DNA Structural Nanotechnology

John Reif

*Duke University*

**DNA Nanostructure Group**
John Reif & Thomas H. LaBean

Graduate Students: Harish Chandran and Nikhil Gopalkrishnan

Recent Graduated Phds: Urmi Majumder, Sudheer Sahu, & Peng Yin
Introduction to DNA Self-Assembly
Self-Assembly in Nature

Spontaneous organization of components into stable superstructures due to local interactions

Figure 3-25. Molecular Biology of the Cell, 4th Edition.
Beauty at the Nanoscale: An Ideal 3D shape for Nanoassembly
Hybridization used for DNA Self-Assembly

Hybridization

3' T T G T T T A A C C T
5'  

3' A A C A A A T T G G A
5'  

3' T T G T T T A A C C T
5'  

3' A A C A A A T T G G A
5'  

HYDROGEN BONDING

LIGATION
Examples of DNA nanostructures

3D Cube
(Chen and Seeman, 91)

2D Periodic Grid Lattices
(Yan et al: Nature 03) Duke Univ
(He et al 05)

2D Hierarchical Assembled Lattices
(Park et al: Angewandte Chemie 06)
Duke Univ

2D Algorithmic Assembly
(Park et al: Angewandte Chemie 06)
Duke Univ

1D Algorithmic Assembly
(Rothemund 06)

3D Cube
(Mao et al: Nature 00) NYU&Duke Univ
(Park, et al 05) Duke Univ

Tube Lattices
(Lai et al PNAS 04) Duke
(Yin et al Science 08) Duke&Caltech

Barcode patterning
(Yan et al: PNAS 03) Duke Univ
Barcode

Origami - 2D Addressable Lattices
(Rothemund 06)
Ned Seeman: Father of DNA Nanotechnology

His Initial Ideas & Motivation for DNA Nanotechnology

Ned Seeman
New York University, USA
**Cube**


- Not rigid geometry
- 3-armed & 4-armed junctions
- Step-wise assembly on solid support

Ned Seeman
New York University

**Truncated Octahedron**

Chengde Mao
Purdue University
DNA Tiles and Lattices
Construction with “Smart Bricks”

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

- Comprehensive Review paper:
  "Challenges and Applications for Self-Assembled DNA Nanostructures",
  [Reif, LaBean, Seeman, 2000]
  
DNA tiles
DNA molecules self-assembled from artificially synthesized single stranded DNA.

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

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

**Self-Assembly from DNA strands, to Tiles, to Lattices**
Double-Crossover (DX) DNA tiles

[Winfree, Seeman 1998]:

- **First DNA crossover molecules self-assembled from artificially synthesized single stranded DNA.**
  - consist of two double-helices fused by crossover strands.

- **Pads:**
  - Total of 4 Pads of single stranded DNA at ends.

**Tile Types:**
- **DAE** contains an **Even** number of helical half-turns between crossover points.
- **DAO** contains an **Odd** number.
- DAO and DAE are double-crossover DX tiles with two anti-parallel crossovers.
Double-Crossover (DX) DNA tiling Lattices

[Winfree, Seeman 1998]:

A

B

~33 nm

2 x 15.98 nm
  – 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.
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.
DNA Tile Design Software
Duke&Caltech’s TileSoft: Sequence Optimization Software For Designing DNA Secondary Structures

UIL: Default window

Tile A (Optimized)

Peng Yin, Bo Guo, Christina Belmore, Will Palmeri, Erik Winfree, Thomas H. LaBean, John H. Reif, TileSoft: Sequence Optimization Software For Designing DNA Secondary Structures, DNA10, 2004
Nanorex’s Nanoengineer (Sims): Commercial Software For Designing DNA Secondary Structures
DNA Tubes & Ribbons
Programming DNA Tube Circumferences

Peng Yin, et al.

Science 321, 824 (2008);
DOI: 10.1126/science.1157312
DX-tile Array with Au Nanoparticles

Hao Yan
Arizona State

Science 323, 112-116 (2009)
3D DNA Lattices
An Application of 3D Regular DNA Tiling Lattices:

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

Ned Seeman
New York University
DNA Six-helix Bundle

Seeman
Pseudohexagonal
2D DNA Crystal

Nano Letters 5, 661-665 (2005)
DNA Tetrahedron Molecular cage

Rapid Chiral Assembly of Rigid DNA Building Blocks for Molecular Nanofabrication

Single-Molecule Protein Encapsulation in a Rigid DNA Cage
C. M. Erben, R. P. Goodman, A. J. Turberfield

Andrew Turberfield
Oxford University, UK
Crossover Tiles
2D & 3D Grid Lattcices
Crossover DNA Tiles and their Lattices

