DNA Conformational Switch

DOI: 10.1002/anie.200604589

Light-Driven Conformational Switch of i-Motif DNA**

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With the advantages of conformational polymorphism and programmable sequence recognition, DNA has now become a powerful tool in nanostructure fabrication[1] and nanodevice design.[2–6] In most reported cases, DNA-based nanodevices are associated with a conformational switch driven by external fuels, such as DNA/RNA strands,[3] acids/bases,[4] enzymes,[5] or chemical oscillators,[7] in contact mode. These driving methods are still far different from the electrical/optical signal that silicon-based microelectronic devices can process and, as a result, limit the integration and further applications of the DNA devices.

Thus, the regulation of DNA conformation by a non-contact stimulator, like photo/electrical signals, has become an important challenge in DNA-nanodevice design. Only a few attempts have been reported, for example, the introduction of azobenzene substitution into DNA strands to serve as a UV-sensitive artificial base pair and influence the strand melting point[8] or remote electronic control over the hybridization behavior of DNA molecules by inductive coupling of a radio-frequency magnetic field to a metal nanocrystal covalently linked to DNA.[9] Recently, a strategy involving temporary masking of the Watson–Crick base pairing with a photolabile protecting group, called "caging", has been developed.[10] However, a photoinduced conformational switch of natural DNA has not been achieved. Herein, we present our approach for achieving a light-induced natural-DNA conformational switch between close-packed quadruplex and random-coil structures with a reversible photo-irradiated pH-jump system.[11]

The mechanism of our light-driven DNA conformational switch system is illustrated in Figure 1. It comprises three main components: 1) a 21mer DNA **X**: 5′-CCCTAACCC TAACCCTAACCC-3′; 2) a light-induced hydroxide ion emitter, molecular malachite green carbinol base (MGCB); 3) a surfactant, cetyltrimethylammonium bromide (CTAB), that is used to improve the solubility of MGCB.[11] It is well known that DNA strands with stretches of cytosine bases can form closely packed four-stranded structures called the "i motif" through protonated cytosine–cytosine ($C:C^+$) base-pair formation under slightly acidic conditions.[12] In our design, the initial solution containing DNA **X**, MGCB, and CTAB shows a slightly acidic pH value that facilitates the formation of the i-motif structure by DNA **X**. In the presence of 302-nm UV light, MGCB gives out OH^- ions, as well as showing an obvious color change, and this leads to an increase in the pH value.[11] Thus, the i-motif structure will deform into random coils with deprotonation of the $C:C^+$ base pairs. After the light is turned off, the malachite green (MG) cation will recombine with the OH^- ions and return to the MGCB form to complete the cycle. Consequently, the pH value decreases and the DNA **X** switches back to the i-motif conformation again. Accordingly, the conformational switch of DNA **X** could be cycled by turning the UV light on and off alternately.

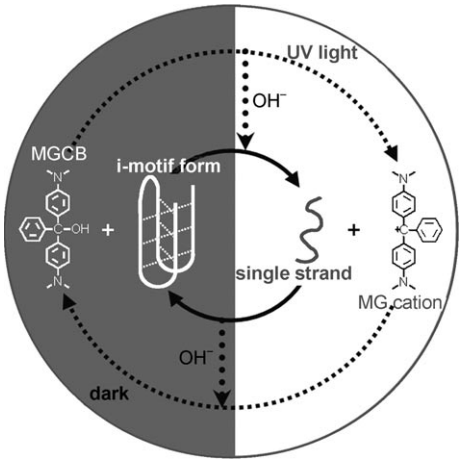

Figure 1. Working mechanism of the light-induced conformational switch of DNA **X**. Inner cycle: The DNA conformational switch between the i-motif and random-coil structures. Outer cycle: The light-induced malachite green based pH jump. The conformational switch of DNA **X** was associated with the on and off phases of UV light through the translation of photoinduced hydroxide-group release by MGCB.

