Regulation of 2D DNA Nanostructures by the Coupling of Tile Curvatures and Arm Twists

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DNA overwinding and underwinding between adjacent Holliday junctions have been applied in DNA origami constructs to design both left-handed and right-handed nanostructures. For a variety of DNA tubes assembled from small tiles, only a theoretical approach of the intrinsic tile curvature was previously used to explain their formation. Details regarding the quantitative and structural descriptions of the intrinsic tile curvature and its evolution in DNA tubes by coupling with arm twists were missing. In this work, we designed three types of tile cores from a circular 128 nucleotide scaffold by longitudinal weaving (LW), bridging longitudinal weaving (bLW), and transverse weaving (TW) and assembled their 2D planar or tubular nanostructures via inter-tile arms with a distance of an odd or even number of DNA half-turns. The biotin/streptavidin (SA) labeling technique was applied to define the tube configuration with addressable inside and outside surfaces and thus their component tile conformation with addressable concave and convex curvatures. Both chiral tubes possessing left-handed and right-handed curvatures could be generated by finely tuning p and q in bLW-E\textsubscript{p/q} designs (bLW tile cores joined together by inter-tile arms of an even number of half-turns with the arm length of p base pairs (bp) and the sticky end length of q nucleotides (nt)). We were able to assign the chiral indices (n,m) to each specific tube from the high-resolution AFM images, and thus estimated the tile curvature angle with a regular polygon model that approximates each tube’s transverse section. We attribute the curvature evolution of bLW-E\textsubscript{p/q} tubes composed of the same tile core to the coupling of the intrinsic tile curvature and different arm twists. A better understanding of the integrated actions of different types of twisting forces on DNA tubes will be much more helpful in engineering DNA nanostructures in the future.
Supporting Information

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S1 Experimental

S1.1 Sample preparation. All linear DNA strands were provided with denaturing polyacrylamide gel electrophoresis (PAGE) purification by Sangon Biotech (www.sangon.com). Structural designs and their corresponding DNA sequences are shown in Section S7. To assemble DNA nanostructures, DNA strands were mixed with a stoichiometric ratio to a volume between 20 and 50 µL at a concentration of 0.2 to 0.5 µM in TAE-Mg buffer (40 mM Tris, 40 mM HAc, 12.5 mM Mg(Ac)₂, 1 mM EDTA, pH 8.0).

S1.2 Preparation of circular DNAs. The c128nt DNA strand was achieved through T4 DNA ligation. Firstly, two linear strands of 5'-phosphorylated 64 nt (3.5 µM) and two corresponding 20 nt splint DNA strands (4.5 µM) were mixed in 80 µL TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The mixture was heated to 95 °C for 5 minutes, then cooled down to room temperature over 2 h. After annealing, 10×T4 buffer (660 mM Tris-HCl, 66 mM MgCl₂, 100 mM DTT, 1 mM ATP, 10 µL) and T4 ligase (300 U/µL, 10 µL) were added, and the mixture was incubated for 16 h at 16 °C, followed by T4 ligase inactivation at 95 °C for 5 minutes. After inactivation of the ligase, 10 µL 10× reaction buffer and 10 µL Exonuclease I (5 U/µL) were added to digest the remaining linear DNA strands of residues by incubation at 37 °C for 30 minutes. The enzyme selectively digested the single-stranded DNA strands, and left the circularized DNA strands intact. The DNA circles were then purified by denaturing PAGE followed by ethanol precipitation. The protocol was as follows: (1) purification of the circularized DNA strands by 6% denaturing PAGE in TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH ~8.3), using a constant voltage of 5V/cm for 2 h; (2) cutting out of the corresponding gel bands by a razor blade under UV light; (3) chopping and crushing of the gel bands into fine pieces and transfer into a 2.0 mL Eppendorf tube; (4) addition of deionized water at least twice the size of the gel volume into the tube and elution at room temperature overnight; (5) filtering to collect the supernatants, recovery of any residual DNAs by rinsing with small volume of deionized.
water and filtering again to combine the supernatant fractions; (6) extraction of the eluent with n-butanol to about 200 μL; (7) addition of 500 μL 100% ethanol, 20 μL 3M NaOAc (pH = 5.2) and storage of the tube at -20 °C for 2 h; (8) centrifugation at 12000 rpm for 10 minutes at 4 °C and discarding of the supernatant; (9) addition of 600 μL of 75% ethanol (-20 °C) and centrifugation again to collect the pellet; (10) drying and storage of the DNA circles at -20 °C.

Figure S1. Purification of c128nt via denaturing PAGE. A denaturing PAGE (6%) photo shows the linear 128 nt band at the left lane and its corresponding circular 128 nt (c128nt) bands on a fluorescent plate under UV irradiation. The circular 128 nt migrates more slowly than its corresponding linear 128 nt. A yield of 40–60% was achieved based on UV-Vis measurement.

S1.3 Annealing ramps. We applied the one-pot assembly protocol for all DNA nanostructures. All samples in PCR (polymerase chain reaction) tubes were annealed in a PCR thermo cycler programmed at 95 °C for 10 min, then with a decreasing rate of 1 °C/5 min to 65 °C, and finally with a decreasing rate of 0.1 °C/10 min to 20 °C. The total annealing time was about 70 h.

S1.4 Native PAGE analysis. The native gel was carried out on a polyacrylamide electrophoresis plate (length × width × depth = 150 mm × 100 mm × 1.5 mm) system from Beijing Liuyi Instrument Factory. To analyze the stability and yield of tile cores, 5 μL of each annealed DNA complex (LW-8T, bLW-8T, or TW-8T) was added with 1 μL loading dye (0.05% bromophenol blue, 0.05% xylene cyanol FF, 60% glycerol, 10 mM Tris-HCl, 60 mM EDTA, pH=7.6) and then subjected to 8% native PAGE in TAE-Mg buffer at 5 volts/cm for about 4 h in an ice water bath. Then the gel was dyed with
GelRed™ about 30 minutes and analyzed under UV light. The gel documentation and analysis were carried out with ImageJ.

**Figure S2. Stability of LW, bLW, and TW tile cores by native PAGE.** The appearance of only one main band in each lane indicates that the three tile cores LW-8T, bLW-8T, and TW-8T are stable complexes. The helix models of LW-8T, bLW-8T, and TW-8T are shown in the right panels, with each helper strand carrying two protruding 8Ts (gray shadowed) on both ends out of the tile core. The yields of tile cores, labeled under their corresponding bands, were processed using ImageJ.

**S1.5 AFM imaging.** AFM images were obtained in both “ScanAsyst mode in air” and “ScanAsyst mode in fluid” (Dimension FastScan, Bruker) with FastScan-C or Scanasyt-Fluid+ tips (Bruker). The sample preparation and imaging protocol was as follows: 2 μL sample were deposited onto a freshly cleaved mica (Ted Pella) and incubated between 2 and 4 minutes, then the specimen was washed twice with 50 μL of deionized water, and finally the specimen was imaged in either air or in fluid mode. The fluid imaging was carried out by adding 80 μL TAE-Mg buffer onto the specimen and 40 μL TAE-Mg buffer onto the AFM tip.

**S1.6 In-situ binding of streptavidin to biotin-labeled lattices.** Streptavidin (SA) was ordered from Sangon Biotech, Shanghai. The SA stock solution was prepared as follows: 1 mL of ultrapure water was added to a tube containing 1 mg of lyophilized SA protein, then incubated for 10 minutes at room temperature and gently mixed to
obtain a 1 mg/mL stock solution (According to the molecular weight of 55,000 Da of streptavidin, the streptavidin concentration of stock solution was calculated to be 18.2 µM), 20 µL of the stock solution were taken out and diluted to 5 µM with ultrapure water, and the ready-for-use SA solution was stored in a fridge. The remaining solution was divided into aliquots and stored frozen at -20°C (to avoid freeze-thaw cycles).

Figure S3. Schematic of the in-situ binding of SAs on biotin-labeled monolayered and double-layered tubes. The geometric relationship of a SA (each SA is represented with a red star in the schematic drawing) relative to its host tile can only be judged on monolayered tubes, but not on double-layered tubes because SAs could bind to both upper and lower layers of the tube. The overlaps of SAs, c1 and c2-frames from upper and lower lattice layers make the distinction very challenging.

S1.7 AFM imaging of in-situ biotin/streptavidin bindings on air-blown DNA nanotubes. In order to define the conformation of tiles in DNA tubes, we developed a technique to blow the DNA nanotubes open. The protocol was as follows: 1) The annealed sample solution (4 µL) was deposited onto a freshly cleaved mica surface (Ted Pella). The DNA nanostructures got adsorbed into the mica for about 2 to 4 minutes. Then the liquid part was removed with filter paper. 2) 70 µL of pure water were added on the specimen and the liquid was absorbed with filter paper, this process was repeated twice to clean off any remaining salts. 3) 70 µL of water were added on the specimen and the sample was dried out with a compressed air to break the tubes either completely or partly into an open form. 4) 70 µL of TAE-Mg buffer were deposited on the specimen and 30 µL of the same buffer on the AFM tip. 5) The streptavidin solution (5 µM) was added in increments of 0.5 µL into the buffer drop, incubated statically between 10 and
20 min after each addition. 6) The sample was imaged after each addition until the density of the streptavidin on open and sealed DNA tubes reached a yield over 70\%, during which period the density of physically adsorbed streptavidin on bare mica as background was very low so that it didn’t interfere with the assignment of specifically bound SAs on DNA tubes. In-situ biotin/SA binding yield over 70\% was high enough to define the geometric relationship of a SA to its host tile. AFM images were obtained in the ScanAsyst mode in fluid (Dimension FastScan, Bruker) with FastScan-C or ScanAsyst-Fluid+ tips (Bruker).

S2 Theoretical estimation of unit cell parameters

Theoretical estimations of the unit cell parameters \((a, b, c, \text{ and } \phi)\) were carried out through the assembly models listed below. Under each schematic assembly model of LW-E21/5, bLW-E21/5, and TW-E21/5, their corresponding unit cell parameters \((a, b, c, \text{ and } \phi)\) were calculated according to the diameter of a helix at 2.5 nm, and the height per base pair rise at 0.324 nm/bp (= 3.4 nm/10.5 bp).

For LW-E21/5,

\[
|a| = 2 \times (32 + 21) \text{bp} \times 0.324 \frac{\text{nm}}{\text{bp}} = 33.9 \text{ nm}
\]

\[
|b| = 4 \times 2.5 \text{ nm} = 10.0 \text{ nm}
\]

\[
|c_1|=|c_2|=\frac{\sqrt{|a|^2+|b|^2}}{2} = 17.7 \text{ nm}
\]

\[
\phi = 180^\circ - 2 \times \arctan\left(\frac{10.0 \text{ nm}}{33.9 \text{ nm}}\right) = 147^\circ
\]

For bLW-E21/5,
assuming that the 10 bp bridge forms a rectangle at the center, with two edges at 3.4 nm (the length of a full turn) and 2.0 nm (the diameter of a helix), then its diagonal length is $\sqrt{3.4 \text{nm}^2 + 2 \text{nm}^2} = 3.9 \text{nm}$,

$$\varphi = 180^\circ - 2 \arctan\left(\frac{2 \text{nm}}{3.4 \text{nm}}\right) = 120^\circ$$

$$| \mathbf{c}_1 | = | \mathbf{c}_2 | = 0.324 \frac{\text{nm}}{bp} \times (32 + 21) \text{bp} + 3.9 \text{nm} = 21.1 \text{nm}$$

$$| \mathbf{a} | = 2 \times | \mathbf{c}_1 | \times \cos 30^\circ = 2 \times 20.9 \text{nm} \times 0.866 = 36.1 \text{nm}$$

$$| \mathbf{b} | = 2 \times | \mathbf{c}_1 | \times \sin 30^\circ = 2 \times 20.9 \text{nm} \times 0.5 = 21.1 \text{nm}$$

For TW-E21/5,

$$| \mathbf{a} | = 2 \times (22 + 20) \text{bp} \times 0.324 \frac{\text{nm}}{bp} = 26.9 \text{nm}$$

$$| \mathbf{b} | = 6 \times 2.5 \text{nm} = 15.0 \text{nm}$$

$$| \mathbf{c}_1 | = | \mathbf{c}_2 | = \frac{\sqrt{| \mathbf{a} |^2 + | \mathbf{b} |^2}}{2} = 15.4 \text{nm}$$
\[ \varphi = 180^\circ - 2 \times \arctan \left( \frac{15.0 \text{ nm}}{26.9 \text{ nm}} \right) = 122^\circ \]

Similarly, the unit cell parameters \((a, b, c, \text{ and } \varphi)\) for each lattice of other assemblies, E-tiling assemblies of LW-E20/4, bLW-E26/6, LW-E31/5, LW-E31/7, TW-E32/6, and three O-tiling assemblies of LW-O26/4, bLW-O26/4, TW-O26/4, could be estimated in the same way. All experimental and theoretical unit cell parameters are listed in Table S1.

**Table S1. Unit cell parameters of all the assemblies in this work.**

| Assembly | \(a\) /nm | \(b\) /nm | \(c\) /nm | \(\varphi\) |
|----------|-----------|-----------|-----------|------------|
| LW-O26/4 | 37.4(37.1)| 12.0(10.0)| 21.1(19.2)| 149(150°)  |
| LW-E21/5 | 34.6(33.9)| 12.0(10.0)| 18.3(17.7)| 142(147°)  |
| bLW-O26/4| 36.3(38.9)| 26.9(22.5)| 22.6(22.5)| 107°(120°) |
| bLW-E20/4| 34.4(35.9)| 21.9(20.7)| 20.4(20.7)| 115°(120°) |
| bLW-E21/5| 35.1(36.1)| 23.7(21.1)| 21.2(21.1)| 112°(120°) |
| bLW-E22/6| 36.3(37.1)| 22.7(21.4)| 21.4(21.4)| 116°(120°) |
| bLW-E31/5| 41.2(42.1)| 25.7(24.0)| 24.3(24.0)| 116°(120°) |
| bLW-E31/7| 41.9(42.1)| 24.2(24.3)| 24.2(24.3)| 120°(120°) |
| bLW-E32/6| 42.2(42.7)| 24.9(24.3)| 24.5(24.3)| 119°(120°) |
| TW-O26/4 | 30.6(30.5)| 14.7(15.0)| 17.0(17.0)| 129°(128°) |
| TW-E21/5 | 27.3(26.9)| 15.2(15.0)| 15.6(15.4)| 128°(122°) |

# The measured parameters from high-resolution AFM images of each assembly are followed with theoretically estimated parameters in brackets. The experimental data were in accordance with theoretical estimations.

**S3 Tube parameters calculated from the chiral indices \((n,m)\)**

The chiral indices \((n,m)\) of a specific tube were used to calculate the tube perimeter, helical angle \(\alpha\), and the tube axial periodicity \(|T|\) as follows:
Figure S4. The chiral indices \((n,m)\) of a specific DNA nanotube and its unit cell parameters.

The grid pattern represents a 2D lattice background, which is defined with the basis vectors \(a\) and \(b\), the primitive vectors \(c_1\) and \(c_2\), and the inter-angle \(\phi = 120^\circ\) at the bottom left corner. The shadowed green rectangle is the radial projection of a DNA nanotube \((3,3)\) unit cell, which can be illustrated with the perimeter vector \(Ch(n,m)\), the tubule axis vector \(T\), and the helical angle \(\alpha\) (defined as the inter-angle between \(Ch(n,m)\) and \(c_2\)). The unit cell parameters can be theoretically estimated from the chiral indices \((n,m)\), \(c\), and the inter-angle \(\phi\).

