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At the Crossroads of Chemistry, Biology, and Materials: Structural DNA Nanotechnology

Review

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Structural DNA nanotechnology consists of combining unusual DNA motifs by specific structurally well-defined cohesive interactions (primarily sticky ends) to produce target materials with predictable 3D structures. This effort has generated DNA polyhedral catenanes, robust nanomechanical devices, and a variety of periodic arrays in two dimensions. The system has been used to produce specific patterns on the mesoscale through designing and combining specific DNA strands, which are then examined by atomic force microscopy. The combination of these constructions with other chemical components is expected to contribute to the development of nanoelectronics, nanorobotics, and smart materials. The organizational capabilities of structural DNA nanotechnology are just beginning to be explored, and the field is expected ultimately to be able to organize a variety of species that will lead to exciting and possibly revolutionary materials.

Introduction

We have recently celebrated the 50th anniversary of the Watson-Crick proposal for the structure of DNA. This proposal provided the chemical basis for our understanding of genetics; indeed, the last half-century of biology has been devoted to the exploitation of this model and to attempts to incorporate it into classical biological phenomena, such as recombination and development. With the culmination of the Human Genome Project as well as the forensic and medical applications of DNA analysis, society is just beginning to feel the impact of DNA. It would not be inappropriate to describe the double helix as a structural emblem for our culture, in much the same way that the pyramids of Egypt, the Great Wall of China, the temples of ancient Greece and Rome, and the cathedrals of medieval Europe were emblematic of those cultures.

Regardless of its central importance in biology, the applications of DNA are not restricted to the biological sciences. DNA is a molecule, and it functions successfully as genetic material because of its chemical properties. These properties include the affinity of complementary sequences, a well-stacked antiparallel double-helical backbone that is largely regular regardless of sequence, a persistence length around 50 nm, and a code that makes it possible to read its sequence from the outside, even when the strands are paired. It natural to ask, "Can these properties be exploited outside of biology?" Structural DNA nanotechnology aims to use the properties of DNA to produce highly structured and well-ordered materials from DNA; it is an effort that has been underway since the early 1980s [1].

The recognition of DNA molecules by their complements can be used for more than the formation of a simple double helix. Genetic engineers recognized in the early 1970s that single-stranded overhangs (termed "sticky ends") could be used to direct the intermolecular associations of different DNA molecules [2]. Sticky-ended cohesion is illustrated in Figure 1A. An important feature of sticky-ended cohesion is that when two sticky ends cohere, they form classical B-DNA [3], as shown in Figure 1B. Thus, if one knows the positions of the atoms of one component of a cohesive pair, one knows the positions of the other component. Hence, sticky-ends provide the most readily programmable and predictable intermolecular interactions known, from the perspectives of both affinity and structure. They can be usefully contrasted with antibody-antigen interactions, whose affinities are well-defined but whose structural interactions are not predictable a priori. Overall antibody structures are well known, but experimental determination of the structure of each antibody-antigen pair is necessary if we are to know as much about their relative orientations as we already know about the structure of DNA molecules that cohere by sticky-ended cohesion.

That's the good news, but there is also bad news: from a topological standpoint, the DNA double helix is just a line. The line may be curved, and closed ones can be knotted or even catenated; nevertheless, concatenating a bunch of lines together end-to-end just results in longer lines. Not very interesting. What would solve this problem? The answer is evident: branched DNA molecules. DNA branched at the level of secondary structure is found in biological systems, most prominently as the ephemeral four-arm Holliday junction, an intermediate in genetic recombination [4]. Although branched DNA of biological origin usually displays symmetry that allows its branch point to migrate, it is a simple matter to design [1] and assemble synthetic DNA sequences that are stable because they lack this symmetry. An example of a stable asymmetric Holliday junction analog lacking this symmetry is shown in Figure 1C. Branched junctions containing three, five, or six arms have also been assembled and characterized [5, 6].

Structural DNA Nanotechnology

The basic notion behind structural DNA nanotechnology is to combine the concepts of stable branched DNA molecules with sticky-ended cohesion or other forms of cohesion that are structurally well defined. This idea is illustrated in Figure 1D, which shows a branched sticky-ended junction that assembles as a group of four units to produce a quadrilateral. The outside of this quadrilateral contains further sticky ends, so in principle, this arrangement could be extended to produce a lattice in two or three dimensions [1]. Once one recognizes that branched DNA molecules can be fused, molecular graphs and connected networks whose edges are DNA helix axes can be constructed in an almost limitless variety.

