Topological Linkage of DNA Tiles Bonded by Paranemic Cohesion

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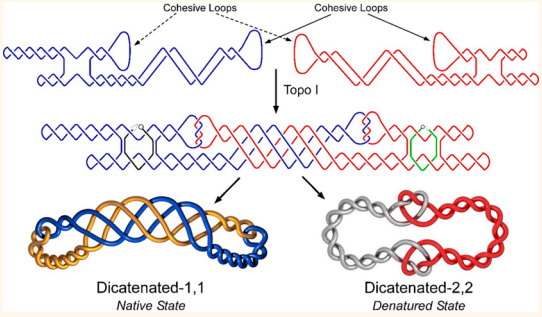
ABSTRACT Catenation is the process by which cyclic strands are combined like the links of a chain, whereas knotting changes the linking properties of a single strand. In the cell, topoisomerases catalyzing strand passage operations enable the knotting and catenation of DNA so that single- or double-stranded segments can be passed through each other. Here, we use a system of closed DNA structures involving a paranemic motif, called PX-DNA, to bind double strands of DNA together. These PX-cohesive closed molecules contain complementary loops whose linking by *Escherichia coli* topoisomerase 1 (Topo 1) leads to various types of catenated and knotted structures. We were able to obtain specific DNA topological constructs by varying the lengths of the complementary tracts between the complementary loops. The formation of the structures was analyzed by denaturing gel electrophoresis, and the various topologies of the constructs were characterized using the program Knotilus.



KEYWORDS: DNA catenane · DNA knot · paranemic cohesion · topoisomers · topological linkage · linking number · 3′,3′ and 5′,5′ linkages

Topological rearrangements of DNA involving strand passage operations play an important role in the manifestation of DNA functions in living systems, including replication, transcription, and recombination.[1] These operations are catalyzed by topoisomerases, which are most familiar for their roles in the relaxation and insertion of negative and positive supercoils into DNA.[2] Structural DNA nanotechnology is predicated on the notion of ligating branched DNA species into larger constructs.[3] In addition to the assembly of conventional DNA constructs, ligating branched DNA molecules is central to some aspects of DNA-based computation.[4] Experimentally, enzymatic ligation in these systems has been found to be of relatively low efficiency.[5,6] Consequently, we have asked whether the linkage of cyclic molecules catalyzed by topoisomerases could provide an alternative to ligation as an effective operation in the context of structural DNA nanotechnology and of DNA-based computation.

Topoisomerase I (Topo I) from *Escherichia coli* is the best studied enzyme among type IA topoisomerases.[7] It is a monomeric metal-binding protein of 97 kDa. It preferentially binds single-stranded (ss) DNA and can cleave ss-DNA sites followed by subsequent ligation. The enzyme forms contacts with both 5′- and 3′-terminal regions of the DNA cleavage site.[1] We have used *E. coli* Topo I to create a topological bond between two DNA constructs, thereby creating interlocked circular DNA structures. The existence of a binding site for single-stranded DNA at the catalytic center of Topo I accounts for cleavage of even short oligonucleotides (seven nucleotides in length) by this enzyme.[1] Cleavage occurs at a distance of three nucleotides from the 5′ end and four nucleotides from the 3′ end of the cleavage site.[1]

Knotted nucleic acids are found frequently in biological systems.[8−11] In addition, knotted topology has been the subject of synthetic chemical interest for almost 60 years,[12−14] first deliberately realized in Sauvage's synthesis of a trefoil knot.[15] The plectonemic nature of the DNA double helix makes it an ideal material for the construction of single-stranded synthetic knots that can be used as model systems for learning the properties of these unusual molecular topologies.[16] We have reported previously

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Received for review July 13, 2015 and accepted September 12, 2015.

Published online September 13, 2015
10.1021/acsnano.5b04333

the construction of trefoil knots and of amphichiral figure-8 knots from synthetic DNA molecules,[17,18] as well as trefoil knots from synthetic RNA molecules.[19] The topologically negative nodes in these knots were derived from double-helical half-turns of right-handed B-DNA, and the positive nodes result from double-helical half-turns of left-handed Z-DNA. Sequences that can form Z-DNA are also capable of forming B-DNA, in the absence of Z-promoting conditions.[20]

Consequently, by varying the solution conditions used during ligation, different knots have been made from the same synthetic strand of DNA.[18] It has also been shown that the ability to synthesize molecules of different topologies from the same strand makes it possible to interconvert them by DNA topoisomerases.[21] If solution conditions are established that favor one topological form over another, a single-strand-specific DNA topoisomerase is able to catalyze the interconversion of these species in the absence of an energy source. In the work reported here, we have used *E. coli* Topo I to create topological bonds between DNA constructs.