Used Corrugation to form 2D Grid Lattices

Also form Tubes & Ribbons

Au Metallization of 4x4 ribbon and Conductivity Measurement

Symmetric Cross DNA Tiles and their Grid Lattices

Symmetric Cross Tile

Figures adopted from He et al, 2005

Symmetric Tile

Corrugation creates enormous lattices

Chengde Mao
Purdue University
Double-decker tiles: Route to Assembly in 3D

4 identical arms

sticky ends

Branched Junction

2 cross tiles held together by branched junctions

Urmı Majumder, Duke
Double-decker tiles: Route to Assembly in 3D

Corrugation cancels curvature of lattice
=> creates enormous lattices

Urmī Majumder, Duke
Double-decker tiles: Route to Assembly in 3D

2D Lattices

2D Programmed Double-Decker Tiles

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

Urmia Majumder, Duke
Double-decker tiles: Route to Assembly in 3D

3D Generalized Corrugation cancels curvature of lattice in all 3 dimensions!

3D Programming of Double-Decker Tiles

Urmia Majumder, Duke
Patterned DNA Lattices
Patterned DNA lattices:

- Allows for Attachment of Nanoparticles at Specific Sites on Lattice

Application: Molecular Electronics:
- Layout of molecular electronic circuit components on DNA tiling arrays.
Molecular Pattern Formation using Scaffold Strands for Directed Nucleation:  
H Yan, T LaBean, L Feng, J. Reif, PNAS (2003).

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

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

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

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

Barcode lattice displays banding patterns dictated by the same sequence of bit values programmed on each layer.
Linear DNA DX lattices with biotin covalently bound to DNA tiles
Can be used for patterning of heteromaterials using DNA-directed assembly.

Hierarchical Assembly of DNA Lattices with 2 D Pattern “DNA”

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 Advantage of 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.
Patterned 2D DNA Lattices using Scaffold Strands Running Through Entire Lattice
Assembling a 2D Pattern by Directed Nucleation:

Self Assembly of Tiles around a DNA Strand Defining a 2D Pattern

Design Idea by LaBean & Reif, early 2000s
DNA Origami
Nature, 2006

Paul W K Rothemund
California Institute of Technology

William Shi
Harvard University, USA


Nanoscale DNA Octahedron
S.M. Douglas, H. Dietz, T. Liedl, B. Högberg, F. Graf and W. M. Shih
Self-assembly of DNA into nanoscale three-dimensional shapes, Nature (2009)
S.M. Douglas, H. Dietz, T. Liedl, B. Högberg, F. Graf and W. M. Shih
Self-assembly of DNA into nanoscale three-dimensional shapes, Nature (2009)
3D Wireframe Icosahedron

William Shi
Harvard

S.M. Douglas, H. Dietz, T. Liedl, B. Högberg, F. Graf and W. M. Shih
Self-assembly of DNA into nanoscale three-dimensional shapes, Nature (2009)
DNA origami design software

Kurt Gothelf
Aurhus UNiv, Denmark

ACS Nano, 2008

www.cdna.dk/origami
Self-assembly of a DNA origami box
Andersen et al Nature 2009, 459, 73

Aurhus Univ,
Denmark
Computational DNA Lattices
Computation with “Smart Bricks”

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.
Computation by Self-assembly of DNA Tilings

- Computation by DNA tiling lattices:
  - First Proposed by [Winfree, 98].
  - First Experimentally demonstrated by

- 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.
Experimental Demonstrations of Computation via Tiling Assembly:

1D DNA Tiling Computation:


- **String Tile Addition Pads:**
  - The sticky end pads on right encode:
    - carry bits coming in and $I_{A_i}$ and $I_{B_i}$ 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.

<table>
<thead>
<tr>
<th>tile</th>
<th>$c_i$</th>
<th>$I_{A_i}$</th>
<th>$I_{B_i}$</th>
<th>$o_i$</th>
<th>$c_{i+1}$</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>2</td>
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<tr>
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<td>1</td>
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<tr>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

- **Pad Programming via Truth Table:**
  - Column $c_i$ gives values for the 3 right-hand pads ($c_{1_i}$, $\sim c_{2_i}$, $c_{3_i}$)
  - Column $c_{i+1}$ gives value for the 3 left-hand pads ($\sim c_{1_{i+1}}$, $c_{2_{i+1}}$, $\sim c_{3_{i+1}}$).
“String Tile” Addition. Example.

