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Construction of Holliday Junction in Small Circular DNA Molecules for Stable Motifs and Two-Dimensional Lattices

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Abstract: Design rules for DNA nanotechnology have been mostly learnt from using linear single stranded (ss) DNAs as the source materials. For example, a typical DAO (Double crossover, Antiparallel, Odd half turns) tile for assembling two-dimensional (2D) lattices is built by only two linear ss-oligonucleotide scaffold strands, like two ropes making a square knot. We demonstrate here a new type of coupled DAO (cDAO) tiles and their 2D lattices using small circular ss-oligonucleotides as scaffold strands and linear ss-oligonucleotides as staple strands. A cDAO tile of cDAO-c64nt, shaped as a solid parallelogram, is constructed with a Holliday junction (HJ) at the center and two HJs at both poles of a circular 64 nucleotides (c64nt); similarly, cDAO-c84nt, shaped as a crossed quadrilateral composed of two congruent triangles, is formed with a HJ at the center and four 3-way junctions at the corners of c84nt. Perfect 2D lattices were assembled from cDAO tiles: infinite nanostructures of nanoribbons, nanotubes, and nanorings, and finite nanostructures. The structural relationship between the visible lattices imaged by AFM and their corresponding invisible secondary and tertiary molecular structures of HJs, inclination angle of hydrogen bonds against the double helical axis, and tile's chirality can be interpreted very well. This work could shed new light on DNA nanotechnology using the unique circular tiles.

Keywords: circular DNA • DNA structure • Self-assembly

Since Nadrian Seeman's pioneer work on DNA nanotechnology in the early eighties of last century, DNA molecules have been used to build fascinating motifs for construction of many kinds of nanostructures. The typical motifs include DAE (Double crossover, Antiparallel, Even half turns often at 4 and 6) and DAO tiles,^[1] star tiles,^[2] ss-tiles and ss-bricks,^[3] and DNA origami.^[4] Among most of the DNA nanostructural assemblies, HJs^[5] play one of the key roles as pivot points for crosslinking four double-stranded (ds) arms and intensifying the rigidity of DNA motifs as well as lattices.^[1b] Till now most of DNA motifs and lattices have been assembled from linear ss-DNAs as the source materials. However, both linear and circular DNAs are widely distributed in biological species, playing a central role in gene replication, transcription, recombination, translation, protein recognition, etc. Why our mother nature chooses both linear and circular DNAs definitely does have her own rationale.

The topological difference of DNAs will bring differentiations in their secondary and tertiary structures of biological key elements such as HJs in conformation, chirality, supercoiling, condensation, etc., and also in their biological functions. Recently a few reports described the use of circular ss-oligonucleotides as scaffolds to build motifs, nanotubes, and DAE lattices.^[6] For conciseness, we define a "circular tile" as a stable DNA complex molecule associated from one circular scaffold and other linear staples of ss-oligonucleotides, while a "linear tile" from a full set of linear ss-oligonucleotides. The following differences can be told for the same design but with circular tile instead of linear tile in DNA nanotechnology: 1) A circular DNA molecule is covalently connected continuously, thus circular tiles and lattices are intensified, compared to linear ones having more nicks. This phenomenon has been observed previously by means of both pre-circularization and post-circularization.^[1c,6d-7] 2) A circular scaffold can be only threaded by linear staples during assembly to form motifs and to weave lattices, i.e., a circular molecule lacks the active twisting spatial free degree and only possesses the adaptive one whereas a linear molecule has both. Referred from the knowledge of the anti, elipsed, and gauche conformations of hydrocarbon molecules, to complete such a threading, every nucleotide in a circular strand should rotate freely along its backbone under certain conditions such as at a higher temperature, otherwise, the circular strand will be twisted to high tension which cannot be released and thus a fully twisted threading cannot be completed. For this reason, patience should be paid to control the sequential dynamic assembly process carefully to avoid the incomplete threading of circular strands. For example, c64nt will be threaded by its complementary linear staple 6 times, and c84nt by its linear partner staple 8 times, after complete association. 3) A molecular crowding environment will exist inside the small circular DNA.^[8] In another sentence, bond lengths and angles inside the circle will be smaller than those outside the circle. However, such geometrical crowding structures in circular tiles might not be so different from linear ones because the looped linear scaffold also resembles the geometrical shape of the circular one. The above subtle differences cannot be distinguished in some motifs and their assemblies such as DAE tiles with E at 4 or 6 half turns and their 2D DAE-O (–O means odd half turns inter-tile distance) lattices, whether they are circular or linear tiles. While for other motifs, the subtle geometrical structural differences will be accumulated and magnified in their DNA assemblies, thus resulting in partly or completely different DNA lattices in structure, size, and appearance.

