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
Reem Mokhtar
Tianqi Song
Tong Niu
Guangjian (Jeff) Du

Prior Recent Graduate Students
Nikhil Gopalkrishnan
Peng Yin
Harish Chandran
Harish Chandran
Urmia Majumder
Organizational 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
Goal of DNA-based autonomous devices

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).
NonAutonomous DNA Nanorobotics

Switch conformation based on environment
Early DNA robotics devices needed external control, so not autonomous.

Rotation

Open/close

Open/close

Open/close

Rotation

Extension/contraction

Extension/contraction

Extension/contraction

(Mao et al 99)

(Yurke et al 00)

(Simmel et al 01)

(Simmel et al 02)

(Yan et al 02)

(Li et al 02)

(Alberti et al 03)

(Feng et al 03)
DNA Tweezers:
- Nonautonomous Device
- Used Strand Displacement
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

Ligase

Walker

Track

A* B C D A

PfM I BstAP I
Evidence of the autonomous unidirectional motion of the walker. a) Experimental design: The six-stage enzyme combinations of the endonucleases PflM I and BstAP I (lane 2: no enzyme; lane 3: with PflM I; lane 4: with BstAP I; lane 5: with BstAP I and PflM I). c) PAGE analysis of the stepwise motion of the walker. Lane 0: labeled 10-bp DNA ladder marker; lane 1: device with no enzyme (control); lanes 2–5: samples corresponding to the stepwise process of Figure 2b. The intensity of the 57-nucleotide band and the appearance of a band corresponding to DNA bands are indicated beside the gels.

Dedicated to the completion of process IV in which B* can be ligated to C to form B*C. The intensity of the 68-nucleotide band increased, and a 57-nucleotide band disappeared. This experiment enabled us to inspect more closely the device to operate in a stepwise fashion (rather than autonomously to the completion of process IV in which B*C is cleaved by BstAP I to generate C*, thus producing a strand of 57 nucleotides. (These stages in the motion of the walker were also observed in a time-course experiment; one more day later, after the enzymatic activity of T4 ligase had ceased, the addition of BstAP I resulted in the disappearance of the 57-nucleotide band and the appearance of a band corresponding to a 68-nucleotide fragment in the sense that they are site specific, only execute reactions at their recognition sites. The unidirectional motion of the walker was also tested by deactivating PflM I, which had some use in DNA computations.

Oligonucleotide lengths (in numbers of bases) correspond to DNA bands shown in Figure 2b. Figure 2c shows an autoradiograph of a 20% denaturing gel which shows the products formed at the end of each process. The walker is expected to be able to continue to give a strand of 57 nucleotides. Subsequently, B* can be ligated to C to form B*C; the expectation that B* can be ligated to both A and C. The half-life of PflM I at 37°C is approximately 8 h (New England Biolabs, unpublished observations). The unidirectional motion of the walker was also tested by deactivating PflM I, which had some use in DNA computations.

In addition, Deoxyribozymes (DNAzymes) are a class of circular oligonucleotides that can be designed with a specific function or activity. Ligation and polymerization of the DNAzyme can then be controlled by ATP. In the study by Yin et al. (2004), A Unidirectional DNA Walker That Moves Autonomously along a Track. Angewandte Chemie International Edition, 43(37), 4906–4911. doi:10.1002/anie.200460522

DNA walker motion

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
DNA wheels setup
DNA wheels motion
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)
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
DNAzyme Calculator
DNA Doctor

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

![Diagram of DNAzyme Device for DNA Doctor](image)
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’
DNA Origami Walker

DNA Origami Walker

DNA Devices that Open Nano-Containers

Bear trap: proximity sensed capture
Bear trap: proximity sensed 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.
• 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

≈0.3 nm

≈2 nm

Trunk

Branch

b1

b2

b3

b4

b5
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)
What we do

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

Self-Assembled DNA Nanostructures:
www.cs.duke.edu/~reif/paper/DNA-NanoscienceTalks/DNA-Nanostructures/DNA-
Nanostructures.pdf

DNA-Based Programmable Autonomous Molecular Robotic Devices:
www.cs.duke.edu/~reif/paper/DNA-NanoscienceTalks/DNA-ProgAutoMolRobotics/
DNA-ProgAutoMolRobotics.pdf