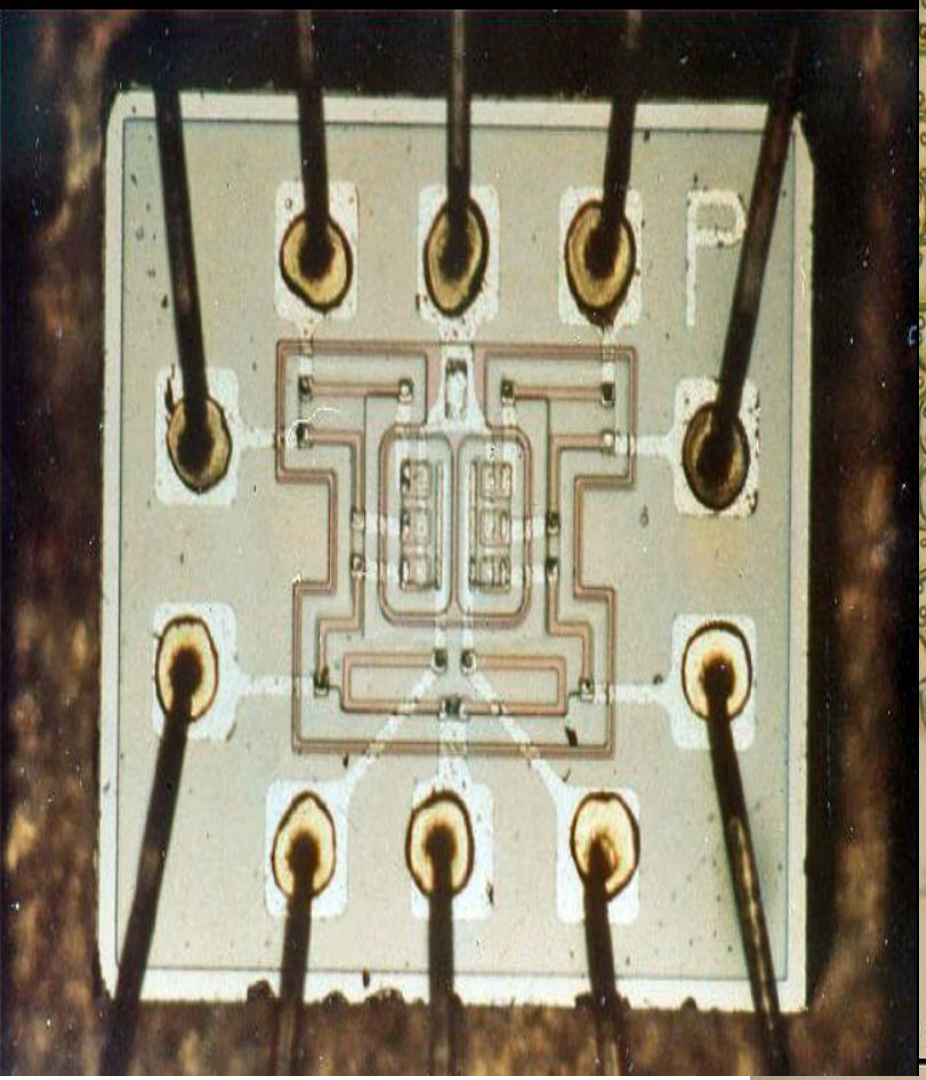

“Engineering Entropy-Driven Reactions and Networks Catalyzed by DNA”

Argyrios Gkionis

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Main story…

1. Intro: The main Idea. “How everything started….”

2. Problems and Network Ideas

3. The Paper

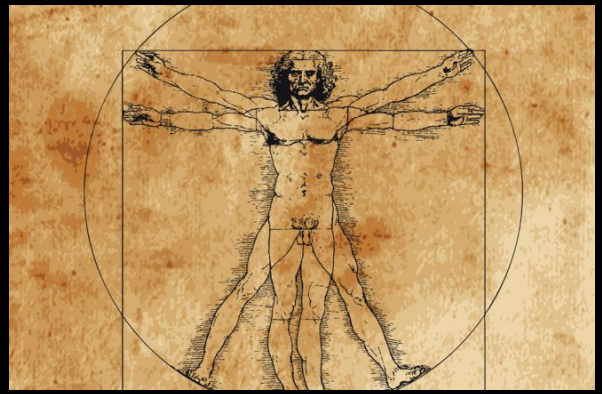
4. Conclusions & Take home message

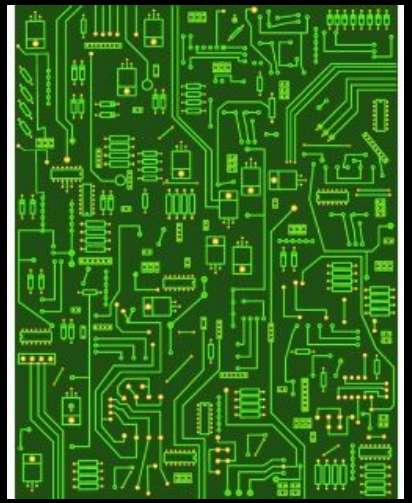
Intro: What is a Biological Circuit/Network?

