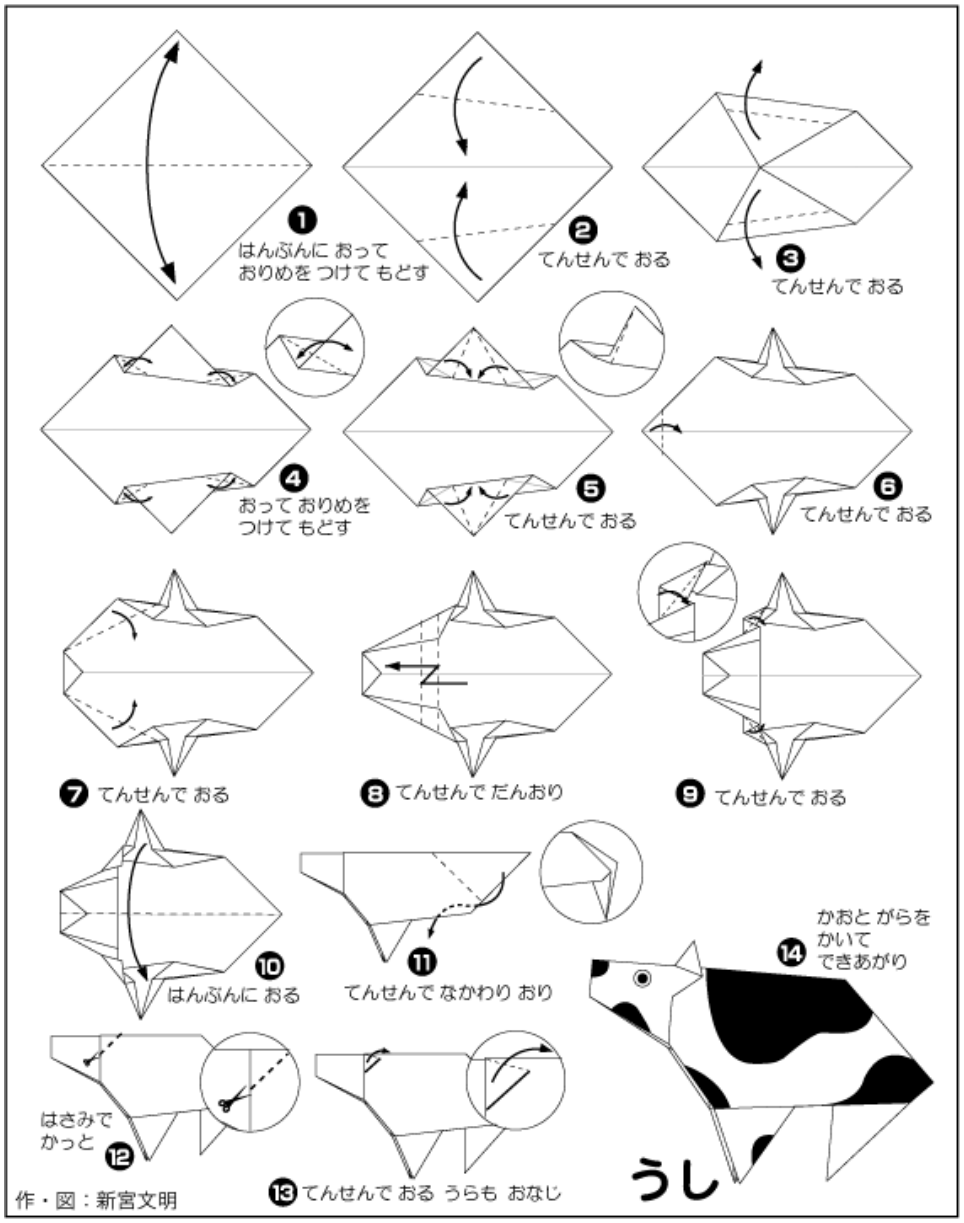
Technical Seminar 22th Jan 2013

DNA Origami

Hitoshi Takizawa, PhD


❶ はんぶんに おって おりめを つけて もどす
❷ てんせんで おる
❸ てんせんで おる
❹ おって おりめを つけて もどす
❺ てんせんで おる
❻ てんせんで おる
❼ てんせんで おる
❽ てんせんで だんおり
❾ てんせんで おる
❿ はんぶんに おる
⓫ てんせんで なかわり おり
⓬ はさみで かっと
⓭ てんせんで おる うらも おなじ
⓮ かおと がらを かいて できあがり
うし
作・図：新宮文明

Agenda

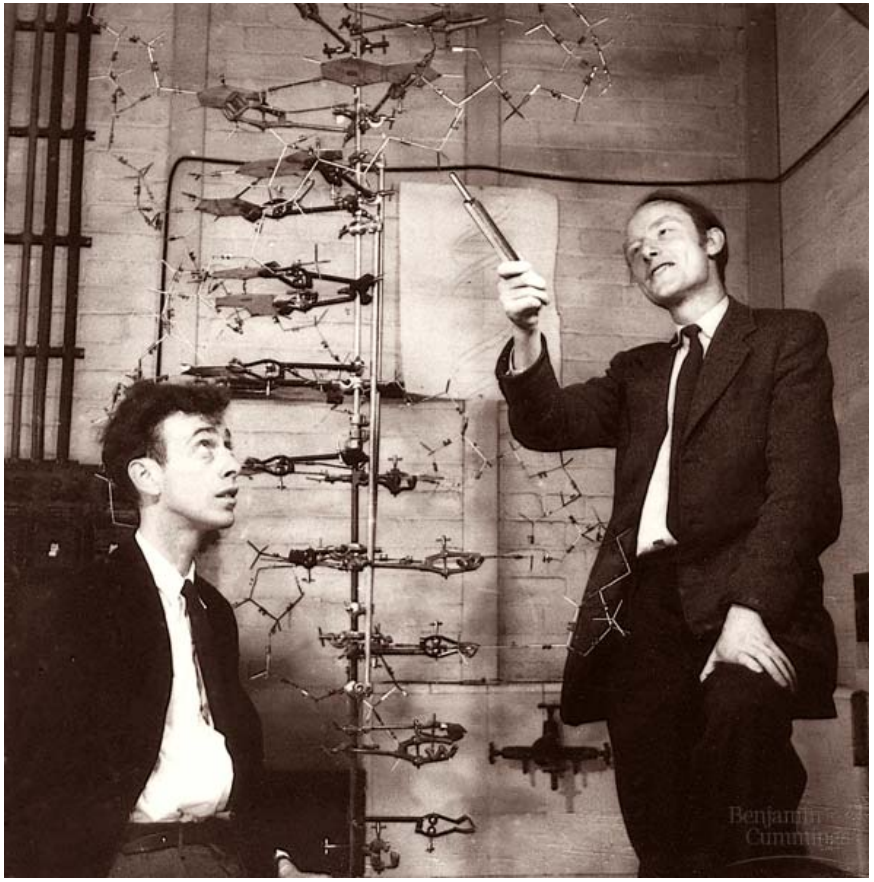
1) Basis of structural DNA nanotechnology

2) DNA origami technique (2D, 3D, complex shape)

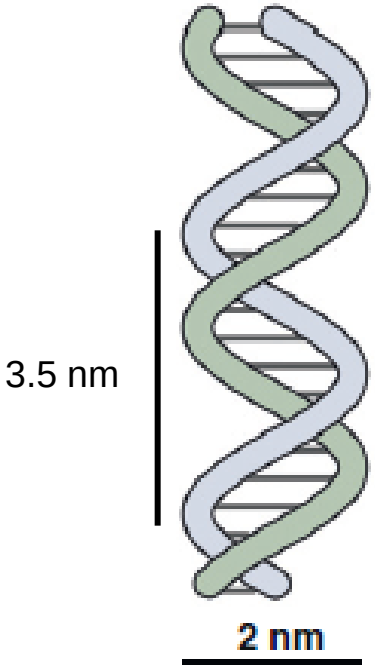
3) Programmable nanofactory

4) Application

Watson-Crick DNA Helix

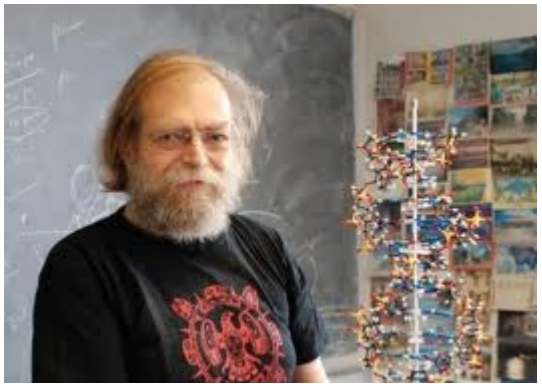


The paper in Nature 1953, Nobel prize in 1962



Simmel FC, Curr Opin in Biotech 2012

Nadrian C. Seeman's thought in 1980's



Crystallographer

Woodcut depth by MC Escher

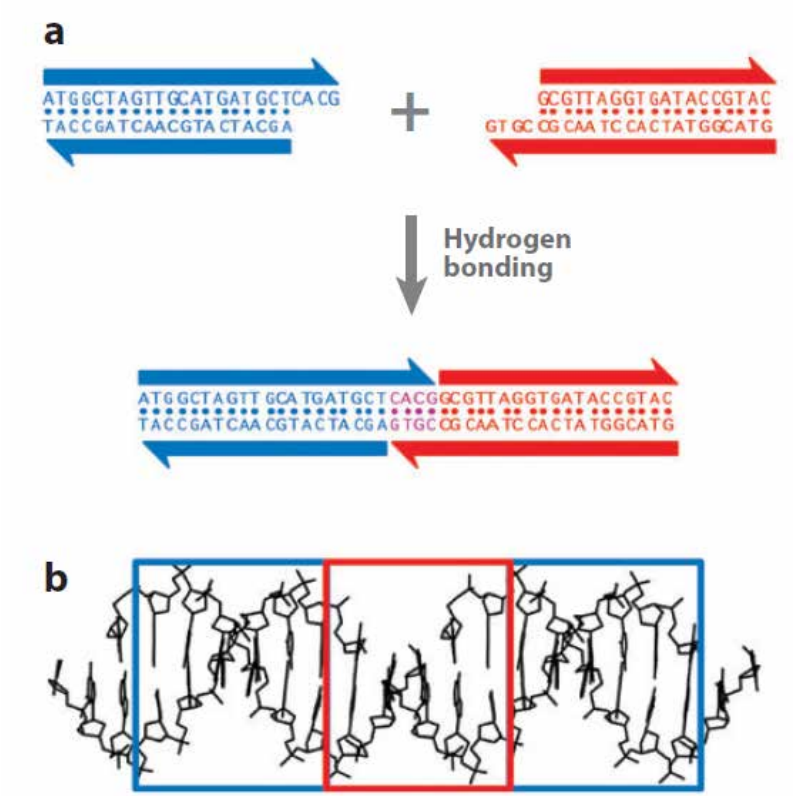
Can DNA be used in a non-biological material – as a material for molecular construction?

