

Conformational Switching of G-Quadruplex DNA by Photoregulation**

Xiaolin Wang, Jing Huang, Yangyang Zhou, Shengyong Yan, Xiaocheng Weng, Xiaojun Wu, Minggang Deng, and Xiang Zhou*

Native, self-assembling nucleic acid nanomachines that can walk, move, or rotate have been developed.^[1] Owing to their ability to form diverse secondary structures, for example, by the highly sequence-specific hybridization of complementary sequences, the hybridization of DNA and RNA through Watson–Crick H bonds, and the assembly of triplexes through Hoogsteen bonds, nucleic acids are ideal building blocks for the construction of nanodevices. Quadruplex architecture is a nucleic acid secondary structure that plays an important role in nanomachine research, particularly in the control of reversible folding and extension of the G quadruplex of DNA in the presence of external stimuli.^[2] Mergny and co-workers reported that a copper(II)-mediated structural switch with a flexible ligand could regulate the conformation of the G quadruplex.^[3] Nanodevices based on a quadruplex-to-duplex-transition that rely on the use of single-stranded DNA as fuels have been shown to perform rotary movements.^[4] Among external stimuli, such as temperature,^[5] pH value,^[6] electrical-field strength,^[7] and molecular recognition,^[8] photoregulation is particularly advantageous for controlling movement and conformation. For example, photoregulation does not require any additional components and does not cause undesirable side reactions. Irradiation is an accurate and simple method, and the timing, location, and strength of light can be controlled readily. Moreover, photoregulation provides a clean source of energy and can be repeated many times without loss of efficiency.^[9–12]

The introduction of a photochromic group into biomolecules, such as peptides, oligonucleotides, sugar scaffolds, and phospholipids, can cause conformational changes that alter the photochemical properties of the biomolecule. Accordingly, various biological processes involving modified biomolecules can be regulated in a straightforward manner by irradiation.^[13] Recently, Ogasawara and Maeda demonstrated the successful photoregulation of G-quadruplex formation through isomerization of a photochromic nucleobase, ^{8FV}G, incorporated in aptamers.^[14] Spada and co-workers introduced a photoactive moiety at the C8 position of a lipophilic guanosine derivative to regulate the existence of G quartets.^[15] However, all these photocontrollers are photochromic modified nucleobases. Specific molecules have not been shown to function as G-quadruplex photocontrollers; thus, we became interested in designing a photoswitch to regulate the formation of G-quadruplex DNA.

The azobenzene moiety is widely used as a photoresponsive molecular tool^[16] because it possesses excellent photochemical characteristics. Specifically, azobenzene isomerizes to predominantly *trans* and *cis* forms under visible (Vis) and ultraviolet (UV) light, respectively. In this study, we synthesized the azobenzene derivative **1** (Scheme 1) to control the movement and conformation of a G quadruplex by irradiation. Our results suggest that the formation and dissociation of G-quadruplex DNA was induced by interconversion of the *trans* and *cis* forms of compound **1**.

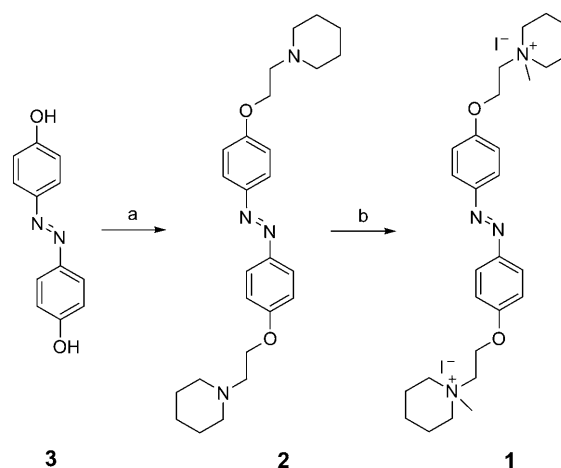
Compound **1** was synthesized by treating 4,4'-dihydroxyazobenzene with 1-(2-chloroethyl)piperidine hydrochloride

