DNA Nanotechnology

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Evolution of DNA Origami

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DNA can be used in nanotechnology to connect or functionalize different nanostructures, such as gold nanoparticles,[1] quantum dots,[2] and single-walled carbon nanotubes.[3] Moreover, it can be used to build nanostructures composed exclusively of DNA.[4] Research in this area, pioneered by Seeman,[5] has afforded two-dimensional[6] (2D) and three-dimensional[7] (3D) structures generated from multiple short strands of DNA. These applications rely on the self-assembly (directed by base complementarity) of DNA strands and the different structural characteristics of single- and double-stranded DNA. Single-stranded DNA is flexible and can be bent easily. On the other hand, short sequences of double-stranded DNA are rigid and straight. On the basis of these properties, short sequences of DNA have been designed to self-assemble into rigid structures under appropriate annealing conditions.

Another approach for the construction of 2D structures is known as DNA origami, whereby DNA is folded in a controlled manner into almost any shape.[8] This method requires a long scaffold of single-stranded DNA, which is folded, and several short sequences that function as "staples" to force the long DNA strand to fold into the desired shape. The "staple" DNA sequences can be designed to bind several regions of the DNA scaffold on the basis of base complementarity. In this way, multiple double-stranded DNA sites are generated, which leads to the desired 2D rigid structure (Figure 1). Several shapes have been created by this approach, including rectangles, stars, and smiley faces. Other significant contributions in this area include the preparation of nanotubes,[9] microarray chips,[10] and dolphin-shaped DNA structures.[11]

The Shih research group at Harvard recently reported two breakthroughs related to DNA origami. They were able not only to build 3D shapes[12] on the basis of the origami strategy, but also to twist and bend DNA bundles with excellent control.[13]

The construction of DNA origami had been limited to two dimensions until recently, when the first three-dimensional DNA-origami-based structures were described;[9,12,14] the construction of a DNA box is an outstanding example.[14] Shih and co-workers[12] have now described a versatile method for the creation of different 3D shapes. The key to obtaining these structures is the use of "staple" strands designed to form Holliday junctions at specific positions. Once the 2D origami is generated by the "staple" sequences, it is forced to refold by Holliday junction formation to give honeycomb-like DNA 3D structures. The location of these Holliday junctions determines the final 3D architecture. When these junction sites are placed at seven-base-pair (bp) intervals along the double-stranded DNA, three junction sites exist in a 21 bp stretch of the DNA. In this case, since a complete turn (360°) in a DNA duplex in the B form requires 10.5 bp, the junctions are separated by 120° in the same helix. As a consequence, this arrangement enables the formation of Holliday junctions with three adjacent DNA duplexes (Figure 2).

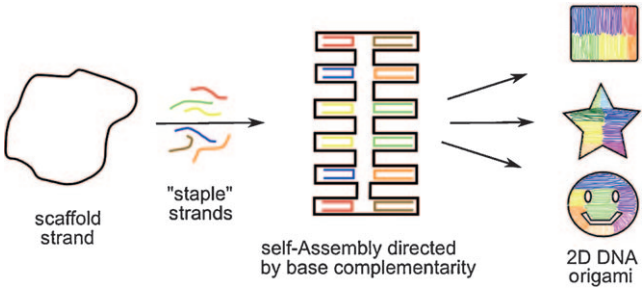

Figure 1. Preparation of DNA origami. Annealing of the long scaffold strand and the "staple" strands gives rise to a self-assembled structure as a result of the favorable interaction between complementary sequences. A variety of shapes can be obtained depending on the design of the sequences.

By deleting or introducing these junctions at specific positions, Shih and co-workers were able to build several 3D structures, such as a monolith, a square nut, a railed bridge, and a slotted cross. The design of a 3D structure on the basis of this method may appear complicated; however, this research group has developed a program that enables the design of a complex structure in one day.[15]

Even more notable is the development by the Shih research group of a way to bend the previously mentioned honeycomb DNA structures to build twisted and curved nanoscale 3D systems.[13] This achievement is significant, since double-stranded DNA forms a stable, straight structure. For this reason, the methods previously reported were mostly limited to the construction of architectures with straight lines;

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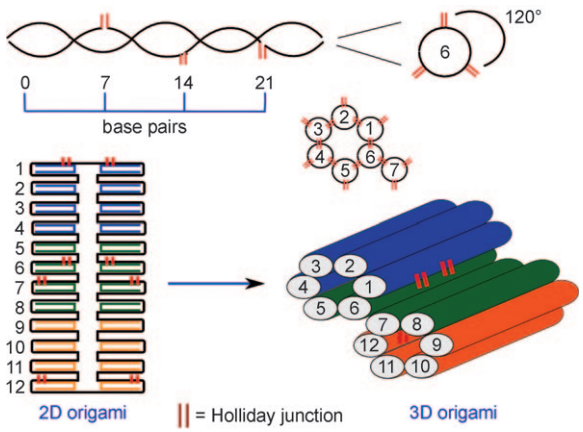

Figure 2. Design of 3D DNA origami, the assembly of which is directed by Holliday junction formation. Since the helical path of the strand rotates 240° every 7 bp, 14 bp gives rise to a rotation of 120° plus 360°, and 21 bp to a rotation of 0° plus two times 360°. Therefore, the Holliday junctions are separated by 120°, which enables the interaction of a DNA duplex with three other DNA duplexes.

curves were not common.[6e,7c] However, in this new study, Shih and co-workers were able to create curves with control of both the direction of the twist and the angle of the bend.

For the construction of these twisted and curved systems, Shih and co-workers designed the honeycomb in the same way as previously, but they changed the length of the "staple" sequences. When a separation of less than 7 bp between Holliday junctions was used (deletion), the structure was forced to shrink, which led to an overtwisted DNA structure. On the other hand, when a separation of more than 7 bp between Holliday junctions was used (insertion), the unit generated had to expand to fit into the DNA array, and undertwisted DNA was formed. As a result of these modifications, the honeycomb system had to change its structure to compensate the forces generated; in this way, the corresponding twisted or curved shapes were created (Figure 3).

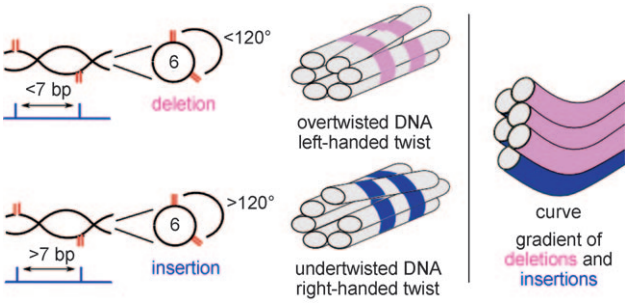

Figure 3. Introduction of twists and curves in a DNA bundle by changing the separation between Holliday junctions. For details see the text.

To study the global twisting of these systems, a model of 60 interconnected DNA duplexes arranged in 10 rows with 6 helices per row was used. Three versions of this model were prepared: A system designed not to twist had the Holliday junctions evenly placed every 7 bp along the 126 bp of the DNA duplexes. In a second version, one base pair was deleted from every third array. In this case, the system contained overtwisted DNA fragments in one third of all the array cells and had a length of 120 bp with an average twist density of 10 bp per turn. The opposite version had an additional base pair in every third array, which gave rise to a structure with undertwisted DNA fragments, a length of 132 bp, and a twist density of 11 bp per turn. Each individual group of DNA arrays was polymerized to give a bigger structure, which could be analyzed by transmission electron microscopy. In the first case, the resulting ribbons were straight with no detectable global twist. In contrast, the systems with over- and under-twisted DNA fragments formed ribbons that clearly twisted, with a global left- and right-handed twist, respectively.

The curved structures were obtained by using balanced gradients of insertions and deletions according to the relationship: the higher the gradient, the bigger the bend angle. By this approach, seven versions of a three-row, six-helix-per-row (3-by-6) bundle were obtained with different bend angles: 0°, 30°, 60°, 90°, 120°, 150°, and 180°.

Finally, the great utility of this approach for the creation of bent structures was illustrated with the construction of nanostructures with different shapes (Figure 4). For example,

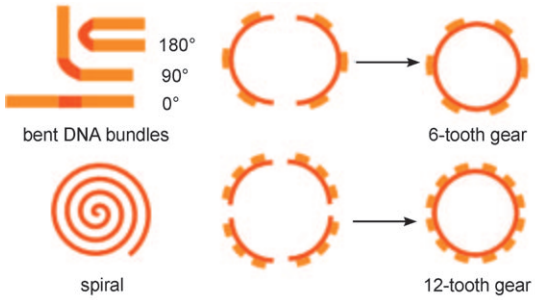

Figure 4. Bending of DNA bundles to give different angles and shapes.

Shih and co-workers prepared two semicircles with three "teeth" and a 25 nm radius that could assemble into a circular structure with six "teeth". Moreover, they were able to tune the DNA bundle to fold into a quarter circle with a 50 nm radius; they then connected four of these bundles to form a circular structure that resembled a gear with 12 teeth. Another interesting example is the preparation of a spiral: each of six segments of a six-helix bundle was programmed to bend into a half circle with increasing radii of curvature. An octahedral structure was also reported, as well as concave and convex triangles.

In summary, the Shih research group has made remarkable contributions to the evolution of DNA origami, in particular in the use of Holliday junctions to fold the 2D origami into 3D shapes, which can be designed with a software program. What is more, they were able to control the bending and twisting of the honeycomb structures in a precise manner. These results provide an opportunity to develop more-complex nanostructures and to investigate the

physical properties of these bent DNA structures, which could one day be key components of nanodevices.

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