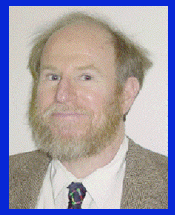
Duke Univ

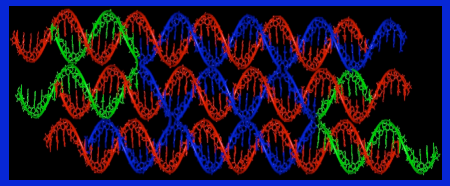


PI: John Reif, Duke University
Subcontractors:
Nadrian C. Seeman, NYU
& Erik Winfree, Caltech

# DARPA BIOCOMP Contract:
# Programmable DNA Lattices: Design, Synthesis and Applications

## Key Technology: The Programmed Self-Assembly of Patterned 2D Lattices

### A New, Powerful Technology for Rendering Patterns at the Molecular Level

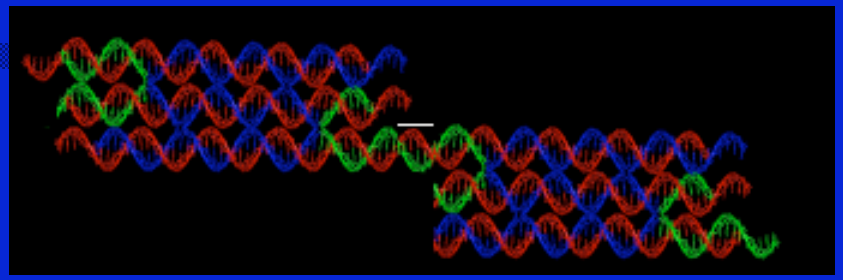


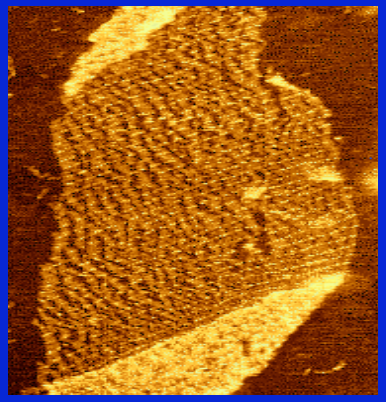
A 2D DNA lattice is constructed by a self-assembly process:

--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:

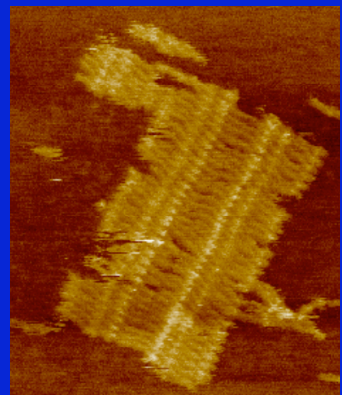
-Tiles have sticky ends that provide programming for the patterns to be formed.

-Or 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.

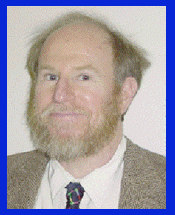
-pixel distances 7 to 14 nanometers

-not diffraction limited



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

Duke Univ



PI: John Reif, Duke University
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Nadrian C. Seeman, NYU
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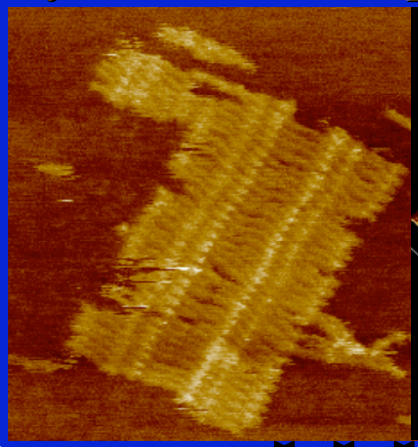
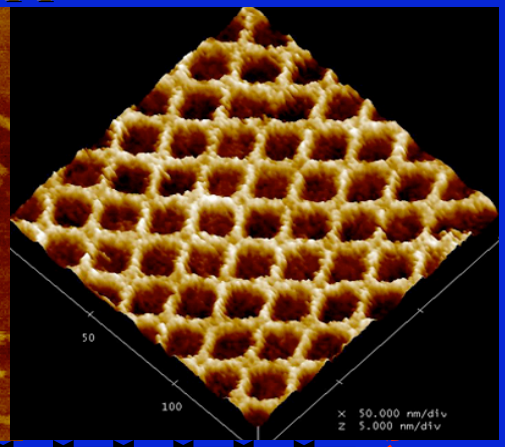
# DARPA BIOCOMP Contract:

## Programmable DNA Lattices: Design, Synthesis and Applications

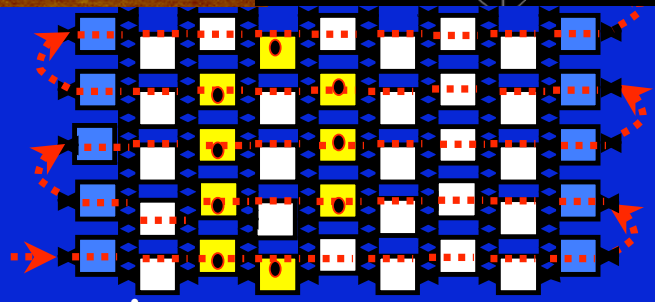





•Tasks and Goals:

1) Patterned DNA Arrays.

- Set of specific tiles which form patterns.
- Assembly around scaffold strands.
- Molecular fabric.

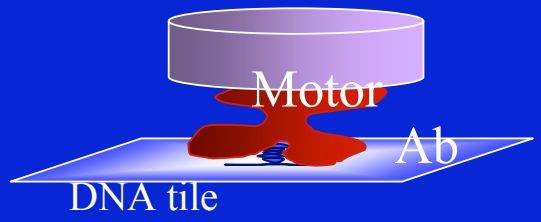


2) Computation via DNA Self-Assembly

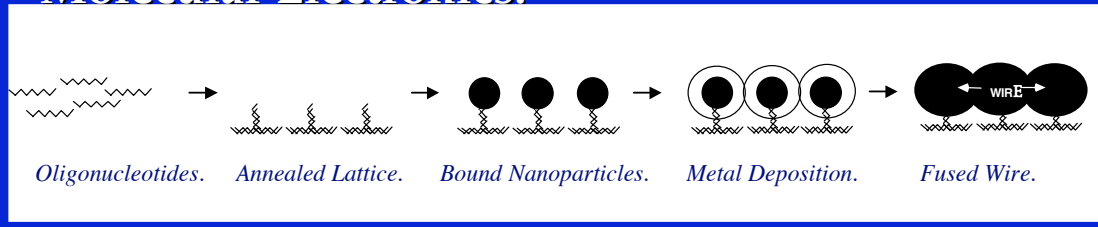
- Reporter strand output (requires ligation).
- Microscopic readout
  (via AFM, TEM, SEM, etc.).

3) Applications of DNA-Based Assemblies.

Molecular motors & actuators:




Molecular Electronics:




4) Software for Design and Simulation of DNA Assemblies.

# Programmable DNA Lattices: Design Synthesis & Applications

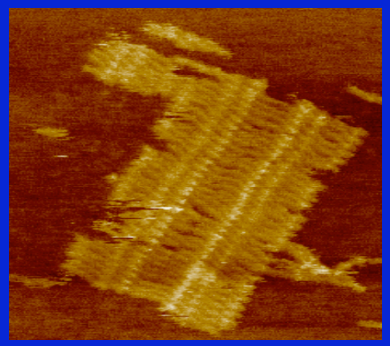

## Duke University (PI John Reif)

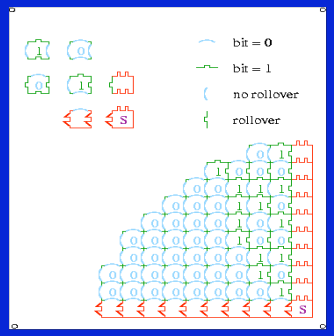
## Subcontracts: NYU (Nadrian Seeman), Caltech (Erik Winfree & Niles Pierce)


### Scientific Objectives:

*Programmable construction of complex nanostructures of supramolecular (10-100 nm) scale.*

- DNA lattices with complex 2D patterning, and
- periodic 3D DNA lattices.






*Tiling Design for Binary Counter*

### New Ideas: *Molecular Self-assembly:*

-DNA strands self-assemble into *DNA tile* nanostructures.
-DNA tiles self-assemble into periodic and aperiodic lattices.

### *Algorithmic Self-assembly:*

-DNA tiles form DNA lattices with complex patterning.
-Methodology is *programmable* by choice of DNA tiles.

### *Nanostructure Templating and Patterning:*

-DNA lattice superstructure for other complex nanostructures.
-Other molecular nanostructures attach to specific DNA strands within DNA lattices.

### Impact to US defense:

Protein structure determination via host-lattice crystals:
-Periodic 3D DNA lattices capture proteins for X-ray diffraction. *Key spin-off:* Structural characterization of antigens from pathogens.

### *Patterned DNA Lattice Technology:*

-DNA nanostructures can serve as scaffolds for molecular sensors and actuators.
*Key Spin-off:* identifying pathogens (e.g., bacteria).
-DNA lattices can be used as scaffolds for positioning molecular electronics components into complex circuits.
*Key Spin-off:* Molecular Nanoelectronics.

### Schedule:

Design of novel DNA tiles & lattices and support software

Optimization algorithms for DNA design implemented.
Patterned 2D DNA lattice: modest size (64 tiles)
Periodic 3D DNA lattices: diffracting to 2.5 Å

Characterization of error rates of self-assemblies
Patterned 2D DNA lattices: moderate size(512 tiles)
Periodic 3D DNA lattice: diffracting to 2.5 Å

Self-assembly of 4-bit demultiplexing RAM
Patterned 2D DNA lattices: thousands of tiles
Periodic 3D DNA lattices: diffracting to 2.5 Å

**2001 2002 2003 2004**

# DUKE PERSONNEL on PROJECT

- John H. Reif
  - Professor (PI)
  - 25% Effort
- Thomas H. LaBean
  - Assistant Research Professor
  - 25% Effort
- Hao Yan
  - Assistant Research Professor
  - 50% Effort
- Liping Feng
  - Research Technician
  - 75% Effort
- Dage Liu
  - Postdoctoral Research Associate
  - 25% Effort

# Major Goals of Duke Group

1) Patterned DNA Arrays.
   - Set of specific tiles which form patterns.
   - Assembly around scaffold strands.
   - Molecular fabric.

2) Computation via DNA Self-Assembly
   - Reporter strand output (requires ligation).
   - Microscopic readout (via AFM, TEM, SEM, etc.).

3) Applications of DNA-Based Assemblies.
   - Molecular and nano-scale electronics.
   - Molecular motors and actuators.

4) Software for Design and Simulation of DNA Assemblies.

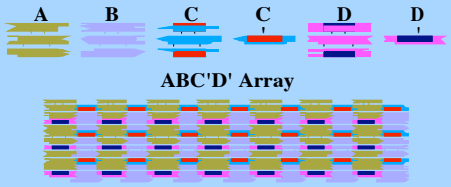
# NYU SUBCONTRACT

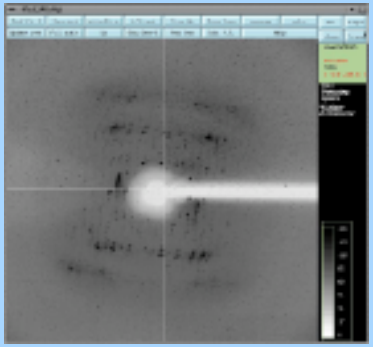
## DIRECTED ASSEMBLY OF 3D NANOSTRUCTURE ARRAYS

NADRIAN C. SEEMAN, NEW YORK UNIVERSITY

## SCIENTIFIC OBJECTIVES:

**DESIGN 3D CRYSTALLINE ARRAYS FROM DNA.**



## NEW IDEAS:

**SELF-ASSEMBLY OF DNA FOR CONTROL OF NANOSTRUCTURE.**

**DESIGNED CONNECTION BETWEEN THE MOLECULAR AND MACROSCOPIC SCALES.**

**DIRECTING THE ASSEMBLY OF BIOLOGICAL AND NANOELECTRONIC MOLECULES BY DNA SCAFFOLDING.**

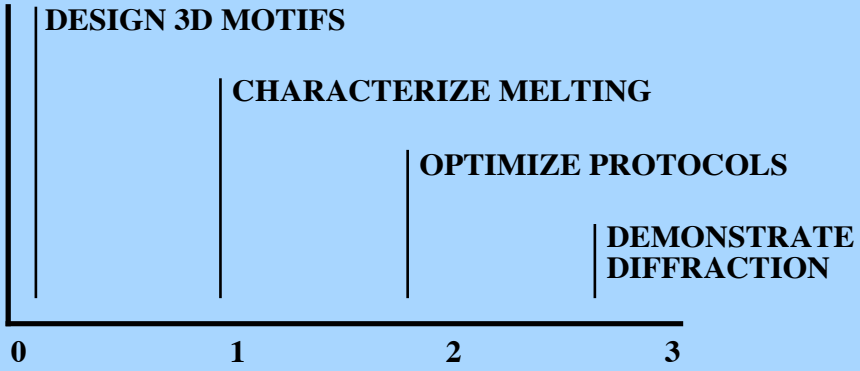
## IMPACT:

**ELIMINATION OF THE MACROMOLECULE CRYSTALLIZATION PROBLEM -- RATIONAL CONSTRUCTION OF CRYSTALS TO SOLVE PATHOGEN PROTEIN 3D STRUCTURES.**

**FACILITATION OF NANOELECTRONICS THROUGH DNA-DIRECTED SCAFFOLDING.**

**IMPLEMENTATION OF NANOROBOTICS.**

## SCHEDULE



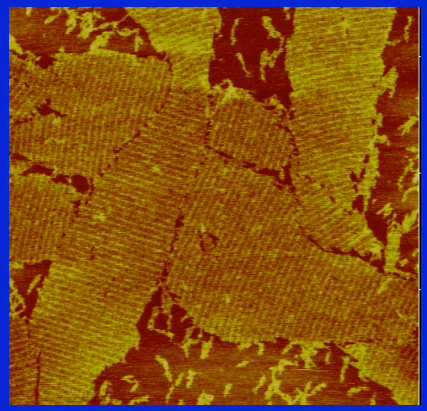
# CALTECH SUBCONTRACT

## Computational and Experimental Design of DNA Devices

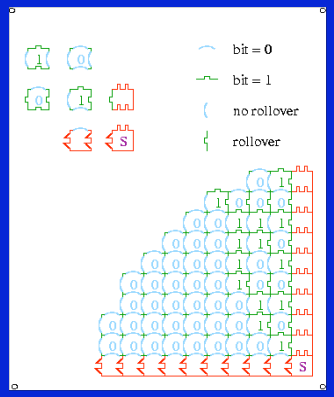
Erik Winfree and Niles Pierce

### Scientific Objectives:

DNA sequence design of self-assembled devices for
Reliable lattice assembly & patterned nanostructures



AFM image of striped lattice




Binary Counter

### New Ideas:

Algorithmic self-assembly of DNA tiles
forming templates for electronic circuits

Thermodynamic partition functions
and dynamic simulation for multi-stranded
DNA systems

Algorithms for positive and negative
DNA sequence design

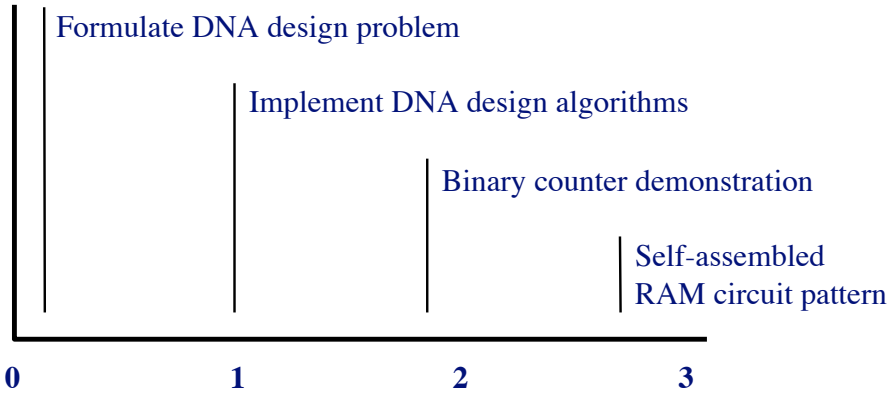
### Impact:

Reliable automated design
of complex molecular systems

Ability to pattern nanostructures using
self-assembled DNA lattice

Nanoelectronics, nanorobotics, nanosensors,
nanoactuators

### Schedule:



# Background Literature on DNA Self-Assembled Tiling Lattices.

- [Winfree and Seeman,98] The first experimental demonstration of self-assembly of DNA to construct 2D lattices consisting of up to a hundred thousand 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] recently experimentally demonstrated for the first time a computation using 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

# MAIN OBJECTIVE

Programmable construction of complex nanostructures of supramolecular (10 - 100 nm) scale.

- Biological paradigm: self-ordering properties of biomacromolecules.
- Exploit DNA as a programmable structural material.
- Engineer artificial DNA sequences which spontaneously self-organize into desired structures.
- Produce DNA tiles and tile sets from which specifically patterned lattices of increasing complexity can be formed.
- Induce desired patterns on other materials (e.g. metallic nanostructures, carbon nanotubes, etc.) via DNA lattice self-assemblies.

# Challenges and Approaches

- Technical Challenges.
  - Decrease difficulties of input/output steps.
  - Construct/debug new component tile structures.
  - Minimize errors during lattice assembly.
  - Increase efficiency of reporter strand ligations.
- Approaches.
  - Computer simulation. Experimental construction and testing.
- Why We Will Succeed.
  - Diverse team whose skill sets overlap and complement each other extremely well. Rich community of creative collaborators with diverse tools and instruments to share.

# DOD and National Security Relevance

- Compact massively parallel computation, cryptography and data storage.
  - Next generation computing.
  - Other nations are developing capabilities.
- Biological DNA as input.
  - Wet database of selected personnel DNA.
  - Possible diagnostics for genetically determined diseases.
  - Systems in which to pose whole genome and transcriptome questions.
- Self-assembling structures for bottom-up nanofabrication.
  - DNA lattices as templates for positioning molecular sensors and actuators.
  - Next generation electronics (nano- and molecular electronics).
  - Possible biosensors and implantable therapeutic robots.

# MILESTONES

- Barcode tile lattice: Propagation of 1D pattern into 2D for visual readout (AFM, TEM, etc.).
  - 0.5 - 1 year. (done)
- Targeted immobilization of hetero materials on DNA tile lattice.
  - 6 months. (done)
- Prototype construction of new tile designs.
  - 1 year. (done)
- Massively parallel XOR computation by “string tile” assembly.
  - 1 year. (done)
- Demonstration of conductivity in metallic nano-wire templated on DNA lattice.
  - 1.5 years. (in process of testing)
- Demonstration of complex pattern formation with tens or hundreds of tile varieties.
  - 2 years. (done for small bar codes)

# METRICS for Evaluating Progress

- Computational measures of goodness of sequence design.
  - Sequence similarity minimization and evaluation. Kinetic simulations.
- Electrophoretic and spectroscopic analyses.
  - Native and denaturing PAGE. UV absorption melting curves. Shows stoichiometry and stability of multi-strand complexes.
- Fluorescence resonance energy transfer.
  - FRET measures distance between labeled components.
- Advanced microscopic techniques (AFM, TEM, SEM, STEM).
  - Imaging demonstrates formation of desired structures.
- PCR amplification of full length reporter strand.
  - Truncated products lack primer binding sites.
- Conductivity of templated nano-wires.
  - Demonstrates complete metallization and particle fusion.

# DELIVERABLES

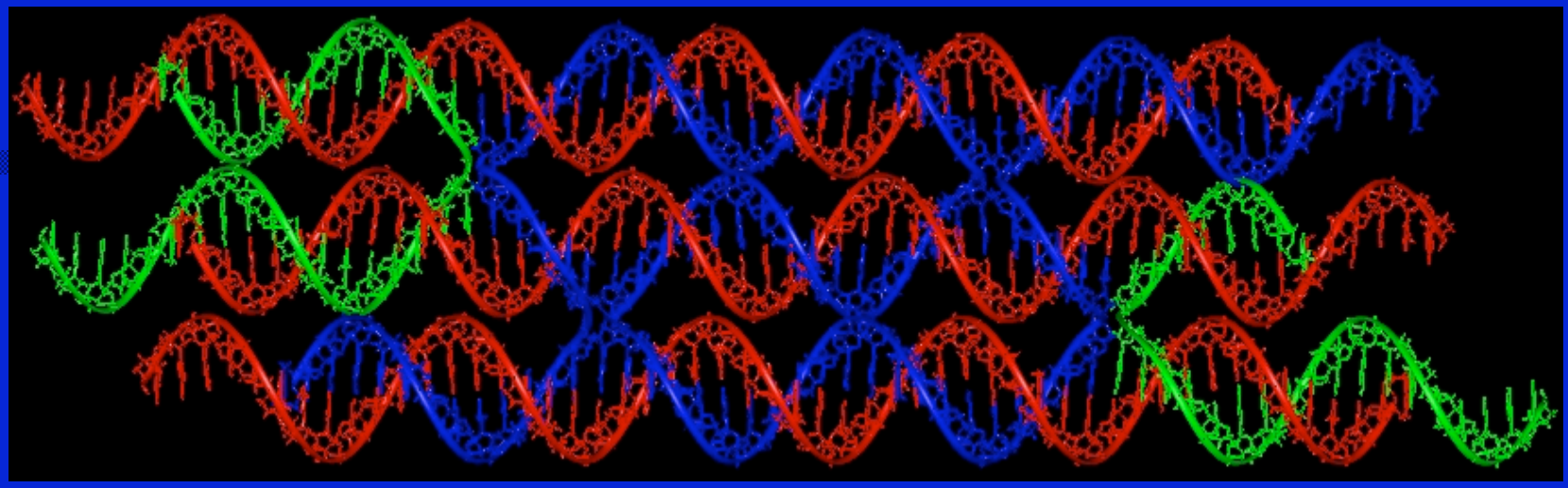
- Software for DNA sequence design.
- Software for simulation of DNA annealing.
- Novel DNA tile structures.
- 2-Dimensional DNA lattices of increased complexity.
- Massively-parallel, self-assembling DNA-based computer.
- Patterned formation of functional metallic nano-wires.
- Publications in high impact scientific journals.

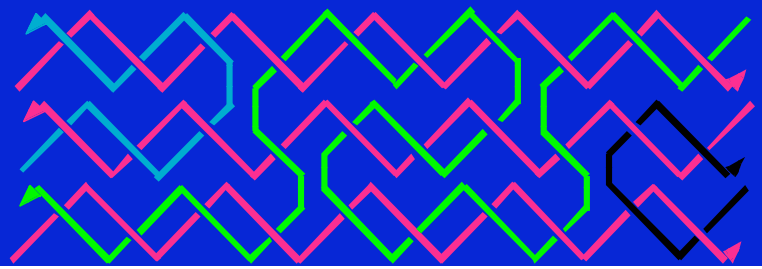
# DNA Nanostructures:

**DNA tiles:** composed of a few strands of DNA that self-assemble (via DNA annealing) into a roughly rectangular shape.

**TX tiles:** 3 double stranded DNA with Holiday junctions

Approx 20 Angstroms wide

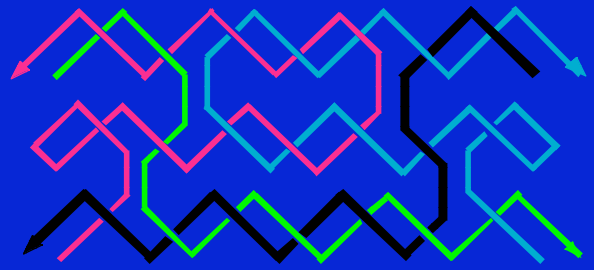




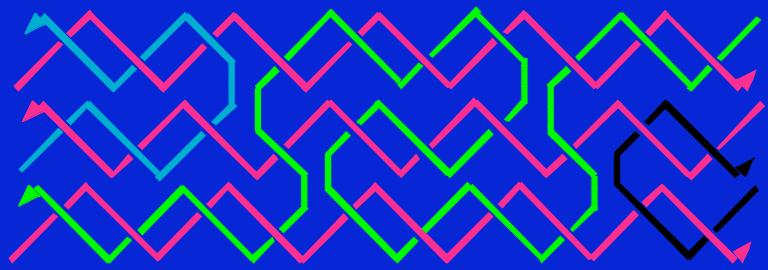
# Plans and Approaches

Building on our recent successful constructions we are incrementally improving and expanding our tools and techniques to include more complex tile types, larger tile sets, and a variety of attachment chemistries for immobilization of other materials.

