

Folding DNA to create nanoscale shapes and patterns

Supplementary Notes 1–11

Paul W. K. Rothemund

Computation and Neural Systems, and Computer Science, California Institute of Technology, Pasadena, California, United States of America

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.

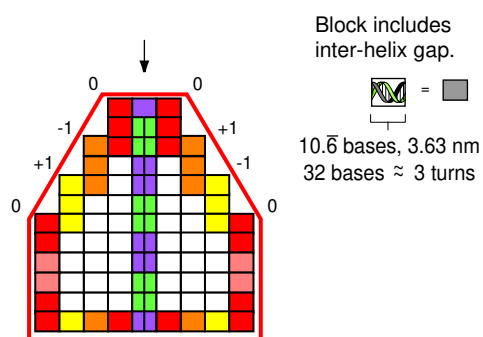
A note on seams: while most seams presented here are vertically aligned (for simplicity and convenience in design), and this necessitates the use of bridges to strengthen seams, it is possible to create staggered seams (as E. Winfree has suggested) so that staple strands naturally cross and bridge the 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.

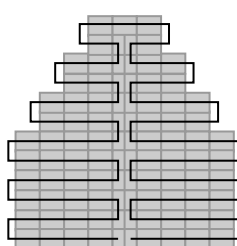
In some shapes it is desired that a strand pass both back and forth through a narrow waist so that it may access different portions of the shape. For example, consider a vertically oriented hourglass shape. If it is desired that a circular scaffold be used, then the scaffold must pass both up and down 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



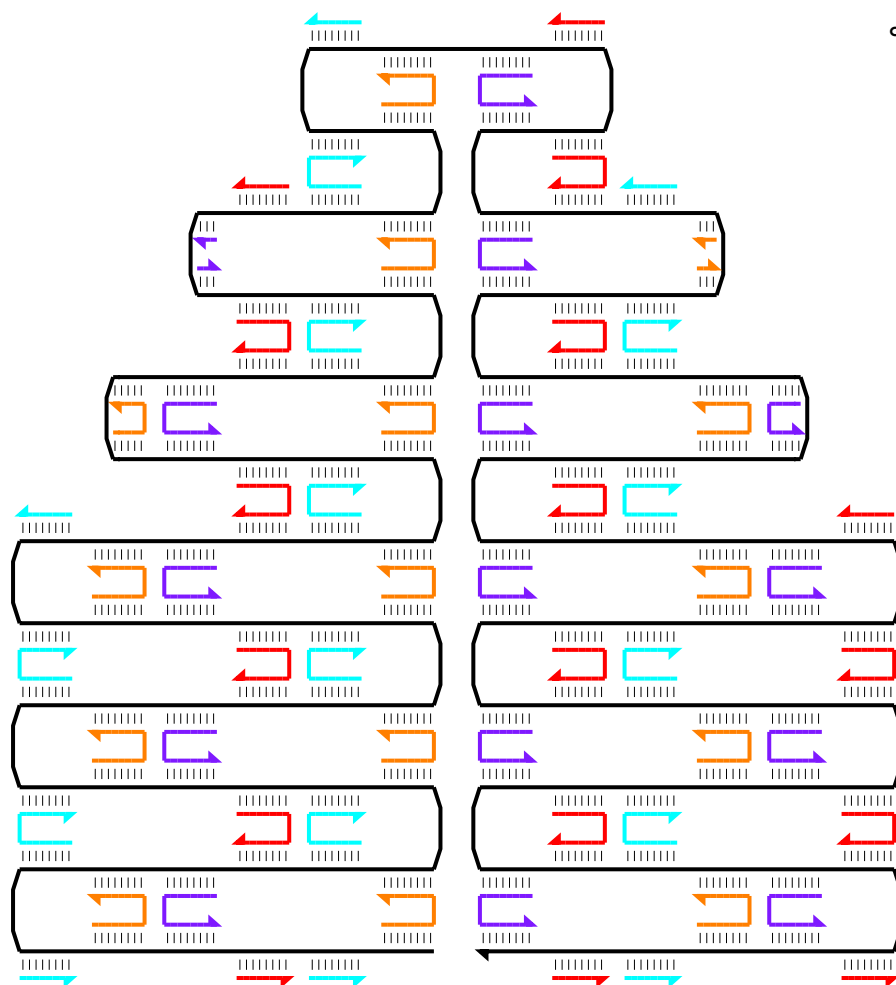
2. Folding path



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
```

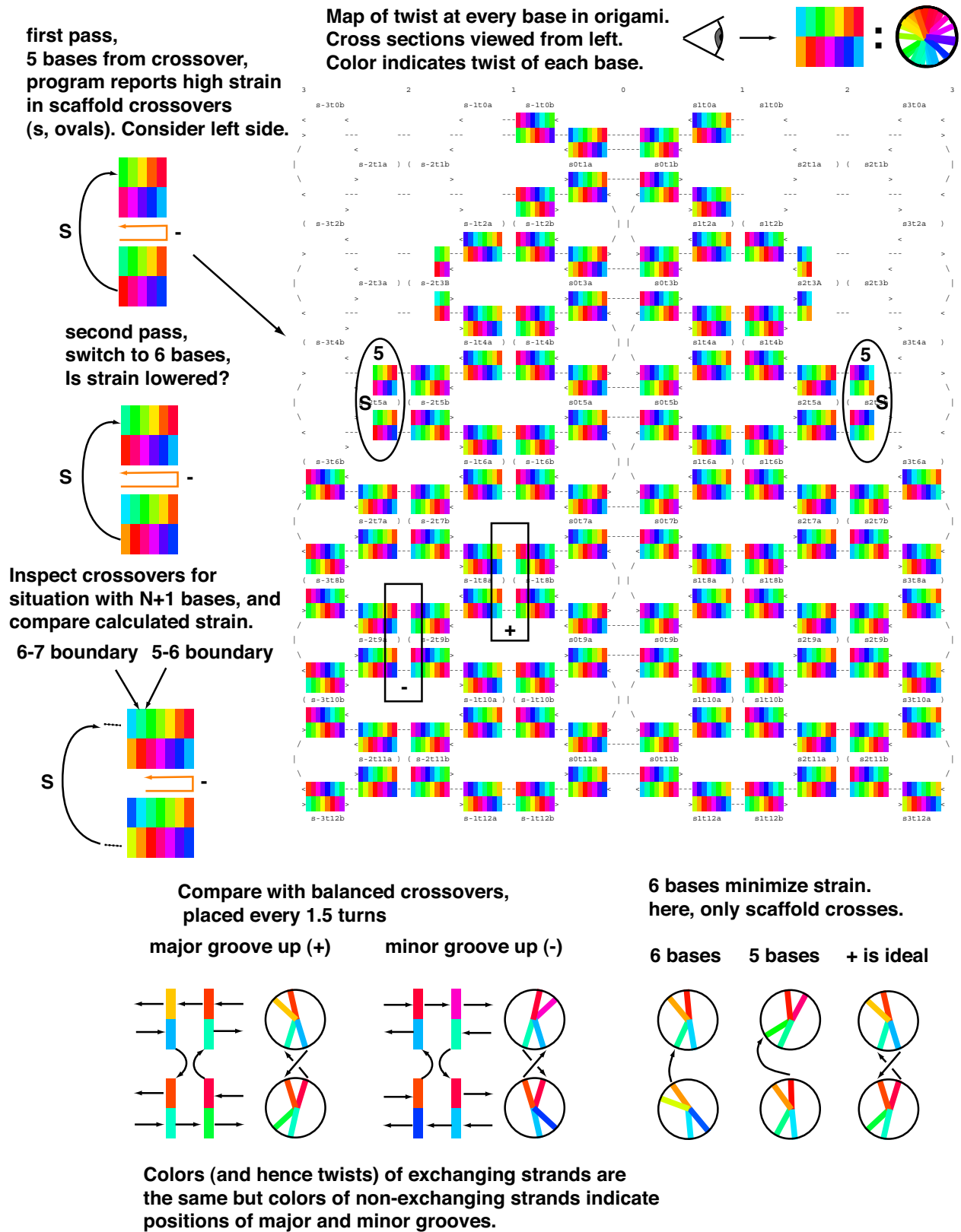
Supplementary Figure S1: First three steps of origami design.

```

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---AGGCGGGT>          >ACTCATT---GGCACCCC---AGGCTTTA---CACTTTAT---GCTTC          ---          >
/          <          <          CCTGG CGAAGCAC<          <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 CGAAAATC>          \ /          >GCTCACAA TTCCACAC>          >
( s-3t6b          s-1t6a ) ( s-1t6b          | |          s1t6a ) ( s1t6b          s3t6a )
CCGATAAG<          <TAAACCGG CTAAAGCC<          / \          <GTCTTTTG TCGATACT<          <CTCGAGCC
>GGCTATTC---TTTTGATT---TATAAGGG---ATTTTGCC---GATTTCGG---AACCACCA>          >ATTTTACA---CAGGAAAC---AGCTATGA---CCATGATT---ACGAATTC---GAGCTCGG>
/          <          <          <AAAATAA ATATTCCC<          <TTGGTGTT-----TAAAGTGT<          <GGTACTAA TGCTTAAG<          \
|          s-2t7a ) ( s-2t7b          s0t7a          s0t7b          s2t7a ) ( s2t7b          |
\          >          >          >GGTTGAGT GTTGTTCC>          >CCACTATT-----GTGCCAAG>          >TCGACTCT AGAGGATC>          /
<GCTCTATC---CCAACTCA---CAACAAGG---TCAAACTC---TGTTCTCA---GGTGATAA<          <CACGGTTC---GAACGTAC---GGACGTCC---AGCTGAGA---TCTCCTAG---GGGCCCCAT<
CGAGATAG>          >AGTTTGA ACAAGAGT>          \ /          >CTTGCAATG CTTGCAGG>          >CCCGGGTA
( s-3t8b          s-1t8a ) ( s-1t8b          | |          s1t8a ) ( s1t8b          s3t8a )
GGGACTAT<          <AACTGCAA CTTCAAGT<          / \          <CAAAATGT TGCAGCAC<          <GCAATGGG
>CCCTGATA---GACGGTTT---TTGCCCT---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---CTTGCTAG---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---TTGGCCG---CTTGCAAC---GCTCTTTC---CTTCCCTT<          <GGCCGTGG---CGAAGACC---ACGGCCTT---TGGTCCGT---TTCCGGGT---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 CGAAAAATC>          \ /          >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>          /
<GCTCTATC---CCAACTCA---CAACAAGG---TCAAACTT---TGTTCTCA---GGTGATAA<          <CGGTCACG---GTTCGAAC---GTACGGAC---GTCCAGCT---GAGATCTC---CTAGGGGC<
CGAGATAG>          >          >          >AGTTTGA ACAAGAGT>          \ /          >CAAGCTTG CATGCCTG>          >GATCCCCG
( s-3t8b      s-1t8a ) ( s-1t8b      | |          s1t8a ) ( s1t8b      s3t8a )
GGGACTAT<          <          <          <AACTGCAA CCTCAGGT<          / \          <ATGTTGCA GCACTGAC<          <TGGGTTGA
>CCCTGATA---GACGGTTT---TTCGCCCT---TTGACGTT---GGAGTCCA---CGTTCTTT>          >CGTCGTTT---TACAACGT---CGTGACTG---GGAAAACC---CTGGCGTT---ACCCAAT>
/          <          <          <CTGCCAAA AAGCGGGA<          <GCAAGAAA-----GCAGCAAA<          <CCTTTTGG GACCGCAA<          \
|          s-2t9a ) ( s-2t9b      s0t9a      s0t9b      s2t9a ) ( s2t9b      |
\          >          >          >CACTACGT GAACCATC>          >TTGGGGTC-----TCGCTATT>          >GGGGGATG TGCTGCAA>          /
<GCTACCGG---GTGATGCA---CTTGGTAG---TGGGTTTA---GTTCAAAA---AACCCAG<          <AGCGATAA---TGCGGTGC---ACCGCTTT---CCCCCTAC---ACGACGTT---CCGCTAAT<
CGATGGCC>          >          >          >ACCCAAAT CAAGTTTT>          \ /          >ACGCCAGC TGGCGAAA>          >GGCGATT
( s-3t10b      s-1t10a ) ( s-1t10b      | |          s1t10a ) ( s1t10b      s3t10a )
TTAGCCCC<          <          <          <GCTAAATC ACGAAATG<          / \          <CGTGGCTA GCGGGAAG<          <GACTTACC
>AATCGGGG---GCTCCCTT---TAGGGTTC---CGATTTAG---TGCTTTAC---GGCACCTC>          >AGAGGGCC---GCACCGAT---CGCCCTTC---CCAACAGT---TGCGCAGC---CTGAATGG>
/          <          <          <CGAGGGAA ATCCCAAG<          <CCGTGGAG-----TCTCCGG<          <GGTTGTCA ACGCGTCG<          \
|          s-2t11a ) ( s-2t11b      s0t11a      s0t11b      s2t11a ) ( s2t11b      |
\          >          >          >GACGGGA AAGCCGGC>          >GAAGGGA-----CTTTCCGG>          >GGAACCA GGCAGC>          /
<ATCTCGAA---CTGCCCTT---TTCGGCCG---CTTGACAC---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---          ---          ---          <
>          >          >          GCGCGTTI>          \ /          >TGCCCAGC          >          >
( s-3t2b          s-1t2e ) ( s-1t2i          | |          s1t2i ) (          s3t2a )
<          <          <          <TTTTTCTT 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>          /
<ATCTCGAA---CTGCCCT---TTCCGCCG---CTTGACCC---GCTCTTTC---CTTCCCTT<          <GAAAGGCC---GTGGCGAA---GACCACGG---CCTTTGGT---CCGTTTCG---CGGTAAGC<
>TAGAGCTT>          >          >          >GAACGTGG---CGAGAAAG>          >CACCGCTT---CTGGTGCC>          >GCCATTGC<
s-3t12f          s-1t12j          s1t12g

```

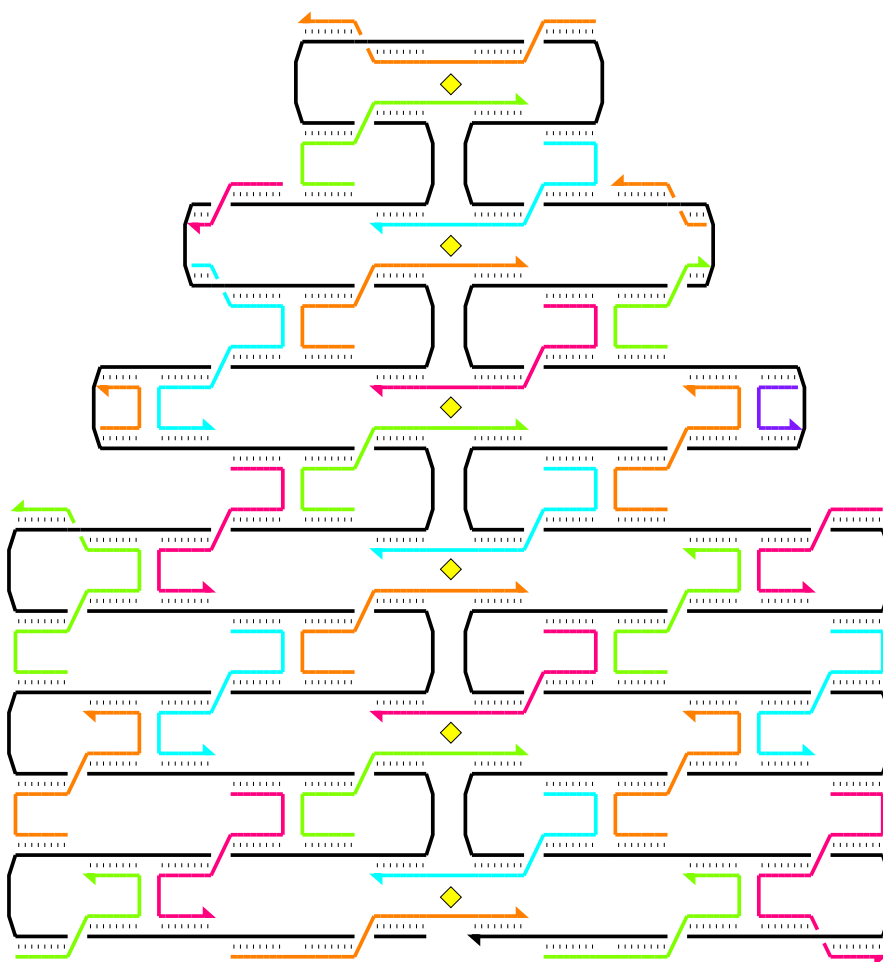
Supplementary Figure S6: Third pass diagram with staple strands after merge (bridged seam).