For a DNA nanotube of given chiral indices \((n,m)\), its perimeter vector is

\[
Ch = (n, m) = nc_1 + mc_2
\]

which has a magnitude (perimeter of the tube)

\[
|C| = C = |c_1|\sqrt{n^2 + m^2 + 2nm \cos \phi} \quad 0 \leq |m| \leq n \quad (1)
\]

When \(\phi = 120^\circ\),

\[
|C| = C = c\sqrt{n^2 + m^2 - nm}. \quad 0 \leq |m| \leq n \quad (2)
\]

Here, to keep both \(n\) and \(m\) of the chiral indices \((n,m)\) in positive integers, we use \(\phi = 120^\circ\), different from the \(\phi = 60^\circ\) for carbon nanotubes.

The diameter, \(D\), of the DNA nanotube is:

\[
D = \frac{c}{\pi} \quad (3)
\]

Similar to carbon nanotubes’ formula, through trigonometry, the helicity \(\alpha\), was obtained as follows:
\[
\cos \alpha = \frac{(2m-n)}{2\sqrt{n^2 + m^2 - nm}} \\
\sin \alpha = \frac{\sqrt{3} n}{2\sqrt{n^2 + m^2 - nm}} \\
\tan \alpha = \frac{\sqrt{3} n}{2(m - n)} \\
60^0 \leq \alpha \leq 150^0 \quad (4)
\]

d, the highest common divisor of (n,m) \\
d_R, the highest common divisor of \((2n+m,2m+n)\) \\
T, length of \(T\) \\
\[
T = \frac{\sqrt{3}C}{d_R} \quad (5)
\]

According to reference\(^1\), by defining \(\phi = 120^\circ\) (in practice, \(\phi\) could vary a little around \(120^\circ\) due to the DNA spring-like structure), all lattice parameters (the helicity \(\alpha\) and the axial periodicity \(T\)) can easily be obtained from the chiral indices \((n,m)\) and \(c\), as shown in equations (1) to (5). Except for the tube perimeters, other parameters are not discussed further here because they are beyond the scope of this work.

**S4 Assignment of the chiral indices \((n,m)\) to a specific tube based on its high-resolution AFM image**

Assignment of the chiral indices \((n,m)\) to a specific tube was made possible by directly counting the number of \(c_1\)- and \(c_2\)-frames to form a perimeter vector according to its high-resolution AFM images. For example, the bLW-E\(_{31/7}\) assemblies were uniform tubes with the same perimeter, which can be defined only to the chiral indices \((3,3)\) by counting the number of \(c_1\)- and \(c_2\)-frames from the well-resolved uniform tubes (block 1 of Figure S18). However, for most of the infinite tubes built by periodic packing of the same tile such as bLW-E\(_{21/5}\), bLW-E\(_{32/6}\), and TW-E\(_{21/5}\), each design generated the same type of infinite tubes with very similar structures but perimeter variations occurred in a narrow window due to small changes of the curvature during the assembly. A cluster of chiral indices \((n,m)\) were assigned to the same type of tubes with small perimeter variations. In Figures S5 to S9, we illustrate the assignments of \((6,3)\), \((8,3)\), \((8,4)\), \((10, 4)\), and \((10, 5)\) by directly counting the number of \(c_1\) and \(c_2\)-frames to form a perimeter vector in their corresponding high-resolution AFM images of the bLW-E\(_{21/5}\) tubes with both open and closed structures.
Figure S5. Assignment of the chiral indices (6,3) for a bLW-E$_{24/5}$ tube. The top layer of the tube appears to have been almost completely removed. Fortunately, the top c$_1$-frames’ traces remained. Thus, (n,m) could be counted directly, as shown in the figure. The measured perimeter is $59.3 \times 2 = 118.6$ nm, which is in accordance with the calculated perimeter of 119.0 nm from equation (1) in Table S2.

Figure S6. Assignment of the chiral indices (8,3) for a bLW-E$_{24/5}$ tube. The double-layered region of the tube exhibits five bottom c$_1$-frames stuck on mica (two edges at the double-layer region were broken by air-drying), and the bottom left region shows a fully open tube with 8 c$_2$-frames. The
measured perimeter is $81.5 \times 2 = 163.0$ nm, which was in accordance with the calculated perimeter of $157.3$ nm from equation (1) in Table S2.

Figure S7. Assignment of the chiral indices (8,4) for a bLW-E$_{21/5}$ tube. This represents an open tube with $c_1$-frames’ traces left on its top. The measured perimeter was $80.7 \times 2 = 161.4$ nm, which was in accordance with the calculated perimeter of $158.8$ nm from equation (1) in Table S2.

Figure S8. Assignment of the chiral indices (10,4) for a bLW-E$_{21/5}$ tube. The upper double layer shows the tube structure in closed form, and the lower monolayer shows the tube structure clearly.
in the open form. Therefore, the chiral indices (10,4) could be clearly counted. The measured perimeter is $95.8 \times 2 = 191.6$ nm, which was in accordance with the calculated perimeter of 196.6 nm from equation (1) in Table S2.

![Image](image.png)

**Figure S9. Assignment of the chiral indices (10,5) for a bLW-E21/5 tube.** The tube’s top layer was clearly imaged, while the texture of its bottom layer could only be seen in the broken region. By shifting a bottom $c_1$-frame in parallel to the tube’s top part, the chiral indices (10,5) could be counted. The measured perimeter was $101.2 \times 2 = 202.4$ nm, which was in accordance with the calculated perimeter of 198.4 nm from equation (1) in Table S2.

**S5 Assignments of a cluster of chiral indices by the numerical approximation method to both bLW-E21/5 and TW-E21/5 tubes**

As shown in the above Section S4, to assign the chiral indices (n,m) to every individual tube by means of its high-resolution AFM images was possible but very time-consuming. An uncertainty also exists for the correct counting of $n$, the number of $c_2$-frames, because we may count 1 fewer than the real number at the top layer of a tube. For most of the bLW-E21/5 tubes with perimeters distributed in the range from 110 to 230 nm, we show the numerical calculation results in Table S2. Combining numerical methods and high-resolution imaging results together, we assigned the (n,m) sets in
Table S2 to match the 4 perimeter windows in Figure 2C(c): 110~150 nm with the abundance of 20/73 = 27.4\% to \(l-(6,2), \, l-(6,3), \, \) and \(l-(7,3); \) 150~190 nm with the abundance of 34/73 = 46.6\% to \(l-(8,3), \, l-(8,4), \, \) and \(l-(9,4); \) 190~230 nm with the abundance of 16/73 = 21.9\% to \(l-(10,4), \, l-(10,5), \, \) and \(l-(11,5); \) and a very few wider tubes with the abundance of 3/73 = 4.1\% to \(l-(12,5)\) and \(l-(12,6).\)

**Table S2. The paraxial cluster of the chiral indices \((n,m)\) for bLW-E21/5 tubes in Figure 2C(c).**

| Perimeter window /nm | 110~150 | 150~190 | 190~230 | 230~270 |
|----------------------|---------|---------|---------|---------|
| Chiral indices       | (6,2), (6,3), (7,3), (7,4), (8,3), (8,4), (8,5), (9,4), (9,5) | (10,4), (10,5), (11,5), (12,5), (12,6) |         |         |
| C(calculated) /nm    | 118, 119, 124, 138, 141, 157, 159, 165, 177, 180 | 197, 198, 217, 236, 238 |         |         |
| Tube No.             | 20      | 34      | 16      | 3       |

# We assign the measured perimeters of the bLW-E21/5 tubes into 4 windows listed in the first row and their corresponding tube numbers in the last row. The perimeter \(C\) of each set of chiral indices was calculated from equation (1) (Section S3) with experimental data of \(c = 21.2\) nm and \(\phi = 112^\circ.\)

We listed the identified (in black), possible (in green), and forbidden (in red) \((n,m)\) sets and their corresponding perimeters in the second and third rows according to the analysis of high-resolution AFM images.

The bLW-E21/5 tubes can be represented with a cluster of chiral indices \((n,m)\), where \(n = 2m+2,\) \(2m+1,\) and \(2m,\) and \(m = 3, 4,\) and \(5,\) as shown in the 2D Cartesian coordinates \((m,n)\) of Figure S10.

Note that in their high-resolution AFM images, we did not find the following chiral indices, \(l-(6,4), \, l-(8,2), \, l-(8,5), \, l-(10,3), \, l-(10,6), \) and \(l-(12,7).\) We suggest that these chiral indices were forbidden to form because the inter-angle \(\phi\) measured at the range \(110^\circ \leq \phi \leq 120^\circ\) in Tables 1 and S1 limits their formation. In addition, \(l-(7,3)\) and \(l-(9,4)\) with \(n\) at the odd numbers of 7 and 9, should occur with higher frequencies because they fall at the region of highly abundant tubes in

![Figure S10. The chiral indices \((n,m)\) with the abundance weighted with the circle size for bLW-E21/5 tubes (n at even numbers represented with a filled black circle and at odd numbers with a filled green circle).](attachment:figure_s10.png)
Figure S10. Small changes of perimeters in an individual bLW-E_{21/5} tube were observed occasionally, which suggested the occurrence of lattice dislocations by switching between adjacent sets of the chiral indices (n,m), for example, from (6,3) to (7,3), (7,3) to (8,3), (8,3) to (8,4), (8,4) to (9,4), and (9,4) to (10,4), etc.

For the TW-E_{21/5} tubes, we judged the chiral indices (n,m) with a limitation of n = m from their high-resolution images (Figures 3B(c) and S21). Therefore, it was straightforward to calculate the perimeters from equation (1) with (n,m) from (4,4) to (9,9), which are listed in Table S3. The tube abundances falling in 6 perimeter windows are presented in Figure 3B(g) and also listed in Table S3.

Table S3. Assignments of a cluster of the chiral indices (n,m) by numerical approximation to TW-E_{21/5} tubes.#

| Perimeter window /nm | 45~60 | 60~75 | 75~90 | 90~105 | 105~120 | 120~135 |
|----------------------|-------|-------|-------|--------|---------|---------|
| Chiral indices       | (4,4) | (5,5) | (6,6) | (7,7)  | (8,8)   | (9,9)   |
| C(calculated) /nm    | 53.7  | 67.1  | 80.5  | 93.9   | 107.3   | 120.7   |
| Tube No.             | 6     | 14    | 21    | 14     | 9       | 3       |

# We assigned the measured perimeters of TW-E_{21/5} tubes into 6 windows listed in the first row and their corresponding tube numbers in the last row. The perimeter C of each set of chiral indices was calculated from equation (1) (Section S3) with experimental data of c = 15.3 nm and \( \phi = 128° \). We listed the chiral indices (n,m) sets and their corresponding perimeters in the second and third rows combining the analysis of their high-resolution AFM images.

S6 Saddle-like tile oligomer model to simultaneously grow “large” and “giant” tubes

The paired group of “orthogonal” tubes in bLW-E_{32/6} assemblies have been seldom observed in other DNA assemblies. Growth of “giant” tubes with their axes along \( \mathbf{b} \) is thermodynamically disfavored. To simultaneously grow such “orthogonal” tubes, we suggested the following saddle-like model of tile oligomers at the initial growth stage as their growth mechanism.
Figure S11. Saddle-like tile oligomer model to grow “large” and “giant” tubes simultaneously.

The mechanism of simultaneous growth of “large” and “giant” tubes is suggested: 1) A saddle-like oligomer forms at the initial assembly stage, 2) the competing closure of ring seeds leads to epitaxial growth of “large” and “giant” tubes.

S7 Additional AFM images

For the LW-E$_{21/5}$ (Figure S12), LW-O$_{26/4}$ (Figure S13), bLW-O$_{26/4}$ (Figure S14), and TW-O$_{26/4}$ (Figure S21) assemblies, more additional zoomed-out and zoomed-in AFM images are provided. For all the E-tiling tubes, we show additional AFM images in different blocks: the 1$^{st}$ block, the schematic tube model(s) in the first panel, followed with more additional zoomed-out and zoomed-in AFM images; the 2$^{nd}$, 3$^{rd}$, or 4$^{th}$ blocks, a schematic brick lattice model with a biotin/SA label at overhang 2 (or 1*, or 1) per tile in $l$-face (blue) or $r$-face (green) representing the inside surface of the designed tube in the first panel, followed with zoomed-out AFM images to confirm the chirality evaluation. Due to the imaging resolution, we only relied on the SA dots overlain on the monolayer lattice to define the chirality of the tube and thus that of its component tile. Sometimes, SA dots overlain on both monolayer and double layer lattices of a tube are also shown. The overlapping of two sets of SA dots and two sets of lattice grids from bottom and top layers on double layered tubes made the geometric relationship of every biotin/SA dot to its host tile very challenging to define.
Figure S12. LW-\text{E}21/5 assemblies of planar ribbons, heterogeneous tubes, and nanofibers. In zoomed-out images (1-5) scanned in air, nanofibers appear simultaneously with planar ribbons, indicating the stiffness of LW tile cores. Zoomed-in images (6-8) in fluid distinguish tiny changes of nanoscale textures of top layers from bottom layers.

