Autonomous Programmable Nanorobotic Devices Using DNAzymes

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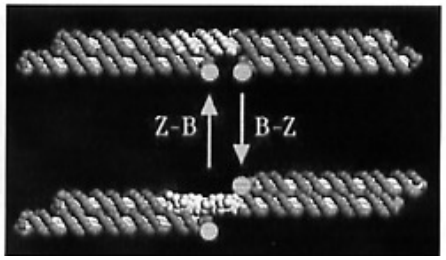
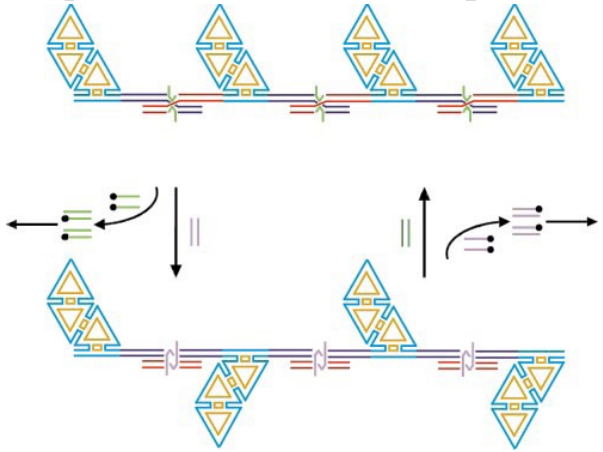
Non-Autonomous DNA based Nanorobotical devices

Advantages of DNA-based synthetic molecular devices:

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

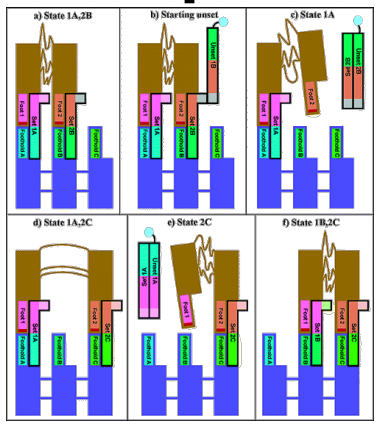


B-Z transition device
[Mao, Seeman 99]



PX-JX transition [Yan et al 02]

DNA-fuelled Molecular machine
[Yurke et al 00]

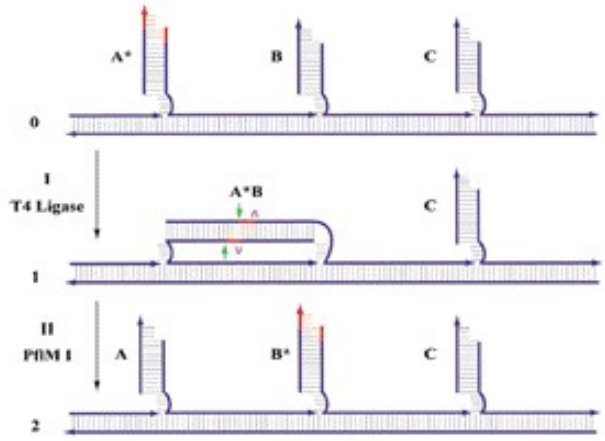


DNA Biped walker
[Sherman et al 04]

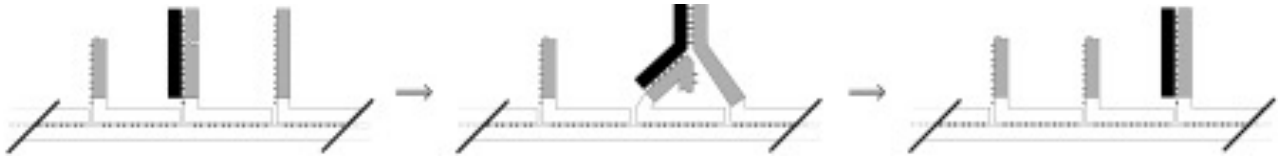
Autonomous DNA based Nanorobotical devices

Major challenges:

- *Autonomous (*without externally mediated changes per work-cycle)
- *Programmable (*their behavior can be modified without complete redesign of the device)

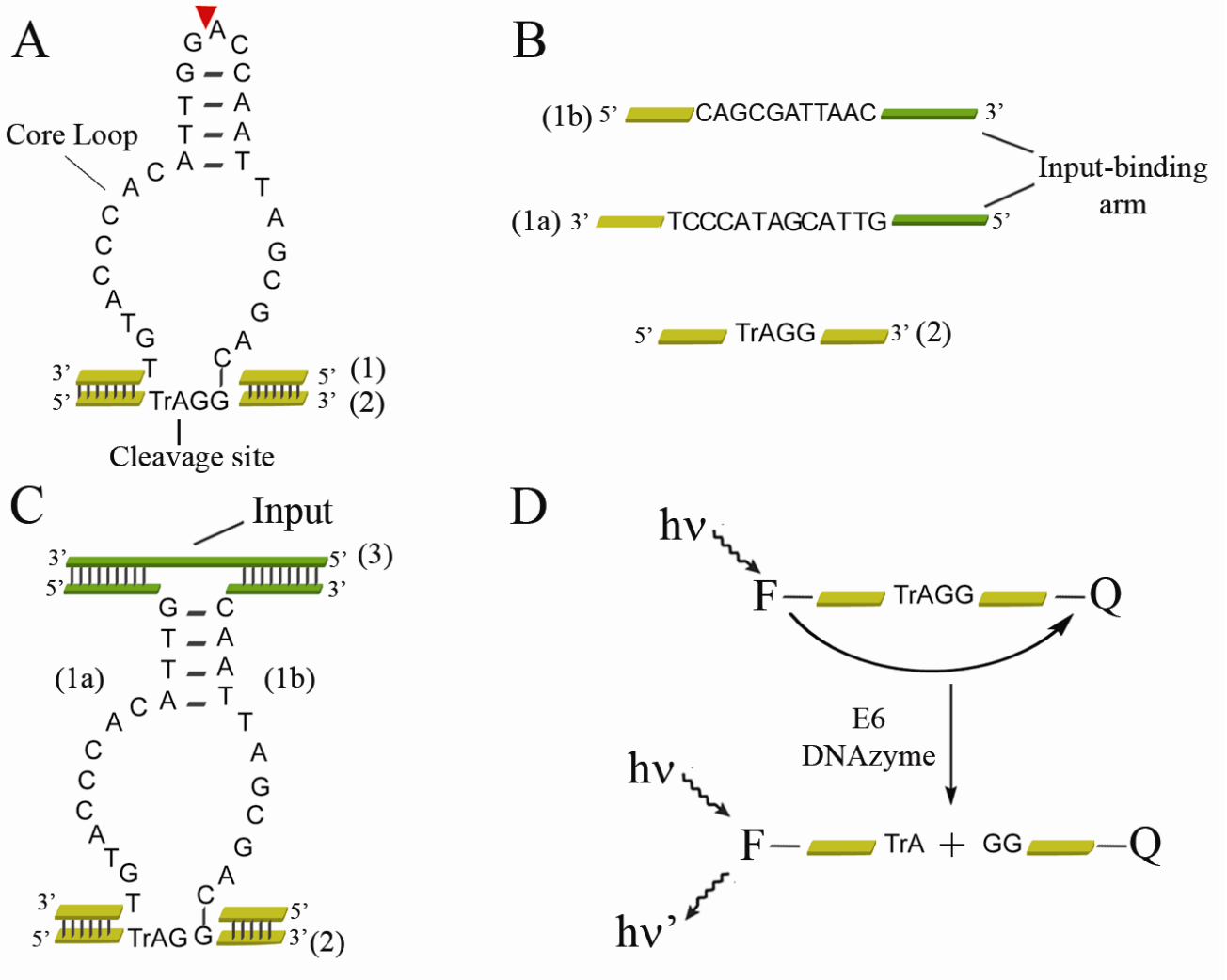


Unidirectional DNA Walker
[Yin et al 04]



DNA motor powered by Nicking enzyme
[Bath et al 05]

DNAzyme

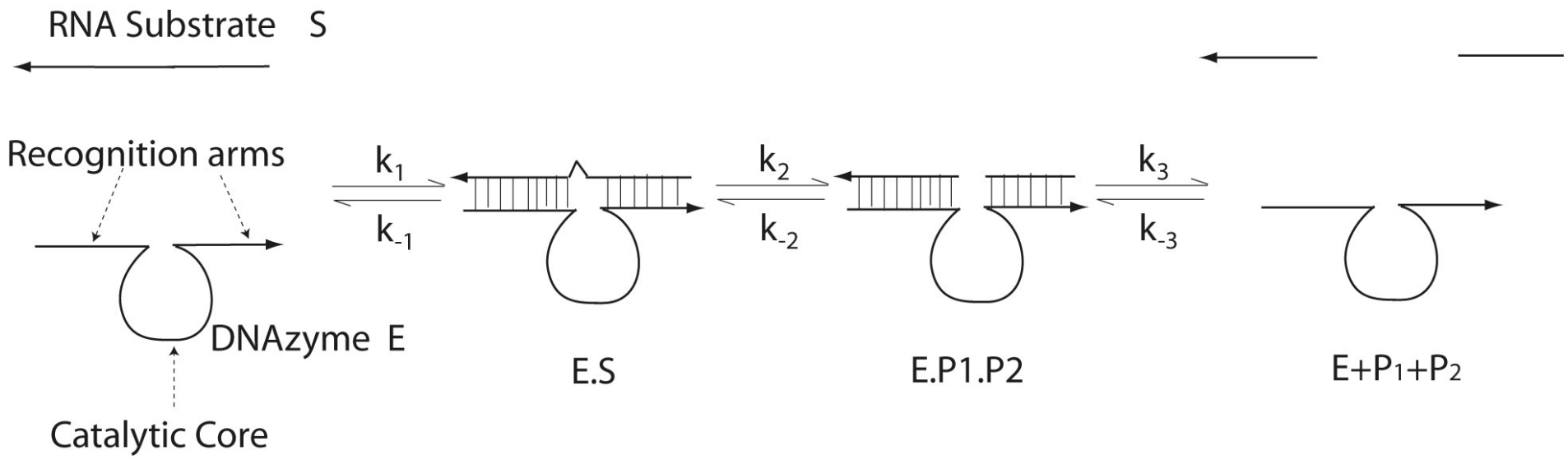


(from Wilner)

DNAzymes are DNA oligonucleotides that can catalyze specific chemical reactions, such as restriction cuts.

