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A Self-Assembled DNA Bipyramid

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Nanometer-scale DNA polyhedra can be created by self-assembly protocols that exploit the specificity of base-pairing interactions. DNA polyhedra consist of 3- or 4-arm branch junctions[1] connected by rigid double-helical edges. The first examples, a cube and a truncated octahedron, were made by Seeman and co-workers using multistep assembly processes.[2] Shih and co-workers designed a DNA octahedron that was largely made from one strand.[3] A different design principle was introduced with the DNA tetrahedron:[4] Tetrahedra can be made rapidly and in high yield from multiple strands in a single assembly step. The efficient synthesis of DNA polyhedra is an enabling step toward their application as molecular cages[5] or as building blocks for three-dimensional nanofabrication.[4a] Herein we show that more complex, less symmetric polyhedra can also be created by one-step assembly: We demonstrate the formation of a trigonal bipyramid.

The structure of the DNA bipyramid is illustrated in Figure 1. Six strands of DNA (synthetic oligonucleotides) each run around one of the faces and hybridize to neighboring strands to form nine 20-basepair edges (two turns of the double helix, 7 nm). Single, unpaired adenosine nucleotides act as spacers, connecting neighboring edges at the vertices. Six of the nine edges contain nicks (breaks in the DNA backbone) where the 5′ and 3′ ends of one strand meet. Figure 1 shows the positions of the nicks in the bipyramid whose formation is reported below; other nick positions are also compatible with efficient assembly (Supporting Information Figure S1). The bipyramid contains three four-arm and two three-arm junctions; all its faces are triangles, so the structure is braced and is expected to be rigid.[4a]

The bipyramid is formed in a single annealing step. The six strands are combined in equimolar amounts, heated to 95 °C and cooled to room temperature over ∼15 min. Bipyramids are the main product of formation (a yield of ∼40% was achieved) and can easily be gel purified. Bipyramids can also be formed by incubation at room temperature, but the yield is reduced (see Supporting Information for details of synthesis and analysis of yield). Figure 2 shows polyacrylamide gel electrophoresis (PAGE) analysis of the products of the single-step annealing process. The first lane from the left in Figure 2a contains all products: a single high-intensity band corresponds to the bipyramid. Byproducts of lower mobility, which probably contain multiple copies of the constituent strands, can be removed by gel purification. The identity of the bipyramid was confirmed by investigating the topological relationships between component strands, as described below.

Fifteen different bipyramids were formed, corresponding to all possible ways of choosing two strands to be 5′-phosphorylated and four strands to be unphosphorylated. After cooling, samples were incubated with T4 DNA ligase (New England Biolabs) and then gel purified (see Supporting Information for details). DNA ligase will ligate (covalently join) a phosphorylated 5′ end to a 3′ end if the two free ends are held together by hybridization to a common "splint" strand: If the bipyramid forms as designed, the 5′ and 3′

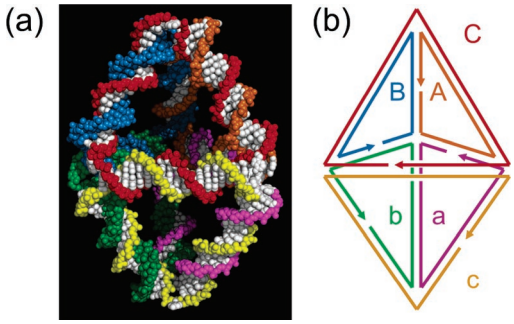

Figure 1. (a) Molecular model of a DNA trigonal bipyramid and (b) positions of the six constituent DNA 63-mers (a−c and A−C).

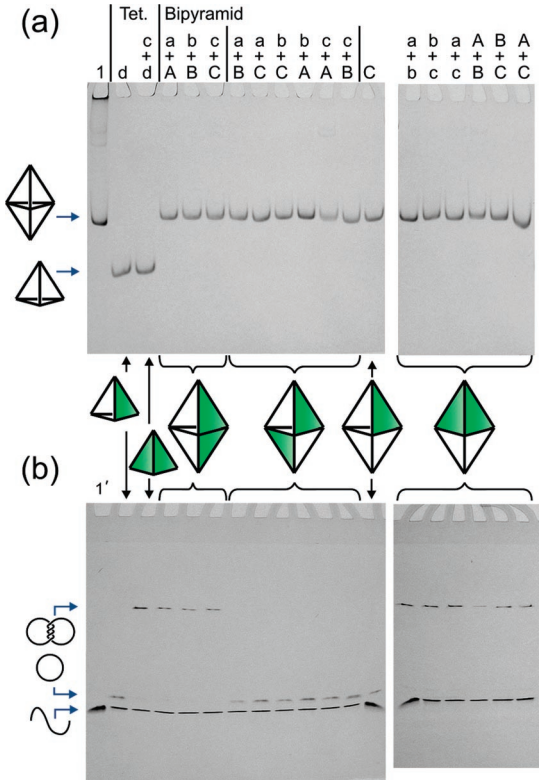

Figure 2. Analysis of the bipyramid by (a) native and (b) denaturing PAGE: Lane 1, unpurified products of bipyramid synthesis; lane 1′, (control) strand a. Subsequent lanes in both native and denaturing gels contain gel-purified tetrahedra (controls, see Supporting Information) and bipyramids with one or two of the constituent strands ligated (ligated strands are identified above each lane). Schematics indicate relationships between faces around which ligated strands are designed to run.

