Nanostructures

DOI: 10.1002/ange.201407729

A Twisting Electronic Nanoswitch Made of DNA**

*Yu Chuan Huang and Dipankar Sen**

Abstract: *Single-stranded DNAs and RNAs that are rich in the nucleobase guanine form four-stranded G-quadruplexes, which are held together by hydrogen-bonded guanine quartets. In aqueous solution, both DNA duplexes and G-quadruplexes are modest conductors of electrical charge. A tight, topologically constrained DNA construct called twDNA is now reported, in which a core of four guanine-rich single strands structurally and electronically links together four DNA double helices. The addition and removal of K^+ or Sr^{2+} cations promote alternative conformers of twDNA, which have strikingly distinct electronic properties. Unlike DNA mechano-electronic switches that require large conformational changes, twDNA requires only modest twisting/untwisting structural attenuations to achieve electronic switching.*

DNA is a remarkably versatile raw material for the self-assembly of molecular machines on a nanometer scale.[1–6] Such structures can be either static or mobile, the latter being capable of structural switching in response to the binding or chemical reaction of a defined "fuel". Short DNA helices (double, triple, or quadruple helices) in aqueous salt solutions conduct electrical charge at low but detectable levels.[7–10] The charge carrier can be either a guanine radical cation ("electron hole") or a thymine or cytosine radical anion ("excess electron"). Of the two kinds of charge conduction, hole conduction has been more extensively studied. A convenient way to initiate hole transfer within a DNA helix is to irradiate a photosensitizer, such as anthraquinone, that is covalently linked and end-stacked upon one terminus of the helix with UV light. The specific path of hole conduction through the DNA can be monitored at nucleotide resolution in several different ways—most conveniently by monitoring the consequences of guanine oxidation. The mobile holes (guanine radical cations: $G^{\bullet+}$) can react with water to give oxidized guanine products in proportion to the equilibrium distribution of $G^{\bullet+}$ across a given DNA helix. These oxidative G lesions can be localized and quantitated by sequential treatment with hot aqueous base (which breaks the DNA backbone at G oxidation sites) followed by inspection and quantitation of the resulting DNA fragments by denaturing gel electrophoresis.[7–9]

Single-stranded DNAs or RNAs that are rich in guanine can form stable and polymorphic four-stranded structures, so-called G-quadruplexes, which contain hydrogen-bonded guanine base quartets.[11–14] The stable formation of G-quadruplexes requires the binding, within one or between successive G-quartets, of certain key cations, notably K^+ and Sr^{2+}, but also Pb^{2+}, NH_4^+, and a few others.[15] Li^+, by contrast, does not specifically stabilize G-quadruplexes.

In recent years, different classes of mechanoelectronic DNA nanoswitches have been reported,[16] including those that exploit ligand–aptamer interactions[17–21] and others that exploit the reversible formation of G-quadruplexes by spatially separated G·G mismatch motifs within DNA duplexes ("contractile duplexes").[22–24] Herein, we describe a new DNA mechanoelectronic switch, which can "twist" and "untwist" between distinct conformational states of differing electronic conductivity. Figure 1a shows the design of twDNA. Four DNA oligomers, strands a–d, are assembled together in a 1:1:1:1 ratio to form an assembly containing four Watson–Crick duplexes (duplexes X and Z are 15 bp long, Y is 17 bp, and AQ is 26 bp long). One pair of duplexes (AQ, Y) is separated from the other (X, Z) by a guanine-rich core made up of a GGGG sequence from each strand (Figure 1b). In an aqueous solution (pH 7.4) containing Li^+ ions but no K^+ or Sr^{2+} ions, the G-rich core might be expected to have a loose structure (Figure 1a, left), whereas the addition of K^+/Sr^{2+} ions could in principle enable the core to twist to a compact quadruple helix. If so, such a transition could be reversed by the addition of cation-specific chelators (18-crown-6 for K^+ and ethylenediaminetetraacetate (EDTA) for Sr^{2+}). To monitor the charge-flow efficiency through different conformers of twDNA, detector GG sequences were located in each duplex arm (5′-G of these dinucleotide sequences labeled as G_{AQ}, G_X, G_Y, and G_Z; Figure 1b; the guanine closer to the 5′ end of the dinucleotide is always more heavily damaged than the other guanine).[7] The a single strand was 14 nt longer than the b single strand, such that in the assembled twDNA a 14-nt oligonucleotide (AQ-P, containing a covalently attached AQ moiety on its 5′ end) could hybridize to the overhang of the a strand. The AQ moiety would then serve as a hole injector into the twDNA. Charge-flow-related guanine oxidation, monitored as DNA strand cleavage at G_X, G_Y, and G_Z relative to G_{AQ}, would provide a quantitative measure of charge flow across twDNA.

