Input-Dependent Induction of Oligonucleotide Structural Motifs for Performing Molecular Logic

Tao Li, Damian Ackermann, Anna M. Hall, and Michael Famulok*

Life and Medical Science (LIMES) Institute, Program Unit Chemical Biology and Medicinal Chemistry, University of Bonn, Gerhard-Domagk-Str. 1, 53121 Bonn, Germany

Supporting Information

ABSTRACT: The K⁺–H⁺-triggered structural conversion of multiple nucleic acid helices involving duplexes, triplexes, G-quadruplexes, and i-motifs is studied by gel electrophoresis, circular dichroism, and thermal denaturation. We employ the structural interconversions for performing molecular logic operations, as verified by fluorimetry and colorimetry. Short G-rich and C-rich cDNA and RNA single strands are hybridized to produce four A-form and B-form duplexes. Addition of K⁺ triggers the unwinding of the duplexes by inducing the folding of G-rich strands into DNA- or RNA G-quadruplex mono- and multimers, respectively. We found a decrease in pH to have different consequences on the resulting structural output, depending on whether the C-rich strand is DNA or RNA: while the protonated C-rich DNA strand folds into at least two isomers of a stable i-motif structure, the protonated C-rich RNA strand binds a DNA/RNA hybrid duplex to form a Y·RY parallel triplex. When using K⁺ and H⁺ as external stimuli, or inputs, and the induced G-quadruplexes as reporters, these structural interconversions of nucleic acid helices can be employed for performing logic-gate operations. The signaling mode for detecting these conversions relies on complex formation between DNA or RNA G-quadruplexes (G4) and the cofactor hemin. The G4/hemin complexes catalyze the H₂O₂-mediated oxidation of peroxidase substrates, resulting in a fluorescence or color change. Depending on the nature of the respective peroxidase substrate, distinct output signals can be generated, allowing one to operate multiple logic gates such as NOR, INH, or AND.

INTRODUCTION

Under standard conditions, two complementary strands of single-stranded (ss) DNA or RNA hybridize to form a Watson–Crick paired double-helix.1 Two types of triple-stranded helices of nucleic acids, parallel (Y·RY) and antiparallel (R·RY), can form when a third strand binds to the major groove of a Watson–Crick duplex via Hoogsteen hydrogen bonding.2,3 A Y·RY triplex is built on the C⁺·GC and T·AT base triplets, of which the formation of C⁺·GC is only allowed at acidic pH (below 6) when cytosine residues become protonated. Under acidic conditions, a protonated C-rich ssDNA strand can fold into a higher-order four-stranded structure called i-motif, consisting of two parallel duplexes whose C⁺·C base pairs are fully intercalated.4 RNA i-motif structures are also found under similar conditions, but these exhibit considerably lower stability than do the DNA counterparts.5 An intramolecular i-motif can adopt more than one folding topology,6 as evidenced by NMR and gel electrophoresis.5,7 Another four-stranded helix, the G-quadruplex (G4), is formed by G-rich ssDNA or RNA in the presence of monovalent cations like Na⁺ or K⁺.8 The G4-motif is built on the Hoogsteen hydrogen-bonded guanine tetrads that stack on one another, stabilized by van der Waals interactions.9,10 Monovalent cations like K⁺ can facilitate the π–π stacking of DNA and RNA G-quadruplex monomers to form stable dimers and trimers.11–14

There are some intrinsic relationships between these helical structures of nucleic acids. Two G-rich and C-rich strands can either hybridize to form a Watson–Crick double helix, or they individually fold into G-quadruplex and i-motif in the presence of K⁺ and H⁺. As a consequence, a potential competition can occur between duplex- and G-quadruplex or i-motif formation, which has been observed in biologically relevant nucleic acids like human telomeric DNA and others.15,16 Similarly, at acidic pH, both the i-motif and a C⁺·GC triplex can form.17 Hence, an additional level of competition, between i-motif- and triplex-formation, and between G-quadruplex and C⁺·GC triplex formation, may occur under certain conditions in response to external stimuli like K⁺ and H⁺. When equipped with appropriate fluorescent or other indicators, the response of nucleic acids to external stimuli can directly be followed. This read-out can be transduced into a Boolean logic operation, in which the presence or absence of a stimulus and the increase or decrease of the readout signal are related to a 1/0 event. Along this route, numerous molecular logic gates have been realized by employing various nucleic acids and read-out formats.18–20 However, a nucleic acid reconfiguration system covering the complexity of the possible interconversions of nucleic acids from homo- and heteroduplexes to triplexes, G-quadruplexes, and i-motifs has not yet been systematically devised.21

