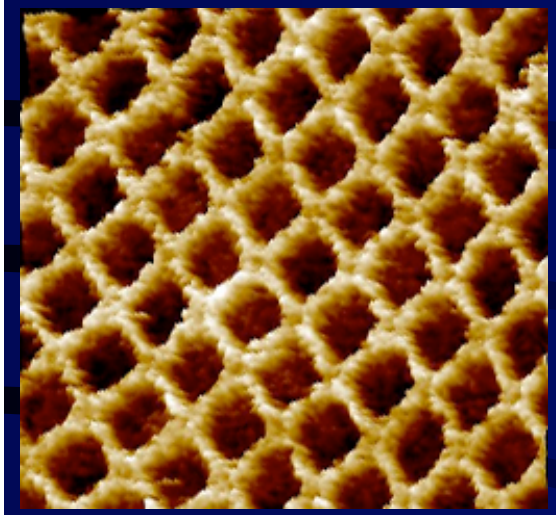
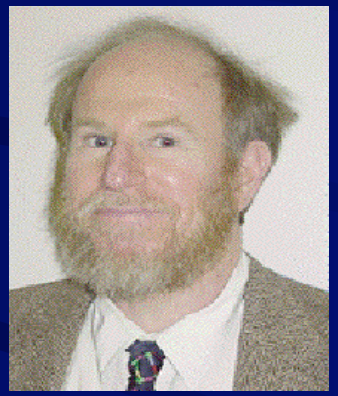
DNA Self-Assembly for Molecular Patterning, Computation and Robotics

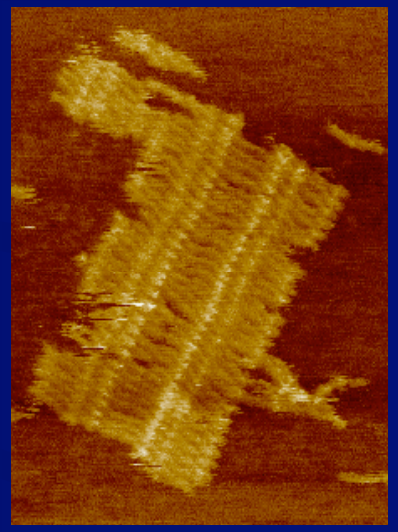


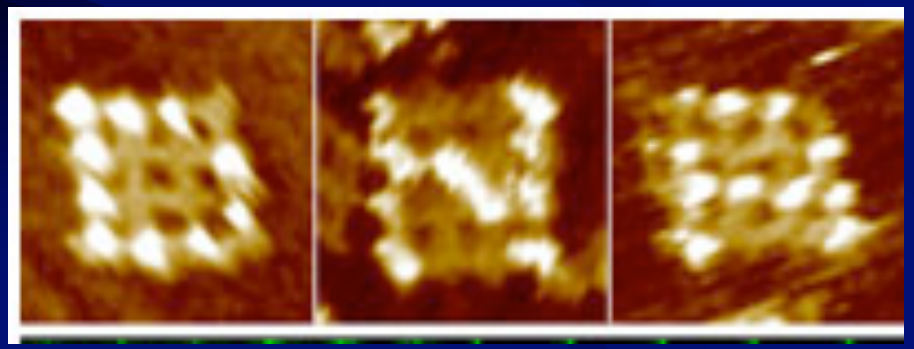
John H. Reif

Computer Science Department

Duke University

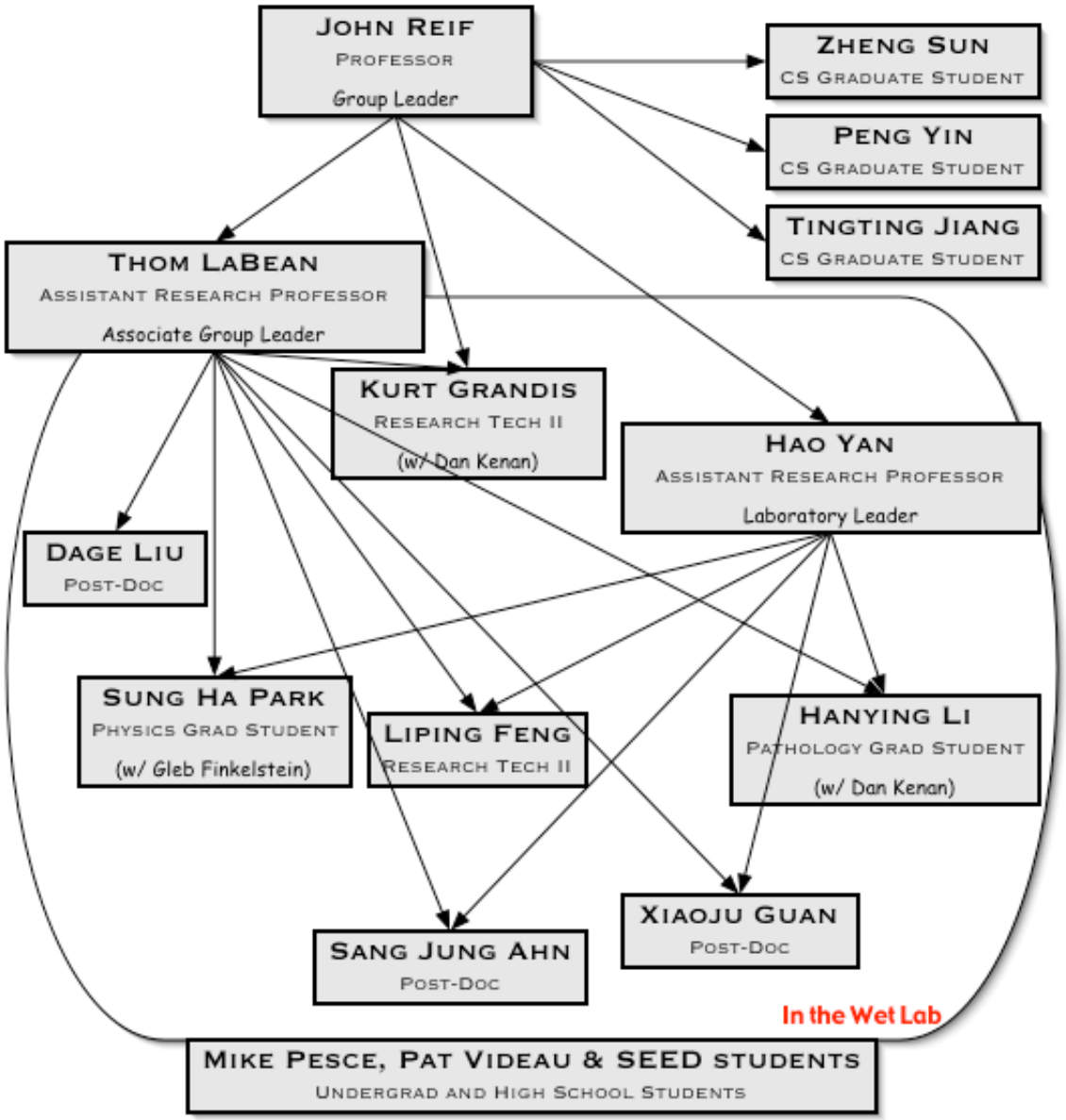






DNA NanoTech Group

Duke University



Reif'sPapers on DNA Self-Assembled Tiling Lattices & Motors

[LaBean, Winfree, Reif & Seeman: J. Am. Chem. Soc. 2000] The construction, analysis, ligation and self-assembly of DNA triple crossover complexes

[Mao, LaBean, Reif, Seeman: Nature 2000] Logical Computation Using Algorithmic Self-Assembly of DNA Triple-Crossover Molecules

[Yan, Feng, LaBean & Reif: JACS, 2003] Constructed DNA Nanotubes and Demonstrated Parallel Molecular Computation of Pair-Wise XOR Using DNA String Tile.

[Yan, LaBean, Feng, and Reif: PNAS, 2003] Experimental Demonstration of Directed Nucleation Assembly of Barcode Patterned DNA Lattices.

[Yan, Park, Finkelstein, Reif & LaBean: Science, 2003] DNA-Templated Self-Assembly of Protein Arrays and Highly Conductive Nanowires.

[Feng, Park, Reif & Yan: Angewandte Chemie 2003] A Two State DNA Lattice Actuated by DNA Motors.

[Li, Park, Reif, LaBean, Yan: J. Am. Chem. Soc. 2004] DNA Templated Self-Assembly of Protein and Nanoparticle Linear Arrays.

[Liu, Reif, LaBean: PNAS 2004] DNA nanotubes self-assembled from triple-crossover tiles as templates for conductive nanowires.

[Yin, Yan, Daniel, Turberfield, Reif: Angewandte Chemie, 2004] A Unidirectional DNA Walker Moving Autonomously Along a Linear Track.

[Park, Yan, Reif, LaBean, Finkelstein: Nanotechnology, 2004] Electronic nanostructures templated on self-assembled DNA scaffolds.

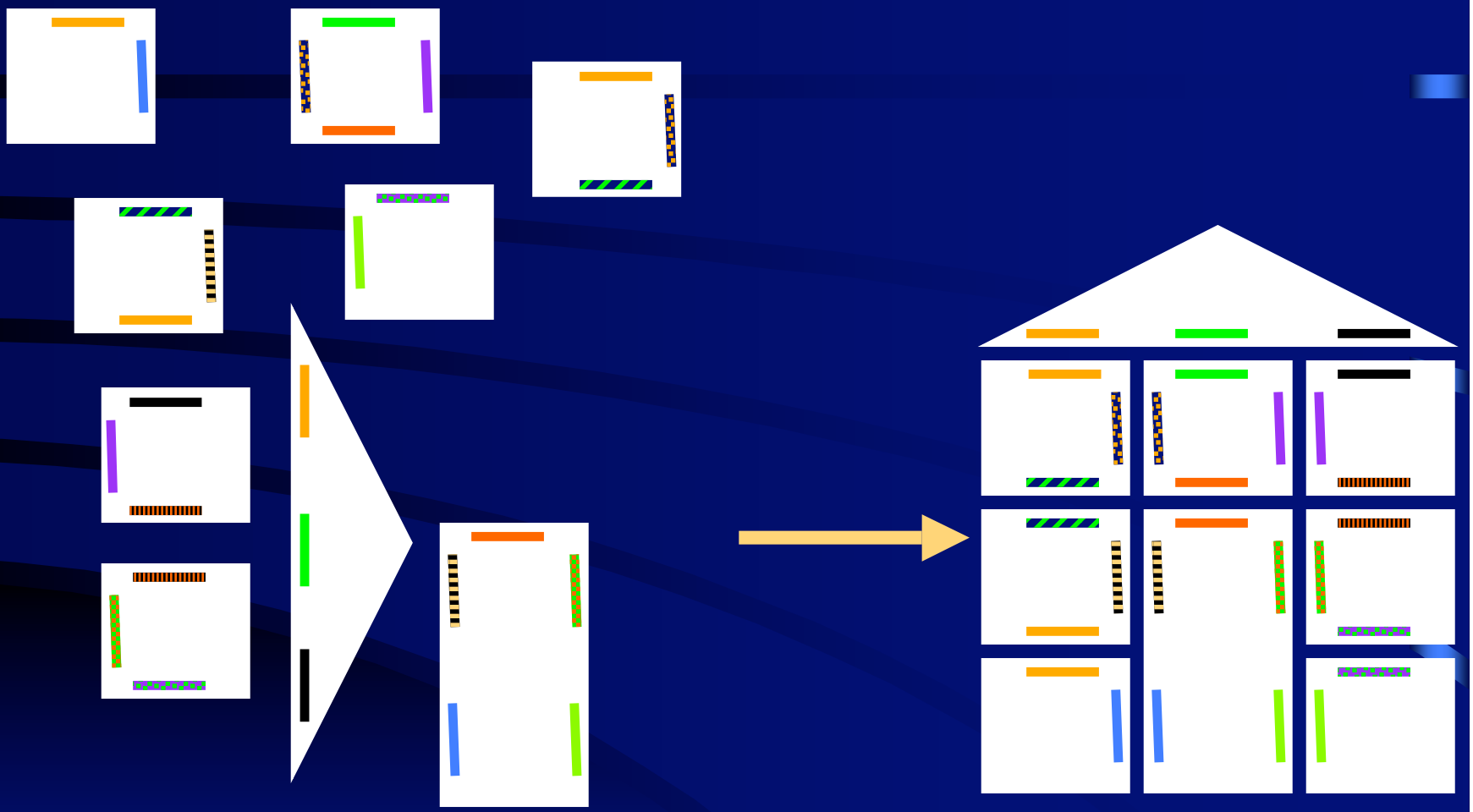
[Park, Yin, Reif, LaBean, Yan: NonoLetters 2005] Programmable DNA Self-assemblies for Nanoscale Organization of Ligands and Proteins

[Park, Barish, Reif , Finkelstein, Yan, LaBean: Nano Letters 2005] Three-Helix Bundle DNA Tiles Self-Assemble into 2D Lattice or 1D Templates for Silver Nanowires

[Park, Barish, Reif, Finkelstein, Yan and LaBean, Nano Letters 2005] Three-Helix Bundle DNA Tiles Self-Assemble into 2D Lattice or 1D Templates for Silver Nanowires, (Communication), Volume 5, Number 4, pp. 693-696 (2005).

[Park, Pistol, Ahn, Reif, Lebeck, Dwyer, and LaBean, Angewandte Chemie 2006] Finite-Size, Fully Addressable DNA Tile Lattices Formed by Hierarchical Assembly Procedures.

Construction with “Smart Bricks”



A tiling assembly using `Smart Bricks' with affinity between colored pads.

Programmable Patterning of DNA Lattices

A New, Powerful Technology

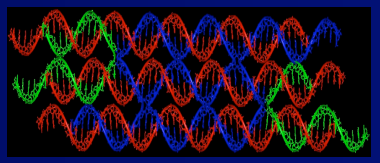
- for the construction of molecular scale structures

- for Rendering Patterns at the Molecular Level.

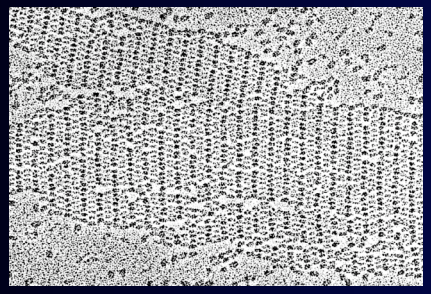
A 2D DNA lattice is constructed by a self-assembly process:

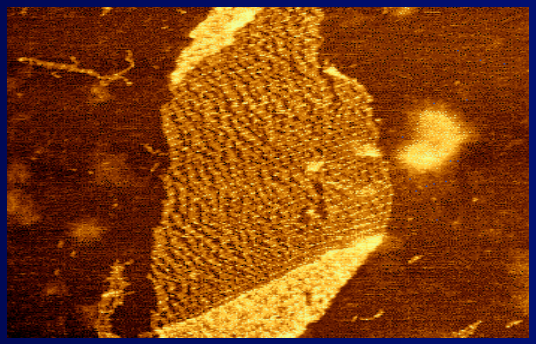
--Begins with the assembly of DNA tile nanostructures:

- DNA tiles of size 14 x 7 nanometers
- Composed of short DNA strands with Holliday junctions



- These DNA tiles self-assemble to form a 2D lattice:





-The Assembly is Programmable:

-Tiles have sticky ends that provide programming for the patterns to be formed.

-Alternatively, tiles self-assemble around segments of a DNA strand encoding a 2D pattern.

- Patterning: Each of these tiles has a surface perturbation depending on the pixel intensity.

-pixel distances 7 to 14 nanometers

-not diffraction limited

Key Applications: Assembly of molecular electronic components & circuits, molecular robotic components, image rendering, cryptography, mutation detection.

Background Literature on DNA Self-Assembled Tiling Lattices.