**TAO35**



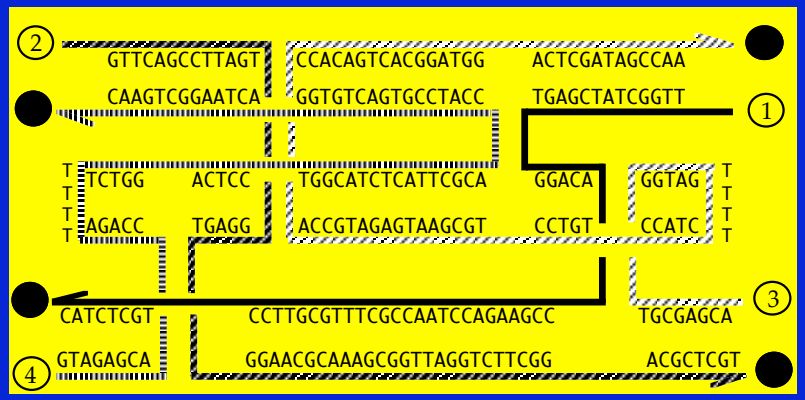
**TAE 44**

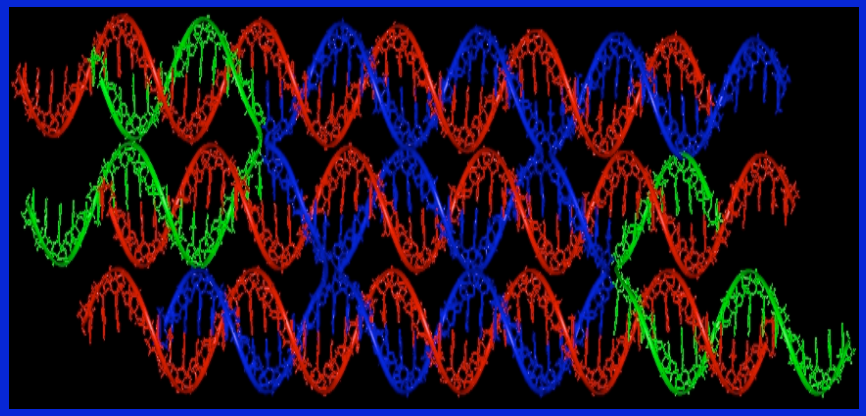


Strand topology traces of example tiles. ‘T’ denotes three DNA helices; ‘A’ denotes anti-parallel crossovers (strand changes direction of propagation at crossover points); ‘O’ and ‘E’ are odd and even, respectively, and refer to the number of helical half-turns between adjacent crossover points.

# DNA TX tile Nanostructures:

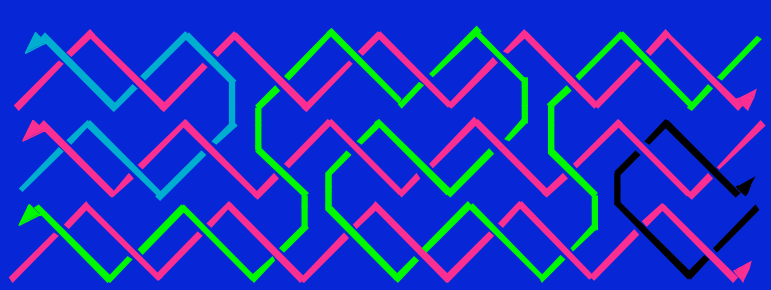
- 3 double stranded DNA with Holiday junctions
- Approx 20 Angstroms wide



TAO35

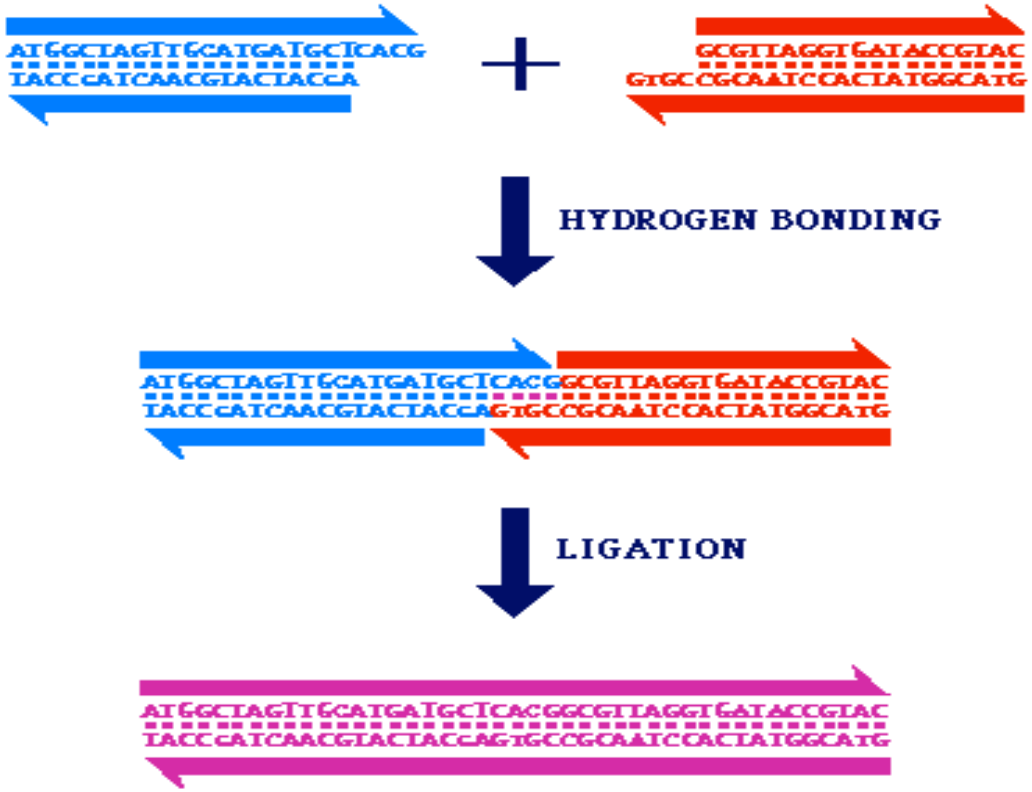
TAE 44



Strand topology traces of TX tiles.

- 'T' denotes three DNA helices
- 'A' denotes anti-parallel crossovers
  (strand changes direction of propagation at crossover points)
- 'O' and 'E' are odd and even, respectively:
  refer to the number of helical half-turns between adjacent crossover points.

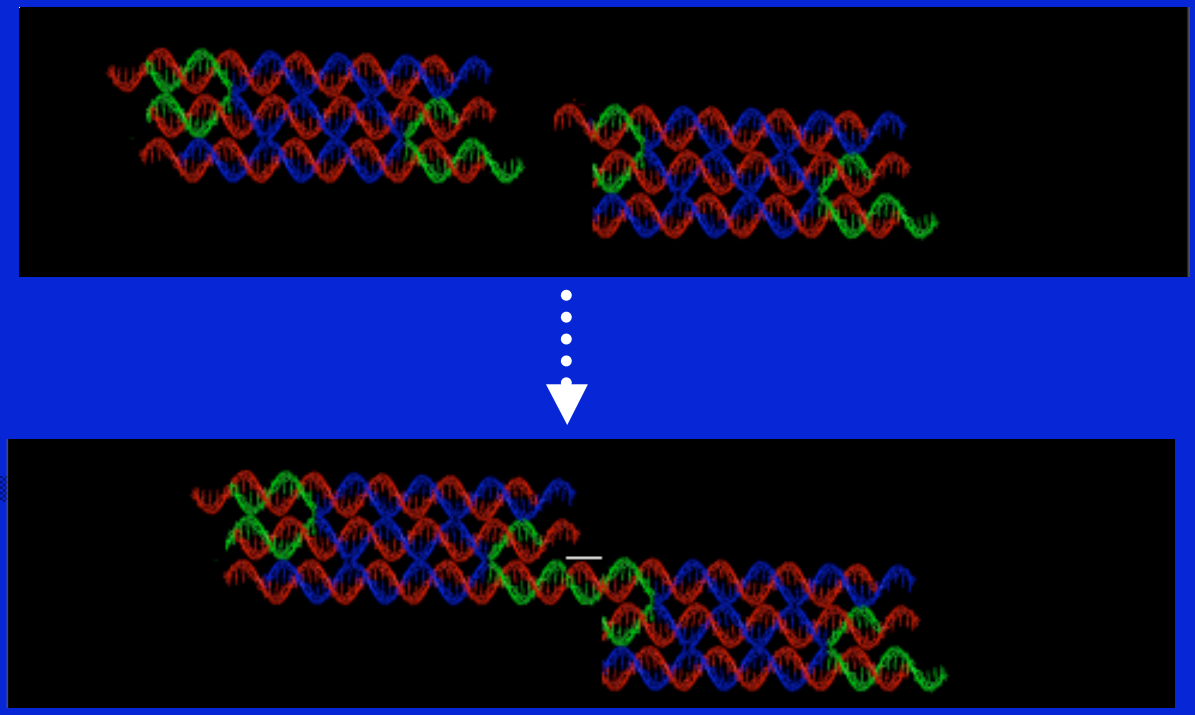
# DNA Hybridization and Ligation Operations.




**Hybridization** of sticky single-strand DNA segments.

**Ligation**: If the sticky single-strand segments that anneal abut doubly stranded segments of DNA, you can use an enzymic reaction known as **ligation** to concatenate these segments.

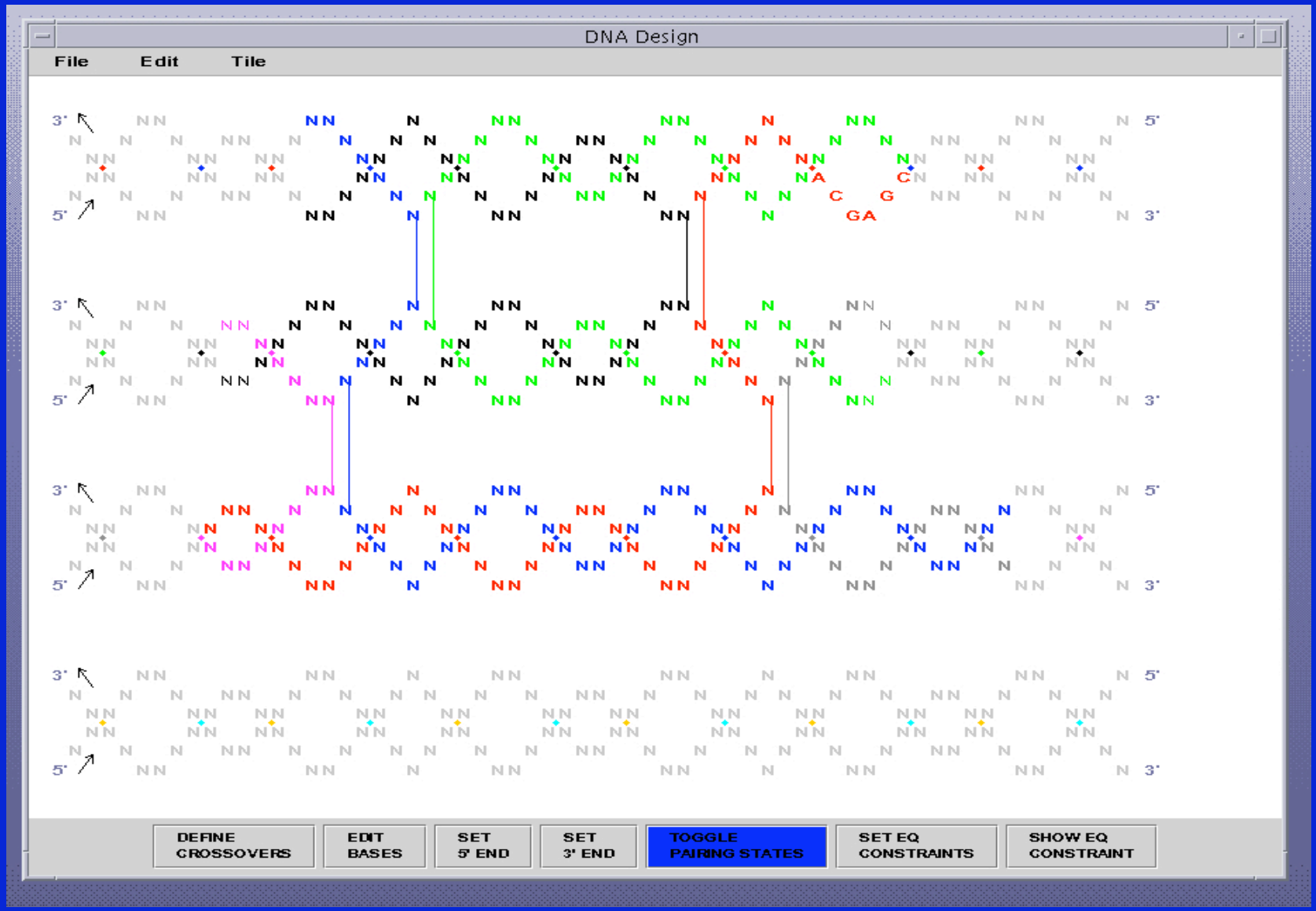
**Unique Sticky Ends on DNA tiles.** Input layers can be assembled via unique sticky-ends at each tile joint thereby requiring one tile type for each position in the input layer.



**Tiling self-assembly:** proceeds by the selective hybridization of the pads of distinct tiles, which allows tiles to compose together to form a controlled tiling lattice (these pads determine the form of the tiling that self-assembles).

**Technical Challenge:** Optimal Design of strands composing tiles.

# Setting WC constraints



# Computer simulations of the Tiling Self-Assembly

Prior to experimental tests,

we also made computer simulations of our protocol for for self-assembly of patterned 2D lattices

Goals:

- to optimize the sequence designs for the DNA tiles and
- to optimize experimental parameters such as the schedule of annealing temperatures.

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.

# Computer simulations of the Tiling Self-Assembly

Prior to experimental tests,

we also made computer simulations of our protocol for for self-assembly of patterned 2D lattices

Goals:

- to optimize the sequence designs for the DNA tiles and
- to optimize experimental parameters such as the schedule of annealing temperatures.

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.

## Methods for Generating: Microscopically Observable Pixels

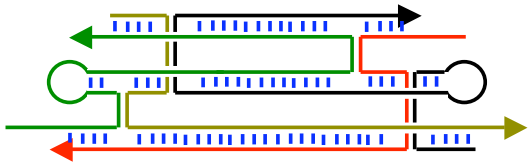
pixels can be imaged by AFM, SEM, or TEM.

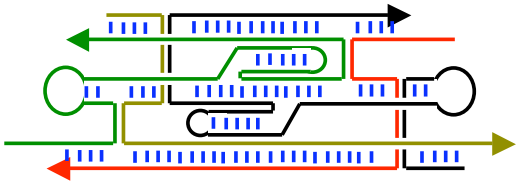
1) Stem-loops.
2) Metallic nanoparticles.
3) Triangles or multi-triangle tiles.
4) Biotin-streptavidin (with or without nanogold).
5) Multi-tile subassemblies.
6) New tile topologies.

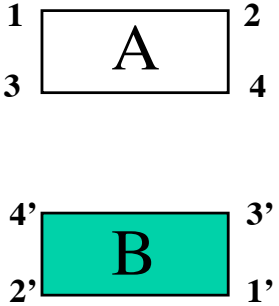
# Stem-loops: Chosen Method for Generating Pixels which are Microscopically Observable by AFM methods

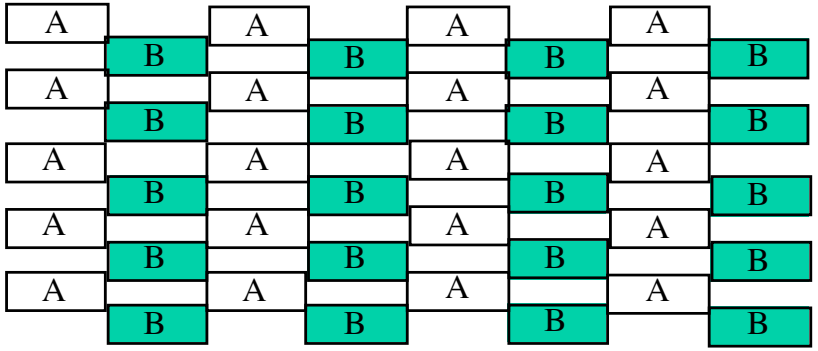
- **DNA tiles with additional stem-loops of 8 to 16 basepairs, directed out of the plane of the tile helix axes, are used in DX and TX lattices to evaluate successful assembly of periodic arrays.**
- **Stem-loops can also be directed orthogonal to the tile helix axes within the tile plane in single layer assemblies.**
- **These loops are used mark binary values on the tiles where the presence of a loop indicates a 1 and the absence indicates 0.**
- **Modification of protruding stems or stem-loops with gold or biotin-streptavidin increases their visibility**

# TAO AB* Lattice



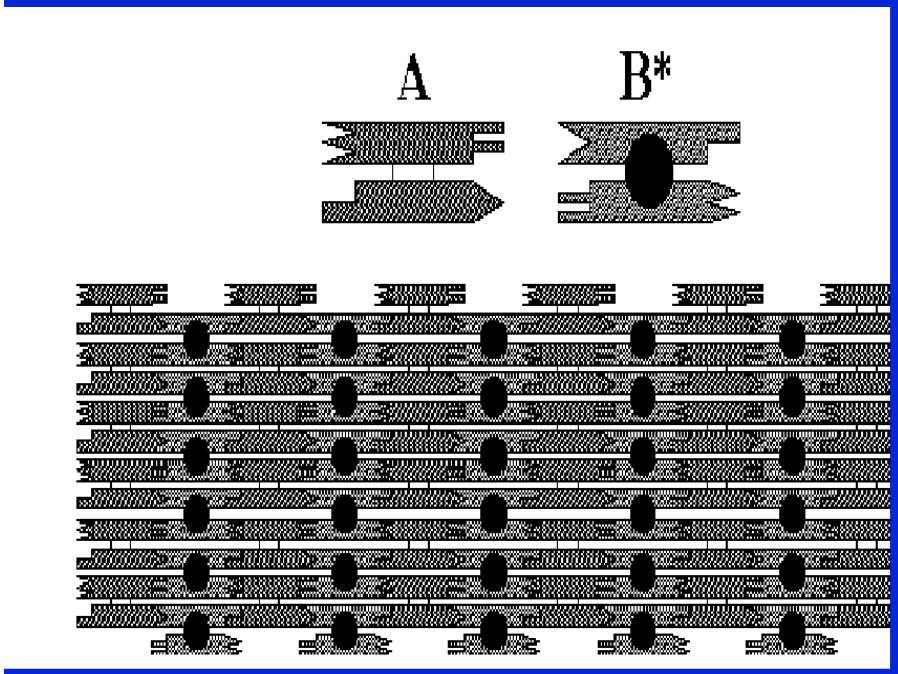




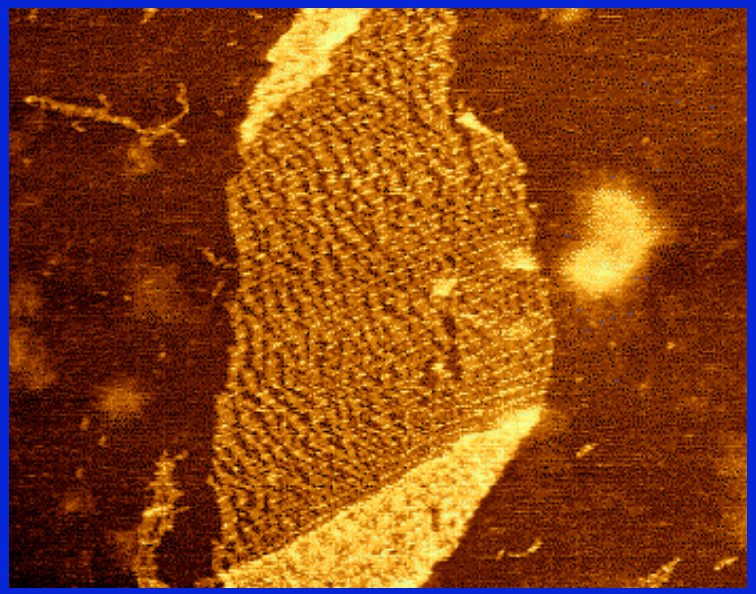



# 2D DNA Self-Assembled Tilings: Rendering Simple Banded Images

B* Tiles with Loops




Atomic Force Microscope Image
Bands Generated by B* Tiles with Attached Beads



# Technology Used For Imaging Molecules

- **AFM: Atomic Force Microscope**
- **SEM: Scanning Electron Microscope**
- **TEM: Transmission Electron Microscope**

## New Technology for Output of the 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 Lattices:

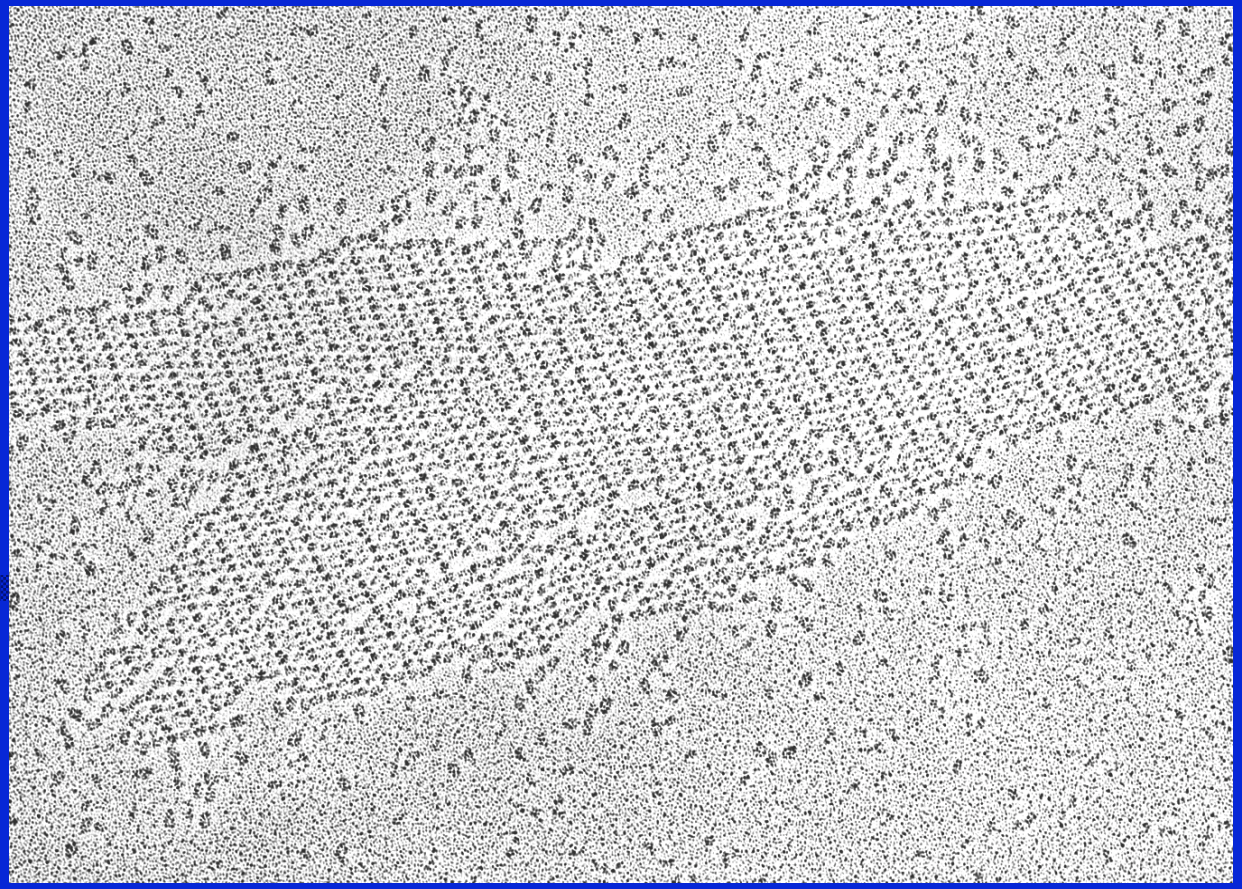
- Tiles (DNA nanostructures) self-assemble around each segment of a DNA strand encoding an image pixel.

