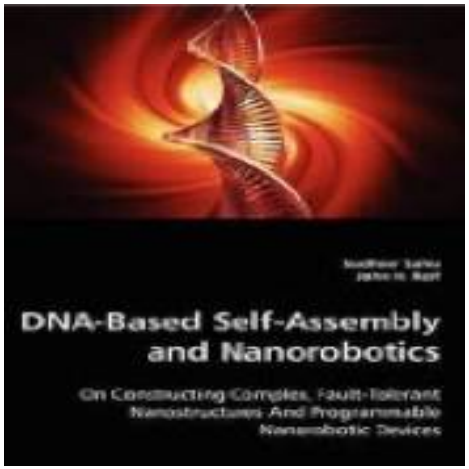
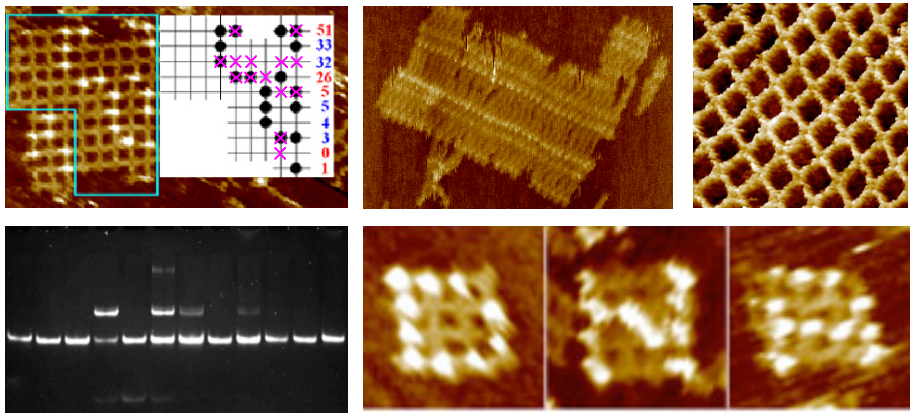
DNA-Based Programmable Autonomous Molecular Robotic Devices

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

Harish Chandran

Urmi Majumder

Peng Yin

Harish Chandran

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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 hybrization-reaction driven device for exact matching of complementary DNA strands

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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,

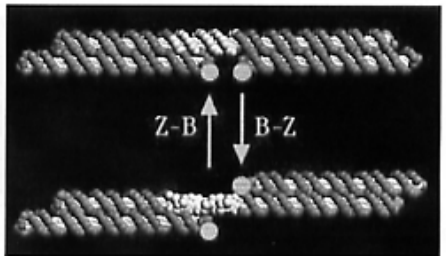
(ii) programmable: the tasks the molecular devices execute can be modified without an entire redesign and

iii) autonomous: they operate without external mediation (e.g. thermal cycling).

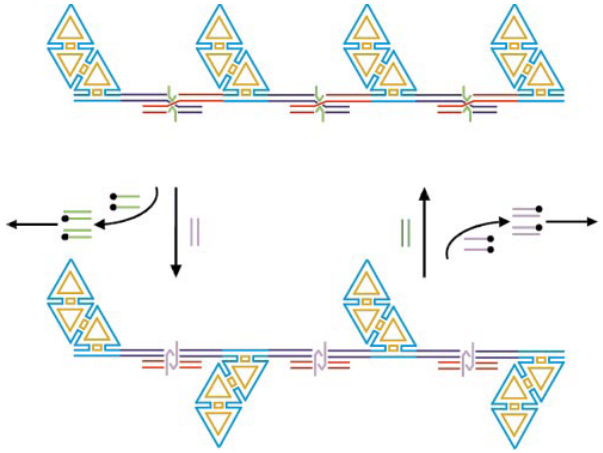
Non-Autonomous DNA based Nanorobotical devices

Advantages of DNA-based synthetic molecular devices:

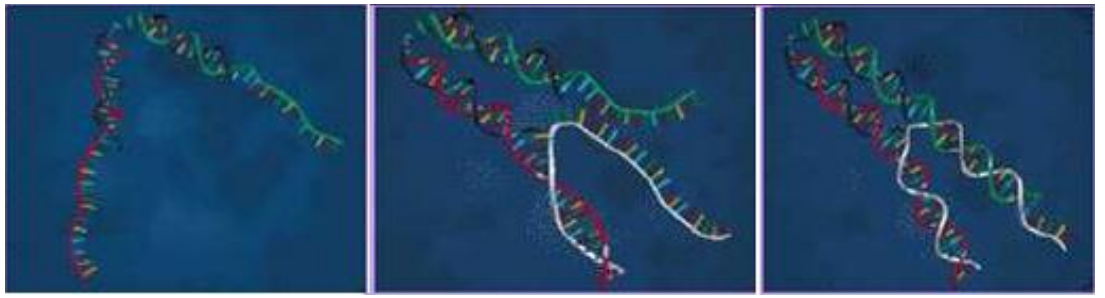
- simple to design and engineer
- well-established biochemistry used to manipulate DNA nanostructures



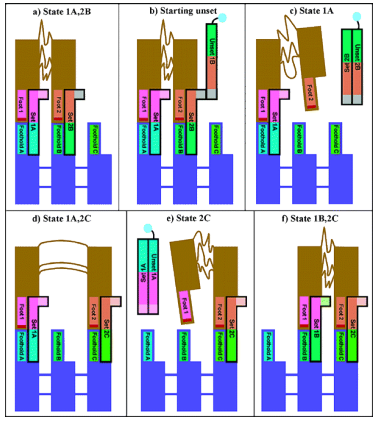
B-Z transition device
[Mao, Seeman 99]



PX-JX transition [Yan et al 02]



DNA-fuelled Molecular machine [Yurke et al 00]



DNA Biped walker
[Sherman et al 04]

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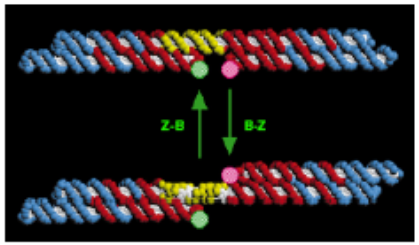
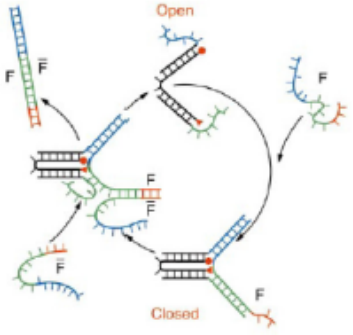
Early DNA robotics devices needed external control, so not autonomous

Rotation



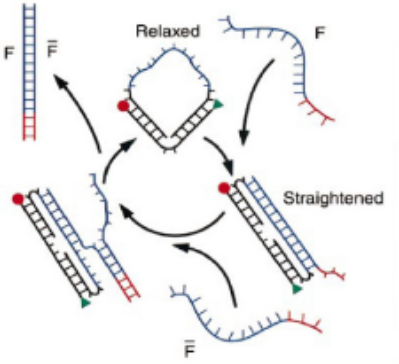
(Mao *et al* 99)

Open/close



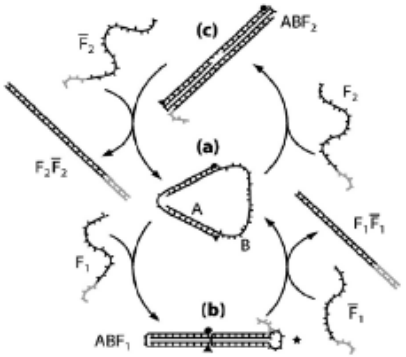
(Yurke *et al* 00)

Open/close



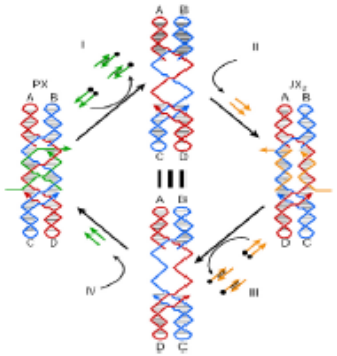
(Simmel *et al* 01)

Open/close



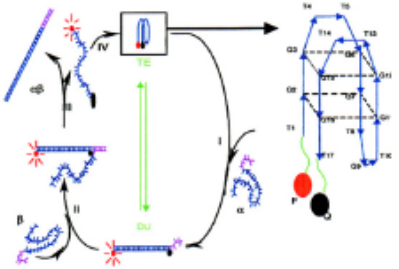
(Simmel *et al* 02)

Rotation



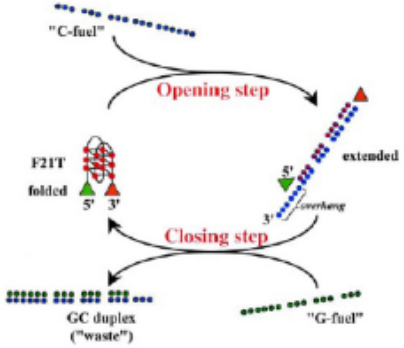
(Yan *et al* 02)

Extension/contraction



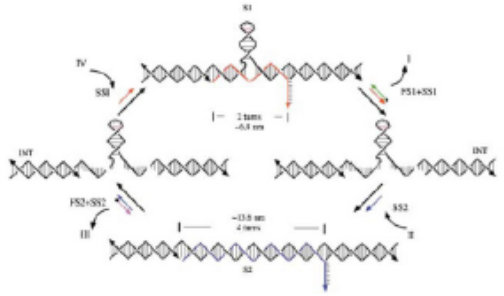
(Li *et al* 02)

Extension/contraction



(Alberti *et al* 03)

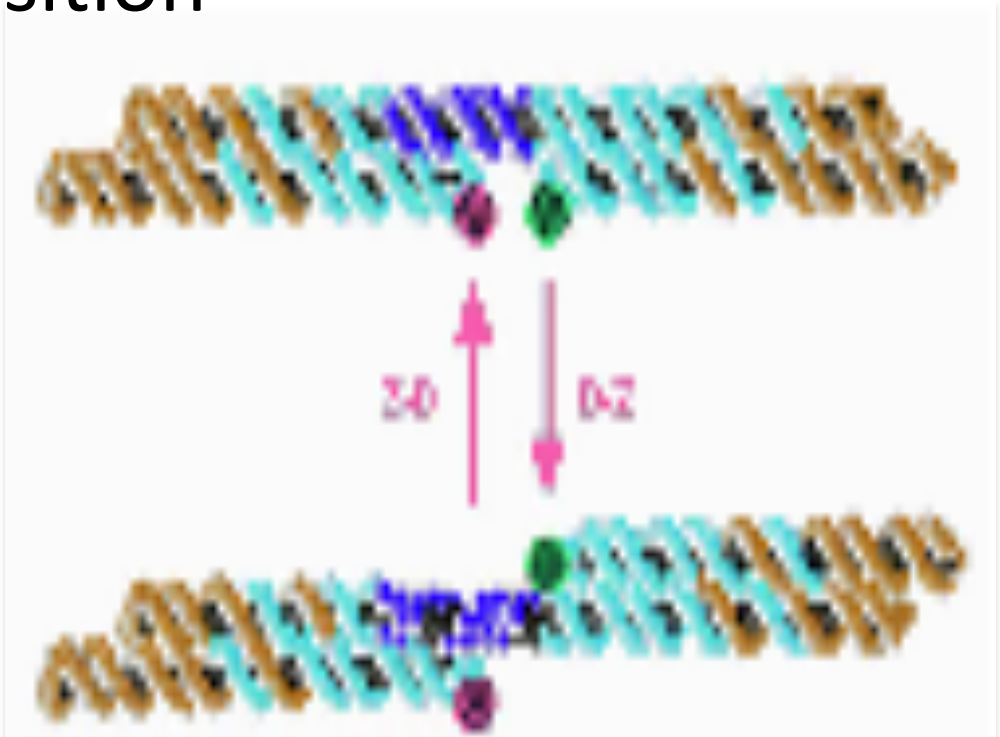
Extension/contraction



(Feng *et al* 03)

NonAutonomous DNA Nanorobotics

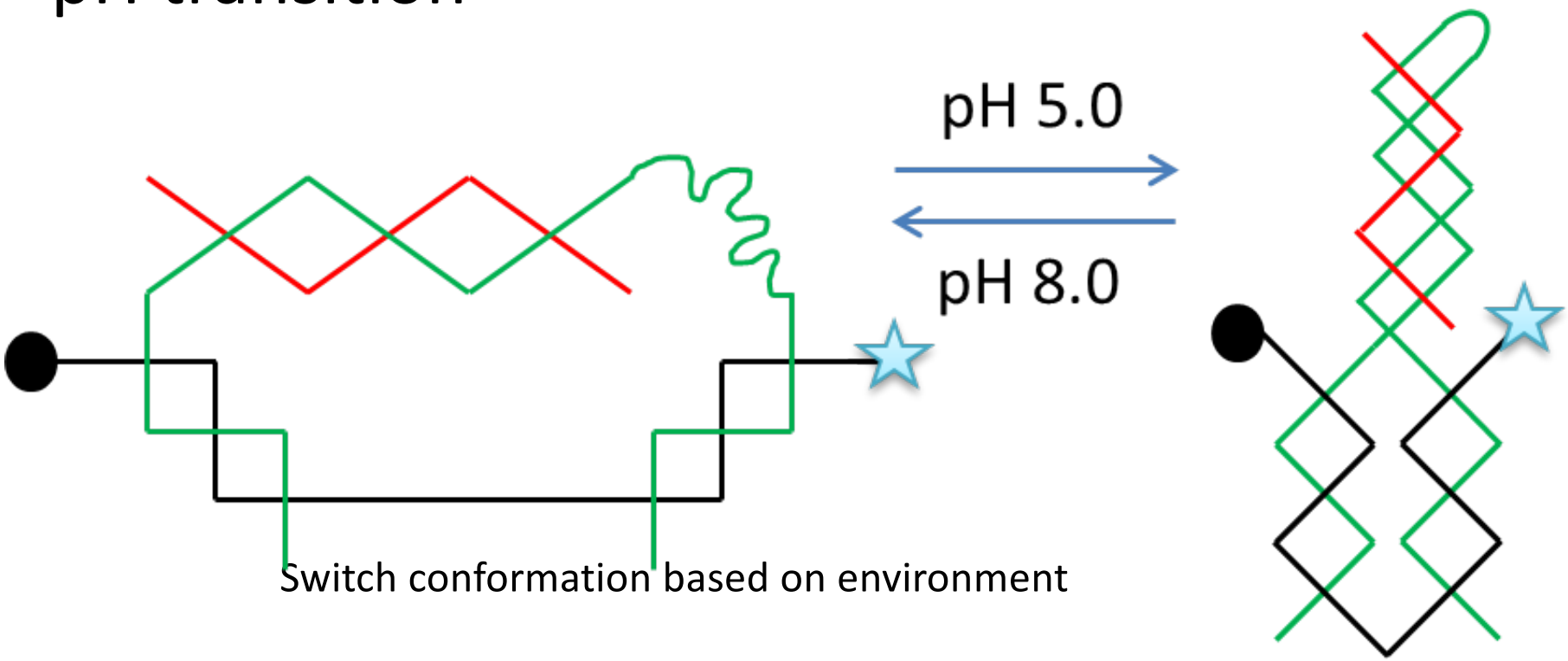
- B-Z transition



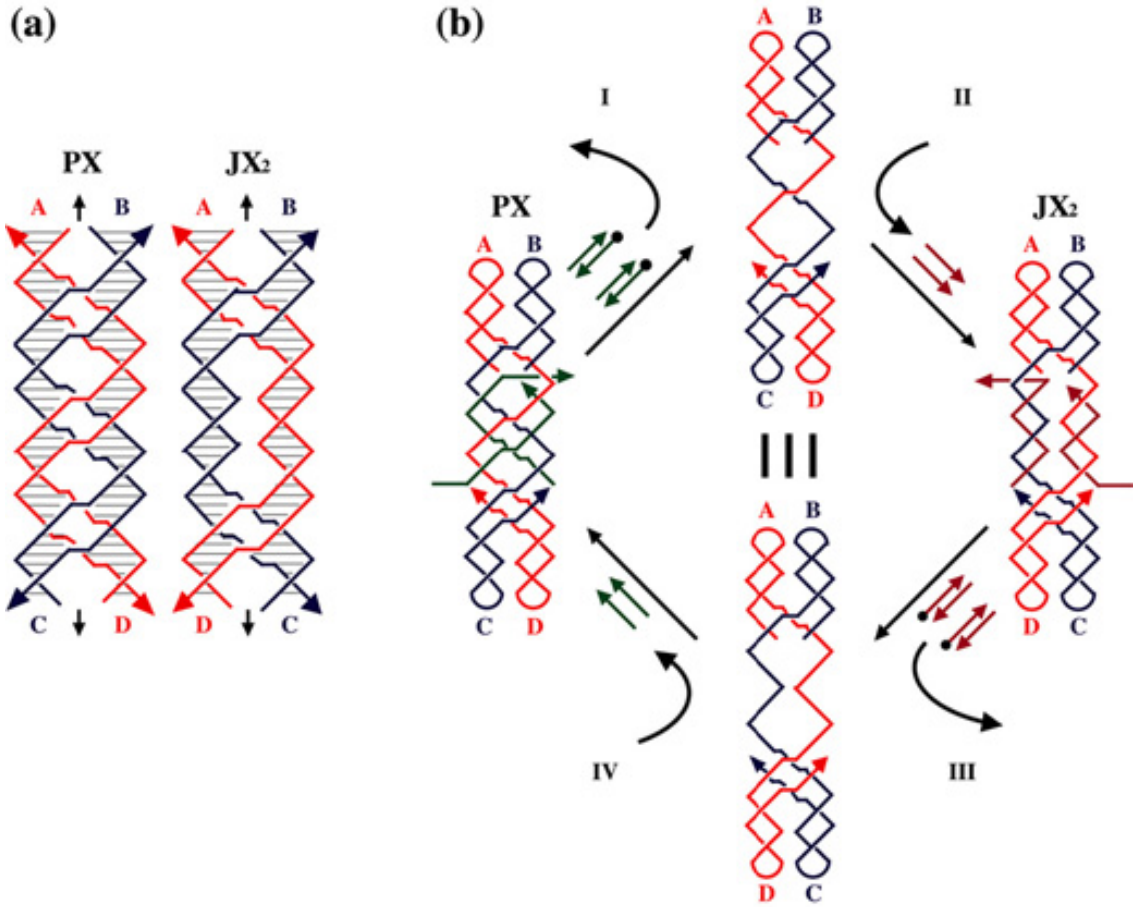
Switch conformation based on environment

NonAutonomous DNA Nanorobotics

- pH transition



Non-Autonomous DNA based Nanorobotical 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 180û 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

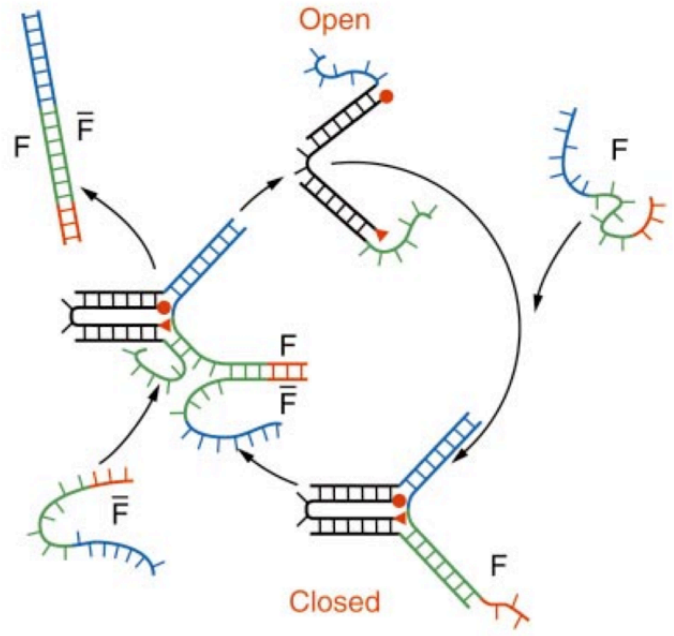
DNA Tweezers:

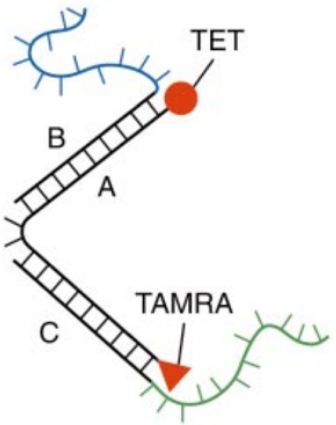
- Nonautonomous Device

- Used Strand Displacement

Bell Labs/Oxford

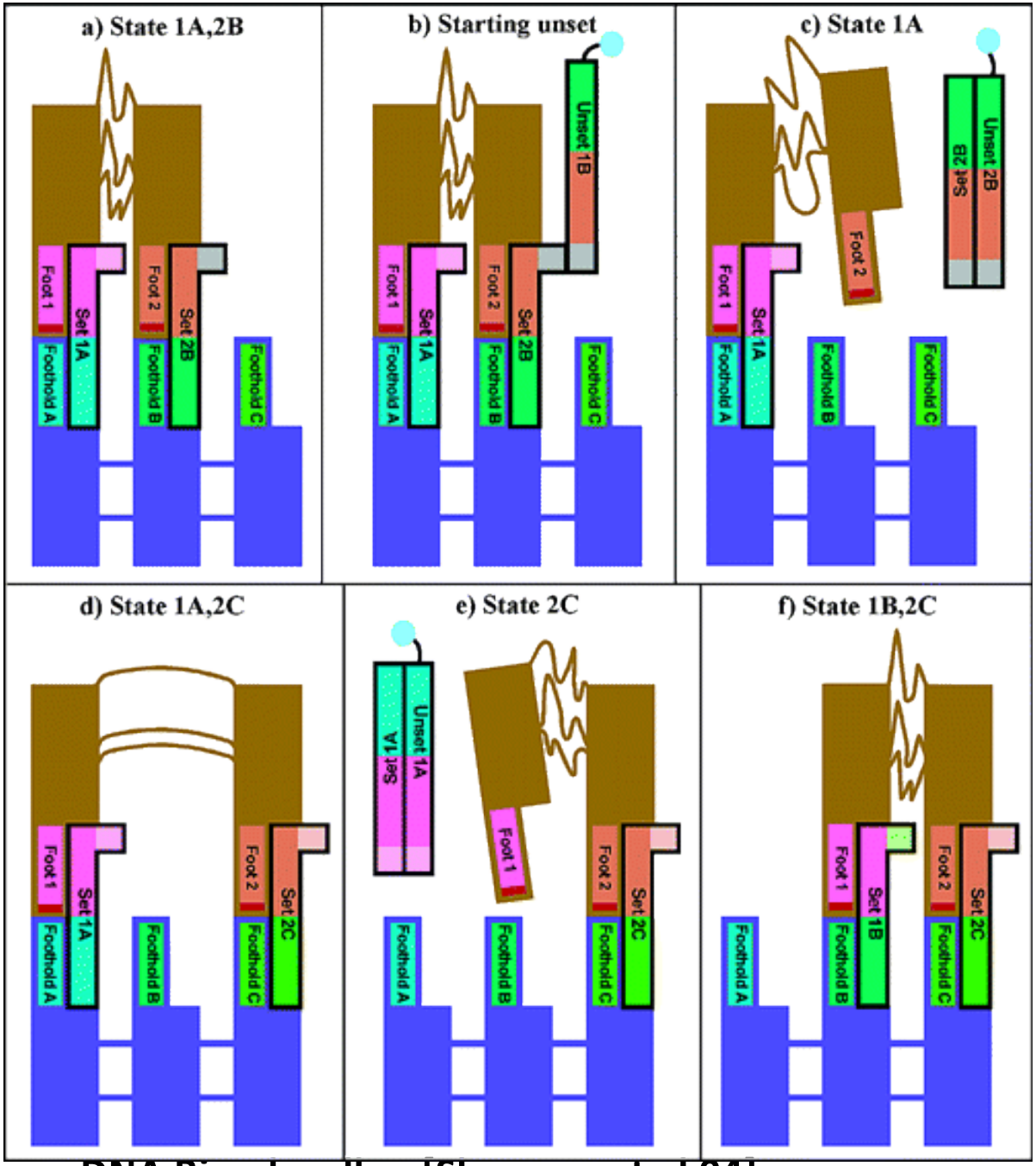
2000





A DNA-fuelled molecular motor made of DNA

Non-Autonomous DNA based Nanorobotical devices



DNA Biped walker [Sherman et al 04]

Non-Autonomous DNA based Nanorobotical devices

DNA Biped walker [Sherman et al 04]

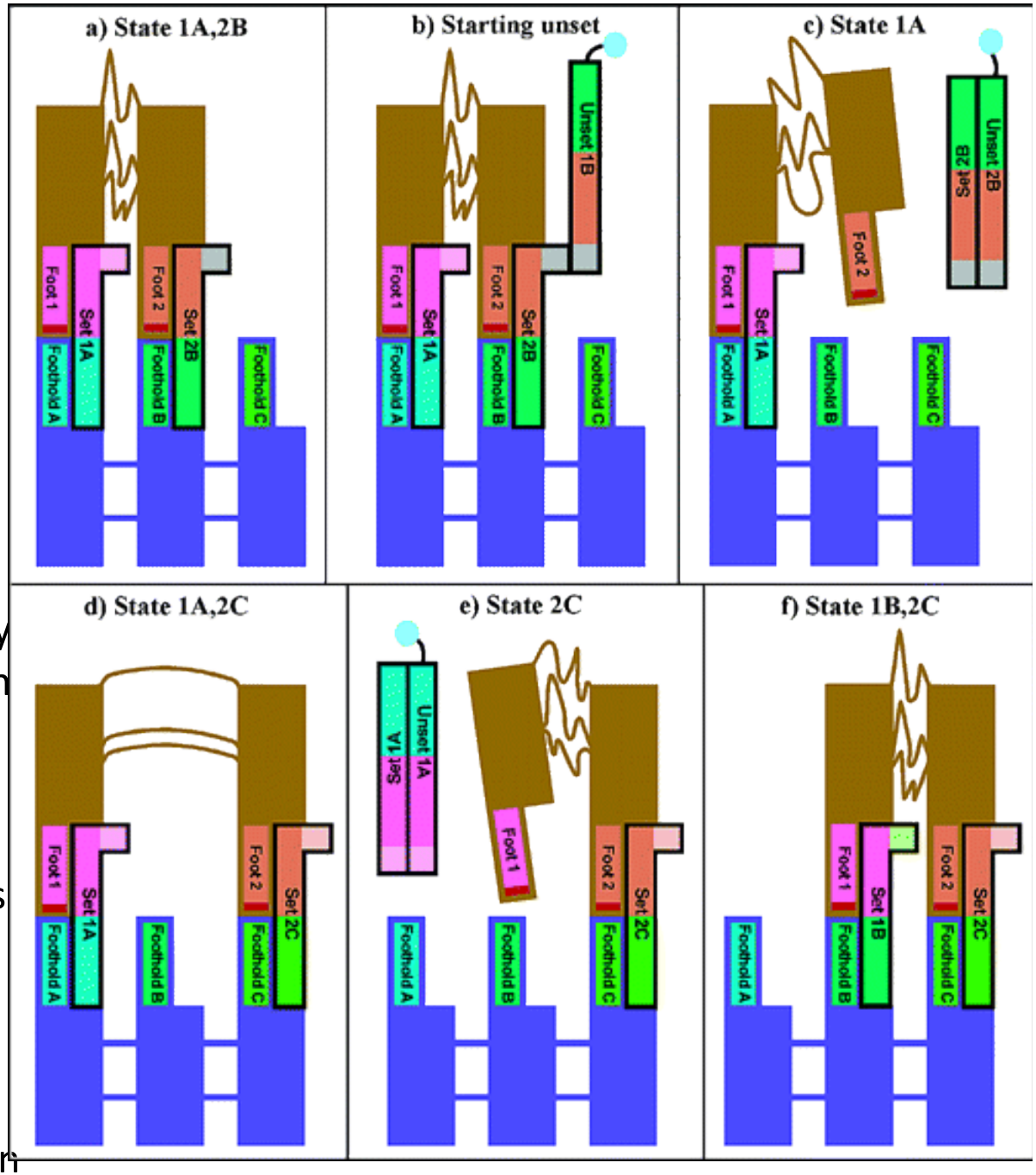
The biped walker moves forward in an inchworm fashion where the relative positions of the leading and trailing leg do not change.

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.



Non-Autonomous DNA based Nanorobotical deviceså

DNA Biped walker [Sherman and Pierce2004]

J-S Shin, N Pierce, A Synthetic DNA Walker for Molecular Transport, Journal of American Chemical Society, vol. 126, no. 35, p. 10834–10835, 2004.