```

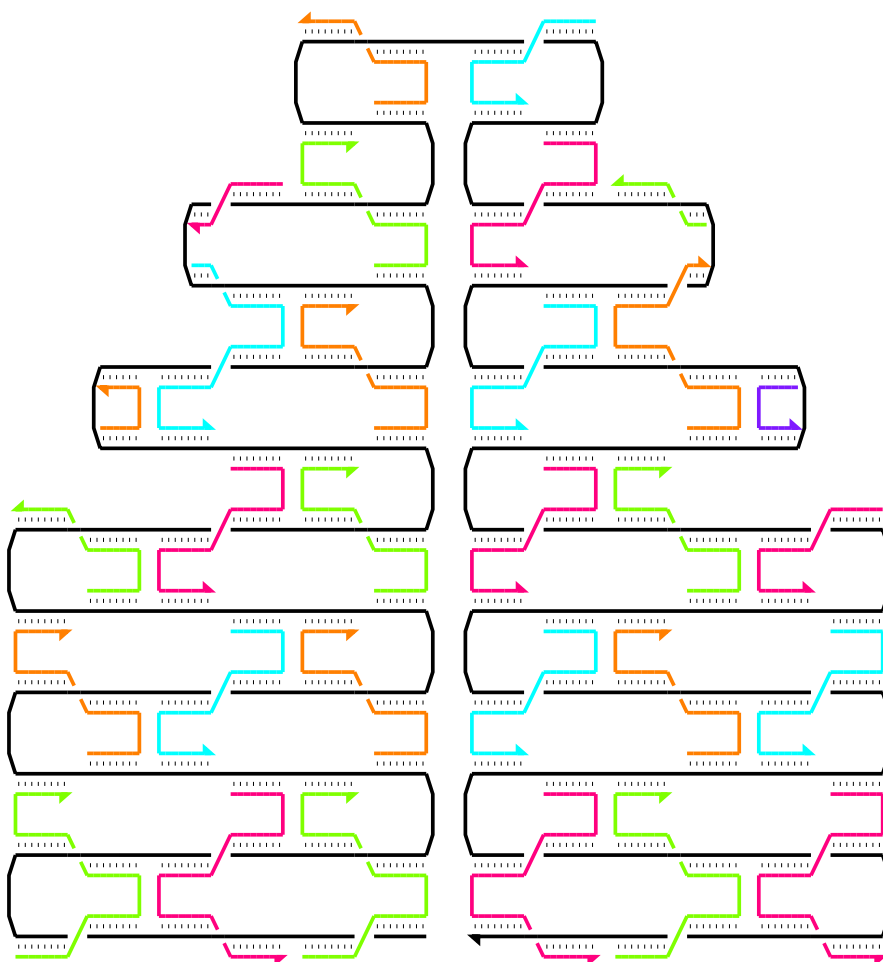
3          2          1          0          1          2          3
s-3t0b          s-1t0a          s1t0e          s1t0b          s3t0a
<          <          <          ---AGAGGGGC<          <AATTACGT---          <          <
>          ---          ---          ---TCTCCCCG---CGCGTTGG-----CCGATTCA---TTAATGCA---          ---          ---          >
/          <          <          <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          |
\          >          >          CACGCT GGTTTGCC>          >CTGTTTGA-----AAATTGTT>          >ACACAACA TACGAG          >          /
<          ---          ---          GTGCGA---CCAAACGG---GGTCGTCC---GCTTTTAG---GACAAACT<          <TTTAACAA---TAGGCGAG---TGTTAAGG---TGTGTTGT---ATGCTC          ---          <
>          >          >          >CCAGCAGG CGAAAATC>          \ /          >ATCCGCTC ACAATTCC>          >
(          s-1t6e ) (          | |          s1t6e ) (          s3t6e )
CGGATAAG<          <          <          <TAAACCG CTAAGGC<          / \          <TTTGTGCA TACTGGTA<          <AGCCATGG
>GGCTATTC---TTTTGATT---TATAAGGG---ATTTTGCC---GATTTTCGG---AAACCACCA>          >CACACAGG---AAACAGCT---ATGACCAT---GATTACGA---ATTCGAGC---TCGGTACC>
/          <          <          <AAAACATA ATATTCCC<          <TTGGTGGT-----GTGTGTCC<          <CTAATGCT TAAGCTCG<          \
|          s-2t7f ) (          s0t7f          s2t7f ) (          |
\          >          >          >GTTGAGT GTTGTTC>          >CCACTATT-----GCCAGTGC>          >CAGGTCGA 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
>CCCTGATA---GACGGTTT---TTCGCCCT---TTGACGTT---GGAGTCCA---CGTTCTTT>          >CGTCGTTT---TACAACGT---CGTGACTG---GGAAAAACC---CTGGCGTT---ACCCAAC>
/          <          <          <CTGCCAAA AAGCGGGA<          <GCAAGAAA-----GCAGCAAA<          <CCTTTTGG GACCGCAA<          \
|          s-2t9f ) (          s0t9f          s2t9f ) (          |
\          >          >          >CACTACGT GAACCATC>          >TTGGGGTC-----TCGCTATT>          >GGGGGATG TGCTGCAA>          /
<GCTACCGG---GTGATGCA---CTTGGTAG---TGGTTTA---GTTCAAAA---AACCCAG<          <AGCGATAA---TGCGGTG---ACCGCTTT---CCCCCTAC---ACGACGTT---CCGCTAAT<
CGATGGCC>          >ACCCAAAT CAAGTTTI>          \ /          >ACGCCAGC TGGCGAAA>          >GGCGATTA
(          s-1t10f ) (          | |          s1t10g ) (          s3t10g )
TTAGCCCC<          <GCTAAATC ACGAAATG<          / \          <CGTGGCTA GCGGGAAG<          <GACTTACC
>AATCGGGG---GCTCCCTT---TAGGGTTC---CGATTTAG---TGCTTTAC---GGCACCTC>          >AGAGGGCC---GCACCGAT---CGCCCTTC---CCAACAGT---TGCGCAGC---CTGAATGG>
/          <          <          <CGAGGGAA ATCCCAAG<          <CCGTGGAG-----TCTCCGGG<          <GGTTGTCA ACGCGTCG<          \
|          ) (          | |          ) (          |
\          >          >          >GACGGGA AAGCCGGC>          >GAAGGGA-----CTTTCCGG>          >GGAACCA GGCAAAGC>          /
<ATCTCGAA---CTGCCCT---TTCGGCCG---CTTGACC---GCTCTTTC---CTTCCCTT<          <GAAAGGCC---GTGGCGAA---GACCACGG---CCTTTGGT---CCGTTTCG---CGGTAAGC<
>TAGAGCTT>          >GAACGTGG---CGAGAAAG>          >CACCGCTT---CTGGTGCC>          >GCCATTG<
s-3t12g          s-1t12g          s1t12g

```

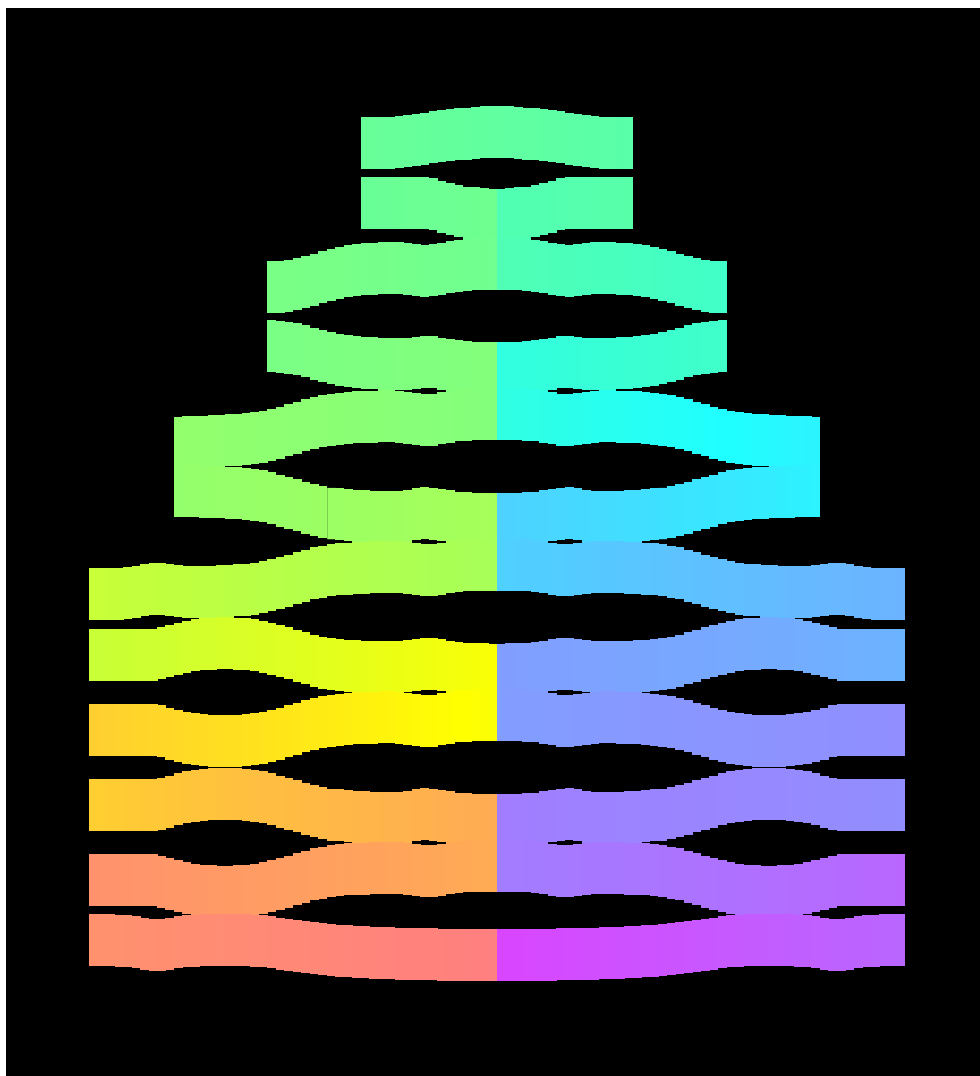
Supplementary Figure S7: Third pass diagram with staple strands after merge (unbridged seam).



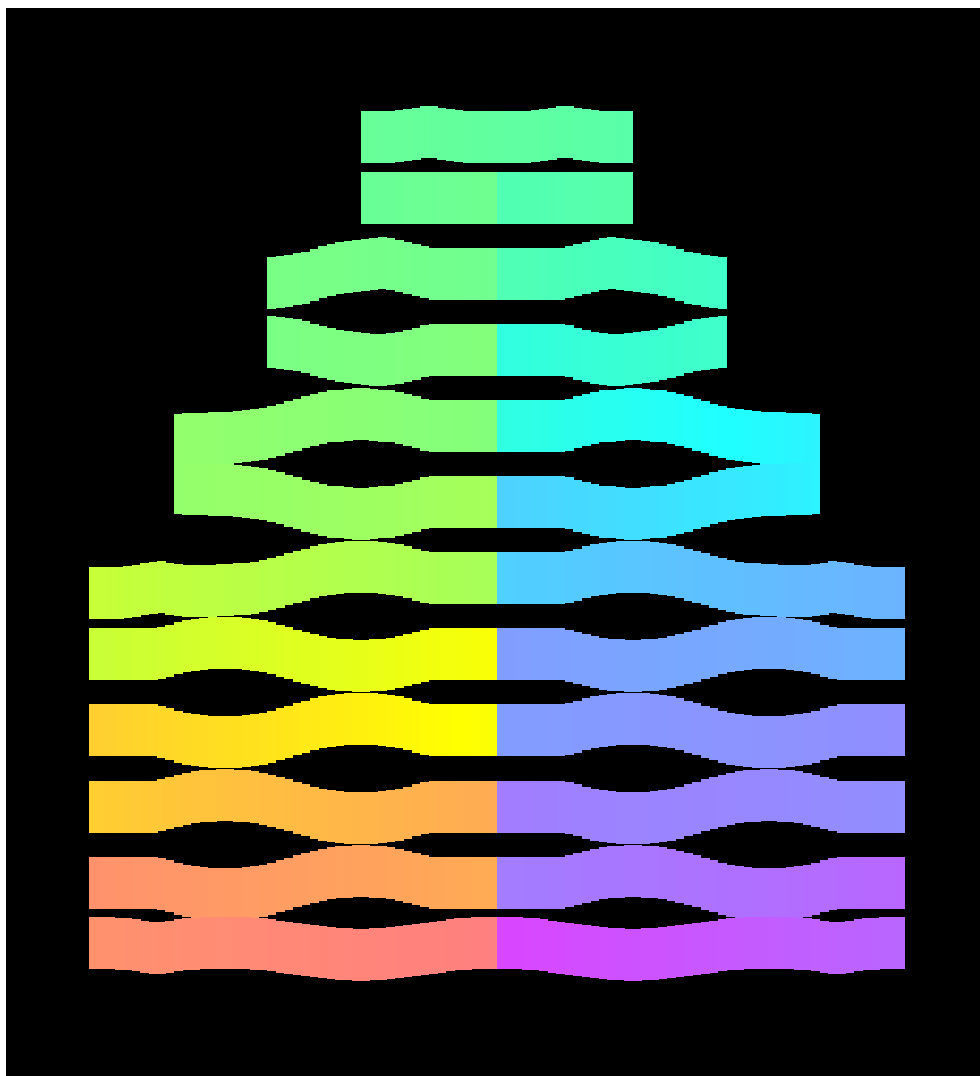
Supplementary Figure S8: Third pass diagram with staple strands after staggered merge.



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



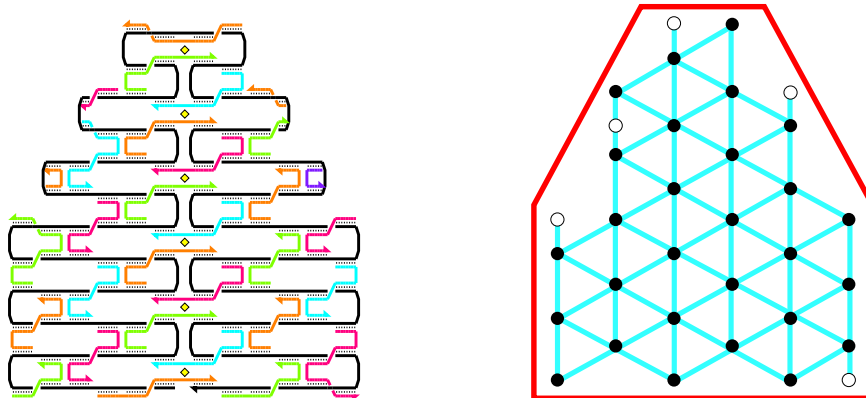
Supplementary Figure S10: Crossover diagram of bridged design.



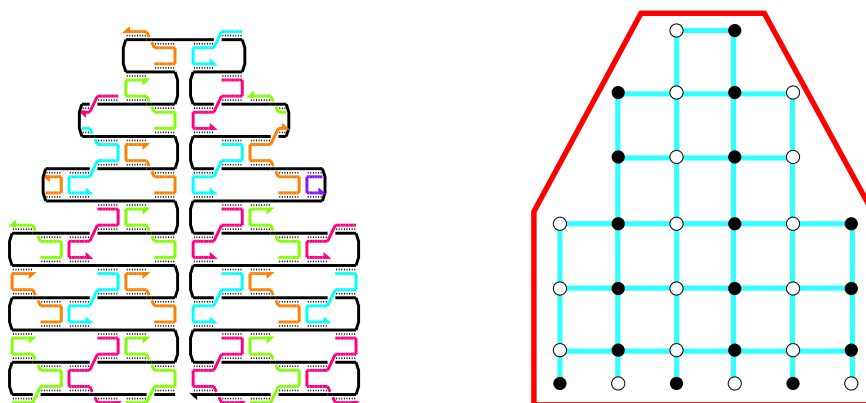
Supplementary Figure S11: Crossover diagram of unbridged design.

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.

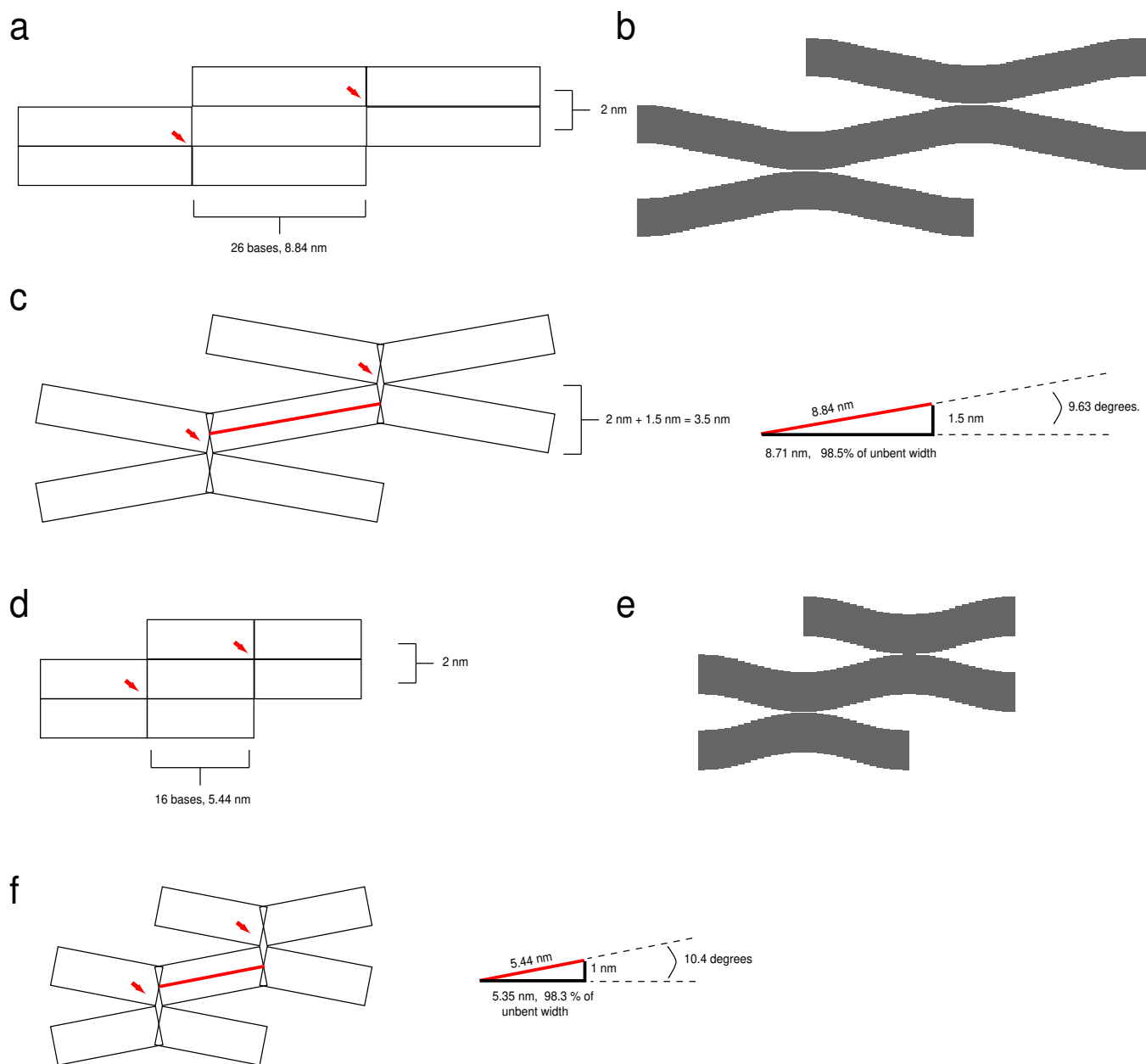
Given an estimate of the inter-helix gap, it would seem *a priori* more difficult to estimate or design the *width* of a DNA origami. To create the inter-helix gap it appears the DNA helices must bend back and forth between the crossovers in which they participate. If one assumes that the contour length 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.

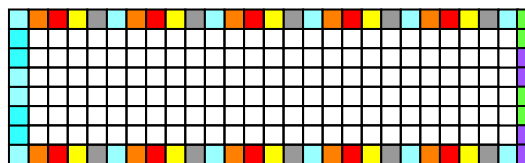


Supplementary Figure S13: A figure that suggests that the effect of helix bending between crossovers contributes little to the width of a DNA origami.

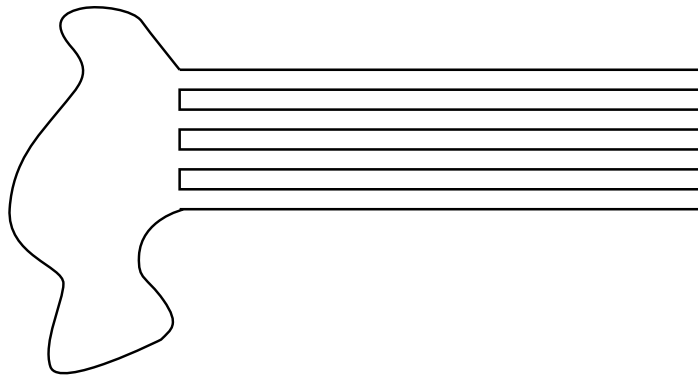
Supplementary Note S3: Designs and sequences

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; GGGAGAGCGGTTCCATCACCACAAT
x4y0B; CCCACTACGTGAATGCGTATTGGGCG
x6y0A; GAGACGGGCAACACACGTCAAAGGG
x6y0B; AGAAGCTGGACTCGCTGATTGCCCTT
x8y0A; GCAAGCGTCCACTGAGTGTGTTCC
x8y0B; CCCGAGATAGGTGCTGGTTGCCCC

x1y1A; ATTGCGTTGCGCTCTACAATTCAC
x1y1B; AAATTGTTATCCGCACTGCCCGCTTT
x3y1A; TGCATTAAATGAATCTCGAATTCGTAA
x3y1B; CCCGGGTACCGAGCGGCCAACGCGCG
x5y1A; CCAGGGTGGTTTTTGCCAAAGCTTGCA
x5y1B; AAACGACGGCCAGTCTTTTACCAGT
x7y1A; CACCGCTGGCCCGTTGGGTAAACGCC
x7y1B; GCAAGGCGATTAAATGAGAGATTGCA
x9y1A; AGCAGGCGAAAATCTTCGTATTAC
x9y1B; GAAGGCGATCGGTGCGGGCCCTGTTTATGATGTTGCCGA

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

x1y3A; CATTAAATGTGAGTAAATCAGTCTCAT
x1y3B; ATTAATTTTTTTCGAGTAACAACCC
x3y3A; CGGATTGACCGTATATTTAAATTGTA
x3y3B; TTGTATAAGCAAAATGGGATAGGTCA
x5y3A; CCGTGCACTGCCCCCGGTTGATAA
x5y3B; TCAATCATATGTAAGTTTGAAGGGAC
x7y3A; CGCACTCCAGCCAACAAGGAATCGA
x7y3B; GAGTCTGGAGCAAGCTTTCCGCGACC
x9y3A; GCGCCATTGCGCAATTTTGAAGATCT
x9y3B; AATGCCGAGAGGGTAGCTATTTAGGCTGCGCAACTGTTGG

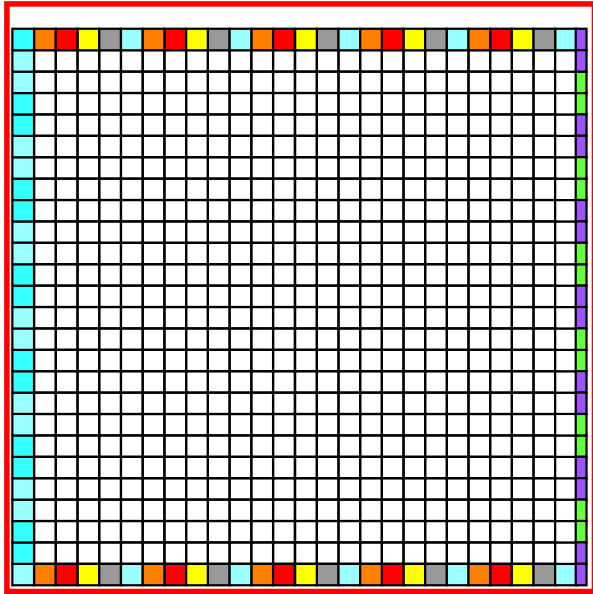
x0y4A; CAATAAATCATACAGGCAAGGGGAACGCCATAAAAATAATT
x0y4B; TTTTAAACCAATACAAAGAATTAGCA
x2y4A; TAAAGCTAAATCGTGTAAAATTCGC
x2y4B; AACGTTAATATTTGTTGTACCAAAAA
x4y4A; GGAGAAAGCCTTTAAAAACAGGAAGA
x4y4B; TCAGAAAAGCCCTTTCAACGCAAGG
x6y4A; TTTTAAATGCAATTAATACTAGCATG
x6y4B; TGAACGTAATCGGCCTGAGTAATGT
x8y4A; AAGGCCGAGACAGGTCAATGCCTGA
x8y4B; ACAAAGGCTATCAGTCAATCACCAT

x1y5A; AAATTAAGCAATAGCTGAAAAGGTGG
x1y5B; ATTTGGGCGCGAAAGCCTCAGAGCA
x3y5A; CATTATGACCCGTATTTCGCAATGG
x3y5B; GACCATTAGATACTAATACTTTTTCG
x5y5A; ATAAAAATTTTACAGTTGATTCCCA
x5y5B; TCATTCCATATAAGAACCCCTCATATA
x7y5A; GTAGGTAAAGATTTGTTTAAATATG
x7y5B; CTGTAGCTCAACACAAAAGGGTGAGA
x9y5A; CAATATGATATTCTTTCCGATGCG
x9y5B; CTCCTTTTGATAAGAGGTCATAACCGTTCTAGCTGATAAATT

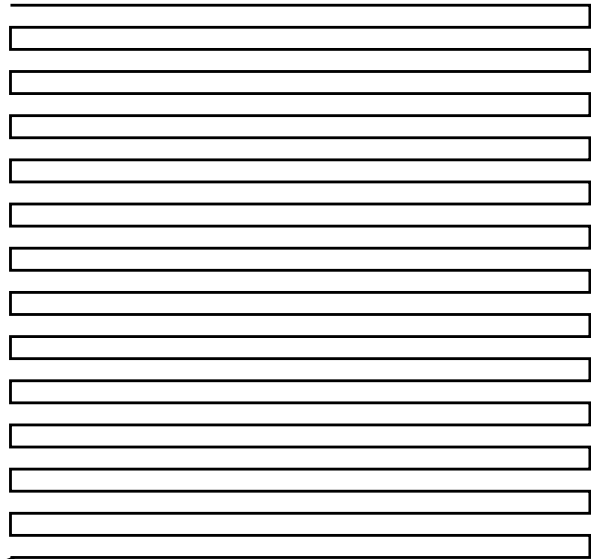
x0y6A; ATGTTTAGACTGGATAGCGTCAATAGTAGCATTAAACATC
x0y6B; CATCAATTCTACTCAATACTGCGGAA
x2y6A; CTCAAATGCTTTATAGCTATATTTTC
x2y6B; TCAATAACCTGTTAACAGTTCAGAAA
x4y6A; AGGTCTTTACCCTGTAGATTAGTTT
x4y6B; ATTCTGCGAACGAGACTATTATAGTC
x6y6A; GATTAGAGGAAGGTGCTGGAAGTT
x6y6B; CAACTAAAGTACGCCGAAAGACTTC
x8y6A; AAAGCGAACGAGCTGAATATAATG
x8y6B; TTAGAGCTTAATTCGGGAAGCAAACT

x1y7C; TCGTCATAAATATTATTGAATCCCC
x3y7C; ACGAGAAATGACCATAAATCAAATAATC
x5y7C; AGAAGCAAAAGCGGATTGCTCAAAAA
x7y7C; AAATATCGCGTTTTAATTGAGCTTC
x9y7C; CCAACAGGTCAGGATTAGAGAGTACCTTTAATTG

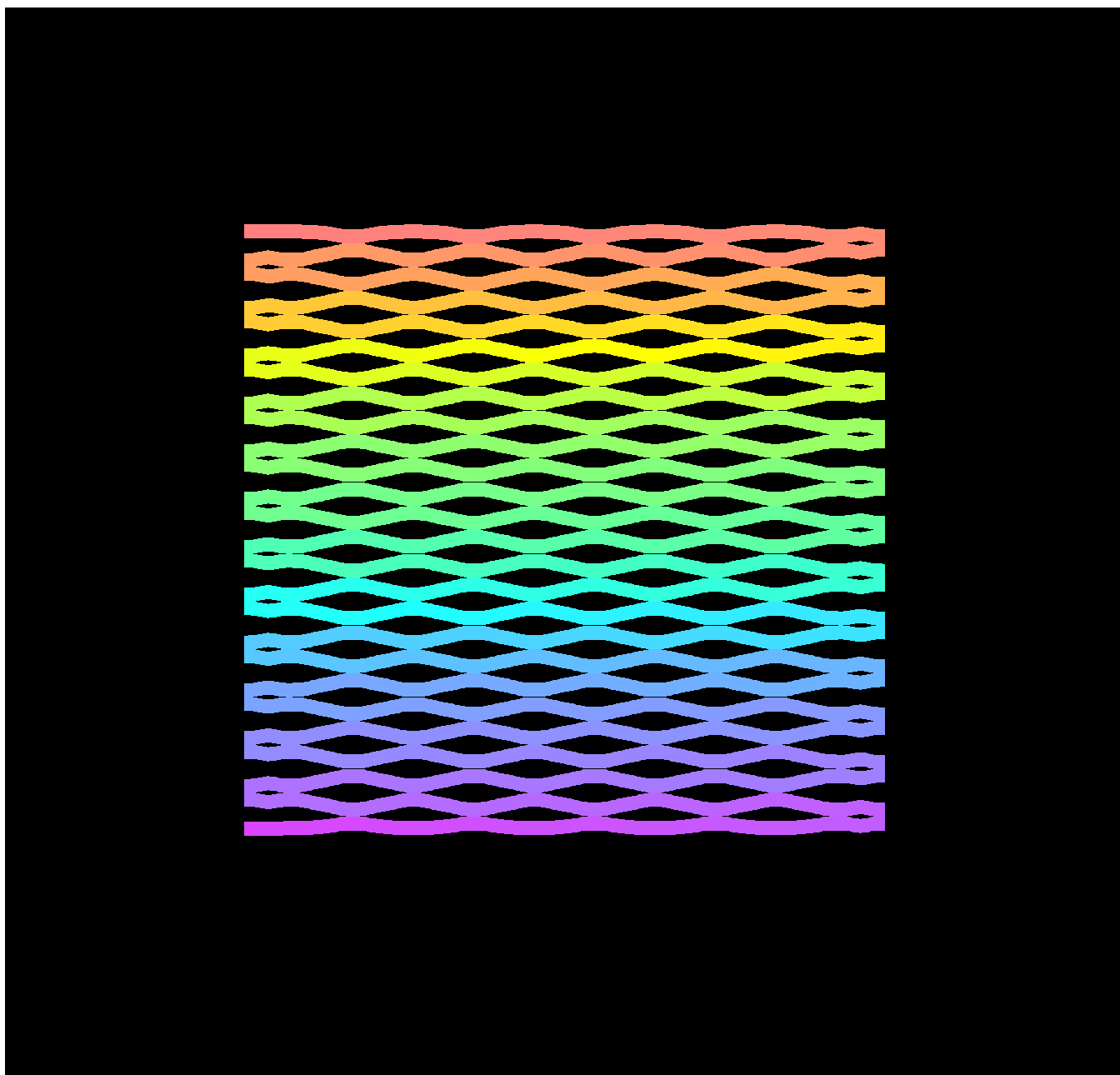
Supplementary Figure S15: Strands used to create $\sim 1/3$ of a square.

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; GAGCCCCGATTAGAGCTTGACGGG
x3y_1C; CAAGTTTTTGGGGTCGAGGTGCGCT
x5y_1C; CGAAGAACCGCTCTATCAGGCGATGG
x7y_1C; AGTTTGAACAAGAGCTCCACTATTA
x9y_1C; AATCGCGAAATCCCTTATAAATCAAAGAATAG

x0y0A; CTGGGGTGGCTAATGAGTGAGCGTGGCGAGAAAGGAAGGAA
x0y0B; GAAAGCCGGCGAACTAACTCACATTA
x2y0A; CCAAGTCGGGAAACGGAAACCTAAAGG
x2y0B; AAAGCACTAAATCTGCTGCTGCCAGC
x4y0A; GGGAGAGCGGGTTCATCACTACCCAAAT
x4y0B; CCACTACGTGAATGCGGTATGGGCG
x6y0A; GAGACGGGCAACACAGCTCAAGGG
x6y0B; AGAAGCTGAGCTGCTGATTCGCCCTT
x8y0A; GCAAGCGGTCCACTGAGTGTGTTC
x8y0B; CCGGAGATAGGGTGTGCTGTTCGCC

x1y1A; ATTGCGTTGCGCTCTCAAAATCCAC
x1y1B; AAATTTGTTATCCGCACTGCCGCTTT
x3y1A; TGCATTAAATGAATCTGAAATCTGTA
x3y1B; CCGCGTACCGAGCGGCCAACCGCGG
x5y1A; CCAAGGTGGTTTTTGGCAAGCTTGCA
x5y1B; AAACGACGGCGAGCTTTTCCACAGT
x7y1A; CACGCGCTGGCCGCTTGGGTAAACCGC
x7y1B; GAGAGCGGCAATTAAGAGAGGTGCA
x9y1A; AGAAGCGGAAATTTCTTCGCTATTAC
x9y1B; GAAAGCGGATCGGTGGCGGCCCTGTTTGTAGTGGTGGTCCGA

x0y2A; CGCGTCTGGGCTTCTGTAGCGGGAACATAAAGTGTAAAGC
x0y2B; ACAACATACGAGCGAGCTTTCAATCA
x2y2A; GTCCGATTCTCCGCTGTTTCCCTGTGT
x2y2B; TCATGTCTACAGCTGGGAAACACCG
x4y2A; CGTTGGTGATAGTACTCTAGAGGATC
x4y2B; TGCGTCTGAGCTCGGCGGCATCGTAA
x6y2A; GAGCAGCAGTATCGTCAACGAGTGTGA
x6y2B; AGCGTTTTCGAGCGCTCAGGAAGAT
x8y2A; GCTTCTGTGCGGAGGGGGATGTGCT
x8y2B; GCGCATCTGGCGAAGAACACGCGCAAA

x1y3A; CATTAAATGTGAGTAAATCAGCTCAT
x1y3B; ATTAATTTTTGTGCGAGTAAACACCC
x3y3A; CGGATTGACCGTATATTTAAATTTGA
x3y3B; TTGTATAAGCAAAATGGGATAGGTCA
x5y3A; CCGTGCACTGCCCCCGGGTGAATA
x5y3B; TCAATCATATGTAAGTTTGAAGGGAGC
x7y3A; CGCAGCTCCAGCCAAACAGAGATCGA
x7y3B; GAGTCTGGAGCAAGCTTTCCGCGACC
x9y3A; GCGCAATCCGCAATTTTGAAGAGATCT
x9y3B; AATGCGGAGAGGGTAGTATTTAGCTGCGCAACTGTTGG

x0y4A; CAATAAATCATACAGGCAAGGGGAACGCCATCAAAATAAAT
x0y4B; TTTTAAACAAATCAAGAATTAGCA
x2y4A; TAAAGCTAAATCGTGTAAATTCGC
x2y4B; AACGTTAAATTTTGTGTACCAAAA
x4y4A; GGAGAGAGCCTTTAAAAAACAGGAAGA
x4y4B; TCAGAAAAGCCCTTTCAACGCAAGG
x6y4A; TTTTAAATGCAATTAACATAGCATG
x6y4B; TGAAGGGTAAATCGGCTGAGTAATG
x8y4A; AAGCGTGAAGCAGCTCATTCGCTCA
x8y4B; ACAAGGCTATCAGTCAATCACCAT

x1y5A; AAATTAAGCAATAGCTGAAAGGGTGG
x1y5B; ATTTGGGGCGCGAAAGCTCAGAGCA
x3y5A; CATTATGACCTGATTTCGCAATGG
x3y5B; GACCATTAGATACTAATACTTTTGGC
x5y5A; ATAAAAATTTTACAGTTGATTTCCA
x5y5B; TCATTTCCATATAAGAACCCCATATA
x7y5A; GTAGGTAAAGATTGTTTAAATATG
x7y5B; CTGTAGCTCAACCAAAAGGGTGAGA
x9y5A; CAATATGATATCTTTTGGGATGGC
x9y5B; CTCCTTTTGATAAGAGGTCTAATCGTTCTAGCTGATAAAT

x0y6A; ATGTTTAGACTGGATAGCGTCAATAGTAGTACCATTAACATC
x0y6B; CATCAATCTACTCAATACTCGCGAA
x2y6A; CTCAATGCTTTATAGCTATATTTTC
x2y6B; TCAATAACCTGTTTATAGCTCAGAAA
x4y6A; AGGCTTTTACCCTGTAGATTAGTTT
x4y6B; ATCTCGGAGCAGAGCTAATTATAGT
x6y6A; GATTAAAGGAGAGGTCTCGGAAGTT
x6y6B; CAACCTAAGTACGCGGCAAGAGCTTC
x8y6A; AAAGCGAACAGAGCTGAATATAAGT
x8y6B; TTAGAGCTTAATTCGGGAAGCAACT

x1y7A; TCGTCATAAATATCGAGAGGCTTTTG
x1y7B; AAAACCAAAATAGCTAATTGAATCCCC
x3y7A; ACGAGAATGACCAAGCAACACTATCA
x3y7B; GAGGCATAGTAAGTAAATCAAAAATC
x5y7A; TAGGAATACCCAGTTCATCAAAA
x7y7A; AAATATCGCGTTTACAGATTAATTAC
x7y7B; AGCAACTAAGCGATAATTTGAGCTTC
x9y7A; CCAACAGGTCAAGCAGTCAAGAGCT
x9y7B; TTTAAGACTGCGCTAATTAATTAGAGAGTACCTTTAATGG

x0y8A; CGGGAACGAGGCGGACGAGCGTGCCAGAGGGGGTAAATAGTAA
x0y8B; CAAAGAAGTTTTTCAATCATAGGGA
x2y8A; ACAGATGAACGGTCCAGACGACGATA
x2y8B; TAACCTCGTTTATGACAGACGAGCG
x4y8A; AGTAATCTTGACACAAAGGAATTAC
x4y8B; AGATACATACGCGGAAACCGGATATT
x6y8A; TGCTCATTCAGTGCATGATTGAGAT
x6y8B; AGGTGAAGAAGATTAAATAGCTTTGCC
x8y8A; TAAATTTGGGCTGTGCTGTTAATAAA
x8y8B; TGGGAAGAAAATAGATGGTTTAATT

x1y9A; ACCGAACGTACGAGCTGATAAATGG
x1y9B; GATTGTATCATCACTTTGAAAGAGG
x3y9A; GCATAGGCTGGCTCCAGCGATTATA
x3y9B; CTCATCTTTGACCGCACTTCATCAAG
x5y9A; CATTACCAAAATCAACCTTAAACGA
x5y9B; ACTACGAAGGCAACGTAACAAAGC
x7y9A; CTGACGAGAAGCATGAGGAAGTTTCC
x7y9B; AAAGACTTTTTTCAACGAGAGGAGTAG
x9y9A; TCAACTTTAATCAGGAAGGAGGAGTAG
x9y9B; TCAGCAGGAAGACGAGTCTTTGGTAATACCTTATGCGAT

x0y10A; AACTTTCAACAGTTTACGCGGACCTGCTCCATGTTACTTATG
x0y10B; TGTCGAATCCGAGTGAAGATAGAA
x2y10A; ATAATTTTTTCAACAAAGTCAACGGA
x2y10B; CCAAGCGGCAAGAGTTGAAATCTCC
x4y10A; TAAITGTATCGGTATACACTTAAACAA
x4y10B; AAGAGGCAAAAGATATACGCTTGCT
x6y10A; GATACCGATAGTTATACGTTAATGCC
x6y10B; ATTAAGCGAGGCAACGCCGACAATGA
x8y10A; ATATATTCGCTCGGCTTTGAGGACT
x8y10B; CAACGCGTACAGACTGAGGCTTCGAG

x1y11A; AGGACCAACTTAATTTACGCGGTTA
x1y11B; AAGTTTTGTGCTCGGAATTCGGAATA
x3y11A; AAAAAGAGGCTTACCTCACAGACA
x3y11B; ACAACGCGTGAAGCAAAAGGAGCCTT
x5y11A; TTGAGGTTGAATTCATGACCTTAAC
x5y11B; CCAATAGGAACCTCTTAAACAGCTT
x7y11A; CAACCAACCTGACGACCTCAGAGCCA
x7y11B; CCTCAGAAAGCGCCACGCGATAACCG
x9y11A; GGAAGTTAAAGGCCACTCAGGAGGTT
x9y11B; CCGGAATAGGTGATACCGCTGCTTTTGGCGGATCGTCAACC

x0y12A; GGAAGCGCAGCTCTGTAATCTGTATGGGATTTGCTAAAC
x0y12B; GTAAATGAATTTTTACGTTCCAGTA
x2y12A; CAGGAGGTGATCGGCGTAACGATCTA
x2y12B; CGCCCTCATGTTAGTAATAGTTTGA
x4y12A; GCGCGTATAAACCAACGAGTCAAACT
x4y12B; ACTGAGTTCTGCTGTTAATGCCCGCT
x6y12A; AAACATGAAGTATCAGGAGTAGCAAGC
x6y12B; CCACCTCATTTTTTAAAGGCTGAGA
x8y12A; CGGGGTTTGTCTCAGAACCGCCCA
x8y12B; AGACCGCCACGAGTACAGGCGGA

x1y13A; AGCGTCATACATGTTGGCCTTGATAT
x1y13B; CGAGGTACAGAGAGCTTTTGTATGATA
x3y13A; ACGGGTCAAGTCCGACGAGGCGC
x3y13B; CGCCACCAAGACCTTGACAGCACT
x5y13A; GCGTATTTGCGAAGCCCTCAGAACCGC
x5y13B; CTCAGAGCGGCCACTTATTTCTGA
x7y13A; CTCCTCAAGAGAAAATACCCGGAA
x7y13B; TCTTTTCAATACGGAATTAGATTAG
x9y13A; TAAAGTCCGTCAGAGGCTTTTGGGT
x9y13B; CAGACTGTAGGCGGTTTTCATAGGGGTTGATATAAGTATAGC

x0y14A; TTAATTTGTCAACAATCAATAGACTCCTCAATTAAGCCAGAA
x0y14B; TCACAAACCAATAAATATTCATATGG
x2y14A; GACATTCACGAGCAAGAGGTTGAG
x2y14B; GCGCGCAGCATTTGTTGAGGAGGGA
x4y14A; TAAAGGTGAATTAACCACTCAGAG
x4y14B; CACCTCAGAGCGCTCCCGCTCACCGA
x6y14A; GCAAAATCAGGCGGAAACGCGCTCC
x6y14B; CAGAGCGCACCATAGCACCATTACC
x8y14A; TGAACCATCATGTTAGCGTTTGGCA
x8y14B; CATAGCCCGCTTAAGCAGACCGGTAA

x1y15A; TTTACCAGCGCCAAAGGTGGCAACA
x1y15B; AAAATACATACATAAGACAAAAGGCG
x3y15A; GGTAAATATTGACATTAAGACTCCTT
x3y15B; AAGAAGTGGAGGAAATTTATTCAT
x5y15A; CTGAGGCTTTGAAGGAAACCGGAG
x5y15B; ACAAGGTTACAGGGAATTAGAGCCA
x7y15A; ATTAGCAAGGCGGTACCGAAGCCCTT
x7y15B; GCAATAGCTTCAAGAGCTCAACAA
x9y15A; TCAGTAGGCAAGGAGTTAAGCCCAA
x9y15B; GAGAGATACCCCAAGAAATTAACAAGTTTGCCTTTAGCGT

x0y16A; AATCAAGATTAGTTGCTATTTCAAAGACACACGGAATAAGT
x0y16B; TATAAAGAACCTGCGACCGAGCTAC
x2y16A; TAACGAGCGCTTTTGAAGCAACGTAG
x2y16B; ATTACGAGTATGTTCCAGAGCTTAAT
x4y16A; ATTTATTTCCCAACGGAATACCCAA
x4y16B; AAACGCAATTAATCAAAATAGAA
x6y16A; GAAATAGCAGCGAGGAGATGGCGGA
x6y16B; TTTAAGAAAAGATTATTCAGAGAGAA
x8y16A; GACGGGGAATTAACCAATAGAAATA
x8y16B; TAAATAGAGCAAGACTGACACCCCTG

x1y17A; AATTTTATCTGAAGGCGTTTATGAGC
x1y17B; TTCTAAGAACGCGATCTTACCACGCG
x3y17A; TTGCGAGTTACACAATAGCAAGCA
x3y17B; TCATTACCGCGCAATTAACAGCCAT
x5y17A; ACGATTTTTTGTTCGAGAAACAGCA
x5y17B; AGTACCGCACTCAACGTCAAAAAT
x7y17A; TAACATAAAAACACTGCTTTCCTTA
x7y17B; AATCAATAATCGGGGGAAGCACTTA
x9y17A; AACAAAGTCAGAGAAATATATCCCA
x9y17B; TAGATAAGTCTGAAACAGAGGATTTGAGCGCTAATATCA

x0y18A; AATAAGAATAAACCCGGAATCGGAGGTTTTGAAGCCTTA
x0y18B; AACCTCCGAGCTCTATAATTTACTAGA
x2y18A; TATACAAATTTCTGGCTTTCCGGTA
x2y18B; ATCAGATATAGAAAACGAGTATAAAGC
x4y18A; AGAATCGCATATTTTATCTGATAGAA
x4y18B; AGCGCTTTTTTATTTTACAAAGCCCA
x6y18A; GAGCGATATAAGGGTATTAACCA
x6y18B; TCATTCAAGAACGAGATATAAAGT
x8y18A; CCAGACGACGACAGATGTAGAAAC
x8y18B; TCCTAATTTACGAATAAACCAATGT

x1y19A; AAAAGCCTGTTTTATAAATTTAATGGT
x1y19B; TCATCTCTCGAGCGTATCATATGCGT
x3y19A; CACGCGTCAACAGGAAGCGGAGAA
x3y19B; ATCCGAGCAAAATAGGCGTTAATGG
x5y19A; CATGTAATTTAGTGGGTTTATATAAC
x5y19B; CTCGCGCTTAGGTGAGGAGTATTC
x7y19A; ACCGACAAAGGTTTTTATCAAAATG
x7y19B; AGTCAATAGTAAAAAGTAATTTCTGT
x9y19A; TCAGCTAATGCAATGAAACATAGCG
x9y19B; TTAATTTCCCTTAGAATCTCAACGCGCTGTTTATCAACA

x0y20A; TTTAAGCTCAGATGAATATACGCTGTGATAAATAAGGCGTTA
x0y20B; TTGAATAACGCGAGTAAACAGTACCT
x2y20A; TTGCGCTGATGCTATTTAGTTAAAT
x2y20B; CTTTTTCAATAATTTTGAATACAGCA
x4y20A; TTCATTCAATATGATGCAAAATCA
x4y20B; TATATGAAATGCGCTGAGCAAGAA
x6y20A; CAAATTAATTAACCTACCTTTTAAAC
x6y20B; TAGGCTGAGAGAAATTAACAAATTC
x8y20A; CAGTACATAAATCAGACGCTGAGAG
x8y20B; ATAGCTTAGATTAAATATATGTTAGG

x1y21A; TTTACATCGGAGTATTGTCACGTA
x1y21B; TACCATATCAAAAACATTAAGCGGA
x3y21A; TTAACAATCGGGTTTGGATTATAC
x3y21B; TATAATCGGTGATGAGGCGGAATA
x5y21A; AGATGATGAAGACATCATATTCCTG
x5y21B; GGAGCGGAATTAACATCAAGAA
x7y21A; ATTTGAATTAACCTGATTAATCATCT
x7y21B; TTTAAAGGTTGATTTTAAATGAA
x9y21A; GAAATACTTGTGCAACACTGTAAT
x9y21B; ATTAGCTTTACAAACAACTCTGTAAATCGTGCCTATTA

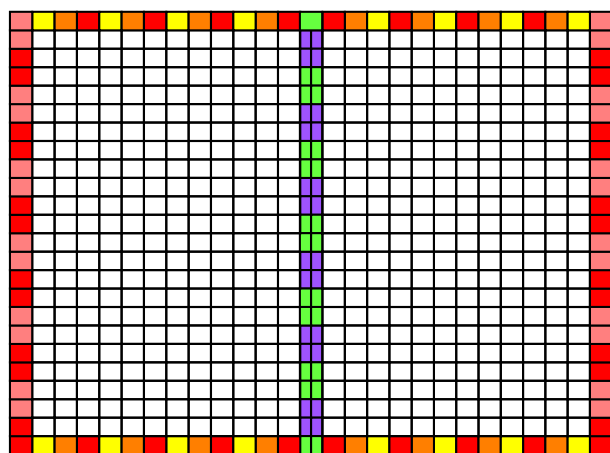
x0y22A; AATACCGAAGCAACACGAGGAATTCGCTAGATTTCAGG
x0y22B; AAACAGAAATAAAGAGGATAAACA
x2y22A; GCGTCAACAGTACGAGGTTAGAAC
x2y22B; TTCTGAATTAATGGCAGCTGAGAGC
x4y22A; TCACCTTGTGAGGGAATTCATCA
x4y22B; ATTAATCAGATGATCCTCAATATCA
x6y22A; GCAAAATCAACAGTGAACACGAGAA
x6y22B; TTTGCGGAACAAATGAAGGAATTGA
x8y22A; AGCACTAACACTGAACGTTTAAAT
x8y22B; AAATCCTTTGCCCAATAGATTAGAGC

x1y23A; GAGGTGAGGCGGTTTATGCTTTAATG
x1y23B; TTTTGAATGCTCAGATTAATCAAC
x3y23A; GAGCAGCAATGACTGACCTGAAAGC
x3y23B; AGATAGAACCTTTAAATCTTAAGCA
x5y23A; ACCCTCAATCAATTCACAGGACGAT
x5y23B; GCAGATTCAACAGATCTGCTGAAGT
x7y23A; GGAAGGTATCTATTTTACGCTCA
x7y23B; GGAATACTCAACAAATCTTTAGG
x9y23A; CTTCAATAGATAAATATTAACGCCA
x9y23B; CTGCTGGTAATTCAGGAAGTATTTAGGATTAGAAGT

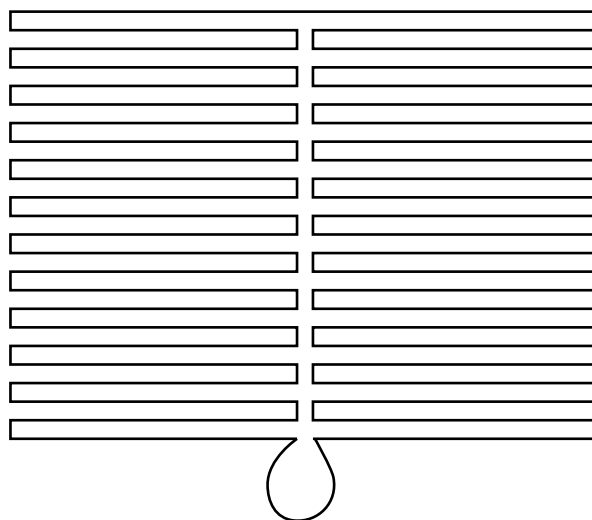
x0y24A; GCGCTTAATGCGCGCTACAGCCATAAACCTCGCCATTA
x0y24B; CGCGAAGTATAGGGCGGTACTATG
x2y24A; GCTTCTCGCTAGCAGCAACATAT
x2y24B; GTAAGAATCTGAGTACAGAGCGG
x4y24A; ATTTAGACAGGATTCTGGCCACAGC
x4y24B; AATAAAGGCAACAGGTCACCGCA
x6y24A; TGAGGCCACGAGATTATTTACATG
x6y24B; TCGTCTGAATTTGTAAGAGTCTGT
x8y24A; AATACTTCTTGAAGAAACGCTCAT
x8y24B; GCCATTGCAACAGTTAGTAATAACAT

x1y25C; GTTGCTTTGACGAGCAGTATAACGT
x3y25C; AGCTAAACAGGAGGCGGATTAAGGG
x5y25C; ATCTCGAAGGTTTATTAATACAG
x7y25C; CCATCAGCAAAATTAACGCTGTAGC
x9y25C; CACTTGGCTGAGTGAAGAACTCAATATCGCG

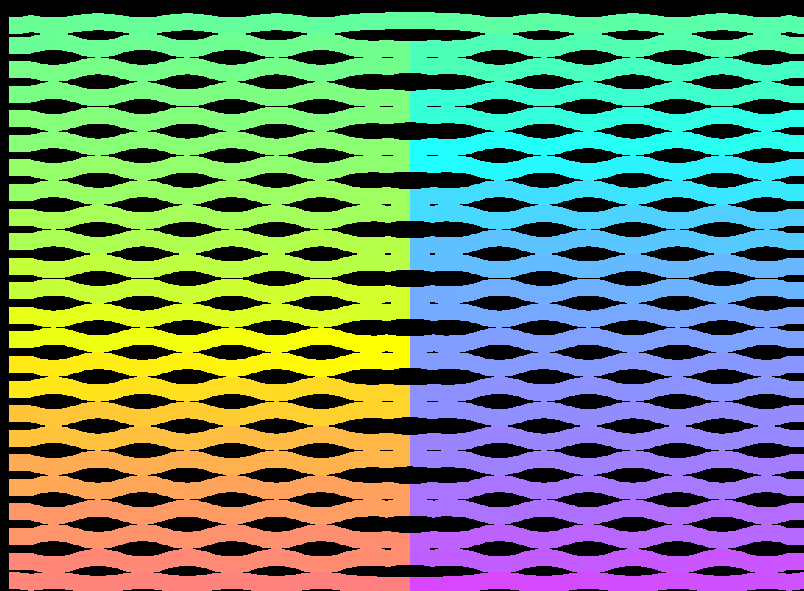
Supplementary Figure S18: Sequences for the square.

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.

Plate number: 1

r1t0g, A1, CAAGCCCAATAGGAACCCATGTACCGTAAACAC
r1t10e, B1, TCTTACCAGCCAGTATACAAAATAAATGAAATA
r1t10f, C1, CCTAATTTTACGCTAACAGAGCGTCTATATCGCG
r1t12e, D1, CTAATTTATCTTTCTTATCATTCATCTCGTAA
r1t12f, E1, ATCGGCTGCGAGCATGTAGAAACACGAGTATAT
r1t14e, F1, AATTACTACAAATTTCTTACAGTAATCCCATC
r1t14f, G1, GCGTTATAGAAAAGCCTGTTTGAAGAGCGGG
r1t16e, H1, TAGAATCCCTGAGAAGAGTCAATAGGAATCAT
r1t16f, A2, TTAAGACGTTGAAAACATAGAGGATTTAAATCA
r1t18e, B2, TTTAACGTTTCGGGAAGAAACATAATTTTCCCT
r1t18f, C2, CTTTACACAGATGAATATACAGTAAGCGCA
r1t20e, D2, GGATTTAGCGTATTAATCTTTGTTTTCAGG
r1t20f, E2, CGACAACCTAAGTATTAGACTTTTACAGCCGGAA
r1t22e, F2, TAGCCCTTACCAGCAGAGATAAAAACATTTGA
r1t22f, G2, ACGAACCACAAACATCGCCATTAATGGTGGTT
r1t24h, H2, CGGCCCTTGCTGGTAAATATCCAGAAAGAACTGA
r1t2e, A3, TGCCCTTGACTGCCATTTCGGAACAGGATAG
r1t2f, B3, AATGCCCGCTAACAGTGGCCGTATGTGAATTT
r1t4e, C3, AACCAGAGACCTCTAGAGCCAGCCGGGTGAG
r1t4f, D3, GAGCGCCCGCACACCGGAGCCGCTAAACACA
r1t6e, E3, ATTGAGGTTAAAGCTGAATTAATCAATCCAGCGG
r1t6f, F3, TTATTTATAGGAGAGGTAAATATTCATTCAGT
r1t8e, G3, GCAATAGCGCAGATAGCGGAACATTAACACCG
r1t8f, H3, AAAAGTAATATCTTACCGAGGCCAACACATAT
r3t0g, A4, CTCAGAGCCACACCTCATTTTTCCTATTATT
r3t10e, B4, TATTTGCTCCCAATCCAAATAAGTAGGTTAA
r3t10f, C4, ATTAATTAACCCAGCTACAATTTTCAGAAAGC
r3t12e, D4, TAAGTCCCTACCAGTACCCGACTCTTAGTTGCG
r3t12f, E4, GGTATTAAGAAACAGAAATAATTAAGAGCA
r3t14e, F4, AGGCGTTACAGTAGGGGCTTAATGACAATAGA
r3t14f, G4, ACGCTCAAAATAAGAAATAAGAAATCAAGATTT
r3t16e, H4, CTGTAATCATAGGTCTGAGAGACGATAAATA
r3t16f, A5, ATCAAAATCGCTGCAATAGATAATCAGAGGTG
r3t18e, B5, ACAGAAATCTTTGAATACCAAGTTCTCTTGCT
r3t18f, C5, CCTGATTGAAAGAAATGCGGTAGACCCGAAAG
r3t20e, D5, AGATTAGATTAAAGAGTTTGAGTACAGCTAAA
r3t20f, E5, TTATTAATGCGCTCAATAGATAATCAGAGGTG
r3t22e, F5, GAATGGCTAGTATTAACACCGCTCAACATAAT
r3t22f, G5, AGGCGGTCAATAGCTTTTAAATGGCGAATATTA
r3t24h, H5, CCGCGAGCCATTTGCAACAGGAAAAATTTTT
r3t2e, A6, AGTGTACTTGAAGAGTATTAGAGGCGCGCACCC
r3t2f, B6, CTGAAGACAGGTAAATAGTTTAAACCCCTCAGA
r3t4e, C6, GTTTGCCACCTCAGAGCGCCAGCAGTACAGG
r3t4f, D6, GCCACCACTCTTTTCATAATCAACCGCTCACC
r3t6e, E6, AGCGCCAACCAATTTGGGAATAGATTATTAGC
r3t6f, F6, GACTTTGAGAGACAAAAGGGCGACAAAGTTACCA
r3t8e, G6, GCCCAATACCGAGGAAATGAGTAAATTTTACC
r3t8f, H6, GAAGGAAATAGAGACGACCAACCAACAGCCAT
r5t0g, A7, CCTTCAGAAACCCGCACTTGAAGCTGAGACT
r5t10e, B7, AGGTTTGAAGCTCAAAATGAAGAGCGCTAAT
r5t10f, C7, TTTTGTTTAAGAGCTTAAATGAGATTCGAGAA
r5t12e, D7, AATGCGAGCCGTTTTTTTTCATCTTGCGGG
r5t12f, E7, CAAGCAAGAGCGCGCTGTTTATCAAGCAATCGC
r5t14e, F7, AATGGTTTACAAACGCCCAACATGTAGTTCAGCT
r5t14f, G7, CATATTTAGAAATACCCAGCGTGTTCCTTTT
r5t16e, H7, AAATCAATGGCTTAGGTTGGGTTTACTAAATTT
r5t16f, A8, TAACCTCCATATGTAGTGAATAAACAACAAATC
r5t18e, B8, AACCTACCCGCAATATTTCATTTCCAGTACAT
r5t18f, C8, GCGCAGAGATATCAAAATATTTCGATCATATC
r5t20e, D8, CTAATAATAGACAAGAAACCAACGGGTTAG
r5t20f, E8, ATTTTGGCTCTTTAGGAGCACTTAACGCAAGT
r5t22e, F8, GCGTAAAGAGAGAGCCAGCAGAAAAGGTTAT
r5t22f, G8, GCCACGCTATACGTGGGAATGACACAGCTCAT
r5t24h, H8, GGAATACTACATTTTGGCGTCACTTCAAGAA
r5t2e, A9, TAAGCGTCAAGGATTAAGGATTAAGTCGCCCA
r5t2f, B9, CCTCAAGAAATACATGGCTTTTGAATAGAACCA
r5t4e, C9, TCGGCACTTCGCCCGCAGCATCTGACGTCCAG
r5t4f, D9, CACCAAGATTTCGGCTCATGCGCCCGCAGCAAA
r5t6e, E9, TCACAATCGTAGCACCATTACCATCTGTTTCA
r5t6f, F9, AATCACCAAATAGAAAATTCATATAACCGGA
r5t8e, G9, ATCAGAGAAAGAACTGGCAATGATTTTATTGG
r5t8f, H9, ATACCCAGATAAACCCACAGAAATAACGATT
r7t0g, A10, TATCACCCTACTCAGAGGTTTATGCGGGGTTT
r7t10e, B10, GAGCGCTTAGAGAAATAACATAAAGAACACCC
r7t10f, C10, CTTTACAGTTAGCGAACTCCCGCAGTAGGAA
r7t12e, D10, CCGAGCAGCGCCCAATAGCAACGAGAACCG
r7t12f, E10, TCATTTACCCGACAAATAACCAATATTTAGGC
r7t14e, F10, TTTTAGTTTTTGGAGCGAGTAAATTAATCTGT
r7t14f, G10, AGAGGCAATAATTTTCACTCTTCTGATTAACATA
r7t16e, H10, TTGAATTTATGCTGATGCAAAATCGACCAATATA
r7t16f, A11, TATGTAAACCTTTTTTAAATGGAAAAATTAACCT
r7t18e, B11, TGGATTATGAAGATGATTAACAAACAAATTTCAAT
r7t18f, C11, GAGCAAAACATCTGGAATTAATGGAAGAGGAG
r7t20e, D11, ATCAACAGTCACTATTTCTGATTTGATTTGTT
r7t20f, E11, CGGAATTAATGGAAGAAATGAGGTTGAAAAAT
r7t22e, F11, GCCAACAGTCACTTCTGCTGAACCTGTTGGCAA
r7t22f, G11, CTAAGCAGAGATAGAACCCCTCTGATATGCTCT
r7t24h, H11, GAAATGGATTATTTACATTTGGCAAGATCTCTG
r7t2e, A12, GGAAGGCGAGAGGCGAGTAAAGTGAAGAGGTG
r7t2f, B12, TGCTCAGTCAGTCTGTAATTTTACAGAGAGGT
r7t4e, C12, TGCTCTTTAGTCAGAGATTGGGCTGCCAGAAAT
r7t4f, D12, TGAGGCGAGCGTCAGACTGATGAGTACGAAGG
r7t6e, E12, ACGCAAGGTCACCAATGAACCAATCAAGTT
r7t6f, F12, CCGGAAACACACACCGGAATAAGTAAGACTCC
r7t8e, G12, TGAACAAACAGTATGTTAGCAATCAAGAA
r7t8f, H12, TTATTCAGGTACAGGGTAAATGATAGCAGC

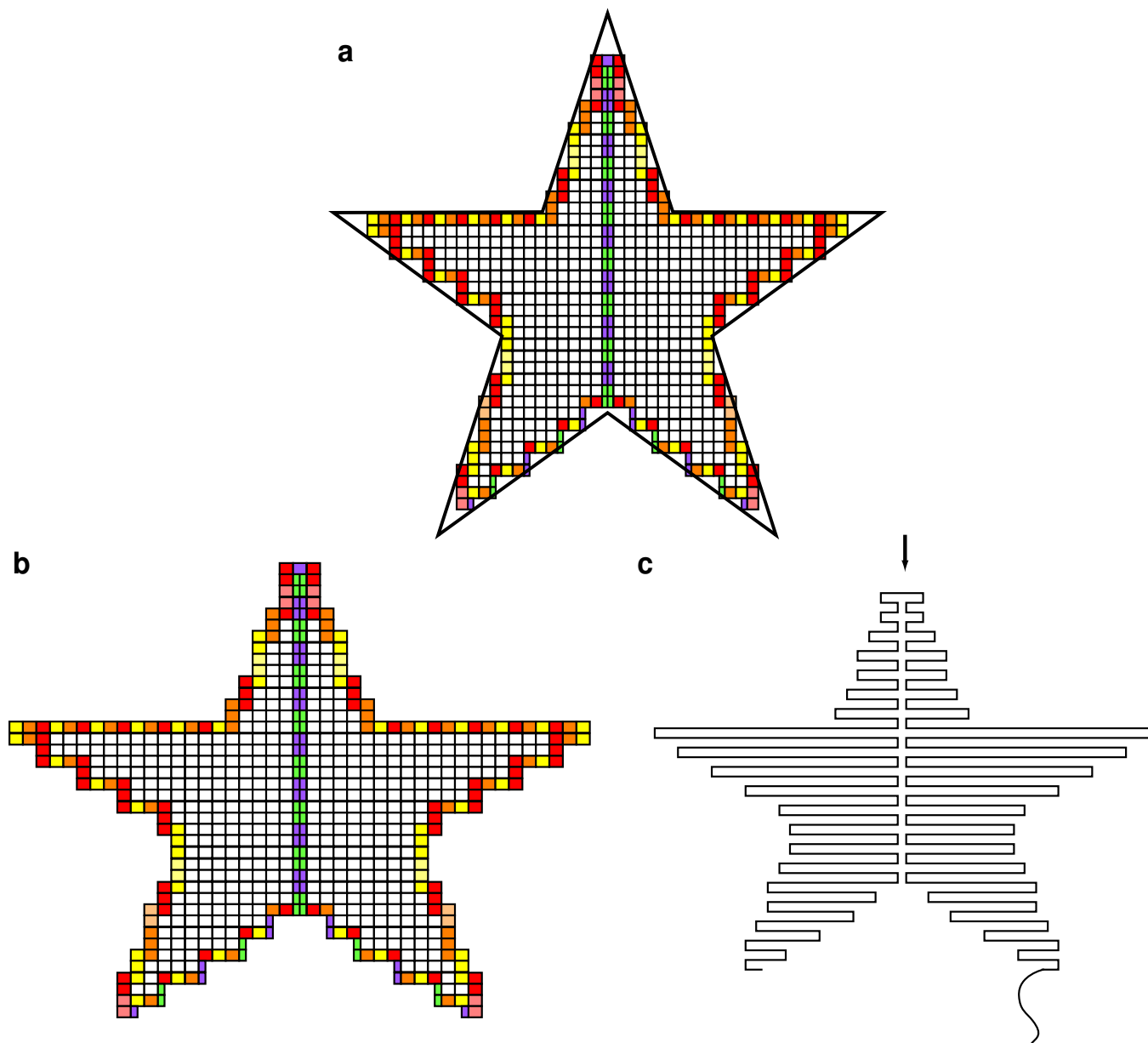
Plate number: 2

r9t0f, A1, TATAAGTATAGCCCGGCGCTGAG
r9t10f, B1, GCGCATTAGCTTATCCGGTATTCTAAATCAGA
r9t12f, C1, TATAGAAAGCGACAAAGGTAAAGTAGAGAAATA
r9t14f, D1, TAAAGTACCCGCGAGAAACCTTTTATCGCAAG
r9t16f, E1, ACAAGAAATTAATTTACATTTTAAACACATCAAG
r9t18f, F1, AAAACAAATTCATCAATTAATCTTATCAGAT
r9t20f, G1, GATGGCAAAATCAATATCTTGGTCCAAATATC
r9t22j, H1, AAACCTCTACCAGTAATAAAAGGAAATTCACAGTACACG
r9t2f, A2, AGGGTTGAATAAATCCTCATTAATGATTATTC
r9t4f, B2, ACAACAAATCAGTAGCGCACAGATCGATAGC
r9t6f, C2, AGCACCTTTAAAGGTGCGCAACATAGTAGAAAA
r9t8f, D2, TACATACAGACGGGAGAAATTAACACAGGGAA
r1t0g, E2, TGAGTTTTCGTACCACAGTACAAACTTAATTGT
r1t10e, F2, TTTTAAATGCCCGAAAGACTTCAATTCAGAG
r1t10f, G2, AAGAGAACGAGCTTCAAGCGAAGATACATT
r1t12e, H2, TTTCAATTTGGTCAATAACCTGTTTAAATCAATA
r1t12f, A3, TCGCAATGGGCGCGAGCTGAAATAATGTGT
r1t14e, B3, AGACAGTCACTTCAAAAGGTGAGATATCATAT
r1t14f, C3, AGGTAAAGAAATCACCATTCAATAATAATTTT
r1t16e, D3, GCTCATTTTCGCATTAATTTTGGAGCTTAGA
r1t16f, E3, GTTAAATTTTAAACAAATTTTGAACACCGCGAC
r1t18e, F3, TTCGCAATGGCGGAAACAGGCAACACAGTAC
r1t18f, G3, GCTTTCGGTCAGGCTGCGCAACTGTTGATATCC
r1t20e, H3, GCATAAAGTTTCCACAACTACGAAACAAAT
r1t20f, A4, GCTCACAAATGTAAGAGCTTGGGGTGGGTTGCC
r1t22e, B4, CCGAAATCGGAAATCCTGTTTGAATACCGA
r1t22f, C4, CCGAGAGGCGCAAACTCCCTTATAAAGCGCGC
r1t24h, D4, GAACGTGGCGAGGAAGGAGGAAACAACTAT
r1t2e, E4, CTTAAACATCAGCTTCTTTCGAGAAACAGT
r1t2f, F4, TCGGTTTAGCTGATACCGATAGTCCAACTTA
r1t4e, G4, CTCACTCTTGAGGCAAAAGAAATACACTCCCTCA
r1t4f, H4, AAACGAAATGACCCCGACGAGTATTTCATTAC
r1t6e, A5, GAATAAGGACTTAACAGAGCTGTCAGAGCGAAA
r1t6f, B5, CCAAACTACTTTCGCTGACGAGCAACGCGCAAAA
r1t8e, C5, CATAACCCGAGGACTGTAGAGGCTTTTAAAG
r1t8f, D5, GGAATTACTCGTTTACAGAGCAGCAAAAGATT
r3t0g, E5, TGTAGCATTCACAGACAGCCCTCATCTCCA
r3t10e, F5, GAAGCAAAAAGCGGATTCATCAGATAAAAA
r3t10f, G5, TCAGAAGCCTCCCAACAGGTACGAGATCTGCGAA
r3t12e, H5, TCAATTTCTTTAGTTTGAACATTACAGAGCCG
r3t12f, A6, CGAGTAGAATAATAGTAGTAGAAGAACCTCA
r3t14e, B6, ACCGTTCTTAAATGCAATGCTGAGAGGTGGCA
r3t14f, C6, TATATTTTAGCTGATAAAATTAATGTTGTATA
r3t16e, D6, AAATAATTTTAAATTTGAAGAGCTGATATCA
r3t16f, E6, GCAAAATTCGCGCTCTGGCCTTCTTGGCCTCAG
r3t18e, F6, GCGCATCGCACTTCAGCGAGCTTTGGCATCAA
r3t18f, G6, GAAGATCGGTGCGGGCCTCTTCGCAATCATGG
r3t20e, H6, GTGAGCTAGTTTCTGTGTGAAATTTGGGAAG
r3t20f, A7, TCAATAGCTACTCAATTAATGGCGCCTGAGA
r3t22e, B7, GAATAGCCGCAAGCGGCTCCAGCTCTCAATAGA
r3t22f, C7, GAGTTGCAAGAGATAGGGTTGAGTAAGGAGC
r3t24h, D7, CCCCAGTTTAGAGCTTGAGCGGGGAAATCAAAA
r3t2e, E7, CAATGACACTCCAAAGAGGCTCTACAGGCC
r3t2f, F7, AAAAAGGACACCACTCGCCCGCGGGGTAAA
r3t4e, G7, GCGAAACATGCCACTACGAGGCACTGCGCGGA
r3t4f, H7, ATACGTAAGAATCAACCGAGAGTTTCACTCAAG
r3t6e, A8, ACGAGTAGTGACAGAAACCGGATATACCAAGC
r3t6f, B8, AGTAATCTTAAATGGGCTTGAGAGAAATACCA
r3t8e, C8, CCAAAATATAATGCAAGATACATAAACACAGGA
r3t8f, D8, CATTCACGCGAGAGGCTTTTGCATATTATAG
r5t0g, E8, CGTAAGCATCTAAAGTTTGTGCTGAATTGCG
r5t10e, F8, TACCTTTAAGGCTCTTACCCCTGACAAAGAAAT
r5t10f, G8, CAAAATCACTTGCCTCTTTTGAATAGTTTCAAT
r5t12e, H8, CAATAATACAGTTGATTTCCCAATTTAGAGAG
r5t12f, A9, TCCATATACATACAGGCAAGCACTTTTATTT
r5t14e, B9, GGTAGCTAGGATAAAAAATTTTGTGTAACATC
r5t14f, C9, CAACGCAATTTTGGAGATCTCATGATAATC
r5t16e, D9, CTTTATCCCAAAAACAGGAAGACCGGAGAG
r5t16f, E9, AGAAAAGCAACATTAATTTGAGCATCTGCCA
r5t18e, F9, CAGCTGCGGAGCAGCAGCATGATCGTAGCCAG
r5t18f, G9, GTTTGAGGAAAGGGGAGTGTGCTAGAGGATC
r5t20e, H9, ACTGCGCGCGAGCTGCAATTCGTATTACCG
r5t20f, A10, CCGCGGTACTTTCAGTTCGGGAAACGGGCAAC
r5t22e, B10, AGTTTGGAGCCCTTACCGGCTGTTGCGCTCT
r5t22f, C10, AGCTGATACAGAGTCACTTATGAGGTGCC
r5t24h, D10, GTAAAGCACTAAATCGGAACCTAGTTGTTCC
r5t2e, E10, ATATATCTTTTTCACGTTGAAAAATAGTTAG
r5t2f, F10, AATAATAAGTTCGCTGAGGCTTGCAGAGACT
r5t4e, G10, CGCCTGATGGAAGTTTTCATTAAACATAACCG
r5t4f, H10, TTTTCATGAAATTTGTGTGCAAAATCTGTACAGA
r5t6e, A11, TATGCAATATAGGCTGCTGACCTTGTATCAT
r5t6f, B11, CCAGGCGCTTAACTATTGTGAAATACAGGAT
r5t8e, C11, TTTGCCAGATCAGTTGAGATTTTAGTGGTTTAA
r5t8f, D11, AAAGATTACGGGGGTAATAGTAAACCATATAAT
r7t0g, E11, ACGTTAGTAAATGAATTTCTGTGAAGCGGAGT
r7t10e, F11, TTTTTCGCGAGAAAACGAGGAATGAGTTTATG
r7t10f, G11, AAACAGTTGATGGCTTAGAGCTTATTAAATAT
r7t12e, H11, CAAAATTAAGTACGGTGTGGAAGAGGCTCA
r7t12f, A12, TGCAACTAAGCAATAAAGCCTCAGTTATGACC
r7t14e, B12, TCAGGTCACTTTTTCGGGAGAGAGCAATAAG
r7t14f, C12, CTGTAAATTTGCTGTGAGAGCTGGAAGAACTAG
r7t16e, D12, ACCCGTCTCATATGTATCCCGGTAAAGGTTAG
r7t16f, E12, CATGTCAAGATTCTCCGTGGGAACCGTTGGTG
r7t18e, F12, ATTAAGTTTCGATCTGTAACCGGTGCGAGTAACA
r7t18f, G12, TAGATAGGGGTTAAGCCAGGGTTGTGCGAAG
r7t20e, H12, GCCAGTGCCTGAGGTCAGCTTCGAGAGGCG

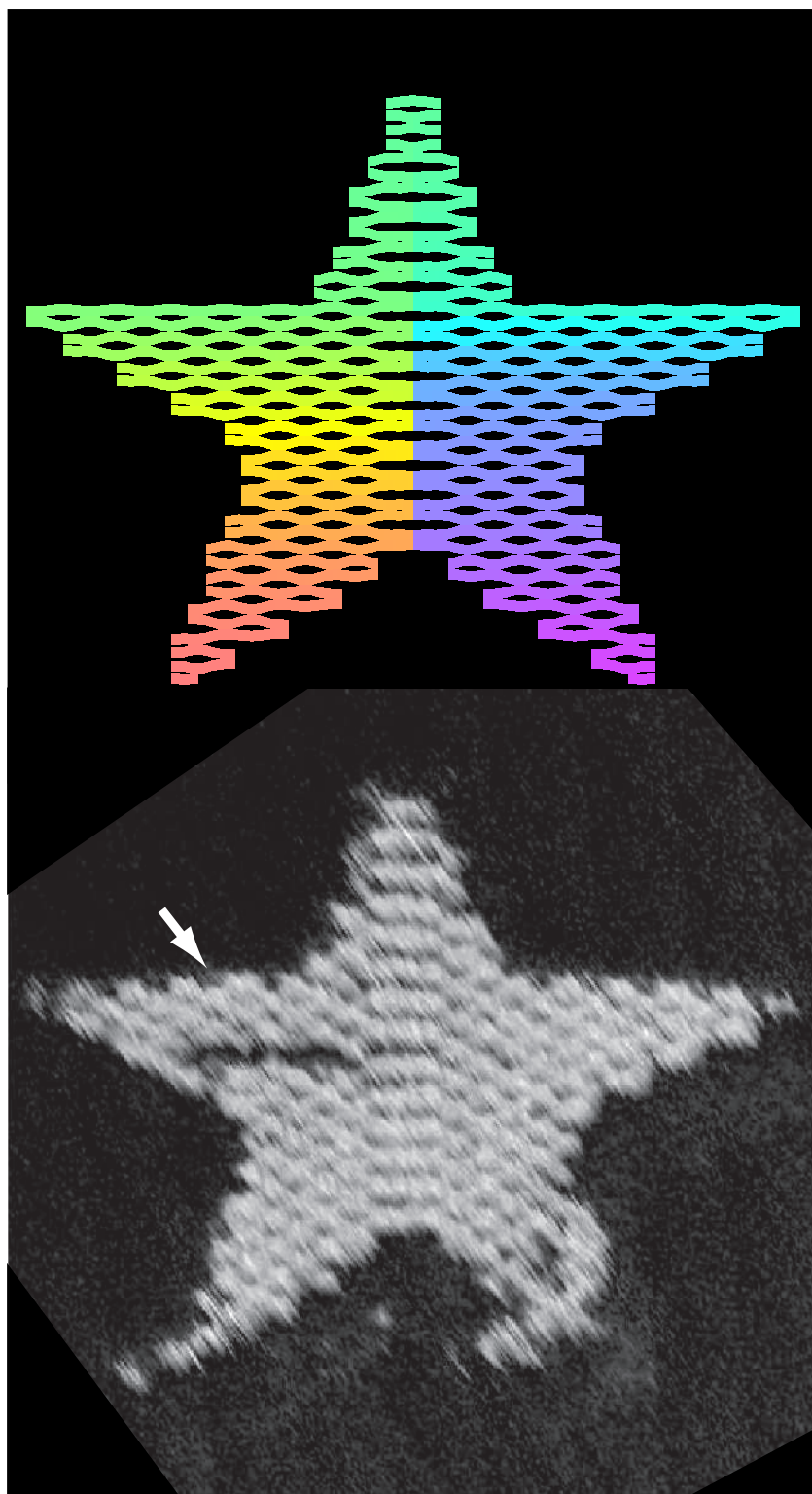
Plate number: 3

r-7t20f, A1, CTTGCATGCATTAATGAATCGGCCGCCAGGG
r-7t22e, B1, TGGACTCCCTTTTCAACAGTGAGACCTGTGCT
r-7t22f, C1, TGGTTTTTAAAGCTCAAAAGGCGAAGAGCAATA
r-7t24h, D1, ACCCAAAATCAAGTTTTTGGGGGTCAAAAGAAC
r-7t2e, E1, AAAGGCGGAAAGGAAACAACTAAAGCTTTCCAG
r-7t2f, F1, GAGAATAGCTTTTGGCGGATCGTCGGGTAGCA
r-7t4e, G1, GCTCCATGAGAGGCTTTGAGGACTAGGAGATT
r-7t4f, H1, ACGGCTACTTACTTAGCCGGAACGCTGACCAA
r-7t6e, A2, CAGATTTTAGAGACAGATGAACGCGCGACCT
r-7t6f, B2, CTTTGAAGAACTGGCTCATTTATTAAATAA
r-7t8e, C2, CAGGATGTAACGGAACAACTATTACCTTATG
r-7t8f, D2, ACGAACTACGCTCCAATCTGCGGAATGCTTT
r-9t10e, E2, ATATAATCGATTGAATCCCCCTCAAACTGCTCA
r-9t12e, F2, GCTAAATCCTGTAGCTCAACATGTTATTGCTGA
r-9t14e, G2, GAGGATCGGTGTACCAAAAACAAAGCAATAA
r-9t16e, H2, GATTAGCCGATGAACGGTAACTGTAAGCAAAA
r-9t18e, A3, CAGCGATGTAATGGGATAGGTCAAAACGGCG
r-9t20e, B3, GGGAGAGGTGTAAAACAGCGGCCATTTCCAGT
r-9t22e, C3, AGGTAAAGAAATCACCATTCAATAATAATTTT
r-9t24e, D3, CGATGGCCCACTACGTAACAAACGCTG
r-9t2i, E3, CAGCGAAAAACCTTCAACAGTTTCTGGGATTTGTAAAC
r-9t4e, F3, ACGGTCAGACAGCATCGGAACGAACCCCTCAG
r-9t6e, G3, GGCGGTGTGCATAAGGGAACCGAAAGAGGCTAG
r-9t8e, H3, TAAATTTGGAAGAAATCTACGACCAAGCTCA
rt-rem1, A4, AACATCATGCTGCTGAAGTAAAGAGACT
rt-rem2, B4, TGTAGCAATATCTTCTTGATTAGTAAT
rt-rem3, C4, CAGCTGCTGCATACGAGTAAATTAACCGT
rt-rem4, D4, ATAACTAGTAGGCGACAGGATGAAGAAAG
rt-rem5, E4, ACGCGAGAGCTTAAAGGAGGAGGTTTTT
rt-rem6, F4, TTAAGGGGATTTTGAAGAGGACCGGT
rt-rem7, G4, AGAGCGGGAGTAAACGAGGAGCGCA
rt-rem8, H4, TATACAGTCTGCTTCCCTCGTGAATATC
rt-rem9, A5, GTACTATGGTTGCTTTGACGAGGACG
rt-rem10, B5, GCGCTTAATGCGCGCTACAGGGCGC

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

s10t15f, A1, AAAGACAAGCAAGGCCGGAAGACGT
s10t17f, B1, AGAGAGAACCACAAGAATTGAGTTCACGGCC
s11t14e, C1, AGCACCATTACCATTAAGGGCGA
s11t16e, D1, CATTCAACATATCAGAGAGATAACTAACATAA
s11t18g, E1, AAACAGGGAACAAGCAAGCCGTTTACCGCACTCATCGAG
s11t18h, F1, AAGCGCATATAGACGGGGGGTAATTGAGCGCTACGATTGAG
s12t15f, G1, GGAGGGAACAGCAAAATCACCAGT
s13t14e, H1, ATTTGGGAATTAGAGCGGTAAATA
s13t16g, A2, TTGACGGACCTGAACAAAGTCAGAGAATTAAGTGAACAC
s13t16h, B2, AATTATTTCATTAAAGGGTCACCGACTTGAGCC
s14t15b, C2, ATCACCTGAATT
s1t0h, D2, ATATAGCCCGGAATAGGTGTATCACCGCTACTCA
s1t10i, E2, CGTTCCAGGAAAGCGCAGTCTCTTTCAACAG
s1t12i, F2, CAGGAGGTCACAGAGCCCGCCGCAAAAGGAG
s1t14i, G2, CCAGAGCCTTTTTCATAATCAAAATATATTCCG
s1t16i, H2, TTAAGACTGAATACCCAAAAGACGAACCGGA
s1t18i, A3, TTATCCTGGCTATTTTGCACCCAGTAATAGTA
s1t20i, B3, ATGTTCCAGGTCCAGAGCAGCAACATCAACAT
s1t22i, C3, ACCATAAATTATTTAGTTAAATTTCACCAAAA
s1t24i, D3, AATTTCTCTCTGTAATCTGTCGAGAGATCT
s1t26i, E3, TTGAATACCAATAAGCGATTTCGCCAAATCAGC
s1t28i, F3, ATGATGGCTATCATCATATTTCTGGGGGACGA
s1t2i, G3, GATATAAGCGGATAAGTGCCGCTCGCACCTCA
s1t30i, H3, ATCTAAAAAGTTGAAAGGAATTAGTTGTAA
s1t32g, A4, GCGCGAATGACAGTTGGCAATCAACTATTCTTTA
s1t4i, B4, GTACCAGGTAGAGTAGCGGGGTCCTCAGA
s1t6i, C4, TTAAGAGGCTATTATCTCGAAGACAGTACCGTA
s1t8i, D4, TTGAGTAATAAGTTTAAAGCGGGCGTAACGA
s1t11f, E4, GTCAGAGCTCATTAAGCGCAGAATTAAAGCGTC
s1t13f, F4, AACCGCCTCCCGCACCAGCAACCTGAGGACG
s1t15f, G4, CGCAGTATATAGCGTTTGCATCAACACCGG
s1t17f, H4, CAACGCTAAACCGCAATAATAAGCCCTTATTA
s1t19f, A5, GAACGCGCTAAATCAAGATTAGTTAATCTTAC
s1t21f, B5, TTGAAATAAAGGTAAGATTATTTCTCTAATGCA
s1t23f, C5, CCTTGAAAAAATCTTTCAAATTAATAGTGT
s1t25f, D5, AAAATCGCGAGTGAATAACCTTGCCTTAGAAT
s1t27f, E5, AATATAATTTTACATCGCGGAGAAACAGGTTAC
s1t29f, F5, GGAGCACTCCGAGAGGAGCGGAATAATTATC
s1t5g, G5, CAAGAGAAGA
s1t7b, H5, TGCCCCAGTTAA
s1t7g, A6, GTATAAACCCTGCTATTTCGGAACCTGAGACTCCT
s1t9b, B6, ATGATACTTTTG
s1t9f, C6, ATACATGGCAGGAGTGTACTGGTACAGTGCCT
s1t10e, D6, ACAACAAATAAATCCATTGGCCT
s1t12e, E6, TGTATTTCCGCCACCACTTCGAGGCCCTCAGA
s1t14e, F6, GCCGCCACCGGTATAGCCCTCTTGTAGCAAA
s1t16e, G6, ACGTAGAACAGCAAGAACCGGAGGAGCGGT
s1t18e, H6, CTTTCCAGGAGGTTTGAAGCGCTCTGTTTAT
s1t20e, A7, CAACATATATAAATTCAGACACCAACCGCT
s1t22e, B7, GTGATAAAGACAAAGACCGGACGATAGCGG
s1t24e, C7, ATAGCTTAATAATCAATATCTGTGACGAGCG
s1t26e, D7, GAATATTATACAGTAACAGTACCTCTCGATTG
s1t28e, E7, TTTGAGATTGGAACAAAGAACCAACACTA
s1t30e, F7, ATAGATTATCAATCAATATCTGTTAGCCCTA
s1t32h, G7, AAACATCGATTTTGAATGSGCTATTAGTCTTTAAT
s1t34a, H7, ATGGAAGCGCTC
s1t13g, A8, CCACCCCTCAGA
s1t15g, B8, CATAAAGGTTTTCATCGGCATTTTCTCAGAAACCG
s1t17f, C8, TTGCCAGTAGCCGACAAAGTTACAAATATA
s1t19f, D8, CTGAACAAACCTCCCGCATTTGCGAGCCTAAT
s1t21f, E8, TAAATAAGGCCAGTAATAAGAGAAGATAAGTC
s1t23f, F8, CGCTGAGATTGCAATCAATCGCATAGGCGGT
s1t25f, G8, TTACCTGATTAATGGAACACAGTACCTAATA
s1t27f, H8, GAATAATGTACAGTCAGATTGAATACATTCAA
s1t29f, A9, AATAGATAAATCATATCATTTTGTATCTTCT
s1t31f, B9, AATACCGACTCAAATATCAACCCGAGCGGTC
s1t33f, C9, ATTTTGACAGCTGGCCACAGCAATCCATTAA
s1t35f, D9, CATTCGAACAGGAAAAATACCTAC
s1t14e, E9, GTCAGACTGTAGCGCGTGGCAACA
s1t16e, F9, TATAAAGAGAAAAAGTAAGCAGATTACAAAA
s1t18e, G9, AAACAGCCCGGAGGCGTTTATCGGAAAAATA
s1t20e, H9, ATATCCCGACGAGGCGATTTTCGAAATAAACA
s1t22e, A10, CCGGAATCTTATGTAAATCTGAAGAGTCA
s1t24e, B10, TAGTGAATATTGGAATTACCTTTTGCAGAAAG
s1t26e, C10, AGATGATGCGTAGATTTTTCAGGTGAAGGGTT
s1t28e, D10, AGAACCTAATTTTAAAGTTTGAATACATTT
s1t30e, E10, GAGGATTCTACACCTCTGTAACAGAAACCA
s1t32e, F10, CCACGAGACTGAAGCGTAGAATGCTCAATC
s1t34e, G10, GTCTGAACAAATATTACCGCCAGC
s1t36a, H10, GAGTCTGTAA
s1t15f, A11, AGACACCAAGATTTCGCTTTAGC
s1t17f, B11, TATCCCAAAACCGAAGCCCTTTTAAACGCGAA
s1t19f, C11, TACGAGCACCGGTTATCTTAGAACAATTAAT
s1t21f, D11, TAGAAAAACCAATGTAAATTATGTCCTAAT
s1t23f, E11, ATCATAGGGGTTGGGTTTATATACATAATTAC
s1t25b, F11, ATTAATAACAA
s1t25f, G11, ATCAAGAATACATTTTAAACATTTCTTATCAA
s1t27b, H11, TAAAACTGACG
s1t27f, A12, AAATATTAGAAATAAGAAATTTGAACAAAC
s1t29f, B12, TAGACTTTTGCAGCAAGCTTATTACCATATCA
s1t31f, C12, CAGAGGTGAATGAATAATCAAGAGAGATT
s1t33f, D12, TTACATTGATAGAACCTTCTGACAGATAAAA
s1t35f, E12, CGCAAAATCTGGTAAATATCAGAAATGGATT
s1t37f, F12, TCAGTAGGCGCACGAGTCAATCA
s1t14e, G12, TCAGTAGGACAGAAATCGGAATA
s1t16e, H12, GTTTATTTAGCAATAGCTATCTTTCCAAATA

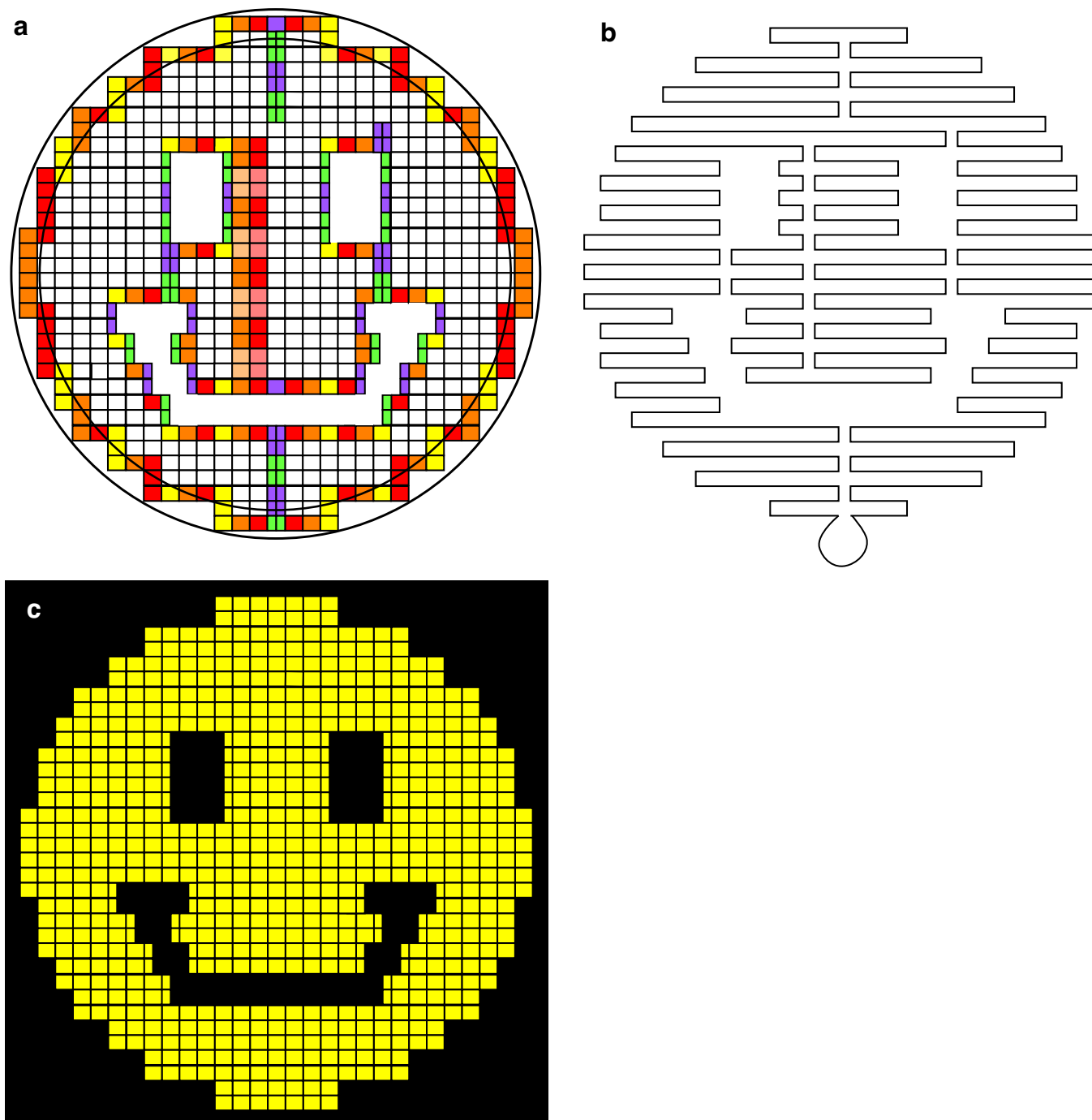
Plate number: 2

s7t18e, A1, AGAAACAGAGATATAGAAGGCTTATTGTAGAAA
s7t20e, B1, CCAATCAAGGCATATTAAACAACGGCCCTGTTT
s7t22g, C1, AGTATCATTTTAACTCCGGCTTATCTTGAGAGACTACCTT
s7t22h, D1, ATCGGTTATACAAATGGCTTAATTAGAGAATCTAATCGGC
s7t28e, E1, CTGCTATTAAATCCTTTACAAACAA
s7t30e, F1, TTCGACAACCTGAGAGCCAGCAGCAAGGCGGTC
s7t32e, G1, AGTATTAAATCTTGCCCAACAGAGGCGAGATT
s7t34e, H1, ACCAGTCACAAACATCTCGGCCCTGGAACCGTTG
s7t36e, A2, TAGCAATAAGAAGTGTTTTTATAA
s7t38a, B2, AATCAGCGTTAG
s8t15f, C2, TCAATAGAGATAGCAGCACCGGTAA
s8t17f, D2, TTAACGTGCGAAGAAACAAATGAAATGTCACAA
s8t19f, E2, TGCTTTTCAATAGCAAGCAAAATCATTTTTGT
s8t31g, F2, ACAGTGCCACG
s8t33h, G2, ATAAAAGGAGCCACCGCCTGCA
s8t35b, H2, CACTTGTAAAT
s8t35g, A3, ATTAGTAACCTGAGTAGAAGAACTCACGACCAGTA
s8t37f, B3, CTAACAGGTAACGCCAGAACTCTGCTTTCTTTG
s8t39f, C3, TATAACGTGCTTTCTCAGCGGAG
s9t14e, D3, CACCAATGAACCATCAAAATTCAT
s9t16e, E3, ATGGTTTAAAGCCCAATATAAGAAAAATGA
s9t18e, F3, AAATAGCAAAATCAATCCGCGCCCTTATCAT
s9t20g, G3, TCAAGAGAACAGCTCAACAGTAGCTTACCAGTAAAGC
s9t20h, H3, CGGGTATTAAACCAAGTATTTCATCGTAGGCCCTTTAC
s9t36e, A4, ATTTAGCAGGAACCGGAGCCGA
s9t38g, B4, TTAAGGGTGTCTTGACGAGCAGCGGCGCTACTATGTT
s10t15f, C4, ATAAATTGACGTAAATGCTCACTAGC
s10t17f, D4, TCATCAGTGGCTCATTAACAGCATATCGCCTG
s11t14e, E4, AAGGCACCAACTTAAAGCCCGGAGA
s11t16g, F4, TTTGTATCCAGGACGTTGGGAAGCAACATATTACAGGT
s11t20g, G4, TTAAGAGGAAGTACAGATTGCATCAAAAAGAAAGAT
s12t15f, H4, CAAAGTACACGAAGGAGCAAAAG
s13t14g, A5, AATACATAAACCACTCCAGCGATTATACCAA
s13t18g, B5, AAACGAATCAACGAAAAAATCTACGTTAATAGCGCGAAA
s14t15a, C5, TGACCCCATCTT
s1t10e, D5, TGATGGGAGTGAAGATAAGAAAGTTCCAGTT
s1t10i, E5, TTTCAAGCTATTTCCTGATGTTATTTGGGA
s1t12e, F5, GAAATCTTGTATCGGTTTATCAGATGACAAC
s1t12i, G5, CCTTTAATCCAAAAAAGGCTTCCAGCATTTGA
s1t14e, H5, AACCATCGGCTTGACGGAGTTAATGGCTGAC
s1t14i, A6, TCGCTGAGCCACGCAATACCGGATCACCGGAA
s1t16e, B6, CTTCACTAACCCAAATCAAGCTAAGGCTTTTG
s1t16i, C6, TATTCATTAGAGTAATCTTGCAATGGCATGA
s1t18e, D6, CAAAAGAAAGAGCTGGATAGGCTCTTAGAGCT
s1t18i, E6, AAATGTTTGTGTTTGGCAGAGGGGCTCAACT
s1t20e, F6, TAAATGCTTATGCAACTTAAAGTACAAAGCCTC
s1t20i, G6, GTTTTAAAGAAATATAATGCTGTAGTAACAAAC
s1t22e, H6, AGAGCATACCTGTAAATCTTTTGTAAATCTGA
s1t22i, A7, CATTATGAAGAAATCTTGAACATGGCATTA
s1t24e, B7, TGCCGGAGTATCAGGCTATTTGCCCTTATTTGT
s1t24i, C7, ACAAGAGCGAGGATGCTATTTTGTATTAAT
s1t26e, D7, TAAATTTCTAACCAATAGGAAGCGCGCGCAT
s1t26i, E7, TATTTGATTTGATTAATTTTGTGTTGATTGCT
s1t28e, F7, GTAACCGTTGCGGCTCAGGAAGATTGGGTAA
s1t28i, G7, CGACAGTAGCATCTGCCAGTTTGAATTATCAG
s1t2i, H7, GAACCGCGGAGGTTTATGACCGGAGGGTT
s1t30g, A8, CGCCAGGGCAGTGCCAAAGCTTGACGCTCACTGCC
s1t30i, B8, ACAGCGGCTTTTCCAGTACAGCAGGAAGGTT
s1t32h, C8, GCTTTCCAGTCGGGAAACCTGCTGCACATTAAT
s1t4g, D8, CCTCATTTTCA
s1t4i, E8, GCCACCAACCTCTCAGAACCGCATTTGCTCA
s1t6i, F8, ACACATGAGCCCAATAGGAACCCATGAAAGTA
s1t8e, G8, TCCACAGATTGTCGTCTTTCCAGGAATTTTC
s1t8i, H8, TCTAAAGTCAGCCCTCATAGTTAGTCAGTGCC
s2t11f, A9, ATAAATTTTGAACAACTAAAGGAAT
s2t13f, B9, CGCCGACACTTGCTTTGAGGTGATGCGAATA
s2t15f, C9, GCATAGGCAAGTCTGCGGAATCGTAACAAAA
s2t17f, D9, TAGCGAGACAAGCTGCTCATTGACACCGGC
s2t19f, E9, CGGATGGCAATCTGCGGAATCGTAACAAAA
s2t21f, F9, TAAGCAATGGTGTCTGGAAGTTTCCATTTTGT
s2t23f, G9, CTAGCTGACGGGAGAAGCGCTTATAGCAAAAT
s2t25f, H9, AACGTTAAGAGAGTCTTGAGCAACAAACCGTT
s2t27f, A10, TGATAGTGCATCAAAAAATTAATCGAAATGTGA
s2t29f, B10, CGATTAGCGCATCCAGCGAGCTCAGCTTGG
s2t31f, C10, TGCGTTGCTGCTGCGAGGTCAGCTTGCAGG
s2t5g, D10, GGGATAGCAAGTTTCGTACCCAGTACATGTAGCAT
s2t7a, E10, AACGCCCACTAC
s2t9a, F10, GTAAATACGTTA
s3t12g, G10, ATTTCTTAAAC
s3t16e, H10, GGTGTACAGTGAATAAGGCTTGCCACCAAGC
s3t18e, A11, ACGATAAACATAAATTTCAATGACCTTTTGA
s3t20e, B11, TAAGAGGATTCCATATTAACAGTTGGCAAGCG
s3t22e, C11, AAAGATTTTTCAACGCAAGGATAACCATCAAT
s3t24e, D11, ATGATATTCAAGAGAACTGATGAAGTATAAGC
s3t26e, E11, AAATATTCTGCTTGCCCTCTCTGTCGTAATG
s3t28e, F11, GGAATAGGTTTCCGCGACCGCTCTGCGAAAGG
s3t30e, G11, GGGATGTGCTAGAGGATCCCGGAGTGAAGTA
s3t32e, H11, GCTAAGTCAAGCTTAATGAAGCAAGCGG
s3t34b, A12, CCCCAGGGTTTG
s3t34e, B12, TCCAGCTCAGGCGAAAAATCCTGT
s4t13g, C12, AGCTTGATACATCTGCACCTCAGCAAGATGAAC
s4t15f, D12, AAGAGAGCGGGAAGACAGCATCG
s4t17f, E12, CTTCTGTTTCTGACGAGAAACCAAACTTTGA
s4t19f, F12, TAATGCTATCCCTCAAATGCTCATATAAC
s4t21f, G12, ATCATACAGATTCCCAATCTTGCGAGTACCT
s4t23f, H12, TCAAAATCAAAATTTTGAACCCCTTCAATAA

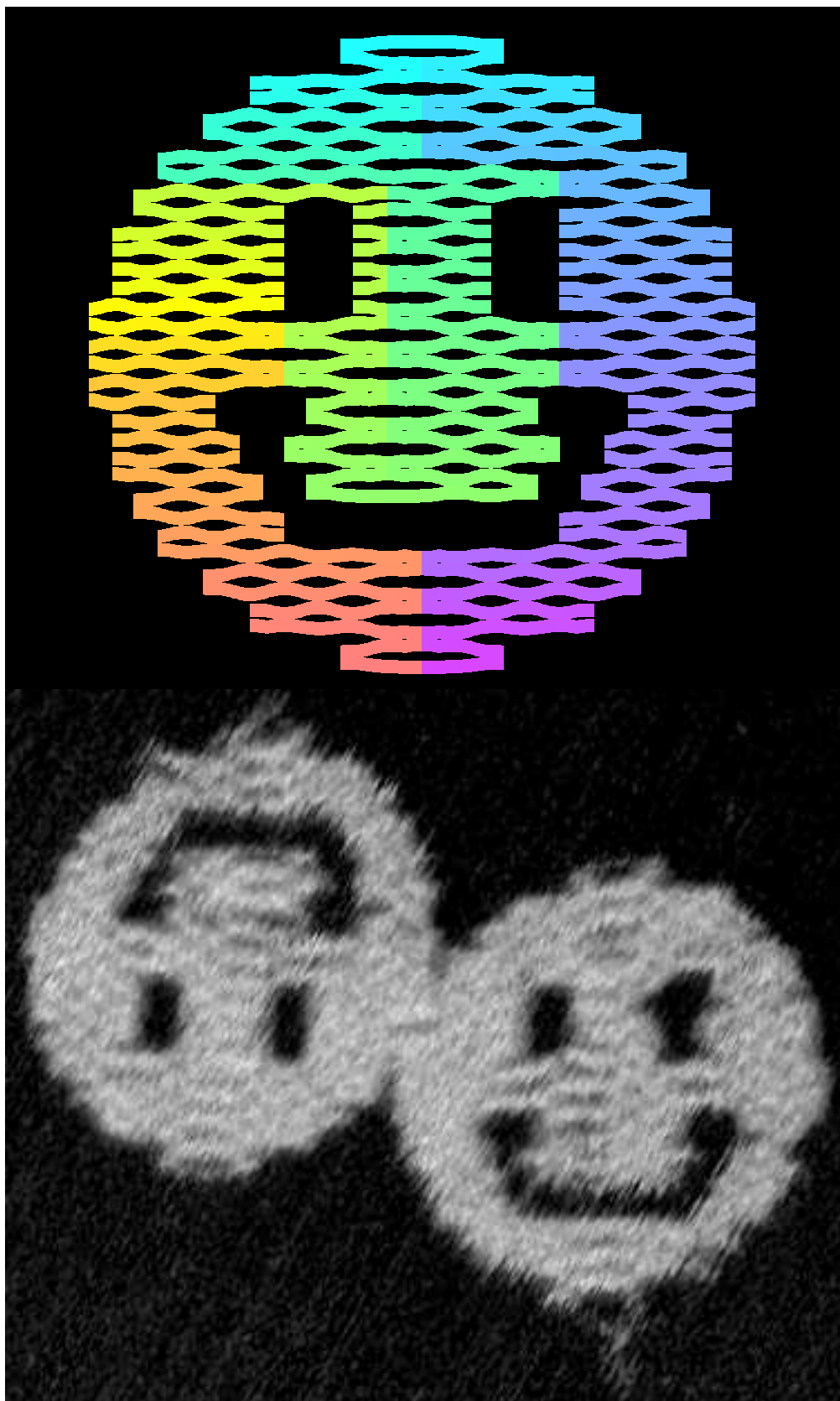
Plate number: 3

s-4t25f, A1, GGAAGATTTCGGTAATCGTAAAACTGGAGACAG
s-4t27f, B1, CGGATTGAAGCGAGCTTTCATCAACAAAAAAG
s-4t29f, C1, GCCAGCTGGGTGCCGGAACAGGACAAACCG
s-4t31f, D1, GGTGCTTATCCAGGCTCGAATTGCTATTAC
s-4t33f, E1, GAGTGTGATCGGCAACGGCGGGAGCGCTGG
s-4t35f, F1, TTGATGGTGGTTCGACCCCTGAGA
s-5t14e, G1, GAACGAGGATAGCAACGGGAACCG
s-5t16e, H1, AACTGACCGAACGAGTAGTAAATTTAGTAAGA
s-5t18e, A2, GCAACACTTTAAACAGTTCAGAAACAGGTCAG
s-5t20e, B2, GATTAGAGAAGAGTAGATTAGTAGTAGTAG
s-5t22e, C2, CATTAAACATATATTTTAAATGCAAGGGTGA
s-5t24e, D2, GAAAGGCCAGCATGTCAATCATATATAATCAG
s-5t26e, E2, AAAAGGCCCATTAATGTAGGACGCGATTCT
s-5t28e, F2, CCGTGGGCAAAAGGCCATTCGCCCGGTGCGG
s-5t30e, G2, GCCTCTTCGTATATCGGTCATAGGGAAGCAT
s-5t32e, H2, AAAGTGTAGAGAGCGGTTTGGTGCCCTTCA
s-5t34e, A3, CCGCCTGGAATCGGCAAAATCCCTTGGACTCC
s-5t36b, B3, AAAACAGGGCG
s-5t36e, C3, AAGCTCAAGCTCTATCAGGGCGAT
s-6t15f, D3, AATCATAGGCTACAGAGGCTTTG
s-6t17f, E3, ACGAGCGAGGCTTGAGTGTGTTTAGACGGTC
s-6t19f, F3, AACTCCAAACGAGATGACCATAAAGAGGAAT
s-6t21f, G3, CTAGTAAATGACCATATAGATACCGGAAGCA
s-6t25a, H3, CGGTTGTATGCC
s-6t27a, A4, ACCCGGTGAACA
s-6t29f, B4, AGGGCGATATTAGGCTGCGCAAC
s-6t31f, C4, AAGAGCTGTTTCCCTGTGATTTGGGA
s-6t33f, D4, AGCTGATTATTTGGGCGAGGTCACACAACA
s-6t35f, E4, AAGAGCATTTAAGTAAACAAAGATCGGGCAAC
s-6t37f, F4, GGCCCATACGTGAACCCACTTAT
s-7t14e, G4, AAGAGCTGACCTTTAGCCGGGAA
s-7t16e, H4, CGAGGCGCAATTTCAACTTTTATCAGATACAG
s-7t18e, A5, AAGCCCAATCAAAATTAAGTCAATCAAGCG
s-7t20g, B5, AACCAGACTTTGCGAATGGTCAAGGAGCTGAAAGGGTGG
s-7t24g, C5, GTAGGTAAAGATTCAAAATGCTGAGTAAATGTCATCAAT
s-7t30g, D5, ATGTTTATCCG
s-7t36e, E5, ACAGCAAGTCACTACCCAAATCAAGATCGGAAC
s-7t38b, F5, CCGATTGAGGCC
s-7t38e, G5, CCTAAAGGTAGAGCTTGACGGGGA
s-8t15f, H5, TGTTACTTTTCATGAGGAGATTTTCC
s-8t17f, A6, ACTAAGTGCATTGGAATTACCTTACTGCTCCA
s-8t19f, B6, TCGAGCTTTACCTGACTATTATCACTTCA
s-8t31h, C6, CTCACAAATCCGTTTCTTTTTT
s-8t33g, D6, CACCGAGTGAAGCCCGGAGATAGGGTATGTTTGG
s-8t35a, E6, TGTTGCGAGTGT
s-8t37f, F6, AGCACTAATTTTTTGGGCTCGAGG
s-9t14e, G6, ATTAACCGGGAATTAATTTGTCGAAA
s-9t16e, H6, TCCGCGACTCGAATTTAAGAACTTGAGATTT
s-9t18g, A7, AGGAATACAGTCAGGAAGGAGCAAGACTTCAAAATACG
s-9t22g, B7, ATTTTCATTTGGGCGTCAACCTGTTAGCTATGCTTTTAA
s-9t40g, C7, CGAGAAGGAAGGAAAGCGCCGCAAGCTGGTGGCGGTAA
s-t-rem1, D7, GCCTTAATGCGCCGCTACA

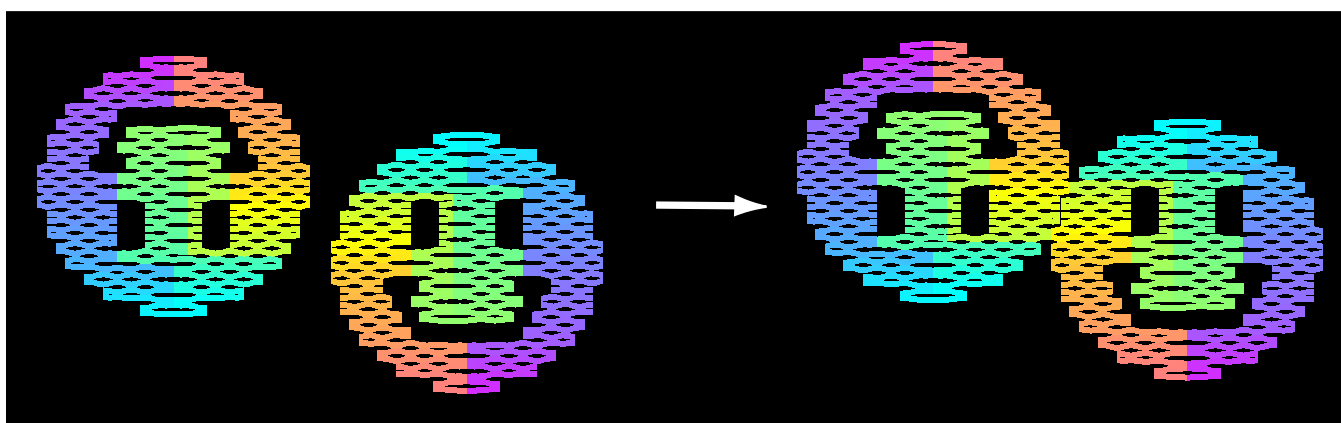
Supplementary Figure S24: Sequences for the star.



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

s0m25k, A1, AAACACCAGACGAGTAGTAAATTTGGGCTTGA
s10m15g, B1, ATATATTTTATG
s10m17g, C1, TCCTTTGAAACAACTTTTTCAA
s10m19g, D1, ATTCATTTCAAATTCCTCTAGAA
s1m0j, E1, ACGCAAAGACACACGGAATAAGTTTATTTTG
s1m10e, F1, CGTACTCAACCTTCAGAACCGCCAGCAGGCC
s1m12e, G1, CAATAGGAAGTACAACTACAAACGAGCTAAC
s1m14e, H1, GATCTAAAAATTTTCTGTATGGGATGTGAGAAT
s1m16e, A2, AGAAAGGATGCTTTTCGAGGTGAATGACAATGA
s1m18e, B2, CAACAACCATCGGAACGAGGGTAGTTTTTCAT
s1m20e, C2, GAGGAAGTAAAACACTCATCTTTGGTACAAACG
s1m22e, D2, GAGATTTGGACCACTTTGAAAGAAAGGCTGGC
s1m24e, E2, TGACCTTCGGCTTCGCCCTGACGAG
s1m28i, F2, GCAACAGTAGGGCGGTGAGTATTAAAGGAGAGG
s1m2i, G2, ATGTTAGCGATTAAAGACTCCTTATACCGATTG
s1m30i, H2, GTAATAAGCGAGATTACCAGTCAATAGGGTT
s1m32i, A3, AGTAGAAGATTAGTAAATACATCAATCGGAAC
s1m34g, B3, GTCTGTCATCAGCATGACAACTACTCTTTGAACTCAA
s1m4i, C3, GAGCAAGAAGATTAGTAAAGCCCTGACGACA
s1m6i, D3, ATATTATTAGCCAGTTACAAAATACCTCAGAG
s1m8e, E3, CCCTGCCCTCGGATAAGTGGCGCTGTGTATCAC
s2m11f, F3, ACCGTAAACCACTCAGAACCGCCGAGGGTTT
s2m13f, G3, GTCTTTCCACTGAGTTTCGTCAACCACTGAT
s2m15f, H3, AGGAATTGAGACGTGTATTAATGAGTTTGTCT
s2m15g, A4, CGAATAATAATTTTAAAGGAGCCTTAAATTGCGATATAT
s2m17f, B4, CGCATAACTATCGGTTTATCAGCTACAACTAA
s2m19f, C4, AACGGGTAGACGCGGAAGACAGCATCGCCCA
s2m1b, D4, AAGGTGTACATA
s2m1f, E4, AAAATACAGCAACATATAAAAGAA
s2m21f, F4, CCTGATAAGGCAAAAGATACACTTTCCATTA
s2m23f, G4, TAATCTTGATTAAGGGAACCGAATATCATCG
s2m25f, H4, CTCATTCAGTGAATAAATCAAGAG
s2m27g, A5, GCGAGCAAAAGAAAATACGAACACAGGAGAGAGAT
s2m29g, B5, TCTGGCCAGATAAAACAGAGGTGCCACGCTGAGAGCCA
s2m31f, C5, CTATCGGCTGGATTATTACATTGAGGGACAT
s2m33b, D5, CCGTTGAATTTAA
s2m3f, E5, AATAGCAACCAAGAACTGGCATAAACGTAG
s2m5f, F5, CAAATAAGGAGAGATAACCCACAACAACTAGA
s2m7f, G5, ACCTATTATTCCAGAGCTCAATTTTCCCAATC
s2m9f, H5, AGTACCGCTTTGCTCAGTACAGGATTTCCGA
s3m16i, A6, GGCTCCAAACAGTTGAAAATCTCCTAACCTCC
s3m18e, B6, TCGGTCCGCGGAGTCTGACACCTCAATACGT
s3m18i, C6, CGCTTTTGTGAGGCTTCGAGGAGTTTAAAA
s3m20b, D6, GGCACCTACGAA
s3m20e, E6, AATGCCACAACTTAAACGAAAGAAATTTGTCT
s3m22e, F6, GAAATCCGCGCGAGACGGTCAATCACAAGAAC
s3m24b, G6, CCAAAATATTAC
s3m24e, H6, CGGATATTCAACGTAAACAAGCTG
s3m2e, A7, AATAATAACGGAATACAGTATC
s3m30g, B7, AGAACCTCTGCTCAATCTGCTGAACTTCTGCTGTAATATCC
s3m32g, C7, AGAACCAATATTACGAGCTACTACTTTTGAAGCTGACCT
s3m4e, D7, TTACCGAATTGAGCGCTTAATTTCAAAACGATT
s3m6e, E7, TTTTGTGTTACGCTATACGAGGTTTCTTGAAA
s3m8e, F7, CATGAAGATTAGGATTAGCGGGAT
s3m8i, G7, GAGAAGGATATTAGAGGCTGGATAGAAGGC
s4m23g, H7, ACTTAGCCGAAGCAGCGACCTGCTCATAGTT
s4m25g, A8, GAGCCGTCAATAGATAAGCAACATAGATATTCTGAACCT
s4m29g, B8, GAAAGCGTCCATATAAAATACCGTAAGACATCACCTTG
s4m3f, C8, TAAGAAAAGAAACGAGGAAACGC
s4m5f, D8, AATGAAACAAAGCTCAGAGGGTAGCCCTTTT
s4m9g, E8, CCAATCAATAATCGGCTACGAGCATGTGAAATAAGAGAA
s5m12e, F8, TATAAAGTATTTTTCGAGCGAGTAACCGGAATC
s5m14g, G8, ATAATTACTAAATAGAATAAACA
