DNA Origami
DNA Origami Papers

1. 2005, Rothemund – Design of DNA Origami
2. Mar 2006, Rothemund – Folding DNA to create nanoscale shapes and patterns
3. May 2009, Shih et al – Self-assembly of DNA into nanoscale three-dimensional shapes
4. Aug 2009, Shih et al – Folding DNA into Twisted and Curved Nanoscale Shapes
5. Apr 2009, Andersen et al - Self-assembly of a nanoscale DNA box with a controllable lid
6. April 2011, Han et al – DNA Origami with Complex Curvatures in Three-Dimensional Space
7. Nov 2011, Arbona et al – Modeling the folding of DNA Origami
8. Nov 2009, Shih et al – Multilayer DNA Origami Packed on a Square Lattice

Other interesting papers (to be posted)
DNA Origami

TOP DOWN

• Cost
• Serial
• Ultra-high vacuum, ultra-clean conditions or cryogenic temperatures

→ Need to exploit properties for directed self-organization (bottom-up)

2006 - Folding DNA to create nanoscale shapes and patterns
Derivation of DX and TX Molecules

DS + DS

a

+ 

2 Reciprocal Exchanges

Resolve Twice

DX

b

+ 

2 Reciprocal Exchanges

Resolve Twice

TX

Derivation of DX and TX Molecules

“Parallel double-crossover molecules with an odd number of half-turns between branch points will contain an excess major or minor groove isolated between the two crossovers. This region must therefore be designed to differ from the number of residues per helical turn, N, by approximately $\phi N$ or $(1 - \phi)N$ residues, where $\phi$ is the golden ratio (0.618), as this is roughly the angular difference between the major and minor grooves of B-DNA (Harel et al., 1986). We use 16 residues between branch points to produce a molecule with an excess major-groove (wide) (DPON) spacing and 14 residues between branch points to produce a molecule with an excess minor-groove (narrow) (DPON) spacing.”

...helpers or by self-interactions.

Origami' because a single long strand is folded, whether by many...design approaches are termed 'multi-stranded', 'scaffolded', composed of one long strand and few or no helpers as in Fig. 1c. Here...as in Fig. 1a, (2) composed of one long 'scaffold strand' (black)...strands, being (1) composed entirely of short oligonucleotide strands...designs may be classified by how they are built up from component...technology paradigm, a couple major distinctions can be drawn. First,...disadvantages of different approaches. Within the DNA nanotechnology...that allows the creation of arbitrary patterns and shapes?

To answer this question, one must understand the advantages and...DNA origami (and is the subject of this paper).

...introduced by double-crossover molecules is simple using scaffolded...more strongly to mica surfaces than helpers do and so excess helpers...helpers, separating them is not difficult (e.g. large origami stick much...in misfoldings). Because origami are easily differentiable from the...prevent reorientation. Because they are multi-stranded structures the cube and truncated octahedron do not suffer from this problem but generalization...problem of equalizing ratios of strands by allowing an excess of...purification may be required. Because, for large and complex designs,...to arbitrary geometries seems difficult (perhaps not enough thought...Newnan and coworkers recently described an algorithmic approach for molecules; this is what algorithmic self-assembly is about.

Since the cube, truncated octahedron and octahedron are all uniquely...comprising a single DNA molecule. We have previously reported that...as the octahedron) do not suffer from this problem but generalization...to exactly equal. If there is not an equal proportion of the various...purposes of this section are two-fold: to review the method and describe some issues in the algorithm...create structures). 3-prime ends (usually written 3', he...creates a unique structure. In this situation Watson-Crick binding directs each strand to...is no ambiguity as to which strands should stick where in a final...is no ambiguity as to which strands should stick where in a final

...Multi-stranded (uses multiple strands to create structures)

- Short strand ratios must be EXACTLY EQUAL

multi-stranded

2006 - Folding DNA to create nanoscale shapes and patterns
- Single-stranded (using a single strand only – without helpers/stapes - to create a structure)

**Fig. 1.** Examples of non-canonical, branched DNA structures. 3-prime ends (usually written 3', here ‘3’) of DNA strands are marked by arrowheads.
• **Origami**
  – Allows excess of helpers to be used
  – Large origami stick more to mica than helpers
  – Uniquely addressable

**Fig. 1.** Examples of non-canonical, branched DNA structures. 3-prime ends (usually written 3’, here ‘3”) of DNA strands are marked by arrowheads.

2006 - **Folding DNA to create nanoscale shapes and patterns**
2005 – Design of DNA Origami

- Origami → folding
- Not yet used term “staples”, called “helpers” (later)
- Describes the design steps (reiterated in 2006 paper). **This paper explains the method much more clearly for CS people like me**
- Used Matlab to design origami (clunky → never released → made obsolete anyway...)
2006 - Folding DNA to create nanoscale shapes and patterns

• Paul Rothemund
• Designed in five steps (first two by hand, last three by computer)
• One-pot reaction
Design: Step 1

- y depends on the gap between helices
- Gap depends on spacing of crossovers
- Can use any odd number of half-turns

10.67 bases ≈ 1 turn
3.6 nm in length

16 bases ≈ 1.5 turns between crossovers along a helix

2006 - Folding DNA to create nanoscale shapes and patterns
Design: Step 2

- Vertical raster reversal, 3 turns
- Raster progression, 4.5 turns

- ‘scaffold crossovers’
- Distance must be an odd number of half-turns
- Seam $\rightarrow$ contour that the path does not cross
Design: Step 2 - Rules

• ‘inter-helix gap’ depends on crossover spacing
• Seeman + Fu → crossover points must be multiples of half-turns
  – Even half-turns on reversal
  – Odd half-turns otherwise
Design: Step 3