To verify that the designed system does work, circular dichroism (CD) spectroscopy has been employed to study the conformational changes of DNA during the cycling. As shown in Figure 2, when the system is in its initial state where the pH value is around 6.0, DNA **X** shows a positive peak near 285 nm, a crossover at around 270 nm, and a negative peak near 260 nm, thereby indicating a typical i-motif conformation.[13] Control experiments showed that MGCB and CTAB have no obvious CD signal in the measured range whether

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[**] We thank Prof. Q. Fan, Prof. L. Jiang, and Prof. M. Irie for helpful discussions and Prof. H. Zhang for providing the micro-pH sensor. This work is partly funded by the Science 100 Program of CAS, the National Natural Science Foundation of China under grant nos. 20573027 and 50625312, and the MOST 973 project under grant nos. 2005CB724701 and 2006CB705601.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

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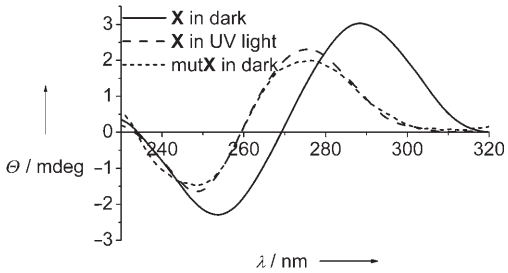

Figure 2. Measurements of DNA **X** conformations in the absence (solid line) and presence (dashed line) of UV light. CD spectra were recorded at 15 °C with 5 μM DNA in 50 mM CTAB, 200 mM NaCl, and 100 μM MGCB. Dotted line: Mutated DNA **X** in CTAB and MGCB solution in the absence of UV light acts as a control that indicates no i-motif structure forms.

under UV light or in the dark (data not shown), a fact that further verifies the formation of the i-motif structure by DNA **X**. By studying the temperature dependence at 285 nm, any influence of MGCB and CTAB on the stability of the i-motif structure formed by DNA **X** has been ruled out (see the Supporting Information). According to the thermal dynamic data achieved, 15 °C was chosen as the working temperature in this experiment to achieve a higher switch efficiency. After exposure to 302-nm UV light for 5 minutes, the pH value of the system could be raised to around 6.5 and an obvious change in the CD spectrum could be observed, which indicates the deformation of the i-motif structure at the higher pH value. During the cycling, the amplitude of pH change in the system gradually decreased (see the Supporting Information). In accordance with Porter's report,[14] we propose that this might be due to the complex photodegradation of MGCB in an aqueous environment.

Multiple cycling of DNA conformation has been achieved by turning the UV light on and off alternately. The CD signals were monitored at 285 nm to reflect the conformational change of DNA **X** (Figure 3). During each cycle, the time for the dissociation and formation of i motif is not the same: 5 minutes with the light on is enough to open the i-motif structure, but a much longer period of 55 minutes is necessary to complete the reverse process. The variation in time may be

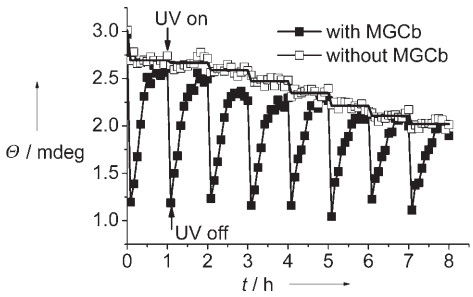

Figure 3. Monitoring of the cycling of the conformational switch of DNA **X** through measurement of the circular dichroism signals at 285 nm. The control experiment in the absence of MGCB shows slight photodegradation of the DNA after each cycle of UV irradiation. The average value of the CD signals at 285 nm for each cycle in the dark state (solid line) shows the stepwise manner of the DNA degradation.

ascribed to the difference in the dissociation and recovery rates of MGCB, which is in agreement with literature data.[11] From the eight cycles shown in Figure 3, we found that the amplitude of the switches decreases slowly with cycling. In the control experiment without MGCB, DNA **X** keeps the i-motif structure but the intensity of the CD signal at 285 nm slightly decreases along with the cycling (see the Supporting Information and Figure 3). The HPLC experiments (see the Supporting Information) further proved the gradual degradation of DNA **X** during cycling. Thus, we could attribute the decrease in the CD signal intensity to complex DNA damage by direct UV irradiation.[15]

In summary, we present herein the conformational switching of i-motif DNA by a coupled light-induced OH^- emitter system in noncontact mode. To the best of our knowledge, this is the first example of controlling the conformation of natural DNA with light. As the i-motif structure has been used as an important component in DNA-nanomachine design that has been demonstrated to facilitate microdevices[16] and drive microcantilever movement,[17] we can therefore expect that this noncontact driving method will promote the application of DNA nanodevices and their integration with microelectrical/optical devices.

