DNA-Based Programmable Autonomous Molecular Robotic Devices

John Reif
Dept CS
Duke University

Reif’s DNA Self-Assembly Group
Current Graduate Students
Hieu Bui
Sudhanshu Garg
Tianqi Song
Tong Niu
Guangjian (Jeff) Du

Prior Recent Graduate Students
Nikhil Gopalkrishnan
Peng Yin
Harish Chandran
Harish Chandran
Urmik Majumder

DNA-Based Self-Assembly and Nanorobotics
Organization of talk

- DNA (non-Autonomous) Motors
- DNA Autonomous Walkers
- DNA Autonomous Devices:
  - DNA Autonomous Devices that Compute as they Walk
  - DNA Devices that Open Nano-Containers
  - Meta DNA: DNA-based meta molecules with molecular machinery replacing enzymes
  - High-fidelity Hybridization Device: A hybridization-reaction driven device for exact matching of complementary DNA strands
DNA-based autonomous biomolecular devices are molecular assemblies and molecular devices that are:

(i) self-assembled: that is they assemble into DNA nanostructures in one stage without explicit external control,

(ii) programmable: the tasks the molecular devices execute can be modified without an entire redesign and

(iii) autonomous: they operate without external mediation (e.g. thermal cycling).
Non-Autonomous DNA based Nanorobotic devices

Advantages of DNA-based synthetic molecular devices:
• simple to design and engineer
• well-established biochemistry used to manipulate DNA nanostructures
Early DNA robotics devices needed external control, so not autonomous.

- **Rotation**
  - (Mao et al 99)

- **Open/close**
  - (Yurke et al 00)
  - (Simmel et al 01)
  - (Simmel et al 02)

- **Extension/contraction**
  - (Yan et al 02)
  - (Li et al 02)
  - (Alberti et al 03)
  - (Feng et al 03)
NonAutonomous DNA Nanorobotics

• B-Z transition

Switch conformation based on environment
NonAutonomous DNA Nanorobotics

- pH transition

Switch conformation based on environment
Non-Autonomous DNA based Nanorobotic devices

A DNA Nanomechanical Device Based on Hybridization Topology

The sequence-dependent device is based on the PX motif of DNA. The PX motif, postulated to be involved in genetic recombination, consists of two helical domains formed by four strands that flank a central dyad axis (indicated by the vertical black arrows). In (a) below, two stands are drawn in red and two in blue, where the arrowheads indicate the 3’ ends of the strands. The Watson-Crick base pairing in which every nucleotide participates is indicated by the thin horizontal lines within the two double helical domains. Every possible crossover occurs between the two helical domains. The same conventions apply to the JX2 motif which lacks two crossovers in the middle. The letters A, B, C and D, along with the color coding, show that the bottom of the JX2 motif (C and D) are rotated 180° relative to the PX motif. (b) illustrates the principles of device operation. On the left is a PX molecule. The green set strands are removed by the addition of biotinylated green fuel strands (biotin indicated by black circles) in process I. The unstructured intermediate is converted to the JX2 motif by the addition of the purple set strands in process II. The JX2 molecule is converted to the unstructured intermediate by the addition of biotinylated purple fuel strands in process III. The identity of this intermediate and the one above it is indicated by the identity sign between them. The cycle is completed by the addition of green set strands in process IV, restoring the PX device.

Up
DNA Tweezers:

- Nonautonomous Device

- Used Strand Displacement
Non-Autonomous DNA based Nanorobotic devices

DNA Biped walker [Sherman et al 04]
Non-Autonomous DNA based Nanorobotical devices

DNA Biped walker [Sherman et al 04]

The biped walker moves forward in an inchworm fashion where the relative positions of the leading and trailing leg do not change.

Parts:
- a track (blue),
- two legs (brown),
- two feet (pink and orange) and
- two footholds (green and turquoise).

The walker progresses along the track by the binding and unbinding of the feet on the footholds.

- The binding occurs when a single stranded set strand binds a foot to its foothold by forming a bridge across them.
- The unbinding occurs when this bridge is stripped away via a toehold due to the strand displacement action of unset strands.
Walker moves in a **foot over foot manner** (like kinesin) - each step the trailing foot swings past the leading foot.