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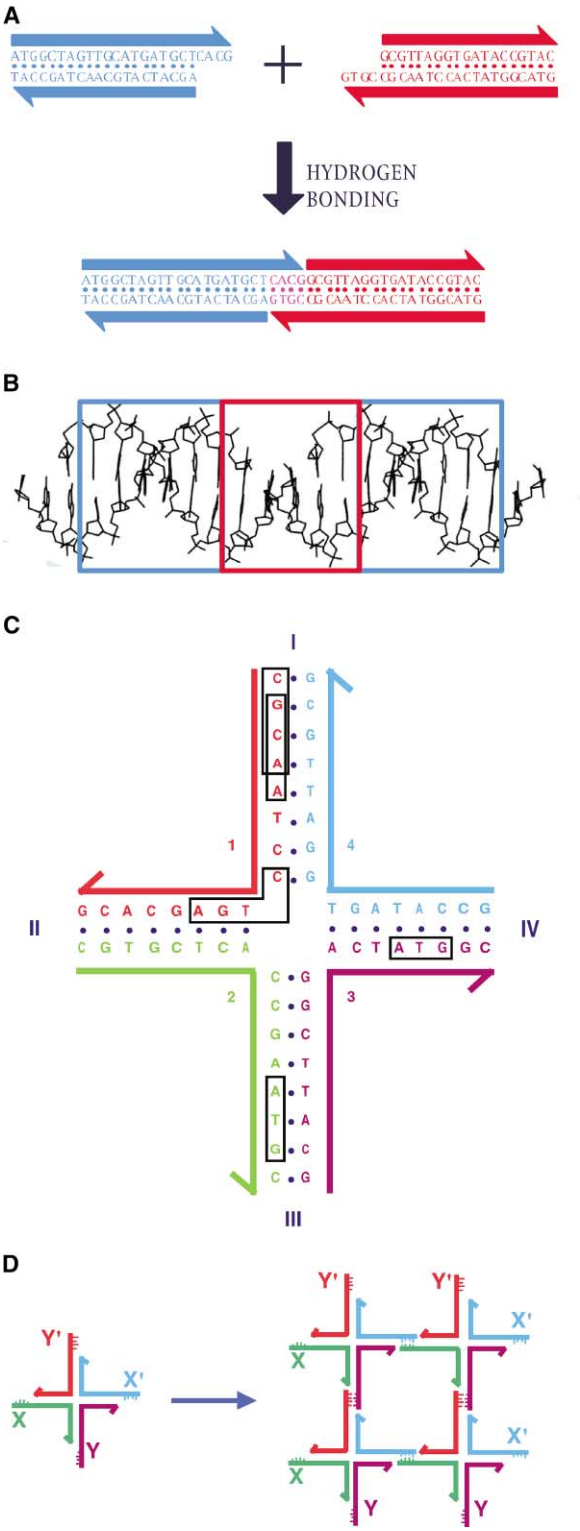

Figure 1. Basics of Structural DNA Nanotechnology

(A) Sticky-ended cohesion. Two double-helical strands with complementary overhangs are shown. Under appropriate conditions, they will cohere in a sequence-specific fashion and can be ligated, if desired.

(B) The structure of the sticky-ended junction. A portion of the crystal structure of an infinite DNA double helix formed by sticky-ended cohesion is shown. The part cohering by sticky ends is in the red box, whereas the blue boxes surround continuous DNA segments. The DNA in all three sections is conventional B-DNA.

(C) A stable branched junction. There is no dyad symmetry flanking the branch point; tetramers, such as the boxed sequences CGCA and GCAA, are unique, and there is no TCAG to complement the CTGA flanking the corner.

(D) Sticky-ended assembly of branched molecules. A branched molecule is shown on the left with four sticky ends, X, complementary to X′, and Y, complementary to Y′. Four of them are shown to assemble to form a quadrilateral, with further sticky ends on the outside, so that an infinite lattice could be formed by the addition of further components.

It is important to distinguish structural DNA nanotechnology from another type of DNA nanotechnology that I will term compositional DNA nanotechnology. Structural DNA nanotechnology uses well-structured components, combined by using both affinity and structure to control geometry or, at least, strand topology; the goal of this approach is structural predictability with a precision (or resolution) of 1 nm or less in the products. By contrast, in compositional DNA nanotechnology, these conditions are not completely met; the components may be flexible or unknown, or the cohesive interactions by which they are combined may be uncharacterized. Consequently, the composition of the product may be known, but its 3D structure may unpredictable. Incompletely characterized forms of cohesion such as paranemic cohesion [7] or the osculating interactions of tecto-RNA [8] will eventually be used conveniently in structural DNA nanotechnology when they are as well characterized as sticky ends.

A detailed description of the work produced by compositional DNA nanotechnology, in which DNA is used largely as “smart-glue,” rather than a precise structural component, is beyond the scope of this article. Nevertheless, numerous laboratories have managed to make useful and valuable materials by this approach. For example, this approach has been used in diagnostics [9], in the organization of DNA nanoparticles on small [10] and large [11] scales, and in the production of DNA-protein aggregates [16]. It has also been used in combination with non-DNA organic components [12, 13]. Although smart-glue approaches do not provide the high-resolution structural features sought in structural DNA nanotechnology, using such approaches to organize nanoparticles can lead to organized products, but with lower precision (10s to 100s of nanometers). In a complementary vein, G-wires [14, 15] are examples of well-structured nucleic acid systems that lack the sequence diversity central to structural DNA nanotechnology.