s5m16i, H8, GGCTTAGTGAGAGACTACCTTTTAAAAAAA
s5m18i, A9, TGGAAACATTTTCAATTGAAATACCTTAAAGGC
s5m22g, B9, TATCAAAATTA
s5m24a, C9, GAACAATTTGCG
s5m28e, D9, CAAATATCTAGCCCTTAAACATCGAAGAAATAC
s5m2e, E9, ACAAAAGTTACCGAAGAGTAGACAG
s5m30g, F9, GTGGCAACAAAAAGCTCATGGAACAGCCATTGCAACAGG
s5m30h, G9, GACAATAATTTTGAATTTTAAATGCGCGAACTGAAACCCCTC
s5m4e, H9, ATAGCGGAACCTGAAACACCTCTGAAATAGCAGC
s5m6j, A10, CTTTACAGACCCGACTACAATTTTCTGTAATCTTCCAAACGCTCA
s5m8i, B10, TTTATCCGGCGAAGCAATCAGATATCTCTCTCA
s6m11f, C10, AAGGTAAAAATATCCCTCATCTTAATTTGTCTTTCT
s6m13f, D10, CGCTGTTTAAATTTAGGCAAGGCGACCGACAA
s6m15f, E10, TATAACTAGTGATAAAATAGGCGTTAGAAAAA
s6m17f, F10, ATCAATATATCAAAATCATAGTCTTGGGTTA
s6m19f, G10, TAATTACATTTTAAACAGTACATA
s6m23f, H10, CCAGAAGGAGAACCTACCA
s6m25f, A11, GAGGATTTTGAATTAACATTTATCATAGAAACCA
s6m27f, B11, AATCAATATATCTTTAGGAGCACTATACATTT
s6m29b, C11, TAGTCTGGCTAT
s6m5b, D11, AAGCGCACAGGG
s6m5f, E11, ACATAAAAAATTAGACGGGAGAATT
s6m7f, F11, GAACGCGATTAGTTGCTATTTTGCAGAGAATA
s6m9f, G11, CTTATCATATTACCGCGCCCAATATATTCTAA
s7m10e, H11, CGGGTATCTGGAACAGAAAATAAGTAAATCTCT
s7m12e, A12, GTCCAGACTAACCAACGCAACATGAGTATCAT
s7m14e, B12, ATGCGTTATTGAAATACCGAGCGTTATTGAAA
s7m16e, C12, TGCTGATGAGTCAATAGTGAATTTATGTGAGT
s7m18e, D12, GAATAACCATCAAGAAAACAAAATTAACGGA
s7m20e, E12, TTGCGCTGTACATCGGGAGAAACATTTGCCACG
s7m22e, F12, TAAACAGGAATAATGAAGGGTTAGCGGAAT
s7m24e, G12, TATCATCAATTAATTTTAAAGTAGAAGTAT
s7m26e, H12, TAGACTTTGGAAGGTTATCAAAATCTGGTCA

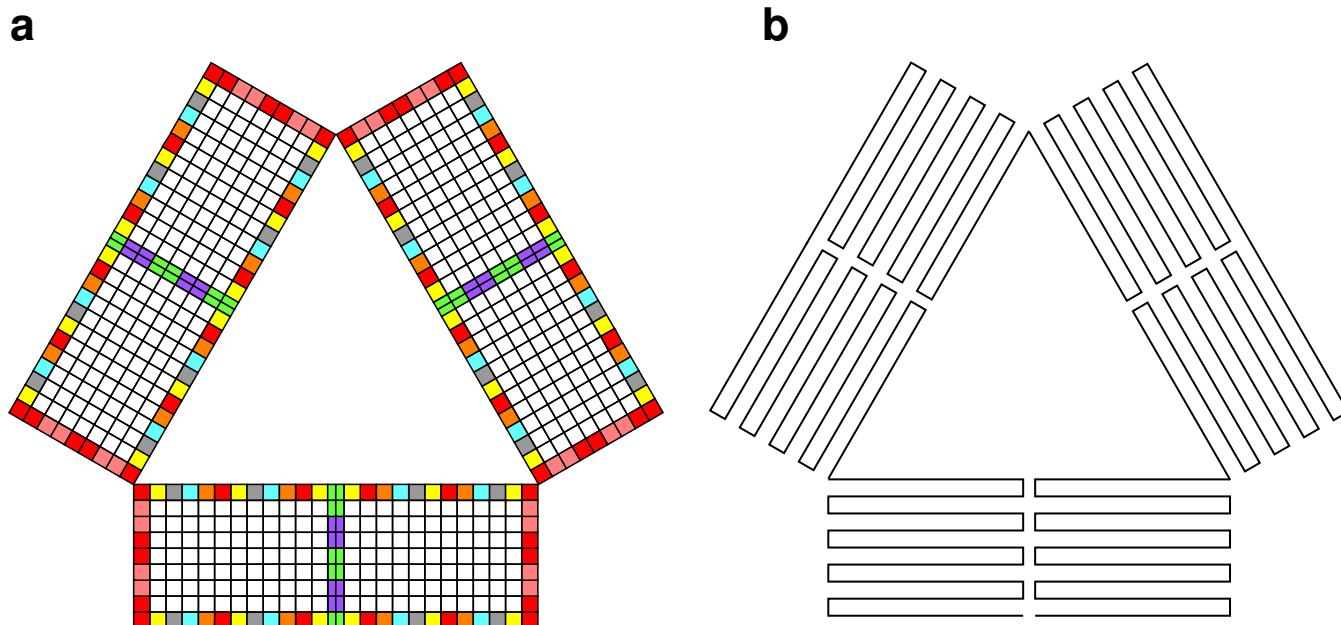
Plate number: 2

s7m28g, A1, GTTGGCAAACTCAACAGTTG
s7m8e, B1, AGCGAACCTTTTCATCGTAGGAATCTCCAAGAA
s8m11f, C1, TAAACCAACCAACATAGATAAGTCAAACCAAG
s8m13f, D1, CTTACAGTAGAGAAATGCCATATTGACGACAA
s8m15f, E1, ATCGCAAGACCTAAATTTAATGGTTACAAATT
s8m17f, F1, GTAATTCGTAAAGCGCTGAGAAGCAAATCCA
s8m19f, G1, GAATACCAAGATGATGAACAAACTTGCCTCT
s8m21f, H1, AAATTCGCGAGTAAACAGTACCTTTATTGCTTT
s8m23f, A2, ATTATCGAGTTTGAGTATGACTTCTAAATAAAG
s8m25b, B2, TTAATATCTCGTA
s8m25f, C2, TCGACAACCTTTGCCGCAACGTTTATTCTCTG
s8m27g, D2, AAAGGAATTGAACAACAA
s8m7g, E2, GGGAGGTTTGAAGCCTTAAATCAAGAGGCGTTTT
s8m9b, F2, AGAACATCATCG
s8m9g, G2, TACCGCACAGCAAGCCGTTTTTATTCCGACTTGC
s9m10e, H2, GAACCGCCCTGTTTATATGTTTCAG
s9m12e, A3, CTAATGCAACAGTAGGCGTTTAAATATAAAGC
s9m14e, B3, CAACGCTCTTAAATTTCACTCTTCTGACAAAGAA
s9m16e, C3, CGCGAGAAATGAGCATAGCTTAGGATCGCTATT
s9m18e, D3, AATTAATTTTACCTGAGCAAGAAAGAAATTCAA
s9m20e, E3, AATCGCGCAGTGAATGAGTATATATAGTTTCT
s9m20f, F3, AGAGGCGGAAT
s9m22g, G3, CAGGTTTAAATATAATCCTGATTGATGGAATTCATC
s10m15g, H3, TAGTATATTCAAGAAAGCCCC
s10m17g, A4, AAAAAAGGAAGACGAGTAAACAA
s10m19g, B4, CCGCTCGGATT
s10m1e, C4, TCACAACTCAATAGAAATACGAGCG
s10m10i, D4, AAATATCGTATAGCCCGGAATAGGAGAGGGTT
s10m10j, E4, GCCACACTTAAGAGGAGCCCGAGATTGCAAT
s10m12i, F4, CAAAAAGACCTCAITTTACGGGATCCCTCAGA
s10m12j, G4, ATTCACAGAGTCGAGGAAAGCGTTACCGCTG
s10m14i, H4, ACTATTATGACAGCCCTCATAGTTTCTGTAGC
s10m14j, A5, ACAACTTTTACAAAATCAGGTTCTACGAGAAT
s10m16i, B5, GACCATTAACACAGTTTTCAGCGGATTTGCTAA
s10m16j, C5, CAGCTTGAAGCTGGATAGGCTCTCAATAGTA
s10m18i, D5, AAATGTTTTTACCGATAGTTGGCCCTTCTTAA
s10m18j, E5, ACAGAGGCTCATACCTCGCTTTTATGTAAGA
s10m20i, F5, GCAACACTTTTGAAGACTTAAGACCAACGGCT
s10m20j, G5, CGATTATAATTTAGGAATACCAAGAATTTCAT
s10m22i, H5, CAGTTGAGCGCAAGCGGCAACAAACCCCGAG
s10m22j, A6, GAACGGTGCAATTATCCAGTCAGGATTTTAAAG
s10m24i, B6, AACTGGCTTACAGACAGCGGCTGAGACAGAT
s10m28i, C6, CGGTTTGAACCTGCCCAACGCGCGACCGCT
s10m2e, D6, CCAAGAGCAAGGTAAATTTGACGAAGGCGCG
s10m2i, E6, AGGGAGGGAAGAGCGGACATTTATCGCAGT
s10m30e, F6, TATAAATCTTCAGTTTGGAACTTTTGTGG
s10m30i, G6, GAGTGTGAAAAGAAATAGCCCGACGACGACCA
s10m32g, H6, GGTGAGGAGGACCCCGATTAGAAAGCCGCGAAGCTGG
s10m32i, A7, CCTAAGAGTGCCGTATACAGCACTACTTGGCTG
s10m34j, B7, CGAGAAGGAAGGGAAGCCACGAGTAAAGAA
s10m4e, C7, AACAGCTTGAAGTATACCAAGTCAACCCCTCAG
s10m4i, D7, CCGTATGCCAATGAACCACTGGAATTAATAA
s10m6e, E7, AACCGCCACGAACCAACGAGGAGTGCCCTT
s10m6i, F7, CGCGCAACCCCTCAGAGCCACCAACGAGCAT
s10m8e, G7, GAGTAAACGCTTTTAAATTCGAGCTAAGACTTC
s10m8i, H7, GATATAAGGTGCCGTATAAACAGTTTAAAGCC
s2m17g, A8, AGAGGGGGAATATCGCGGAATCGTTTAAACAGTTGAGAA
s2m19f, B8, ACAGAGCAACAGACGAGCATAAAGTTTGGC
s2m1a, C8, ATGGTTATTCAT
s2m21f, D8, AGGTAGAATTCAACTAATGAGAGTAAGGAATT
s2m23f, E8, CTTATGCGAGCTTGGGAAGAAAAATTATTAC
s2m25f, F8, GATGGTTTAAATTTCAATGAATTAC
s2m27g, G8, GCGAGCTGCATTAAGTTATTGGGCGCCAGGGAAATCCCT
s2m29g, H8, AATCGCATGGTTTTTCTTTTCACTCGGGAACCTGTGCT
s2m3a, A9, CGGGGAGCTGTA
s2m3f, B9, CAAATAGCGAAATTTATCATTAA
s2m5f, C9, GAGCGCGCAGTTTGCTTTAGCGTCAACCATTA
s2m7f, D9, ACAGGGTCCCGCGCGCAGCATTTGACTCCCTCA
s2m9f, E9, TCAAAGCAACGACAGCAAGTTTAA
s3m16i, F9, CAAATGCTATAAATATTCAATTGAGAGTCTGG
s3m18i, G9, CAAAGAAAGAACAAATAGCGAGATTTTAAAC
s3m20a, H9, CGCCAAACATAA
s3m24a, A10, TCATTGCTTTAA
s3m2e, B10, GGTGAATTATCAACGTCGAAATC
s3m30g, C10, TTGATGGTAACGTGGACTCCAACGGGCCACTAGTGAAC
s3m32g, D10, CATCAACCAAAATCAAGAGTCCACTATTAAAGGTTCCGA
s3m4e, E10, AACTAGTACGAGCTGTAGCGCGTTCCACCAAC
s3m6e, F10, GGAACGCGCAGAGGTTGAGGCGAGGAGTGTA
s3m8e, G10, CTGGTAACTCGGAAGCAAACTCCAA
s4m11f, H10, GTAGCATTTTTCATTTCATATAACGTACGGTG
s4m13f, A11, TTTTGAAGAACTCAATAAATCATATAGTA
s4m15g, B11, CCCTCATATATTTTAAATAAAAAAT
s4m21g, C11, GAACTAACGGAACAACTCTACGTTTAAATAAAC
s4m27g, D11, TTGCGCTTCTTCTGTGTGAATTTGTCATGGTCATAGCTGT
s4m27h, E11, ACTGCGCGCTTCCGAGTGGAGCGGCAACATCTCTGT
s4m29f, F11, CAGGCGAAGCTGATTGCCCTCATAAATTCGG
s4m3f, G11, TAGAGCCACACCGACTTGAAGCAT
s4m5f, H11, AACAGAGTTTCATCGGATTTTCTGTTGGGAAT
s4m7f, A12, ATGATACAGTCAGAGGATTTGGCTTATCAACGG
s4m9f, B12, TCTGGAAGAGCTGAGGATAGAGGGCTTTTG
s5m10e, C12, CAATCAAAGTTGATTTCCCAATTCGTGGCATC
s5m12e, D12, AATCTACTACAGGCAAGGAAAGTTATTATCA
s5m14e, E12, ACGAAGGATGCAATGCTGAGTAAGATCTAC
s5m16e, F12, AAGGCTAAGAGAACTGATGAACGATTGCGAT
s5m16i, G12, AGCAACACTCAGGCTCATTTGCTGAATCCCCCT
s5m18e, H12, TAAATTTTACGCCATCAAAAAATA

Plate number: 3

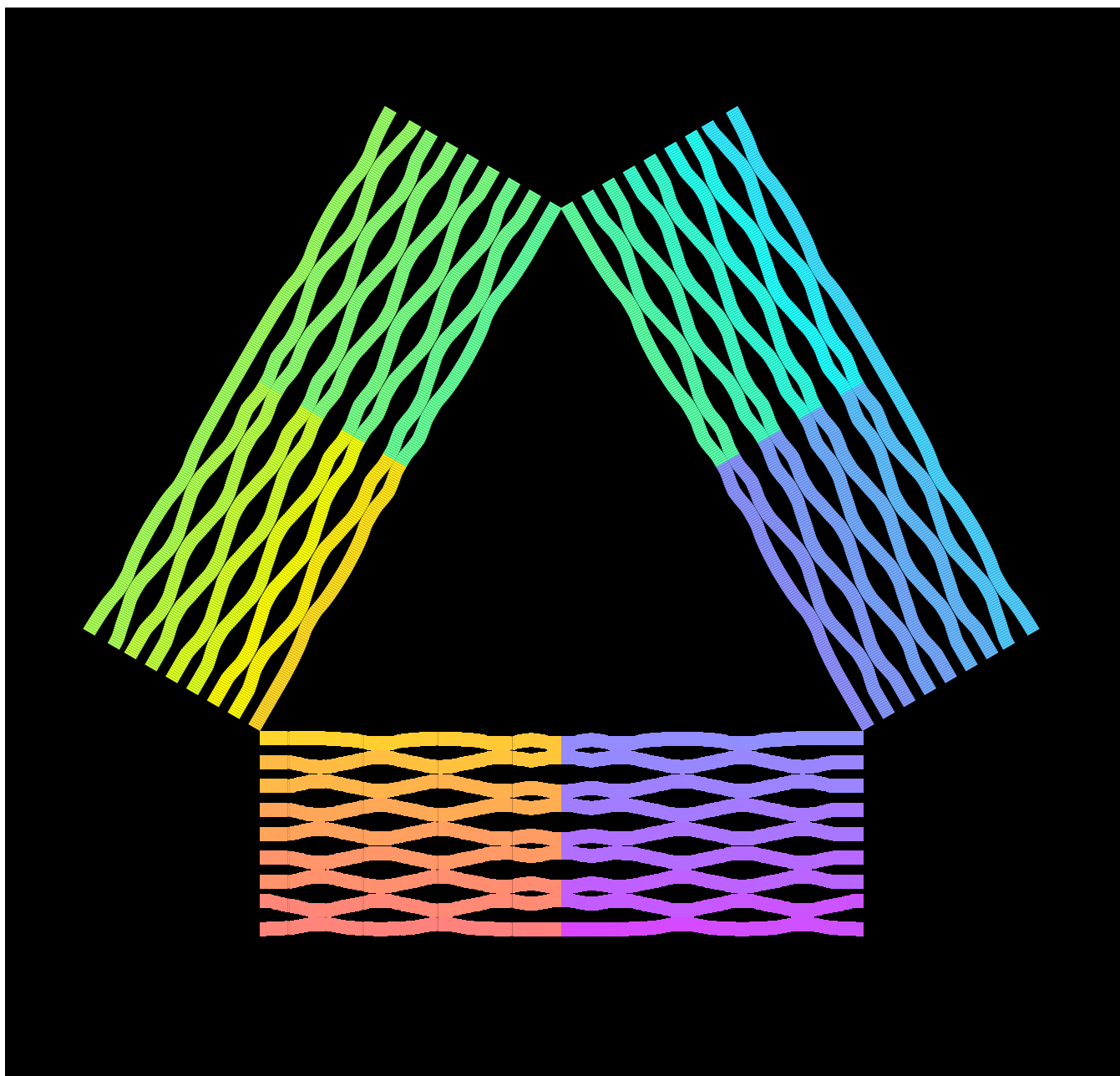
s5m18i, A1, CAATAGGATGTAAATCAGCTCATGGCTTTTG
s5m22g, B1, GTGCGGGAACCTGTGCTGC
s5m24b, C1, GGGTAAATAGTT
s5m24e, D1, AAGGCGATCGCCAGGGTTTTCCAGAGCTCGA
s5m26e, E1, ATTCGTAATTATCCGCTCACAATTTGTGAGTGA
s5m28g, F1, ACTCACATCCCGCTGGCCCTGAGAGCGGTCCAGCTGTT
s5m32g, G1, CGCTATCAGGCGGATTCAAAGGCGAAAACTGCCCCAG
s5m4e, H1, GTCATAGCCCCCTTATCTTTTCA
s5m6e, A2, TAATCAATGATATTCAACAAACAAAGTAAGCG
s5m8e, B2, TCATACATAGTACCTTTAATGCTTAAATATG
s6m11f, C2, CTGAAAAGTCGGAACGAGTATTGTAGCTCA
s6m13f, D2, AGAAGCCTAATAGCAAAATTAAGGCGCGGAG
s6m15f, E2, TTTTGTAGATTGTAGTAAAGATTTTTCGGGG
s6m17f, F2, TTGTTAAAGTAATCGTAAACATAGGATAGCTA
s6m19f, G2, GTCACGTTTTCGCTGTGGCCTTCTTAATATT
s6m21f, H2, CTTTCCGGGGGTAGATGGCGCATGGGATAG
s6m21g, A3, CACCGCTTCTG
s6m23f, B3, AAGGGGAGCAGGCAAGCGCCATCCAGCCAG
s6m25f, C3, CGGGTACCGTACGAGCTGTAAAGCTTGGCGA
s6m27f, D3, CCTAATGACCAACACATACGAGAGGATCCC
s6m29a, E3, CAGCAAGAGTTG
s6m5a, F3, TTGCGATAGGCT
s6m7g, G3, ACGGTTCCATAAATCCTCTATAAAGCCAGATGGA
s6m9f, H3, ACATGTTTCCCTTTTGTAAAGAGTCTGAAATTT
s7m10e, A4, GTTAAACGCTGTAGCCAGCTTTCAGGCGGATT
s7m12e, B4, TCATTTGGCAATAAAGCCTCAGAGATAGCCCT
s7m14e, C4, GTTAATCTTCAAAGGGTGAGGATTTAAATG
s7m16e, D4, CCGGAGAGCATGTCAATCATATGTAATTAAT
s7m18e, E4, GTTAAACGCTGTAGCCAGCTTTCAGGCGGATT
s7m20e, F4, GAGCGTAATGTACCGGTGCATCTTCAGGAAG
s7m22e, G4, CAGTCACTCGCATTTAGGCTGCGCTTCGCTA
s7m24e, H4, TACGCGCAACGAGCGGATGCCATGCAAGCTG
s7m26e, A5, GACTCTCGGGAAGCAT
s8m11f, B5, CTTGTTAGTCAATTTCCGAAATGG
s8m13f, C5, AAAACATTTCAAAAGTCAATGCTGCAATAAC
s8m15f, D5, AGCTGATAGGCGGAGACGATGATGTACCA
s8m17f, E5, AAGCAAAATCCCGGTTTGAATACACGCTTCT
s8m19f, F5, GAACAACTCAACATTAATGTGAGATTGTAT
s8m21f, G5, TATGGCCCGCAGTTTGAAGGGAGCTCCGTGG
s8m25a, H5, CATGCGAGCTTG
s8m27g, A6, AAGTGAAGGCTGGGGTG
s8m7g, B6, AAGCGGCACTCATTTTTCGGGATGGCTGCTGAAT
s8m9a, C6, CTTAATTTAGAG
s9m14e, D6, ATCAACCATCAA
s9m24g, E6, CGATCGCTGCGGGCCTCAACTGTTGGGAAGGGACGACAG
sm-rem1, F6, CCTGAGAGAGTGTTTTATAATCAGTAG
sm-rem2, G6, ATTTAGACAGGAACGCTGACCGCAGAT
sm-rem3, H6, GAGACTTAACAGAGGCGGCAATTAAGGG
sm-rem4, A7, AGCTGCTTCTCGTTGATGACGAGCG
sm-rem5, B7, ACTATGTTGCTTTGACGAGCAGGTATA
sm-rem6, C7, CGCGCTTAATGCGCGCTCAGGGCGCGT

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

e0t11d, A1, CATAGTTAGCGTAAATCGATGAACGG
e0t13c, B1, GCGCAAAATGACGATTAATCTTTT
e0t13d, C1, GAGCAACAGAGACGATCTAAAGTT
e0t15c, D1, ACTCATCTTTGACTCAATCTCATATA
e0t15d, E1, AAACATATTAGCAACACACCTCGCCC
e0t17c, F1, AATCACGCTAACATATTAGTAGCA
e0t17d, G1, GTGTCTGGAAGTTCGCCAGCGATTAT
e0t19c, H1, CTAATGCAATACATACGCAAAAG
e0t19d, A2, TCTTTACCCCTGACAGCTGCTCATTC
e0t1d, E2, CAATAGTGAATTTTCTCTAGTACGAG
e0t21d, C2, TAACACCCGCTGCAATCGCCGAGAG
e0t23c, D2, CCAGCTGGCGAAAGAGAGCGGAA
e0t23d, E2, TACCAAGTTACAAGATTCTCCGTGGG
e0t25c, F2, AGCTAACTCACATACCTCAATATCA
e0t25d, G2, CAAAGAAACACCGGGGGATGTGCTG
e0t27c, H2, CGGCAAAATCCCTTTAAAGGGACATT
e0t27d, A3, ATCACTCTTGCTGATGATTCGGTGGC
e0t29c, B3, GAAAGGAAGGGAATTAATCAGTGAGG
e0t29d, C3, ACACGACCGAGTAATAATAACAAAG
e0t3c, D3, GTATCATATTCGCTTATCAACACAAA
e0t3d, E3, ATTAGCGGGGTTATCAAAATCATAG
e0t5c, F3, CATTCCTAATTTAATCAGTTTGGCTG
e0t5d, G3, GATTGGCCTTGATATACAAATCTT
e0t7c, H3, CTATTTTGCACCCAGTTTATTTTGT
e0t7d, A4, CAGTAGGACAGACGAGCATGTAGAA
e0t9c, B4, AGAGAGATAACCCCAAGAAATTTGAGT
e0t9d, C4, GACACACGGAATAGCTACAATTTTA
e1t10e, D4, TAAGCCCAATAATAGAGACGAGAAAC
e1t12a, E4, TAATCGTAAATCGCTGAGAGTCTG
e1t12b, F4, CTATCAGGTCATTCAGTGTCAATCA
e1t14a, G4, GCGGGAAGAGCTTCGGTGTACCA
e1t14b, H4, GCATAAAGCTAAATATTTCACACGCA
e1t16a, A5, CAGTTGATTTCACACAAATAGATGAG
e1t16b, B5, TGTTTAAATATGATTTTCGCAACGA
e1t18a, C5, AGCAAAAGCGGATATCAAAATCAGG
e1t18b, D5, AGAATGACCATAGACATCAAAAGAT
e1t20e, E5, GAATTACGAGCAATGTAGAGCAAC
e1t22a, F5, CGAATTTATCATTTGATTTGCTTGA
e1t22b, G5, TAACGCTTTCGCTCATTAATCTGAG
e1t24a, H5, TTATCATCATATTTTATTTTTCGGGAA
e1t24b, A6, TTGAGTAACTTACTGATTTATCAGAA
e1t26a, B6, AACCTCTCAATCAAAAAATCAAAAGC
e1t26b, C6, CCAGCAGCAAGTGTATCTGGTCAGTT
e1t28a, D6, CTGGCCACAGAGAGATTACCAGCTG
e1t28b, E6, TATTTACATTTGGCAGTAAGACCTTCT
e1t2a, F6, GCGGATAAGTGGCGAGAGGATTTAGG
e1t2b, G6, TGAGACTCTCTCAAGTCGAGAGGGTTG
e1t30e, H6, CCACCGAGTAAAGAGTCTGTCCATC
e1t4a, A7, TAAATCCTCATTAAGGACGTCAGAC
e1t4b, B7, TGACGAGGAGTTGAAGCCAGATGGA
e1t6a, C7, TTAGCGTCAGACTCGACACCGTAAAT
e1t6b, D7, GAAACCATCGATGATAGCGGTTTTTC
e1t8a, E7, CACAACTAATAGAAAGAACGCAAA
e1t8b, F7, GTGGCAACATATAAATTCATATGTT
e2t11e, G7, TATGTACCCCGGTTGATAATCAGAAA
e2t13a, H7, AGGATAAAAAATTAGATCTACAAAGG
e2t13b, A8, AGCTATTTTGTAGTAGAAGCTTCAT
e2t15a, B8, GTAGATTATGATTATAAAGCCTCAGA
e2t15b, C8, GCAAAATTAAGCAGACATTAGATAC
e2t17a, D8, TAAGGAGAGCCCTGTAGCTCAACA
e2t17b, E8, GCTGAATATAATGAAAGAGCTTCAAA
e2t19a, F8, ACTATCATATAACCGTTCAGAAAAGC
e2t19b, G8, AAATGCTTTAAATCTGTTTACCAGAC
e2t1e, H8, ATATAAGTATAGCCCGGAATAGTGTT
e2t21e, A9, CAAAGAGAGATGATGAACAAACATC
e2t23a, B9, TGAATGCAATCTCGGAGAGAAACAA
e2t23b, C9, AGTACCTTTTATCATCAATATAATCTT
e2t25a, D9, GGCAAATCAACAGTAATTTTAAAGAT
e2t25b, E9, GCGCGAAGCTTATTTGAAGGAATTG
e2t27a, F9, GACCTGAAAGCTGCGCAGCTGAGAG
e2t27b, G9, CGCCTGCAACAGTAAGAAATAGTGGC
e2t29a, H9, ACGCAAAATTAACGCTGTAAATGGAAT
e2t29b, A10, TTGACGCTCAATCGTTGTAGCAATAC
e2t3a, B10, AAGCGCAGTCTCTAGTATTAGAGGCG
e2t3b, C10, TCTGAACATGAAGATTTTACCCTTC
e2t5a, D10, ATCGGCATTTTTCGCCGCCGCCAGCAT
e2t5b, E10, ACCACACCCAGAGGTCATAGCCCTCT
e2t7a, F10, TTACGAGCGCAAAAAGCTGCACCAAT
e2t7b, G10, TTAGCAAGGCCGGAAGCAAAAGGGCG
e2t9a, H10, AATGAAATAGCAATACATACATAAAG
e2t9b, A11, CAAACGTAGAAATAGACTTACTTACC
e3t10e, B11, GAAGCCCTTTTAAAGAAAGTAAGCA
e3t12a, C11, AGCCCCAAAACATGCGCGAGAGGGT
e3t12b, D11, GCTGATAAATTAAGGAAGATTGTATA
e3t14a, E11, ATATTTTAAATGCGAGCAAGAATTA
e3t14b, F11, AATCATACAGGCAAACTGCTGAGTAA
e3t16a, G11, ATTTGCGAAATGGTTAGAGCTTAAT
e3t16b, H11, TTTTGGGATGGCTCAATTAACCTGTT
e3t18a, A12, TATCGCTTTTAAATTAATCCCTCTC
e3t18b, B12, TCATAAATATTATTCAGAGCTTCAAA
e3t20e, C12, GACGATAAAACCAAAATAGCGAGAG
e3t22a, D12, AAGAAACAAATATAATACAGTAAC
e3t22b, E12, TTAACGTGAGATGATATACATTTAA
e3t24a, F12, GATTGTTTGGATTATTAATATCTTTT
e3t24b, G12, ATTGACACTGATACCTTCTGAATA
e3t26a, H12, AGGAAGGTTATCTCTAGTATTAAACAC

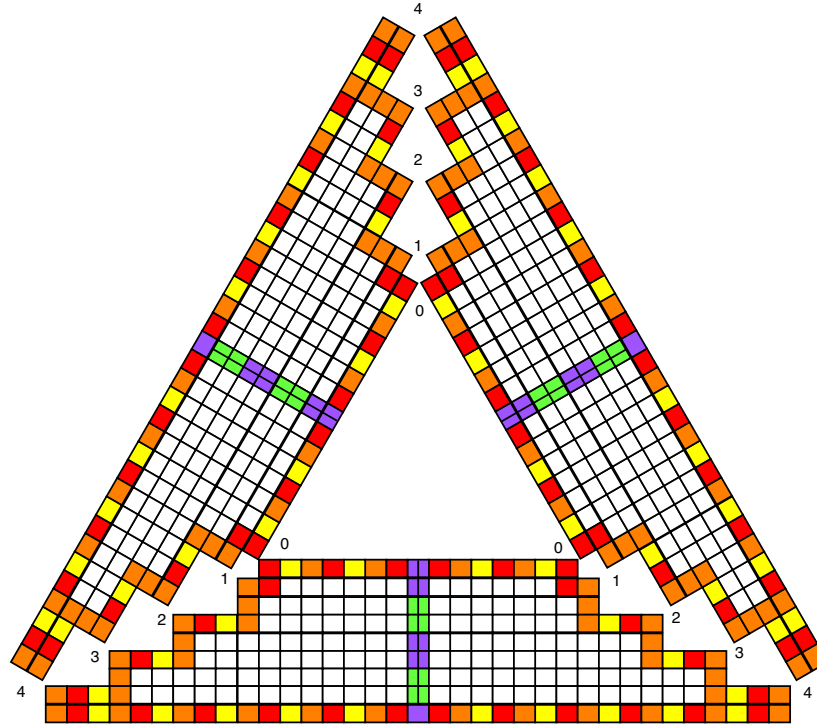
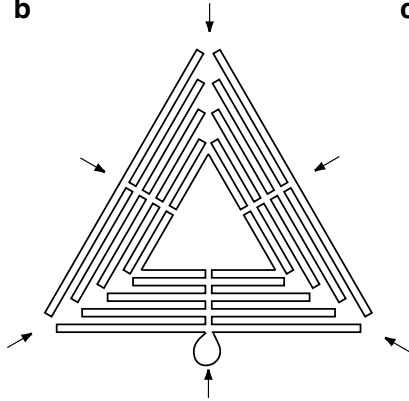
Plate number: 2

e3t26b, A1, AGAGGTGAGCGGGAATATCTTTAG
e3t28a, B1, ACAGACAATATTAAATNCTCAATT
e3t28b, C1, AAAAGCTCATGTTGTAAGTGCTATT
e3t2a, D1, ATCACGCTACTCACGGAAGCTTATT
e3t2b, E1, CCCCTGCTATTTTGGAGTTTGTAGTAC
e3t30e, F1, TTCTTTGATTAGTAATAACATCACTT
e3t4a, G1, CAGTAGGCTCATAGCCGCCACGAGCA
e3t4b, H1, CCACACCTCCAGACATGGCTTTTGA
e3t6a, A2, TATTAGCGTTTCGACAGCAATATACCA
e3t6b, B2, CAAAATCACAGTCATCTTTTCAATA
e3t8a, C2, ACATTAACAGCATGCCAGTATGTTAG
e3t8b, D2, AGACTCTCTTATTATAGGGAGGGAAG
e4t11e, E2, AGCAAAATTTAAATTTGTAACGTTA
e4t13a, F2, TGTGTAGTAAAGTCAACCGTCTTA
e4t13b, G2, CATCAATATGATAATTTCAAAGGGTG
e4t15a, H2, TAGCTATATTTTCTTAACATCCAATA
e4t15b, A3, AATAGTAGTAGCAATTTGGGGCGGA
e4t17a, B3, GCGAAGCAGACCCGATTAAGAGGTCAT
e4t17b, C3, AATTGCTCTTTTGAAGCAACATCCA
e4t19a, D3, GCTTTTGCAAAAGTACTGCGGAATG
e4t19e, E3, GGATAGCGTCCAAAAGTTTGGCAGAGGGGGTAAATAGTA
e4t1e, F3, CGCCACCTCTCAGAACCGCCACCTCA
e4t21e, G3, CAATTTTCAATTTGAATTTCTTTTAA
e4t23a, H3, ATGGAAGGGTGTAGATGTTTTCAGGT
e4t23b, A4, TAAAGAAATTTGCGAACCTACCATATC
e4t25a, B4, GAGCACTAACACGACCTTTACAAAACA
e4t25b, C4, TTTAGAGTATTATAATAGATTAGAG
e4t27a, D4, AGTCTTTAATGCCGAGAGATAAAAC
e4t27b, E4, AAGCAACACCCAGGACCTCATGATGCC
e4t29a, F4, GCTCTGAGTAGAAGCATTGCAACAGGA
e4t29e, G4, TATTACCGCCAGCACTCAAACTCTCGCCCTTGTCTGGTA
e4t3a, H4, TGATACAGGAGTGAACAGCTTAATGCG
e4t3b, A5, ACAGTGCCTGTTACTTGGTAAATAAG
e4t5a, B5, TCAAAATCACCGGGCCACCTCTCAGAG
e4t5b, C5, CACCTCTCAGAACCAACGAGGACCC
e4t7a, D5, GTAAATATTGACGGAATTAGAGCGAG
e4t7b, E5, TTGAGCCTTTGGGAATTTATTCATT
e4t9a, F5, GATAGCCGAACAACTGGCATGATTA
e4t9e, G5, AATACCCCAAAAAGAGTTTACAGAGGAAACCGGAGAAAC
e5t12a, H5, ATATTTTGTAAAAAGTCAAACTCAC
e5t14a, A6, AGAAAGCGCGAGCATCAATCTTACT
e5t16a, B6, GCTGAAAAGGTGAGAGAGTTAGCTTT
e5t18a, C6, ACAGGTGAGATTAAGATTGTAGACT
e5t22a, D6, ATGGAACAGTACCGTAAAAAGCAAAA
e5t24a, E6, AAAATTTTGTCAATATGATTTGAGGA
e5t26a, F6, CCGTCAATAGATAATTTAAATATCCG
e5t28a, G6, CTAACCACTGAGATCTCCAGAACACA
e5t2a, H6, GAACGCCCACTCTAGTGGCTGAGTA
e5t4a, A7, TTTTAAAGGCTGCTCTCAGAGCGCG
e5t6a, B7, ACCGGAACCGCTCTCAGCTCAGCAGC
e5t8a, C7, AAAGGTGAATTTATGCAATTAATACGG
e1t10e, D7, TCAGAGGGTAAATGAGCGCTAATATC
e1t12a, E7, CGCTGTAGCATTCAGATCTGATTA
e1t12b, F7, TTGTGCTCTTTCCCAACAGACCGCT
e1t14a, G7, TTAACACGCTTGAATTTTTCGCTGCT
e1t14b, H7, AGCATTAACCGATTACCGATAGTTGG
e1t16a, A8, AAAGAGGCAAAAGTAAGTACACAGG
e1t16b, B8, ACCAAGCGGCAAAAATAGCATTAAC
e1t18a, C8, GACAAGAACCGGATGCTCTCAGAGGA
e1t18b, D8, AGTGAATAGGCTTATTCATTACCA
e1t20e, E8, TTGAGATTTAGAATACCAATTTCA
e1t22a, F8, TTTTCAATCAATTTTGAACGTAATGG
e1t22b, G8, AACAAACGGCGGAAATTTTTCGCTG
e1t24a, H8, TCGGTGCGGCGCTTTGGGTAAAGCGA
e1t24b, A9, CAGGCGGATTAGCTTTCGATTTACG
e1t26a, B9, GTAAAGCCTGGGTTTCCAGTCGGGAA
e1t26b, C9, CTCACCTGCGCTTGGCTTAATGAGTG
e1t28a, D9, ATCTGTTTGTATGAGGGTTGAGTGT
e1t28b, E9, AATAGCCCGAGATGTGGTCCGAAAT
e1t2a, F9, GCTTAGATTAGACCTTTTAACTCT
e1t2b, G9, GTCTGAGAGACTACGCTGAGGAAGAT
e1t30e, H9, ACGGGAAGCGCGCGACGCTGGCGGA
e1t4a, A10, CATATAATTAGACACACGCTCAACAG
e1t4b, B10, ACCAGTATAAAGCAAAAGCCTGTTTA
e1t6a, C10, AAGTCTCTGAACACGCGCTGCTTTCC
e1t6b, D10, ACCAATCAATAATGAAATAATATC
e1t8a, E10, TTGAAGCTTTAAACACGCTTAACGAG
e1t8b, F10, TCTGAACTTACTCAAGATTAGTTG
e2t11e, G10, GTTTCGTACCAAGTACAACTACAC
e2t13a, H10, ATCAGTTTGTCTTTGGGATTTTGTCA
e2t13b, A11, TGAATTTTCTGTACAGGTTGAATTC
e2t15a, B11, CACTACGAGGCAAGTTAAAGGCCCG
e2t15b, C11, GAGGCTTGCGAGGCAATAAAGC
e2t17a, D11, GGCTGACCTTCATCGCTGATAAAT
e2t17b, E11, AGATTTGTATCATCAAGATTAATCTT
e2t19a, F11, TTATTACAGGTAGGTAGTAATTTGGG
e2t19b, G11, AACACAGAACGAAAGATTATCATCG
e2t1e, H11, TTAGAATCTTGAACATAGCGGATA
e2t21e, A12, TCGCGTCTGGCTTCTCTGTAGCAGC
e2t23a, B12, GGCTGCGCACTGGGTTGTAGATGGCG
e2t23b, C12, GATAGGTACAGTTTGGGGAAGGCGCA
e2t25a, D12, CAACATACGAGCCACGACGTTGTAA
e2t25b, E12, GGGTTTTCCAGTGGAGCATATAAGT
e2t27a, F12, ACAGTGGTTTGGCCTGCTAATTAAG
e2t27b, G12, ACCTGTGCTGCCACGACGAGCGAA
e2t29a, H12, AAAGGGAGCCCCCAAGAGTCCACT

Plate number: 3

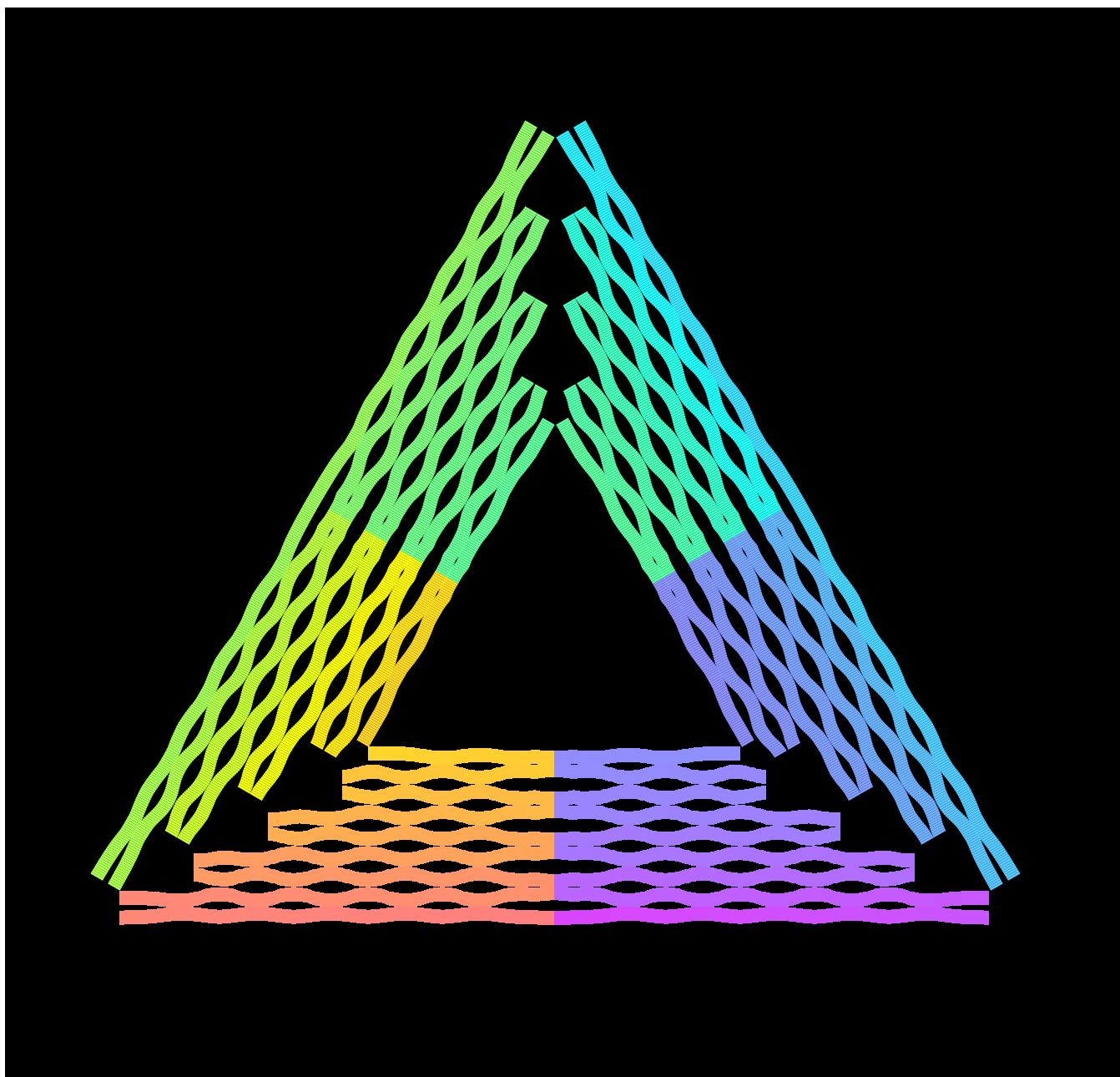
e2t29b, A1, GTTCCAGTTTGGAGATTAGAGCTTG
e2t3a, B1, CGTAAATAAGAAGTTATATAACTAT
e2t3b, C1, CGGCTTAGGTTGTGAACACCGGAAT
e2t5a, D1, AACGCGCTGTTTAAAGATCCGATAT
e2t5b, E1, TAGGCGTTAATTTGATCAACATAGAT
e2t7a, F1, GCGAACCTCCCGAACCGGTTATTA
e2t7b, G1, TTATCATTTCAAGCTTCGGGAGGAT
e2t9a, H1, GAATTAATGAACTCAATTTTGGCAG
e2t9b, A2, CGTCTTTCCAGAGACCTGGAACAAAG
e3t10e, B2, AAACAGGGAAGCGGATTAGCGGGGA
e3t12a, C2, TAGGAACCATGTAGTTTCAGCGGAG
e3t12b, D2, AACAACTTCCACACCGGTAACATGA
e3t14a, E2, AAAGGAGCTTTTACACCTCAGAGC
e3t14b, F2, TTTTGGGATCGATTGATTCGGTTT
e3t16a, G2, CATTAACCGGTACGACTGCTTCAT
e3t16b, H2, GTGTGGAATCCGAATACGTAATG
e3t18a, A3, CGGTGTAGCAGCAATTTCACTCTTTA
e3t18b, B3, CTGAGATGGTTTAGGCGCATAGGCT
e3t20e, C3, AATTAACAGACTTAACGGAACA
e3t22a, D3, CCAATAGAACCGGATCTGCCAGTT
e3t22b, E3, CATGTAACCGCTCATCAAAATAAT
e3t24a, F3, CAGGCAAGCGCGGCAAGCTTGTAT
e3t24b, G3, AACGAGCGGCGGATCTCGGCATTCA
e3t26a, H3, AATTGTTACCGCGGAGAGGCGGG
e3t26b, A4, ATTGGTCAACCGGTCAACATTTCCACA
e3t28a, B4, CCGTGAAGGTTGAGCTCCACGCTCA
e3t28b, C4, ATTAAGAACGTCGACGACAGCGGCTC
e3t2a, D4, AATCGTCGCTATTGCAATCCAAT
e3t2b, E4, ATGAGTCTGCAAAATGATTTTTC
e3t30e, F4, GCGGTAAGACCTAATCGGAACCTC
e3t4a, G4, AAATACAGCGCTCATGAATTTTAGG
e3t4b, H4, TTAACACCGCAAGTGAATAAAGG
e3t6a, A5, ATAAACACATGTTTCTGAGCAAGT
e3t6b, B5, CCAAGTACCGCATCGCTAATGAG
e3t8a, C5, TATTCTTAAGCAAGCATATATTATT
e3t8b, D5, TTAACAAATTAACCGGAGGCGTTTTA
e4t11e, E5, CTCATTTTAGGATAGCAAGCCCA
e4t13a, F5, TGAAATCTCCAAAGAACCACTAAAGG
e4t13b, G5, TAGGAATAGCAAGTAAAGGCGCTCA
e4t15a, H5, TAAAGACTTTTTCGGAAACGAGGGA
e4t15b, A6, TAGGAATAGCAAGTAAAGGAGTTTC
e4t17a, B6, ACCAATCTTGAAGAACGAGGCGAGA
e4t17b, C6, GTACTTACGCGGAGAGGAGTGA
e4t19b, D6, ATCAATTTGGAATGAAATATCTAGCT
e4t1e, E6, TGTGAGTGAATTAACCTTGTCTTGTGA
e4t21e, F6, TTTTGTAAATCAGCTCAATTTTAA
e4t23a, G6, TCCGCGACCGCTTACAGATTCTCGGCT
e4t23b, H6, TGAGGAGACAGCTGCTGGCGGGA
e4t25a, A7, CATGGTCAAGCTCTCTAGAGAGTCC
e4t25b, B7, GCCTGCAAGTGGATTCTCTGTGTGA
e4t27a, C7, CAGCTGATTGCGCCGAGGAGTGGT
e4t27b, D7, TTTGCGATTGGGTTACCGCGGAGT
e4t29b, E7, AAGGGAACAACTTGGGCTCGGCT
e4t3a, F7, TCTTCTGACCTACGCGGAGAAATCT
e4t3b, G7, GCAAGCAAGAAATTTAATGGTTTG
e4t5a, H7, AAGATTAATCTGTGAGGCAAGTAA
e4t5b, A8, CAGAGGCTTTTCCGACAGACATCA
e4t7a, B8, CAAATCAGATATAATTTTCATCGTGA
e4t7b, C8, GCAAGCTGTTTGAAGGCTTCTCGC
e4t9b, D8, ATCCCAATCCAAAGAGCAATAACATTA
e5t10e, E8, AAAATGAAATAGAGCCTTTACAGTAAGAAACGATT
e5t12b, F8, AATTGCGAATATCAGGACGACCC
e5t14b, G8, GCAAGCTACAGAAATTTTTCAGCT
e5t16b, H8, CGGTCAATCAAAAGGCTTTGAGGAC
e5t18a, A9, TTAAGACTGGCTGGGACCGCACTG
e5t20e, B9, CATTAACAGTACGAGCGTGGGAAACCTTATGCGATT
e5t22b, C9, CAGGAAGATCGCAATTCGATTAAT
e5t24b, D9, CGGCTACCGAGCCTCCAGCAGCTT
e5t26b, E9, TTTCTTTTACCATCGAATTCGTAAT
e5t28b, F9, GATGGCCCATACGCTGAGACGGGCA
e5t2b, G9, TTTCAATATATTAATTAATCAATATA
e5t30e, H9, GTGAACCATCACCCAAATCAAGTTTTCGTCTATCAGGGC
e5t4b, A10, GAGAAATTAAGTTTAGTTAAATTTCA
e5t6b, B10, GAATCAATTACCGCAGCAGAAAGGT
e5t8b, C10, TTTGTTTAACTGCGCCCAATAGCAAG
et-rem1, D10, ACGCCAGAACTCCTGAGAAGTGTTTT
et-rem2, E10, TTAAGGGAATTTTAGACAGGAACGCT
et-rem3, F10, AGAGCGGAGCTTACACAGGAGCGGCA
et-rem4, G10, TATAAGCTGTTTCTCTGTAGAAATC
et-rem5, H10, GTACTATGGTTCTTGTAGCAGCAGC
et-rem6, A11, CGCCTTAATGCGCCGCTACAGGCGCG

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,	TTAATTGTATTTCACCAGAGCC
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,	CGGTGCGGCCTTCCAAAAACATT
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
t1s18h, D1, AATACTGCGGAATCGTAGGGGTAATAGTAAATGTTTAGACT
t1s28h, E1, TCTTTGATTAGTAATAGTCTGTCTCATCAGCAAAATAACGGTT
t1s8h, F1, CAGAAGAAACCGAGGTTTTTAAAGAAAGTAGCAGATAGCCG
t1s219c, G1, GGATAGCGTCC
t1s29c, H1, GTAGCAATACT
t1s29c, A2, AACAAAGTTAC
t1s10g, B2, GACGGGAGAAATTAACTCGGAATAAGTTTATTTCCAGCGCC
t1s12i, C2, TCATATGTGTAATCGTAAAACTAGTCATTTTC
t1s14i, D2, GTGAGAAAATGTGTAGGTAAAGATACAACCTTT
t1s16i, E2, GGCATCAAAATTTGGGGCGGAGCTAGTTAAAG
t1s18i, F2, TTCGAGCTAAGCATTTCAAAATCTCGGGAACGAG
t1s20g, G2, GAATACCACTTTCAACTTAAGAGGAAGCCCGATCAAAAGCG
t1s22i, H2, TCGGGAGATATACAGTAAACAGTACAATAAATTT
t1s24i, A3, CTTGATTAAAGGAGCGGAATTTATCTCGGCTC
t1s26i, B3, GCAAACTCACTCAATCAATTTCTGCAGTTCGA
t1s28i, C3, CGACCAGTCAATTTGCAGATTTCACTGCTTGC
t1s2i, D3, CGGGTTTCTCTCAAGGAAGATTTTGAATTA
t1s30g, E3, TTGACGAGACGATATCACTGAAATGAGTATTTAAATAAAG
t1s4i, F3, AGCGCTCATGTCTTGAATTTTCCAGCATCTCT
t1s6i, G3, TTCATAATCCCTTATTAGCGTTTTCCTTACCT
t1s8i, H3, ATGGTTTATGTCAACAATCAATAGATATAAAC
t2s11g, A4, AGAAAGCCCAAAAGAGTCTCGAGCAACAATCAACCATT
t2s13g, B4, ACAGTCAAGAGAGATCGATGAACGACCCCGTTGATAATC
t2s15f, C4, ATAGTAGTATCAATGCTCGAGATTCGCGGAG
t2s17f, D4, AACGAGACCTTTAGCTATATTTTCTTCTACTA
t2s19g, E4, GATAAGTGCCTGCGAGTGAACGATTAACAGGATTACAGGAG
t2s21g, F4, CTTGATTGCTTTGAATTTGCGTAGATTTTCAGGCATCAATA
t2s23g, G4, TGGCAATTTTAACTGCTCAAGTAAAGCAATAACGGATTTCG
t2s25f, H4, AAGGAATTTCAAGAAACACCAAGTCAGATGA
t2s27f, A5, GGACATTTCACTCAATTTCAACATCGAGCTTGA
t2s29g, B5, TTTGATGATTAAAGAGCTGAGACTTCTCGTACACGAGCG
t2s31f, C5, CCGGAACCGAAGTGAAGAGCGCAACATCTCA
t2s33g, D5, AAAGACAACATTTTCGCTCATAGCCAAATCA
t2s35g, E5, GTGAGAGGCTAATTTGATGCAACATTAAGAGCGATTGAG
t3s14e, F5, CAATATGACCTCATATATTTTAAAGCATTTAA
t3s16e, G5, CATCCAATAAATGCTCAATTAACCTCGGAAGCA
t3s18g, H5, AACTCCAAGATTGCTCAAAAGATTAATCGAGATACATAA
t3s20g, A6, CGCCAAAAGAAATACAGTCAGAGCAAGCGCAGGTGAG
t3s22e, B6, TAATCTGATTATCATTTTTCGGAAGAGGAGG
t3s24e, C6, TTATCTAAAGCATCACTTCTGATGCGCAAC
t3s26g, D6, AGAGATAGTTTGACCTCAATCTGACGTCTTCTCGTT
t3s28g, E6, AGAATCAGAGCGGAGATGAAATACCTACATAAACCTTC
t3s30g, F6, TGATCTGGAATCTCTTAAAGCAGAGCCAC
t3s32e, G6, CACCGGAAAGCGGTTTTCATCGGAAGGCGA
t3s34g, H6, CATTCACAAGCAAGCAAGCAAGCAACCTTGAACAAA
t4s11g, A7, GCAAAATTTAAATTTAGATTCACAAAGCTACTGATAAA
t4s13g, B7, CGTTCTAGTCAGGTCTTGCCTGACAGGAAGATTGTATAA
t4s15f, C7, CAGGCAAGATCACTTCTGAGGAGGAGG
t4s17f, D7, GATTAGAGATTAGATCATTTTTCGCAATCATTA
t4s19g, E7, TAGCCCGGAATAGGTGAATGCCCTTGTGTGCTAGT
t4s21g, F7, GCGCAGAGCGGAATTAATTTTTCGACGTAAATTTCTGAAT
t4s23g, G7, GATTATACAGAAATAAAGAAATACCAAGTTTACAAAATC
t4s25f, H7, TAGGAGCATAAAAGTTTGAGTAAACATTTGTTTG
t4s27f, A8, TGACCTGACAAATGAGAAATCTAAAATATCTT
t4s3g, B8, TTTAACGTTTCGGAACCTATTATTAGGTTTATGATAAAGTA
t4s5f, C8, CTCAGAGCATATTTCACAAACCAAGTAATTAAGT
t4s7f, D8, GGAGGGAATTTAGCGTCAGACTTGTCTCGCTTCA
t5s10g, E8, GATAACCCACAAGATTTGATGCAACATGAAATAATTTTC
t5s14e, F8, TTAATGCTCTTATTTCACGCGAGGCAAGAAAG
t5s16e, G8, TTAGCAAAATAGATTTCATGAGGCACTCTT
t5s18g, H8, TAATTTGCTTTAACCCTGACTATTATGAGGCTAGTAAAGAGC
t5s20g, A9, AACACTATCATAAACCATCAAAATCAGGTCTCCTTTTGA
t5s24e, B9, AATGGAAGCGAAGTTTAAATTTTCAACCAAC
t5s26e, C9, TAATAGATCGCTGAGAGCCAGCAGAAAGCGTAA
t5s28g, D9, GAATACGTAAACGAAAAAGCTCTCAACAGGAGGCGGA
t5s30g, E9, TTAAGGGATTTTAGATACCGCCAGCATTTCGCGCACAGA
t5s4e, F9, CTTTGAGTCAGAGATTGGCTTTGCGCCACCC
t5s6e, G9, TCAGAACCCAGAAATCAAGTTTGGCGTAAATA
t5s8g, H9, TTGACGGAATACATACATAAAGGGCGCTAATATCAGAGA
t6s13c, A10, CTATTTTGTAG
t6s15g, B10, ATAAAGCCTTTTCGCGGAGAGCGCTGAGAGGGGTAG
t6s17f, C10, TAAGAGGTCAATTTTCGGAACGAGATTAAAGCA
t6s23c, D10, ACCATATCAAA
t6s25g, E10, TCAATAGATATTAAATCCTTTGCCGGTTAGAACCT
t6s27f, F10, CAATATTTGCTGTCAACAGTGCATAGAGCGG
t6s3c, G10, TATAAACAGTT
t6s5g, H10, CAGAGCCGAGAGGTTGAGGCGAGTAAAGCTGCCG
t6s7f, A11, ATTAAGGCGGTAATCAGTAGCGAGGCCCTCT
t7s10g, B11, ATAAAGCAAGAAACATGGCATGATTAAAGACTCCGACTTG
t7s14e, C11, ATGACCTGTATAATCTTCAGAGCA
t7s16e, D11, TAAAGCTATATAACAGTTGATTCCCATTTTTT
t7s18g, E11, CGGATGGCAGGAAATGACCATTAATGTTTACAGAGCAC
t7s20g, F11, GATAAAAAACAAAATATTAAACAGTTTCAGAAATTAGAGCT
t7s24e, G11, ACAATTCGACCACTGTAATCAT
t7s26e, H11, TTGAGGATGCTCAGTATTAAACCTTTGAATGG
t7s28g, A12, CTATTAGTATATTCGAGACAAATTCAGGAACGTAAGCCA
t7s30g, B12, GAATCTCTGAGAGGTGATCGGCTTGTGCTGACTTTAAATG
t7s4e, C12, GCGCGCAGATTGACACACCTTC
t7s6e, D12, AGAGCCGACCATCGATAGCAGCATGAATTAAT
t7s8g, E12, CACCGTCACCTTATTAGCAGTATTGATTAAAGCCCAATA
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, ACTAAAGTACGGGTGCGAATATAA
t9s18g, F1, TGCTGTAGATGCCCCCTCAATGCTGCGAGAGGCTTTTGCA
t9s20h, G1, AAAGAAGTTTGGCAGCATAAATATTCAATTGACTCAACATGTT
t9s26e, H1, ACCACAGCAGAAAGTATGATGCC
t9s28g, A2, TAAACATTAGAGAACCTCAACCTTTTATAATCAGTGAG
t9s30h, B2, GCCACGAGTAAAGAACATCACTTGCTGAGCGCCATAAAA
t9s6e, C2, CCATTGACAGGCGGGGGGAATTA
t9s8g, D2, GAGCCAGCGAATACCCAAAAAGCAATGAAATAGCAATAGC
t10s17h, E2, ACCAACCTAAAAAATCAACGTAAACAAATAAATTTGGGCTTGAGA
t10s27h, F2, AACTCAACATTATTAGTGTGTTTCCAGAAACCGCTTATCAGGG
t10s7h, G2, AGCACAATAATCCCGACTTCCGGGAGATCTCGAATCTTACCA
t11s18e, H2, ATAAGGCTTTC
t11s28e, A3, ACTATTAAAGA
t11s8e, B3, TCAAGATTAGT
t12s19h, C3, CCGCAGAGAAACACCAAGCAGTAGGCTGCTCATTCACTGTA
t12s29h, D3, AGCTGAGCTCAACGCTCAAGGGGCAATTTGGAACAGAGTCC
t12s9h, E3, TGCTATTTTGACCCAGCTCAATTTTGTGTTTGAAGCCTTAA
t1s10e, F3, AGAGAATAACATAAAAAAGGAGGAAGCGCATTA
t1s12i, G3, AGGGATAGCTACAGGCCACCAACCCCATGTCAA
t1s14e, H3, ATTTTCTGTCAGCGAGGTGAGAAATACCGATAT
t1s14i, A4, CAACAGTTTATGGGATTTTGTCTAATCAAAAGG
t1s16e, B4, ATTCGCTTTCGCGGATGCTCACCCGAAATTCG
t1s16i, C4, CGCGCTTTGCTGAGGCTTCAGGGGAAAGGT
t1s18g, D4, CGACCTCGGCTCAATCATAAAGGAACGGAACCAACTATT
t1s18i, E4, CGCGAGACTTCATGTTACTTATGCGGCTTTAA
t1s20e, F4, ACAGTGAAGAAAGATTCACTCAGTTGAGATTAG
t1s22i, G4, CGCGTCTGATAGGAACGAGTCAACCTTTTCA
t1s24e, H4, CAGTTTGAAGCAGCTCCAGCGAGCTAAACGAGC
t1s24i, A5, AGGAAGATGGGAGCAGCAGCATGATCATATT
t1s26e, B5, GCGACGTGCATCCCGGGTACCAGGTTTCTTCT
t1s26i, C5, CTCTAGAGCAACCTGTCGATCGTGGTCAGTTG
t1s28g, D5, TTTCCAGCAGCTGGCCCTGAGAGAAAGCGCGCAAGCTGG
t1s28i, E5, CTTCCAGCTGAGAGCGGCGCAACAGCATGACCA
t1s21f, F5, CTTTTCATTTTAAACAAATTTTCATGAGATTAG
t1s30e, G5, CGAGAAAGGAAGGAGGCGCTATGTTGTTCT
t1s4e, H5, TTATCAACCGGCTTAGTGTGGTAAAGCTGT
t1s4i, A6, TTTAACTCATGATGCTCGAGAGTTCGAGATTCCAGTA
t1s6e, B6, TTAGATTCGCAACGCTCAACAGTTCGCTGTC
t1s6i, C6, AGTATAAAATATGCTGTATACAAAGCATCTT
t1s8g, D6, TTTCTTACGATCATTCGAGAAACATAGCAGCTTTACAG
t1s8i, E6, CAACTACCTATTCCAAGACGAGAAATTCAT
t2s11g, F6, CTTCAAGACCGCCCAAGGCCCAATAGGAACGTAAATGA
t2s13g, G6, AGACGTTCACATGATACCGTTCAACCCCTCAGAACCGCCCA
t2s15f, H6, CAGCATCAAGAAAGAACCACTAAGCTTTTCC
t2s17f, A7, ATTTGTTCTCAGACGCGAAGACGAGTCACTCGCC
t2s19g, B7, AAAACAAAATTAATTAATGAGTACGATCACTTTAGTGAAT
t2s21g, C7, CTTCAATTTTAAACCGCTTCTGTCAGGAGGATCTGCTC
t2s23g, D7, GTAAACGCTCTTTCATCAACATTTAAATTTTGTATAATCA
t2s25f, E7, AGCTTGTATTCGCGGACCGCTTCTCGGCGCTG
t2s27f, F7, CCAGGGTGGCTCGAATTCGATTCGAGTCAAG
t2s3g, G7, AGAGTCAAAAATCAATATATGTTGATGAAAACCAATCAAG
t2s5f, H7, ACTAGAAATATATACTATATGACGCTGAGA
t2s7f, A8, TCAATTAATAGGCTTAAATGGAACATATAATT
t3s10g, B8, AACGTCAAAAATGAAAGCAAGCGCTTTTATGAAACCAA
t3s14e, C8, GTTTTGTGAGGAATTCGCAATAACCGAAT
t3s16e, D8, GACAACAAGCATCGGAACGAGGCTGAGATTG
t3s18g, E8, TATCATGTTGAAAGGAGCAGATGGAAGAAAATCTACG
t3s20g, F8, TTAATAAAAGCAATTAACCGAATCGCAACCTCTGATATA
t3s24e, G8, GTGATAGTGGGTGCGGAAACCGAGGAACGCGAG
t3s26e, H8, GTTTTTCATGCTGATAGCTGTTTGAAGGGG
t3s28g, A9, GTTTGCTCAGCTGCTGTTTTCGCGGAGGAGCGCCGATT
t3s30g, B9, TAGAGCTTGAAGGGAGTTCGAGCAAGCGGTCTATGGGCG
t3s4e, C9, GATTAAAGAAATGCTGATGCAAAATCAGAAATAA
t3s6e, D9, CACCGGAATCGCATATTTCACAAAATTTACG
t3s8g, E9, AGCATGTATTTCATGTTAGGAATCAAAAGATTTTTGTGTT
t4s11g, F9, AGGTTTATGATCGGCTAGGTTTCGTCACCGAGGATCAAA
t4s13g, G9, AGCGTAACTACAAATCAACAGCGCTATCAACGCTCTGAGG
t4s15f, H9, TAGTTGCGAAATTTTTTCACTGTTGATCATAGTT
t4s17f, A10, GTACACGAGCAACCGCTACAGAGGATACCGA
t4s19g, B10, GAGCAAAAGAGATGAGTGAATAACCTTGCTTATAGTCTA
t4s21g, C10, GTTAAAAATTCGATTAATGTTGAGCGAGTAAACACAGTTTG
t4s23g, D10, GGATAGGTACCGCTCGAATTTCTCTAAAAGCTTAATATTT
t4s25f, E10, AGTTGGGTCAGAGCGCAATTCGCGCGTAATG
t4s27f, F10, CGCGCGGGCTGTGTGAAATTTGTTGGGATTA
t4s3g, G10, ACATAGCGCTGTAATGCTGCTGCTATTCAATTAACCT
t4s5f, H10, GTTAAATCAATCGCAAGCAAGGCTTGAAG
t4s7f, A11, CCGATCTCGCCAAATGTAAATTTAAATAGGCG
t5s10g, B11, TCCCAATCCAAATAAGATTACGCGCCCAATAAATAATAT
t5s12e, C11, TGTAGCATTTCC
t5s16e, D11, AACAGCTTGTCTTGTAGAGACTAAAGGATTATA
t5s18g, E11, CCAAGCGCAGGCGCATAGGCTGGCAGAACTGGCTCAATTAT
t5s20g, F11, ACCAGCTCAGGAGTGTGGAACGCGTACAGACCGGAACAA
t5s22e, G11, GTGGGAACAA
t5s26e, H11, TGCTGCAATTCGCTCACAATTTCCAGCTGCA
t5s28g, A12, TTAATGAAGTTTGTAGTGGTTCGAGGTCGCGTAAAGCA
t5s2e, B12, TTAATTAATTT
t5s30g, C12, CTAATTCGGAACCTTAAGCAGGCAAAATCTTCCGCGCA
t5s6e, D12, GTGTGATAGGCGAGAGGATTTTCAGTCTGTA
t5s8g, E12, ACAAGAGGAGCAAGCAATCAGATAACGCGCTATTATTTA
t6s13f, F12, ACAGCAGCGCAATCTTCAAAAAAATTTCTTTA
t6s15c, G12, CGAGGTGAGGCTTCAAGAGGAGCC
t6s17f, H12, ACCCCAGAGCTTTTTCATGAGGAACCTGCTTT

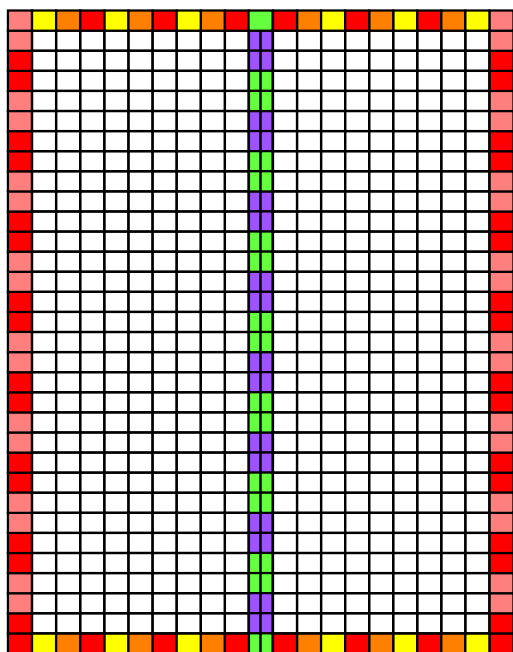
Plate number: 3
t6s23f, A1, CGGCGGATTGAATTGAGGCTGCGCAACGGGGGAGTG
t6s25c, B1, TGGCGAAATGTTGGGAAGGCGAT
t6s27f, C1, TGCTGTGACACACATACGAGCCAGCGCCAGC
t6s3f, D1, TGCTGTGAAATAACCGGAGAAATTTTACCGAAC
t6s5c, E1, GTTTGAAATTTCAAAATATATTTTGG
t6s7f, F1, AATAGATAGGCGCAGTAATAAGAGATTAAATG
t6s10g, G1, CGCAGTTACAAAATAATAGAGGCTTATCCGGTTTACAA
t6s14e, H1, TTTAATTTGAT
t6s18g, A2, AAAACACTTAATCTTGACAAAGCAATTAATCATTTGTGAATT
t6s20g, B2, ACCTTATGCGATTTTATGACCTTCATCAAGAGCATCTTTG
t6s24e, C2, CGGTGCGGGCC
t6s28g, D2, TTCCAGTCTTTATAAATCAAAAGAGAACCATCACCAAAAT
t6s30g, E2, CAAGTTTTTGGGGTGAAATCGGCAAAATCCGGGAACCC
t6s4e, F2, TTAATTTTCATC
t6s8g, G2, CGCGCTGTTATTTCAAGAACGCGATTCCAGAGCCTAATTT
t6s8g, G2, CGCGCTGTTATTTCTAAGAACGCGATTCCAGAGCCTAATTT
t6s15f, H2, CGGTTTATCAGGTTTCCATTAACCGGGAATACACT
t6s17c, A3, GCGTATGCTTAAATAGTAAATGCTG
t6s25f, B3, TCTTGCATTTTGAAGCAATAGGTTGATGCCGCT
t6s27c, C3, GCGCTCAAGAGCTTGGGGTGCTA
t6s5f, D3, TTTGACCTTAAATATAAAGTACCGGACTCGAGAAC
t6s7c, E3, CAGCTTAAAGAGGTAAGTGAATTT
t6s10g, F3, AGCTTAAAGAGGCTTGGCGTTTTCAGCAACCAACATGT
t6s16g, G3, ACTCAAGAGC
t6s20g, H3, TGTTTAAATTTCAACTCGGATATTCAATACCCAGAAAGA
t6s26e, A4, ATGAGTGTAGCT
t6s30g, B4, CGATGCGCCACTACGTATAGCCCGAGATGGGATTGCGTT
t6s4e, C4, CTGTCAGACG
ts-rem1, D4, GCGCTTAATGCGCGCTACAGGGC

Bridges between trapezoids
t5s2e-t6s23c-3T, A6, TTAATTAATTTTTTACCATATCAAA
t7s4e-t8s25c-2T, B6, TTAATTTTCATCTTACCATTTTACAA
t9s6e-t10s27c-1T, C6, CTGTCAGACGTATACGGAACGA
t11s8e-t12s29c-0T, D6, TCAAGATTAGTGTAGCAATACT

t5s12e-t6s3c-3T, E6, TGTAGCATTCCTTTTTATAAACAGTT
t7s14e-t8s5c-2T, F6, TTTAATTTGTATTTTCACACAGAGCC
t9s16e-t10s7c-1T, G6, ACTACGAAGGCTTAGCACCATTA
t11s18e-t12s9c-0T, H6, ATAGGCTTGCACAAAGTTTAC

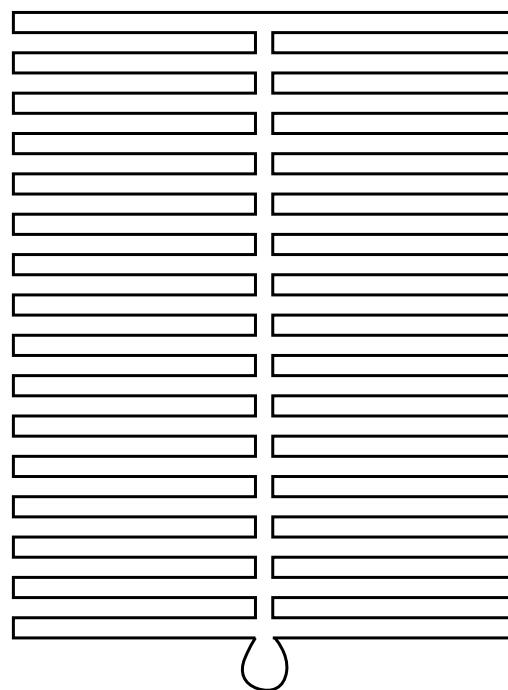
t5s22e-t6s13c-3T, A7, GTGGGAACAAATTTCTATTTTGTAG
t7s24e-t8s15c-2T, B7, CGGTGCGGCGCTTCCAAAAAATTT
t9s26e-t10s17c-1T, C7, ATGAGTGTAGCTTTTAAATATGCA
t11s28e-t12s19c-0T, D7, ACTAATTAAGAGGATAGCGTCTC

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

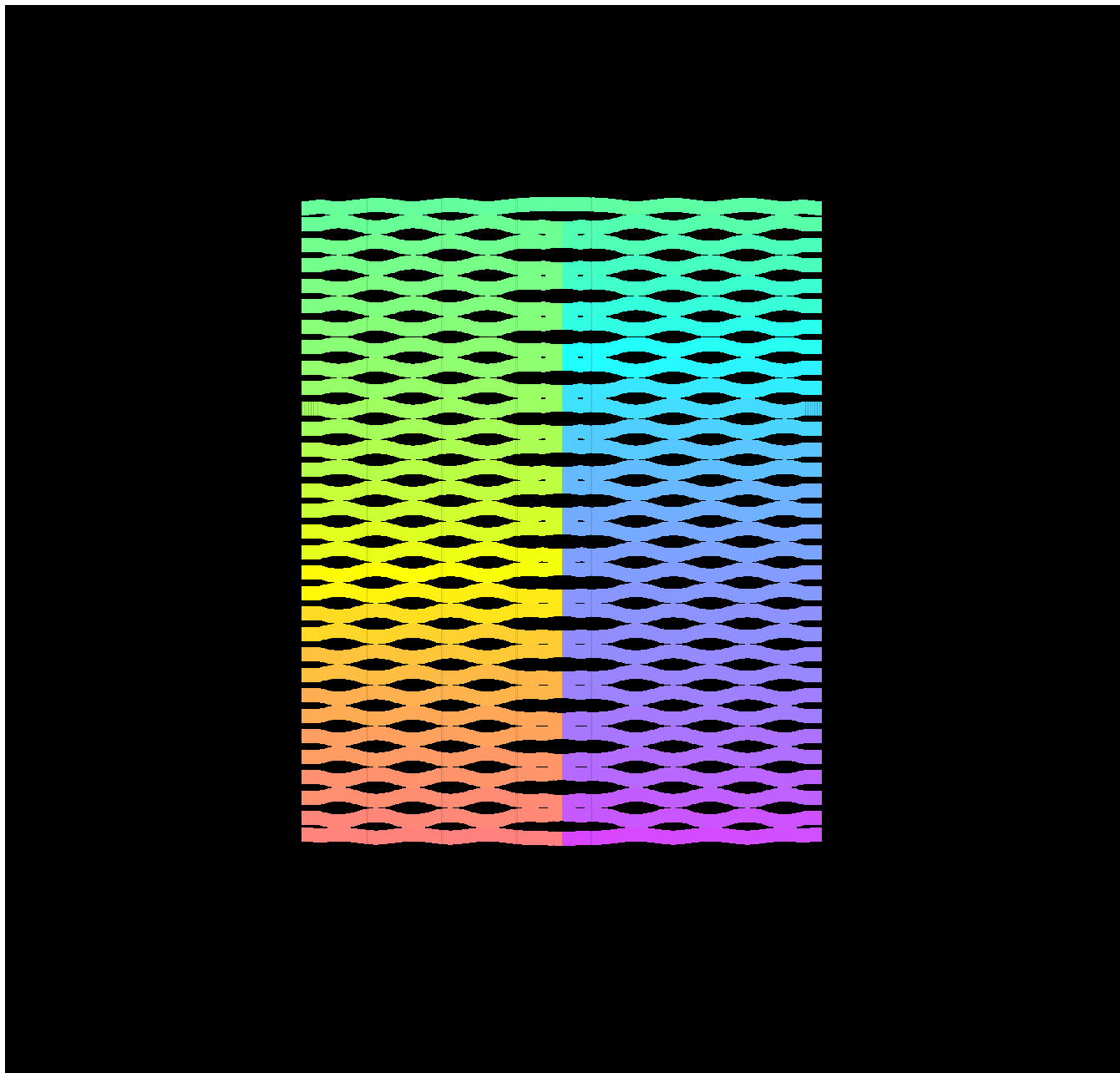
a

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

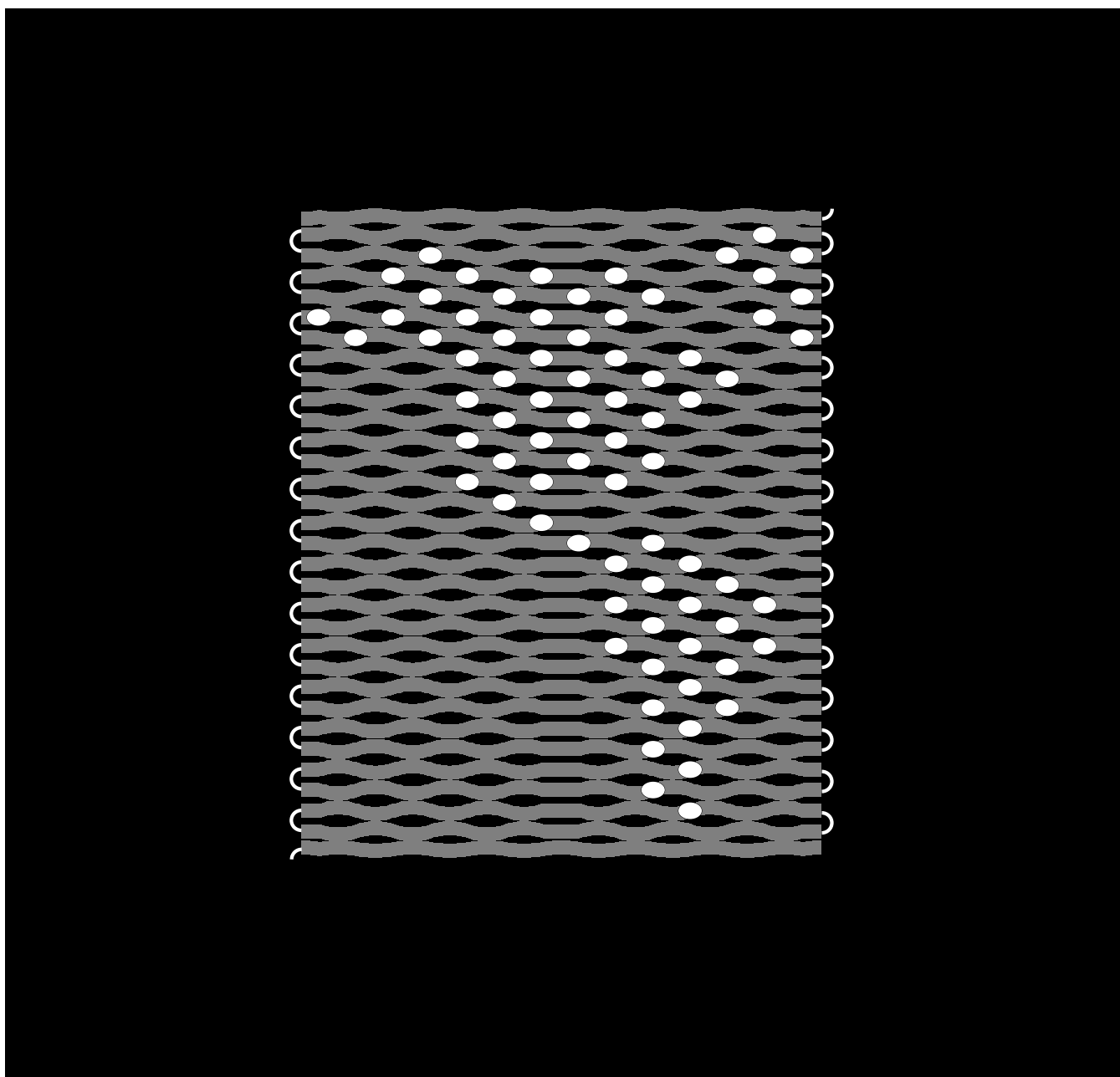
32 helices tall

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, AGGGTGTGATATAAGTATAGCCCGGAATAGGTG
t1r10e, B1, TGAACAAAGATAACCCCAAGAAATAAGACTCC
t1r10f, C1, ATCAGAGAGTCAGAGGGTAAATTAACACAGTCA
t1r12e, D1, TATTTTGCACCGTAAACGAGCGCTCTGAACACCC
t1r12f, E1, TCTTACCACCCAGCTACAAATTTAAAGAAAT
t1r14e, F1, ATCGGCTGACCAAGTACCGCACTTTAGTTGTC
t1r14f, G1, GGTATTAATCTTTCTTATCATTCATATCGCG
t1r16e, H1, CATATTAATTTTCTGAGCCAGTAATAAATCAATA
t1r16f, A2, AGAGGCATACACGCCCAACATGTATCTGCGAA
t1r18e, B2, ACAAGAAAAATTTCACTCTCTGACAGAAATCGC
t1r18f, C2, TTTTAGTTTCGCGAGAAACCTTTTTTATGACC
t1r20e, D2, AAATCAATCGTCGCTATTAATTAATTAATGCAAG
t1r20f, E2, CTGTAATATATGTGAGTGAATAAAAGGCTA
t1r22e, F2, TTTAACGTTTCGGGAGAAACAATAACAGTACAT
t1r22f, G2, CTTTACACAGATGAATATACAGTGCACATA
t1r24e, H2, TTATTAATGAACAAAGAACACCTTTTTCAGG
t1r24f, A3, ATTTTTCGCTTTAAAGTTTGTAGTACCGGACCC
t1r26e, B3, CTAAGCAAAATCAATATCTGGTACCCGAAAGC
t1r26f, C3, AAACCTCTTACACCTTGCTGAACCTAGAGATC
t1r28e, D3, GCCAACAGATAGTGGCAGACAAATGAACAAAT
t1r28f, E3, GCGTAAGAAGATAGAACCTCTTGAAGCGCGC
t1r2e, F3, TAAGCGTCGGTAATAAGTTTAAACGCGTCAG
t1r2f, G3, AGTGCTACTATACATGCGCTTTTGATCTTTCCAG
t1r30e, H3, GTTGATGCGCTGAGTAGAGAACTACATCTCTG
t1r30f, A4, ATCACTTGAATACTCTTTGATAGTTGTCTTC
t1r32h, B4, TACAGGCGCGTATGTGTGTGCTATTAATTAACC
t1r4e, C4, AACCAGAGACCCCTCAGAACCGCCACGTTCCAG
t1r4f, D4, GAGCCGCCCCACCAACGAGACCGTGCGCCGA
t1r6e, E4, GACTTTGAGGTAGCACCATTACCATATACACGG
t1r6f, F4, AATCACCAACATTTGGGAATTTAGACCAACCTA
t1r8e, G4, TTATTACGTTAAAGGTGGCAACATACGCTACCC
t1r8f, H4, TACATACACAGTATGTGTAGCAAACTGTACAGA
t3r0g, A5, TGCTCAGTACACGCGGATAAGTGGGGGTGAC
t3r10e, B5, GCGCATTAATAAGAGCAAGAAACAATAACGGA
t3r10f, C5, GCCCAATAGACGGGAGAAATTAATTTTCAGAG
t3r12e, D5, AGGTTTTCGCGCACTTACAAAATACAGGGAA
t3r12f, E5, CCTAATTTAAGCCTTAATCAAGAAATCGAGAA
t3r14e, F5, CTAATTTACCGTTTATTTATTTCTGCGGG
t3r14f, G5, CAAGCAAGCAGCATGTAGAAACACAGAGAAATA
t3r16e, H5, ACGCTCAACGACAAAGAGTAAAGTATCCATC
t3r16f, A6, TAAAGTACCAGTAGGGCTTAATTGCTAAATTT
t3r18e, B6, TATGTAAAGAAATACCGACCGTGTAAAGCCA
t3r18f, C6, AATGGTTTGTGCTGATGCAAACTCATTTCCCT
t3r20e, D6, TTGAATTAATGAAACATAGCATTTAAACTA
t3r20f, E6, TAGAATCCCTTTTAAATGAAACGGAATTCG
t3r22e, F6, ACAGAAATCTTTGAATACCAAGTTAATTTTCAT
t3r22f, G6, CCTGATTTGAAAGAAATTCGCTGAGAGAAAGAG
t3r24e, H6, CGACAACCTTCATCATATTTACGTACGTAAJA
t3r24f, A7, CGGAATTACGTATTAATCTTTGTGTTGCCAA
t3r26e, B7, GCCACGCTTTGAAAGAAATTTGAGGAACAATTT
t3r26f, C7, ATCAACAGAGAGCGTATGAGCAAAAATATTTTT
t3r28e, D7, GTCAACAGATTAGTCTTTAATGCGCAACAGT
t3r28f, E7, GAATGGCTACCAAGTAATAAAGAGGCAAACTAT
t3r2e, F7, GGAAAGCGGTAACAGTCCCGCTATTCGGGGTTT
t3r2f, G7, TGCCCTTGACAGTCTCTGAATTTACCCCTCAGA
t3r30e, H7, GTAAAGAGCTGTTGAATATTCAGAAATTCACCA
t3r30f, A8, CGGCTTGTGCTGTCCATCAGCATGTAGCAGAG
t3r32h, B8, CACGTATAACGTGCTTCTCTGTGATGACACCA
t3r4e, C8, GTTTCGCCACTCAGAGCGCCACCGCCAGAAAT
t3r4f, D8, GCCACCACTCTTTTCAATATCAATATGACAGG
t3r6e, E8, TTATTATGTCACCAATGAACCATTAATTAGC
t3r6f, F8, CCGGAACTAAAGGTGAATATCATCAAAAGAA
t3r8e, G8, ATACCCAAACACCCAGGAAATAGTACGGGAA
t3r8f, H8, ACGCAAAAGAAAGTGGCATGTTTGTAGTTAA
t5r0g, A9, CCTCAAGAGAAAGATAGAGATTAAGAAACAGTT
t5r10e, B9, CTTTACAGTATCTTACAGGACCGAGTTACCA
t5r10f, C9, GCAATAGCAGAGAAATACATAAAACAGCCAT
t5r12e, D9, GAGCGCTTTCCCAATCAAAATAGATAGCAGC
t5r12f, E9, ATTAATTTATAGCGAACCTCCCGCAGTAGGAA
t5r14e, F9, TAAGTCTGCGGCCCAATAGCAGCAAGAACGC
t5r14f, G9, TCAATACCGGAACAGAAAAATAAATTTCTGT
t5r16e, H9, GCGTTATACGACAAATGAACATACAAATAGA
t5r16f, A10, CCAGACGCAAAATCTTACACAGTAGATAAATA
t5r18e, B10, TAACTTCAATAAGAAATAAACCATTCATAT
t5r18f, C10, AGCGCTTAGGCTTAGTTGGGTTAAGCTTAGA
t5r20e, D10, AAAACAAACTGAGAAAGGTCAATATACCTTTT
t5r20f, E10, TTAAGACGATTAATTAATTTTAAACAAAAATC
t5r22e, F10, AACCTACCGCGAAATTAATCTTACATCAAG
t5r22f, G10, GCGCAGAGATATCAAAATTAATTTGATACAGAT
t5r24e, H10, GGAATTAGTTAGTCAATTAATTCACAGGTTAG
t5r24f, A11, GATGCGAAAGATATTAGACTTTACACAGTTAT
t5r26e, B11, AGCGGCTCTCTTTAGGAGCACTAAACATTTGA
t5r26f, C11, CTAATAATAGTATTAACACCGCTCTGACACTGA
t5r28e, D11, GAAATGGAACCACTGCCATTAAGACAGGGTG
t5r28f, E11, TAGCCCTATTAATTAATTTGSCAGCAATATTA
t5r2e, F11, ACAAACTACTGCTATTTTCGAGCACTGAGACT
t5r2f, G11, AATGCCCAATAAATCTCTTAATAAGAACACAC
t5r30e, H11, AGAAGTGTCAATTCGACAGGAAAAATCTGCT
t5r30f, A12, CCGCCAGCTTTTAAATACAGTGAAGAAATCAG
t5r32h, B12, AGCGGAGCTTAAACAGGAGCGGAGAACTCG
t5r4e, C12, TCGGCATTTCCGCGCGCAGCATGATGATATTC
t5r4f, D12, CACCGAGTTTCGCTGATACCCCTCATGATAGC
t5r6e, E12, ATTGAGGGAATCAGTAGCAGCAGACGCTTTTCA
t5r6f, F12, AGCACCTGTAGGGAAGTAAATTTTATTTTGT
t5r8e, G12, GAAGAAAAATAGAAATTCATATTTTCAACCG
t5r8f, H12, TCACAATCCCGAGGAACGCAATAATGAAATA

Plate number: 2

t7r0f, A1, TGAAGATTAAAGAGGCTATTATT
t7r10f, B1, AAAAGTAAACGCTCAAAATGAAAAACGACT
t7r12f, C1, TTTTGTGTTGCTATTCGCGTATTTCAAACTCAGA
t7r14f, D1, TATAGAAAGCAGCGCTGTTTATCAGTTTACGCT
t7r16f, E1, AATGCAGAGAAAAAGCTGTTTAGGGAATCAT
t7r18f, F1, AATTAATCATAGTGTCTGAGAGACGTGAATTT
t7r20f, G1, ATCAAAATGAAGATGATGAACAAAAATTAACCT
t7r22f, H1, GAGCAAAAATCTCTGAATAATGGATGATTTGT
t7r24f, A2, TGGATTATGCGCTCAATAGATTAATCAACTAAT
t7r26f, B2, AGATTAGACCCAGAGAAATAAAAATACCGA
t7r28f, C2, ACGAACCACTACATTTTTCAGCTCACGCTCAT
t7r2f, D2, CTGAAACAGTCAGACGTTTGCGCTCAGGAGGT
t7r30f, E2, GGAAATACAGGAACGCTGACCCATTAAGGGATT
t7r4f, F2, TGAGGACGCGCTCAGACTGTAGCGATCAAGTT
t7r6f, G2, TGCTTTTAAGCAAAAAGGGCAGAGGTTTACC
t7r8f, H2, AGCGCAACGAGATAGCCGAACATTTTAAAG
t1r0g, A3, TATCACCGTACTCAGGAGGTTTAGATAGTAGTAG
t1r10e, B3, GGACGTTGAGAACTTGGCTCATATTATGCGCTAAT
t1r10f, C3, CGATTTTAGGAAAGAAAACTTACGGAATAAAAA
t1r12e, D3, TTTGCGAGCGAGAGGCTTTTGCAATCTGAA
t1r12f, E3, CCAAAATAGGGGGTAATAGTAAAAAAGATTT
t1r14e, F3, TTTTAATTTGCCCAAGGACTTCAACAGAGAGC
t1r14f, G3, AAGAGAACGAGCTTCAAGGCAAGAGTTTCAT
t1r16e, H3, CAGTAGAACAAGTTGATTTCCCAATTTTAGGCG
t1r16f, A4, TCAATATATTAGTTTGAACCATTAAGCATATA
t1r18e, B4, CTGTAATAGGTTGTACCAAAACACAAATATA
t1r18f, C4, GCTAAATCTTTTTCGGGAGAGACCGGAGAG
t1r20e, D4, TCAGGTCAATTTTGTAGAGATCTACCTCTGCT
t1r20f, E4, GGTAGCTATTGCTGAGAGTTCTGGTTAAATCA
t1r22e, F4, AAATAATTTTAAACCAATAGGAACACAGTAC
t1r22f, G4, GCTCAATTTGCGCTCTGCGCTCTTCCGCGCTAG
t1r24e, H4, GCTTCTGGCACTTCAGCCAAACACAAATCAT
t1r24f, A5, GAAGATCTGCGCGGAAACAGGAGTGCACAG
t1r26e, B5, CCGCGGTCTGCGAGTCTGACTTCAAAATAG
t1r26f, C5, CTTGCTGCGGAGCTCGAATTCGTCTGTGCT
t1r28e, D5, GGGAGAGGCATTAAATGAATCGCCACCTGA
t1r28f, E5, GCCAGCTGCGGTTTTCGCTATTGGGAATCA
t1r2e, F5, ACGTTAGTTTCTAAAGTTTGTCTGATACAGG
t1r2f, G5, CGTAACGAAATGAATTTTCTGTAGTGAATTT
t1r30e, H5, AGTTTGGACGAGATAGGTTTGAAGTAAATAC
t1r30f, A6, GAATAGCCACAAGAGTCCACTATTAAAGCGCGC
t1r32h, B6, GAACGTGCGGAGAAAGGAGGAATGCGCGCG
t1r4e, C6, CAATGACAGCTTGATACCGATAGTCTCCCTCA
t1r4f, D6, CTTAAACAAACACCATCGCCACGCGGGTAA
t1r6e, E6, AAACGAAATGCCACTACGAAAGCAGCGACAA
t1r6f, F6, ATACGTAAAGGCAAAAGAAATACACTGACCAA
t1r8e, G6, CCAGGCGCAGGACAGATGAACGGGTGAJAAAA
t1r8f, H6, CTTTGAJAAATAGGCTGCGTGAACCTTTATG
t3r0g, A7, CCCTCAGAACCGCCACCTCAGAJAACACGCGC
t3r10e, B7, ACGAATCTATTATCATCTGTGAATTTTCATCAAG
t3r10f, C7, TTTCACTACGGAACACATATTAAACACTAT
t3r12e, D7, ACTGATATCGTTTACCAGACGACTTAATAJAA
t3r12f, E7, CATAACCCGCGTCAATCATGCGGTATTATAG
t3r14e, F7, GAAGCAAAAAGCGGATTCATCAATGTTTAG
t3r14f, G7, TCAGAAAGCTTCAACAGCTCAGGATTTAAJATA
t3r16e, H7, TCGCAATAAGTACGTTGTCTGGAACGAGACCG
t3r16f, A8, TGCAACTAGGTCAATTAACCTGTTTAGAJATTAG
t3r18e, B8, CAACGCAAGCAATAAAGCTCTAGGATATCACT
t3r18f, C8, CAJAAATTAGGATAAJAAATTTTAGGATATTCA
t3r20e, D8, AGAJAATCAGCTGATAAJAATTAATGCTTTATTT
t3r20f, E8, ACCGTTCTGATGAACGJTAATCTGTAAATTTT
t3r22e, F8, CTTTCACTCTGCAATTAJATTTTGGACAJAJAA
t3r22f, G8, GTTAAAJAATCAATTAJATTTGAGCATCTGCCA
t3r24e, H8, TTTGCGCATGGACGACGAGTATCTGAGCCAG
t3r24f, A9, GTTTGAGGTGAGGCTGCGCAACTGTTTCCCAAGT
t3r26e, B9, TCAATAGCTTGTAAACGACGCGCAAJAGCGCCA
t3r26f, C9, CACGAGCTGTTTCTGTGTGAJATTTGCGGCTC
t3r28e, D9, TGGTTTTTTCTTTCAGTTCGGAJAJAJATCATGG
t3r28f, E9, ACTGCGCGCTTTTACCAGCTGAGATGGTGGTT
t3r2e, F9, TGCTAJAATCTCACAGACAGCTTCTTACCGCCA
t3r2f, G9, TGTAGCATAJAATTTCAACAGTTTCTAJATTGTA
t3r30e, H9, TGGACTCTCGCAAJAATCCCTTATACGCGAGGG
t3r30f, A10, CCGAJAATCAAGCTCAAJAGGGCAAJAGGGGAGC
t3r32h, B10, CCCCAGATTTAGAGCTTGAAGGGGAJAJAJAGC
t3r4e, C10, ATAJATTCTCAGCTTGCTTTGAGTGGGATTT
t3r4f, D10, TCGGTTTAGTCTGCTGAGGCTTGCAAAGACT
t3r6e, E10, CTCATCTTGAAGTTTTCATTAJAJAATCAACCG
t3r6f, F10, TTTCAATGATGACCCCGAGGATTAAGCGCGAG
t3r8e, G10, AGTAATCTTCAAJAGGAGACGAACTAJAJAA
t3r8f, H10, ACGGTCAAJAGCAAGACGGAATAGGTTTAA
t5r0g, A11, CTCAGAGCCACCCCTCATTTTTCGTAJACAC
t5r10e, B11, AAAGATTCTAJAATTTGGGCTTGAJATTCAATC
t5r10f, C11, ACGAGTAGATCAGTTGAGATTTAGCGCCAJAJAA
t5r12e, D11, TAAAJATTGAGGCAJAGTAAAGCACTAGGATG
t5r12f, E11, GAAATTACCAATGAATCCCCCTCACAJAJAAT
t5r14e, F11, TACCTTTAGGTTCTTTACCCCTGAJAACTGCTA
t5r14f, G11, CAJAAATCAATTTGCTCTTTTGAJATTTGCTGA
t5r16e, H11, TTTCAATTTCTGATGCTCAAJATGTTTGAJAGAG
t5r16f, A12, ATAJAATGGGGGCGGAGCTGAJATTAACATC
t5r18e, B12, TATAATTTTACAJAGGCAJAGCAAJAGCTAJAT
t5r18f, C12, CAATAAJAATGAATGCAATGCTGAGAJAGGCGCG
t5r20e, D12, CATGTCAJAAATCACCATCAAJATAAJACCTCA
t5r20f, E12, AGACAGTCTCATATGTACCCCGGTTTGTATAAJ
t5r22e, F12, ACCGCTGTTAJAATTTGAJACGTTAJAJAAGT
t5r22f, G12, GCAAJATGATTTCTCGTGGGAACCGTTGGTG
t5r24e, H12, GCGCATGCGCATCTGAACCGTGCAGTAAJAA

Plate number: 3

t-5r24f, A1, TAGATGGGTGCGGGCTCTTCGCGCAAGGGC
t-5r26e, B1, GCTCAAGGGTAAACGCCAGGGTTTTGGGGAAG
t-5r26f, C1, ATTAAGTTTTCCACCAACATACGCCATAATGA
t-5r28e, D1, AGCTGATTACTACATTAATTCGGTGTATCTC
t-5r28f, E1, GTGAGCTAGCCCTTACCGCTCGGGGTTGGC
t-5r2e, F1, GAGAAATAGTACACAGTCAAACTCCGCCACCC
t-5r2f, G1, TGAGTTTCAAGGAACAACTATGAATCTCCAA
t-5r30e, H1, GAGAAATAGTACACAGTCAAACTCCGCCACCC
t-5r30f, A2, CCAGAGCGGATGGCCCACTACGTGAGGTCGC
t-5r32h, B2, GTAAAGCACTAAATCGGAACCTAAACCCGTC
t-5r4e, C2, AAAGGCCGCTCAAAGAGGACCTTAGCGGAGT
t-5r4f, D2, AAAAGAGGCTTTTTCGGGATCGTCGGGTAGCA
t-5r6e, E2, GCGAAACAAGAGGCTTTGAGGACGTGAGGAGTT
t-5r6f, F2, ACGGCTACAGTACACAGCGCAACTTCGGACCT
t-5r8e, G2, CCAATCATTAATAGCCGGAACGTACCAACG
t-5r8f, H2, GCTCCATGACGTAAACAGGCTGTACACCAJAA
t-7r10e, A3, CATTCAACCTTGCCCTGACGAGAAACATTGACT
t-7r12e, B3, AAAAGGCTTTTTCGGGATCGTCGAGATAGATACCA
t-7r14e, C3, TTTTTCGCGAGCAACAGAAATGAATGCTTT
t-7r16e, D3, TTTGCTGATGGCTTAGCGGATCGTCAAGGCTCA
t-7r18e, E3, AGGTAAAGCACTAATAGTAGTAGCAAGGTGGCA
t-7r20e, F3, TCAATTTGATGGCTTAGCGGATCGTCAACAGCGC
t-7r22e, G3, GATTGACCCCAAAAAAGGAGGAATGATTAATC
t-7r24e, H3, CAGCGTGGCTTTTTCGGGATCGTCAACAGCGC
t-7r26e, A4, GCATTAAGGAAGAGGGGATGCTTATTACGCG
t-7r28e, B4, GAATAGCAATGTGTCGAAATCTGTATCAT
t-7r2f, C4, AATAATAATAGGAACCATGTACGAGGATAGCAAGCCAA
t-7r30e, D4, ACCAAAGCTGAACGGGCTGACGCTCCCTGAG
t-7r32e, E4, CAGGTTTTTGGGGTGCAGCAACT
t-7r4e, F4, CAGCGAATTTTTCAGTTGAGGAATTTGGC
t-7r6e, G4, CGCCTGATGACAGCATGCGCAAGCAAGCTCAG
t-7r8e, H4, GAATAGGAATGTGTGCGAAATCTGTATCAT
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.

Supplementary Figure S39: New England Biolabs sequence for M13mp18 used to design the staples for all origami. It is based on an unpublished resequencing of M13mp18, F.J. Stewart 5/28/02.

Supplementary Note S4: Experimental Methods

Supplementary Note S4.1: Design, synthesis, and sample preparation.

