DNA based Non-Autonomous Nanorobotic Devices

John H. Reif

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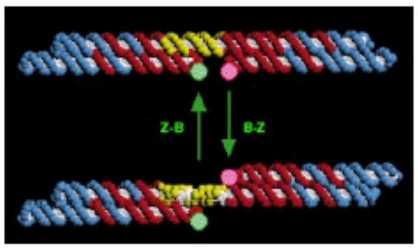
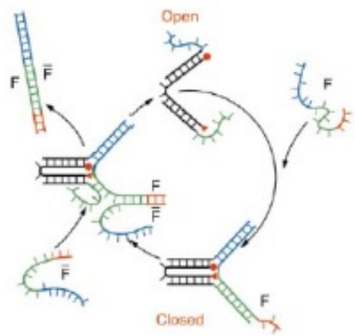
Early DNA robotics devices needed external control, so not autonomous

Rotation



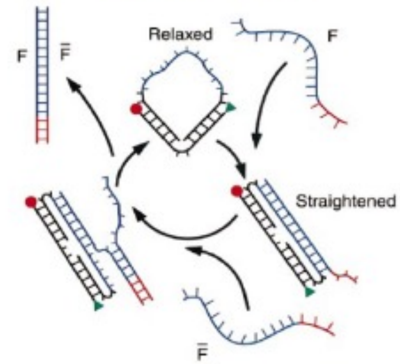
(Mao *et al* 99)

Open/close



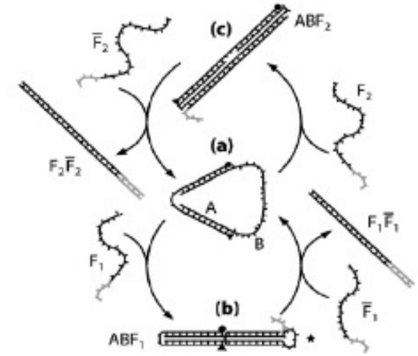
(Yurke *et al* 00)

Open/close



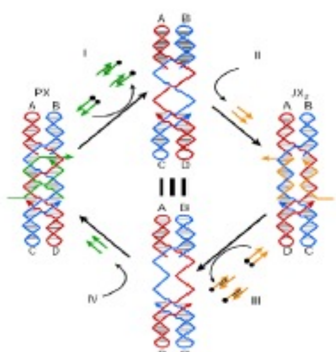
(Simmel *et al* 01)

Open/close



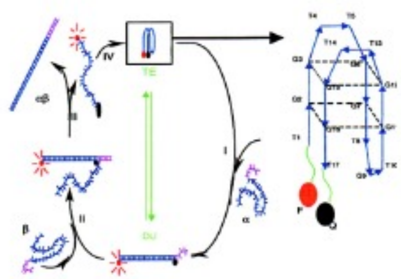
(Simmel *et al* 02)

Rotation



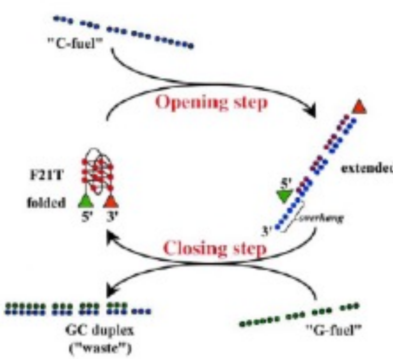
(Yan *et al* 02)

Extension/contraction



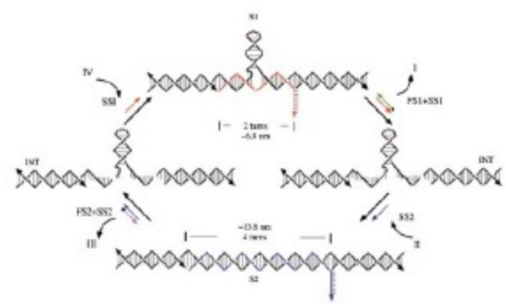
(Li *et al* 02)

Extension/contraction



(Alberti *et al* 03)

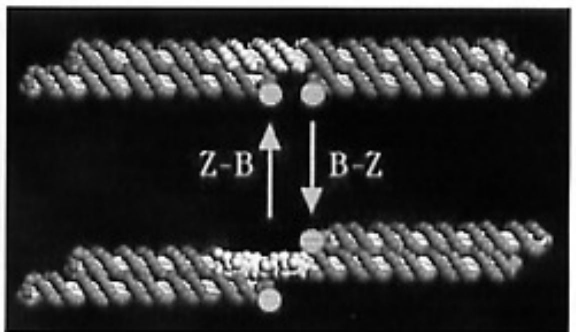
Extension/contraction



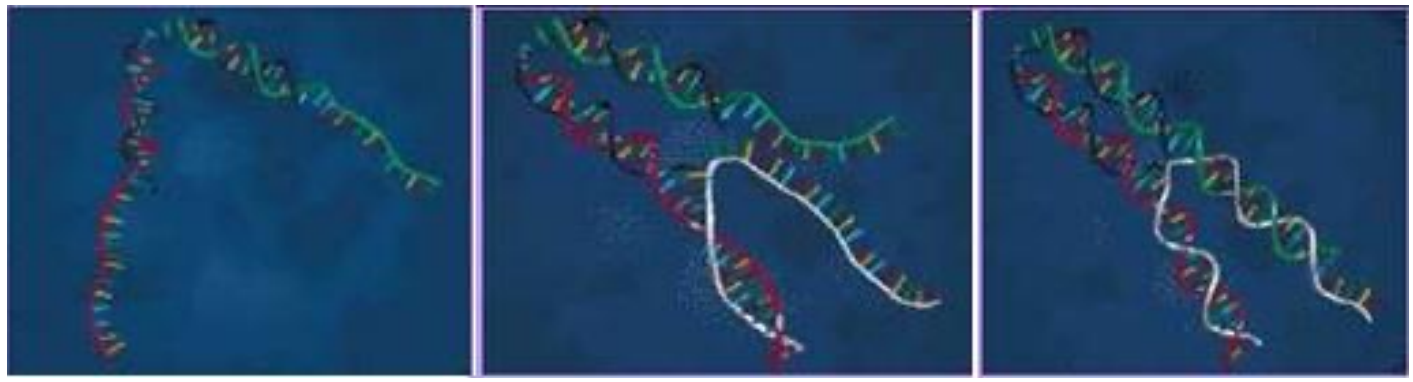
(Feng *et al* 03)

Non-Autonomous
DNA based Nanorobotical devices

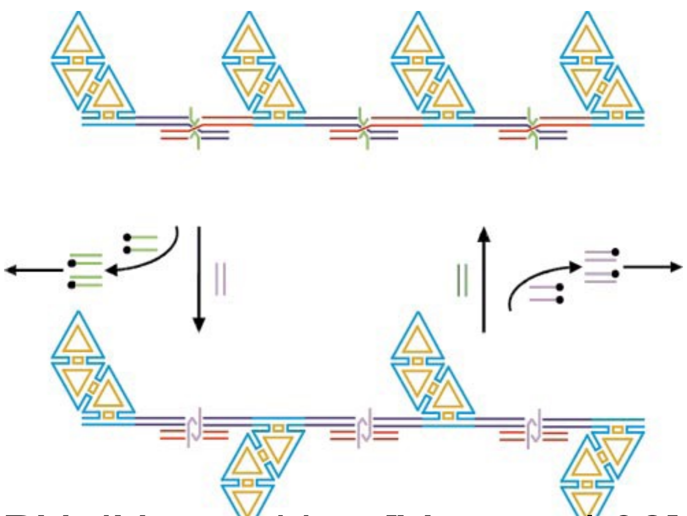
Advantages of DNA-based synthetic molecular devices:
- **simple to design and engineer**
- **well-established biochemistry used to manipulate DNA nanostructures**



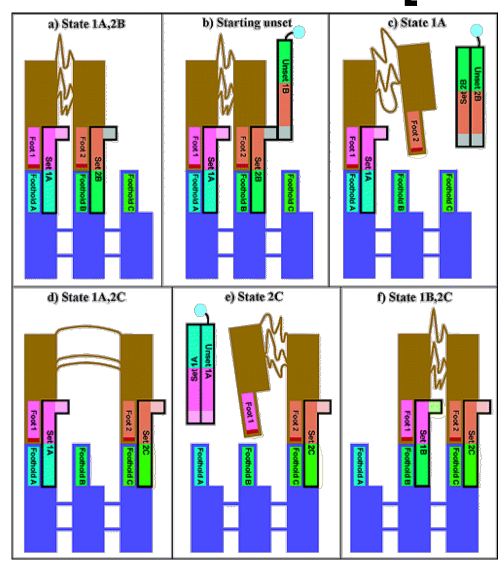
B-Z transition device [Mao, Seeman 99]



DNA-fuelled Molecular machine [Yurke et al 00]

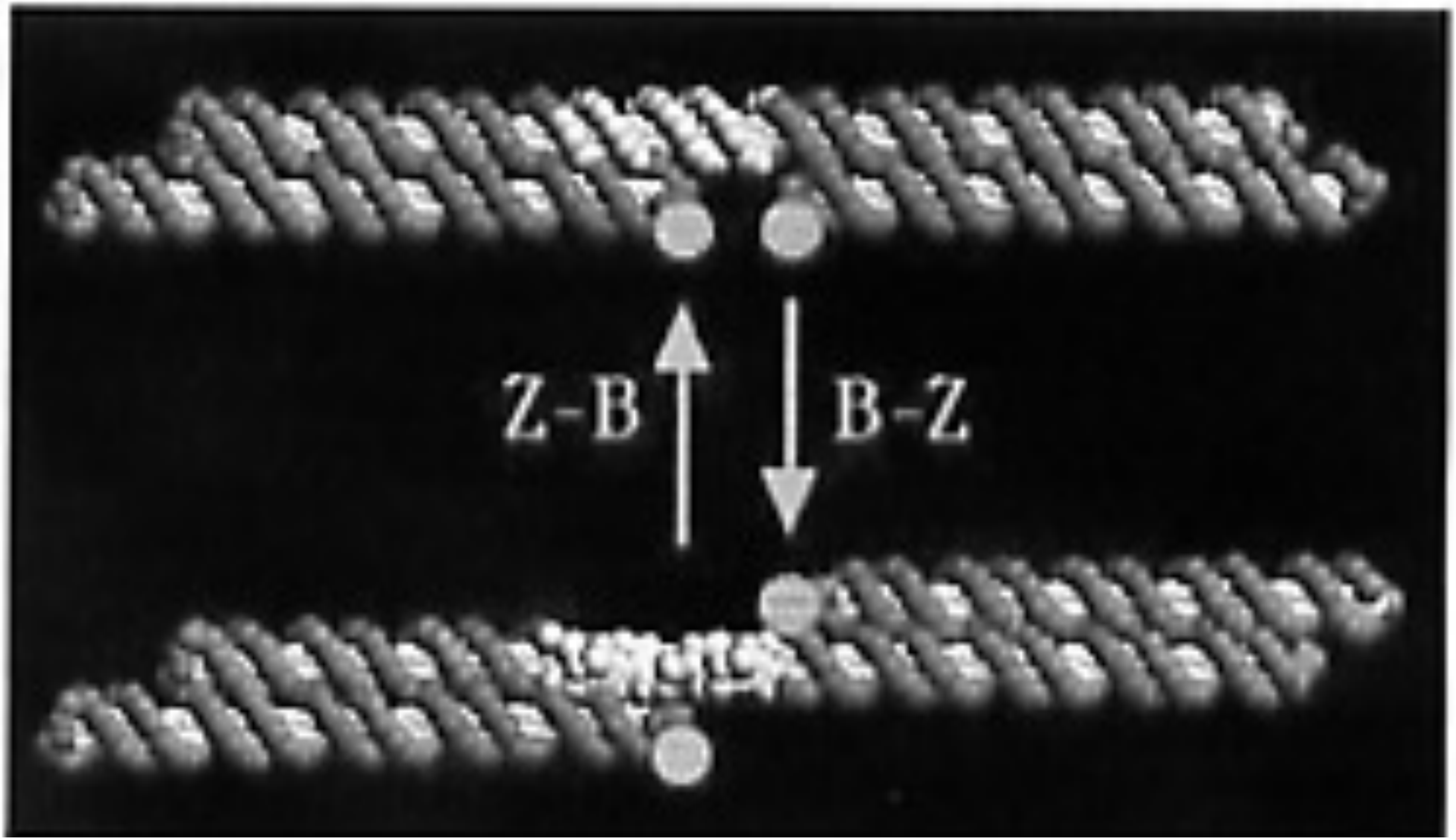


PX-JX transition [Yan et al 02]



