DNA Junctions

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Substrate-Assisted Assembly of Interconnected Single-Duplex DNA Nanostructures**

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A general methodology for the creation of arbitrary shapes and patterns is one of the ultimate goals of nanostructural studies. One approach to this goal is the formation of building blocks that cover nearly all varieties of shapes. Recently, DNA has become known as a new material for nanoscale structures. By using the Watson–Crick complementarity of the double helix, we can design 2D and 3D DNA nanostructures and self-assemble them in solution. The basic methodology of this structural DNA nanotechnology is to design a rigid 'motif' that comprises several DNA junctions and can be used as a building block. Although the variety of motifs depends on the structural geometry of the junctions, almost all the structures reported to date have been based on a limited choice of crossover junctions. This restriction limits the available shapes of DNA nanostructures, which are mainly typified by parallel-packed duplexes. Herein, we report a novel interconnected single-duplex-based T-shaped junction and its use in the assembly of motifs, and therefore show how various structures can be designed and created without the use of conventional junctions. The use of these motifs overcomes the limitations in the varieties of available DNA nanostructures and provides a well-defined geometry for these structures. We show that motifs with T-shaped junctions are able to assemble into various structures in solution, such as orthogonal coordinated ladders, lattices, and polar coordinated wheels. Moreover, we also report that these structures can grow dramatically over the whole substrate surface in a new substrate-assisted assembly method.

The invention of DNA double-crossover molecules[1] has allowed the creation of diverse DNA nanostructures, which gave rise to a new field in which DNA is used as a nanoscale building block. Immobilized stacked X formation of Holliday (crossover) junctions[4,5] was used to create a molecule with rigid parallel axes, thus defining a method to utilize the rigidity of DNA to form structures in solution. The formation of a rigid 'motif' with several combined crossover junctions and subsequent connection of the junctions with single-stranded complementary sticky ends has become a generic design principle of DNA nanostructures. Although many other molecules[2,3] and various structures[6–17] with several crossover junctions have been designed and self-assembled to date, these examples are essentially based on the same geometry. An alternative junction structure along with a new method to enhance rigidity is therefore required for the further development of DNA nanotechnology.

Herein, we report novel T-shaped junctions that provide a right-angled geometry, overcome the current design limitations, and expand the varieties of DNA nanostructures. 'T-shaped' junctions consist of two single-duplex DNA helices. One duplex has a single-stranded bulge section in the center, and the second duplex has a 'sticky end' at the terminus. The two single-stranded regions undergo Watson–Crick base pairing to leave no unmatched bases. After hybridization, the first duplex is bent perpendicularly and the second duplex is inserted into the bend, thus resulting in an interconnection comprising a branched T-shaped junction (Figure 1 and Figure S8 in the Supporting Information). Thus, a motif based on this junction is termed a 'T-motif'. A branch can be placed anywhere on the major groove of the trunk by using five or six nucleotides as a single-stranded region. The length of interconnection is derived from the distance between the backbones of complementary bases and the axial length of the base pairs (Figure 1 in the Supporting Information). Note that the T-junction itself plays the roles of both junctions and sticky ends, whereas the junctions and sticky ends are located at different regions in conventional crossover-junction-based motifs.

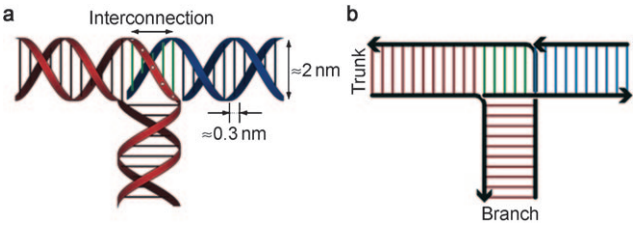

Figure 1. A single-duplex-based T-junction. a) Helical representation. The red and blue parts indicate the two double helices and the green lines indicate the sticky end (5 bp in the figure). b) Schematic representation of the motif. The arrows indicate the 5′–3′ direction. The horizontal double helix is labeled 'trunk' and the vertical helix is labeled 'branch'.