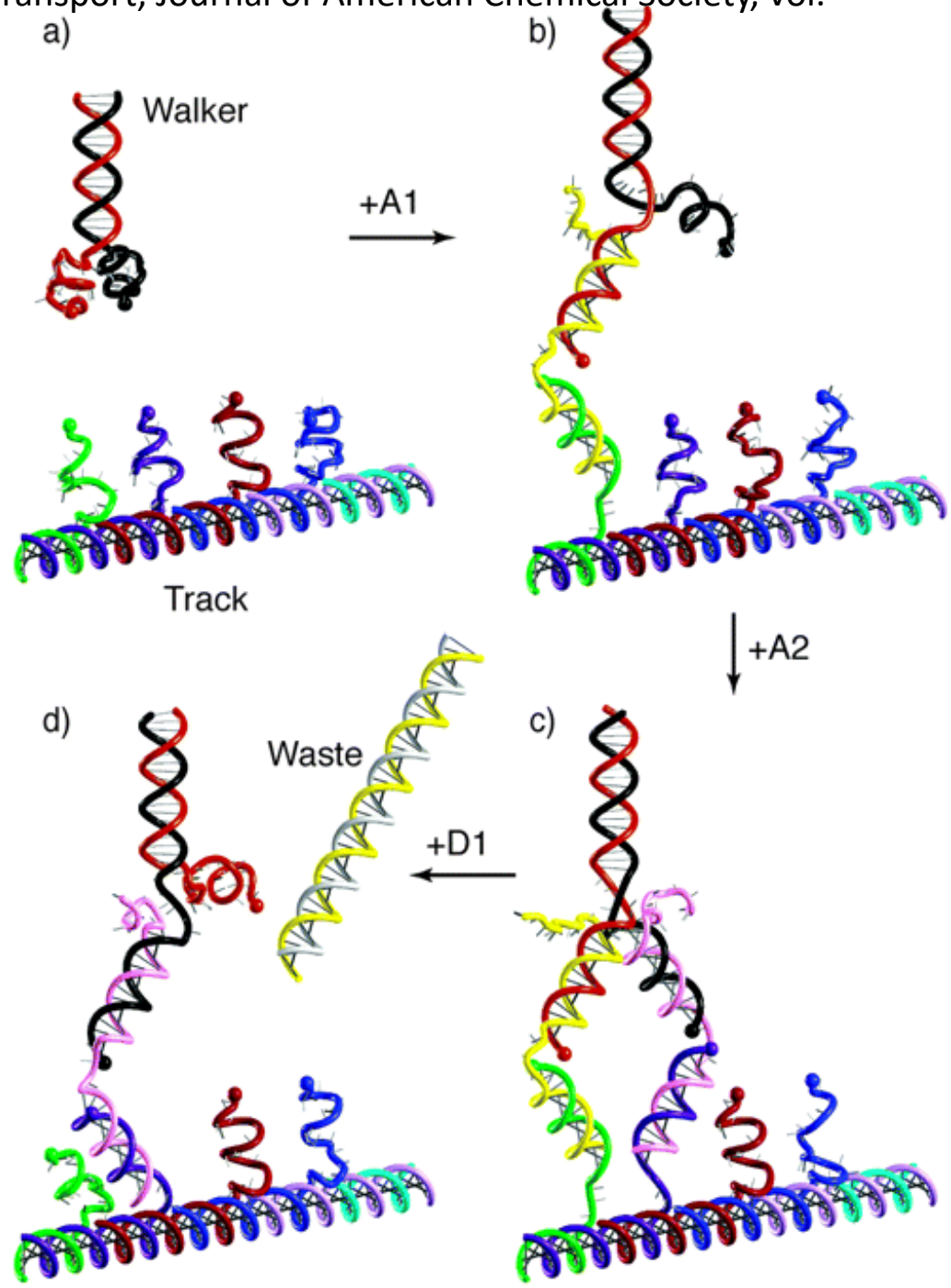
Walker moves in a **foot over foot manner** (like kinesin) - each step the trailing foot swings past the leading foot.

- 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.



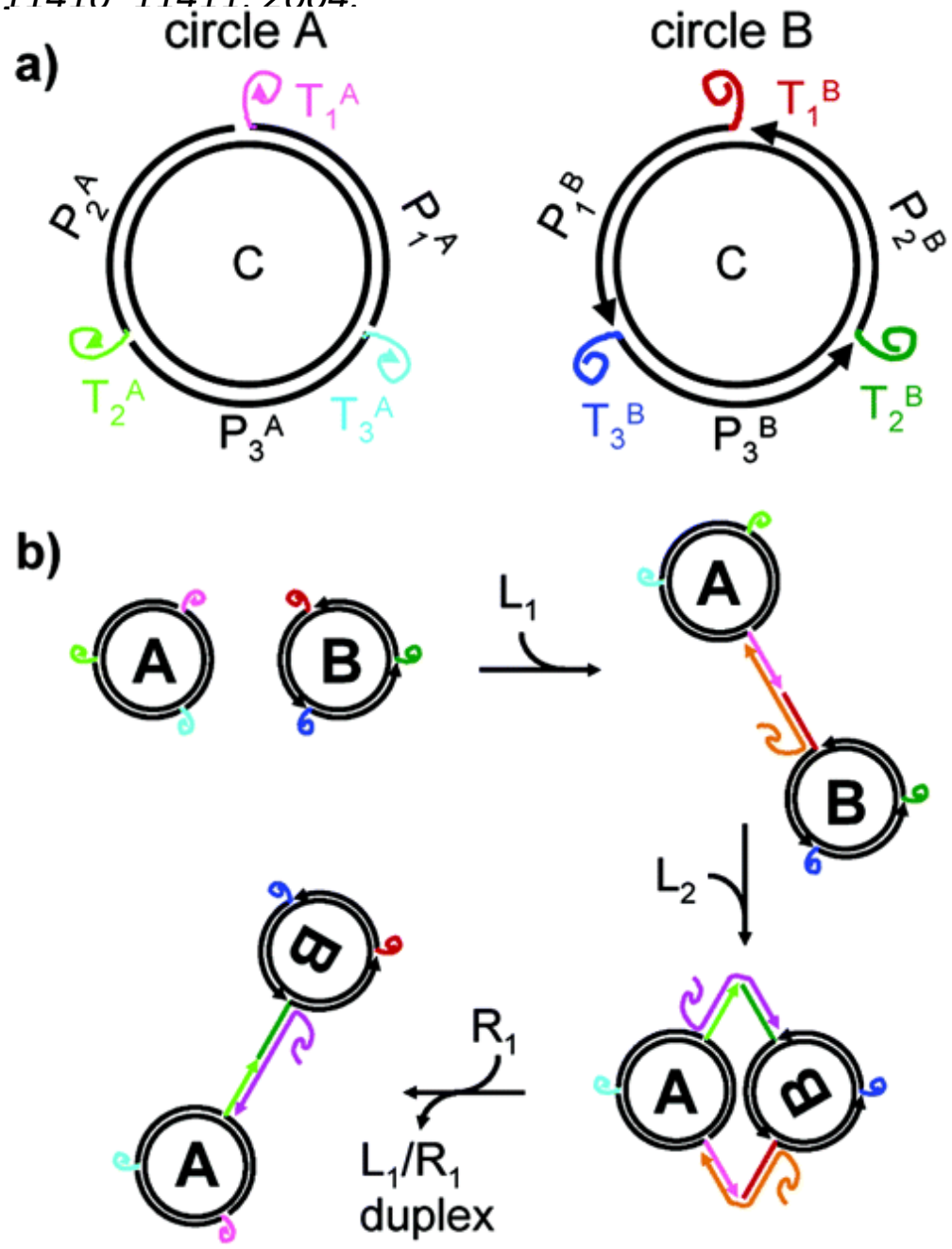
Non-Autonomous DNA based Nanorobotic devices

DNA Biped walker Tian&Mao2004]

Y Tian, C Mao, Molecular Gears: A Pair of DNA Circles Continuously Rolls against Each Other, Journal of American Chemical Society, vol. 126, no. 37, p. 11410–11411, 2004.

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.



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Autonomous DNA Walkers:
DNA Devices that Walk on DNA Nanostructures

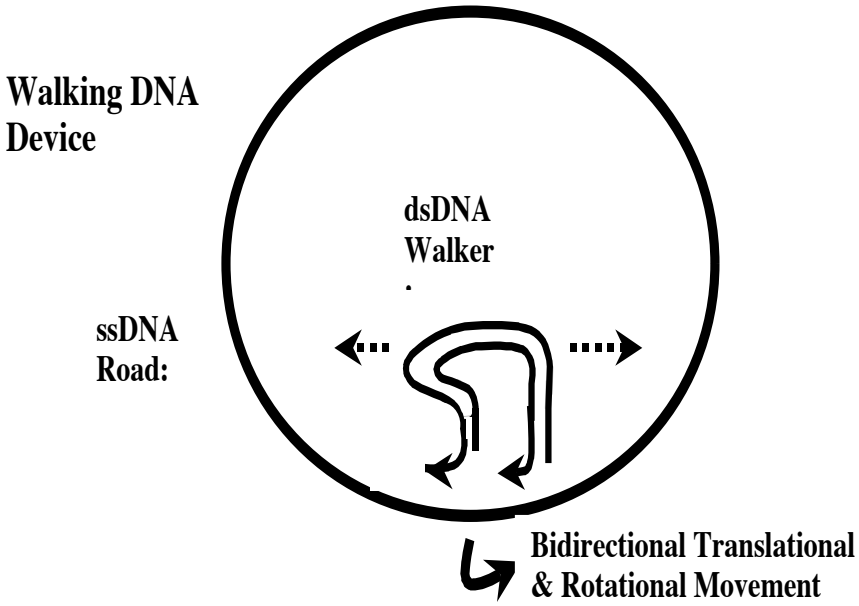
First DNA Walker Devices: Formulation & First Designs

[Reif, 2002]

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

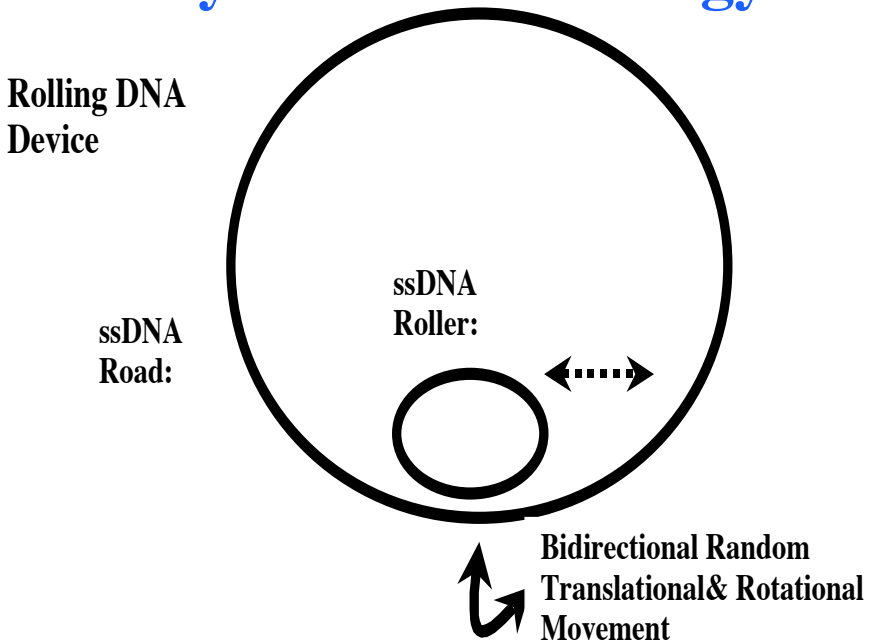
Walking DNA device

Use ATP consumption



Rolling DNA device

Use hybridization energy



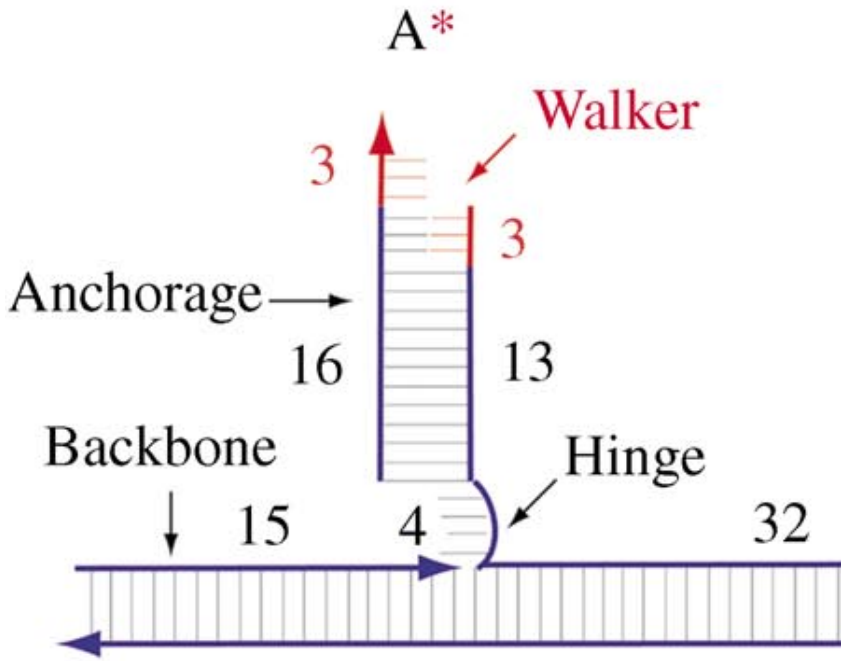
These DNA devices translate across a circular strand of ssDNA and rotate simultaneously.

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



Yin, P., Yan, H., Daniell, X. G., Turberfield, A. J., & Reif, J. H. (2004). A Unidirectional DNA Walker That Moves Autonomously along a Track. *Angewandte Chemie International Edition*, *43*(37), 4906–4911. doi:10.1002/anie.200460522

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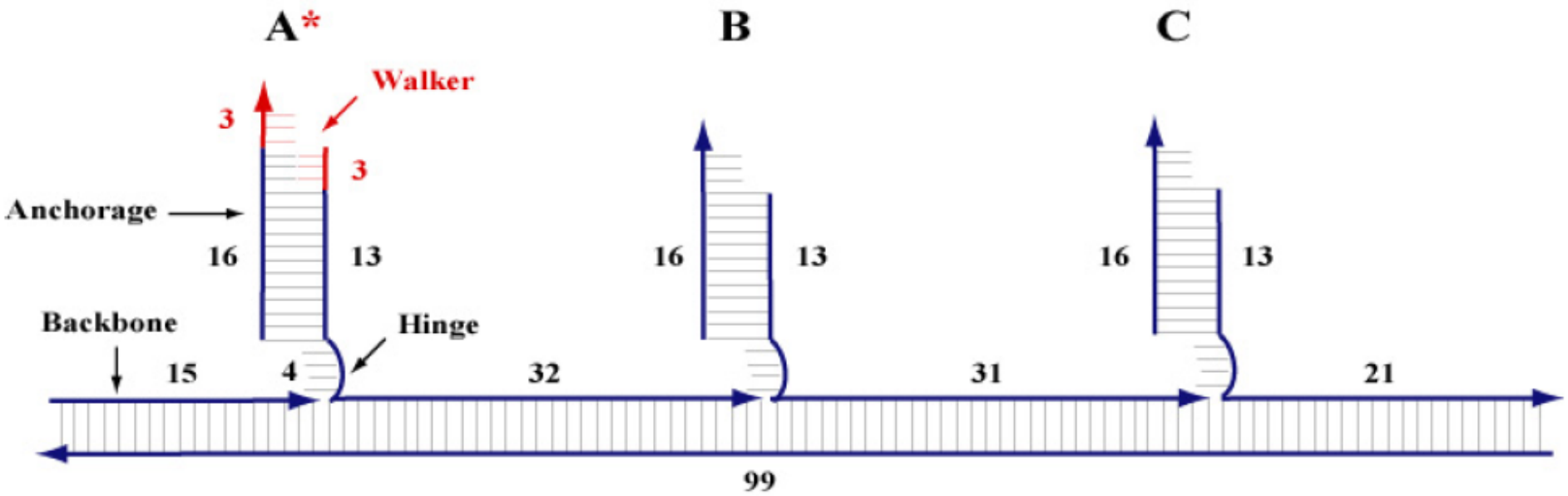
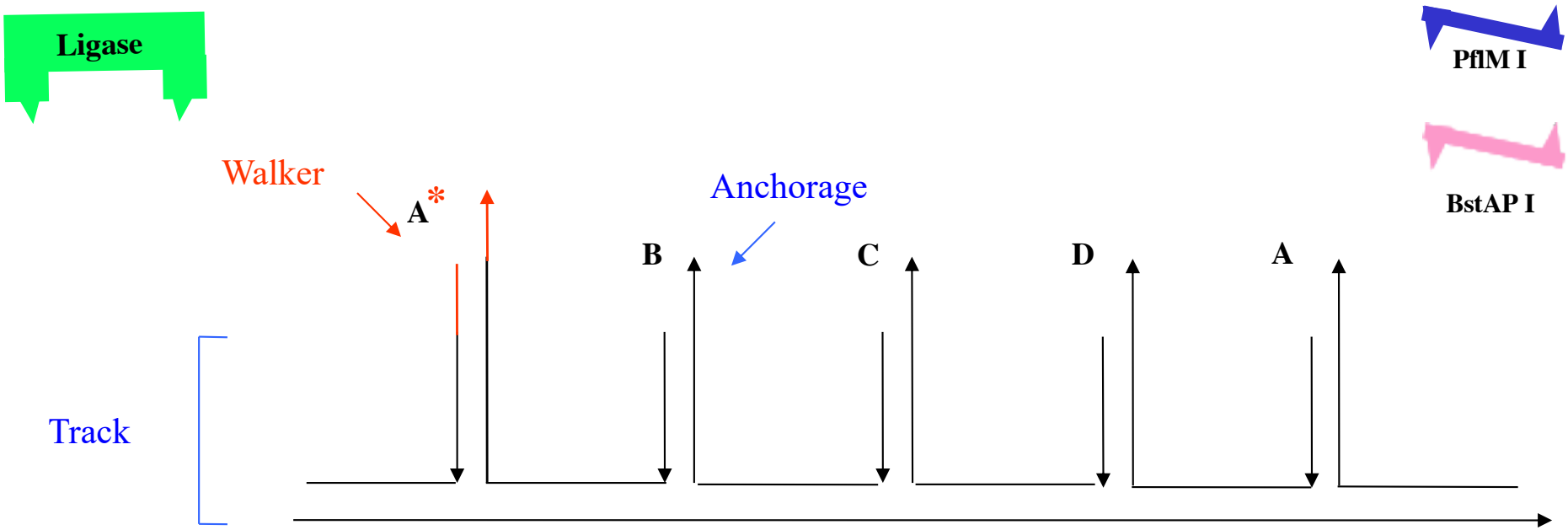
Our work: DNA walker
First autonomous DNA robotic device

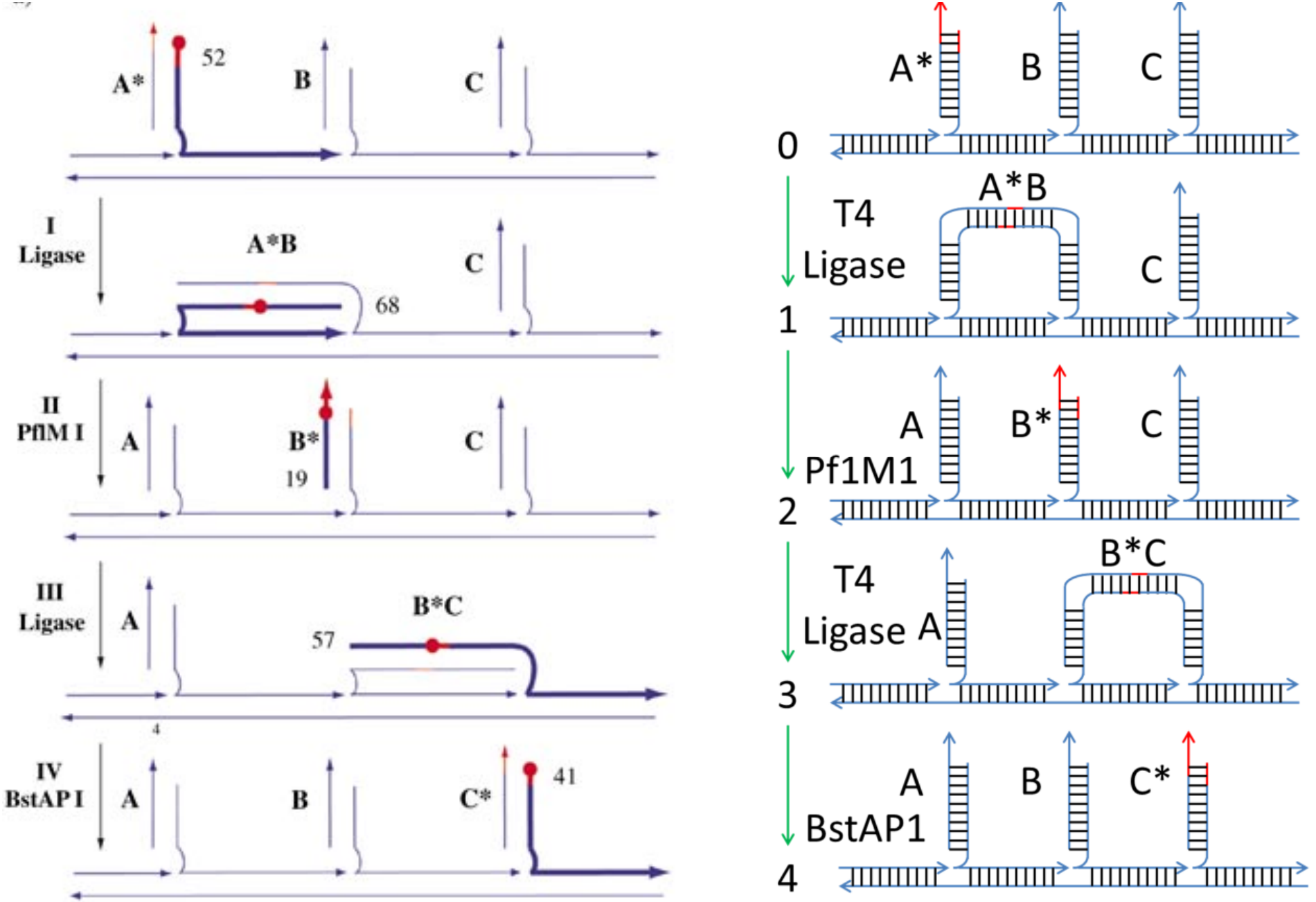


- Very first design for DNA walker
- Series of stators (blue)
- One walker (red)
- Use of ligase and restriction enzymes

Demonstrated First Autonomous DNA Walker:

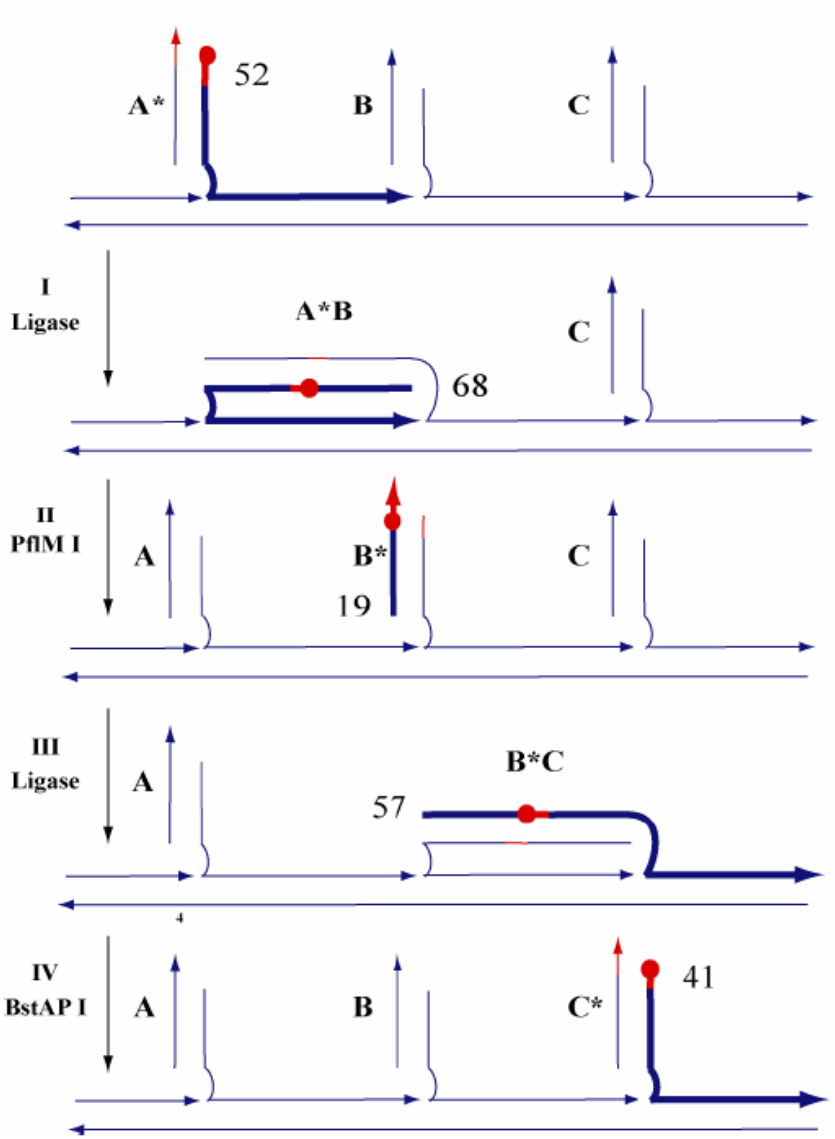
Peng Yin, Hao Yan, Xiaoju G. Daniel, Andrew J. Turberfield, John H. Reif, A Unidirectional DNA Walker Moving Autonomously Along a Linear Track, Angewandte Chemie Volume 43, Number 37, Sept. 20, 2004, pp 4906-4911.