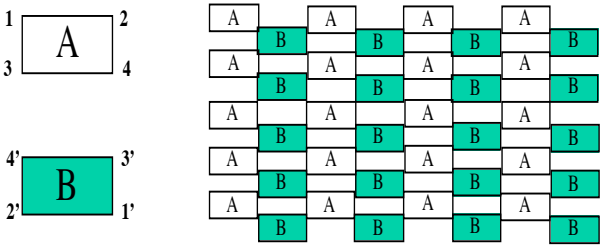
- Each tile has a surface perturbation depending on pixel intensity.

- The tiles then self-assemble into a 2D tiling lattice.

- Scalable to extremely large patterns
- - not diffraction limited
- - by an Atomic Force Microscope
- Example Application:
  - 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 cm on a side.

# TEM Image of TAO AB* Lattice



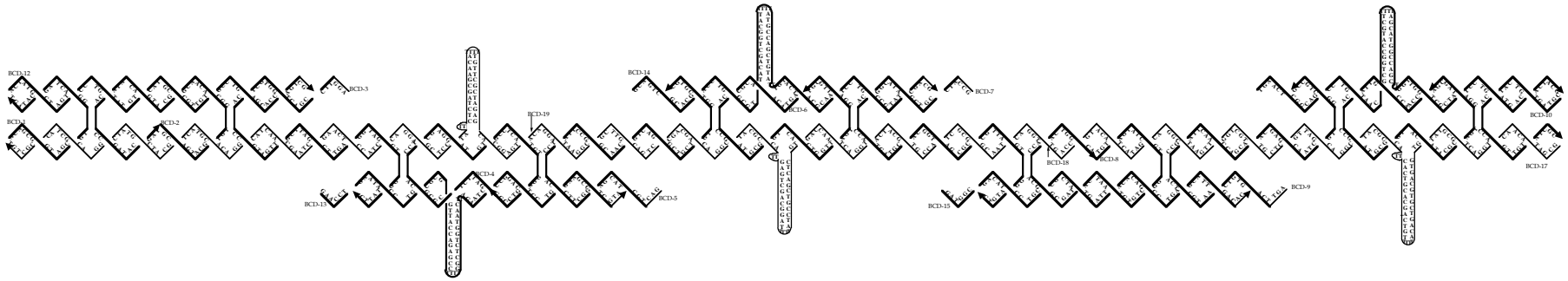



**Cartoon of DNA lattice composed of two types of TAO tile: B with (dark) and A without (light) stem-loops directed out of the lattice plane.**

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

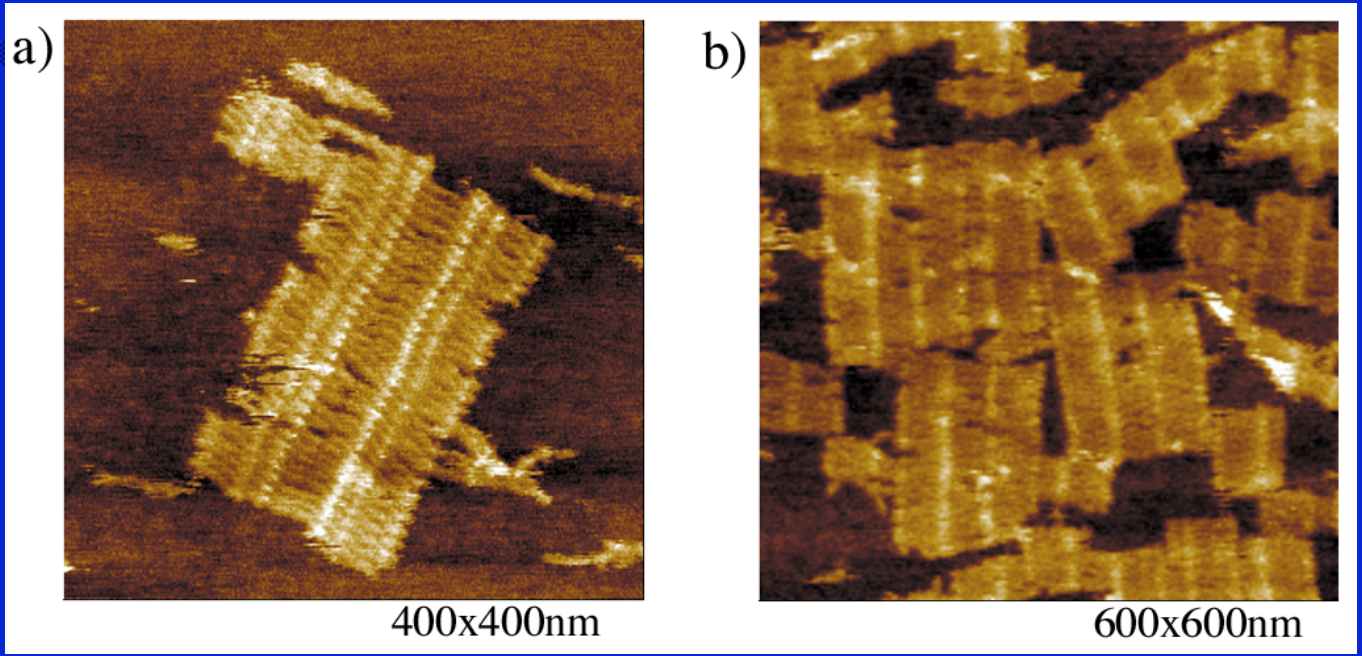
- **A tiles (lighter) only associate with B tiles (darker) and vice versa.**
- **B tiles appear darker due to increased platinum deposition on an extra loop of DNA directed out of the lattice plane.**

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


BCD-12
BCD-1
BCD-2
BCD-3
BCD-13
BCD-4
BCD-19
BCD-5
BCD-14
BCD-6
BCD-7
BCD-15
BCD-18
BCD-8
BCD-9
BCD-16
BCD-17

# Directed Nucleation Assembly of Barcode Patterned DNA Lattices

Hao Yan, Thomas H. LaBean, Liping Feng, John H. Reif*




- **Aperiodic patterned DNA lattice (Barcode Lattice) by directed nucleation self-assembly of DNA tiles around a scaffold DNA strand.**
- **A first step toward implementation of a visual readout system capable of converting information encoded on a 1-D DNA strand into a 2-D form readable by advanced microscopic techniques.**

- **Three major strategies for formation of patterned DNA tiling lattice self-assemblies:**

  - ***Unmediated Algorithmic Self-assembly***

  - ***Sequential Step-wise Assembly Techniques***

  - ***Directed Nucleation Assembly Techniques***

# Directed Nucleation Assembly:

A method for assembly of complex patterns

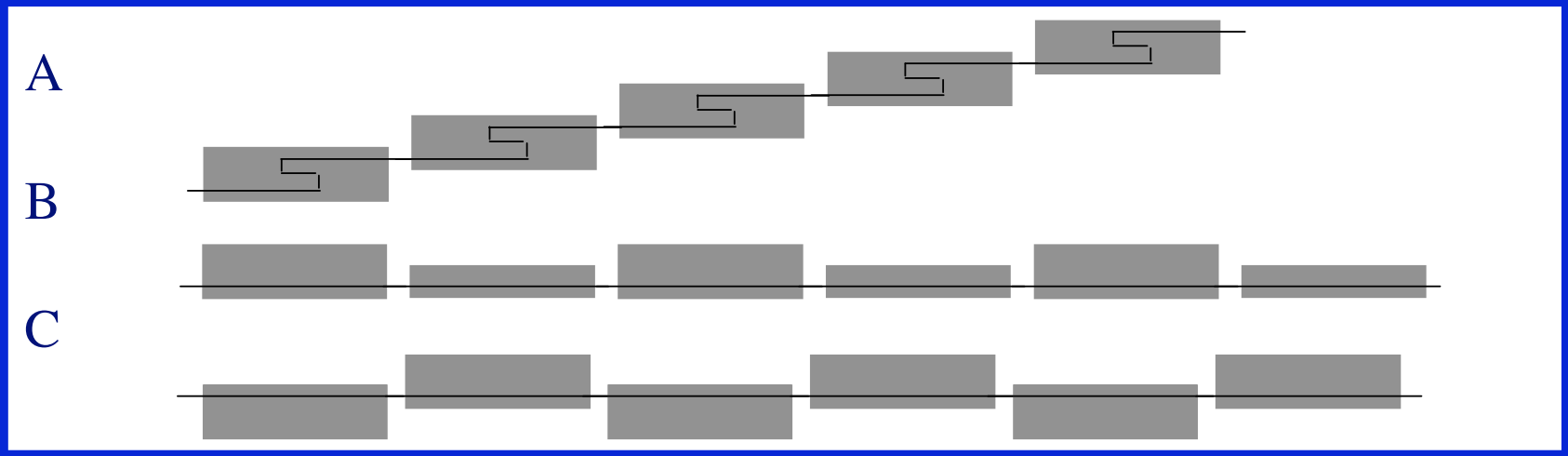
- Use artificially synthesized DNA strands that specify the pattern and around which 2D DNA tiles assemble into the specified pattern.
- The permanent features of the 2D pattern are generated uniquely for each case.

# 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:

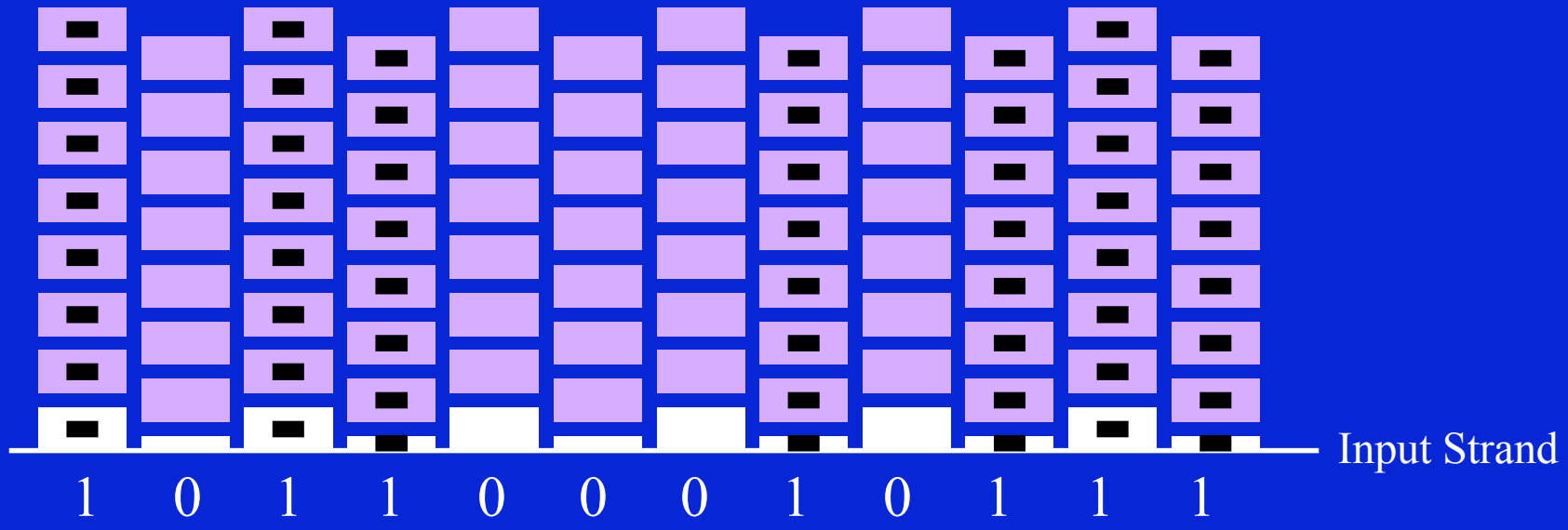
- Multiple tiles of an input layer can be assembled around a single, long DNA strand we refer to as a scaffold strand (shown as black lines in the figures).




- 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.

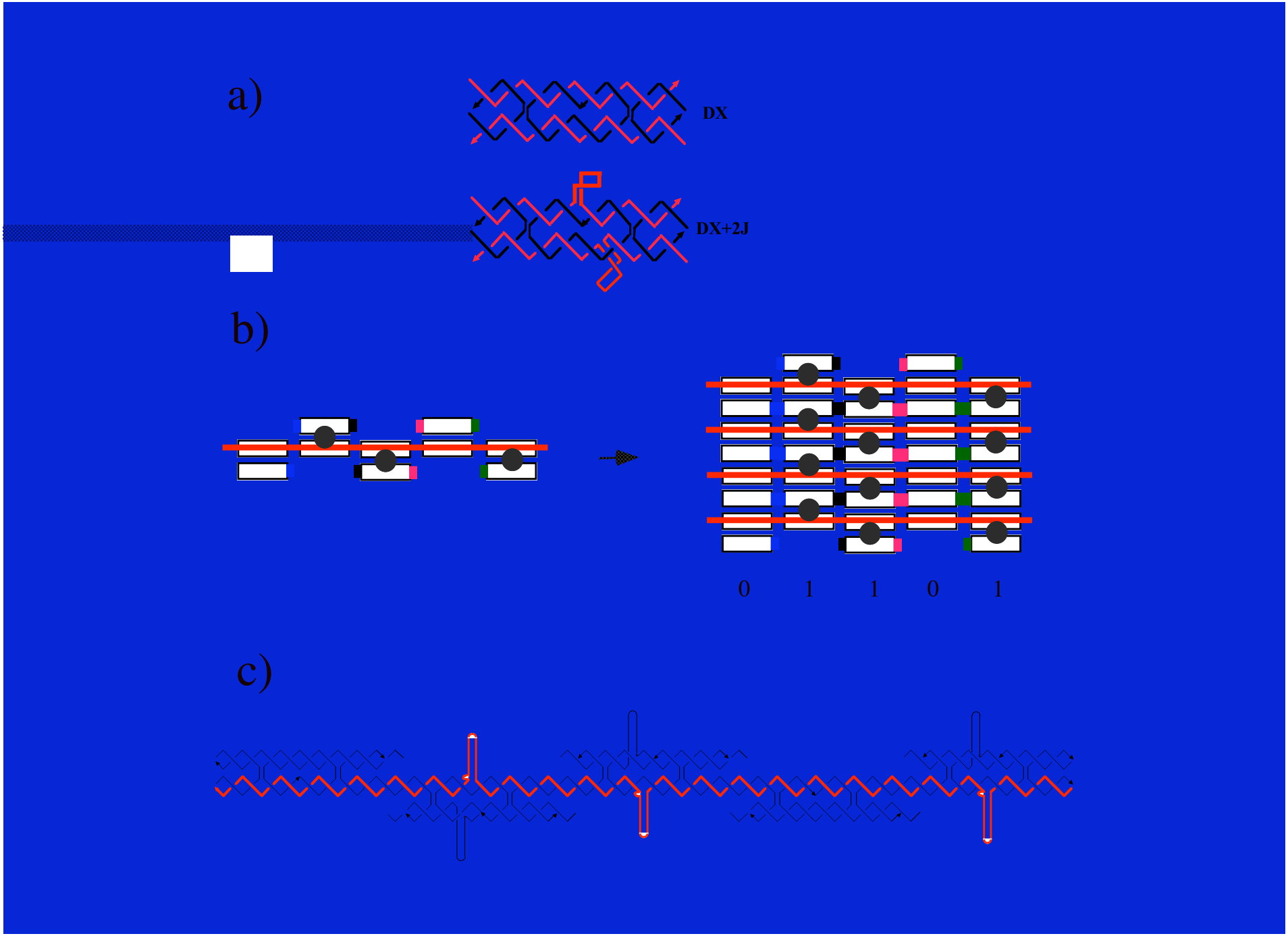
# Directed Nucleation Technique for 1 D Patterns: Barcode Lattice for Readout




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

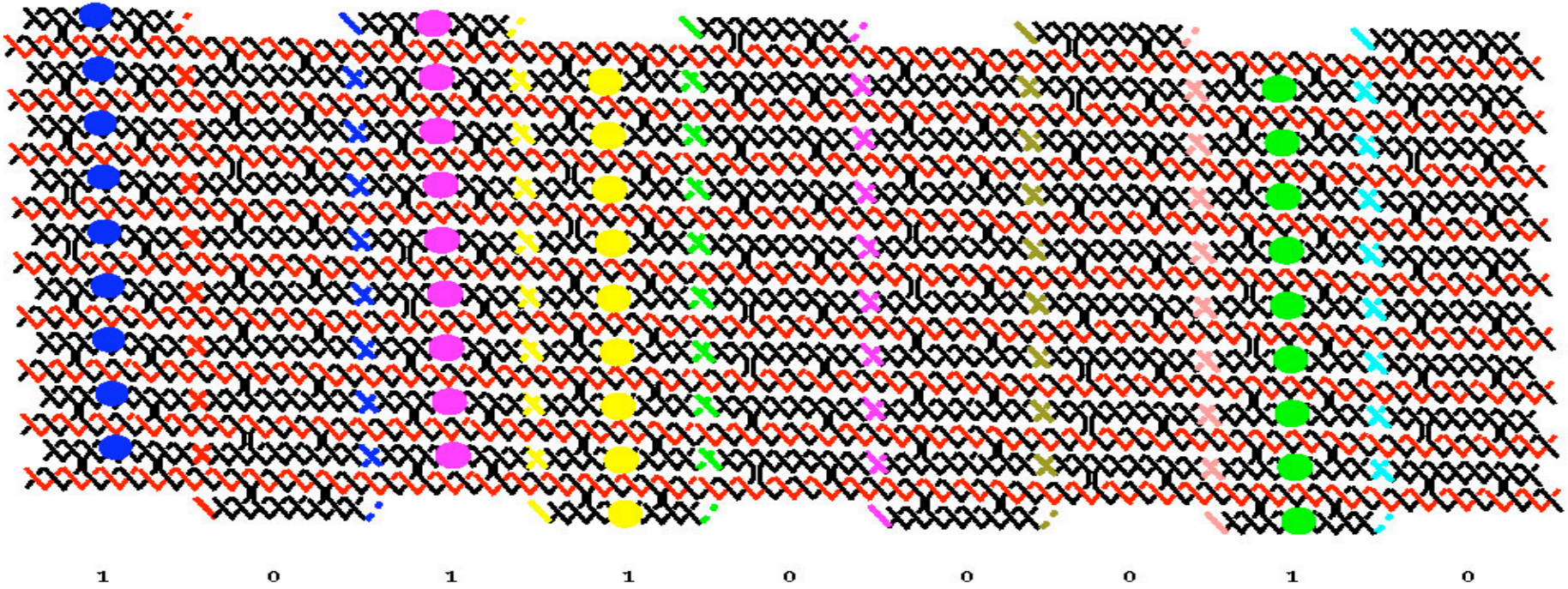
**Extends 2D arrays into simple aperiodic patterning:**

- **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.**


a)
DX
DX+2J
b)
0 1 1 0 1
c)

# Barcode Lattice for Rendering 1 D Patterns:

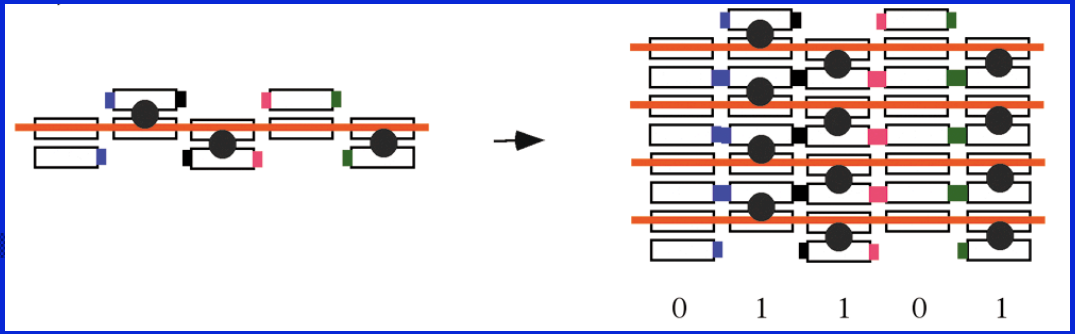
Unit (9 tiles, filled dot i.e. loop represent 1, 8 pairs of asymmetric stickyends represented by colored lines, The long red strand are the input)

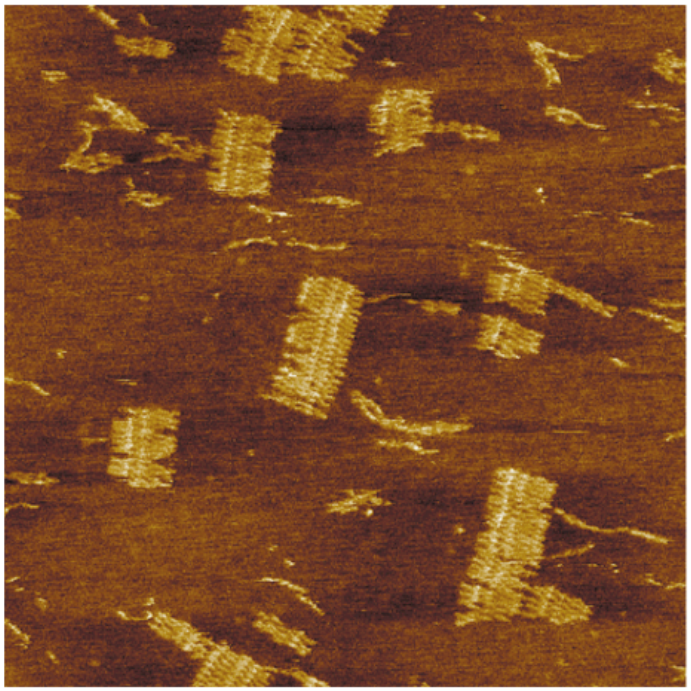



A typical 9X9 periodic array assembled from the unit, there will not be frameshift because the different pairs of stickyend

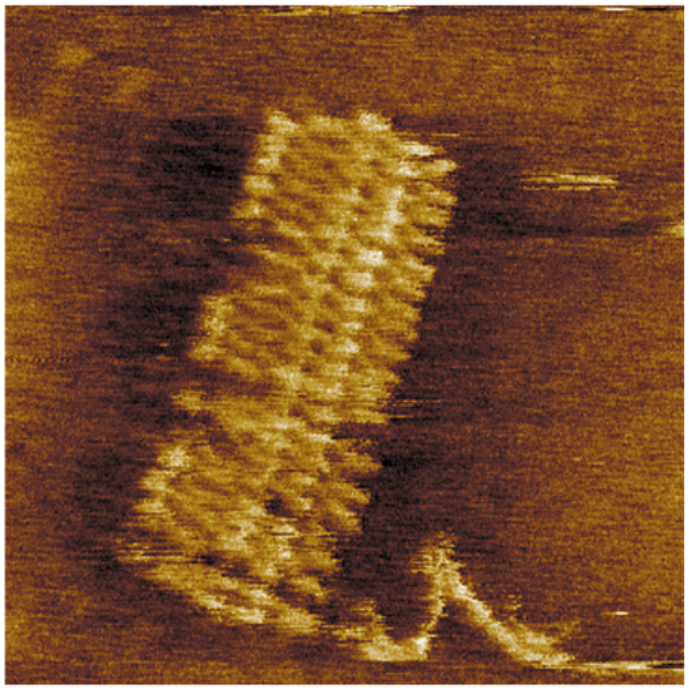
**Barcode lattice displays banding patterns dictated by the same sequence of bit values programmed on each layer.**

01101



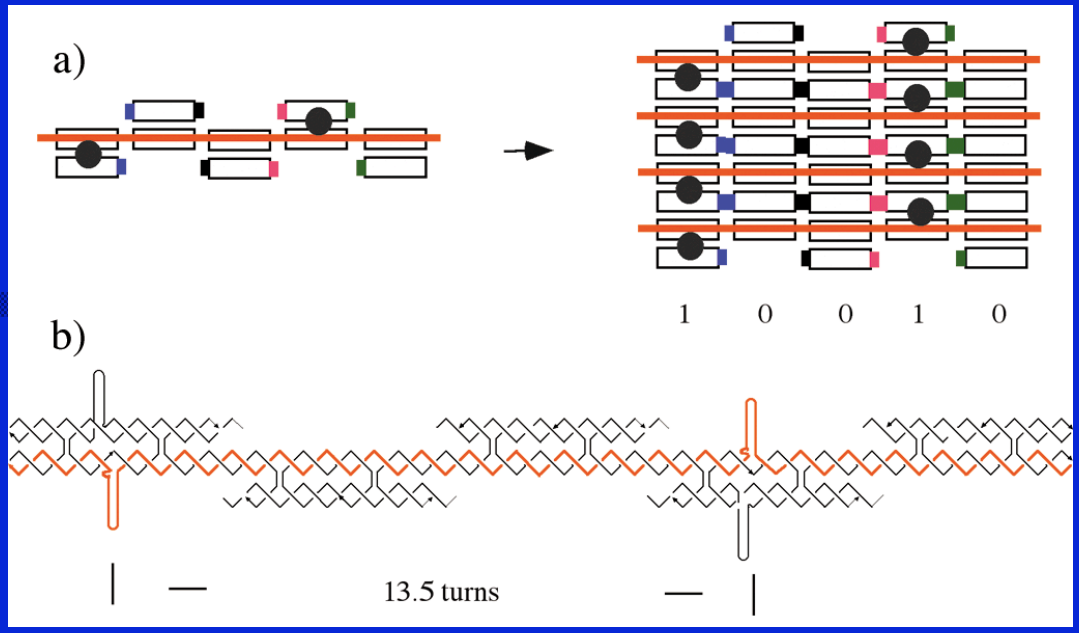



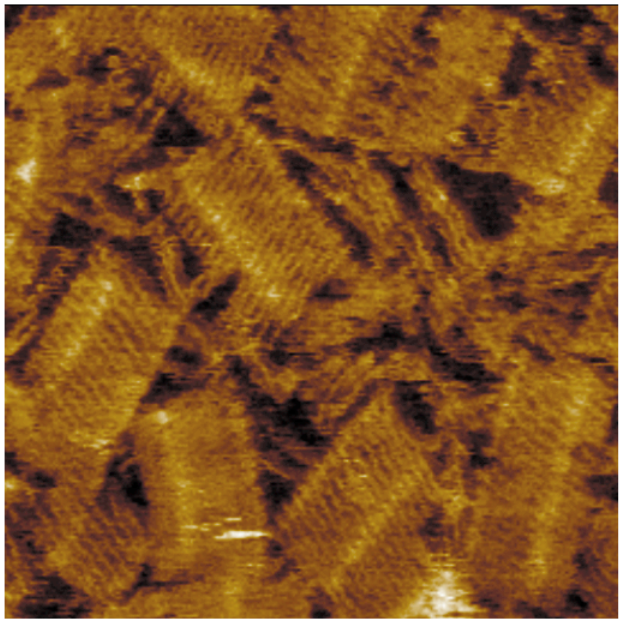
800x800nm



250x250nm

10010

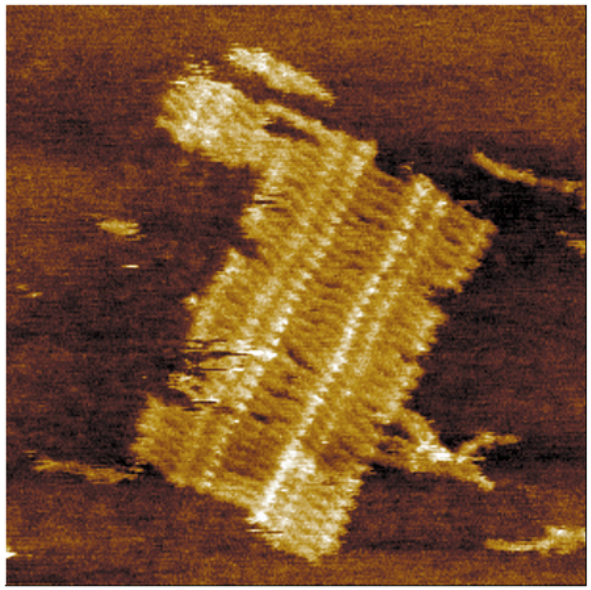


400x400nm

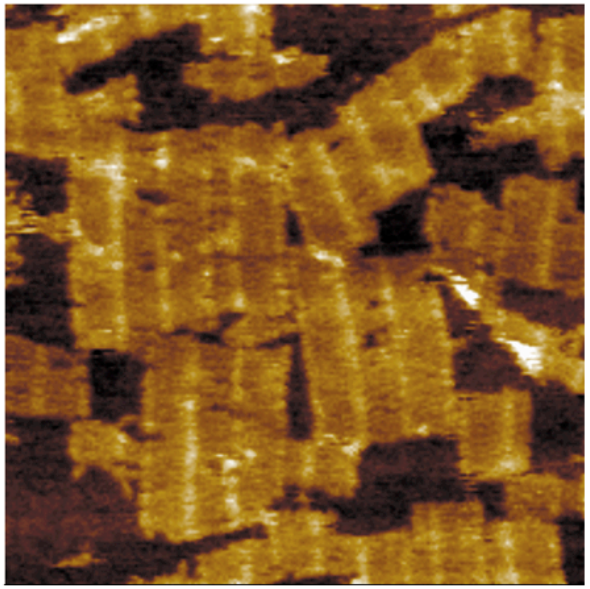
# Blunt-end Helix Stacking Effect in DNA Self-assembly

a)



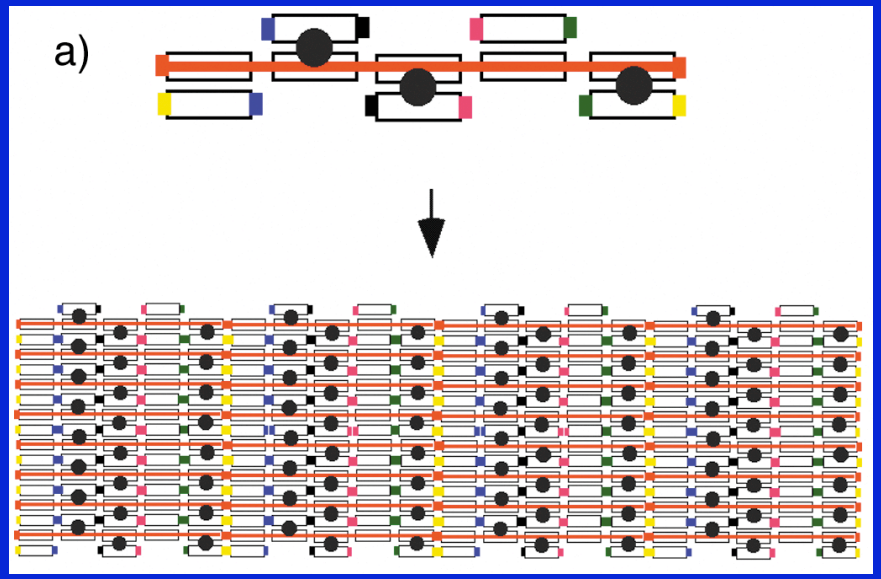
400x400nm

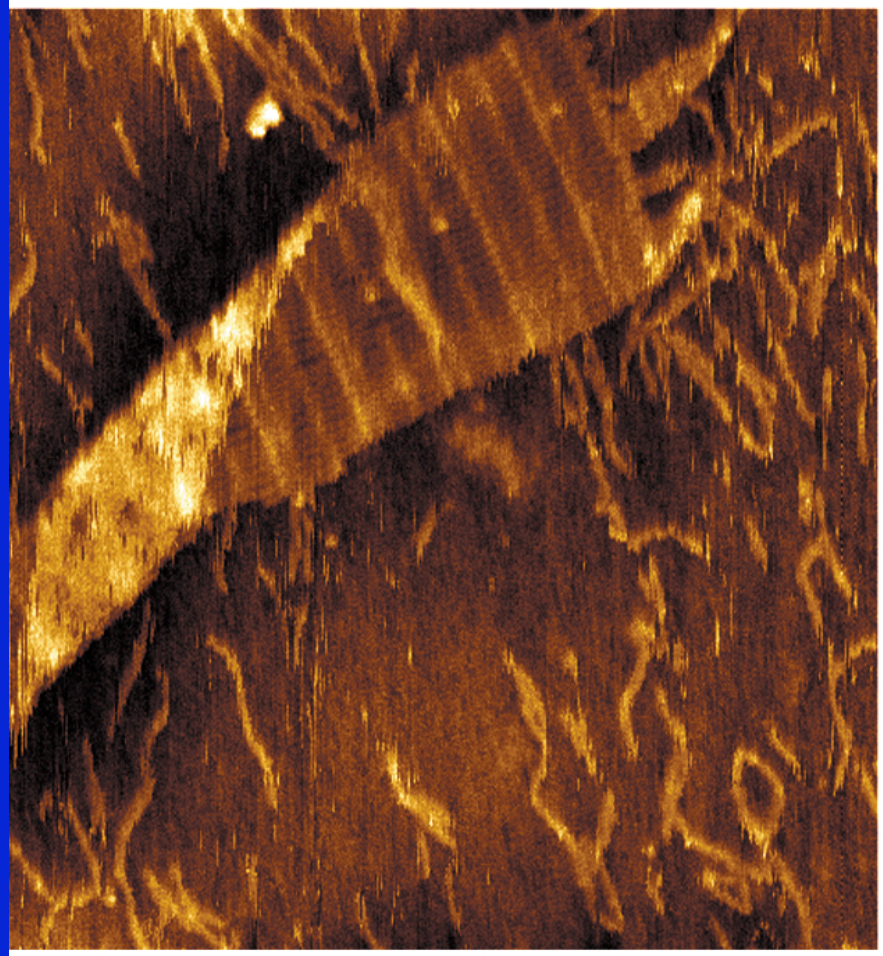
b)



600x600nm

# Self-assembly of a Ribbon Lattice from Repeating DNA Barcode Units



750x750nm

## 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:

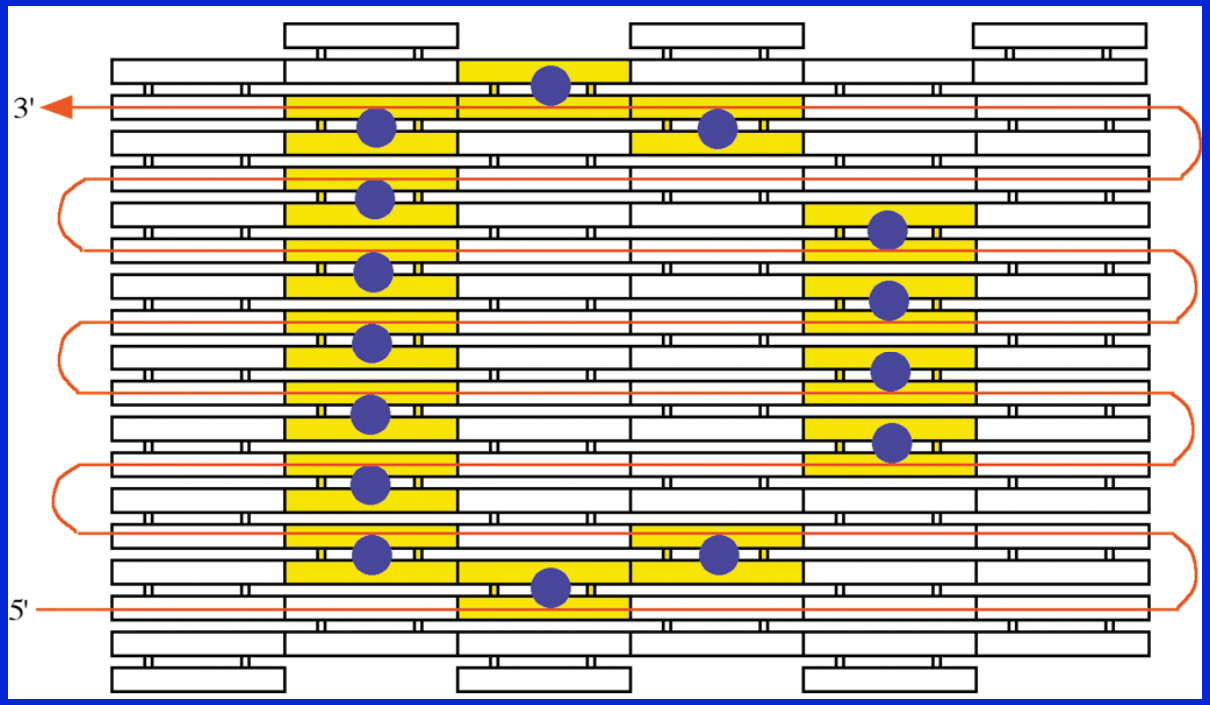
**- Molecular Scale Patterning of Molecular Electronics and Molecular Motors.**

- 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.

# Future Work:

- Apply directed nucleation technique to DNA computing.

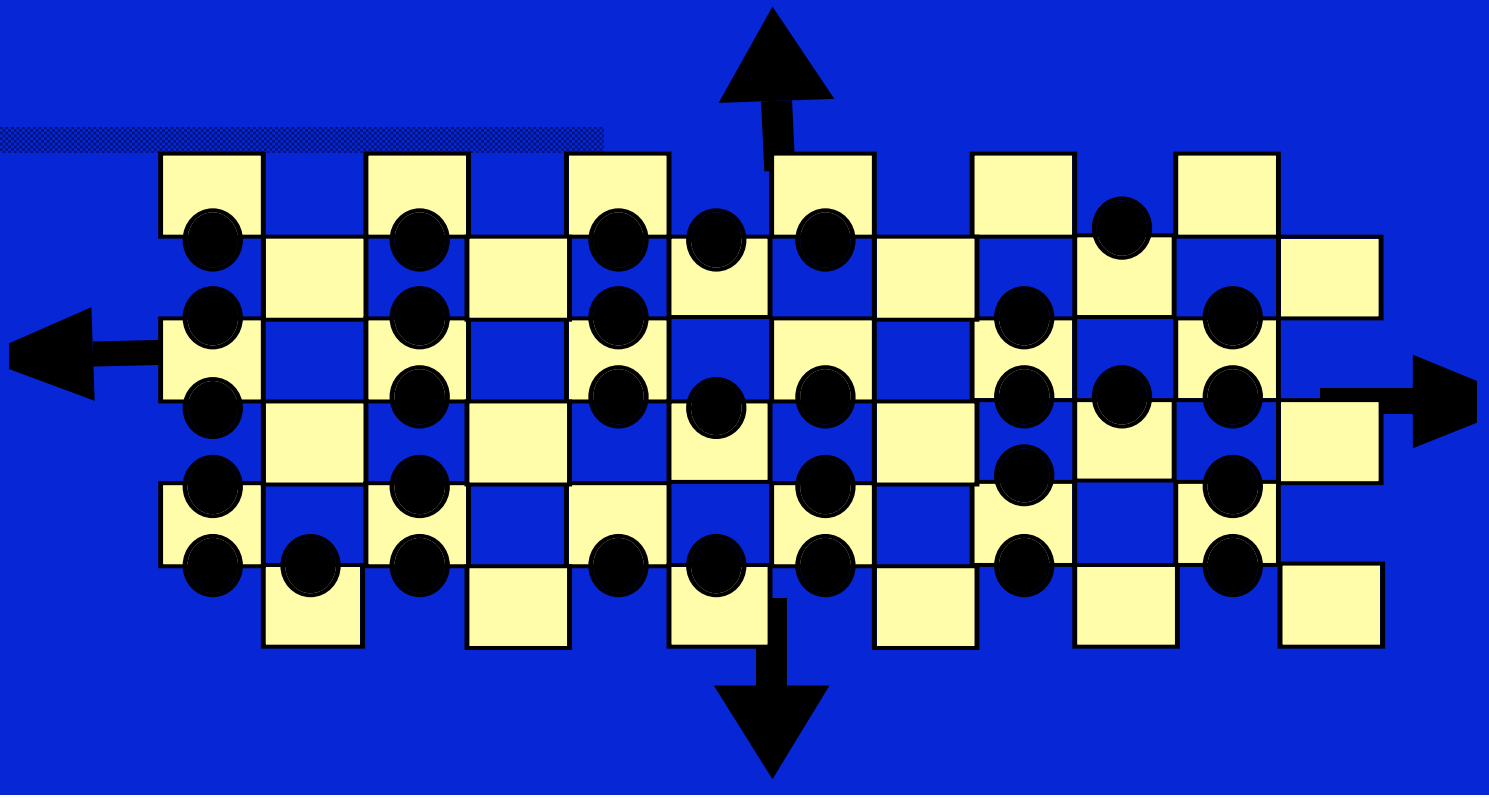
- 2D patterns might also be encoded in the same way using a scaffold strand.



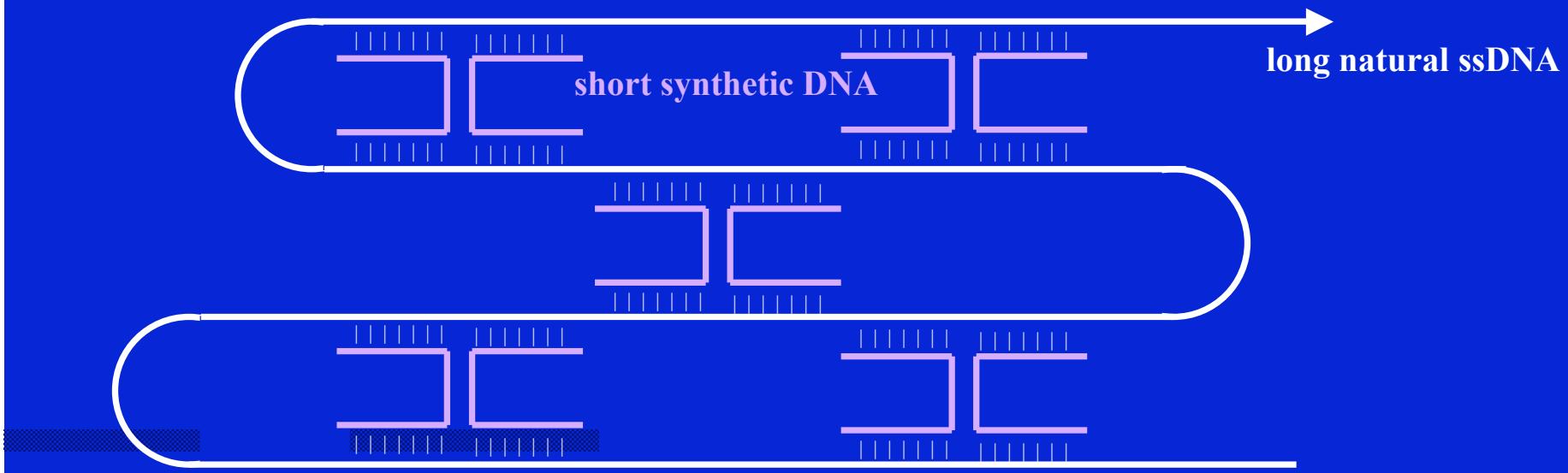
# Planned Demonstration of use of Patterned DNA tiling

## Molecular Nanofabracation of repeated "USA" 2D lattice.

- Repeated unit of T= 3 x 12 = 60 distinct DX tiles
- Each tile has 120 + p 20 Base pairs, where p is number (0, 1, or 2) of stem loops(pixels).
- Number of pixels P = 32. (Can have another pattern on other side)
- Number of DNA Base Pairs = 120 T+ P20 = 7200+640 = 7840.



# Molecular Fabric by Crossover Assembly within DNA from Biological Sources




**Alternative Technique for Molecular Pattern Formation:**

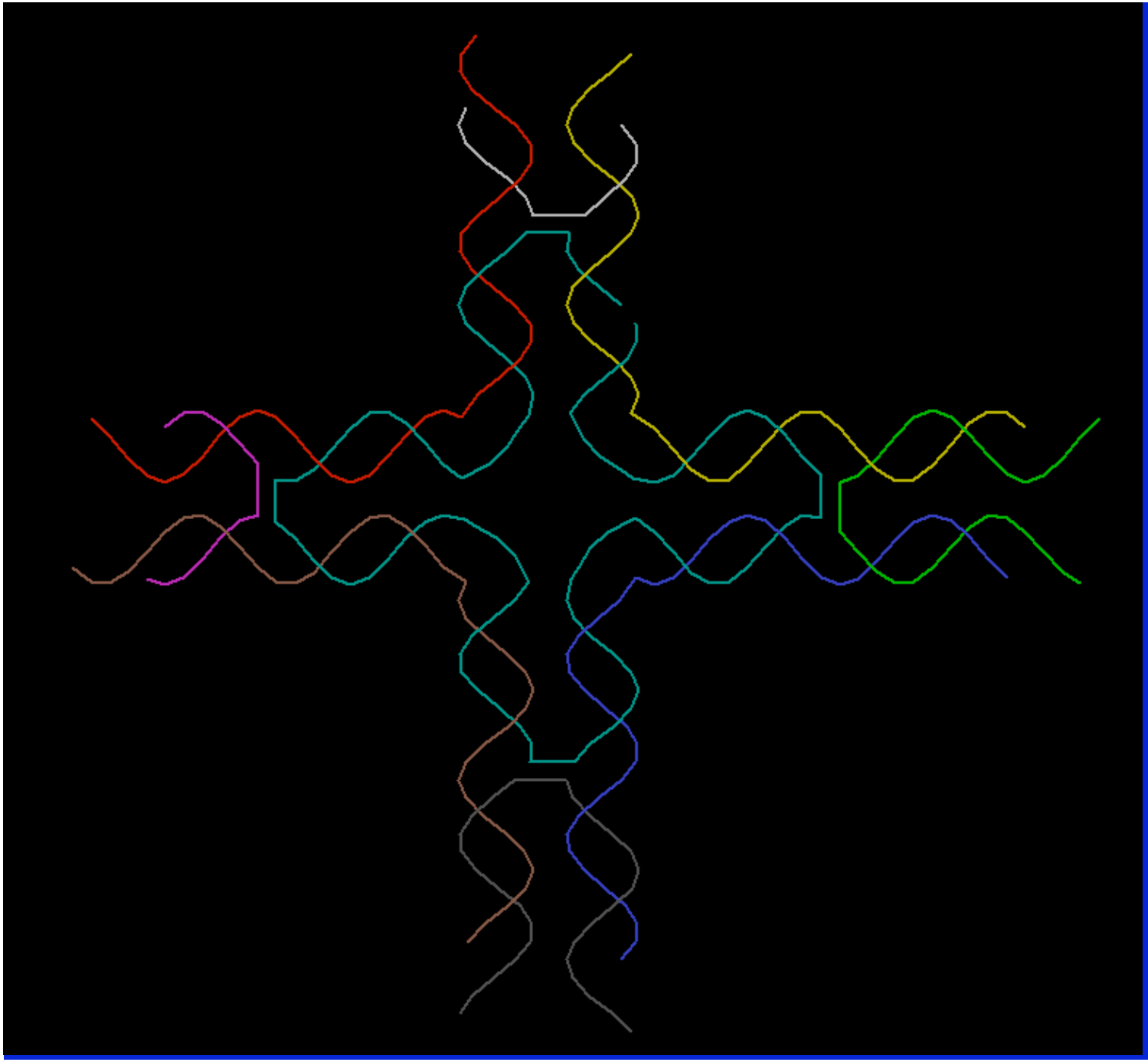
**Single strand DNA acquired from a biological source is annealed with custom synthetic oligonucleotides specifically designed to basepair with the long natural DNA strand such that a 2D lattice is formed.**

# 4x4 DNA Tile and Lattices: Design, Self-Assembly Characterization and Metallization of a Novel DNA Nanostructure Motif

Hao Yan, Sung Ha Park, Liping Feng, John Reif, Thomas H. LaBean*

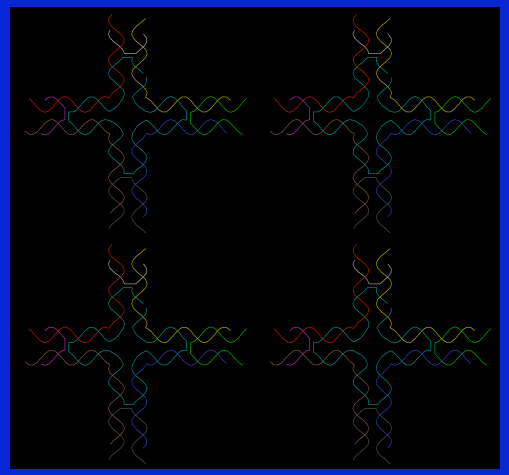
# Design and Self-assembly of Four-way DNA Triple Crossover Molecule

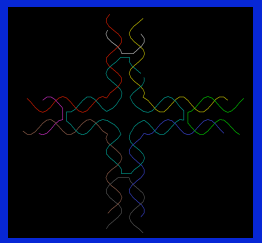
Liping Feng, Hanying Li, Thomas LaBean, John Reif, Hao Yan*

