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DNA Tubes forming Helices with Controlled Diameters and Chirality

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Abstract

Multihelical DNA bundles could enhance the functionality of nanomaterials and serve as model architectures to mimic protein filaments on the molecular and cellular level. We report the self-assembly of micrometer-sized helical DNA nanotubes with widely controllable helical diameters ranging from tens of nanometers to a few micrometers. Nanoscale helical shapes of DNA tile tubes (4, 6, 8, 10 and 12-helix tile tubes) are achieved by introducing discrete amounts of bending and twist through base pair insertions and/or deletions. Microscale helical diameters, which require smaller amounts of twist and bending, are achieved by controlling the intrinsic "supertwist" present in tile tubes with uneven number of helices (11, 13 and 15-helix tile tubes). Supertwist fine-tuning also allows us to produce nanotubes of defined chirality.

Keywords

DNA nanotechnology; DNA self-assembly; helical nanotubes; chirality; protein filament mimics

Helical motifs can be found throughout biological micro- and nanostructures. Famous examples are the double helix of DNA¹, cellulose fibrils of tendrils² or the shell of holoplanktonic mollusks³ to name just a few. At the macroscopic world, screws and springs, both helical structures, serve essential mechanical functions in classical engineering such as conversion of rotational into linear motion, or storing and releasing mechanical energy by using a combination of rigidity and elasticity. Miniaturizing these concepts to the micro- and nanoscale would not only allow the construction of novel materials, which can function as mechanical nanotools, but also provide an adjustable helical building block to mimic hierarchically assembled structures such as protein filaments. Top-down fabrication methods

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Author contributions

A.M.M., and T.L. conceived the experiments. A.M.M. produced the structures, performed and analyzed the measurements. A.M.M., and T.L. interpreted the results and wrote the manuscript.

Competing financial interests

The authors declare no competing financial interests.

have so far produced micro- and nanohelices by strain-induced self-scrolling,^{4–6} glancing angle deposition^{7–10} of evaporated or sputtered materials, and 3D direct laser writing^{11, 12} in photoresists. Micro- and nanoscrews fabricated by these methods were recently used for the propulsion of artificial micro- and nanoswimmers,^{5, 8, 13–16} which are foreseen to achieve future biomedical tasks such as *in vivo* drug delivery or blood clot removal.^{17–19} Micro- and nanosprings were found to possess extraordinary properties, such as super-elasticity^{6, 20, 21} and high mechanical strength²² making them promising candidates for the construction of shape memory materials^{6, 20} and composites with improved mechanical integrity.²³ However, large-scale production of three-dimensional structures at the nanometer scale is challenging for conventional techniques and many of the employed materials are not biocompatible, which hampers their potential for biomedical applications. Bottom-up DNA self-assembly, on the other hand, offers the above-mentioned features while enabling the design of structures with nanometer precision^{24–28}. For example, DNA origami allows the construction of rigid nanostructures with designed amount of bending and twist.²⁹ We recently showed that several micrometer-long helical DNA nanotubes are assembled in a simple one-pot reaction from only eight different oligonucleotides.¹⁴ By systematically testing the various parameters of DNA tile design, such as the number and relative positions of inserted or deleted basepairs and the relative position of tiles, we now establish a set of rules for the rational design of custom-shaped DNA tube helices of defined chirality and helical diameters.

In DNA tile tube assembly, single-stranded DNA oligonucleotides (tiles) assemble into repetitive segments of n -helix tubes that can polymerize into tubes of micrometer length²⁴. If the number of DNA double helices of a given tube design is even, the sheet of parallel double helices can roll up into a tube (see Supporting Figure S?) with zero offset along its two long edges, as exemplarily illustrated for a 12-helix tile tube (12HT) in Figure 1a. This design results in straight and unstressed tubular structures^{14, 24}. When a base pair is inserted into or deleted from a selected double helix (Figure 1b), local stress and strain is generated and distributed over the whole tube structure as was previously demonstrated for similar DNA constructs^{14, 29, 30}. For example, in the case of a base pair insertion at one position, this particular double helix gets longer (Figure 1b, c red parts) and pushes the neighbouring double helices *via* the cross-over points towards opposite directions. At the same time the same cross-over points are twisted in respect to each other and the whole tube structure both bends and twists, adopting a helical shape (Figure 1c). Note that we refer to twist along the tube's primary axis only.