Figure S13. LW-\text{O}_26/4 assemblies of planar ribbons. Depending on the scanning conditions such as the tip sharpness, planar ribbons (panels 1, 2, 6, 7) did not show clear nanoscale textures. Repeating scans more than 4 times can help distinguish nanoscale textures clearly, but such repeating scans removed some tiles and left empty pores on the lattices (panels 3, 5, 8).
Figure S14. bLW-O$_{26/4}$ assemblies. The bLW-O$_{26/4}$ assemblies mostly appeared as DNA tubes with heterogeneous perimeters, probably due to the flip-over of the intrinsic curvature of bLW tile cores behaving like the flip-over of contact lenses.
Figure S15. **bLW-E$_{21.5}$ assemblies.** 1st block: panel 1 shows a schematic of the typical tube model of l-(8,4) with tiles’ $l$-faces inside and $r$-faces outside (top) and a schematic of its component brick tile model illustrating the $l$-face and 4 overhang ($1^*$, 2, and $2^*$) positions (bottom); panels 2 to 4 are zoomed-out images in fluid without air-drying showing relatively homogeneous tubes in high
yield; panels 5 to 8 are zoomed-out images in air after air-drying showing tubes in both open and closed forms, in which open tubes in panels 7 and 8 demonstrate that nearly all tubes’ axes are parallel to $c_2$-frames. 2nd block: panel 1 is a schematic of the lattice model of $l$-faced tiles with each tile carrying a biotin/SA label at overhang 2; panels 2 through 11 represent SA dots overlain on air-blown tubes showing the clockwise rotation relationship from each SA dot sitting on a $c_2$-framing arm to its adjacent two $c_1$-framing arms via the acute angle on monolayer lattices. 3rd block: panel 1 is a schematic of the lattice model of $l$-faced tiles with each tile carrying a biotin/SA label at overhang 1*; panels 2 through 11 represent SA dots overlain on air-blown tubes showing the counterclockwise rotation relationship from each SA dot sitting on a $c_1$-framing arm to its adjacent two $c_2$-framing arms via the acute angle on monolayer lattices. 4th block: descriptions are similar to the 3rd block, with a difference that each tile carries a biotin/SA label at overhang 1. In both the 3rd and 4th blocks with biotin/SA labels at overhangs 1* and 1, respectively, SA dots of both designs are located on $c_1$-framing arms. We couldn’t distinguish the two overhangs with SA dots inside the monolayer lattice. However, an additional imaging feature helped us to assign SA dots at overhang 1* or overhang 1. The feature was that a linear array of dangling SA dots protruded out of one $c_2$-framing edge of each open tube strip, while no dangling SA dots could be found at the other $c_2$-framing edge; this was due to the breaking-up of the sticky end cohesion at both edges and the biotin label sticking to its own overhang 1* or 1. Therefore, the dangling SA dots at overhang 1* in the 3rd block images were located at the opposite edge of the open tube strip relative to those at overhang 1 in the 4th block images. The chirality of the edge-dangling SA dots enabled us to distinguish the biotin/SA labels at overhang 1* or 1 possible.
Figure S16. bLW-E$_{20/4}$ assemblies. 1<sup>st</sup> block: panel 1 shows a schematic of the unique tube $l$-(4,2) (top) and a schematic of its component tile illustrating the $l$-face and 4 overhang positions (bottom); panel 2 is a zoomed-out image in fluid showing the low product yield of tubes; panels 3 and 4 are zoomed-in images in fluid after air-drying showing the unique tube $l$-(4,2) structure. 2<sup>nd</sup> block: panel 1 is a schematic of the lattice model of $l$-faced tiles with each tile carrying a biotin/SA label at overhang 2; panels 2 through 6 represent SA dots overlain on air-blown tubes showing the clockwise rotation relationship from each SA dot sitting on a $c_2$-framing arm to its adjacent two $c_1$-framing arms via the acute angle on monolayer lattices. 3<sup>rd</sup> block: panel 1 shows a schematic lattice model of $l$-faced tiles with each tile carrying a biotin/SA label at overhang 1*; panels 2 through 6 represent SA dots overlain on air-blown tubes showing the counterclockwise rotation relationship from each SA dot sitting on a $c_1$-framing arm to its adjacent two $c_2$-framing arms via the acute angle on monolayer lattices.
Figure S17. bLW-E22/6 assemblies. 1st block: panel 1 shows a schematic of the typical tube \( r-(6,6) \) (top) and a schematic of its component tile illustrating the \( r \)-face and 4 overhang positions (bottom); panels 2 through 4 represent zoomed-out and zoomed-in images in fluid after air-drying, demonstrating a low product yield of tubes. 2nd block: panel 1 is a schematic of the lattice model of \( r \)-faced tiles with each tile carrying a biotin/SA label at overhang 2; panels 2 through 5 represent SA dots overlain on air-blown tubes showing the counterclockwise rotation relationship from each SA dot sitting on a \( c_2 \)-framing arm to its adjacent two \( c_1 \)-framing arms via the acute angle on monolayer lattices. 3rd block: panel 1 is a schematic of the lattice model of \( r \)-faced tiles with each tile carrying a biotin/SA label at overhang 1*; panels 2 through 5 represent SA dots overlain on air-blown tubes showing the clockwise rotation relationship from each SA dot sitting on a \( c_2 \)-framing arm to its adjacent two \( c_1 \)-framing arms via the acute angle on monolayer lattices.

Figure S18. bLW-E31/5 assemblies. 1st block: panel 1 shows a schematic of the typical tube \( l-(10,5) \)
(top) and a schematic of its component tile illustrating the $l$-face and 4 overhang positions (bottom); panels 2 and 3 are zoomed-out images in fluid before air-drying showing a low product yield of tubes; panel 4 is a zoomed-in image showing the typical $l$-(10,5) tube structure. 2nd block: panel 1 is a schematic of the lattice model of $l$-faced tiles with each tile carrying a biotin/SA label at overhang 2; panels 2 through 6 represent SA dots overlain on open tubes showing the clockwise rotation relationship from each SA dot sitting on a $c_2$-framing arm to its adjacent two $c_1$-framing arms via the acute angle on monolayer lattices.

Figure S19. **bLW-E$_{31/7}$ assemblies.** 1st block: panel 1 shows three projections of a schematic unique tube $r$-(3,3) in different directions (top) and a schematic of its component tile illustrating the $r$-face and 4 overhang positions (bottom); panels 2 through 4 are zoomed-out images in fluid before air-drying showing a high product yield of tubes; panels 5 to 7 are zoomed-in images in fluid before air-drying demonstrating the unique $r$-(3,3) tube structures with different projection directions. 2nd block: panel 1 is a schematic of the lattice model of $r$-faced tiles with each tile carrying a biotin/SA label at overhang 2; panels 2 through 6 show SA dots overlain on open tubes showing the counterclockwise rotation relationship from each SA dot sitting on a $c_2$-framing arm to its adjacent two $c_1$-framing arms via the acute angle on monolayer lattices. 3rd block: panel 1 is a schematic of
the lattice model of \( r \)-faced tiles with each tile carrying a biotin/SA label at overhang \( 1^* \); panels 2 through 6 show SA dots overlain on open tubes showing the clockwise rotation relationship from each SA dot sitting on a \( c_1 \)-framing arm to its adjacent two \( c_2 \)-framing arms via the acute angle on monolayer lattices.

**Figure S20.** \( \text{b LW-E}_{32,6} \) assemblies. 1st block: panel 1 shows a schematic of the “giant” tube \( l-(23,23) \) with its component tile’s \( l \)-face (upper part) and a schematic of the “large” tube \( r-(16,16) \) with...
its component tile’s \( r \)-face (lower part); panels 2 through 7 show two groups of zoomed-out and zoomed-in images of co-existing “large” and “giant” tubes in fluid before air-drying (one group is from 2 to 4, and the other group is from 5 to 7). Two highly qualified zoomed-in images show one “large” tube \( r-(19,19) \) (panel 4) and one “giant” tube \( l-(24,-24) \) (panel 7), in which \( n \) and \( m \) in the chiral indices \((n,m)\) are counted directly from their images. 2\textsuperscript{nd} block: panel 1 shows a schematic of the “giant” tube assembly model of \( l \)-faced tiles with each tile carrying a biotin/SA label at overhang 2; panels 2 through 6 show SA dots overlain on air-blown tubes showing the clockwise rotation relationship from each SA dot sitting on a \( e_2 \)-framing arm to its adjacent two \( c_1 \)-framing arms via the acute angle on monolayer lattices. 3\textsuperscript{rd} block: panel 1 shows a schematic of the “large” tube assembly model of \( r \)-faced tiles with each tile carrying a biotin/SA label at overhang 2; panels 2 through 11 show SA dots overlain on air-blown tubes showing the counterclockwise rotation relationship from each SA dot sitting on a \( e_2 \)-framing arm to its adjacent two \( c_1 \)-framing arms via the acute angle on monolayer lattices. 4\textsuperscript{th} block: panel 1 shows a schematic of the “giant” tube assembly model of \( l \)-faced tiles with each tile carrying a biotin/SA label at overhang 1\*; panels 2 through 5 show SA dots overlain on open tubes showing the counterclockwise rotation relationship from each SA dot sitting on a \( c_2 \)-framing arm to its adjacent two \( c_1 \)-framing arms via the acute angle on monolayer lattices. 5\textsuperscript{th} block: a schematic of the “large” tube assembly model of \( r \)-faced tiles with each tile carrying a biotin/SA label at overhang 1\*; panels 2 through 4 show SA dots overlain on open tubes showing the clockwise rotation relationship from each SA dot sitting on a \( c_1 \)-framing arm to its adjacent two \( e_2 \)-framing arms via the acute angle on monolayer lattices.
Figure S21. TW-O$_{26/4}$ assemblies of planar handkerchief-like lattices. TW-O$_{26/4}$ assemblies often show handkerchief-like multilayers. More than 4 scans were needed to achieve individual tile textures in zoomed-in images (panels 5 through 8).
Figure S22. TW-E21/5 assemblies. 1st block: panel 1 shows a schematic of the typical tube r-(6,6) (top) and a schematic of its component tile indicating a biotin label position and the direction at the l-face (dark dot) and another biotin label at the r-face (gray dot); panels 2 through 4 are zoomed-out images showing a high product yield of tubes; panels 5 through 7 are zoomed-in images in fluid
with individual tile textures achieved through repeated scans more than 4 times. 2nd block: panel 1 is a schematic of the tube assembly model with each tile carrying a biotin/SA label at overhang 2 protruding out of its \( l \)-face; panels 2 through 11 show SA dots on air-blown tubes only appearing on the tops of double layers, but not on monolayer surfaces. 3rd block: panel 1 is a schematic of the tube assembly model with each tile carrying a biotin/SA label at overhang 1\(^*\) protruding out of its \( r \)-face; panels 2 through 11 show SA dots on air-blown tubes only appearing on monolayer surfaces, but not on the tops of double layers. 4th block: panel 1 is a schematic of the tube assembly model with each tile carrying both biotin/SA labels at overhang 2 and overhang 1\(^*\) protruding out of its both \( l \)-face and \( r \)-face; panels 2 through 11 show SA dots on air-blown tubes appearing on both surfaces of monolayers and double layers. The above images indicate that the intrinsic tile curvature of TW-E21/5 tubes follows the right-hand grip rule.

**S7 Sequence information**

**LW-O_{26/4}**

\[
c_{128\text{nt}}: \quad 5' - TAAGATGAAGATAGCGCACAATGGTGCCGATCCTCGTCTCTGTCAA \\
CTCGTCTATGCAAGCCCTGCTCAGCTGTGATCATATGCTAGTCC \\
TGTAGGTCGACGCACCTGCGGTTCGCATGGGCTATC -3'
\]

\[
\text{H1}: \quad 5' - TGCGACTCGTAACTGTGATACGGCATCCG -3'
\]

\[
\text{H2}: \quad 5' - GGATGTGTCGACCTAGCGTGCTG -3'
\]

\[
\text{H3}: \quad 5' - CGCACCGATGCAACCCACGTAGTACCCGGA -3'
\]

\[
\text{H4}: \quad 5' - TGATTGCGGCCGCTGGCTGGCTGGCTG -3'
\]

\[
\text{M1}: \quad 5' - GGCTCAGCACGCTGACGCTATCTTCAGGCTGCTAGT -3'
\]

\[
\text{M2}: \quad 5' - TGCCGTATCGAGCCAGGTCGTGCGACCGA \\
ATCCGACCATTGTGTGGGCAGCATCCGGA -3'
\]

\[
\text{M3}: \quad 5' - AGCCCAACGACGAGTGACAGAGA -3'
\]
CGTACAGGACTAGCATAGGGTTGCATCGG -3'
M4: 5’- GGTACTACGTGTATGATCACAGCTGAGCA
     GGGCTTGGCATAGACGCGGCAATCATCAC -3’
LW-E21/5

LWE-H1
LWE-M2
LWE-H3
LWE-M4
LWE-M1
LWE-H2
LWE-M3
LWE-H4

c128nt: 5’- TAAGATGAAGATAGCGCACAATGGTGGATTCGATTCCGTCTCTGTCAA
     CTCGTCTATGCCAAGCCCTGCTAGCTGATGACATACATGCTAGTCC
     TGTAGGTCGACACGTGGCGTGGCATTGACCTATC -3’
H1: 5’- ACTGCAGTGTAAGCTCTTACGTCA -3’
H2: 5’- GTTTGAGACTGTAGCC -3’
H3: 5’- CCATCCCACAGAATGCGTTCGTGCAC -3’
H4: 5’- ACCATTACACTACTAG -3’
M1: 5’- GATGGGGCTACAGCGCTATCTTCATCTCTT
     AGATAGGCCATGCGAATTCACACTCTCTT -3’
M2: 5’- TAAGAGCTGCCAGGTCGTGCAGACCAAT
     CCGAACCAGTGTCTCAACACGCTAC -3’
M3: 5’- GCACTAGTCTAGTCTCGAGTTGACAGAGA
     CGTACAGGACTAGCATAGCTCGTGG -3’
M4: 5’- GAACGCATTATGATCACAGCTGACGCA
     GGGCTTGGCATAGATGGTTGACG -3’

bLW-O26/4
c128nt: 5’- TAAGATGAAGATAGCGCACAATGGTCGGATTTCCGTCTCTGTGCTAACTTCGTCTATGCCAAGCCCTGCTCAGCTGTGATCATACTATGCTAGTCCCTGTCGACGACCTGGGCCGTTCGCATGGCCCTACATC -3’

H1: 5’- TGCGACTCGTAACTGTCGATACGGCATCCG -3’

H2: 5’- GGATGGTCGCCATCAGCGTGCTG -3’

H3: 5’- CGCACCCGTGCAAACCCACGTAGTGACCCGGGA -3’

H4: 5’- TGATTGCCGCGTCTGGCTCGTG -3’

M1: 5’- GGCTCAGCAGCGCTGACGAGTTGACACAGAGACGGAATCCAGCCATTGTGCAGTTACGAGT -3’

M2: 5’- TGCCGTATCGACGCTATCTTCATCTTACAAGGGCTTGGCATAGATGGCGACATCCGTGA-3’

M3: 5’- AGCCCAACCGAGCCATGATCAGCTAAGGGATAGGCCATGCGAACGGTTGCATCGG-3’

M4: 5’- GGTACTACGTGGCCAGGTCGTGCGACCTACAGGACTAGCATAGCGCGGCAATCATCAC-3’

bLW-E_p/q
c128nt: 5'- CCCGAACACCTCCCATGCAGTCTCCCTGCTCTGACTACGTCAA
       GCGTCGGTGTGATGGTTATAAGGTCCCCTCTTTCAATTCCGAA
       ATACCTCACGGATCATCCGTAGTGCTCCTCCAAGAGGAGGTGA-3'

bLW-E20/4
H1:  5'- CCATCCCACGAGATGCGTTCGAGC-3'
H2:  5'- GGCTACAGTCTCAAAAC-3'
H3:  5'- ACTGCAGTGTGAAGCTCTTACGCA-3'
H4:  5'- CTAGTAGTGTAATGGT-3'
M1:  5'- CAGTGTTTGAGAATGGGAGGTGTTCGGGTCACTCCTCTTGGAGGCTCGTGGG-3'
M2:  5'- GAACGCATCACTACGGATGATCCGGTCATACGACAGAGCAGGGAGACTGCCTGTAGCCTGCG-3'
M3:  5'- ATGGACCATTACGACGCTTGACGTAGTCGTCGTATGACTGAGGTATTTCGGAATTCACACTG-3'
M4:  5'- TAAGAGCTTGAAAGAAGCGGGACCTTATAACCATCACACCACTACTAGGCTC-3'
H2-biotin: 5'-/Biotin/ GGCTACAGTCTCAAAAC-3'
H4-biotin: 5'-/Biotin/ CTAGTAGTGTAATGGT-3'

bLW-E21/5
H1:  5'- CCATCCCACGAGATGCGTTCGTCAGC-3'
H2:  5'- GGCTACAGTCTCAAAAC-3'
H3: 5’- ACTGCAGTGTAAGGCCGCTTTAGCAGTCA-3’
H4: 5’- CTAGTAGTGTAATGGT-3’
M1: 5’- GCAGTGTTTGAGAATGGGAGGTGTTC
      GGGTCACCTCCTTTGGAGGAGGTCTCGTG-3’
M2: 5’- GAACGCACCATCAGGGATGATCCGTA
      CATACGACAGGCAGGGAGATGCCTGTAGAGCTG-3’
M3: 5’- GATGGACCATACGAGCTTGGACGTA
      GTCGTCGATTAGTGAGGTATTTCGGAAATTTTCACACT-3’
M4: 5’- TAAGAGCTTTGAAAGAGCGGGGACCT
      TATAACCATCACCACACTAGGCTAC-3’
H1-biotin: 5’-/Biotin/ CCATCCCACGAGAATGCGTTCGTAGCGC-3’
H2-biotin: 5’-/Biotin/ GGCTACAGTCTCAAAC-3’
H4-biotin: 5’-/Biotin/ CTAGTAGTGTAATGGT-3’

**bLW-E22/6**

H1: 5’- CCATCCCACGAGAATGCGTTCGTAGCGC-3’
H2: 5’- GGCTACAGTCTCAAAC-3’
H3: 5’- ACTGCAGTGTAAGGCCGCTTTAGCAGTCA-3’
H4: 5’- CTAGTAGTGTAATGGT-3’
M1: 5’- TGCAGTGTTTGAGAATGGGAGGTGTTC
      GGGTCACCTCCTTTGGAGGAGGTCTCGTG-3’
M2: 5’- CGAACGCACCATCAGGGATGATCCGTA
      CATACGACAGGCAGGGAGATGCCTGTAGAGCTG-3’
M3: 5’- GATGGACCATACGAGCTTGGACGTA
      GTCGTCGATTAGTGAGGTATTTCGGAAATTTTCACACT-3’
M4: 5’- TAAGAGCTTTGAAAGAGCGGGGACCT
      TATAACCATCACCACACTAGGCTAC-3’
H2-biotin: 5’-/Biotin/ GGCTACAGTCTCAAAC-3’
H4-biotin: 5’-/Biotin/ CTAGTAGTGTAATGGT-3’

**bLW-E31/5**
H1: 5'- GTGCAGCAAGTGCTTAAACCATTGGTACAAG-3'
H2: 5'- GAATGAACTAGGGATAATAAGAGGACACAGA-3'
H3: 5'- ACCGACTAAATGATTGAGTCTCTAAACGCTAC-3'
H4: 5'- TACAAAAGTTCAACCATAAGCAGATAAACACCACC-3'
M1: 5'- GCCCTCTTATTATATGGTGAGGTGC
       GTCACTCCTCCTTGGAGGGACTTGCTGCACGCTGG-3'
M2: 5'- ACCAATGTTAAACCACACTACGAGATCCCGGATCAGCGCAGA-3'
M3: 5'- TTTATCGTCTATGGACGCTTTGACGTAGTCTCGTGATG
       ACTGAGGTATTTCGGAATTCTTAGTGTTCTTTGTG-3'
M4: 5'- CGTTAGGACTCAATGAAAGAAGCGGG
       ACCTTATAACCACACCGTTGAACCTTTGTACTCGT-3'
H2-biotin: 5'-/Biotin/ GAATGAACTAGGGATAATAAGAGGACACAGA -3'

bLW-E31/7

H1: 5'- GTGCAGCAAGTGCTTAAACCATTGGTACAAG-3'
H2: 5'- GAATGAACTAGGGATAATAAGAGGACACAGA-3'
H3: 5'- TACAAAAGTTCAACCATAAGCAGATAAACACCACC-3'
H4: 5'- ACCGACTAAATGATTGAGTCTCTAAACGCTAC-3'
M1: 5'- CCTCTTATTATATGGTGAGGTGC
       GTCACTCCTCCTTGGAGGGACTTGCTGCACGCTGG-3'
M2: 5'- CAATGTTAAACCACACTACGAGATCCCGGATCAGCGCAGA-3'
M3: 5'- TTAGGACTCAATGACGCTTGACGTAGTCTCTCGTGATG
       ACTGAGGTATTTCGGAATTGAACTTTGTATCTGTGC-3'
M4: 5'- TATCGTCTATGGTGAAAGAAGCGGGACCT
       TATAACCACATCACCACCATTAGTGCTTGTTGT-3'
H2-biotin: 5'-/Biotin/ GAATGAACTAGGGATAATAAGAGGACACAGA -3'
H4-biotin: 5'-/Biotin/ ACCGACTAAATGATTGAGTCTCTAACGCTAC-3'

bLW-E32/6
H1: 5’- GTGCAGCAAGTGTTATATGGTACAAGG-3’
H2: 5’- GAATGACTAGGGATAATAAGAGGGCACAGAC-3’
H3: 5’- ACCGACTAAATGATTGAGTCCTACTACGGTCTACG-3’
H4: 5’- TACAAATTTCAACCATAGACGATAAACCACCG-3’
M1: 5’- GCCCTCTTTATTATGGGAGGTGTTCGGGT
CAGTAGTATTTGCTGACCAGGCTGG-3’
M2: 5’- ACCAATGGTTAACCACCTACGGATCCTCAGGCTAGCCTACG
ACAGAGCAGGAGACTGCCCCTAGTTCATTCCGCTAGA-3’
M3: 5’- TTTATCGTCTATGGGACGTTCAGTCGTCGTA
TGACTGAGGTATTTGGGAAATTATTTACATTTAGTGCGTGTCTGT-3’
M4: 5’- CGTTAGGACTCAATGAAAGAAGCGGGACCTTATAA
CCACGACCTCGTTGAATCTTGTACCTTGT-3’
H2-biotin: 5’/-Biotin/ GAATGACTAGGGATAATAAGAGGGCACAGAC-3’
H4-biotin: 5’/-Biotin/ TACAAATTTCAACCATAGACGATAAACCACCG-3’
TW-O26/4

TW-O26/4

H1: 5’- GCTACAGACTAATTCTCAAACG -3’
H2: 5’- CTAGTAGTCCTTTGGTAATGGTCGAG -3’
H3: 5’- GATTGCGCTCGCTGTTTACGTCTC - 3’
H4: 5’- ACCACGAGATCCTATGCGTTCGCTTT -3’
H5: 5’- GAAGTGTAACATCGCTTTACCTCTTG - 3’

TW-O26/4

TW-O26/4

c128nt: 5’- TAAGATGAAGATAGCGCACAATGGTGGATCCGCTCTCTCTCTGCTCA
CTCGTCTATGCAAGCCCTGCTCAGCTGATGCATCACTACATGCTAGTCC
TGAGGGTGCAACTGGGCGTTCGGCCTATGCCCCTAC -3’
H1: 5’- GCTACAGACTAATTCTCAAACG -3’
H2: 5’- CTAGTAGTCCTTTGGTAATGGTGAG -3’
H3: 5’- GATTGCGCTCGCTGTTTACGTCTC - 3’
H4: 5’- ACCACGAGATCTATGGTGCTTT -3’
H5: 5’- GAAGTGTAACATCGCTTTACCTCTTG - 3’
H6: 5’- GGAGGTAAATTGGTGAGGGCTACTA -3’
M1: 5’- CGAACGCATAGATAGACGAGTTATT
    GTGCCTAAAGTCTCGTAGCGTCTG -3’
M2: 5’- GTTGGGAGTCTCGTGCTGCTG -3’
M3: 5’- GGATAGCAGCGAGGAGGAGCG
    AATCTGGAGACTACTAGGAGA -3’
M4: 5’- ACCATTACCAAGATAGCGACCTACA
    GCTGAGGTTTCACACTCAAAG -3’
M5: 5’- GCCTTCACCACAGAGACTATCGT
    GGCCATGGAGCGCAATCTAGT -3’
M6: 5’- CCGAAACAGCCAGCAGCCAGGCA
    AGTATGAATTTTACCCAGA -3’

TW-E21/5

c128nt: 5’- TAAGATGAAGATAGCGCACAATGGTCGGATTCCGTCTCTGTCAA
    TCTCGTCTATGCCAAGCCCTGCTGCTCAGCTGTATGATCATACTATGCTAGTCC
    TGTAAGGTCGACACCTGGCCGTCGATGCGCTGCTTCTAATC -3’
H1: 5’- TACAGACTAATTCTCAAACA-3’
H2: 5’- TTCTAGTAGTCCTTTGGTAATGGTCG-3’
H3: 5’- TTGATATGCGCTGCTGTGTCTTTA -3’
H4: 5’- TCAAACGAGATCCTATGCGTCGAC -3’
H5: 5’- GTGTGAAACTCAGCTCTT -3’
H6: 5’- AGAAGGTTAAATTGGTGAGGTGTT -3’
M1: 5’- ACGCATAGATAGACGAGTTATTGTGCCTAAGTGCTGAGCCA -3’
M2: 5’-TGAGAATTTCTTCACGACCGACAGCTTGGCGATCTCGT-3’
M3: 5’-CGTCAAGAGCTGACAGGGAGACGGAATCTCTTTAAGGACTACT-3’
M4: 5’-CATTACCAAGATAAGCGACCTACAGCTGAGGTTTCACACTGGC-3’
M5: 5’-CCTCACCATCAGGACTATCGTGCCATGGAGCGCAGCAA-3’
M6: 5’-GACGTAACACAGCCGAAACGCCAGGGCATAATGATGAATTTAACC-3’
M2-biotin: 5’-TGAGAATTTCTTCACGACG
ACAGCT/iBiodT/GGCGATCTCGT-3’
M5-biotin: 5’/-Biotin/ CCTCACCATCAGGACTATCGTGCCATGGAGCGCAGCAA-3’

1. M. S. DRESSELHAUS; G. DRESSELHAUS; SAITO, R., Physics of carbon nanotubes. *Carbon* 1995, 33(7), 883-891.
Regulation of 2D DNA Nanostructures by the Coupling of Tile Curvatures and Arm Twists

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ABSTRACT: DNA overwinding and underwinding between adjacent Holliday junctions have been applied in DNA origami constructs to design both left-handed and right-handed nanostructures. For a variety of DNA tubes assembled from small tiles, only a theoretical approach of the intrinsic tile curvature was previously used to explain their formation. Details regarding the quantitative and structural descriptions of the intrinsic tile curvature and its evolution in DNA tubes by coupling with arm twists were missing. In this work, we designed three types of tile cores from a circular 128 nucleotide scaffold by longitudinal weaving (LW), bridging longitudinal weaving (bLW), and transverse weaving (TW) and assembled their 2D planar or tubular nanostructures via inter-tile arms with a distance of an odd or even number of DNA half-turns. The biotin/streptavidin (SA) labeling technique was applied to define the tube configuration with addressable inside and outside surfaces and thus their component tile conformation with addressable concave and convex curvatures. Both chiral tubes possessing left-handed and right-handed curvatures could be generated by finely tuning p and q in the bLW-Ep/q designs (bLW tile cores joined together by inter-tile arms of an even number of half-turns with the arm length of p base pairs (bp) and the sticky end length of q nucleotides (nt)). We were able to assign the chiral indices (n,m) to each specific tube from the high-resolution AFM images, and thus estimated the tile curvature angle with a regular polygon model that approximates each tube’s transverse section. We attribute the curvature evolution of the bLW-Ep/q tubes composed of the same tile core to the coupling of the intrinsic tile curvature and different arm twists. A better understanding of the integrated actions of different types of twisting forces on DNA tubes will be much more helpful in engineering DNA nanostructures in the future.

INTRODUCTION

For the past 40 years, the field of DNA nanotechnology has relied on the programmability of Watson-Crick base pairs (bp) to construct many versatile DNA nanostructures and nanodevices.1–8 The organization of 1D, 2D, and 3D DNA nanostructures can be divided into two hierarchically coherent architectures, namely weaving and tiling. Weaving relies on winding soft, thread-like, single-stranded DNAs into double-stranded helixes (hereafter “helix” will be used to indicate “double-stranded helix”) and crossovers mainly three- and four-branched junctions, forming small tiles of nanoscale dimensions,9,13 larger origami tiles of tens of nanometers,1 or single-stranded tile and brick lattices of (sub)micrometers.15–17 Each tile can be categorized into two sets: a tile core surrounded by the scaffold strand including boundary junctions, and the outer overhangs carrying the helix stems and joints of the sticky ends. Tiling, a term originated from the construction architecture, refers here to the assembly of DNA tiles into either finite or infinite nanostructures by joining tiles with inter-tile arms via sticky end cohesion. In our current work, we term the joining with the arm length of an even number of half-turns as E-tiling and that of an odd number of half-turns as O-tiling. In tiles, the intrinsic tile curvature is originated from weaving, whereas the arm twist is generated from tiling. Coupling of both will finely tune the 2D DNA
nanostructures into different morphologies and chirality. From 1D to 3D periodic DNA nanostructures, Bravais lattices can be abstracted, with tile cores as lattice points, joining arms as bonds, and the smallest repeating structures as unit cells. Thus, the 2D planar and tubular lattices in this work will be described via crystal terms and analyzed using crystal theory.

Design strategies
A small DNA tile, such as a double crossover (DX) tile or a multi-arm junction tile composed of several oligonucleotides, is often woven via a few four-branched Holliday junction (HJ) crossovers to only one specific weaving pattern because of the space limits of its weaving architecture. Recently, small circular DNA molecules have been used as scaffolds to build cDAO tiles (coupled double crossovers composed of two antiparallel helixes with an odd number of half turns between adjacent crossovers), 1D nanowires, 2D nanoribbons as well as nanotubes. The cDAO tile assembled from a circular 64 nt strand (abbreviated as c64nt) is composed of two DAO motifs coupled in series. However, with a circular 84 nt scaffold (c84nt), the center-crossover motif HJ-c84nt failed to form two DAE (double crossovers composed of two antiparallel helixes with an even number of half turns between the crossovers) tiles coupled in series, and thus it was unable to form 2D lattices. The reason was that the incoming and outgoing nucleotides of each helper strand at its corresponding pole forms a nick, and the nick was located inside and restrained by the c84nt scaffold loop. Such a failure drove us to think about weaving a circular DNA tile with two DAE motifs coupled in parallel. Increasing the size of the circular scaffold to 128 nt (c128nt, twice of c64nt), we folded it into an H-shape and an X-shape, and obtained three weaving architectures to build the following tiles and 2D nanostructures: 1) traditional longitudinal weaving (LW) along the H-shaped scaffold to generate LW tile cores and LW-E_{p/q}/LW-O_{p/q} (E_{p/q} refers to an arm length of an even number of half-turns composed of p base pairs (bp) and a sticky end length of q nucleotides (nt), and O_{p/q} refers to an arm length of an odd number of half-turns composed of p bp and a sticky end length of q nt) nanostructures, 2) bridging longitudinal weaving (bLW) along the X-shaped scaffold by inserting a 10 bp helix at the center to yield bLW tile cores and bLW-E_{p/q}/bLW-O_{p/q} nanostructures, 3) transverse weaving (TW) of the H-shaped scaffold to produce TW tile cores and TW-E_{p/q}/TW-O_{p/q} nanostructures.

Definition and estimation of tile curvature
Because of the spring-like structure of DNA, a well-known phenomenon in DNA nanotechnology is that DNA tubes and planar ribbons are often observed when E-tiling and O-tiling are applied, respectively; this has been attributed to the intrinsic tile curvature. Following the canonical winding phase of B-DNA, E-tiling requires that all tile faces to be aligned identically, and O-tiling requires that adjacently joined tile faces to be aligned alternately. By assuming that the arms in both E-tiling and O-tiling are straight and do not generate any torque, the intrinsic tile curvatures will be accumulated by E-tiling to generate tubes, whereas they will be cancelled out by O-tiling both locally and globally to produce planar ribbons. Such qualitative explanations regarding tile curvature have been proven to be successful in most cases. However, details and quantitative descriptions about its contents and scopes have been lacking such as the tile conformation (concave or convex?), the curvature angle of the tile with a defined orientation, and the curvature transition and transformation (does a tile curvature evolve gradually or flip-over evenly?) with changing arm lengths and/or sticky end lengths. The absence of such data in the design toolbox of DNA nanotechnology has constituted a hurdle to realize precise and diverse DNA nanostructures efficiently.

A well-established strategy to design curled DNA nanostructures with addressable surfaces is the periodic insertion or deletion of base pairs between adjacent HJs, resulting in net torsional strains on the segments and thus leading to the formation of right- or left-handed beams and tubes. Such curving effects with overwinding and underwinding strategies have been applied in DNA origami and single stranded tile nanostructures via the accumulation of periodically biased torques to yield left-handed and right-handed constructs, respectively. In the two systems of DNA origami and single-stranded tiles, DNA weaving and tiling architectures are integrated together, in which the DNA tile cores and E/O-tiling arms cannot be clearly distinguished. While for a large number of DNA tubes assembled from small tiles via E-tiling, only a very few reports mentioned about their addressable inside and outside...
Many questions regarding the intrinsic tile curvature and its evolution with changing arm and sticky end lengths still remain unanswered. In DNA tubular structures of DAE-E tubes,\textsuperscript{35} six helix bundle tubes,\textsuperscript{37-38} and single-stranded tile tubes,\textsuperscript{16, 39} the universal polygon model has been applied to explain the curving of tile arrays to tubes. Such polygon models are based on the B-DNA winding criteria including the major/minor grooving effect at crossovers by presupposing that all helixes are rigid, tangent between adjacent helixes, and parallel to the tube axis. However, a precise physical description of the tile curvature and its cooperation with arm-twisting forces have not yet been illustrated clearly in the polygon models mentioned above.

In this work, in order to quantify the tile curvature, we first relied on high-resolution AFM images of each type of 2D lattices with a lateral resolution at about 2.0 nm to abstract its 2D Bravais lattice of centered rectangle and precisely measured the unit cell parameters (Section S2, S3 and S4). It is obvious that only the E-tiling tubes are adequate for the curvature analysis. Secondly, we described each specific tube with the chiral indices (n,m)\textsuperscript{33, 40} based on its high-resolution AFM image. The correspondence of the tube perimeters from theoretical calculations based on (n,m) and lattice linear and angular constants to experimental measurements confirmed the correct assignment of (n,m). Thirdly, we projected the tube unit cell possessing the chiral indices (n,m) (in this work, we limited our analysis to two types of stable tubes, n = 2m and n = m) onto its transverse section and approximate the projection to a regular polygon model. The exterior angle of the regular polygon is assigned as the tile curvature angle. We define this semi-quantified curvature as the global tile curvature, which integrates the intrinsic tile curvature and the arm twist together. We define the intrinsic tile curvature ideally as the curvature of a free tile, which depends on its weaving architecture. However, its measurement is beyond our current capabilities. In practice, we could assign the global tile curvature defined from an E-tiling tube with an arm length of 21 bp and a sticky end length of 5 nt (specified as E\textsubscript{21:5}) as the intrinsic tile curvature, which is based on the postulation that the E\textsubscript{21:5} arm is straight and does not yield any additional torque.\textsuperscript{22} Under such a postulation, we also considered the intrinsic tile curvature as the intrinsic curvature of the tile core.

We relied on the combination of both biotin/SA labeling and high-resolution AFM imaging techniques to define the configurations of the E-tiling tubes and thus the conformations of the DNA tiles, primarily for the stable and minimally distorted tubes wound according to the canonical B-DNA helixes. We represent the stereo-conformation (i.e. chirality) of a tile with the right-hand or left-hand grip rule. To demonstrate, with the thumb points towards the inside surface of the tube or the concave face of the tile, the rotation direction of c128nt from 5’ to 3’ can be followed by either right-hand or left-hand grip. For concise notation, we use l- or r- as the prefix of the chiral indices (n,m) of a specific tube as l-(n,m) or r-(n,m) to represent its left-handed or right-handed curvature. Similarly, we separated and identified the two opposite faces of a tile using either the l-face or the r-face. Moreover, the prefix of l- or r- in front of (n,m) indicates a clear geometry of the tube, whereas l- or r- in front of “face” only indicates one of the two opposite faces of a tile exclusively.

RESULTS AND DISCUSSION

Synthesis of c128nt and tile stability

The circular c128nt was synthesized using two linear, phosphorylated 64 nt oligonucleotides and their two corresponding splints by T4 ligation. The c128nt was purified through denaturing polyacrylamide gel electrophoresis (PAGE) with a yield of 40–60% (Section S1.2 and Figure S1). We used the native PAGE to test the stability and yield of three tile cores of LW, bLW, and TW (Section S1.4 and Figure S2). For each core, a single band confirms its stability. The yield was 80% for each core based on an analysis with the software “imageJ” of the gel band intensity in each lane.

A unified 2D Bravais lattice of centered rectangle
From both theoretical designs and experimental results of AFM images, we applied a unified 2D Bravais lattice of centered rectangle to describe all the 2D DNA lattices originated from c128nt-derived tiles. For example, in the brick assembly model of Figure 1A(a), a compound unit cell of centered rectangle containing two tiles is defined by the mutually orthogonal basis vectors \( a \) and \( b \) with unit lengths \( a \) and \( b \), respectively, and a primitive cell of rhombus containing one tile is superimposed by the primitive vectors \( c_1 \) and \( c_2 \) with unit length \( c \) and inter-angle \( \phi \). By achieving a lateral resolution of about 2.0 nm in AFM images, we were able to measure the unit cell constants of each 2D lattice. The measured lattice constants were in line with the simple theoretical estimations of our designs well (theoretical descriptions refer to Section S2 and S3, data refer to Table 1 and Table S1).

2D arrays of longitudinally woven (LW) tiles

In Figure 1, both designs of LW-E21/5 and LW-O26/4 are shown with their brick assembly models (A(a) with face-identical packing and B(a) with face-alternating packing), helix tile models (A(b) and B(b)), zoomed-out (A(c) and B(c)) and zoomed-in (A(d) and B(d)) AFM images (more images are shown in Figures S12 and S13). In the AFM images of the LW-E21/5 and LW-O26/4 arrays, we found mostly planar ribbons and very few wide tubes. In contrast with the general rule that the E-tiling results in tubes, the LW-E21/5 assemblies represented mostly planar ribbons and lengthy monolayer fibers (Figure S12), meaning that the longitudinal weaving of H-shaped c128nt resulted in tightly folded, crowded, rigid, and planar LW cores with minimal curvature, similar to our previously reported cDAO-c64nt system.20 According to the traditionally faceted crystal growth theory by means of bond directions, lattice edges along \( c_1 \) and \( c_2 \), so-called flat faces, would appear thermodynamically, whereas edges along \( a \) and \( b \) (the so-called stepped faces) would occur dynamically. In both the LW-E21/5 and LW-O26/4 designs, either \( c_1 \) or \( c_2 \) only had a small inter-angle (less than 20°) with respect to \( a \), and the aspect ratios of \( a/b \) were larger than 1.5, thus, the growth of their 2D lattices was dynamically favored. The lengthy edges (stepped faces) of planar ribbons and the axes of tubes in their AFM images are usually along \( a \).

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**Figure 1. Longitudinal weaving and tiling systems of LW-E21/5 and LW-O26/4.** (A) LW-E21/5: a) an assembly model for E-tiling with all the brick modules colored in blue (the \( l \)-face), meaning having face-identical packing; b) a helix tile model; c and d) zoomed-out and zoomed-in AFM images. (B) LW-O26/4: a) an assembly model with adjacent brick modules along the horizontal direction colored blue for the \( l \)-face and green for the \( r \)-face alternately, which indicates face-alternating packing; b) a helix tile model; c and d) zoomed-out and zoomed-in AFM images. In all the tile helix models of Figures 2A(b) and 2B(b), the blue strand c128nt acts as the central scaffold and rotates clockwise in the 5’→3’ direction, thus, the \( l \)-face always faces up, and its opposite \( r \)-face always faces down; the same number pairs of (1, 1*) or (2, 2*) will cohere together to form arms whose length is defined as the inter-tile distance between adjacent HJs. In both the brick assembly models of Figures 1A(a) and 1B(a), the tile brick module is divided into two parts of deep-colored inner core and light-colored outer overhangs carrying double helix stems and sticky end joints, and decorated with several carved short bars representing HJs; a centered rectangle unit cell (defined by the basis vectors \( a \) and \( b \)) and a plain rhombus unit cell (defined by the primitive vectors \( c_1 \) and \( c_2 \)) with an inter-angle \( \phi \) are
superimposed. In the zoomed-out AFM images, each inset shows the organization of 9 tiles in the Cartesian coordinates of \(a\) and \(b\), clearly illustrating the individual tile cores and their joining regions. The measured lattice constants are \(a = 34.6\) nm, \(b = 12.0\) nm, \(c = 18.3\) nm, \(\phi = 142^\circ\) for LW-E21/5; and \(a = 37.4\) nm, \(b = 12.0\) nm, \(c = 21.1\) nm, \(\phi = 149^\circ\) for LW-O26/4.

Because we did not use any AFM imaging markers on the LW-derived tiles, their densely woven 2D arrays (as well as TW-E21/5 and TW-O26/4 assemblies which are described later) were primarily imaged in fluid as brighter patches of planar ribbons, handkerchief-like structures, or blurring tubes without any texture, shown in Figures 1A(c), 1B(c), S12, S13 (as well as in Figures 3A(c), 3B(b), S21, and S22). To obtain high-resolution images with nanoscale textures, we employed two imaging skills: 1) On a wide tube, the top monolayer sits flatways on a bottom monolayer and both layers are electrostatically repulsive. The nanoscale rhombus texture of the top layer was easily resolved using between 1 to 3 scans, shown in Figure 1A(d) and magnified in the left bottom insert. In comparison with the brick assembly model of Figure 1A(a), each bright intersection point represents a LW core, each edge a 21 bp joining arm, and each dim pit a rhombus center torn a little bit apart by scanning. 2) For the LW-O26/4 monolayer array which was strongly adsorbed on the hard substrate of mica, its high-resolution image in Figure 1B(d) was achieved through at least 4 scans. However, repeated scans removed some tiles from the lattice patch and generated scattered empty pores. The hierarchical and distinctive imaging features in Figure 1B(d) are read as follows: First, the bright tile array and the dim fine line, parallel to each other along \(b\), alternate with each other. The periodic distance of the pattern corresponds to a tile length of \(a/2\), and the fine line corresponds to the sticky end cohesion column (4 bp or 0.32 nm \(\times\) 4 = 1.3 nm wide) of Figure 1B(a). Secondly, the magnification of three bright tile arrays in the insert of Figure 1B(d) shows sub-tile details, in which the \(a\)-parallel, bright, short bar, corresponding to either the upper or the lower two helixes of a tile, alternates with the dim valley, corresponding to the gap between double-helixes. Moreover, the central HJ connecting both the upper and lower halves of a tile could occasionally be imaged as a bright dot. We hypothesize that the strong adsorption of the DNA helixes on hard mica enabled the upper and lower halves of a tile to be resolved. The measured unit cell parameters of both the LW-E21/5 and LW-O26/4 lattices are listed in the caption of Figure 1, which are in line with their theoretical estimations (Section S2 and Table S1). The high-resolution imaging details also confirm the correct Bravais lattice assignment of the centered rectangle.

**Chiral indices and curvature evolution of the bLW-E\(_{p/q}\) tubes**

The center-bridging strategies to build 2D bLW-derived nanostructures are demonstrated in Figure 2. The insertion of a flexible 10 bp helix instead of the central HJ releases more space and provides more flexibility for the bLW-derived tiles so that they can regulate themselves during the assembly.

We show the O-tiling bLW-O\(_{26/4}\) assemblies first. Figure 2A(a) shows an assembly model with face-alternating brick modules and Figure 2A(b) shows a helix tile model. Figure 2A(c) illustrates one of the widest tubes with a perimeter of 1.6 \(\mu\)m (twice the width of the squashed double layer). The zoomed-in image in Figure 2A(d) illustrates the tile details. At each intersection point of the lattice, two tiny faint pores can be seen separated by the 10 bp bridge. Thus, the geometry of the bLW core is defined as an X-shape with two pairs of opposite angles of around 60\(^\circ\) and 120\(^\circ\). The X-shaped geometry can be explained by the fact that the 2 three-branch junctions at both sides could be kinked more easily than the two continuous helixes without any breaks at the top and bottom, as shown in the helix tile model of Figure 2A(b). The bLW-O\(_{26/4}\) assemblies provide the dominant tubes with scattered perimeters (Figure S14).

In Figure 2B, we indicate three sets of chiral indices \((n,m)\) to specify the DNA tubes. The chiral indices have been widely used in defining the carbon nanotube structures, but they have rarely been used in describing the DNA tube structures. On the hexagonal lattice background \((\phi = 120^\circ)\) of the bLW-E\(_{p/q}\) assemblies with \(l\)-faces facing up, we drew the Bravais unit cells at the bottom left, and three sets of chiral indices: \((8,4)\) in purple shadow representing a typical bLW-E\(_{21/5}\) tube unit cell, \((3,3)\) in green shadow representing the unique bLW-E\(_{31/7}\) tube unit cell, and \((23,-23)\) in blue shadow representing a typical “giant” bLW-E\(_{32/6}\) tube unit cell. The chiral indices \((n,m)\) are abstracted from \(n c_1 + m c_2\), which is the chiral (or perimeter) vector \(Ch(n,m)\) originated from the origin \(O\).
For example, (8,4) and (3,3) are abstracted from $Ch(8,4) = 8c_1 + 4c_2$ and $Ch(3,3) = 3c_1 + 3c_2$, respectively. The tube unit cell of (23,-23) is represented with repeating units at the top right, in which vectors of 23$c_1$ and -23$c_2$ are omitted for illustration purposes. The unit cell parameters of $c$ and $\varphi$ were measured directly from the AFM images, thus, the chiral indices (n,m) could precisely define many tube unit cell parameters, including the perimeter (diameter and radius), the helical angle of the inter-angle between $c_2$ and $Ch(n,m)$, and the axial periodicity ($T$) (Section S3 and Figure S4), and also the global tile curvature being investigated in our study.40

Combining both the biotin/SA labeling and the AFM imaging techniques, we analyzed in detail the E-tiling tubes of bLW-E21/5 in Figures 2C(a-f), bLW-E31/7 in Figures 2D(a-e), and bLW-E32/6 in Figures 2E(a-e). The evolution of the perimeters of the bLW-Epq tubes, their configurations, their tile conformations, and their global tile curvatures by manipulating both the arm and sticky end lengths enabled us to gain a much deeper understanding of the tube formation mechanism. For the bLW-E21/5 tubes, Figure 2C(a) shows the schematic brick model of the typical tube (8,4) (top) and the helix model of its component tile (bottom) with a biotin label at overhang 2. Figure 2C(b) provides a zoomed-out AFM image containing many tubes (top) and a zoomed-in image of only one typical tube (bottom). Densely distributed tubes in the top panel suggest a high yield for the products. Figure 2C(c) shows an abundance of 73 tubes falling in 4 perimeter windows, in which tube perimeters were measured from more than 5 batches of assemblies (Section S5, Table S2, and block 1 of Figure S15). The tubes were clearly seen as mostly homogeneous, falling within the perimeter window of 110.0 to 230.0 nm. An obvious feature of the bLW-E21/5 tubes in all the zoomed-in images (Figures 2C(b,d), Figures S4-9 and S15) was that the two groups of $c_1$ and $c_2$-frames were not symmetric. One group of frames were aligned nearly parallel to the tube axis, and the other group curled around the tube axis with left-handed rolling. Figure 2C(d) shows the $l$-face brick assembly model with each tile carrying a biotin/SA label (top), and the real zoomed-in image of the inside surface of a tube (bottom). The ideal match of the periodic structure of the SA dots (each SA dot is around 5.5 nm in diameter)39 overlying on the background lattice between the real image and the model refers that the intrinsic tile curvature follows the left-hand grip rule.