Advantages of DNA for Structural Nanotechnology

The primary advantage of DNA for these goals lies in its outstanding molecular-recognition properties, enabling precise structural alignment of diverse DNA molecules that piggyback various molecular species. DNA appears to be unique among biopolymers in this regard. As noted above, other systems may lead to specific binding, but only with nucleic acids (and currently only with sticky-ended cohesion) are the detailed 3D geometries known

in advance; this is because sticky ends form B-DNA when they bind to link two molecules together [3].

The convenience of chemical synthesis [17] is another key advantage of DNA; "vanilla" DNA is available from a number of vendors, and DNA synthesizers are readily capable of generating a number of varied molecules based on commercially available phosphoramidites. A variety of enzymes are commercially available to manipulate DNA and to trouble-shoot errors. For example, DNA ligases enable the covalent joining of complexes held together by sticky-ended cohesion; exonucleases are useful in purifying cyclic target molecules from linear-failure products [18]; restriction endonucleases are useful both to trouble-shoot syntheses and to create cohesive ends from topologically closed species [18, 19].

We noted above several of DNA's features that help it to perform its genetic functions. One of these was the persistence length, about 500 Å [20], leading to a predictable overall structure for the short (70–100 Å) lengths typically used. Another is the external DNA code that can be read even when the double helix is intact [21]; thus, if DNA is used for scaffolding, absolute positions can be addressed within a predesigned cavity. The ability to pack nanoelectronics very tightly is likely to be aided by the high density of functional groups (every 3.4 Å or so) on DNA; consequently, DNA tile motifs with dimensions of 10–20 nm do not place an inherent limit in the close packing of components that can be scaffolded by DNA.

We have exploited DNA in almost all of this work. However, we are not limited to the DNA molecule evolved in nature for use as genetic material. A vast number of DNA analogs have been produced and analyzed for therapeutic purposes (e.g., [22]). This means that systems prototyped by conventional DNA may ultimately be converted to other backbones and bases, as required by specific applications. For example, it is unlikely that nanoelectronic components will be scaffolded successfully by conventional polyanionic DNA molecules. However, there are numerous neutral analogs, such as PNA, that may be much better suited to act as scaffolds for this purpose.

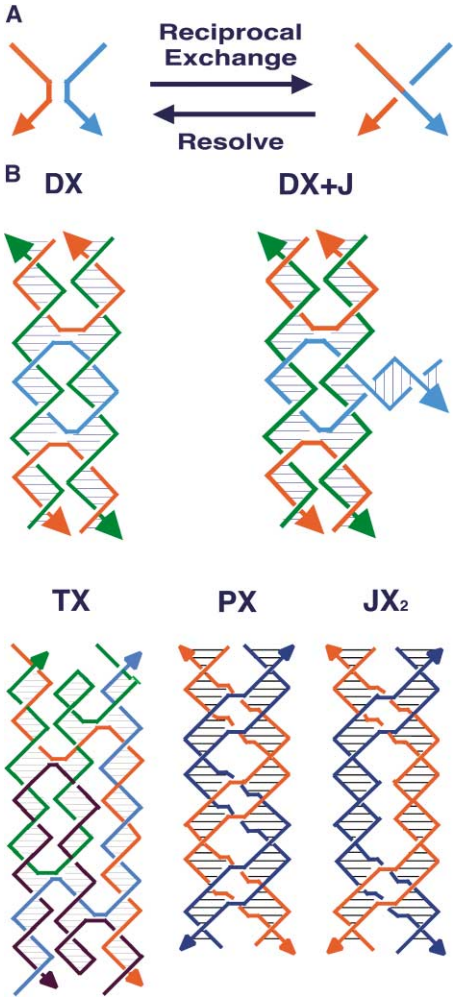

Figure 2. Motif Generation

(A) The process of reciprocal exchange. A red strand and a blue strand exchange to form a red-blue strand and a blue-red strand.

(B) Motifs used in structural DNA nanotechnology. Two reciprocal exchanges between strands of opposite polarity yield the DX molecule shown. The DX+J motif, usually made with the extra helix roughly perpendicular to the plane of the other two, is made by combining a DNA hairpin and a DX molecule. The TX motif results from combining the DX molecule with another double helix. The PX motif is derived by performing reciprocal exchange between two helices at all possible positions where strands of the same polarity come together. The JX_2 motif is similar to the PX motif except that reciprocal exchange is omitted at two adjacent juxtapositions.

Motif Generation and Design

The formation of a square-like quadrilateral from four junctions in Figure 1D rests on the assumption that the 4 arm branched molecule is rigid and looks like a simple crossroads. Neither of these is correct. The structure is a right-handed cross involving two stacking domains [23, 24] but, more seriously, the connection between the domains is somewhat flexible. Fortunately, one of the mechanisms that biology uses to produce the Holliday junction provides a general mechanism to derive stiff motifs. This mechanism is known as reciprocal exchange [25] and is illustrated in Figure 2A. It can be performed multiple times between strands of the same polarity or of opposite polarity, leading to different products. Although the process of reciprocal exchange is often complicated in living systems, nanotechnological systems do not actually have to undergo reciprocal exchange to produce new motifs; once the structural design process is complete, new motifs are generated directly via a sequence assignment procedure to design strands that will self-assemble into the motif. Figure 2B illustrates the DX molecule (opposite polarity) [26], the DX+J molecule [27], the TX molecule (opposite polarity) [28], and the PX and JX_2 molecules (same polarity) [25]. The DX molecule is known to be about twice as stiff as conventional linear duplex DNA [29]; the DX, DX+J, and TX motifs have been used to produce patterned 2D DNA arrays [28, 30], and the DX, PX, and JX_2 motifs have been used as components of DNA nanomechanical devices [31, 32]. The rigidity of these motifs is key to their utility in these applications.

