DNA: Beyond the Double Helix

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Summary: Reciprocal exchange can be used to produce DNA motifs based on branching at the level of secondary structure. These motifs can be combined by sticky-ended cohesion to produce a variety of structures. Stick polyhedra and nanomechanical devices have been produced by self-assembly from motifs based on branched DNA. Periodic arrays with tunable surface features has also been produced; aperiodic arrangements have been used for DNA-based computation.

Keywords: branched DNA, DNA-based computation, periodic arrays, self-assembly, stick polyhedra, surface features, tunable nanomechanical devices

Introduction

We are all aware that DNA is the genetic material of living organisms. It is able to function in that capacity because of its chemical nature. The key features of its chemistry are the specificity of one strand for its complement, the stiffness of the double helix, and the predictability of the DNA structure that two antiparallel strands will form. For over 20 years, our group has been engaged in using these features to exploit the architectural properties of DNA.

"Architectural properties!" What architectural properties? After all, the DNA double helix axis is just a line, topologically speaking, so concatenation of sticky-ended fragments of DNA double helices ought not be very interesting from an architectural standpoint. Not exactly. Biological DNA indeed is a molecule with a linear helix axis, a property necessary for the complement of any strand to be well-defined.[1] Nevertheless, branched molecules arise naturally as Holliday junction[2] intermediates in the process of genetic recombination; these intermediates are necessarily ephemeral. However, chemical DNA synthesis[3] can be used to produce stable branched species. It is straightforward to define sequences of DNA that will associate to form more complex topologies.[4] An example of a stable branched junction[5] is shown in Figure 1a. Similarly, the biological operation of reciprocal exchange provides a direct method to design a variety of motifs that can serve as basic units of DNA nanoconstruction.[6]

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This operation is illustrated in Figure 1b: Two strands, one gray and one black are combined to make two hybrid strands, one gray-black and one black-gray. This operation is shown in a larger context in Figures 1c and 1d; the difference between these two figures is that in Figure 1c the operation takes place between strands of the same polarity, whereas in Figure 1d the strands are of opposite polarity. For a single exchange event, the two products are simply conformers of each other, but for two or more operations distinct species result.

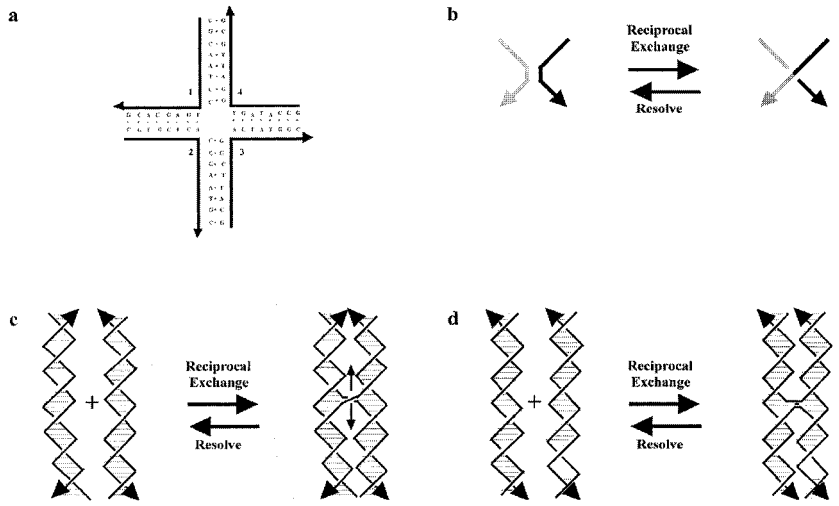

Fig. 1. (a) A four-arm DNA branched junction made from synthetic strands whose sequences have been designed. (b) The reciprocal exchange operation. Two juxtaposed strands, gray and black are exchanged to produce two hybrids, a gray-black strand and a black-gray strand. (c) Reciprocal exchange between two strands of the same polarity. (d) Reciprocal exchange between two strands of opposite polarity.