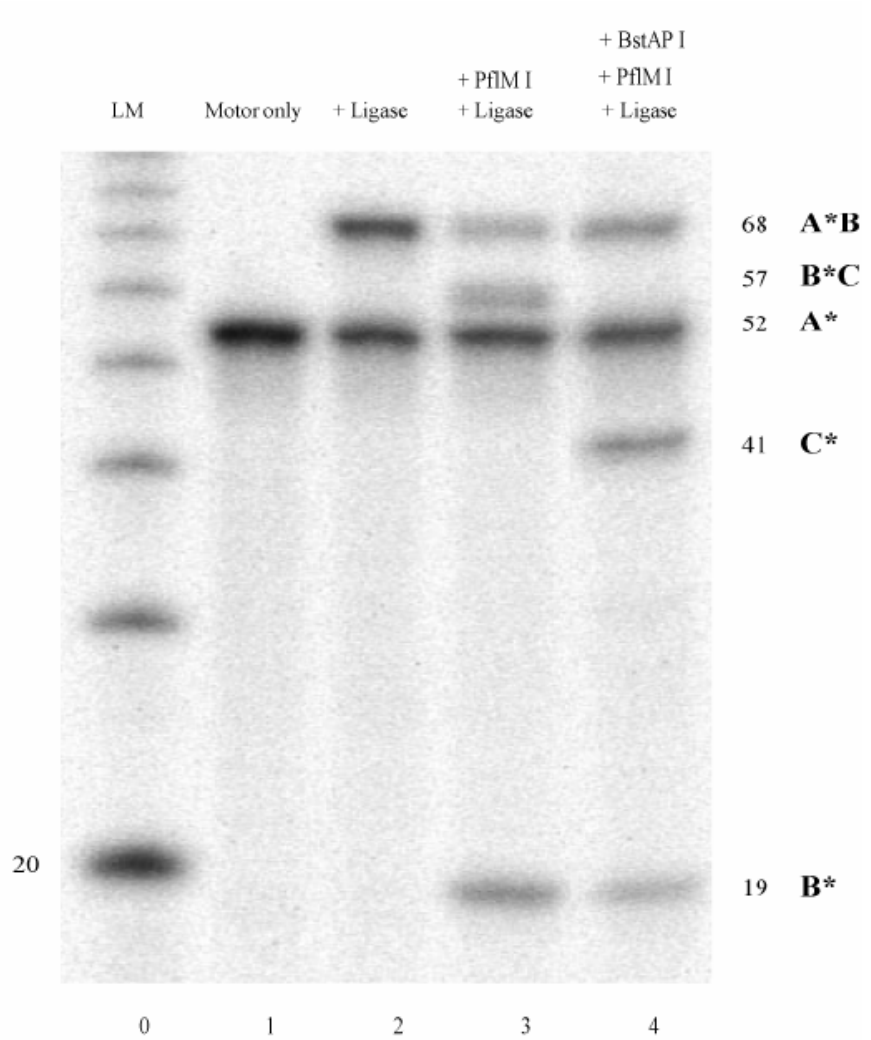




Yin, P., Yan, H., Daniell, X. G., Turberfield, A. J., & Reif, J. H. (2004). A Unidirectional DNA Walker That Moves Autonomously along a Track. *Angewandte Chemie International Edition*, *43*(37), 4906–4911. doi:10.1002/anie.200460522

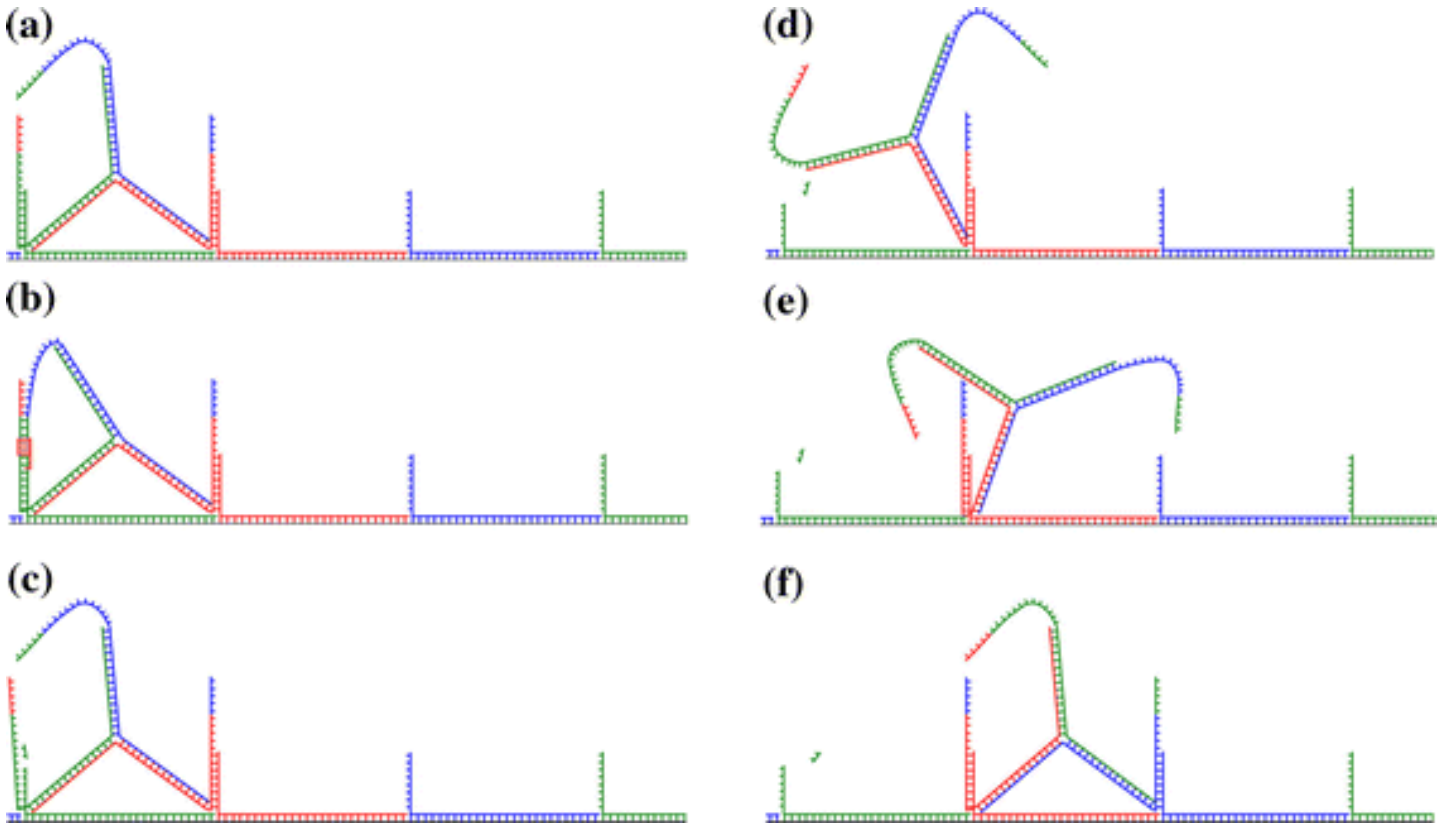
DNA walker motion





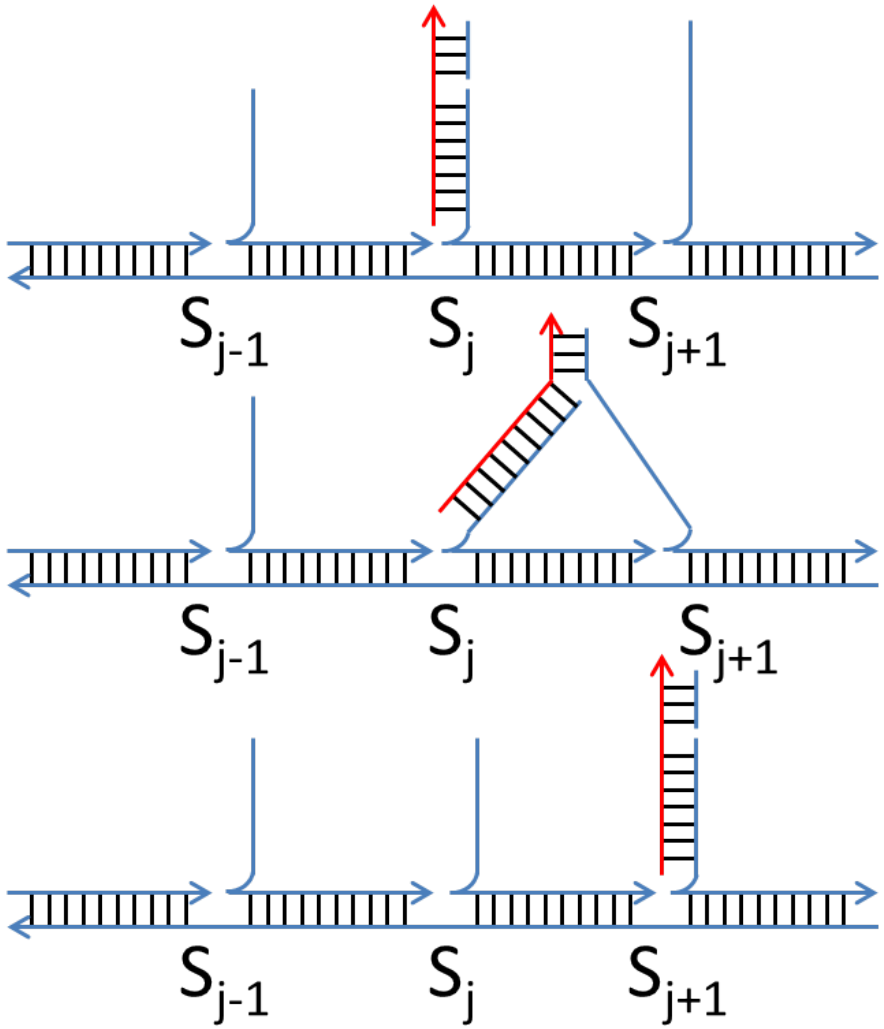
Peng Yin, Hao Yan, Xiaoju G. Daniel, Andrew J. Turberfield, John H. Reif, **A Unidirectional DNA Walker Moving Autonomously Along a Linear Track**, Angewandte Chemie [International Edition], Volume 43, Number 37, Sept. 20, 2004, pp. 4906-4911

Other Walkers powered by Restriction Enzymes:



H Sekiguchi, K Komiya, D Kiga, M Yamamura, A Design and Feasibility Study of Reac- tions Comprising DNA Molecular Machine that Walks Autonomously by Using a Restriction Enzyme, Natural Computing, vol. 7, no. 3, pp. 303-315, 2008.

Other Walkers powered by Restriction Enzymes:

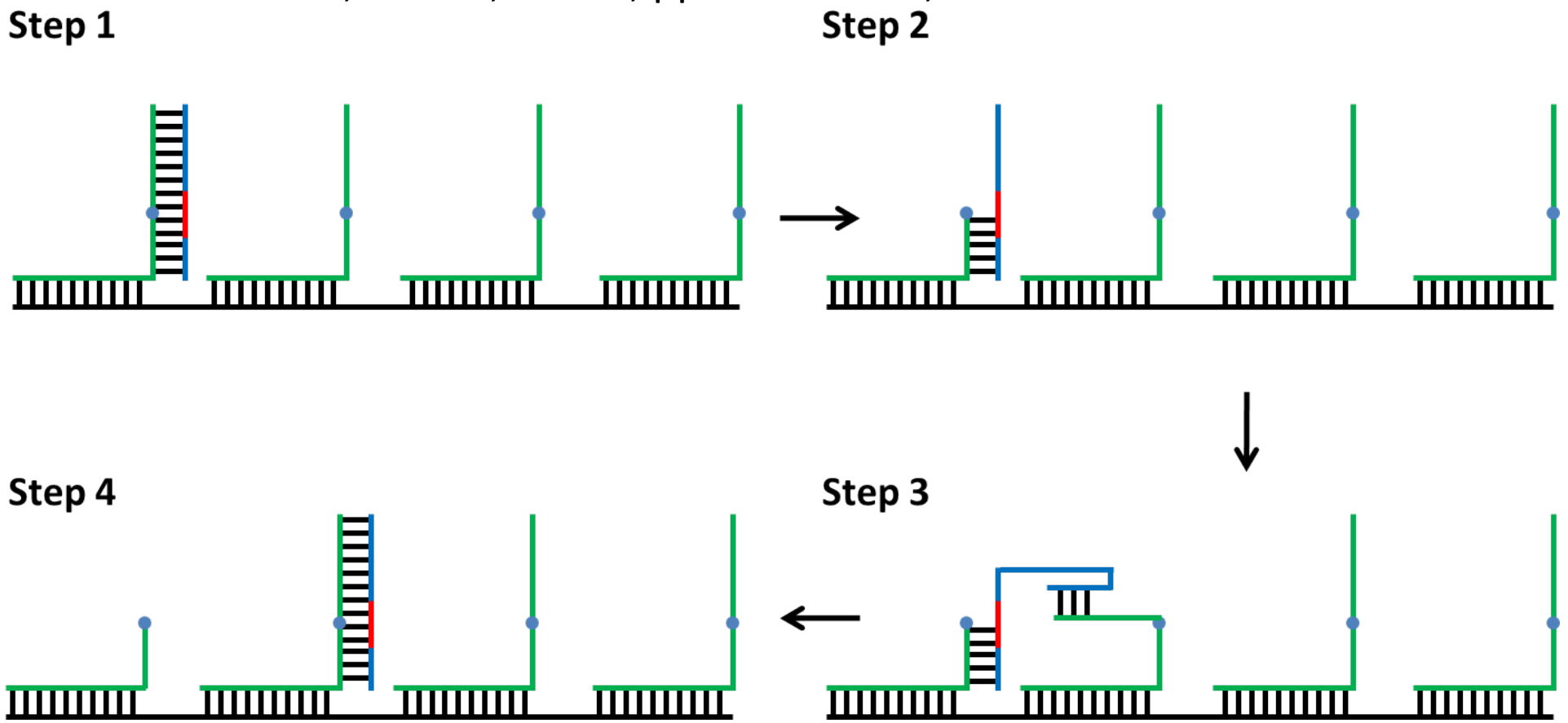


J Bath, S Green, A Turberfield, A Free-Running DNA Motor Powered by a Nicking Enzyme, Angewandte Chemie International Edition, vol. 44, no. 28, pp. 4358-4361, 2005.

Other Walkers powered by DNAenzymes:

[Tian & Mao 2005]

Y Tian, Y He, Y Chen, P Yin, C Mao, A DNAzyme That Walks Processively and Autono- mously along a One-Dimensional Track, Angewandte Chemie International Edition, vol. 44, no. 28, pp. 4355-4358, 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

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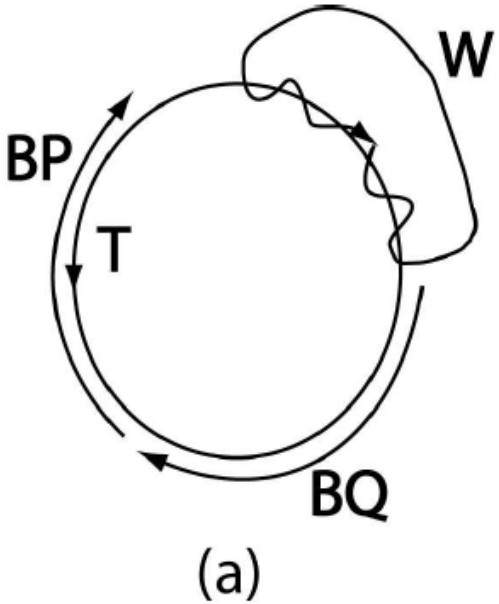
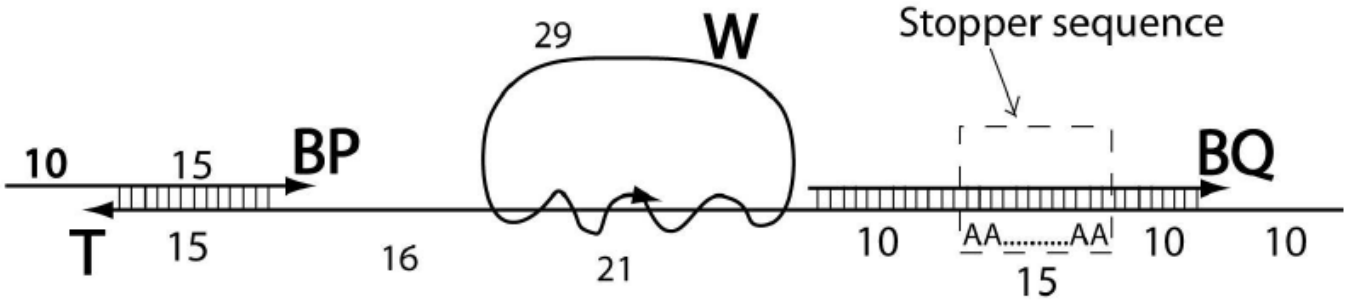
DNA Wheels

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)



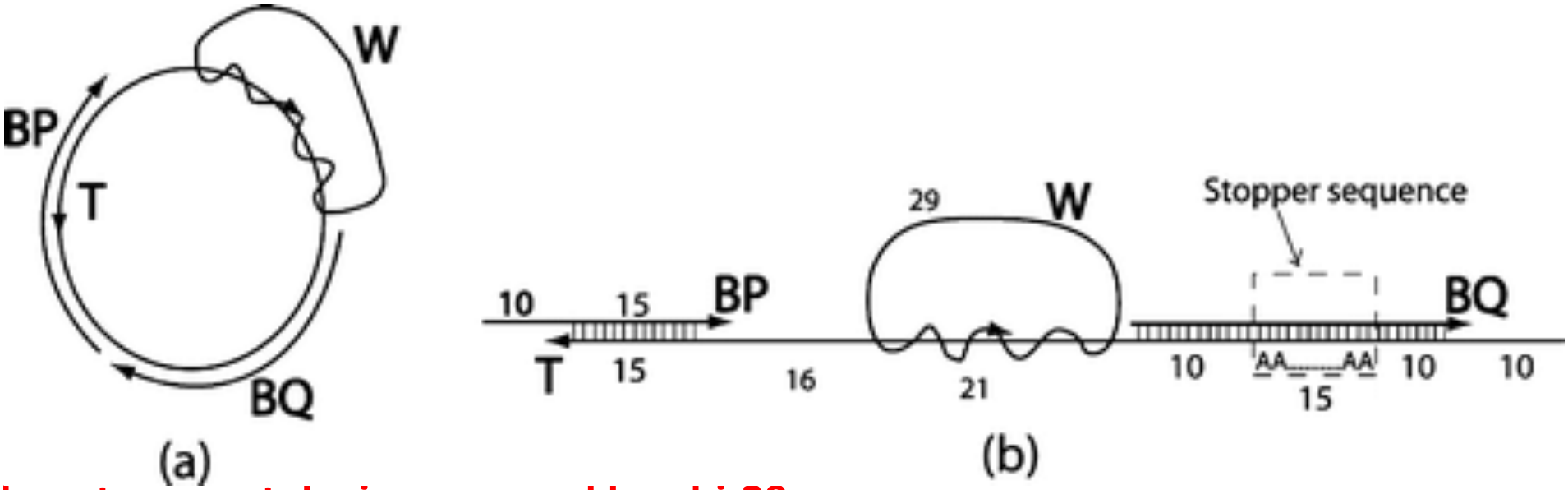
(a)



(b)

- phi-29 strand displacing polymerase
- Pushes cargo strand around a circular track

Walker powered by Polyerase:



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.

S

Sahu, T LaBean, J Reif, A DNA Nanotransport Device Powered by Polymerase ϕ, Nano Letters, vol. 8, no. 11, pp. 3870-3878, 2008.

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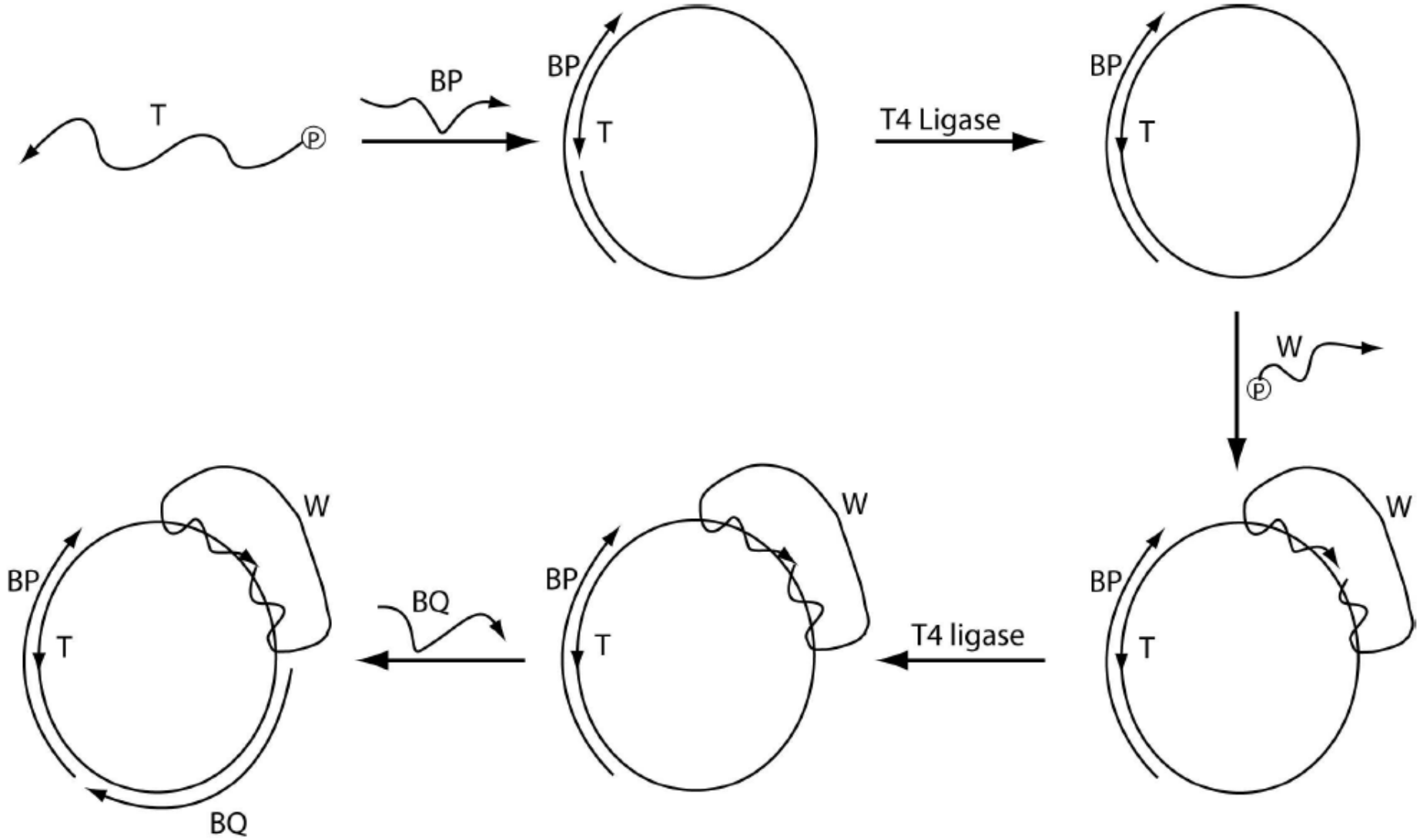
DNA wheels setup



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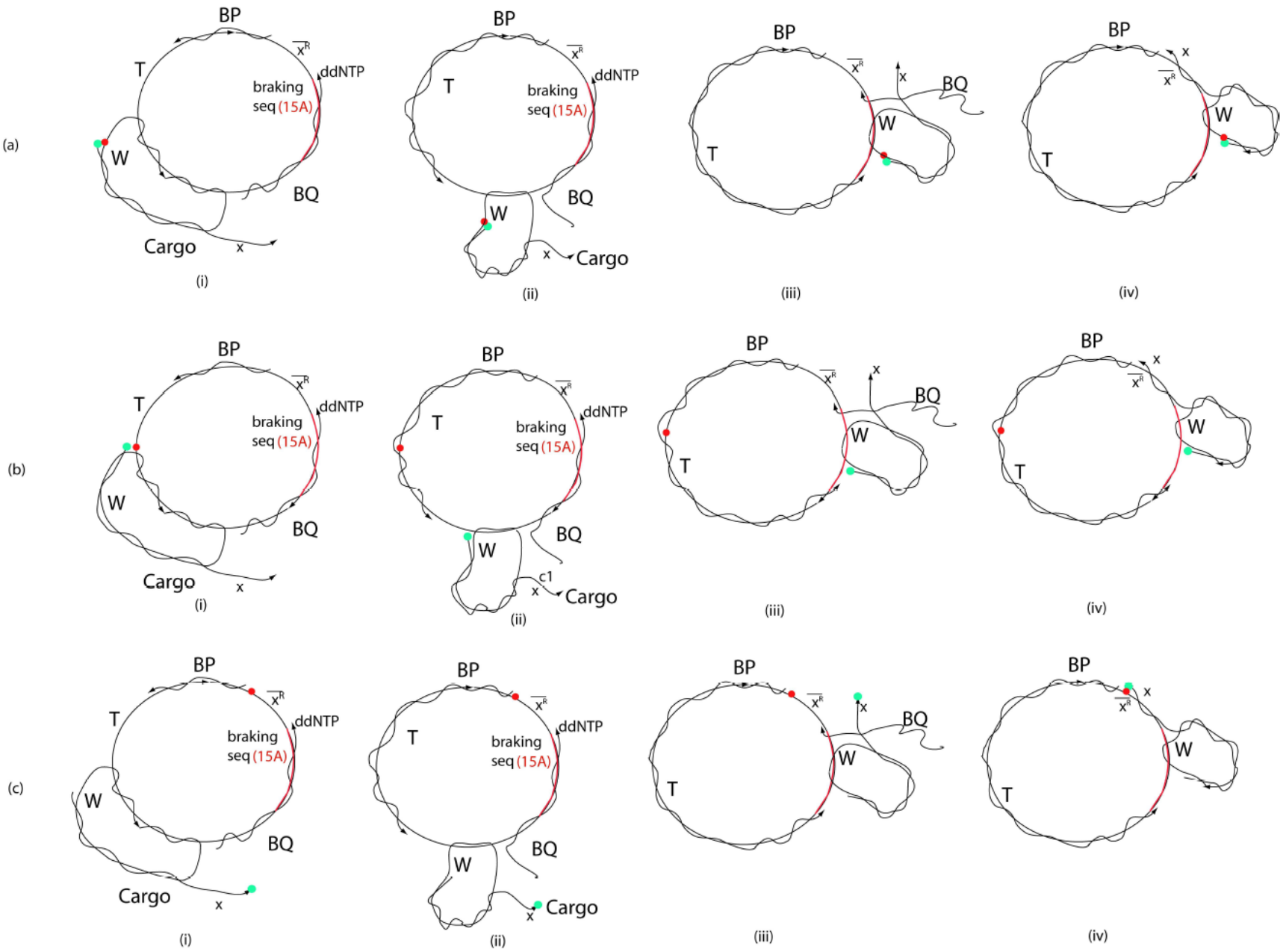
DNA wheels motion



DUKE
COMPUTER
SCIENCE

DNA wheels motion

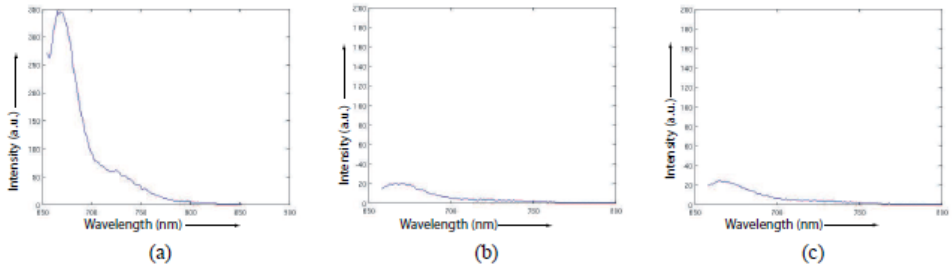


Fig. 6. (a) The fluorescence shown by the assembly in absence of the cargo containing the quencher (b) The fluorescence quenched by the assembly of cargo containing the quencher (c) The fluorescence remains quenched even after the activity of the polymerase $\phi 29$, which indicates that the cargo is not dislodged from the wheel W

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)

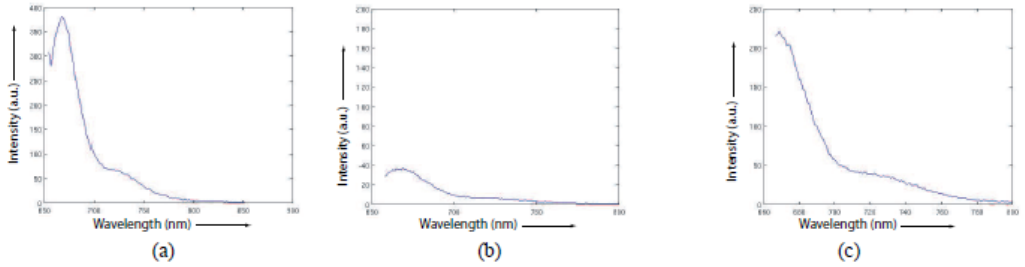


Fig. 7. (a) The fluorescence is shown by the assembly in absence of the cargo containing the quencher (b) The fluorescence is quenched after the assembly of the cargo containing the quencher (c) The fluorescence reappears after the polymerase $\phi 29$ pushes the wheel containing the quencher

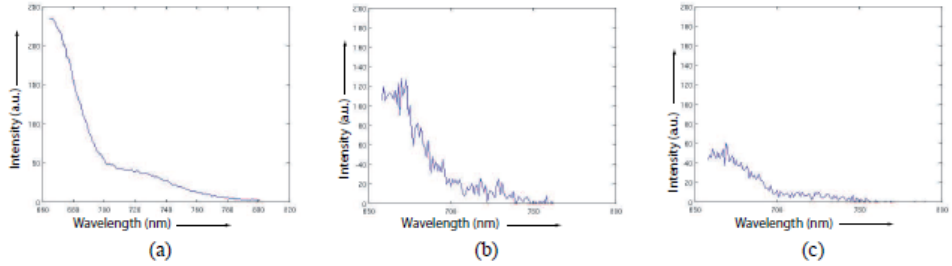
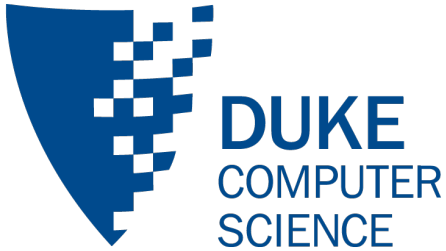


Fig. 8. (a) The fluorescence is shown by the assembly in absence of the cargo containing the quencher (b) The fluorescence remains after the assembly of the cargo containing the quencher, away from the fluorophore (c) The fluorescence quenches after the polymerase $\phi 29$ pushes the wheel before it stops at stopping sequence, and the sticky end of the cargo hybridizes with the track to quench the fluorescence



Autonomous DNA Devices using no Enzymes: Fueled by Strand Displacement

Autonomous DNA Biped walker Turberfield2008]

S Green, J Bath, A Turberfield, Coordinated Chemomechanical Cycles: A Mechanism for Autonomous Molecular Motion, Physical Review Letters, vol. 101, no. 23, 2008.

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.

