

Chapter 4

Tile-Based DNA Nano-assemblies

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Abstract Tile-based DNA nanostructures have played an important role in the evolution of the field of structural DNA nanotechnology over the last three decades. These structures are formed using multiple short synthetic oligonucleotide strands that are specifically designed to self-assemble into desired configurations. Successful assembly of a variety of planar DNA tiles has been demonstrated including crossover tiles, multi-armed tiles, weave tiles, etc. These tiles have then been used to form one- and two-dimensional arrays or lattices. In addition, three-dimensional tile-based structures have been developed using helix bundle tubes, finite-sized closed polyhedral structures, and a three-dimensional crystalline lattice. This chapter chronicles the progress thus far in the design and synthesis of tile-based DNA nano-assemblies. Furthermore, it details the steps involved in the successful design, synthesis, and characterization of such structures. It also reviews the applications of these tiles in molecular computation and in programmed assembly of other nanoscale materials such as proteins, metallic nanoparticles, and aptamers.

Contents

4.1	Genesis of DNA Tiles	72
4.2	Crossover Tiles with Parallel Helices	73
4.3	Multi-armed Tiles	74
4.4	Helix Bundle Tiles	75
4.5	Noncanonical Tiles	76
4.6	Finite-Sized 3D Structures	77
4.7	Three-Dimensional Lattices Using DNA Tiles	79
4.8	Design of DNA Tiles	80
4.9	Experimental Synthesis of DNA Tiles	82

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4.10	Characterization of DNA Tiles and Lattices	84
4.11	Applications of DNA Tiles	85
4.12	Conclusion and Future Directions	88
	References	89

4.1 Genesis of DNA Tiles

Nucleic acid strands can be designed such that they hybridize with their complementary sequences and thus fold and assemble into well-defined secondary structures, building blocks, and lattices. This capacity for programmed molecular recognition makes DNA a versatile material with which to design and build nanometer-scale structures. Normal, double-helical DNA is a linear, antiparallel, and unbranched complex of two molecules. It is, however, possible to make DNA structures containing branched junctions; that is, the constituent molecules are linear (i.e., unbranched) but the complex displays a branched topology. For example, a four-arm junction can be made using four individual DNA strands which are complementary to each other in the correct pattern. Using Watson–Crick complementarity, portions of the strands base pair with one another to form two domains of duplex DNA that are linked at one crossover point by exchange of two of the four strands. Such a four-arm junction is an immobile form of the Holliday junction, which is a biologically important structure that exists in cells during homologous recombination (Holliday 1964; Seeman 1982). Topological branch junction structures are an effective strategy for creating multivalent complexes from the normally bivalent and linear DNA double helix. Branched DNA structures have been used to create a wide variety of DNA complexes with useful properties.

In 1982, Nadrian Seeman was the first to propose that DNA building blocks containing branch junctions could be used to construct ordered arrays (Seeman 1982). His vision for this new area of research was to assemble DNA into three-dimensional crystalline lattices to scaffold biological macromolecules, nanodevices, and nanoelectronic components within periodic arrays. After initial experiments involving synthesis of four-arm junctions (Kallenbach et al. 1983; Petrillo et al. 1988) and development of design theories regarding formation of DNA nanostructures (Seeman and Kallenbach 1983), Seeman’s group successfully demonstrated the formation of a quadrilateral shape from DNA branched junctions (Chen et al. 1989) followed by five- and six-arm junctions (Wang et al. 1991) and, much later in 2007, of 8- and 12-arm junctions (Wang and Seeman 2007).

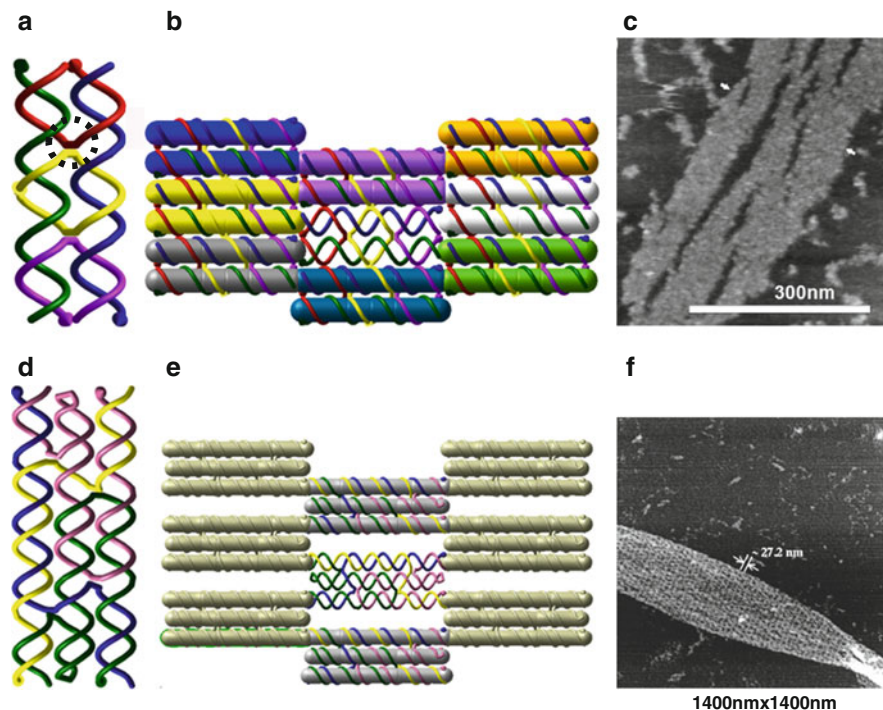


Fig. 4.1 (a) A double crossover (DX) tile (shown with vertical helix axes). The two helices are held parallel by crossover junctions (*circled*). (b) Sticky ends of a double crossover tile can be suitably programmed to form two-dimensional lattices, shown here with horizontal helix axes. The adjacent tiles are identical but distinctly colored for clarity. (c) AFM image of the DX tile array. (d) A triple-crossover (TX) tile, which is a planar tile with three parallel helices held together by crossovers. (e) Two sets of TX tiles, identified by *color*, appropriately equipped with sticky ends will form two-dimensional arrays as demonstrated in the AFM image in (f) (Fu and Seeman 1993; Winfree et al. 1998; LaBean et al. 2000)

4.2 Crossover Tiles with Parallel Helices

In 1993, it was shown for the first time that DNA strands could be designed such that, when annealed, they formed a structure in which the axes of the DNA duplexes were constrained to be parallel and coplanar (Fu and Seeman 1993). This complex is called a double crossover tile (DX tile or simply DX), because it contains two strand-exchange crossover points linking two double helices (Fig. 4.1a). The main advantage of this design is that the junctions are constrained to a single orientation as opposed to being flexible as observed in the four-arm junction. In such a structure, two strands typically “cross over” from one duplex to the neighboring duplex to hold them together. Such *crossover* motifs, which are the same as immobile Holliday junctions, have since become an integral part of DNA nanostructures (Fig. 4.1a), and joining them together in novel ways with DX-like pairings have generated a large diversity of tile types.

Characterization of DNA nanostructures by atomic force microscopy (AFM) was published for the first time in 1998 (Winfrey et al. 1998). Micrometer scale two-dimensional lattices were constructed by equipping DX tiles with sticky ends (Fig. 4.1a–c). This strategy immediately paved the way for the use of DNA as building material for wide ranging nanoscale self-assemblies. Once the success of the strategy was established, research in this direction quickly gained momentum. A number of different structures were reported in the next few years most notably the DNA triple-crossover tile (TX tile), which is a planar tile containing three axially parallel DNA double helices (LaBean et al. 2000) (Fig. 4.1d–f). Additional tile designs include PX (paranemic crossover) and JX_2 (juxtaposed) tiles (Shen et al. 2004). In PX tile, the two helices on one side of the central junction are rotated about a half-turn from their positions when compared to the JX_2 tile. Following this, a few bulkier tiles were also prototyped, including double–double crossover tile (DDX), also known as the quad-crossover (QX), consisting of four parallel DNA double helices (Reishus et al. 2005) and tiles containing 8 and 12 helices (all with parallel and coplanar helix axes) (Ke et al. 2006).

4.3 Multi-armed Tiles

In 1999, Seeman’s group designed a two-dimensional DNA lattice from Holliday junction analogues containing two helical domains twisted relative to each other to mimic the approximately 60° angle observed in crystals of individual junctions. Four such junctions were fused to form a rhombus-like tile structure (Mao et al. 1999a). These rhomboid structures were then self-assembled into two-dimensional periodic arrays. This assembly distinguished itself from the assembly of crossover tiles as the lattice growth was achieved in both x and y directions independently (helix stacking in both dimensions) as opposed to the asymmetric growth of the previous lattices (helix stacking only in a single dimension). This concept was further developed when Yan et al. used crossovers in each arm of a four-arm building block giving rise to a cross-tile, also known as the 4×4 tile (Yan et al. 2003a), which also had the characteristic of helix stacking in both x and y directions (Fig. 4.2a). Equipping the tile with sticky ends on both helices in each of the four arms resulted in a beautiful two-dimensional periodic lattice with square aspect ratio (Fig. 4.2b, c). The original 4×4 tile design had some inherent curvature within the tiles, but the effect of this on the overall curvature of the 2D lattice was eliminated by using a corrugation strategy (which involved flipping adjacent tiles).

Mao et al. constructed three-, four- and six-point stars using the same overall design principle but with the added characteristic of symmetric arms (i.e., identical nucleotide sequences on each of the multiple tile arms) (He et al. 2005a, b, 2006). Since the sequences of each arm were identical, this entailed a significant reduction in the number of different DNA strands required per tile type. Moreover, it also ensured that any possible geometric distortions and asymmetric, sequence-specific

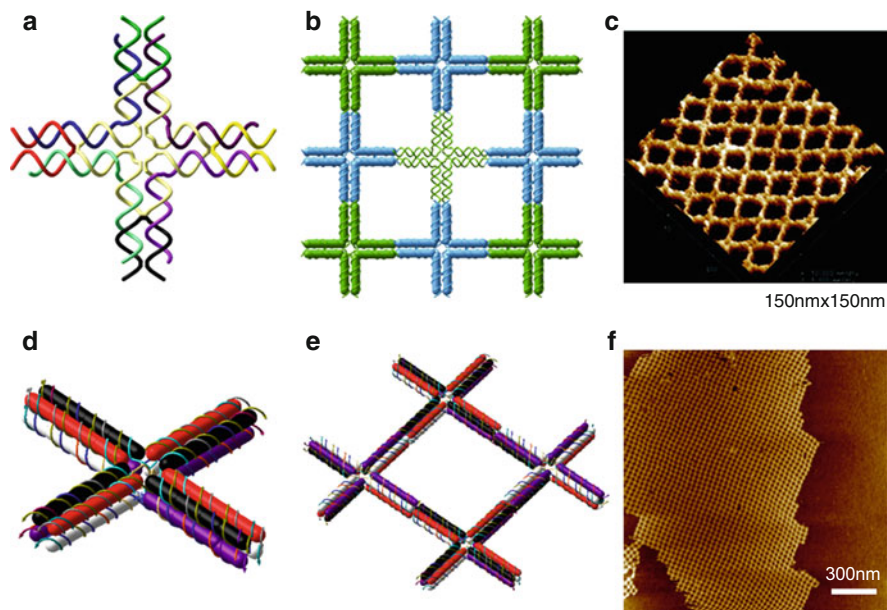


Fig. 4.2 (a) 4×4 tile with sticky ends. (b) Two-dimensional lattice formed by using corrugation strategy with 4×4 tiles. Adjacent tiles are flipped such that the same-colored tiles face same direction in the array. (c) AFM image of the 4×4 tile lattice. (d) Double-decker tile with sticky ends. All four arms are symmetric. (e) Two-dimensional lattice formed by using corrugation strategy with double-decker tiles. (f) AFM image of the double-decker lattice with several micrometer in each dimension (Yan et al. 2003a; Majumder et al. 2011)

curvature in the tile would be cancelled out. By adopting sequence symmetry in the cross-tile, it was possible to obtain much larger lattices compared to the asymmetric tiles with edge lengths on the millimeter scale (He et al. 2005b). Double-decker tile is another example of a cross-tile (Majumder et al. 2011). It comprised of two 4×4 tiles, lying one on top of the other and linked by two crossovers in each arm perpendicular to the plane of the tile (Fig. 4.2d). The four arms of the double-decker tile were also symmetric, and large two-dimensional lattices of tens of micrometers in size were observed when the corrugation strategy was employed (Fig. 4.2e, f).