- **Basic Techniques of DNA nanostructures developed by Seeman at NYU in 1980s.**

- **[Winfree and Seeman,98] The first experimental demonstration of self-assembly of DNA to construct 2D lattices consisting of up to tens of thousands of DNA tiles.**

- **[LaBean, Winfree, Reif, & Seeman, 2000] constructed a useful class of DNA nanostructures known as TX tiles which have a number of individual DNA strands that run through the tiles.**

  J. Am. Chem. Soc. 122, 1848-1860 (2000).

  www.cs.duke.edu/~reif/paper/DNAtiling/tilings/JACS.pdf

- **[Mao, LaBean, Reif, Seeman,2000] Experimentally demonstrated for the first time a computation Used self-assembled DNA lattices of TX tiles that self-assembled around input strands running through the tiles:**

  Nature, Sept 28, p 493-495 (2000).

  www.cs.duke.edu/~reif/paper /SELFASSEMBLE/AlgorithmicAssembly.web.pdf

- **Comprehensive Review paper:**

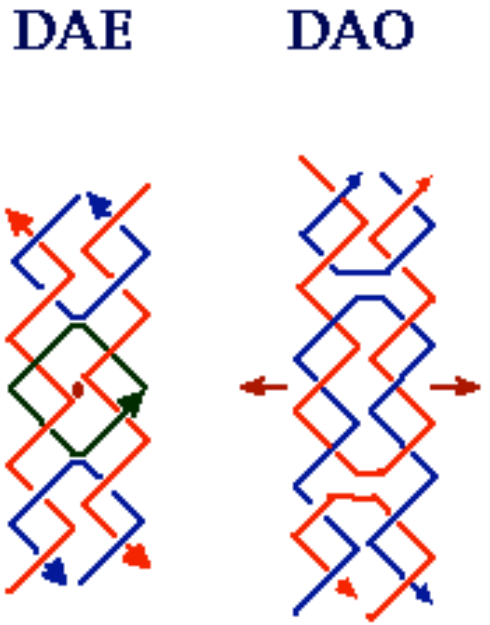
  "Challenges and Applications for Self-Assembled DNA Nanostructures",

  [Reif, LaBean, Seeman, 2000]

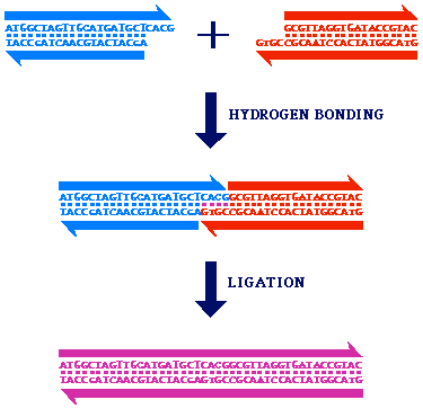
  www.cs.duke.edu/~reif/paper /SELFASSEMBLE/selfassemble.pdf

DNA tiles

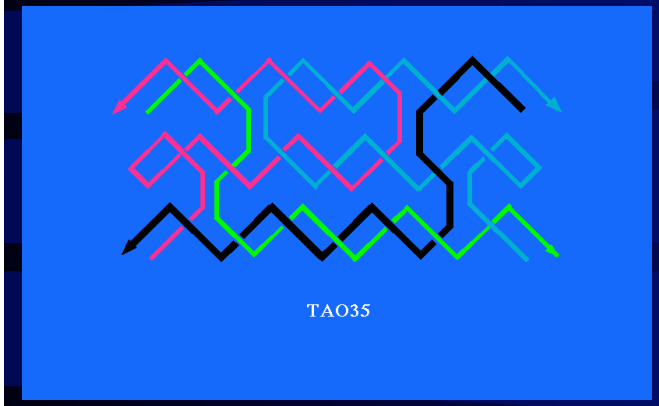
- DNA crossover molecules self-assembled from artificially synthesized single stranded DNA.
- Double-crossover (DX) Tiles [Winfree, Seeman]:
  - consist of two double-helices fused by crossover strands.
  - DAE contains an Even number of helical half-turns between crossover points.
  - DAO contains an Odd number.

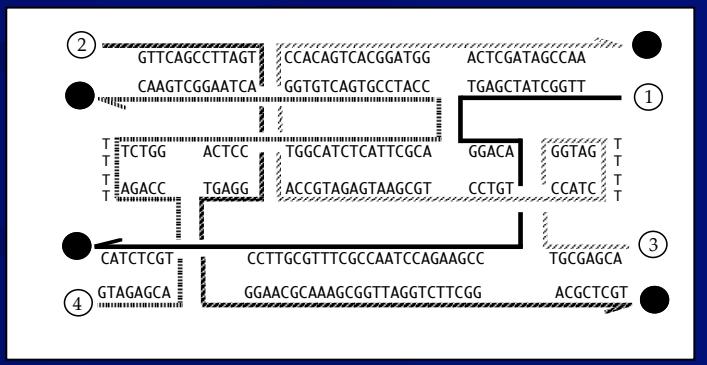


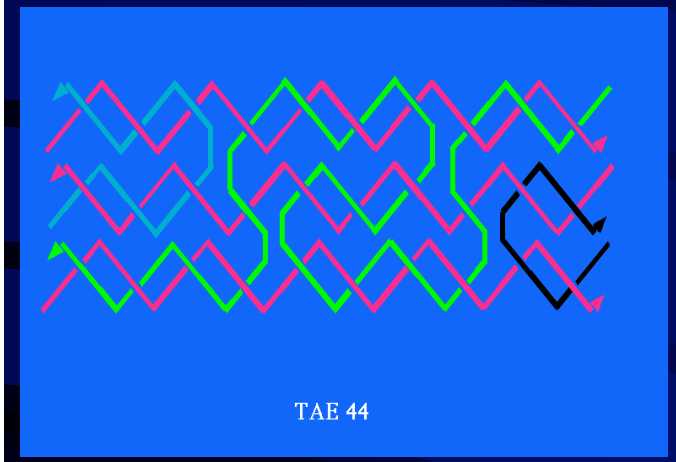
- Anti-parallel crossovers:
  - cause a reversal in direction of strand propagation through the tile following exchange of strand to a new helix.
  - DAO and DAE are double-crossover DX tiles with two anti-parallel crossovers.
- Pads:
  - Tiles have sticky ends that preferentially match the sticky ends 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.

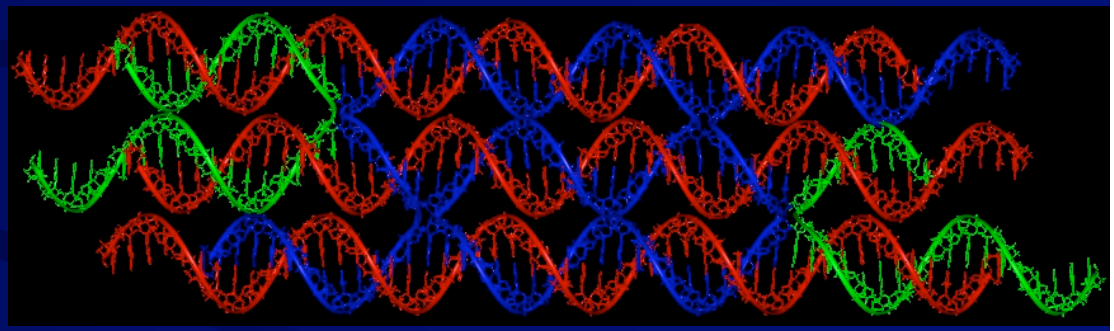


TX Tiles



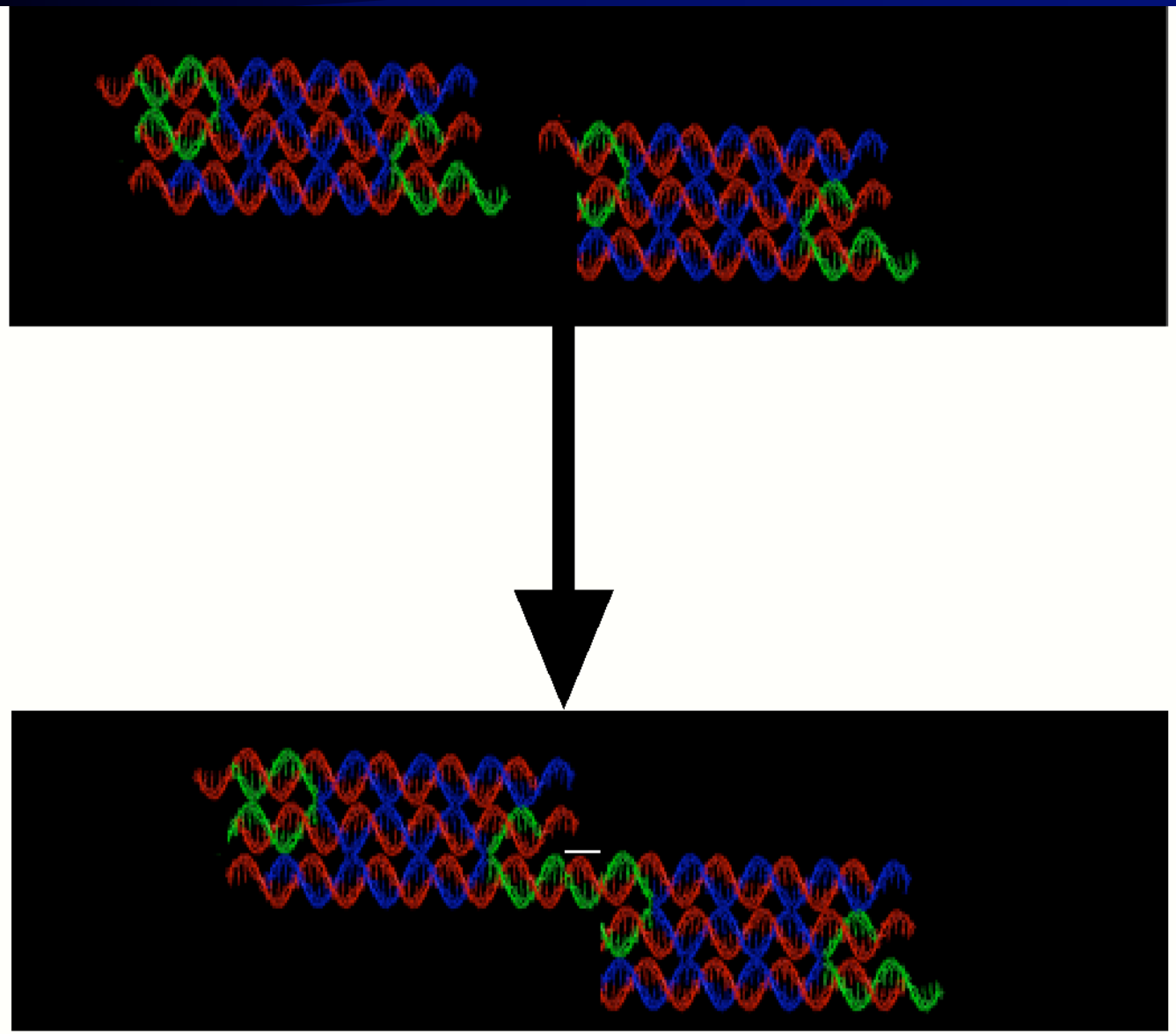




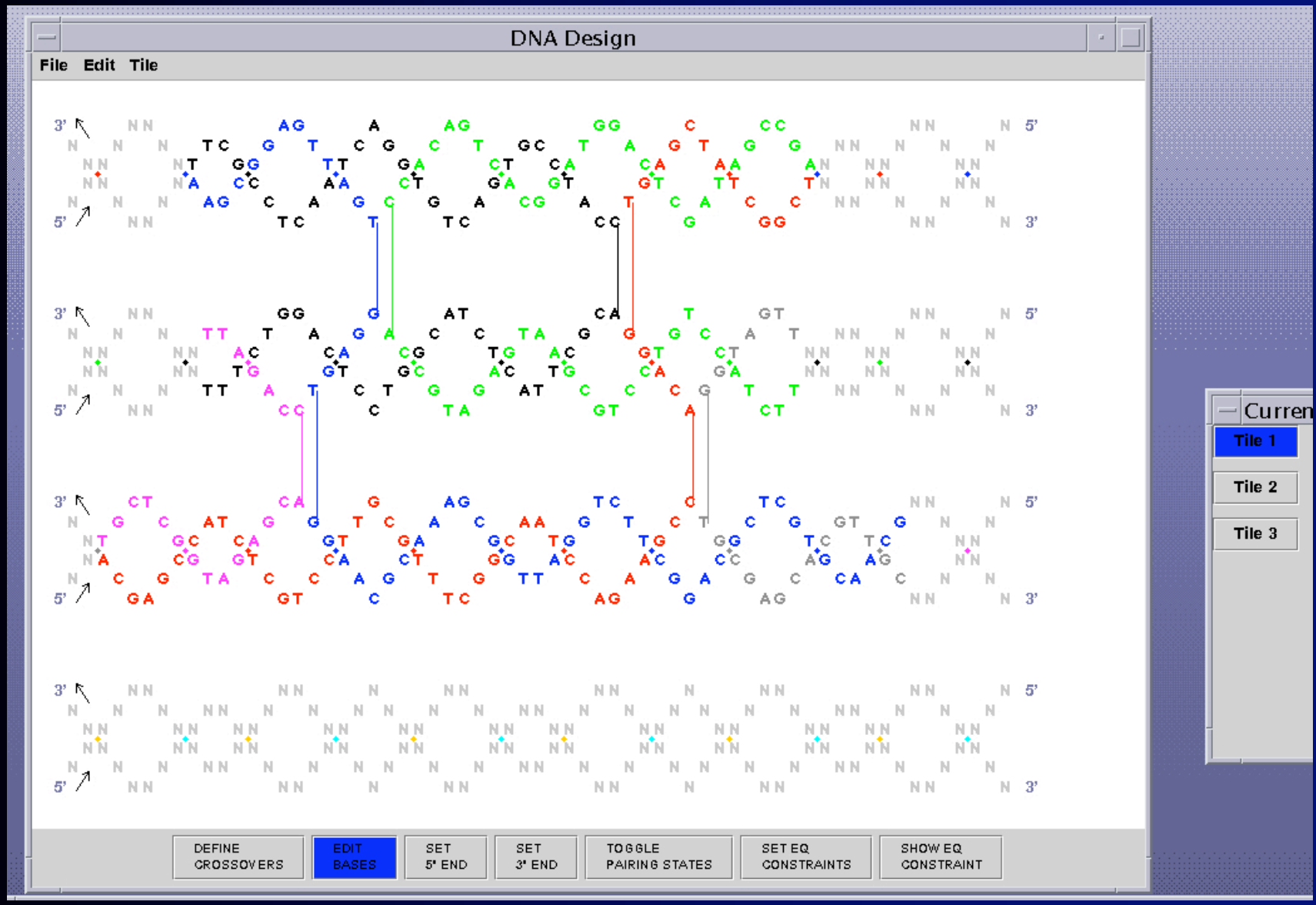


- Triple-crossover (TX) Tiles [LaBean, et al, J. Am. Chem. Soc., 2000]:
  - consist of three double-helices fused by crossover strands.
  - TAE contains an Even number of helical half-turns between crossover points.
  - TAO contains an Odd number.
- Total of 6 Pads of single stranded DNA at ends.

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.**



Tile A (Optimized)



Computer Simulation of Self-Assembly

Prior to experimental tests,

- **we made computer simulations of our protocol for for self-assembly of patterned 2D lattices**
- **Goals:**
  - approximate the kinetics of self-assembly chemistry.
  - **to optimize the sequence designs for the DNA tiles and**
  - **to optimize experimental parameters such as the schedule of annealing temperatures.**

- Discrete time simulation of the tiling assembly processes [Winfree98]:
  - Used approximate probabilities for insertion or removal individual tiles from the assembly.
  - Does not allow tilings to combine(assume low concentrations).

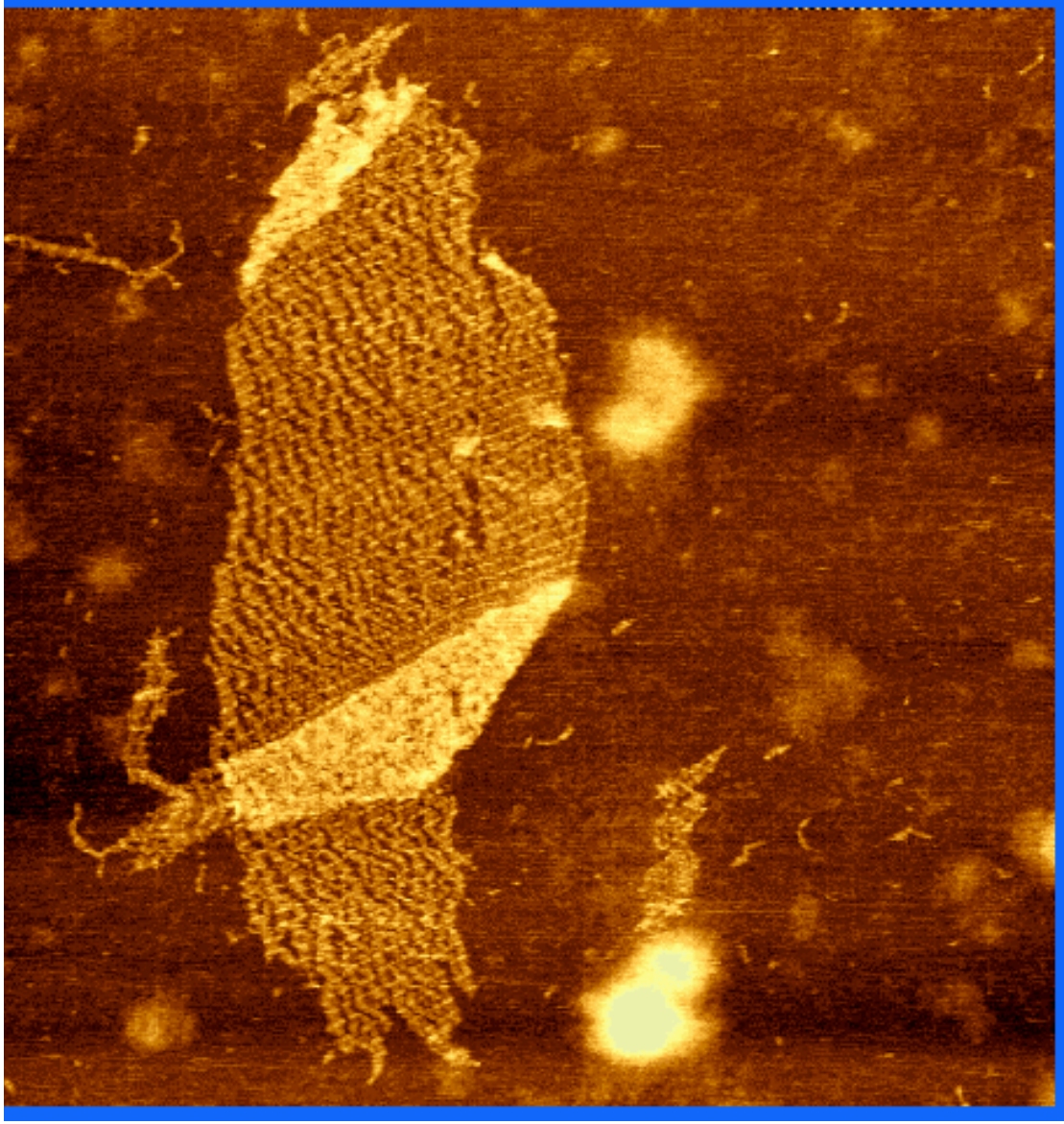
Our computer simulation of the tiling:

- **uses a multistage process where the tiling occurs in stages**
- **allows distinct hybridization melting temperatures for the distinct stages.**

- Improved simulation software with a Java interface [Yuan at Duke, 2000]
  - Speed up by use of an improved method of Winfree for computing on/off likelihoods.
  - Example tilings: string tilings for integer addition and XOR computations.
  - URL: www.cs.duke.edu/~reif/SIMULATIONS/demo.html

Large Scale DNA Self-Assembled Tilings

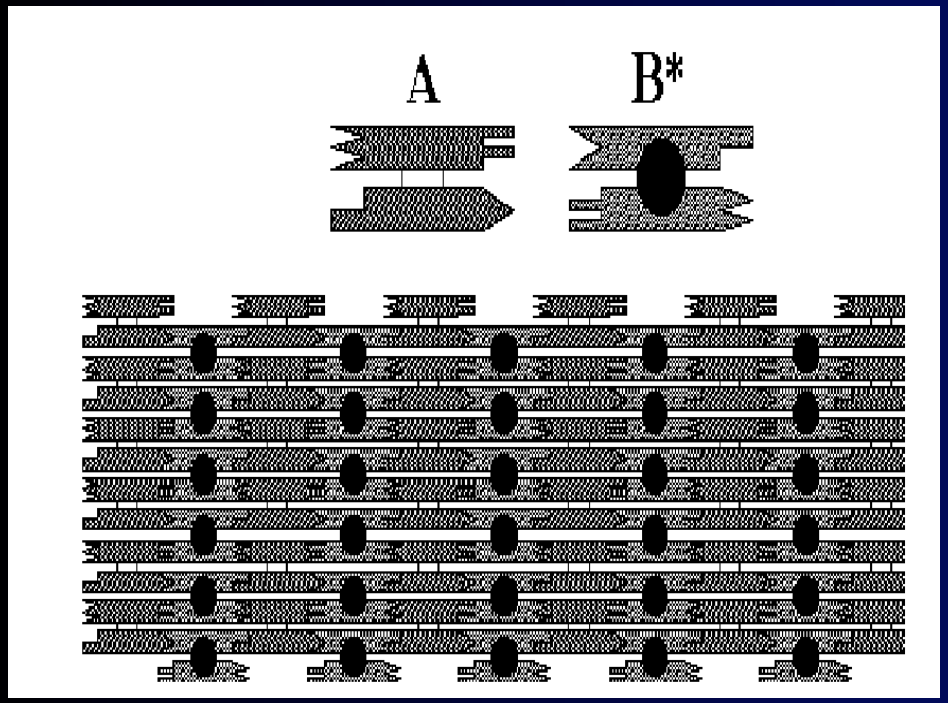
Visualization by Atomic Force Microscope.



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 ~28 nm.

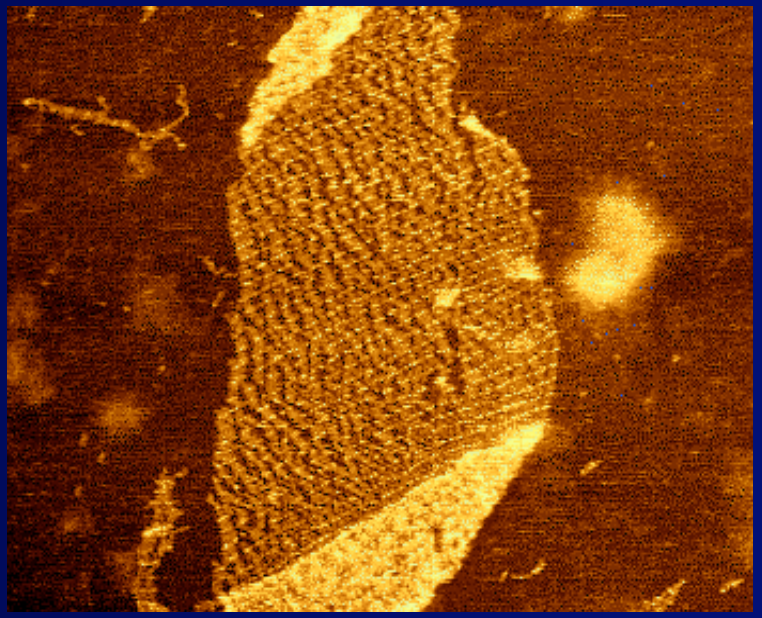
2D DNA Self-Assembled Tilings: Rendering Simple Banded Images

B* Tiles with Loops

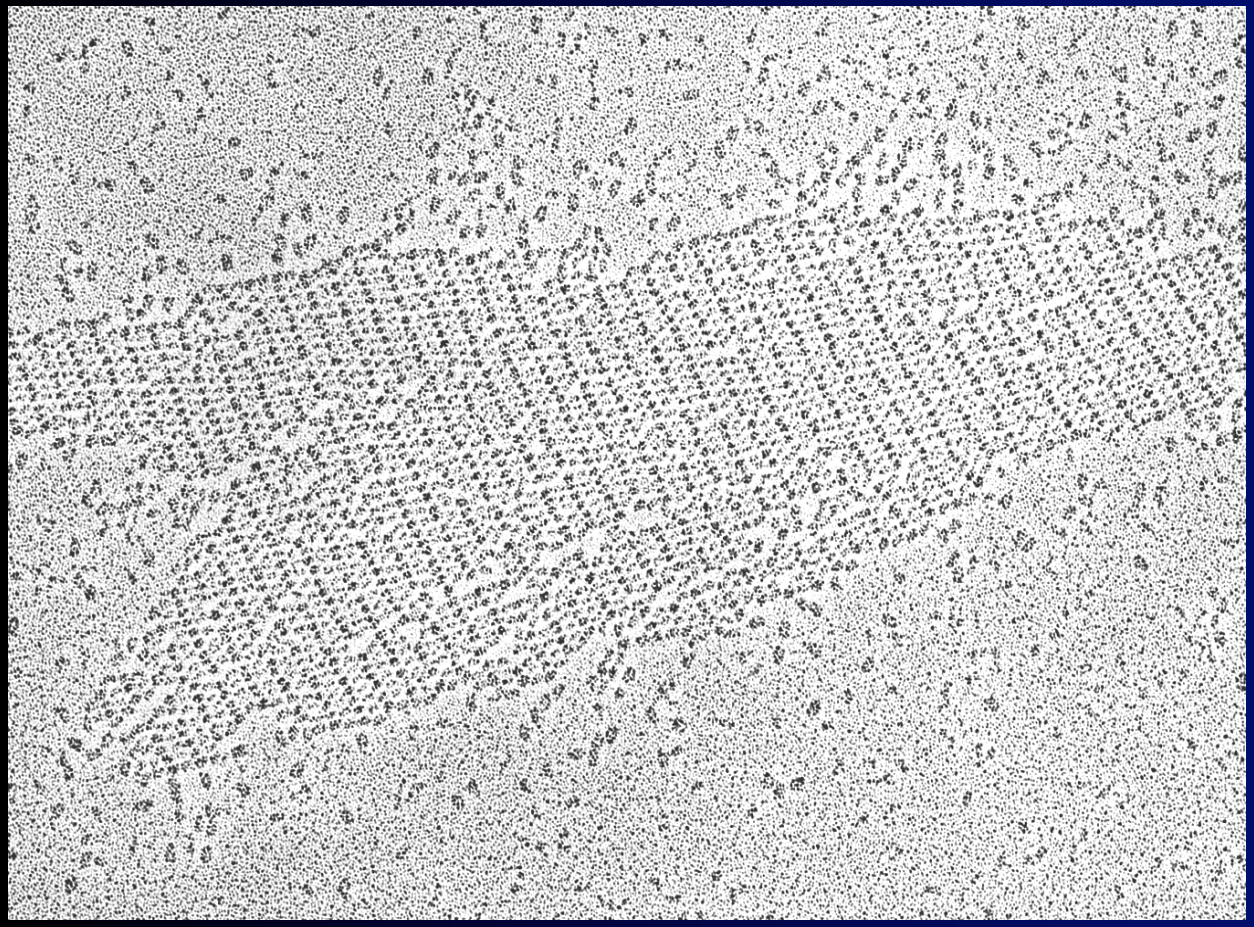


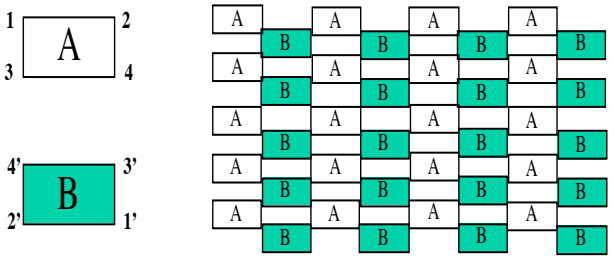
Atomic Force Microscope Image

Bands Generated by B* Tiles with Attached Loops



TEM Image of TAO AB* Lattice





Cartoon of DNA lattice composed of two types of TAO tile: B with (dark) and A without (light) stem-loops directed out of the lattice plane.

Platinum rotary-shadow TEM image of DNA lattice assembled by stoichiometric annealing of 8 oligos designed to form two tile types (A and B):

- **A tiles (lighter) only associate with B tiles (darker) and vice versa.**
- **B tiles appear darker due to increased platinum deposition on an extra loop of DNA directed out of the lattice plane.**

Stripes of dark B tiles have approximately 28 nm periodicity, as designed.

Directed Nucleation Assembly:

A method for assembly of complex patterns

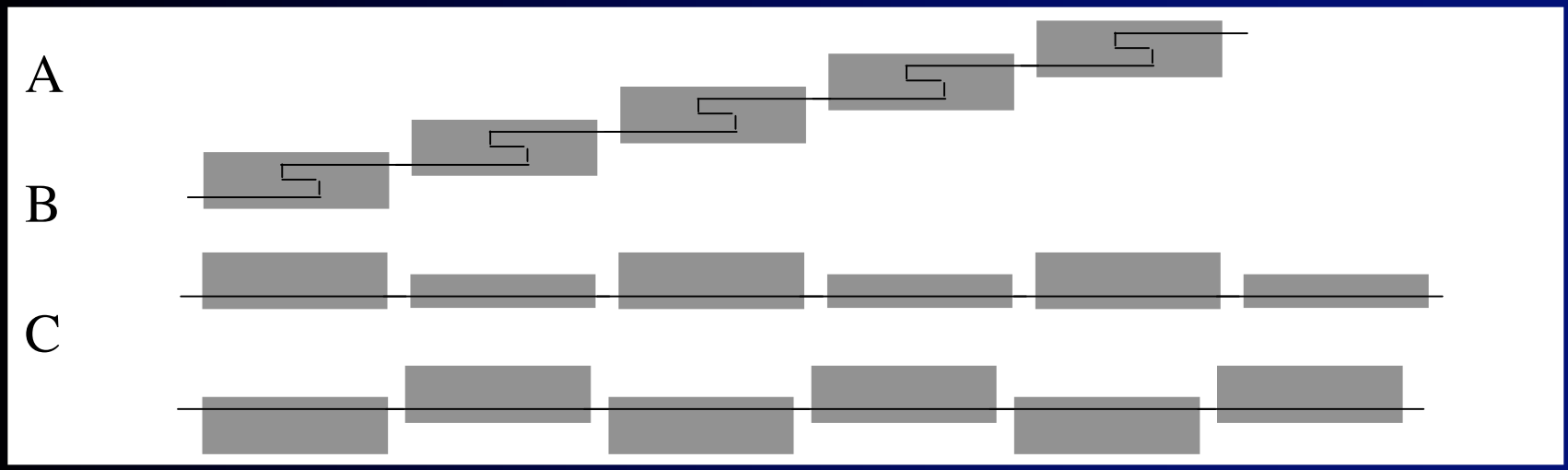
- Use artificially synthesized DNA strands that specify the pattern and around which 2D DNA tiles assemble into the specified pattern.
- The permanent features of the 2D pattern are generated uniquely for each case.

Directed Nucleation Self Assembly Steps:

- an input DNA strand is synthesized that encodes the required pattern
- then specified tiles assemble around blocks of this input DNA strand, forming the required 1D or 2D pattern of tiles.

Molecular Pattern Formation using Scaffold Strands for Directed Nucleation:

- 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).



- Examples of Arrangements of Scaffold Strands :
  - (A) Diagonal TAO layer which partially defines binding slots for tiles of the next successive layer.
  - (B) Horizonal layer of alternating TAE and DAE tiles.
  - (C) crenellated horizontal layer which could be comprised of TAE or DAE tiles.

  Structures in B and C completely define binding slot for tiles on next layers.

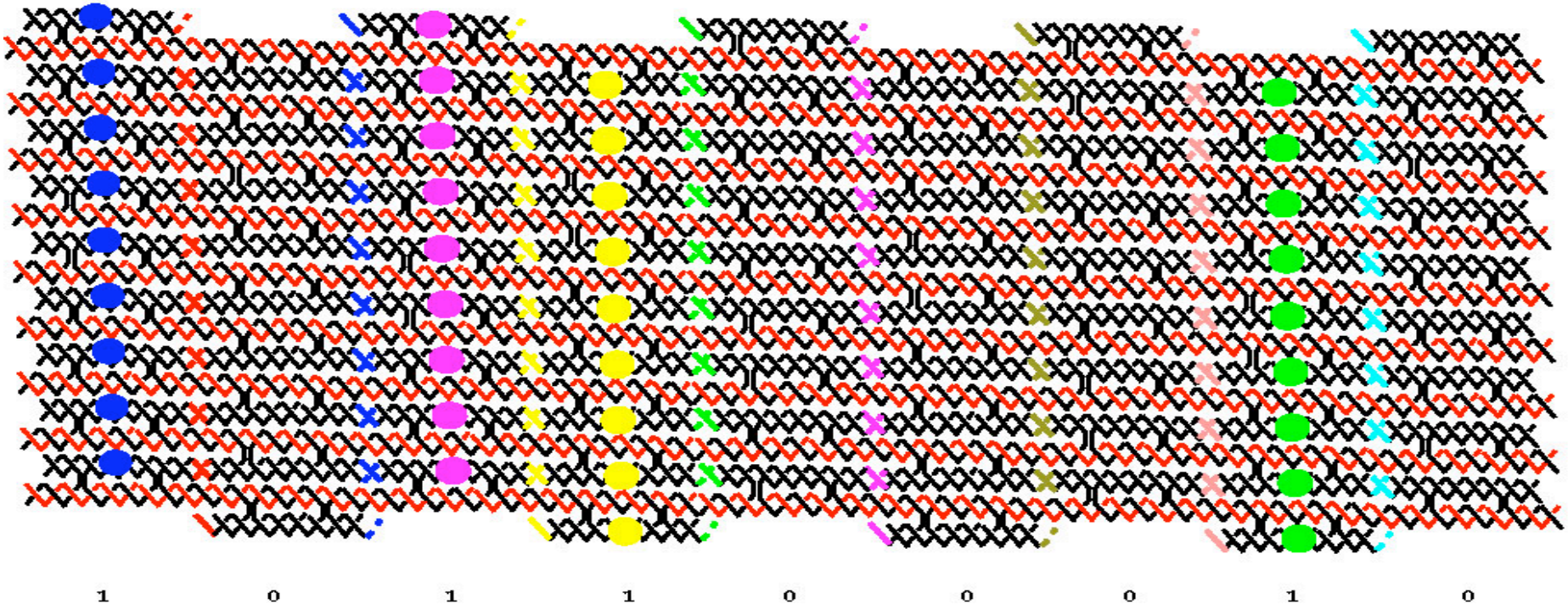
Directed Nucleation Technique for 1 D Patterns: Barcode Lattice for Readout

