DNA Computing and Robotics Using DNAymes

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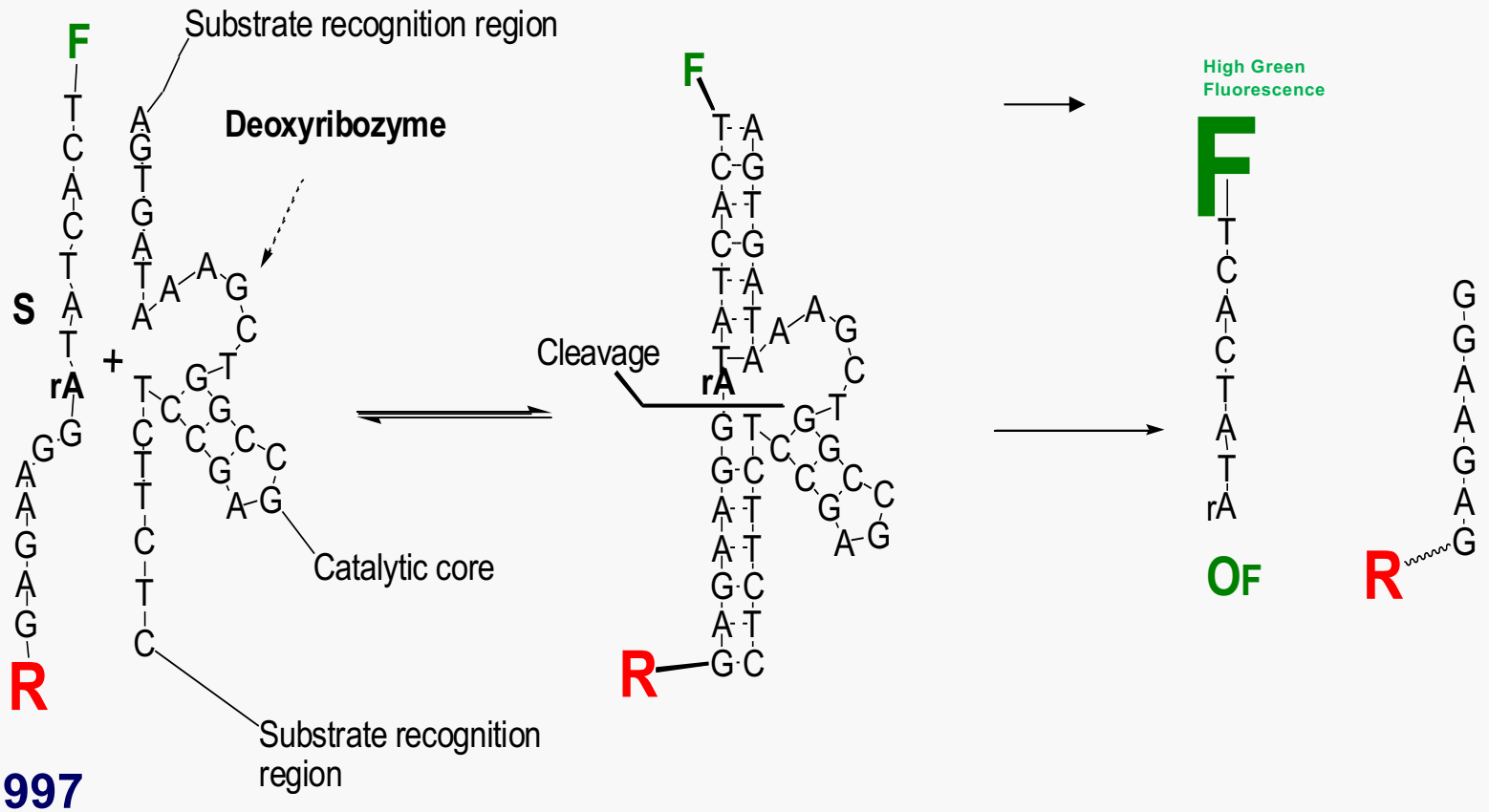
MCBC

Ribozymes used to cut double stranded DNA at Recognition Sites:



Joyce 1995, 1997

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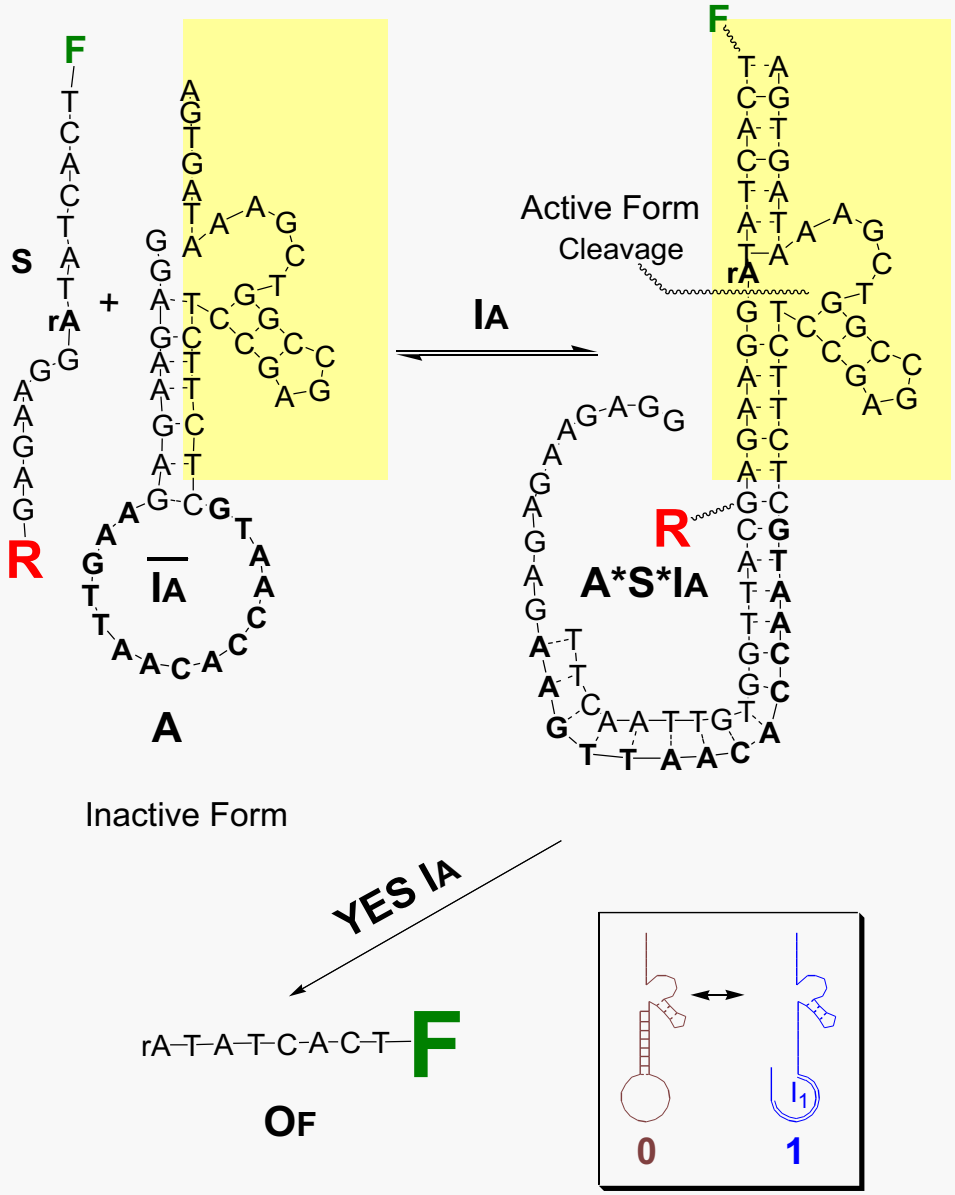
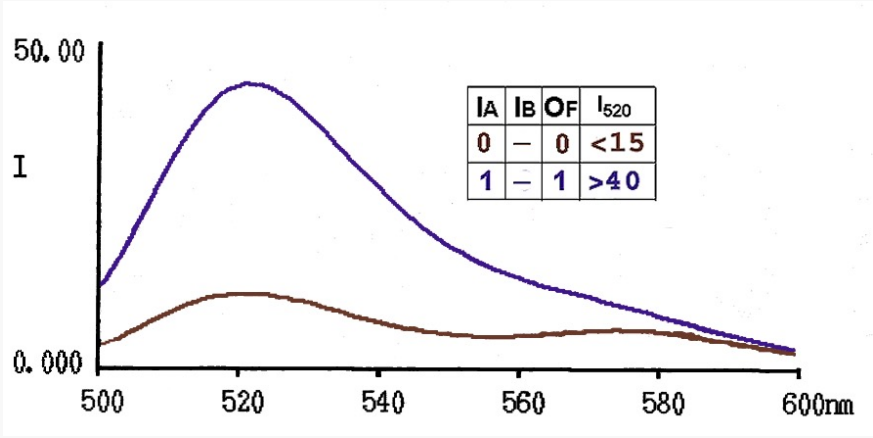
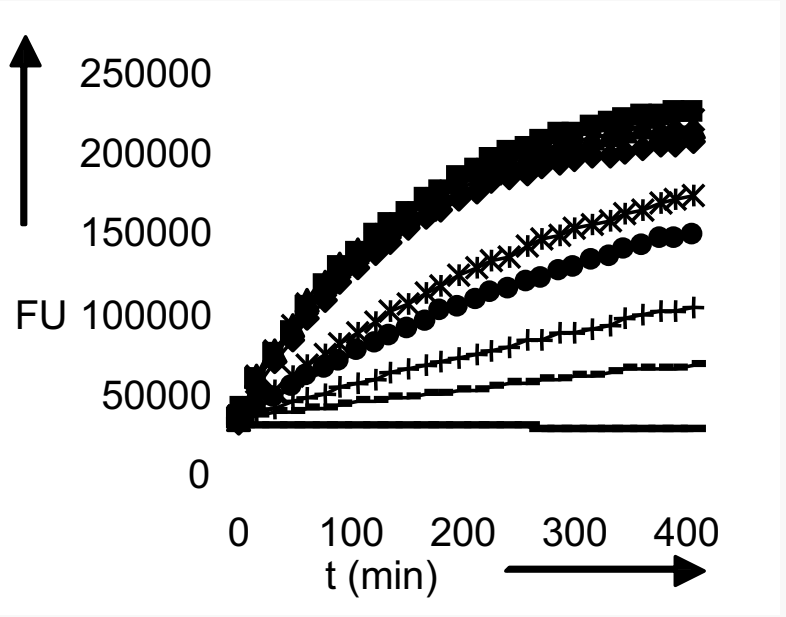
Columbia University

Catalytic Molecular Beacons as Sensor Gates Detector



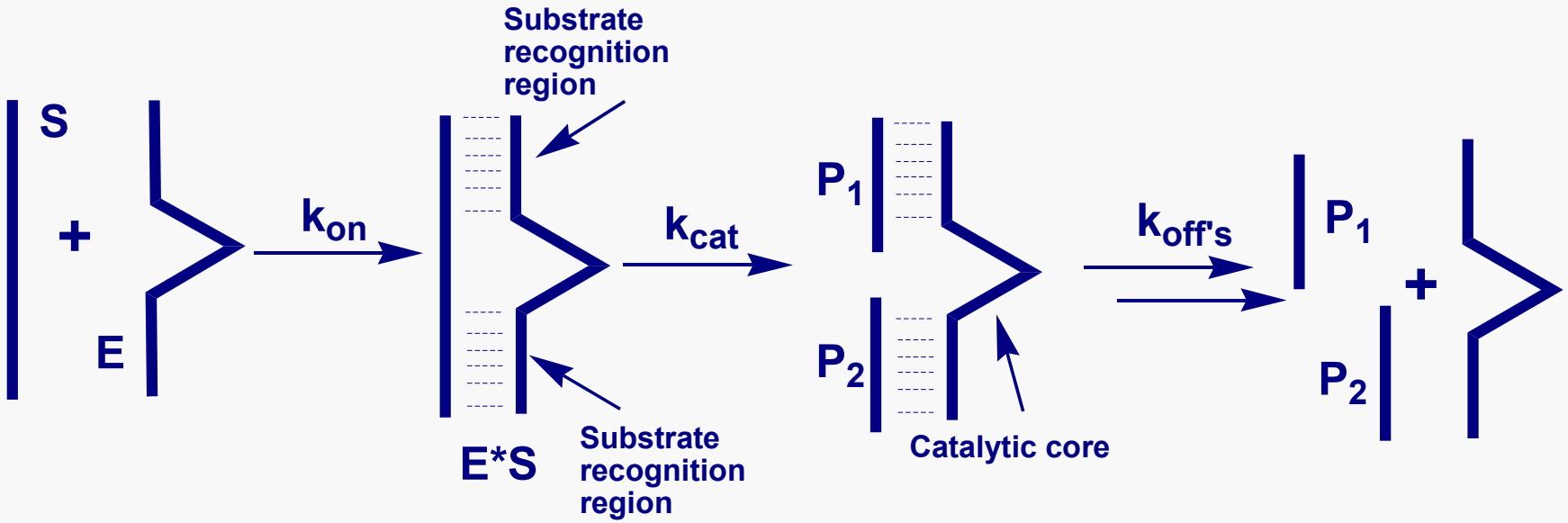


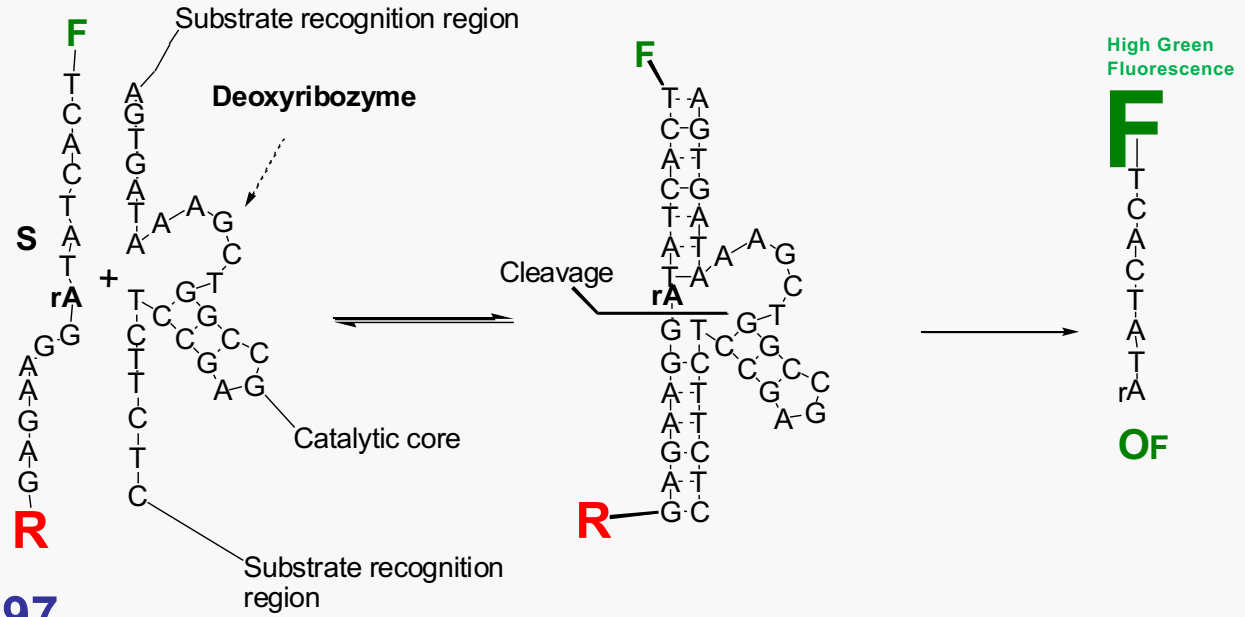


Joyce 1995, Breaker 1999, Tyagi, Kramer 1996

Stojanovic et al., *ChemBioChem* **2001**
Stojanovic et al., *J. Am. Chem. Soc.* **2002**

Ribozymes used to cut double stranded DNA at Recognition Sites:





Joyce 1995, 1997

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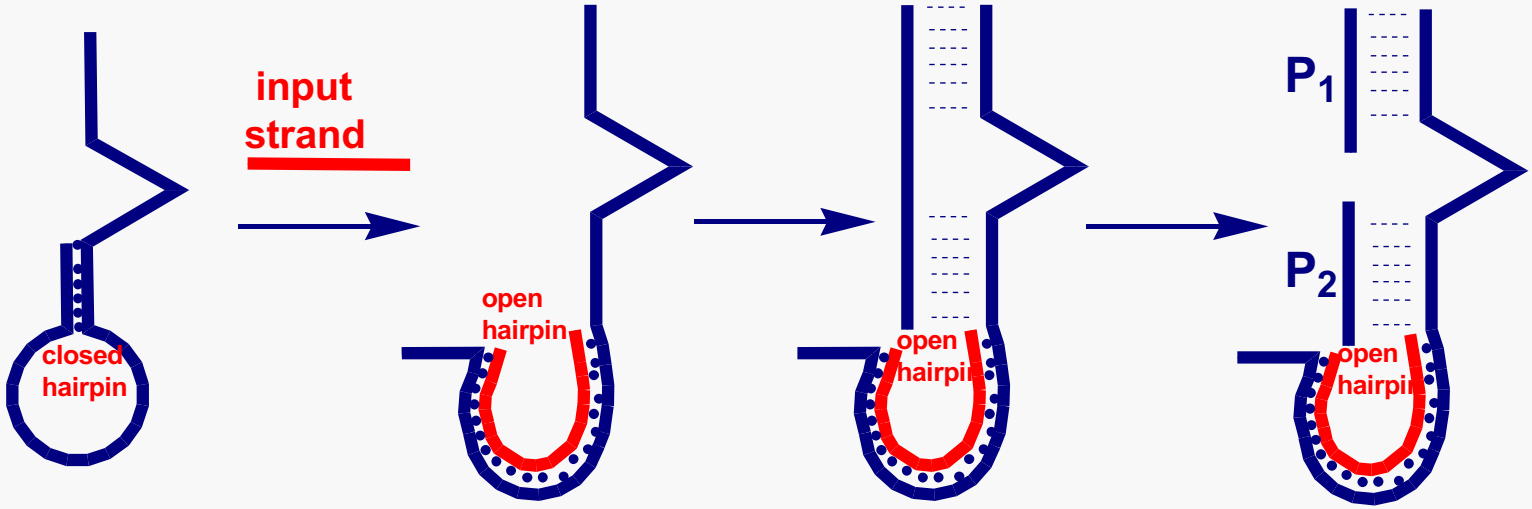
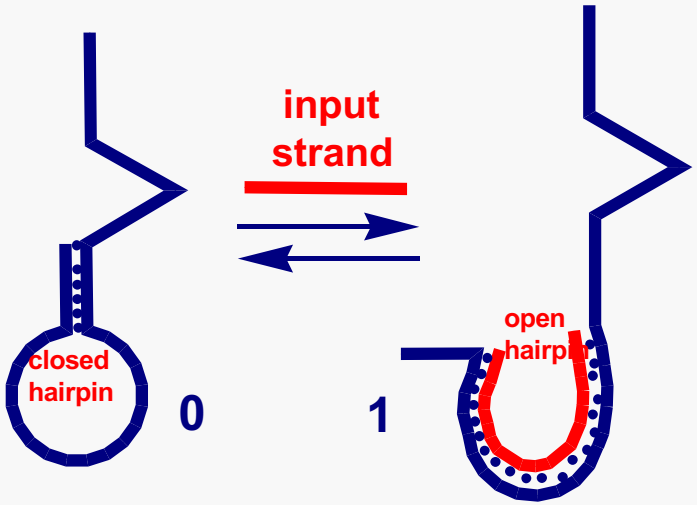
We can combine Ribozymes with stem loops: into a two-state switch:

Details of Reactions:



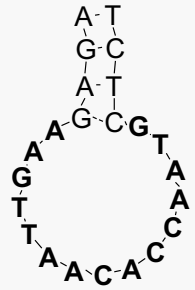
Summary of Reactions:



Detector gate
or
sensor
or
YES gate
or
…

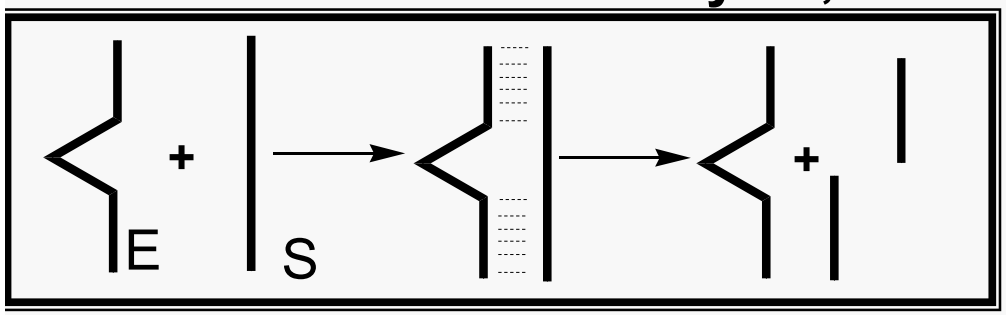
Recipe for Computing Primitives:

1. Take Recognition Regions,

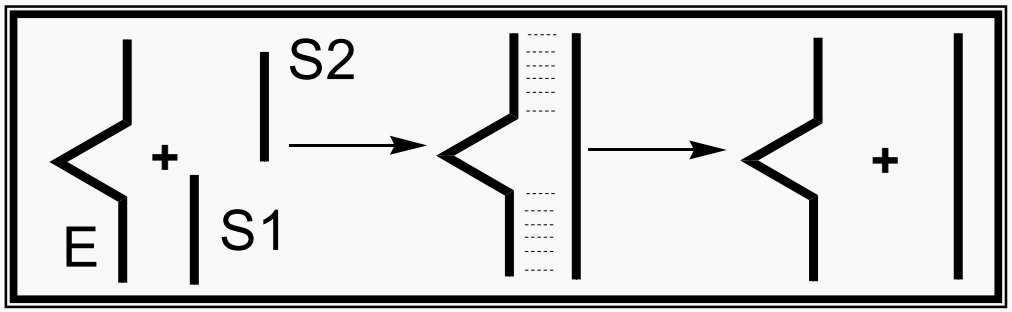


Beacons (Tyagi and Kramer)

2. Take Nucleic acid catalysts,

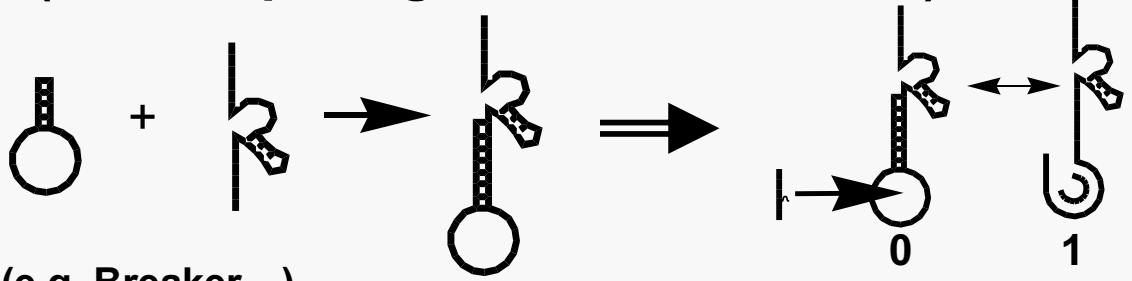


Phosphodiesterase (Joyce)



Ligase (Szostak)

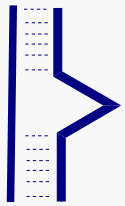
3. And combine (for computing elements, at least) them in:



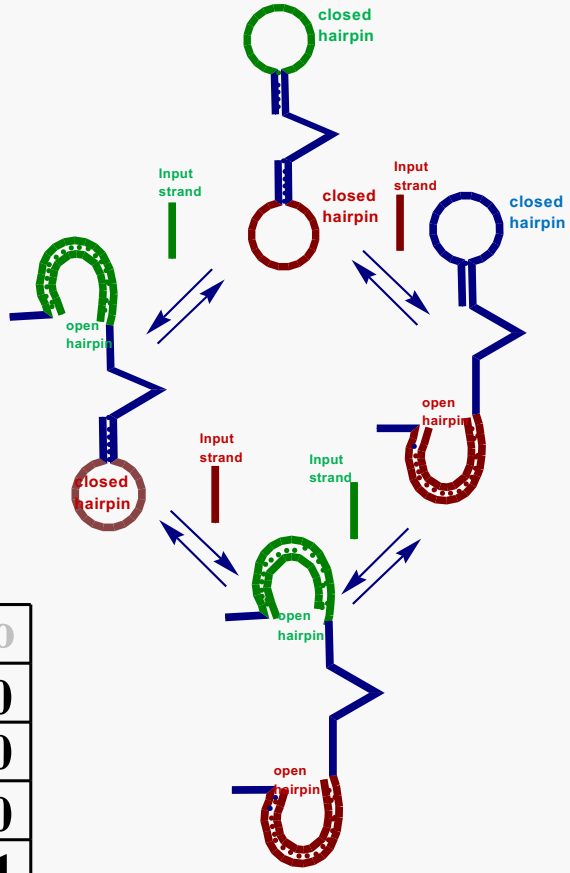
Cf. modular design (e.g. Breaker…)

Complete set of Boolean Logic switches:

DNAzyme



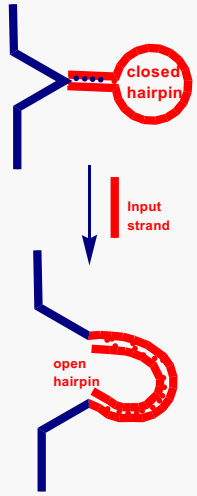
AND Gate:



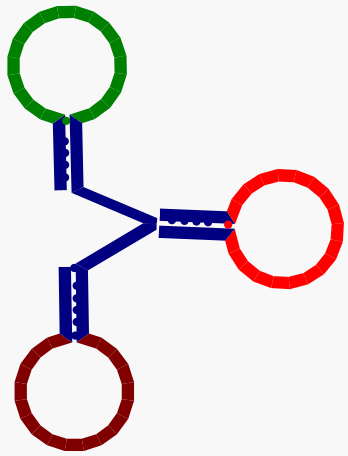
i_1	i_2	0
0	0	0
1	0	0
0	1	0
1	1	1

NOT Gate:

i_1	0
0	1
1	0

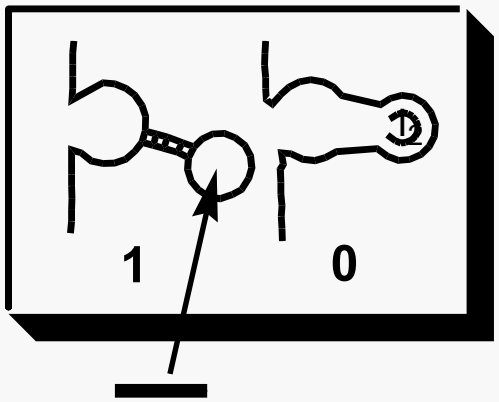


ANDANDNOT:

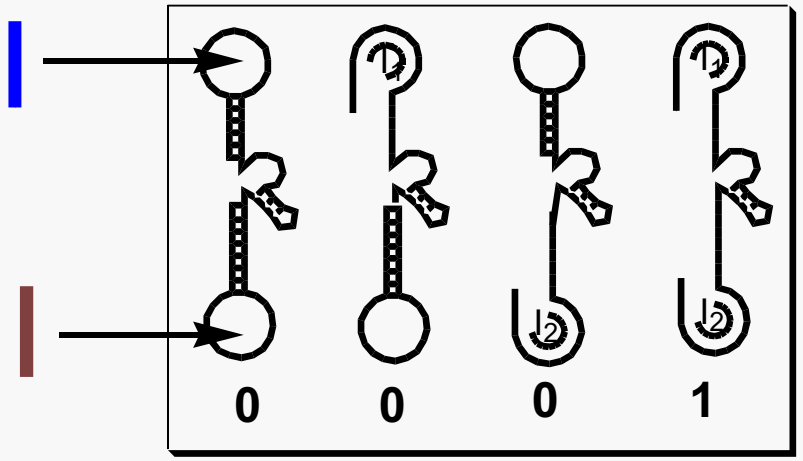


i_1	i_2	i_3	0
0	0	0	0
1	0	0	0
0	1	0	0
0	0	1	0
1	1	0	1
1	0	1	0
0	1	1	0
1	1	1	0

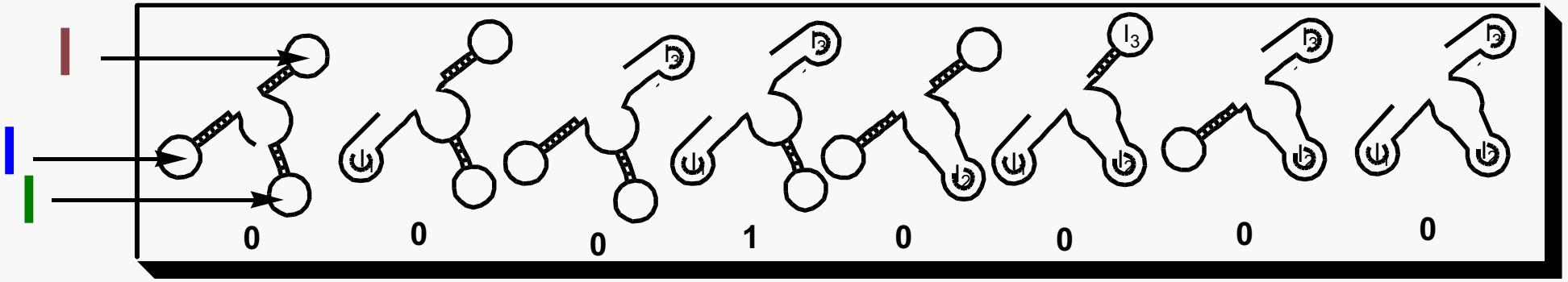
Switching Primitives (Logic Gates):



NOT

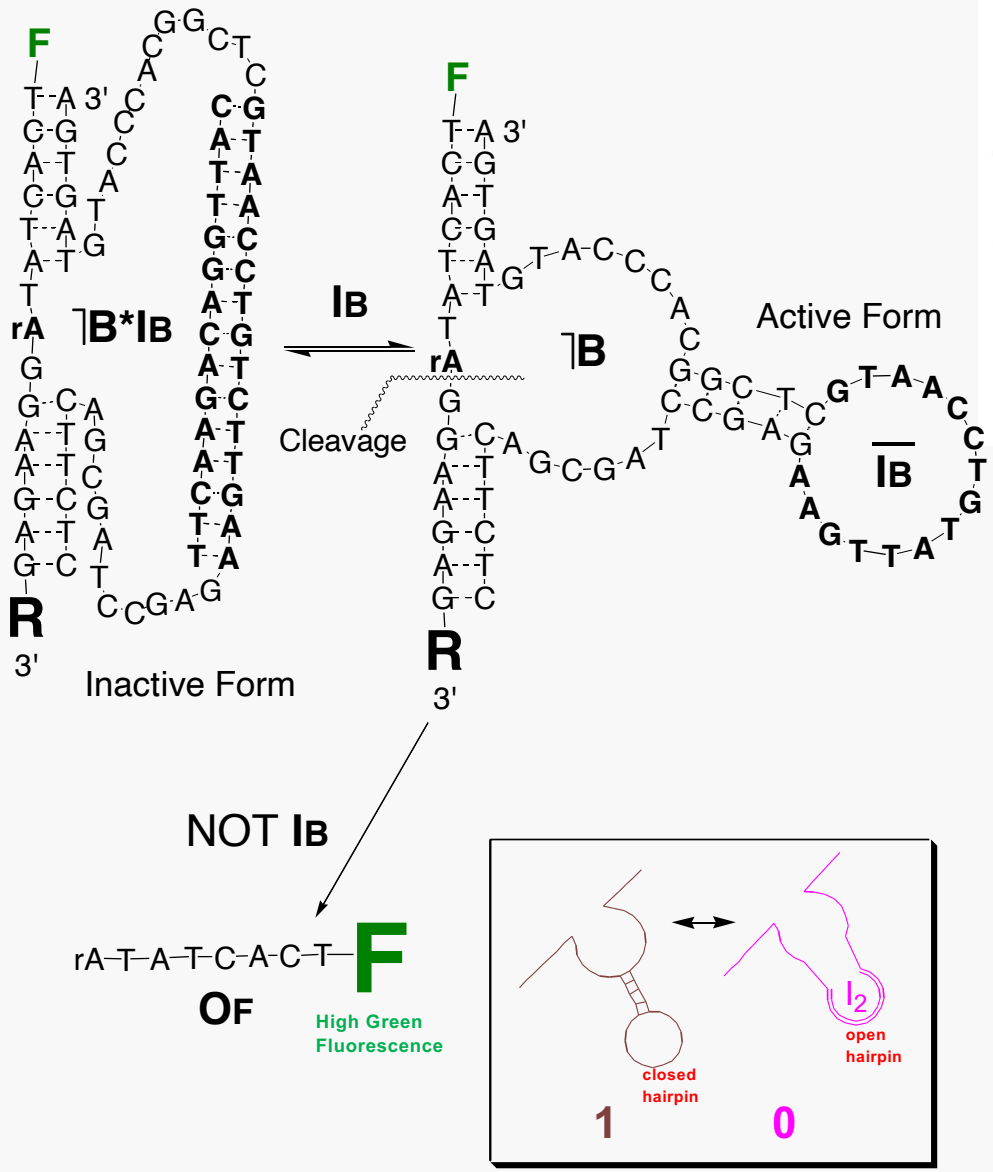


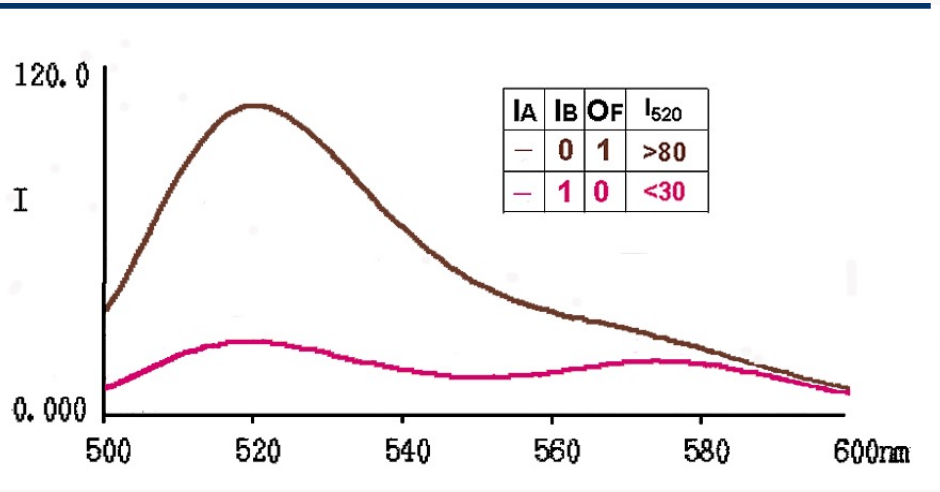
AND

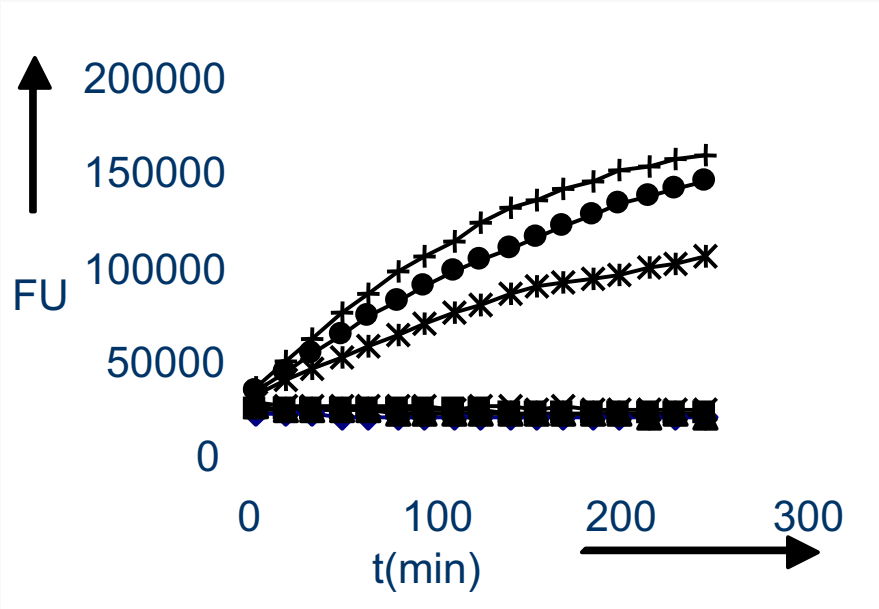


Three-input Gates

NOT Gates – Inverters:

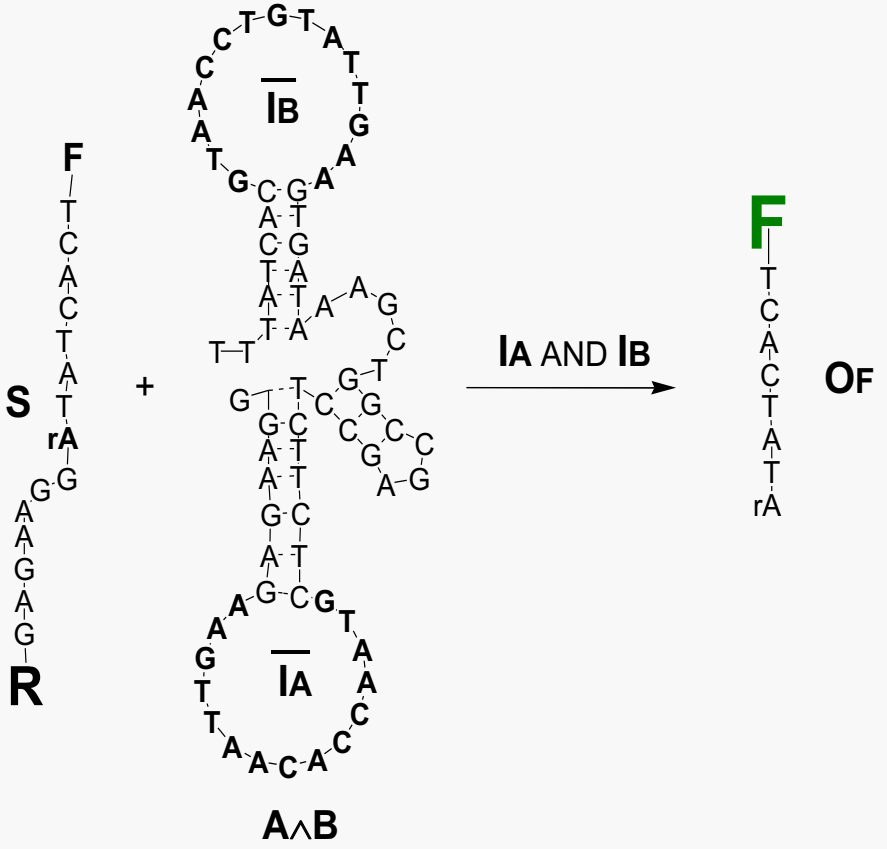


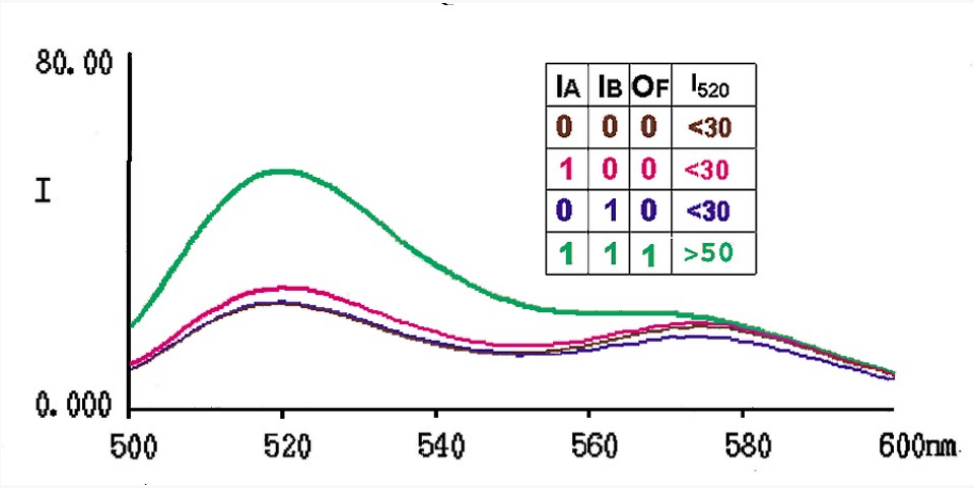


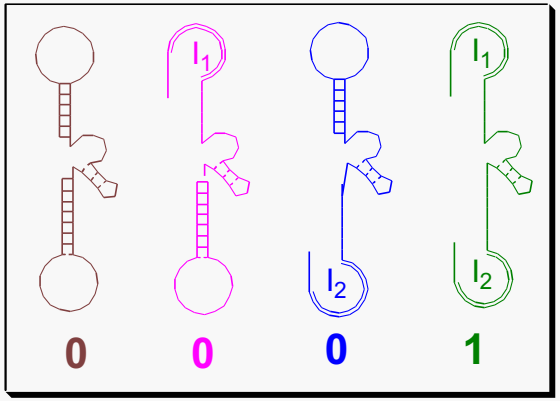


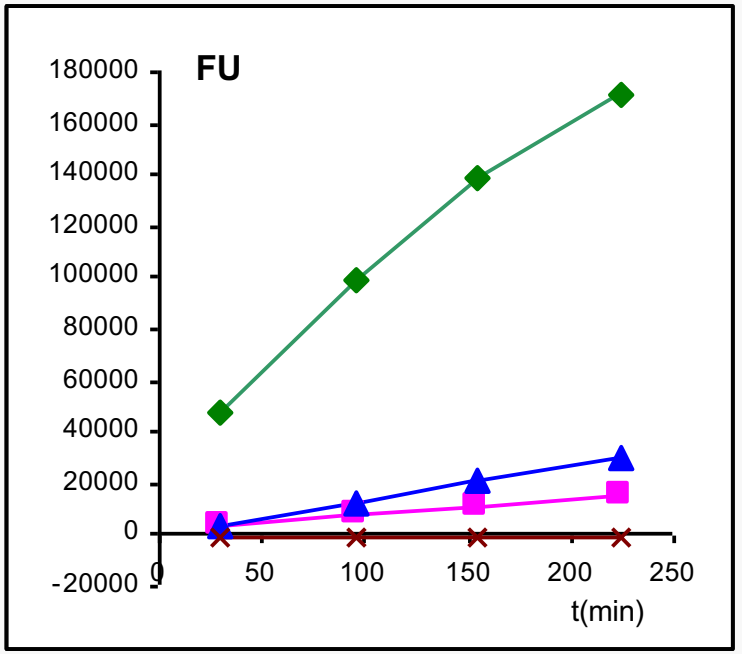
Stojanovic et al., *J. Am. Chem. Soc.* **2002**

Dual Input Molecular Computation Elements: AND Gate

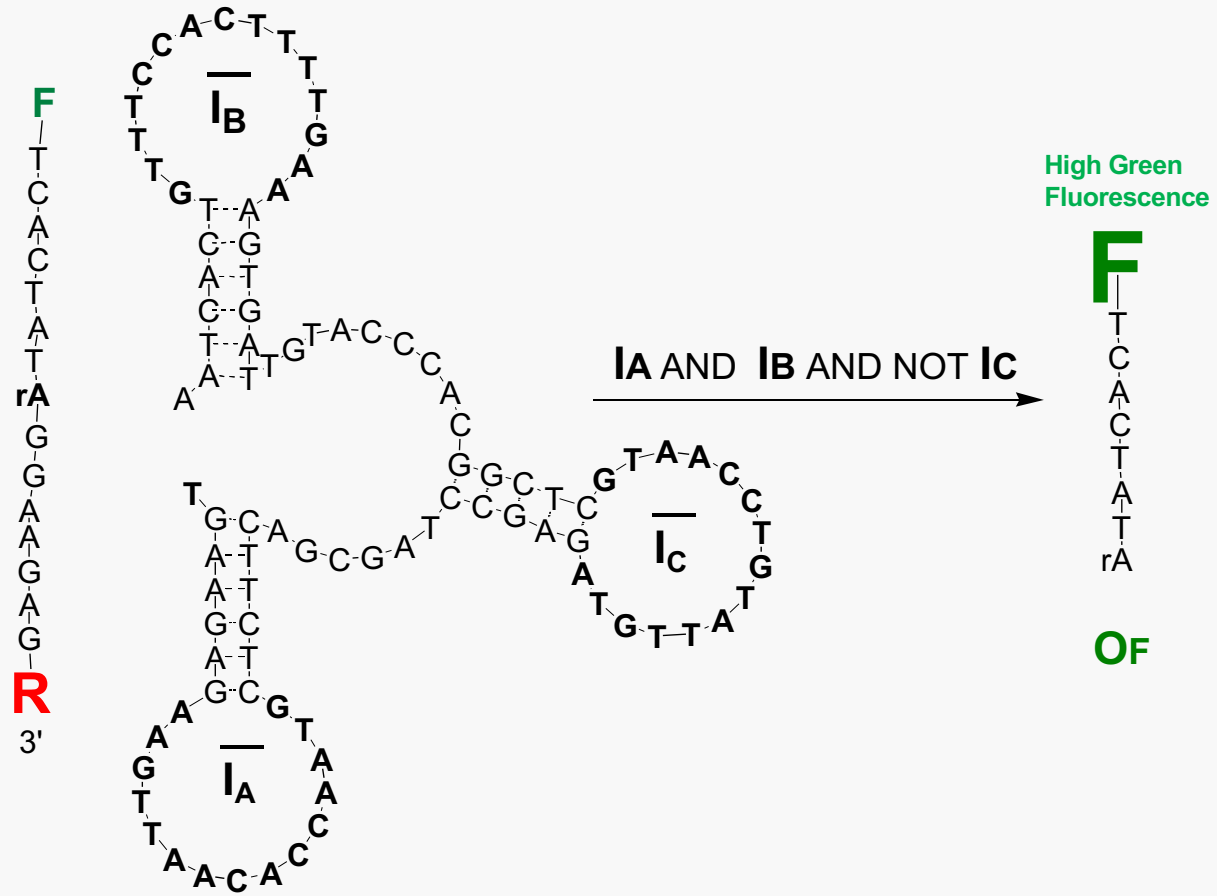


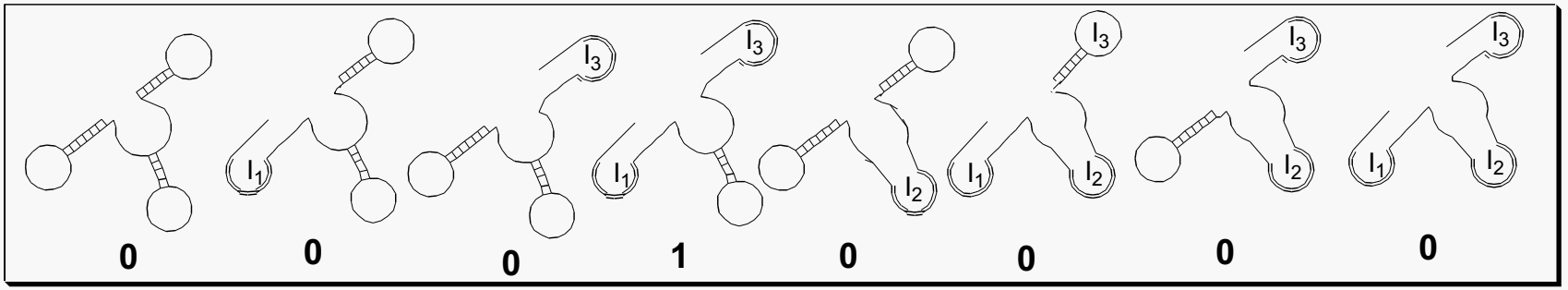




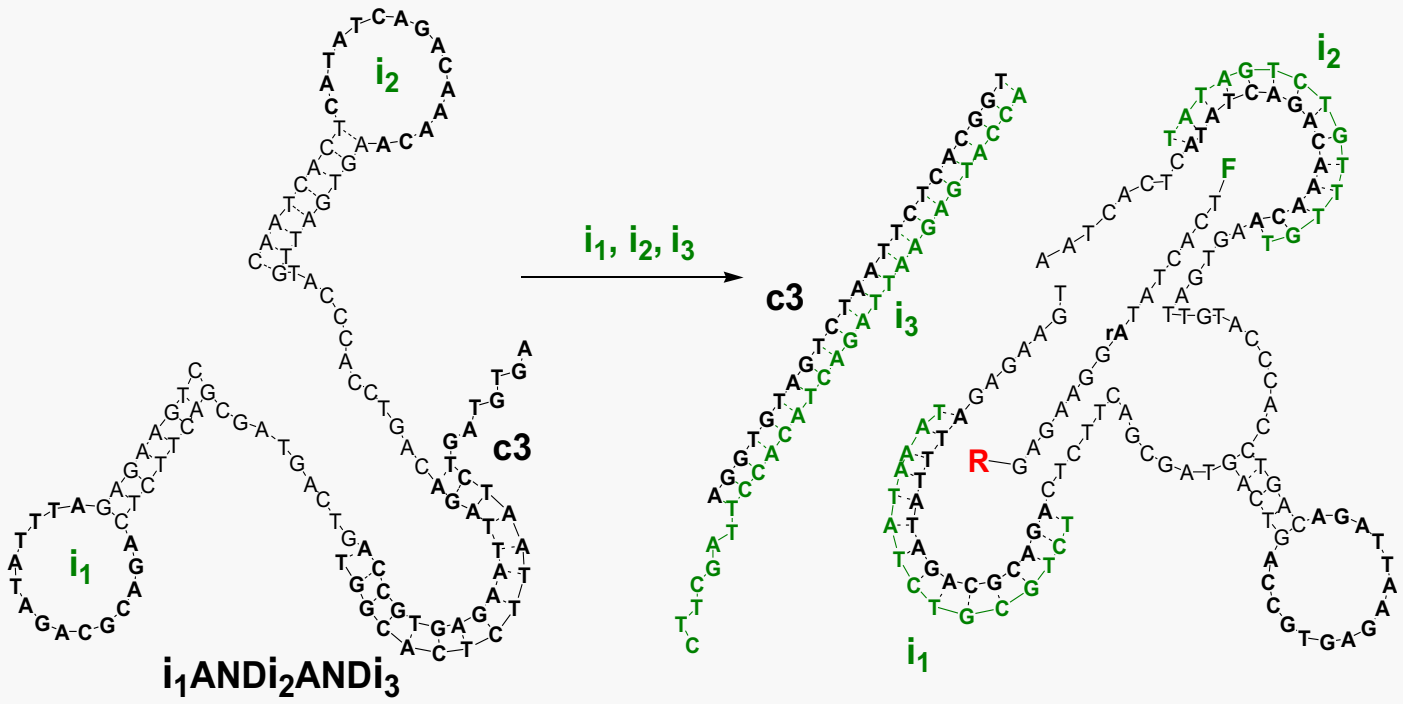


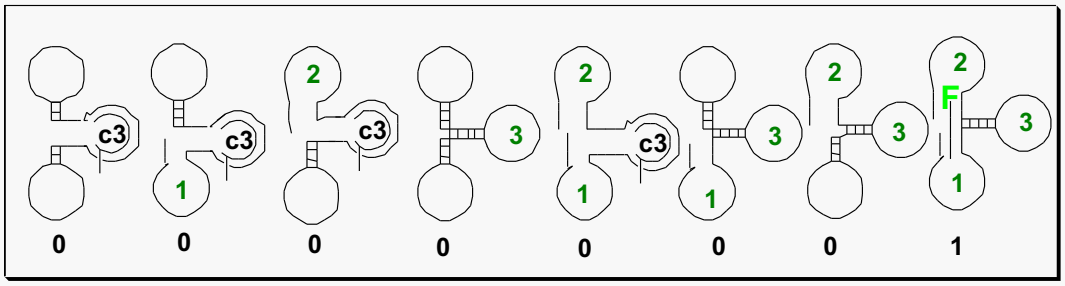
Triple Input Elements: ANDANDNOT (INHIBIT)