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

TAE Assemblies for XOR Computation

XOR via TAE Computational Complex with Visual Readout

2D DNA Tiling Computation:

Assembly of Binary Counter

Eric Winfree
Caltech
Design of Self-assembled RAM

Eric Winfree
Caltech
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 LaBean99] -- 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**
Error-Correction of DNA Lattices
Example of a Computational Error

Eric Winfree
Caltech
Compact Error Correction in DNA Computational Lattices

Initial Computational Tiles:

Duke Design for Compact Error Resilient DNA Tiles

Self-propagation of Error Detection Makes Erroronious Assembly Unstable

Sudheer Sahu & Peng Yin
Activatable Tiles
Urmi Majumder, Thomas H LaBean, John H Reif

- Activatable tiles utilize a novel protection/deprotection strategy to enforce directional growth in tiling assembly
  - Each tile has an additional capability of holding and transitioning between states
  - Prior to binding with other tiles, activatable tiles are in an inactive state when the tile’s output pads are protected from binding with other tiles
  - After other tiles bind to this tile’s input pads the tile transitions to an active state.

- Can provide compact error-resilience to algorithmic assemblies

- Other applications
  - Concentration and Sensing Systems
  - Reaction Catalyzation
One Dimensional (1D) Directional Assembly

Start Tiles
- S1
- S2

Intermediate Tiles
- 11
- 12

Output Tile
- O

Direction of crystal growth
- a
- b

if S1, 11, 12 and O are present in the test tube then resultant assembly is the following:

With S1 as the start tile
- O
- 11
- S1

With S2 as the start tile
- O
- 12
- S2

Achieved using a novel protection scheme

Desired System

Erroneous System (in absence of protection)

Urmia Majumder
1D Directional Assembly with Activatable Tiles:

Reaction Pathway:

Stage 0:
- Protection Layer
- 11
- c' a'
- 12

Stage 1:
- Protection Layer
- Primer
- c' a'
- 11
- SI

Stage 2:
- Protection Layer
- c'
- 11
- a
- SI

Stage 3:
- Protection Layer
- c'
- 11
- a'
- SI

Back to Stage 1:
- Another step of de-protection begins here, which is basically going back to Stage 1 again.

Urmi Majumder
Activatable Tiles for 1D Directional Assembly: DNA Design

[Diagram of DNA tiles with annotations]

- Primer (P)
  - 3' to 5'
- Template for extension by Polymerase ((M)[Protection Strand])
  - Template T=S1A'+P++S1B'+M+S2'
- Complement of the Primer in Solution (P')
  - 3' to 5'
- Quencher (DABCYL)
- Fluorophore (FAM)
- Toehold
- Sticky End
- 5' to 3'

Urmia Majumder
Extension to 2D: Basic Scheme for Deprotection with Activatable Tiles and Error Resilience

TWO DIMENSIONAL DEPROTECTION AND ASSEMBLY

Signal for Output Deprotection by DNA polymerization

Urmila Majumder
Using Activatable Tiles for Concentration of Target Molecules

Applications of Activatable Tiles:
(beyond Computational Tiling)

Urmi Majumder
Applications of Activatable Tiles
(beyond Computational Tiling)

Using Activatable Tiles for Reaction Catalyzation

Urmia Majumder
DNA Robotic Devices
Nonautonomous DNA Motors

B-Z transition device
[Mao, Seeman 99]

DNA-fuelled Molecular machine
[Yurke et al 00]

DNA Non-Autonomous Biped walker
[ Sherman et al 04]
Autonomous DNA Robotic Devices
DNA Walker Devices: Formulation & First Designs
[Reif, DNA2002]

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

Walking DNA device
Use ATP consumption

Rolling DNA device
Use 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 $O(n^{1/2})$. 
First Autonomous DNA Walker 2004:

Peng Yin, Hao Yan, Xiaoju G. Daniel, Andrew J. Turberfield, John H. Reif, A Unidirectional DNA Walker Moving Autonomously Along a Linear Track,

Restriction enzymes

Ligase

Walker

Anchorage

Track

PfM I

BstAP I

Peng Yin

Andrew Turberfield
Oxford
Autonomous Motion of the Walker
A “Molecular-Racecar” on Circular Track
Used Power of Strand-displacing Polymerase


- Protector BQ prevents W from moving on its own
- Powerful strand displacement capability of Phi 29 during polymerization dislodges BQ from track
  => much faster & forceful movement than other DNA Walkers
- Experimental Demonstrations via FRET and Gel data

Used Polymerase Phi 29 to push wheel W on circular track T

Polymerase Phi 29

Replicative polymerase from bacteriophage Phi29
Phi29 polymerase can travel at the rate of 2000 nucleotides per minute at room temperature
This polymerase has exceptional strand displacement and processive synthesis properties

Sudheer Sahu
Other Autonomous Molecular Walkers

Mechanism for coordinated motion of a synthetic molecular motor
S.J. Green, J. Bath and A.J. Turberfield


T Omabegho, R Sha, and N Seeman, Bipedal DNA Brownian Motor with Coordinated Legs, Science (2009)

Andrew Turberfield
Oxford University, UK

Ned Seeman
New York University
DNAzyme based nanomechanical devices

Mao’s DNAzyme Crawler
• autonomous,
• no protein enzymes.

