Physical Models for Exploring DNA Topology

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Abstract

Two types of physical models have been developed for treating DNA molecules whose topology is of interest. The two model motifs combine jacks-and-straws molecular representations with flexible tubing in different proportions. Both motifs present a low-resolution construct of DNA that retains helix axes, strand individuality and the distinguishability of the major and minor grooves. Molecules whose double helix axes are branched are modelled by stiff double helices and flexible branch sites. Supercoiled and knotted DNA molecules are modelled on a smaller scale, in a system in which a flexible backbone is supported by a series of stiff helical struts; removal of this scaffolding immediately reveals the linking of the strands. The models are light and easy to construct. They may be used either for demonstrations or as a research tool that assists the interpretation data.

Introduction

Molecular models of DNA abound in classrooms and laboratories throughout the world, as befits the central role of this molecule in the chemistry of genetics. Most of these models are designed to demonstrate the static features of about one turn of double helix: the twofold symmetry of the phosphate-sugar chains, the major and minor grooves, the complementarity of the purine and pyrimidine bases (1), and the recognition sites available for regulatory proteins (2). The large expense associated with accurate ball and stick or space-filling models of DNA has recently led to frequent replacement of physical models by computer graphics molecular representations (e.g., 3). This is appropriate for those applications in which DNA may be treated as a static or nearly-static structure.

As our knowledge of DNA grows, new concepts have come into play, for which inexpensive physical models are necessary. Chief among these new areas are those involving new topology for DNA strands or helix axes: supercoiling (e.g., 4), DNA branched junctions in recombination chemistry (e.g., 5-7) or macromolecular engineering (8), and DNA knot and catenane formation (e.g., 9-11). Topology is frequently more important than actual structure when treating these concepts: It is the linking, knotting or braiding of DNA which is the feature of interest, particularly in molecules for which the actual 3-D structures involved are either unknown or not unique. All

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Legends for Color Figures

Figure 1: Isomerizations of Holliday Recombination Intermediates. The Holliday recombination intermediate in the parallel Sigal-Alberts conformation and its transformations are illustrated. This structure consists of four strands (red, green, orange and blue), each of which participates in two double helices. The 5' end of each strand is indicated by the small piece of tubing perpendicular to the circumferential segment on one end. Note that in this figure trigonal clusters have been distorted, so that the minor groove is approximately 150°, and the major groove is approximately 210°. The Sigal-Alberts conformations shown are characterized by the stacking of two of the double helices on each other, with one of the strands unperturbed in each helix; here, the unperturbed strands are the red and the orange strands. Two conformers of this crossover isomer are shown, the braided structure (a), in which the green and blue strands are braided at the crossover point, and (b) the unbraided structure, in which the green and blue strands cross over between helices but are not otherwise braided. (a) and (b) can be interconverted by a 360° rotation of one double helix (either red or orange) perpendicular to its axis, about the central horizontal through the crossover. Isomerization between alternative Holliday crossover isomers is illustrated in panels b-f. The five panels illustrate the qualitative steps necessary to undergo isomerization from one crossover structure to the other, without generating a braided structure, such as shown in (a). These steps are similar, but in a different sequence from those outlined by Meselson and Radding (24): We start with (b), a Sigal-Alberts crossover structure with parallel helix axes, in which the green and blue strands form the crossover. In (c) the helix axes have been reoriented antiparallel, by rotating the left (red) double helix. In (d), the stacks have been broken, so that the four helix axes are distinct. In (e), the stacks have been reformed, with the blue and green strands continuous, and the red and orange strands forming the crossover, while the helix axes are antiparallel. In (f), the top helix has been rotated a half-turn to make the helix axes parallel, thus completing the isomerization.

