DNA Nanotechnology

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Three-Dimensional Nanoconstruction with DNA**

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DNA structures · nanostructures · nanotechnology · self-assembly

When nanotechnology visionaries generated computer models of geometric objects composed of atoms and molecules some 15 years ago, only few people could believe that there would ever be a "technology" capable of molecular nanoconstruction. Even though original proposals of mechanical "nanoassemblers" have not been realized so far, some of the goals of nanotechnology have now almost been achieved—with the help of self-assembling DNA molecules.[1] In a series of recent breakthroughs it was shown that DNA can be used to produce not only two-dimensional patterns of essentially arbitrary shape,[2,3] but also mechanically stable three-dimensional nanoobjects.[4–9]

The field of DNA nanotechnology was founded by Nadrian Seeman in the early 1980s. Already in his seminal proposal in 1982,[10] he envisioned three-dimensional molecular construction with DNA. However, early attempts to produce 3D structures[11] were hampered by the lack of rigidity of the DNA building blocks. A number of important conceptual advances have now resulted in rigid assemblies and also provided more efficient assembly strategies with an impressively high yield of correctly formed structures.

Programmable self-assembly with DNA molecules is based on the principle that two single strands of DNA bind together only when their base sequences are complementary. On the nanometer-length scale, double-stranded DNA is a relatively rigid, linear molecule, whereas single-stranded DNA is comparatively flexible. So, in principle, by choosing the right sequences, one can "program" the interactions between DNA molecules and use them to generate molecular networks of rigid and flexible elements. To achieve long-range two-dimensional or even three-dimensional order, however, simple components such as DNA duplexes or Holliday crossover structures were found to be too flexible. This led to the development of inherently rigid building blocks for DNA nanoconstruction such as the double- or multiple-crossover motifs,[12] which are composed of several DNA duplexes woven together by shared strands. One such construction is a bundle of six DNA helices, in which adjacent helices are connected through Holliday junctions and three neighboring helices form an angle of 120°.[5] Another way to impose rigidity on a structure is to rely on triangular building blocks such as in 2D tensegrity assemblies,[13] in tetrahedra,[14] octahedra,[4] or bipyramids.[15]

Apart from such improvements in "architecture", major advances in the assembly procedure itself have been accomplished. Traditionally, DNA-based nanostructures self-assemble from a number of oligonucleotides with carefully chosen base sequences. The oligonucleotides are mixed in buffer solution and annealed by heating the solution to a high temperature (around 95 °C) and slowly letting it cool to room temperature. During the cooling process—depending on the melting temperature of the individual components of the structure—complementary sequences hybridize with each other. To obtain large and defect-free structures, all oligonucleotides must be present in exact equimolar stoichiometry, and annealing must be performed over a long period of time (up to several days).

A few years ago, a radically different approach towards DNA assembly was developed by Rothemund[3] (and previously proposed by Yan et al.[16]), which is based on folding a single, long "scaffold" strand into a desired shape with the help of multiple "staple" strands. In contrast to the traditional assembly approach, there is no need to observe strict stoichiometric ratios, and assembly occurs quite quickly and with an extraordinary yield. This is because after initial attachment of the staple strands to the scaffold, folding occurs locally within the scaffold. When a large excess of staple strands are used, mismatches in the assembly are "healed" by strand displacement. The scaffolded origami technique was recently employed by Shih and co-workers[7] to fold a long scaffold strand derived from bacteriophage M13 into six-helix bundles with a length of 410 nm (Figure 1).

Related to scaffolded origami is an assembly technique based on intramolecular folding of a long single strand.[4] Shih and colleagues created a 1669 nt long strand, which—in the presence of five additional 40 nt long helper strands—intramolecularly hybridized with itself to form the backbone of an "unfolded" octahedron. The branched intermediate structure could then fold intramolecularly into a three-dimensional octahedral structure by paranemic cohesion.[17] Here formation of a 3D structure with high yield was favored by utilizing intramolecular folding. As a "deltahedron", the octahedron is composed of intrinsically stiff triangular building blocks, which ensure mechanical stability of the product.