As noted above, stable branched junctions are pro-

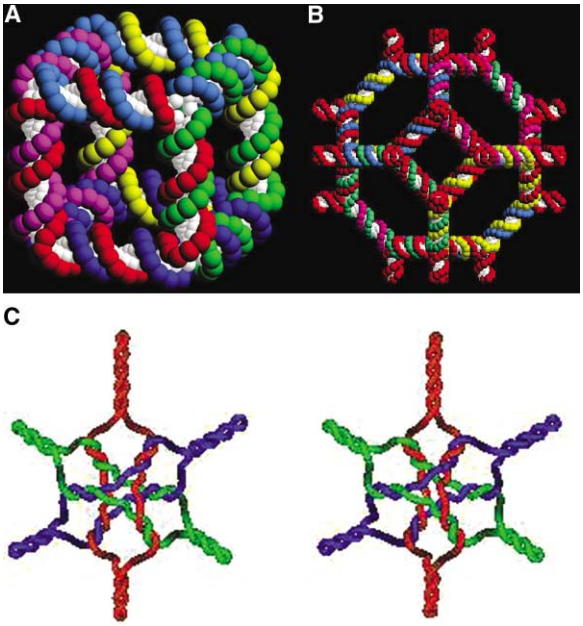

Figure 3. Ligated Products from Flexible DNA Components

(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. There are two turns of DNA between the vertices of each polyhedron, making them, respectively, a hexacatenane and a 14-catenane. (C) Borromean rings. This is a stereo view of Borromean rings. A right-handed 3 arm junction is in front, and a left-handed 3 arm junction is at the rear; if any of the circles is cleaved at one of its equatorial hairpins, the other two circles dissociate.

duced from synthetic molecules, so there must be a method for assigning sequences to them. We have been successful in using the method of sequence symmetry minimization [1, 33] to design sequences. The basic idea is that DNA strands will maximize the double-helical structures that they form. Although an early approach [34] involved calculating a likelihood of formation from nearest-neighbor equilibrium thermodynamic parameters, no constructs built to date appear to require a calculation this extensive. The method used can be understood readily by reference to Figure 1C. This molecule contains four 16-mers, labeled 1, 2, 3, and 4. We break up each of these single strands into a series of 13 overlapping tetramers, such as the CGCA or GCAA that have been boxed; we insist that each of these be unique. In addition, we insist that each tetramer that spans a branch point, such as the boxed CTGA, not have its linear complement (TCAG) present; this restriction results in these tetramers being unable to form linear double helices. Consequently, competition with the four octamer double-helical targets can occur only from trimers, such as the boxed ATG sequences.

Prototype Systems for New Materials

The idea of using DNA sequences to direct the assembly of new materials has been demonstrated many times. The first examples were DNA stick polyhedra, where the edges are double-helical DNA and the vertices correspond to the branch points of DNA branched junctions. A cube-like molecule [18] and a truncated octahedron [35] are shown in Figure 3. Although the double helices flanking a branch point are quite stiff, the angles between them appear to be much floppier [5]. Consequently, these polyhedra can be described and characterized only on the topological levels of branching and linking but not on the level of structural geometry with well-defined coordinates. With edges whose lengths contain an exact number of double-helical turns (two for each polyhedral edge), each face corresponds to a cyclic single strand. Thus, the cube is a hexacatenane, and the truncated octahedron is a 14-catenane. The cube was synthesized in solution, but the truncated octahedron was assembled step-wise by a solid support method [19].

If a catenane or a knot is drawn in a 2D representation, there will be a number of positions where one strand crosses over another. These are the nodes (or "unit tangles" [36]) of the system. Catenanes and knots are characterized by the numbers and locations of their nodes. A half-turn of DNA corresponds to a node in a knot or catenane [37]. Owing to the close relationship between catenanes and knots [38], DNA also has been used to design and synthesize [39] a variety of single-stranded knot molecules. In addition, it has been relatively simple to construct a long-time topological target, Borromean rings [40]. DNA appears to be the most convenient system with which to achieve topological targets.

Catenanes and knots are individual objects that demonstrate the versatility and convenience of DNA branched junctions as a system for the construction of difficult targets. However, it is necessary to produce larger, well-structured DNA systems, such as periodic arrays, to generate interesting materials. The floppiness of individual branched junctions makes them unsuitable as components for such constructions. However, as noted above, DX molecules (Figure 2B) are branched species that are roughly twice as stiff as linear DNA [27, 29] and are therefore substantially stiffer than simple branched junctions. They, and TX molecules (Figure 2B), are therefore well-suited to serve as components for periodic arrangements.