Design of the Catenation System. The system utilized here consists of the two circular molecules shown in Figure 1a. Each molecule consists of a DX domain,[22] a PX tail,[23] and two complementary loops. The two cyclic structures can cohere *via* PX cohesion[24] and contain complementary sequences between opposing loops. We used a 6:5 PX motif[23] for the system. The circular molecules have one section consisting of a DAE-DX motif.[22] The small circles at the centers of the DX units (black and green in Figure 1) are not sealed. Each DX component is tailed by a half-PX segment attached to its lower domain (Figure 1a) that can cohere with the opposite half-PX segment on the other circular molecule by PX complementarity.[24,25] On one side, the top of the DX domain is extended by a 16 nucleotide loop flanked by two dT_5 tracts, while the other side is sealed with two dT_4 loops. The half-PX tail is likewise extended with a 16 nucleotide loop also flanked by two dT_5 tracts. The sequences of the two loops on each circular structure are complementary to the sequences of the two loops on the other circular structure. The loops were designed to be oriented roughly perpendicular to the plane containing the circular molecules. These DNA circles formed by PX interactions melt around 58 °C.[26] To overcome this instability and to keep the circles together, the hydrogen-bonded interaction between the circles was transformed into a topological bond through Topo I catalysis, which enables strand passage of the cohesive loops.

Design of the Knotted System. We constructed a knot from PX-cohesive tiles that first get ligated into a circle (Figure 1b). The knots were obtained after treatment with Topo I. Tile κ has a pair of sticky ends emanating from the DX domain with $3'-3'/5'-5'$ linkages, whereas tile Ω has regular sticky ends coming out at the tip of the PX tail (detailed schemes in Figures S6 and S7). Each tile is made of one long strand that is obtained by ligating shorter component strands along with one short linear central DX strand.

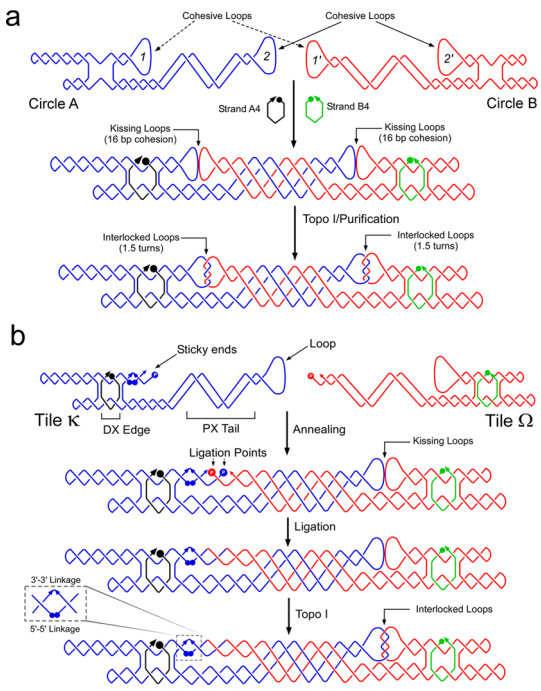

Figure 1. (a) Schematic drawing showing the annealing of circles A and B. PX cohesion of the circles with two sets of cohesive loops. The green strand forms the central strand of the DX region. There is a 16 base complementarity between the loops. (b) Schematic diagram showing the ligation followed by knotting of the PX-cohering tiles. The fused arrowheads represent 3′,3′ linkages, and the fused circles represent 5′,5′ linkages.

Tile κ has the following features:

(1) a DX domain that is closed with T_4 loops on one side at the top and the bottom
(2) a PX tail attached at the bottom of the DX domain
(3) a 26 nucleotide loop at the tip of the PX tail
(4) sticky ends at the top of the DX domain with $3'-3'$ and $5'-5'$ linkages

Tile Ω has the following features:

(1) a DX domain that is closed with T_4 loops on one side at the top and the bottom
(2) a PX tail attached at the bottom of the DX domain
(3) a sticky-ended extension at the tip of the PX tail
(4) a 26 nucleotide loop at the top of the DX domain

The two tiles have PX sequence complementarity[25] through the PX tails and Watson–Crick complementarity through the complementary loops and the sticky-ended region.

RESULTS AND DISCUSSION

Formation of Catenanes. As shown in Figure 2b, before Topo I treatment of the complex, the kissing loops can

