

Folding DNA to create nanoscale shapes and patterns

Supplementary Notes 1–11

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Supplementary Note S1: Design of DNA origami

The program used for designing DNA origami, `multishapes.m`, may be downloaded from:

<http://www.dna.caltech.edu/SupplementaryMaterial/>

Below is a description of how design proceeds using this program. It is not meant to be a manual but rather to show the level of abstraction at which the origami are designed, and to show the various types of diagrams that the program can draw to aid in design. If scaffolded DNA origami becomes widely used, a better CAD design tool will have to be written. Given a desired shape (for example the red outline in Fig. 1a) design of a DNA origami to approximate it proceeds in five phases (two manual design steps and three passes of the program):

1. **Generation of a block diagram.** By hand, a rough geometric model is generated. It is comprised of rectangular blocks in which each block is taken to be one turn of DNA wide and one DNA helix plus the inter-helix gap in height. (Such a block diagram sloppily overestimates the height of a structure by one inter-helix gap.) An example block diagram is in Supplementary Fig. S1 step 1.

This step is performed with an eye towards the next step (generation of a folding path), in some cases the block diagram is conceived almost simultaneously with the folding path. The phase of the underlying periodic crossover lattice is chosen as well; generally this phasing is chosen so that seams and long edges of the shape align with columns of periodic crossovers. For blocks on the edge of the diagram, it is useful to keep track of the relationship of such blocks to the underlying crossover lattice. For an origami with 1.5-turn spacing between crossovers there are 3 possible offsets that an edge may have with respect to the underlying lattice—call them 0, +1 and -1 (Origami with 2.5 turn spacing have 5 possible offsets). For designs with a central seam, blocks on edges of offset 0 are colored red and blocks on edges of offset +1 and -1 are colored yellow and orange, depending on whether they occur to the left or right of the central seam. At this point the placement of seams may already be apparent; if so, half-blocks are used along seams. Adjacent half-blocks involved in the same scaffold crossover are colored the same (one of either green or purple) but adjacent half-blocks that participate in different crossovers are given different colors.

2. **Generation of a folding path,** by raster fill, through the block diagram. For a given shape there are many compatible raster fill patterns; currently the raster fill pattern must be hand-designed. For any helical domain in which the scaffold is to start and end on the same side of the helix (top or bottom), an integral number of turns (blocks) is traversed. For any helical domain in which the scaffold starts and ends on opposite sides of the helix, the scaffold traverses an odd number of half-turns (half-blocks). An example folding path is in Supplementary Fig. S1 step 2.
3. **Generation of a first pass design** based on the block diagram and folding path. The lengths of various helical domains, in units of DNA turns, are implied by the block diagram and folding paths; these are what is input to the computer program. For every design with seams presented here *except* the 3-hole disk, a single vertical seam was used in the design and so a simple matrix representation of the domain lengths could be used. Supplementary Fig. S1 step 3 shows a matrix (`design_turns`) of these domain lengths that is input to the program. (The equilateral and sharp triangle have a single seam *in each domain* that could be similarly specified by a single matrix. For the 3-hole disk, the position of seams was entered as a separate matrix and the routing of the scaffold between these seams as a matrix slightly more complicated than the `design_turns` matrix.) Column C0 gives the total number of turns in a particular row of the design. Column C1 and C4 give the total number of turns to the left and right of the seam, respectively. (Column C0, the sum of C1 and C4, was thus redundant and was used for checking the design.) Columns C2 and C3, unused for this design, give the offset (in number of turns) from the seam of the left and right helical domains, respectively; this feature is not used for the house design here, but is used for the bottom “legs” of the star design. The program converts lengths given (by the user) in turns to numbers of bases (matrix `design_lengths`) and outputs a first-pass design.

The first pass design can be output either as a line diagram (as in Supplementary Fig. S1 step 3) or as a detailed diagram showing the sequence of the scaffold layed out along the folding path, and the sequence of the staples where they appear in the final folded structure (Supplementary Fig. S2). In the detailed design diagram staple strands are indexed by xy position of the adjacent crossover and the designation ‘a’ or ‘b’ depending on whether the staple falls to the left or right of the crossover, respectively. Here names are of the form **sXtYP** where **X** is the x position, **Y** is the y position, and **P** the position with respect to the adjacent crossover.

4. **Refinement of the helical domain lengths** to minimize strain in the design. In a third sort of diagram (Supplementary Fig. S3), the computer program displays the predicted twist at each base position as a color (assuming the spacing of crossovers represents an exact number of half turns). Red indicates that the base at a particular position is pointing up, blue that it is pointing down. At crossover points, strand backbone positions should fall at the tangent point between helices; thus bases from the helix above a crossover should be blue and those from the helix below a crossover should be red. (More specifically, the color of bases at the crossover should be spaced equally clockwise and counterclockwise of the colors blue and red, that themselves should occur exactly at the tangent point.) All of the crossovers internal to the design are spaced 16 bases apart and related by a glide symmetry that should balance strain. This can be observed for the two types of crossovers (‘+’, major-groove up and ‘-’ major-groove down) that occur internally in a shape, shown in both the boxed regions and at the lower left of Supplementary Fig. S3. (A naive view is that the bases of the crossover will actually be centered around the tangent point between helices. In reality, twist strain might be relieved by distortion of the crossover but the idea is that if so, such a distortion will be balanced by that of neighboring symmetrically-placed crossovers.)

Ideally, at all other crossovers in the design, the orientation of bases would be similar to that desired for the balanced internal crossovers. However, because of the non-integral number of bases in a single turn, and the major-minor groove angle, it is not possible to put all crossovers in this optimal orientation. Crossovers along the edges of the shape, in particular, must be adjusted to minimize strain. The program computes a “strain energy” along the edges of the design, and so positions of predicted high strain can be identified. (For a given strand passing through a crossover, the computed strain energy is just the sum of the squared angular deviation from the tangent point for the base before and the base after the crossover.)

By hand, helical domain lengths are changed by single bases until the strain energy is minimized. The map of twists aids in this process. For example, high twist strain occurs in a couple scaffold crossovers in the first pass design (marked by ‘s’ labels inside ovals in Supplementary Fig. S3). These crossovers were initially designed to be 5 bases away from the nearest internal crossover (a ‘-’ crossover). At bottom right, the situation for one such crossover may be compared to that which would occur if the distance to the nearest internal crossover were changed to 6 bases, as well as the ‘ideal’ situation for this type of crossover, that of a ‘+’ type crossover. The 6-base distance creates the least strain.

Once the appropriate adjustments are decided, a matrix of adjustments is input to the design program, with the original design (Supplementary Fig. S4, **design{ADJUSTMENTS}**, top). The matrix gives adjustments for the left and right edges of each helical domain in the design, columns C1 and C2 for domains to the left of the seam and columns C3 and C4 for columns to the right of the seam. The program updates **design_lengths** (Supplementary Fig. S4, bottom) accordingly and a second pass diagram is generated (Supplementary Fig. S5).

5. **Breaking and merging of strands.** The merging of strands is specified by giving a pairwise list of the names to be merged (i.e. **s-2t9b** and **s-1t8a**) along with the name of the new strand (i.e. **s-1t8e**). The program checks to see that all strands to be merged have adjacent 3’ and 5’ bases. The position of strands to be broken is specified by the name of a strand and the position along its length at which it is to be broken. The pattern of merges is not unique. Supplementary Fig. S6, Supplementary Fig. S8, and Supplementary Fig. S10 show three different diagrams (full sequence, line drawing, and crossover map) of a design that features bridging staple strands across the seam. Supplementary Fig. S7, Supplementary Fig. S9, and Supplementary Fig. S11 show three different diagrams (full sequence, line drawing, and crossover map) of a design that has no bridging staples. Diagrams are interleaved to allow comparison of the differences between these two designs. Using a PDF viewer, flip back and forth between two diagrams of the same type to see the effect of different merge patterns. Particularly interesting are the crossover maps, Supplementary Fig. S10 and Supplementary Fig. S11. The addition of bridging staples creates a characteristic pattern of “bars” down the center of the design which is observed in experiments that using bridging staples (and not observed in experiments that don’t use bridging staples.) Supplementary Fig. S12 highlights the the basic type of grid underlying each merge pattern and the implications for applying pixels to the pattern.

The design method given here is a generalization of that developed by Nadrian Seeman for creating rigid molecules out of parallel helical domains (here helices are technically ‘antiparallel’ in the standard terminology), which was first elaborated for the creation of double-crossover molecules (molecules with two parallel helices, ref. 16) and later extended to triple crossover molecules (molecules with three parallel helices,²⁴). The main principle used in these works is that **crossovers may be used to hold helices rigidly in a parallel orientation**. More specifically, **wherever the twist of two parallel helices bring the backbones of the two helices sufficiently close, reciprocal strand exchange can be used to incorporate a crossover**. Further, an amazing aspect of the principle is that such a crossover does not disturb base pairing in either helix; the crossover appears to contain only single phosphate from each strand. The basic principle can be extended to many general schemes with a variety of crossover spacings and crossover types (parallel or antiparallel), as was mentioned in ref. 16. Here I explore a scheme that uses a regular grid of antiparallel crossovers (spaced an odd number of half-turns apart) in the bulk of a shape but on the edges and seams of a shape admits the placement of a crossovers with arbitrary offsets (in integral numbers of turns) from the underlying crossover grid.

The composition of double crossovers into periodic two dimensional crystals²⁵ showed that, through the use of sticky-end interactions, arbitrary numbers of helices could be held in a parallel arrangement by crossovers. Because the natural equilibrium length for a single turn of DNA appears to be close to 10.5 base pairs^{26, 27}, and because DNA backbones are not symmetrically spaced around the helix (there is a major and minor groove), designs of such two dimensional DNA nanostructures (which must use integral numbers of DNA bases) invariably incorporate features that should cause strain. That is, the design assumes a DNA geometry slightly different than that of a single isolated helix with 10.5 bases per turn with ‘normal’ major/minor groove angles. This difficulty appears to have manifested itself experimentally. A number of 2D DNA nanostructures form tubes rather than sheets^{15,22}.

The solution to this problem was first articulated to me by Erik Winfree, and was implicit in the design of DAO-E double crossover lattices²⁵: **crossovers (and nicks) in extended structures of parallel helices should be placed so that they have symmetries which balance strain**. This principle is described at length in ref. 15 and its supplemental materials; it is also often described as ‘corrugation’²². The principle has demonstrably inhibited tube formation in at least one system²².

For DNA origami this criterion was used in the placement of crossovers; after merging it does not hold true for nicks in some designs. The use of 16 bases to represent 1.5 turns of DNA (in the 1.5-turn crossover spacing structures) or 26 bases to represent 2.5 turns of DNA (in the 2.5-turn crossover spacing structures) means that the helical domains between crossovers are slightly overtwisted or undertwisted, respectively. To balance this strain, alternating columns of staple crossovers are related by a glide symmetry—the local configuration of crossovers in one column is identical to that of crossovers in the next column over after a translation and a ‘flip’ (a rotation about one of the crossovers in-plane axes). Cross-section 1 of Fig. 1d shows the presumed orientation of backbones through one column of crossovers in the lattice, and the top two helices of cross-section 2, the presumed orientation of crossovers in an adjacent column 1.5 turns away (alternatively the diagrams for ‘+’ and ‘-’ crossovers in Supplementary Fig. S3). This symmetry should tend to balance strain in the origami and cause them to be, on average, flat. (So far, no experimental evidence has demonstrated that the origami are flat, however).

The use of a glide symmetry means that large regions of a DNA origami should have balanced strain. However, at seams and edges this is not necessarily true, even where a seam or edge lines up with the underlying crossover lattice. At seams or edges, because DNA has a major and minor groove, a crossover involving staple strands is in tension with an adjacent crossover involving the scaffold strand. Such a configuration of crossovers in tension has never before been used in DNA nanostructures. For example, in Fig. 1d the cross-section through a seam (cross-section 2) has been drawn so that the staple crossover is relaxed (top two helices of three) and the scaffold crossover (bottom two helices of three) is highly strained. Perhaps both crossovers assume some intermediate conformation.

How the strain is actually relieved is unknown, the final base pairs of each helix may be distorted. Strain at seams or edges does not appear to cause any gross defects in the origami; bases at the end of the helices are highly available for stacking against other DNA origami which suggests that the last base pair does form and assumes a planar configuration. If, in the future, strain associated defects should be detected at edges, then one or two scaffold bases could be left unpaired and allowed to form a hairpin that should relax the crossover.

Another place that the design of scaffolded DNA origami currently breaks with normal DNA nanotechnology is in its use of a wide range of sequences for its antiparallel crossovers. Customarily, crossover sequences are drawn from one of a few sequences that both form an immobile branched junction²⁸ and have well-characterized geometry. Such junctions have been designed with minimal symmetry so that the junction cannot branch migrate back and forth. Because the crossover sequences in the DNA origami demonstrated here are determined by the M13mp18 DNA sequence, and hundreds of them are used, a few probably have symmetries that would allow them to branch migrate a few bases; the crossover sequences have not been inspected for such properties. Further, different crossover sequences show a varying tendency to assume one of two different stacked-X conformers^{29, 30}, one of which is incompatible with

the DNA origami's intended structure at every crossover.

It is hoped that the juxtaposition of multiple crossovers in DNA origami inhibits both branch migrations and conformer isomerizations; isomerization or migration to an undesired form at one junction would tend to increase strain between adjacent junctions. A study of a pair of symmetric antiparallel junctions juxtaposed with an asymmetric antiparallel junction has shown that the asymmetric junction can prevent the adjacent symmetric junctions from branch migrating³¹. But the same study showed that two symmetric antiparallel junctions juxtaposed next to each other *can* branch migrate. Thus it seems possible that several symmetric junctions near each other might conspire and migrate. Indeed it seems likely that some local rearrangements of junctions in origami happen; since they are likely to be smaller than a few nanometers, they cannot be observed by AFM. Eventually higher resolution structural information on DNA origami will determine if such isomerizations occur. Importantly, I note that there is no reason why better characterized, well-behaved junction sequences should not be incorporated into DNA origami designs if it helps to create more precise structure. The incorporation of specific crossover sequences will require the use of a synthetic scaffold rather than a natural one, a practical inconvenience for very long scaffolds.

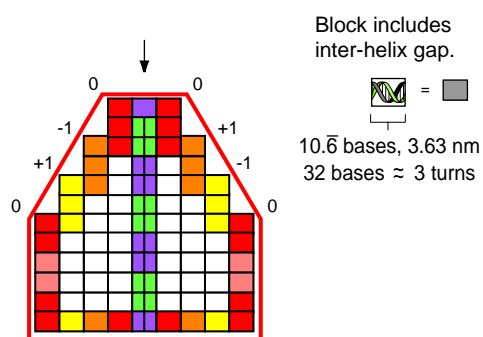
A note on seams: while most seams presented here are vertically aligned (for simplicity and convenience in design), and this necessitates the use of bridges to strengthen seams, it is possible to create staggered seams (as E. Winfree has suggested) so that staple strands naturally cross and bridge the seam vertically (between two adjacent helices) and no creation of horizontal bridges would be required. In these cases the addition of horizontal bridges across some parts of the seam might still add additional strength. A small instance of staggered seams occurs in the smiley face design. Above the right hand eye a small 2-helix seam appears that, because it is not aligned with other seams would not necessarily need bridges. An experiment in which the staples at this position were rearranged to remove horizontal bridging gave smileys of (not surprisingly) similar quality.

A note on folding paths and arbitrary shapes: there may be additional constraints on DNA origami that limit the family of shapes that can be approximated a little. In particular, shapes with lots of long thin projections or thin "waists" connecting two different parts of the shape may not form very well. As presented here, the minimum allowable width of a vertically oriented structure (such as a tall thin rectangle), if the scaffold rasters progresses in one direction vertically, is 1.5 turns, or about 5.4 nm wide. I have not tested the formation of such a narrow structure. The narrowest equivalent structure occurs at the jaw hinge of the smiley, 4.5 turns wide, about 16 nm wide. While most smileys are well formed, a significant number have dislocations along these 4.5 turn waists and it appears to be a weak spot.

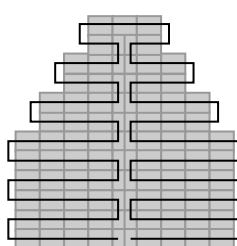
In some shapes it is desired that a strand pass both back and forth through a narrow waist so that it may access different portions of the shape. For example, consider a vertically oriented hourglass shape. If it is desired that a circular scaffold be used, then the scaffold must pass both up and down the narrow waist of the hourglass. As presented here, the minimal allowable width of the waist, which would accomodate the scaffold going up and down, would be 3 turns or about 11 nm. This width is the width of the top 4 helices of the star and because they do not image well, it seems that such a narrow waist may be floppy (in isolation). Clearly the analogous waist down the center of the smiley, which is 6 turns or 22 nm wide forms well and is mechanically stable in the context of the larger structure. Note that it is asymmetric and is composed of 1.5 turn and 4.5 turn wide vertical rasters.

Similarly, consider a horizontally oriented hourglass. For a circular scaffold to pass both left and right through the waist of the hourglass would require two helices, and so in principle the waist could be about 5 nm wide. However, I am unsure how well such a skinny waist would form. Again the smiley gives the best example of the smallest such waist so far. Below each eye is a four helix waist, about 11 nm wide which forms well and is stable.

1. Block diagram



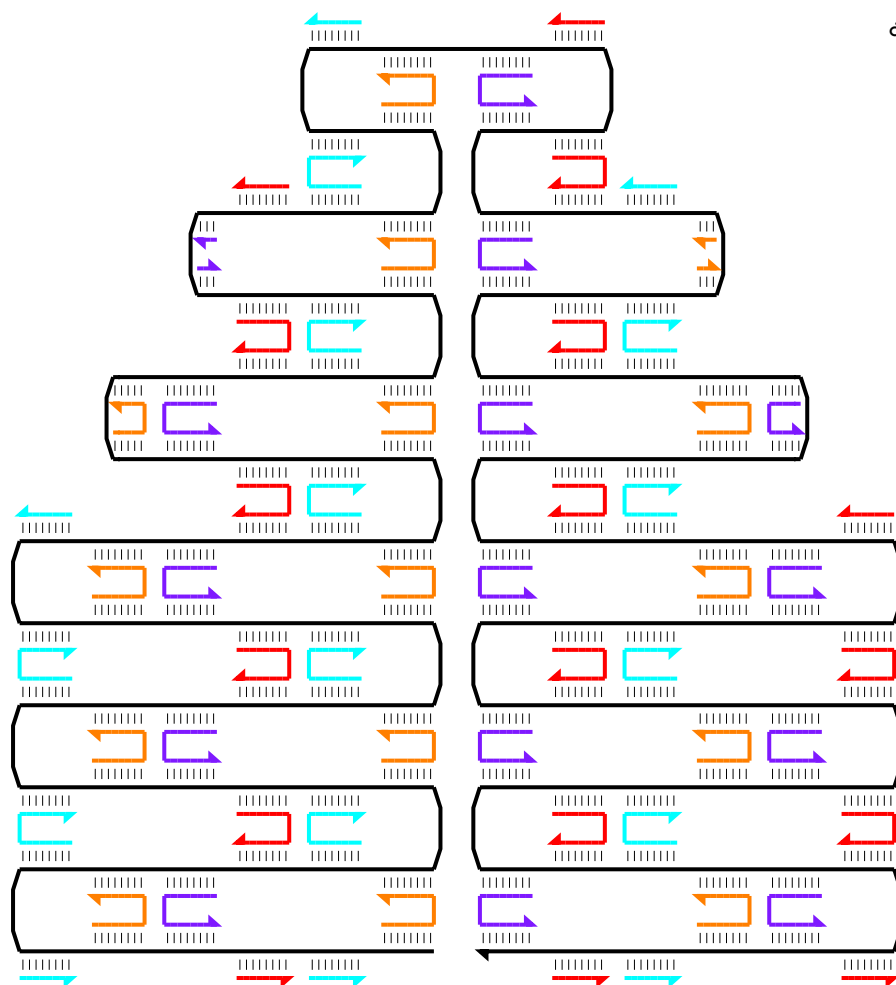
2. Folding path



3. First pass design

```
% C0 C1 C2 C3 C4
design_turns = [
[3 1.5 0 0 1.5]; %H1
[3 1.5 0 0 1.5]; %H2
[5 2.5 0 0 2.5]; %H3
[5 2.5 0 0 2.5]; %H4
[7 3.5 0 0 3.5]; %H5
[7 3.5 0 0 3.5]; %H6
[9 4.5 0 0 4.5]; %H7
[9 4.5 0 0 4.5]; %H8
[9 4.5 0 0 4.5]; %H9
[9 4.5 0 0 4.5]; %H10
[9 4.5 0 0 4.5]; %H11
[9 4.5 0 0 4.5]; %H12
];
```

Program outputs first pass design:



```
design_lengths =
C0 C1 C2 C3 C4
32 16 0 0 16 %H1
32 16 0 0 16 %H2
54 27 0 0 27 %H3
54 27 0 0 27 %H4
74 37 0 0 37 %H5
74 37 0 0 37 %H6
96 48 0 0 48 %H7
96 48 0 0 48 %H8
96 48 0 0 48 %H9
96 48 0 0 48 %H10
96 48 0 0 48 %H11
96 48 0 0 48 %H12
```

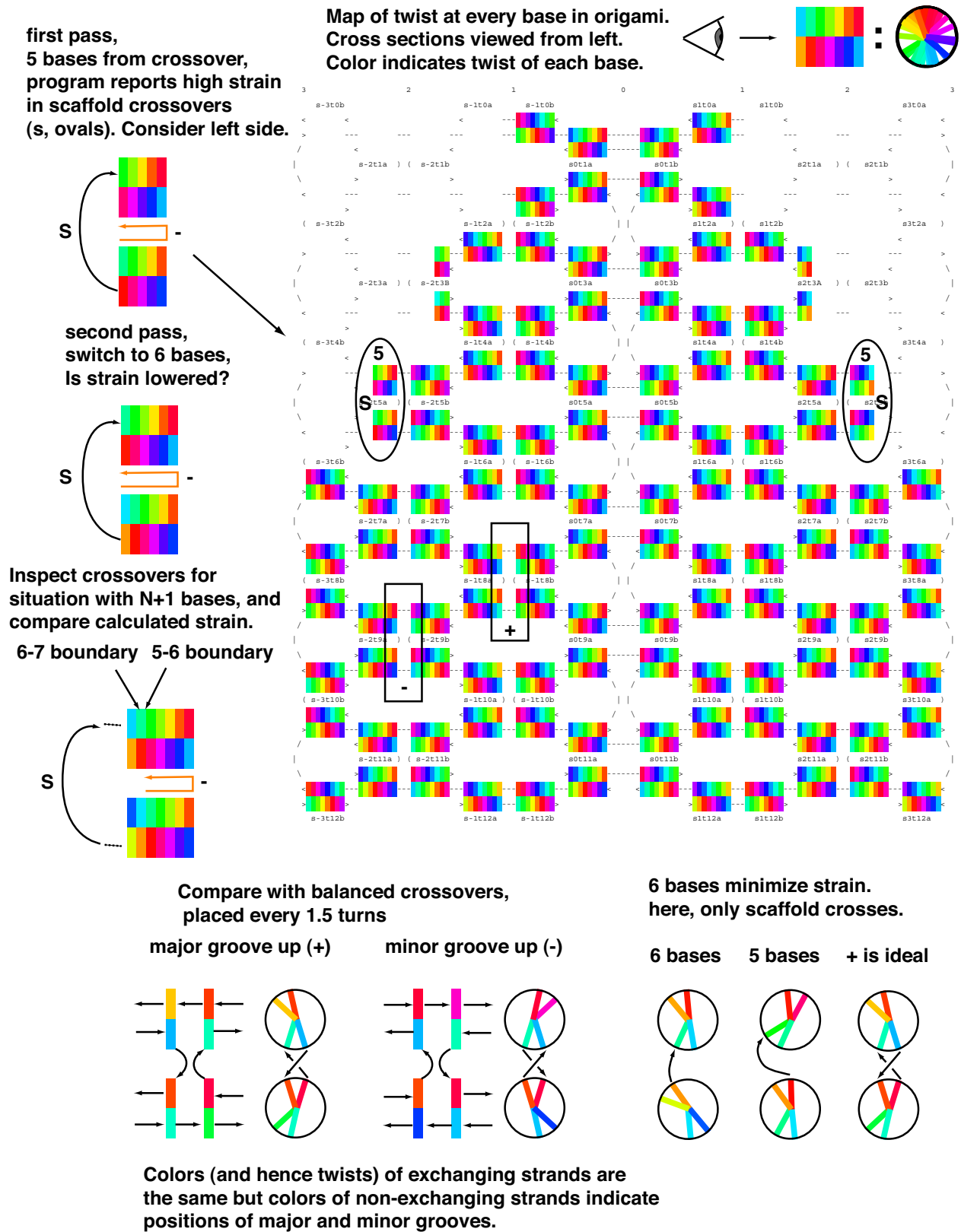
Supplementary Figure S1: First three steps of origami design.

```

3          2          1          0          1          2          3
s-3t0b          s-1t0a    s-1t0b          s1t0a    s1t0b          s3t0a
<          <          <          ---GGAGAGGG<          <GTAATTAC---          <          <
>          ---          ---          ---CCTCTCCC---CGCGCGTT-----GGCCGATT---CATTAATG---          ---          ---          >
/          <          <          <GCGCGCAA-----CGGCTAA<          <          <          \
|          s-2t1a ) ( s-2t1b          s0t1a          s0t1b          s2t1a ) ( s2t1b          |
\          >          >          >GTATTGGG-----CCTGTCGT>          >          >          /
<          ---          ---          ---GCCAAACG---CATAACCC<          <GGACAGCA---CGGTCGAC---          ---          ---          <
>          >          >          CGGTTTGC>          \ /          >GCCAGCTG          >          >
( s-3t2b          s-1t2a ) ( s-1t2b          | |          s1t2a ) ( s1t2b          s3t2a )
<          <          <          <CACTTTTC TTTTGGT<          / \          <GACCTTTC GCCCGTCA<          <
>          ---          ---          CTG---GTGAAAAG---AAAAACCA---CCCTGGCG>          >TTTCCCGA---CTGGAAAG---CGGGCAGT---GAG          ---          ---          >
/          <          <          GAC<          <GGGACCGC-----AAAGGGCT<          <CTC          <          \
|          s-2t3a ) ( s-2t3B          s0t3a          s0t3b          s2t3A ) ( s2t3b          |
\          >          >          TGA>          >TTGCCCTT-----GAGCTAAC>          >GCG          >          /
<          ---          ---          ACT---CTGCCCGT---TGTCGACT---AACGGGAA<          <CTCGATTG---AGTGTAAAT---TAACGCAA---CGC          ---          ---          <
>          >          >          >GACGGGCA ACAGCTGA>          \ /          >TCACATTA ATTGCGTT>          >          >
( s-3t4b          s-1t4a ) ( s-1t4b          | |          s1t4a ) ( s1t4b          s3t4a )
<          <          <          <GTTGAGAG AGTCCCGG<          / \          <CCGTGGGG TCCGAAAT<          <
>          ---          ---          GGACC---GCTTGCTG---CAACTCTC---TCAGGGCC---AGGCGGTG>          >ACTCATT---GGCACCCC---AGGCTTTA---CACTTTAT---GCTTC          ---          >
/          <          <          CCTGG CGAACGAC<          <TCCGCCAC-----TGAGTAAT<          <GTGAAATA CGAAG          <          \
|          s-2t5a ) ( s-2t5b          s0t5a          s0t5b          s2t5a ) ( s2t5b          |
\          >          >          ACGCT GGTTTGCC>          >CTGTTTGA-----TGTTATCC>          >AACATACG AGCCG          >          /
<          ---          ---          TCGCA---CCAAACGG---GGTCGTCC---GCTTTTAG---GACAAACT<          <ACAATAGG---CGAGTGTT---AAGGTGTG---TTGTATGC---TCGGC          ---          <
>          >          >          >CCAGCAGG CGAAAAATC>          \ /          >GCTCACAA TTCCACAC>          >          >
( s-3t6b          s-1t6a ) ( s-1t6b          | |          s1t6a ) ( s1t6b          s3t6a )
CCGATAAG<          <TAAACGG CTAAAGCC<          / \          <GTCTTTG TCGATACT<          <CTCAGAGCC
>GGCTATTC---TTTTGATT---TATAAGGG---ATTTTGCC---GATTTCGG---AACCACCA>          >ATTTTACA---CAGGAAC---AGCTATGA---CCATGATT---ACGAATTC---GAGCTCGG>
/          <          <          <AAAATAA ATATTCCC<          <TTGGTGTT-----TAAAGTGT<          <GGTACTAA TGCTTAAG<          \
|          s-2t7a ) ( s-2t7b          s0t7a          s0t7b          s2t7a ) ( s2t7b          |
\          >          >          >GGTTGAGT GTTGTTC>          >CCACTATT-----GTGCCAAG>          >TCGACTCT AGAGGATC>          /
<GCTCTATC---CCAACTCA---CAACAAGG---TCAAACT---TGTTCTCA---GGTGATAA<          <CACGGTTC---GAACGTAC---GGACGTCC---AGCTGAGA---TCTCCTAG---GGGCCCCAT<
CGAGATAG>          >AGTTTGA ACAAGAGT>          \ /          >CTTGCAATG CCTGCAGG>          >CCCGGGTA
( s-3t8b          s-1t8a ) ( s-1t8b          | |          s1t8a ) ( s1t8b          s3t8a )
GGGACTAT<          <AACTGCAA CCTCAGGT<          / \          <CAAAATGT TGCAGCAC<          <GCAATGGG
>CCCTGATA---GACGGTTT---TTCGCCCT---TTGACGTT---GGAGTCCA---CGTTCTTT>          >TGGCCGTC---GTTTTACA---ACGTCTGT---ACTGGGAA---AACCTGG---CGTTACCC>
/          <          <          <CTGCCAAA AAGCGGGA<          <GCAAGAAA-----ACCGGCAG<          <TGACCCCTT TTGGGACC<          \
|          s-2t9a ) ( s-2t9b          s0t9a          s0t9b          s2t9a ) ( s2t9b          |
\          >          >          >CACTACGT GAACATC>          >TTGGGTC-----TATTACGC>          >GATGTGCT GCAAGGCG>          /
<GCTACCGG---GTGATGCA---CTTGGTAG---TGGGTTTA---GTTCAAAA---AACCCAG<          <ATAATGCG---GTCGACCG---CTTTCCCC---CTACACGA---CGTTCCCG---TAATTCAA<
CGATGGCC>          >ACCCAAAT CAAGTTTT>          \ /          >CAGCTGGC GAAAGGGG>          >ATTAAGTT
( s-3t10b          s-1t10a ) ( s-1t10b          | |          s1t10a ) ( s1t10b          s3t10a )
TTAGCCCC<          <GCTAAATC ACGAAATG<          / \          <CGGGCGTG GCTAGCGG<          <GTCGGACT
>AATCGGGG---GCTCCCTT---TAGGGTTC---CGATTTAG---TGCTTTAC---GGCACCTC>          >GCGAAGAG---GCCCCGAC---CGATCGCC---CTTCCCAA---CAGTTGCG---CAGCCTGA>
/          <          <          <CGAGGGAA ATCCCAAG<          <CCGTGGAG-----CGCTTCTC<          <GAAGGGTT GTCAACGC<          \
|          s-2t11a ) ( s-2t11b          s0t11a          s0t11b          s2t11a ) ( s2t11b          |
\          >          >          >GACGGGA AAGCGGCG>          >GAAGGAA-----CCGGCACC>          >ACCAGGCA AAGCGCCA>          /
<ATCTCGAA---CTGCCCTT---TTCGGCCG---CTTGCAAC---GCTCTTTC---CTTCCCTT<          <GGCCGTGG---CGAAGACC---ACGGCCTT---TGGTCCGT---TTCGCGGT---AAGCGGTA<
>TAGAGCTT>          >GAACGTGG---CGAGAAAG>          >GCTTCTGG---TGCCGGAA>          >TTCGCCAT<
s-3t12b          s-1t12a    s-1t12b          s1t12a    s1t12b          s3t12a

```

Supplementary Figure S2: First pass diagram, staple strands with xy labels and explicit bases.



