Supporting Information

One-pot synthesis of defined-length ssDNA for multi-scaffold DNA origami

Willem E. M. Noteborn[†], Leoni Abendstein[†] & Thomas H. Sharp[†,*]

[†]Department of Cell and Chemical Biology, Leiden University Medical Center, 2300 RC Leiden, The Netherlands

AUTHOR INFORMATION
*Corresponding Author: E-mail: t.sharp@lumc.nl. Tel: 0031-7152-69499

Materials

M13mp18 was obtained from Bayou Biolabs/Tebu-Bio. pUC19 and pEGFP-C1 were synthesized in-house. Lambda DNA was purchased from Thermo Fisher Scientific. All staples and PCR primers were synthesized and purified using standard desalting procedures by Integrated DNA Technologies (IDT). Primers containing phosphate and phosphorothioate modifications were HPLC purified by IDT. Tris base, EDTA, $MgCl_2$, PEG 8000, and NaCl were purchased from Sigma-Aldrich. T7 exonuclease, Lambda exonuclease, ApoI-HF restriction endonuclease, proteinase K, Q5® High-Fidelity 2X Master Mix, and Monarch® PCR & DNA Cleanup Kit (5 µg) were obtained from NEB/Bioké. AccuStart Taq DNA Polymerase HiFi was purchased from Quantabio. Amicon® Ultra 0.5 mL Centrifugal Filters (MWCO: 100 kDa) were obtained from Merck.

Polymerase chain reactions

Double-stranded DNA production was performed using the Q5® High-Fidelity 2X Master Mix. The enzyme was used according to the manufacturer's specified buffer and protocol for standard PCR. Briefly, 25 ng of template was mixed with both primers (1 µM each), PCR master mix or individual PCR buffer and components (e.g. $MgSO_4$, dNTPs), and H_2O in a total volume of 50 µL. The annealing temperature for all reactions was 60°C. The PCR protocol using the Q5® enzyme consisted of a 30 s initial denaturation at 98°C, followed by 30 cycles of 10 s at 98°C, 30 s at 60°C, and 45 s per kilobase at 72°C. Afterwards, PCR reactions were evaluated using 0.8 – 1.0 % agarose gel electrophoresis or directly used for ssDNA production. Asymmetric PCR was performed in a similar way to the standard PCR protocol using AccuStart Taq DNA Polymerase HiFi and primer combinations that were specially designed for aPCR with a final concentration of 1 mM of sense primer and 20 nM of antisense primer.[1]

T7 exonuclease digestion

Freshly made PCR reactions (50 µL each) were directly used for ssDNA generation without purification. To each reaction, 5 µL of NEB buffer 4 and 1 µL T7 exonuclease (10 units) was added and incubated overnight at 25°C. Afterwards, ssDNA was purified using a Monarch® PCR & DNA Cleanup Kit (5 µg DNA binding capacity), quantified using a Nanodrop 1000 spectrophotometer, and stored at -20°C. Alternatively, after digestion 1 µL of proteinase K solution (10 mg/mL) was added to the mixture and incubated for 30 min at 37°C to inactivate any DNA modifying enzyme. In this way, the ssDNA could directly be used in DNA origami folding in a one-pot fashion.

Lambda exonuclease digestion

Lambda exonuclease-based digestion was performed in a similar fashion to the T7 exonuclease-based method. 5 µL of lambda exonuclease buffer and 1 µL (5 units) of lambda exonuclease were added to a PCR reaction (50 µL each) and incubated overnight at 37°C. Better digestion was obtained by first purifying the PCR product using a Monarch® PCR & DNA Cleanup Kit (5 µg DNA binding capacity). Afterwards, the reactions were purified again using the same protocol as with T7 exonuclease.

Restriction endonuclease digestions

M13mp18 ssDNA (1 pmol) was combined with two oligonucleotides containing a complementary sequence to two ApoI restriction sites (20 pmol each) in 50 µL of 1X NEB CutSmart® buffer (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 100 μg/mL BSA, pH 7.9) and thermally annealed from 85°C to 25°C at a rate of 1°C per min in a thermal cycler. Afterwards, restriction enzyme ApoI-HF (1 μL, 20 units) was added and the mixture was incubated overnight at 37°C. Digestion was examined using 1 % agarose gel electrophoresis.

DNA origami folding and purification

DNA origami folding solutions were prepared in PCR tubes by mixing the appropriate ssDNA scaffold (20 nM) with ssDNA staples (200 nM of each staple) in DNA origami folding buffer (20 mM Tris, 5 mM NaCl) supplemented with 20 mM $MgCl_2$ (31-DDH and 42-DDH were prepared using 25 mM $MgCl_2$) in a total volume of 50 μL. DNA origami structures were thermally annealed in a Bio-Rad C1000 Touch™ Thermal Cycler using the following protocol: 80°C down to 76°C at a rate of 5 min/°C, 75°C down to 30°C at a rate of 13.75 min/0.5 °C, 29°C down to 20°C at a rate of 10 min/°C. Folded DNA origami were purified and concentrated using Amicon® Ultra 0.5 mL Centrifugal Filters (MWCO: 100 kDa) or using PEG precipitation.[2]

Agarose gel electrophoresis

PCR products, ssDNA digestion products, and freshly folded DNA origami samples were loaded onto a 1 % agarose gel in 1× TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA) supplemented with 12 mM $MgCl_2$. Gels were run on a BioRad Mini-PROTEAN Tetra Cell electrophoresis device at 4 °C for 1 h under a constant voltage of 100 V. Afterwards, gels were stained with GelRed® and imaged using a Bio-Rad's Gel Doc XR+ system.

Assessing heat degradation of ssDNA

Lambda DNA was used to synthesise ssDNA of length 2,342 nt, 5,000 nt and 10,000 nt (Figure S9 and Table S14) before 20 nM solutions of each ssDNA was heated at either 40°C, 65°C, or 95°C in a thermal cycler. Samples were taken at specific time intervals of 15 min, 30 min, 1 h, 2 h, 4 h, and 24 h. Directly afterwards, the freshly drawn samples were stored at -20°C to stop further heat treatment until all samples from all time points could be analysed simultaneously on a 1 % agarose gel. For this, a 50 ng aliquot of each sample was loaded onto 1 % agarose gels and run for 1 h at 100 V. Afterwards, the gels were stained using GelRed® and visualized under UV light.

Electron microscopy and image analysis

Five freshly prepared 50 μL folding reactions with a scaffold concentration of 20 nM were combined, purified and concentrated to a volume of 25 μL using an Amicon Ultra 0.5 ml centrifugal filter (MWCO: 100 kDa). A three-microliter aliquot was applied onto a freshly glow-discharged TEM grid (Cu, 200 Mesh) with a continuous carbon support film and incubated for 2 min. The sample was stained with a 2 % uranyl formate solution for 1 min. Imaging was performed on a Tecnai T12 BioTWIN operating at 120 kV. Micrographs were collected at a 68,000x magnification using an Eagle 4k x 4K CCD camera. Single-particle picking and class averaging was performed using the image processing software package EMAN2.[3]

Supplemental Figures

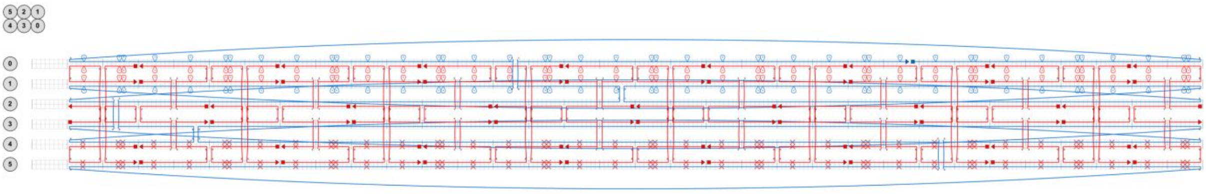

Figure S1. Schematic design of the 38 nm toroid. Grey circles represent DNA helices on the square lattice. Scaffold and staples are blue and red, respectively.

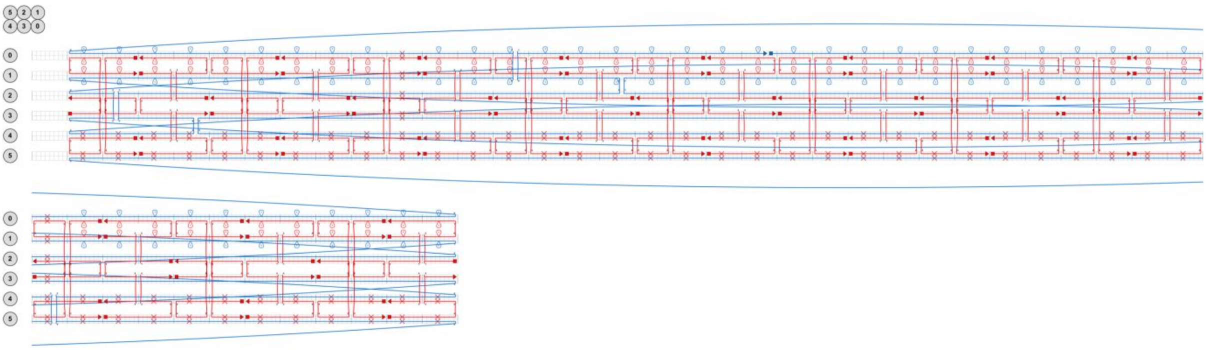

Figure S2. Schematic design of the 49 nm toroid. Grey circles represent DNA helices on the square lattice. Scaffold and staples are blue and red, respectively**.**

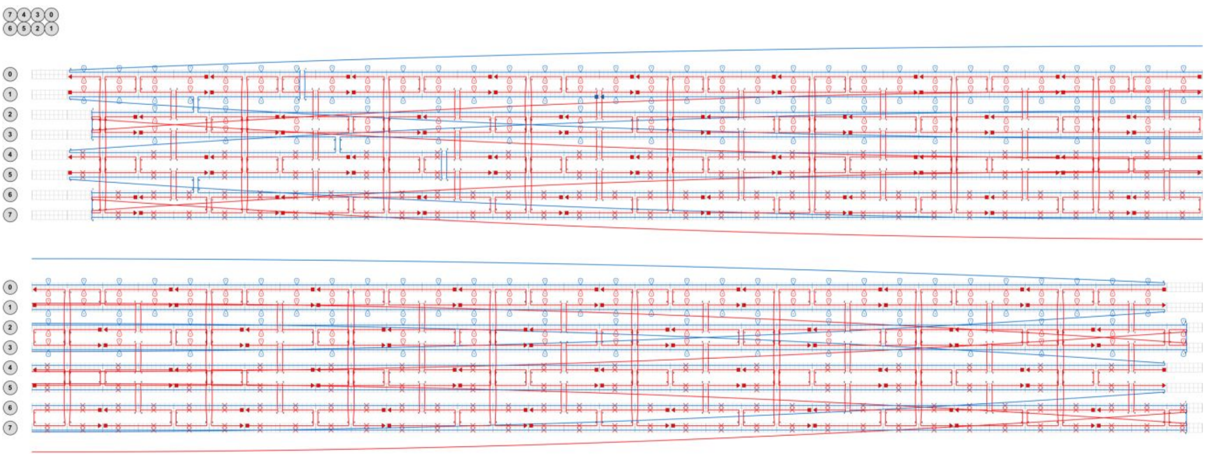

Figure S3. Schematic design of the 62 nm toroid. Grey circles represent DNA helices on the square lattice. Scaffold and staples are blue and red, respectively**.**

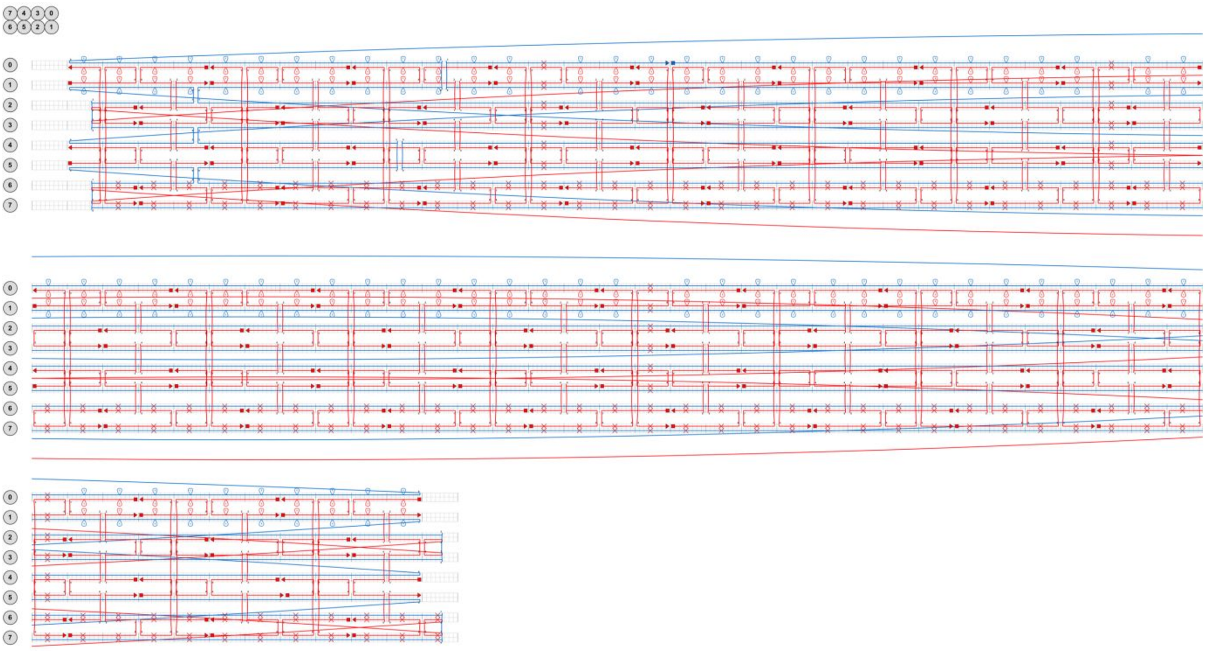

Figure S4. Schematic design of the 74 nm toroid. Grey circles represent DNA helices on the square lattice. Scaffold and staples are blue and red, respectively**.**