DNA 'sticky ends' have a central role in genetic engineering: These are short single-stranded overhangs can be used to direct the associations of two different DNA molecules.[7] They produce cohesive hydrogen bonded interactions, interactions that can be made covalent with the use of a DNA ligase. We combine synthetic versions of branched molecules with sticky ends. This enables us to generate new shapes of DNA, that lead to objects, arrays and devices. This concept is illustrated in Figure 2, where four branched junctions are combined to produce a quadrilateral that could be extended by the sticky ends on the outside. An extended complex

would result in a 2-dimensional lattice. However, this system is not limited to two dimensions. The angle between the double helices shown in Figure 2 is a function of their separation. Thus, the drawing showing the DNA backbones as parallel lines is valid only for to an exact number of double helical half-turns. The relative orientations are a function of the separation, just like two wing-nuts on a screw.

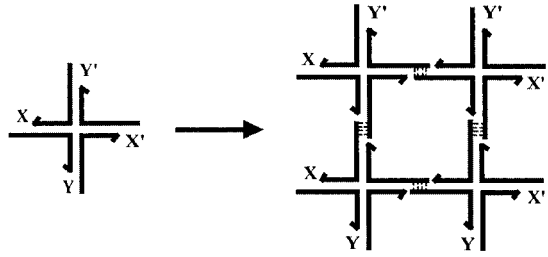

Fig. 2. A four-arm DNA branched junction with two sets of complementary sticky ends (X and X', Y and Y') forms a quadrilateral by self-assembly. The open valences on the outside can lead to the self-assembly of periodic arrays.

Constructions

In the course of our research, we have used DNA topological schemes to generate a variety of branched molecules in both flexible and stiff motifs.[8] Combining flexible motifs with sticky ends has enabled us to produce DNA molecules connected like geometrical stick figures, a cube and a truncated octahedron, as well as a variety of knots and Borromean rings (Figure 3).

More recently, we have used stiff motifs to produce a variety of two-dimensional arrays with programmable patterns from these building blocks. These arrays include patterns formed from double crossover molecules and from triple crossover molecules. The double crossover (DX) molecules can be decorated by a DNA hairpin perpendicular to the plane of the helix axes, to generate a topographic marker that is visible in the atomic force microscope (AFM). Different patterns may be programmed and modified (Figure 4).[9,10]

Similar arrays can be made from triple crossover (TX) molecules, containing three double helical domains, rather than two.[11] A more intriguing application of TX molecules is that we have used them to perform a prototype cumulative XOR calculation by self-assembly.[12] The components of this assembly are shown in Figure 5, and the way that the answer is extracted is shown in Figure 6.

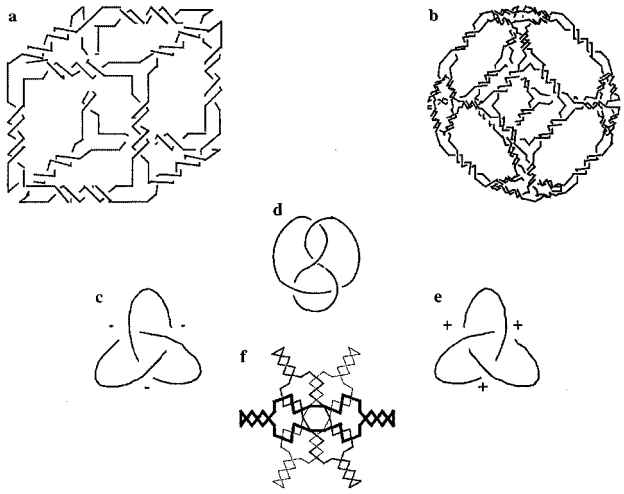

Fig. 3. Ligated products from flexible DNA components. (a) A stick cube and (b) a stick truncated octahedron. The drawings show that each edge of the two figures contains two turns of double helical DNA. The twisting is confined to the central portion of each edge for clarity, but it actually extends from vertex to vertex. Both molecules are drawn as though they were constructed from 3-arm junctions, but the truncated octahedron has been constructed from 4-arm junctions, which has been omitted for clarity. (c-e) Knots Constructed from DNA. The signs of the nodes are indicated. (c) A trefoil knot with negative nodes. (d) A figure-8 knot. (e) A trefoil knot with positive nodes. (f) Borromean rings. Scission of any of the three rings shown results in the unlinking of the other two rings.

