DNA-Based Programmable Autonomous Molecular Robotic Devices

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DNA-based autonomous devices

- Are molecular assemblies and molecular devices that are:
  - **Self-assembled:** that is they assemble into DNA nanostructures in one stage without explicit external control,
  - **Programmable:** the tasks the molecular devices execute can be modified without an entire redesign and
  - **Autonomous:** they operate without external mediation (e.g. without thermal cycling).
Autonomous DNA Walkers: DNA Devices that Walk on DNA Nanostructures
First DNA Walker Devices: Formulation & First Designs


Designs for the first autonomous DNA nanomechanical devices that execute cycles of motion without external environmental changes (made random walks)

**Walking DNA device**
(Used ATP consumption)

**Rolling DNA device:**
(Used DNA hybridization only)

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 $O(n^{1/2})$. 
Autonomous DNA Walkers using Enzymes
First autonomous unidirectional DNA walker
[Yin, Reif, 2004]

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

First autonomous unidirectional DNA walker
[Yin, Reif, 2004]

First autonomous DNA walker:

• One walker (red)

• Attached to the DNA nanotrack are a series of stators (dark blue) with single stranded DNA available for hybridization.

• Walker operation uses enzymes: ligase and restriction enzymes
Autonomous DNA Walker:

Operation uses enzymes:

- **ligase** and
- **restriction enzymes** PflM I and BstP I

Walker (Red) moves left to right
Walker (Red) moves left to right


Note: Restriction Cut Indicated by Red Scissors
Gel Determination of DNA walker motion:

Another Unidirectional autonomous DNA Walker powered by Restriction Enzymes: [Bath&Turberfield,2005]

Walker (Red) moves left to right

Another Unidirectional autonomous DNA Walker, but powered by DNAzymes: [Tian&Mao,2005]


Steps of a walker powered by DNAzymes:
• Walker moves left to right
• The DNAzyme region of the strand is shown in red.
Another Unidirectional autonomous DNA Walker powered by Restriction Enzymes: [Sekiguchi&Yamamura,2008]


Restriction Cut Indicated by Red Scissors
Autonomous DNA Racetrack
Runners using Polymerase:
DNA Devices that Walk on
Circular DNA Nanostructures
Nano transport device powered by phi-29:

- **Uses phi-29 strand displacing polymerase**
- The polymerase extends the primer BP, and pushes the wheel W on circular track T.
- Protector strand BQ prevents the wheel from moving on its own but is dislodged by polymerase extension of BP on left.
Setup for Circular DNA Walker

1. Non-circular DNA
2. Add a BP
3. T4 Ligase
4. Walker W
5. Add BQ
6. T4 Ligase
Motion of Circular DNA Walker
Fluorescence Evidence of Motion of Circular DNA Walker

Fig. 6. (a) The fluorescence shown by the assembly in absence of the cargo containing the quencher (b) The fluorescence quenched by the assembly of cargo containing the quencher (c) The fluorescence remains quenched even after the activity of the polymerase φ29, which indicates that the cargo is not dislodged from the wheel W

Fig. 7. (a) The fluorescence is shown by the assembly in absence of the cargo containing the quencher (b) The fluorescence is quenched after the assembly of the cargo containing the quencher (c) The fluorescence reappears after the polymerase φ29 pushes the wheel containing the quencher

Fig. 8. (a) The fluorescence is shown by the assembly in absence of the cargo containing the quencher (b) The fluorescence remains after the assembly of the cargo containing the quencher, away from the fluorophore (c) The fluorescence quenches after the polymerase φ29 pushes the wheel before it stops at stopping sequence, and the sticky end of the cargo hybridizes with the track to quench the fluorescence
Assume:

- $n_1 > n_2$ (where $n = n_1 + n_2$)

Molecular Gears: A Pair of DNA Circles Continuously Rolls against Each Other

Ye Tian and Chengde Mao
JACS 2024

Scheme of the rolling process of gears. (a) Structures of the individual gears. C and P indicate DNA strands, and T indicates teeth. (b) Operation of the gears. L and R represent linker and removal strands, respectively. L₁ and R₁ are complementary to each other. Both circles remain intact during the rolling process. The only changed strands are the linker (L) and removal (R) strands. Note that no twisting motion will be generated to the central stands during the rolling process.
Autonomous DNA Devices
(Using No Enzymes: Fueled by Strand Displacement)
Autonomous DNA Walker using no enzymes


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, and

(ii) The lifting of the left foot reveals a toehold domain.

