Robust multicellular computing using genetically encoded NOR gates and chemical ‘wires’

Alvin Tamsir[1], Jeffrey J. Tabor[2], and Christopher A. Voigt[2]

Kathryn Atherton and Michael Mechikoff

[1]Department of Biochemistry and Biophysics, University of California, San Francisco
[2]Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco

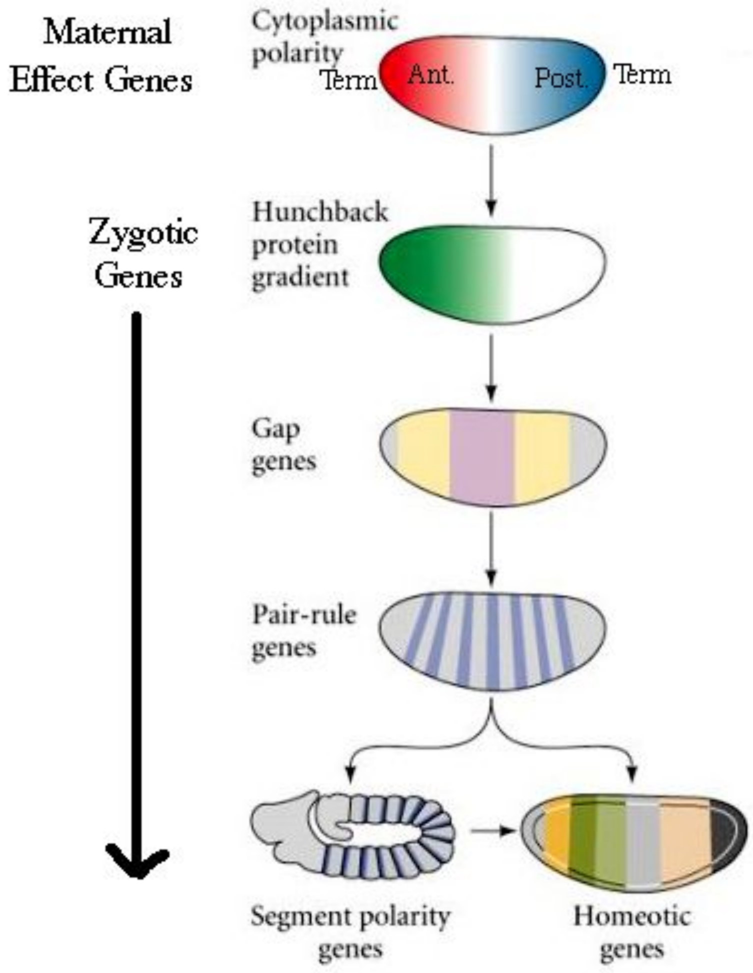
Project Goals:

Understand and harness natural cellular computational power to produce more reliable and complex synthetic computations (how cells make decisions)

Construct difficult two-input synthetic logic gates

Cells naturally perform computations to create complex structures and outputs.

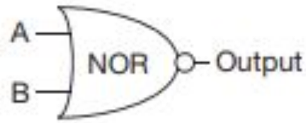
- Example: developmental biology
- Cells “calculate” gradients of chemicals to determine cell type and body plan



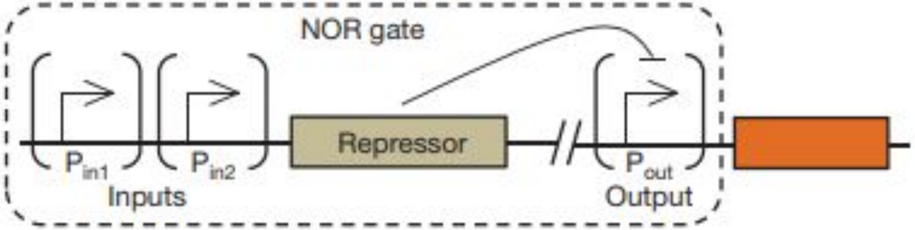
http://courses.biology.utah.edu/bastiani/3230/DB%20Lecture/Lectures/a9FlyMaternal.html

Construction of a NOR gate is simplest solution for more predictable and robust synthetic logic gate construction.