In fact, the previously reported circular DAE is structurally similar to a linear DAE, with a negligible difference that the former has a continued circular scaffold while the latter a looped scaffold carrying a nick.^[6e] How about building a DAO tile from a small circular strand? It seems impossible because the core structure

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of DAO is made by only two linear scaffolds like two ropes making a square knot, where each scaffold makes a U-turn at one crossover and routes in and out of the DAO loop at the other crossover, while a circular scaffold restrains its extension out of the scaffold loop at both crossovers. Such a dilemma can be solved by inserting a HJ at the center of c64nt, then a pair of two coupled DAO tiles can be formed by making two additional polar HJs at both poles. This new cDAO motif and its derivatives are stable and rigid enough to assemble up to 5 μm long 2D DNA lattices, whereas the same-sequenced linear cDAO tiles cannot.

We previously reported the use of c64nt as scaffolds to assemble DNA nanotubes.^[6d] Continuing the work, we placed a HJ at the center of c64nt and set central symmetric nick pairs at different positions of the two main staples (Figure S4), and found structural polymorphism of annealed monomers and polymers, where monomers could be used as rigid motifs for assembling DNA lattices. Schematic double helical drawings of the key circular monomer motifs and polymers are shown in Figure 1. c64bp and c84bp are two control samples, each is considered as a continued ds-loop with a nick at any site of its staple. We will illustrate the phase analysis for HJ-c64nt, HJ-c84nt, and tHJ-c84nt as examples. Other motifs' phases can be easily analyzed following the same way. Since we only deal with the 2D nanostructures here, we will discuss the helical phases in the paper plane by projecting all the double helical nucleotides to this plane. The coordinates of base pairs, nicks, HJs, and 3-way junctions are defined according to the number segments in Figure 1. As shown in HJ-c64nt, a central HJ is located at the c64nt center with coordinates of (16, 17, 16', 17'), and two nicks are at both poles. The four HJ-making nucleotides of the two main staples must phase to the maximum distant positions from their upper and lower helical axes inside the c64nt loop respectively. To meet the Watson-Crick helical phasing criteria for a 16 bp distance, each main staple must route in and out of c64nt at the same pole from and to the maximum distant positions from their helical axes respectively, which are labeled with yellow hexagons at 1' and 32 of the right pole, and 1 and 32' of the left pole. The circular tile of cDAO-c64nt can be easily built by extending the two main staples outside both poles of HJ-c64nt to make two extra polar HJs and to associate with two helper staples respectively. The asymmetric tile of aHJ-c64nt, a twin tile of HJ-c64nt, is built with 34 bp at the upper strand and 30 bp at the lower strand. Similar to cDAO-c64nt built from HJ-c64nt, the asymmetric acDAO-c64nt is derived from aHJ-c64. Although c64 nanowire was designed as a monomer in Figure S4 with two central symmetric nicks at (8, 9) of the upper strand and (8', 9') of the lower strand, it is actually folded as nanowires, schematically drawn in Figure 1 and also in Figure S6 with a full set of sequences repeated twice. While for HJ-c84nt, the helical phasing of the central HJ with coordinates of (21, 22, 21', 22') requires the four HJ-making nucleotides of the two main staples route to the maximum distant positions from their upper and lower helical axes inside the c84nt loop respectively. Due to the routing distance of two full turns of 21 bp, each main staple must route from and to the maximum distant positions from their helical axes at the same pole inside the c84nt loop respectively, forming a nick labeled with a sky blue hexagon. Since the two