- **DNAzymes are discovered by In vitro selection or In vitro evolution**
- **DNAzymes also named: Deoxyribozymes,DNA enzymes or catalytic DNA.**

DNAzyme kinetics

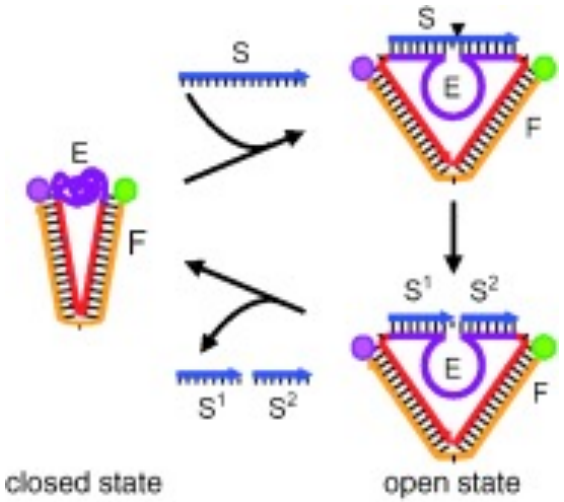


- 2^{nd} step is rate determining
- Requires metal ion as cofactor
- $k_2 >> k_{-2}$, $k_1 >> k_{-1}$, $k_3 >> k_2$

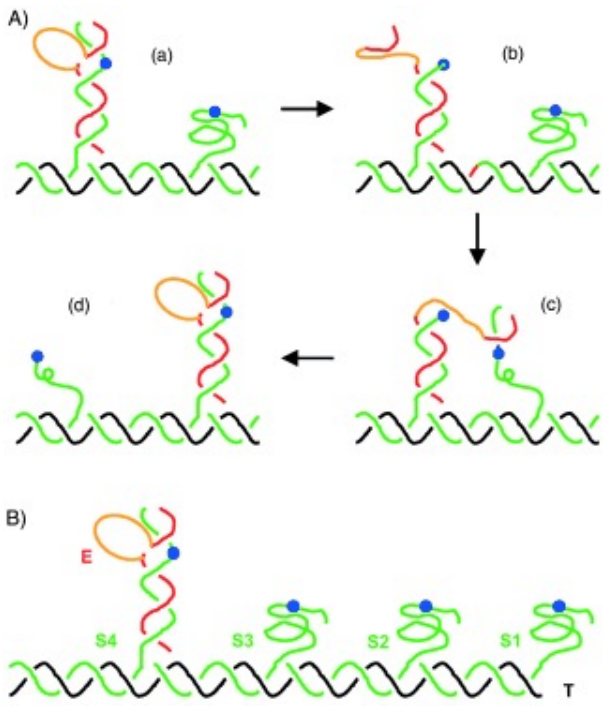
[Santoro]

DNAzyme based nanomechanical devices

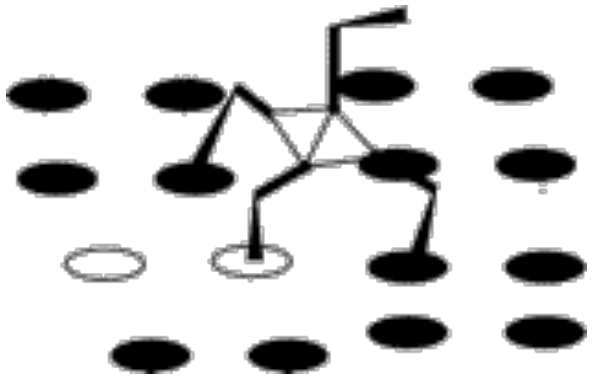
- Autonomous
- Programmable
- Require no protein enzymes



DNAzyme tweezer
[Chen et al 04]

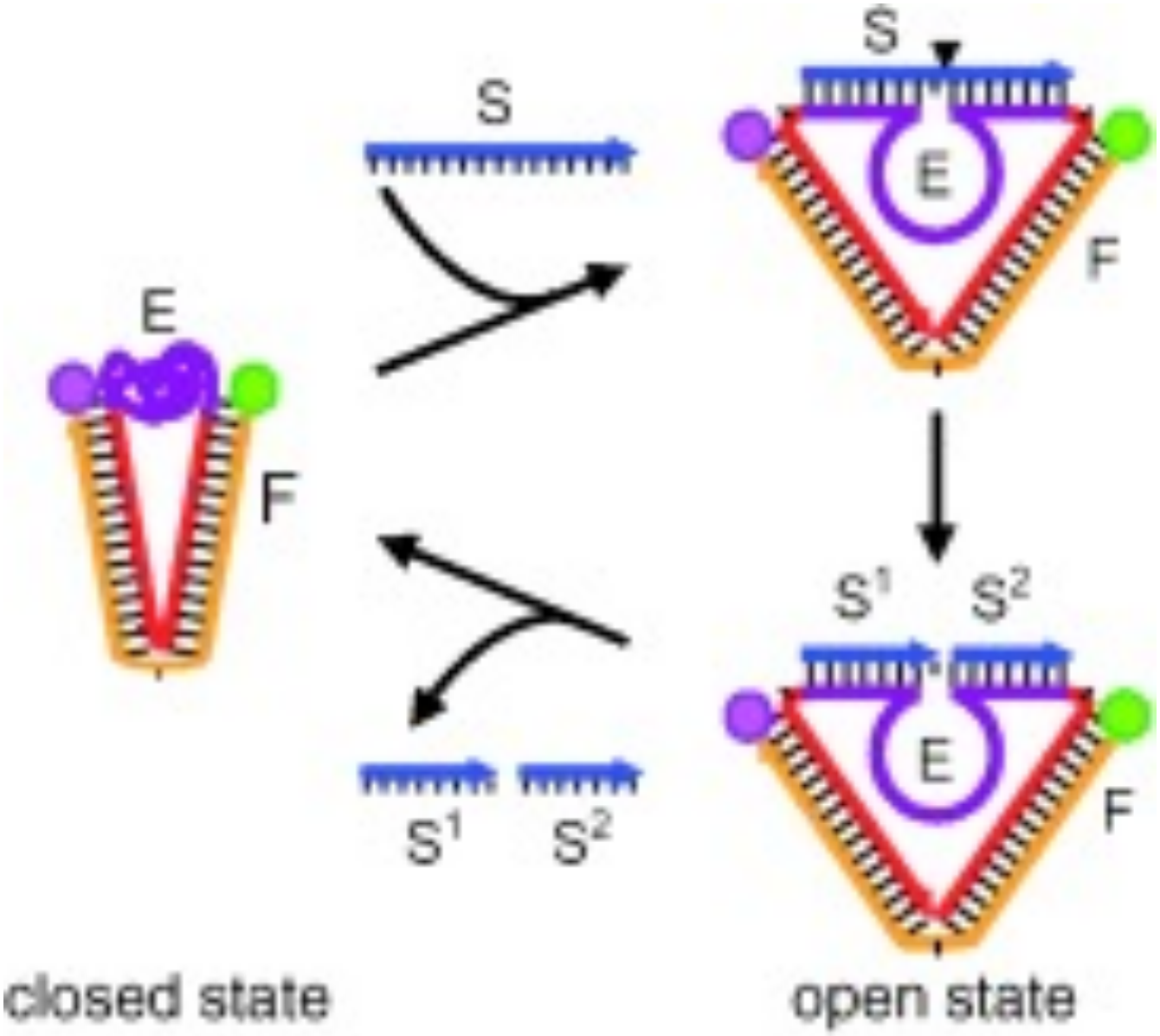


DNAzyme crawler
[Tian et al 05]

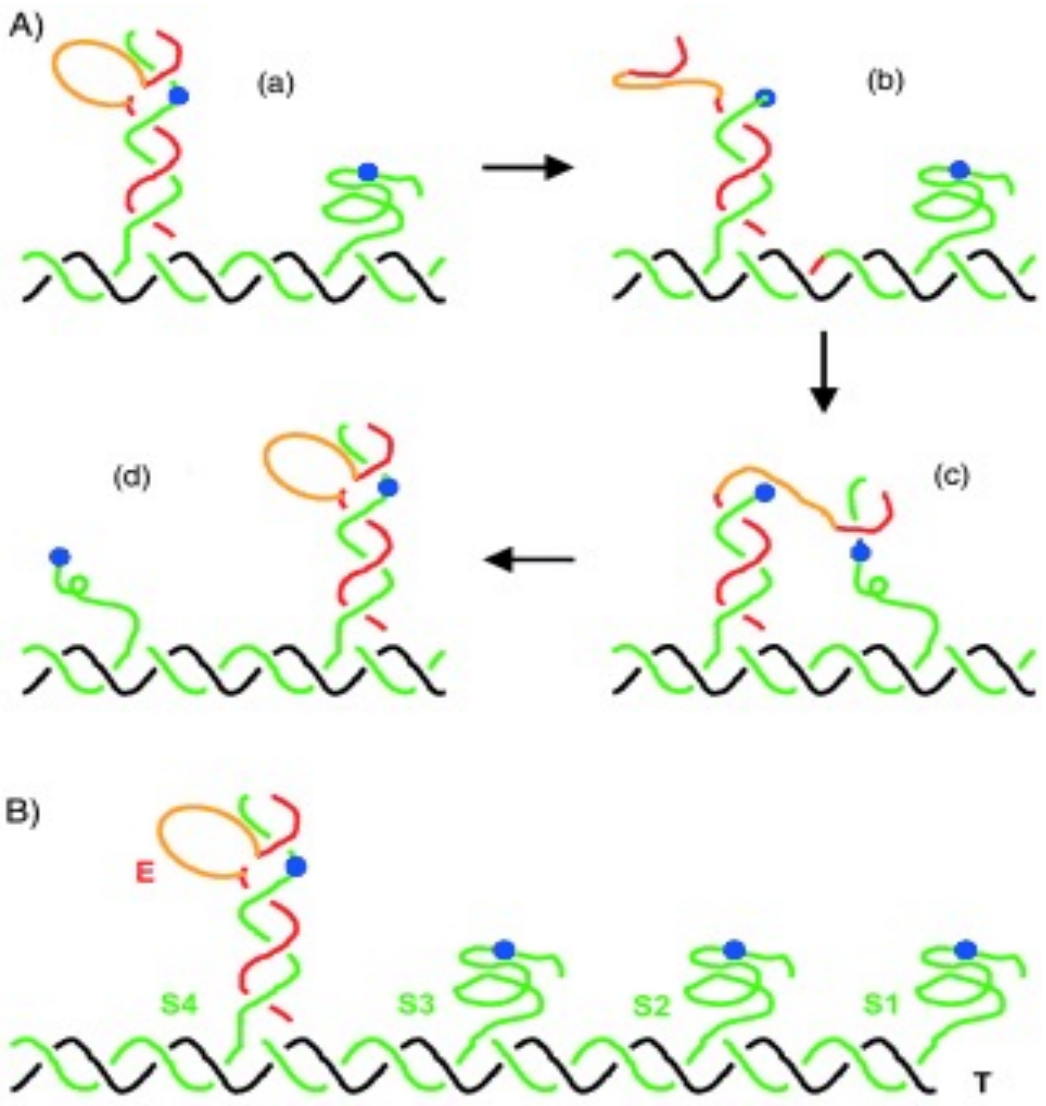


Polycatalytic Assemblies
[Pei et al 06]

DNAzyme Tweezer [Chen et al 2004]



DNAzyme Crawler [Tian et al 2005]