The use of T-motifs brings several favorable properties, which are derived from the structural characteristics of T-

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shaped junctions, to DNA nanostructures. The first and most obvious property is that a T-shaped junction can provide geometries from which a wide variety of DNA nanostructures can be created. The basic principle for creating shapes and patterns with DNA is the use of motifs as units that define the final appearance of the structure. Although some shapes are well approximated by the use of motifs comprising helices with parallel axes, many other shapes, such as porous lattices and polar coordinated structures, are difficult to realize because of the lack of available geometrical variation. Moreover, the previously reported bulged three-arm junctions do not provide fixed arm angles,[18] therefore it is difficult to define a fixed geometry with these junctions alone. By combining several T-shaped, right-angled junctions to form a motif that can be found in RNA nanostructures,[19] we were able to design and create various new shapes and patterns, which is difficult to accomplish by using motifs with conventional geometries. The second property is an improvement in structural resolution. The junction can define the position of the branch itself because of its rigidity; therefore, if we define the structural resolution as the length (number of base pairs, bp) of a minimal fixed structure, the spatial resolution of a T-motif would be $l+1$ (6 or 7 bp), where l is the length of the interconnection. On the other hand, crossover-based motifs generally need more than two junctions to fix their conformation, and therefore require at least one full turn (10.5 bp) as a minimum. The higher resolution of T-motifs allows the formation of smaller, 'nanoscopic' details by DNA nanostructures to be advanced. The third advantage is the rigidity and flexibility of the structure. Generally, it is difficult to form structures from single-duplex-based branched motifs[20] without using non-DNA components because of their lack of structural integrity.[18,21] Consequently, the current basic strategy to build such structures is to combine several duplexes[22] as a rigid motif, or to directly design rigid crystalline tubes with single strands.[23] On the other hand, the provision of some degree of flexibility at the expense of maintaining rigidity is effective in avoiding stress inside the structures.[24] These requirements can be satisfied to some extent with T-motifs because the absence of unpaired bases at the T-junction provides rigidity and the single-duplex section between junctions ensures flexibility. The fourth property is the avoidance of electrostatic repulsion among DNA backbones. T-motif-based nanostructures consist of a branched single duplex; thus, in principle, there is clearance between helices, and the resulting nanostructure can avoid the distortion that arises from helices aligned in parallel.[13,25,26]

T-motif-based nanostructures were designed by two-dimensional graphical abstraction (see the Supporting Information for details). Two types of 'track' (ladder), two types of orthogonal coordinate porous '2D lattice', and a polar coordinate 'wheel' structure were designed using this method and self-assembled. Firstly, two types of track structures were designed to show configurational control by the T-motifs (Figure 2a,b, rows 1 and 2). The connection was based on T-shaped junctions, although the ladders' opposite 'handrail' directions were inverted in two designs. The 'rung' length of a ladder determined this inversion adjustment. Secondly, two types of two-dimensional lattice structures were designed to examine base-pair parameters (Figure 2c,d, rows 1 and 2). These designs showed that even a small change in duplex length can lead to drastic structural differences. The 'brick-wall' type was obtained by changing the helix length (half-turn difference) of the track structures' 'handrail'. This planar structure was designed to provide a resolution that was more than three times higher in area than in crossover-based porous structures.[9] The 'windmill' type is another pattern with two sizes of pores that show different combinations of junctions, in which motifs were connected to each other with a 90° rotation. The wheel structure utilized the flexibility of the T-motif's single duplex-based structure (Figure 2e, rows 1 and 2). Compared with the ladder design, one side of the 'handrail' was shortened and the other was extended; thus, this length difference determined the curvature of the assembled ring structure (Figure S2 in the Supporting Information).

Each set of equimolar strands was mixed in buffer solution and slowly annealed from 95 °C to room temperature or 4 °C in order to self-assemble the nanostructures (Figure S4 in the Supporting Information). Besides ordinary annealing in solution, we examined a new method: substrate-assisted self-assembly. A substrate that binds to DNA was annealed together with the strands, and was expected to function as a 2D planar template for maintaining DNA motifs near the surface, and to assist the self-assembly of DNA into the desired 1D or 2D structures. A freshly cleaved mica fragment was chosen as the substrate material because it is well known for the binding of divalent cations, and was also used for DNA observation by atomic force microscopy (AFM) because of its atomic flatness. Solution and mica-assisted self-assembly were both carried out under the same conditions, except the mica-assisted assembly method was performed at a lower final DNA concentrations (0.5 μM for solution assembly, 0.1 μM for mica-assisted assembly).