Seeman NC, J. Theor Biol 1982

- Two molecule of DNA pair to form a double helix when their sequences are complementary.
- High affinity of two complementary DNA strands

"Structural DNA nanotechnology"

Hybridization



Key aspects of sticky-ended cohesion are:

High specificity

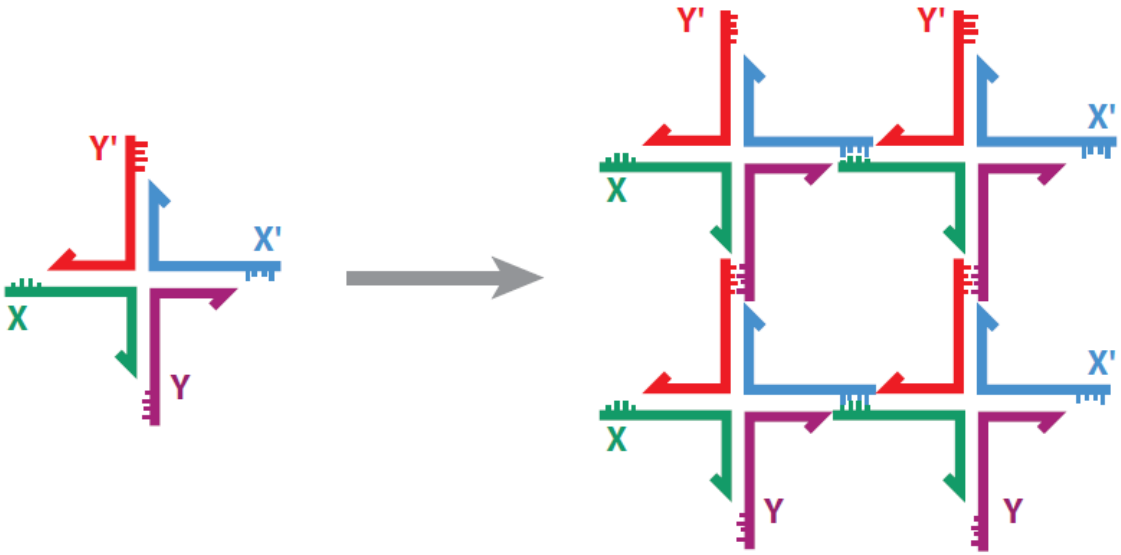
High spatial precision

High spatial and flexibility

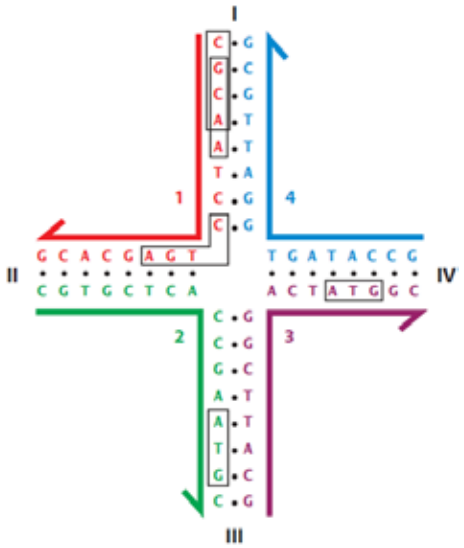
We can predict the local product structure formed when sticky ends cohere.
-> no need to determine crystal structure

Seeman NC, Ann. Rev. Biochem 2010

Self-assembly of Branched DNA (“Holliday Junctions”)



Essential to form infinite lattices



Sequence design and sequence symmetry minimization

Each strands are broken up to series of 13 overlapping tetramer

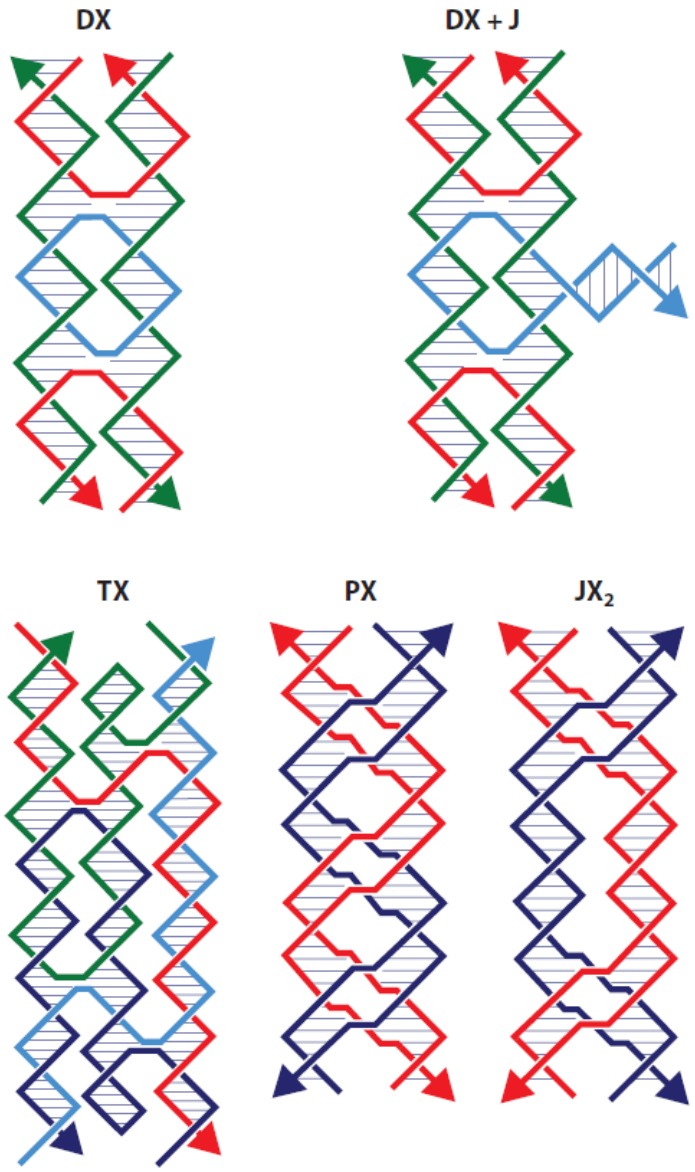
- Each tetramer needs to be unique (out of 256 tetramers)
- To avoid formation of linear duplex DNA, linear complements to each of 12 tetramers flanking the branch point are also forbidden.
- Homology sequence between trimers can be ignored as the free energy between octamers win out.

….

Seeman NC, Ann. Rev. Biochem 2010

Motifs of DNA Lattices

- **Polarity**
- **Topology**

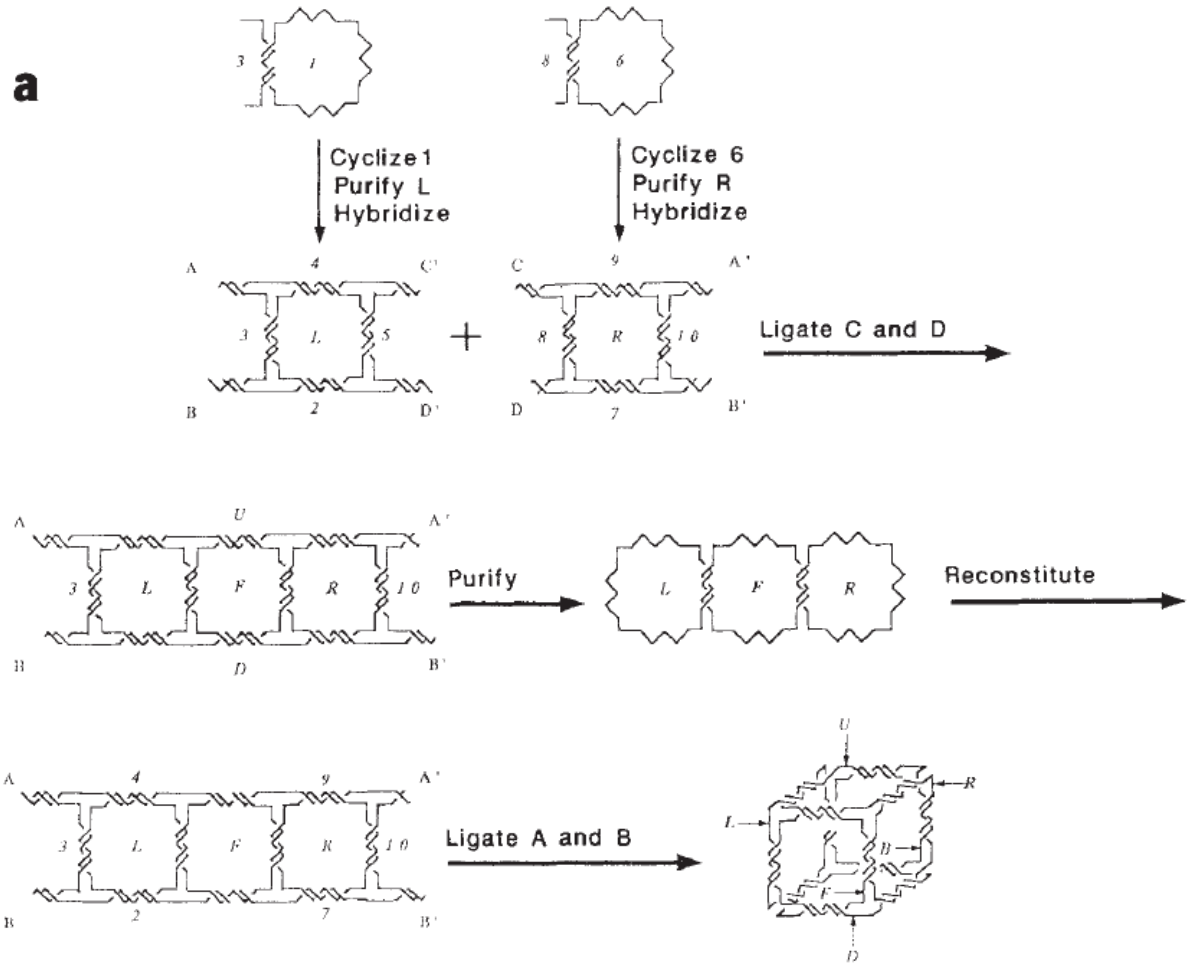


Seeman NC, Ann. Rev. Biochem 2010

NATURE · VOL 350 · 18 APRIL 1991

Synthesis from DNA of a molecule with the connectivity of a cube

Junghuei Chen & Nadrian C. Seeman



However, technical limitations are:

- Involves a large number of short oligonucleotides and complicated construction process
- the yield of complete structures is highly sensitive to stoichiometry (relative ratios of strands)

Breakthrough in 2006

NATURE|Vol 440|16 March 2006

ARTICLES

Folding DNA to create nanoscale shapes and patterns

Paul W. K. Rothemund[1]

Several restrictions in previous method:

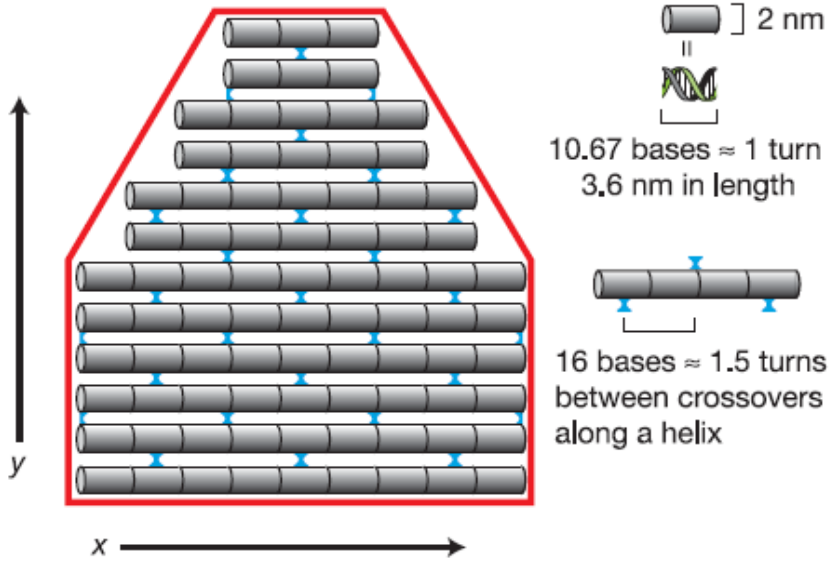
1) Sequences must be optimized to avoid secondary structure or undesired binding interactions

2) Strand must be highly purified

3) Strand concentrations must be precisely equimolar

The design of DNA origami

1st step: to built a geometric model of a DNA structure that will approximate the desired shape by even number of parallel double helices