(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.

Walker moves left to right
Autonomous DNA Walker using no enzymes


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.

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

Walker moves left to right
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.

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Biped Autonomous DNA Walker using no enzymes

The DX nanotrack 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.

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

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

- With addition of F2 alone, 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 fuel-grabbing sequence c restored, the walker transitions to RS-4, incorporating another F1 into the track, thereby kicking L-O off of T3.
**Biped Autonomous DNA Walker using no enzymes**


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.
A DNA motor inspired by bacterial pathogens like Rickettsia rickettsia:

• 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.
The DNA walker undergoes cartwheeling movements on a nanotrack of complementary oligonucleotides.

Measured a stepping rate constant approaching 1 s\(^{-1}\), which is 10- to 100-fold faster than prior DNA walkers.

Used single-particle tracking to demonstrate movement of the walker over hundreds of nanometres within 10 min, in quantitative agreement with predictions from stepping kinetics.
Autonomous DNA Walkers that Navigate Networks (Using Nicking Enzyme)
A DNA-based molecular motor that can navigate a network of tracks

Shelley F. J. Wickham, Jonathan Bath, Yousuke Katsuda, Masayuki Endo, Kumi Hidaka, Hiroshi Sugiyama & Andrew J. Turberfield


**Operation:** uses a nicking enzyme

- ‘Block’ strands with unique address domains (magenta/green) prevent the motor (black) from stepping when it reaches a junction.

- The selected path is unblocked by an instruction strand that hybridizes to the toehold on the selected block strand (green) to initiate a strand displacement reaction that removes it from the stator.

- The walker (black) can then step to the unblocked stator.

- The resulting duplex contains a new recognition site for the nicking enzyme.

- Enzyme cleavage of the stator, and subsequent dissociation of the cut stator fragment, generates a 6 nt toehold that initiates migration of the motor onto the next intact stator.

- Repetition of this cycle of step and cut drives the motor along the programmed path.

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**Removal of Block’ strand**

**Forward Movement of Motor**

**Walker moves left to right**

- Walker moves left to right
- Nanotrack
- Waste
- Walker
- Nanotrack
- Walker
- Nanotrack
- Walker
- Nanotrack
- Walker
- Nanotrack
A DNA-based molecular motor that can navigate a network of tracks[Wickham&Turberfield2012]
Shelley F. J. Wickham, Jonathan Bath, Yousuke Katsuda, Masayuki Endo, Kumi Hidaka, Hiroshi Sugiyama & Andrew J. Turberfield

The DNA track network is assembled on a rectangular DNA origami substrate:
Selective displacement of blocking strands from junction stators (colored crosses) opens just one path.
The motor (black circle) travels down the open path, destroying the track behind it.

Tracks decorated with excess motor visualized by AFM (scale bars, 50 nm):
- A reference marker (white square) is used to confirm the orientation of the track.
Autonomous DNA Walkers that Navigate Networks (Using No Enzymes: Fueled by Strand Displacement)
Solving mazes with single-molecule DNA navigators [Chao 2019]

https://www.nature.com/articles/s41563-018-0205-3
Autonomous DNA Devices that Deliver Cargo as They Walk
(Using No Enzymes: Fueled by Strand Displacement)
Cargo-sorting DNA robots

- **Design:** Encode recognition between cargos and their destinations.
  - NanoRobots are single-stranded DNA with:
    - The nanoRobot has one leg and two foot domains for walking
      - The nanoRobot performs a random walk without any energy supply.
    - nanoRobot has one arm and one hand domain for picking up and dropping off cargos.
      - A single NanoRobot can repeatedly sort multiple cargos.

- **Each NanoRobot’s Actions:**
  - Explores a two-dimensional testing ground on the surface of DNA origami,
  - Picks up multiple cargos of two types that are initially at unordered locations and
  - Delivers cargos to specified destinations, until all molecules are sorted into two distinct piles.