Following these design principles, we expect that for tubes with larger number of helices n the effect of insertions and deletions will be distributed over more double helices and thus become smaller. Within a tube of a given number of double helices, we expect to get a stronger effect for more insertions and deletions. To test these hypothesis, we changed the number of insertions or deletions $N_{ins,del}$ for a variety of tube sizes n (for design details and tile sequences see Supplementary Note S1 and Figure S1).

Figure 2a shows transmission electron microscopy images of tile tubes for decreasing tube size n (from 12HT to 4HT) and constant number of insertions ($N_{ins} = 1$). All tubes revealed an undulatory shape of nanometer dimensions that became more pronounced for smaller

tube sizes. Exact quantification of the helix parameters, such as helical diameter, pitch and chirality, was not possible as TEM sample preparation resulted in a 2-D confinement of the original helical shape of the tubes. However, Nam *et al.* recently proposed that such squeezed conformations, so-called *squeelices*, have almost the same curvature ω as their unconfined 3D predecessors.³¹ In place of the helical diameter and pitch, we therefore extracted the radius of curvature $r_c = 1/\omega$ from the 2D confined helical shape (see inset of Figure 2a) and compared it for the different tube types (detailed information on the statistical analysis are given in Figure S2 and Table S1). We found that r_c steadily decreased with decreasing tube size n for both, tubes with insertions (Figure 2b) and deletions (Figure 2c) from 560 nm to 88 nm and from 457 nm to 65 nm, respectively (TEM images of tubes with deletions are shown in Figure S3).

Figure 2d shows images of tile tubes with increasing amount of insertions N (from 1 to 6) and constant tube size $n = 12$. We chose the 12-helix tile tube (12HT) for these experiments as its total interhelical angle is $\sim 360^\circ$ by the geometry of DNA so that no additional bending and twisting is required to close (illustrated in Figure S4)²⁴. Insertions and deletions in the 12HT were placed in such a way that they successively increase the total amount of bending. We found that r_c steadily decreased for increasing amounts of insertions (Figure 2e) or deletions (Figure 2f) from 554 nm to 155 nm and from 556 nm to 110 nm, respectively (TEM images of 12HT tubes with deletions are shown in Figure S5). To achieve even stronger bending, we constructed tubes with the same number of insertions and deletions located on opposite double helices (Figure 2g).

Indeed, r_c decreased drastically for all five tube types. The minimum radius that we achieved with this approach was 77 nm (Figure 2h). At higher numbers of insertions and deletions proper folding of the tubes was inhibited. In previous studies on 6- and 4-helix bundles, such arrangements led to the formation of rings,³⁰ however, we observed that preferentially helical structures formed. This discrepancy can be explained by the greater persistence length of the 12HT^{32–34} which reduces the probability of fluctuation-induced ring closure during tube growth. An asymmetric design with N insertions and $N-1$ deletions is shown in Figure 2i (TEM images are shown in Figure S6). Its radii were consistently smaller than those for tubes with only insertions or deletions and larger than for tubes with both insertions and deletions.

In order to determine whether this technique is generally applicable for the controlled construction of nanoscale helical structures, we compared our results to radii predicted from energetic considerations using a DNA toy model recently presented by Dietz *et al.*²⁹ It expresses the energy stored within the DNA tile tube as a sum of stretch (S) and bend energy (B) (for detailed information see Supplementary Note S2). Remarkably, the energetic predictions (presented as black symbols in the graphs of Figure 2, respectively) show good agreement and only slightly overestimate the experimental data in all of the tubes examined. A similar overestimation also occurred for large r_c in the original DNA toy model study.²⁹ We can only speculate that the comparatively strong deviations for the first values in all graphs are the result of a buffering effect in DNA nanostructures. In contrast to perfect double-stranded DNA, which are assumed in the model, our structures have nicks and cross overs. The additional flexibility that these features give to the structures could absorb some

of the insertion- or deletion-induced bending. As this effect needs to be overcome only once, it would affect tubes with few insertions or deletions more than those with many insertions or deletions. For small r_c , the deviations can be attributed to a structural change in the cross section of the modified tile tubes from a perfect circle (see Supplementary Figure S7 for detailed information). In conclusion, we were able to generate DNA tile tubes with radii of curvature ranging from 65 nm to 560 nm using the insertion and deletion method.