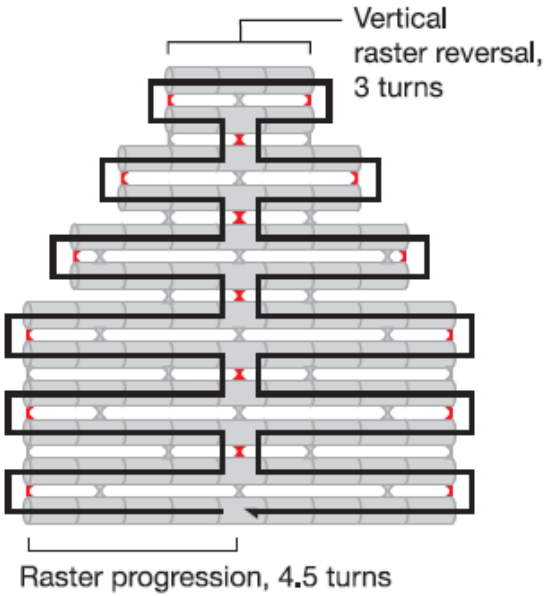


The design of DNA origami

1st step: to built a geometric model of a DNA structure that will approximate the desired shape by even number of parallel double helices

↓

2nd step: to fold a single long scaffold DNA strand back and forth in a raster fill pattern



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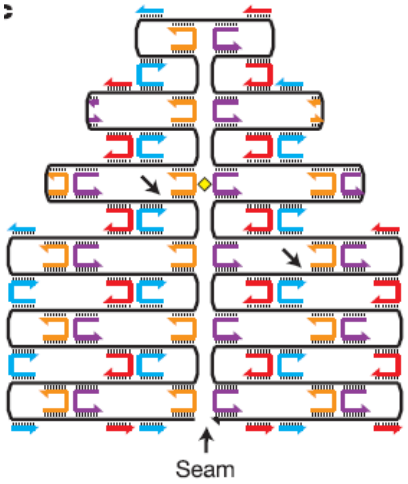
2nd step: to fold a single long scaffold DNA strand back and forth in a raster fill pattern

↓

3rd step: to design a set of “staple strands” that provide Watson-Crick complements, and create the periodic crossovers to arrange the crossovers in alternating directions in alternating columns



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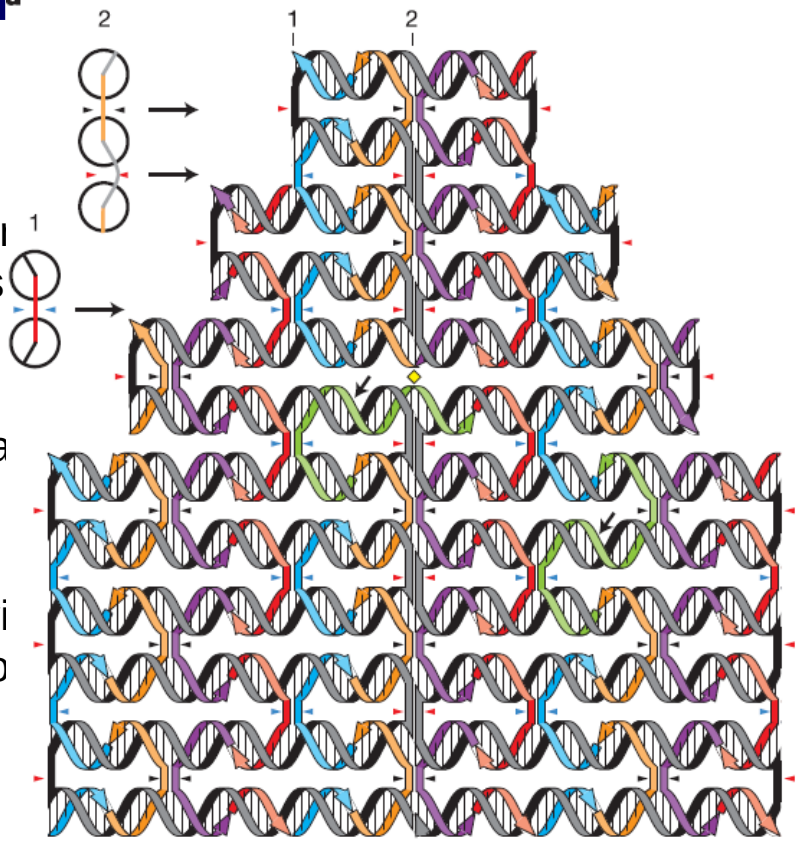

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3rd step: to design a set of “staple strands” that provi
create the periodic crossovers to arrange the crosso
alternating columns

4th step: to minimize the twist tension between scaffold crossovers by changing their position

The design of DNA origami

1st step: to built a geometric model of a DNA structure t
by even number of parallel double helices

↓

2nd step: to fold a single long scaffold DNA strand back

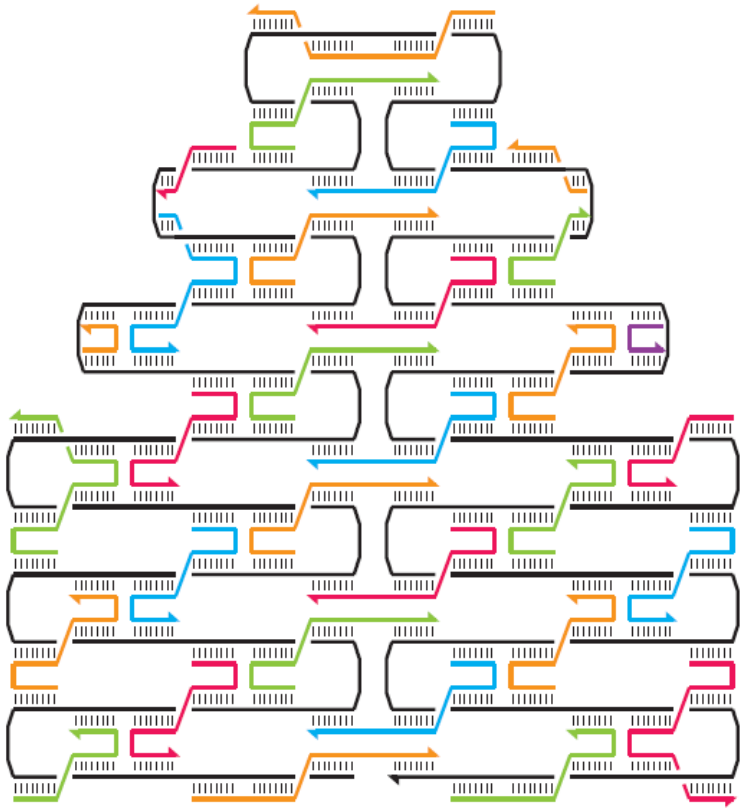
↓

3rd step: to design a set of “staple strands” that provide
create the periodic crossovers to arrange the crossover
alternating columns to minimize the tension caused by

↓

4th step: to minimize the twist tension between scaffold

↓

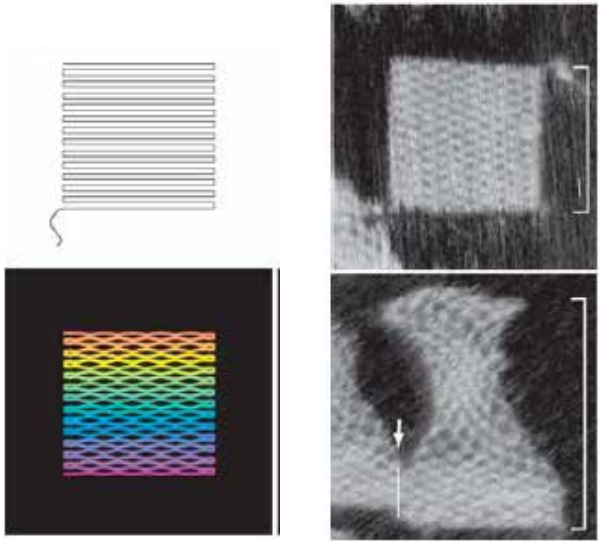


5th step: pairs of adjacent staples are merged across niches to achieve higher binding specificity and higher biding energy which results in higher melting temperatures

Validation

Material:
- Virus M13mp18 (single stranded 7,249-nt)
- 100-fold excess of 200-250 staple and “reminder strands” to fold the unused sequence

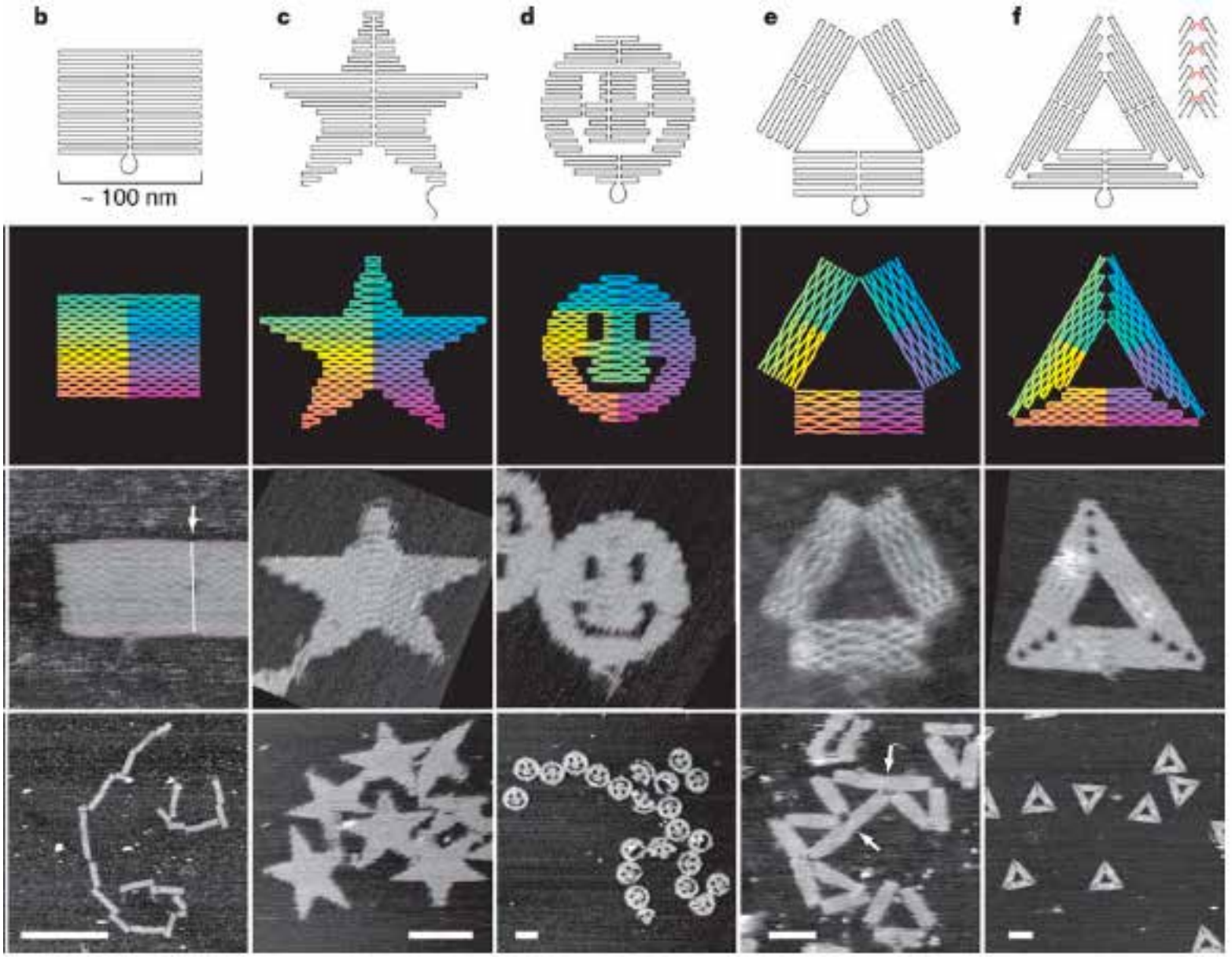
Method:
•All are mixed and annealed from 95 to 20 degree C in <2h
•Samples were deposited on mica and only folded DNA stuck to the surface while others remained in solution
•Atomic force microscope (AFM) without purification



13% well formed with ratio of 1.00 to 1.07 (W vs H)

25% rectangular form

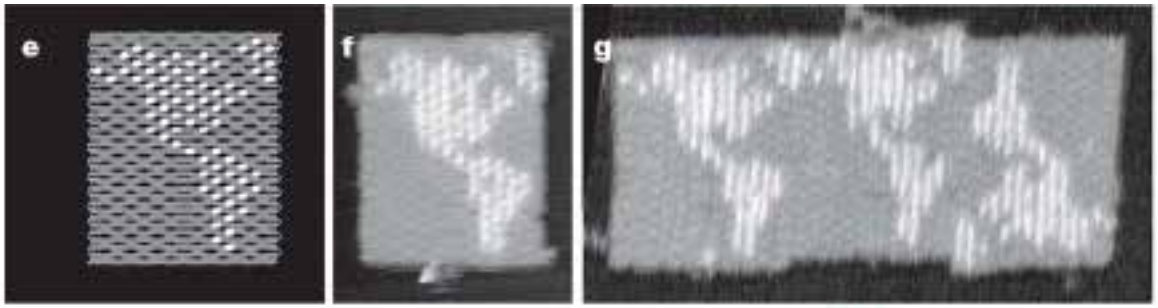
25% hourglass shape



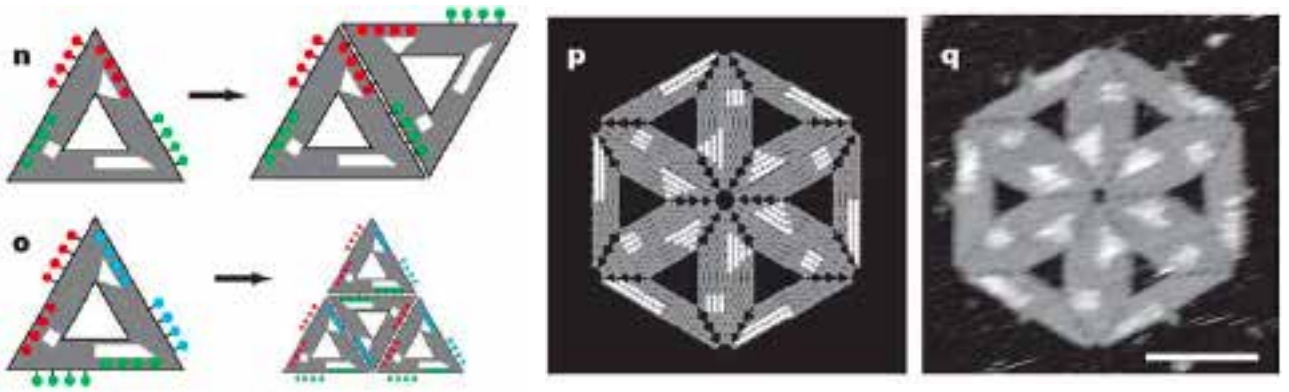
Well formed	90%	63%	70%	<1%	88%

Binary pixels using labeled staples

Labeled staples (3nm above the mica) -> light ‘1’ pixels
Unlabeled staples (1.5nm) -> dark ‘0’ pixels



Folding error is similar to unpatterned origami
Most defects were “missing pixels” although only 6% error



Summary of novel DNA origami method

Important factors for the novel DNA origami folding are:

1) Strand invasion that allows correct binding of excess staples

2) An excess of staples to displace unwanted secondary structure

3) Cooperative effects in which correct addition of each staples organizes the scaffold for subsequent binding of adjacent staples

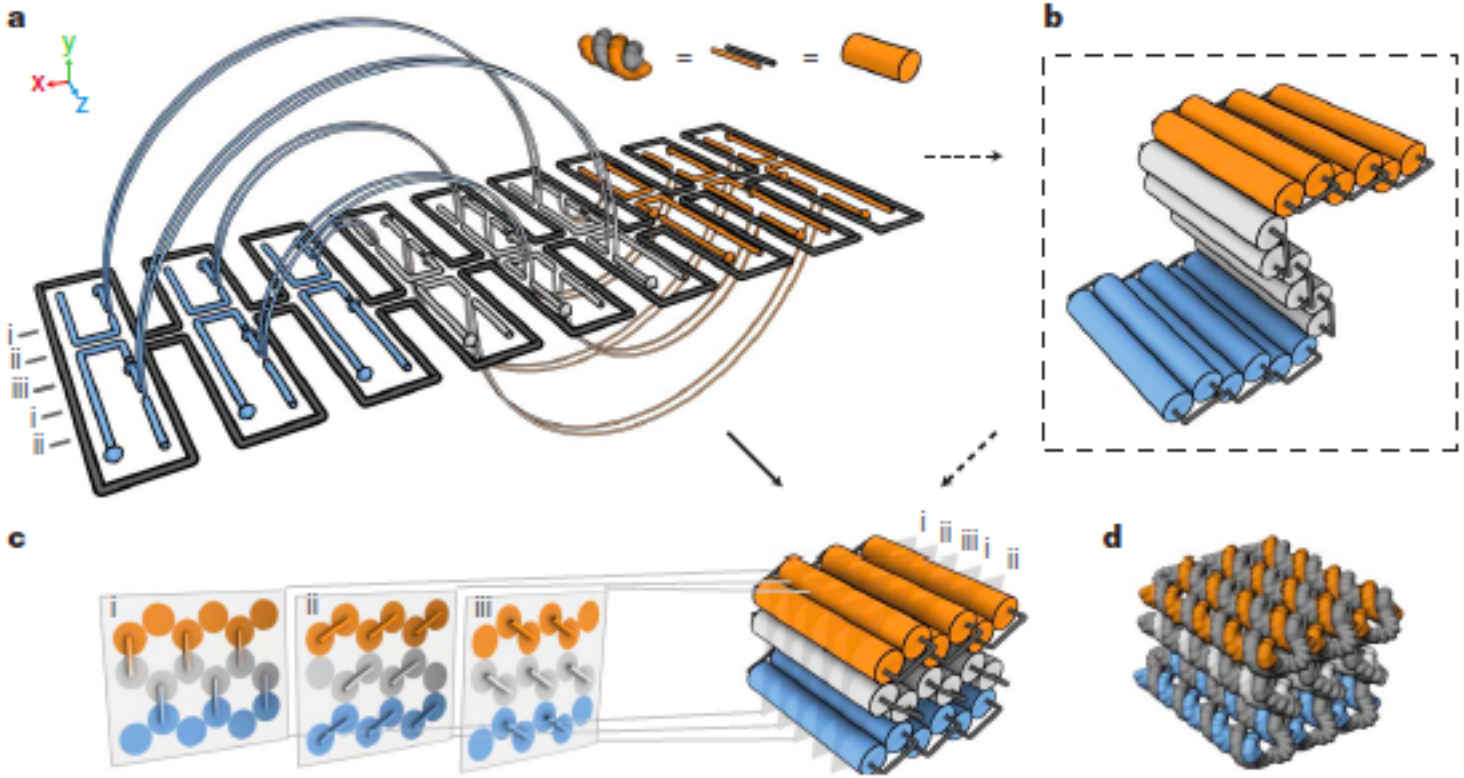
4) Design that intentionally does not rely on binding between staples

3D

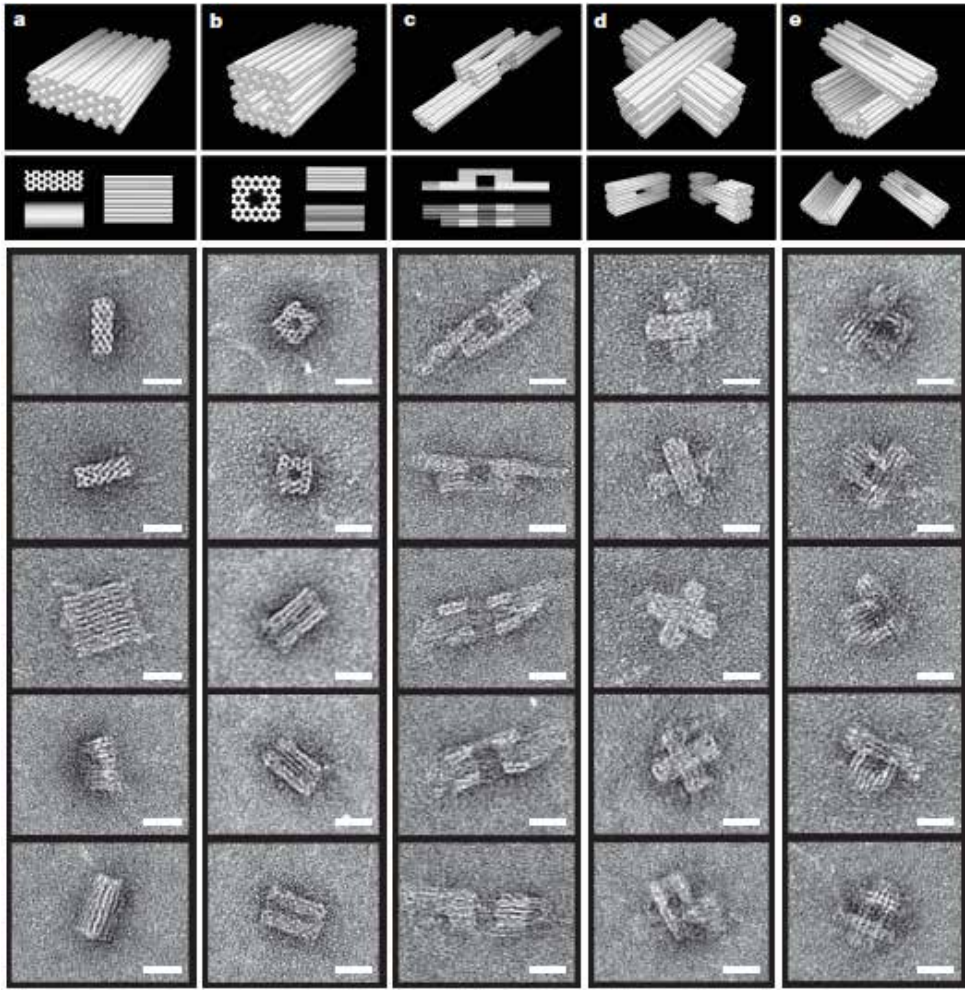
nature Vol 459|21 May 2009|doi:10.1038/nature08016