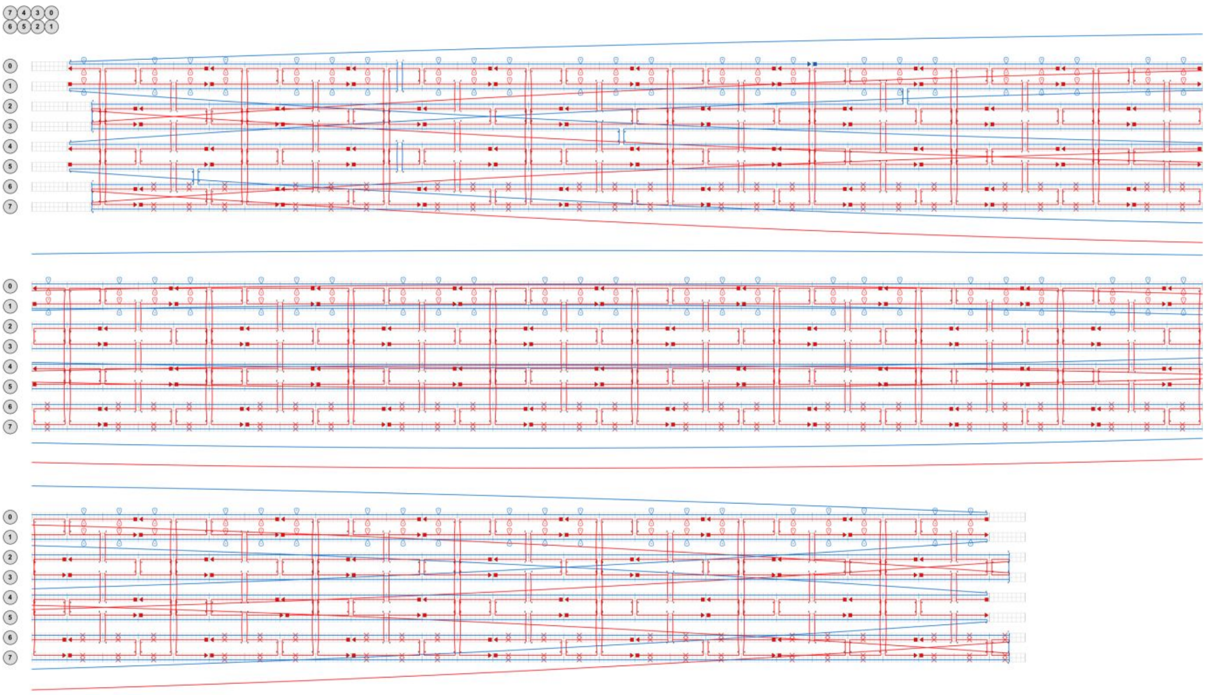

Figure S5. Schematic design of the 90 nm toroid. Grey circles represent DNA helices on the square lattice. Scaffold and staples are blue and red, respectively**.**

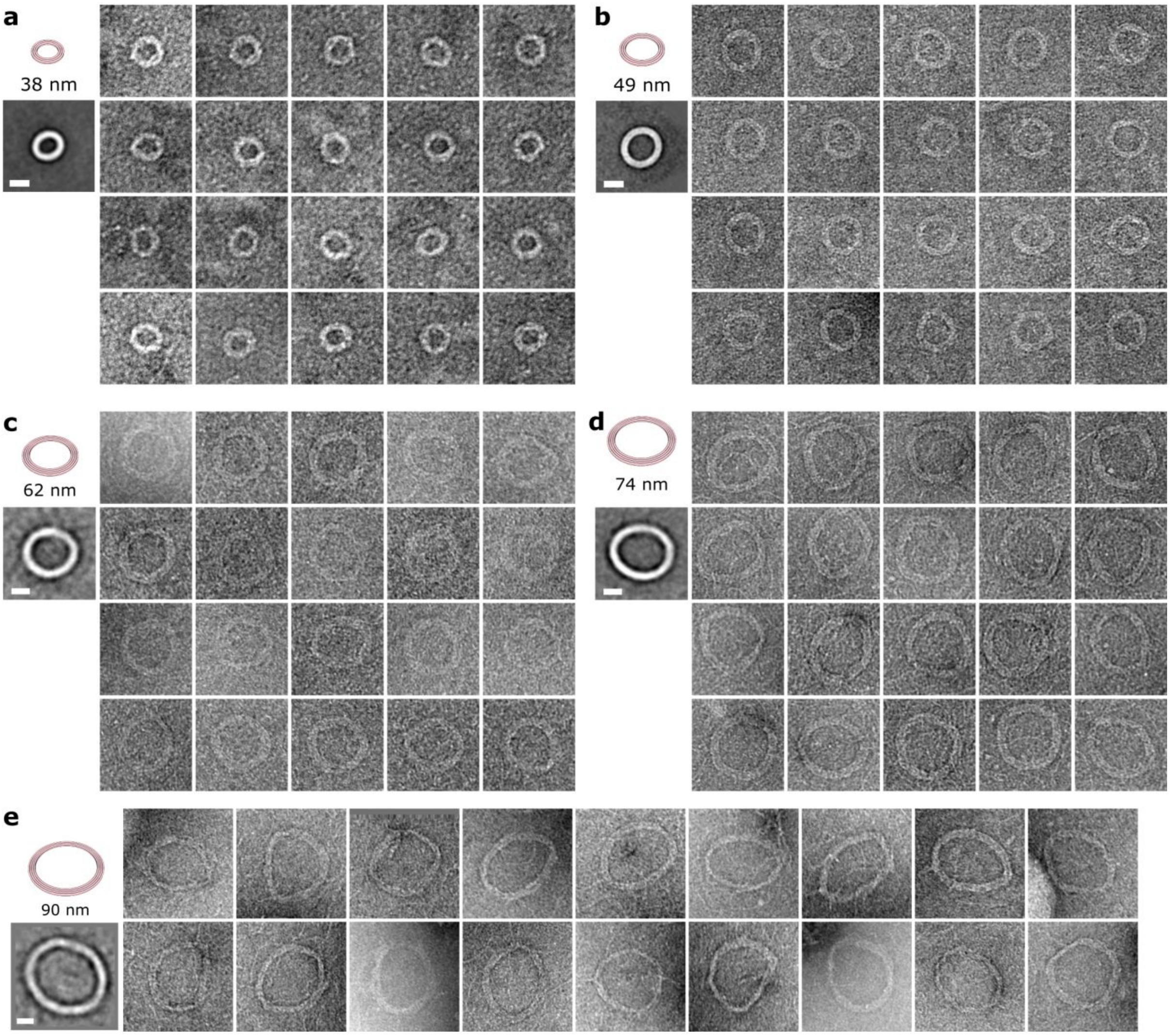

Figure S6. Negative stain EM images of the toroid designs. Computer designs, class averages and negative-stained individual toroids of diameter; (a) 38 nm, (b) 49 nm, (c) 62 nm, (d) 74 nm, (e) 90 nm. Scale bars are 25 nm for all panels.

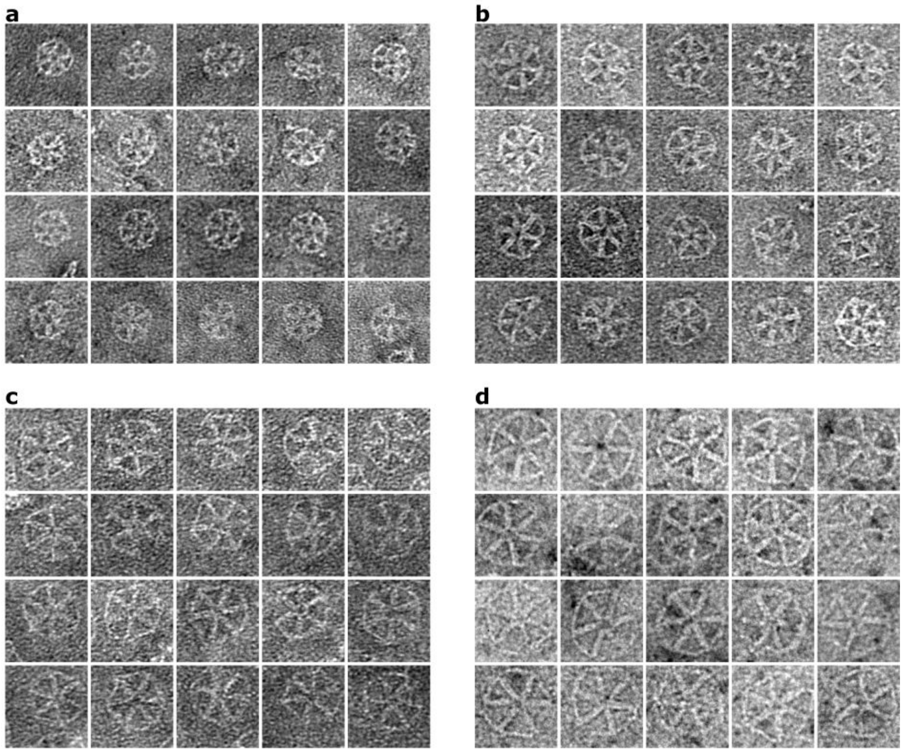

Figure S7. Negative stain EM images of double-decker hexagon (DDH) designs. (a) 1229-DDH. (b) 1512-DDH. (c) 1872-DDH. (d) 2268-DDH. Each box has an edge length of 100 nm.

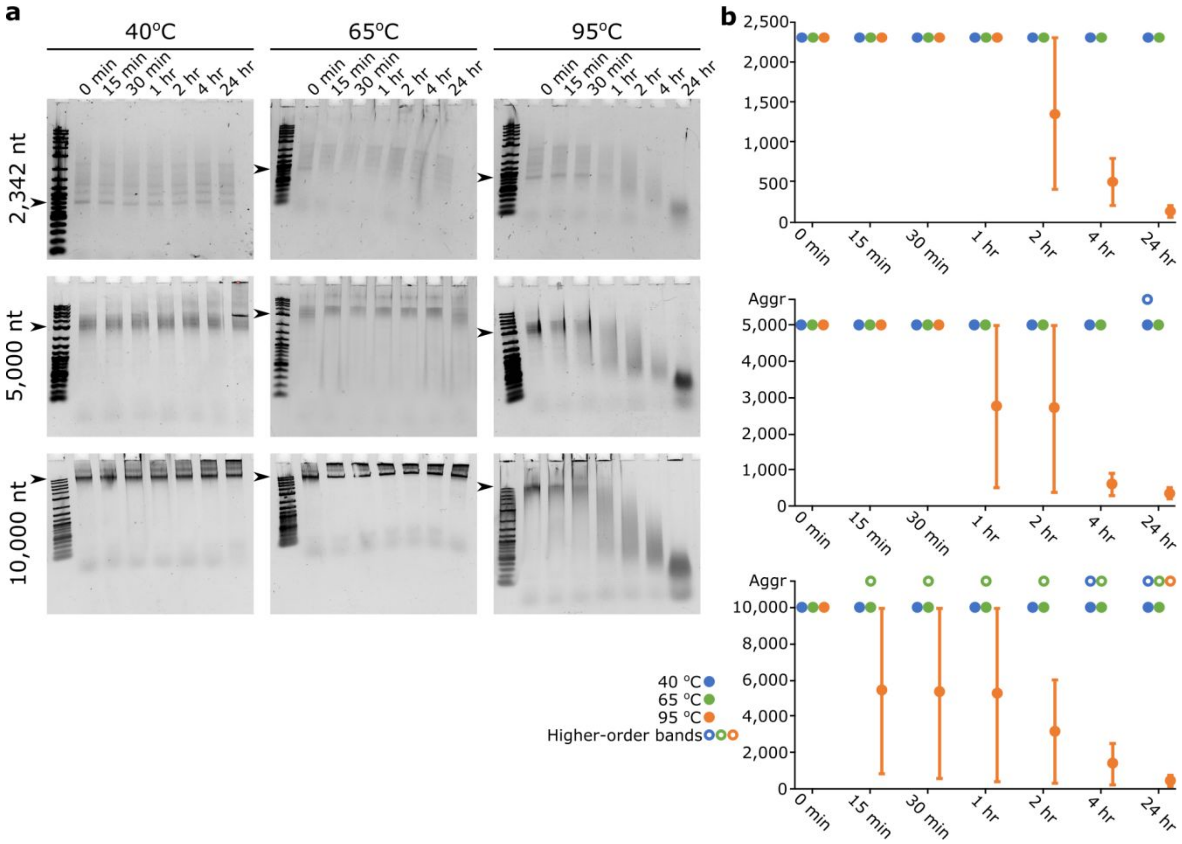

Figure S8. Thermal degradation of ssDNA is proportional to length and temperature. (a) Incubation at 95 °C degrades ssDNA 10,000 in length within 15 min. (b) Thermal degradation of ssDNA is proportional to length. See Table S14 for primer sequences and template information.

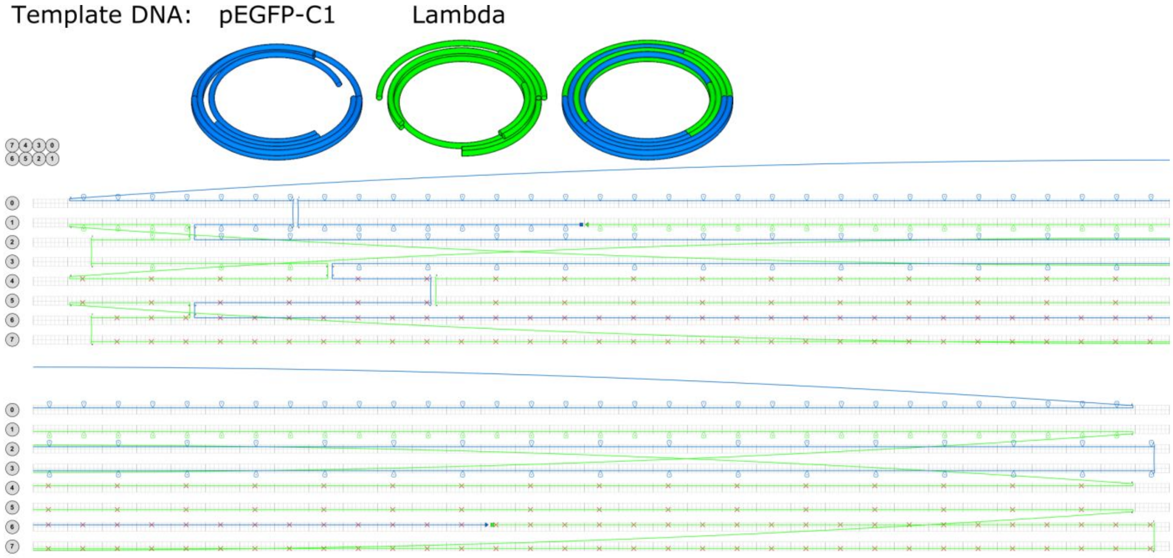

Figure S9. Schematic design of the 62 nm toroid split into two scaffold strands using different template DNA. Scaffold strands are blue and green. The route of each scaffold within the completed origami is shown at the top, along with the complete structure and the source of the template DNA. Grey circles represent DNA helices on the square lattice. Staple strands are not shown and follow the same scheme as Figure S3**.**