[*] X.-L. Wang,^[†] J. Huang,^[†] Y.-Y. Zhou, S.-Y. Yan, X.-C. Weng, X.-J. Wu, M.-G. Deng, Prof. X. Zhou
College of Chemistry and Molecular Sciences
Key Laboratory of Biomedical Polymers, Ministry of Education
Wuhan University, Hubei, Wuhan 430072 (P.R. China)
Fax: (+86) 27-8733-6380
E-mail: xzhou@whu.edu.cn
Prof. X. Zhou
State Key Laboratory of Natural and Biomimetic Drugs
Peking University and
State Key Laboratory of Applied Organic Chemistry
Lanzhou University (P.R. China)

[†] These authors contributed equally.

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Scheme 1. Synthesis of compound **1**: a) 1-(2-chloroethyl)piperidine hydrochloride, dry acetone, K₂CO₃, argon, reflux, 59%; b) CH₃, CHCl₃, 45 °C, 49%.

to give **2**, which was subsequently transformed into the final product by methylation (Scheme 1). Compounds **1** and **2** were fully characterized by NMR spectroscopy and HRMS methods (see the Supporting Information).

Initially, the isomerization of compound **1** was investigated by photoillumination. The azobenzene moiety is known to undergo *trans*-to-*cis* isomerization under UV/Vis light. UV/Vis and ^1H NMR spectroscopy were used to characterize the isomerization of compound **1**.

The absorption spectra of the *trans* and *cis* azobenzene were confirmed to be distinct.^[17,18] As shown in Figure 1, photoisomerization depends on the duration of UV/Vis

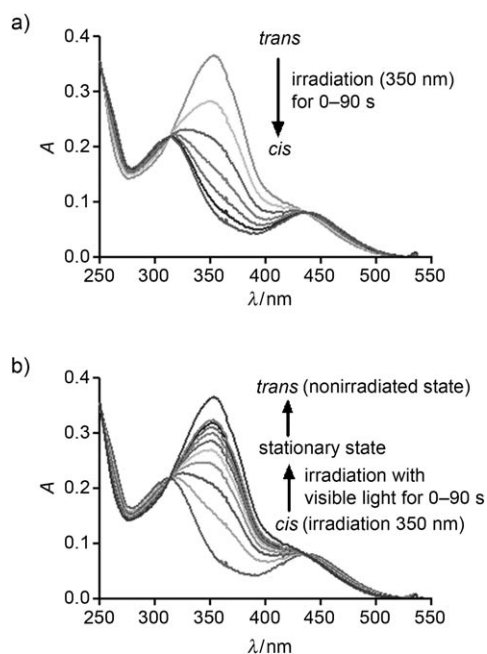


Figure 1. UV/Vis spectra of compound **1** (25 μM) in 10 mM Tris/HCl buffer with EDTA (1 mM) at pH 7.4. a) UV/Vis absorbance of compound **1** under UV irradiation at 350 nm at various reaction times (0–90 s). b) UV/Vis absorbance of compound **1** prior to irradiation (top line) and under visible light at various reaction times (0–90 s) following UV irradiation at 350 nm. EDTA = ethylenediaminetetraacetic acid, Tris = 2-amino-2-hydroxymethylpropane-1,3-diol.

irradiation. Upon the irradiation of a dilute solution of **1** (25 μM in 10 mM Tris/HCl buffer with EDTA (1 mM), pH 7.4) at 350 nm for approximately 60 s, compound **1** underwent isomerization to the *cis* form. However, irradiation with visible light, did not completely reisomerize the *cis* to the *trans* form, even when irradiation was continued for more than 30 minutes. The reaction system attained a photostationary state after irradiation with visible light for about 60 seconds. These results are similar to those of previous studies^[9,17,19] and suggested that repeated photoconversion between the *cis* form and the photostationary state could be induced by irradiating **1** alternately with visible and UV light (350 nm), each time for 1 minute.