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

- **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.**

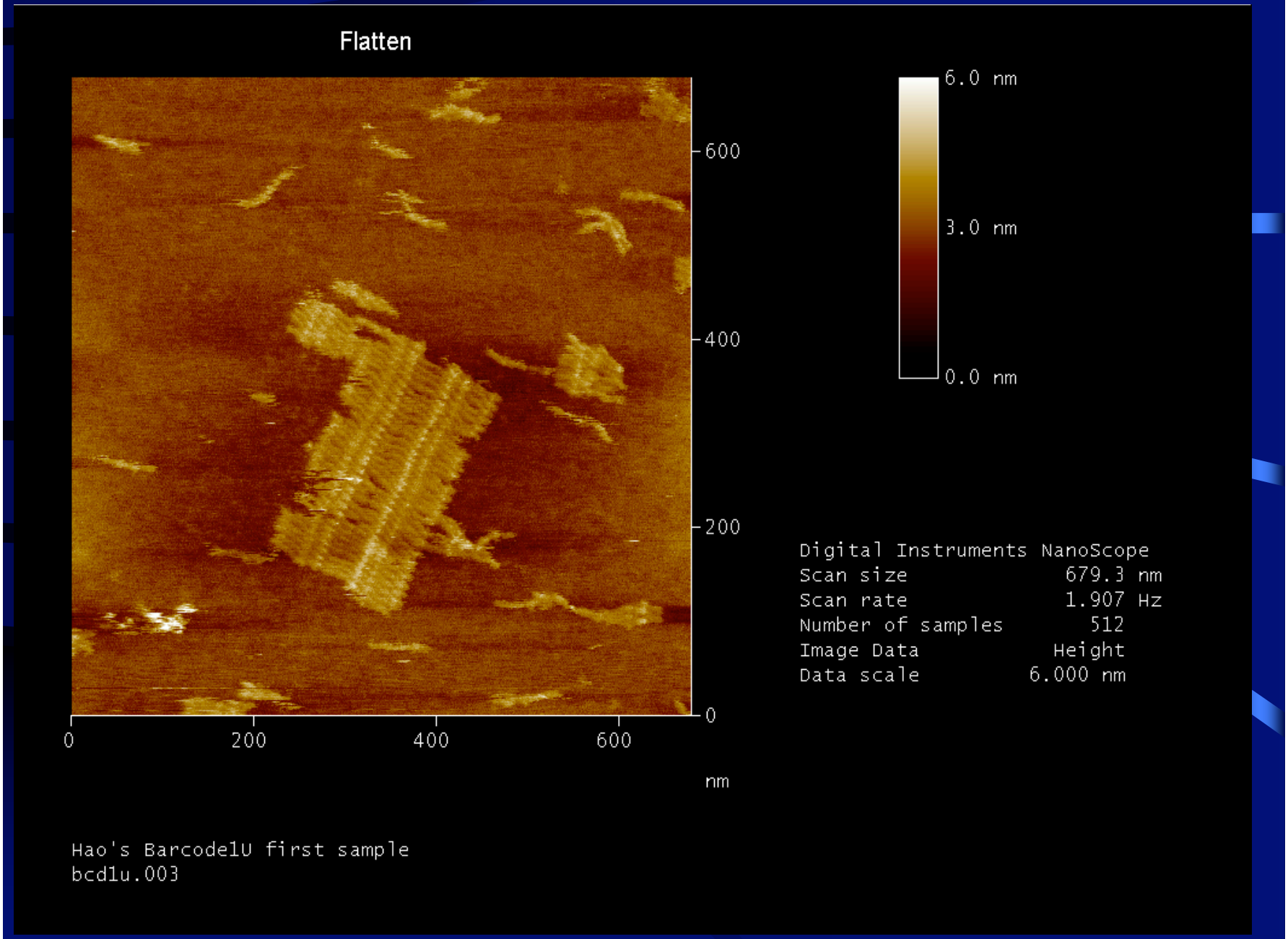
Barcode Lattice for Rendering 1 D Patterns:

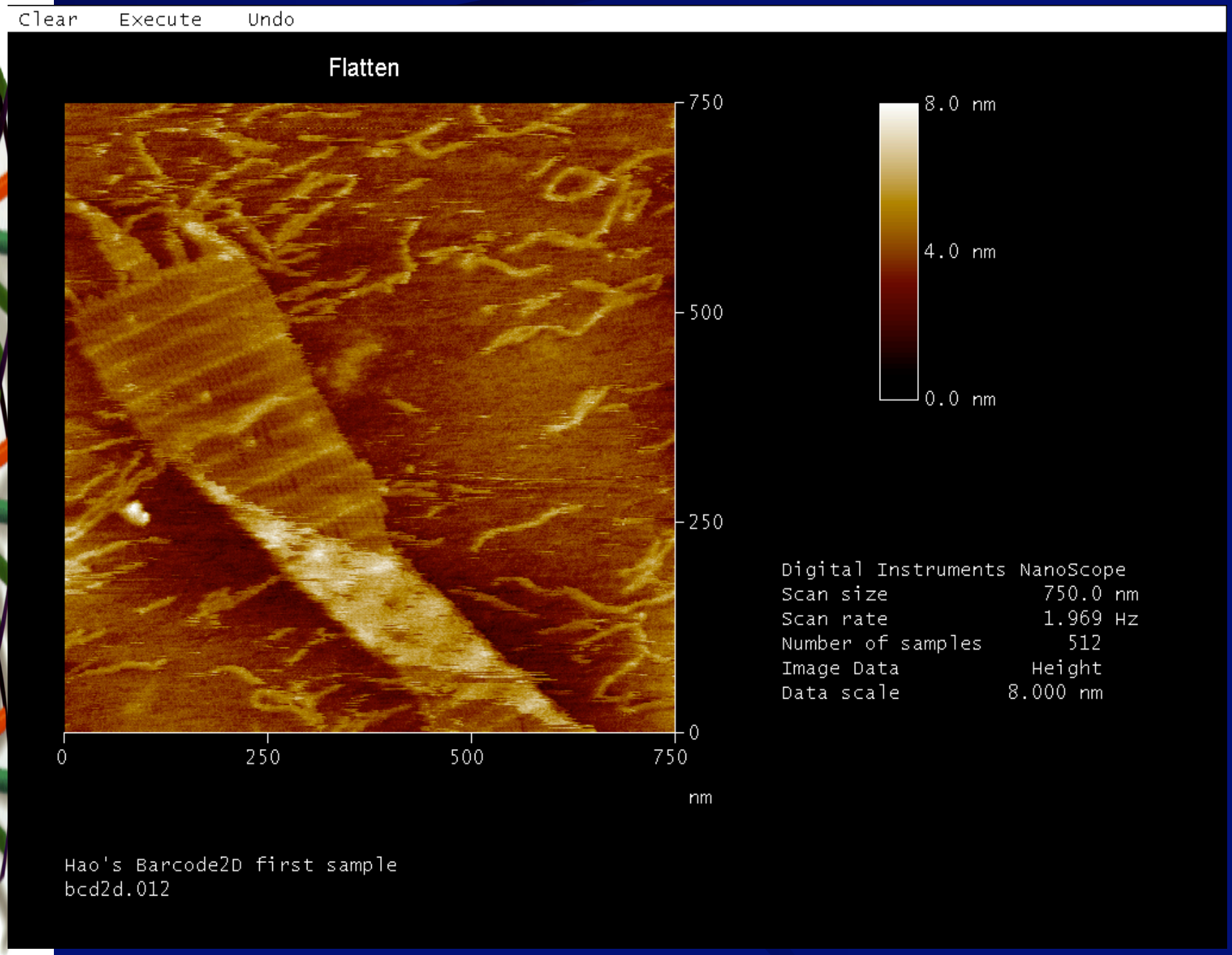
Unit (9 tiles, filled dot i.e. loop represent 1, 8 pairs of asymmetric stickyends represented by colored lines, The long red strand are the input)



A typical 9X9 periodic array assembled from the unit, there will not be frameshift because the different pairs of stickyend

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


Flatten
6.0 nm
3.0 nm
0.0 nm
600
400
200
0
0
200
400
600
nm
Digital Instruments NanoScope
Scan size 679.3 nm
Scan rate 1.907 Hz
Number of samples 512
Image Data Height
Data scale 6.000 nm
Hao's Barcode1U first sample
bcd1u.003


Clear
Execute
Undo
Flatten
750
500
250
0
0
250
500
750
nm
8.0 nm
4.0 nm
0.0 nm
Digital Instruments NanoScope
Scan size 750.0 nm
Scan rate 1.969 Hz
Number of samples 512
Image Data Height
Data scale 8.000 nm
Hao's Barcode2D first sample
bcd2d.012

Directed Nucleation Technique for Output of 2D Patterns:

- A DNA strand encodes a 2D Pattern.
- Render pattern as a 2D lattice at the molecular scale
  - approximately 20 Angstroms per pixel (1 Angstrom= 1 ten-billionth of a meter).

Self-Assembly of Patterned 2D Lattice:

- Tiles (DNA nanostructures) self-assemble around each segment of a DNA strand encoding an image pixel.
- Each tile has a surface perturbation depending on pixel intensity.
- The tiles then self-assemble into a 2D tiling lattice.
- Scalable to extremely large patterns
  - not diffraction limited
  - by an Atomic Force Microscope
- Major Applications:

  - Molecular Scale Patterning of Molecular Electronics and Molecular Motors.

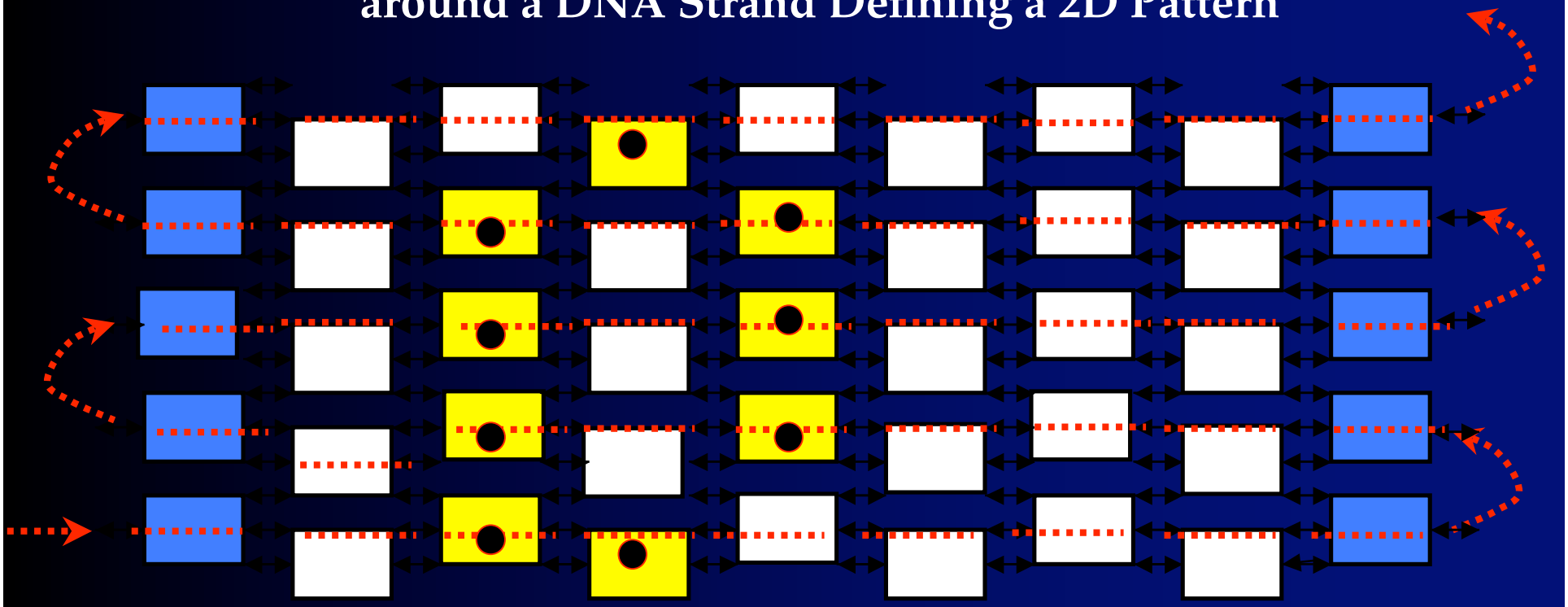
- Other Applications: Image Storage
  - a region 100km x 100km imaged by a satellite to 1 cm resolution
  - resulting image is of size 1,000,000 x 1,000,000, containing 10^{12} pixels
  - requires a DNA lattice of size 2 millimeters on a side.

2D DNA Self-Assembled Tiling

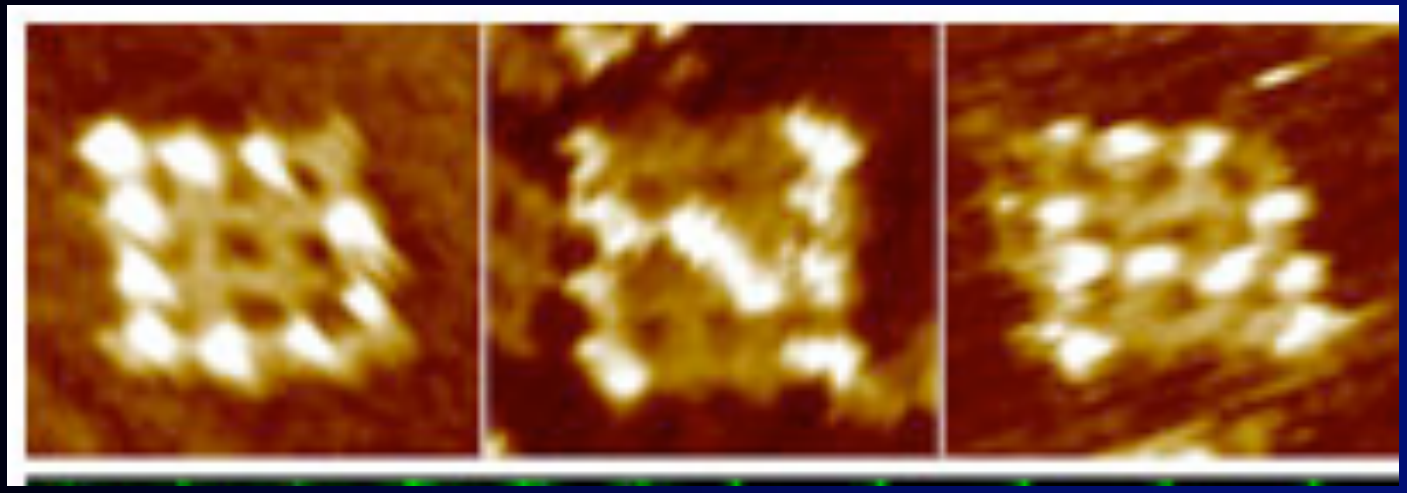
The Process of Assembling a 2 D Pattern by Directed Nucleation :

Self Assembly of Tiles

around a DNA Strand Defining a 2D Pattern



Duke's Hierarchical Assembly of DNA Lattices with 2 D Pattern "DNA"



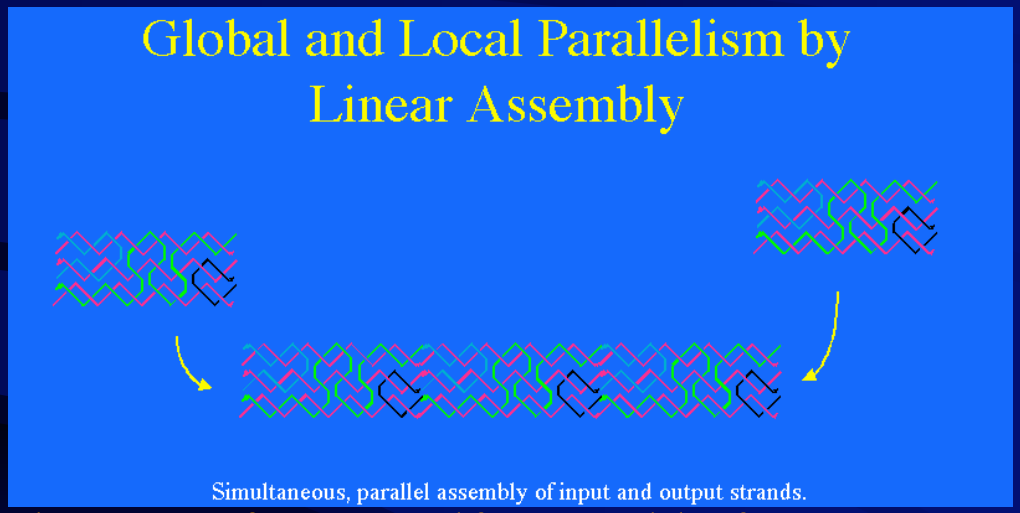
Sung Ha Park, Constantin Pistol, Sang Jung Ahn, John H. Reif, Alvin R. Lebeck, Chris Dwyer, and Thomas H. LaBean, Finite-Size, Fully Addressable DNA Tile Lattices Formed by Hierarchical Assembly Procedures, Angewandte Chemie [International Edition], pp. 735-739, Volume 45, Issue 5, January 23, 2006.

Computation by Self-assembly of DNA Tilings

- Tiling Self-assembly can:
  - Provide arbitrarily complex assemblies using only a small number of component tiles.
  - Execute computation, using tiles that specify individual steps of the computation.
- Computation by DNA tiling lattices:
  - First Proposed by [Winfree, 98].
  - First Experimentally demonstrated by
    - [Mao, et al 2000] Mao, C., T.H. LaBean, J. H. Reif, and N.C. Seeman, An Algorithmic Self-Assembly, Nature, Sept 28, p 493-495 (2000).

