DNA Components for Molecular Architecture

NADRIAN C. SEEMAN*

Department of Chemistry, New York University, New York, New York 10003

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It is hard to imagine a modern chemist lacking an appreciation for the aesthetics of molecular architecture. Usually, we think of individual molecules when we think of molecular architecture, but in its most general meaning, architecture also applies to arrangements of atoms and molecules that fill space to form crystals. For years, synthetic chemistry has aimed at building molecules with particular structures, usually because those structures were associated with a particular functional end point. If one is lucky enough to crystallize such a product, one's synthetic efforts usually can be confirmed by crystallography; the beauty of infinite 3-D arrays of atoms and molecules immediately impresses all those who have examined crystal structures. By contrast to the conventional crystallization experiment, our group has been working toward the goal of using DNA components to fabricate crystals directly. We have not reached this goal yet, but our journey in this direction has resulted in the assembly of numerous unusual DNA molecules. DNA consists of alternating sugar−phosphate backbones; attached to each sugar is a base, adenine (A), guanine (G), cytosine (C), or thymine (T). It is well-known that two antiparallel backbones wrap around each other to form a (topologically) linear double helix held together by specific hydrogen bonded base pairs, A-T and G-C.

Architects, like artists, work in particular mediums: wood, brick, steel, concrete, animal hides, sod, stone, and adobe have all been used to construct dwellings, often as a compromise between ideal and available materials. Why do we use DNA as our primary medium for assembling chemical architecture? The key advantage of using DNA is the ability to specify intermolecular associations, by means of "sticky ended" association.[1] An example of sticky ended association is seen in Figure 1, where two double helices are shown interacting by means of overhanging strands. This feature means that DNA is the molecule with the most readily predictable and programmable *intermolecular* interactions. A useful feature of the interactions visible in Figure 1 is that DNA duplexes

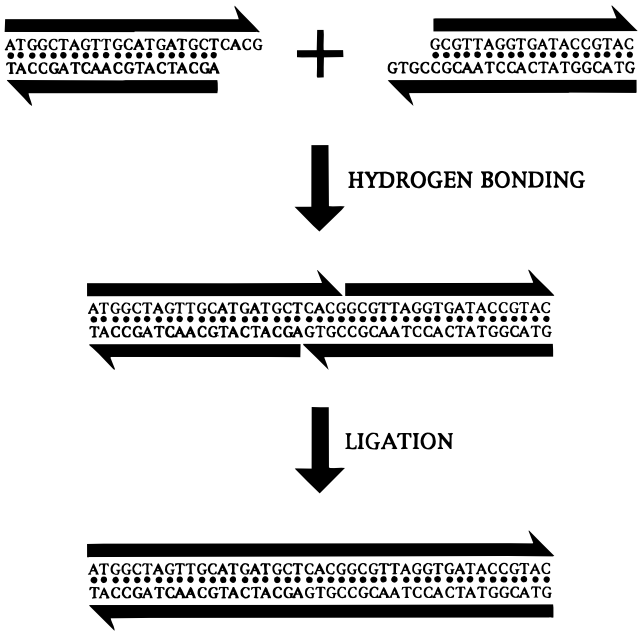

FIGURE 1. Sticky-ended cohesion and ligation. Two linear double-helical molecules of DNA are shown at the top of the drawing. The antiparallel backbones are indicated by the black lines terminating in half-arrows. The half-arrows indicate the 5′ → 3′ directions of the backbones. The right end of the 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 the drawing shows that they can be ligated to covalency by the proper enzymes and cofactors.

associate in a specific fashion by hydrogen bonding; complementary sticky ends cohere to produce a specific local structure, B-DNA, whose structural parameters are well-known.[2] Figure 1 also indicates that sticky ended association can be annealed to covalency by DNA ligase.[1]

Using natural DNA for molecular or crystalline architecture has a fundamental problem: Molecules with unbranched helix axes are logically equivalent to line segments, practical only for the assembly of long lines and circles, or perhaps knots and catenanes. To make stick figures or lattices with complex connectivity, it is necessary to incorporate branch points that act as vertices in the system. Branched DNA molecules are found as ephemeral four-arm intermediates, known as Holliday junctions,[3] in the biological process of recombination.[4,5,6] The position of the branch point in naturally occurring Holliday junctions is unstable because the branch point is flanked by 2-fold sequence symmetry; this symmetry permits it to relocate, by an isomerization called branch migration.[7,8] Fortunately, it is possible to design synthetic DNA mol-

Nadrian C. Seeman was born in Chicago in 1945. Following a B.S. in biochemistry from the University of Chicago, he received his Ph.D. in biological crystallography from the University of Pittsburgh in 1970. His postdoctoral training, at Columbia and MIT, emphasized nucleic acid crystallography. He obtained his first independent position at SUNY/Albany, where his frustrations with the macromolecular crystallization experiment led him one day to the campus pub. There, he realized that the similarity between six-arm DNA branched junctions and the periodic array of flying fish in Escher's "Depth" might lead to the rational approach to crystallization described here. He has been trying to implement it ever since, for the last 9 years at NYU. Further information is available at http://seemanlab4.chem.nyu.edu.

