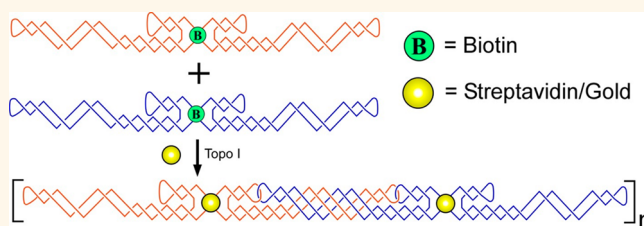# Covalent Linkage of One-Dimensional DNA Arrays Bonded by Paranemic Cohesion

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ABSTRACT The construction of DNA nanostructures from branched DNA motifs, or tiles, typically relies on the use of sticky-ended cohesion, owing to the specificity and programmability of DNA sequences. The stability of such constructs when unligated is restricted to a specific range of temperatures, owing to the disruption of base pairing at elevated temperatures. Paranemic (PX) cohesion was developed as an alternative to sticky ends for the cohesion of large topologically closed

species that could be purified reliably on denaturing gels. However, PX cohesion is also of limited stability. In this work, we added sticky-ended interactions to PX-cohesive complexes to create interlocked complexes by functionalizing the sticky ends with psoralen, which can form cross-links between the two strands of a double helix. We were able to reinforce the stability of the constructs by creating covalent linkages between the 3′-ends and 5′-ends of the sticky ends; the sticky ends were added to double crossover domains *via* 3′—3′ and 5′—5′ linkages. Catenated arrays were obtained either by enzymatic ligation or by UV cross-linking. We have constructed finite-length one-dimensional arrays linked by interlocking loops and have positioned streptavidin—gold particles on these constructs.

KEYWORDS: paranemic cohesion · catenanes · topological linkage · UV cross-linking · one-dimensional DNA arrays · 3′,3′ and 5′,5′ linkages · ligated DNA constructs

Recent years have witnessed substantial advances in the use of DNA as a smart material to construct periodically patterned structures.[1,2] DNA has also been used to direct the assembly of other functional molecules by the use of appropriate attachment chemistries. The diversity of materials that can be attached covalently to DNA considerably enhances the attractiveness of DNA nanostructures for the assembly of other materials.[3] Self-assembling DNA tiles represent a versatile system for nanoscale construction. Prominent among the DNA tiles are the double-crossover (DX) tile[4] and the paranemic crossover (PX) tile.[5] The most commonly used DX tile consists of two double helices joined twice by reciprocal exchange between strands of opposite polarity[6] (known as DAE or DAO depending on the separation of the crossovers).[4] The PX tile also consists of two double helices, but they are joined by reciprocal exchange between strands of the same polarity;[5,6]

surprisingly, when this fusion occurs, the result is a molecule that looks like two interwrapped paranemic double helices that can be separated without strand passage operations.[7,8]

Novel scaffolds for the immobilization of nanoparticles are targets of intensive research in the broader field of nanotechnology, because of their potential applications in the areas of catalysis, biosensing, and nanomaterials.[9,10] We have shown previously that DNA can act as a robust polymer to organize nanoparticles on one-dimensional and two-dimensional arrays.[2] The different types of scaffolds built so far rely on Watson—Crick base pairing. The ligation of one-dimensional DNA arrays was performed[11] in the last century and involved the ligation of DNA triangles containing double-crossover molecules. The possibility of creating polycatenated scaffolds is expected to open new avenues for the organization of nanoparticles on DNA arrays.
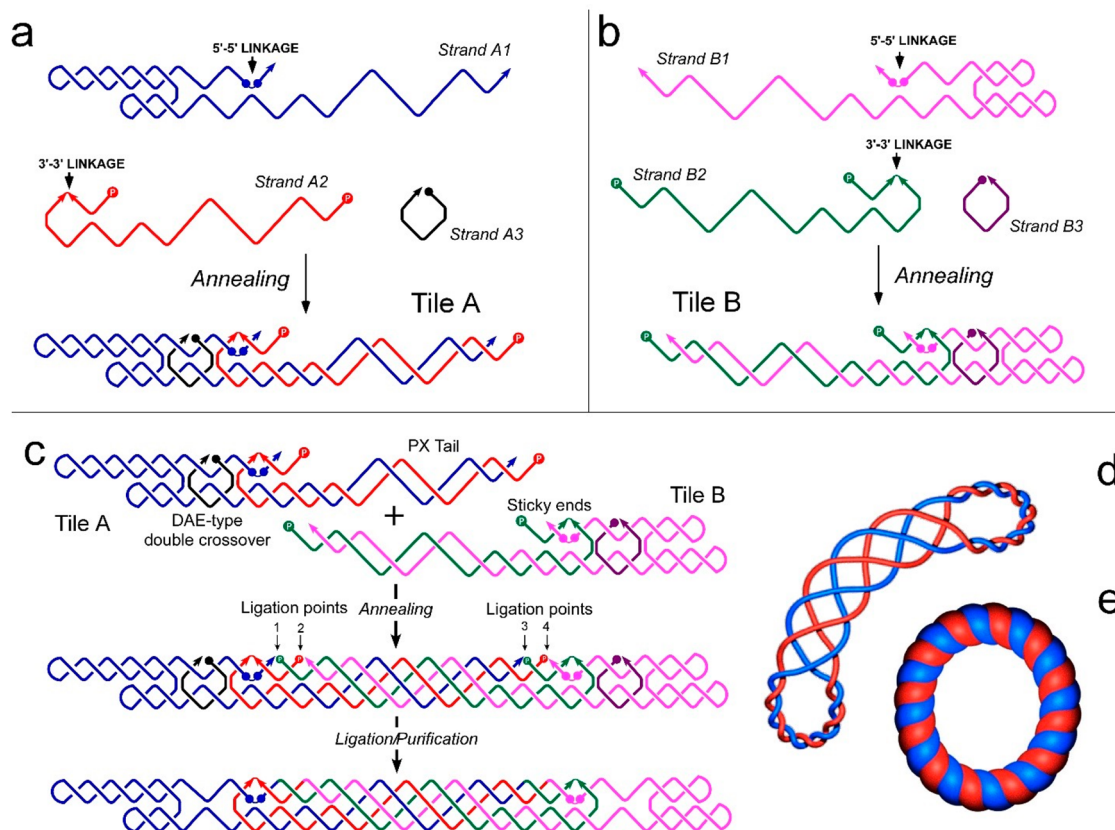
Figure 1. Scheme illustrating the formation of tiles A (a) and B (b), respectively. Each tile is made of three strands, of which one strand has a $5'-5'$ linkage (represented by two connected circles) and another has a $3'-3'$ linkage (represented by two connected arrowheads). One strand completes the structure of a DAE-type DNA double-crossover (DX) motif. One edge of the tile is closed both at the top and at the bottom domain by dT_4 loops. The top and bottom domains form a DAE double crossover. The other edge of the tile contains a half-PX loop that is extended with single-stranded DNA that forms two sticky-ended portions. (c) The two tiles are complementary both by PX cohesion and by sticky ends and can be ligated to form a catenane. (d) Pov-ray[19] image of the catenane in its native state. (e) The same structure as a torus.