- 250 staples, each 32 bases long.
- Provide complements for scaffold

2006 - Folding DNA to create nanoscale shapes and patterns
Design: Step 4

Folding DNA to create nanoscale shapes and patterns
Design: Step 5

Conceptually, the second step (illustrated in Fig. 1b) proceeds by folding a single long scaffold strand (900 nucleotides (nt) in Fig. 1b) back and forth in a raster fill pattern so that it comprises one of the two strands in every helix; progression of the scaffold from one helix to another creates an additional set of crossovers, the 'scaffold crossovers' (indicated by small red crosses in Fig. 1b). The fundamental constraint on a folding path is that the scaffold can form a crossover only at those locations where the DNA twist places it at a tangent point between helices. Thus for the scaffold to raster progressively from one helix to another and onto a third, the distance between successive scaffold crossovers must be an odd number of half-turns. Conversely, where the raster reverses direction vertically and returns to a previously visited helix, the distance between scaffold crossovers must be an even number of half-turns. Note that the folding path shown in Fig. 1b is compatible with a circular scaffold and leaves a 'seam' (a contour which the path does not cross). Once the geometric model and a folding path are designed, they are represented as lists of DNA lengths and offsets in units of half-turns. These lists, along with the DNA sequence of the actual scaffold to be used, are input to a computer program. Rather than assuming 10.5 base pairs (bp) per turn (which corresponds to standard B-DNA twist), the program uses an integer number of bases between periodic crossovers (for example, 16 bp for 1.5 turns). It then performs the third step, the design of a set of 'staple strands' (the coloured DNA strands in Fig. 1c) that provide Watson–Crick complements for the DNA lattices.

Figure 1

Design of DNA origami.

a, A shape (red) approximated by parallel double helices joined by periodic crossovers (blue).
b, A scaffold (black) runs through every helix and forms more crossovers (red).
c, As first designed, most staples bind two helices and are 16-mers.
d, Similar to c with strands drawn as helices. Red triangles point to scaffold crossovers, black triangles to periodic crossovers with minor grooves on the top face of the shape, blue triangles to periodic crossovers with minor grooves on bottom. Cross-sections of crossovers (1, 2, viewed from left) indicate backbone positions with coloured lines, and major/minor grooves by large/small angles between them. Arrows in c point to nicks sealed to create green strands in d. Yellow diamonds in c and d indicate a position at which staples may be cut and resealed to bridge the seam.
e, A finished design after merges and rearrangements along the seam. Most staples are 32-mers spanning three helices. Insets show a dumbbell hairpin (d) and a 4-T loop (e), modifications used in Fig. 3.

2006 - Folding DNA to create nanoscale shapes and patterns
Design of DNA origami.

With strands 2006	
-­‐
  -­‐
 2006
 10.5 base pairs (bp) per turn (which corresponds to standard B-DNA
2006
 16 March 2006

In this study, data are consistent with an inter-helix gap of 1 nm
2006
16 March 2006

once the geometric model and a folding path are designed, they
2006
16 March 2006

Once the geometric model and a folding path are designed, they
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

Raster progression, 4.5 turns
2006
16 March 2006

2006 - Folding DNA to create nanoscale shapes and patterns
Scaffold and create the periodic crossovers. Staples reverse direction at these crossovers; thus crossovers are antiparallel, a stable configuration well characterized in DNA nanostructures. Note that the crossovers in Fig. 1c are drawn somewhat misleadingly, in that single-stranded regions appear to span the inter-helix gap even though the design leaves no bases unpaired. In the assembled structures, helices are likely to bend gently to meet at crossovers so that only a single phosphate from each backbone occurs in the gap (as ref. 16 suggests for similar structures). Such small-angle bending is not expected to greatly affect the width of DNA origami (see also Supplementary Note S2).

The minimization and balancing of twist strain between crossovers is complicated by the non-integer number of base pairs per half-turn (5.25 in standard B-DNA) and the asymmetric nature of the helix (it has major and minor grooves). Therefore, to balance the strain caused by representing 1.5 turns with 16 bp, periodic crossovers are arranged with a glide symmetry, namely that the minor groove faces alternating directions in alternating columns of periodic crossovers (see Fig. 1d, especially cross-sections 1 and 2). Scaffold crossovers are not balanced in this way. Thus in the fourth step, the twist of scaffold crossovers is calculated and their position is changed (typically by a single bp) to minimize strain; staple sequences are recomputed accordingly. Along seams and some edges the minor groove angle (150°) places scaffold crossovers in tension with adjacent periodic crossovers (Fig. 1d, cross-section 2); such situations are left unchanged.

Wherever two staples meet there is a nick in the backbone. Nicks occur on the top and bottom faces of the helices, as depicted in Fig. 1d. In the final step, to give the staples larger binding domains with the scaffold (in order to achieve higher binding specificity and higher binding energy which results in higher melting temperatures), pairs of adjacent staples are merged across nicks to yield fewer, longer, staples (Fig. 1e). To strengthen a seam, an additional pattern of breaks and merges may be imposed to yield staples that cross the seam; a seam spanned by staples is termed 'bridged'. The pattern of merges is not unique; different choices yield different final patterns of nicks and staples. All merge patterns create the same shape but, as shown later, the merge pattern dictates the type of grid underlying any pixel pattern later applied to the shape.

Folding M13mp18 genomic DNA into shapes

To test the method, circular genomic DNA from the virus M13mp18 was chosen as the scaffold. Its naturally single-stranded 7,249-nt sequence was examined for secondary structure, and a hairpin with a 20-bp stem was found. Whether staples could bind at this hairpin was unknown, so a 73-nt region containing it was avoided. When a linear scaffold was required, M13mp18 was cut (in the 73-nt region) by digestion with BsrBI restriction enzyme. While 7,176 nt remained available for folding, most designs did not fold all 7,176 nt; short (~25 nt) ‘remainder strands’ were added to complement unused sequence. In general, a 100-fold excess of 200–250 staple and remainder strands were mixed with scaffold and annealed from

---

**Figure 2 | DNA origami shapes.** Top row, folding paths. a, square; b, rectangle; c, star; d, disk with three holes; e, triangle with rectangular domains; f, sharp triangle with trapezoidal domains and bridges between them (red lines in inset). Dangling curves and loops represent unfolded sequence. Second row from top, diagrams showing the bend of helices at crossovers (where helices touch) and away from crossovers (where helices bend apart). Colour indicates the base-pair index along the folding path; red is the 1st base, purple the 7,000th. Bottom two rows, AFM images. White lines and arrows indicate blunt-end stacking. White brackets in a mark the height of an unstretched square and that of a square stretched vertically (by a factor >1.5) into an hourglass. White features in f are hairpins; the triangle is labelled as in Fig. 3k but lies face down. All images and panels without scale bars are the same size, 165 nm × 165 nm. Scale bars for lower AFM images: b, 1 μm; c–f, 100 nm.
Method?