Received: November 19, 2011
Published: January 31, 2012
Horseradish peroxidase (HRP) and catalyzes the H$_2$O$_2$-dependent oxidation of peroxidase substrates. This detection relies on the specific binding of the catalytic quadruplex complex in the presence of peroxidase substrates. Depending on the applied conditions, the G/C-rich oligonucleotides can potentially rearrange into a variety of structural motifs. These range from A- and B-type homo- and heteroduplexes, a C$^+$-GC triple helix, G-quadruplexes, and i-motif structures. Under certain conditions, these oligonucleotides engage in various structural motifs that have the potential to interconvert from one to another. To establish conditions for interconversion and to analyze the various motifs formed, we designed short G-rich and C-rich cDNA- and RNA-oligonucleotides D1, D2, R1, and R2 that easily hybridize to form four Watson–Crick duplexes. Upon addition of K$^+$ and H$,^+$ different structural conversions are expected to occur to these duplexes (Figure 1a), due to the difference in the stability of various structural motifs.

We employed PAGE under nondenaturing conditions to analyze the structures formed by the different pairs of oligonucleotides under different conditions (Figure 2, lanes 1–4), with four constituent strands as the controls (lanes 5–8). At pH 8.5 and in the absence of K$^+$-ions, only a single band appears for each duplex (Figure 2a, lanes 1–4), indicating that each pair of complementary strands forms a stable double helical structure. The corresponding CD spectra show that the DNA duplex (D1D2) has a positive peak near 270 nm and another smaller one around 220 nm (Figure 3a), consistent with the CD characteristics of a B-form conformation. The RNA duplex (R1R2) has a negative peak at 213 nm and a positive one near 270 nm with a shoulder around 250 nm, indicating that this duplex adopts a A-form conformation.

The CD characteristics of R1D2 are more similar to those of A-form RNA than to those of B-form DNA, indicating that the global conformation of this DNA/RNA hybrid duplex is A-form. However, the CD spectrum of D1R2 shows it appears to adopt an intermediate conformation, neither B-form nor A-form. A similar phenomenon also occurred to other DNA/RNA hybrid duplexes. The RNA and DNA/RNA hybrid duplexes all have a lower mobility than the DNA duplex (Figure 2a), consistent with previous observations. Under the same conditions at pH 8.5 and in the absence of K$^+$-ions, the C-rich control strands R2 and D2 have typical CD characteristics of poly-C single strands, with a positive band near 275 nm (Figure 3a). The two G-rich strands, especially R1, have CD features of parallel G-quadruplexes, with a positive band near 260 nm and negative one around 240 nm. This suggests that D1 and R1 fold into parallel G-quadruplexes even in the absence of K$^+$. The melted D1 runs slowly in PAGE (Figure 2a, lane 7), while D1 is smeared (lane 5), presumably due to the poor thermal stability of its G-quadruplex structure (no obvious $T_m$ see Figure S1 in the Supporting Information) in the absence of metal cations. The intact but widened band of folded D1 without K$^+$ is only observed at acidic pH (Figure 2c,
Upon incubation with K⁺, some dramatic changes are observed in the electrophoretic behavior of the different oligonucleotides (Figure 2b). More than one band is clearly observed in lanes 1 and 4, with the same mobility as the oligonucleotides (Figure 2b). More than one band is clearly observed in the electrophoretic behavior of the different phenomena are observed in PAGE (Figure 2c). In lanes 1 and 3, there is one band with the same mobility as D1 or R1, respectively, and a band with the same mobility as D2 (see lanes 5, 7, 8). This demonstrates that both duplexes D1D2 and R1D2 completely unwind at pH 4.5, presumably by forming i-motif structures. Formation of the i-motif is evidenced by CD spectroscopy (Figure 3c). Both D1D2 and R1D2 display a positive band near 290 nm in the corresponding CD spectra (Figure 3c, left panel). The CD spectrum of the D2 control has a dominant positive band near 290 nm and a negative one around 260 nm (Figure 3c, right panel), consistent with the characteristics of an i-motif structure.24 In contrast, the CD characteristics of R2 still remain as those of an unfolded single strand, because the i-motif structure of C-rich RNA is so unstable that it only exists at millimolar strand concentrations and at low temperature.5 Note that the Tm value of folded D2 is increased as the strand concentration increases (see Figure S3 in the Supporting Information), which is a typical characteristic of an intermolecular structure.34 That is, D2 forms the intermolecular i-motif structure at acidic pH, which should be attributed to its three short loops (each composed of one T residue) not compatible with the intramolecular folding of i-motif.7 In principle, an intramolecular i-motif has four possible folding configurations,6 and two bands of i-motif isomers can be observed in gel electrophoresis.7 Similarly, two bands of D2 are always observed in PAGE at acidic pH (Figure 2c and d, lanes 1, 3, 8). Considering only one phase transition in the melting profile of folded D2 (see Figure S1), the two bands of D2 suggest that this intermolecular i-motif also has more than one possible folding configuration, as described previously (see Figure S4 in the Supporting Information for the proposed structures of the intermolecular i-motif).7 In case of D1R2, we observe two strong bands in purple under acidic conditions (Figure 2c, lane 4), and a faint band in cyan between them, presumably corresponding to D1 folded into G4 (red asterisk; compare with lane 5). This band indicates that a small amount of D1R2 unwinds, but neither of the other two bands (purple) corresponds to R2 (compare lane 6). On the other hand, the corresponding CD spectrum of D1R2 in Figure 3c (line in}

