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Light-Driven DNA Nanomachine with a Photoresponsive Molecular Engine

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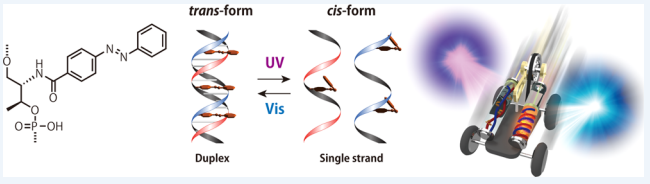
CONSPECTUS: DNA is regarded as an excellent nanomaterial due to its supramolecular property of duplex formation through A−T and G−C complementary pairs. By simply designing sequences, we can create any desired 2D or 3D nanoarchitecture with DNA. Based on these nanoarchitectures, motional DNA-based nanomachines have also been developed. Most of the nanomachines require molecular fuels to drive them. Typically, a toehold exchange reaction is applied with a complementary DNA strand as a fuel. However, repetitive operation of the machines accumulates waste DNA duplexes in the solution that gradually deteriorate the motional efficiency. Hence, we are facing an "environmental problem" even in the nanoworld. One of the direct solutions to this problem is to use clean energy, such as light. Since light does not contaminate the reaction system, a DNA nanomachine run by a photon engine can overcome the drawback of waste that is a problem with molecular-fueled engines.



There are several photoresponsive molecules that convert light energy to mechanical motion through the change of geometry of the molecules; these include spiropyran, diarylethene, stilbene, and azobenzene. Although each molecule has both advantages and drawbacks, azobenzene derivatives are widely used as "molecular photon engines". In this Account, we review light-driven DNA nanomachines mainly focusing on the photoresponsive DNAs that we have developed for the past decade. The basis of our method is installation of an azobenzene into a DNA sequence through a D-threoninol scaffold. Reversible hybridization of the DNA duplex, triggered by *trans*−*cis* isomerization of azobenzene in the DNA sequences by irradiation with light, induces mechanical motion of the DNA nanomachine. Moreover we have successfully developed azobenzene derivatives that improve its photoisomerizaition properties. Use of these derivatives and techniques have allowed us to design various DNA machines that demonstrate sophisticated motion in response to lights of different wavelengths without a drop in photoregulatory efficiency.

In this Account, we emphasize the advantages of our methods including (1) ease of preparation, (2) comprehensive sequence design of azobenzene-tethered DNA, (3) efficient photoisomerization, and (4) reversible photocontrol of hybridization by irradiation with appropriate wavelengths of light. We believe that photon-fueled DNA nanomachines driven by azobenzene-derivative molecular photon-fueled engines will be soon science rather than "science fiction".

INTRODUCTION

Beyond its biological importance, DNA is recognized as an exciting material in nanotechnology due to facile programmability. Various DNA nanoarchitectures have been designed by assembling DNA in combination with organic molecules to supply missing functions to DNA.[1−7] Among DNA-based nanoarchitectures, nanosized motional machines have been developed by using DNA as a scaffold for nanobodies such as tweezers, walkers, and gears.[8−15] Like machines in the macroscopic "meter-scale world", nanomachines are also subject to the law of conservation of energy. One type of engine used to drive these DNA-based nanomachines is fueled by spontaneous hybridization of DNA with its complementary strand. Yurke et al. first demonstrated tweezer-like motion using an engine driven by reversible opening and closing of two handles (Figure 1).[9] The two handles close upon hybridization of two overhangs with the added DNA fuel **F**. Closed tweezers are reset to the initial open state by the toehold exchange with another DNA fuel **F̲** producing **F**/**F̲** waste duplexes. Most nucleotide-based nanomachines are driven by the toehold exchange method. However, repetitive motions accumulate **F**/**F̲** waste duplexes in the solution, which contaminates the microenvironment and retards driving efficiency.[9] Hence, the "nanoworld" faces environmental problems similar to those of the "meter world". One of the solutions to environmental problems is use of clean energy sources that do not contaminate the nanoenvironment such as electricity, protons, or light. pH-driven nanomachines equipped with pH-sensitive DNA architectures such as i-motif minimize contamination.[15,16] Among the energy sources listed above, light is the most promising, because light does not contaminate the reaction system, and its use makes spatiotemporal control of bioreactions possible.[17] In this Account, we first introduce several photochromic compounds with potential as photon engines and then focus on recent progress on the conversion of light energy into mechanical motion.

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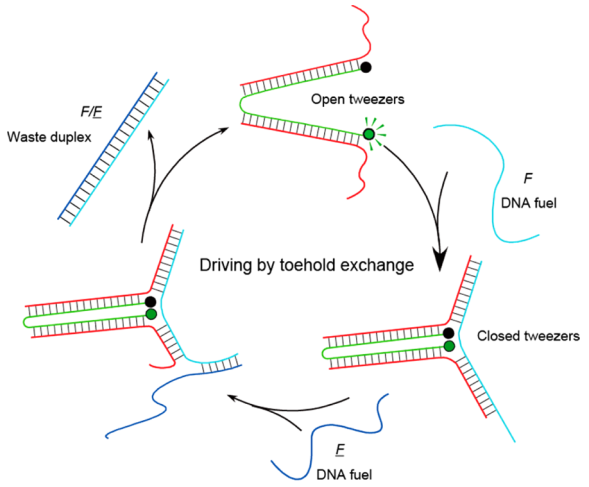

Figure 1. Schematic illustration of a DNA nanomachine powered by DNA fuel on the basis of the toehold exchange reaction developed by Yurke et al.[9] Adapted with permission from ref 9. Copyright 2000 Nature Publishing Group.