DNA Biped walker [Sherman et al 04]

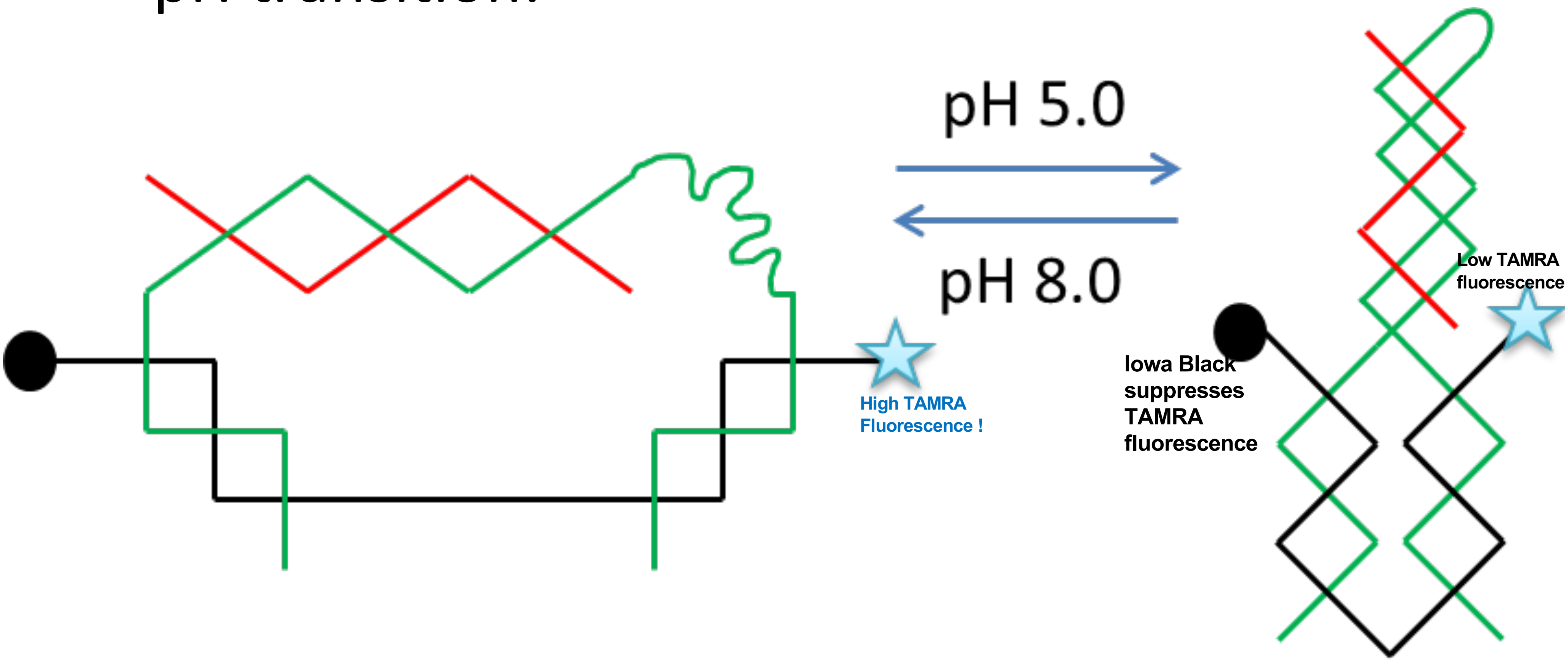
Non-Autonomous DNA based Nanorobotical device Using pH dependant transitions between B-form and Z-form Duplex DNA



B-Z transition device [Mao, Seeman 99]

NonAutonomous DNA Nanorobotic Device using Change in pH

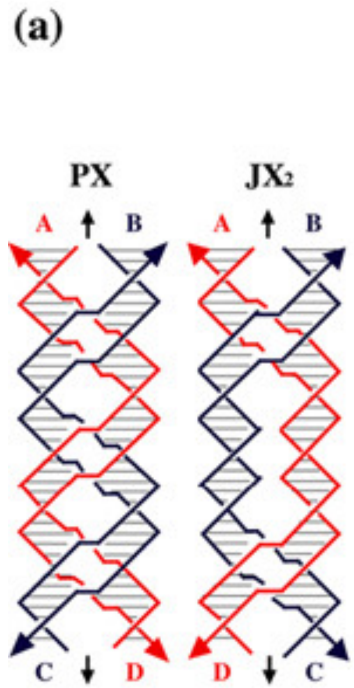
- pH transition:

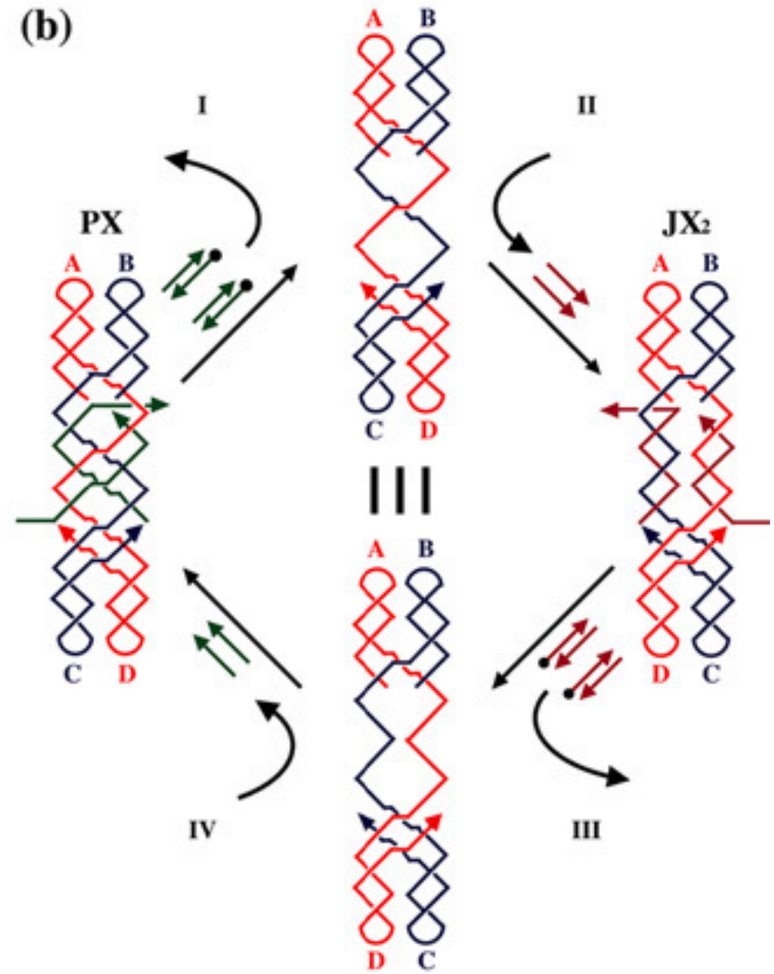


Switch conformation based on environment (Ph)

Non-Autonomous DNA based nanorobotic device using fuel strands to control PX to JX transition

[Seeman]





(a) illustrates PX and JX motifs of DNA:

The PX motif of DNA:

- Two stands are drawn in red and two in blue, where the arrowheads indicate the 3' ends of the strands.
- Has two helical domains formed by four strands that flank a central dyad axis (indicated by the vertical black arrows).
- 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 JX2 motif of DNA:

- Similar to PX, but 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 device operation:

- On the left is a PX molecule.

In process I: Green strands are removed by addition of biotinylated green fuel strands (biotin indicated by black circles).

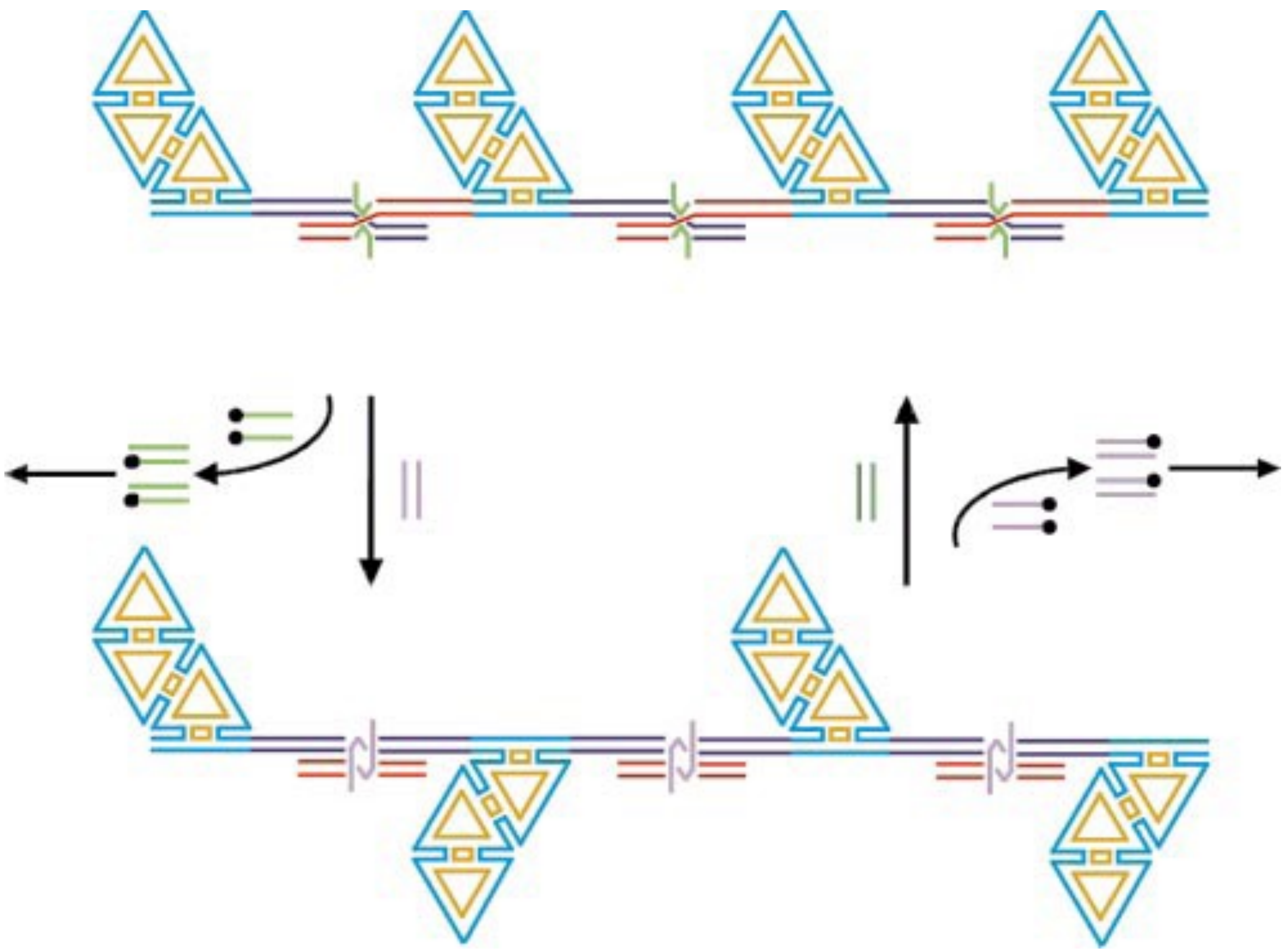
In process II: The unstructured intermediate is converted to the JX2 motif by the addition of the purple set strands.

In process III: The JX2 molecule is converted to the unstructured intermediate by the addition of biotinylated purple fuel strands.

- The identity of this intermediate and the one above it is indicated by the identity sign between them.

In process IV: The cycle is completed by the addition of green set strands, restoring the PX device.