main staples cannot protrude out of c84nt for tile connections, HJ-c84nt cannot be used to assemble 2D lattices. An alternative path for the two main staples routing out of c84nt is shown in tHJ-c84nt. Both main staples route in and out of c84nt, at a 16 bp distance from the central HJ, from and to the maximum distant positions from their helical axes respectively, labeled with four yellow hexagons at 6' and 37 of the right side, and at 6 and 37' of the left side; both helper staples associate with the rest of two 10 nt segments of 38-to-5' and 5-to-38' respectively; such a folding strategy makes a crossed quadrilateral composed of two congruent isosceles triangles. cDAO-c84nt can be easily built by extending the main and helper staple strands out of tHJ-c84nt and with correlated strands paired together respectively. cDAO-c84nt is so-called because the four 3-way crossovers (or 3-way junctions) at the four corners with coordinates of (37, 38), (5', 6'), (37', 38'), and (5, 6) are coupled by the central 4-way crossover (central HJ) together. cDAO-c74nt is folded as half part of a solid parallelogram as in cDAO-c64nt and half part of a hollow triangle as in cDAO-c84nt. Similar to c64 nanowire, c84 nanowire was designed as a monomer with two central symmetric nicks at (10, 11) of the upper strand and (10', 11') of the lower strand, but it is actually folded as nanowires, schematically drawn in Figure 1 and also in Figure S7 with a full set of sequences repeated twice.

Native PAGE is the primary experimental method to examine the stable complex DNA molecule that could be used as a motif or tile for DNA nanotechnology. Shown in Figure 2A, sample names are listed on the top. We explain the PAGE results with the well-known DNA reptation model in electrophoresis.^[9] The linear ds-helix can be pictured as moving through impenetrable gel fibers, with the motion mediated by a snake-like reptation; while the circular ds-helix in our case should be modified with the number and size of pores inside. Four fully associated c64nt duplexes with designed nick(s) on staples were run in the left four lanes. c64bp migrated as a wriggling oblong loop with a medium sized pore inside, where the pore was penetrated by gel fibers, thus c64bp was retarded to a band corresponding to a linear 74 bp of the markers. HJ-c64 can be pictured as a wriggling lanky Arabic number of 8 with two small sized pores, whereas aHJ-c64, with a similar shape to HJ-c64, is bent somehow towards the short 30 bp side. Their migration bands correspond to a linear 64 bp around. The migration band of c64nt nanowire is at the slot entrance, indicating a polymer, but not a monomer as designed. c64nt nanowire was also imaged by AFM in Figure 2C. In fact, we have investigated a series of central symmetric nick pairs with coordinates from {(2, 3), (2', 3')} to {(14, 15), (14', 15')} by native PAGE (Figure S4). Among all the nick pairs, only the three pairs of {(32', 1), (32, 1')}, {(1, 2), (1', 2')}, and {(2, 3), (2', 3')} close to both poles at the balanced positions presented stable monomer tiles like HJ-c64nt; the pair of {(3, 4), (3', 4')} gave an unknown intermediate monomer according to a linear 140 bp, which could be explained by migration retardation of two larger pores; the eight pairs of {(4, 5), (4', 5')} to {(11, 12), (11', 12')}, away from the three balanced positions, generated polymers to avoid torsional and shear stresses; and the final three pairs of {(12, 13), (12', 13')} to {(14, 15), (14', 15')} close to the center provided monomers like c64bp without forming a central HJ respectively.