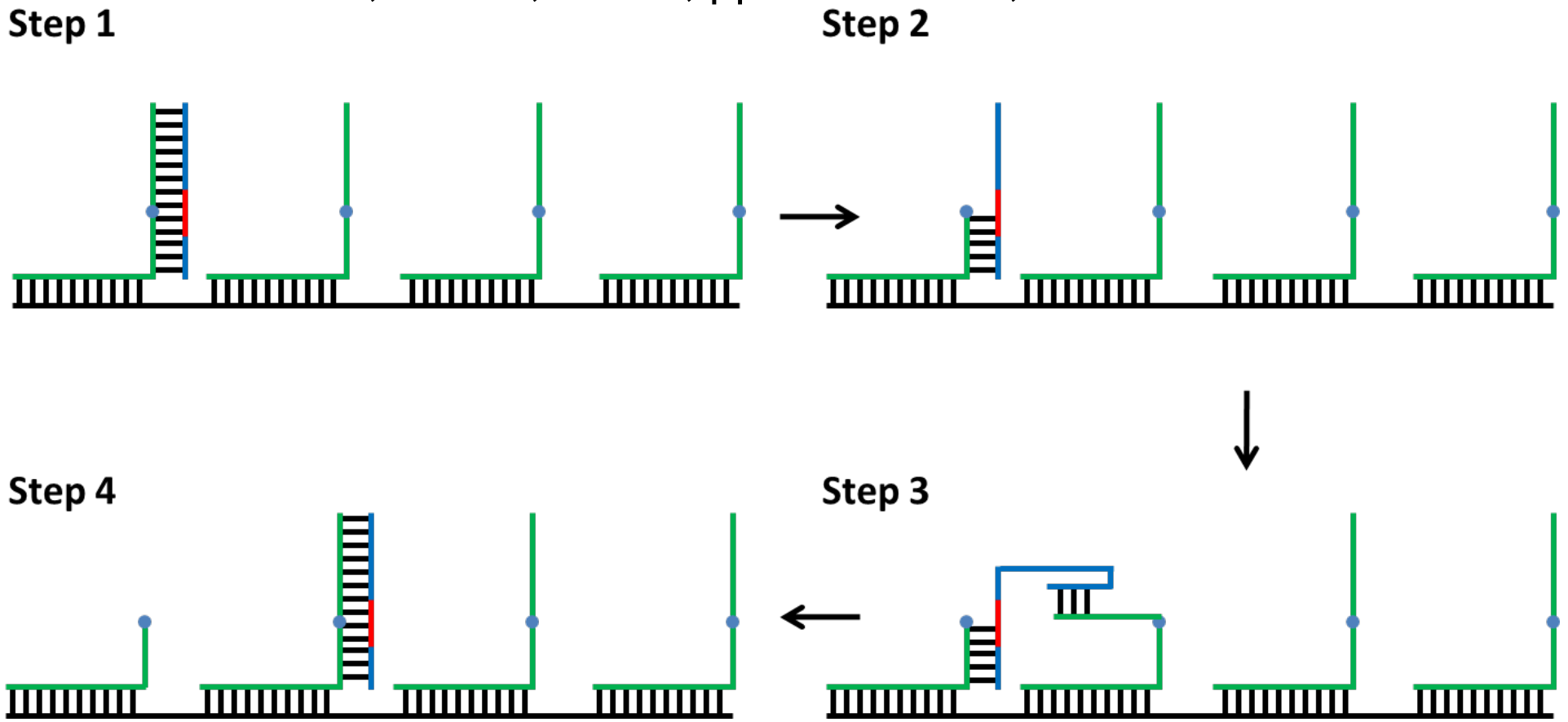


DNAzyme crawler
[Tian et al 05]

Other Walkers powered by DNAenzymes:

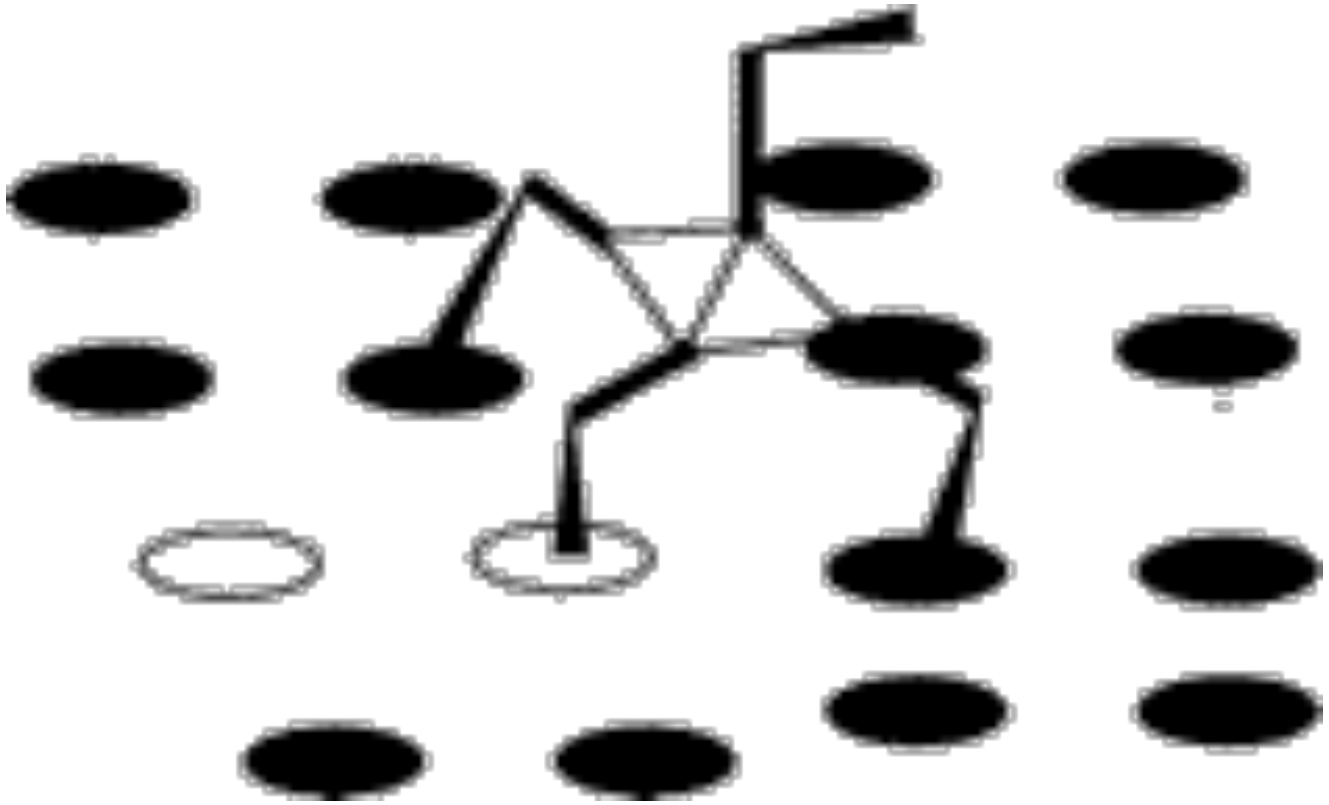
[Tian & Mao 2005]

Y Tian, Y He, Y Chen, P Yin, C Mao, A DNAzyme That Walks Processively and Autonomously allong 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.

DNAzyme based Spider Walker [Stojanovic]



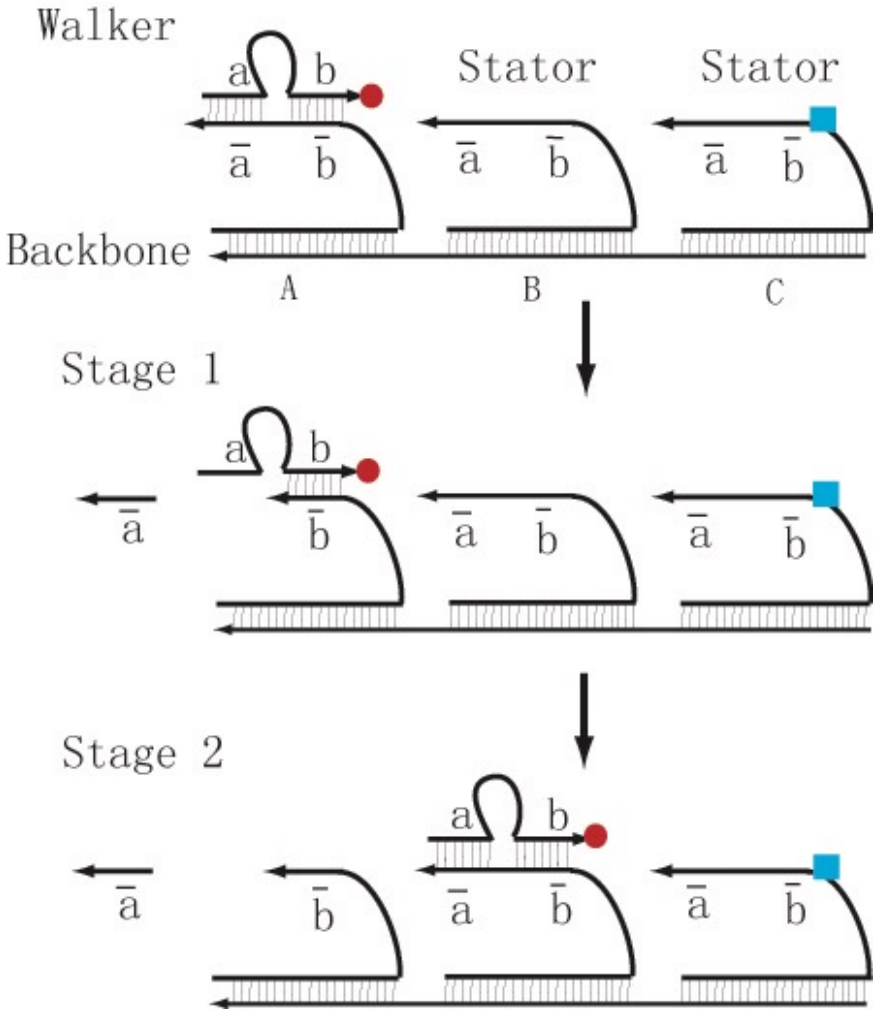
Polycatalytic Assemblies [Pei et al 06]

DNAzyme Computing (Sahu& Reif)

1. *DNAzyme FSA:* a finite state automata device, that executes finite state transitions using DNAzymes
   - extensions to probabilistic automata and non-deterministic automata,
2. *DNAzyme Router:* for programmable routing of nanostructures on a 2D DNA addressable lattice
3. *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
   - operates without use of any protein enzymes.