The positioning of streptavidin—gold particles on DNA arrays involves biotin-containing DNA nucleotides (typically thymidine) protruding from the arrays. In this regard we have attached streptavidin—gold nanoparticles directly to biotin groups positioned into the strands that form part of a DX[4] portion of the tiles. DNA self-assembly begins with the chemical synthesis of single-stranded DNA molecules, which self-assemble into branched DNA DX motifs, complexes that here further cohere by PX cohesion.[4] We avail ourselves of the activity of *E. coli* topoisomerase I (Topo I), which brings DNA systems to equilibrium by catalyzing strand passage operations that lower the overall free energy. We report here DNA tiles made from circular molecules that can be catenated by Topo I activity, thereby forming one-dimensional (1D) DNA arrays with interlocked rings, intact and readily renatured, despite exposure to denaturing conditions. These arrays provide an excellent template for spatially positioning other molecular species.

RESULTS AND DISCUSSION

Each tile consists of a DX domain and a PX cohesive tail (Figure 1). Note that by a "PX tail" we mean an incompletely paired double-stranded structure that is designed to cohere with another complementary structure by means of PX cohesion.[12,13] The outer ends of the DX domain are closed by dT_4 loops, and the other side is extended by sticky ends on the top helix, whereas the bottom helix extends as a PX tail. The open part of the PX tail is also extended by sticky ends. Each tile is composed of three strands: one strand makes up part of the DX domain, a second strand makes part of the PX tail, and a third strand tethers together the top and bottom parts of a DAE-type DNA double-crossover molecule (Figure 1a and b).[4] Because of the difference in phasing between the DAE domain and the PX domain, the sticky ends are parallel to each other. To solve this structural problem, the polarities of the sticky ends were reversed from one of the domains (either PX or DX) by creating $3'-3'$ and $5'-5$ linkages.[14,15]