The annealed solutions were observed on the mica surface by AFM imaging. All designs formed the expected structures upon annealing in solution (Figure 2a–e, row 3; Figure S6a,c,g in the Supporting Information). Both the 1D tracks were well formed (Figure S5e,f in the Supporting Information), although their length was restricted to about several hundred nanometers. The 2D brick-wall lattice was also formed with a preference for the growth direction. This directed growth could have been caused by the motif's flexibility, which led to tubular structures as the lattice grew until a particular size was reached, because the kinetic effect results in the preferential formation of a tube rather than a planar structure in solution. On the other hand, the windmill, which is another type of 2D lattice, failed to form large structures in solution. We successfully observed a closed wheel structure, although its yield was not high (Figure S3 in the Supporting Information).

The results of the substrate-assisted self-assembly were qualitatively different compared to those obtained in solution (Figure 2a–e, rows 4 and 5 and Figure 6b–h in the Supporting Information). The track ladders were much longer (up to the micrometer scale), and, in addition, the ladder structures were aligned in a particular direction on the mica surface. Furthermore, although some gaps were found, almost the

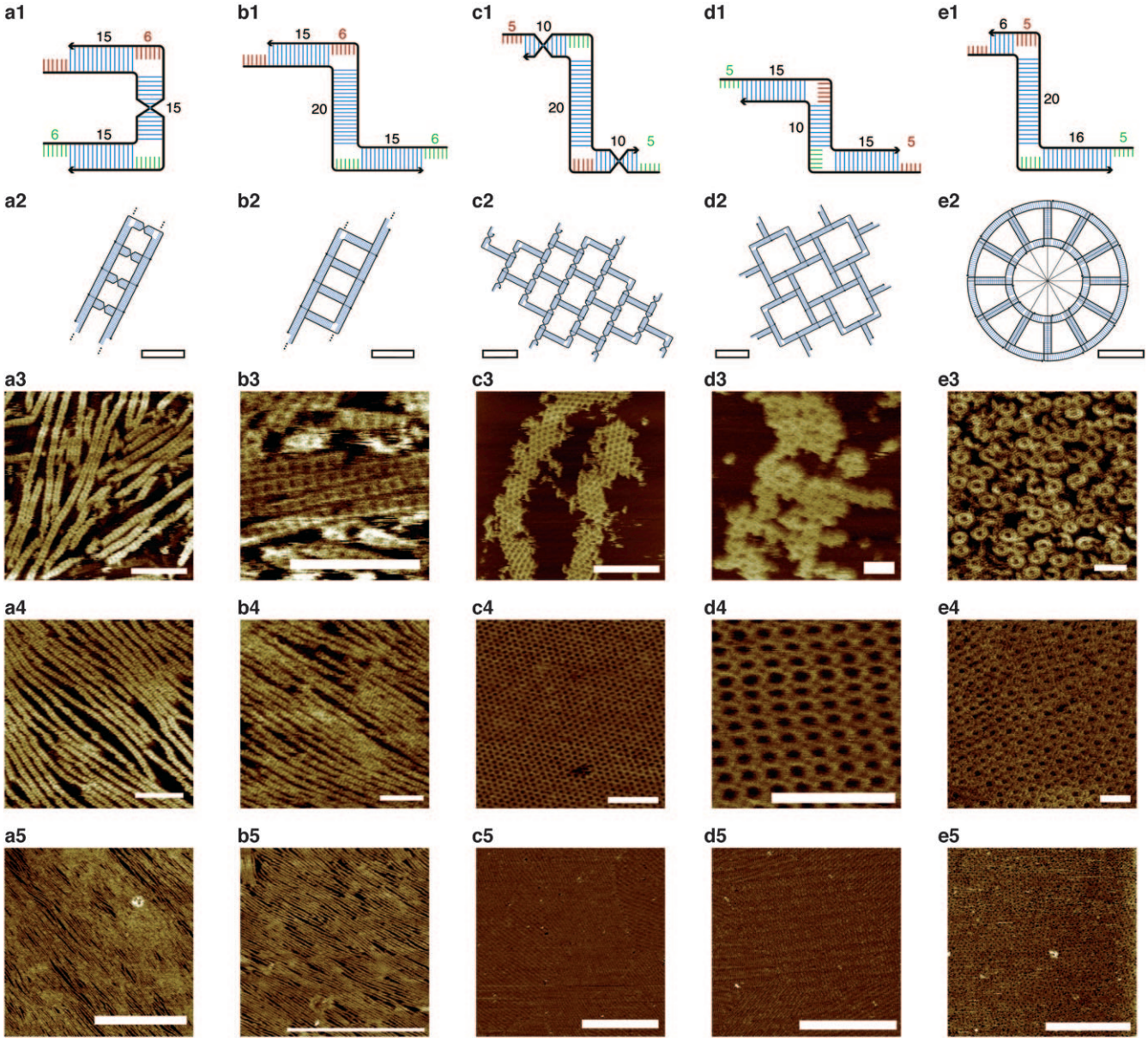

Figure 2. T-motif-based nanostructures and their lengths (bp). a) 1D ladder 15–15–15, b) 1D ladder 15–20–15, c) 2D brick-wall lattice, d) 2D windmill lattice, and e) wheel. Row 1: schematic representation of the motif. The red and green bases correspond to complementary sticky ends. Note that some crossed sequences are drawn in such a way as to show the arrangement of the sticky-end positions. Row 2: schematic representation. Row 3: solution self-assembly results (AFM images). Rows 4, 5: mica-assisted self-assembly results (AFM images). Scale bars: row 2 = 10 nm, rows 3, 4 = 100 nm, row 5 = 1 μm.

entire substrate surface was covered by the structure. Two types of 2D lattices were also successfully formed (Figure 5 a–d in the Supporting Information). A flat planar structure comprising a number of a few micrometer-sized monocrystals that were connected to each other was obtained in both cases (Figure S9 in the Supporting Information). In addition, the monocrystal size was enlarged by a cyclic annealing protocol (Figure S7 in the Supporting Information). The structures were also observed to exist without any gaps over the entire mica surface. We propose that these monocrystals grow independently on the surface until they meet each other, subsequently, parts of the growth fronts are connected and form a patchwork-like appearance. Although some of the wheels had defects, most of them were successfully formed by mica-assisted self-assembly. In addition, the wheels on the surface were aligned in hexagonal-close-packed formation. These results imply that substrate assistance stabilizes a T-motif self-assembly and leads the structure to align in a packed formation.

