Molecular Programming

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INRIA Scientific Board, Paris, 2011-11-18
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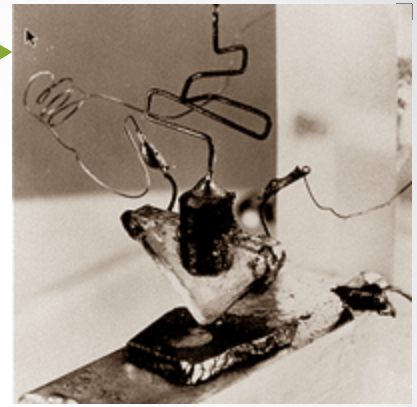
Smaller and Smaller

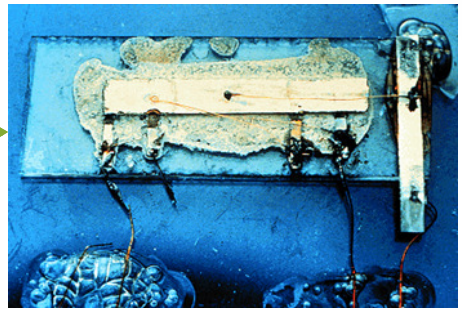
First working transistor

John Bardeen and Walter Brattain , Dec. 23, 1947.



First integrated circuit

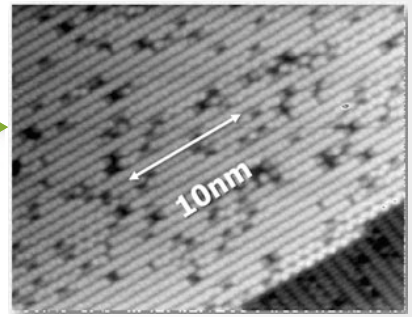
Jack Kilby, Sep. 1958.



50 years later

25nm NAND flash

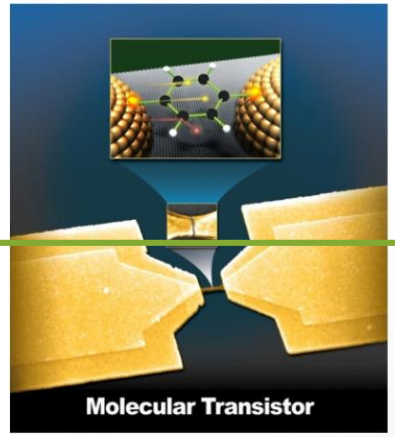
Intel&Micron, Jan. 2010. ~50atoms.



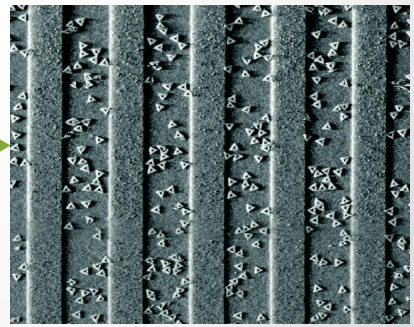
Scanning tunneling microscope image of a silicon surface showing 10nm is ~20 atoms across

Single molecule transistor

Observation of molecular orbital gating.
Nature, 2009; 462 (7276): 1039



Molecules on a chip

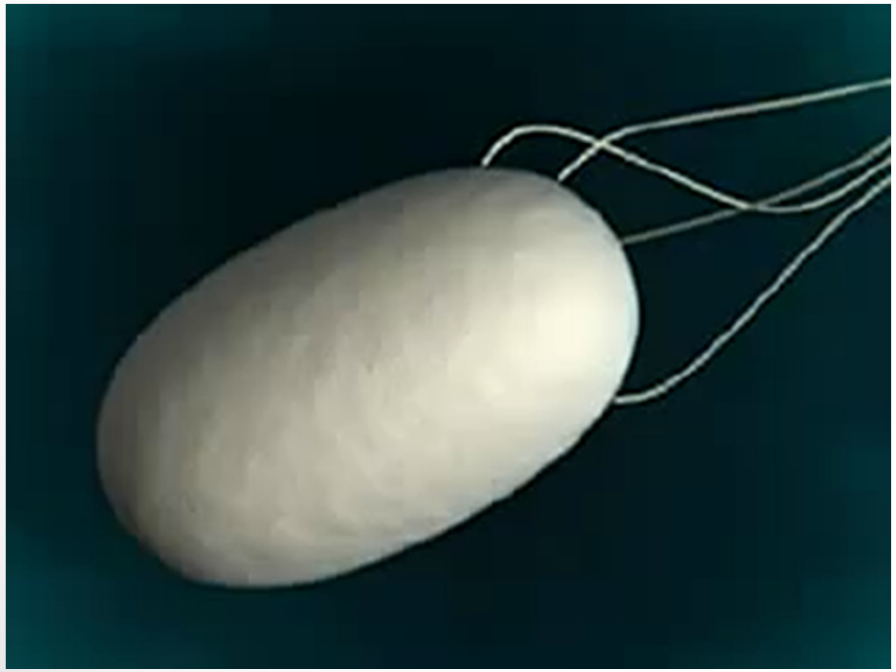


~10 Moore's Law cycles left!

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

Building The Small*est* Things

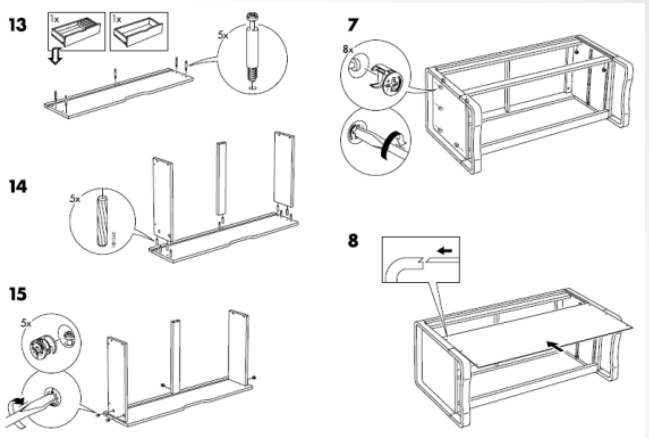
- How do we build structures that are by definition smaller than your tools?
- Basic answer: you can't. Structures (and tools) should build themselves!
- By *programmed self-assembly*.



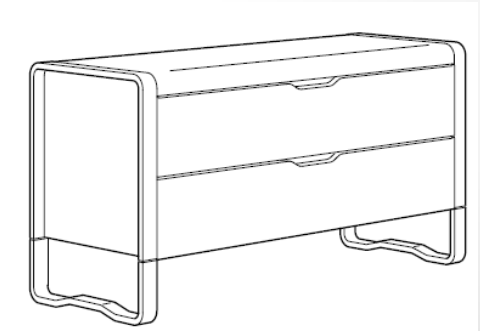
www.youtube.com/watch?v=Ey7Emmddf7Y

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…



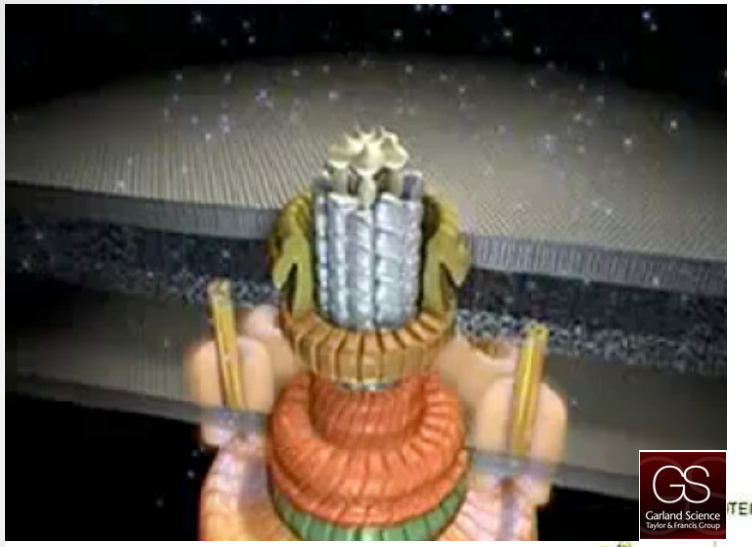
Add water

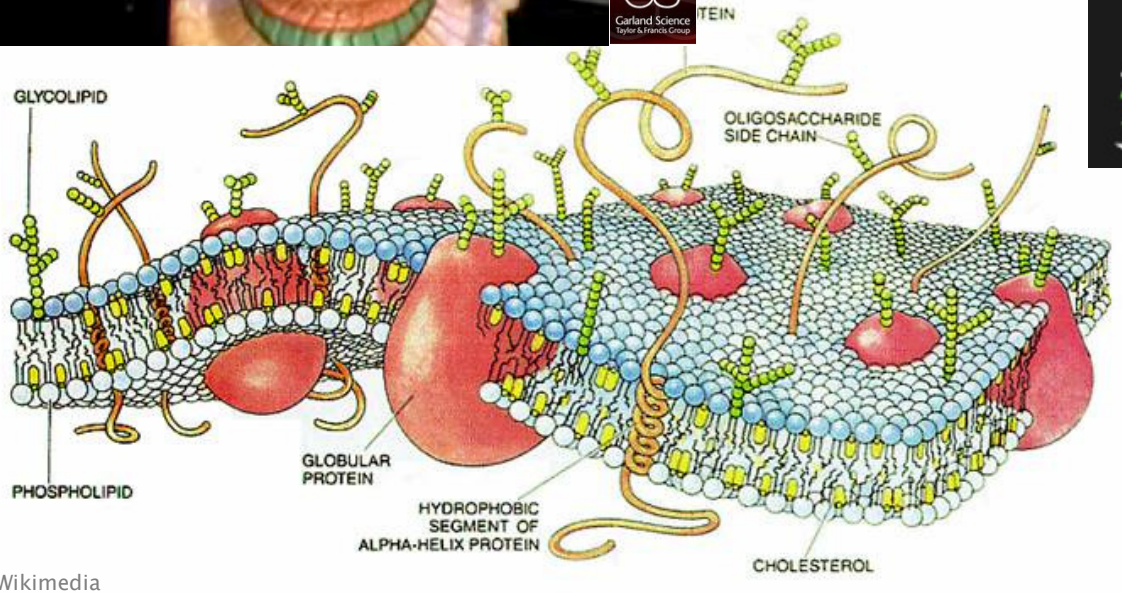


http://www.ikea.com/ms/en_US/customer_service/assembly_instructions.html

Programmed Self-Assembly

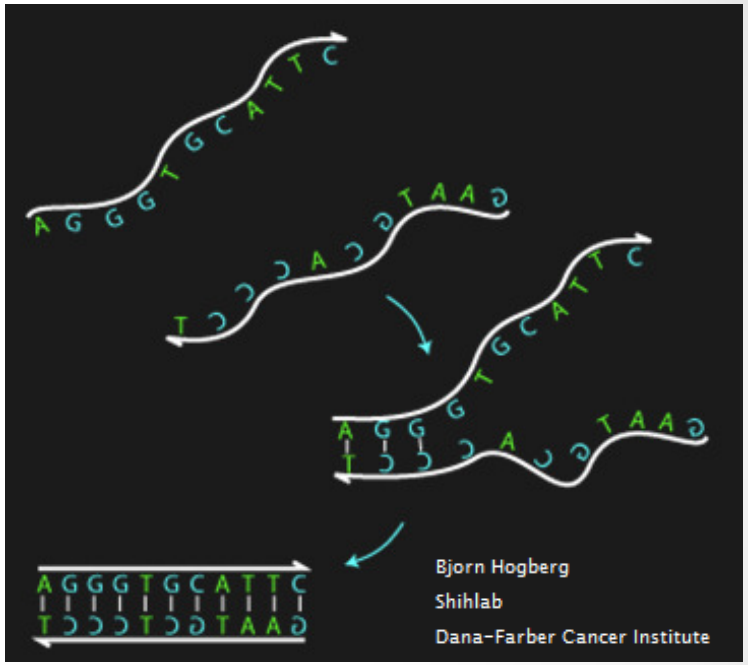
Proteins





Wikimedia

DNA/RNA



Membranes

Molecular Languages

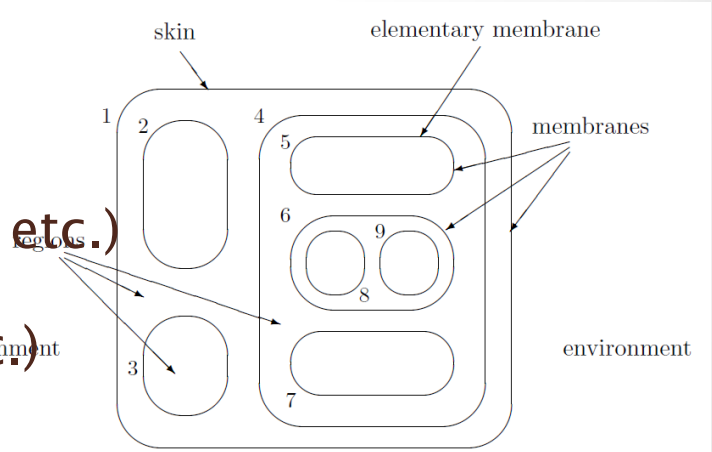
- modeling languages -

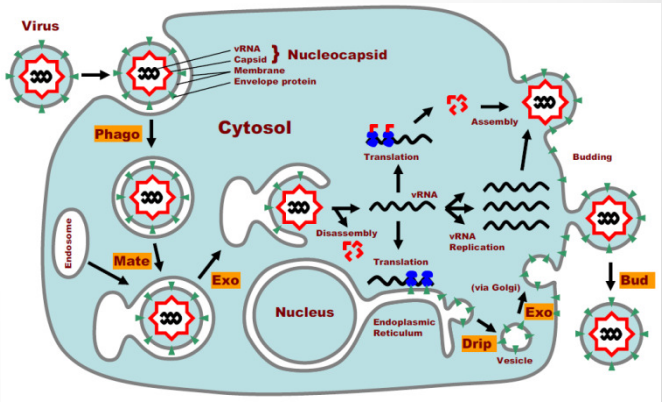
Chemistry

- Chemical reactions
  - $A + B \rightarrow_r C + D$ (a program)
- Ordinary Differential Equations
  - $d[A]/dt = -r[A][B]$ … (a semantics)
- Rich analytical techniques based on Calculus
- But prone to combinatorial explosion
  - E.g., due to the peculiarities of protein interactions

High(er)-Level Languages

- Gene Networks
  - Synchronous Boolean networks
    - Stewart Kauffman, etc.
  - Asynchronous Boolean networks
    - René Thomas, etc.
- Protein Networks
  - Process Algebra (stochastic π-calculus etc.)
    - Priami, Regev-Shapiro, etc.
  - Graph Rewriting (kappa, BioNetGen etc.)
    - Danos-Laneve, Fontana & al., etc.
- Membrane Networks
  - Membrane Computing
    - Gheorghe Păun, etc.
  - Brane Calculi
    - Luca Cardelli, etc.

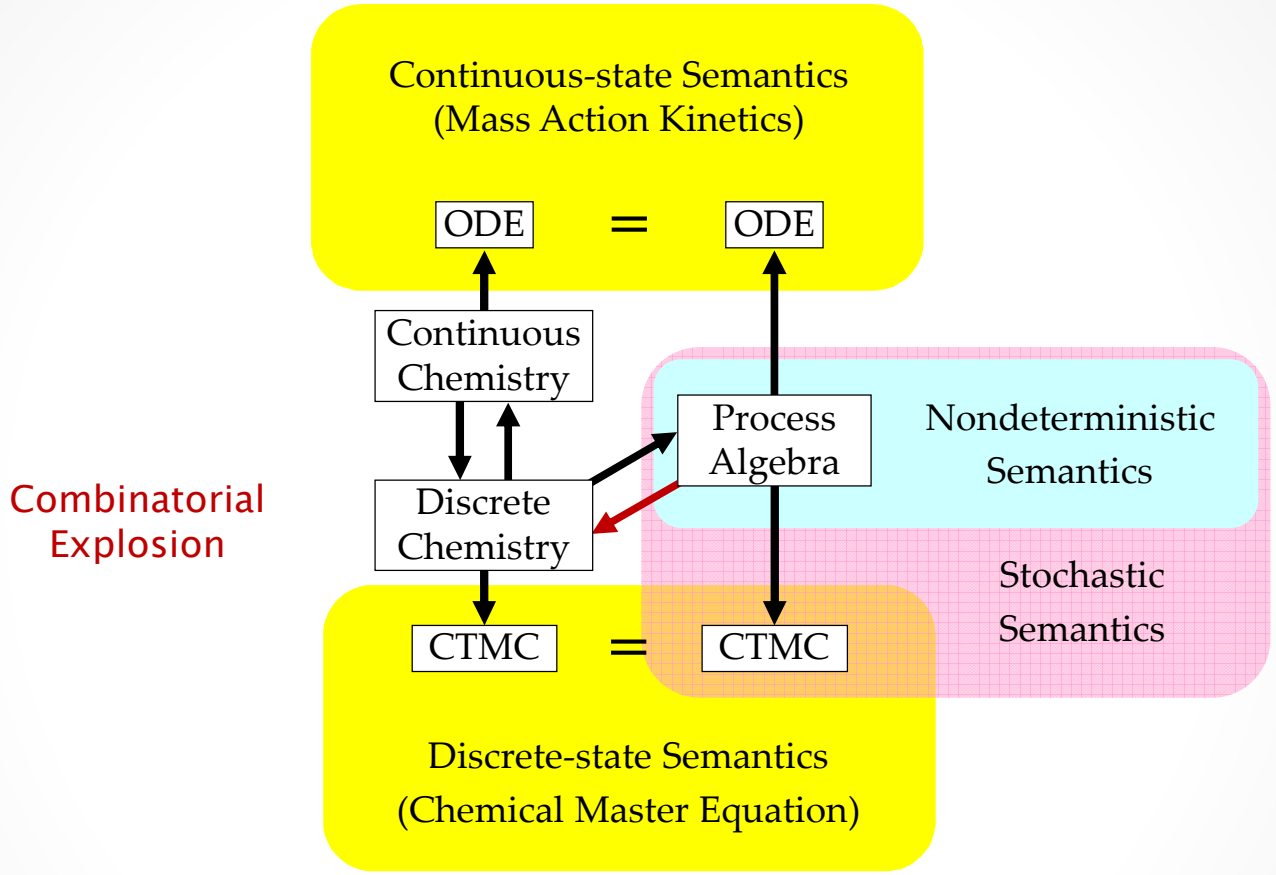




Molecular Languages

- Reaction-Based (A + B → C + D) (Chemistry)
  - Limited to finite set of species (no polymerization)
  - Practically limited to small number of species (no run-away complexation)
- Interaction-Based (A = !r; C) (Process Algebra)
  - Reduces combinatorial complexity of models by combining independent submodels connected by interactions.
- Rule-Based (A{-}:B{p} → A{p}:B{-}) (Logic, Graph Rewriting)
  - Further reduces model complexity by describing molecular state, and by allowing one to 'ignore the context': a *rule* is a reaction in an unspecified (complexation/phosphorylatio) context.
  - Similar to informal descriptions of biochemical events ("narratives").
- Syntactic connections
  - The latter two can be translated (to each other and) to the first, but doing so may introduce an infinite, or anyway *extremely large*, number of species.

Semantic Connections



These diagrams commute via appropriate maps.

L. Cardelli: "On Process Rate Semantics" (TCS)

L. Cardelli: "A Process Algebra Master Equation" (QEST'07)

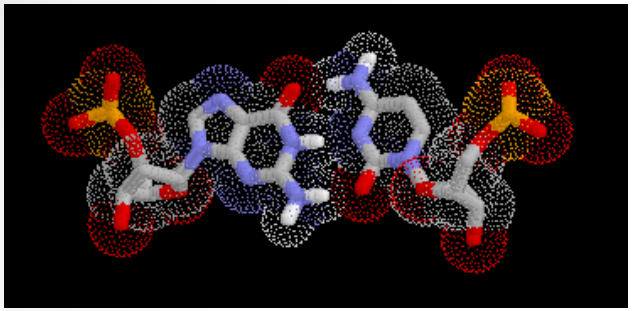
But what about Execution?

- Chemistry is not easily executable
  - Please Mr Chemist, execute me these reactions that I just made up.
- Similarly, the molecular languages seen so fare are descriptive (modeling) languages
- How can we actually execute molecular languages? With real molecules?

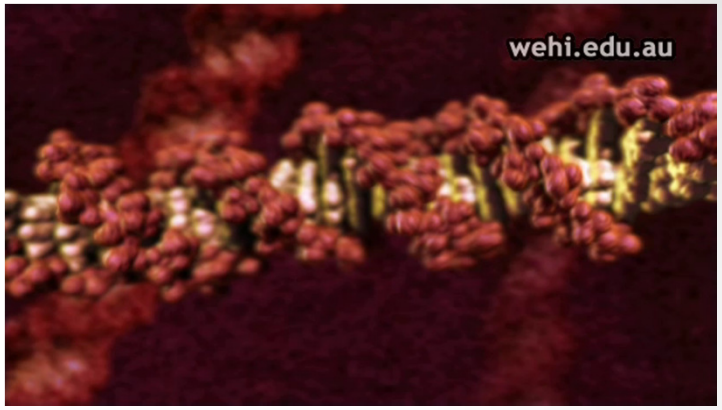
Molecular Languages

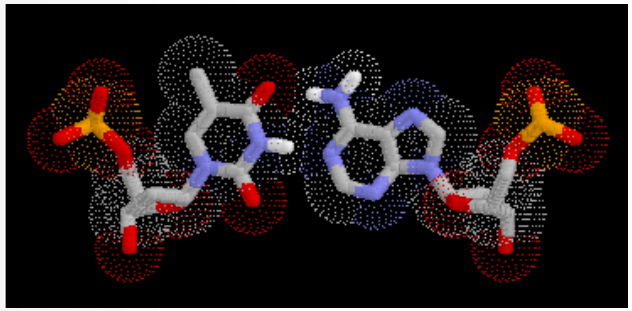
- executable languages -

DNA



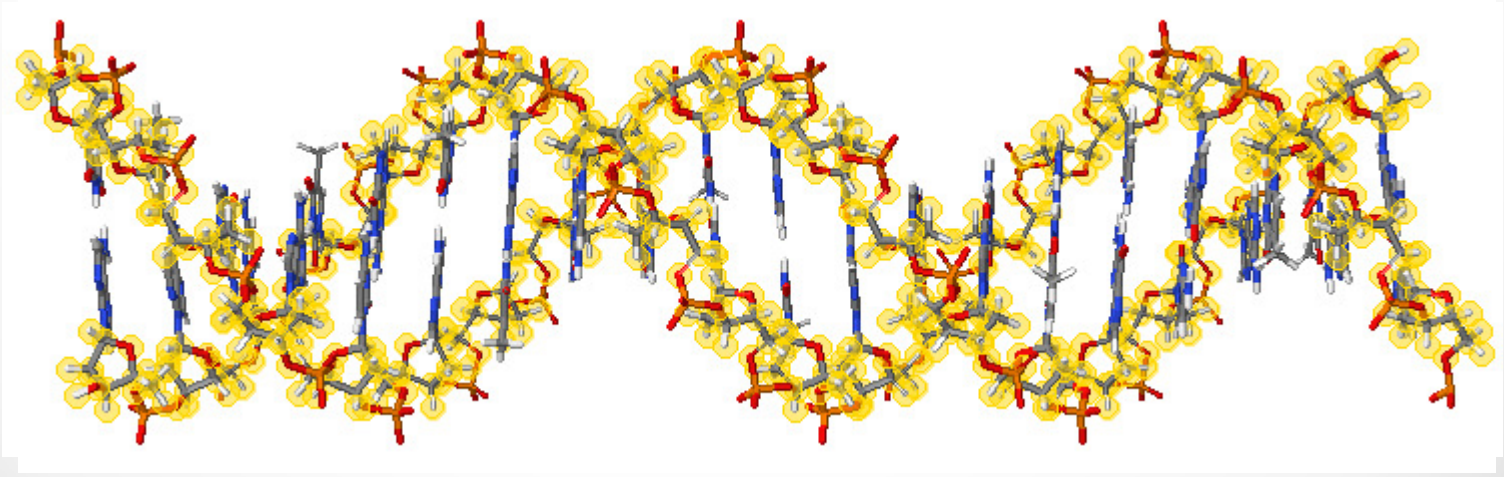
GC Base Pair
Guanine-Cytosine





TA Base Pair
Thymine-Adenine

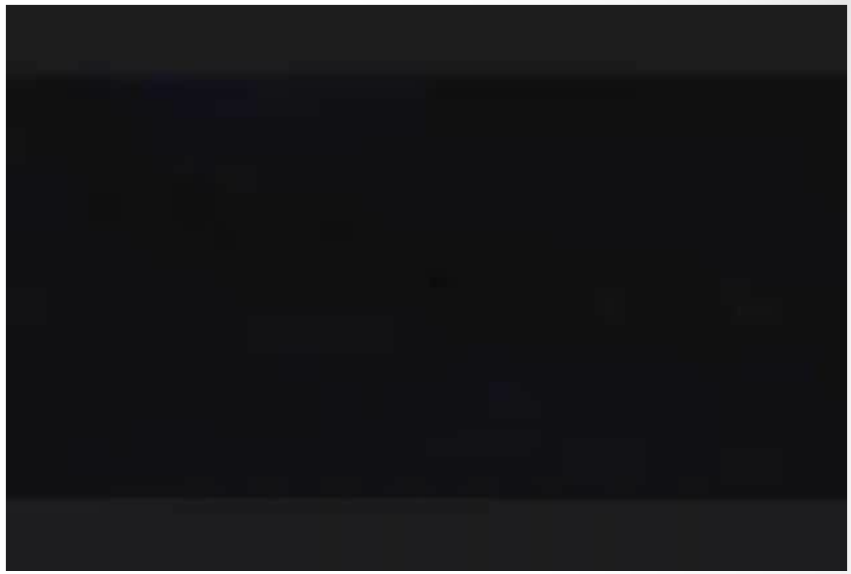
Interactive DNA Tutorial
(http://www.biosciences.bham.ac.uk/labs/minchin/tutorials/dna.html)



Sequence of Base Pairs (GACT alphabet)

Robust, and *Long*

- DNA in each human cell:
  - 3 billion base pairs
  - 2 meters long, 2nm thick
  - folded into a 6μm ball
  - 750 MegaBytes
- 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

wehi.edu.au

Andromeda Galaxy
2.5 million light years away

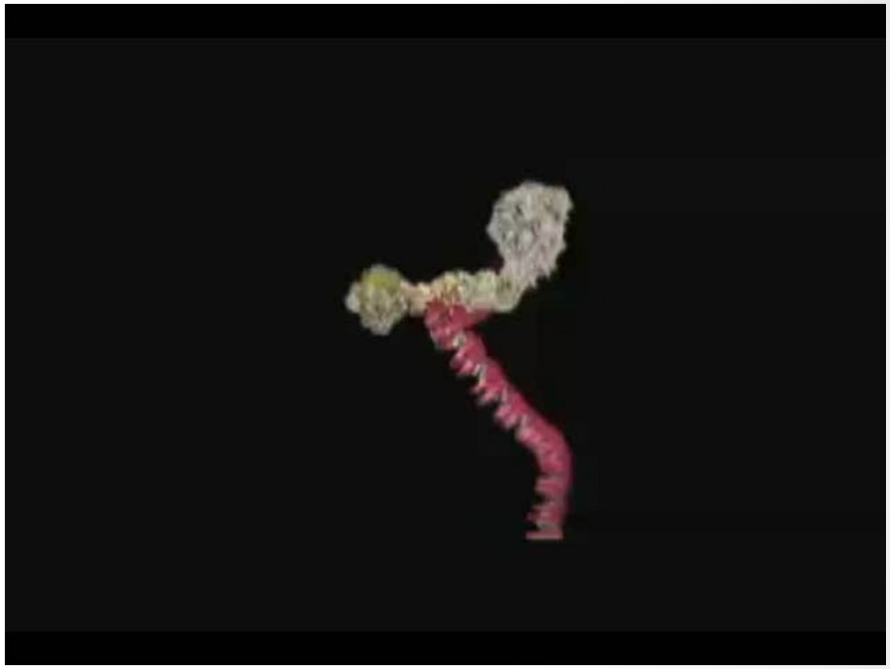
Natural DNA Operation

- 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)



DNA transcription in *real time*

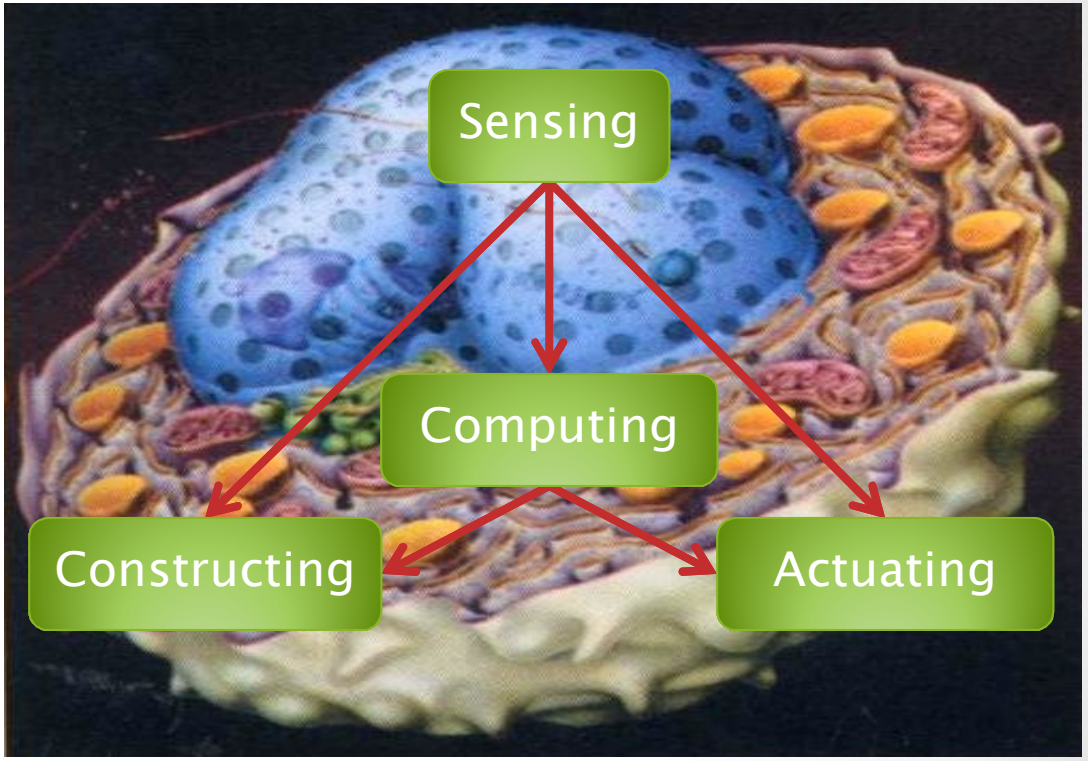
RNA polymerase II:
15-30 bases/second

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

Unnatural DNA Operation

- Sensing
  - Reacting to forces
  - Binding to molecules
- Actuating
  - Releasing molecules
  - Producing forces
- Constructing
  - Chassis
  - Growth
- Computing
  - Signal Processing
  - Decision Making

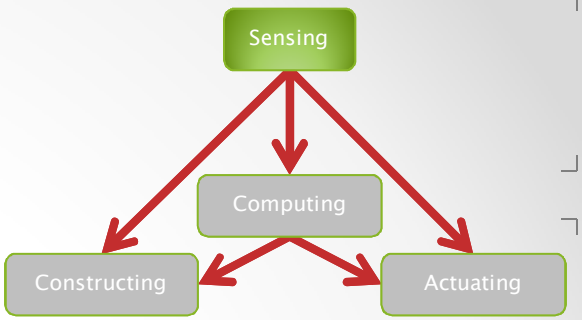
Nanoscale Control Systems



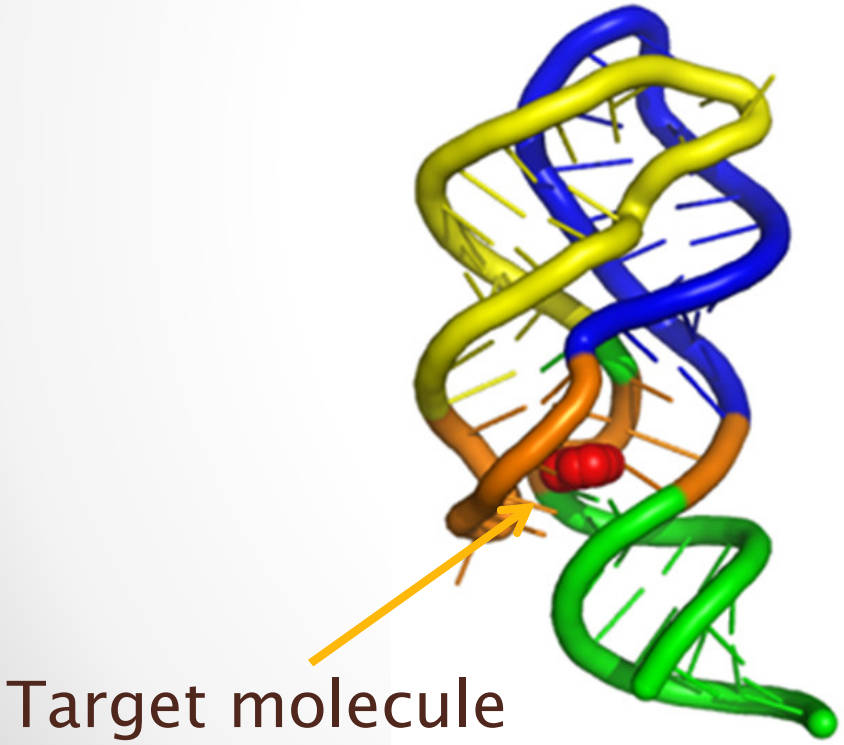
Nucleic Acids can do all this.

And interface to biology.

Sensing



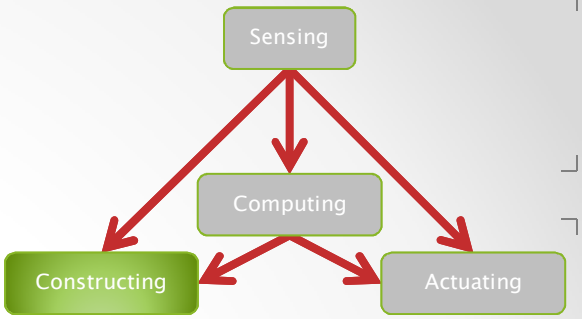
Aptamers: natural or artificially evolved DNA molecules that stick to other molecules (highly selectively).



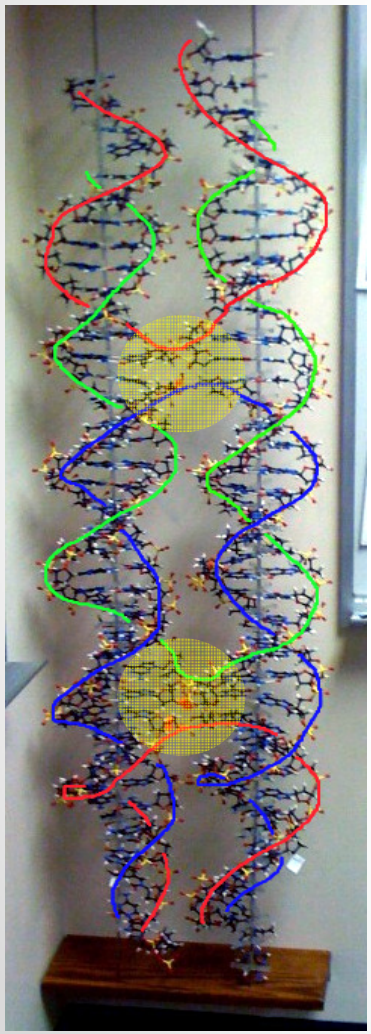
Adenine riboswitch aptamer

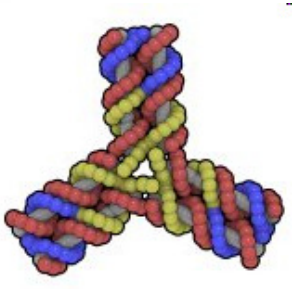
Structural basis for discriminative regulation of gene expression by adenine- and guanine-sensing mRNAs. Chem Biol. 2004 Dec;11(12):1729-41.

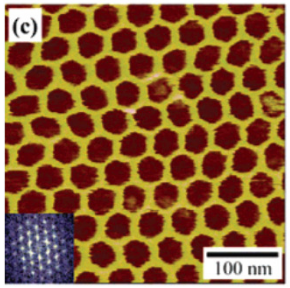
Constructing



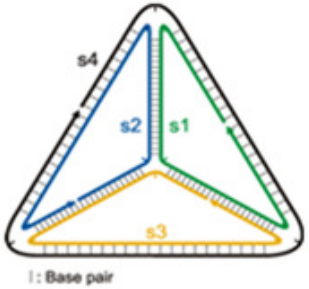
Crosslinking

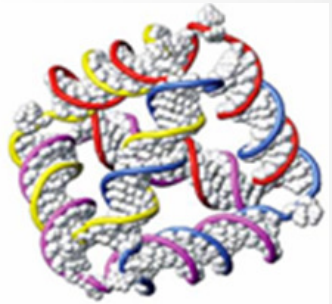






Chengde Mao, Purdue





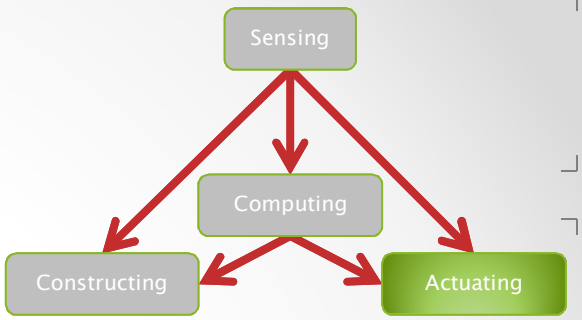
Andrew Turberfield, Oxford

Folding DNA into Twisted and Curved Nanoscale Shapes

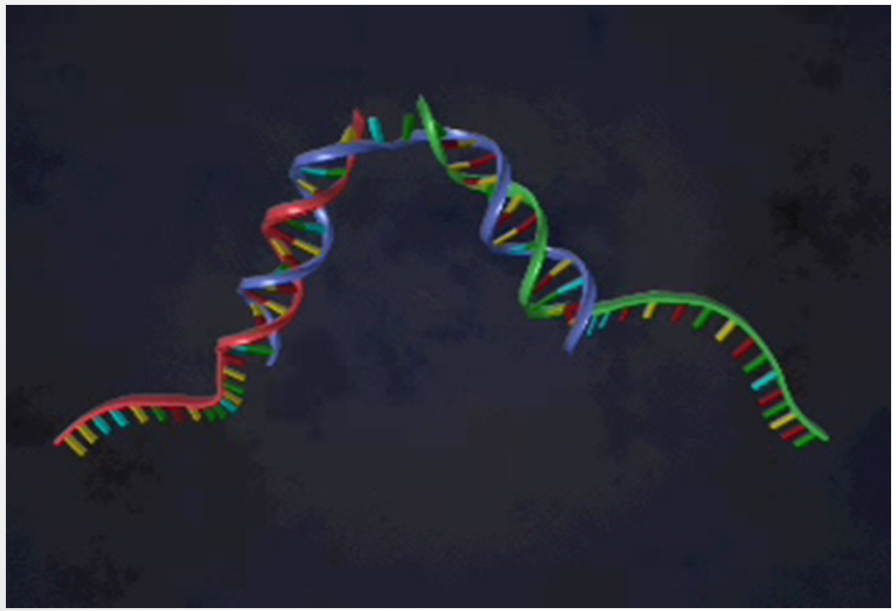
Hendrik Dietz, Shawn M. Douglas, & William M. Shih

Science, 325:725–730, 7 August 2009.

Actuating

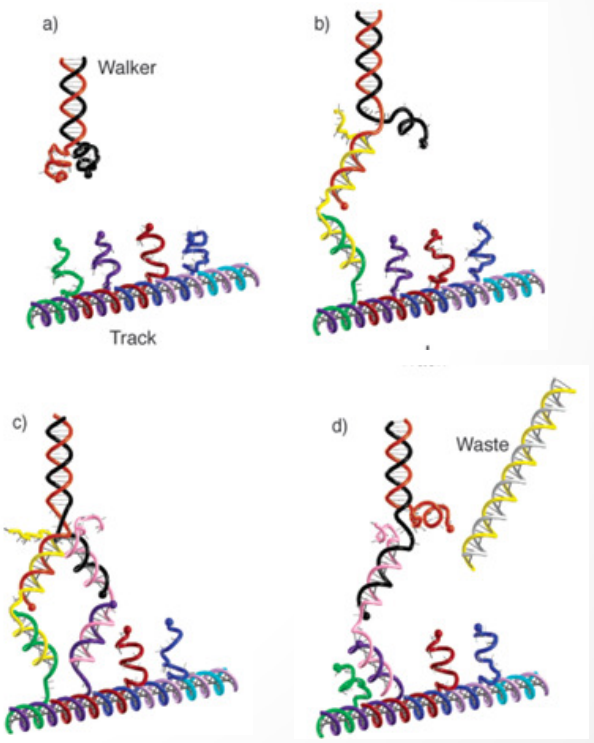


DNA tweezers

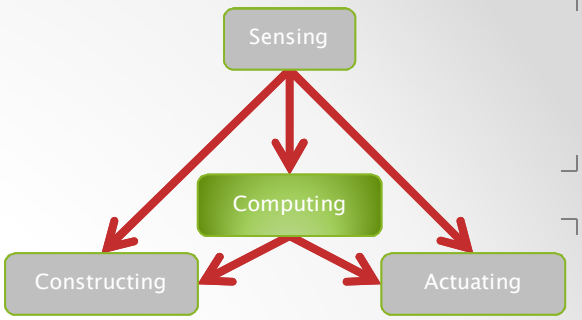


Bernard Yurke, Boise State

DNA walkers



Computing



- Sensors and Actuators at the 'edge' of the system
  - They can use disparate technologies and phenomena
- Computation in the 'kernel' of the system
- Compositionality in the kernel
  - The components should use uniform inputs and outputs
  - The components should be ‘computationally complete’

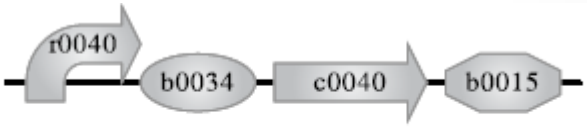
“Embedded” Computing

(Synthetic Biology)

- Using bacterial machinery (e.g.) as the hardware.
  Using embedded gene networks as the software.

- MIT Registry of Standard Biological Parts

- GenoCAD
  - Meaningful sequences [Cai et al.]

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r0040:prom; b0034:rbs; c0040:pcr; b0015:ter
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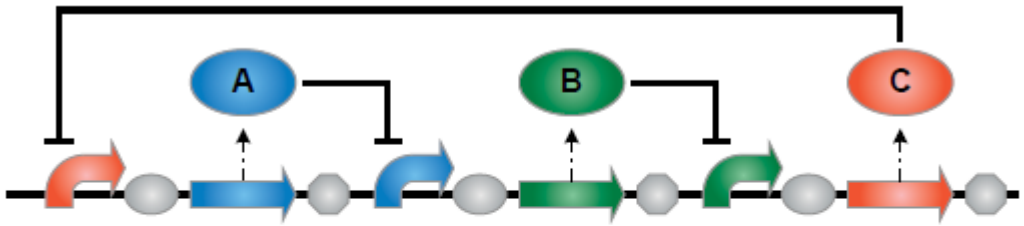
- GEC
  - [Pedersen & Phillips]



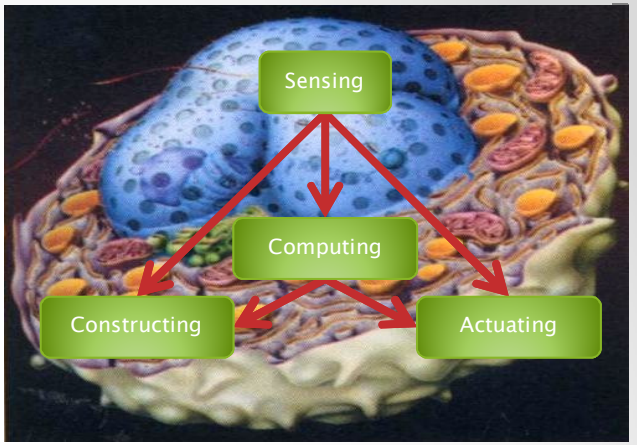

```
prom<neg(C)>; rbs; pcr<codes(A)>; ter;
prom<neg(A)>; rbs; pcr<codes(B)>; ter;
prom<neg(B)>; rbs; pcr<codes(C)>; ter
```

“Autonomous” Computing

(Nano-engineering)

- Mix & go
  - All (or most) parts are synthesized
  - No manual cycling (cf. early DNA computing)
  - In some cases, all parts are made of DNA (no enzyme/proteins)
- Self-assembled and self-powered
  - Can run on its own (e.g. environmental sensing)
  - Or be embedded into organisms, but running ‘separately’

Curing



A doctor in each cell

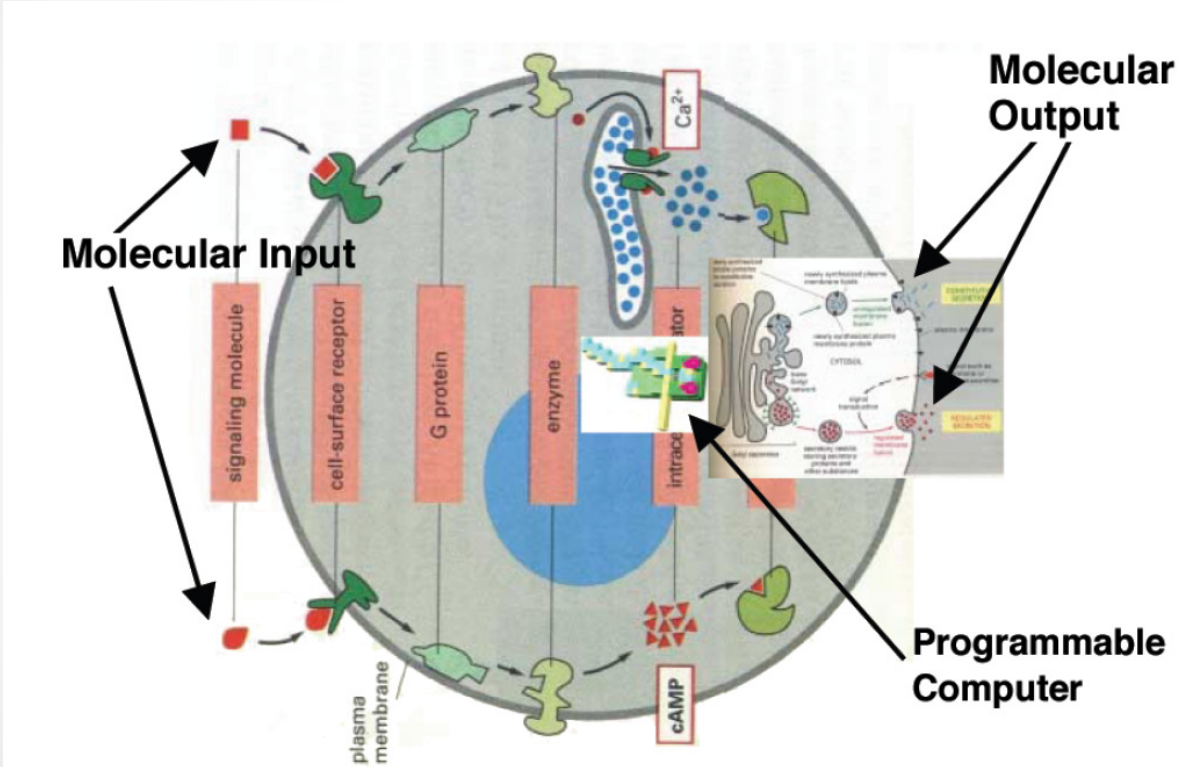


Fig. 1 *Medicine in 2050: "Doctor in a Cell"*

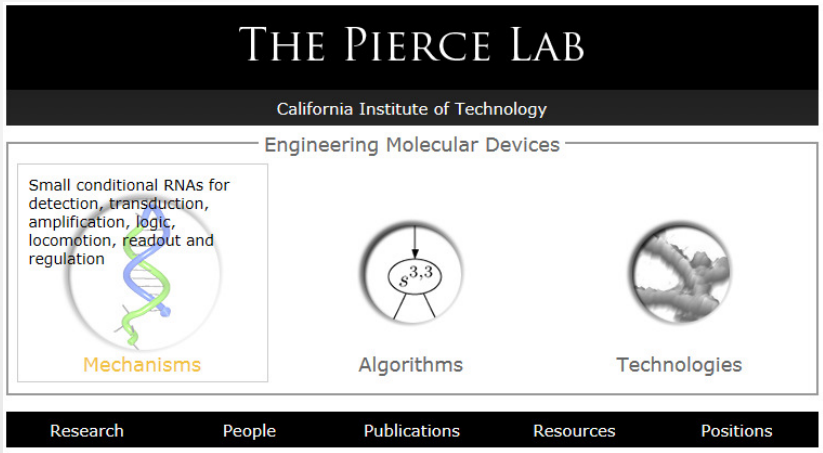
Ehud Shapiro

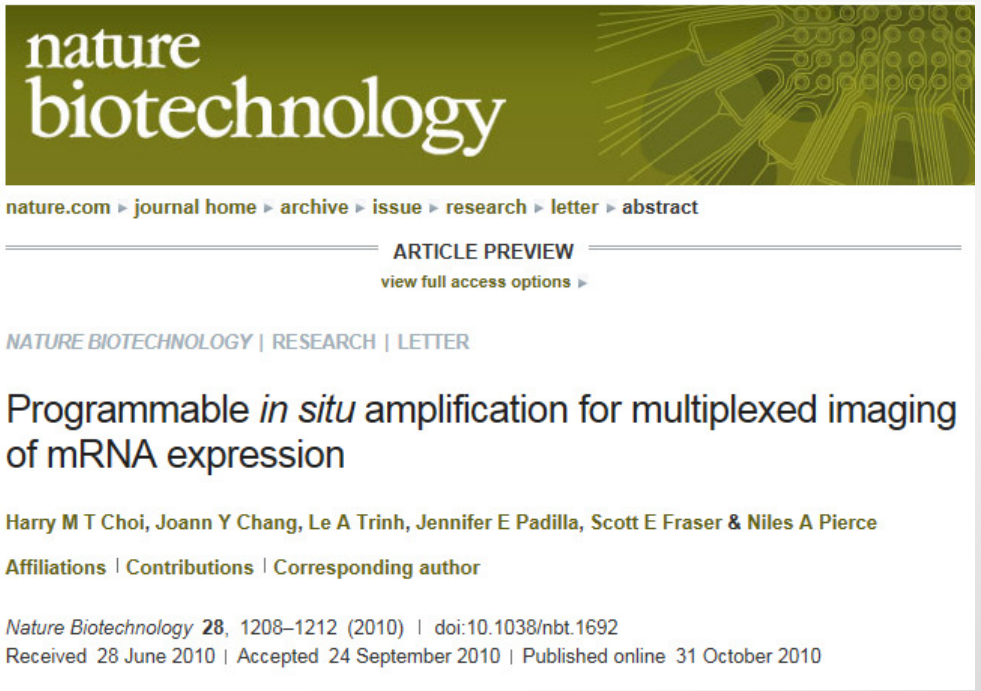
Rivka Adar
Kobi Benenson
Gregory Linshitz
Aviv Regev
William Silverman

Molecules and computation

RNA operation in (dead) cells

- Using RNA Hybridization Chain Reaction for imaging of mRNA expression.
  - The programmability of orthogonal RNA reactions enables spatial imaging with 5 simultaneous targets.





Molecular Computation

DNA Computing

- Non-goals
  - Not to solve NP-complete problems.
  - Not to replace electronics.
  - Not necessarily using genes or producing proteins.

- For general ‘molecular programming’
  - To precisely control the organization and dynamics of matter and information at the molecular level.
  - To interact algorithmically with biological entities.
  - The use of DNA is “accidental”: no genes involved.
  - In fact, no material of biological origin.

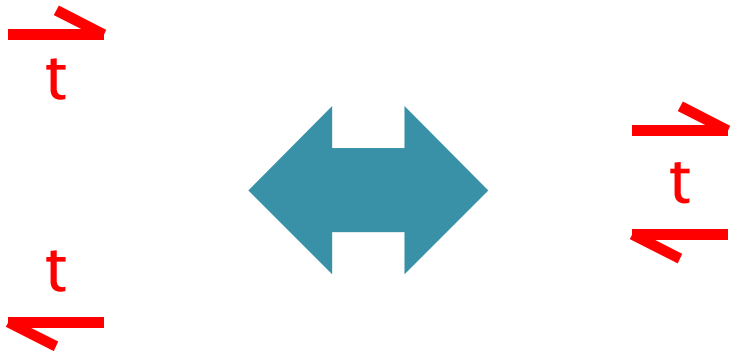
Domains

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

- I.e., 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.

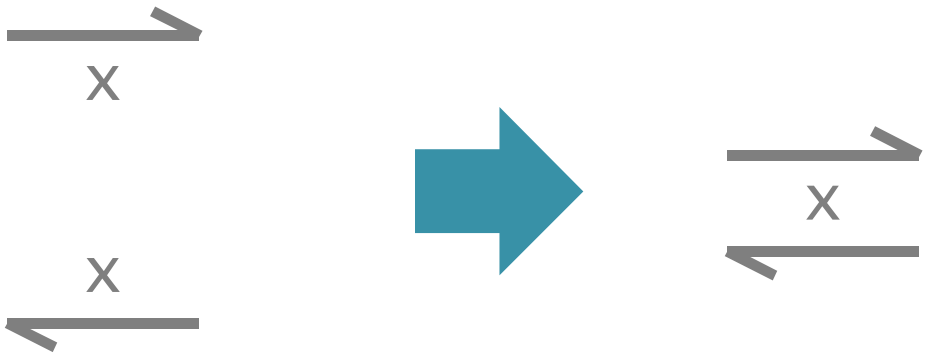
- Choosing domains (subsequences) that are suitably independent is a tricky issue that is still somewhat of an open problem (with a vast literature). But it can work in practice.

Short Domains



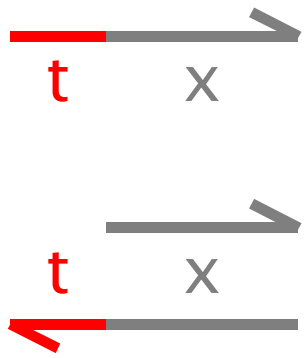
Reversible Hybridization

Long Domains



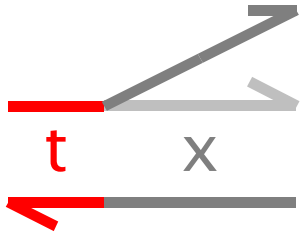
Irreversible Hybridization

Strand Displacement



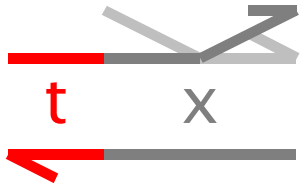
"Toehold Mediated"

Strand Displacement



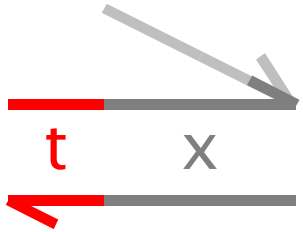
Toehold Binding

Strand Displacement



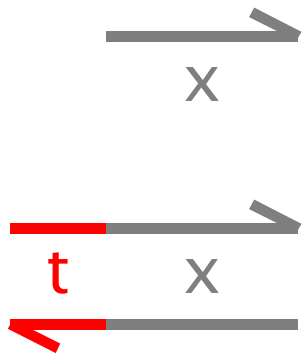
Branch Migration

Strand Displacement



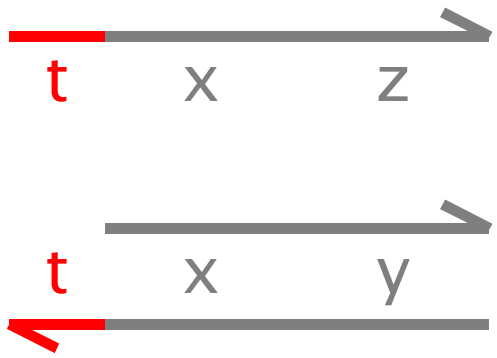
Displacement

Strand Displacement

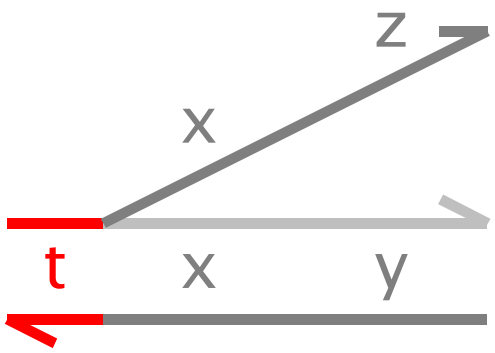


Irreversible release

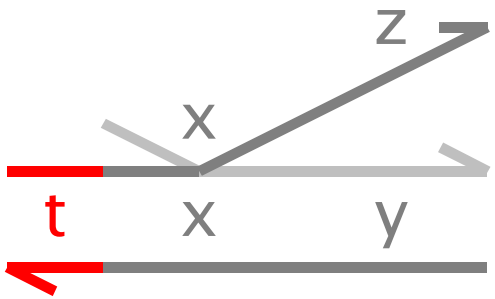
Bad Match



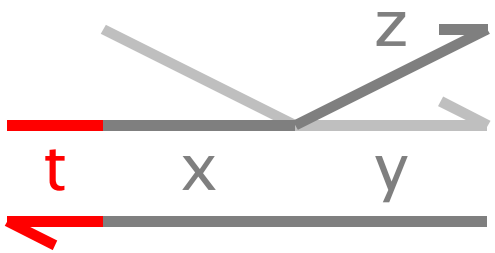
Bad Match



Bad Match



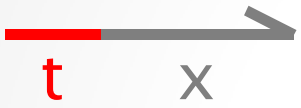
Bad Match



Cannot proceed
Hence will undo

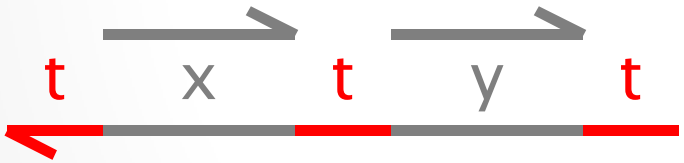
Two-Domain Architecture

- Signals: 1 toehold + 1 recognition region



Garbage collection
"built into" the gates

- Gates: "top-nicked double strands"
(or equivalently double strands with open toeholds)

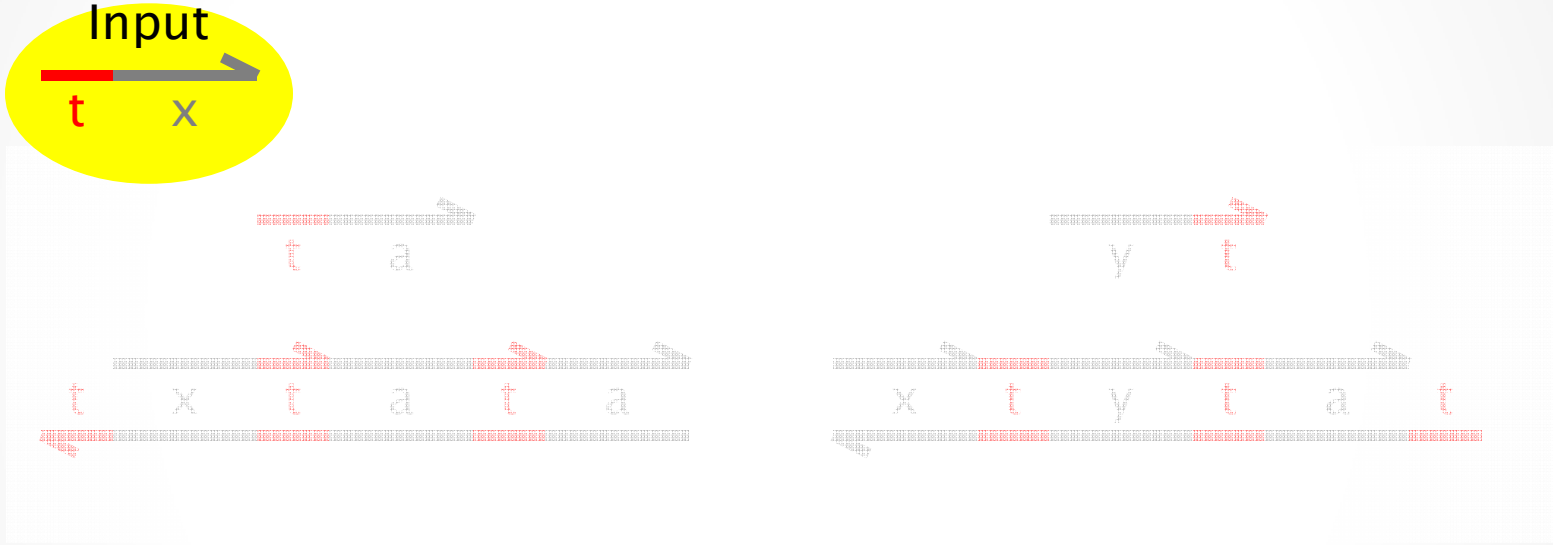


Two-Domain DNA Strand Displacement

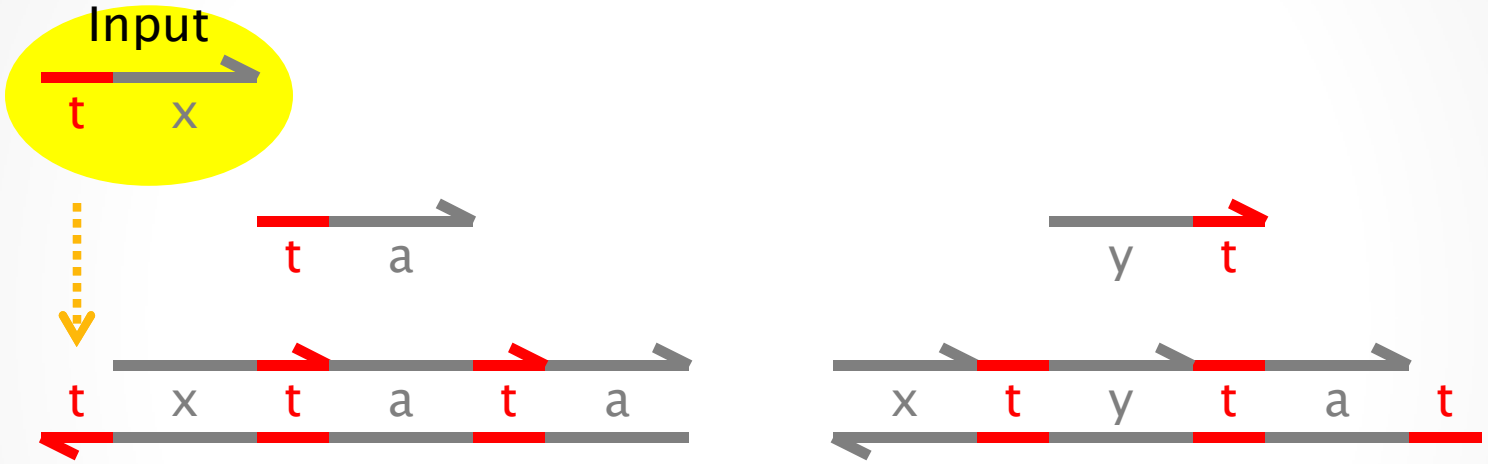
Luca Cardelli

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

Transducer x→y



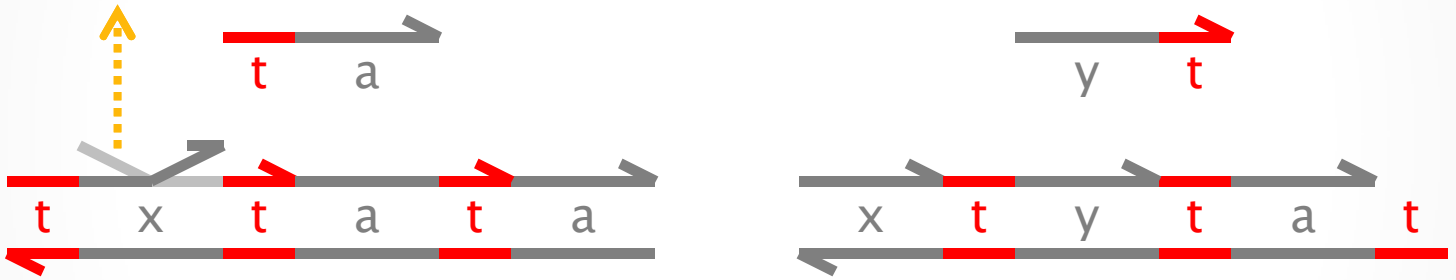
Transducer x→y



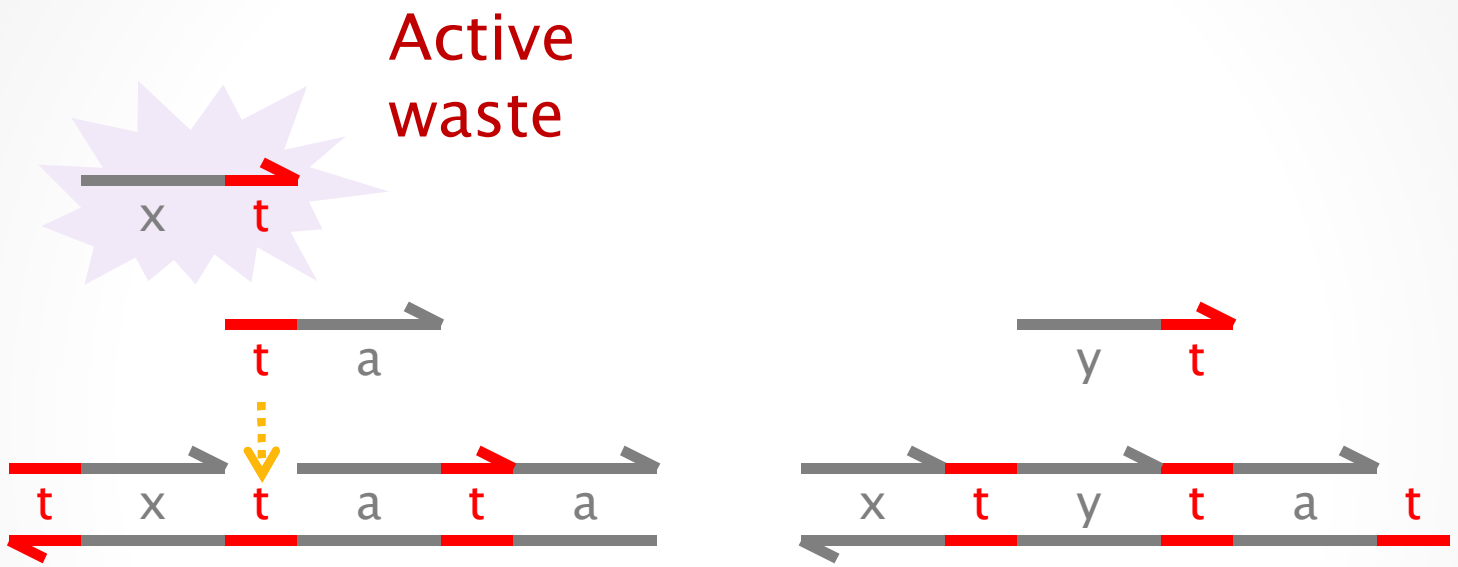
Built by self-assembly!

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

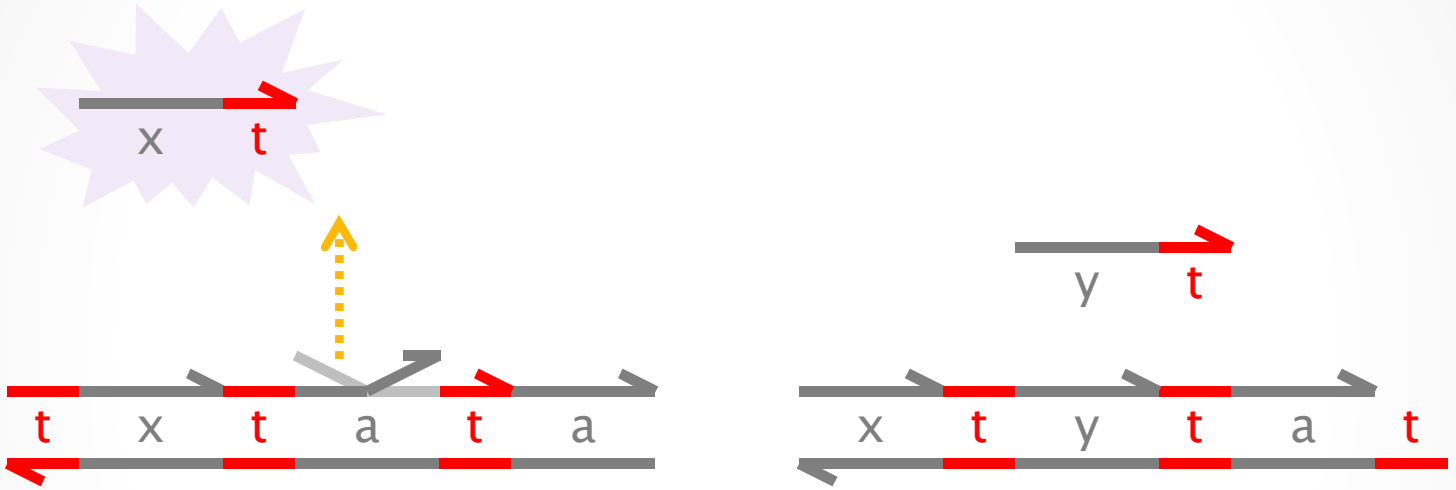
Transducer x→y



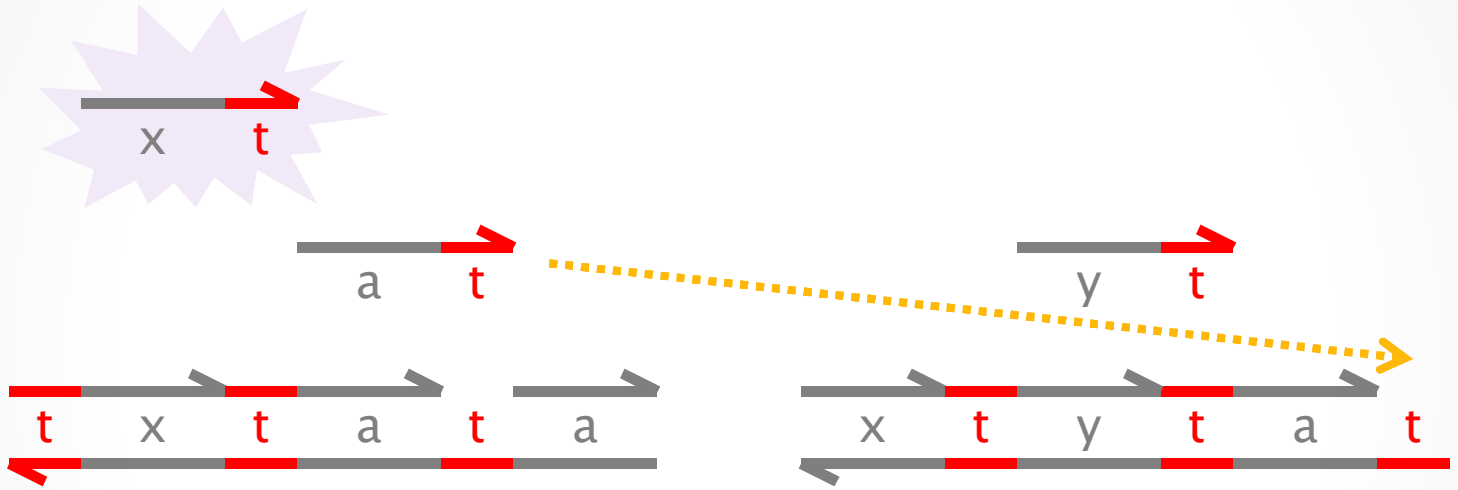
Transducer x→y



Transducer x→y

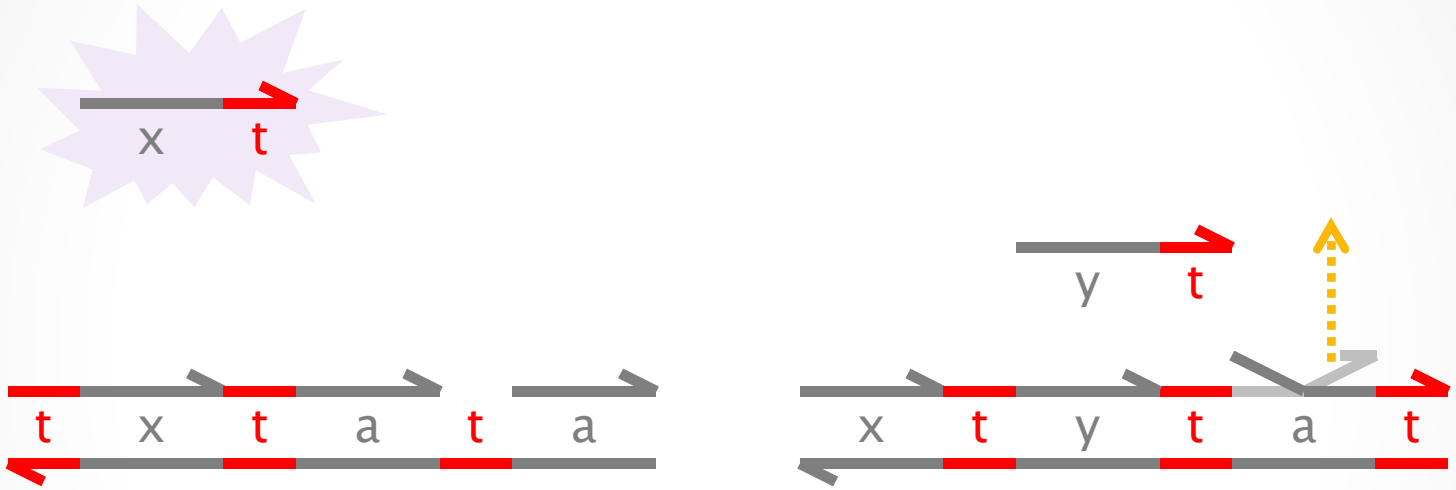


Transducer x→y

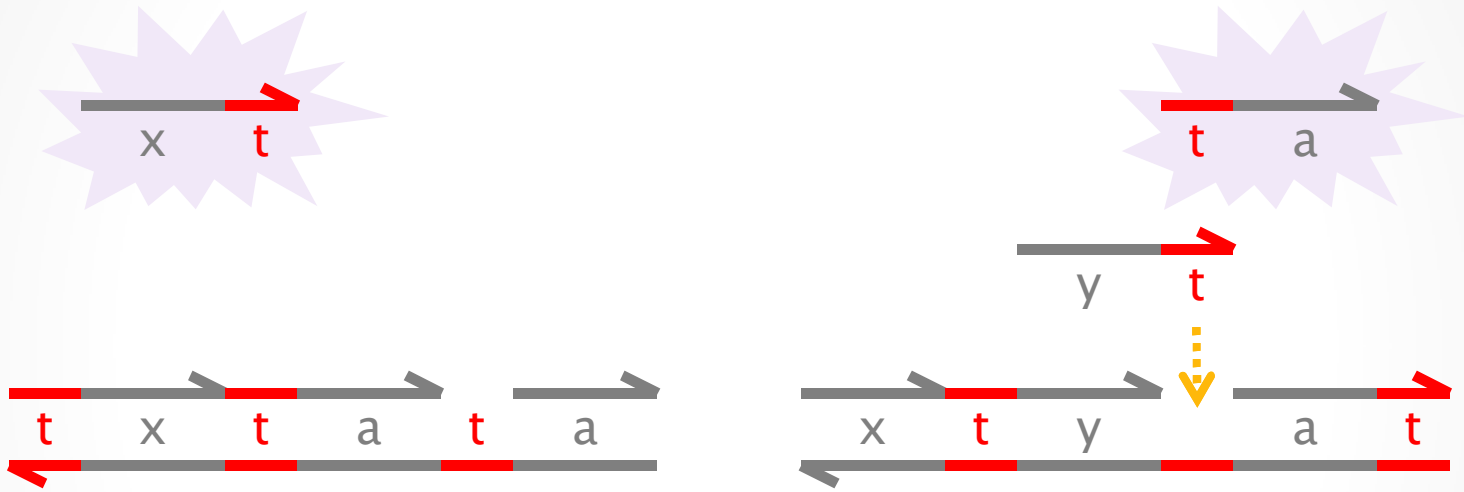


So far, a tx *signal* has produced an at *cosignal.*
But we want signals as output, not cosignals.

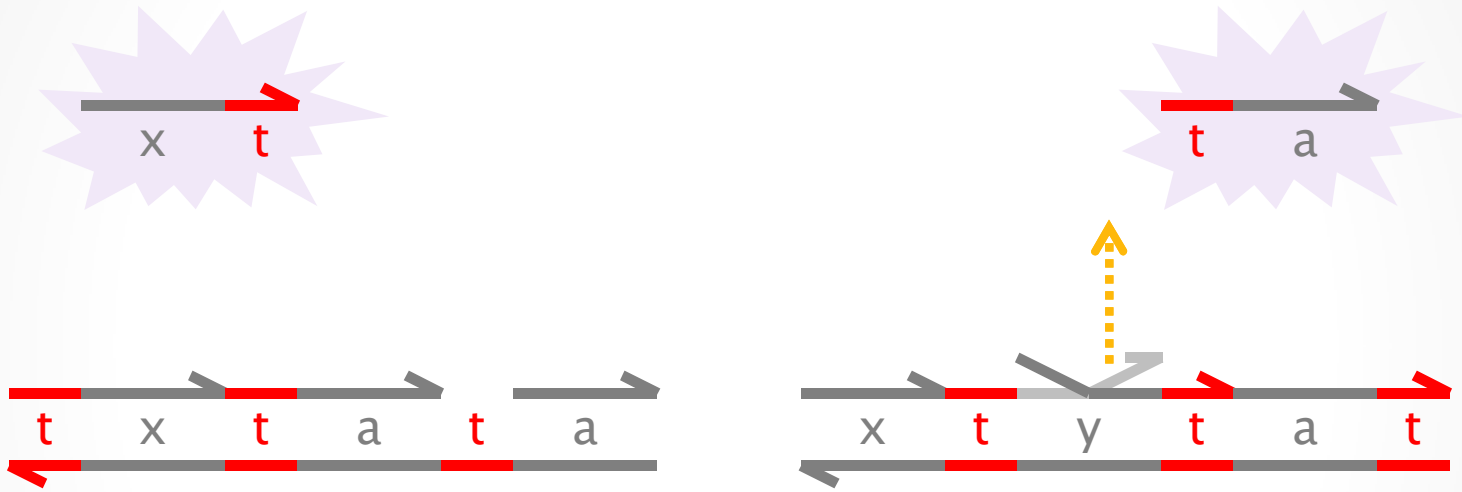
Transducer x→y



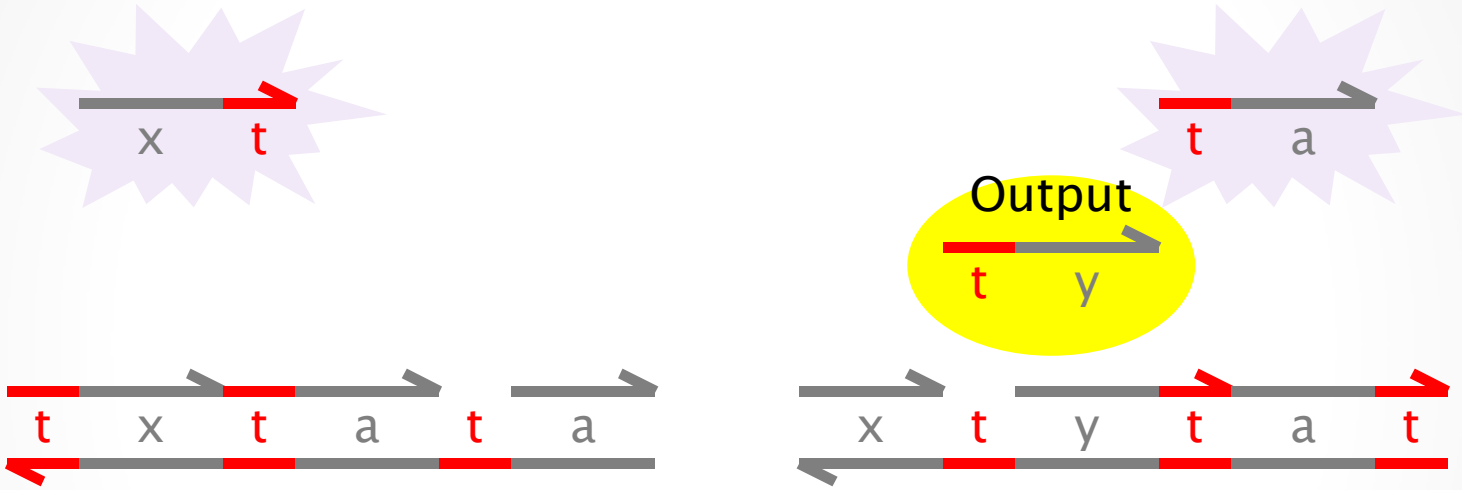
Transducer x→y



Transducer x→y



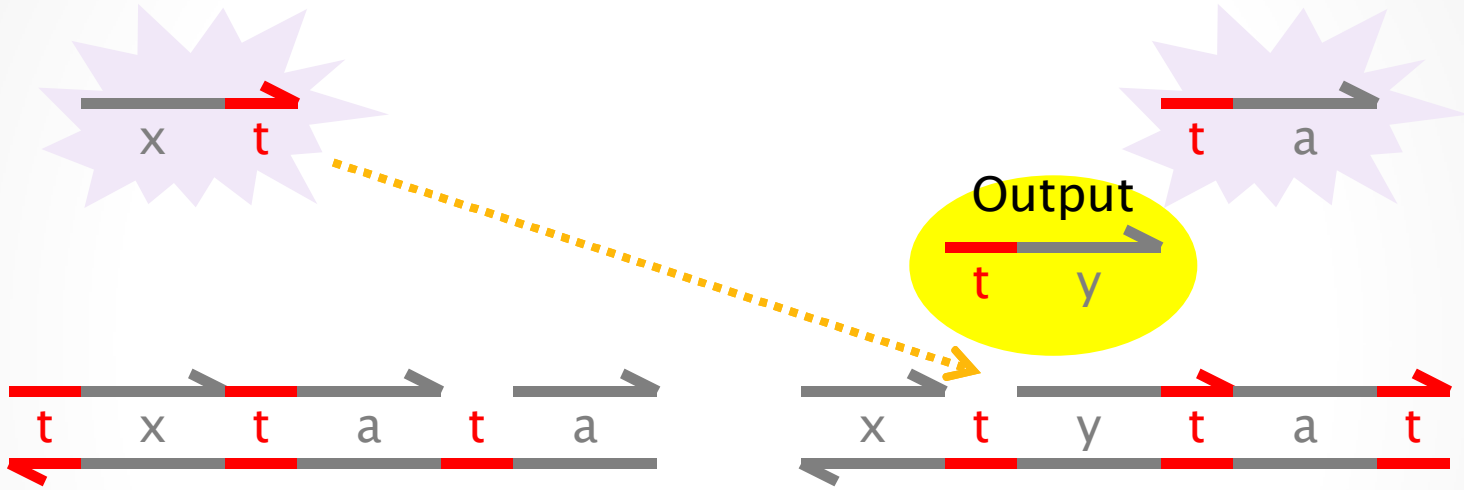
Transducer x→y



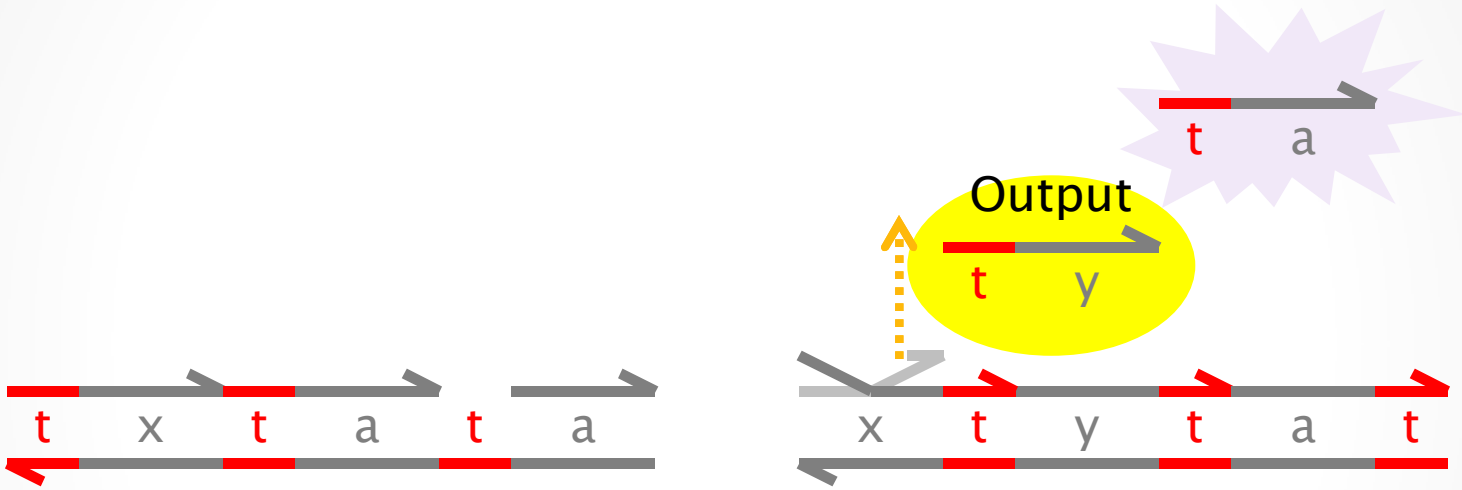
Here is our output ty *signal*.

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).

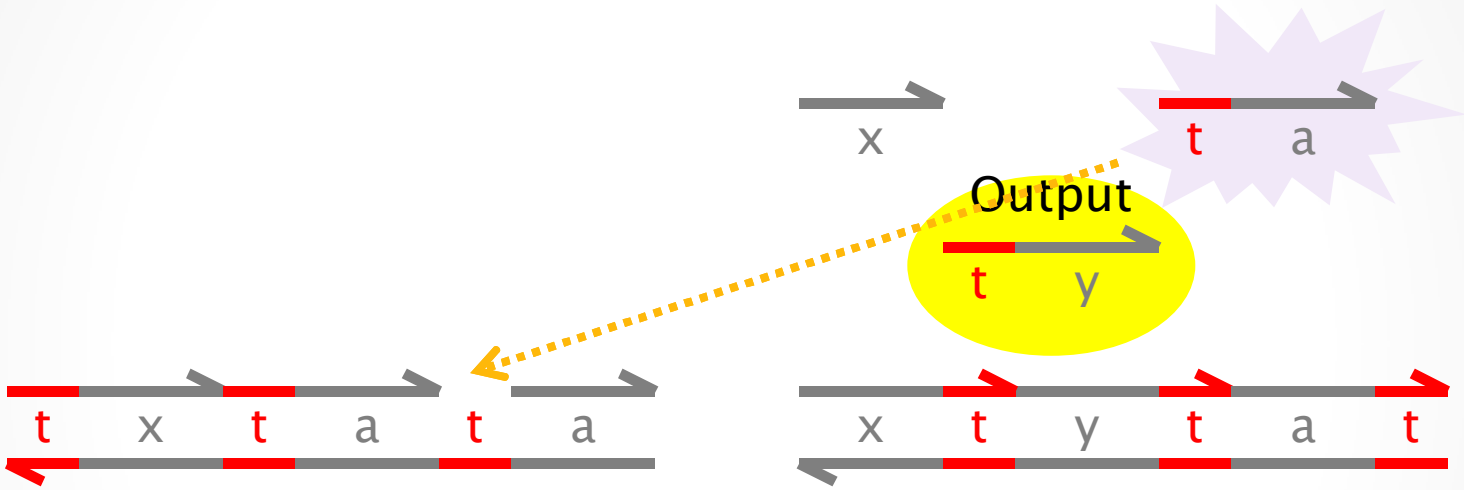
Transducer x→y



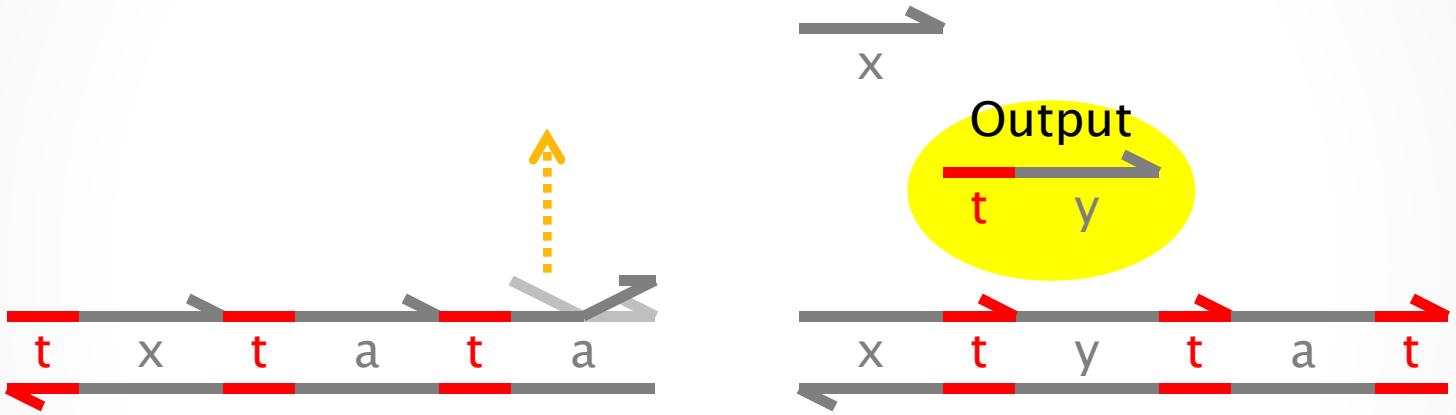
Transducer x→y



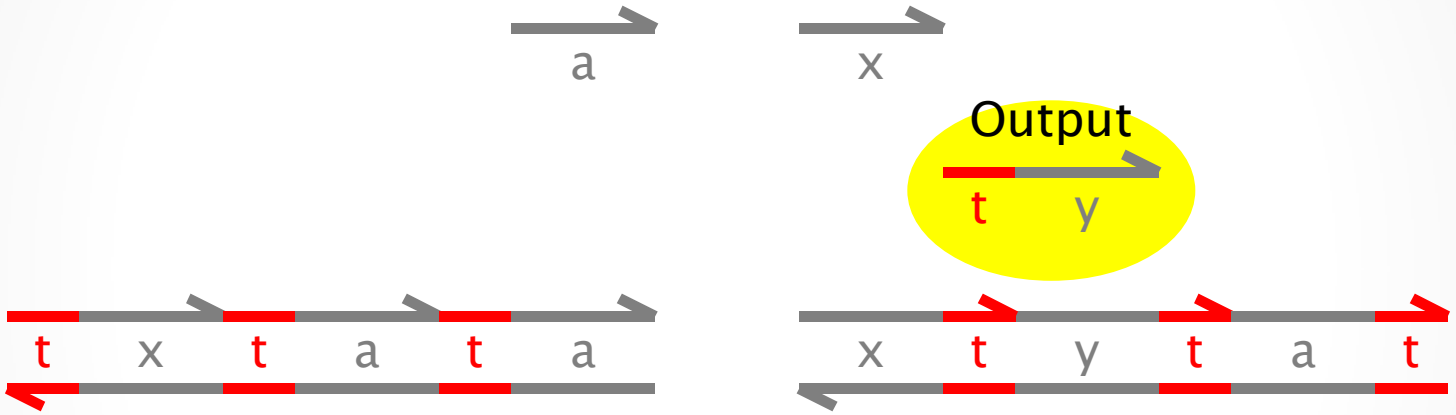
Transducer x→y



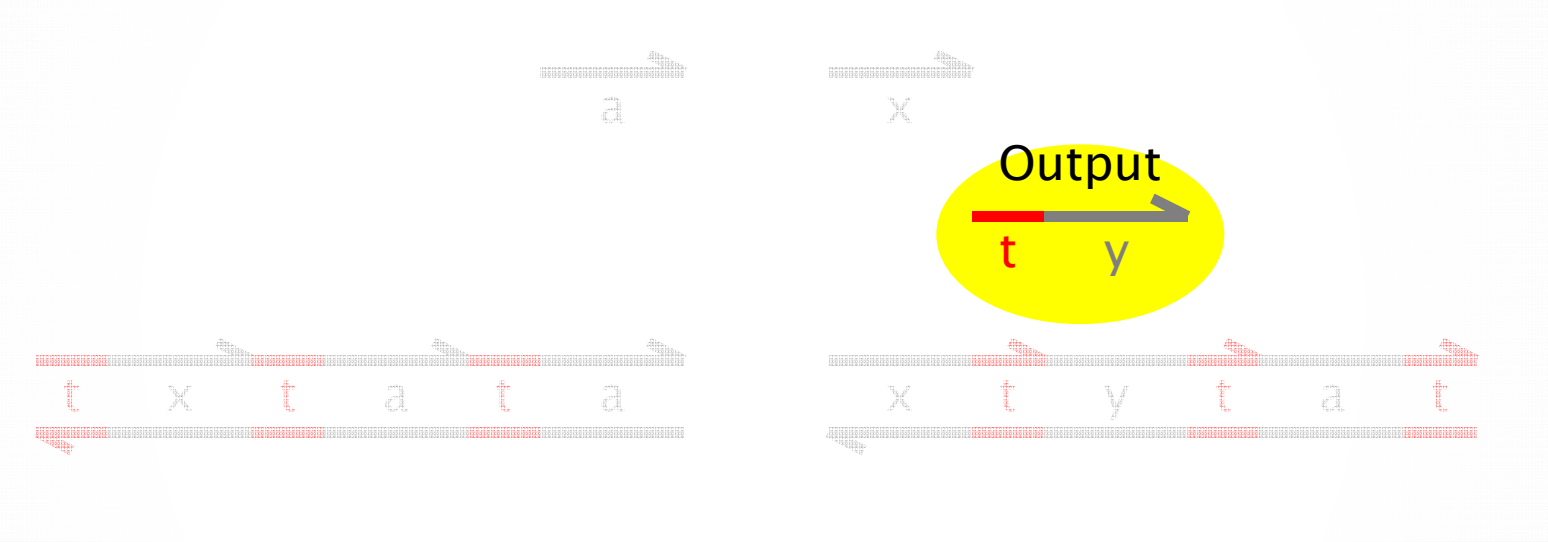
Transducer x→y



Transducer x→y

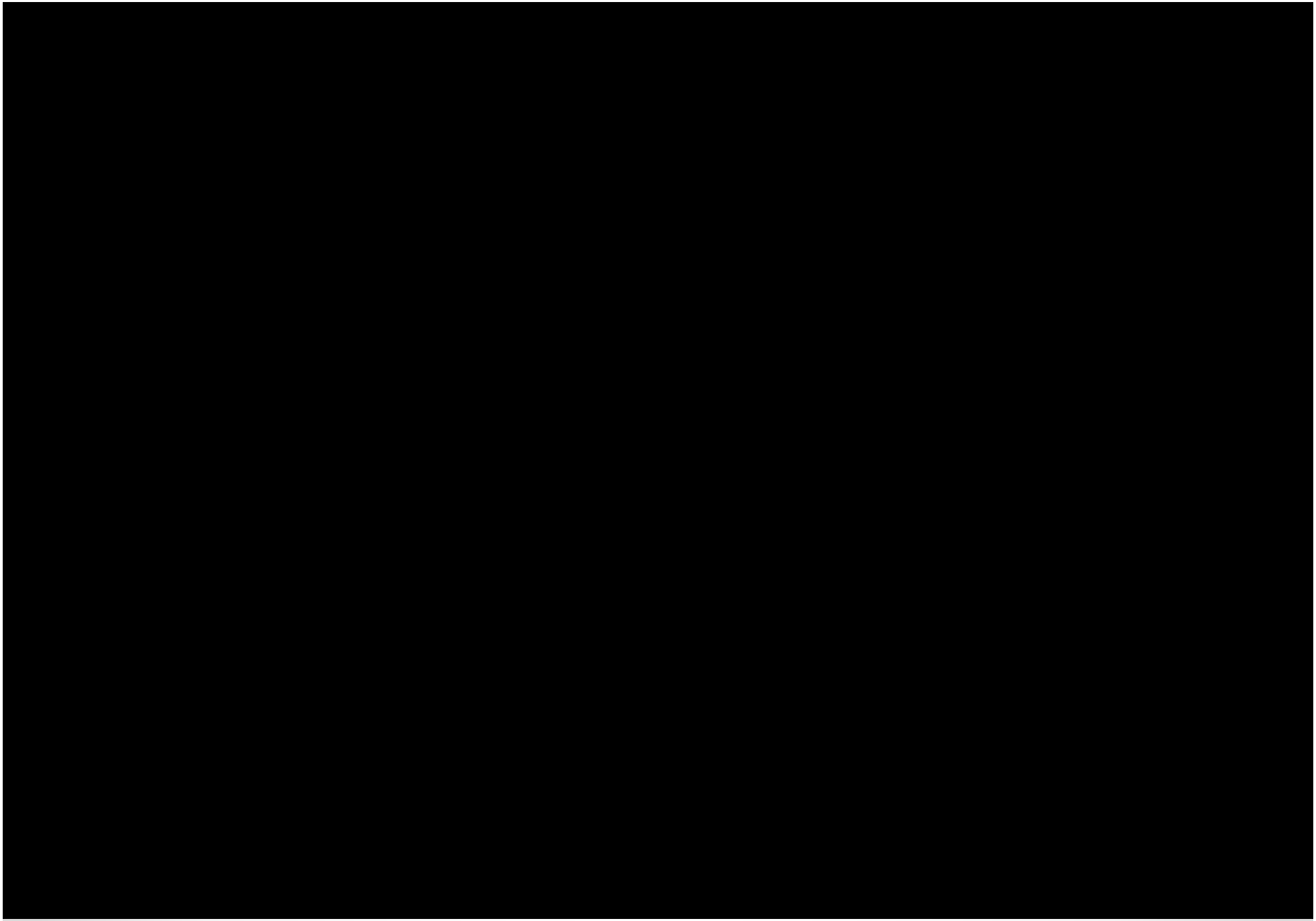


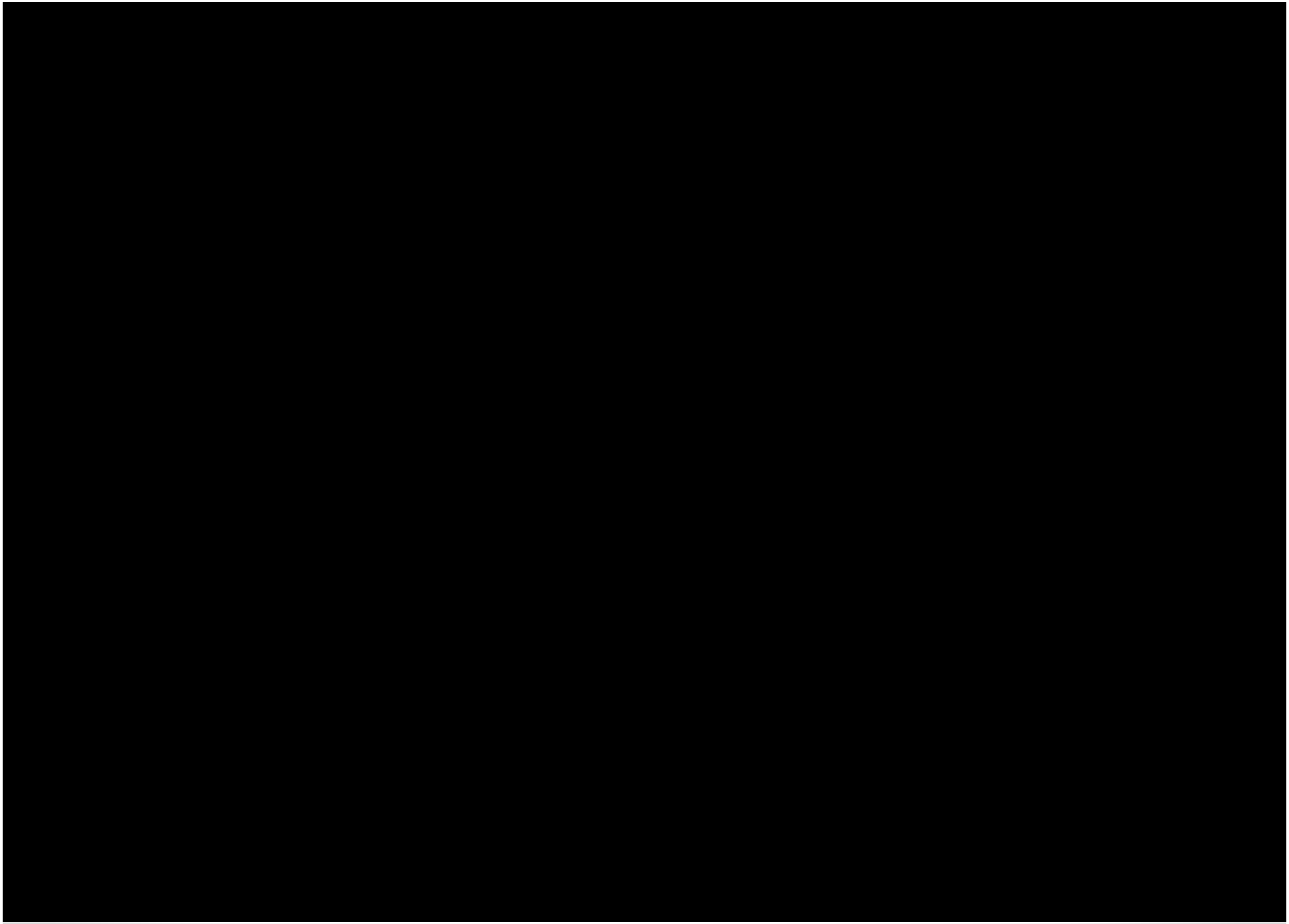
Transducer x→y



Done.

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





General n×m Join-Fork

- Easily generalized to 2+ inputs (with 1+ collectors).
- Easily generalized to 2+ outputs.

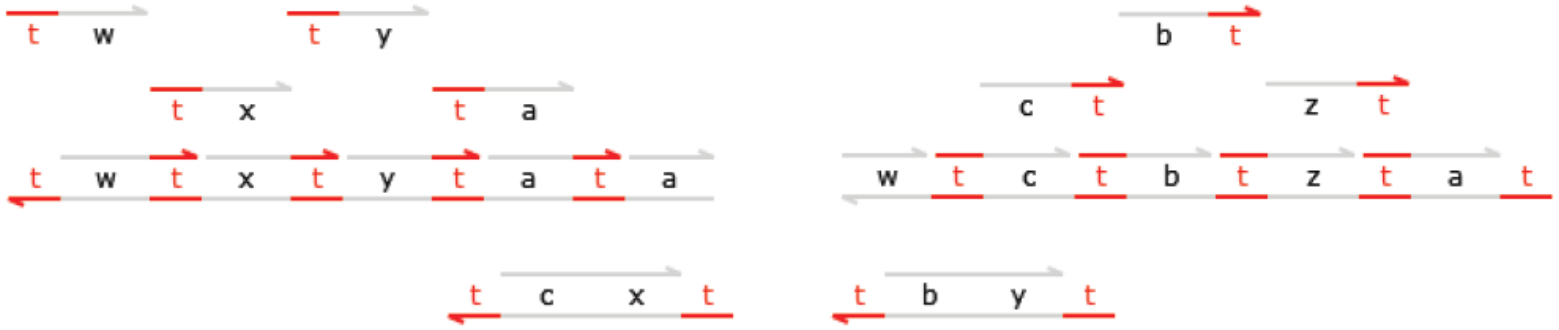
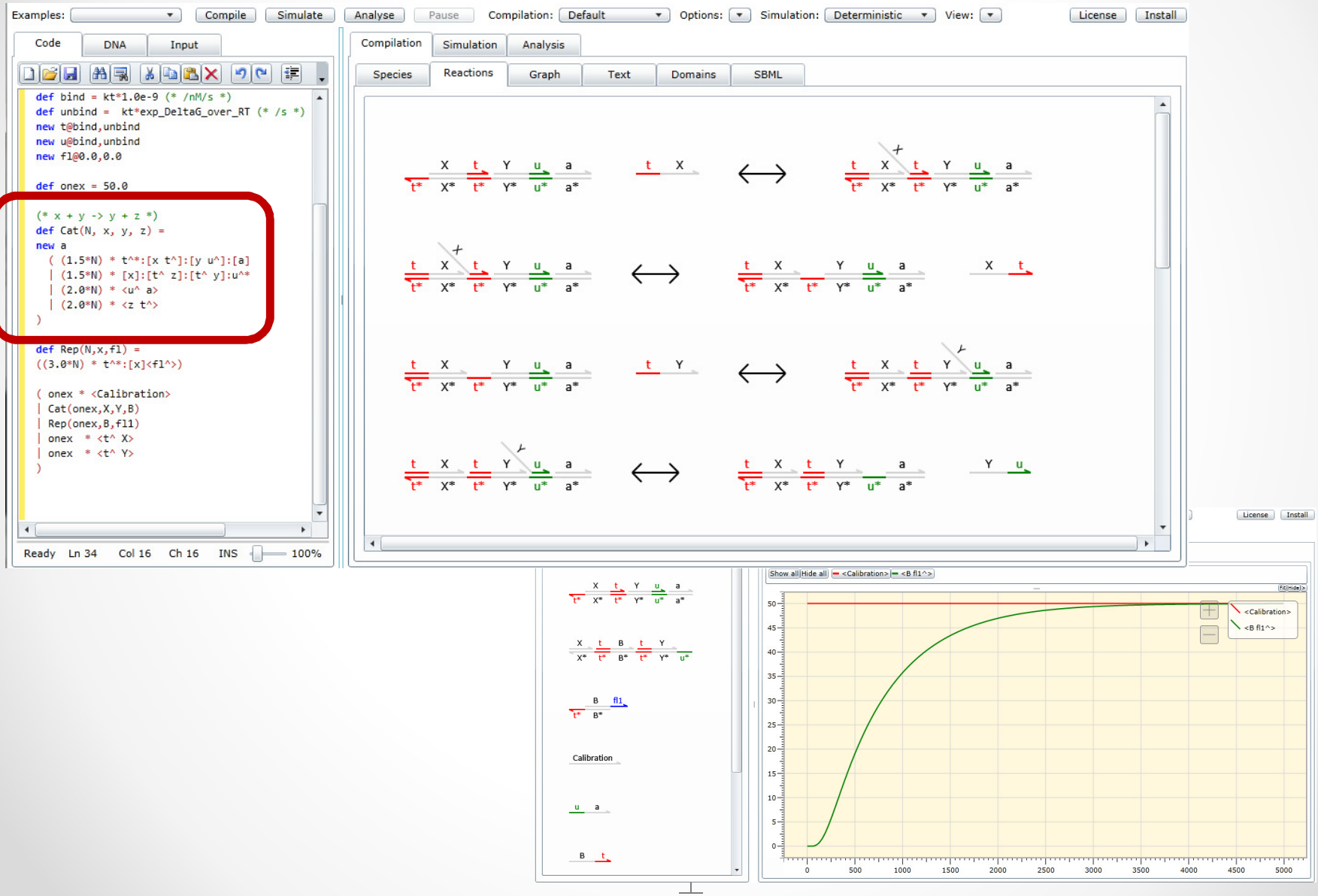


Figure 9: 3-Join $J_{wxyz} \mid tw \mid tx \mid ty \longrightarrow tz$: initial state plus inputs tw, tx, ty.

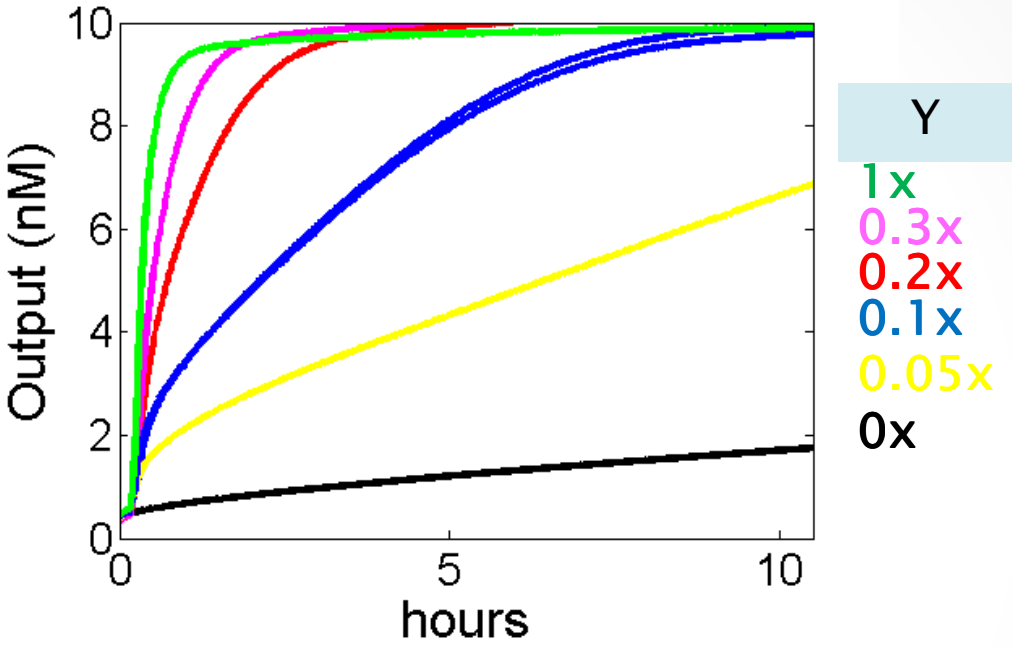
DNA Programming



Experiments

Two-domain gate for $X+Y \rightarrow Y+B$

X+Y→Y+B
35C
1x = 50nM



Yuan-Jyue Chen and Georg Seelig
U.Washingon.

X+Y→Y+B		Concentration
LG1		1.5x
LG2		1.5x
input		1x
Catalyst		0x, 0.05x,0.1x,0.2x,0.3x,1x
~B		2x
R1		2x
B readout		3x

Verification

Verification Issues

- Environment
  - The nano-environment is messy (stochastic noise, failures, etc.)
  - But we should al least ensure our designs are *logically correct*
- Verifying Components
  - Reversible reactions (infinite traces)
  - Interferences (deadlocks etc.) between copies of the same gate
  - Interferences (deadlocks etc.) between copies of different gates
  - Removal of active byproducts (garbage collection) is tricky
- Verifying Populations
  - Gates come in (large) populations
  - Each population *shares private domains* (technologically unavoidable)
  - Correctness of populations means proofs with large state spaces

Correctness

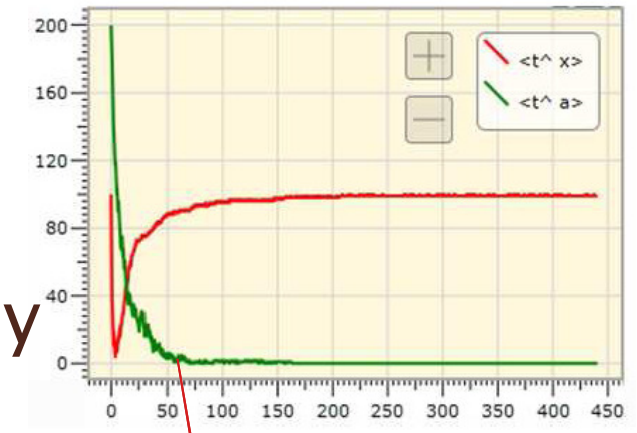
- The spec of a transducer:

$$x.y \mid x \rightarrow y$$

  - Is it true at all?
  - Is it true *possibly, necessarily,* or *probabilistically ?*
  - Is it true in the context of a *population of identical transducers?*
  - Is it true *in all possible contexts?*
  - If false, does it become true for *infinite populations*?

Interfering Transducers

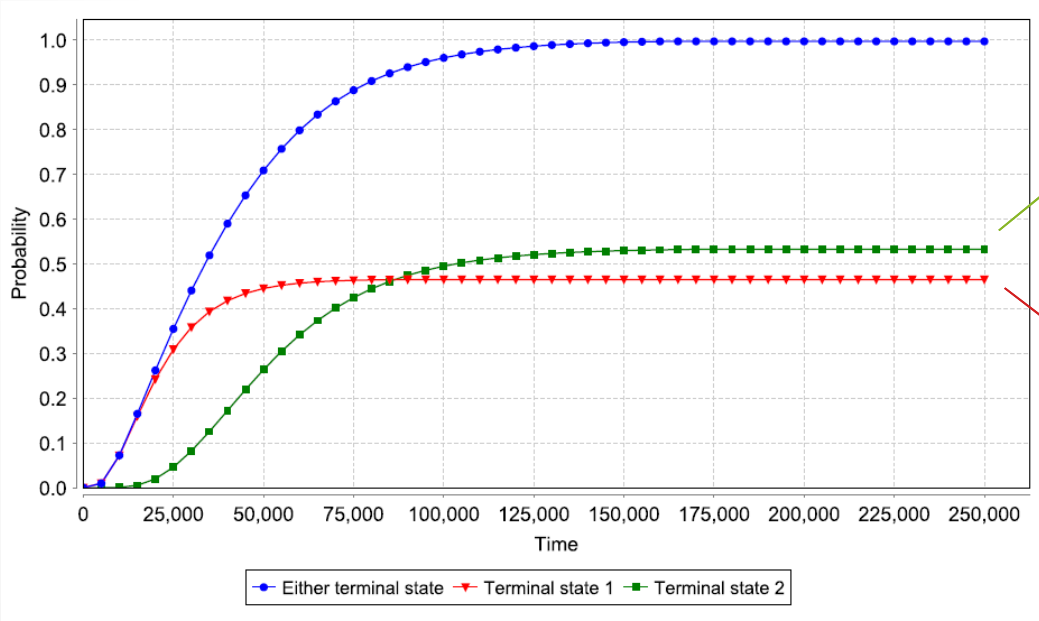
- Let a be the private transducer domain, but let's share it between x.y and y.x
- Interference: $x._a y \mid y._a x \mid x \not\rightarrow^{\forall} x$
- But still: $x._a y \mid y._a x \mid x \mid y \rightarrow^{\forall} x \mid y$
- A large population of such gates in practice does not deadlock easily.
- The wisdom of crowds: individuals can be wrong, but the population is all right.



Stuck gates in a population of 200

Modelchecking DNA Systems

- Using the PRISM stochastic modelchecker
  - Termination probability of interfering transducers $x \mid x._a y \mid y._a z$



Correct Termination

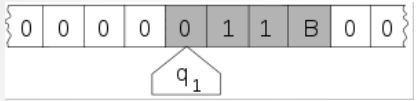
Incorrect Termination

L. Cardelli, M. Kwiatkowska, M. Lakin, D. Parker and A. Phillips.
Design and Analysis of DNA Circuits using Probabilistic Model Checking.
http://qav.comlab.ox.ac.uk/papers/dna-pmc.pdf. September 2010

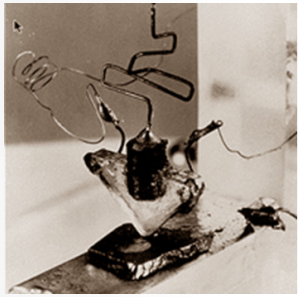
Conclusions

A Brief History of DNA

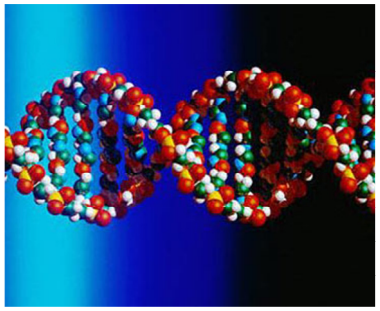
Turing Machine, 1936



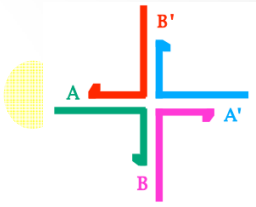
Transistor, 1947



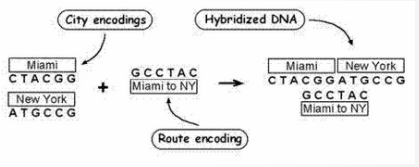
DNA, ~3,800,000,000



Structural DNA, 1982



DNA Algorithm, 1994



Digital ~~Computers~~

Computer programming

Software

systematic manipulation of information

20th century

Matterware??

systematic manipulation of matter

21th century

DNA ~~Computers~~

Molecular programming

Acknowledgments

- Microsoft Research
  - Andrew Phillips
- Caltech
  - Winfree Lab
- U.Washington
  - Seelig Lab