PHOTORESPONSIVE MOLECULES FOR PHOTON ENGINES

In the "meter world", light energy is converted to electric power via a solar cell to drive electric motors.[18−20] It is not possible to simply downsize a meter-scale motor powered by electricity to nanosize. A molecular-sized photon engine that can directly convert light energy into mechanical motion must be incorporated into a nanomachine. Reversibly photoisomerizable organic molecules that undergo large structural changes are suitable for these photon engines. Readily accessible photoresponsive molecules include spiropyran, diarylethene, stilbene, and azobenzene derivatives; the photochemical properties of these molecules are described in the following.

Spiropyran[21]

The chemical structure of typical spiropyran is shown in Figure 2a. Irradiation with UV light (300−400 nm) isomerizes a bulky closed spiropyran into a planar, open merocyanine structure with an extended π-conjugate system having λ_{max} at 500−600 nm. The spiropyran form is nonpolar; the merocyanine form has a zwitterionic structure that is more hydrophilic. In a hydrophobic solvent, merocyanine can be isomerized back to spiropyran either thermally or by irradiation with visible light (λ > 490 nm).[21] Hence, photoisomerization induces both huge structural changes and polarity changes that can be advantageous in various applications. Spiropyran has several drawbacks, however, when it is used in an aqueous environment. Spiropyran preferentially adopts the open merocyanine structure in polar aqueous media due to its hydrophilic nature. In aqueous solution, excitation of the merocyanine form with visible light results in isomerization to the colorless closed form, but it thermally reverts to the open form within less than 30 min. To stabilize the closed form, it is necessary to irradiate with visible light continuously or at intervals. Moreover, UV irradiation of the closed form does not induce isomerization to the open form of spiropyran under aqueous conditions; isomerization of spiropyran to merocyanine occurs only thermally. Inconveniently, merocyanine gradually decomposes in water. These drawbacks of spiropyran prevent conjugation to biomolecules and severely limit applications in aqueous media. Our group first conjugated spiropyran to the 5′-termini of DNA to examine its photoswitchable ability (Figure 2a),[22] and Wagenknecht's group incorporated it into an oligodeoxynucleotide via an acyclic linker.[23] Very large polarity changes are observed in modified DNA due to spiropyran−merocyanine isomerization, and the modification also alters the melting temperature (T_m) of a DNA duplex. Although results indicate that spiropyran is potentially an excellent photon engine for a DNA nanomachine, the limitations mentioned forced us to abandon further development. Recently, however, Heckel's group developed a modified spiropyran that is photoisomerized from closed to open form by UV-light irradiation even in an aqueous environment (Figure 2a).[24] If the drawbacks of spiropyran can be overcome, we believe it will be an excellent photon engine.

Diarylethene

Diarylethene is another well-characterized photochromic molecule. The open structure of colorless diarylethene undergoes electrocyclization upon irradiation with UV light to form a closed ring structure that can be converted back to the open structure by visible light irradiation (Figure 2b).[25] Unlike spiropyran and azobenzene (*vide infra*), open-to-closed and closed-to-open isomerization occurs only upon light irradiation because both forms are thermally stable. Since the light-induced structural change in diarylethene is not as large as the change induced in spiropyran, fewer diarylethene derivatives have been conjugated to biomolecules. Jäschke's group first reported diarylethene-modified nucleosides, and Wagenknecht's group first synthesized diarylethene-functionalized DNA via phosphoramidite chemistry (Figure 2b).[26−28] Jäschke et al. activated a transcription reaction upon induction of the closed ring conformation of diarylethene conjugated to a thymine located in the T7 promoter sequence (Figure 2b).[29] Although the limited conformational change of diarylethene may not produce enough mechanical power to drive a nanomachine, these derivatives may be useful in other applications such as photoregulation of redox potential to control hole or electron conductivity.

Stilbene

This compound reversibly isomerizes between a planar *trans*-form and a nonplanar *cis*-form upon UV light irradiation. Since the *cis*-form is thermally stable, *cis*-to-*trans* isomerization does not occur thermally but only upon light irradiation. These properties are suitable for converting light energy to mechanical motion, and Lewis et al. were first to report on the photoregulation of stilbene-tethered DNA.[30] Stilbene has two drawbacks as a photoswitch. One is that *trans*-to-*cis* isomerization is induced by 340 nm light, but reversion to the *trans*-form requires UV light of shorter wavelength (300 nm > λ), which causes unfavorable photoreactions of biomolecules, such as formation of thymine dimers. The other drawback is related to the photoisomerization mechanism. Photoisomerization of stilbene proceeds via a rotation route that involves disruption of a carbon−carbon π bond and rotation around the remaining σ bond (Figure 2c).[31] Significant space is needed to allow the rotational movement of the benzene ring. When the stilbene moiety is in a narrow space or a restricted position, as it is when intercalated in a DNA duplex, photoisomerization does not occur.[32] To avoid the second drawback, Maeda's group designed a guanine analogue that is conjugated with styrene at the 8 position for the photoregulation of G-quadruplex formation.[33,34]