Direct evidence for the *cis* and *trans* ligand in the photostationary state of compound **1** was provided by ^1H NMR spectroscopy. In previous studies,^[20] ^1H NMR spec-

tra clearly indicated that the chemical shifts of the aromatic hydrogen atoms in *trans* and *cis* azobenzenes are distinct. Before and after illumination at 350 nm, we recorded the ^1H NMR spectra of compound **1** in $\text{H}_2\text{O}/\text{D}_2\text{O}$ (1:9; see the Supporting Information). Irradiation of **1** (*trans* isomeric form) for 30 minutes completely shifted the signals for 3-H, 3'-H, 5-H, 5'-H (azobenzene *meta* hydrogen atoms) and 2-H, 2'-H, 6-H, 6'-H (azobenzene *ortho* hydrogen atoms) from $\delta = 7.0$ and 7.7 ppm to $\delta = 6.8$ and 6.9 ppm, respectively. The resulting chemical shifts correspond to the *cis* isomeric state of compound **1**. However, when compound **1** was irradiated with visible light following UV irradiation, the ^1H NMR spectra of **1** were not restored to their original state (as observed prior to irradiation), in agreement with the results of UV/Vis spectroscopy. According to the ^1H NMR spectra, a mixture of *trans* and *cis* forms was present following irradiation with visible light, and the efficiency of photoconversion was approximately 84.6%.

Next, the folding and stretching motions of the G quadruplex of the proposed photocontroller were evaluated. Circular dichroism (CD) is a reliable method for determining the conversion of the quadruplex into the unfolded conformation. Unfolded telomere DNA ($\text{d}(\text{TTAGGG})_4$) appears as a positive CD peak at 257 nm. In the presence of the *trans* form of compound **1**, the CD spectrum of $\text{d}(\text{TTAGGG})_4$ indicated a parallel G-quadruplex structure, which is characterized by a positive peak at 265 nm and a negative peak at 240 nm (Figure 2a). Upon irradiation with UV light at 350 nm for 1 minute, formation of the *cis* form led to the disappearance of the signal at 265 nm. The open and closed forms of the $\text{d}(\text{TTAGGG})_4$ DNA were interconverted by UV/Vis photo-regulation (Figure 2b). Figure 2c shows the spectra of $\text{d}(\text{TTAGGG})_4$ throughout 10 continuous cycles of CD during UV/Vis photoillumination. No changes in absorptivity were observed for the signal at 265 nm, which suggested that the exchange process was totally reversible, even after 30 cycles. Thus, the conformation of the G quadruplex alternated between the folded and unfolded forms. These results revealed that the proposed nanodevice converted light directly into mechanical work.

The results indicated that the folding and stretching motion of G-quadruplex devices could be induced by UV/Vis photoillumination (Figure 3). The folding–stretching motion of telomere DNA was initiated by formation of the G quadruplex in the presence of compound **1**. Next, the folded conformation of the G quadruplex was dissociated by irradiation of the solution with UV light at 350 nm for 1 minute. The stretched oligomer was folded into the G quadruplex by irradiation with visible light for 1 minute. By irradiating the solution alternately with UV and visible light, each time for 1 min, the folding and stretching of the DNA could be repeated (Figure 2d).

The thermal stability of the telomere DNA ($\text{d}(\text{TTAGGG})_4$) in the presence of the *trans* and *cis* forms of **1** was determined by monitoring the melting point of the oligomer. The results suggested that the thermal stability of DNA was dependent on the configuration of compound **1**: further evidence for conformational changes in the G quadruplex. In these experiments, the CD signal at 265 nm was

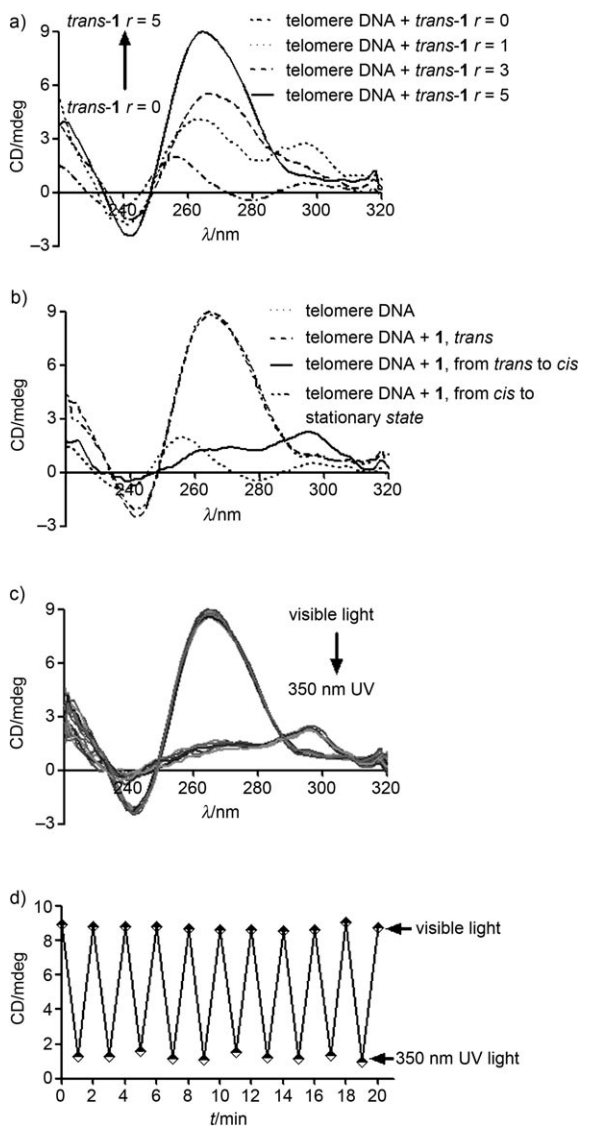


Figure 2. CD spectroscopic studies of telomere DNA in 10 mM Tris/HCl buffer with EDTA (1 mM) at pH 7.4. a) Titration of *trans*-1 ($r = [\text{compound } 1]/[\text{DNA strand}]$). b) Spectra of telomere DNA in the absence or presence of compound **1** ($r = 5$) without or with photoirradiation. The irradiation time with UV or visible light was 1 min. c) Reversible stretching and folding motion of the G quadruplex upon photoirradiation (10 cycles). d) Cycling of the photomediated structural conversion of telomere DNA by irradiation alternately with UV and visible light, each time for 1 min, as monitored by the CD intensity at 265 nm.

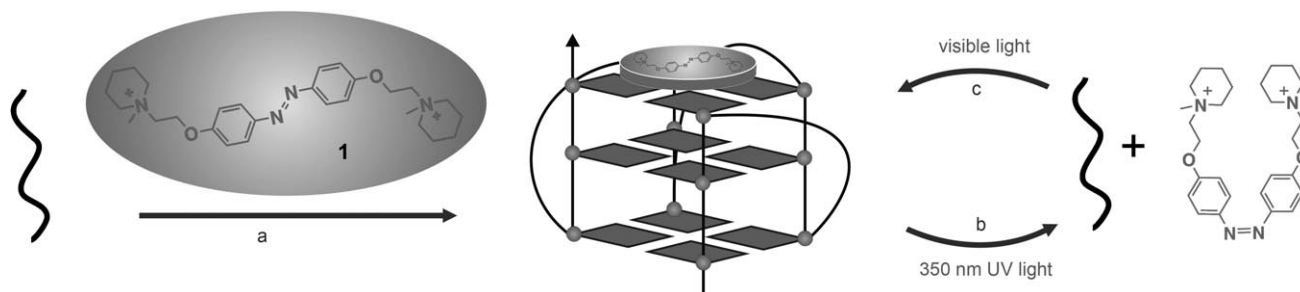


Figure 3. Reversible folding and stretching motion of the G quadruplex was induced by the photoresponsive compound **1**: a) the telomere DNA was induced to form a G quadruplex; b) the folded G quadruplex was disassociated by irradiation with UV light at 350 nm for 1 min (stretching); c) the stretched oligomer folded into the G quadruplex again upon illumination with visible light for 1 min (folding).

also monitored. In a solution of *trans*-**1**, $d(\text{TTAGGG})_4$ showed a large variation in absorbance over a wide range of temperatures ($T_m = 55.8^\circ\text{C}$). This behavior indicated the formation of a G-quadruplex secondary structure. In a solution of the *cis* form of **1**, the melting profile of $d(\text{TTAGGG})_4$ resembled that of control DNA and thus indicated that the G-quadruplex structure had completely dissociated (data shown in the Supporting Information).