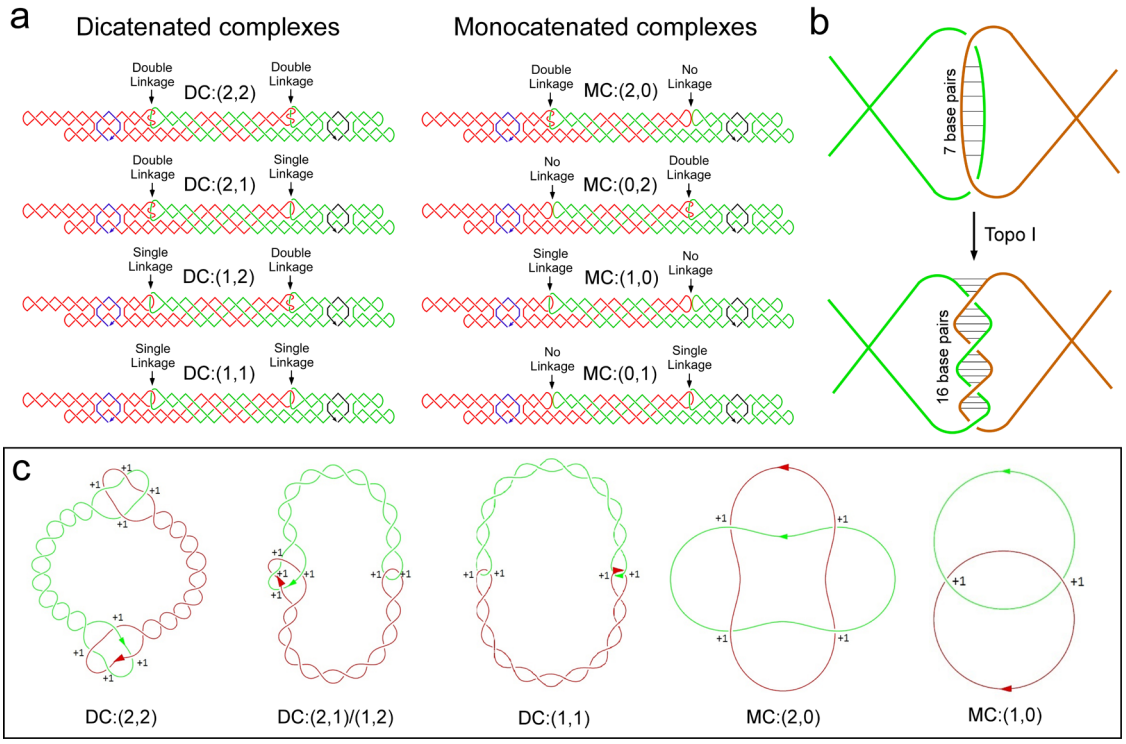

Figure 2. (a) Various singly and doubly linked products of the Topo I treatment. Doubly linked catenanes are represented as DC and single-linked as MC. The linkage between the two pairs of loops is denoted by the linking numbers in parentheses. (b) Loops before Topo I treatment: the cohesive loops are kissing on a 7 base tract, while the other bases remain unpaired. After Topo I action, the cohesive loops can have more base complementarity. (c) Knotilus rendering of the various linked structures with their sign notation.

cohere by about a half-turn of DNA (7 nucleotides). The rest of the complementary bases do not cohere because of steric/topological constraints. Topo I treatment of the kissing loops breaks one of the single strands from one of the kissing loops, passes it through the other loop, and reseals it, thus enabling full base pairing between the two complementary loops. The driving force behind the action of the Topo I enzyme is an increase in the base pairing of the system, thereby stabilizing the complex and lowering the overall free energy.

Topo I Treatment of Cohesive Complexes. Figure 3a shows a 5% denaturing gel of the PX complex that was treated with Topo I. Lanes 1 and 4 contain the individual circular molecules A and B; lane 2 contains the PX complex treated with Topo I, and lane 3 shows the products of Exo I plus Exo III treatment of the catenated complex. The annealed circles were treated with *E. coli* Topo I for 24 h at 37 °C. The catenated product was denatured at 90 °C for 1 h to show that the catenane contains both circles A and B (lane 2, Figure 3b). The generation of multiple products stems from two factors: (i) the complex can be either doubly or singly linked, depending on whether both or only one of the loops are catenated; (ii) the presence of a 16 base pair complementary tract in the loop can give a catenation product that is linked either once or twice.

Mono- and Dicatenated Products. As shown in the different drawings of Figure 2a, the complex formed by

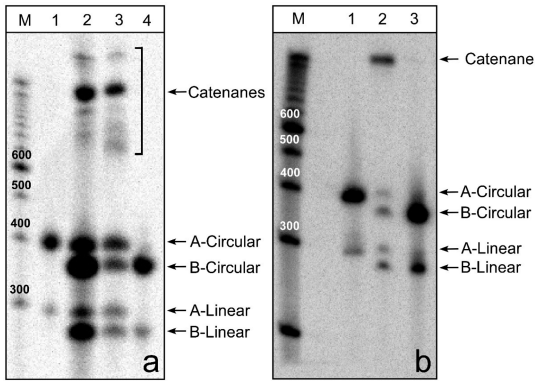

Figure 3. (a) Autoradiogram of the denaturing gel showing the results of the Topo I reaction. Both circles have been radioactively labeled. Lane 3 contains the results of the Topo I reaction after treatment with Exo I and III. Both lanes 2 and 3 show multiple catenated products. Lanes 1 and 4 have the individual circles A and B for comparison. (b) Heat denaturation of the catenated product. Lane 2 contains the catenane that was denatured at 90 °C for 1 h. Breakdown products show that the catenane is composed of both circles A and B in lanes 1 and 3.

the PX-cohesive circles with complementary loops can yield many types of catenated products depending on the reactivity of the loops. If both sets of loops get interlocked, there are three possible cases: (i) both sets of loops can be catenated (dicatenated: DC) with a linking number of 2, DC-(2,2); (ii) one set of loops is catenated with a linking number of 2, while the other

set is catenated with a linking number of 1, DC-(2,1), and *vice versa*, DC-(1,2); (iii) both sets of loops can be catenated with a linking number of 1, DC-(1,1). However, if only one set of loops gets interlocked (monocatenated: MC), the linking between the two loops can be either MC-(2,0) and MC-(0,2) with a linking number of 2 or MC-(1,0) and MC-(0,1) where the linking number is 1. The Knotilus[27,28] renderings (Figure 2c) of the different topological constructs based on the physical models[29] of the five possible topoisomers of the Topo I reaction products give a clearer idea of the structure of the catenanes. Based on the results given by Knotilus, the more compact structures are expected to move faster on denaturing gels,[30] a notion borne out by experiment (Figure 4).