Supplementary Figure S3: First pass diagram, twists displayed as colors for examination of strain.

In the second pass a matrix of adjustments (in nucleotides) is defined:

```

%      C1  C2  C3  C4
design{ADJUSTMENTS} = [
    [0    0    0    0];    % H1
    [0    0    0    0];    % H2
    [0    0    0    0];    % H3
    [0    0    0    0];    % H4
    → [1    0    0    1];    % H5
    → [1    0    0    1];    % H6
    [0    0    0    0];    % H7
    [0    0    0    0];    % H8
    [0    0    0    0];    % H9
    [0    0    0    0];    % H10
    [0    0    0    0];    % H11
    [0    0    0    0];    % H12
];

```

And the design lengths are updated accordingly.

```

design_lengths =
    C0      C1      C2      C3      C4
    32      16       0       0      16
    32      16       0       0      16
    54      27       0       0      27
    54      27       0       0      27
    → 76      38       0       0      38
    → 76      38       0       0      38
    96      48       0       0      48
    96      48       0       0      48
    96      48       0       0      48
    96      48       0       0      48
    96      48       0       0      48
    96      48       0       0      48

```

Supplementary Figure S4: Adjustments to applied during the second pass.


```

-3          -2          -1          0          +1          +2          +3
s-3t0b          s-1t0a          s-1t0b          s1t0a          s1t0b          s3t0a
<          <          <          ---AGAGGGGC<          <AATTACGT---          <          <
>          ---          ---          ---TCTCCCCG---CGCGTTGG-----CCGATTCA---TTAATGCA---          ---          ---          >
/          <          <          <GCGCAACC-----GGCTAAGT<          <          <          \
|          s-2t1a ) ( s-2t1b          s0t1a          s0t1b          s2t1a ) ( s2t1b          |
\          >          >          >GCGTATTG-----AACCTGTC>          >          >          /
<          ---          ---          ---CCGCCAAA---CGCATAAC<          <TTGGACAG---CACGGTCG---          ---          ---          <
>          >          >          GCGCGTTT>          \ /          >GTGCCAGC          >          >
( s-3t2b          s-1t2a ) ( s-1t2b          | |          s1t2a ) ( s1t2b          s3t2a )
<          <          <          <CTTTTCTT TTTGGTGG<          / \          <CCTTTCGC CCGTCACT<          <
>          ---          ---          GGT---GAAAGAA---AAACCACC---CTGGCGCC>          >TCCCGACT---GGAAAGCG---GGCAGTGA---GCG          ---          ---          >
/          <          <          CCA<          <GACCGCGG-----AGGGCTGA<          <CGC          <          \
|          s-2t3a ) ( s-2t3B          s0t3a          s0t3b          s2t3A ) ( s2t3b          |
\          >          >          AGT>          >GATTGCC-----GTGAGCTA>          >TTG          >          /
<          ---          ---          TCA---CTCTGCCC---GTTGTGCA---CTAACGGG<          <CACTCGAT---TGAGTGTA---ATTAACGC---AAC          ---          ---          <
>          >          >          >GAGACGGG CAACAGCT>          \ /          >ACTCACAT TAATTGCG>          >
( s-3t4b          s-1t4a ) ( s-1t4b          | |          s1t4a ) ( s1t4b          s3t4a )
<          <          <          <TGAGAGAG TCCCGGTC<          / \          <GTGGGGTC CGAAATGT<          <
>          ---          ---          GACCGC---TTGCTGCA---ACTCTCTC---AGGGCCAG---GCGGTGAA>          >TCATTAGG---CACCCAG---GCTTTACA---CTTTATGC---TTCCGG          ---          >
/          <          <          CTGGCG AACGAGCT<          <CGCCACTT-----AGTAATCC<          <GAAATACG AAGGCC          <          \
|          s-2t5a ) ( s-2t5b          s0t5a          s0t5b          s2t5a ) ( s2t5b          |
\          >          >          CACGCT GGTTCGCC>          >CTGTTTGA-----AAATTGTT>          >ACACAACA TACGAG          >          /
<          ---          ---          GTGCGA---CCAAACGG---GGTCGTCC---GCTTTTAG---GACAAACT<          <TTTAACAA---TAGGCGAG---TGTTAAGG---TGTGTTGT---ATGCTC          ---          <
>          >          >          >CCAGCAGG CGAAAATC>          \ /          >ATCCGCTC ACAATTCC>          >
( s-3t6b          s-1t6a ) ( s-1t6b          | |          s1t6a ) ( s1t6b          s3t6a )
CCGATAAG<          <          <          <TAAACGG CTAAAGCC<          / \          <TTTGTGCA TACTGGTA<          <AGCCATGG
>GGCTATTC---TTTTGATT---TATAAGGG---ATTTTGCC---GATTTCGG---AACCACCA>          >CACACAGG---AAACAGCT---ATGACCAT---GATTACGA---ATTCGAGC---TCGGTACC>
/          <          <          <AAAATAA ATATTCCC<          <TTGGTGTT-----GTGTGTCC<          <CTAATGCT TAAGCTCG<          \
|          s-2t7a ) ( s-2t7b          s0t7a          s0t7b          s2t7a ) ( s2t7b          |
\          >          >          >GGTTGAGT GTTGTTC>          >CCACTATT-----GCCAGTGC>          >CAGGTCGA CTCTAGAG>          /
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CGAGATAG>          >          >          >AGTTTGA ACAAGAGT>          \ /          >CAAGCTTG CATGCCTG>          >GATCCCCG
( s-3t8b          s-1t8a ) ( s-1t8b          | |          s1t8a ) ( s1t8b          s3t8a )
GGGACTAT<          <          <          <AACTGCAA CCTCAGGT<          / \          <ATGTTGCA GCACTGAC<          <TGGGTTGA
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/          <          <          <CTGCCAAA AAGCGGGA<          <GCAAGAAA-----GCAGCAAA<          <CCTTTTGG GACCGCAA<          \
|          s-2t9a ) ( s-2t9b          s0t9a          s0t9b          s2t9a ) ( s2t9b          |
\          >          >          >CACTACGT GAACCATC>          >TTGGGGTC-----TCGCTATT>          >GGGGGATG TGCTGCAA>          /
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CGATGGCC>          >          >          >ACCCAAAT CAAGTTTT>          \ /          >ACGCCAGC TGGCGAAA>          >GGCGATT
( s-3t10b          s-1t10a ) ( s-1t10b          | |          s1t10a ) ( s1t10b          s3t10a )
TTAGCCCC<          <          <          <GCTAAATC ACGAAATG<          / \          <CGTGGCTA GCGGGAAG<          <GACTTACC
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/          <          <          <CGAGGGAA ATCCCAAG<          <CCGTGGAG-----TCTCCGG<          <GGTTGTCA ACGCGTCG<          \
|          s-2t11a ) ( s-2t11b          s0t11a          s0t11b          s2t11a ) ( s2t11b          |
\          >          >          >GACGGGA AAGCCGGC>          >GAAGGGA-----CTTTCCGG>          >GGAACCA GGCAGC>          /
<ATCTCGAA---GTGCCCCT---TTCGGCCG---CTTGCAAC---GCTCTTTC---CTTCCCTT<          <GAAAGGCC---GTGGCGAA---GACCACGG---CCTTTGGT---CGGTTTCG---CGGTAAGC<
>TAGAGCTT>          >          >          >GAACGTGG---CGAGAAAG>          >CACCGETT---CTGGTGCC>          >GCCATTCC<
s-3t12b          s-1t12a          s-1t12b          s1t12a          s1t12b          s3t12a

```

Supplementary Figure S5: Second pass diagram with staple strands before merge.

```

-3          -2          -1          0          +1          +2          +3
s-3t0b          s-1t0a          s1t0g          s1t0b          s3t0a
<          <          <          ---AGAGGGGC<          <AATTACGT---          <          <
>          ---          ---          ---TCTCCCCG---CGCGTTGG-----CCGATTCA---TTAATGCA---          ---          ---          >
/          <          <          <GCGCAACC-----GGCTAAGT<          <          <          \
|          s-2t1a ) ( s-2t1b          >          s2t1a ) ( s2t1b          |
\          >          >          >GCGTATTG-----AACCTGTC>          >          >          /
<          ---          ---          ---CCGCCAAA---CGCATAAC<          <TTGGACAG---CACGGTCG---          ---          ---          <
>          >          >          GGCGGTTI>          \ /          >TGCCCAGC          >          >
( s-3t2b          s-1t2e ) ( s-1t2i          | |          s1t2i ) (          s3t2a )
<          <          <          <CTTTTCTT TTTGGTGG<          / \          <CCTTTCGC CCGTCACT<          <
>          ---          ---          GGT---GAAAAGAA---AAACCACC---CTGGCGCC>          >TCCCAGCT---GGAAAGCG---GGCAGTGA---GCG          ---          ---          >
/          <          <          CCA<          <GACCGCGG-----AGGGCTGA<          <CGC          <          \
|          s-2t3a ) ( s-2t3g          >          s2t3c ) ( s2t3b          |
\          >          >          AGT>          >GATTGCC-----GTGAGCTA>          >TTG          >          /
<          ---          ---          TCA---CTCTGCCC---GTGTGCGA---CTAACGGG<          <CACTCGAT---TGAGTGTA---ATTAACGC---AAC          ---          ---          <
>          >          >          >GAGACGGG CAACAGCT>          \ /          >ACTCACAT TAATTGCG>          >
( s-3t4b          ) ( s-1t4i          | |          s1t4i ) ( s1t4f          s3t4a )
<          <          <          <TGAGAGAG TCCCGGTC<          / \          <GTGGGGTC CGAAATGT<          <
>          ---          ---          GACCGC---TTGCTGCA---ACTCTCTC---AGGGCCAG---GCGGTGAA>          >TCATTAGG---CACCCAG---GCTTTACA---CTTTATGC---TTCCGG          ---          >
/          <          <          CTGGCG AACGACGT<          <CGCCACTT-----AGTAATCC<          <GAAATACG AAGGCC          <          \
|          s-2t5a ) (          >          ) ( s2t5b          |
\          >          >          CACGCT GGTTTGCC>          >CTGTTTGA-----AAATTGTT>          >ACACAACA TACGAG          >          /
<          ---          ---          GTGCGA---CCAAACGG---GGTCGTCC---GCTTTTAG---GACAAACT<          <TTTAACAA---TAGGCGAG---TGTTAAGG---TGTGTTGT---ATGCTC          ---          <
>          >          >          >CCAGCAGG CGAAAATC>          \ /          >ATCCGCTC ACAATTCC>          >
(          s-1t6e ) ( s-1t6i          | |          s1t6i ) ( s1t6f          s3t6e )
CGGATAAG<          <          <          <TAAACGG CTAAAGCC<          / \          <TTTGTGCA TACTGGTA<          <AGCCATGG
>GGCTATTC---TTTTGATT---TATAAGGG---ATTTTGCC---GATTTTCGG---AACCACCA>          >CACACAGG---AAACAGCT---ATGACCAT---GATTACGA---ATTCGAGC---TCGGTACC>
/          <          <          <AAAATAA ATATTCCC<          <TTGGTGGT-----GTGTGTCC<          <CTAATGCT TAAGCTCG<          \
|          ) (          >          ) (          |
\          >          >          >GGTTGAGT GTTGTTC>          >CCACTATT-----GCCAGTGC>          >CAGGTGCA CTCTAGAG>          /
<GCTCTATC---CCAACTCA---CAACAAGG---TCAAACCT---TGTTCTCA---GGTGATAA<          <CGGTCAGC---GTTCGAAC---GTACGGAC---GTCCAGCT---GAGATCTC---CTAGGGGC<
CGAGATAG>          >          >          >AGTTTGA ACAAGAGT>          \ /          >CAAGCTTG CATGCCTG>          >GATCCCCG
( s-3t8g          s-1t8e ) ( s-1t8i          | |          s1t8i ) ( s1t8f          s3t8e )
GGGACTAT<          <          <          <AACTGCAA CCTCAGGT<          / \          <ATGTTGCA GCACTGAC<          <TGGGTTGA
>CCCTGATA---GACGGTTT---TTCGCCCT---TTGACGTT---GGAGTCCA---CGTTCTTT>          >CGTCGTTT---TACAACGT---CGTGACTG---GGAAAAACC---CTGGCGTT---ACCCAAT>
/          <          <          <CTGCCAA AAGCGGGA<          <GCAAGAAA-----GCAGCAAA<          <CCTTTTGG GACCGCAA<          \
|          ) (          >          ) (          |
\          >          >          >CACTACGT GAACCATC>          >TTGGGGTC-----TCGCTATT>          >GGGGGATG TGCTGCAA>          /
<GCTACCGG---GTGATGCA---CTTGGTAG---TGGTTTA---GTTCAAAA---AACCCAG<          <AGCGATAA---TGCGGTCG---ACCGCTTT---CCCCCTAC---ACGACGTT---CCGCTAAT<
CGATGGCC>          >          >          >ACCCAAAT CAAGTTTT>          \ /          >ACGCCAGC TGGCGAAA>          >GGCGATTA
( s-3t10f          s-1t10e ) ( s-1t10i          | |          s1t10i ) ( s1t10f          s3t10g )
TTAGCCCC<          <          <          <GCTAAATC ACGAAATG<          / \          <CGTGGCTA GCGGGAAG<          <GACTTACC
>AATCGGGG---GCTCCCTT---TAGGGTTC---CGATTTAG---TGCTTTAC---GGCACCTC>          >AGAGGGCC---GCACCGAT---CGCCCTTC---CCAACAGT---TGCGCAGC---CTGAATGG>
/          <          <          <CGAGGGAA ATCCCAAG<          <CCGTGGAG-----TCTCCGG<          <GGTTGTCA ACGCGTCG<          \
|          ) (          >          ) (          |
\          >          >          >GACGGGA AAGCCGGC>          >GAAGGGA-----CTTTCCGG>          >GGAACCA GGCAAAGC>          /
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>TAGAGCTT>          >          >          >GAACGTGG---CGAGAAAG>          >CACCGCTT---CTGGTGCC>          >GCCATTGC<
s-3t12f          s-1t12j          s1t12g

```

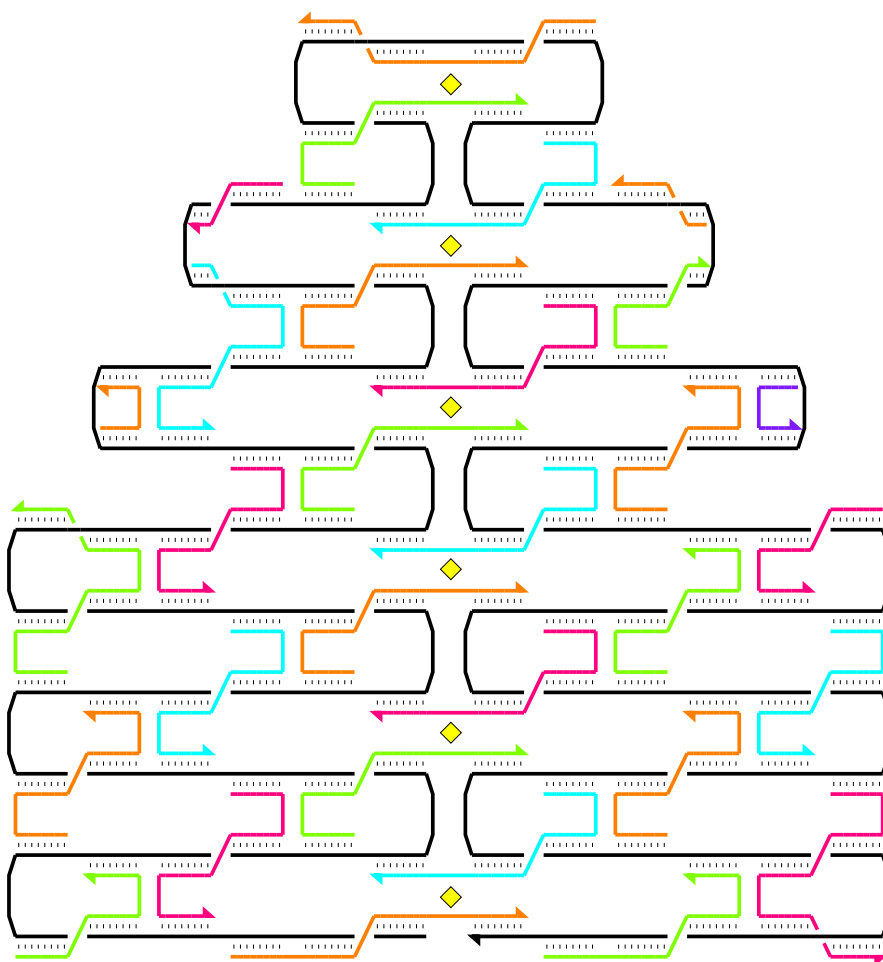
Supplementary Figure S6: Third pass diagram with staple strands after merge (bridged seam).

```

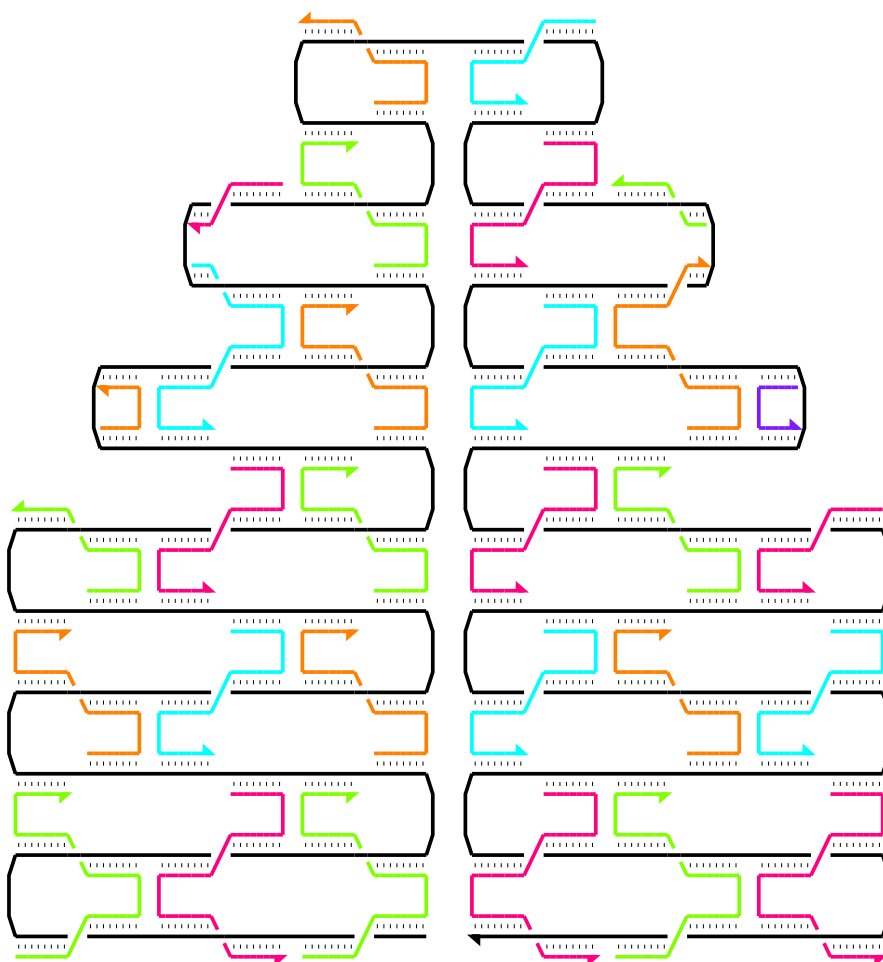
3           2           1           0           1           2           3
s-3t0b      s-1t0a      s1t0e      s1t0b      s3t0a
< < < ---AGAGGGGC< <AATTACGT--- < <
> --- --- --- ---TCTCCCCG---CGCGTTGG-----CCGATTCA---TTAATGCA--- --- --- >
/ < < <GCGCAACC-----GGCTAAGT< < < \
| s-2t1a ) ( s-2t1b s0t1f s2t1a ) ( s2t1b |
\ > > >GCGTATTG-----AACCTGTC> > > /
< --- --- --- ---CCGCCAAA---CGCATAAC< <TTGGACAG---CACGGTCG--- --- --- <
> > >GGCGGTTI> \ / >GTGCCAGC > >
( s-3t2b s-1t2e ) ( | | s1t2e ) ( s3t2a )
< < <CTTTTCTT TTTGGTGG< / \ <CCTTTGCG CCGTCACT< <
> --- --- GGT---GAAAAGAA---AAACCACC---CTGGCGCC> >TCCCGACT---GGAAAGCG---GGCAGTGA---GCG --- --- >
/ < <CCA< <GACCGCGG-----AGGGCTGA< <CGC < \
| s-2t3a ) ( s-2t3g s0t3f s2t3c ) ( s2t3b |
\ > >AGT> >GATTGCC-----GTGAGCTA> >TTG > /
< --- --- TCA---CTCTGCCC---GTGTGCGA---CTAACGGG< <CACTCGAT---TGAGTGTA---ATTAAACGC---AAC --- --- <
> > >GAGACGGG CAACAGCT> \ / >ACTCACAT TAATTGCG> >
( s-3t4b ) ( | | s1t4e ) ( s3t4a )
< < <TGAGAGAG TCCCGGTC< / \ <GTGGGGTC CGAAATGT< <
> --- GACCGC---TTGCTGCA---ACTCTCTC---AGGGCCAG---GCGGTGAA> >TCATTAGG---CACCCAG---GCTTTACA---CTTTATGC---TTCCGG --- >
/ < <CTGGCG AACGACGT< <CGCCACTT-----AGTAATCC< <GAAATACG AAGGCC < \
| s-2t5a ) ( s0t5f s2t5g ) ( s2t5b |
\ > >CAGCGT GGTTTGCC> >CTGTTTGA-----AAATTGTT> >ACACAACA TACGAG > /
< --- GTGCGA---CCAAACGG---GGTCGTCC---GCTTTTAG---GACAAACT< <TTTAACAA---TAGGCGAG---TGTTAAGG---TGTGTTGT---ATGCTC --- <
> > >CCAGCAGG CGAAAATC> \ / >ATCCGCTC ACAATTCC> >
( s-1t6e ) ( | | s1t6e ) ( s3t6e )
CGGATAAG< <TAAACCGG CTAAAGCC< / \ <TTTGTGCA TACTGGTA< <AGCCATGG
>GGCTATTC---TTTTGATT---TATAAGGG---ATTTTGCC---GATTTTCGG---AACCACCA> >CACACAGG---AAACAGCT---ATGACCAT---GATTACGA---ATTCGAGC---TCGGTACC>
/ < <AAAACATA ATATTCCC< <TTGGTGGT-----GTGTGTCC< <CTAATGCT TAAGCTCG< \
| s-2t7f ) ( s0t7f s2t7f ) ( |
\ > >GGTTGAGT GTTGTTC> >CCACTATT-----GCCAGTGC> >CAGGTGCA CTCTAGAG> /
<GCTCTATC---CCAACTCA---CAACAAGG---TCAAACCT---TGTTCTCA---GGTGATAA< <CGGTCAGC---GTTCGAAC---GTACGGAC---GTCCAGCT---GAGATCTC---CTAGGGGC<
CGAGATAG> >AGTTTGA ACAAGAGT> \ / >CAAGCTTG CATGCCTG> >GATCCCCG
( s-1t8e ) ( | | s1t8e ) ( s3t8e )
GGGACTAT< <AACTGCAA CCTCAGGT< / \ <ATGTTGCA GCACTGAC< <TGGGTTGA
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/ < <CTGCCAAA AAGCGGGA< <GCAAGAAA-----GCAGCAAA< <CCTTTTGG GACCGCAA< \
| s-2t9f ) ( s0t9f s2t9f ) ( |
\ > >CACTACGT GAACCATC> >TTGGGGTC-----TCGCTATT> >GGGGATG TCGTGCAA> /
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( s-1t10f ) ( | | s1t10g ) ( s3t10g )
TTAGCCCC< <GCTAAATC ACGAAATG< / \ <CGTGGCTA GCGGGAAG< <GACTTACC
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| ) ( ) ( |
\ > >GACGGGA AAGCCGGC> >GAAGGGA-----CTTTCCGG> >GGAACCA GGCAAAGC> /
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s-3t12g s-1t12g s1t12g

```

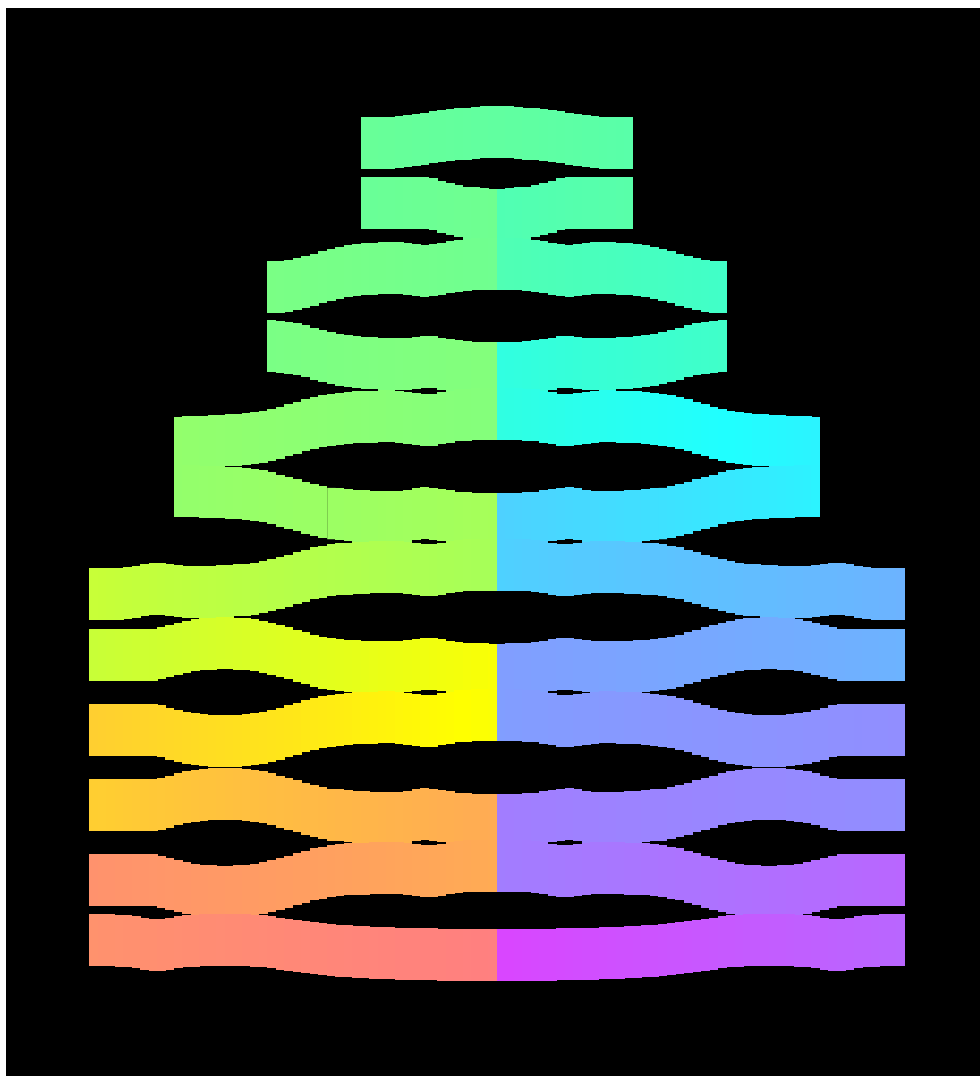
Supplementary Figure S7: Third pass diagram with staple strands after merge (unbridged seam).