The first examples of two-dimensional periodic arrays were DX arrays that contained the capability to produce patterns [30]. Figure 4A illustrates an array produced from a DX molecule and a DX+J molecule. The dimensions of these 2 nm-thick tiling components are about 4 nm $\times$ 16 nm. The DX+J molecule has its extra domains rotated out of the plane of the array so that they can act as topographic markers for the atomic-force microscope. Thus, a series of striped features, separated by about 32 nm, should appear on the pattern, as seen on the right of the drawing. To demonstrate the level of control over the pattern, a second DX array is shown in Figure 4B. Here, three DX tiles are combined with a DX+J tile to produce a pattern where the stripes are separated by 64 nm.

TX molecules also can be used to produce 2D arrays. One can take the same pattern-making approach used with the DX arrays by using a TX+J tile [28]. However, the TX tile offers a convenient way to insert specimens within the array; it is possible to produce a gap in a continuous lattice by connecting individual tiles 1–3, i.e., by designating sticky ends that connect the first domain

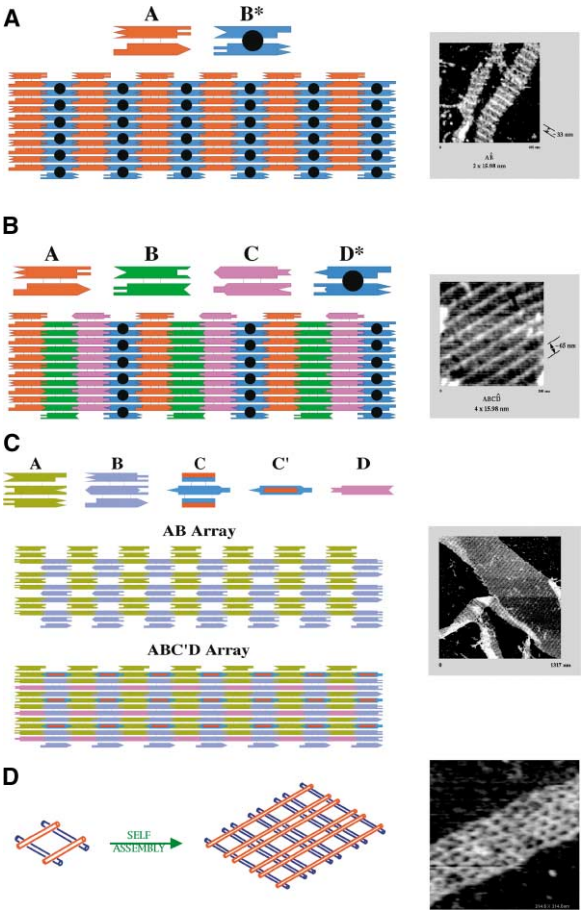

Figure 4. DNA Arrays

(A) Two DX molecules tile the plane. A conventional DX molecule, A, and a DX+J molecule, B*, are seen to tile the plane. The extra domain on B* leads to stripes. The molecules are 4 × 16 nm, so the stripes are approximately 32 nm apart, as seen in the AFM image at the right.

(B) Four DX molecules tile the plane. This arrangement is similar to that in (A), but there is only one DX+J molecule, D*, so the stripes are separated by approximately 64 nm, as seen on the right.

(C) A TX Array. Two TX tiles, A and B, are connected by complementarity between their first and third double-helical domains, resulting in spaces between the tiles. D is a linear duplex that fits in the yellow rows, and C is a TX rephased by three nucleotide pairs; it fits into the gray rows and extends helices beyond the AB plane in both directions, as shown in the micrograph at the right.

(D) A DNA parallelogram array. Four Holliday junction analogs form a parallelogram that is extended to produce a periodic array. The sizes of the cavities in the array may be tuned. Those in the array the right are approximately 13 nm × 20 nm.

to the third domain, as illustrated in the AB array of Figure 4C. One way to demonstrate a robust insertion into this array is to rephase a third TX tile (C in Figure 4C) by three nucleotide pairs (aproximately 102°), so that it is roughly perpendicular to the AB array (designated C′ in Figure 4C). This tile fits into the blue-gray column in the AB array by cohesion of its central domain. A fourth component, a piece of linear duplex DNA (D in Figure 4C) inserted into the gold array is also shown.

An unlikely but effective system results from combining four 4 arm branched junctions into a parallelogram [41], as illustrated in Figure 4D. As noted above, the 4 arm branched junction assorts its four arms into two double-helical domains [23], which are twisted with respect to each other [24]. The twist can be 40°–70° from either antiparallel [24, 41, 42] or (with 3′,3′ and 5′,5′ linkages in the crossover strands) parallel [43], so that a variety of parallelograms can be produced. The parallelograms can be connected through sticky-ended cohesion to produce a cavity-containing array, such as the one shown in Figure 4D. It is straightforward to alter the sizes of the cavities, so the porosity of this system is readily tunable.