• 250 helper strands, each 32 bases long
• 5 ml drop from each strand solution
• Buffer (pH control)
• Magnesium salt (Magnesium Mg++ ions neutralize negative charges on the DNA and allow the single-stranded DNA to come together and form the double helix)
  - AFM imaging under buffer w/out purification.
  - Samples deposited on mica
    - only folded DNA structures stuck to the surface
    - excess staples remained in solution
• Heating to 90 C then cooling to 20 C in under 2 hrs (in 2006 papers, says 95 C in over 2 hours).

2006 - Folding DNA to create nanoscale shapes and patterns
sequences, or rearrangement of helper strands to bridge seams, should will be preferred. Similarly, the merging of helper strands into longer acceptable in order to better approximate a desired curve; within a strain should be similarly automatic and similarly subject to some routing around voids. The adjustment of some raster-fill algorithms should be used to sign software. Users should be able to specify a shape and the structure are decided by the user and specified in excruciating detail. the geometrical model and folding path, these perturbations to the minimize twist strain, or to join or to break helper strands. Like humans don't naturally think in terms of a double helix, made worse the placement of seams, the raster-fill algorithm should probably take some user preferences con bearing on the mechanical properties of the final structure through none have ever been integrated into a DNA design package. Much more interesting is the generalization of DNA origami present departures from the regular lattice and the makes the problem somewhat better—the configuration of twists can point. If two helices were properly aligned, it would seem that this tangent line could be off by roughly 34 degrees (in each helix) the twist of two backbones at the position of closest approach to groove mean that the backbone of the DNA strands cannot always be one turn apart along the helices. However, the combination of the non-integral number of bases per turn and the existence of a major/minor from 90 C to 20 C.

Fig. 6. A cartoon depicts folding of DNA origami as temperature changes from 90 C to 20 C.

2005 – Design of DNA Origami
Figure 3 | Patterning and combining DNA origami. a, Model for a pattern representing DNA, rendered using hairpins on a rectangle (Fig. 2b). b, AFM image. One pixelated DNA turn (~100 nm) is 30× the size of an actual DNA turn (~3.6 nm) and the helix appears continuous when rectangles stack appropriately. Letters are 30 nm high, only 6× larger than those written using STM in ref. 3; 50 billion copies rather than 1 were formed. c, d, Model and AFM image, respectively, for a hexagonal pattern that highlights the nearly hexagonal pixel lattice used in a–i. e–i, Map of the western hemisphere, scale 1:2 × 10^15, on a rectangle of different aspect ratio. Normally such rectangles aggregate (h) but 4-T loops or tails on edges (white lines in e) greatly decrease stacking (i). j–m, Two labellings of the sharp triangle show that each edge may be distinguished. In j–u, pixels fall on a rectilinear lattice. n–u, Combination of sharp triangles into hexagons (n, p, q) or lattices (o, r–u). Diagrams (n, o) show positions at which staples are extended (coloured protrusions) to match complementary single-stranded regions of the scaffold (coloured holes). Models (p, r) permit comparison with data (q, s). The largest lattice observed comprises only 30 triangles (t). u shows close association of triangles (and some breakage). d and f were stretched and sheared to correct for AFM drift. Scale bars: h, i, 1 μm; q, s–u, 100 nm.
End Result

Templated self-assembly of DNA into custom two-dimensional shapes on with a multiple-kilobase ‘scaffold strand’ that is folded into a flat array of antiparallel helices by interactions with hundreds of oligonucleotide ‘staple strands’
Discussion

• Prior to Rothemund, attempts were made, but inhibited.
• Yield is 70%
• He ignored 3 criteria in his method:
  – Sequences must be optimized to avoid secondary structure or undesired binding interactions
  – Strands must be purified
  – Strands must be equimolar
    (legacy of Seeman)
Discussion

• Prior to Rothemund, attempts were made, but inhibited.
• Yield is 70%
• He ignored 3 criteria in his method:
  – Sequences must be optimized to avoid secondary structure or undesired binding interactions
  – Strands must be purified
  – Strands must be equimolar
(legacy of Seeman)
cont. Discussion

- Suggested factors that helped this succeed:
  1. strand invasion
  2. an excess of staples (why? proposed 5:1)
  3. cooperative effects
  4. design that intentionally does not rely on binding between staples.

DNA origami may be viewed as a ‘nanobreadboard’ to which diverse components can be added.

2006 - Folding DNA to create nanoscale shapes and patterns
Shih et al - Self-assembly of DNA into nanoscale three-dimensional shapes

• Extended Rothmund’s method to 3D pleated (folded) layers of helices constrained into a honeycomb lattice

• Approximated 6 shapes
Complementary staple strands wind in an antiparallel direction around the scaffold strands to assemble B-form double helices.

Shih et al - Self-assembly of DNA into nanoscale three-dimensional shapes
Hierarchical assembly of DNA-origami nanostructures can be achieved through the assembly of DNA origami monoliths into higher-order constructs. 

- **Monolith** (a): A basic building block composed of scaffold and staple strands, forming a cylindrical structure.
- **Square nut** (b): A modified monolith with additional staple strands, forming a square cross-section.
- **Railed bridge** (c): An intermediate structure with staples forming a zigzag pattern across the bridge.
- **Stacked cross** (d): A complex structure with multiple layers of staples, forming a cross shape.
- **Slotted cross** (e): A structure with a central slot and additional staple strands, forming a slot cross.

**Genie bottle** (d): A more complex structure with a central cross and additional staple strands, resembling a genie bottle.