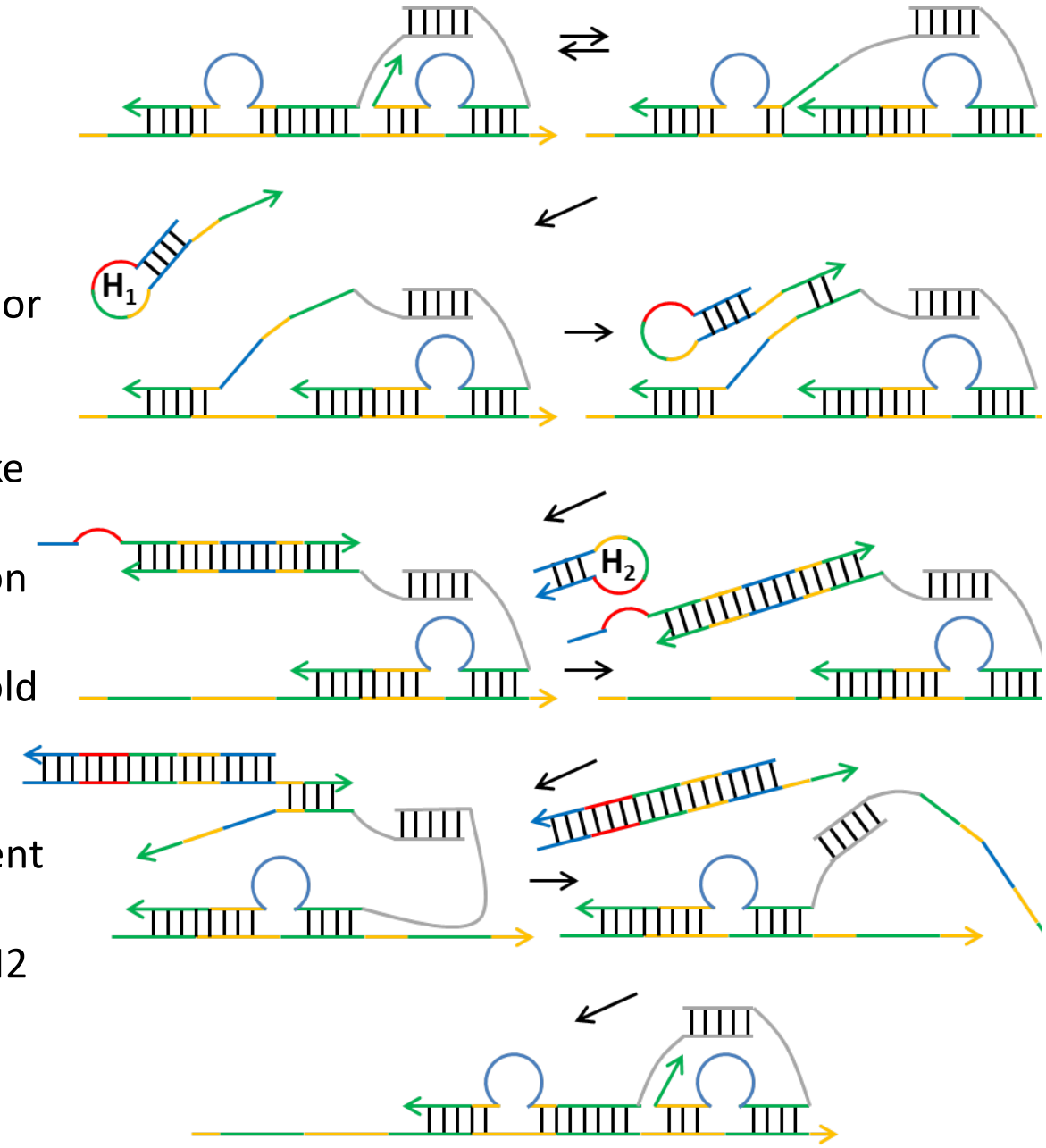
The trailing foot is more likely to detach from track, and equally likely:

- Swings forward ahead of leading foot or
- Reattachs 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]

S. J. Green, J. Bath, and A. J. Turberfield, Coordinated Chemomechanical Cycles: A Mechanism for Autonomous Molecular Motion, Physical Review Letters, 101, 238101 (2008).

Two-part fuel: complementary hairpins H1 and H2.

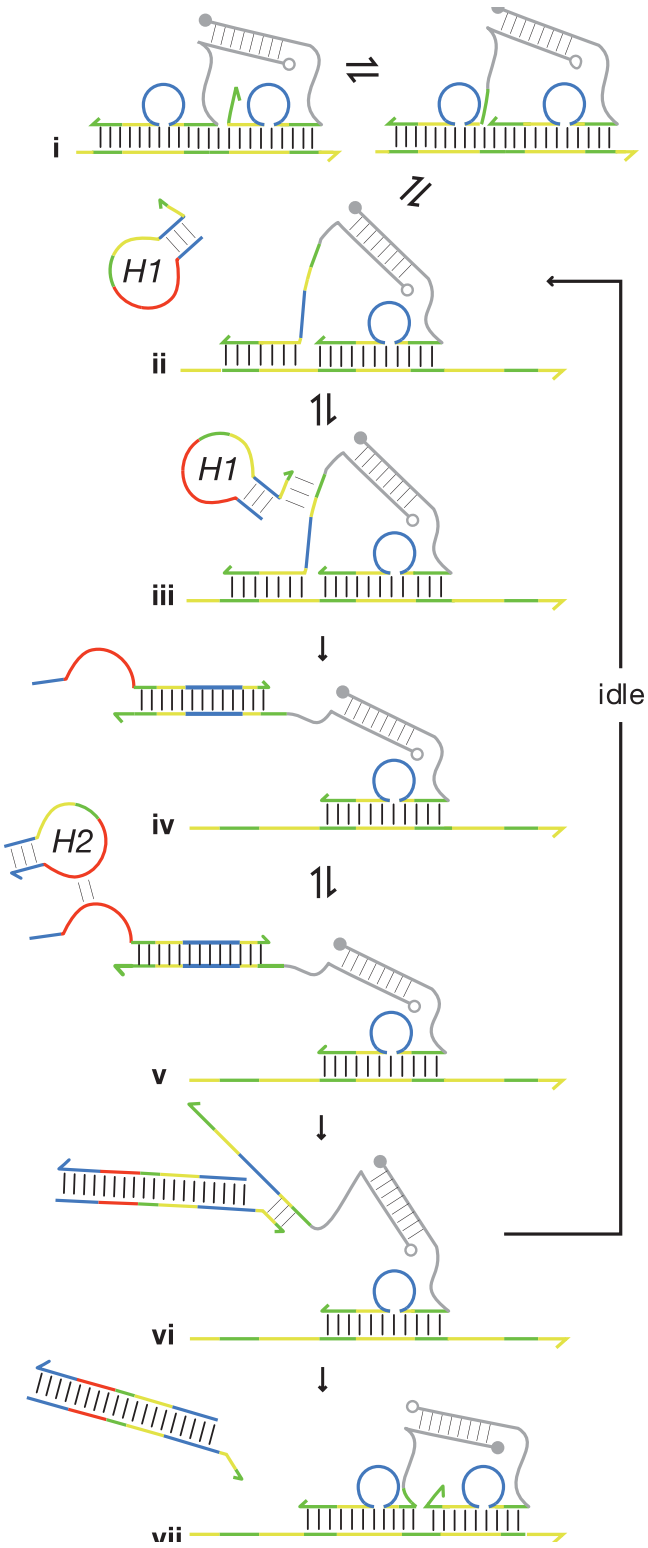
Walker Operation:

(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,
Nature, vol. 451, no. 7176, pp. 318-322, 2008.

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

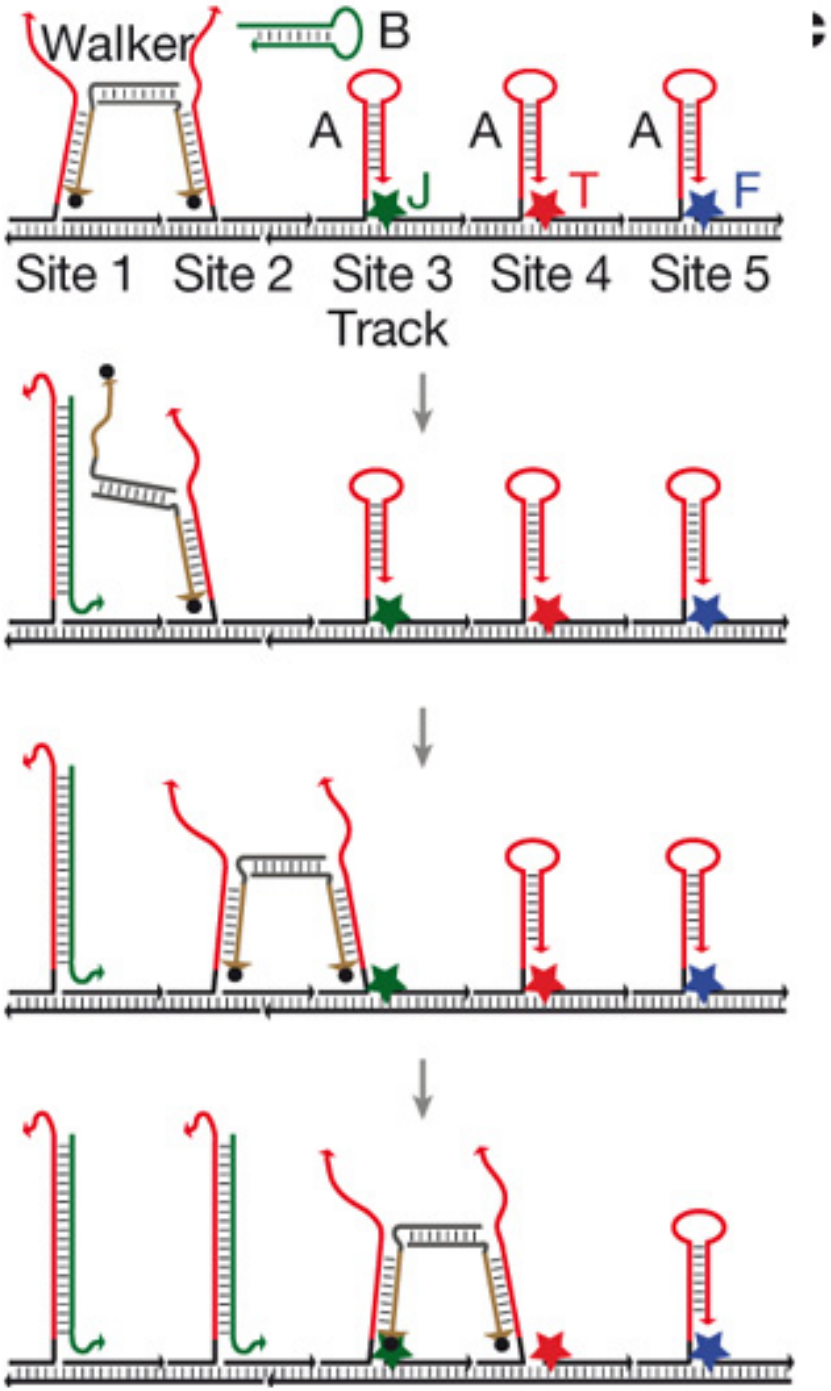
Stators are in the form of hairpins

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:

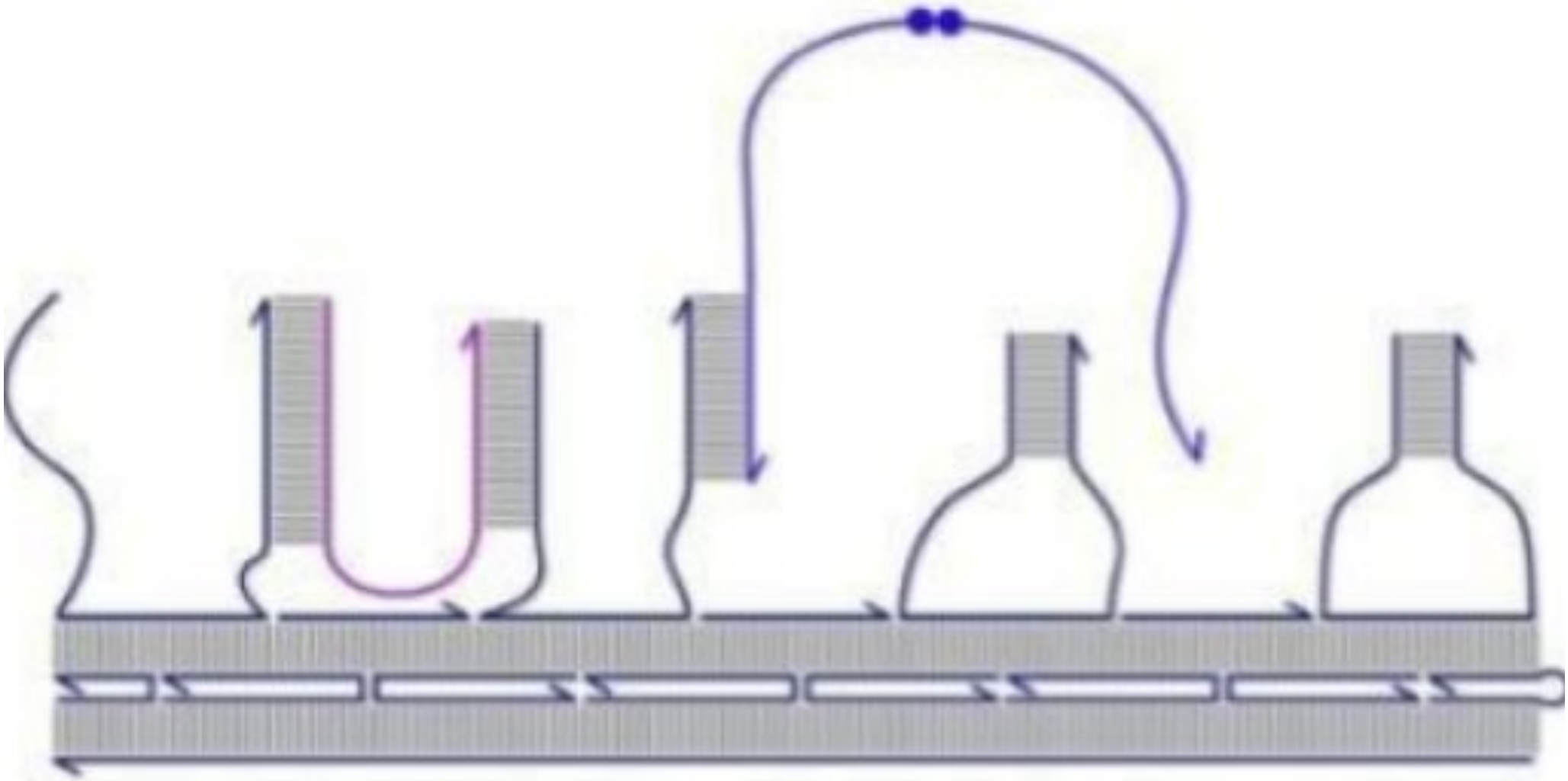
- 50% chance at each step that the leading foot is detached from the stator, in which case the walker halts.
- slight probability that both the legs of the walker detach from the track.



Autonomous DNA based Nanorobotic devices

Autonomous DNA Biped walker [Seeman2009]

Tosan Omabegho, Ruojie Sha, and Nadrian C. Seeman. A Bipedal DNA Brownian Motor with Coordinated Legs. Science, 2009; 324 (5923): 67 DOI: 10.1126/science.1170336



Autonomous DNA Biped walker [Seeman2009]

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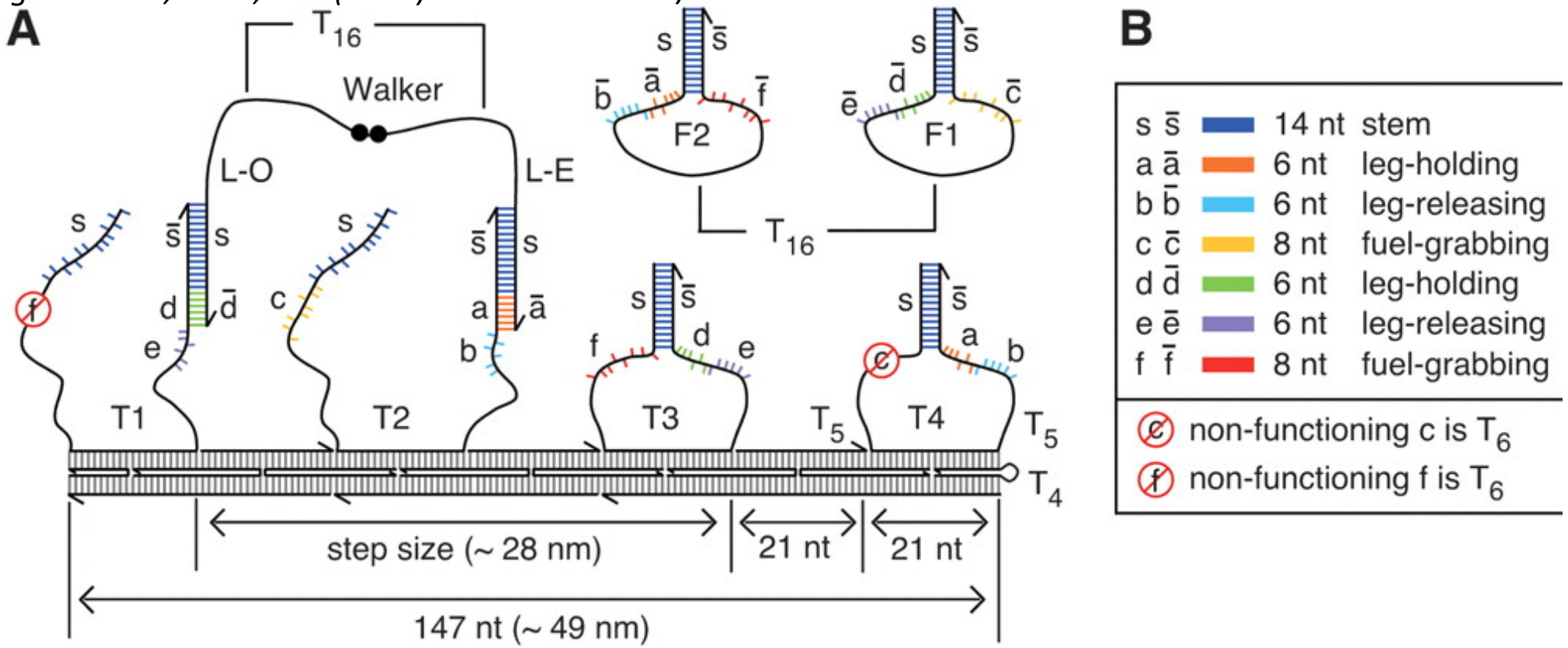


Illustration of the DX track structure with the walker on it.

•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.

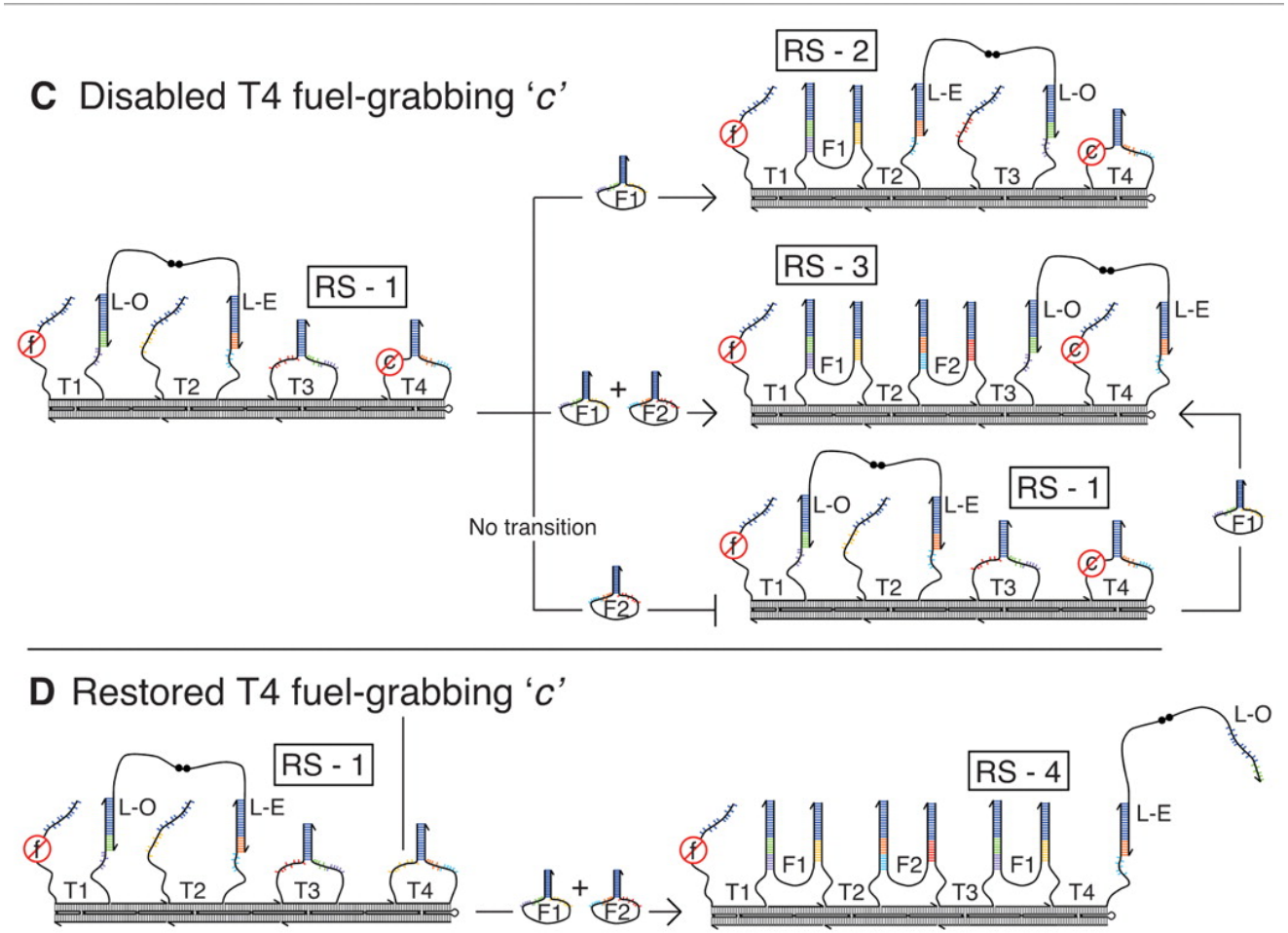
Autonomous DNA Biped walker [Seeman2009]

(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]

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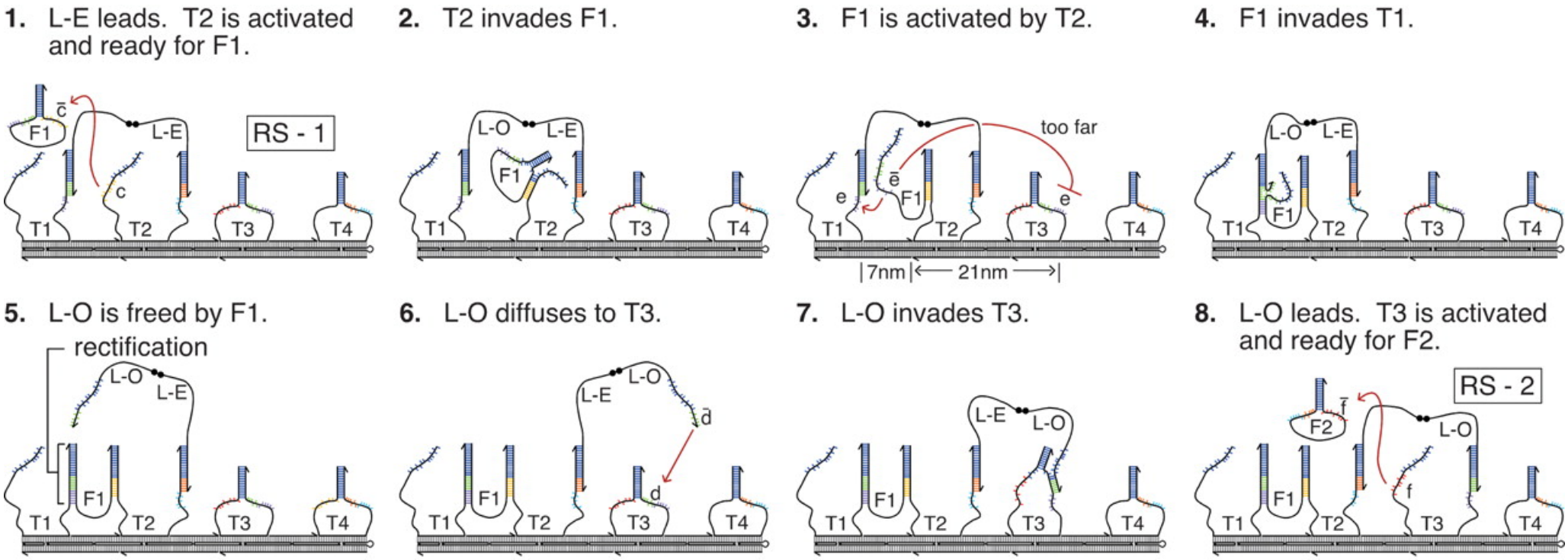
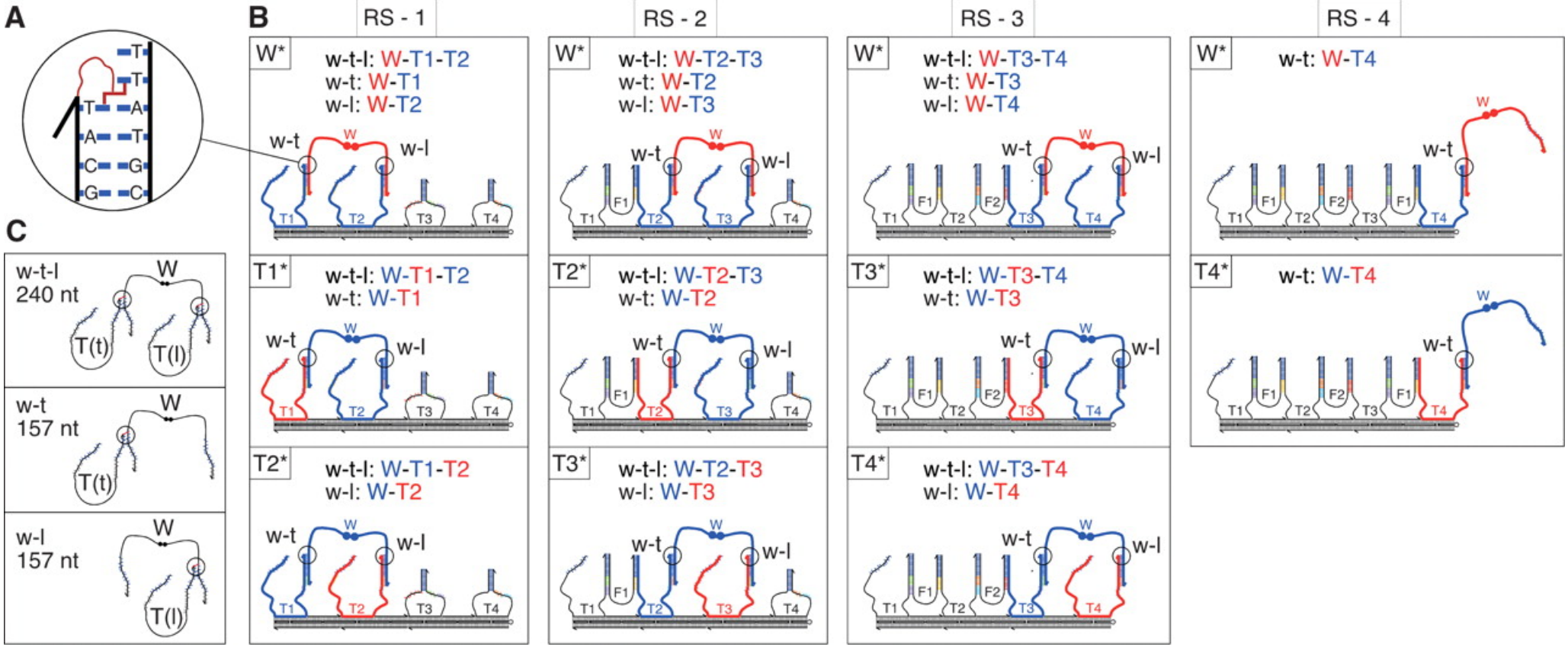


Transition from RS-1 to RS-2: In eight sequential frames, this illustration depicts the biped taking one step.

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

Autonomous DNA Biped walker [Seeman2009]



Psoralen cross-linking and 32P labeling.