* E-mail: Ned.Seeman@NYU.edu.

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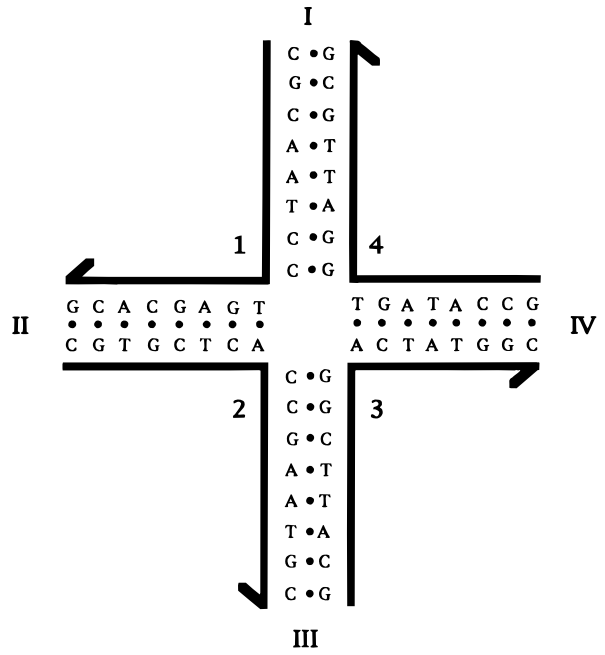

FIGURE 2. Stable DNA branched junction. The junction shown is composed of four strands of DNA, labeled with Arabic numerals. The 3′ end of each strand is indicated by a half-arrow. Each strand is paired with two other strands to form a double-helical arm. There is no homologous 2-fold sequence symmetry flanking the central branch point, thereby stabilizing its position.

ecules (called DNA branched junctions) without 2-fold sequence symmetry, so that the position of the branch point is fixed.[9,10] An example of a stable branched molecule is shown schematically in Figure 2. In addition to eliminating 2-fold sequence symmetry, other features of sequence symmetry often are minimized, to ensure that branched molecules are the favored products.[9,10] Four-arm branched junctions have been characterized extensively because of their relationship to Holliday junctions.[11,12] In addition to Holliday analogues containing four arms, branched junctions containing three,[13] five, and six[14] arms have been constructed.

The idea behind our work is very simple. We wish to combine the sticky ended methodology of molecular biotechnology[1] with branched DNA molecules, to produce objects and lattices with particular structures.[9,15,16] An example is illustrated in Figure 3, where we show a four-arm branched junction with complementary sticky ends assembling into a quadrilateral. Beyond the quadrilateral, the system appears capable of forming an infinite 2-D lattice because of the unsatisfied sticky ended "valences" on its periphery. This leads us to the key goal of this work: the assembly of 3-D DNA crystals that can be used

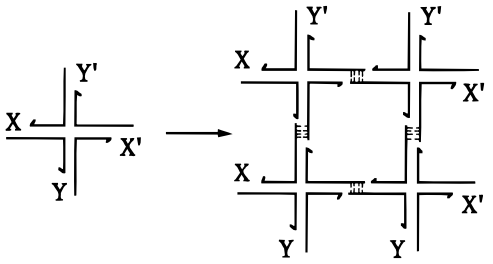

FIGURE 3. Formation of a two-dimensional lattice from an immobile junction with sticky ends. X is a sticky end, and X′ is its complement. The same relationship exists between Y and Y′. Four of the monomeric junctions on the left are complexed in parallel orientation to yield the structure on the right. X and Y are different from each other, as indicated by the pairing in the complex. As shown in Figure 1, ligation by DNA ligase can close the gaps left in the complex. The complex has maintained open valences, so that it could be extended by the addition of more monomers.

like macromolecular-scale zeolites to host biological guest molecules of interest. Whereas DNA has a code on its outside surface as well as its inside,[17] it is possible that we will be able to tether and orient the guests in parallel orientations, so that they will be suitable for X-ray crystallographic analysis. No periodic arrays have been constructed yet, but we have assembled DNA molecules whose helix axes have the connectivities of a cube[18] and a truncated octahedron.[19]