Guanines in both single-stranded and Watson–Crick base-paired double-stranded DNAs can be methylated at the

[*] Dr. Y. C. Huang, Prof. D. Sen
Department of Molecular Biology & Biochemistry
Simon Fraser University
Burnaby, British Columbia V5A 1S6 (Canada)
Prof. D. Sen
Department of Chemistry, Simon Fraser University
Burnaby, British Columbia V5A 1S6 (Canada)
E-mail: sen@sfu.ca

[**] This work was supported by the Natural Science and Engineering Research Council of Canada (NSERC) through a Discovery Grant (RGPIN 105785-2012). We are grateful to Dr. Jeff Warren for his valuable comments.

Supporting information for this article, including protocols for DNA purification and preparation, twDNA assembly, chemical protection assays, twDNA switching experiments with Sr^{2+} and EDTA, and data analysis, is available on the WWW under http://dx.doi.org/10.1002/anie.201407729.

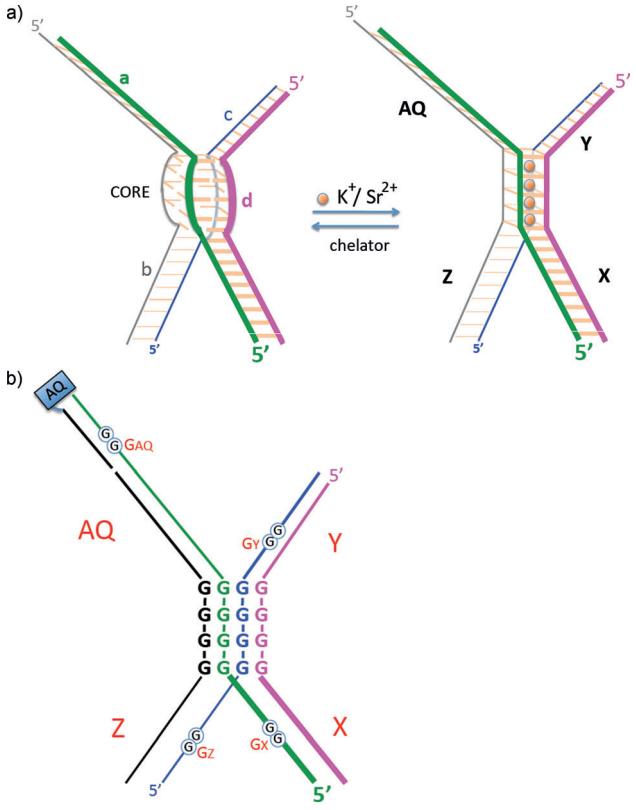

Figure 1. Design of twDNA. a) Design and conception of a prototypical twDNA. Four single-stranded DNAs, a, b, c, and d, self-assemble to give twDNA, made up of a guanine-rich core and four duplex arms: AQ, X, Y, and Z. In the absence of K^+ or Sr^{2+} ions (left), the four strands of the core are expected to have a loose single-stranded character. In the presence of K^+ or Sr^{2+} ions, however (right), the guanine-rich core should twist to form a quadruple-helical (right-handed) G-quadruplex, stabilized by K^+ or Sr^{2+} ions bound to the cavities between successive quartets. b) The specific twDNA used in this study. Altogether, five single-stranded DNA oligomers are used, with the base-pairing complementarities shown. A short DNA strand (AQ-P) derivatized with anthraquinone at its 5′ terminus forms a portion of the AQ arm. Strand a has two sets of specifically positioned GG dinucleotide "reporters"; the guanines closest to the 5′ end of these GG sequences are labeled G_{AQ} and G_X. Likewise, strand c has two reporters, with the guanines closest to the 5′ end labeled G_Y and G_Z.

guanine N7 position by dimethyl sulfate (DMS). However, guanines within G-quartets are not methylated by DMS because the N7 positions are involved in hydrogen bonding. Treatment with hot aqueous piperidine breaks the DNA backbone at the sites of G methylation. The resultant cleavage pattern within a given strand (5′-labeled with ^{32}P phosphate) can then be analyzed and quantitated by denaturing gel electrophoresis. To follow charge transfer in all four duplex arms as well as in the twDNA core, either the a or the c strand was singly ^{32}P-labeled.