We describe here more experimental details for our reasoning process. First, the tubes must be in the open form to image both the background lattice structure and the overlying SA dots clearly, as shown in the bottom panel of Figure 2C(d) and in blocks 2-4 of Figure S15. DNA tubes are often reported to be open after repeated scans.42-44 Herein, we report another experimental solution to prepare DNA tubes mostly in the open form or in both the open and closed forms by more conveniently regulating the air-blowing strength. After a 2–4 min period of incubation of the sample drop on mica to let the DNA tubes get adsorbed, we washed the sample specimen with water and blew the water drop away with air streams (Section S1.6 and Figure S3). The top layers of the tubes were either partly or completely removed with water due to the surface tension interactions, whereas the bottom layers were strongly adsorbed and remained intact on the mica. Then, the AFM scanning in either air or buffer mode was carried out. The completely broken and partly broken bLW-E21/5 tubes were imaged mostly as monolayer strips, which were either locally straight or kinked at some sites. The monolayer strips exposing the inside surfaces of the tubes outside are a requirement to define the direction of the curvature. For conciseness purposes, we only show the results of biotin/SA labeling at the 5’-end of the outer helper strand (green strand) of overhang 2 for bLW-E21/5, bLW-E31/7, and bLW-E32/6 in Figure 2 (additional AFM images of each design are shown in Figures S15, S19, and S20). The other biotin/SA labeling results at overhang 1 and 1* are also shown in Figures S15-20. The in-situ binding of the SAs on biotin-labeled lattices was carried out under a dilute SA solution according to previously reported protocols.18,39 Although the biotin-labeled nucleotide is located at the buried $r$-face in Figure 2C(d), high yield of binding of the SAs (70–90 %) was confirmed by AFM imaging because the empty space between the arms allowed biotins to be bent and stand up from the mica, exposed their functional groups and bound the SAs easily. From the massive SA dots overlying on bLW-E21/5, bLW-E31/7, and bLW-E32/6 monolayer lattice strips, we could in most cases precisely define the position of an SA dot relative to its corresponding tile. For example, in the bottom panel of Figure 3C(d), the SA dots always sit on the longitudinal $c_2$-frames along the tube axis. With the center of each X-shaped tile as a lattice point, via the acute angle from each SA dot at an overhang 2 of a $c_2$-frame to its two adjacent
of tile oligomers, which indicated that the deviation of ±1 bp of arms from the canonical B-DNA would disturb the tube formation and arm twist and the intrinsic tile curvature. bLW-E20/4 would enhance the left-handed curling to the unique tube otherwise, if they have the right-handed curvature, via the acute angle the rotation direction from the SA-located E20/4 tube was assigned to the chiral indices assayed over 60 individual bLW-E21/5 tubes at high-resolution from more than 5 batches with biotin/SA labels on overhangs 1, 1*, and 2, all imaging results supported the left-handed curvature (more AFM images are in Figure S15).

Quantitative analysis of the curvature angle of a specific tube relies on assigning the chiral indices (n,m) to the tube. In practice, we could assign (n,m) to a perforated bLW-Ep/q tube based on its high-resolution AFM images illustrating the tube lattices in both the open and closed forms, such as in Figures S5-9. The high-resolution AFM images indicated that around 90% of the bLW-E21/5 tubes had 4, 5, or 6 tube-axis-parallel c2-frames from the bottom layer stuck on the mica, and a few other irregular tubes (around 10%), had c2-frames either clearly tilted against the tube axis or had wider perimeters. By directly counting the chiral indices (n,m) from a highly resolved tube in both the open and closed forms (Figures S4-9), we were able to assign tubes with 4 bottom c2-frames stuck on the mica mostly to l-(6,2), l-(6,3), and l-(7,3) (Figures S5, S10 and Table S2), 5 bottom c2-frames mostly to l-(8,3), l-(8,4), and l-(9,4) (Figures S6, S7, S10, and Table S2), and 6 bottom c2-frames to l-(10,4), l-(10,5), and l-(11,5) (Figures S8-10, and Table S2). We also applied the numerical approximation method (Section S3-5, Table S2, and Figure S10) to match the chiral indices to the tube perimeter windows of Figure 2C(c). The numerical calculations led to the conclusion that the bLW-E21/5 tubes in Figure 2C(c) could be represented with a paraxial cluster of the chiral indices (n,m) along the linear segment n = 2m+1, where m is 3, 4 and 5 in the 2D Cartesian coordinates (m,n) (Figure S10). The range of the curvature angle was estimated from these clustered tubes, which was sufficient to support our claim of semi-quantitation of the tile curvature. We took the most abundant bLW-E21/5 tube of l-(8,4) (Figure 2C(c), Table S2, and Figure S10), as an example to describe the quantitation approach. First, we define the curvature of a tile as the bending of two halves of a tile along an axis passing through the tile center to a dihedral angle, which could clearly be compared to a butt hinge with a pair of leaves bending along a shaft. It is reasonable to assume that the arms along c1 in the bLW-E21/5 tube l-(8,4) bend at their centers of sticky end cohesion sites with the same curvature as the tile core. Thus, we approximated the transverse section of the perforated tube l-(8,4) by projecting its tube unit cell in Figure 2B to a 16-gon, as shown in Figure 2C(e). The physical appearance for the 16-gon was recognized as follows: 8 tile centers (lattice points) and 8 arm centers (lattice bond centers) along c1 are projected to 16 vertices, and 16 straight half-arms (half lattice bonds) along c1 to 16 edges. Therefore, the curvature angle θ of l-(8,4) was estimated as the exterior angle 360°/16 = 22.5°, schematically shown in Figure 2B(f). Similarly, a 12-gon was approximated from the narrowest tube l-(6,3) and a 24-gon from the widest tube l-(12,6), and their curvature angles were estimated at 30° and 15°, separately.

From the above approximation approaches, the intrinsic tile curvature angle of the bLW-E21/5 tubes was estimated to be limited in the window of 22.5° ± 8°.

Furthermore, we investigated the evolution of the global tile curvature in both the bLW-E20/4 and bLW-E22/6 tubes, which have 1 bp deletion and 1 bp insertion from bLW-E21/5 in both the arms and sticky ends, respectively. The biotin/SA labeling results (Figure S16) showed that the bLW-E20/4 tubes possessed a left-handed curvature (6 individual tubes from 2 batches were measured), whereas the bLW-E22/6 tubes (Figure S17) had a right-handed curvature (6 individual tubes from 2 batches were measured). The unique bLW-E20/4 tube was assigned to the chiral indices l-(4,2), and the typical bLW-E22/6 tube to r-(6,6). The opposite curvatures can be explained as follows: 1) The arm in bLW-E20/4 with a helical twist density of 10.0 bp/turn (less than 10.5 bp/turn of canonical B-DNA) and 4 nt sticky end cohesion would generate a left-handed arm twist, whereas the arm in bLW-E22/6 with a helical twist density of 11.0 bp/turn (larger than 10.5 bp/turn) and 6 nt sticky end cohesion would yield a right-handed arm twist. 2) Depending on the coupling of the arm twist and the intrinsic tile curvature, bLW-E20/4 would enhance the left-handed curling to the unique tube l-(4,2) (Figure S16), while bLW-E22/6 would flip over the intrinsic tile curvature from left-handed to right-handed, thus resulting in tubes r-(5,5), r-(6,6), and r-(7,7), among which, r-(6,6) was the most abundant one (Figure S17). Both tubes coexisted with many other residual fragments of tile oligomers, which indicated that the deviation of ±1 bp of arms from the canonical B-DNA would disturb the tube formation.
process to some degree. In the bLW-E20/4 tube of \(l-(4,2)\), \(c_1\) and \(c_2\)-frames played their respective framing roles as in bLW-E21/5 (i.e., \(c_1\)-frames curled around the tube axis with left-handed rolling and \(c_2\)-frames aligned nearly parallel to the tube axis). For the bLW-E22/6 tubes of \(r-(5,5)\) to \(r-(7,7)\), both the \(c_1\)- and \(c_2\)-frames were symmetric and played equal framing roles.

We also extended the bLW-E_{pq} assemblies to the arm length to three full turns and the sticky end length from 5 to 7 nt. We tested the assemblies of bLW-E_{11/5}, bLW-E_{31/7}, and bLW-E_{32/6}. For the bLW-E_{31/5} assemblies, the helical twist density of \(31/3 = 10.3\) bp/turn (less than 10.5 bp/turn) in the arm and 5 nt sticky end cohesion would generate a left-handed arm twist. Similar to bLW-E_{20/4}, the coupling of both the left-handed twisting forces from the arms and the tile cores generated the typical tube with the chiral indices \(l-(10,5)\), where the left-handed curvature was defined from the biotin/SA labeling results (8 individual tubes from 2 batches were measured) and the yield for tube products was moderate (Figure S18).

The bLW-E_{31/7} assemblies gave high yield products of uniform tubes with the chiral indices \((3,3)\), as shown in Figure 2D(b) and in block 1 of Figure S19. Investigation of the tile conformation with biotin/SA labeling was not as straightforward as for the bLW-E_{21/5} tubes because the bLW-E_{31/7} tubes were much narrower. Controlling the strength of compressed air to generate proper monolayer structures for tile conformation assignment was challenging. We list here some of the difficulties that we encountered: 1) When the tubes were broken by strong air-blowing, the open monolayer strips seldom bound SAs because labeled biotins were most probably blown to stick tightly on the mica, 2) gentler blowing of compressed air couldn’t properly open the tubes well because of the tightly bonded narrow tube structure with the stronger sticky end cohesion of 7 nt, 3) even properly open monolayer strips were often discontinuous and their maximum strip width of 3 to 4 tiles (lattice points) in either \(c_1\) or \(c_2\) direction was the minimum threshold for tile conformation assignment. We tried to image more than a hundred of individual tubes, but were only able to clearly define 15 tubes’ configuration with the right-handed curvature from more than 5 batches, as shown in Figure 2D(c) and in blocks 2 and 3 of Figure S19. With the regular polygon approximation method, projection of the \(r-(3,3)\) tube unit cell elements to its transverse section could be approximated with a 12-gon, and the exterior angle (i.e. the global tile curvature angle) was 30°. The global tile curvature of the bLW-E_{31/7} tube of \(r-(3,3)\) is schematically shown in Figure 2D(d), with the dihedral axis along \(a\).

Schematic and AFM images of the bLW-E_{32/6} tubes and their curvatures are shown in Figures 2E(a-e). Much different from previously assembled tubes with similar configurations, two types of tubes were achieved with high yield and high quality (a very few lattice fragments were imaged on mica), the “large” tube (bottom of Figure 2E(a) and its AFM images at the bottom of Figure 2E(b) and in block 1 of Figure S20) with its axis along \(a\) having an average perimeter of 407.1 nm, represented with the chiral indices \((16,16)\), and the “giant” tube (top of Figure 2E(a) and its AFM images at the top of Figure 2E(b) and in block 1 of Figure S20) with its axis along \(b\) having an average perimeter of 970.8 nm, represented with the chiral indices \((23,-23)\). Because their tube widths and lengths were large enough to form well organized 2D monolayer lattices in both lateral and longitudinal directions, their tile conformations could be easily defined with the biotin/SA labeling technique. “Giant” tubes were shown to have the left-handed curvature (Figure 2E(c) and block 3 of Figure S20), whereas “large” tubes possessed the right-handed curvature (Figure 2E(d) and block 2 of Figure S20) (over 20 individual tubes of each type from 3 more batches were imaged). The global tile curvature angle of the “large” tube \(r-(16,16)\) was estimated at 9° from a regular 64-gon (bottom of Figure 2E(c)), and that of the “giant” tube \(r-(23,-23)\) at 3.9° from a regular 92-gon (top of Figure 2E(c)). The widest “giant” bLW-E_{32/6} tube reached up to 1600.0 nm in circumference and 5.0-8.0 μm in length, commensurate in their width and even longer in length when compared to most of the well-behaved planar lattices assembled from small tiles via O-tiling.

For the bLW-E_{32/6} assemblies, “large” tubes with the right-handed curvature further support the flip-over effect on the intrinsic left-handed curvature of the bLW tile core. “Giant” tubes keep their left-handed curvature while growing with their tube axes along \(b\), which are not thermodynamically favored. We propose the following mechanism to simultaneously grow both types of tubes: 1) Both arm length of 32 bp with a helical twist density of \(32/3 = 10.7\) bp/turn (> 10.5 bp/turn of the canonical B-DNA) and 6 nt sticky end cohesion would generate an integral right-handed twisting force. 2) The intrinsic tile curvature of the bLW cores would generate a
left-handed curving force. 3) There should be a delicate balance between the two opposite curving forces at the initial assembly stage, which drives the tile oligomers to a saddle-like shape (Section S6 and Figure S11). In the saddle-like oligomers, the deeper curvature surrounding $a$ is driven by the right-handed curving force and the other shallower curvature surrounding $b$ is driven by the left-handed curving force. 4) With further growth, depending on which curved surface closes first to form a ring as a tube seed, which ends up growing epitaxially to a full tube. The saddle-like model of the tile oligomers is strongly supported by two evidences: 1) The directions of growth of the tube axes (“large” tubes along $a$ and “giant” ones along $b$) are perpendicular to each other. 2) The global tile curvatures (“large” tubes with the right-handed curvature and “giant” ones with the left-handed one) are opposite to each other. We named the specifically paired group of “large” and “giant” tubes as “orthogonal” tubes. The phenomenon of tube widening with $b$ as the tube axis had also been observed in the DAE-E tubes. However, the authors addressed that the mechanism of formation and configuration of the tube were not clearly described. In our case, the saddle-like oligomer model reasonably explains how “giant” tubes overcome the higher energy barrier to form a ring seed and finally grow up epitaxially with their tube axes along $b$. 
Figure 2. Bridging longitudinal weaving and tiling systems and tile curvature transformation. (A) bLW-O_{26/4} tubes: a) an assembly model with tile brick modules, b) a helix tile model, c and d) zoomed-out and zoomed-in AFM images. (B) Three sets of chiral indices representing typical E-tiling tubes: a typical bLW-E_{21/5} tube unit cell of (8,4) shadowed in gold with its tube axis along \( c_2 \), the unique bLW-E_{31/7} tube unit cell of (3,3) shadowed in green with its tube axis along \( a \), and a "giant" bLW-E_{32/6} tube unit cell of (23,-23) shadowed in blue with its tube axis along \( b \). (C) bLW-E_{21/5}: a) a brick tube model of \( l-(8,4) \) with \( l \)-faces colored blue inside and \( r \)-faces colored green outside (top) and a helix tile model (bottom) with a biotin label at overhang 2 pointing toward the \( r \)-face, b) zoomed-out (top) and zoomed-in (bottom) AFM images of the bLW-E_{21/5} tubes, c) perimeter distribution of 73 tubes, d) a brick assembly model carrying biotin/SA labels (top) and its corresponding zoomed-in AFM image (bottom) of an inside surface lattice with biotin/SA dots, e) the approximation of the transverse section of tube \( l-(8,4) \) with 16-gon, f) the intrinsic tile curvature represented with a dihedral angle along the axis \( c_2 \) following the left-hand grip rule. (D) bLW-E_{31/7} tubes: a) a brick tube model of \( r-(3,3) \) with \( l \)-faces in blue outside and \( r \)-faces in green inside (top) and a helix tile model with
a biotin label at overhang 2 (bottom) pointing toward the l-face, b) zoomed-out (left) and zoomed-in (right) AFM images, c) a brick assembly model carrying biotin/SA labels (left) and its corresponding zoomed-in AFM image (right) of an inside surface lattice with biotin/SA dots, d) global tile curvature of r-(3,3) represented with a dihedral angle along the a axis following the right-hand grip rule. E, bLW-E_{22/6} tubes: a) a brick model of a “giant” tube l-(23,-23) (top) and that of a “large” tube r-(16,16) (bottom), and the helix tile model of bLW-E_{22/6} with a biotin/SA label at overhang 2 is similar to bLW-E_{32/6} and thus omitted, b) AFM images of both “large” and “giant” tubes and their corresponding zoomed-in images in insets illustrating their different tube axes, c) a zoomed-in AFM image of the inside surface lattice of a “giant” tube with biotin/SA dots and its corresponding l-faced brick model (inset), d) a zoomed-in AFM image of an inside surface lattice of a “large” tube with biotin/SA dots and its corresponding r-faced brick model (inset), e) the global tile curvature of the “giant” tube (top) represented with a dihedral along the axis b following the left-hand grip rule and that of the “large” tube (bottom) with a dihedral angle along the a axis following the right-hand grip rule.

In Table 1, we summarize the linear and angular constants of 2D lattice (a, b, c, and ϕ) of the bLW-E_{pq} tubes, followed by their chiral indices (n,m), perimeters (C), curvature angles (θ), and length ranges of the most abundant tube for each design.

Table 1. Experimental unit cell parameters and tube parameters of the bLW-E_{pq} tubes.

| Tube       | a/ nm  | b/ nm  | c/ nm  | ϕ/ °    | Ch (n,m) | C/ nm  | θ (axis) | length/μm |
|------------|--------|--------|--------|---------|----------|--------|----------|------------|
| bLW-E_{20/4} | 34.4   | 21.9   | 20.4   | 115°    | l-(4,2)  | 74.3   | 45°(c2)  | 2.0-5.0    |
| bLW-E_{21/5} | 35.1   | 23.7   | 21.2   | 112°    | l-(8,4)  | 158.8  | 22.5°(c2) | 3.0-6.0    |
| bLW-E_{22/6} | 36.3   | 22.7   | 21.4   | 116°    | r-(6,6)  | 136.2  | 15°(a)  | 2.0-5.0    |
| bLW-E_{31/5} | 41.2   | 25.7   | 24.3   | 116°    | l-(10,5) | 219.0  | 18°(c2)  | 4.0-7.0    |
| bLW-E_{31/7} | 41.9   | 24.2   | 24.2   | 120°    | r-(3,3)  | 72.7   | 30°(a)  | 6.0-10.0   |
| bLW-E_{32/6} | 42.2   | 24.9   | 24.5   | 119°    | l-(23,-23) | 970.8 | 4°(b) | 4.0-8.0    |
| (giant)     |        |        |        |         |          |        |         |            |
| bLW-E_{32/6} | 42.3   | 25.4   | 24.7   | 118°    | r-(16,16) | 407.1 | 5.6°(a) | 4.0-8.0    |
| (large)     |        |        |        |         |          |        |         |            |

For all six designs, bLW-E_{20/4} and bLW-E_{31/7} have their corresponding unique tubes with the chiral indices Ch(n,m) of l-(4,2) and r-(3,3), respectively; bLW-E_{21/5}, bLW-E_{22/6}, and bLW-E_{31/5} are represented with their most abundant tubes l-(8,4), r-(6,6), and l-(10,5), respectively; and the “giant” and “large” tubes in bLW-E_{32/6} are represented with l-(23,-23) and r-(16,16) at their average perimeters, respectively. The tube perimeter C was calculated as C = c\sqrt{n^2 + m^2 + 2nm \cos \phi}, where c and \phi measured experimentally are listed in the same row (refer to Section S3 of SI). The curvature angle θ correlated with the tile dihedral axis was estimated according to the following approximation rule: For each bLW-E_{pq} tube represented with (n,m), when n = 2m, θ was calculated as the exterior angle of a regular 2n-gon; when n = |m|, θ was calculated as the exterior angle of a regular 4n-gon.

2D arrays of transversely woven (TW) tiles

Figures 3A and 3B show the transverse weaving of the H-shaped c128nt scaffold with six helper strands to a stable tile core, and further with O-tiling leading to TW-O_{26/4} planar lattices and E-tiling leading to TW-E_{21/5} tubes. In the TW core, each helper strand weaves in the transverse direction over and under every other scaffold strand four times, forming the densest HJs in 2D DNA lattices with a density of 2HJ per turn.\(^{45}\) Compared with LW cores having totally 5 HJs and bLW cores totally 4 HJs, the TW core possesses totally 12 HJs.

The face-alternating O-tiling assembly of TW-O_{26/4} provides a planar handkerchief-like lattice with dimensions up to 4×4 μm\(^2\), shown in Figures 3A(c) and S21. To achieve high-resolution images with individual tile textures, as shown in Figure 3A(d) and in Figure
S21, more than 4 scans were needed. However, repeated scans moved some tiles away and left dim pores clearly showing shadows in the lattice. In contrast with the LW-E21/5 and LW-O26/4 ribbon-like lattices having ragged edges (stepped faces) along their longitudinal directions, TW-O26/4 lattices presented sharp vertices and straight edges (flat faces) along the \(c_1\) and \(c_2\) directions, due to the strong bonds with three helixes integrated together as an arm for sticky end cohesion.\(^{46}\) As shown in Figure 3(d), with assignments of \(c_1\) and \(c_2\) along the sharp edges, \(a\) and \(b\) were easily defined.

The E-tiling assembly of TW-E21/5 shows relatively homogenous tubes (Figure 3B(b) and block 1 of Figure S22). Although the TW core has the highest HJ density, formation of tubes instead of planar arrays was an indication that the TW core has an intrinsic curvature, probably due to the lack of a central HJ and therefore the bending of both half-parts along \(a\) passing through the TW core. Similarly, we achieved high-resolution AFM images with individual tile textures by repeated scans in the same region more than 4 times. As shown in Figure 3B(c) and block 1 of Figure S22, parts of the top layers were torn open along the flat face of \(c_1\) or \(c_2\). The TW-E21/5 tube axis is along the direction of the arm helix extension of \(a\) because it is thermodynamically favored. The number of counted tiles along \(c_1\) and \(c_2\) in Figure 3B(c) were 10, thus, this tube was assigned to the chiral indices (5,5).

The tile conformation was defined with the in-situ biotin/SA labeling technique too. In contrast with the perforated bLW-Ep/q tubes, TW-E21/5 assemblies were sealed tubes without any space between the tiles. In this case, the biotin orientation on mica-supported lattices was key for the in-situ binding of the SAs. When biotins were exposed on the top of the DNA lattice, the SAs bound to biotins efficiently, whereas when biotins were buried in a monolayered lattice, no binding occurred. We applied three biotin-labeling strategies: 1) an iBiodT label at overhang 2 pointing toward the \(l\)-face of every TW core, 2) a biotin label at the 5’-end of a helper strand at overhang 1\* pointing toward the \(r\)-face of every TW core, 3) both types of biotin labels at the \(r\)- and \(l\)-faces of every TW core, as shown in the TW-E21/5 tile helix model of Figure 3B(a) (bottom). After in-situ binding of SAs, the biotin labels exposed on the \(l\)-faces bind SAs only at the top of the double-layers (i.e. sealed tubes), but not on the monolayers (i.e. open tubes), as shown in Figure 3B(d) and block 2 of Figure S22; the biotin labels exposed on the \(r\)-faces bind SAs only on the monolayers but not at the top of the double-layers, as shown in Figure 3B(e) and block 3 of Figure S22; the biotin labels exposed on the \(r\)- and \(l\)-faces bind SAs both on monolayers and at the top of the double-layers, as shown in block 4 of Figure S22. For each labeling design, we have imaged more than 30 individual tubes from 3 separate batches. All imaging results support that the intrinsic tile curvature of TW-E21/5 followed the right-hand grip rule.

We measured the tube perimeters of 67 tubes in more than 5 different batches by doubling the width. The abundances of different perimeter windows are plotted in Figure 3B(g), indicating a nearly normal distribution from 45 to 135 nm. Combining both the direct counting of the chiral indices \((n,m)\) from high-resolution AFM images and the numerical approximation method in Section S3, we assigned the most abundant tube \((21/67 = 31.3\%)\) at the perimeter window of 75.0–90.0 nm to \(r-(6,6)\), the second most abundant tubes \((14/67 = 21\%)\) at 60.0–75.0 nm to \(r-(5,5)\) and at 90.0–105.0 nm to \(r-(7,7)\), and the less abundant tubes at 45.0–60.0 nm to \(r-(4,4)\) \((6/67 = 9.0\%)\), at 105.0–120.0 nm to \(r-(8,8)\) \((9/67 = 13.4\%)\), and at 120.0–135.0 nm to \(r-(9,9)\) \((3/67 = 4.5\%)\) (Section S5 and Table S3). Because the TW-E21/5 tubes have their helixes closely juxtaposed without any space between them, and the arm helixes are parallel to the tube axis, there will be no bending occurring in the arm regions. Therefore, we approximated the transverse section of the most abundant \(r-(6,6)\) to a regular 12-gon, that of the narrowest \(r-(4,4)\) to a regular 8-gon, and that of the widest tube \(r-(9,9)\) to a 18-gon. The curvature angle window was estimated to be around \(30^\circ \pm 15^\circ\), which is schematically represented in Figure 3B(f).
**Figure 3. Transverse weaving and tiling systems.** (A) TW-O26-4: a) an assembly model with tile brick modules, b) a helix tile model, in which the two centered helixes are 20 bp and the four other helixes (upper two and lower two) are 22 bp in length within the tile core along \(a, c\) and d) zoomed-out and zoomed-in AFM images with lattice constants of \(a = 30.6\ nm, b = 14.7\ nm, c = 17.0\ nm,\) and \(\varphi = 129^\circ.\) (B) TW-E21-5: a) a brick assembly model (top) and a helix tile model with a 5'-biotin label (gray dot) pointing toward the \(r\)-face and an iBiodT (dark dot) pointing toward the \(l\)-face (bottom), b) a zoomed-out image, c) a zoomed-in image with lattice constants of \(a = 27.3\ nm, b = 14.3\ nm, c = 15.3\ nm,\) and \(\varphi = 128^\circ,\) d) a brick assembly model of the tube \(r-(6,6)\) with biotin/SA labels on the \(l\)-faces and its corresponding zoomed-in images with SA dots only bound at the top of the double-layers, e) a brick assembly model of the tube \(r-(6,6)\) with biotin/SA labels on the \(r\)-faces and its corresponding zoomed-in images with SA dots only bound on the monolayers, f) the intrinsic tile curvature of TW-E21-5 tubes represented with a dihedral angle along the \(a\) axis following the right-hand grip rule, (g) 67 tubes distributed in 6 different perimeter windows.

**COMPRESHENSIVE DISCUSSION**

In this work, the most interesting phenomenon was the modification of the global tile curvatures of bLW-E\(_{p/q}\) tubes with different arm lengths at 4 or 6 DNA half-turns and sticky end lengths in the range of 4 to 7 nt. As shown, many parameters influence the global tile curvature such as the intrinsic tile curvature, the arm length, the sticky end length, the tile orientation vs the tube axis. We simplified the multiple factors into a model in which the tube configuration would be tuned with two twisting forces. One of them results from the intrinsic tile curvature and the other emanates from the arm twist, which was affected by both the arm and the sticky end length. The intrinsic tile curvature of a tile core depended on its weaving architecture. For example, the bLW cores had an intrinsic left-handed curvature, while the TW cores possessed the intrinsic right-handed curvature, both of which were inferred from the bLW-E\(_{21/5}\) and TW-E\(_{21/5}\) tubes without any extra twist in the arm. The extra arm twist nearly follows the overwinding/underwinding rule\(^{22}\) by regulating the inter-tile arm lengths between two adjacent HJs at the exception of the assembly of the bLW-E\(_{21/5}\) tubes. In briefly describing the overwinding/underwinding rule, when the helical twist density in an arm is at 10.5 bp/turn (10.44 bp/turn to be precise)\(^{22}\) of the canonical B-DNA, the arm would be straight; when it is less than 10.5 bp/turn, the arm would gain a left-handed twisting torque; and when it is larger than 10.5 bp/turn, the arm would gain a right-handed twisting torque.\(^{22}\) In our case, according to the experimental results, the rule needed small modifications: When a sticky end cohesion exists in the arm and the sticky end length is at 4 or 5 nt, the extra twisting torque generated from the sticky end cohesion could be ignored, thus, the above overwinding/underwinding rule was observed; however when the sticky end length was at either 6 or 7 nt, the extra right-handed twisting torque
generated from the sticky end cohesion needed to be counted.\textsuperscript{47-48} Taking the bLW-E\textsubscript{31/7} tubes as the example, although its helical twist density of the arm of 31/3 = 10.3 bp/turn was less than 10.5 bp/turn, its much stronger 7 nt sticky end cohesion could generate an extra right-handed twisting torque. As a consequence the coupling of the right-handed arm twist and the intrinsic left-handed tile curvature generated the unique bLW-E\textsubscript{31/7} tube \( \tau (3,3) \) in high yield with the right-handed curvature.

We grew tubes using a very slow annealing process over 70 hours, allowing for the assembly of all the lattice structures to be thermodynamically controlled. From the AFM imaging results, we found out that the bLW-E\textsubscript{pq} tubes always presented either one of two clusters of the chiral indices \((n,m)\) with 1) \( n \approx 2m \) and 2) \( n \approx m \) when \( 0 \leq m \leq n \). When \( n \approx 2m \), tubes possessing the left-handed curvature were governed by the intrinsic tile curvature, while when \( n \approx m \), the tubes followed the right-handed curvature due to the control by the extra right-handed arm twist. The flip-over of the tile curvature could be compared to that of a contact lens. We suggest that the bLW tile core is always curved due to its rigidity, and its intrinsic left-handed curvature can be forced by stronger opposite arm twists to switch to the right-handed curvature during the assembly process. The two types of chiral indices \((n,m)\) of \( n \approx 2m \) and \( n \approx m \) should represent the most stable structures with global energy minima for tubes possessing the left-handed and right-handed curvatures, respectively. Other intermediate tubes with \( n \) and \( m \) \( (0 \leq m \leq n) \) deviating from \( n \approx 2m \) and \( n \approx m \) should possess higher deformation energies and would not be thermodynamically favored. In fact, we imaged a few of these tubes in the bLW-E\textsubscript{21/5} assemblies, but they represented less than 5\% of the total. In all the tested bLW-E\textsubscript{pq} tubes, we only achieved three tube assemblies in high yield, bLW-E\textsubscript{21/5}, bLW-E\textsubscript{31/7}, and bLW-E\textsubscript{32/6}. Thus, the tube assembly performance depends not only on a delicate balance between the intrinsic tile curvature and the arm twists, but also on the helix winding phase and geometry match between the tile cores and the arms.

Formation of nonhomogeneous tubes assembled from small tiles by O-tiling is often attributed to the decrease of the global free energy of the system. We observed dominant tubular structures and minor planar lattices in the bLW-O\textsubscript{26/4} assemblies, which might be caused by the rigidity of bLW tile cores which always possess a curvature. The curvature flip-over effect observed in the E-tiling tubes might also occur in the O-tiling assemblies. Once a ring seed spontaneously forms, epitaxial extension of the tube lattices could force the oppositely curled tiles in the bLW-O\textsubscript{26/4} assemblies to switch to the same curled ones. As for the less rigid TW and DX tiles, the intrinsic tile curvature is more flexible, thus leading O-tiling of TW and DAE tiles to generate dominant planar ribbons and minor nonhomogeneous tubes with random stoichiometric ratios, suggesting that the global curling bias is much weaker on the TW-O\textsubscript{26/4} and the DAE-O\textsubscript{26/4} assemblies\textsuperscript{5} than on the bLW-O\textsubscript{26/4} ones. Overall, coupling of shallower intrinsic tile curvatures with weaker arm twisting torques would generate either larger lattices or wider tubes easily in higher probability. On the other hand, the coupling of the deeper intrinsic curvatures with stronger arm twisting torques to generate large arrays would be difficult to achieve, and would most probably yield ill-behaved fragments of tile oligomers, narrower tubes, and even fibers rather than well-behaved DNA lattices. Other couplings between the two extremes would generate moderate structures in a complicated way.

In summary, we not only realized three weaving architectures to construct three new tile cores, LW, bLW, TW, and their 2D lattices, but we also deciphered each tube configuration and their component tile’s conformation with the biotin/SA labeling technique. By abstracting the 2D Bravais lattice of centered rectangle for each assembly, we introduced the chiral indices to quantify the global tile curvature. We used a simplified model to analyze in detail the curving forces acting on the two types of DNA tubes (the intrinsic tile curving force and the extra arm curving force), The coupling of these two forces allowed us to understand the mechanism of formation of the DNA tubes into a plethora of different shapes, diameters, and configurations. Such detailed investigation of the DNA tube curvatures including structure and quantitation will on one hand be helpful in the future for engineering DNA nanostructures with high yield and high quality, and for the investigation of the different physicochemical properties and biological functionalities of the DNA chiral assemblies on the other hand.

ASSOCIATED CONTENT
Supporting Information available. Supporting Information Available: experimental methods, a discussion about the theoretical estimation of lattice linear and angular constants, a discussion about the tube parameters calculated from the chiral indices (n,m), a discussion about the assignment of the chiral indices (n,m) to a specific tube based on its high-resolution AFM image, a discussion of assignments of a cluster of chiral indices by the numerical approximation method to both bLW-E_{21/5} and TW-E_{21/5} tubes, a discussion of the saddle-like tile oligomer model to simultaneously grow “large” and “giant” tubes, additional AFM images of each assembly with descriptions in its figure caption, and sequence information. These materials are available free of charge via the Internet.

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Notes

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REFERENCES

(1) Seeman, N. C., DNA in a Material World. Nature 2003, 421 (6921), 427-431.
(2) Winfree, E.; Liu, F.; Wenzler, L. A.; Seeman, N. C., Design and Self-Assembly of Two-Dimensional DNA Crystals. Nature 1998, 394 (6693), 539-544.
(3) Rothemund, P. W. K., Folding DNA to Create Nanoscale Shapes and Patterns. Nature 2006, 440 (7082), 297-302.
(4) Seeman, N. C.; Sleiman, H. F., DNA Nanotechnology. Nat. Rev. Mater. 2017, 3 (1), 17068.
(5) Hong, F.; Zhang, F.; Liu, Y.; Yan, H., DNA Origami: Scaffolds for Creating Higher Order Structures. Chem. Rev. 2017, 117 (20), 12584-12640.
(6) Ramezani, H.; Dietz, H., Building Machines with DNA Molecules. Nat. Rev. Genet. 2020, 21 (1), 5-26.
(7) Aldaye, F. A.; Palmer, A. L.; Sleiman, H. F., Assembling Materials with DNA as the Guide. Science 2008, 321 (5897), 1795-1799.
(8) Platnich, C. M.; Hariri, A. A.; Sleiman, H. F.; Cosa, G., Advancing Wireframe DNA Nanostructures Using Single-Molecule Fluorescence Microscopy Techniques. Acc. Chem. Res. 2019, 52 (11), 3199-3210.
(9) Fu, T.; Seeman, N., DNA Double-Crossover Molecules. Biochemistry 1993, 32 13, 3211-20.
(10) Liu, F.; Sha, R.; Seeman, N. C., Modifying the Surface Features of Two-Dimensional DNA Crystals. *J. Am. Chem. Soc.* 1999, 121 (5), 917-922.

(11) Mao, C.; Sun, W.; Seeman, N. C., Designed Two-Dimensional DNA Holliday Junction Arrays Visualized by Atomic Force Microscopy. *J. Am. Chem. Soc.* 1999, 121 (23), 5437-5443.

(12) Zheng, J.; Birktoft, J. J.; Chen, Y.; Wang, T.; Sha, R.; Constantiou, P. E.; Ginell, S. L.; Mao, C.; Seeman, N. C., From Molecular to Macroscopic via the Rational Design of a Self-Assembled 3D DNA Crystal. *Nature* 2009, 461 (7260), 74-77.

(13) Liu, D.; Wang, M.; Deng, Z.; Walulu, R.; Mao, C., Tensegrity: Construction of Rigid DNA Triangles with Flexible Four-Arm DNA Junctions. *J. Am. Chem. Soc.* 2004, 126 (8), 2324-2325.

(14) Shih, W. M.; Quispe, J. D.; Joyce, G. F., A 1.7-Kilobase Single-Stranded DNA that Folds into a Nanoscale Octahedron. *Nature* 2004, 427 (6975), 618-621.

(15) Zhang, C.; He, Y.; Chen, Y.; Ribbe, A. E.; Mao, C., Aligning One-Dimensional DNA Duplexes into Two-Dimensional Crystals. *J. Am. Chem. Soc.* 2007, 129 (46), 14134-14135.

(16) Yin, P.; Hariadi, R. F.; Sahu, S.; Choi, H. M. T.; Park, S. H.; LaBean, T. H.; Reif, J. H., Programming DNA Tube Circumferences. *Science* 2008, 321 (5890), 824-826.

(17) Ke, Y.; Ong, L. L.; Shih, W. M.; Yin, P., Three-Dimensional Structures Self-Assembled from DNA Bricks. *Science* 2012, 338 (6111), 1177-1183.

(18) Yan, H.; Park, S. H.; Finkelstein, G.; Reif, J. H.; LaBean, T. H., DNA-Templated Self-Assembly of Protein Arrays and Highly Conductive Nanowires. *Science* 2003, 301 (5641), 1882-1884.

(19) Tian, C.; Li, X.; Liu, Z.; Jiang, W.; Wang, G.; Mao, C., Directed Self-Assembly of DNA Tiles into Complex Nanocages. *Angew. Chem. Int. Ed.* 2014, 53 (31), 8041-8044.

(20) Guo, X.; Wang, X.-M.; Wei, S.; Xiao, S.-J., Construction of a Holliday Junction in Small Circular DNA Molecules for Stable Motifs and Two-Dimensional Lattices. *ChemBioChem* 2018, 19 (13), 1379-1385.

(21) Guo, X.; Wang, X. M.; Xiao, S. J., Stable DNA Motifs, 1D and 2D Nanostructures Constructed from Small Circular DNA Molecules. *J. Vis. Exp.* 2019, (146).

(22) Dietz, H.; Douglas, S. M.; Shih, W. M., Folding DNA into Twisted and Curved Nanoscale Shapes. *Science* 2009, 325 (5941), 725-730.

(23) Ke, Y.; Douglas, S. M.; Liu, M.; Sharma, J.; Cheng, A.; Leung, A.; Liu, Y.; Shih, W. M.; Yan, H., Multilayer DNA Origami Packed on a Square Lattice. *J. Am. Chem. Soc.* 2009, 131 (43), 15903-15908.

(24) Woo, S.; Rothemund, P. W., Programmable Molecular Recognition Based on the Geometry of DNA Nanostructures. *Nat. Chem.* 2011, 3 (8), 620-7.

(25) Maier, A. M.; Bae, W.; Schifflers, D.; Emmerig, J. F.; Schiff, M.; Liedl, T., Self-Assembled DNA Tubes Forming Helices of Controlled Diameter and Chirality. *ACS Nano* 2017, 11 (2), 1301-1306.

(26) Wei, B.; Dai, M.; Myhrvold, C.; Ke, Y.; Jungmann, R.; Yin, P., Design Space for Complex DNA Structures. *J. Am. Chem. Soc.* 2013, 135 (48), 18080-18088.

(27) Marchi, A. N.; Saaem, I.; Vogen, B. N.; Brown, S.; LaBean, T. H., Toward Larger DNA Origami. *Nano Lett.* 2014, 14 (10), 5740-5747.

(28) Benn, F.; Haley, N. E. C.; Lucas, A. E.; Silvester, E.; Helmi, S.; Schreiber, R.; Bath, J.; Turberfield, A. J., Chiral DNA Origami Nanotubes with Well-Defined and Addressable Inside and Outside Surfaces. *Angew. Chem. Int. Ed.* 2018, 57 (26), 7687-7690.

(29) Sun, S.; Yang, Y.; Li, D.; Zhu, J., Large Chiral Nanotubes Self-Assembled by DNA Bricks. *J. Am. Chem. Soc.* 2019, 141 (50), 19524-19528.

(30) Mathieu, F.; Liao, S.; Kopatsch, J.; Wang, T.; Mao, C.; Seeman, N. C., Six-Helix Bundles Designed from DNA. *Nano Lett.* 2005, 5 (4), 661-665.

(31) Hong, F.; Jiang, S.; Lan, X.; Narayanan, R. P.; Sule, P.; Zhang, F.; Liu, Y.; Yan, H., Layered-Crossover Tiles with Precisely Tunable Angles for 2D and 3D DNA Crystal Engineering. *J. Am. Chem. Soc.* 2018, 140 (44), 14670-14676.
(32) Qian, H.; Tian, C.; Yu, J.; Guo, F.; Zheng, M.-S.; Jiang, W.; Dong, Q.-F.; Mao, C., Self-Assembly of DNA Nanotubes with Defined Diameters and Lengths. Small 2014, 10 (5), 855-858.

(33) Mitchell, J. C.; Harris, J. R.; Malo, J.; Bath, J.; Turberfield, A. J., Self-Assembly of Chiral DNA Nanotubes. J. Am. Chem. Soc. 2004, 126 (50), 16342-16352.

(34) Liu, X.; Zhao, Y.; Liu, P.; Wang, L.; Lin, J.; Fan, C., Biomimetic DNA Nanotubes: Nanoscale Channel Design and Applications. Angew. Chem. Int. Ed. Engl. 2019, 58 (27), 8996-9011.

(35) Rothemund, P. W. K.; Ekani-Nkodo, A.; Papadakis, N.; Kumar, A.; Fygenson, D. K.; Winfree, E., Design and Characterization of Programmable DNA Nanotubes. J. Am. Chem. Soc. 2004, 126 (50), 16344-16352.

(36) Ke, Y.; Liu, Y.; Zhang, J.; Yan, H., A Study of DNA Tube Formation Mechanisms Using 4-, 8-, and 12-Helix DNA Nanostructures. J. Am. Chem. Soc. 2006, 128 (13), 4414-4421.

(37) Sherman, W. B.; Seeman, N. C., Design of Minimally Strained Nucleic Acid Nanotubes. Biophys. J. 2006, 90 (12), 4546-4557.

(38) Wang, T.; Schiiffels, D.; Cuesta, S. M.; Fygenson, D. K.; Seeman, N. C., Design and Characterization of 1D Nanotubes and 2D Periodic Arrays Self-Assembled from DNA Multi-helix Bundles. J. Am. Chem. Soc. 2012, 134 (3), 1606-1616.

(39) Woods, D.; Doty, D.; Myhrvold, C.; Hui, J.; Zhou, F.; Yin, P.; Winfree, E., Diverse and Robust Molecular Algorithms Using Reprogrammable DNA Self-Assembly. Nature 2019, 567 (7748), 366-372.

(40) Dresselhaus, M. S.; Dresselhaus, G.; Saito, R., Physics of Carbon Nanotubes. Carbon 1995, 33 (7), 883-891.

(41) Saito, R.; Fujita, M.; Dresselhaus, G.; Dresselhaus, M. S., Electronic Structure of Chiral Graphene Tubules. Appl. Phys. Lett. 1992, 60 (18), 2204-2206.

(42) Barish, R. D.; Schulman, R.; Rothemund, P. W. K.; Winfree, E., An Information-Bearing Seed for Nucleating Algorithmic Self-Assembly. Proc. Natl. Acad. Sci. U.S.A. 2009, 106 (15), 6054-6059.

(43) Mohammed, A. M.; Schulman, R., Directing Self-Assembly of DNA Nanotubes Using Programmable Seeds. Nano Lett. 2013, 13 (9), 4006-4013.

(44) Jorgenson, T. D.; Mohammed, A. M.; Agrawal, D. K.; Schulman, R., Self-Assembly of Hierarchical DNA Nanotube Architectures with Well-Defined Geometries. ACS Nano 2017, 11 (2), 1927-1936.

(45) Minev, D.; Wintersinger, C. M.; Ershova, A.; Shih, W. M., Robust Nucleation Control via Crisscross Polymerization of Highly Coordinated DNA Slats. Nat. Commun. 2021, 12 (1), 1741.

(46) Reishus, D.; Shaw, B.; Brun, Y.; Chelyapov, N.; Adleman, L., Self-Assembly of DNA Double-Double Crossover Complexes into High-Density, Doubly Connected, Planar Structures. J. Am. Chem. Soc. 2005, 127 (50), 17590-17591.

(47) Lee, J. Y.; Kim, Y.-J.; Lee, C.; Lee, J. G.; Yagyu, H.; Tabata, O.; Kim, D.-N., Investigating the Sequence-Dependent Mechanical Properties of DNA Nicks for Applications in Twisted DNA Nanostructure Design. Nucleic Acids Res. 2018, 47 (1), 93-102.

(48) Jung, W. H.; Chen, E.; Veneziano, R.; Gaitanaros, S.; Chen, Y., Stretching DNA Origami: Effect of Nicks and Holliday Junctions on the Axial Stiffness. Nucleic Acids Res. 2020, 48 (21), 12407-12414.