4.4 Helix Bundle Tiles

DNA helix bundles constitute another category of DNA tiles. In these tiles, multiple double helices are aligned using crossovers so that they are axially parallel (but not coplanar), and they are typically closed around their circumference. Using this strategy, three-, four- and six-helix bundles have been created (Park et al. 2005;

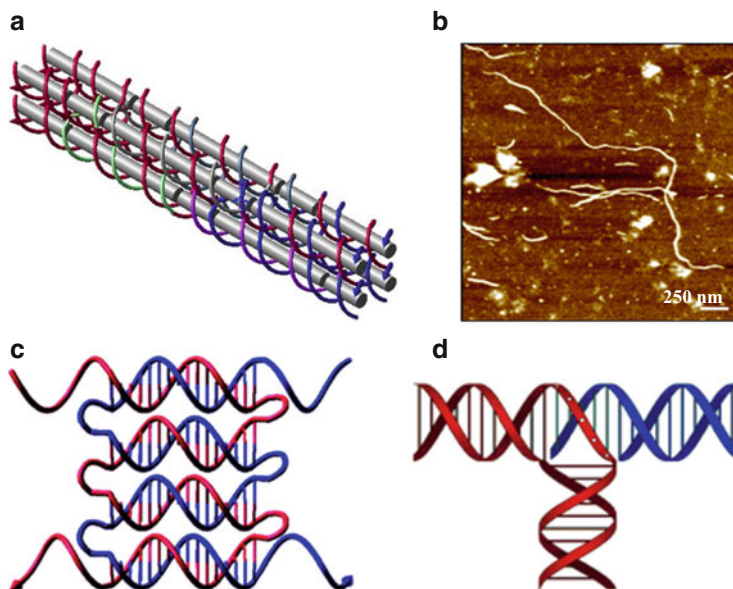


Fig. 4.3 (a) DNA four-helix bundle. (b) One-dimensional filaments constructed with the four-helix bundle. (c) Four-helix weave tile. Adjacent helices are linked via flexible T_4 loops. (d) T-junction. Weave tile and T-junction are examples of noncanonical tiles (Rangnekar et al. 2011; Hansen et al. 2010; Hamada and Murata 2009)

Rangnekar et al. 2011; Mathieu et al. 2005). The formation of helix bundle tiles added a new dimension to the field of DNA nanotechnology. When such bundles were attached along their axes using sticky ends, they formed uniform filaments micrometers or tens of micrometers long. By suitable programming of the sticky ends, formation of regular two-dimensional lattices using these bundle tiles was also shown. Figure 4.3a, b illustrates four-helix bundle tile and one-dimensional arrays formed using it (Rangnekar et al. 2011). Later, Seeman's group demonstrated the formation of six- and eight-helix bundles using half-bundle tiles (Kuzuya et al. 2007). They also designed a tile consisting of a six-helix bundle sheath that encompassed a central DNA double helix (Wang et al. 2009).

4.5 Noncanonical Tiles

The presence of multiple crossovers connecting parallel double helices has been repeatedly shown to provide stability and rigidity to tile-based DNA nanostructures. A new building block design, known as the weave tile strategy, was recently reported (Hansen et al. 2010). In this strategy, only two DNA strands are used per tile, rather than the four to nine strands used in the previously discussed tiles. The sequences were designed such that complementary regions weave back and forth to

mimic the strand routing of origami architectures (see Chap. 5), thereby forming a weave-like tile with rigid double-helical domains tethered to neighboring domains by flexible single-stranded T_4 loops (Fig. 4.3c). This strategy avoids the use of crossover junctions, thereby providing greater structural flexibility to the DNA nanostructure without compromising overall stability of the constructs. While weave tiles were only able to assemble into small, somewhat faulty lattices, they were shown to serve as aptamer-display platforms for designing highly effective enzyme inhibitors with substantial anticoagulant activity in blood plasma clotting assays (Rangnekar et al. 2012).

Weave tiles and Holliday junction-based tiles have design limitations affecting the available shapes of DNA nanostructures which are mainly typified by parallel-packed duplexes. However, another DNA branch junction structure known as T-junction motif provides a right angle geometry at the branch point between the connected duplexes (Hamada and Murata 2009). One junction consists of two DNA duplexes. One duplex has a sticky end at the terminus which is complementary to a single-stranded bulge section in the center of the second duplex. After hybridization, the first duplex is inserted into the bulge via its sticky end causing the second duplex to bend at about a 90° angle, thus resulting in an interconnection comprising a branched T-shaped junction (Fig. 4.3d). These T-motifs were used to build nanostructures displaying several different two-dimensional architectures. Rigid triangles from flexible DNA four-arm junctions were also designed using a tensegrity strategy and were then assembled into one- and two-dimensional arrays (Liu et al. 2004).

Recently, Yin et al. (2008) synthesized filaments analogous to DNA helix bundle tiles with monodisperse and programmable circumference. They synthesized “DNA tubes” with circumferences of 4, 5, 6, 7, 8, 10, and 20 DNA helices that displayed increasing stiffness and persistence length with increasing circumference. However, they used single-stranded tiles to build the filaments which involved half-crossovers where only one strand crosses over between helices at the junction points (rather than two strands in the Holliday junction-like crossovers). This single-stranded tile strategy was entirely different from the one adopted by earlier studies using multi-stranded tiles and gave rise to structures with significantly reduced (but tunable) structural rigidity. The structural tunability of the single-stranded tiles was further exploited to create finite but complex and addressable two dimensional shapes as well as three-dimensional structures with sophisticated surface features and intricate interior cavities (Wei et al. 2012; Ke et al. 2012; see Chap. 5 for further details on this type of DNA nanostructures).