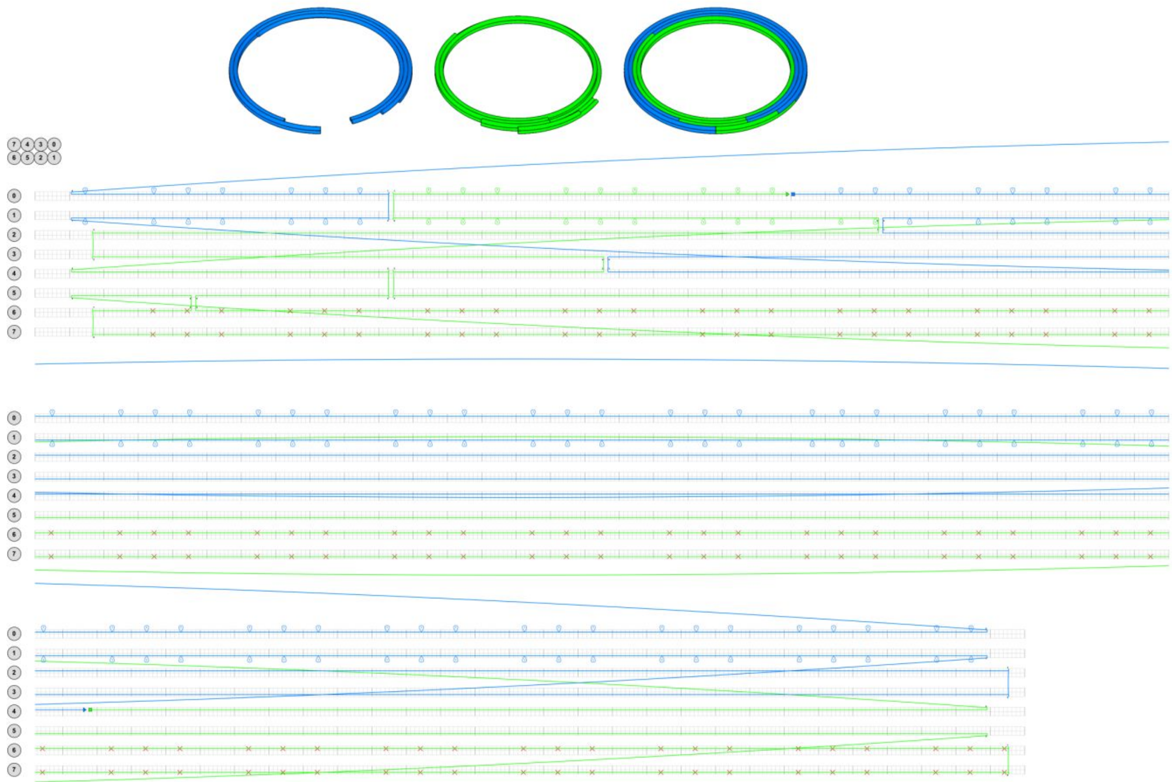

Figure S10. Schematic designs of the 90 nm toroid split into two scaffold strands. Scaffold strands are blue and green. The route of each scaffold within the completed origami is shown at the top, along with the complete structure. Grey circles represent DNA helices on the square lattice. Staple strands are not shown and follow the same scheme as Figure S5**.**

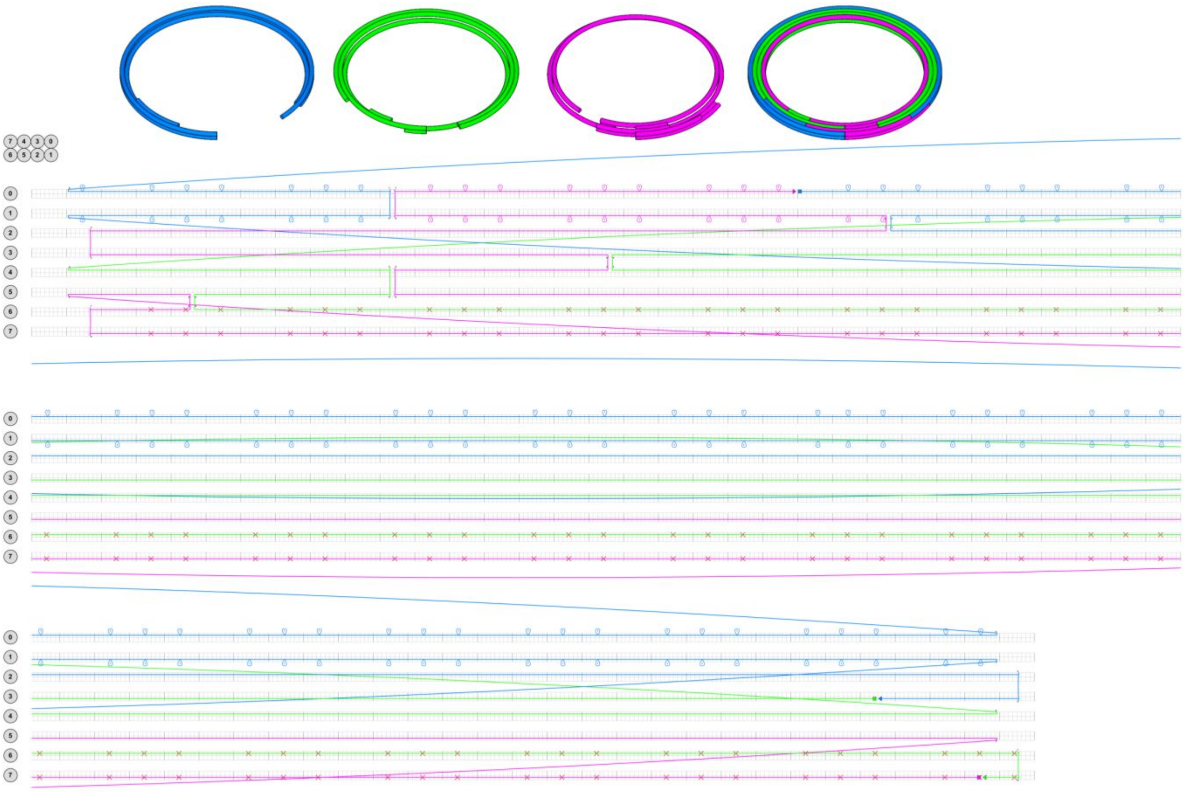

Figure S11. Schematic designs of the 90 nm toroid split into three scaffold strands. Scaffold strands are blue, green and purple. The route of each scaffold within the completed origami is shown at the top, along with the complete structure. Grey circles represent DNA helices on the square lattice. Staple strands are not shown and follow the same scheme as Figure S5.

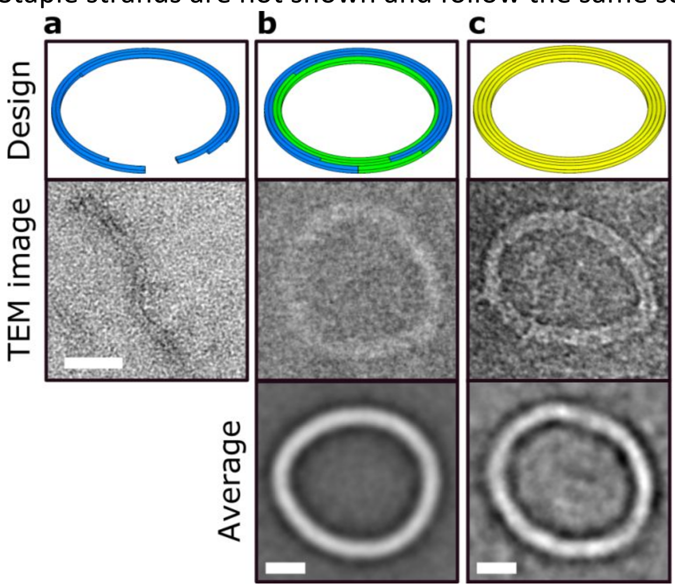

Figure S12. TEM analysis of the 90 nm toroid formed from two scaffolds. (a) Structure formed from half of the required scaffold showing the route of the scaffold within the completed origami design (blue; top) and a representative TEM image of the resulting amorphous structure. (b) Structure formed from both of the required scaffolds, showing the route of each scaffold (blue and green) within the completed origami design (top), a representative TEM image of the resulting toroid, and a class average of the structures found in TEM. (c) Comparison with the 90 nm toroid formed from a single scaffold (yellow). Scale bars: a, 50 nm; b & c, 20 nm

Supplemental Tables

Table S1. Primer sequences for Figure 1b. Template was M13mp18 ssDNA. Phosphorothioated nucleotides shown in bold.

For PCR followed by T7 exonuclease digestion:	
1536_Fwd_PS	**GACGG**GTTGTTACTCGCTC
1536_Rev	GTTTCTCGTCAGGGCAAGC

Table S2. Primer sequences for Figure 1c. Template was M13mp18 ssDNA. ApoI recognises the dsDNA sequence RAATTY (where R and Y are purine- and pyrimidine-containing bases, respectively), which are underlined, and the cleavage sites are indicated with ▼. Phosphorothioated nucleotides shown in bold and phosphate modification by /5Phos/.

For restriction endonuclease digestion by ApoI-HF:	
5917/1332 nt primer 1	AGAGTCAATAGT<u>G▼AATTT</u>ATCAAAATCATA
5917/1332 nt primer 2	ACAATCAATAGA<u>A▼AATTC</u>ATATGGTTTACC
For aPCR:	
1229_Fwd	GCACTGACCCCGTTAAAACTTA
1229_Rev	GACTTGCGGGAGGTTTTGAAG
For PCR followed by lambda exonuclease digestion:	
1229_Fwd_PS	**GCACT**GACCCCGTTAAAACTTA
1229_Rev_Phos	/5Phos/GACTTGCGGGAGGTTTTGAAG
For PCR followed by T7 exonuclease digestion:	
1229_Fwd_PS	**GCACT**GACCCCGTTAAAACTTA
1229_Rev	GACTTGCGGGAGGTTTTGAAG

Table S3. Scaffold sequences for the DDH designs. Template was M13mp18 ssDNA. Primer locations are underlined and phosphorothioated nucleotides shown in bold.

1229 nt for the 1229-DDH
<u>**gcact**gaccccgttaaaactta</u>ttaccagtacactcctgtatcatcaaaagccatgtatgacgcttactggaacggtaaattc agagactgcgctttccattctggctttaatgaggatttatttgtttgtgaatatcaaggccaatcgtctgacctgcctcaacc tcctgtcaatgctggcggcggctctggtggtggttctggtggcggctctgagggtggtggctctgagggtggcggttctgagg gtggcggctctgagggaggcggttccggtggtggctctggttccggtgattttgattatgaaaagatggcaaacgctaataag gggggctatgaccgaaaatgccgatgaaaacgcgctacagtctgacgctaaaggcaaacttgattctgtcgctactgattacgg tgctgctatcgatggtttcattggtgacgtttccggccttgctaatggtaatggtgctactggtgattttgctggctctaatt cccaaatggctcaagtcggtgacggtgataattcacctttaatgaataatttccgtcaatatttaccttccctccctcaatcg gttgaatgtcgcccttttgtctttggcgctggtaaaccatatgaattttctattgattgtgacaaaataaacttattccgtgg tgtctttgcgtttcttttatatgttgccacctttatgtatgtattttctacgtttgctaacatactgcgtaataaggagtctt aatcatgccagttcttttgggtattccgttattattgcgtttcctcggtttccttctggtaactttgttcggctatctgctta cttttcttaaaaagggcttcggtaagatagctattgctatttcattgtttcttgctcttattattgggcttaactcaattctt gtgggttatctctctgatattagcgctcaattaccctctgactttgttcagggtgttcagttaattctcccgtctaatgcgct tccctgtttttatgttattctctctgtaaaggctgctattttcatttttgacgttaaacaaaaaatcgtttcttatttggatt gggataaataatatggctgtttattttgtaactggcaaattaggctctggaaagacgctcgttagcgttggtaagattcagga taaaattgtagctgggtgcaaaatagcaactaatcttgatttaagg<u>cttcaaaacctcccgcaagtc</u>
1512 nt for the 1512-DDH
<u>**gcctc**aacctcctgtcaat</u>gctggcggcggctctggtggtggttctggtggcggctctgagggtggtggctctgagggtggcg gttctgagggtggcggctctgagggaggcggttccggtggtggctctggttccggtgattttgattatgaaaagatggcaaac gctaataagggggctatgaccgaaaatgccgatgaaaacgcgctacagtctgacgctaaaggcaaacttgattctgtcgctac tgattacggtgctgctatcgatggtttcattggtgacgtttccggccttgctaatggtaatggtgctactggtgattttgctg gctctaattcccaaatggctcaagtcggtgacggtgataattcacctttaatgaataatttccgtcaatatttaccttccctc cctcaatcggttgaatgtcgcccttttgtctttggcgctggtaaaccatatgaattttctattgattgtgacaaaataaactt attccgtggtgtctttgcgtttcttttatatgttgccacctttatgtatgtattttctacgtttgctaacatactgcgtaata aggagtcttaatcatgccagttcttttgggtattccgttattattgcgtttcctcggtttccttctggtaactttgttcggct atctgcttacttttcttaaaaagggcttcggtaagatagctattgctatttcattgtttcttgctcttattattgggcttaac tcaattcttgtgggttatctctctgatattagcgctcaattaccctctgactttgttcagggtgttcagttaattctcccgtc taatgcgcttccctgtttttatgttattctctctgtaaaggctgctattttcatttttgacgttaaacaaaaaatcgtttctt atttggattgggataaataatatggctgtttattttgtaactggcaaattaggctctggaaagacgctcgttagcgttggtaa gattcaggataaaattgtagctgggtgcaaaatagcaactaatcttgatttaaggcttcaaaacctcccgcaagtcgggaggt

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1872 nt for the 1872-DDH

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aatgaaaatatagctaaacaggttattgaccatttgcgaaatgtatctaatggtcaaactaaatctactcgttcgcagaattg
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2268 nt for the 2268-DDH

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cctcaaagcctctgtagccgttgctac

Table S4. Staple sequences for the 1229-DDH design.

GAAGCTTTTTCCTTTTTAAGATTCATATGGTTTTTTTTACCAGCGCGCACCA
CAGCCTTTTTATATTATTTAAATAAGAAACGTTTTTATTTTTTGTTCCCACA
CCAGTTACAACAATAGCTAT
CCAATAACCGAGAAGGAAATAA
AGAATTTTTTTGAGTTAAGCGAGCAAGAAACTTTTTAATGAAATAGAATAAA
AATAGTTTTTCAGCCTTTACACATAAAAACATTTTTGGGAAGCGCAGAATTA

TAACGTCAAAAGAGAGATAA
CAAAGAAAGAATACCCATCAGA
ACTGATTTTTACACCCTGAAGGGTAATTGAGTTTTTCGCTAATATCAATGAA
ATCCTTTTTTCATTAAAGCCCTCAGAGCCGCTTTTTCACCAGAACCCCGCCG
TTAGACAAACATTCACGGGA
AAACGTTTTTTAGAAAATACGGCAACATATATTTTTAAAGAAACGCGAATAAGTTTATTTTTTTTTGTCACAGATAGC
GGTTGCAGTATATTACGAGGCA
CCAGCTTTTTATTGACAGGAGGTCAGACGATTTTTTTGGCCTTGATAAATAA
CGGAATTTTTCCGCCTCCCTCAGACTGTAGCTTTTTGCGTTTTCATGGTCAT
ACCACAGAGCGAACCCAGAG
GTTTGAAAGGTATACATCCATC
AGCCCTTTTTCCTTATTAGCTTTTCATAATCTTTTTAAAATCACCGCACCAC
CGGCATAAAGTTCATTTTTC
TATTGTTTTTACGGAAATTAGTGAATTATCATTTTTCCGTCACCGATTGGGA
AGGGAACCACGAAAGACGGGAA
CGAACTTTTTAAAGTTACCAGGAAACGCAATTTTTTAATAACGGAACTGGCATGATTTTTTTAAGACTCCTTGTTAGC
AAAAGTAAGCAATCAATAGAAA
GAAACTTTTTCATCGATAGCAGTAGCGACAGTTTTTAATCAAGTTTCCCTCAGAACCTTTTTGCCACCCTCACGCAGT
CCGGAAACGTCCAGTGCCGAGC
TTACCTTTTTATTAGCAAGGGTCTTTCCAGATTTTTGCCTAATTTGCTTACC
CAAAGACAAAATCACCAGTA
ATTAGTTTTTAGCCAGCAAAAGGGCGACATTTTTTTCAACCGATTGGGTAAA
CTTGAGTAATCAGCACCGCCAT
CAGAGCCGCCAGCCTTTAGCGT
CTCTGTTTTTAATTTACCGTCATACATGGCTTTTTTTTTGATGATAGGTAATAAGTTTTTTTTTAACGGGGTACCAAT
AGAATGGAAAGGAGCCACCACC
AGAGAAAGCGTTCCAGTGAATA
TCCCATGTACTCAGGAGATCCA

Table S5. Staple sequences for the 1512-DDH design.