We have demonstrated that it is possible to establish links between circular PX-containing molecules having loops that can pair. The denaturing gel run after Topo I treatment of the AB complex shows the formation of catenated molecules. Since the complex has two sets of complementary loops, two systems of PX-cohesive circles were designed to contain one set of complementary loops. The goal was to test the catenation reaction separately on each set of complementary loops.

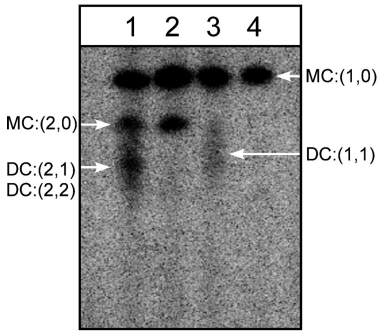

Figure 4. Denaturing gel showing the various single- (MC) and double-linked (DC) products of the Topo I treatment. Lane 1 has two sets of cohesive loops with 16 base cohesion. Lane 2 has one set of cohesive loops with 16 base cohesion. Lane 3 has two sets of cohesive loops with 11 base cohesion. Lane 4 has one set of cohesive loops with 11 base cohesion.

Influence of the Length of Cohesive Tracts. In the first complex, the complementary loops 1 and 1′ of Figure 1a have been replaced by T_4 loops to yield circle G and circle H, respectively, of Figure 5a. As a result, the GH complex contains only one set of complementary loops (2 and 2′). Figure 5b is a 5% denaturing gel of the G−H complex treated with the Topo I enzyme. Lanes 1 and 4 contain the individual circular molecules, and lanes 2 and 3 contain the PX complex that has been treated with Topo I at 20 or 37 °C, respectively. It is clear from the gel that the two complementary loops tested do form a catenated complex. The reaction run at 37 °C yielded two topoisomers, whereas the reaction run at room temperature yielded only one catenated product.

In the second complex, the complementary loops 2 and 2′ have been replaced by T_4 loops (Figure 6a). As a result, the IJ complex contains only one set of complementary loops (1 and 1′). Figure 6b shows the IJ complex treated with Topo I at 20 °C (lane 2) and 37 °C (lane 3). Similar to the GH complex, the reaction run at 37 °C yields two topoisomers, whereas the reaction run at room temperature yields only one catenated product.

A third complex ST was designed in which all four complementary loops were replaced by T_4 loops (Figure 7). This was a control to test whether Topo I could act on the cohesive complex formed by the PX

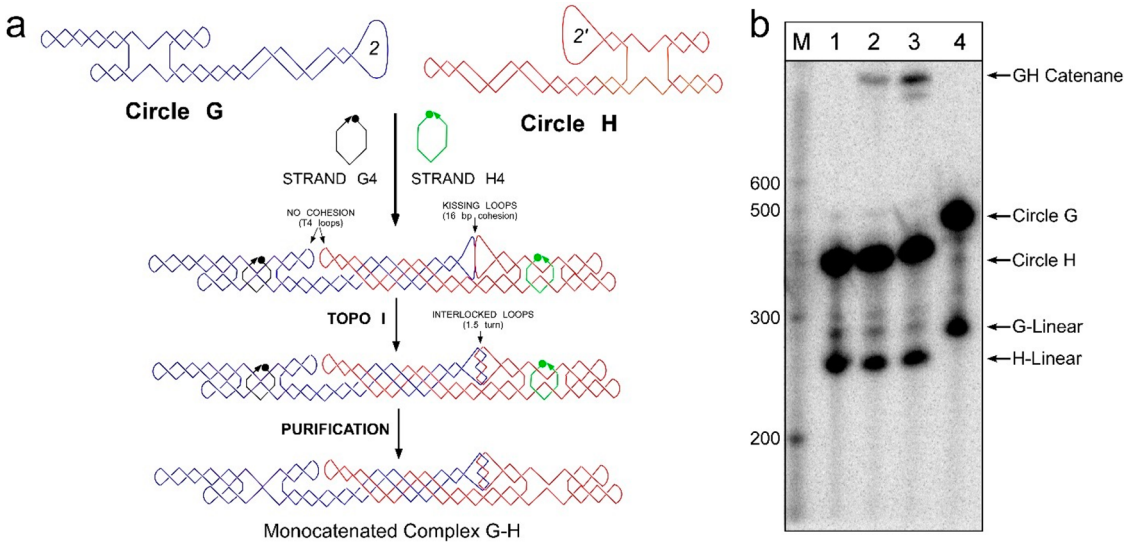

Figure 5. (a) Schematic drawing showing the results of the catenation of the GH complex. (b) Denaturing gel showing the results of the Topo I treatment of the GH complex. Only circle H is radioactively labeled. The reaction was run at 37 °C (lane 3) and 20 °C (lane 2).The lanes labeled 1 and 4 contain the individual circular molecules. The reaction run at 37 °C yielded two topoisomers, whereas the reaction run at room temperature yielded only one catenated product.