- Has 2 single stranded legs partially hybridized together, leaving single stranded attachment regions on each.
- The track is a double stranded helix with single strand stators jutting out at periodic intervals.

**Locomotion** is achieved by hybridizing and denaturing the legs to the stators in a precise sequence.

Legs are anchored to the first two stators by the use of bridging DNA strands.

- The trailing leg is then pried loose by using a detachment strand to strand displace away its bridging strand via a toehold, then swings over and binds to the next stator, representing a step of the walker.
- The new trailing leg is now also pried loose in the same manner.
Non-Autonomous DNA based Nanorobotic devices

**DNA Biped walker Tian&Mao2004**


Same as the walker of Shin and Pierce except cargo walks along a circular track and returns to its original position after three steps.

Due to the symmetry of the design, the cargo and the track have the same geometric circular structure.
Autonomous DNA Walkers: DNA Devices that Walk on DNA Nanostructures
First DNA Walker Devices: Formulation & First Designs
[Reif, 2002]

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 $O(n^{1/2})$. 
Unidirectional Autonomous Walker

Peng Yin, Hao Yan, Xiaoju G. Daniell, Andrew J. Turberfield, and John H. Reif

Molecular-Scale device in which an autonomous walker moves unidirectionally along a DNA track, driven by the hydrolysis of ATP

Our work: DNA walker
First autonomous DNA robotic device

- Very first design for DNA walker
- Series of stators (blue)
- One walker (red)
- Use of ligase and restriction enzymes
Demonstrated First Autonomous DNA Walker:


Restriction enzymes

<table>
<thead>
<tr>
<th>Ligase</th>
<th>Walker</th>
<th>Anchorage</th>
<th>Track</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A*</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td></td>
</tr>
</tbody>
</table>

Restriction enzymes:

- PfuM I
- BstAP I
That Moves Autonomously along a Track.

DNA walker motion

Other Walkers powered by Restriction Enzymes:

Other Walkers powered by Restriction Enzymes:

Other Walkers powered by DNAenzymes:

[Tian & Mao 2005]

Steps of a walker powered by DNAzymes. The DNAzyme region of the strand is shown in different shade.
Autonomous DNA Racetrack Runners:
DNA Devices that Walk on Circular DNA Nanostructures
DNA Wheels

Sudheer Sahu, Thomas H. LaBean and John H. Reif, A DNA Nanotransport Device Powered by Polymerase φ29, Nano Letters, 2008, 8 (11), pp 3870–3878, (October, 2008)

- phi-29 strand displacing polymerase
- Pushes cargo strand around a circular track
Nano transport device powered by phi-29.

Polymerase extends the primer BP, and pushes the wheel W on the track T.

Protector strand BQ prevents the wheel from moving on its own but is dislodged by polymerase extension of BP on left.

DNA wheels setup
DNA wheels motion

(a) BP  
\( T \) braking seq (15A)  
\( W \) Cargo  
\( BQ \)  

(b) BP  
\( T \) braking seq (15A)  
\( W \) Cargo  
\( BQ \)  

(c) BP  
\( T \) braking seq (15A)  
\( W \) Cargo  
\( BQ \)  

(i)  

(ii)  

(iii)  

(iv)  

(i)  

(ii)  

(iii)  

(iv)  

(i)  

(ii)  

(iii)  

(iv)
Sudheer Sahu, Thomas H. LaBean and John H. Reif, A DNA Nanotransport Device Powered by Polymerase \( \varphi \text{29} \), Nano Letters, 2008, 8 (11), pp 3870–3878, (October, 2008)
Autonomous DNA Devices using no Enzymes: Fueled by Strand Displacement
Autonomous DNA Biped walker Turberfield2008]


Acts as a Brownian ratchet: Walker moves along a linear track with asymmetric bias towards one end of track, with aid of fuel supplied by DNA hairpins.

The trailing foot is more likely to detach from track, and equally likely:
- Swings forward ahead of leading foot or
- Reattaches back at its original position.

=> Walker is biased towards stepping forward rather than back, and behaves like a Brownian ratchet.

