription, and RNase H activity have also been demonstrated with photoresponsive DNAs.[20–22] Photoregulation efficiency can be amplified by the introduction of multiple azobenzene residues onto the DNA.[23] For example, nine azobenzene groups were introduced onto a DNA strand 20 nucleotides (nt) in length, and the clear-cut photoswitching of DNA duplex formation was observed without loss of sequence specificity. Photoregulation of the opening of a DNA hairpin as a simple nanomachine by invasion of a DNA opener was also recently demonstrated.[24] All these results prompted us to propose a new strategy for building photon-fuelled DNA nanomachines that are "environmentally friendly" without producing DNA waste during operation. Herein we report a simple, inexpensive, clean, and long-lived photoresponsive DNA nanomachine that can be operated continuously by reversibly photoswitching its mechanical motion with light irradiation.

A photoresponsive DNA machine composed of four strands (**A**, **B**, **C**, and **F**) was designed based on the DNA-fuelled tweezers reported by Yurke et al.,[8a] as illustrated in Figure 1. Strand **A** is hybridized with strands **B** and **C** to form two stiff double-stranded arms that are 22 base pairs long and sufficiently stable at the operating temperature. Tetrachlorofluorescein (TET) and carboxytetramethylrhodamine (TAMRA) were attached respectively to the 5′ and 3′ ends of strand **A**. When strand **F** is hybridized with the overhangs of strands **B** and **C** (left side of Figure 1 A), the tweezers are closed, and the fluorescence emission from TET at ~540 nm (λ_{ex} = 514.5 nm) is quenched by resonant intramolecular energy transfer to TAMRA due to the close proximity of these two dyes. However, when strand **F** is dissociated (right side of Figure 1 A), the tweezers are open, and the fluorescence from TET is recovered. Here, 12 azobenzene moieties are introduced onto a 32-nt-long strand **F** ($\mathbf{F}_{12X}$) so that opening and closure of the tweezers can be photo-controlled by the dissociation and hybridization of strand **F** ($\mathbf{F}_{12X}$) with **B** and **C**. What we expect is as follows: the tweezers are closed after visible light irradiation (azobenzene *cis*→*trans* isomerization), whereas UV light irradiation (*trans*→*cis* isomerization) opens them due to the destabilization effect of *cis*-azobenzene.

When strands **A**, **B**, and **C** were mixed in the absence of strand **F** at 50 °C, strong fluorescence from TET was observed because the tweezers were completely open (Figure 2: – – –). In the presence of $\mathbf{F}_n$ (the native form of strand **F**), however, the fluorescence decreased dramatically because the tweezers

[a] *Prof. Dr. H. Asanuma*
Graduate School of Engineering, Nagoya University
Furo-cho, Chikusa-ku, Nagoya 464-8603 (Japan)
Fax: (+81) 52-789-2528
E-mail: asanuma@mol.nagoya-u.ac.jp

[b] *Prof. Dr. X. Liang,[+] H. Nishioka,[+] N. Takenaka*
Core Research for Evolution Science and Technology (CREST)
Japan Science and Technology Agency (JST)
Kawaguchi, Saitama 332-0012 (Japan)

[+] *These authors contributed equally to this work.*

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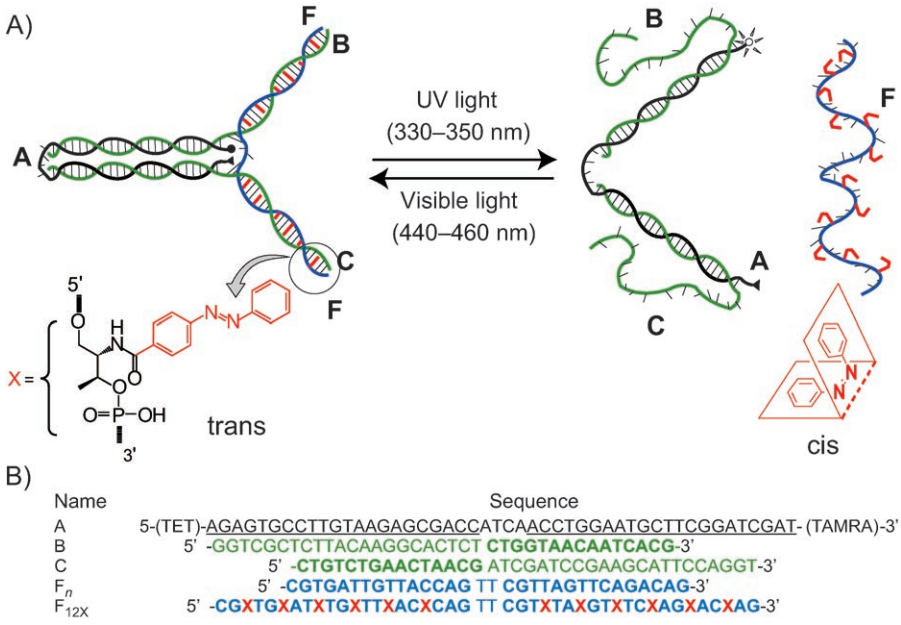