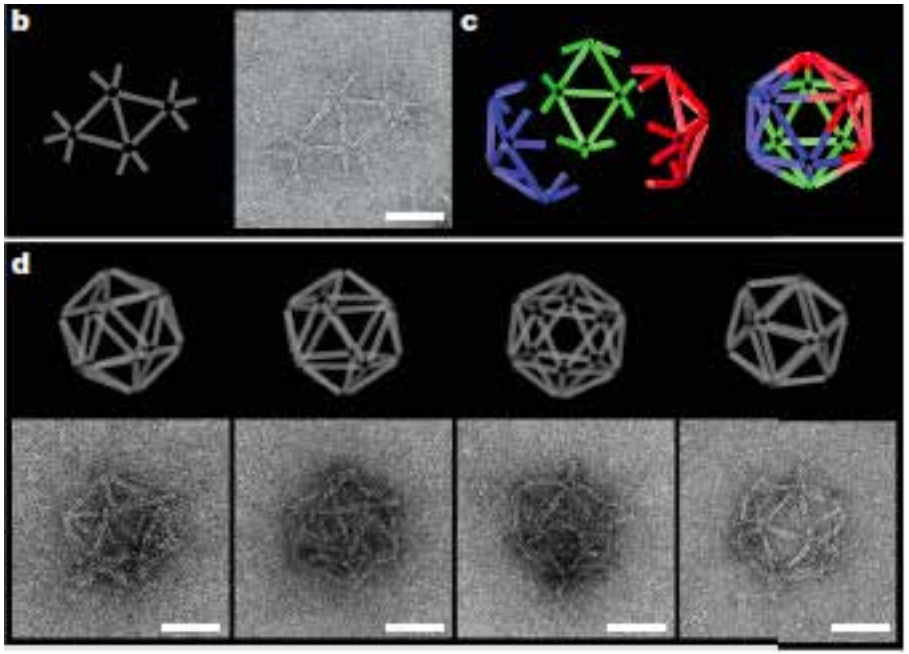
Self-assembly of DNA into nanoscale three-dimensional shapes

Shawn M. Douglas[1,2,3], Hendrik Dietz[1,2], Tim Liedl[1,2], Björn Högberg[1,2], Franziska Graf[1,2,3] & William M. Shih[1,2,3]

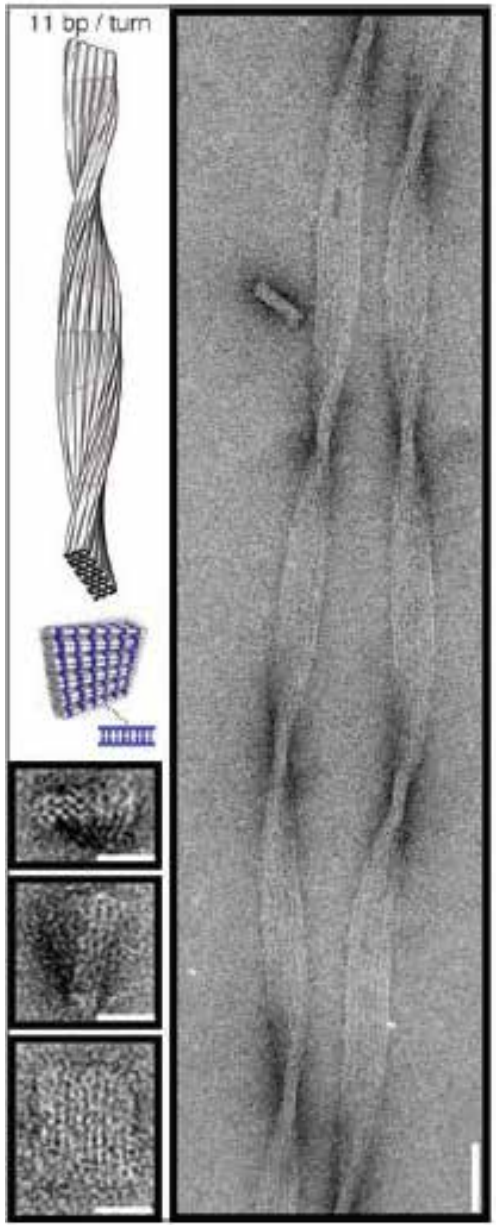


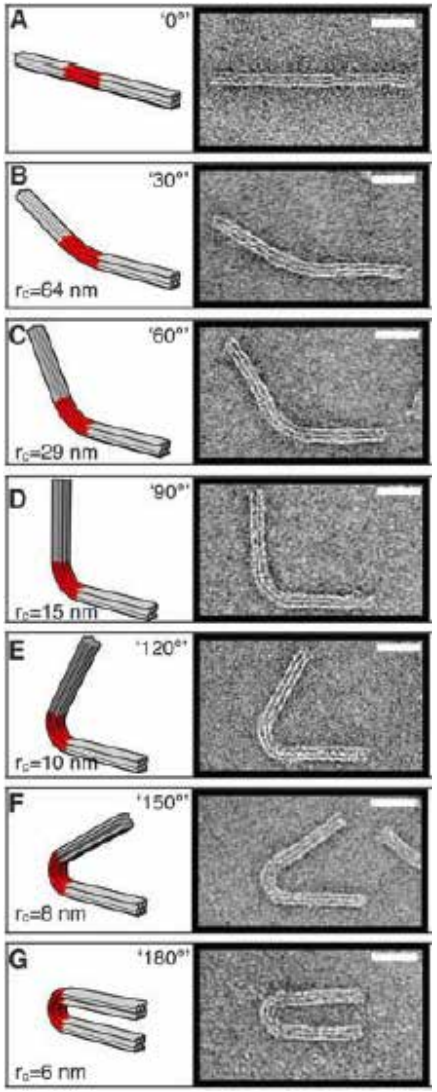
3D

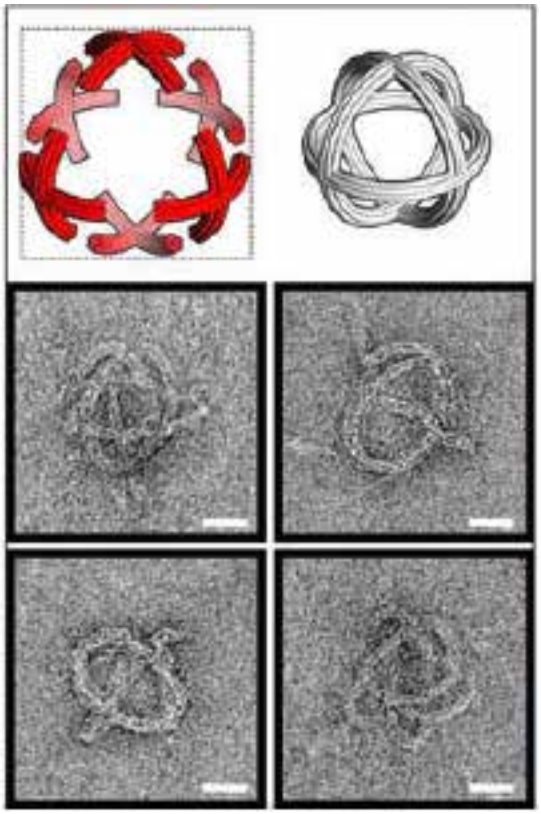




More complicated shape: Twisted and curved







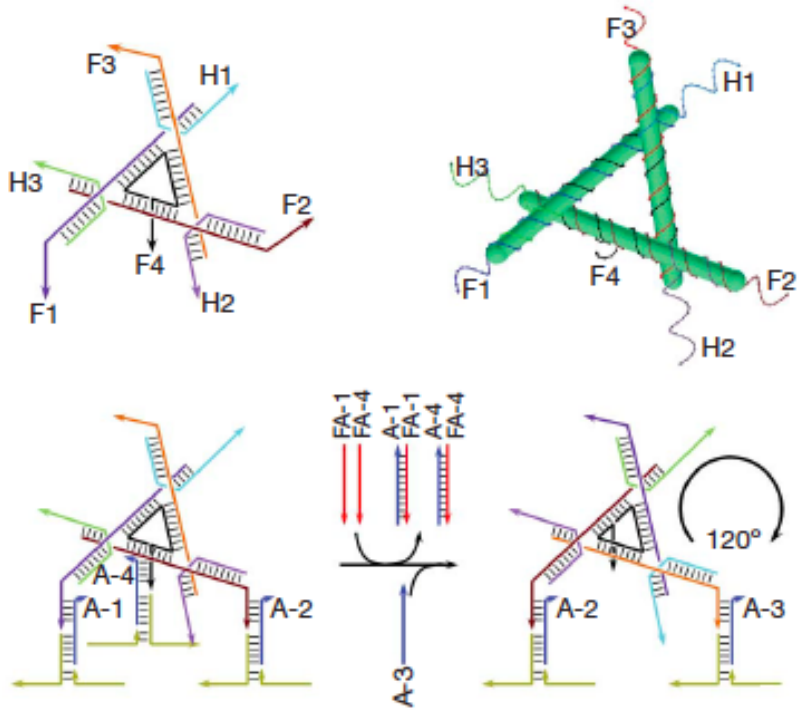
Folding DNA into Twisted and Curved Nanoscale Shapes
Hendrik Dietz *et al.*
Science **325**, 725 (2009);
DOI: 10.1126/science.1174251

Science
AAAS

What is missing?

DNA walker

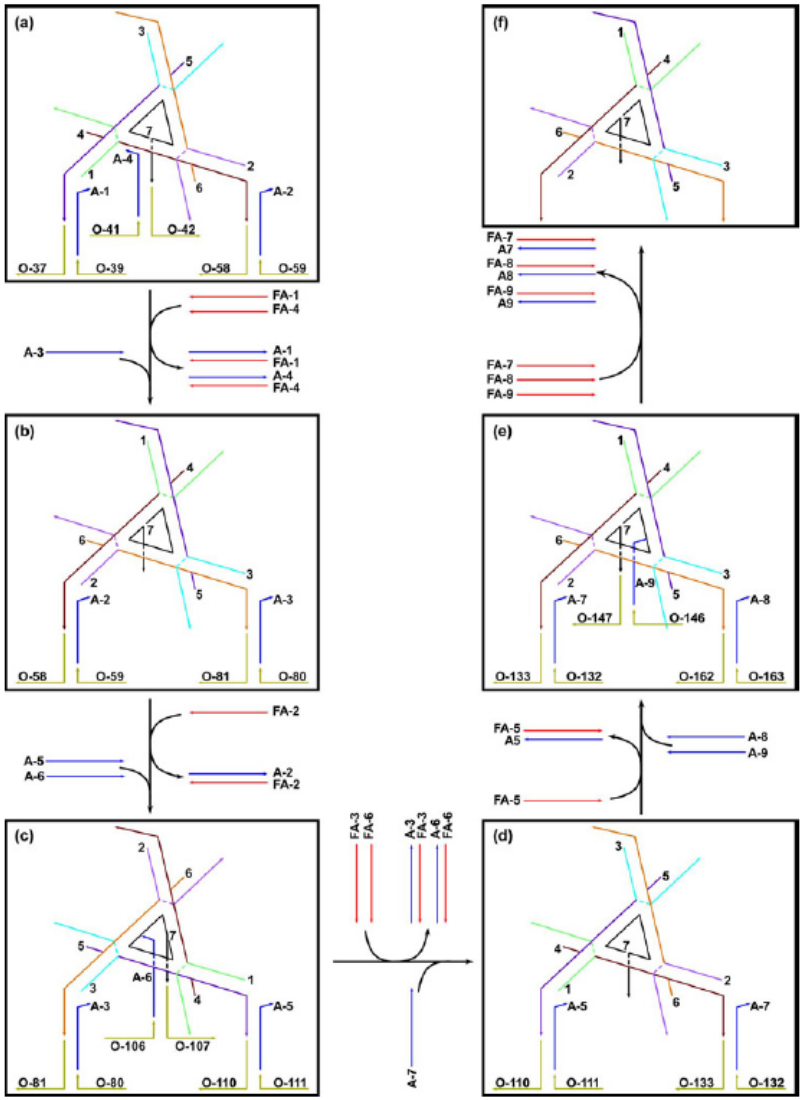
Walking device



1) 2h R.T. incubation with "fuel DNA"
2) 2h R.T. incubation with "step DNA"