K^+, Sr^{2+}, and Pb^{2+} ions are known to support G-quadruplex formation.[15] We examined whether these ions supported G-quadruplex formation by the core within the topological constraints of twDNA. Figure 2a shows guanine methylation protection data of twDNA in Tris buffer (25 mM Tris-HCl, pH 7.4) supplemented with Li^+ (100 mM, "Li"), K^+ (10, 100, or 250 mM, K1–K3), Pb^{2+} (1, 5, or 10 mM, Pb1–Pb3), or Sr^{2+} (1, 5, or 10 mM, Sr1–Sr3). The G lanes represent guanine sequence ladders for strand a (very left), and strand c (very right). The guanines shown inside a red rectangle in Figure 2a represent the G_4 core of a given strand. In Li^+ solutions, every guanine within the a and c strands of twDNA reacted with DMS. In K^+ and Sr^{2+} solutions, however, the core G_4 (but no other G) was protected from methylation (relative to Li^+) in both the a and c strands. Therefore, even within the topological constraints of twDNA, all core guanines from the four participating strands are able to participate in G-quadruplex formation in K^+ and Sr^{2+} solutions. In a solution containing Pb^{2+}, only the two central guanines of the core G_4 show levels of protection from DMS, consistent with an incomplete G-quadruplex formation within the Pb^{2+}–twDNA complex.

Figure 2b shows the results of charge-conduction-generated guanine oxidative damage. Inspection of the band intensities at G_X, G_Y, G_Z reveals that in neither Li^+ nor Pb^{2+} solution, charge flow was enabled to these sites. In contrast, the G_X, G_Y, G_Z intensities in K^+ or Sr^{2+} solution are significantly higher than in the Li^+ or Pb^{2+} solutions. Curiously, oxidative damage to G_{AQ} was also higher in the presence of K^+ and Sr^{2+} than in the presence of Li^+. One possibility is that this reflects an additional stabilization of the AQ duplex within twDNA by the formation of a stable G-quadruplex at the adjacent core. Alternatively, in the Li^+ and Pb^{2+} solutions, oxidative damage occurred preferentially in the relatively unstructured guanines of the core (yielding oxidized products that are less efficiently cleaved with piperidine relative to those formed in duplexes).[25] The band intensities at G_X, G_Y, and G_Z as either percentages of the total density of bands in a given gel lane (left) or as G_N/G_{AQ} ratios (right) are shown in Figure 2c (N = X, Y, or Z). Mean values obtained from 3–6 independent experiments are shown, and error bars represent one standard deviation. The G_N/G_{AQ} ratios (right), in particular, show that the efficiency of charge flow into the X, Y, and Z arms of Sr^{2+}–twDNA occurs at levels generally higher than in K^+–twDNA. It can further be seen that although charge flow to all three output duplexes (X, Y, and Z) was enhanced by K^+ and Sr^{2+}, the greatest enhancement occurred for the Y duplex. This likely due to the fact that passage of charge from the AQ duplex to the Y duplex requires a short transit through the core G-quadruplex, whereas passage to the other duplexes requires charge flow through the entire G-quadruplex. The higher level of charge flow to the Y duplex does not result from a direct interaction with the AQ moiety at the end of the AQ duplex as the Y arm is 17 bp and the AQ arm is 26 bp long. Even if these two duplexes were disposed to lie side-by-side in space, the AQ moiety would still be removed by as much as approximately 31 Å away from the "top" of the Y duplex. Thus, no direct interaction between the AQ moiety and the Y duplex is plausible.