Four fully associated c84nt duplexes with designed nick(s) on staples were run in the right four lanes beside the Marker (Figure 2A). c84bp, similar to c64bp but carrying a large sized pore, was penetrated and retarded by massive gel fibers and migrated much more slowly with a band according to a linear 130 bp. HJ-c84, similar to HJ-c64 while with two little larger pores inside and two nicks at both poles, has a clear band according to a linear 84 bp, indicating a monomer, while a slot entrance band shows other conformers too because even the stable HJ-c84nt in Figure 1 could possess some strains regarding the unhappy base pairing at both poles. The stable triangular tile of tHJ-c84nt without overhangs is proven by a clear band according to a linear 90 bp. C84nt nanowire is confirmed as polymers with a band at the slot entrance, and also with nanowires imaged by AFM in Figure 2D. c64 nanowire is more straight and shorter than c84 nanowire, due to a central HJ in c64nt. Though both c64 nanowire and c84 nanowire could have other folding structures such as nanotubes,^[6d] they are assigned to nanowires because their AFM imaging heights in air were measured to be only 1.2 nm respectively.

In the middle panel (Figure 2B), the three assemblies of cDAO-c64nt, acDAO-c64nt, and cDAO-c84nt carrying four 10 bp blunt-ended overhangs are proven to be stable monomer tiles with only one clear band respectively. Both cDAO-c64nt and acDAO-c64nt having 104 bp ran surprisingly faster, according to a linear 42 bp around, which can be explained by a reptation model as a straight and tight rod without pores inside. Their high rigidity is attributed to the synergistic effect of three HJs in combination, which will be proven later by their lattice appearance and persistence length calculation. cDAO-c84nt migrated as a linear 190 bp, much more slowly than its precursor tile of tHJ-c84nt. Such a huge retardation effect can be interpreted as its four 10 bp overhangs increased the tile volume greatly.

To discuss, the tile stability can be well illustrated with a clear band in native PAGE, whereas the tile rigidity cannot be correlated to the migration distance in a straightforward way. Rather the relationship between rigidity and migration distance of a tile is complicated by the tile geometries, such as its skeleton, e.g., the solid rod of (a)cDAO-c64nt or the hollow integral rigid frame of cDAO-c84nt, its pore number and size, and its occupied volume.

The 2D infinite lattices assembled from two-tile systems of cDAO-c64nt-E and cDAO-c64nt-O are shown in Figure 3A and B with AFM images, and in Figure 3E and F with their corresponding schematic assembling models respectively. cDAO-c64nt-E and cDAO-c64nt-O differ by the inter-tile distances: -E means even numbers of 4 half turns, and -O means odd numbers of 5 half turns. The former requires all the tiles to be aligned identically, thus all c64nt scaffolds rotate in the same direction, which is indicated with curved arrows in Figure 3E. The latter, with the schematic assembly model in Figure 3F, asks alternation of the two neighboring tiles' faces along the horizontal helical axes, the cDAO-c64nt tile core of solid parallelogram has a C_i symmetry, thus the rotation directions of the two neighboring c64nt scaffolds are opposite; while the tiles' alignment along the vertical arrays remains the same; such a cDAO-c64nt-O assembly follows the so-called "corrugation rule".^[2e] Both assemblies yield planar nanoribbons.