The results for the T-motifs and the substrate-assisted assembly method suggest that several important possibilities are made available by these nanostuctures. The first possibility is that the number of DNA nanostructure varieties are widened by using this new junction. The ability to design and self-assemble orthogonal and polar coordinate structures implies that varieties of porous shapes and patterns can be

designed from this motif, including 3D structures such as periodic crystals and spheres, and algorithmic self-assemblies.[6,27] Drug-delivery cages, nanofilters, and nanopores are some examples of the applications that can be realized by this new junction. Secondly, the substrate-assisted assembly can be readily utilized for large-scale DNA self-assembly. The binding of DNA to substrates in order to enhance their rigidity can be used in addition to the rigidity of the motif itself. Large-scale DNA nanostructure assembly on the substrate will provide new aspects and practical examples of assemblies for various fields, such as supramolecular chemistry, crystallography, and molecular nanotechnology. In addition, this new assembly method will also open up possibilities for various future applications that use DNA nanostructures as templates, including bottom-up and top-down hybrid devices, such as nanocircuits, high-density memories, metamaterials, and protein arrays for nanoreactors.

Experimental Section

Oligonucleotides: All DNA sequences were designed using DNA Design software, developed by Winfree Lab at Caltech (Pasadena, CA; see the Supporting Information for specific sequences). Oligonucleotides were synthesized and purified by HPLC by Sigma–Aldrich Japan K.K.

Formation: DNA strands were mixed in an equimolar ratio with tris-acetate-EDTA/Mg^{2+} (TAE/Mg^{2+}, 12.5 mM tris = tris(hydroxymethyl)aminomethane, EDTA = ethylenediaminetetraacetic acid) buffer. Mixed samples were gradually annealed from 95°C to room temperature or 4°C over 2 days in solution or on mica. The mica-assisted self-assembly method was performed under the same conditions except the mica substrate was annealed with all DNA samples. Samples were then directly observed by AFM.

AFM Imaging: Observations were carried out in tapping mode on a Multimode AFM with Nanoscope IIIa controller (Veeco) using NP-S cantilevers.

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