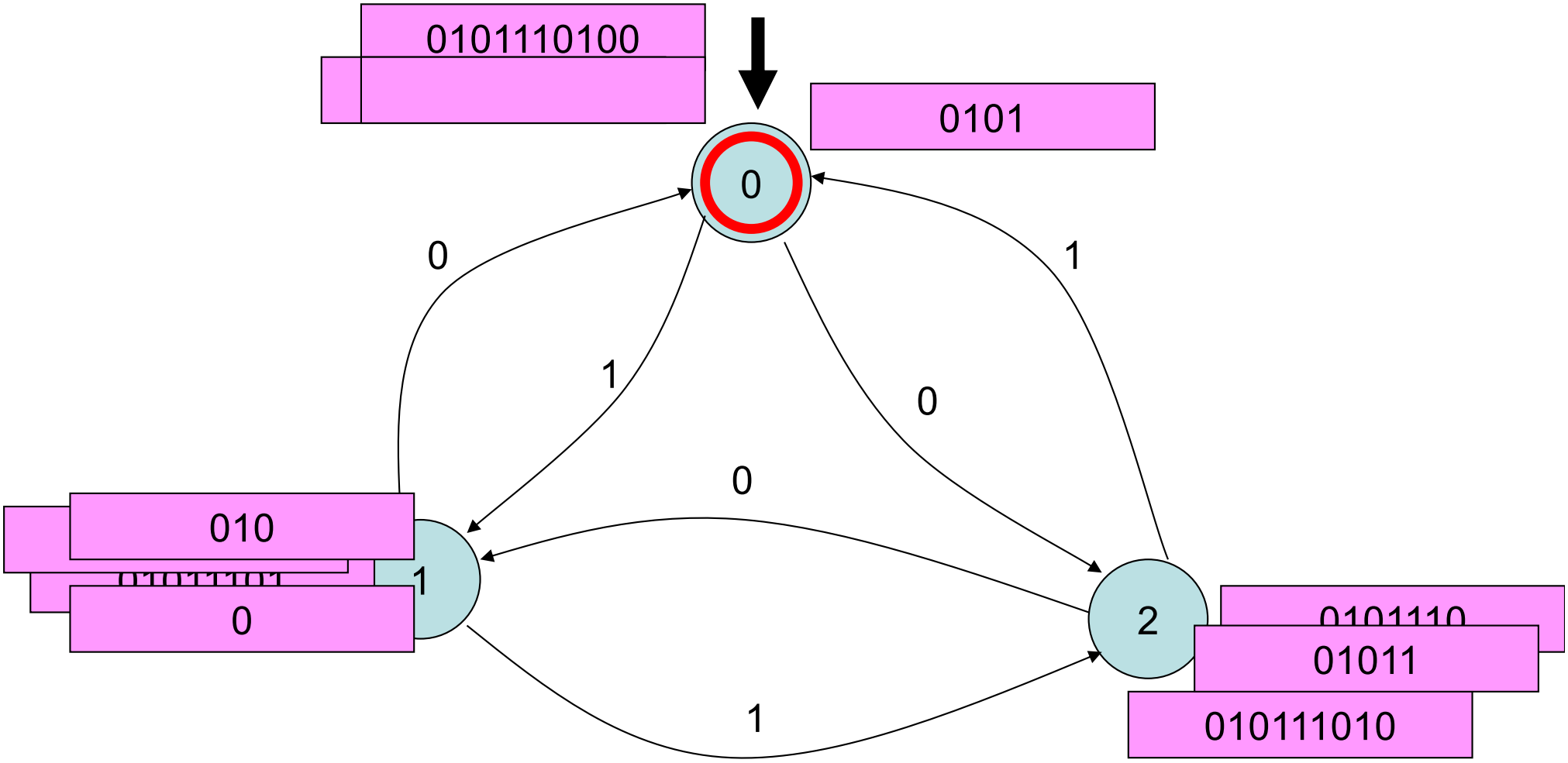
DNAzyme Based Crawler

Basic Actions:
- Cleaving by DNAzyme
- Strand displacement



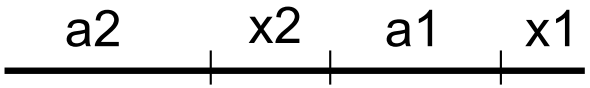
[Tian et al 05]

Finite State Automata (FSA)

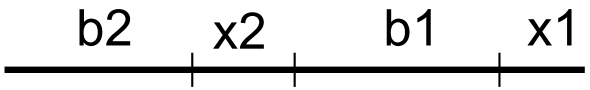


Inputs to DNAzyme FSA

Encoding Input bits:



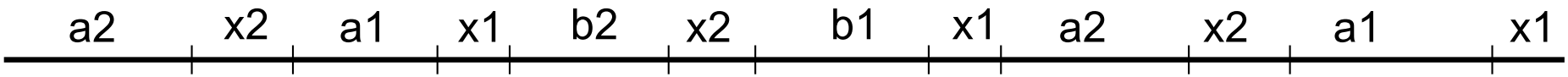
0



1

Note: x1 and x2 will be used for protection

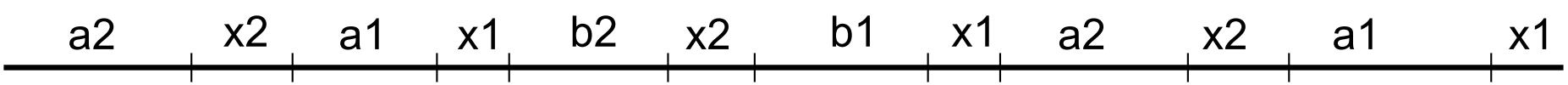
Input sequence of bits:



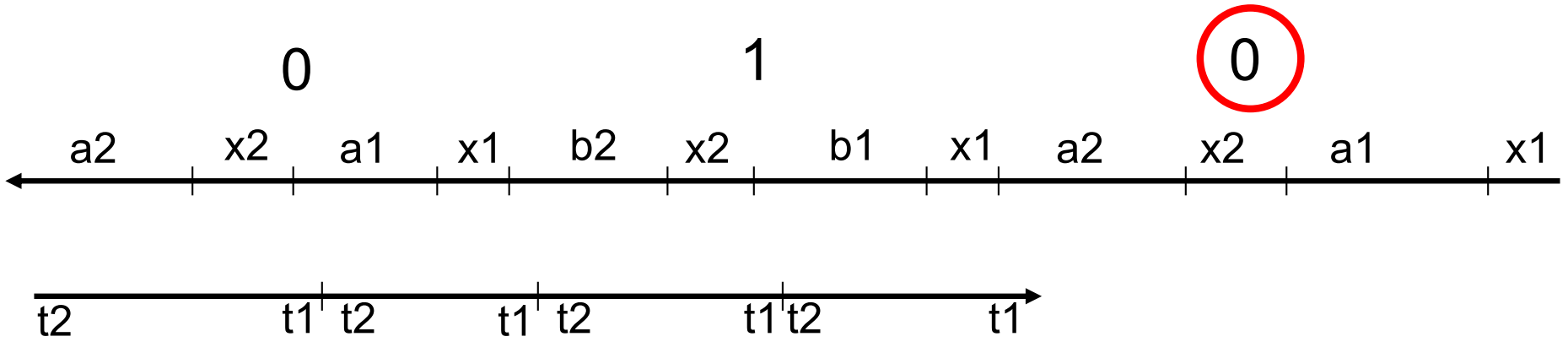
0 1 0

Input Protection

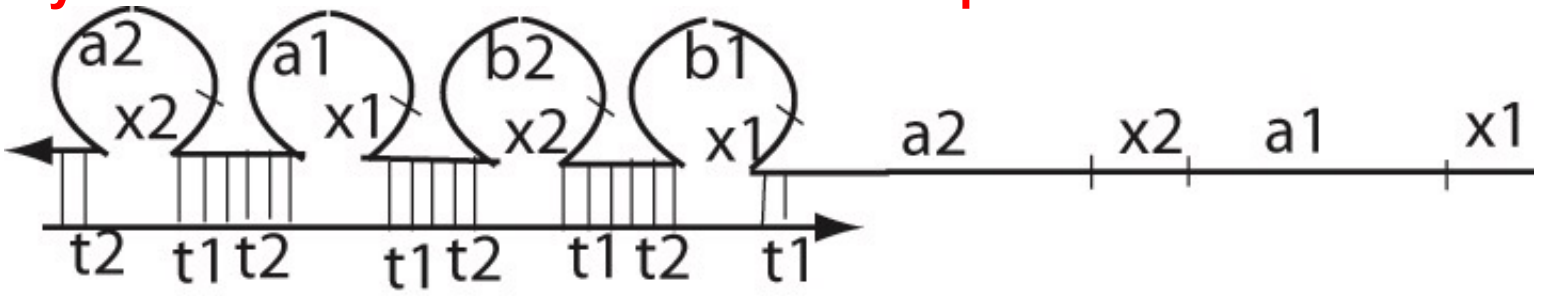
Active Input strand: **The input that is being currently read by state machine**



Add Protection strand to Input strand:



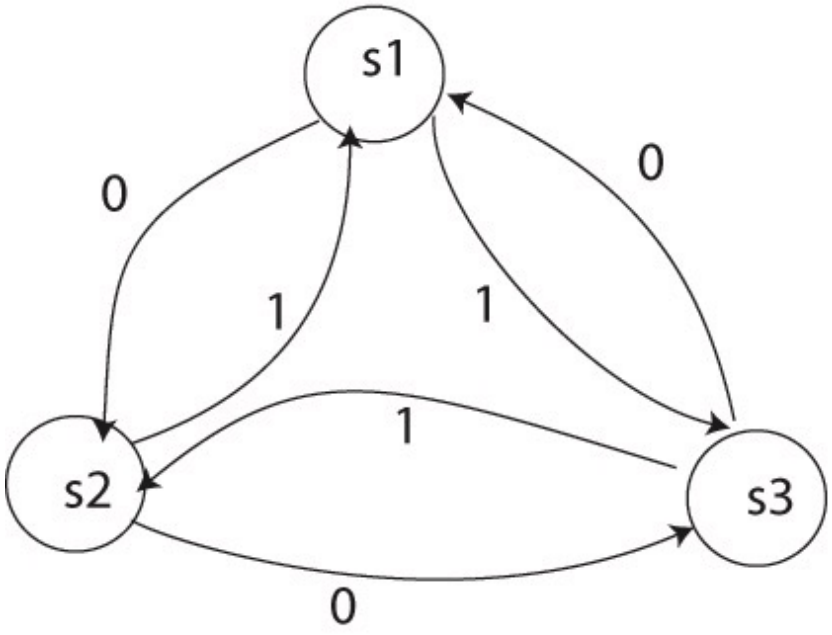
Hybridization of Protection strand to Input strand:



