Molecular Devices

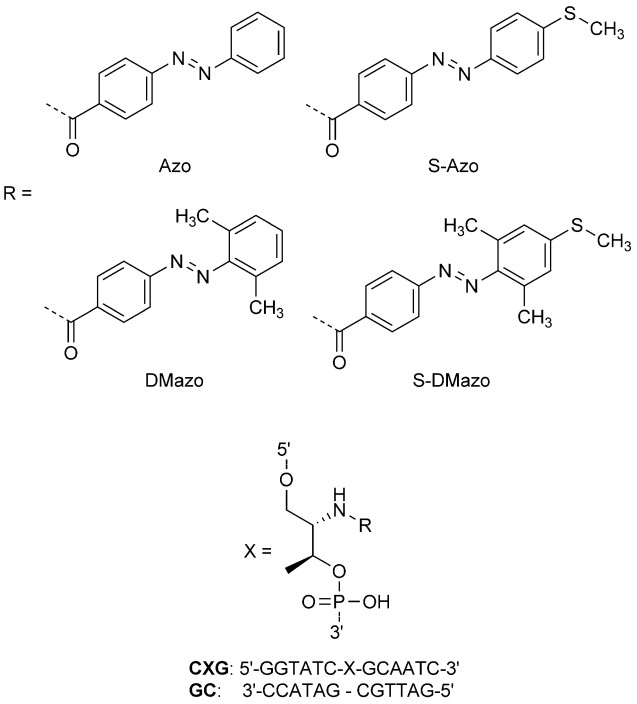
DOI: 10.1002/anie.201106093

A Photon-Fueled DNA Nanodevice that Contains Two Different Photoswitches**

Hidenori Nishioka, Xingguo Liang, Tomohiro Kato, and Hiroyuki Asanuma**

DNA is a promising, self-assembling nanomaterial for the construction of delicate nanostructures and nanodevices.[1–5] However, when short DNA oligonucleotides are used as the fuel to drive a nanodevice, the waste gradually accumulates and lowers the operating efficiency.[3,4] Accordingly, efforts have been made to construct new nanodevices that use "clean" fuels. For example, Liu and co-workers utilized electric signals to reversibly drive a DNA switch.[6] We constructed photon-fueled nanomachines that did not deteriorate after many working cycles.[7]

A photon-fueled DNA nanodevice has been constructed based on the reversible photoregulation of DNA hybridization in azobenzene-modified DNA. The planar *trans*-azobenzene intercalates between adjacent base pairs and stabilizes the duplex through stacking interactions, whereas the nonplanar *cis*-azobenzene destabilizes the duplex by steric hindrance.[8,9] To date, photoswitches have essentially contained a single species, such as azobenzene-4′-carboxylic acid (Azo) or 2,6-dimethylazobenzene-4′-carboxylic acid (DMazo; Scheme 1). Ultraviolet (310–370 nm) or visible light (λ greater than 400 nm) induces reversible isomerization between the *trans* and *cis* forms of such compounds. We sought to develop a photoswitch that photoisomerizes upon irradiation at other wavelengths to diversify the photoswitches and to make them applicable for various purposes where UV light should be avoided. In this study, we synthesized an azobenzene derivative that photoisomerizes reversibly upon irradiation with visible light. When tethered onto D-threoninol and linked to DNA, 2,6-dimethyl-4-(methylthio)azobenzene-4′-carboxylic acid (S-DMazo, Scheme 1) had a sufficiently high *cis* content, acceptable thermal stability, and a high photoregulatory efficiency. By combining Azo and S-DMazo, a photon-fueled DNA nanomachine that moved with a seesaw-like motion upon irradiation with light of different wavelengths was produced.



Scheme 1. Structures of azobenzene derivatives introduced into DNA. The sequence of a modified DNA (**CXG**) and its complementary sequence (**GC**) used in the model system of photoregulation of DNA hybridization are also shown.

