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Experiments in Structural DNA Nanotechnology: Arrays and Devices

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Abstract

In recent years, the chemistry of DNA has expanded from biological systems to nanotechnology. The generalization of the biological processes of reciprocal exchange leads to stable branched motifs that can be used for the construction of DNA-based geometrical and topological objects, arrays and nanomechanical devices. The information in DNA is the basis of life, but it can also be used to control the physical states of a variety of systems, leading ultimately to nanorobotics; these devices include shape-changing, walking and translating machines. We expect ultimately to be able to use the dynamic information-based architectural properties of nucleic acids to be the basis for advanced materials with applications from nanoelectronics to biomedical devices on the nanometer scale.

Keywords

DNA Structure; Periodic Designed DNA Arrays; DNA Nanomechanical Devices; 2D DNA Crystals; 3D DNA Crystals; Control of Structure; Branched DNA Motifs

1. INTRODUCTION

DNA is the genetic material of all living organisms; it serves as the repository for all of the information needed for the organism to grow, to replicate, to respond to its environment, and (for eukaryotes) to develop from a zygote to an adult organism. DNA is able to perform in this way because of its chemical properties. However these same molecular properties also can be exploited for chemical ends on the nanometer scale. The DNA molecule is inherently a nanoscale species: The diameter of the double helix is about 2 nm and its helical repeat is about 3.5 nm. However, such dimensions could easily describe a variety of proteins. Why focus on DNA?

The key feature of DNA that makes it useful as a nanoscale building block is its specificity and programmability in intermolecular interactions. For over a half century, the notion of hydrogen bond-mediated Watson-Crick base pairing has dominated thinking about DNA. This pairing consists of adenine (A) paired specifically with thymine (T) and guanine (G) paired specifically with cytosine (C) within the context of the DNA double helical backbone. It is also possible to get two different double helices to cohere end-to-end by having short overhangs at the ends of the strand; these overhangs are called 'sticky ends', and the way that they bring helices together is illustrated in Figure 1a. The top drawing in

Figure 1a shows two double helices with 4-residue sticky ends that are complementary to each other. The middle drawing shows that these two molecules can cohere by hydrogen bonding to form a single molecular complex. The bottom drawing illustrates that it is possible to ligate these molecules to form one double helical complex.

The predictable specificity of sticky-ended affinity is only one part of the story of sticky ends. The other part is structure. It has been shown that sticky ends cohere to form the conventional structure of DNA, known as B-DNA. The importance of this fact becomes evident when we consider other systems from which specific affinity might be derived. For example, antigen-antibody complexes have high specificity, but the geometrical description of the system is not predictable; it requires experimental work, e.g., crystallography, to be determined for each individual case. By contrast, the structure of sticky-ended DNA complexes is well-defined, at least locally. Given that the persistence length of DNA is about 50 nm,² the local structure in the vicinity of a combining site is very well-known. Thus, sticky ends can be used to join DNA molecules together. One might think of an analogy to Velcro, but Velcro is floppy. The strongly cohesive pieces of a jigsaw puzzle or, perhaps, of Lego blocks are a better analogy.

The foregoing describes a way to assemble linear DNA molecules. It is not terribly interesting to join linear DNA molecules for nanotechnological purposes, because they cannot produce precise complex systems in 2D or 3D. To do that, it is necessary to work with branched DNA molecules, rather than linear DNA molecules. Fortunately, branching is a concept familiar in nucleic acid chemistry and molecular biology. For example, ephemeral branch points are found as Holliday junction intermediates in the process of genetic recombination.³ Branching can occur when exchange occurs between two DNA strands, as illustrated in Figure 1b.⁴ Two strands are shown, one outlined, and one filled. When reciprocal exchange occurs between such molecules, new species are generated; in Figure 1b, the products are a filled-outlined molecule, and an outlined-filled molecule.

