DNA Nanotechnology

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Automatic Molecular Weaving Prototyped by Using Single-Stranded DNA**

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One of the goals of molecular-based nanoscience is the organization of matter into strong molecular structures that display the advantageous properties of analogous macroscopic structures.[1] A woven fabric is an example of such a macroscopic structure, but deliberately braided woven molecules have not been reported, because nodes of designated[2] and alternating signs must be placed specifically. Owing to its double helical structure, DNA is an ideal programmable molecule to build synthetic topological targets.[3] A half-turn of DNA, about six nucleotide pairs, corresponds to a node or a crossing point in a knot or a catenane.[4] Deliberate trefoil knots,[5,6] a figure-eight knot,[7] polyhedral catenanes,[8,9] specifically linked electrophoretic mobility standards,[10] and Borromean rings[11] are examples of previous DNA topological constructs. Nevertheless, a woven arrangement requires an even greater level of control over the placement of nodes. Here, we have prototyped a planar woven arrangement by using the B-DNA conformation for all nodes by strategically combining D-nucleotides and L-nucleotides. This work represents the first step on the way to automatic molecular-scale weaving. A previous use of L-nucleotides in DNA nanotechnology has been reported,[12] indicating that opposite-handed deviations from ideal structures occur in uniformly L-nucleotide DNA. However, there is no prior report of using a combination of D-nucleotides and L-nucleotides in the same strands for topological or nanotechnological purposes.

An image of a woven arrangement is illustrated in Figure 1a. This Roman mosaic in Conimbraga, Portugal, shows a simple braid. The arrangement contains alternating positive and negative nodes, as emphasized in Figure 1b. The node signs are generated relative to a group of vertical helix axes; were the picture to be rotated 90° within its plane, the signs would switch. In Figure 1c, each node has been drawn flanked by six horizontal lines, representing the base pairs of a half-turn of DNA.