![Diagram](image-url)
cyan) at pH 4.5 appears to be very similar to that of the A-form duplex D1R2 observed at pH 8.5 (Figure 3a, left panel, cyan line). However, the main difference between D1R2 at pH 8.5 vs 4.5 is revealed by their melting behavior: the $T_m$ of D1R2 at pH 4.5 is increased by 7.8 °C as compared to the $T_m$ measured at pH 8.5 (Figure 4). The Δ$T_m$ of 7.8 °C suggests the formation of a triple helix structure R2⁺·D1R2 that accommodates the protonated R2 strand. This notion is supported by the fact that the cytosine base is easily protonated under acidic conditions, allowing the formation of either an i-motif or a C⁺·GC triplex.3,35 Our CD measurements (Figure 3c, right panel, green line) indicate that the C-rich R2 oligonucleotide prefers to form the single strand structure rather than the RNA i-motif structure. The protonated, single-stranded R2 can readily bind to the major groove of the duplex D1R2 by forming C⁺·GC base triplets, resulting in a Y·RY parallel triplex.2,3 To investigate this hypothesis, we added an equivalent of R2 to the D1R2, and an equivalent of R2 to the R1R2 under the same conditions (Figure 2c, lanes 9 and 10). Analysis by PAGE revealed a significant increase of the band that corresponds to
the band in lane 4 displaying the slowest electrophoretic mobility (lane 9, Figure 2c). A second band migrates exactly with the duplex in lane 4. Only a very small amount of single-stranded R2 is observed under these conditions (compare lane 6). This strongly suggests that R2 can combine with D1R2, and the resulting structure is an R2·D1R2 triplex. Although the duplex coexists with the triplex R2·D1R2, only one transition is observed in the melting profile of D1R2 under acidic condition (Figure 4). Interestingly, the T<sub>m</sub> under acidic conditions increases by 7–8 °C as compared to the curve obtained at pH 8.5. This indicates the formation of a structure that exhibits higher stability than the heteroduplex, in accordance with triplex formation.

In contrast, the band pattern obtained in lane 10 of Figure 2c corresponds to that of the R1R2 duplex and the unpaired R2, indicating that the excess R2 oligonucleotide does not engage in triplex formation with the R1R2 duplex. A likely explanation is that the D1R2 triplex is in a parallel G-quadruplex rather than triplex (Figure 3d; see also Supporting Information), which enables the hemin catalytic activity toward the H<sub>2</sub>O<sub>2</sub>-mediated oxidation of Sc, AR, and TMB under appropriate conditions (see Figure S6 in the Supporting Information). In the presence of hemin, both D1 and R1 have a moderate catalytic activity toward the H<sub>2</sub>O<sub>2</sub>-mediated oxidation of Sc, AR, and TMB under appropriate conditions (see Figure S6 in the Supporting Information), which enables the hemin–G-quadruplex complex to serve as the reporter in this logic system.