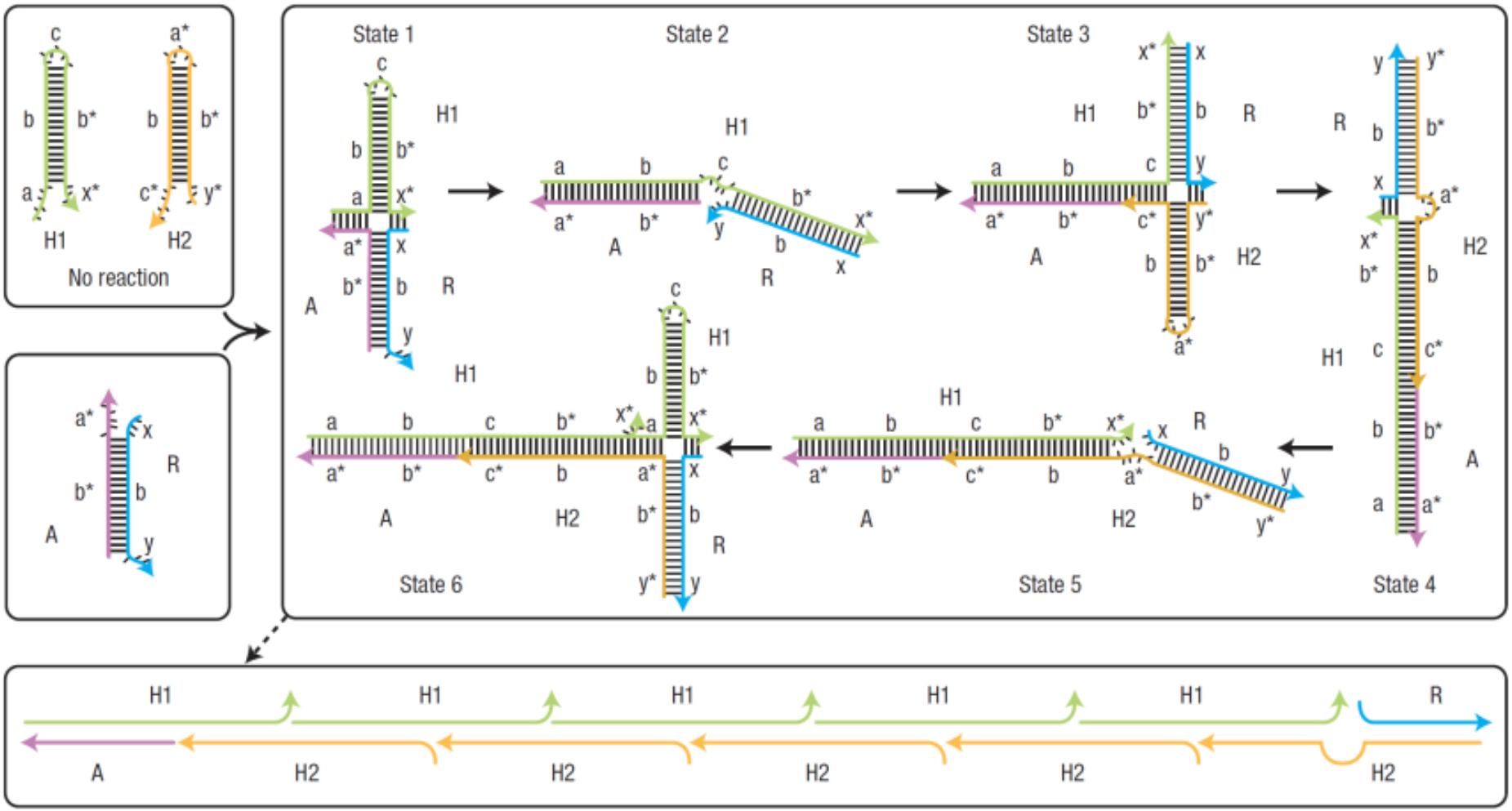
(A) A detailed picture of the UV-activated psoralen crosslinking reaction between the track stem loops and the walker. The psoralen on the stem loops covalently links to the thymidines on the walker's legs just outside the duplex formed by the stem loops and the walker's legs.

(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.

DNA Motor Fueled by Hybridization [Venkatarama2007]

S Venkataraman, R Dirks, P Rothemund, E Winfree, N Pierce, An Autonomous Polymerization Motor Powered by DNA Hybridization, Nature Nanotechnology, vol. 2, pp. 490-494, 2007.



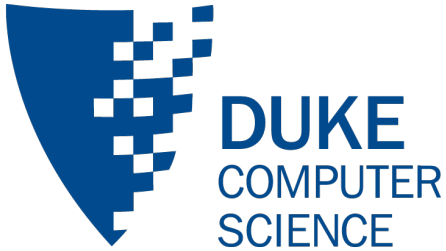
A DNA motor inspired by bacterial pathogens like Rickettsia rickettsii.

•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

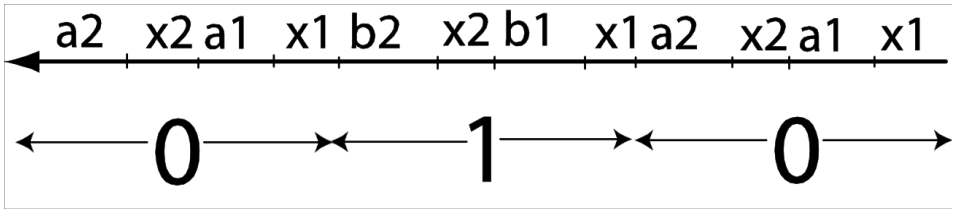
Sudheer Sahu

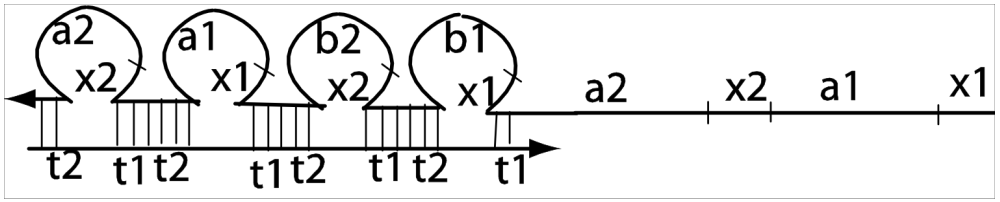
- *DNAzyme calculator* : a limited ability computational device
- *DNAzyme FSA:* a finite state automata device, that executes finite state transitions using DNAzymes
  - extensions to probabilistic automata and non-deterministic automata,
- *DNAzyme router:* for programmable routing of nanostructures 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

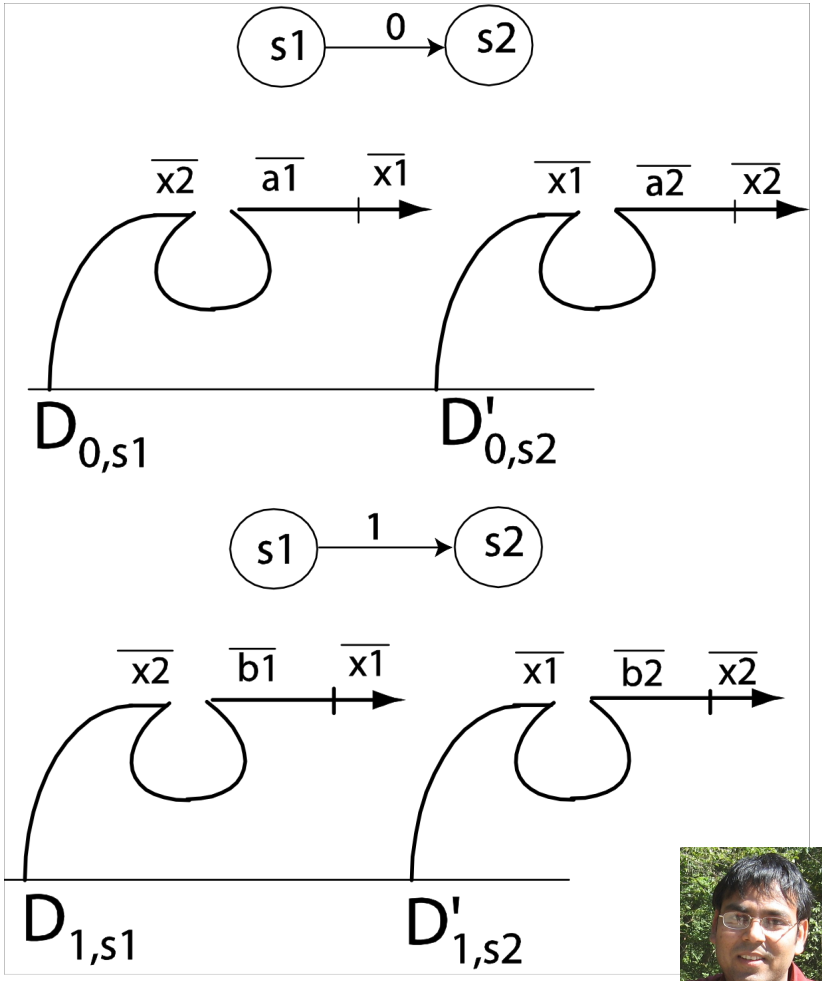
All Devices:

- Autonomous, programmable, and no protein enzymes.
- The basic principle involved is inspired by Mao's DNAzyme Walker

DNAzyme FSA (inputs, transitions)

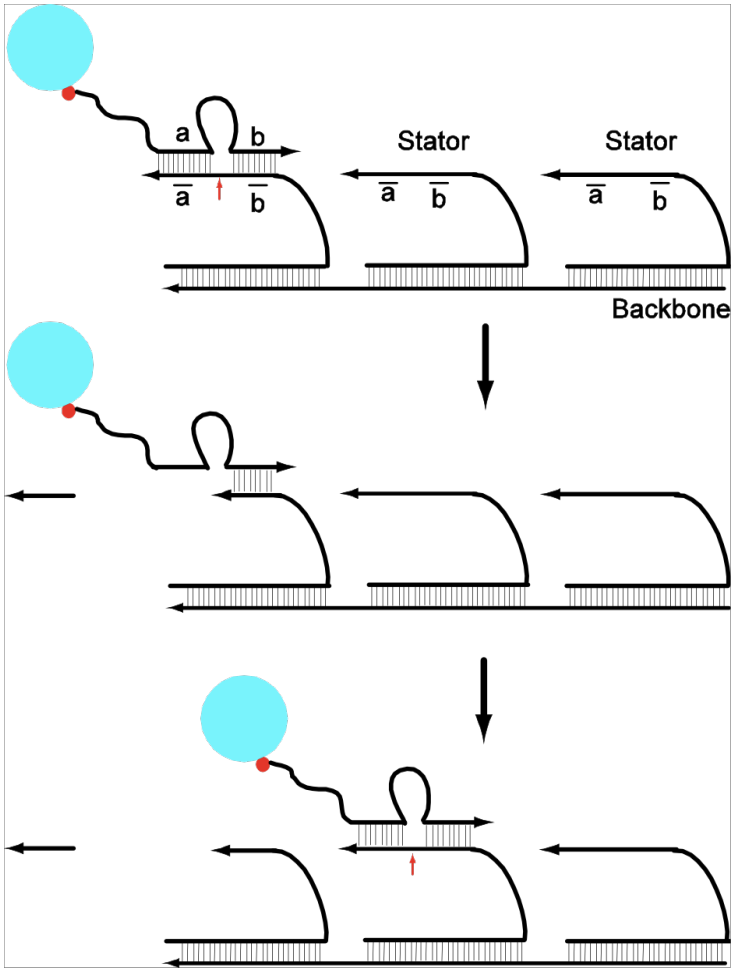


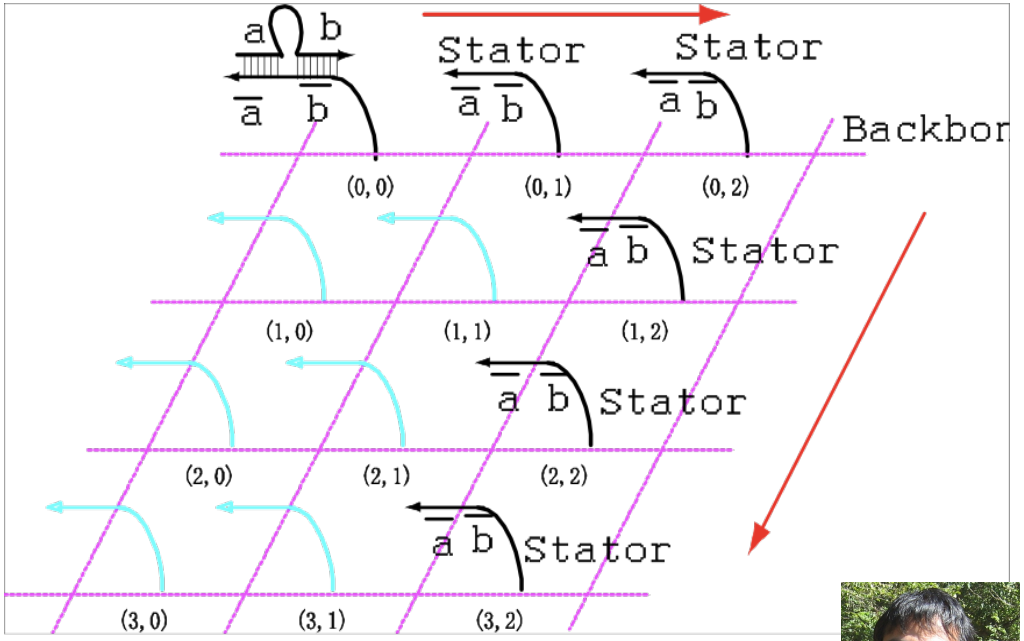




Sudheer Sahu

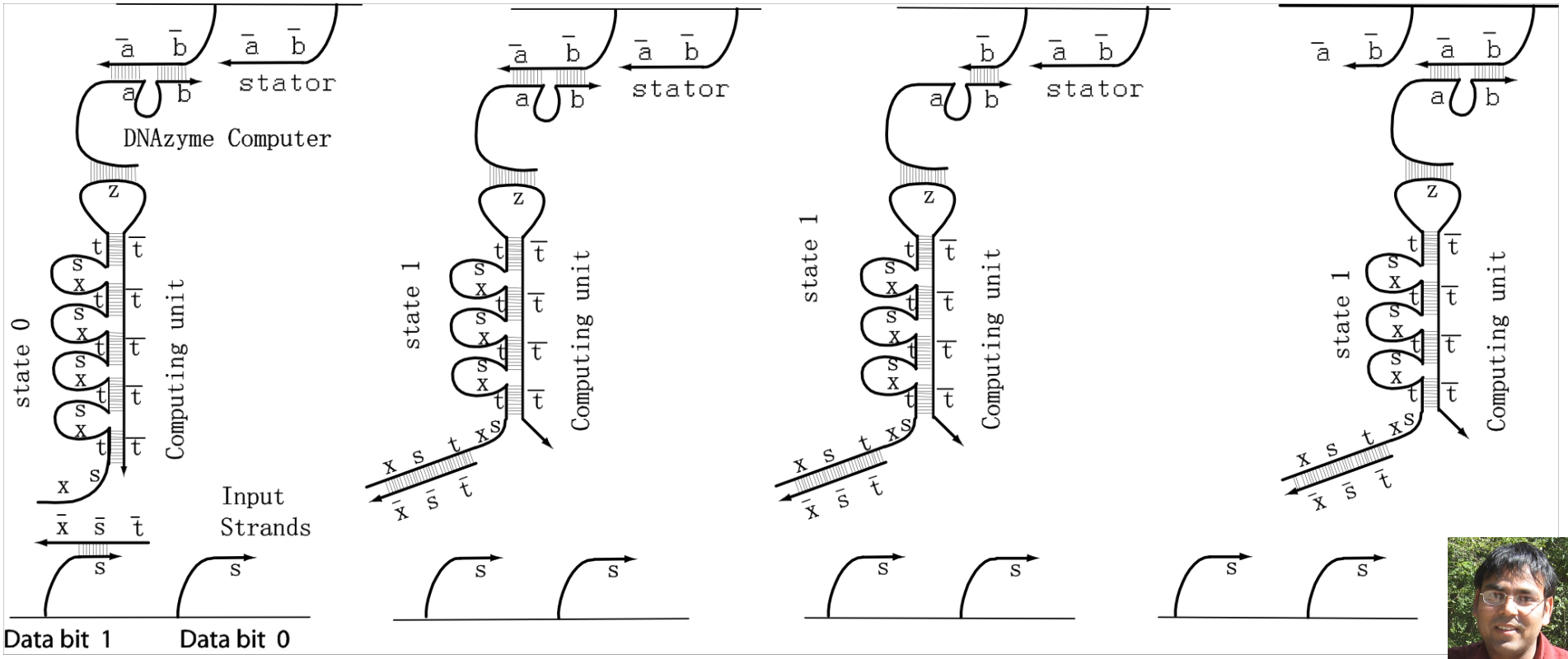
DNAzyme Crawler





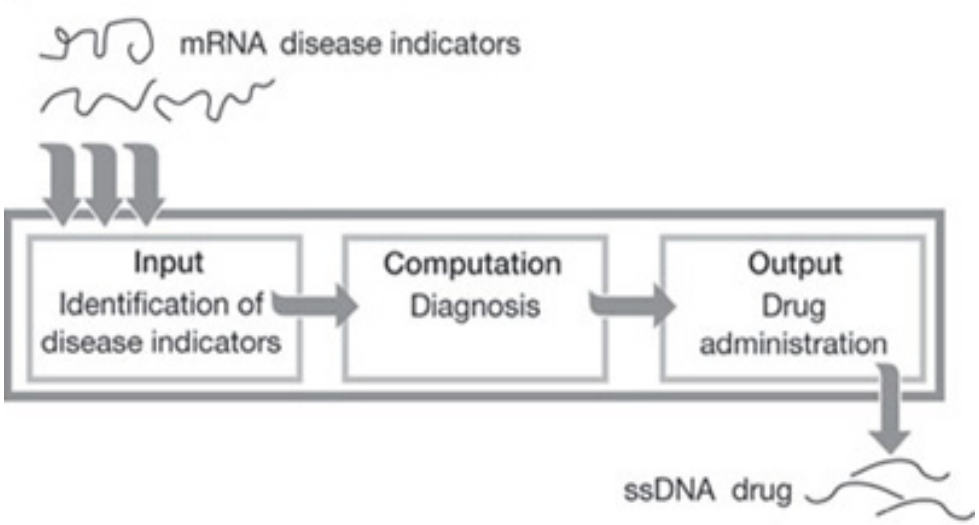
Sudheer Sahu

DNAzyme Calculator

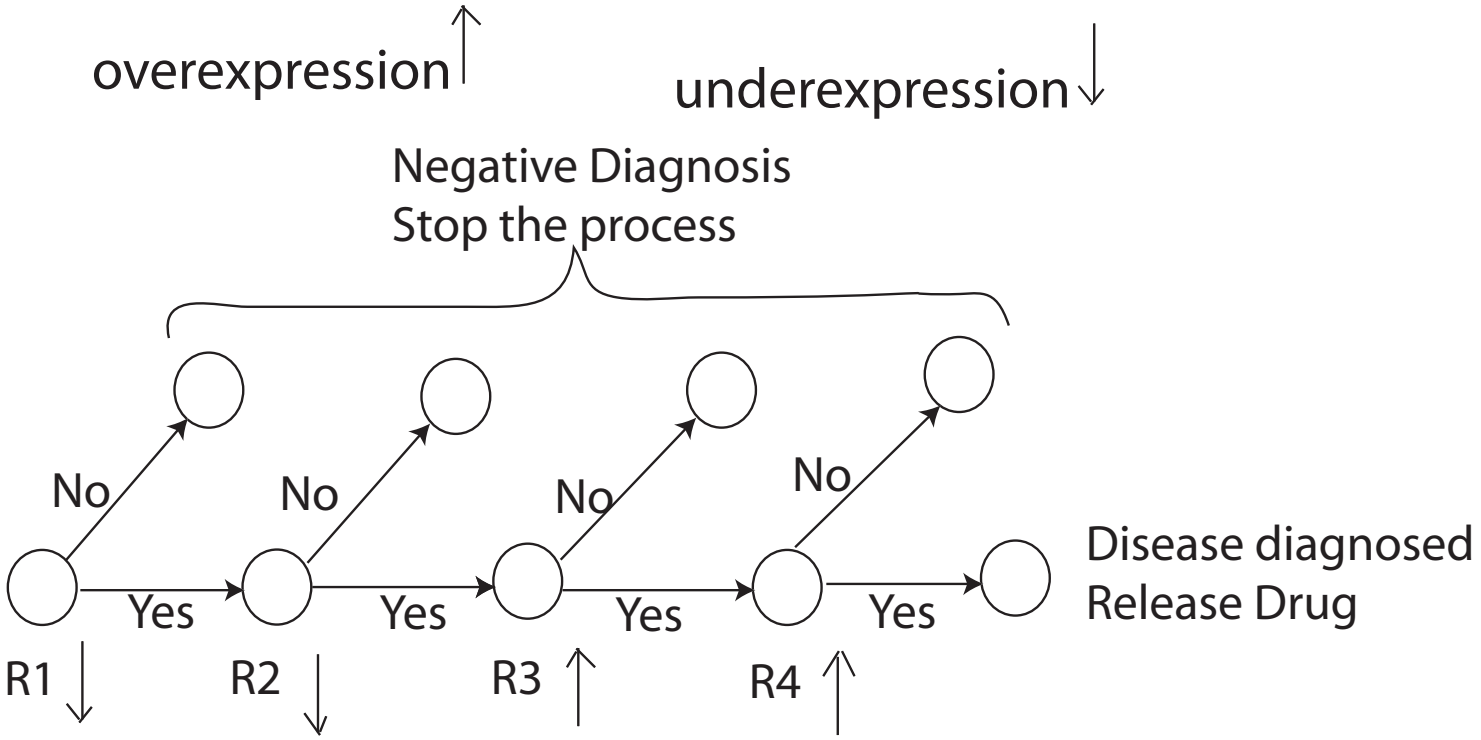


Sudheer Sahu

DNA Doctor



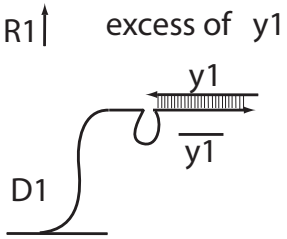
Y. Benenson *et al., An autonomous molecular computer for logical control of gene expressionNature* 429, 423-429 (2004)

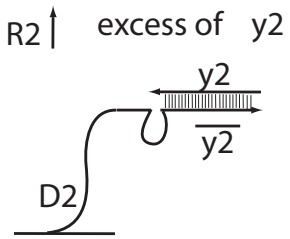


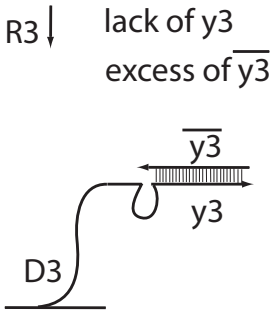
DNAzyme Device for DNA Doctor
John H. Reif and Sudheer Sahu, 2006)

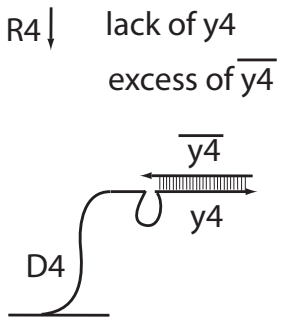
Detecting RNA Expression:
Senses expression of sequence of RNAs y_1, y_2, y_3, y_4

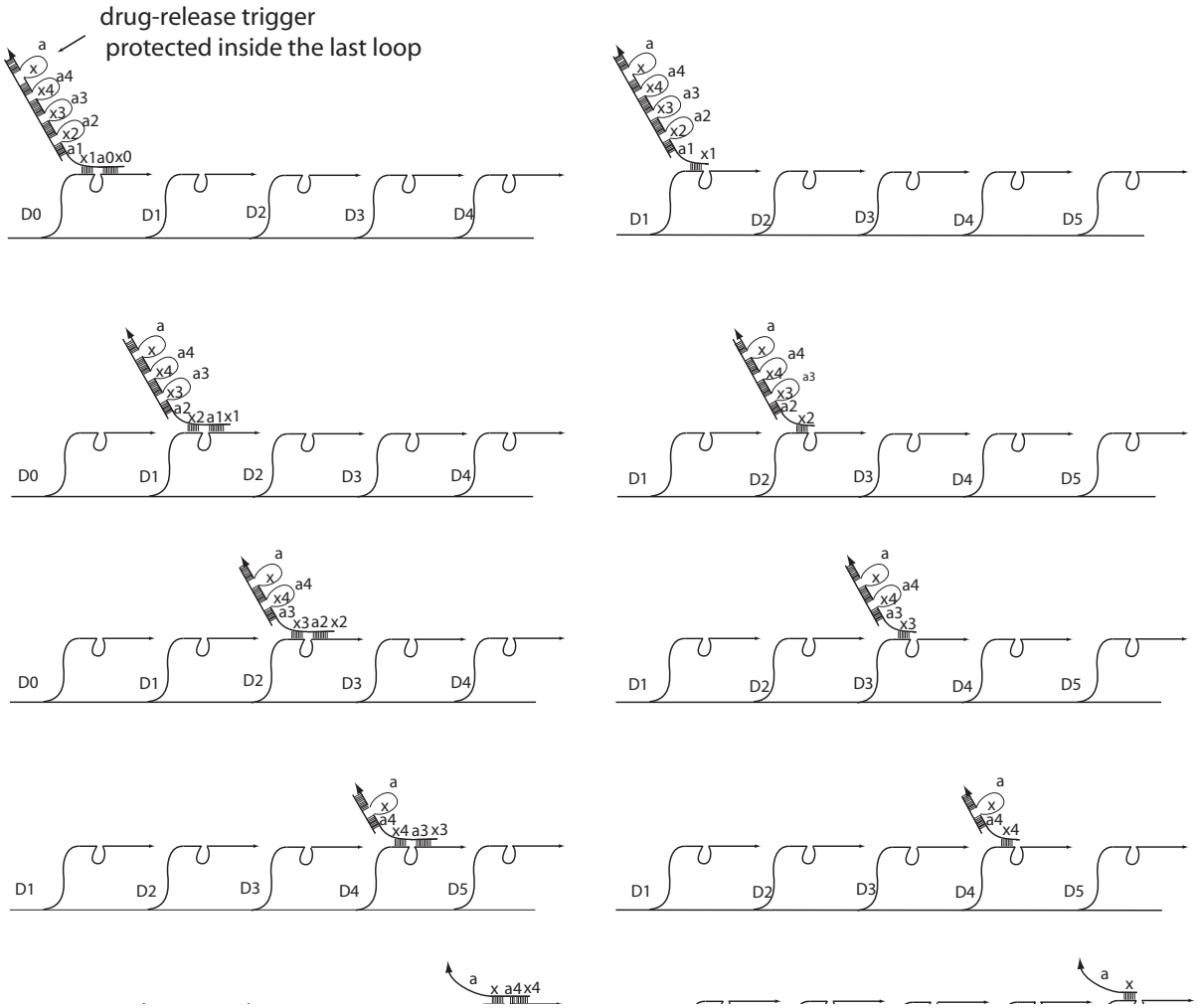
A threshold concentration of complement of y_1, y_2, y_3, y_4 is added to the solution, therefore lack of y_3, y_4 causes excess of complement of y_3 and y_4, respectively.