ends of each phosphorylated strand will be ligated to form a closed circle. If and only if the two phosphorylated strands run along a common edge of the bipyramid then the two circles will be linked (with linking number 2). Figure 2a shows that all 15 purified DNA

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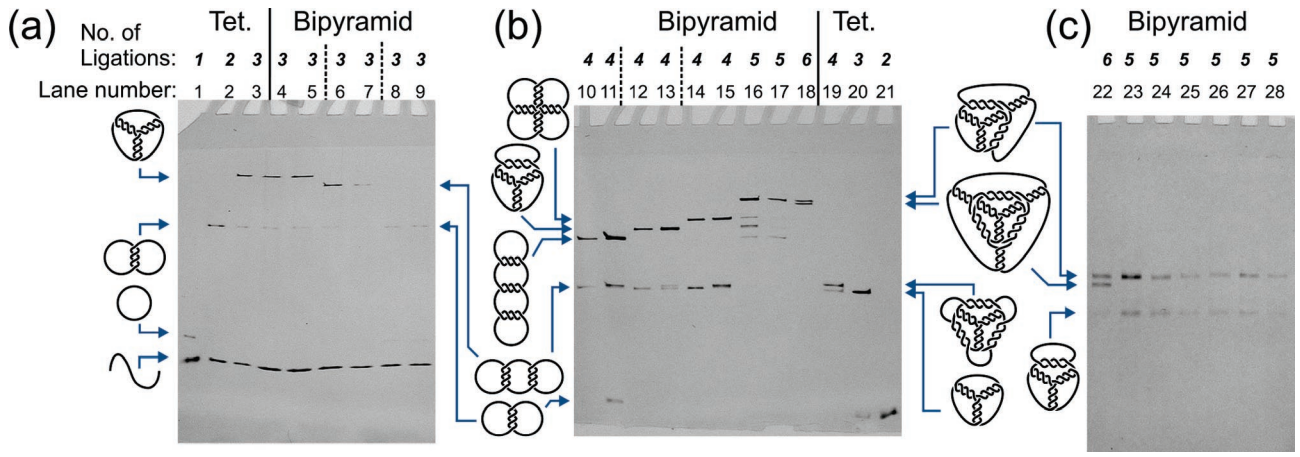

Figure 3. Denaturing PAGE analysis of bipyramids with more than two phosphorylated strands. Control lanes contain DNA tetrahedra (Tet.) in which the pattern of linkage between strands is known.[4a] All samples were incubated with T4 DNA ligase and purified from native polyacrylamide gels. The number of phosphate modifications and hence ligated nicks is given above the lane for each sample. Dashed vertical lines separate samples with the same number of linked circles but different patterns of linkage. Phosphorylated strands in the bipyramid samples are (lane 4) A,B,C; (lane 5) a,b,c; (lane 6) a,c,A; (lane 7) b,B,C; (lane 8) a, B, C; (lane 9) b, c, A; (lane 10) b,c,A,C; (lane 11) a,c,A,B; (lane 12) b,A,B,C; (lane 13) a,b,c,C; (lane 14) a,b,A,B; (lane 15) a,c,A,C; (lane 16) all but B; (lane 17) all but c; (lane 18 and lane 22) all; (lane 23) all but B; (lane 24) all but A; (lane 25) all but C; (lane 26) all but c; (lane 27) all but b; (lane 28) all but a.

bipyramids migrate with approximately the same mobility in a native gel. (Two control lanes contain purified 20-basepair DNA tetrahedra[4a] with one and two phosphorylated strands ligated, producing single circles and linked pairs of circles, respectively.) Figure 2b shows the same samples on denaturing gels in which the base pairing between DNA strands is disrupted. Bands corresponding to unligated strands, single closed circles and linked pairs of circles can be identified by comparison with control lane 1′ and the partially ligated tetrahedron controls, respectively. As expected, ligation of any of the nine pairs of strands that are designed to share an edge leads to the formation of linked circles and ligation of the six pairs of strands which are designed not to share an edge leads to unlinked circles. This result confirms that the topological relationship between each pair of strands is as designed.

Ligation of more than two strands creates more complex catenanes. Within the bipyramid there are examples of three different ways to link (or not link) three circles and three ways to link four circles. We have created each of these linkage patterns twice, by ligating different subsets of strands that are expected to produce the same pattern. Figure 3a,b shows denaturing PAGE analysis of these bipyramids, which were purified from a native gel. (Faster bands correspond to a smaller number of linked circles created by ligation failures.) Catenanes containing the same number of circles differently linked have different mobilities, but catenanes produced by ligation of different subsets of strands that are designed to have the same pattern of linkage have the same mobility, as expected.

Figure 3c is a low-percentage denaturing polyacrylamide gel that demonstrates the small mobility difference between the bipyramid in which all six nicks have been ligated and the six possible constructs with any one of the six strands not ligated. Six interlinked circles have a higher mobility than five. Only the fully ligated construct survives digestion by exonuclease III (Supporting Information Figure S3): This confirms that the bipyramid incorporates all six strands.

In summary, we have demonstrated that a trigonal bipyramid with 20-basepair edges can be formed in high yield in a single self-assembly step from six DNA strands. This shows that the assembly scheme developed for DNA tetrahedra can be extended to larger polyhedra. It will be fascinating to explore the possibilities and limitations of single-step assembly of multiple oligonucleotides further, to establish design rules for robust assembly in three dimensions and to enable applications as molecular building blocks and cages.

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Supporting Information Available: Materials and methods; bipyramid formation with different nick positions and at different temperatures, results of exonuclease digestion. This material is available free of charge via the Internet at http://pubs.acs.org.

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