Single-stranded M13mp18 DNA (New England Biolabs), resequenced in 2002, was quantitated by UV absorbance at 260 nm. (Different clones of M13mp18 have small sequence differences, with the potential to affect folding; by AFM no qualitative difference was observed for a Bayou Biolabs clone—see Supplementary Note .) Staple and remainder strands were purchased unpurified (Integrated DNA Technologies; the manufacturer’s mass spectrophotometry indicated that strands had a few percent $n - 1$ truncation products.) in water at 100 μ M or 150 μ M and stored at -20°C. The desired set of up to 273 short strands was mixed with M13mp18 (typically 160 nM of each short strand, 1.6 nM M13mp18 circular or linear, a 100-fold excess of short strands) in a 100 μ l volume of 1X Tris-Acetate-EDTA (TAE) buffer with 12.5 mM magnesium acetate (pH=8.3) and annealed from 95°C to 20°C in a PCR machine (Eppendorf) at a rate of 1°C/minute in .1°C steps. When composing sharp triangles into hexagons or lattices the best results were obtained when staples that mediated the interaction, i.e. extended staples, were used in only 4-fold excess. To create linear DNA for the square and star, circular single-stranded M13mp18 DNA was incubated in restriction buffer with a short complementary strand at 37°C for 15 minutes, then linearized by digestion with BsrB I (New England Biolabs), phenol-chloroform extracted, and ethanol precipitated.

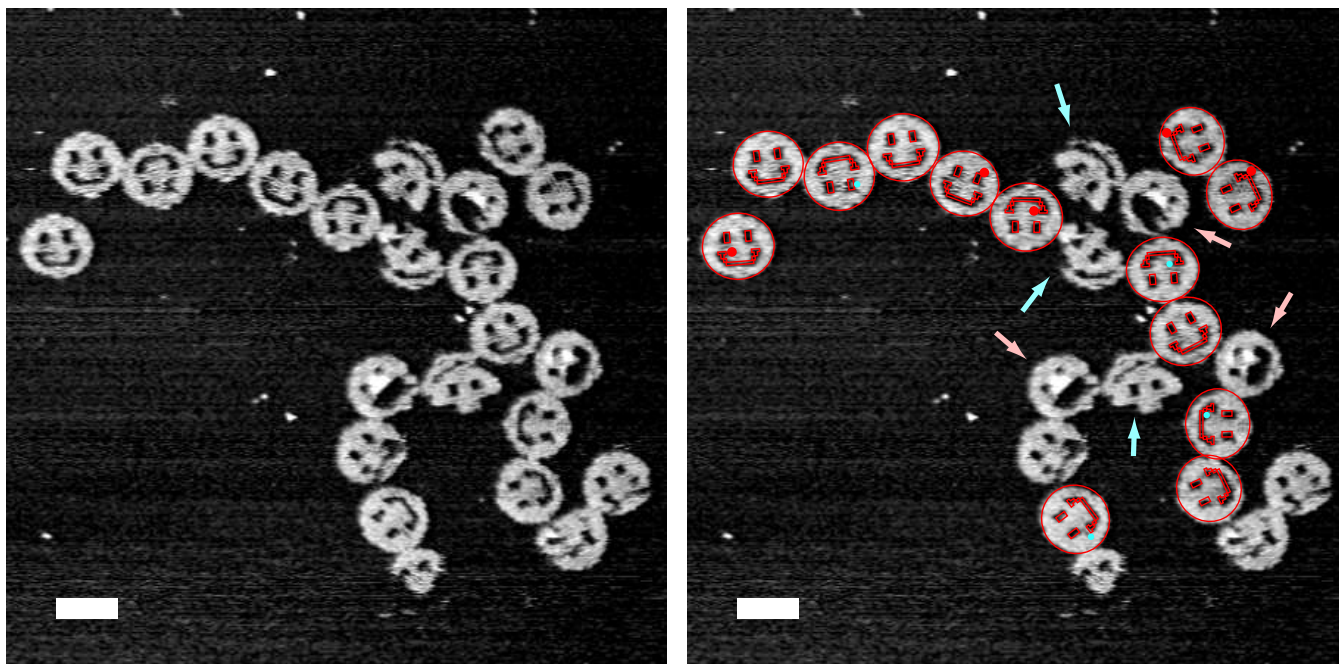
Supplementary Note S4.2 Atomic force microscopy.

Imaging was performed in Tapping Mode under TAE/Mg²⁺ buffer on a Digital Instruments Nanoscope III Multimode AFM (Veeco) with a nanoAnalytics Q-control III (Asylum Research) and a vertical engage J-scanner, using the $\approx$ 9.4 kHz resonance of the narrow 100 μ m, 0.38 N/m force constant cantilever of an NP-S oxide-sharpened silicon nitride tip (Veeco). Samples were prepared by deposition of $\approx$ 5 μ l onto freshly-cleaved mica (Ted Pella).

After self-assembly was complete, samples were prepared for AFM imaging by deposition of $\approx$ 5 μ L onto a freshly-cleaved mica surface (Ted Pella) attached by hot melt glue to a 15 mm metal puck; an additional 30 μ L of buffer was added to both sample and cantilever (mounted in the standard Tapping Mode fluid cell) before the sample and fluid cell were positioned in the AFM head. The tapping amplitude setpoint, after engage, was typically 0.2 - 0.4 volts, the drive amplitude was typically 100-150 millivolts, scan rates ranged from 2-5 Hz. Fine structure associated with crossovers was most clearly resolved for low amplitude setpoint and high drive amplitude values. However, under such conditions, the greatest damage is done to the sample and the hairpin labels are less distinct, sometimes disappearing entirely. Thus, to prevent damage to samples, drive amplitude was minimized subject to the constraint that fine structure was still visible; this often involved lowering the amplitude setpoint. After this procedure, the drive amplitude was typically 70-90 millivolts and the amplitude setpoint .15-.25 volts. Drive amplitude was often further reduced through use of the Q-control.

The single most important factor in acquiring high resolution images appears to be the quality of the AFM tip. In a single AFM session only 1 in 10 NP-S AFM tips might prove capable of revealing fine structure in origami or distinguishing individual hairpin labels. Practically the best approach seems to be to change tips as quickly as possible until the desired resolution is achieved.

Images were flattened by subtracting a low-order polynomial from each scan line, or by adjusting each scan line to match intensity histograms. In Fig. 2:a₃-f₃ the scale of AFM images was adjusted (by < 5%) so that the width of experimental structures matched the theoretical widths of origami. Theoretical widths were calculated assuming .34 nm/base. The width change due to bending of helices by small angles between crossovers ($\sim$ 10 degrees) was ignored; simple geometry suggests it would change theoretical widths by <2% (Supplementary Note 2).



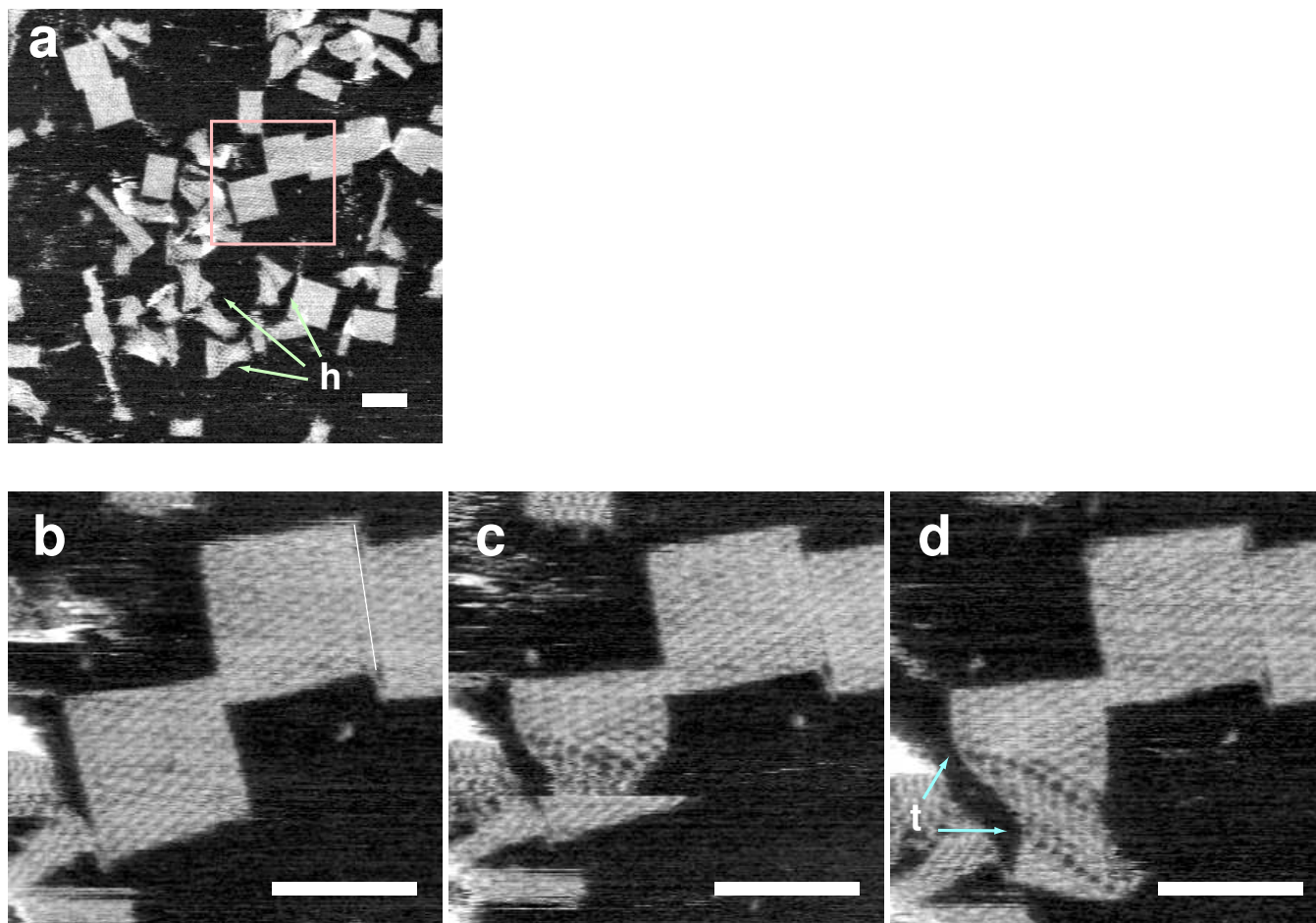
Supplementary Figure S40: Scale bar, 100 nm.

Supplementary Note S5: Experimental variations and controls

In this note I give a text or data for any experimental variation, control, or phenomena that was mentioned in the text but not shown.

Supplementary Note S5.1: Counting well-formed structures

Supplementary Fig. S40 shows one of the fields (reproduced from Fig. 2:d₄) used to obtain the frequency of well-formed structures smileys (here a smiley variant that has the seam above its right eye unbridged). Red dots are 15 nm in diameter, blue dots are 10 nm in diameter. These dots help determine the size of holes. Deciding which apparent holes are defects and which are correct high-resolution crossover structure (see Supplementary Fig. /refsmileyrainbow) is a matter of judgment. At right, templates of smileys are superimposed over experimental structures. To better fit the smileys some templates have been stretched up to 10%. In this field 13 structures have been judged well-formed, and 10 structures have been judged malformed. Two characteristic deformations of smileys are indicated: pink arrows indicate structures in which the ‘upper lip’ of the smiley has been flipped up onto the nose; light blue arrows indicate structures in which the ‘jaw’ has become dislocated, landing over the ‘forehead’ of the smiley.



Supplementary Figure S41: Scale bars, 100 nm.

Supplementary Note S5.2: Stretching of squares into hourglasses

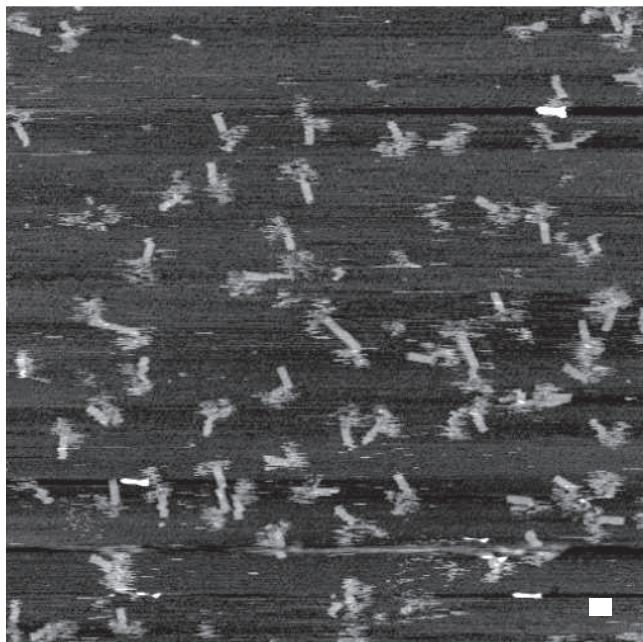
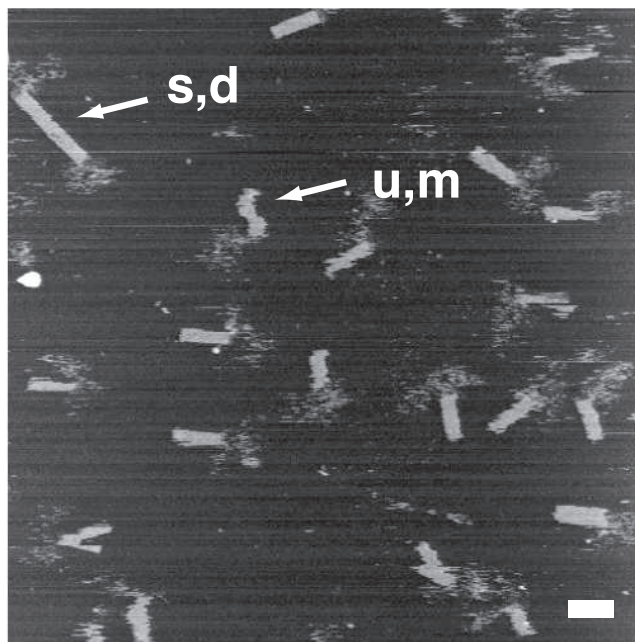
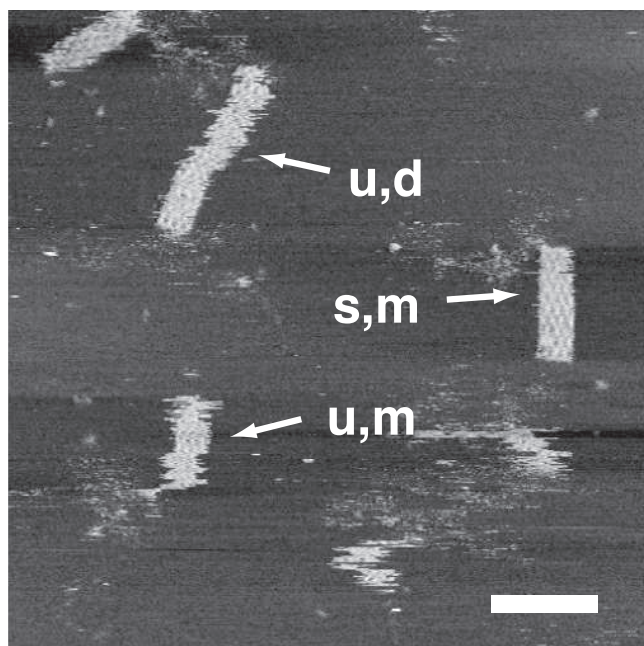
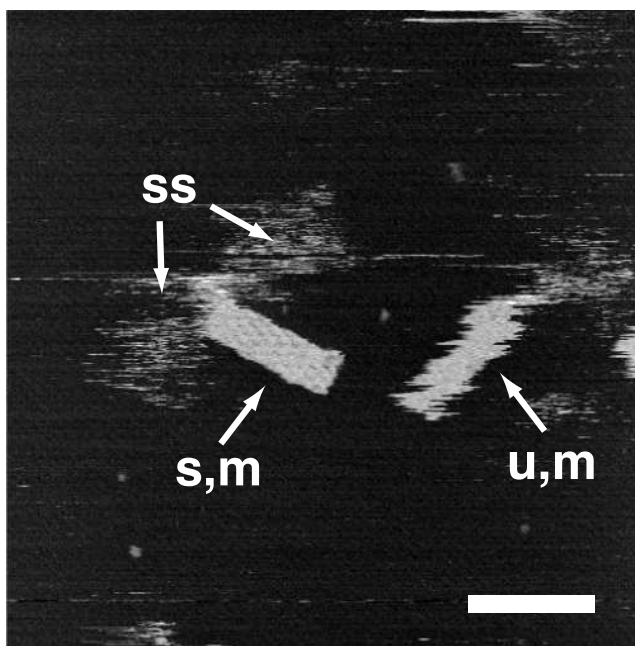
Supplementary Fig. S41a shows a typical field of 26-helix squares. A few structures are square, but many others are rectangular fragments or hourglass shapes (green arrows, marked 'h'). Supplementary Fig. S41b–d document the stretching of a square, over the course of three AFM scans, into an hourglass shape. The hourglass shape that results has what appear to be transitions between the normal folded lattice, and stretched lattice, along diagonals in the structure (blue arrows marked 't'). This is in contrast with the hourglass shown in the main text (Fig. 2:a₄) which appears to have a continuous deformation of the lattice.

Supplementary Note S5.3: One third of the square

The first test of the scaffolded DNA origami method, before the creation of the full square, was the creation of the bottom $\sim 1/3$ of the square (Supplementary Fig. S42). Also, a circular M13mp18 scaffold DNA was used rather than a linearized one, because the corners of the rectangle were close enough that the unfolded portion of the M13mp18 scaffold DNA could easily bridge the corners without deforming the rectangle. No remainder strands were used on the $\sim 2/3$ of M13mp18 DNA left unfolded. Apparently long, unfolded single-stranded sections of the scaffold do not adversely affect folding and remainder strands (where they are used for other designs) are probably unnecessary.

The $1/3$ squares were observed singly (Supplementary Fig. S42, structures marked ‘m’ for monomer) or as dimers (Supplementary Fig. S42, structures marked ‘d’ for dimer). Dimers always appeared to be the result of stacking of $1/3$ squares by vertical edges *opposite* from the unfolded single scaffold. Thus the unfolded scaffold appeared to prevent stacking at adjacent vertical edges. Single-stranded scaffold takes on a ‘cloud-like’ appearance that varies from AFM image to AFM image (Supplementary Fig. S42, marked ‘ss’).

Coplanar helices in DNA nanostructures appear to bind mica cooperatively; the larger a DNA nanostructure, the more tightly it appears to bind mica. This trend is apparent in the mobility of DNA nanostructures deposited on mica and imaged by tapping mode AFM under buffer. Most of the structures described in the main paper move infrequently during imaging, occasionally rotating by a few degrees or slipping by a few tens of nanometers (as judged by stationary structures around the mobile structure). The $1/3$ squares, however, were more difficult to image because they often slipped, as shown by structures marked ‘u’ (for “unstable”) in Supplementary Fig. S42. In the same field, other structures may stick well (and are thus labelled ‘s’ for “stable”); when structures are stable, their fine structure (the lattice of crossovers) can often be observed. Sometimes, a structure that appears stable in one image moves in the next (data not shown). Thus, as for DNA nanotubes¹⁵ and other DNA nanostructures, the interaction of scaffolded DNA origami with the mica surface (under buffer) is complex and dynamic and must be taken into account when interpreting AFM images.

a**b****c****d**

Supplementary Figure S42: The first experiment performed for this paper was to create $\sim 1/3$ of the 2.5-turn spacing square by adding $\sim 1/3$ of the staples for the square to circular M13mp18 DNA. Structures that appear to be moving under imaging are marked with a 'u' for "unstable"; structures that appear to be stable are marked with an 's'. Monomers of the $1/3$ square are marked with an 'm'; dimers are marked with a 'd'. Single-stranded scaffold, unfolded by staples is marked with an 'ss'. Scale bars, 100 nm.

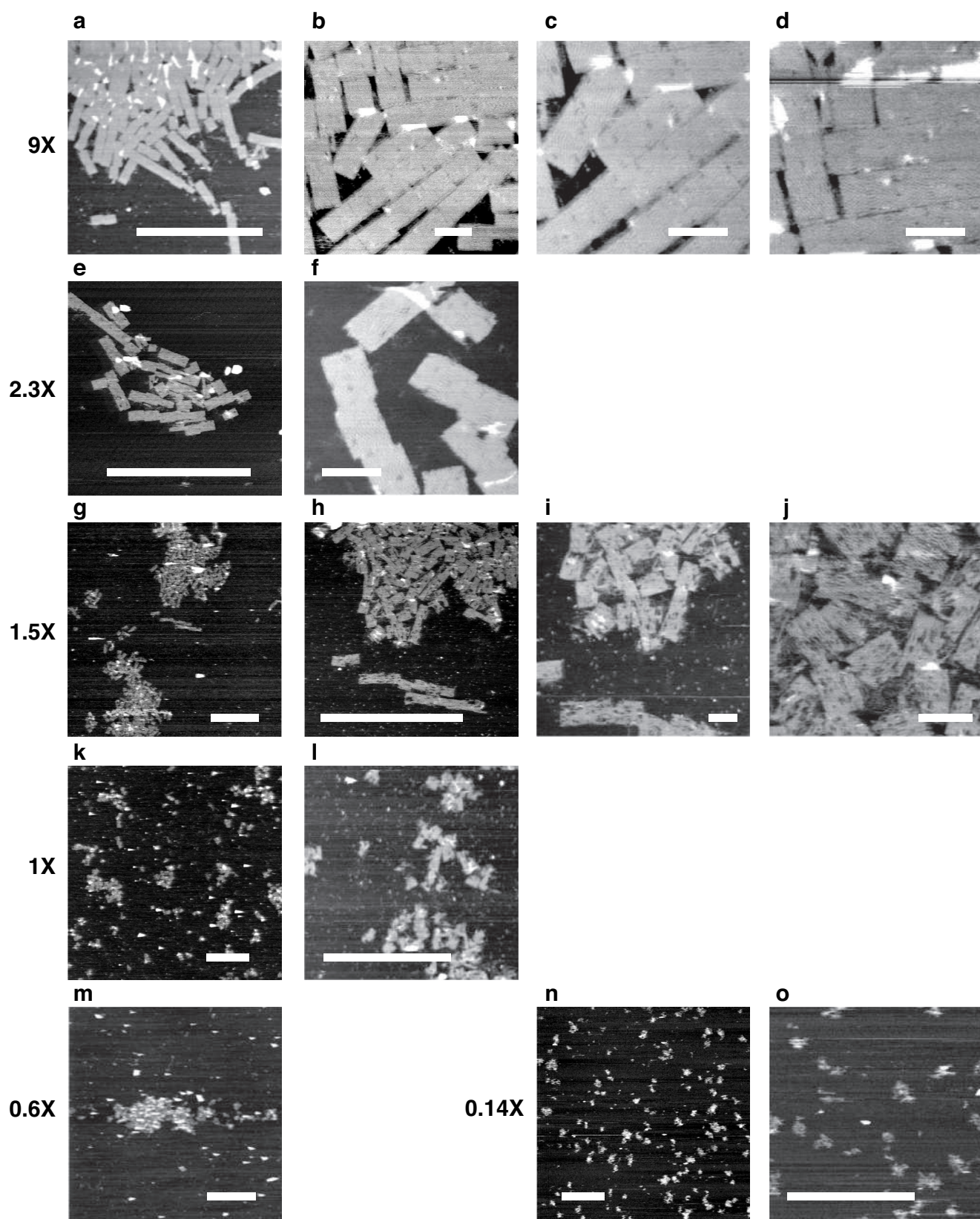
Supplementary Note S5.4: Stoichiometry

Oligos were received from the manufacturer (Integrated DNA technologies) at a nominal concentration of 100 or 150 μM as determined by UV absorbance at 260 nm. To estimate the error in stoichiometry, the concentration of 10 staple strands were remeasured by UV absorbance upon receipt by diluting 4 μl of stock solution with 196 μl of distilled water. This modelled the type of pipetting errors that occurred in the experiments since typically 3-7 μl of each staple stock solution was used when staples were mixed. (In a given experiment a fixed volume of each staple strand was used so no renormalization was performed.) Concentrations were calculated based on extinction coefficients calculated according to a nearest neighbor model³⁵. Assuming that the nearest neighbor model is correct, errors in absolute concentrations ranged from -5% to +13% and averaged +6% with a standard deviation of 6%. This may have reflected a systematic difference between the manufacturer's and the laboratory's spectrophotometers or measurement of volume. Nevertheless, this means that errors in relative concentration had a range of roughly 20%, and thus I estimate the error in concentration to be $\sim 10\%$. The M13mp18 scaffold strand was similarly quantitated but variable volumes of it were used to achieve a desired concentration in the final experiment. Pipetted in small 1-2 μl volumes, its stoichiometry relative to the staple strands is assumed to have, similarly, at least $\sim 10\%$ error.

In most experiments, a vast excess of staple strands (100 to 300-fold) over the scaffold were used. A question becomes, how small an excess can be effectively used to create DNA origami. Supplementary Fig. S43 shows AFM images of samples in which a smaller staple:scaffold ratio (from 9-fold down to .14-fold) was used. At a 9-fold excess (Supplementary Fig. S43a-d), rectangles are indistinguishable from those at higher molar excess of staple strands. Small 'hole' defects were sometimes observed but these defects are often observed upon repeated high resolution scanning even at high (100 to 300-fold) molar excess of staple strands. Rectangles created with just a 2.3-fold excess (Supplementary Fig. S43e-f) were difficult to distinguish from rectangles created with higher staple strand excess – that is, I am not sure they are distinguishable. It appeared that fewer long stacked chains formed, and that there were more malformed rectangles, but I made no attempt to quantify this.

In contrast, at a 1.5-fold excess of staple strands (Supplementary Fig. S43g-j), clear differences from normal rectangles were observed. Rectangles take on a "moth-eaten" appearance and have holes that often cover over 10% of their area. Linear stacks of rectangles may still be observed, however (bottom of Supplementary Fig. S43h). At a 1:1 staple:scaffold ratio (Supplementary Fig. S43k,l) no clear stacking is observed and it requires imagination to make out rectangular structures. At a 0.5 staple:scaffold ratio (Supplementary Fig. S43m) aggregates of partially folded scaffold strands still form but at a 0.14 ratio (Supplementary Fig. S43n,o) no large aggregates are formed; Instead, structures that appear to contain 1-3 scaffold strands are observed and they have a "streaky" appearance characteristic of structures that are mobile on the mica surface.

Because of the sloppiness with which strands were quantitated, no precise conclusions should be drawn from these experiments. However, I conclude that a 10-fold excess of staple strands is a "safe" concentration to work with for DNA origami containing 32-mer staple strands and that, because of the structures observed at a 2.3-fold excess, the use of a *vast* excess of staple strands should not be seen as a fundamental requirement of scaffolded DNA origami. Better control of concentrations may allow a 2-fold excess to be used for the construction of well-formed origami.



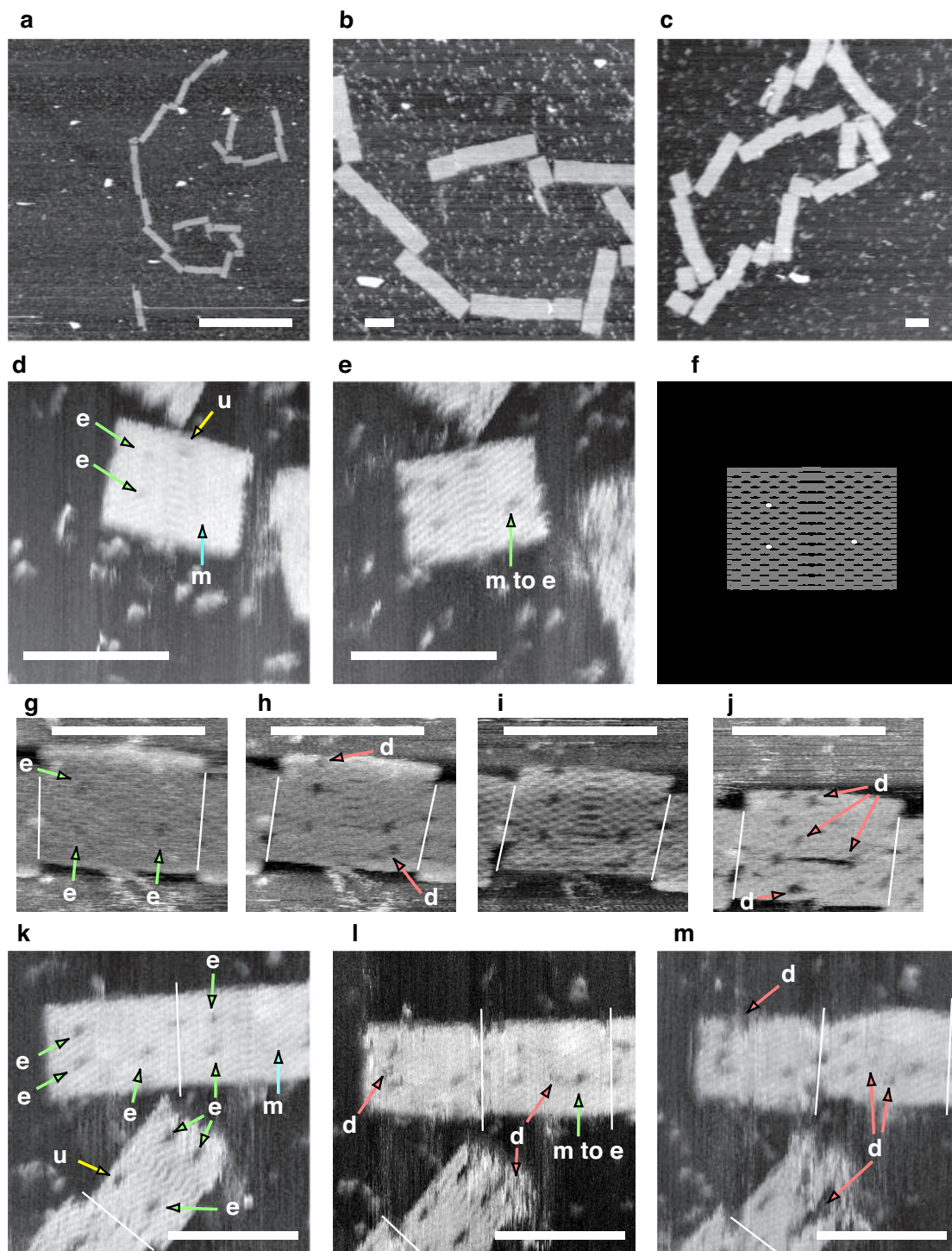
Supplementary Figure S43: Changing the concentration of staple strands relative to the scaffold. Scale bars in **a,e,g,h,k-o** are 1 micron. Scale bars in **b-d, f, i, j** are 100 nm

Supplementary Note S5.5: Removal of a strand

To test the ability of atomic force microscopy to detect defects in origami, rectangles were prepared with three staple strands intentionally omitted (**r-5t8e**, **r-5t16e** and **r5t14f**). The positions of the omitted strands in the rectangle are diagrammed in Supplementary Fig. S44f as white dots. At low magnification, no defects could be observed (Supplementary Fig. S44a–c). Even at high magnification, in images of typical resolution, no defects were observed; the majority of AFM tips did not provide resolution that allowed imaging of the defects. Roughly 1 in 5 tips were sufficiently sharp to allow ‘holes’ at the position of the omitted strands to be observed (features marked ‘e’ for ‘expected’ in Supplementary Fig. S44d–e, and g–m), although not all rectangles showed all three holes immediately upon imaging (features marked ‘m’ for ‘missing’). It sometimes took repeated imaging for holes to appear (features marked ‘m to e’), as if repeated scanning of the AFM tip was enlarging the defect.

A difficulty is that unexpected defects (features marked ‘u’) were sometimes observed during initial scans and further, repeated scanning often created holes and tears in structures (features marked ‘d’). Two examples, in which (upon an initial scan) holes appear in the expected positions without other holes elsewhere, convinced me that the observed defects were associated with the omission of strands. These examples were not culled from hundreds of images, only about a half-dozen high-resolution examples of the sample were obtained. Thus these examples appear to be statistically significant (although I have made no attempt to calculate their significance based on the rate of appearance of holes in high-resolution images of pristine rectangles).

I conclude that, while omitted strands are difficult to observe, any strands systematically missing from structures (say because of bad secondary structure in a staple strand or the scaffold) should be detectable in multiple high-resolution images.



Supplementary Figure S44: Visualization of the positions of omitted strands in rectangles. **a–c** show that at low magnification and resolution, defects due to omitted strands cannot be observed. **f** diagrams the position of the three omitted strands. **d–e** and **g–m** show high resolution images of rectangles with omitted strands. ‘e’ marks expected defects, ‘u’ marks unexpected defects, ‘m’ marks a missing defect, and ‘d’ marks AFM damage. Defects on the vertical edges of rectangles (ragged edges) are so common that I do not mark them. Such defects seem to be created or enhanced easily by AFM imaging. White lines mark boundaries between rectangles. Rectangles do not always land in the ‘canonical’ orientation and under imaging roughly 50% appear flipped. Thus **d–e** and **k–m** have been flipped left to right so that the rectangles they contain may be more easily compared with **f**. In **k–m** the diagonally oriented rectangle at bottom is flipped with respect to **f**. Inspect **f** and note that the singleton omission on the right hand side is slightly raised with respect to the pair of omissions on the left; AFM defects show a similar pattern. Scale bar in **a**, 1 micron. Scale bars in **b–e** and **g–m** are 100 nm.

Supplementary Note S5.6: Unbridged seams

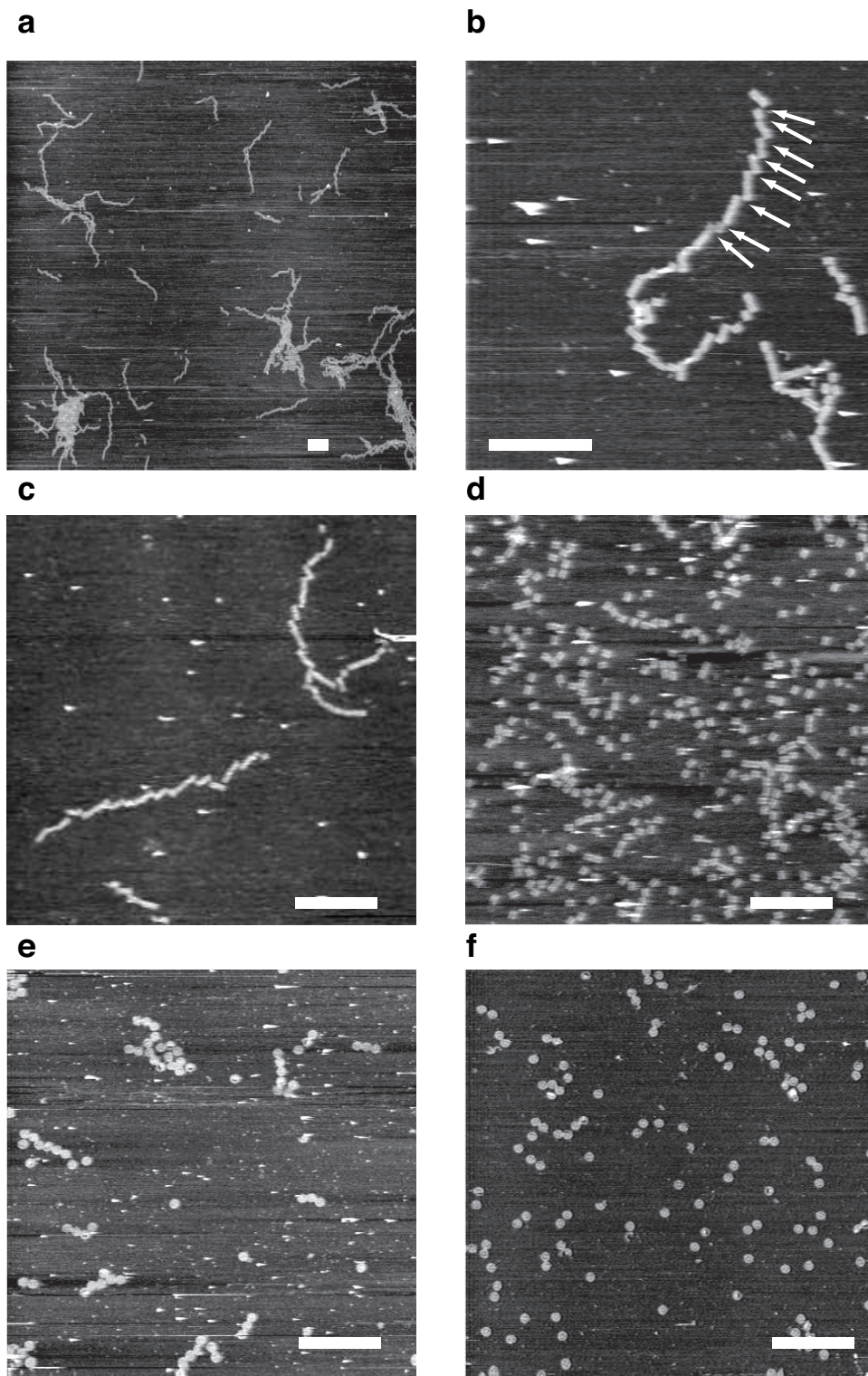
Unbridged seams in scaffolded origamis are those held together only by stacking interactions. Sometimes such seams dislocate; this is best observed in patterned rectangles because the two distinct halves of a rectangle can be unambiguously identified, as snowflake patterns in Supplementary Note S6 show. Supplementary Fig. S53d shows a small dislocations along an unbridged seam. Large dislocations at unbridged seams are also common. Supplementary Fig. S53c has white arrows that point to dislocations in which the two halves of the origami are completely separated.

Supplementary Note S5.7: Prevention of stacking

Stacking interactions based on blunt-ended helices can be quite strong; rectangles which have many parallel blunt-ends along their left and right edges stack so strongly that they may form long chains over 5 microns in length (Supplementary Fig. S45a–c). As deposited on mica, such chains exhibit frequent ~ 75 nm dislocations, every few rectangles along the chain (arrows, Supplementary Fig. S45b). I hypothesize that in solution rectangles in such chains are completely stacked and no such offsets occur.

To avoid aggregation based on stacking interactions, several methods can be employed. First, the staple strands along the edges of a shape may be simply left out, and the scaffold left unstructured along these edges. Supplementary Fig. S45d shows rectangles that have been disaggregated in this way. Simply omitting staple strands out leaves unstructured scaffold at the edges of the rectangle and decreases the size of the potential pixel array by two columns. Thus a second, more aesthetically pleasing, method is that employed in Fig. 3e: the addition of 4T hairpin loops to staple strands on the edges of a shape. (The use of 4T hairpin loops to disaggregate DNA nanostructures was first demonstrated by Rebecca Schulman for her “zig-zag boundaries”.) A third very similar method is to add 4T tails to staple strands that have ends on the edge of the shape. Supplementary Fig. S45e shows the normal amount of aggregation for 3-hole disks. Supplementary Fig. S45f shows that the addition of just a small number (12) of 4T tails to the 3-hole disks causes almost complete disaggregation.

These experiments show that, when it is desirable to do so, stacking and the aggregation it induces can be almost entirely suppressed.



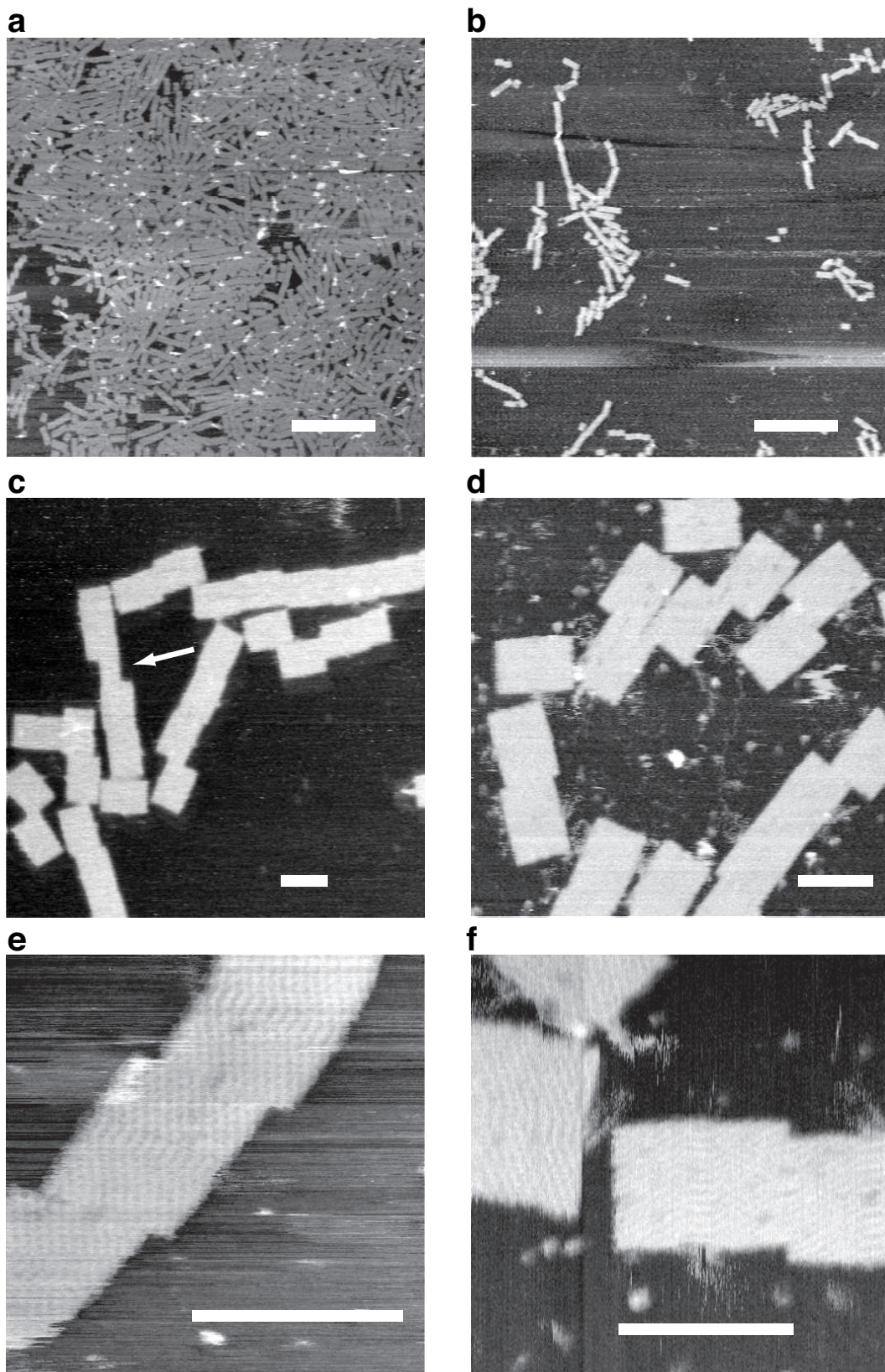
Supplementary Figure S45: Methods to discourage stacking of DNA origami. **a–c** In addition to large aggregates, rectangles often form very long stacked chains. Arrows indicate dislocations of rectangles by a single rectangle width (roughly 75 nm). **d** Stacking (and aggregation) are almost completely inhibited by omitting staples on the left and right edges of the rectangle. **e** Normal 3-hole disks aggregate strongly; almost no 3-hole disks occur as singletons. **f** The simple addition of 4T-tails to the 6 blunt-end helices its left and right edges of the 3-hole disks almost completely abolishes stacking and aggregation. Scale bars, 1 micron.

Supplementary Note S5.8: A different M13mp18 clone

While all viruses labelled M13mp18 are supposed to have identical DNA sequences, in practice this does not appear to be the case. The originally deposited sequence for M13mp18 in Genbank (accession X02513, 7249 bases long,³⁶) appears to have an error that was corrected by adding a ‘T’ at position 900 (accession M77815, 7250 bases long). Amersham Biosciences gives the sequence of M13mp18 (evidently their clone) as a 7249 sequence that differs from X02513 by a pair of compensatory frame shifts (bounding the region from 977 to 1556) and 3 point mutations outside of the frameshift. New England Biolabs gives a 7249 sequence (Supplementary Fig. 39) for their clone (resequenced in 2002) that differs from the Amersham sequence by a pair of compensatory frameshifts (bounding the region 900-977) and 23 point mutations outside of the frameshift.

The staple strands given in this paper were created using the New England Biolab’s sequence and all experiments save those described here were performed with New England Biolab’s M13mp18 single-stranded DNA (Catalog number: N4040S). To test whether small differences in sequence could be detected by AFM, staples for the rectangle were used to fold a sample of M13mp18 DNA from Bayou Biolabs (which reports that their sequence is the same as that of Amersham, although it has not been recently resequenced). Thus the staple strands should have had mismatches with the scaffold at 23 positions and a 78 base section should have been shifted by 1 base with respect to the scaffold. Qualitatively, no differences were observed between rectangles created with Bayou Biolabs M13mp18 DNA and New England Biolabs M13mp18 DNA (Supplementary Fig. S46).

While this seems to suggest that small sequence differences do not matter, I am uncomfortable suggesting that the staple sequences described in this paper be used for M13mp18 DNA with a sequence that is known to be different. Thus I suggest either generating new staple strand sequences, or using New England Biolabs M13mp18. Given that the difference (in point mutations) between the New England Biolabs sequence and Amersham sequence is 0.32% of the M13mp18 genome, and that the sequencing error rate in both these sequences could easily be 1%, this suggestion is probably based in irrational fear.

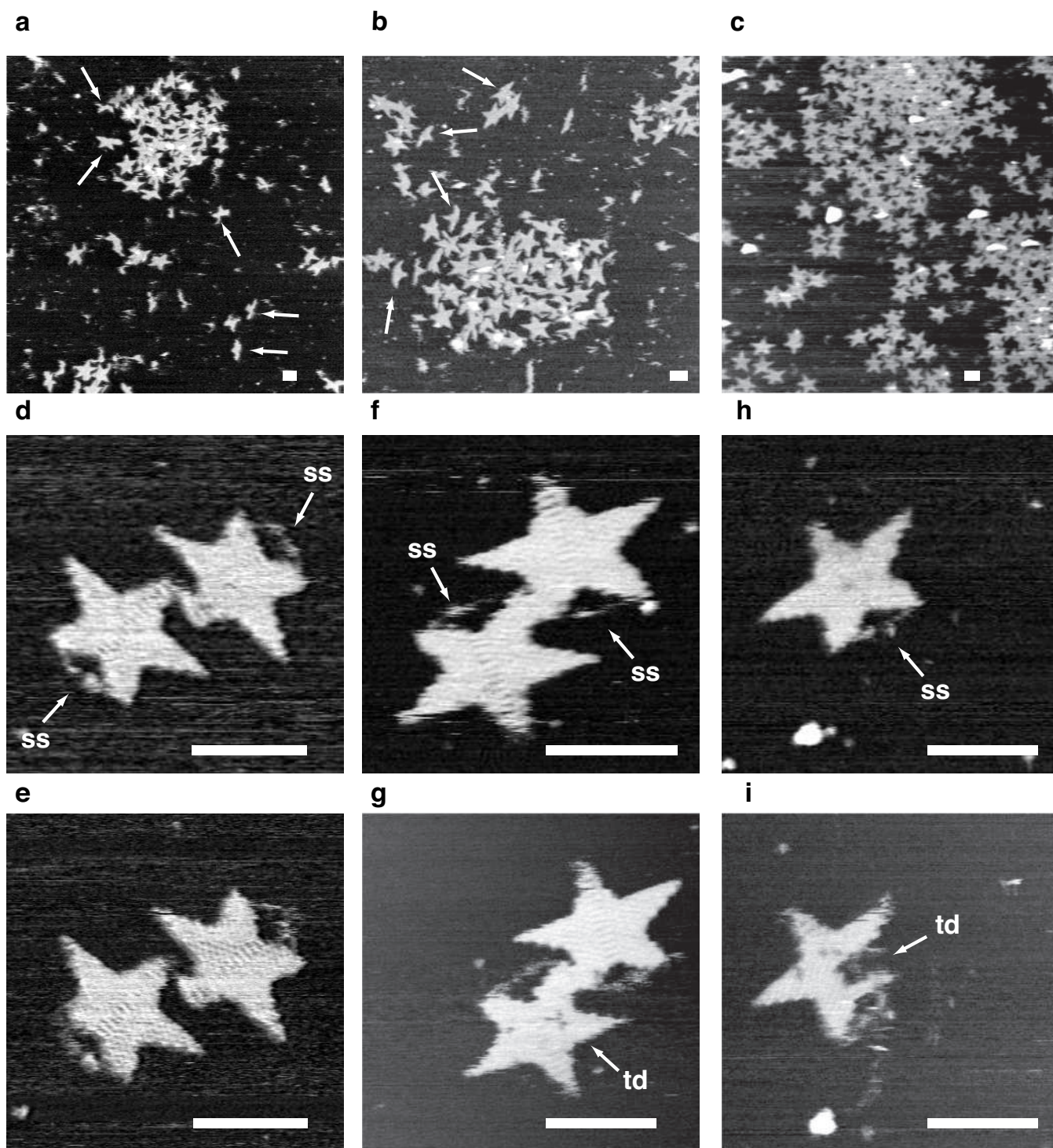


Supplementary Figure S46: Rectangles created using Bayou Biolabs M13mp18 DNA.

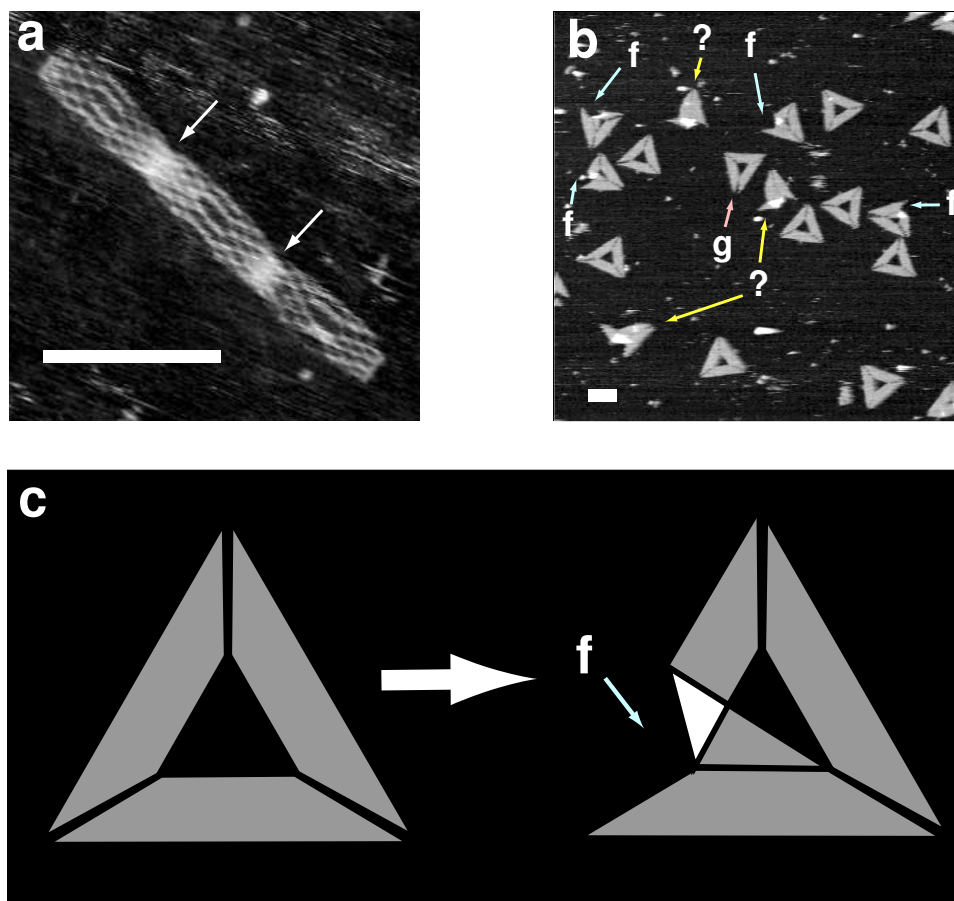
Supplementary Note S5.9: Use of circular scaffold for Stars

Use of circular scaffold with stars (Supplementary Fig. S47c–i) appears to give better results than with linear scaffold (Supplementary Fig. S47a,b). Fewer structures appear to be fragments of stars (arrows in Supplementary Fig. S47a,b). However, it is difficult to tell whether the lower two points of the star, which the circular scaffold bridges and often image poorly, are well-formed. The circular scaffold that bridges the two lower points is easily visualized, however, as a single, somewhat diffuse, arc (labelled as ‘ss’ in Supplementary Fig. S47d,f, and h). The fact that circular scaffold appeared to give better results may be attributed to the high purity of the circular scaffolds. After linearization, the quality of linear scaffold, in terms of the percentage of strands that were full length, was not assessed and a large percentage of the strands may have not been full length.

Of incidental interest, Supplementary Fig. S47d–i show the range of outcomes that is observed when a structure is imaged more than once in an attempt to get higher resolution images. (In an attempt to get higher resolution, the tip-sample interaction is increased by increasing the tapping amplitude or decreasing the amplitude setpoint.) Sometimes a structure (Supplementary Fig. S47d) is imaged with higher resolution (Supplementary Fig. S47e.) Sometimes a structure (Supplementary Fig. S47f) sustains a small amount of damage from the tip (labelled ‘td’ for “tip-damage” in Supplementary Fig. S47g). Sometimes a structure (Supplementary Fig. S47h) sustains severe damage (Supplementary Fig. S47i).



Supplementary Figure S47: The difference between stars created using a circular scaffold and those created using a linear scaffold. **a,b** Stars created using a linear scaffold. Arrows indicate example fragments; not all fragments are labelled. **c-i** Stars created using a circular scaffold strand.



Supplementary Figure S48: Deformation of triangles. **a** Triangles made from rectangle domains sometimes break and form 3-domain rectangles based on stacking interactions (white arrows). **b** Sharp triangles made from trapezoidal domains but without bridges. 'g' and a pink arrow mark a gap between trapezoids in a non-equilateral triangle. 'f' and green arrows mark places where tips of trapezoids have folded into the interior of sharp triangles. '?' and yellow arrows mark structures for which no sensible interpretation has been made. **c** Interpretation of the sharp triangles with folded trapezoids from **b**. Gray indicates a single thickness of DNA structure, white a region of double thickness. Scale bars, 100 nm.

Supplementary Note S5.10: Deformation of triangles and unbridged sharp triangles

In experiments designed to create triangles from three rectangular domains, 88% of structures (of 199 structures observed) had roughly triangular shapes but < 1% (a single structure) were equilateral. The remaining 12% of structures took a variety of forms but, strikingly, 3% of structures took the form of three-domain rectangles held together by stacking interactions (Supplementary Fig. S48a). This suggests that the scaffold may break preferentially at the vertices of the triangle.

A large percentage (55%) of sharp triangles (created from trapezoidal domains) remain well-formed when bridges are not used along the seams between trapezoidal domains (Supplementary Fig. S48b). Typical defects take the form of trapezoids that fold back away from seams ('f' labels) into the interior of the triangle. Some structures cannot easily be interpreted ('?' labels).

Supplementary Note S6: Hairpins for creating patterns

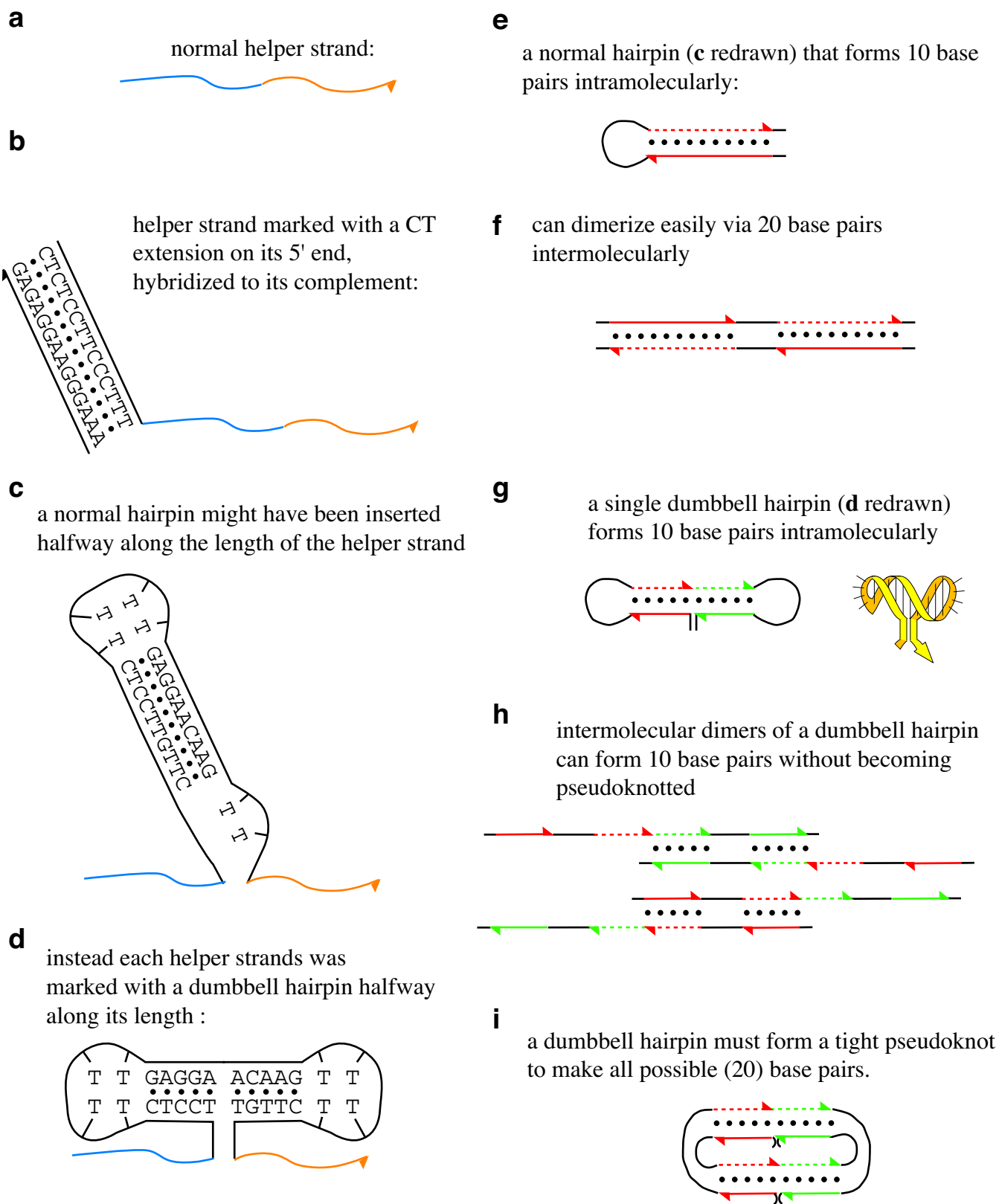
To label a DNA nanostructure, DNA hairpins are often added to increase the height of the nanostructure at a desired location. DNA hairpins have a tendency to dimerize, and, at the high concentrations at which they occur (up to 40 μ M if all positions were labelled by the same hairpin) might inhibit formation of the shapes. Thus a new type of hairpin, a *dumbbell* hairpin was designed (Supplementary Fig. S49d) that, in order to dimerize, must form a presumably strained pseudoknotted structure (Supplementary Fig. S49i). Dumbbell hairpins were inserted between nt. 16 and 17, for all 32-mers in the rectangle design *except* strands **r9t0f** and **r-9t24e** which had the hairpins inserted between nt. 8 and 9. Note that (1) no normal hairpins were tested, they might have worked without difficulty and (2) the idea that dumbbell hairpins to form fewer dimers has not yet been tested (by say gel electrophoresis comparing normal and dumbbell hairpins at high concentration).

In an attempt to allow post-labelling of the shapes after formation a second type of marker was explored (Supplementary Fig. S49b): a 14 base mixed C and T tail was added to the 5' end of each staple strand. The idea was that this polypyrimidine addition would not hybridize with any staple strands or to itself. After formation of a shape, the corresponding 14 base polypurine strand was added to create duplex at desired positions, which it was assumed would yield good AFM height contrast. Instead the duplex markers imaged very poorly in a manner that was highly scan angle dependent. Multiple scans from multiple angles suggest that the poly-CT tails do in fact get labelled by poly-AG complements but imaging was very difficult (data not shown). The duplex markers, because they are attached to the origami by only one covalent bond, appear to be flexible. Thus such a method may be useful for attaching gold balls or other materials to the shapes but serves as a warning that verification of the correct structure by AFM may be difficult.

Despite the apparent complexity of the patterns, physically selecting and mixing strands was relatively simple. Because the set of '0' strands and set of '1' strands (bearing dumbbell hairpins) are complementary, and '0' and '1' strands are stored in characteristic positions of matched 96 well plates, two complementary pipette tip boxes are easily constructed, one for selecting the '0' strands and the other for selecting '1' strands. This is accomplished by taking a full tip box, and, for each position of a desired '1', moving that tip to the same position in an empty tip box. The originally full tip box becomes a box for selecting the '0' strands, and the originally empty tip box a box for selecting the '1' strands. A multichannel pipettor or robotic workstation can then be used to apply the tip boxes to the 96 well plates. The pipettor gets a tip if and only if the corresponding position in the tip box has a tip, and thus performs only the desired pipetting operations. This idea is diagrammed in Supplementary Fig. S50 and a worksheet for creating the snowflake design is given in Supplementary Fig. S51.

To give a better feeling for the range of structures that were observed in the pattern experiments, I give a page of images for each structure that was observed and include some zoom-outs of the images used in the paper (Supplementary Fig. S52, 53, and 54). Note the presence of flipped structures with hairpins down that image poorly. Note also that the "DNA" and "snowflake" patterns were rendered on a rectangle with 18 $\times$ 12 pixels and that the "Map" was rendered on a completely different rectangle with 14 $\times$ 16 pixels.

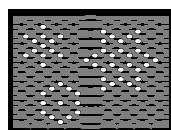
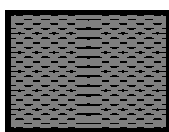
The resolution of patterns was measured directly from staggered patterns. Distances measured between pairs of '1' pixels in alternating columns (two pixel widths: $11.5 \pm .9$ nm s.d., $n = 26$) and adjacent rows (one pixel height: $6.6 \pm .5$ nm s.d., $n = 24$) are consistent with an expected pixel size of 5.4 nm $\times$ 6 nm. I note that the measured pixel width is exactly that expected given the measured width of the rectangle. The measured width was 104.14 nm and the theoretical width 97.92 nm. Assuming that the scale is off (by 6.36%) and that the latter value is correct, then the width of two columns of pixels is $10.8 \pm .85$ nm, spot on. The same holds true for the measured pixel height. The measured height was 77.08 nm and from our estimation of the gap (based on many experiments we would expect a height of 71 nm. Assuming the latter was actually correct the scale is off (by 8.56%) then one pixel height is $6.1 \pm .46$, very close to the expected value.



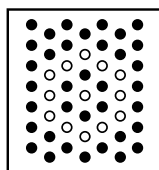
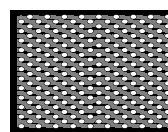
Supplementary Figure S49: Markers for AFM contrast, possible unintended interactions.

Creating arbitrary patterns

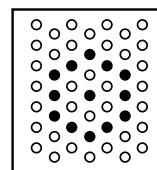
original shape strands



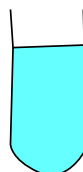
new strands with hairpin labels



From a full box (left) the desired pattern of tips (black dots) to an empty box (right) so that the boxes hold complementary patterns of tips.

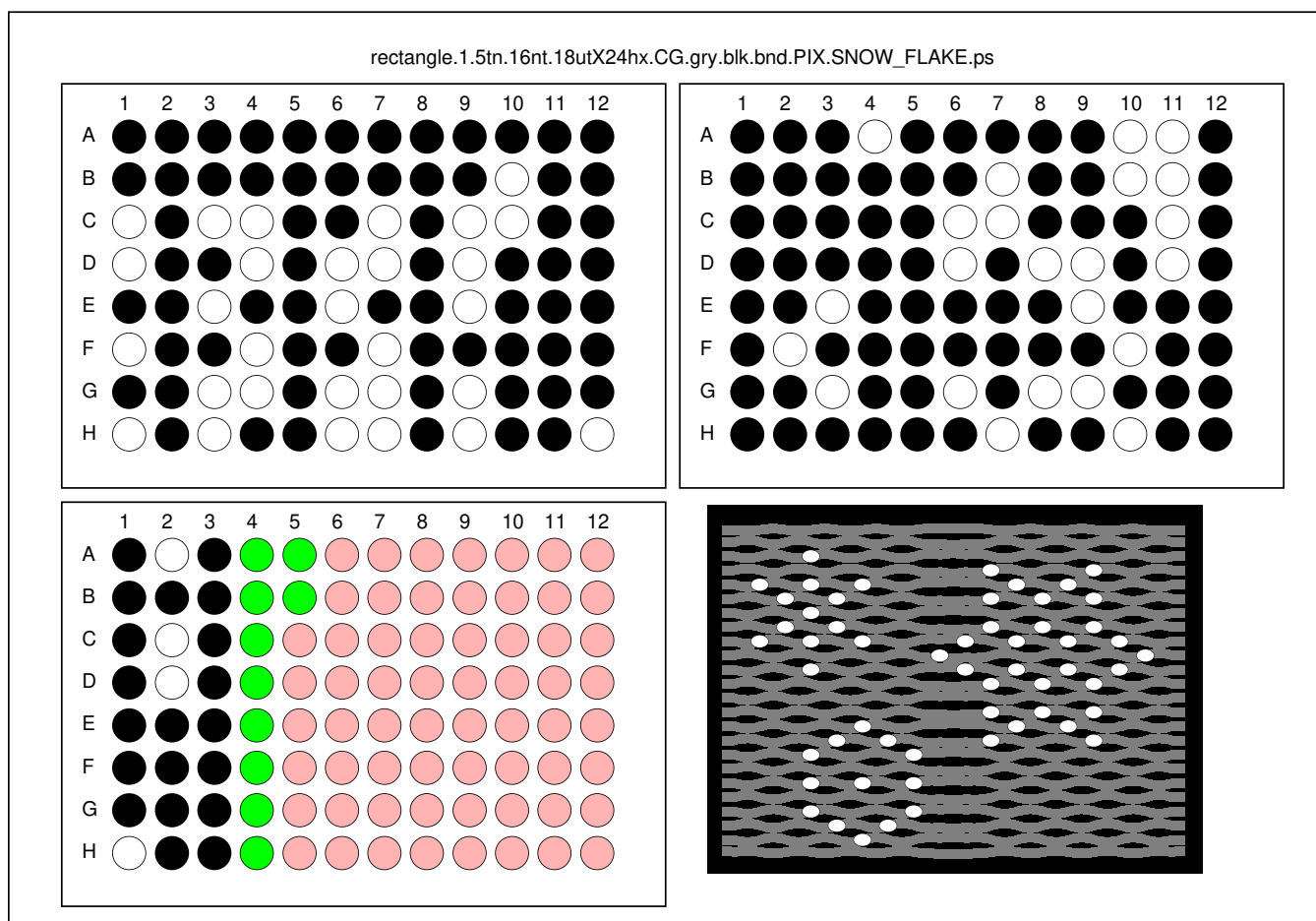


The formerly full box is used to draw from plates holding the original strands.

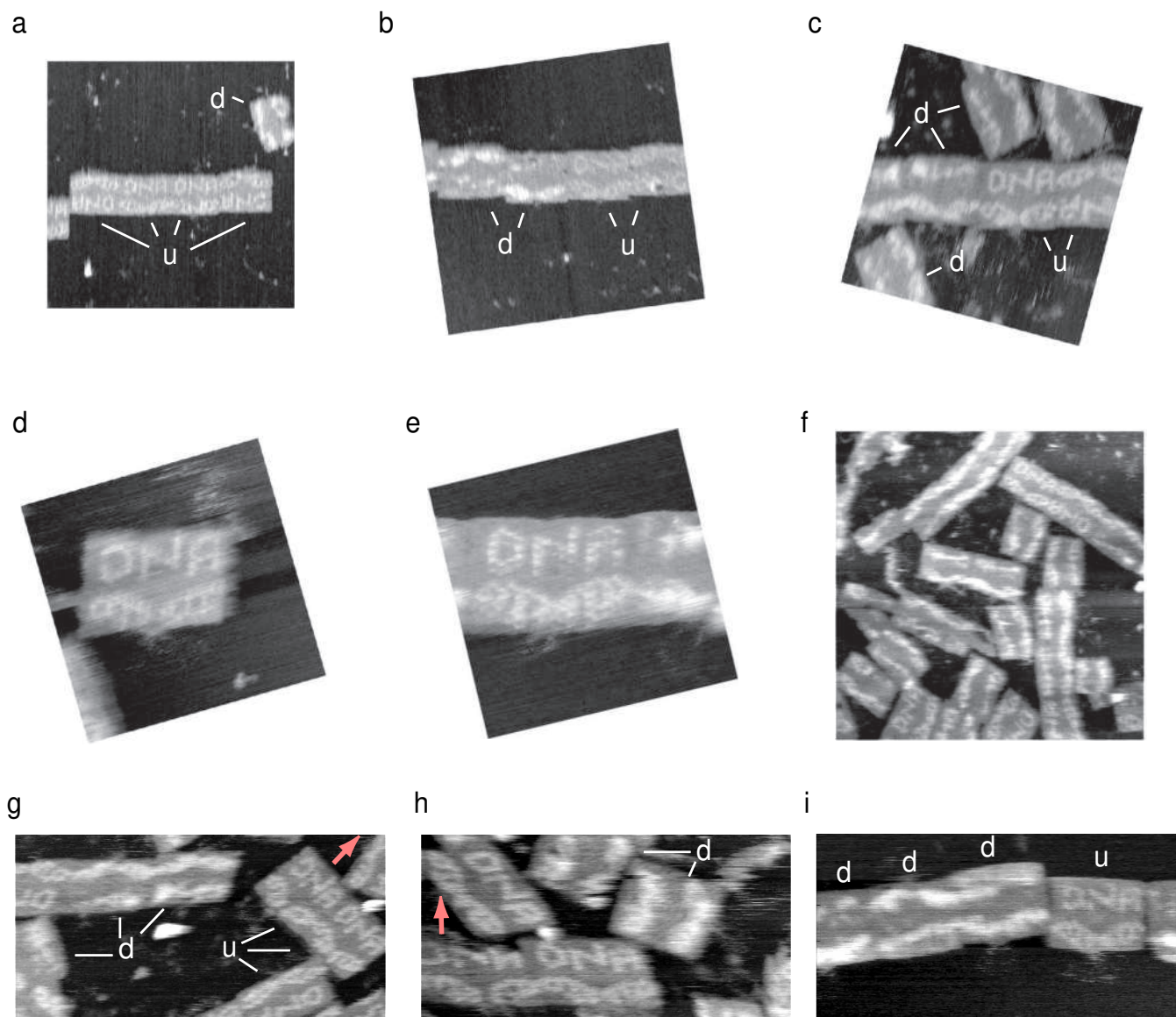


The formerly empty box is used to draw from plates holding the new strands with hairpins.

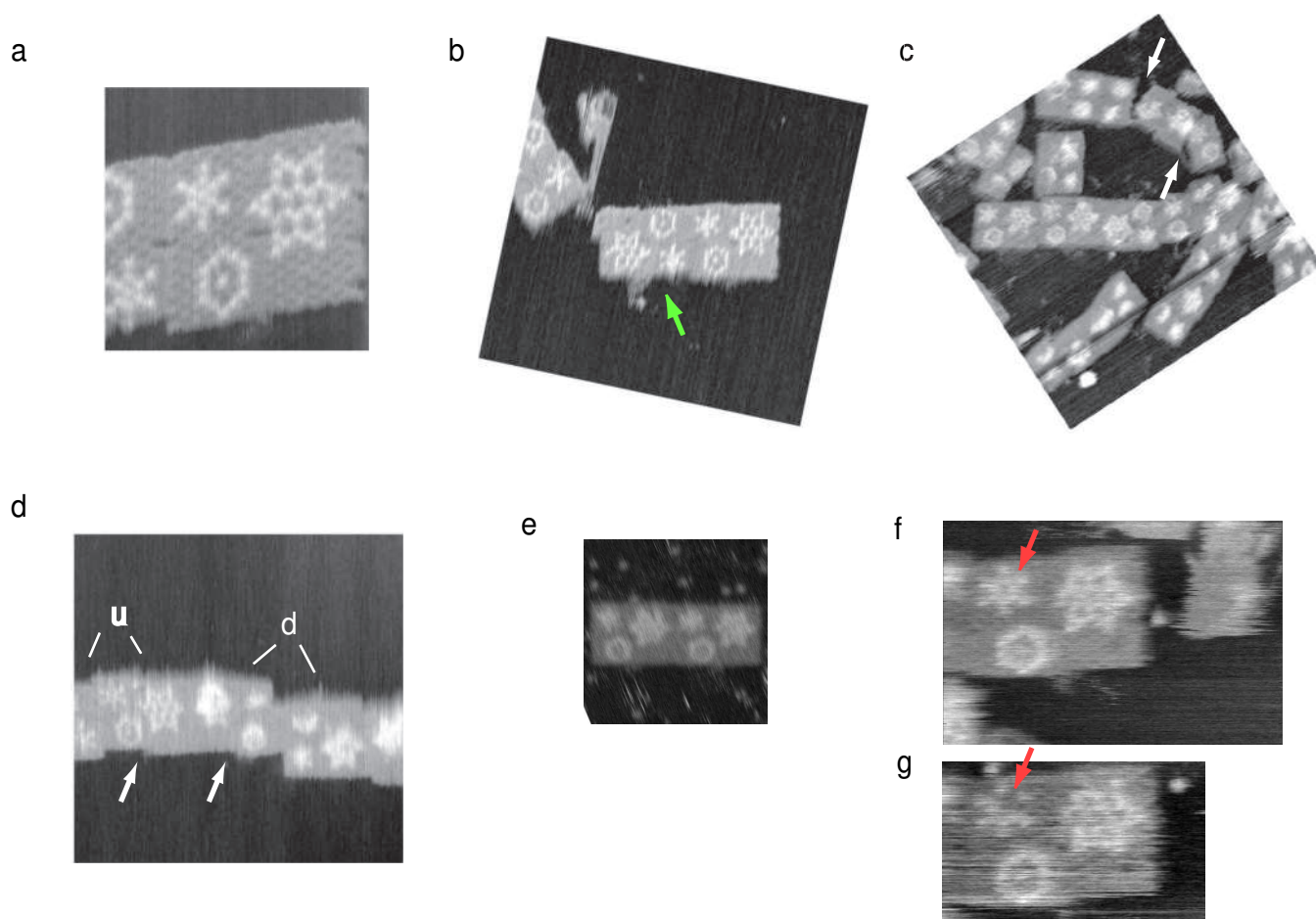
Supplementary Figure S50: Making a pattern on DNA origami.



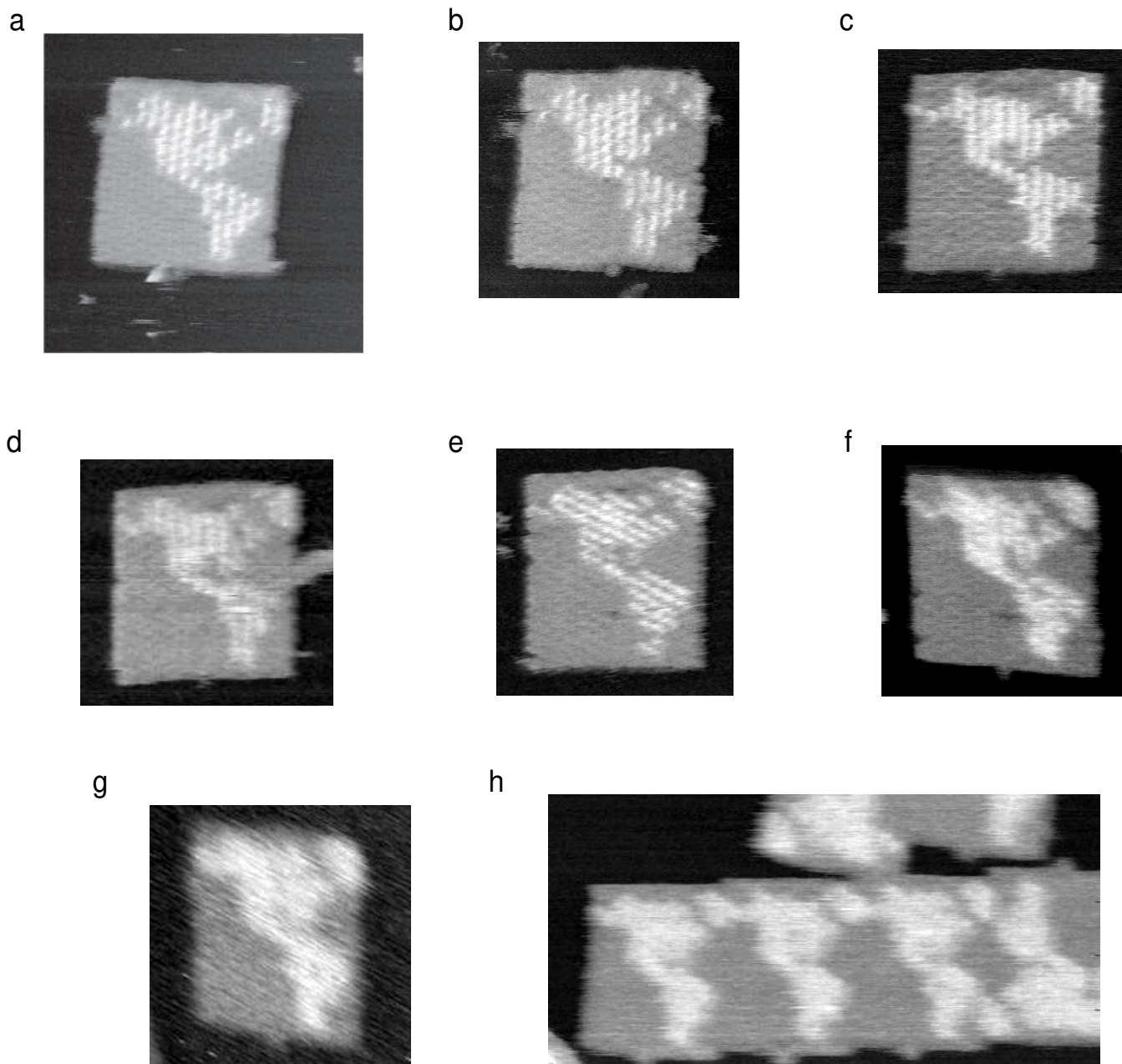
Supplementary Figure S51: A worksheet that allows easy preparation of a DNA pattern. Three 96 well plates are represented. Black circles mark wells that should be sampled from a set of 3 plates with unlabelled staple strands. White circles mark wells that should be sampled from a set of 3 homologous plates with dumbbell hairpin labels. Green circles represent the remainder strands that should be drawn independent of what pattern is being created. The pattern created using this mix of strands is diagrammed in the fourth quadrant.



Supplementary Figure S52: Extra images of 'DNA' pattern. **a** Enlarged view of Fig. 3b. **b-i** Other relatively high resolution images. 'u' indicates a rectangle with hairpins facing up; 'd' a rectangle with hairpins facing down. 42% ($S = 55$) of rectangles with the 'DNA' pattern were face-up. Pink arrow indicates the position and orientation of the same position in two images (**g** and **h**).



Supplementary Figure S53: Extra images of 'snowflake' pattern. **a** An uncorrected version (unsheared and unstretched) of Fig. 3d; this original was heavily distorted by AFM drift. **b** An uncorrected zoom out (none was required) of the structures from Fig. 3d showing surrounds. Green arrow indicates an area of damage in a neighboring structure, probably caused by the tip. **c–g** Extra relatively high resolution images. 'u' indicates hairpins are facing up; 'd' that hairpins are facing down. Rectangles in all these images have unbridged seams that occasionally dislocate slightly (white arrows, **d**) or grossly (white arrows, **c**). Sometimes a hairpin irreversibly disappears, see pink arrows in **f**, **g**.



Supplementary Figure S54: Extra images of the 'Map' pattern. **a** An uncorrected version (unsheared and unstretched) version of Fig. 3f; this original was distorted by AFM drift. **b-f** Other high resolution maps for comparison. Observe that there is no correlation between missing pixels. Depending on the batch of AFM tips used (Veeco NP-S, oxide sharpened), only 1 tip in 5 to 1 tip in 30 provides such resolution. **g,h** More typical (but still good) resolution.

Supplementary Note S7: Composition of shapes

To give sharp triangles specific binding interactions, staple strands along their edges were cut and pasted to yield two new types of staple strands: (1) extended staple strands that project 8 bases from the edge of the triangle which served as ‘donors’ and (2) truncated staple strands that left an 8 base section of the scaffold single-stranded available as an ‘acceptor site’. Given a sharp triangle as drawn in the design (with a particular face of the triangle facing up out of the page), extended staple strands were always positioned on the righthand half of the sharp triangle’s edge and truncated staple strands were always positioned on the lefthand half the sharp triangles edge. In this way, whenever the edges of two triangles met (with the faces of the triangle pointing in the same direction) the position of extended staple strands on one edge matched up with the position of the truncated staple strands on the other edge (and vice versa). If the sequences of the 8-base donor sections of the extended staple strands complemented the single-stranded acceptor portions of the scaffold, then the two edges could bind. Sequences are given in Supplementary Fig. S55.

The composition of sharp triangles into hexagons and lattices was only of limited success. While the interactions between sharp triangles were specific and as designed, the formation of hexagons and lattices were not quantitative. Several factors are likely to influence yields: (1) the stoichiometry of extended staple strands (2) the formation temperature of sharp triangles relative to the formation of inter-triangle bonds (3) the flexibility of inter-triangle bonds. There is much room for the optimization of these factors and quantitative composition of origami shapes into larger structures remains an open problem.

Because extended staple strands are not like normal staple strands in that they do not bind exclusively to a single scaffold strand, the stoichiometry of the extended staple strands immediately becomes important. For example, if a full 100-fold excess of extended staple strands were used, then excess staple strands could fill up all of the available single-stranded vacancies left by truncated staple strands. Ideally, there would be exactly 1 copy of each extended staple strand for each scaffold strand. Practically, because the stoichiometries of staple strands were uncertain, I used a range of extended staple:scaffold ratios (approximately 100, 20, 4 and 1) and retained the normal 100-fold excess for the rest of the staple strands. As expected, at a high relative excess of extended staple strands (100-fold) few triangles associated into hexagons. At a somewhat lower excess (20-fold) some hexagons formed. No matter what the ratio of extended staples to scaffold the yield of hexagons was low (Supplementary Fig. S56); the best results were obtained with a ratio of 4.

The formation temperature of sharp triangles relative to the formation of inter-triangle bonds is probably very important to the correct composition of triangles. Ideally, over the course of annealing, sharp triangles would form completely at a high temperature, and then only at a much lower temperature would weak inter-triangle bonds be strong enough to bring triangles together. If the bonds between triangles are too strong, then they will form at a temperature near that at which the sharp triangles themselves form and the sharp triangles may still be partially melted, disordered and floppy. This would seem to result in poorly formed structures.

The strength of inter-triangle interactions can be tuned by the number of extended staple strands that are used. I tried variations in which 2, 4, 8, and 16 staple strand bridges should have formed between sharp triangle edges (using subsets of the sequences in Supplementary Fig. S55). Hexagons and lattices formed with 4 and 8 staple strand bridges between edges but not for 2 and 16 staple strands. The experiments suggest that 2 bridges are too weak, and 16 bridges are too strong, for proper composition of triangles. These experiments are preliminary, however. Given the poor stoichiometry in the experiments, it is possible that on average 2 acceptor sites per edge were filled with excess extended staple strands. If this were true then it would explain why in 2-bridge experiments, few triangles bound each other. Further, the 16-bridge experiments are not really comparable with the others. In the 16-bridge experiments, the acceptor (left side) of a sharp triangle is left almost completely single-stranded and floppy because 8 truncated staple strands occur in a single row. This is in sharp contrast to the 4 and 8 bridge experiments in which truncated staple strands alternate with normal staple strands on the acceptor side of the edge; the normal staple strands potentially make the edge more rigid.

Finally, I note that the hexagon of triangles may, at 30 megadaltons, hold the record for the largest man-made molecular complex. It has $6 \times (7249 + 7156 + 980) = 92310$ nt (counting scaffold, staples and 35 hairpins/triangle at 28 bases each, not counting remainder strands) for a total molecular weight of 30.46 megadaltons (counting 330 daltons per nt). Individual unpatterned origami are all ~ 4.7 megadaltons. For comparison, the molecular weight of the eukaryotic ribosome is 4.2 megadaltons.

Truncated versions of the right hand side bottom row strands. To make lattices of sharp triangles, or hexagons these strands are used to create acceptor sites. Potentially all of them might be used for a lattice (since acceptors must be placed on 3 sides) but only 2/3 of them might be used for hexagons (since acceptors must be placed on 2 sides). The actual number depends on the number of donor/acceptor pairs desired. Here, the name of the strand indicates the position in the sharp triangle plates that it is meant to replace.

```
t1s10g-TR-P1-B2, A5, AATTAACGCGAATAAGTTTATTTCAGCGCC
t1s20g-TR-P1-G2, B5, CATTCAACTTAAGAGGAAGCCGATCAAAGCG
t1s30g-TR-P1-E3, C5, CACGTATACTGAAATGGATTATTTAATAAAG
t3s10g-TR-P1-E5, D5, GTAATTGATGGCAACATATAAAGCGATTGAG
t3s18g-TR-P1-H5, E5, AACTCCAAGATTGCATCAAAAGATAATCGAG
t3s20g-TR-P1-A6, F5, GGAATTACAGTCAGAAAGCAAAGCGCAGGTCAG
t3s28g-TR-P1-D6, G5, AGAGATAGTTTGACGCTCAATCGTACGTGCTT
t3s30g-TR-P1-E6, H5, AGCGGGAGATGGAATAACCTACATAACCCCTTC
t3s8g-TR-P1-H6, A6, CATTCAACAAACGCAAGACACCGAAGACCC
t5s10g-TR-P1-E8, B6, ACAAGAATGTTAGCAACGTAGAAAATTATTC
t5s18g-TR-P1-H8, C6, TAATTGCTTTACCTGACTATTATGAGGCATA
t5s20g-TR-P1-A9, D6, CATAACCCATCAAAAATCAGGTCTCCTTTTGA
t5s28g-TR-P1-D9, E6, GAATACGTAAACAGGAAAAACGCTCCTAAACAG
t5s30g-TR-P1-E9, F6, ATTTTAGATACCGCCAGCCATTGCGGCACAGA
t5s8g-TR-P1-H9, G6, TTGACGGAATACATACATAAAGGGCGCTAAT
t7s10g-TR-P1-B11, H6, AAGAAACATGGCATGATTAAGACTCCGACTTG
t7s20g-TR-P1-F11, A7, CCAAAATATTAAACAGTTTACAGAAATAGAGCT
t7s18g-TR-P1-E11, B7, CGGATGGCAGGAAATGACCATATCGTTTAC
t7s28g-TR-P1-A12, C7, CTATTGATATATCCAGAACATATCAGGAACG
t7s30g-TR-P1-B12, D7, AGAAGTGATATCGGCTTGCTGGTACTTTAATG
t7s8g-TR-P1-E12, E7, CACCGTCACCTTATTACGCGATTGAGTTAA
t9s10h-TR-P2-D1, F7, CGAAGCCCAACCAATAATAACGAAATCACCGAG
t9s18g-TR-P2-F1, G7, TGCTCTAGATCCCCCTCAAATGCTGCCAGAG
t9s20h-TR-P2-G1, H7, TTTGCCAGCATAAATATTCATTGACTCAACATGTT
t9s28g-TR-P2-A2, A8, TAAACATTTAGAAGAACTCAAACCTTTTATAA
t9s30h-TR-P2-B2, B8, GTAAAGAACATCACTTGCTGAGCGCCATTAAAA
t9s8g-TR-P2-D2, C8, GAGCCAGCGAATACCCAAAGAACATGAAATA
```

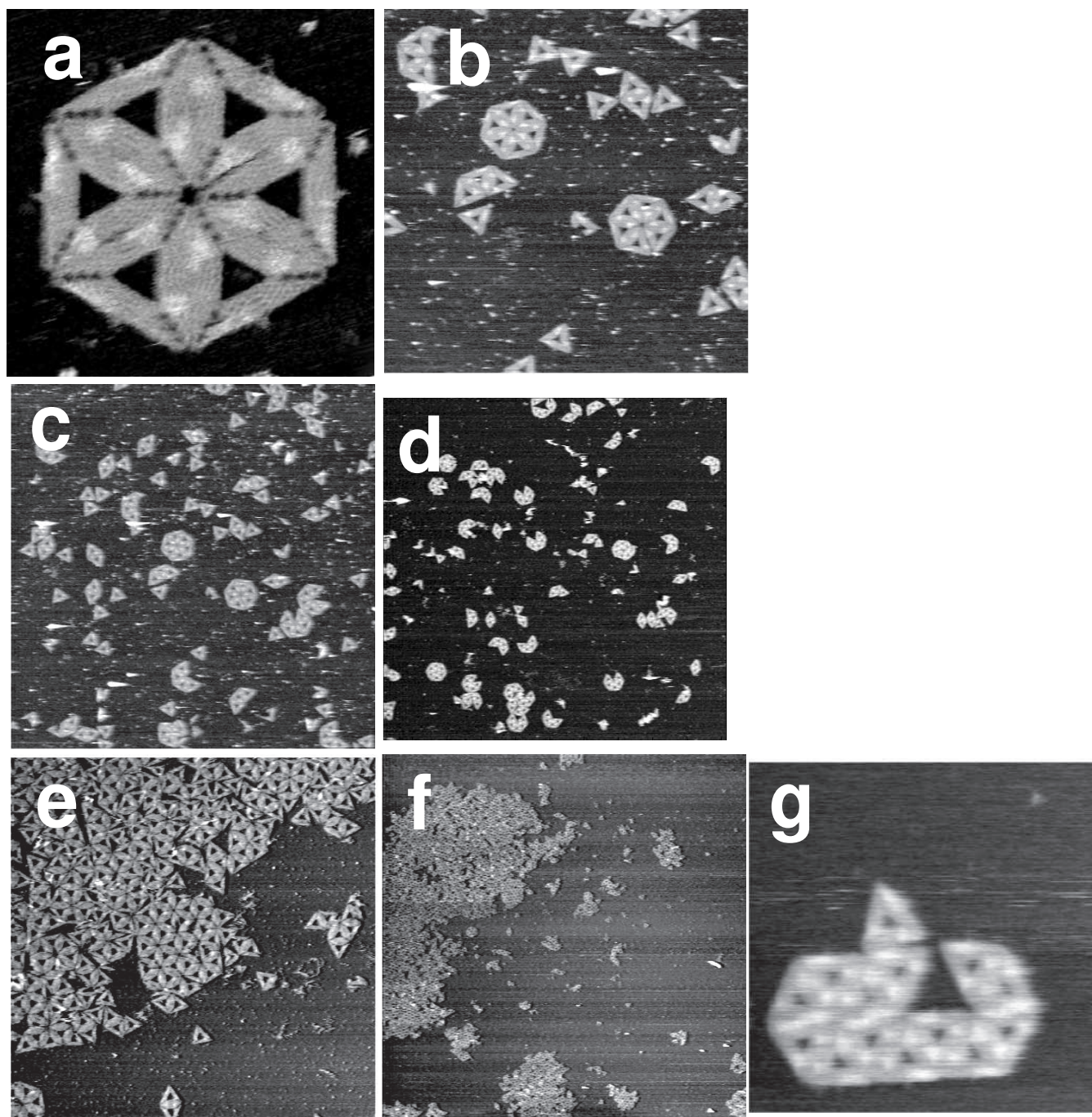
These strands have been extended to provide donors that make triangular lattice.
Here again the name of the strand gives its provenance.