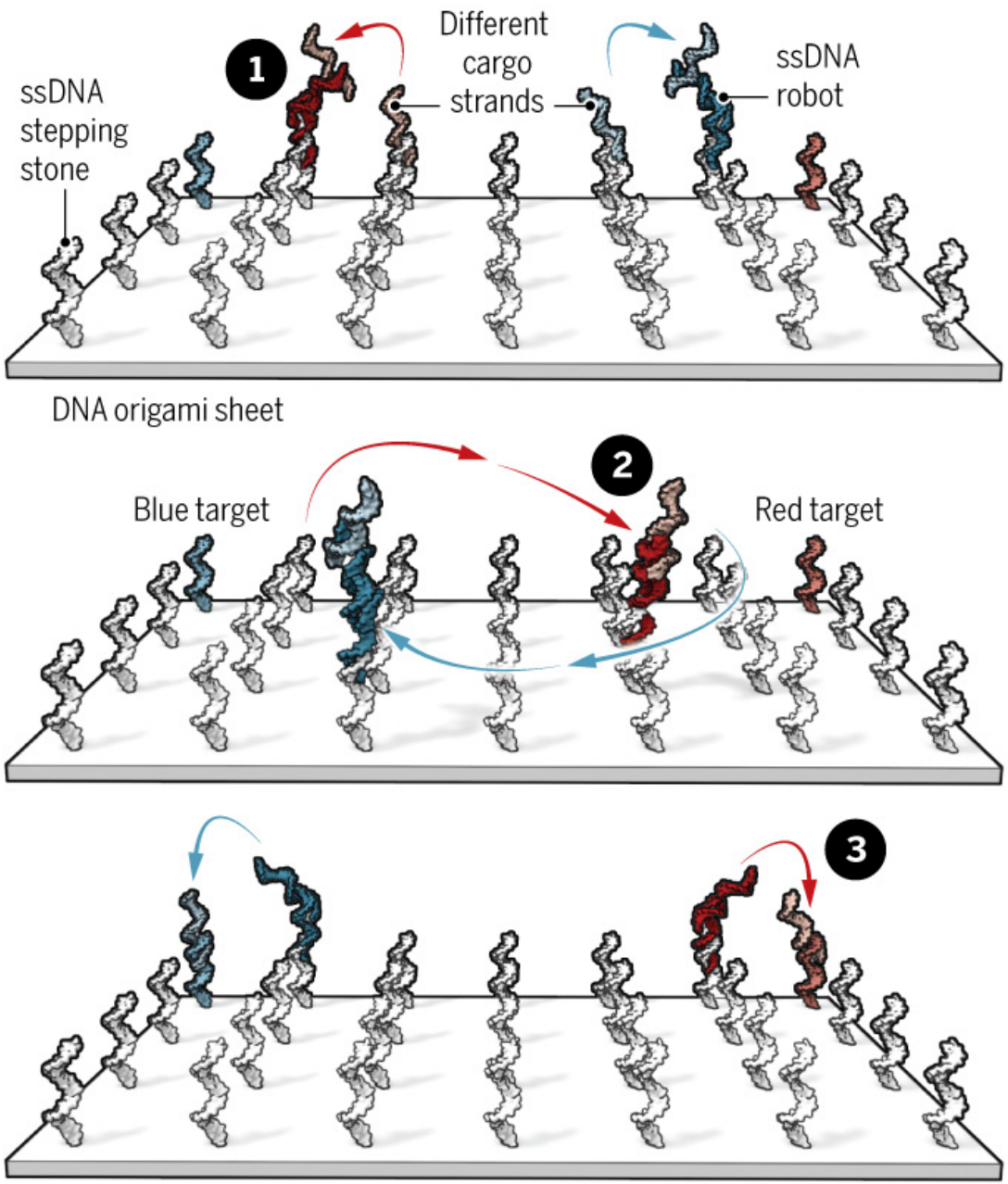
Cargo-sorting DNA robots

Thubagere, et al, A cargo-sorting DNA robot, Science 2017

- They demonstrate three modular building blocks for a DNA robot that performs cargo sorting at the molecular level.
- A simple algorithm encoding recognition between cargos and their destinations allows for a simple robot design, a single-stranded DNA with one leg and two foot domains for walking, and one arm and one hand domain for picking up and dropping off cargos.
- The robot 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 them to specified destinations, until all molecules are sorted into two distinct piles.
- The robot is designed to perform a random walk without any energy supply.
- Exploiting this feature, a single robot can repeatedly sort multiple cargos.
- Localization on DNA origami allows for distinct cargo-sorting tasks to take place simultaneously in one test tube, or 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.



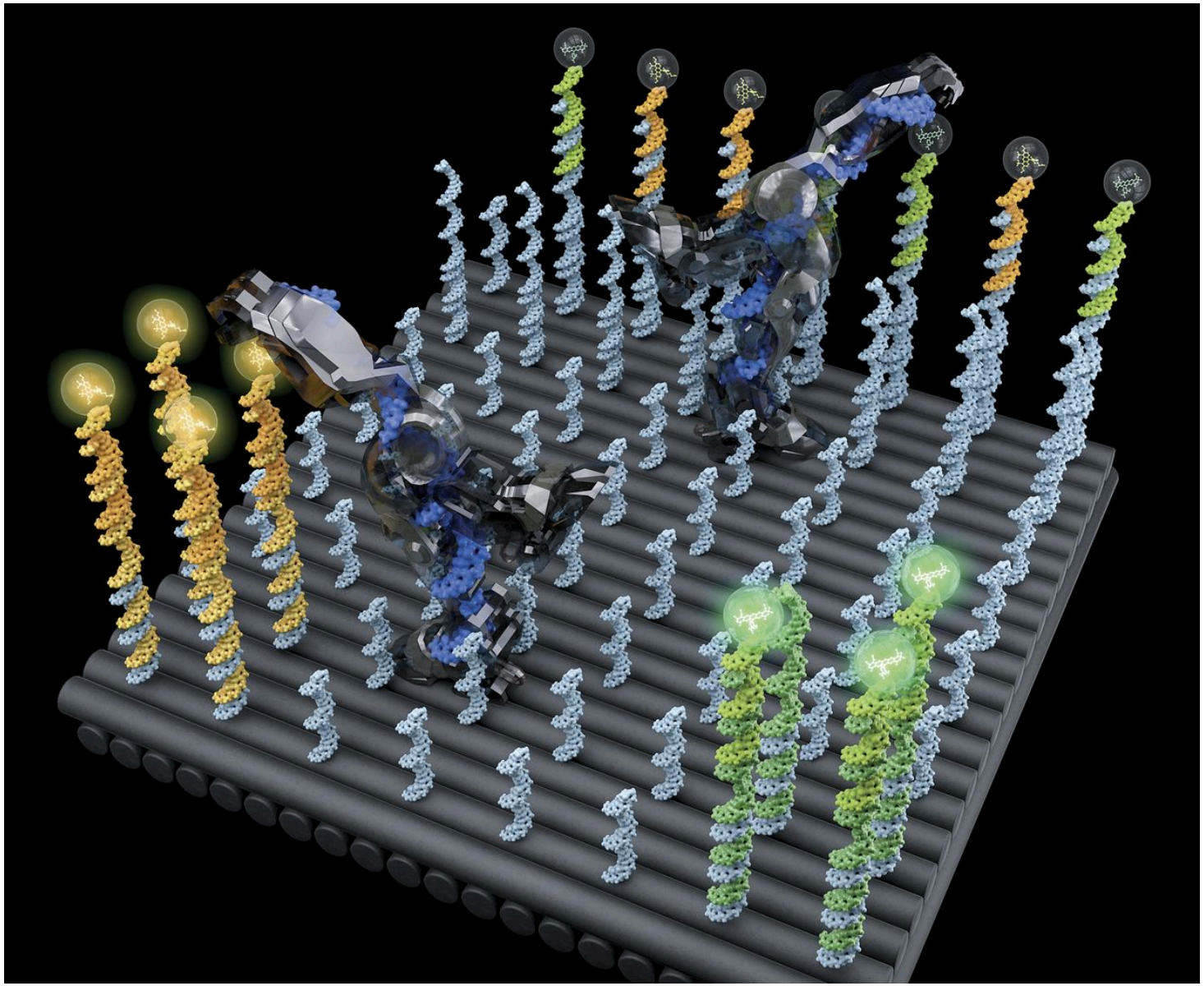
1. Robot picks up cargo at location on DNA origami.

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.

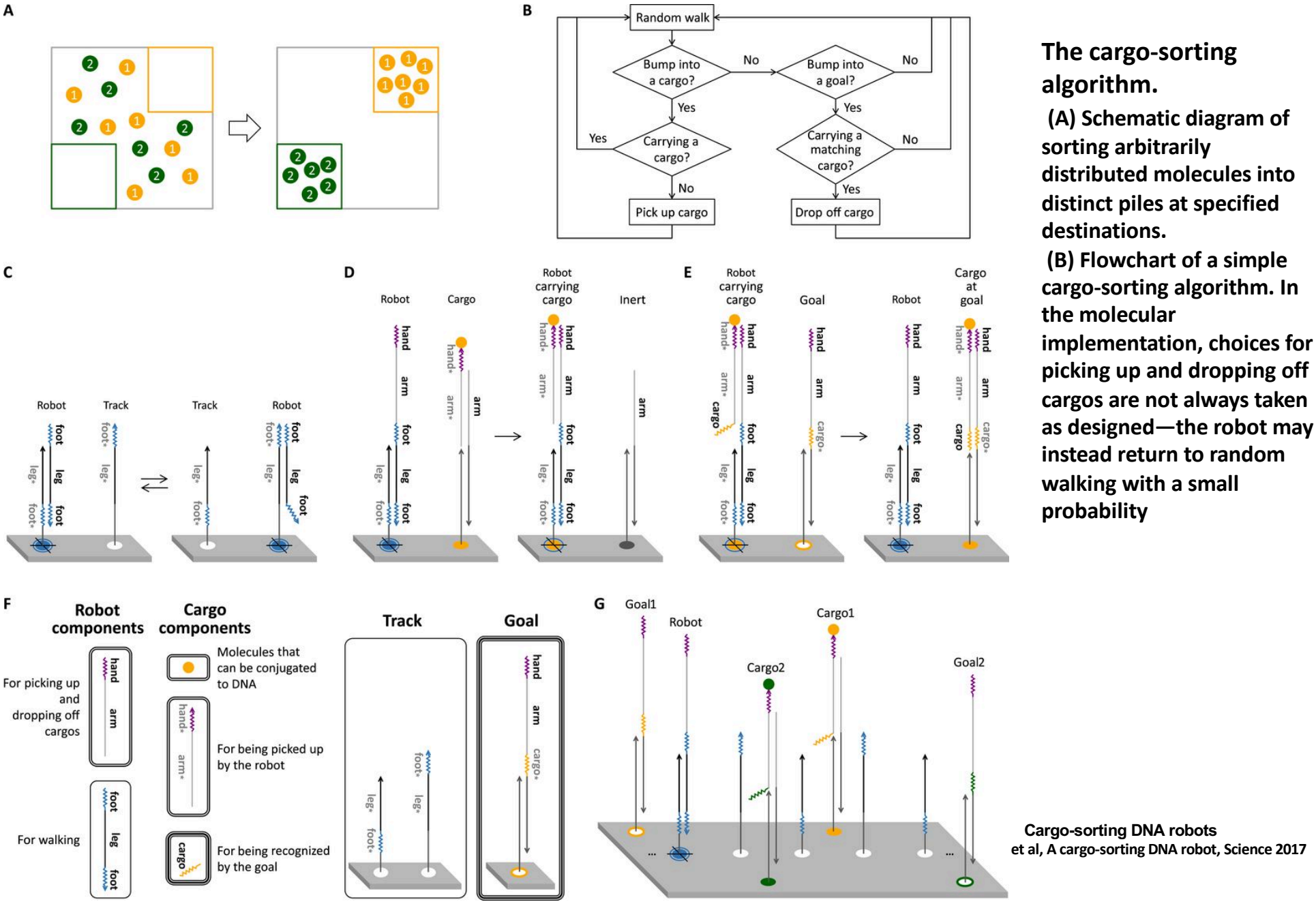
Cargo-sorting DNA robots

Thubagere, et al, A cargo-sorting DNA robot, Science 201



Cargo-sorting DNA robots
Thubagere, et al, A cargo-sorting DNA robot, Science 2017

Conceptual illustration of two DNA robots.
The robots are collectively performing a cargo-sorting task on a DNA origami surface, transporting fluorescent molecules with different colors from initially unordered locations to separated destinations.



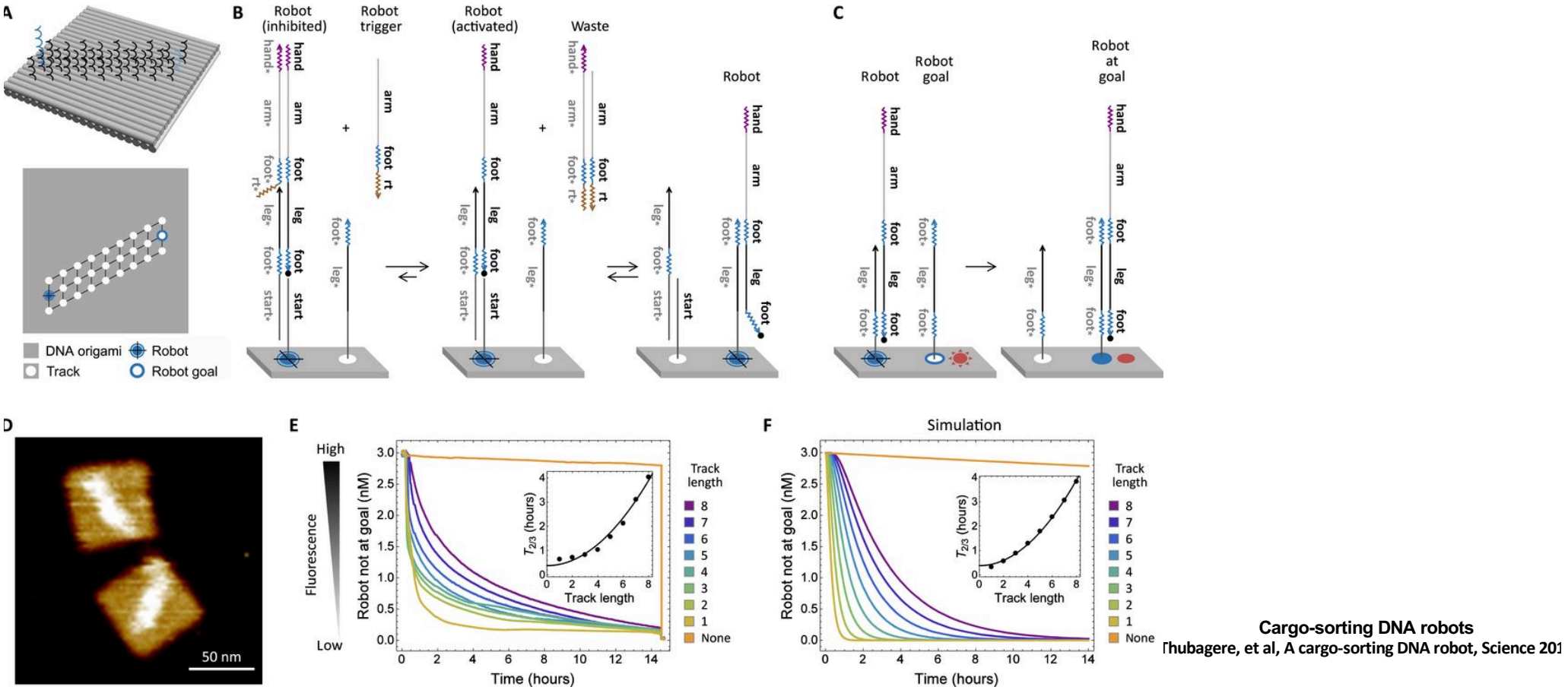
The cargo-sorting algorithm.

(A) Schematic diagram of sorting arbitrarily distributed molecules into distinct piles at specified destinations.

(B) Flowchart of a simple cargo-sorting algorithm. In the molecular implementation, choices for picking up and dropping off cargos are not always taken as designed—the robot may instead return to random walking with a small probability

Cargo-sorting DNA robots
et al, A cargo-sorting DNA robot, Science 2017

The cargo-sorting algorithm. Mechanism of the three building blocks for the (C) random walk, (D) cargo pickup, and (E) cargo drop-off. (F) Composability of the three building blocks. Three types of outlines highlight the components used in the three building blocks. (G) Implementation for sorting multiple types of cargos. Squiggled lines indicate short toehold domains and straight lines indicate long branch migration domains in DNA strands, with arrowheads marking their 3' ends



Cargo-sorting DNA robots
Thubagere, et al, A cargo-sorting DNA robot, Science 201

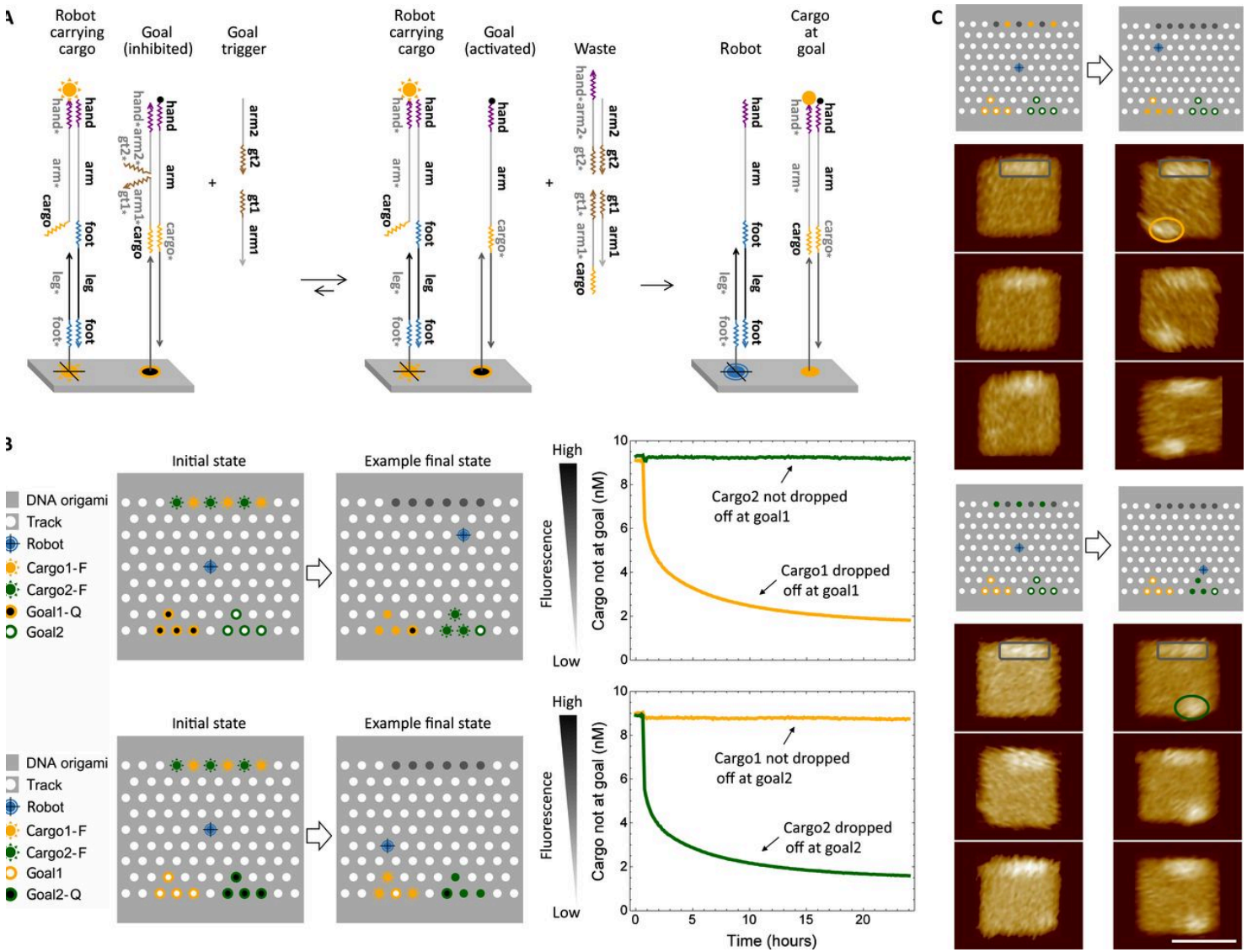
The random-walk building block.

(A)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 (fig. S4A).

(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 × higher conc. than the inhibited robot.

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

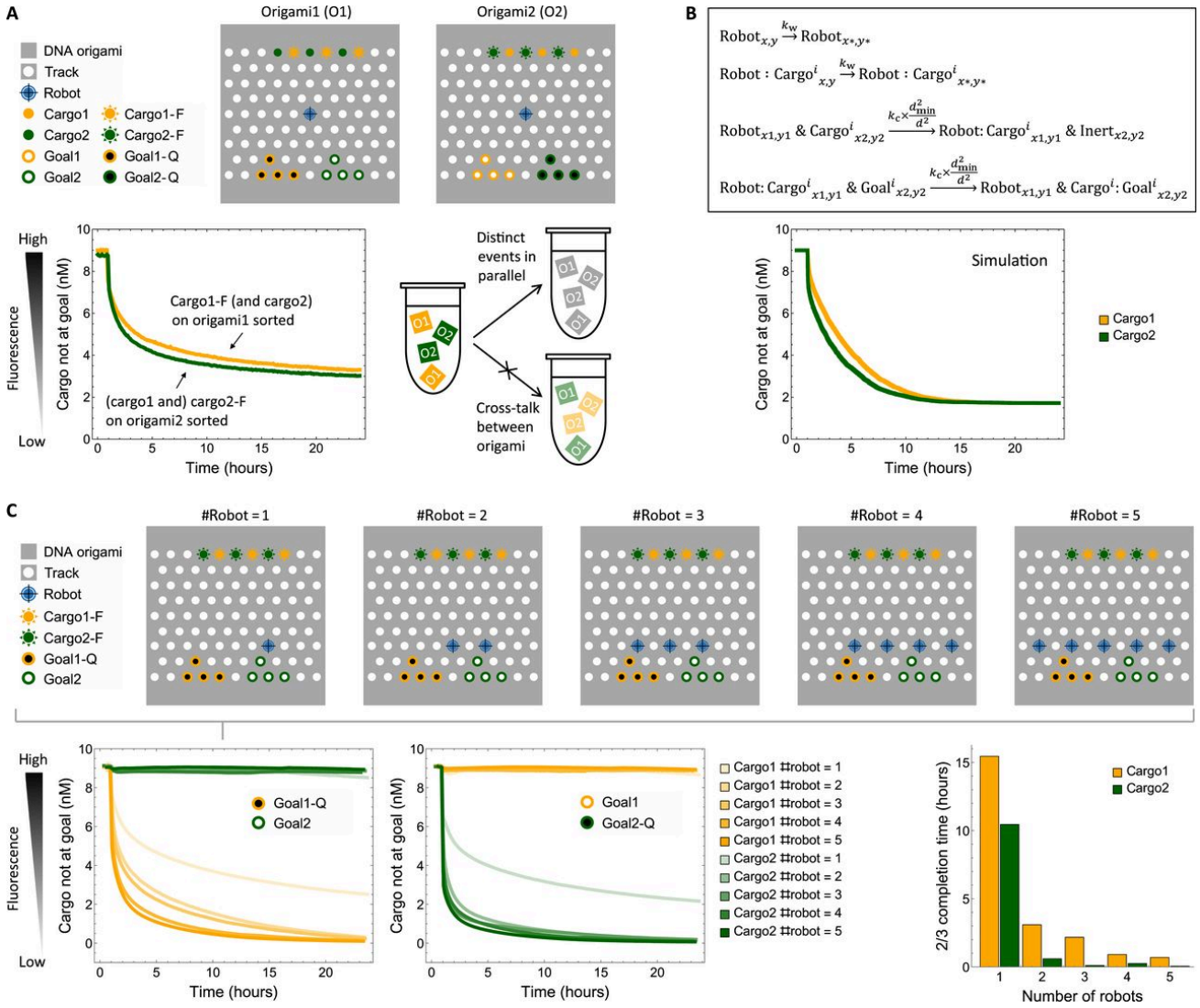
(D) AFM image of the double-layer DNA origami with a track of length 8. (E) Fluorescence kinetics data of random-walk experiments with eight distinct track lengths and a negative control with no track. A 20-fold excess of free-floating robot strands, relative to the origami concentration, was added at the end of the experiments to measure the maximum possible completion level.



Cargo-sorting DNA robots
Thubagere, et al, A cargo-sorting DNA robot, Science 201

Demonstration of cargo sorting.

(A) 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 in fig. S8A. (B) 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. (C) 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



Cargo-sorting DNA robots
Thubagere, et al, A cargo-sorting DNA robot, Science 2017

Exploring the 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. Robotx,y indicates a robot at an arbitrary track location (x, y). (x*, y*) is a neighboring location of (x, y). Cargoi and Goali indicate specific types of cargo and goal, respectively. d is the Euclidean distance between (x1, y1) and (x2, y2). dMin is the Euclidean distance between a robot and a cargo or goal at its immediate neighboring location.

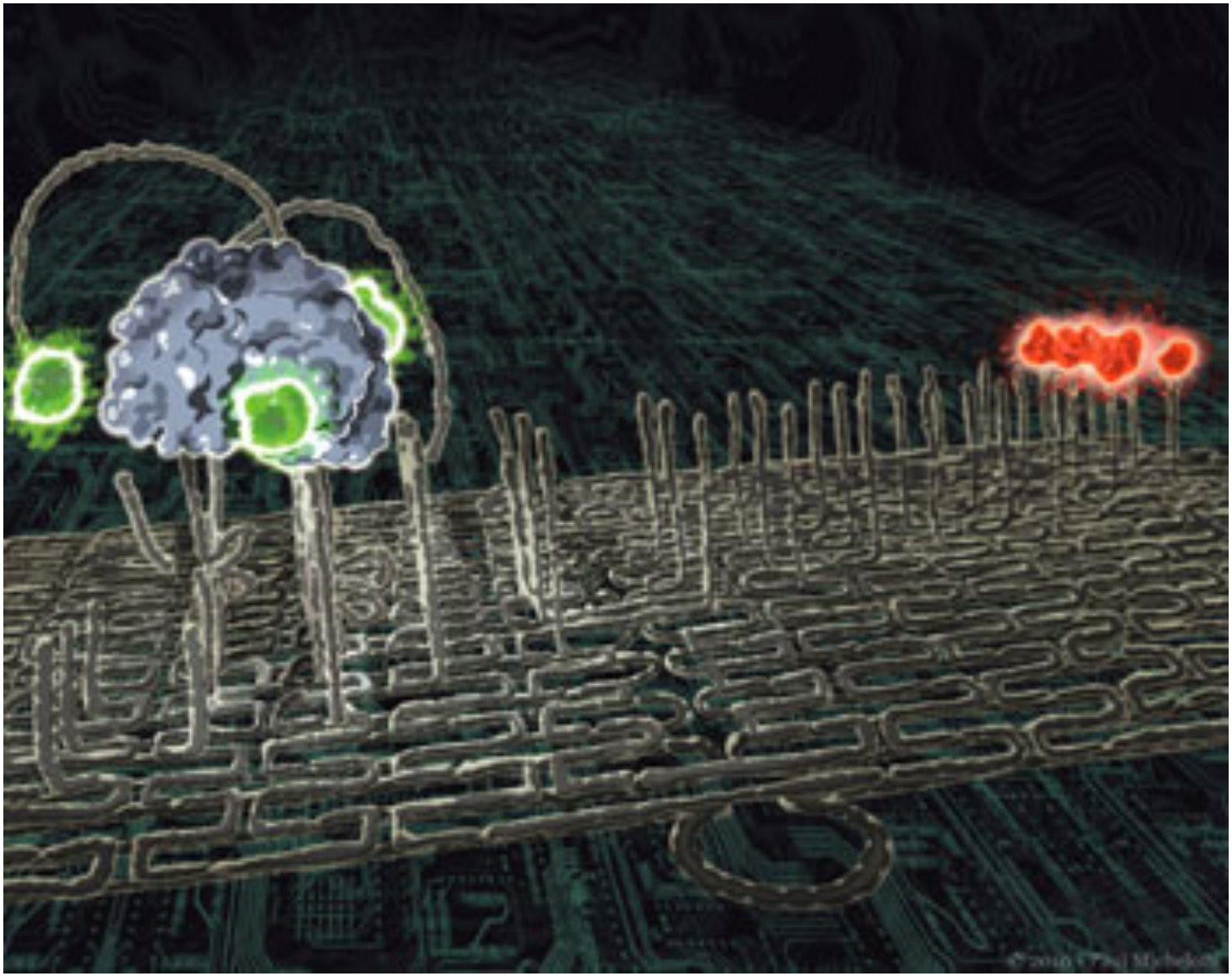
(C) Fluorescence kinetics experiments with multiple robots collectively performing a single cargo-sorting task.

A DNA nanoscale assembly line

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

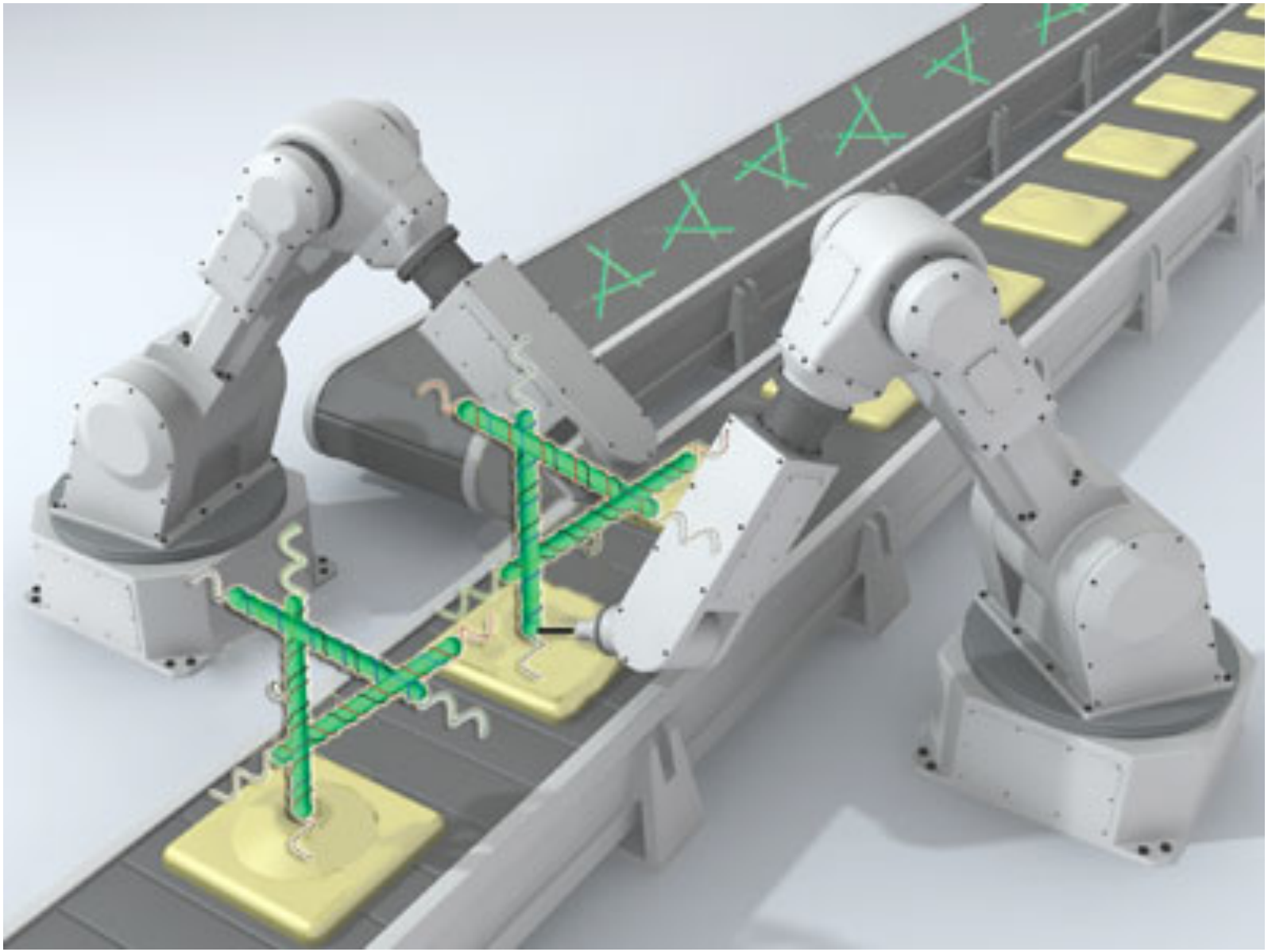
A walker that moves along an origami tile, with programmable cassettes that transfer cargo (gold nanoparticles) to the walker's 'hands'

A DNA nanoscale assembly line



Gu, H., Chao, J., Xiao, S.-J., & Seeman, N. C. (2010). A proximity-based programmable DNA nanoscale assembly line. *Nature*, *465*(7295), 202–205. doi:10.1038/nature09026

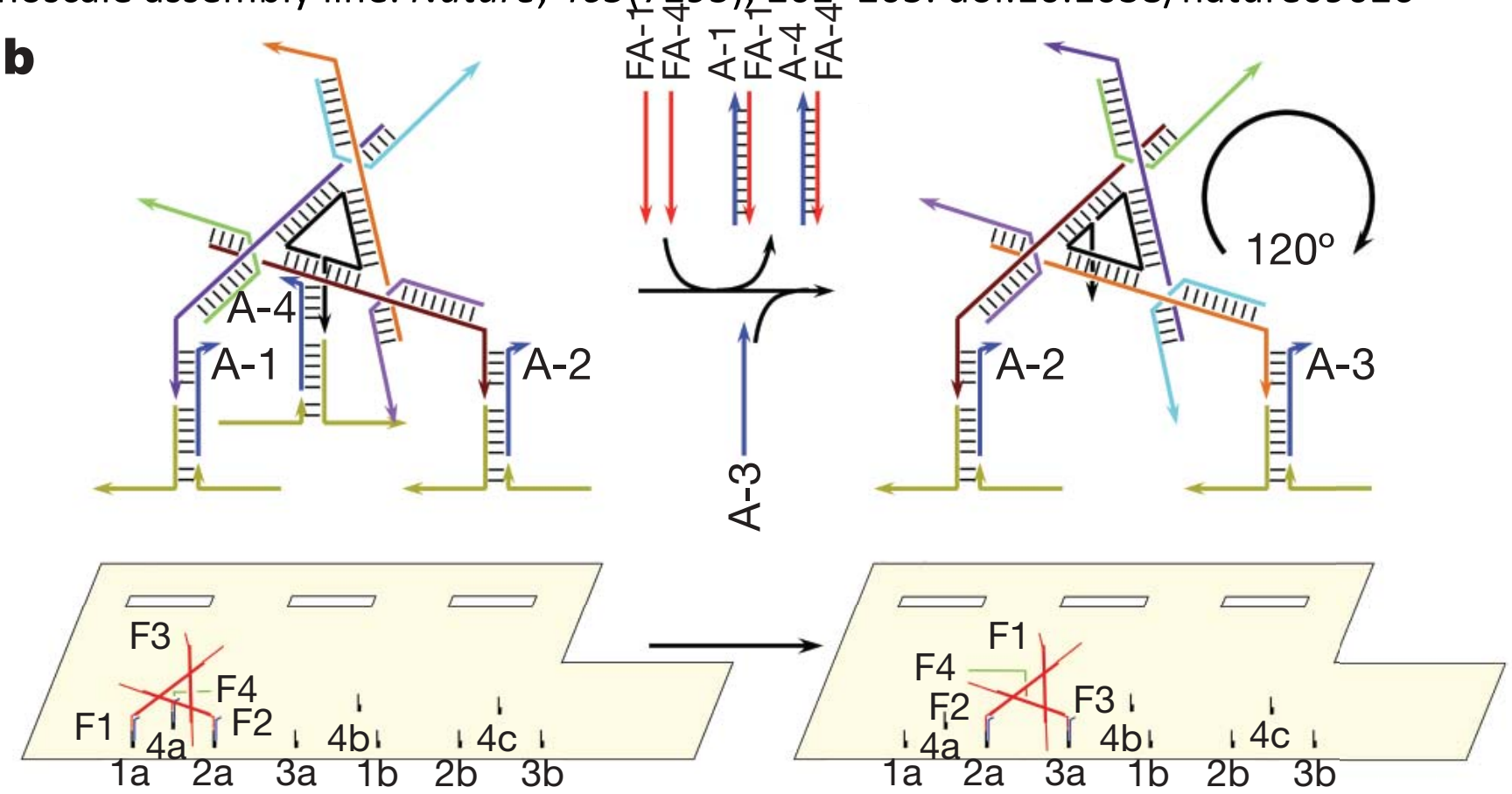
A DNA nanoscale assembly line



Gu, H., Chao, J., Xiao, S.-J., & Seeman, N. C. (2010). A proximity-based programmable DNA nanoscale assembly line. *Nature*, *465*(7295), 202–205. doi:10.1038/nature09026

DNA Origami Walker

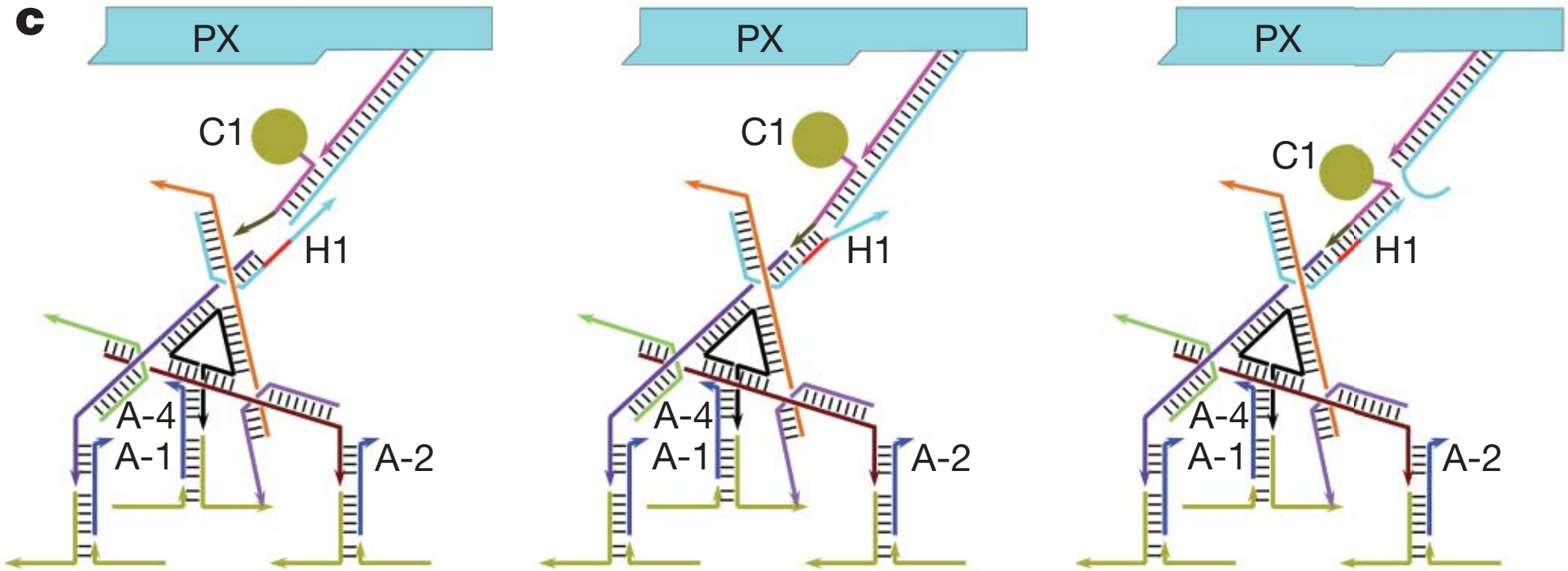
Gu, H., Chao, J., Xiao, S.-J., & Seeman, N. C. (2010). A proximity-based programmable DNA nanoscale assembly line. *Nature*, *465*(7295), 202–205. doi:10.1038/nature09026



- 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

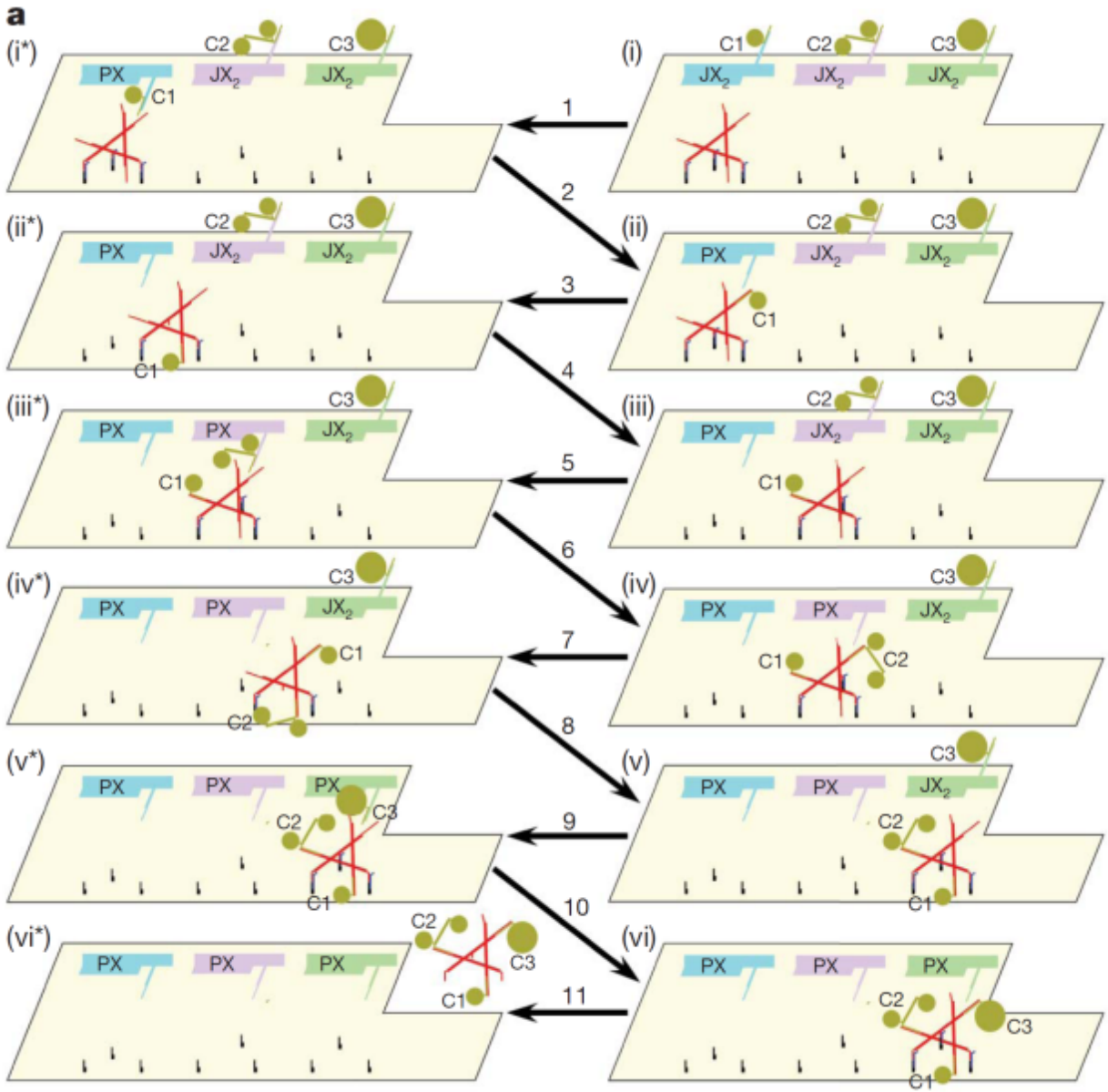
Gu, H., Chao, J., Xiao, S.-J., & Seeman, N. C. (2010). A proximity-based programmable DNA nanoscale assembly line. *Nature*, *465*(7295), 202–205. doi:10.1038/nature09026



- 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



Gu, H., Chao, J., Xiao, S.-J., & Seeman, N. C. (2010). A proximity-based programmable DNA nanoscale assembly line. *Nature*, *465*(7295), 202–205. doi:10.1038/nature09026

DNA Devices that Open Nano-Containers

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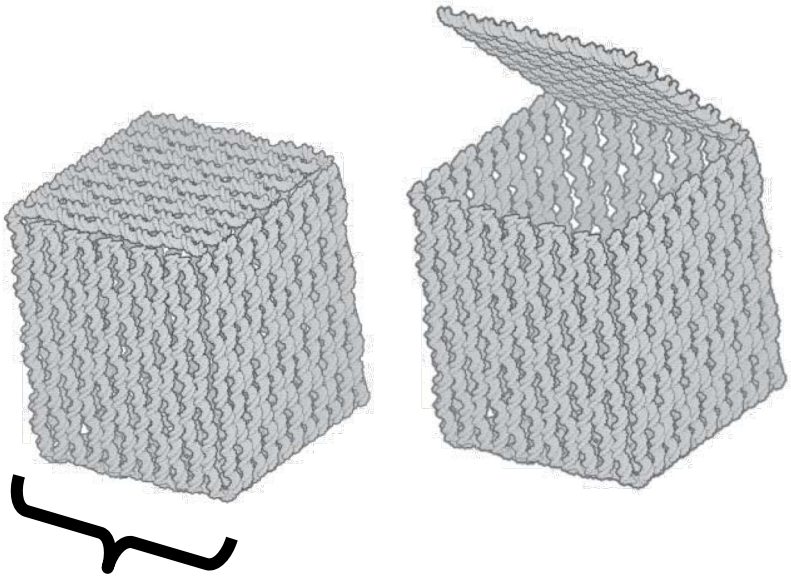
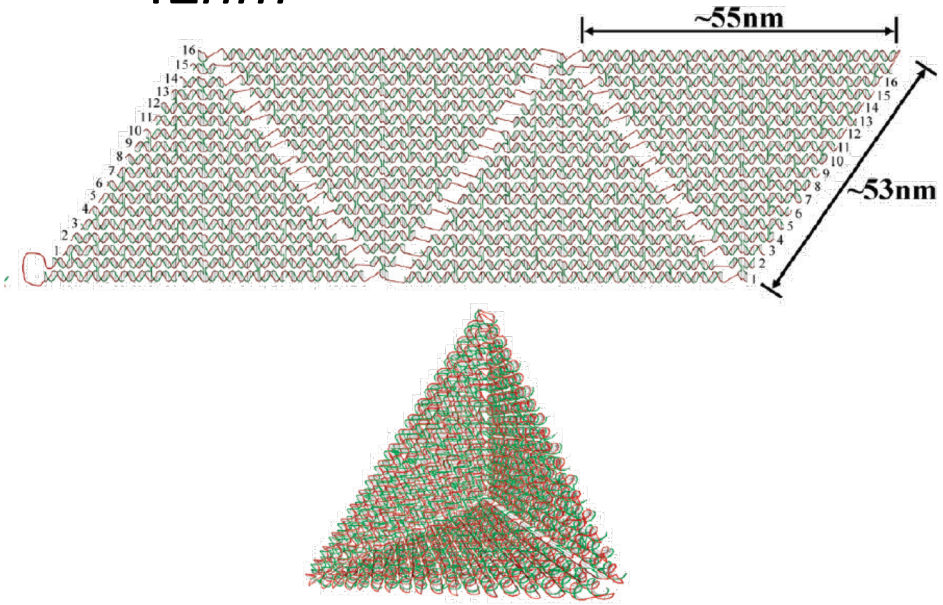
3D DNA origami – tetrahedron

Self-assembly of a nanoscale DNA box with a controllable lid: E. S. Andersen, M. Dong, M. M. Nielsen, K. Jahn, R. Subramani, W. Mamdouh, M.M. Golas, B. Sander, H. Stark, C.L.P. Oliveira, J.S. Pedersen, V. Birkedal, F. Besenbacher, K.V. Gothelf & J. Kjems.

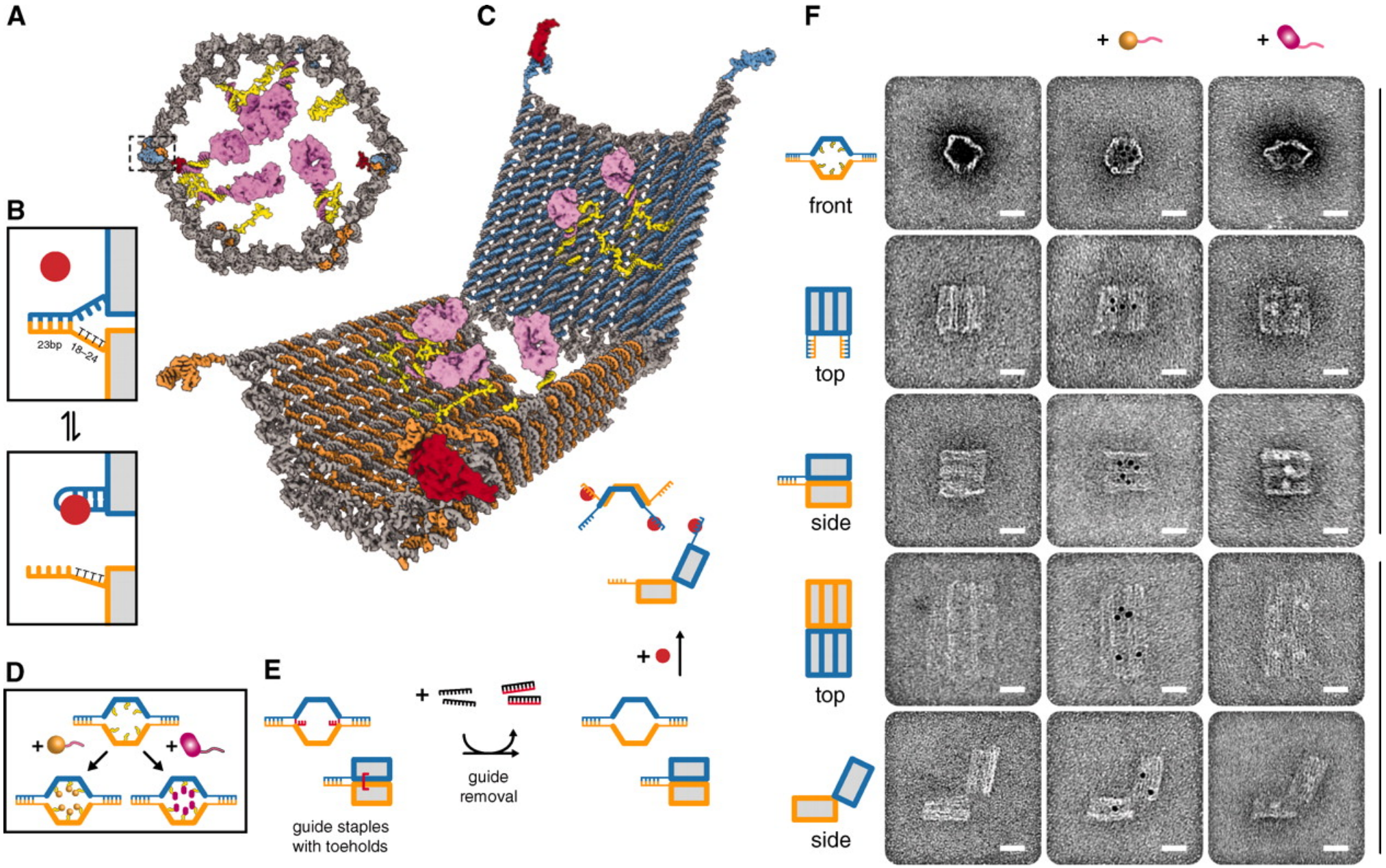


Scaffolded DNA Origami of a DNA Tetrahedron Molecular Container: Y. Ke, J. Sharma, M. Liu, K. Jahn, Y. Liu and H. Yan



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



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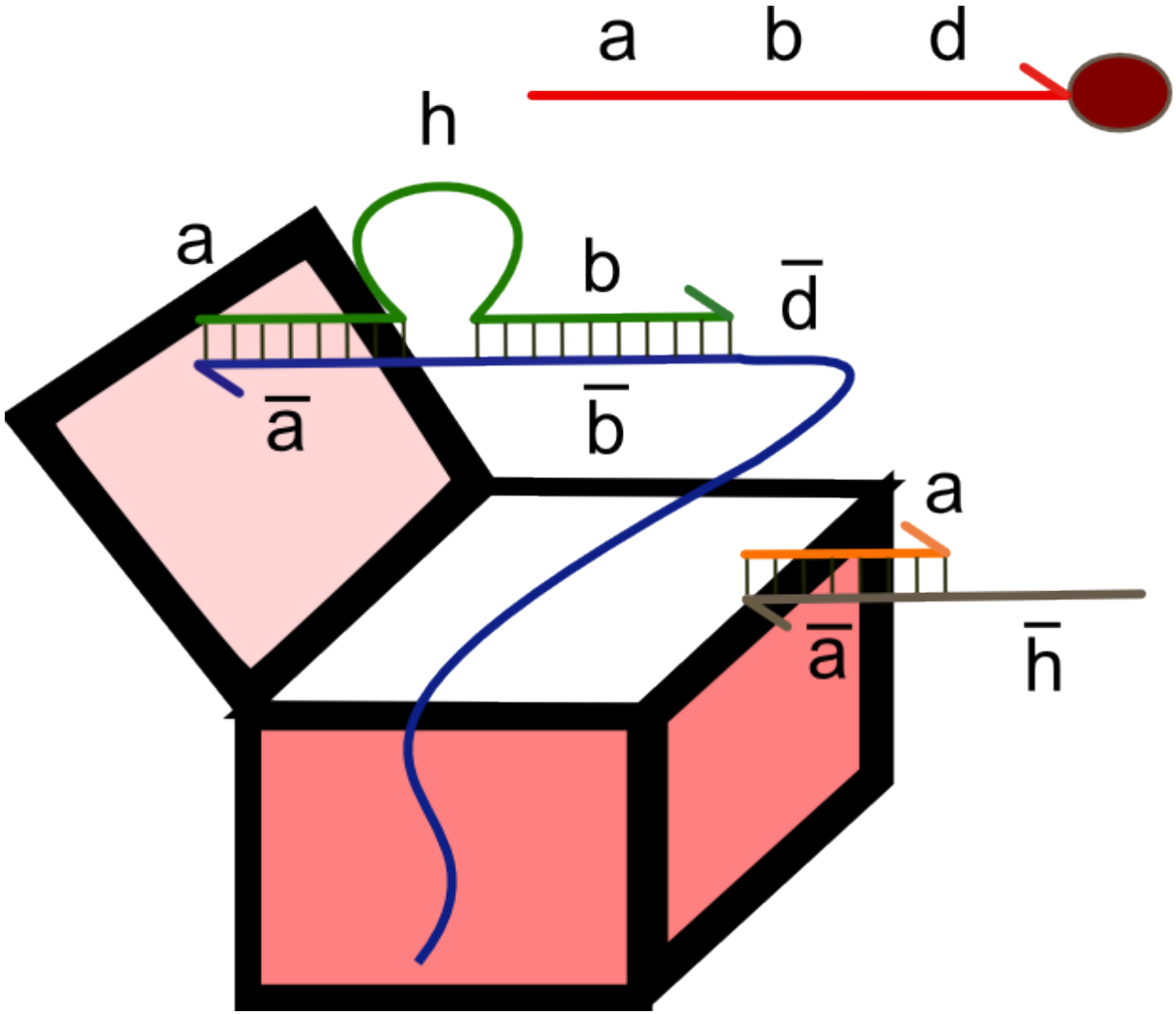
Bear trap: Proximity Sensed Molecular Capture



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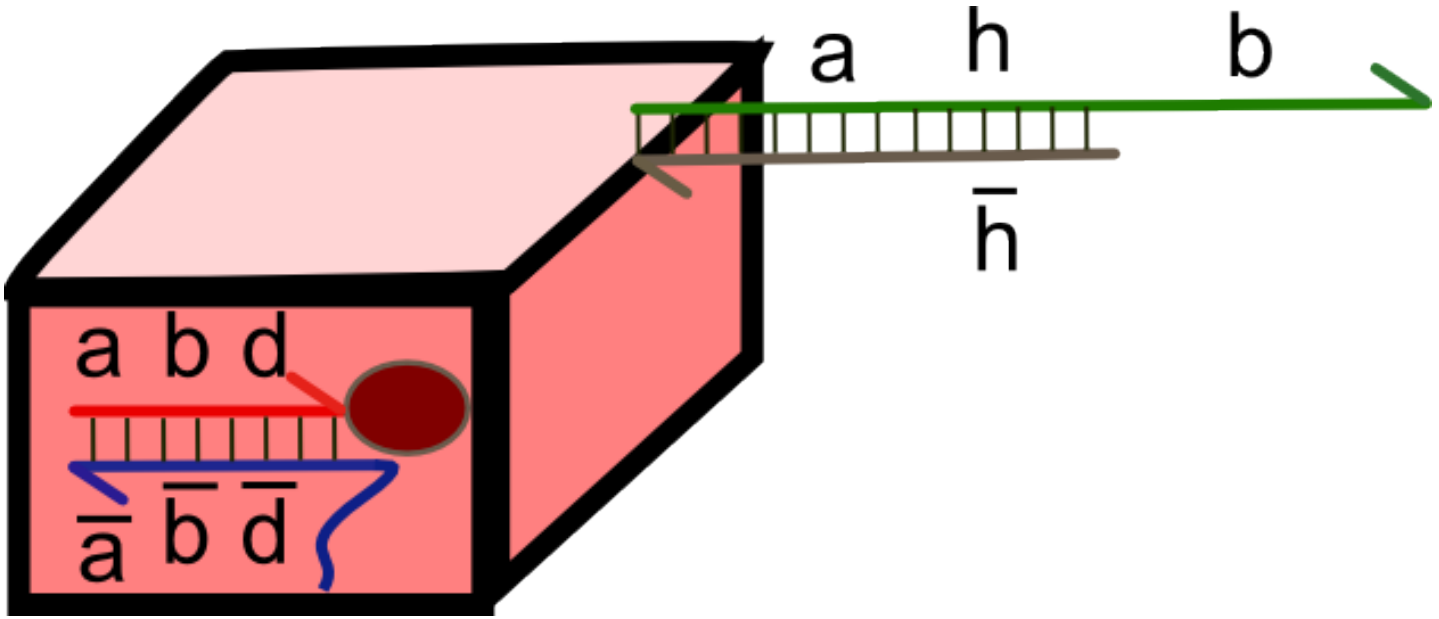
Bear trap: Proximity Sensed Molecular Capture



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Meta-DNA:

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

Harish Chandran, Nikhil Gopalkrishnan, Bernard Yurke, John Reif, Meta-DNA: Synthetic Biology via DNA Nanostructures and Hybridization Reactions, Journal of the Royal Society Interface, (published online Jan., 2012), pp. 1742-5662 doi: 10.1098/rsif.2011.0819

An expanded version appears as Meta-DNA: A DNA-Based Approach to Synthetic Biology, Chapter in Systems and Synthetic Biology: A Systematic Approach, edited by K. Raman, G.B. Stan and V. Kulkarni, published by Springer, to appear (2015).