The cDAO-c64nt-E ribbon is 1.2 nm high, 2.0 μm wide, and 5.0 μm long (Figure 3A, S19), and the cDAO-c64nt-O ribbon is 1.2 nm high, 0.6 μm wide, and 5.0 μm long (Figure 3B, S20). The indent parallel stripes are located at the sticky ended cohesion regions, ~ 1.7 nm wide, according to 5 to 6 bp's length. The periodic distance of stripes for cDAO-c64nt-E is 18.2 nm and for cDAO-c64nt-O is 19.7 nm, in line with their theoretical ones of $(32+21)$ bp \times 0.34 nm/bp = 18.0 nm and $(32+26)$ bp \times 0.34 nm/bp = 19.7 nm respectively assigning per base rise of 0.34 nm. The linear DAE-E system^[10] was reported to yield nanotubes because the 4 half turns inter-tile distance asks the curved DAE tiles to be aligned identically, leading to the closing of a patch of tile array to form a nanotube. The planar nanoribbons from cDAO-c64nt-E exclude the tile curvature and confirm our previous claim that cDAO-c64nt behaves as a straight and tight rod, much more rigid and planar than linear DAE tiles with E at 4 or 6; from the tile rigidity and the dense woven texture, the helical axes can be assigned to be along the ribbon's longitudinal axis, thus a tilt angle of 83° of the stripes against the helical axes was measured. The chiral model of cDAO-c64nt is suggested according to inclination of base-paired hydrogen bonds against the double helical axis, which was reported previously in HJ-related DNA single crystals^[11,5d] and also in a RNA assembly system^[12]. The tilt angle of 83° is attributed to the synergistic effect of three HJs of cDAO-c64nt in combination. The absolute tile conformation or chirality in Figure 3E can be aligned directly to its lattice image in Figure 3A. While for the cDAO-c64nt-O ribbon, the parallel stripes were measured to be perpendicular to the ribbon's longitudinal axis, i.e., the helical axes (Figure 3B, F). The perpendicular structure is explained by the assembly model with staggered arrangement of chirality-alternated tiles along the helical axes. In this case, both upper and lower surfaces of a ribbon are structurally equal. We also designed a finite lattice of 5×6 patches of cDAO-c64nt-O (Figure 3C, G), including 32 tiles with the same circular scaffold of a c64nt while with different sequenced overhangs (Figure S14). All the four edges of the finite lattice have six thymine ending overhangs to prevent the inter-lattice π - π edge-stacking.^[3a,3b] Its length was measured at ~ 82 nm, and its width at ~ 40 nm, corresponding to respective theoretical estimations of $((32+26) \times 5 - 26)$ bp \times 0.34 nm/bp = 89.8 nm, and 2.6 nm/helix \times (6 \times 2) helix + 1.5 nm \times 5 = 38.7 nm if we assign per helix diameter to 2.6 nm and the buckling gap between tiles to 1.5 nm in aqueous media.^[4a] The yield for the perfect 5×6 patches of cDAO-c64nt-O can be estimated at $\sim 30\%$ from their agarose gel band percentage by strength (Figure S16) and at $\sim 28\%$ by statistical analysis of their area occupancies in AFM images (Figure S24). As the asymmetric tile of acDAO-c64nt is as stable and rigid as cDAO-c64nt, its predictable curvature is accumulated to curved acDAO-c64nt-E (-E was designed as linear connections in Figure S13) nanowires including nanorings, nanoarcs, and nanospirals (Figure 3D, H). Statistically, the one-tile wide curved nanowires are mostly composed of 10 to 20 tiles. From the well-known worm-like-chain model, the rigidity of a polymer can be well characterized quantitatively by the persistence length along its longitudinal axis. We applied an open-source software of FiberApp^[13] to estimate the persistence length of acDAO-c64nt-E at ~ 700 nm (details in Section 2 of SI), 14 times of 50 nm of a linear double helix's persistence length,

which is reasonable because it is in line with the tile's rigid and straight rod model, and also comparable to the previously reported persistence length in the range of 2.0 to 30.0 μm for nanotubes built from DAE-E and DNA origami.^[10,14]