**pEGFP-N1** and **p7308**: These are DNA constructs used in the folding process, with different sequences for different shapes.

**Shih et al** - Self-assembly of DNA into nanoscale three-dimensional shapes
Tools
Adobe Illustrator/ad hoc programs (prior to caDNAno) -> since ported.

Ex: square-nut

Shih et al - Self-assembly of DNA into nanoscale three-dimensional shapes
Method

One-pot reaction

1. Rapid heating → slow cooling
2. 10 nM scaffold strands derived from M13 bacteriophage
3. 50 nM of every oligonucleotide staple strand
   (5x to 1x...why?) purified by reverse-phase cartridge
4. Buffer, salts → 5 mM Tris + 1 mM EDTA (pH 7.9 at 20 uC), 16 mM MgCl2
5. Thermal-annealing ramp from 80 C to 60 C → 80 min then 60 C to 24 C → 173 h.
6. 2% agarose gel containing 45 mM Tris borate + 11 mM EDTA (pH 8.3 at 20 C), and 11 mM MgCl2 at 70 V → Monomer bands were excised
7. Objects were electrophoresed
   DNA was recovered by physical extraction from the excised band
   negative-staining by uranyl formate
8. Objects were imaged using TEM

Shih et al - Self-assembly of DNA into nanoscale three-dimensional shapes
Assembly of a target three-dimensional shape using the honeycomb-pleat-based strategy described here can be conceptualized as laying down the scaffold strand into an array of antiparallel helices (Fig. 1a) where helix $m+l$ has a preferred attachment angle to helix $m$ of $\pm 120$ degrees with respect to the attachment of helix $m−l$ to helix $m$ (Fig. 1b, c); this angle is determined by the relative register along the helical axes of the Holliday-junction crossovers that connect helix $m+l$ to helix $m$ versus those that connect helix $m−l$ to helix $m$.

Shih et al - Self-assembly of DNA into nanoscale three-dimensional shapes
Figure 1 | Design of three-dimensional DNA origami. a, Double helices comprised of scaffold (grey) and staple strands (orange, white, blue) run parallel to the z-axis to form an unrolled two-dimensional schematic of the target shape. Phosphate linkages form crossovers between adjacent helices, with staple crossovers bridging different layers shown as semicircular arcs.
Crossovers between adjacent staple helices are restricted to intersections between the block and every third layer of a stack of planes orthogonal to the helical axes, spaced apart at intervals of seven base pairs or two-thirds of a turn.

**Shih et al** - Self-assembly of DNA into nanoscale three-dimensional shapes
Three-dimensional DNA origami shapes. The first and second rows show perspective and projection views of cylinder models, with each cylinder representing a DNA double helix. a, Monolith. b, Square nut. c, Railed bridge. d, Slotted cross. e, Stacked cross. Rows three to seven show transmission electron microscope (TEM) micrographs of typical particles. For imaging, samples were adsorbed (5 min) onto glow-discharged grids pre-treated with 0.5 M MgCl₂, stained with 2% uranyl formate, 25 mM NaOH (1 min), and visualized with an FEI Tecnai T12 BioTWIN at 120 kV. f, Top,
f, Top, field of homogeneous and monodisperse stacked-cross particles.

Bottom, expanded view of boxed area from above.

Shih et al - Self-assembly of DNA into nanoscale three-dimensional shapes
In this Letter, Figure 3 was printed incorrectly. The corrected figure is presented below.

In a recent Letter (1), we described the use of cyclic staple strands in a scaffold strategy to fold DNA origami structures containing dozens of helices (2). The staple design was based on a subset of the C-shaped domain from the tobacco mosaic virus (TMV) coat protein crystal structure (3). Here we report on our efforts to fold DNA-origami nanostructures of increasing complexity, including a model of a wireframe icosahedron with a diameter of about 100 nm (4). For the 1.2 h ramp, the temperature was lowered from 95°C to 20°C at a rate of 1.6 min°C⁻¹. For the 3 h, 6 h, 12 h, 18 h, 37 h, 74 h and 173 h ramps, the temperature was lowered from 80°C to 60°C at 4 min°C⁻¹, and then from 60°C to 24°C at rates of 5, 10, 20, 30, 60, 120 or 280 min°C⁻¹, respectively.

*Figure 3*| Gel and TEM analysis of folding conditions for three-dimensional DNA origami. **a**, Cylinder models of shapes: monolith, stacked cross, railed bridge, and two versions of genie bottle, with corresponding scaffold sequences. Labels indicate the source of scaffold used for folding the object (for example, p7560 is an M13-based vector of length 7,560 bases). **b**, Shapes were folded in 5 mM Tris + 1 mM EDTA (pH 7.9 at 20°C) and 16 mM MgCl₂ and analysed by gel electrophoresis (2% agarose, 45 mM Tris borate + 1 mM EDTA (pH 8.3 at 20°C), 11 mM MgCl₂) using different thermal-annealing ramps. For the 1.2 h ramp, the temperature was lowered from 95°C to 20°C at a rate of 1.6 min°C⁻¹. For the 3 h, 6 h, 12 h, 18 h, 37 h, 74 h and 173 h ramps, the temperature was lowered from 80°C to 60°C at 4 min°C⁻¹, and then from 60°C to 24°C at rates of 5, 10, 20, 30, 60, 120 or 280 min°C⁻¹, respectively. **c–e**, TEM and gel analysis of influence of MgCl₂ concentration on folding quality. **c**, The fastest-migrating bands in the 4 mM MgCl₂ lanes were purified and imaged, revealing gross folding defects. **d**, Shapes were folded with a 173 h ramp in 5 mM Tris + 1 mM EDTA (pH 7.9 at 20°C) and MgCl₂ concentrations varying from 0 to 30 mM. **e**, As in **c**, leading bands were purified from the 16 mM MgCl₂ lanes and found to exhibit higher-quality folding when analysed by TEM. **f**, Excess NaCl inhibits proper folding. Shapes were folded with 173 h ramp in 5 mM Tris + 1 mM EDTA (pH 7.9 at 20°C), 16 mM MgCl₂, and varying NaCl concentrations.
structures from DNA.

that is not accessible by flat structures, including those requiring

have been identified as being particularly well-behaved.

16 mM MgCl$_2$...
Shih et al - Folding DNA into Twisted and Curved Nanoscale Shapes

- Goal: quantitatively control the degree of curvature
- A radius of curvature as tight as 6 nanometers was achieved.
- Bend angles ranged from 30° to 180°

→ Realized something close to the extreme bending of DNA found in the nucleosome

Source: Wikipedia.org/wiki/Nucleosome
Fig. 1. Design principles for controlling twist and curvature in DNA bundles. (A) Double helices are constrained to a honeycomb arrangement by staple-strand crossovers. Semi-transparent crossover planes mark the locations of strand crossovers between neighboring helices, which are spaced at 7-bp intervals along the helical axis. From left to right, each plane contains a class of crossovers rotated in-plane by 240° clockwise with respect to the preceding plane. The crossover planes divide the bundle conceptually into helix fragments that can be viewed as residing in array cells (one cell is highlighted). (B) Array cell with default content of 7 bp, which exerts no stress on its neighbors. (C) Above, array cell with content of 5 bp, which is under strain and therefore exerts a left-handed torque and a pull on its neighbors. Below, array cell with content of 9 bp, which is under strain and therefore exerts a right-handed torque and a push on its neighbors. Force vectors are shown on only two of the four strand ends of the array-cell fragment for clarity.

Shih et al - Folding DNA into Twisted and Curved Nanoscale Shapes
(D) (Left) Site-directed deletions installed in selected array cells indicated in orange result in global left-handed twisting with cancellation of compensatory global bend contributions; (right) site-directed insertions in selected array cells (shown in blue) result in global right-handed twisting. (E) Site-directed base-pair deletions (indicated in orange) and base-pair insertions (indicated in blue) can be combined to induce tunable global bending of the DNA bundle with cancellation of compensatory global twist contributions.
Fig. 2. Deviations from 10.5 bp per turn twist density induce global twisting. (A to C) (Top left) Models of a 10-by-6–helix DNA bundle (red) with 10.5, 10, and 11 bp per turn average double-helical twist density, respectively, and models of ribbons when polymerized (silver). (Bottom left) Monomeric particles as observed by negative-stain TEM. Scale bars, 20 nm. (Right) Polymeric ribbons as observed by TEM. Scale bars, 50 nm. (D)
(D and E) Tilt-pair images of twisted ribbons polymerized from 11 bp per turn (D) and 10 bp per turn (E); 10-by-6–helix bundles, recorded at goniometer angles of 40° and −40°. Arrows indicate the observed upward (for 11 bp per turn) or downward (for 10 bp/turn) direction of movement of the twisted-ribbon nodes. The dashed line provides a reference point (ends of ribbons remain stationary on goniometer rotation). CCD, charge-coupled device. (F) Ethidium-bromide–stained 2% agarose gel, comparing migration of unpurified folded bundles. (G) Histograms of the observed node-to-node distance in twisted ribbons, as observed in negative-stain TEM micrographs. Left- and right-handed ribbons undergo half-turns every 235 ± 32 nm (n = 62 internode distances measured) and 286 ± 48 nm (n = 197), respectively (numbers after the ± sign indicate SD). (H) Plot of observed global compensatory twist per turn versus double-helical twist density initially imposed by design. A value of 0.335 nm per bp was used to calculate global twist per turn from values obtained in (G). Error bars indicate SD.
Fig. 3. Combining site-directed insertions and deletions induces globally bent shapes. (A to G) Models of seven 3-by-6–helix-bundle versions programmed to different degrees of bending and typical particles, as observed by negative-stain TEM. $r_c$, radius of curvature. Scale bars, 20 nm.

Shih et al - Folding DNA into Twisted and Curved Nanoscale Shapes
**Fig. 4.** Bending enables the design of intricate nonlinear shapes. Red segments indicate regions in which deletions and insertions are installed. Scale bars, 20 nm. (A) Model of a 3-by-6–helix DNA-origami bundle designed to bend into a half-circle with a 25-nm radius that bears three non-bent teeth. Monomers were folded in separate chambers, purified, and mixed with connector staple strands to form six-tooth gears. Typical monomer and dimer particles visualized by negative-stain TEM.
(B) 3-by-6–helix bundle as in (A), modified to bend into a quarter circle with a 50-nm radius. Hierarchical assembly of monomers yields 12-tooth gears. (C) A single scaffold strand designed to fold into a 50-nm-wide spherical wireframe capsule resembling a beach ball and four typical particles representing different projections of the beach ball. The design folds as six bent crosses (inset) connected on a single scaffold. (D) A concave triangle that is folded from a single scaffold strand. The design can be conceptualized as three 3-by-6 bundles with internal segments designed to bend by 60°. (E) A convex triangle assembled hierarchically from three 3-by-6 bundles designed with a 120° bend (Fig. 3E). (F) A six-helix bundle programmed with varying degrees of bending folds into a spiral-like object.

Shih et al - Folding DNA into Twisted and Curved Nanoscale Shapes
Andersen et al - Self-assembly of a nanoscale DNA box with a controllable lid

• Extend the DNA origami method into three dimensions by creating an addressable DNA box 42 X 36 X 36 nm^3 in size that can be opened in the presence of externally supplied DNA ‘keys’.