Suitability of DNA as a Macromolecular Construction Material

The success of the construction in Figure 3 depends on the rigidity of the DNA segments that form the edges of the quadrilateral, as well as the rigidity of the junctions, which form the angles at the corner. If either were flexible, the quadrilateral would not be the exclusive product, and the regular array suggested by the figure would be unlikely to result. DNA is often considered to be highly flexible because most pictures of it represent very long molecules. However, it has a persistence length of 500 Å or more,[20,21] under conventional solution conditions, which makes it locally a relatively stiff molecule. This is analogous to cooked spaghetti, which appears very floppy when we confront it on a plate in 30 cm lengths; however, segments a few millimeters long are not readily bent. Short pieces of DNA, two or three turns (ca. 70−100 Å) long, can be regarded as stiff building components. Thus, the edges of DNA stick figures appear to be sufficiently rigid for the purposes of construction.

What about the angles between junction arms? The rigidity of the angles is usually assayed in a ligation-closure experiment.[13,22−25] In this experiment, two arms of a branched junction contain complementary sticky ends;

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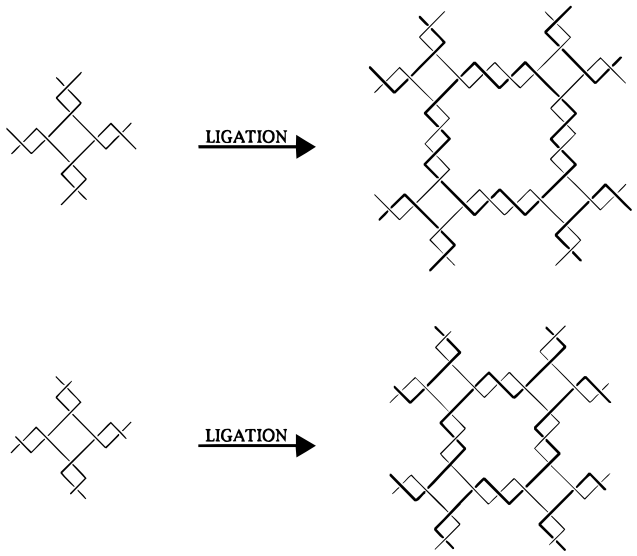

FIGURE 4. Topological consequences of ligating DNA molecules containing even and odd numbers of DNA half-turns in each edge. These diagrams represent the same ligation shown in Figure 3. However, they indicate the plectonemic winding of the DNA and its consequences. The DNA is drawn as a series of right-angled turns. In the top panel, each edge of the square contains two turns of double helix. Therefore, the central dark strand is a cyclic molecule. Strands are drawn with alternate thick and thin lines. In a lattice, all of the strands shown would form cyclic strands. In the bottom panel, each edge of the square contains 1.5 turns of DNA. Therefore, the strands do not form cycles, but extend infinitely in a warp and weft meshwork.

many molecules are ligated together, to produce linear and cyclic products. The production of a unique cyclic product would suggest a fixed angle between the two arms. In all experiments involving branched junctions, cyclic products ranging from dimers to oligomers (ca. decamers) are seen.[13,22,23] These experiments have been interpreted to suggest that the angles between the branches are flexible. Although no constraints have been noted in experiments sensitive to the flexure of angles about the branch points, torsional constraints are present involving the twist through the junction.[22] Thus, the DNA branched junction does not provide us directly with a macromolecular analogue of the rigid tetrahedral or trigonal valence cluster, so familiar to chemists. This is the key problem in this system; possible solutions are discussed below.

The Topological Aspects of DNA Construction

Figures 1−3 all represent the DNA backbone as a pair of antiparallel lines. This is often a useful approximation, but the true structural flavor of the DNA molecule is lost if the wrapping of the double-helical strands around each other is ignored. For example, Figure 4 illustrates the same ligation shown in Figure 3, but now with topology taken into account. The top panel shows a junction ligated to form a quadrilateral containing two turns per edge, and the bottom panel shows the formation of a quadrilateral with 1.5 turns per edge. The underlying strand structures are shown as thick and thin lines. The quadrilateral with two turns per edge contains a cyclic molecule and four others paired with it directly; an infinite lattice of such squares would be a network of linked

