Electron Transport in DNA

DOI: 10.1002/anie.201202141

Transporting Excess Electrons along Potential Energy Gradients Provided by 2′-Deoxyuridine Derivatives in DNA**

Takeo Ito, Yuta Hamaguchi, Kazuhito Tanabe, Hisatsugu Yamada, and Sei-ichi Nishimoto**

Charge transfer through peptides and proteins is one of the most important reactions in the processes of photosynthesis, signal transduction, respiration, and some enzymatic activities.[1–5] DNA duplexes can also transport holes and excess electrons over a distance,[3–23] and such charge-transfer reactions in DNA might be involved in the recognition of damaged DNA bases by DNA repair enzymes.[24] In addition, it has been demonstrated that electron-transporting DNA could be used as a device for genotyping and for single-nucleotide polymorphism analysis.[25–29]

To date, studies on hole transfer (HT) through the highest occupied molecular orbital (HOMO) of DNA bases have demonstrated that holes on DNA migrate over long distances mainly between guanine–cytosine (G-C) base pairs and partially between adenine–thymine (A-T) base pairs. Recently, highly efficient HT was achieved by replacing the A-T base pair with the 7-deazaguanine–T base pair, which has a higher HOMO energy level than the A-T base pair.[13]

Electrons injected into DNA also migrate along the duplex through the lowest unoccupied molecular orbital (LUMO), most likely between C and T by means of a thermally activated hopping mechanism at ambient temperature.[17] In contrast with the HT in DNA, the efficiency of excess electron transfer (EET) from a photoinduced electron donor over a distance through DNA bases has been reported as being low. This is partly explained by the fast charge recombination between the DNA-tethered electron donor and the excess electron, and kinetically competitive proton transfer between the radical anion of C ($C^{-\bullet}$) and its complementary G.[30,31] If one considers the redox stability of DNA bases, nanoscale electronic devices based on EET chemistry are seemingly preferable, because HT in DNA results in the oxidation of G. Although many issues remain regarding the durability of redox chemical reactions, one of the strategies for developing novel DNA-based devices is to use modified DNA analogues that overcome the aforementioned shortcomings of natural DNA bases.

In this study, we developed DNA containing uracil (U) derivatives with different LUMO energy levels, and examined the regulation and directional control of EET in DNA. Our temporal goal is to construct molecular diode-like DNA nanostructures[32–34] in which the direction and efficiency of EET could be arbitrarily controlled depending on the chemical structures of the intervening DNA bases. We investigated photoinduced electron transport from the DNA-tethered photoinduced electron donor phenothiazine (PTZ; $E_{ox}^{*} = -2.7$ V vs SCE)[35] to the co-inserted 5-bromouracil (^{Br}U) through the intervening U derivatives. Product analysis clearly showed that injected electrons migrated according to the potential energy gradient of the LUMOs of U derivatives, which is, to the best of our knowledge, the first example of the manipulation of the direction of EET using DNA analogues.

As candidates for replacing T in DNA as excess electron carriers, we chose four 2′-deoxyuridine derivatives: 2′-deoxyuridine (dU), 5-fluoro-2′-deoxyuridine ($d^{F}U$), 5-hydroxy-2′-deoxyuridine ($d^{OH}U$), and 2′-deoxypseudouridine ($d^{P}U$). Our preliminary density functional theory calculation (B3LYP/6-31G*) suggested that the LUMO levels of the derivatives are sufficiently high for transporting excess electrons: LUMO level of dT, −1.18 eV; of dU, −1.28 eV; of $d^{F}U$, −1.53 eV; of $d^{P}U$, −1.39 eV and of $d^{OH}U$, −1.36 eV (Figure 1). Also, the electron affinities (EAs) of T, U, and 5-fluorouracil (^{F}U) have been previously reported as 1.56 eV (T), 1.62 eV (U), and 1.82 eV (^{F}U), respectively.[36]

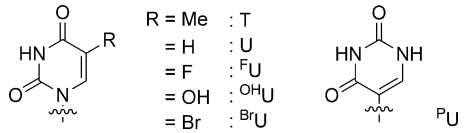

Figure 1. Chemical structures of thymine (T), uracil (U), 5-fluorouracil (^{F}U), 5-hydroxyuracil (^{OH}U), 5-bromouracil (^{Br}U), and pseudouracil (^{P}U).