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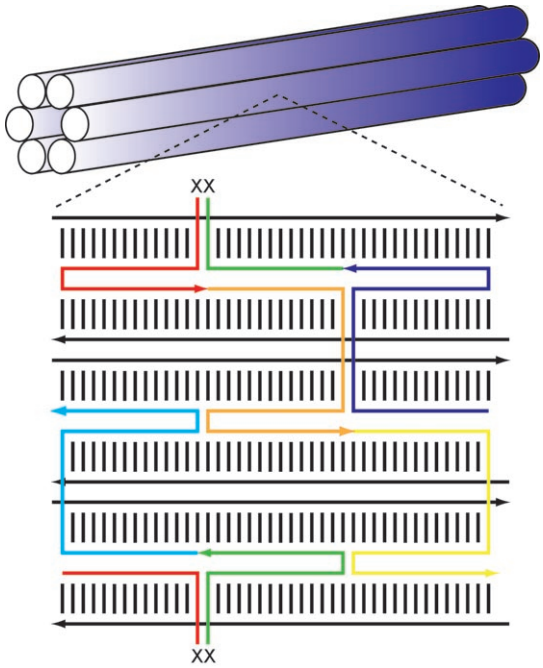

Figure 1. Origami assembly of six-helix bundles 410 nm in length (not drawn to scale).[7] In DNA origami, one long single strand derived from the genome of a phage is "folded" into the desired shape by a large number of "staple strands". The helix bundle is composed of six DNA helices connected by crossover junctions. The distance between junctions is chosen such that the angle formed by three neighboring helices is 120°. Shown is a schematic depiction of the basic structural motif, which is repeated 28 times along the axis of the bundle. The viral template strand is shown in black. Six 42 nt staple strands per unit are needed for assembly (shown in color), resulting in a total of 168 staples for the whole structure. Strands in the uppermost and lowermost helix are connected as indicated by the "x".

An alternative strategy towards efficient assembly of DNA objects is the "hierarchical" assembly method. Here, the DNA strands composing the object form a prestructured complex at high temperatures, which intramolecularly folds into the target structure at a lower temperature. The length and sequences of the different parts of the structure define the "hierarchy" of structure formation. Longer complementary sequences form stable complexes at higher temperatures than shorter ones do. As in the origami technique, the final structure is formed in a first-order process through local interactions. Turberfield and co-workers utilized this strategy to produce nanoscale DNA tetrahedra.[6] The structures were formed from four strands of DNA in a rapid assembly step with a yield of 95% (Figure 2). The mechanical rigidity of these structures was proven in AFM experiments.

A different method to control connectivity in DNA-based assemblies was introduced by von Kiedrowski and co-workers a few years ago.[18] They synthesized trisoligonucleotides in which three strands of DNA are covalently connected to an organic linker molecule with a threefold symmetry axis. Using 20 distinct trisoligonucleotides as vertices, they recently succeeded in assembling mechanically stable DNA-based molecular dodecahedra.[9]

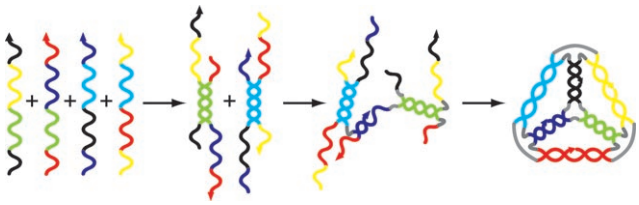

Figure 2. Hierarchical assembly of a tetrahedron.[6] The tetrahedron is formed by four strands. First, two strands associate with each other over a length of 20 bp. As soon as two such intermediates bind together through the remaining unpaired strands, the target structure assembles rapidly by intracomplex interactions. The result can be ligated to form a covalently closed, stable structure.

As in the last example, typically the assembly of more complex structures requires a larger number of DNA strands with distinct base sequences. The sequences must be chosen carefully to avoid unwanted interactions between the strands, and this makes sequence design challenging. Recently, Mao and co-workers could show that the requirement of unique sequences and interactions can be relaxed in some cases.[19] Starting with a DNA crossbar motif developed by Yan et al.,[20] which is composed of nine distinct strands of DNA (Figure 3a), they developed a related structure, which can be formed by only three types of DNA strands—but now with symmetric sequences (Figure 3b).

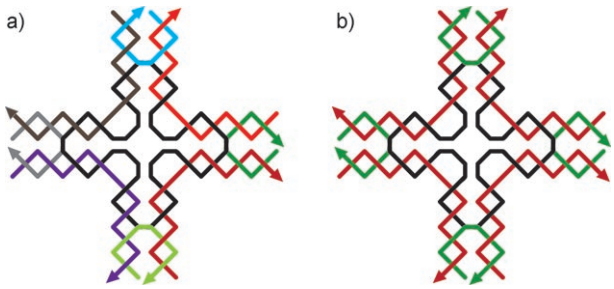

Figure 3. Sequence symmetry reduces the complexity of assemblies. a) A DNA crossbar structure composed of four Holliday junctions, which are formed by nine strands of DNA with distinct sequences.[20] b) A crossbar structure that is formed by nine DNA strands with symmetric sequences. The number of strands with distinct base sequences is reduced to three.[19]

When equipped with sticky ends, DNA crossbar structures can be used to assemble large two-dimensional lattices. Sequence symmetry results in a number of advantageous features: Apart from simpler sequence design and less synthesis effort, larger structures with less assembly errors can be formed. For a smaller number of strands it is easier to maintain exact stoichiometry. In addition, the high symmetry of the crossbar tile results in reduced stress in the assembly, favoring the formation of very large structures.