Programming Self-assembly of DNA Tilings = Design of Pads of DNA Tiles.

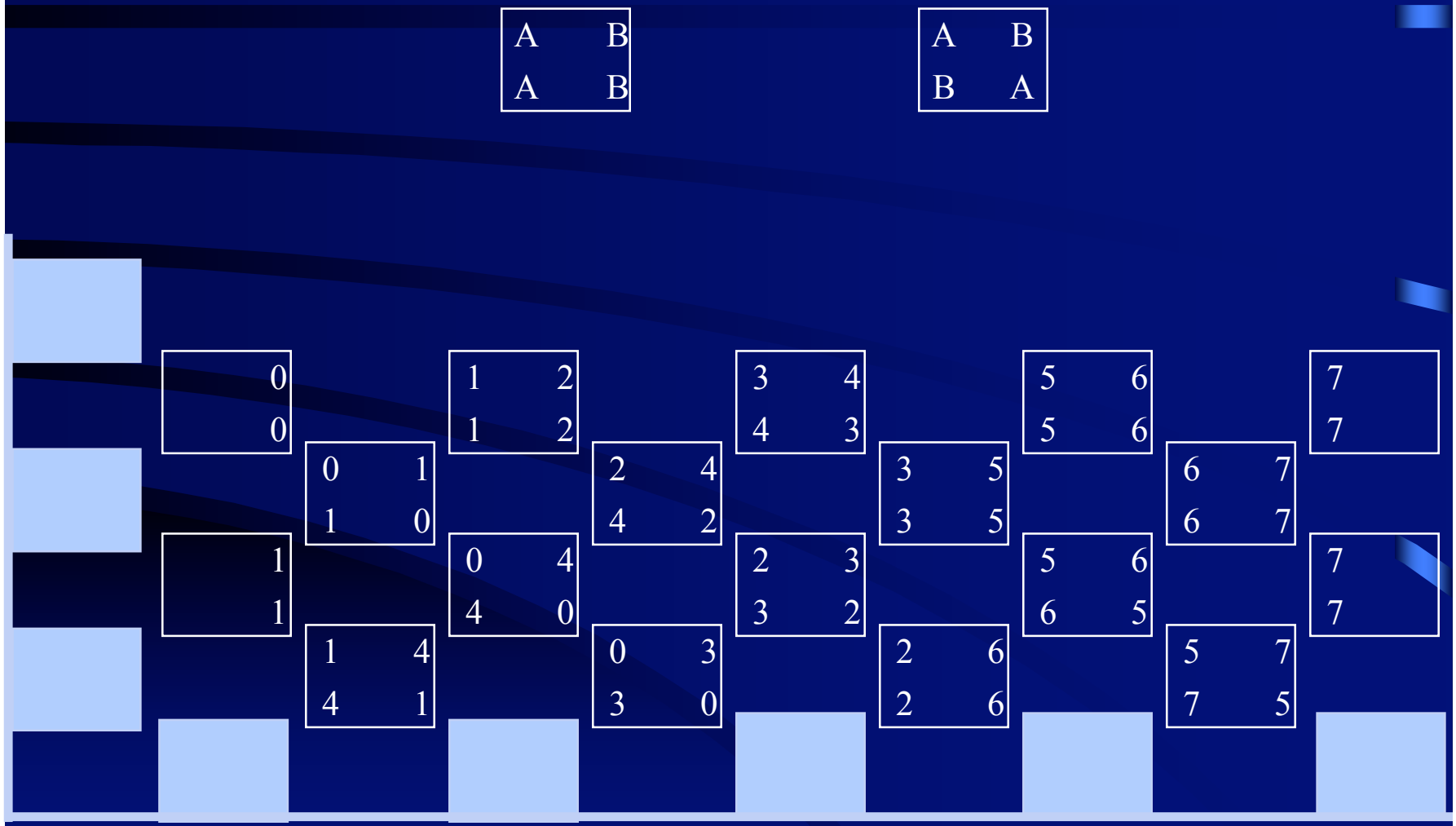
- Pads: complementary base sequences determining neighbor relations of tiles in final assembly
- Large-Scale Computational Tilings formed during assembly:
  - encode valid mappings of input to output.
  - local tile association rules insure only valid computational lattices form regardless of temporal ordering of binding events.



- Key Advantageof DNA Self-Assembly for DNA Computing:
  - Use a sequence of only 4 laboratory procedures:
    - mixing the input oligonucleotides to form the DNA tiles,
    - allowing the tiles to self-assemble into superstructures,
    - ligating strands that have been co-localized, and
    - performing a single separation to identify the correct output.

Computation with “Smart Bricks”

Sorting



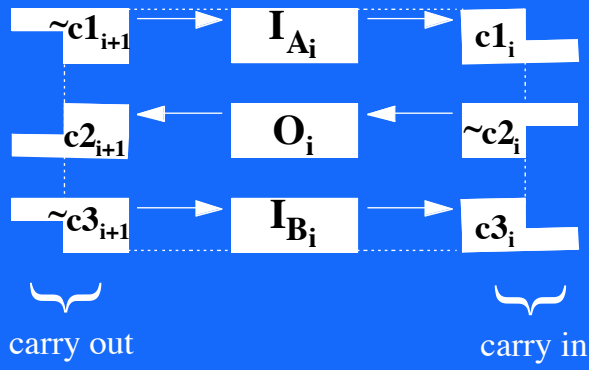
A tiling assembly using `Smart Bricks' to Sort 8 Keys.

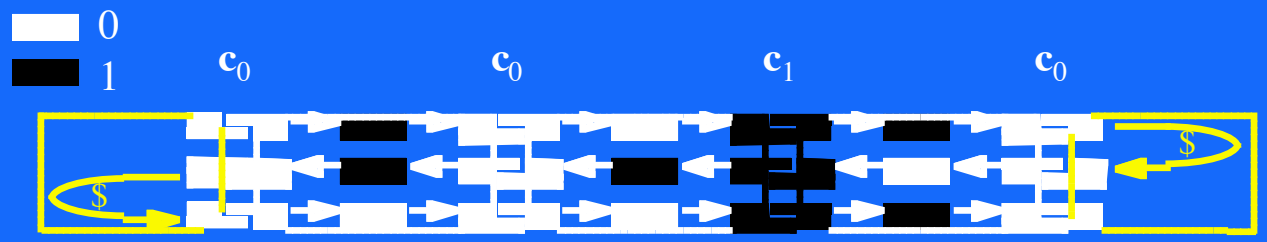
Domino Tiling Problems

- Defined by Wang [Wang61] (Also see [Grunbaum, et al, 87]).
- Input:
  - a finite set of unit size square tiles,
  - Tile pads: each of whose sides are labeled with symbols over a finite alphabet.
  - initial placement of a subset of certain tiles,
  - dimensions of the region where tiles must be placed.
- Domino Tiling Problem:
  - assuming arbitrarily large supply of each tile
  - place the tiles to completely fill the given region
  - each pair of abutting tiles must have identical symbols on their contacting sides.
- [Berger66]: Undecidable Domino Tiling problems:
  - over an infinite domain with a constant number of tiles
  - tiling patterns simulate single-tape Turing Machines
- [LewisPapa81, Winfree98, Moore00] :
  - NP-complete finite-size tiling problems
- Program-size Complexity (Number of Tiles) of Tiling Self-assembly
  - [Rothemund & Winfree, 2000]: Assembly of an n x n square uses O(log n /log log n) distinct tiles.
  - [Adleman,et al 2002]

- String Tile Addition Pads:
  - The sticky end pads on right encode:
    - carry bits coming in and I_{Ai} and I_{Bi} encode the two input bits.
  - Left-hand pads pass new carry value on to next step
  - Reporter strands indicated by arrows; O_i encodes: output bit.

tile	c_i	I_{A_i}	I_{B_i}	O_i	c_{i+1}
1	0	0	0	0	0
2	0	0	1	1	0
3	0	1	0	1	0
4	0	1	1	0	1
5	1	0	0	1	0
6	1	0	1	0	1
7	1	1	0	0	1
8	1	1	1	1	1



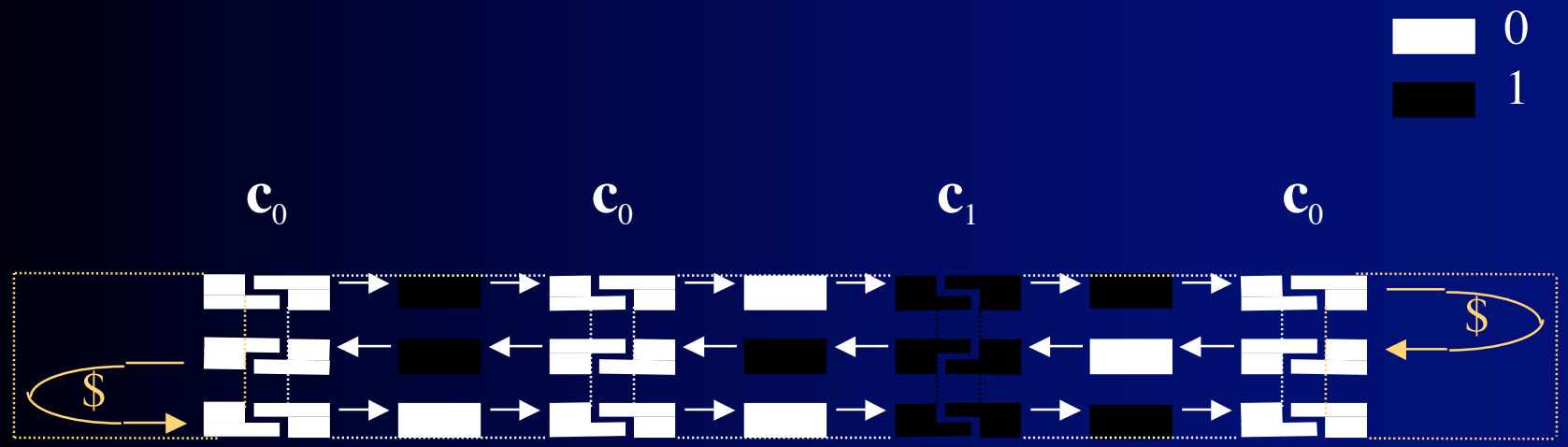


1 0 1 $ 0 1 1 $ 0 0 1 = I_A $ O^R $ I_B

101
+001
110

- Pad Programming via Truth Table:
  - Column c_i gives values for the 3 right-hand pads ($c1_i$, $\sim c2_i$, $c3_i$)
  - Column c_{i+1} gives value for the 3 left-hand pads ($\sim c1_{i+1}$, $c2_{i+1}$, $\sim c3_{i+1}$).

“String Tile” Addition. Example.

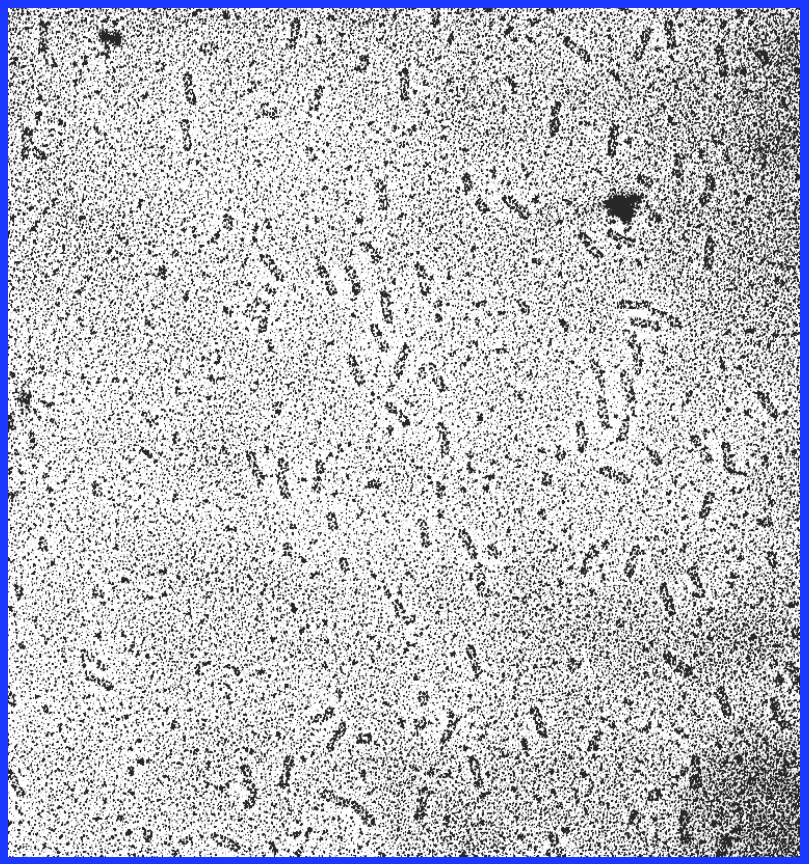


- Anneal strands to form assembly.
- Ligate reporter strand segments.
- Purify reporter strand and read values by PCR.