The two tiles were assembled in a solution consisting of 40 mM Tris-HCl (pH 8.0), 20 mM acetic acid, 2 mM EDTA, and 12.5 mM Mg acetate (Figure S2). Figure S3 illustrates a 6% nondenaturing gel containing the two tiles and their cohesive complex. The lanes labeled 1 and 3 contain the individual tiles, and the lane labeled 2 contains their 1:1 stoichiometric mixture. It is clear that
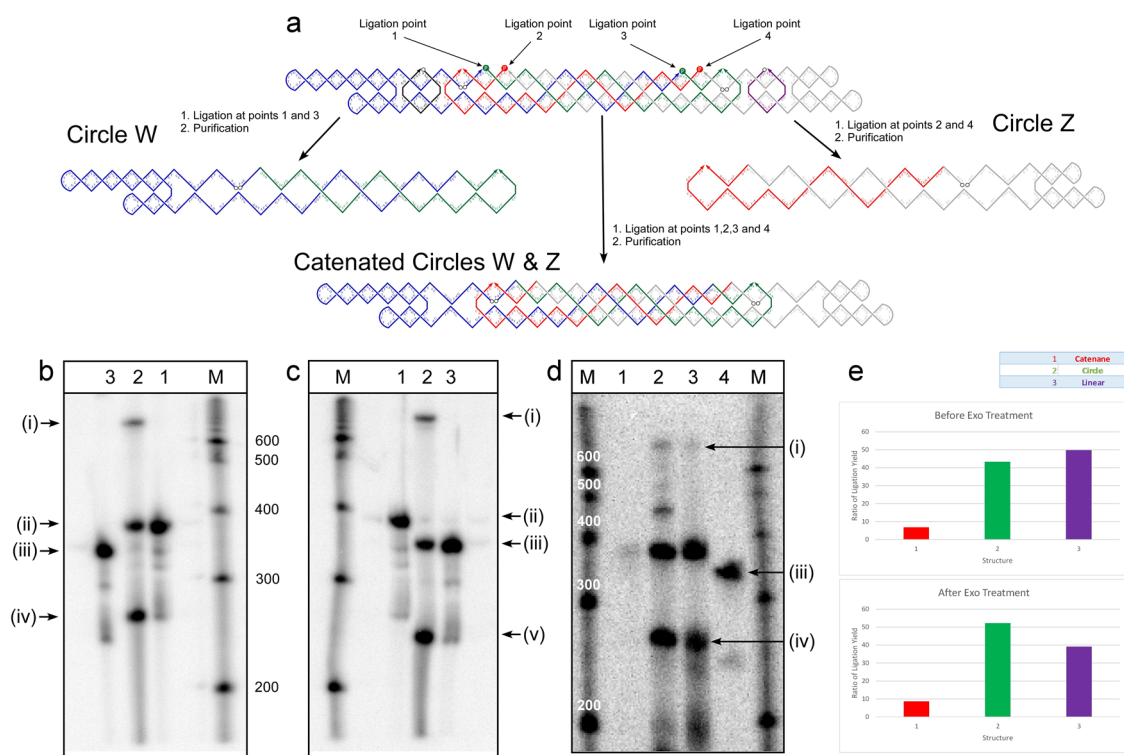
Figure 2. (a) Schematic showing the generation of the circle W, circle Z, and the catenated complex. The two circles and the canetane are formed when ligation happens simultaneously at the points indicated by the arrow. Circle W is obtained when strands A1 and B2 (see Figure 1) are ligated at ligation points 1 and 3. Circle Z is obtained when strands B1 and A2 (see Figure 1) are ligated at ligation points 2 and 4. (b) Denaturing gel showing the formation of the catenane by ligation of four nicks with strand A1 radioactively labeled. (c) Denaturing gel showing the formation of the catenane by ligation of four nicks with strand B1 radioactively labeled. (d) Denaturing gel showing the results of the Exo I and Exo III treatment of the ligated catenane. Lane 2 shows the results of the catenation before the Exo I and Exo III treatment, and lane 3 shows the results of the Exo I and Exo III treatment. Lanes 1 and 4 contain the circular markers W and Z. We can see that the linear strand has been degraded in lane 3 and that the circle and the catenane survived the Exo I and Exo III treatment. (e) Analysis of the ligation results. The graphs show the ratios of the yield of ligation before and after Exo treatment. Notations: (i) catenane, (ii) circle W, (iii) circle Z, (iv) W-linear, (v) Z-linear.

the two tiles have formed a 1:1 complex on the gel. The single bands in the individual tile lanes 1 and 3 act as controls for the possibility of nonspecific binding.

The introduction of the 3′−3′ and 5′−5′ linkages in the sticky-end region of the DX domain allows these DNA segments to be antiparallel to the complementary sticky ends at the tip of the PX tails. The presence of free 3′- and 5′-ends owing to the sticky ends allows for various terminal modifications of the strand. The hydrogen-bonded base-pairing interaction between the two tiles can be converted to a topologically linked interaction by creating a covalent bond between the OH group on the 3′-end and the phosphate group at the 5′-end by enzymatic ligation (Figure 1a). Another possibility would be to introduce a chemical functionality on either the 5′-end, the 3′-end, or both, to allow for directed chemical reactions in PX-cohesive DNA nanostructures. The shape of the catenated products was modeled through Knotilus[16,17] after obtaining the Gauss code.[18] Figure 1d shows the Pov-Ray[19] rendering of the structure in the native state. The Knotilus[16,17] rendering of the catenane in its denatured state is shown in Figure 1e. The structure has 24 crossings.

The denaturing gels in Figure 2 show the formation of DNA catenanes by the ligation of PX-cohesive tiles that also cohere through sticky ends. The distribution of linear products (one point ligated, so the structure is nicked), circular products (two points ligated), and the catenated target product (four points ligated) is about 60%, 30%, and 10%, respectively. A three-point ligation would yield both a circular and a linear product. Our results demonstrate that it is possible to generate catenated DNA circles from DNA tiles that bind before ligation via both PX cohesion and sticky-ended cohesion. This opens the possibility of generating various types of catenated circles by adding functional groups at the 3′- and 5′-ends of the sticky ends.