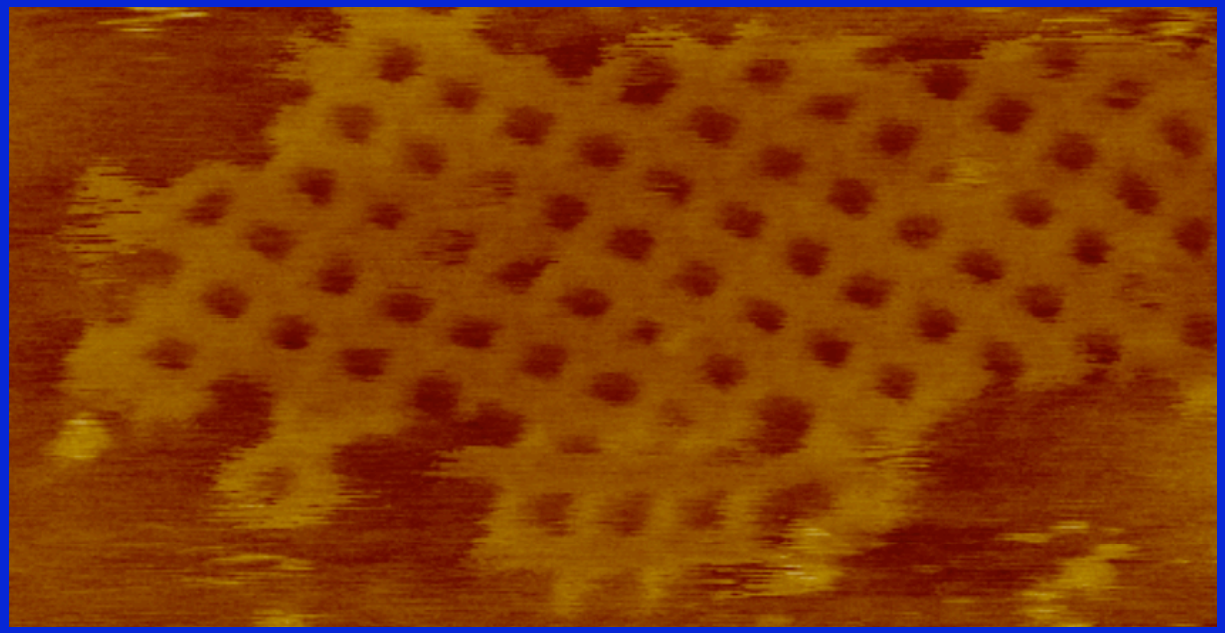


# 4 x 4 Tile

# A Lattice of 4 x 4 Tiles

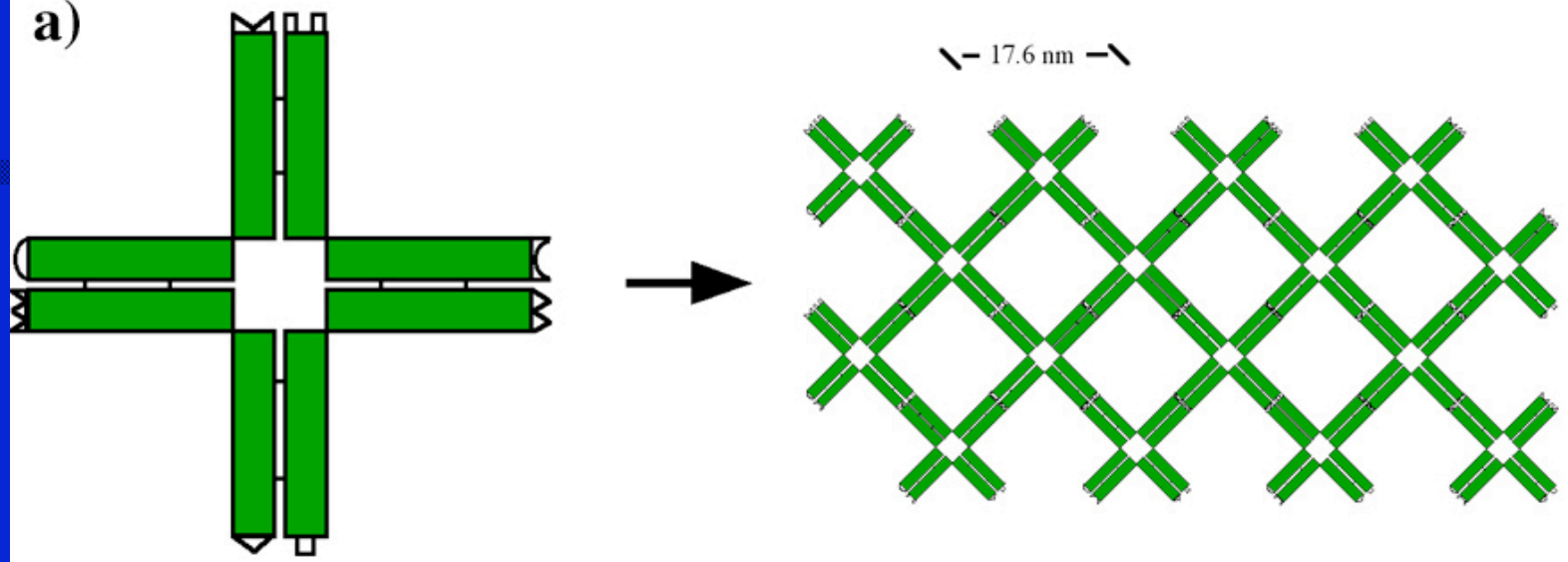




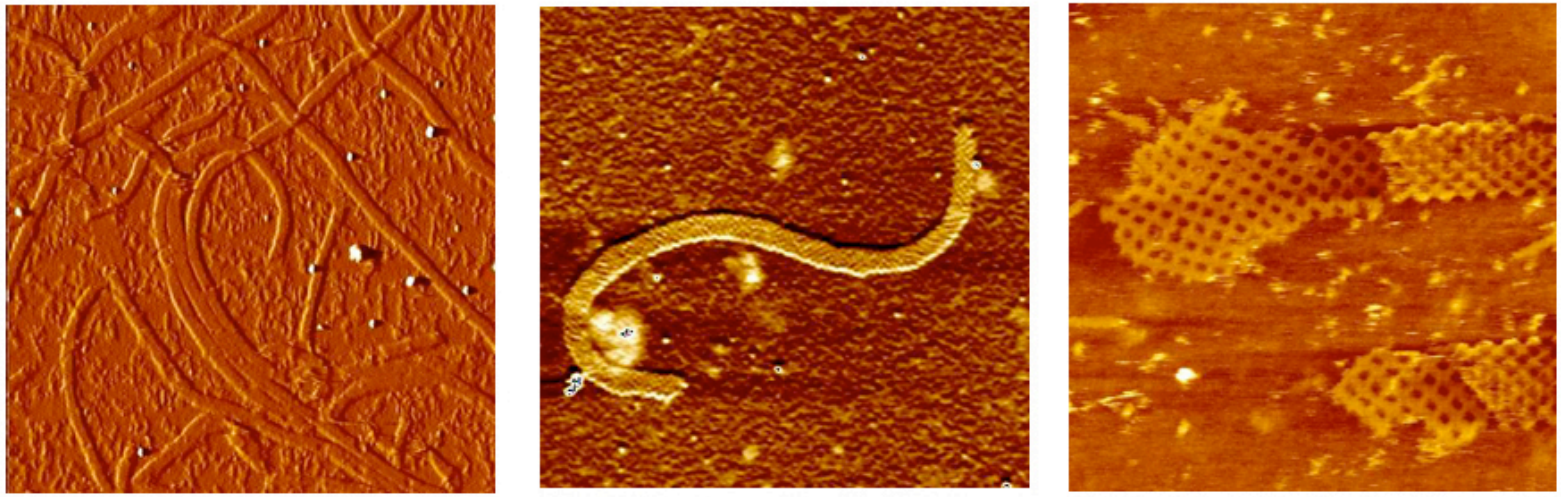


# Uncorrugated 4 x 4 tile

a)




b)

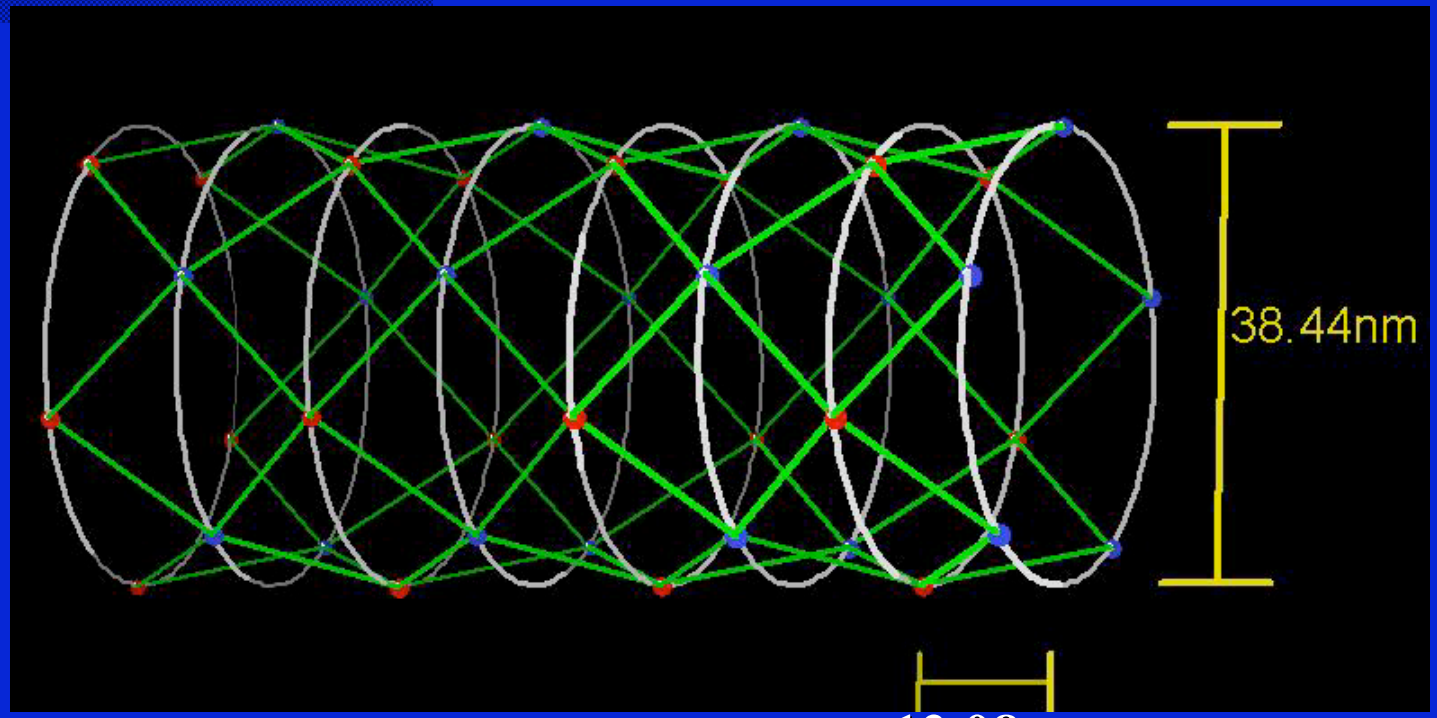


3x3 um

1x1um

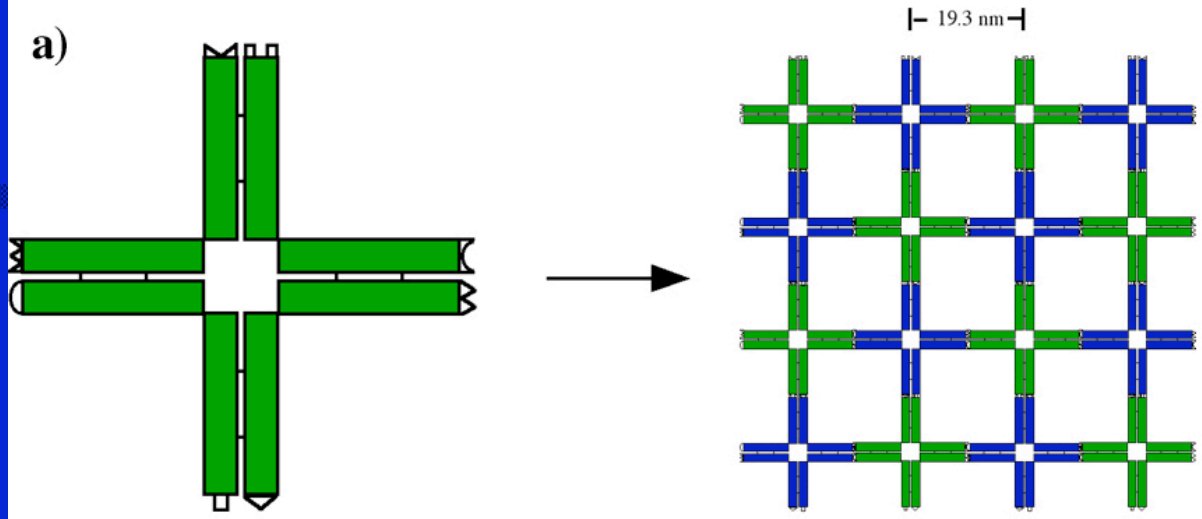
500x500 nm

# Tubes Self-Assembled from Uncorrugated 4 x 4 tile

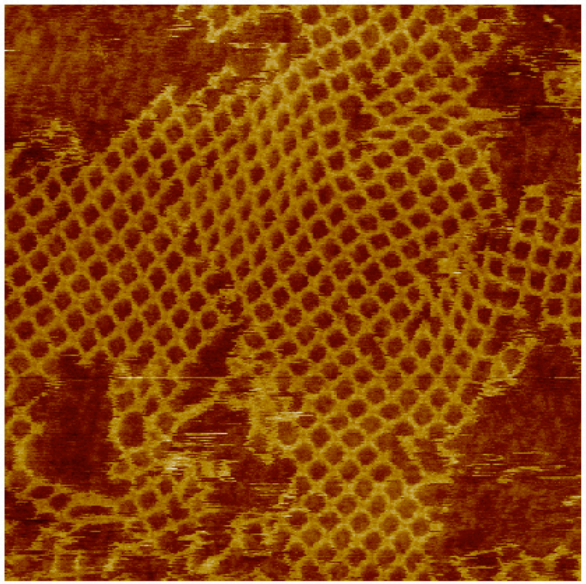


# Corrugated 4 x 4 tile

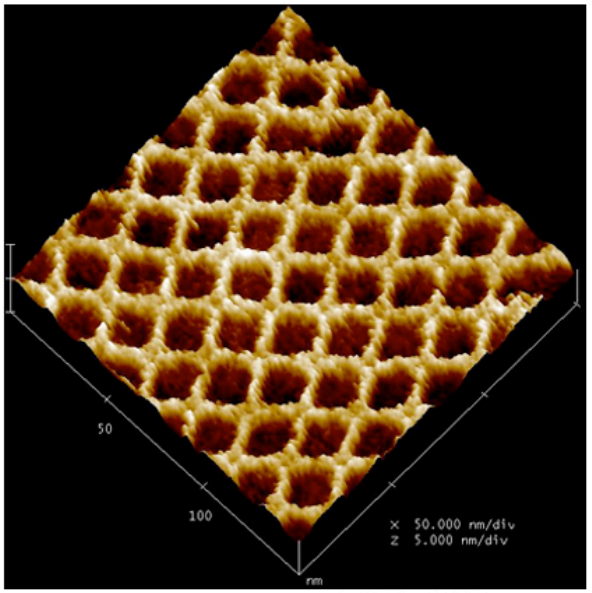
a)



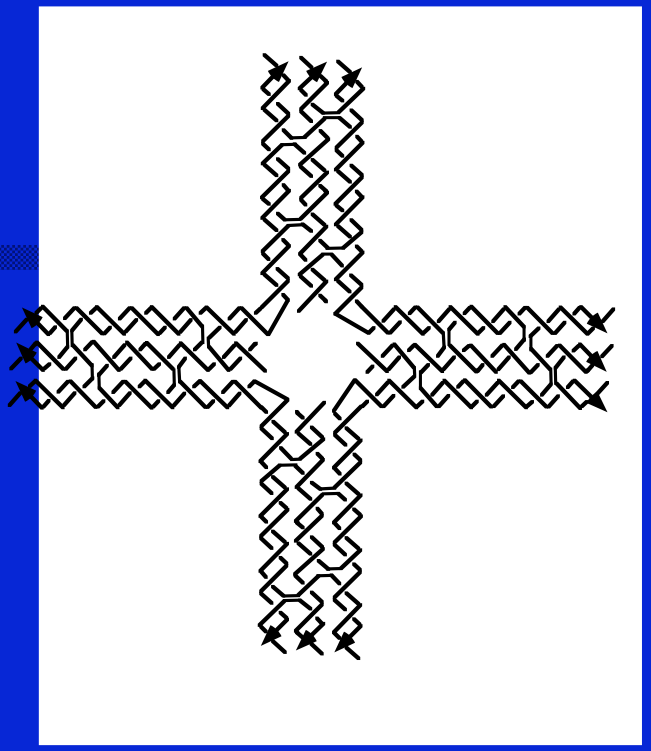

b)



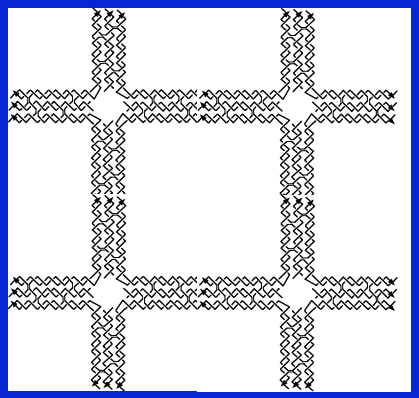
500x500nm

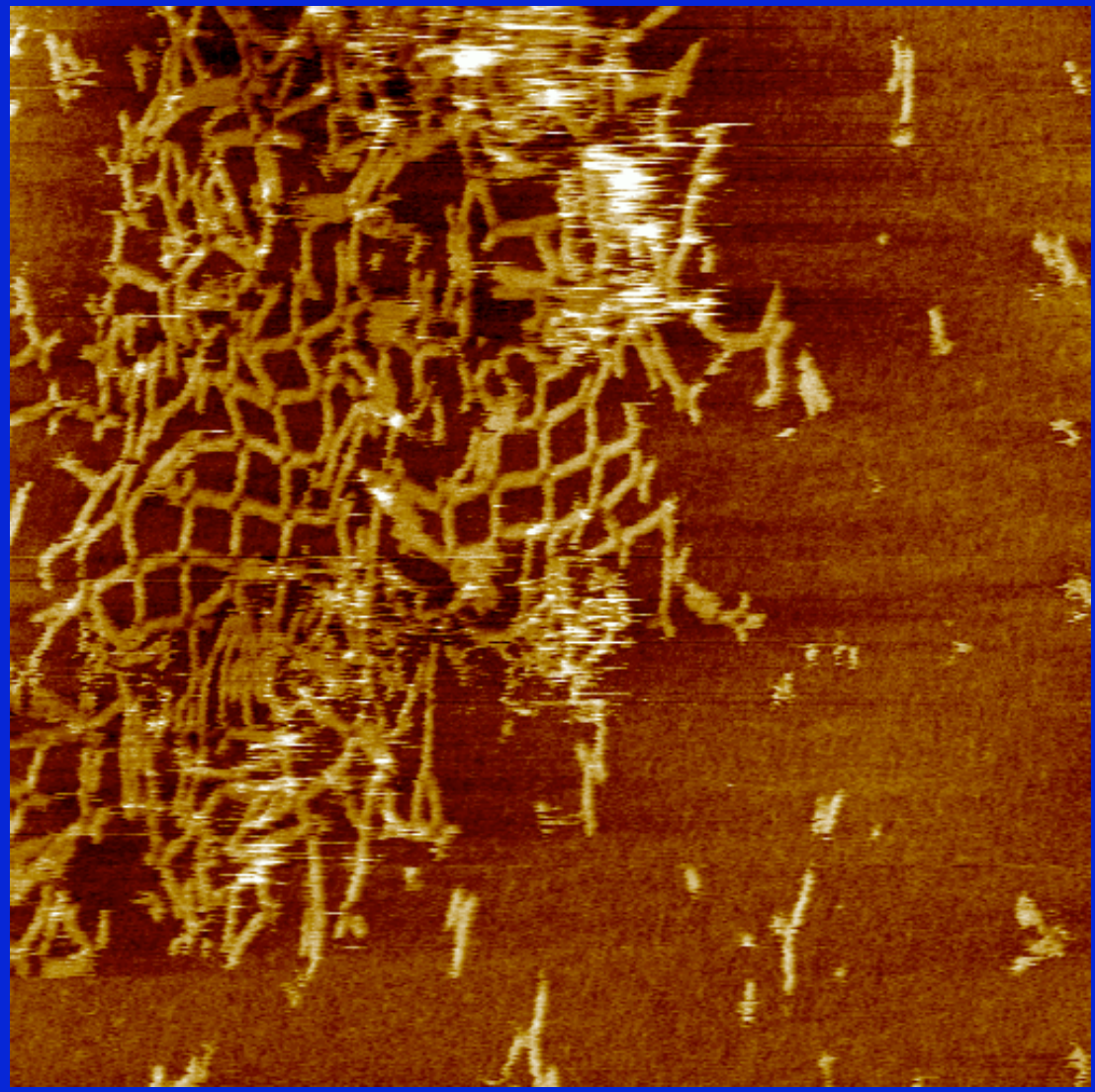



150x150nm



|– 38nm –|





800x800nm

# TX x 4 tile Assemblies

# DNA Attachment Chemistry: Linking various types of molecules to DNA lattices.

- DNA could serve as a selective assembly glue between DNA and device configurations.

- Applications:
  - Molecular Electronics:
    - Links to organic molecular electronic compounds [WST+00], [Tour and Bunz, to appear]
      - via organic synthesis methods.
    - Links to gold balls and gold wires:
      - via DNA attachment chemistry.
  - Molecular Robotics:
    - Between DNA and Protein Motor Devices
    - via links from DNA to expressed proteins.
  - Molecular Carbon Nanoassembly:
    - Between DNA and Carbon Nanotubes
    - via end-linkers.

# Applications of DNA lattices as a substrate for:

- Molecular Electronics:
  - Layout of molecular electronic circuit components on DNA tiling arrays.
- DNA Chips:
  - ultra compact annealing arrays.
- X-ray Crystallography:
  - Capture proteins in regular 3D DNA arrays.
- Molecular Robotics:
  - Manipulation of molecules using molecular motor devices arranged on DNA tiling arrays.