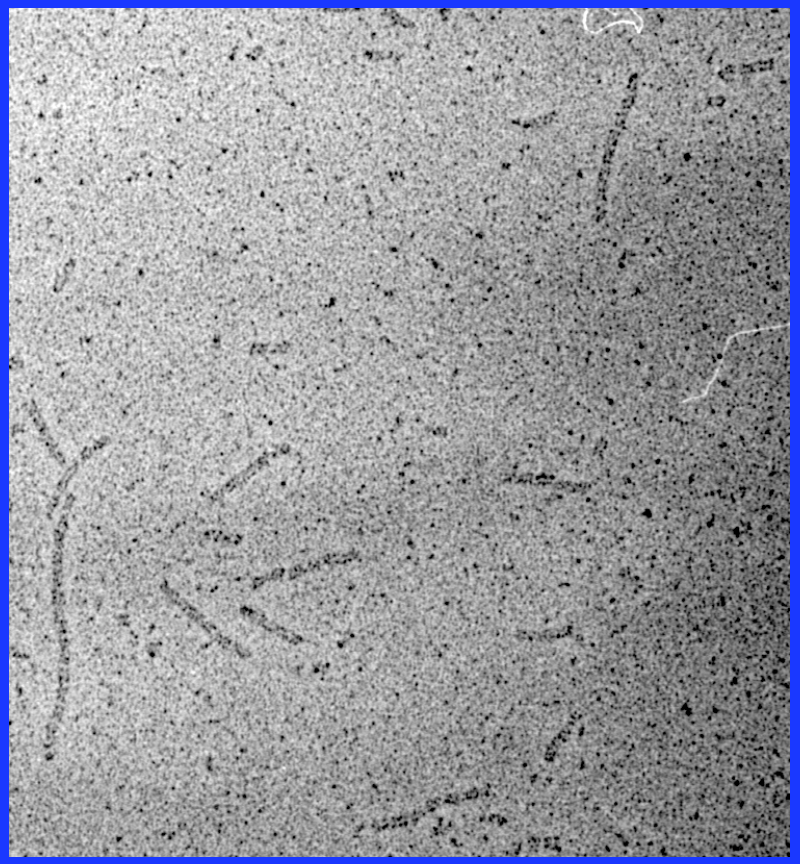
1 0 1 $ 0 1 1 $ 0 0 1 = I_A $ O^R $ I_B

$$\begin{array}{r} 101 \\ +001 \\ \hline 110 \end{array}$$

TAE Assemblies for XOR Computation



LC-RC 1:1



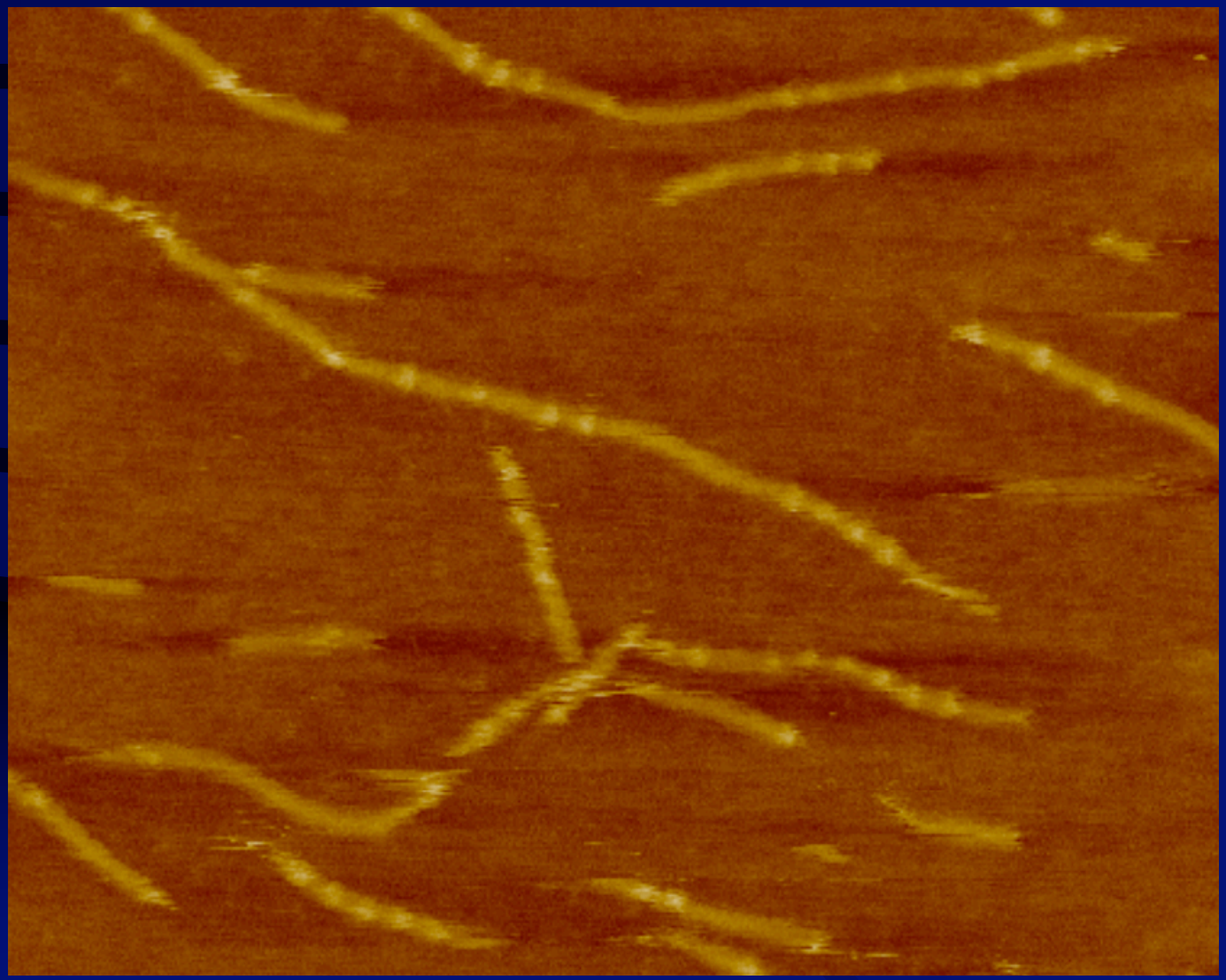
LC-AX-RC 1:8:1

TAE Assemblies for XOR Computation

Transmission electron microscope (TEM) images of platinum rotary-shadowed samples on mica. The left panel shows two-tile complex formed by stoichiometric annealing of 12 oligonucleotides comprising left corner (LC) and right corner (RC) tiles of the string tile computer described above. The right panel shows multi-tile complex resulting from a similar annealing containing LC, RC, and computational tile (AX) with 8-fold excess AX versus corner tiles. Note the distribution of complex sizes due to probabilistic assembly.

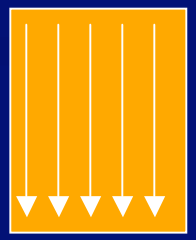
Ligation of reporter strands in the complex has thus far not been efficient enough to obtain digital readout. While optimization of ligation conditions continues, a visual output method is being tested. Oligos holding bit value information have been replaced by strands containing extra DNA loops oriented in the lattice plane. Presence of a loop indicates a value of one; absence of a loop represents a value of zero. Tile assemblies incorporating these value-holding loops are being examined by TEM and AFM for visual readout.

XOR via TAE Computational Complex with Visual Readout



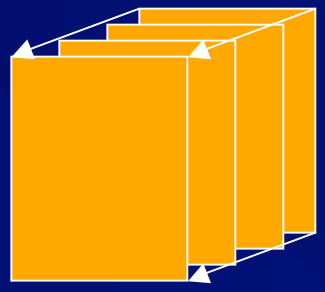
Future Challenges for Computational Tiling Self-Assemblies:

- Two Dimensional DNA Tiling Computations:
  - Apply known VLSI systolic array architecture designs
    - Example: Integer multiplication via repeated additions



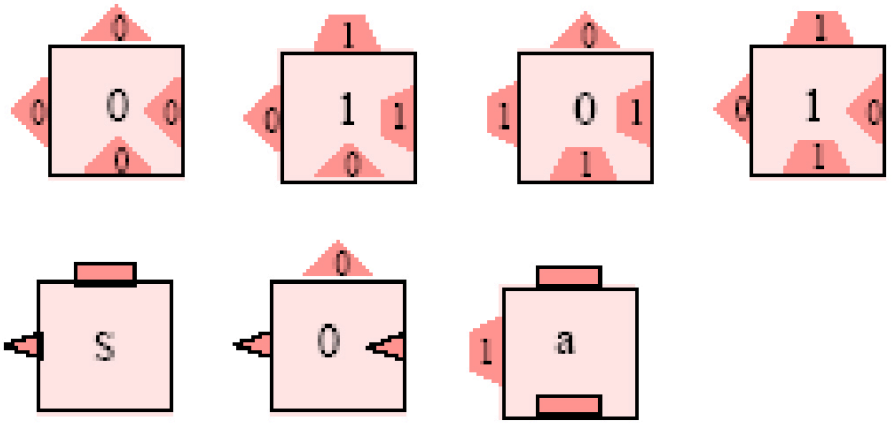
  - Logical processing
    - SAT [Lagoudakis and LaBean,99] -- but only to moderate scale.
    - evaluating Boolean queries and circuits

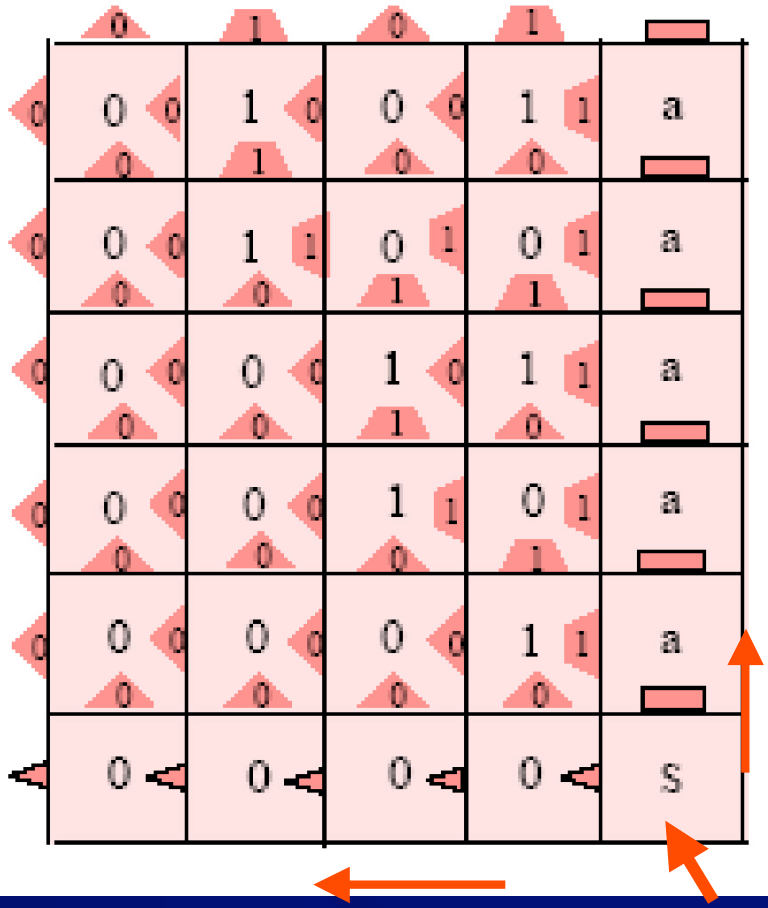
- Three Dimensional DNA Tiling Computations:
  - time-evolution (time is the third dimension of the tiling) of a two dimensional cellular automata
  - Example: simulation of fluid flow.



- Error-Resilient Design

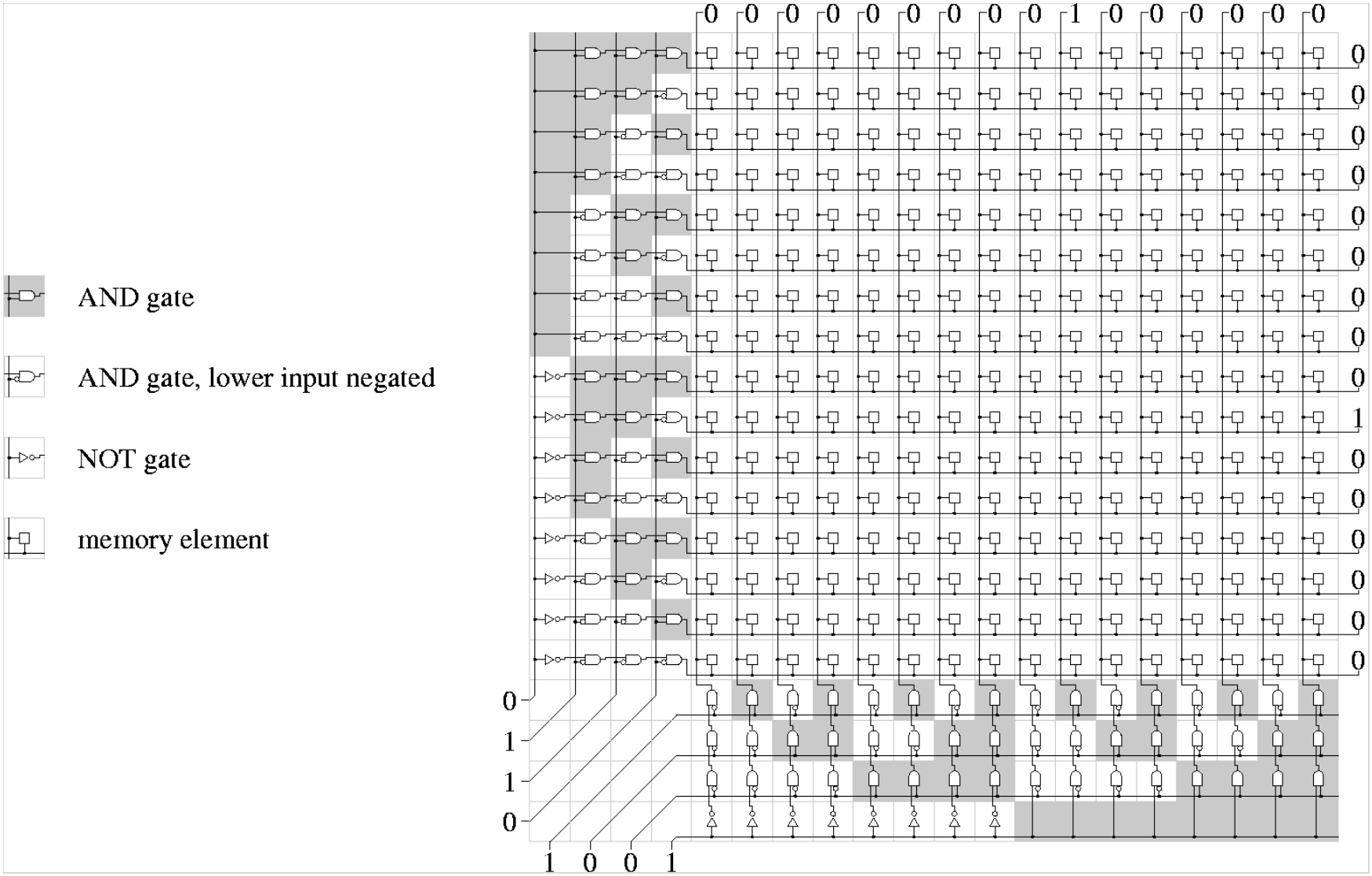
Assembly of Binary Counter (Winfree)





Binary counter

Design of Self-assembled RAM Circuit



Applications of DNA lattices as a substrate for:

(1) Molecular Electronics:

- Layout of molecular electronic circuit components on DNA tiling arrays.

(2) DNA Chips:

- ultra compact annealing arrays.

(3) X-ray Crystallography:

- Capture proteins in regular 3D DNA arrays.

(4) Molecular Robotics:

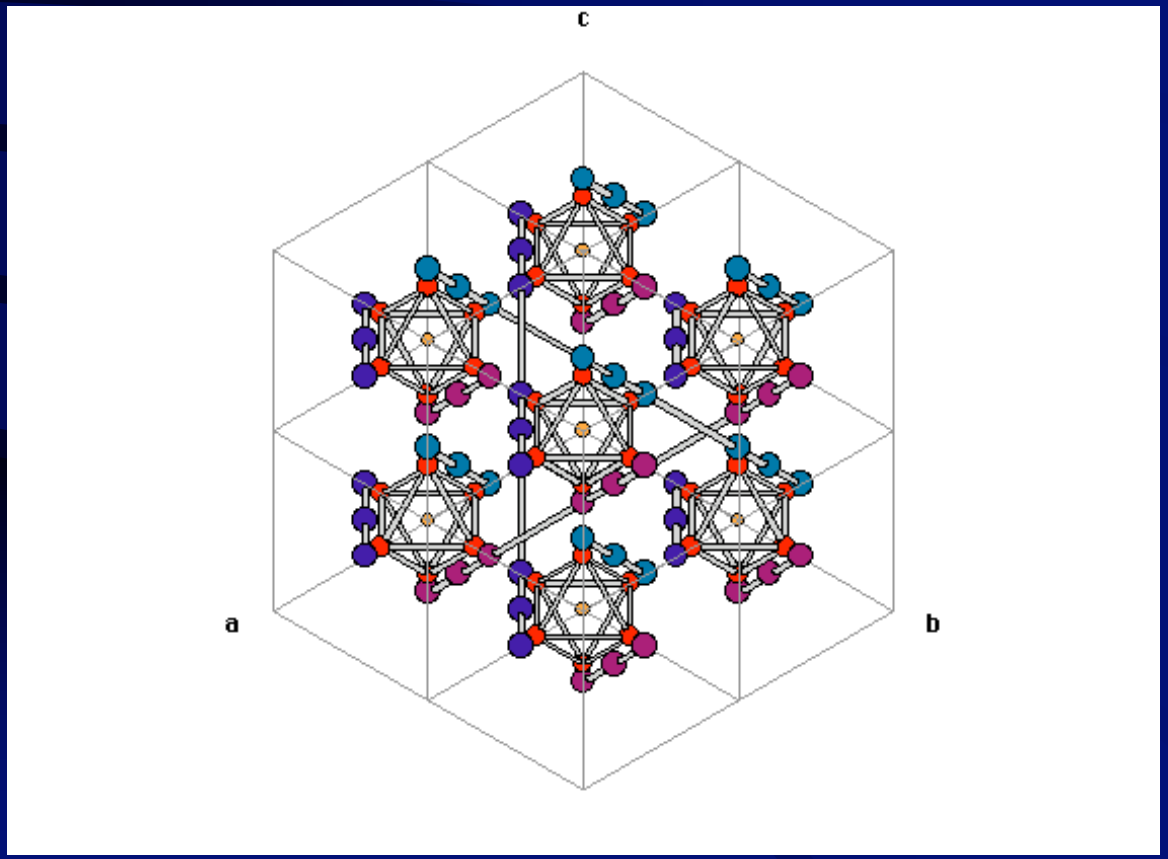
- Manipulation of molecules using molecular motor devices arranged on DNA tiling arrays.

An Application of DNA lattices:

- Molecular Electronics:
  - Layout of molecular electronic circuit components on DNA tiling arrays.

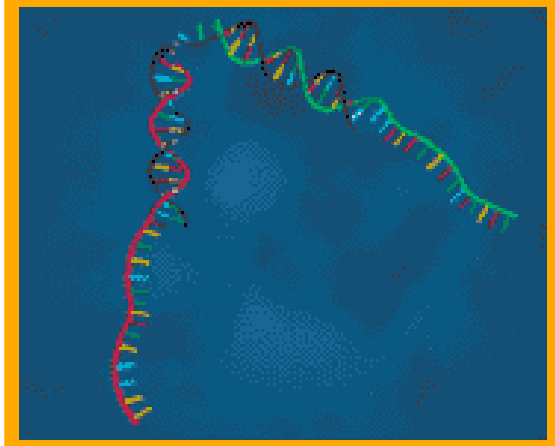
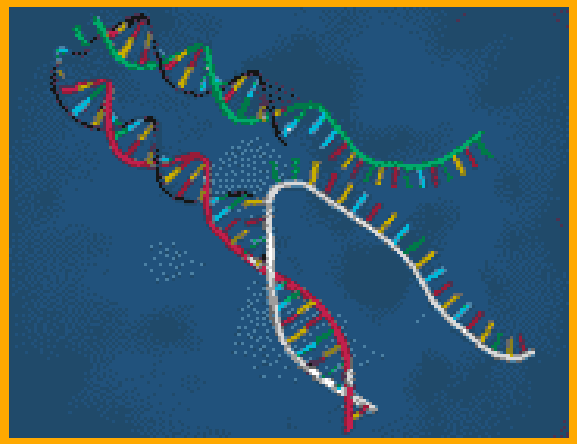
An Application of 3D Regular DNA Tiling Lattices:

- As a substrate for Capturing Proteins
- for X-ray Crystallography [Seeman]



Applications of DNA lattices as a substrate for Molecular Robotics

- Re-Engineering Biological Molecular Motors
  - Construction of these biological molecular motors and their linking chemistry to DNA arrays:
    - Protein motors are modular and can be re-engineered to accomplish linear or rotational motion of essentially any type of molecular component.
    - Motor proteins have well known transcription sequences.
    - There are also well known proteins (binding proteins) that provide linking chemistry to DNA.
    - Protein motors and attached linking elements might be synthesized from sequences obtained by concatenation of these transcription sequences.
- Programmable Sequence-Specific Control of NanoMechanical Motion.
  - an array of molecular motors would be more useful if they can be selectively controled.
  - Manipulate specific molecules: do chemistry at chemically identical but spatially distinct sites.
- Applications of Molecular Motors to to DNA arrays:
  - Manipulation of molecules using molecular motor devices arranged on DNA tiling arrays.
  - Molecular Babbage Machines:
    - A DNA array of motors, may offer a mechanism to do DNA computation of arrays whose elements (the tiles) hold state.
  - Parallel Cellular Automata computations may be executed:
    - arrays of finite state automata each of which holds state.
    - The transitions of these automata and communication of values to their neighbors might be done by conformal (geometry) changes, again using this programmability.
    - Cellular Automata can do computations for which tiling assemblies would have required a further dimension.