EET efficiency was investigated by product analysis using polyacrylamide gel electrophoresis (PAGE). PTZ was placed in the duplex DNA by conventional phosphoramidite chemistry, and ^{Br}U was used as a chemical probe for detecting excess electrons that migrated from PTZ (Figure 2).[18] Once an electron is captured by ^{Br}U, the spontaneous ($k \approx 10^{9}\ s^{-1}$ for isolated ^{Br}U)[37] release of a bromide anion yields the corresponding uracil-5-yl radical, which in turn abstracts one hydrogen from the 5′-adjacent deoxyribose. The sugar radical

[*] Dr. T. Ito, M. Sc. Y. Hamaguchi, Dr. K. Tanabe, Dr. H. Yamada, Prof. Dr. S. Nishimoto
Department of Energy and Hydrocarbon Chemistry
Graduate School of Engineering, Kyoto University
Kyoto Daigaku Katsura, Nishikyo-ku, Kyoto 615-8510 (Japan)
E-mail: takeoit@scl.kyoto-u.ac.jp
niqda894@kitc.city.kyoto.lg.jp

[**] This work was supported by a Grant-in-Aid for the Global COE program "International Center for Integrated Research and Advanced Education in Materials Science" from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of the Japanese Government. A part of this study was performed with the support of the Radioisotope Research Center of Kyoto University.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201202141.

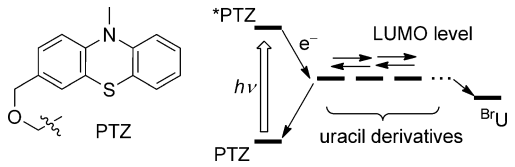

Figure 2. Chemical structure of phenothiazine (PTZ). Excess electrons injected from photoexcited PTZ (PTZ*) migrate through T and U derivatives and react irreversibly with ^{Br}U or recombine with the PTZ radical cation ($PTZ^{+\cdot}$).

eventually affords alkali-labile products as a result of reactions with water.[38–40] Thus, the excess electrons that migrated from PTZ to ^{Br}U could be evaluated by quantifying the amount of strand-cleavage products obtained after piperidine-catalyzed hydrolysis of the photoexposed DNA.

To evaluate the relative electron-transfer efficiency through the U derivatives, the photoinduced electron-transfer reaction was investigated in DNA containing four consecutive U derivatives between PTZ and ^{Br}U (PTZ-ODN 1/X-ODN 1; Figure 3 a). DNA was exposed to UV light at 365 nm under an

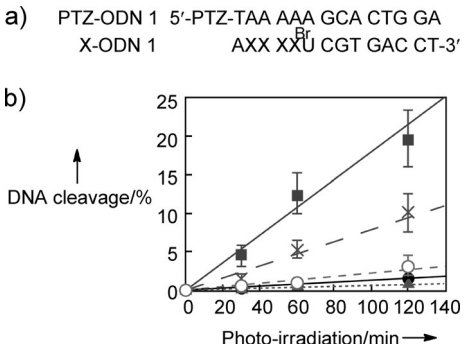

Figure 3. EET from photoexcited PTZ to ^{Br}U through four uracil derivatives (X). a) DNA sequences of PTZ-ODN 1 and X-ODN 1; b) ^{32}P-radiolabeled duplex DNA (PTZ-ODN 1/X-ODN 1; X = (●) T, (×) U, (■) ^{F}U, (▲) ^{OH}U, (○) ^{P}U) in buffer solution (10 mM phosphate, 90 mM NaCl, pH 7.0) was exposed to UV light (365 nm) for the indicated periods at 4 °C, followed by treatment with piperidine at 90 °C for 30 min. DNA fragments that corresponded to electron-transfer products were quantified using PAGE.

N_2 atmosphere and then treated with piperidine at 90 °C. Uracil derivatives, including ^{Br}U, do not absorb UV light at wavelengths above 350 nm (Figure S1 in the Supporting Information). PAGE of the products revealed the formation of electron-transfer products with a yield that varied depending on the sequences of the inserted U bases (Figure S2 in the Supporting Information). The electron-transfer efficiency was remarkably higher in the ^{F}U- or U-containing duplexes (PTZ-ODN 1/F-ODN 1 and PTZ-ODN 1/U-ODN 1) than it was in the other duplexes (Figure 3 b). The thermal stability of the duplexes, as evaluated by melting temperature (T_m), suggests that ^{P}U and ^{OH}U slightly destabilize the duplex (Table S1 in the Supporting Information). Therefore, local structural distortion at the ^{P}U or ^{OH}U stretches should diminish the apparent electron-transfer efficiency.[35] Another reason for the inefficient electron transfer in the ^{P}U-containing duplexes might be the difference in the LUMO distribution between ^{P}U and the other U derivatives.