What about 3D materials? This system should be as amenable to producing 3D materials as it is to producing 2D materials. It is relatively simple to design a series of 3D arrangements, but the formation of 3D systems is subject to all of the problems that confront conventional crystallization experiments. For example, a defect caused by creation of a gap in a 2D array can be filled in at any time because the missing element can be inserted from the third dimension. However, in 3D, once a gap is incorporated into the lattice, it can be corrected in this manner only so long as the layer containing the gap remains on the surface. Another thing the differentiates 2D from 3D is the form of analysis; the arrays shown in Figure 4 are all atomic-force micrographs, with a typical resolution of 4–7 nm. X-ray diffraction is the primary technique to be used in 3D; diffraction to 10 Å resolution is of only marginal value in establishing the structure, and 2–3 Å resolution is the minimal standard for effective structural analysis. Several 3D arrays have been constructed, but they have not yet diffracted to better than approximately 10 Å resolution. Nevertheless, they do grow large: 2D arrays usually have dimensions of the order of 1–2 μm, but it is not hard to grow 3D arrays with dimensions of 10–100 μm or larger.

Is periodicity the only option for materials? Definitely not. Winfree [44] has suggested that DNA self-assembly can be used for computation. Perhaps more importantly, he has suggested that algorithmic assembly can be used to produce new materials with specific structures that are more complex than simple periodicity. For example, he has shown that in the presence of border tiles, four tiles can count, so that the dimensions of an array could be dictated [45]. To prototype an algorithmic assembly, we have demonstrated that a cumulative exclusive OR (XOR) calculation can be performed by self-assembly of TX tiles [46]. This is shown in Figure 5. The component "tiles" are shown as input tiles (blue), initiation tiles (green), and gating tiles (red). The bottom domain of the gating tiles is flanked by sticky ends that represent the four possible input value pairs for an XOR calculation; these value pairs are (0,0), (1,1), (0,1), and (1,0). The first two value pairs generate a tile whose value is 0, and the second two produce a tile whose value is 1. The value of a tile is established by the presence of a recognition site for one of two possible restriction enzymes on the tile. The "reporter" strand (the thick red strand in the tile at the top of Figure 5) containing the site extends over the diagonal of the tile. When the reporter strands are ligated together, the input is connected to the output in a long strand; partial restriction of this product strand yields the answer to the calculation. Algorithmic assem-

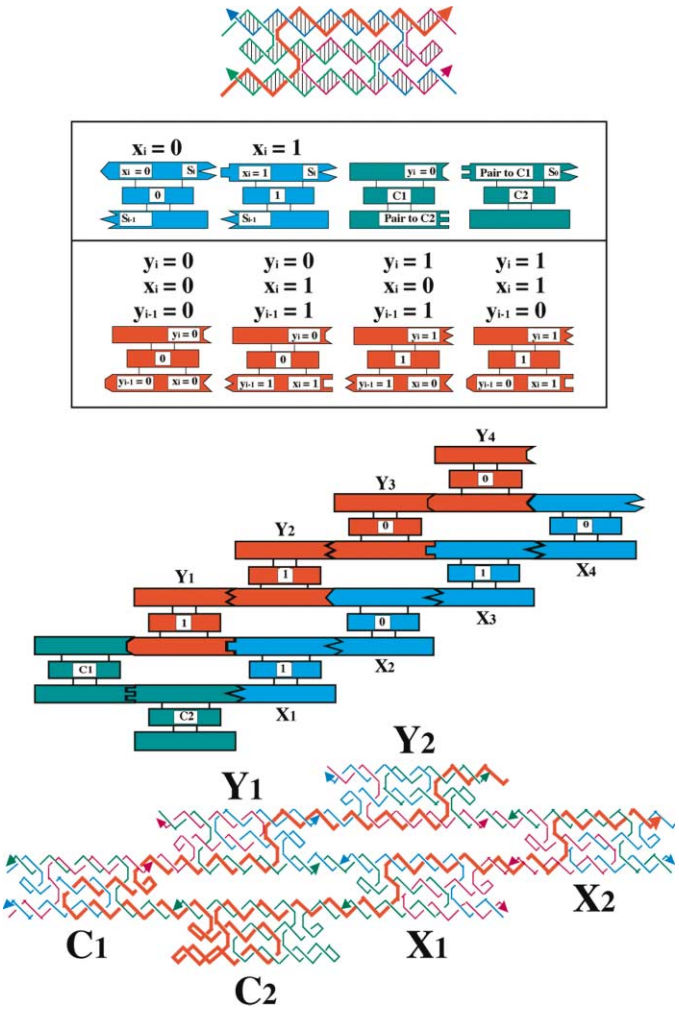

Figure 5. Four-Bit XOR Algorithmic Self-Assembly of TX DNA Tiles

At the top of the panel is a TX tile with its reporter strand emphasized with a thick red line. Below this are schematics of the input tiles (blue), initiator tiles (green), and gating tiles (red). The four possible inputs to the XOR gate correspond to sticky ends on the bottom domains of the red tiles. The schematic tiles are shown to self-assemble to produce the output arrangement of red tiles in the schematic below this. At the bottom the answer is extracted by ligation of the reporter strands, which are later subjected to partial restriction analysis.

bly is in its early stages, and it will take a great deal of development and tinkering before it can be performed reliably, particularly in 3D.

