Self-Assembled DNA Nanostructures

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DNA-Based Self-Assembly and Nanorobotics
On Constructing Complex, Fault-Tolerant Nanostructures and Programmable Nanorobotic Devices
Organization of talk

- Overview of DNA & DNA Self-Assembly

- Novel self-assembly DNA nanostructures: DNA Tiles and DNA Lattices

- Programmable Molecular Patterning via DNA Lattices

- 2D & 3D DNA Origami

- 3D lattices via double decker tiles
Introduction to DNA Self-Assembly
Feynman’s Ill-Conceived Top-Down Approach to Nanotechnology

Feynman ("Plenty of room at the bottom", 1959):
• Can the doctor be swallowed? (Albert Hibbs)
• Can we build tiny factories that can arrange atoms the way we want?
• Can we write the 24 volumes of the Encyclopedia Brittanica on the head of a pin?

=> Suggested a Top-Down Approach to Nanotechnology

“This fact - that enormous amounts of information can be carried in an exceedingly small space - is, of course, well known to the biologists, and resolves the mystery which existed before we understood all this clearly, of how it could be that, in the tiniest cell, all of the information for the organization of a complex creature such as ourselves can be stored. All this information—whether we have brown eyes, or whether we think at all, or that in the embryo the jawbone should first develop with a little hole in the side so that later a nerve can grow through it - all this information is contained in a very tiny fraction of the cell in the form of long-chain DNA molecules in which approximately 50 atoms are used for one bit of information about the cell.”
Self-assembly in nature

Spontaneous organization of components into stable superstructures due to local interactions

From microscopic living cells to gigantic galaxies

Figure 3-25. Molecular Biology of the Cell, 4th Edition.
Why study self-assembly?

• Plays a fundamental role in biology, especially in formation of living cell
  • Attempt to understand life must include a thorough study of SA

• One of the few known methods for the construction and manipulation of nanostructures

• Any Turing-computable function can be computed via self-assembly of Wang tiles
  • New paradigm of computing
  • Lower bounds proved in theoretical self-assembled systems can be translated (by appropriate reductions) to Turing systems

• Brings about order from disorder
  • Interesting at a philosophical level
Double Stranded DNA

Source: Wikipedia.com

Source: http://www.coriell.org/assets/images/personalized-medicine/dna-genes-snps-enlarged.jpg
Overview

• Why DNA?
  1. Natural nanoscale material
  2. Ability to carry information can be exploited in self-assembly process
  3. Well established base-pairing model in which the stability of a base-pair depends on their identity (A-T, C-G)
Overview

Adenine

Thymine

5’ end

3’ end

Phosphate-
deoxyribose
backbone

Guanine

Cytosine

5’ end
Key to DNA Self-Assembly

Hybridization

3’ TTGTTTATACCT 5’

5’ AACAAATTGGGA 3’

3’ TTGTTTATACCT 5’

5’ AACAAATTGGGA 3’
What is DNA Self-Assembly?

Programming DNA strands to organize themselves into nanoscale shapes, patterns, and devices through Watson-Crick base-pairing.
DNA Nanotechnology

Seeman 1982:

- “It is possible to generate sequences of oligomeric nucleic acids which will preferentially associate to form migrationally immobile junctions, rather than linear duplexes, as they usually do.”

Some results of DNA self-assembly

NYU 1991
The Electrophoretic Properties Of A DNA Cube And Its Substructure Catenanes : Mao And Seeman

Purdue 2005
Self-assembly Of Hexagonal DNA Two-dimensional (2D) Arrays: He, Chen, Liu, Ribbe, And Mao

NYU 1991
The Electrophoretic Properties Of A DNA Cube And Its Substructure Catenanes : Mao And Seeman

Purdue 2005
Self-assembly Of Hexagonal DNA Two-dimensional (2D) Arrays: He, Chen, Liu, Ribbe, And Mao

2004
Algorithmic Self-assembly Of DNA Sierpinski Triangles: Rothemund, Papadakis, Winfree

2006
Folding DNA To Create Nanoscale Shapes And Patterns: Rothemund

2009
Harvard
Self-assembly Of DNA Into Nanoscale Three-dimensional Shapes: Douglas, Dietz, Liedl, Hogberg, Graf, Shih

2003
Directed Nucleation Assembly Of DNA Tile Complexes For Barcode-patterned Lattices: Yan, Labeau, Feng, Reif

2006
Finite-size, Fully-addressable DNA Tile Lattices Formed By Hierarchical Assembly Procedures : Park, Pistol, Ahn, Reif, Lebeck, Dwyer, Labeau

