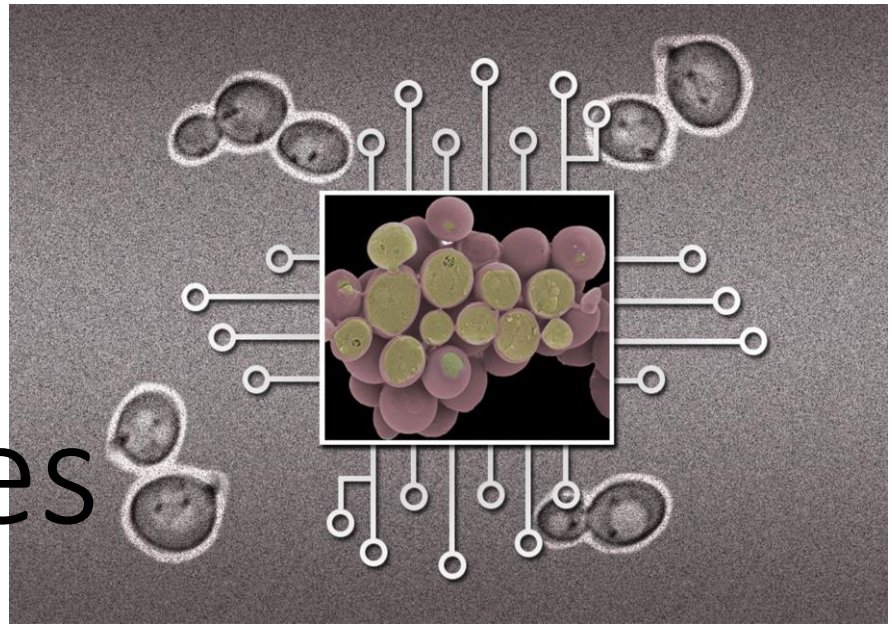
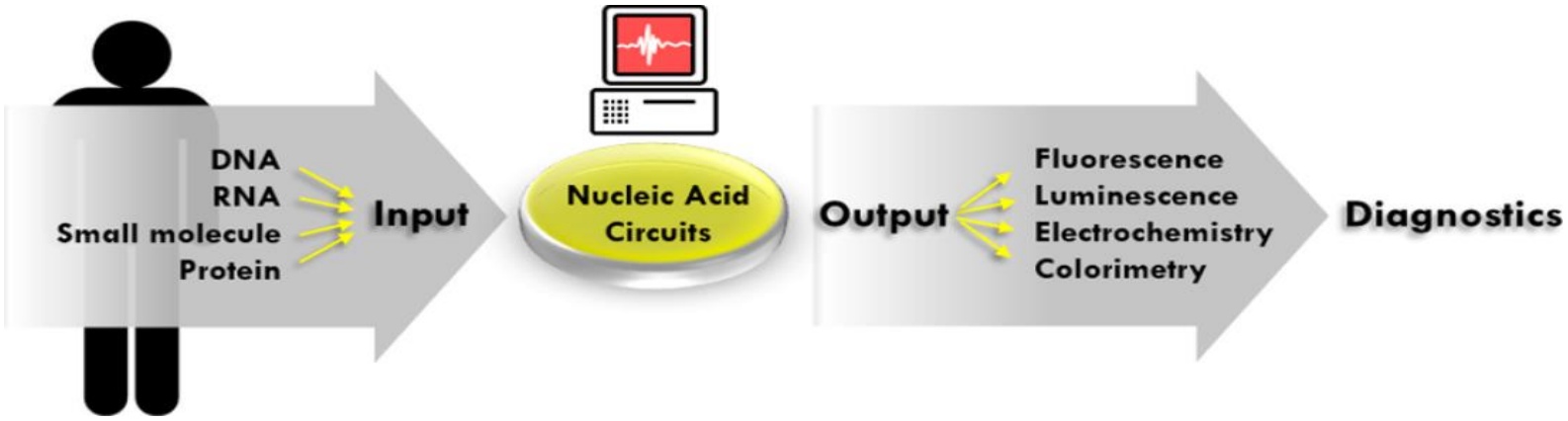


DNA Machines

Biophysics of Systems

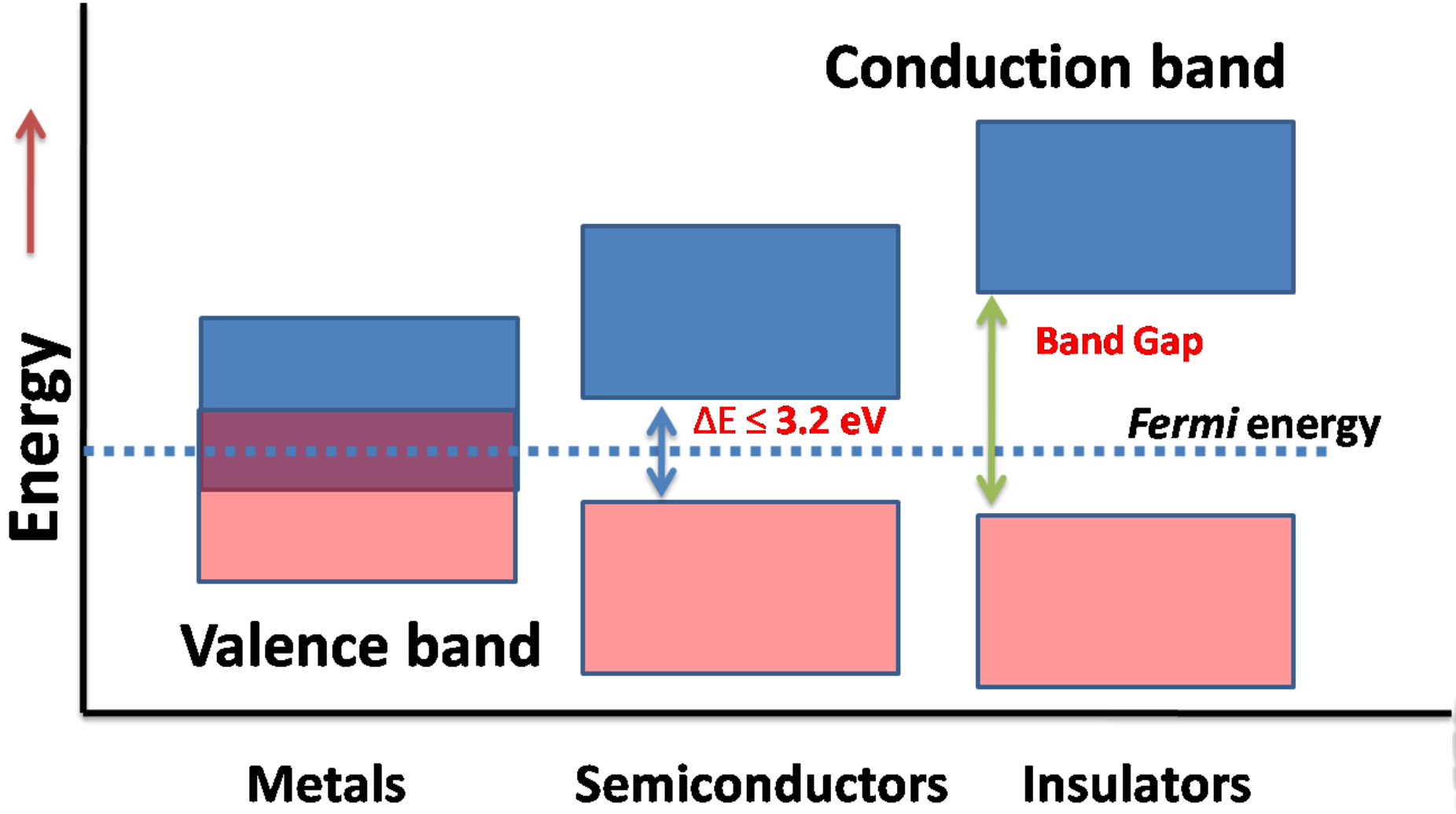
Girnar Goyal, Achim Theo Brinkop

Contents

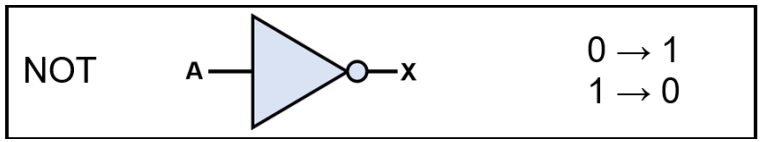


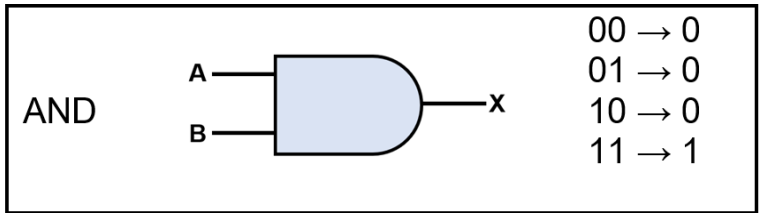
Jung, C., & Ellington, A. D. (2014)

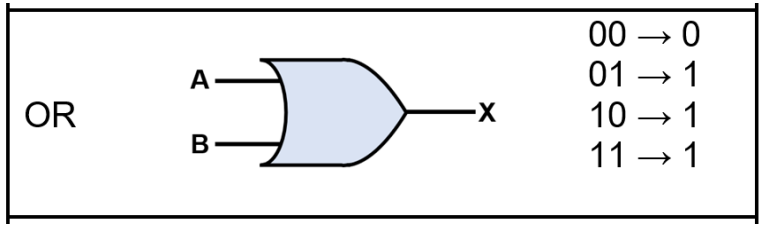
The Story

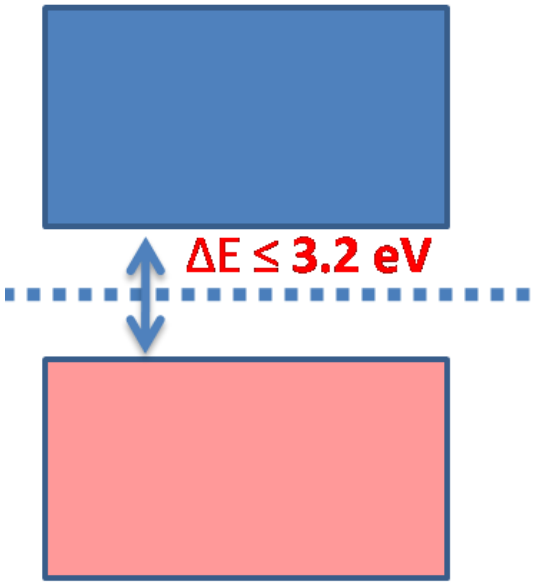

Conduction band
Energy
Band Gap
ΔE ≤ 3.2 eV
Fermi energy
Valence band
Metals
Semiconductors
Insulators