Figure 3 shows a denaturing gel with the products of the result of the ligation of the complex using circular templates. Both routes depicted in Figure 3a lead to the formation of catenated structures having the same mobility, with a yield of the catenation around 30%. Figure 4a illustrates the design of the two tiles used for 1D array formation. The two tiles are complementary by both PX cohesion and sticky-ended cohesion. The two tiles were reconstituted individually with the
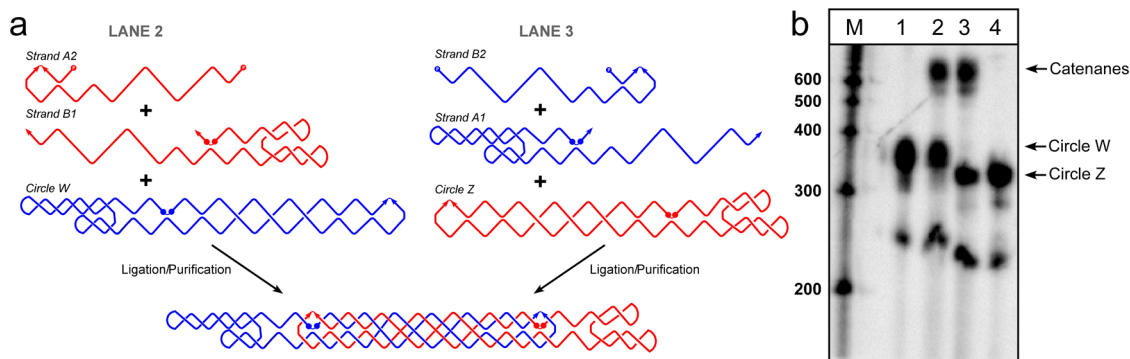
Figure 3. Denaturing gel showing the formation of catenanes by ligation of two nicks using circular templates. In lane 2, circle W is radioactively labeled, while in lane 3 circle Z is radioactively labeled.
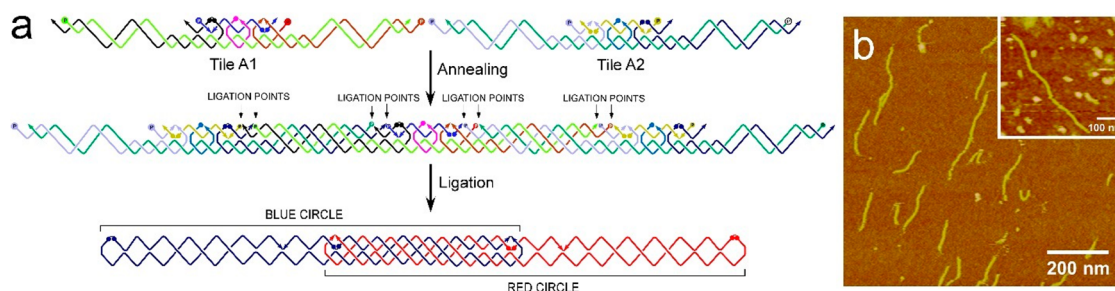

Figure 4. (a) Schematic drawing of the formation of the PX-cohesive one-dimensional array by ligation of sticky ends. (b) AFM image showing the ligated linear array after phenol extraction of the ligase, which can denature the DNA. Inset: The length of the ligated linear array can reach 600 nm.

20-mer DX unclosed, but nominally cyclic strands, in a solution consisting of 40 mM Tris-HCl (pH 8.0), 20 mM acetic acid, 2 mM EDTA, and 12.5 mM Mg acetate and used for visualization under an atomic force microscope (AFM), as seen in Figure 4b.

A topologically unique ladder-like polycatenane was prepared from two different tiles followed by ligation to form the mechanically interlocked 1D array. The two tiles were annealed as described above and observed under the AFM. After removal of DNA ligase, we can observe the presence of catenated linear arrays (Figure 4b), confirming the mechanically bonded nature of the 1D product array. The ligation of the sticky ends of PX cohesive tiles at four ligation points creates two interlocked circular structures (Figure 2a and b). The length of each circular tile is about 51 nm in its native state. The AFM image of the 1D array shown in the inset in Figure 4b shows an array about 560 nm in length, which corresponds to 11 linearly interlocked circular structures.

Removal of the DNA ligase by phenol extraction and subsequent ethanol precipitation adds extra steps prior to assessing the results of the reaction. An alternative nonenzymatic method for generating DNA catenanes is by cross-linking the DNA tiles with psoralen.[20] DNA cross-linking refers to the covalent linkage between the complementary strands of duplex DNA (interstrand cross-linking) or between bases of a single strand of DNA (intrastrand cross-linking).