```
t-10s17h-LT-P2-E2, A1, ACCAACCTAAAAATCAACGTAACAAATAAATGGGCTTGAGAAAAAGGT
t-10s27h-LT-P2-F2, B1, AACTCACATTATTGAGTGTGTTCCAGAAACCGTCTATCAGGGGCCACCGA
t-10s7h-LT-P2-G2, C1, ACGACAATAAATCCCGACTTGCGGGAGATCCTGAATCTTACCATATCTTTAC
t-1s18g-LT-P2-D4, D1, CGACCTGCGGTCAATCATAGGGAACGGAACAACATTATTGAATACCA
t-1s28g-LT-P2-D5, E1, TTTCCAGCCTTGCGCCTGAGAGAAAGCCGCGAAGCTGGTTGACGAG
t-1s8g-LT-P2-D6, F1, TTTCTTTAGCACTCATCGAGAACAATAGCAGCCTTTACAGGACGGGAG
* t-3s10g-LT-P2-B8, G1, TGAACAAAAAGCTCAAAAATGAAAGCAAGCCGTTTTTATGAAACCAA
t-3s18g-LT-P2-E8, H1, TATCATCTGTTGAAAGAGGACAGATGGAAGAAAAATCTACGCGCCAAAA
* t-3s20g-LT-P2-F8, A2, ATACATAATTAAATAAAGCAACTAACCGAAGTACCAACCTCCTGATAA
t-3s28g-LT-P2-A9, B2, GTTTGCGTCACGCTGGTTTGCCCAAGGGAGCCCGGATTAGAAATCAG
* t-3s30g-LT-P2-B9, C2, TCCTCGTTTAGAGCTTGACGGGGAGTTGCAGCAAGCGGTCAATTGGGCG
t-3s8g-LT-P2-E9, D2, AGCATGTATTTCATCGTAGGAATCAAACGATTTTTTGTGTTGTCAGAGG
* t-5s10g-LT-P2-B11, E2, ATCAGAGATCCCAATCCAAATAAGATTACCGCGCCCAATAAATAATAT
t-5s18g-LT-P2-E11, F2, CCAAGCGCAGCGCATAGGCTGGCAGAACTGGCTCATTATAACACTAT
* t-5s20g-LT-P2-F11, G2, GTAAGAGCAGCAGTACGAGCTTGGAAACGGTGTACAGACCGAAACAAA
t-5s28g-LT-P2-A12, H2, TTAATGAACTTTGATGGTGTCCGAGGTGSCCTAAAGCATTAAGGG
* t-5s30g-LT-P2-C12, A3, GAGGCGGACTAAATCGGAACCTTAAGCAGGCGAAATCCTTCGCCCAA
t-5s8g-LT-P2-E12, B3, ACAAGAAAGCAAGCAAAATCAGATAACGACCATATTATTAGATAACCC
* t-7s10g-LT-P3-G1, C3, GCCCAATAGCCAGTTACAAAATAATAGAAGGCTTATCCGGTTATCAAC
t-7s18g-LT-P3-A2, D3, AAAACACTTAATCTTGACAAGAACTTAATCATTGTGAATTGATAAAAA
* t-7s20g-LT-P3-B2, E3, CAGACGACACCTTATGCGATTTTATGACCTTCATCAAGAGCATCTTTG
t-7s28g-LT-P3-D2, F3, TTCCAGTCCTTATAAATCAAAAGAGAACCATCACCCAAATGAATCCTG
* t-7s30g-LT-P3-E2, G3, GTACGCCACAAGTTTTTTGGGGTCGAAATCGGCAAAATCCGGGAAACC
t-7s8g-LT-P3-G2, H3, GCGCCTGTTATTCTAAGAACGCGATTCCAGAGCCTAATTTATAAGAGC
* t-9s10g-LT-P3-F3, A4, GCAATAGCAGCTAACGAGCGTCTGGCGTTTTAGCGAACCCCAACATGT
t-9s20g-LT-P3-H3, B4, CTTTTGCATGGTTTTTAATTCAGCTCGGATATTCATTACCCACGAAAGA
* t-9s30g-LT-P3-B4, C4, TCAGTGAGCGATGGCCCACTACGTATAGCCCGAGATAGGATTCGCTT
```

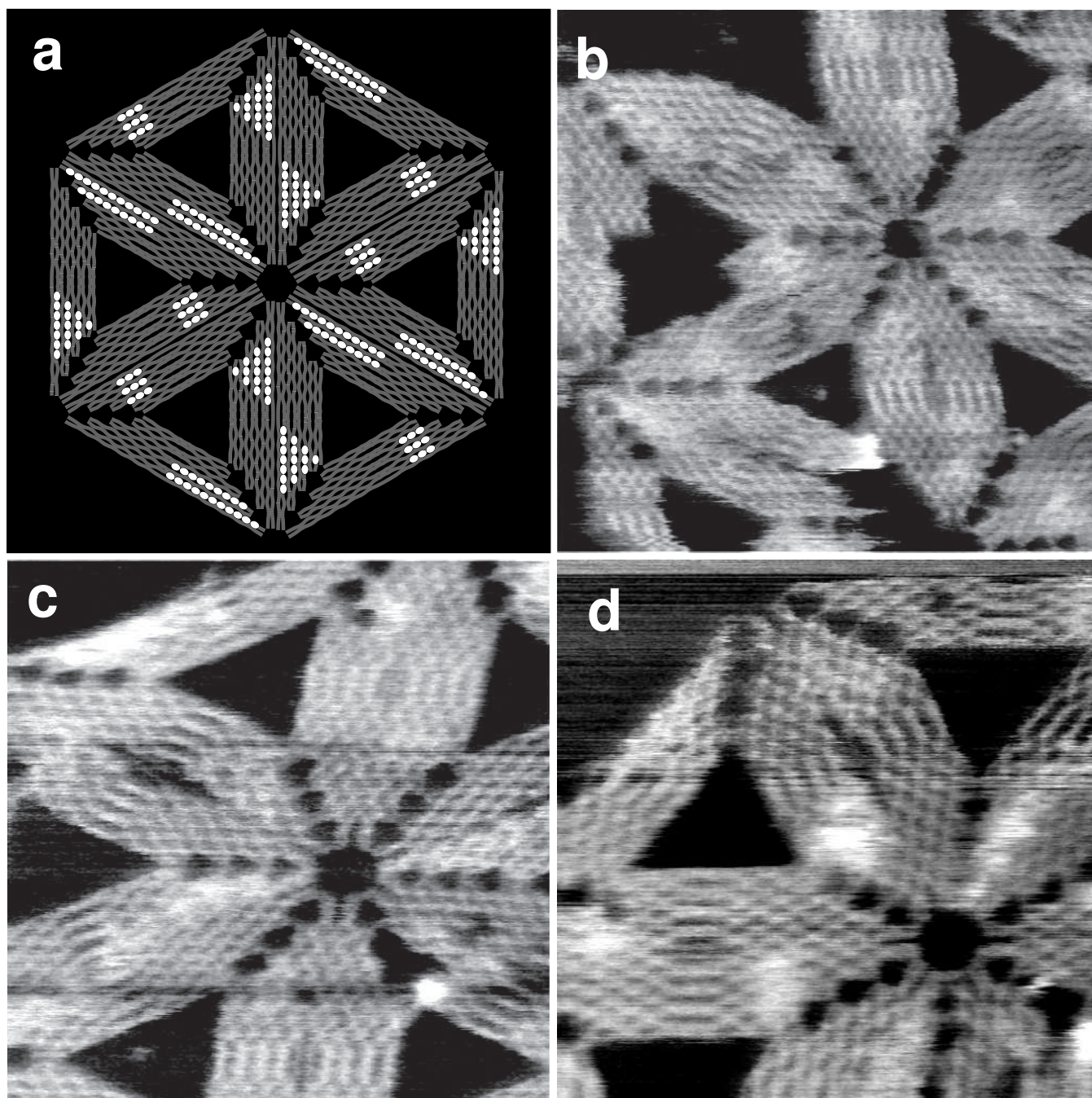
These strands have been extended to provide donors that make hexagons.