This simple protocol can lead to a variety of branched species, some of which are illustrated in Figure 1c. A single reciprocal exchange event can produce a Holliday junction-like molecule (HJ), shown at the left of Figure 1c. This is a 4-arm branch, which is stabilized by ensuring that there is no twofold symmetry around its branch point; minimizing sequence symmetry is key to the design of all unusual DNA motifs.^{5,6} In addition, to 4-arm junctions,⁷ 3-arm junctions⁸ and 5-arm and 6-arm junctions have been reported.⁹ A double exchange between DNA double helices produces double crossover (DX) molecules,¹⁰ and double exchanges between three successive helices produced triple crossover (TX)¹¹ molecules. These molecules are all shown in Figure 1c. They have been produced by reciprocal exchange between strands of opposite polarity. Strands with the same polarity can also undergo reciprocal exchange. An example of this, shown with exchange events at every possible position is the paranemic crossover (PX) molecule.¹² A PX molecule lacking two exchanges, called JX₂, is shown next to the PX molecule. These last two motifs are used in a nanomechanical device described below.

Figure 1d illustrates the combination of branched motifs with sticky-ended association. A 4-arm junction, shown in a cruciform arrangement, has its arms tailed in sticky ends, X and its complement X', and Y and its complement Y'. The panel on the right illustrates four of these molecules arranged parallel to each other to form a quadrilateral. Note that there are unsatisfied sticky ends. Those on the outside of the quadrilateral can participate in further interactions to yield a 2D periodic array. A variety of 2D DNA arrays have been produced, and they are described below. The helical nature of the DNA molecule means that constructs are not restricted to two dimensions. In principle, motifs can be rotated out of the plane to produce 3D designed periodic arrangements; this 3D crystals have been produced, but their

quality is limited to 10 Å resolution. In addition, a number of objects have been generated that are certainly incompatible with planar graphs. These include trefoil and figure-8 knots,^{13,14} and Borromean rings,¹⁵. Polyhedral catenanes whose helix axes have the connectivities of a cube¹⁶ and of a truncated octahedron¹⁷ were early successes in this program.

2. DNA ARRAYS

Being able to produce a series of topological targets, such as the knots, Borromean rings and polyhedral catenanes is of little value unless functionality can be included in the system. The essence of nanotechnological goals is to place specific functional species at particular loci, using the architectural properties of DNA. Functionality includes the use of periodic DNA arrays to scaffold molecular arrangements of other species, algorithmic assemblies that perform computations, and the development of DNA-based nanomechanical devices. Algorithmic assembly is generally regarded as aperiodic assembly for purposes of computation or construction; it is beyond the scope of this article. In addition to the intermolecular specificity described above, the key architectural property that is needed to build and demonstrate these arrays and devices is high structural integrity in the components; even if their associations are precise, the assembly from marshmallow-like components will not produce well-structured materials. Single-branched junctions, such as the HJ structure in Figure 1c are relatively flexible.^{8,18} Fortunately, the DX molecule is considerably more rigid, apparently stiffer than even double helical DNA.¹⁹ The TX and PX molecules appear to share this rigidity²⁰ Consequently, it has been possible to use these molecules as the building blocks of both arrays and devices. Arrays are useful both as the basis of DNA computation by self-assembly and as frameworks to mount DNA nanomechanical devices.

Figure 2 illustrates 2D arrangements that entail the use of DX molecules to produce periodic patterns. Figure 2a illustrates a two-component array that can tile the plane. One tile is a DX molecule labeled **A** and the second is a DX molecule labeled **B***. **B*** contains another DNA domain that projects out of the plane of the helix axes. This other domain serves as a topographic marker for the atomic force microscope (AFM) when the **AB*** array is deposited onto the surface of mica. The dimensions of the two DX tiles in Figure 2a are about 4 nm tall × 16 nm wide × 2 nm thick. Thus, the **B*** markers in the 2D array shown should appear as stripe-like features separated by ~32 nm, which has been confirmed by experiment. Figure 2b shows a 4-tile arrangement that should produce stripes separated by ~64 nm, also confirmed by experiment.²¹ Thus, it is possible to design and produce patterns using DNA components; these patterns contain predictable features, based on the sticky-ended cohesion of individual motifs. In addition to forming arrays from DX molecules, it is also possible to produce periodic arrays from TX molecules.¹¹ A variety of DNA parallelograms have been used to produce arrays, as well.^{22–24} These motifs are produced by combining four HJ-like branched junctions. Unlike the DX, TX and PX molecules, the two domains of the HJ molecule are not parallel to each other. As a function of the sequence and backbone connections at the crossover point, they adopt angles of ~60°, ~70°, or ~40°, thus producing a diversity of parallelogram angles.