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(2) DNA Chips:

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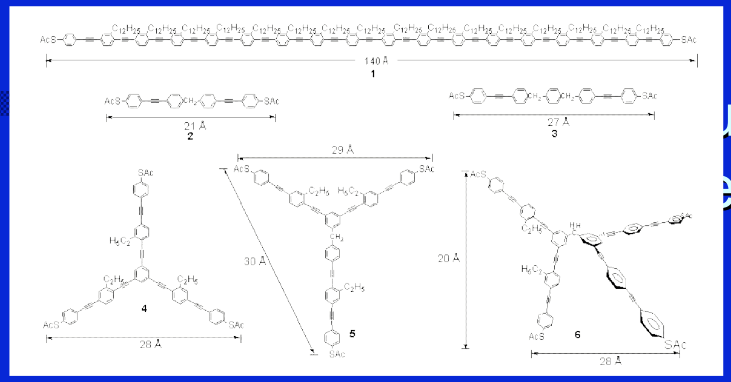
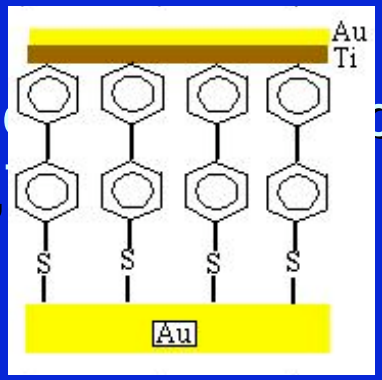
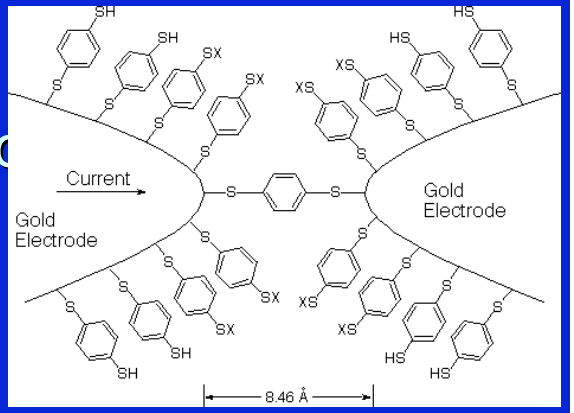
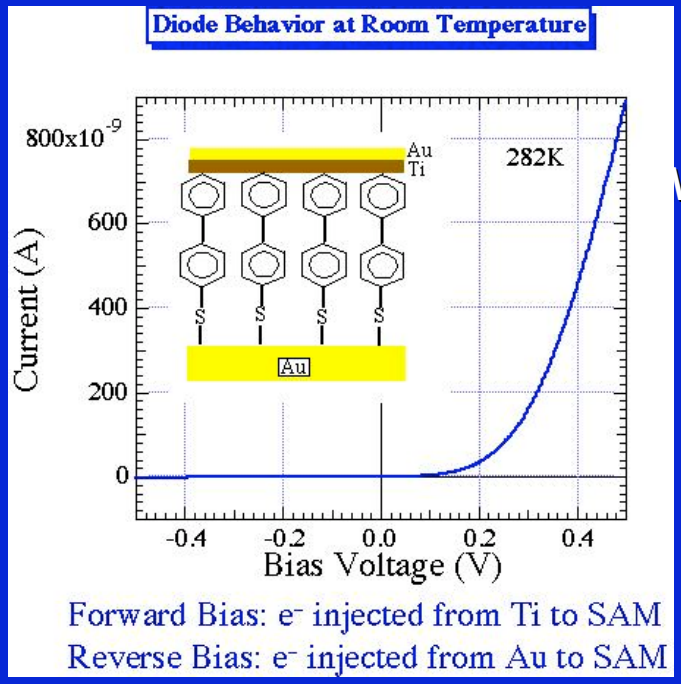
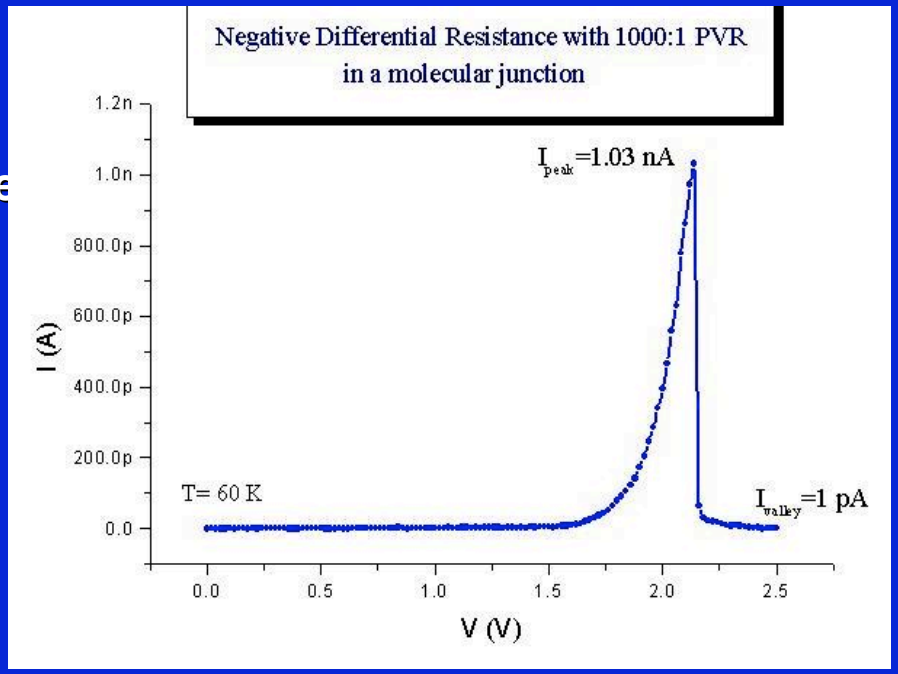



Au
Ti
Au

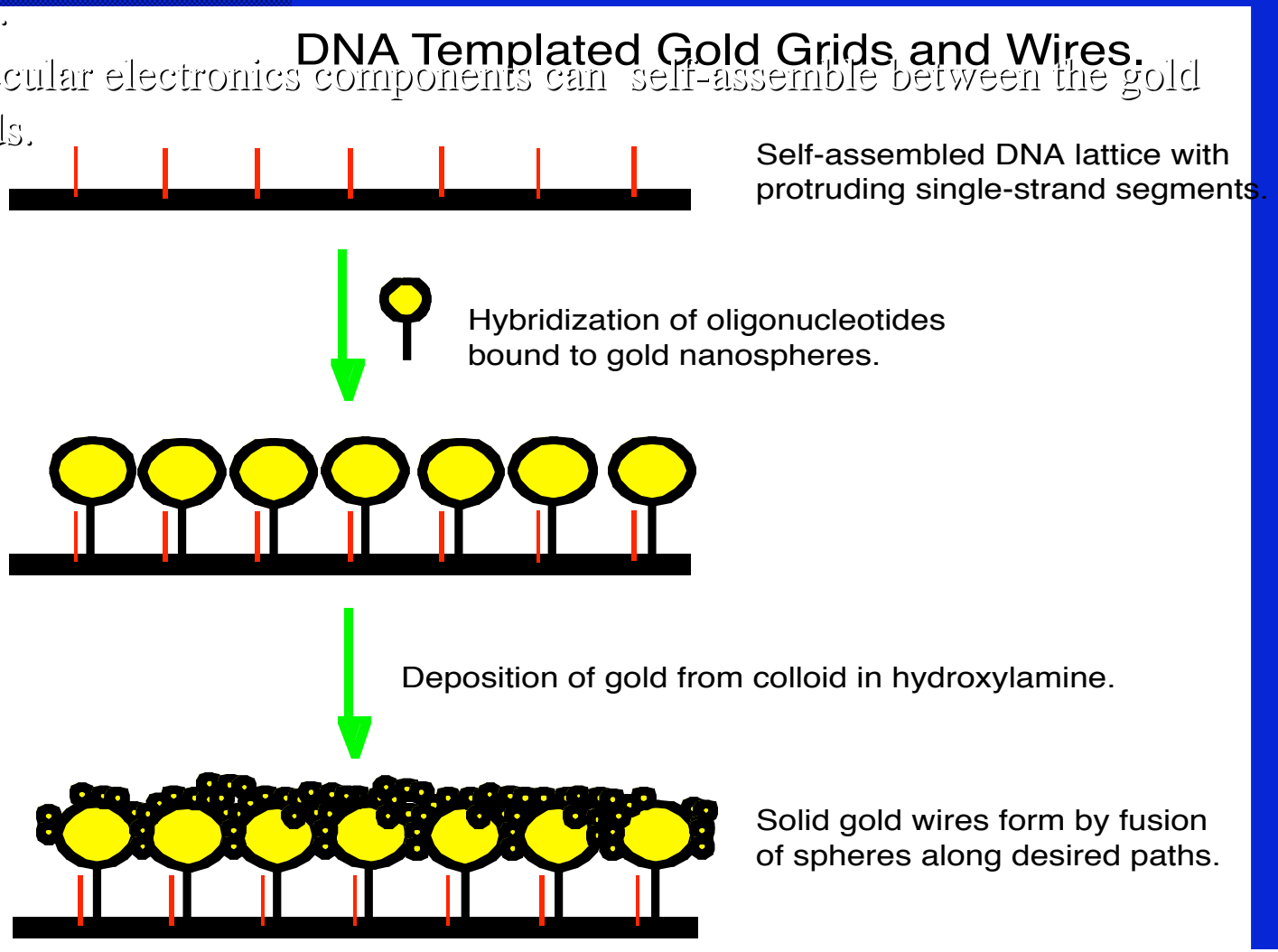

Current
Gold Electrode
Gold Electrode
8.46 Å


Diode Behavior at Room Temperature
800x10-9
600
400
200
0
Current (A)
Au
Ti
282K
Au
-0.4
-0.2
0.0
0.2
0.4
Bias Voltage (V)
Forward Bias: e- injected from Ti to SAM
Reverse Bias: e- injected from Au to SAM


Negative Differential Resistance with 1000:1 PVR
in a molecular junction
1.2n
1.0n
800.0p
600.0p
400.0p
200.0p
0.0
I (A)
Ipeak=1.03 nA
T= 60 K
Ivalley=1 pA
0.0
0.5
1.0
1.5
2.0
2.5
V (V)

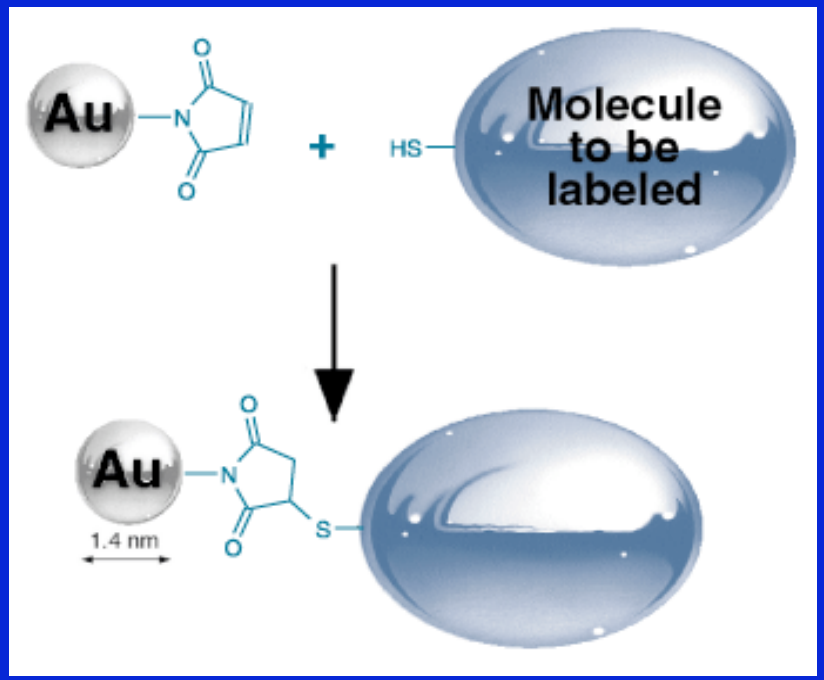
# Forming Gold Wires on DNA Tiling Lattices:

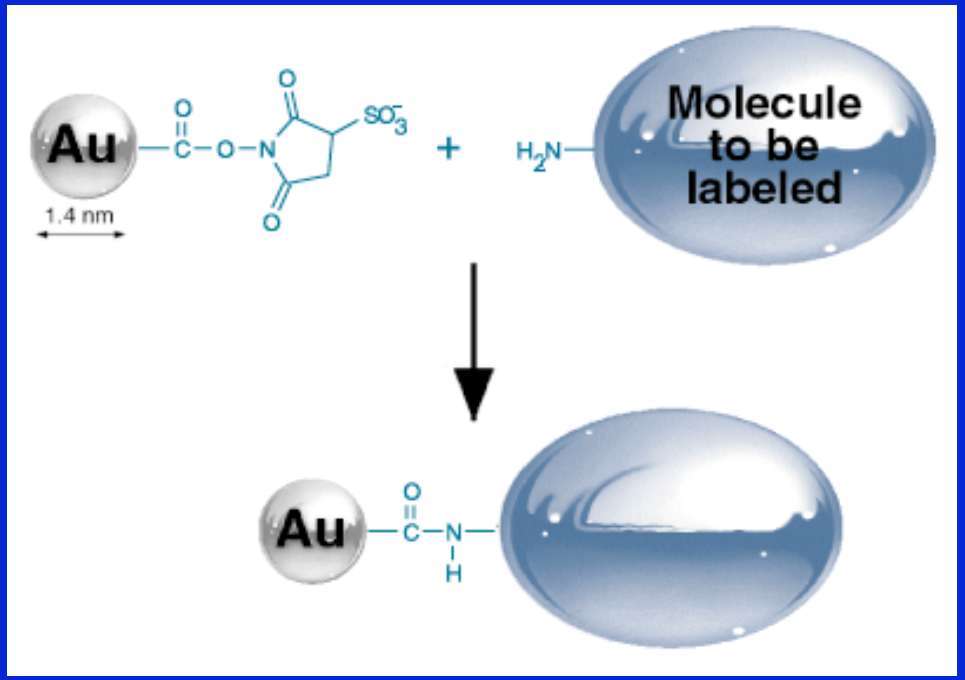
- DNA strands attached to gold beads hybridize at selected tiles of DNA array.
- Gold wires forms by fusion of free gold beads to beads attached to DNA array.
- Molecular electronics components can self-assemble between the gold breads.



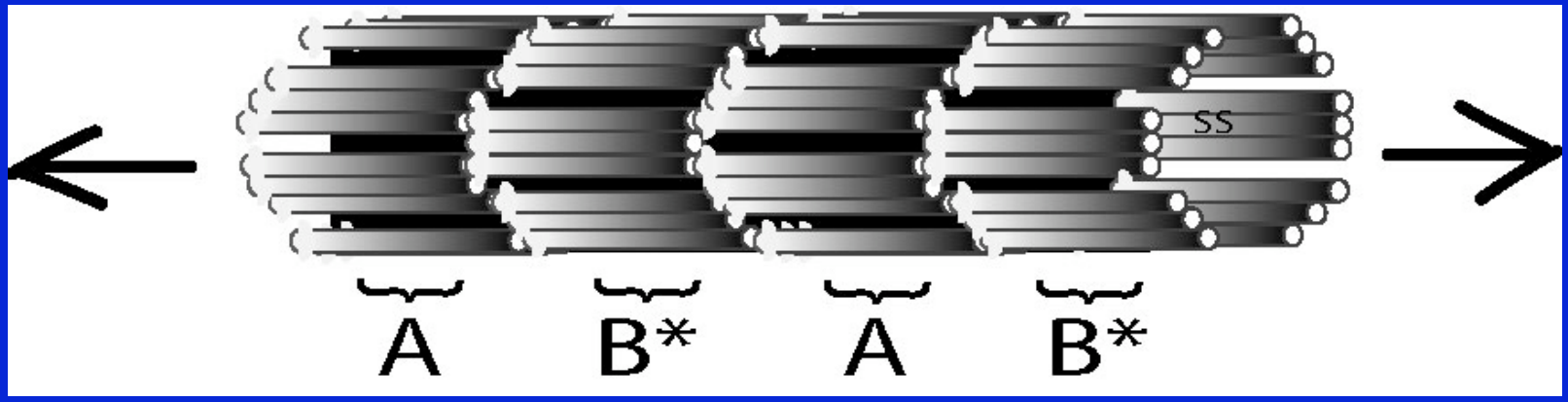
# Attachment Chemistries

- Monomaleimido nanogold + thiol (SH)
- Mono-NHS nanogold + amino ($NH_2$)
- Other (biotin/avidin, Au/S, etc.)



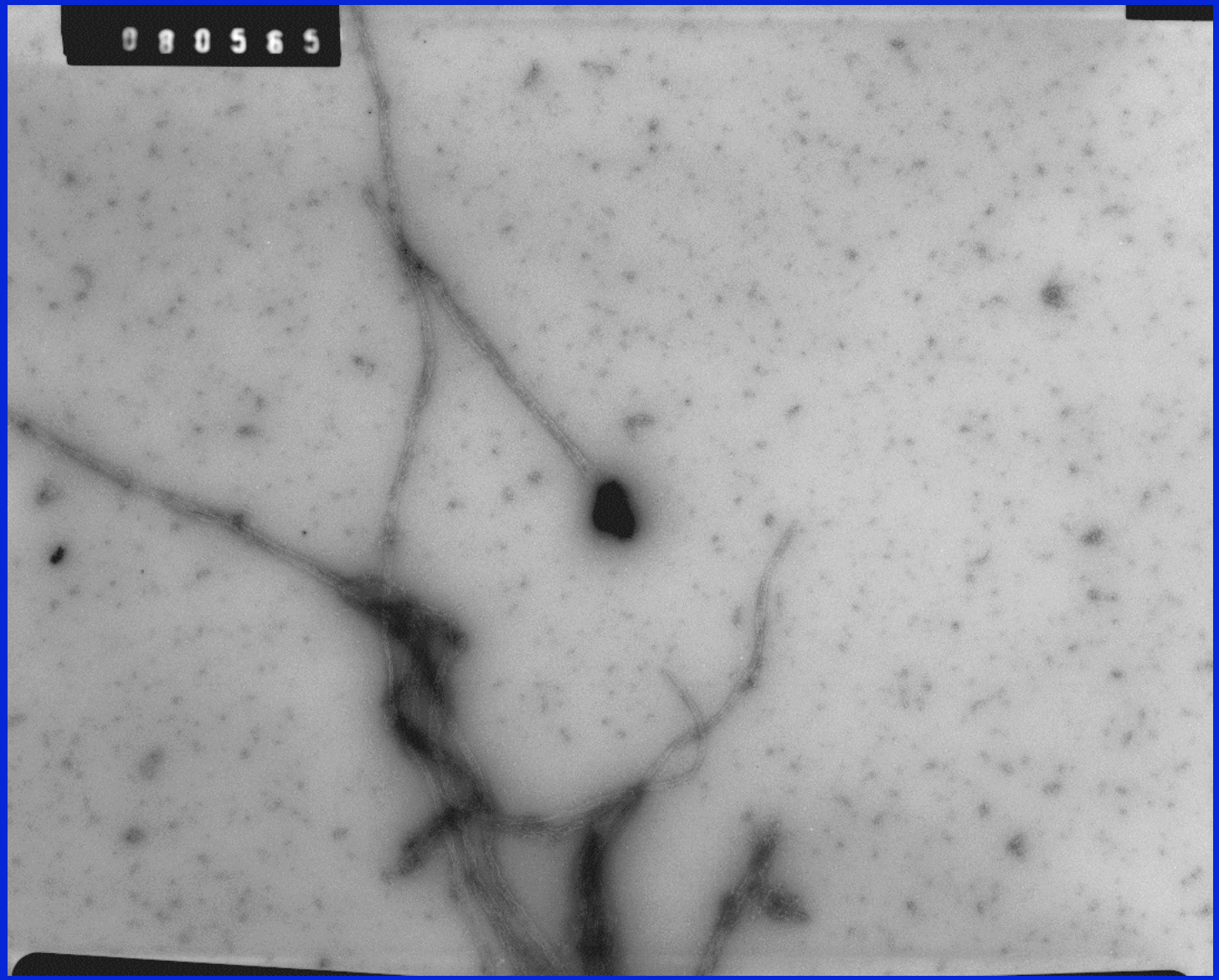



# Model of TAO Tiles within Nanotubes




- Helix axes parallel with nanotube axis
- Even number of tiles per layer (at least 6 or 8)

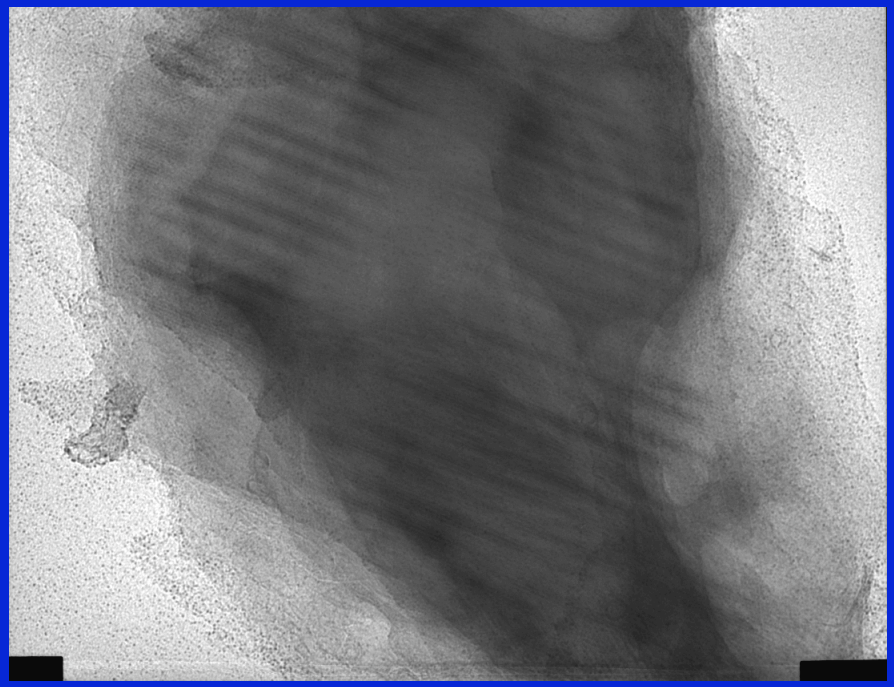
# Tile Nanotube Binding to Colloidal Gold

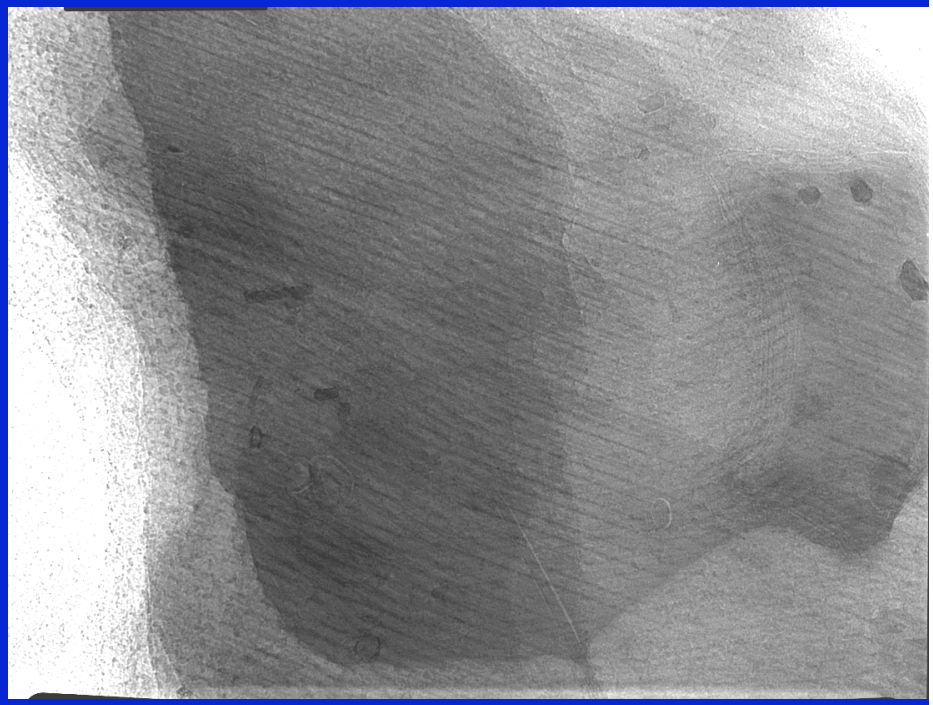



Preformed gold nanoparticles targeted via Au-S interactions.