2003
4x4 DNA Tile And Lattices: Characterization, Self-assembly And Metallization Of A Novel DNA Nanostructure Motif : Yan, Park, Finkelstein, Reif And Labeau

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Unpublished Data:
Majumder, Reif
Design & Experimental Demonstration of DNA Tiles and Lattices
Example: Self-assembly of DNA lattices

- Driven by Watson-Crick base pairing: \( A \leftrightarrow T \) & \( C \leftrightarrow G \)
- Leads to energy minimization of the final structure
  - Base pairing and base stacking
- Programmability:
  - AGTGC sticks to GCACT (reverse complement)
DNA tiles
DNA molecules self-assembled from artificially synthesized single stranded DNA.

- **Anti-parallel crossovers:**
  - cause a reversal in direction of strand propagation through the tile following exchange of strand to a new helix.

- **Pads:**
  - Tiles have sticky ends that preferentially match the sticky ends of certain other DNA tiles.
  - The sticky ends facilitate the further assembly into tiling lattices.
  - **Total of 4 Pads** of single stranded DNA at ends.

**Self-Assembly from DNA strands, to Tiles, to Lattices**
• TX tile – extension of the DX tile

• Three helices made of 4 strands
• **Triple-crossover (TX) Tiles** [LaBean, Reif et al, J. Am. Chem. Soc., 2000]:
  
  – consist of three double-helices fused by crossover strands.
  – TAE contains an Even number of helical half-turns between crossover points.
  – TAO contains an Odd number.

• Total of 6 Pads of single stranded DNA at ends.
Unique Sticky Ends on DNA tiles. Input layers can be assembled via unique sticky-ends at each tile joint thereby requiring one tile type for each position in the input layer.

Tiling self-assembly:

proceeds by the selective annealing of the pads of distinct tiles, which allows tiles to compose together to form a controlled tiling lattice.
TX lattices
TX lattices
Large Scale DNA Self-Assembled Tilings
Visualization by Atomic Force Microscope.

$AB^*$ Lattice. An atomic force microscope image of DNA lattice formed by two TAO tiles one of which contains an extra loop directed out of the plane. These loops form the visible stripe features with the expected spacing of $\sim 28$ nm.

Crossover DNA Tiles and their Lattices

Used Corrugation to form 2D Grid Lattices

Also form Tubes & Ribbons
DNA Tubes & Ribbons
TX tubes
TX tubes

AFM

TEM
Au Metallization of 4x4 ribbon and Conductivity Measurement

Patterned DNA Lattices
Programmable Patterned DNA Nanostructures

NOT Patterned

Patterned
Patterned DNA lattices:

• Allows for Attachment of Nanoparticles at Specific Sites on Lattice

• Application: Molecular Electronics:
  – Layout of molecular electronic circuit components on DNA tiling arrays.

Multiple tiles of an input layer can be assembled around a single, long DNA strand we refer to as a scaffold strand (shown as black lines in the figures).

Barcode lattice displays banding patterns dictated by the sequence of bit values programmed on the input layer.

- Extends 2D arrays into simple aperiodic patterning:
  - The pattern of 1s and 0s is propagated up the growing tile array.
  - The 1-tiles are decorated with a DNA stem-loop pointing out of the tile plane (black rectangle) and 0-tiles are not.
  - Columns of loop-tiles and loopless-tiles can be distinguished by AFM as demonstrated with periodic AB* lattice.
Barcode Lattice for Rendering 1 D Patterns:

H Yan, T LaBean, L Feng, J. Reif, PNAS (2003).

Barcode lattice displays banding patterns dictated by the same sequence of bit values programmed on each layer.
Barcoded lattices

C

\[ \text{--- 4.5 turns ---} \quad \text{--- 9 turns ---} \]

D

800x800nm

250x250nm
Cross tile
Cross tile

(a) [Image of a cross tile diagram]

(b) [Image of a cross tile experiment result]
Cross tiles: Grid Assembly in 2D

Cross Tile

Figures adopted from He et al., 2005

Symmetric Tile

Corrugation creates enormous lattices
Cross Tile Lattices:
Highly uniform molecular scale lattices
far below VLSI scales
Uncorrugated cross tile tubes
Corrugated cross tile

Scan size: 155x155 nm

1.4 nm

19.0 nm
Hierarchical cross tile
Hierarchical Assembly of cross tiles
Addressable cross tile
Molecular Scale Patterning using Hierarchical Assembly of cross tiles
Hierarchical Assembly of DNA Lattices with 2 D Pattern “DNA”

