Supporting Information for

# *PCR-based scaffold preparation for the production of thin, branched DNA origami nanostructures of arbitrary sizes*

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## *Materials and Methods*

**Materials.** M13mp18, lambda DNA, and streptavidin-coated magnetic beads were purchased from New England Biolabs. Staple strands for DNA origami folding were purchased from Sigma-Aldrich or Operon and diluted to ~100 µM in TE buffer. PCR primers were purchased from Sigma-Aldrich or Operon. PCR purification kits were obtained from Qiagen. DNA polymerase and PCR buffers were acquired from Invitrogen.

**Generation of PCR-based scaffolds.** PCR was performed (Techne TC-3000 thermal cycler) with 1 µg of each primer and between 20-80 ng of template DNA (either M13mp18 or lambda DNA). Platinum *Pfx* polymerase (2.5 units) was added to the solution containing primers, template, 1x *Pfx* amplification buffer, 2 mM $MgSO_4$, and a mixture of dNTPs (500 nM each) in a 100 µL volume. The following program was used: 95 ºC for 2.5 min, 30 cycles of 95 ºC for 45 s, 58-60 ºC for 45 s, 68 ºC for 1-5 min, and a final extension at 68 ºC for 4-6 min. The annealing temperature was chosen to be 1-2 ºC below the melting point of the primers. The PCR product was purified with a QIAquick PCR purification kit (Qiagen). The product was also run on a 0.7% agarose gel, and if multiple bands were present, the desired band was separated from the rest through gel purification.

**Single-stranded scaffold separation and purification.** Streptavidin-coated magnetic beads (800 ng) were rinsed 3 times (by mixing with solution, pelleting the beads with a magnet, and removing the supernatant) with 200 µL bead buffer (20 mM Tris-HCl, 1 mM EDTA, 0.2 M NaCl), and then combined with 200 µL bead buffer and 20-50 µL purified PCR product. The samples were gently mixed 15-30 min (depending on the length of the scaffold) to bind the DNA to the beads. The beads were pelleted, the supernatant (which contained all unbound DNA) was removed, and the beads were rinsed 2-3 times with 200 µL bead buffer. NaOH (0.2 M, 150 µL) was added to the pelleted beads, and the sample was gently mixed 6 min to denature the DNA. The supernatant (containing the desired ssDNA) was collected and combined with 100 µL of 5 M ammonium acetate (pH 7.6) to neutralize the pH. The product was purified either with a spin column (QIAquick PCR purification kit) or by ethanol precipitation. DNA concentration was measured with a Nanodrop ND-1000 spectrophotometer using 260 nm extinction coefficients of 50 ng·cm/µL for dsDNA and 33 ng·cm/µL for ssDNA.

**Origami Folding.** Purified single-stranded scaffold was mixed with staple strands in a 1:100 molar ratio in 1x TAE-$Mg^{2+}$ buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, 12.5 mM magnesium acetate). Each design was folded by denaturing (95 ºC for 3 min) and slowly annealing from 75 ºC to 4 ºC in 70 min for the rectangular origami and 95 ºC to 4 ºC in 90 min for the branched shapes.

**AFM imaging.** DNA origami samples were deposited onto freshly-cleaved mica, rinsed with water, and dried using compressed air or nitrogen. The samples were imaged in air using tapping

mode on a Digital Instruments Nanoscope IIIa MultiMode AFM (Veeco) with silicon force modulation AFM tips (Vistaprobes, 3 N/m, 60 kHz).

**Design Program.** The DNA origami folding program developed at BYU is available upon request from dna_origami@byu.edu.

***Branched structure flexibility***

The flexibility of the thin, branched structures presented here may affect their range of uses. The following figures (Figure S1 and Figure S2) demonstrate some flexibility in the top of the 'T' shape, which is three helices wide, compared to the shorter base, which is four helices wide. They also demonstrate some flexibility in the square junction.