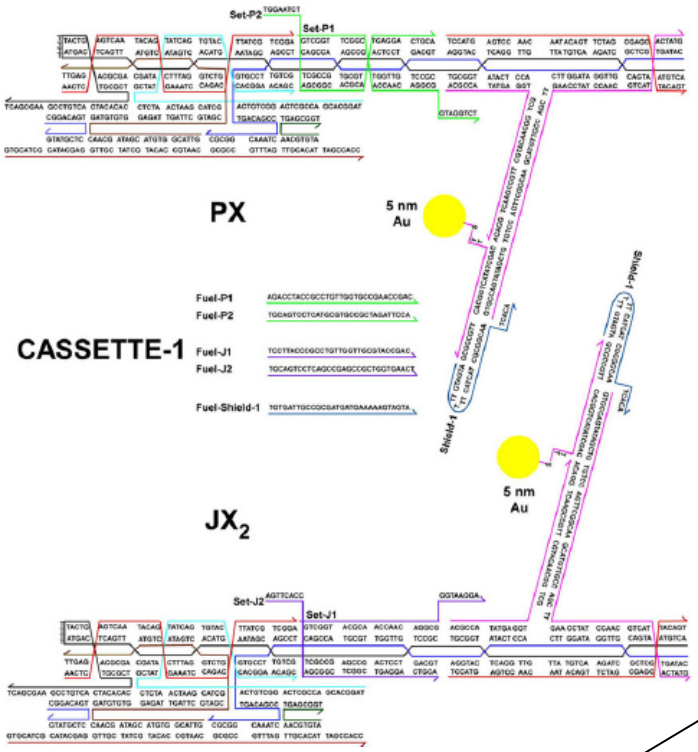
Gu H et. al., Nature 2010

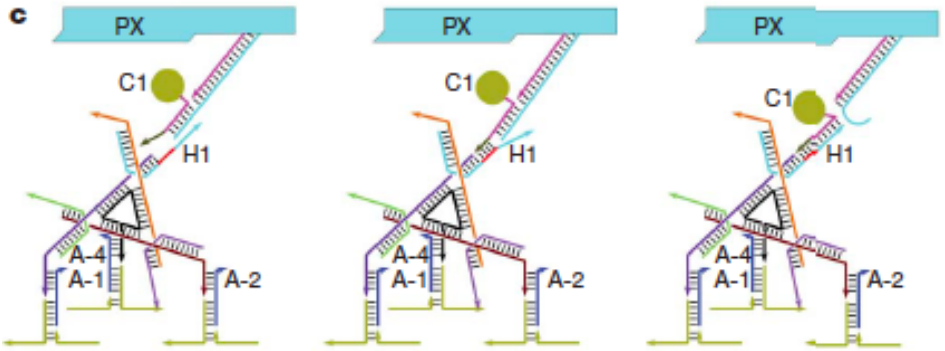
“Big” Four Steps in a Nanoscale



Gu H et. al., Nature 2010

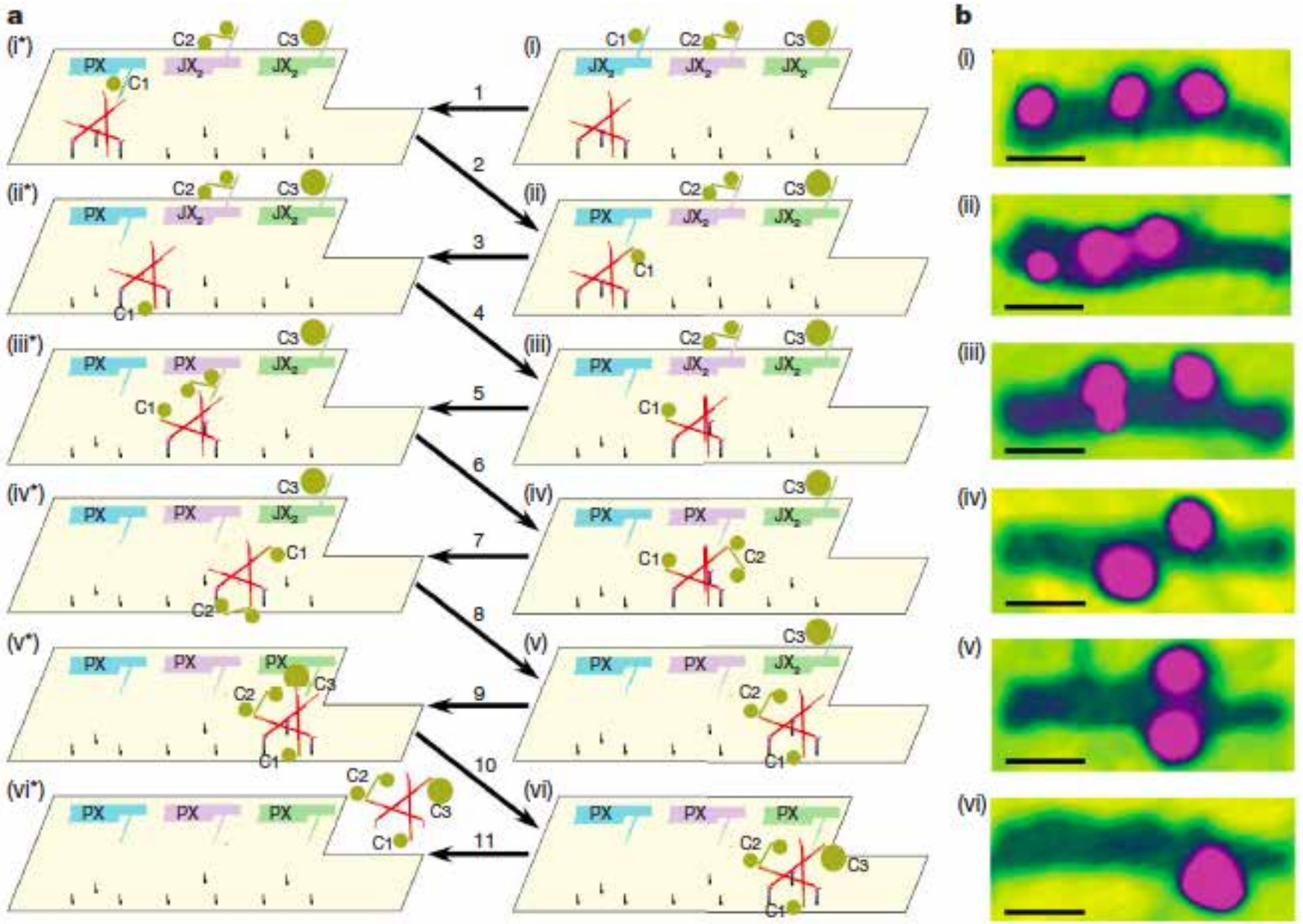
Cargo pick-up station





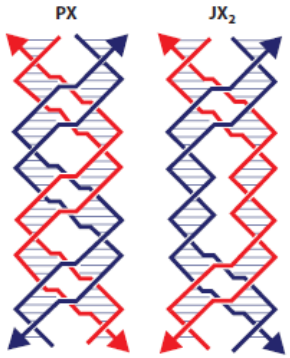
Gu H et. al., Nature 2010

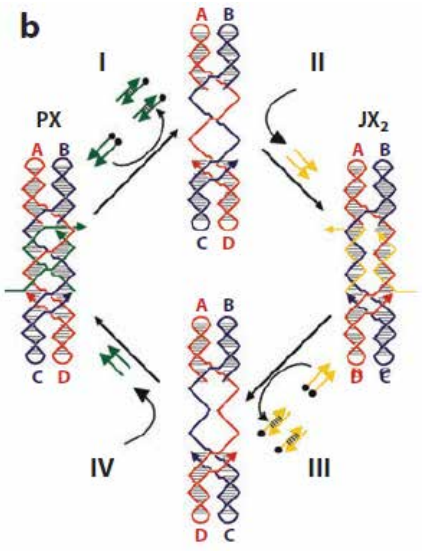
Programmable assembly line “Nanofactory”

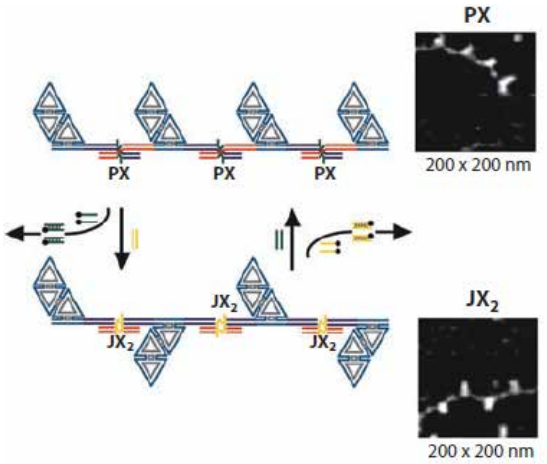


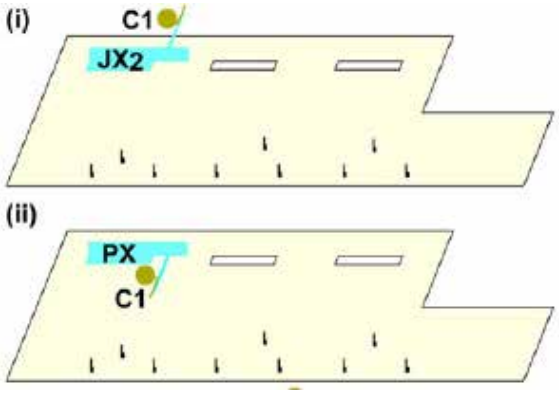
Gu H et. al., Nature 2010

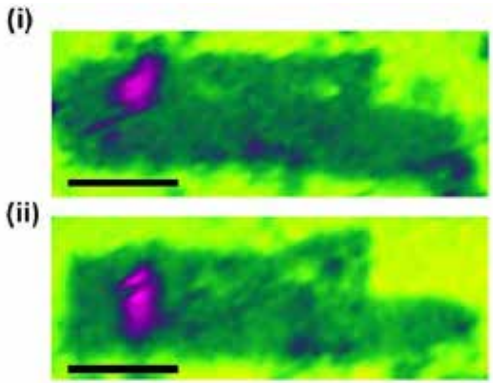
Programmable cargo-pick up station











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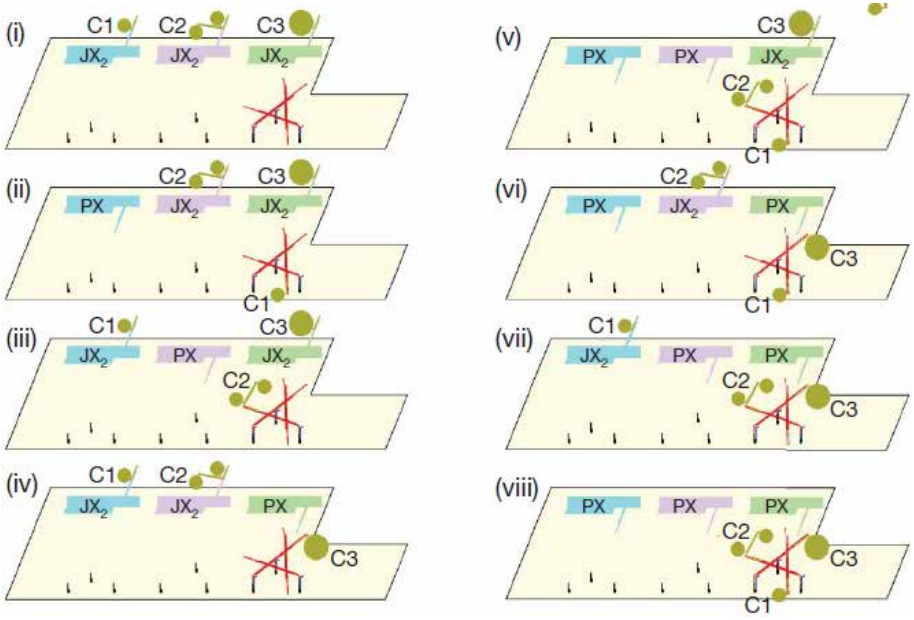

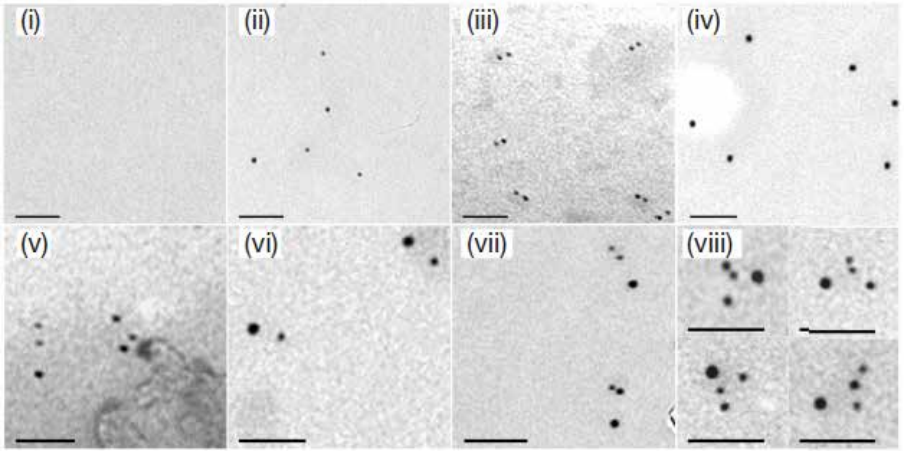

Gu H et. al., Nature 2010

Summary of Nanofactory

This system adds elements of both programmability and temporal control to DNA assisted assembly

As a perspective,

with some modification it would allow the construction of new chemical species that are not readily synthesized by other means.