In certain applications such as microswimmers, filaments with microscale helical diameters are desirable as this provides stronger propulsion forces.¹⁴ This requires even smaller amounts of twist and bending than those arising from single basepair insertions or deletions. We therefore introduced a novel technique referred to as *tile shifting*, which relies on the stepwise reduction of the intrinsic *supertwist* of DNA tile tubes to generate tubes with smallest quantities of twist along the tube's primary axis. Due to the alternating tile alignment, DNA tile tubes with an odd number of double helices close with a discrete offset resulting in a supertwisted helix bundle (Figure 3a). This supertwist can be attenuated in small steps by reducing the offset of closure through shifting of single tiles one nucleotide (nt) position at a time within the structure (Figure 3b). This also allows the predefinition of the chirality of the supertwist. In the depicted case of a tile-shifted 13-helix tube (stw13HTs) it is left-handed for geometrical reasons (for design details and tile sequences see Supplementary Note S3 and Figure S8 and S9).

Smaller quantities of bending were achieved by modifying the tube structures with defined arrangements of Cy3 dyes (see Supplementary Figure S1 for Cy3-modified tile sequences). This also enabled us to visualize the tubes in solution via fluorescence microscopy. In our previous work we noticed that a Cy3-modified 6-helix tile tube exerted quantities of bending so small that it resulted in a helical microscale diameter of the overall tube structure.³² A Cy3 dye in the vicinity of a DNA duplex blunt end is known to induce a stretch and a right-handed twist deformation of the DNA helix (Figure 4a) similar to that of a basepair insertion.^{35, 36} In accordance with this observation, we found that the amount of bending in a 6HT strongly depends on the arrangement of the Cy3 dyes on the tube. Figure 4b demonstrates that Cy3-modifications on one side of the tube give stronger bending - and an associated smaller helical diameter of the tube - than modifications on opposing sides of the tube. Such opposing arrangements are therefore promising to construct tubes with small quantities of bending.

By combining the bending through Cy3 modifications with the fine-controlled supertwisting via *tile shifting*, we were able to stepwise control the microscale helical diameter of DNA tile tubes (Figure 5a). Fluorescence microscopy images of Cy3-modified supertwisted 13HTs (stw13HTs) with increasing amount of tile shift (from 0 to 6 nt) are shown in Figure 5b. Without tile shift (0 nt), the 13HT had a small helical diameter, which was barely resolvable with light microscopy (see Supplementary Figure S10 for TEM images of this structure). A stepwise tile shift (from 0 to 4 nt), however, resulted in a stepwise increase of the helical diameter to a maximum of $\sim 2 \mu\text{m}$ at 4nt. Further tile shifting (5 nt and 6 nt) decreased the helical diameter again. Helical diameters for 1 nt, 2 nt, 3 nt and 5 nt are $\sim 0.4 \mu\text{m}$, $\sim 1 \mu\text{m}$, $\sim 1.5 \mu\text{m}$ and $\sim 0.6 \mu\text{m}$, respectively.

To understand this behaviour we considered the total amount of bending and twist present in the 13HTs. While keeping the amount of Cy3-induced bending constant in all tubes, the overall twist - a superposition of supertwist and Cy3-induced twist - was changed in small steps through tile shifting (from 0 to 6 nt). For small shifts (< 4 nt), the left-handed supertwist dominated over the right-handed Cy3-induced twist. As more tile shift was introduced (from 0 to 4 nt), the supertwist decreased which resulted in a reduced overall twist and therefore larger helical diameters. A helical maximum diameter of $\sim 2 \mu\text{m}$ was reached at a 4 nt shift, which suggests that the total twist is minimal. By further reducing the supertwist (> 5 nt), the Cy3-induced right-handed twist became dominant over the supertwist and again reduced the tube's helical diameter.

We tested this hypothesis by determining the chirality of these tube structure by taking "tomographic" slices of their 3D shapes via fluorescence microscopy. For each sample a set of images was taken while the focal plane was moved through the sample volume. Figure 6a exemplarily shows fluorescence images of stw13HTs with a 2 nt tile shift in three distinct positions of the focal plane: slightly beneath (left), centred (middle) and slightly above (right) the centre of the helical tile tube. The contours of each plane yielded a distinct pattern, which allowed us to determine the tubes chirality. Furthermore, a 3D reconstruction of the structures could be generated from the set of images (Figure 6b). In the depicted sample the chirality was determined to be left-handed. We successively applied this technique to the tile-shifted stw13HTs from Figure 4 and additionally to tile-shifted stw11HTs and stw15HTs (see Supplementary Figure S11). In all three cases a change of chirality from left- to right-handed was observed for increasing amounts of tile shift (see Supplementary Figure S12).