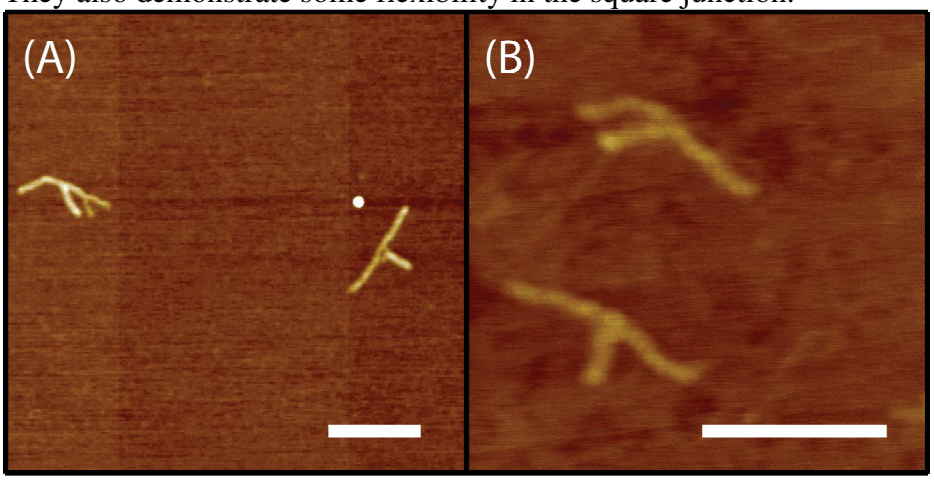


**Figure S1**. AFM images of the 'T' design, showing some flexibility in the top arms. In (A), one 'T' is bent considerably, while the other is straight. In (B), two different 'T's show flexibility in the top arm. The scale bar in each image is 200 nm.

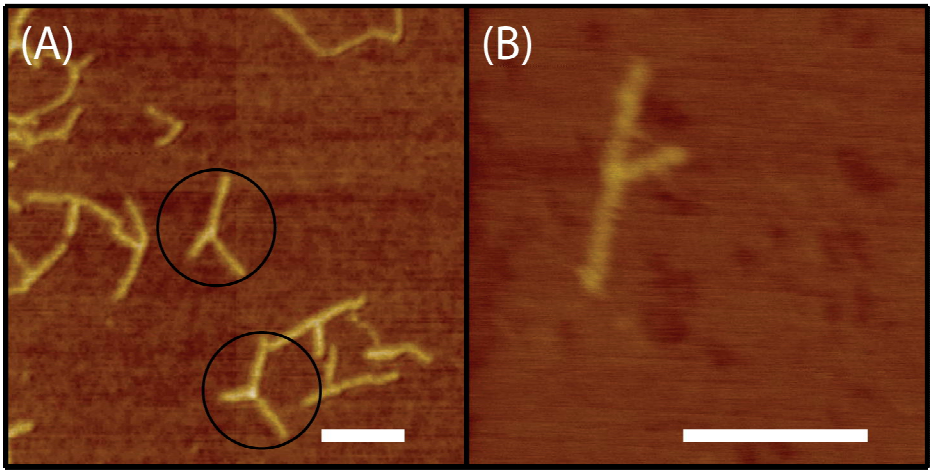


**Figure S2.** AFM images of the 'T' design, showing flexibility in the square junction; (A) shows several structures with nearly axisymmetric junctions (circled), while (B) shows a 'T' with straight arms, but having the base connecting at an obtuse angle. Both scale bars are 200 nm.

***Scaffold and staple strand sequences for the origami designs***
(All sequences are given 5' to 3')

**Lambda rectangle**

*Scaffold:*
Lambda phage DNA, bases 2868-3623, 756 bp

*PCR Primers:*
GGTGCT GACACGGAAG AAAC
[BioTEG]ATCATCAGCAGATTGTTCTTTATTC

*Staple sequences:*
TTTTATTCTGAACTAATTCCGTGTCAGCACC
TCATAACGTCCGGTTTCAACGTCACAACGTCTC
TTCACATCGTGTTAGTTTACTGAGAGCAT
TTTGATAATTCATTACTAAAAACGATAACACCGTG
TAAATTCTCAAGAACGATGGGTTACAA
TGCTAAAGCAGGAGTTTTCACACGCATCTTATAGAAA
GTGAAAACATTCCTAATATTTGATAGGTTGAAATCAAGAGAA
CGTCCTATGACATAAA
TTGAAACGATAAGGTTGTAAATGT
TTAATAAAAGGACTTAAAAAAAACGATAATGCAAACTACG
GGCTCAGGTTGCACAGATAATA
ACATTATATTTACTATCTAGCCCATTTTTAAAGAAATATTCG
ATCATCAGCAGATTGTTTAGACTGTGAATTG
ATCAAGTGCGAAAAGATTCTTTATTCATTTTGT
AAAATATTACTTCAAAAGGTTTTACCAAT
CGCCCTCGTATCACATGGATCTTTCTGTATGAAGA
GTTGTATTTCCCTCAGGTCAGACCAGA
TCATCAACAAAACACAAGGCAGAATGCCAGCAGGACC
CGCTCCATGCGCTTGCTCTTCATCTAGCGGTT
TTTGAGCACGTTGGCCTTACATACATCTGTCG
GCACTTTGTTACGCAACCAATACTATTAA
ATCACATTTCAGCAATACAGGGAAAATCT
ATATCCATGAACATAAAAGATATTACTATACC
TCAGAACACTACACAAATCTTTCCACGCTAAA