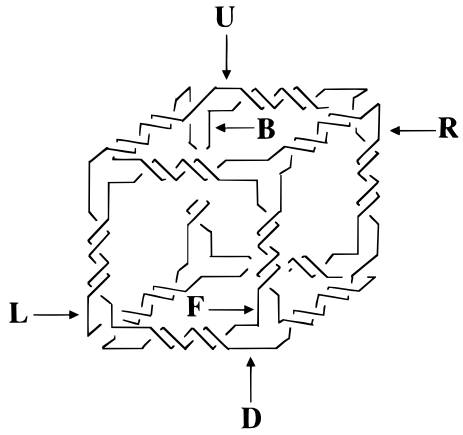

FIGURE 5. DNA molecule whose helix axes have the connectivity of a cube. The molecule shown consists of six cyclic strands that have been catenated together in this particular arrangement. They are labeled by the first letters of their positional designations, up, down, front, back, left and right. Each edge contains 20 nucleotide pairs of DNA, so we expect that their lengths will be about 68 Å. From model building, the axis-to-axis distance across a square face appears to be about 100 Å, with a volume (in a cubic configuration) of approximately 1760 nm^3, when the cube is folded as shown.

circles. By contrast, the structure with 1.5 turns per edge contains no cycles, but rather a woven meshwork of long strands.[15,16]

The double-helical nature of DNA also places constraints on the lengths of edges in DNA figures. Two branched junctions at either end of a double-helical DNA edge are related to each other like wing nuts on a screw: their relative orientation is a function of the length of the intervening DNA. Although it appears possible to design objects with edges of any length,[15,16] the simplest architectures derive from the use of an integral number of double-helical half-turns. All geometrical objects built from DNA to date have been designed with this constraint in mind.[18,19,24,26]

The difficulties of designing molecules from double-helical components are more than compensated by the properties of the products and by new windows of synthetic control that are opened by this feature.[27] Geometrical objects constructed from DNA are not weakly associated hydrogen bonded complexes. Rather, they are robust catenanes, whose components are topologically bonded to each other. If each edge of a DNA polyhedron contains an integral number of double-helical turns, each face corresponds to a cyclic single strand of DNA, linked to each of its neighboring faces once for each turn in the edge that they share. The catenation between strands permits rigorous topological analysis of products under denaturing conditions.[18,19,28] As a logical extension, it is possible to design periodic arrays from DNA whose topology is that of molecular chain mail.[16,27,29]

Perhaps equally important is the fact that the half-turn of DNA provides a convenient chemical means of building

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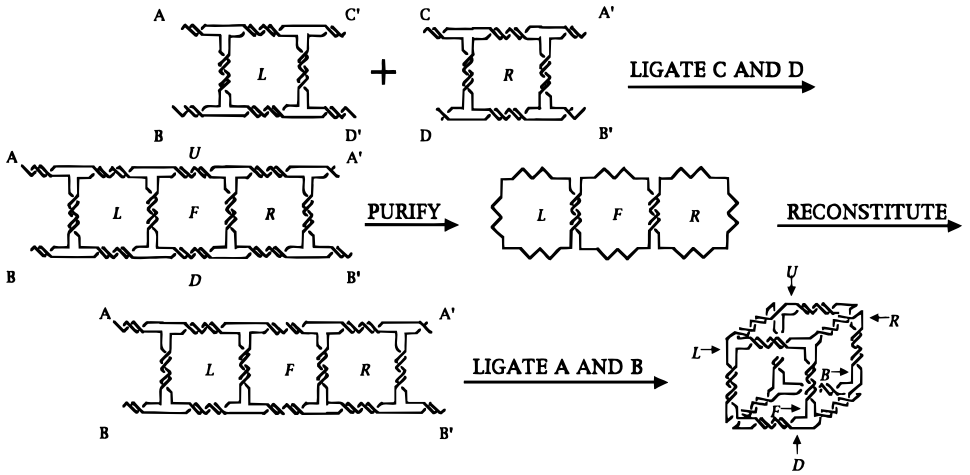

FIGURE 6. Synthesis of a DNA cube. The molecule is built from 10 chemically synthesized strands, two 80-mers, and eight strands containing about 40 nucleotides. These are hybridized to form two quadrilaterals in the first step. Two ends (C and D) are ligated to form a belt-like molecule that must be denatured and reconstituted in order to purify it from side products. The belt-like molecule is then cyclized to form the cubelike molecule.