# Our Nanogold Patterning of a DNA Lattice

## TEM images of TAO lattices

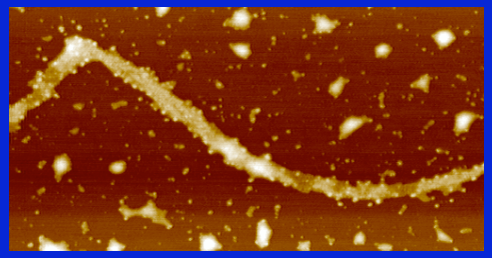




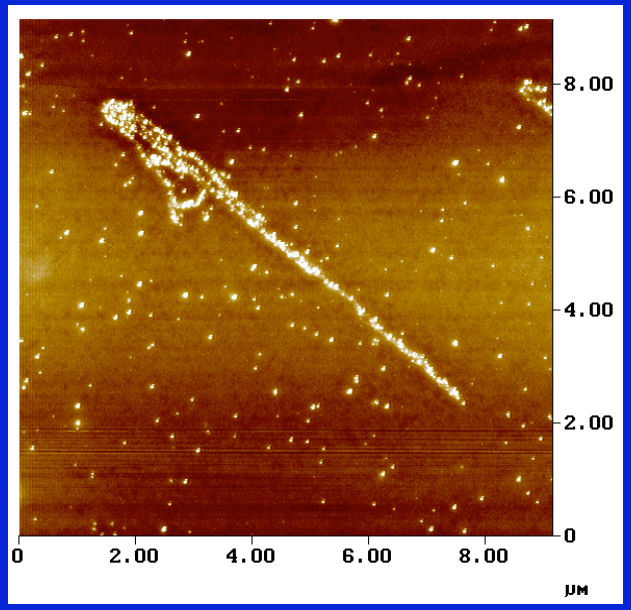
**AB* TAO Lattice** **ABCD* DX Lattice**

- **Moire` interference patterns indicates patterned gold nanoparticles are**
- **present in multiple layers.**
- **Electron Diffraction Patterns indicates nanogold is bound in lattice pattern**

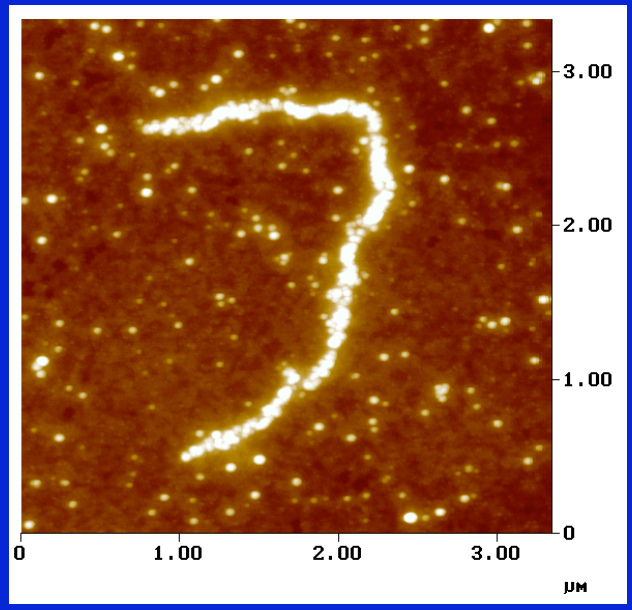
# Targeted Metallization of AB* Nanotubes



A



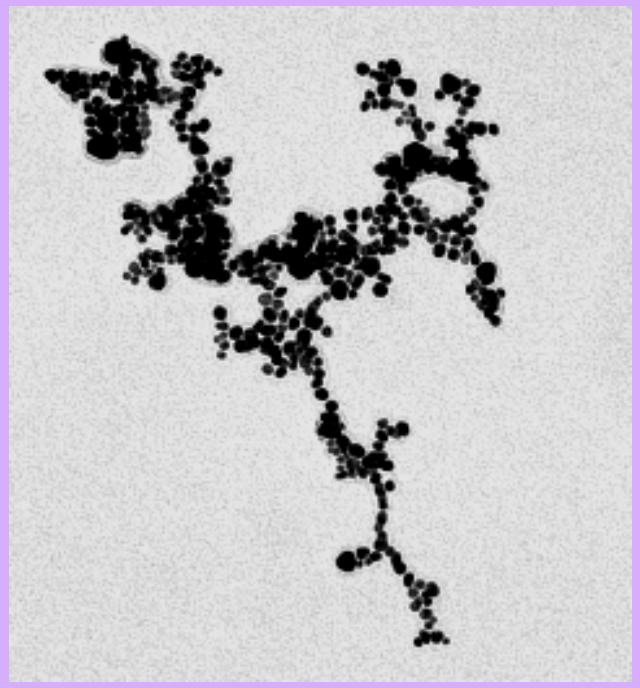

B

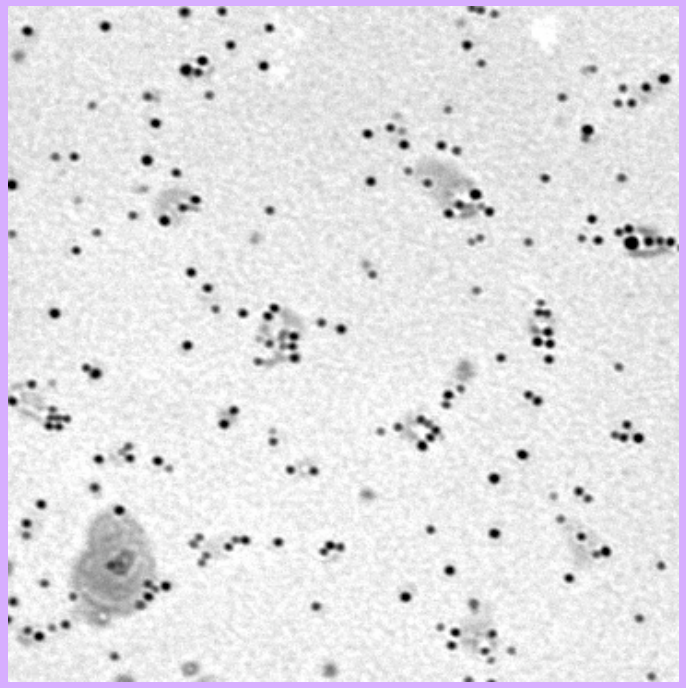



C

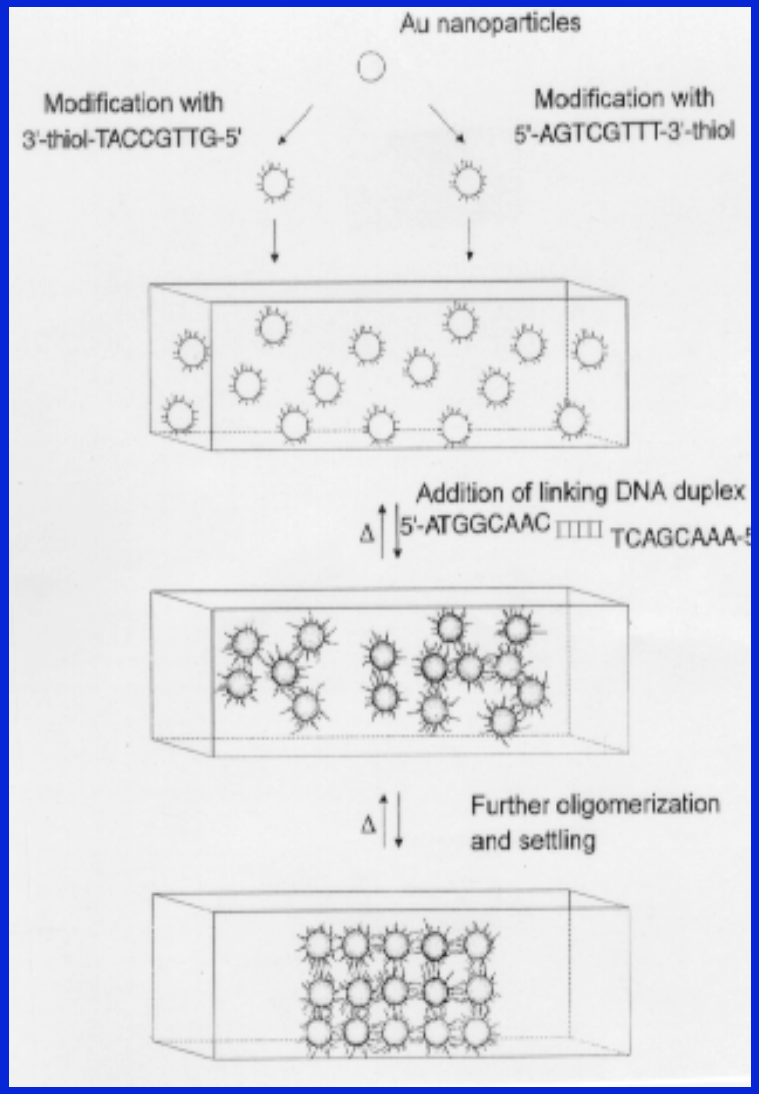
AFM images of progressive metallization of AB*$(SH)_2(NH_2)$ Fibers.
Panel A: Monofunctional nanogold (1.4 nm) bound to $NH_2$ groups on surface of fibers.
Panel B: Silver Enhanced staining deposited silver on the bound gold (2 minutes).
Panel C: Silver Enhance staining (5 minutes). Note nearly continuous metal wire.

# Nano-gold Particles without and with Bound Oligonucleotide

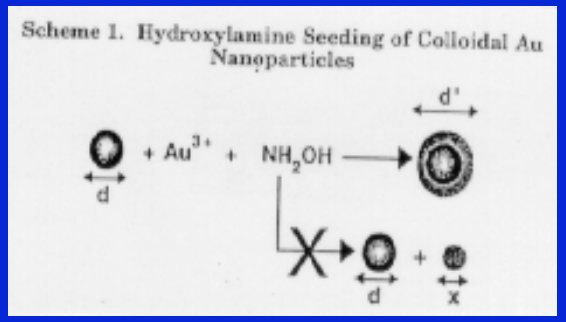




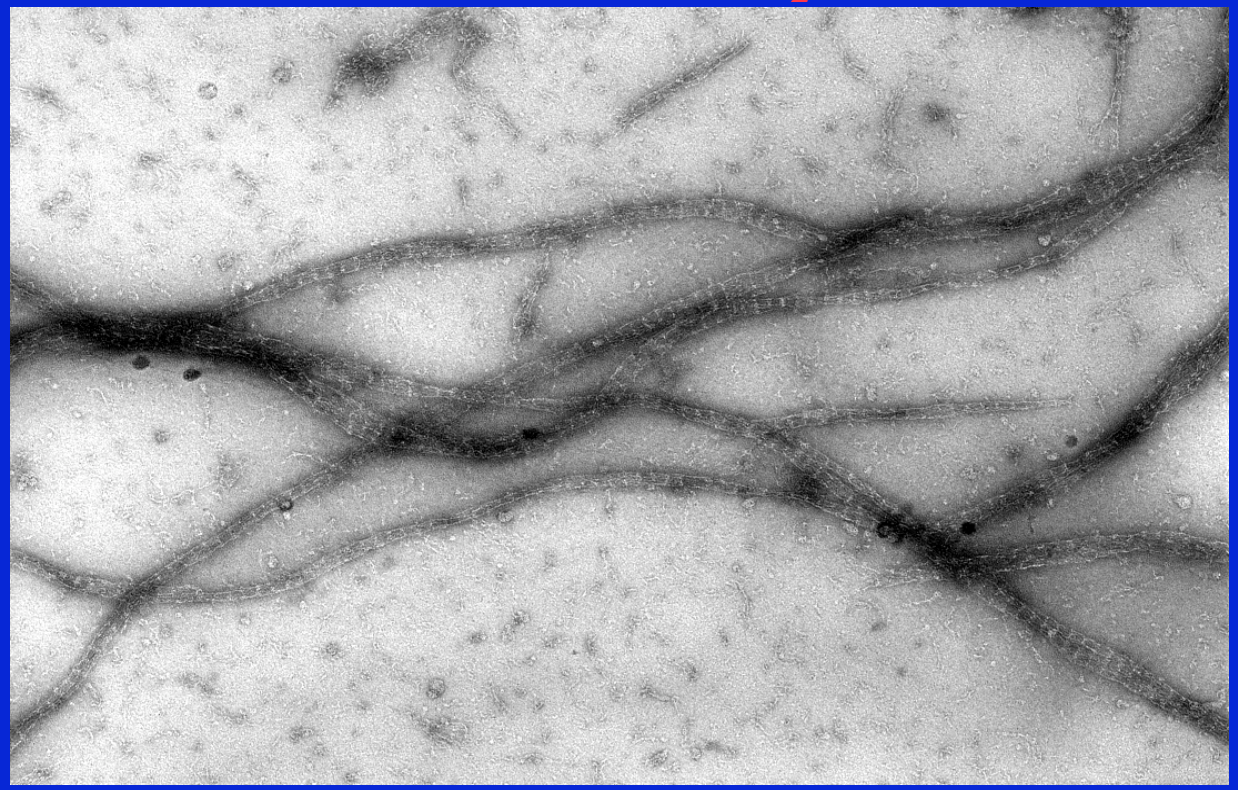
Gold nano-spheres of approximately 4 nm diameter were formed by reaction of gold chloride and sodium borohydride in dilute aqueous solution. The left panel shows a TEM image of bare gold particles while the right panel shows gold particles coated with the thiol containing oligo CGCGGATATT. Note the decreased clumping of the oligo-bound gold.




DNA lattice may be useful as foundation upon which to grow nano-scale gold wires. Figure to left shows labeling and targeting of gold nano-spheres using DNA (Mirkin et al, Nature 382, 607, 1996). We are currently testing the annealing of oligo-bound gold particle on the B tiles of the lattice shown above. Wire formation proceeds by fusion of neighboring spheres as show in the figure below -- deposition of gold from colloid onto immobilized nano-spheres (Brown & Natan, Langmuir 14, 726, 1998)



# DNA Fibers from AB*$(SH)_2$ Tile Lattice



We have reproducibly fabricated fibers of between 5 and 10 microns in length with uniform width (~25 nm) from TAO tiles. The fibers result from annealing reactions containing two tile types, A and B*, in which the B* tiles carry a dsDNA stem orthogonal to the tile plane and terminating with a thiol (SH) group on the end of both protruding strands. It appears that the thiols associate with other thiols on neighboring tiles and cause a characteristic curling of the lattice resulting in formation of tubes instead of sheets. An addition dsDNA stem protruding from the "underside" of B* tiles produces the stripes visible on the outside of the tubes.

# Procedure of Electrical Measurement of DNA-Based Metallized Nanotubes

*Sung Ha Park*

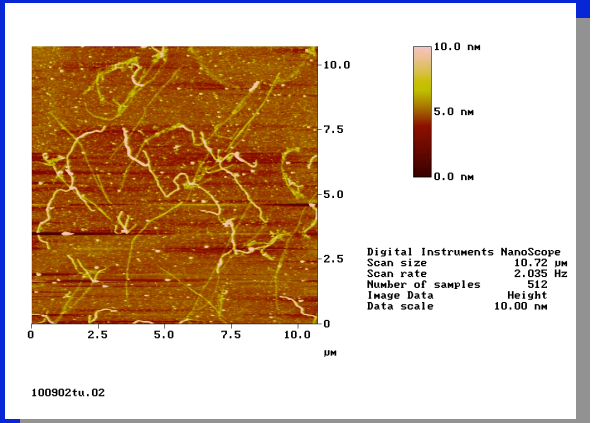
*Graduate Student, Duke University*

**Process of experimental Setup for measuring conductivity of DNA-based devices**

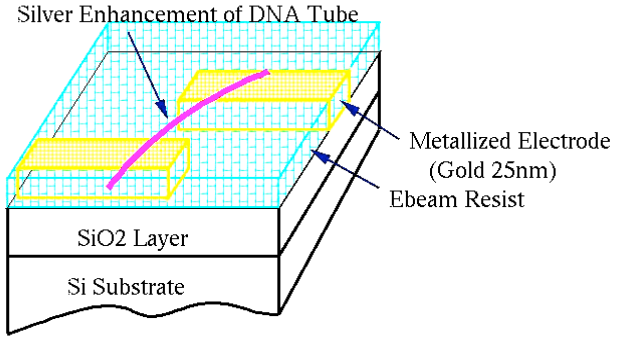
- **Compact electronic circuit is possible using nanometer-scale DNA nanotubes**
- **Position controllable using carefully designed DNA bases**

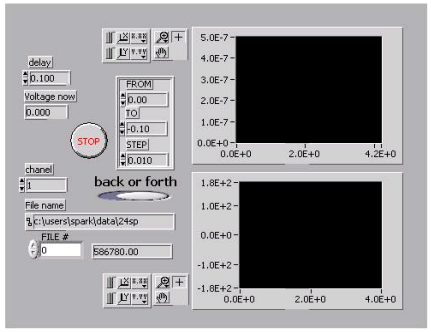
# Two Processes of Conductivity Measurement for Metallized DNA Nanotubes

## *1. Random Deposition Nanotubes with Electron Beam Lithography process*

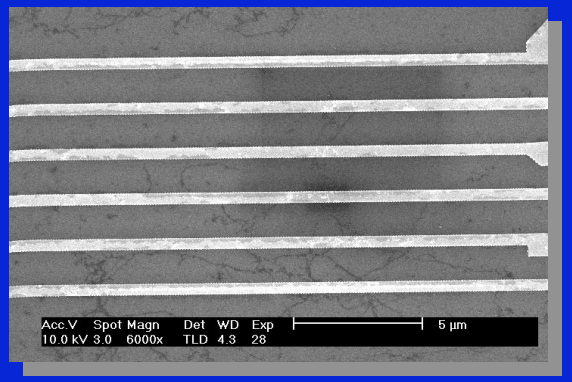



(a)

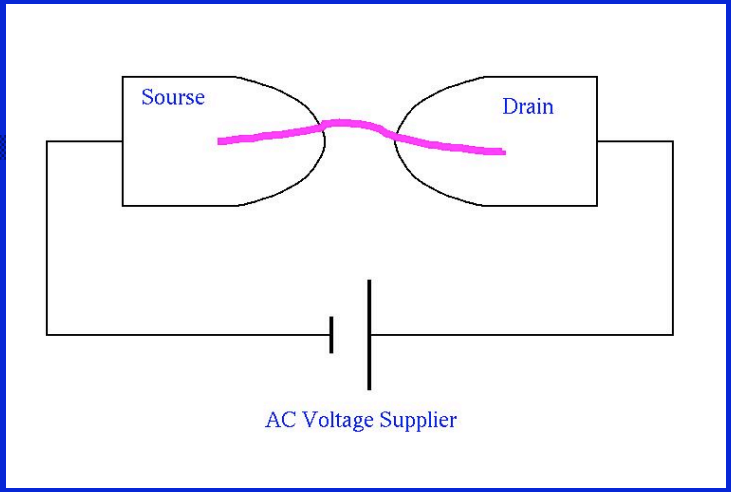


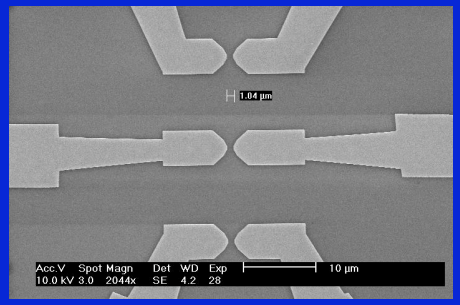

- (a) Preparation Metallized DNA Tubes on Silicon Substrate
- (b) Electron Beam Patterning
- (c) Electric Measurement using LabView Interface



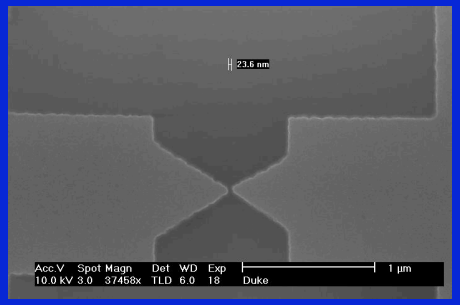
# 2. Trapping DNA Nanotubes using AC Voltage