Bernard Yurke's Molecular Tweezers (Bell Labs):

Composed of DNA and powered by DNA hybridization.

-Two dsDNA arms are connected by a ssDNA hinge

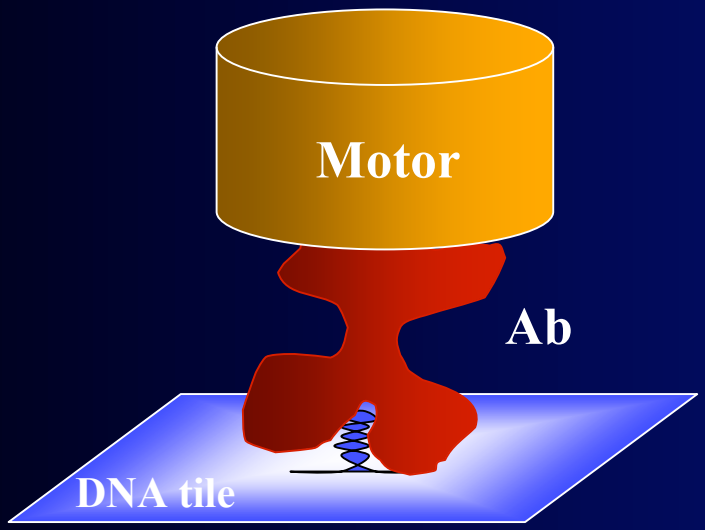
-Two ssDNA "handles" at the ends of the arms.

To close tweezers:

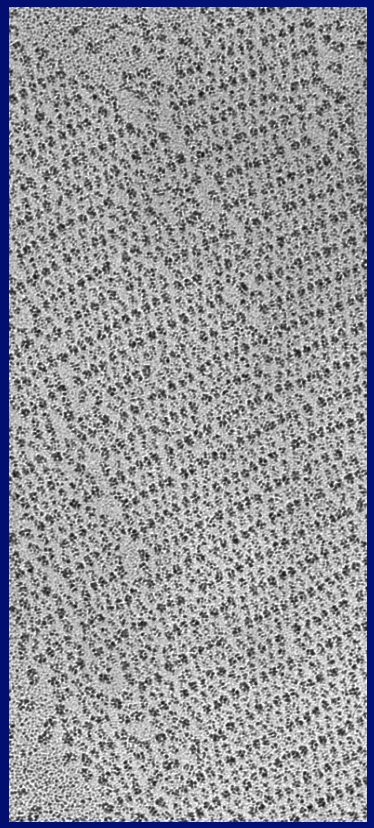
-Add a special "fuel" strand of ssDNA.

-The "fuel" strand attaches to the handles and draws the two arms together.

DNA Tile Lattice for Templating Molecular Motors



A **bifunctional antibody (Ab)** is shown bound to a DNA aptamer on a tile and to a motor protein, thus immobilizing the motor onto the tile.



An example **DNA lattice**

More complex patterns of motors on lattices can allow for sophisticated molecular robotics tasks.

Nanomechanical Devices built of DNA:

[Seeman, et al 2001]

- used rotational transitions of dsDNA conformations between the B-form (right handed) to the Z-form (left-handed) controlled by ionic effector molecules and
- extended this technique to be DNA sequence dependant

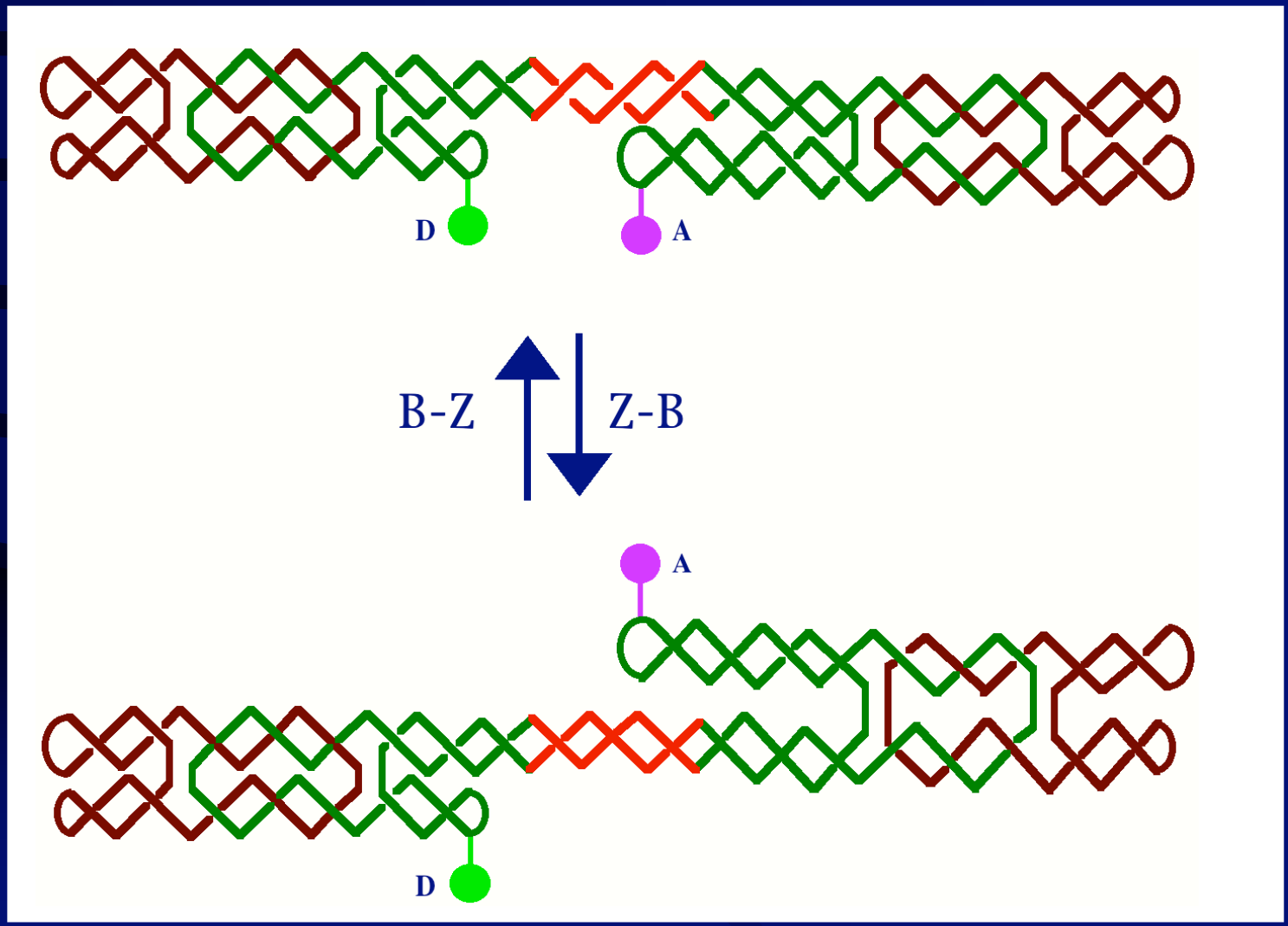
[Yurke and Turberfield, 2000]

- used a fuel DNA strands acting as a hybridization catalyst to generate a sequence of motions in another tweezers strand of DNA
- extended this technique to be DNA sequence dependant
- the two strands of DNA bind and unbind with the overhangs to alternately open and shut the tweezers.

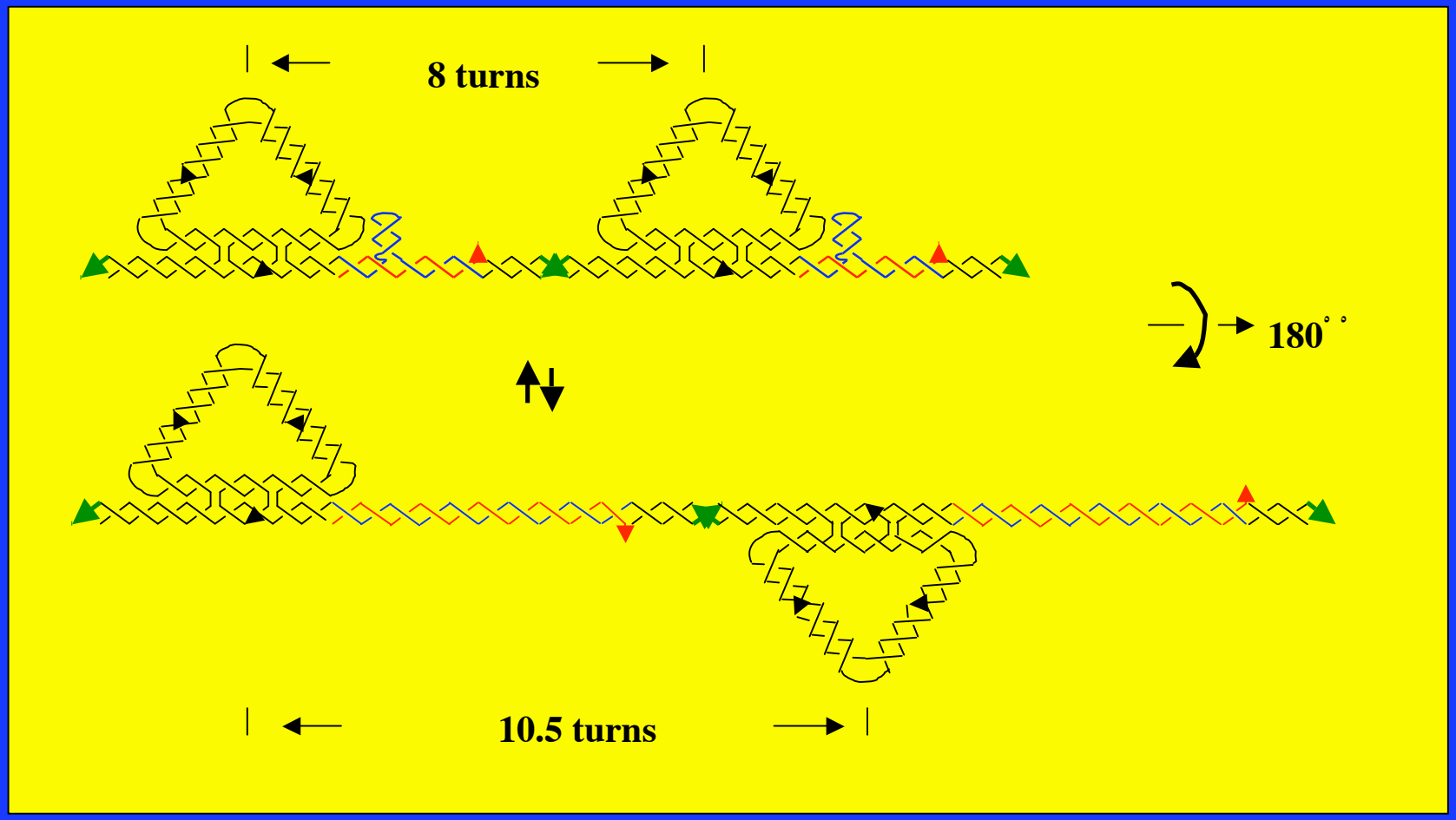
[Reif, 2002] Design of Autonomous DNA Nanomechanical Devices:

Walking and Rolling DNA

B-Z DNA Nanomechanical Device [Seeman, 1999]



DNA Nanomechanical Device



Walking Triangles: By binding the short red strand (top figure) versus the long red strand (bottom figure) the orientation of and distance between the triangular tiles is altered. These changes are observable by AFM.

Applications: Programmable state control for nanomechanical devices.

Also as a visual output method.

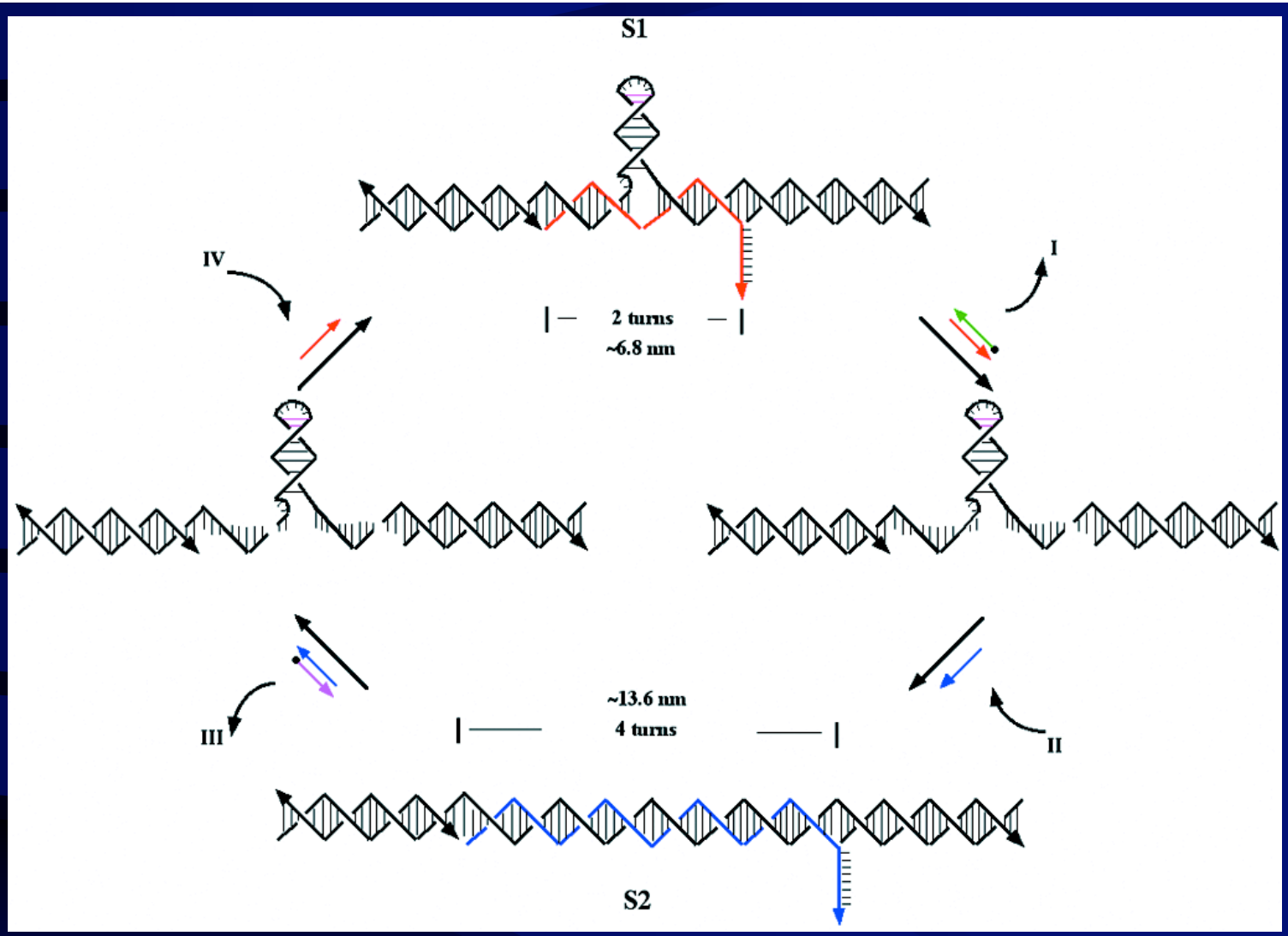
A Switchable Two-State DNA Lattice Controlled by DNA Nano-actuators

- We have constructed and incorporated a robust DNA nano-actuator device into 2D DNA lattices.

- The nano-actuator device constructed here results in a linear translational motion of ~6.8 nm.