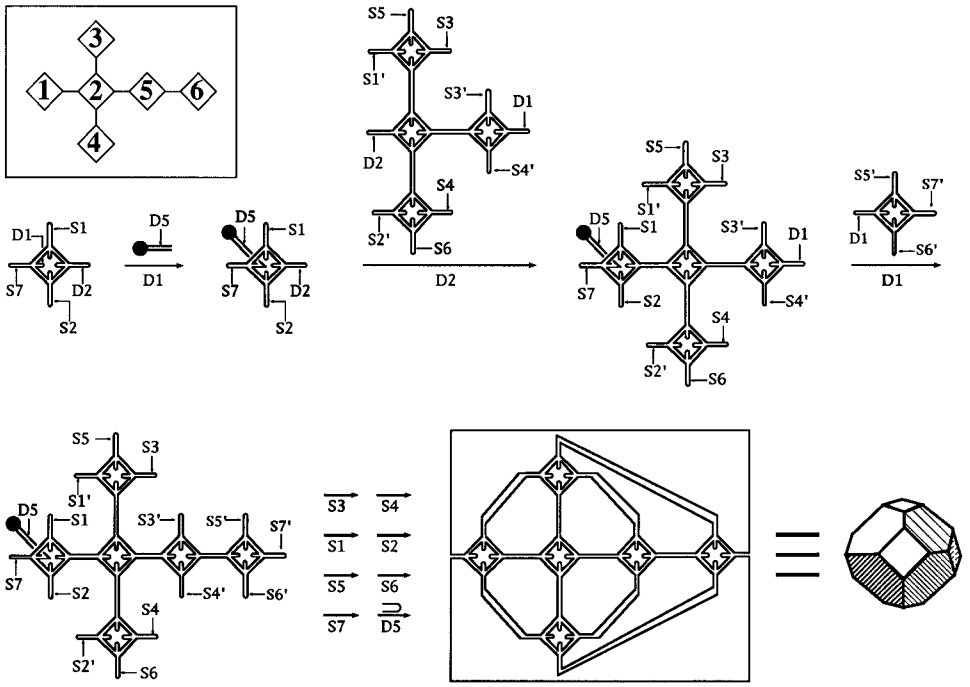

FIGURE 7. Synthetic scheme used to synthesize a truncated octahedron on a solid support. The boxed diagram in the upper left corner indicates the numbering of the individual squares. Each square in the rest of the diagram is shown with its restriction sites indicated. Symmetrically cleaving restriction sites are named "S", indicated in pairs, with one of the pairs being primed; restriction sites that are cut distally by type IIs restriction enzymes are named "D"; restriction sites on the exocyclic arms are not indicated. The arms that will eventually be combined to form edges of the object are drawn on the outside of each square, and the exocyclic arms are drawn on the inside of the square. A reaction is indicated by a line above a restriction site name: This means that the restriction enzyme (or enzyme pair for those labeled "S") is added, protecting hairpins are removed and then the two sticky ends are ligated together. The product is shown in two forms. On the left, the S1−S6 closures are shown as triple edges, to emphasize their origins; the two strands of the edge formed by the S7 closure are separated to maintain the symmetry of the picture. On the right, a slightly rotated front view of a polyhedral representation of a truncated octahedron is shown without the exocyclic arms; the symmetry of the ideal object is evident from this view.

a topological crossing.[30] This is the fundamental building block of all topological species derived from strands in 3-D, such as knots and catenanes; it is sometimes called a node or a unit tangle.[31] Nodes can be positive or negative: negative nodes correspond to the crossings of right-handed B-DNA, and positive nodes correspond to the crossings of left-handed Z-DNA.[32] Utilizing the relationship between DNA half-turns and unit tangles, single-stranded DNA trefoil knots of both signs have been made,[33,34] as well as a figure-8 knot,[35] which is a topological rubber glove.[36] An RNA knot has also been constructed, leading to the discovery of RNA topoisomerase activity.[37]

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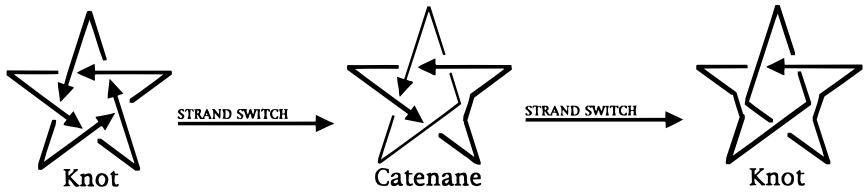

FIGURE 8. Interconversions of knots and catenanes by switching strands at a node. The structure shown on the left is a 5_1 knot. The strand direction is indicated by the arrowheads appearing along the strand. When the two strands entering the lower node on the right exchange outgoing partners, the node disappears. This converts the knot to a catenane, shown in the middle; the two linked cycles are drawn so as to retain their shapes, but they are drawn with pens of different thicknesses. When the lower left node of the catenane undergoes a strand switch, the structure is converted to a trefoil knot (right).