Assembling a 2D Pattern by Directed Nucleation:
Self Assembly of Tiles around a DNA Strand Defining a 2D Pattern

Design Idea by LaBean & Reif, early 2000s
DNA Origami
Paul W K Rothemund’s DNA Origami

© 2006 Nature Publishing Group - Direction. As noticed before in DNA lattices, parallel helices in such structures are not close-packed, perhaps owing to electrostatic repulsion. Thus the exact y-resolution depends on the gap between helices. The gap, in turn, appears to depend on the spacing of crossovers. In Fig. 1a crossovers occur every 1.5 turns along alternating sides of a helix, but any odd number of half-turns may be used. In this study, data are consistent with an inter-helix gap of 1 nm for 1.5-turn spacing and 1.5 nm for 2.5-turn spacing, yielding a y-resolution of 6 or 7 nm, respectively.

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.

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
Fig. 6. A cartoon depicts folding of DNA origami as temperature changes from 90°C to 20°C.
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 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.
3D DNA Origami


doi:10.1038/nature08016
3D Shaped DNA Origami

Folding DNA into Twisted and Curved Nanoscale Shapes.
3D Shaped DNA Origami

Double Decker Tiles: A Rout to 3D DNA Lattices

Urmia Majumdar, Abhijit Rangnekar, Kurt V. Gothelf, John H Reif and Thomas H LaBean, Design and Construction of Double-Decker Tile as a Route to Three-Dimensional Periodic Assembly of DNA, Journal American Chemical Society (JACS), Vol. 133, no. 11, pp. 3843—3845 (Feb. 2011)
3D lattices via double decker cross tiles

- 2D lattices out of DNA tile
  - (a) DX tiles
  - (b) Four arm junction
  - (c) Three arm junction
  - (d) Five arm junction
  - (e) Six arm junction
  - (f) T-junction

- 3D lattices
  - (g) Tensegrity lattice

- Application of 3D lattices:
  - Imaging proteins
  - Organizing molecular electronic components
  - Organizing functional inorganic materials
  - Tile based computing
Double-decker tiles: Route to Assembly in 3D

4 identical arms

sticky ends

2 cross tiles held together by branched junctions

Branched Junction

Urmi Majumder, Duke
Double decker tiles

Four fold sequence symmetry
Double decker tiles

Four fold sequence symmetry
Double-decker tiles: Route to Assembly in 3D

2D Pad Programming of Double-Decker Tiles

Corrugation cancels curvature of lattice
=> creates enormous lattices

Urmia Majumder, Duke
2D lattice design

Corrugation
2D lattice design

Corrugation
Highly regular 2D lattices via double-decker cross tiles

Atomic force microscopy images of the double-decker 2D lattice with corrugation. The scale bars are (A) 10 μm, (B) 300 nm (C) 200 nm. (D) Fluorescence microscopy image of the same sample. The scale bar is 20 μm. The lattices are tens of micrometers in size.

Urmı Majumder, Abhijit Rangnekar, Kurt V. Gothelf, John H. Reif and Thomas H. LaBean, 
Double-decker tiles: Route to Assembly in 3D

2D Lattices

2D Programmed Double-Decker Tiles

Yields:
- Extremely Large, Regular
- 2D Grids with Predominant Unidirectional Banding

Urmi Majumder, Duke
3D staggered lattices
2D staggered lattices

Corrugated
2D staggered lattices AFM
3D staggered lattices
Double-decker tiles: Route to Assembly in 3D

3D Generalized Corrugation cancels curvature of lattice in all 3 dimensions!
• **John Reif**  
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• **PhD Candidates:**  
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• **2nd Year Graduate Students:**  
  – Tong Niu  
  – Guangjian (Jeff)
What we do

• John: interested in all things
• Hieu: building a DNA-origami-based circuit
• Sudhanshu: exponentially auto-catalytic system
• Tianqi: analog computer using DNA
• Reem:
  – Designing a self-reconfigurable DNA origami nanorobot
  – Building a software that can simulate DNA hybridization reactions using Graph Grammars, along with methods from scientific computing (and machine learning)
Reif Papers on the Web

Reif Papers on DNA nanoscience on the Web:

- **Survey on DNA Computation:**

Other Reif Papers on the Web:
Talk Locations on Reif’s Website

-  [www.cs.duke.edu/~reif/paper/DNA-NanoscienceTalks](http://www.cs.duke.edu/~reif/paper/DNA-NanoscienceTalks)

**DNA Computing: Theory, Experiments & Software:**

**Self-Assembled DNA Nanostructures:**

**DNA-Based Programmable Autonomous Molecular Robotic Devices:**