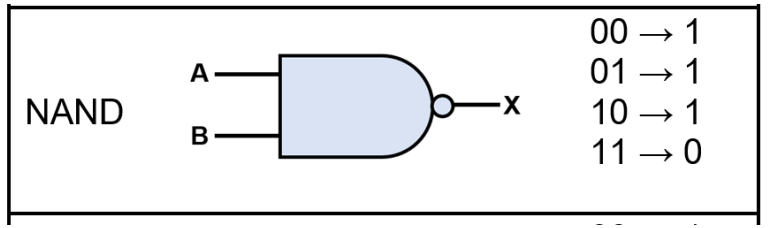
The Story

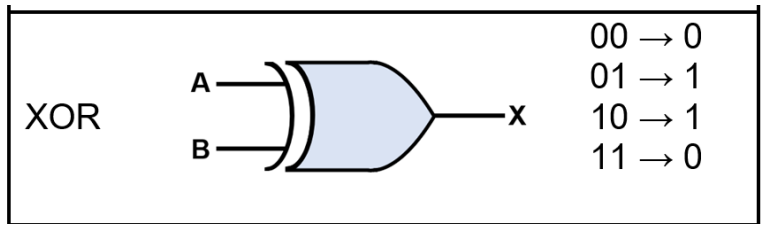

NOT
A
X
0 → 1
1 → 0


AND
A
B
X
00 → 0
01 → 0
10 → 0
11 → 1

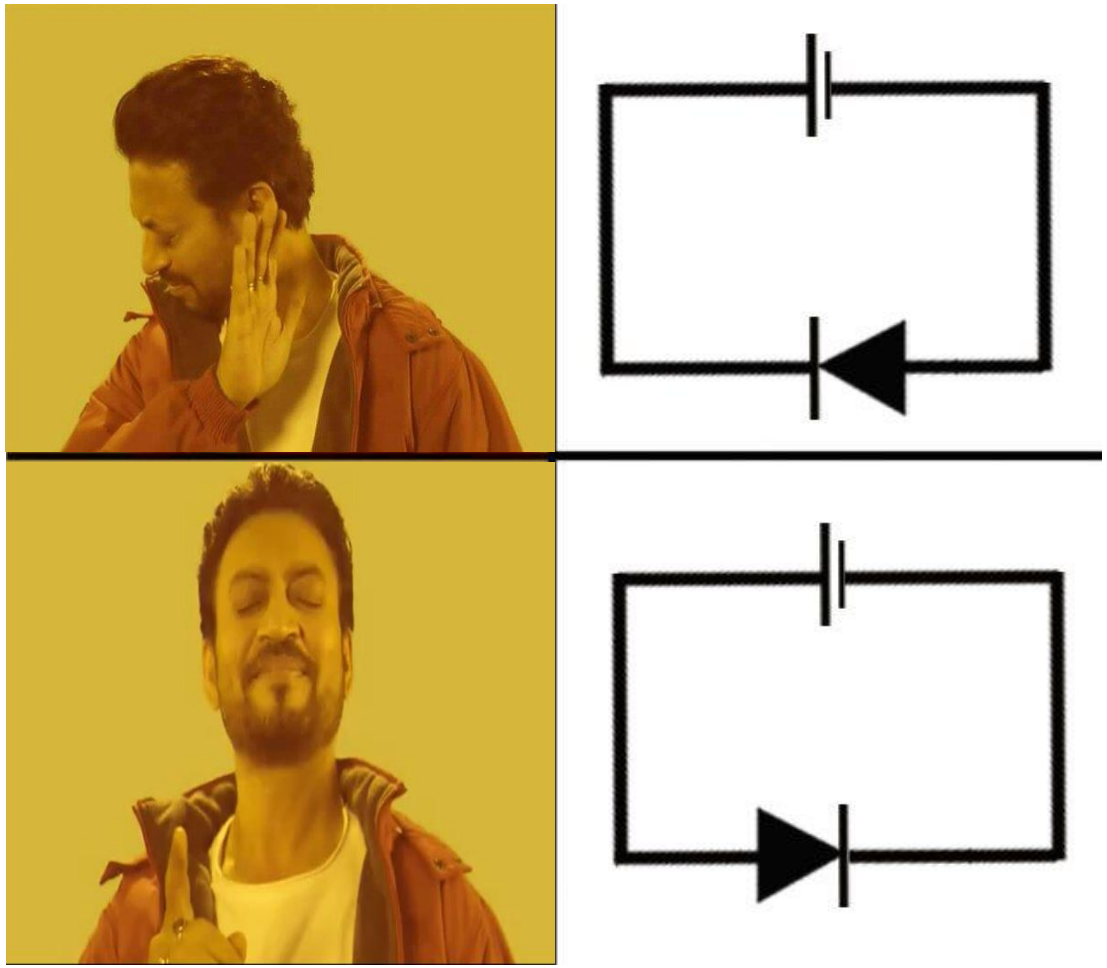

OR
A
B
X
00 → 0
01 → 1
10 → 1
11 → 1

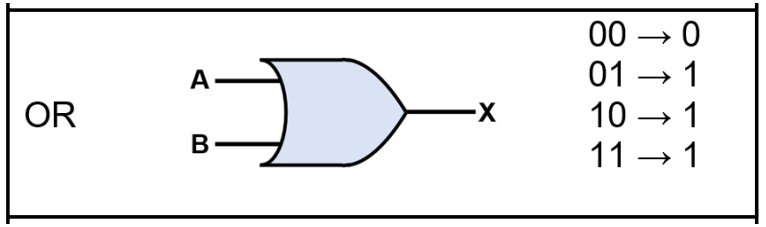

ΔE ≤ 3.2 eV
Semiconductors


NAND
A
B
X
00 → 1
01 → 1
10 → 1
11 → 0

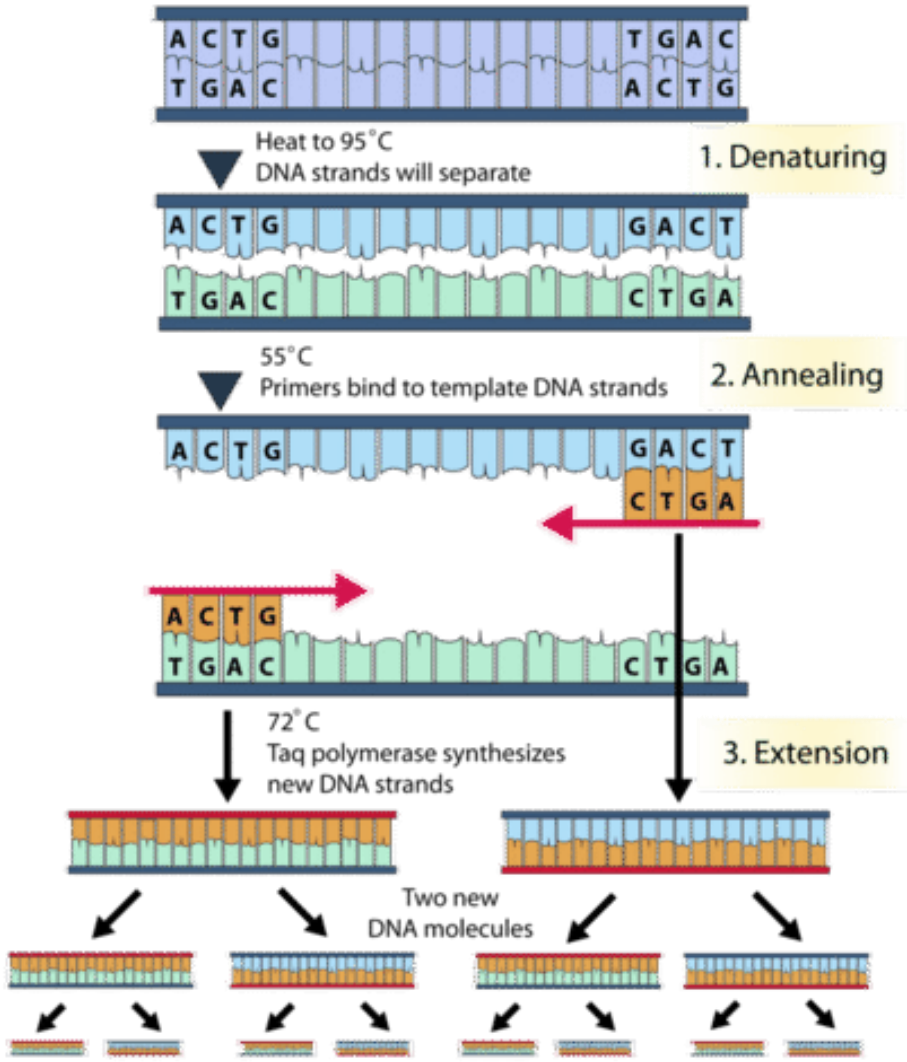

XOR
A
B
X
00 → 0
01 → 1
10 → 1
11 → 0

The Story





PCR Technique



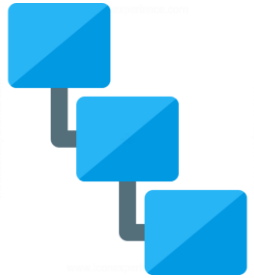
- used to amplify DNA sequences.
- The technique can produce a billion copies of the target sequence in just a few hours.

Nutshell

Specified input oligonucleotide

Watson-Crick base pairing specificity.

Strategic Design Principles



Signal cascading between gates

Modular Biochemical Circuits

Specified output oligonucleotide

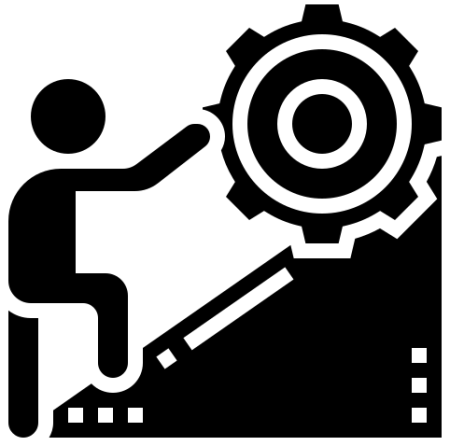
Hybridization based systems (non-catalytic gates)

DNA catalyzed Entropy Driven systems (catalytic gates)

Zhang et. al., Science (2007)

Existing Challenges

- Distinct chemical species for distinct signals.
- Modular Gate Designs.
- Robust and fast Catalytic Mechanism (Signal gain).
- Need for arbitrary complexity in biochemical circuits.
- Undesired leaks and crosstalks.



Zhang et. al., Science (2007)

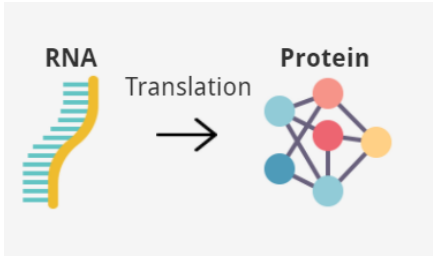
RNA World

- Chicken-egg Problem (Protein-DNA).
- RNA enzymes (ribozymes) can catalyze chemical reactions.
- Replication with specificity. (A-U, G-C)
- Ability to form stable duplexes.
- Complex structures of tRNA => RNAs as catalysts.
- Suggests that sophisticated biochemical organization can be achieved with nucleic acids alone.

Doudna et. al., Nature, 1989
Narlikar and Hershlag, 1997

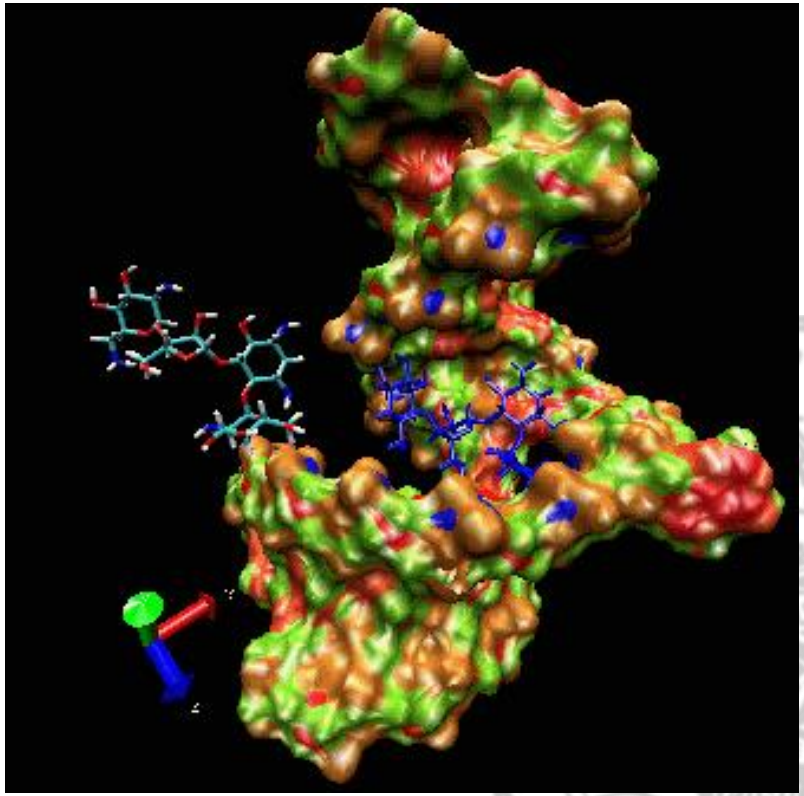
RNA World

What might have caused proteins to ultimately take over?



- **Favorable folding properties**, relative to RNA.