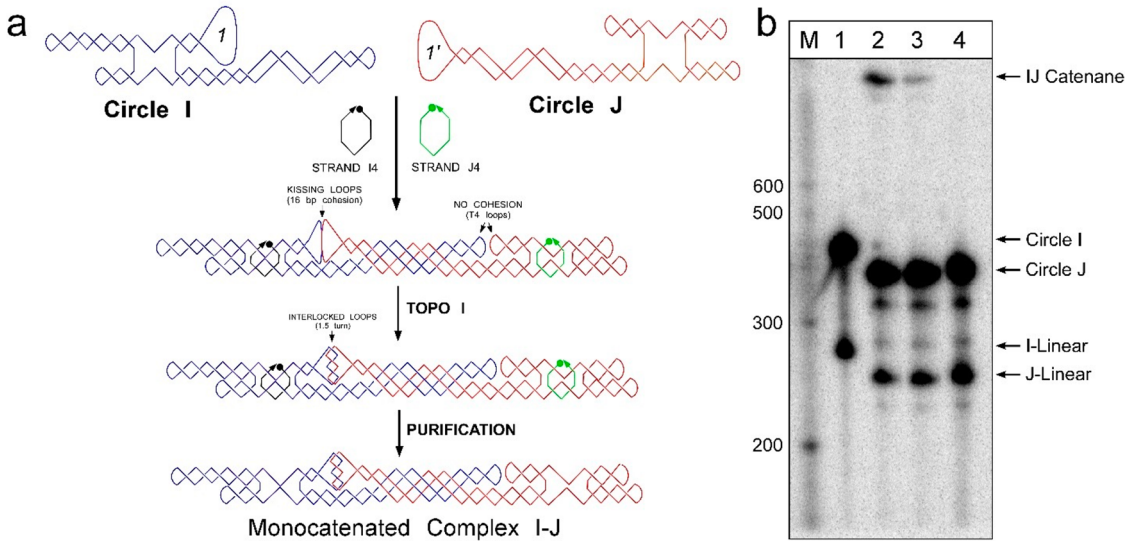

Figure 6. Schematic drawing of molecules I and J. The second system named I−J complex is made of two circular species I and J. One pair of cohesive loops was replaced by T_4 loops. (b) Denaturing gel showing the results of the Topo I treatment of the IJ complex. Only circle J is radioactively labeled. The reaction was run at 37 °C (lane 2) and 20 °C (lane 3). We see the presence of two topoisomers in lane 2.

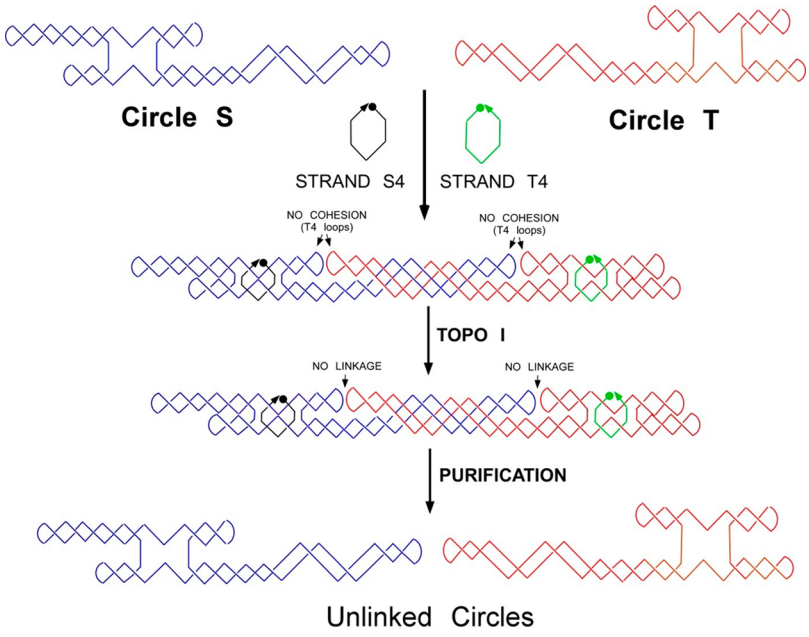

Figure 7. Schematic representation of the control system ST with T_4 loops.

tails to yield a catenated complex. Figure 8 shows a denaturing gel of the ST complex that has been treated with Topo I. No bands were observed that correspond to a catenane, which further demonstrates that in this system the Topo I enzyme acts only on the complementary loops.

Furthermore, the αB complex (Figure S8) was formed by two PX-cohesive circular molecules that have only one turn of cohesion in both sets of loops and yield the (1,1) complex. Circle α has been redesigned from circle A, where the complementary tracts have been reduced from 16 to 11 nucleotides. The $\alpha\beta$ complex is formed by two PX-cohesive circular molecules that have only one set of complementary loops (Figure S9). Circle β has been redesigned from circle B, where the cohesion from strand B2 has been eliminated by creating mismatches along the complementary tract. The designed $\alpha\beta$ complex thus contains one set of complementary loops with a pairing tract 11 nucleotides long and forms only a monocatenated complex. The results of Topo I treatment of the αB and $\alpha\beta$ complexes are shown in Figure S10.