CGTTTTTATTTTTTTTTCATCGTAGGTAGACGGGAGATTTTTATTAACTGAA
AACATGTAATTTTTTTTTAGGCAGAGGCCAGTAATAAGTTTTTAGAATATAAA
ATCGGCTGTCTTTTTTTTTCCTTATCAGGGTATTAAACTTTTTCAAGTACCGC
ATTCTGTCCAGTTTTTACGACGACAAGTTCAGCTAATTTTTTGCAGAACGCG
AACAATAGATATTTTTAGTCCTGAACATATCCCATCCTTTTTTAATTTACGA
TAGCCCCCTTATTTTTTTTAGCGTTTGAGGCCGGAAACTTTTTGTCACCAATG
GCGTCTTTCCATTTTTGAGCCTAATTATCCCAATCCATTTTTAATAAGAAACCAGCCTTTACATTTTTGAGAGAATAA
TAGCAGCACCGTTTTTTAATCAGTAGAAGTTTGCCTTTTTTTTTAGCGTCAGA
AAAGGTGAATTTTTTTTATCACCGTCAGTTTATTTTGTTTTTTCACAATCAAT
TATGGTTTACCTTTTTAGCGCCAAAGGACATTCAACCTTTTTGATTGAGGGA
CACAAGAATTGTTTTTAGTTAAGCCCTAGCTATCTTATTTTTCCGAAGCCCT
CAAGCAAATCATTTTTGATATAGAAGTTAGCGAACCTTTTTTCCCGACTTGCTGCTATTTTGCTTTTTACCCAGCTAC
TAAGACTCCTTTTTTTTATTACGCAGTGGTGGCAACATTTTTTATAAAAGAAATTAGAGCCAGCTTTTTAAAATCACCA
CTGGCATGATAGGAGGTTGAGGCCAACGCTCACCCAAAAGAA
GAAACGCAATATTTTTATAACGGAATAACAGTAGGGCTTTTTTTAATTGAGA
AGTAAGCAGATTTTTTAGCCGAACAAGTAATTGAGCGTTTTTCTAATATCAG
TACATAAAATGTTAGCAAACGTAGAGAAATTTTAAAAATACA
CATTTGGGAACGCAAAGACACCACGGAATAACCGACTTGAGC
CACCGGAACCATTTTTGAGCCACCACGAACCGCCACCTTTTTCTCAGAGCCAAGAGCCGCCGCTTTTTCAGCATTGAC
TAATCAAAATGTAGCACCATTACCATTAGCACCATCTTTTCA
CACCCTCACGGAACCGCCTCCCTCAACATTAAACAGAGCCGC
CCACCACCCCACCCTCAGAGCCGCTCGAGCATTTCACCAGAA
GAGGCGTTGCTTATCCGGTATTCTAGAACTTCCAAAGAACGC
AGATTAGTGGGAGGTTTTGAAGCCAAATAAAGAATTAAATCA
GCTAACGAAATTTTATCCTGAATCGAATCCGACATTACCAAC
TATTATTTTGCCAGTTACAAAATAAGGGCACAAAAACAGCCA
GAAAATAGGATTTTTTGTTTAACGTAACCAGAGATCAAAAAT
CCCAATAGCATAAAAACAGGGAAGCGCATAATCATTACCGCG
CATATTTAACAACGCCACTCATCGAGAACAAGCAAGCATCGC
GACAAAAGGTAAAGTAGCATGTAGAAACCAATCAATAGTACC
TCGGCATTTTCGGTCACTGTAGCGCGTTTTCATTATCCCTGT
CACGGAAATTATTCATTGGGAAGGTAAATATTGATCGAAAAC
AAACAATGAAATAGCAAAATAATAAGAGCAAGAATTCAAGAA
ACCAGAAGGAAACCGAGCACCCTGAACAAAGTCAGAGGAGTT

Table S6. Staple sequences for the 1872-DDH design.

AGCGAAAGACATTTTTGCATCGGAACTGACCTTCATCTTTTTAAGAGTAATC
TCGTCACCAGTTTTTTACAAACTACATAGTTAGCGTATTTTTACGATCTAAA
ACACTGAGTTAGGCCGCTTT
TTCGGTCGTCGCCCACGCATAACGGTAAAAT
CGCCGACAATGTTTTTACAACAACCACTGAGGCTTGCTTTTTAGGGAGTTAA
AATTTTCTGTATTTTTTGGGATTTTGGAGAATAGAAATTTTTGGAACAACTA
TTAGTAAATGAATTTCTTAA
TATCGGTTCTCCAAAAGGAGCCTAAGAATAC
TGAAAATCTCCTTTTTAAAAAAAAGGTATCAGCTTGCTTTTTTTTCGAGGTG
TGTCTGGAAGTTTTTTTTCATTCCATGAACCAGACCGTTTTTGAAGCAAACT
TGTTTTAAATAATAATTTTTTCACGTAAGG
GATTTGTATCATTTTTTCGCCTGATATACTTAGCCGGTTTTTAACGAGGCGCAACTTTGAAAGTTTTTAGGACAGATG
GCTTAATTTCATTTTTGCGGATGAACAAAGT
ATTGCTCCTTTTTTTTTGATAAGAGGGCTGAATATAATTTTTTGCTGTAGCT
TTTACCCTGACTTTTTTATTATAGTCTTTGCAAAAGATTTTTAGTTTTGCCA
GAATGACCATATAGAGAGTACCTTTACCAAC
CCCCCTCATCGTCATAAATATTCGCGACCTG
ATAGCGTCCAATTTTTTACTGCGGAAAATGCTTTAAATTTTTCAGTTCAGAA
TGAGATGGTTTAAATGTTTAGACTGGGAGGG
CACCAGAACGATTTTTGTAGTAAATTATTGTGAATTATTTTTCCTTATGCGA
ACGAGAAATCAGTGAATAAGGCTGGGAACCG
ACTAAAGACTTTTTTTTTTCATGAGGCCACTACGAAGTTTTTGCACCAACCTACTCATCTTTGTTTTTACCCCCAGCG
CGGCTACAGAAGACCAGGCGCATAGGCTGGCGAGGGTAGCAA
AATGCAGATACTTTTTATAACGCCAAATCATAACCCTTTTTTCGTTTACCAGAGAGGAAGCCCTTTTTGAAAGACTTC
TAGGAATACCAGGCAAGGCAAAGCCACCCTCCAGTTGAGATT
ACATTCAACTTAAATCATAC
ATTACAGGTAGTTTTTAAAGATTCATATTTTCAGGGATTTTTTAGCAAGCCC
TTACCCAAATCGTTAATAAA
GACGTTGGGAATTTTTGAAAAATCTACAACGTAACAATTTTTAGCTGCTCAT
GCAACACTAAGGAATTACGAGGCACATTATA
CCAGTCAGTTTTAAGAACTGGCTTAGTAAGA
GGATTGCATCAAACCAAAATAGCGAGAGGCTAGAAGCAAAGC
AAAAAGATTAACGACGATAA
TAGATTTAGTTTTTTTTGACCATTAGGCTATATTTTCTTTTTATTTGGGGCGAGTAGTAGCATTTTTTTAACATCCAA
TTCCCAATTCTTTTAATTCGAGCTTCAAAGCATAACAGTTGA
TGCGAACGAGAAATATCGCG
CCTGTTTAATACATTTCGCAAATGCAGTTTC
AGCGGAGTCTAAACAACTTTCAAGTCAATAA
CTACTAATCGAGCTGAAAAGGTGGCACAGAC
AGCCCTCAACGCCTGTAGCATTCCATCAATT
ACGTAATGAAGTTTCCATTAAACGCGATATA
ACTAAAACAAAACGAAAGAGGCAATTAATTG
ACAACGGAATTATACCAAGCGCGAGCTTAGA
CTCCATGTAATTGTGTCGAAATCCATTGAAT
AACTGACCAGACGGTCAATCATAATGCCCTG
CTTTGAGGAACGGTGTACGG
GAACCCATGTACCGTATGCGGGATCGTCACCCTCAGCAATAG
TGTCGTCTTTCCAGACGACAGCTTGATACCGATAGTTGGTTT
AATTGCGAATATGCAACTAAAGTACGGCAACA
AGGTCAGGATAATCAAAAATCAGGTCAACGA
GGTAATAGTAAATTTCAACTTTAATCGGGCT
CTAACGGAACAACATTTTGACAAGAACCGGATATTCAACGAA

Table S7. Staple sequences for the 2268-DDH design.

AAGAACTGGCTTTTTTCATTATACCAAGCGAACCAGATTTTTCCGGAAGCAA
TACGTAATGCCTTTTTACTACGAAGGCACTAAAACACTTTTTTCATCTTTGA
CCACATTCTTCATCAGTTGAGATTACACCAGA
ACGAGTAGCTTGCCCTGACGAGAATAGGAATA
AACTAATGCAGGTAGAAAGA
CTGCTCATTCATTTTTGTGAATAAGGTAAATTGGGCTTTTTTTGAGATGGTT
ATTTGTATCATTTTTTCGCCTGATAAACTTAGCCGGATTTTTACGAGGCGCA
CCTCGTTTTAGTAAGAGCAACACTGCGCATAG
GCTGGCTGACGGTGTACAGACCAGATCATAAC
ACCAGACGACTTACGAGGCA
ACTTTGAAAGATTTTTGGACAGATGAACCTTCATCAATTTTTGAGTAATCTT
GGATAGGTCACTTTTTGTTGGTGTAGATTTTGTTAAATTTTTATTCGCATTA
TCAATCATAAGGAACAAACGGCGGATTGTCGG

GACCGTAATGGTAACAACCC
CTGGATAGCGTTTTTTCCAATACTGCGAAAACGAGAATTTTTTGACCATAAACATCAAAAAGATTTTTTTAAGAGGAA
AATAGTAAAAAAGAAGTTTTGCCACCTGTAGC
CAGCTTTCATTCGCGTCTGGCCTTGAGGGGGT
AATGTTTAGAAGGCTTTTGC
ATAGGAACGCCTTTTTATCAAAAATAATCAACATTAATTTTTATGTGAGCGA
ACAAGAGAATCTTTTTGATGAACGGTATGCAATGCCTTTTTTGAGTAATGTG
TTGTTAAATCAGGTCATTGCCTGAGAGTCTAC
TCTGGAGCAATTTTGAGAGA
AATGCTTTTAAATATTCATTGAATTTCTAGCT
GATAAATTATATGATATTCAACCGCCCCCTCA
AAACAGTTCAGGAATCGTCA
GAGACAGTCAATTTTTATCACCATCAAATGCCGGAGATTTTTGGGTAGCTAT
AAAGATTCAAAGGAAGTTTCATTCCATCTAAA
ATAACAGTTGAAATATGCAA
TGCTGTAGCTCTTTTTAACATGTTTTATTCCCAATTCTTTTTTGCGAACGAG
CAGAAGCAGGTCTTTACCCTGACTTAATTGCT
GAATATAACGGATGGCTTAGAGCTATTATAGT
AAGCGGATTGTCAAAAATCA
TAACGGAACAATTTTTCATTATTACAGATACATAACGTTTTTCCAAAAGGAAGATAAAAACCATTTTTAAATAGCGAG
GGGAAGAAAAGCGTTTTAATTCGAGCTTCAAGTCAGGACGTT
TAAAACGAACGCCCGAAAGACTTCAAATATCATCTACGTTAA
AAATTAAGCAATTTTTTAAAGCCTCATTGCGGGAGAATTTTTGCCTTTATTTAATCAGAAAAGTTTTTCCCCAAAAAC
TAAATCATACGGTAACGCCAGGGGTAGCAACTTAACATCCAA
ATTCTACTAATTTTTTAGTAGTAGCAGGCTACAGAGGTTTTTCTTTGAGGAC
AAATGGTCAATTTTTTAACCTGTTTATTTGATAAGAGTTTTTGTCATTTTTG
TATGACCCCTAAATCGGTTGTACCATTAGATA
CATTTCGCTAGATTTAGTTTGACCAAAAACAT
TAGCATGTCATAGAACCCTCATATATTTTAAAATCGTAAAAC
ACGACAGTATCTTTTTGGCCTCAGGACAAAGCGCCATTTTTTTCGCCATTCACCAGCTGGCGATTTTTAAGGGGGATG
GTAACCGTGCATTTAAATTGTAAACGTTAATATGGGCGCATC
CTGGTGCCCCAGCCAGCTTTCCGGCGACCTGC
TCCATGTTATTGTGTCGAAATCCGCACCGCTT
GGCCTCTTCTGTTGGGAAGGGCGAAAGAGGCA
AAAGAATACACCAACCTAAAACGATCGGTGCG
TTACCTTATGCGATTTTTAAAGACTTTTTCATGAGGAATGAA
AAATCAACGTAACAAAGCCCCCAGCGATTATACCAAGCACCC
TACCTTTAATTGCTCCTGCTATATTTTCATTTGGGGCGAGAG
AATTAGCATGCTGCAAGGCGATTAAGTTGAGGCAAGGCAAAG
TAATACTTGAGCATAAAGTG
CGGTTGATCAACGCAAGGATAAAAATTTTATCATATGTACCC
AGGGGACGAGGAAGATTGTATAAGCAAATATCTGCCAGTTTG
AAACCAGGAGATCGCACTGG
CTATTACGGGCTGCGCAACG
TTCAACTTTAATCATTGGTTTCCATTAAACGGGTAAAATAAT
AGAACCGGATATTCATTGCGAAACAAAGTACAACGGAGGACA
ATTCTCCGTGGGGAACCGAACTGACCAGACGG
AAAGGCTATCAGCTCATTTTTTAACCAAATTT
GTACGGTGTCTAGGGTGAGAAAGGCCGTAGGT
CAACAGGTCAGGATTAGCGAGCTGAAAAGGTGGCATCAACTC

Table S8. Scaffold sequences for the individual toroid designs. Template was M13mp18 ssDNA. Primer locations are underlined and phosphorothioated nucleotides shown in bold.