The goal of this work is to connect specific regions of nucleic acid structure via organic moieties. Specifically, we want to produce interstrand cross-links across PX cohesive tiles to connect two different domains of the tiles. This strategy is expected to increase DNA thermal stability by covalently locking the two tiles together. So as to form covalent cross-links between two PX cohesive tiles, the two strands with $3'-3'$ linkages in both tiles were modified by introducing psoralen groups (shown as Ψ in Figures S4 and S5) at both 5'-ends of the strands. The catenanes are created by exposing the complex to UV light ($\lambda = 350$ nm). When the two cohesive PX segments are annealed together, the psoralen group at the 5'-end of the strand can link to the thymidine on the complementary sticky end. It has been reported that the interstrand complex exposed to UV light is formed with nearly 50% yield,[20] although better cross-linking yields can be obtained with cinnamate[21] or ^{cnv}K cross-linking.[22]

Tiles A and B were modified for psoralen cross-linking (renamed tiles K and L, respectively, Figures S4 and S5) as follows: On the 3'-ends of the strands containing the $5'-5$ linkages, the sequences were modified to be 5'-GT-3'. For the 5' region of the $3'-3'$ strands, the sequences were modified to be 5'-AC-3'. This was done to increase the efficiency of the cross-linking reaction. The original system has been designed to form a PX-cohesive complex with one A-T pair at each of the four cross-linking points. Tile K (Figure S4) and tile
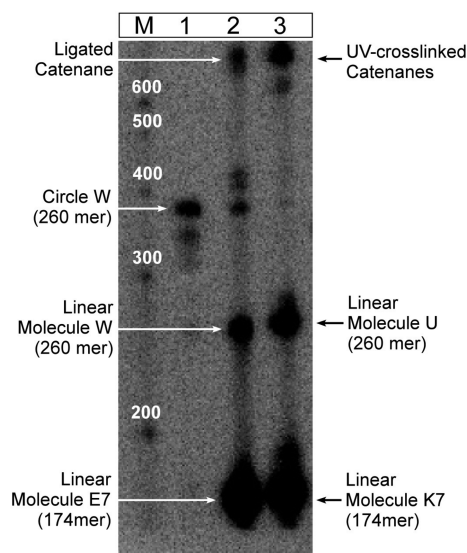
Figure 5. Denaturing gel comparing the formation of the catenane by enzymatic ligation and UV cross-linking.

Figure 6. Melting of annealed and UV-cross-linked 1D arrays at different temperatures. The preannealed tiles were mixed together at 37 °C and slowly cooled to 20 °C for 24 h and then to 4 °C overnight. The samples were deposited onto a mica surface, heated to various temperatures, and visualized using atomic force microscopy. Scale bars: 200 nm.

L (Figure S5) consist of a strand with a 5′–5′ linkage, a second strand with a 3′–3′ linkage, and a third strand that makes up the domain of a DAE-type DNA double-crossover molecule.[4]

The KL system we have designed has two PX-cohesive tiles that when annealed together has four psoralen groups at each of the 5′-ends of the sticky ends; these, in turn, can cross-link with the thymidine nucleotide attached to the 3′-ends of the complementary sticky ends. The results of cross-linking the system with two cross-linking points were compared with the catenation of a system by ligation (Figure 5). The system with two cross-linking points yields, as expected, three types of catenated structures: The middle band appears to correspond to the singly cross-linked product of the psoralen group with the thymidine in the continuous strand. The singly cross-linked product of the psoralen group with the thymidine in the complementary strand is the lower band, and the upper band is the doubly linked psoralen. We ran various control experiments on this system. In the first system (MN, Figure S6) all the thymines (pyrimidines) at the 3′-ends were replaced by guanines (purines). In the second system (OP, Figure S7), one thymine on one of the tiles was replaced by a guanine. In the third system (QR, Figure S8), one thymine on each tile was replaced by a guanine. As seen in Figure S9, no fully cross-linked complex was observed on the denaturing gel in the MN, OP, and QR lanes.

DNA plays an increasingly significant role for the design and construction of highly ordered nanostructures, which can template the organization of functional molecules such as proteins and nanoparticles.[23,24] The advantage of using a nonenzymatic technique allows for direct visualization of the constructed array wit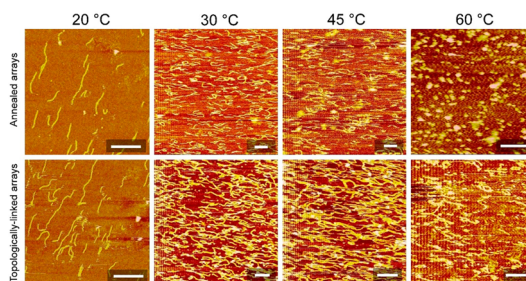hout the need to remove the debris of the enzymatic reaction. Moreover, the time needed to link the tiles by UV radiation (15–30 min) is much shorter than the use of DNA ligase (typically 16 h). Figure 6 shows the AFM images of the melting of the annealed 1D arrays and the topologically connected arrays *via* UV-cross-linking of psoralen groups.