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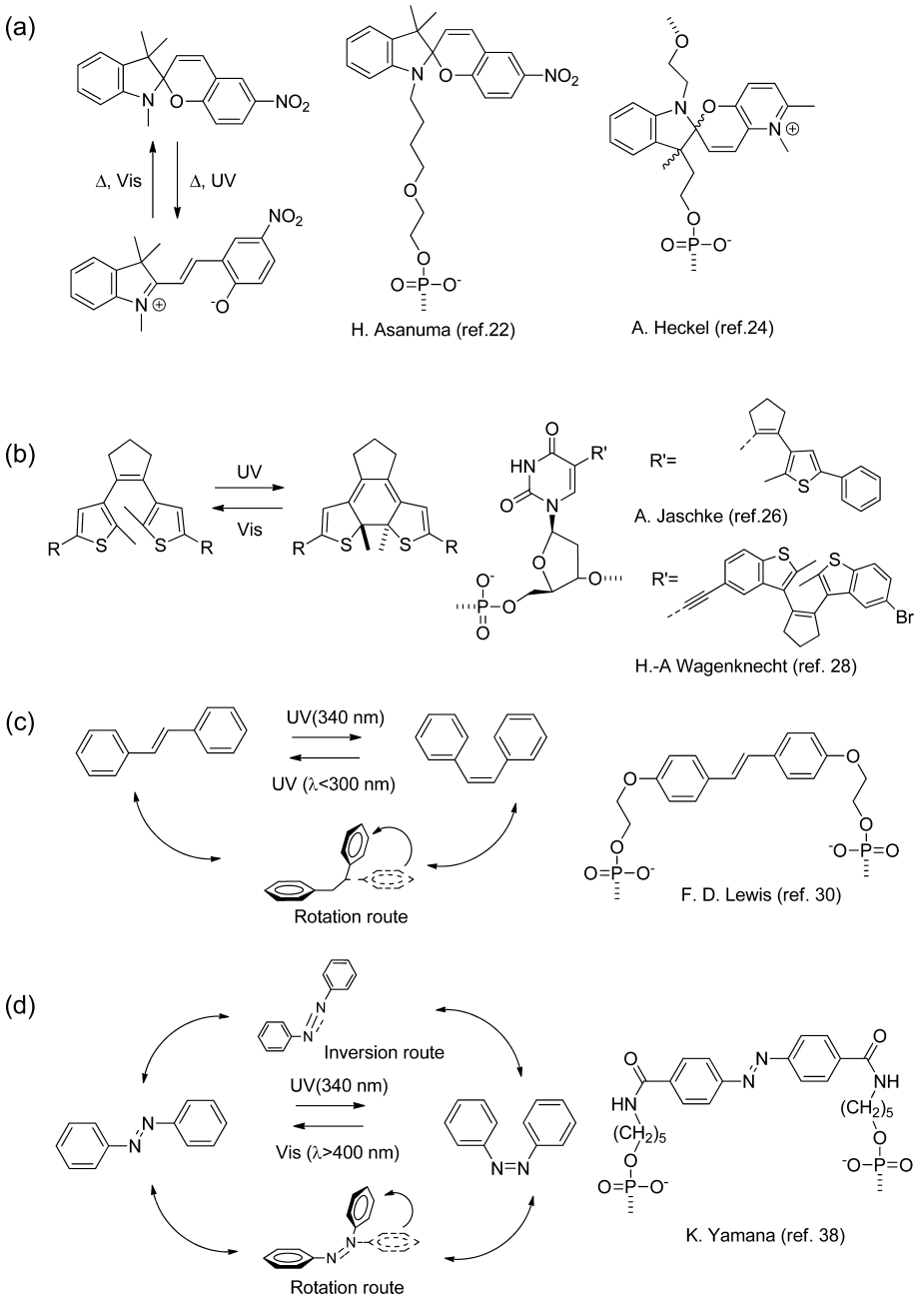

Figure 2. Typical photoresponsive molecules that have been incorporated into DNA as a reversible photoswitch for the photoregulation of DNA functions: (a) spiropyran, (b) diarylethene, (c) stilbene, and (d) azobenzene.

Azobenzene

Among the photoresponsive molecules, azobenzene derivatives are the most popular photoswitch for versatile applications.[35−37] Like stilbene, azobenzene reversibly photoisomerizes between a planar *trans*-form and a nonplanar *cis*-form upon light irradiation (Figure 2d). Azobenzenes have several advantages as photoswitches with respect to other molecules. One is its availability. Chemical syntheses of azobenzene derivatives are simple, and some are commercially available. Second is chemical stability. Repetitive *trans*−*cis* photoisomerization does not cause any decomposition even in aqueous conditions. Third, *trans*-to-*cis* isomerization occurs with irradiation at around 350 nm, and the *cis*-to-*trans* isomerization is induced by harmless visible light so that biomolecules are not damaged. Yamana et al. first synthesized azobenzene-tethered DNA as a photoisomerizable linker (Figure 2d).[38] Photoisomerization of azobenzene occurs either via an inversion route or through rotation as shown in Figure 2d.[31] Inversion proceeds through a transition state in which one of the nitrogen atoms is sp hybridized. Inversion does not require a large amount of space so that even intercalated azobenzenes can be photoisomerized,[31,39,40] although isomerization yield is lower than when the azobenzene can freely rotate at the photostationary state. A limitation is that *cis*-azobenzene is thermally unstable compared with the *trans*-form, and it gradually reverts to *trans* in the dark. Usually, the maximum yield of the *cis*-form induced by light irradiation is around 70−80% at the photostationary state; 100% photoisomerization to the *cis*-form is impossible.

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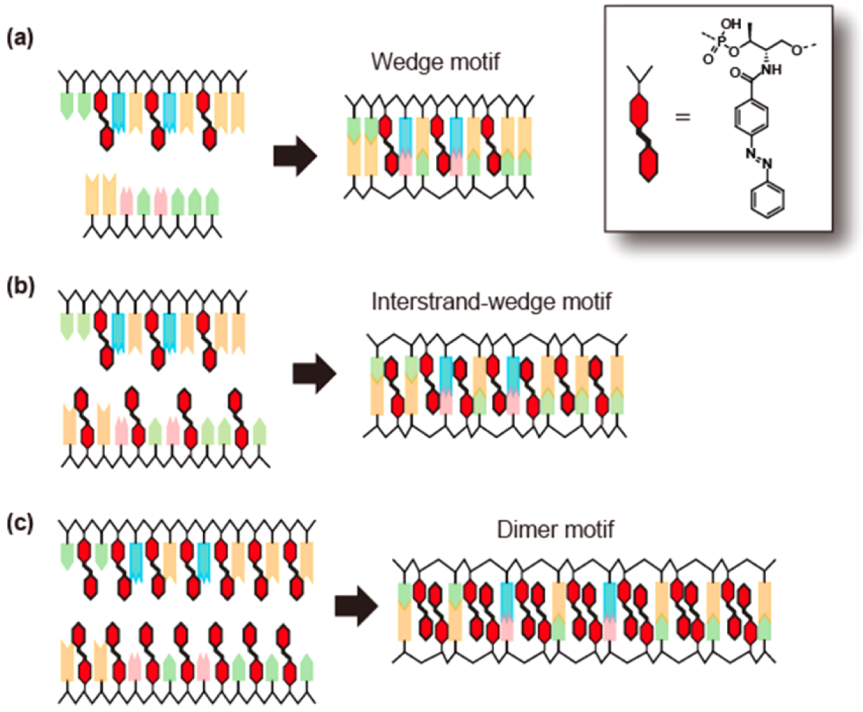

