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Folding and cutting DNA into reconfigurable topological nanostructures

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Abstract

Topology is the mathematical study of the spatial properties that are preserved through the deformation, twisting and stretching of objects. Topological architectures are common in nature and can be seen, for example, in DNA molecules that condense and relax during cellular events¹. Synthetic topological nanostructures, such as catenanes and rotaxanes, have been engineered using supramolecular chemistry, but the fabrication of complex and reconfigurable structures remains challenging². Here, we show that DNA origami³ can be used to assemble a Möbius strip, a topological ribbon-like structure that has only one side^{4–6}. In addition, we show that the DNA Möbius strip can be reconfigured through strand displacement⁷ to create topological objects such as supercoiled ring and catenane structures. This DNA fold-and-cut strategy, analogous to Japanese kirigami⁸, may be used to create and reconfigure programmable topological structures that are unprecedented in molecular engineering.

Structural DNA nanotechnology⁹, which makes use of DNA as an information-coding polymer capable of programming nano-structure assembly, has proven its utility in creating sophisticated nanoscale geometrical objects^{10–16}. The recent development of DNA origami³, a method that uses short DNA oligos as staples to fold single-stranded genomic DNA scaffolds into geometrically defined two- (ref. 3) and three-dimensional nanoarchitectures^{17–21}, has opened up great opportunities for directed assembly of chemical and biomolecular species with exquisite positional control^{22–26}.

Engineering topologically complex molecular architectures represents an appealing challenge for chemists and materials scientists, because structures such as catenanes, rotaxanes and knots often display unique material properties². Topological structures have been successfully generated through organic and supramolecular synthesis^{27,28}. Earlier works from Seeman and colleagues have exploited enzymatic ligation of simple, branched DNA junctions to create configurations such as various knots²⁹ and a Borromean ring structure³⁰, which were predicated on the relationships between topological crossing points and half-turns within B-DNA or Z-DNA. However, progress using DNA to achieve

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Author contributions H.Y. and D.H. conceived and designed the experiment. D.H., S.P. and Y.L. performed the experiments. D.H., Y.L., S.P. and H.Y. analysed the data. All authors discussed the results. H.Y., Y.L. and D.H. wrote the manuscript.

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topological nanostructures has been quite limited in the past decade, and new strategies³¹ need be developed to meet the challenge of engineering complex synthetic DNA geometries at the nanoscale.

Here, we demonstrate a DNA origami technique that can be used to create sub-100-nm topological architectures that are reconfigurable. We chose the Möbius strip as our demonstration target, not only because it is artistically inspiring, but also because it will likely display unique material properties^{4,5} that may be applied to create novel molecular devices⁶. The Möbius strip has several interesting characteristics. Topologically speaking, it is a surface with only one side and only one boundary. A model can be easily created by taking a paper strip and giving it a half-twist, and then joining the ends of the strip together to form a loop. Cutting along the centre line of the loop destroys a Möbius strip, yielding a twice longer loop with two full twists. Cutting creates a second independent edge, the two halves of which were on either side of the scissors. If the Möbius strip is cut along about a third of the way in from the edge, it creates two strips interlocked together. One is a thinner Möbius strip, which is the centre third of the original strip, comprising one-third of the width but the same length as the original strip. The other is a longer, thinner strip with two full twists, comprising one-third of the width and twice the length of the original strip. In this work, we experimentally demonstrate all of these interesting features of the Möbius strip using DNA fold-and-cut technology, showing the versatility of structural DNA nanotechnology in creating highly programmable, topologically controlled architectures.

Figure 1 illustrates our design for engineering ~100-nm Möbius strips using single-stranded M13mp18 viral DNA as scaffolding strands and 164 short DNA oligos as staple strands. Each strip contains eleven DNA double helices assembled, in parallel, within a common plane when it is open. The overall length of the strip is ~210 nm (61.5 helical turns) in length and the width is ~25 – 30 nm (eleven helices plus gaps between the helices). The strip is twisted 180° along its central axis and connected back to itself, seamlessly. In Fig. 1a, each coloured band represents a different DNA double helix. Note that, although the strip contains eleven DNA helices, only six different colours are used. The central helix (red) circles around the length of the strip once and each of the other five helices spans the length of the strip twice while also twisting around the central helix by 180° degrees before reconnecting to itself. Figure 1b shows a small fraction of the Möbius strip with DNA helix details, in which the scaffold strand is blue and the staple strands are different colours. The entire Möbius strip consists of 20.5 units like this.

Figure 1c shows a generalized unit in the Möbius DNA strip to illustrate the folding path. The ends of the helices are labelled from 1 to 11, with the ends labelled with the same number being continuously connected with each other. In the Möbius strip structure, the pink dashed box shown in Fig. 1c is the basic design unit that will be repeated 20 times in a row and connected back to the starting point. The design unit used in the experiment contains nearly the same folding path of the scaffold strand as shown in Fig. 1c, except that 5 out of 20 such units contain a crossover point for the scaffold strands. One representative folding path unit is shown in Fig. 1d that has a scaffold strand crossing over between helices 8 and 9 (see Supplementary Information for detailed layout and strand sequences of each design unit).

In the initial design of the Möbius strip structure we used 32 base pairs (bp) for every three full helical turns or 10.67 bp per turn, which closely resembles B-type DNA, which has a twist of ~10.5 bp per turn. No extra twist is introduced along the helices. Of the scaffold strand, 99.5% is hybridized to the staple strands. The extra ~33 nucleotides are left as a single-stranded loose loop dangling across helix 1. We minimized the number of crossovers

for the scaffold strand and distributed these necessary scaffold crossover points evenly along the length of the strip to create a seamless connection.

Möbius strip structures were assembled by mixing scaffold strands with helper strands in a ratio of 1:10 at a concentration of 10 nM in tris-acetate-EDTA (TAE) buffer containing 12.5 mM Mg^{2+} , then annealed from 90 to 4 °C over 10 h (see Supplementary Information for materials and methods). The formation of Möbius strips was characterized by gel electrophoresis (Supplementary Fig. S12) and verified by atomic force microscopy (AFM) and transmission electron microscopy (TEM) imaging (Fig. 2 and Supplementary Figs S1–S11), revealing that the structure formation was highly efficient. An analysis of the relative intensities of the bands in the gel reveals a 57% yield of the desired product (see Supplementary Fig. S12 for details).

During AFM and TEM imaging, each Möbius strip was flattened on the surface, as DNA has a tendency to maximize the contact with the hydrophilic mica surface or glow discharged TEM grid. They often showed a truncated triangular-shaped pattern with overlapping at the three vertexes. This can be easily modelled by pressing a paper Möbius strip on a flat surface. Both the widths and overall lengths of the Möbius strips measured under AFM and TEM conformed to the designed parameters. The topologies of the structures were also distinguished using amplitude-mode AFM imaging (Fig. 2d), because the amplitude of the tip vibration is sensitive to the edges (sudden height changes) of the surface feature. In addition, under TEM imaging of the negatively stained samples, stain-induced structural contrasts (for example, edges versus inner surfaces between layers) provided further evidence of the topology of the structure (Fig. 2c and Supplementary Fig. S4).

In our design, the Möbius strips had a tendency to form structures with both left-handed and right-handed chirality. Models for both forms are illustrated in Fig. 2c,d. The chirality can be distinguished in carefully executed amplitude-mode AFM images in both trace and retrace directions (Fig. 2d and Supplementary Fig. S2) and in the contrast of negatively stained TEM imaging of the samples (Supplementary Fig. S4). The ratio between the left-handed and right-handed Möbius strips was approximately 1:1.4 as determined by counting ~360 Möbius strips from a collection of TEM images (see Supplementary Information for statistical analysis).

The nearly equal distribution of left- and right-handed chiralities indicates that the handedness of the final structures is dominantly determined randomly during the self-assembly process. This observation can be explained by the following proposed mechanism. In the design, we kept the distribution of the scaffold crossover points along the Möbius strip and the nick points between the staple strands evenly distributed. In any local section of the structure, a number of staple strands cooperatively assemble to the scaffold strand. During the nucleation step, the first few DNA strands that hybridize with the scaffold strand do not experience the presence of other strands and are not affected by the overall twist that arises from other parts of the assembly. As more staple strands bind to their proper positions, the folding path of the scaffold is soon determined and fixed. The free energy changes that govern the formation of right- or left-handed Möbius strips are expected to be very similar and include (i) the free energy gained from the number of base pairs formed, (ii) the energy paid to twist the parallel DNA helix bundle to form the 180 or -180° turn, and (iii) the energy to bend the DNA helix bundle to form a loop. The free energy gained from maximizing the number of base pairs dominates the assembly process and is equivalent in both chiral constructs. The energies required to bend the DNA helices to form the loops are also expected to be equivalent. The energy differences occurring when creating right- or left-handed twists in the DNA helix bundle are therefore the only possible determining

factor for the chirality of the final structures. Indeed, for a right-handed DNA helix with 10.67 bp per turn (>10.5 bp per turn), a right-handed twist is expected to form due to over-twisting²¹. Our observation of the nearly 1:1.4 distribution of left-handed and right-handed chirality of the structure indicates that the distribution of the chirality of the final structures may not be determined by the overall enthalpy changes, but by kinetic factors. This is because, once staple sequence recognition has commenced and determined the folding path of the scaffold, and before the twisting energy has taken hold, the chirality becomes inevitable because the energy barrier to reverse the chirality soon becomes too high to overcome.

The Möbius strip can be reconfigured into various topologies by cutting along the length of the strip at different positions. The addressability of the self-assembled DNA Möbius strip incorporates convenient 'cutting' sites along selected DNA helices, which enable DNA strand displacement. The DNA strand displacement technique has been previously used to reconfigure DNA nanomechanical devices⁷ and structures¹⁷. In the present work, we used the simultaneous displacement of a large set of DNA strands (40 and 81) to demonstrate a 'DNA fold-and-cut' methodology (we call this 'DNA kirigami') using two examples. Briefly, we showed that we can make a DNA origami ring structure that has two full turns along its axis, and we can also efficiently create a catenane of two interlocking rings without using enzymatic ligation.

A three-dimensional illustration of the Möbius strip before and after it is cut in the middle is shown in Fig. 3a (we call this design a kirigami-ring). After cutting, the resulting structure is a circle that is half as narrow and twice as long as the original Möbius strip, contains a twist of 720° (four half turns) around its middle axis, and then loops back to itself. This topology is no longer considered a Möbius strip, because it has two surfaces and two edges (along helix 1 and helix 5). The cutting is achieved by a DNA strand displacement technique⁷, which removes the staple strands that are hybridized to the scaffold strand at helix 6 (the middle helix). We designed a set of staple strands with dangling single-stranded tails (toehold tails), which extended out from the DNA structure along helix 6. As illustrated in Fig. 3b (see also Supplementary Information), every unit framed within the pink dashed box contains four single-stranded toehold tails. Each is 8 nucleotides (nt) in length; 3' end tails are depicted in orange and 5' end tails in green, respectively. The addition of displacement strands (16 nt each, coloured in red) that are fully complementary to toehold-bearing staple strands will displace the staple strands from the scaffold strand so that the upper half of the Möbius strip is separated from the lower half to form the longer, twisted ring structure that is twice as long as the original Möbius strip. To facilitate the strand displacement, we used a thermal cycling protocol (see Supplementary Information for details).

The formation of Möbius strips containing toehold strands was first verified by AFM imaging (Supplementary Fig. S8), and the yield of the kirigami-ring structure was found to be ~66% (see Supplementary Fig. S12 for details). The AFM and TEM images shown in Fig. 3c,d (see additional AFM and TEM images in Supplementary Figs S6,S7) revealed that some of the kirigami rings exhibited open loop structures with two to three kinks as they were pressed down on the substrate surface, while many others resemble figure-of-eight-like structures. The figure-of-eight conformation results from the relaxation of the twist within the kirigami ring, which forms a supercoil. At the intersection, the height of the cross point, as measured under AFM, was about twice that of the remaining portion lying in the rings, which indicated that one part of the ring was laid on top of the other. Because of the resolution limitation of AFM, we were not able to clearly visualize the single-stranded DNA of helix 6 after structural reconfiguration.

As described earlier, the Möbius strip may also be cut along its length approximately one-third of the way into its width. This results in the catenane topology illustrated in Fig. 3e (we refer to this structure as a kirigami catenane). The underlying design (Fig. 3f and Supplementary Information) is similar to the kirigami ring, except that we used only one set of exclusively 3', single-stranded tails to reduce the amount of displacement strands needed for cutting. After addition of the displacement strands, the crossovers of the staple strands between neighbouring helices 4 and 5 are released (the same thermal cycling protocol used for creating the kirigami ring structure was applied here). As a result, helices 1 to 4 conjoin to form the larger ring, and helices 5 and 6 conjoin to form the smaller ring.

The formation of Möbius strips containing this set of toehold strands was again verified by AFM imaging (Supplementary Fig. S8), and the yield of the kirigami-catenane structure was found to be ~74% (see Supplementary Fig. S12 for details). AFM images of the kirigami catenane clearly reveal two interlocking rings comprising each structure. Figure 3g shows the height images (upper panel) and the amplitude images (lower panel) of the catenane. The height images depict two overlapping loops, and the amplitude images reveal that one loop penetrates the other rather than merely lying on top. Figure 3h shows TEM images of the structure, further verifying the structure formation of the kirigami catenane. It is worth pointing out that the only way to connect two individual DNA loops within Euclidean space involves cutting at least one of them in order to link it to the other. After that, we would have to seal the cleavage point to create a catenane. However, DNA kirigami technology allows us to take advantage of the properties of the Möbius strip to easily engineer a catenane without linearizing either of the two components.

It is foreseeable that the combination of DNA self-assembly and reconfiguration will further increase the topological complexity of two-dimensional or three-dimensional DNA nanostructures to create knots and catenanes that are otherwise difficult to make using other nanofabrication methods. For example, by introducing two full turns of twist into the Möbius strip and then cutting along the middle of the strip surface, the knot topology can be achieved. The strategies demonstrated here may generate unique topological nanostructures with novel material properties that may be further enhanced and functionalized through directed self-assembly with other materials^{25,26,32}.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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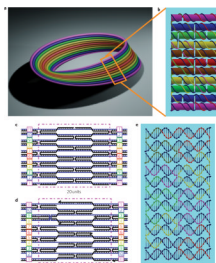


Figure 1. Design of a Möbius DNA strip

a, Three-dimensional illustration of the Möbius DNA strip. Each coloured band represents a different DNA double helix. **b**, A fraction of the Möbius strip in **a** is illustrated in the DNA helical model. The whole Möbius strip is composed of 20.5 of these units. **c**, Generalized folding path of a unit in the Möbius strip with the scaffold strand (blue) running through the entire structure and staple strands (black) helping to fold it into the designed structure. **d**, Design used in the experiment for one of the 20 units. The scaffold strand crosses over between helices 3 and 4. **e**, DNA helical strand model for the unit shown in **d**.

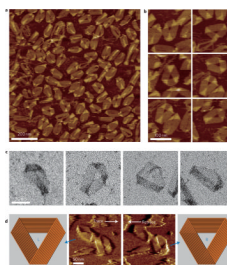


Figure 2. Visualization of the Möbius DNA strips with AFM and TEM imaging
a,b, Zoom-out and zoom-in AFM images of the Möbius DNA strip. **c**, TEM images of the Möbius DNA strip negatively stained with uranyl formate. The two images on the left have a right-handed chirality, and the two images on the right have a left-handed chirality. **d**, AFM amplitude images with the tip scanning in trace and retrace directions indicating the co-existence of both left-handed and right-handed chiral structures in the sample. Schematics are shown to illustrate the chirality of the Möbius strip.

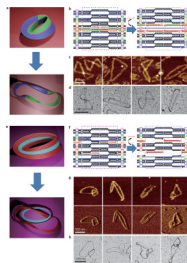


Figure 3. DNA kirigami to achieve reconfigurable topologies from the Möbius strip
a–d, DNA kirigami-ring structure: design schematics (**a,b**), AFM height images (**c**) and TEM images (**d**). **e–h**, The DNA kirigami-catenane structure: design schematics (**e,f**), AFM height (**g**, upper panels) and amplitude (**g**, lower panels) images, and TEM images (**h**).