The two-tile infinite lattices of cDAO-c84nt-E and cDAO-c84nt-O are shown in Figure 4A and B with AFM images, and in Figure 4D and E with their corresponding schematic assembling models respectively. cDAO-c84nt-E requires all the tiles to be aligned identically, which results in uniform nanotubes with a diameter of 120 nm, corresponding to 24 cDAO-c84nt tiles rolled up with zig-zag connections. Not like the rigid and planar cDAO-c64nt tiles assembling nanoribbons, cDAO-c84nt possesses a curved face and two asymmetric growth axes along its two crossed sides, which lead to the closing of a patch of tile array to form a nanotube, somehow similar to the linear DAE-E system.^[10] The double layered nanotube is 2.5 nm high, and is from 5 to 15 μm long (Figure 4A, S21). Theoretically, the lattice unit cell should correspond to a rhombus with a side length of $(32+21) \text{ bp} \times 0.34 \text{ nm/bp} = 18.0 \text{ nm}$. However careful measurements from open nanotubes confirms that the unit cell is a parallelogram, with an acute interior angle of 69° and two side lengths of 18.2 and 16.0 nm (Figure 4A, D, S21). The parallelogram lattice is certainly attributed to the secondary and tertiary tile structure of cDAO-c84nt, whose detailed chiral model is suggested to be a pair of two congruent hollow triangles forming a crossed quadrilateral (Figure 4D, F), with two parallel bases tilted 70.9° towards the horizontal (Figure S5). The chirality of cDAO-c84nt is attributed to the synergistic effect of the central HJ and other four 3-way junctions in combination. Of five junctions the central HJ should play the main alignment role.^[6d] The measured interior angle of 69° of the parallelogram lattice, larger than the apex angle of 54.2° in the suggested model (Figure S5), indicates the unit cell of the seemingly parallelogram is actually a polygon, and 69° is a collective angle by simplifying the polygon into a parallelogram. The absolute tile conformation in cDAO-c84nt-E including its chirality and the rotation direction of c84nt can be deduced directly from its lattice structure, thus the red and green segments connected strands in Figure 4D are assigned to the right-handed and left-handed twisting netting twines of the nanotube in Figure 4A respectively. cDAO-c84nt-O follows the corrugation folding rule, therefore planar nanoribbons, accompanied with nanotubes, were easily obtained (Figure 4B, E, S22). The formation of nanotubes is similar to that in the linear DAE-O system,^[1b] because the folding of a planar lattice to a nanotube is thermodynamically favored. In view of the lattice strength, cDAO-c84nt is not as rigid as cDAO-c64nt, and the inter-tile connections are spatially isolated, thus the whole cDAO-c84nt-O lattice is much softer, and can be rolled randomly to nanotubes with non-uniform diameters. The width of planar nanoribbons is from 0.5 to 3.0 μm , and its length is between 2 to 5 μm (Figure S22). The rhombic lattice constant is 19.3 nm, in line with its theoretical length of $(32+26) \text{ bp} \times 0.34 \text{ nm/bp} = 19.7 \text{ nm}$. The acute interior angle of the rhombic unit cell is 83° . From the corrugation rule, both upper and lower surfaces of a cDAO-c84nt-O ribbon are structurally equal. To demonstrate their affordable assembly diversities, a finite lattice of 5×6 patches of cDAO-c74&c84nt-O (sequences in Figure S15) is shown in Figure 4C with an AFM image and in Figure 4F with its assembly model. Its length was

measured to be $\sim 150.0 \text{ nm}$, corresponding to its theoretical estimation of $(32+26) \text{ bp} \times 0.34 \text{ nm/bp} \times \sin(83^\circ/2) \times 12 = 156.8 \text{ nm}$, and its width at $\sim 60.0 \text{ nm}$, corresponding to $(32+26) \text{ bp} \times 0.34 \text{ nm/bp} \times \cos(83^\circ/2) \times 4 = 59.1 \text{ nm}$. All the four edges are ended with six protruding thymine overhangs. To intensifying both edges along the horizontal, we used the asymmetric tile of cDAO-c74nt, which carries half part of a hollow triangle and half part of a solid parallelogram (Figure 4F), and set the half part of solid parallelogram towards the outside. Only one sequenced c84nt and one sequenced c74nt were used as scaffolds respectively, while the two circular tile cores were connected with different sequenced overhangs. The yield for the comparably sized 5×6 patches of cDAO-c74&c84nt-O can be estimated at $\sim 23.5\%$ from their agarose gel band percentage by strength (Figure S16).

To sum, structural polymorphism of monomers and polymers of fully associated c64nt or c84nt with a central HJ and a pair of two central symmetric nicks was found with native PAGE. Using the new type of cDAO tiles of cDAO-c64nt, cDAO-c74nt, and cDAO-c84nt, we have successfully achieved perfect 2D lattices of planar nanoribbons and nanotubes by even and odd half turns inter-tile connections respectively. The visible lattice structures and their corresponding invisible molecular structures of HJs, inclination angle, and tile's chirality can be correlated very well. The high rigidity of the twin tiles of cDAO-c64nt and acDAO-c64nt was probed by native PAGE with a surprisingly fast migration speed, by AFM imaging with dense textured planar nanoribbons for cDAO-c64nt-E(O) and with curved nanowires scaled with a persistence length at $\sim 700 \text{ nm}$ for acDAO-c64nt-E. The topological constraint should be the main parameter to be concerned in construction of DNA motifs and lattices using circular DNA tiles instead of linear ones. This work illustrates some advantages of circular tiles for DNA nanotechnology.