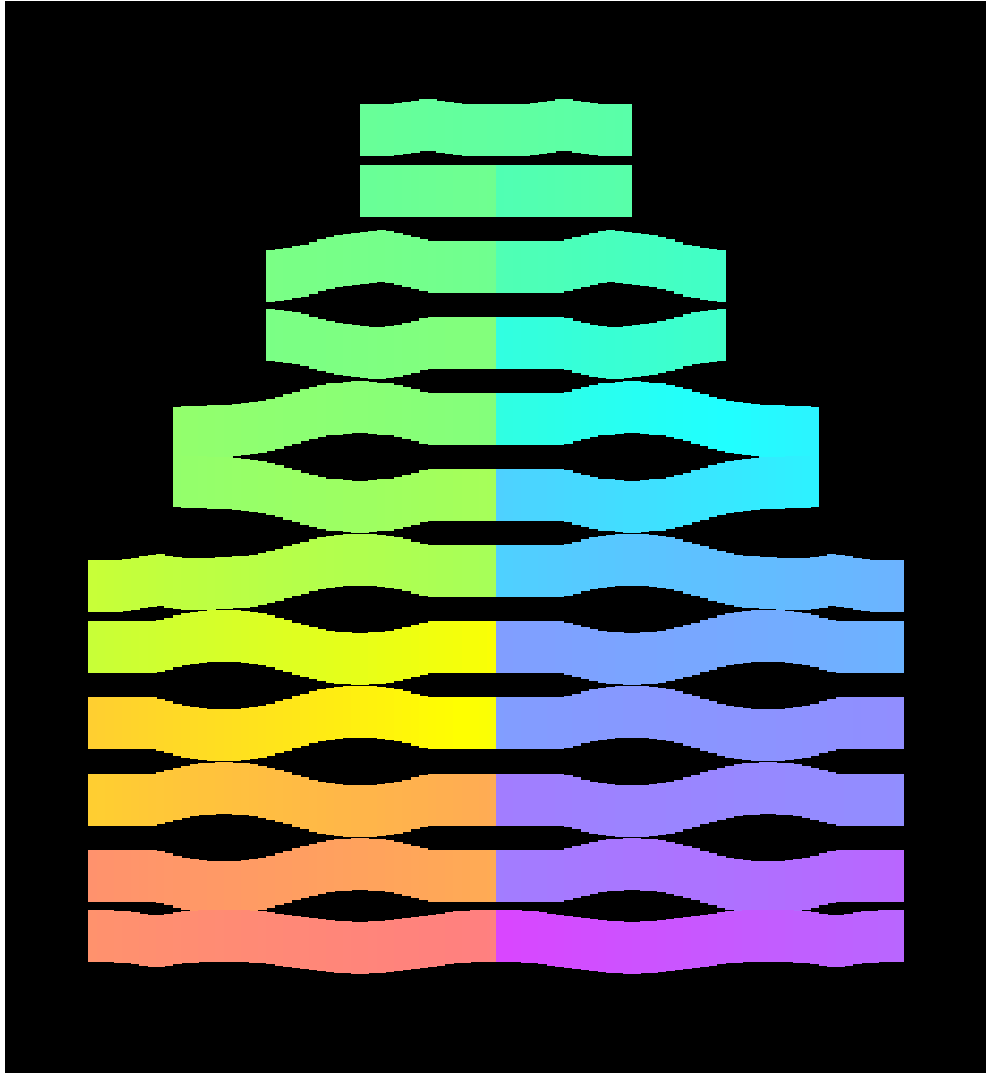
Supplementary Figure S8: Third pass diagram with staple strands after staggered merge.



Supplementary Figure S9: Third pass diagram with staple strands after rectilinear merge.



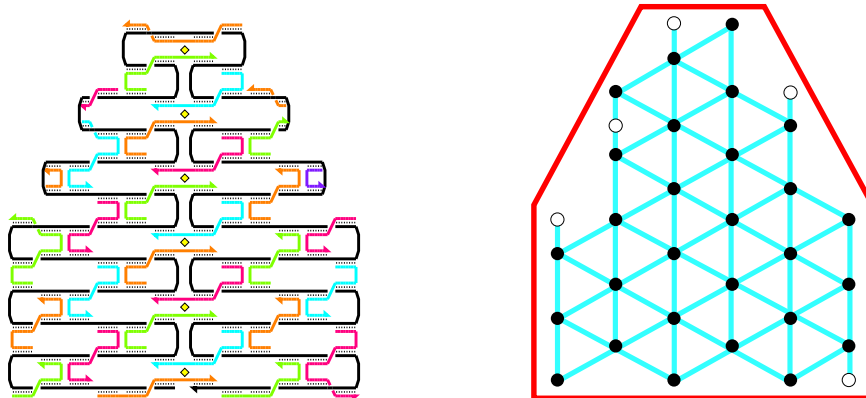
Supplementary Figure S10: Crossover diagram of bridged design.



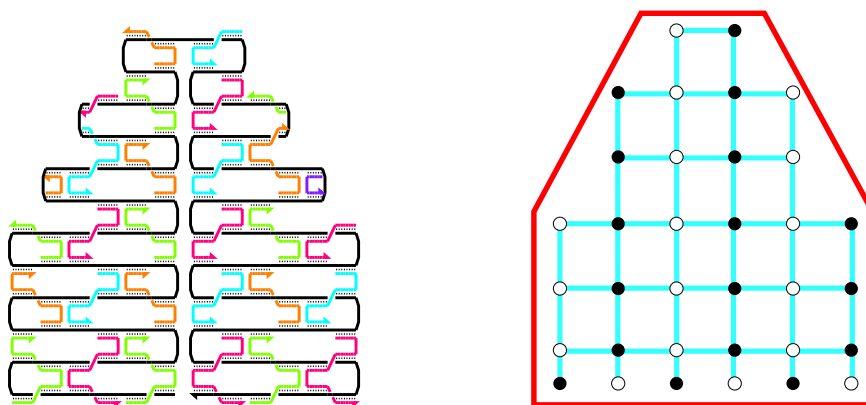
Supplementary Figure S11: Crossover diagram of unbridged design.

a

Staggered merge pattern

**b**

Rectilinear merge pattern



Supplementary Figure S12: Different patterns of merges yield different types of grids for any pixel pattern. Black dots indicate merges made on the top face of the structure, white dots indicate merges made on the bottom face. To create a ‘1’ pixel a hairpin is added at the position of one of the merges. Special cases on the edge of the shape are not normally used for pixels. **a** A staggered pattern of merges. In this case all modifications made to the middle of a staple strand fall on the same face of the lattice. **b** A rectilinear pattern of merges. In this case a modifications made to the middle of a staple strand fall on alternating faces of the lattice, depending on the column in which they occur. While the structure in **a** has a bridged seam, and the structure in **b** has an unbridged seam, the basic pattern of merges is independent of whether or not the seam is bridged.

Supplementary Note S2: Effects of inter-helix gaps and DNA bending on the length and width of DNA nanostructures

When the first planar DNA nanostructures based on parallel double helical domains were made (DNA tile lattices based on double-crossover molecules²⁵) a few assumptions were made about their structure. It was assumed (1) that the helices would be lie close-packed and (2) that the helices would be without bends. Implicit in these assumptions were two more: (3) the length of DNA nanostructures with parallel helices (measured perpendicular to the helices) was assumed to be given by $2h$ nanometers where h is the number of helices and (4) the width was assumed to be $.34n$ nanometers where n was the number of nucleotides in the structure. Here I review what has been learned about these assumptions. (1) turns out to be incorrect, at least for structures imaged by AFM on mica under buffer. Because of this, (2) appears to be incorrect and (3) is not a good approximation for the length (top to bottom) of a DNA nanostructure. While (4) is probably inexact it turns out to remain a useful approximation for the width of a DNA nanostructure.

When a DNA nanostructure with parallel helices bound by together crossovers is imaged by AFM, the result does not model a series of close-packed cylinders. Instead, AFM seems to reveal gaps between helices, typically 1-2 nm wide, whose position and length follow the pattern of crossovers in the underlying structure. Wherever two helices have a crossover, no gap is observed; a few nanometers away from a crossover, an inter-helix gap is observed.

The source of the inter-helix gap is unknown, it may be electrostatic repulsion between helices (as first, to my knowledge, suggested by Rizal Hariadi), or detailed geometry of the crossovers (free crossovers, when not constrained by adjacent crossovers in a multi-crossover molecule, assume an angle of approximately 60 degrees^{32, 33}). It remains for the gap to be measured on different substrates, or in solution, or by a different imaging technique such as TEM, or for it to be measured as a function of salt concentration which might be expected to change the gap by changing the screening of electrostatic interactions.

Whatever the source, the width of the gap appears to depend on the spacing of crossovers: here origami with 16 nt spacing (about 1.5 turns) between crossovers have a ~ 1 nm gap, origami with 26 nt spacing (about 2.5 turns) appear to have a ~ 1.5 nm gap. I note that the relationship between crossover spacing and gap width is not yet proven. Here, all structures with 2.5-turn spacing have one pattern of nicks—that of Fig. 1c—that yields staples that connect only 2 helical domains; on the other hand, all structures with 1.5 turn spacing have a pattern of nicks—that of Fig. 1e that connect 3 helical domains. Thus it is possible that 2.5-turn spacing structure with a nick pattern like that of Fig. 1e might have a different spacing than the 2.5-turn structures explored here. To test whether the pattern of nicks has an effect one could re-render the 2.5-turn spacing square with 3-helix spanning staple strands as in Fig. 1e.

Because the interhelix gap appears to set the aspect ratio of DNA nanostructures constructed from parallel helices, we can use it to attempt to engineer the length and width of DNA origami. With an estimate of the gap in hand, it is simple to design DNA origami with a desired *length* (if a roughly periodic pattern of crossovers is used): the length of the structure should be $2h + (h - 1)g$ nm where h is the number of 2 nm wide helices and g is the inter-helix gap. Lengths measured by AFM are typically within 5% of the predicted length by this formula; it assumed that this error is caused by AFM drift or miscalibration. Note that the formula predicts lengths roughly 50% and 75% greater than those that would be predicted assuming close-packed helices.

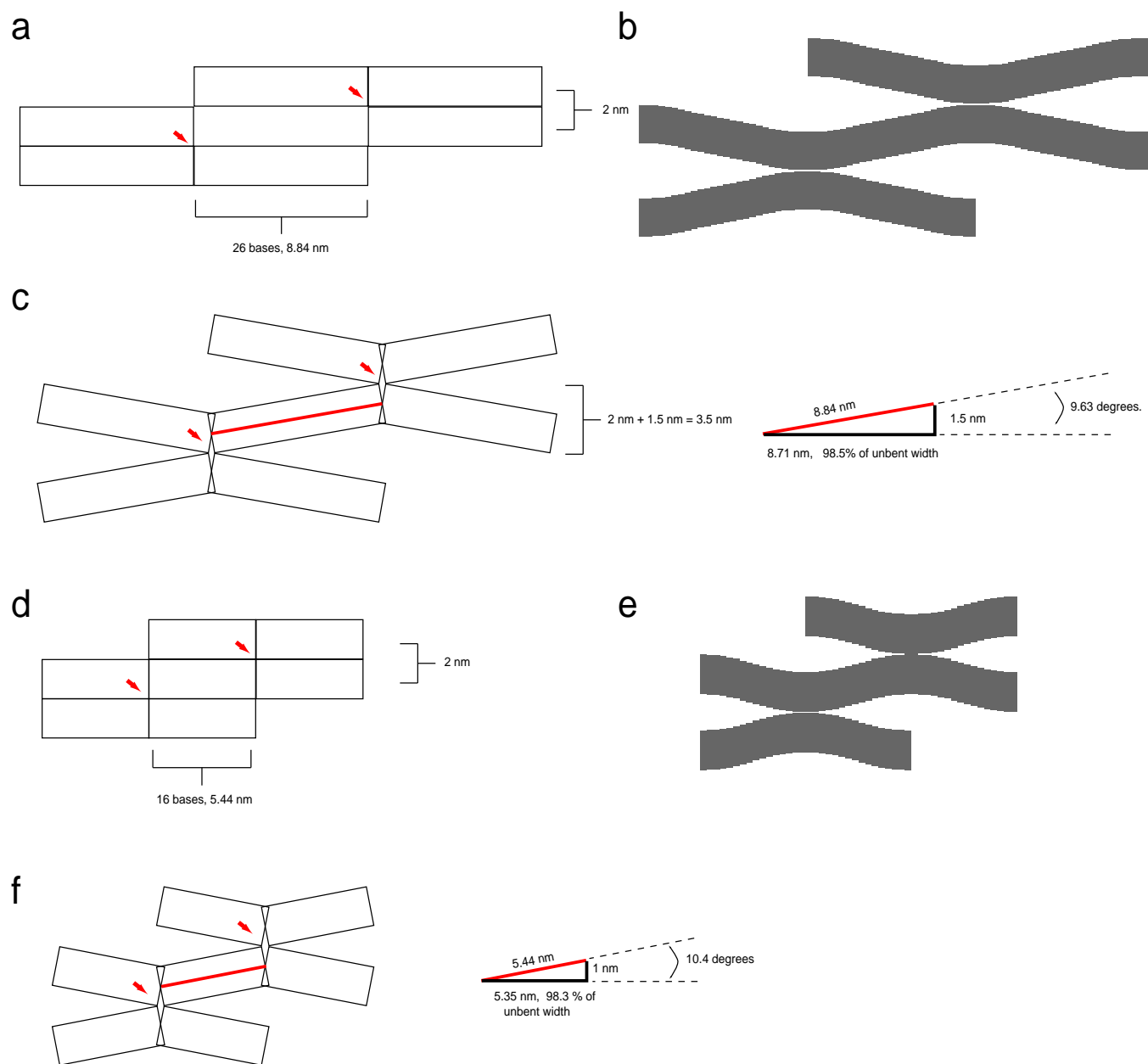
Given an estimate of the inter-helix gap, it would seem *a priori* more difficult to estimate or design the *width* of a DNA origami. To create the inter-helix gap it appears the DNA helices must bend back and forth between the crossovers in which they participate. If one assumes that the contour length of a helix of DNA does not change as it bends and follows a curve, then the end to end distance of a DNA helix following such a curve must be shorter than the end to end distance of a straight helix of the same number of nucleotides. That is, to get a correct estimate for the width of an origami, one must take the bend into account.

However, very little is known about the nature of the bending. So far, few AFM images of DNA have a resolution high enough for the contour of the helix to be traced explicitly and so it seems there is not enough data to model it accurately. Exactly what curve is followed by the helix is probably affected by electrostatic repulsion between the DNA backbones, mechanics of DNA bending, the amount of supercoiling between crossovers, and detailed geometry of the junctions. In the schematic drawing of DNA origami (row 2, Fig. 2) I give a cartoon version of the bending that seeks to reproduce structures seen in AFM images based on the pattern of crossovers in the design. Zooms of the curve used, (based on the sums of exponentials that decay away from crossovers) are giving in Supplementary Fig. S13b and e. There is no reason to believe that these curves are physically accurate.

As a very rough estimate of the change in width due to helix bending, close-packed versions of the 2.5 turn spacing and 1.5 turn spacing lattices (Supplementary Fig. S13 a and d) were deformed by bending the helical domains between crossovers an amount appropriate to create the inter-helix gap (≈ 10 degrees). The projection of these bent domains on the x-axis was then calculated and taken as the new width between crossover. The width between crossovers

changed less than -2% in both cases (Supplementary Fig. S13 c and f), because of the small angles involved. I note that because I use 32 nt to cover 3 helical turns in the 1.5 turn spacing designs, the DNA in most designs is overtwisted (relative to 10.5 bases/turn) by 1.5%. Thus it is possible that relaxation of supercoiling might have a compensatory effect (relative to the effect of bending) on the width of DNA origami. (On the other hand, 52 bases are used to cover 5 turns in the 2.5 turn spacing designs and they are 1% undertwisted with respect to 10.5 bases/turn.)

Finally, the experimental widths of DNA origami are typically within 10% of that predicted using the $.34n$ nanometers approximation. Thus while helix bending appears to happen to accomodate the inter-helix gap, the width of structures is predicted by the formula $.34n$ nanometers to within AFM error.

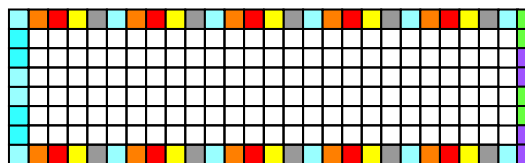


Supplementary Figure S13: A figure that suggests that the effect of helix bending between crossovers contributes little to the width of a DNA origami.

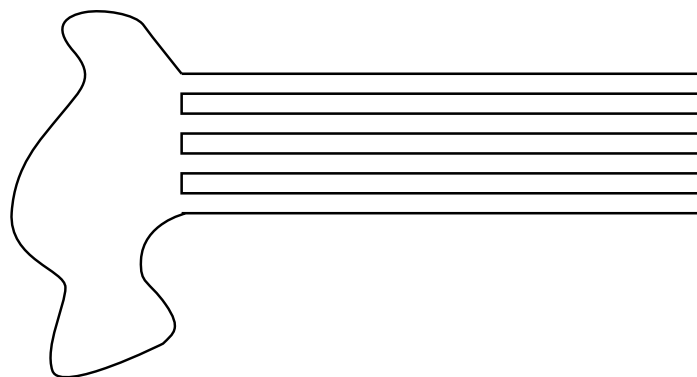
Supplementary Note S3: Designs and sequences

In this note, for all large designs I include: (1) a block diagram and reproduction of the folding path (2) an enlargement of the schematics used to diagram the effects of crossover position on helix bending as in Fig. 2, row 2 and (3) the list of sequences used. A few comments:

1. **Because they are very large and do not print well, the full designs with staple and scaffold sequences explicitly written out appear in a separate file as Supplementary Note 12, not here.**
2. For the 1/3 square, the crossover diagram is not included but is similar to that for the full square.
3. For the smiley and star I include high-resolution AFM that correponds well to the crossover models for comparison. For the smiley I include a model of how smileys can maximize stacking interactions.
4. For the tall rectangle, two different crossover diagrams are given, one for a bridged seam, and one for the unbridged seam (as used in Fig. 3e-i).
5. At the end of this note the full sequence of the New England Biolabs clone of M13mp18 used in this paper is included (Supplementary Fig. S39). The sequence is unpublished and appears to be available only from the NEB web site.

a

26.5 turns wide at 10.4 bases/turn -> 276 bases
8 helices tall

b

Supplementary Figure S14: Schematics for $\sim 1/3$ of the square (8 helices) in Fig. 2a used in the first origami experiments (Supplemental Note S5.3). **a** Block diagram. Designed for 2.5-turn spacing blocks have 5 different offsets with respect to the underlying lattice of crossovers, hence the 5 different hues of blocks in different columns. As in other block diagrams, orange block/red block boundaries have an offset of 0 turns with respect to the underlying lattice of crossovers. **b** Folding path. A circular scaffold is used.

1y_1C; GAGCCCCGATTTAGAGCTTGACGGG
x3y_1C; CAAGTTTTTTGGGGTCGAGGTGCCGT
x5y_1C; CGAAAAACCGTCTATCAGGGCGATGG
x7y_1C; AGTTTGGAACAAGAGTCCACTATTAA
x9y_1C; AATCGGCAAAATCCCTTATAAATCAAAGAATAG

x0y0A; CTGGGGTGCCTAATGAGTGAGCGTGGCGAGAAAGGAAGGAA
x0y0B; GAAAGCCGGCGAACTAACTCACATTA
x2y0A; CCAGTCGGGAAACGGAAACCTTAAAGG
x2y0B; AAAGCACTAAATCCTGTCGTGCCAGC
x4y0A; GGGAGAGCGCGTTCCATCACCCAAT
x4y0B; CCCACTACGTGAATGCGTATTGGGCG
x6y0A; GAGACGGGCAACACACGTCAAAGGG
x6y0B; AGAAGCTGGACTCGCTGATTGCCCTT
x8y0A; GCAAGCGGTCCACTGAGTGTGTTCC
x8y0B; CCCGAGATAGGTGCTGGTTGCCCC

x1y1A; ATTGCGTTGCGCTCTCACAAATCCAC
x1y1B; AAATTGTTATCCGCACTGCCCGCTTT
x3y1A; TGCATTAAATGAATCTCGAATTCGTAA
x3y1B; CCCGGTACCGAGCGGCCAACGCGCG
x5y1A; CCAGGGTGGTTTTTGCCAAAGCTTGCA
x5y1B; AAACGACGGCCAGTCTTTTCAACAGT
x7y1A; CACCGCTGGCCCGTTGGGTAAACGCC
x7y1B; GCAAGGCGATTAAATGAGAGATTGCA
x9y1A; AGCAGGCGAAAATCTTCGTATTAC
x9y1B; GAAGGCGATCGGTGCGGGCCCTGTTTGTGTTGGTTCCGA

x0y2A; CGCGTCTGGCCTTCCTGTAGCCGAAGCATAAAGTGTAAGC
x0y2B; ACAACATACGAGCCAGCTTTCATCAA
x2y2A; GTCGGATTCTCCGTGTTTCTGTGTG
x2y2B; TCATGGTCATAGCTGGGAACAAACGG
x4y2A; CGTTGGTGTAGATACTCTAGAGGATC
x4y2B; TGCCTGCAGGTGCGGGCGCATCGTAA
x6y2A; GACGACAGTATCGTCACGACGTTGTA
x6y2B; AGGGTTTTCCAGGCCTCAGGAAGAT
x8y2A; GCTTCTGTGCGGAGGGGATGTGCT
x8y2B; GCCAGCTGGCGAAGAACCGGCAAA

x1y3A; CATTAAATGTGAGTAAATCAGCTCAT
x1y3B; ATTAATTTTTTGTGAGTAACAACCC
x3y3A; CGGATTGACCGTATATTTAAATTGTA
x3y3B; TTGTATAAGCAAAATGGGATAGGTCA
x5y3A; CCGTGCACTTGCCCCCGGTTGATAA
x5y3B; TCAATCATATGTAAGTTTGAAGGGAC
x7y3A; CGCACTCCAGCCAACAAGGAATCGA
x7y3B; GAGTCTGGAGCAAGCTTTCGGCACCC
x9y3A; GCGCCATTGCGCATTTTGAGAGATCT
x9y3B; AATGCCGAGAGGGTAGCTATTTTCAAGCTGCGCAACTGTTGG

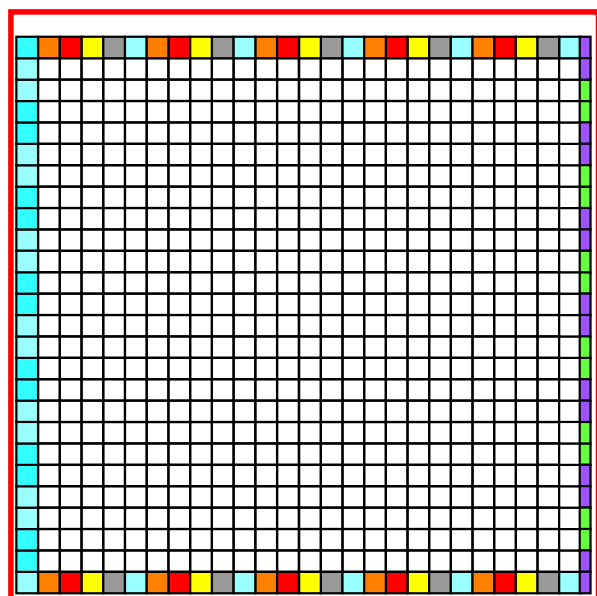
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x0y4B; TTTTAAACCAATACAAAGAAATTAGCA
x2y4A; TAAAGCTAAATCGTGTAAAAATTCGC
x2y4B; AACGTTAATATTTGTTGTACCAAAAA
x4y4A; GGAGAAAGCCTTTAAAAAACAGGAAGA
x4y4B; TCAGAAAAGCCCTTTCAACGCAAGG
x6y4A; TTTTAAATGCAATTAATACTAGCATG
x6y4B; TGAACGTAATCGGCCTGAGTAATGT
x8y4A; AAGGCCGAGACAGGTCAATTGCCTGA
x8y4B; ACAAAGGCTATCAGTCAAAATCACCAT

x1y5A; AAATTAAGCAATAGCTGAAAAGGTGG
x1y5B; ATTTGGGCGCGAAAGCCTCAGAGCA
x3y5A; CATTATGACCCGTATTTCGCAATGG
x3y5B; GACCATTAGATACTAATACTTTTGCG
x5y5A; ATAAAAATTTTACAGTTGATTCCCA
x5y5B; TCATTCCATATAAGAACCTCATATA
x7y5A; GTAGGTAAGATTTGTTTAAATATG
x7y5B; CTGTAGCTCAACACAAAAGGGTGAGA
x9y5A; CAATATGATATTCTTTTCGGATGCG
x9y5B; CTCCTTTTGATAAGAGGTCATAACCGTTCTAGCTGATAAATT

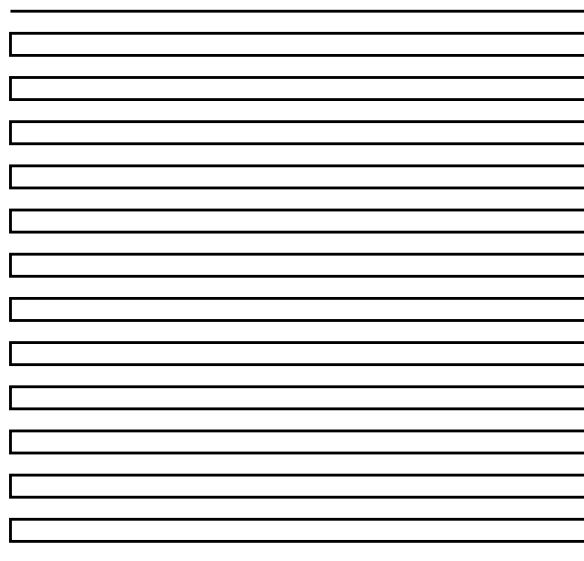
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x0y6B; CATCAATTCTACTCAATACTGCGGAA
x2y6A; CTCAAATGCTTTTATAGCTATATTTTC
x2y6B; TCAATAACCTGTTAACAGTTTCAAGAA
x4y6A; AGGTCTTTACCTGTAGATTAGTTT
x4y6B; ATTTCTGCGAACGAGACTATTAGTTC
x6y6A; GATTAAAGGGAAGGTGCTTGGAGTT
x6y6B; CAACTAAAGTACGCCGAAAGACTTC
x8y6A; AAAGCGAACGAGCTGAATATAATG
x8y6B; TTAGAGCTTAATTCGGAAGCAAACT

x1y7C; TCGTCATAAATATTCAATGAATCCCC
x3y7C; ACGAGAAATGACCATAAATCAAAAAATC
x5y7C; AGAAGCAAAAGCGGATTGCTCAAAAA
x7y7C; AAATATCGCGTTTTAATTGAGCTTC
x9y7C; CCAACAGGTCAGGATTAGAGAGTACCTTTAATTG