4.6 Finite-Sized 3D Structures

Along with two-dimensional structures, Seeman also commenced efforts to build three-dimensional structures. His initial work included construction of a DNA cube (Chen and Seeman 1991) and a DNA-truncated octahedron (Zhang and Seeman

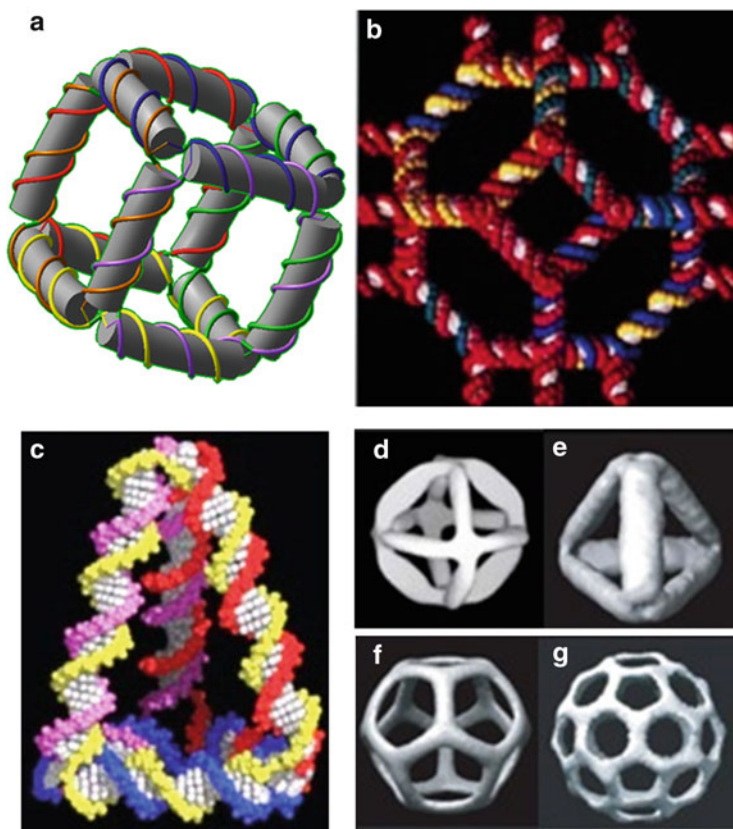


Fig. 4.4 Finite-sized three-dimensional DNA structures. (a) DNA cube. (b) Truncated octahedron. (c) Tetrahedron. (d–g) Cryo-electron microscopy reconstruction images of (d) octahedron, (e) tetrahedron, (f) dodecahedron, and (g) buckyball (Chen and Seeman 1991; Zhang and Seeman 1994; Goodman et al. 2005; Shih et al. 2004; He et al. 2008)

1994). The DNA cube consisted of 12 equal-length double-helical edges arranged about eight vertices (Fig. 4.4a). The vertices were branch points of three-arm junctions. This was the first demonstration of a closed polyhedral structure made from DNA. The truncated octahedron had 36 edges arranged about 24 vertices which were branch points of four-arm junctions (Fig. 4.4b). However, due to the lack of available characterization techniques at the time as well as the extremely small amount of construct synthesized (e.g., on the order of femtomoles), it was impossible to visualize and difficult to conclusively prove the formation of these structures.

In 2004, Shih et al. were able to characterize a three-dimensional DNA nanostructure—a DNA octahedron—by using cryo-electron microscopy (cryo-EM) for the first time (Shih et al. 2004) (Fig. 4.4d). The octahedron was made using a long, specially designed, biosynthesized DNA strand which was

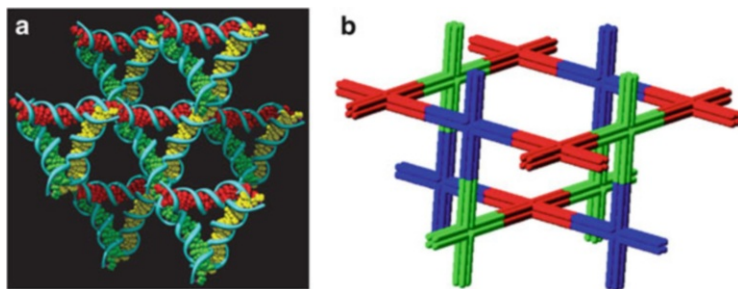


Fig. 4.5 (a) Three-dimensional crystal lattice formed by DNA tensegrity *triangles*. The cavities are rhombohedral in shape. (b) Double-decker tile can also potentially be used to form three-dimensional lattice structures. The cavities in this case would be cubic and larger than the rhombohedral cavities in (a) (Zheng et al. 2009; Majumder et al. 2011)

subsequently folded using a small number of short oligodeoxynucleotide strands. Synthesis of a DNA tetrahedron was also reported later (Goodman et al. 2005) (Fig. 4.4c). More recently, Mao et al. demonstrated the self-assembly and characterization using cryo-EM of tetrahedra, dodecahedra, and buckyball-like structures (He et al. 2008) (Fig. 4.4e–g). The building blocks for all the structures in the Mao study were the three-point stars. Different structures were obtained by assembling three-point star tiles at different concentrations.

4.7 Three-Dimensional Lattices Using DNA Tiles

In 2009, 27 years after founding the field of DNA nanotechnology in an effort to create periodic matter based on DNA crystals, Seeman et al. demonstrated, for the first time, well-ordered macromolecular three-dimensional crystalline lattices using the DNA “tensegrity” triangle (Zheng et al. 2009). As mentioned above, the tensegrity triangle is a rigid DNA motif with threefold rotational symmetry (Liu et al. 2004). The resulting three-dimensional lattice has periodic rhombohedral cavities of approximately 103 nm^3 in size (Fig. 4.5a). Several other DNA tiles have also been proposed for use in the formation of three-dimensional lattices. Sticky ends of the double-decker tile (Majumder et al. 2011) may be programmed to form three-dimensional lattice containing cubic cavities with a periodicity of $\sim 60 \text{ nm}$ (Fig. 4.5b). DNA four-helix bundle (Rangnekar et al. 2011) may also be used to form an octahedral structure which could be further assembled into three-dimensional cubic lattice. Triple-crossover tile (TAE tile) (LaBean et al. 2000) and double-double crossover tile (DDX tile) (Reishus et al. 2005) may also be programmed so as to yield periodic arrays in three dimensions, although only the triangle tile crystals have been successfully documented so far.

4.8 Design of DNA Tiles

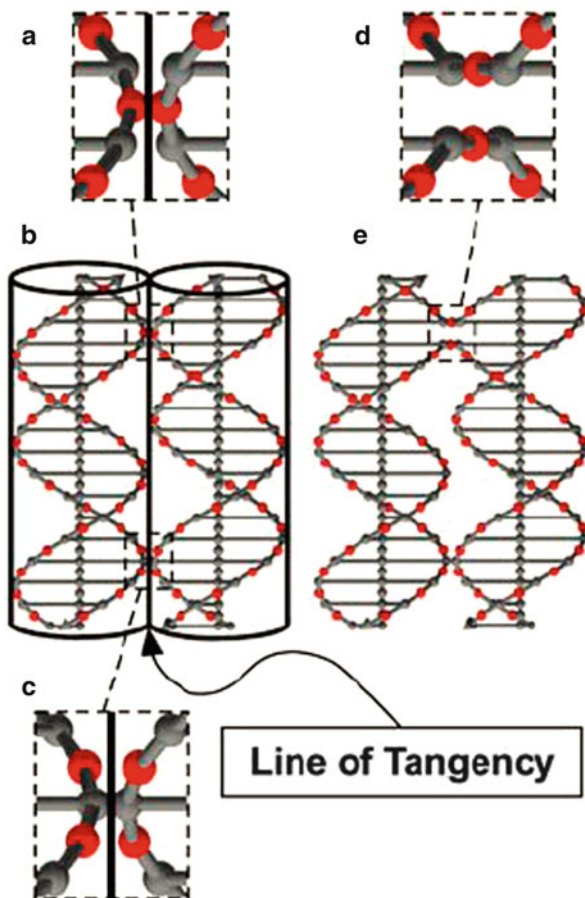
There are essentially three steps to successfully form any DNA nanostructure—design, experimental assembly, and post-assembly characterization. Nanostructure design includes geometric structure design as well as nucleotide sequence design. Structure design constitutes a positive design paradigm (Dirks et al. 2004) and maximizes the probability of forming the target structure by energy and strain minimization. Sequence design constitutes positive and negative design paradigms (Dirks et al. 2004) and optimizes specificity for the target structure by sequence-symmetry minimization (SSM) and other constraints on the sequences of the participating DNA strands in order to decrease the probability of forming alternative structures.

A major component of many DNA tiles is the crossover junction. Placement within the design and especially spacing between crossovers is of primary importance. A small stretch of DNA double helix can be considered as a rigid cylinder with almost fixed dimensions. The pitch of the B-form DNA double helix is approximately 10.5 bases/turn (Wang 1979). Thus, with the rise of each nucleotide, there is an angular displacement of 34.28° in the plane perpendicular to the helical axis. A starting assumption in calculating the geometry of DNA nanostructures is that all the bases are essentially identical. Given this assumption, the precise positions of the nucleotides can be modeled. The crossovers can then be designed geometrically by determining the locations where the phosphodiester linkage would cross over from one helix to the other.

In 2006, Sherman and Seeman presented a theoretical framework for designing DNA tiles based on the crossover motif, where each double-helical domain is axially parallel and connected to the neighboring domain via Holliday junction-like reciprocal exchanges (Sherman and Seeman 2006). They defined nucleoside end midpoints (NEMids), which are located at the midpoints between consecutive C3' atoms, and not at the locations of the phosphates (Fig. 4.6). The two linkages in a crossover always flank one NEMid on each of the helices involved. A properly designed crossover does not usually disrupt the base structure since the section of the DNA backbone involved with the crossover is the phosphodiester linkage between two bases. A minimally strained crossover should have the NEMids involved directly on the tangency line between the two helical domains (Fig. 4.6). When nucleosides, instead of the NEMids, are on the tangency line, strand exchange at the crossover would be strained and therefore should be avoided. Crossovers between helices can thus be designed by aligning the helical axes parallel to each other, followed by aligning the NEMids by rotating the individual helices around their axes.

Each arm of a multi-arm tile contains parallel helices bound by crossovers. The arms can then be connected within the core of the tile using single-stranded poly-T regions, for example. The length of the poly-T strand often determines the geometry and shape of the DNA tile. In 4×4 tile and six-point star, it is four bases (Yan et al. 2003a; He et al. 2006), whereas in three-point star, it is three bases (He et al. 2005a). Moreover, as mentioned earlier, Mao et al. used three-point star as motif to create several finite-sized three-dimensional structures (He et al. 2008).

Fig. 4.6 Design of crossover positions. The nucleosides are represented as *gray spheres* and the nucleoside end midpoints (NEMids) are represented as *red spheres*. (a) NEMids are aligned in the first step at the line of tangency between two parallel helices as shown in (b). (c) The point of contact of nucleosides in the adjacent helices at the line of tangency should not be used to design crossover. (d) Aligned NEMids are then used to create crossover, where one strand in each helix crosses over to the adjacent helix as shown in (e) (Sherman and Seeman 2006)



The shape and size of the resulting structures was dictated by the concentration of the individual three-point star tiles, as well as the length of the poly-T loop at the center of the tile. Flexible poly-T loops may also be used to connect parallel helices in lieu of more rigid crossovers as has been demonstrated in the case of DNA weave tiles (Hansen et al. 2010).

Small DNA tiles are often used to form large lattice structures in one, two and three dimensions. The lattice structures are formed via sticky end cohesion between the tiles. Sticky ends (single-strand DNA) are designed so that they are sufficiently stable in keeping the lattice intact at the characterization temperature. Moreover, the sticky end regions between adjacent tiles must maintain the double-helical twist consistent with the tile design and must not lead to over- or under-winding of the DNA double helix and negatively affect the stability of the target lattice structure. Enthalpic and entropic contributions to the free energy of binding between DX tiles have been measured in order to study the relative effects of rigidity and flexibility in multivalent binding associations (Nangreave et al. 2011).

The next step in DNA tile design is to generate and optimize nucleotide sequences for the component strands. The most important tool to achieve this is SSM (Seeman 1990). SSM means that the sequences are selected with the goal of minimizing sequences with similarities between segments of molecules. Thus, the chances of undesired associations are decreased, and control over secondary structure is improved. The sequence design process assigns sequences that assemble into otherwise unlikely structures by making the maximization of Watson–Crick base-pairing contingent upon their formation. The basic premise underlying this concept is that DNA will form continuous, perfectly paired, double-helical segments in preference to other arrangements.

