Nanofabrication with 3D DNA Origami

2010 FNANO
William Shih
Monday, 2010 April 26
Rothemund PW Nature

100 nm
Shawn Douglas
Wyss Technology Development Fellow


100 nm
Flat sheets can be folded into 3D shapes

Origami
Japanese art of paper folding
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Japanese art of paper folding
Flat sheets can be folded into 3D shapes

Satoshi Kamiya

Robert Lang

Tuesday, April 27, 2010
Rothenmund PW Nature
“Monolith”

Shawn Douglas

“Genie Bottle”

Franziska Graf
“Square Nut”  Shawn Douglas

“Railed Bridge”  Tim Liedl
“Slotted Cross”

Björn Högberg
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Björn Högberg
Three Environmental Determinants of Successful Folding

Folding Time  
(1 to 173 hours)

Divalent Cation Concentration  
(0 to 30 mM)

Monovalent Cation Concentration  
(320 to 0 mM)


Tuesday, April 27, 2010
Design challenges

Steep learning curve

Ad hoc methods are tedious and error prone

New shapes can require days or weeks of effort
http://cadnano.org/

NMR alignment medium
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800 nm-long DNA nanotubes

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...concentrated to 25 mg/mL

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weakly align proteins in the presence of detergent

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800 nm-long DNA nanotubes

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weakly align proteins in the presence of detergent

enabling measurement of global angular restraints

Douglas SM, Chou JJ, Shih WM. 
Exploring shape dependence of DNA-particle cellular uptake

Franziska Graf

Katrina Galkina
Closeups

100 nm
3D Wireframe Icosahedron
“Floating compressions”
Kenneth Snelson

Kenneth Snelson
Trigonal Tower, 1962-63
Aluminum and Steelon wire
65" X 31" X 28"
Pre-stressed tensegrity prisms

High porosity and surface area for delivery of morphogens

Potential for mechanical actuation
Chimeric Actuatable Materials that integrate into ECM

Switch stem-cell fate (e.g. bone, fat) by mechanics

Elaine Gee, Franziska Graf, Don Ingber

Tuesday, April 27, 2010
Figure 1. Forcing DNA-origami blocks into nonlinear shapes.

(A) Double helices are constrained to a honeycomb arrangement by strand crossovers. Semi-transparent crossover-lattice planes mark the locations of strand crossovers between neighboring helices, which are spaced at 7 bp intervals along the helical axis. Each consecutive plane contains a class of crossovers rotated in-plane by 240º with respect to the preceding plane. The crossover-lattice planes divide the block conceptually into helix fragments that can be viewed as residing in unit cells containing by default 7 bp (one unit cell is highlighted in red).

(B) Inserting an additional base pair into every unit cell locally underwinds and compresses the helix fragments, resulting in compensatory global right-handed twist.

(C) Deleting a base pair from every unit cell locally overwinds and stretches each fragment, resulting in compensatory global left-handed twist.

(D) Targeted base-pair insertions and deletions to individual unit cells can be combined to induce tunable global bending of the DNA block while compensatory global twist contributions cancel out.

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(D) Targeted base-pair insertions and deletions to individual unit cells can be combined to induce tunable global bending of the DNA block while compensatory global twist contributions cancel out.

Combining site-directed insertions and deletions induces globally bent shapes. (A-G) Models of seven 36-helix-block versions programmed to different degrees of bending and typical particles as observed by negative-stain TEM. Scale bars: 20 nm.

(H) Ethidium-bromide-stained 2% agarose gel comparing migration of unpurified folding products of the seven differently bent blocks.

(I, J) Low-magnification TEM micrographs of the block versions programmed to bend by 30º and 150º, respectively. Scale bars: 100 nm.

(K) Histograms of bend angles as observed in individual particles for the seven different block versions. Average bend angles were determined to be 0º±(3º s.d.) (N=74); 30.7º±(5.4º s.d.) (N=212); 62.4º±(5.9º s.d.) (N=208); 91.3º±(5.2º s.d.) (N=206); 121º±(8.4º s.d.) (N=212); 143.4º±(9º s.d.) (N=131); 166º±(9º s.d.) (N=106). Insets: Plot of average double-helical twist density through the cross section of the bent segment that results from the pattern of insertions and deletions installed to induce bending.
bp/turn

cross-sectional row
spiral

beach ball

corner

concave

convex
6-tooth gear

12-tooth gear
Stretched out ssDNA substrate for ssDNA translocases to be studied using single-molecule fluorescence

Adjustable contour length of ssDNA
~600 nt for ~7 pN
~900 nt for ~2 pN
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Conclusions

We can self-assemble arbitrary 3D-origami DNA nanostructures.

Precise control over self-assembly of 3D DNA nanostructures will be useful.

Support
NIH New Innovator Award
DFCI Barr Award in Innovative Basic Cancer Research
Wyss Institute for Biologically Inspired Engineering

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Franziska Graf
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Tim Liedl, Ph.D.
Adam Marblestone

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• **caDNAno Exercise #1**: Build a six-helix bundle in caDNAno-HC without any knowledge about the structure of DNA

  - Select helices in left-hand panel
  - Extend scaffold strands
  - Connect scaffold strands
  - Delete excess scaffold
  - Autostaple
  - Introduce staple breaks
  - Introduce scaffold break
  - Add sequence
**caDNAno Exercise #2**: Understand the logic of caDNAno-HC default behavior

- **HC-Q1**: Why does caDNAno-HC only allow staple crossovers to occur on planes spaced seven base pairs apart?

- **HC-Q2**: Why does caDNAno only allow scaffold crossovers +/- five base pairs from allowed staple crossover positions?

- **HC-Q3**: What is the crossover density imposed by caDNAno with the "autostaple" function?

- **HC-Q4**: What staple-strand lengths will caDNAno highlight as a warning?
• **caDNAno Exercise #3**: Build an 18-helix bundle in caDNAno-HC

- Select helices in left-hand panel
- Extend scaffold strands
- Connect scaffold strands
- Delete excess scaffold
- Autostaple
- Introduce staple breaks
- Introduce scaffold break
- Add sequence
- Force crossover option
• **caDNAno Exercise #4**: Download published multilayer origami designs at

• [http://cadnano.org/gallery.html](http://cadnano.org/gallery.html)

• and inspect in caDNAno
• **caDNAno Exercise #5**: Understand the logic of caDNAno-SQ default behavior

  - HC-Q1: Why does caDNAno-SQ only allow staple crossovers to occur on planes spaced eight base pairs apart?
  - HC-Q2: Why does caDNAno only allow scaffold crossovers +/- 5 or 16 base pairs from allowed staple crossover positions?
  - HC-Q3: What is the crossover density imposed by caDNAno with the "autostaple" function?
  - HC-Q4: What staple-strand lengths will caDNAno highlight as a warning?
• **caDNAno Exercise #6**: Build a four-helix bundle in caDNAno-SQ

- Select helices in left-hand panel
- Extend scaffold strands
- Connect scaffold strands
- Delete excess scaffold
- Autostaple
- Introduce staple breaks
- Introduce deletions
- Introduce scaffold break
- Add sequence
• **caDNAno Exercise #7**: Build a six-helix flat sheet in caDNAno-SQ

- Select helices in left-hand panel
- Extend scaffold strands
- Connect scaffold strands
- Delete excess scaffold
- Autostaple
- Introduce staple breaks
- Introduce deletions
- Introduce scaffold break
- Add sequence
caDNAno Exercise #8: Exploring design choices in caDNAno

- What is the ideal pattern of scaffold crossovers to use for multilayer origami?
- What is the ideal crossover density for multilayer origami?
- What is the ideal pattern of staple-strand breaks and staple-strand length distribution to employ?
- If we could use any scaffold sequence we wanted, what would be the design rules for building that sequence?
- What are the ideal solution components for folding multilayer origami?
• **caDNAno Exercise #9**: Understand how to create curvature with targeted deletions and insertions
• caDNAno Exercise #10: Building an six-helix bundle 45 degreee arc