DNA Recognition

DOI: 10.1002/ange.200503822

# Molecular Recognition of a Three-Way DNA Junction by a Metallosupramolecular Helicate**

*Aneta Oleksi, Alexandre G. Blanco, Roeland Boer, Isabel Usón, Joan Aymamí, Alison Rodger, Michael J. Hannon,* and Miquel Coll**

Although much current drug research focuses on agents that inhibit the action of proteins, nucleic acids are attractive therapeutic targets because of their potential for controlling

[*] Professor M. J. Hannon
School of Chemistry
University of Birmingham
Edgbaston, Birmingham B15 2TT (UK)
Fax: (+44) 121-414-7871
E-mail: m.j.hannon@bham.ac.uk

Dr. A. Oleksi, A. G. Blanco, Dr. R. Boer, Professor M. Coll
Institut de Biologia Molecular de Barcelona-CSIC and
Institut de Recerca Biomèdica
Parc Científic de Barcelona
Josep Samitier 1–5, 08028 Barcelona (Spain)
Fax: (+34) 93-403-4979
E-mail: mcoll@ibmb.csic.es

Prof. I. Usón
Institut de Biologia Molecular de Barcelona-CSIC and
Institució Catalana de Recerca i Estudis Avançats
Jordi Girona 18, 08034 Barcelona (Spain)

Dr. J. Aymamí
Departament d'Enginyeria Química
Universitat Politècnica de Catalunya
Diagonal 647, 08028 Barcelona (Spain)

Professor A. Rodger
Department of Chemistry
University of Warwick
Coventry, CV4 7AL (UK)

[**] This work was supported by an EU Marie Curie Research Training Network (HPRN-CT-2002-00175) and by grants (to M.C.) from the Ministerio de Educación y Ciencia and the Generalitat de Catalunya. M.J.H. is the Royal Society of Chemistry Sir Edward Frankland Fellow 2004-5. A.G.B. is the recipient of a fellowship from the Ministerio de Sanidad y Consumo. We thank Dr. J. Kerckhoffs and J. Peberdy for helicate synthesis.

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

disease at the source. Indeed, DNA is already the target for widely used anticancer molecules such as intercalators and platinum drugs; consequently the field of DNA recognition has attracted much interest. Existing synthetic agents that bind to DNA do so essentially in one of five distinct modes:[1] They bind covalently (or through coordination bonds) to the DNA bases (as cisplatin), intercalate between the bases, bind in the major groove (often with formation of hydrogen bonds to the bases), bind in the minor groove (as polyamide groove binders), or bind to the sugar–phosphate backbone. In spite of many efforts to redesign their chemical structure and narrow down their binding capacities to specific DNA sequences, DNA-binding drugs (particularly those which have reached the clinic) are, in general, nonspecific drugs with broad cytotoxic effects. The ability to recognize a specific unusual DNA structure—in contrast to strict sequence recognition—is an attractive alternative for gaining specificity. Herein we present a completely new mode of DNA recognition through an atomic resolution X-ray crystal structure of a three-way DNA junction in complex with a synthetic tetracationic supramolecular helicate that fits perfectly into the central-trigonal hydrophobic cavity of the DNA junction. This is not only without precedent as a mode of DNA recognition, but reveals a three-way DNA junction as a well-defined potential structural target for novel, highly specific drugs.

DNA junctions are unique branched structures that consist of several double strands converging at one point. The best-characterized DNA junction is the four-way junction, also known as a Holliday junction, a key intermediate in homologous recombination.[2] Three-dimensional structures of free four-way DNA junctions[3] and different complexes with proteins have been solved.[4] In contrast, three-way junctions, although being the simplest and most abundant nucleic acid branched structures, are not so well characterized. Three-way junctions occur both in RNA and DNA. In RNA they are involved in crucial biological functions such as splicing[5] and translation,[6] and in DNA they are formed transiently during DNA replication (the replication fork).[7] They are also intermediate structures during triplet repeat expansions,[8] anomalies associated with several human genetic diseases such as myotonic dystrophy type 1 and Huntington's disease.[9] Three-way junctions are present in the inverted terminal repeats of certain viral genomes[10] and are intermediates during phage genetic recombination.[11]