**M13 rectangle**

*Scaffold:*
M13mp18, bases 5870-6625, 756 bp

*PCR Primers:*
CCACCATCAAACAGGATTTTCGCC
[BioTEG]TTCTCCGTGGGAACAAACGGC

*Staple sequences:*
GGTACCGAGCTCGAATCCTGTTTGATGGTGG
CCCCAGCAGGCGAAAATTCGTAATCATGGTCAT
ACAATTCCACACAACCCGCCTGGCCCTGA
ACAGCTGATTGCCCTTCAATACGAGCCGGAAGCAT
GCTAACTCACATTATATTGGGCGCCAG
CGGGGAGAGGCGGTTTGCGATTGCGTTGCGCTCACTG
TTCAGGCTGCGCAACTGTTGGCCAGTCGGGAAACCTGTCGTG
CCCGCTTTGAAGGGCG
TGGCGAAAGGGGCCTAATGAGTGA
AAAGTGTAAAGCCTGGGGTGGATGTGCTGCAAGGCGATTA
ACGTTGTAAAAGTTATCCGCTC
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TTCTCCGTGGGAACAATCTAGAGGATCCCCG
CATGCCTGCAGGTCGACACGGCGGATTGACCGT
CATCGTAACCGTGCATTTTCCCAGTCACG
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ACGACGACAGTATCGGCCTCAGGAAGATCGCA
CCGGAAACCAGGCAAAGCGCCATTCGCCA
CCAGCTGCATTAATGAATCGGCCAACGCG
GGTGGTTTTTCTTTTCACCAGTGAGACGGGCA
GAGAGTTGCAGCAAGCGGTCCACGCTGGTTTG

**T Design**

*Scaffold:*
M13mp18, bases 5734-1442, 2958 bp

*PCR Primers:*
CTGATAGACGGTTTTTCGCCC
[BioTEG]CATAACCGATATATTCGGTCGC

*Staple sequences:*
GCATCAATTCTACTAATA
GGTAGAAAGTCTATCAG
TACCACATACGTGGACTCCAACGTCAAAGGGC
CAAAAGGATCCAGTTTGGAACAAGAGTCCACT
ACTATCATAAAGAATAGCCCGAGATAGGGTTG
AAAAACCAGTTCCGAAATCGGCAAAATCCCTT
GAAGTTTTGCCCCAGCAGGCGAAAATCCTGTT
TTTAGACTAGAGAGTTGCAGCAAGCGGTCCAC
CGTCATAAAACAGCTGATTGCCCTTCACCGCC
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AATTGGGCTTGAGATGGTTTAATTACGACGAT
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TGTGAGCGATTGTATAAGCAAATATTTAAATT
CCTTCCTGTAATATTTTGTTAAAATTCGCATT

**U Design**

*Scaffold:*
Lambda phage DNA, bases 37501-41340, 3840 bp

*PCR Primers:*
TCAACCTCAAGCCAGAATGC
[BioTEG]CGCGTCTGAATATCCTTTGG

*Staple sequences:*
TAAGCTGCGGTTGCGTTTCTGGCTTGAGGTTGA
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**B design**

*Scaffold:*
Lambda phage DNA, bases 37501-42308, 4808 base pairs long

*PCR Primers:*
TCAACCTCAAGCCAGAATGC
[BioTEG]CGACGCTTTCTTGTTCG

*Staple sequences:*
AAGATATCTGATTCCAGGCTTCCCCCACTTGCTGCCGCTCTG
TCTTCTGCTGTTGGCTTTAGCC
GTGGTGTAATTCCCTCGCCCGATGGTGCGATAGTCTTCA
CGGTTTTCTGGCTGAAATGGTCATC
GCTTCGGTTCATCAGCTCTGATGCCAATCCAC
ACAGTATTTGGTGAAGGGAACGAGTTTTAAGCGGATATCGTTAGCCCACCCAGCAA
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TTACCTCTTCCGCATGAGATGTTTTTAT
ATATTCATTCTGACGAGTAGAGCGGGGTTATTTA
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GAACCATATGTAAGTATTTCCTTAGATAACAA
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CCCAGGTAGGTTGACTGGCCTATTCAACCTGT
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CAGCTTTCTGCCATTTTAGCCGCGGCCTGATT
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GCCATTCCGGCGCACAGAAGCTATTATGCGTC
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TCCAGAGCAGAGGTCTACCGCCCATGACAGGA
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CGTGGTGACGCTGACGTTTGGCAGTCCGGCGG
GCTACGCGGCTTCTTCCCGGCACCATGCAACAAACTGCCCGGGTG
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