The 2D packing of natural objects is usually hexagonal. Making hexagonal arrays was long an unfulfilled goal of DNA nanotechnology: The three-arm junction is floppy, and even stiffer motifs were incapable of producing an hexagonal array. In addition, the single sticky end is susceptible to variations of twist. Doubling the thickness of a bulged-junction triangle to produce a doubly-thick motif proved to be the key to reaching this goal.²⁵ Figure 3 illustrates two slightly-different triangles combining to produce a trigonal-pseudo-hexagonal array. An AFM image of this array is also shown in Figure 3. The honey-comb nature of the

arrangement is evident from this image. A key control experiment was to remove the sticky ends from one of the two helices in each direction: No array was found, demonstrating that the presence of DX cohesion helps to solve such geometrical problems as exist (e.g., twist problems) when only a single helix is used.

The key goal, of course, is not 2D arrays, but 3D arrays. This is a somewhat trickier system, because the analytical tool to be brought to bear is much more demanding: 2D arrays are analyzed by AFM, a technique whose ultimate resolution is of the order of 2–3 nm in most cases. By contrast, 3D arrays, i.e., crystals, are examined by x-ray diffraction; the resolution of this technique is limited only the wavelength of the light, usually around 1 Å. X-ray diffraction patterns that extend only to, say, 10 Å are not generally regarded as very good, as the nature of atomic structure in macromolecules is only revealed around 2–3 Å resolution. Thus, the criteria for successful arrays are much higher for 3D than they are for 2D.

Trouble-shooting the formation of 3D crystals is notoriously difficult, because the interactions that determine the crystal structure are not usually known until after the structure has been determined. However, designed crystals offer analytical approaches that are not available to traditional crystallization. If a motif inherently spans 3-space, and has sticky ends along each of the three directions, it is possible to analyze the interactions in the three different sections that result from using sticky ends only in two directions. For the motif shown in Figure 4a, a three-section set of such arrays is shown in Figure 4b. It is evident from these images that the middle direction has no geometrical problems, but that the other two arrays show a tendency to curl, rather than to lie flat on the surface. A second example is shown in Figure 5. Here, we illustrate a 6-helix bundle motif and the three arrays that result from it.

Programmed arrays bear on several aspects of DNA nanotechnology. First, they appear to be appropriate for the scaffolding of other molecules. One suggestion is that periodic arrays could be used to arrange biological macromolecules into crystalline arrangements⁵ (Figure 6a). A second suggestion is that they could be used to arrange the components of nanocircuitry²⁶ (Figure 6b). Similarly, arrays may be used as a supporting surface to organize nanomechanical switches and devices. With an eye to using DNA as scaffolding, we other components have been attached to these arrays, such as gold nanoparticles²⁷ and nylon subunits²⁸. In an attempt to make arrays from neutral components, peptide-nucleic acid (PNA) backbones have been incorporated into the array shown in Figure 2a. The helicity of B-DNA is about 10.5 nucleotide pairs per turn, but we found in this work that the helicity of PNA-DNA hybrid molecules is about 15.5.²⁹

3. DNA NANOMECHANICAL DEVICES

Nanomechanical action is a central target of nanotechnology. The first DNA-based devices were predicated on structural transitions of DNA driven by small molecules. The first deliberate DNA device entailed the extrusion of a DNA cruciform structure from a cyclic molecule.³⁰ The position of the branch point was controlled by the addition or removal of an intercalating dye to the solution. This system was not very convenient to operate, and the large size of the DNA circle made it unwieldy to handle. Nevertheless, it demonstrated that DNA could form the basis of a two-state system.