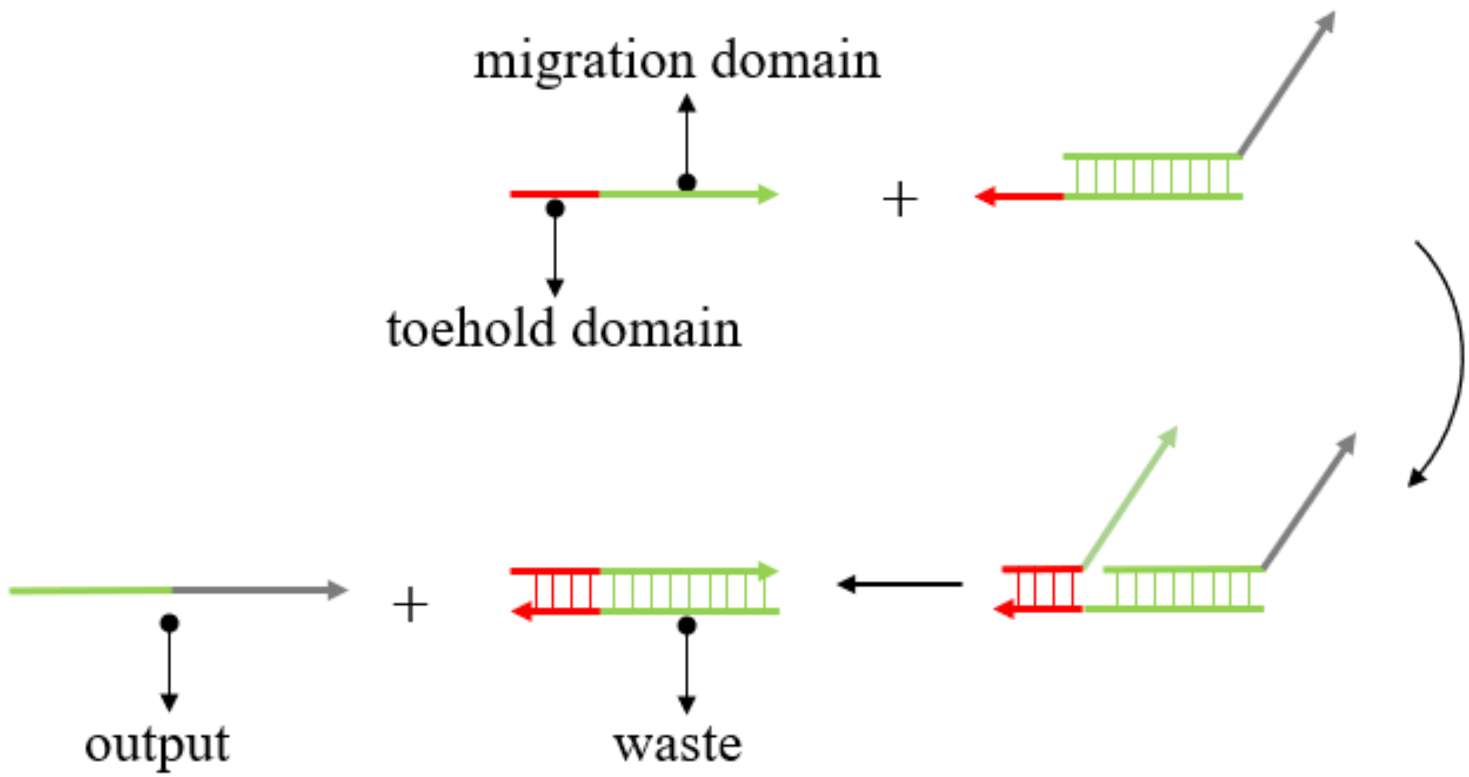
Another Non-Autonomous DNA Nanorobotical device using PX-JX transition



PX-JX transition [Yan et al 02]

DNA Tweezers: [Yurke2000]

- Nonautonomous Device
- First use of Toehold-Mediated Strand Displacement

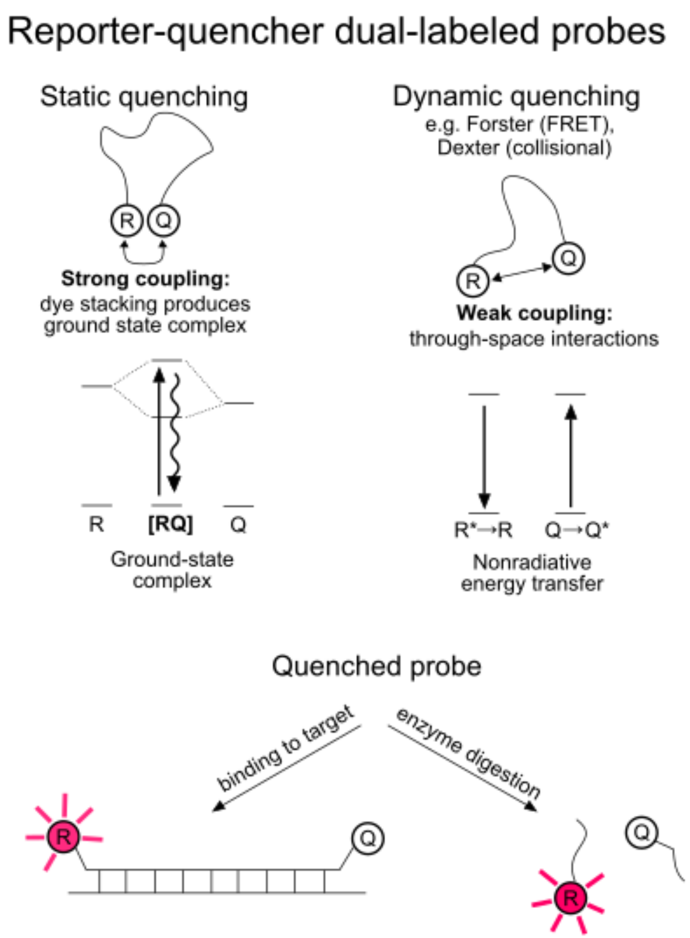


Toehold-Mediated Strand Displacement

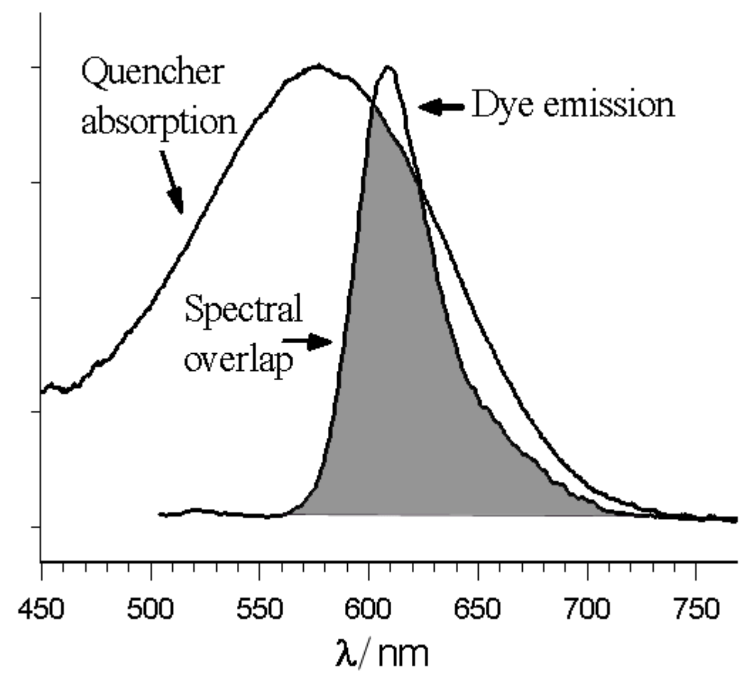
DNA Tweezers: [Yurke2000]

Uses two fluorophores:
- a donor dye (5 ' -**TET**) and
- an acceptor dye (3 ' -**TAMRA**)

Quenching:
- is a process which decreases the intensity of a fluorescent die.
- is the basis for Förster resonance energy transfer (FRET) assays



Donor emission and quencher absorption spectral overlap



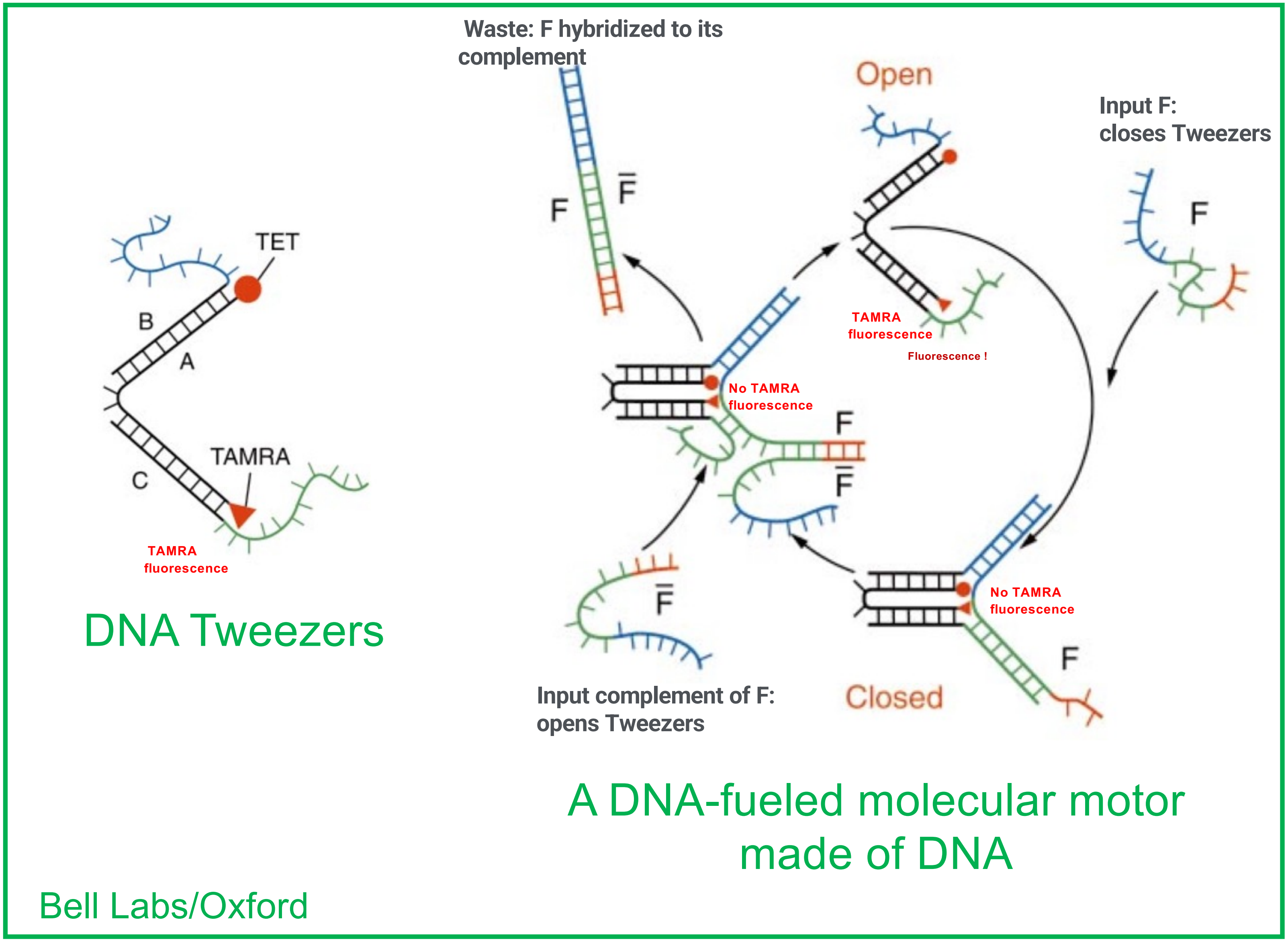
DNA Tweezers: [Yurke2000]

- Nonautonomous Device
- Used Strand Displacement

Uses two fluorophores:

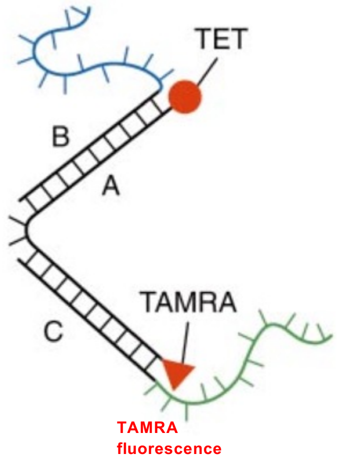
● a donor dye (5 ' -**TET**) and

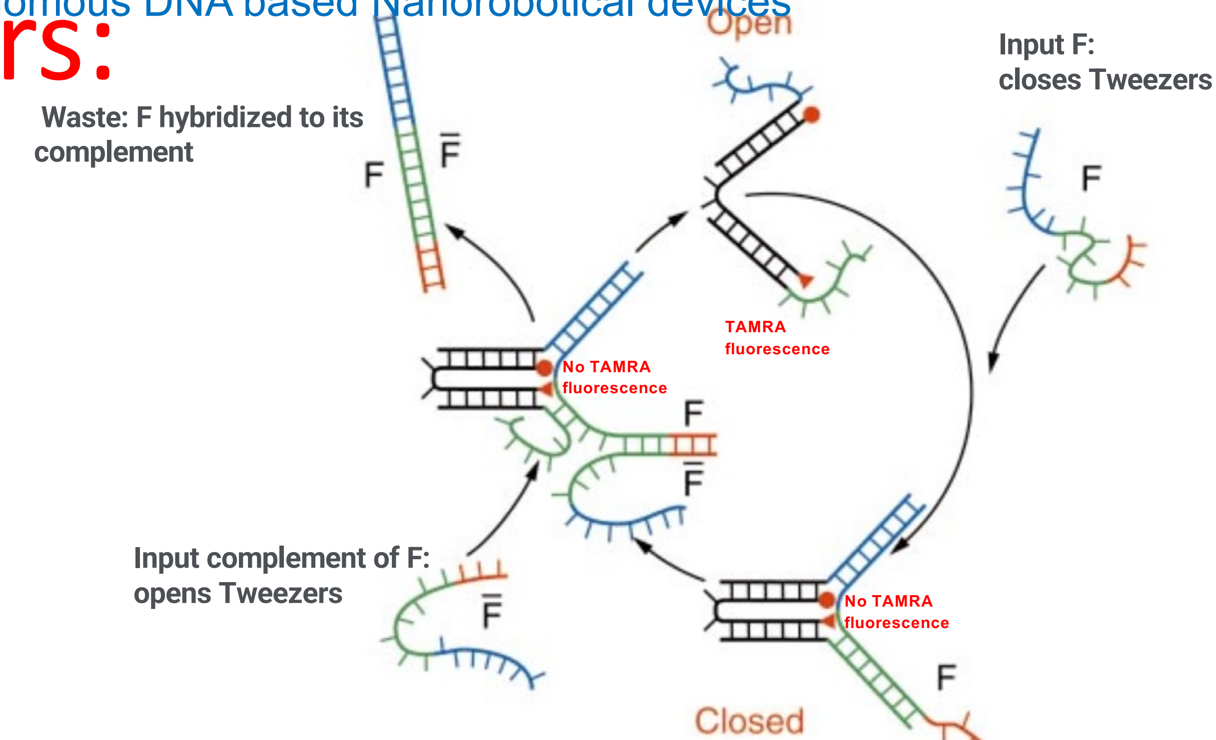
▲ an acceptor dye (3 ' -**TAMRA**)



DNA Tweezers:

Non-Autonomous DNA based Nanorobotical devices





Two double stranded DNA arms linked via a flexible single stranded region
Single stranded sticky regions extend beyond the ends of the arms.

States:

(1) Open state when the sticky ends are unbound

(2) Closed state when the sticky regions are hybridized to a fuel strand F.

Non-Autonomous DNA Nanorobotical device Operation:

State Transitions (controlled by adding fuel strand F or its complement):

(a) from the open to the closed state via the hybridization of the fuel strand F.

(b) from closed to open state when the complement to F binds to F via a toehold and strand displaces it, freeing the sticky regions of the tweezers.

Non-Autonomous DNA based Nanorobotical devices

DNA Biped walker [Sherman et al 04]

W Sherman, N Seeman, A Precisely Controlled DNA Biped Walking Device, Nano Letters, vol. 4, pp. 1203-1207, 2004.

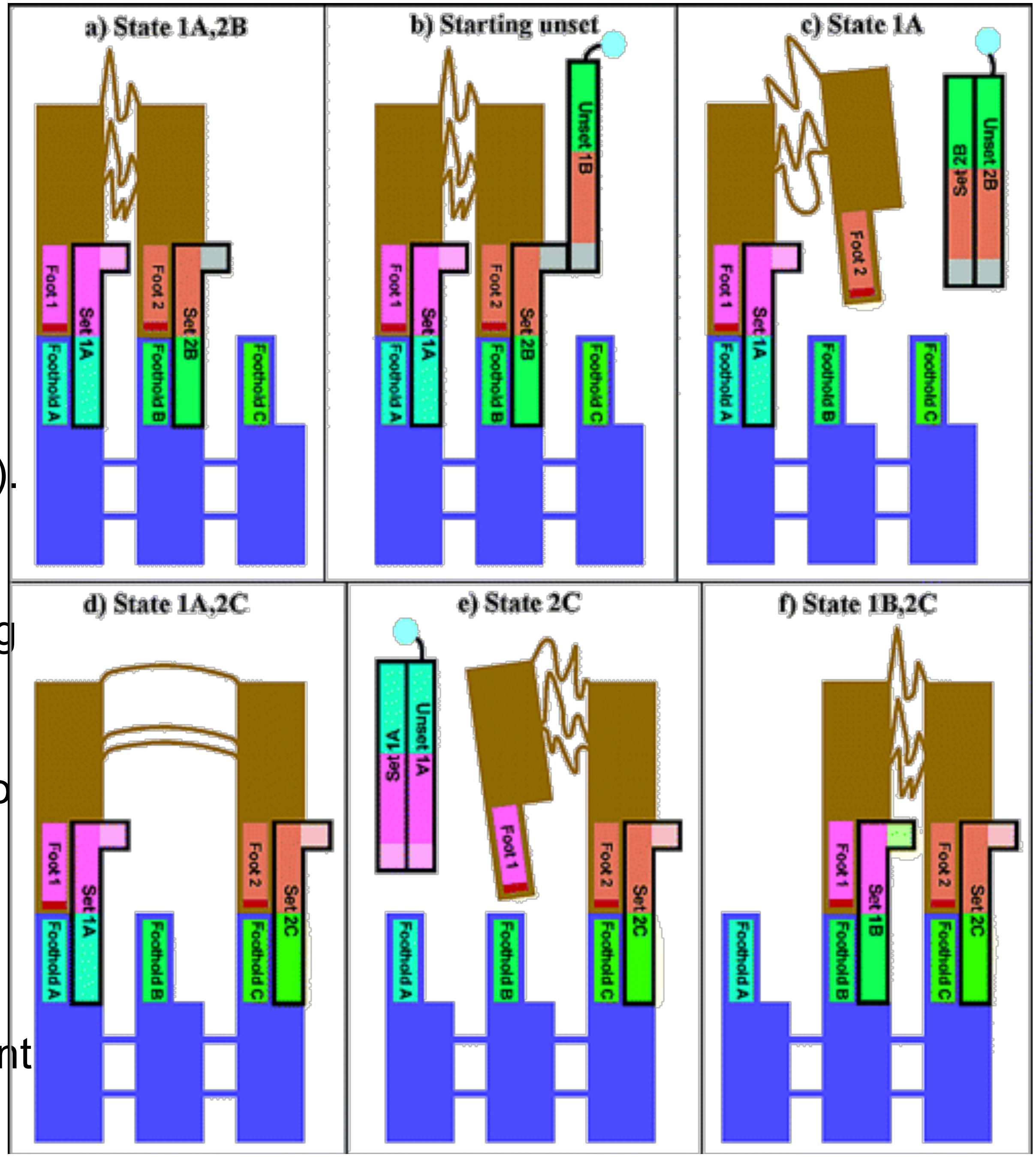
- The biped walker moves forward in an inchworm fashion.
- The relative positions of leading and trailing leg do not change.

Parts:

- Track (blue),
- Two legs (brown),
- Two feet (pink and orange) and
- Two footholds (green & turquoise).

Walking:

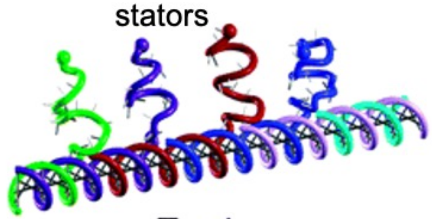
- The walker progresses along the track by the binding and unbinding of the feet on the footholds.
- **Binding:** occurs when a single stranded *set* strand binds a foot to its foothold by forming a bridge across them.
- **Unbinding:** occurs when this bridge is stripped away via a toehold due to strand displacement action of *unset* strands.



Non-Autonomous DNA based Nanorobotic Walker

DNA Biped walker [Shin&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.



DNA Sequences for the Walker System

Walker strands (W)	
W1	5′-GGCTGGTTTCTGCTCTCTAGTTCGCGAGGTGCAATCTCCTATC-3′-BHQ1
W2	IBRQ-5′-GTCTGGGATGCTGGATACTGAACCTAGAGAGCAGAAACCAGCC-3′
Track strands (T)	
T1	HEX[c]-5′-GCTGTACTTTGGTTACTGAAAGGGAGTGGCTCGGA-3′
T2	5′-TCCGAGCCACTCCCTGGACACCATCTACAAACTTGTATGGGACGTAGCGT-3′-Cy5
T3	FAM[d]-5′-TCCACATCGGACTCTGTAATGCAAAGACACGTTACTTGTAGATGGTGTCC-3′
T4	5′-GTAACGTGTCTTTGCTCTCAAACATACGCTCTTCATGGCATTCGTCACCA-3′-Texas Red
T5	5′-AACTCTTAGCCAAGATCGTAAGCGTATGTTTGAGA-3′
T6	5′-TACGATCTTGGCTAAGAGTT-3′
Attachment strands (A)	
A1	5′-AGTAACCAAAGTACAGCACTGCGATAGGAGATTGCACCTCCAATTTACCC-3′
A2	5′-GACTGTTACGGTATCCAGCATCCCAGACGGTCAACGCTACGTCCCATACA-3′
A3[e]	5′-ACAGAGTCCGATGTGGAAGTCAGATAGGAGATTGCACCTCATCATTGTCG-3′
A4[e]	5′-GGATCAGTTAGTATCCAGCATCCCAGACCTAAGTGGTGACGAATGCCATG-3′

A1 hybridizes to red leg of Walker

A2: hybridizes to black leg of Walker

DNA Biped walker [Shin&Pierce2004]

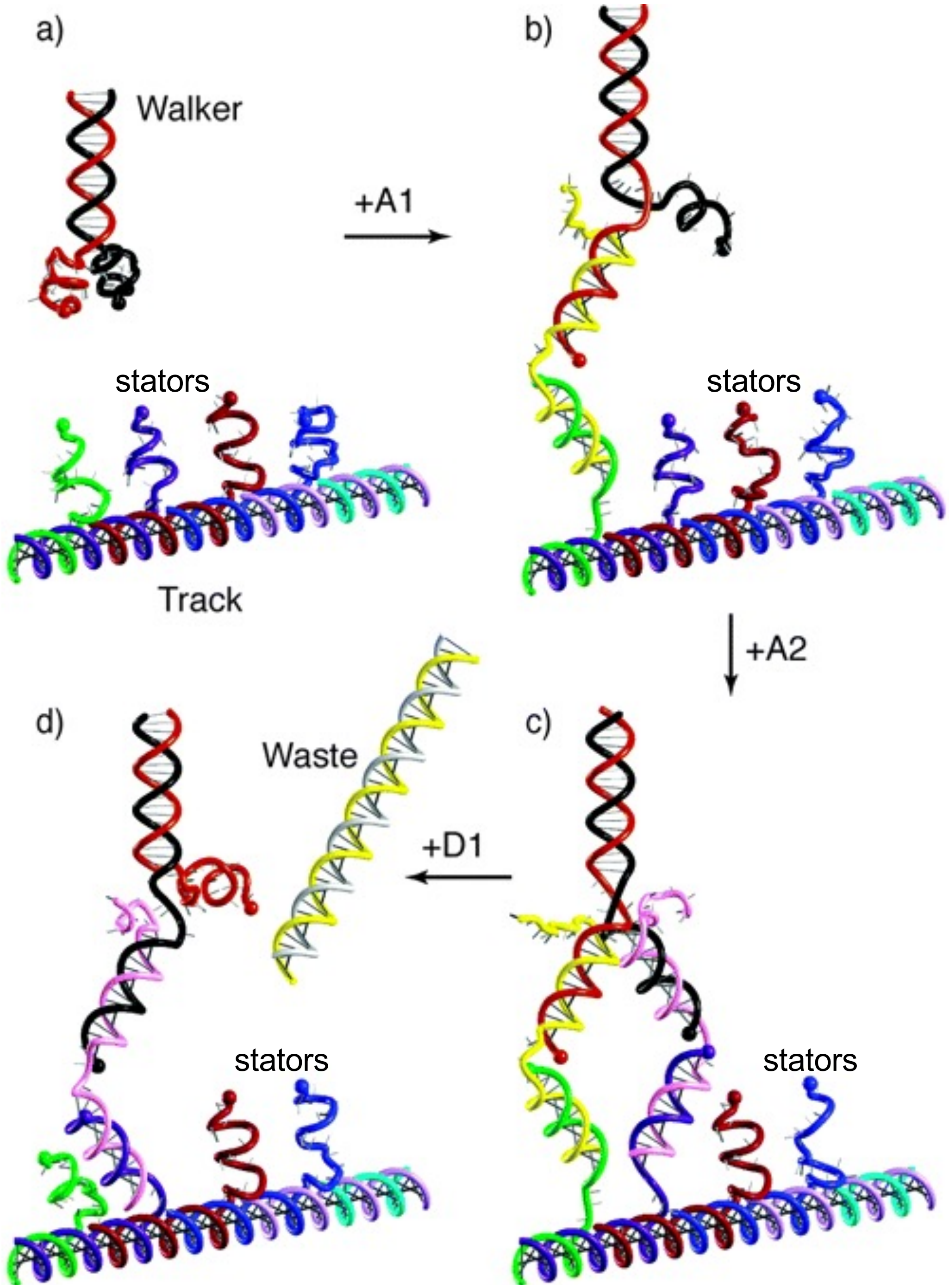
J-S Shin, N Pierce, A Synthetic DNA Walker for Molecular Transport, Journal of American Chemical Society, vol. 126, no. 35, p. 10834–10835, 2004.