The *trans* isomers of azobenzene and its alkyl derivatives have absorption maxima (λ_{max}) at around 320 nm (π–π^* transition), and the *cis*-rich form can be obtained by irradiation with light of wavelengths between 310–370 nm.[10] An azobenzene derivative with a large bathochromic shift to the visible region can be obtained by the introduction of an electron-donating group at the *para* position. However, the overlap of π–π^* and n–π^* transitions (at ca. 450 nm) makes it difficult to obtain the *cis*-rich form, and the stronger conjugation as well as the electronic effects caused by these substituents decrease the thermal stability of the *cis* form.[11] To avoid large overlap of the π–π^* and n–π^* transitions in the *trans* form and to ensure a high thermal stability in the *cis* form, the ideal azobenzene derivative would have a λ_{max} at around 400 nm. We designed S-DMazo to satisfy these requirements. In S-DMazo, the methylthio group at the *para* position of the distal benzene ring causes a bathochromic shift in the λ_{max} value of the *trans* form to 400 nm.[12] The two methyl groups at the *ortho* positions of the distal ring enhance the photoregulatory efficiency and the

[*] Dr. H. Nishioka, Prof. Dr. X. Liang, T. Kato, Prof. Dr. H. Asanuma
Department of Molecular Design and Engineering
Graduate School of Engineering, Nagoya University
Furo-cho, Chikusa-ku, Nagoya 464-8603 (Japan)
E-mail: liang@mol.nagoya-u.ac.jp
asanuma@mol.nagoya-u.ac.jp
Prof. Dr. X. Liang
School of Food Science and Engineering
Ocean University of China
Yushan-lu 5, Shinanqu, Qingdao 266003 (P.R. China)
E-mail: liangxg@ouc.edu.cn

[**] This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (Japan, nos. 20750132, 21241031, and 18001001).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201106093.

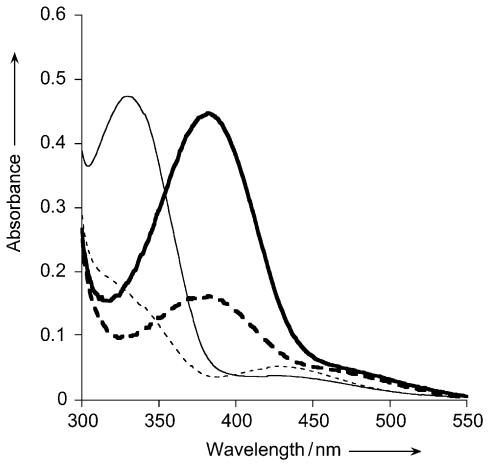


Figure 1. Absorption spectra of S-DMazo (thick lines) and Azo (thin lines) tethered onto **CXG** in either the *trans* form (solid lines) or in the *cis*-rich state (dotted line). Conditions: **CXG** (20 μM), NaCl (100 mM), phosphate buffer (10 mM, pH 7.0), 60°C. Wavelengths of light for isomerization of S-DMazo and Azo to their *cis* forms are 400 nm and 340 nm, respectively. The λ_{max} values for *trans*-S-DMazo and *trans*-Azo are 382 nm and 330 nm, respectively.

thermal stability of the *cis* form.[13] We introduced S-DMazo with a D-threoninol linker into the DNA sequence **CXG** (Scheme 1) by using standard phosphoramidite chemistry (Schemes S1 and S2 in the Supporting Information).[8b] For comparison, Azo, DMazo, and 4-(methylthio)azobenzene-4′-carboxylic acid (S-Azo) were also introduced into **CXG**.

Figure 1 shows the UV/Vis spectra (recorded at 60°C) of Azo- and S-DMazo-modified **CXG** in the absence of its complementary sequence **GC** (Scheme 1). The λ_{max} of *trans*-S-DMazo was 382 nm, which is 52 nm higher than that of *trans*-Azo (λ_{max}, 330 nm). At 25°C, the λ_{max} shifted to 391 nm, and shifted further to 397 nm when **CXG** was hybridized to **GC** at 25°C (Figures S1 and S2 in the Supporting Information). Upon irradiation at 400 nm, 66% of *trans*-S-DMazo isomerized to the *cis* form (Figure 1). Upon irradiation of *trans*-S-DMazo at 370 nm, 390 nm, 450 nm, or 520 nm, the *cis* content was 66%, 66%, 17%, and 25%, respectively (Table S1 in the Supporting Information). Interestingly, *trans*-rich S-DMazo (58% *trans* form) was obtained after irradiation at 340 nm.