Figure 2: Macrocyclic Tetramers Formed by Ligation of Nucleic Acid Junctions. Antiparallel Sigal-Alberts structures have been used for these constructions. (a) This square structure is formed from the ligation of 4-arm nucleic acid junctions which have been separated by two full turns of DNA. Note the continuous red strand which forms a closed cycle. The presence of closed cyclic strands (assayed by resistance to the enzyme Exonuclease III) is the experimental evidence for the closure depicted (25). (b) Two successive corners are highlighted, and the black reporter-straw, attached near each corner, indicates that the two junctions have formed a similar structure, although rotated 90°. (c) Ligation of identical 4-arm junctions separated by 1.5 turns of DNA, but not formed into a macrocycle. Reference to the black reporter-straw shows that all the junctions have the same structure. (d) A square structure is formed from the ligation of 4-arm nucleic acid junctions which have been separated by 1.5 turns of DNA. Note that the red strand again forms a continuous cyclic chain. (e) The black reporter-straw highlights the fact that two successive junctions adopt very different structures. It was only through use of these models that a structural model for the experimentally observed cyclization of the tetrameric species was obtained.
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Figure 3: Negatively Supercoiled Circular DNA (a) A circular DNA molecule. The opposite strands are color coded, black and yellow. The helix axis is white, but interrupted once a turn by a green segment. (b) This molecule has been negatively supercoiled. (c) Local denaturation has been emulated by removal of the scaffolding. Note that this has relaxed the entire molecule. (d) The denatured segments of (c) have been formed into a cruciform structure, which is an available option if the sequence has two-fold symmetry. Note that the transition from (b) to (c) to (d) has been accomplished by altering the scaffolding (helix axis + radial struts), but without breaking the Tygon backbone structures.

Figure 4: Exploration of Knotting Structures of DNA. This figure illustrates that one can use the scaffolded system as an experimental system for the exploration of the knotted structures which result when DNA molecules are passed through one another. (a) A "Double-Shamrock" molecule formed by ligating together two 4-arm junctions which have three closed loops at the ends of their arms. (b) The top two loops have been passed through each other. (c) The scaffolding has been removed, and the knot which has been formed is revealed. Passing different loops of this structure through each other results in different knots.

Figure 5: A Simple Trefoil Knot Made from DNA or RNA. This knot is made from a cyclic single strand of DNA or RNA containing four pairing regions, each approximately a single turn long, interspersed with non-pairing DNA. The first region (red) is complementary to the third (blue), and the second region (orange) is complementary to the fourth (green). (a) The intact model, with its twofold symmetry axis approximately vertical in the plane of the figure. (b) The scaffolding has been removed to reveal that this system forms a trefoil knot.
three of these areas deal with large DNA molecules, too large for the convenient display of atomic concepts, such as the hydrogen-bonding complementarity of the bases. Although nucleic acid branched junction constructs have been modelled with computer graphics (12), the molecular features of systems with unknown structures are most easily treated with physical models.

An ideal physical model accurately represents the structural and dynamic features of the molecule on the scale selected. All molecular scientists are familiar with jacks-and-straws models, which are useful in directly representing the geometry of nuclear centers in small molecules. However, models in which every atom is represented are not convenient for exploring the properties DNA molecules whose topology is a significant feature. Supercoiled DNA has been represented traditionally with interwound flexible tubing. Such models are often confusing, when used to demonstrate that topological consequences derive from the helical nature of DNA.

I present here two types of models, combining jacks and straws with tubing. In one type of model, stiff helices made from jacks and straws are the dominant motif, with flexible tubing representing short joints between them. This model is useful in modelling the various features of Holliday recombination intermediates (13), as well as other nucleic acid junction constructs (8). In the second type of model, jacks and straws form a scaffolding for the assembly of tubing; removal of the scaffolding reveals the topology of the structure. Superhelical and knotting properties are readily modelled with this system. The details presented apply to classical B-DNA (1), but simple modifications permit construction or incorporation of other forms of DNA, such as left-handed Z-DNA (14).