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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

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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

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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.

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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

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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

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Meta DNA

- 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

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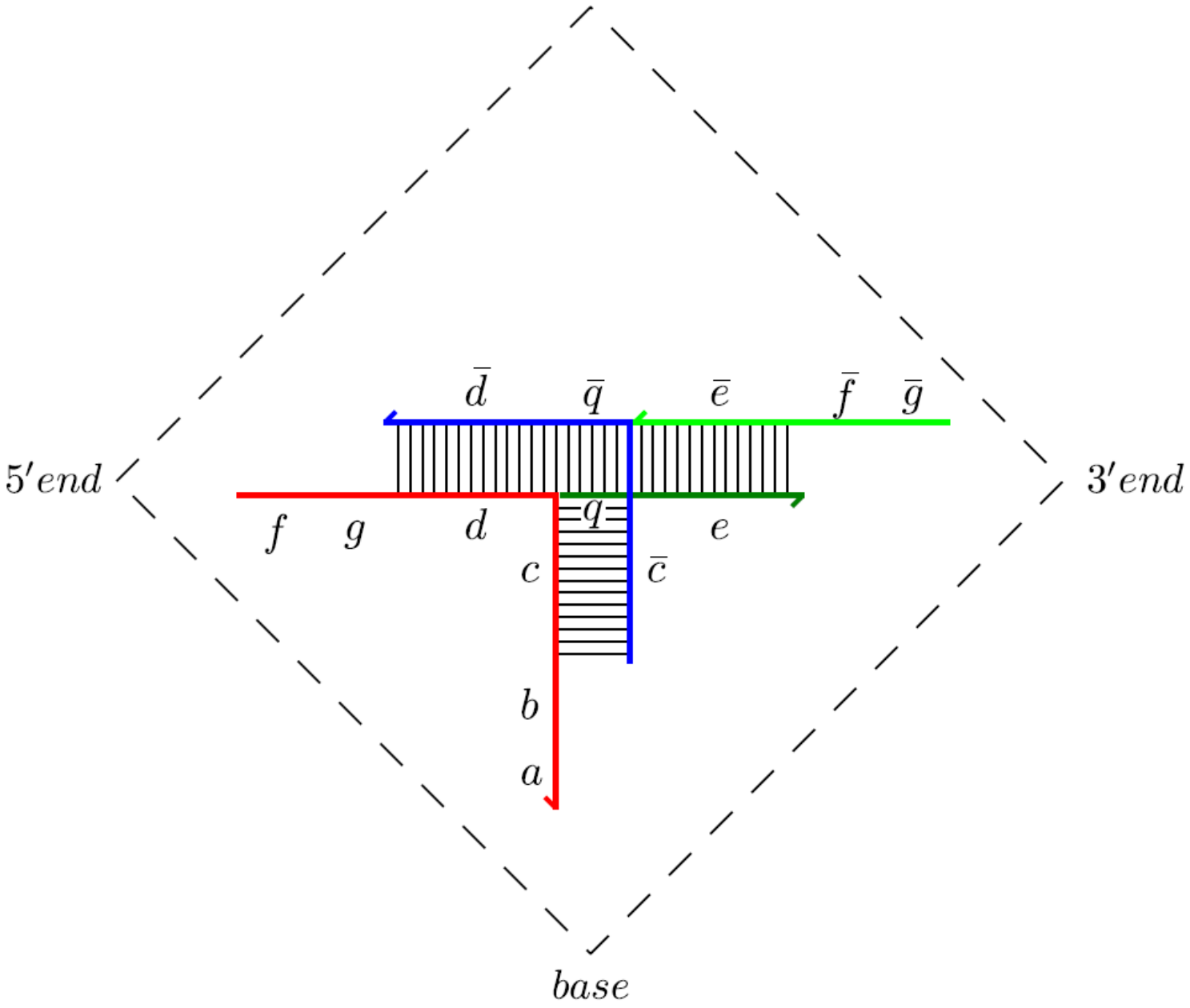
Internals of a Meta nucleotide



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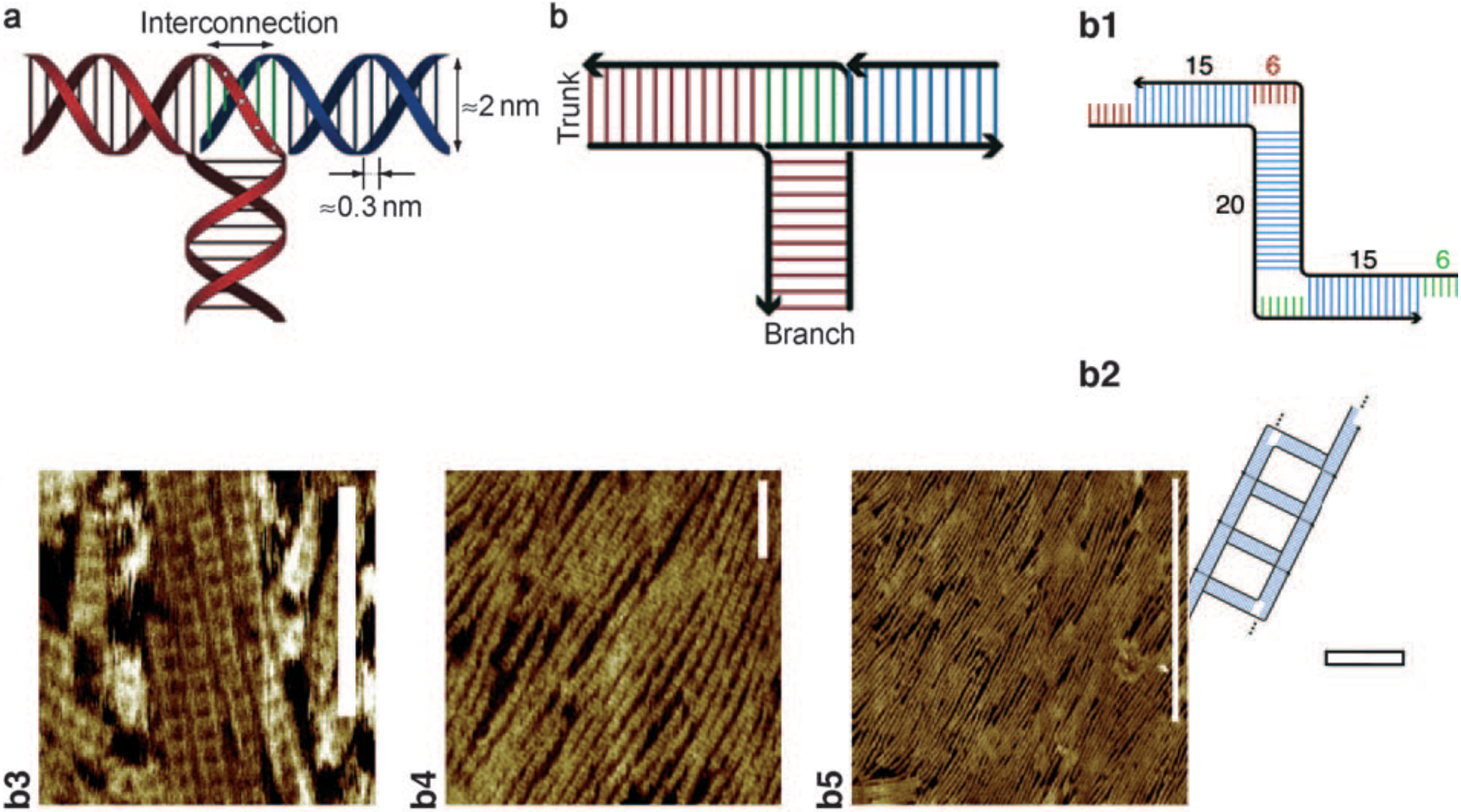
The T-junction



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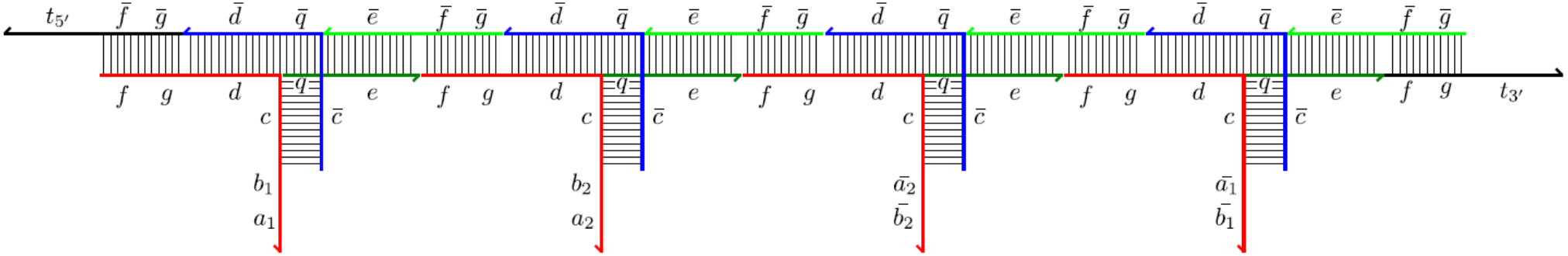
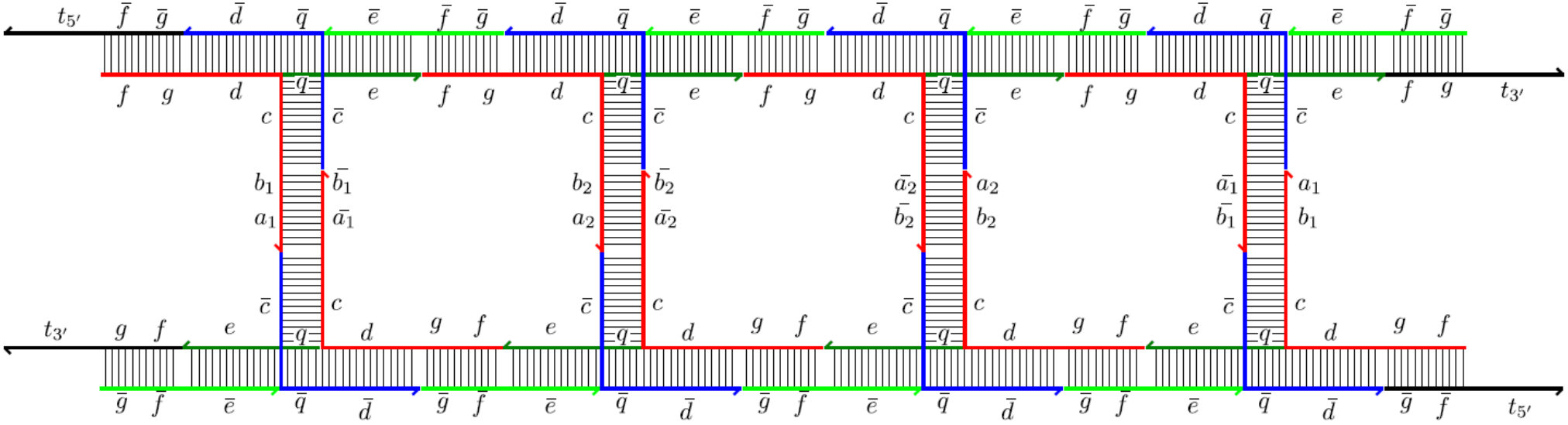
Internals of a ssMetaDNA and dsMetaDNA



(a) *Internals of a single stranded mDNA.*



(b) *Internals of a double stranded mDNA.*

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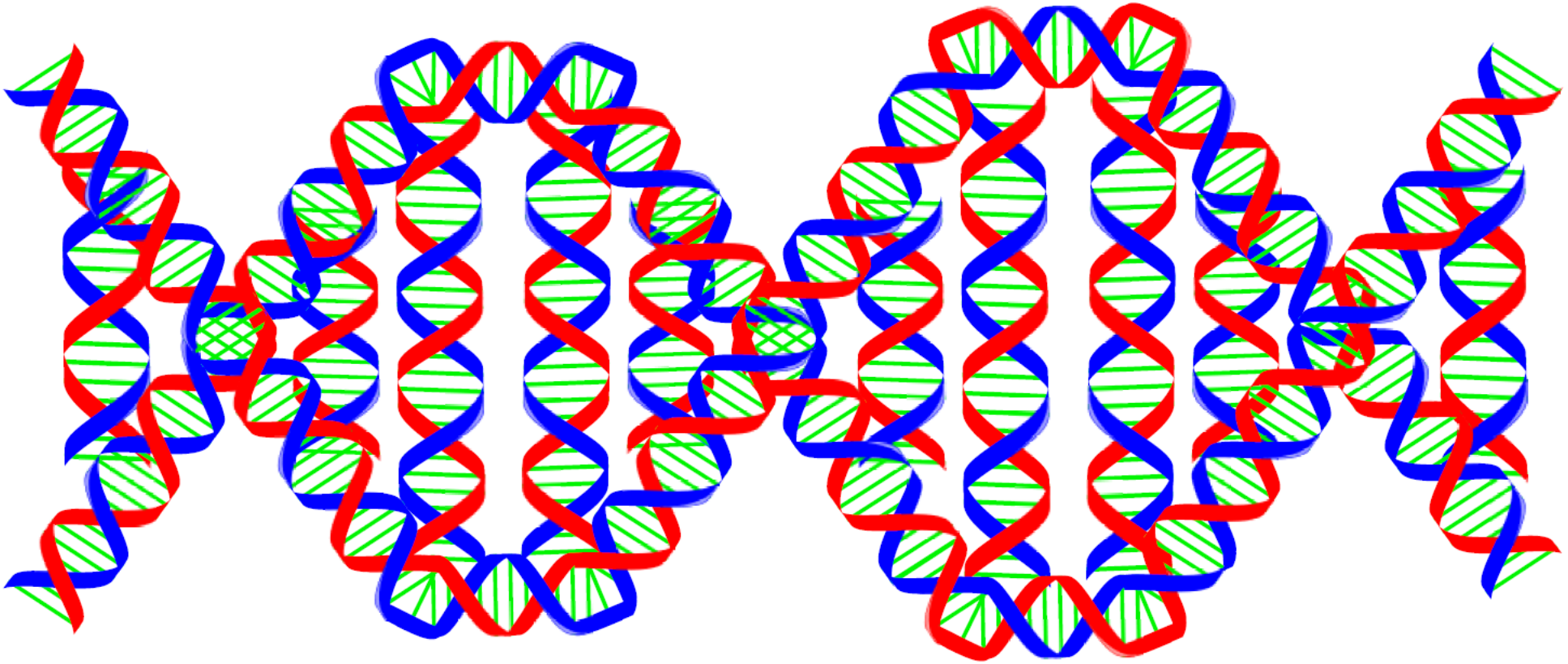
Artistic impression of the tertiary structure of the Meta double helix



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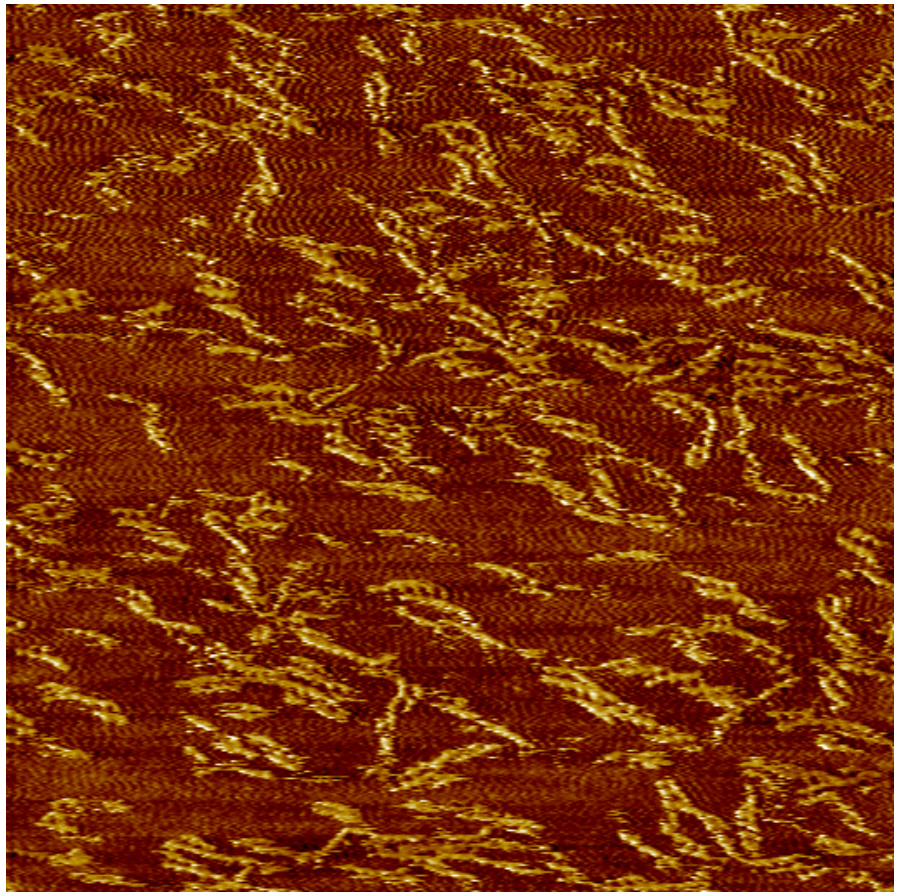
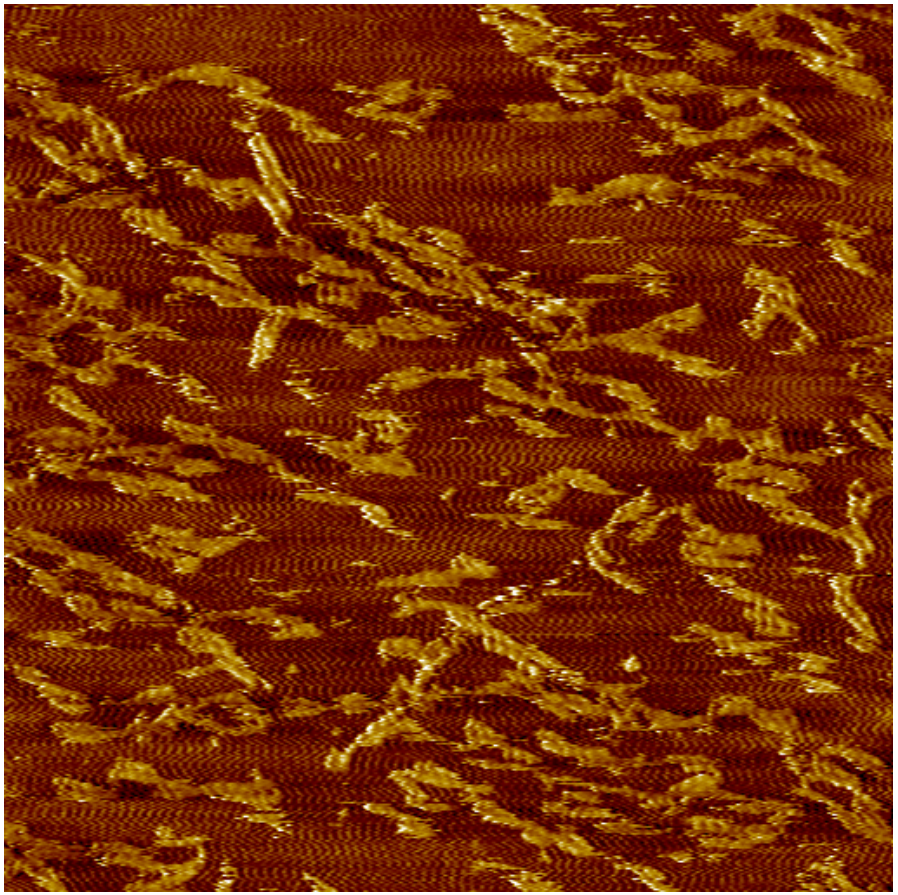
AFM images of the MetaDNA double helix





Yan lab

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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

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

- Transport devices
- Molecular motors
- Detection
- Signaling
- Computing systems

Hi-fidelity DNA Hybridization

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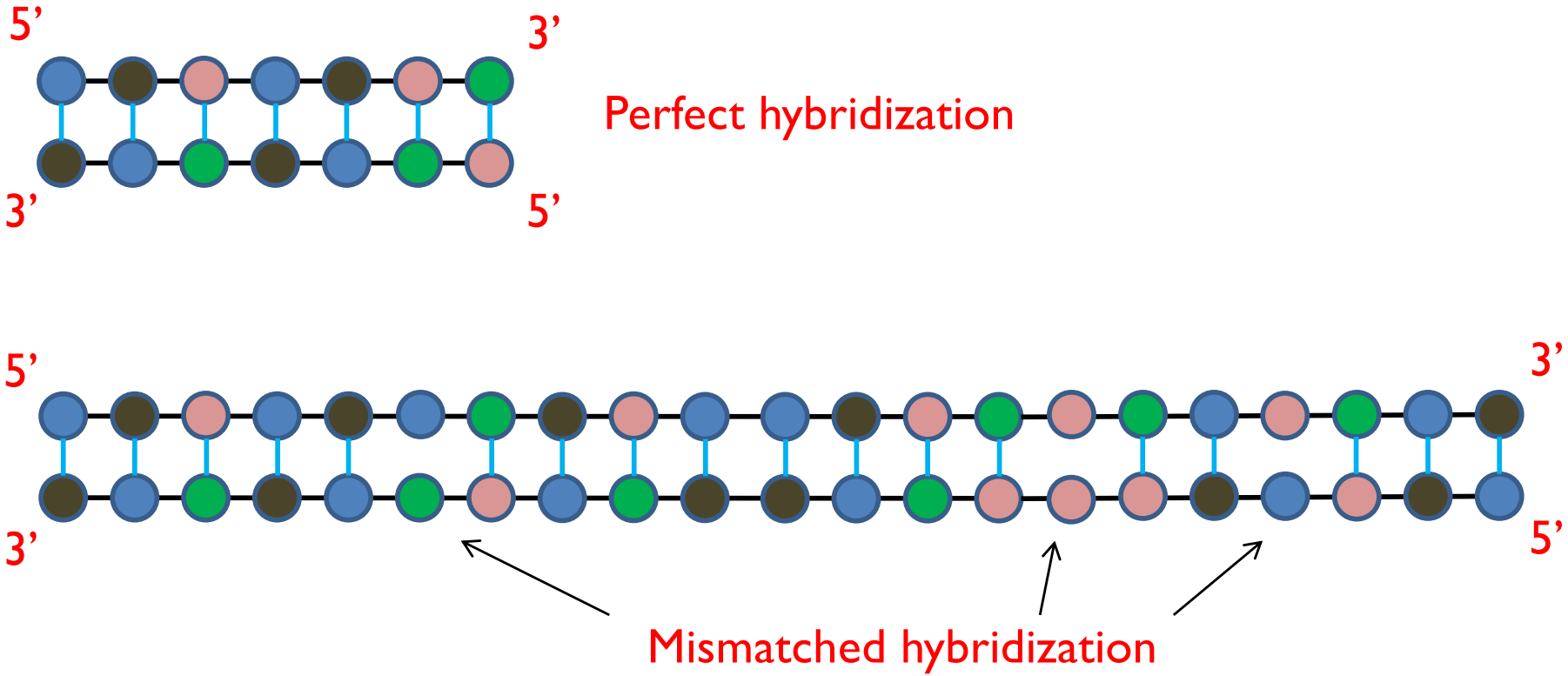
Hi-fidelity DNA hybridization



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

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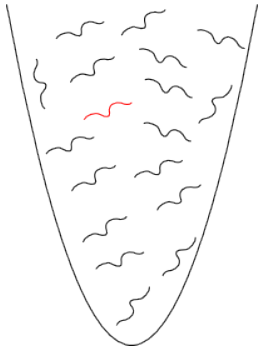
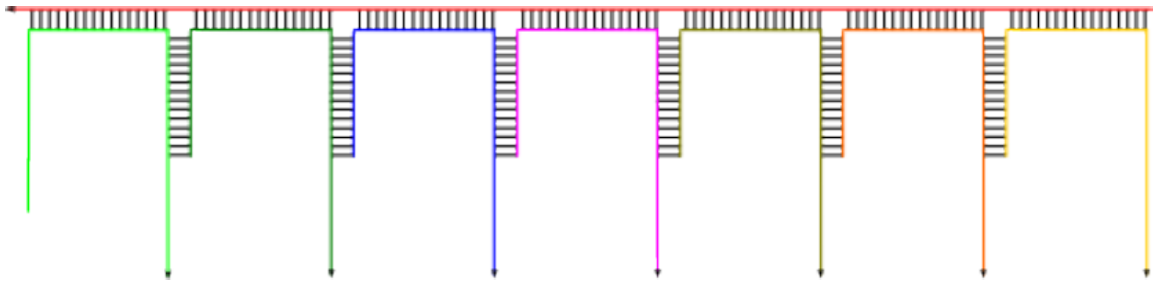
Exact hi-fidelity hybridization



- 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



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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 ≈ 95%** and **b ≈ 1/10**th of length of **s**

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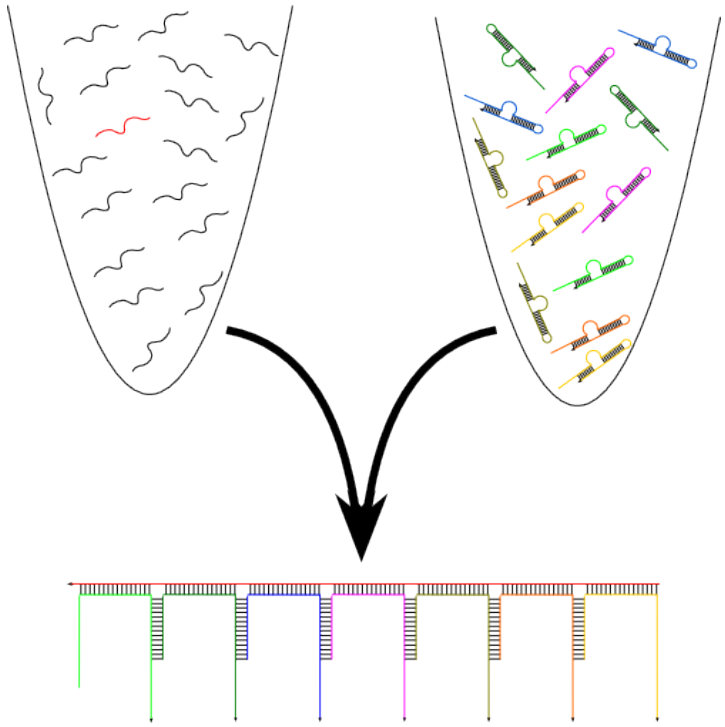
Our results

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

- Nikhil Gopalkrishnan, Harish Chandran and John Reif, **High-Fidelity DNA Hybridization using Programmable Molecular DNA Devices**, International Conference on DNA Computing and Molecular Programming, (DNA16) pp 59-70.



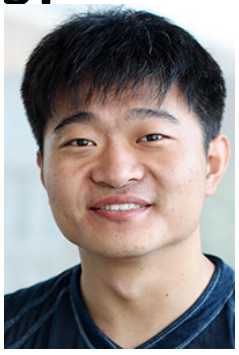
Reif Lab

- 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)

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Reif Papers on the Web

Reif Papers on DNA nanoscience on the Web:

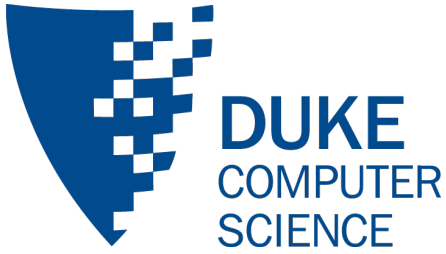
- http://www.cs.duke.edu/~reif/vita/papers.html

- **Survey on DNA Computation:**

Hieu Bui, Harish Chandran, Sudhanshu Garg, Nikhil Gopalkrishnan, Reem Mokhtar, Tianqi Song and John H Reif, DNA Computing, Chapter in Section 3: Architecture and Organization, Volume I: Computer Science and Software Engineering (Edited by Teofilo F. Gonzalez), The Computer Science Handbook, Third Edition (Editor-In-Chief Allen B. Tucker), Taylor & Francis Group, (2014).

Other Reif Papers on the Web:

- http://www.cs.duke.edu/~reif/vita/papers.html



Talk Locations on Reif's Website

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

DNA Computing: Theory, Experiments & Software:

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