We evaluated the photoregulatory efficiency of S-DMazo by monitoring the change in the melting temperature (ΔT_m) of modified DNA duplexes after the *trans*–*cis* isomerization. As shown in Table 1, the difference in the melting temperature between the **CXG/GC** duplexes that contain the *cis* and *trans* forms of S-DMazo is

Table 1: T_m values for duplexes **CXG**/**GC** that contain various azobenzene derivatives and the thermal stability of their *cis* forms.[a]

Azobenzene derivative (R)	T_m [°C][b] *trans*	*cis*	ΔT_m[d]	$\tau_{1/2}$ of *cis* [h][c]
S-DMazo	48.3	34.9	13.4	6.4
Azo[e]	48.9	43.2	5.7	3.3
S-Azo	46.1	45.2	0.9	0.42
DMazo[e]	50.9	36.3	14.6	25

[a] Solution conditions: DNA (5 μM), NaCl (100 mM), phosphate buffer (10 mM, pH 7.0). [b] T_m value of the unmodified DNA duplex is 47.7°C. [c] Half-lives of *cis*-azobenzene derivatives for the thermal isomerization at 60°C. [d] Change of T_m value induced by *cis*–*trans* isomerization. [e] For comparison, data for Azo and DMazo duplexes are also listed.

13.4°C (see Figure S3 in the Supporting Information for the actual melting curves). This difference is twofold larger than that of the corresponding Azo-modified duplexes. For this experiment, the *trans*-to-*cis* and *cis*-to-*trans* photoisomerizations were carried out at 400 nm and 450 nm, respectively; thus, the photoregulation of DNA hybridization with only visible light was achieved. The two methyl groups at the *ortho* positions of the distal ring were essential as the duplex that was modified with S-Azo had a ΔT_m value of only 0.9°C. In contrast, DMazo, without the methylthio group, had a ΔT_m of 14.6°C.[13b] The steric bulk of the methylthio group in the *para*

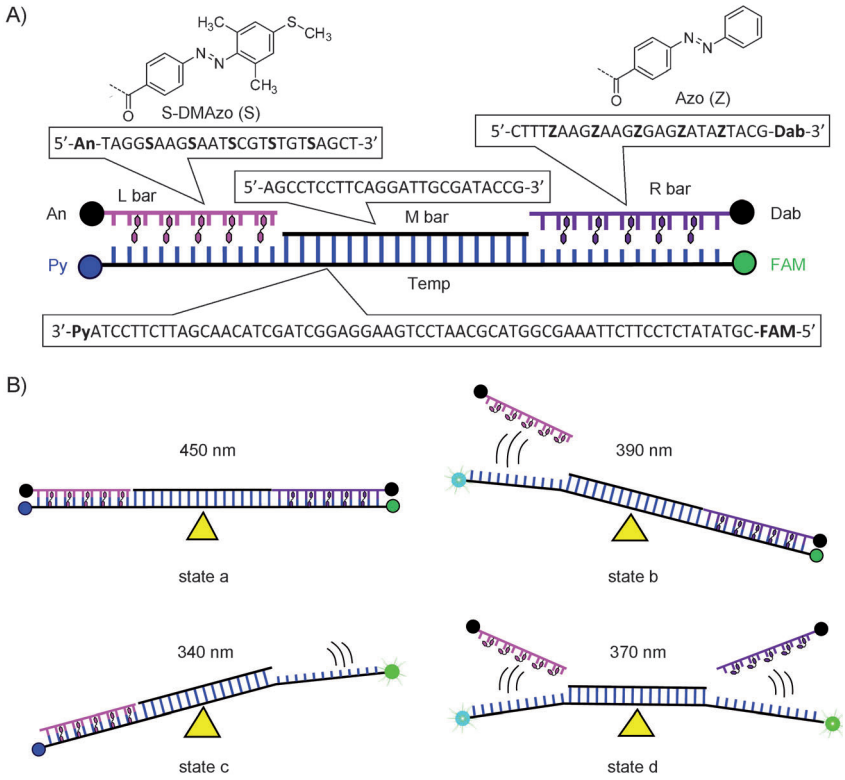


Figure 2. a) Illustration of the design of a DNA nanodevice that contains S-DMazo- and Azo-modified DNAs. b) Four possible states (a, b, c, and d) of the nanodevice are achieved by irradiation at four wavelengths (340 nm, 370 nm, 390 nm, and 450 nm, respectively). The 5′ and 3′ termini of the long oligonucleotide (**Temp**) are modified with fluorophores FAM and Py, respectively. The oligonucleotide **M bar** hybridizes with the central region of **Temp**. Dab is attached at the 3′ end of the Azo-modified oligonucleotide **R bar**, and An is attached at the 5′ end of the S-DMazo-modified oligonucleotide **L bar**.

position in the S-Azo-modified duplexes hindered the hybridization of **CXG** with the complementary strand. The duplex with *trans*-S-DMazo had a T_m of 48.3 °C, which is comparable to the native DNA duplex with the same sequence (T_m = 47.7 °C, Table 1) and is important for constructing stable nanostructures.

We examined the thermal stability of *cis*-S-DMazo. The half-life of *cis*-S-Azo was approximately 25 min at 60 °C, which is much shorter than the half-life of *cis*-Azo (ca. 200 min), because the electron-donating methylthio group greatly reduces the thermal stability of the *cis* form (Table 1). Although *trans*-S-Azo could also be isomerized to the *cis* form with 400 nm light (Figure S4 in the Supporting Information), neither the thermal stability nor the photoregulatory efficiency was acceptable for actual applications. Interestingly, the half-life of *cis*-S-DMazo was twofold longer than that of *cis*-Azo (Table 1), which shows that the presence of two methyl groups greatly improves the thermal stability of *cis*-S-DMazo.[13b]

By combining S-DMazo and Azo, a light-driven DNA nanodevice that moves like a seesaw was designed. As shown in Figure 2 A, the DNA nanodevice consists of four oligonucleotides: a 20 nucleotide (nt) oligonucleotide that contains five S-DMazo residues (**L bar**); a 25 nt unmodified DNA (**M bar**); a 20 nt oligonucleotide modified with five Azo residues (**R bar**); and **Temp**, a 65 nt DNA. The working principle of the nanodevice is illustrated in Figure 2 B. After irradiation at 450 nm, both *cis*-S-DMazo and *cis*-Azo isomerize to the *trans* forms so that both the **L bar/Temp** and the **R bar/Temp** duplexes are formed (state a). Upon irradiation of the device at 390 nm (or 400 nm), *trans*-S-DMazo isomerizes to the *cis* form and Azo remains in the *trans* form so that the **L bar/Temp** duplex dissociates and the **R bar/Temp** duplex remains stable (state b). After irradiation at 340 nm, *cis*-S-DMazo isomerizes to the *trans* form and *trans*-Azo isomerizes to the *cis* form so that the **L bar/Temp** duplex reforms and the **R bar/Temp** duplex dissociates (state c). Upon irradiation at 370 nm, *trans*-Azo and *trans*-S-DMazo both isomerize to the *cis* forms and both **L bar/Temp** and **R bar/Temp** duplexes dissociate (state d). The native duplex in the middle (**M bar/Temp**) is a rigid spacer that is a stable duplex in all states. Manipulation of this DNA nanodevice is carried out simply by irradiating with the appropriate wavelengths of light.

Two fluorophore/quencher systems, pyrene/anthraquinone (Py/An)[14] and (6-fluorescein-6-carboxamido)hexanoate/4-dimethylaminoazobenzene-4′-carboxylic acid (FAM/Dab, Figure 2), were used to quantitatively analyze the hybridization of the duplexes. The two fluorophores (FAM and Py) were attached to each end of the **Temp** sequence (Figure 2). The quenchers An and Dab were attached at the 5′ end of **L bar** and at the 3′ end of **R bar**, respectively. The formation of the duplexes that consist of **L bar/Temp** and **R bar/Temp** should quench emissions from FAM (emission at 500–560 nm) and Py (emission at 370–450 nm), respectively. As shown in Figure 3, the fluorescence intensity of FAM decreased after irradiation at 390 nm or 450 nm, which indicated that the **R bar/Temp** duplex was formed. The fluorescence of FAM increased after irradiation at 370 nm or 340 nm, which indicated that the **R bar/Temp** duplex was dissociated. The **L bar/Temp** duplex formed after irradiation at 340 nm or 450 nm, whereas irradiation at 370 nm or 390 nm caused the **L bar/Temp** duplex to dissociate. The fluorescence changes are consistent with the results of the photoisomerization experiments (Figure 1, see also Tables S1 and S2 in the Supporting Information) and T_m measurements (Table 1, see also Table S3 in the Supporting Information). In these experiments, all irradiations with light were carried out at 47.5 °C, the same temperature that was used during the measurements of the fluorescence.[15] These results indicate that all four states of the DNA nanodevice shown in Figure 2 B were obtained.

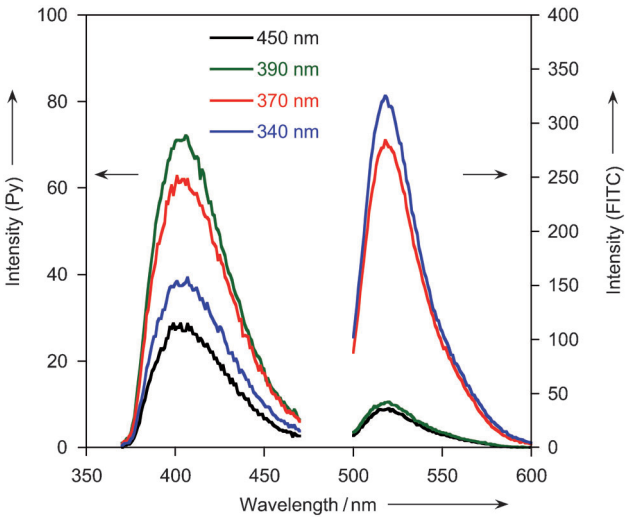


Figure 3. Quantitative analysis of the manipulation of the DNA nanodevice by monitoring the fluorescence of Py (370–450 nm) and FAM (500–560 nm) after irradiation with the indicated wavelengths of light. Conditions: DNA (0.1 μM), NaCl (100 mM), phosphate buffer (10 mM, pH 8.0), 47.5 °C. Before fluorescence measurements, samples were irradiated for 15 min at 47.5 °C.

The photoregulatory efficiency of S-DMazo was also quantitatively evaluated by analyzing the changes in the fluorescence (Figure 3). For example, after irradiation at 340 nm, 60 % of the **L bar** strands were in the duplex form. After irradiation at 390 nm, 79 % of the **L bar** strands were dissociated and 94 % of the **R bar** strands were in the duplex form. Furthermore, repetitive switching between state b and state c was achieved by alternately irradiating the system at 340 nm and 390 nm. As shown in Figure 4, irradiation at 340 nm caused the **R bar/Temp** to dissociate and the **L bar/Temp** duplex to form, whereas irradiation at 390 nm induced the dissociation of the **L bar/Temp** duplex and formation of the **R bar/Temp** duplex. Thus, seesaw-like movement was successfully achieved by irradiation with light (see Figure S5 in the Supporting Information for polyacrylamide gel electrophoresis (PAGE) data).

In conclusion, the hybridization of DNA can be efficiently photoregulated by visible light with the introduction S-DMazo into an oligonucleotide. *Trans*-S-DMazo isomerizes efficiently to the *cis* form upon irradiation at 400 nm, and the thermal stability of *cis*-S-DMazo was higher than that of *cis*-Azo as a result of two methyl groups at the *ortho* positions of

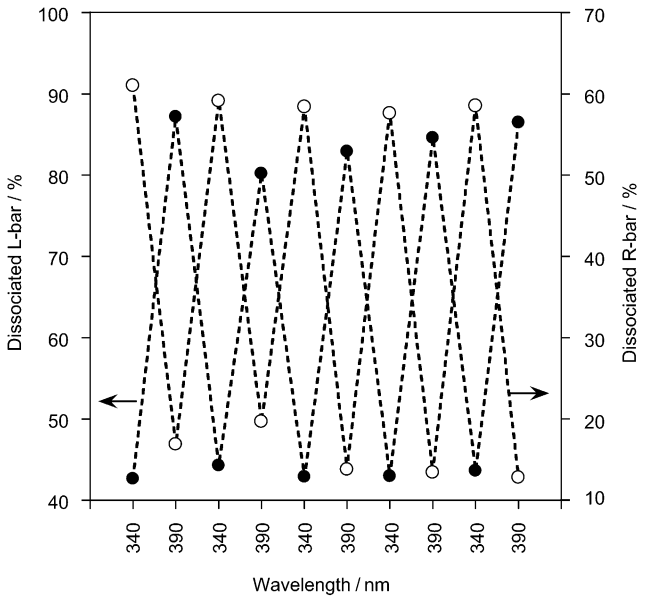


Figure 4. Repetitive seesaw-like movement of the DNA nanodevice between state b and state c induced by alternating light irradiation at 340 nm and 390 nm. Ratios of dissociated **L bar/Temp** (black circles) and **R bar/Temp** (white circles) are shown. Conditions: DNA (0.1 μM), NaCl (100 mM), phosphate buffer (10 mM, pH 8.0), 47.5 °C. Each irradiation was 10 min long.

the distal ring. By combining Azo and S-DMazo, a DNA nanodevice with seesaw-like movement that is driven by light was constructed. These results demonstrate that DNA that is modified with azobenzene derivatives is an excellent nanomaterial with which to build elaborate nanodevices. Because UV light shorter than 380 nm is harmful to some biomolecules and should be avoided for biological applications, the S-DMazo-modified DNA derivatives are promising candidates for the photoregulation of bioreactions in vivo.

Received: August 28, 2011
Revised: October 31, 2011
Published online: December 15, 2011

Keywords: azo compounds · DNA structures · isomerization · molecular devices · photoregulation

[1] a) N. C. Seeman, P. S. Lukeman, *Rep. Prog. Phys.* **2005**, *68*, 237 – 270; b) M. K. Beissenhirtz, I. Willner, *Org. Biomol. Chem.* **2006**, *4*, 3392 – 3401; c) A. Condon, *Nat. Rev. Genet.* **2006**, *7*, 565 – 575; d) T. J. Bandy, A. Brewer, J. R. Burns, G. Marth, T. Nguyen, E. Stulz, *Chem. Soc. Rev.* **2011**, *40*, 138 – 148.

[2] a) C. A. Mirkin, R. L. Letsinger, R. C. Mucic, J. J. Storhoff, *Nature* **1996**, *382*, 607 – 609; b) E. Winfree, F. Liu, L. A. Wenzler, N. C. Seeman, *Nature* **1998**, *394*, 539 – 544; c) W. M. Shih, J. D. Quispe, G. F. Joyce, *Nature* **2004**, *427*, 618 – 621; d) P. W. K. Rothemund, *Nature* **2006**, *440*, 297 – 302.

[3] a) B. Yurke, A. J. Turberfield, A. P. Mills, F. C. Simmel, J. L. Neumann, *Nature* **2000**, *406*, 605 – 608; b) T. Liedl, T. L. Sobey, F. C. Simmel, *Nano Today* **2007**, *2*, 36 – 41.