**Stiff Helices with Flexible Joints**

It is often desirable to construct a low-resolution model of DNA in which as many features as possible are preserved from our higher-resolution knowledge of the structure (15). The model described in this section represents every residue of the double helix by three straws: one along the helix axis, one along the radius, and one along the helix circumference. All straws and jacks used here are Framework Molecular Models (16), purchased from Prentice-Hall. The model has been constructed from jacks and straws on the 1cm/Angstrom scale. The helix axis is formed from trigonal (bipyrimidal) valence clusters, using the "p-orbital" pair of prongs for the addition of 3.4 cm straws that correspond to the helical translation. A 12-cm straw strut is then attached to two of the three trigonal directions corresponding to radii of the double helix; using different colors for individual strands clarifies the model. Use of the 12-cm length generates a van der Waals radius for the DNA (17), with the resultant helical pathway beyond the actual phosphate radius. These two radial straw struts, plus the 3.4 cm helical translation straw represent the internal portion of the nucleotide pair: the base and the sugar, except for those atoms which form the 6 backbone torsion angles (e.g., 18).

The 120° angle between the two 12 cm straws corresponds to the minor groove of the DNA, while the 240° angle also formed by the straws generates the major groove.
This is not a large distortion from the actual angles (about 146° and 214° (19)); the proper angle may be generated with two pairs of pliers, if necessary. However, as seen below, the trigonal cluster may be used directly for many applications. The use of trigonal clusters also permits the modelling of triple-stranded DNA molecules (20). Octahedral clusters should be substituted by those wishing to use this system to model the four-stranded DNA structures which have been postulated (21,22).

An octahedral or trigonal cluster containing a "T" is then attached to the end of each pair of straw struts. These struts are next joined to their similarly colored mates by another straw, 8.2 cm long. This straw corresponds to the helical contour length from residue to residue (for a classical 10-fold DNA) at this radius. These final segments model the backbone of the double helix, which is not represented by the radial struts. One must be careful to twist the individual links in the proper direction to get a right-handed helix. It is also useful to remember that, when looking at the vertical helix axis, from the minor groove side, the strand on one's right has its 5' end at the top and its 3' end at the bottom; by the twofold symmetry of the molecule, the strand on the left has its 5' end at the bottom and its 3' end at the top. Because these components are not inherently chiral, it is often useful to hook a short straw on an unused valence direction to denote the 5' end (Figure 1).

Flexible segments may be incorporated into the structure by the introduction of flexible tubing in intimate contact with the straws. I use Tygon tubing, 1/8" i.d., 1/4" o.d., 1/16" wall. A short piece of straw (about 1 cm) is inserted into each end of the piece of tubing. This piece is used to replace a backbone segment at a site where flexibility is desired. Because the straw domains are so large and so stiff, the tubing behaves as a totally flexible connector, a universal joint with no angular constraints. The fixed length of the flexible segment adds the one constraint which corresponds to existing knowledge: fixed maximum extent of chain.

Figure 1 illustrates the use of these models to explain central features in recombination chemistry. Figure 1 shows a braided (a) and unbraided (b) Holliday intermediate in the "closed" or parallel-helix Sigal-Alberts conformation (23). These two structures differ with regard to their crossover strands which are braided (linked) (Figure 1a) or not (Figure 1b) at the crossover site. Braided structures are not believed to exist. Panels b-f of Figure 1 demonstrate the qualitative steps involved in the isomerization between two alternative crossover structures. The large number of steps involved are necessary to avoid forming a braided structure, such as the one in Figure 1a (24).

Nucleic acid junctions have recently been suggested for use as the structural scaffolding in a biochip memory device (25). Figure 2 illustrates a simpler macromolecular engineering application, the formation of closed macrocyclic structures from ligated nucleic acid junctions (5,26). Figure 2a shows a cyclic structure formed from 4 nucleic acid junctions separated by two turns of DNA, while Figure 2b indicates that each of the junctions forming a corner of the square is identical. Figure 2c illustrates a linear structure with the same junction structures, but now separated by 1.5 turns of DNA, rather than 2. Figure 2d shows a macrocyclic square formed from this linear molecule. For this case, the alternate corners of the second structure are different, as highlighted in Figure 2e.
Scaffolded Flexible Tubing Models