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Conflict of interest

The authors declare no conflict of interest.

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Figure 1. Schematic double helical models of monomer circular tiles of c64bp, HJ-c64nt, aHJ-c64nt, cDAO-c64nt, acDAO-c64nt, c84bp, HJ-c84nt, tHJ-c84nt, cDAO-c74nt, cDAO-c84nt, and polymers of c64nt nanowire and c84nt nanowire. Both c64nt nanowire and c84nt nanowire are represented with their simplest folding unit cells respectively, where the three aligned dots above and

below each of the two unit cells mean the infinite alignment of unit cells vertically up and down with equal distance between duplexes to form nanowires. Double straight and folded number segments with key ordinals in bright blue above and below HJ-c64nt, HJ-c84nt, and tHJ-c84nt indicate sequential base pairs in the upper and lower duplexes of the circular scaffold respectively. In each tile of the second and third columns, the circular scaffolds of c64nt, c74nt, and c84nt are colored with deep blue, two main staples forming the central HJ are colored with emerald green in the right half and maroon in the left half. In each tile of the third column, two helper staples constructing the polar junctions at both ends of the tile are colored with auburn at the right and olive green at the left.

Figure 2. Native PAGE photos of DNA motifs with sample names listed above each lane (A and B) and AFM images of c64nt nanowire (C) and c84nt nanowire (D).

Figure 3. AFM images of infinite cDAO-c64nt-E (A), infinite cDAO-c64nt-O (B), finite 5x6 patches of cDAO-c64nt-O (C), infinite acDAO-c64nt-E (D), and their corresponding schematic assembly models (E to H). In the schematic assembly models, the cDAO-c64nt tile core with a C_i symmetry is represented with a solid parallelogram and its two different faces are distinguished by sky blue and deep blue in (E) to (G), the inter-tile connections are drawn as red and green segments, the acDAO-c64nt tile core is drawn as a solid reverse trapezoid colored with deep blue in (H). Curved arrows in (E) to (G) point to the rotation direction of circular scaffolds respectively. The lattice parameters are labeled on the assembly models of (E) and (F) respectively. Schematic parallelogram tiles of cDAO-c64nt from (E) to (G) are aligned with their longitudinal axes to the horizontal.

Figure 4. AFM images of infinite cDAO-c84nt-E (A), infinite cDAO-c84nt-O (B), finite 5x6 patches of cDAO-c74&c84nt-O (C), and their corresponding tile assembly models (D to F). The finite lattice of cDAO-c74&c84nt-O (C and F) is made of 12 asymmetric cDAO-c74nt tiles on both left and right sides, and 20 cDAO-c84nt tiles inside. The schematic cDAO-c84nt tile core model in (D) to (F) is represented with a pair of two congruent hollow triangles forming a crossed quadrilateral, whose left and right sides are tilted 70.9° towards the horizontal (Figure S2); the schematic asymmetric cDAO-c74nt tile core model in (F) is represented with two parts combined together: one by a hollow triangle and the other by a solid parallelogram. The lattice parameters are labeled on the assembly models of (D) and (E) respectively. In (D) to (F), curved arrows point to the rotation direction of circular scaffolds, the inter-tile connections are labeled with red and green segments, and the two different faces of cDAO-c84nt are distinguished by sky blue and deep blue respectively, schematic cDAO-c84nt and cDAO-c74nt tiles are aligned with their longitudinal axes to the horizontal.

Entry for the Table of Contents

COMMUNICATION

Design rules for DNA motifs and lattices have been mostly learnt from using linear single stranded DNA molecules as the source materials. Here we report a new type of coupled DAO (cDAO) motifs and lattices constructed from small circular DNA molecules as scaffolds.

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Construction of Holliday Junction in Small Circular DNA Molecules for Stable Motifs and Two-Dimensional Lattices

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