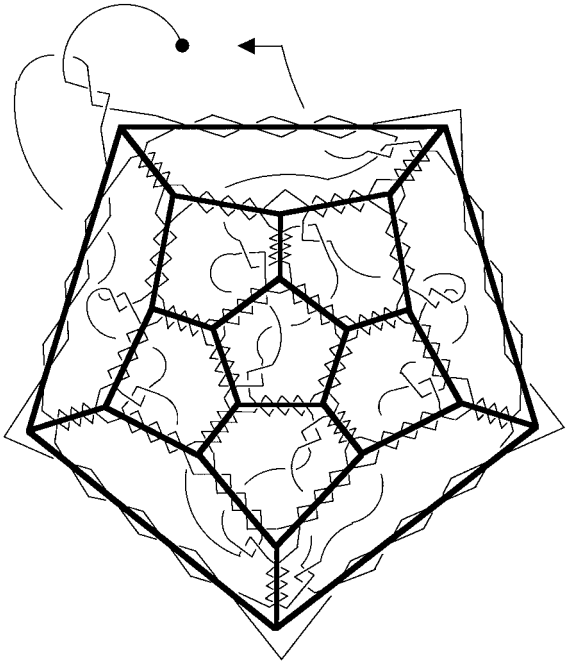

FIGURE 9. Single-stranded representation of a pentagonal dodecahedron. A pentagonal dodecahedron is illustrated with 12 exocyclic arms, in a Schlegel diagram. The Schlegel diagram of the dodecahedron is shown in the thickest lines. Wrapped around these lines are two turns of DNA per edge, drawn with very thin rectilinear lines. In addition to the DNA on the edges, each pentagon contains an exocyclic double-helical arm a single turn long. The DNA representing each of the individual faces has been connected by curved lines to a neighboring face via the exocyclic arms, so that the entire representation is a single long strand. The 3′ end of the strand is denoted by the arrowhead and the 5′ end by a filled circle. Each exocyclic double-helical segment would be designed to contain a restriction site, so that it could be severed from the connecting DNA upon formation of the structure. No attempt at topological representation is made here: all connecting DNA (curved lines) lies behind the polygonal DNA.

Most recently, this same approach has been used to construct Borromean Rings from single-stranded DNA.[38]

The Construction of DNA Geometrical Objects

As noted above, it is not possible to rely on the rigidity of branched DNA molecules as an aid either in the construction of geometrical targets or in their characterization. Consequently, the construction of all objects has relied on the specificity of DNA sticky-ended recognition; likewise, all characterization has been topological, rather than geometrical. Sticky-ended specificity can be used for the assembly of finite objects because each edge of an object can be associated with a particular pair of sticky ends. We have used this method successfully as the basis of the synthesis of a DNA molecule whose helix axes have the connectivity of a cube.[18] A schematic of this molecule is shown in Figure 5; every edge contains a unique restriction site, permitting proof of synthesis by cleavage to yield target subcatenanes.[18]

A simplified scheme for the synthesis is shown in Figure 6. Ligation at sticky ends C−C′ and D−D′ could be controlled because only those ends contained phosphate groups. As one might imagine, the purification and reconstitution steps in Figure 6 were not planned in the original synthetic scheme. They were necessitated because a sticky-ended ligation in a solution containing a picomole of flexible squares is different from ligation on paper, where only two squares are drawn: the ladder-like target intermediate was produced in low yield, and it was necessary to remove the byproducts that included ligations on the C and D edges to different molecules.

It is possible to eliminate most of the problems involved in solution synthesis by moving to a solid-support methodology.[39] Its key advantage is that growing molecules are isolated from one another on the support, so that the simplicity and specificity of sticky-ended ligation is not jeopardized by the presence of many copies of the same molecule in solution. In addition, the method permits control over the synthesis of individual edges of an object; control derives from the restriction endonuclease digestion of hairpin loops forming each side of the new edge. Each cycle of the procedure creates an object that is covalently closed and topologically bonded to itself, enabling some purification to take place on the support. As with other syntheses using solid supports,[40] this methodology permits convenient removal of reagents and catalysts from the growing product. The strategy permits the separate execution of steps involving additions and cyclizations, which are optimized under different conditions.