Experimental Section

Oligonucleotide **X** with the sequence 5′-CCCTAACCCTAACCC TAACCC-3′ and mutated **X** with the sequence 5′-C*T*CTAACCC TAACCCTAACCC-3′ were purchased from SBS Genetech and purified by denaturing polyacrylamide gel electrophoresis. MGCB was bought from Sigma–Aldrich.

CD spectra were collected on a JASCO J-810 CD spectrometer. UV/Vis studies were performed on a Varian Cary 100 spectrometer. All CD and UV/Vis spectra were measured at 15 °C as maintained by the temperature-control units affiliated to the spectrometers. To minimize any influence on the sample solution by the spectrometer's UV source during the course of the CD or UV/Vis measurement, the scan rate used was 1000 nm min^{-1} and a shutter was added between the sample cell and the UV source for time-course studies. The pH value was recorded with a commercial pH meter (Sanxin, Shanghai). An 8-W UV lamp with single-wavelength output at 302 nm was used as the UV light source (Amersham, USA).

In a typical procedure, stock solutions of DNA **X** (200 μM), CTAB (200 mM), NaCl (4 M), and MGCB (100 mM in dimethylsulfoxide) were mixed and diluted with water to give final concentrations of 5 μM **X**, 50 mM CTAB, 200 mM NaCl, and 100 μM MGCB. The pH value of this solution was slightly acidic. The cycles of switching were accomplished by alternately irradiating this solution under UV light for 5 min and then leaving it in dark for 55 min. The solution temperature was kept at 15 °C with a cold-water bath.

Received: November 10, 2006
Revised: January 19, 2007
Published online: February 26, 2007

Keywords: conformational changes · DNA structures · nanotechnology · photochromism

[1] a) L. Feng, S. H. Park, J. H. Reif, H. Yan, *Angew. Chem.* **2003**, *115*, 4478–4482; *Angew. Chem. Int. Ed.* **2003**, *42*, 4342–4346; b) W. M. Shih, J. D. Quispe, G. F. Joyce, *Nature* **2004**, *427*, 618–621; c) D. Reishus, B. Shaw, Y. Brun, N. Chelyapov, L. Adleman,

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J. Am. Chem. Soc. **2005**, *127*, 17590 – 17591; d) S. H. Park, C. Pistol, S. J. Ahn, J. H. Reif, A. R. Lebeck, C. Dwyer, T. H. LaBean, *Angew. Chem.* **2006**, *118*, 749 – 753; *Angew. Chem. Int. Ed.* **2006**, *45*, 735 – 739.

[2] a) C. Mao, W. Sun, Z. Shen, N. C. Seeman, *Nature* **1999**, *397*, 144 – 146; b) R. P. Fahlman, M. Hsing, C. S. Sporer-Tuhten, D. Sen, *Nano Lett.* **2003**, *3*, 1073 – 1078.

[3] a) B. Yurke, A. J. Turberfield, A. P. Mills, Jr., F. C. Simmel, J. L. Neumann, *Nature* **2000**, *406*, 605 – 608; b) J. J. Li, W. Tan, *Nano Lett.* **2002**, *2*, 315 – 318; c) H. Yan, X. Zhang, Z. Shen, N. C. Seeman, *Nature* **2002**, *415*, 62 – 65; d) A. J. Turberfield, J. C. Mitchell, B. Yurke, A. P. Mills, M. I. Blakey, F. C. Simmel, *Phys. Rev. Lett.* **2003**, *90*, 118102; e) P. Alberti, J.-L. Mergny, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 1569 – 1573; f) M. K. Beissenhirtz, I. Willner, *Org. Biomol. Chem.* **2006**, *4*, 3392 – 3401.

[4] a) D. Liu, S. Balasubramanian, *Angew. Chem.* **2003**, *115*, 5912 – 5914; *Angew. Chem. Int. Ed.* **2003**, *42*, 5734 – 5736; b) Y. Chen, S. H. Lee, C. Mao, *Angew. Chem.* **2004**, *116*, 5449 – 5452; *Angew. Chem. Int. Ed.* **2004**, *43*, 5335 – 5338.

[5] Y. Chen, M. Wang, C. Mao, *Angew. Chem.* **2004**, *116*, 3638 – 3641; *Angew. Chem. Int. Ed.* **2004**, *43*, 3554 – 3557.

[6] a) P. Yin, H. Yan, X. G. Daniell, A. J. Turberfield, J. H. Reif, *Angew. Chem.* **2004**, *116*, 5014 – 5019; *Angew. Chem. Int. Ed.* **2004**, *43*, 4906 – 4911; b) Y. Tian, C. Mao, *J. Am. Chem. Soc.* **2004**, *126*, 11410 – 11411; c) J.-S. Shin, N. A. Pierce, *J. Am. Chem. Soc.* **2004**, *126*, 10834 – 10835; d) W. B. Sherman, N. C. Seeman, *Nano Lett.* **2004**, *4*, 1203 – 1207; e) J. Bath, S. J. Green, A. J. Turberfield, *Angew. Chem.* **2005**, *117*, 4432 – 4435; *Angew. Chem. Int. Ed.* **2005**, *44*, 4358 – 4361.

[7] T. Liedl, F. C. Simmel, *Nano Lett.* **2005**, *5*, 1894 – 1898.

[8] a) H. Asanuma, T. Ito, T. Yoshida, X. Liang, M. Komiyama, *Angew. Chem.* **1999**, *111*, 2547 – 2549; *Angew. Chem. Int. Ed.* **1999**, *38*, 2393 – 2395; b) H. Asanuma, X. Liang, T. Yoshida, A. Yamazawa, M. Komiyama, *Angew. Chem.* **2000**, *112*, 1372 – 1374; *Angew. Chem. Int. Ed.* **2000**, *39*, 1316 – 1318; c) H. Asanuma, K. Shirasuka, T. Yoshida, T. Takarada, X. Liang, M. Komiyama, *Chem. Lett.* **2001**, 108 – 109; d) X. Liang, H. Asanuma, M. Komiyama, *J. Am. Chem. Soc.* **2002**, *124*, 1877 – 1883; e) K. Takahashi, S. Yaegashi, H. Asanuma, M. Hagiya in *The 11th International Meeting on DNA Computing* (Eds.: A. Carbone, M. Daley, L. Kari, I. McQuillan, N. Pierce), London, Ontario, Canada, **2005**, pp. 147 – 156.

[9] K. Hamad-Schifferli, J. J. Schwartz, A. T. Santos, S. Zhang, J. M. Jacobson, *Nature* **2002**, *415*, 152 – 155.

[10] a) L. Kröck, A. Heckel, *Angew. Chem.* **2005**, *117*, 475 – 477; *Angew. Chem. Int. Ed.* **2005**, *44*, 471 – 473; b) A. Heckel, G. Mayer, *J. Am. Chem. Soc.* **2005**, *127*, 822 – 823; c) G. Mayer, L. Krock, V. Mikat, M. Engeser, A. Heckel, *ChemBioChem* **2005**, *6*, 1966 – 1970; d) P. Wenter, B. Furtig, A. Hainard, H. Schwalbe, S. Pitsch, *Angew. Chem.* **2005**, *117*, 2656 – 2659; *Angew. Chem. Int. Ed.* **2005**, *44*, 2600 – 2603; e) C. Höbartner, S. K. Silverman, *Angew. Chem.* **2005**, *117*, 7471 – 7475; *Angew. Chem. Int. Ed.* **2005**, *44*, 7305 – 7309; f) A. Heckel, M. C. R. Buff, M.-S. L. Raddatz, J. Müller, B. Potzsch, G. Mayer, *Angew. Chem.* **2006**, *118*, 6900 – 6902; *Angew. Chem. Int. Ed.* **2006**, *45*, 6748 – 6750.

[11] M. Irie, *J. Am. Chem. Soc.* **1983**, *105*, 2078 – 2079.

[12] J. L. Leroy, M. Gueron, J. L. Mergny, C. Helene, *Nucleic Acids Res.* **1994**, *22*, 1600 – 1606.

[13] N. Berova, K. Nakanishi, R. W. Woody, *Circular Dichroism: Principles and Applications*, 2nd ed., Wiley-VCH, New York, **2000**.

[14] J. J. Porter, *Text. Res. J.* **1973**, *43*, 735 – 744.

[15] C. J. Burrows, J. G. Muller, *Chem. Rev.* **1998**, *98*, 1109 – 1152.

[16] D. Liu, A. Bruckbauer, C. Abell, S. Balasubramanian, D.-J. Kang, D. Klenerman, D. Zhou, *J. Am. Chem. Soc.* **2006**, *128*, 2067 – 2071.

[17] W. Shu, D. Liu, M. Watari, C. K. Riener, T. Strunz, M. E. Welland, S. Balasubramanian, R. A. McKendry, *J. Am. Chem. Soc.* **2005**, *127*, 17054 – 17060.

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