We have successfully constructed cross-linked one-dimensional linear DNA arrays by UV treatment of cohesive tiles bearing psoralen groups on the 5′-ends of the sticky ends. The AFM images shown provide direct evidence that the use of psoralen groups for the construction of DNA arrays provides a useful tool in DNA nanotechnology. Melting studies on the AFM show that the linear constructs are quite robust and still retain their cohesion and shape at 60 °C. The advantage of using this method over enzymatic ligation is 3-fold: (i) the time it takes to create these cross-linked catenanes (30–45 min) is much less than the time to covalently link DNA tiles *via* DNA ligase (16–24 h); (ii) the result of the cross-linking reaction by UV treatment can be visualized directly by AFM; in the case of the ligation reaction, an extra step at the end of the reaction to remove the enzyme is necessary for AFM visualization; (iii) a third advantage of the UV cross-linking reaction has to do with the conditions of the reaction: the optimal temperature for the ligation reaction is 16 °C, although the reaction can also take place at room temperature with a lower efficiency owing to less cohesion. It must be noted though that due to the heat generated by the UV lamp, the reaction was done in the cold room at 4 °C to compensate for the potential denaturing effect of the heat on the annealed DNA assemblies. We have demonstrated that it is possible to generate catenated DNA circles from DNA tiles that cohere *via* both PX cohesion and sticky-ended cohesion. This opens the possibility of generating a variety of catenated circles by modifying the functionality at the 3′- and 5′-ends of the sticky ends.

Formation of One-Dimensional Gold Arrays. Figure 7a illustrates the design of two circular species. Each molecule consists of a central DX domain flanked by two PX-cohesive tails. The top part of the DX domain is
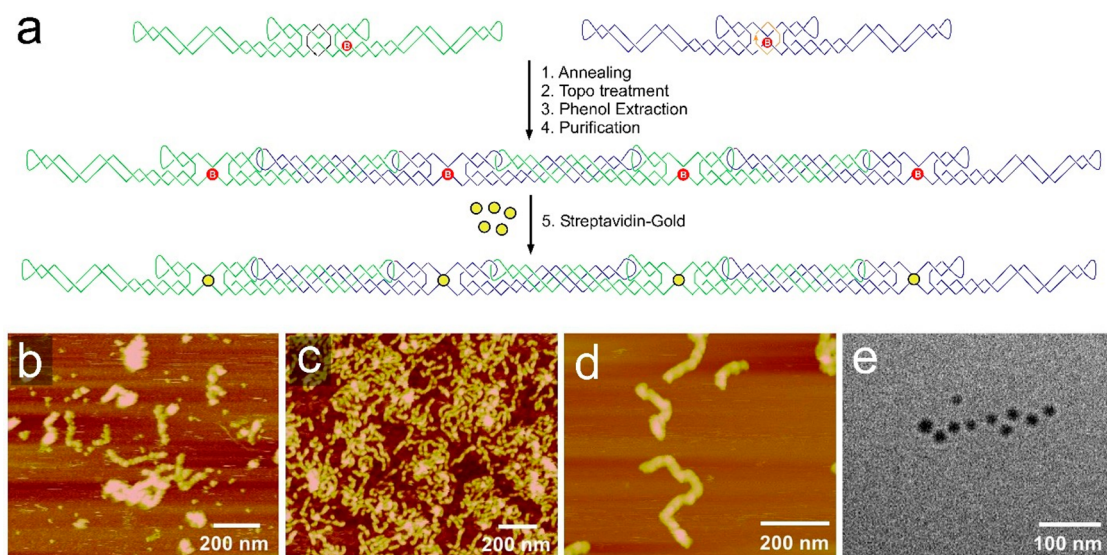
Figure 7. (a) Schematic of the formation of the catenated one-dimensional array. (b) AFM image of arrays before Topo I treatment. (c) AFM image showing the catenated arrays after Topo I treatment. (d) AFM image of the arrays after heat denaturation at 90 °C. (e) TEM image of the one-dimensional array with DNA-streptavidin-conjugated nanogold.
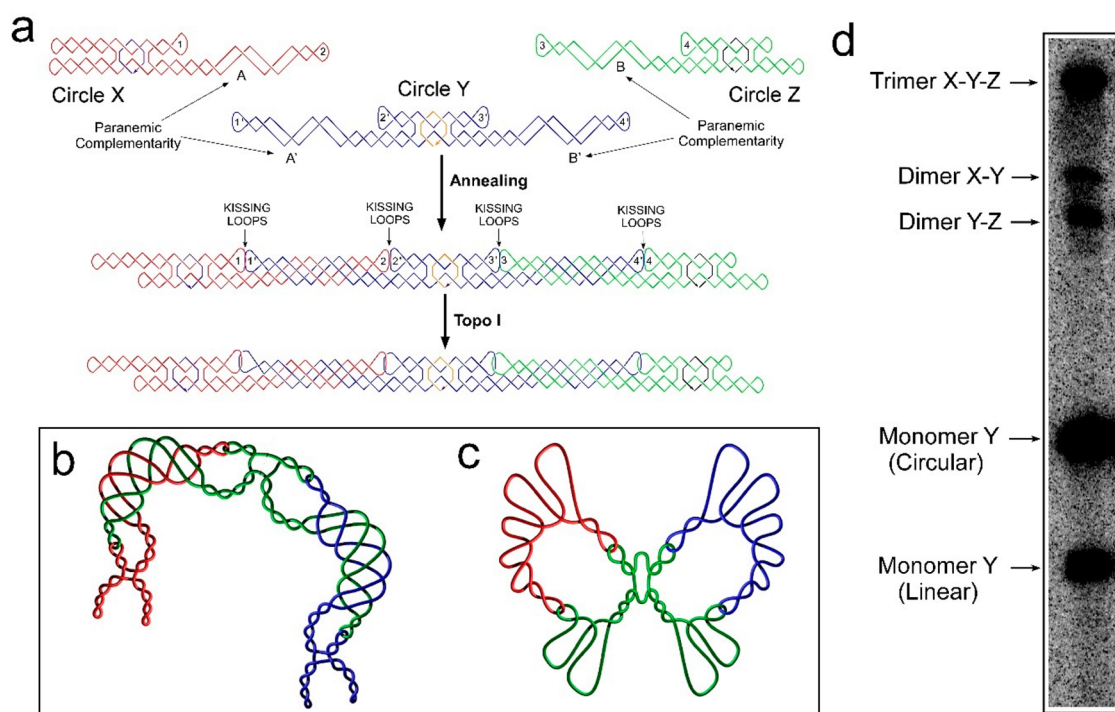