As a test of this methodology, we have constructed a truncated octahedron. This is a 14-sided figure, composed of six squares and eight hexagons. Each edge contains two turns of DNA, so the molecule is a 14-catenane of DNA, and each strand is linked twice to each of its neighbors. Each vertex of a truncated octahedron is connected to three other vertices, making it a three-

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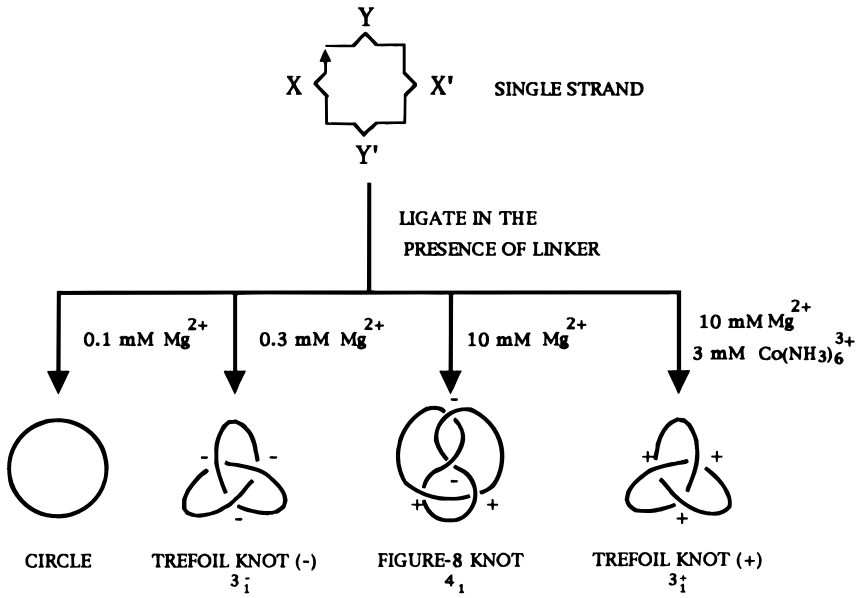

FIGURE 10. Four target topologies from a single strand of DNA. The top of this scheme indicates the molecule from which the target products are produced. The four pairing regions, X and its complement X′ and Y and its complement Y′, are indicated by the bulges from the square. The 3′ end of the molecule is denoted by the arrowhead. The nick is between helical domains and, therefore, requires a linker complementary to the 3′ and 5′ ends of the strand. The molecular topologies are shown at the bottom.

connected object.[41,42] However, the object we made was constructed from four-arm branched junctions, leaving an extra arm attached to each vertex. We had hoped to link truncated octahedra together through these arms, but the polyhedron proved to be an inappropriate starting material. The synthesis is summarized in Figure 7. As in the case of the cube, proof of synthesis relies on using restriction endonucleases to digest the product to yield specific subcatenanes. The construction of the truncated octahedron by the solid support methodology demonstrates that this is an effective means for the assembly of DNA polyhedra.

The Assembly of Topological Targets

There is a close relationship between catenanes and knots. As illustrated in Figure 8, it is possible to create a catenane from a knot and *vice versa* by a simple operation on a node: one can regard a node as consisting of four polar strands connected in pairs, two before the node and two following the node; switching the connected pairs, while maintaining polarity, destroys the node and alters the relationship of the strands involved between catenation and knotting. The solid support methodology utilizes the reverse of this procedure to create nodes from hairpins and also to switch from (usually trivial) knots to catenanes.[27]

One would imagine that the use of DNA as a construction medium could be coupled conveniently to biological synthesis of one's target molecules. Unfortunately, branched molecules cannot be replicated by biological means such as cloning the DNA or by the polymerase chain reaction.[43] This is because DNA polymerases do not replicate branched structures. However, one can imagine joining the strands of a polyhedron together with linkers, to make a long strand that might then fold to yield the target pairing. The extra linkers could then be removed by restriction endonucleases, leaving a polyhedron ready to ligate to other polyhedra. A pentagonal dodecahedron of this sort is illustrated in Figure 9.

To test control of DNA folding in a tractable context, we have made a series of topological constructs. We have noted above that the relationship between a half-turn of DNA and a node in a knot can lead to the rational construction of DNA knots. The ability to control the B−Z transition by solution conditions has permitted us make several different knots from the same strand, as shown in Figure 10. Knots are characterized by electrophoretic mobility, sedimentation, Ferguson analysis, and susceptibility to restriction endonucleases.[33−35] Once the knots have been formed, one can change the preferred knot by altering solution conditions; when this is done, the knots can be interconverted by topoisomerases.[44]

A different test of topological control is provided by the construction of Borromean rings from DNA. This is a topological link, involving three strands that contains no catenated pairs. Figure 11 shows that two three-arm branched junctions, one of B-DNA and one of Z-DNA can be ligated to form this link. In fact, it is *easier* to make the link shown, using three-half-turns of DNA at each strand contact, than to make classical Borromean rings containing a single node at each crossing. Proof of synthesis relies on individual cleavage of the three rings by restriction endonucleases, yielding only single circles and no catenated molecules.[45]

The Search for Rigidity

The motivating end point for DNA nanoconstruction is the rational assembly of periodic matter from DNA. There

