Genetic programs constructed from layered logic gates in single cells

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Presented by Paula and Natalie

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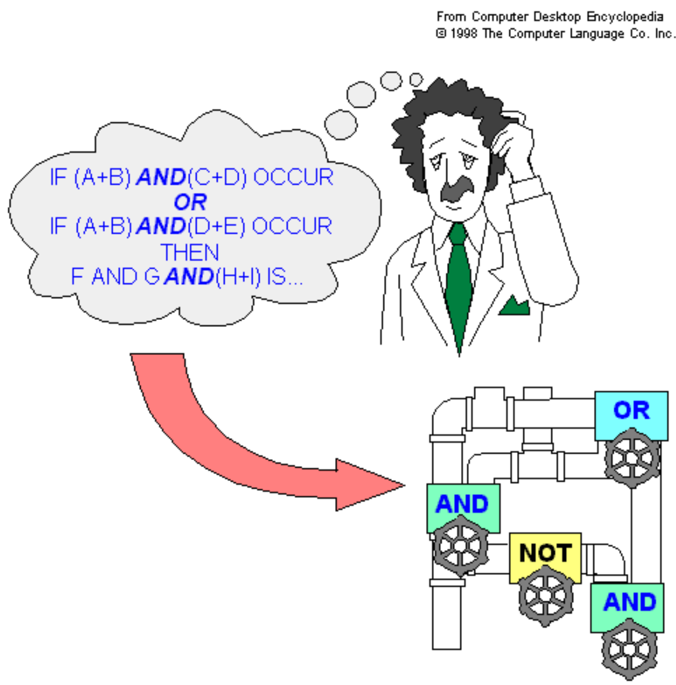
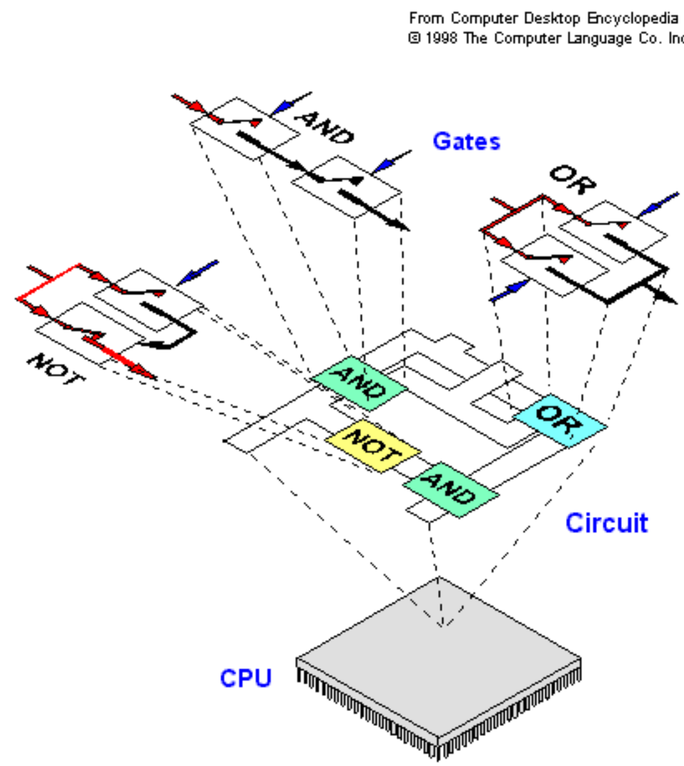
20.385

Nature 491, 249–253 (08 November 2012) doi:10.1038/nature11516

Applications of Layered Logic Gates

With the proper arrangement of Boolean logic gates, it's possible to create memory. Two NOT gates where the output feeds back to the input of the first NOT gate, and in this way the gate will "remember" the input value.

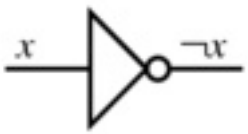




With a few simple logic gates, it's possible to make a wide variety of useful memory circuits

Boolean Logic Gates

NOT



In (A)	Out
0	0
1	0
0	1

OR

In (A)	In (B)	Out
0	0	0
0	1	1
1	0	1
1	1	0

AND

In (A)	In (B)	Out
0	0	0
0	1	0
1	0	0
1	1	1

NOT + AND = NAND

NOT + OR = NOR

Building a molecular AND gate

AND

In (A)	In (B)	Out
0	0	0
0	1	0
1	0	0
1	1	1

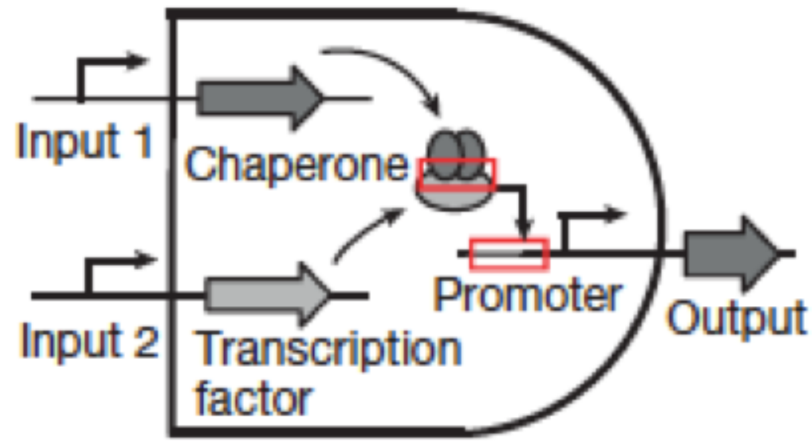
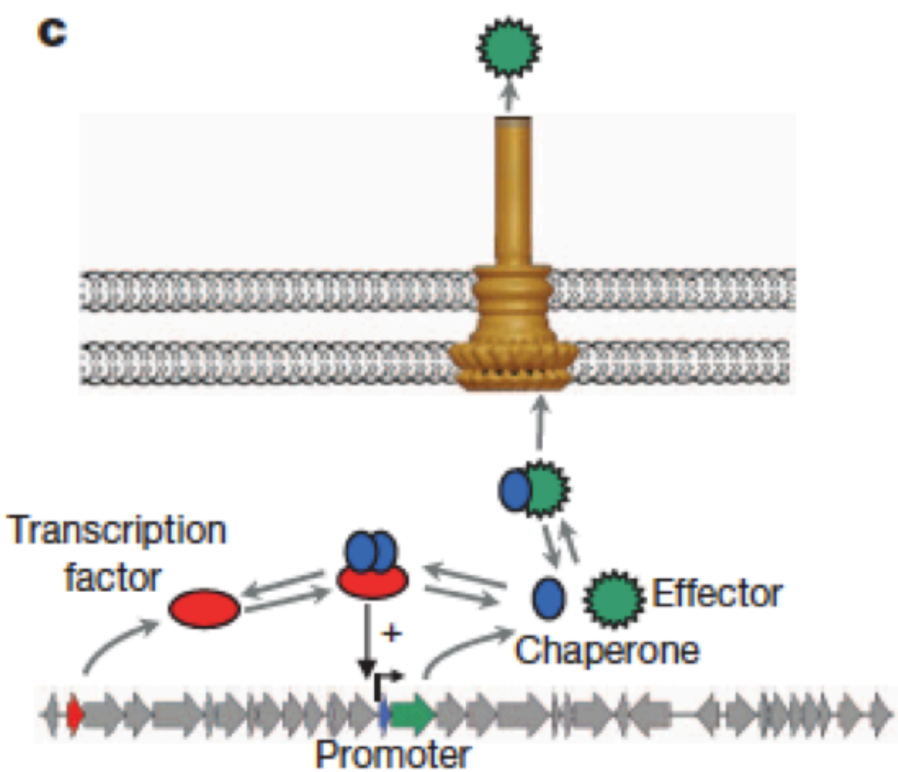


Figure 1b

Source	Transcription Factor	Chaperone	Promoter
Salmonella	InvF	SicA	PsicA

Type Three Secretion System



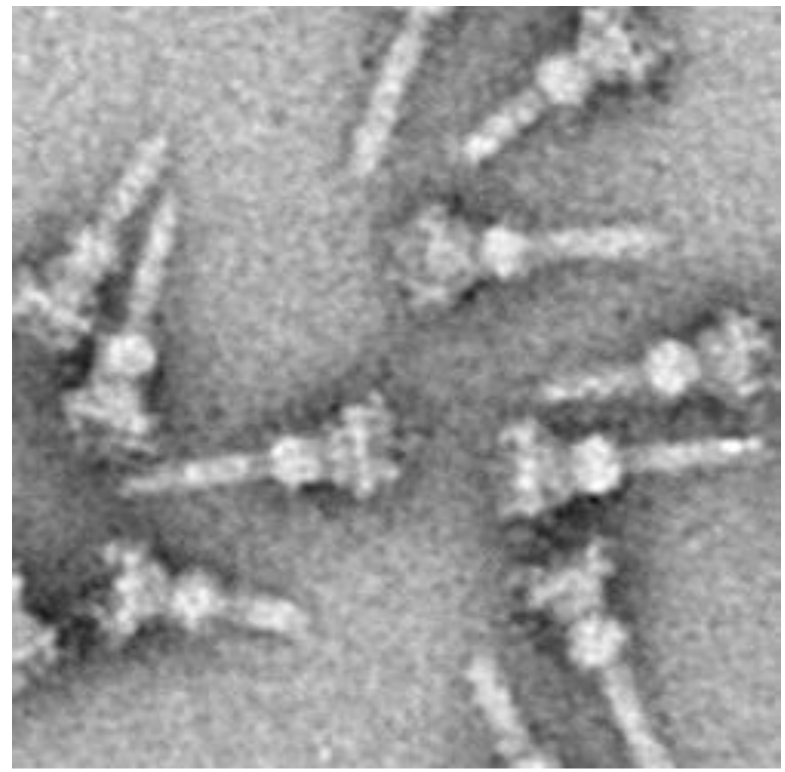


Figure 1c

Orthogonal Pairs from other T3SS

Source	Transcription Factor	Chaperone	Promoter
Salmonella	InvF	SicA	P(sicA)
Shigella	MxiE	IpgC	P(ipaH)
Yersinia	YsaE	SycB	P(syc)
Pseudomonas	ExsA	ExsC	P(exsC)

Orthogonal Pairs: sequence identity

Source	Transcription Factor	Chaperone	Promoter
Salmonella	InvF (100%)	SicA (100%)	P(sicA)
Shigella	MxiE (27%)	IpgC (54%)	P(ipaH)
Yersinia	YsaE (15%)	SycB (50%)	P(syc)
Pseudomonas	ExsA (10%)	ExsC (13%)	P(exsC)

Orthogonal Pairs: down to three

Source	Transcription Factor	Chaperone	Promoter
Salmonella	InvF (100%)	SicA (100%)	P(sicA)
Shigella	MxiE (27%)	IpgC (54%)	P(ipaH)
~~Yersinia~~	~~YsaE (15%)~~	~~SycB (50%)~~	~~P(syc)~~
Pseudomonas	ExsA (10%)	ExsC (13%)	P(exsC)

"Yesinia parts were non-functional and were not pursued further."

Orthogonal Pairs: optimizing InvF/SicA

Source	Transcription Factor	Chaperone	Promoter
Salmonella	InvF*	SicA	P(sicA)
Shigella	MxiE	IpgC	P(ipaH)
Pseudomonas	ExsA	ExsC	P(exsC)

Annotated InvF MSFSESR
Correct InvF MLNTQEVLKEGEKRKIRSPEAWFIQTCSAQKLHMSFSESR

Figure 2a

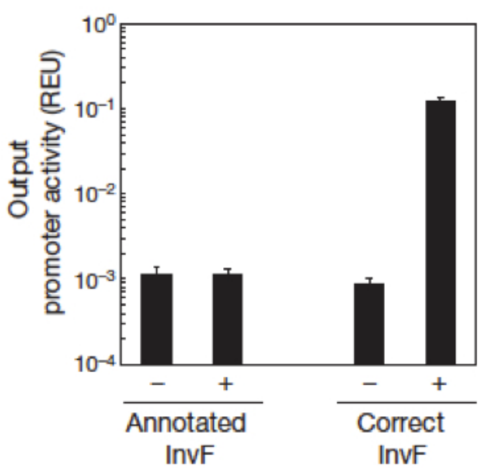


Figure 2b

Orthogonal Pairs: optimizing MxiE/IpgC

Source	Transcription Factor	Chaperone	Promoter
Salmonella	InvF*	SicA	P(sicA)
Shigella	MxiE	IpgC	P(ipaH)*
Pseudomonas	ExsA	ExsC	P(exsC)

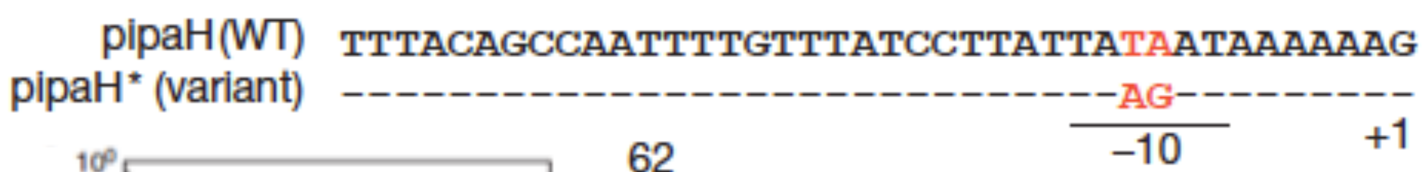


Figure 2a

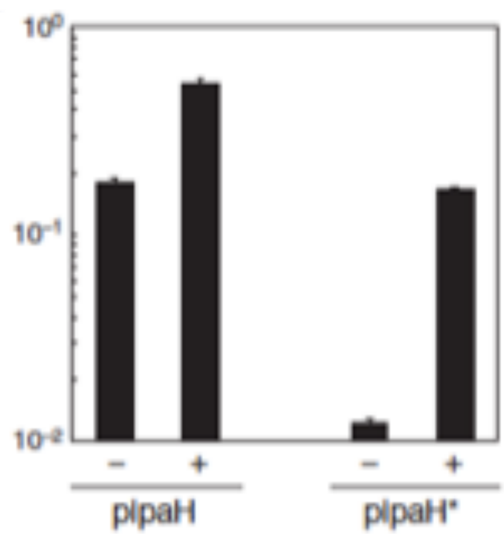
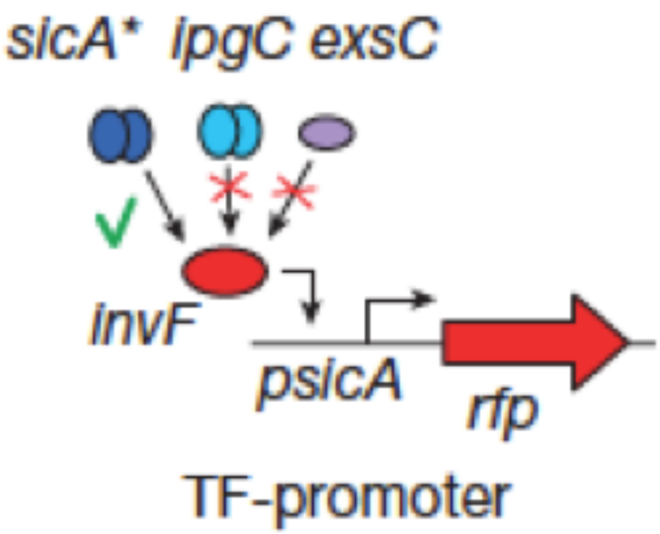


Figure 2c

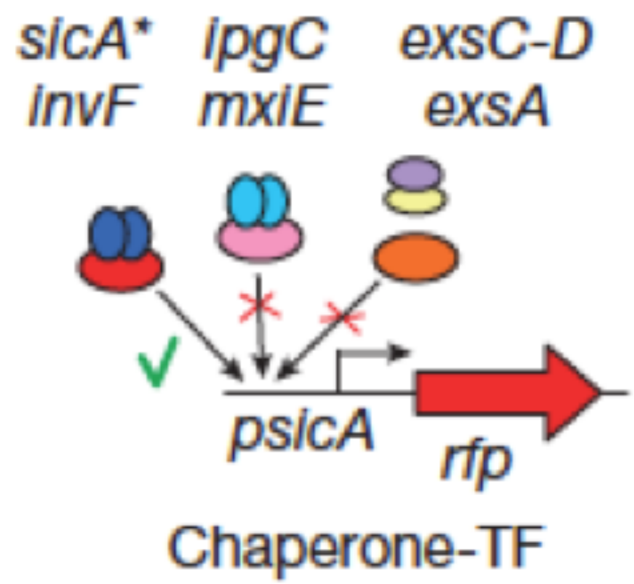
Cross talk: defined

“In electronics, **crosstalk** (**XT**) is any phenomenon by which a signal transmitted on one circuit or channel of a transmission system creates an undesired effect in another circuit or channel.”

One kind of cross talk



Another kind of cross talk



Orthogonal Pairs: reducing SicA crosstalk

Source	Transcription Factor	Chaperone	Promoter
Salmonella	InvF*	SicA*	P(sicA)
Shigella	MxiE	IpgC	P(ipaH)*
Pseudomonas	ExsA	ExsC	P(exsC)

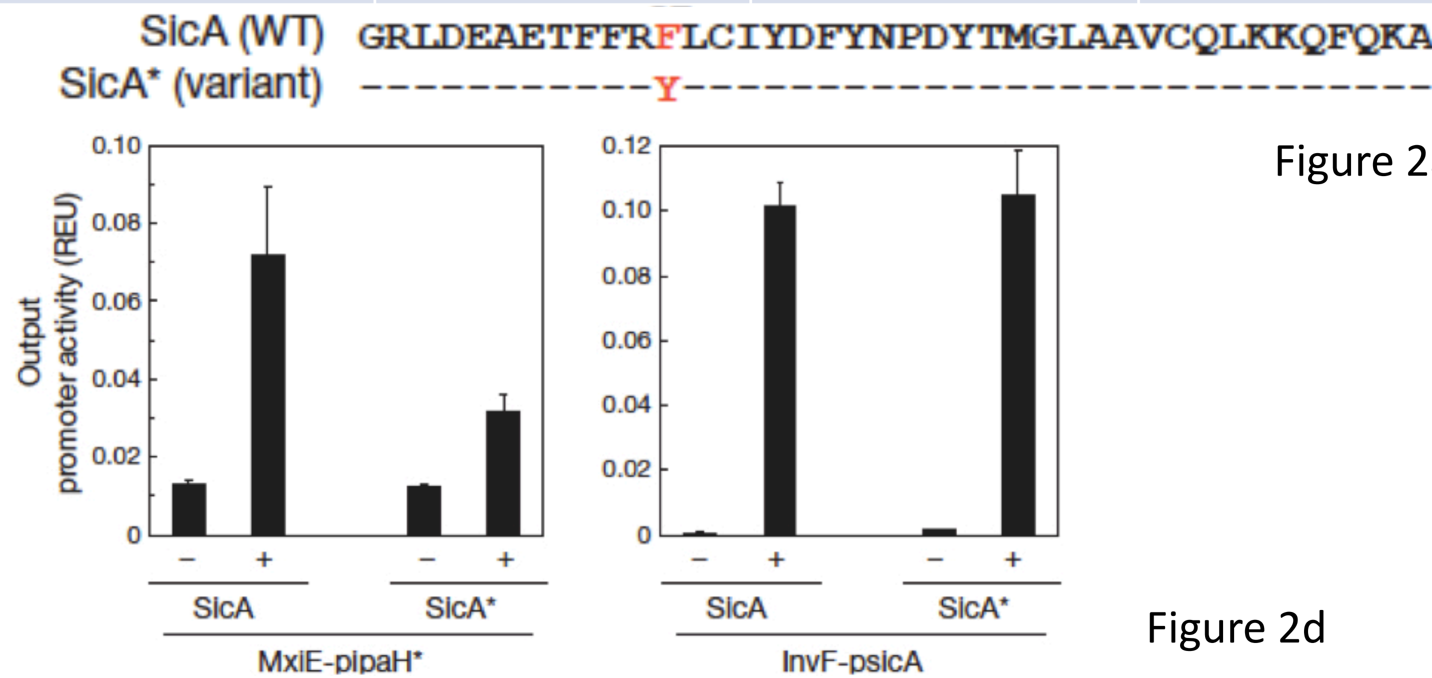
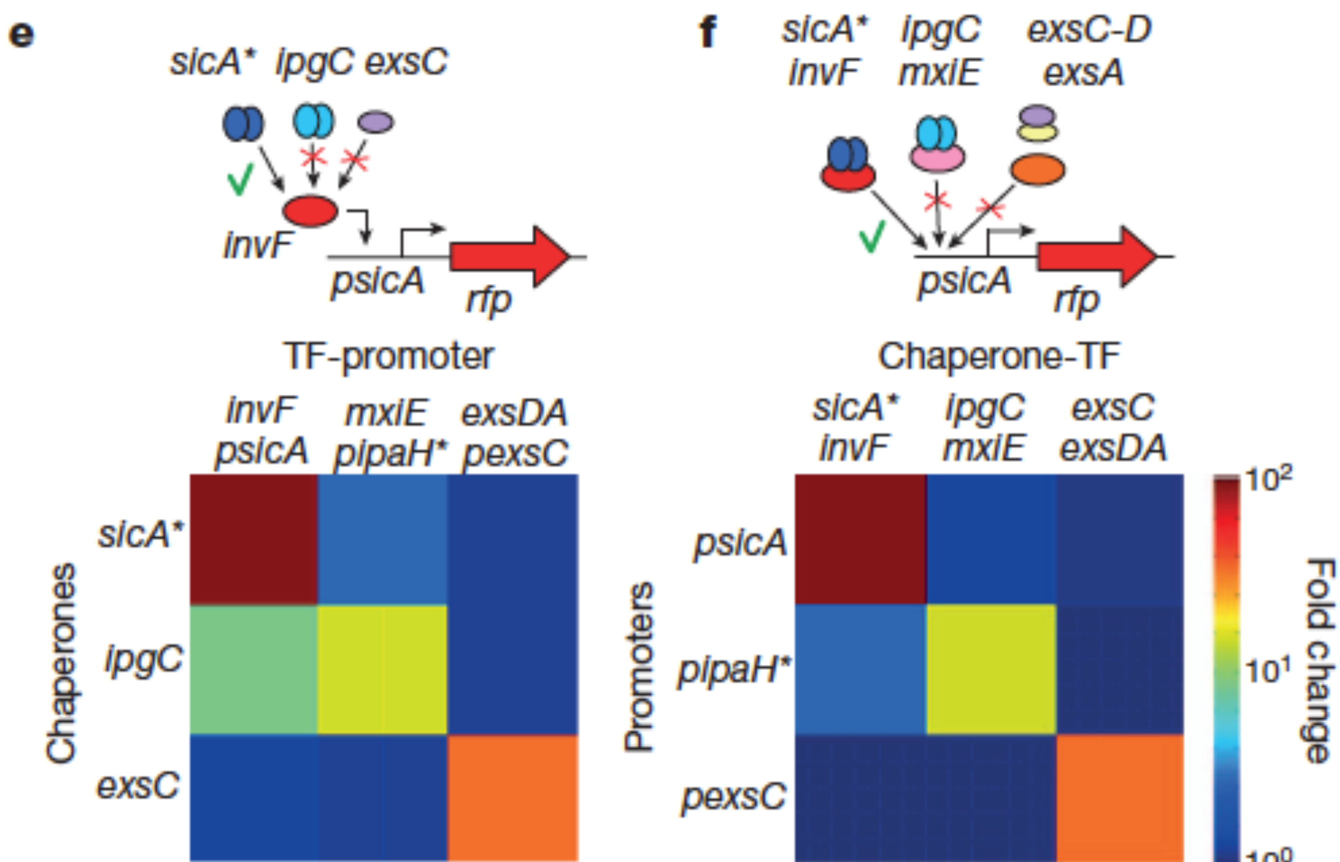


Figure 2a

Figure 2d

Three Orthogonal Pairs

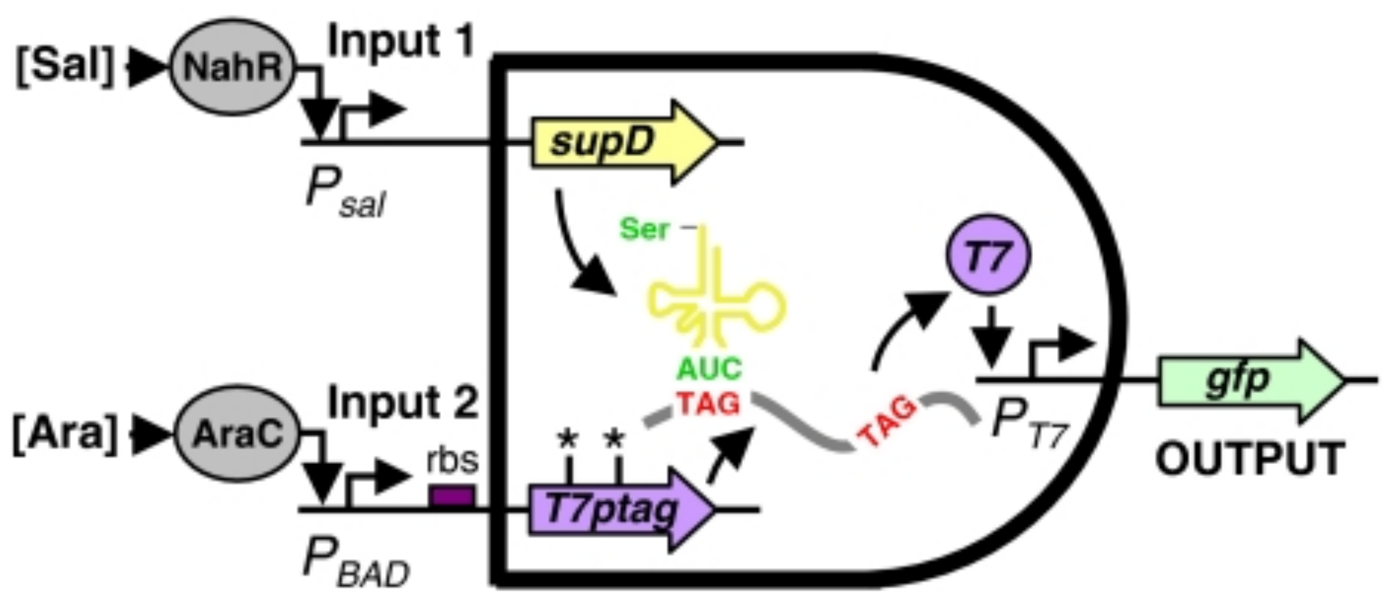


Alternative AND gate

Mol Syst Biol. 2007;3:133. Epub 2007 Aug 14.

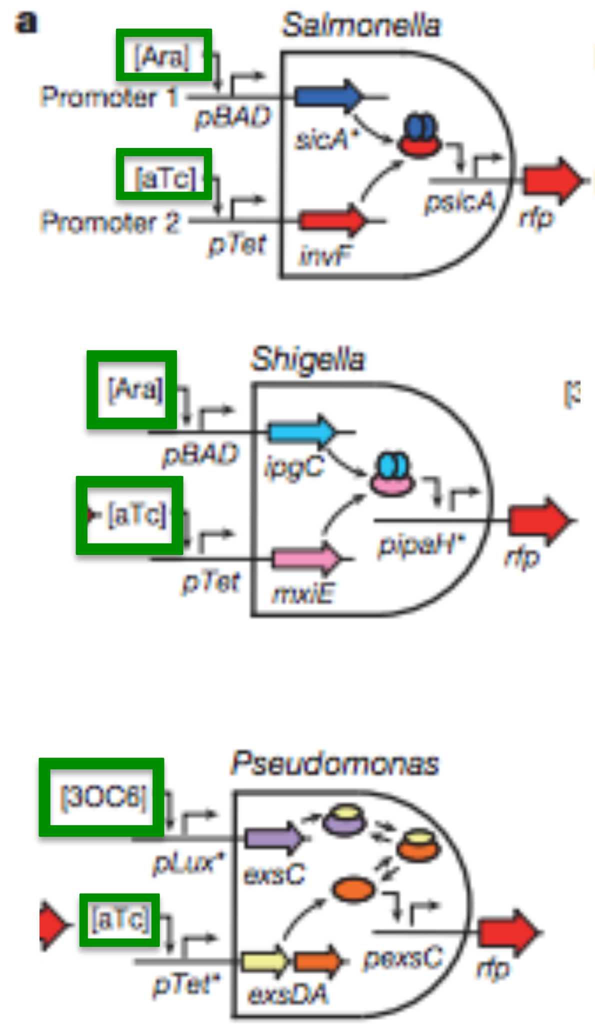
Environmental signal integration by a modular AND gate.

Anderson JC, Voigt CA, Arkin AP.

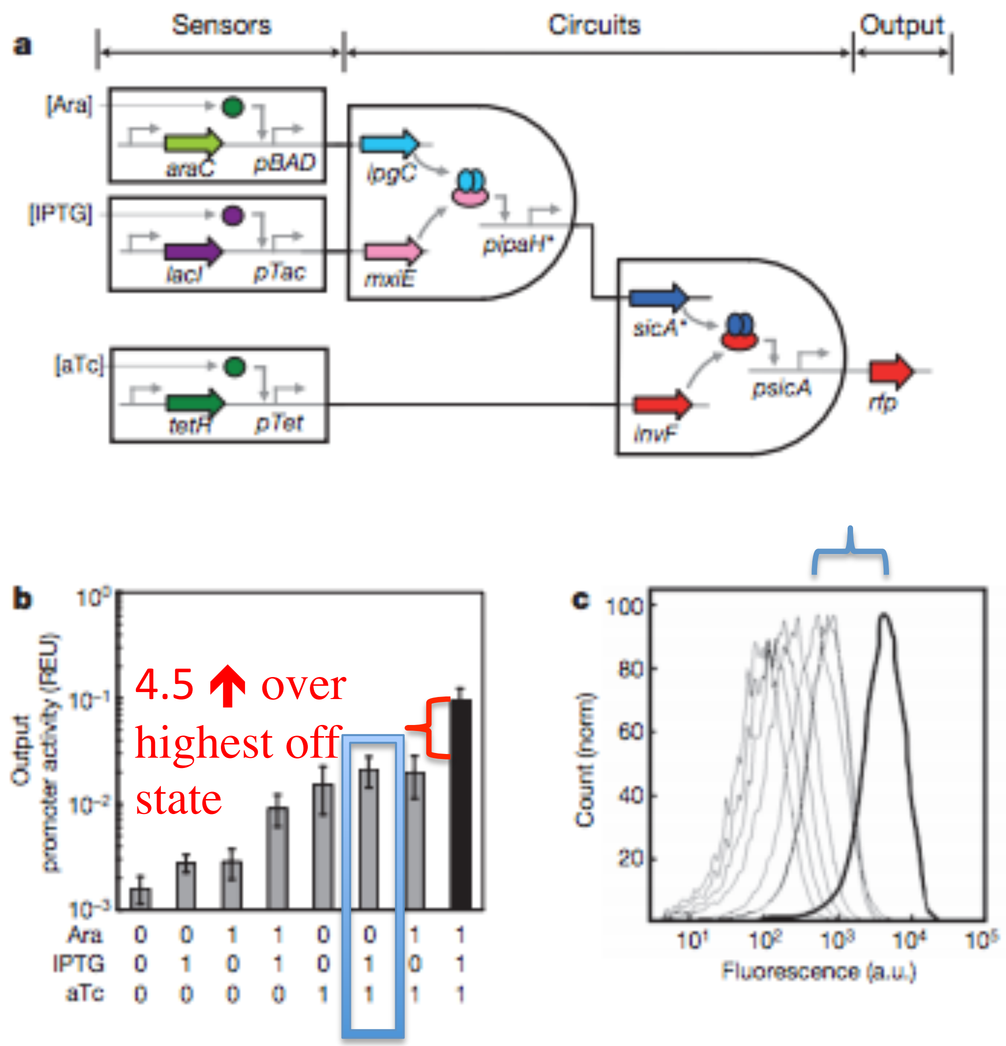


Three 2-input AND gates constructed

- Two promoters chosen:
  - *Salmonella/Shingella*
    - Arabinose (pBAD)
    - aTc (pTet)
  - *Pseudomonas*
    - AI-1 (pLux*)
    - aTc (pTet)
- Output promoter transcriptionally fused to RFP
- Gates characterized by normalization to a constitutive promoter

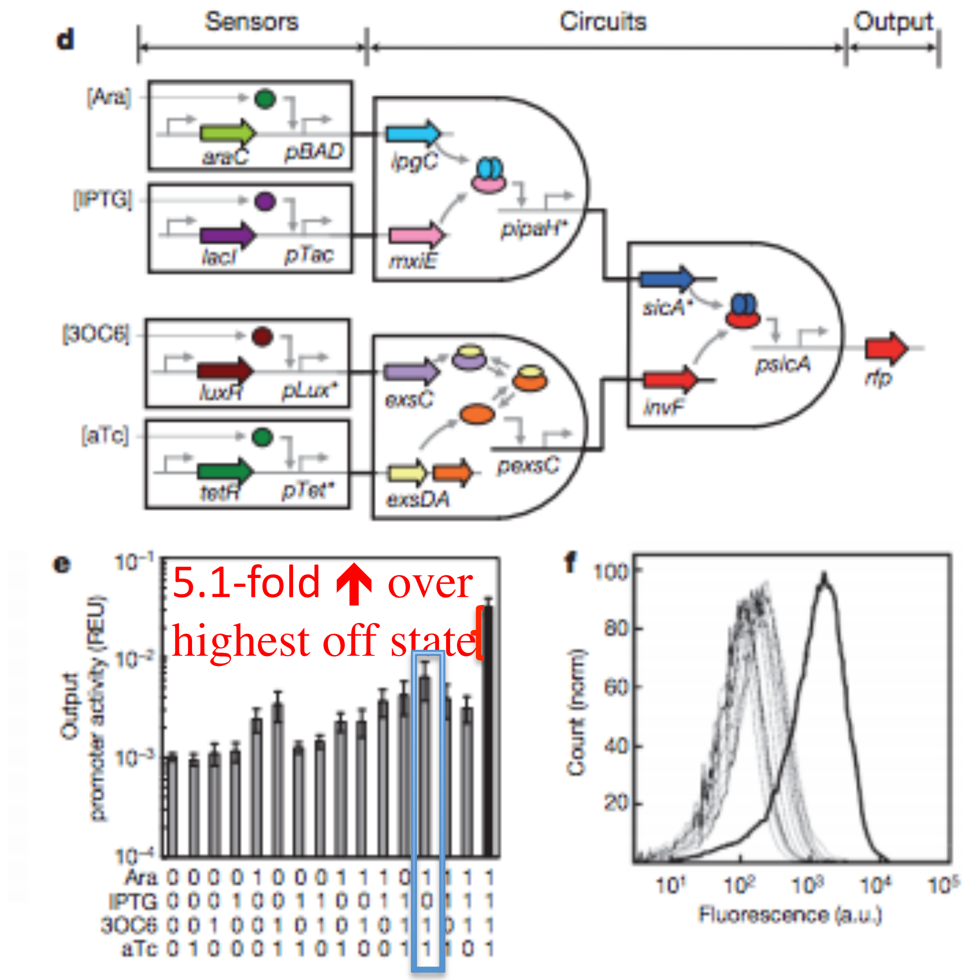


Three-input system exhibits 4.5-fold increase in fluorescence output



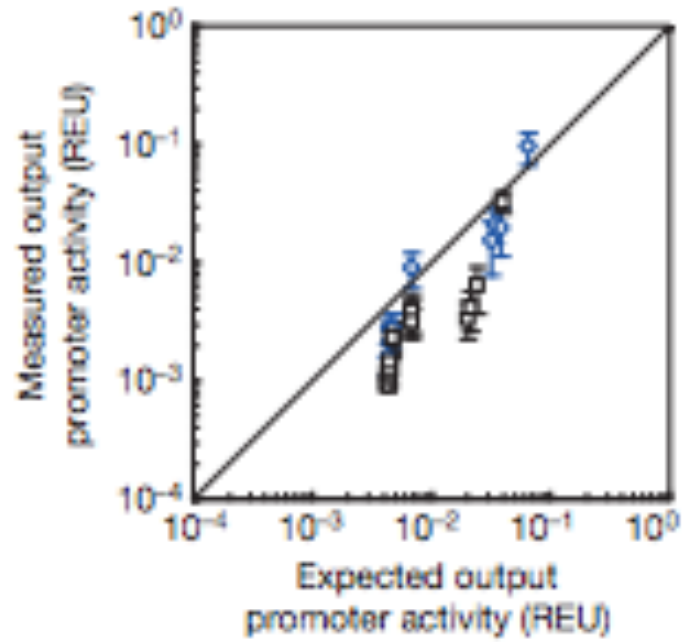
Four-input system increases expression 5.1-fold

- Constructed using:
  - 7 genetic devices
  - 11 regulatory proteins
- Largest and most complex genetic program ever constructed



Characterization for individual circuits sufficient to predict performance for the program

- Could differ due to:
  - Genetic context
  - Interference between circuits
  - Combined impact on cellular resources
- Tested using data for individual gates and inducible systems
- Calculated expected response matched measured values



4-input system

3-input system

Constructed circuits displayed synchronous behavior

- Potential for asynchronous behavior due to
  - Delays between layers
  - Dynamics of each circuit consists of mixed timescales
- kinetic model to predict potential asynchronous behavior:

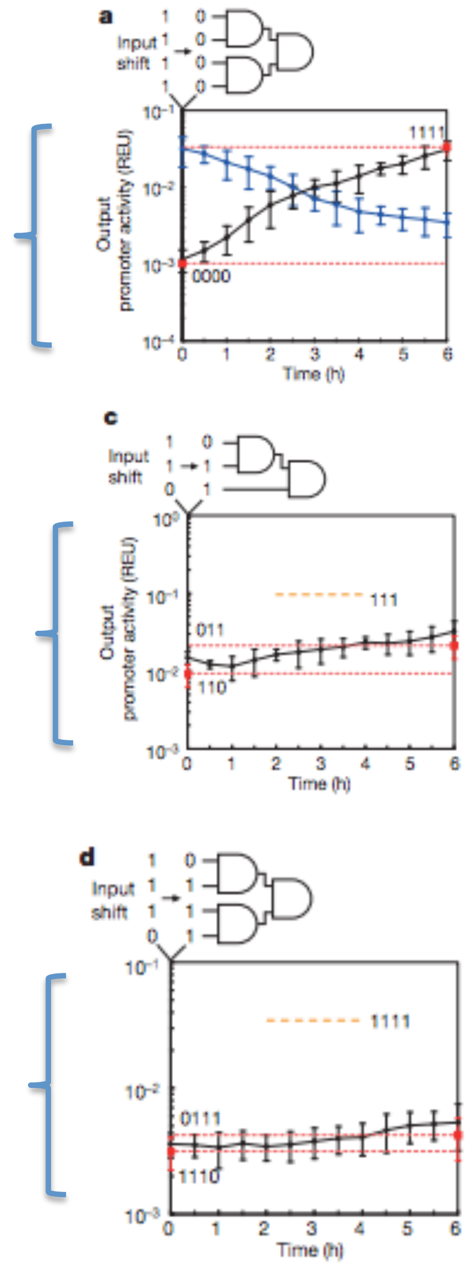
$$t_d >> \frac{\gamma_x \gamma_y}{K \alpha_x \alpha_y \gamma_g},$$

- Chaperone proteins degrade more quickly that transcription factors expressed = NO ASYNCHRONOUS BEHAVIOR

4 input gate

3 input gate

4 input gate



Assumptions

- Possible to minimize any potential crosstalk
- Parts from different species would work similarly in an E. coli chassis (ex. Chaperone protein still required for folding of activator proteins)
- By choosing all type 3 secretion systems, could match kinetics (all gates work the same way so kinetics of the system are consistent)
- Through modeling, can accurately correlate fluorescence unit outputs with input promoter activity
- Layering on of more gates is not affecting cellular limits of protein production

Limitations

- Limited number of inducer molecules
- Rate of degradation and of cellular processes like protein folding
- Reliance on cell for producing all of the raw materials
- Potential cross-talk for more complex systems in the future

Significance & Future work

- Improved part mining and directed evolution
- Computer-aided design
- Understanding circuit interaction and host effects
- Minimization of environment, evolution, and genetic context on circuit performance

Discussion Questions

- How would trying other types of biological parts to make AND logic gates?
- Could you use different cells containing different logic gates to create more complex systems?