Def. 1: **Molecular circuits** operating in cells consist of molecular species (genes, proteins, etc.) that interact with one another in specific ways. For example, a given gene can be transcribed to produce a corresponding mRNA, which can in turn be translated to produce a specific protein.

Def. 2 (wiki): **Synthetic biological circuits** are an application of synthetic biology where biological parts inside a cell are designed to perform logical functions mimicking those observed in electronic circuits.

The Idea: Understanding by Building

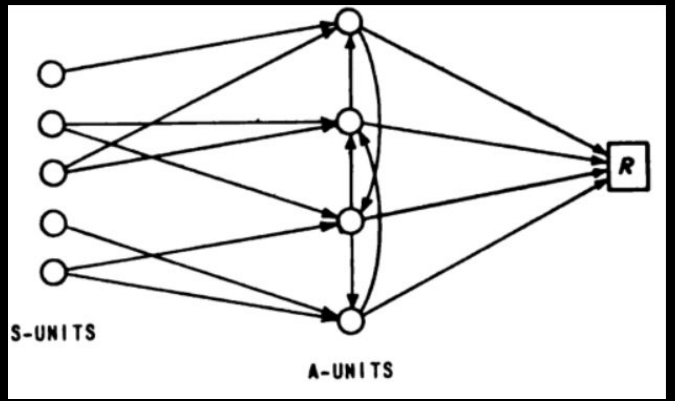




Associative Memory Network

$$E = -\frac{1}{2}\sum_{i,j} w_{ij} s_i s_j - \sum_i \theta_i s_i$$

Question:How much information can we pack in a very small space and how much energy is needed?

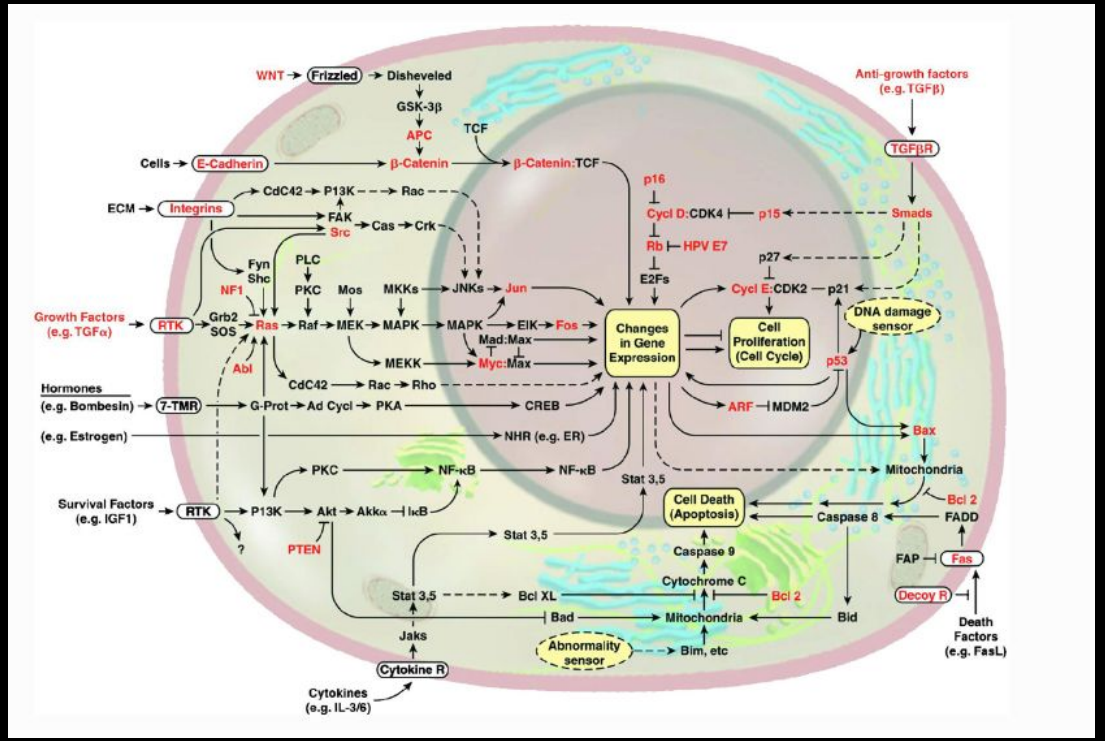


Design & Problems

- Protein circuits,Genetic circuits,RNA/DNA circuits.