[4] a) Y. He, M. Su, C. Zhang, A. E. Ribbe, W. Jiang, C. Mao, *Nature* **2008**, *452*, 198 – 202; b) S. Modi, M. G. Swetha, D. Goswami, G. D. Gupta, S. Mayor, Y. Krishnan, *Nat. Nanotechnol.* **2009**, *4*, 325 – 330.

[5] M. Endo, H. Sugiyama, *ChemBioChem* **2009**, *10*, 2420 – 2443.

[6] Y. Yang, G. Liu, H. J. Liu, D. Li, C. H. Fan, D. S. Liu, *Nano Lett.* **2010**, *10*, 1393 – 1397.

[7] a) X. G. Liang, H. Nishioka, N. Takenaka, H. Asanuma, *ChemBioChem* **2008**, *9*, 702 – 705; b) M. G. Zhou, X. G. Liang, T. Mochizuki, H. Asanuma, *Angew. Chem.* **2010**, *122*, 2213 – 2216; *Angew. Chem. Int. Ed.* **2010**, *49*, 2167 – 2170.

[8] a) H. Asanuma, T. Ito, T. Yoshida, X. G. Liang, M. Komiyama, *Angew. Chem.* **1999**, *111*, 2547 – 2549; *Angew. Chem. Int. Ed.* **1999**, *38*, 2393 – 2395; b) H. Asanuma, X. G. Liang, H. Nishioka, D. Matsunaga, M. Liu, M. Komiyama, *Nat. Protoc.* **2007**, *2*, 203 – 212.

[9] a) S. Uno, C. Dohno, H. Bittermann, V. L. Malinovskii, R. Häner, K. Nakatani, *Angew. Chem.* **2009**, *121*, 7498 – 7501; *Angew. Chem. Int. Ed.* **2009**, *48*, 7362 – 7365; b) X. G. Liang, T. Mochizuki, H. Asanuma, *Small* **2009**, *5*, 1761 – 1768; c) X. G. Liang, H. Nishioka, T. Mochizuki, H. Asanuma, *J. Mater. Chem.* **2010**, *20*, 575 – 581; d) F. Tanaka, T. Mochizuki, X. G. Liang, H. Asanuma, S. Tanaka, K. Suzuki, S. Kitamura, A. Nishikawa, K. Ui-Tei, M. Hagiya, *Nano Lett.* **2010**, *10*, 3560.

[10] G. Zimmerman, L. Y. Chow, U. J. Paik, *J. Chem. Phys.* **1958**, *80*, 3528 – 3531.

[11] a) N. Nishimura, T. Sueyoshi, H. Yamanaka, E. Imai, S. Yamamoto, S. Hasegawa, *Bull. Chem. Soc. Jpn.* **1976**, *49*, 1381 – 1387; b) T. Asano, T. Okada, *J. Org. Chem.* **1986**, *51*, 4454 – 4458.

[12] a) T. Fujii, H. Kashida, H. Asanuma, *Chem. Eur. J.* **2009**, *15*, 10092 – 10102; b) P. C. Chen, Y. C. Chieh, J. C. Wu, *J. Mol. Struct.: Theochem* **2005**, *715*, 183 – 189.

[13] a) H. Nishioka, X. G. Liang, H. Kashida, H. Asanuma, *Chem. Commun.* **2007**, 4354 – 4356; b) H. Nishioka, X. G. Liang, H. Asanuma, *Chem. Eur. J.* **2010**, *16*, 2054 – 2062.

[14] H. Asanuma, H. Hayashi, J. Zhao, X. G. Liang, A. Yamazawa, T. Kuramochi, D. Matsunaga, Y. Aiba, H. Kashida, M. Komiyama, *Chem. Commun.* **2006**, 5062 – 5064.

[15] Azobenzene was photoisomerized from *trans* to *cis* in the duplex state, although the ratio of the *cis* form obtained was lower relative to the isomerization in the single-stranded state. See Refs. [8b] and [9b].