Inspired by nature, we have been exploring a new approach for synthetic DNA recognition through the design of agents that possess similar nanoscale molecular surfaces to those of biomolecular DNA-recognition motifs.[12,13] In particular, we have used metallosupramolecular chemistry to generate nanoscale synthetic agents, such as the tetracationic supramolecular helicate (Figure 1 a) $[Fe_2L_3]^{4+}$, which is formed from three bis-pyridylimine organic strands wrapped about two $Fe^{2+}$ ions. We have previously described the binding of this agent to natural polymeric DNAs and the remarkable intramolecular DNA coiling that results.[12] To gain more information we have been attempting to crystallize the agent with oligonucleotides. We report herein the complex with a DNA palindromic hexanucleotide: 5′-d(CGTACG)-3′. To our surprise, we observe molecular recognition not of duplex DNA but rather of a singular DNA structure, a three-way junction (Figure 1 b), which defines a unique triangular-shaped hydrophobic binding site.

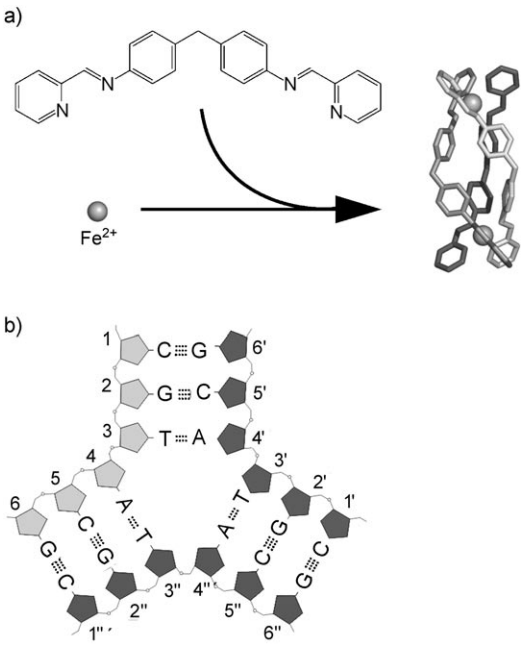


***Figure 1.*** a) The $[Fe_2L_3]^{4+}$ ($L = C_{25}H_{20}N_4$) tetracationic supramolecular helicate with the $Fe^{2+}$ ions represented as spheres; b) The three-way junction of this study showing the DNA sequence used, 5′-d(CGTACG)-3′, and the base-pair arrangement.

The structure of the DNA–supramolecule complex is shown in Figure 2. The DNA has formed a three-way Y-shaped junction and the central hollow space of the junction is occupied by the tetracationic supramolecular compound. The structure of the helicate drug does not suffer any significant conformational change upon binding to DNA (see the Supporting Information). This helicate has remarkable dual characteristics: on one hand it has high positive charge because of the two $Fe^{2+}$ ions axially located at both ends of the drug and, on the other hand, it displays a large hydrophobic surfaces due to the presence of 12 aromatic rings. Both characteristics are driving forces for the noncovalent recognition of the DNA junction by the helicate. First, an electrostatic-dipole interaction occurs between the positively charged supramolecule and the negatively charged DNA phosphates (Figure 3 a). Second, the phenyl rings at the centre of the helicate form extensive π-stacking interactions with the thymine and adenine bases at the junction. Considering a single organic strand, one phenyl ring (B) is stacked on T3, whereas the other phenyl ring (C) is stacked on A4 (Figure 3 b). Because of the threefold symmetry of the drug, which matches the threefold symmetry of the three-way junction, this double stacking interaction is repeated three times. Also, strikingly, the twist between the two phenyl rings B and C of the drug is exactly 60°, which matches the 60° kink in the DNA strand between bases T3 and A4. This therefore allows a perfect stack between consecutive bases and consecutive phenyl rings.

  

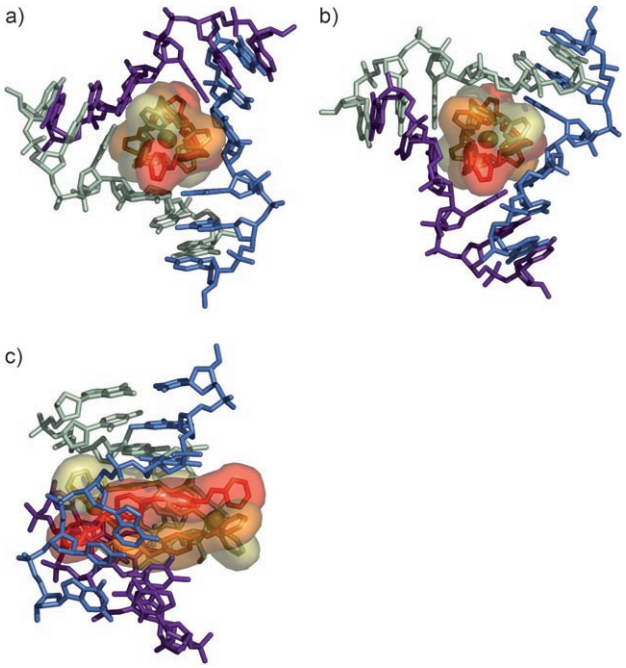


***Figure 2.*** Three-dimensional structure of the $[Fe_2L_3]^{4+}$–DNA complex. The drug is shown with both sticks and surface representations, whereas the $Fe^{2+}$ ions are shown as spheres. a) Major groove side view; b) Minor groove side view; c) Lateral view. One of the $Fe^{2+}$ ions and three terminal phenyl rings stick out from the junction major groove side.

The three pyridine rings (D) at one end of the drug are located at the minor-groove side of the junction and are again twisted 60° with respect to phenyl rings C. This permits them to occupy the DNA minor grooves sandwiched between two opposite DNA-backbone sugar moieties, namely A4 of one strand and C5 of the complementary strand (Figure 3c). This interaction is reminiscent of those observed in minor-groove binders.[14] Similar to the interaction between minor-grove binders and A-tract DNA sequences, the minor groove narrows down, allowing strong van der Waals interactions between the drug and the DNA sugar–phosphate backbone. At the other end of the prismatic supramolecule, the three pyridine rings (A) occupy the major groove sticking away from the DNA and do not interact with it (Figure 2c).

No conventional hydrogen bonds are present between the drug and the DNA because the external surfaces of the drug contain only C–H units. However, short C–H···X interactions are observed between the imino C–H adjacent to rings D and the N3 nitrogen of A4; the (putative) hydrogen atoms are in the appropriate orientation for a C–H···X hydrogen bond (C···N3(A4) = 3.3 Å).

It is noteworthy that, out of several oligonucleotides differing in length (6, 8, 10, and 12 mers) and sequence, we have only obtained complex crystals when the central nucleotides are thymine and adenine (TA). The other crystals diffract to low resolution and thus are not reported further herein, despite that the data are similarly consistent with a three-way junction structure. Although this study is not exhaustive, it suggests that $[Fe_2L_3]^{4+}$ may bind selectively to three-way junctions with a central TA sequence.

A more-detailed analysis of the three-way DNA junction itself is revealing. Its structure corresponds to the open, nonstacked Y-shaped junction.[15–17] It has threefold symmetry which, in this case, corresponds to a crystallographic symmetry favored by the palindromic nature of the oligonucleotide sequence used. The three arms of the junction are therefore identical and there is no coaxial helix-to-helix stacking between them. Instead, the angle between the arms is 110°, with folding in an almost-flat pyramidal arrangement (Figure 2). All the bases are paired in a Watson–Crick manner and the backbone sugar moieties are in the C2′-*endo* conformation. The helical parameters correspond to B-DNA, with an average twist of 35°. The TA bases at the junction point are, at one of their faces, not stacked with other DNA bases, leaving a large triangular hydrophobic tunnel where the drug binds. When going along a single strand, a dramatic kink is apparent at the central T3–A4 segment, where the helical axis suddenly deviates by 60°. This structural feature imposes large deformations of the DNA-backbone geometry, namely an unsusual backbone $\beta$ and glycosidic $\chi$ torsion angles (Figure 3). The three-way junction defines two distinct sides or faces, a major groove side (Figure 2a) and a minor groove side (Figure 2b) in which the three respective major or minor grooves converge. At the arms, the minor

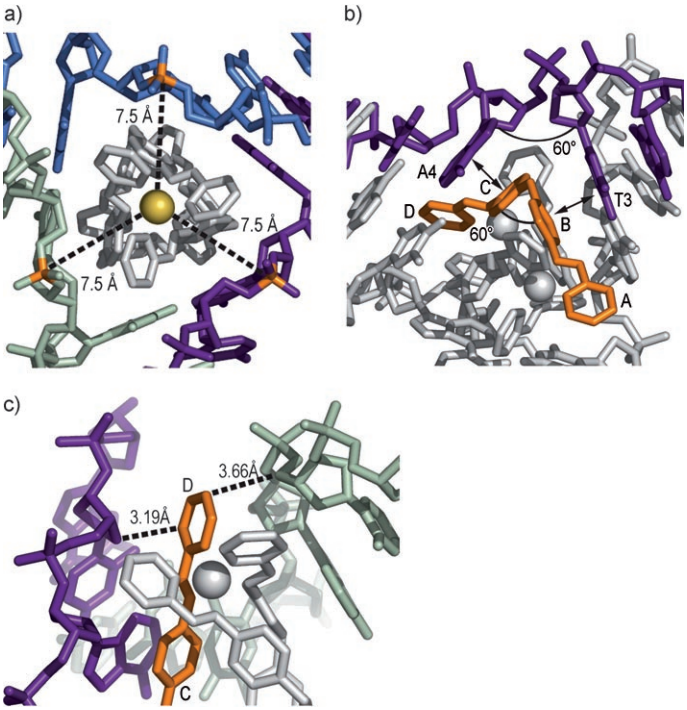


***Figure 3.*** a) View along the junction threefold axis showing the iron–phosphate electrostatic interactions at the minor groove side; b) Stacking interactions (double arrows) between the phenyl rings B and C of the drug and the central T3 and A4 bases of the junction. The DNA strand shows large distortions at the junction point, unstacking of the bases, an unusual backbone $\beta$ torsion angle (O5′(T3)-C5′(T3)) of 78°, and a glycosidic $\chi$ torsion angle of −150° at thymidine T3 ($\beta \approx 180°$ and $\chi \approx -110°$ in regular B-BNA); c) Interaction of the terminal phenyl ring D of the drug at the narrow minor groove of the DNA, reminiscent of minor groove-binding drugs. The $Fe^{2+}$ ions are represented as spheres.

  

groove has as narrow a width as the one observed in adenine-track DNA segments,[14] with room only for a single-water spine of hydration. The major groove has a regular B-DNA width.

Excitingly, the only previous example of an X-ray structure of a 3-way junction (of the Cre recombinase protein bound to a three-way junction)[18,19] shows DNA in the same Y conformation as the one we have found (Figure 4). Indeed, the structures are almost superimposable, which indicates that the three-way junction is not significantly perturbed by the drug binding but rather that it is an almost-perfect fit between both structures. Thus the present three-way DNA-junction structure is not an artefact of the crystal packing but rather a structural recognition target for both proteins and drugs.

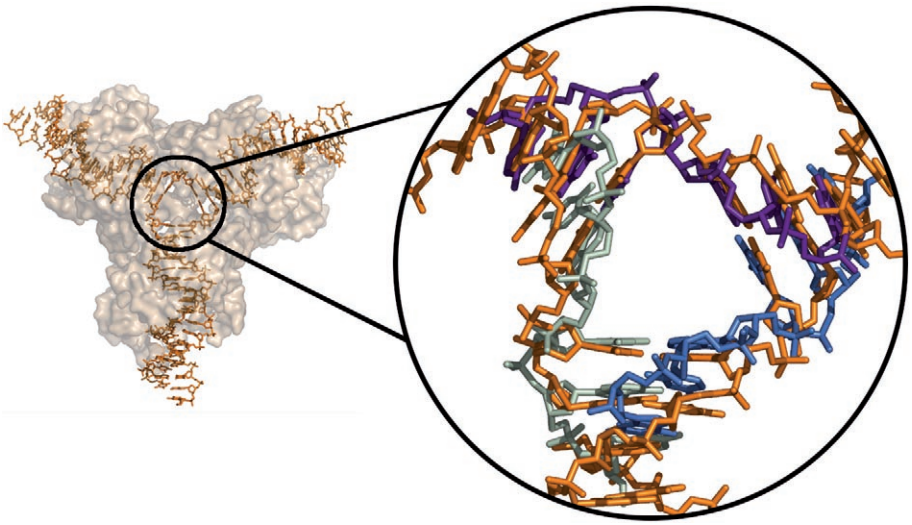

***Figure 4.*** The three-way DNA junction (orange sticks) in the complex structure with Cre recombinase (surface)[18] and (inset) its superimposition with the DNA three-way junction reported herein (colored as in Figure 2). Both junctions have similar unstacked Y forms.

It is pertinent to consider the formation of the junction structure from this palindromic DNA hexanucleotide rather than the more-common double-stranded (ds) B-DNA structure. For a palindromic sequence, full Watson–Crick hydrogen bonding can, in principle, be satisfied within any oligomeric formulation (ds-DNA, three-way junction, four-way junction etc.). Palindromic DNA thus represents a potential dynamic combinatorial library[20] of oligomers, although entropic considerations would normally dictate adoption of a ds-structure. In this instance the triple-helical antiprism selected the member of the library (the three-way junction) to which it binds most effectively, therefore driving the equilibrium exclusively to this structure. Such an effect has implications for the emerging field of DNA nanotechnology.

The key feature in biomolecular DNA-recognition motifs is the size and shape of the molecular surfaces which match to the size and shape of the DNA target and provide a variety of recognition points. The structure herein reveals that our nanoscale supramolecular triple-helical antiprism is similarly matched to the size and shape of the cavity in the three-way DNA junction. It demonstrates that such a three-way DNA-junction structure is an appropriate target for the design of a new family of ligands with a high structural specificity and strong-binding characteristics. Furthermore, this work reveals an unprecedented mode of drug binding to DNA where four kinds of interactions act sinergically: $[Fe_2L_3]^{4+}$ provides both electrostatic interactions, face-to-face π-stacking intercalation-type interactions, C–H···X type H-bond interactions and minor-groove sandwiching interactions. These findings open the way for the development of anti-DNA therapeutic agents with completely novel characteristics and we are actively exploring this further.

## *Experimental Section*

Crystallization and data collection: The synthesis of the helicate $[Fe_2L_3]Cl_4$ ($L = C_{25}H_{20}N_4$) (Figure 1) has been described previously.[12] Crystals were grown at room temperature from sitting drops that contained an aqueous solution of $[Fe_2L_3]Cl_4$ (0.5 μL of 10 mM), d(CGTACG) (1 μL of 3 mM), and reservoir solution (1.5 μL) that contains magnesium acetate (0.08 M), Tris-HCl (2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride; 0.05 M; pH 8.5), and PEG 400 (5%). After one week, purple opaque octahedral crystals that measured $0.2 \times 0.2 \times 0.2\ mm^3$ appeared. For freezing, crystals were harvested in reservoir solution that contained glycerol (25%). A multiple wavelength anomalous dispersion (MAD) experiment was performed at the Fe–K-absorption edge at the European synchrotron radiation facility beamline BM16 (Grenoble). Furthermore, a high-resolution data set was collected at beamline ID14-EH2. All data sets were indexed and integrated with XDS[21] and scaled and merged with XSCALE (see the Supporting Information).

Structure determination and refinement: Six Fe atoms were located with SHELXD[22] from anomalous difference data. Initial MAD phases obtained by Harker construction and density modification with SHELXE,[23] allowed us to calculate an experimental 2.6 Å resolution map where one drug–DNA complex was readily interpretable. A second complex was located on a twofold axis, perpendicular to the helicate axis, and therefore disordered in two opposite orientations. The first complex was build by using the X-VIEW graphic software.[24] Once completed, this complex was used to build the second disordered complex in the two alternative conformations. This was done through the superimposition of the metal ions, which had been located clearly. Refinement of the structure followed by using SHELXL97,[25] with restraints for the disordered complex that was linked to the ordered complex and with half occupancy. Bulk solvent correction was applied and atomic isotropic temperature factors refined. TURBO-FRODO graphic software[26] was used for further model building and the introduction of water molecules. The final refinement and model statistics are described in the Supporting Information.

Atomic coordinates: The atomic coordinates have been deposited with the PDB at the European Bioinformatics Institute (EBI) under accession code 2ET0.

Received: October 28, 2005

**Keywords:** bioinorganic chemistry · biosupramolecular chemistry · DNA recognition · DNA structures · helical structures

  

[1] For examples, see: P. B. Dervan, B. S. Edelson, *Curr. Opin. Struct. Biol.* **2003**, *13*, 284 – 299; *Cisplatin, Chemistry and Biochemistry of A Leading Anti-Cancer Drug* (Ed.: B. Lippert), Wiley-VCH, Weinheim, **1999**; J. Reedijk, *Chem. Commun.* **1996**, 801 – 806; Z. J. Guo, P. J. Sadler, *Adv. Inorg. Chem.* **2000**, *49*, 183 – 306; S. Neidle, *Nucleic acid structure and recognition*, Oxford University Press, Oxford, **2002**; S. J. Lippard, J. M. Berg, *Principles of Bioinorganic Chemistry*, University Science Books, Mill Valley, CA, **1994**; J. B. Chaires, *Curr. Opin. Struct. Biol.* **1998**, *8*, 314 – 320.

[2] R. Holliday, *Genet. Res.* **1964**, *5*, 282 – 304.

[3] M. Ortiz-Lombardia, A. Gonzalez, R. Eritja, J. Aymami, F. Azorin, M. Coll, *Nat. Struct. Biol.* **1999**, *6*, 913 – 917; B. F. Eichman, J. M. Vargason, B. H Mooers, P. S. Ho, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 3971 – 3976.

[4] T. Biswas, H. Aihara, M. Radman-Livaja, D. Filman, A. Landy, T. Ellenberger, *Nature* **2005**, *435*, 1059 – 1066; S. M. Roe, T. Barlow, T. Brown, M. Oram, A. Keeley, I. R. Tsaneva, L. H. Pearl, *Mol. Cell* **1998**, *2*, 361 – 372; D. N. Gopaul, F. Guo, G. D. Van Duyne, *Embo J.* **1998**, *17*, 4175 – 4187; M. Ariyoshi, T. Nishino, H. Iwasaki, H. Shinagawa, K. Morikawa, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 8257 – 8262; Y. Chen, U. Narendra, L. E. Iype, M. M. Cox, P. A. Rice, *Mol. Cell* **2000**, *6*, 885 – 897.

[5] C. Guthrie, B. Patterson, *Annu. Rev. Genet.* **1988**, *22*, 387 – 419.

[6] A. Nikulin, A. Serganov, E. Ennifar, S. Tishchenko, N. Nevskaya, W. Shepard, C. Portier, M. Garber, B. Ehresmann, C. Ehresmann, S. Nikonov, P. Dumas, *Nat. Struct. Biol.* **2000**, *7*, 273 – 277; B. T. Wimberly, D. E. Brodersen, W. M. Clemons, R. J. Morgan-Warren, A. P. Carter, C. Vonrhein, T. Hartsch, V. Ramakrishnan, *Nature* **2000**, *407*, 327 – 339; N. Ban, P. Nissen, J. Hansen, P. B. Moore, T. A. Steitz, *Science* **2000**, *289*, 905 – 920.

[7] M. R. Singleton, S. Scaife, D. B. Wigley, *Cell* **2001**, *107*, 79 – 89.

[8] C. E. Pearson, M. Tam, Y. H. Wang, S. E. Montgomery, A. C. Dar, J. D. Cleary, K. Nichol, *Nucleic Acids Res.* **2002**, *30*, 4534 – 4547.

[9] R. R. Sinden, *Nature* **2001**, *411*, 757 – 758.

[10] C. J. Leonard, K. I. Berns, *Prog. Nucleic Acid Res. Mol. Biol.* **1994**, *48*, 29 – 52; J. S. Ren, X. G. Qu, J. B. Chaires, J. P. Trempe, S. S. Dignam, J. D. Dignam, *Nucleic Acids Res.* **1999**, *27*, 1985 – 1990.

[11] F. Jensch, B. Kemper, *Embo J.* **1986**, *5*, 181 – 189.

[12] M. J. Hannon, V. Moreno, M. J. Prieto, E. Molderheim, E. Sletten, I. Meistermann, C. J. Isaac, K. J. Sanders, A. Rodger, *Angew. Chem.* **2001**, *113*, 903 – 908; *Angew. Chem. Int. Ed.* **2001**, *40*, 879 – 884; I. Meistermann, V. Moreno, M. J. Prieto, E. Moldrheim, E. Sletten, S. Khalid, P. M. Rodger, J. C. Peberdy, C. J. Isaac, A. Rodger and M. J. Hannon, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5069 – 5074; M. J. Hannon, C. L. Painting, A. Jackson, J. Hamblin, W. Errington, *Chem. Commun.* **1997**, 1807 – 1808.

[13] C. Uerpmann, J. Malina, M. Pascu, G. J. Clarkson, V. Moreno, A. Rodger, A. Grandas and M. J. Hannon, *Chem. Eur. J.* **2005**, *11*, 1750 – 1756.

[14] M. Coll, C. A. Frederick, A. H. Wang, A. Rich, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 8385 – 8389.

[15] D. R. Duckett, D. M. Lilley, *Embo J.* **1990**, *9*, 1659 – 1664.

[16] This three-way junction differs from other three-way junctions derived by NMR (B. N. van Buuren, F. J. Overmars, J. H. Ippel, C. Altona, S. S. Wijmenga, *J. Mol. Biol.* **2000**, *304*, 371 – 383; V. Thiviyanathan, B. A. Luxon, N. B. Leontis, N. Illangasekare, D. G. Donne, D. G. Gorenstein, *J. Biomol. NMR* **1999**, *14*, 209 – 221; B. Wu, F. Girard, B. van Buuren, J. Schleucher, M. Tessari, S. Wijmenga, *Nucleic Acids Res.* **2004**, *32*, 3228 – 3239) in which two arms are stacked coaxially. However, the DNA sequences used in those studies, with unpaired bulged out bases, explains the marked differences compared with our structure.

[17] It is interesting that the three-way junction herein has a completely different geometry from the unliganded four-way junction (reference [3]). That junction was found in the X-stacked form, that is, with the arms coaxially stacked pairwise in two continuous helices rotated 45° and with a very compact structure at the crossing point. In contrast with the three-way junction reported herein, such a four-way junction leaves no room or hollow for ligand binding, except for the DNA grooves. However, the four-way junction is quite flexible and can respond structurally to binding events. Open, nonstacked square-planar four-way junctions are observed in the protein–DNA complex of RuvA and Cre recombinase (reference [4]). Also, several studies indicate that four-way DNA junctions can undergo a transition from a closed, X-stacked conformation to an open, nonstacked planar conformation when lowering the $Mg^{2+}$ or other cation concentrations (D. R. Duckett, A. I. H. Murchie, S. Diekmann, E. Von Kitzing, B. Kemper, D. M. J. Lilley, *Cell* **1988**, *55*, 79 – 89). This is not the case in three-way DNA junctions, where variation in the counterion concentration does not affect the structure, which always remains in the open, nonstacked Y form (reference [15]). The presence of only three double strands drastically restrains the conformation possibilities of the three-way junction when compared with four-way junctions.

[18] K. C. Woods, S. S. Martin, V. C. Chu, E. P. Baldwin, *J. Mol. Biol.* **2001**, *313*, 49 – 69.

[19] Another three-way DNA junction has been reported in complex with the DNA repair protein Ku (J. R. Walker, R. A. Corpina, J. Goldberg, *Nature* **2001**, *412*, 607 – 614). In this case, a DNA junction was used only for the purpose of stabilizing the protein–DNA complex formation and the DNA used was not the natural substrate. Nevertheless, the same nonstacked Y conformation was observed for the junction.

[20] J. M. Lehn, *Chem. Eur. J.* **1999**, *5*, 2455 – 2463.

[21] W. Kabsch, *J. Appl. Crystallogr.* **1993**, *26*, 795 – 800.

[22] G. Sheldrick, H. Hauptmann, C. Weeks, R. Miller, I. Usón in *International Tables for Crystallography F* (Eds.: E. Arnold, M. Rosmann), Kluwer Academic Publishers, Dordrecht, **2001**, pp. 333 – 351.

[23] G. Sheldrick, *Z. Kristallogr.* **2002**, *217*, 644 – 650.

[24] D. E. McRee, *J. Struct. Biol.* **1999**, *125*, 156 – 165.

[25] G. Sheldrick, T. Scheider, *Methods Enzymol.* **1997**, *277*, 319 – 343.

[26] A. Roussel, C. Cambilleau in *Silicon Graphics geometry partners directory* 77–79, Silicon Graphics, Mountain View, CA, **1989**.


  