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Paranemic Cohesion of Topologically-Closed DNA Molecules

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Structural DNA nanotechnology entails the self-assembly of unusual DNA motifs by directed cohesive interactions.[1] A diversity of motifs based on branched DNA molecules has been used to produce a variety of constructs: DNA stick polyhedra, such as a cube[2] and a truncated octahedron;[3] topological constructs, such as knots[4] and Borromean rings;[5] nanomechanical devices;[6,7] and noncovalent two-dimensional periodic[8−11] or one-dimensional algorithmic[12] arrays. These nanoconstructions were facilitated by sticky-ended cohesion, sometimes followed by ligation. In our experience, only topologically closed objects can be purified readily from failure products, because denaturing gels are required to effect the purification;[2] restriction of hairpins can produce short sticky ends. However, naturally occurring restriction enzymes typically produce sticky ends no longer than four or five nucleotides; this is long enough for ligated species, but lattice components must cohere noncovalently. Sticky ends this short have proved too weak to bond noncovalent arrays of large objects, such as DNA triangles[13,14] containing upward of 150 nucleotide pairs; longer sticky ends (6 or 7 nucleotides) are strong enough to assemble such species.[14] Methods to produce long sticky ends from hairpins have been advanced by Zhang and Taylor[15] and by us.[16] However, neither of these is convenient; the former requires special reagents, and the latter involves tedious biochemistry. A nicking restriction enzyme[17] might be able to emulate Zhang and Taylor's reagent.

Here, we present a solution to the problem of large-object noncovalent DNA assembly that circumvents sticky ends. The essence of this method is the paranemic cohesion of two topologically closed molecules by use of a motif known as paranemic crossover (PX) DNA.[18] PX DNA has been used in a nanomechanical device,[7] and it has been suggested as a possible participant in genetic recombination.[18,19] The PX motif arises from the fusion of two parallel double helices by reciprocal exchange at every possible point where the two helices come in contact. The left of Figure 1 derives the PX motif by forming purple crossovers between parallel red and blue DNA double helices; the panel to the right of the identity shows that the product is two interwrapped double helices. The remarkable consequence of this type of crossover structure is that the strands of the two interwrapped helices are completely unlinked. Thus, PX molecules can be built from DNA dumbbells, as shown in the right panel of Figure 1.[19] The PX structure contains alternating half-turns of DNA that correspond to minor groove or major groove spacings; the minor groove spacing is five nucleotide pairs, and the major groove spacing may contain six to eight pairs.[19] There are no sequence requirements beyond Watson−Crick base pairing in each helical domain.

The paranemic nature of the PX structure suggests that it can be used to produce a new form of DNA cohesion. Inspection of the right panels of Figure 1 shows that the red or blue strands pair with strands of the same color for a half-turn, and then they separate and pair with strands of the opposite color before pairing again with strands of the same color. Thus, the red strands are paired to each other for a half-turn, to the blue-strands for a half-turn, and then again to themselves. The intercolor pairing promotes intermolecular cohesion, and it also the leads to specificity. The overlapping PX domains can be made as long as needed to attain the cohesive strength and specificity desired. The only other paranemic cohesion system of which we are aware consists of binding between RNA hairpin loops.[20]

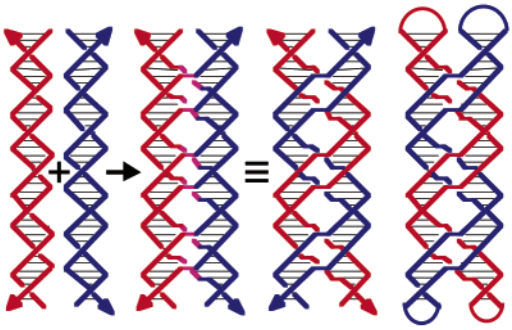

Figure 1. PX motif. The origin of the motif is shown at the left, where a red and a blue double helix exchange strands at every point, where they come into contact, to produce purple crossovers. The covalent strand structure is shown to the right of the identity sign, where a red and a blue double helix are seen to interwrap. The paranemic relationship of the interwrapped helices is emphasized on the right where the blue and red helices are capped in hairpin loops.