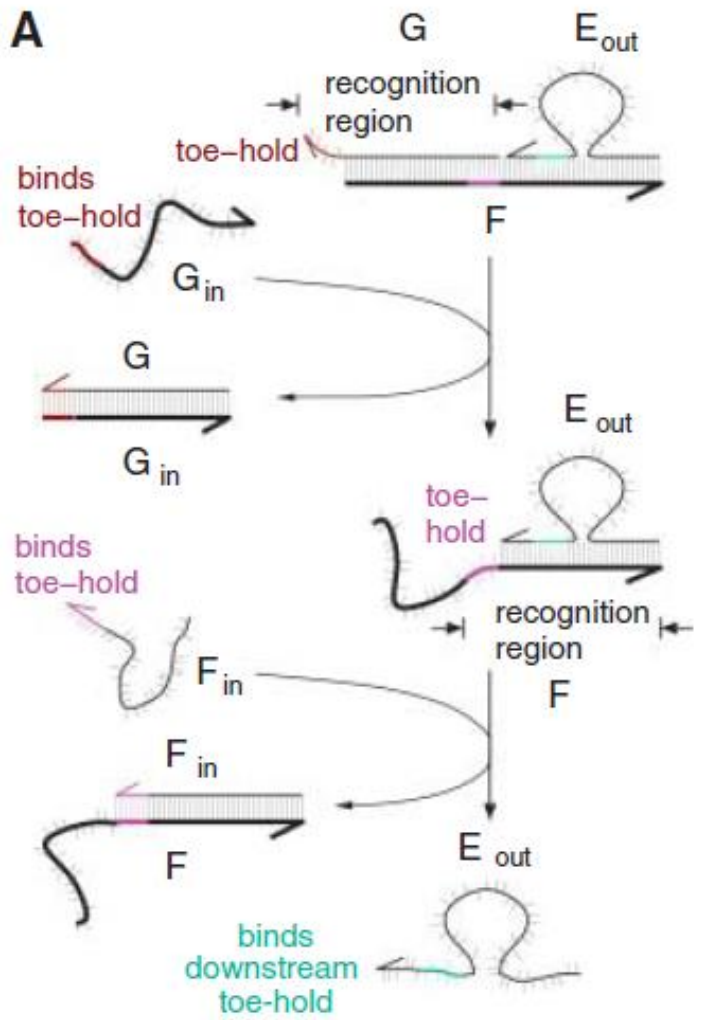
- Large number of more catalytically effective and **diverse side chains of proteins**.
- **Speed:** Rate constant of ligand binding for RNA << Rate constant of ligand binding for protein limit ~ diffusional limit.



Doudna et. al., Nature, 1989
Narlikar and Hershlag, 1997

Hybridization based systems (non-catalytic)

- Single stranded nucleic acids as I/O.
- Mechanism relies on sequence recognition and strand displacement.
- AND, OR, NOT gates.
- Signal amplification is a bottleneck.
- Indicates need for catalytic systems.

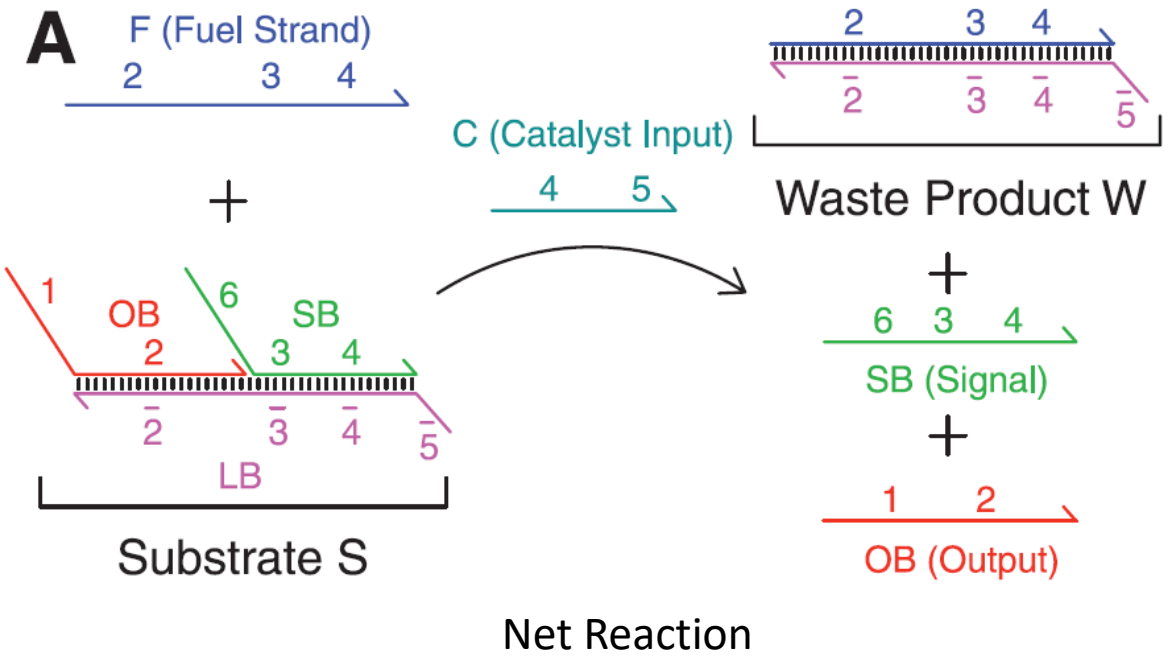


	REACTANTS	PRODUCTS
1	EFG, NO INPUTS	SAME AS REACT.
2	EFG + F_{in}	SAME AS REACT.
3	EFG + G_{in}	EF + GG_{in}
4	EFG + F_{in} + G_{in}	E + FF_{in} + GG_{in}

Seelig et. al., Science (2006)

Entropy driven systems (catalytic)

- Simpler. Faster. Modular.
- Reaction driven by entropic gains.
- AND, OR, NOT gates.
- Output of one gate can serve as input to another.
- C and OB can be entirely independent in sequence => Modularity.
- Doesn't require unusual secondary structures (pseudoknots, kissing loops etc).



Zhang et. al., Science (2007)

Over to Achim

Content

- Proposed catalytic pathway
  - Reaction principles
  - Properties
  - Stability
- Experimental analysis
  - PAGE-analysis of products
  - Fluorescent analysis of the reaction time-course
- Examples of more advanced networks

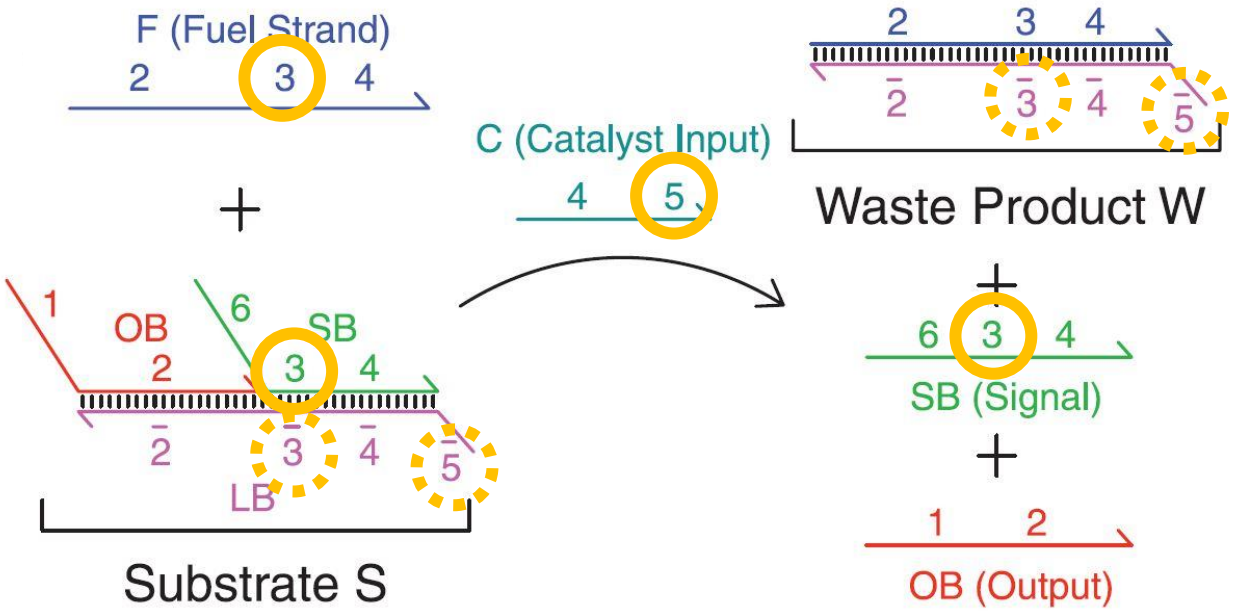
Proposed catalytic pathway

Nomenclature

F: fuel strand SB: signal strand C: catalyst
OB: output strand LB: linker strand

Domains:

- Specific sequence
- Toehold domains: 3, 5
  - Domains where strands bind/unbind
- Specificity domains: 1, 2, 4, 6
  - Determine identities/function of *C*, *OB*, *SB*
  - Prevent wrong binding



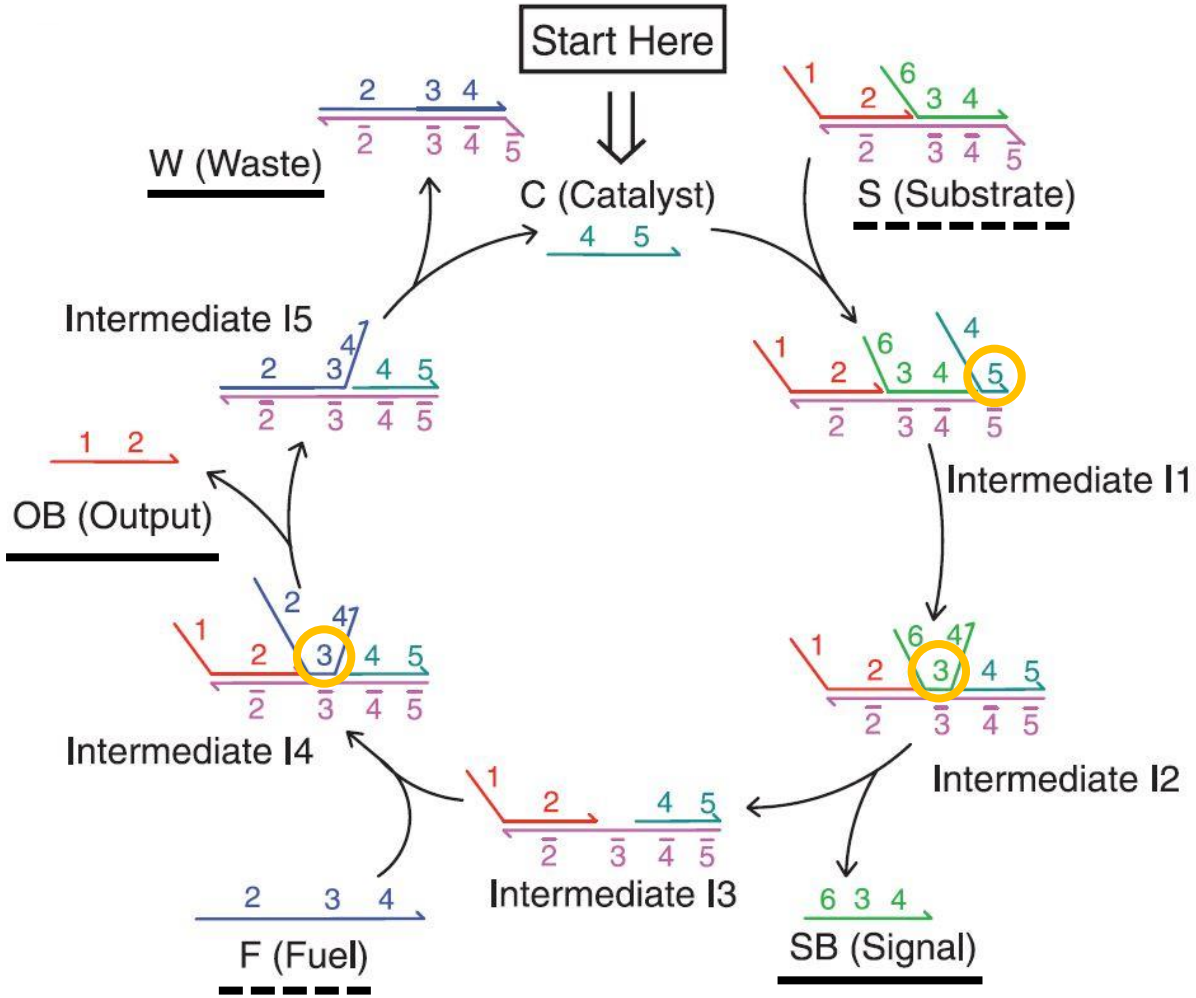
$\bar{n}$: complementary strand to n

Source: Zhang et al., *Science* (2007)