Trailing and leading feet are in competition for the same subsequence on the track.
- If trailing foot loses, it exposes a toehold by which fuel strand H1 invades and detaches it.
  => Gives asymmetry making detachment of the trailing foot much more likely.
- Once detached, a further fuel strand H2 takes away H1 and allows the foot to attach back to the track, either at the same location or a forward step.
Autonomous DNA based Nanorobotic devices
DNA walker [Tuberfield2008]


**Two-part fuel:** complementary hairpins H1 and H2.

**Walker Operation:**

(i) Competition between feet for binding to the track can lift part of the left foot from the track to reveal a toehold domain (ii).

(iii) This can bind the complementary toehold domain of H1, initiating a strand-displacement reaction that opens the neck of H1 and displaces the left foot from the track (iv).

(v) Part of the opened loop H1 can act as a second toehold to initiate hybridization with H2 to form a stable waste product (the H1 H2 duplex),

(vi) displacing H1 from all but the initial toehold domain of the lifted foot and allowing the foot to rebind the track to the left or right with equal probability
Autonomous DNA based Nanorobotic devices

DNA Biped walker [Yin2008]

P Yin, H Choi, C Calvert, N Pierce,
Programming Biomolecular Self-assembly Pathways,

A biped walker walks hand over hand along stators attached to a double stranded linear track.

Stators are in the form of hairpins

The process is autonomous because the stators have identical sequence and the two legs of the walkers have the identical complementary sequences

The walker is driven forward when its trailing leg is detached from the stator by the fuel strand B via a toehold-mediated strand displacement process and the leg swings over to the next stator in line.

Detachment Possibilities:
• 50% chance at each step that the leading foot is detached from the stator, in which case the walker halts.
• slight probability that both the legs of the walker detach from the track.
Autonomous DNA based Nanorobotic devices

Autonomous DNA Biped walker [Seeman2009]

Illustration of the DX track structure with the walker on it.

- The walker is shown on stem loops T1 and T2.
- The walker’s 5′, 5′ linkage is denoted by two black dots and its 3′ ends by half arrows.
- T16 denotes flexible polythymidine linkers on the walker and two fuel hairpins, F1 and F2.
- Two T5 regions provide flexibility at the base of the track stem loops.
Autonomous DNA Biped walker [Seeman2009]

(C) The walker is programmed to take two steps from RS-1 to RS-3 with the addition of F1 and F2 simultaneously (middle).

A single step is made from RS-1 to RS-2 with the addition of F1 alone (top).

- With the addition of F2 alone, the walker does not move.
- Only with the further addition of F1 does the walker make the transition from RS-1 to RS-3 (bottom).

(D) With the T4 fuelgrabbing sequence c restored, the walker transitions to RS-4, incorporating another F1 into the track, thereby kicking L-O off of T3.
Autonomous DNA Biped walker [Seeman2009]


1. L-E leads. T2 is activated and ready for F1.
2. T2 invades F1.
3. F1 is activated by T2.
4. F1 invades T1.
5. L-O is freed by F1.
6. L-O diffuses to T3.
7. L-O invades T3.
8. L-O leads. T3 is activated and ready for F2.

Transition from RS-1 to RS-2: In eight sequential frames, this illustration depicts the biped taking one step.

Illustrations 1 to 5 depict the activation of F1 by T2 and the release of L-O from T1 by F1. The freed leg L-O then begins the catalyzed release of L-E from T2 (illustrations 6 to 8).

Key to directionally biasing the biped, illustration 3 shows how the activated fuel strands are spatially restricted to act on the stem loop 7 nm away rather than the stem loop 21 nm away.
Psoralen cross-linking and 32P labeling.

(A) A detailed picture of the UV-activated psoralen crosslinking reaction between the track stem loops and the walker. The psoralen on the stem loops covalently links to the thymidines on the walker’s legs just outside the duplex formed by the stem loops and the walker’s legs.

(B) Visualizing the cross-link products with 32P. The three cross-linked products w-t (walker linked to the stem-loop on its trailing leg), w-l (walker linked to the stem-loop on its leading leg), and w-t-l (walker linked on both its trailing and leading leg) are shown forming in each experiment (W*, T1*, T2*, T3*, and T4*) that they are visible for each resting state (RS-1, RS-2, RS-3, and RS-4) of the system. The radioactive strand is drawn in red and the nonradioactive strands that are part of the cross-linked complex are drawn in blue. The constituent components of the products formed are listed in each box.

