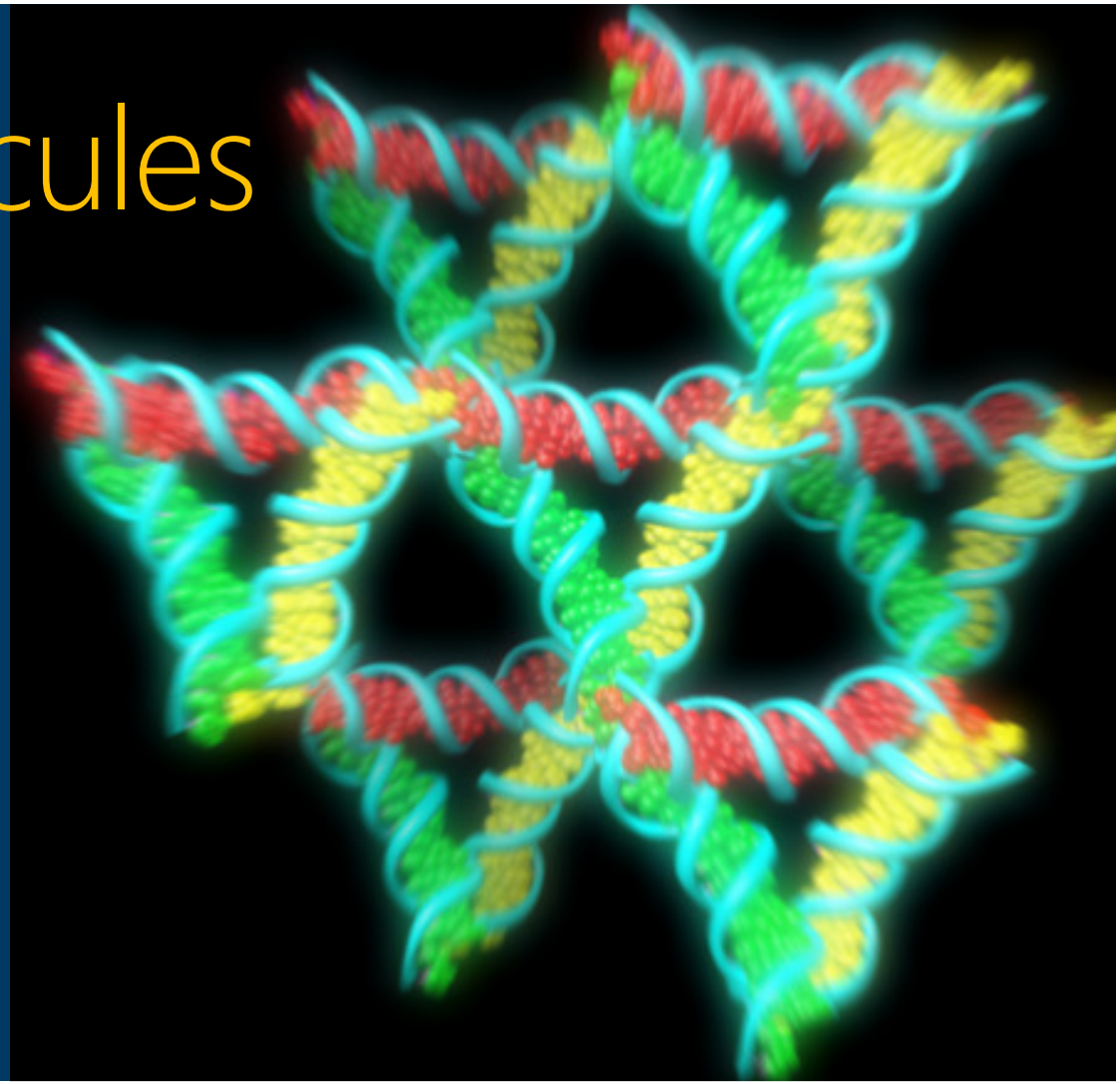
Telling Molecules What To Do

The programmatic manipulation of matter

Luca Cardelli

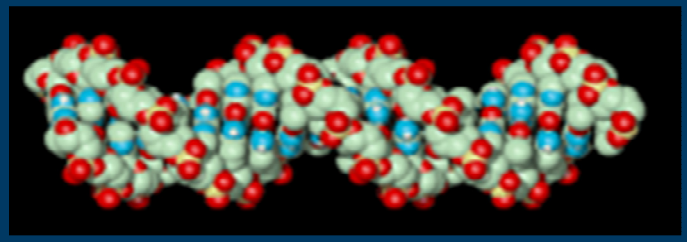
Microsoft Research &
University of Oxford

Distinguished Lecture 2016
Lisbon, 2016-09-28



Objectives

- The promises of Molecular Programming:
  - In Science & Medicine
  - In Engineering
  - In Computing
- The current practice of Molecular Programming
  - DNA technology
  - Molecular languages and tools
  - Example of a molecular algorithm



Molecular Programming:
The Hardware Aspect

Smaller and smaller things can be built

Smaller and Smaller

First working transistor
John Bardeen and Walter Brattain , Dec. 23, 1947

First integrated circuit
Jack Kilby, Sep. 1958.

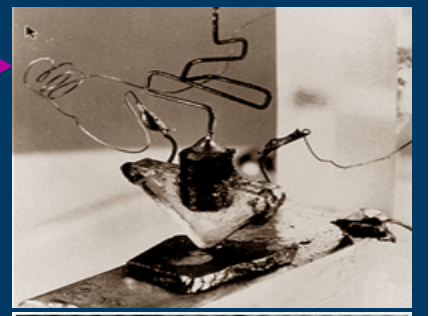
50 years later

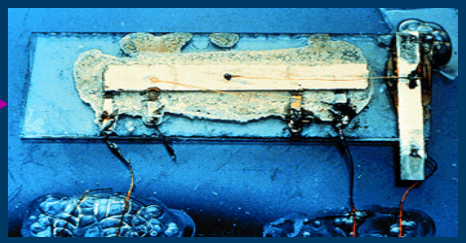
25nm NAND flash
Intel&Micron, Jan. 2010. ~50atoms

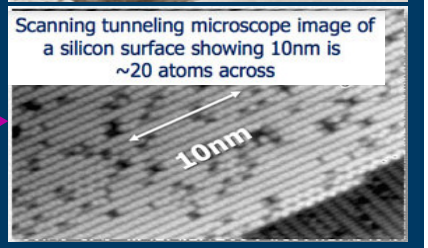
Single molecule transistor
Observation of molecular orbital gating
Nature, 2009; 462 (7276): 1039

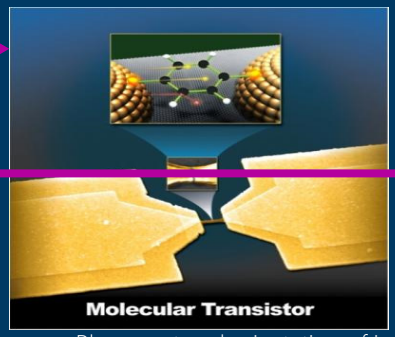
Molecules on a chip

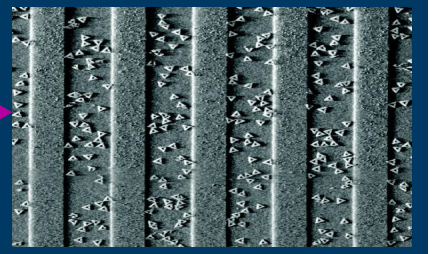
~10 Moore's Law cycles left!











Placement and orientation of individual DNA shapes on lithographically patterned surfaces. Nature Nanotechnology 4, 557 - 561 (2009).

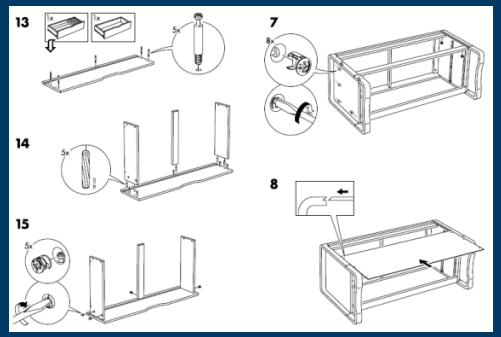
Building the *Smallest* Things

- How do we build structures that are by definition smaller than your tools?
- Basic answer: you can't. Structures (and tools) should build themselves!
- By *programmed self-assembly*

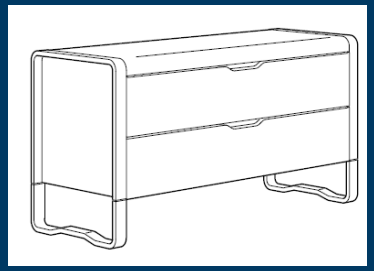
www.youtube.com/watch?v=Ey7Emmddf7Y

Molecular IKEA

- Nature can self-assemble.
  Can we?
- *"Dear IKEA, please send me a chest of drawers that assembles itself."*
- We need a magical material where the pieces are pre-programmed to fit into to each other.
- At the molecular scale many such materials exist…



Add water

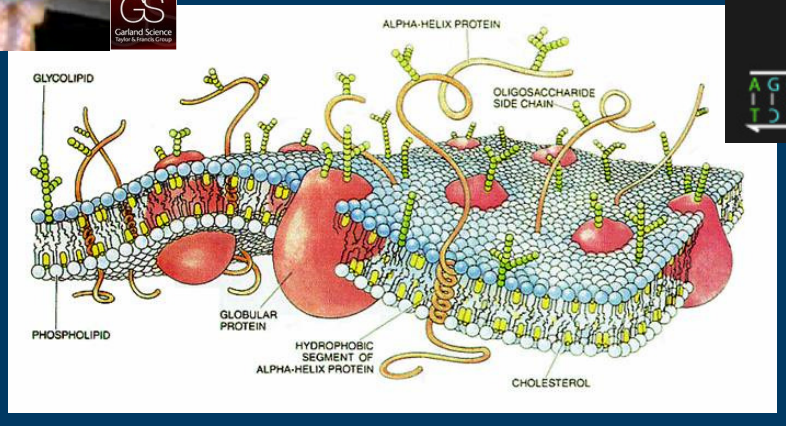


http://www.ikea.com/ms/en_US/customer_service/assembly_instructions.html

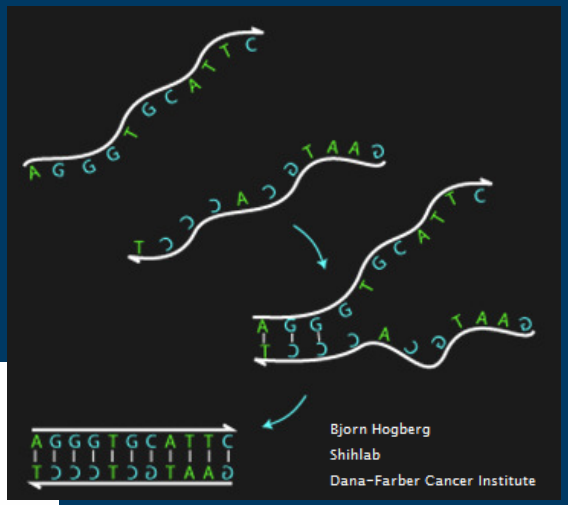
Programmed Self-Assembly

Proteins

Membranes



DNA/RNA

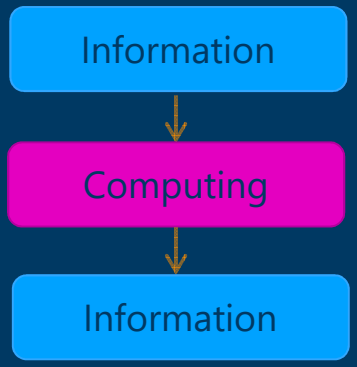


Molecular Programming:
The Software Aspect

Smaller and smaller things can be programmed

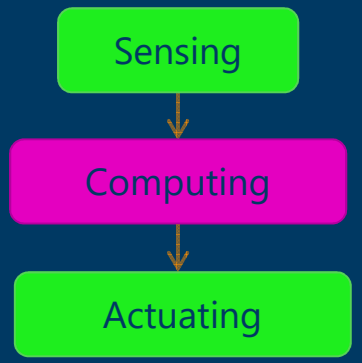
We can program…

- Information
  - Completely!



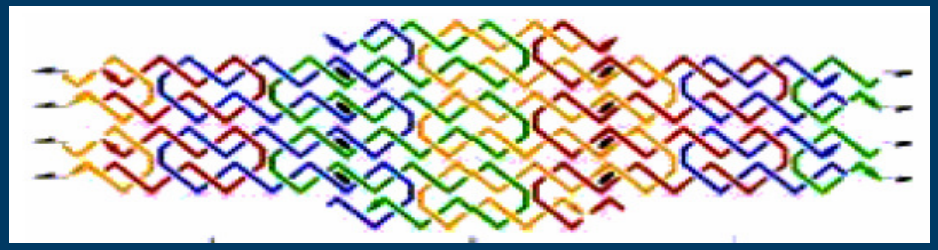
We can program…

- Forces
  - Completely!
    (Modulo sensors/actuators)

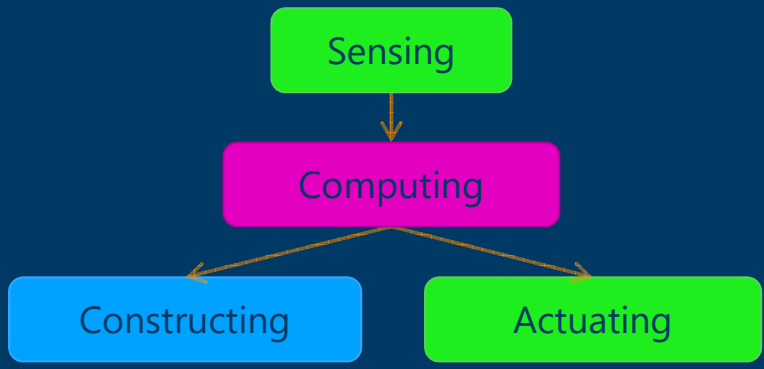


We can program…

- Matter
  - Completely and directly! By self-assembly.
  - Currently: only DNA/RNA.

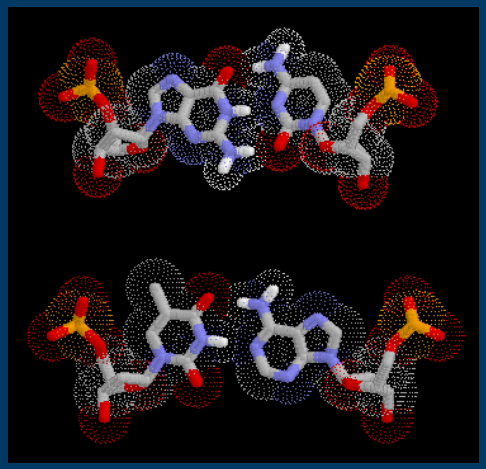


  - But DNA is an amazing *material*



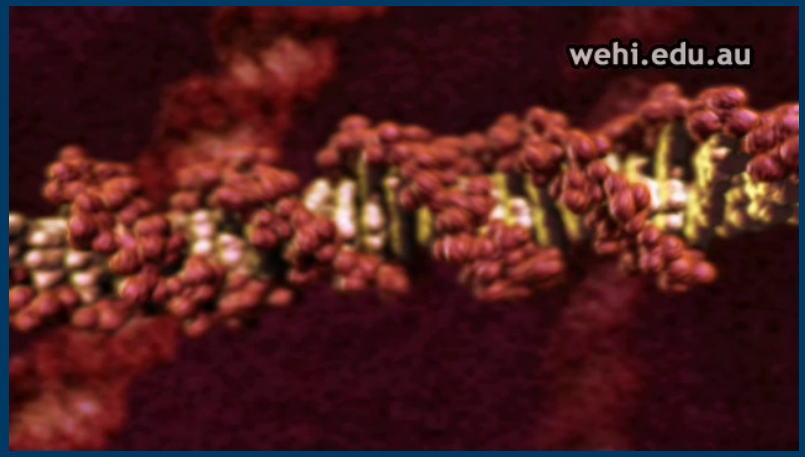
It's like a 3D printer without the printer!
[Andrew Hellington]

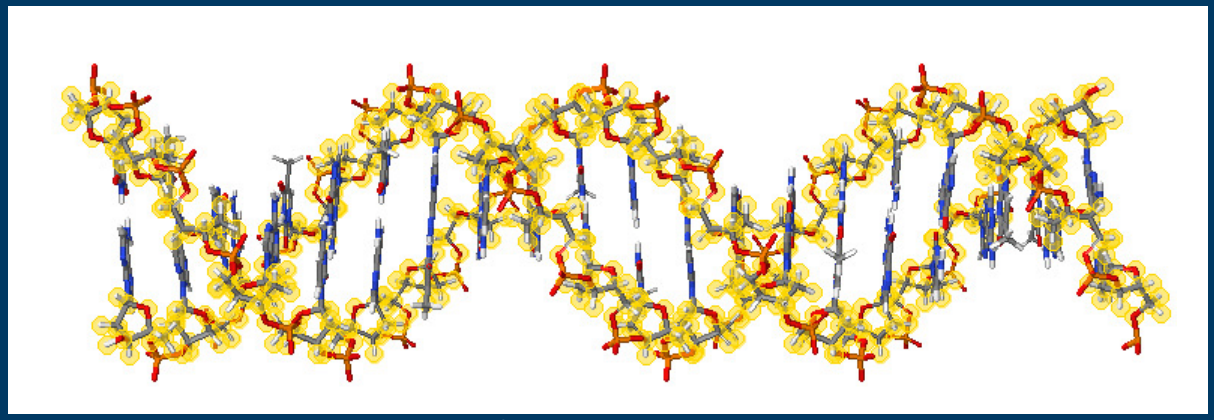
DNA



G-C Base Pair
Guanine-Cytosine

T-A Base Pair
Thymine-Adenine





Sequence of Base Pairs (GACT alphabet)

Interactive DNA Tutorial
(http://www.biosciences.bham.ac.uk/labs/minchin/tutorials/dna.html)

Astronomical

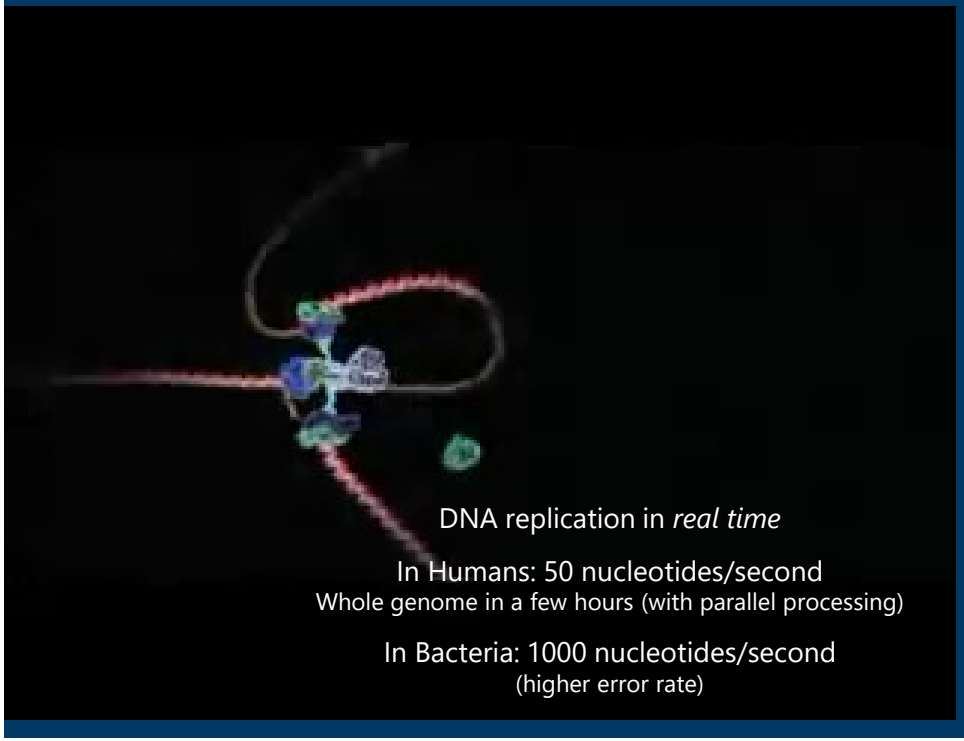
- DNA in each human cell:
  - 3 billion base pairs
  - 2 meters long, 2nm thick
  - 750 megabytes
  - folded into a 6μm ball, 140 exabytes (million terabytes)/mm^3
- A huge amount for a cell
  - Every time a cell replicates it has to copy *2 meters of DNA* reliably.
  - To get a feeling for the scale disparity, compute:
- DNA in human body
  - 10 trillion cells
  - 133 Astronomical Units long
  - 7.5 octabytes
- DNA in human population
  - 20 million light years long

DNA wrapping into chromosomes

Andromeda Galaxy
2.5 million light years

Zipping Along

- DNA can support structural and computational complexity.



DNA replication in *real time*

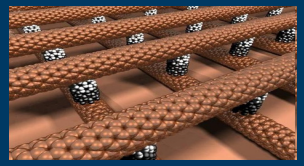
In Humans: 50 nucleotides/second
Whole genome in a few hours (with parallel processing)

In Bacteria: 1000 nucleotides/second
(higher error rate)

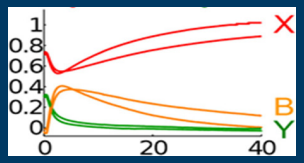
Drew Berry
http://www.wehi.edu.au/wehi-tv

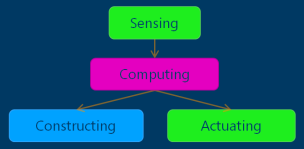
What is special about DNA?

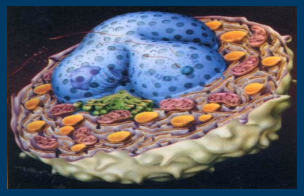
- There are many, many nanofabrication techniques and materials



- But only DNA (and RNA) can:
  - Organize ANY other matter [caveats apply]
  - Execute ANY kinetics [caveats: up to time scaling]
  - Assemble Nano-Control Devices
  - Interface to Biology



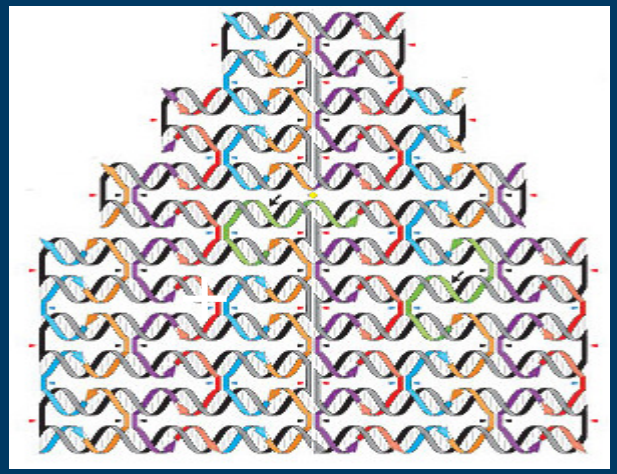




H.Lodish & al. Molecular Cell Biology 4th ed.

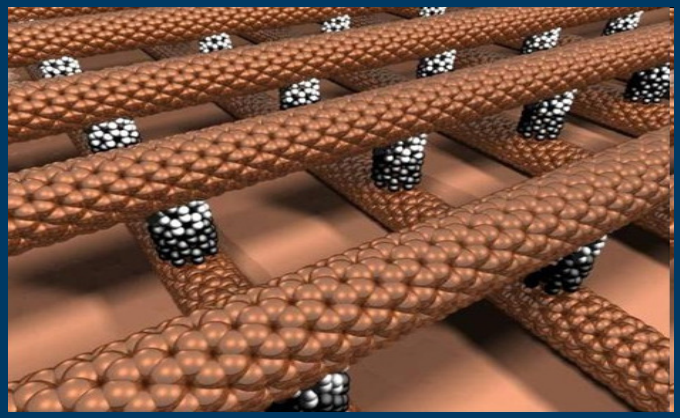
Organizing Any Matter

- Use one kind of programmable matter (e.g. DNA).
- To organize (almost) ANY matter through it.



PWK Rothemund, *Nature* 440, 297 (2006)

6 nm grid of individually addressable DNA pixels



European Nanoelectronics Initiative Advisory Council

"What we are really making are tiny DNA circuit boards that will be used to assemble other components."
Greg Wallraff, IBM

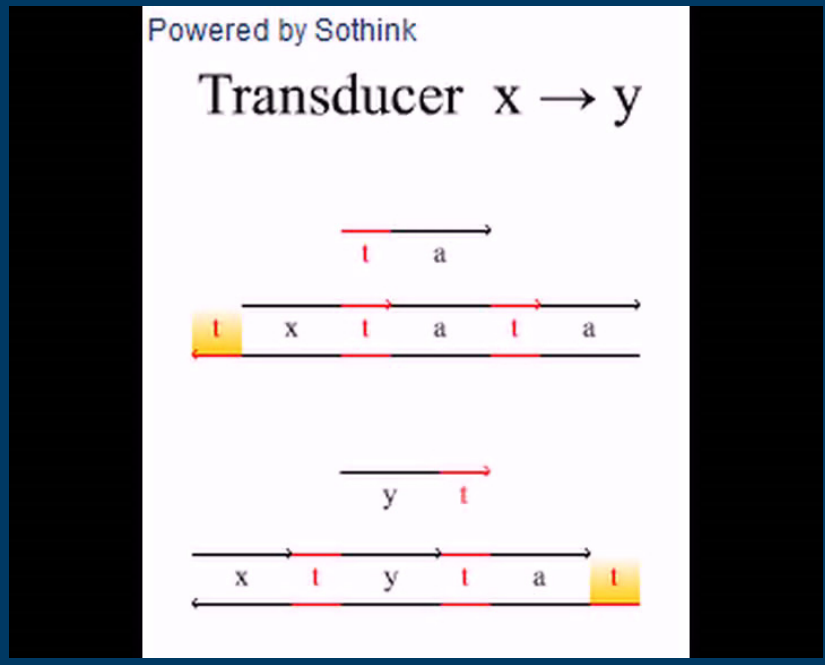
Executing Any Kinetics

- The kinetics of any finite network of chemical reactions, can be implemented (physically) with especially programmed DNA molecules.

- Chemical reactions as an executable programming language for dynamical systems!

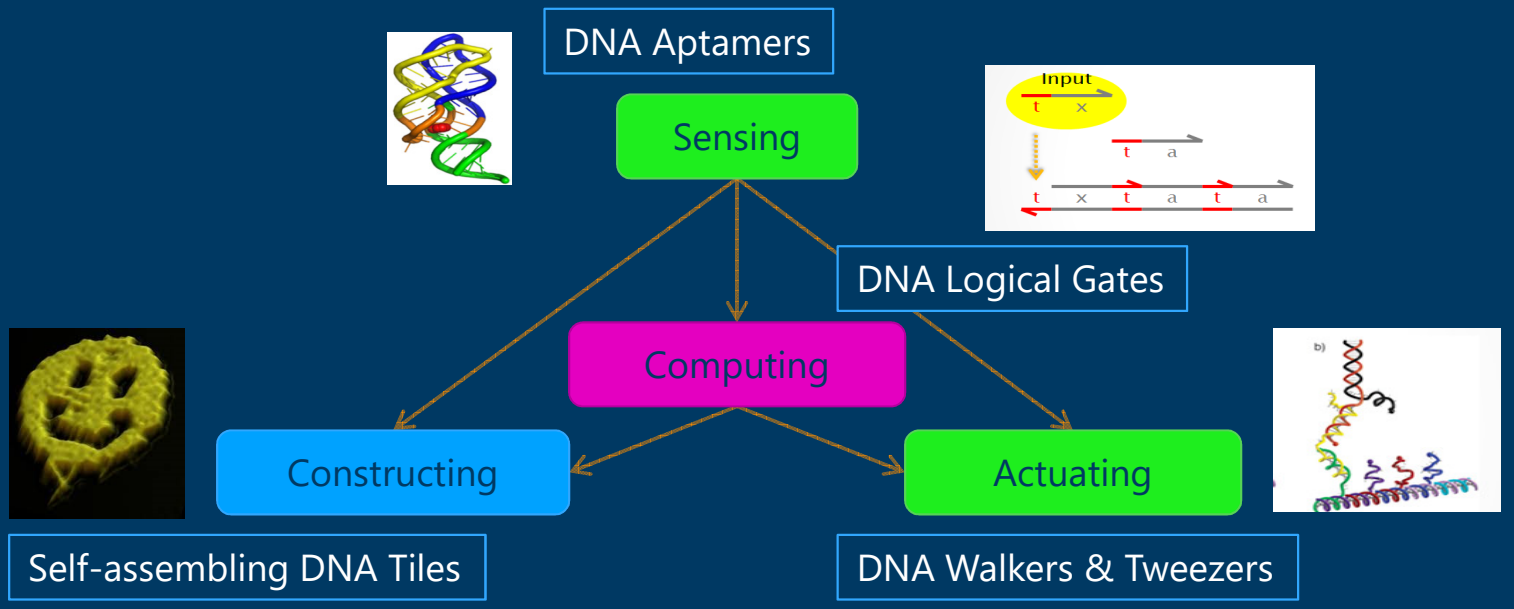
DNA as a universal substrate for chemical kinetics PNAS

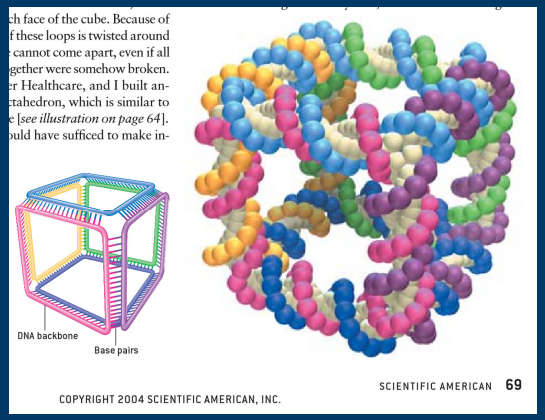
David Soloveichik[a,1], Georg Seelig[a,b,1], and Erik Winfree[c,1]

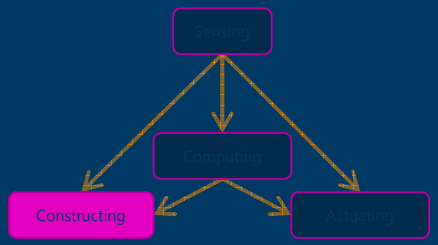


Building Nano-Control Devices

- All the components of nanocontrollers can already be built entirerly and solely with DNA, and interfaced to the environment

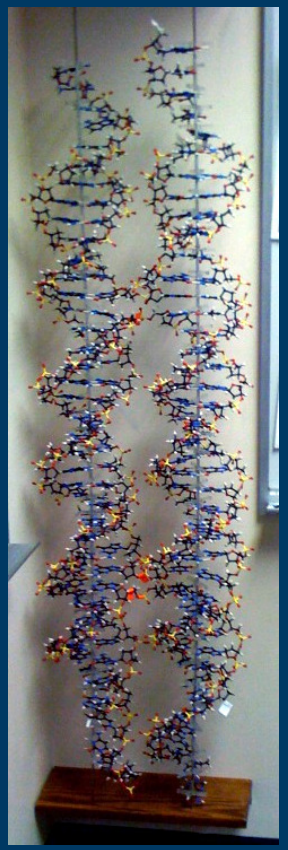


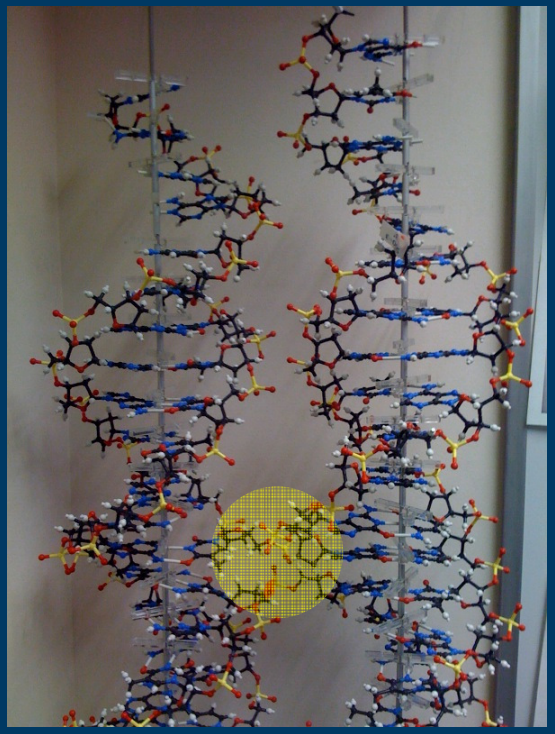




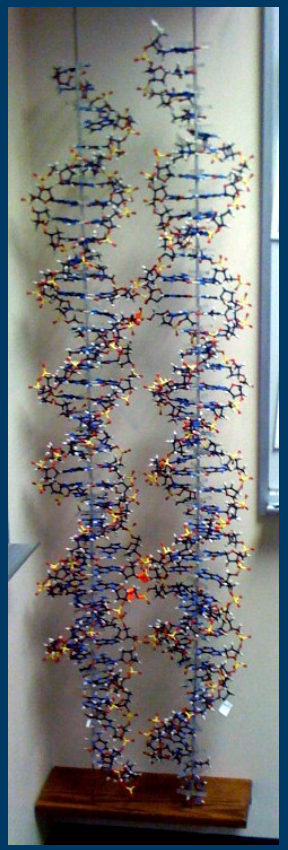
Constructing

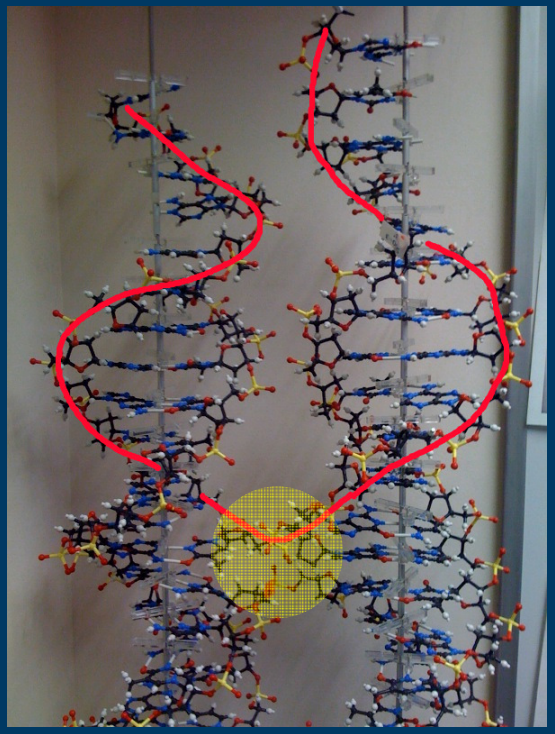
Crosslinking





Crosslinking





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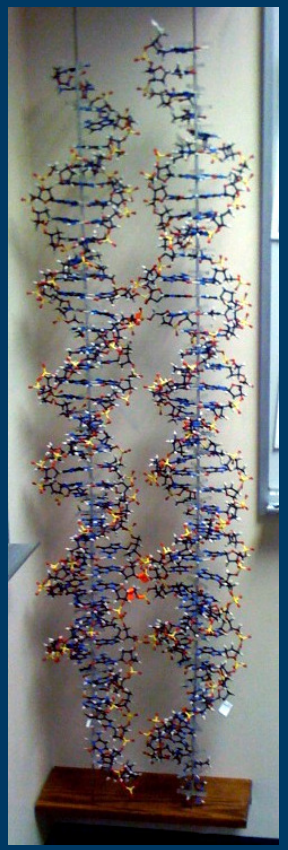

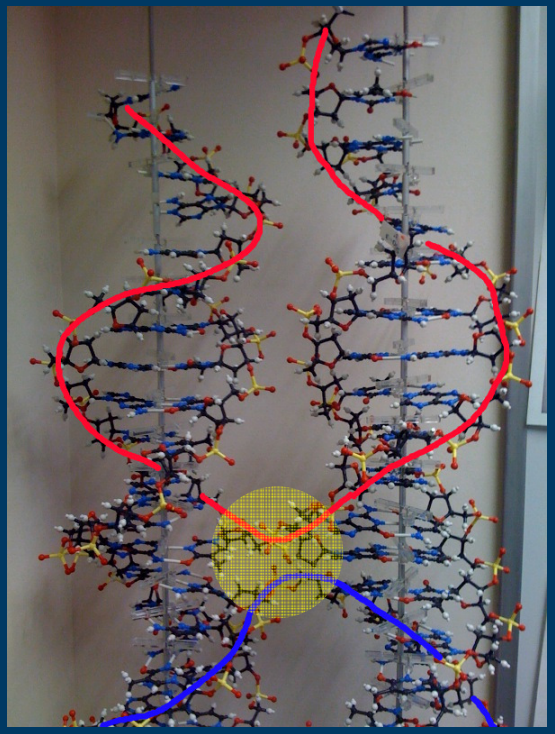

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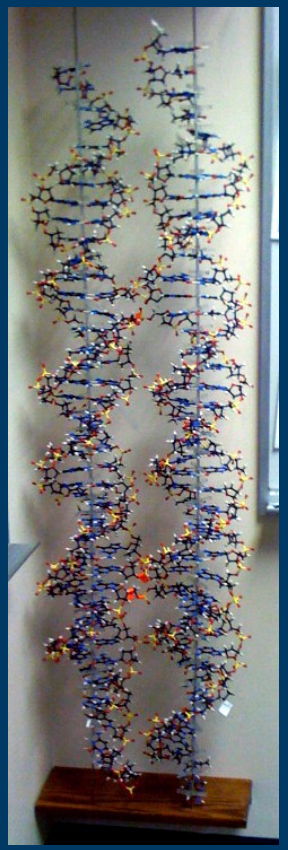

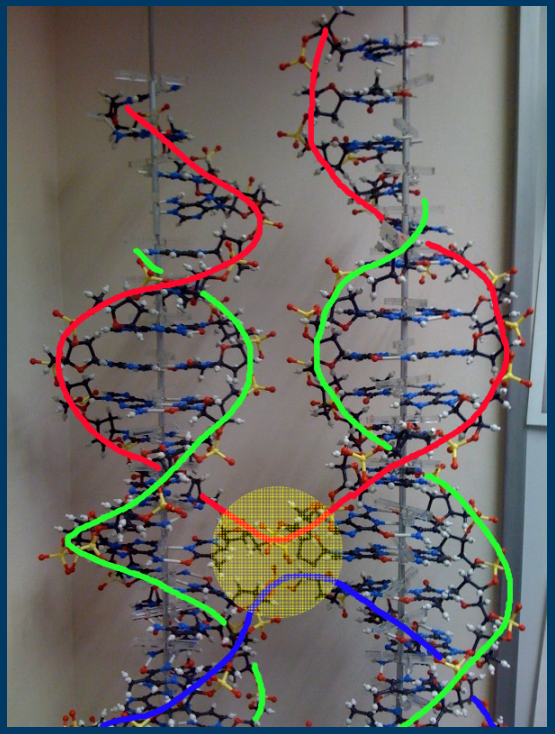

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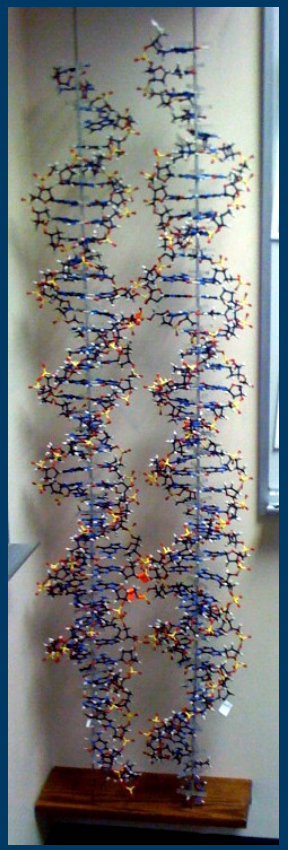
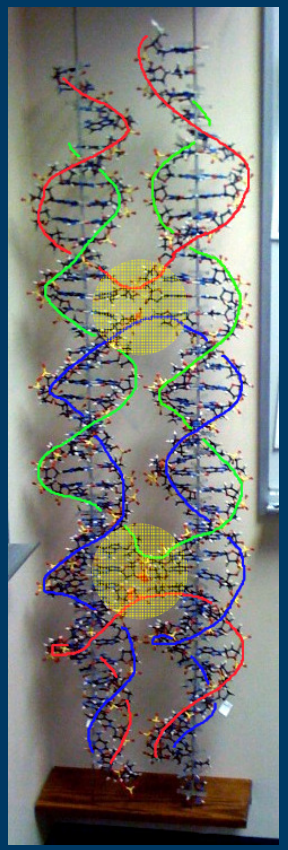
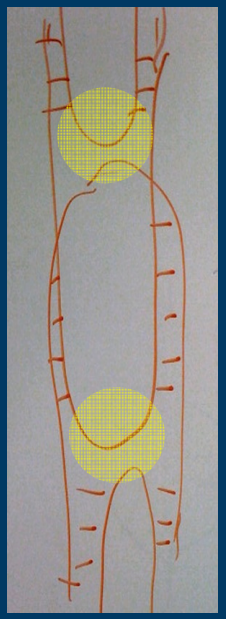


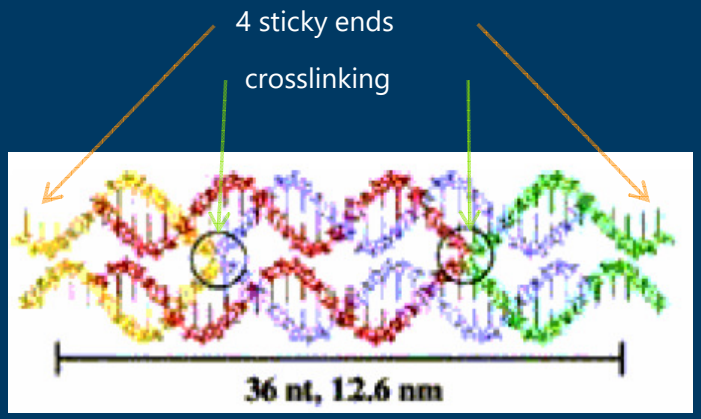


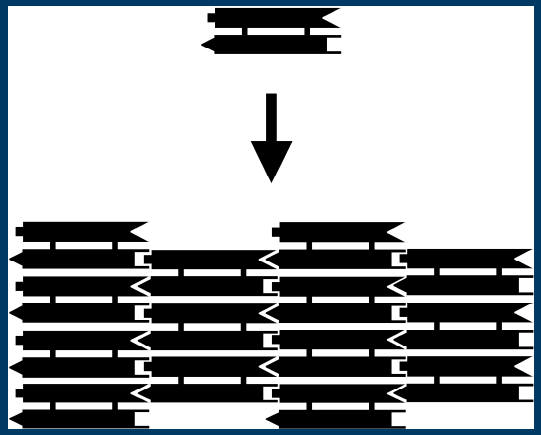
In nature, crosslinking is deadly (blocks DNA replication).

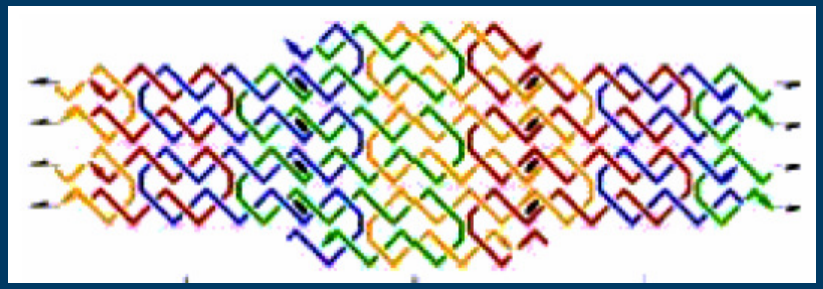


In engineering, crosslinking is the key to using DNA as a construction material.

DNA Tiling







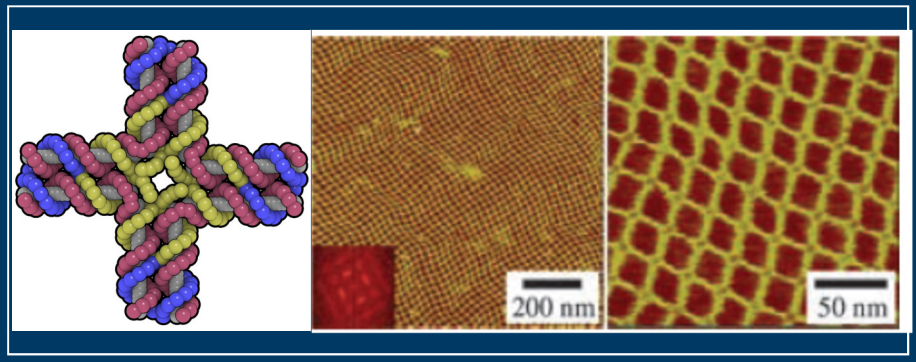
Construction and manipulation of DNA tiles in free space

Pankhudi

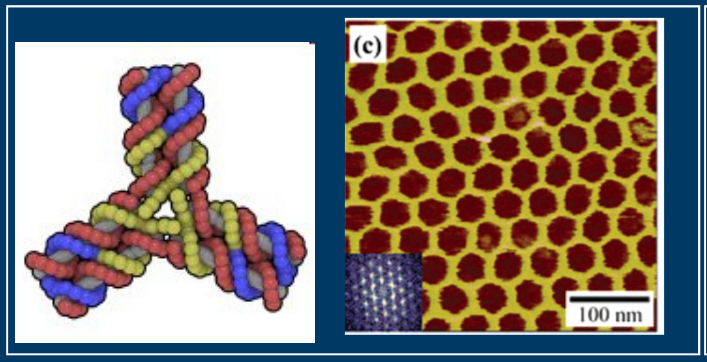
2D DNA Lattices

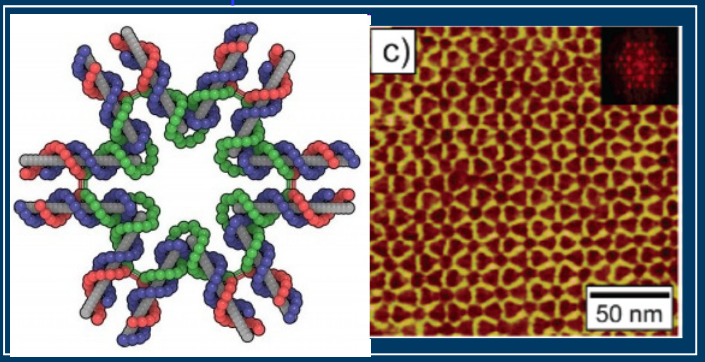
Chengde Mao

Purdue University, USA



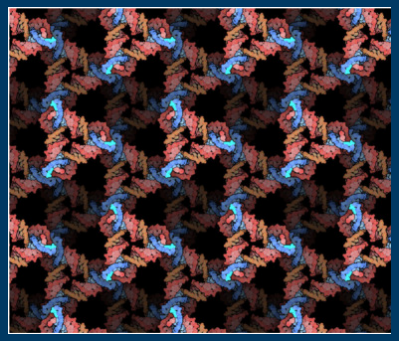
N-point Stars

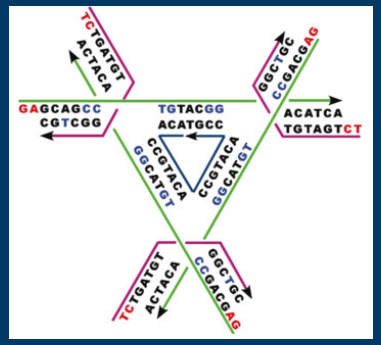




3D DNA Structures

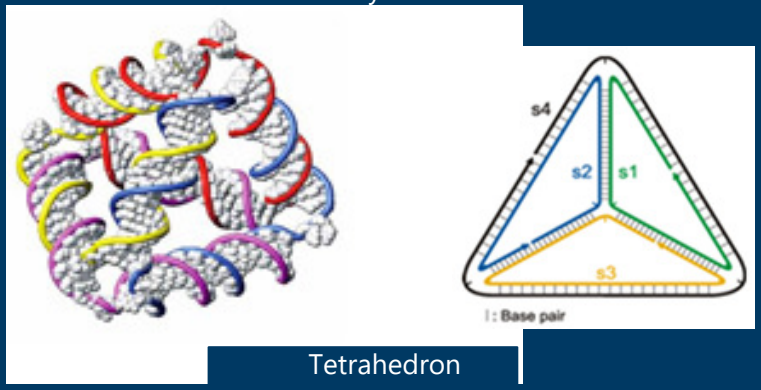
Ned Seeman
NYU





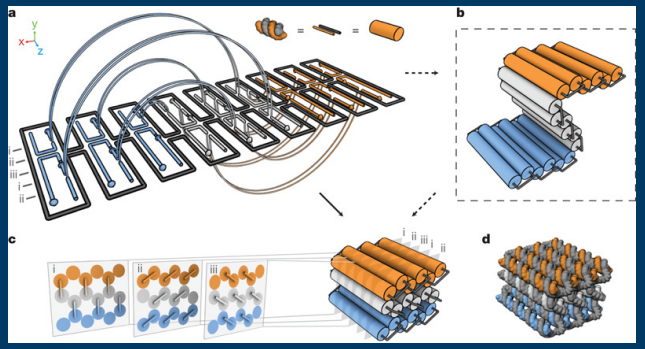
3D Cyrstal

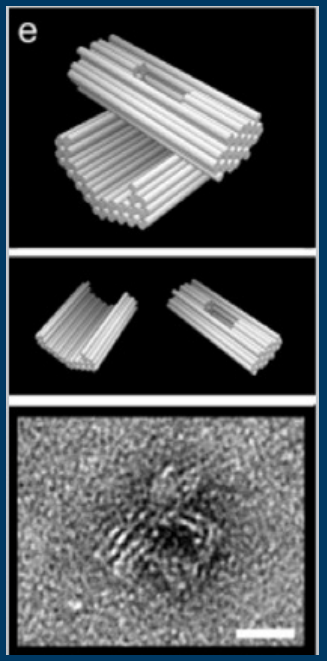
AndrewTuberfield
Oxford

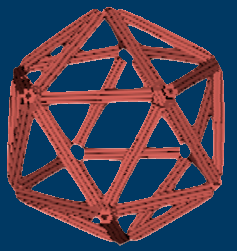


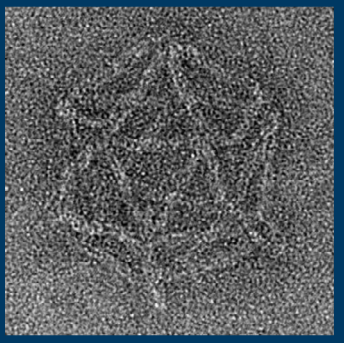
Tetrahedron

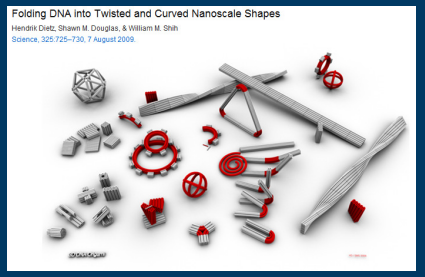
CADnano











William Shih
Harvard

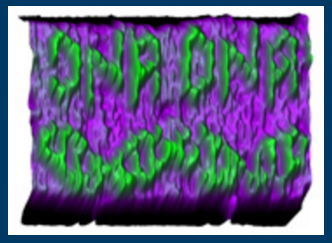
https://www.youtube.com/watch?v=Ek-FDPymyyg

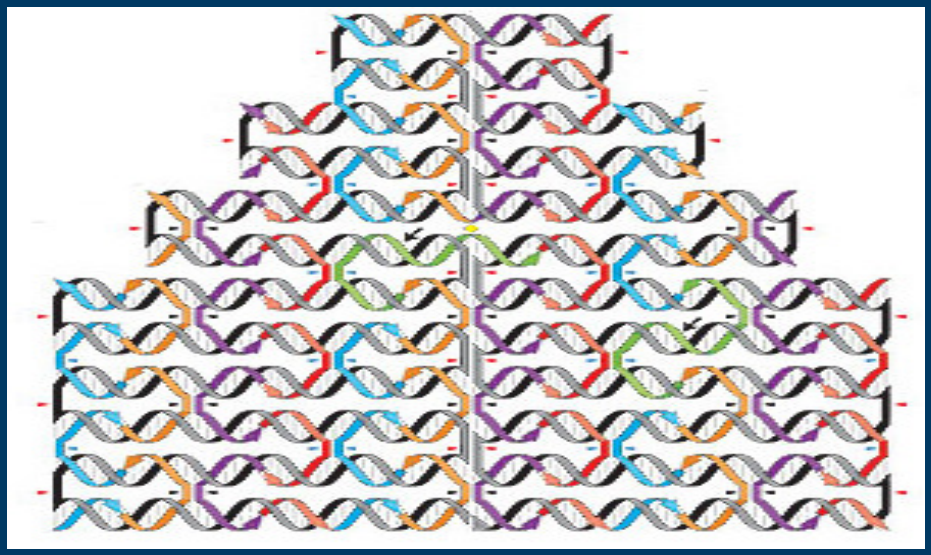
S.M. Douglas, H. Dietz, T. Liedl, B. Högberg, F. Graf and W. M. Shih
Self-assembly of DNA into nanoscale three-dimensional shapes, Nature (2009)

DNA Origami

Folding long (7000bp) naturally occurring (viral) ssDNA
By lots of short 'staple' strands that constrain it

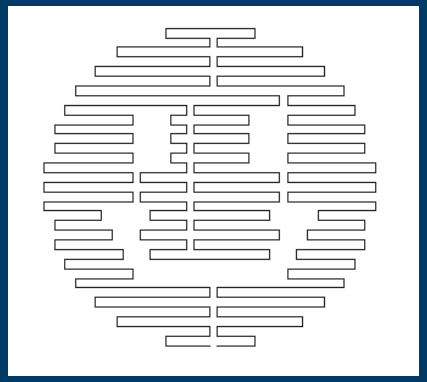
Paul W K Rothemund
California Institute of Technology

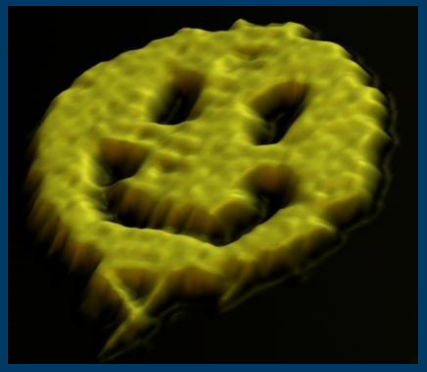




PWK Rothemund, *Nature* 440, 297 (2006)

Black/gray: 1 long viral strand (natural)
Color: many short staple strands (synthetic)

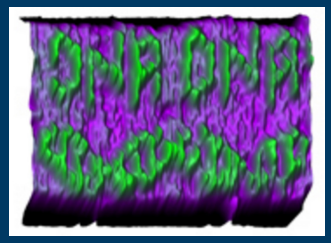




Paul Rothemund's "Disc with three holes" (2006)

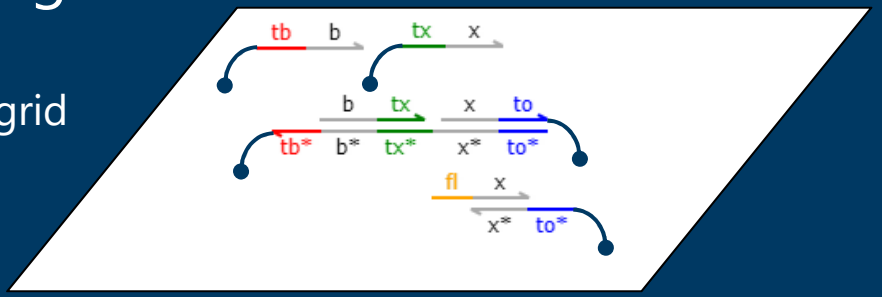
DNA Circuit Boards

- DNA origami are arrays of uniquely-addressable locations
  - Each staple is different and binds to a unique location on the origami
  - It can be extended with a unique sequence so that something else will attach uniquely to it.



Some staples are attached to "green blobs" (as part of their synthesis) Other staples aren't

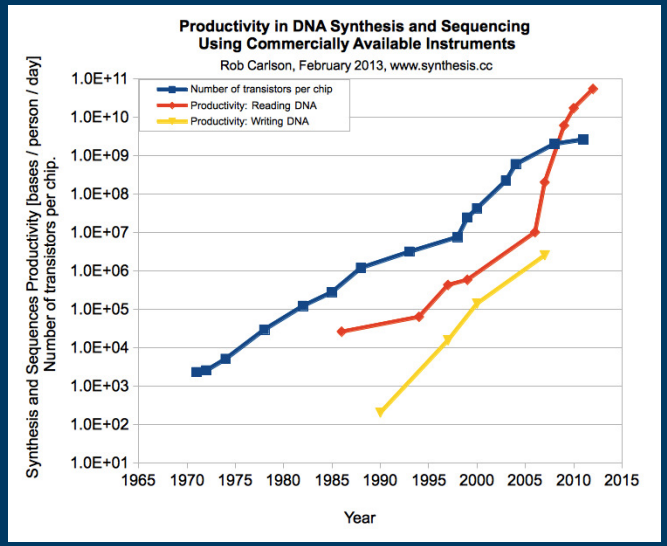
- More generally, we can bind "DNA gates" to specific locations
  - And so connect them into "DNA circuits" on a grid
  - Only neighboring gates will interact



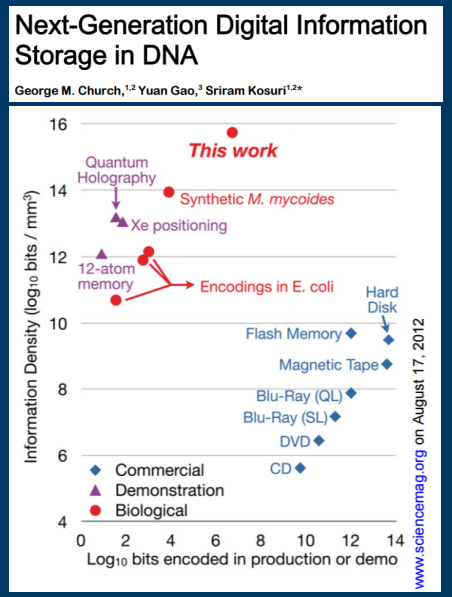
Dalchau, Chandran, Gopalkrishnan, Reif, Phillips. 2014

DNA Storage (Read/Write)

DNA has a data density of 140 exabytes (1.4×10^{20} bytes) per mm^3 compared to state-of the art storage media that reaches ~500 megabytes (5×10^{8} bytes) per mm^3
DNA has been shown to be stable for millions of years

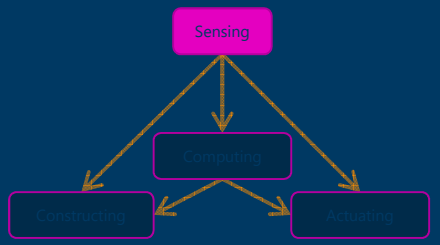


The Pace and Proliferation of Biological Technologies
March 4, 2004 by Rob Carlson



We have machines that can read (sequence) and write (synthesize) DNA. The Carslon Curve of "productivity" is growing much faster than Moore's Law.