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: 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.



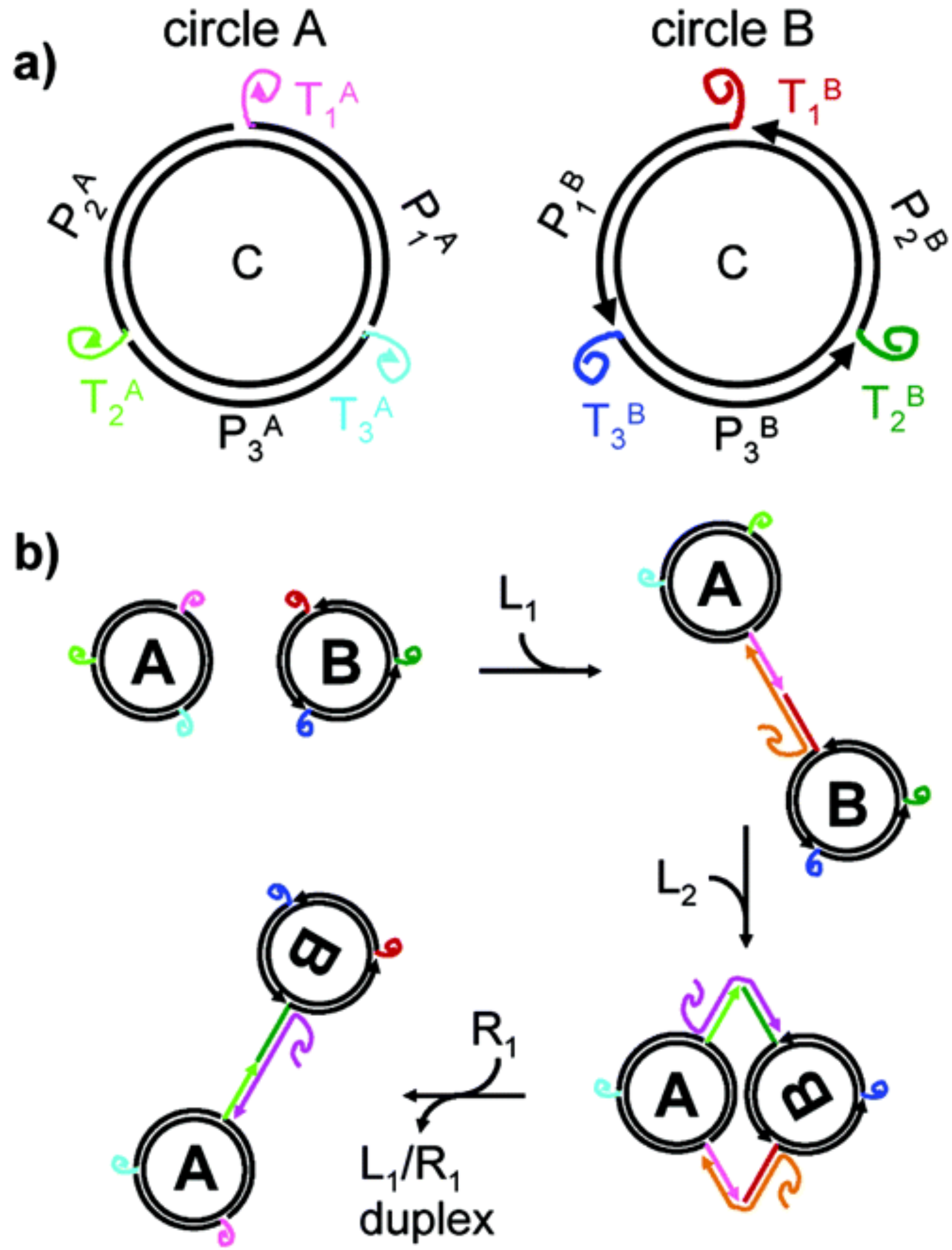
DNA Biped walker [Tian&Mao2004]

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.

Operation:

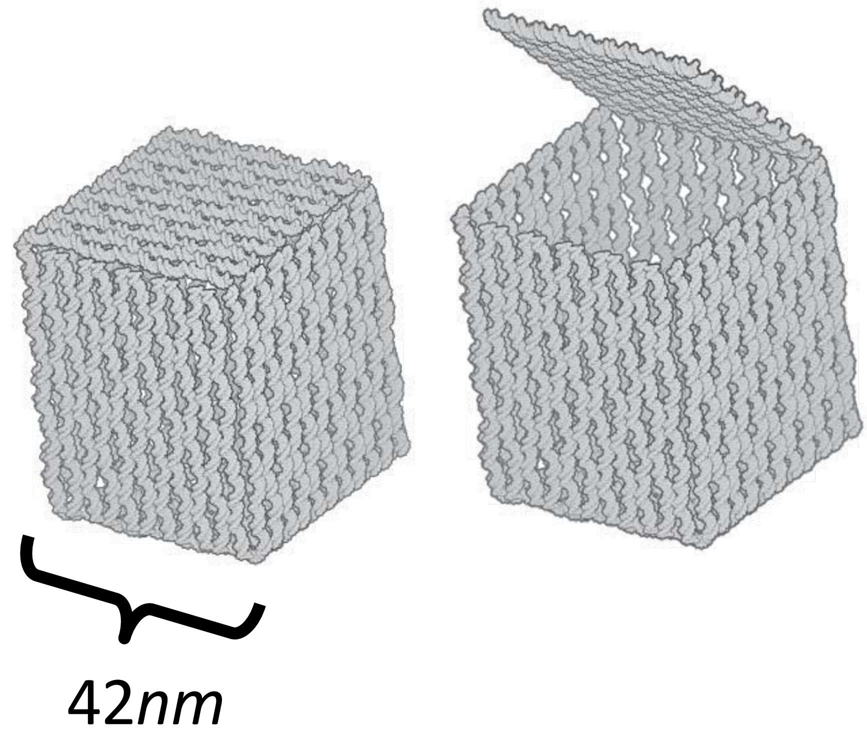
Same as the walker of Shin and Pierce except cargo walks along a circular track and returns to its original position after three steps.

Due to the symmetry of the design, the cargo and the track have the same geometric circular structure.



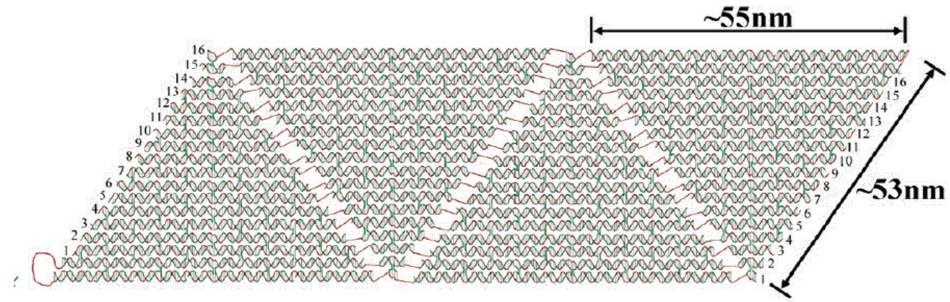
DNA Devices that Open Nano-Containers

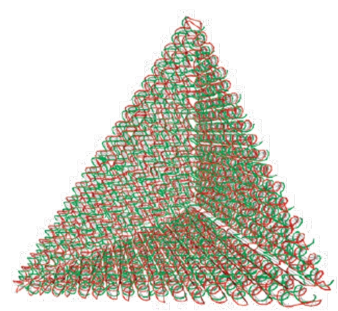
3D DNA Origami Containers



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.