- **Localization on DNA origami:**
  - Allows for distinct cargo-sorting tasks to take place simultaneously in one test tube, or
  - Allows for multiple robots to collectively perform the same task
Parallel Cargo Transport Using Multiple DNA Robots

DNA robots independently execute operations (cargo pickup, random movement to adjacent stepping stones, cargo drop off) via hybridization reactions.

1. Robot picks up cargo at location on DNA origami.

2. Robot randomly moves across DNA stepping stones on origami sheet, to its target location.

3. Each transported cargo is dropped off at its target location on the DNA origami.

Cargo-sorting DNA robots
Conceptual illustration of two DNA NanoRobots:

- The NanoRobots are collectively performing a cargo-sorting task on a DNA origami surface. They transport fluorescent molecules with different colors from initially unordered locations to separated destinations.

Cargo-sorting DNA robots
Task of Sorting Cargo

Flowchart of a cargo-sorting NanoRobots

Cargo-sorting DNA robots

Note: Squiggled lines indicate short toehold domains and straight lines indicate long branch migration domains in DNA strands, with arrowheads marking their 3’ ends
(B) Mechanism of protecting the robot from interactions with tracks and activating the robot only at the beginning of an experiment. The activation reaction is biased forward by using trigger strands at $20 \times$ higher conc. than the inhibited robot.

(C) Mechanism of the robot reaching a goal location.

Cargo-sorting DNA robots
Mechanism of protecting a goal from interactions with cargos and activating the goal only at the beginning of an experiment. The layout of the two types of tracks in all cargo-sorting systems is shown.
3D and 2D schematic diagrams of an eight-step long track on a double-layer DNA origami. The lines between adjacent track locations indicate possible moves of the robot: The two types of track strands are in a checkerboard pattern, and for each step, the robot can only move between two distinct types of tracks. Thus, the hexagonal grid is functionally a square grid for the movement of the robot.
(D) AFM image of the double-layer DNA origami with a track of length 8.

(E) Fluorescence kinetics data of random-walk experiments with eight distinct track lengths and a negative control with no track. A 20-fold excess of free-floating robot strands, relative to the origami concentration, was added at the end of the experiments to measure the maximum possible completion level.

Cargo-sorting DNA robots
Fluorescence kinetics data of cargo-sorting experiments with two distinct types of cargos. In the initial states, cargo1-F and cargo2-F indicate cargos labeled with fluorophores, and goal1-Q and goal2-Q indicate goals labeled with quenchers. The final states show a random choice of the locations of the robot and an unoccupied goal.

AFM images of each type of cargos at their initial locations and delivered to their goal locations, respectively. All images are at the same scale, and the scale bar in the bottom right image is 50 nm.
Demonstrating parallelism with mixed populations of DNA origami and with multiple robots on individual DNA origami surfaces:

(A) Fluorescence kinetics experiments with two mixed populations, each with two types of cargos sorted separately.

(B) Stochastic simulation of sorting two types of cargos as a continuous-time Markov chain. Robot\(_{x,y}\) indicates a robot at an arbitrary track location \((x, y)\). \((x^*, y^*)\) is a neighboring location of \((x, y)\). Cargo\(_i\) and Goal\(_i\) indicate specific types of cargo and goal, respectively. \(d\) is the Euclidean distance between \((x_1, y_1)\) and \((x_2, y_2)\). \(d_{\text{Min}}\) is the Euclidean distance between a robot and a cargo or goal at its immediate neighboring location.

(C) Fluorescence kinetics experiments with multiple robots collectively performing a single cargo-sorting task.
Social DNA Nanorobots

Ming Yang, and John H. Reif
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Challenge

The diversity of the types of activities nanorobots can perform

The practical limitations on the complexity of individual nanobot
• Defined by Wilson in 1975
• Behaviors of social insects:
  • work together to gain resources
  • share their findings with each other
  • defend their home when under attack
  • attack other insects for territory and food
  • communicate by mechanical, optical, or chemical signals (e.g., pheromone)
  • waggle dance by honey bee
  • voting

Waggle Dance by Honey Bee to Communicate direction of Flowers
Activities of Social Insects