DNA Nanomechanical Devices: On the Road to Smart Materials

There are many uses for arrays whose structures are fixed; these will be discussed below. However, the informational content of DNA ought to allow for the ability to produce very smart materials, materials that can demonstrate a flexible response to their environment in much the same way that cells are able to respond to changes in their environments. The earliest DNA devices did not have informationally based features. The first of these, illustrated in Figure 6A, consisted of a circular molecule with a fixed cruciform containing four mobile (symmetric) nucleotide pairs at its base [47]. If the circular molecule is negatively supercoiled, the four mobile nucleotide pairs will be held in the extruded form. If an intercalating dye such as ethidium is added to the solution, the circle will relax, and the four nucleotides will reinsert into the circle. In addition to its lack of informational content, this device was not convenient to operate, did not have a well-defined 3D structure, and could not be incorporated readily into an organized DNA superstructure.

The second device [48] solved a lot of these problems, although it too was not sequence specific. It was based on the transition between conventional right-handed B-DNA and left-handed Z-DNA [49]. Z-DNA has two requirements, a sequence that is capable of forming Z-DNA [particularly, $(CG)_n$] and conditions to promote the transition, such as high ionic strength, or an effector that emulates high ionic strength, such as $Co(NH_3)_6Cl_3$. As shown in Figure 6B, the device consists of two DX molecules connected by a shaft. The yellow nucleotides on the shaft represent 20 nucleotide pairs that are capable of undergoing the B↔Z transition. In the absence of Z-promoting conditions, these nucleotides will be in the B state, as shown at the top of the panel, but in the presence of Z-promoting conditions they convert to Z-DNA. This conversion effectively results in the rotation of one DX component relative to the other of 3.5 turns, placing it on the opposite side of the shaft. The transition was demonstrated by measuring FRET differences between the two states.

The first potentially sequence-specific nanomechanical device was built by Yurke and his colleagues [50]. It consisted of DNA tweezers that could be transformed

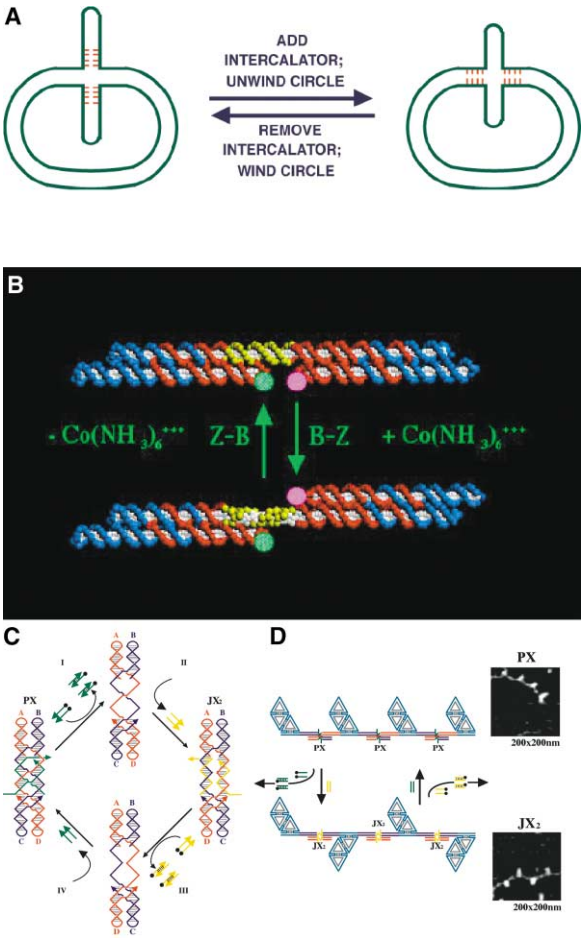

Figure 6. DNA Devices

(A) A mobile control device. The cruciform structure on the left contains four mobile base pairs at its base. Addition of an intercalator unwinds the circle and moves them into the circle. Removal of the intercalator reverses the action of the device.

(B) A DNA nanomechanical device based on the B-Z transition. The device consists of two DX molecules connected by a shaft containing 20 nucleotide pairs (yellow) capable of undergoing the B-Z transition. Under B conditions the short domains are on the same side of the shaft, but under Z-conditions (added $Co(NH_3)_6^{3+}$) they are on opposite sides of the shaft. The pink and green FRET pair are used to monitor this change.

(C) The machine cycle of a PX-JX_2 device. Starting with the PX device on the left, the green strands are removed by their complements (process I) to leave an unstructured frame. The addition of the yellow strands (process II) converts the frame to the JX_2 structure, in which the top and bottom domains are rotated a half turn relative to their arrangement in the PX conformation. Processes III and IV reverse this process to return to the PX structure.