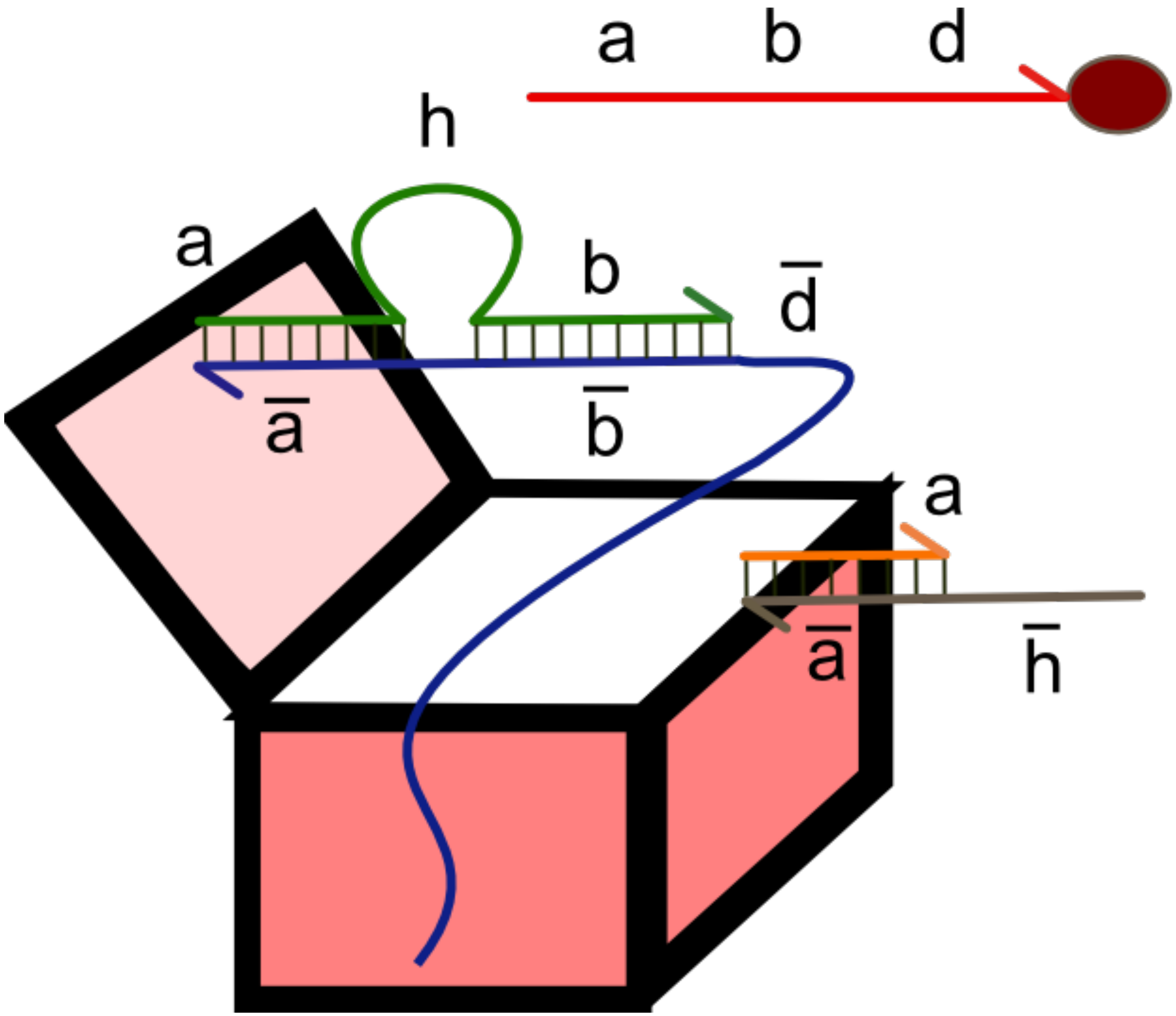




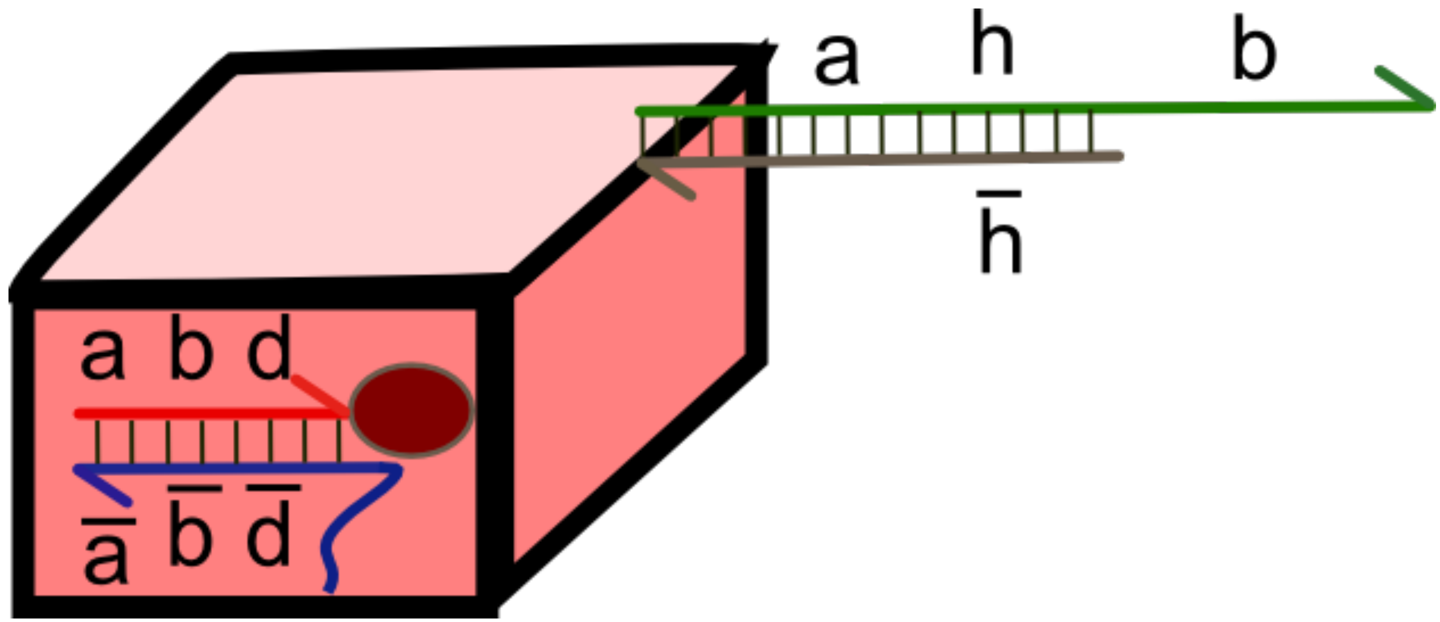
Scaffolded DNA Origami of a 3D DNA Tetrahedron Molecular Container:

Y. Ke, J. Sharma, M. Liu, K. Jahn, Y. Liu and H. Yan

Bear trap: Proximity Sensed Molecular Capture

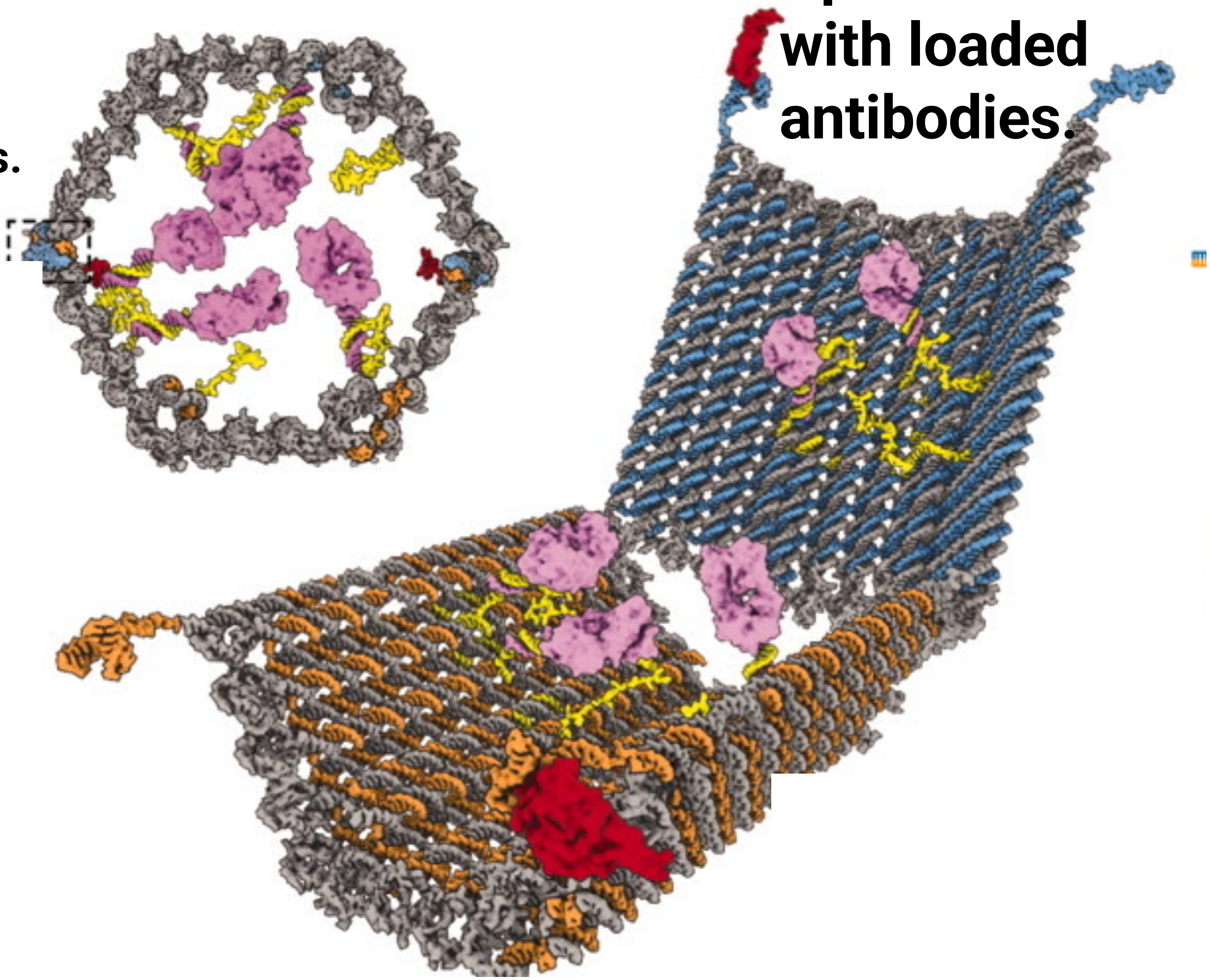


Bear trap: Proximity Sensed Molecular Capture



DNA Nanorobot with 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



A logic-gated DNA nanorobot for encapsulation and display of cargo

DNA Nanorobot with Proximity Sensed Molecular Release

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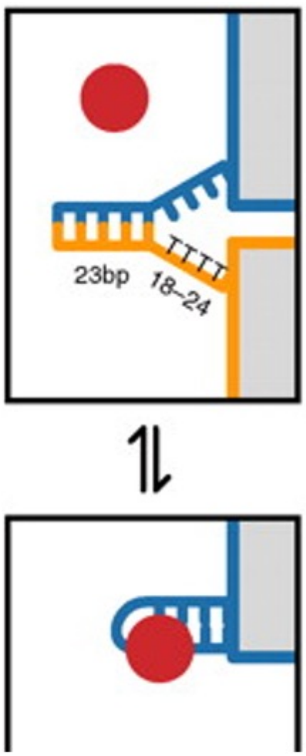
Using an Aptamer with a Molecular Target to Dehybridize Double stranded DNA



Target Molecule (in red)

DNA aptamer (in blue)
hybridized with
partial complement DNA
(in orange)

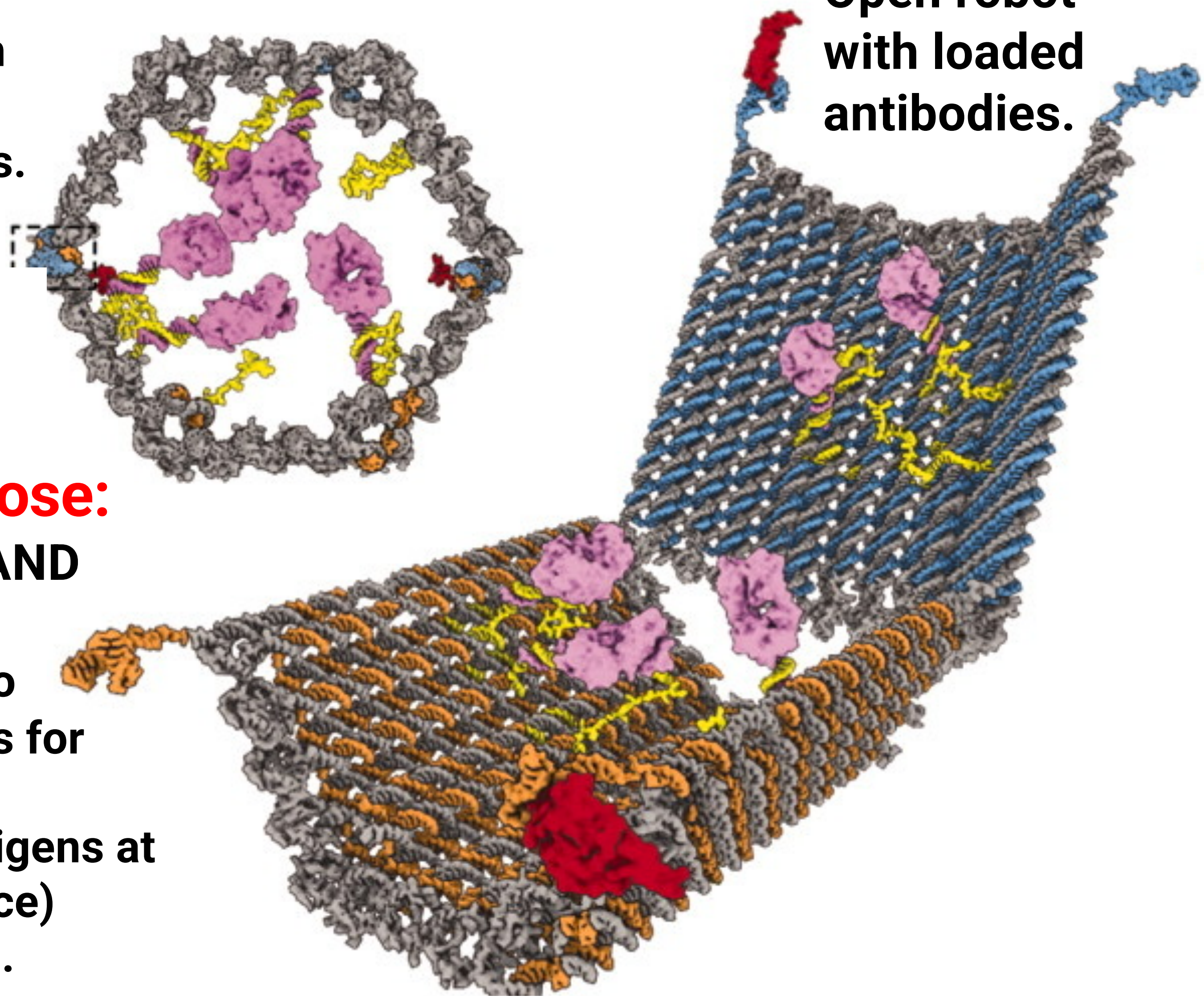
DNA aptamer binding with target

DNA Nanorobot with 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

Closed robot with loaded antibodies.