Is twDNA capable of switching its conduction? Figure 3 shows that sequential back and forth switching between twDNA conformers is achievable by serial additions of $SrCl_2$ and EDTA. Lanes labeled "S" in Figure 3 report on solutions

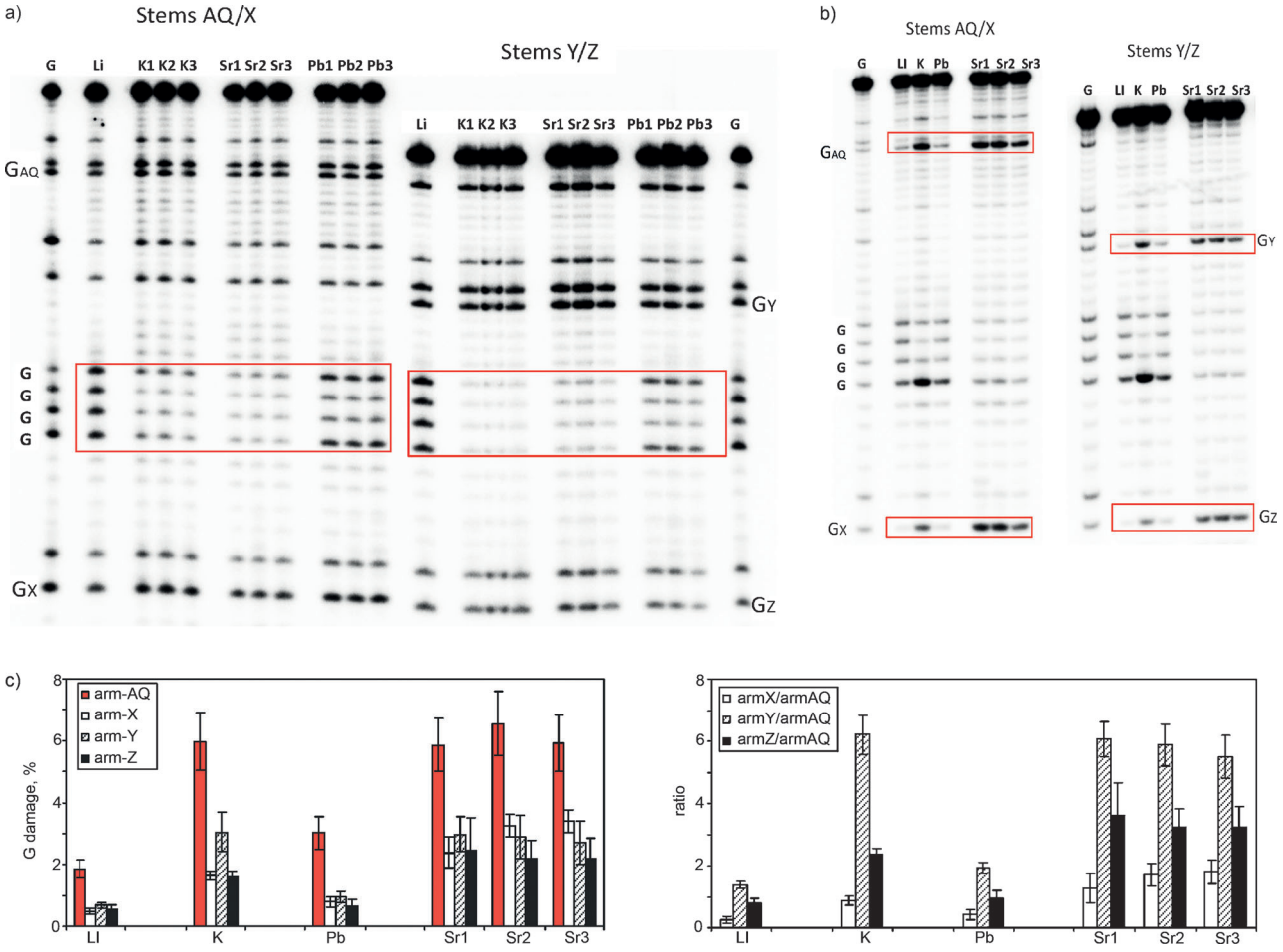

Figure 2. Methylation protection and charge-flow patterns within twDNA in the presence of specific cations. a) Dimethyl sulfate protection pattern of twDNA 5′-^{32}P-labeled on the a strand (reporting on stems AQ and X as well as the core; left) and the 5′-^{32}P-labeled c strand (reporting on stems Y and Z as well as the core; right). The four consecutive bands marked "G" refer to the guanines of the core. Lanes marked "G" show sequence ladders generated by DMS-mediated methylation of single-stranded strand a (very left) and strand c (very right). Li lanes show results obtained using Li^+ solutions (100 mM), K1, K2, and K3 those obtained with K^+ solutions (10, 100, and 250 mM, respectively), lanes labeled Sr1/Pb1–Sr3/Pb3 show results obtained with Sr^{2+}/Pb^{2+} solutions (1, 5, and 10 mM). b) Mapping of the charge-flow-generated guanine oxidative damage in twDNA. Li lanes: Li^+ (100 mM); K: K^+ (100 mM); Pb: Pb^{2+} (10 mM); Sr1, Sr2, and Sr3: Sr^{2+} (1, 5, and 10 mM, respectively). c) Plots of guanine oxidation levels at the G_{AQ}, G_X, G_Y, and G_Z sites. The data are presented in two formats; left: the intensity of the G_N band (where N = AQ, X, Y, or Z) as a percentage of the total intensity of bands in a given lane. Thus, the number plotted for stem AQ represents the band intensity at G_{AQ} divided by the total intensity of bands in that lane. Right: data shown as G_X/G_{AQ} (stem X/stem AQ), G_Y/G_{AQ}, and G_{YZ}/G_{AQ}. The error bars represent one standard deviation from the mean.

with a net excess of Sr^{2+} ions, whereas "E" lanes correspond to solutions with a net excess EDTA (see the Supporting Information for details). Figure 3 shows that the band intensities at G_X, G_Y, G_Z and G_{AQ}, as well as at the core guanines, recapitulate patterns seen in Figure 2b, with the lane E data resembling those from Li^+–twDNA, and the lane S data resembling Sr^{2+}–twDNA. Thus, it is indeed feasible to switch twDNA back and forth between conductive and less conductive conformers. Under the experimental conditions that we have tried quenching of Sr^{2+} by EDTA is not complete; however, it may be possible to find conditions where such quenching processes are more comprehensive. In the above experiments, the change in charge flow between the S and E states (Figure 3) is approximately three- to four-fold and thus sensitive enough to be detected both biochemically (as in Figure 3) as well as by chip-based methods such as cyclic voltammetry.[24]

To view twDNA as a prototype from which practical mechanoelectronic devices may be designed, it is key to investigate whether it is possible to predetermine the relative conductivities of the three recipient arms. One way to attempt this is to make substitutions of the core guanines. The most conservative change possible is to substitute individual guanines with hypoxanthine/inosine (I). Not only can I form mixed quartets with G without major destabilization,[26] but it also has a higher oxidation potential than G (1.5 V vs. 1.29 V, both relative to the normal hydrogen electrode (NHE)).[27,28] Therefore, we used selective G→I substitutions within the twDNA core to see whether this substitutions led to changes in the relative levels of charge flow to the three recipient

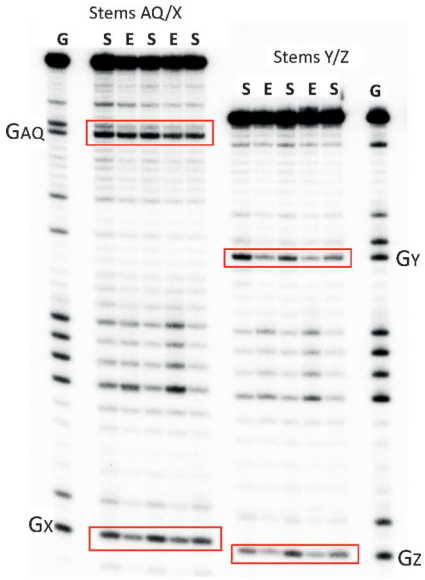

Figure 3. Reversible switching of the charge flow through twDNA by addition and sequestration of Sr^{2+} ions. Charge-conduction patterns through twDNA, in the presence of a net excess of Sr^{2+} ("S") or EDTA ("E").

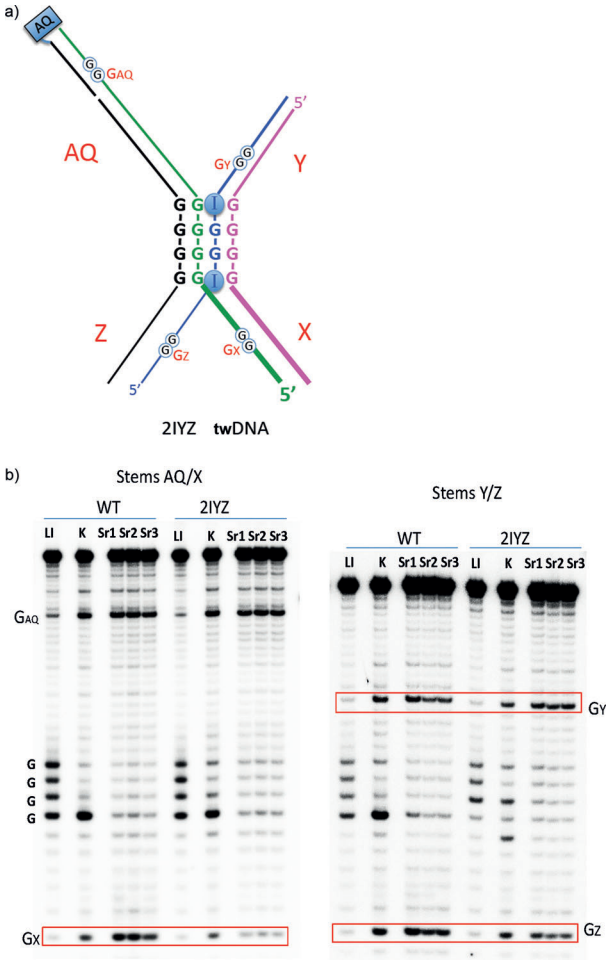

Figure 4. The effect of core inosine substitutions on the charge-flow patterns. a) A diagram showing the sites of guanosine to inosine substitution in strand c within 2IYZ twDNA. b) Charge-flow patterns in unmodified twDNA relative to inosine-modified 2IYZ twDNA.

duplex arms. Figure 4a shows the design of a doubly I-modified twDNA (2IYZ). This is one of many possible variants of the G-rich core; our initial choice was to investigate G→I substitutions within a single constituent strand within twDNA. Parallel charge flow experiments were now carried out on 2IYZ twDNA and on the unmodified twDNA ("WT"), in Sr^{2+} solution (1, 5, or 10 mM; Figure 4b). The control WT twDNA data conform with data shown in Figure 2, with poor charge flow to the G_X, G_Y, and G_Z sites in Li^+ solution and enhanced flow to all those sites in K^+ and Sr^{2+} solutions. However, with 2IYZ twDNA, the lanes show an interesting difference. In Li^+ solution, the G-oxidation patterns within strand a (Stems AQ/X, Figure 4b) are largely similar between the WT and 2IYZ complexes. Considering the oxidation patterns, the relative levels of charge transfer to the G_X, G_Y, and G_Z sites in WT compared to those for 2IYX, especially in the Sr lanes, are particularly interesting. Whereas the G_{AQ} and G_Y damage levels are comparable for the two DNA complexes, a marked decrease in charge transit to G_Z and complete abolition of charge transfer to G_X can be seen in 2IYZ relative to WT. Why this set of G→I substitutions leads to this particular conductive outcome is not immediately clear in the absence of high-resolution structural data. However, experiments with further G→I substitutions should provide deeper insights into the optimized conduction paths within twDNA.

In summary, we have described an unusual DNA switch, with one input lead and three output leads as well as a central core whose conformation (and conductivity) can be controlled by the binding or removal of specific cations, such as K^+ or Sr^{2+}. twDNA is both structurally and topologically a more complex assembly than other DNA electronic/mechatronic switches described before.[16,24] Control of the gross conductivity of twDNA is clearly achievable by addition and removal of metal ions such as Sr^{2+}. However, finer (as well as more directional) switching may be feasible by strategic doping, for example, with inosine, in combination with the incorporation of different "sensing" DNA elements, such as ligand–binding aptamers, into the design of twDNA. Although in this work, the data on twDNA conduction was presented in the form of charge-flow-promoted oxidative damage to guanines, prior experience with other DNA nanoswitches built from DNA helices is that their activity, although initially defined on the basis of guanine oxidative damage patterns, can be transferred to chip-based devices that may be studied by cyclic voltammetry or impedance spectroscopy.[17,18,24] An immediate utility of the study of conduction patterns through various G→I substituted twDNAs should be the fascinating insights into the optimal paths of hole conduction through G-quadruplexes.

Received: July 29, 2014
Revised: September 3, 2014
Published online: October 21, 2014

Keywords: cations · DNA structures · electrophoresis · G-quadruplexes · nanostructures

[1] N. C. Seeman, *Annu. Rev. Biochem.* **2010**, *79*, 65.
[2] F. A. Aldaye, A. L. Palmer, H. F. Sleiman, *Science* **2008**, *321*, 1795.
[3] C. K. McLaughlin, G. D. Hamblin, H. F. Sleiman, *Chem. Soc. Rev.* **2011**, *40*, 5647.
[4] J. Bath, A. J. Turberfield, *Nat. Nanotechnol.* **2007**, *2*, 275.
[5] C. Teller, I. Wilner, *Curr. Opin. Biotechnol.* **2010**, *21*, 376.
[6] Y. Krishnan, F. C. Simmel, *Angew. Chem. Int. Ed.* **2011**, *50*, 3124; *Angew. Chem.* **2011**, *123*, 3180.
[7] N. Turro, J. K. Barton, *J. Biol. Inorg. Chem.* **1998**, *3*, 201.
[8] B. Giese, *Annu. Rev. Biochem.* **2002**, *71*, 51.
[9] G. B. Schuster, *Acc. Chem. Res.* **2000**, *33*, 253.
[10] Y. C. Huang, A. K. Cheng, H.-Z. Yu, D. Sen, *Biochemistry* **2009**, *48*, 6794.
[11] D. Sen, W. Gilbert, *Nature* **1988**, *334*, 364.
[12] H. J. Lipps, D. Rhodes, *Trends Cell Biol.* **2009**, *19*, 414.
[13] M. Tarsounas, M. Tijsterman, *J. Mol. Biol.* **2013**, *425*, 4782.
[14] P. Murat, S. Balasubramanian, *Curr. Opin. Genet. Dev.* **2013**, *25C*, 22.
[15] "G-Quadruplexes and Metal Ions": N. H. Campbell, S. Neidle in *Interplay between Metal Ions and Nucleic Acids*, *Metal Ions in Life Sciences 10* (Eds.: A. Sigel, H. Sigel, R. K. Sigel), Springer, Amsterdam, **2012**, pp. 119 – 134.
[16] Y. Tang, B. Ge, D. Sen, H.-Z. Yu, *Chem. Soc. Rev.* **2014**, *43*, 518.
[17] Y. C. Huang, B. Ge, D. Sen, H.-Z. Yu, *J. Am. Chem. Soc.* **2008**, *130*, 8023.
[18] J. M. Thomas, B. Charaborty, D. Sen, H. Z. Yu, *J. Am. Chem. Soc.* **2012**, *134*, 13823.
[19] R. P. Fahlman, D. Sen, *J. Am. Chem. Soc.* **2002**, *124*, 4610.
[20] C. G. Sankar, D. Sen, *J. Mol. Biol.* **2004**, *340*, 459.
[21] J. M. Thomas, H. Z. Yu, D. Sen, *J. Am. Chem. Soc.* **2012**, *134*, 13738.
[22] R. P. Fahlman, M. C. Hsing, C. S. Sporer-Tuhten, D. Sen, *Nano Lett.* **2003**, *3*, 1073.
[23] Y. C. Huang, D. Sen, *J. Am. Chem. Soc.* **2010**, *132*, 2663.
[24] B. Ge, Y. C. Huang, D. Sen, H. Z. Yu, *Angew. Chem. Int. Ed.* **2010**, *49*, 9965; *Angew. Chem.* **2010**, *122*, 10161.
[25] C. J. Burrows, J. G. Muller, *Chem. Rev.* **1998**, *98*, 1109.
[26] J. Gros et al., *Nucleic Acids Res.* **2007**, *35*, 3064.
[27] S. Steenken, S. V. Jovanovic, *J. Am. Chem. Soc.* **1997**, *119*, 617.
[28] N. S. Hush, A. S. Cheung, *Chem. Phys. Lett.* **1975**, *34*, 11.