In addition, the durability of the U radical anions was confirmed by checking stability during the radiation-induced reduction of the derivatives in aqueous solution, because irreversible trapping of excess electrons results in the retardation of long-range electron transfer. One-electron reduction of T, U, ^{F}U, ^{Br}U, and ^{OH}U by hydrated electrons (e_{aq}^-) generated as a consequence of water radiolysis under oxygen-free conditions was examined, and decomposition of U derivatives was monitored using HPLC. However, no remarkable decomposition was observed, except in the cases of ^{Br}U and ^{OH}U (Figure S3 in the Supporting Information). Considering the chemical reactivities of the U derivatives, we chose T, U, and ^{F}U as electron-transporting molecules for constructing artificial DNA. EET efficiency through the consecutive U bases increased (F-ODN 1 > U-ODN 1 > T-ODN 1) as the LUMO energy level of the uridine bases decreased (T > U > ^{F}U).

Encouraged by these results, we next prepared a DNA containing PTZ in the middle of the duplex, four consecutive U derivatives on both the 3′ and 5′ sides of PTZ, and two ^{Br}U bases as electron-transfer probes. For this experiment, enantiomeric (*R*)- and (*S*)-PTZ phosphoramidites were synthesized and inserted into the duplex to evaluate the effect of the localization of PTZ in DNA (Figure 4 a). As summarized in Figure 4 b, the electron-transfer efficiencies through the U bases in the 5′→3′ direction (through the YYY sequence) and in the 3′→5′ direction (through the ZZZ sequence) were compared. When Y and Z were identical, electron migration in the 3′→5′ direction was predominant (in the case of TT-ODN 2, the yields of the electron-transfer

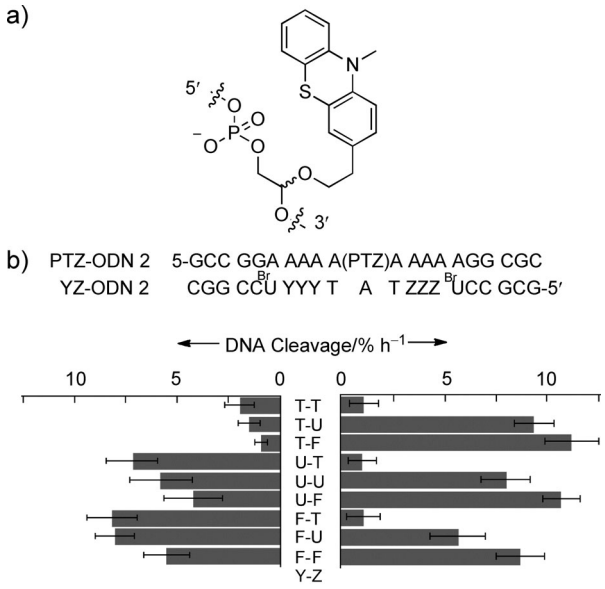

Figure 4. Asymmetrical EET from photoexcited PTZ to ^{Br}U through uracil derivatives. a) Chemical structure of (*R*)- and (*S*)-PTZ inserted in the middle of oligodeoxynucleotides; b) ^{32}P-radiolabeled duplex DNA ((*R*)-PTZ-ODN 2/YZ-ODN 2; Y, Z = T, U, ^{F}U) in buffer solution (10 mM phosphate, 90 mM NaCl, pH 7.0) was exposed to UV light (365 nm) at 4 °C, followed by piperidine treatment.

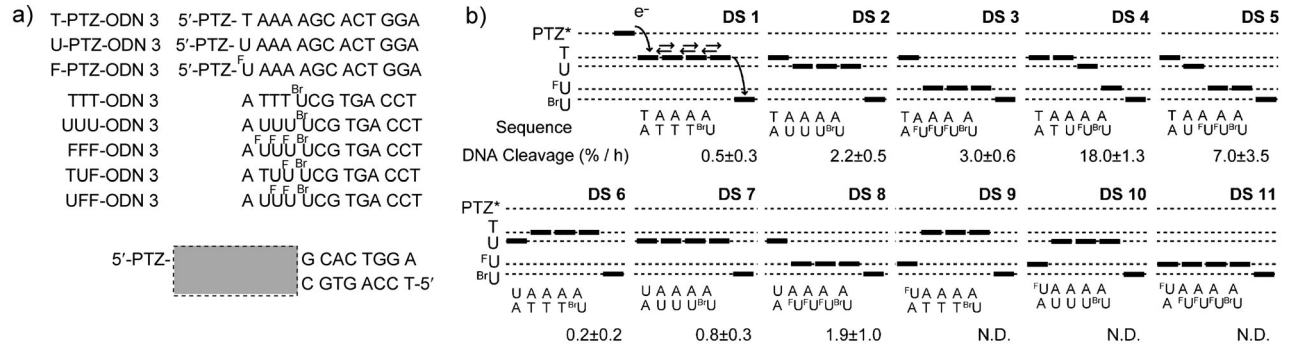

Figure 5. EET through various sequences of uracil derivatives. a) DNA sequences used in this experiment; b) sequence-dependent EET efficiencies as determined from the yield of DNA-cleavage products. The sequence in the gray box was changed as indicated in the diagram, which depicts the calculated LUMO levels of uracil derivatives and PTZ*.

products were too low for the efficiencies to be compared), which is in accordance with the results of previous reports.[31,41] The intrinsic electron flow could be reversed by inserting U derivatives, that is, electrons moved to U derivatives with higher EA. For example, electron transfer in the 3′→5′ direction was predominant in the TU-ODN 1-containing duplex, but migration in the other direction became predominant when the sequence was replaced with UT-ODN 1. Asymmetrical localization of PTZ in the duplexes seemed less likely, because the enantiomeric (*R*)- and (*S*)-PTZ-containing ODNs showed essentially the same electron-transport properties (Figure S4 in the Supporting Information). Nonlocalization of the photoinduced electron donor has also been confirmed in the case of diaminonaphthalene-tethered DNA duplexes.[31]

Mechanistic investigation of DNA-mediated charge transfer has revealed that excess electrons hop between nucleobases, most likely between pyrimidine bases. Moreover, it has been suggested that back electron transfer to the radical cation of the electron donor occurs rapidly and efficiently.[42–45] Thus, the remarkable enhancement of the apparent electron-transfer efficiency observed in the cases of U- or ^{F}U-containing DNA could be explained by retardation of the back electron-transfer process; in other words, an electron injected from PTZ* to the adjacent T either migrates further to the next U bases or recombines with $PTZ^{+\cdot}$. Therefore, the LUMO energy gap between the adjacent T and the next consecutive U derivatives should affect the relative yield of back electron transfer. It is not clear why electron transfer from 3′ to 5′ of pyrimidine bases is preferable, but it has been suggested that asymmetrical orbital overlap around the electron-deficient nucleobase intermediate may affect the direction of HT in DNA.[46] Such directionality should be considered if we develop DNA devices for genotyping based on the charge-transfer properties of DNA.

Finally, we explored the construction of DNA containing U derivatives to produce various LUMO energy potential gradients along the strands (Figure 5). In this experiment, the PTZ-adjacent nucleobase was changed (T-, U-, and F-PTZ-ODN 3) and three U derivatives were inserted on the opposite strands (TTT-, UUU-, FFF-, TUF-, and UFF-ODN 3). Eleven duplex DNAs (**DS 1**–**11**; Figure 5b) obtained by the combination of the oligodeoxynucleotides were used. As depicted in Figure 5b, excess electron hopping through U stretches in which the LUMO energy level is flat (**DS 1**, **DS 7**, and **DS 11**) or upstream from the 3′→5′ direction (**DS 6**, **DS 9**, and **DS 10**) was apparently inefficient compared with that observed through **DS 2**–**DS 5** and **DS 8**, probably because of fast charge recombination. It was also apparent that the multistep energy gradient enhanced the charge separation, as observed for **DS 4** and **DS 5**. The role of the nucleobase at the electron-injection moiety seems to be important in preventing initial charge recombination,[44] because insertion of ^{F}U at the 3′-adjacent base of PTZ apparently lowered the electron-transfer efficiency compared with the case of PTZ-ODN 1/F-ODN 1. Back and forth EET between intervening U bases with a flat energy level may enhance the lifetime of excess electrons on the duplex and result in back electron transfer to $PTZ^{+\cdot}$. Interaction between adjacent DNA bases could alter their LUMO levels to some extent; nevertheless, the current study demonstrates that it is possible to estimate electron-transfer efficiency based on the LUMO levels of the isolated uracil derivatives.

In conclusion, we have synthesized artificial DNA duplexes containing U derivatives as alternative pyrimidine bases to T to modulate the electron-transport properties of DNA. Our successful control of the directionality of the electron transfer by using T, U, and ^{F}U may widen the potential applications of artificial DNA as a novel electronic device; for example, the control of electron transfer on multidimensional DNA duplexes might be possible by using branched duplexes containing modified DNA bases.

Received: March 18, 2012
Published online: June 13, 2012

Keywords: DNA · electron transport · photochemistry · radical ions · radical reactions

[1] J. Stubbe, D. G. Nocera, C. S. Yee, M. C. Y. Chang, *Chem. Rev.* **2003**, *103*, 2167–2201.
[2] M. Cordes, B. Giese, *Chem. Soc. Rev.* **2009**, *38*, 892–901.
[3] F. Boussicault, M. Robert, *Chem. Rev.* **2008**, *108*, 2622–2645.
[4] H.-A. Wagenknecht, *Charge transfer in DNA: From mechanism to application*, Wiley, New York, **2005**.
[5] J. C. Genereux, J. K. Barton, *Chem. Rev.* **2010**, *110*, 1642–1662.

[6] S. O. Kelley, J. K. Barton, *Science* **1999**, *283*, 375 – 381.
[7] P. T. Henderson, D. Jones, G. Hampikian, Y. Kan, G. B. Schuster, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 8353 – 8358.
[8] F. D. Lewis, X. Liu, J. Liu, S. E. Miller, R. T. Hayes, M. R. Wasielewski, *Nature* **2000**, *406*, 51 – 53.
[9] D. Porath, A. Bezryadin, S. De Vries, C. Dekker, *Nature* **2000**, *403*, 635 – 638.
[10] G. B. Schuster, *Acc. Chem. Res.* **2000**, *33*, 253 – 260.
[11] a) B. Giese, J. Amaudrut, A. K. Kohler, M. Spormann, S. Wessely, *Nature* **2001**, *412*, 318 – 320; b) B. Giese, *Annu. Rev. Biochem.* **2002**, *71*, 51 – 70.
[12] a) Y. C. Huang, D. Sen, *J. Am. Chem. Soc.* **2010**, *132*, 2663 – 2671; b) B. Ge, Y. C. Huang, D. Sen, H.-Z. Yu, *Angew. Chem.* **2010**, *122*, 10161 – 10163; *Angew. Chem. Int. Ed.* **2010**, *49*, 9965 – 9967.
[13] K. Kawai, H. Kodera, Y. Osakada, T. Majima, *Nat. Chem.* **2009**, *1*, 156 – 159.
[14] E. J. Merino, A. K. Boal, J. K. Barton, *Curr. Opin. Chem. Biol.* **2008**, *12*, 229—237.
[15] H.-A. Wagenknecht, *Nat. Prod. Rep.* **2006**, *23*, 973 – 1006.
[16] A. Schwögler, L. T. Burgdorf, T. Carell, *Angew. Chem.* **2000**, *112*, 4082 – 4085; *Angew. Chem. Int. Ed.* **2000**, *39*, 3918 – 3920.
[17] Z. L. Cai, Z. Y. Gu, M. D. Sevilla, *J. Phys. Chem. B* **2000**, *104*, 10406 – 10411.
[18] T. Ito, S. E. Rokita, *J. Am. Chem. Soc.* **2003**, *125*, 11480 – 11481.
[19] C. Behrens, M. K. Cichon, F. Grolle, U. Hennecke, T. Carell, *Top. Curr. Chem.* **2004**, *236*, 187 – 204.
[20] T. Ito, S. E. Rokita, *J. Am. Chem. Soc.* **2004**, *126*, 15552 – 15559.
[21] P. Daublain, A. K. Thazhathveetil, Q. Wang, A. Trifonov, T. Fiebig, F. D. Lewis, *J. Am. Chem. Soc.* **2009**, *131*, 16790 – 16797.