Parameters of the Catenation Reaction. Negatively supercoiled DNA has a higher free energy than its relaxed equivalent owing to torsional strain. In this study, Topo I has been used to relieve the strain on a

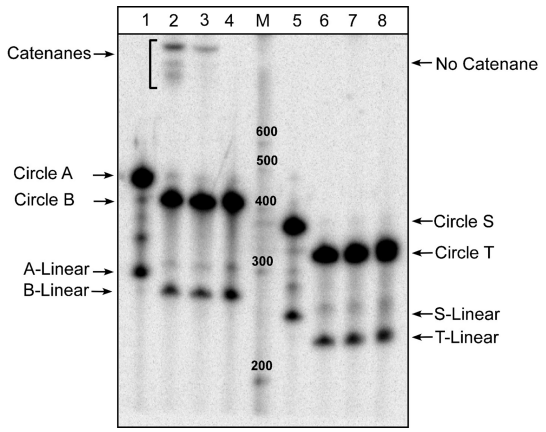

Figure 8. Autoradiogram comparing the fully linked AB system and the control system ST (without any cohesive loops). Lanes 1 and 4 contain circles A and B as a reference. Lanes 2 and 3 show the catenated products. Only circle A was radiolabeled in lane 1, and only circle B was radiolabeled in lanes 2, 3, and 4. Lanes 5 and 8 show the circles S and T, and lanes 6 and 7 are the Topo-treated ST complex. Only circle S was radiolabeled in lane 5, and only circle T was radiolabeled in lanes 6, 7, and 8. No catenated products are observed in either lane 6 or 7.

DNA complex by catenation of cohesive loops. The product of the action of Topo I on the system was a number of relaxed structures. As shown here, the 16 nucleotide sequence yields catenated circles that are linked either once or twice. The 11 nucleotide sequence yields only one type of catenated structure containing only a single link. Therefore, we also analyzed the different parameters of the loops and their design to look into the minimum base pairing length needed between the complementary loops for the catenation reaction to occur. One other parameter we analyzed was the transition between species with Lk = 1 and Lk = 2 and the role of the T tracts flanking the complementary loops in the catenation reaction.

We tested the effects of the T tracts that flank the complementary loops on the results of the catenation reaction (Figure S11). Twenty-two circular molecules forming 11 pairs of PX-cohesive circles with various lengths of T tracts (dT_1 to dT_{10}) were designed. In all of the 11 complexes, only one set of loops contains tracts with complementary sequences. The other set of loops is not complementary. The complementary loops 1 and 1′ were used for the study. The T_n tracts appear to have a stretching effect on the complementary tracts as some catenation occurs even when $n = 0$ (Figure S12). The second linkage appears when $n = 5$. As we keep increasing the length of the T tracts from 5 to 10, more of the catenanes get doubly linked species.

A system of complementary loops with a half-turn of cohesion was also designed. Figure S13 shows the molecule used for the study. The loops on strands $\sigma 1$ and $\sigma 2$ contain complementary tracts that have a half-turn cohesion with loops 1 and 2 on circle A. Catenation occurs only when the loops are one-turn

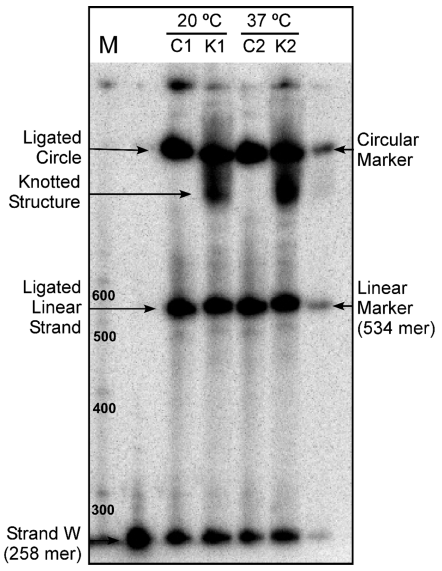

Figure 9. Denaturing gel comparing the ligation of the circle and formation of the knot.

long (Figure S14). The minimum base pairing between the complementary loops for the catenation reaction to take place is around 7−8 nucleotide pairs (Figure S15). The doubly linked species appear in the 16 base cohesion system.

Formation of Knots. Figure 9 illustrates a denaturing gel of the PX complex that was treated either by DNA ligase or by DNA ligase followed by Topo I enzyme. Lanes C1 and C2 contain the cyclic structures obtained after ligation. Lanes K1 and K2 show the knotted structures that are obtained when the ligation is followed by Topo I treatment of the annealed complex.