```
t-10s17h-HX-P2-E2, A6, ACCAACCTAAAAATCAACGTAACAAATAAATGGGCTTGAGATATCTTAC
t-10s7h-HX-P2-G2, B6, ACGACAATAAATCCCGACTTGCGGGAGATCCTGAATCTTACCAAAAGAAGT
t-1s18g-HX-P2-D4, C6, CGACCTGCGGTCAATCATAGGGAACGGAACAACATTATTGACGGGAG
t-1s8g-HX-P2-D6, D6, TTTCTTAGCACTCATCGAGAACAATAGCAGCCTTTACAGGAATACCA
* t-3s10g-HX-P2-B8, E6, ATACATAAAACGCTCAAAAATGAAAGCAAGCCGTTTTTATGAAACCAA
t-3s18g-HX-P2-E8, F6, TATCATCGTTGAAAGAGGACAGATGGAAGAAAAATCTACGGTCAGAGG
* t-3s20g-HX-P2-F8, G6, TGAACAAATTAATAAAACGAACTAACCGAACTGACCAACTCCTGATAA
t-3s8g-HX-P2-E9, H6, AGCATGTATTTTCATCGTAGGAATCAACGATTTTTTGTTCGCCAAAA
* t-5s10g-HX-P2-B11, A7, GTAAGAGCTCCCAATCCAAATAAGATTACCGCGCCCAATAAATAATAT
t-5s18g-HX-P2-E11, B7, CCAAGCGCAGGCGCATAGGCTGGCAGAACTGGCTCATTATGATAACCC
* t-5s20g-HX-P2-F11, C7, ATCAGAGAACCGCTCAGGACGTTGGAACGGTGTACAGACCCGAAACAAA
t-5s8g-HX-P2-E12, D7, ACAAGAAAGCAAGCAATCAGATAACAGCCATATTATTAAACACTAT
* t-7s10g-HX-P3-G1, E7, CAGACGACGCCAGTTACAAAATAATAGAAGGCTTATCCGGTTATCAAC
t-7s18g-HX-P3-A2, F7, AAAACACTTAATCTTGACAAGAACTTAATCATTGTGAATTATAAGAGC
* t-7s20g-HX-P3-B2, G7, GCCCAATAACCTTATGCGATTTTATGACCTTCATCAAGAGCATCTTTG
t-7s28g-HX-P3-G2, A8, GCGCCTGTTATTCTAAGAACGCGATTCCAGAGCCTAATTTGATAAAAA
* t-9s10g-HX-P3-F3, B8, CTTTTGCAACGCTAACGAGCGTCTGGCGTTTTAGCGGAACCCCACTGT
* t-9s20g-HX-P3-H3, C8, GCAATAGCTGGTTTTAATTTCAACTCGGATATTCATTACCCACGAAGA
```

Supplementary Figure S55: Staple strand variants for creating hexagons and lattices from triangles. Truncations provide acceptor sites, extended versions, donor sites. Stars (*) indicate extended staples that were used for the 8-bridge hexagons (8 starred strands) and 8-bridge lattices (12 starred strands) described in this paper. Four extended staples per side were used. Corresponding truncated strands were also used, and may be derived from the design.



Supplementary Figure S56: Extra images of the composition of triangles into hexagons and lattices. All structures shown here use 4 donor and 4 acceptor sites per edge (as depicted in Fig. 3n and o), for a total of 8 staple strand bridges between each pair of edges; all structures also use a 100-fold excess of normal staple strands. **a** Higher resolution image of hexagon from Fig. 3q. The tip-sample interaction force has been increased and hairpins marking the hexagon are less visible (but finer details appear.) **b** and **c**, Zoom-outs of Fig. 3q show that the fraction of hexagons formed is quite low $< 2\%$; the excess of extended staples is about 20-fold. **d** Another sample with a lower concentration of extended staples (about 4-fold excess) has fewer free triangles but no greater a number complete hexagons. **e** and **f**, Zoom-out of Fig. 3t shows large aggregates of triangles with only small well-ordered domains; the extended staple:scaffold ratio was roughly 1:1. **g** A well-formed 13-triangle aggregate at low resolution; the resolution is barely sufficient to show that the matching rules are correct in this structure. The extended staple excess was about 4-fold.



Supplementary Figure S57: High resolution images of the composition of triangles into lattices. Compare the fine structure of the model to AFM images. **a** Six triangles arranged in the bonding pattern used for the lattice. **NOTE:** this is **not** the hexagon, it is the inner segment of Fig. 3r rotated by 30 degrees. **b** a reproduction of Fig. 3u, only larger. **c** and **d** two more high resolution views. While damaged and suffering from AFM scan artifacts, these images show fine structure associated with crossovers well.

Supplementary Note S8: M13mp18 scaffold and staple strand secondary structure

Repetition of sequences (and their complements) in the scaffold and staple strands may cause them to have undesired binding to each other or to themselves (secondary structure). How much repetition can be tolerated is an interesting question. Clearly a poly-A scaffold cannot work, but could scaffolds limited to an AT alphabet fold robustly? Understanding such limits will require solving difficult combinatorial and thermodynamic problems. Here, I give examples of secondary structure and other undesired binding interactions in the M13mp18 scaffold and the staple strands, structure that was not too difficult to overcome. I argue that, for its length and base composition, M13mp18's sequence is not special in this regard—it is not a particularly “lucky” sequence with little secondary structure.

To get a feeling for M13mp18 secondary structure, I used Michael Zuker's DNA Mfold³⁷

<http://www.bioinfo.rpi.edu/applications/mfold/old/>

to obtain predicted foldings for 6000 base sections of M13mp18 sequence, as well as predicted foldings of 6000 base random sequences of similar base composition. All folds were computed at 20°C, 40 mM Na⁺ and 12 mM Mg²⁺. Rather than use the M13mp18 sequence reported in Genbank, I used the sequence found by New England Biolabs (NEB) the last time their M13mp18 clone was resequenced, (F.J. Stewart, NEB, 5/28/02).

Lowest energy folds for seven 6000 base segments of the M13mp18 sequence (using a sliding window, starting at $n = 1, 1001, 2001, 3001, 4001, 5001, 6001$ and 7001) were obtained. (I would have folded all 7249 bases at once, but 6000 is the Mfold server's limit). The strongest structure ($n=4001$, -1003 kcal/mole) and weakest structure ($n = 6001$, -904 kcal/mole) are shown in Supp. Fig. S58 and Supp. Fig. S59. I noticed strong secondary structure around base 5500. This structure, a series of several strong hairpins, is well-known structure of biological significance and occurs in the intergenic region (5500-6000) of M13³⁸. Of particular interest is a strong 20 base hairpin, [A], that has been enlarged in the inset of Supp. Fig. S58.

To get a quantitative measure of the predicted secondary structure I averaged free energies of folding (for the lowest energy structures). For the seven sections examined the average is -965 ± 37 kcal/mole. The large variation in energy is due to the fact that these 6000 base segments can be classed into two types. (1) Those that span the intergenic region ($n = 1001, 2001, 3001, 4001, 5001$) with its strong, biologically relevant secondary structure; they have an average energy of -990 ± 12 kcal/mole. (2) Those that don't span the intergenic region completely ($n = 1, n=6001, n=7001$) which have an average energy of -924 ± 24 .

To evaluate whether M13mp18's sequence has unusually strong or weak secondary structure, ten random 6000 base sequences were generated to have a base composition similar to the M13mp18 sequence (24.4% A 21.1% C 21.2% G 33.4% T, fixed at 1462 A's, 1266 C's, 1270 G's, 2002 T's). Supp. Fig. S60 shows the lowest energy fold for one of these sequences; visually it appears that the M13mp18 sequence (Supp. Fig. S58 and 59) has secondary structure similar to that of a random sequence of similar length. However, the average calculated energy for random sequences is significantly less than that for M13mp18's sequence, -867 ± 13 kcal/mol. Thus it seems that M13mp18's sequence has somewhat stronger secondary structure than would be expected.

The secondary structure of M13mp18 DNA does appear to be less strong than that predicted for sequences of even base composition. For comparison, ten 6000 base sequences of composition A=G=C=T=1500 have an average calculated free energy of -1080 ± 21 . To explore the strong effect that base composition has on secondary structure, I looked at two more examples. For A=G=T=2000 and C=0 the predicted average energy is -157 ± 13 . And for A=C=T=2000 and G=0 the predicted average energy is -93 ± 6 . In the event that secondary structure becomes a limiting factor in the creation of DNA origami, such skewed base compositions might be used but at the cost of specificity in staple-scaffold binding.

Of all the potential secondary structure that the M13mp18 sequence has, only the [A] loop was deemed worrisome enough to be avoided. Mfold predicts the structure of loop [A] as a hairpin, (20 nt stem, 4 G-T mismatches, $\Delta G = -14.4$ kcal/mole) at positions 5515-5557. The sequence at these positions is:

GGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCC

In designing DNA origami, I decided to avoid the entire 73 base section (5515-5587).

GGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCGCTCCTTTTCGCTTTC

This allowed me to linearize M13mp18 DNA by incubating with the complement of bases (5558-5587)

GAAAGCGAAAGGAGCGGGCGCTAGGGCGCT

and cutting with BsrB I between positions 5573 and 5574. (BsrB I has recognition sequence, CCGCTC at 5571-5576; it leaves a blunt end between bases 3 and 4.) Because I did not purify the linearized DNA away from the complement used to linearize it, in all designs I avoided the entire 73 base section. I don't know if this is necessary.

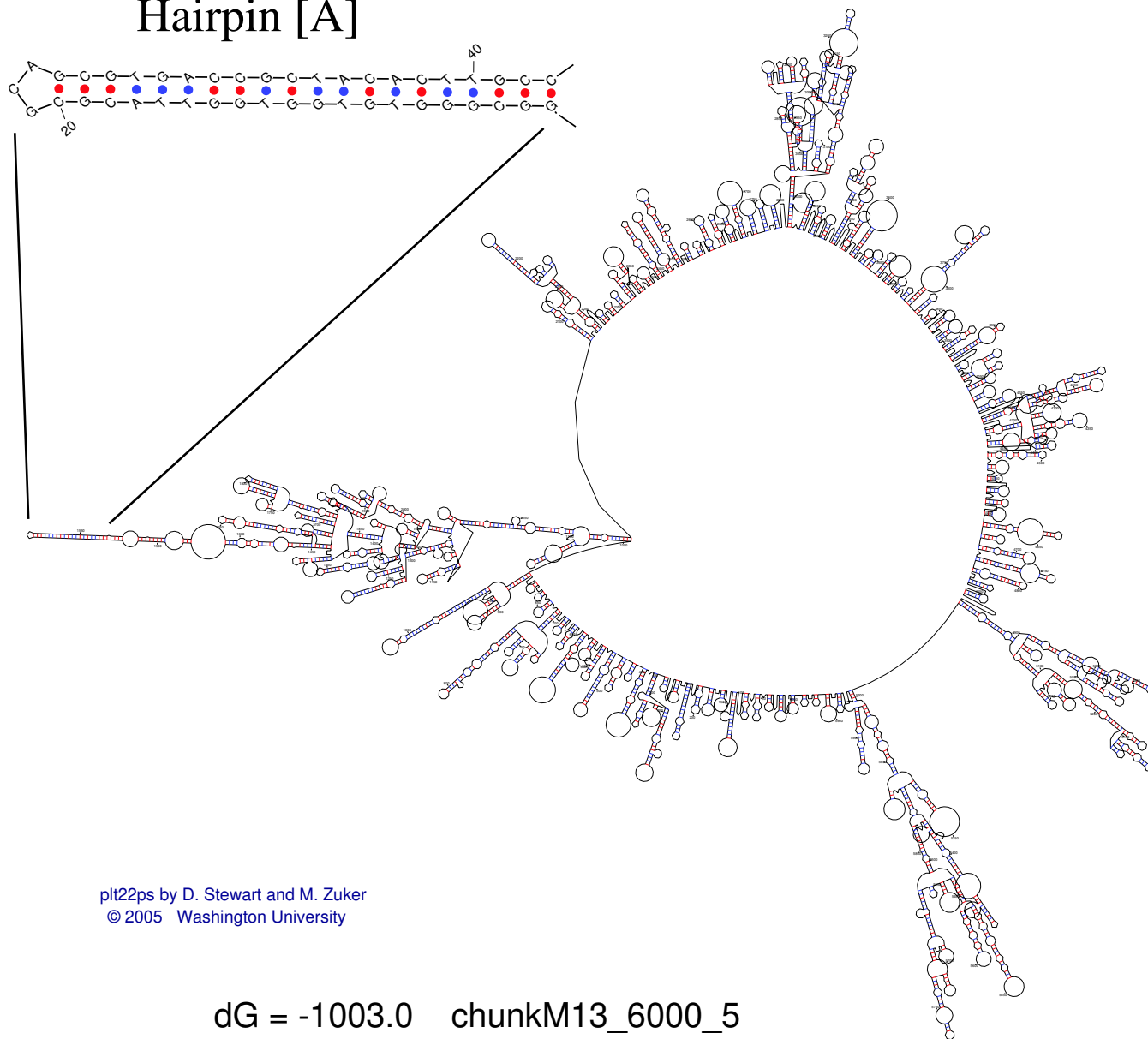
Other less strong but well known secondary structure in the scaffold did not seem to cause problems. For example, the hairpin [C] GGGTGATGGTTCACGTAGTGGGCCATCGCCC has a 14 base-pair stem. Occurring at bases 5704-5734, this sequence is 116 nucleotides into the origami structures. It occurs, for example in the rectangle on the bottom edge of the lower left corner, a position that suffers no apparent defects.

Finally, I note that staple strands themselves may have unintended secondary structure or binding interactions. By concatenating the sequences of staple strands with 'NNNN' linkers between them and folding the resulting sequence with Mfold, I was able to find a couple potential bindings between different staple strands (with lengths of 10 and 11 nucleotides and having single G-T mispairs) as well as some secondary structure within single staple strands. For example, the rectangle staple strand r7t22e, GCCAACAGTCACCTTGCTGAACCTGTTGGCAA can form an 8 base hairpin:

```
GCCAACAGTCACCTT\
|||||||      |
AACGTTGTCCAAGTCG/
```

Such secondary structure would normally be considered unacceptable in the design a DNA nanostructure. The next section explores reasons for why scaffold and staple secondary structure might not cause problems for the formation of scaffolded DNA origami.

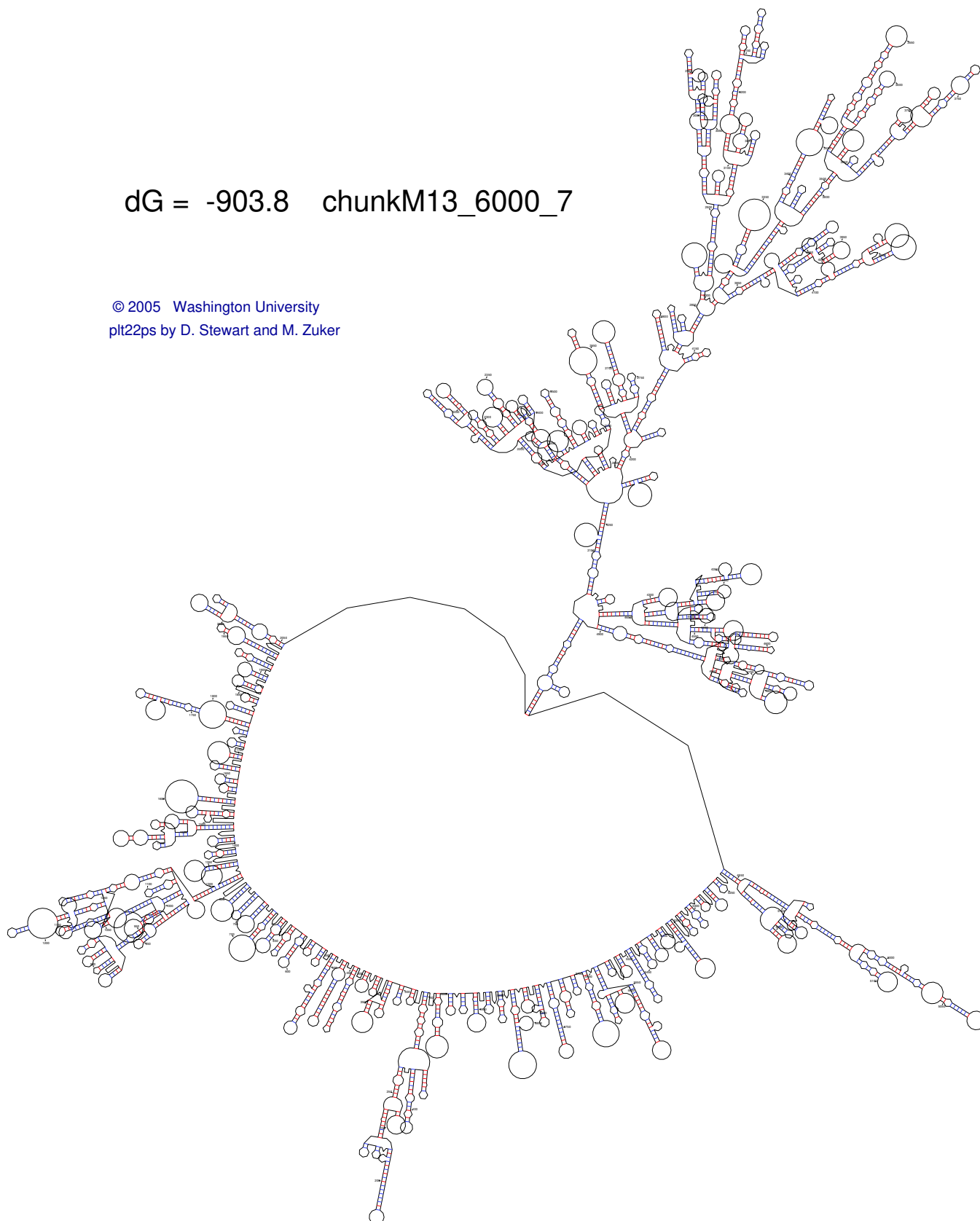
Hairpin [A]



Supplementary Figure S58: A 6000 base chunk of M13mp18 sequence spanning the intergenic region.

dG = -903.8 chunkM13_6000_7

© 2005 Washington University
plt22ps by D. Stewart and M. Zuker



Supplementary Figure S59: A 6000 base chunk of M13mp18 sequence that skips the intergenic region.

dG = -858.4
rand_M13A_6000_2

plt22ps by D. Stewart and M. Zuker
© 2005 Washington University



Supplementary Figure S60: A 6000 base random sequence with the same base composition as M13mp18's sequence.

Supplementary Note S9: Why does scaffolded DNA origami work?

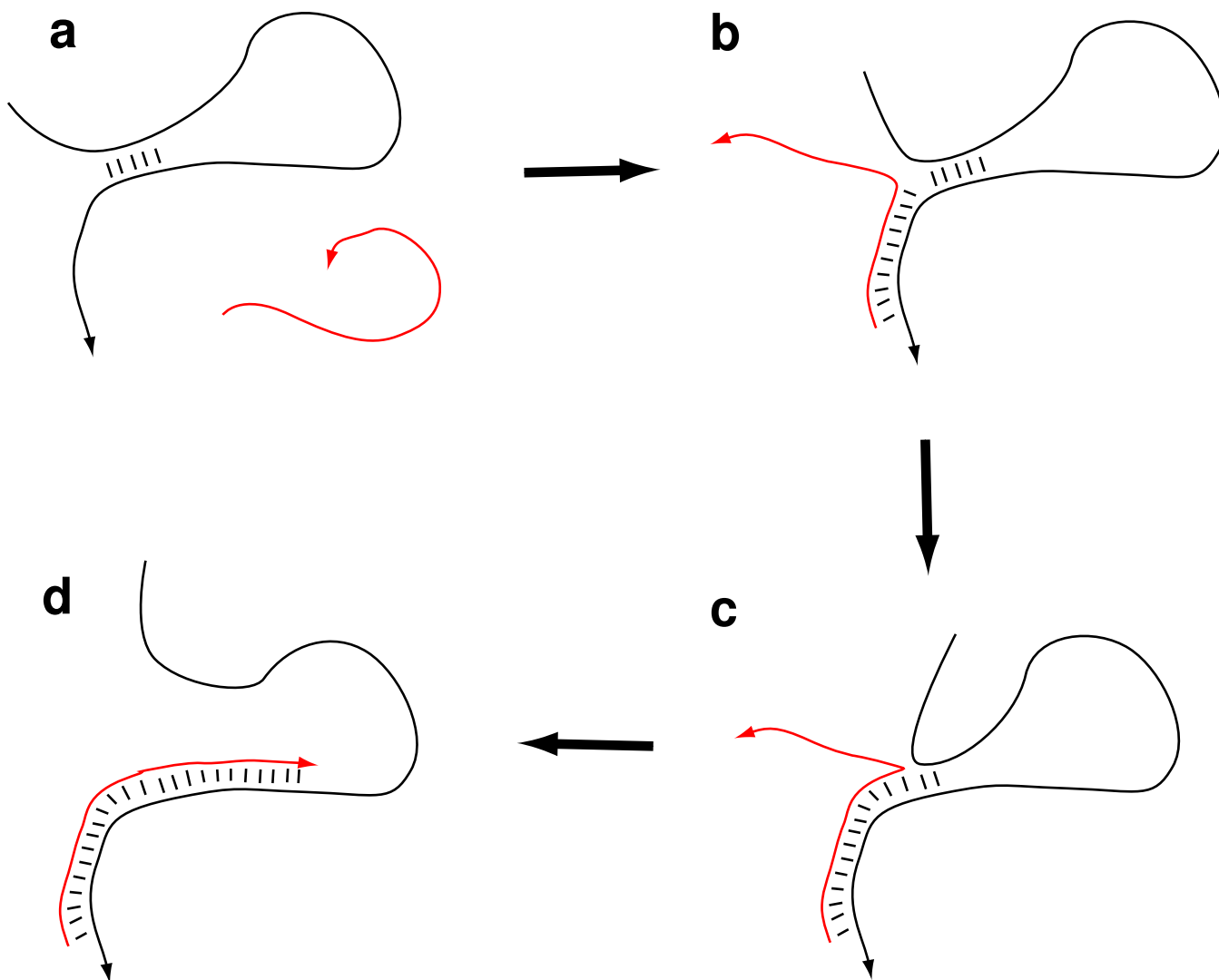
Consider any secondary structure that the scaffold might assume. It is unlikely that this secondary structure perfectly blocks the binding sites for all the staple strands that should bind its sequence. Thus staple strands may bind by partial matches at first (to gain a ‘toehold’), and then participate in a branch migration that displaces the secondary structure (Supplementary Fig. S61). A longer region of complementarity between the staple and the scaffold stabilizes the staple-scaffold interaction over the scaffold secondary structure. The excess of staple strands may help drive this process. Explicit use of strand displacement to actuate nanomachines appears in^{39, 40, 41, 42}. (It was Bernie Yurke’s work on DNA motors that convinced me that strand invasion might make scaffolded DNA origami possible.)

Another factor that may work against scaffold secondary structure is the role of staple strands as intramolecular bridges. Each successful addition of a staple strand organizes the scaffold for subsequent binding of adjacent staple strands and constrains the scaffold in a way that precludes a large set of undesired secondary structures. Thus one might expect the binding of staple strands to be highly cooperative. To see why intramolecular interactions may be important, consider cutting a scaffolded shape into a multi-stranded structure based on unique tiles (for which the minimum free energy state should be the scaffolded shape, just with more backbone nicks). For such a system the addition of a tile at any one position does not significantly constrain the global structure.

Next consider the interactions of staple strands with themselves. Many strong complexes exist between them; none is a perfect match, however. The scaffold can displace such structure and gain a required staple strand.

Now consider purity. A truncated staple strand might bind to the scaffold. However, because of the excess of staple strands, there exist many full length length staple strands that can bind and displace the truncated strand. This means that only the purity of the scaffold matters; because the scaffold is derived from a biological source, it is very pure.

In a similar way, because *staple strands do not bind to each other*, the relative stoichiometry between the staple strands does not matter. With staple strands in excess over the scaffold, the remaining relevant concentration is the effective local concentration of scaffold in intramolecular events. Here the intramolecular nature of scaffold folding enforces a kind of equimolarity—any two sections of the scaffold that are brought together by a staple strand are by definition, equimolar. Again, such could not be said for the same sections if the scaffolded structure were cut into multi-stranded unique tiles. This highlights a crucial difference between the scaffolded method shown here and that previously proposed^{17,18}. In the latter scheme the scaffold runs through *every other* helix; the structure is held together by interactions between multi-stranded tiles and so the *staple strands must bind to each other*. For such schemes precise equimolarity is likely important.



Supplementary Figure S61: Opening of scaffold secondary structure by strand displacement. **a** Five bases of undesired secondary structure in the scaffold occur in the middle of the binding site for the red staple. **b** The red staple strand can still bind by 10 bases adjacent to the hairpin stem and gain a ‘toehold’. **c** A random walk at the junction between the staple and hairpin allows the staple strand to gain three more basepairs. **d** Eventually the random walk results in the hairpin opening, which allows the rest of the staple to bind.

Supplementary Note S10: Cost

The going rate for oligonucleotides synthesized unpurified, in plates, at the 100 nmole scale is between \$.18 and \$.25 per nucleotide (USD). Thus a 32-mer oligonucleotide costs about \$7 for about 80 nmol of material. (Additional PAGE purification costs \$40-80 per oligonucleotide and yields, on average, 10 nmol of material. Thus per nmol, purified oligonucleotides are about 50× as expensive as unpurified ones.) The total cost then, for about 80 nmol of each staple strand to complement the 7249 base pair M13mp18 scaffold is \$1500; staples thus cost about \$19/nmol. Four picomoles (10 micrograms) of scaffold was purchased from New England Biolabs for \$30; scaffold thus cost \$7500/nmol. Using a 100-fold excess of staple strands, the cost of DNA origami (of the current size) would be $\$7500 + \$1900 = \$9400$ per nmol of which the scaffold strand is 80% of the cost.

Recent controls have shown two things: 1) results using only a 10-fold excess of staple strands are indistinguishable from those with a 100-fold excess and 2) results using a 10-fold cheaper source of M13mp18 DNA (Bayou Biolabs) yield results indistinguishable from those with New England Biolabs. The net result of these observations is that, if these two modifications to the protocol were implemented, the cost of DNA origami would be closer to \$1000 per nmol but that the scaffold strand would still be 80% of the cost of the origami.

In sum, the price of synthetic oligonucleotides does not, as one might expect, dominate the cost of DNA origami. In principle excess staple strands might be recycled—removed from the reaction after folding of the scaffold is complete—further decreasing their contribution to the cost of origami.

Supplementary Note S11: Additional references

- [24] LaBean, T., Yan, H., Kopatsch, J., Liu, F., Winfree, E., Reif, J., and Seeman, N. Construction, analysis, ligation, and self-assembly of DNA triple crossover complexes. *J. Am. Chem. Soc.* **122**, 1848–1860 (2000).
- [25] Winfree, E., Liu, F., Wenzler, L., and Seeman, N. Design and self-assembly of two-dimensional DNA crystals. *Nature* **394**, 539–544 (1998).
- [26] Wang, J. C. Helical repeat of DNA in solution. *Proc. Nat. Acad. Sci. USA* **76**, 200–203 (1979).
- [27] Rhodes, D. and Klug, A. Helical periodicity of DNA determined by enzyme digestion. *Nature* **286**, 573–578 (1980).
- [28] Kallenbach, N., Ma, R.-I., and Seeman, N. An immobile nucleic-acid junction constructed from oligonucleotides. *Nature* **305**, 829–831 (1983).
- [29] Murchie, A., Clegg, R., von Kitzing, E., Duckett, D., Diekmann, S., and Lilley, D. Fluorescence energy transfer shows that the four-way DNA junction is a right-handed cross of antiparallel molecules. *Nature* **341**, 763–766 (1989).
- [30] Duckett, D., Murchie, A., Diekmann, S., von Kitzing, E., Kemper, B., and Lilley, D. The structure of the Holliday junction, and its resolution. *Cell* **55**, 79–89 (1988).
- [31] Sha, R., Liu, F., and Seeman, N. Direct evidence for spontaneous branch migration in antiparallel DNA Holliday junctions. *Biochemistry* **39**, 11514–11522 (2000).
- [32] von Kitzing, E., Lilley, D., , and Diekmann, S. The stereochemistry of a four-way dna junction: a theoretical study. *Nucleic Acids Research* **18**(9), 2671–2683 (1990).
- [33] Eis, P. and Millar, D. Conformational distributions of a four-way junction revealed by time-resolved fluorescence resonance energy transfer. *Biochemistry* **32**(50), 13852–13860 (1993).
- [34] Jr., M. R., Anderson, C., and Lohman, T. Thermodynamic analysis of ion effects on the binding and conformational equilibria of protein and nucleic acids: the roles of ion association or release, screening, and ion effects on water activity. *Quarterly Reviews of Biophysics* **11**, 103–178 (1978).
- [35] Puglisi, J. and I. Tinoco, J. Absorbance melting curves of RNA. In *Methods in Enzymology: RNA Processing*, Dahlberg, J. and Abelson, J., editors, volume 180, 304–325 (Academic Press, New York, 1989).
- [36] Yanisch-Perron, C., Vieira, J., and Messing, J. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**(1), 103–119 (1985).
- [37] Zuker, M. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research* **31**(13), 3406–3415 (2003).
- [38] Zinder, N. and Horiuchi, K. Multiregulatory element of filamentous bacteriophages. *Microbiological Reviews* **49**(2), 101–106 (1985).
- [39] Yurke, B., Turberfield, A., Mills, Jr., A., Simmel, F., and Neumann, J. A DNA-fuelled molecular machine made of DNA. *Nature* **406**, 605–608 (2000).
- [40] Simmel, F. and Yurke, B. Using DNA to construct and power a nanoactuator. *Physical Review E* **63**, 041913–1–5 (2001).
- [41] Yan, H., Zhang, X., Shen, Z., and Seeman, N. A robust DNA mechanical device controlled by hybridization topology. *Nature* **415**, 62–65 (2002).
- [42] Turberfield, A., Mitchell, J., Yurke, B., Mills, Jr., A., Blakey, M., and Simmel, F. C. DNA fuel for free-running nanomachines. *Physical Review Letters* **90**(11), 118102–1–4 (2003).