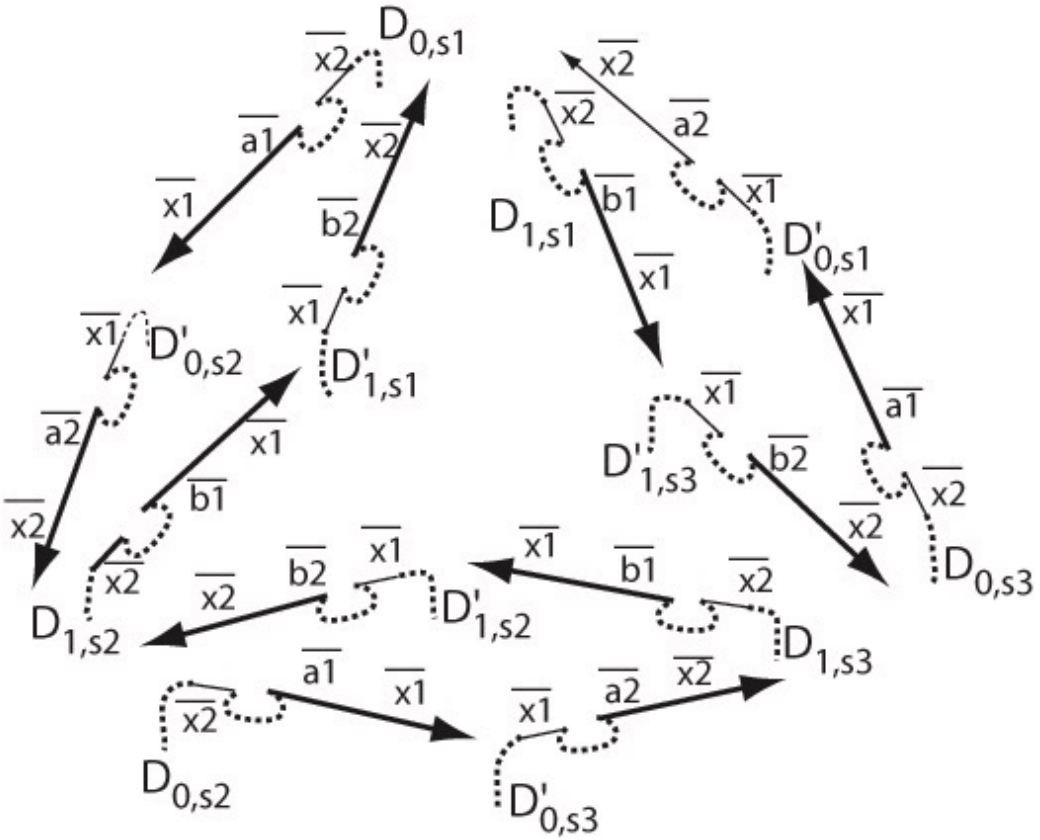
0 1 0

Complete Finite State Machine

State Transitions:



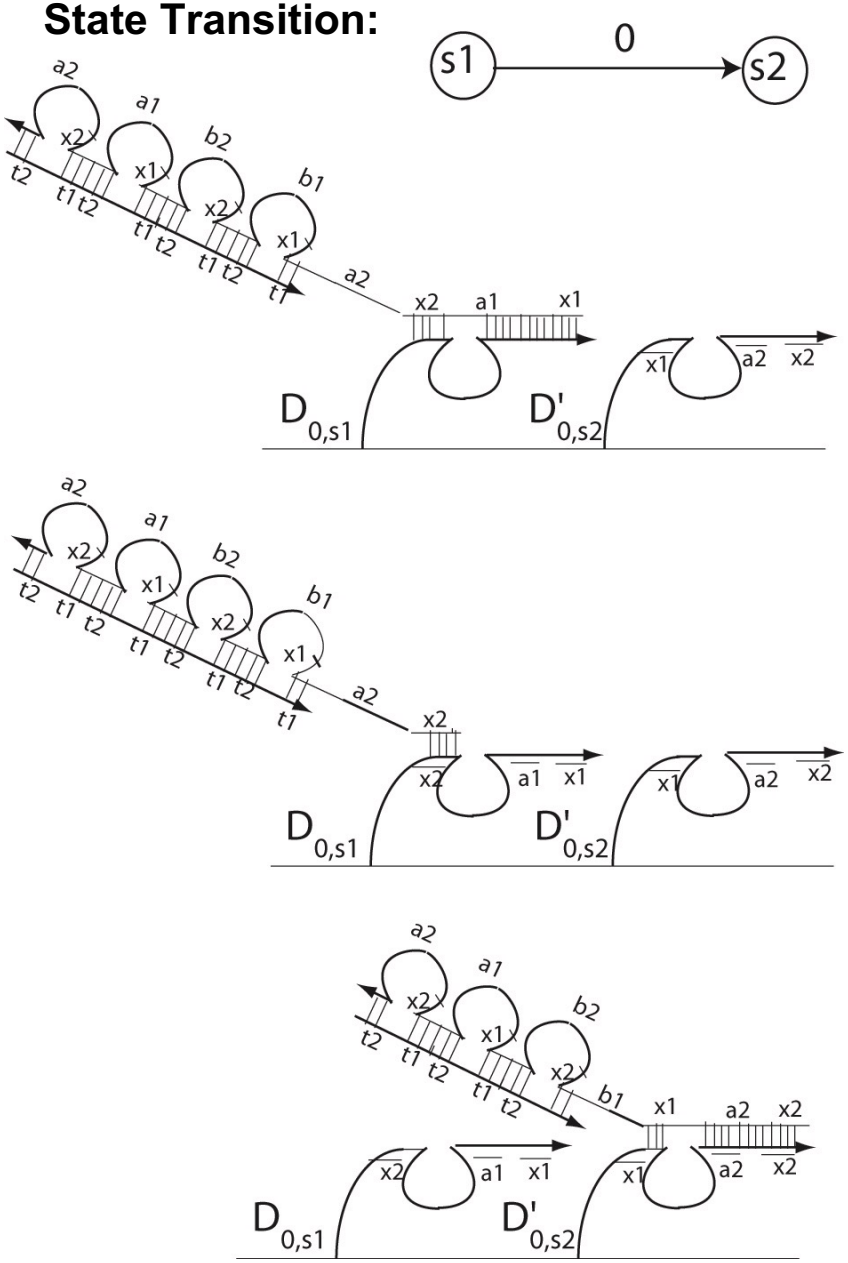
DNAzyme Implementations of Distinct State Transitions:



Step by step execution of a 0-transition

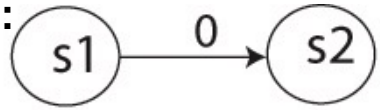
DNAzyme Implementation of State Transition:

State Transition:

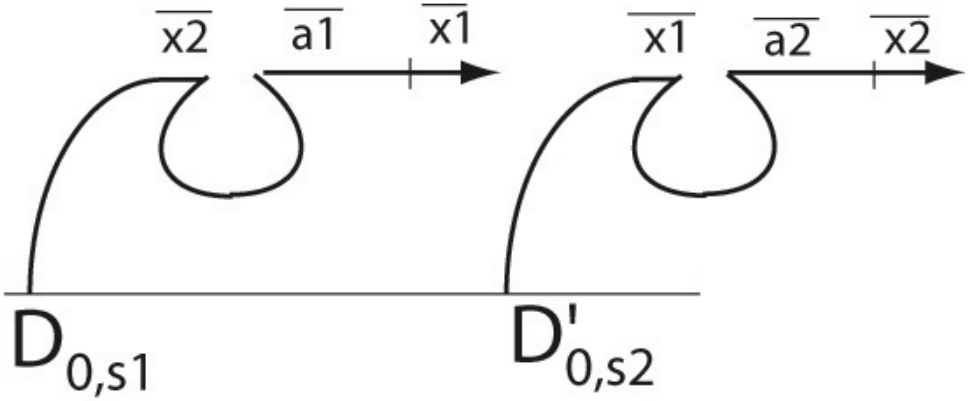


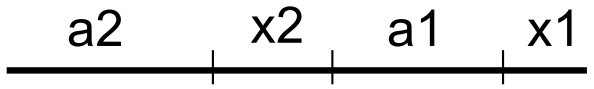
State Transitions of DNAzyme FSA

State Transition:



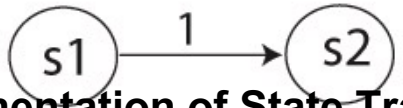
DNAzyme Implementation of State Transition:



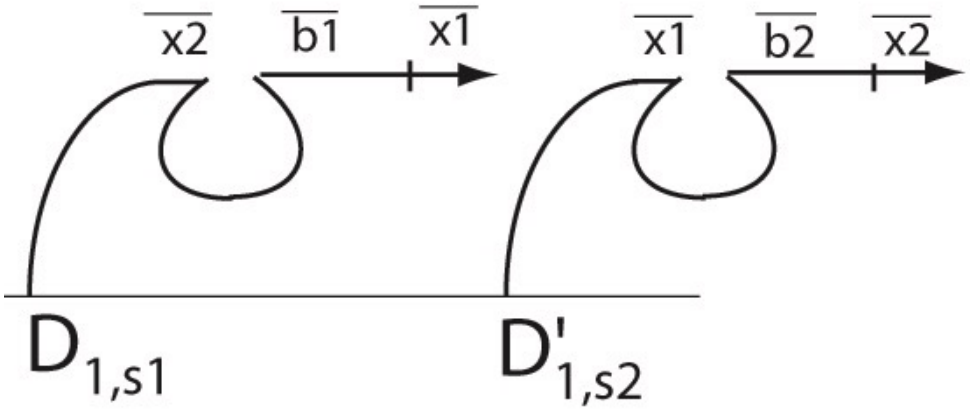


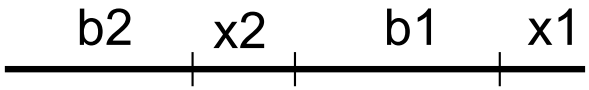
0

State Transition:



DNAzyme Implementation of State Transition:

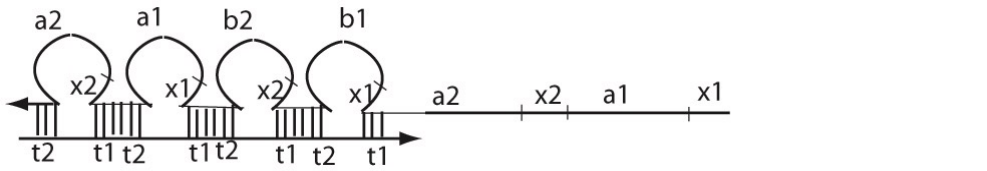


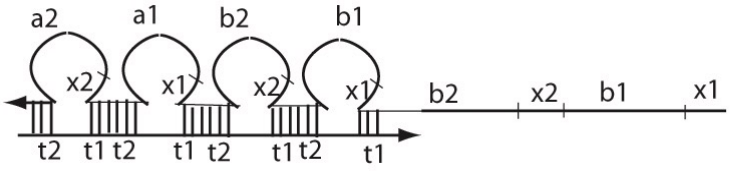


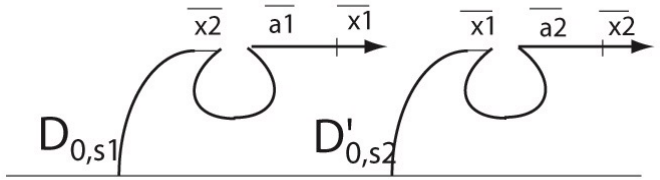
1

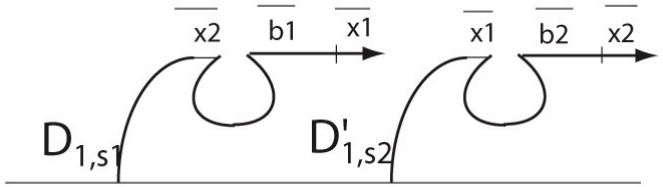
Transition specificity

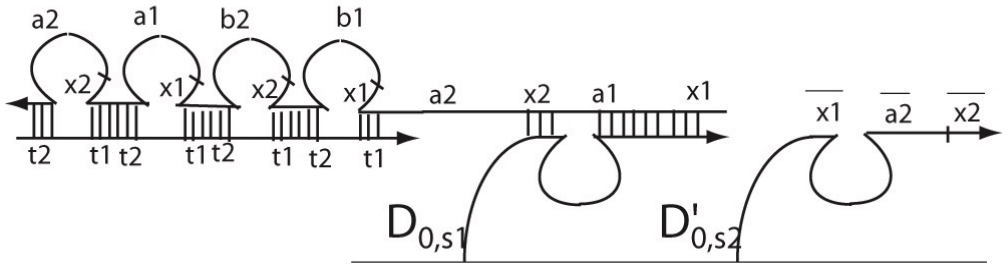
DNAzyme Implementations of Distinct State Transitions:

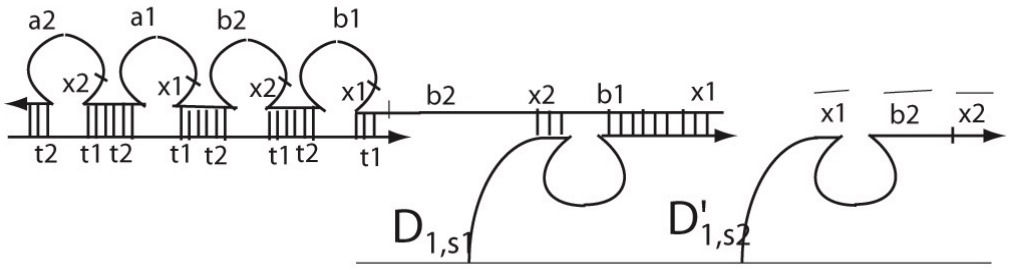








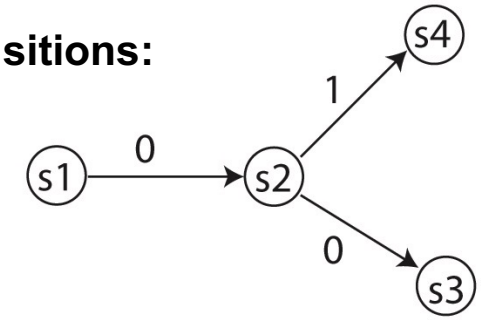


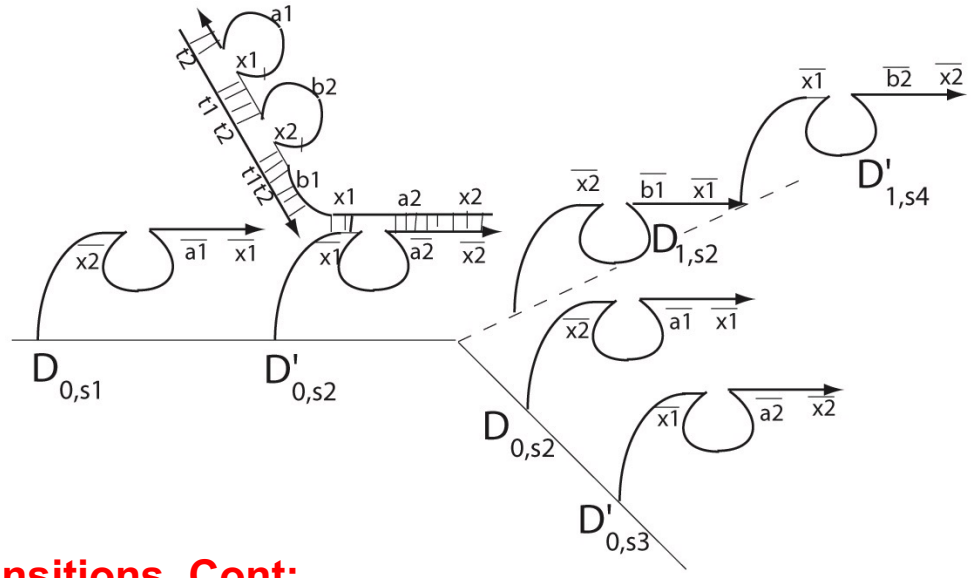


Choosing next state transition

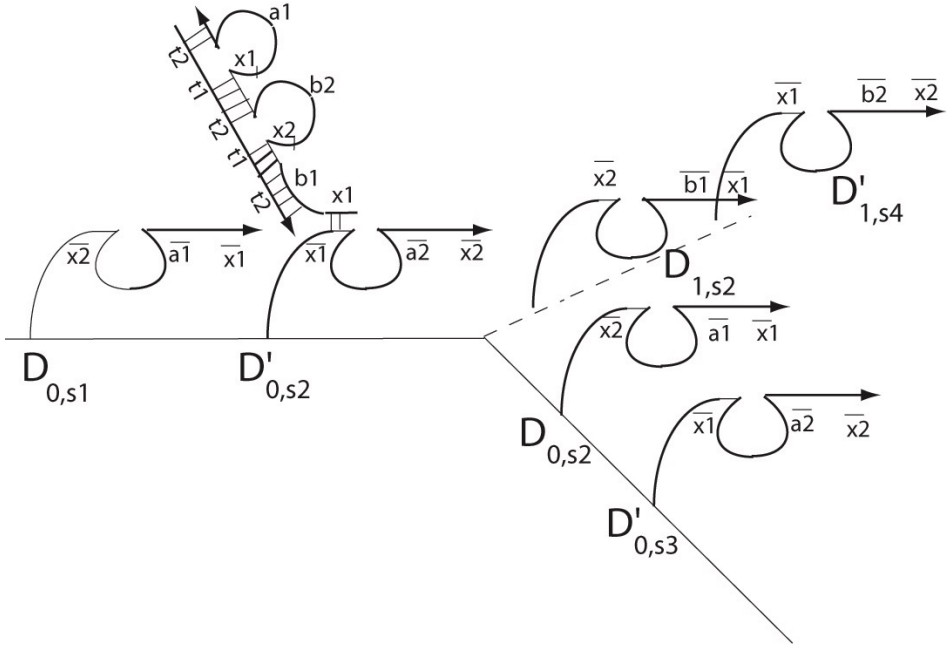
DNAzyme Implementations of Distinct State Transitions:

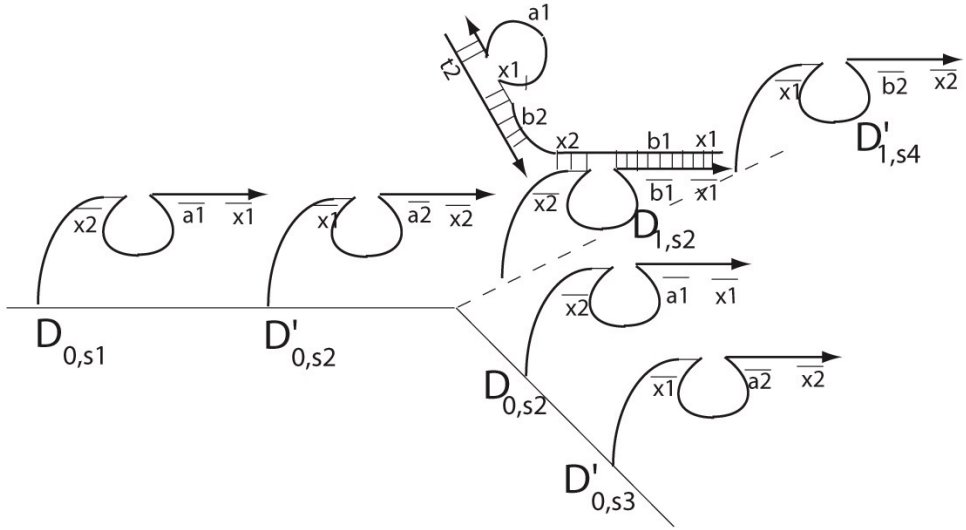
State Transitions:





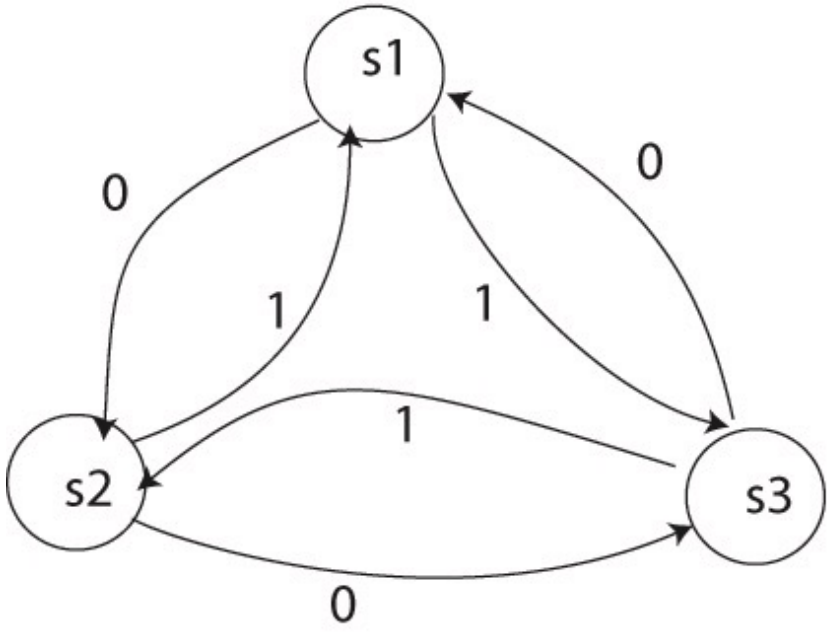
DNAzyme Implementations of Distinct State Transitions, Cont:



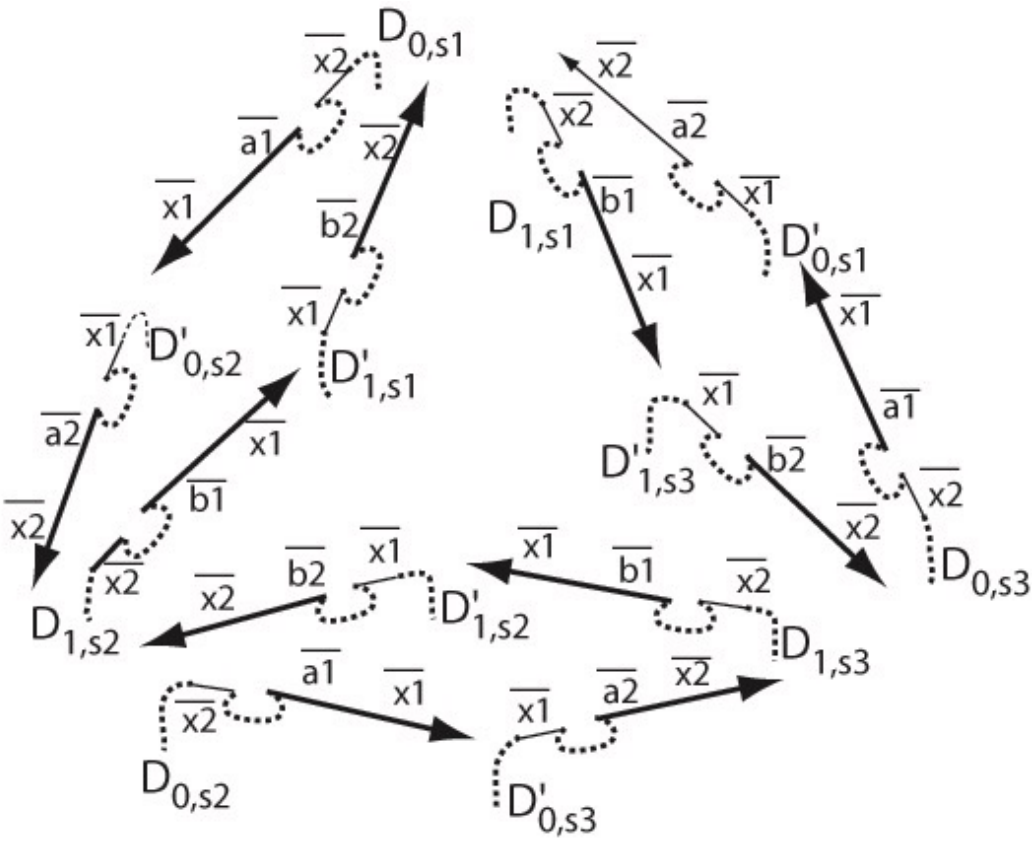


Complete Finite State Machine

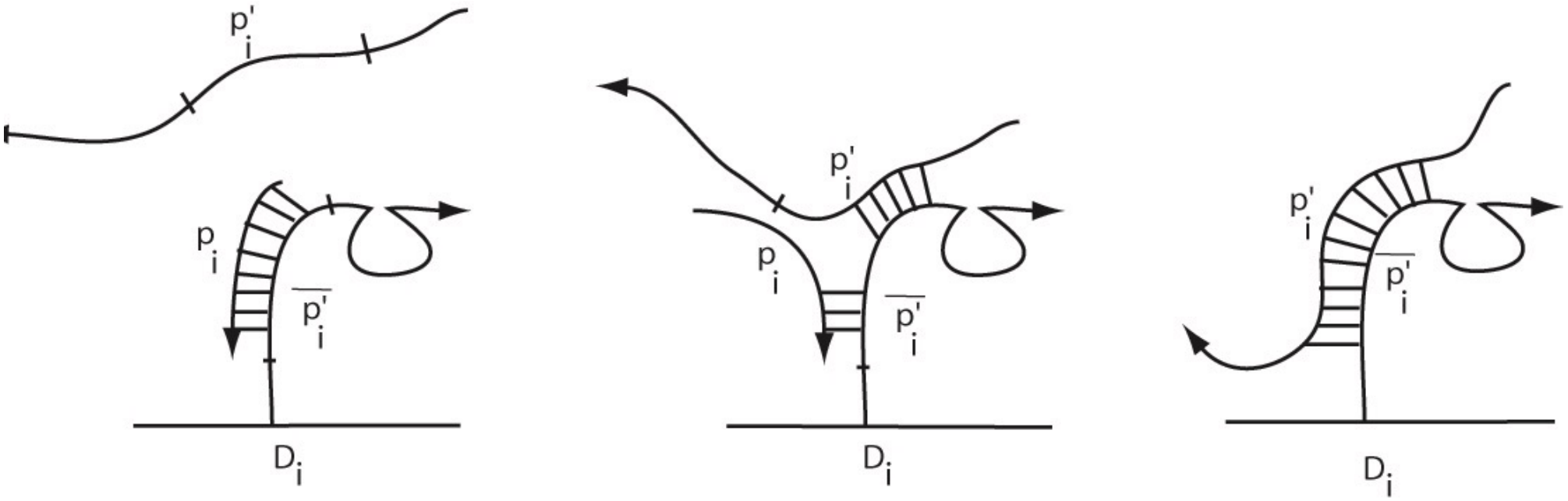
State Transitions:



DNAzyme Implementations of Distinct State Transitions:



Output Detection using Fluorescent In-Situ Hybridization(FISH)

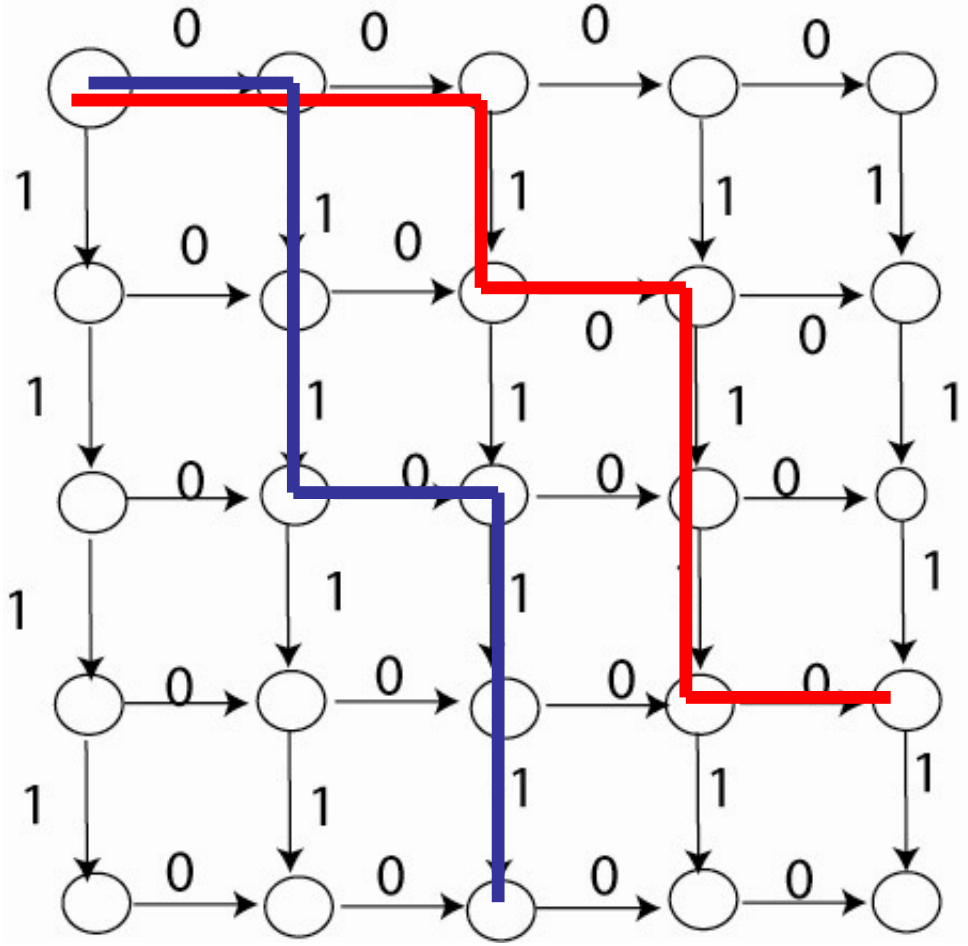


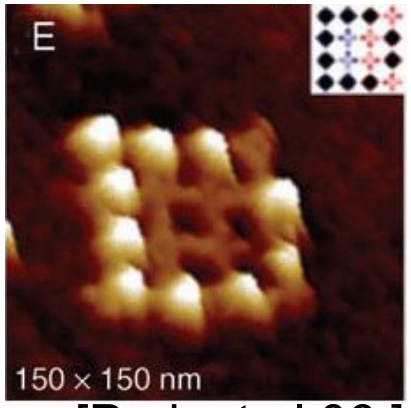
- p_i s are the fluorescent probes
- *Reporting sequence* in the last bulge loop of input nanostructure
- A section of reporting sequence displaces fluorescent probe from the DNAzyme depicting the output state

Summary of DNAzyme FSA

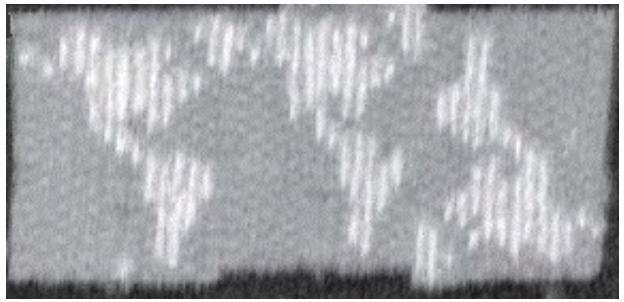
- Non-deterministic finite automata
- Probabilistic automata
  - identical DNAzyme sequences result in uniform state-transition probabilities
  - partially complementary sequences to obtain arbitrary state-transition probabilities (ratio of hybridization probability is in accordance with transition probabilities)
- Reusable system
- No. of DNAzymes required is proportional to the no. of transitions (proportional to no. of states for binary input) in FSA
- Question: whether this scheme can be extended to non-planar layouts

DNAzyme Router





[Park et al 06]



[Rothemund 05]

0 Go right
1 Go down

Input: 110110

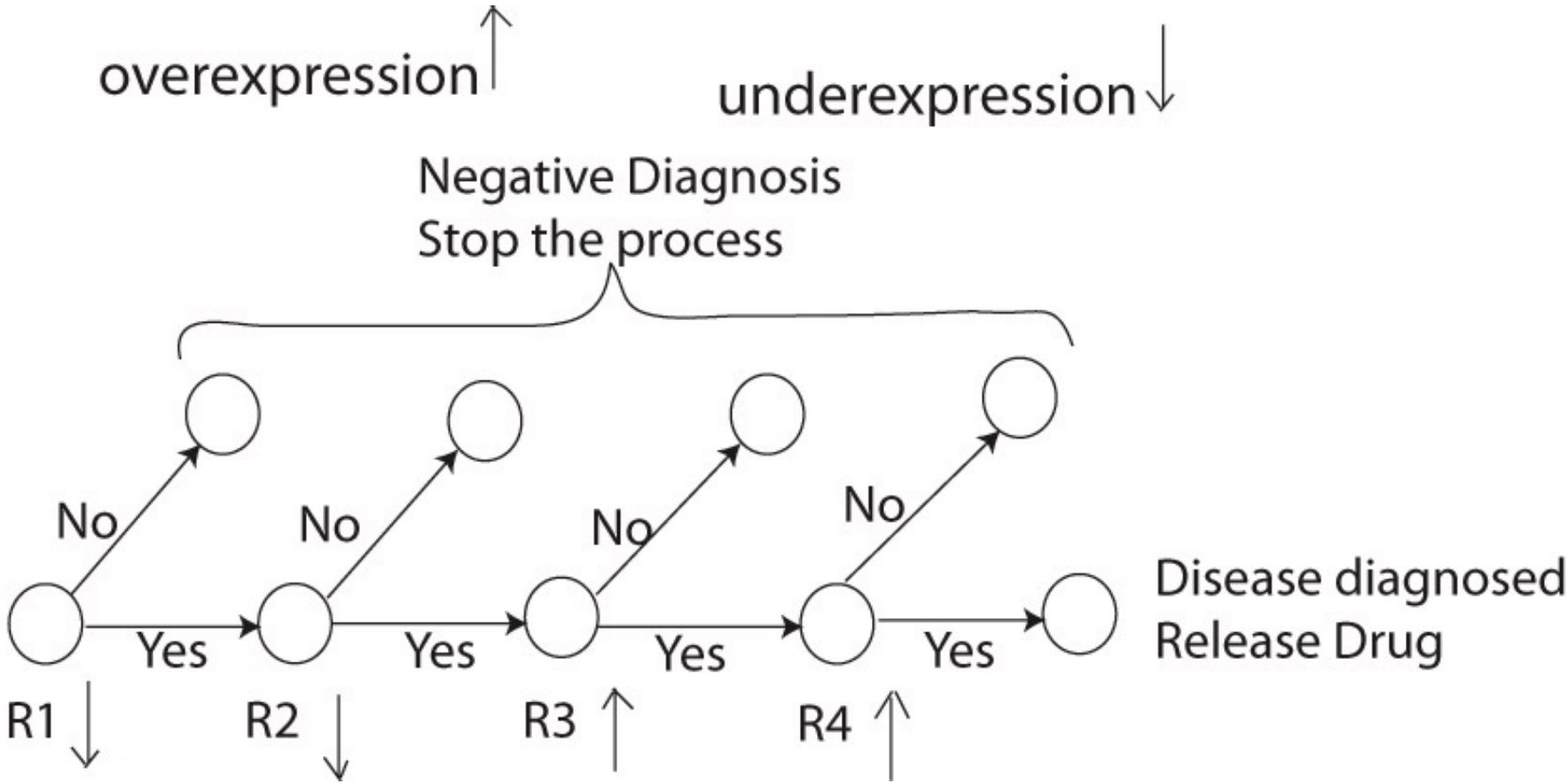
Input: 0110100

DNAzyme Router

- Input string acts as program for the robot
- Non-destructive
- Multiple robots walking together

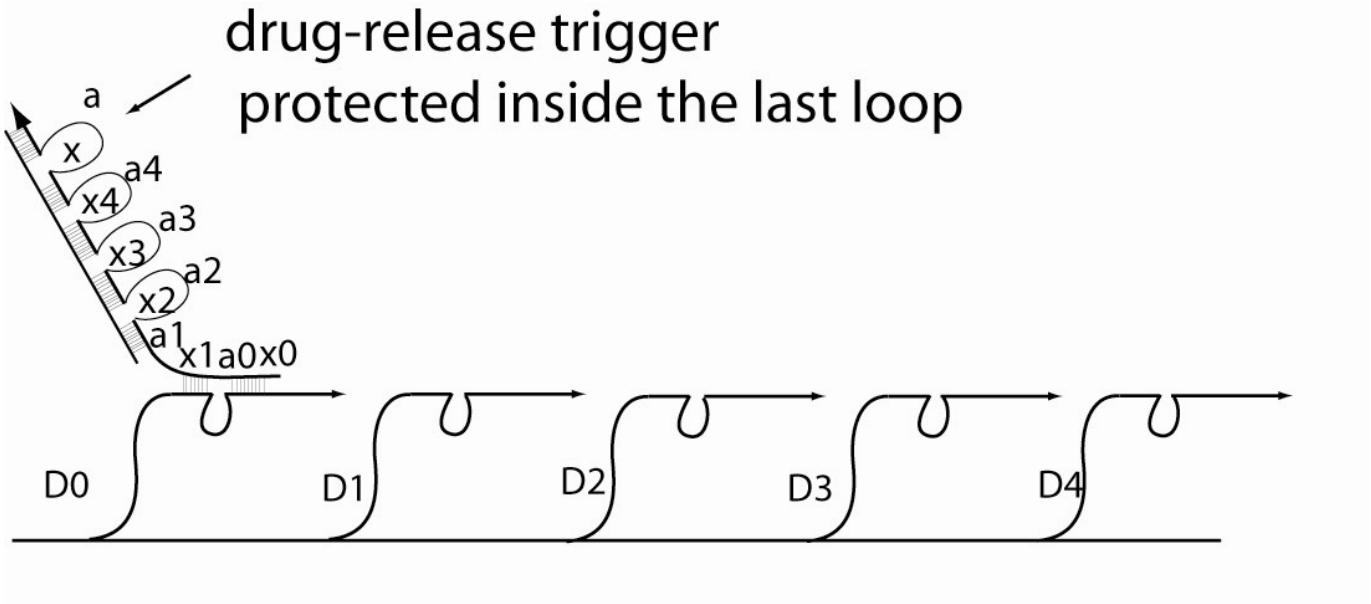
DNAzyme Doctor (state diagram)

- Shapiro Device [uses protein enzymes]



Design Principle

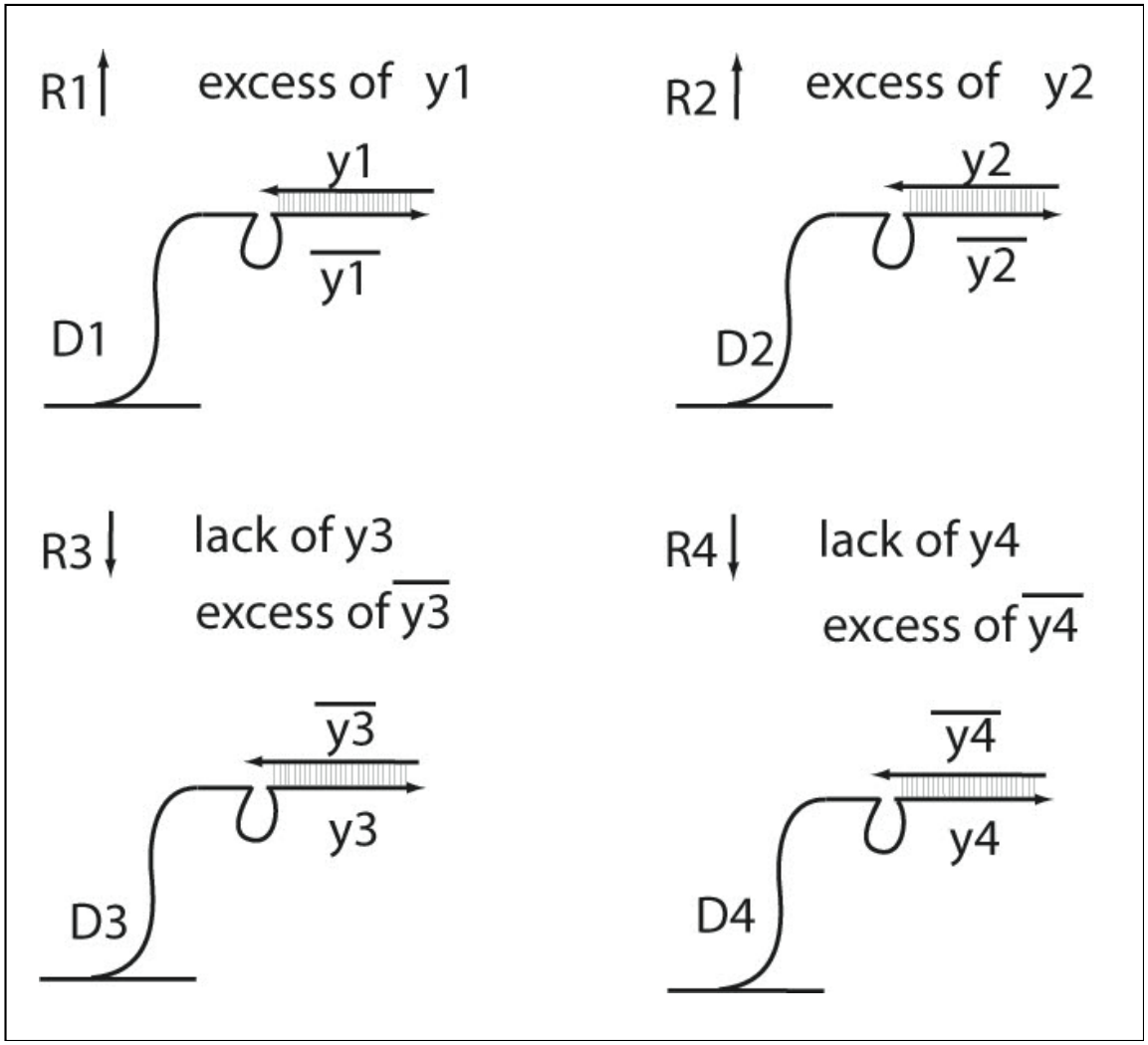
- We need AND operation
- We need a way to test for the under-expression and over-expression conditions



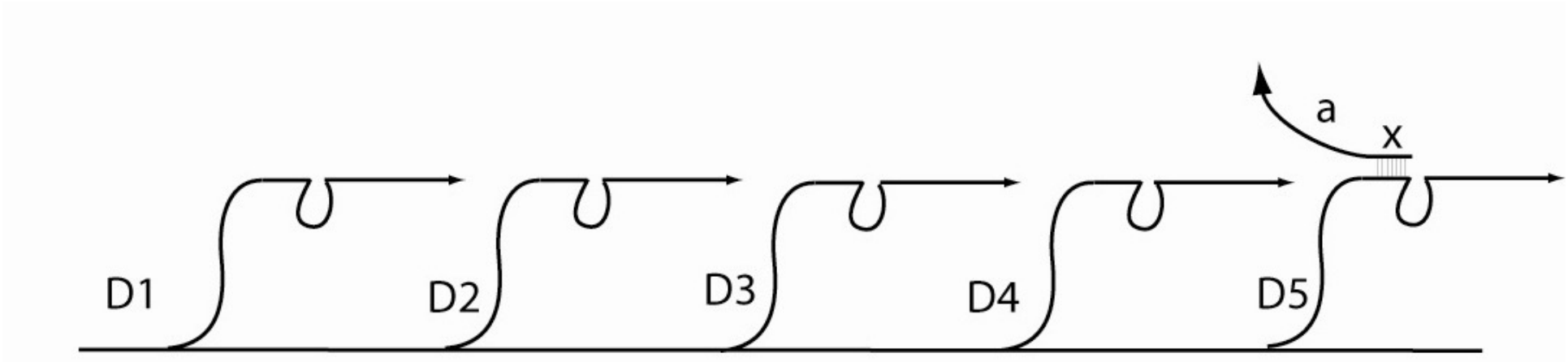
Detecting RNA Expression

sequence of RNAs y_1, y_2, y_3, y_4

A threshold concentration of $\overline{y_1}$, $\overline{y_2}$, $\overline{y_3}$, $\overline{y_4}$ is added to the solution, therefore lack of y_3, y_4 causes excess of $\overline{y_3}$ and $\overline{y_4}$, respectively.



DNAzyme Doctor : In Action



Summary

DNAzyme based systems:

- Autonomous
- Programmable
- Protein Enzyme Free
- Easily extended to interesting applications
- Only 4 different sequences of DNAzymes required