(D) AFM demonstration of the operation of the device. A series of DNA trapezoids are connected by devices. In the PX state, the trapezoids are in a parallel arrangement, but when the system is converted to the JX_2 state, they are in a zigzag arrangement.

between closed and open states. The transformation was achieved by adding "set" strands that had short unpaired segments; pairing with the full complements to the set strands (including the unpaired segments) removed them and thereby restored the original state. Unfortunately, this clever device was not robust; dimers and other multimers were produced between transitions. The first robust sequence-specific device is based on interconversion of the PX and JX_2 motifs via the Yurke mechanism [32]. The PX and the JX_2 motifs differ by a half-turn rotation (see Figure 6C), so interconversion between the two motifs rotates the ends of the device by a half turn. The device consists of a frame in which one of the red-and-blue strands has been interrupted and replaced with a green "set" strand. Variation of this part of the frame leads to numerous devices, each controlled by its own set strands. The machine cycle is shown in Figure 6C. Starting at the PX state, addition of the full complements (termed "fuel" strands) to the green set strands results in their removal from the PX complex, leaving a poorly structured frame; the black dots on the complements represent biotin groups, so the duplexes of the set strands and their complements can be removed from solution by streptavidin beads. Addition of the yellow set strands converts the frame to the JX_2 structure; addition of the yellow fuel strands again produces the frame, and addition of the green set strands restores the PX state. Variation of the sequence in the area bound by the set strands can result in a number of different devices in this system. Figure 6D illustrates AFM experiments that follow the operation of this device. A series of three-triangle DNA trapezoids have been connected by PX-JX_2 devices. When this system is in the PX state, the trapezoids are parallel to each other; when it is in the JX_2 state, a zigzag structure results.

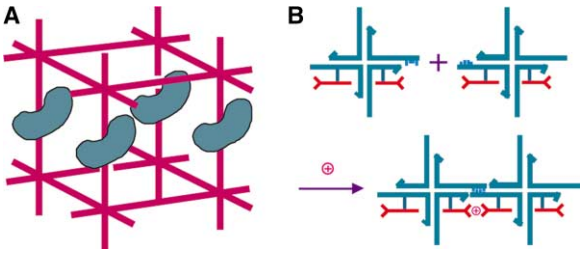

Figure 7. Applications of Structural DNA Nanotechnology

(A) Scaffolding of biological macromolecules for crystallographic purposes. A DNA box (magenta) is shown with sticky ends protruding from it. Macromolecules are organized parallel to each other within the box, rendering them amenable to crystallographic structure determination.

(B) Scaffolding nanoelectronics. Blue branched DNA junctions direct the assembly of attached nanoelectronic components (red) to form a molecular synapse stabilized by the presence of an ion.

Goals for Structural DNA Nanotechnology

How do the capabilities demonstrated above lead to practical and useful goals? The motivating application of this system is to scaffold biomolecular crystallization by 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 [1]. Indeed, the author's frustration with the biological-crystallization experiment is what led to this entire program. The basic idea is shown in Figure 7A. Of course, if one can imagine organizing biological macromolecules into an array, one can imagine organizing other molecules as well. Prominent among these are the components of

molecular electronics [51]. There are many species that appear to be well-suited to nanoelectronics, e.g., metallic nanocrystals, quantum dots, and carbon nanotubes, but it is very difficult to organize them into arrays that function as effective hardware. Structural DNA nanotechnology can probably provide the organizational capabilities necessary to arrange these units into functional entities. A schematic diagram illustrating this concept is shown in Figure 7B.

Another species to organize into arrays is the robust sequence-dependent nano-device described above. If we can incorporate N different species of these two-state devices in 2D or in 3D, we should be able to generate 2^N different structural states. Short-range goals include creating a molecular pegboard and, possibly, using species derived from this system for producing a molecular assembly line. Multiple structural states are a necessary concomitant of nanorobotics, so this system seems capable of leading to DNA-based nanorobotics.

Concluding Remarks

I have described a new architectural system on the nanometer scale that is derived from the central biological molecule, DNA. However, instead of emphasizing its role in biological systems, I have stressed its chemical features because they facilitate the development of new and exciting systems in structural DNA nanotechnology. Conventional linear cellular DNA is not a propitious system to use to generate exciting and diverse systems, systems that would lead to valuable new materials. Consequently, we have concentrated on branched systems, topologies derived from biology but derived from the process of recombination rather than information storage. It is a great pleasure for the author to see that labs other than his own are now beginning to publish work in structural DNA nanotechnology. In particular, the recent work of Reif, Yan, and their colleagues [52–54] are exciting additions to the literature; similarly, the work of Rothemund et al. [55], producing 2D Sierpinski triangles from DNA, indicates a level of control over algorithmic assembly that is likely to have broad implications for new materials. In current work we and the other practitioners of structural DNA nanotechnology have used only Watson-Crick base pairing. However, many new tertiary interactions are being discovered (e.g., [56]), and some of these are being used for the purpose of generating new nucleic acid-based materials [8]. As we learn about the thermodynamics and structural requirements of these interactions, they ultimately will lead to a whole new generation of capabilities for structural nucleic acid nanotechnology. Likewise, as we learn more about the metabolism of DNA, we may discover new motifs that are currently exploited by living systems and that can be used by the materials sciences to generate still more numerous types of new materials. Structural nucleic acid nanotechnology is in its infancy, but it seems to be capable of remarkable versatility in the organization of matter on the nanoscale.

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