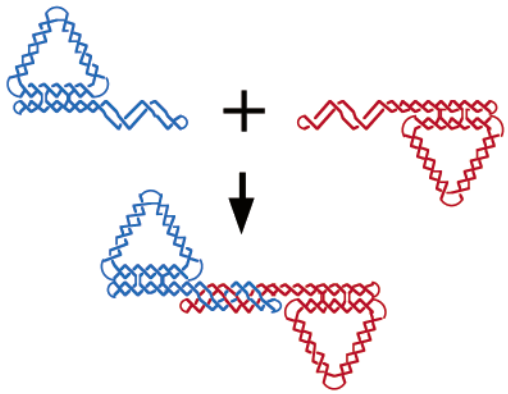

Figure 2. Paranemic cohesion. The blue triangle on the left and the red triangle on the right have one edge consisting of a DX motif. The small circle at the center of the DX is not sealed. Each nontriangular DX domain is tailed in a segment that can pair with the opposite segment on the other triangle by PX complementarity.

Figure 2 illustrates our test system for PX-based cohesion. We have chosen a system that has proved intractable to cohesion by the short sticky ends that can be produced by the restriction of a DNA hairpin.[14] This system consists of a pair of DNA triangles, one of whose edges is a double crossover (DX) molecule,[21] as used

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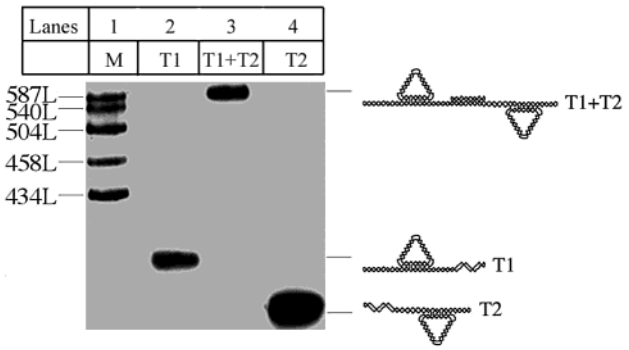

Figure 3. Test of paranemic cohesion. Autoradiogram of a 6% (19:1) nondenaturing polyacrylamide gel. The lane labeled M contains marker double helices from a *Hae*III digest of pBR322 (Sigma). The two triangles are in the lanes labeled T1 and T2. Their 1:1 PX cohesion product is in lane 3. The triangles are annealed separately from 90 to 37 °C as follows: 90 °C (5 min), 65 °C (30 min), 45 °C (30 min), 37 °C (30 min). Then they are mixed at 37 °C and annealed at 37 °C for another 30 min, followed by 1 h at room temperature and 1 h at 4 °C. The molecules were labeled and ligated as described previously,[13] except that ligation took place at room temperature in a buffer supplied by the vendor of the T4 DNA ligase (USB).

previously.[13] Except for the small central strand, the molecules are closed topologically. However, the long extensions on the extra helices are designed to be complementary in the PX sense discussed above.[22] The expectation is that the two triangles will cohere when introduced into the same solution under appropriate conditions.

The PX part of this system has been designed with six nucleotide pairs in the major groove. The sequences of the two triangles have been designed using the program SEQUIN,[23] which aims to minimize sequence symmetry. The strands have been synthesized by routine phosphoramidite procedures[24] and purified by denaturing gel electrophoresis. Individual strands have been fused to form the longer strands and catenated circles by enzymatic ligation. The triangles are different sizes (450 and 492 nucleotides), so that they can be differentiated on gels.

The two triangles have been purified on denaturing gels and reconstituted with the 20mer unclosed circle, in a solution consisting of 40 mM Tris-HCl (pH 8.0), 20 mM acetic acid, 2 mM EDTA, and 125 mM Mg acetate. The high concentration of Mg^{2+} was a parameter that was optimized.

Figure 3 illustrates a 6% nondenaturing gel containing the two components and their cohesive complex. The lanes labeled T1 and T2 contain the individual triangles, and the lane labeled T1+T2 contains their 1:1 stoichiometric mixture. It is clear that the two triangles have formed a 1:1 complex on the gel; on the basis of a log(MW) vs mobility plot, the MW of the complex contains one copy of each component (not shown). Note the single bands in the individual triangle lanes (T1 or T2) control for the possibility of nonspecific binding.

We have demonstrated that it is possible to combine large, topologically closed, DNA molecules through paranemic cohesion. These interlinked double helices offer a new tool for the cohesion of DNA nanostructures. We expect that this form of cohesion will have widespread applications in biotechnology, because the half-PX components are unlikely to form unwanted secondary structures in the way that single strands do. We have shown elsewhere that PX molecules are sufficiently stiff to form linear oligomeric constructs of DNA motifs.[7] Paranemic cohesion offers a new way to combine DNA molecules for purposes of self-assembly, molecular recognition, and nanosystems.

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Supporting Information Available: The sequences of the triangle molecules (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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