Proposed catalytic pathway

F: fuel strand SB: signal strand C: catalyst
OB: output strand LB: linker strand

- Toehold domains: 3, 5
  - Accelerate the initiation of strand displacement reactions
  - *Length* determines kinetics: Short length (4-10nts) → weak binding
- Specificity domains: 1, 2, 4, 6
  - Ensure specific interactions
  - *Length*: any length sufficient to ensure thermal stability



Source: Zhang et al., *Science* (2007)

Catalytic activity

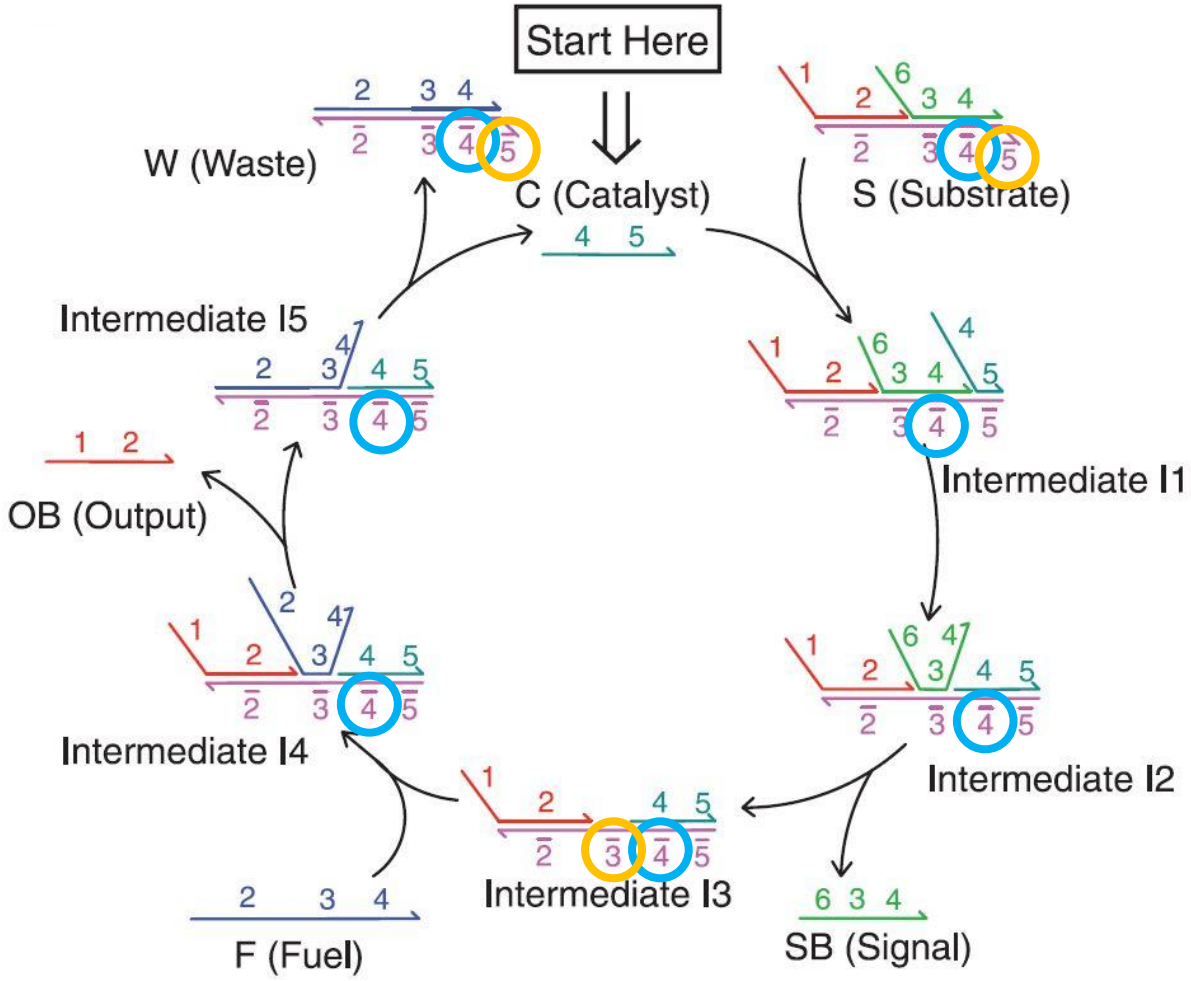
F: fuel strand SB: signal strand C: catalyst
OB: output strand LB: linker strand

Alternative interactions must not interfere:

→Design principle: "toe-hold exchange"

→Complements of specificity domains never appear in their single-stranded form

→Expectation: catalytic gate functions for most choices of domain sequences

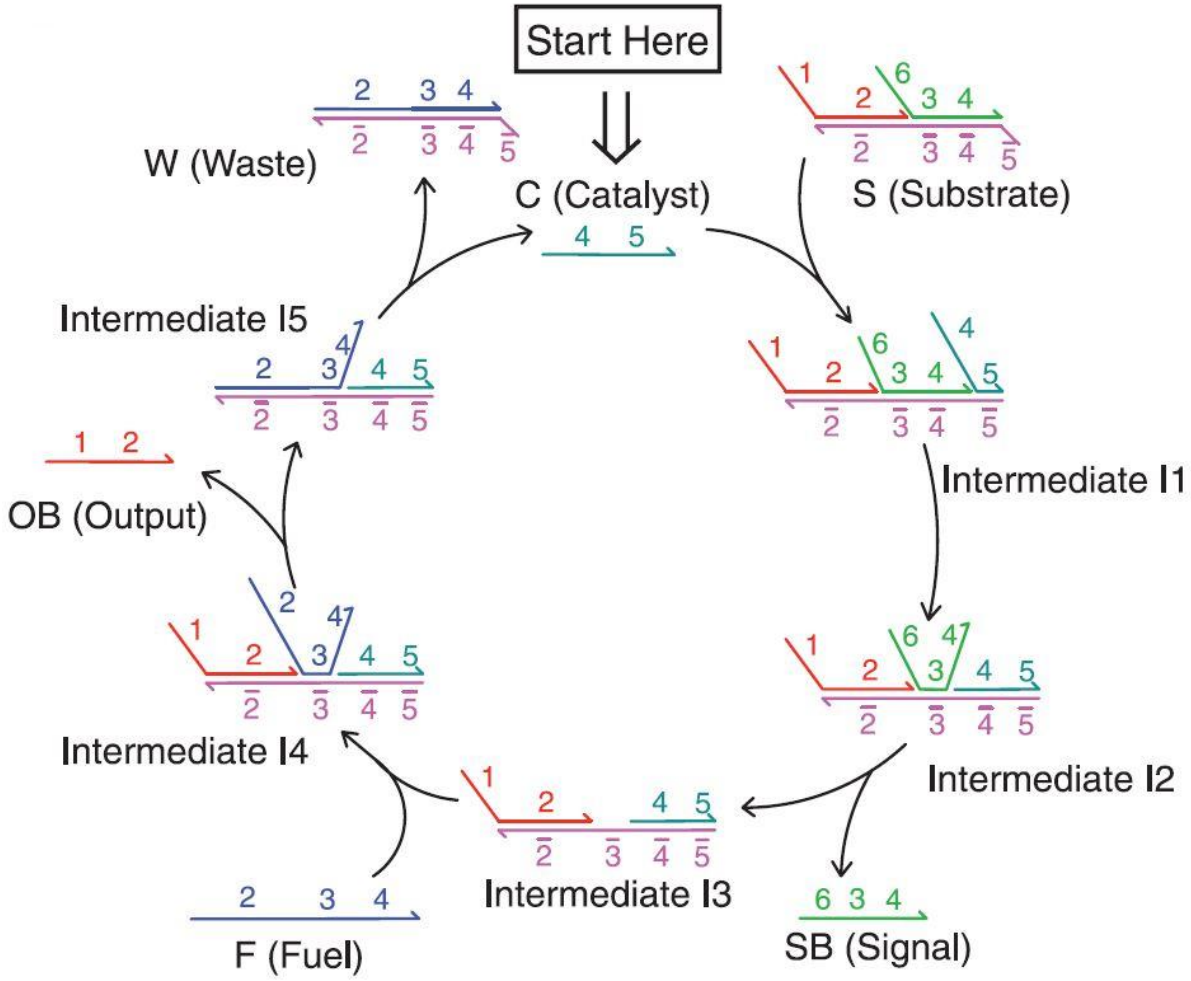


Source: Zhang et al., *Science* (2007)

Properties of catalytic gate

- Speedup of target reaction
  → Detection of *C* possible
- Re-release of *C* to allow for multiple turn-over
- Reaction mechanism:
  - Based on branch migration
  - Driven by entropy
- Different from catalysis in biological organisms:
  - No enzyme is required
  - No covalent bonds are altered

F: fuel strand SB: signal strand C: catalyst
OB: output strand LB: linker strand



Source: Zhang et al., *Science* (2007)

Stability

F: fuel strand SB: signal strand C: catalyst
OB: output strand LB: linker strand

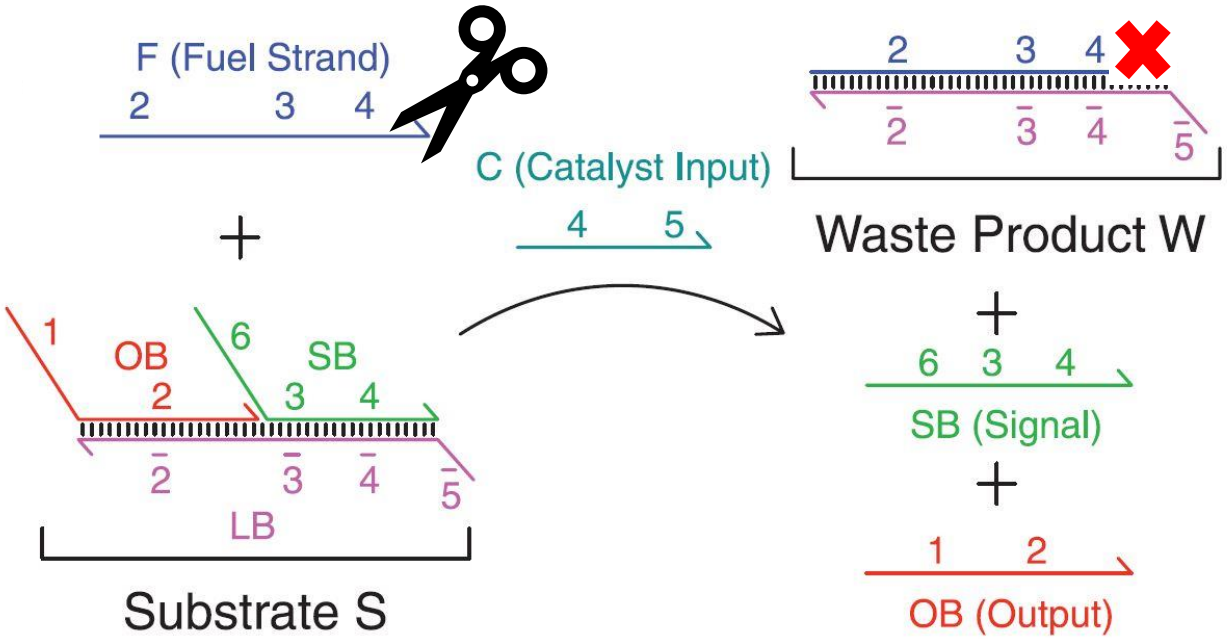
- Each broken base pair is replaced by similar one
  → free energy change small
- **Test:** truncate length of *F*

→ Products energetically less favorable

→ Still, ***W*** favored at equilibrium

→ Reaction driven by entropy

- Robust to environmental conditions that alter strength of DNA hybridization, e.g.
  - Temperature
  - Salt concentration



Source: Zhang et al., *Science* (2007)