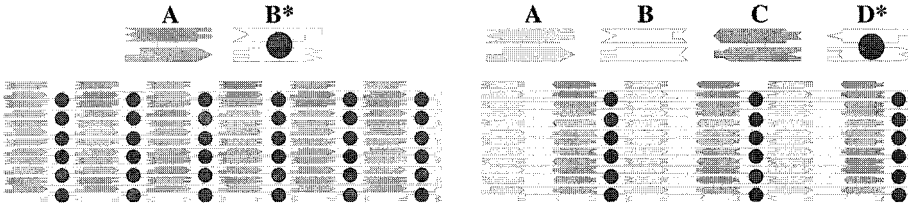

Fig. 4. Two dimensional DNA double crossover arrays. At the top of each panel are shown DX molecules, A and B* or A, B, C and D*. Their sticky ends are represented by geometrical shapes. It is clear from the patterns below these drawings that this set of molecules can tile the plane. Likewise, it is clear that different patterns can be programmed by changing the assignments of the sticky ends. The stripe-like pattern generated by the features in the B* and D* tiles can be visualized in the atomic force microscope. The stripes in the AB* array are separated by 32 nm, whereas those in the ABCD* array are separated by 64 nm. The patterns may be modified by restriction, ligation or hydrogen bonded association.

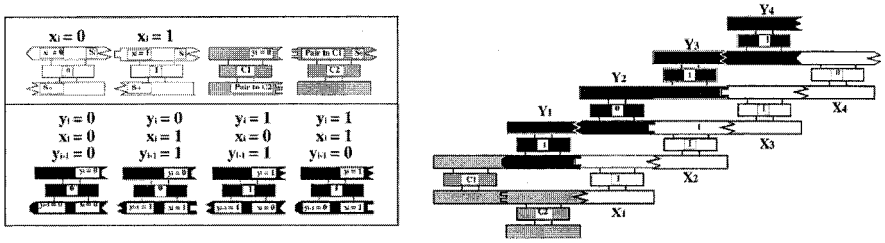

Fig. 5. The components of a DNA-based cumulative XOR calculation are shown on their left, and their self-assembly is shown on the right. The light gray TX tiles are the input to the calculation, the dark gray tiles are initializers, and the black tiles act as the four XOR gates. They contain the four possibilities on their bottom helical domains as sticky ends. The input and initializers have longer sticky ends, so they assemble first.

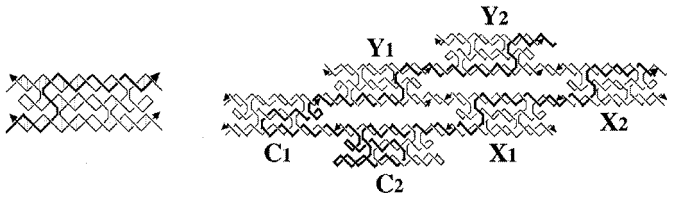

Fig. 6. Extracting the answer from the self-assembly. The strand structure of the TX tile is shown on the left. The value of the tile, 0 or 1, derives from a restriction site (one of two alternatives) on the dark strand of the tile. The strand structure of the assembly is shown on the right. The dark strands have been ligated, thereby connecting the input with the output. The long ligated dark strand is extracted and then subjected to restriction by the enzymes in separate experiments; the answer can be read off the resulting gel.

Stiff DNA motifs have also been used to produce two different DNA devices. One device is based on the transition between right-handed B-DNA and left-handed Z-DNA;[13] the second is based on controlling motif topology in a sequence-specific fashion (Figure 7).[14] The first device has a global trigger, the addition of a small molecule $Co(NH_3)_6^{3+}$, so all molecules will react in the same way. The sequence-dependent trigger of the second device allows us to address each species of device separately, creating a panoply of robust structural states.

Objectives

What are the objectives of this program? The first potential application of this system is to scaffold biomolecular crystallization, using a nucleic acid host lattice to organize a biological

macromolecular guest into a crystal that can diffract X-rays and thereby enable structure determination.[4] Of course, if one can imagine organizing biological macromolecules into an array, one can imagine organizing other molecules as well. Prominent amongst these are the components of molecular electronics.[15] There are many species that are well-suited to nanoelectronics, e.g., metallic nanocrystals or carbon nanotubes, but it is difficult to organize them into functional arrays. We expect that structural DNA nanotechnology will be able to provide the organization needed for this purpose. Schematic diagrams of these goals are shown in Figures 8 and 9.

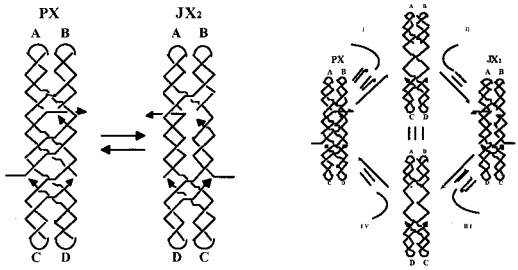

Fig. 7. A sequence-dependent DNA nanomechanical device. The left side shows the two end-state molecules of the device. The one on the left, a PX motif, contains crossovers at every point in the central region. The molecule on the right, known as JX_2, lacks two of those crossovers in the central portion of the molecule. The molecules differ by the relative positions of the C and D markers, although A and B are the same; thus JX_2 is a half-turn unwound, relative to PX. The strands containing the horizontal extensions (called set strands) set the conformation to one of the two states. The right panel illustrates the machine cycle. Removing the set strands by adding their full complements allows the opposite set strands to bind, changing the state of the molecule.

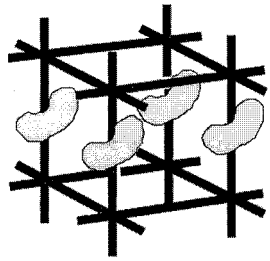

Fig. 8. Macromolecular guests in a DNA host lattice. The cube-like structure represents a polyhedron constructed from DNA, and the extensions represent sticky ends. The irregular blobs attached to it represent biological macromolecules arranged parallel to each other.

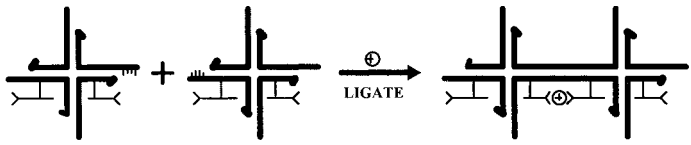

Fig. 9. DNA branched junctions organizing nanoelectronic components. Two branched junction structures are shown. They have complementary sticky ends, and a nano-wire is hanging from each of their horizontal arms. The idea illustrated here is that when the DNA structures cohere *via* their sticky ends, the nano-wires will be directed to make contact, as well. An ion is shown as an additional organizing element in this scheme.

A third species to organize into arrays is the sequence-dependent nano-device that we have already produced. If we can incorporate N different species of these 2-state devices, we should be able to generate 2^N different structural states. Short-range goals include molecular pegboards, and molecular assembly lines. Multiple structural states are a necessary concomitant of nanorobotics, so this system may lead to a future DNA-based nanorobotics.

The primary advantage of DNA for these goals is its outstanding molecular recognition properties, allowing precise structural alignment of diverse intermolecular species. DNA appears to be unique among biopolymers in this regard. Although there are other systems that lead to specific binding, only in the case of nucleic acids are the structures formed known in advance: Sticky ends form B-DNA.[16] If one considers a related cohesive system, antigen-antibody interactions, affinity can still be predicted, but the structure of every individual complex must be determined by experiment (e.g., crystallography) before it can be used in nanoconstruction.

Other advantages of DNA are the convenience of chemical synthesis,[3] and the presence of enzymes to manipulate it and trouble-shoot errors. For example, DNA ligases enable covalentbonding of complexes held together by sticky-ended cohesion.[17] Exonucleases can be used to purify cyclic target molecules from linear failure products. Restriction endonucleases can be used both to trouble-shoot syntheses and to create cohesive ends from topologically-closed species.[18,19] The persistence length of DNA is about 500 Å,[20] leading to a predictable overall structure for the short (70-100 Å) lengths we use. There is an external code on DNA that can be read, even when the double helix is intact.[21] The packing of nanoelectronics very

tightly will be aided by the high density of functional groups (every 3.4 Å or so) on DNA, regardless of tile size.

The enzymes noted above make it convenient for us to prototype our constructions with conventional DNA. However, we are not limited to the molecule evolved in nature for use as genetic material. A vast number of DNA analogs have been produced and analyzed for therapeutic purposes.[22,23] This means that systems prototyped by conventional DNA ultimately may be converted to other backbones and bases, as required by specific applications.

Acknowledgments

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