(C) Denatured topologies and size of the three walker–stem-loop cross-link products w-t, w-l, and w-t-l.
A DNA motor inspired by bacterial pathogens like Rickettsia rickettsii.

- The motor transports a single stranded cargo by (non-enzymic) polymerization, with the cargo always located at the growing end of the polymer.
- The system consists of two meta-stable hairpins H1 and H2 and an initiator strand (A) which carries the cargo (R)
- Initiator triggers a chain reaction building a linear double stranded polymer, with each hairpin unfolding to attach as a bridge between two hairpins of the other type.

The byproduct of the polymerization is the transport of the cargo relative to the initiator strand.
Autonomous DNA Devices that Compute as They Walk
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
DNA Doctor

DNAzyme Device for DNA Doctor
John H. Reif and Sudheer Sahu, 2006

Detecting RNA Expression:
Senses expression of sequence of RNAs $y_1$, $y_2$, $y_3$, $y_4$

A threshold concentration of complement of $y_1$, $y_2$, $y_3$, $y_4$ is added to the solution, therefore lack of $y_3$, $y_4$ causes excess of complement of $y_3$ and $y_4$, respectively.
Multi-Foot Programmable DNA Walkers
A DNA nanoscale assembly line

Hongzhou Gu, Jie Chao, Shou-Jun Xiao & Nadrian C. Seeman

A walker that moves along an origami tile, with programmable cassettes that transfer cargo (gold nanoparticles) to the walker’s ‘hands’
A DNA nanoscale assembly line

A DNA nanoscale assembly line

DNA Origami Walker


- DNA walkers have seven 'limbs':
- Four DNA strands are used as feet
- The other three are used to carry the cargo donated by the DNA modules, which are anchored to a DNA origami tile that acts as the DNA walker's track.
- Walker is moved by externally controlled 'fuel' strands that are added to displace the feet, so they move to other positions.
Using DNA Origami Walker for A DNA nanoscale assembly line


- DNA walker travels along a path with three DNA 'modules' at fixed intervals in an assembly line arrangement.

- The modules hold a cargo of gold nanoparticles and are individually programmed to either donate or keep their cargo, so as the DNA walker passes by it can be loaded with cargo resulting in eight possible end products.
A DNA nanoscale assembly line

DNA Devices that Open Nano-Containers
3D DNA origami – tetrahedron


Proximity Sensed Molecular Release

Douglas SM, Bachelet I, Church GM. A logic-gated nanorobot for targeted transport of molecular payloads. Science 2012; 335:831-4
Bear trap: Proximity Sensed Molecular Capture
Bear trap: Proximity Sensed Molecular Capture
Meta-DNA:

DNA Nanostructures with hybridization reactions that provide molecular machinery mimicking conventional DNA enzymic reactions


Synthetic biology

- Goal: design and assemble synthetic systems that mimic biological systems.

- Fundamental challenge: synthesizing synthetic systems for artificial cells

- Impact:
  1. a better understanding of the basic processes of natural biology
  2. re-engineering and programmability of synthetic versions of biological systems
Prior protein-based approaches to synthetic biology

- Key aspects of modern nucleic acid biochemistry: extensive use of protein enzymes
  - originally evolved in cells to manipulate nucleic acids
  - later adapted for laboratory use.

- Limited extent of the programmability of the available chemistry for manipulating nucleic acids

- Very difficult to predictively modify the behavior of protein enzymes.

- Thus methods for synthetic biology based on synthesis of novel proteins enzymes are very difficult
Our general approach of DNA-based meta-molecules

- Our approach: synthesize artificial biochemical systems
  - Provide the same functionality of nucleic acids, enzymes and other proteins
  - Use a very limited number of types of base molecules with a very limited chemistry
  - We call these Meta-Molecules

- Meta-Molecules:
  - Molecules that are constructed of DNA
  - But have the properties of natural biological molecules such as proteins and nucleic acids (DNA and RNA)
  - Programmable matter that simulates a number of the most basic and important biochemical reactions that act on DNA
  - Reactions that have an affect similar to protein-based reactions but are entirely based on DNA hybridization reactions.
Meta DNA

• A first baby step in design of complex synthetic biological systems

• Biological systems (or any physical system for that matter) can be viewed as information processors

• We believe DNA is a versatile molecule that can store and process information to ultimately support complex systems

• As biochemists: list out key properties and reactions of DNA

• As computer scientists: abstract these properties and develop notations to capture the complexity of various DNA reactions

• As engineers: design subsystems and interactions that yield an approximation of our abstraction
Meta DNA

• Based entirely on strands of DNA as the only component molecule.

• Prior work on self-assembled DNA nanostructures

• Far easier to re-engineer and program for desired functionality
  • Entirely DNA-based

• Each base of MetaDNA is a DNA nanostructure

• MetaDNA bases are paired similar to DNA bases
  • Much larger alphabet of bases
  • Increased power of base addressability
The MetaDNA bases self-assemble to form flexible linear assemblies
  - Single-stranded MetaDNA, abbreviated as ssMetaDNA Analogous to single stranded DNA
  - Hybridize to form stiff helical structures
    - Duplex MetaDNA, abbreviated as dsMetaDNA Analogous to double stranded DNA
    - Can be denatured back to ssMetaDNA

We discuss experimentally demonstrations (by Hao Yan’s group at ASU) of the self-assembly of ssMetaDNA and dsMetaDNA from MetaDNA bases
Internals of a Meta nucleotide
The T-junction

Interconnection:

- Trunk
- Branch

b1 and b2 diagrams with measurements.
Internals of a ssMetaDNA and dsMetaDNA

(a) Internals of a single stranded mDNA.

(b) Internals of a double stranded mDNA.
Artistic impression of the tertiary structure of the Meta double helix
AFM images of the MetaDNA double helix

Yan lab
Potential applications of MetaDNA and their reactions for in vitro biochemical systems

- Detailed sequence level protocols for:
  - MetaDNA synthesis
  - MetaDNA Hybridization, MetaDNA Denaturatation & MetaDNA Strand Displacement
  - MetaDNA Polymerization
  - MetaDNA Restriction
  - MetaDNA Helicase Denaturation
  - MetaDNA Replication

- The protocols operate without the use of enzymes, based only on hybridization reactions and are largely isothermal and autonomous
Potential applications of MetaDNA and their reactions for in vitro biochemical systems

- Transport devices
- Molecular motors
- Detection
- Signaling
- Computing systems
Hi-fidelity DNA Hybridization
Hi-fidelity DNA hybridization

- Hybridization fidelity depends on length
- Errors in hybridization
- Noise: Strands with sequence similar to the target
Exact hi-fidelity hybridization

- Test tube: ensemble of distinct sequences
- Target sequence \( s \)
- Problem statement: Completely hybridize all copies of \( s \) and don’t hybridize any other sequence
- Multiple strands may bind to \( s \) and cooperatively hybridize it
Approximate hi-fidelity hybridization

• Hybridization Error
  • \( b \) bases may mismatch: \( b \)-hybridized

• Failure probability
  • probability of \( b \)-hybridization at least \( p \)

• Problem statement: \( b \)-hybridize each copy of \( s \) with probability at least \( p \) and no other sequence is \( b \)-hybridized with probability greater than \( 1 - p \)

• \( p \approx 95\% \) and \( b \approx 1/10 \)th of length of \( s \)
Our results

• Detailed sequence level protocols (2) for approximate High-Fidelity Hybridization

• John Reif
  www.cs.duke.edu/~reif/

• PhD Candidates:
  – Sudhanshu Garg (~sgarg)
  – Hieu Bui (~hbui)
  – Reem Mokhtar (~reem)
  – Tianqi Song (~stq)

• 2nd Year Graduate Students:
  – Tong Niu
  – Guangjian (Jeff)
Reif Papers on the Web

Reif Papers on DNA nanoscience on the Web:

- Survey on DNA Computation:

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

- www.cs.duke.edu/~reif/paper/DNA-NanoscienceTalks

DNA Computing: Theory, Experiments & Software:

Self-Assembled DNA Nanostructures:

DNA-Based Programmable Autonomous Molecular Robotic Devices: