

Supplementary Information

Programing Molecular Topologies from Single-stranded Nucleic Acids

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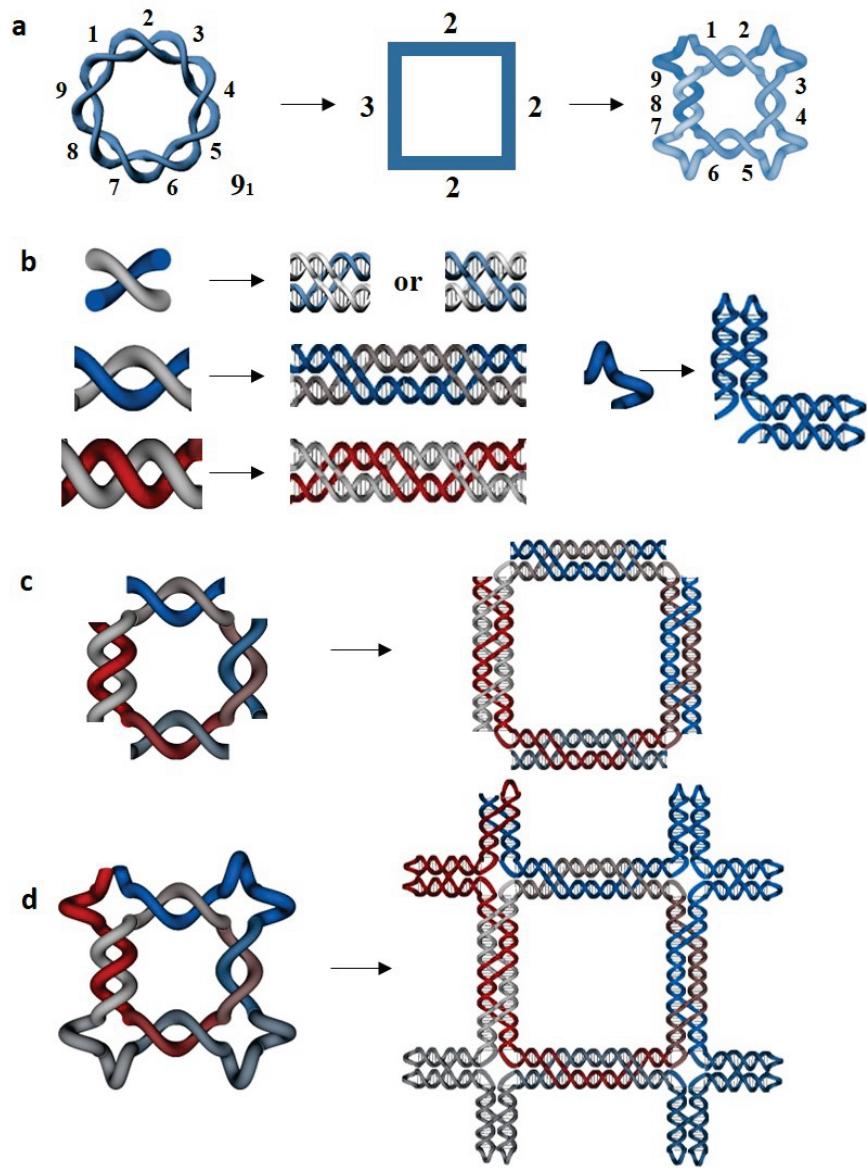
†These authors contributed equally to this work

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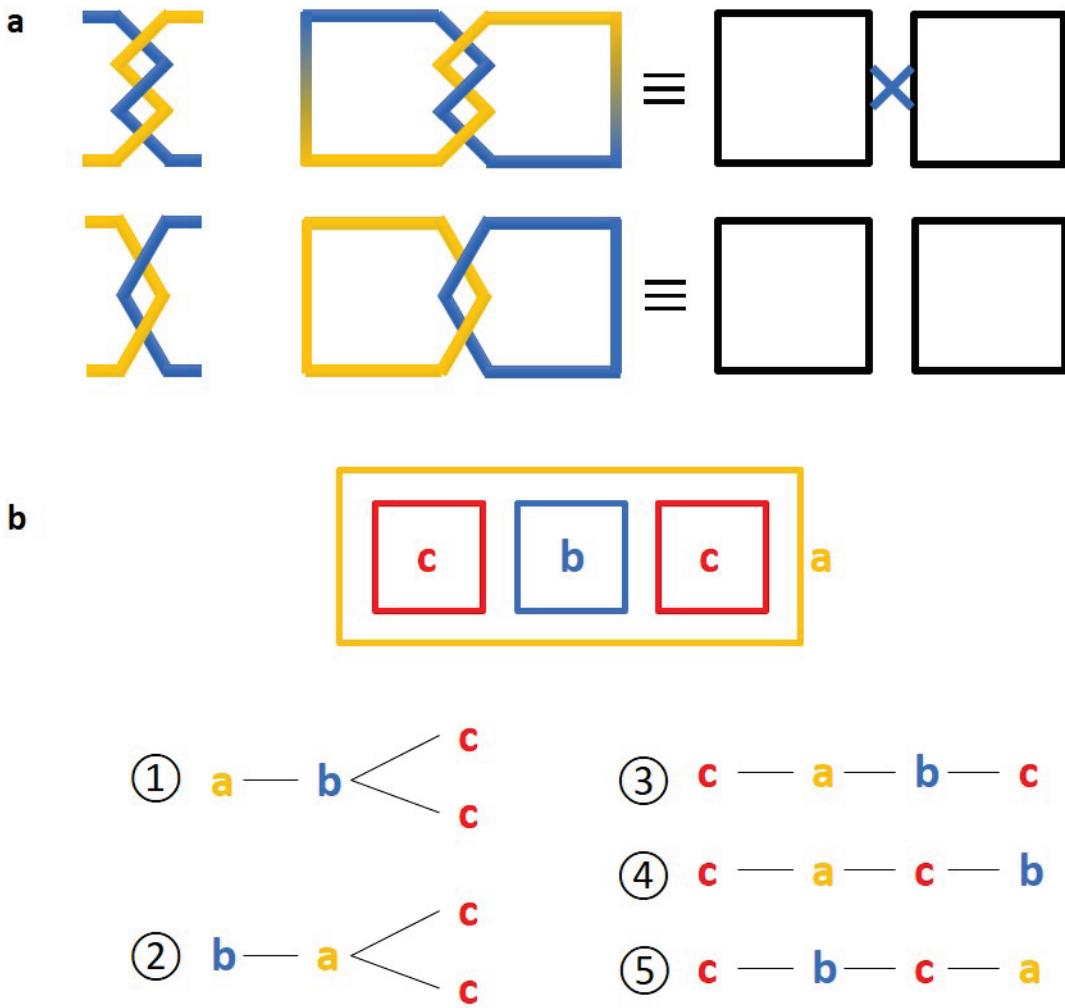
Supplementary Figures 1 to 19

Supplementary Tables 1 to 8

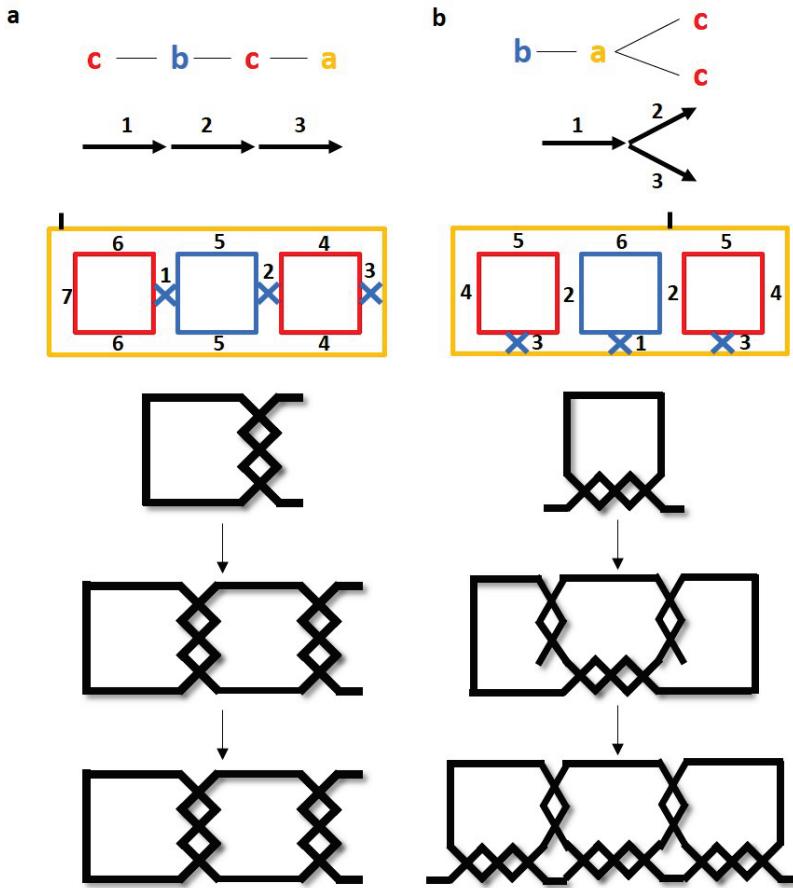
DNA Sequences



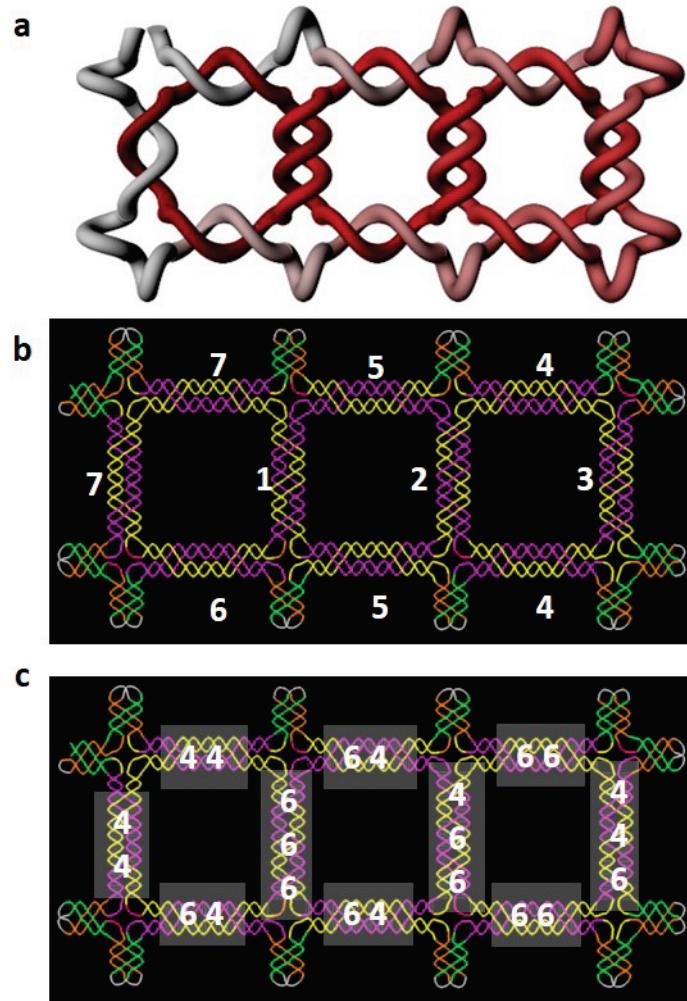
Supplementary Figure 1. The design of a DNA knot 9₁. **a.** The schematics show how to assign 9 crosses on a square geometry. As each edge has the same length, well-distributed crosses are preferred in order to maintain the stability of the DNA structure. **b.** Paranemic cohesion (PX) interaction, each contains two parallel crossovers with 4 bp or 6 bp. These base pairs are used to represent the cross in our knot design schematics. The distance between the adjacent PX crosses are designed as integer multiples of one DNA helical turn (10, 11, 21 or 32 bp). We also designed a small linking structure in order to connect DNA strands in each outer corner. **c.** An arrangement of each of the edges of the square with the corresponding DNA structures. **d.** Adding small linking structures at the vertexes finishes the design of the structure. The DNA strands in the inner corners are connected directly using poly T loops (T_4 is used for a 90 degree turn in the square). The outer corners are linked with the linking structure containing one PX.



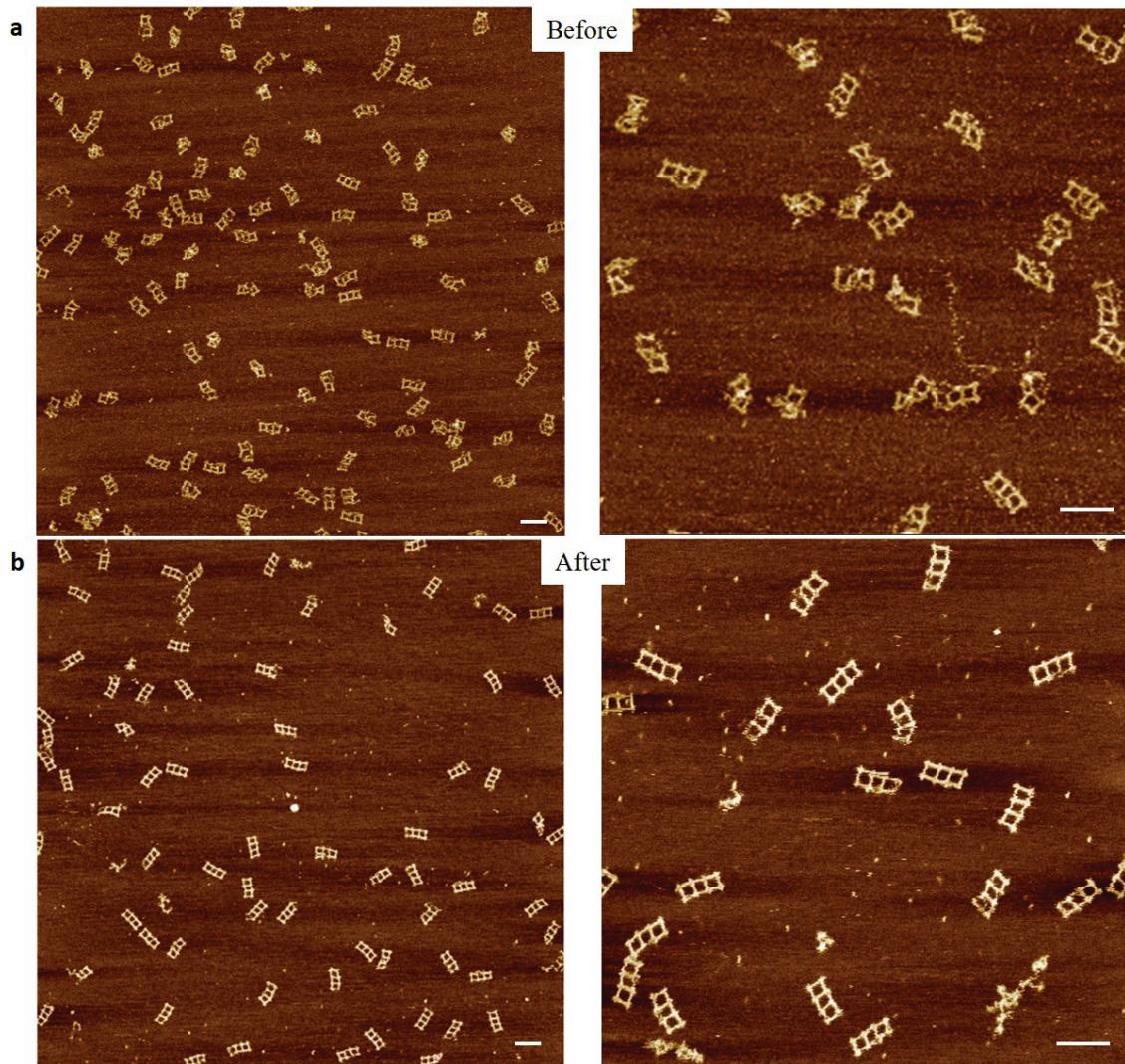
Supplementary Figure 2. A schematic of the folding pathway design. **a.** An edge with an odd numbers of crosses (1, 3, 5...) will allow the two loops that form the edge to connect into one large loop, i.e. to form a knot structure, while an edge with even numbers of crosses (2, 4, 6...) will produce a link between two separate loops. The schematics show an edge with 3 crosses that formed a knot of 3_1 , and an edge of 2 crosses that resulted in a link structure called a Hopf link. **b.** In order to form a knot structure instead of a link, we needed to create an odd number of crossings among the individual loops and choose different orders of connections to link the four loops (a, b, c) to get the target knot. We named the loops involved according to their geometric relationships, and the two c loops are the same due to their symmetry. The largest loop a can be connected with either loops b or c , while c can only connect with loops a or b , not with the other loop c . We listed all of the possible orders of connections among the loops, which represent different folding pathways that could be used in order to form the target knot structures. Pathways 1 and 3 are branched and pathways 2, 4, and 5-8 are linear.



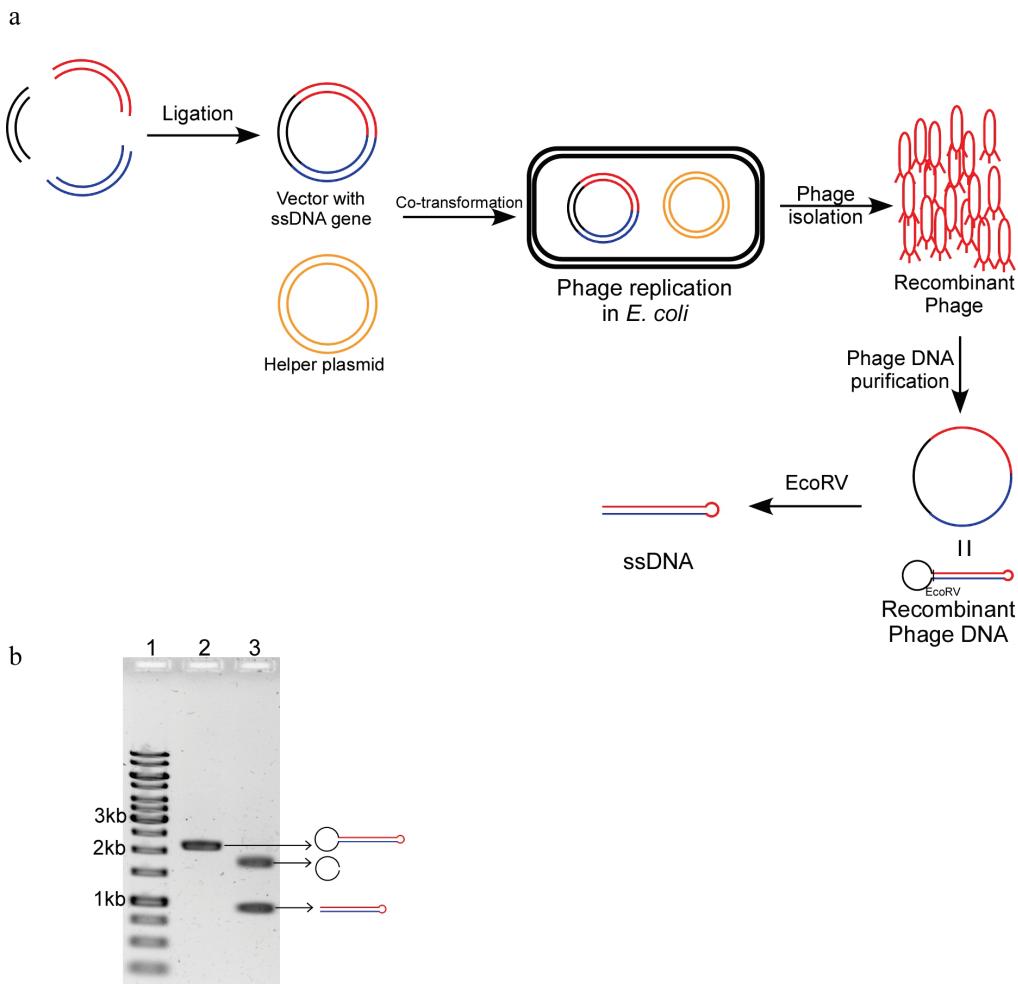
Supplementary Figure 3. A comparison between a linear folding pathway (a) and a branched folding pathway (b). According to the connection relationship between the loops, *a* to *c*, we assigned the edges with either 3 crosses or 2 crosses, (with the total number of the crosses being an odd number), to form the knot. The edges with 3 crosses are marked with an X. These edges should form earlier than the edges with 2 crosses, (due to the annealing step used and the difference in the strength of the paramecic cohesions involved). The pathways represent the order of the formation of the three cross edges, i.e. the formation of the corresponding loops. The direction of the linear pathway *c-b-c-a* (**a**) can be reversed as *a-c-b-c* without changing the relationships of loop connection. However, these two linear pathways are not equivalent. We selected *c-b-c-a* as a preferred direction because the two ends will not need to thread into any preformed loops during the early steps, which is when the unfolded strand is still long. For the branched pathway (**b**), the two ends need to be separated after forming the first 3-cross edges. Each end then travels individually and threads through a preformed loop (the central one) in order to create the 2-cross edges to form the loops on the sides. Therefore, the branched path is expected to be less favorable than the linear one because the formation of the 2-cross edges is expected to occur later than the 3-cross edges due to thermodynamic reasons. Among the linear paths, the best path should avoid threading through pre-formed structures when the unfolded strand is long. Due to these reasons, path 5 (illustrated here) is better than path 3 and 4 as shown in Supplementary Figure 2.