Experimental analysis

PAGE analysis

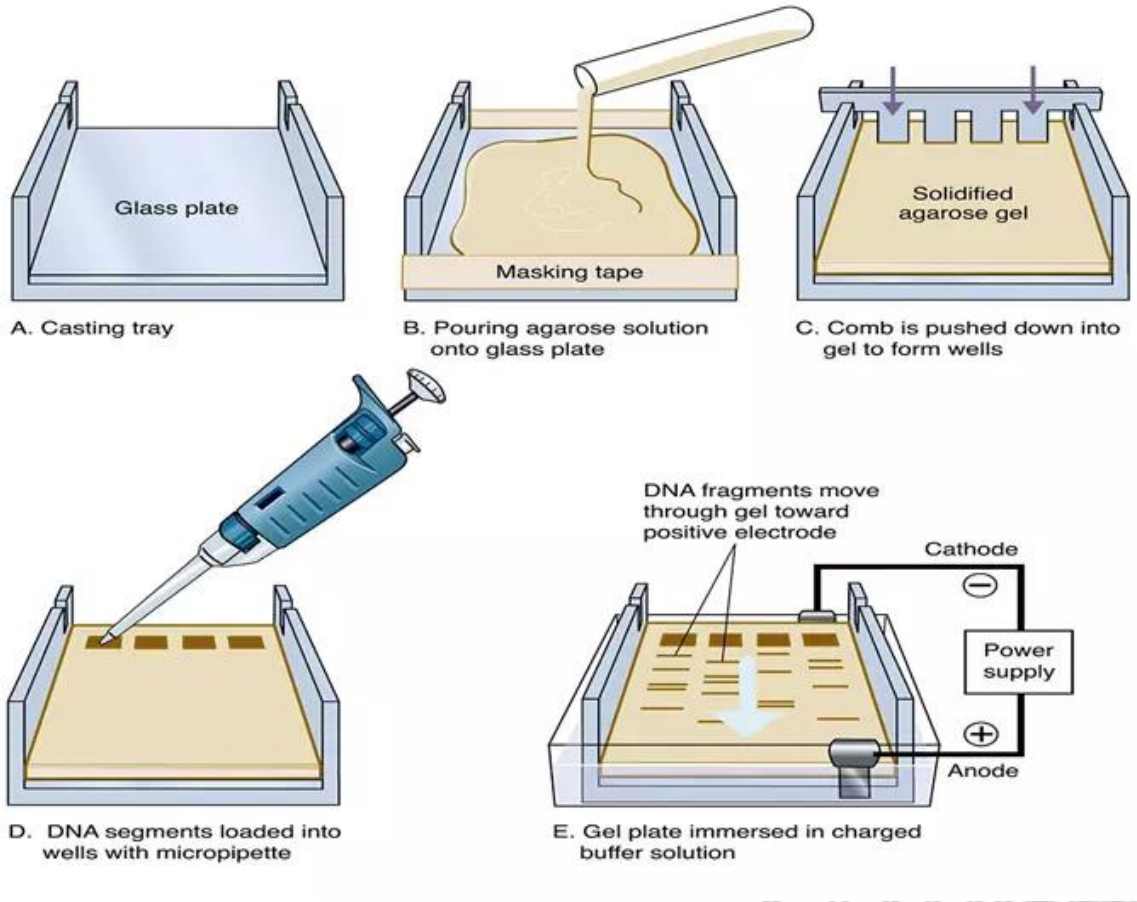
Goal: Test…

- …if the proposed pathway is correct
- …how fast the reactions take place

PAGE: Polyacrylamide gel electrophoresis

- Load sample into gel
- Apply voltage

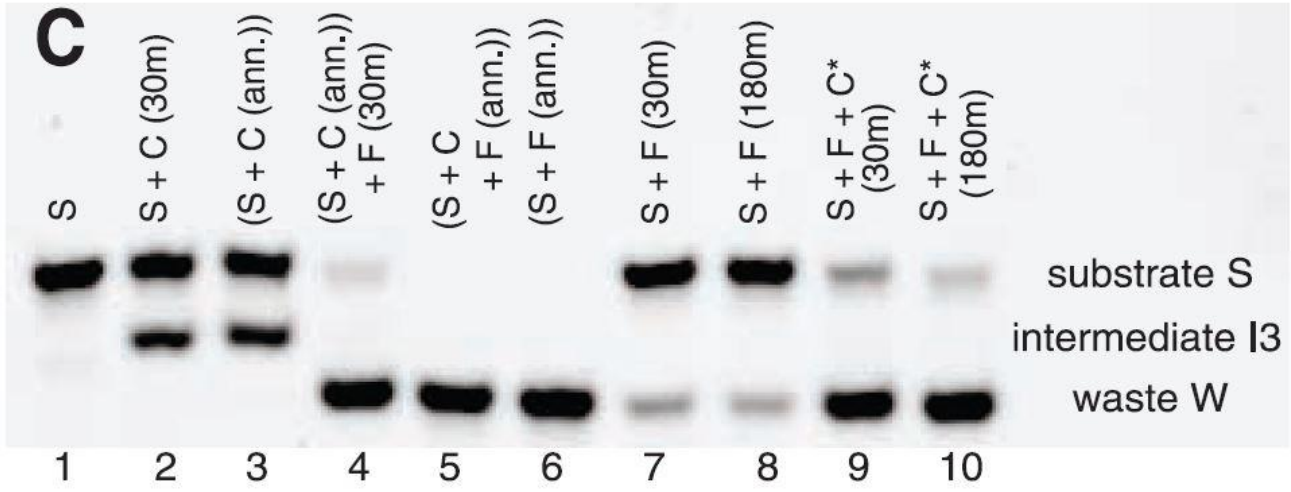
→ Separation by electrophoretic mobility



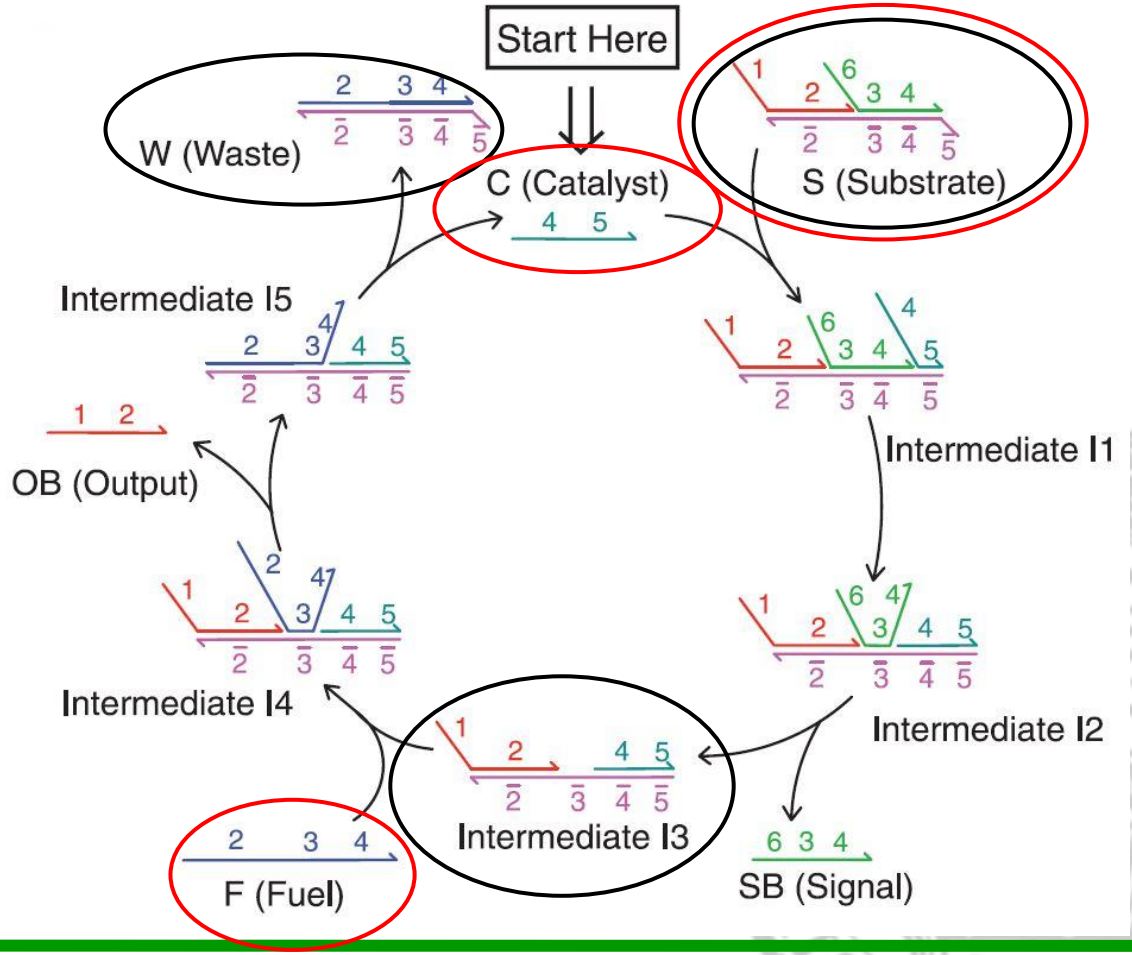
Source: *https://www.smacgigworld.com/blog/instrumentation-and-methodology-of-electrophoresis.php*

PAGE analysis

F: fuel strand SB: signal strand C: catalyst
OB: output strand LB: linker strand



*C**: substochiometric concentration of *C* (0.1x)



Source: Zhang et al., *Science* (2007)

Rate equations (reduced reaction model)

F: fuel strand SB: signal strand C: catalyst
OB: output strand LB: linker strand

$$S + F \xrightarrow{k_0} SB + OB + W$$

$$S + C \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} I3 + SB$$

$$I3 + F \xrightarrow{k_2} I5 + OB$$

$$I5 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C + W$$

$$OB + OR \xrightarrow{k_{ROX}} \text{Fluorescence}$$

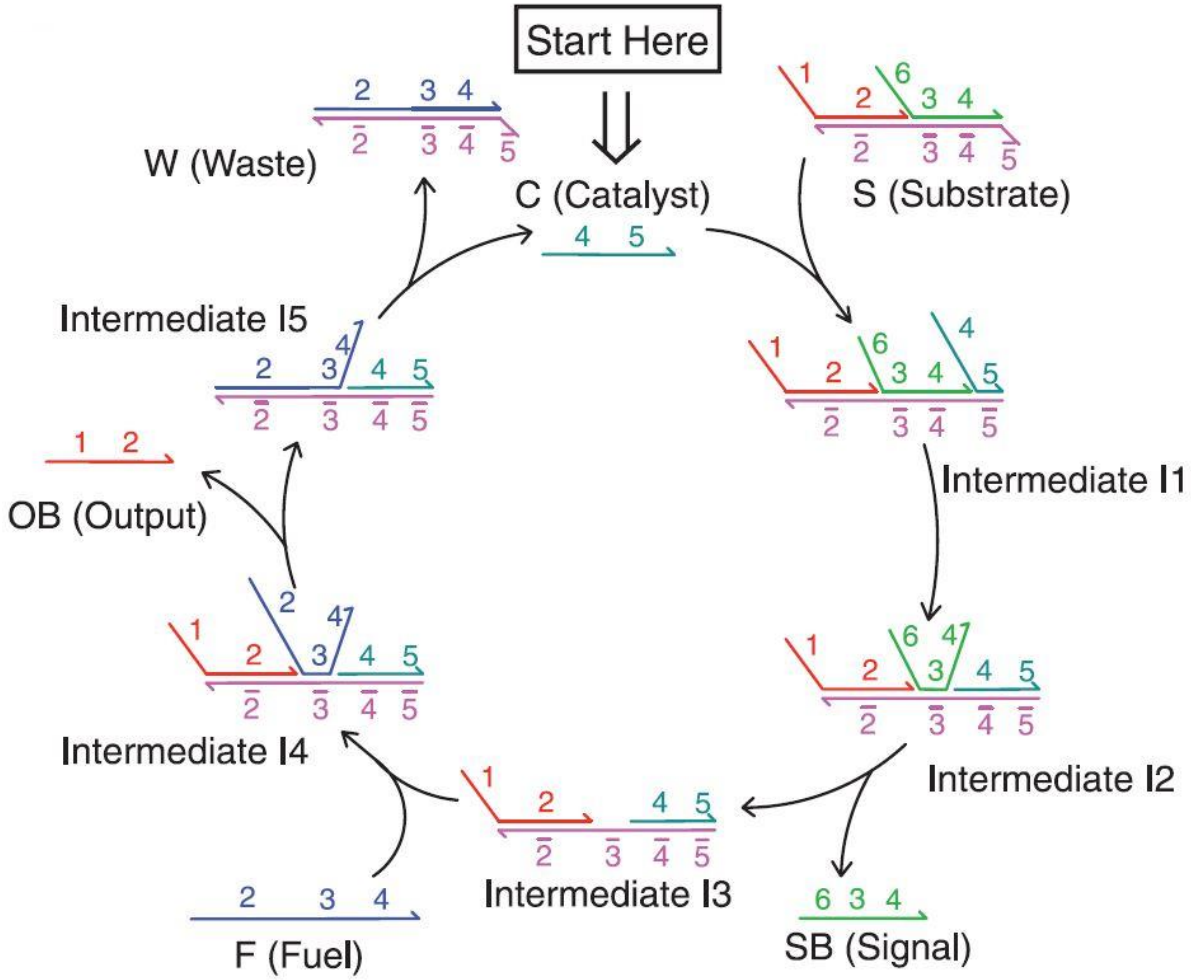
where $k_0 = 2.3 \cdot 10^1 \text{M}^{-1}\text{s}^{-1}$,