[22] D. Fazio, C. Trindler, K. Heil, C. Chatgilialoglu, T. Carell, *Chem. Eur. J.* **2011**, *17*, 206 – 212.
[23] M. J. Park, M. K. Fujitsuka, K. Kawai, T. Majima, *J. Am. Chem. Soc.* **2011**, *133*, 15320 – 15323.
[24] E. Yavin, A. K. Boal, E. D. A. Stemp, E. M. Boon, A. L. Livingston, V. L. O'Shea, S. S. David, J. K. Barton, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 3546 – 3551.
[25] E. M. Boon, D. M. Ceres, T. G. Drummond, M. G. Hill, J. K. Barton, *Nat. Biotechnol.* **2000**, *18*, 1096 – 1100.
[26] M. Inouye, R. Ikeda, M. Takase, T. Tsuri, J. Chiba, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 11606 – 11610.
[27] J. Hihath, B. Q. Xu, P. M. Zhang, N. J. Tao, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 16979 – 16983.
[28] T. Takada, M. Fujitsuka, T. Majima, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 11179 – 11183.
[29] K. Kawai, H. Kodera, T. Majima, *J. Am. Chem. Soc.* **2010**, *132*, 14216 – 14220.
[30] Z. Cai, X. Li, M. D. Sevilla, *J. Phys. Chem. B* **2002**, *106*, 2755 – 2762.
[31] T. Ito, S. E. Rokita, *Angew. Chem.* **2004**, *116*, 1875 – 1878; *Angew. Chem. Int. Ed.* **2004**, *43*, 1839 – 1842.
[32] S. Yasutomi, T. Morita, Y. Imanishi, S. Kimura, *Science* **2004**, *304*, 1944 – 1947.
[33] M. Elbing, R. Ochs, M. Koentopp, M. Fischer, C. von Hänisch, F. Weigend, F. Evers, H. B. Weber, M. Mayor, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 8815 – 8820.
[34] I. Díez-Pérez, J. Hihath, Y. Lee, L. Yu, L. Adamska, M. A. Kozhushner, I. I. Oleynik, N. Tao, *Nat. Chem.* **2009**, *1*, 635 – 641.
[35] a) T. Ito, A. Kondo, S. Terada, S. Nishimoto, *J. Am. Chem. Soc.* **2006**, *128*, 10934 – 10942; b) T. Ito, A. Hayashi, A. Kondo, T. Uchida, K. Tanabe, H. Yamada, S. Nishimoto, *Org. Lett.* **2009**, *11*, 927 – 930.
[36] S. D. Wetmore, R. J. Boyd, L. A. Eriksson, *Chem. Phys. Lett.* **2001**, *343*, 151 – 158.
[37] E. Rivera, R. H. Schuler, *J. Phys. Chem.* **1983**, *87*, 3966 – 3971.
[38] G. P. Cook, M. M. Greenberg, *J. Am. Chem. Soc.* **1996**, *118*, 10025 – 10030.
[39] K. Fujimoto, Y. Ikeda, S. Ishihara, I. Saito, *Tetrahedron Lett.* **2002**, *43*, 2243 – 2245.
[40] R. Tashiro, A. Ohtsuki, H. Sugiyama, *J. Am. Chem. Soc.* **2010**, *132*, 14361 – 14363.
[41] M. Tanaka, B. Elias, J. K. Barton, *J. Org. Chem.* **2010**, *75*, 2423 – 2428.
[42] F. D. Lewis, X. Liu, S. E. Miller, R. T. Hayes, M. R. Wasielewski, *J. Am. Chem. Soc.* **2002**, *124*, 11280 – 11281.
[43] P. Daublain, A. K. Thazhathveetil, V. Shafirovich, Q. Wang, A. Trifonov, T. Fiebig, F. D. Lewis, *J. Phys. Chem. B* **2010**, *114*, 14265 – 14272.
[44] K. Tainaka, M. Fujitsuka, T. Takada, K. Kawai, T. Majima, *J. Phys. Chem. B* **2010**, *114*, 14657 – 14663.
[45] T. Ito, T. Uchida, K. Tanabe, H. Yamada, S. Nishimoto, *J. Photochem. Photobiol. A* **2011**, *219*, 115 – 121.
[46] M. A. O'Neill, J. K. Barton, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 16543 – 16550.