A Versatile Molecular Logic Device Built on Nucleic Acid Helices. The structural conversion of nucleic acid helices may find applications in some fields. As a proof-of-concept experiment, we devise multiple logic gates based on nucleic acid structural conversion, where two triggering ions (K<sup>+</sup> and H<sup>+</sup>) serve as the inputs (Figure 1b). The logic output of this system relies on the peroxidase activity of DNA and RNA G-quadruplexes combined with a catalytic cofactor hemin. There are some appropriate peroxidase substrates such as scopoletin (Sc), Amplex Red (AR), and 3,3′,5,5′-tetramethylbenzidine (TMB) for different detection means (Figure 5b). In the presence of hemin, both D1 and R1 have a moderate catalytic activity toward the H<sub>2</sub>O<sub>2</sub>-mediated oxidation of Sc, AR, and TMB under appropriate conditions (see Figure S6 in the Supporting Information), which enables the hemin–G-quadruplex complex to serve as the reporter in this logic system.

Sc is a fluorescent substrate that can be oxidized to a nonfluorescent product of unknown structure, catalyzed by HRP (Figure 5b). Here, it also proves as a substrate suitable for the HRP-mimicking hemin–G-quadruplex complex (see Figure S6a in the Supporting Information). Two additional features make this substrate interesting with respect to being used for logic-gate operations: (1) Sc is H<sub>2</sub>O<sub>2</sub>-resistant in the absence of catalysts; and (2) it is highly sensitive to pH; at low pH its fluorescence is remarkably reduced as compared to pH above 8. These factors would make Sc suitable for application in a NOR logic gate operation, as the two inputs K<sup>+</sup> and H<sup>+</sup>
both cause a decrease in the fluorescence intensity of Sc during the structural conversion.

Figure 6 shows different logic behaviors of four duplexes at four input modes in the presence of Sc. Without any input, fluorescence intensity of Sc is high in four cases, because no hemin–G-quadruplex complex is formed. Upon input of K⁺, the fluorescence decreases sharply in two cases (D1D2 and D1R2), resulting from the oxidation of Sc catalyzed by hemin–D1 complex. This means the K⁺-triggered unwinding of two duplexes and the formation of G-quadruplex, consistent with the observations in PAGE and CD measurements. In contrast, the fluorescence does not decreases so obviously in the other two cases (R1R2 and R1D2), attributed to no or only partial unwinding of these duplexes as evidenced by PAGE. In the presence of H⁺, the fluorescence intensity is always low due to acid quenching. With a threshold of 0.5 for logic output (1/0), both D1D2 and D1R2 behave as a two-input NOR logic gate, while R1R2 and R1D2 behave as an INV gate inverting the logic input (1/0) of H⁺.43 The corresponding truth tables of these logic gates are shown in Table 1.

AR is another peroxidase substrate for fluorometric H₂O₂ determination catalyzed by HRP,44 and has also been applied to the hemin–G-quadruplex complex.45 Unlike Sc, AR itself has no fluorescence behavior, but its enzymatic oxidation product (resorufin) is highly fluorescent (Figure 5b).46 Similarly, the fluorescence of resorufin is sensitive to pH and quenched by acids.46 These factors enable the utilization of AR as another peroxidase substrate to devise an INH logic gate based on the K⁺–H⁺-triggered structural conversion of four duplexes. Figure 7 shows that in the absence of K⁺ and H⁺, the fluorescence intensity is always low in four cases. Under this condition, the background fluorescence originates from slow catalysis by unbound hemin. Upon input of K⁺, the fluorescence increases sharply in two cases (D1D2 and D1R2), attributed to the production of resorufin from AR oxidation catalyzed by hemin–G-quadruplex complex. In contrast, R1R2 and R1D2 cannot cause an obvious increase in fluorescence intensity, because they are unable to completely unwind to release the G-quadruplex. In the presence of H⁺, the fluorescence intensity is always close to zero due to acid quenching. With the output threshold of 0.5, the logic behaviors of D1D2 and D1R2 are consistent with a two-input INH gate, while both R1R2 and R1D2 behave as a ZERO gate that always outputs 0.43 The truth tables of these logic gates are shown in Table 1.