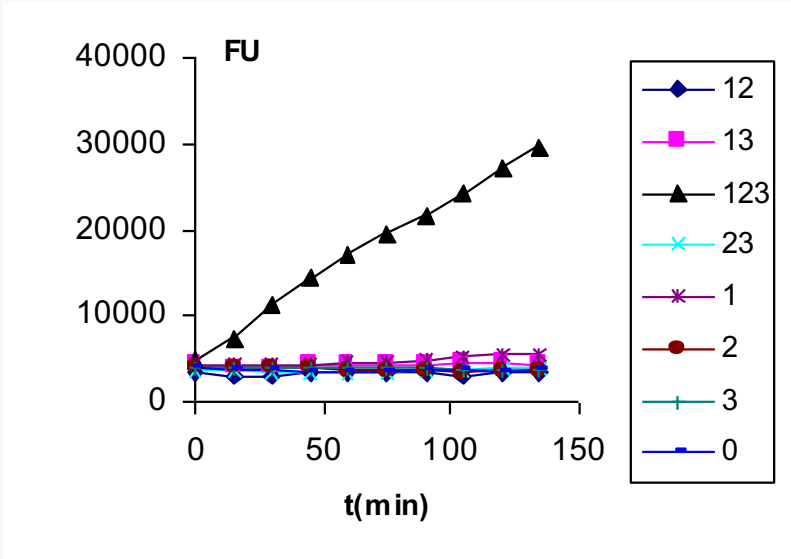




Triple Input Elements: ANDAND



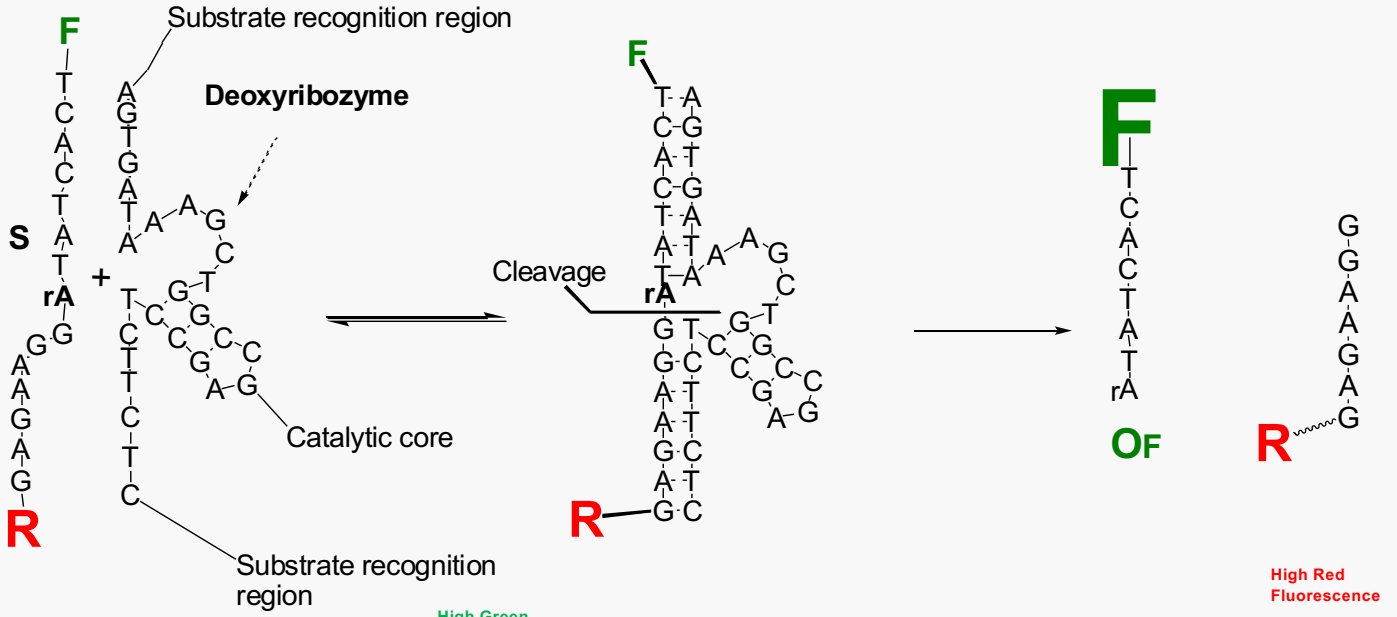


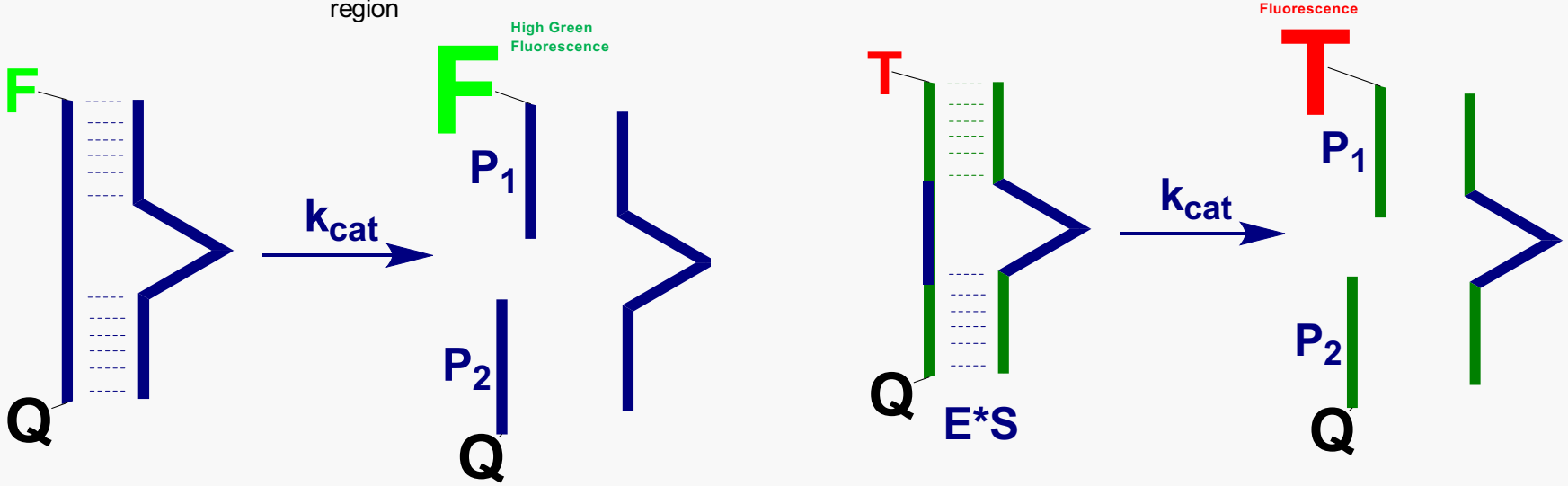


Harvey Lederman

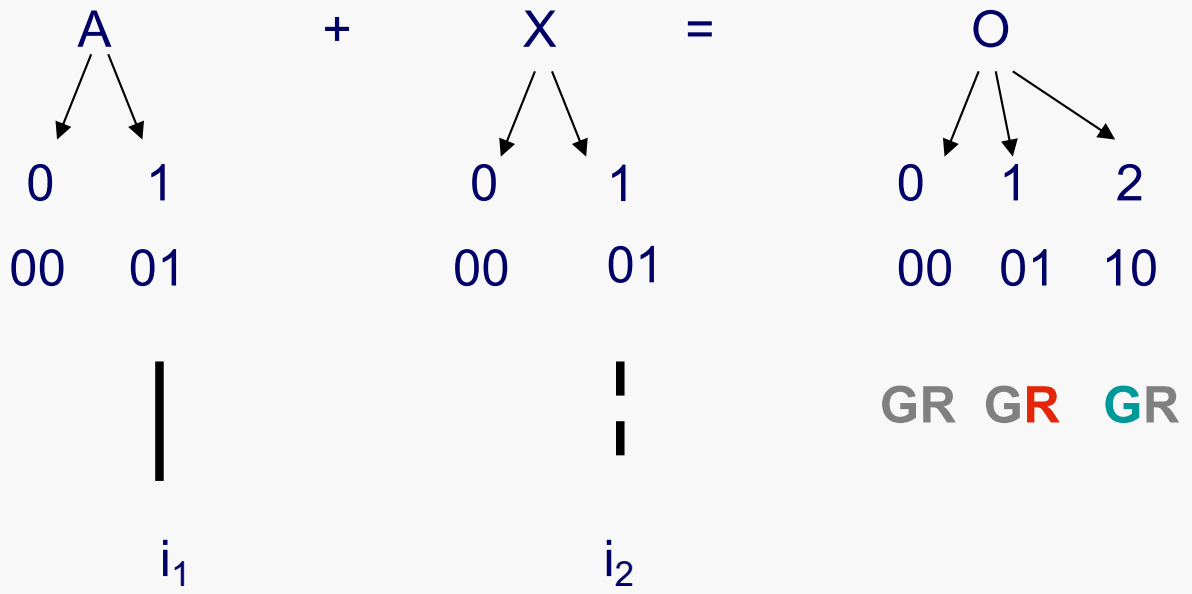
Before we give examples of gates, a reminder:

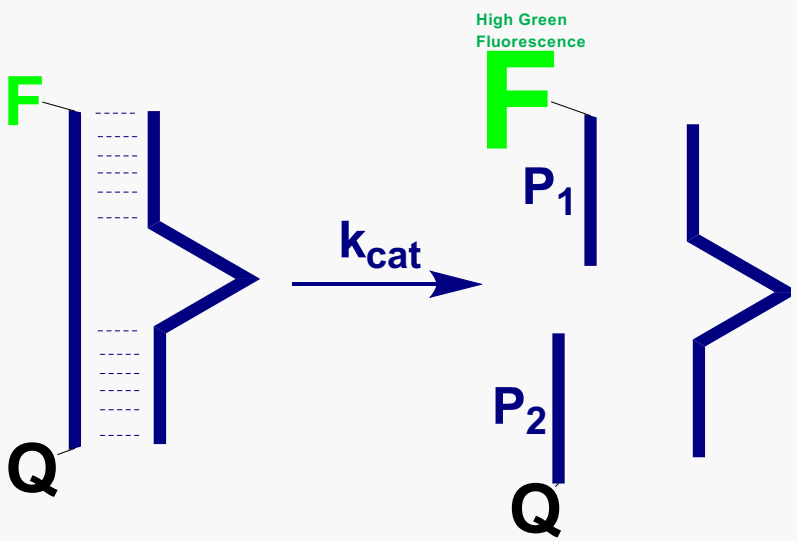
1 is an increase in fluorescence, 0 no such increase!



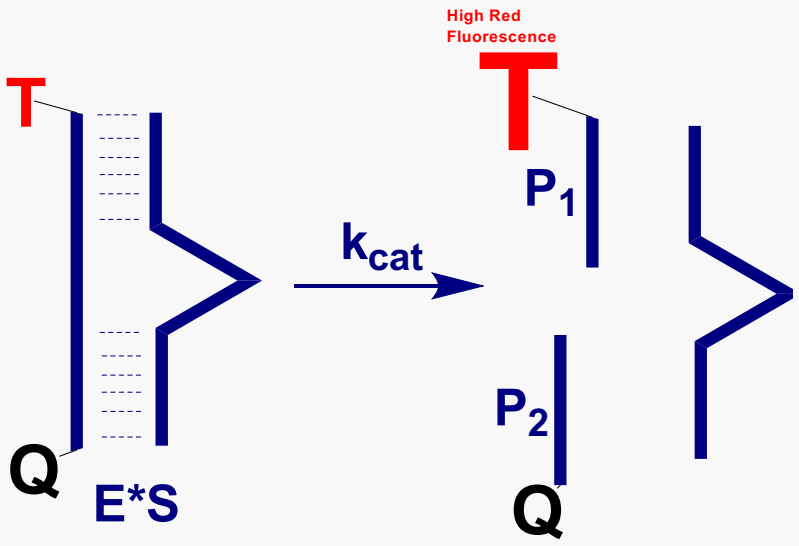


Example:
add A (0 or 1) and X (0 or 1) and get O (0 or 1, or 2):



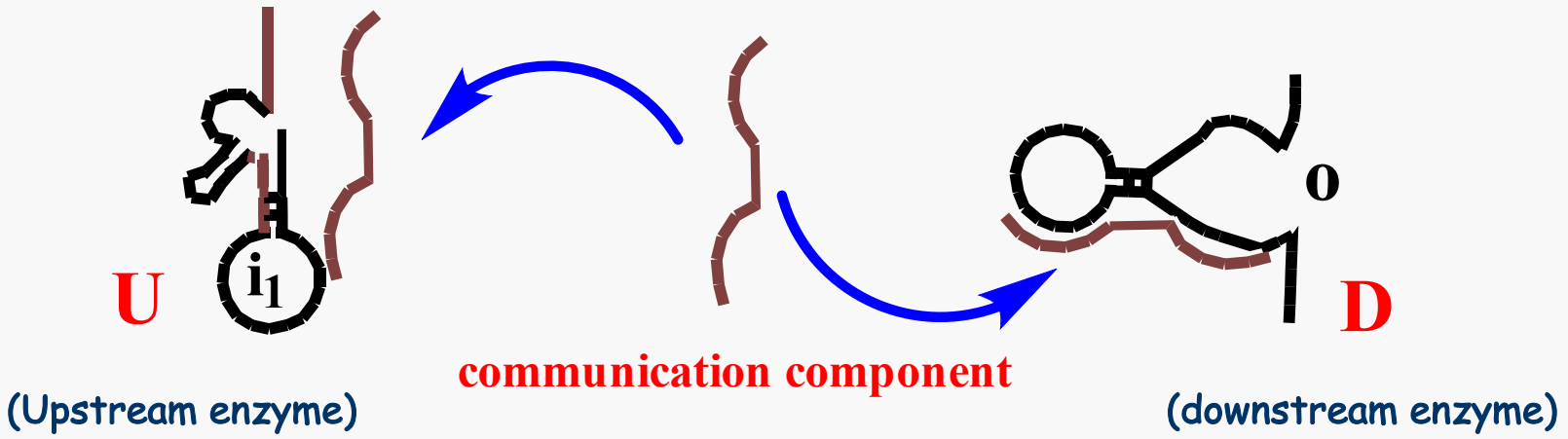


First deoxyribozyme

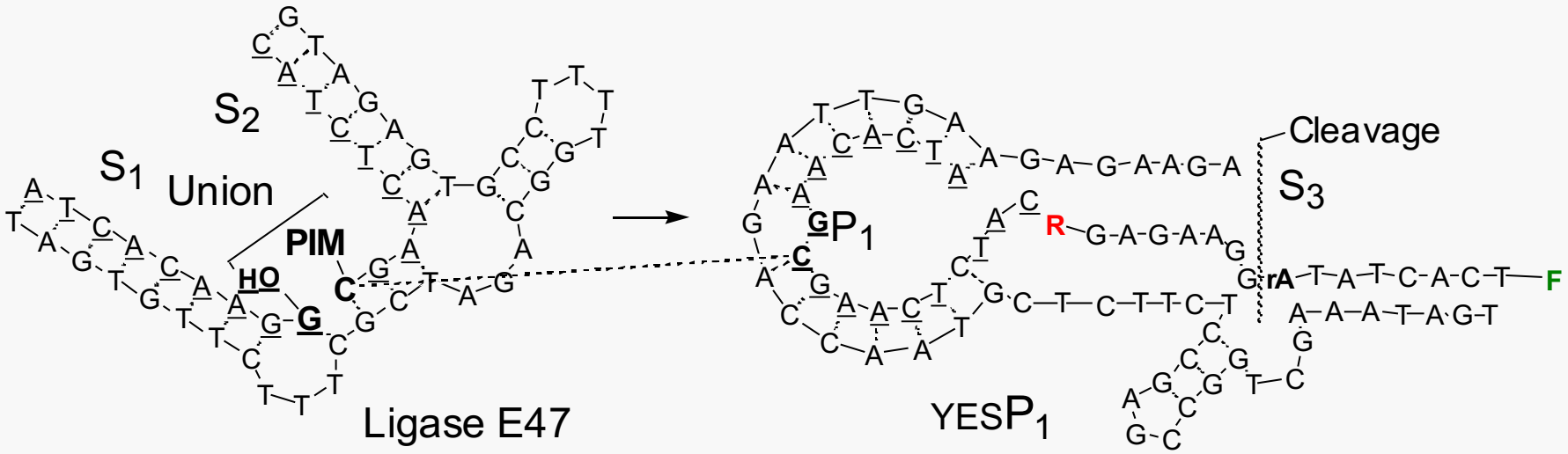


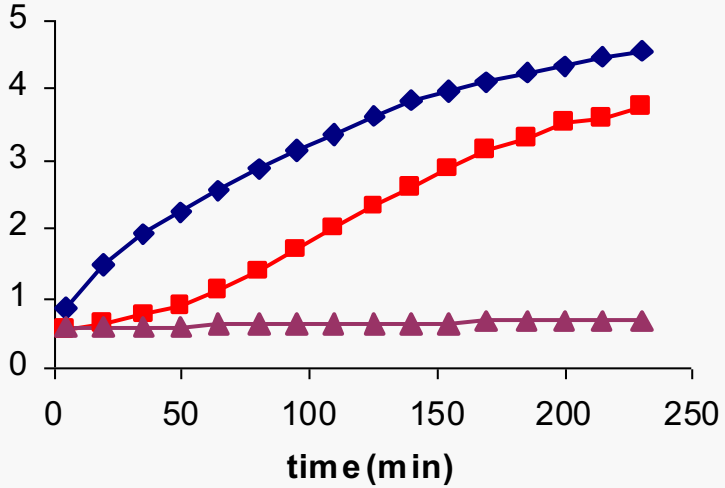
Second deoxyribozyme

Is it possible to have serial circuits?



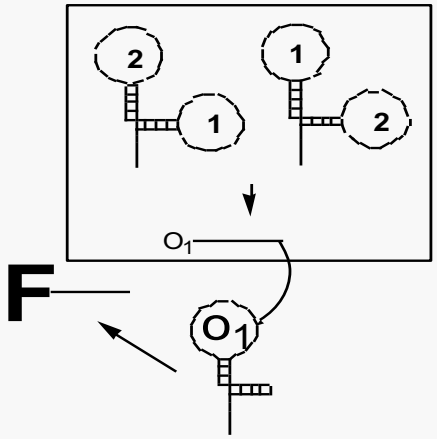
Intergate Communication : Ligase-Cleavase



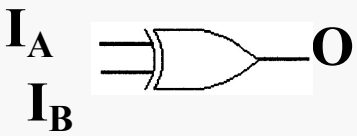


Stanka Semova, Dmitry Kolpashchikov

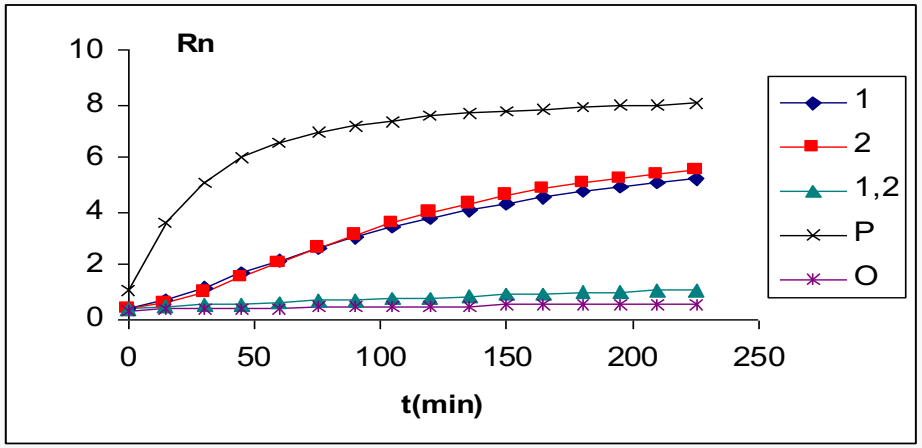
Ligase-cleaves XOR circuit:



XOR

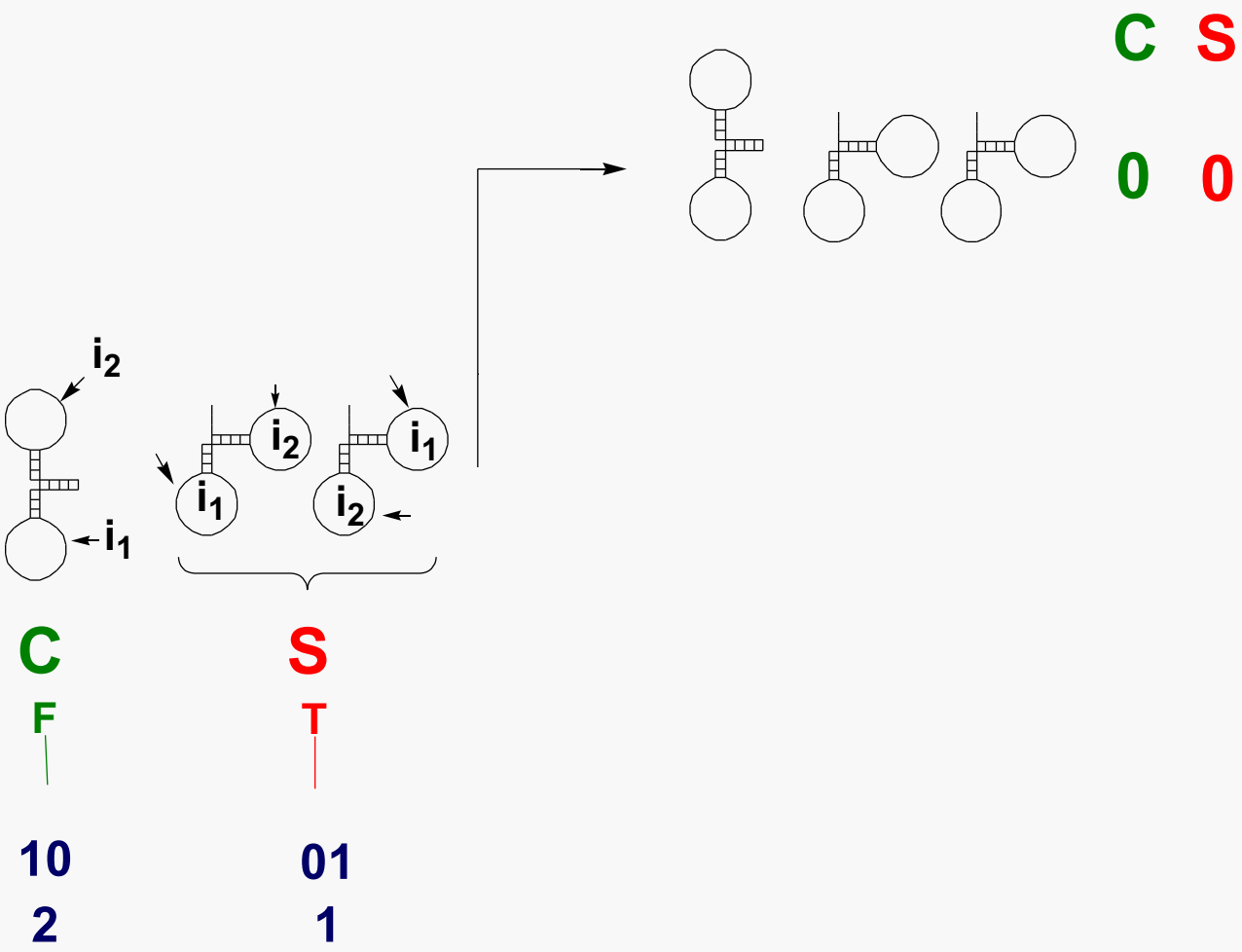


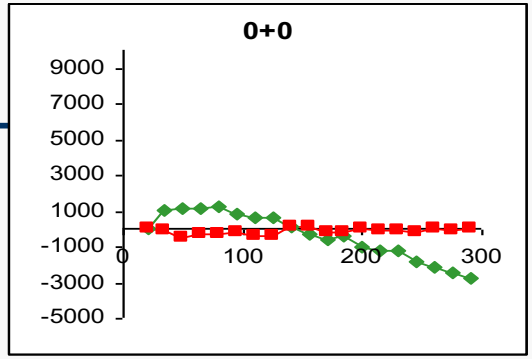
I_A	I_B	O
0	0	0
1	0	1
0	1	1
1	1	0

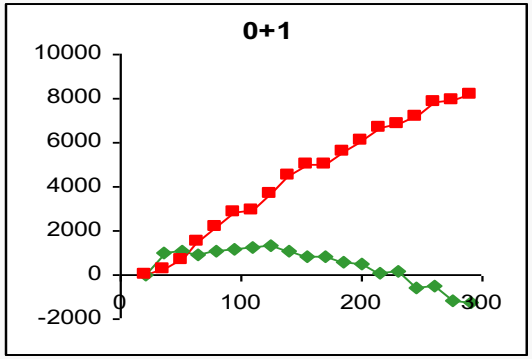


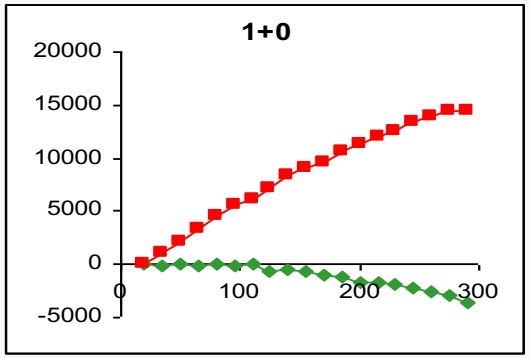
JACS **2005**

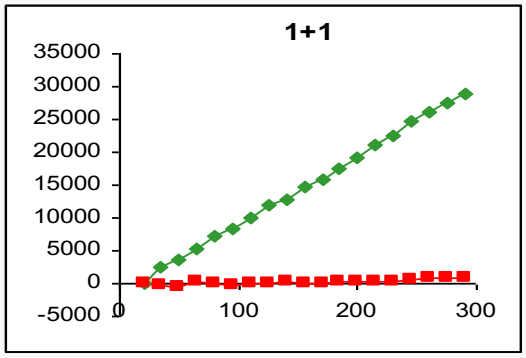
Implement addition with gates:







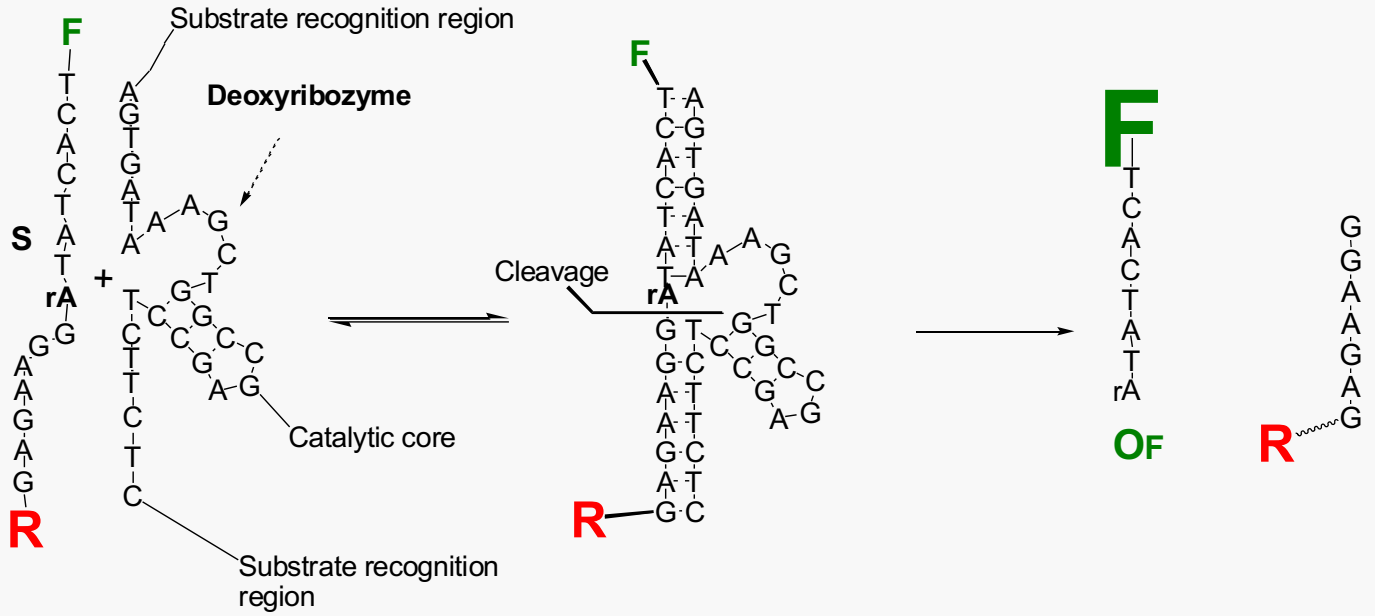


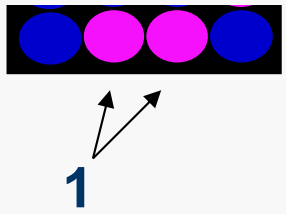


Stojanovic & Stefanovic, *J. Am. Chem. Soc.* ***2003***

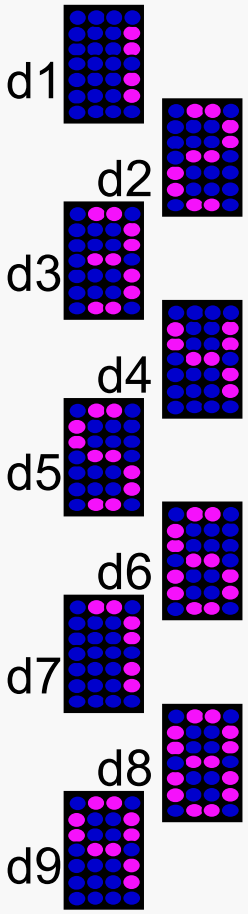
Now, a twist:

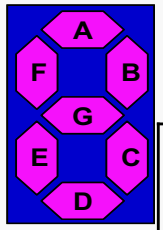
1 is an increase in fluorescence, 0 no such increase!





Example: 7-segment display:





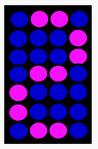
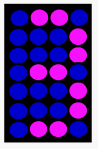
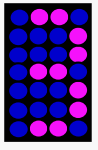
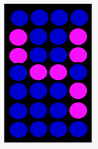
Segments required

	A	B	C	D	E	F	G
1	✗	✓	✓	✗	✗	✗	✗
2	✓	✓	✗	✓	✓	✗	✓
3	✓	✓	✓	✓	✗	✗	✓
4	✗	✓	✓	✗	✗	✓	✓
5	✓	✗	✓	✓	✗	✓	✓
6	✓	✗	✓	✓	✓	✓	✓
7	✓	✓	✓	✗	✗	✗	✗
8	✓	✓	✓	✓	✓	✓	✓
9	✓	✓	✓	✗	✗	✓	✓

More arithmetical operations:

Test by constructing simple **2+2-bit adder**

Designate 4 oligonucleotides as binary inputs

	Binary inputs A_1 A_0 + X_1 X_0	Non general Display output
1+1:	0 1 + 0 1 =	
1+2:	0 1 + 1 0 =	
2+1:	1 0 + 0 1 =	
2+2:	1 0 + 1 0 =	

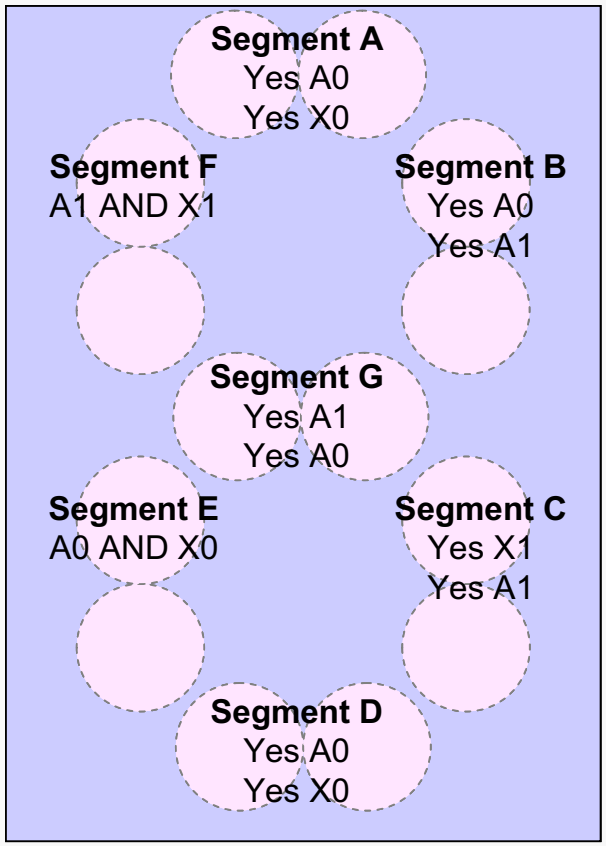
Expected display

Adder result "hard-wired" into decoder

Adding 2+2 with visual display:

- Determine logic gates required, and layout in wells (via Microsoft Excel)

	Binary inputs A_1	A_0	+	X_1	X_0	Non general Display output
1+1:	0	1	+	0	1	=
1+2:	0	1	+	1	0	=
2+1:	1	0	+	0	1	=
2+2:	1	0	+	1	0	=



Very simple logic gate arrangement required:

- 4 YES gates
- 2 AND gates

Fully constructible from reagents in freezer

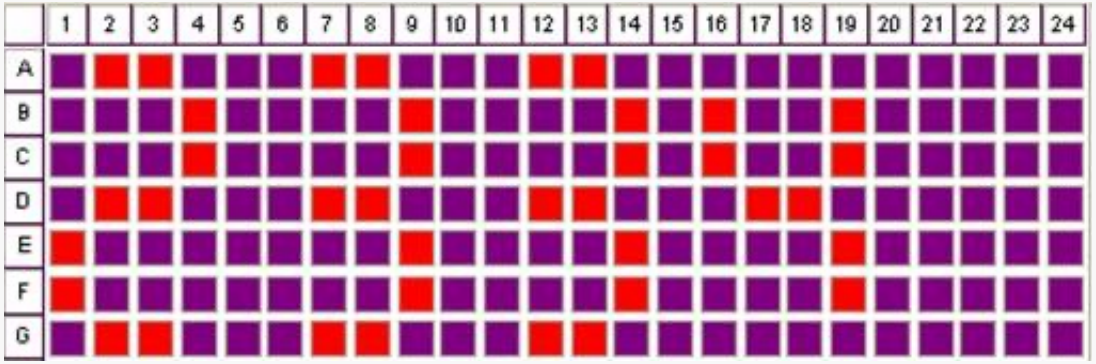
Tested with all input combinations….

Adding 2+2 with visual display:

$(A_1A_0 + X_1X_0 \Rightarrow \text{display})$

Binary inputs: **01+01=** **01+10=** **10+01=** **10+10=** ***No inputs***

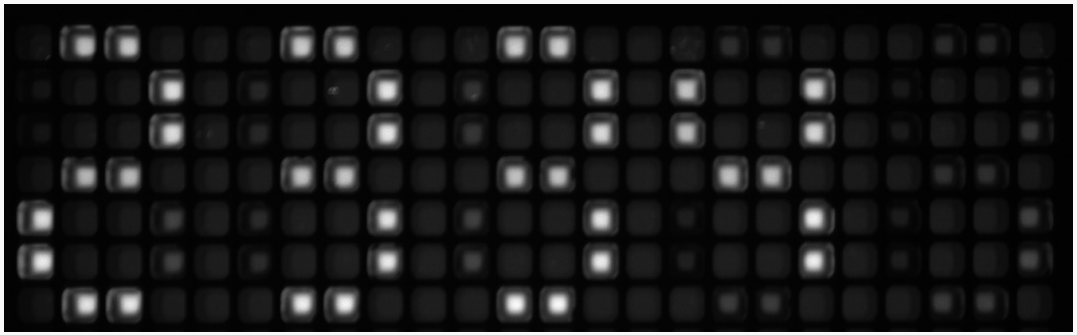
Constructed & tested in a single day



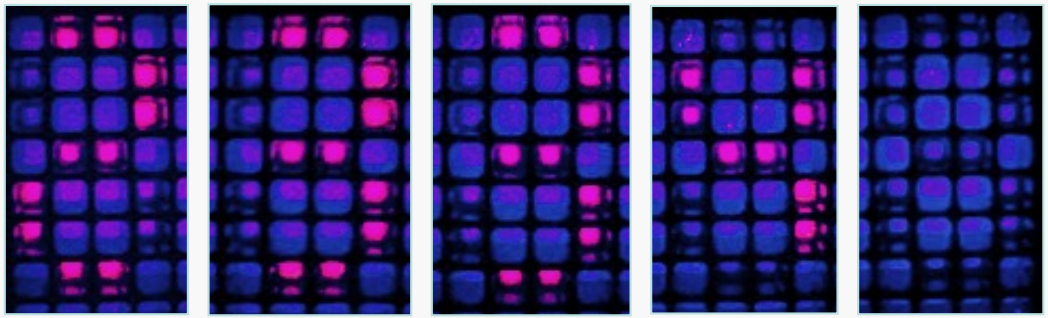
Perfect digital behavior

Fluorescence reader ~20-30mins

7-segment Display



B&W imager within ~1hr



Color photograph ~5hrs

Arithmetic ***(1+1=2)*** ***(1+2=3)*** ***(2+1=3)*** ***(2+2=4)*** ***Background***

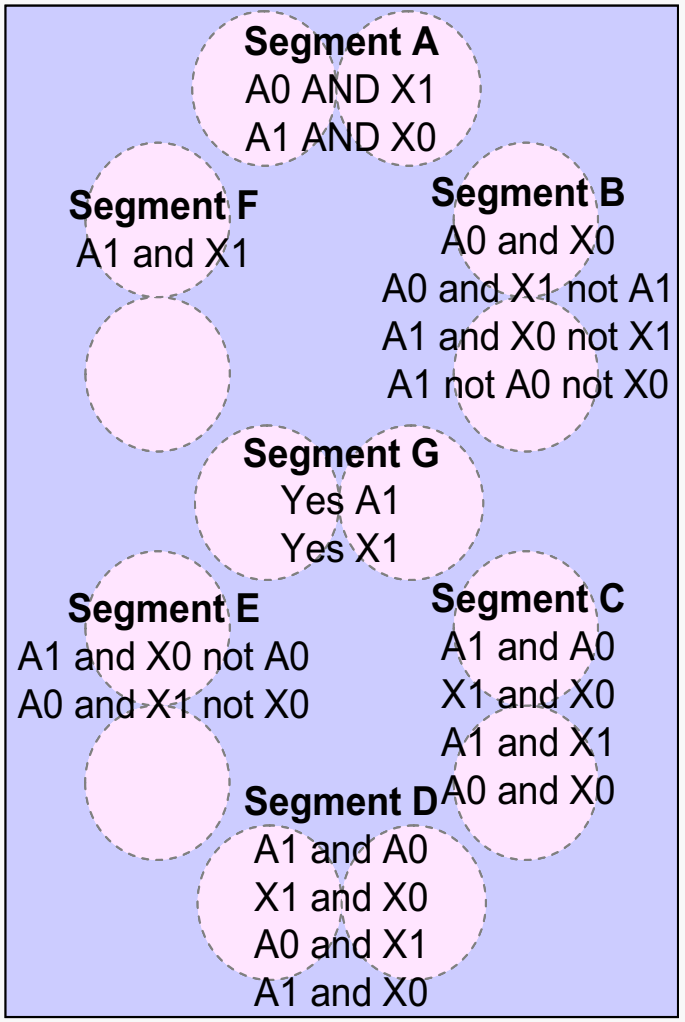
J. Macdonald, Columbia University; DNA 13, 2007

2x2 multiplier with visual display:

Gate arrangement

19 gates required

$(A_1A_0 \times X_1X_0 \Rightarrow \text{display})$



2x2 multiplier with visual display:

Color photography

($A_1A_0 \times X_1X_0 \Rightarrow$ display)

Binary inputs: 01 × 01 = 01 × 10 = 01 × 11 = 10 × 01 = 10 × 10 =

7-segment Display

Arithmetic *(1 × 1=1)* *(1 × 2=2)* *(1 × 3=3)* *(2 × 1=2)* *(2 × 2=4)*

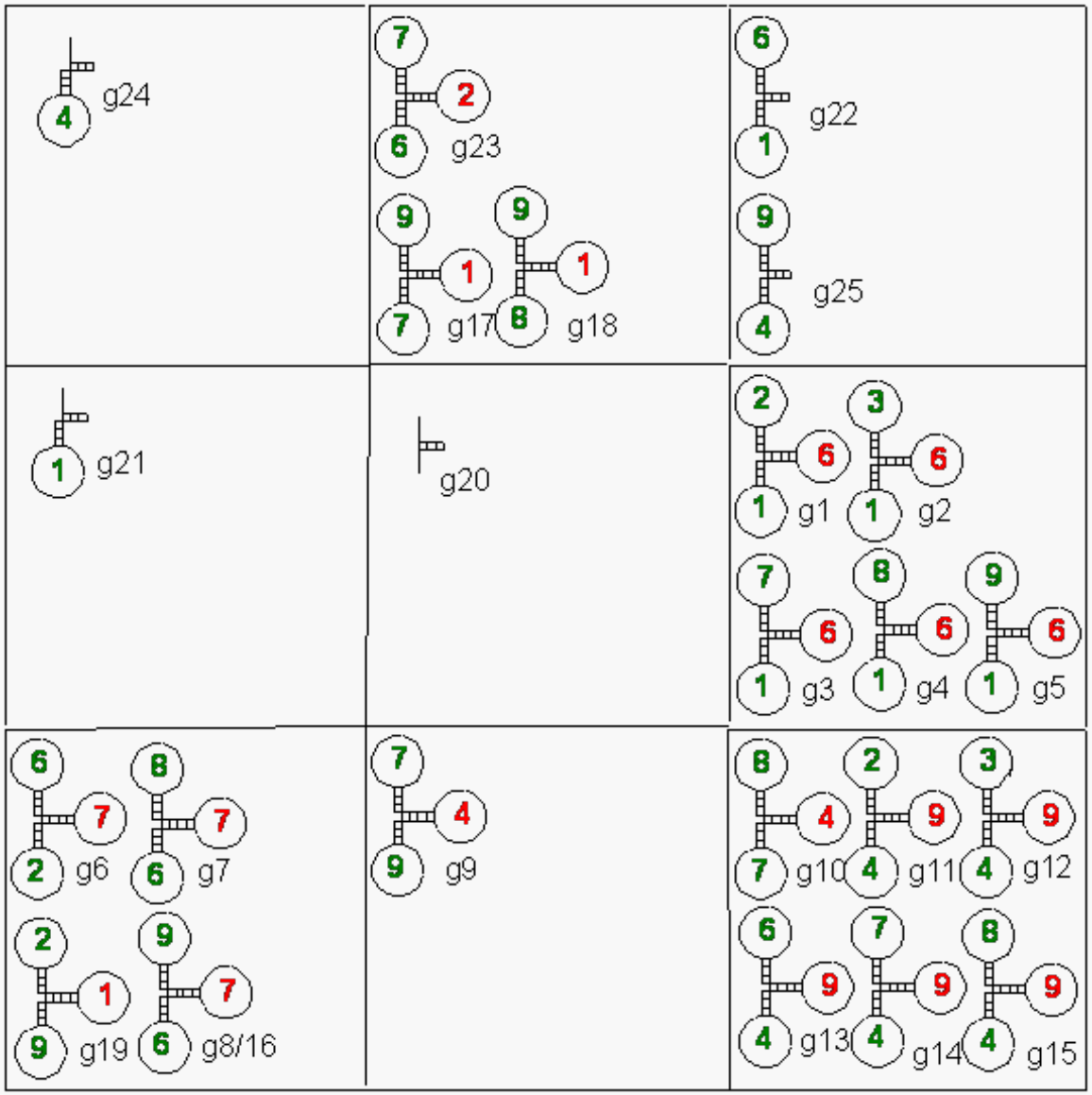
Binary inputs: 10 × 11 = 11 × 01 = 11 × 10 = 11 × 11 = (00 × 00)

7-segment Display

Arithmetic *(2 × 3=6)* *(3 × 1=3)* *(3 × 2=6)* *(3 × 3=9)* *no input control*

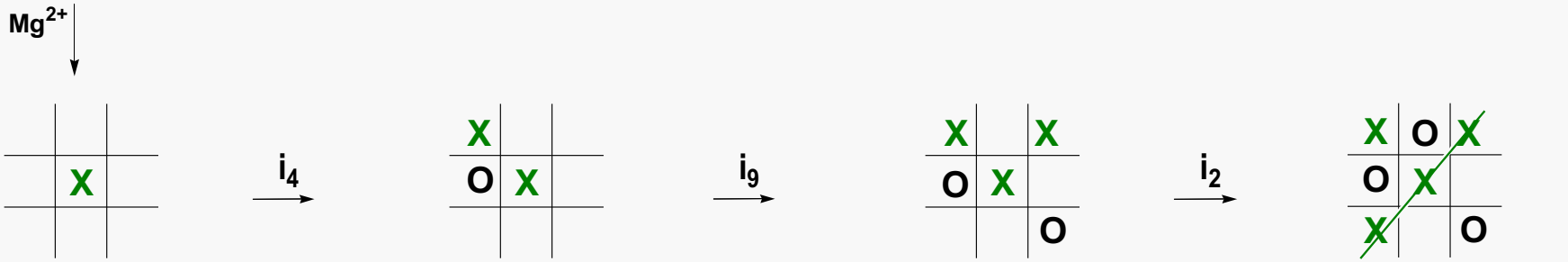
J. Macdonald, Columbia University

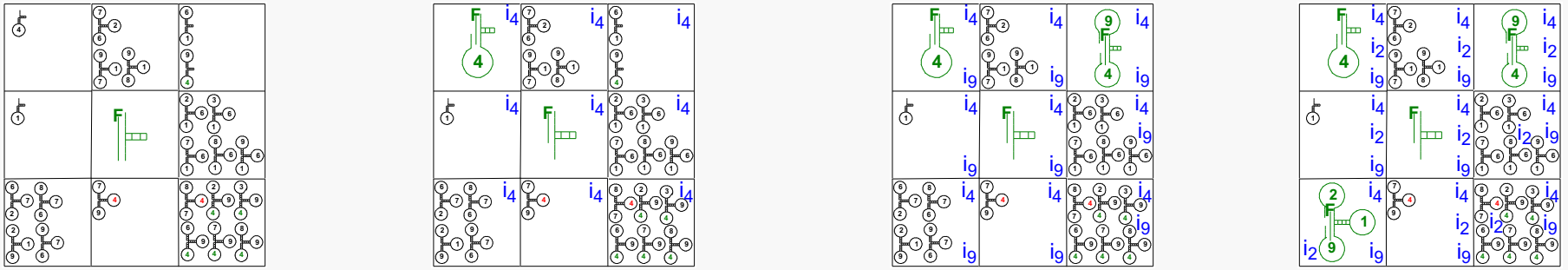
Implementation of tic-tac-toe playing algorithm with deoxiribozymes:
Molecular Array of YES and ANDANDNOT Gates (MAYA)



Stojanovic, Stefanovic, *Nat. Biotech.* **2003**

MAYA *vs.* Milan: Losing Game





MAYA *vs.* Milan: losing game

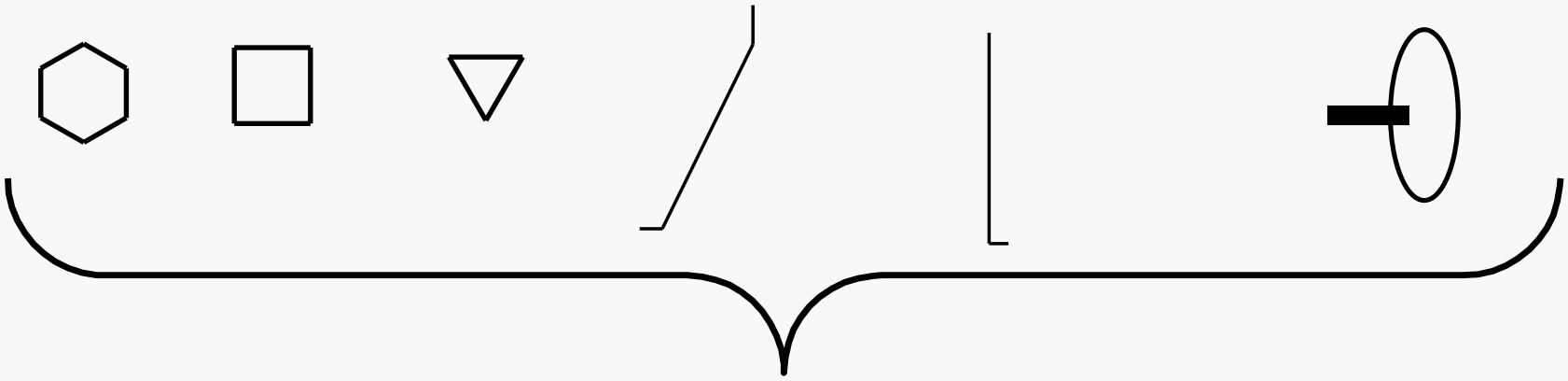
From Sci. Am. 2008

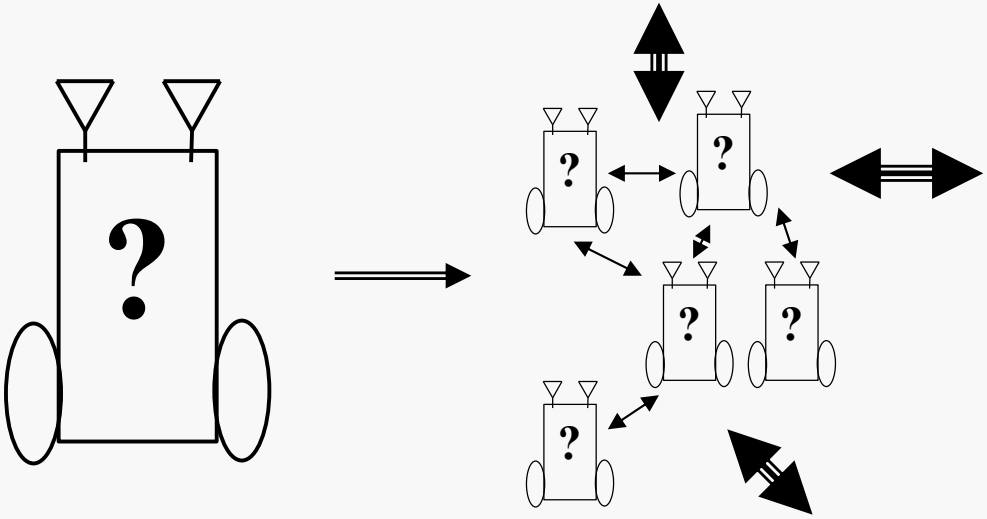
Suppose we have a set of primitives:

Sensor primitives:

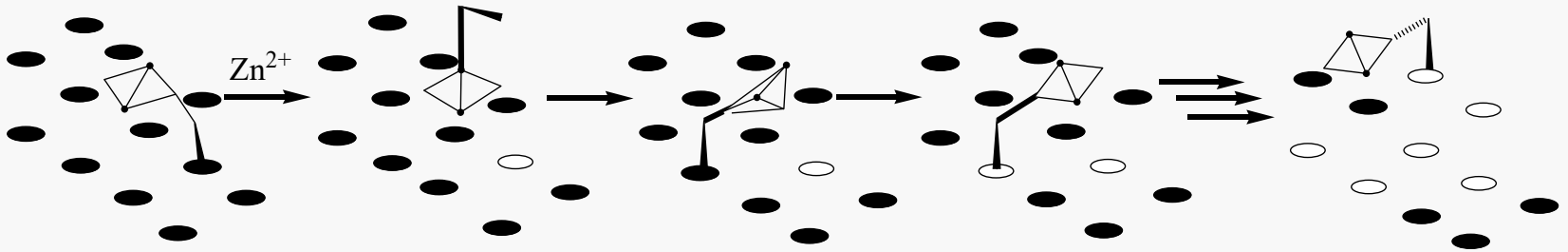
Computing primitives:

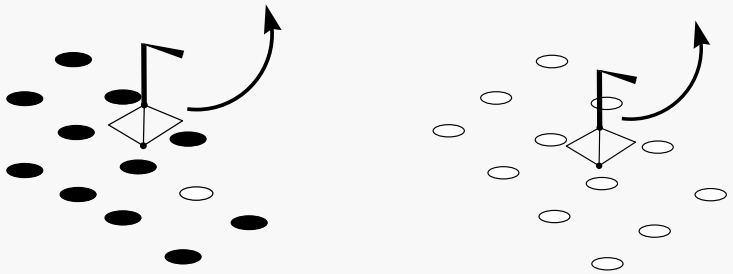
Moving primitives:



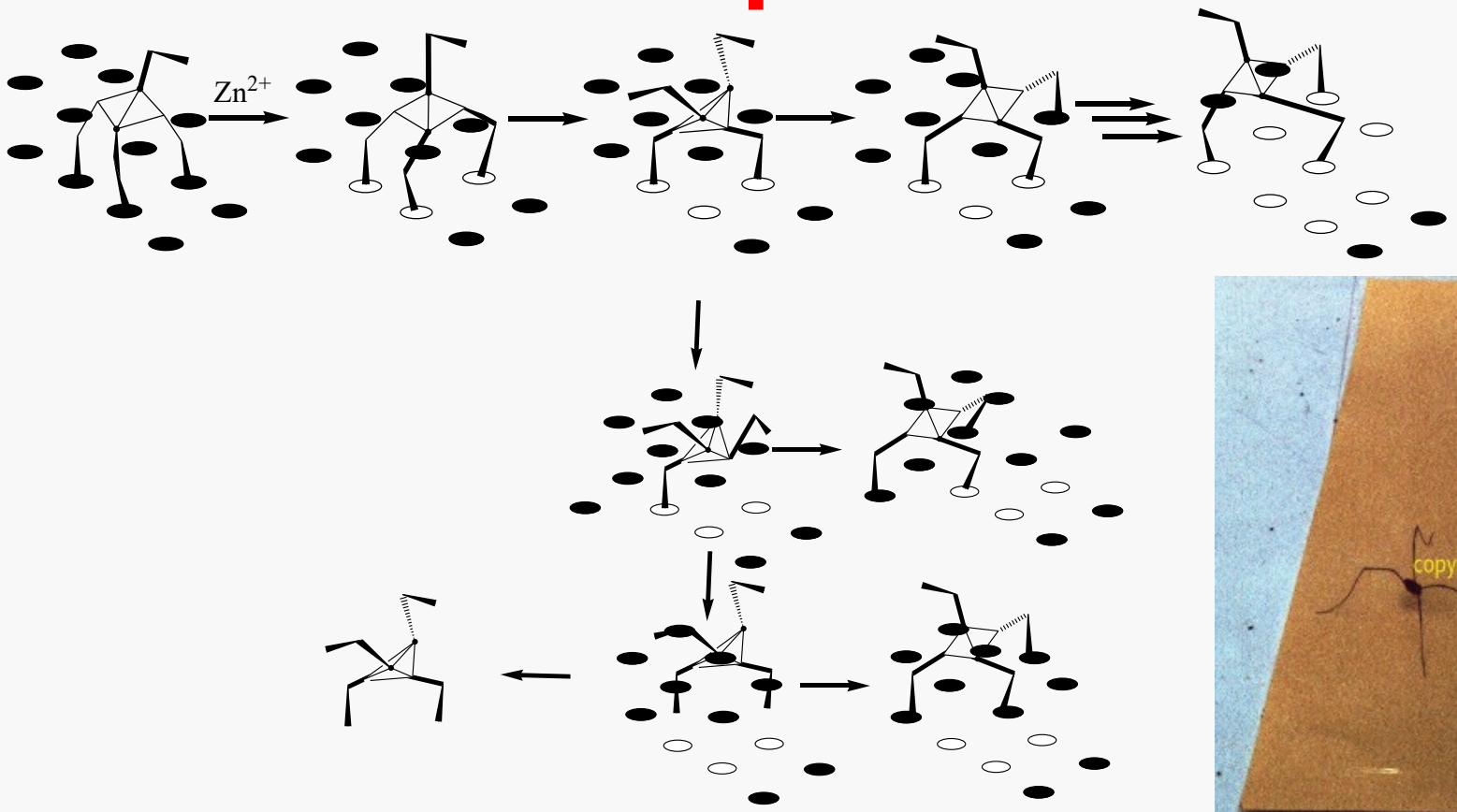


The Simplest Moving Primitive:



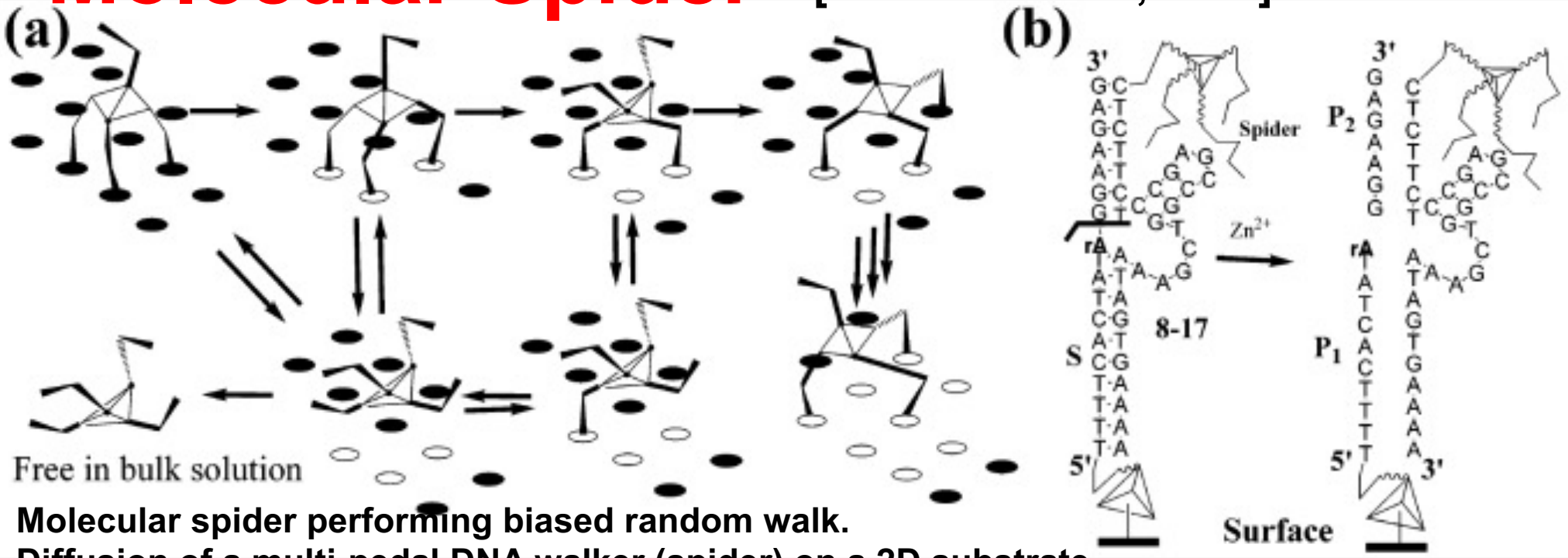


But, what about multivalent design? => Molecular Spider



[Pei et. al *JACS*, 2006]
R Pei, S Taylor, D Stefanovic, S Rudchenko, T Mitchell, M Stojanovic,
Behavior of Poly- catalytic Assemblies in a Substrate-displaying Matrix,
Journal of the American Chemical Society, vol. 128, no. 39, pp. 12693-12699, 2006.

Molecular Spider [Pei et. al *JACS*, 2006]



Molecular spider performing biased random walk.
Diffusion of a multi-pedal DNA walker (spider) on a 2D substrate.

The legs form duplexes - double stranded links - to the complementary strands in the surface.

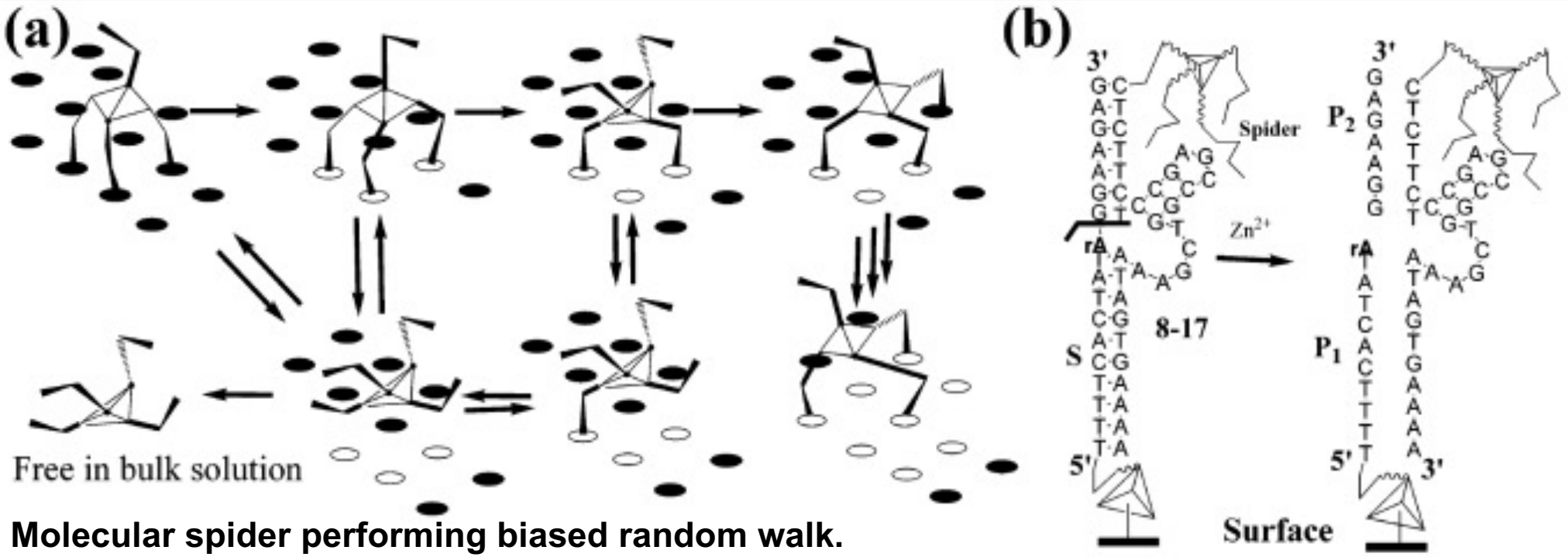
One of the duplexes is cleaved and the leg explores the surrounding surface forming another duplex at another position.

If the leg binds to a position already visited, its stay there is brief as it 'remembers' the process; for new positions, the cleavage takes longer.

By repeating this process the walker moves along the surface independently.

At the end of the track the walker binds to uncleavable DNA strands and stops.

Molecular Spider [Pei et. al *JACS*, 2006]

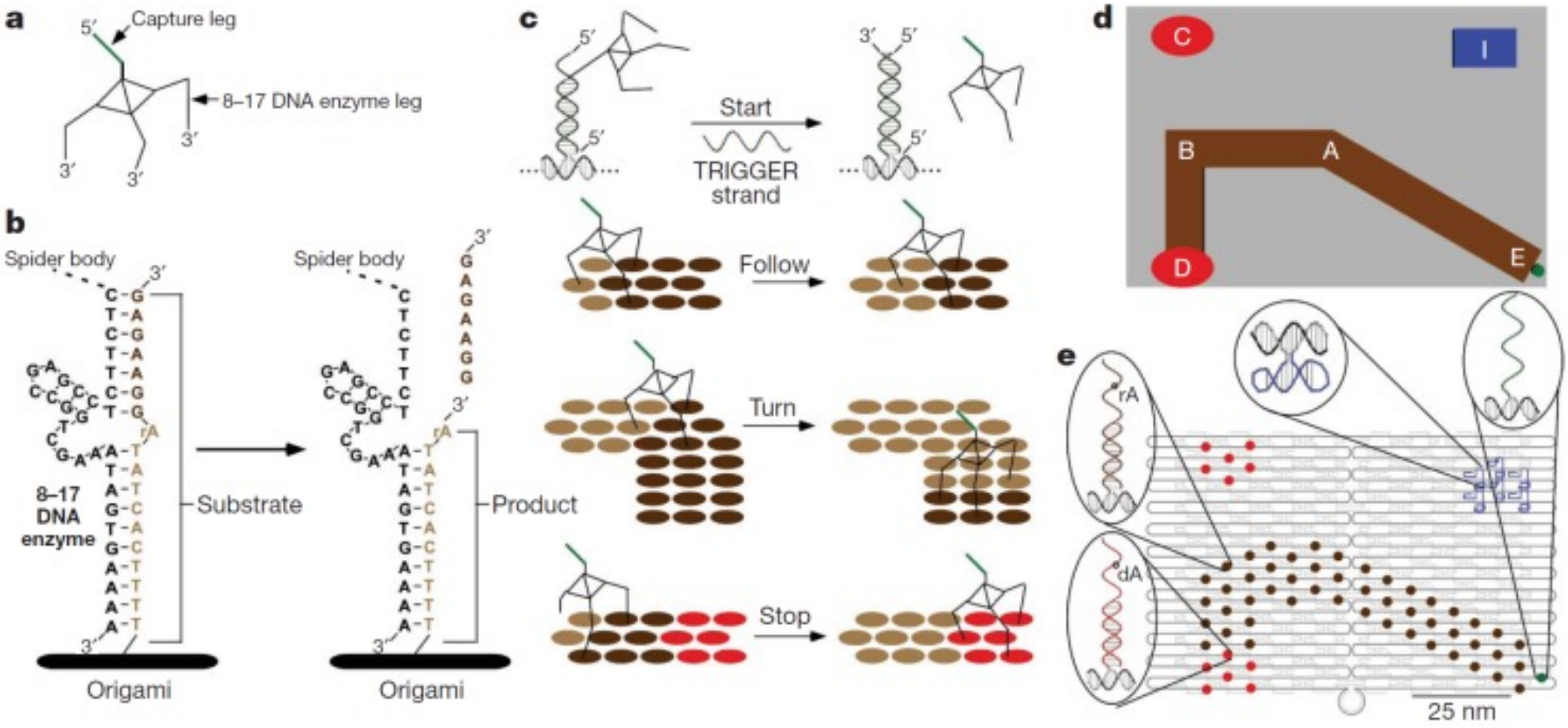


Molecular spider performing biased random walk.
Diffusion of a multi-pedal DNA walker (spider) on a 2D substrate.

- Body of spider is a molecule of streptavidin, and
- The 4 legs are DNAzyme molecules attached to the body.

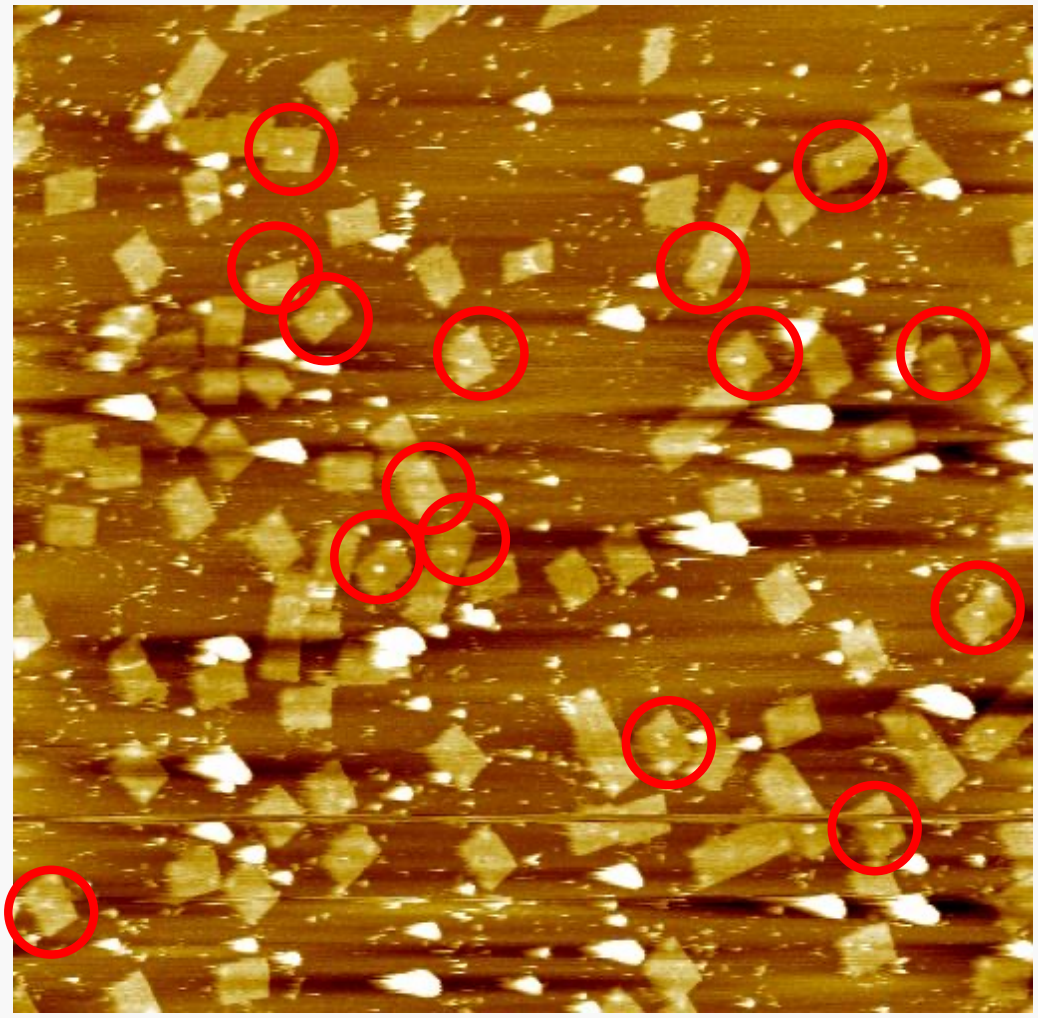
- The spiders crawls a surface by attaching and detaching from RNA substrates via DNAzyme.
- Leg attachment occurs via DNA-RNA hybridization while the detachment is via the catalytic restriction of the RNA stator by the DNAzyme followed by spontaneous denaturation from short strands due to entropic effects.
- Once a leg detaches from a substrate it binds (with high probability) to a new substrate and the process continues.
- The spider is biased towards binding unvisited substrates.

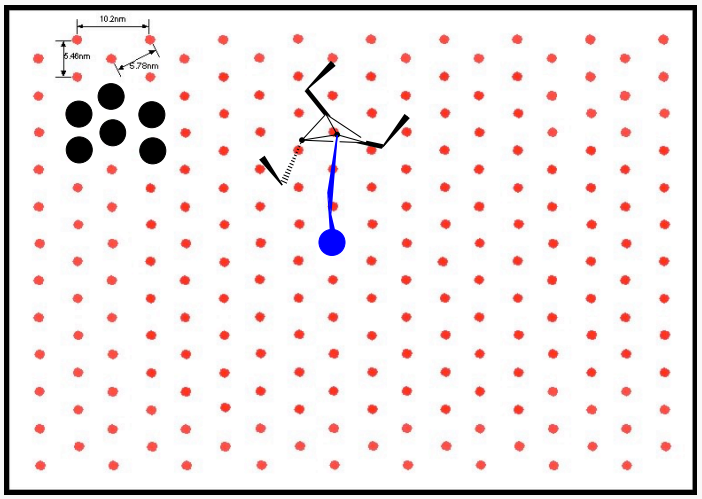
Molecular Spider [Pei et. al *JACS*, 2006]

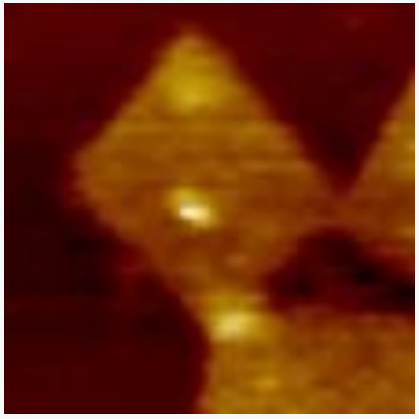


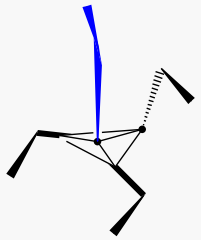
Molecular spider performing biased random walk.
Diffusion of a multi-pedal DNA walker (spider) on a 2D substrate.

AFM Images of Spider at Starting Point



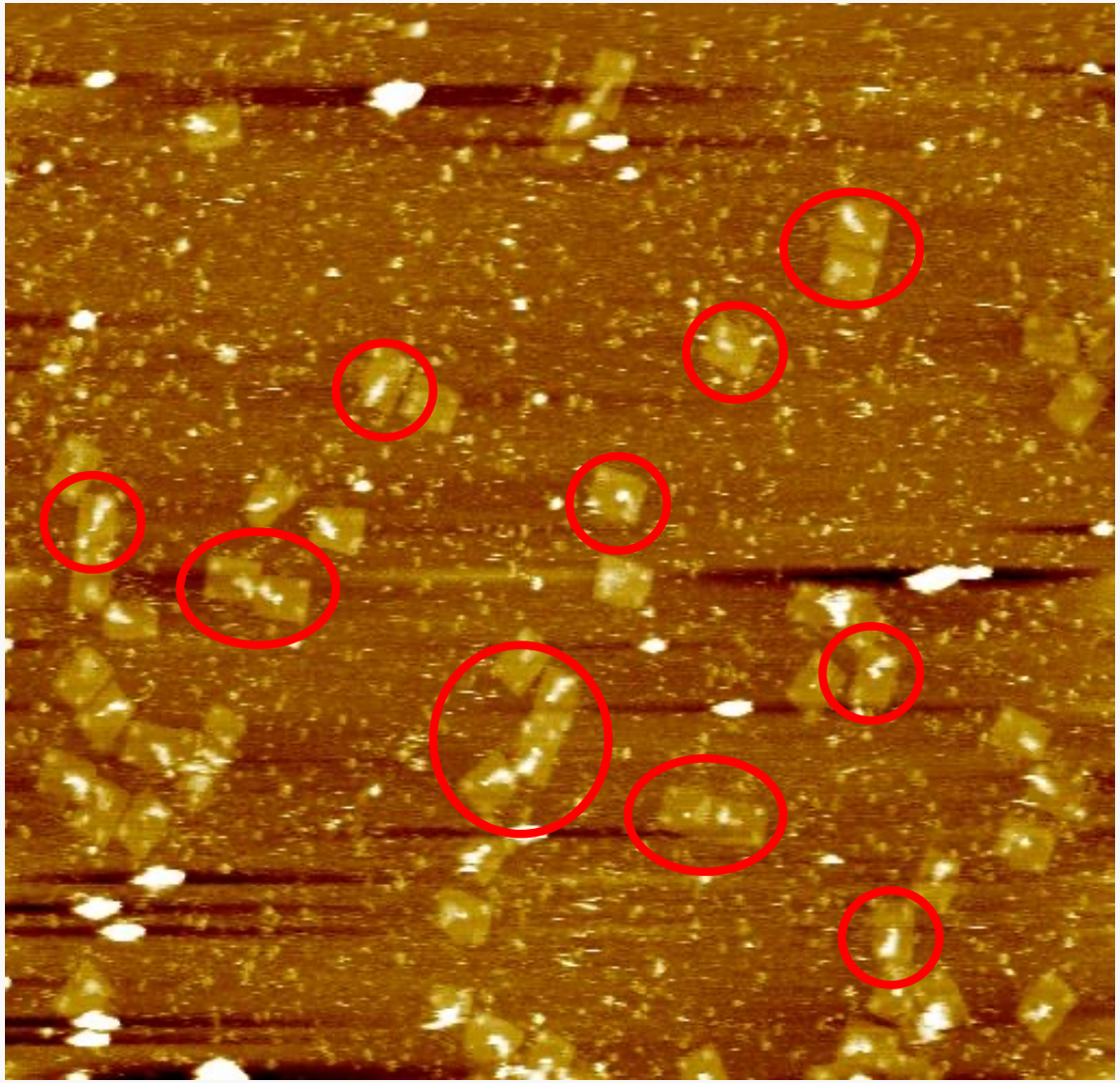


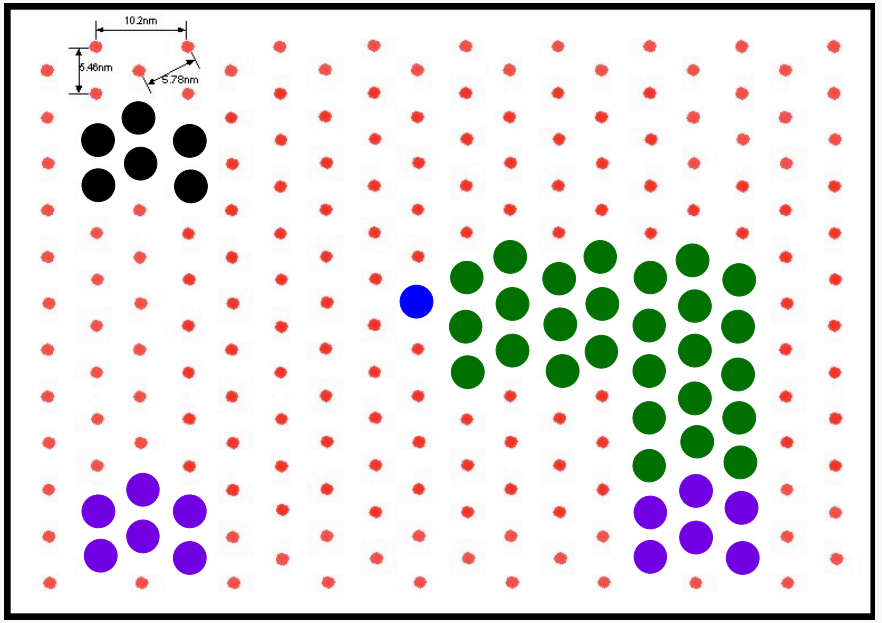


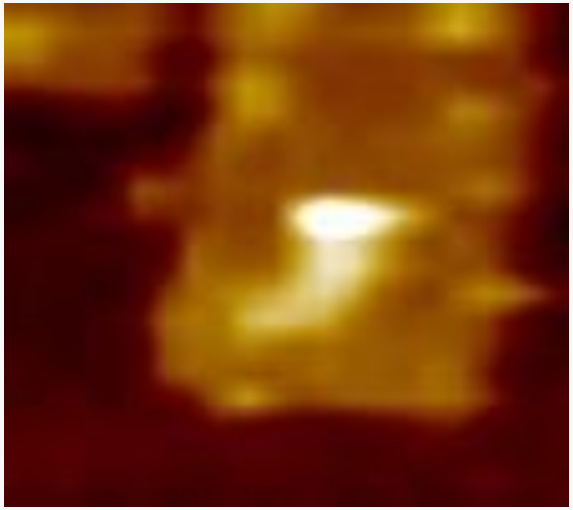


Kyle Lund, Hao Yan, video now: with Nadine Dabby, Erik Winfree

AFM images of Spider on Lanes of Substrates



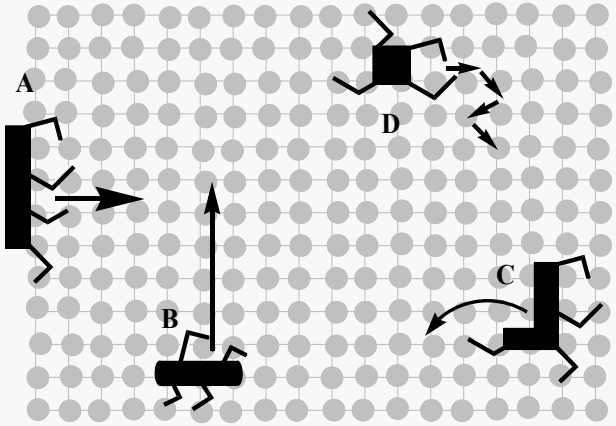


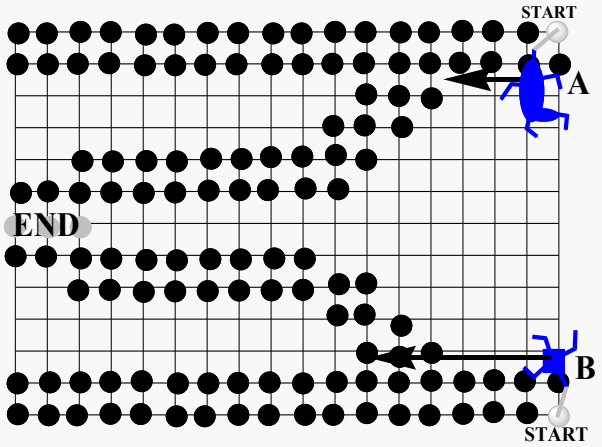


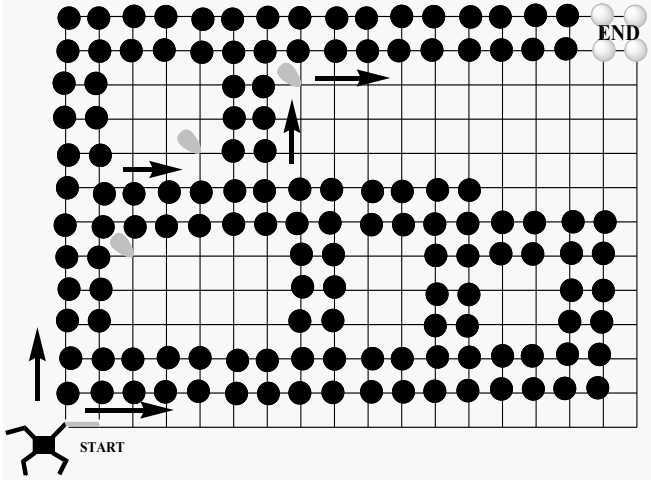
Kyle Lund, Hao Yan

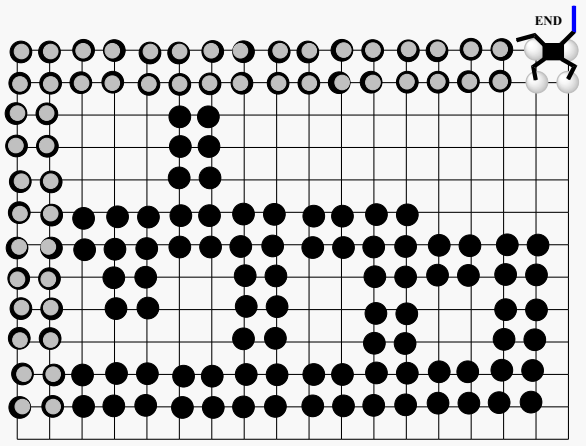
Video with: Nadine Dabby, Erik Winfree

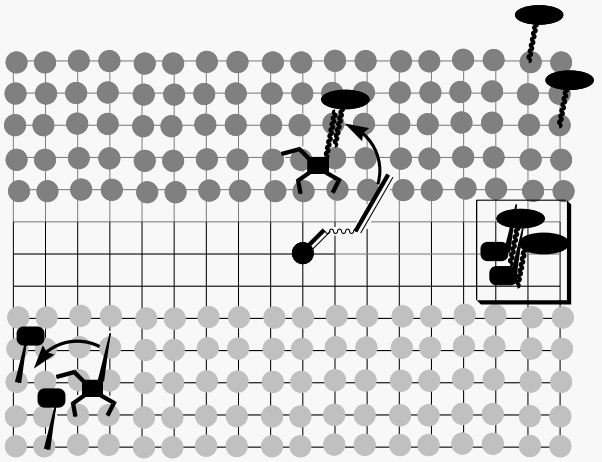
Programming Spider Walking Paths





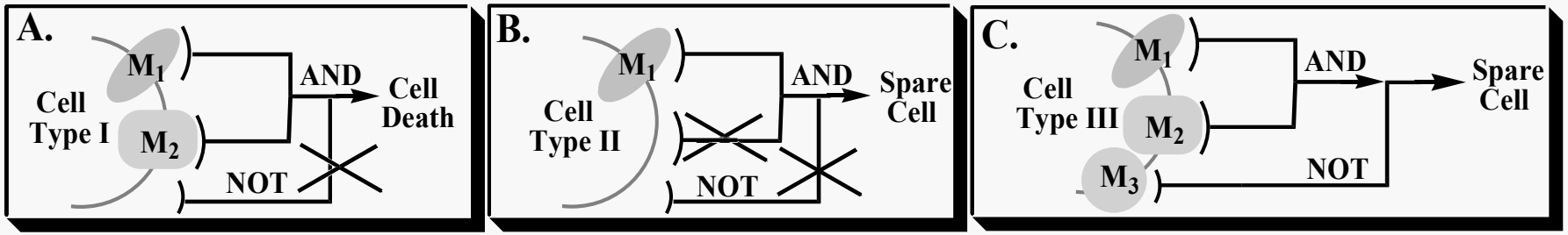






Potential practical applications:

1. Cell-death by Boolean Calculations:



2. Glucose-triggered movement of catalytic nanoassemblies: