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DNA Nicks and Nodes and Nanotechnology

Nadrian C. Seeman*

Department of Chemistry, New York University, New York, New York 10012

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ABSTRACT

The properties that make DNA such an effective molecule for genetic material also make it a superb molecule for nanotechnology, when stable, stiff branched species are combined with cohesive interactions. Reciprocal crossovers between single strands of duplex or complex molecules lead to a panoply of motifs that can be used for DNA nanotechnology. A general derivation of these molecules is shown here, including components of devices and periodic or algorithmic arrays.

The molecular properties of the DNA molecule that allow it to act as the repository of genetic information also confer on it the characteristics of an outstanding nanoscale synthon for biomimetic nanotechnology. This fact has led to the development of both DNA nanotechnology[1,2] and the closely related field of DNA-based computation[3] by algorithmic self-assembly.[4] Both endeavors rely on Watson−Crick base pairing, but the backbone structures involved are complex species, not simple linear duplex molecules. These DNA motifs have been inspired by a variety of sources, and we have shown that they lead naturally to a generalization of Watson−Crick complementarity.[5] The purpose of this letter is to derive DNA motifs by a general procedure, reciprocal exchange between DNA molecules. Reciprocal exchange is a phenomenon that occurs naturally in genetic recombination, although the mechanisms are far more intricate than the distilled version we present here. Nevertheless, it is important to realize that crossovers occur rarely and ephemerally in nature, despite their utility in generating nanotechnological motifs.

There are three logical steps to reciprocal exchange: First, two DNA molecules are juxtaposed; the space between the two molecules is called a zero node.[6] Second, the two strands are nicked, thereby breaking them into two pieces each. Third, the strands are rejoined in a new combination. The rejoining creates a signed node (±1), and it respects strand polarity. The nicking of DNA molecules, and their recombination to form new nodes, is central to the generation of new motifs with potential nanotechnological utility.

The left of Figure 1a shows a red hairpin and a blue hairpin with a zero node between them. Their helix axes are horizontal, and the dyad axis between them is vertical. The right of Figure 1a shows that after reciprocal exchange the two hairpins have been converted into a single duplex molecule, with the colors indicating that the new molecules are combinations of the old ones. Figure 1b shows the same operation, but now looking down the central dyad axis of the molecule. It is clear in this example that two short molecules have been extended to form one long molecule, although the complexity of the motif is not increased. The reverse operation, called resolution, is also shown in Figure 2. One may think of resolution as replacing a signed node (−1 along B-DNA, +1 at the branch point of the Holliday junction[7]) with a zero node.

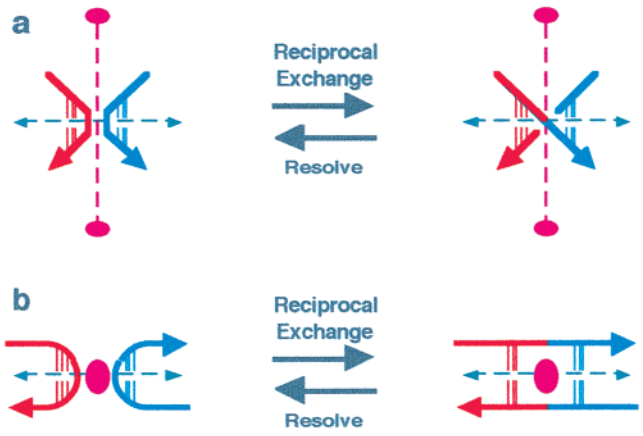

Figure 1. *Reciprocal exchange between two DNA hairpins. (a) A view normal to the dyad axis.* A red and a blue hairpin are shown. The helix axis is horizontal and the dyad axis is vertical. Arrowheads on strands indicate the 3′ end. A negative node is formed in the rightward reaction, where the strands have retained their initial colors. *(b) A view down the dyad axis.* The view in *(a)* has been rotated 90° about the horizontal. This operation is related to the construction system used in the solid-support methodology to DNA nanoconstruction.[25]

DNA nanotechnology entails combining motifs derived from branched DNA molecules with intermolecular sticky-

* To whom correspondence should be addressed. E-mail: ned.seeman@nyu.edu.

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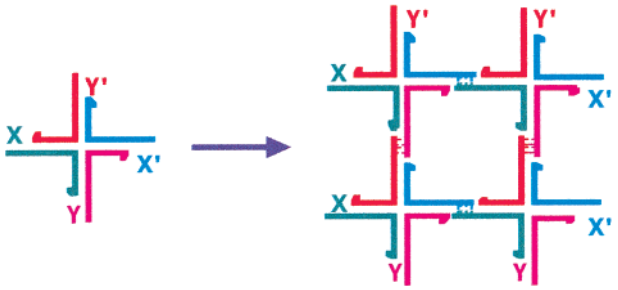

Figure 2. *Assembly of four 4-arm branched junctions into a quadrilateral.* A branched junction is shown on the left, with four sticky ends, X, Y, and their complements, X′ and Y′. Assuming rigid parallelism, the four are shown assembled into a quadrilateral; the open "valences" on the outside suggest that this motif could be assembled further into a two-dimensional array.

ended cohesion. There are two key features to sticky-ended cohesion that make it important for the applications of DNA nanotechnology discussed here, predictable affinity and structure. It is evident that the recognition of one sticky end by a complementary sticky end leads to predictable affinity. Sometimes overlooked, however, is the fact that the local product structure (B-DNA) is known ahead of time;[2] this contrasts with, say, antigen−antibody interactions, in which structural experiments must be conducted to ascertain the relative orientations of the two components. An example of sticky-ended association is shown in Figure 2, which illustrates a 4-arm branched junction whose sticky ends associate to produce a quadrilateral that could be extended to yield a two-dimensional periodic array. There are a lot of ways to think about generating 4-arm branched junctions, but here, we will do it by reciprocal exchange. It is clear from Figure 1 that we cannot do this by the longitudinal exchange illustrated there. Rather, we must perform a transverse operation between helix axes, rather than along them. This operation is shown in Figure 3.

To understand Figure 3 fully, it is important to remember that the double helix consists of two antiparallel strands of DNA. Consequently, there are two different ways in which the two strands can be combined in a transverse fashion. Reciprocal exchange can take place between strands of the same polarity, as shown in Figure 3a, or it can occur between strands of opposite polarity, as shown in Figure 3b. As noted above, we preserve polarity in these operations (although it can be violated),[8] so the structures of the two branch points shown are quite different: The branch point in Figure 3a contains one strand that passes over the other, and the dyad axis relating the two helices lies parallel to the helix axes; by contrast, the branch point in Figure 3b contains strands that reverse their directions, and the dyad axis is normal to the plane of the page. For the case of the single crossover illustrated in this diagram, there is no fundamental difference between the products in Figure 3a and 3b: They are simply different conformations of the same molecule. It is worth pointing out that the difference between Figures 3a and 3b results from a different vertical phasing (a half-turn) between the two duplexes that are juxtaposed before the exchange.

The 4-arm junction is one of a number of branched junctions that can be envisioned. 3-arm[9], 5-arm, and 6-arm[10]

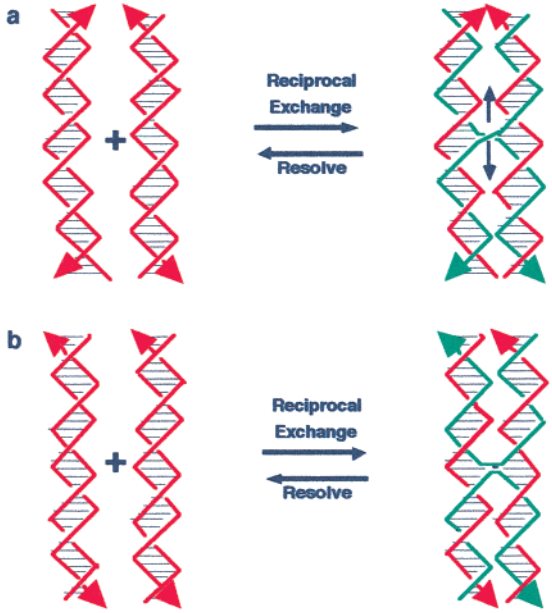

Figure 3. *Reciprocal exchange generates a four-Arm junction from two double helices.* Two juxtaposed double helices exchange strands to produce 4-arm junctions. *(a)* shows exchange between strands of the same polarity, and *(b)* shows exchange between strands of opposite polarity. Symmetry elements are shown, and the dyad symmetry in each product is emphasized by the color coding, so that the red strands are related by 2-fold symmetry to the red strands and the green strands to the green strands.

branched junctions have been produced experimentally, and it has been suggested that there is no limit to the number of arms that can flank a branch point.[11] Arms can be added to junctions individually by reciprocal exchange between the tip of a hairpin and a junction, as shown in Figure 4. Here, a 4-arm junction in the antiparallel conformation of Figure 3b is combined by reciprocal exchange with the tip of a hairpin to produce a 5-arm junction. It is important to be able to make branched junctions with arbitrary numbers of arms, because the connectivity[12,13] of a structure or lattice built from DNA is limited by the number of arms that flank its junctions.

Major recent successes of DNA nanotechnology include the formation of 2D periodic arrays[14−16] and 1D algorithmic arrays,[17] as well as the construction of a robust nanomechanical device.[18] For these purposes, it has been necessary to employ DNA motifs that are much more rigid than branched junctions are known to be;[9,19] nevertheless, branched junctions combined into parallelograms have also been used to produce lattices.[8,20] Rigid motifs[21] were developed for arrays and devices, in particular DNA double[22] and triple[16] crossover molecules. The double crossover (DX) molecule contains two helical domains, connected twice, and the triple crossover (TX) molecule contains three domains, each joined to its neighbor(s) twice. The derivation of the double crossover molecule from two helices is shown on the left of Figure 5. As was the case with the single crossover formed in Figure 3, there are two fundamentally different ways that the two reciprocal exchanges can be effected, either by joining strands of the same polarity (Figure 5a), or by joining strands with opposite polarity (Figure 5b). In contrast to the

Figure 4. *Reciprocal exchange between a hairpin and a 4-arm junction produces a 5-arm junction.* A 4-arm junction is shown on the left, with each strand colored individually. The dark green hairpin fuses with the purple strand to produce a 5-arm junction. The green and purple strand colors are conserved to show the origin of the strands constituting the fifth arm. A symmetrized version of the molecule is shown at the far right.

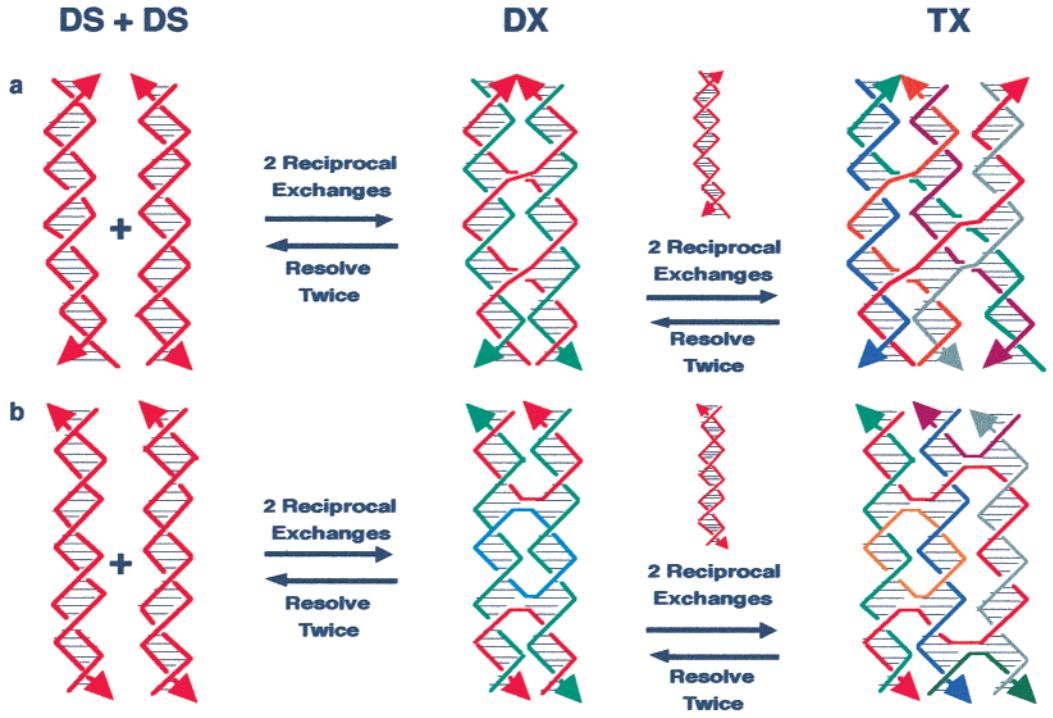

Figure 5. *DNA double and triple crossover molecules produced by two reciprocal exchanges between two double stranded molecules. (a)* Illustrates double strand (DS) fusions between strands of the same polarity, and *(b)* illustrates fusions between strands of opposite polarity. Double crossover (DX) molecules are shown in the central portion of the drawing; their colored strands are related to each other by dyad symmetry. Triple crossover (TX) molecules are shown on the right of the drawing, formed by fusing a double helix to the double crossovers in the middle panels. The six colors used for the triple crossover in *(a)* and the seven colors used in *(b)* indicate the loss of global symmetry, although there is local symmetry in some regions of the structures.

single crossover cases shown in Figure 3, these molecules are not interconvertable by a change in conformation; they are distinct motifs. In fact, there are a total of 5 different topological motifs that can be described for DX molecules, depending on the separations of their crossovers.[22] In addition to the use of antiparallel DX molecules in DNA arrays,[14,15] and devices,[18] Barton and her colleagues have investigated their electrical conducting properties[23] and Yurke et al. have used them implicitly in a sequence-specific DNA device.[24] Likewise, Fahlman and Sen have suggested the use of parallel DX molecules in nanotechnological applications.[25] The right side of Figure 5 shows the addition of a third domain to the DX molecules to form TX molecules, again with two reciprocal exchanges. We have continued to perform exchanges with molecules of the same polarity in Figure 5a and with the opposite polarity in Figure 5b, although we could have switched them. One of the exchanges in Figure 5a involves one of the strands that is already involved in a crossover, and the other one does not. This was also an arbitrary choice. To date, the only triple-crossover molecules constructed involve reciprocal exchanges between strands of opposite polarity.

It is not necessary to restrict oneself only to two crossovers in motifs composed of two domains. Figure 6 illustrates what happens when one performs a reciprocal exchange at every possible strand juxtaposition. Figure 6a illustrates the same-polarity case, and Figure 6b shows the opposite-polarity case. The opposite-polarity molecule in the middle is a polycatenane, which has not been explored yet in the laboratory. However, the structure shown in the middle of Figure 6a is

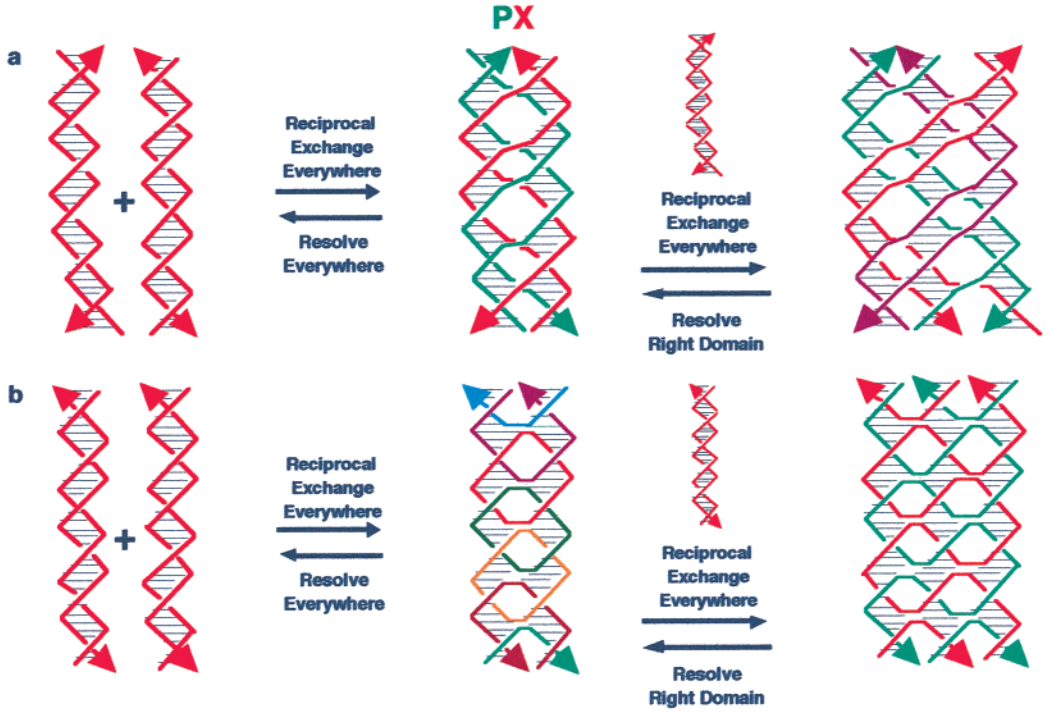

Figure 6. *The generation of PX DNA and its opposite polarity equivalent by reciprocal exchange.* The left side of *(a)* illustrates the consequences of performing a crossover at every possible juxtaposition in the same-polarity case. The result is the remarkable PX structure, drawn with green and red strands, which are related to each other by a dyad axis vertical in the page. This is a paranemic joining of two backbone structures, and it is very stable. The far right of *(a)* contains the 3-domain equivalent of the PX structure, with three interwound duplexes. The left part of *(b)* illustrates the opposite polarity version of *(a)*, and the far right of *(b)* shows the 3-domain generalization of this structure; except for the ends, it is composed only of two strands. As yet, neither of these 3-domain structures has been made in the laboratory.

a remarkable molecule. It consists of two double helices, one red and one green, that are bound together paranemically, so this structure is known as paranemic crossover DNA, or PX DNA. It has been suggested that PX DNA could be involved in homology recognition in homologous recombination.[26] It is also possible to use this motif as the basis for a molecular device that will be discussed elsewhere (H. Yan, X. Zhang & NCS, in preparation). Although DX molecules of the sort shown in Figure 5a behave poorly in solution,[22] the PX molecules shown in Figure 6a are very well behaved.[26] It is evident that both of these molecules can be generalized by the addition of a third helix, as we did with two crossovers in Figure 5. These additions are shown on the right of Figure 6; neither has been produced successfully in the laboratory. It goes without saying that many topologies lie between the extremes of two reciprocal exchanges and all possible reciprocal exchanges. The paranemic nature of the PX molecule suggests that it can be used in another way: To replace sticky ends as long cohesive units, as illustrated in Figure 7; this diagram shows two DNA triangles[27] held together by paranemic contacts. So far, all of the motifs involving fused helices with parallel or antiparallel axes have been found to be sufficiently rigid for nanoconstruction.

A further, related, motif is shown in Figure 8. This is the DX+J motif, formed by combining a hairpin with a one of the duplex arms of a DX molecule. It is worth pointing out that we have drawn this motif, and the various three-domain

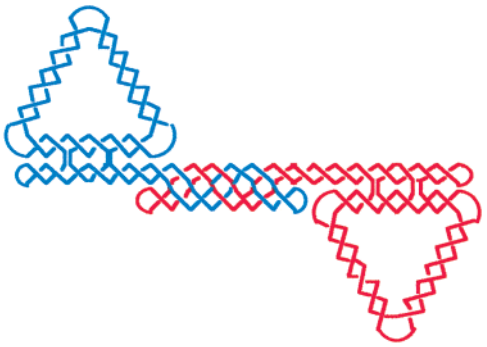

Figure 7. *Paranemic cohesion between DNA triangles.* The red triangle and the blue triangle are topologically closed structures that are robust enough to be isolated under denaturing conditions.[24] The PX cohesion shown between them can be arbitrarily strong, thereby obviating the need to produce sticky ends by restriction of hairpins.[25]

motifs discussed above, to have all of their helix axes coplanar. However, that feature is certainly not necessary. The main use of DX+J motifs has been with the extra DNA domain oriented as nearly as possible normal to the plane defined by the DX helices.[14,15] The same is true for the related TX+J motif.[16] In a similar spirit for motifs consisting exclusively of helices with parallel (or antiparallel) helix axes, we have constructed 6-helix bundles of DNA, wherein six helices are held together by means of reciprocally exchanged strands (C. Mao, F. Mathieu, K. C. Kinnally & NCS, in preparation).

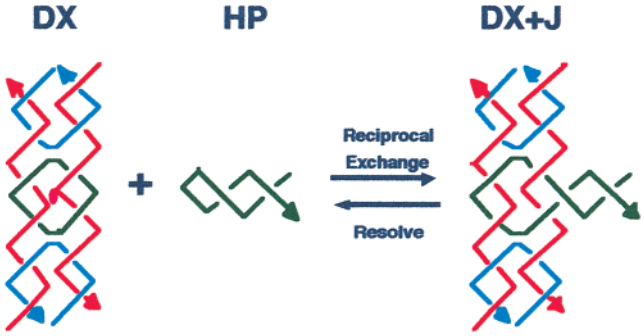

Figure 8. *Reciprocal exchange between DX and hairpin molecules generates the DX+J motif.* The green hairpin molecule is fused by reciprocal exchange with the central cyclic strand of this DX molecule, also drawn in green. The result is a DX+J molecule that has been used as a topographic marker in the atomic force microscopy of 2D DNA arrays.[14−16]

All of the DNA motifs described here, except for conventional duplexes, share the feature of complementarity interrupted by a nick.[5] Conventional nucleic acid complementarity entails two continuous polynucleotide backbones: The sequence on one strand is one-for-one complementary to the sequence on the other strand. The discontinuous continuity that enables us to produce branched structures leads to an ambiguity in the definition of the complement because any number of nucleotides or nucleotide pairs can be inserted at the nick in the complement. This feature seems with good reason to be avoided in the storage of biological information, but it is central to our ability to exploit DNA nanotechnology to the fullest.

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