• Used package at [http://cdna.au.dk/software/](http://cdna.au.dk/software/) (link not the same as paper)
**Figure 1 | Design of a DNA origami box.** a, Sequence map of the circular, single-stranded DNA genome of the M13 bacteriophage with regions used to fold the six DNA sheets shown as coloured arrows (A–F). Base numbering starts from a 44-nucleotide spacer region between sheets A and B that contains a stable hairpin structure. Spacers of 33 nucleotides are positioned between each face. b, c, Molecular models of the six DNA sheets in a flat and cubic higher-order structure, respectively. The six DNA sheets are colour-coded as in a.
Figure 2 | AFM imaging of two- and three-dimensional DNA origami structures. a, AFM image of a sample in which the six DNA sheets were folded along the M13 backbone. Inset, magnified view of a preferred arrangement of the six sheets. b, AFM image of a sample in which the edges of the DNA sheets were linked to form a box. Inset, magnified view of a box-like particle. c, AFM image of a sample in which one lid of the DNA box was left open. Inset, magnified view of a structure in which the lid is protruding from the body of the box. The colour scale shows the height above the surface in the range 0–15 nm.
Supplementary Figure 4. Collapse of box structure during AFM imaging. a, AFM images of the DNA box. Two images on top show box-like dimensions. The two images below show particle-structures that upon AFM imaging changed shape into double- or single-layer DNA sheets. Cartoon below illustrate how the AFM tip might distort the soft and hollow DNA box. b, Four representative images of DNA box particles with an open lid. The cartoon below illustrates how the elongated main part of the particle is likely the result of a sideway collapse of main box, while the lid is laying flat on the surface.

Explanation for disparity in height of box between 4-12 nm → Over time structures were observed to collapse from 3D into flat structures.
In conclusion, the AFM images (Fig. 2c), which probably corresponds to a sideway collapse of the assembled an open DNA box in which the staple strands closing lid D results in their collapse into flat structures one or two DNA sheets in height with a one-sheet extension of dimensions similar to lid D observed that prolonged scanning of individual 3D DNA structures which is most likely caused by distortion of the hollow, soft DNA box.

To gain stronger evidence for the formation of the DNA box, we performed cryogenic transmission electron microscopy (cryo-EM) on a sample of the assembly. Individual DNA boxes showed prominent dense edges could be used to measure the short and long side lengths of features of the DNA box (Fig. 3c). For 337 class averages, electron-images were subjected to single-particle image processing, and class averages were consistent with the class averages and the corresponding two-dimensional re-projections of the 3D cryo-EM map of the DNA box was consistent with the class averages with three different side lengths with the Debye background added.

**Cry-EM:**
- [http://www.youtube.com/watch?v=BJKkC0W-6Qk](http://www.youtube.com/watch?v=BJKkC0W-6Qk)
- [http://www.eicn.ucla.edu/cryoem](http://www.eicn.ucla.edu/cryoem)

**SAXS:**
- [http://www.youtube.com/watch?v=2QOsh2vgY2Q](http://www.youtube.com/watch?v=2QOsh2vgY2Q)

**Figure 3 | Characterization of DNA origami box by cryo-EM and small-angle X-ray scattering (SAXS).**

- **a,** Single-particle cryo-EM images of box-shaped assemblies.
- **b,** Single-particle reconstruction of the DNA box applying D2 symmetry. Left, theoretical model. Middle, surface representation of the cryo-EM map. Right, cut-open view showing the interior cavity of the cryo-EM map. 
- **c, d,** Comparison of the class averages of the DNA boxes (c) with the corresponding two-dimensional re-projections of the 3D cryo-EM map (d).
- **e,** Experimental SAXS data (circles) with corresponding fits from different approaches: red dashed curve, fit using the theoretical atomic coordinates for the box; blue dotted curve, typical Debye background for modelling the excess oligonucleotides; dash–dot curve, fit using the theoretical atomic coordinates for the box with the Debye background added; solid curve, fit using a semi-analytical model for a box with three different side lengths with the Debye background added. $I(q)$, SAXS intensity; $q$, momentum transfer modulus (Methods). Inset, semi-analytical box model with the estimated side lengths and wall thickness.
Figure 3 | Characterization of DNA origami box by cryo-EM and small-angle X-ray scattering (SAXS). a, Single-particle cryo-EM images of box-shaped assemblies. b, Single-particle reconstruction of the DNA box applying D2 symmetry. Left, theoretical model. Middle, surface representation of the cryo-EM map. Right, cut-open view showing the interior cavity of the cryo-EM map. c, d, Comparison of the class averages of the DNA boxes (c) with the corresponding two-dimensional re-projections of the 3D cryo-EM map (d). e, Experimental SAXS data (circles) with corresponding fits from different approaches: red dashed curve, fit using the theoretical atomic coordinates for the box; blue dotted curve, typical Debye background for modelling the excess oligonucleotides; dash–dot curve, fit using the theoretical atomic coordinates for the box with the Debye background added; solid curve, fit using a semi-analytical model for a box with three different side lengths with the Debye background added. \( I(q) \), SAXS intensity; \( q \), momentum transfer modulus (Methods). Inset, semi-analytical box model with the estimated side lengths and wall thickness.
Lock: DNA duplexes attached to both faces B,D (lid and front lateral) with a toehold on the helix attached to B.

Key: signaling DNA sequence that displaces the lid’s part of the lock.

Figure 4 | Programmed opening of the box lid. a, b, Illustrations of the unlinked faces of the box (a) and the controlled opening of the box lid (b). The emission from the Cy5 and Cy3 fluorophores are marked with red and green stars, respectively. Loss of emission from Cy5 is denoted by a red circle and the independent lock–key systems are indicated in blue and orange.
Here we functionalized lid D of the DNA box with a dual lock–key mechanism and SAXS, which probe a large ensemble and can therefore determine the small and large faces. The dimensions of the 3D reconstruction are concave bending of the faces may reflect the difference in design of structures, providing further evidence of the successful self-assembly. The data showed that the scattering derives mainly from box-shaped particles, which can satisfactorily describe the scattering data and gave overall dimensions of 46 nm, which was in good agreement with the atomic model of the scattering data was in good agreement with the atomic model of the intended 3D design.

To analyse the native DNA origami box in solution and without a potential disruptive sample fixation, we used dynamic light scattering measurements of the closed box before (black curve) and 35 min after (red curve) the addition of keys. The fluorescence intensity of the Cy5 and Cy3 fluorophores are marked with red and green stars, respectively. Loss of emission from Cy5 is denoted by a red circle and the independent lock–key systems are indicated in blue and orange.