Figure 3. Schematic representations of (a) wedge motif, (b) interstrand-wedge motif, and (c) dimer motif. Azobenzenes were incorporated into DNA using a D-threoninol scaffold.

COVALENT INTRODUCTION OF AZOBENZENE TO DNA AS A MOLECULAR PHOTON ENGINE

From the viewpoint of constructing DNA-based nanomachines, modulating DNA hybridization in response to light is a simple approach. For example, Nakatani's group designed a photoswitchable "molecular glue" in which azobenzene was incorporated between two naphthylidine heterocycles that reversibly bind to a DNA duplex containing a GG mismatch.[41] Hybridization and dehybridization of the duplex are induced by alternating photoirradiation between UV and visible light.

Direct incorporation of the photoresponsive molecule into the DNA allows photoregulation of hybridization at a single-molecule level. Usually, incorporation of photochromic compounds is conducted on natural nucleotides.[35] However, such modification on nucleotides has several synthetic difficulties: four (A, G, C, T) modified phosphoramidite monomers have to be synthesized via laborious protection−deprotection processes. To avoid such synthetic complexity, we designed a base-surrogate tethering azobenzene on not L- but D-threoninol as a scaffold; this is easily synthesized and allows for multiple introductions of azobenzenes at any position and in any number via solid-phase synthesis.[42−46] A single azobenzene inserted into a 12-base pair DNA does not destabilize the duplex in *trans*-form, whereas *cis*-azobenzene significantly lowers the stability of the duplex.[47] Our NMR analyses[48] revealed that *trans*-azobenzene, which is planar, stabilizes the DNA duplex due to stacking with the adjacent base pair and that the nonplanar *cis*-azobenzene destabilizes the duplex due to steric hindrance. Repetitive regulation of hybridization and dissociation of the DNA duplex can be promoted by alternating between UV and visible light. Photocontrol of the hybridization of DNA/RNA and RNA/RNA duplexes and triplex DNA can be also performed by introducing the azobenzene as a photoresponsive switch.[49,50]

In general, *trans*-azobenzene cannot be completely *cis*-isomerized upon UV-irradiation. Particularly, far below the T_m, *trans*−*cis* isomerization is suppressed (20−40%) due to the stacking interactions at the photostationary state.[40] In order to drive a DNA nanomachine efficiently, almost complete photoregulation of hybridization and dissociation of a sufficiently long DNA duplex is crucially important. We therefore inserted multiple azobenzenes into a long duplex. Motifs included a wedge, an interstrand wedge, and a dimer (Figure 3). In the wedge motif, azobenzenes (X) are multiply introduced into one strand at every two native nucleotides (N), 5′-$(NNX)_n$-NN-3′, whereas the complementary strand is composed of only native nucleotides (Figure 3a).[46] Here, azobenzenes are not exchanged with natural nucleotides but additionally introduced so that the obtained duplex involves single bulge-like wedges every two base pairs. Interestingly, when in the *trans*-form, the azobenzenes do not interfere with duplex formation even though a number of unnatural nucleotide analogues are asymmetrically present. In contrast, the T_m of the duplex with azobenzenes in the *cis*-form is dramatically decreased, and photoregulatory efficiency increases with the number of azobenzenes inserted into the strand. This motif is useful when one strand must be native or is difficult to modify, such as would be the case when photoregulation of an enzymatic reaction is desired.[51]

In interstrand-wedge[52,53] and dimer motifs,[54] azobenzenes are introduced into the both strands of the duplex. The interstrand-wedge motif has azobenzene insertions every two natural nucleotides in each strand. In the duplex of 5′-$(NNX)_n$-NN-3′ and 3′-$(NXN)_{n+1}$-5′, azobenzenes and native base pairs alternate (Figure 3b). In the dimer motif, both oligonucleotides have alternating natural nucleotides and azobenzenes, and hybridization results in dimerized azobenzenes alternately aligned with natural base pairs (Figure 3c). Both motifs are much more stable than the corresponding natural duplexes