Figure 1. A) Schematic illustration of the nanotweezers photoswitched by light irradiation, and B) oligonucleotide sequences. The tweezers are closed (left side of panel A) when the azobenzene groups are in the *trans* configuration as a result of irradiation with visible light. Irradiation with UV light opens the tweezers through *trans*→*cis* isomerization (right). Strands **A** and **F** are shown in black and blue, respectively, and strands **B** and **C** are colored green. Residue X with the azobenzene moiety is shown in red. Underlined letters in strand **A** indicate the regions that hybridize with strands **B** and **C**. Complementary regions between **F** ($\mathbf{F}_n$ and $\mathbf{F}_{12X}$), and **B** and **C** are shown in boldface. Strand $\mathbf{F}_{12X}$ is the photoresponsive DNA bearing 12 azobenzene moieties, and $\mathbf{F}_n$ is the native control sequence without azobenzene modification.

were closed by the hybridization of overhangs of **B** and **C** with $\mathbf{F}_n$ (Figure 2: •••••). Similarly, the presence of photoresponsive $\mathbf{F}_{12X}$ lowered the fluorescence to almost the same level as that of $\mathbf{F}_n$ after irradiating with visible light at 50 °C for 1 min (——), demonstrating that *trans*-azobenzene-modified $\mathbf{F}_{12X}$ (*trans*-$\mathbf{F}_{12X}$) could close the tweezers as efficiently as $\mathbf{F}_n$ did.[25,26] In contrast, fluorescence was significantly recovered by UV light irradiation of the solution containing **A**, **B**, **C**, and *trans*-$\mathbf{F}_{12X}$ at 50 °C for 5 min (–•–•): its spectra was close to that of the open tweezers consisting of only **A**, **B**, and **C** (compare ––– with –•–•).[27–29] These results clearly demonstrate that opening and closure of the tweezers with photoresponsive $\mathbf{F}_{12X}$ are switched simply by light irradiation. Photoinduced opening and closing of the tweezers also took place at other temperatures, although the photoregulation efficiency depends greatly on the operation temperature (Figure S1). According to our previous study, an efficient photoregulation of DNA hybridization should be carried out at a temperature between the melting temperature (T_m) of the duplex containing *trans*-azobenzenes and that of the duplex with *cis*-azobenzenes.[23,30] Because the T_m values of **B**, **C**, and $\mathbf{F}_{12X}$ in *cis* and *trans* configurations were 35.0 and 59.2 °C, respectively (Figure S2), the photon-fuelled tweezers designed in the study presented herein worked most efficiently at around 50–55 °C. Although the working temperature range was limited and the tweezers did not work at ambient temperature, the T_m value can be lowered by changing the sequence of the **F** strand and the number of azobenzene groups introduced.

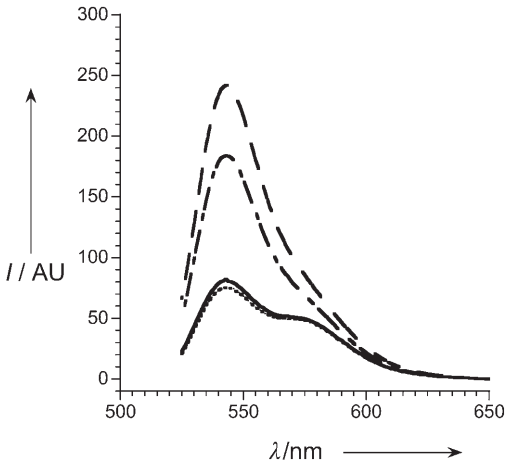

Figure 2. Closure and opening of the DNA tweezers with light irradiation at 50 °C, monitored by fluorescence intensity. The tweezers are completely open with strands **A**, **B**, and **C** present in solution (–––). Also shown are data for the inclusion of strand $\mathbf{F}_{12X}$ after UV light irradiation for 5 min (–•–•), or after visible light irradiation for 1 min (——), or upon the addition of strand $\mathbf{F}_n$ (•••••).

As described above, our photon-fuelled nanomachine produces no waste during operation. In the original report of such tweezers,[8a] closure was carried out by the addition of strand **F** with an eight-nt-long overhang, and opening (removal of strand **F**) was done by the addition of strand **Fc**, which is complementary to **F** (Figure S3). Therefore, successive opening and closing required successive additions of strands **F** and **Fc**, and this inevitably produced a large amount of **F**–**Fc** duplex waste. As a result, cycling efficiency of the original tweezers decreased by about 40% after seven cycles owing to the successive addition of DNA fuel and accumulation of waste duplex.[8a] In our system, opening and closure are carried out by successive irradiation of the solution containing photoresponsive $\mathbf{F}_{12X}$ with UV and visible light. Figure 3 shows the change in fluorescence intensity as the tweezers were cycled ten times between the open and closed states by successive irradiation with UV and visible light at 50 °C. For every cycle, almost all the tweezers were closed after visible light irradiation for 1 min, and the tweezers were open after UV light irradiation for 4 min. The cycling efficiency did not decrease after 10 open/close iterations (Figure 3), because no extra oligonucleotides were added.

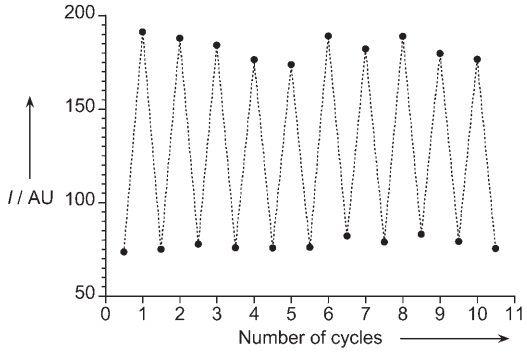

Figure 3. Repeated opening and closing of the photoresponsive molecular tweezers at 50 °C by alternate irradiation with visible light for 1 min and UV light for 4 min. When the tweezers are closed, resonant energy transfer from the TET dye to TAMRA decreases the fluorescence intensity. The solution containing the tweezers was irradiated outside the fluorometer and then the cell was placed into the holder to measure the fluorescence after 30 s ($\lambda_{em} = 543$ nm).

Therefore, the efficiency of photoregulation does not change with the number of operation cycles as long as the DNA strands involved are not destroyed. In fact, no decomposition of the introduced azobenzene residues was detected under the light irradiation conditions used, although continuous irradiation with visible light for a long time (>30 min) was found to destroy the fluorophore TET (data not shown). Thus, these photoresponsive tweezers are expected to endure many open/close cycles without loss in working efficiency.

Another point of concern is the duration of irradiation time required to open or close the tweezers. In the case of visible light irradiation at 50 °C, we found that irradiation for 1 min was sufficient to reach equilibrium (Figure 4A), whereas UV

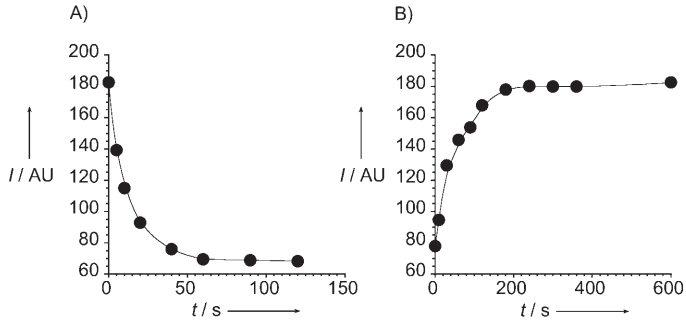

Figure 4. Time course for A) closing the tweezers with visible light irradiation and B) opening them with UV light irradiation at 50 °C ($\lambda_{em} = 543$ nm).

irradiation for 3 min was necessary to open the tweezers (Figure 4B). Therefore, an efficient open/close photoswitching cycle can be performed with UV and visible light irradiation for 3 and 1 min, respectively (see Figure S4 for fluorescence spectra). Although the switching time depends on light intensity, it was similar to that used for the DNA-fuelled machine under the irradiation conditions employed earlier.[8a]

In conclusion, an efficient photon-fuelled molecular machine was constructed with photoresponsive azobenzene-modified DNA. Further studies underway include improvement in operation efficiency and decreasing the number of molecular engines by using modified azobenzenes, improving the thermal stability of *cis*-azobenzene, and designing new nanomachines with hairpin structures.

Experimental Section

Materials: The azobenzene-containing oligonucleotides were supplied by Nihon Techno Service Co., Ltd. (Tsukuba, Japan), and purified by polyacrylamide gel electrophoresis. The oligonucleotides containing only native bases (strands **B**, **C**, and $\mathbf{F}_n$) and strand **A** modified with TET and TAMRA were supplied by Integrated DNA Technologies, Inc. (Coralville, USA). Oligonucleotides **B, C**, and $\mathbf{F}_n$ were used directly after desalting. Oligonucleotide **A** was purified by RP HPLC. The concentrations of all oligonucleotides were determined by UV/Vis spectroscopy within 10% error. The molecular extinction coefficient (ε) of an azobenzene residue is $1.095 \times 10^4\ \text{M}^{-1}\text{cm}^{-1}$. Stock solutions of 25 μM in TE buffer (10 mM Tris pH 8.0, 1.0 mM EDTA) were prepared.