The second DNA-based device (Figure 7a) was a marked advance. It was relatively small, and included two rigid components, DX molecules like those used to make the arrays of Figure 2. It, too, relied on the addition or removal of a small molecule. The basis of the device was the transition between right-handed (conventional) B-DNA and left-handed Z-DNA. There are two requirements for the formation of Z-DNA, a 'proto-Z' sequence capable of forming Z-DNA readily (typically a (CG)_n sequence), and conditions (typically

high salt or molecules like $\text{Co}(\text{NH}_3)_6^{3+}$ that emulate the presence of high salt) to promote the transition³¹ The sequence requirement enables us to control the transition in space, and the requirement for special conditions allows us to control the transition, and hence the device, in time. As shown in Figure 4a, the device consists of two DX molecules connected by a shaft containing a proto-Z sequence that consists of $(\text{CG})_{10}$. In B-promoting conditions, both of the DX helices not collinear with the shaft are on the same side of the shaft. In Z-promoting conditions, one of these helices winds up on the other side of the shaft. This difference is the result of converting a portion of the shaft to Z-DNA, which rotates one DX motif relative to the other by 3.5 turns. The motion is demonstrated by fluorescence resonance energy transfer (FRET) measurements that monitor the difference between dye separations on the DX motifs³².

The problem with the B–Z device is that it is activated by an unspecific molecule, $\text{Co}(\text{NH}_3)_6^{3+}$. Thus, a number of such devices, embedded in an array, would all respond similarly, at least within the limits of a small amount of chemical nuance¹⁴ Thus, N two-state devices would result in essentially two structural states; clearly it would be of much greater value to have N distinct 2-state devices capable of producing 2^N structural states. It is evident that a sequence-dependent device would be an appropriate vehicle for the goal of achieving multiple states. The method for devising sequence-dependent devices was worked out by Yurke and his colleagues.³³ It entails setting the state of a device by the addition of a ‘set’ strand that contains an unpaired tail. When the full complement to the set strand is added, the set strand is removed, and a different set strand may be added. This system has been adapted to the PX and JX_2 motifs (Figure 1)²⁰ Figure 7b shows how these two states can be inter-converted by the removal of one pair of set strands (processes **I** and **III**) and the addition of the opposite pair (processes **II** and **IV**). The tops and bottoms of the two states differ by a half-rotation, as seen by comparing the **A** and **B** labels at the tops of the molecules, and the **C** and **D** labels at the bottoms of the molecules. A variety of devices can be produced by changing the sequences of the regions where the set strands bind.

We have exploited this idea by combining two such devices to produce a nanomechanical device that performs translation. This device is shown in Figure 8, where its component parts are compared with those of a ribosome, the cellular translation device. Two different PX-JX_2 devices are shown coupling a diamond and two double-diamonds, labeled in Roman numerals. The set strands for the PX-JX_2 devices are the message to be translated. The Arabic numerals indicate sticky ends; those along the top row are set by the states of the two devices. The translation takes place through the mediation of DX molecules, which act in the same way as aminoacyl-tRNA molecules. The top strand of the DX will be incorporated into the product analogous to the amino acid. The bottom portion of the DX, like the tRNA, binds to the top row of the device through its sticky ends, labeled ‘i’ and ‘j’. There are six different DX molecules, corresponding to the possible device slots in the four states that the translation device can assume. The correct molecules are incorporated, regardless of which coded set strands are added; this is easily established by sequencing the product. The coding between the sequences of the set strands and the sequences of the product is arbitrary.

Another device that exploits the Yurke method of adding and removing strands from a DNA motif is a bipedal walker that walks on a sidewalk.³⁵ The operation of this ‘nanorobot’ is illustrated in Figure 9. The walker is attached non-covalently to the sidewalk by a pair of set strands (Figure 9a) that can be removed by the introduction of their complete complements (unset strands) into the solution. The leading leg of the walker is removed in this fashion (Figure 9b–c). Addition of a new set strand can bind the leading leg to a new position on the sidewalk (Figure 9d). The trailing leg then can be removed from the sidewalk by the addition of its unset strand, (Figure 9e) and a new set strand can be added to bind it to its new position (Figure 9f). The walker can be walked in both directions. Its pathway is

demonstrated by crosslinking aliquots of the walker-sidewalk complex at each step and analyzing the components on a denaturing gel.

