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Electron Transfer

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# Photoinduced Reductive Electron Transfer in LNA:DNA Hybrids: A Compromise between Conformation and Base Stacking**

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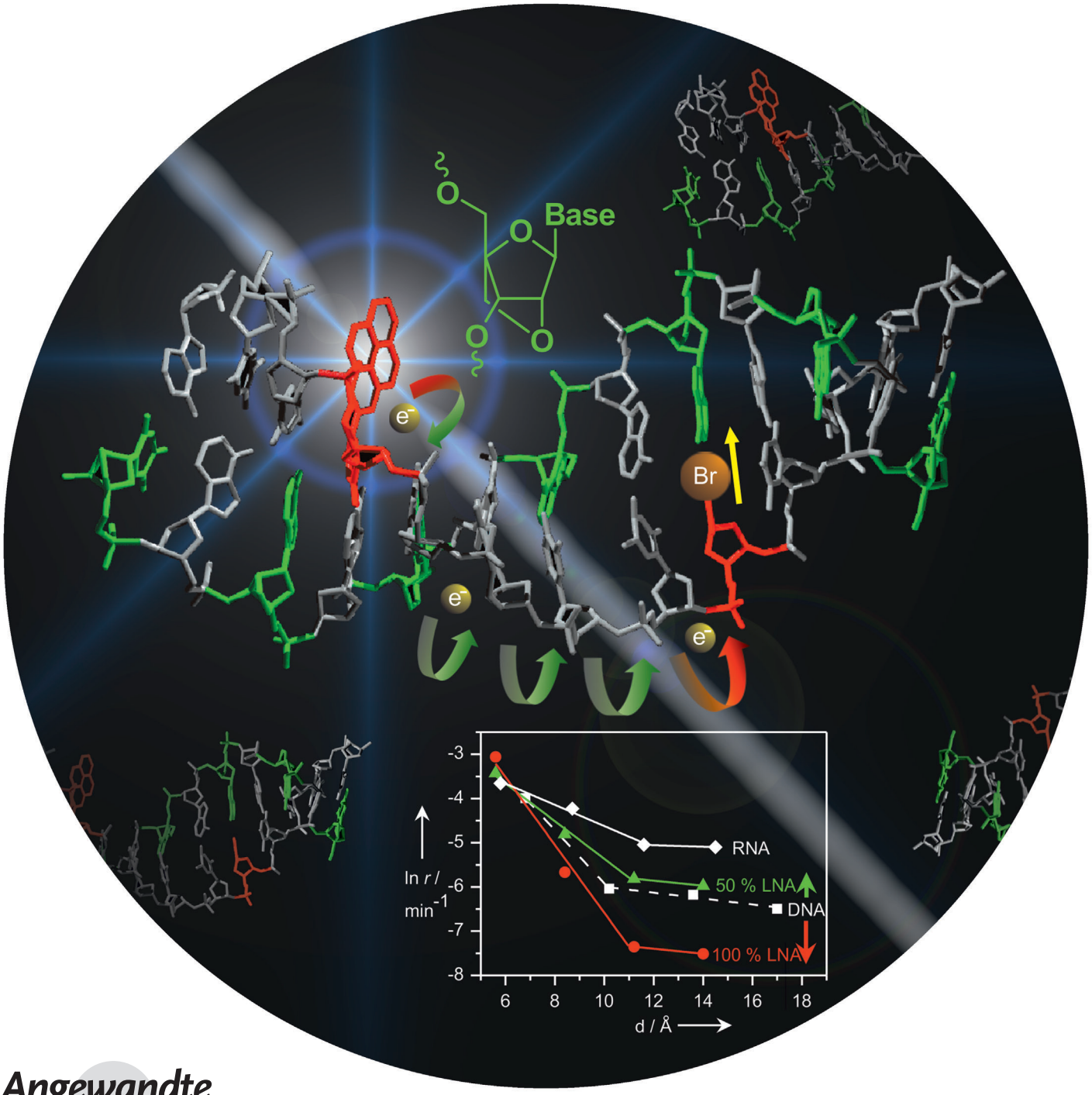


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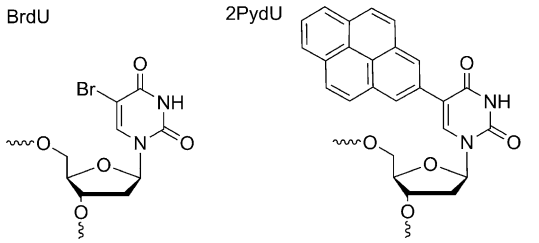
Nucleic acids and their synthetic analogues are fascinating architectures with respect to molecular electronics owing to the spontaneous self-assembly into networks and complex 2D and 3D structures.[1] Photoinduced charge-transfer reactions through DNA have been studied thoroughly; the oxidative mode mainly in the 1990s,[2] and the reductive type in the last decade.[2,3–8] Hopping mechanisms have been elucidated, not only experimentally over long ranges,[2–6] but also theoretically over short ranges.[9] Hole transfer is accompanied by oxidative guanine damage. This major disadvantage makes reductive electron transfer (ET) through DNA much more promising for nanoelectronics.[7,8] In fact, ET rate constants for charge hopping over thymines are faster ($4\times10^{10}$ s$^{-1}$)[10] than the rate constants for hole hopping ($10^4$–$10^{10}$ s$^{-1}$).[11,12] However, rates show only how fast an ET process occurs, but do not indicate much about the efficiency of ET. The efficiency gives information how much charge arrives (in comparison) at the acceptor, which is an important parameter for molecular electronics.

The efficiency of ET through natural DNA is too low to be considered seriously for nanoelectronics.[7] Current research focuses how the ET can be improved while keeping the spontaneous self-assembly as the most attractive feature of nucleic acid architectures. It is known that ET in DNA is gated by the conformation of the double helix.[8,13,14] A-type double helices are an important and promising alternative. It been shown that charge transfer in RNA can be more efficient than in DNA.[15] Other studies with RNA,[16] including those with opposite results[17] (also with LNA:DNA hybrids[18]), bring about the question as to whether the more flexible structure is solely responsible for possibly higher ET efficiency. The helix conformation (A-type) with its shorter base stacking distance could bring also an advantage. On the other hand, the chemical instability of RNA diminishes its great potential for electronics. To solve these issues, we present herein ET experiments with locked nucleic acid (LNA) between 5-(2-pyrenyl)-2′-deoxyuridine (2PydU) and 5-bromo-2′-deoxyuridine (BrdU) as the electron donor–acceptor pair.[19] In LNA the sugar pucker is locked in the C3′-*endo* type, thereby stabilizing the A-type helix in LNA:DNA hybrids. 40% LNA nucleotides in one strand diminish the average rise to 2.8 Å and the twist to 32 ± 1 Å.[20] Further modifications leads to smaller twist, but the rise remains constant.[21] With respect to base stacking, LNA:DNA hybrids are structurally very similar to DNA:RNA helices, however the flexibility is reduced. Owing to the methylene bridge in particular, the phosphate backbone of LNA is very rigid.

We[8,22] and others[23] have used 5-(1-pyrenyl)-2′-deoxyuridine (1PydU) to study reductive ET. The strong electronic coupling between the two aromatic parts, however, yields intramolecular exciplex states[22] that interfere with electron injection into DNA. 2PydU[24] is a better electron injector, as the attachment at the 2-position decouples the pyrene. On the acceptor side, BrdU has been applied very often for probing reductive ET in DNA[5,7,8,23] because the chemical reactivity of the BrdU radical anion is fast enough to resolve differences of ET efficiencies.[25]

Based on our[26] and others[27] previous assumptions that thymines are the major electron carrier, up to four intervening T-A base pairs were placed between donor (2PydU) and acceptor (BrdU) in DNA III-0 to III-4 (Figure 1). DNA II is the reference without BrdU, and DNA I is the reference without any modification. Four different complementary strands were used: DNA, RNA, 50%LNA/50%DNA (lna), and 100% LNA.




I 3' - CAC - GCT - TTT - TTT - TTA - TTA - CG - Fluo - 5'

II 3' - CAC - GCT - TTT - TT(2Py)dU - TTA - TTA - CG - Fluo - 5'

III-*n* 3' - CAC - GC(T)$_{5-n}$ - dU(Br)(T)$_n$dU(2Py) - TTA - TTA - CG - Fluo - 5'

DNA 5' - GTG - CGA - AAA - AAA - AAT - AAT - GC - 3'

RNA 5' - GUG - CGA - AAA - AAA - AAU - AAU - GC - 3'

lna 5' - GTG - CGA - AAA - AAA - AAT - AAT - GC - 3'

LNA 5' - GTG - CGA - AAA - AAA - AAT - AAT - GC - 3'

***Figure 1.*** Structure of 2PydU and BrdU, and oligonucleotide sequences. Underlined letters: LNA units (Fluo = fluorescein marker; see the Supporting Information).

The duplexes were first characterized by their melting temperatures ($T_m$; Table 1). The introduction of the 2PydU modification causes a destabilization of approximately 5 °C, regardless if it is in pure DNA duplexes (I_DNA vs. II_DNA), RNA duplexes (I_RNA vs. II_RNA) or hybrids with 50% LNA (I_lna vs. II_lna). This is a typical value for a modification at the 5-position of dU. The second modification, BrdU, does not introduce any additional destabilization. All of the doubly modified duplexes in each set show comparable thermal stability of ca. 58–59 °C (III-0_DNA to III-4_DNA), 53–54 °C (III-0_RNA to III-4_RNA), and 71–72 °C (III-0_lna

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***Table 1:*** Melting temperatures $T_m$ of oligonucleotides.[a]

| Duplex | $T_m$ [°C] | Duplex | $T_m$ [°C] | Duplex | $T_m$ [°C] |
|---|---|---|---|---|---|
| I_DNA | 62.9 | I_RNA | 58.3 | I_lna | 75.4 |
| II_DNA | 58.1 | II_RNA | 52.8 | II_lna | 70.9 |
| III-0_DNA | 59.2 | III-0_RNA | 53.8 | III-0_lna | 71.2 |
| III-1_DNA | 58.7 | III-1_RNA | 53.3 | III-1_lna | 72.2 |
| III-2_DNA | 58.9 | III-2_RNA | 53.6 | III-2_lna | 71.0 |
| III-3_DNA | 58.7 | III-3_RNA | 53.7 | III-3_lna | 71.1 |
| III-4_DNA | 58.8 | III-4_RNA | 53.9 | III-4_lna | 72.0 |

[a] $\lambda = 260$ nm, 20–90 °C, rate 0.7 °C min$^{-1}$, 2.5 μM DNA in 10 mM $NaP_i$-buffer (pH 7.0), 250 mM NaCl.

to III-4_lna). All of the hybrids with pure LNA counterstrands exhibit melting temperatures that are higher than 90 °C.[28] The major differences between the $T_m$ ranges of pure DNA and DNA:LNA hybrids can be attributed to the preorganization of the LNA single strand and better base stacking. It is important to mention that the thermal stabilities of all modified duplexes are sufficiently high to perform irradiation at RT with guaranteed double stranded samples.

Irradiations were performed with a 200 W Hg/Xe lamp using a 320 nm cutoff filter to ensure selective excitation of 2PydU outside both the nucleic acid and BrdU absorption range. All of the oligonucleotides for ET (III-0 to III-4) were labeled at the 5′-terminus with fluorescein to allow quantification by fluorescence after polyacrylamide gel electrophoresis (PAGE) analysis. As expected, based on previous reports,[5,7,8,23,25] PAGE of photoirradiated samples that were treated with piperidine showed the formation of cleavage products at the BrdU site, which were combined in the quantitative analysis (Table 2). Under these conditions, all of

***Table 2:*** Strand cleavage of duplexes after 30 min of irradiation.

| Counter-strand | Strand cleavage [%] | | | |
|---|---|---|---|---|
| | III-1 | III-2 | III-3 | III-4 |
| DNA | 44.6 | 7.3 | 6.1 | 4.7 |
| RNA | 58.4 | 40.5 | 19.2 | 18.7 |
| lna | 67.4 | 24.2 | 8.3 | 7.4 |
| LNA | 76.5 | 9.7 | 1.9 | 1.9 |

the oligonucleotide samples with 2PydU and BrdU adjacent to each other (III-0_DNA, III-0_RNA, III-0_lna, III-0_LNA) showed no ET-induced strand cleavage, which is probably due to very fast charge recombination. In the samples with intervening base pairs between donor and acceptor, cleavage drops with growing distance, regardless of which counterstrand has been used. With four intervening T-A pairs, only RNA:DNA hybrids show a strand decomposition of nearly 20%. Hybrids with counterstrands that consist of 50% LNA units (III-3_lna/III-4_lna) exhibit higher strand breakage yields than DNA, whereas those with pure LNA complements (III-3_LNA/III-4_LNA) show lower yields.

A semi-quantitative analysis can be obtained from the monoexponential fitting of the strand cleavage yields and the subsequent linear fitting of the initial phase (see the Supporting Information). These chemical rates are plotted against distance between 2PydU and BrdU (Figure 2). It is important to mention that these rates do not represent ET rates but allow a simple analysis of ET efficiencies in our

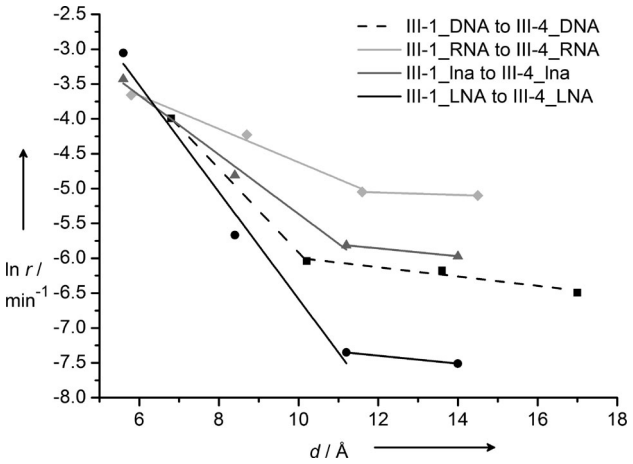


***Figure 2.*** Analysis of the dependence of the strand cleavage rate $r$ on distance $d$. Helical rises: 3.4 for DNA; 2.9 for DNA:RNA,[31] and 2.8 for DNA:LNA.[32]

experiments. First, the four hybrid sets show an initially stronger distance dependence that changes to a weaker dependence. This dual behavior has been observed previously for oxidative hole transfer[29] and reductive ET.[12] The distance dependence of strand cleavage rates in our RNA:DNA hybrids is the weakest. More remarkable however is the observation that photoinduced cleavage at the BrdU site is also enhanced in those hybrids with 50% LNA in the complement, whereas it is diminished in hybrids with 100% LNA.[30]

Previous results have revealed that electron injection through the single C–C bond in 1PydU is very fast (2 ps$^{-1}$)[8] and thus should not differ in the different hybrids. Second, it is accepted knowledge that the amount of cleaved strands reflects relative efficiencies of ET.[5,7,8,23,25] Based on both assumptions, the observed differences can be attributed solely to the two parameters discussed at the beginning: The helix type, A or B, influences ET by different base stacking; or the different flexibility modulates the ET. In the literature, efficient charge transfer in DNA:RNA hybrids was nearly exclusively attributed to the more flexible structure in comparison to pure DNA.[16] Our results show clearly that this cannot be the single reason. DNA:LNA hybrids, regardless if they contain 50 or 100% LNA, are more rigid than DNA, but III-1_lna to III-4_lna exhibit better ET efficiency than III-1_DNA to III-4_DNA. Therefore, this difference must be attributed to the key characteristics of the A-helix, which are smaller base distance and helical twist[31] together causing better base stacking.[20,21,32] This property overrides the negative effect of the locked sugar pucker in the double helices with 50% LNA units and yields better ET efficiencies than in pure DNA of the B-type.

In conclusion, LNA helped to elucidate the different roles of conformational flexibility and A-type base stacking with respect to ET efficiency. Not surprisingly, the conformational

  

locking of LNA units has clearly a negative effect on ET rate[18] and efficiency (see the results of III-1_LNA to III_4_LNA). On the other hand, LNA forces the nucleic acid structure into the A-type, which is very similar to RNA, leading to better base stacking and thereby promoting ET. It becomes clear that a good compromise between supporting A-type double helix and conformational flexibility is providing the best architecture for increasing the ET efficiency through nucleic acids architectures. This is an important result for the future design of nucleic acids for ET and molecular electronics, as the significant advantage of LNA in contrast to RNA is the high chemical stability. Cleverly arranged LNA nucleotides are able to improve the efficiency of ET over short ranges and thereby potentially also over longer ranges. Together with metal ions or chromophores as artificial stepping stones for long-range ET, LNA provides great potential for the development of nucleic-based architectures for molecular nanoelectronics.

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[1] E. Stulz, *Chem. Eur. J.* **2012**, *18*, 4456 – 4469.
[2] *Top. Curr. Chem.* **2004**, *236* ("Longe-Range Charge Transfer in DNA I"); *Top. Curr. Chem.* **2004**, *237* ("Longe-Range Charge Transfer in DNA II").
[3] B. Giese, *Bioorg. Med. Chem. Lett.* **2006**, *14*, 6139 – 6143.
[4] a) C. Behrens, M. K. Cichon, F. Grolle, U. Hennecke, T. Carell, *Top. Curr. Chem.* **2004**, *236*, 187 – 204; b) A. Kumar, M. D. Sevilla, *Chem. Rev.* **2010**, *110*, 7002 – 7023.
[5] T. Ito, S. E. Rokita, *J. Am. Chem. Soc.* **2004**, *126*, 15552 – 15559.
[6] J. C. Genereux, A. K. Boal, J. K. Barton, *J. Am. Chem. Soc.* **2010**, *132*, 891 – 905.
[7] a) C. Wagner, H.-A. Wagenknecht, *Chem. Eur. J.* **2005**, *11*, 1871 – 1876; b) A. Manetto, S. Breeger, C. Chatgilialoglu, T. Carell, *Angew. Chem.* **2006**, *118*, 325 – 328; *Angew. Chem. Int. Ed.* **2006**, *45*, 318 – 321; c) B. Elias, J. C. Genereux, J. K. Barton, *Angew. Chem.* **2008**, *120*, 9207 – 9210; *Angew. Chem. Int. Ed.* **2008**, *47*, 9067 – 9070; d) T. Ito, A. Hayashi, A. Kondo, T. Uchida, K. Tanabe, H. Yamada, S.-i. Nishimoto, *Org. Lett.* **2009**, *11*, 927 – 930; e) R. Tashiro, A. Ohtsuki, H. Sugiyama, *J. Am. Chem. Soc.* **2010**, *132*, 14361 – 14363.
[8] P. Kaden, E. Mayer-Enthart, A. Trifonov, T. Fiebig, H.-A. Wagenknecht, *Angew. Chem.* **2005**, *117*, 1662 – 1666; *Angew. Chem. Int. Ed.* **2005**, *44*, 1636 – 1639.
[9] a) E. Hatcher, A. Balaeff, S. Keinan, R. Venkatramani, D. N. Beratan, *J. Am. Chem. Soc.* **2008**, *130*, 11752 – 11761; b) F. C. Grozema, S. Tonzani, Y. A. Berlin, G. C. Schatz, L. D. A. Siebbeles, M. A. Ratner, *J. Am. Chem. Soc.* **2008**, *130*, 5157 – 5166; c) T. Kubař, U. Kleinekathöfer, M. Elstner, *J. Phys. Chem. B* **2009**, *113*, 13107 – 13117.
[10] M. J. Park, M. Fujitsuka, K. Kawai, T. Majima, *J. Am. Chem. Soc.* **2011**, *133*, 15320 – 15323.
[11] a) F. D. Lewis, X. Liu, J. Liu, S. E. Miller, R. T. Hayes, M. R. Wasielewski, *Nature* **2000**, *406*, 51 – 53; b) P. T. Henderson, D. Jones, G. Hampikian, Y. Kan, G. B. Schuster, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 8353 – 8358; c) T. Takada, K. Kawai, X. Cai, A. Sugimoto, M. Fujitsuka, T. Majima, *J. Am. Chem. Soc.* **2004**, *126*, 1125 – 1129; d) S. M. M. Conron, A. K. Thazhathveetil, M. R. Wasielewski, A. L. Burin, F. D. Lewis, *J. Am. Chem. Soc.* **2010**, *132*, 14388 – 14390; e) C. Wan, T. Fiebig, S. O. Kelley, C. Treadway, J. K. Barton, A. H. Zewail, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 6014 – 6019.
[12] F. D. Lewis, H. Zhu, P. Daublain, T. Fiebig, M. Raytchev, Q. Wang, V. Shafirovich, *J. Am. Chem. Soc.* **2006**, *128*, 791 – 800.
[13] L. Valis, Q. Wang, M. Raytchev, I. Buchvarov, H.-A. Wagenknecht, T. Fiebig, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 10192 – 10195.
[14] M. A. O'Neill, J. K. Barton, *J. Am. Chem. Soc.* **2004**, *126*, 11471 – 11483.
[15] T. Takada, Y. Otsuka, M. Nakamura, K. Yamana, *Bioorg. Med. Chem.* **2011**, *19*, 6881 – 6884.
[16] a) M. A. O'Neill, J. K. Barton, *J. Am. Chem. Soc.* **2002**, *124*, 13053 – 13066; b) E. M. Boon, J. K. Barton, *Bioconjugate Chem.* **2003**, *14*, 1140 – 1147; c) D. T. Odom, J. K. Barton, *Biochemistry* **2001**, *40*, 8727 – 8737.
[17] V. Sartor, P. T. Henderson, G. B. Schuster, *J. Am. Chem. Soc.* **1999**, *121*, 11027 – 11033.
[18] K. Kawai, M. Hayashi, T. Majima, *J. Am. Chem. Soc.* **2012**, *134*, 9406 – 9409.
[19] Based on excited state redox potential of pyrene ($E^* = -1.9$ V) and of BrdU ($E = -1.8$ V) vs. NHE.
[20] K. E. Nielsen, S. K. Singh, J. Wengel, J. P. Jacobsen, *Bioconjugate Chem.* **2000**, *11*, 228 – 238.
[21] A. Eichert, K. Behling, C. Betzel, V. A. Erdmann, J. P. Fürste, C. Förster, *Nucleic Acids Res.* **2010**, *38*, 6729 – 6736; Å. K. E. Nielsen, J. Rasmussen, R. Kumar, J. Wengel, J. P. Jacobsen, M. Petersen, *Bioconjugate Chem.* **2004**, *15*, 449 – 457.
[22] a) M. Raytchev, E. Mayer, N. Amann, H.-A. Wagenknecht, T. Fiebig, *ChemPhysChem* **2004**, *5*, 706 – 712; b) A. Trifonov, I. Buchvarov, H.-A. Wagenknecht, T. Fiebig, *Chem. Phys. Lett.* **2005**, *409*, 277 – 280.
[23] S. T. Gaballah, J. D. Vaught, B. E. Eaton, T. L. Netzel, *J. Phys. Chem. B* **2005**, *109*, 5927 – 5934.
[24] C. Wanninger-Weiß, H.-A. Wagenknecht, *Eur. J. Org. Chem.* **2008**, 64 – 71.
[25] D. Fazio, C. Trindler, K. Heil, C. Chatgilialoglu, T. Carell, *Chem. Eur. J.* **2011**, *17*, 206 – 212.
[26] B. Giese, *Annu. Rev. Biochem.* **2002**, *71*, 51 – 70.
[27] R. Huber, T. Fiebig, H.-A. Wagenknecht, *Chem. Commun.* **2003**, 1878 – 1879.
[28] M. A. Campbell, J. Wengel, *Chem. Soc. Rev.* **2011**, *40*, 5680 – 5689.
[29] a) B. Giese, J. Amaudrut, A.-K. Köhler, M. Spormann, S. Wessely, *Nature* **2001**, *412*, 318 – 320; b) F. D. Lewis, H. Zhu, P. Daublain, B. Cohen, M. R. Wasielewski, *Angew. Chem.* **2006**, *118*, 8150 – 8153; *Angew. Chem. Int. Ed.* **2006**, *45*, 7982 – 7985.
[30] The small but reverse effect of strand cleavage efficiences observed for the shortest distance (one base pair) must be caused by local structural pertubation introduced by the pyrene modification.
[31] G. L. Conn, T. Brown, G. A. Leonard, *Nucleic Acids Res.* **1999**, *27*, 555 – 561.
[32] a) See Ref. [20]; b) see Ref. [21].

  