$k_1 = 6.5 \cdot 10^5 \text{M}^{-1}\text{s}^{-1}$,

$k_2 = 4.2 \cdot 10^5 \text{M}^{-1}\text{s}^{-1}$,

$k_3 = 4 \cdot 10^{-3}\text{s}^{-1}$ (fitted), and

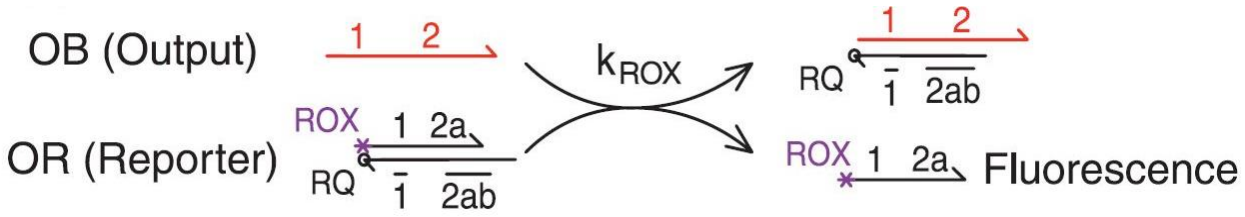
$k_{ROX} = 4 \cdot 10^5 \text{M}^{-1}\text{s}^{-1}$



Model: acceleration of reaction by over four orders of magnitude ($k_2/k_0 = 1.8 \cdot 10^4$) by addition of *C*

Source: Zhang et al., *Science* (2007)

Time course of catalyzed reaction

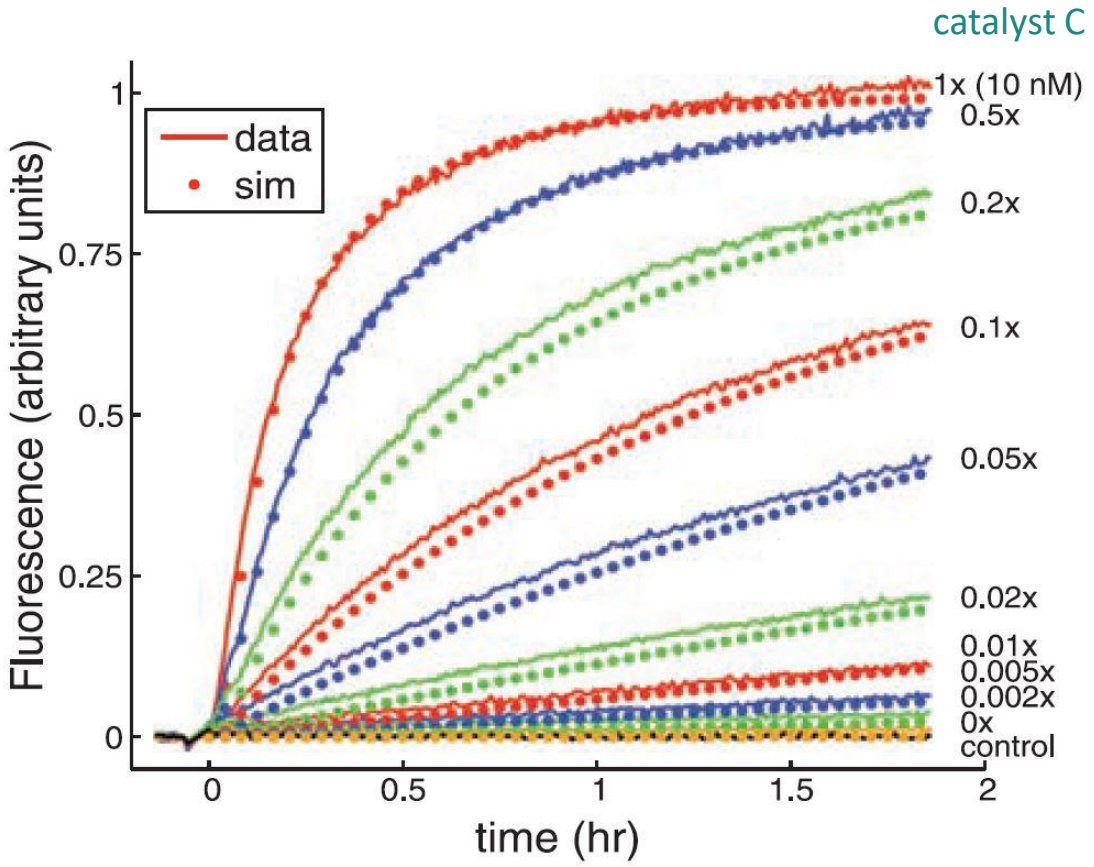


$$OB + OR \Rightarrow RQ + ROX$$

OR: Indirect reporter complex
RQ: Quencher
ROX: fluorophore-labeled strand

→Close to model prediction

→20pM resolution

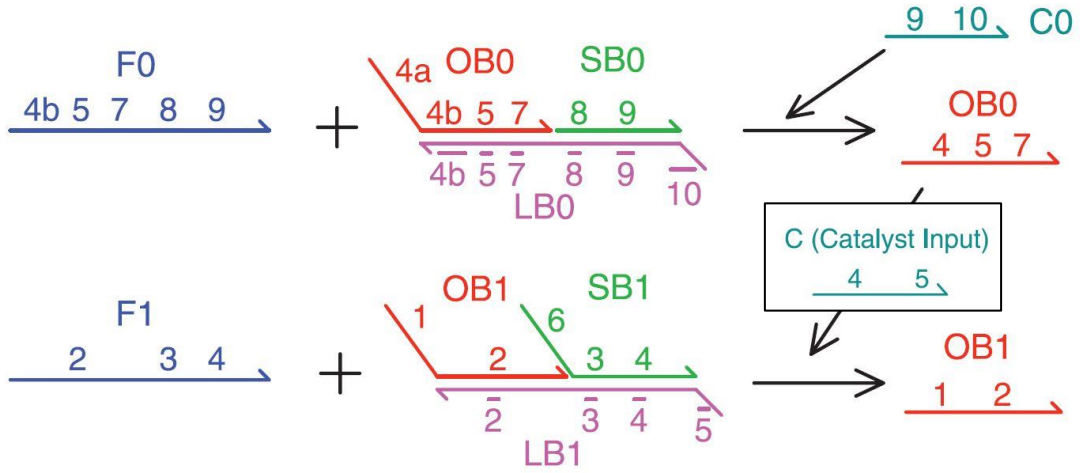


Source: Zhang et al., *Science* (2007)

Infinite options!!!

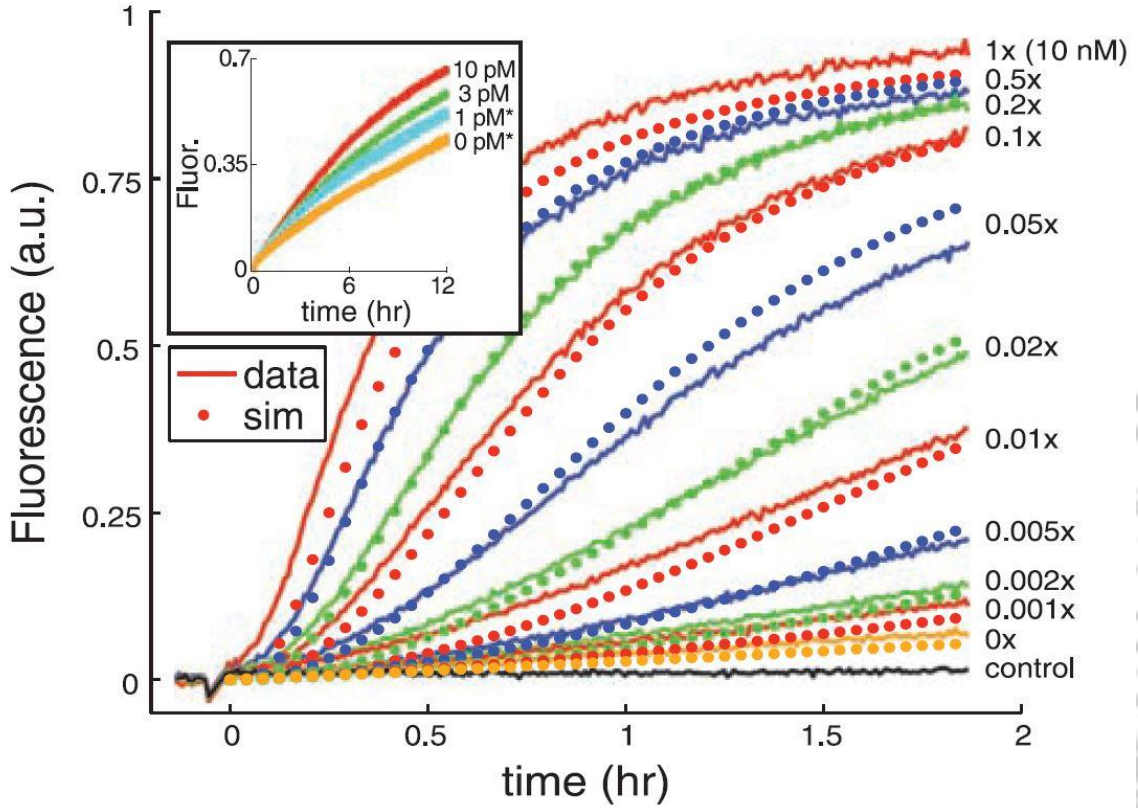
Two-layer feedforward network

F: fuel strand SB: signal strand C: catalyst
OB: output strand LB: linker strand



- Amplifier to detect small quantities of *C0* (quadratic increase)
- Reliable distinction between 1 pM and 0 pM of *C0*

→ 900x amplification

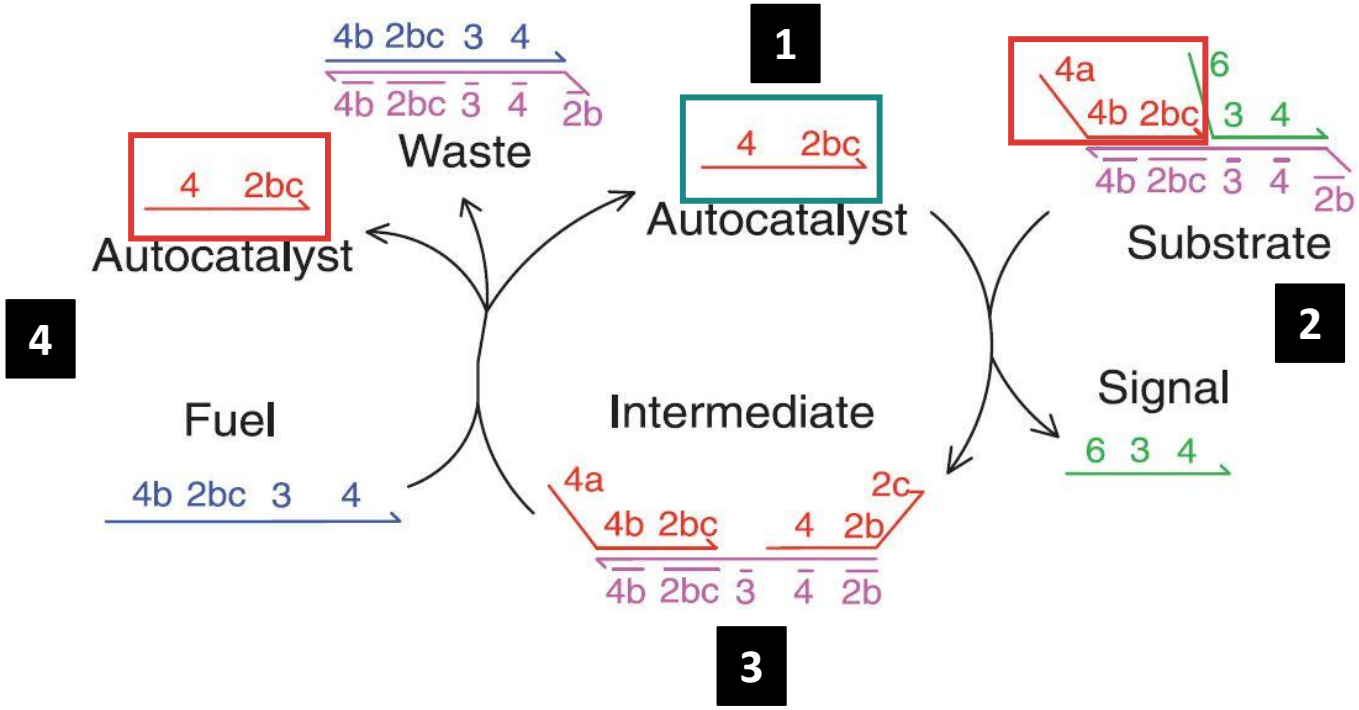


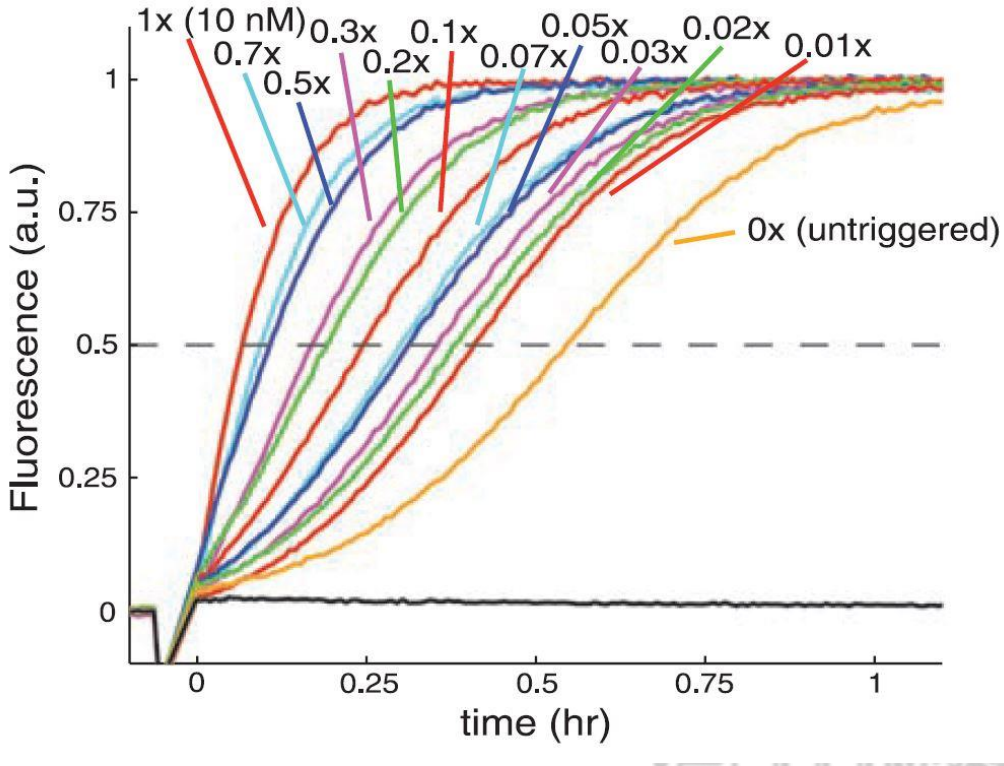
Source: Zhang et al., *Science* (2007)

Autocatalyst system

F: fuel strand SB: signal strand C: catalyst
OB: output strand LB: linker strand

- Output *OB* contains catalyst *C* as subsequence
- Exponential increase of *C*

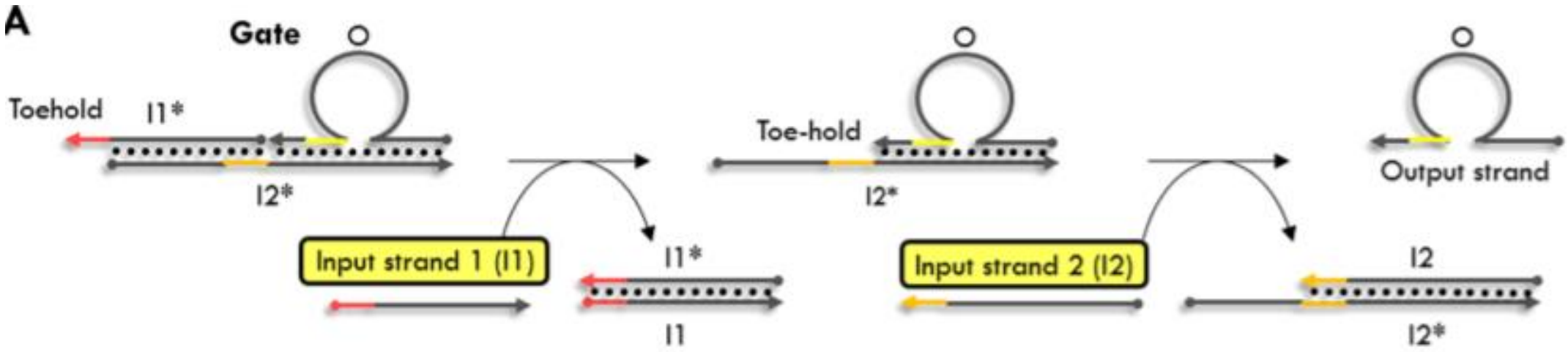




Source: Zhang et al., *Science* (2007)

back to Girnar

Entropy driven catalytic AND Gate

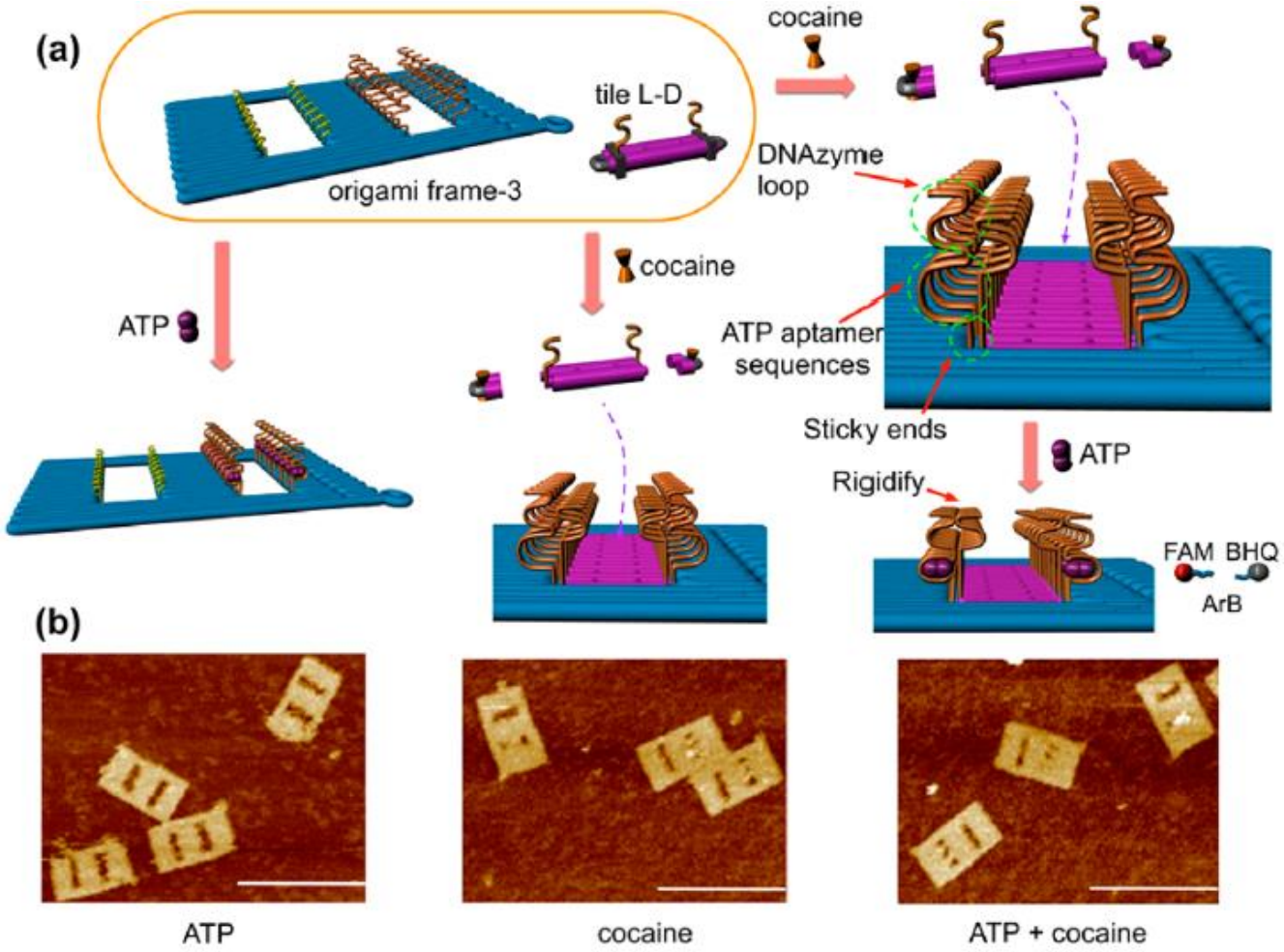


- Multiplicative 'AND' like behaviour.
- A two-input AND gate complex consisted of the O, I1*, and I2* oligonucleotides

Jung, C., & Ellington, A. D. (2014).

More Robust systems – DNA Origami

- Geometric Nanostructures
- Operations of the “AND” logic gate using a DNA origami pattern
- Time-dependent fluorescence intensity changes with different inputs.
- unexpected fluorescence leakages owing to the unspecific tile filling.



Yang et. al., ACS AMI (2016)

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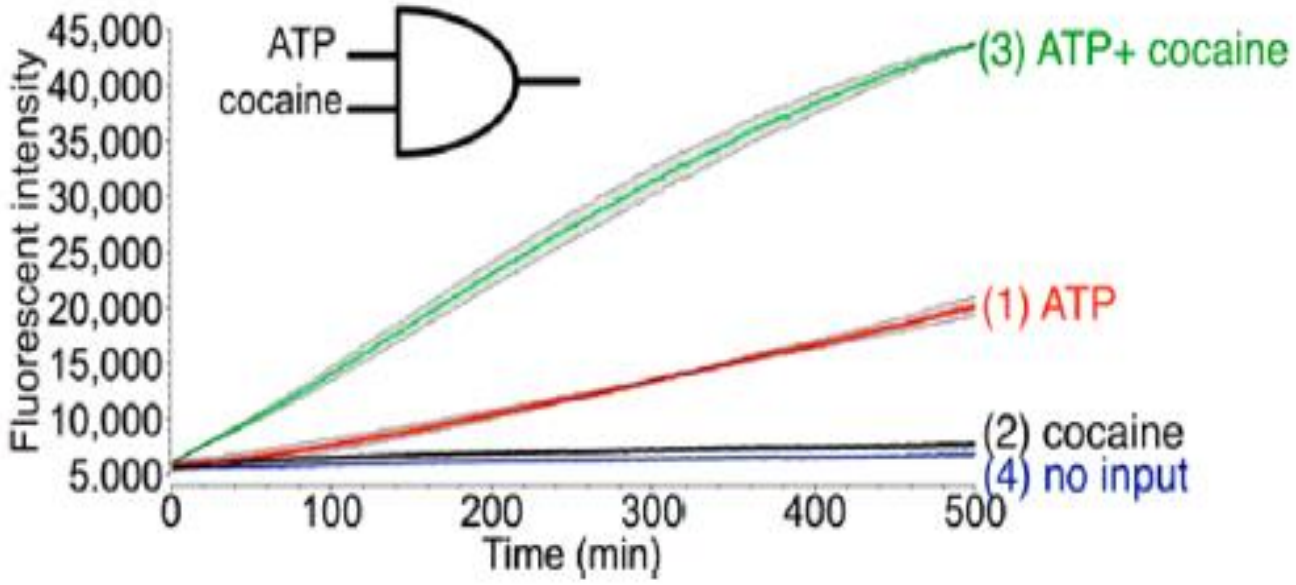
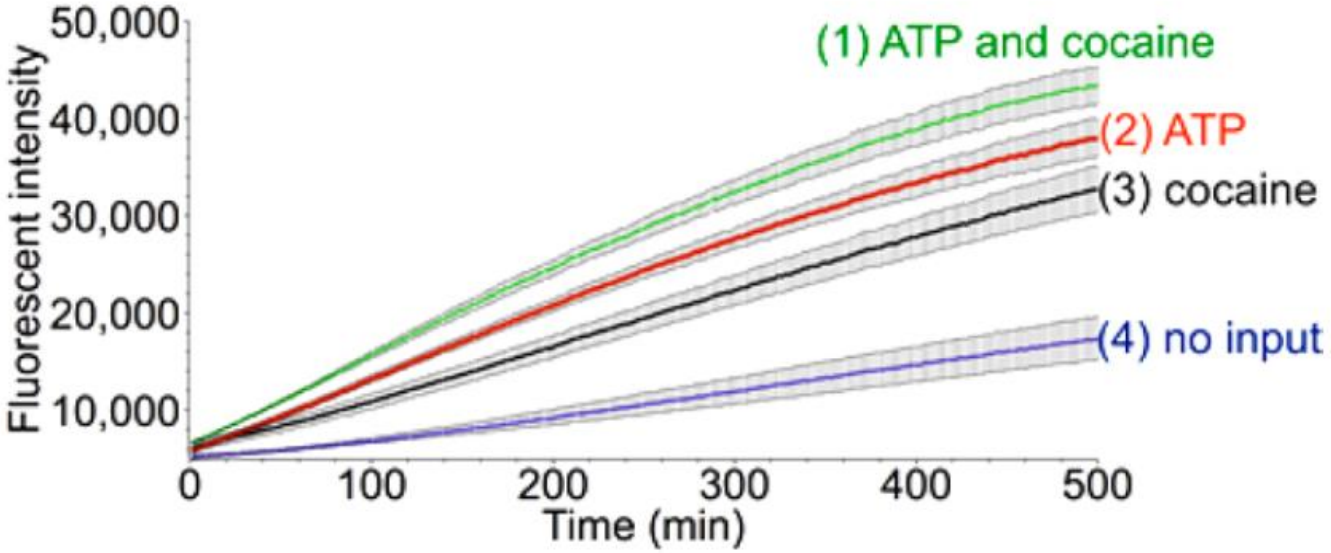


Inp 1	Inp 2	Out
0	0	0
0	1	0
1	0	0
1	1	1

Yang et. al., ACS AMI (2016)

More Robust systems – DNA Origami

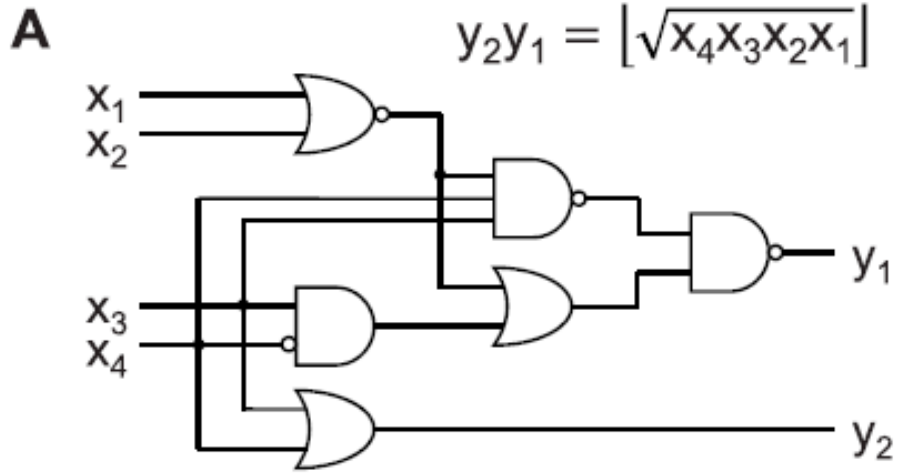
- Geometric Nanostructures
- Operations of the “OR” logic gate using a DNA origami pattern
- Time-dependent fluorescence intensity changes with different inputs.
- unexpected fluorescence leakages owing to the unspecific tile filling.



Yang et. al., ACS AMI (2016)

More Robust systems

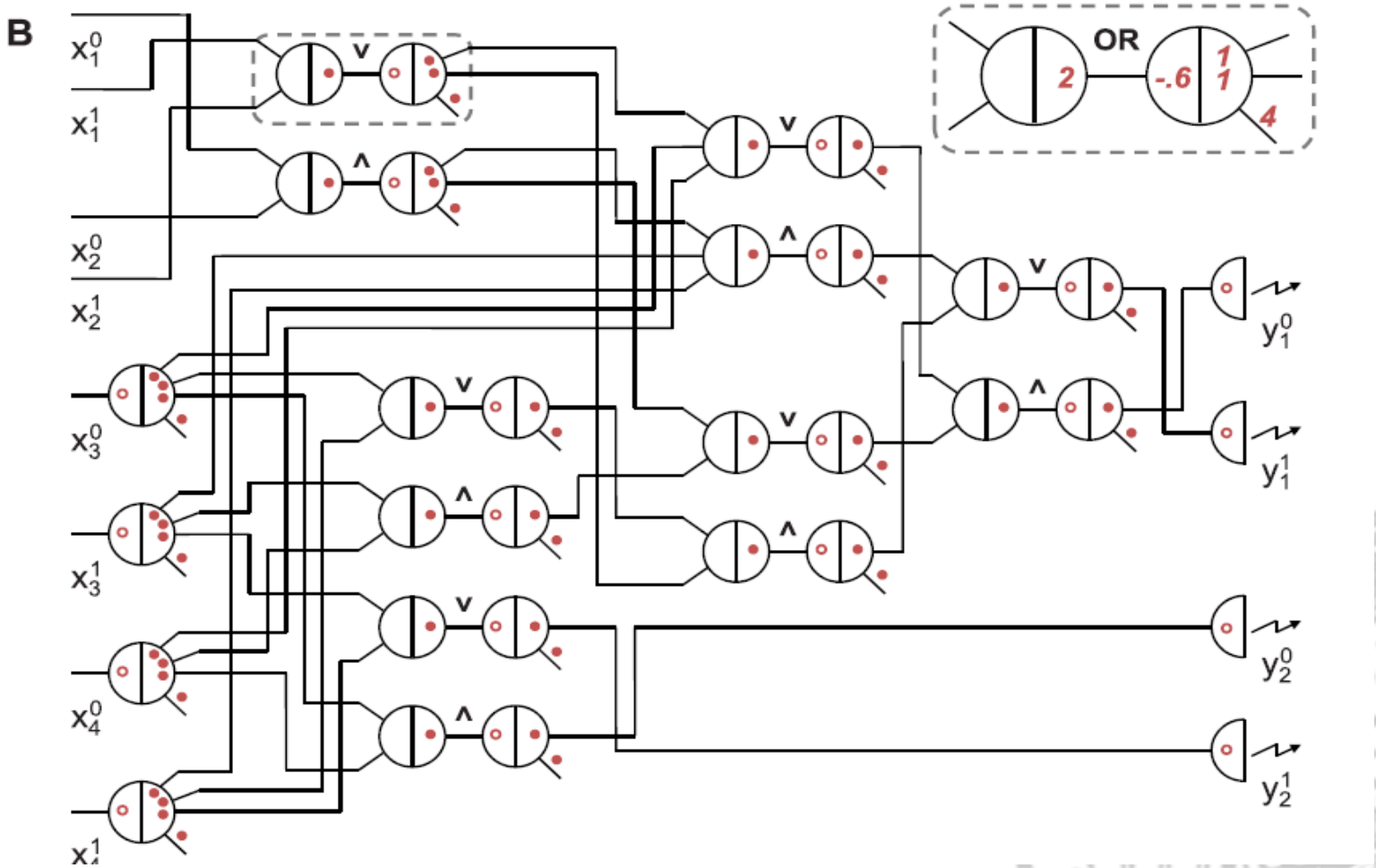
- A square-root circuit implemented with the seesaw DNA motif.
- A digital logic circuit that computes the floor of the square root of four-bit binary numbers.



Qian et. al., Science (2011)

More Robust systems

- A square-root circuit implemented with the seesaw DNA motif.
- 74 initial DNA species.
- 130 different DNA strands while running in one test tube.



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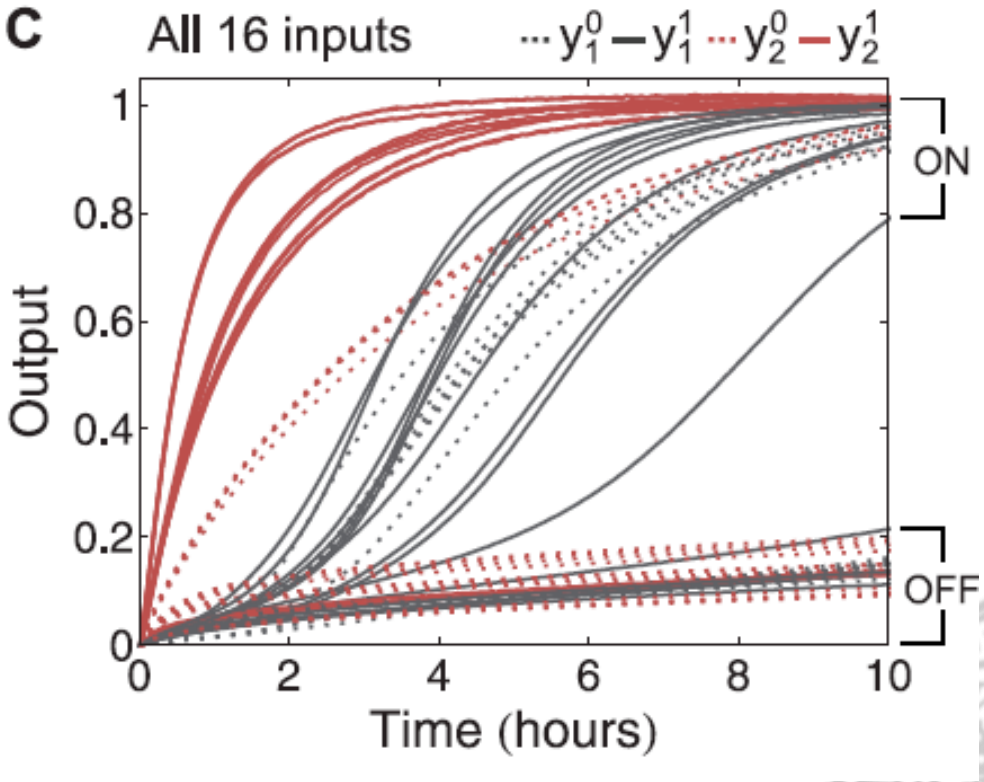

Qian et. al., Science (2011)

Key Takeaways

- Reaction driven by configurational entropy.
- Robust Methodology (T, salt concentration etc).
- ~900 fold signal amplification. (1pM catalyst)
- High Modularity => larger and wide range of chemical circuits.
- Circuits interfaced to molecular sensors and actuators.
- DNA Origami or DNA ‘Software engineering’. New gen analytical hardwares.

Zhang et. al., Science (2007)
Yang et. al., ACS AMI (2016)

References

[1]. Zhang, D. Y., Turberfield, A. J., Yurke, B., & Winfree, E. (2007). Engineering Entropy-Driven Reactions and Networks Catalyzed by DNA. Science, 318(5853), 1121–1125. doi:10.1126/science.1148532

[2]. Seelig, G., Soloveichik, D., Zhang, D. Y., & Winfree, E. (2006). Enzyme-Free Nucleic Acid Logic Circuits. Science, 314(5805), 1585–1588. doi:10.1126/science.1132493

[3]. Qian, L., & Winfree, E. (2011). Scaling Up Digital Circuit Computation with DNA Strand Displacement Cascades. Science, 332(6034), 1196–1201. doi:10.1126/science.1200520

[4]. Jung, C., & Ellington, A. D. (2014). Diagnostic Applications of Nucleic Acid Circuits. Accounts of Chemical Research, 47(6), 1825–1835. doi:10.1021/ar500059c

[5]. Yang, J., Jiang, S., Liu, X., Pan, L., & Zhang, C. (2016). Aptamer-Binding Directed DNA Origami Pattern for Logic Gates. ACS Applied Materials & Interfaces, 8(49), 34054–34060. doi:10.1021/acsami.6b10266

Thanks!