- Problems:

1. function(s) are often totally unclear.
2. Crosstalk among the components.
3. Noise (Stochasticfluctuations)
4. etc.



Paper Time!

- Artificial Biological circuits.
- Oligonucleotides as a substance for our circuit.
- Entropy driven
- Different circuits with different kinetics.

Engineering Entropy-Driven Reactions and Networks Catalyzed by DNA

David Yu Zhang,[1]† Andrew J. Turberfield,[2] Bernard Yurke,[3]* Erik Winfree[1]†

Artificial biochemical circuits are likely to play as large a role in biological engineering as electrical circuits have played in the engineering of electromechanical devices. Toward that end, nucleic acids provide a designable substrate for the regulation of biochemical reactions. However, it has been difficult to incorporate signal amplification components. We introduce a design strategy that allows a specified input oligonucleotide to catalyze the release of a specified output oligonucleotide, which in turn can serve as a catalyst for other reactions. This reaction, which is driven forward by the configurational entropy of the released molecule, provides an amplifying circuit element that is simple, fast, modular, composable, and robust. We have constructed and characterized several circuits that amplify nucleic acid signals, including a feedforward cascade with quadratic kinetics and a positive feedback circuit with exponential growth kinetics.

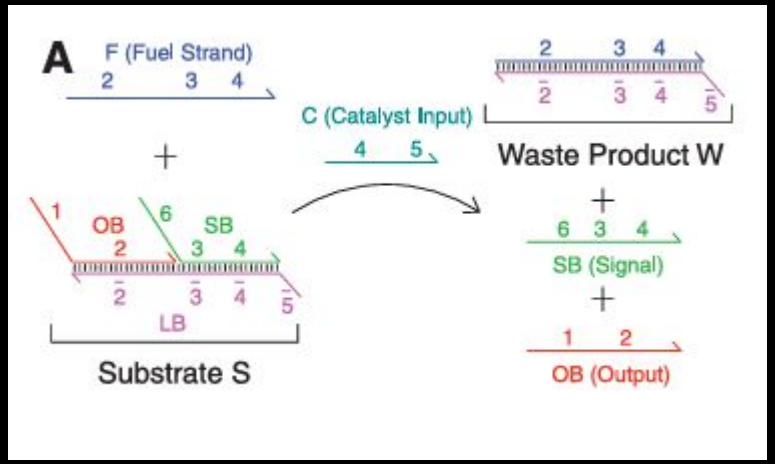
The development of modular biochemical circuit elements poses several challenges. First, distinct signals must be carried by distinct chemical species, motivating the use of information-carrying molecules whose sequences can be used to encode signal identity. Second, "wiring up" a gate to specified inputs and outputs involves the design and synthesis of new molecules; this calls for modular gate designs. Third, a fast and robust catalytic mechanism must be identified and coupled to a suitable energy source in order to create gates with signal gain. Fourth, it must be possible to construct circuits of arbitrary complexity that can produce an unlimited variety of dynamical behaviors. Finally, there should be no leak or crosstalk between distinct signals and gates. It is difficult to meet all these challenges simultaneously.

Nucleic acids are attractive for this purpose because the combinatorial sequence space allows for an enormous diversity of signal carriers, and the predictability and specificity of Watson-Crick base pairing facilitate the design of gate architectures. The "RNA world" hypothesis further suggests that sophisticated biochemical organization can be achieved with nucleic acids alone (*1*), and nucleic acids have indeed been shown to be a versatile construction material for engineering molecular structures and devices (*2*, *3*), including catalytic (*4*–*8*) and logical (*9*–*12*) control elements and circuits (*13*–*17*). Engineering (deoxy)ribozyme-based logic gates has been very effective, resulting in systems containing over 100 gates operating independently in parallel (*10*) as well as systems demonstrating cascading of a signal between two gates (*13*, *15*, *16*). Alternatively, hybridization-based systems, usually driven by the energy of base-pair formation, have proven especially suitable for cascading signals, as demonstrated by a circuit five layers deep (*17*). That work, relying primarily on noncatalytic logic gates, identified amplification and signal gain as essential for scaling up to large cascaded circuits. We provide a solution to this problem.