A “vocabulary element” is defined as the set of nucleotides which is not repeated anywhere else in the structure (Seeman 1990). Depending on the length of the vocabulary element, one can define the maximum number of possible vocabulary elements—64 for trimers, 256 for tetramers, etc. The length of the vocabulary element, in turn, depends upon the size of the structure to be designed. A program called SEQUIN, based on SSM algorithm, was developed by Seeman (1990) in FORTRAN in order to assist with the design of sequences. This program is used to assign sequences to design helices, crossovers, single-stranded loops, connectors, etc. SEQUIN generates adequate sequences, without attempting an exhaustive search for the best possible sequence. The same approach and criteria is extended to the design of sequences for the sticky ends. Additional factors, such as G + C percentage, eschewing runs of poly-G, avoidance of polypurine tracts, may also be taken into consideration while assigning sequences. It is also crucial to ensure the absence of inverted repeats while designing sequences, since these would lead to self-pairing. In case of tiles with symmetric arms, such as three-, four- and six-point stars (He et al. 2005a, b, 2006), the SSM principle is applied to one arm only and the same sequence is then used for all the arms. It must, however, be ensured that the specificity for the target structure is maintained and not affected due to undesired interactions between strands.

4.9 Experimental Synthesis of DNA Tiles

In any tile-based assembly involving multiple strands, ensuring purity and balancing stoichiometry of the participating strands are vitally important. Impure strands may lead to the formation of improperly or partially assembled tiles which would hamper further formation of 1D, 2D, or 3D lattices. Incorrect stoichiometry could also have the same effect. However, in the case of origami-based structures, the staple strands are usually provided in large excess compared to the scaffold strand (see Chap. 5 for further details on origami-based DNA nanostructures). Thus, relative stoichiometry of the staple strands becomes less vital. Moreover, impure strands can also be used under the assumption that in the limit of equilibrium assembly, incorrect strands in the structure would be displaced by the correct strand via strand displacement (Rothemund 2006).

DNA tile and lattice formation in aqueous solution is critically dependent on the presence of counterions. DNA strands have phosphate groups in the backbone thus imbuing them with net negative charge. For DNA strands to bind and form duplex, the electrostatic repulsion between the negatively charged backbones must be offset by the addition of cations in the solution which would act as counterions. DNA tiles containing closely packed helices also benefit from the presence of counterions to reduce the electrostatic repulsion between neighboring duplexes. Mg^{++} is the most commonly used counterion, although Na^+ and Ni^{++} are also used. Divalent cations bridge neighboring negatively charged phosphate groups within and between helices. Mg^{++} ions are most often used in tris-acetate-EDTA buffer (pH typically between 7.4 and 8.2) for DNA tile assembly. In some experiments, especially in the presence of gold nanoparticles, the presence of Mg^{++} ions may be undesirable, since it can lead to aggregation and precipitation of the colloidal gold. In such cases, monovalent cations, such as Na^+ , may be used. At such times, Mg^{++} and Na^+ should be optimized in combination to minimize solubility problems and increase the overall stability of the system components and final target structure (Chandran et al. 2013).

After mixing the participating strands at appropriate stoichiometry in the presence of counterions and buffer, the next step is to assemble the structure. This is achieved by first subjecting the DNA strands to denaturing conditions, so as to destroy any existing secondary structure and then gradually reducing the denaturing conditions so as to form the structure by maximizing Watson–Crick base pairing. Typically, self-assembly is achieved using thermal annealing, wherein, the reaction mixture is first heated to temperatures in excess of 90 °C and then cooled slowly to room temperature. Substrate-assisted annealing has also been done in which the mixture is thermally annealed in the presence of a solid substrate (e.g., mica) which functions as a 2D planar template for maintaining DNA structures near the surface and assists the self-assembly of DNA into the desired 1D or 2D structures (Hamada and Murata 2009).

It may be desirable to assemble the structure at a constant temperature or to use starting solution conditions with greater capacity to denature DNA base pairs. In such cases, the self-assembly of DNA nanostructures can be achieved via isothermal annealing (Jungmann et al. 2008). Here, a denaturing agent (e.g., urea or formamide) is used instead of or along with high temperature to break the secondary structure of the DNA strands. The concentration of denaturing agent in the solution is slowly reduced (by dialysis) to aid the formation of the nanostructure. Although fundamentally similar, there is an important difference between thermal and isothermal annealing strategies. At and near the melting temperature during thermal annealing of the target structure, when the structure formation takes place, the participating strands have much higher kinetic energy owing to elevated temperature. It is possible that such increased local motion could contribute to assembly errors, although this has not been directly tested.

The duration of annealing can also be crucial. Individual tiles do not need long annealing times; the self-assembly can be accomplished by using thermal annealing in 5 min to 2 h. DNA lattices, assembled from individual tiles, often need longer

time periods for annealing. According to design and experimental evidence, the tiles form first during annealing and then the tiles assemble to form the lattice structure. As mentioned earlier, the tile formation takes place mainly at or near its melting temperature. The sticky ends bind to each other at a lower temperature. Tile can be much bulkier than individual strands, thus their motion in solution is far slower than that of individual strands. Moreover, at lower temperature, the kinetic energy of the tile is also less. Hence, it becomes essential to incubate the annealing mixture for longer time periods at lower temperatures in order to form tile-based lattice structures. In such cases, Newton's Law of Cooling (Burmeister 1993) provides an ideal strategy to perform annealing, which is usually done over 16–24 h. The annealing solution is incubated in a hot water bath (temperature $> 90\text{ }^{\circ}\text{C}$), and the water bath is provided sufficient insulation so that it comes down to room temperature in 16–24 h. For tile-based lattice structures, after annealing, the sample is often incubated in the refrigerator ($4\text{ }^{\circ}\text{C}$) overnight to allow the lattice sample to slowly approach its structural equilibrium (Park et al. 2006).

Hierarchical assembly of the DNA nanostructure may also be performed (Park et al. 2006, 2008). This strategy is preferred when more than one type of tile is needed to form the lattice and different tiles share some common strands. It is then desired to assemble individual tiles first followed by mixing and reannealing them. Reannealing is accomplished by heating the mixture to a temperature just above the melting temperature of the sticky ends but well below the melting temperature of the tile so as not to disintegrate and scramble the tiles.

4.10 Characterization of DNA Tiles and Lattices

The next step is the characterization of the assembled DNA nanostructure. For small structures, characterization is usually performed by non-denaturing polyacrylamide gel electrophoresis (PAGE) (see, for example, LaBean et al. 2000). The DNA structures migrate in the gel under applied electric field towards the positive electrode. The speed of migration depends on the molecular weight of the structure as well as some dependence on the overall shape of the complex. More massive, bulkier, and distended structures move slower than smaller, less bulky, and more spherical structures. For larger structures, agarose gel electrophoresis is often employed (Shih et al. 2004). Gel electrophoresis does not provide conclusive structural evidence and thus it becomes essential to either compare the designed structure with a known structure (Mathieu et al. 2005) or to form the structure step by step (by adding one DNA strand at a time) and compare the structures formed after each step (Rangnekar et al. 2011). The latter type of analysis is known as a formation gel.

For large planar structures, such as 1D or 2D lattices, AFM is an invaluable tool in characterization. For AFM imaging, DNA nanostructures are deposited on a substrate surface. The most commonly used substrate is freshly cleaved mica, which is negatively charged (Eaton and West 2010). Thus, the positive counterions,

bound to the DNA structure, aid in the binding of the nanostructure to the mica surface. Imaging is usually performed with tapping mode under a drop of liquid (buffer solution). This ensures that the structure remains stable during the imaging procedure. AFM provides conclusive evidence for formation of the target structures. Fluorescence microscopy can also be used for characterization of very large structures (He et al. 2005b). The annealed structure is treated with a fluorescent dye, which intercalates into the DNA double helix and results in increased fluorescence of the dye. Fluorescence microscopy does not reveal the detailed features. Hence, it is mostly used to determine the approximate size of the nanostructure.

Larger 2D DNA nanostructures may be characterized by transmission electron microscopy (TEM). The annealed structure is negative stained with a heavy metal compound (typically uranyl formate or acetate) which increases the opacity of the sample for the incident electron beam, thus revealing the shape of the DNA structure. TEM of DNA nanostructures does not have as high resolution as AFM; therefore, AFM is usually preferred for 2D nanostructures. Cryogenic transmission electron microscopy (cryo-EM) provides the most convincing evidence for finite-sized 3D nanostructure formation. During cryo-EM, the sample is first frozen to preserve the structural integrity of the nanostructure (Shih et al. 2004). Then TEM is performed, and the images of the structure in different orientation are captured. Software can then be used to combine images and reconstruct a 3D model of the nanostructure. For high-resolution structure determination of 3D crystal lattices, X-ray crystallography has been used (Zheng et al. 2009).

4.11 Applications of DNA Tiles

One of the first applications of DNA tiles was demonstrated in 2000 in the field of molecular computation when the triple-crossover tiles were used to perform computations via algorithmic self-assembly (Mao et al. 2000). The tiles were used to execute four steps of cumulative XOR operations on a string of binary bits. Construction of an aperiodic, patterned lattice displaying barcode information was also reported later using a process of self-assembly by directed nucleation of DX tiles around a scaffold DNA strand (Yan et al. 2003b) (Fig. 4.7a, b). Winfree et al. made DNA crystals that could perform copy operations and also crystals that could count in binary as they grew (Barish et al. 2005). DNA has also been utilized for the construction of functioning molecular devices. The very first such device was a nanomechanical B-Z device made of two DX tiles which could undergo transitions from one conformational state to another based on the solution conditions (Mao et al. 1999b). The switching of DNA between B-form and Z-form and back was shown to cause the structural transition of the device. Later, a PX-JX₂ device was constructed which would undergo transition using DNA strands as the trigger (Yan et al. 2002).

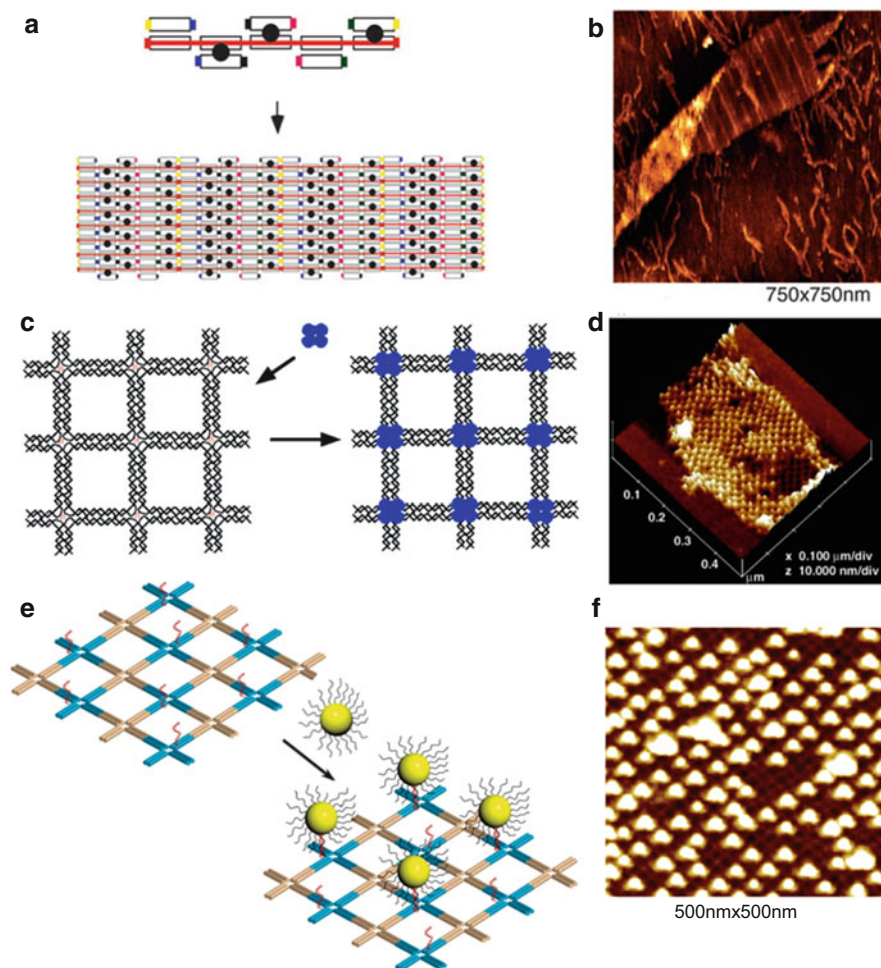


Fig. 4.7 (a) Repeating DNA barcode units, made from DX tile array, form a ribbon lattice. *Black circles* represent hairpin loops that serve as topographic marker. (b) AFM image of the barcode lattice, where the hairpin stripes act as the readout. (c) 4×4 tile lattice were functionalized with biotin at the center, which was then used to form periodic assembly of streptavidin molecules (represented as *blue tetramers*). (d) AFM image of the streptavidin assembly templated on the lattice. (e) The 4×4 tile lattice was also constructed with protruding strand at the center of each tile. This strand acted as host for gold nanoparticles conjugated with the complementary strand, resulting in the programmed assembly of the nanoparticles. (f) AFM image of the gold nanoparticle arrays (Yan et al. 2003a, b; Zhang et al. 2006)

Self-assembled tile-based DNA lattices have also been used as templates for creating programmable and periodic assembly of other molecules and nanostructures. Implementing existing strategies of DNA-protein conjugation

in vitro on DNA tiles provides opportunities for numerous applications utilizing controlled protein assemblies. The most commonly used strategy for DNA–protein conjugation is the use of biotin-functionalized DNA for binding to streptavidin. A DNA strand can be synthesized with a covalent biotin modification at a desired location, which then acts as the binding site for the streptavidin protein. The biotin–streptavidin binding is one of the strongest known non-covalent interactions (Livnah et al. 1993). One of the first such demonstrations was shown in 2003 when Yan et al. used biotin-functionalized 4×4 tile lattice for templated assembly of streptavidin molecules (Yan et al. 2003a) (Fig. 4.7c, d). They further used this strategy to create templated arrays of streptavidin on TX tile arrays (Li et al. 2004). It is possible to fuse other proteins of interest with streptavidin through recombinant DNA technology, and consequently, functional assembly of any protein may be achieved. Covalent coupling of proteins or peptides to DNA by hetero-bifunctional cross-linkers was also exploited to synthesize peptide nanoarrays using DX tile array as scaffold (Williams et al. 2007).

DNA-binding proteins can also bind specifically to the DNA nanostructures without requiring covalent modification of the DNA strand. Proteins which take part in the process of homologous recombination inside the cell have intrinsic binding affinity for Holliday junctions, and therefore they bind to crossovers in DNA nanostructures. One such bacterial recombination protein (RuvA) was used for specific binding to two-dimensional DNA templates to form ordered protein arrays (Malo et al. 2005).

DNA aptamers may also be used for binding proteins to DNA tiles which also do not require any covalent modification to the DNA strand. Aptamers can be incorporated in the DNA tiles at desired locations which then act as the binding site for the specific protein targets of the aptamer. Using this strategy, single-chain antibodies were assembled on DNA arrays (Li et al. 2006). Moreover, it was also demonstrated that two thrombin-binding aptamers could be placed with optimal spacing on a DNA tile to create a bivalent construct for the enhanced binding of thrombin molecule to the DNA nanostructure (Rinker et al. 2008). When four copies of thrombin-inhibiting aptamer were displayed on a weave tile, the resultant anticoagulant activity of the construct was much higher than that of free aptamers (Hansen et al. 2010). The weave tile was used in this case to increase the local concentration of the aptamer, thereby increasing its anticoagulant potential. It was further shown by LaBean et al. that the DNA weave tile can also be used to organize two distinct thrombin-binding aptamers with optimum spacing and orientation (Rangnekar et al. 2012). By judicious engineering of the DNA tile, they created a functional DNA nanostructure which was a multi-aptamer enzyme inhibitor with significantly better anticoagulant activity compared to individual aptamers. They also achieved reversal of thrombin inhibition by using single-stranded DNA antidotes, thus enabling significant control over blood coagulation.

DNA tiles also provide a platform to create programmable assemblies of inorganic nanostructures such as nanoparticles. Yan et al. showed conjugation of 5 nm gold nanoparticles with streptavidin and used biotin–streptavidin conjugation

strategy to achieve linear assembly of gold nanoparticles on a linear TX tile array (Li et al. 2004). The same year, two-dimensional assembly of gold nanoparticles was demonstrated using 2D DNA lattice made from DX tiles (Le et al. 2004). Subsequently, 2D assembly of gold nanoparticles was achieved using 4×4 tile lattice by employing three different strategies. In the first strategy, one of the participating strands of the 4×4 tile was thiolated and conjugated with a gold nanoparticle followed by annealing resulting into the formation of the lattice with templated nanoparticles (Sharma et al. 2006). In the second approach, 4×4 tile lattice was constructed with periodically protruding strands which acted as the host for gold nanoparticles conjugated with the DNA strand complementary to the protruding strands (Zhang et al. 2006) (Fig. 4.7e, f). The third strategy made use of gold-binding peptides covalently fused to DNA strands that were part of the intrinsic 4×4 tiles (Carter and LaBean 2011). Seeman et al. used triangular DNA tiles to form two-dimensional assemblies of gold nanoparticles of two different sizes (Zheng et al. 2006). Yan et al. subsequently used gold nanoparticles to fold 2D DX tile array into tubules of various 3D architectures, ranging in shape from stacked rings to single spirals, double spirals, and nested spirals (Sharma et al. 2009). The shape of the tubules was controlled by varying the size of the nanoparticles.

4.12 Conclusion and Future Directions

Tile-based designs have been a popular architectural strategy for assembling molecular constructs within the field of structural DNA nanotechnology. Tile building blocks and lattices formed from them remain useful for forming large, periodic structures such as might be desired as nanoscale metamaterials. Although the addressability of DNA origami as well as its reliable, high-yield assembly has taken some of the interest away from DNA tile assemblies, there remain a number of areas in which tile-based designs cannot easily be replaced. First, the size of origami structures is limited by the size of ssDNA strands available for use as scaffold, so while the largest origami is on the order of a couple hundred nanometers on an edge, tile lattices on the millimeter scale have already been prepared. Second, for medical applications, such as the anticoagulant enzyme inhibitor discussed above, DNA tiles are on the right size scale for binding to individual protein molecules. Finally, for applications in molecular computation, tile-based systems provide the ability to generate tile sets with fairly large numbers of unique tile types without extremely high costs for synthesis of the component strands. Many future applications of DNA tiles are possible both in the realm of electronics nanofabrication as well as designer molecular medicine.

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