1536 nt for the 38 nm toroid

gacgggttgttactcgctcacatttaatgttgatgaaagctggctacaggaaggccagacgcgaattatttttgatggcgttc
ctattggttaaaaaatgagctgatttaacaaaaatttaatgcgaattttaacaaaatattaacgtttacaatttaaatatttg
cttatacaatcttcctgtttttggggcttttctgattatcaaccggggtacatatgattgacatgctagttttacgattaccg
ttcatcgattctcttgtttgctccagactctcaggcaatgacctgatagcctttgtagatctctcaaaaatagctaccctctc
cggcattaatttatcagctagaacggttgaatatcatattgatggtgatttgactgtctccggcctttctcacccttttgaat
ctttacctacacattactcaggcattgcatttaaaatatatgagggttctaaaaatttttatccttgcgttgaaataaaggct
tctcccgcaaaagtattacagggtcataatgtttttggtacaaccgatttagctttatgctctgaggctttattgcttaattt
tgctaattctttgccttgcctgtatgatttattggatgttaatgctactactattagtagaattgatgccaccttttcagctc
gcgccccaaatgaaaatatagctaaacaggttattgaccatttgcgaaatgtatctaatggtcaaactaaatctactcgttcg
cagaattgggaatcaactgttatatggaatgaaacttccagacaccgtactttagttgcatatttaaaacatgttgagctaca
gcattatattcagcaattaagctctaagccatccgcaaaaatgacctcttatcaaaaggagcaattaaaggtactctctaatc

ctgacctgttggagtttgcttccggtctggttcgctttgaagctcgaattaaaacgcgatatttgaagtctttcgggcttcct
cttaatctttttgatgcaatccgctttgcttctgactataatagtcagggtaaagacctgatttttgatttatggtcattctc
gttttctgaactgtttaaagcatttgaggggggattcaatgaatatttatgacgattccgcagtattggacgctatccagtcta
aacattttactattaccccctctggcaaaacttcttttgcaaaagcctctcgctattttggtttttatcgtcgtctggtaaac
gagggttatgatagtgttgctcttactatgcctcgtaattccttttggcgttatgtatctgcattagttgaatgtggtattcc
taaatctcaactgatgaatctttctacctgtaataatgttgttccgttagttcgttttattaacgtagatttttcttcccaac
gtcctgactggtataatgagccagttcttaaaatcgcataaggtaattcacaatgattaaagttgaaattaaaccatctcaag
cccaatttactactcgttctggtgtttctcgtcagggcaagc

2119 nt for the 49 nm toroid

gacgggttgttactcgctcacatttaatgttgatgaaagctggctacaggaaggccagacgcgaattatttttgatggcgttc
ctattggttaaaaaatgagctgatttaacaaaaatttaatgcgaattttaacaaaatattaacgtttacaatttaaatatttg
cttatacaatcttcctgtttttggggcttttctgattatcaaccggggtacatatgattgacatgctagttttacgattaccg
ttcatcgattctcttgtttgctccagactctcaggcaatgacctgatagcctttgtagatctctcaaaaatagctaccctctc
cggcattaatttatcagctagaacggttgaatatcatattgatggtgatttgactgtctccggcctttctcacccttttgaat
ctttacctacacattactcaggcattgcatttaaaatatatgagggttctaaaaatttttatccttgcgttgaaataaaggct
tctcccgcaaaagtattacagggtcataatgtttttggtacaaccgatttagctttatgctctgaggctttattgcttaattt
tgctaattctttgccttgcctgtatgatttattggatgttaatgctactactattagtagaattgatgccaccttttcagctc
gcgccccaaatgaaaatatagctaaacaggttattgaccatttgcgaaatgtatctaatggtcaaactaaatctactcgttcg
cagaattgggaatcaactgttatatggaatgaaacttccagacaccgtactttagttgcatatttaaaacatgttgagctaca
gcattatattcagcaattaagctctaagccatccgcaaaaatgacctcttatcaaaaggagcaattaaaggtactctctaatc
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cccaatttactactcgttctggtgtttctcgtcagggcaagccttattcactgaatgagcagctttgttacgttgatttgggt
aatgaatatccggttcttgtcaagattactcttgatgaaggtcagccagcctatgcgcctggtctgtacaccgttcatctgtc
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gatttcgacacaatttatcaggcgatgatacaaatctccgttgtactttgtttcgcgcttggtataatcgctgggggtcaaag
atgagtgttttagtgtattcttttgcctctttcgttttaggttggtgccttcgtagtggcattacgtatttttacccgtttaat
ggaaacttcctcatgaaaaagtctttagtcctcaaagcctctgtagccgttgctaccctcgttccgatgctgtctttcgctgc
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ttgttgtcattgtcggcgcaactatcggtatcaagctgtttaag

4069 nt for the 62 nm toroid

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ctattggttaaaaaatgagctgatttaacaaaaatttaatgcgaattttaacaaaatattaacgtttacaatttaaatatttg
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gagggttatgatagtgttgctcttactatgcctcgtaattccttttggcgttatgtatctgcattagttgaatgtggtattcc
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ggcacttatccgcctggtactgagcaaaaccccgctaatcctaatccttctcttgaggagtctcagcctcttaatactttcat

gtttcagaataataggttccgaaataggcagggggcattaactgtttatacgggcactgttactcaaggcactgaccccgtta
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gaataccggataagccttctatatctgatttgcttgctattgggcgcggtaatgattcctacgatgaaaataaaaacggcttg
cttgttctcgatgagtgcggtacttggtt

4846 nt for the 74 nm toroid

gattacgaattcgagctcggtacccggggatcctctagagtcgacctgcaggcatgcaagcttggcactggccgtcgttttac
aacgtcgtgactgggaaaaccctggcgttacccaacttaatcgccttgcagcacatccccctttcgccagctggcgtaatagc
gaagaggcccgcaccgatcgcccttcccaacagttgcgcagcctgaatggcgaatggcgctttgcctggtttccggcaccaga
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5904 nt for the 90 nm toroid

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ttgctgctggc

Table S9. Staple sequences for the 38 nm toroid design.

AGGATTAGAGAGTCATAAATACGAACTA	GTGAGCGAGTATTTGGGGTAAATCGGGAAGTT
CCTTTTGATAAGAGTTAAACAAAAGATTC	TTTTAACCAAAAAAAAGGCGCCATCATACTGC
CACTATCATAACCCGTCCAATACAGGCA	ATGATATTCCATAAAGCCGCGAGCTGCGAGAG
ATGGCTTAGAGCTTATTCACATTCAAC	GGCATCAACTGTAGCCAGAGCTGATAAATTAATGCCG
CAGACCGGAAGCATGACTAATAAAAAT	ACACCAGAAAAATGGTCGTAATACTTCAAAAA
CGACGATAAAAACAATAGTCAATAAAG	TGCAATGCCTTATTTCAGTTTGACCAGCGAAC
GCTTTTGCAAAAGCCCGAAATTGTACCA	ACGGAACAGGTTGATAACAAAAACAGGAAGATTGTA
CGTTTTAATTCGAGGATTGCATTTGCGGG	CATTTCGCCGAGTAGTAGTAGGTAAAGATTCAAAA
AAACGAGAATGACACCTTTATGGAAGTT	CTGGCCTTCTTCTACTAAAATTAAGAAAATGT
TTAGACTGGATAGCTCGTTAGCATTAA	AGAAGCCTTGAGTAATGTAATTGGGCTTGAGATGGTT
GGAATCGTCATAAATAAAGTAGGCATTTG	TAATGCAGGCCTGAGAGTTGTTAAATCAGCTCATT
CAGAAGCAAAGCGCTTCAAATTAGATA	CGAACGAGCTTTAATCATAACCCTCATATATTTTAAA
CCCCTCAAATGCTGTCATTATGTTTTA	TAAGCAAATACTAAAGTACAGGTAGGTTCAGA
GATTAAGAGGAAGACTTCAAGTTTAGCT	CTGAATATCATTAAATTTTCTGGAGCAAACAAGAGAA
TCAGGTCTTTACCCAACTCCACAATTCTG	AAAACATTGGCCGGAGATCGCTTGCCCTGACGAGAA
TTGCCAGAGGGGGTCAAAATAGAAAAGGT	CATCCAATTAGGAACGCAGAGATCTACAAAGGCTAT
GGGTGAGAAAATGACCCTAATAACCTATATCG	TCATTCCACTGAAGCCCTCAGAAGCTCATTATACCA
TATGTACCCCACATTATTACGGTGTCATTGCT	GTCAGGACGAATCTACGTTGATTCCACAGGTC
TTTAGAAATTGGGATAGTGTGAATTACCTTATGCG	CCTCAGAGAACCGTTCTCTTTCATCAACATTAAAT
CAGGTCATTATACATAAAATTACGAAGAGCAA	GAGAGGGTAAATTAGCAATAGTAGTTACCAGA
TAAAATTCGAATGCTGTGAATACCATTGAATC	ATATTTTCAACAACCCGCAGTCAAATCACCATCAAT
TAATTTCAATAGATTTAACGCAAGGTTATAGT	AGGCAAAGGCTATTTTTGCATCAAAAATAATTCGCGT
ATCAGTTGGGTAATCGTAGTAAACGTTAATATTTTGT	AATATGCAATTTAAATTAAACTAGCATGTCAATCA
ATTTTAAGAATATAACAGTTAATAAACAAAAA	TCGATGAACAGATTTAGAGCTCAACTTTGCGG

Table S10. Staple sequences for the 49 nm toroid design.

TTAATAAAACGAACCTCATTCATTTAGTT	ACATTATGAATTGCTGAAGCGGATTAGTACAA
GAGGCATAGTAAGAAAGAGGATCATGAGG	ATCGCGTTCAAAAACAGTAAAAATTTTTAGAACCC
AGATTTAGGAATACAGAGTAACGAAAGAC	TTTAGACTCCATCGCCCTTTAGCTATATTTTCATT
CGATTTTAAGAACTGGGCTTGAAAGTACG	GCAAGGCAAAAGTTTCAAAAACGAGTATACCA
CCTAAAACGAAAGAATAAGGGCTCCAACA	GAAGTTTTAGGGGGTAGCGGAGACTTGCAGGGAGT
GCAGATACATAACGAGGCGCAGGCTACAG	TGACAACAAGGATAGCGTTCGCAAACAAATCA
TATTACAGGTAGAACATTACCTGGTCAAT	TATTTAAATGAGGAAGCTTTTTGCGGCGACCT
CGGAGATTTGATTGAATCTATCTGTAGC	AGACCGGAAATCATATGATGCCTGAGTAATGTGTA
CTAAAACACTCATCAGCCGGAATTGCTCC	GGTAAAGATATTAAACGGTAATGCCGCACCAA
GTCAGGACGTTGGGCGAGAAAAACAGTTG	TATAGTCTTTTTGTATCGGTTGTACCAAAA
ATTATACCAAGCGCGAAATCCGATGGCTT	AACCGGGACTTTTGCTGACGCATAACCGATATATT
TGATAAATTGTGTCGAAACAAGCATCAAA	TCAACATGTAAAGCTAATAAATCAGCTCATTTTTT
TTAATTTCAACTTTTGAATTACTGACTAT	TGGGGCGCGAGATACATTCCAATACACAACAT
ACGAGTAGTAAATTGGCTCATAATGACCA	CAAAGGCAATAGCGTAGCAACTAGGCTG
GCAGACGGTCAATCGGCAAAAAGCGAACC	AGCATCGGAATTAATGCCTATTTTTGAGAGATCTA
AAGGCTTGCCCTGAAAGAAAACCTCAAAT	GCTTTAAAGTAGCCAGCATCCAATAAATCATACAG
ACGGTGTACAGACCCCAAAAGCGACGATA	TTTTGATAACGCAAGGAGAAGATTGTATAAGCAAA
CTGACCAACTTTGAGCAGAAGACTACACA	CTATCATAAGAATCGATTGAGAAAGGCCGGAGACA
GCTGACCTTCATCACACATTCTTGCAAAA	AACCAATAGAAATCAGGATGCAACTAGATGGT
AAGAACCGGATATTAGATTCAGTAAAATG	GTCAAATCAAGGACTAATTACCAGAGAATTAC
ACGTAACAAAGCTGTAACGGATGCGGAAT	GGTCAGGATTAAATGCATACCCCGGTTGATAATCA
GCTCCATGTTACTTTTTGACCCTTCAAAT	GCAAACAAGACCCTCGTAGACTTTTCAGATGA
GAAAAGCCCTTAATTCGTACCTTTAACGAGGC	CGCATTAAAAGAAGCAAATATAATGCATCGCC

TGACCATTAGCTGAAAATCATAGTTGCGCCGACAA
TCATATATTTTAGAGAGAGCTTCAAGAATACA
TTTATTTCAAGAGGTCACCGAAAGACCCAGCG
GTGTCTGGAGAATTAGCCAAAAATAATTCGCGTCT
ATTCCCAAAGCATTAACTTTCATCAACATTAAATG
TAAAGGCCGTCGTCACCGGGTAATATCAGTTG
AAAACCAATATCAGGTCTGATATTCAACCGTTC
CGGTCGCTGGCCAGAGGCTCAGCAGTCTTGAC
CTCAGAGCATTTTAAATTCTTTACCCCTTATG
GGCCTTCCTCAGTTCAGTTCCATATCACCAGA

TAAATCAAGAACGCCATAAAATTAAGCAATAAAGC
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TAGCATGTCAGCAATACGGTAAAAAAAACCGAA
AATAGTAGTTTCTGCGATGAATCCCATCTACG
AGGCTTTGCCATCAATAATTGCCTGAGAGTCTGGA
TAGCTGATAAACGAGGGAGAGGCTTAACTAAT
AGAGCTTACCCTGTAATTAATATTTTGTTAAAATT
TGAGCGAGTATATTCATACGAGTAGAGTGAAT
CGTCATAAAACAACCCGGGTGGCATCAATTCTACT
AAGATTAATGTAAACGTACTTTTGCGGGAGAAGCC

Table S11. Staple sequences for the 62 nm toroid design.