**Figure 4 | Programmed opening of the box lid.** a, b, Illustrations of the unlinked faces of the box (a) and the controlled opening of the box lid (b). The emission from the Cy5 and Cy3 fluorophores are marked with red and green stars, respectively. Loss of emission from Cy5 is denoted by a red circle and the independent lock–key systems are indicated in blue and orange.


<table>
<thead>
<tr>
<th>Dye</th>
<th>Absorbance Max</th>
<th>Emission Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cy3</td>
<td>550 nm</td>
<td>570 nm</td>
</tr>
<tr>
<td>Cy5</td>
<td>649 nm</td>
<td>670 nm</td>
</tr>
</tbody>
</table>

**Source:** Wikipedia.org/wiki/Cyanine
Supplementary Figure 1. Mechanism for signal-induced opening of the DNA box. a, An atomic model of the DNA box held closed by “locks” (orange and blue) that are double helices formed by two short strands protruding from the lid and the main box, respectively. Each “lock” has a small sticky-end where a “key” sequence signal can bind and open the “lock” by strand displacement. If both “locks” are opened the lid of the box is effectively opened (b). The reporter system for detecting the lid opening is a Cy3-Cy5 FRET system. In the closed state the two fluorophores are in close proximity resulting in FRET emission from Cy5 (red star) when Cy3 is excited. In the open state the two fluorophores are far apart and excitation of Cy3 only results in emission from Cy3 (green star).
Methods

- SARSE program for creating realistic 3D models, which facilitated the design of the 3D edge-to-edge staple strand crossovers.
- Deposited the samples on a mica surface and performed the AFM imaging in a buffer solution.
- Cryo-EM. The sample was adsorbed on carbon film and plunge-frozen in liquid ethane.
- SAXS. Collected data using a high-resolution set-up of a laboratory-based small-angle x-ray scattering.
- FRET. To detect the opening process, we functionalized the box with Cy3 and Cy5 fluorophores and a lock–key system.
Han et al – DNA Origami with Complex Curvatures in Three-Dimensional Space
Fig. 1. Design principles for DNA origami with complex curvatures in 3D space. (A) A parallel arrangement of DNA double helices to make multihelical DNA nanostructures. The distance between consecutive crossovers connecting adjacent helices (L1, L2, and L3) is constant and generally corresponds to 21 or 32 bps (about two or three full turns of B-form DNA). (B) Bending of DNA helices into concentric rings to generate in-plane curvature. The distance between crossovers in the outer rings are greater than in the inner rings (L3 > L2 > L1). This distance is not required to be regular, or exactly equal to a whole number of full turns of B-form DNA for every helix. (C) Schematic diagram of a three-ring concentric structure. The long single-stranded DNA scaffold is shown in cyan, and short oligonucleotide staple strands are shown in various colors. Two scaffold crossovers are required between adjacent rings to achieve the three-ring arrangement. They are located far apart, on opposite sides of the rings. Five periodic, staple-strand crossovers connect the outer and middle rings and the middle and inner rings, respectively, constraining the three bent double-helical DNA rings to the same 2D plane. (D) Helical and cylindrical view of the three-ring concentric structure. (E) A general method to introduce out-of-plane curvature in a multihelical DNA structure. All DNA helices exhibit a natural B-form conformation. There are 10 possible values of θ ranging from ~34° to ~343°. Due to steric hindrance, not all values are allowed. Only a few of these values are demonstrated here. (F) Various views of the structure shown in (E), viewed along the helical axes, tilted by 135°, and perpendicular to the helical axes.

To produce a complex 3D object, it is necessary to create curvatures both in and out of the plane. Out-of-plane curvature can be achieved by shifting the relative position of crossover points between DNA double helices (Fig. 1, E and F). Typically, two adjacent B-form helices (n and n + 1) are linked by crossovers that are spaced 21 bps apart (exactly two full turns), with the two axes of the helices defining a plane. The crossover pattern of the two-helix bundle and those of a third helix can be offset by any discrete number of individual nucleotides (not equal to any whole number of half turns, which would result in all three helices lying in the same plane), and in this way, the third helix can deviate from the plane of the previous two. However, with B-form DNA, the dihedral angle (θ)—the angle between the planes defined by n and n + 1, and n + 1 and n + 2—can not be finely tuned, and \( \sim 34.3^\circ/\text{bp} \) is the smallest increment of curvature that can be achieved.

Fig. 2. Curved 2D DNA nanostructures with various structural features. (Upper panels) Schematic designs. (Middle panels) Zoom-in AFM images with 50-nm scale bars. (Lower panels) Zoom-out AFM images with 100-nm scale bars. (A) Nine-layer concentric ring structure. Only 3600 of 7249 nucleotides of the scaffold strand are used in this structure, and the remaining single-stranded loop is left unpaired, attached to the outer ring (often visible due to formation of secondary structures). (B) Eleven-layer modified concentric square frame structure with rounded outer corners and sharp inner corners.

Table 1. Design parameters for the nine-layer concentric-ring structure. The number of bps in each ring, number of crossovers between adjacent helices, conformation of the double helical DNA in bps/turn, and radius are listed, respectively.

<table>
<thead>
<tr>
<th>Ring no.</th>
<th>bps</th>
<th>No. of crossovers</th>
<th>bps/turn</th>
<th>Radius (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>5</td>
<td>10</td>
<td>10.3</td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>5</td>
<td>10</td>
<td>12.9</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>10</td>
<td>10</td>
<td>15.5</td>
</tr>
<tr>
<td>4</td>
<td>350</td>
<td>10</td>
<td>11.7</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>400</td>
<td>10</td>
<td>10</td>
<td>20.6</td>
</tr>
<tr>
<td>6</td>
<td>450</td>
<td>10</td>
<td>9</td>
<td>23.2</td>
</tr>
<tr>
<td>7</td>
<td>500</td>
<td>10</td>
<td>10</td>
<td>25.8</td>
</tr>
<tr>
<td>8</td>
<td>550</td>
<td>10</td>
<td>11</td>
<td>28.4</td>
</tr>
<tr>
<td>9</td>
<td>600</td>
<td>–</td>
<td>10</td>
<td>30.9</td>
</tr>
</tbody>
</table>