In a recent breakthrough, Mao et al. combined several of the concepts introduced above.[8] Molecular tetrahedra, dodecahedra, and bucky balls were built from intrinsically stiff crossover structures with sequence symmetry. Assembly was accomplished using a hierarchical strategy. The DNA polyhedra were all constructed from a three-point star motif

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that had previously been used for the assembly of 2D crystals.[21] From these studies it was known that the star structure possesses a considerable intrinsic curvature. For two-dimensional assembly the curvature had to be compensated by arranging neighboring units such that they faced opposite sides of the assembly. For 3D assembly, the units were now arranged to face in the same direction in order to sum up the curvature rather than to compensate for it. To adjust the flexibility of the vertices, single-stranded loops were introduced into the three-point star (see Figure 4). In

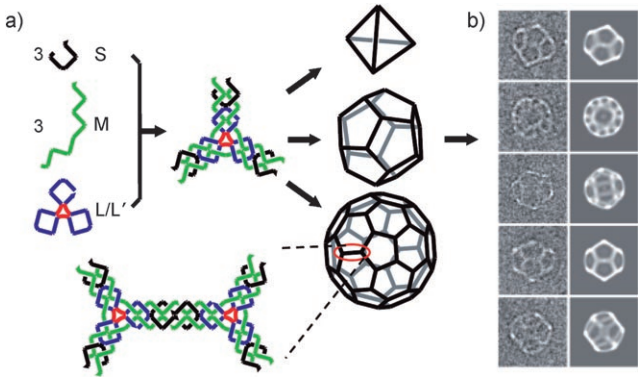

Figure 4. Hierarchical assembly of DNA-based 3D objects.[8] a) A sequence-symmetric three-point-star unit was assembled from three copies of strand M, three copies of strand S, and one central strand L. The central strand contains a single-stranded loop (red) of length 3 nt or 5 nt, which provides flexibility. Depending on loop length and total DNA concentration, the formation of tetrahedra (90% yield), dodecahedra (76% yield), or bucky balls (69% yield) is favored. b) Cryo-electron microscopic images of DNA dodecahedra and corresponding projections expected from this structure. The figure is composed of Figure 1 and Figure 3 d of Ref. [8] (Copyright: Nature Publishing Group; reproduced with kind permission from the authors).

contrast to the conditions for the assembly of 2D crystals, the concentrations of the DNA strands were reduced in order to favor intracomplex interactions. Depending on the loop length and the total concentration of the DNA strands, the different polyhedra were formed in high yield. Tetrahedra formed from four three-point star units with a more flexible 5 nt loop. Dodecahedra formed from 20 star units with a 3 nt loop at a low concentrations, whereas at higher concentrations, 60 of these units assembled into "bucky balls". The polyhedra assembled by Mao et al. are considerably larger in size than the tetrahedra discussed above. The diameters of the tetrahedra, dodecahedra and bucky balls are 20, 50, and 80 nm, respectively.

As summarized in the preceding paragraphs, three-dimensional nanoconstruction based on DNA molecules has made huge advances in recent years. More mechanically robust structural motifs have been introduced, and novel assembly strategies have been developed. With these, a variety of rigid molecular objects have now been constructed. The powerful DNA origami technique allows for the construction of arbitrarily shaped objects; however, this comes at the cost of a large synthesis effort. Typically, hundreds of DNA "staple" strands have to be used for origami assembly. In contrast, symmetric assemblies such as those developed by Mao et al. require very only few distinct DNA sequences. Depending on the structural complexity required for an application, one can choose between these two complementary strategies.

A variety of applications have been envisioned for DNA-based 3D assemblies. Already Seeman's original proposal[10] aimed at the realization of three-dimensonal networks for the arrangement of nanoscale objects. Arranging proteins in such an artificial crystal could be very useful for structural studies. In fact, Shih and colleagues already utilized their origami-based six-helix bundles for NMR structure determination. Liquid-crystalline matrices formed by the DNA bundles were used to induce alignment of membrane proteins, which helped to improve measurements of their NMR residual dipolar couplings.[7]

A different area of applications lies in the construction of "nanocontainers", which might be used for storage and controlled release of molecules or nanoscale objects. Turberfield and colleagues were able to show that a protein could be placed into the cavity of a DNA tetrahedron,[22] and they recently also demonstrated a switchable version of this nanocontainer.[23]

Another fascinating possibility would be the construction of self-assembled 3D electronic networks. So far, however, long-range three-dimensional order based on DNA assemblies has not been realized. With the novel architectures and techniques highlighted in this article, this might be just a matter of time.

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