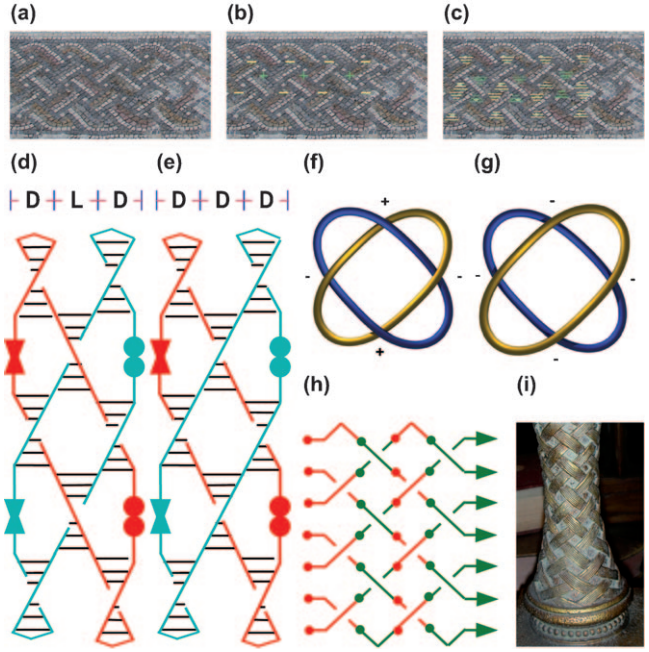

Figure 1. Representations of braided topologies. a–c) A section of a Roman mosaic located in Conimbraga, Portugal, illustrating a braided structure. b) Relative to a vertical helix axis, nodes have been assigned a sign, + (green) or − (yellow), depending on the nature of the crossing. c) The equivalence between a half-turn of DNA (6 nucleotide pairs) and a node in a knot is emphasized, by replacing node signs by a 6 horizontal lines that correspond to base pairs; the same color coding is used as in (b). d–g) The molecules made in this work. d,e) The molecule in (d) is a prototype of a woven or braided catenane. The need to maintain antiparallelism in the DNA strands forming the double helical segments results in needing to make 3′,3′ linkages, drawn as bow-tie features on the left, and 5′,5′ linkages, drawn as fused circles on the right. The molecule in (e) is the same as in (d), except it does not contain L-nucleotides. f,g) Simplifications of these molecules, drawn by Knotilus[20] where the nature of (f) as a toroidal Solomon link[21] is evident. h,i) A woven tube; h) positive nodes are in green and negative nodes are in red, relative to a vertical helix axis. 5′ ends of strands are indicated by filled circles of the same color as the strands. 3′ ends are indicated by arrowheads, but only on the right, for clarity. i) A lamp base decorated in a woven tube.

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The nodes produced by conventional right-handed double helical B-DNA are negative nodes.[2] In the cases where positive nodes have been needed in the past,[6,7,11] left-handed Z-DNA has been used to produce them. The disadvantages of using Z-DNA include the lack of sequence diversity that typically characterizes Z-DNA-forming sequences[13] and the difficulty of forming Z-DNA within very short segments. In addition, Z-DNA is not exactly a mirror image of right-handed B-DNA, exhibiting both a different helical twist and a

different pitch from B-DNA, besides the fact that it contains D-nucleotides.[14] L-Nucleotides do not have these drawbacks, and we use them here in this new topological application of DNA. Thus, the negative (right-handed) nodes built here are made using conventional D-deoxyribose nucleotide pairs, and the positive (left-handed) nodes are made using L-deoxyribose nucleotide pairs.

Figure 1d and e illustrate a schematic of the deliberately braided structure, compared with one made of exclusively D-nucleotide DNA. Figure 1d shows the interwoven self-assembled structure made from a mixture of D- and L-nucleotides and Figure 1e shows the structure that would result using only D-nucleotides. Each molecule consists of two cyclic strands, one red and one blue. Each of the crossings corresponds to a half-turn of DNA, similar to the representations in Figure 1c. The molecules consist of three vertical double-helix-containing domains. The central domain in the molecule shown in Figure 1d contains L-nucleotides, and it is flanked by two domains containing D-nucleotides. All three domains in the molecule shown in Figure 1e contain D-nucleotides. The differences between the two molecules are striking: Figure 1d contains an interwoven catenane, whereas Figure 1e contains two juxtaposed cyclic strands that are not linked at all. A simplified image that depicts the backbone topology of the braided molecule in Figure 1d is shown in Figure 1f and a similar image showing the topology of Figure 1e is shown in Figure 1g. The unlinked molecules in Figure 1e and g constitute an ideal control for the braided molecules in Figure 1d and f.

An alternative to the planar braided arrangement shown in Figure 1a is the tube-like braided structure shown in Figure 1h and 1i, where the two edges meet one another at the rear of the image to form a cylindrical pattern. Figure 1h is a schematic showing how alternating positive (green) and negative (red) nodes can match up behind the plane, to form a cylindrical or tube-like structure; an example of such a cylindrical structure is shown in Figure 1i, a lamp base that consists of a tube-like weave. Remarkably, the planar arrangement targeted in this work requires more synthetic effort than the tube-like arrangement would require. This difficulty is a consequence of the polarity of the backbone strands and the need to enforce antiparallel strands on each DNA half-turn domain. Both of the systems shown in Figure 1d and 1e contain strand polarity indicators: The bow-tie structures on the left sides of the molecules represent 3′,3′ linkages and the filled double circles on the right sides of the structures represent 5′,5′ linkages. These unusual linkages are hardly unprecedented in synthetic DNA molecules,[15,16] but they do make the synthesis of the strands containing them more involved than the synthesis of strands made exclusively of 5′,3′ linkages.