Potential application for Other fields

1) Novel biological experiments that aims at modelling complex protein assemblies and examining the effects to spatial organization
2) Molecular electronic or plasmonic circuits by attaching nanowires, carbon noanotubes or gold nanoparticles
3) Nanoelectronics (e.g. RAM)
4) Nanophotonics
5) Coordination chemistry (Gartner JZ, J Am Chem Soc 2002)

Potential application for Biology

1) NMR structure determination (Douglas SM, PNAS 2007)

membrane proteins are encoded by 20-35% but represent only <1% known protein structure

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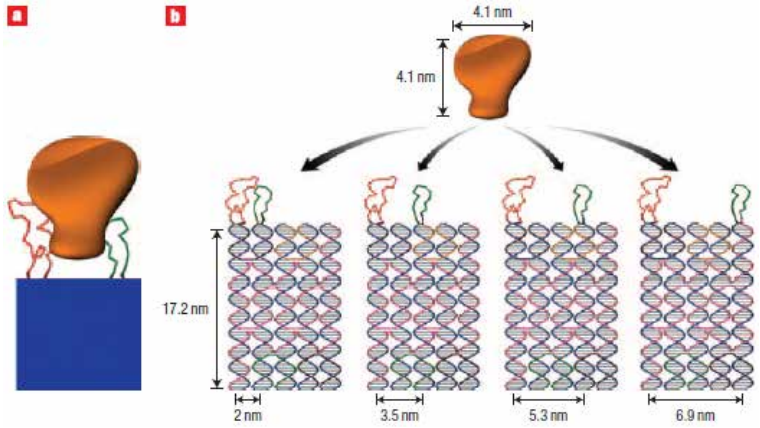

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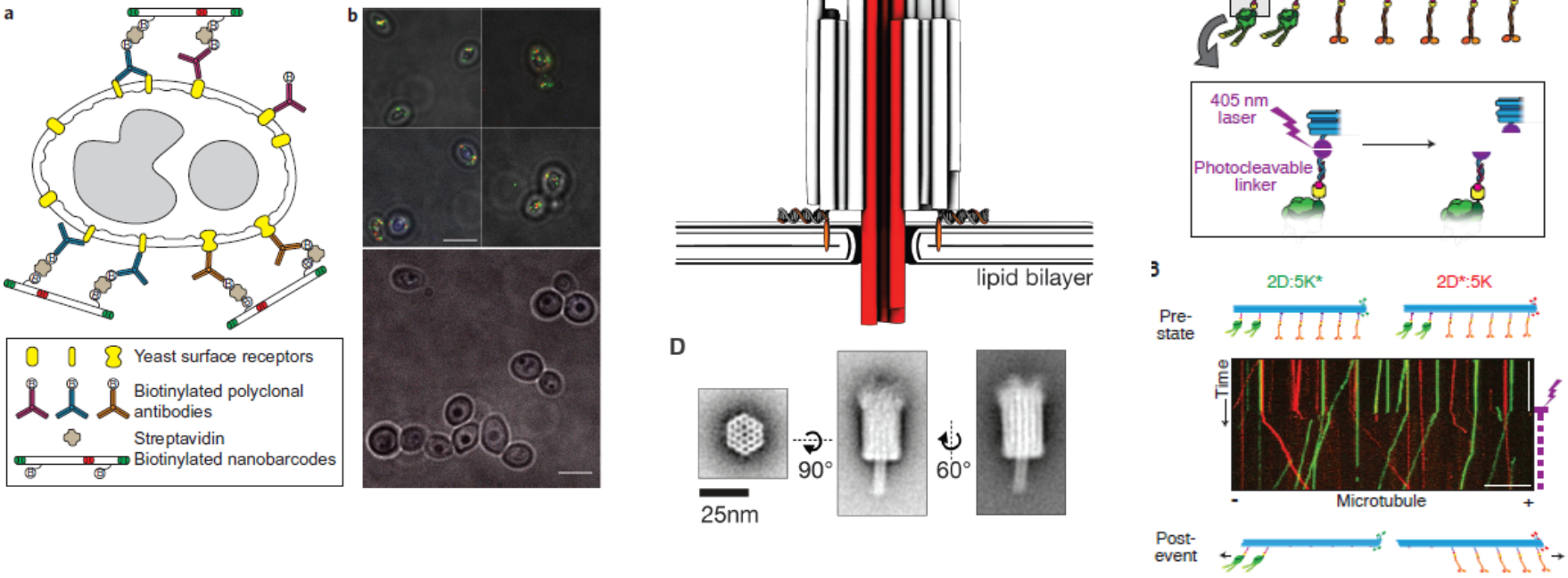
3) Molecular probe for single cell or molecule imaging (Lin C, Nat Chem 2012; Derr, Science 2012; Acuna GP, Science 2012)



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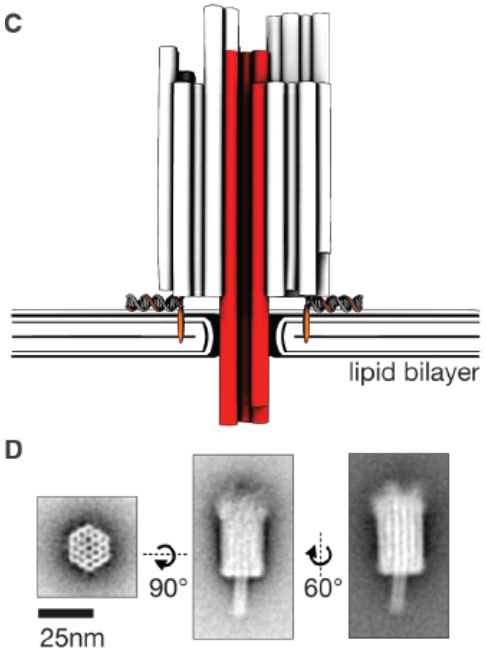
4) Generation of artificially synthesized new molecule (Langecker, Science 2012)



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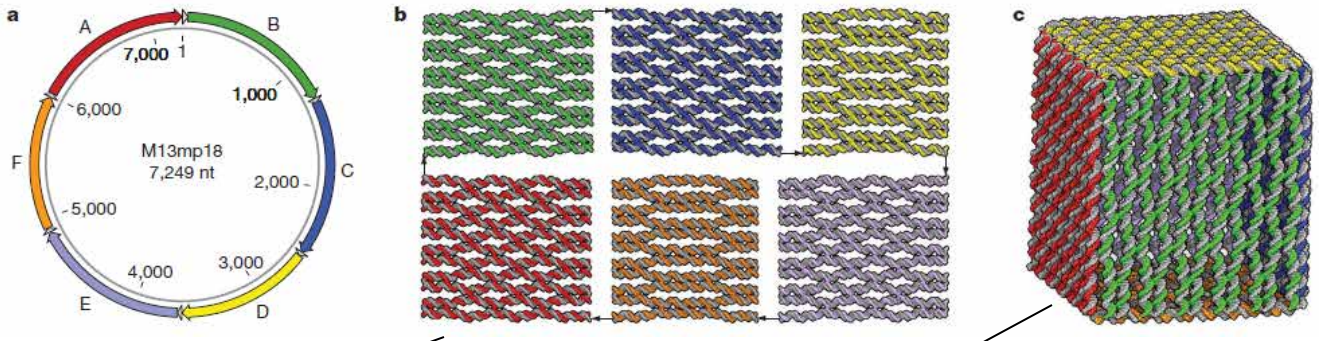
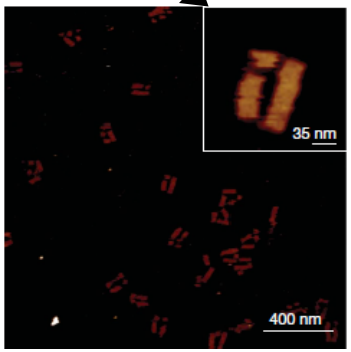
Targeting Delivery System for Nanomedicine

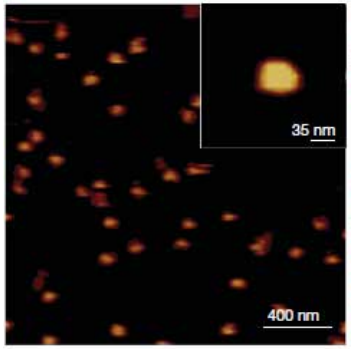
Vol 459|7 May 2009|doi:10.1038/nature07971 nature

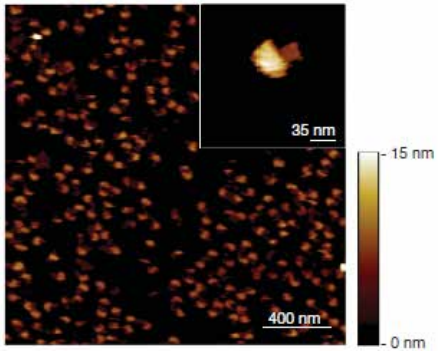
Self-assembly of a nanoscale DNA box with a controllable lid

Ebbe S. Andersen[1,2,3], Mingdong Dong[1,2,4]†, Morten M. Nielsen[1,2,3], Kasper Jahn[1,2,3], Ramesh Subramani[1,2,4], Wael Mamdouh[1,2,4], Monika M. Golas[5,8], Bjoern Sander[6,8], Holger Stark[8,9], Cristiano L. P. Oliveira[2,7], Jan Skov Pedersen[2,7], Victoria Birkedal[2], Flemming Besenbacher[1,2,4], Kurt V. Gothelf[1,2,7] & Jørgen Kjems[1,2,3]