We hypothesized that UV/Vis light could act as a switch for exonuclease I, which catalyzes hydrolysis reactions, on the basis of the conversion of a folded G quadruplex into an unfolded conformation by photoillumination. The exonuclease I hydrolysis assay described by Tan and co-workers was used to evaluate the stability of the quadruplex in the presence of compounds known to interact with DNA.^[21a] When the 3' end of T24G4 was folded into a G quadruplex in the presence of K^+ ions, exonuclease I could not degrade DNA (Figure 4). Moreover, in the presence of compound **1**

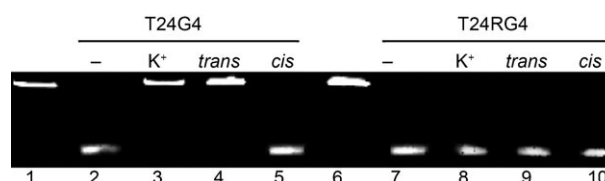


Figure 4. Quadruplex formation by T24G4 and resistance to hydrolysis by exonuclease I. Lane 1: T24G4; lane 2: negative control (without T24G4); lanes 3–5: T24G4 treated with K^+ (100 mM), *trans*-**1** (1.25 μM), and *cis*-**1** (1.25 μM), respectively; lane 6: T24RG4; lane 7: negative control (without T24RG4); lanes 8–10: T24RG4 treated with K^+ (100 mM), *trans*-**1** (1.25 μM), and *cis*-**1** (1.25 μM), respectively.

and visible light, the reaction was inhibited as a result of G-quadruplex formation. However, upon irradiation with UV light at 350 nm, the exonuclease was switched on, and hydrolysis occurred. The assay was also conducted with mutant T24RG4, which is not able to form a secondary structure. In this case, the digestion of DNA was not affected by irradiation. Overall, our results suggested that the folding and unfolding of the G quadruplex are regulated by photoillumination.

The binding affinity of the isomers for the quadruplex was determined through a UV/Vis-absorption titration experiment. The binding data determined by UV titration were

analyzed quantitatively by using the Scatchard equation^[22] (see the Supporting Information). The results showed that *trans*-**1** induced significant hypochromicity in the telomere-DNA titration system, and that the binding constant of *trans*-**1** and the telomere DNA was about $(3.9 \pm 0.5) \times 10^6 \text{ M}^{-1}$.

Finally, the *trans* and *cis* forms of compound **1** were optimized on the basis of the PM3 method of Gaussian03. The primitive molecular-modeling result was used for calculations with Accelrys Discovery Studio 2.1, which provided more information on the binding interactions between the isomers of **1** and telomere DNA (see Figure S4 in the Supporting Information). The *trans* isomer displayed a planar azobenzene core, which participated in π - π stacking with the guanine quartets (see Figure S4C in the Supporting Information). The positively charged side chains linked to the azobenzene core displayed a high degree of relaxation and approached the phosphate backbone. In the *cis* isomer, the benzene rings of the azobenzene core formed a v-shaped groove. This configuration reduced π - π stacking and the rotary flexibility of the side chain. Thus, the *cis* isomer and telomere DNA could not interact sufficiently to effect a conformational change.

In summary, the azobenzene derivative **1** was synthesized, and its photoisomerization properties were evaluated by UV/Vis and ¹H NMR spectroscopy. CD spectroscopy, thermal denaturation studies, a UV/Vis-absorption titration experiment, and an exonuclease I hydrolysis assay were performed to demonstrate that the formation and dissociation of G-quadruplex DNA could be photoregulated successfully by compound **1**. In agreement with our hypothesis, a *trans* azobenzene moiety induced the formation of G quadruplexes, whereas a *cis* azobenzene moiety caused dissociation of this conformation. Compound **1** is the first small molecule that has been used to induce reversible stretching and folding in a G quadruplex on the basis of photoirradiation. This nanodevice directly converts light into mechanical work and could be applied as a nanounit that moves or works by photoregulation. Thus, compound **1** may enable the development of new DNA-nanodevice applications.