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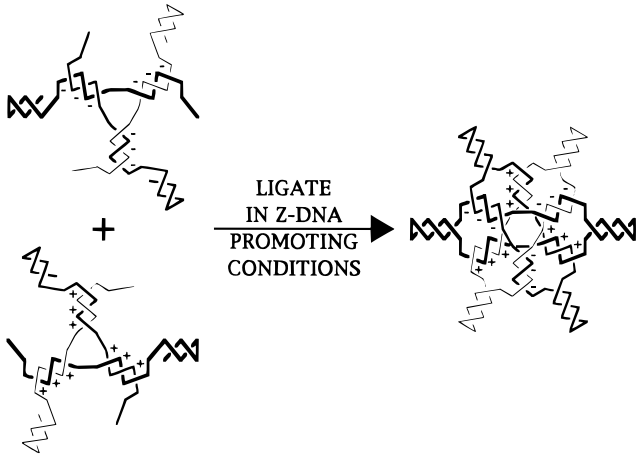

FIGURE 11. Synthesis of Borromean rings. The two components (left) of the link are a three-arm branched junction with right-handed (negative) B-DNA nodes (top) and a three-arm branched junction with left-handed (positive) Z-DNA nodes (bottom). The three ultimate strands are drawn with lines of three different thicknesses. Extending from each arm are a duplex hairpin and an octanucleotide complementary to a hairpin on the other junction. When mixed and ligated under Z-promoting conditions, the link on the right forms. The B-DNA junction is drawn in front of the Z-DNA junction in the link. Inspection of the link reveals that elimination of any cycle frees the other two from each other.

are at least three properties necessary for the components of systems where this is possible: (1) the predictable specificity of intermolecular interactions between components; (2) the structural predictability of intermolecular products; and (3) the structural rigidity of the components.[23] We have pointed out above that branched DNA satisfies the first two criteria well, but fails on the third criterion. A system unable to satisfy the third criterion cannot be used reliably to make periodic matter because it can cyclize on itself, thereby poisoning crystal growth. One can imagine using solid-support schemes with cycles of deprotection, leading to hierarchical 3-D assemblies of subunits.[46] However, this is not an efficient means to generate new 3-D periodic materials. Ideally, one would like to produce starting materials by the solid-support methodology, release topologically closed components, and then free them by restriction, so that they can associate to form crystalline arrays in solution.[24]

Consequently, we have sought rigid DNA motifs, inspired by biological systems. The bulged three-arm DNA branched junction appeared initially to provide a more rigid motif than the unbulged junction.[23] However, this initial success was not borne out with more rigorous testing.[24] Fortunately, we have discovered recently that another biologically derived motif, the double-crossover molecule,[47] appears to have the desired properties.[25] A double-crossover molecule with antiparallel domains is

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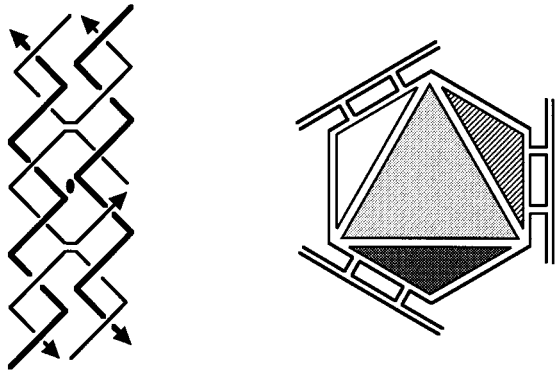

FIGURE 12. Double-crossover molecule and its incorporation into a deltahedral motif. The left side of the figure shows a double-crossover molecule, which has been shown to be stiff in ligation-closure experiments. This particular motif is shown on the right side of the drawing to be added to three edges of an octahedron. The three helices shown will span 3-space.

shown on the left of Figure 12. Deltahedra are known to be rigid,[48] providing another aspect of structural rigidity. The right side of Figure 12 shows how the double-crossover molecule could be combined with a deltahedral component, so that it would lead to a 3-D array.[25]

Concluding Comments

The ability to control the structure of matter on the nanometer scale is likely to lead to new materials of interesting and valuable properties, such as molecular chain mail in two or three dimensions. The components of the DNA systems described here are expected eventually to generate periodic species that can aid in diffraction studies of biological macromolecules[9] and possibly in DNA computing.[29] There are structural transitions of DNA molecules that should be adaptable to produce simple nanomechanical devices.[46] The suggestion has even been made that DNA can be used as scaffolding to direct the assembly of molecular electronic components to produce memory devices.[49] This system represents the combination of synthetic chemistry, topology, and 3-D structure. The excitement is just beginning.

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