The Entropy Driven reaction

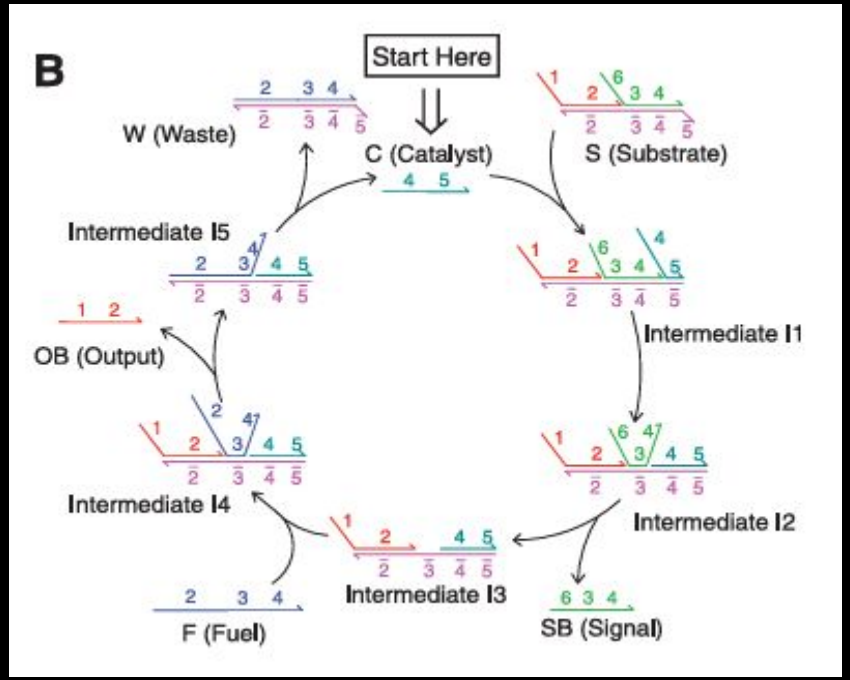
- All (F,S,W…) are DNA molecules.
- Strands subdivided in domains.