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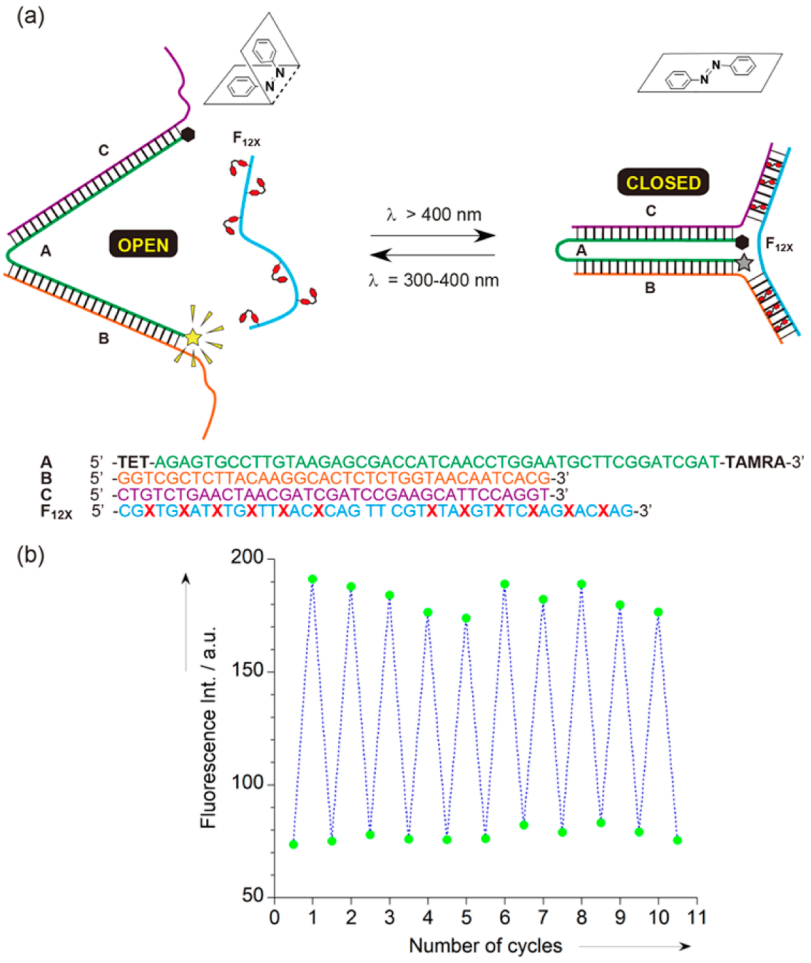

Figure 4. Photoresponsive DNA tweezers convert between open and closed conformations upon light irradiation.[55] (a) Schematic representation of photoresponsive DNA tweezers. (b) Repetitive open and close motion of DNA tweezers upon alternate irradiation with visible and UV light monitored by fluorescent intensity. When the tweezers are closed, resonant energy transfer from the TET to TAMRA decreases the fluorescence intensity. Adapted with permission from ref 55. Copyright 2008 Wiley-VCH Verlag GmbH & Co. KGaA.

without azobenzenes. Unlike the wedge motif, in the dimer motif, both strands are symmetrical with similar numbers of azobenzenes, and stacking interactions between azobenzenes and base pairs remarkably stabilize the duplex. Duplexes with the dimer motif are stable compared with those containing the interstrand-wedge motif due to the stacking interaction with *trans*-azobenzenes. Since these two motifs involve many azobenzenes, complete dissociation of the duplex is possible even with lower *cis*-isomerization of azobenzene at the photostationary state. Furthermore, introduction of azobenzene into the DNA in either the interstrand-wedge or dimer motif does not affect sequence specificity of hybridization.[54]

MECHANICAL OPEN-CLOSE MOTION OF DNA NANOMACHINES WITHOUT WASTE PRODUCTS

In order to demonstrate a photon-driven DNA nanomachine, we constructed photoresponsive tweezers (Figure 4a).[55] With the toehold exchange-driven machines, waste **F**/**E** duplexes accumulate after repeated cycles in solution, contaminating the microenvironment and retarding driving efficiency. To solve this nanoenvironmental problem, we designed a photoresponsive $\mathbf{F}_{12X}$ containing azobenzenes. In our system, photoresponsive $\mathbf{F}_{12X}$ functions as a photon engine; *trans*-$\mathbf{F}_{12X}$ facilitates hybridization with overhangs in a wedge motif to close the handles of the tweezers. UV light irradiation isomerizes azobenzenes from *trans*-to-*cis* in the $\mathbf{F}_{12X}$ resulting in dissociation from the overhangs to open the tweezers. Unlike toehold exchange, this cycle does not produce any waste. To demonstrate that the DNA tweezers were opening and closing upon irradiation, tetrachlorofluorescein (TET) and carboxytetramethylrhodamine (TAMRA) were attached at the 5′ and 3′ termini of strand A, respectively, and open and closed states were inferred based on the behavior of TET analyzed by FRET. Exactly as we expected, efficiency of the photoregulated mechanical motion did not change significantly after 11 operation cycles (Figure 4b), unambiguously demonstrating the superiority of the light-driven tweezers relative to the toehold exchange-driven tweezers.

In this design of photoactivatable tweezers, the engine ($\mathbf{F}_{12X}$) must dissociate from the main body, and the open−close motion is based on not an intramolecular but on an intermolecular reaction. Hence, the operation efficiency depends on the concentration of DNA, and therefore, this machine cannot be operated at the single-molecule level. To drive a DNA nanomachine at a single-molecule level, all the components must be covalently incorporated into the nanomachine. It should be noted that such covalent incorporation is impossible for toehold-driven DNA nanomachines because toehold exchange is an intermolecular reaction. It is possible to design an intramolecular photon engine, however. We attached

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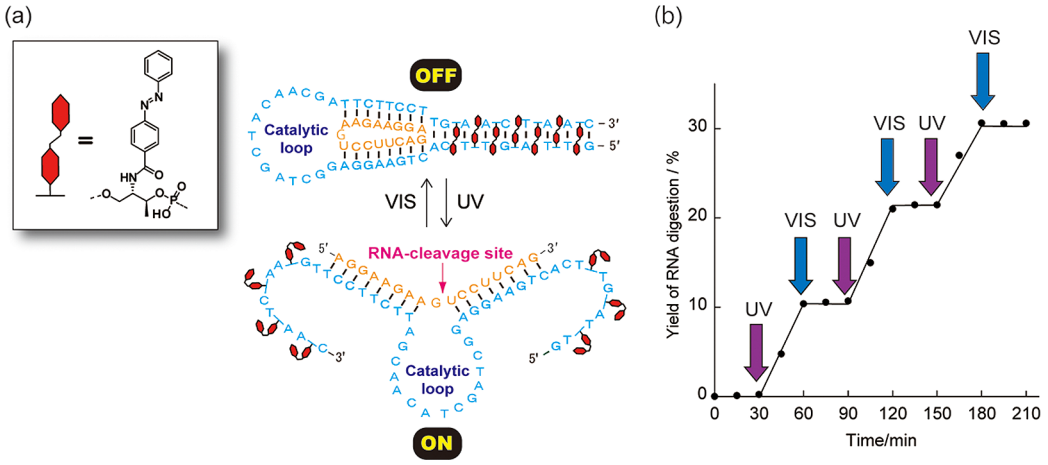

Figure 5. Photoresponsive RNA scission by 10-23 DNAzyme.[56] (a) Open and closed conformation of DNAzyme upon isomerization of azobenzenes. (b) Alternate photoirradiation between UV and visible light turns RNA cleavage activity on and off. Adapted with permission from ref 56. Copyright 2010 Wiley-VCH Verlag GmbH & Co. KGaA.

the interstrand-wedge motif at the termini of a hairpin DNA, and the hairpin (closed structure) to random coil (open structure) transition was reversibly photoregulated by light irradiation.[53] Since the open−close movement is based on the intramolecular reaction between the two arms, this light-driven nanomachine can be operated at a single-molecule level.

An interesting application of DNA nanomachines is photocontrol of a reaction. We designed a photoresponsive DNAzyme that depends on machine-like open−close motion.[56,57] Conformational changes in the catalytic loop sandwiched between two arm regions lead to the on−off switching of RNA cleavage (Figure 5). Azobenzenes were introduced into the extended regions on 5′ and 3′ ends of the DNAzyme. When in *trans*-form, an interstrand-wedge duplex is formed, and in this closed state, the DNAzyme is inactive. Upon UV irradiation and isomerization to the *cis*-form, dissociation of the stem region results in formation of the active conformation of the DNAzyme inducing RNA cleavage. Alternate irradiation with UV and visible light clearly cause reversible functioning of the DNAzyme (Figure 5b).

SYNTHESIS OF THE AZOBENZENE DERIVATIVES FOR MORE EFFECTIVE PHOTOREGULATION

When the wedge motif is used, only a limited number of azobenzenes can be introduced into a duplex. In some cases, such as photoregulation of enzymatic reaction, the number of introduced azobenzenes must be minimized to avoid extreme structural alternation. Accordingly, new azobenzenes that can induce much larger differences in melting temperature (ΔT_m) between *trans*- and *cis*-forms are required. We found that modification with a methyl group at an *ortho* position of the distal benzene ring of azobenzene to create 2-methylazobenzene (2-Me-Azo, Figure 6a) results in a stabilization of DNA duplex in *trans*-form by a stacking interaction and destabilization of the duplex in *cis*-form relative to the parent azobenzene modification.[47] As shown in Figure 7a, the T_m of DNA modified with 2-Me-Azo increased in *trans*-form and decreased in *cis*-form relative to the parent azobenzene-modified duplex. In addition, we found that substitution of azobenzene with methyl groups at both *ortho* positions (2,6-dimethylazobenzene, DM-Azo) remarkably retarded the thermal isomerization from *cis*- to *trans*-form and further increased the ΔT_m relative to

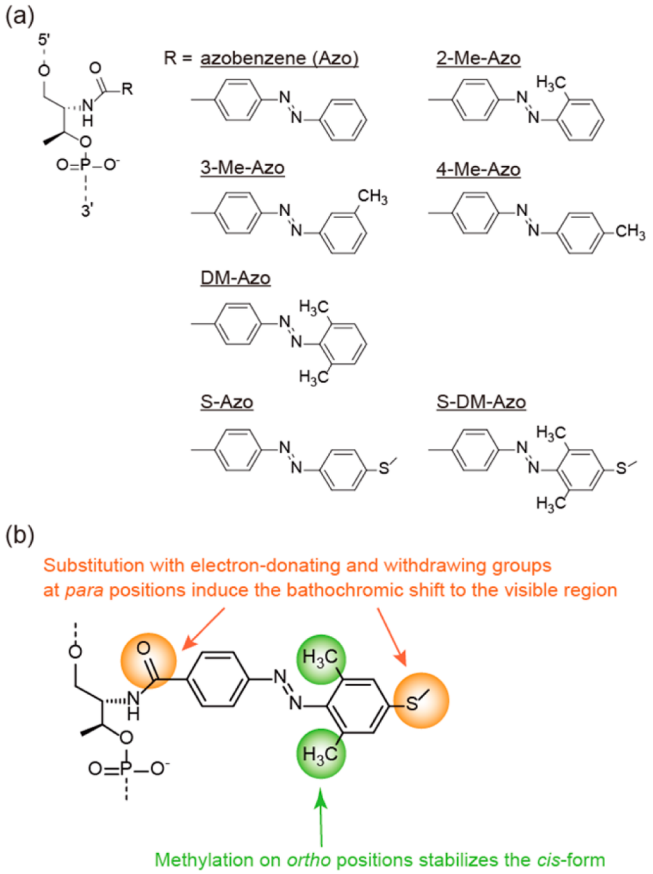

Figure 6. (a) Chemical structures of azobenzene derivatives. (b) Molecular design of azobenzene derivative with enhanced photoregulatory efficiency and isomerization by only visible light irradiation.

the parent modification. The restriction of *cis*-to-*trans* photoisomerization via the inversion process might be caused by steric hindrance due to the bulky *ortho* substituents to the sp orbital of the nitrogen lone pair in the transition state. This steric hindrance does slightly decrease the ratio of *cis*-form to *trans*-form after UV irradiation at the photostationary state.[47]

Although DNA and RNA do not absorb UV light in the range of 300−400 nm, irradiation with strong UV light may damage biological macromolecules. Therefore, we developed an

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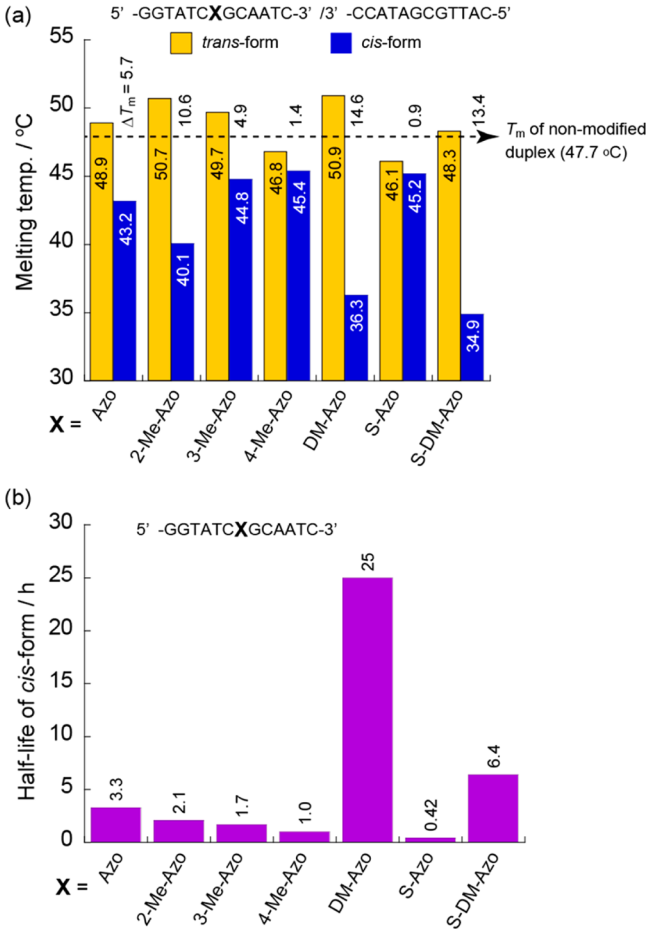

Figure 7. Effect of modifications of azobenzene on (a) T_m of the duplex involving the azobenzene derivative in *trans*- or *cis*-form in 10 mM phosphate buffer, pH 7.0, 100 mM NaCl, and (b) half-life of thermal *cis*-to-*trans* isomerization of the azobenzene in single-stranded DNA at 60 °C under the same buffer conditions.

azobenzene derivative that isomerizes upon visible light irradiation. In order to induce a large bathochromic shift into the visible region, electron-donating and -withdrawing groups were introduced at *para* positions of each benzene ring.[58] Overlap of $\pi-\pi^*$and $n-\pi^*$ transitions (at ca. 450 nm) should be avoided because it leads to low *cis* content. In the new azobenzene derivative, 2,6-dimethyl-4-(methylthio)-azobenzene-4′-carboxylic acid (S-DM-Azo), a methylthio group and a carboxylate were incorporated at *para* positions of the azobenzene scaffold; the derivative has an absorption maximum (λ_{max}) at 400 nm.[59] Usually, push−pull substitution of azobenzene decreases the thermal stability of the *cis*-form. Indeed, methylthio substitution at a *para* position of azobenzene (S-Azo) significantly shortened the half-life of the *cis*-form (Figure 7b). This drawback was solved by two methyl substitutions at *ortho* positions. Thus, the methylthio substitution at a *para* position of azobenzene in conjunction with methylation of both *ortho* positions allows photoisomerization with light above 400 nm with sufficient thermal stability of *cis*-form (Figure 6b and 7b). DNA modified with S-DM-Azo using the D-threoninol scaffold exhibited a sufficiently high *cis* content, acceptable thermal stability, and a high photoregulatory efficiency.

DNA NANOMACHINE SWINGING LIKE A SEESAW CONTROLLED BY AZOBENZENE DERIVATIVES THAT SENSE DIFFERENT WAVELENGTHS OF LIGHT

Our S-DM-Azo isomerizable only by visible light is useful not only for biological applications but also in nanomachines with sophisticated motion. Figure 8 illustrates a DNA nanomachine

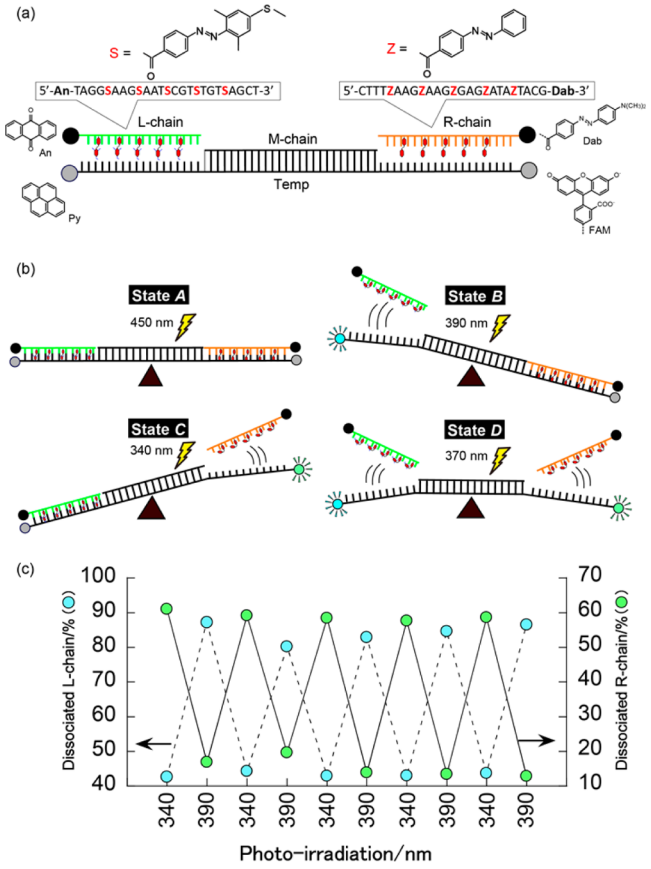

Figure 8. Concept of seesaw-like motion involving the two kinds of photon engines.[60] (a) Schematic representation of components of the DNA seesaw. (b) Four states of "DNA seesaw" obtained upon irradiation with indicated wavelengths of light. (c) Photoresponsive motion of the DNA seesaw by alternate irradiation with 340 and 390 nm light. Adapted with permission from ref 60. Copyright 2012 Wiley-VCH Verlag GmbH & Co. KGaA.

that has seesaw-like motion prepared by combining Azo and S-DM-Azo.[60] In this machine, a 65-nucleotide DNA (Temp) was hybridized with a 20-nucleotide DNA containing five S-DM-Azo modifications at the 5′ terminus (L-chain), a 20-nucleotide DNA containing five Azo modifications at the 3′ terminus (R-chain), and a 25-nucleotide unmodified DNA (M-chain) in the central region (Figure 8a). Because Azo and S-DM-Azo are photoisomerized at different wavelengths, we can create all four possible states by irradiation with suitable wavelengths of light (Figure 8b).

To quantify the hybridization of the duplexes in each state, we used two fluorophore/quencher systems. Anthraquinone (An) and pyrene (Py) were attached at the 5′ terminus of the L-chain and at the 3′ terminus of Temp as a quencher and a fluorophore, respectively. The R-chain was modified at the 3′ end with 4-dimethylaminoazobenzene-4′-carboxylic acid (Dab) as a quencher, and the 5′ terminus of Temp was modified with

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the fluorophore (6-fluorescein-6-carboxamido)hexanoate (FAM). Upon irradiation with 450 nm light, both S-DM-Azo and Azo isomerize to the *trans*-forms and both L- and R-chains are hybridized with Temp (Figure 8b, state A). After irradiation of the nanomachine at 390 nm (or 400 nm), S-DM-Azo isomerizes to the *cis*-form while Azo remains *trans* so that only the L-chain dissociates from the duplex and the R-chain/Temp duplex remains stable (state B). Subsequent irradiation at 340 nm results in isomerization of *cis*-S-DM-Azo to the *trans*-form and *trans*-Azo to the *cis*-form so that the L-chain hybridizes with Temp and the R-chain/Temp duplex dissociates (state C). Upon irradiation at 370 nm, both Azo and S-DM-Azo isomerize to the *cis*-forms resulting in dissociation of both L and R chains from Temp (state D). Alternating irradiation between 340 and 390 nm promotes mutual emission of Py and FAM, indicating that repetitive switching between states B and C is achieved without deterioration (Figure 8c).

EQUIPPING MOTIONAL MACHINES WITH PHOTORESPONSIVE MOLECULAR ENGINES

Since most DNA-based nanomachines are driven by hybridization, use of our azobenzene-tethered photoresponsive DNA changes them to light-driven nanomachines. Famulok's group demonstrated a mechanical motion of DNA rotaxane architecture in which both the dumbbell-shaped molecule and the macrocycle were made of double-stranded DNA.[61,62] They used our photoresponsive DNA carrying DM-Azos in the wedge motif on their macrocycle and observed reversible photoswitching of a double-stranded DNA rotaxane architecture between an immobile pseudorotaxane state and a state in which the macrocycle was free along the axle.

Mao's group designed buckyball-shaped DNA capsules by hybridization of polyhedrons derived from three-branched structures called three-point-star motifs.[63] Here, introduction of our azobenzene-tethered DNA into the sticky ends (in a dimer motif) of the three-point-star motifs stabilizes duplex DNA; the three-point star motifs are disrupted upon UV irradiation.[64] The photocontrollability of the DNA capsules was clearly visualized by using fast scanning atomic force microscopy (AFM). Sugiyama's group also visualized conformational changes upon irradiation in hexagonal DNA origami structures equipped with our photoresponsive DNAs by fast scanning AFM.[65] Other groups have also demonstrated the superiority of our photoresponsive DNA photon engines.[66−69]

PERSPECTIVE

As demonstrated herein, DNA nanomachines involving azobenzenes work efficiently without contaminating the nanoenvironment. Although we believe that photon engines are promising tools for driving nanomachines, there remain several problems: low quantum yields of photoisomerization resulting in high fuel cost, slow hybridization−dehybridization, and restricted *trans*-to-*cis* photoisomerization in the duplex. These difficulties cannot be overcome only by azobenzene modification. Optimization of the scaffold,[70] the use of DNA chaperones, and laser techniques (i.e., tunable femtosecond pulse laser) might be necessary. At present, alternating irradiation with UV and visible light is needed to switch between two states, duplex and single-stranded DNA. If coherent oscillation of the two states could be achieved by continuous light irradiation of a specific wavelength, we can say light energy is converted to mechanical energy in a real sense. Furthermore, a "real photon engine", such as a light-powered DNA motor that can rotate like a rotary engine or a kind of DNA linear motor that moves along a DNA rail, has yet to be developed. The movie "Fantastic Voyage" released in 1966 was only science fiction. In the future, however, we believe that sophisticated light-driven nanomachines will partly realize this fiction by assembling pieces of functional elements together.

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Notes

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Hiroyuki Asanuma received his Ph.D. (1989) from The University of Tokyo. In 1989, he joined Fuji Photo Film Co. Ltd. as a researcher. He moved to The University of Tokyo in 1995 as Assistant Professor. He was promoted to Associate Professor in 2000. In 2005, he moved to Nagoya University as a full Professor. His research interests include (1) photoregulation of DNA and RNA functions, (2) design of new XNAs, (3) new fluorescent probes for practical applications, (4) design of nucleic acid-based drugs, and (5) assembled functional molecules.

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