DNA based Non-Autonomous Nanorobotic Devices

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Early DNA robotics devices needed external control, so not autonomous.

Rotation

Open/close

Open/close

Open/close

(Mao et al 99)

(Yurke et al 00)

(Simmel et al 01)

(Simmel et al 02)

Rotation

Extension/contraction

Extension/contraction

Extension/contraction

(Yan et al 02)

(Li et al 02)

(Alberti et al 03)

(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:

  - pH 5.0: High TAMRA Fluorescence
  - pH 8.0: Iowa Black suppresses TAMRA fluorescence

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 180° 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
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 dye.
- is the basis for Förster resonance energy transfer (FRET) assays
DNA Tweezers: [Yurke2000]
- Nonautonomous Device
- Used Strand Displacement

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

A DNA-fueled molecular motor made of DNA

Bell Labs/Oxford
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.
DNA Biped walker [Sherman et al 04]


- 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.
DNA Biped walker [Shin&Pierce2004]


DNA Sequences for the Walker System

<table>
<thead>
<tr>
<th>Walker strands (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1 5'-GGCTGGTTTCTGCTCTCTAGTTTGCGAGGTGCAATCTCCTATC-3' - BHQ1</td>
</tr>
<tr>
<td>W2 5'-IBRQ-5'-GTCTGGGATGCTGGGATCAGACCTAGAGAGCAAGAACCAGCC-3'</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Track strands (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 5'-HEX-5'-GCTGTACTTGGTTACTGAAAGGGAGTGGCTCGGA-3'</td>
</tr>
<tr>
<td>T2 5'-TCCGAGCCACCTCCCTGGACACCCATCTCAAAAAATCTTTGGATGCGACTGCTCC-3' - Cy5</td>
</tr>
<tr>
<td>T3 5'-FAM-5'-TCCACATCAGACTCTGTAATGCAAGACACCTTTACTTGTAGAGTGGGTCC-3'</td>
</tr>
<tr>
<td>T4 5'-GTAACCGTGTCCTGCTGCATCAACATCAGCTTTCATGATCCTGCATTGCTCACA-3' - Texas Red</td>
</tr>
<tr>
<td>T5 5'-ACCTTAAAGGGAAGATCTAGAGATGATGTTTGAGA-3'</td>
</tr>
<tr>
<td>T6 5'-TACGATCTTTGCTAAGATT-3'</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Attachment strands (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 5'-AGTAACCAAAAGTACACGACTCCGTAGGAGATTGACCCCTCAATTACCCT-3'</td>
</tr>
<tr>
<td>A2 5'-GACGTTAGATGTCACCTGCAACCCAGCCTACGCTCAGTTACGATA-3'</td>
</tr>
<tr>
<td>A3 5'-ACAGAGGCAGCTGTGGAAGTGAGATGATGCTACCTCATATTGCG-3'</td>
</tr>
<tr>
<td>A4 5'-GGATCGTTTGTATCCAGCATTCCCAGATCATAGTGGTGAGCAGATG-3'</td>
</tr>
</tbody>
</table>

A1 hybridizes to red leg of Walker
A2: hybridizes to black leg of Walker

red leg of Walker
black leg of Walker
stators
Track
Non-Autonomous DNA based Nanorobotic Walker

DNA Biped walker [Shin&Pierce2004]

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.
Non-Autonomous DNA based Nanorobotical devices

DNA Biped walker [Tian&Mao2004]

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:

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

Closed robot with loaded antibodies.

Open robot with loaded antibodies.

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

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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
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


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