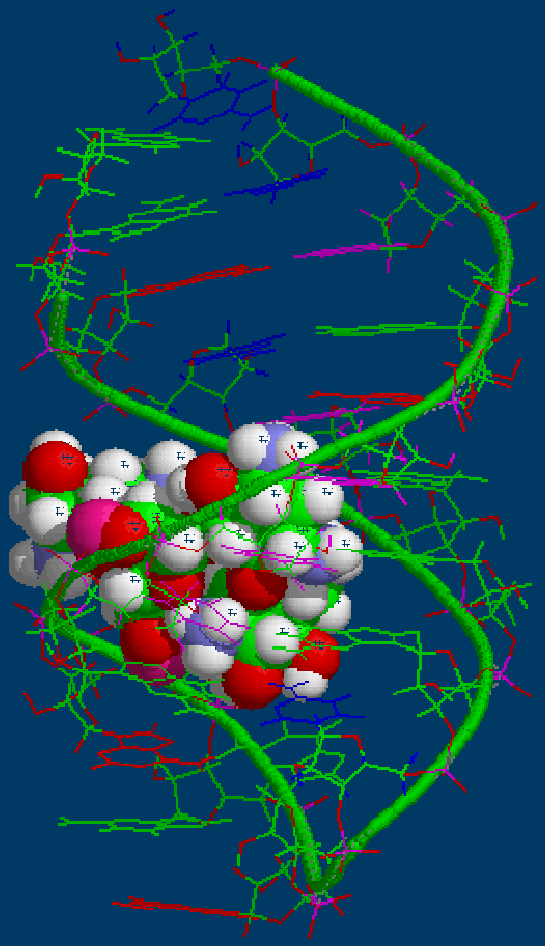
Cost of sequencing is decreasing rapidly ($1000 whole human genome), while cost of synthesis is decreasing very slowly.
[Rob Carlson, www.synthesis.cc]



Sensing

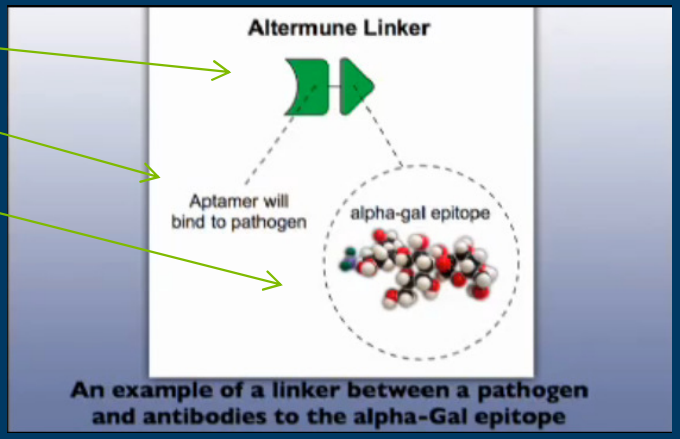
Aptamers

Artificially evolved DNA molecules that stick to anything you like highly selectively

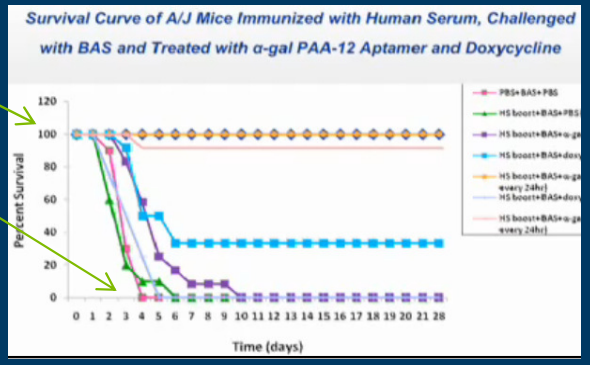


Pathogen Spotlights

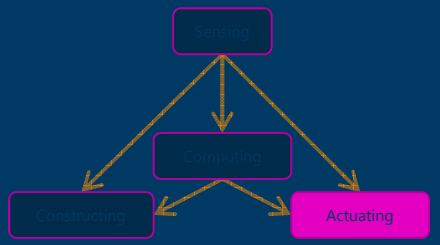
- DNA aptamer binds to:
  - A) a pathogen
  - B) a molecule our immune system (when allergic) hates and immediately removes (eats) along with anything attached to it!



- Result: instant immunity
  - Mice poisoned with Anthrax plus aptamer (100% survival)
  - Mice poinsoned with Anthrax (not so good)

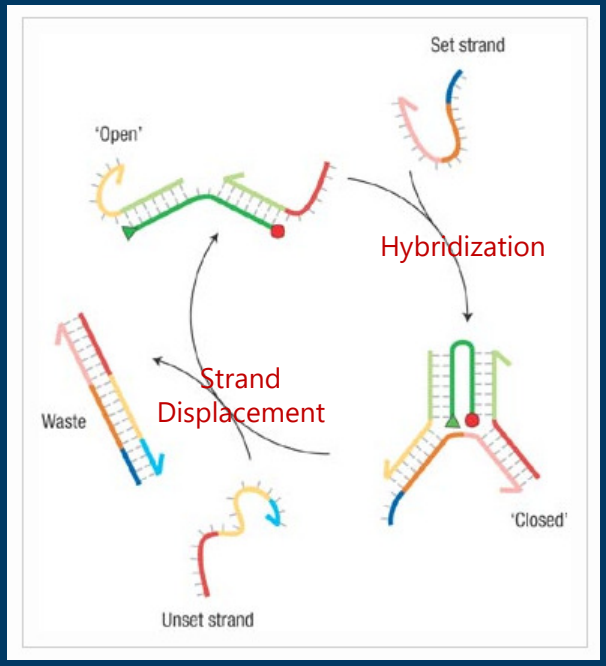


Kary Mullis (incidentally, also Nobel prize for inventing the Polymerase Chain Reaction)



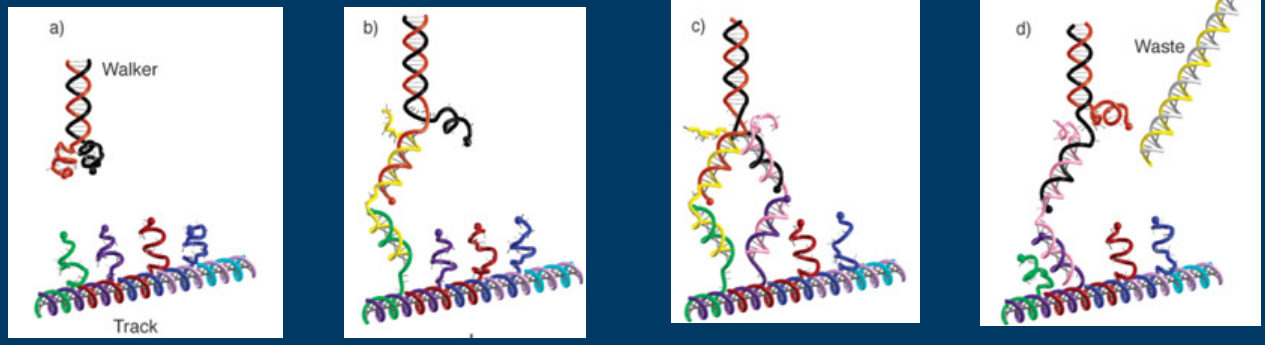
Actuating

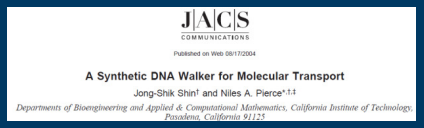
DNA Tweezers



DNA nanomachines
Jonathan Bath & Andrew J. Turberfield
Nature Nanotechnology **2**, 275 - 284 (2007)
doi:10.1038/nnano.2007.104

DNA Walkers





J|A|C|S
COMMUNICATIONS

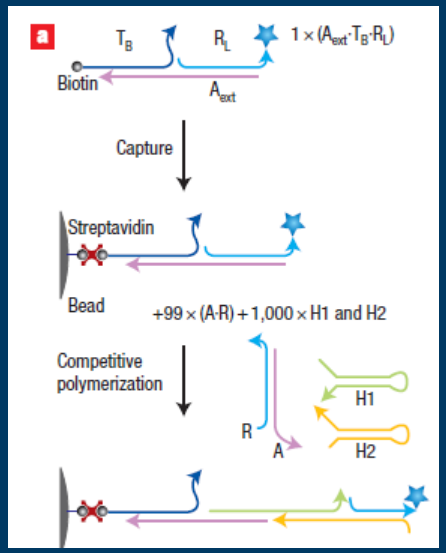
Published on Web 08/17/2004

A Synthetic DNA Walker for Molecular Transport

Jong-Shik Shin† and Niles A. Pierce*,†,‡

Departments of Bioengineering and Applied & Computational Mathematics, California Institute of Technology, Pasadena, California 91125

Polymerization Motor



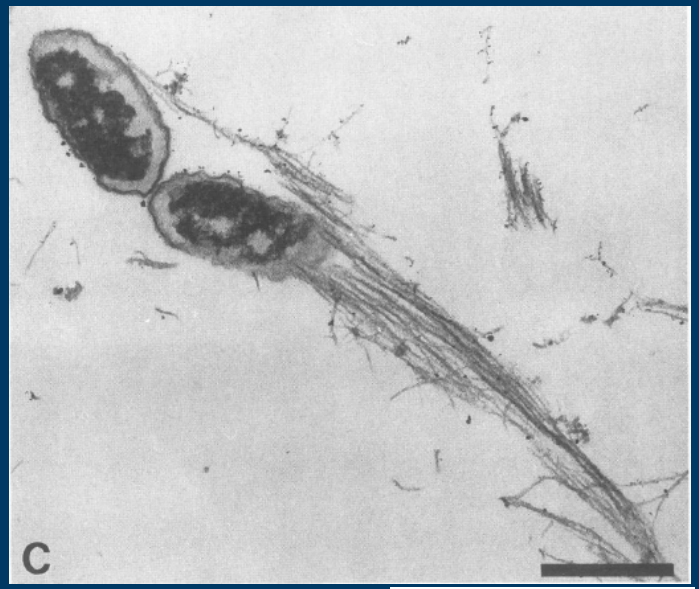
An autonomous polymerization motor powered by DNA hybridization

SUVIR VENKATARAMAN[1], ROBERT M. DIRKS[1], PAUL W. K. ROTHEMUND[2,3], ERIK WINFREE[2,3] AND NILES A. PIERCE[1,4]*

Triggered amplification by hybridization chain reaction

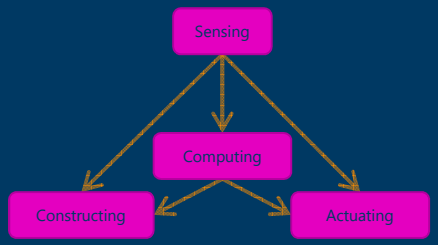
Robert M. Dirks† and Niles A. Pierce‡,§

Rickettsia (spotted fever)



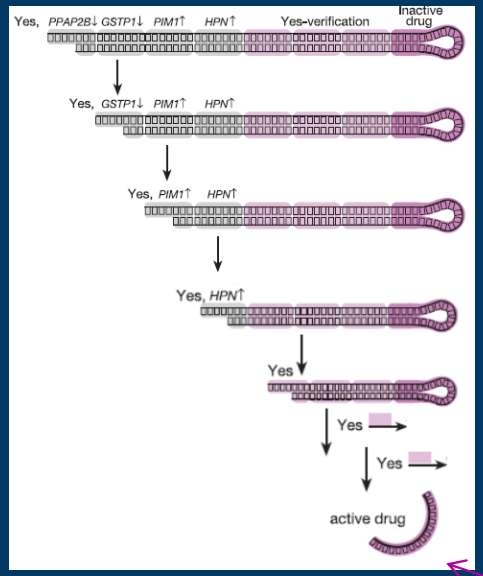
Directional Actin Polymerization Associated with Spotted Fever Group Rickettsia Infection of Vero Cells

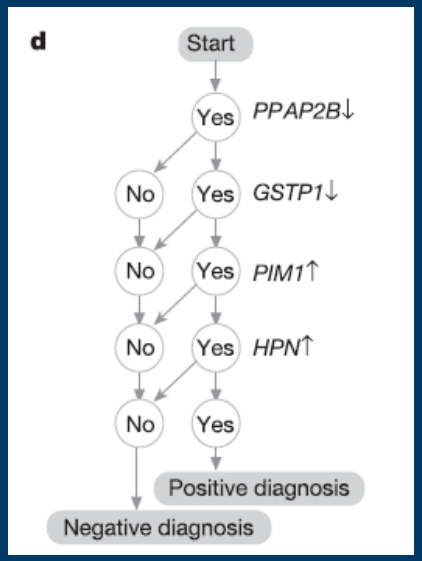
ROBERT A. HEINZEN, STANLEY F. HAYES, MARIUS G. PEACOCK, AND TED HACKSTADT*



Curing...

Computational Drugs





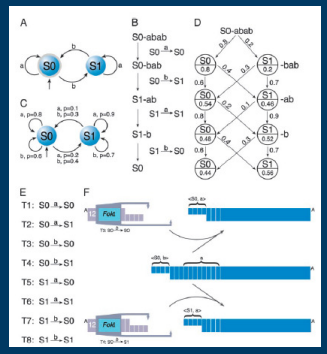
Vitravene (GCGTTTGCTCTTCTTCTTGCG)

- An automaton sequentially reading the string PPAP2B, GSTP1, PIM1, HPS (known cancer indicators) and sequentially cutting the DNA hairpin until a ssDNA drug (Vitravene) is released.

An autonomous molecular computer for logical control of gene expression

Yaakov Benenson[1,2], Binyamin Gil[2], Uri Ben-Dor[1], Rivka Adar[2] & Ehud Shapiro[1,2]

Based on restriction enzymes



Stochastic computing with biomolecular automata

Rivka Adar*†, Yaakov Benenson*†‡, Gregory Linshiz*‡, Amit Rosner§, Naftali Tishby¶§, and Ehud Shapiro*‖

Interfacing to Biology

- A doctor in each cell

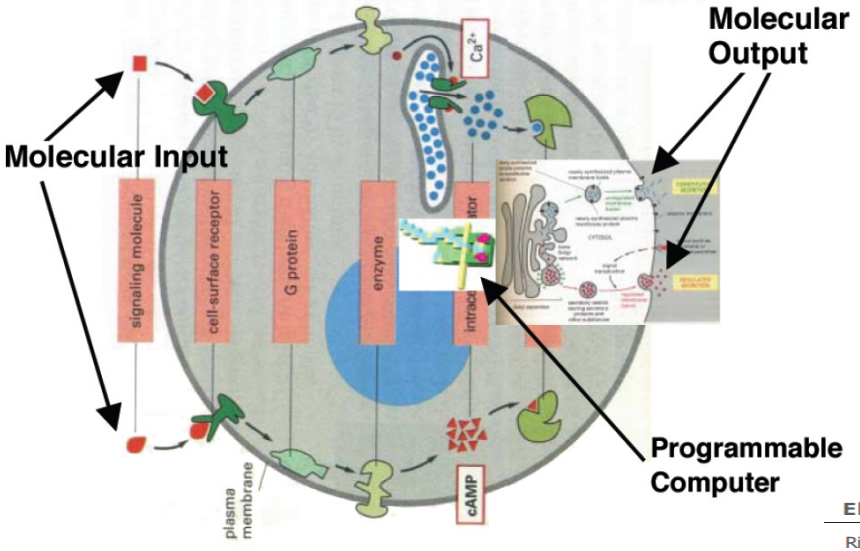


Fig. 1 Medicine in 2050: "Doctor in a Cell"

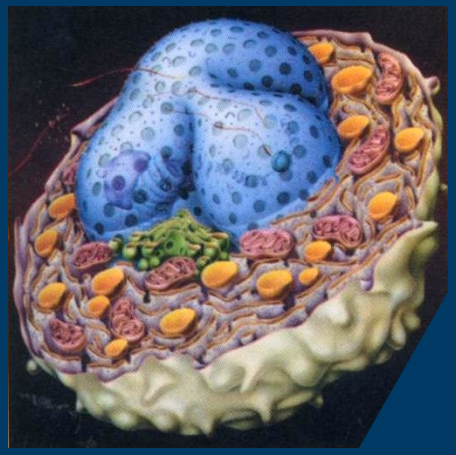
Ehud Shapiro
Rivka Adar
Kobi Benenson
Gregory Linshitz
Aviv Regev
William Silverman

Molecules and computation

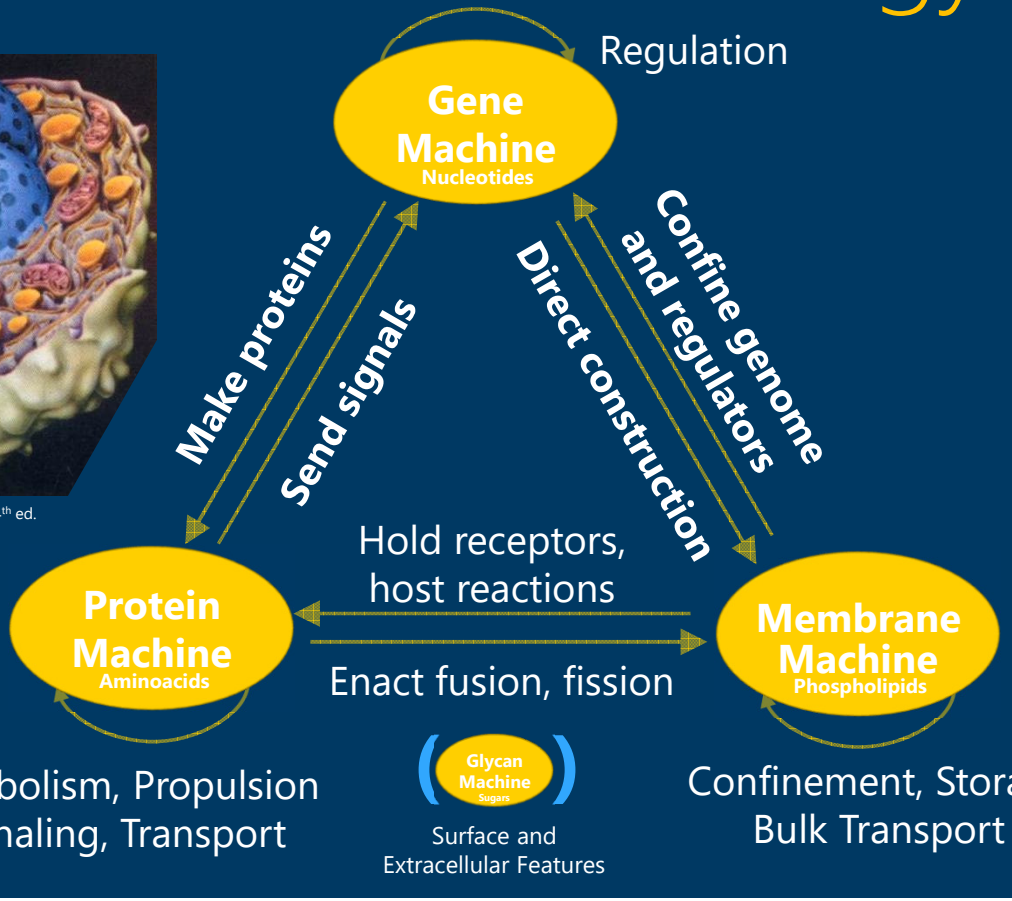
Molecular Programming:
The Biological Aspect

Biological systems are already
'molecularly programmed'

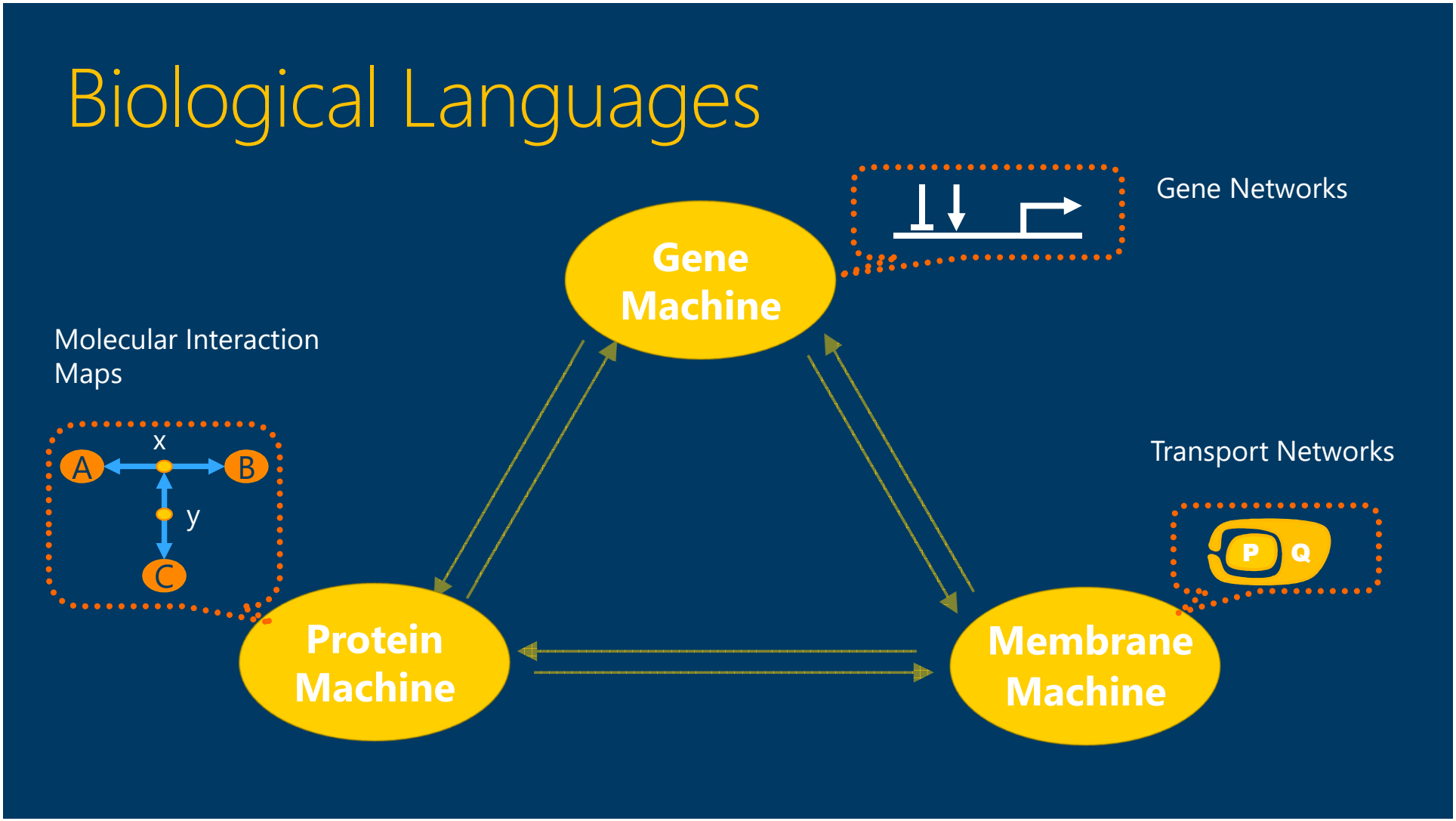
Abstract Machines of Biology



H.Lodish & al. Molecular Cell Biology 4th ed.



Biological Languages



But …

- Biology is programmable, but (mostly) not by us!

- Still work in progress:
  - Gene networks are being programmed in synthetic biology, but using existing ‘parts’
  - Protein networks are a good candidate, but we cannot yet effectively design proteins
  - Transport networks are being investigated for programming microfluidic devices that manipulate vesicles

Molecular Languages

… that we can execute

Our Assembly Language: Chemistry

- A Lingua Franca between Biology, Dynamical Systems, and Concurrent Languages
- Chemical Reaction Networks
  - $A + B \rightarrow_r C + D$ (the program)
- Ordinary Differential Equations
  - $d[A]/dt = -r[A][B]$ … (the behavior)
- Rich analytical techniques based on Calculus
- But prone to combinatorial explosion
  - E.g., due to the peculiarities of protein interactions

How do we "run" Chemistry?

- Chemistry is not easily executable
  - "Please Mr Chemist, execute me this bunch of reactions that I just made up"
- Most molecular languages are not executable
  - They are descriptive (modeling) languages
- How can we execute molecular languages?
  - With real molecules?
  - That we can design ourselves?
  - And that we can buy on the web?

Molecular Programming with DNA

Building the cores of programmable molecular controllers

The role of DNA Computing

- Non-goals
  - Not to solve NP-complete problems with large vats of DNA
  - Not to replace silicon
- Bootstrapping a carbon-based technology
  - To precisely control the organization and dynamics of matter and information at the molecular level
  - DNA is our engineering material
    - Its biological origin is “accidental” (but convenient)
    - It is an information-bearing programmable material
    - Other such materials will be (are being) developed

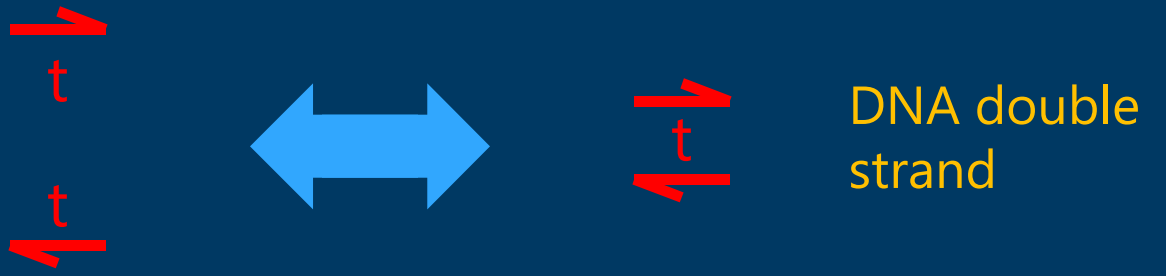
Domains

- Subsequences on a DNA strand are called domains
  - *provided* they are "independent" of each other

oriented DNA single strand

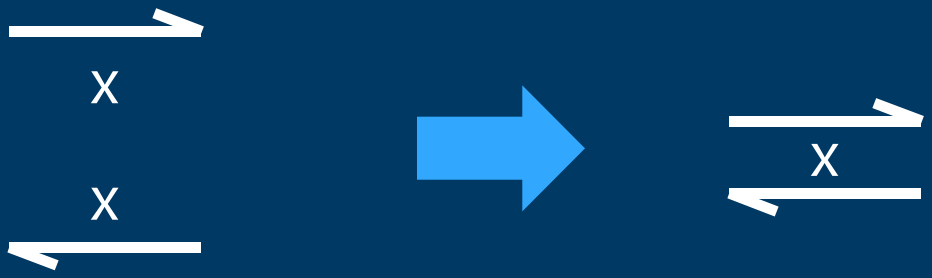
- Differently named domains must not hybridize
  - With each other, with each other's complement, with subsequences of each other, with concatenations of other domains (or their complements), etc.

Short Domains



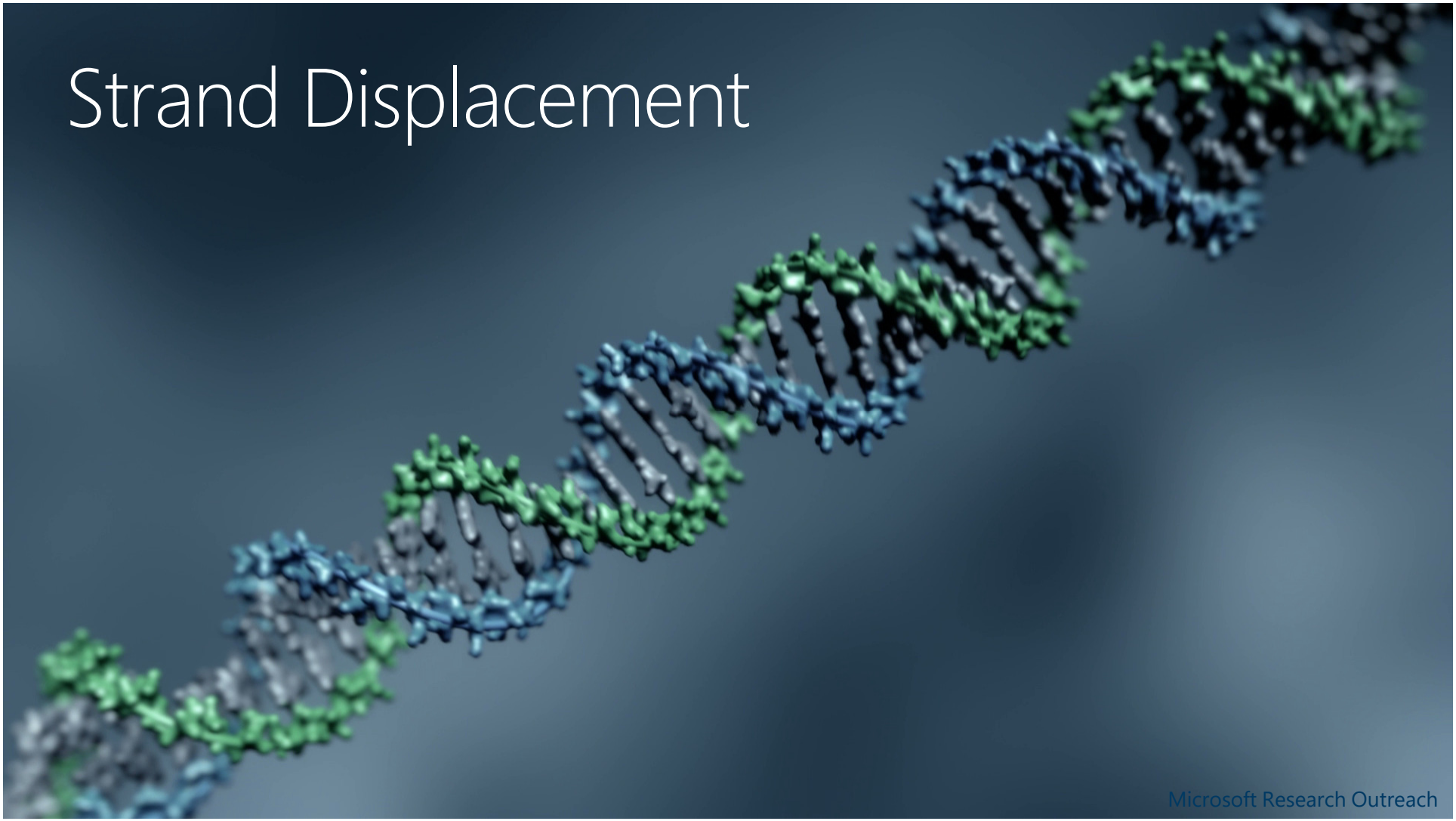
Reversible Hybridization

Long Domains

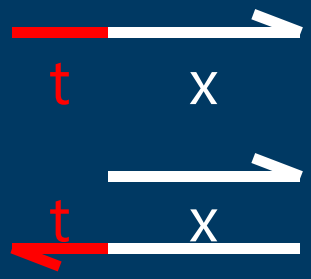


Irreversible Hybridization

Strand Displacement

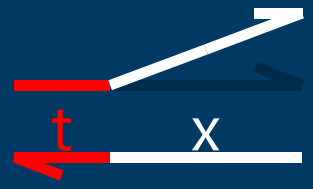


Strand Displacement



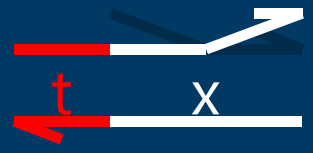
"Toehold Mediated"

Strand Displacement



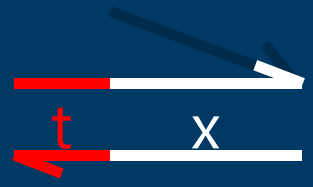
Toehold Binding

Strand Displacement



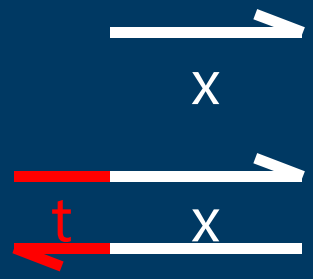
Branch Migration

Strand Displacement



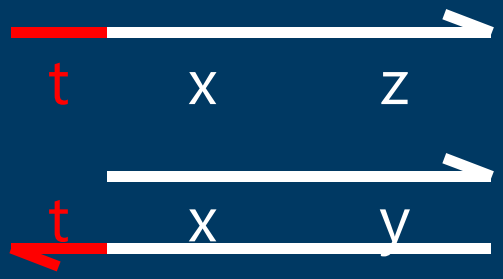
Displacement

Strand Displacement

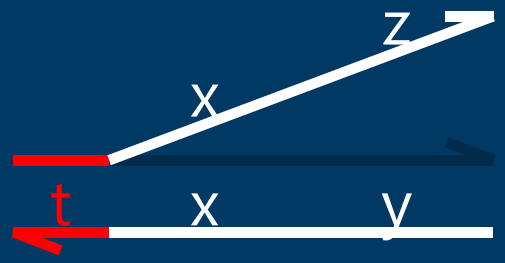


Irreversible release

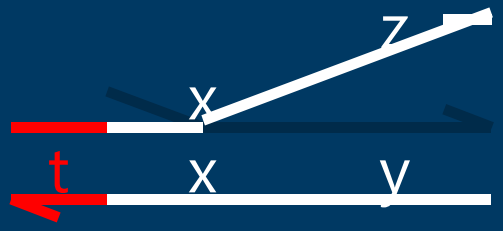
Bad Match



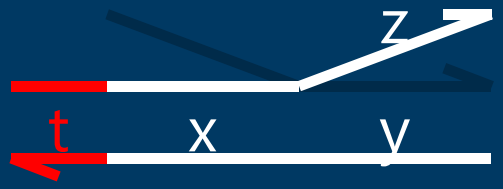
Bad Match



Bad Match



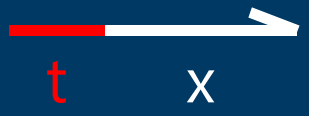
Bad Match



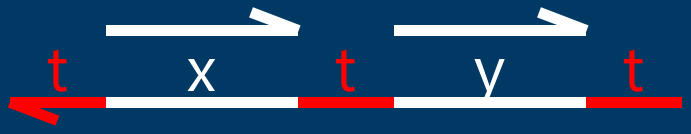
Cannot proceed
Hence will undo

Two-Domain Architecture

- Signals: 1 toehold + 1 recognition region



- Gates: "top-nicked double strands" with open toeholds



Garbage collection "built into" the gate operation

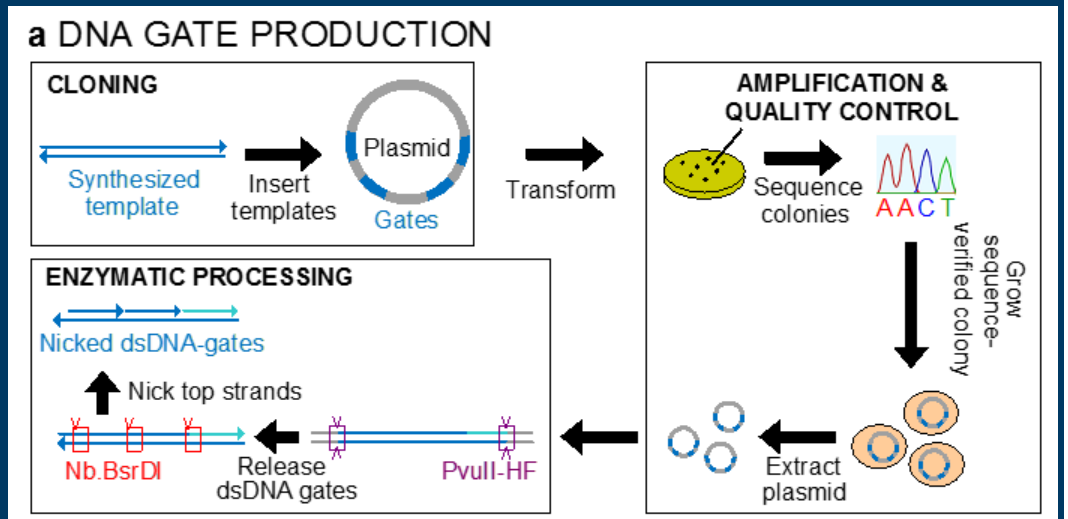
Two-Domain DNA Strand Displacement

Luca Cardelli

In S. B. Cooper, E. Kashefi, P. Panangaden (Eds.): Developments in Computational Models (DCM 2010). EPTCS 25, 2010, pp. 33-47. May 2010.

Plasmidic Gate Technology

- Synthetic DNA is length-limited
  - Finite error probability at each nucleotide addition, hence ~ 200nt max
- Bacteria can replicate plasmids for us
  - Loops of DNA 1000's nt, with extremely high fidelity
  - Practically no structural limitations on gate fan-in/fan-out



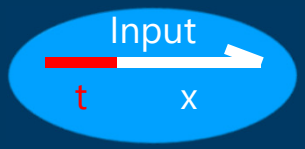
Programmable chemical controllers made from DNA

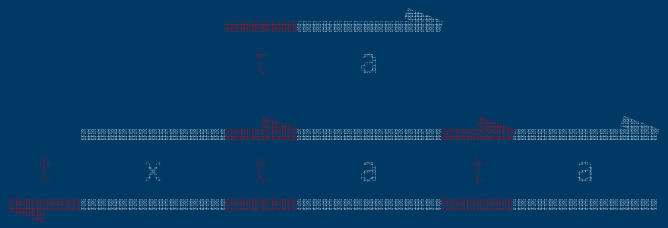
Yuan-Jyue Chen[1], Neil Dalchau[2], Niranjan Srinivas[3], Andrew Phillips[2], Luca Cardelli[2], David Soloveichik[4], and Georg Seelig[1,5]

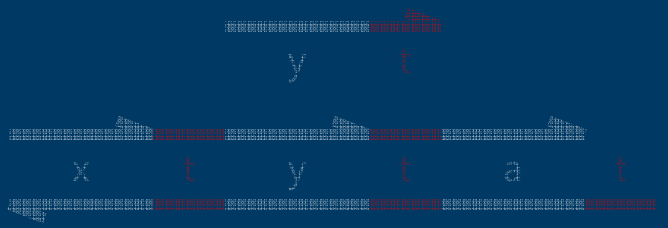
Only possible with two-domain architecture

Transducer

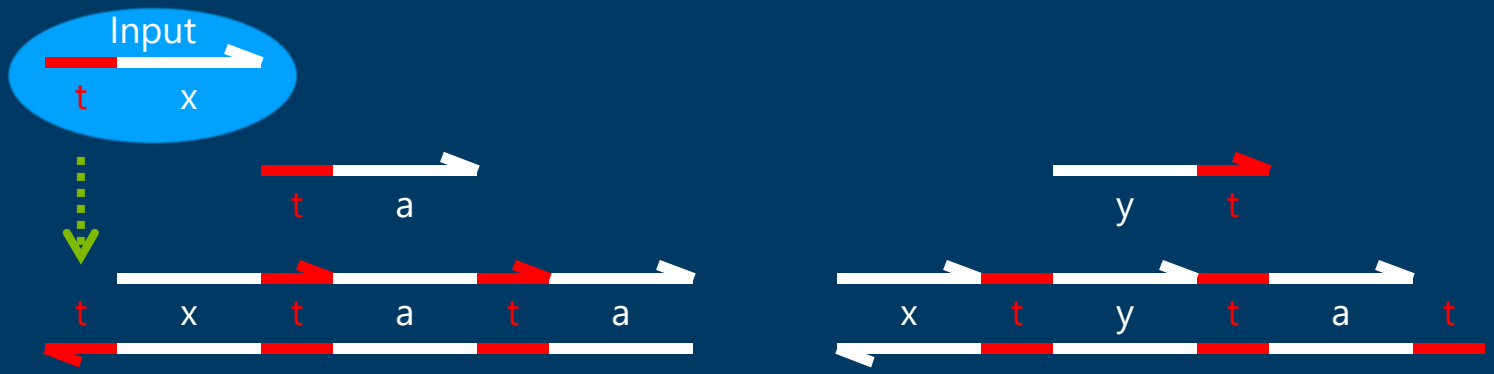
Transducer x→y







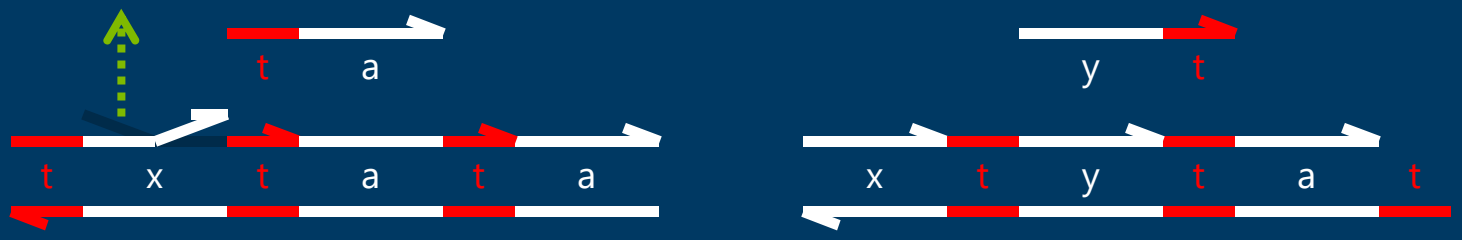
Transducer x→y



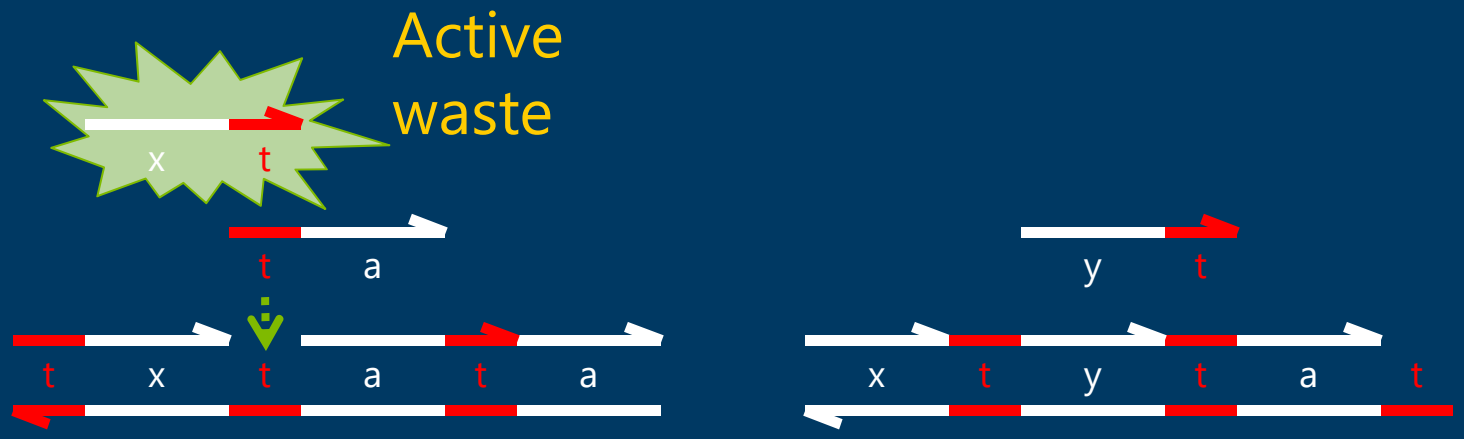
Built by self-assembly!

ta is a *private* signal (a different ‘a’ for each xy pair)

Transducer x→y

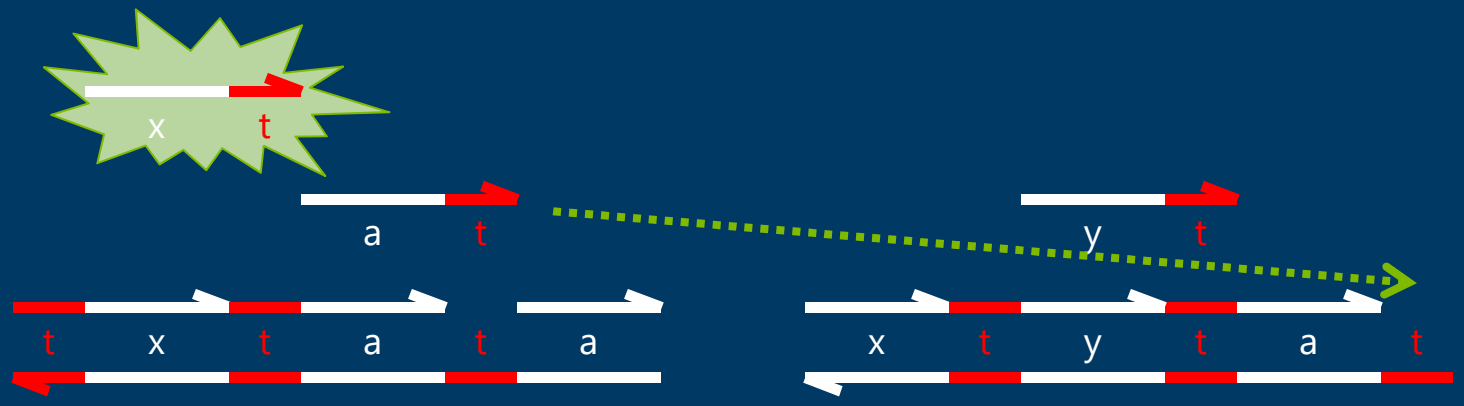


Transducer x⟶y



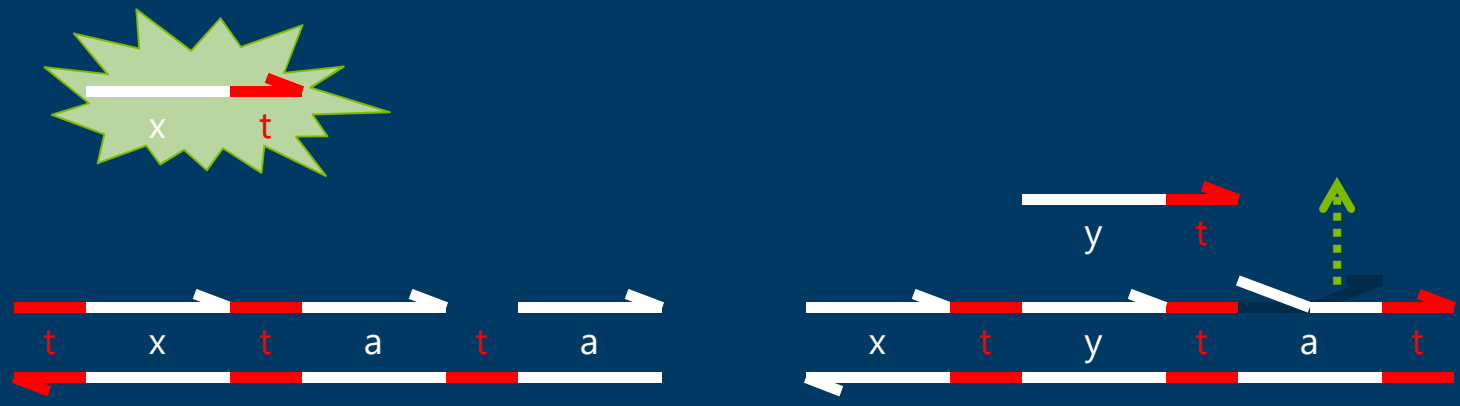
Transducer x→y

Transducer x→y

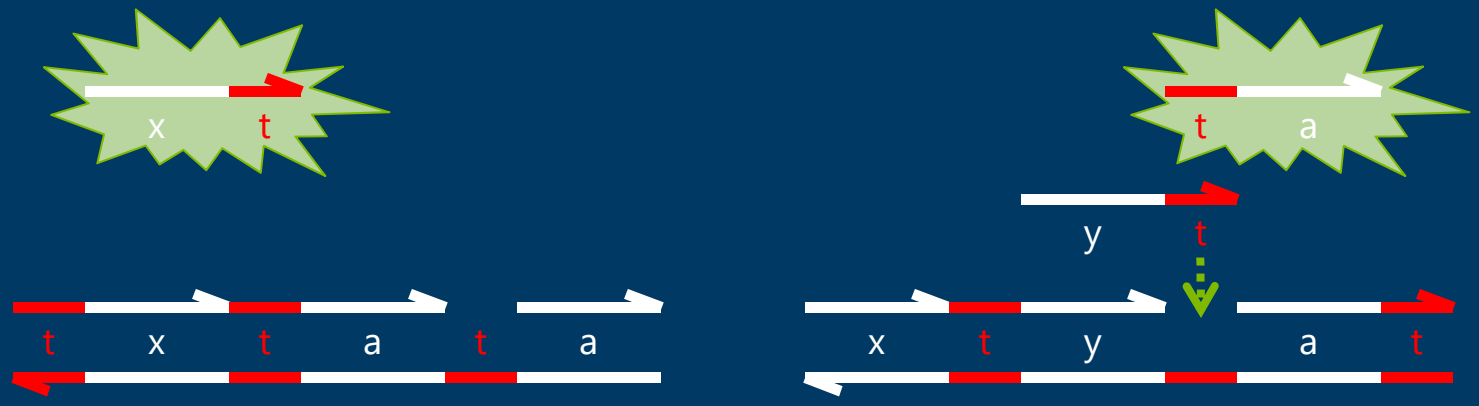


So far, a **tx** *signal* has produced an **at** *cosignal*.
But we want signals as output, not cosignals.

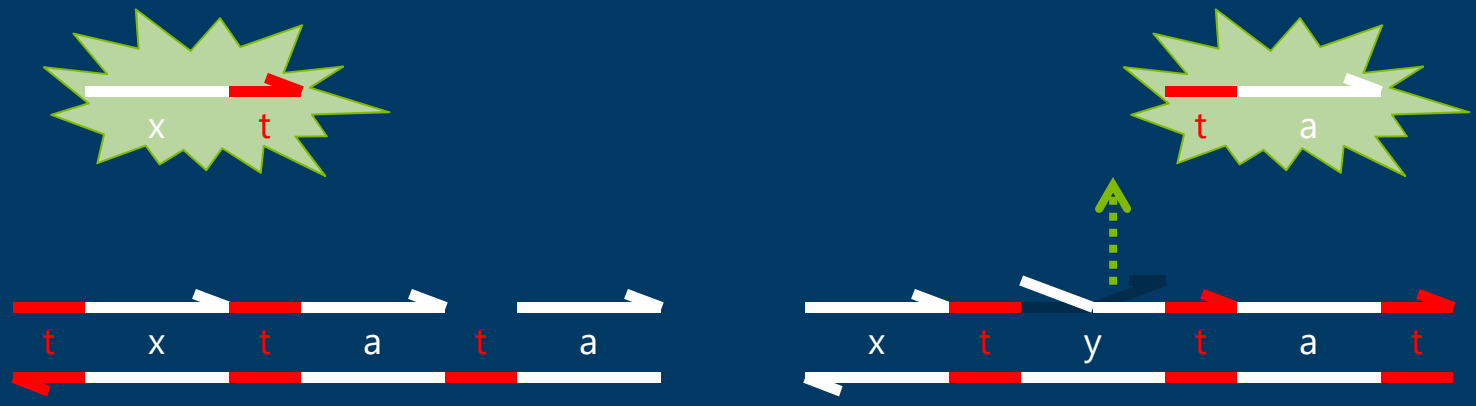
Transducer x→y



Transducer x→y



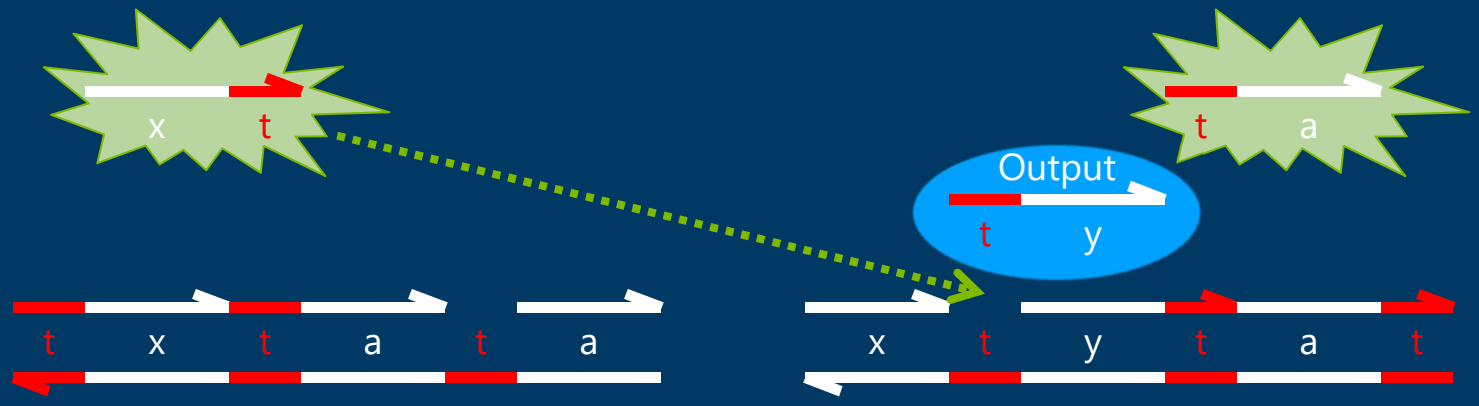
Transducer x⟶y



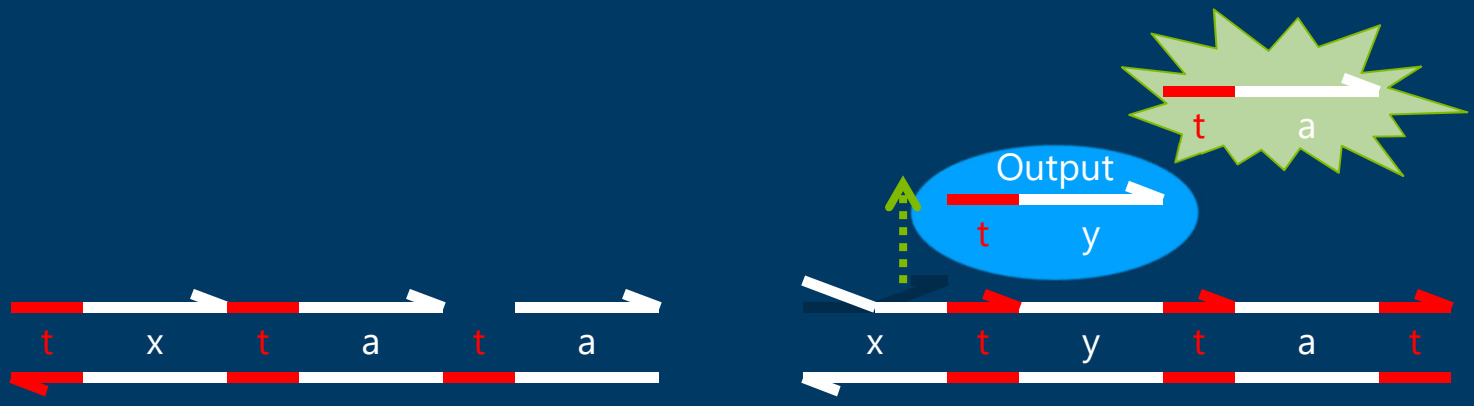
Transducer x⟶y

Here is our output **ty** *signal*.
But we are not done yet:
1) We need to make the output irreversible.
2) We need to remove the garbage.
We can use (2) to achieve (1).

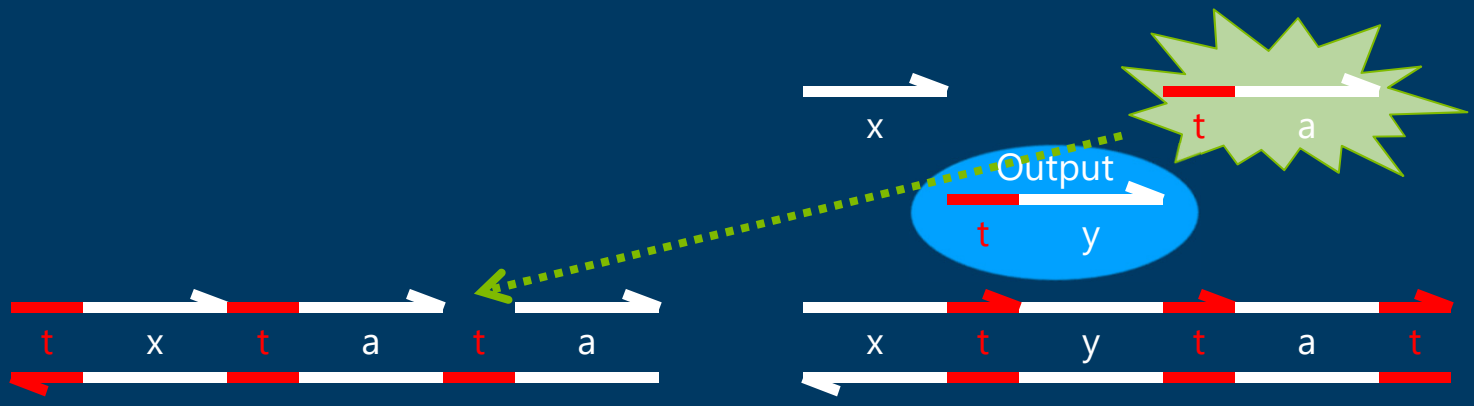
Transducer x→y



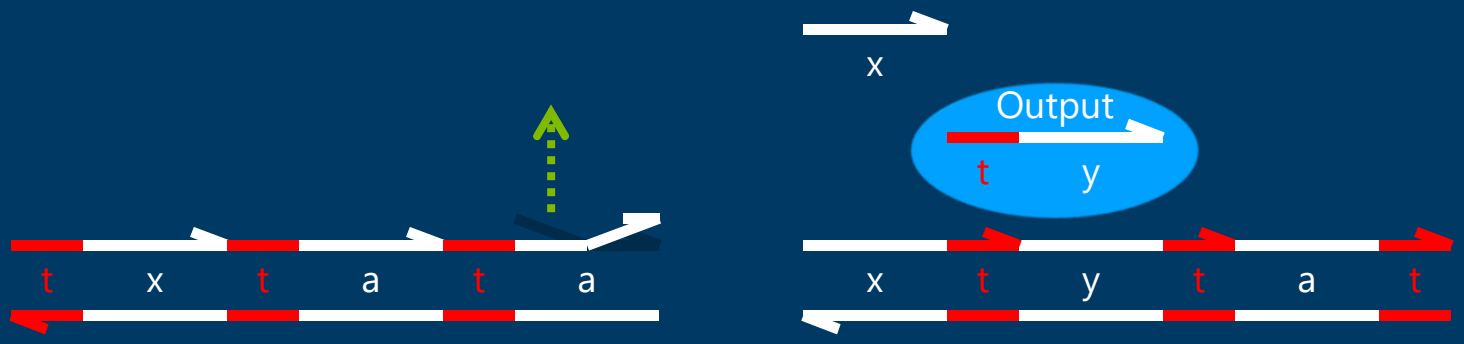
Transducer x⟶y



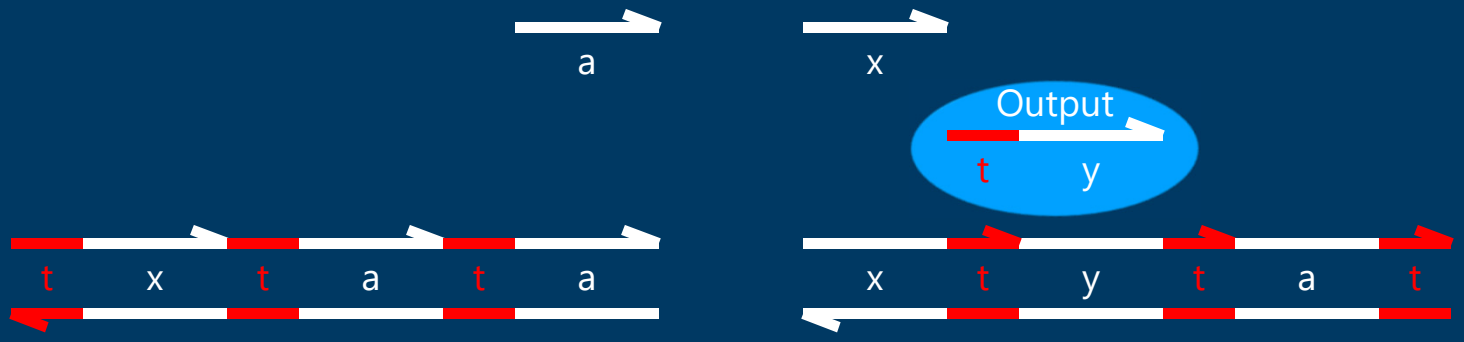
Transducer x⟶y



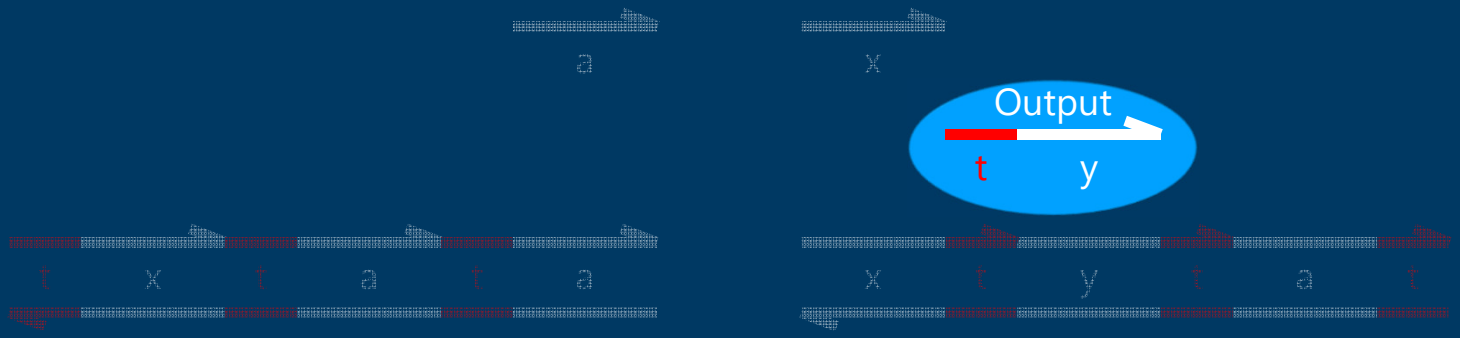
Transducer x⟶y



Transducer x→y



Transducer x⟶y



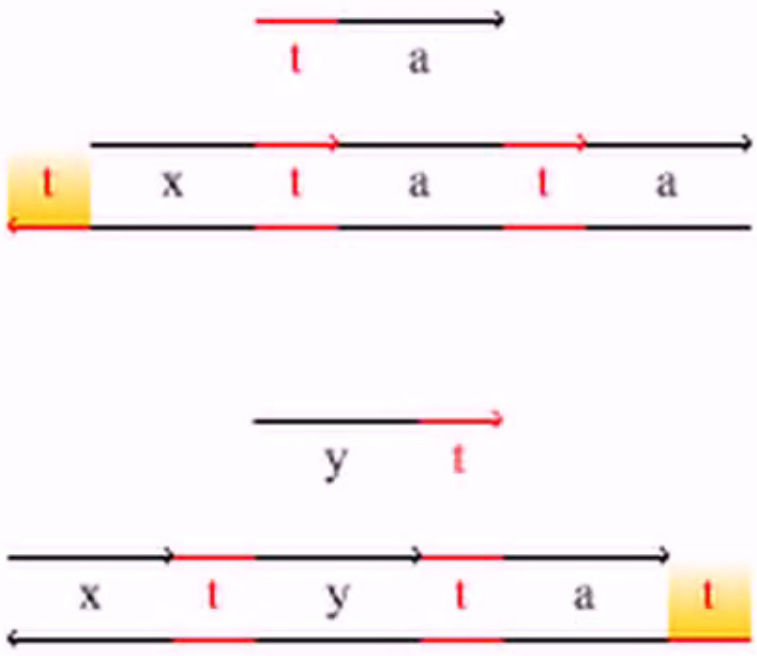
Done.

N.B. the gate is consumed: it is the energy source

(no proteins, no enzymes, no heat-cycling, etc.; just DNA in salty water)

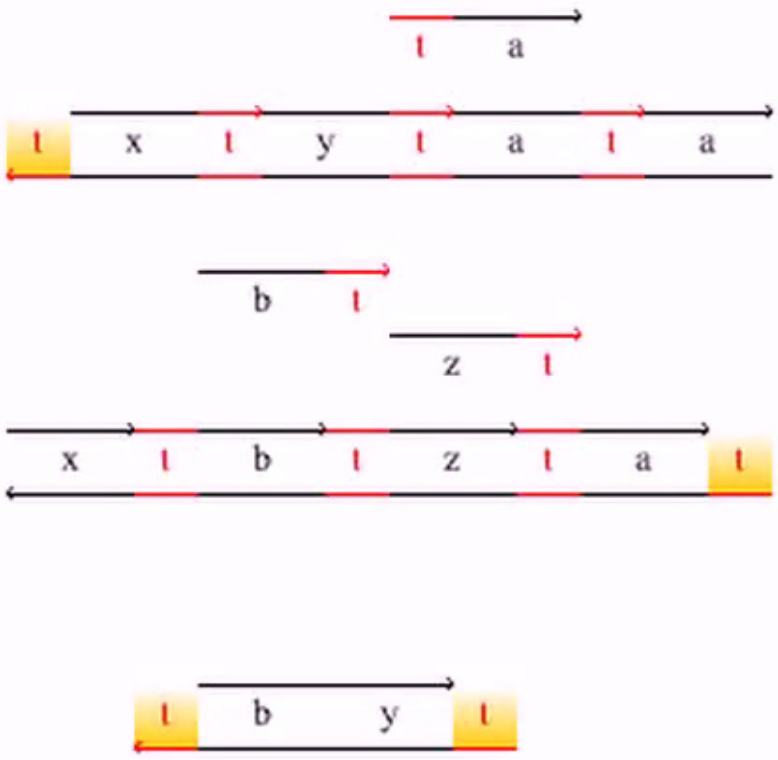
Powered by Sothink

Transducer x → y



Powered by Sothink

Join x+y → z

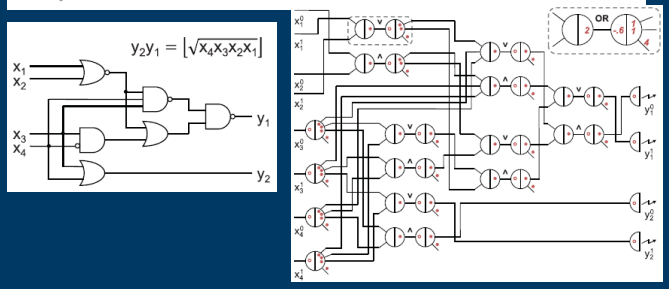


Large-scale Circuits (so far…)

3 JUNE 2011 VOL 332 SCIENCE

Scaling Up Digital Circuit Computation with DNA Strand Displacement Cascades

Lulu Qian[1] and Erik Winfree[1,2,3]*



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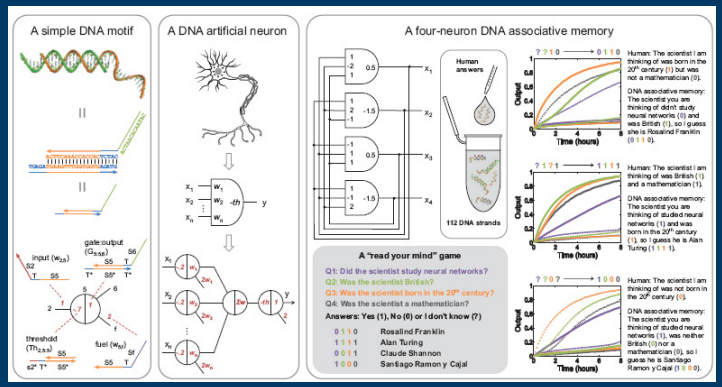
Neural network computation with DNA strand displacement cascades

Lulu Qian[1], Erik Winfree[1,2,3] & Jehoshua Bruck[3,4]

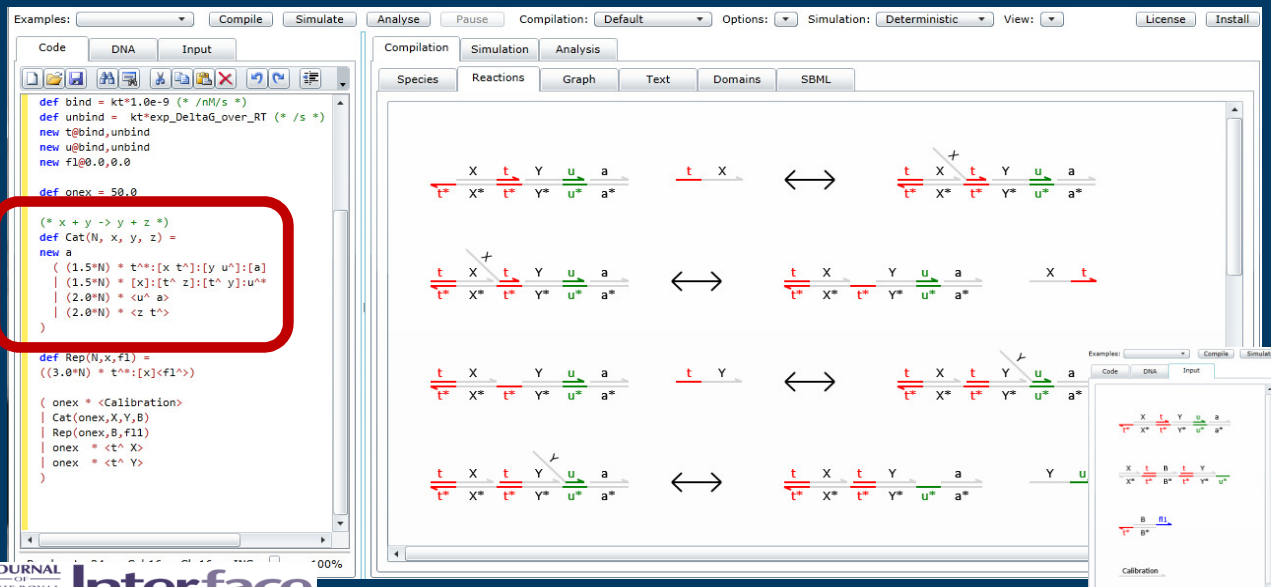


Tools and Techniques

A software pipeline for Molecular Programming

Development Tools

MSRC Biological Computation Group



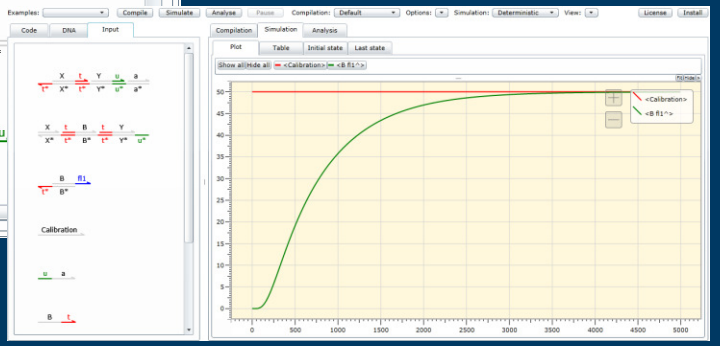
JOURNAL OF THE ROYAL SOCIETY Interface

A programming language for composable DNA circuits

Andrew Phillips and Luca Cardelli

Visual DSD

A Development Environment for DNA Strand Displacement



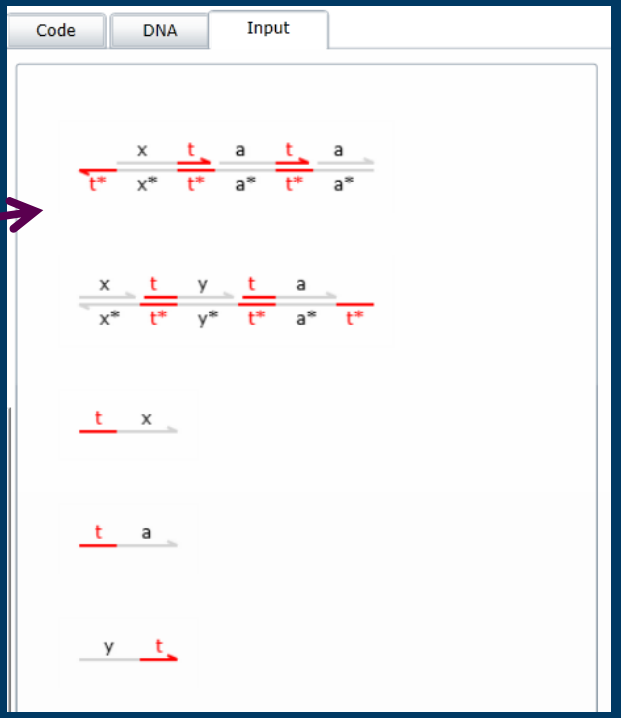
Execution

A wetlab pipeline for Molecular Programming

Output of Design Process

- Domain structures
  - (DNA sequences to be determined)

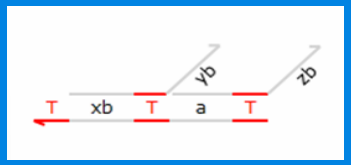
"Ok, how do I run this for real"

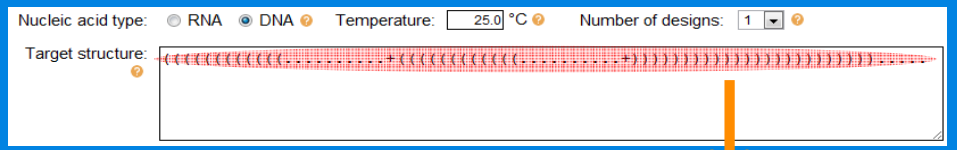


From Structures to Sequences

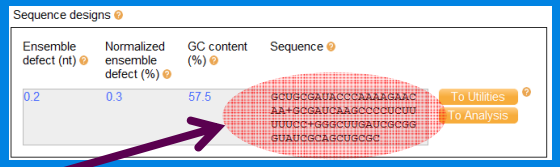
www.nupack.org

DSD Structure → "Dot-Paren" representation

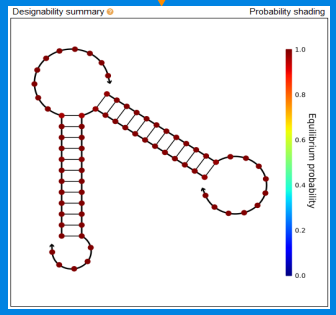




Output Sequences

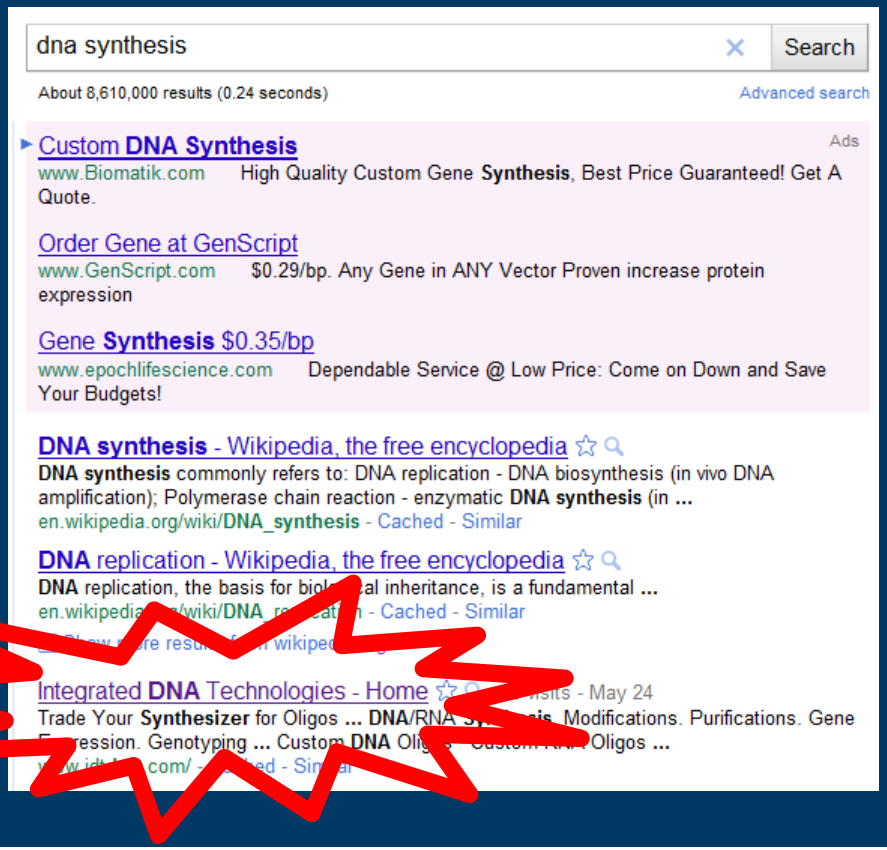


Thermodynamic Synthesis



"Ok, where do I buy these?"

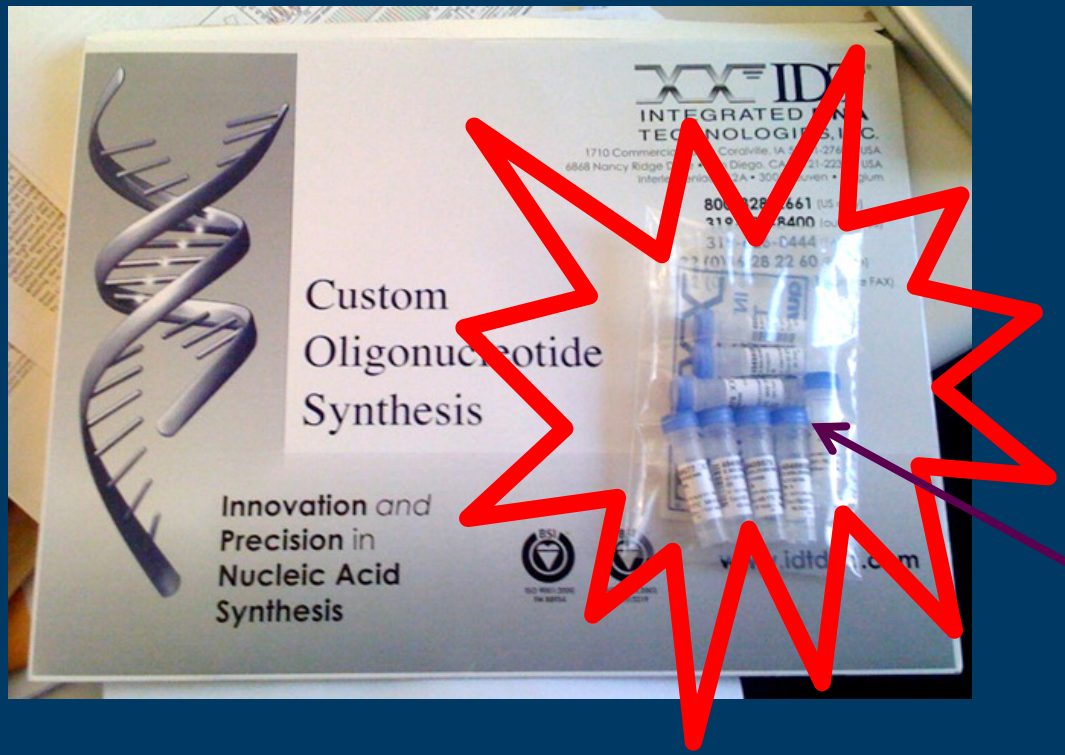
"DNA Synthesis"



From Sequences to Molecules

- Copy&Paste from nupack

Molecules by FedEx

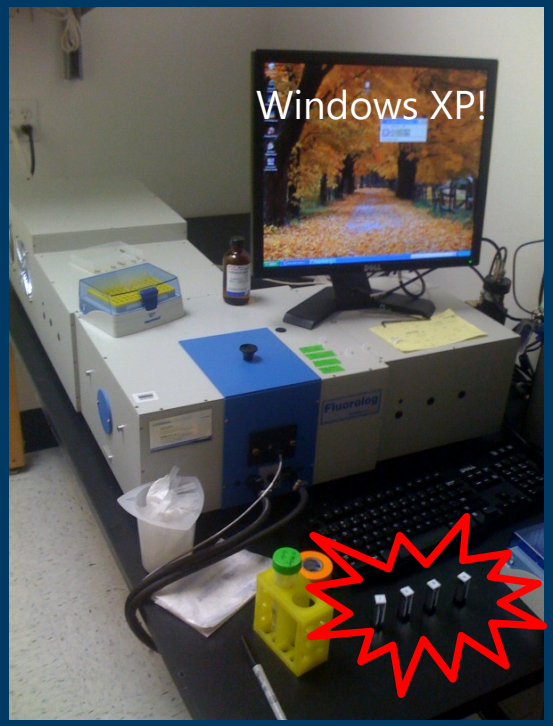


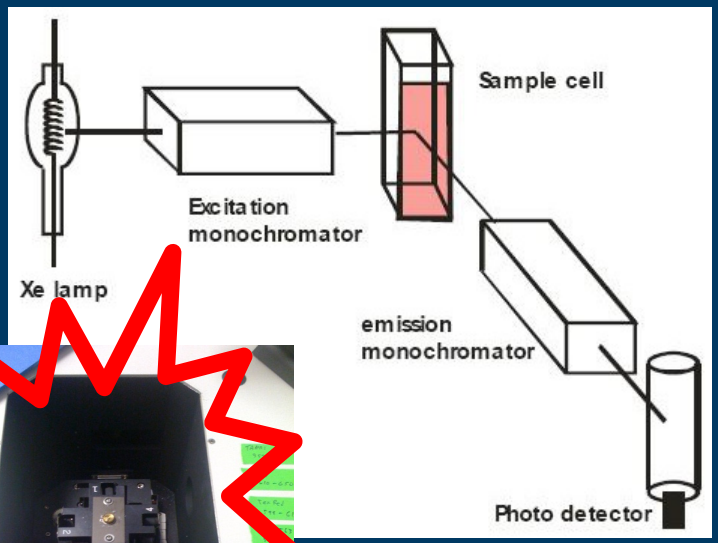
"Ok, how do I run these?"

Add Water

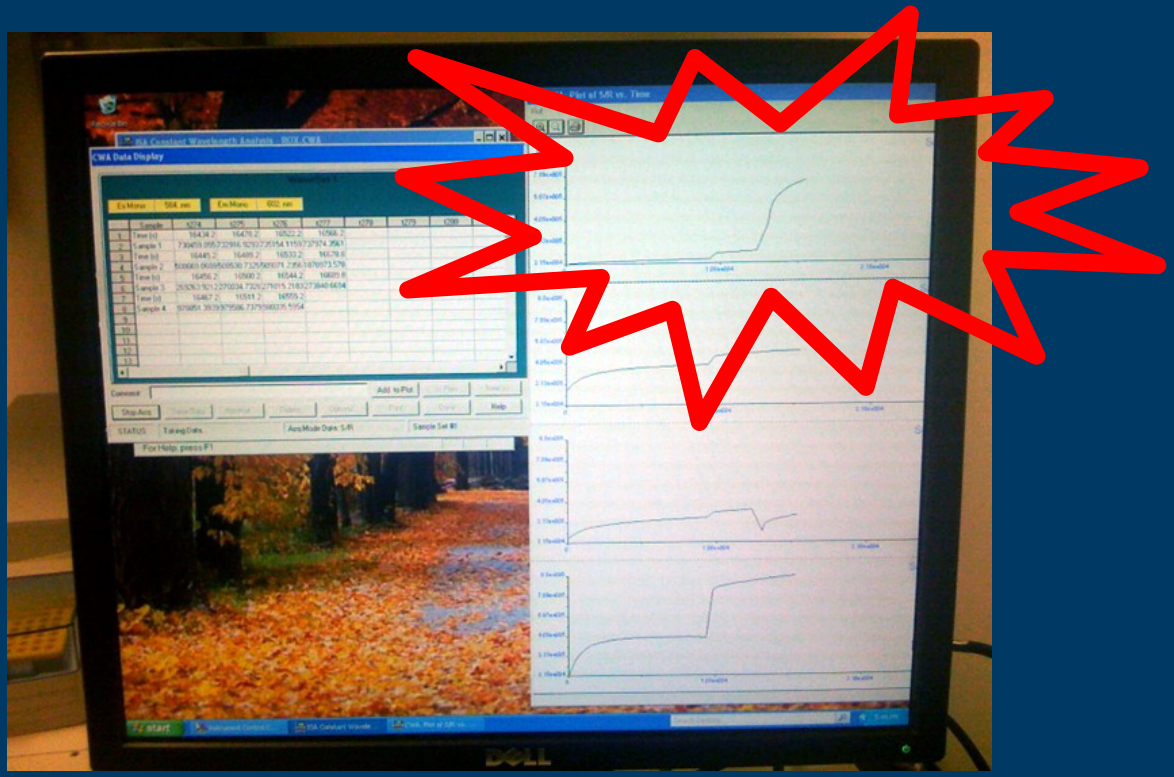
Execute (finally!)

- Fluorescence is your one-bit ‘print’ statement





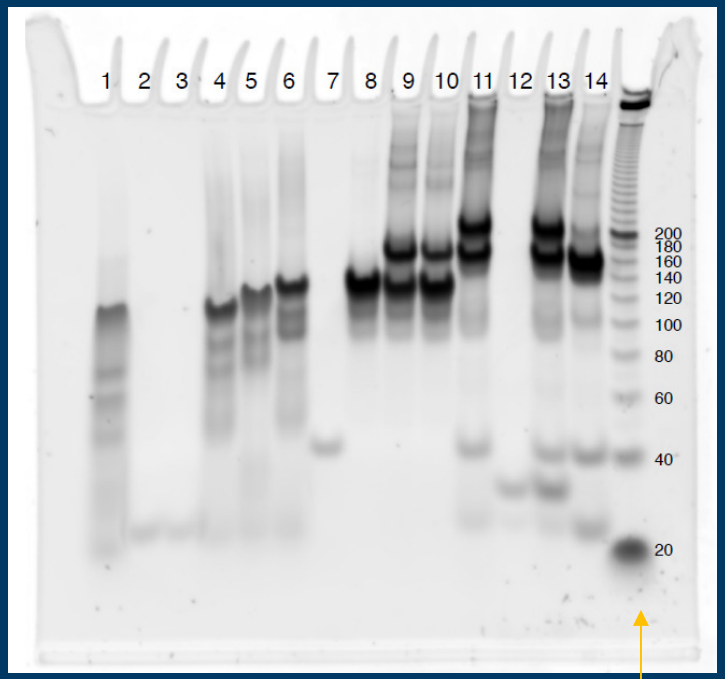
Output



Debugging

- A core dump

DNA strand length

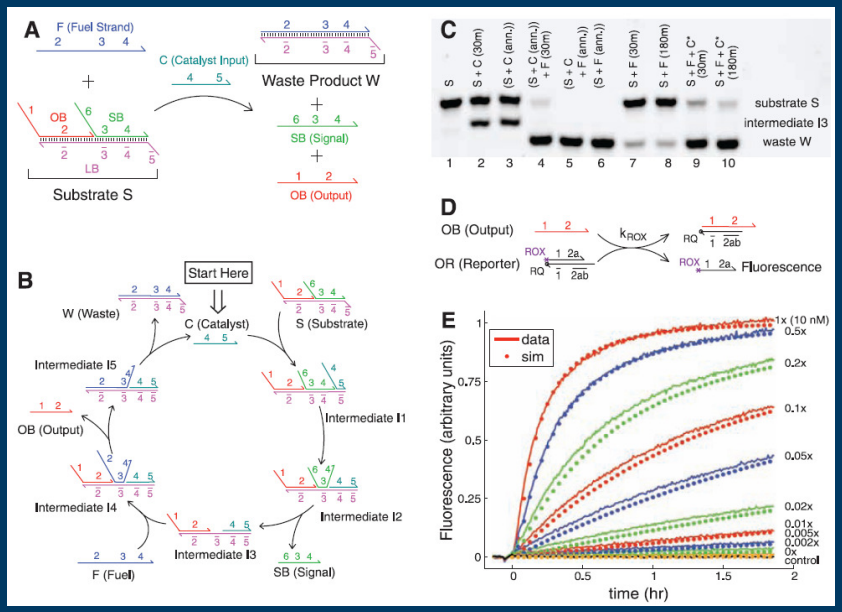


Various processing stages

Calibration scale

Delivery!

Engineering Entropy-Driven Reactions and Networks Catalyzed by DNA
David Yu Zhang, *et al.*
Science **318**, 1121 (2007);
DOI: 10.1126/science.1148532



A Molecular Algorithm

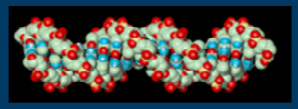
Running something interesting with DNA

Approximate Majority Algorithm

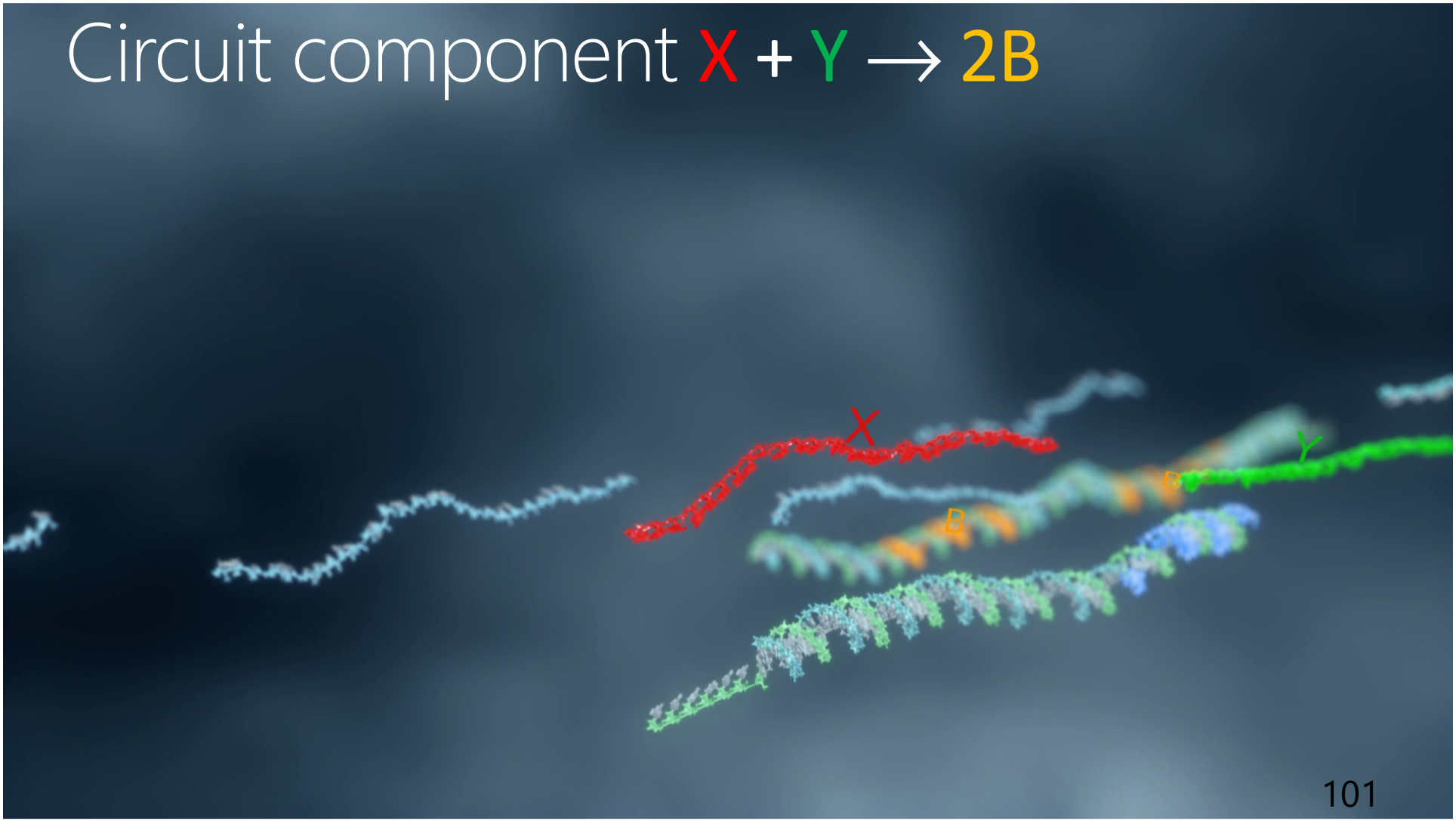
- Given two populations of agents (or molecules)
  - Randomly communicating by radio (or by collisions)
  - Reach an agreement about which population is in majority
  - By converting all the minority to the majority
    [Angluin et al., Distributed Computing, 2007]

- 3 rules of agent (or molecule) interaction
  - $X + Y \rightarrow B + B$
  - $B + X \rightarrow X + X$
  - $B + Y \rightarrow Y + Y$

"our program" →

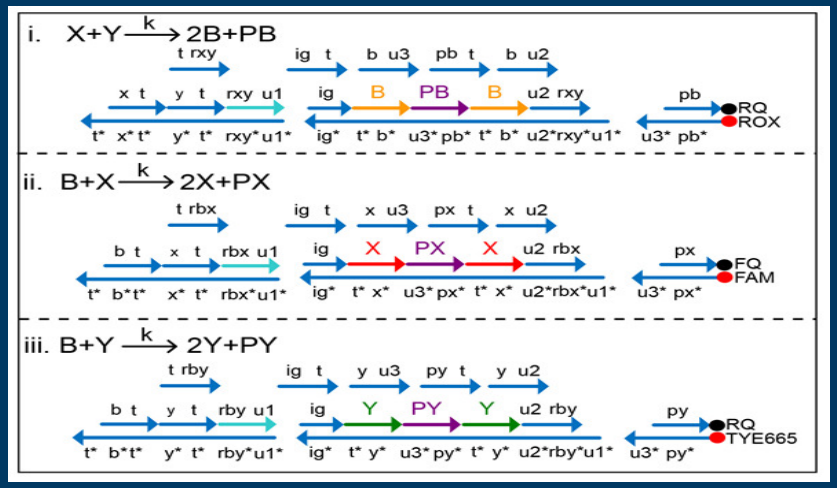


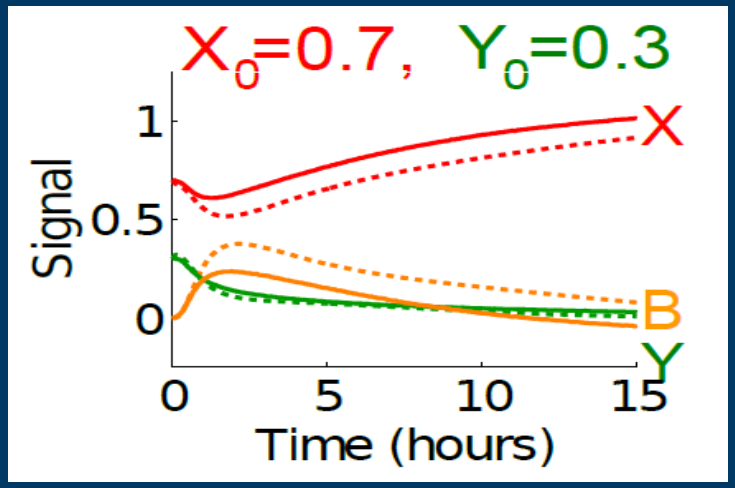
Circuit component X + Y → 2B



DNA Implementation, at U.W.

- Programmable chemical controllers made from DNA
  [Yuan-Jyue Chen, Neil Dalchau, Niranjan Srinivas, Andrew Phillips, Luca Cardelli, David Soloveichik and Georg Seelig]

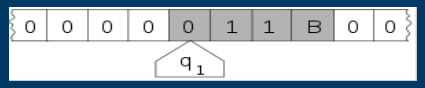




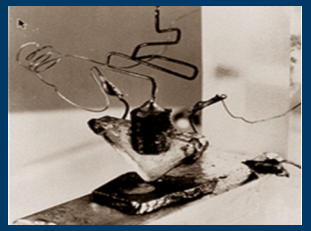
Final Remarks

A Brief History of DNA

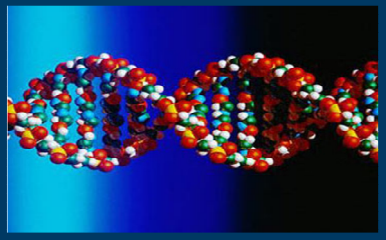
Turing Machine, 1936



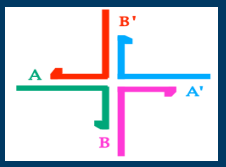
Transistor, 1947



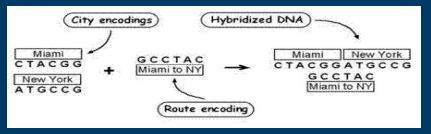
DNA, -3,800,000,000



Structural DNA Nonotech, 1982



DNA Algorithm, 1994



Computer programming

20th century

Systematic manipulation of information

Systematic manipulation of matter

21th century

Molecular programming

Acknowledgments

- Microsoft Research
  - Andrew Phillips, Biological Computation Group
- Caltech
  - Winfree Lab
- U.Washington
  - Seelig Lab

Questions?

Resources

- Biological Computation Group at MSR
  https://www.microsoft.com/en-us/research/group/biological-computation/
- Molecular Programming Project at Caltech
  http://molecular-programming.org/
- Georg Seelig's DNA Nanotech Lab at U.W. CS&E
  http://homes.cs.washington.edu/~seelig/
- "DNA Computing and Molecular Programming" Conference Proceedings
  http://www.dna-computing.org/