Open robot with loaded antibodies.



To open & close:

Uses Boolean AND gating with

- **Two-lock (two aptamer locks for robot) and**
- **Two-key (antigens at the cell surface) combinations.**

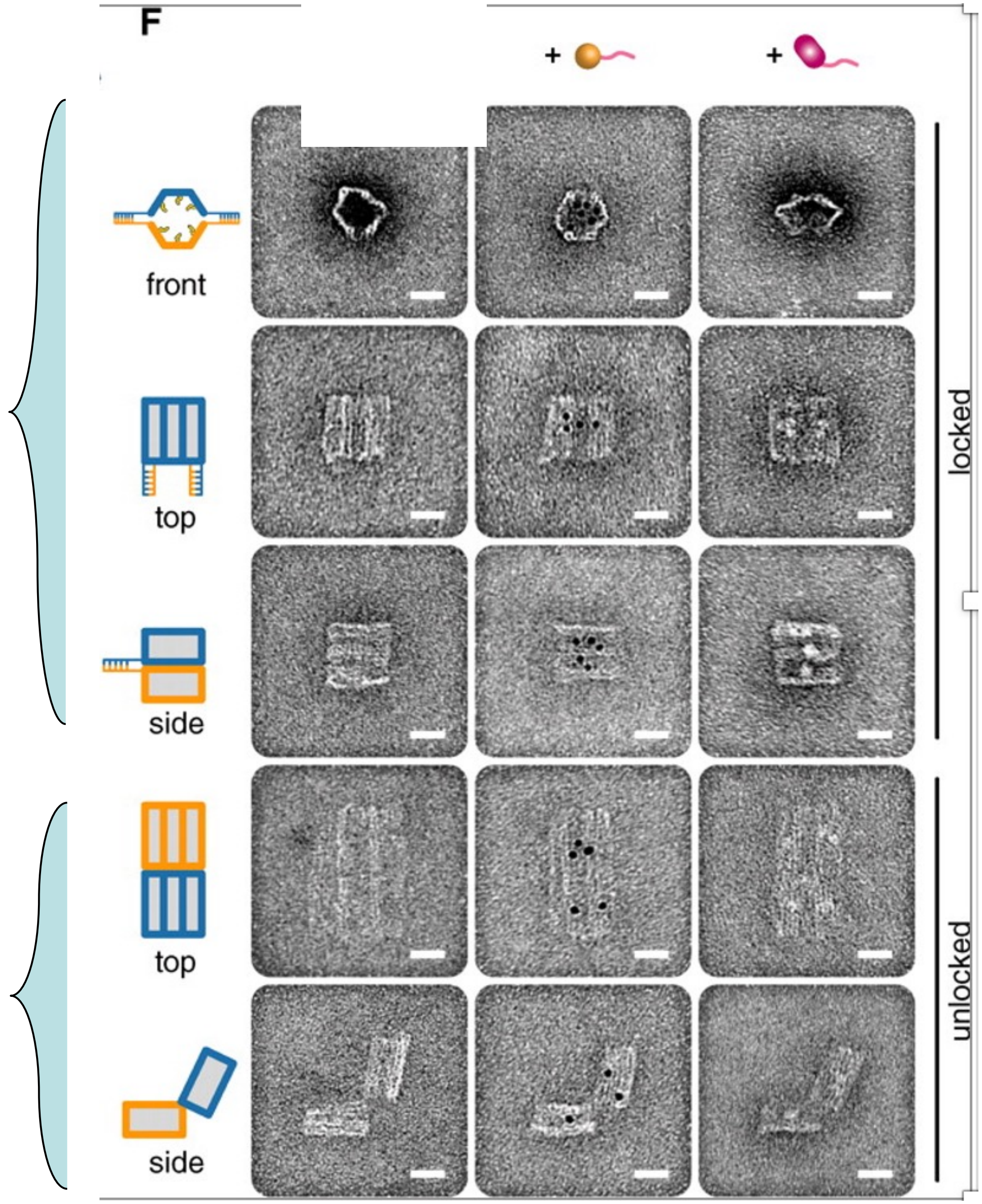
A logic-gated DNA nanorobot for encapsulation and display of cargo.

DNA Nanorobot with 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

Locked NanoContainer

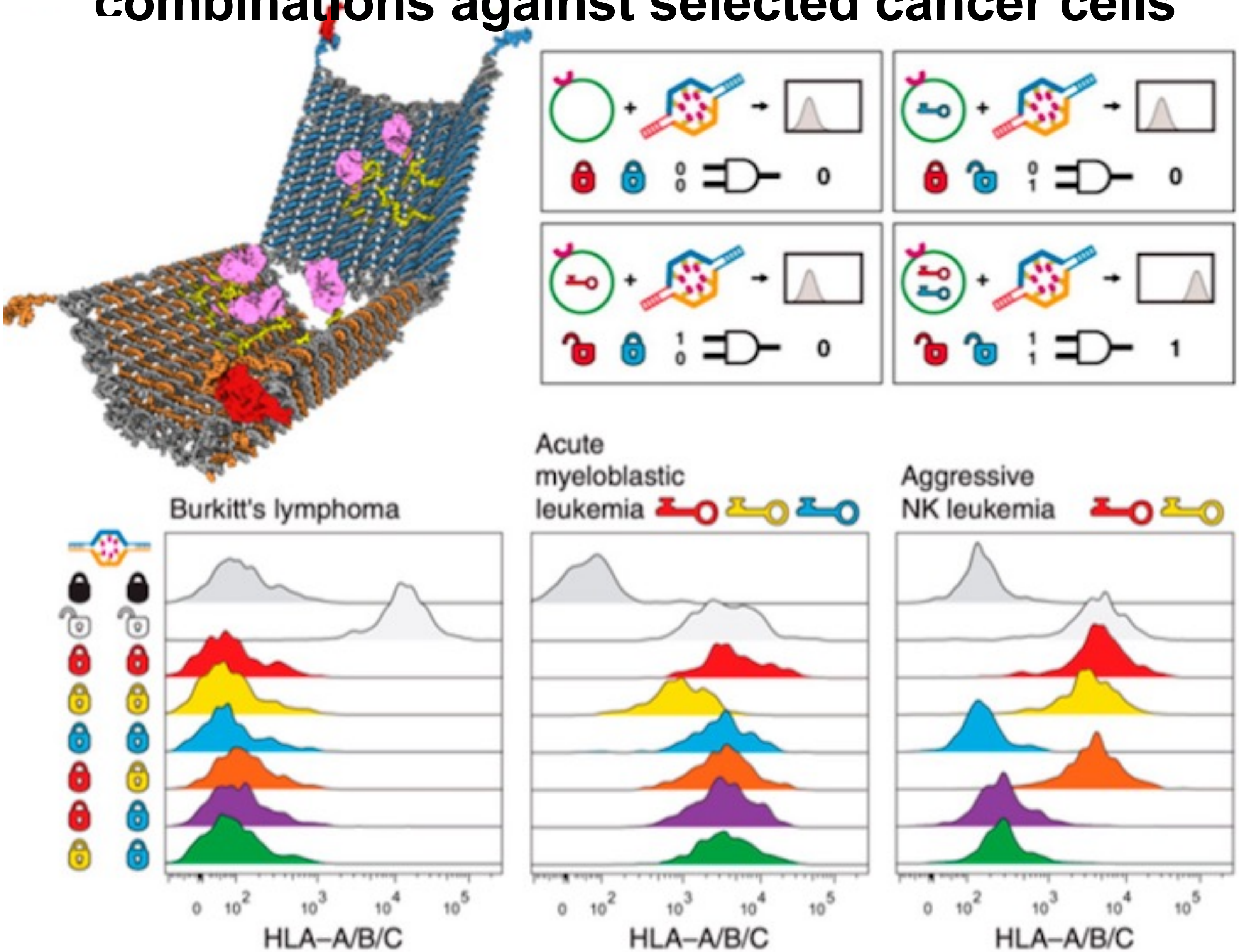
Unlocked NanoContainer



DNA Nanorobot with 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

Logic-gating in action: different lock-key combinations against selected cancer cells

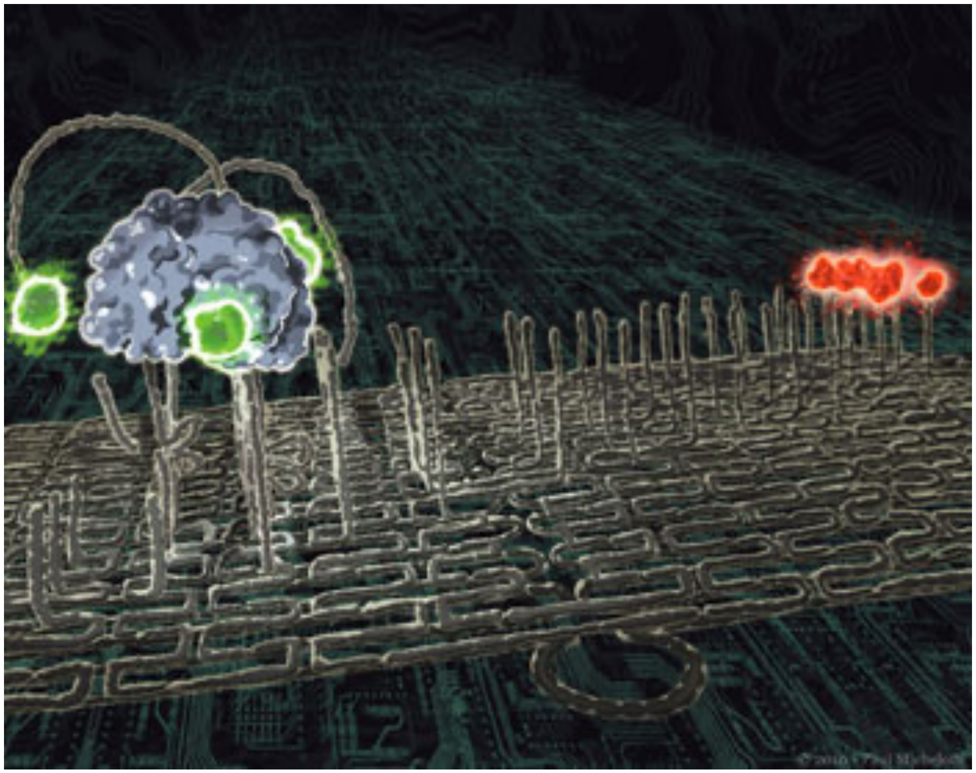


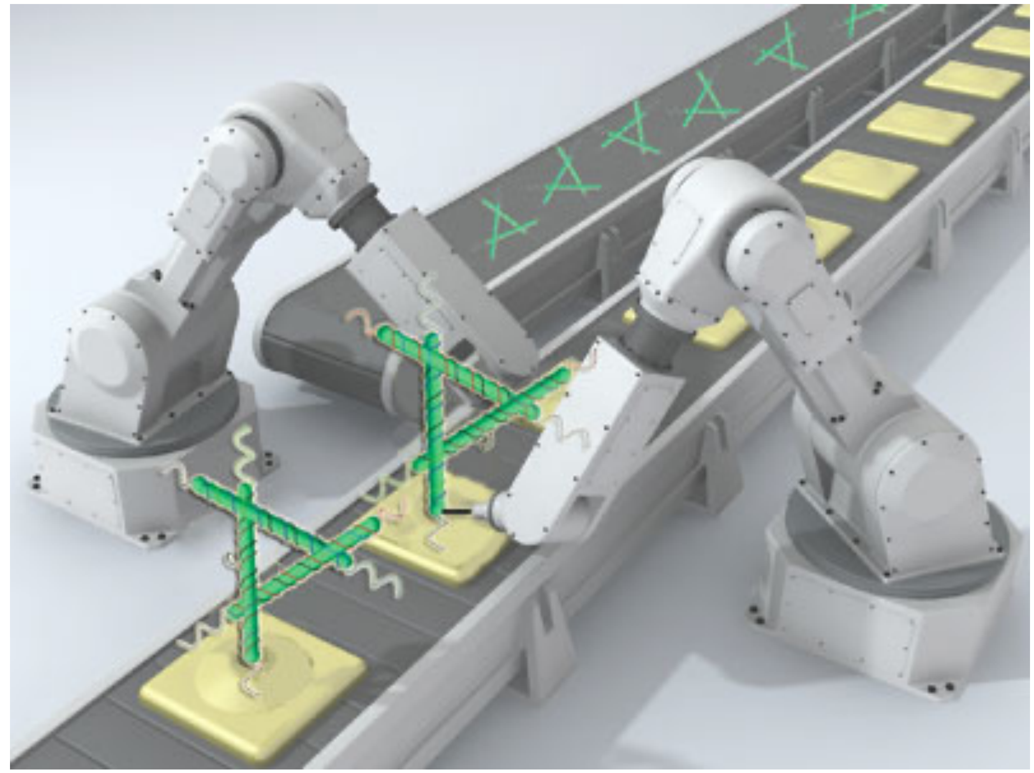
DNA Devices that Deliver Cargo as They Walk

A Proximity-based DNA nanoscale assembly line (Seeman group 2010)

- **A Non-autonomous walker that moves along an origami tile using four feet.**
- **The origami tile has programmable 'cassettes' that transfer cargo (gold nanoparticles) to the walker's 'hands'.**
- **Uses No Enzymes: Fueled by Strand Displacement**

A Proximity-based DNA nanoscale assembly line (Seeman group 2010)

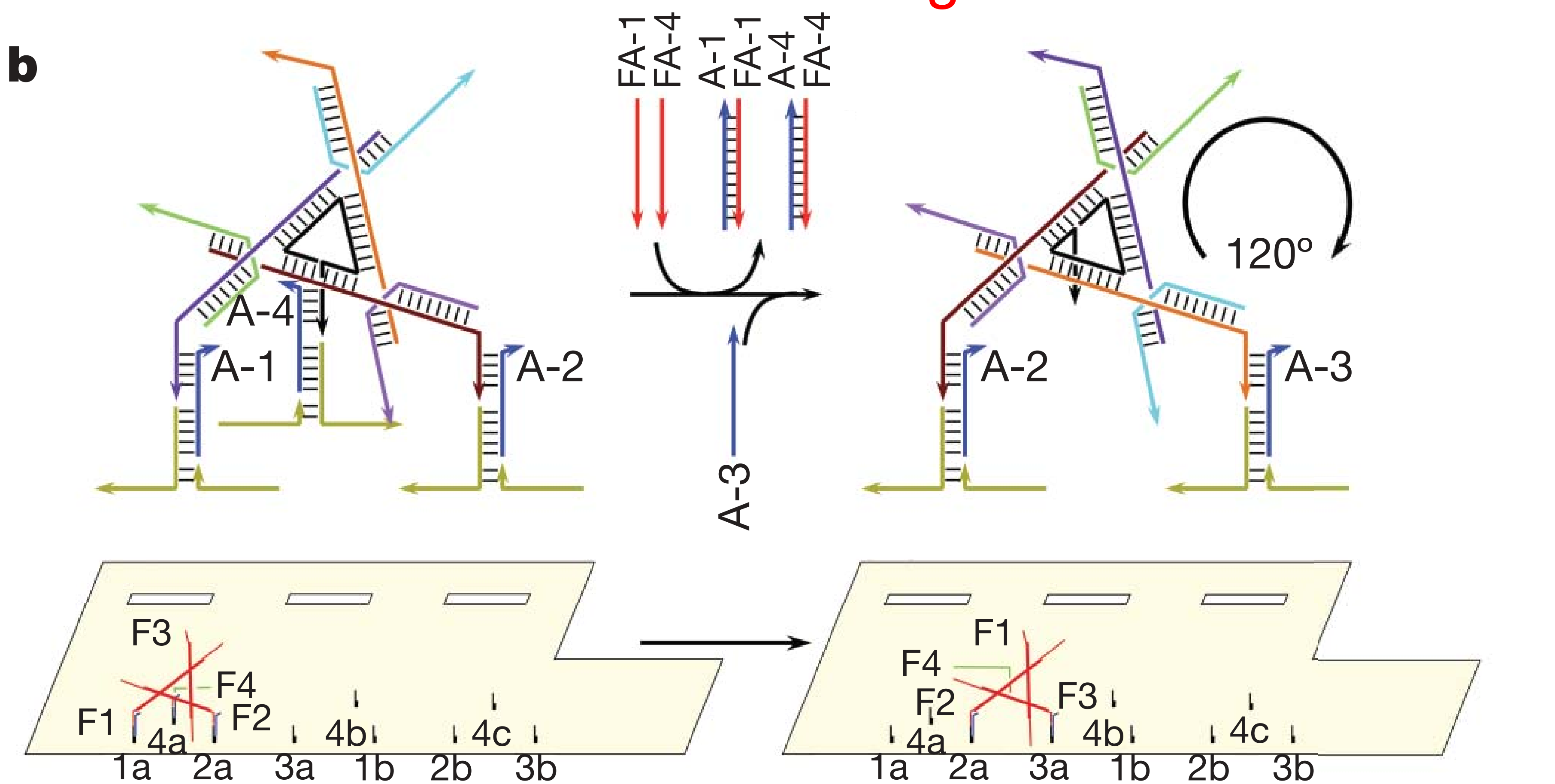




Inaccurate, but evocative Illustration

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 nanoscale assembly line
Uses Nonautonomous DNA Origami Walker



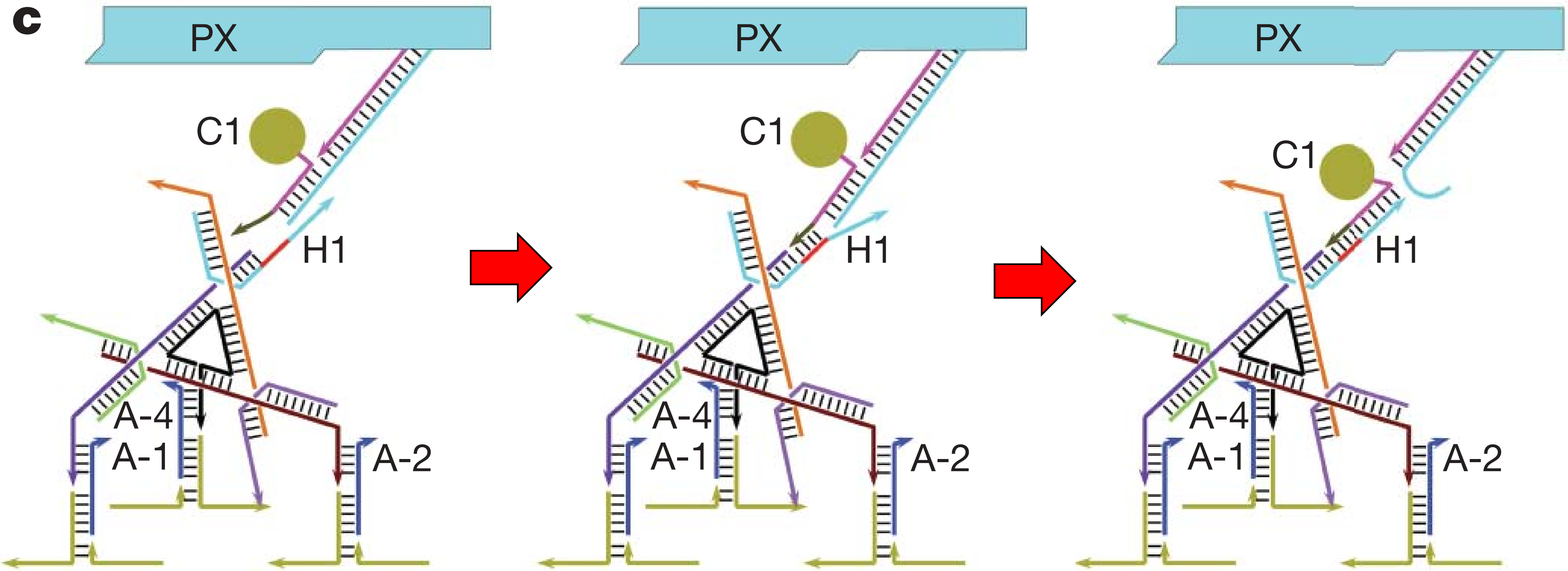
DNA Walker Design:

- **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 Nonautonomous 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

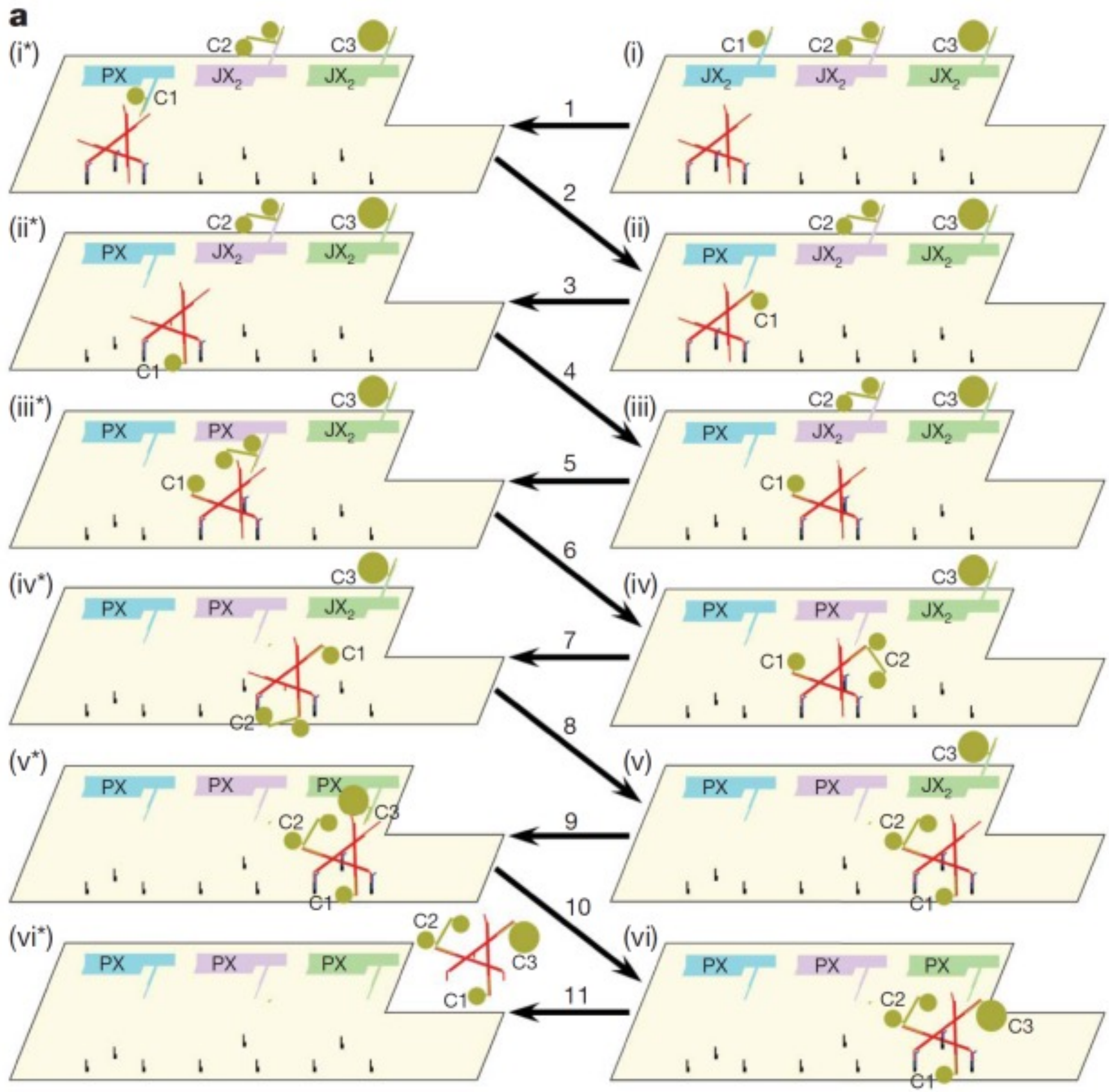


Operation:

- **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.**

Operation of 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