Toehold Specificity

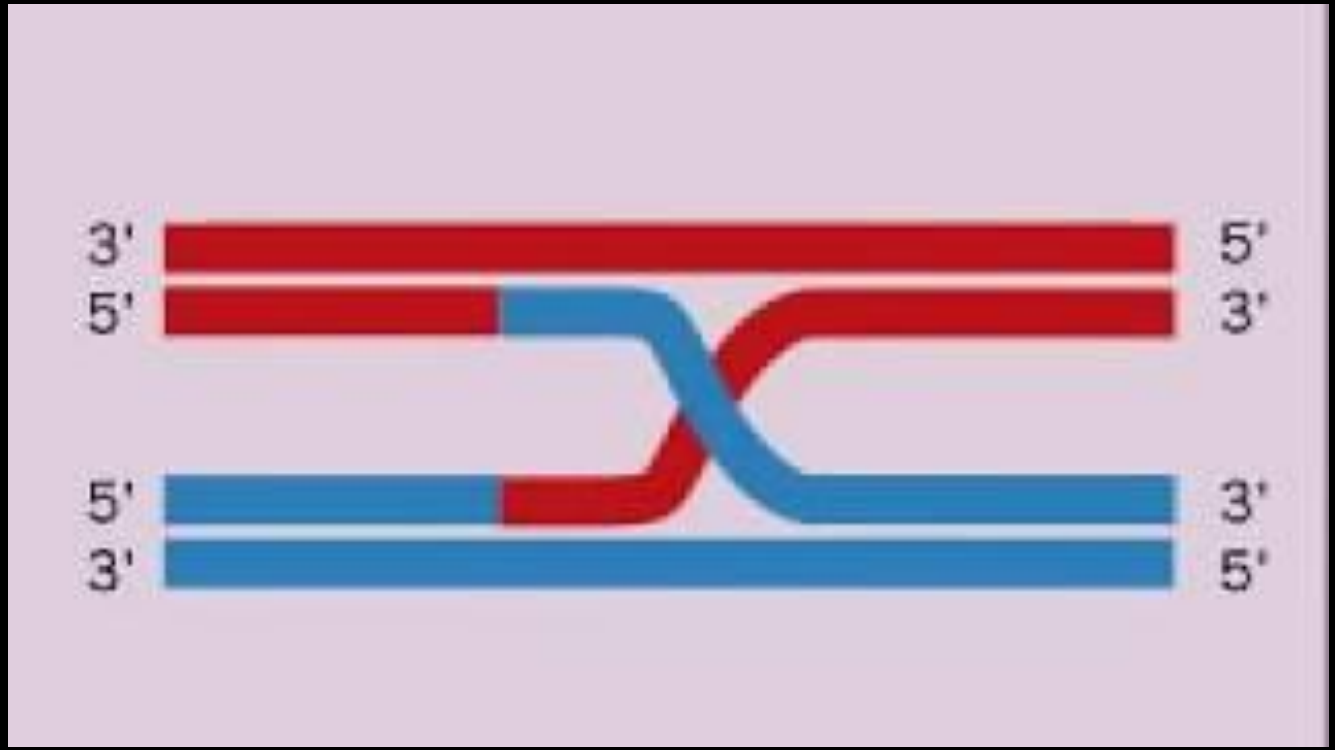


Domain	Sequence	Length (nt)
1	5′-CTTTCCTACA-3′	10
2a	5′-CCTACG-3′	6
2b	5′-TCTCCA-3′	6
2c	5′-ACTAACTTACGG-3′	12
3	5′-CCCT-3′	4
4	5′-CATTCAATACCCTACG-3′	16
5	5′-TCTCCA-3′	6
6	5′-CCACATACATCATATT-3′	16

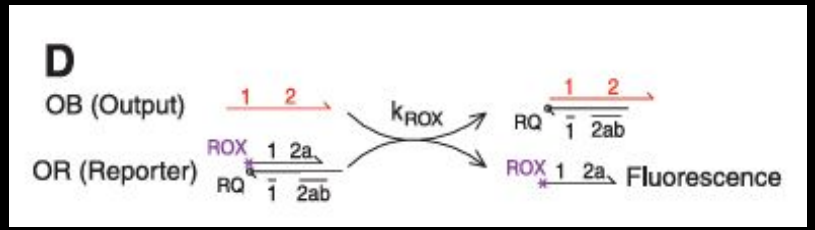
Catalytic activity and Toehold exchange



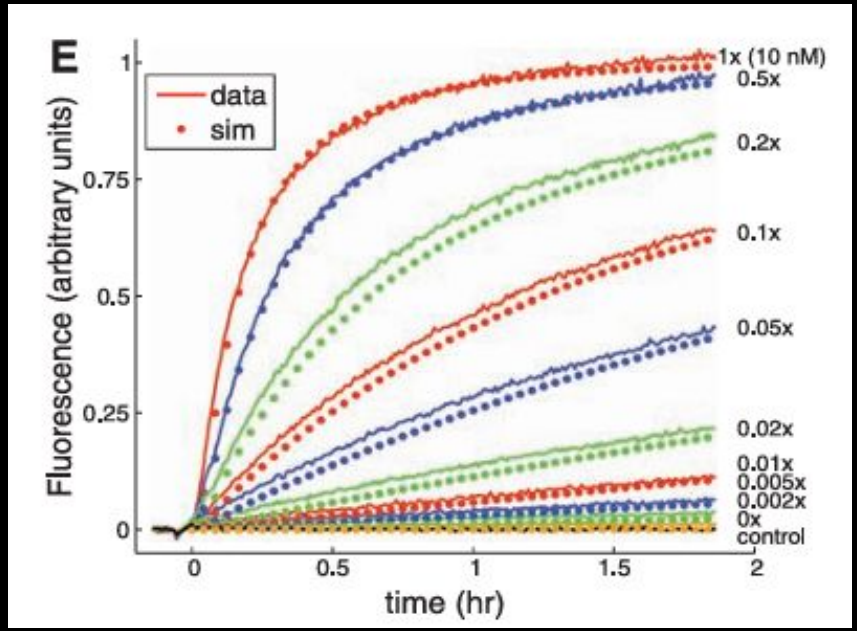
Branch migration ?



Measuring the Time course/ Demonstration of Catalysis (Kinetics)



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



Our Model and a little “thermodynamic” engineering

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

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

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

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

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

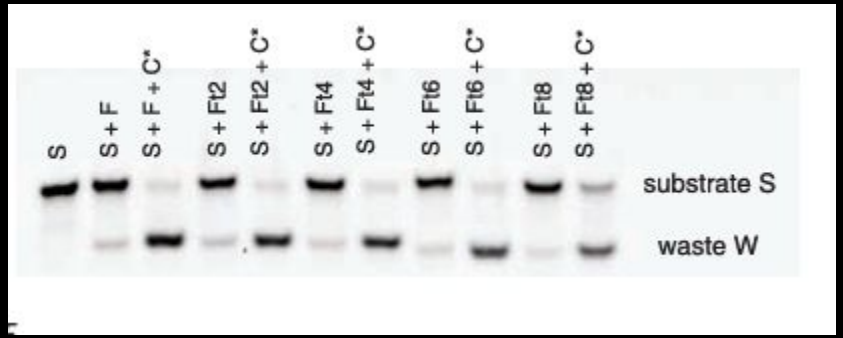
where $k_0 = 2.3 \cdot 10^1 \mathrm{M}^{-1}\mathrm{s}^{-1}$,

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

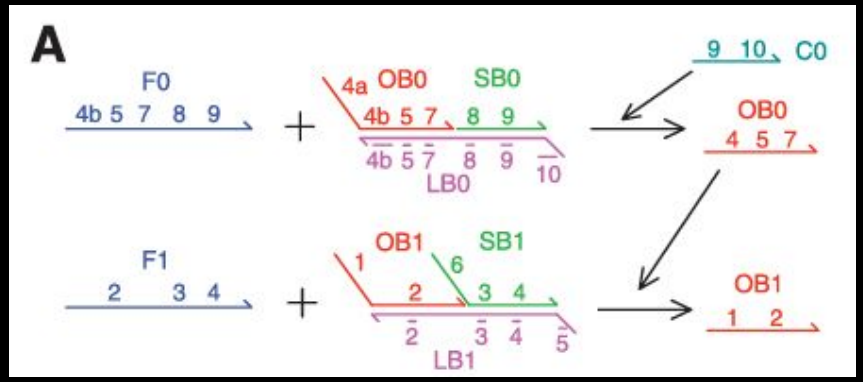
$k_2 = 4.2 \cdot 10^5 \mathrm{M}^{-1}\mathrm{s}^{-1}$,

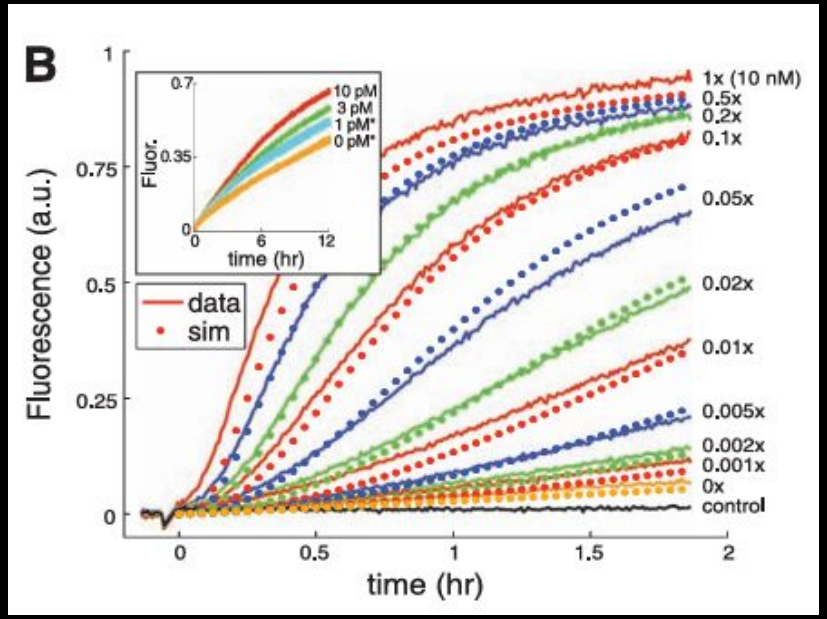
$k_3 = 4 \cdot 10^{-3}\mathrm{s}^{-1}$ (fitted), and

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



2-layer Cascaded network

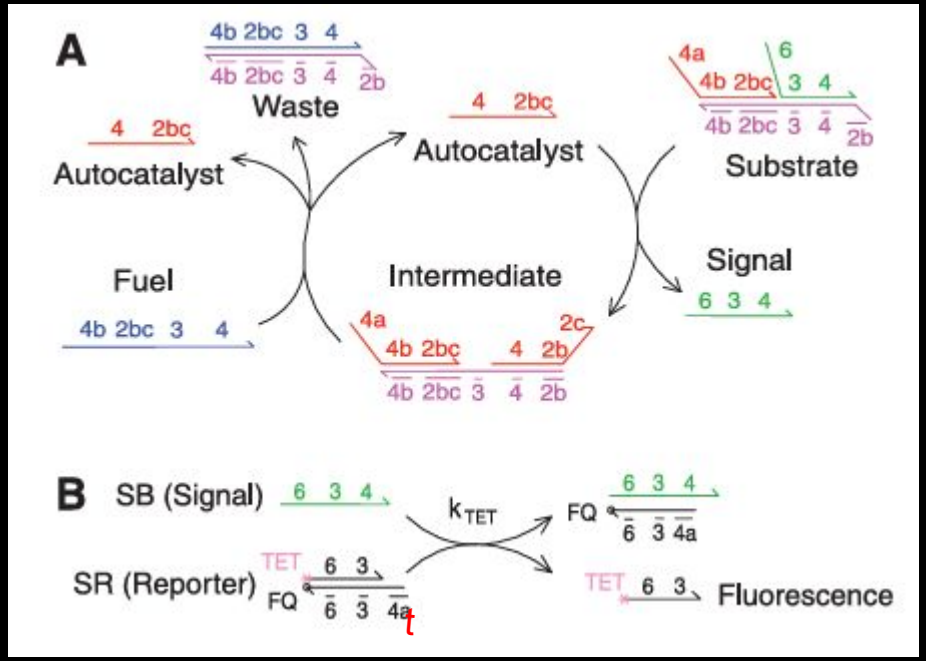


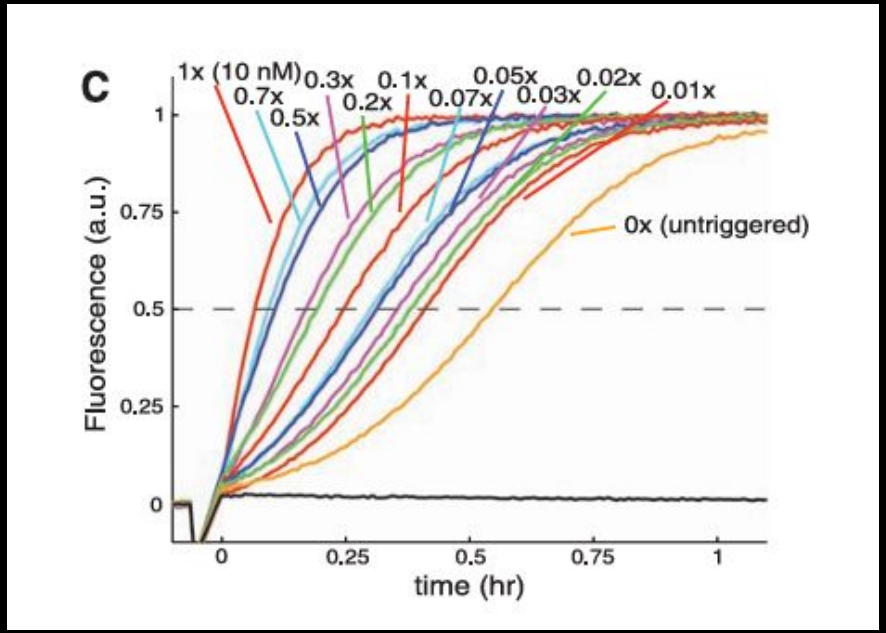


Dom.	Sequence	Length
1	5'- CTTTCCTACA -3'	10
2a (=x)	5'- CCTACG -3'	6
2b (=y)	5'- TCTCCA -3'	6
2c	5'- ACTAACTTACGG -3'	12
3	5'- CCCT -3'	4
4a	5'- CATTCAATAC -3'	10
4b (=x)	5'- CCTACG -3'	6
5 (=y)	5'- TCTCCA -3'	6
6	5'- CCACATACATCATATT -3'	16
7	5'- TACTTATTAGCC -3'	12
8	5'- GACA -3'	4
9a	5'- CTACTTTCAC -3'	10
9b (=x)	5'- CCTACG -3'	6
10 (=y)	5'- TCTCCA -3'	6

Table S2: Domain sequences

Can we achieve exponential growth kinetics?

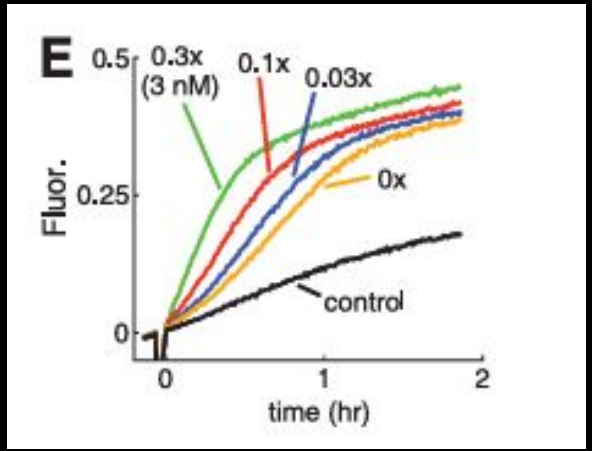




$$SB + SR \xrightarrow{k_{TET}} Fluorescence$$

A little experiment for the end…

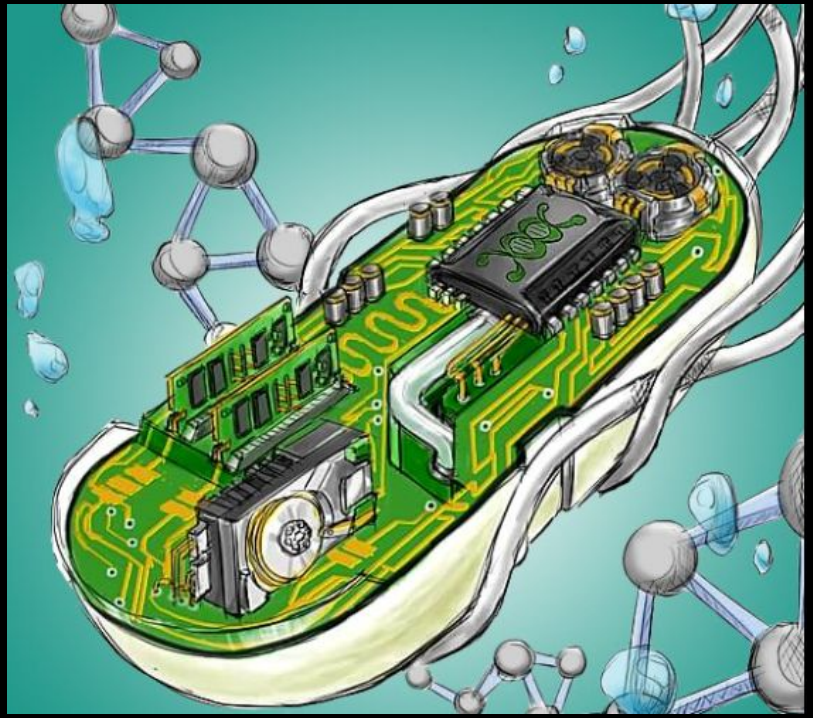
- Tested the autocatalytic system
- Mouse liver RNA
- Rabbit reticulocyte lysate.



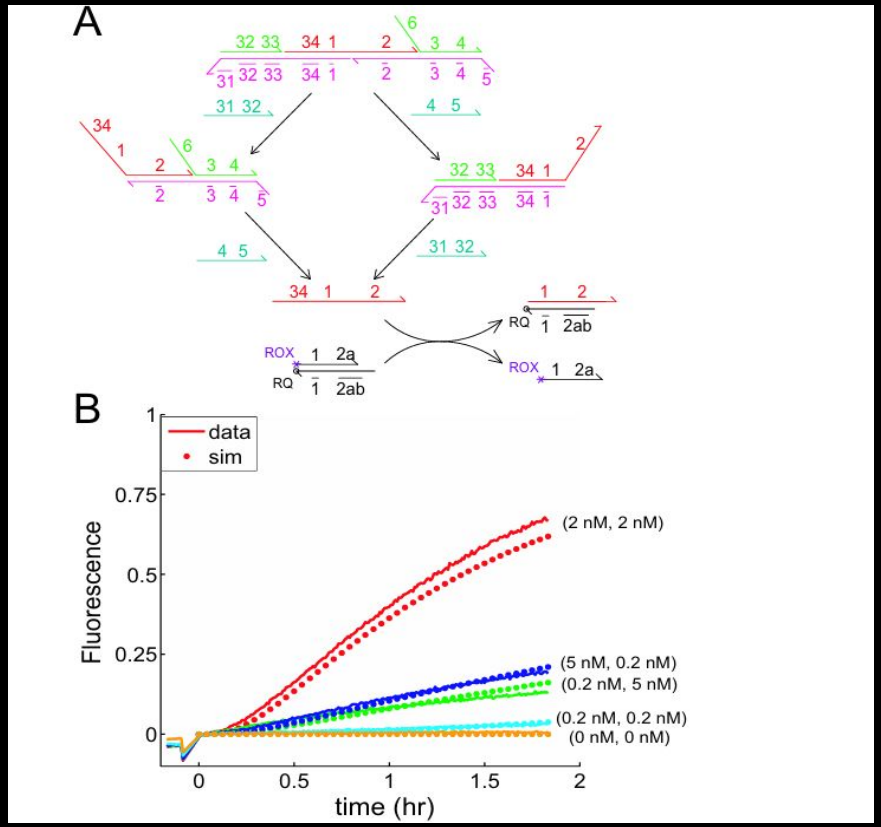
Conclusions

- Better than self- replication reactions that have been proposed in the past.
- Experiment: RNA but not cell lysate did not show the observed drift. We need the catalyst!
- Their Autocatalytic system better, reduces the spontaneous activity of the circuit!
- Nucleic Acids are great for constructing circuits.
- Development ⟶ Great Biomedical applications in the near Future!

THE END (AND)…..



EXTRA: Catalytic AND gate



Dom.	Sequence	Length
31	5'- CACACA -3'	6
32	5'- ACTTCAGTCATTAAGC -3'	16
33	5'- AGAC -3'	4
34	5'- CCATACAAGTATCA -3'	14

Table S4: New domain sequences for the catalytic analog AND gate

THANK YOU!