These models use the same materials as those described above. The proportion of flexible Tygon tubing segments has increased, so that the backbones are all tubing; the straws only constitute helix axes and radial struts between the axis and the tubing backbones. Of course short pieces of straw are still inserted at the ends of each piece of tubing to connect it to the jacks. These models are built to a smaller scale, and they allow actual model-building experiments, in order to explore the topological and structural consequences of various actions on the DNA molecule. The materials chosen have convenient flexibilities, so that the molecule responds appropriately in supercoiling relaxation demonstrations. Using the same tubing in larger proportion, and on a smaller scale makes it behave as a stiffer component of the system. Knot formation and the topological results of supercoiling are readily seen to be consequences of the Watson-Crick helical turns of the DNA molecules.

The models are constructed on the 2 mm/Angstrom scale. It is not possible to represent every residue on this smaller scale, as was possible with the first models. I have chosen to include about 3 radial struts per turn, rather than 10, as above. More than 2 struts are necessary, in order to retain the chirality of the helix within a single turn. Retention of the trigonal clusters from the previous motif results in retention of the distinguishability of the major and minor grooves, even on this scale. I use 2.2 cm helix axis segments, 2.4 cm struts and 6.2 cm lengths of tubing. A certain amount of experimentation (+/- a few millimeters) is necessary to get lengths which fit the builder’s personal quirks in putting things together.

An understanding of the role of supercoiling has become essential for comprehension of the regulation of the genetic information carried by DNA (e.g., 27). Figure 3a shows a circular double helical DNA molecule built with this modelling system. Figure 3b shows the same molecule which has been negatively supercoiled. Note that the axes of the double helices form a right-handed helical arrangement, as they are supposed to (28) with negative supercoiling. Figure 3c shows how denaturing a short region of the molecule relaxes the supercoiling. Figure 3d indicates that the same relaxed structure can be made more stable if right-handed cruciforms are formed from the denatured region, if this is appropriate for the sequence (29-31).

Knotted and concatenated structures are becoming increasingly important aspects of the replication and recombination of DNA molecules (9-11). The relationship between coils, interwinds and knots is very difficult to grasp without the help of models. Figure 4 shows how this modelling system can be used to figure out the topology which results when a branched structure has two loops interwound, as can be done by a topoisomerase, given large enough loops (32). In Figure 4a, I show a Double-Shamrock, formed by ligating together two 4-arm nucleic acid junctions. In Figure 4b, I have passed one strand through another, to create an interwound system. In Figure 4c, the scaffolding has been removed, and the knot structure of the system is evident from inspection. Different structures, with interlocks separated by different numbers of coils will yield different knots.
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Figure 5a shows another nucleic acid (DNA or RNA) knotting system, a single-stranded circle, containing four sequences one turn long, interspersed with non-pairing regions. The first sequence (red) is complementary to the third (blue), while the second sequence (orange) is complementary to the fourth (green). The three dimensional structure can be twofold symmetric; this is evident from Figure 5a. Removal of the scaffolding in Figure 5b shows that this arrangement defines a backbone structure containing a trefoil knot.

Discussion

The two types of models described above are extremely simple and reasonably inexpensive to make with commercially available parts. Besides being easy to construct, these models are very light; for example, the model in Figure 1 weighs less than 200 grams; it can be dismantled to fit in a coat pocket and reassembled in less than 1 hour. Thus, they are easy to transport for demonstrations and lectures. They become "working" models, rather than heavy, rigid edifices consigned to the insides of glass cases. If damage occurs, repair is very easy to accomplish.

Since the topological properties of DNA are global, rather than local, it is necessary to make models on a small enough scale to form complete closed molecules. Some of the structural details of the DNA helix have been elided in these models, and some are slightly distorted, but the relevant features which larger DNA systems require have been conserved. Thus, we have taken a "forest, rather than trees" approach, while maintaining awareness of the basic structural aspects which generate the topological phenomena in the first place. Exploration of this system can lead to fruitful model-building exercises which clarify the nature of the complex topological systems critical to the function of DNA as genetic material.

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References and Footnotes


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