[Tian et al 05]

Chengde Mao
Purdue University

DNA Spiders
[Pei et al 06]

Milan N. Stojanovic
Programmable Autonomous DNA Nanorobotic Devices
Using DNAzymes

John H. Reif and Sudheer Sahu

- *DNAzyme calculator*: a limited ability computational device
- *DNAzyme FSA*: a finite state automata device, that executes finite state transitions using DNAzymes
  - extensions to probabilistic automata and non-deterministic automata,
- *DNAzyme router*: for programmable routing of nanostructures on a 2D DNA addressable lattice
- *DNAzyme porter*: for loading and unloading of transported nano-particles
- *DNAzyme doctor*: a medical-related application to provide transduction of nucleic acid expression.
  - can be programmed to respond to the under-expression or over-expression of various strands of RNA, with a response by release of an RNA

All Devices:
- Autonomous, programmable, and no protein enzymes.
- The basic principle involved is inspired by Mao’s DNAzyme Walker
DNAzyme FSA (inputs, transitions)
DNAzyme Crawler

Sudheer Sahu
DNAzyme Calculator

Sudheer Sahu
DNA Doctor

DNAzyme Device for DNA Doctor
(John H. Reif and Sudheer Sahu, 2006)
Impact of structural DNA nanotechnology in science

Nature and Science publications
<table>
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<th>S.No.</th>
<th>Title</th>
<th>Journal</th>
<th>Authors</th>
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<tr>
<td>5.</td>
<td>DNA-Templated Self-Assembly of Protein Arrays and Highly Conductive Nanowires</td>
<td>Science (2003)</td>
<td>H Yan, S H Park, G Finkelstein, J H Reif, T H LaBean</td>
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<td>13.</td>
<td>Self-Assembled Water-Soluble Nucleic Acid Probe Tiles for Label-Free RNA Hybridization Assays</td>
<td>Science (2008)</td>
<td>Y Ke, S Lindsay, Y Chang, Y Liu, H Yan</td>
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Applications of DNA Nanotechnology

Current Applications:
- Sequencing (pacific biosciences)*
- Drug development (Vipergen).*
- DNA tags*
- Mirkins DNA-nanoparticle-based sensor *
- Shi’s NMR work

* Commercial

Potential applications:
- Sensing application.
- Positioning of aptamers for multitarget molecular sensing
- Positioning of catalysts and reactants for molecular scale reaction platform (for advanced fuel cell technologies)
- Crystallization of host molecules (membrane proteins)
- Molecular cages for drug delivery
- Positioning of photonic components within a molecular scale platform for advanced solar cell technologies and for advanced sensing systems.
- Assembly of molecular electronic devices and circuits.
- DNA Machines instead of PCR.
- Nanomachines that respond to biological inputs
- DNA doctors
Vision for Applications of Structural DNA Nanotechnology

Hao Yan
Arizona State University, USA
Hao Yan
Arizona State
Vipergen’s Yoctoreactor
DNA Nanotechnology for drug development

1. Combinatorial library assembly and chemical reactions
2. Primer extension
3. Selection - binding to target - washing
4. DNA amplification and compound identification by sequencing
5. Rolling Translation

Encoded carrier units

Diversity = 1.6 \times 10^9

Display product

DNA-encoded molecule synthesized by the YoctoReactor®

"What we are really making are tiny DNA circuit boards that will be used to assemble other components."
--Greg Wallraff, IBM
DNA-based sensors

DNAzyme-Functionalized Au Nanoparticles for the Amplified Detection of DNA or Telomerase Activity

probe | analyte | output

hybridization probe | DNA, RNA | colour change

aptamers | proteins, small molecules, ions | enzyme-linked pigment production, nanoparticle aggregation, SERS

output DNA signal

drug release .........
DNA Nanostructure Cages for Capturing Small Molecules

Application: Drug Delivery

- biocompatible drug containers
- can interact with DNA, RNA, or proteins to trigger release

Single-Molecule Protein Encapsulation in a Rigid DNA Cage

Self-assembly of a nanoscale DNA box with a controllable lid
E. S. Andersen et al., Nature 459, 73-76 (2009)
An Application of 3D Regular DNA Tiling Lattices:

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

Ned Seeman
New York University
Shi’s DNA-Origami Nanotube

Induced alignment of membrane proteins for NMR structure determination


enabling measurement of global angular restraints

weakly align proteins in the presence of detergent
DNA Motor Devices for Transport and Assembly

In Cells, molecular motors are used to:
- assemble the cytoskeleton
- change cells shape
- transport cargoes

DNA Walking Devices
these move along DNA tracks
Use for transport and arrangement of molecular systems

Mechanism for coordinated motion of a synthetic molecular motor
S.J. Green, J. Bath and A.J. Turberfield
DNA Doctor