Closed form Open form

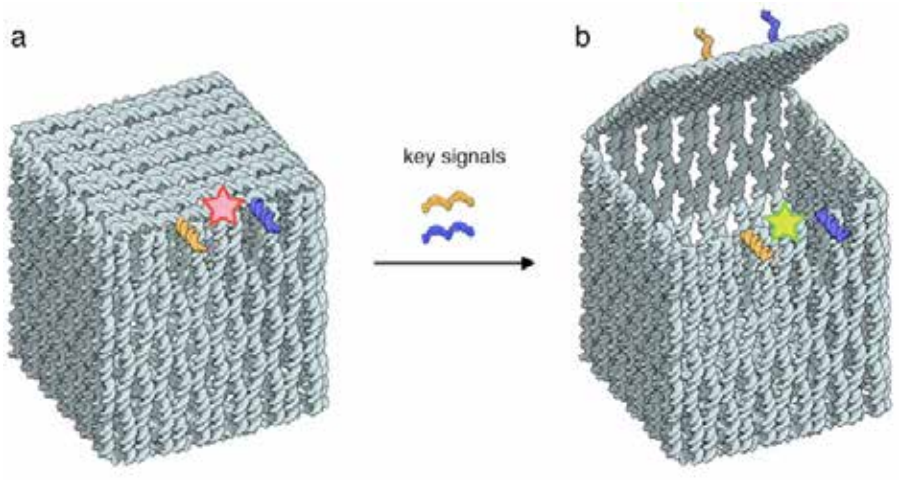
Design:

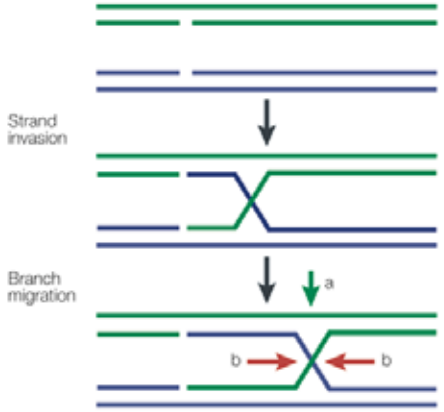
H, 42; W, 36; L, 36 nm

Experimental data:

H, 46+/- 2; W, 38 +/- 1; L, 30+/- 1 nm

Principle of key lock system

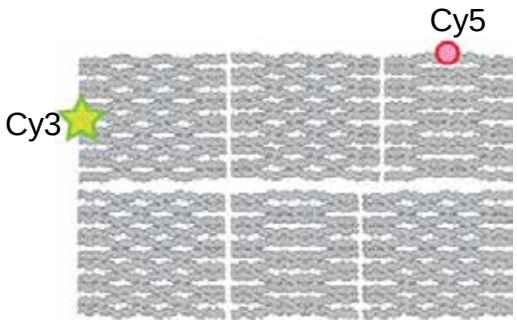


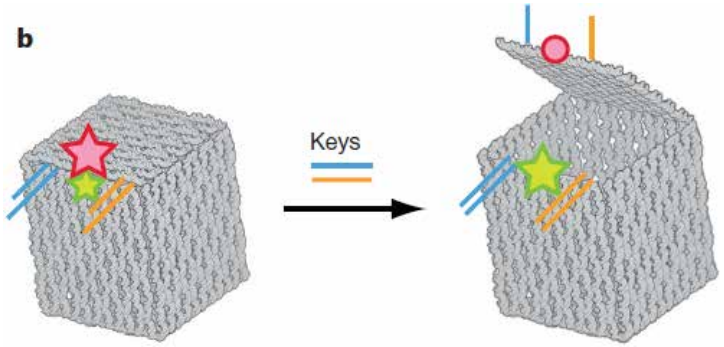



```
B-Lock1       5'-BOX-GGCAGCTCGACTGATG-3'
D-Lock1       3'-BOX-CCGTCGAGCTGACTACGCTGACGT-5'


Key1             5'-GGCAGCTCGACTGATGCGACTGCA-3'


B-Lock2       5'-BOX-TTCTAGGCATCGTAAG-3'
D-Lock2       3'-BOX-AAGATCCGTAGCATTCCATCATGG-5'


Key2             5'-TTCTAGGCATCGTAAGGTAGTACC-3'
```

Initial biding with 8-nt initiates branch migration that removes Lock strand and add key strand with complete complement

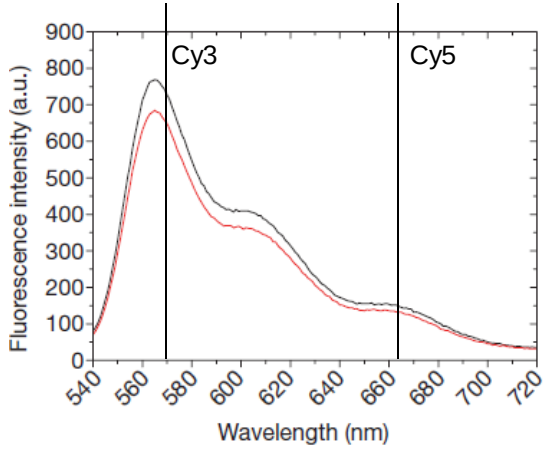
Dynamic control and programmability of the box lid



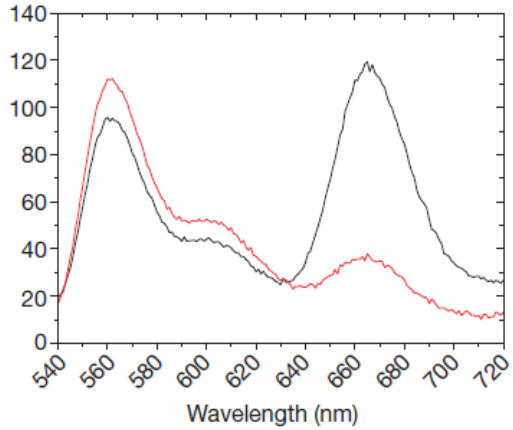


FRET measurement

Box with unlinked faces



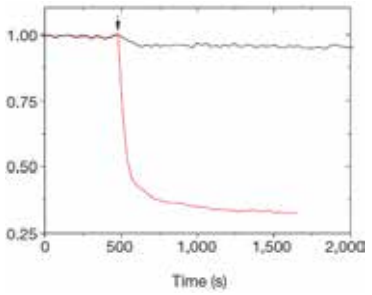
Box with linked faces



Unrelated seq.

Related seq.

Kinetics



Before key

After key

Response - ca. 40s

Discussion

The application of ‘nanorobotic’ device could be restricted to:

1) transport of material in or out of the box in a controlled fashion

2) packing of biological active component as enzymes to control access to their relevant substrates

3) delivery of hazardous drug or diagnostic sensor to specifically target tissue or cell

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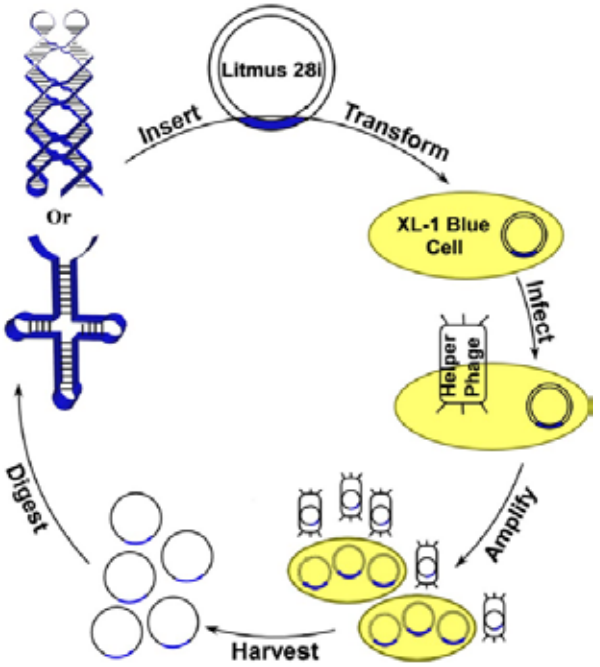
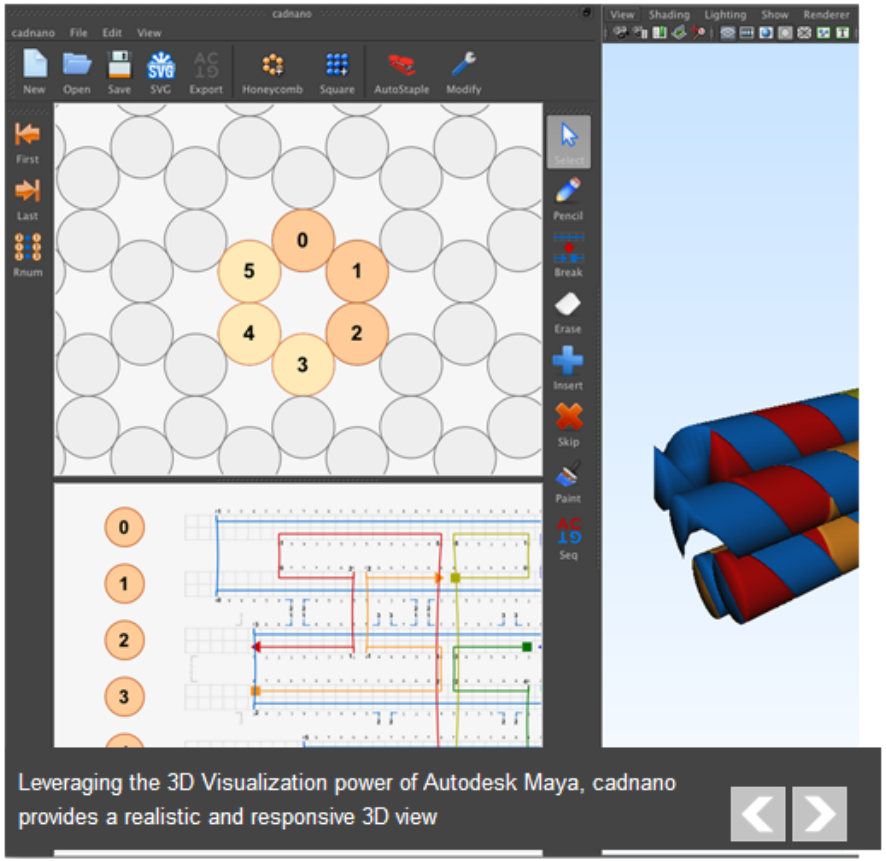
6) In vivo cloning of nanostructure (Lin C, PNAS 2008)





Leveraging the 3D Visualization power of Autodesk Maya, cadnano provides a realistic and responsive 3D view

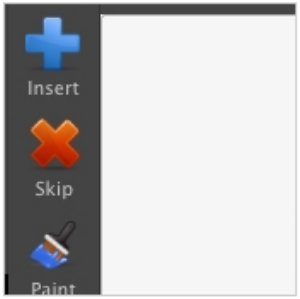
cadnano simplifies and enhances the process of designing three-dimensional DNA origami nanostructures. Through its user-friendly 2D and 3D interfaces it accelerates the creation of arbitrary designs. The embedded rules within **cadnano** paired with the finite element analysis performed by cando, provide relative certainty of the stability of the structures.

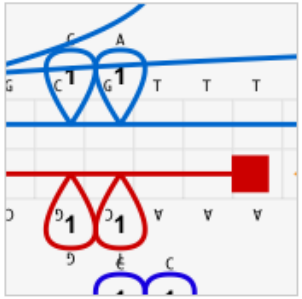
cadnano features:

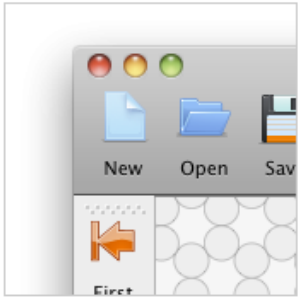
- Platform independent (tested in Windows, OSX and Linux)
- Visual cues aid design process for stable structures
- 3D interface powered by Autodesk Maya*
- Open architecture for plug-in creation
- Free and open source (MIT license)

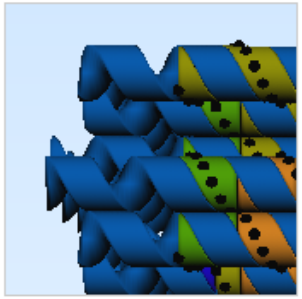
DOWNLOAD CADNANO
It's free and open source.

latest screenshots (click here for more)









Thank you!