Experimental Section

Exonuclease I and oligomers labeled with carboxytetramethylrhodamine (TAMRA) were purchased from TaKaRa Biotech (Dalian, China). Other oligomers used in this study were purchased from Invitrogen (China). ¹H and ¹³C NMR spectra were recorded on Varian Mercury 300 and 600 spectrometers, respectively. Chemical shifts were reported as δ values relative to the internal standard tetramethylsilane. CD spectra were recorded and CD melting experiments carried out on a Chirascan CD spectrometer (Applied Photophysics, UK) equipped with a Peltier temperature controller. UV spectra were recorded on a Shimadzu 2550 UV-Vis double-beam spectrophotometer.

For the sample-irradiation experiments with UV light at 350 nm, a 50 W high-pressure mercury lamp was used as the light source, and a filter was employed to extract light of wavelength 350 nm with a 15 nm peak width at half height. For the sample-irradiation experiments with visible light (> 400 nm), the sample was irradiated under an incandescent lamp (Philips) with an 11 W E27 cool day light bulb. UV or visible light was focused on the sample through an aperture at a distance of 3 cm.

¹H NMR spectroscopic experiments with the ligands for configuration analysis were performed on Varian Mercury 300 spectrometers and carried out at 20 °C in an H₂O/D₂O (1:9) mixture containing Tris/HCl (10 mM) and EDTA (1 mM; pH 7.4). A standard jump-return pulse sequence was used for water suppression with a relaxation delay of 2 s. The corresponding changes in the molecular configuration upon photoillumination were determined by ¹H NMR spectroscopy.

The samples for CD spectroscopy were recorded at 20 °C with a quartz cell with an optical path length of 1 mm. All CD spectra were baseline-corrected for signal contributions of the buffer. Neither the *trans* nor the *cis* form of compound **1** contributed to the CD signal between 220 and 320 nm under our experimental conditions. For the melting-temperature experiment, the signal at 265 nm was monitored between 20 and 90 °C.

Previously reported conditions were used for the exonuclease I hydrolysis experiments.^[21] A mixture of T24G4 (5'-TAMRA-T₂₄(G₅T₂A)₃G₃-3'; 0.25 μM) or T24RG4 (5'-TAMRA-T₂₄GTGTGAGTGGAGGTGTGAGGT-3'; 0.25 μM) and compound **1** was heated to 94 °C and then cooled down. The mixture of DNA and compound **1** was irradiated with UV light at 350 nm to form the *cis* configuration. After irradiation, all procedures were performed in the dark.

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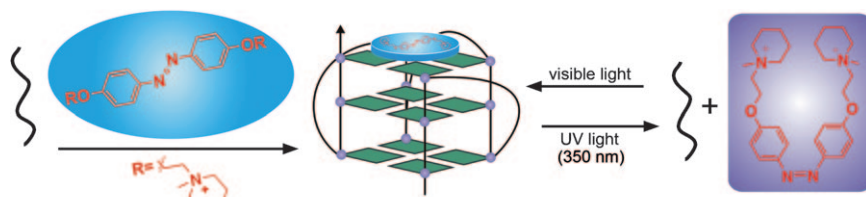
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Communications

DNA Structures

X.-L. Wang, J. Huang, Y.-Y. Zhou, S.-Y. Yan,
X.-C. Weng, X.-J. Wu, M.-G. Deng,
X. Zhou* 

Conformational Switching of G-
Quadruplex DNA by Photoregulation



Bend and stretch... bend and stretch... An azobenzene derivative was used to induce reversible stretching and folding of G-quadruplex DNA upon photoirradiation (see picture). The G quadruplex formed in the presence of the *trans*

isomer was dissociated by irradiation with UV light, and the resulting open oligomer was refolded into a G quadruplex under visible light. This nanodevice thus converts light directly into mechanical work.