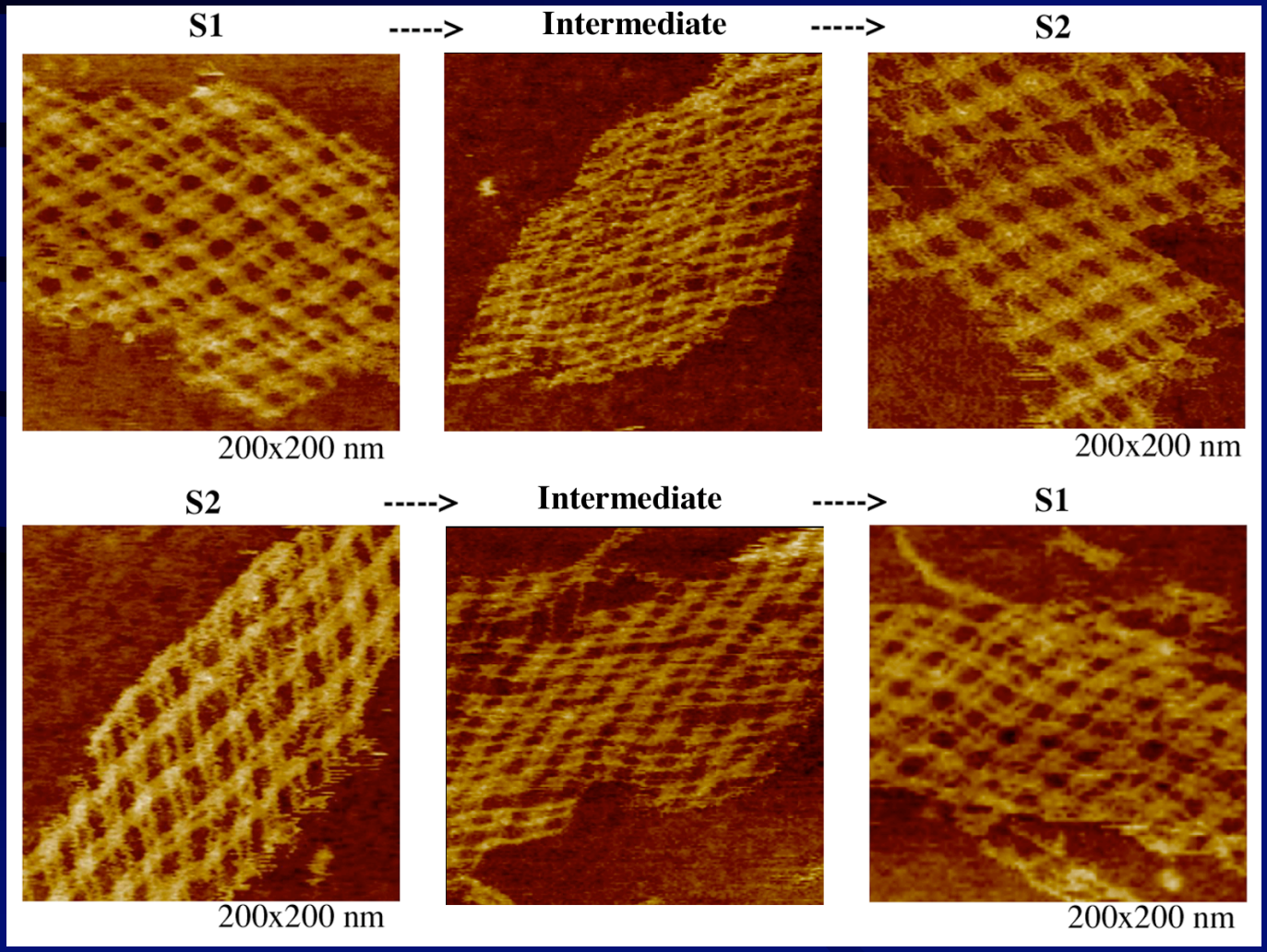
Introduction

- **Controlled mechanical movement in molecular-scale devices is one of the key goals of nanotechnology.**
- **DNA is an excellent candidate in construction of such devices due to the specificity of base pairing and its robust physicochemical properties.**
- **a major challenge is to implement molecular machines into two-dimensional (2D) or three-dimensional (3D) patterned arrays.**
- **Applications:** 1) The size and shape of the lattice can be programmed through the control of sequence-dependent devices, leading to controlled nanofabrication of molecular nanoelectronic wires with on and off states. 2) Molecules or nanoparticles can be selectively manipulated, e.g. sorted and transported, using molecular motor devices arranged on DNA tiling arrays, which may lead to programmed chemical synthesis. 3) It may offer a mechanism to do DNA computation of arrays whose elements (the tiles) hold state.



Schematic drawing of the design and operation of the nano-actuator device.

AFM evidence for the two state DNA lattice actuated by DNA nano-actuator devices



[Reif, 2002]: DNA Motor Devices:

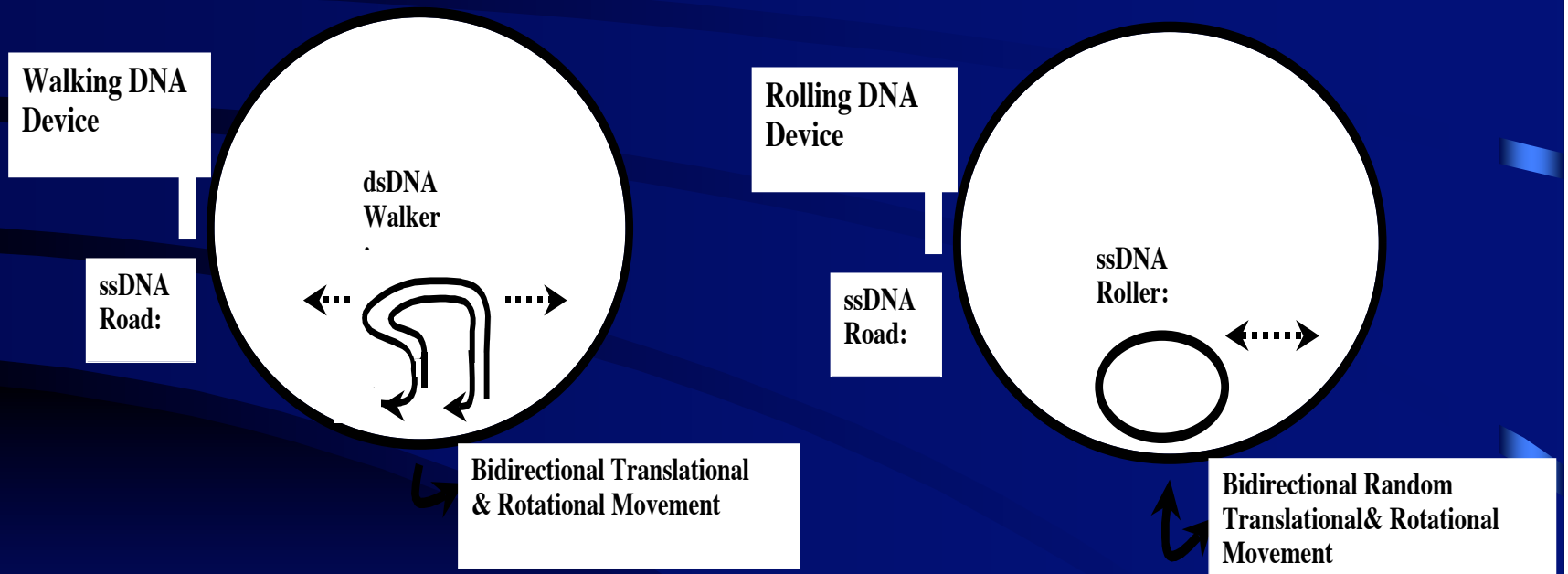
Designs for the first autonomous DNA nanomechanical devices that execute cycles of motion without external environmental changes.

Walking DNA device

Uses ATP consumption

Rolling DNA device

Uses hybridization energy

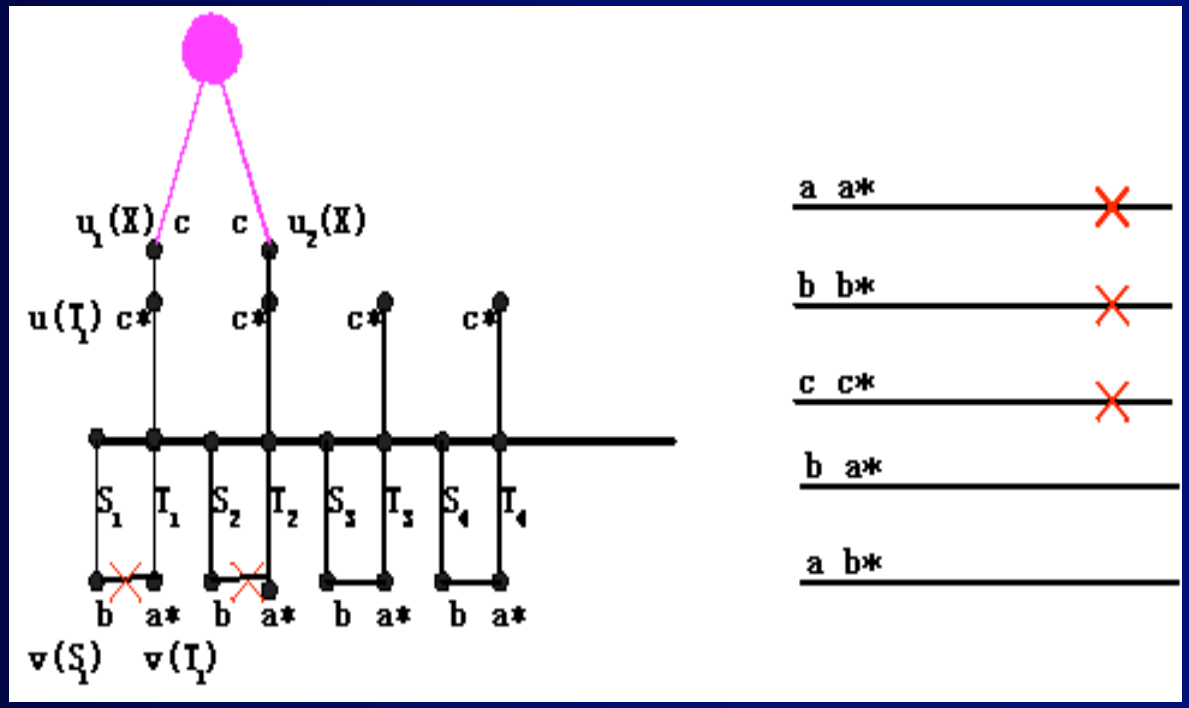


These DNA devices translate across a circular strand of ssDNA and rotate simultaneously.

Generate random bidirectional movements that acquire after n steps an expected translational deviation of $O(n^{1/2})$.

DNA Walker With Attachment Property :
Basic Design

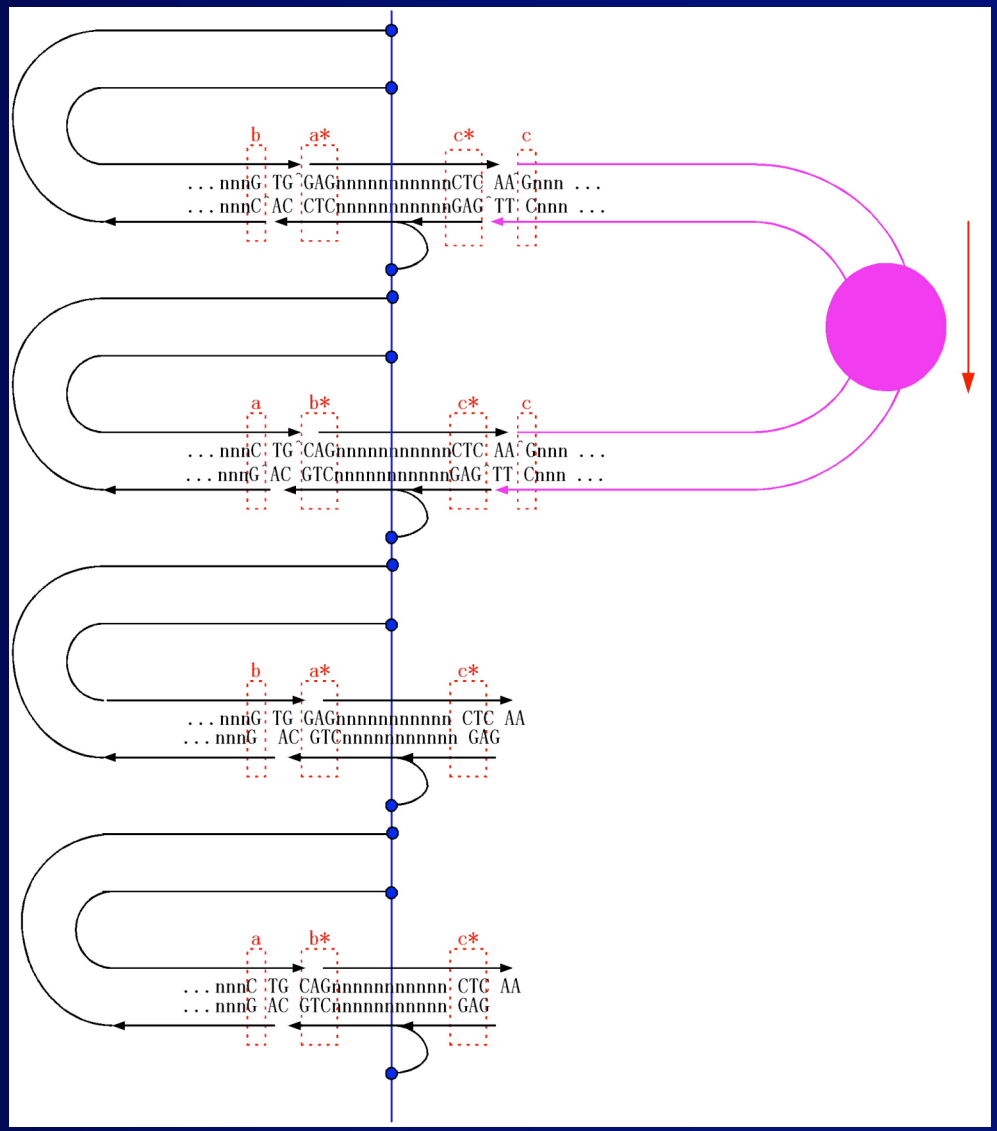
- **Signaling Machinery:**
  - **- Transducer, T**
  - **- Switcher, S**
- **The movement of robot:**
  - **-Autonomous**
  - **- Unidirectional**
  - **- Always attached to track**
  - **- Occlusion free**



DNA Walker With Attachment Property : **Implementation**

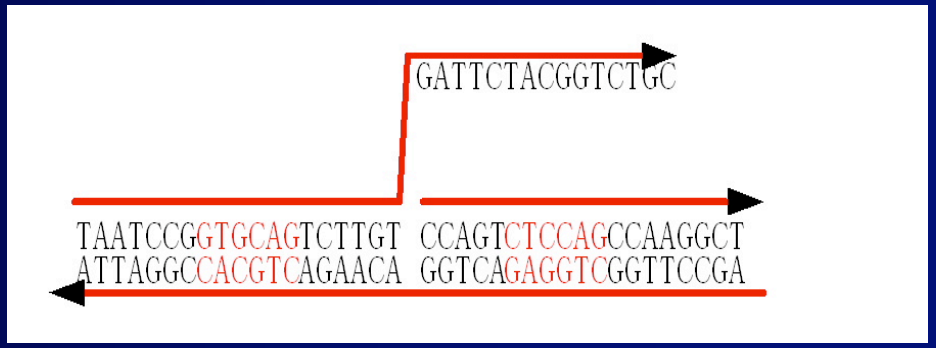
- Implementation with Endonucleases and T4 Ligase

Endo-nuclease	**Sequences**
BpmI	5' …CTGGAG$(N)_{16}$^… 3' 3' …GACCTC$(N)_{14}$^… 3'
BsgI	5' …CTGCAG$(N)_{16}$^… 3' 3' …GACGTC$(N)_{14}$^… 3'
BpuEI	5' …CTTGAG$(N)_{16}$^… 3' 3' …GAACTC$(N)_{14}$^… 3'

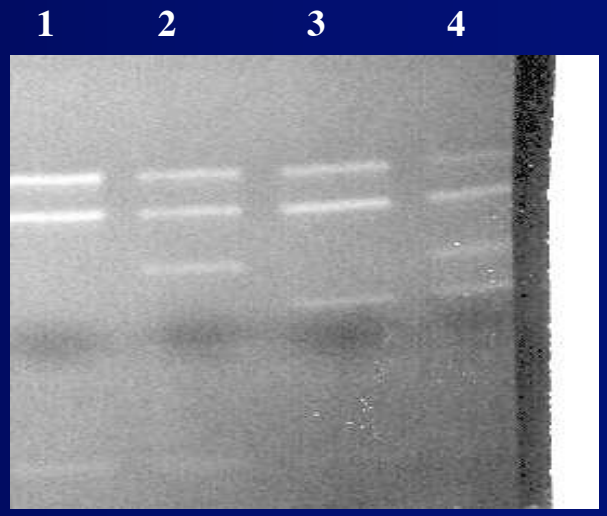


DNA Walker With Attachment Property : **Preliminary Experimental Result**

- A key assumption:
  The desired restriction is not affected by a dangling single strand DNA between endonuclease recognition site and restriction site.



- Preliminary experimental result validates the assumption.



Lane 1: Control
Lane 2: Bpm I
Lane 3: Bsg I
Lane 4: Bpm I + Bsg I