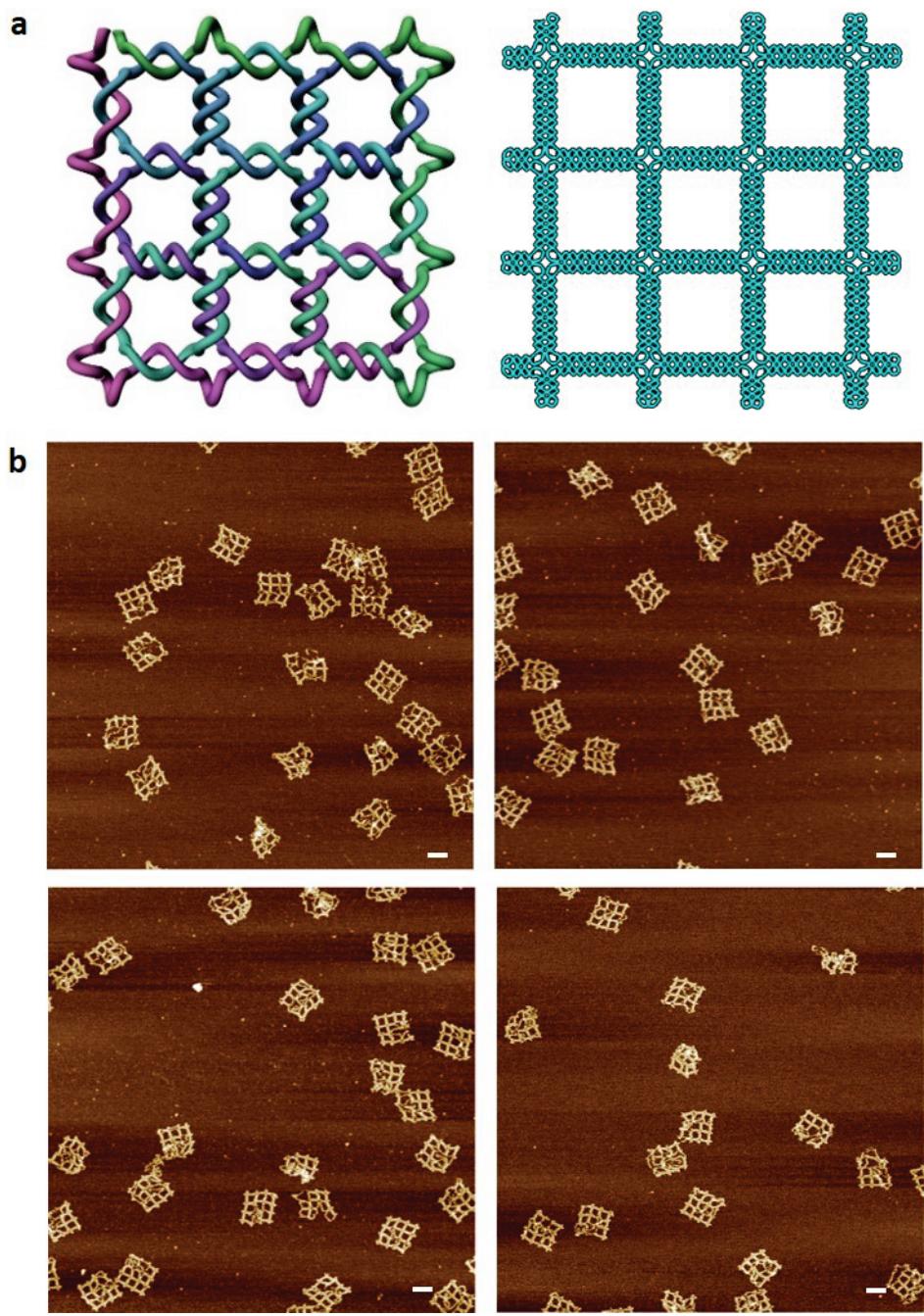
Supplementary Figure 4. The sequence design for the hierarchical folding based on the selected best pathway. **a.** A schematic of the cross section of the *c-b-c-a* folding path (as shown in Supplementary Figures 2-3). The ends of the partially folded dsDNA are located at the upper left corner. The gradual color change from red to grey represents the order of the looping. **b.** The folding order of all of the edges are labeled as steps 1 to 7. The edges that are marked as 1-3 are the 3-cross edges while 4-7 are the 2-cross edges. **c.** A DNA structure that represents the target topological geometry. The white numbers are the length (bp) of each paranemic cohesion interaction.



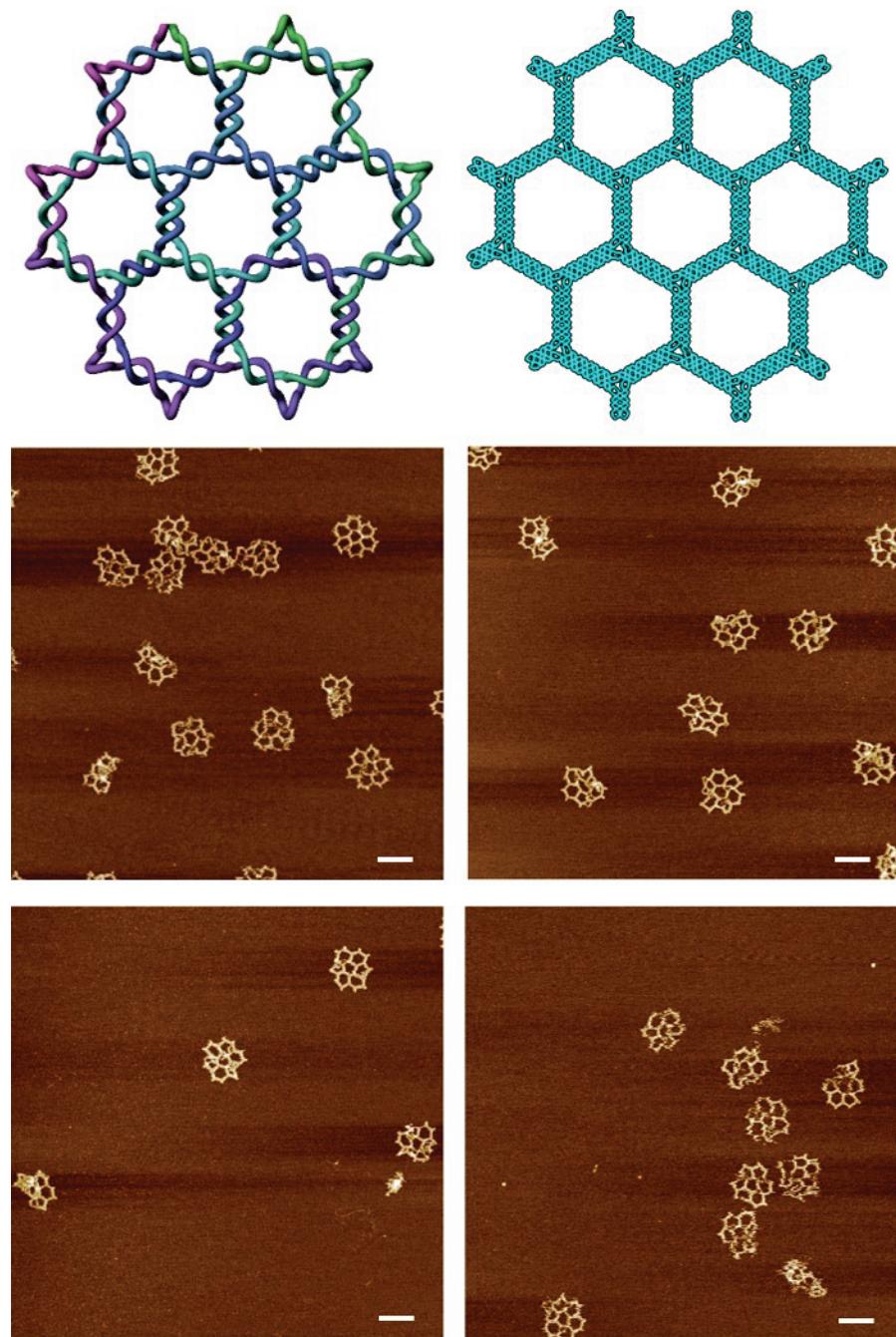
Supplementary Figure 5. A comparison between the yield of the three-square knot structure 9_1 and the different folding pathways, via the use of AFM imaging. With an unfavorable folding pathway (**a**), the folding yield is only 0.9% (2/221). With the best folding pathway (**b**), the folding yield is increased to 57.9% (124/214). Scale bars are 100 nm.



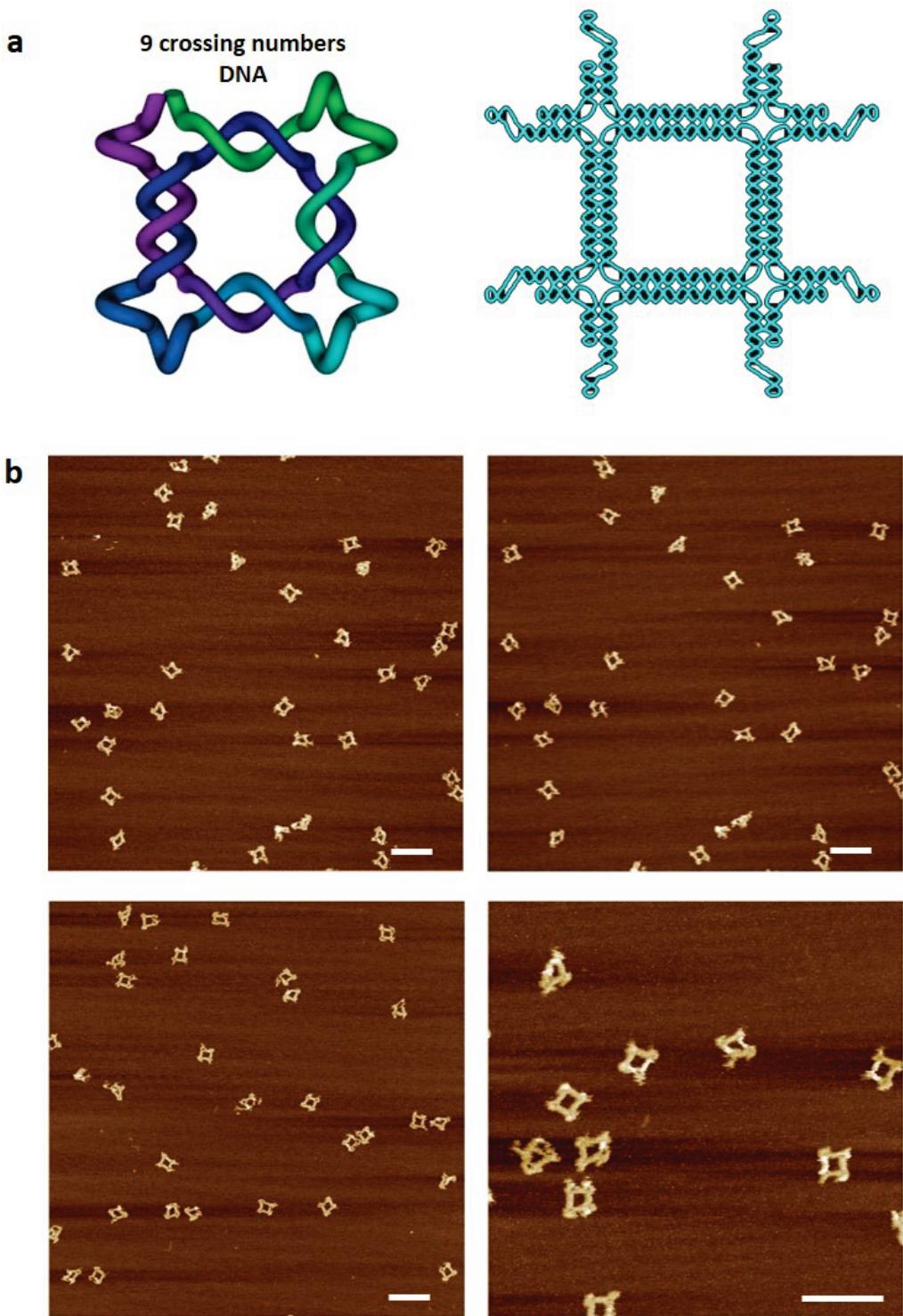
Supplementary Figure 6. The replication and production of an ssDNA by using a recombinant M13 phage. **a.** The ssDNA replication process. First, the two halves of the ssDNA gene (red and blue), were obtained by restriction enzyme digestion from a commercially synthesized plasmid. Then, the custom synthesized ssDNA gene was ligated into a phagemid vector pGEM-7zf(-) (black) by T4 DNA ligase (New England Biolabs) and co-transformed into *E. coli* DH5 α competent cells (New England Biolabs) with the helper plasmid pSB4423.(1) During the phage replication, the ssDNA sequence (red and blue) was packed into the phage capsid as its genome. Recombinant phages were then harvested from the *E. coli* medium and the recombinant phage genomic DNA was isolated and purified. The EcoRV restriction sites were initially designed at the ends of the ssDNA and the phage DNA digestion by EcoRV restriction enzyme produced the ssDNA molecule (partially paired and folded into a hairpin, with the 5' and 3' ends meeting each other and the unpaired bubbles as paramecic cohesion sites). **b.** An example of the 1800 nt ssDNA purification by gel electrophoresis. Lane 1 represents the 1 kb dsDNA ladder. Lane 2 contains the purified phage DNA without EcoRV cleavage. After EcoRV digestion, the 1800 nt ssDNA molecule (lower band) is separated from the vector DNA (upper band) in lane 3. The 1800 nt ssDNA molecule runs slightly faster than the 1 kb dsDNA (2000 nt).



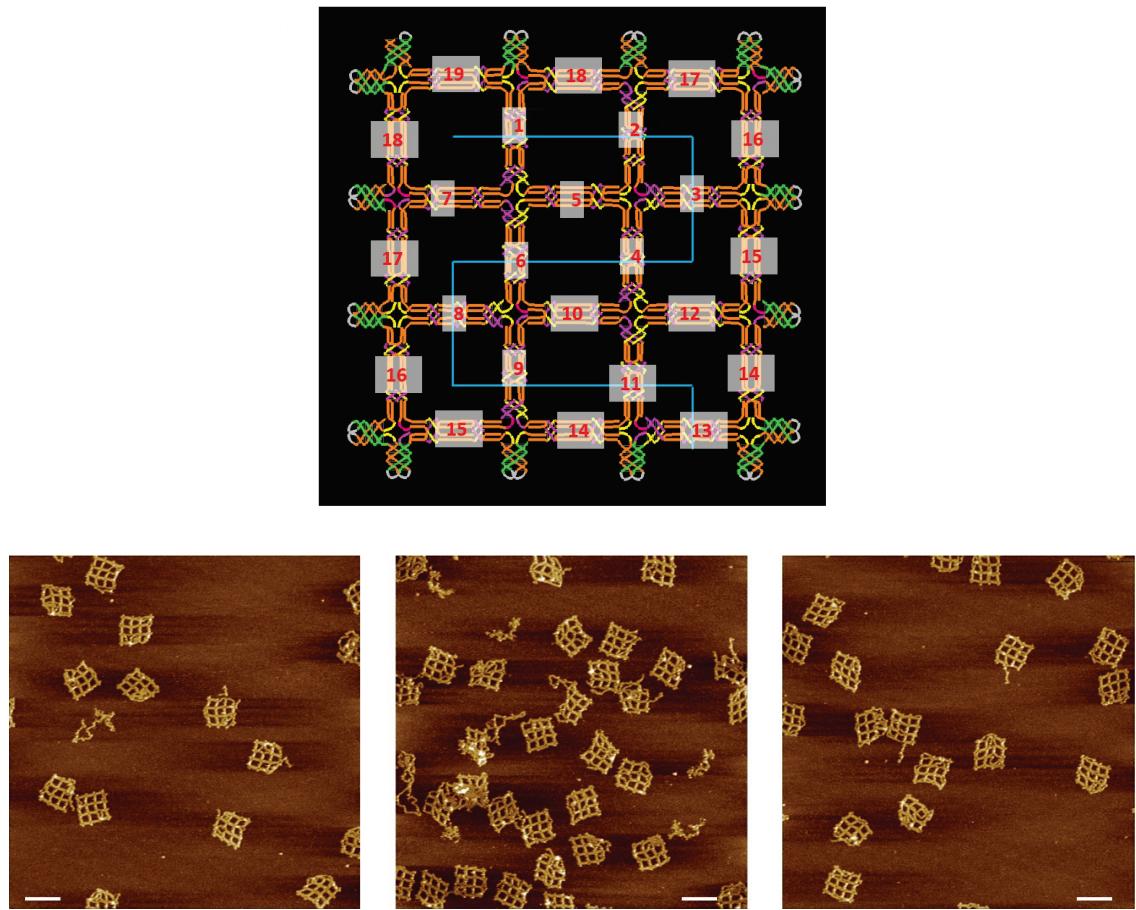
Supplementary Figure 7. The design and characterization of the 9-square knotted DNA structure. **a.** Design schematic. **b.** AFM images. Scale bars are 100 nm.



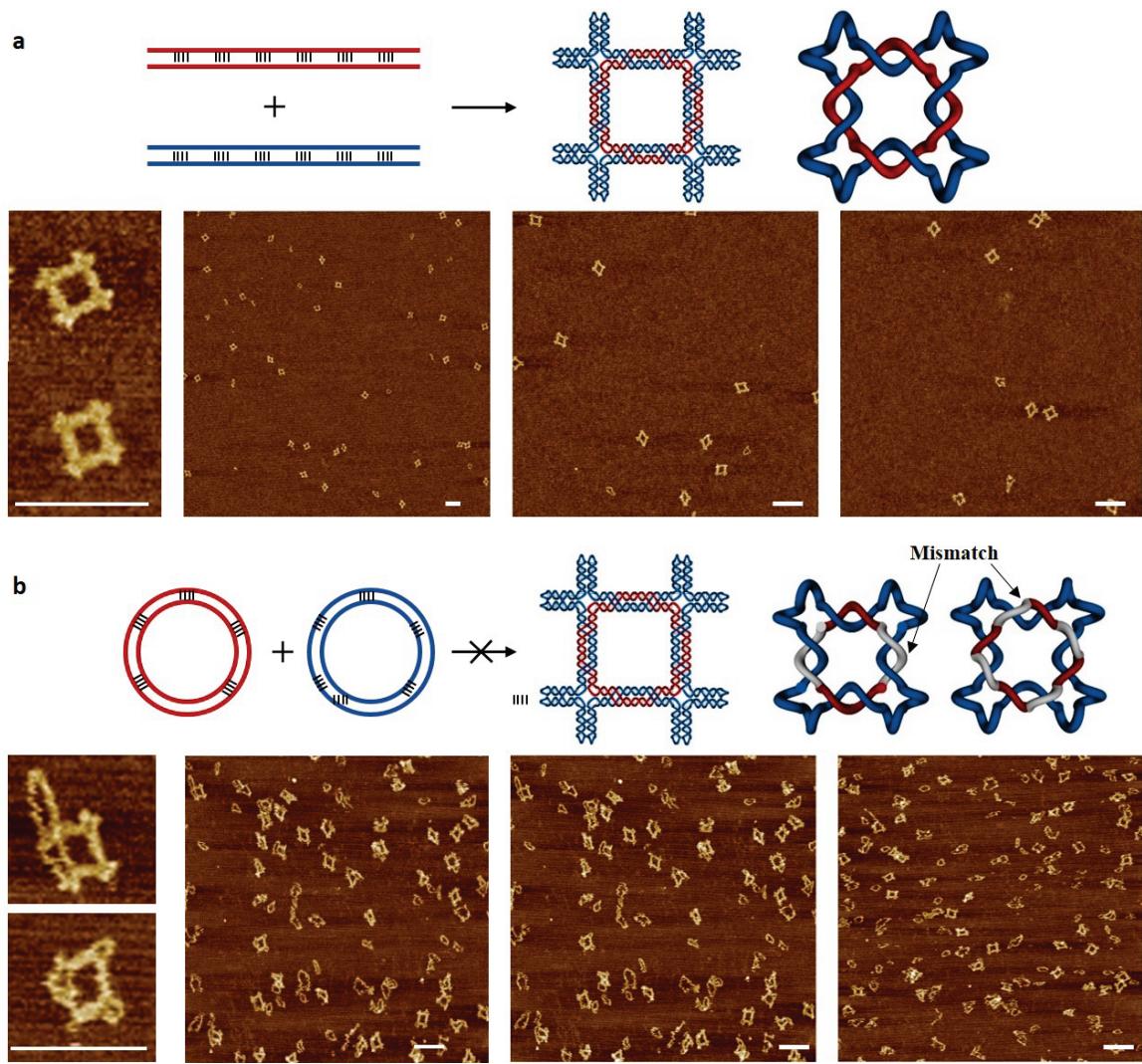
Supplementary Figure 8. The design and characterization of the hexagonally knotted DNA structure. Design schematic (top) and AFM images (bottom). Scale bars are 100 nm.



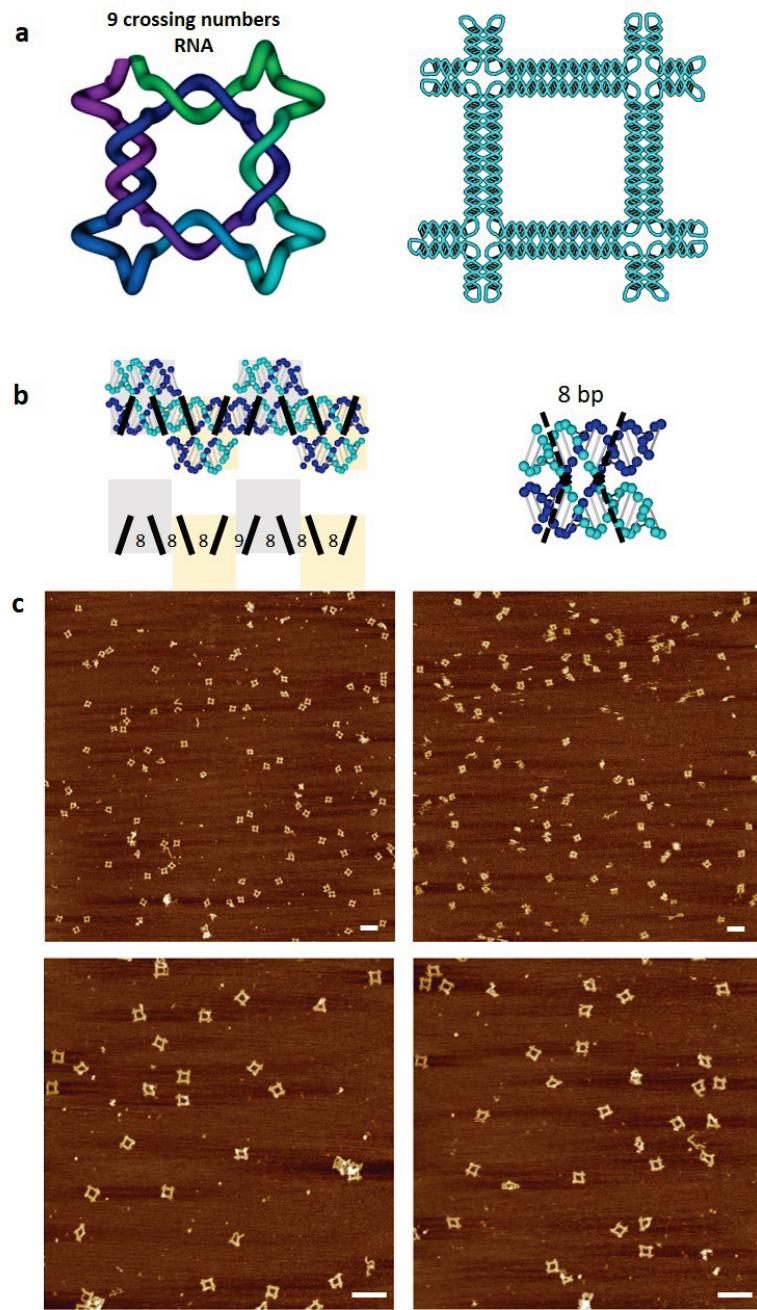
Supplementary Figure 9. The design and characterization of the square knotted DNA structure 9₁. **a.** Design schematic. **b.** AFM images. Scale bars are 100 nm.



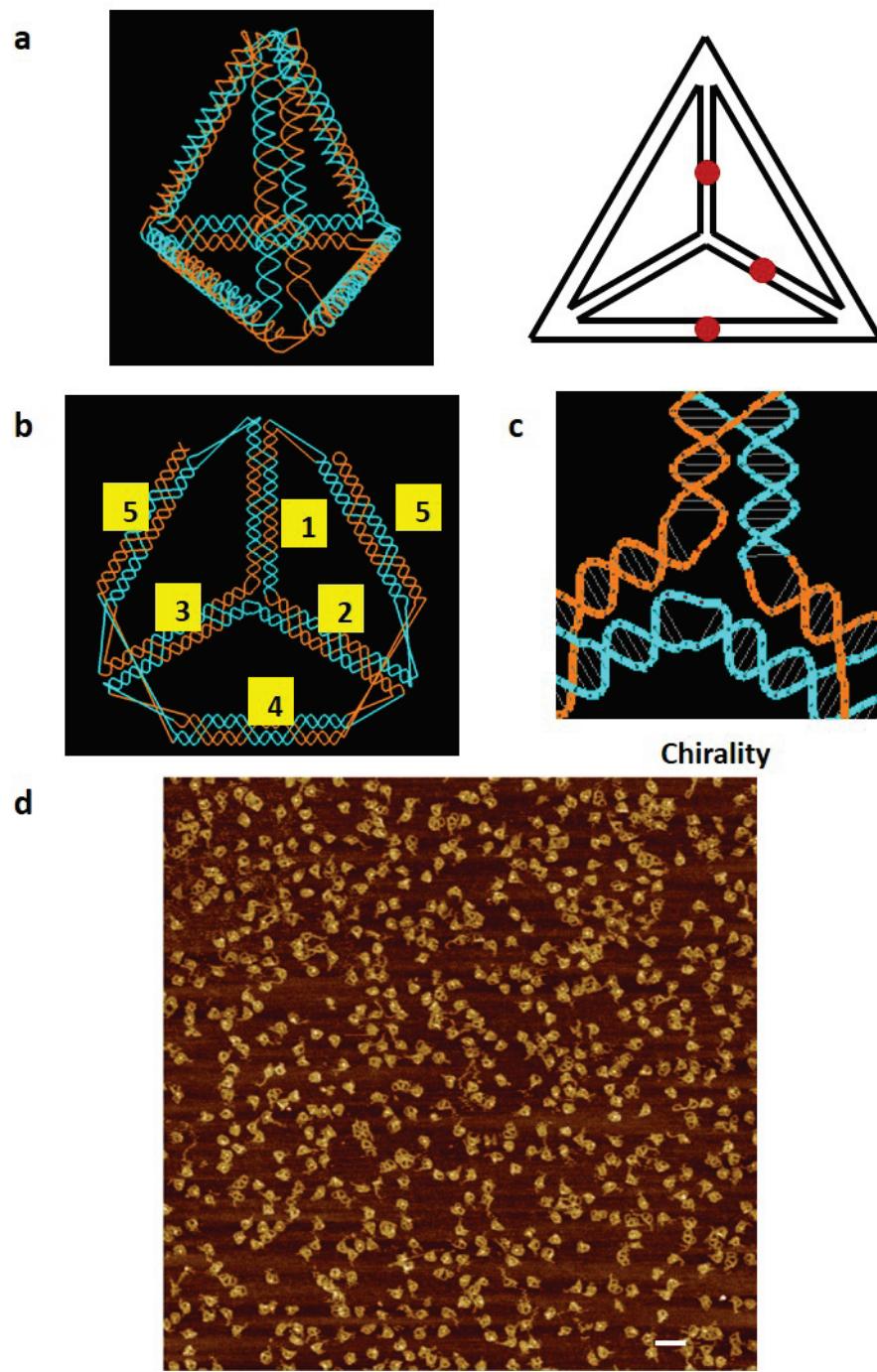
Supplementary Figure 10. The design and characterization of the 9-square knotted DNA structure with hierarchical folding. (Top) Folding pathway design. The numbers on the edges mark the anticipated order of the formation of the crosses on the edges, based on the designed sequences. (Bottom) AFM images of the folded knot structure (with 57 crossings). A majority (if not all) of the structures formed show some degree of errors. It seems that if the crossings in some of the earlier steps did not form properly, the crossings in the later steps could still form, but that the errors would be permanently trapped and there would be no chance of correcting the errors. Since each crossing may have a certain rate of error, with 57 crossings, the final product is expected to have a low yield. Nevertheless, the stepwise yield is quite high (>90%) with the overall structures having a high resemblance to the expected design. Most of the structures have more than half of their edges properly formed. The scale bars are 100 nm.



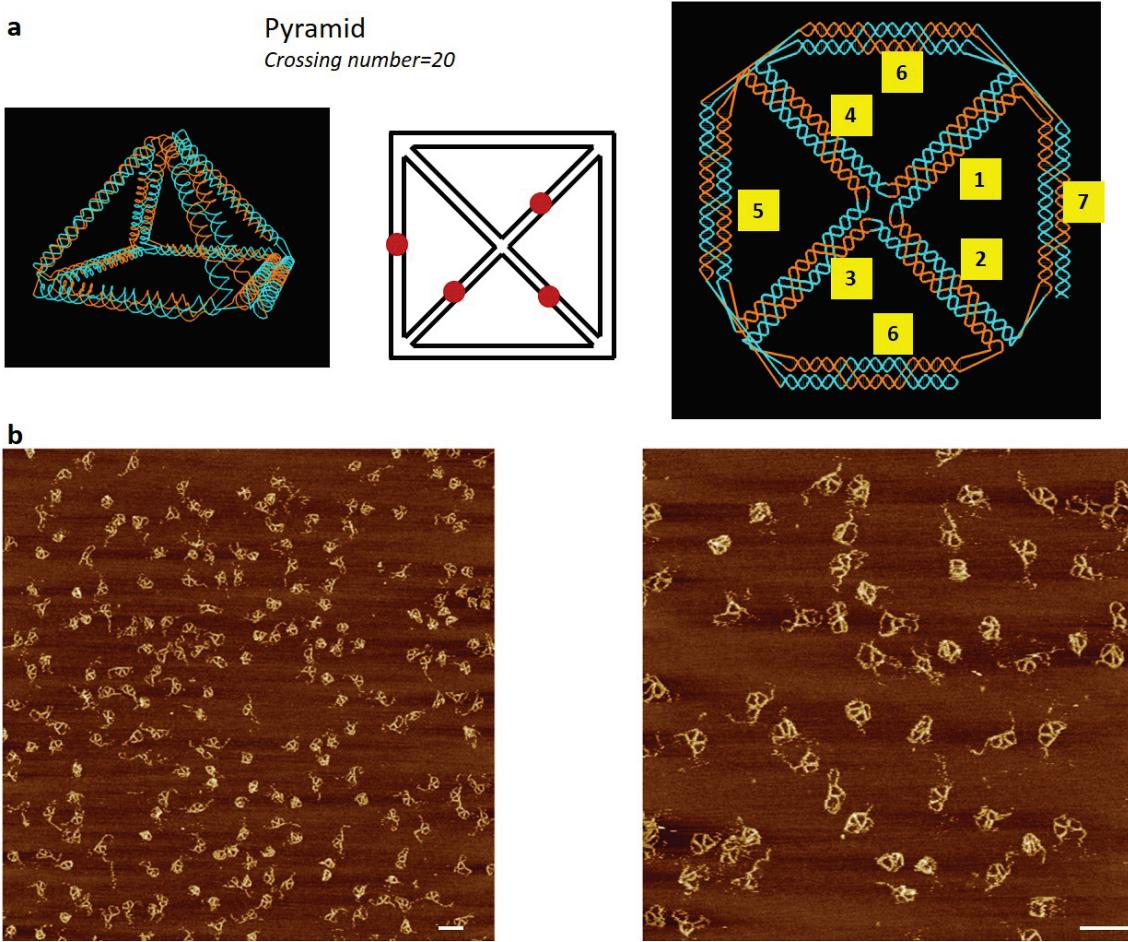
Supplementary Figure 11. Topological control experiments with linear and circular dsDNA. A DNA link structure with linking number of 8 was designed and constructed from four linear ssDNA. **a. Two linearly annealed and partially paired dsDNAs (with internal loops for PX cohesions between the two DNA), can self-assemble into the designed structure with a high yield, as shown in the AFM images. **b**. After circularization, although the two dsDNA rings could still bind with each other partially through some of the paramecium cohesion interactions, extensive defects were observed in all of the structures under high resolution AFM. The two circular dsDNA molecules cannot form the correctly interlocked nanostructure. All scale bars are 100 nm.**



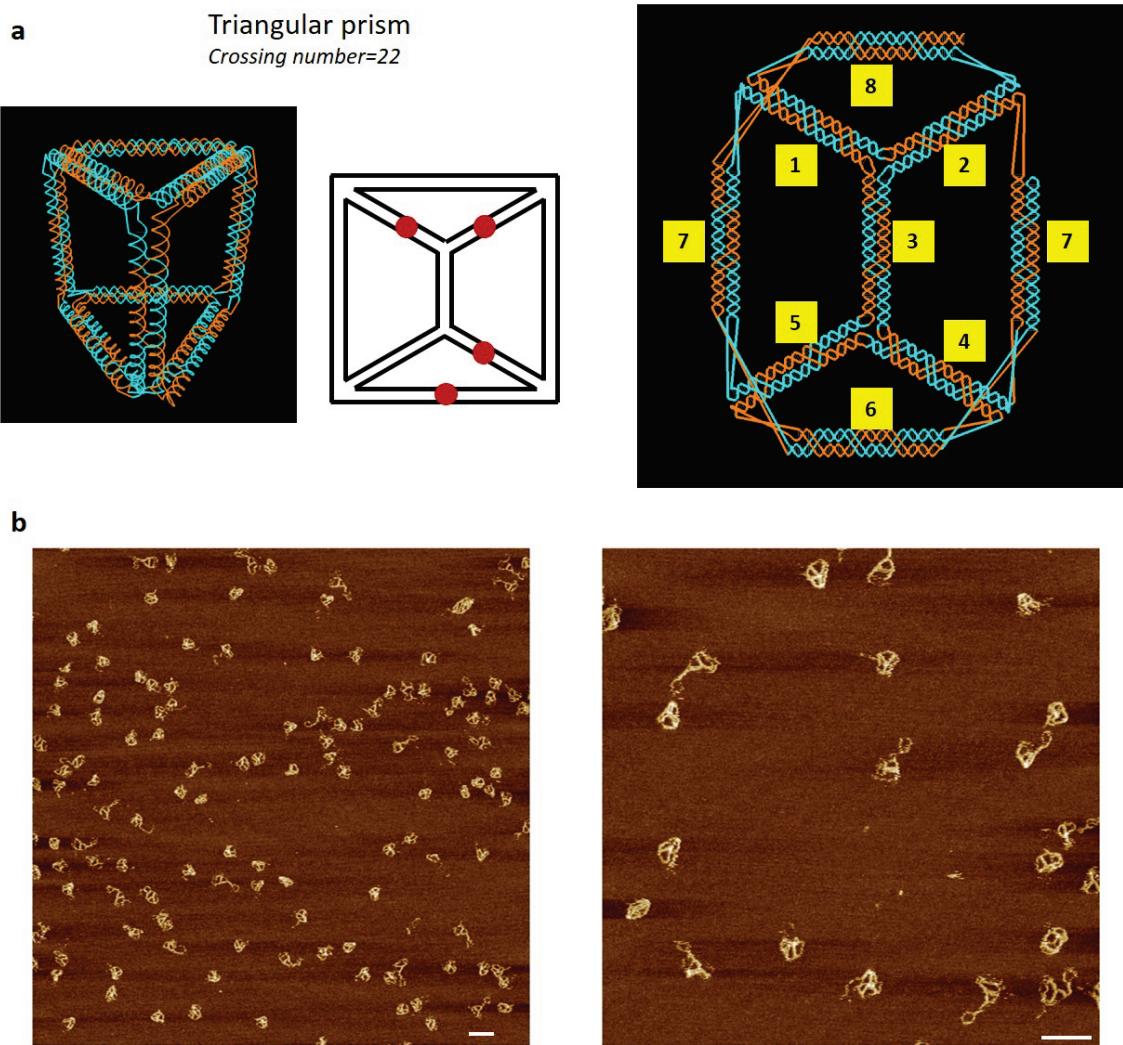
Supplementary Figure 12. The design and characterization of the square knotted RNA structure 9₁. **a.** The design schematics. An a-form double helix is used as the structural model. **b.** This image shows an RNA paramecic cohesion design that was based on A-form double helices. 8 bp was chosen as the length of the PX cohesion for RNA; 33 bp was chosen as the length of the repeating unit (3 full turns). **c.** AFM images illustrating a high yield (~ 60%) of the expected structure. All scale bars are 100 nm.



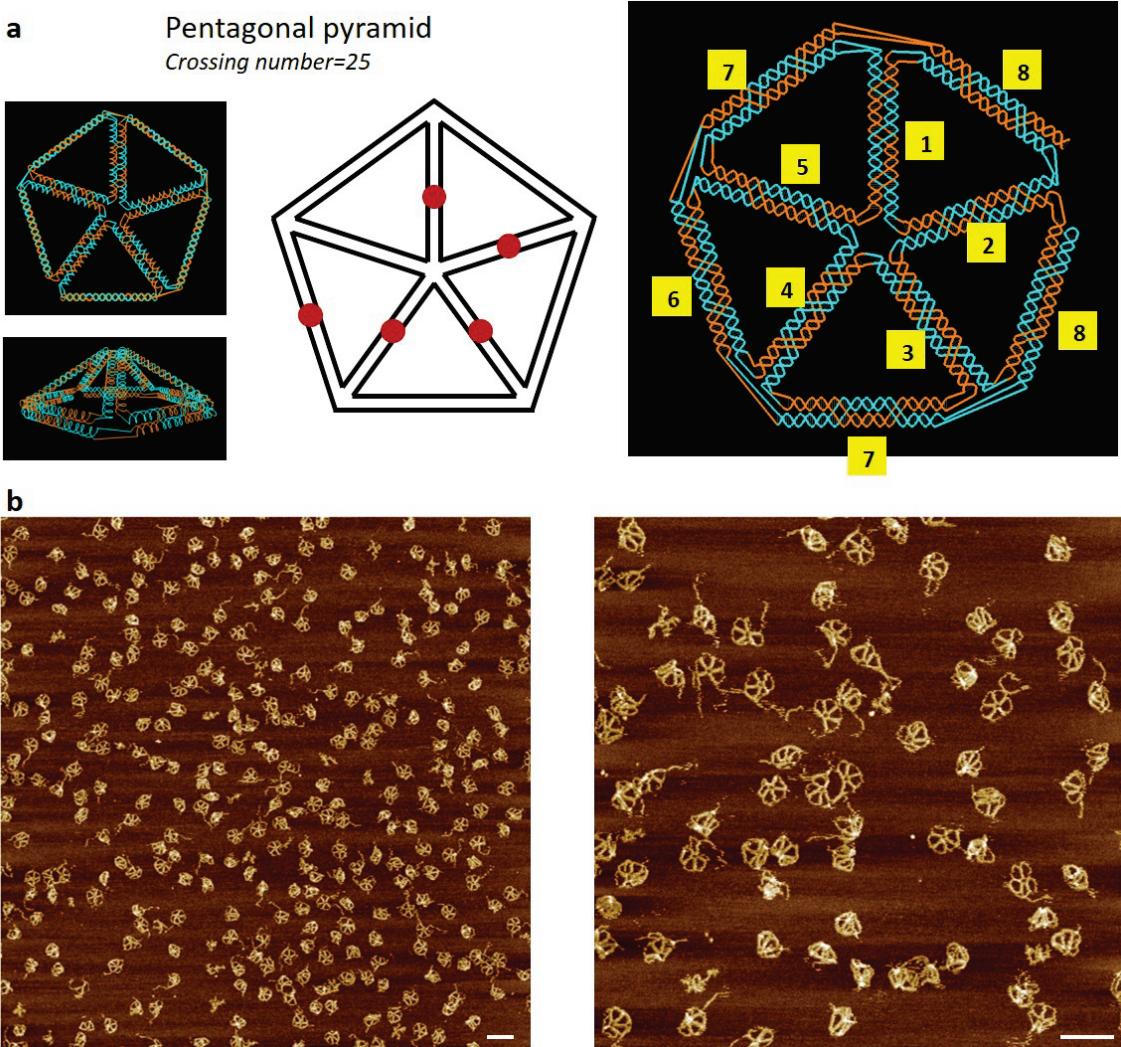
Supplementary Figure 13. The design and characterization of the tetrahedron knotted DNA structure. **a.** The design schematics of the folding pathway. In the middle 2D diagram, the red dots mark the edges of the tetrahedron that have the 3 crossing numbers. **b.** The number on the edges mark the anticipated order of formation of the edges. **c.** The vertexes all show the illustrated chirality. **d.** AFM image. Most of the structures in the image display the expected structure. However, some of the structures shown are missing one or two edges. The scale bar is 100 nm.



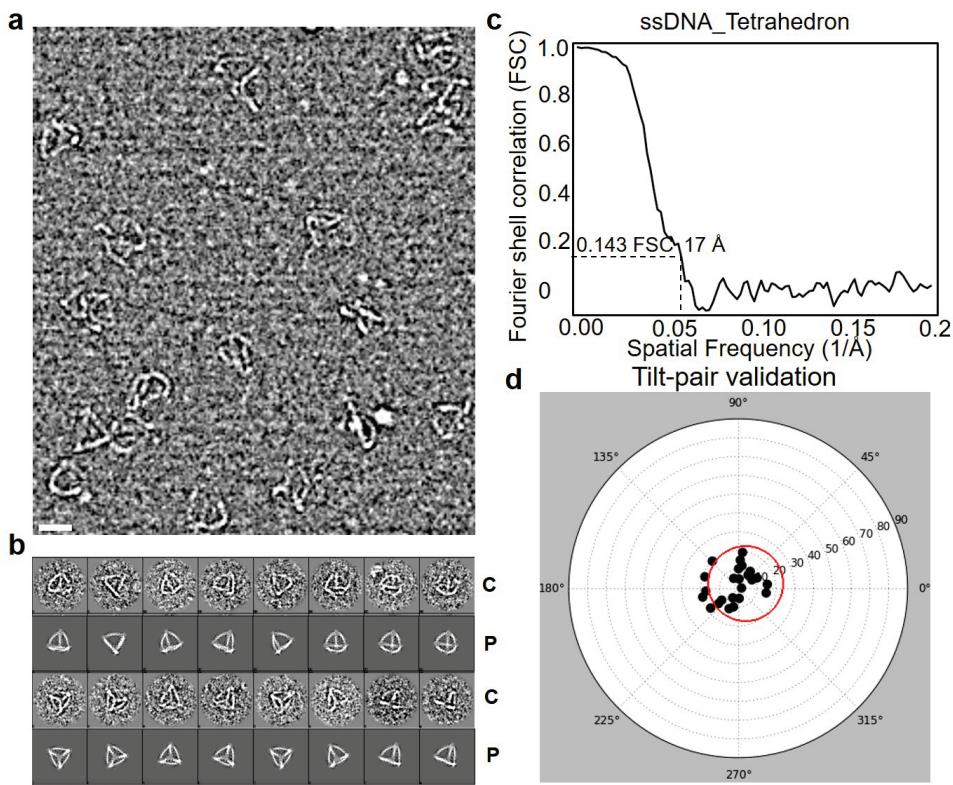
Supplementary Figure 14. The design and characterization of a pyramid knotted DNA structure (crossing number = 20). a. The design schematics of the folding pathway. **b.** AFM images. However, although many of the structures that are shown are distorted or broken, due to interactions with the substrate surface, a majority of the edges shown are well formed. The scale bars are 100 nm.



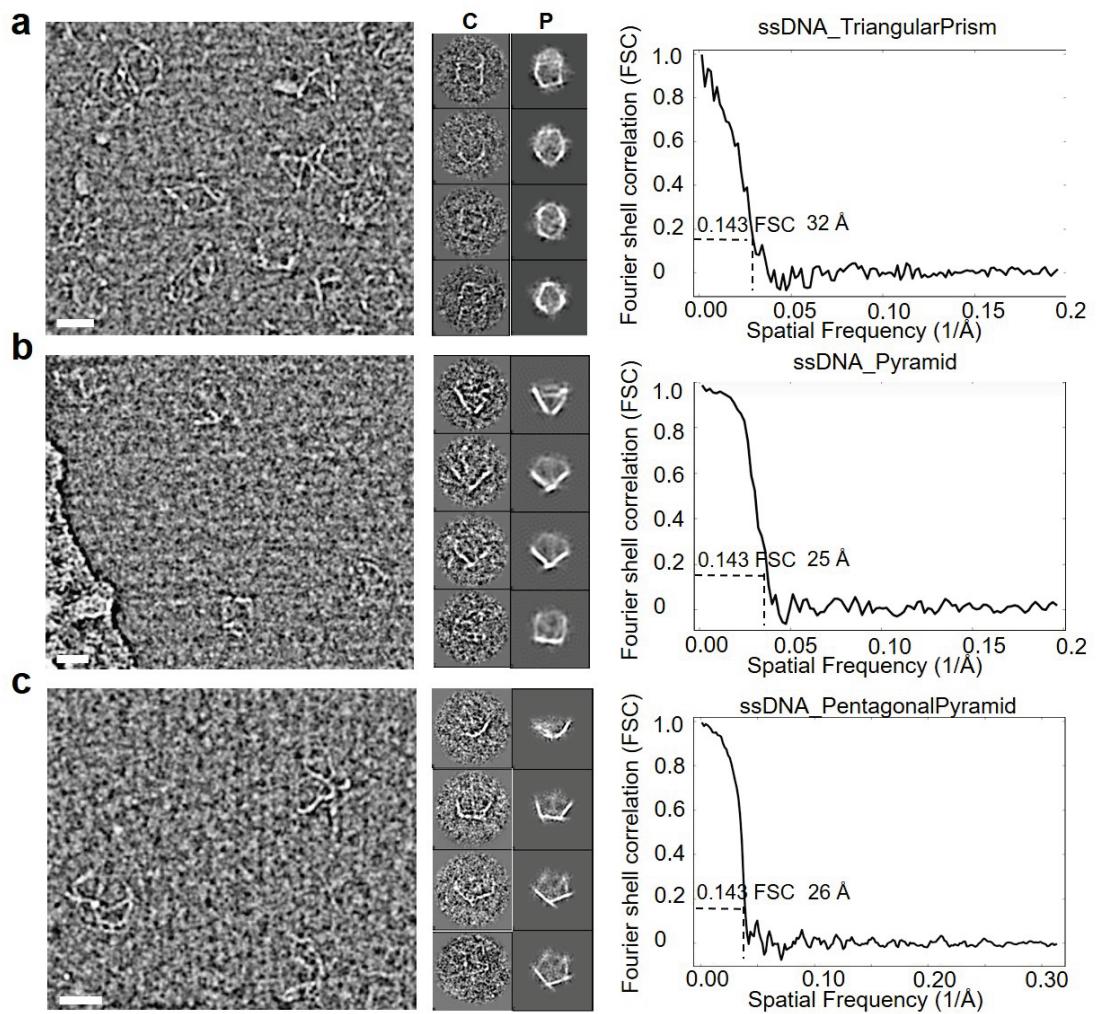
Supplementary Figure 15. The design and characterization of the triangular prism with the knotted DNA structure (crossing number = 22). **a.** The design schematic that includes the folding pathway. **b.** AFM images. The scale bars are 100 nm.



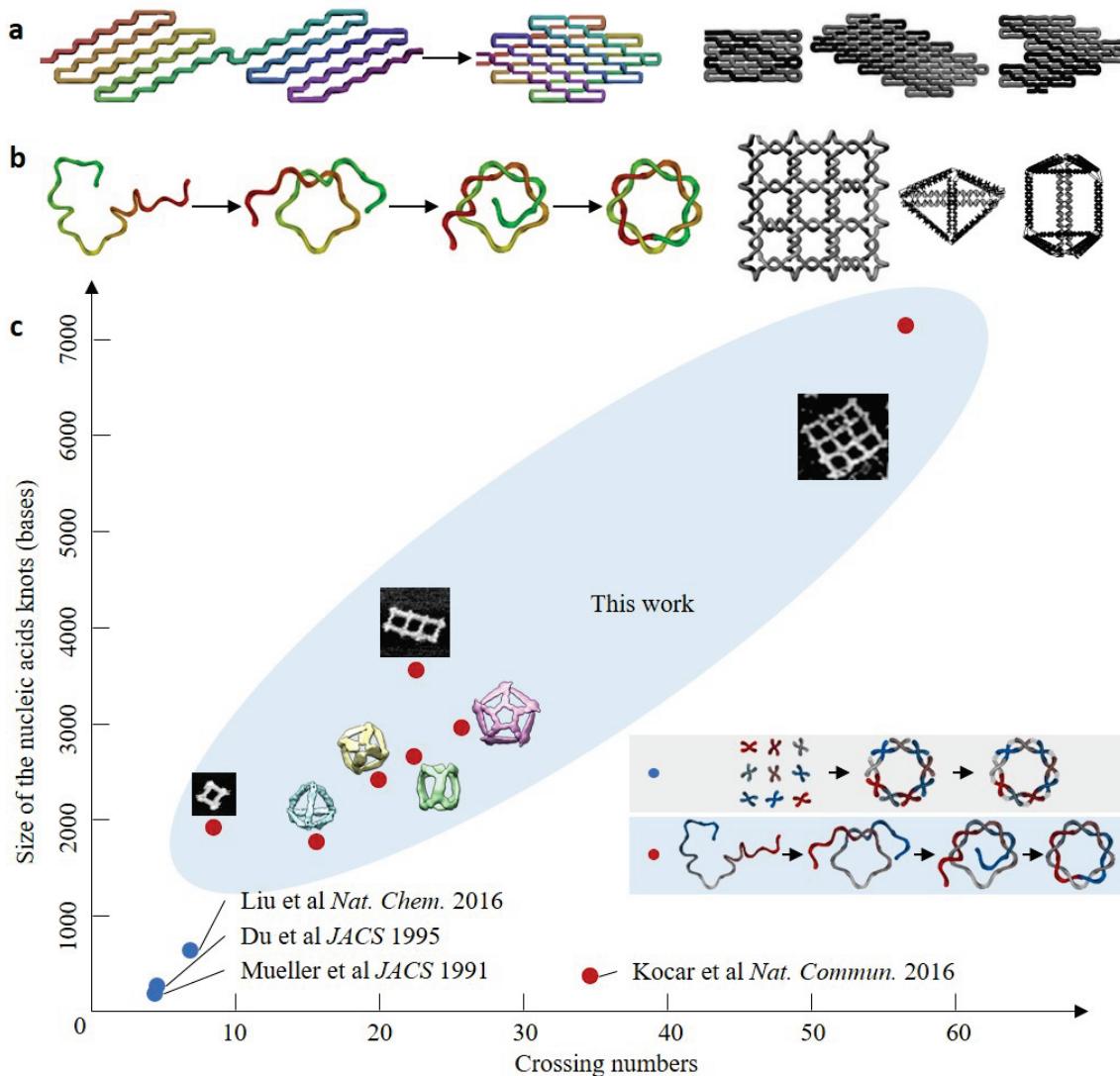
Supplementary Figure 16. Design and characterization of a pentagonal pyramid that has a knotted DNA structure (crossing number = 25). a. The design schematics of the folding pathway. **b.** AFM images. The scale bars are 100 nm.



Supplementary Figure 17. Cryo-EM characterization of a single stranded DNA tetrahedron. **a.** This image shows a raw cryo-EM micrograph with visible particles. Scale bar is 20 nm. **b.** These images show the reference-free 2D class averages and projections of the final 3D reconstruction. **c** This graph is a gold-standard FSC plot for the final 3D reconstruction of the particles. **d.** A tilt-pair validation result. The red circle shows particle pairs that cluster around the experimental tilt geometry.



Supplementary Figure 18. Cryo-EM characterization of an ssDNA triangular prism (a), pyramid (b) and pentagonal pyramid (c). For each structure, a raw cryo-EM micrograph is shown with visible particles. There are also images on the right in smaller boxes, showing the raw particles. Furthermore, a Gold-standard FSC plot is also shown for the 3D reconstruction of these particles. All scale bars are 20 nm.



Supplementary Figure 19. Comparation of self-assembled topological DNA/RNA nanostructures. A knot can be constructed via two strategies by using DNA. The top two rows show that knot can be assembled by either connecting 9 right-handed X-shaped junction tiles together (blue dot), or by threading a single chain through itself 9 times (red dot). Prior to this study, it is a grand challenge to fold a single DNA/RNA strand into a complex topology with high crossing numbers and long length of nucleic acids in a programmable and controllable way.

Supplementary Table 1. A summary table of the design parameters for the structure in Supplementary Figure 4, including the number of bases in each PX cohesion, and the GC content of the PX cohesions in each edge.

Step	1	2	3	4	5	6	7
Cross number	3	3	3	2	2	2	2
length	666	664	644	66	64	64	44
bp	36	32	28	24	20	20	16
GC %	69	66	61	58	60	55	50

Supplementary Table 2. A summary table of the length of the PX cohesion, the number of the base pairs involved, and the GC content of the base pairs for the structure in Supplementary Figure 10.

Step	Length	bp	GC%
1	666	36	67
2	466	32	66
3	466	32	62
4	446	28	64
5	66	24	67
6	444	24	63
7	66	24	58
8	444	24	58
9	444	24	54
10	66	24	54
11	444	24	54
12	66	24	50
13	444	24	50
14	46	20	60
15	46	20	55
16	46	20	50
17	46	20	45
18	44	16	50
19	44	16	44

Supplementary Table 3. A summary table of the design parameters for the structure in Figure 3.

Step	1	2	3	4	5	6	7
Cross#	3	3	3	2	2	2	2
length	666	664	644	66	64	64	44
bp	36	32	28	24	20	20	16
GC%	69	66	61	58	60	55	50

Supplementary Table 4. A summary table of the sequence design parameters that facilitate the folding order of structure in Supplementary Figure 13.

Step	1	2	3	4	5
Cross number	3	3	2	3	2
length	666	664	66	444	46
bp	36	32	24	24	20

Supplementary Table 5. A summary table of the sequence design parameters that facilitate the folding order of structure in Supplementary Figure 14.

Step	1	2	3	4	5	6	7
Cross number	3	3	3	2	3	2	2
length	666	664	644	66	444	46	44
Bp	36	32	28	24	24	20	16

Supplementary Table 6. A summary table of the sequence design parameters that facilitate the folding order of structure in Supplementary Figure 15.

Step	1	2	3	4	5	6	7	8
Cross number	3	3	2	3	2	3	2	2
length	666	664	66	444	66	444	46	44
bp	36	32	24	24	24	24	20	16

Supplementary Table 7. A summary table of the sequence design parameters that facilitate the folding order of structure in Supplementary Figure 16.

Step	1	2	3	4	5	6	7	8
Cross number	3	3	3	3	2	3	2	2
length	666	664	644	644	66	444	46	44
bp	36	32	28	28	24	24	20	16

Supplementary Table 8. Tilt-pair validation of single stranded DNA tetrahedron

# Total particle pairs	49
# Particle pairs in cluster	15
Fraction in cluster (%)	30.6
Mean tilt angle ($^{\circ}$)	9.47
RMSD tilt angle ($^{\circ}$)	4.00
Mean tilt axis ($^{\circ}$)	42.4
RMSD tilt axis ($^{\circ}$)	54.9
Experimental tilt angel ($^{\circ}$)	9.9

Sequences:

9 crossing number square knotted DNA

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23 crossing number 3-square knotted DNA before hierarchical design

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23 crossing number 3-square knotted DNA after hierarchical design

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57 crossing number 9-square knotted DNA before hierarchical design

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57 crossing number 9-square knotted DNA after hierarchical design

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67 crossing number hexagonal knotted DNA

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9 crossing number square knotted RNA

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 GUGAUGUCCAGCGAGUAGCAGUAUCUUAGAGAGAGUCGAUUCUCGCACUGGG
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15 crossing number DNA tetrahedron

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20 crossing number DNA pyramid

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22 crossing number DNA triangular prism

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ATGCCAGAGGACTACTGGATCCGATTCAACTATGGACTCATCACTGCTT
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25 crossing number DNA pentagonal pyramid

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 CACAGTAGCCGTAGCGTGTATGTCGACTGTGCACCAGAGCTTTGGTAAC
 GCTTTAGGTAGACGGGAACCGGGAAAACGTGTGACATGTTAACCAATCTG
 CCATATACGAGGAACGTCCCAGTGCAGCAGAACATCATACAGCTCCA
 TGACTGGCACGTCCCGAAGTCGGTTCGACGCACCTGGTTGGTTCCGTCCC
 TTATAATGTGGTGGAGTACCACTAGCAATAAGTCGGGTTCTAGGCTCGCAG
 AGTTCTATCCATGTGCCGACTATGGGACCGTCTCAGCAGGGAGTAGGTACGA
 GACCTGACCCCTCGGGCAGTGGGAATCTGCGTTCTCTAGTTTTCTAGAGAAC
 GCAGATTCATGTTGCCGAGGGTCAGGGTCTCGTACCTAGGTTCTGCTGAGAC
 GGTCCCATAGTCTTGGCACATGGATAGAACTAGTGGAGCCTAGGAACCCGA
 CTTATGATCCATGGTACTCCACCACATTATAAGGGACTTGGAAACCAACCA
 AGGTGGTGTGAACCGACTTCGCGGACAGGTCACTGGAGCTGTATCTGCT
 TCTGCGTCACTTCGGGACGTTCTCGTATATCACTGGTTGGTTAACATGTCAC
 ACAGTTTCGTTGCACCCGTCTACCTAGCGTTACCAAAAGCCAGGCAGCACA
 GTCGAATGGGACACGCTACGGCTACTGCCGACAGTCGTGCGGCTTTGTCA
 GTT TAGGTTAACAGAGGCCAGTGGATCTGAGCTGAGTTAACTATTGCC
 ACTCACACGGCTGGCATTGCTTGTCTTAGTGGAAATATGTCGGCTAGTGTCCA
 TTCCTCTACGTGGCTGTCTTATGGCTTAGAGGGAGTAAGGTCTTCGTCATT
 CCGATGCAAATCAGGCATCTCTCTAACACCGTCTCATGACCGTTCGTACGGT
 CTTAAAGTGTGCATAAGTTGCTTGTCTAACAAACCGCTCAGGTAAAGTACT
 GATACAACGTGAATGACTTGCAAGAACGATTGGCATCAGAATTGATCTTAC
 TCTCTCCTGGACAGTTGCATATGGTCCAGCCAACATGTTGGCTCAGAA
 CAACCGGGCAAGTGACGTGCAGCTGCAACATGCCACTGGTGGCCTTCATA
 TGCCAGTGTGTTGTAATAGCAATAGGGAGTTAGCAAGTGTACCCAAAAGC
 CCTTCAGAGTACACCCGCGGTACAAATGCACCGATGTGGACGCGAGGTCTT
 CCCACCAAAAGACAGGGACAGATACTCCTTCGGGCTCAGCAGGAGGGACC
 TATGACCAGGGCTAAGGGTCCAGGTTACGTTAGATTGTGTCTCTGGC
 AATTGGAAAGGAGAGGGAAAAGTAAGGATCACTGGGAGTGGAGTACTGCGTT
 TGTTCATGAGCGATGCCACGTGTAACATTGCTTGAAGCTGCCCTA
 CTGGGCTTAAAGATGCTTCAAAGTCGAGCCCGTCCCCAGCGTTGTTAGTCC
 GTTCTGTGCTCATCCACGTCTACTCACTCCGTGACGTTGTTAACATTGCTT
 TCTGGTCTAGCCCGTACATCCAGTGAATAGAGCGTACTGCCAGCTGTGTACC
 CAGCGGTCCCAACTGACGACACGCACTACGTCCTCTCATCGTGAGAGGTG
 CTACTCAATTGCACTTGATGACTATACGAAGTTCAAGAGCCAACCTCCGGT
 GTGAGTGACTCGATCGAAAGCTGACGGGCTGATGATCGCAGATGTGTTAT
 CGGGATTAAAGAGATATGTGTACCCCAAGCCTAGCCATAGGTACATTAGCA
 ATGAAGCGAGGAGTCCGGCTACGCATGAT

Topological control strands:

1:

GAACAGGTGAGCTCATAATGGCGTACGTTCGTACCCATTTCGTAGACACTCC
 TCAGTTTTCAAGAGAGTGGCGTGTGAGACTACAACAGGTTTTGTCA
 TGTAGTAGATCTTCTGGCTTAAATCAGGTGCCGGCATCTGATACTGGCA
 TCAGGCTGTGACGGACAAATCAACTTGTACAAAGAGCACAGGGTTTTG
 ATACTGCTCGAGCTCGGGAAAGCGAGTTGGTTTTGAAGTTGCTTCCGGG
 TTTGCAAGATAAGAGGACCCCTAGCCTCAGCGCAGCAATTATCGTTGTA

CGAAACGCAGTCCGTTCTCCAAGGTACATAGGTTTGCAACGTACCCAT
GGTCCTGACATGTTAGGTTTGCCTGACATGTGTCGGTTAACAGAGTGG
TGGACACGGACGTCACCTGAAGTCTGATGCACAACCTGGACCCATGTGTA
TCATTAGATACGAGCAATCCGTTTGAGGGTGCTCGCCCCTGGAAAGAGA
CAGTGCCTTCTCCTGTCTGGATTCTCGTCTCCACAA
ATGACC

2:

CACCTGTTCGGTATTGTGACACGTCGAGAACAGTCCCTGCCACTGTACGAGAGTT
TTTCCGCAGTACAGCTCCTTACGGGCCTCTCCCTCTTTTCGGATAGA
AGGTATCTTGATACACATGGGTCGTTGATGTGCATCAGACTCAAGGTGAC
GTCGAATTGCACCACTCTCCGACTACACGCACGCTTTCTTAACGTGTAC
AAGGTTTACCATGCCAGGGTTGCTTTCTATCCTGGTTGGAGCGGACTG
CGTTCTGCTGGCCAAATAATTGCTGCGCTGAGGCTAGGCCTGGCTATCT
TGCCCCGATGGATAACTCTTTCCAATATCCACCCGATTTGAGCTCT
GCGTATCTTTCCCTGGCAGATTGCTGTTGATTGTCCGTAGTCCGCTGA
TGCCAGTATCAGATGCCGGCGAGTCACGTTAACGCCAGGGATCTCGATTGGT
ACTTTTCTGTCAATCGCTCAATTTCACGCCACCAGTCTGTTTCTGA
GCTGGTTCTACGTGGTACGAACGTACAGACAGATGAGCT

3:

CATGACGGAGATTACCTCGAACTCCAGCTGGATAGGTTGAGGACTACAA
CGTGTAAATATTGCCCTAAGGGTAGCAATCCTGTCTAGCTAAAACACGTAGT
TTTCGAGCTCTGACGTGACGCCATCGATACCTCAGCGTATCGCCTCGGACTCT
ACCACAGAGGGTATTTCCTATGCGCCCAACGGTGTGGCTCCGTGCACCA
ATTCCGCCAGCGTTGCTTACAGCGACTTTGCGCTCGACTCAATTCTCCACT
GATC

4:

TCCGTCATGGATCAGTGGACGTGTCAGTCAGCGCGTCGCTGTAAGCAACCA
ACAAAGGAATTGGTGCACGGAGCCACACACGTGCCTGCGCATAAGGTACCC
CTGTGGTAGCACAGCAGGCGATACGCTGAGGTATCGATGGCCCTGATTAGA
GCTCGCTACGTGTTAGCTGCCATTGCTAACCCCTAGGCAAAATATTG
AAGCATGTAGTCCTCCCTATCCGAGCTGGACAGAGTGGTAATC

Reference:

1. G. Aad *et al.*, Search for a new resonance decaying to a W or Z boson and a Higgs boson in the [Formula: see text] final states with the ATLAS detector. *Eur Phys J C Part Fields* **75**, 263 (2015).