- **Random Walking**: where insects of the colony make random walks.
- **Flocking**: a group of insects of the colony follow a selected leader individual insect.
- **Guarding**: a group of the insects of the colony follow, and guard from attack by another group, a particular individual insects of the colony.
- **Attacking**: a group of the insects of the colony attack another group.
- **Communication**: between insects of the colony.
- **Democratic Group Decision Making** (**Voting**): among groups of insects of the colony.
- **Foraging**: a select foraging group of the insects of the colony leave the colony and attempt to discover new sources of food, and then report back to the colony their discoveries.
- **Harvesting**: a harvesting group of insects of the colony travel (navigating by either (a) following successful foragers or (b) following their chemical trail, or (c) via instruction from successful foragers) to the new sources of food and harvest it.
Social DNA nanorobots:
Autonomous mobile DNA devices that execute a series of pair-wise interactions between simple individual DNA nanorobots, causing a desired overall outcome behavior for the group of nanorobots which can be relatively complex.
Motivation

The diversity of the types of activities nanorobots can perform

The practical limitations on the complexity of individual nanobot

DNA nanorobots + social insects
Social DNA nanorobots

execute a series of pair-wise interactions that determine an over-all desired outcome behavior as a group.
Possible Example Behaviors of Social DNA Nanorobots:

- **Walking**, where a group of DNA nanorobots make random traversals of a 2D nanotrack.
- **Self-avoiding Walking**, where a group of DNA nanorobots walk on a 2D nanotrack and avoid the locations visited by themselves or any other DNA nanorobots.
- **Flocking**, where a group of DNA nanorobots follow the movements of a designated leader DNA nanorobot.
- **Guarding**, where a group of DNA nanorobots follow and guard by a particular DNA nanorobot from attack by another group of DNA nanorobots.
- **Attacking**, where a group of DNA nanorobots attack another group of DNA nanorobots.
- **Communication** between pairs of nearby DNA nanorobots: where a finite amount of information is transferred between a pair of nearby DNA nanorobots.
- **Voting by Assassination**, a process where there are originally two unequal size groups of DNA nanorobots; when pairs of DNA nanorobots from distinct groups collide, one or the other will be assassinated (by getting detached from the nanotrack); eventually all members of the smaller groups of DNA nanorobots are assassinated with high likelihood.
- **Foraging** and **Harvesting**, where a group of designated foraging DNA nanorobots randomly walk on the 2D nanotrack and can transform to a “discovery state” when they discover a target molecule (e.g., a group of gold nanoparticles attached to 2D surface; a group of harvesting DNA nanorobots which follow the trail of foraging DNA nanorobots in discovery state, pick up the detected target molecules and deliver the target molecules to a designated region of the 2D nanotrack.
Social DNA Nanorobots

• **Goal:** to increase the complexity of the various tasks the nanorobots can execute and at the same time preserve a low design complexity for individual nanorobots.

• **Results:** Give detailed designs for social DNA nanorobots that perform novel behaviors of Self-avoiding Walking, Flocking, and Voting by Assassination.

• **Simulations:** Their behaviors were simulated in the 2D surface CRN model.
Social DNA Nanorobots
Yang, and John H. Reif, Social DNA Nanorobots, pp 371-396, Invited Chapter for Book on DNA Nanotechnology at 40 for the next 40 – A Tribute to Nadrian C. Seeman, (edited by Natasha Jonoska and Erik Winfree, book series Natural Computing, Springer (2023)).

• **Results:** designs for social DNA nanorobots that walk over a 2D nanotrack and collectively exhibit various programmed behaviors. These employ only hybridization and strand-displacement reactions, without use of enzymes.

• **List of types of social DNA nanorobots designed:**
  – **Self-avoiding Random Walking:** where a group of DNA nanorobots randomly walk on a 2D nanotrack and avoid the locations visited by themselves or any other DNA nanorobots.
  – **Flocking:** where a group of DNA nanorobots follow the movements of a designated leader DNA nanorobot.
  – **Voting by Assassination:** a process where there are originally two unequal size groups of DNA nanorobots; when pairs of DNA nanorobots from distinct groups collide, one or the other will be assassinated (by getting detached from the 2D nanotrack and diffusing into the solution away from the 2D nanotrack); eventually all members of the smaller groups of DNA nanorobots are assassinated with high likelihood.