Figure 8. (a) Schematic drawing showing the annealing of the three-component complex. (b) Pov-Ray rendering of the catenated complex in its native state and (c) denatured state. (d) Denaturing gel autoradiogram showing the results of the catenation of the three-component complex. Only circle Y is radioactively labeled.

closed by two 26-nucleotide loops, one on each side. Likewise the tips of the PX tails are also extended by 26-nucleotide loops similar in design to the ones flanking the upper part of the DX domain. The DX domain is tethered by a short 20-mer linear strand and makes up a DAE-type DNA double-crossover molecule. The system of two circular molecules is designed to be an AB−AB copolymer whose component monomers can be mechanically interlocked using Topo I

enzyme to form a polycatenated one-dimensional linear array. A biotin group shown in red in the sequences was attached during the synthesis of the DNA strand to a thymidine located in the DX region of the tiles.

We demonstrate by nondenaturing gel electrophoresis and by AFM that we can combine tiles that cohere *via* PX cohesion to form linear arrays. The AFM image in Figure 7b shows the arrays formed by the
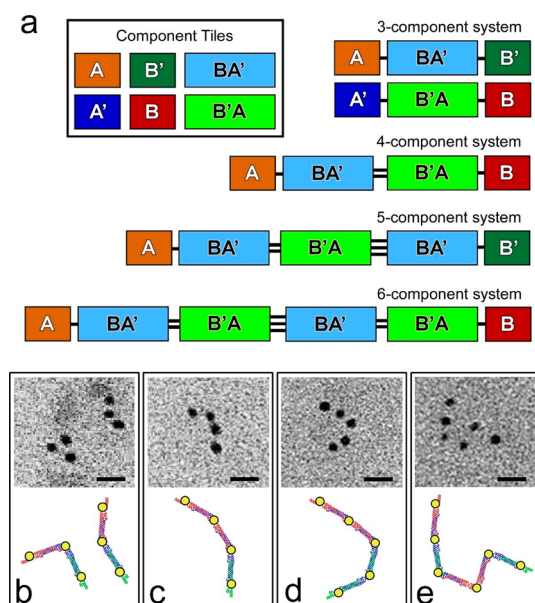
Figure 9. (a) Algorithm for the assembly of an *n*-component system with four starting tiles. By choosing from a pool of six unique tiles (four end tiles, A, B, A′, and B′, and two connecting tiles, BA′ and B′A), we can construct catenated arrays of any desired length. (b–e) TEM images of the three-, four-, five-, and six-component complexes with DNA streptavidin-conjugated nanogold arrays. Scale bars: 50 nm.

PX cohesion of DNA circles before Topo I treatment. Figure 7c shows the AFM image of the catenated arrays after treatment with Topo I, and Figure 7d shows the arrays after heating them at 90 °C for 15 min. The TEM image (Figure 7e) provides direct evidence that nanoparticles can be organized on biotin groups located on the strands that make up the DNA array and not on a loop that protrudes from the plane of the array. The number of gold nanoparticles that can be seen by TEM on a single linear array is about 7–9 on average.

A PX-cohesive system made up of two circular molecules yields a catenated complex that can be readily restored following exposure to denaturing factors such as temperature and chemical denaturants when these components need to be applied. We made a three-component system that coheres *via* a paranemic interaction (Figure 8a). The design of the central component of the system is based on a motif developed previously[25] that has two PX tails flanking a middle DX domain. This central circular molecule coheres with two separate circular molecules in a paranemic fashion. Figure 8b and c show the Knotilus[16,17] rendering of the three-component catenane in its native and denatured states.

The denaturing gel in Figure 8d shows the PX complex that has been treated with the Topo I enzyme. In this case, the central component Y was radioactively labeled. It is clear that the three circular molecules have formed a catenated complex on the gels. We also analyzed gels where the first component X or the third component Z was radioactively labeled (Figure S14). We have demonstrated by a previously developed method[26] wherein one component of the system is radioactively labeled at a time that each of them appears in the band that corresponds to the triply catenated complex. However, due to the complex topology of the catenated product, the complex does not migrate very far into the gel and remains near the top of the lane. We used the same strategy to create a four-component system (Figure S15). Figure 9a shows an algorithm for making finite DNA arrays. Tiles connected by a single black line are annealed first, followed by the second step where annealed tiles are mixed together (connected by two black lines) and the third annealing step shown as three black lines. For the formation of the four-component system, tiles A and BA′ were annealed in one pot and tiles B′A and B are annealed in a separate pot before being mixed together to form finite arrays (see Supporting Information for formation of five- and six-component tiles). Figure 9b–e show TEM images of the nanoparticles arranged on the three-, four-, five-, and six-component system.

CONCLUSION

The combination of PX cohesion and topological bonding of cohesive loops *via* Topo I offers a new way to combine DNA molecules for purposes of self-assembly, molecular recognition, and nanosystems. We have demonstrated that one-dimensional DNA arrays assembled from PX-cohesive circular tiles can be catenated by the action of the Topo I enzyme on the cohesive loops. The AFM images show that the linear array conserved its cohesive nature even when exposed to denaturing conditions. This novel type of catenated scaffold offers new ways to organize nanoparticles and offers the possibility to extend the method for the construction of finite two-dimensional and potentially finite three-dimensional structures. The use of catenated 1D DNA arrays as a scaffold for metal nanoparticles potentially offers fundamental advantages over other self-assembly approaches with regard to the precision, rigidity, and programmability of the assembled nanostructures.

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)).

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 . 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.

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

Polyacrylamide Gel Electrophoresis. *A. Denaturing Polyacrylamide Gel Electrophoresis.* The 5−20% acrylamide (19:1, acrylamide/bis(acrylamide)) gels contained 8.3 M urea and were run at 55 °C. 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, and 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.

B. Nondenaturing Polyacrylamide Gel Electrophoresis. Gels contain 6% acrylamide (37.5:1, acrylamide/bis(acrylamide)) and buffer consisting of 40 mM Tris-HCl (pH 8.0), 20 mM acetic acid, 2 mM EDTA, and 125 mM magnesium acetate (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, exposed to a Molecular Dynamics 11 in. × 9 in. exposure cassette for up to 15 h, and visualized using a Molecular Dynamics PhosphorImager Storm 840.

Enzymatic Reactions. *A. 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 $\gamma-^{32}P$-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.

B. Ligation. 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. T4 Polynucleotide ligase (US Biomedical) in the amount of 30 units is added, and the reaction is allowed to proceed at 16 °C for 16 h. The reaction is stopped by phenol/chloroform extraction. Samples are then ethanol precipitated.

C. Exonuclease Treatment. To the ligation mixture, 100 units of exonuclease III (Exo III) (US Biochemical) was added directly, and the reaction is 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.

D. Topoisomerase I Treatment. To the annealed complex were added directly 0.2 μg of bovine serum albumin (New England Biolabs) and 10 units of *E. coli* topoisomerase I (New England Biolabs), and the reaction was 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.

Atomic Force Microscopy. The 1D DNA arrays were imaged in tapping mode AFM in buffer. A 5−7 μL amount of the annealed sample was spotted on freshly cleaved mica (Ted Pella, Inc.). An additional 25 μL of fresh buffer was added to both the mica and the liquid cell. The AFM imaging was performed on a Nano-Scope IV (Digital Instruments, Veeco Metrology Group) in tapping in buffer mode, using Veeco's SNL (Sharp Nitride Lever) probe.

Formation of the 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 T4 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.

UV Cross-Linking Reactions. The one-dimensional DNA array formed from the annealed tiles was exposed to UV light at $\lambda = 350$ nm for 30 min in the cold room, and the results were observed by AFM.

Streptavidin-Conjugated Nanogold Binding to a Catenated Linear Array. Catenated DNA arrays containing biotinylated oligo were annealed as described in the complex assembly section above. The concentration ratio of the DNA complex to streptavidin was 1:3. After adding streptavidin to the annealed DNA linear array, the solution was incubated overnight at 4 °C before imaging.

Transmission Electron Microscopy. TEM imaging was performed using a Jeol 1200 EXII instrument operated at 60 kV. The sample was prepared on 400 mesh foamvar-coated nickel grids by dropping 5 μL of sample solution on grids and then wicking off excess solution using filter paper after 10 min. All grids were dried at least for 2−3 h to overnight at 4 °C.

Melting Experiments on AFM. To test for the formation of the cross-linked DNA arrays, the melting behavior of the annealed arrays *versus* the cross-linked arrays was studied on the AFM. For both the annealed and the UV cross-linked arrays, samples were deposited on the mica and the temperature of the surrounding bath was progressively increased from 20 °C to 60 °C. Scans of the arrays were taken as the temperature was increased.

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.5b04335.

Additional experimental results and DNA sequences (PDF)

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