We notice that in the presence of Sc and AR, the structural conversion of nucleic acid helices occurring at acidic pH cannot be exactly reflected by fluorescence change, due to their pH-sensitive properties. In principle, this is achievable using a pH-resistant substrate instead of them. However, most fluorescent peroxidase substrates are sensitive to pH and lose their fluorescent activity under acidic conditions. Therefore, we turned to colorimetric substrates instead of fluorogenic ones to be able to signal at acidic pH. TMB is one of the proper candidates. In contrast to Sc and AR, TMB is an acid-favored...
peroxidase substrate, which can be oxidized to colored products (Figure 5b) catalyzed by HRP47 or the hemin−G-quadruplex complex48 under acidic conditions. Note that the enzymatic oxidation of TMB is very slow and results in two colored products, blue and yellow, over different reaction periods.47 Under our conditions, the time-dependent reaction shows that the initial blue product of TMB is dominant within several hours (see Figure S7 in the Supporting Information). The reaction solution then gradually turns to green, a mixture of the initial blue product and final yellow product.47

With TMB as the substrate, the existence of hemin−G-quadruplex complex can be detected in the presence of both H⁺ and K⁺, which meets the need of devising an AND logic gate (Figure 8). However, both hemin−D1 and hemin−R1 exhibit the peroxidase activity independent of K⁺ (see Figure S6c in the Supporting Information), because D1 and R1 can fold into G-quadruplex even in the absence of metal cations (Figure 3c, right panel). As a result, D1D2 and R1D2 always output 1 in acidic pH and 0 in basic pH, consistent with an ID logic gate that always identifies with the logic input of H⁺.43 In the presence of H⁺ alone, D1R2 forms a triplex and only releases a small amount of G-quadruplex. In this case, the catalytic activity is not so high (output 0), and thus D1R2 behaves as a two-input AND logic gate. For R1R2, it is still consistent with a ZERO gate. The truth tables of these logic gates are shown in Table 1. Note that reversible logic gates might be obtained simply by reverting the H+- and K+-triggered structural conversions.49 This might provide access to more complex computing circuits.

### CONCLUSIONS

We have utilized a nucleic acid system consisting of G-rich and C-rich complementary strands to study the K⁺−H⁺-triggered conversion of multiple helical structures involving duplexes, triplexes, G-quadruplexes, and i-motif. Four A-form and B-form duplexes are formed by hybridizing two complementary strands, evidenced by PAGE and CD. Upon addition of K⁺, the DNA and DNA/RNA hybrid duplexes are subject to unwinding (albeit not completely in some cases), resulting from the formation of G-quadruplexes by G-rich strands. The multimers (dimer and trimer) of DNA and RNA G-quadruplexes were observed in PAGE, together with monomers. H⁺ is also able to trigger the unwinding of DNA and DNA/RNA duplexes, attributed to the formation of an i-motif...
structure or a Y-RY parallel triplex. PAGE evidences at least two isomers of an intermolecular i-motif. At acidic pH, the formed triplex also undergoes a structure conversion upon addition of K⁺, because the G-rich strand is prone to fold into G-quadruplex. In all cases, the RNA duplex is always kept unchanged due to high stability.

Further, the K⁺—H⁺-triggered structural changes of nucleic acid helices have been utilized to build a versatile molecular logic device. Here, K⁺ and H⁺ serve as two inputs, and the released G-quadruplex behaves as the reporter for signal output. After being bound by the cofactor hemin, DNA and RNA G-quadruplexes exhibit peroxidase activity in the presence of substrates Sc, AR, and TMB, resulting in a fluorescence or color change. Multiple logic gate operations (NOR, INH, AND, etc.) are achieved with fluorometry and colorimetry, by means of different catalytic behaviors of hemin–G-quadruplex complex in three peroxidase substrates.

Our study provides rich and useful information for the interconversion of nucleic acid helical structures, which helps to further devise molecular machines and nanodevices built on nucleic acids. For example, this trigger-switched system may function in the context of larger DNA-nanoarchitectures like DNA rotaxanes or interlocked dsDNA-nanostructures such as DNA interlocked rotaxanes or catenanes. From the point of view of molecular computing, the development of versatile logic devices will facilitate constructing more advanced molecular calculators or computing circuits.

**ASSOCIATED CONTENT**

**Supporting Information**

Melting profiles of G-quadruplexes and i-motif, proposed structures of G4 multimers and i-motif isomers, concentration-dependent Tm of D2, CD spectral change of four duplexes, catalytic behaviors of D1 and R1, time-dependent H2O2-mediated oxidation of TMB, and raw spectral data for logic operations. This material is available free of charge via the Internet at http://pubs.acs.org.

**AUTHOR INFORMATION**

Corresponding Author
m.famulok@uni-bonn.de

Notes
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This work is supported by grants from the Alexander von Humboldt Foundation, the ERC, the ESF, and the SFB 624.

**REFERENCES**

1. Watson, J. D.; Crick, F. H. Nature 1953, 171, 737–738.
2. Fox, K. R. Curr. Med. Chem. 2000, 7, 17–37.
3. Frank-Kamenetski, M. D.; Mirkin, S. M. Annu. Rev. Biochem. 1995, 64, 65–95.
4. Gehring, K.; Leroy, J. L.; Gueron, M. Nature 1993, 365, 561–565.
5. Snoossi, K.; Nonin-Lecomte, S.; Leroy, J. L. J. Mol. Biol. 2001, 309, 139–153.
6. Leroy, J. L.; Gueron, M.; Mergny, J. L.; Helene, C. Nucleic Acids Res. 1994, 22, 1600–1606.
7. Mergny, J. L.; Lacroix, L.; Han, X. G.; Leroy, J. L.; Helene, C. J. Am. Chem. Soc. 1995, 117, 8887–8898.
8. Sen, D.; Gilbert, W. Nature 1990, 344, 410–414.
9. Gellert, M.; Lipsett, M. N.; Davies, D. R. Proc. Natl. Acad. Sci. 1962, 48, 2013–2018.
10. Williamson, J. R. Annu. Rev. Biophys. Biomol. Struct. 1994, 23, 703–730.
11. Sen, D.; Gilbert, W. Biochemistry 1992, 31, 65–70.
12. Smargiasso, N.; Rosu, F.; Hsia, W.; Colson, P.; Baker, E. S.; Bowers, M. T.; De Pauw, E.; Gabelica, V. J. Am. Chem. Soc. 2008, 130, 10208–10216.
13. Martadinata, H.; Phan, A. T. J. Am. Chem. Soc. 2009, 131, 2570–2578.
14. Collie, G. W.; Parkinson, G. N.; Neidle, S.; Rosu, F.; De Pauw, E.; Gabelica, V. J. Am. Chem. Soc. 2010, 132, 9328–9334.
15. Pan, A. T.; Mergny, J. L. Nucleic Acids Res. 2002, 30, 4618–4625.
16. Risitano, A.; Fox, K. R. Biochemistry 2003, 42, 6507–6513.
17. (a) Choi, J.; Majima, T. Chem. Soc. Rev. 2011, 40, 5893–5909. (b) Lacroix, L.; Mergny, J. L.; Leroy, J. L.; Helene, C. Biochemistry 1996, 35, 8715–8722.
18. Kolpakchikov, D. M.; Stojanovic, M. N. J. Am. Chem. Soc. 2005, 127, 11348–11351.
19. Elbaz, J.; Lioubabashevski, O.; Wang, F.; Remacle, F.; Levine, R. D.; Willner, I. Nat. Nanotechnol. 2010, 5, 417–422.
20. Win, M. N.; Smolke, C. D. Science 2008, 322, 456–460.
21. Szalicskii, K. Chem. Rev. 2008, 108, 3481–3548.
22. Travascio, P.; Li, Y.; Sen, D. Chem. Biol. 1998, 5, 505–517.
23. Travascio, P.; Bennet, A. J.; Wang, D. Y.; Sen, D. Chem. Biol. 1999, 6, 779–877.
24. Kyp, J.; Kejnovska, I.; Renciuk, D.; Vorlickova, M. Nucleic Acids Res. 2009, 37, 1713–1725.
25. Salazar, M.; Fedoroff, O. Y.; Miller, J. M.; Ribeiro, N. S.; Reid, B. R. Biochemistry 1993, 32, 4207–4215.
26. Bhattacharyya, A.; Murchie, A. I.; Lilley, D. M. Nature 1990, 343, 484–487.
27. Roberts, R. W.; Crothers, D. M. Science 1992, 258, 1463–1466.
28. Ratmeyer, L.; Vaninay, R.; Zhong, Y. Y.; Zon, G.; Wilson, W. D. Biochemistry 1994, 33, 5298–5304.
29. Gulik, A.; Inoue, H.; Luzzato, V. J. Mol. Biol. 1970, 53, 221–238.
30. Scheheragen, M. A.; Bokma, J. T.; Vlaanderen, C. A.; Blok, J.; van Grondelle, R. Biopolymers 1986, 25, 1419–1448.
31. Hazel, P.; Huppert, J.; Balasubramanian, S.; Neidle, S. J. Am. Chem. Soc. 2004, 126, 16405–16415.
32. Rachwal, P. A.; Findlow, I. S.; Werner, J. M.; Brown, T.; Fox, K. R. Nucleic Acids Res. 2007, 35, 4214–4222.
33. Bugaut, A.; Balasubramanian, S. Biochemistry 2008, 47, 689–697.
34. Mergny, J. L.; Lacroix, L. Oligonucleotides 2003, 13, 515–537.
35. Gueron, M.; Leroy, J. L. Curr. Opin. Struct. Biol. 2000, 10, 326–331.
36. Han, H. Y.; Dervan, P. B. Proc. Natl. Acad. Sci. U.S.A. 1993, 90, 3806–3810.
37. Escude, C.; Francois, J. C.; Sun, J. S.; Ott, G.; Sprinzl, M.; Garestier, T.; Helene, C. Nucleic Acids Res. 1993, 21, 5547–5553.
38. Lesnik, E. A.; Freier, S. M. Biochemistry 1995, 34, 10807–10815.
39. Barone, F.; Cellai, L.; Matzeu, M.; Mazzei, F.; Pedone, F. Biophys. Chem. 2000, 86, 37–47.
40. Kong, D. M.; Yang, W.; Wu, J.; Li, C. X.; Shen, H. X. Analyst 2010, 135, 321–326.
41. Cheng, X.; Liu, X.; Bing, T.; Cao, Z.; Shangguan, D. Biochemistry 2009, 48, 7817–7823.
42. Miller, R. W.; Sirois, J. C.; Morita, H. Plant Physiol. 1975, 55, 35–41.
43. Rurack, K.; Triftlunger, C.; Koval’chuk, A.; Daub, J. Chem.-Eur. J. 2007, 13, 8998–9003.
44. Zhou, M. J.; Diwu, Z. J.; PanchukVoloshina, N.; Haugland, R. P. Anal. Biochem. 1997, 253, 162–168.
45. Li, C. I.; Liu, K. T.; Lin, Y. W.; Chang, H. T. Anal. Chem. 2011, 83, 225–230.
46. Towne, V.; Will, M.; Oswald, B.; Zhao, Q. J. Anal. Biochem. 2004, 334, 290–296.
(47) Josephy, P. D.; Eling, T.; Mason, R. P. J. Biol. Chem. 1982, 257, 3669–3675.
(48) Li, T.; Li, B.; Wang, E.; Dong, S. Chem. Commun. 2009, 3551–3553.
(49) (a) Liu, D.; Balasubramanian, S. Angew. Chem., Int. Ed. 2003, 42, 5734–5736. (b) Sanneho, Y.; Endo, M.; Katsuda, Y.; Hidaka, K.; Sugiyama, H. J. Am. Chem. Soc. 2010, 132, 16311–16313.
(50) (a) Teller, C.; Willner, I. Trends Biotechnol. 2010, 28, 619–628. (b) Willner, I.; Allen, V. Org. Biomol. Chem. 2006, 4, 3381–3382. (c) Alberti, P.; Bourdoncle, A.; Saccà, B.; Lacroix, L.; Mergny, J. L. Org. Biomol. Chem. 2006, 4, 3383–3391.
(51) (a) Rasched, G.; Ackermann, D.; Schmidt, T. L.; Broekmann, P.; Heckel, A.; Famulok, M. Angew. Chem., Int. Ed. 2008, 47, 967–970. (b) Mayer, G.; Ackermann, D.; Kuhn, N.; Famulok, M. Angew. Chem., Int. Ed. 2008, 47, 971–973. (c) Ackermann, D.; Schmidt, T. L.; Hannan, J. S.; Purohit, C. S.; Heckel, A.; Famulok, M. Nat. Nanotechnol. 2010, 5, 436–442. (d) Ackermann, D.; Rasched, G.; Verma, S.; Schmidt, T. L.; Heckel, A.; Famulok, M. Chem. Commun. 2010, 46, 4154–4156.
(52) (a) Weizmann, Y.; Braunschweig, A. B.; Wilner, O.; Cheglakov, Z.; Willner, I. Proc. Natl. Acad. Sci. U.S.A. 2008, 105, 5289–5294. (b) Schmidt, T. L.; Heckel, A. Nano Lett. 2011, 11, 1739–1742.