4. WHAT'S NEXT?

This article has discussed the current state of structural DNA nanotechnology, emphasizing the individual components, their assembly into periodic arrays, and their manipulation as nanomechanical devices. Where is this area going? The achievement of several key near-term goals will move structural DNA nanotechnology from an elegant structural curiosity to a system with practical capabilities. First among these goals is the extension of array-making capabilities from 2D to 3D, particularly with high order. Likewise, heterologous molecules must be incorporated more reliably into DNA arrays, so that the goals both of orienting biological macromolecules for diffraction purposes and of organizing nanoelectronic circuits may be met. A DNA nanorobotics awaits the incorporation of the PX-JX₂ device into arrays. The development of self-replicating systems using branched DNA appeared until recently to be somewhat oblique,³⁶ but the recent results from Shi *et al.*³⁷ in making a replicable DNA octahedron constitute a landmark breakthrough in this area. Currently, structural DNA nanotechnology is a biokleptic pursuit, stealing genetic molecules from biological systems; ultimately, it must advance from biokleptic to biomimetic, not just using the central molecules of life, but improving on them, without losing their inherent power as central elements of self-assembled systems.

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Figure 1. Components of Structural DNA Nanotechnology

(a) *Sticky Ended Cohesion*. Two linear double helical molecules of DNA are shown at the top of panel (a). The antiparallel backbones are indicated by the black lines terminating in half-arrows. The half-arrows indicate the 5'→3' directions of the backbones. The right end of the left molecule and the left end of the right molecule have single-stranded extensions ('sticky ends') that are complementary to each other. The middle portion shows that, under the proper conditions, these bind to each other specifically by hydrogen bonding. The bottom of panel (a) shows that they can be ligated to covalency by the proper enzymes and cofactors. (b) *Reciprocal Exchange of DNA Backbones*. Two strands are shown on the left, one filled, and one unfilled. Following reciprocal exchange, one strand is filled-unfilled, and the other strand is unfilled-filled. (c) *Key Motifs in Structural DNA Nanotechnology*. On the left is a Holliday junction (HJ), a 4-arm junction that results from a single reciprocal exchange between double helices. To its right is a double crossover (DX) molecule, resulting from a double exchange. To the right of the DX is a triple crossover (TX) molecule, that results from two successive double reciprocal exchanges. The HJ, the DX and the TX molecules all contain exchanges between strands of opposite polarity. To the right of the TX molecule is a paranemic crossover (PX) molecule, where two double helices exchange strands at every possible point where the helices come into proximity. To the right of the PX molecule is a JX₂ molecule that lacks two of the crossovers of the PX molecule. The exchanges in the PX and JX₂ molecule are between strands of the same polarity. (d) *The Combination of Branched Motifs and Sticky Ends*. At the left is a 4-arm branched junction with sticky ends. On the right four such molecules are combined to produce a quadrilateral. The sticky ends on the outside of the quadrilateral are available so that the structure can be extended to form a 2D lattice.

Figure 2. Tiling the Plane with DX Molecules

(a) *A Two-Tile Pattern.* The two helices of the DX molecule are represented schematically as rectangular shapes that terminate in a variety of shapes. The terminal shapes are a geometrical representation of sticky ends. The individual tiles are shown at the top of the drawing; the way tiles fit together using complementary sticky ends to tile the plane is shown at the bottom. The molecule labeled **A** is a conventional DX molecule, but the molecule labeled **B*** contains a short helical domain that protrudes from the plane of the helix axes; this protrusion is shown as a black dot. The black dots form a stripe-like feature in the array. The dimensions of the tiles are 4 nm $\times$ 16 nm in this projection. Thus, the stripe-like features should be about 32 nm apart. (b) *A Four-Tile Pattern.* The same conventions apply as in (a). The four tiles form an array in which the stripes should be separated by about 64 nm, as confirmed by AFM.

Figure 3. The Assembly of a Trigonal Lattice by DX Cohesion

The upper part of the panel shows two triangles made from DX components. They combine in the lower left to produce a pseudo-hexagonal trigonal lattice. The lower right shows an AFM image of the array.

Figure 4. A 3D DX Triangle and each of its 2D Periodic Sections

(a) The motif is shown in a 3D representation; three DX molecules are arranged in an overlapping pattern. (b) The three 2D sections that one gets if one puts sticky ends only on helices in two directions.

Figure 5. A Six-Helix Bundle and each of its 2D Periodic Sections

(a) Transverse (left) and longitudinal (right) views of a schematic of the six-helix bundle. Referring to (a), the bundles can span 3-space with DX connections by connecting the front top pair of helices to the back bottom pair, the front lower-right pair to the back upper-right pair and the front lower-left pair to the back upper-left pair. (b) AFM images of the three 2D sections in which each of these units has been left deliberately with blunt ends.

Figure 6. Applications of DNA Periodic Arrays

(a) *Biological Macromolecules Organized into a Crystalline Array.* A cube-like box motif is shown, with sticky ends protruding from each vertex. Attached to the vertical edges are biological macromolecules that have been aligned to form a crystalline arrangement. The idea is that the boxes are to be organized into a host lattice by sticky ends, thereby arranging the macromolecular guests into a crystalline array, amenable to diffraction analysis. (b) *Nanoelectronic Circuit Components Organized by DNA.* Two DNA branched junctions are shown, with complementary sticky ends. Pendent from the DNA are molecules that can act like molecular wires. The architectural properties of the DNA are seen to organize the wire-like molecules, with the help of a cation, which forms a molecular synapse.

Figure 7. DNA-Based Nanomechanical Devices

(a) *A Device Predicated on the B-Z Transition.* The molecule consists of two DX molecules, connected by a segment containing proto-Z-DNA. The molecule consists of three cyclic strands, two on the ends drawn with a thin line, and one in the middle, drawn with a thick line. The molecule contains a pair of fluorescent dyes to report their separation by FRET. One is drawn as a filled circle, and the other as an empty circle. In the upper molecule, the proto-Z segment is in the B conformation, and the dyes are on the same side of the central double helix. In the lower molecule, the proto-Z segment is in the Z conformation, and the dyes are on opposite sides of the central double helix. (b) *A Sequence-Dependent Device.* This device uses two motifs, PX and JX₂. The labels A, B, C and D on both show that there is a 180° difference between the wrappings of the two molecules. There are two strands drawn as thick lines at the center of the PX motif, and two strands drawn with thin lines at the center of the JX₂ motif; in addition to the parts pairing to the larger motifs, each has an unpaired segment. These strands can be removed and inserted by the addition of their total complements (including the segments unpaired in the larger motifs) to the solution; these complements are shown in processes I and III as strands with black dots (representing biotins) on their ends. The biotins can be bound to magnetic streptavidin beads so that these species can be removed from solution. Starting with the PX, one can add the complement strands (process I), to produce an unstructured intermediate. Adding the set strands in process II leads to the JX₂ structure. Removing them (III) and adding the PX set strands (IV) completes the machine cycle. Many different devices could be made by changing the sequences to which the set strands bind.

Figure 8. A DNA Nanomechanical Device that Executes Translation

The device is shown at top. It consists of a DNA diamond (labeled I) and two double diamonds, (labeled II and III and labeled IV and V). These motifs are connected by PX-JX₂ devices, leading to four possible states. The Arabic numerals indicate sticky ends. The four states correspond to different sticky end pairs along the top row, 1–2, 4–6 (shown); 1–2, 4–7; 1–3, 5–6; and 1–3, 5–7. Six DX molecules with sticky ends *i* and *j* corresponding to these pairs are in solution, and bind to the sticky ends set by the set strands of the PX-JX₂ devices. The set strands are analogous to the message codons in ribosomal translation, the thick strand of the DX is analogous to the amino acid, and the rest of the DX is analogous to the tRNA molecule that acts as an adaptor. The ‘amino acid’ strands are linked together and then can be sequenced.

Figure 9. A DNA Bipedal Walker that Walks on a Sidewalk

The steps of the walk are shown. In the first stage (a), the walker is attached to the sidewalk by set strands 1A and 2B. In the second and third steps (b and c), unset strand 2B removes set strand 2B, and the flexible linker allows the right leg of the device to move in solution. In the fourth step (d), a new set strand, 2C, attaches this leg to the rightmost point on the sidewalk. In the fifth step (e), set strand 1A is removed by unset strand 1A, freeing the left leg. In the final step (f), set strand 1B attaches the left leg to the sidewalk. The net result is that the walker has moved from the left side of the sidewalk to the right side.