Our DNA-based self-assembly scheme allows large-scale production of biocompatible helical nanotubes with precise control over their micro- and nanoscale helical diameter and chirality. Incorporation of basepair insertions and deletions enables us to adjust the radius of curvature within the nanometer regime in accordance with an energetic model. Microscale helical diameters of defined chirality are achieved through fine controlling the intrinsic supertwist *via* tile shifting in Cy3-modified tubes. Design rules derived from this work and the good correspondence of our experimental results with a simple theoretical model can be utilized to design and construct predefined helical shapes for future applications. For example, micrometer-sized helical constructs can be applied to optimize the swimming behaviour of artificial microswimmers, which allows researchers to gain further insights into the swimming strategies of microorganisms. For our helical tubes to serve as nanosprings, force spectroscopic analysis and Cryo-electron microscopy could be applied to determine their mechanical properties such as their spring constants and to obtain additional information over their helical diameter and pitch. Our novel design techniques demonstrate the flexibility and simplicity of DNA self-assembly for the construction of fully biocompatible nanoscrews and -springs, which may find their way into nanorobots for non-invasive biomedical therapies and in other fields of nanoengineering.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Design principles for the construction of helical DNA tile tubes.

a) DNA tile tubes with an even number of double helices (here a 12-helix tile tube design is shown) close with zero offset of the edges, resulting in a straight overall shape. **b)** Insertion of an additional base pair into a double helix generates expansion (red arrows) and a right-handed torque of the helix. Deletion of a base pair leads to compression (blue arrows) and a left-handed torque. **c)** Expansion respectively compression leads to bending while torque results in twisting of the overall tube structure. The combination of both effects forces the tube into a helical shape.

Figure 2. Construction of helical DNA tile tubes with controlled nanometer-sized radius of curvature.

a) DNA tile tubes with decreasing size n (from 12HT to 4HT) and constant number of insertions $N=1$ as observed by TEM. Inset: Radius of curvature r_c extracted from the 2D-confined undulatory shape. **b)** Relationship between r_c and tubes of different size with insertions (red triangles) and with **c)** deletions (blue triangles). **d)** Tubes with constant size ($n=12$) and increasing amounts of insertions N (from 1 to 6) as observed by TEM. Relationship between r_c and the amount of **e)** insertions (red triangles) **f)** deletions (blue triangles) in tubes of constant size. **g)** Tubes with increasing amounts of both insertions and deletions N (from 1 to 5) as observed by TEM. Relationship between r_c and tubes of **h)** symmetric (red triangles) and **i)** asymmetric arrangements of insertions and deletions.

Theoretical predictions of r_c from an energetic model are shown in black symbols for each tube type. Scale bars: 500 nm.

Figure 3. Design principles for controlling the supertwist of DNA tile tubes

a) Tile tubes with an odd number of tubes close with a discrete offset resulting in a *supertwisted* shape of the structure. **b)** By shifting single tiles in the design, the offset can be reduced stepwise as illustrated for the case of a 3 nt (left) and 6 nt tile shift (right) in a supertwisted 13-helix tile tube (stw13HT). Here, the *tile-shifting* gradually reduces the overall amount of supertwist in the structure.

Figure 4. Cy3-induced bending and twisting of DNA tile tubes

a) A Cy3-modification on a DNA tile tube can act similar to a base pair insertion and lead to a helical shape of the structure. **b)** 6-helix tile tubes (6HTs) with three different arrangements of Cy3 dyes showed different bending and twisting as observed by fluorescence microscopy. Note that opposing Cy3 modifications reduced the total amount of bending whereas the induced twisting added up, which resulted in a helical shape with increased diameter and pitch. Scale bar: 2 μm

Figure 5. Construction of helical DNA tile tubes with controlled micrometer-sized diameter
a) Scheme of a DNA tile tube, which combines *tile shifting* and Cy3 modifications for a controlled bending and twisting of the structure. **b)** Fluorescence microscopy images of stw13HTs with increasing amount of *tile shifting* (from 0 to 6 nt). The tubes were constructed from three Cy3-modified and ten unmodified tiles. Helical diameters of up to $\sim 2 \mu\text{m}$ were observed for a 4 nt tile shift. The decrease of the helical diameter after reaching this maximum already suggests a change in the chirality of the tube shape. Scale bar: 2 μm .

Figure 6. Determination of the chirality of DNA tile tubes

a) Fluorescence microscopy “tomographic” slices of tile-shifted stw13HTs at three different z-positions of the focal plane (left to right: slightly below, centred and slightly above the centre of the helix). The distinct patterns from the images were used to determine the chirality of the tube. In the depicted case of stw13HTs with a 2 nt tile shift, the chirality was determined to be left-handed. **b)** A 3D reconstruction was generated from the entire set of images. Scale bars: 5 μm .