90% yield

To produce a complex 3D object, it is necessary to create curvatures both in and out of the plane. Out-of-plane curvature can be achieved by modifying a square frame. A modified square frame (Fig. 2B) was designed to determine whether sharp and rounded features could be included in the final design scheme, whereas each corner could be offset by any discrete number of bps (Fig. 2C). We found that a double helix (Fig. 2D) could maintain structural integrity with various structural features when the range of DNA conformations amenable to our current design was investigated. In our studies, we constructed a series of DNA nanostructure arrangements (Fig. 2E). Several additional 2D designs were generated (Fig. 2F). Watson-Crick base pairing is generally 16 to 60 nts long and reverses direction if the orientation can not be determined. (Fig. 2G) A TEM image of the nanoflask. Scale bar is 75 nm. (I) TEM images of the nanoflask, randomly deposited on TEM grids. The cylindrical neck and rounded bottom of the flask are clearly visible in the images. Scale bar is 50 nm.
Arbona et al – Modelling the folding of DNA origami

Fig. 1. (a) Derivative $d\theta/dT$ of the degree of pairing with respect to temperature for the three DNA origamis represented in the insets. (b) $d\theta/dT$ for a model where

Fig. 2. (a) Schematic representation of the connectivity of the small origami. (b) B1 staple is in the 'outer' position, (c) B2 staple in the 'inner' position. (b) and (c) show that the binding of staples in the outer (b) or inner (c) positions are very different.
Besides the existence of this entropic hindrance, located on the same side of the bound moities (Fig. b). In the "inner" position. The di staple contains two contiguous parts, 16b long, that bind to avoid mispairing with the 64b template B0. They have close B2m, designed with chemical sequences di the melting curve. The third set has two staples, B1m and 91 whereas B2 only contains G or C nucleotide. Accordingly, positions: the sequence of B1 only contains A or T nucleotide whereas B2(GC), cases (C,E) with B2 already folded; b) experimental data on B2 without B1; c) experimental data on B1m; d) experimental data on B2m

Fig. 3. The derivative $d\theta/dT$ reported in the four figures corresponds to the folding of the dotted staple. a) experimental data on the folding of B1(AT) cases (A,B,D) in the absence of B2(GC), cases (C,E) with B2 already folded; b) experimental data on B2 without B1; c) experimental data on B1m; d) experimental data on B2m

Fig. 4. Computing the entropic penalty for the three different local intermediate states (LIS). The staple to be inserted is represented by the dotted line, the scaffold by the continuous line. (a) LIS1 (b) LIS2 (c) LIS3. Here, we assume that, because of the curvature constraints imposed by this configuration, the staple remains partly unfolded. (d) A typical situation where two types of LIS (LIS1 at the right side of the staple, LIS3 at the left side) can be attributed to a given crossover.

The third LIS, LIS3, corresponds to the inner position found in the hybridization of the three strands. This LIS is characterized by the presence of an internal asymmetric loop, which is formed when the staple hybridizes to the free ends of the two strands (Fig. 3a).

In LIS1, the staple hybridizes to the free ends of the strands, forming an internal asymmetric loop (LIS2, Fig. 3). In a first approximation, the longer the region of the staple, the stronger the instability characteristic of this LIS. This is due to the difficulty for a staple to hybridize non-contiguous parts of theDNA sequence.

The local contribution of the diSSold and the non hybridized part of the staple (Fig. b) is taken into account. For each of these partial states, the equilibrium constant for a partial folded configuration is calculated as explained in the energy model. Once solved, they allow to determine the fraction of partial configuration folded in this environment $p(N_0(S_i), T + dT)$. Then, we can calculate the total fraction of each configuration folded $p(S_i(m, n), (T + dT)$, as the sum of the fraction of those configurations in the different local states, weighted by the probability of each state.

The annealing-melting process is not symmetrical, the hysteresis between the two phases of a cycle is such that the energy per base depending bases stabilizing term is proportional to the number of neighbourising bases in the case where Mg is dominant (see §2).

Indeed, in the folded structure of origamis, double-helix sections such as the small origamis has to be invoked. With the protocol detailed in SI Text, the degree of pairing has been merged two by two in four areas (coloured in black red). Each staple includes a hole [24] and presents the same 8-16-8 pattern. O3 has the same connectivity pattern as O1, but some staples have been merged two by two in four areas (coloured in black red). O2 is another rectangular origami that in-cludes a hole [24] and presents the same 8-16-8 pattern. O2 has only a small shift between annealing-melting, whereas O4 8-16-8 patterns. O2 is another rectangular origami that in-

Fig. 6. Derivative of the pairing degree versus temperature. The data corresponding to annealing are in red, melting in green, the model is in blue for both processes.

between the annealing and melting processes, as well as the model is satisfactory. The model captures the hysteresis annealing curves observed experimentally and computed with ramp (0.4 was extracted as a function of temperature. The temperature protocol detailed in SI Text, the degree of pairing performed coupled to UV-absorption measurements. With a subset of the sca old (M13mp18 virus) and about 200 staples. O1 is in 6c), so that the typical staple pattern is 8-16-16-16-8. Finally the number of bases folded is converted to a theoretical absorbance [23].

The energy per base stabilizing term is proportional to the number of neighbour- as is the case when DNA condensates [20]. This electrostatic interactions are separated by distances of the order of 1nm. It is then reasonable to think that mechanisms such as correlations be-

totaled.

We considered four different melting curves corresponding to two permutations with the lowest and highest annealing temperatures.

Fig. 7. (a) Distribution of melting temperatures (annealing and melting) as a function of the order of the circular permutation of the scaffold strand. (b) two different melting curves corresponding to two permutations with the lowest and highest annealing temperatures.

Fig. 8. Annealing curves of the O1 and O5 origamis. The two origamis correspond to the same scaffold pattern, but different staple pattern (solid line = experimental data, dashed line = theoretical curves)