GCGCGAAACAAAGTAGTGCCGTAAGGGA
TATCATCGCCTGATCGGGGTTCGAGGAA
TGCTTTCGAGGTGAATAGAAAATACATA
TTCCATTAAACGGGGAGCCACACCATTA
TTAAAGGCCGCTTTCCTCATAAACCGAT
CCACTACGAAGGCAACCGCCAGAAACCA
CCTCAGCAGCGAAAACAACGCACGGAAA
CAACAACCATCGCCGAATTTTGTCACAA
AAGAGGCAAAAGAACAGGAGGAGCGACA
AGGACTAAAGACTTCAAGCCCAGAGCCA
AGCCTTTAATTGTAAATAATAACGCAGT
ATATTCGGTCGCTGTTTGTCGACCAGCG
TTGATACCGATAGTAACTTTCCGCAAAG
CATCTTTGACCCCCTAGCCCGAGACTGT
GGCTGAGACTAAAACCAACCTAAAGAAC
GAGGGTAGCAACGGACACTGAACCGTCA
ATTCCACAGACAGCTGCGGGAGCCTTGAT
TAACGATCTAAAGTAGGCTTGATTAAAGC
TGTATCACCGTACTTACACTAGTTTGCCA
CACCAGTACAAACTGACAGCAGACAGGAG
GGATTTTGCTAAACTGCGCCGGGCTTTTG
AGGATTAGGATTAGAAAAAGTCATGATTG
AGACGTTAGTAAATCACGCATGAATTTAC
GTACCAGGCGGATAACAACGGCATGTTAC
TCAGCGGAGTGAGAATTTCTTATAAGTTT
CCGCCACCCTCAGACCAACCTGGAACCAG
CTAAAGGAATTGCGTCGGTTTAACAGTGC
CACGTTGAAAATCTAAGGCTCCTGCCTAT
AACCGCCACCCTCATAAAATACTCAGAGC
ATTTTCAGGGATAGTTTCATGCTCAGAGC
ACCCATGTACCGTACTACAGAAGAACCAC
GGTTGATATAAGTAAGCGATTGGCATTTT
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ATGATACTTTAAACAGAGATACATATATTTCAAC
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TGTCGAATTTAGCTATCCTTTTTAAAATAGTAGT
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AGTTTGACGAACGAGGGTCAATCATCGAGAG
TAGTAAAAAACAGTTATCCTTATTATTTTTT
CCGTATATGTTTAGACGACGACGATCTCAGAGCA
ACACCACGACTAATGCTTCAGAAAGTCAAAAAT
ACCTTTAACTCAGAGCTGGGAATTAATAGGA
AGGACAGATTTCGACGCGCAATCATACCAA
GGCTGGCTGTTTGCCTTATTAGCAAACACT
ATGTTAGCGTTTACCATGGATAGCTAACTGAAC
TCAAATGCAGGAGTGTAAAAGAAAAACAGTT
GAAAATAGCAGCCTTTACAGAAGCCTTACGCCAAA
AGATTAAGAACAAATACGACATTCGTTAGCG
GTTGAGGATTCGAGCTTAAGAACTGTGATAAATT
ATTACCTTATAAAGGTCAGCATTTCGGAAC
ACCATAAAGTAAGCGTTTTATTTTCTGTATG
CTGTAATACTTTTGCGGGAGAGAGAATGAATCCCCC
AATGCCGGAGAGGGTAGCTCAAGATTAAACCAGACC
TCTTTTCGTGTCTGGACAAGAGTAAAGCAAATAT
AAATCAGATATAGAAGGCCGGTAATCGTTGCCCTG
AAAGGCTATCAGGTCATTAACCTCCCGTTAGAGAGT
CCGACTTGTTAATTTCGGTCAGGAACTTGCGGG
TGAGGGAGGGACGTTGGAAAGACTATCTTACCA
GCAAGGCCCATTCAGTCTTAATTGATAGCAAGC
CAAGAAACCCTATTATAATACCCACAAGAGA
TGCGGAATGTCAGTGCGAAAATACGGAACAA
AAAGTACGATAATCAATAATCAGTTTTAGTA
ATTCACAAGGAAGCCCGGAAGAAAACAAATCACC
ACGCAATAACCGAAGCATTCCCAAGTTAAGTTC
ATAACCTGATCCGCGAAAGGAAACTTGCTCA
AAGCCCCAAAAACAGGAAAGCAAGCCGTATGCAACT
TTGCGGATCTCAGAACGCACCATTCACCCTC
CGTTCCATCAAAAATCGTTGAGATTATATATTTT
ATTATAGTGAAAGCGCTATGGTTTTCTTTCC
ACGCTAACGAGCGTCTTTGGAGACAGTATCTACGT
CCACATTCAGAATAAGCATACATACAATGA
TTCGGAAAATAGAAGTTGCAAAGAAGCAAGGCAA
AAAAGGGTGAGAAAGGCCCCAGAGCCTGCATCAAAA
CACCAGAACTCCAACAAACTTTAATGAGATCTAC
AGGAATTACAACATATACTGGTAAAACAGC
ACAGGGAAGCGCATTAGAACCAAAAACACACTATC
AGCAGATAGTTACCAGCCTGCTCAGATTTG
CGCGTCTGGCCTTCCTGTCAGCTCATTTTTGAAAG
GCAAGGATAAAAATTTTTTTGTTTAACACGAGAATG
CCGCACTCATCGAGAACAGATTGTATATCTTGACA
GAATCAAGACCTTCATAGTTTCATAACCAAGTA
CAAATAAGAAACGATTTTAGAACCCTCTAGGAATA
TAACGGGCGTCATAAAGTAAGAGCAATTATGACC
ACGCGAGGCGTTTTAGCGGCCTGAGAGATTGGGCT
TTGCACCCAGCTACAATTCGTTCTAGCGCTCATTA
AAAGCTGCTGGAAACGCGCCTCCCGTAATG
ACCCAAAGCCGAAGAACATTTCGCAAAATAATT
TGGCATGAGAGGCTTTTTTGCCAGAGCGCTAAT
TCGATAGCTATTCATTGTTTTAAATTTTTATTT
CGGTCATTCCCAATTCTACAGACCAAAAATTCGC
ATTAAATTTTTGTTAAATAGCCAGCTTAGTAGATTT
AAATAGCGATTAAGACATGCCCCCAAAAGG
ATCAATATGATATTCAACTTATCCTGATCAAATATC
AACAAGAGAATCGATGAATTATCCGGTAGGTCATTT
GCAAAATCACCAGAACTTGATAAGATTCTAAGA
CATGTCAATCATATGTACCCGCGCCCACTGAATATA
ATCAGAGAGATAACCCACATCATACAGATAGCAAT
TGAGATGGTAGCCATTCGCCACCGGCTTTG
TTAGCCGCATTAGATACTGACCAACTTTTAACCA
ACCCTGAACAAAGTCAGACAATAAAGCAAAAACCA
AGCGCGTTGAACGGTGTGCGAACGTCATCAACA
AGCATTAACATCCAATAAAAGAATTGATAATAAGAG
GCGTTTTACAGGTCAGAAATATTGCTGTAGC
TAATAAAACAAAAGGGAATCCTCCAGGGAG
CACCACCTTGCTCCTTGAGTAGTAATCTGGAGCA
AGAATTAGCAAAATTAAGGGGTAATTGAGGGGGTAA
AGGTTTTGAAGCCTTAAATATTTTTGACATTGTGA
CGCCACCGGCTTAGAGGAATAAGGCTAAAACTAG
GTTACAAAATAAACAGCCATGTGTAGGTTATTACA
ACGAGAAACACCAGTACGCCACCAGGAAGT
TTAAATTGTAAACGTTAACAACCCGTCTCCATATAA
AAGGTGGCGAGGCATATATTCATTAACATAAAA
TTATTCATTGCGATTTTCAAAGCGGTTGCTATT
TACCAGTCAGGAAGGTACGATTGTCGTCAC
AAATGCAATGCCTGAGTAATATTATTTACCCTGACT
CAGTTGATAGCCCCCTTTAGCGTCGAATAGG
GGTAGAAAGAAATTCAAGTCTCTAACCGAT
AGCTATCTTATAACGGTCTGAAAATTAAGA
CCAAAGACGAACTAACAGCGGATTAATTTGCCA
ATTTGGGGCGCGAGCTGAAATTCTACTGAAAAGTA
AGAACCGGAAGCACCGAATCACCAAAACGA
TTAAATGTGAGCGAGTAATATTTTGTTGGCGCATA
ATAGGAACGCCAGGCATCAAAGGTTCAAAATGGTCA
CAGAATGCAGAAGCAAGGAACAACATAAAGATTC
TCAATAGAATTCATCAAGGTCTTTATCCCAATC
AGCCACCGCTCAACATACCCAAATCTAATCAGAA
TCATCGTAGGAATCATTACCCGGTTGAAACGTAAC
TAAAGCTAAATCGGTTGTCGGGAGAATGTCCAATAC

Table S12. Staple sequences for the 74 nm toroid design.

AATAAACAGCCATATTATCTCATTTTTCTTTTGAT
GGAACGCCATCAAAAATACTAATTTGCTTAAATAT
TAAAACTAGCATGTCAATGGTTGATAACTACGTTA
AATTGTAAACGTTAATATACGTCAAAAAGCCTTTA
TACCGACAAAAGGTAAAGGTCGACTCTACATAACG
GAAACGATTTTTTGTTTATTTGTTAAATAAAAACA
AAAAATAATATCCCATCCGGCGAAAGGTTTTGCAA
ATAGAAGGCTTATCCGGTCGTGCATCTACCCTGAC
CCAAGAACGGGTATTAAACCATTCGCCACTGCGGA
AAACCAATCAATAATCGGGCGATCGGTTAGTAAAA
TCTGGAGCAAACAAGAGATGTATAAGCATTATACC
TGCAAGGCGATTAAGTTGAGATAAGTCCCTTTATT
AGGGGACGACGACAGTATATAGCAAGCAGGTGGCA
GCGCAACTGTTGGGAAGGCTGTCTTTCGAGCATAA
ACAGTAGGGCTTTACCCCCATATGAATATGCCGGA
GTTTTAGCGAACCTCCCGGATAGGTCATTGCATCA
GACAATAAACAACATGTTAAAACGACGAAGAGCAA
GAGAACAAGCAAGCCGTTCGGCACCGCCCCCTCAA
CCGGGTACCGAGCTCGAATAATAAGAGGAAAGGCC
CGAGCGTCTTTCCAGAGCATTCGCGTCAGGTCAGG
TTCGCTATTACGCCAGCTTAATTTACGATTATGAC
AGTAACAACCCGTCGGATGTTGCTATTTCTGCGAA
GAGGCATTTTCGAGCCAGTTCGTAATCATTTAGGA
TGTAGCCAGCTTTCATCAGAATCTTACGTTTCATT
AGATGGGCGCATCGTAACATTCTAAGAAGCTATAT
GCGGATTGACCGTAATGGACTTGCGGGGATACATT
GCACTCCAGCCAGCTTTCTTTATTTTCATTAACAT
AAGCTTGCATGCCTGCAGTAATTCTGTAGTAATGT
AAATTTTTGTTAAATCAGTTATCCCAATTGCTGAA
AGCCTTAAATCAAGATTATCTCCGTGGGACTTCAA
TCCCAGTCACGACGTTGTCAGCTAATGAGAACCCT
CCCCAAAAACAGGAAGATATCGATGAATACAAAGG
ATACAAATTCTTACCAGTCAACATGTAATGATATT
CGGAAACCAGGCAAAGCGCCAAGTACCAAGAATTA
CTTGAGCCTTCAACTAAAGGGTGAAATATAAAG
TTTTCATATTAAATGCGGCATAGTGCCAGTGCC
GCCAAAGACAAAATAGGGGAGAAGCTGAACAAG
GGTAATAATTTAGTTTGCCCGAAAGAACAAACG
ACTTTAATCGTTGGGAGAGAGATCCGGTAATCG
CGCCGCCAAAGCAATACGTCCAATATTCAGGCT
GTAACAGTACAGTTGACGAGCTTCATGTGAGCG
CCAAAAGACAAAAATCAGCTGAAAAAATCAGAT
TGAGGGAGTAACCCTCAAATTTTTCAGAACGCG
TCAGACGATCATACAGATTGAATCTTCTGGTGC
TTACGCAGAACAGTTCGTAGTAGCATCGTAGGA
ATAATAAGGGAAGCAAGTCTGGAACAACGCTAA
CAGTCTCTGGGGCGCGAGGTCTTTGCCAGTTTG
TAGCTATCTTTTAATTTTCCCAATTTGCACCCA
CTCAGAGCCGGTTGTAGGGGGTAAGCGGGCCTC
AGAGATAAGTACCTTTAACATGTTCAGTTACAA
GTCAGACTTCAAATCACAGTTGAGATATGCGTT
CGGTCATAAGATTCAAATGCAGATAGAGGATCC
TTAGACGGCATTAAGATGAATAGCATGAAAGAG
ACCACCGGAAGGATAAGTTTACCACCAGGGTTT
ATACATGGGGTCAATAAAAGCGGACGTTGGTGT
CCCTCAGAACTTTTGCCGAGAGGCGGGATGTGC
TCACCAGTAGATTCATCCATCAATATTTAGGCA
CAAAGACACTGGATAGAAGCCTCACTTATCATT
CAGTAGCGTCTAGCTGGAACAACACAACGCTCA
CAATCAATTTGCCAGACCAAAAACAGCATGTAG
ATTCATTAAATTACGAAATGCCTGCCAGACGAC
AGAAGGATATAGCTTAGGATGACAATTCGCATT
TGACGAGATCATTGCCACTGGCTCAAATATTTA
GGCTTGAGCTATTTTTAGAAAAATTCAGAAAAG
TAAATCCTTACTAATAAGAAAACGAGGAAGATC
AAAGTCAGATTTTTGCGAGCTTAATCCAAATAA
CGGAAACGAACTAACGATAAATTATGAGAATCG
ATACATAAAAATATTCGCAAGGCAGCACTCATC
CCTGCCTAAGTACGGTACTCCAACTGGCCTTCC
GAAACCGATCAGAAGCACCTGTTTACGCGAGGC
GTAAGCAGAAGAGGAAGACCATTAAGGTTTTGA
TGAAAGTTGTAGCTCAATTGCTCTAACCAATA
ATACCACAATTTGGGATTTTCATCCCAGGCG
ATGCTTTATATGTTAGGATATTCAAACACTC
CATATATTATCAAAATAATATTGATTTCGTC
CCTGTAATACCGCCACCATATGGTTTAGCGT
TGTTTAGACCACGGAAGAACCACCGATTTGT
AGGTAGAAAGCACCATCAAGTTTGCAAGAGT
CAGAGAGATAGGATTAACTGAACACGAGAGG
CCAAAAGGAAGGTGAAATTAGCGTGAAAGAG
ATAAAACGTCACCAATATAGCAGCTTCATTA
GCAACTAATTTCGGAAAATTGAGTTGCGCCG
TATAATGCATTAAGAGTGAGCGCTCACGCAT
CTATCAGGAACACCAGATTACCTTAATAGGT
ATAAAAACCAAAAGGGTCCCTCAGTTAGCCG
CCAATAAATTGGCCTTCAAACGTAACAGTTT
AAGAAGTTAGAAAATTCCTCAGAGTCGAAAT
CCATAAATACTGGCATGCCAGAATAAACGAA
CACTATCAGGAAGGTACACCGGAATCATAAG
AGTCAGGACATTGTGAAACGAGTATGCTCAT
AGCTAAATCGCCACCATAAGTTTACTTTCCA
AAAAGATTATAGCCGAGATACAGGGGAAGTT
GCAAAATTGCATTGACACATATAATGTATGG
GGGAAGCGGAGAATTAGCGGGGTTACCAGGC
TCGCAAATCTTTTGATACAAAGTTAAGGCTC
GTAGGTAAGCCCCCTTTTATCACCATAGGAA
GGAGACAGGTAGCGCGATTAGAGCACCCTCA
ATTAGAGACCCACAAGCCTATTATCGTCACC
GAGGGTAGATGCATCGGAAACGTTTTAGTAC
TTTCATTTGAATTTACAATAATAATTTTTTC
AAGAGGTCAGGGTAATGCTGAGACAGGGAGT
ACCAGACCAGCAAGAATAAACAGTCGGAACG
ATATCGCGTTACCGAAGGGGTCAGGCTTTGA
CGAGTAGAGTTTTAACGCCCTTTTTCGGTTT
ATCGTCATAGGTGGCAAGGAGGTTTACCAAG
TATTATAGGGAAACGCCGTTCCAGGTAATGC
CCATATTTAACAACGCATAAAGCTTATTAC
ATCATTACCGCGCCCACGGCCTCAGAATGA
CCTGTTTATCAACAATGGTAACGGACGACG
GCTACAATTTTATCCTACATTAAAAAGCGA
TCAATTCCATTAAAGATTAAGGAACAAC
CCATATAGCCCGTAACAATGAATTTCTT
CAACCGTACAGAATTACCATTCCTCAGA
TCAACGCAACCGCCCGACATTTGTAGCA
ACAATGACAACAACGCGGGATTCTGAAACA
ACCGCCACCCTCAGCCTTCATCCTTTAGC
AAACAGCTTGATACACAGCATTAATGCCC
GACGTTAGTAAATGCAACGGAACCAGAGC
CCCATGTACCGTAACAACTTTTTGCCATC
CGCCACCCTCAGAACCGGATAACCGTAAT
CAAAAGGAGCCTTTTTCATGAAGTGTACT
TAAAGGAATTGCGACAACCTAGGAAAGCG
ACCAGTACAAACTACGGTCAACCAGAGCC
AACGATCTAAAGTTAATTGTGCCACCACC
GATTTTGCTAAACAGCGATTAGAGGCAGG
ATCAGCTTGCTTTCTACAGAGTGCCTTGA
CAGCGGAGTGAGAAACACTAACAAACAAA
AACCGATATATTCGGGCTTGCTCCTCAAG
TTCCACAGACAGCCATGTTACAGCCGCCA
GTTGATATAAGTATTTGCAGTTTGCTCCC
TTTTCAGGGATAGCGTACAGAGGCATTTT
GTATCACCGTACTCACAAAGCGTAAATTG
ACGTTGAAAATCTCAAAATACTAAGCGTC
GGACTAAAGACTTTAATTGTATAAGAAAA

CATAGGCTGGCTGAAGCCACCCAGCAAAA
TAAAGGCCGCTTTTCATCGCCAATATCAG
AGGGTAGCAACGGCGAGGTGAAATAGCAA
AGAGGCAAAAGAATTAGAAAGACTCCTTA
CACTACGAAGGCACATAATAACGGAATAC
ATCATCGCCTGATATTGTCGTTTTTGTCA
GAACGAGGCGCAGACAACGCCCAACCGAT
AATCTTGACAAGAACCGCCACAGCAAGGC
CCGCGACCTGCTCCCTCATAGTTACCAGC
CTCAGCAGCGAAAGCGATAGTTAAGCCCA
TCAGTGAATAAGGCAGCCCGGATGCGATT
GGATAAGTGCCTGAGTCGCGTCCCTGAAC
GGAACCGAACTGACCACTGAGCGGAAATT
CCCAAATCAACGTAAGGAGGTTAATTTCA
ATCTTTGACCCCCAACTTTCAGAAAATAC
GACAGATGAACGGTAAGCCCAGTCACCGA
TCCATTAAACGGGTCAAAAAAACCAGAAG
CGCGAAACAAAGTAAATTTTCAAGAAACG

Table S13. Staple sequences for the 90 nm toroid design.

ACCAGGCAAAGCGCCATTAGTTTCATTGTTTAGAC
GCTTGCATGCCTGCAGGTGAGCTTCAAAAGATTAA
TTCAAATAGAGAGGCTGAGGCAAAATCGCACTC
CTAAAACACCACCCTCTTAATTTCATCACCG
AAAATAGCTATTTTAGATTTTCAGTTATCCCA
AGAAGCAACTTAGAATAGTTTCAGTAAGAACG
AATGCTGTAGCTCAACATCGGTGCGTGAGGACT
AAAATCAGGATTAAGATCTGTATGCCGACTTG
CAGTCACGACGTTGTAAAAACAGGTCATTATAGTC
AAAGCGCAGATGAATATGTTTGGATTTTTGAGA
CCGGAATCATGCAGATAAATTGTGCGGCGGATT
AACTGTTGGGAAGGGCGATGTTTTAAATCGTCATA
AGTATCATCAGTTGAGTTACTTAGGAGTAACAA
TTGAGGATTTAGAAGTATCAAATATCTTTCGAG
TTCAACTAATAATTACGCCCGGAAGGAAGCGC
TCACCGTACGCCTGATACATAACGTAGTAGTAG
TATATAACGTCCAATAGGAAGTTTAGGCTGCGC
TGGATAGCTATATGTACAAACTACCTAACGAG
GGTTGATAGCTCCATGATTTAGGACAAGGCAAA
CTGACCAAAGGATTAGAACAGTAGCAAAGAC
CATAACCGAGGAACAAATCGTCGCCGTTTGC
AAAGTACGGTGTCTGGACGCCATTCCCATTAAA
AGGGAGTTCTTTCAACCCTTGAAAGGCATTT
AATCAATATCTGGTCAGTCGTCAATAGATTATCAT
ACAATTTCCACCAGAATCAGCTTGCAAACCCTC
TTTAAATGCAATGCCTGATATTTAAACCGGATA
CTGAATAAGCCTTGATATTTACCGGAGCATGT
GACGATTGTGGAAGGGCGTAGATTGAATCGATG
TAAGAGAAAATCATTGTGACAAGAATTGTAAAC
GTGATAAAGGCATAGTAAGTACAATGTAGATGG
CATTCAGTTAATAAGTGACGACAAAACGCAA
CGGGTACCGAGCTCGAATATCCTTTGCTAATTTA
AAAGACTTCACAGACACCGGCTTAACCAATG
CGCGACCTTAAGTATATAGAAAAAACCAGCG
CCTTGAGTCCAAATCATTGAGATGTAGGTAAAG
CCAACCTAAGGAACCCAACGCGAGAATTAGA
AGTTTCGTATACGTAAAGTAAAATCCATATAAC
CATTAACATCCAATAAAGGAACAAATCGAAATC
GAATAGAAATATATTCGCATCAAAAGCGAACCA
TCAATATATAAAGCAAACCTGAGCTGTCTTTC
AAAGAAACATTTGAATAGCCTTTACAAGCAAG
CGGGTAAACACCAGTAAATGCTGACCATTAC
TAACGATCACGGCTACCCCTCAAAGCTGAATAT
GCATCACCTTGCTGAACCTTAGACTTTTGCGGAAC
CAGCCAGCTTTCCGGCACGCGAACGAGAGAAGTTT
ACCTGTTTAGCTATATTTCTGCCAGAGCGATTA
AGCTTTCATCAACATTAACAATAAAGCAGGTAGAA
CCTGTAATACTTTTGCGTAACCAATATGAACGG
TAGCATTCTTTCATGACTGCGGAATATGCAACT
GAACTAACCAACGCTCGATTAGCGGAGCGCTA
TACCCCGGTTGATAATCAAGGCCGGAGACCAGAAC
AACCTTGCCCGAAAGAACAACCATGAGGATCCC
AACGGTAATCGTAAAACTTTCAACCGTTACCATAT
TCATCAAGGCCTATTTATTTTCGAAGTATGT
AGTACCTTTAATTGCTCTAACGCCAGGATCGTC
AGCGTCATAGAAATTGTTAGAACCTCTAGCTGA
TCAACTTTTATAAAGTCCGTATAATACCGAAG
GAGTAGTAGTCCAGACTTTAACGGAGATAGCC
GACCGTAATGGGATAGGTTTCTACTAACCAAAAGG
ACCTAAATGTTTACCATGACCCCCTTTGAGGGG
GGTTACCATTGAATTAGGAGCGGAATAATACAT
AAAGTTTGATAAATCATTGAAAATGAATCATT
GCTATTACGCCAGCTGGCAGCTTAATTTGCTTTAA
ATGTTCAGTTTCCTGACTTGCGCAAATCATATG
AACTAATAGATTAGAGCTGGCAAATGATTCGCC
TAACGTCAGTCTCTGAATTCACAAAGCATTG
CAAAATTACTAATGCATTTGATGAAAGAAAAA
AAAGGAATTGAGGAAGGTATCTTTAGGGCAATTCA
ACAGTTCATTATCAAATGTCGTCTATCAAGAT
TAACCCTCTTAATGGTTCAGAACCTTGTTTAA
GAAACATGCCAGGCGCCATTATACTTTATTTCA
ATAGCTTAGTCTTTACCTTTTGCGGGGTTTTCC
GGGTAGCATAAAGTTTATCATAGGAATCAGT
TAGTGAATGAAAACGAGACAGCATGATGTGCTG
GACCGGAAGCAAACTCCACGACGGCAGGCTTGC
GTGAATTTCCAAAAGGTACCTTTTTCCCTCA
ATTATTCAGGGAGAAAAGATGATGAGCACTAAC
ACTACCTTTTGAATCCAGAGGCTTGGCCTCTTC
AGTTGATTCCCAATTCTCGCTTCTGCGAAGGCA
AATATTCATTTAACCTGCCCTCATCTACAATT
CTCAGTACACGGTCAAATTATTACCTCAGAGCA
ATAAAGCGGAACAACTCATAAGGCCTGTAGCC
TGCCAGAGAGACAAAGATGTACCGGCCAGTTA
TAATTTAGAACTGGCTATAGGCTGAAATTTTTG
TCAAGAGACTTTGAAATCTACGTTCATTATGAC
TTCATTACAACAGTGCACCGACAAACTGGCA
ATTTTAAGGCAGAGGCCGGAACCTAAGAAACA
CAAGGCGATTAAGTTGGGCTTTTGATAATAAATCA
GATCTACAAAGGAAAATTATCTCTACAGTACCT
AATTACGATAAGGCGTGTTTAGTACCTTTACA
TGATTGCTAGTTACAAAAATTAATCACCCTC
TGCCCCCTAGTAATCTTGAATTACCTCATATAT
ACGCAAGGATAAAAATTTTCGCATTGCTGACCT
GTTAGTAACAGCGAAAGAATGACCAGAGGTCAT
ACCCTCAGATGAATTTCGCTGAGATTAGCGT
ATTCAAAAGGGTGAGAAGAAAAGCCAAGCTGCT
TGTACTGGGAATAAGGCGAGAAACACAGTCAAA
CAATCGCAGGGGTAATTGCCACTAGTGCCGGAA
TGTACAGAAAAGTATTTAACAACGCATAAAG
TGAGAATCAAGAAAAAGAGGACAGAGGAACGCC
CTCAGAACGCGAAACAAAGAGCAAGGCGCGAGC
TTAAATCAGCTCATTTTTGGAGAAGCCCAGTCAGG
TCACCATCAATATGATAAGCATGTCCGTAAAAC
CTAAACAAAAAGGCCGCCTGACTAGGATTAGAG
AGCCCAATAAACGAAATTTGCAAATAGATTTAG
TTTGACCATTAGATACATCAGGAAGAGAATACA
TACCAAGCCGCCACCCTTGAAATATGACGGA
TAAAGCTAAATCGGTTGCTGGCCTTGAACCGAA
TTTACATCTTTCAATTAAGAAGATCCACCAG
ATCCTCATATCCTGATTACAGTAATCAGGTCAT
GAGGAAGCTTCTGTAACTAAAGGAATCAGATA
TGAAAAGGTGGCATCAACACGTTGGCGGAGATT
ACGTTGGGGCCATATTAAGAGGCTAGAATTGA
CATATTCCAGAAAACAAATCGCGCATTAAACC
AATTTTCCAGCGGATTGGTCGCTGCAGTGCCAA
TGCCTGAGAGTCTGGAGCGGGTAGCTATTATACTT
GAATTAGCAAAATTAAGATGTGAGCCCGGAACG
TTTTGCGGATGGCTTAGGAAAGGGGCGGAACGA

AGGCGCAGCAGGCGGATACAAATTTCACAAT
TGCGCCGATTTTCACGATATATGTCCGGAAC
AGATTCATATGCGTTATAAGTGCCAACACCCT
AGAAATAAACATGGCTGAACGCGCAACAATA
TGTATCATCTCAGGAGTAAATAAGCAACCGA
AAAAGGCTCTTAAACATATCATTTACAAACAAT
GTTAATATTTTGTTAAAATTTAGAACCCTTATGCG
GCGCATCGTAACCGTGCATTCATTTGGCACTATCA
TAATAATTCAATGACACTTGTTATCCGAACCAA
TCGACAACTCGTATTAATCGTAATCCCGATAGT
CCCGTCGGATTCTCCGTGTCATACAGGATACCACA
TAAATTAATGCCGGAGAAAACAAGATTCAGGTT
CAGAGCCACTCATCTTGACGACGATGGTCAATA
GGAAGATTGTATAAGCAAAGTAATGTGGTTTAATT
GTAATTCTAATTGGGCACGTAACACCAAAAACA
ACGACGACAGTATCGGCCTTTCGCAAATAAAAACC
ATCAAAAATAATTCGCGTTACCAAAAAAATAAAAC
AAACATCATGATTATCCAATAACGCAACAGTTG
AACAGTACAGTAACATGCTTGATAAAATCTAAA
ATATCGCGTTTTAATTCCGACTCTACGCCCACG
CTTATCATTCCAAGAGAGCCGGATGAAAC
GAGAGAATAACATACGACATTAATAAACA
TAGAAGGCTTATCCTTATTAGTATTAATT
CCGTTTTTATTTTCAACCGCCTTAATGGA
CAAAATAAACAGCCATTTGGGAAAACTTT
GAACAAAGTCAGAGTATTTTGCTTACCAGT
ATTAGACGGGAGAAATGGTTTGCCTGTTT
CGAGGCGTTTTAGCTTTCATCACATAGCG
ATCCAAATAAGAAAGTGAATTATCTTCTG
ATATCAGAGAGATAAGAAACGGGCTTAAT
CGGGAGGTTTTGAATTTGCCTAGAGTCAA
TAGTTGCTATTTTGGCACCGTTCTGAGAG
ACCGCGCCCAATAGAAAATCAGAGTGAAT
TAATATCCCATCCTAGGTATCCTGTTTCA
TTATCCTGAATCTTAAACGTCGGTTGGGT
CGTCAAAAATGAAATAAATATCCGACCGT
AAGTACCGCACTCAGAACCGCTACATTTA
CGTCTTTCCAGAGCAGTAGCATGCAAATC
GTTAAGCCCAATAAATACATACCAACATG
ATGAAATAGCAATAATTACGCGCCAGTAA
CCCTTTTTAAGAAACAAAAGAAAGGTAAA
AGAAACCAATCAATCGCCGCCACAAATAA
GAACAAAGTTACCACCGAGGATAAACAAC
AATTATTCATTAAAGCGATTTTGCCACCCT
AGCGACAGAATCAAGGCCTTAATTCCAGAC
TGATTAAGACTCCTTGCTATCTACAGTTAA
CATCTTTTCATAATCCAAGCAAATTGCGAA
TAATAACGGAATACCAGTAAGCGGTCAGTG
AGAGCCACCACCCTCAACGGGTAGAGGCGA
ACCACGGAATAAGTTGGTAATTGGGTTTTG
GTGGCAACATATAAAACCCACAGAGACTCC
GCCAGCAAAATCACCCTAATTTTAACACTG
ACAGGAGGTTGAGGCAATTTACTTCCAGTA
GATAAGTCCTGGAAAGAAGAACTACAGGAG
CAGAGCCACCACCGGATCGTAGCTCCAAAA
TTGAGGGAGGGAAGGATAGCAGCCGCCACC
CATTAGCAAGGCCGGACCAACGAACGCCTG
AACCACCACCAGAGCAATCGGCCAGAATGG
AAACCATCGATAGCACACCCAGAGTTAGCG
CAGACTGTAGCGCGTGAACCTCGGATTTTG
TCACCGACTTGAGCCATATTATGGATAGCA
GAGCCGCCACCCTCATCGAGAAATTGTATC
TAGCAAACGTAGAAATAAGAGCATTATTCT
TCGGTCATAGCCCCCGGTATTCCGGAGTGA
CCAAAGACAAAAGGGAAAACAGTAGGTGTA
CAATAGAAAATTCATTTAACTGGTCGAGAG

Table S14. Primer sequences for Figures 4 and S9. Template was M13mp18 ssDNA for 2,342 nt ssDNA and Lambda DNA for 5,000, 10,000 and 15,072 nt ssDNA. Phosphorothioated nucleotides shown in bold.

2,342 nt ssDNA (Template = M13mp18):	
2342_Fwd	**GATTA**CGAATTCGAGCTCGGTAC
2342_Rev	GACTTTTTCATGAGGAAGTTTCC
5,000 nt ssDNA (Template = Lambda DNA):	
5000_Fwd	**CGTCA**TAAAATGGTATGCCG
5000_Rev	GGGATTTACGTGCATCCAGT
10,000 nt ssDNA (Template = Lambda DNA):	
10000_Fwd	**CGTCA**TAAAATGGTATGCCG
10000_Rev	ATCCGGATCGCTGAAAAACA
15,072 nt ssDNA (Template = Lambda DNA):	
15072_Fwd	**CGTCA**TAAAATGGTATGCCG
15072_Rev	GCTGATCTCCTGAGAAACCA

Table S15. Scaffold sequences for the split 62 nm Toroid shown in Figures 5a & 5b. Template was either Lambda DNA or pEGFP-C1 DNA as indicated. Primer locations are underlined and phosphorothioated nucleotides shown in bold.

2048 nt ssDNA part 1. Template = Lambda DNA

CGTCATAAAATGGTATGCCGAAAGGGATGCTGAAATTGAGAACGAAAAGCTGCGCCGGGAGGTTGAAGAACTGCGGCAGGCCA
GCGAGGCAGATCTCCAGCCAGGAACTATTGAGTACGAACGCCATCGACTTACGCGTGCGCAGGCCGACGCACAGGAACTGAAG
AATGCCAGAGACTCCGCTGAAGTGGTGGAAACCGCATTCTGTACTTTCGTGCTGTCGCGGATCGCAGGTGAAATTGCCAGTAT
TCTCGACGGGCTCCCCCTGTCGGTGCAGCGGCGTTTTCCGGAACTGGAAAACCGACATGTTGATTTCCTGAAACGGGATATCA
TCAAAGCCATGAACAAAGCAGCCGCGCTGGATGAACTGATACCGGGGTTGCTGAGTGAATATATCGAACAGTCAGGTTAACAG
GCTGCGGCATTTTGTCCGCGCCGGGCTTCGCTCACTGTTCAGGCCGGAGCCACAGACCGCCGTTGAATGGGCGGATGCTAATT
ACTATCTCCCGAAAGAATCCGCATACCAGGAAGGGCGCTGGGAAACACTGCCCTTTCAGCGGGCCATCATGAATGCGATGGGC

AGCGACTACATCCGTGAGGTGAATGTGGTGAAGTCTGCCCGTGTCGGTTATTCCAAAATGCTGCTGGGTGTTTATGCCTACTT
TATAGAGCATAAGCAGCGCAACACCCTTATCTGGTTGCCGACGGATGGTGATGCCGAGAACTTTATGAAAACCCACGTTGAGC
CGACTATTCGTGATATTCCGTCGCTGCTGGCGCTGGCCCCGTGGTATGGCAAAAAGCACCGGGATAACACGCTCACCATGAAG
CGTTTCACTAATGGGCGTGGCTTCTGGTGCCTGGGCGGTAAAGCGGCAAAAAACTACCGTGAAAAGTCGGTGGATGTGGCGGG
TTATGATGAACTTGCTGCTTTTGATGATGATATTGAACAGGAAGGCTCTCCGACGTTCCTGGGTGACAAGCGTATTGAAGGCT
CGGTCTGGCCAAAGTCCATCCGTGGCTCCACGCCAAAAGTGAGAGGCACCTGTCAGATTGAGCGTGCAGCCAGTGAATCCCCG
CATTTTATGCGTTTTCATGTTGCCTGCCCGCATTGCGGGGAGGAGCAGTATCTTAAATTTGGCGACAAAGAGACGCCGTTTGG
CCTCAAATGGACGCCGGATGACCCCTCCAGCGTGTTTTATCTCTGCGAGCATAATGCCTGCGTCATCCGCCAGCAGGAGCTGG
ACTTTACTGATGCCCGTTATATCTGCGAAAAGACCGGGATCTGGACCCGTGATGGCATTCTCTGGTTTTCGTCATCCGGTGAA
GAGATTGAGCCACCTGACAGTGTGACCTTTCACATCTGGACAGCGTACAGCCCGTTCACCACCTGGGTGCAGATTGTCAAAGA
CTGGATGAAAACGAAAGGGGATACGGGAAAACGTAAAACCTTCGTAAACACCACGCTCGGTGAGACGTGGGAGGCGAAAATTG
GCGAACGTCCGGATGCTGAAGTGATGGCAGAGCGGAAAGAGCATTATTCAGCGCCCGTTCCTGACCGTGTGGCTTACCTGACC
GCCGGTATCGACTCCCAGCTGGACCGCTACGAAATGCGCGTATGGGGATGGGGGCCGGGTGAGGAAAGCTGGCTGATTGACCG
GCAGATTATTATGGGCCGCCACGACGATGAACAGACGCTGCTGCGTGTGGATGAGGCCATCAATAAAACCTATACCCGCCGGA
ATGGTGCAGAAATGTCGATATCCCGTATCTGCTGGGATACTGGCGGGATTGACCCGACCATTGTGTATGAACGCTCGAAAAAA
CATGGGCTGTTCCGGGTGATCCCCATTAAAGGGGCATCCGTCTACGGAAAGCCGGTGGCCAGCATGCCACGTAAGCGAAACAA
AAACGGGGTTTACCTTACCGAAATCGGTACGGATACCGCGAAAGAGCAGATTTATAACCGCTTCACACTGACGCCGGAAGGGG
ATGAACCGCTTCCCGGTGCCGTTCACTTCCCGAATAACCCGGATATTTTTGATCTG

2068 nt ssDNA part 2. Template = pEGFP-C1 DNA

GGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGAC
CCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTA
TTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAAT
GGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATT
ACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCAT
TGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAA
TGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGTCAGATCCGCTAGCGCTACCGGTCG
CCACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCAC
AAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCT
GCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGC
ACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACC
CGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACAT
CCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGA
ACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGC
CCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGT
CCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTCCGGACTCAGATCTCGAGCTCAAG
CTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACCGGATCTAGATAACTGATCATAATCAGCCATACCACATTT
GTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAA
CTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATT
CTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTAACGCGTAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAA
TTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAG
GGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTAT
CAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCC
TAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGG
GCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACA

Table S16. Staple sequences for the split 62 nm toroid design.

CCGCCCAGACCTCGGCGGGTGTTCGGGTGGA	CAGTAAAGTATTATGACACGCTGTCAATGG
TTACGAAGGTTGCTCAGGGCATGTGTGAAG	AGTGGTCGCGGGCGCTGCCACGCCCTTCCCTTC
TATGCTCTATAAAGTAGGGCAGCATTTTAAGCTGC	GGCATTATGAGTAAAAGTCCAGCACTATGG
GAAAGGGCAGTGTTTCCCATTTAACGCCTTTGTCGC	TCAGGTAGTCCCACGTTCATAACCCCGATTTAG
CGTTCGTACTCAATAGTTTCGTTCTCACGTGGGTTT	GTTGTACACGAATAGTCTTTCCTCAAGTCGATGG
CTCGATGATGGTGAGCGGTCAGGTAAATGCGGTT	TGAGCTGATTTAACAAAAAGCGCCCTTACGGGCAT
AGGTCACACGACTTGTGGTGGGCGAACTAA	GTGGTGAACATCCCGGCTGCACGGGGTTAT
AACGTCGGTCGTGCTGGATCGCGCGCGATGA	TCCTGTGCGTCGGCCTGCTTTATGACGAGCAGCGAC
GCGATCCGCGACAGCACGTTTCGCTTTCATTAGTGA	CAGGGTGGGCGTGGAGCGCTGTCCAGCAGCCTGT
ACTCAACCCTATCTCGGTGCTCCGGCCTCTTCACC	CATTGATGAGTTTGGACACCACATTCAATTTGTGA
GCCGTTTGAGGCCAAATGCTGGCGGTGGCCCGCT	CGGATTCTTTCGGGAGATGGCCTATTGGCAATGCGG
GGTATCAGTTCATCCAGCTGGGCCATCTCACCCAGG	GAACTTCGCACCAGAAGAATAATGCATTTCACCT
CGACACGGGCAGACTTCAAACCACAACGAAAAAAAT	CTCGCCCAGATACTGCGCAGATATACCTGGTATG
TGGGGTCTTTTTACGTCTGTTCAAACTTGATTA	TCACTTTTGTCACGAGACAGCTCGCTGGGCA
CCATCCCCATTCTTCTGTGCCCCACAGCCC	TCACTGGCTTGCCGGTCTTGAGCTCCGTCAT
CAAAAGCATAGCCTTCGGGCGGACCTGCCAA	GGCCGTCGCACTTCAGAGTTTTTTCCGTCAAGC
GGGCAGCTGCACGCTCGGCTCAATCTGAACAGTG	CACCGACTTCCTTGAACGGGCAGCCAAACCG
CGCCCTAGCGCCCGCTCCAAAGTACAGAGCCACAC	GTAGCGGAGCCTTCAATGACAATCTAGCAACCCC
CGAGAGTGGGGCTGTACCACGGATGTCCACGTT	GCTTTGATGATATCCCGTCCCCAAAAATATCATCAT
CAGATCCCGCCCGGGCGTAGGTGGCCAGGC	GTCCATTTACGTCGCCCCTCTACAACCCATT
CTGCAGAAAAACCAGAGCATAAAATGATTTATA	CCAGACCGCTGAAGCACGGCGGTCAAGTAGG

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TCGCTGGCCTGCCACCCACATAAACGCCGTCGGCAA
TTGATGGTTCTCGGCAGCGGCCCATAGATCTGCC
GGGTGATGGTTCACGTAGGCGGCTGCTTCCCCTTT
CGGTGGATGTCTTTTCTCCTCCCCGTTAAAAAA
TATGGCTGCCAGCTCCCGGCGTCTGAATTTTAA
TCCCTTTCGGCATACCATGCACGCGTACCCGGCCC
CAAATTTATCGCCGGATCAGTTATCAAGTGG
ACCTCCCGGCGCAGCTTTCCTGGCTGGAATAATCT
TCTGCACGGGTGTTGCTAACCATTATGGAATAAC
TCTAAATCGGGGGCTCCCAAACGCCGCGTTCGCCA
CGGTTTTCCAGTTCCGGATTTAGGGTTCGCCACATC
AGGGGGAGCCCGTCGAGACGGCTTTCCGCCGCTTTA
GCAGGCAAAGCTTGCCCCGCGGTATTGGCAT
AGCGAAGCCCGGCGCGGAGTTCCAAACAGGTGCCTC
CCAGCAGGCCAGTCTTTACGCTTGGCCCTGATA
GGTCAGGAAGCGAGCTCAGGGTGCATGCTG
TAACCTGACTGTTCGATAGACGTTGGAGGACTTTGG
TATAGACGTCAGCCAGCGGCTCAAATTTCAGCA
TGTAGTCGCTGCCCATCGAATTTACGCTCATCCGGC
TGCTATTGCAGGGGGAGGTGAACCCCTTGC
TCAGGTTCTTTATTTGGCTGCAGTTAGAATGCT
ATTCAACGGCGGTCTGTGCTATTCTTTTGCGGGGAT
TCCACCACTTCAGCGGAGGACCGCTACCCGGTGCTT
GTCCAACAGTTAAGTGTCACCATCAGTTCTTCA
CTTTAATAGTGGACTCTTCAAAATGCCGATGTGAA
TCATAAAGCCTCATCCCAGCGTCTCTCCCAC
TGGGAGTCGTGGCGGAGTCGATGGATGCCC
AGGGATTTTGCCGATTTCAGTAATTAGCACGGGTC
GATGGGCTGTACACGCGATAAAGAACCTCACGGA
GAAGAAGAGAGCCTTCTTTCCCGTATTGTTCATG
CAGGGTCCATGAAAACGAATGCCATCATCCGCCC
GACGGTTTTTCGCCCTTTTATTCACTCGCACCCAG
CAAAATATTAACGCTTACCATTCATGAATGACGCA
ATTTTCGCCTGGTTGTGAAGATGGCGGTAT
CTGGACGGCAAGTTCACTCACCGAGTCAACATGT
AATAAACAAACAATTGCGGGTATTCCCAGC
GGTGGTTACGCGCAGCGTTCTCTGGCAGGTCCAGC
GCTTTATTACCACCCCGGTGTGGGGACCGGT
GCCGGTCAATTGGCAGACACTGGCACAATG
CGTTTTCATACCATGTCTTCATGGAAGCGG
CTTTCTCGCCACGTTCGCATACTGGCATCTTTCCG
CCAGATAACATTCCGGCATTCATTTCACTAA
TTAAAGCACTCGCAGATGGAGGGGGTTAAGATA
GCCGTCCACCACGGGGTTTCGTAGCTTCTTCAGT
TGCTTTACGGCACCTCGATTCAGGAAACGTGGTGT
TGATGCCGTACGCGCACCAGCGCCGGCGGGTGT
GGATGACGATTCGAAGGGTGCAGGTAACGC
CGATGTTGATACCGGCGTGTTATCACTTGCCAG
GGAATATCTCCAGCTTGCTTGTCGTGCGTCA
TTTGCCATTCCTTGAATCTTGAAGATTTTGG
CTCTGCCATCCGATGGCGGGTCAAACCCC
TGAGTCCGTGTCAGGTAATCTGACTGGAACAAC
GCCGTCGTTTCACGGTCATCCGGACTGCACCGAC
AACGCTTCCGGTTCACGCACGCTGCCAAAAC
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Table S17. Primer sequences for the two scaffold strands required to form the split 90 nm Toroid shown in Figure S9. Template was M13mp18 ssDNA. Phosphorothioated nucleotides shown in bold.

2952 nt ssDNA part 1:	
2952_part1_Fwd	**GATTA**CGAATTCGAGCTCGGTAC
2952_part1_Rev	CCGGAATAGGTGTATCACC
2952 nt ssDNA part 2:	
2952_part2_Fwd	**GCTAT**ACTTATATCAACCCTCTC
2952_part2_Rev	GCCAGCAGCAAATGAAAAAT

Table S18. Primer sequences for the three scaffold strands required to form the split 90 nm Toroid shown in Figure 5c. Template was M13mp18 ssDNA. Phosphorothioated nucleotides shown in bold.

1968 nt ssDNA part 1:	
1968_Fwd	**GATTA**CGAATTCGAGCTCGGTAC
1968_Rev	AACAAAGCTGCTCATTCAGT
1966 nt ssDNA part 2:	
1966_Fwd	**ACGTT**GATTTGGGTAATGAA
1966_Rev	CAGAAGGAAACCGAGGAAAC
1949 nt ssDNA part 3:	
1949_Fwd	**GTAAC**TTTGTTCGGCTATCTG
1949_Rev	GCCAGCAGCAAATGAAAAAT

Supplemental References

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(3) Tang, G., Peng, L., Baldwin, P. R., Mann, D. S., Jiang, W., Rees, I., and Ludtke, S. J. (2007) EMAN2: an extensible image processing suite for electron microscopy. *J Struct Biol 157*, 38-46.