FRET assays to monitor DNA nanomachine operation: The photoresponsive tweezers were prepared by mixing stoichiometric quantities of four strands **A**, **B**, **C**, and **F** in SPSC buffer (50 mM Na_2HPO_4 pH 6.5, 1.0 M NaCl) to a final concentration of 1.0 μM. The solution was added to a 3-mm square quartz cuvette, and the fluorescence spectra of TET were measured with a JASCO FP-6500 fluorescence spectrometer (JASCO, Tokyo, Japan) excited at 514.5 nm (3 nm bandwidth). For data shown in Figures 3 and 4, the fluorescence intensity emitted at 543 nm was used. The irradiation was carried out with a xenon light source (MAX-301, Asahi Spectra Co., Ltd. Tokyo, Japan) equipped with an interference filter (half bandwidth 9 nm) centered at 341.5 nm for UV light irradiation ($0.5\ \text{mW cm}^{-2}$) and an interference filter (half bandwidth 9 nm) centered at 449.5 nm for visible light irradiation ($90\ \text{mW cm}^{-2}$). During irradiation, the cuvette containing the tweezers was put in a water bath to maintain a fixed temperature (±2 °C). After irradiation, the cuvette was immediately moved into the fluorescence spectrometer that was kept at the same temperature to measure fluorescence. During the fluorescence measurements, further UV or visible light irradiation was not carried out.

T_m measurements: Solutions consisting of strands **A**, **B**, **C**, and **F** in SPSC buffer (50 mM Na_2HPO_4 pH 6.5, 1.0 M NaCl) were prepared to give a final DNA concentration of 0.5 μM. After holding at 90 °C for 3 min, the solution was gradually cooled to 10 °C ($1.0\ ^\circ\text{C min}^{-1}$). Melting curves were obtained at a heating rate of $1.0\ ^\circ\text{C min}^{-1}$. Peak temperatures in the derivative curves (dA/dT) were designated as melting points. UV spectra and UV melting profiles (260 nm) were recorded by a JASCO model V-530 spectrometer equipped with a programmable temperature controller.

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[25] The FRET efficiency in the completely open state (without **F** strand) at 50 °C would be ~0.25 as estimated from the averaged distance between the TET and TAMRA chromophores, reported to be 6 nm in ref. [8a]. Based on this value, the FRET efficiency in the closed state could be evaluated as 0.79.
[26] As shown in Figure 2, the fluorescence intensity remained around 70 AU after closing the tweezers at 50 °C. According to Yurke et al.,[8a] rather strong fluorescence remained even in the completely closed state at 20 °C. In our case, the fluorescence intensity was around 60 AU at 20 °C (data not shown). Therefore, we think that the residual fluorescence after closing the tweezers can be attributed mainly to the low FRET efficiency.
[27] In Figure 2, the fluorescence spectrum after UV irradiation (–•–•) does not completely overlap that of the completely open state (–––) due to the incomplete dissociation of *cis*-$\mathbf{F}_{12X}$. We recently synthesized a modified azobenzene which has much more efficient photoresponsiveness: H. Nishioka, X. G. Liang, H. Kashida, H. Asanuma, *Chem. Commun.* **2007**, 4354–4356. With this new modified azobenzene, complete dissociation can be expected.
[28] Although the backbone of strand $\mathbf{F}_{12X}$ is much longer than that of the complementary parts due to the additional incorporation of azobenzene moieties (X residues, Figure 1), both the stability of the duplex formed and the specificity for recognizing the complementary sequence are similar to (or slightly lower than) the native strand $\mathbf{F}_n$ in the case of *trans*-$\mathbf{F}_{12X}$: T_m of **A**/(**B**,**C**) and $\mathbf{F}_n$ was 63.0 °C, whereas $T_m = 59.2$ °C for *trans*-$\mathbf{F}_{12X}$ (see Figure S2). When fewer azobenzene residues (four or eight X residues) were used, photoregulation was also attained but with lower efficiency.
[29] No clear resonant intramolecular energy transfer from TET to *trans*- or *cis*-azobenzene moieties was observed.
[30] If the temperature is too high, the duplex dissociates in both cases of *trans* and *cis* configuration; if the temperature is too low, the duplex is formed in both cases.

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