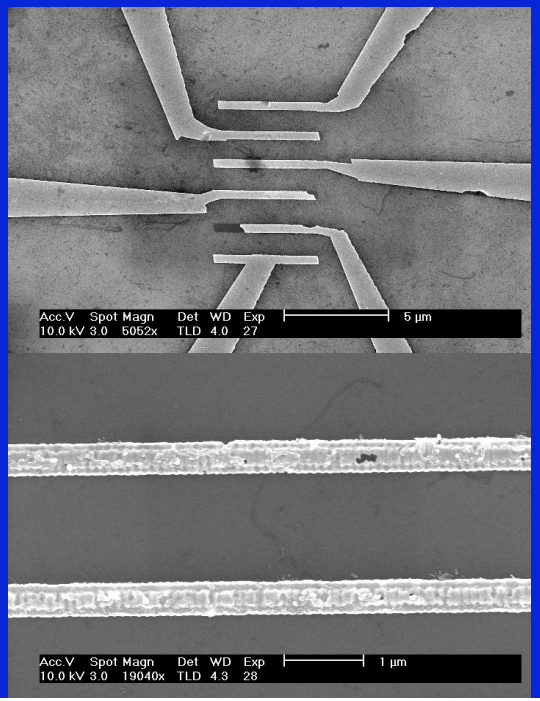

**(a) Preparation of Metal Electrodes**



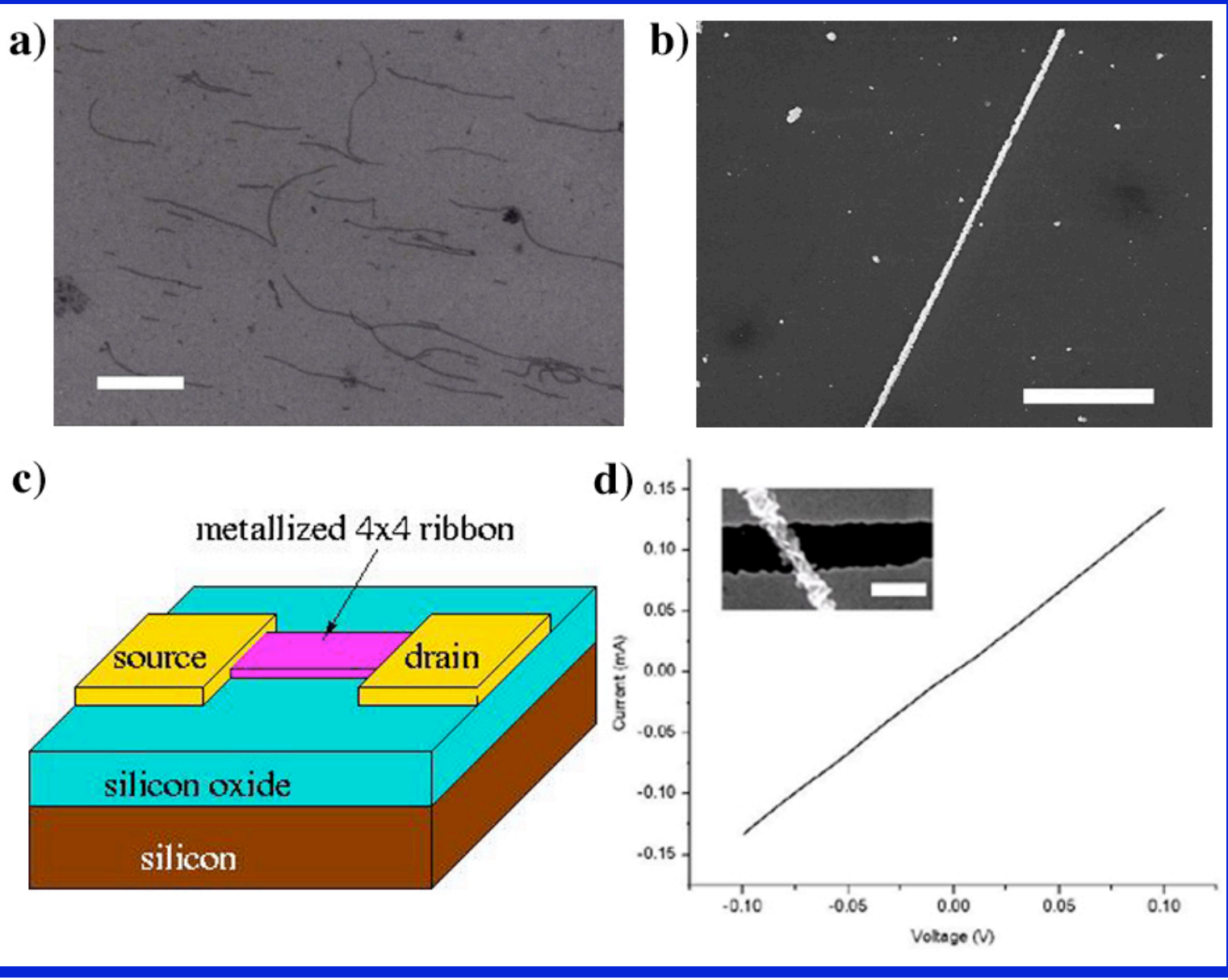

**(b) Trapping Tubes ; Apply AC Voltages (e.g., 1V, 50kH, 30sec)**




**(c) After Trapped Tubes**

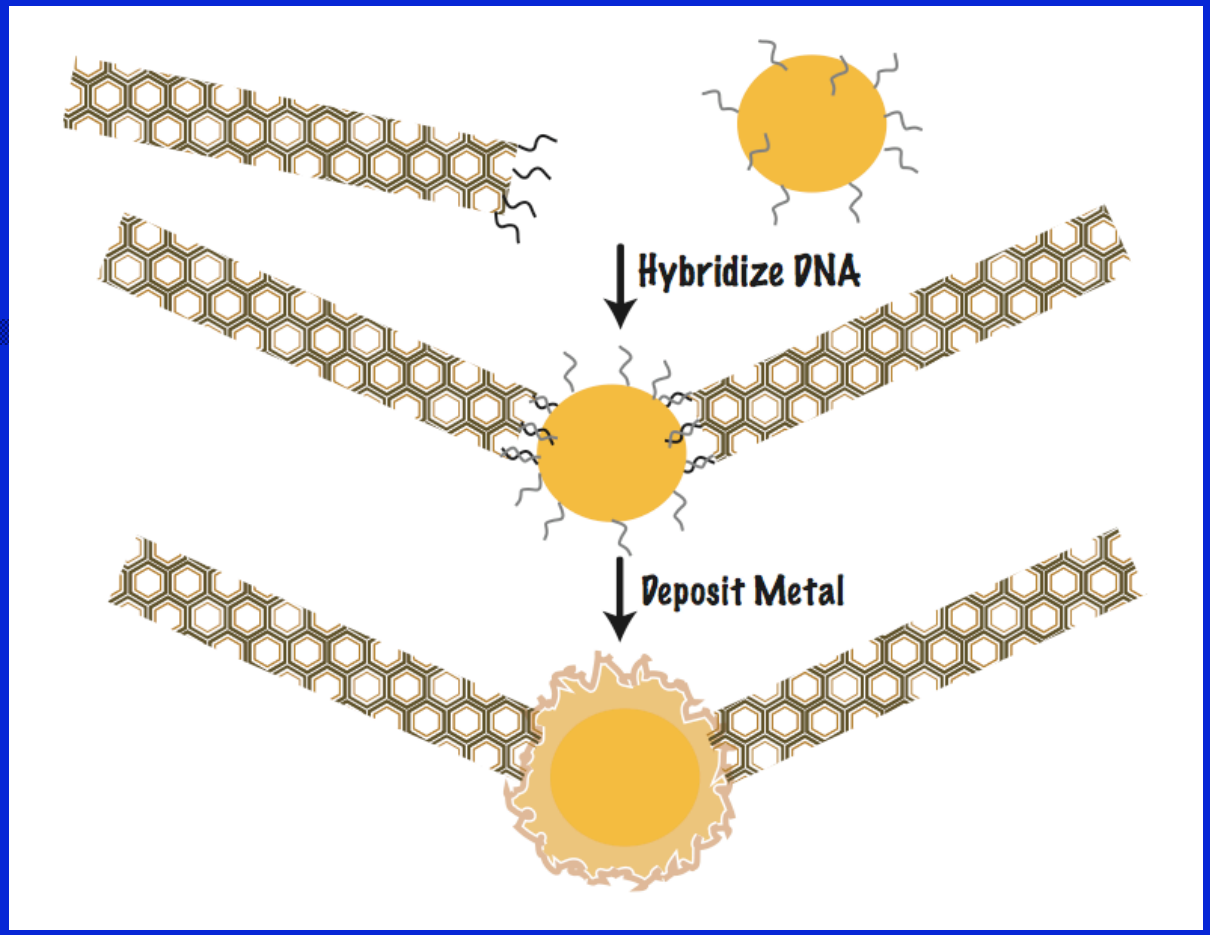


# 2-Step procedure Au Metallization of 4x4 ribbon and Conductivity Measurement



# New Directions in Computer Architecture enabled by DNA NanoTech

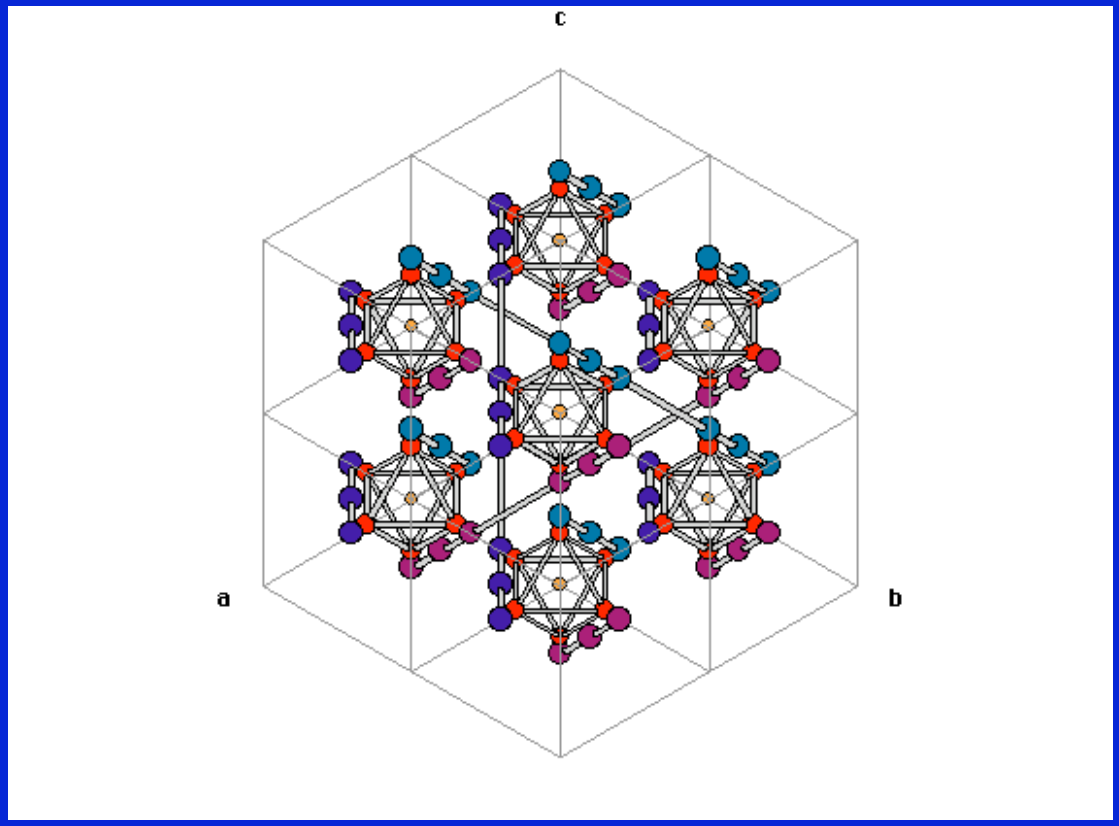
Alvin Lebeck, CS
Dan Sorin, ECE




Using DNA for Targeted SWNT Connections

# Application of 3D Regular DNA Tiling Lattices:

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

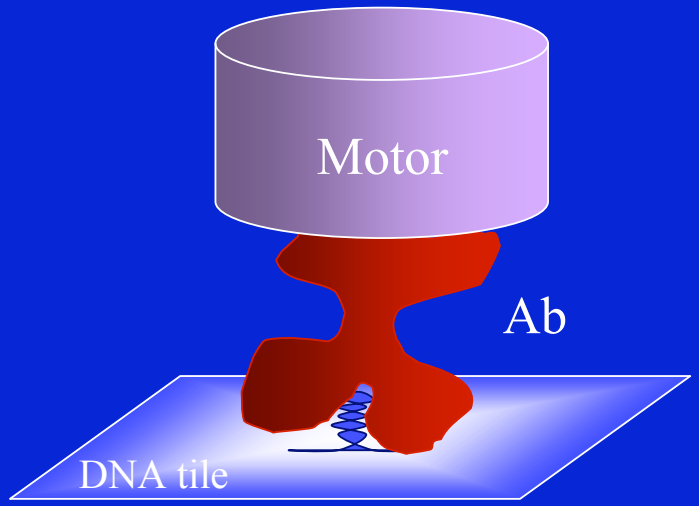


# Applications of DNA lattices as a substrate for Molecular Robotics

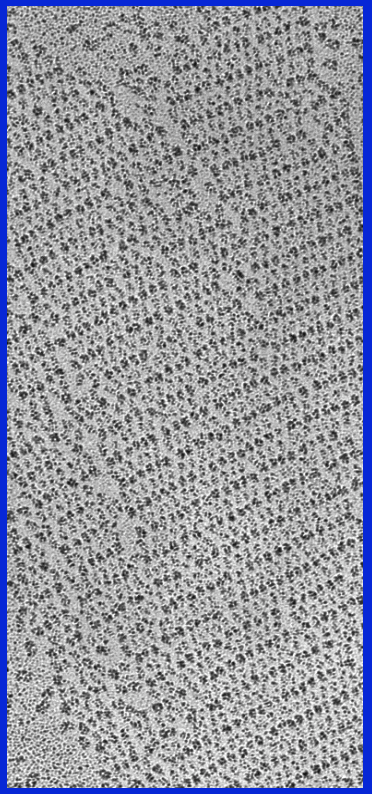
- Challenge: Re-Engineering Biological Molecular Motors
  - Construction of these biological molecular motors and their linking chemistry to DNA arrays:
    - Protein motors are modular and can be re-engineered to accomplish linear or rotational motion of essentially any type of molecular component.
    - 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.
- Challenge: 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.

# DNA Tile Lattice for Templating Molecular Motors



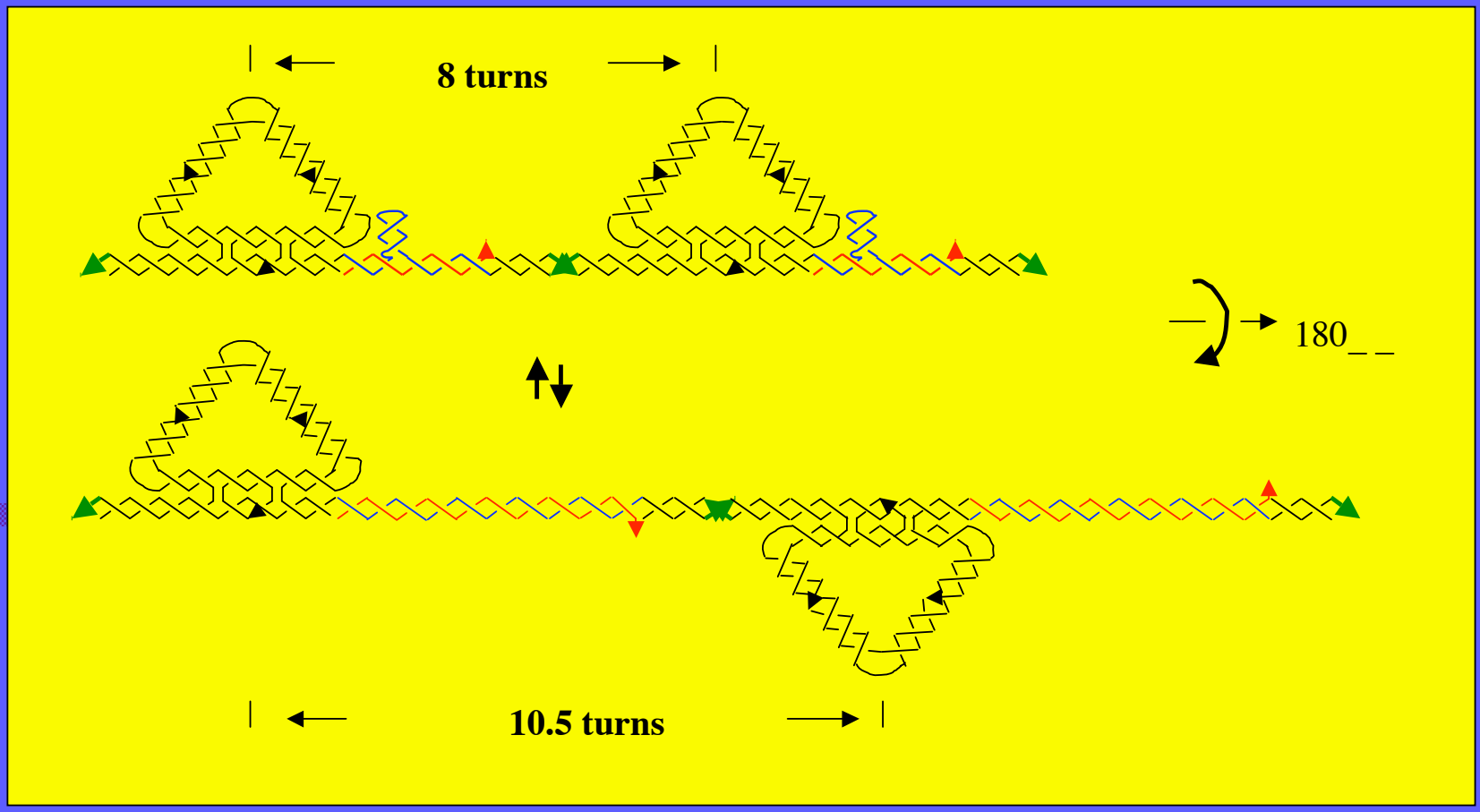

A bifunctional antibody (Ab) is shown bound to a DNA aptamer on a tile and to a motor protein, thus immobilizing the motor onto the tile.



An example DNA lattice

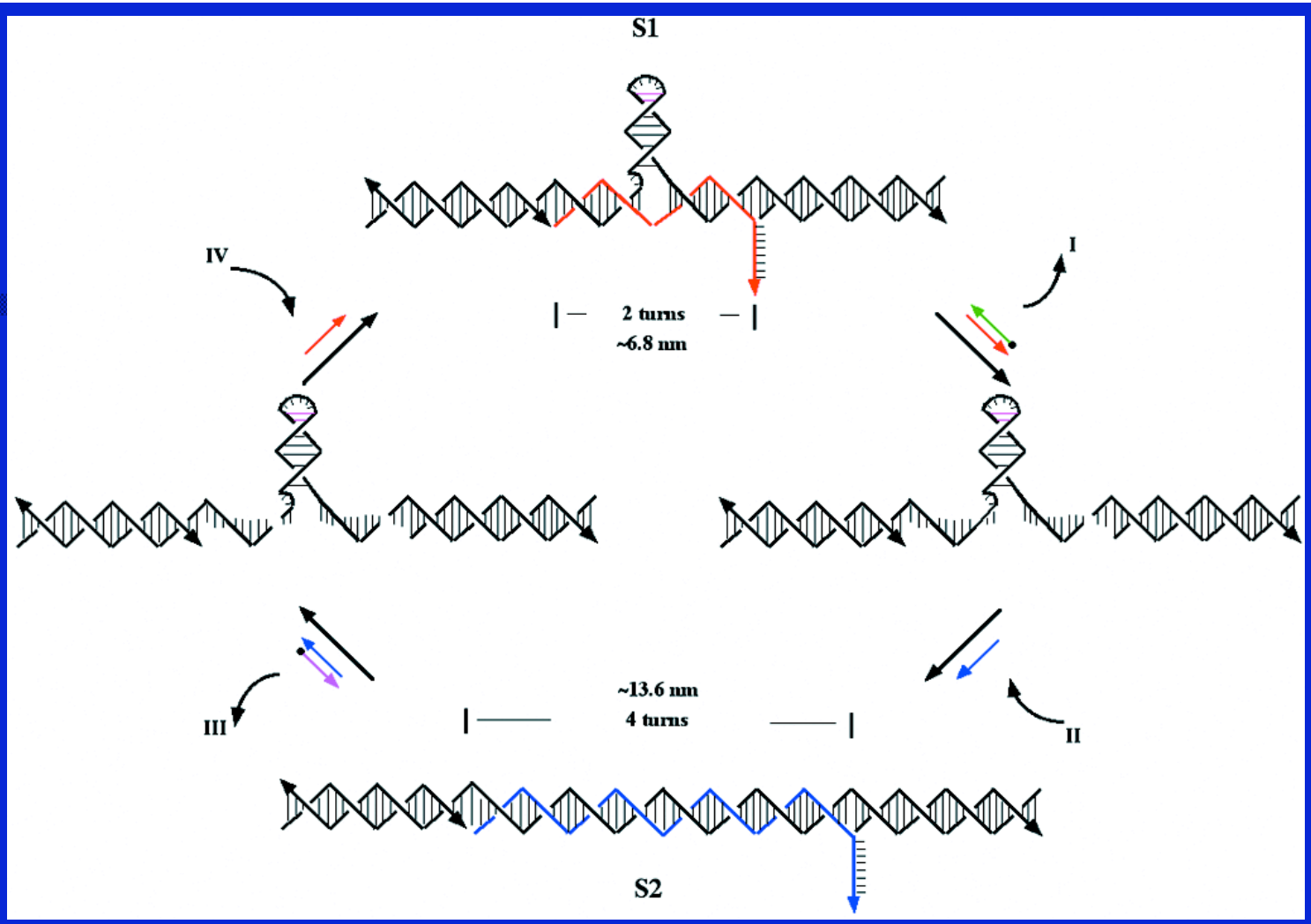
More complex patterns of motors on lattices can allow for sophisticated molecular robotics tasks.

# DNA Nanomechanical Device

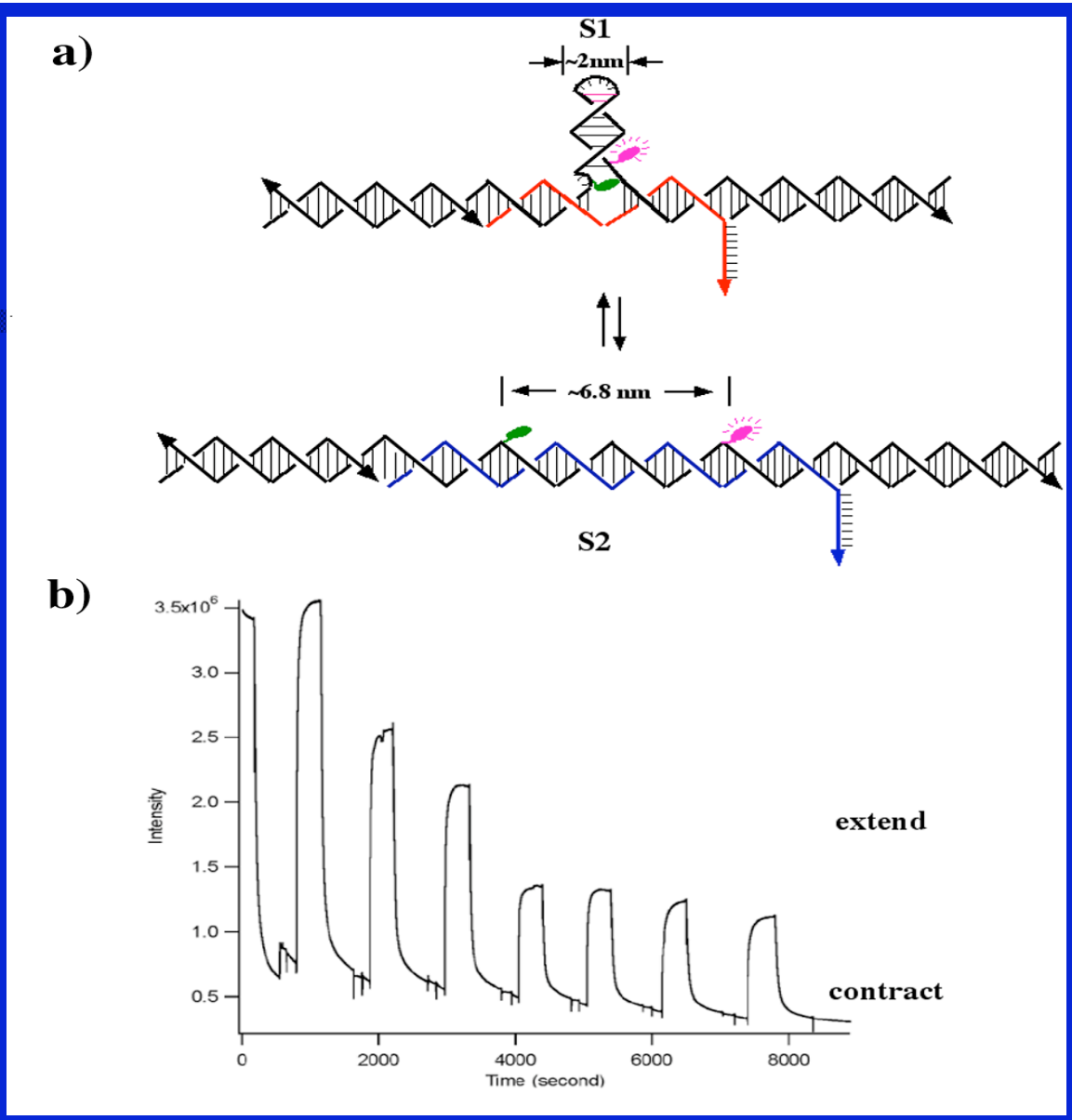



Walking Triangles: By binding the short red strand (top figure) versus the long red strand (bottom figure) the orientation of and distance between the triangular tiles is altered. These changes will be observable by AFM.

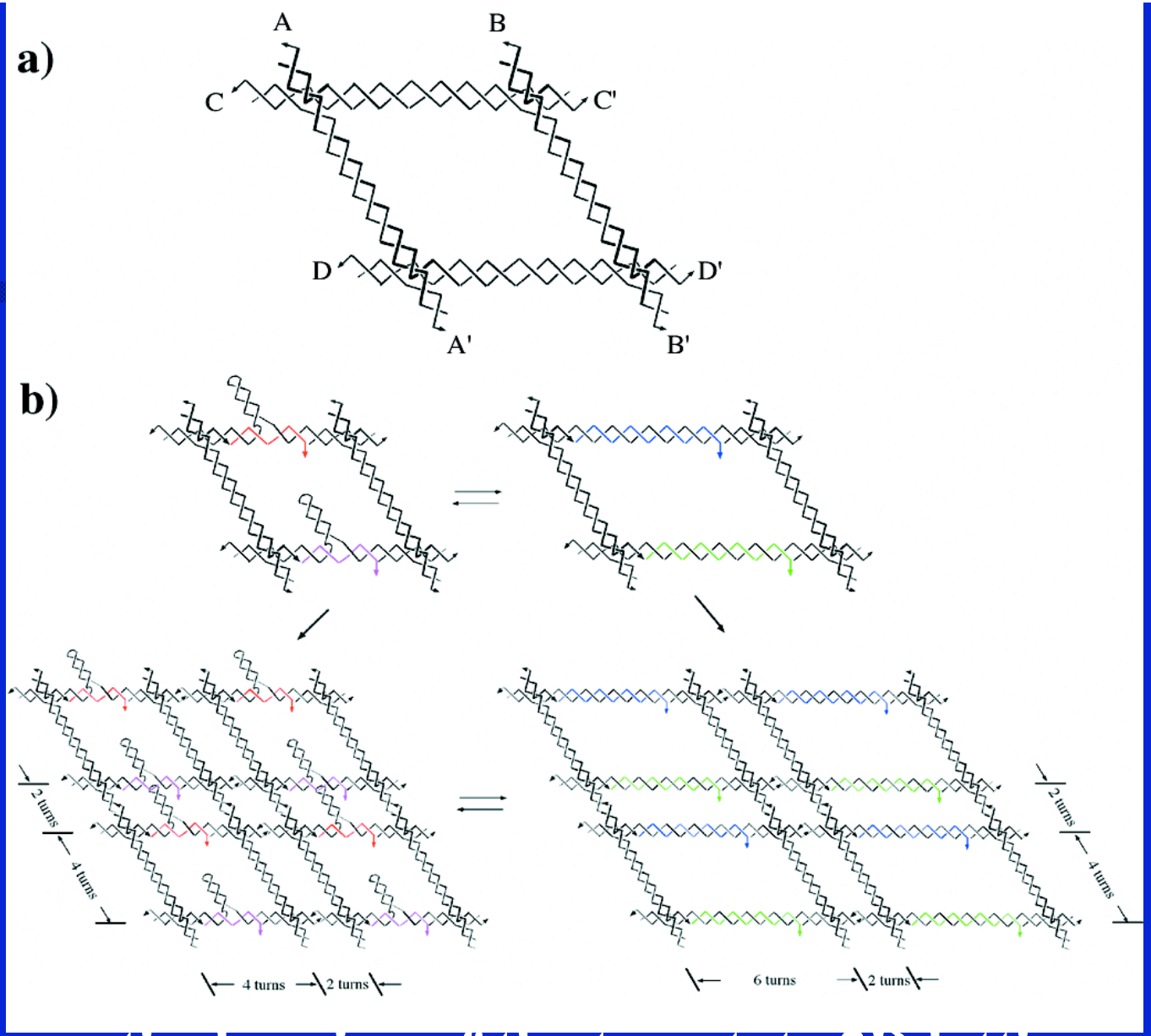
Applications: Programmable state control for nanomechanical devices.
Also as a visual output method.




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




Fluorescent resonance energy transfer spectroscopy measurements for the cycling of the nano-actuator device




**Schematic drawing of the two state 2D lattices actuated by DNA nano-actuator devices**

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