• **Simulation of these social DNA nanorobots:** used a surface-based CRN simulator.
Surface CRN Simulator

Written by Samuel Clamons.
This simulator is a companion to [1]. Simulates chemical reaction networks on a surface, as described by Lulu Qian and Erik Winfree in [2].
You can also run this simulator locally using the python package surface_crs.
For questions, comments, and/or bug reports, contact the author at sclamons@caltech.edu with "surface CRNs" in the subject line.


Paper Examples:
Enter a manifest or choose an example

Miscellaneous Examples:
Enter a manifest or choose an example

What's a surface CRN?
In brief, a surface CRN is a stochastic chemical reaction network where individual molecules are tethered to fixed positions on a surface such that they can only interact with neighbors; stated another way, a surface CRN is an asynchronous cellular automata with transition rules that resemble those of unimolecular and bimolecular chemical reactions.

A surface CRN consists of a number of sites with states on an arbitrary lattice (we usually use a square grid, but a surface CRN could use any graph as its lattice), along with transition rules of the form $A \rightarrow B$ or $A + B \rightarrow C + D$, with a real-valued reaction rate for each transition rule. States change stochastically according to the transition rules for the surface CRN, with expected time specified by the reaction rate for each reaction.

For example, a site with state $A$ can undergo $A \rightarrow B$ to instantaneously switch to state $B$.

https://www.dna.caltech.edu/Surface_CRN_Simulator/srv/
Known Design for Bidirectional Random DNA Nanorobot Walker

Based on cartwheeling bidirectional DNA walker:
Jieming Li & Nils Walter, Exploring the speed limit of toehold exchange with a cartwheeling DNA acrobat, August 2018, Nature Nanotechnology 13(8) DOI: 10.1038/s41565-018-0130-2
Mpeg of Simulation of Random Walking by Nanorobot
(using Surface CRN Simulator)

$T = 0.08$

- Background (O)
- Head (W)
- Line (L)
Design for Self-avoiding Bidirectional Random DNA Nanorobot Walker

**Self-avoiding Random Walking:** where a group of DNA nanorobots randomly walk on a 2D nanotrack and avoid the locations visited by themselves or any other DNA nanorobots.
Mpeg of Simulation of Self-Avoiding Walking by Nanorobot
(using Surface CRN Simulator)

T = 0.25
Design for Flocking by a Group of Nanorobots following a Leader:

A group of DNA nanorobots (type $W_2$) follow the movements of a leader DNA nanorobot (type $W_1$).
Mpeg of Simulation of Flocking by Group of Nanorobots following a Leader
(using Surface CRN Simulator)

\[ T = 0.02 \]

- Background \((O)\)
- Walker1\&2 \((W)\)
- Walker1 \((W1)\)
- Walker2 \((W2)\)
- Walker2 Trace \((T)\)

Green grids show the trace of the Walker2
Design of DNA Nanorobots that Vote by Assassination

There are initially two unequal size of DNA nanorobots; when pairs of DNA nanorobots from distinct groups meet each other, one or the other is assassinated; eventually all members of the smaller group are assassinated with high likelihood.
Design of DNA Nanorobots that Vote by Assassination

**Voting by Assassination:** a process where there are originally two unequal size groups of DNA nanorobots; when pairs of DNA nanorobots from distinct groups collide, one or the other will be assassinated (by getting detached from the 2D nanotrack and diffusing into the solution away from the 2D nanotrack); eventually all members of the smaller groups of DNA nanorobots are assassinated with high likelihood.
Mpeg of Simulation of DNA Nanorobots that Vote by Assassination
(using Surface CRN Simulator)
Number of Walker1: \( N_1 = 10 \),
Number of Walker2: \( N_2 = 6 \)

\[ T = 0.25 \]
Mpeg of Simulation of DNA Nanorobots that Vote by Assassination
(using Surface CRN Simulator)

Number of Walker1: $N_1 = 8$,
Number of Walker2: $N_2 = 8$

$T = 0.17$
Graphs of Simulations of DNA Nanorobots that Vote by Assassination
(using Surface CRN Simulator)

Assume: initial $n_1 > n_2$ ($n_2/n_1$ in $[0,1]$)
Compare different $n$ (where $n=n_1+n_2$)