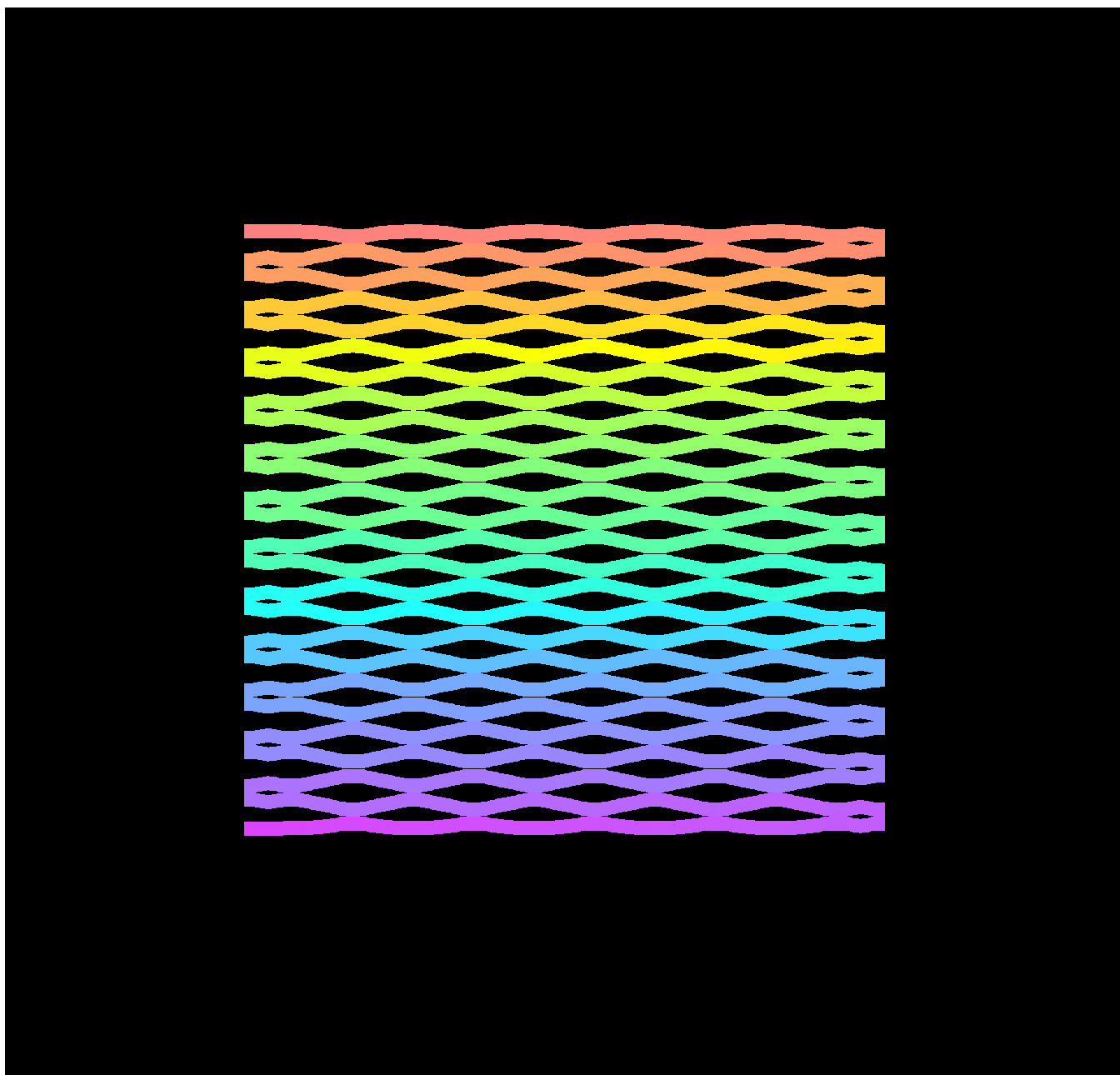
Supplementary Figure S15: Strands used to create $\sim 1/3$ of a square.

a

26.5 turns wide at 10.4 bases/turn -> 276 bases
 26 helices tall

b

Supplementary Figure S16: Schematics for the square in Fig. 2a. **a** Block diagram. Designed for 2.5-turn spacing, blocks have 5 different offsets with respect to the underlying lattice of crossovers, hence the five different hues of blocks in different columns. As in other block diagrams, orange block/red block boundaries have an offset of 0 turns with respect to the underlying lattice of crossovers. The red square highlights our prediction for how well the design is expected to approximate a square. **b** Folding path.



Supplementary Figure S17: Crossover induced structure of square.

1y_1C: GAGCCCCGATTTAGAGCTTGACGGG
x3y_1C: CAAAGTTTTGGGGTCGAGGTCGCCCT
x5y_1C: CGAAGAAACCGTCTTATCAGGCGATGG
x7y_1C: AGTTTGGACAAAGAGTCCACTATTAA
x9y_1C: AATCGGCAAAATCCCTTATAAATCAAAGAATAG

x0y0A: CTGGGGTGGCTAATGAGTGAGCGTGGCGAGAAAGGAAGGGAA
x0y0B: GAAAGCCGGCGAACTAACTACACATTA
x2y0A: CCAAGTCCGAAACGGAAACCTAAAGG
x2y0B: AAAGCACTAAATCTGCTGCTGCCAGC
x4y0A: GGGAGAGCGGCTTCCATCACCCAAAT
x4y0B: CCCACTACGTGAATCGGTATTGGGCG
x6y0A: GAGACGGGCAACCAACAGCTCAAAGG
x6y0B: AGAAGCTGGAATCGCTGATTGCCCTT
x8y0A: CCAAGCGCTCCACTGAGTGTGTTC
x8y0B: CCCGAGATAGGGTCTGCTGTGCCCC

x1y1A: ATTGCGTTGCGCTCTCAAAATTCAC
x1y1B: AAATTTGTTATCCGCATGCCCGCTTT
x3y1A: TGCAATTAATGAATCTGAAATCTGTAA
x3y1B: CCCGCTACCGAGCGGCAACCGCGG
x5y1A: CCAGGGTGGTTTTTGCGAAGCTTGCA
x5y1B: AAACGACGGCGAGCTTTTCCACAGT
x7y1A: CACCGCTGGCCGCTGGGTAAACCGC
x7y1B: GCAAGCGGTAATAGAGAGAGTGTGA
x9y1A: AGAAGCGGAAATTTCTTGGCTATTAC
x9y1B: GAAAGCGGATCGGGTCGGGCGCTGTGTTGATGGTGGTCCGA

x0y2A: CGCGCTCGGCTTCTGTAGCGGAGCATAAAGGTAAAGC
x0y2B: ACAACATACGAGCCAGCTTTCAATCAA
x2y2A: GTCCGATTTCTCGCTGTTTCTCTGTG
x2y2B: TCATGTGTACAGCTGGGACAAACGG
x4y2A: CGTTGGTGTAGATACTCTAGAGGATC
x4y2B: TGCTGTGAGCTCGGGGCGCATCGTAA
x6y2A: GACGACAGTATCGTCAACGAGTGTGA
x6y2B: AGGGTTTTCCGAGCGCTCAGGAAGAT
x8y2A: GCTTCTGTGCGGAGGGGGATGTGCT
x8y2B: GCGCATGTGCGGCGGCAACAGGCGAA

x1y3A: CATTAAATGTGAGTAATCAGCTCAT
x1y3B: ATTAATTTTTGCTCGAGTAAACACCC
x3y3A: CGGATTAAGCGTATATTTAAATTTGA
x3y3B: TTGTATAAGCAAAATGGGATAGGTCA
x5y3A: CCGTGCACTGCGCCCGCGTGAATA
x5y3B: TCAATCATATGTAAGTTTGAAGGGAC
x7y3A: CGCACTCCGACCAACAGAGATCGA
x7y3B: GAGTCTGAGAGCACTTTCCGGCACCC
x9y3A: GCGCCATTCGCCATTTTGAAGAGATCT
x9y3B: AATGCGGAGAGGGTAGCTATTTACGCTGCGCAACTGTTGG

x0y4A: CAATAAATCATACAGGCAAGGGGACGCCATCAAAATAAT
x0y4B: TTTTAAACCAATCAAGAATTAGCA
x2y4A: TAAAGCTAAATCGTGTAAATTCGCC
x2y4B: AACGTTAATATTTGTTGATCCAAAA
x4y4A: GGAGAGGCTTTAAAAAAACAGGAAGA
x4y4B: TCAGAAAAAGCCCTTTCAACGCAAGG
x6y4A: TTTTAAATGCAATTAACACTAGCATG
x6y4B: TGAAGCGTAATCGCGCTGAGTAATG
x8y4A: AAGCGCGAGAGCGGCTATGCGCTGA
x8y4B: ACAAGGCTATCAGTCAATCACCAT

x1y5A: AAATTAAGCAATAGCTGAAAGGGTGG
x1y5B: ATTTGGGGCGCGAAAGCTCAGAGCA
x3y5A: CATTATGACCGTGAATTCGCAAAATGG
x3y5B: GACCATTAGATACTAATACTTTTGGC
x5y5A: ATAAAAATTTTACAGTTGATTTCCCA
x5y5B: TCATTCATATAAGAACCCCTCATATA
x7y5A: GTAGGTAAAGATTTGTTTAAATATG
x7y5B: CTGTAGCTCAACCAAAAGGGTGAGA
x9y5A: CCAATATGATATCTTTTGGGATGGC
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x0y6A: ATGTTTAGACTGGATAGCGTCAATAGTAGTACCATTAACATC
x0y6B: CATCAATCTACTCAATACTCGCGGAA
x2y6A: CTCAAAATGCTTTATGCTATATTTTC
x2y6B: TCAATAAGCTTTTAAAGCTTCAAGAA
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x4y6B: ATTTCTGCGAACGAGACTATTATAGTC
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x6y6B: CAACTAAAGTACGCGGCAAGACTTC
x8y6A: AAAGCGAACAGAGCTGAATATAAGT
x8y6B: TTAGAGCTTAATTCGGAGGCAAACT

x1y7A: TCGTCATAAATATCGAGAGGCTTTTG
x1y7B: AAAACCAAAATAGTCAATGAATCCCC
x3y7A: ACGAGAATGACCAAGCAACACTATCA
x3y7B: GAGGCTAGTAAGTAAATCAAAAAATC
x5y7A: TTAGGAATACCAAGCTTCACTCAAAA
x5y7B: AAATATCGCGTTTACAACTATTATC
x7y7A: AGCAACTAAAGGATAATTCGAGCTTC
x7y7B: CCAACAGCTCAGCGCAGTCAGAGCT
x9y7B: TTTAAGACTGCGCTATTATAATAGAGAGTACCTTTAATGG

x0y8A: CGGGAACGAGGCGGACGCTGCCAGAGGGGTAAATAGTAA
x0y8B: CAAAAGAGTTTTCATCATTAAGGA
x2y8A: ACAGATGAACGGTTCAGACGACGATA
x2y8B: TAAACCTCGTTTATGACAGACGACGG
x4y8A: AGTAATCTTGAACAAGGAATTAAC
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x6y8A: TGCTCATTCAGTGCATGTTGAGAT
x6y8B: AGGTGAAGAAGATTAATAGGCTTGCC
x8y8A: TAAATGGGCTGTACTGCTTAAATAA
x8y8B: TGGGAAGAAAAATAGATGGTTTAATT

x1y9A: ACCGAACTGACGAGCTGATAAATGG
x1y9B: GATTTGTATCATCATCTTGAAGAGGG
x3y9A: GCATAGGCTGGCTCCAGCGGATTATA
x3y9B: CTCATCTTTGACCGACTTTCATCAAG
x5y9A: CATTACCAAACTCAACCTTAAACGA
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x7y9A: CTGACGAGAAACATGAGGAAGTTTCC
x7y9B: AAAGACTTTTTTACGAGAGAGTAGT
x9y9A: TCAACTTTAATCAGGAAGAGGGTAG
x9y9B: TCAGCAGCGAAGCAAGACTCTTGGAATACCTTATGCGAT

x0y10A: AACTTTCAACAGTTTACGCGGACCTGCTCCATGTTACTTAG
x0y10B: TGTCGAATCCGAGTGAAGATAGAA
x2y10A: ATAATTTTTCACAAAGTCAACGGA
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x7y11B: CCTCAGAACCGCCACGCAATACCG
x9y11A: GGGATTTAAAGGCTCAGGAGGTT
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x0y12A: GGAAGCGCAGTCTCTGAATCTGTATGGGATTTGCTAAAC
x0y12B: GTAAATGAATTTTACGTTCCAGTA
x2y12A: CAGGAGGTGTACTGGCGTAACGATCTA
x2y12B: CGCCCTCATTTAGTATTAAGTTTGA
x4y12A: GCGCGTATAAACACAGTACAAACT
x4y12B: ACTGAGTCTGCTGTTAATGCCCTCT
x6y12A: AACATGAAGTATCAGGATAGCAAG
x6y12B: CCACCTCATTTTGAAGGCTGAGA
x8y12A: CGGGGTTTTGCTCTCAGAACCGCCAC
x8y12B: AGGACCGCAACCAAGTACAGCGGGA

x1y13A: AGCGCTCATACATGTTGGCCTTGATAT
x1y13B: CGAGGTACAGAGGCTTTGATGATA
x3y13A: ACGGGTCAGTGCCACGACGAGCC
x3y13B: CGCCACCAAGCACTGTAGTAAGT
x5y13A: GCCTATTTCCGGAACCTCAGAACCGC
x5y13B: CTCAGAGCGCCACTATTAATCTGA
x7y13A: CTCCTCAAGAGAAAAATCACCGGAA
x7y13B: CTCTTTCATATCGGATAGGATTAG
x9y13A: TAAGTGCGCTCAGAGGCAATTTGCGT
x9y13B: CAGACTGTAGCGGCTTTTATAGGGTGTATATAAGTATAGC

x0y14A: TTAATTTGTCAACAATCAATAGACTCTCATTAAGCCAGAA
x0y14B: TCACAAACCAATAAAATTCATATGG
x2y14A: GACATTCACGCAAGCAGAGGTTGAG
x2y14B: GCGCGCAGCAATTTGTTGAGGAGGGAA
x4y14A: TAAAGGTGAATTAACCACTCAGAG
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x8y14A: TGAACCACTGATTAAGCGTTTGCCA
x8y14B: CATAGCCCTTTAAGCAGACCGGTAA

x1y15A: TTTACAGCGCCAAAGGTGGCAACA
x1y15B: AAAATACATACTAAGACAAAAGGCG
x3y15A: GGTAAATATTGACATTAAGACTCCTT
x3y15B: AAGAACTGGCATTTGATTAATTCAT
x5y15A: CTGAGGCAATTTGAAGGAAACCGGAG
x5y15B: ACAAGTTTACAGGAAATAGAGCCA
x7y15A: ATTAGCAAGGCGTACCGAACGCTCT
x7y15B: GCAATAGCTCTTGAAGGCTCACAA
x9y15A: TCAGTAGCAGGAGGTTAAGCCCAA
x9y15B: GAGAGATAACCCCAAGAAATTAACAGTTTGCCTTTAGCGT

x0y16A: AATCAAGATTAGTTGCTATTTCAAAGACACCCACGGAATAAGT
x0y16B: TATAAAGAACCGTGACCCAGCTAC
x2y16A: TAACGAGCGTCTTTTGAAGCAAGCTAG
x2y16B: ATTACGAGTATGTTGACAGGCTAAT
x4y16A: ATTTATTTCCCAACGGAAATCCCA
x4y16B: AAACGCAATTAATCAAAATAGAA
x6y16A: GAAATAGCAGCGACGAGTACCGGA
x6y16B: TTTAAGAAAAGTATTTCAGAGAGAA
x8y16A: GACCGGAGAAATTAACAAATGAATA
x8y16B: TAAATAGAGCAAGACTGAACCCCTG

x1y17A: AATTTTATCTGAAGCGGTTTATGCG
x1y17B: TTCTAAGAACCGCATTTACCAACGC
x3y17A: TTGCGAGTTACACAAATAGCAAGCAA
x3y17B: TCATTACGCGCAATTAACAGCCAT
x5y17A: ACGATTTTTGTTTGCAGAGAACGA
x5y17B: AGTACCGCATCTAACGTCAAAAAT
x7y17A: TAACATAAAAACTGCTTTCTCTTA
x7y17B: AATCAATAATCGGGGAGAGCGATTA
x9y17A: AACAAAGTCAGAGAAATTAATCCCA
x9y17B: TAGATAAGTCTTGAACCAAGAGATTTAGGCGCTAATATCA

x0y18A: AATAAGAATAAACCCGGAATCGGAGGTTTTGAGCCCTTA
x0y18B: AACCTCCGACTCTCATTAATCTAGTA
x2y18A: TTACAAATTTCTGGCTTATCCGGTA
x2y18B: ATAGATATAGAAAACAGTATAAAGC
x4y18A: AGAATCGCATATTTTATCTGTAGGAA
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x6y18A: GAGCGCTATTAAGGGTATTAACCA
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x8y18A: CCAGACACGACAGCATGTAGAAAC
x8y18B: TCCTAATTTACGAATAAACCAATGT

x1y19A: AAAAGCGTGTTTTATAAATTAATGGT
x1y19B: TCATCTCTGAGCGGTATCATATGCTG
x3y19A: CAAAGCTCAACGAGAACGAGCAAG
x3y19B: ATCCGAGACCAATAGGGCTTAATGG
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x5y19B: CTCGCGCTTAGGTGAGGGCATTTTC
x7y19A: ACCGACAAAGAGTTTTTATCAAAATC
x7y19B: AGTCAATAGTAAAAAGTAATTTCTGT
x9y19A: TCAGCTAATCAGTGAACCAATAGCG
x9y19B: TTAATTTCCCTTAGAATCTCAACGCGCTGTTTATCAACAA

x0y20A: TTTAACGTCAGATGAATATACCGTGTGATAAATAAGGCGTTA
x0y20B: TTGAATAACGACAGTAAACAGTACCT
x2y20A: TTGCGCTGATGCAATTTTAGTTAAAT
x2y20B: CTTTTTCAAAATTTTGAATACAGCA
x4y20A: TTCATTTCAATATGATGCAAACTCA
x4y20B: TATATGAATAGCTTGAAGCAAGAA
x6y20A: CAAATTAATTAACCTACCTTTTAAAC
x6y20B: TAGGCTGAGAGAAATTAACAAATTC
x8y20A: CAGTACATAAATCAGACGCTGAGAG
x8y20B: ATAGCTTAGATTAAATATATGTAGG

x1y21A: TTTACATCGGAGTATTTTGCAGCTA
x1y21B: TACCATATCAAAAAACATAAACGGA
x3y21A: TTAACAAATCGCGGTTTGGATTATAC
x3y21B: TATACTGATGTAGAGCGGCTGAGTA
x5y21A: AGATGATGAACACATTCATATTCCTG
x5y21B: GGAGCGGAATTAACCATCAAGAAAT
x7y21A: ATTTGAATTAACCTGTAACTATCAT
x7y21B: TTTAAAGGTTTGTATTTTAAAGGAA
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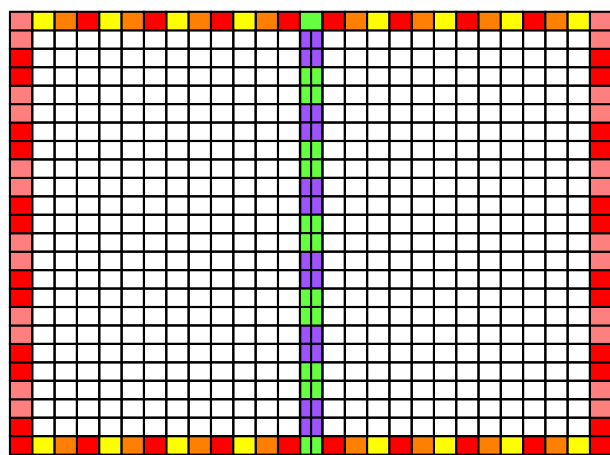
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x4y22B: ATTAATCAGATGATCTCAAAATCAA
x6y22A: GCAAACTCAACAGTGAACGACGAGAA
x6y22B: TTTGCGGAAACAAATGAAGGAATGA
x8y22A: AGCACTAACAACTGAAGCTTATTAAT
x8y22B: AAATCCTTTGCCCAATAGATTAGAGC

x1y23A: GAGGTGAGGCGGTTTATGCTTTAATG
x1y23B: TTTTGAATGGCTACAGATTAACACCC
x3y23A: CGCAGCAAAATGACTGACTGAAAGC
x3y23B: AGATAGAACCTTTAAATATCAAGCA
x5y23A: ACCCTCAATCAATTCACAGACAGAT
x5y23B: GCAGATTCAACAGATCTGGTCAAGTTG
x7y23A: GGAAGGTTATCTATTTTACGCTCAA
x7y23B: GGAATAACCTCAAAATATCTTTAGG
x9y23A: COTCAATAGATAAAATATTAACGCCA
x9y23B: CTTGCTGTAATATTCAGAAATTTAGGATTAGAAGT

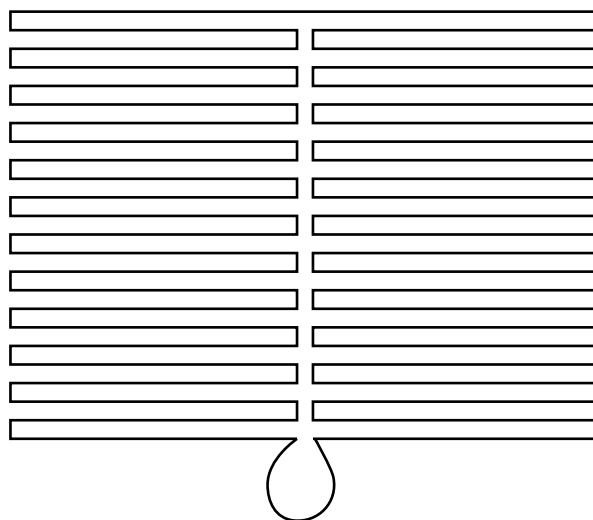
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x0y24B: CGCGAACTGATAGGCGCTACTATG
x2y24A: GCTTCTCGCTTAGCACAGCAATAT
x2y24B: GTAAGAATACGTGGAATCAGAGCGG
x4y24A: ATTTAGACAGGATTCTGGCCACAGC
x4y24B: AATAAAGGGCAACCGGTACGCCGA
x6y24A: TGAGGCCACAGAGATTATTACATGG
x6y24B: TCGTCTGAATGGTAAAGAGTCTGT
x8y24A: AATACTTCTTTGAGAAAAAGCTCAT
x8y24B: GCCATTGCAACAGTTAGTATAACAT

x1y25C: GTTGCTTTGACGAGCAGTATAACGT
x3y25C: AGCTAAACAGGAGGCGGATTAAGGG
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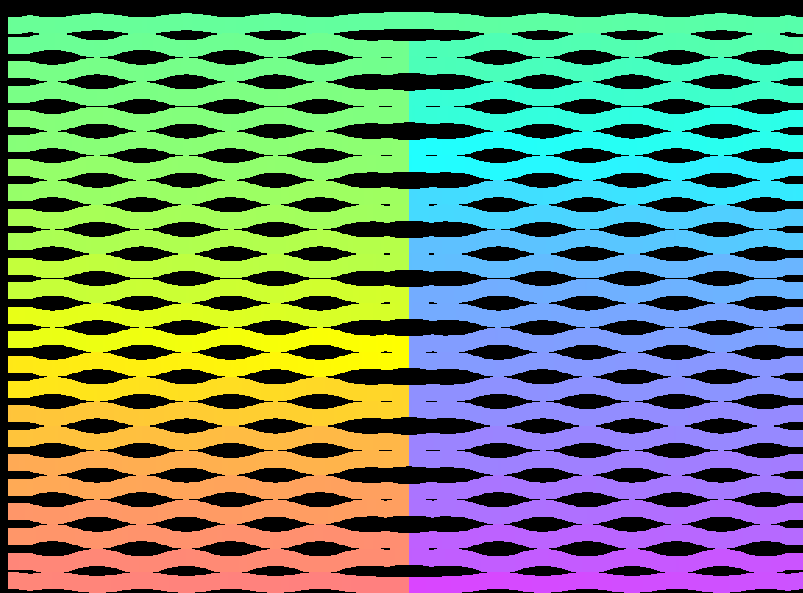
Supplementary Figure S18: Sequences for the square.

a

27 turns wide at 10.666 bases / turn -> 288 nt
 24 helices tall

b

Supplementary Figure S19: Schematics for the rectangle Fig. 2b. **a** Block diagram. **b** Folding path.



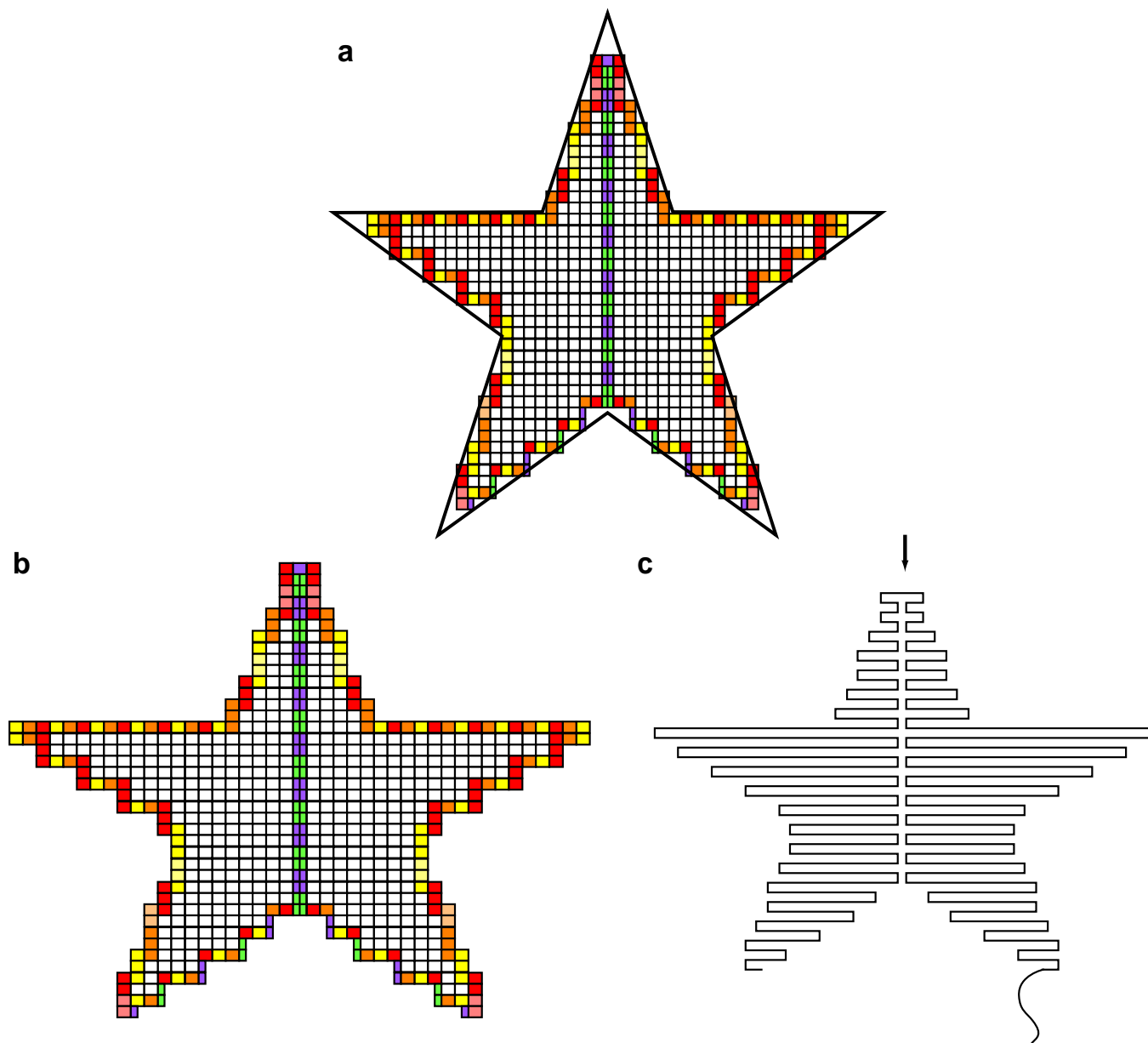
Supplementary Figure S20: Crossover diagram for the rectangle.

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r1t10f, C1, CCTAATTTTACGCTAACAGAGCGCTCTATATCGCG
r1t12e, D1, CTAATTTATCTTTTCCCTTATCATTCATCTCGTAA
r1t12f, E1, ATCGGCTCGGAGCATGTAGAAAGAACCCAGTATAT
r1t14e, F1, AATTACTACAAATTTCTTACCGATTAATCCCATC
r1t14f, G1, CGGTTATAGAAAAGCCTGTTTACGAGGCGCG
r1t16e, H1, TAGAATCCCTGAGAAGAGTCAATAGGAATCAT
r1t16f, A2, TTAAGACGTTTGAAGACATAGAGATTTAAATCA
r1t18e, B2, TTTAAGCTTCGCGGAGAAACAAATATTTTCCT
r1t18f, C2, CTTTATACAGATGAATATACAGTAAGCGCA
r1t20e, D2, GGATTTAGCGTATTAATATCTTTGTTTTCAGG
r1t20f, E2, CGACAACCTAAGTATTAGACTTTTACAGCCGGAA
r1t22e, F2, TAGCCCTTACAGCAGAGATTAACAAATTTGA
r1t22f, G2, ACGAACCAAAACATCGCCATTAATGGTGGTT
r1t24h, H2, CGGCTTGTCTGTGTAATATCCAGAAACGAACTGA
r1t2e, A3, TGCCCTTGACTGCCTATTTCGGAACAGGAGTAG
r1t2f, B3, AATGCCCGGTAAACAGTGGCGCTGTGTGAATTT
r1t4e, C3, AACCAGAGACCTCTCAGAACCGCCAGGGGTGAG
r1t4f, D3, GAGCCGCCCCACACCGGAGACCGCTTAAACAA
r1t6e, E3, ATTGAGGCTAAAGCGTGAATTTAATCAATCACCGG
r1t6f, F3, TTATTTATAGGAGAGGTAAATATTCATTCAGT
r1t8e, G3, GCAATAGCGAGATAGCGGAACAAATCAACCG
r1t8f, H3, AAAAGTAAATATCTTACCGAGGCCAACATAT
r3t0g, A4, CTCAGAGCCACACCTCATTTTCCCTATTATT
r3t10e, B4, TATTTTGTCTCCCAATCCAAATAAGTAGGTTAA
r3t10f, C4, ATTTATTTAAACCCAGCTACAATTTTCAAGAAAG
r3t12e, D4, TAAGTCTTACCAAGTACCCGACTCTTAGTTGTC
r3t12f, E4, GGTATTAAGAAACAGAAATAAATAAAGGCCA
r3t14e, F4, AGGCGTTTACAGTAGGGCTTAATTCGCAATAGA
r3t14f, G4, ACGCTCAAAATAGCAATAGAAATCAACCGCTGAATTT
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r3t16f, A5, ATCAAAATCTCGCTCAATTAATTAAGCAAGTTG
r3t18e, B5, ACAGAAATCTTTGAATACCAAGTTCTTGTCT
r3t18f, C5, CCTGATTGAAAGAAATTCGCTAGAACCGGAACG
r3t20e, D5, AGATTAGATTTAAAGAGTTTGAGTACAGCTAAA
r3t20f, E5, TTATTAATGCGCTCAATAGATTAATCAGAGGTG
r3t22e, F5, GAATGGCTAGTATTAACACCGCTCTCAACTAAT
r3t22f, G5, AGGCGGTCAATAGTCTTTAATAGCGCAATATTA
r3t24h, H5, CCGCCAGCCATTTGCAACAGGAAATAATTTTT
r3t2e, A6, AGTGTACTTGAAGAGTATTAAAGAGCGCCGACC
r3t2f, B6, CTGAACAGGTAAATAGTTTAAACCCCTCAGA
r3t4e, C6, GTTTGCCACCTCAGAGCGCCGACGATACAGG
r3t4f, D6, GCCACCACTCTTTTCAATACCAACCGCTCAC
r3t6e, E6, AGCGCCAAACCAATTTGGGAATTAGATTATTAGC
r3t6f, F6, GACTTTGAGAGACAAAAGGGGAGCAAGTTACCA
r3t8e, G6, GCCCAATACCGAGGACCTCAGATTTTACC
r3t8f, H6, GAAGGAAATAGAGCAGACGAACCAACAGCCAT
r5t0g, A7, CCTTCAGAAACCGCCACCTCAGACCTGAGACT
r5t10e, B7, AGGTTTGAACCTCAAAATGAAGAGCGCTAAT
r5t10f, C7, TTTTGTTTAAGCCTTAAAGAGTATTGATTTG
r5t12e, D7, AATGCGAGCCGTTTATTTTATCTTGTGCGGG
r5t12f, E7, CAAGCAAGAGCGCGCTGTTTATCAGCAATCGC
r5t14e, F7, AATGGTTTACAAACCGCAACATGTAGTTACGCT
r5t14f, G7, CATATTTAGAAATACCGACGCTGTATCTTT
r5t16e, H7, AAATCAATGGCTTAGGTGGGTACTTAAATTT
r5t16f, A8, TAACTCTCATATGTAGTGAATAAACAATAATC
r5t18e, B8, AACTTACCGCAATATTATTTATTTCCAGTACAT
r5t18f, C8, CGCGAGAGATATCAAAATTTTTCAGTATTC
r5t20e, D8, CTAAATATAGAACAGAAACCAACCGGTTAG
r5t20f, E8, ATTTTGGCTCTTTAGGAGACTTAAGCAACAGT
r5t22e, F8, GCGTAAAGAGAGAGCCAGCAGCAAAAGGTTAT
r5t22f, G8, GCCACGCTATACGTGGGACACAGACCGCTCAT
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r7t20f, E11, CGGAATTTATGGAAGGAATTTGAGGTGAAAAAT
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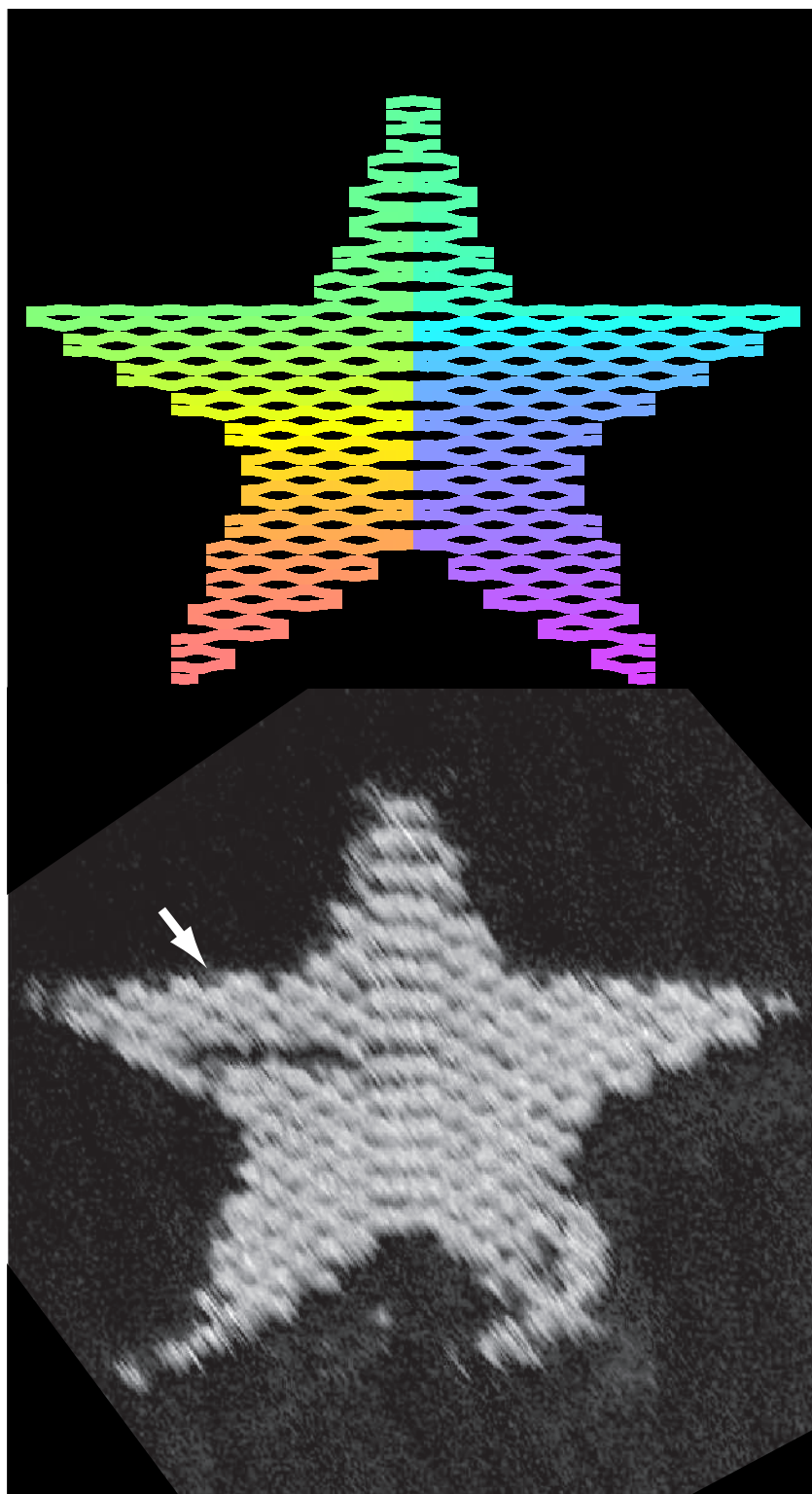
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r9t18f, F1, AAAACAAATTCATCAATATTAATCTTATCAGAT
r9t20f, G1, GATGGCAAAATCAATATCTGGCTCAAAATATC
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r-5t14e, B9, GGTAGCTAGGATAAATAATTTTGTAGTAAATC
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r-7t12f, A12, TGCAACTAAGCAATAAAGCCTCAGTTATGACC
r-7t14e, B12, TCAGGTCACTTTTTCGGGAGAGAGAGATTAAG
r-7t14f, C12, CTGTGAATTTGCTGAGAGCTGCGAAAACTAG
r-7t16e, D12, ACCGCTGCTCATATGTACCCCGTAAAGGATTA
r-7t16f, E12, CATGTCAAGATTCTCCGTGGGAACCGTTGGTG
r-7t18e, F12, ATTAAGTTTGCATCTGTAAACCGGTGCGAGTAA
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r-7t22f, C1, TGGTTTTTAACTGCAAAAGGCGAAGAGCAAT
r-7t24h, D1, ACCCAAAATCAAGTTTTTGGGGTCAAAAGAAC
r-7t2e, E1, AAAGGCGAAAGGAAACACTAAAGCTTTCCAG
r-7t2f, F1, GAGAATAGCTTTTGGCGGATCTGCGGGTAGCA
r-7t4e, G1, GCTTCATGAGAGGCTTTGAGGACTAGGAGATT
r-7t4f, H1, ACGGCTACTTACTTAGCGGGAACGCTGACCAA
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r-9t24e, D3, CGATGCGCACTACGTAAACCGCTG
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r-9t4e, F3, ACGGTCAGACAGCATCGGAACGAACCCCTCAG
r-9t6e, G3, GAGCGGTGTCATAAGGAGAACGGAAGCGCGAG
r-9t8e, H3, TAAATTTGGAAGAGATTTACGACCACTCA
rt-rem1, A4, AACATCACTGCTGGAATGAGAGAACT
rt-rem2, B4, TGTAGCAATCTTCTTGTATTAGTAA
rt-rem3, C4, AGCTCTGCTCATCAGCAATTTAACCGT
rt-rem4, D4, ATCATCAGTGAGGCGACGAGGATAAAG
rt-rem5, E4, ACGTACGCTTACAGGAGGTGTTTTT
rt-rem6, F4, TTAAGGGGATTTTAGACAGGACCGGT
rt-rem7, G4, AGAGCGGAGTAAACAGGAGGCGCA
rt-rem8, H4, TATGAGCTGCTTCTCTCTGTTAGATTC
rt-rem9, A5, GTACTATGGTTGCTTTGACGAGCACT
rt-rem10, B5, GCGCTTAAAGCGCGCTACAGGGCGC

Supplementary Figure S21: Sequences for the rectangle.



Supplementary Figure S22: More design details for the star **a**, Original block diagram assuming that each block (1 turn of DNA) would have an aspect ratio of roughly 1:1 (3.5 nm per turn:3.5 nm per helix) based on the inter-helix gap for 2.5 turn spacing. In reality 1.5-turn spacing appears to have a ratio of roughly 1.2:1 (3.6 nm per turn:3 nm per helix) and so the stars were somewhat squat **b**. **c** reproduces the folding path for reference. In **a**, turns that occur between columns of red blocks and orange blocks have offset 0 with respect to the underlying crossover lattice. Other turns on the left and right outer edges have +1 or -1 offsets depending on which side of the star they occur. Purple and green half-blocks show that turns (in the scaffold) made on the seam or on the interior of the bottom left and right star arms are made an odd number of 1/2 turns (DNA half-turns) away from turns (in the scaffold) on the outer edges.



Supplementary Figure S23: Crossover diagram for the star (top) and high resolution AFM (bottom, taken by E. Winfree) which shows the crossover structure in great detail. Defects are probably tip damage. White arrow points to a section of helix that does not image well and appears to be a hole. On edges where helices can move unimpeded by neighbors helices often disappear at high tapping amplitude.

Plate number: 1

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s11t16e, D1, CATTCAACATATTCAGAGAGATAACTAACATAA
s11t18g, E1, AAACAGGGAACAAGCAAGCCGTTTTCACGCACTCATCGAG
s11t18h, F1, AAGCGCATTTAGACGGGGGTATTTGAGCGCTACAGATTGAG
s12t15f, G1, GGAGGGAACAGCAAAATCACCAAT
s13t14e, H1, ATTTGGGAATTTAGAGCGGTAAATA
s13t16g, A2, TTGACGGAACCTGAACAAAGTCAGAGAATTTAACTGAACAC
s13t16h, B2, AATTATTTTCATTTAAAGGTCACCGACTTGAGCC
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s1t18i, A3, TTATCTCGCTATTTTTCACCCAGTAATAGTA
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s1t24i, D3, AATTTCTCTCTGTAAATCTGTCGAGAGATCT
s1t26i, E3, TTGAATACCAATAAGCGATTTCGCCAAATCAGC
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s1t9b, B6, ATGATACTTTTG
s1t9f, C6, ATACATGCGAGGAGTGTACTGTACAGTGCC
s1t10e, D6, ACAACAAATAAATCCATTTGCGCT
s1t12e, E6, TGATATTTGCGCACCCAGCTTCAGAGCCCTCAGA
s1t14e, F6, GCCGCCACCGGTATAGTACCCCTTGTTAGCA
s1t16e, G6, ACGTAGAACAGAGGAACCAAGGACGAGCGT
s1t18e, H6, CTTTCCAGGAGGTGTTTGAAGCGCTCTGTATTA
s1t20e, A7, CAACATATATAAAGTACGACACCAAGCCGCT
s1t22e, B7, GTGATAAAGAGCAAGAAAGCGAGACATAGCG
s1t24e, C7, ATAGCTTAAATAATCAATATTTAGTGTGAGAGCG
s1t26e, D7, GAATATTTTACGATTAACAGTACCTCTCGATTG
s1t28e, E7, TTTGAGTTTGGAGAACAGAAACCAACACTA
s1t30e, F7, ATAGATTTATCAATCAATATCTGTGTAGCCCTA
s1t32h, G7, AAACATCGATTTTGAATGCTATTAGTCTTTAAT
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s1t31f, B9, AATCCGACCTCAATATCAACACCGAGCGCTC
s1t33f, C9, ATTTTGAACGCTGCGACCAACCCGATTAATA
s1t35f, D9, CATTGCAACAGGAAAAATACCTTAC
s1t14e, E9, GTCAGACTGTGAGCGTGCAGCA
s1t16e, F9, TATAAAGAGAAAAGTAAGCAGATTACAAAAAT
s1t18e, G9, AAACAGCCCGGAGGCGTTTTCGCGAAAAATA
s1t20e, H9, ATATCCCGAGGAGGCTTTTTCGAAATAAACA
s1t22e, A10, CCGGAATCTATATGTAAATCTGAAGAGTCA
s1t24e, B10, TAGTGAATATTTGAATTTACCTTTTTCGAAAGAA
s1t26e, C10, AGATGATGCGGTAGATTTTTTCGTTGAAGGGTT
s1t28e, D10, AGAACCTTAATTTTAAAGTTTGAAGTACATTT
s1t30e, E10, GAGGATTTCTGATCACTCTGTAACAGAAACCA
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s1t34e, G10, GTCGTGAACAAATATTACGCCAGC
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s1t17f, B11, TATCCCAAACCGAAGCCCTTTTAAAAAGCGAA
s1t19f, C11, TACGAGCAGCGGTATTTTCAGAAACATTAAT
s1t21f, D11, TAGAAAAACCAACATGTAATTTAGTCTTAAT
s1t23f, E11, ATCATAGGGGTGGGTTATATAACATAATTAC
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s1t29f, B12, TAGACTTTTTCGCGCAAGCTTTATACCATATCA
s1t31f, C12, CAGAGGTGAATGAATAATTAAGAGAGATTA
s1t33f, D12, TTAACATTTAGTAAGACCTTTCTGACAGATAAAA
s1t35f, E12, CGCAATTTCTGTTAAATATCAGAAATGATTAT
s1t37f, F12, TCAATGAGGCCACGAGTCCATCA
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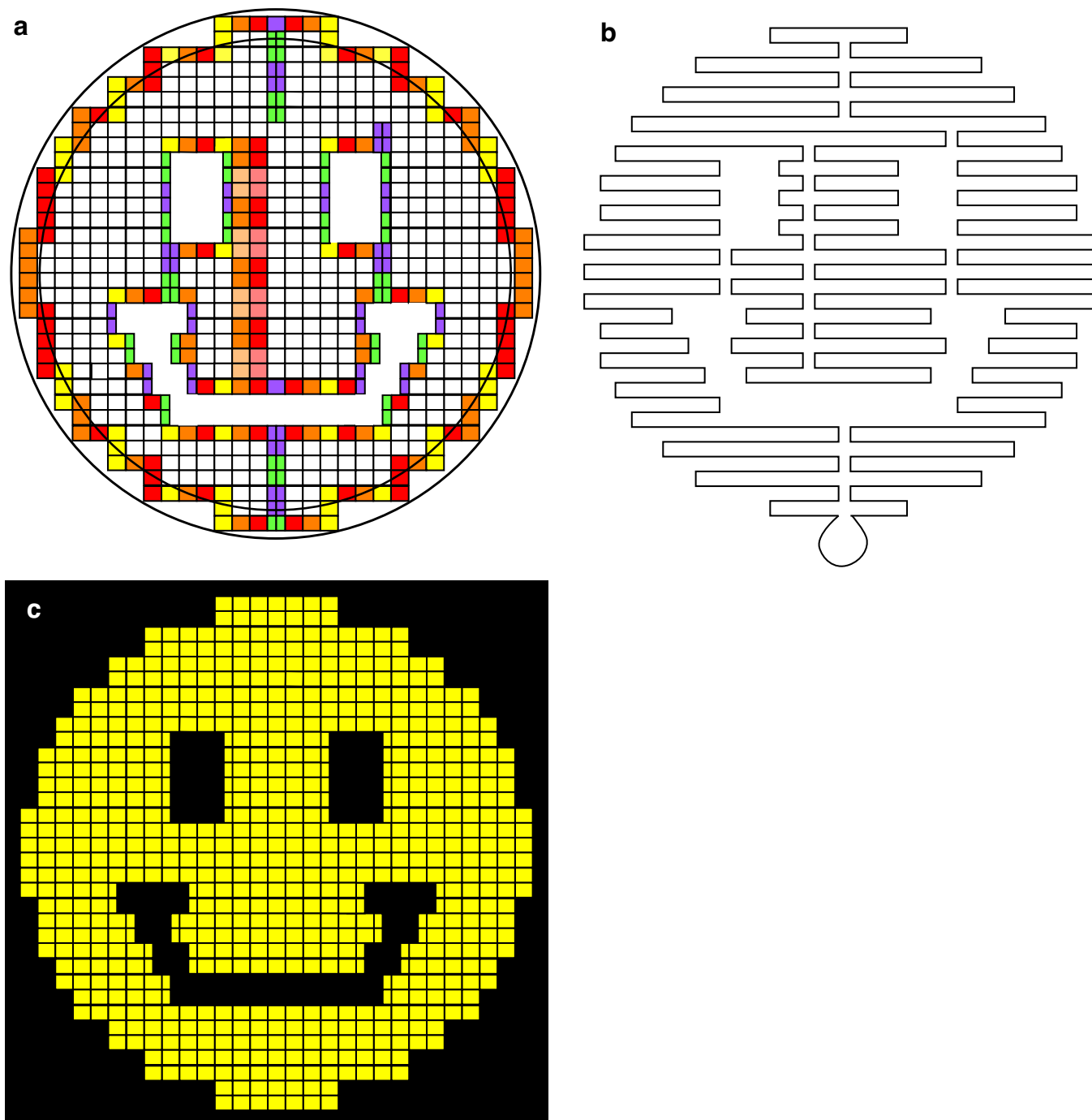
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s7t22h, D1, ATGCGTTATACAAATTTGGCTTAATTTGAGAACTTAATCGGC
s7t28e, E1, CTGCTATTAATCTTTACAAACAA
s7t30e, F1, TTGCAACCTGAGAGCCAGCAGCAAGGCGGTC
s7t32e, G1, AGTATTAATTTCTGGCCACAGAGGCGAGATTC
s7t34e, H1, ACCAGTCAACAACTATCGCCCTTGAACCGGTTG
s7t36e, A2, TAGCAATAAGAAGTGTTTTTATAA
s7t38a, B2, AATCAGCGTTAG
s8t15f, C2, TCAATAGAGATAGCAGCACCGGTAA
s8t17f, D2, TTAACGTGCGAAGAAACAATGAAATGTCACAA
s8t19f, E2, TGTCTTTCAATAGCAAGCAAAATCATTTTTTGT
s8t31g, F2, ACAGTGCACCG
s8t33h, G2, ATAAAAGGAGCCACCGCCGTGCA
s8t35b, H2, CACTTGTAACT
s8t35g, A3, ATTAGTAACCTGAGTAGAAGAACTCACGACCAGTA
s8t37f, B3, CTAACAGGTAAGCCAGATCTGCTGCTTTCTTGT
s8t39f, C3, TATAACGTGCTTTCTTCAGCGGAG
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s9t16e, E3, ATGGTTTAAAGCCCAATATAAGAAAAATGA
s9t18e, F3, AAATAGCAAACTATACCGGCCCTTATCAT
s9t20g, G3, TCCAAGAACACCGCTCAACAGTAGTACCAGTATAAAGC
s9t20h, H3, CGGGTATTAACCAAGTATTTTCATCTGATGGGCCCTTAC
s9t36e, A4, ATTTTAGACAGGAAGTGGGCGCGA
s9t38g, B4, TTAAGGGTGTCTTGAAGAGCAGCGCGCTACTATGTT
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s14t15a, C5, TGACCCCACTTT
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s1t10i, E5, TTTCAAGCATTTTCTGTAAGCACTGAAATTTTAC
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s1t20e, F6, TAAATGCTTATGCAATCAAGATCAAGGCTC
s1t20i, G6, GTTTTAAAGAAATAATGCTGTAGTAACCAAC
s1t22e, H6, AGAGCATACCTGTAAATCTTTTGAATTTGA
s1t22i, A7, CATTATGAAGAACTTACTGTTGATCTTTCTG
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s1t8e, G8, TCCACAGATTTGTGCTCTTTCCAGGAATTTTC
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s2t11f, A9, ATAAATTTTGAACAACTAAAGGAAT
s2t13f, B9, CGCGCACTTTGCTTTGAGGTGATGCGAATA
s2t15f, C9, GCATAGGCGAGGCGCTTTTGGCGGATAGTTG
s2t17f, D9, TAGCGAGACAAGGCTGCTCATTCAGACCAAGC
s2t19f, E9, CGAGTGGCAATCTGCGGAATGTAACCAAAA
s2t21f, F9, TAAGCAATGTTGTCTGGAAGTTTCCATTTTGT
s2t23f, G9, CTAGCTGACGGGAGAGGCTTTATAGCAAAAT
s2t25f, H9, AACGTTAAGAGAGTCTGAGCAACAAACCGTT
s2t27f, A10, TGTAGATGCACTAAAAATTAATGGAATTTGTA
s2t29f, B10, CGATTAGGCGCACTCCAGCGAGCTCAGCTTGG
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s2t5g, D10, GGGATAGCAAGTTTGTGTCACAGTACATGTAGCAT
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s3t12g, G10, ATTTCTTAAAC
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s4t13g, C12, AGCTTGATACGCTGCTACCTCCAGCAAGATGAAC
s4t15f, D12, AAGAGAGCGCAAGAGCAGCATG
s4t17f, E12, CCTCGTTTCTGACGAGAAACCAAACTTTGA
s4t19f, F12, TAATGTCTATCCCTCAAATGCTATCATTAAC
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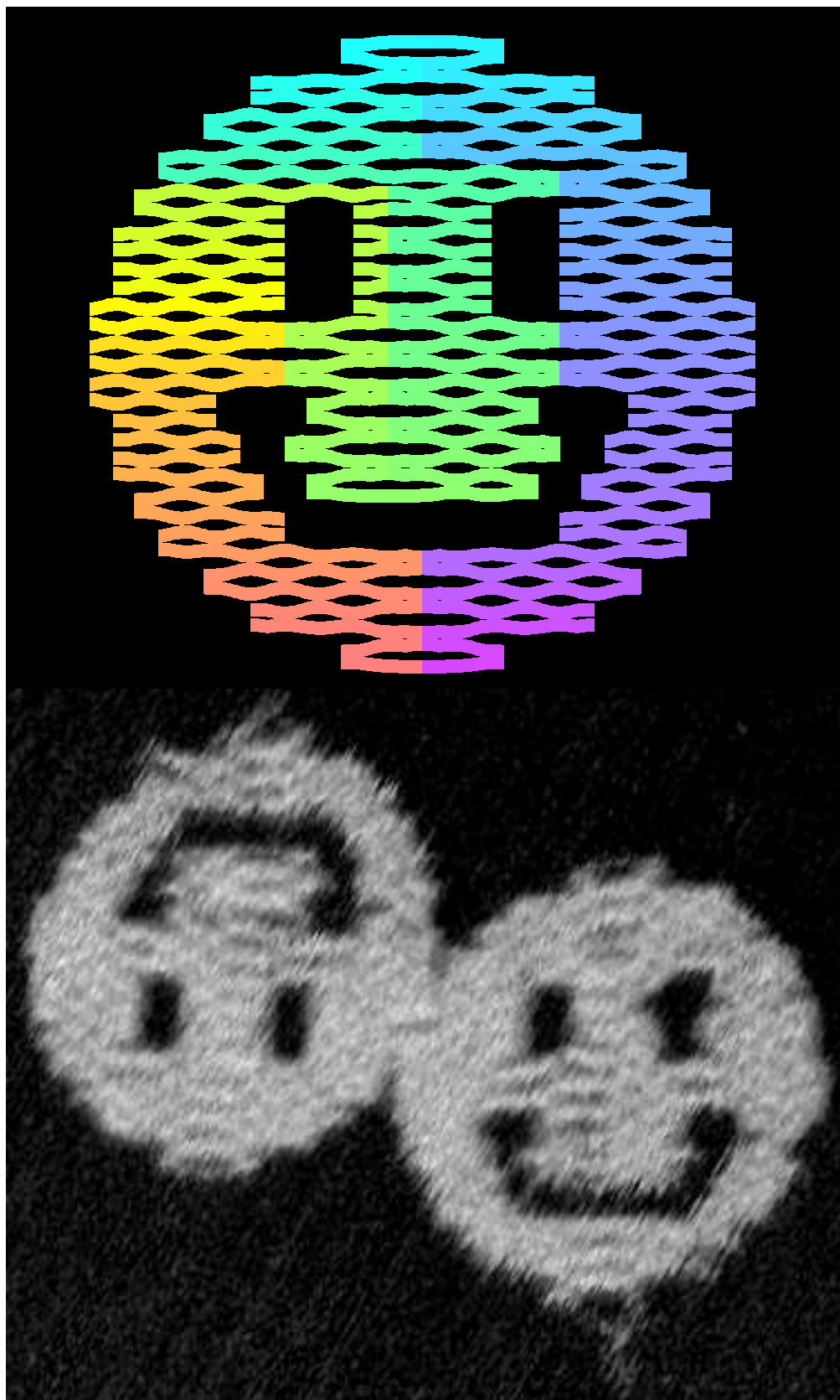
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s4t33f, E1, GAGTGTGATCGGCAACGCGGGAAGCGCTGG
s4t35f, F1, TTGATGGTGGTTCGACCCCTGAGA
s5t14e, G1, GAACGAGGAGTGAACGCGGAACCG
s5t16e, H1, AACTGACCGAACGAGTAGTAATTTAGTAAGA
s5t18e, A2, GCAACACTTTAAACAGTTCAGAAACAGGTGAG
s5t20e, B2, GATTAGAGAAGAGTAGATTAGTAGTAGTAG
s5t22e, C2, CATTAAACATATATTTTAAATGCAAGGTTGA
s5t24e, D2, GAAAGGCCAGCATGTCAATCATATATAATCAG
s5t26e, E2, AAAAGCCCCATTAATGTGAGCGACGATTCT
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s5t30e, G2, GCCTCTTCTGTAATCATGGTCAATGGGAAGCAT
s5t32e, H2, AAAGTGTAGAGAGGCGGTTTGGCTGCCCTTCA
s5t34e, A3, CCGCCTGGAATCGGCAAAATCCTTGGACTCC
s5t36b, B3, AAAACAGGGCG
s5t36e, C3, ACGCTCAAGCTCTTACAGGCGCAT
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s6t19f, F3, AACTCCAAACGAGATGACCATTAAGAGGAAT
s6t21f, G3, CTAGTAAATGAGGATAGATACAGGAAGCA
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s7t38f, F5, CCGATTTGAGCCG
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s8t15f, H5, TGTACTTTTATGAGGAGGATTTCC
s8t17f, A6, ACTAATGATTTGTAATTAACCTTACTGCTCCA
s8t19f, B6, TCGAGCTTTACCTGACTATTTATCATTCA
s8t31h, C6, CTCACAATTCGTTTTTTTTTT
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s9t16e, H6, TCCGCGACTGCGAATTTAAGAACTTGAGATTT
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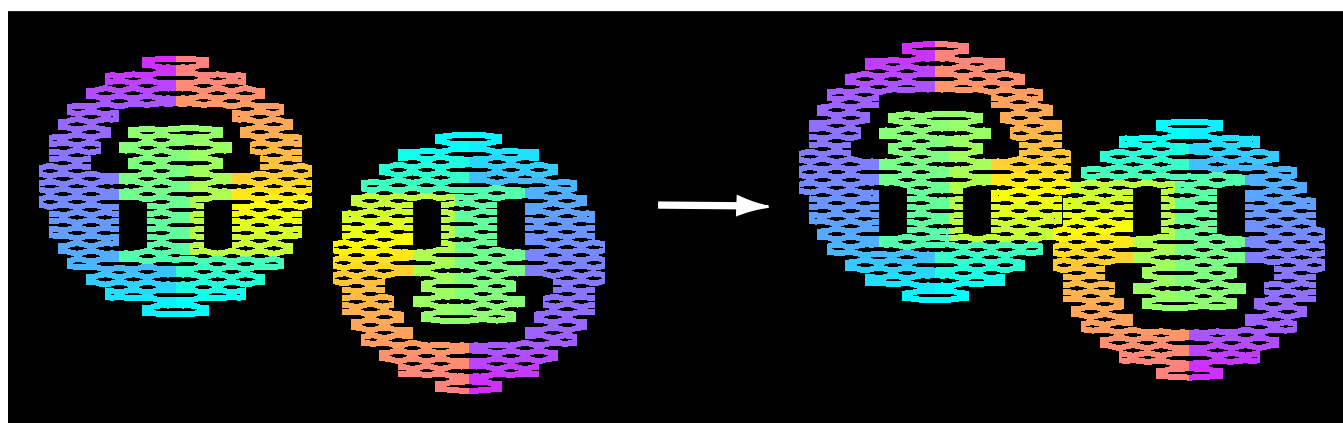
Supplementary Figure S24: Sequences for the star.



Supplementary Figure S25: More design details of the 3-hole disk (smiley). **a**, Block diagram. **b**, Reproduction of the folding path. **c**, Block diagram with all colors and notations removed. In **a**, turns that occur between columns of red blocks and orange blocks have offset 0 with respect to the underlying crossover lattice. Other turns on the left and right outer edges have +1 or -1 offsets depending on which side of the smiley they occur. Purple and green half-blocks show that scaffold turns made at most seams or on the interior of voids are an odd number of $1/2$ DNA turns away from scaffold turns on the outer edges. A pair of columns with alternating light and dark orange and red blocks marks a seam of 0 offset, placed 1.5 turns to the left of the central seam.



Supplementary Figure S26: Crossover diagram for the disk with holes (smiley) with a high resolution zoom out of Fig. 2: d_3 for comparison of fine structure. Some tip damage has occurred to the right eye of the righthand smiley.



Supplementary Figure S27: Diagram showing how the smileys stick together to maximize the number of blunt end stacking interactions. Compare to previous Supplementary Fig. S26.

Plate number: 1

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s10m19g, D1, ATTTCATTTCAATTTCCCTTAGAA
s1m0j, E1, ACGCAAAAGACACACGGAATAAGTTTATTTTG
s1m10e, F1, CGTACTCAACCTTCAGAACCGCCAGCAAGCC
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s1m14e, H1, GATCTAAAAATTTCTGTATGGGATGTGAAGAT
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s1m18e, B2, CAACAACCATCGGAACGAGGCTAGTTTTTCAT
s1m20e, C2, GAGGAAGTAAAACTCATCTTTGGTACAAACG
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s2m1b, D4, AAGGTGTACATA
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s2m33b, D5, CCGTTGAATTTAA
s2m3f, E5, AATAGCAACCAAGAACTTGGCATAAACGTAG
s2m5f, F5, CAAATAGGAGAGATAACCCCAACAAACATGA
s2m7f, G5, ACCTATTTATTTCCAGAGCTCAATTTTCCCAATCG
s2m9f, H5, AGTACCGCTTTTGCAGTACAGGATTTTCGGA
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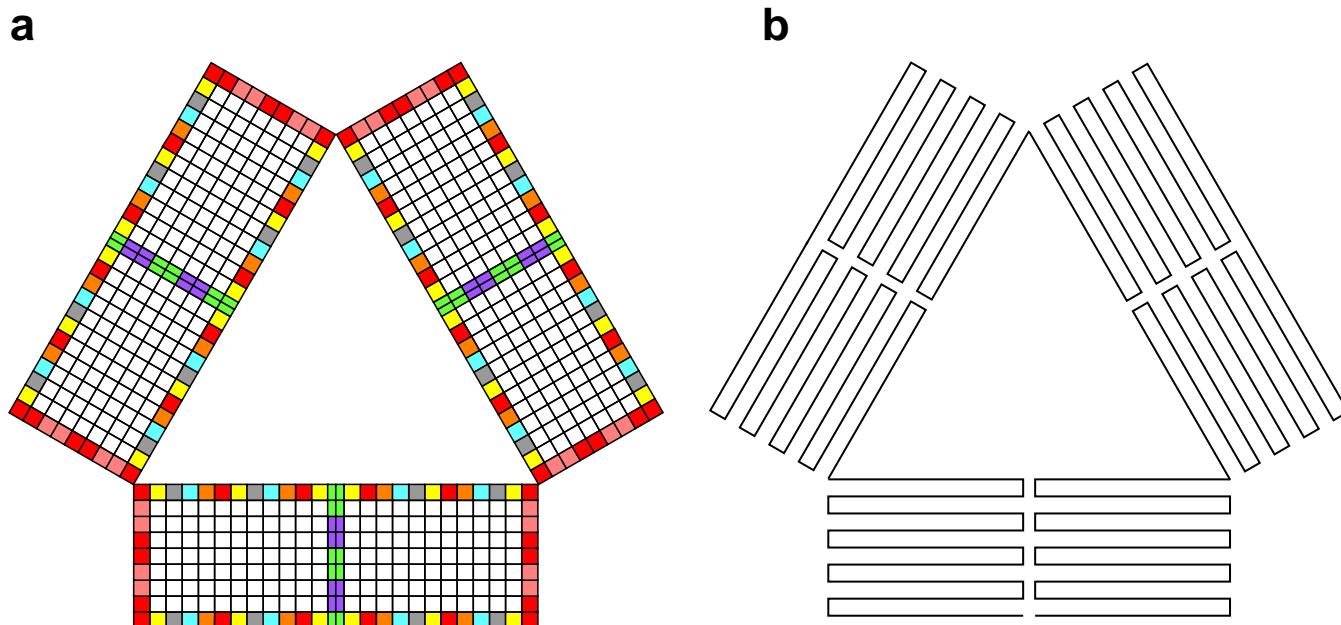
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s10m19g, B4, CCGCTCGGAT
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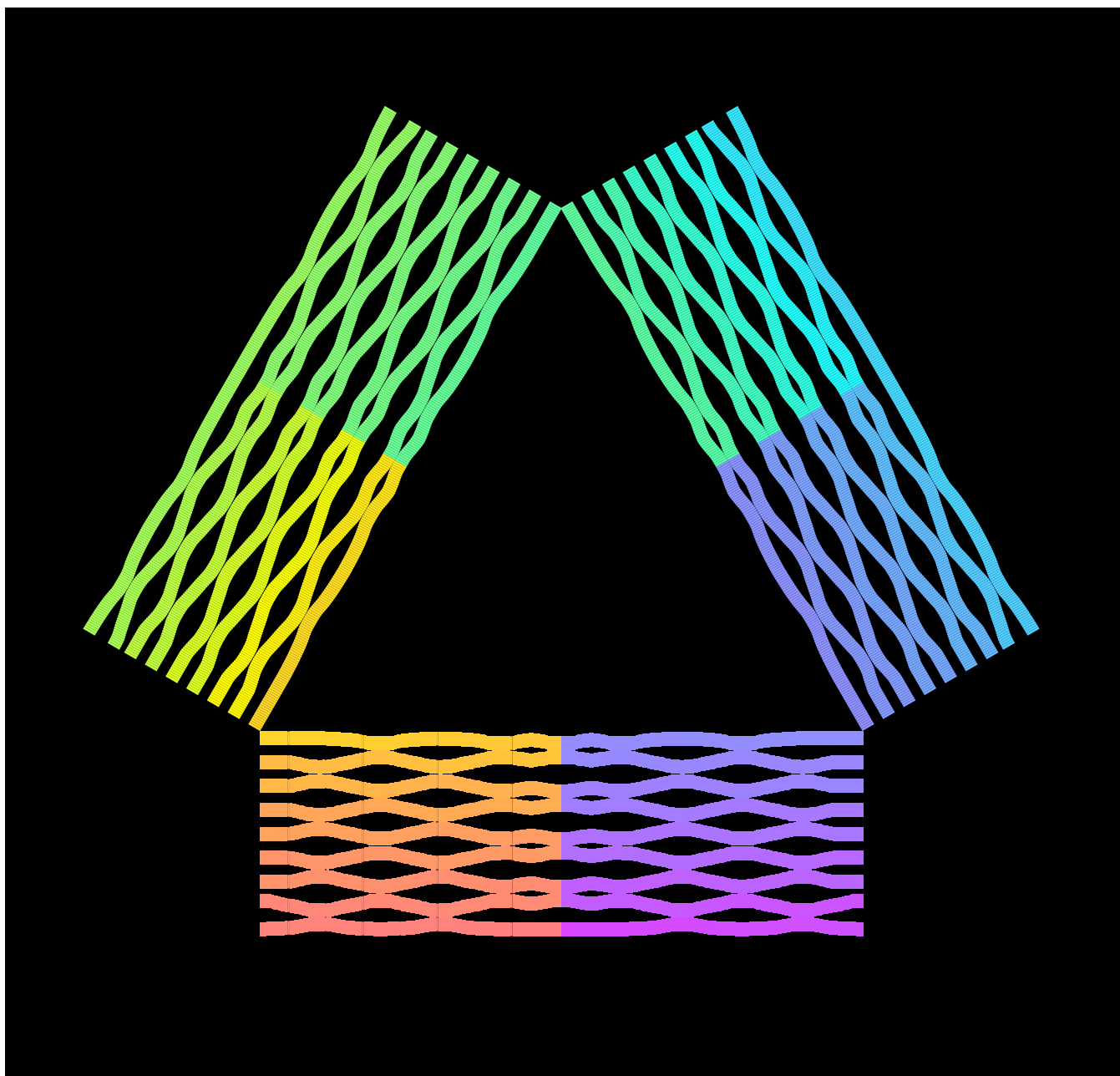
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s6m27f, D3, CCTAATGACCAACCAACATACGAGAGGATCCC
s6m29a, E3, CAGCAAGAGTTG
s6m5a, F3, TTGCCATAGGCT
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sm-rem2, G6, ATTTTAGACAGGAACGCTGACCGCAGAT
sm-rem3, H6, GGAGCTTAACAGAGGCGCGGATTAAGGG
sm-rem4, A7, ATGCTGCTTCTCGTTGATGACGAGCG
sm-rem5, B7, ACTATGTTGCTTTGACGAGCAGGTATA
sm-rem6, C7, CGCGCTTAATGCGCGCTTACAGGCGCGCT

Supplementary Figure S28: Sequences for the disk with holes (smiley).



25 turns wide at 10.4 bases/turn -> 260 bases
9 helices / domain, 27 helices in all

Supplementary Figure S29: Schematics for the triangle Fig. 2e. **a** Block diagram. Designed for 2.5-turn spacing blocks have 5 different offsets with respect to the underlying lattice of crossovers, hence the 5 different hues of blocks in different columns. As in other block diagrams, orange block/red block boundaries have an offset of 0 turns with respect to the underlying lattice of crossovers. **b** Folding path.



Supplementary Figure S30: Crossover diagram for the equilateral composed of rectangular domains.

Plate number: 1

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e0t13d, C1, GAGCAACAGGACGAGTCTAAAGTT
e0t15c, D1, ACTCATCTTTAGCTCATTTCCATATA
e0t15d, E1, AAACATTTAGCAACCAACCATCGCCC
e0t17c, F1, AATCAACGTACATATTTAGTCAAGA
e0t17d, G1, GTGTCTGGAAGTTCGCCAGCGAATAT
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e0t25d, G2, CAAAGAAACACCGCGGGGATGTGCTG
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e1t18b, D5, AGAATGACCATAGACATCAAAAGAT
e1t20e, E5, GAATTACGAGCGCATTTAGAGCAAC
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e1t22b, G5, TAACGGAATTCGCCCTCAATTTACCTG
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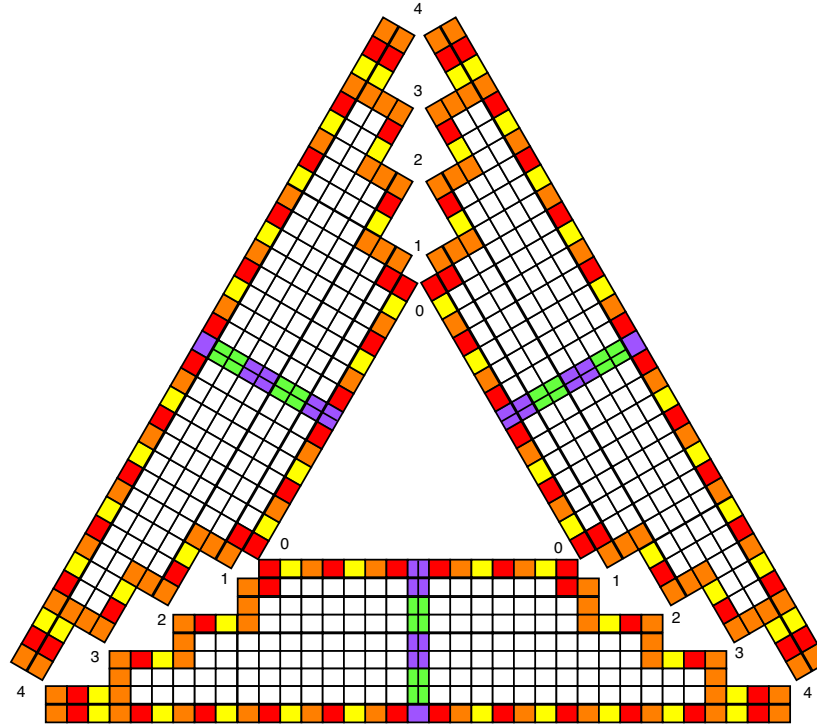
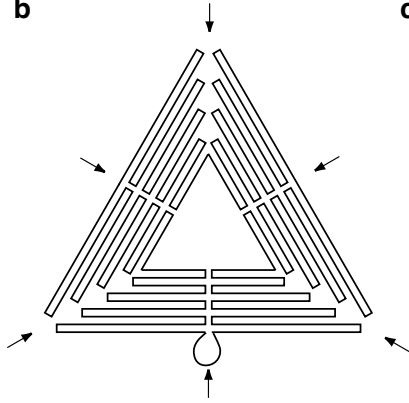
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e1t8a, E10, TTGAAGCTTTAAACACGCTCAACGAG
e1t8b, F10, TCTGAACTTTACTCAAGATTTAGTTG
e2t11e, G10, GTTTCTGACAGTCAAACTACAAAC
e2t13a, H10, ATCAGCTTGTCTTTTGGGATTTTGTCA
e2t13b, A11, TGAATTTTCTGACAGATGAAATTTCT
e2t15a, B11, CACTACGAAGCAAGTTAAAGGCGCG
e2t15b, C11, GAGGCTTTCGAGGCGCAATTAAGAG
e2t17a, D11, GGCTGACCTTTCATCGCTGATAAAT
e2t17b, E11, AGATTTGTATCATCAAGAGTAACTCT
e2t19a, F11, TTATTAACAGGTAGTGTAAATTTGGG
e2t19b, G11, AACACAGAGCAAGAAAGATTTCATCAG
e2t1e, H11, TTAGAATCTTTGAAACATAGCGATA
e2t21e, A12, TCGGCTTTCGCGCTTCTGTTAGCGACA
e2t23a, B12, GGCTGCGCAACTGGGTTGTAGATGGCG
e2t23b, C12, GATAGGTCAAGTTTGGGGAAGGCGGA
e2t25a, D12, CAACATACGAGCCACGAGCTGTGTAA
e2t25b, E12, GGGTTTTCAGTGGGAGCATAAAGT
e2t27a, F12, ACGGTGTTTTCGCGCTGCTAATTAATGA
e2t27b, G12, ACCTGTCTGTCGACCAAGGAGCGGAA
e2t29a, H12, AAAGGAGGCCCAACAGAGTCTCACT

Plate number: 3

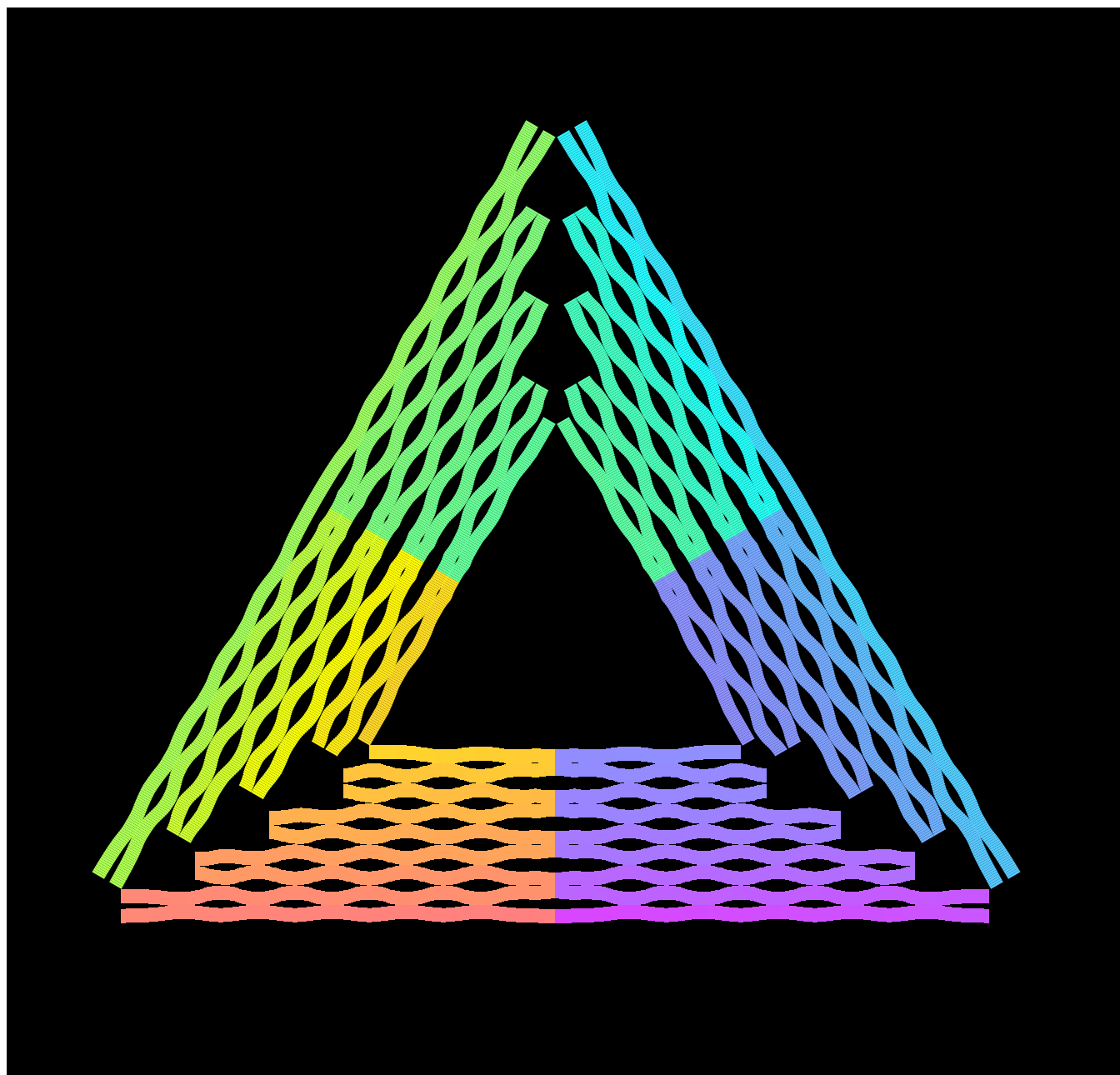
e2t29b, A1, GTTCCAGTTTGGAGATTTAGAGCTTG
e2t3a, B1, CGTTAAATAGAAGTTATATAACTAT
e2t3b, C1, CGCGTTAGGTTGTGAACACCGGAAT
e2t5a, D1, AACGCGCTGTGTTTGAATCGCCATAT
e2t5b, E1, TAGGCGTTTAAATGATCAACATAGAT
e2t7a, F1, GCGAACCTTCGAAACGCGGATTTAA
e2t7b, G1, TTATCATTTCAAGCTTCGCGGAGGTT
e2t9a, H1, GAATTAATGAACTTAAGTTTGGCCAG
e2t9b, A2, GGTCTTTTCAGAGAGCCCTGAACAAAG
e3t10e, B2, AAAACAGGGAAGCGATTTAGAGCGGGA
e3t12a, C2, TAGGAACCATGTAGTTTCAAGCGGAG
e3t12b, D2, AACCAATCTTCAACACCGTAACACTGA
e3t14a, E2, AAAGGAGCTTTTATCACCTCTAGCAG
e3t14b, F2, TTTTTCGCGGATCGATTTGTATCGGTTT
e3t16a, G2, CATTAACCGGTTTACGACCTCTCTCCAT
e3t16b, H2, GTGTGGAATTCGAAATCTCAATGATCG
e3t18a, A3, CGGTGTAGTACGCAATTTCAACATTTA
e3t18b, B3, CTGTGAGTGGTTTGAAGCGCATAGGCT
e3t20e, C3, TAATTAACAGGCTTACCGGAACAACA
e3t22a, D3, CCAATAGAGCAAGCGCATCTGCCAGTT
e3t22b, E3, CAGTGTAAACCGTCTCAAAATTAAT
e3t24a, F3, CCAGGCAAGCGCGGAGGATTTGAT
e3t24b, G3, AACGAGCGGCACTCATTCGCAATTTCA
e3t26a, H3, AATTGTTATCCGCGGGAGAGGCGG
e3t26b, A4, AATAGCAACCGCTCATCAATTTCCACA
e3t28a, B4, CCGTGAAGAGTTTGACCTCAACGCTCA
e3t28b, C4, TTTAAAGACGTTGAGAGAGCGGCTCC
e3t2a, D4, AATCGTCGCTATTTCGAATCTCAACT
e3t2b, E4, GTATTTCTGCAAAATTTATTTTCCC
e3t30e, F4, GCGGTAAAGCATTAATCGGAACCTCT
e3t4a, G4, AATAGCAACCGCTCATCAATTTTAGG
e3t4b, H4, TTAACCAACCGCAAGTGAATAAAGAGG
e3t6a, A5, ATAAACCAACGATTTCTGAGAAACAA
e3t6b, B5, CCAAGTACCGCACTCAAGTATAAGCAG
e3t8a, C5, GTATTTCTAAGCAACGACATATTATTT
e3t8b, D5, TTCAAAATTAACCGGAGGCGGTTTTA
e4t11e, E5, CTCATTTTTCAGGAGTATCGAGGCGCA
e4t13a, F5, TGAATATCTCAAGCAACCACTAAAGG
e4t13b, G5, TGAGAAATGAAGAAATAAGGCTCCCA
e4t15a, H5, TAAAGACTTTTTCGGAACAGGAGGTA
e4t15b, A6, TGAGAAATGAAGCAATATGAGAAAGTTT
e4t17a, B6, ACCAATTTTGAAGAACAGGCGGAGCA
e4t17b, C6, TTACTACTTGGCGGAGGAGCAGATGAA
e4t19b, D6, ATCATTTGGAATTTGAAATTTCTACGT
e4t1e, E6, TGTGAGTGAATTAACCTTCTGTTGTGA
e4t21e, F6, TTTTGTTAATCAAGCTCAATTTTTTAA
e4t23a, G6, TCCGACCGCTTACAGATTTTCGGCT
e4t23b, H6, TGAGGGAGCAGCGCTGGTGCGGAA
e4t25a, A7, CAGTGTCAATCTCTAGAGAGTCC
e4t25b, B7, GCGTGCAGGTCGAGTTTCTGTGTGA
e4t27a, C7, CAGGATGATTGCCCGCGGAGGCTGTT
e4t27b, D7, TTTGCGTATTGGGTTCAACGCGCTGAG
e4t29b, E7, AAGGCGCAAAACTTGGGAGTCCGCG
e4t3a, F7, TCTTCTGACCTAACGCGGAAACTTT
e4t3b, G7, GCAAGACAAGAAATTTAATGGTTTG
e4t5a, H7, AAAGTAAATCTGTGAGCGAGTAAATA
e4t5b, A8, CAGAGGCTATTTCGCAAGACGATCA
e4t7a, B8, CAAATCGATATAATTTTTCATCGTAC
e4t7b, C8, GCAAGCGTATTTTTGAAGGCTTTAGC
e4t9b, D8, ATCCCAATCCAAAGAGAAATAACAT
e5t10e, E8, AAAAATGAATATGACAGCTTTTACAGTAAAGAACGATTT
e5t12b, F8, AATTGCGAATTTCAAGGCGCAACCC
e5t14b, G8, GCAACGATCAAGAAATTTTTCAGGT
e5t16b, H8, CGGTCAATCAAAAGGCTTTTGAAGCAG
e5t18b, A9, TTAAGAACTGGCTGGGAAACGCACTG
e5t20e, B9, CATTAACAGTACGAGCGTTGGGAAACCTTATGCGATT
e5t22b, C9, CAGGAAGATGCAATTCGCAATTAAT
e5t24b, D9, CCGGTTACCGAGCTCCAGAGCAGCTT
e5t26b, E9, TTTCTTTTCAACATCGAATTCGTAAT
e5t28b, F9, GATGGCCCACTACGTGAGAGCGGCA
e5t2b, G9, TTTCAATATATAATTAATCAATATA
e5t30e, H9, GTGAACCATCACCCAAATCAAGTTTTCGTCTATCAGGGC
e5t4b, A10, GAGAAATATAAGTTTAGTTAAATTTCA
e5t6b, B10, GAATCAATTACCGCAACGAGCAAAAGGT
e5t8b, C10, TTTGTTTAACTGCGCCCAATAGCAAG
et-rem1, D10, ACCCGCAAGATCTTGAGAGGTGTTTT
et-rem2, E10, TTGAAGGATTTTGAACAGGAACGGT
et-rem3, F10, AGAGCGGAGCTTAAACAGGAGGCGGCA
et-rem4, G10, TATAACGTGCTTCTCGTTAGAAATC
et-rem5, H10, GTACTATGTTGCTTTCGAGAGCAGC
et-rem6, A11, GCGCTTAACTGCGCCGCTACAGGCGCG

Supplementary Figure S31: Sequences for the equilateral composed of rectangular domains.

a**b****c**

For bridges between trapezoidal slant edges		
contact type,	name,	and sequence
1,	t-5s2e-t6s23c-3T,A6,	TTAATTAATTTTACCATATCAAA
2,	t-7s4e-t8s25c-2T,B6,	TTAATTTTCATCTTAGACTTTACAA
3,	t-9s6e-t10s27c-1T,C6,	CTGTCCAGACGTATACCGAACGA
4,	t-11s8e-t12s29c-0T,D6,	TCAAGATTAGTGTAGCAATACT
1,	t-5s12e-t6s3c-3T,E6,	TGTAGCATTCCCTTTTATAAACAGTT
2,	t-7s14e-t8s5c-2T,F6,	TTTAATTGTATTTCACACAGAGCC
3,	t-9s16e-t10s7c-1T,G6,	ACTACGAAGGCTTAGCACCATT
4,	t-11s18e-t12s9c-0T,H6,	ATAAGGCTTGCAACAAAGTTAC
1,	t-5s22e-t6s13c-3T,A7,	GTGGGAACAAATTTCTATTTTGTAG
2,	t-7s24e-t8s15c-2T,B7,	CGGTGCGGGCCTTCCAAAAACATT
3,	t-9s26e-t10s17c-1T,C7,	ATGAGTGAGCTTTTAAATATGCA
4,	t-11s28e-t12s19c-0T,D7,	ACTATTAAAGAGGATAGCGTCC

Supplementary Figure S32: More details of sharp triangle design. **a** Block design for the sharp triangle composed of trapezoidal domains. Contacts between trapezoids on their slant faces are of type 0, 1, 2, 3, and 4. Contacts of type 0 are bridged by the scaffold strand as shown in the folding path (**b**). Other contacts are bridged by special staples (**c**) that each replace two of the staple strands in the staple sequences (Supplementary Fig. S34), identified by the composite names of the bridging staples. Assuming a 1 nm inter-helix gap and 32 bases/3 turns, I calculated that the contacts would have gaps of width of 1.5696 nm, 1.0822 nm, 0.5944 nm, 0.1070 nm for contact types 1,2,3, and 4. (The gap widths drawn in the block diagram above are not accurate; contact type 4 has essentially no gap given an inter-helix gap of 1 nm.) Assuming that an unpaired thymine can bridge .43 nm (the length per base-pair of single stranded DNA³⁴), this would require adding 3.7, 2.5, 1.4, or 0.25 T's in the bridging staple for each contact point. In fact, 4T loops are often used to bridge 2 nm wide double helices to make them into hairpins assuming .5 nm per T. I used 3, 2, 1, and 0 T's for contacts of type 1, 2, 3, and 4 as can be seen inserted into the middle of the sequence in **c**. As in other block diagrams, in **a**, turns that occur between columns of red blocks and orange blocks have offset 0 with respect to the underlying crossover lattice. Other turns on the left and right outer edges have +1 or -1 offsets depending on which side of the trapezoid they occur. Purple and green half-blocks show that scaffold turns made at the central seams are an odd number of DNA 1/2 turns away from scaffold turns on the outer edges.



Supplementary Figure S33: Crossover diagram for the sharp triangle composed of trapezoidal domains.

Plate number: 1

t10s17c, A1, TTAAATATGCA
t10s27c, B1, ATACCGAACGA
t10s7c, C1, TAGCACCATTA
t11s18h, D1, AATACTGCGGAATCGTAGGGGTAATAGTAAATGTTTAGACT
t11s28h, E1, TCTTTGATTAGTAATAGTCTGTCTCATCACGCAAAATAACGGTT
t11s8h, F1, CAGAAGAAACCGAGGTTTTTAAAGAAAGTAGCAGATAGCCG
t12s19c, G1, GGATAGCGTCC
t12s29c, H1, GTAGCAATACT
t12s9c, A2, AACCAAGTTAC
t1s10g, B2, GACGGGAGAAATTAACTCGGAATAAGTTTATTTCCAGCGCC
t1s12i, C2, TCATATGTGTAATCGTAAAACTAGTCATTTTC
t1s14i, D2, GTGAGAAAATGTGTAGGTAAAGATACAACCTTT
t1s16i, E2, GGCATCAAAATTTGGGGCGGAGCTAGTTTAAAG
t1s18i, F2, TTCGAGCTAAGCATTTCAAAATCTCGGGAACGAG
t1s20g, G2, GAATACCACTTTCAACTTAAGAGGAAGCCCGATCAAAAGCG
t1s22i, H2, TCGGGAGATATACAGTAAACAGTACAATAAATTT
t1s24i, A3, CTTGATTAAAGGAGCGGAATTATCTCGGCTTC
t1s26i, B3, GCAAACTCACCTCAATCAATATTCTGCAGGTGCA
t1s28i, C3, CGACCAGTCAATTTGCAGATTTCACTCTGCTTCG
t1s2i, D3, CGGGGTTTCTCTCAAGGAAGATTTTGAATTA
t1s30g, E3, TTGACGAGACGATATCTGAAATGAGTTATTTAAATAAAG
t1s4i, F3, AGCGCTCATGTCTTGAATTTTACCGACTACCTT
t1s6i, G3, TTTCAATCCCTTATTTAGCGTTTTCCTTCTTCT
t1s8i, H3, ATGGTTTATGTCAACAATCAATAGATATTAAC
t2s11g, A4, AGAAAGCCCAAAAGAGTCTCGAGCAACAATCACCATT
t2s13g, B4, ACAGTCAAGAGAGATCGATGAACGACCCGGTTGATATTC
t2s15f, C4, ATAGTAGTATGCAATGCTCGAGTAGCGCGAG
t2s17f, D4, AACCAAGCTTTAGCTATATTTTCTTCTACTA
t2s19g, E4, GATAAGTGCCTGCGTGAAGTCTCGAGATGAAGCTTACAGGAG
t2s21g, F4, CTTGATTGCTTTGAATTTGCGTAGATTTTCAGGCTCATATA
t2s23g, G4, TGGCAATTTTAACTGCTCAAGTAAAGTAAACGAGTTTCG
t2s25f, H4, AAGGAATTTCAAGAAACACCAAGTCAAGTGA
t2s27f, A5, GGACATTCACTCTCAATATGCAAACTCAAGTTGA
t2s29g, B5, TTTGATGATTAAAGAGTCTGAGACTTCTCTGATACCGAGCG
t2s31f, C5, CCGGAACCGAAGTGAAGAGCGCAACATCTCA
t2s33g, D5, AAAGACAACATTTTCGCTCATAGCCAAATCA
t2s35g, E5, GTCAGAGGCTAATTTAGTGCAACATGAAGGCGATTGAG
t3s14e, F5, CAATATGACCTCATATATTTTAAAGCACTTAA
t3s16e, G5, CATCCAATAAATGCTCAATTAACCTCGGAAGCA
t3s18g, H5, AACTCCAAGATTGCAATCAAAAGATTAATCGAGATACATAA
t3s20g, A6, CGCCAAAAGGAATACAGTCAGAGCAAGCGCAGGTGAG
t3s22e, B6, TAATCTGATTATCATTTTTCGGAAGAGGAAGG
t3s24e, C6, TTTATCTAAAGCATCACTTCTGATGGCCCAAC
t3s26g, D6, AGAGATAGTTTGACGCTCAATCGTACGTCTTCTCGTT
t3s28g, E6, AGAATCAGAGCGGAGATGAAATACCTACATAAACCTTC
t3s4e, F6, TGTAATCGGAATCTCTTAAGAGCAGGACCA
t3s6e, G6, CACCGGAAGCGCGTTTTCATCGGAAGGGCGA
t3s8g, H6, CATTCACAACAAACGCAAGACACAGAACCCCTGAACAAA
t4s11g, A7, GCAAAATTTTAAATTTAGATATCTACAAAGCTAGTATAAA
t4s13g, B7, CGTTCTAGTCAGGTCTTGGCTGACAGGAAGATTGTATAA
t4s15f, C7, CAGGCAAGATATAAGTTTTCATCGGAAGGCTG
t4s17f, D7, GATTAGAGATTAGATACATTTTTCGCAAAATCATTA
t4s19g, E7, TAGCCCGGAATAGGTGATGCGCCCTGCTATGCTGCTAGT
t4s21g, F7, GCGCAGAGCGGAATTAATTTATTTGCAAGTAAATTTCTGAAT
t4s23g, G7, GATTATACAGAAATAAAGAAATACCAAGTTTACAAAATC
t4s25f, H7, TAGGAGCATAAAAGTTTGAGTAAACATTTGTTTG
t4s27f, A8, TGACCTGACAAATGAGAAATCATATAATATCTT
t4s3g, B8, TTTAATCGTTTCGGAACCTATTATTAGGGTGTATATAAGTA
t4s5f, C8, CTCAGAGCATATTCAACAACCAATTAATTAAGT
t4s7f, D8, GGAGGGGAATTTAGCGTCAGACTGTCTCGCTCTT
t5s10g, E8, GATAACCCACAAGAAATGTTAGCAACAGCTGAAAATTTATC
t5s14e, F8, TTAATGCTCTTATTTCACCGAGGCGCAAGAAAG
t5s16e, G8, TTAGCAAAATAGATTTCATGAGGCAAGTACTCTT
t5s18g, H8, TAATTTGCTTTAACCCTGACTATTATGAGGCTAGTAAAGAGC
t5s20g, A9, AACACTATCATAAACCATCAAAATCAGGCTCTCCTTTTGA
t5s24e, B9, AATGGAAGCGAAGCTTATTAATTTCTTAACCAAC
t5s26e, C9, TAATAGATCGCTGAGAGCCAGCAGAAAGCGTAA
t5s28g, D9, GAATACGTAAACGAGAAAACGCTCTTAACACAGGAGCGCA
t5s30g, E9, TTTAAAGGATTTTAGATACCGCCAGCAATTGCGGCACAGA
t5s4e, F9, CTTTGAGTCAGAGATTGGCTTTGCGCCACCC
t5s6e, G9, TCAGAACCCAGAAATCAAGTTTGGCGGTAAATA
t5s8g, H9, TTGACGGAAATACATACATAAAGGGCGCTAATATCAGAGA
t6s13c, A10, CTATTTTGTAG
t6s15g, B10, ATAAAGCCTTTTGGCGGAGAGCGCTGGAGAGGGTAG
t6s17f, C10, TAAGAGGTCAATTTTCGCAACGAGATTAAAGCA
t6s23c, D10, ACCATATCAAA
t6s25g, E10, TCAATAGATATTAAATCCTTTGCCGGTTAGAACCT
t6s27f, F10, CAATATTTTGCTCTGCAACAGTGCCATAGAGCCG
t6s3c, G10, TATAAACAGTT
t6s5g, H10, CAGAGCCGAGAGGTTGAGGCGAGTAAACGTGCCG
t6s7f, A11, ATTAAGGCGGTAATCAGTAGCGAGGCCCTCT
t7s10g, B11, ATAAAGCAAGAAACATGGCATGATTAAAGACTCCGACTTG
t7s14e, C11, ATGACCTGTATAACTTTTCAGAGCA
t7s16e, D11, TAAAGCTATATAACAGTTGATTTCCATTTTTTG
t7s18g, E11, CGGATGGCAGGAAATGACCATATGTTTACAGAGCAGC
t7s20g, F11, GATAAAAAACCAAAATATTAACAGTTTCAGAAATTAGAGCT
t7s24e, G11, ACAATTCGACCACTCGTAAATCAT
t7s26e, H11, TTGAGGATGCTCAGTATTAAACCTTTGAATGG
t7s28g, A12, CTATTAGTATATCTCAGAACAAATCAGGAACGTAAGCCA
t7s30g, B12, GAATCTCTGAGAGGTGATCTGGCCTTGTGCTACTTTAATG
t7s4e, C12, GCGCGCAGCTTGAACACACCTTC
t7s6e, D12, AGAGCCGACCATCGATGAGCAGATGAATTAAT
t7s8g, E12, CACCGTCACTTATAGCAGTATTGAGTTTAAAGCCCAATA
t8s15c, F12, CCAAAAAACATT
t8s17g, G12, TAATTTGCTTGAAGTTTATTTTCCAAATCGGTTGTA
t8s25c, H12, AGACTTTACAA

Plate number: 2

t8s27g, A1, CGCGAACTAAAAACAGAGGTGAGGCTTAGAAGTATT
t8s5c, B1, CCACCAGAGGCC
t8s7g, C1, AGCCATTTTAAACGTCCCAATGAACACCCAGAACCA
t9s10h, D1, TATCTTACCAGAGGCCCAACGCAATTAACCGAAAAATCACCAG
t9s16e, E1, ACTAAAGTACGGGTGTGCAATATAA
t9s18g, F1, TGCTGTAGATGCCCCCTCAATGTGCGAGAGGCTTTTGCA
t9s20h, G1, AAAGAAGTTTGGCAGCATAAATATTCATTGACTCAACATGTT
t9s26e, H1, ACCACCAGCAGAAAGTATGATGCC
t9s28g, A2, TAAACATTTAGAGAATCTCAACCTTTTATAATCAGTGAG
t9s30h, B2, GCCACGAGTAAAGAACATCACTTGCCCTGAGCGCCATTAAAA
t9s6e, C2, CCATTGACAGGCGGGGGAATTA
t9s8g, D2, GAGCCAGCGAATACCCAAAAGAACATGAAATAGCAATAGC
t10s17h, E2, ACCAACCTAAAAAATCAACGTAAACAAATAAATTTGGGCTTGAGA
t10s27h, F2, AACTCAACATATTAGTGTGTTTCCAGAAACCGCTTATCAGGG
t10s7h, G2, AGCACAATTAATCCCGACTTCCGGGAGATCTCGAATCTTACCA
t11s18e, H2, ATAAGGCTTGC
t11s28e, A3, ACTATTAAAGA
t11s8e, B3, TCAAGATTAGT
t12s19h, C3, CCGCAGAGAAACACCAAGCAGTAGGCTGCTCATTCACTGTA
t12s29h, D3, ACGTGAGCTCCAAAGTCAAGGGGCAATTTGGAACAGAGTCC
t12s9h, E3, TGCTATTTTGCAACCGACTGCAATTTGTTTGAAGCCTTAA
t1s10e, F3, AGAGAATAACATAAAAAACAGGGAAGCGCATTA
t1s12i, G3, AGGGAATAGCTCAGGCCACCAACCCCATGTTCAA
t1s14e, H3, ATTTTCTGTGACGGGAGTGAAATACCGATAT
t1s14i, A4, CAACGATTTTATGGGATTTTGTCTAATCAAAAGG
t1s16e, B4, ATTTGCTGTGCGGAGTGTCAACCCAAATCCG
t1s18i, C4, CGCGCTTTGCTGAGGCTTCAGGGGAAAGGT
t1s18g, D4, CGACCTGCGGTCAATCTAAGGAAGCGAAACCAATATT
t1s18i, E4, CGCGCTTGTAGGAGTGTACTTATGCGGCTTTAA
t1s20e, F4, ACAGGTAGAAAGATTCATCAGTTGAGATTAG
t1s22i, G4, CGGCTGTAGTAGGAACGATGCAACTTTTACA
t1s24e, H4, CAGTTTGAAGCAGCTCCAGCCAGCTAAACGAGC
t1s24i, A5, AGGAAGATGGGAGCAGCAGCATTAATCATATT
t1s26e, B5, GCGAGTGCATCCCGGGTACCAGGTTTCTTCT
t1s26i, C5, CTCTAGAGCAACCTGTGATCGTGTGCGAGTTG
t1s28g, D5, TTTCCAGCGCTGGCCCTGAGAGAAAGCGCGCAACGTGG
t1s8i, E5, CTTCAGCGTGAAGCGGCGCAACAGCGATCACA
t1s21f, F5, CTTTTCATTTTAAACAAATTTTCATGAGATTAG
t1s30e, G5, CGAGAAAGGAAGGAGCGCATATGTTGCTGT
t1s4e, H5, TTTATCAACCGGCTTAGTGTGGTAAAGCTGT
t1s4i, A6, TTTAACTCTATAGGTCTGAGAGTTCACAGTA
t1s6e, B6, TTAGATATGCGCAACGCTCAACAGTTCGCTGTC
t1s6i, C6, AGTATAAAATATGCGTTATACAAAGCATCTT
t1s8g, D6, TTTCTTACGACTCATCGAGAACATAGCAGCCTTTACAG
t1s8i, E6, CAACTACCTCATTTCAAGAACCGGAAATTCAT
t2s11g, F6, CTTCAAGAACCGCCCAAGGCCAATAGGAACGTAAATGA
t2s13g, G6, AGACGTTTACATGTACCGTCAACACCCCTCAGAACCGCCAC
t2s15f, H6, CAGCATCAAGAAAGAACCACTAAGCTTTTCC
t2s17f, A7, ATTTGCTGTACAGCAGCGAAGACAGCTCGCC
t2s19g, B7, AAAACAAAATTAATTAATGAGTACGATCACTTAGTGAAT
t2s21g, C7, CTGCTATTTTAAACCGGCTTCTCGCTAGGAGCATCTGC
t2s23g, D7, GTAAACCGTCTTTCATCAACATTTAAATTTTGTATAATCA
t2s25f, E7, ACGTTGATTTCGCGGACCGCTTCTCGGCGCTG
t2s27f, F7, CCAGGGTGGCTCGAATTCGTAATCCAGTCAAG
t2s3g, G7, AGAGTCAAAAATCAATATATGTAGTGAAGAAACCAATCAAG
t2s5f, H7, ACTAGAAATATATACTATATGTACGCTGAGA
t2s7f, A8, TCAATTAATAGGCTTAAATGGAACATATAATT
t3s10g, B8, AACGTTAAAAATGAAAGCAAGCGCTTTTATGAAACCAA
t3s14e, C8, GTTTTGTGAGGAATTCGCAATAATCCGAACAT
t3s16e, D8, GACAACAAGCATCGGAACGAGGGTGAGATTG
t3s18g, E8, TATCATGTTGAAAGGAGCAGATGGAAGAAATCTACG
t3s20g, F8, TTAATAAAAGCAATTAACCGAATCGCAACCTCTGATATA
t3s24e, G8, GTGATAGTGGGTGCGGAAACCGAGGAACGCGAG
t3s26e, H8, GTTTTTCATGCTGATAGCTGTTTGAAGGGG
t3s28g, A9, GTTTGCTGAGCTGTTTTCGCGGAGGAGCGCCCGATT
t3s30g, B9, TAGAGCTTGAAGGGAGTTGCGAGCAAGCGGTCTAGGGCG
t3s4e, C9, GATTAAAGAAATGCTGATGCAAAATCAGAAATAA
t3s6e, D9, CACCGGAATCGCATATTAAACAAAATTTACG
t3s8g, E9, AGCATGTATTTCATCGTAGGAATCAACGATTTTGTGTT
t4s11g, F9, AGGTTTAGTACCGCATGAGTTTCGTCACCGAGGATCAAA
t4s13g, G9, AGCGTAACTACAACTACAAACGCTTATCAACGCTCTCAGG
t4s15f, H9, TAGTTGCGAAATTTTTTCACTGTGATCATAGTT
t4s17f, A10, GTACAAAGAGCAACCGCTACAGAGGATACGA
t4s19g, B10, GAGCAAAAGAGATGAGTGAATAACCTTGCTTATAGTCTTA
t4s21g, C10, GTTAAAAATTCGATTAATGTGAGCGAGTAAACACAGTTTG
t4s23g, D10, GATAGGATACCGCTCGAATTTCTCTAAAAGCTTAATATTT
t4s25f, E10, AGTTGGGTCAAAAGCGCAATTCGCCCCGTAATG
t4s27f, F10, CGCGCGGGCTGTGTGAAATTTGTTGGCGATTAA
t4s3g, G10, ACATAGCGCTGTAATGCTGCTGCTATTCTTTCAATTACCT
t4s5f, H10, GTTAAATCAACTCGCAAGCAAGAGCCTTGAA
t4s7f, A11, CCGACTCTCGCAACATGTAAATTTAAATGAAGC
t5s10g, B11, TCCCAATCCAAATAGATTAACGCGCCCAATAAATAATAT
t5s12e, C11, TGTGACTTCC
t5s16e, D11, AACAGCTTGCTTTGAGGACTAAAGCGATTATA
t5s18g, E11, CCAAGCGCAGGCGCATAGGCTGGCAGAACTGCTCATTAT
t5s20g, F11, ACCAGTTCAGGAGCTGTGGAACGCTGACAGACGAAACAAA
t5s22e, G11, GTGGGAACAA
t5s26e, H11, TGCTGCAATTCGCTCACAATTTCCAGCTGCA
t5s28g, A12, TTAATGAAGTTTGAATGTTGCTTCGAGGTCGCTAAAGCA
t5s2e, B12, TTAATTAATTT
t5s30g, C12, CTAAATCGGAACCTTAAGCAGGCGAAATCTTCCGCGCAA
t5s6e, D12, GTGTGATAGGCGAGAGGATTTTCAGTCTGA
t5s8g, E12, ACAAGAAAGCAGCAATCAGATAACGCGCATTTATTTTA
t6s13f, F12, ACAGCAGCGCAATCTTCAAAAAGAAATTTCTTA
t6s15c, G12, CAGGTTGAGGCTTCAAAAGGAGCC
t6s17f, H12, ACCCCAGAGCTTTTTCATGAGGAACCTGCTTT

Plate number: 3

t6s23f, A1, CGGCGGATTGAATTCAGGCTGCGCAACGGGGGAGT
t6s25c, B1, TGGCGAAATGTTGGGAAGGCGAT
t6s27f, C1, TGCTGTGACACACACATACGAGCCAGCCGAGC
t6s3f, D1, TCCCTTGAATAAACCGGAGAAATTTTACCGACC
t6s5c, E1, GTTTGAAATTTCAATATATTTTGG
t6s7f, F1, AATAGATAGGCGCAGTAATAAGAGATTAAATG
t7s10g, G1, CGCAGTTACAAAATAATAGAGGCTTATCCGGTTATCAAC
t7s14e, H1, TTTAATTTGAT
t7s18g, A2, AAAACACTTAATCTTGACAAAGAACTTAATCATTTGTGAATT
t7s20g, B2, ACCCTTATGCGATTTTATGACCTTCATCAAGAGCATCTTTG
t7s24e, C2, CGGTGCGGGCC
t7s28g, D2, TTTCACTCTTTATAAATCAAAAGAGAACCATCACCCAAAT
t7s30g, E2, CAAGTTTTTTGGGGTGAAATCGGCAAAATCCGGGAACCC
t7s4e, F2, TTAATTTTCATC
t7s8g, G2, CGCGCTGTTATTTCTAAGAACCGGATTCCAGAGCCCTAATTT
t7s8g, G2, CGCGCTGTTATTTCTAAGAACCGGATTCCAGAGCCCTAATTT
t8s15f, H2, CGGTTTATCAGGTTTCCATTAACCGGGAATACACT
t8s17c, A3, GCGTATGCTTAAATAGTAAATGCTG
t8s25f, B3, TCTTGCCTTTTGAAGCATATAAGGTTGATGCCCGCT
t8s27c, C3, GCGCTCACAAGCGCTGGGGTGCTCA
t8s5f, D3, TTTGACCTTAAATATAAAGTACCGGACTCGAGAAC
t8s7c, E3, CAGCTTAAAGAAAGTAAAGTAAAT
t9s10g, F3, AGCTTAAGCGAGGCTGTGGCGTTTATGAGCAACCAACATGT
t9s16e, G3, ACTACAGAGC
t9s20g, H3, TGTTTAAATTTCAACTCGGATATTCTATCCACGAAAGA
t9s26e, A4, ATAGGTGAGCT
t9s30g, B4, CGATGCGCCACTACGTATAGCCCGAGATAGGAGTTGCGTT
t9s6e, C4, CTGCTCAGAGC
ts-rem1, D4, GCGCTTAATGCGCGCTACAGGGC

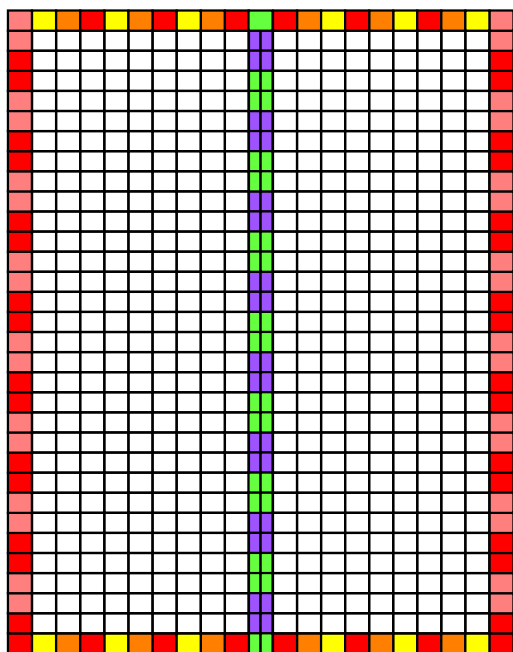
Bridges between trapezoids

t-5s2e-t6s23c-3T, A6, TTAATTAATTTTTTACCATATCAAA
t-7s4e-t8s25c-2T, B6, TTAATTTTCACTCTGAGCTTTTACAA
t-9s6e-t10s27c-1T, C6, CTGCTCAGACGTATACGGAACGA
t-11s8e-t12s29c-0T, D6, TCAAGATTAGTATGCAATACT

t-5s12e-t6s3c-3T, E6, TGTAGCATTCCTTTTTATAAACAGTT
t-7s14e-t8s5c-2T, F6, TTTAATTTGATTTCACACAGAGCC
t-9s16e-t10s7c-1T, G6, ACTACGAAGGCTTAGCACCATT
t-11s18e-t12s9c-0T, H6, ATAGGCTTGCACAAAGTTTAC

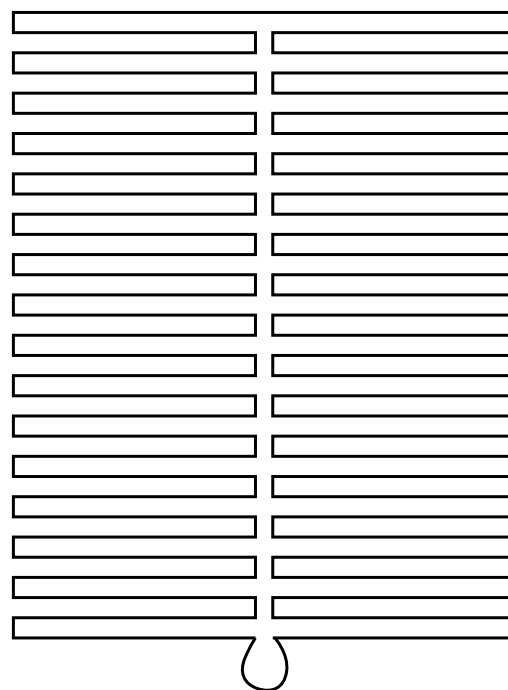
t-5s22e-t6s13c-3T, A7, GTGGGAACAAATTTCTATTTTGTAG
t-7s24e-t8s15c-2T, B7, CGGTGCGGCGCTTCCAAAACATTT
t-9s26e-t10s17c-1T, C7, ATGAGTGAGCTTTTAAATATGCA
t-11s28e-t12s19c-0T, D7, ACTAATTAAGAGGATAGCGTCTC

Supplementary Figure S34: Sequences for the sharp triangle composed of trapezoidal domains.

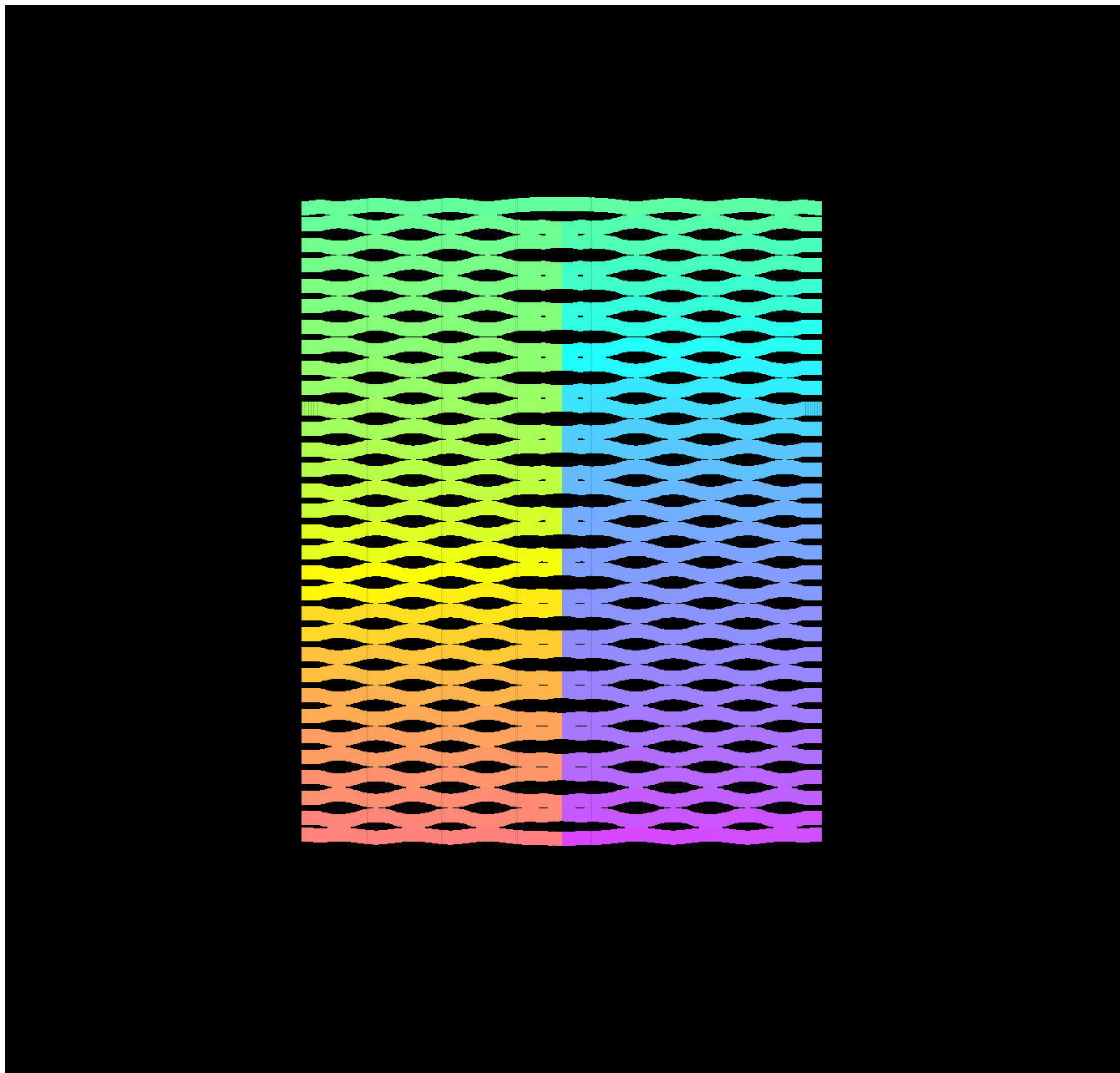
a

21 turns wide at 10.666 bases / turn -> 224 nt

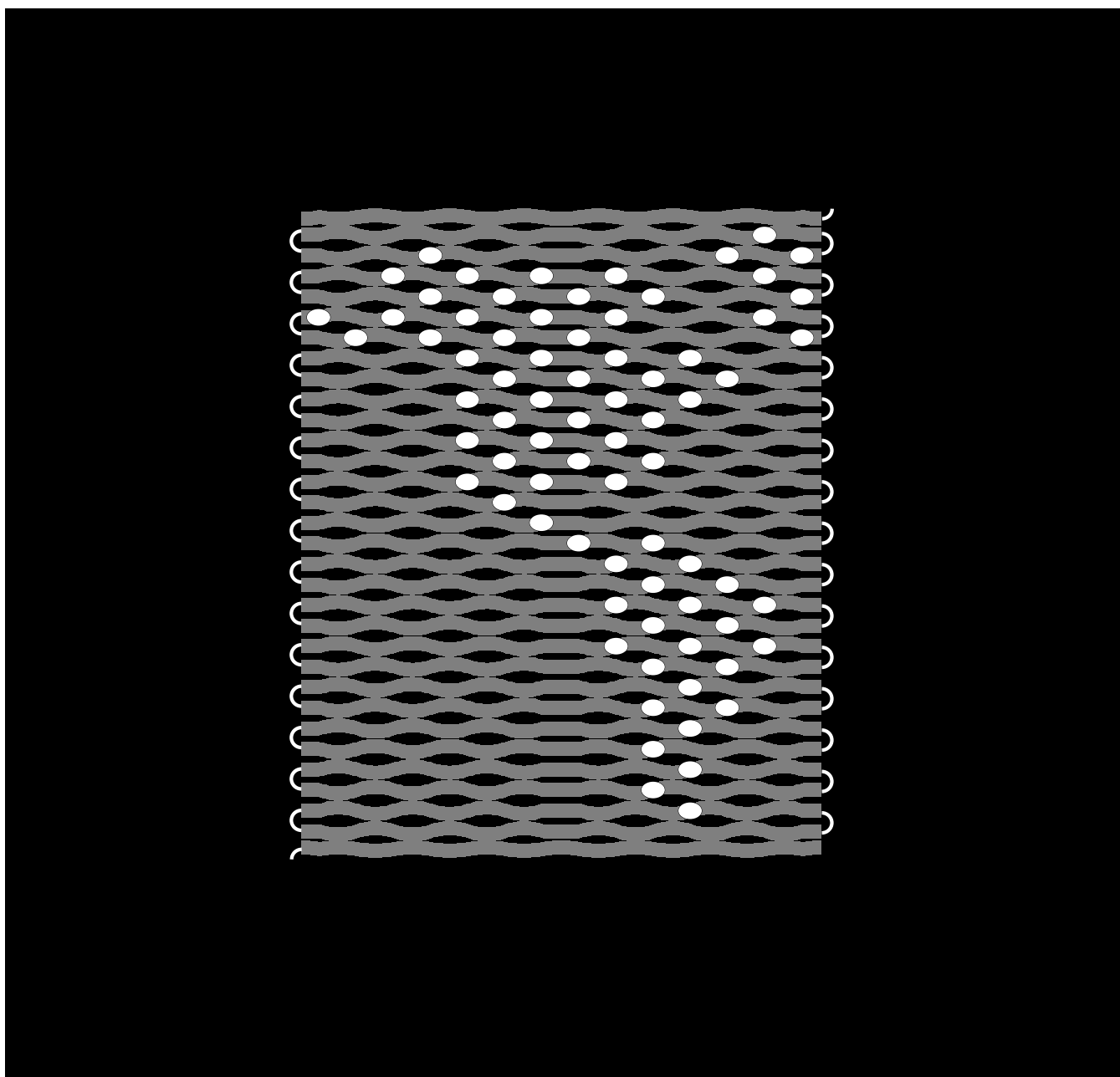
32 helices tall

b

Supplementary Figure S35: Schematics for the “tall” rectangle used for the map pattern shown in Fig. 4e-i. **a** Block diagram. **b** Folding path.



Supplementary Figure S36: Basic crossover diagram for the “tall” rectangle used for the map pattern shown in Fig. 3e-i. Note, here I show the crossovers at the seam as bridged by staple strands. In the experiments shown in Fig. 3f and g, the seam was unbridged and only stacking interactions held it together, as diagrammed in Fig. 3e. For comparison, Fig. 3e is reproduced in the next figure, Supplementary Fig. S36



Supplementary Figure S37: Actual crossover diagram for the “tall” rectangle used for the map pattern shown in Fig. 3e-i. The seam is unbridged and staple strands with ‘TTTT’ loops have been added to the edges to discourage blunt-end stacking. At two corners 4-T tails have been added.

Plate number: 1

t1r0g, A1, AGGGTTGATATAAGTATAGCCCGGAATAGGTG
t1r10e, B1, TGAACAAAGATTAACCCACAAGATAAGACTCC
t1r10f, C1, ATCAGAGAGTCAAGAGGTTAAITGAACAGTCA
t1r12e, D1, TATTTTGCACCGTAACGAGCGCTCTGAACACCC
t1r12f, E1, TCTTTACACCCAGCTACAAITTTAAAGAAGT
t1r14e, F1, ATCGGCTGACCAAGTACCGCACTCTTAGTTGC
t1r14f, G1, GGTATTAATCTTTCTTATCATTCATATCGCG
t1r16e, H1, CATATTTATTTCTTGAGCCAGTAATAAATCAATA
t1r16f, A2, AGAGGCATACAAACGCCAACATGTATCTGCGAA
t1r18e, B2, ACAAGAAAAATTTCTATCTTCTGACAGAAATCGC
t1r18f, C2, TTTTAGTTTCGCGAGAAAACTTTTTTTATGACC
t1r20e, D2, AAATCAATCGTCGCTATTATTAATTAATGCAAG
t1r20f, E2, CTGTAAATATATGTGAGTGAATAAAAGGCTA
t1r22e, F2, TTTAACGTTTCGGGAGAAACAAATACAGTACAT
t1r22f, G2, CTTTACACAGATGAATATACAGTGCATCAA
t1r24e, H2, TTATTAATGAACAAAGAAACCACTTTTTCAGG
t1r24f, A3, ATTTTGGCTTTTAAAGTTTGTAGTACCGGCAAC
t1r26e, B3, CTAAGCAAAATCAATATCTGTGTCACCGCAAGC
t1r26f, C3, AAACCTCTTACACTTGCTGAACCTAGAGGATC
t1r28e, D3, GCCAACAGATACGTGGCAGACAACTAAGAAAT
t1r28f, E3, CGCTAAGAGATAGAAACCTCTGGAAGCGCGC
t1r2e, F3, TAAGCGTCTGATTAAGTTTAAACGCGTACG
t1r2f, G3, AGTGCTACTATACATGCGCTTTTGATCTTTCAG
t1r30e, H3, GTTGAGCCCTGAGTAGAGAAATAGACATCTG
t1r30f, A4, ATCACTTGAATACTCTTTGATTAGTTGTTC
t1r32h, B4, TACAGGCGCGTATTTGGAAATAGACATTAAC
t1r4e, C4, AACAGAGACCCCTCAGAACCGCCACGTTCCAG
t1r4f, D4, GAGCCGCCCAACCAAGGTAAGTTTACGCGCGA
t1r6e, E4, GACTTTGAGGTAGCACCATTACATATACCGG
t1r6f, F4, AATCACCACTATTTGGAAATAGACATTAACCTA
t1r8e, G4, TTATTAAGTAAAGGTGGCAACATACGTCACCC
t1r8f, H4, TACATACAGATGTATGTACAACTGTACAGA
t3r0g, A5, TGCTCAGTACACGAGCGATAGTGGGGGTACG
t3r10e, B5, GCGCATTAATAAGAGCAAGAACTAATACGGA
t3r10f, C5, GCCCAATAGACGAGGAAATTAATCTTTCAGAG
t3r12e, D5, AGGTTTGGCCAGTTACAAAGTAAACAGGGAA
t3r12f, E5, CCTAATTTAAGCCTTTAAATCAAGAAATGAGAA
t3r14e, F5, CTAATTTACCGTTTATTTTCTTCTGCGGG
t3r14f, G5, CAAGCAAGGAGCATGTAGAAACACAGAGAAATA
t3r16e, H5, ACGCTCAACGACAAAGGTAAGTATCCCATC
t3r16f, A6, TAAAGTACCAAGTAGGCTTAATGTCTAAATTT
t3r18e, B6, TATGTAAAGAAATACCGACCGTGTAAAGCCA
t3r18f, C6, AATGGTTTGTCTGATGCAAACTCATTTTCCCT
t3r20e, D6, TTGAATTAATGAAACATAGCAATTAATACTA
t3r20f, E6, TAGAATCCCTTTTAAATGAAACGGAATTCG
t3r22e, F6, ACAGAAATCTTTGAATACCAAGTTAATTTTCAT
t3r22f, G6, CCTGATTTGAAAGAAATTCGCTGAGAGAAAGGAG
t3r24e, H6, CGACAACTTCATCATCTTCTGATACAGTAAJAA
t3r24f, A7, CGGAATTAGCTTTAAATCTTTGGTTTGGCAA
t3r26e, B7, GCCACGCTTTTGAAGGAAATTTGAGGAAACAAT
t3r26f, C7, ATCAACAGGAGAGCGACAGCAAAATATTTTT
t3r28e, D7, GTCAACAGATTAGTCTTTTAAATGCGCAACAGT
t3r28f, E7, GAATGGCTACCAAGTAATAAAGAGGCAAACTAT
t3r2e, F7, GGAAAGCGGTAACAGGTCGCTGATCGGGGTTT
t3r2f, G7, TGCCCTTGACAGTCTCTGAATTTTACCCCTCAGA
t3r30e, H7, GTAAAGAGCTGTGATTAATTCAGAAATTCACCA
t3r30f, A8, CGGCGCTTGCTGCTGTCATCAGCATGTACGAGAG
t3r32h, B8, CAGCTATAACGTGCTCTTCTGCTTGCACACCGA
t3r4e, C8, GTTGTGCCACTCAGAGCGCCACCGCCAGAGAT
t3r4f, D8, GCCACCACTCTTTTCTATAATCAAAATGACAGG
t3r6e, E8, TTATTTATGTACCAAAATGAACATTTATTAGC
t3r6f, F8, CCGGAAACTAAAGGTGAATTTATATAAGAA
t3r8e, G8, ATACCCAAACACCCAGGAAATAGTACGAGGAA
t3r8f, H8, ACGCAAAAGAGAACTGCGATGATTTTGTAGTTAA
t5r0g, A9, CCTCAAGAGAAAGATAGGATTAAGAAACAGTT
t5r10e, B9, CTTTACAGTATCTTACGAGGCGCAGTTACCA
t5r10f, C9, GCAATAGCAGAGAAATACATAAAACAGCCAT
t5r12e, D9, GAGCGGTTTCCCAATCAAAATAGATAGCAGC
t5r12f, E9, ATTATTTTATTAGCGAACTCTCCGAGCTAGGAA
t5r14e, F9, TAAGTCTGCGGCCCAATGACAGCAAGAACGCG
t5r14f, G9, TCAATCCGGAACAGAAAAATATAATTTCTGT
t5r16e, H9, GCGTTATACGACAAATAGCAATACATACATAGA
t5r16f, A10, CCAGACGACAAATCTTTCACAGTAGATAAATA
t5r18e, B10, TAACTTCAATAAGATAAACAACCTATCATAT
t5r18f, C10, AGCGGTTAGGCTTAGGTGGTTTAACTTAGA
t5r20e, D10, AAAACAAACTGAGAAAGAGTCAATATACCTTTT
t5r20f, E10, TTAAGACGATTAATTTACATTTTAAACAAAAATC
t5r22e, F10, AAACCTACCGGAAATTTTCAATTTTCAATCAAG
t5r22f, G10, GCGCAGAGATATCAAAATTTATTTGTATCAGAT
t5r24e, H10, GGAATTTAGTTCATCAATTAATTCAGGGTTAG
t5r24f, A11, GATGCGAAAGATTTAGACTTTTACAGGTTAT
t5r26e, B11, AGGCGGTCTCTTTAGGAGCACTTAAACATTTGA
t5r26f, C11, CTAATAATAGTATTAACACCGCTCTGACACTGA
t5r28e, D11, GAAATGGAACCACTGCGCATTAAGACAGGGTG
t5r28f, E11, TAGCCCTATTTATTTACATTTGCGCAATATTA
t5r2e, F11, ACAACCACTGCGCTATTTTTCGAGCACTGAGACT
t5r2f, G11, AATGCCCAATAAATCTCTATTAAGAGAACAC
t5r30e, H11, AGAAGTGTCAATGCAACAGGAAAAATCTGCT
t5r30f, A12, CCGCCAGCTTTTATAATCAGTGAAGAGATCGT
t5r32h, B12, AGCGGAGCTTAAACAGGAGCGTGAAGATCTCG
t5r4e, C12, TCGGCATTTCCGCGCGCAGCATTTGATGATATTC
t5r4f, D12, CACGAGGTTTGGTATGATACCCCTCATGAGC
t5r6e, E12, ATTGAGGAACTCAGTAGGACGACAGCTTTTCA
t5r6f, F12, AGCACCGTAGGAAAGTAAATTTTATTTTGT
t5r8e, G12, GAAGAGAAAAATAGAAATTTCAATTTTCAACCG
t5r8f, H12, TCACAATCCCGAGGAAACGCAATTAATGAAATA

Plate number: 2

t7r0f, A1, TGAAGTATTAAAGAGCTATTATT
t7r10f, B1, AAAAGTAAACGCTCAAAATGAAAAACGATT
t7r12f, C1, TTTTGTGTGCTATTCGCGTATTTTAAATCAGA
t7r14f, D1, TATAGAAGACGCGCTGTTTATCAGTTTACGCT
t7r16f, E1, AATGCAGAGAAAAAGCTGTTTAGGGAATCAT
t7r18f, F1, AATTAAGTACATAGTCTGAGAGACGTGAATTT
t7r20f, G1, ATCAAAATGAAGATGATGAAACAAATTAACCT
t7r22f, H1, GAGCAAAAACTTCTGAAATTTGATGATTTGTT
t7r24f, A2, TGGAATATGCGCTCAATAGATTAATCACTTAAT
t7r26f, B2, AGATTAGACAGCAGAGAAATAAAAATACCGA
t7r28f, C2, AGCAACCACTACATTTTTCAGCTCACGCTCAT
t7r2f, D2, CTGAAACAGTCAGACAGTTTGCGCTCAGGAGT
t7r30f, E2, GGAATATCAGGAAACGTCGCCATTAAGGGGATTTAGA
t7r4f, F2, TGAGGACGCGCTCAGACTGTAGCGATCAAGTT
t7r6f, G2, TGCTTTTAAAGCAAAAGGGCGACAGGTTTACC
t7r8f, H2, AGCGCAAGCAGATAGCGGAACATTTTAAAG
t1r0g, A3, TATCACCGTACTCAGGAGGTTTAGATAGTAGTAG
t1r10e, B3, GGACGTTGAGAACTGGCTCATTTATGCGCTAAT
t1r10f, C3, CGATTTTAGAGAAAGAAATCTACGGAATAAAA
t1r12e, D3, TTTGCGAGCGAGAGGCTTTTGCAATCTGTAA
t1r12f, E3, CCAAAATAGGGGGTATAGTAAAAAAGGATT
t1r14e, F3, TTTTAAATGCGCCGAAGACTTCAACAGAGAGT
t1r14f, G3, AAGAGAAACGAGCTTCAAGAGCAAGATTTTCAT
t1r16e, H3, CAGTAGAACAAGTTTGTTCCTCAATTTTAGGCG
t1r16f, A4, TCCATATATTTAGTTTTCGCAATTAAGCATATA
t1r18e, B4, CTGTAATAGGTTTGCACAAACACAAATATA
t1r18f, C4, GCTAAATCTTTTTCGGGAGAGGCGCGAGAG
t1r20e, D4, TCAGGTCAITTTTGTAGAGATCTTACCTTGCTT
t1r20f, E4, GTTAGCTATTTGCTGAGAGTCTGTTTAAATCA
t1r22e, F4, AAATAATTTTAAACCAATAGGAACAACAGCTA
t1r22f, G4, GCTCATTTGCGCTCTGCGCTCTCTGCGCTCAG
t1r24e, H4, GCTTTTCGGCTTGCAGCCAGCTTCAATCTATC
t1r24f, A5, GAAGTCTGCGCGGAAACAGGACAGTGCACAG
t1r26e, B5, CCGGCTACCTTGAGGTGCACTCTCAAAATCT
t1r26f, C5, CTTGCTAGCCGAGCTCGAATTCGTCCTGCTGCT
t1r28e, D5, GGGAGAGGCATTAAATGAATCGGCCACCTGAJAA
t1r28f, E5, GCCAGCTCGCGTTTTCGCTATTTGGAATCAJAAA
t1r2e, F5, ACGTTAGTTTCTAAAGTTTGTGCTGATACAGG
t1r2f, G5, CGTAACGAAAAATGAATTTTCTGTAGTGAATTT
t1r30e, H5, AGTTTGGACGAGATAGGTTTGAAGTTAATAAC
t1r30f, A6, GAATAGCCCAAGAGTCCACTATTAAAGCGCGC
t1r32h, B6, GAACGTGCGGAGAAAGGAGGAAATGCGCGCG
t1r4e, C6, CAATGACAGCTTGATACCGATAGTCTCCCTCA
t1r4f, D6, CTTAAACCAACCACTGCGCCACGCGGGTAA
t1r6e, E6, AAACGAAATGCCACTACGAGGCGAGCGAGCAA
t1r6f, F6, ATACGTAAAGGCAAAAGAAATACACTGACGAA
t1r8e, G6, CCAGGCGCGAGGACAGATGAACGGGTGAJAAA
t1r8f, H6, CTTTGAJAAATAGGCTGGCTGACCTCACTTATG
t3r0g, A7, CCCTCAGAACCGCCACCTCTCAGAAACAGCGCC
t3r10e, B7, ACGAACTATTAATCATTTGTGAATTTTCATCAAG
t3r10f, C7, TTTCAACTACGGAACAACATTTTAAACACTAT
t3r12e, D7, ACTGATATGCTTTTACCAGACGACTTAATAJAA
t3r12f, E7, CATAACCCGCGTCAATATCTCGGTTATTATAG
t3r14e, F7, GAAGCAAAAAAGCGGATTTGCATCAATGTTTAG
t3r14f, G7, TCAGAAAGCTTCCAAACAGTTCAGGATTTAAJATA
t3r16e, H7, TCGCAAAATAGTACGTTGCTGGAACAGAGCG
t3r16f, A8, TGCAACTAGGTCAATTAACCTGTTTAGAAATTAG
t3r18e, B8, CAACGCAAAAGCAATAAAGCCTCAGGATACAT
t3r18f, C8, CAAATTAGGATAAAAAATTTTATAGGATATTCA
t3r20e, D8, AGAGAACTCAGCTGATAAATTTATGCTTTATTT
t3r20f, E8, ACCGTTCTGATGAACGGTAACTGCTAAATTTT
t3r22e, F8, CTTTCACTTCGCAATTAATTTTGTAGCAJAAA
t3r22f, G8, GTTAAATTAACATTTAAATGTGAGCATCTGCCA
t3r24e, H8, TTCGCCATGGAACGACAGCATGTCTGAGCCAG
t3r24f, A9, GTTTGAGGTGAGGCTGCGCAACTGTTTCCAGT
t3r26e, B9, TCAATAGCTTGTAAACAGAGCGCCAAAGCGCCA
t3r26f, C9, CACGAGCTGTTTCTGCTGTGAAATTTGCGCTC
t3r28e, D9, TGGTTTCTTTCTTCAGTTCGGGAAJAAATCATG
t3r28f, E9, ACTGCGCGCTTTTACCAGCTGAGATGTTGGTGT
t3r2e, F9, TGCTAAACTCCACAGACGCCCTCTCACCGCCA
t3r2f, G9, TGTAGCATAACTTTCAACAGTTTCTTAATTTGTA
t3r30e, H9, TGGACTCCGCGCAAAATCCCTTATACGCGAGG
t3r30f, A10, CCGAAATCAACGCTCAAAGGCGAAJAGGGAGC
t3r32h, B10, CCCCAGTTTAGAGCTTTCAGCGGGAJAAAGAGC
t3r4e, C10, ATATATTTCTCAGCTTGCTTTTTCGAGTGGGATTT
t3r4f, D10, TCGGTTTAGTGTGCTGAGGCTTTCAGAGAGCT
t3r6e, E10, CTCATCTTGAAGTTTTCATTAJAAACATTAACCG
t3r6f, F10, TTTCAATGATGACCCCGAGCATTAAGCGCGAG
t3r8e, G10, AGTAATCTTCAATAGGGAACGAACTAAJAAA
t3r8f, H10, ACGGTCAATGACAAAGACCGGAATATGTTTAA
t5r0g, A11, CTCAGAGCCACCACTCTCATTTTTCGTAACAC
t5r10e, B11, AAAGATTCTAAATTTGGGCTTGAGATTCAATAC
t5r10f, C11, ACGAGTAGATCAGTTTGAAGTTTTCGCGCAJAAA
t5r12e, D11, TAAATATTGAGGCAATAGTAAGAGCAAGTATAG
t5r12f, E11, GAAATTAACCATTTGAATCCCCCTCACATAAAT
t5r14e, F11, TACCTTTTAAAGTCTTTTCACTGACATCTGCTA
t5r14f, G11, CAAAAATCAITGCTCTTTTGTATATTTGCTGA
t5r16e, H11, TTTCAITTTCTGTAGCTCAACATGTTTGAAGAG
t5r16f, A12, ATATAATGGGGGCGGAGCTGAAATTAACATC
t5r18e, B12, TATATTTTCAATACAGGCAAGCAAGCTATATAG
t5r18f, C12, CAATAAATAAATGCAATGCTGAGAGGCGCGG
t5r20e, D12, CATGTCAAAATACCACTCAATTAATACCCCTCA
t5r20f, E12, AGACAGTCTCATATGTATCCCCGGTTTGTATATA
t5r22e, F12, ACCGCTCGTTAAATTTGTAAACGTTTAAACCTAG
t5r22f, G12, GCAAAATAGATTTCTCGTGGGAAACCGTTGGTG
t5r24e, H12, GCGCATGCGCATCTGAACCGTGCAGTAAACA

Plate number: 3

t-5r24f, A1, TAGATGGGTGCGGGCTCTTCGCGCAAGGCG
t-5r26e, B1, GCTCAAGAGGTAAACGCGAGGTTTTCGGGAAG
t-5r26f, C1, ATTAAGTTTTCACACAAACATACGCTCAATGA
t-5r28e, D1, AGCTGATTCTCACATTAATTCGCTGTTATCC
t-5r28f, E1, GTGAGCTAGCCCTTCACGCGCTGGGTTTGCC
t-5r2e, F1, GAGAAAGTTCACAGTACAACTCCGCGAAC
t-5r2f, G1, TGAGTTTCAAAGGAAACAACTTAAGATCTCCAA
t-5r30e, H1, GAGAAAGTTCACAGTACAACTCCGCGAAC
t-5r30f, A2, CCAGACGCGATGGCCCACTACGTGAGGTGCC
t-5r32h, B2, GTAAAGCACTAAATCGGAACCTTAAACCCGTC
t-5r4e, C2, AAAGGCGCTCCAAAGAGGACCTTAGCGAGGT
t-5r4f, D2, AAAGAGGCTTTTCGCGGATGTCGCGGTAGCA
t-5r6e, E2, GCGAAACAGAGGCTTTGAGGACATAGGAGTT
t-5r6f, F2, ACGGCTACAGTACAAACGCGAGATTCGAGACT
t-5r8e, G2, CCAATCATTAATAGCCGGAACGTACCAACG
t-5r8f, H2, GCTCCATGACGTAAACAGGCTGCTACACCA
t-7r10e, A3, CATTAACCTTGCCCTGACGAGAAACATTAAGT
t-7r12e, B3, AAACAGGTTTATCGAGTACATGAAGATACCA
t-7r14e, C3, TTTTTCGCGAGGAAACAGAGATGAATGCTTT
t-7r16e, D3, TCAATTCGTAGGCTTATGAGTTTAAAGGCTCA
t-7r18e, E3, AGGTAAAGCAATATAGTAGTAAAGGATGTGTA
t-7r20e, F3, AGCAATGATGAAAGGTTGAGATATGCGCT
t-7r22e, G3, GATTGACCCCAAAAAACAGGAAGATGATTAATC
t-7r24e, H3, CAGCGTGGCTTTTTCGCGTGTGCTAAACGCGG
t-7r26e, A4, GCATTAAGGAAAGGGGAGTCTGTTTATACG
t-7r28e, B4, GAATTAAGAAATTTGCGCTGAGGCTGCGGAG
t-7r2f, C4, AATTAATAATAGGAACCATGTACAGAGGATAGCAAGCCAA
t-7r30e, D4, ACCCAATGCAAGCGGCTCAACGCTCCCTGAG
t-7r32e, E4, CAGGTTTTTGGGGTCAGCAACATC
t-7r4e, F4, CAGGAAATTTTTCGAGTGTGAAGAAATTGCG
t-7r6e, G4, CGCCTGATGACAGCATGCGCAACGAGCTCAG
t-7r8e, H4, GAATTAAGAAATTTGCGCTGAGGCTGCTATCAT
t-rem1, A5, GCGCTTAA

Supplementary Figure S38: Sequences for the “tall” rectangle used for the map pattern shown in Fig. 3e-i. These sequences are for a bridged seam.