We have demonstrated that it is possible to create a DNA knot from PX-cohesive tiles that have both complementary loops and sticky ends. As shown on the denaturing gel, in the first step, the action of DNA ligase on the sticky ends creates a circular structure. The linear version of the circular structure and the ligated circular molecule migrate according to their size in comparison to topological markers of the same size. In the second step, the action of the Topo I enzyme on the complementary loops creates a knotted structure that migrates faster than the circular structure on the gel, owing to its greater compactness. It must be noted that the tiles were annealed in two ways: (i) by forming the tiles individually by slow cooling from 90 to 37 °C for 24 h and then annealing from 37 to 20 °C (room temperature) overnight; and (ii) by mixing all the components of the tiles together at 90 °C and slow cooling to 20 °C over 48 h. There was no noticeable difference in the results obtained from these two methods. Also, increasing the length of the complementary tract of the loops from 11 nucleotide pairs (one turn of DNA) to 16 nucleotide pairs (1.5 turns of DNA) creates a second type of DNA knot that migrates faster than the first DNA knot because of an increase in

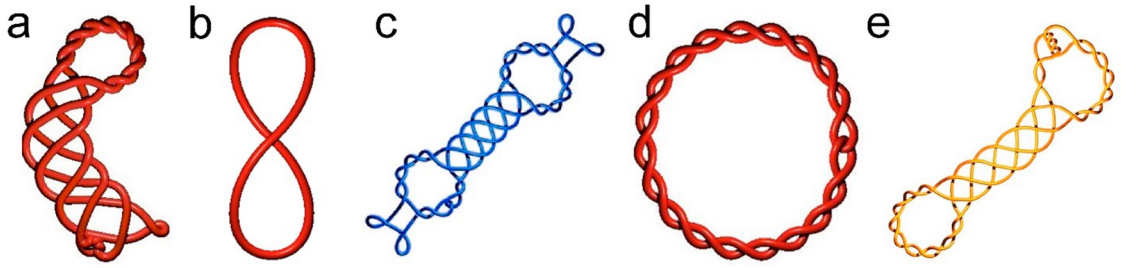

Figure 10. Knotilus rendering of the ligated circular structure in its (a) native state, (b) reduced denatured state, (c) knotted structure (Lk = 1) with the nugatory nodes in its native state, (d) knotted structure in its denatured state, and (e) knotted structure (Lk = 2) in its native state.

the number of links from 1 to 2 (Figure S16). The breakdown of the circular and the two knotted structures at 90 °C yields the expected linear product which corresponds to the open form of both the circle and the knot (Figure S17). Knotilus renderings of the various knotted structures are shown in Figure 10.

CONCLUSIONS

PX cohesion represents a programmable cohesive interaction between cyclic single-stranded molecules that does not require cleavage for base pairing to join molecules together. It can be used conveniently in structural DNA nanotechnology, but it is not as well-characterized as sticky ends.[31] We have demonstrated that it is possible to link PX-cohesive circular molecules topologically when they contain complementary loops. The denaturing gel run after the Topo I treatment of the AB complex shows the formation of catenated molecules. The use of Topo I to create these interlinked circles offers a new tool to fuse DNA nanostructures. We expect that this form of cohesion will have widespread applications in nanoconstruction, as the catenated components are more resistant to hostile conditions, such as high temperatures and chemical denaturants. The combination of paranemic cohesion and topological bonding of complementary loops *via* Topo I offers a new way to combine DNA molecules for purposes of self-assembly, molecular recognition, and nanosystems. We obtained a 50% yield for the catenation reaction *via* Topo I *versus* 30% for a control system that cohered *via* PX and sticky ends that was catenated *via* enzymatic ligation, suggesting that catenation may be more efficient in certain cases.

METHODS

Sequence Design. The sequences have been designed by applying the principles of sequence symmetry minimization,[1,2] insofar as it is possible to do so within the constraints of this system. The crossover points on each strand are predetermined in a PX molecule with an asymmetric sequence: crossover isomerization[3] would produce mispairing because major groove unit tangles would become minor groove unit tangles and *vice versa.* The PX molecules used in this study are PX 6:5, which contain five nucleotide pairs in the minor grooves and six nucleotide pairs in the major grooves.

Synthesis and Purification of DNA. All DNA molecules in this study have been synthesized on an Applied Biosystems 394 automatic DNA synthesizer, removed from the support, and deprotected, using routine phosphoramidite procedures.[4] DNA strands were purified by denaturing polyacrylamide gel electrophoresis containing 6−20% acrylamide (19:1 acrylamide/bis(acrylamide)). Bands corresponding to DNA strands of expected size were excised from denaturing gels stained with ethidium bromide. DNA was eluted in a solution containing 500 mM ammonium acetate, 10 mM magnesium acetate, and 1 mM EDTA at 4 °C overnight. The eluates were then extracted with butanol to remove ethidium, and the DNA was recovered by ethanol precipitation. The amount of DNA was estimated by OD_{260}.

Formation of Hydrogen-Bonded Complexes. Complexes were formed by mixing a stoichiometric quantity of each strand, as estimated by OD_{260}, in a solution containing 40 mM Tris−HCl, pH 8.0, 20 mM acetic acid, 2 mM EDTA, and 12.5 mM magnesium acetate (TAEMg). This mixture was then heated to 90 °C for 5 min and cooled to the desired temperature by the following protocol: 30 min at 65 °C, 30 min at 45 °C, 30 min at 37 °C, 30 min at room temperature, and (if desired) 2 h at 4 °C. Stoichiometry was determined by titrating pairs of strands designed to hydrogen bond together and visualizing them by native gel electrophoresis; absence of monomer indicates the end point.

PX Cohesion. Two circular molecules were annealed with the two 20-mer linear DX strands together in Topo I buffer, which contained 40 mM Tris−HCl, pH 8.0, 20 mM acetic acid, 2 mM EDTA, and 10 mM magnesium acetate, and slowly cooled from 90 to 20 °C in a Styrofoam box for 48 h, followed by 24 h at 4 °C in the cold room.

Polyacrylamide Gel Electrophoresis. *Denaturing Polyacrylamide Gel Electrophoresis.* These gels contained 8.3 M urea and were run at 55 °C. Gels contained 5−20% acrylamide (19:1, acrylamide/bis(acrylamide)). The running buffer consisted of 89 mM Tris−HCl (pH 8.0), 89 mM boric acid, and 2 mM EDTA (TBE). The sample buffer consisted of 10 mM NaOH, 1 mM EDTA, containing 0.1% each of bromophenol blue and xylene cyanol FF tracking dye. Gels were run on a Hoefer SE 600 electrophoresis unit at 55 °C (31 V/cm, constant voltage). After electrophoresis, the gel was exposed to a Kodak X-OMAT (Fisher Scientific) for up to 16 h to obtain the autoradiogram. The gel area containing the band was excised and mixed with a solution containing 500 mM ammonium acetate, 10 mM magnesium acetate, and 1 mM EDTA to elute the target samples at 4 °C overnight. The purified samples were then recovered by precipitation from ethanol.

Nondenaturing Polyacrylamide Gel Electrophoresis. Gels contained 6% acrylamide (37.5:1, acrylamide/bis(acrylamide)) and a buffer consisting of 40 mM Tris−HCl (pH 8.0), 20 mM acetic acid, 2 mM EDTA, and 125 mM TAEMg. The DNA was dissolved in 10 μL of TAEMg. Tracking dye (1 μL) containing TAEMg, 50% glycerol, and 0.2% each of bromophenol blue and xylene cyanol FF was added to the sample buffer. Gels were run

on a Hoefer SE 600 gel electrophoresis unit at 4 V/cm at 4 °C and exposed to a Molecular Dynamics 11″X9″ exposure cassette for up to 15 h and visualized using a Molecular Dynamics PhosphorImager Storm 840.

Enzymatic Reactions. *Radioactive Labeling.* In 25 μL of a solution containing 66 mM Tris−HCl (pH 7.6), 6.6 mM $MgCl_2$, and 10 mM dithiothreitol (DTT), 10 pmol of an individual strand of DNA was dissolved and mixed with 6 μL of 2.2 μM γ-^{32}P-ATP (10 mCi/mL) and six units of polynucleotide kinase (US Biochemical) for 80 min at 37 °C. Radioactive labeling was followed by the addition of 1 μL of unlabeled 10 mM ATP, and incubation proceeded for another 5 min. The reaction was stopped by heating the solution to 90 °C for 10 min followed by phenol extraction and gel purification.

Ligations. Ligations were performed in the kination buffer, which had been brought to 1 mM in ATP. All strands were mixed stoichiometrically, and the solution was then heated to 90 °C for 7 min and cooled slowly to room temperature. The T_4 polynucleotide ligase (US Biomedical) in the amount of 30 units was added, and the reaction was allowed to proceed at 16 °C for 16 h. The reaction was stopped by phenol/chloroform extraction. Samples were then ethanol precipitated.

Exonuclease Treatment. To the ligation mixture was added 100 units of exonuclease III (Exo III) (US Biochemical) directly, and the reaction was allowed to proceed for 0.5−2 h at 37 °C. The solution was heated to 90 °C for 5 min and cooled on ice for 2 min to generate single-stranded DNA. Then, 10 units of exonuclease I (exo I) (USB) was added, and the digestion was continued for 0.5−2 h at 37 °C. The reaction was stopped by phenol extraction.

Topoisomerase I Treatment. To the annealed complex were added 0.2 μg of bovine serum albumin (New England Biolabs) and 10 units of *E. coli* topoisomerase I (New England Biolabs) directly, and the reaction wa allowed to proceed for 24 h at 37 °C. The solution was heated to 90 °C for 5 min, and the enzyme was removed by phenol extraction.

Formation of Circular Structures. Each of the two circular molecules was obtained by ligating their three-component strands with the help of the short linear DX strands. After slow annealing, each solution containing the respective circles was brought to 1 mM in ATP and 10 units of T_4 polynucleotide ligase (USB) was added. The ligation proceeded at 16 °C for 16 h. The circular structures were isolated by denaturing gel and recovered by butanol extraction and ethanol precipitation.

Conflict of Interest: The authors declare no competing financial interest.

Supporting Information Available: The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsnano.5b04333.

Experimental procedure, additional experimental results, and DNA sequences (PDF)

Acknowledgment. This research has been supported by the following grants to N.C.S.: GM-29554 from NIGMS, CMMI-1120890, EFRI-1332411, CCF-1117210, and CCF-1526650 from the NSF, MURI W911NF-11-1-0024 from ARO, N000141110729 and N000140911118 from ONR, DE-SC0007991 from DOE for DNA synthesis and partial salary support, and 3849 from the Gordon and Betty Moore Foundation.

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