Figure 2 shows the sequences of the molecules synthesized for this study. The base sequences are identical, but the topology in the braided molecule in Figure 2a is different from the molecule in Figure 2b. Cyclic molecule 1 is longer than cyclic molecule 2, and both contain unique restriction sites. When restricted, the two different components can be identified readily by the lengths of the product strands. In

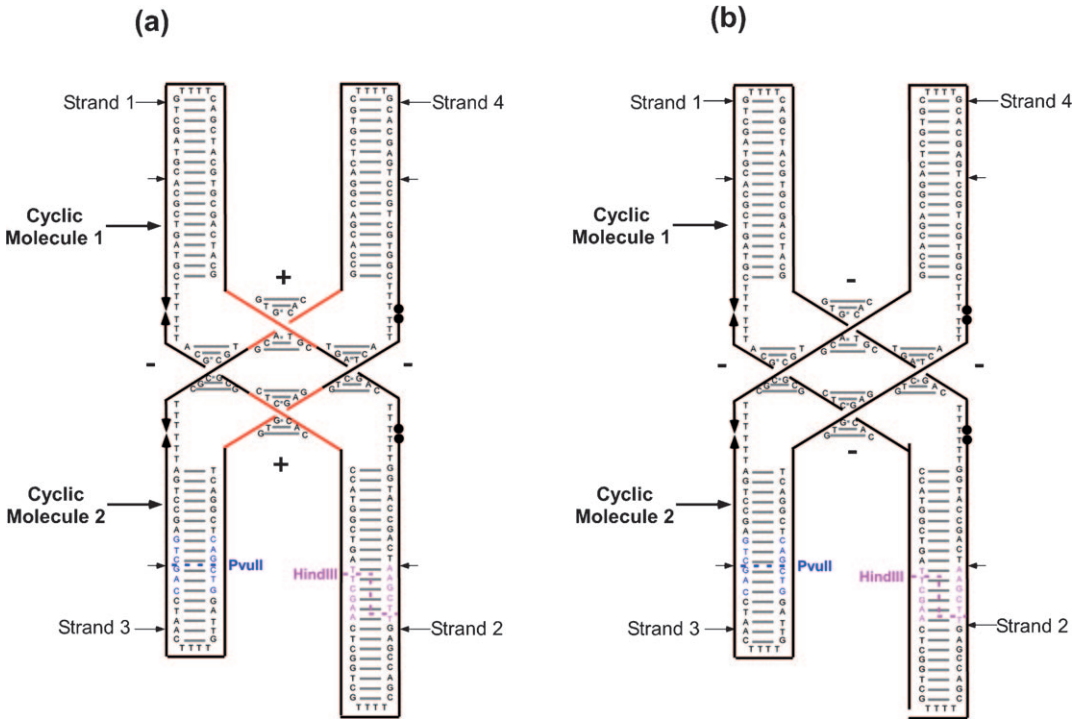

Figure 2. The sequences of the molecules used. Backbone segments drawn in black contain D-nucleotides and backbone segments drawn in red contain L-nucleotides. The molecule in (a) is the braided molecule, and the molecule in (b) is the unbraided control. The 3′,3′ linkages are indicated by arrowheads pointing at each other, and the 5′,5′ linkages are indicated by pairs of filled circles. Topology-determining nodes are indicated at the center of each molecule, where the helical nature of the strands is indicated by crossings whose signs are shown. Restriction sites are color coded and node signs are indicated. Ligation sites are denoted by small arrows pointing at the backbones. The two cyclic molecules are of different lengths.

each of the two systems, cyclic molecule 1 results from ligating strand 1 and strand 2; cyclic molecule 2 results from ligating strand 3 and strand 4.

Figure 3 shows the results of ligation and analysis of the molecules depicted in Figure 2. The samples were both stained and radioactively labeled. Figures 3a and 3c show stained gels of the molecules in Figure 2a and b, respectively; Figures 3b and 3d are the corresponding autoradiograms. There is a band corresponding to the woven DNA catenane at the top of each lane in Figure 3a and b, except for lanes 7 and 8 of Figure 3b, where nothing has been labeled. A variety of other products is visible. Lane 1 contains the ligation products and lane 2 contains the ligation products after treatment with exonucleases I and III. The top two bands, ascribed to the circular strands remain intact in both lanes. Lanes 3 and 4 contain the same materials, but only the long circular strand has been labeled. It is clear that the top circular band remains in the autoradiogram, but not the bottom. Exonuclease treatment results in the disappearance of the band ascribed to the long linear strand (Figure 3b). Lanes 5 and 6 are again treated the same way, except that the short circular strand is labeled; only the short circular and linear strands are visible in lane 5, and the short linear strand is digested in lane 6 (Figure 3b). Figure 3c and d contain the exact same treatments and labeled materials as Figure 3a and b, respectively, except that the complex of Figure 2b is used. The results are identical, except for the key difference of the absence of the bands corresponding to the woven DNA catenane.

Figure 4a is an autoradiogram containing the restriction analysis of the woven catenane. Lane 1 contains catenane isolated from the gels of Figure 3a and 3b. The large circle is restricted in lane 2, leaving the small circle and the long linear molecule. The small circle is restricted in lane 3, leaving the large circle and the short linear molecule. Lanes 4 and 5 contain large and small circular markers. It is clear that the top bands consist of a catenane of the large and small circles. The absence of the top band in Figure 3c and d confirms that the L-nucleotides are necessary for the catenane to form, and that no accidental linking of the two circles occurs in the course of the ligation process. The 10% gels shown here do not resolve singly linked from doubly linked catenanes. A 6% gel shown in the Supporting Information shows that a small amount of singly linked catenane contaminates the primary doubly linked product. L-nucleotides produce positive nodes using only a single half-turn of DNA; in previous work with positive nodes,[6,7,11] two or three nodes were used, because a half-turn of Z-DNA within a larger context was of marginal stability.

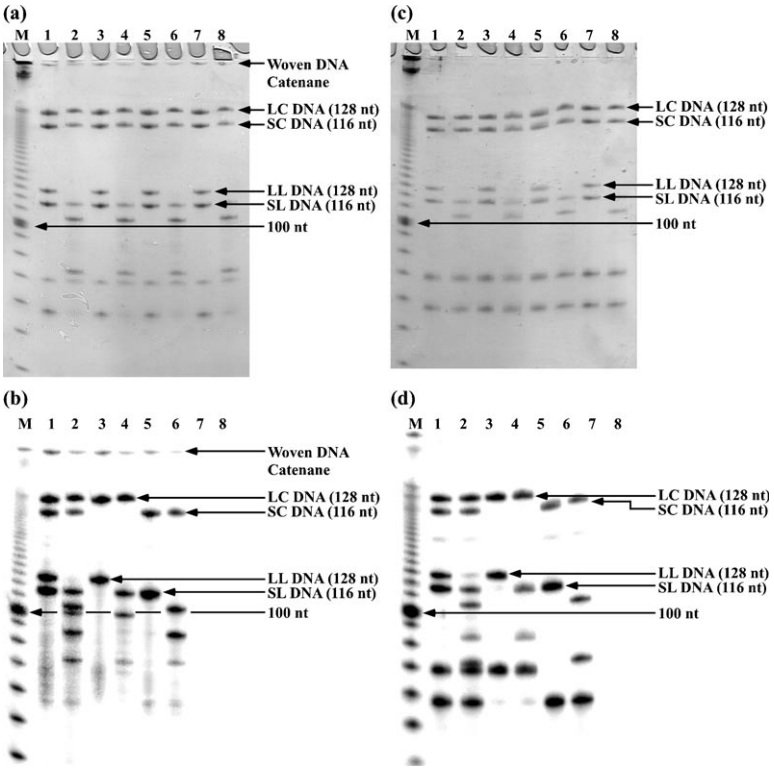

Figure 3. Gels showing the ligation products. Panels (a) and (c) are 10% denaturing gels stained with stains-all dye; the woven DNA catenane is loaded in panel (a) and the D-nucleotide control is in panel (c). Panels (b) and (d) are autoradiograms of the same gels shown in (a) and (c), respectively. In each panel the following contents are loaded: Lane M is a 10-base-pair marker; in lane 1, both large and small circular DNA strands are radioactively labeled; in lane 2, the sample from lane 1 has been treated with exonucleases I and III; in lane 3, only the large circular DNA strand is radioactively labeled; in lane 4, the sample from lane 3 has been treated with exonucleases I and III; in lane 5, only the small circular DNA strand is radioactively labeled; in lane 6, the sample from lane 5 has been treated with exonucleases I and III; in lane 7, none of the DNA strands are radioactively labeled; in lane 8 the sample from lane 7 has been treated with exonucleases I and III. LC DNA refers to the long circular DNA strand, SC refers to the short circular DNA strand, LL DNA refers to the long linear DNA strand, and SL DNA refers to the short linear DNA strand. The 100 nt position on the marker is emphasized.

We have demonstrated that it is possible to form a molecular weave with DNA. It is clear from this prototype that more complex braided or woven patterns are now feasible. The key difficulty of working with such structures on the molecular scale is the demonstration of their formation. The presence or absence of linked species is adequate for demonstrating the topology in the present case, but it would be inadequate to demonstrate the formation of a more complexly woven pattern, which would require direct structural evidence of the topology of the species. Given the small amount of contamination from singly linked catenanes in this study, the presence of by-products seems likely when topological targets of greater complexity are attempted. Nevertheless, the value of building macroscopic materials woven on the molecular scale, even if they contain a few errors, should not be underestimated. These constructs should demonstrate at least some of the features of macroscopically woven materials, and are expected to be quite robust. In addition to DNA constructs themselves, it is important to realize that DNA appears capable of directing molecular topology when it is one rail of a ladder polymer

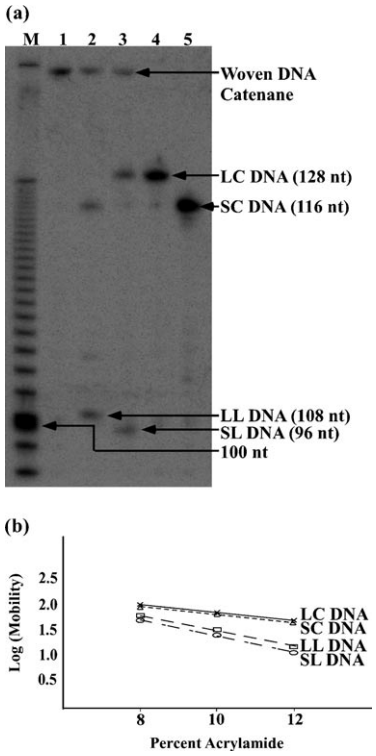

Figure 4. Restriction analysis of the woven DNA catenane. a) 10% denaturing gel where lane M contains a 10-base-pair marker ladder, lane 1 contains purified braided catenane DNA, lane 2 contains the braided catenane DNA after restriction of the large circular DNA strand with Hind III, lane 3 contains the braided catenane DNA after restriction of the small circular DNA strand with Pvu II, lane 4 contains a marker of the large circular strand, and lane 5 contains a marker of the small circular strand. The same abbreviations apply as in Figure 3. b) The Ferguson plot of the key breakdown species on this gel.

(i.e., a polymer in which two polymers are joined unit by unit through a linking group). Recent work has demonstrated that it is possible to connect other polymers, such as nylon,[17] to DNA, and new, strong materials could result either from the ladder polymer, or from the ladder with the DNA rail removed.

Experimental Section

DNA molecules used in this work were designed using the program Sequin[18] and were synthesized by phosphoramidite techniques using L-phosphoramidites (ChemGenes, Wilmington, MA) when needed.[19] 5′,5′ and 3′,3′ linkages were produced as described previously.[15,16] Radioactive phosphorylation, ligation, restriction, and Ferguson analysis were performed as described in the Supporting Information.

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