- NOR or NAND can be layered to create any other logic gate operation.
- Previously used combinations of AND, OR, and NOT, but layering these gates is not very predictable (i.e. impedance matching)
- Using one type of gate to produce any logic operation is more predictable.
- NOR gate is simpler to produce than NAND.
    - Add a second input promoter to a NOT gate



Inputs		
in1	in2	Output
0	0	1
0	1	0
1	0	0
1	1	0

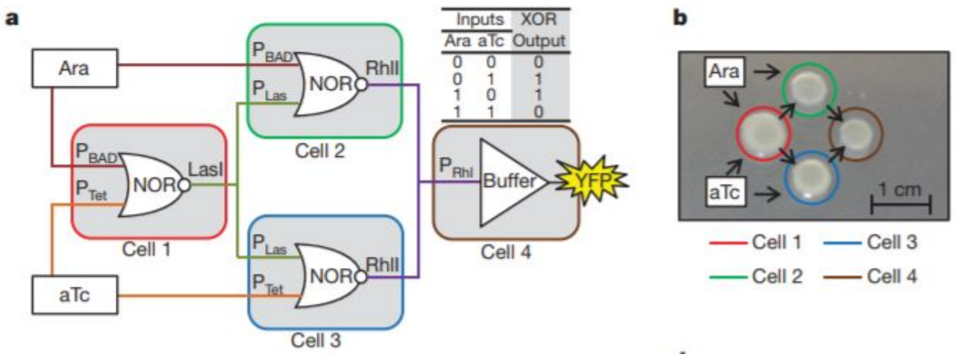


Methods:

The authors implement complex logic by having multiple logic gates feed into each other: combinations of NOR, OR, NOT, and YES.

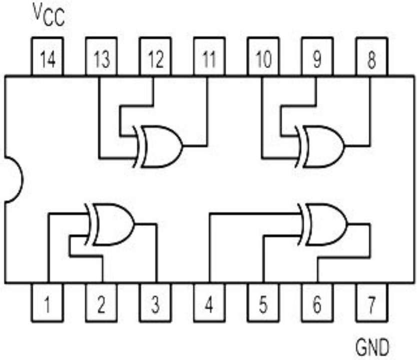
Using *E. coli* allowed the authors to program the different logic gates into different strains and then utilize natural signaling ‘wires’.

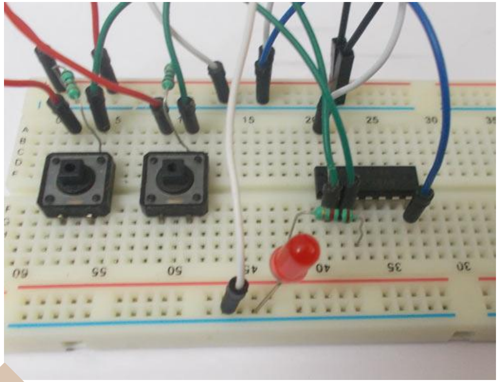
- Quorum sensing
  - The regulation of gene expression in response to **cell density** through intercellular signaling molecules
- Acyl homoserine lactone (AHL) cell-cell communication devices served as the signaling wires
  - LasI and RhlI
- The output of one gate served as the input to the next gate

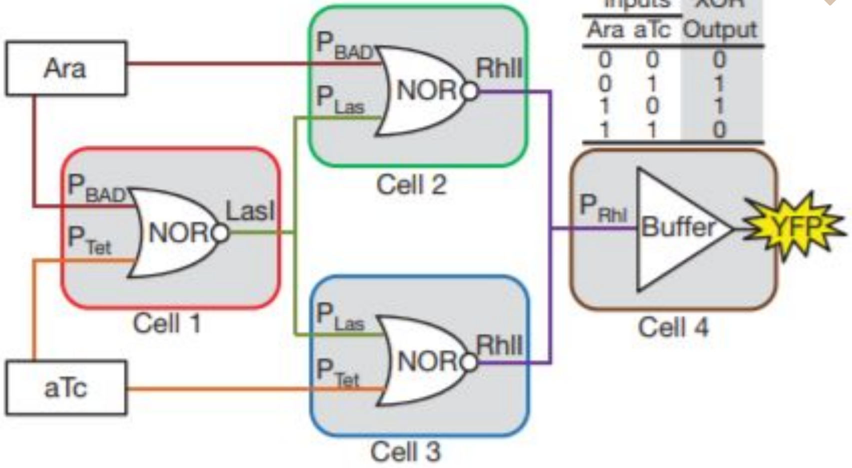


Cell-cell communication was based on electrical circuit boards.

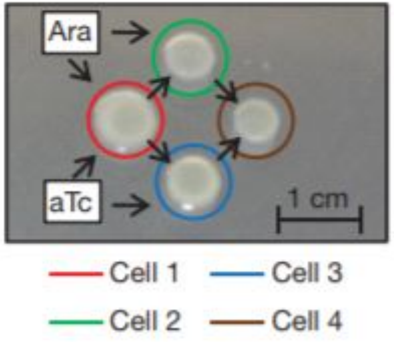
https://circuitdigest.com/electronic-circuits/xor-gate-circuit-diagram







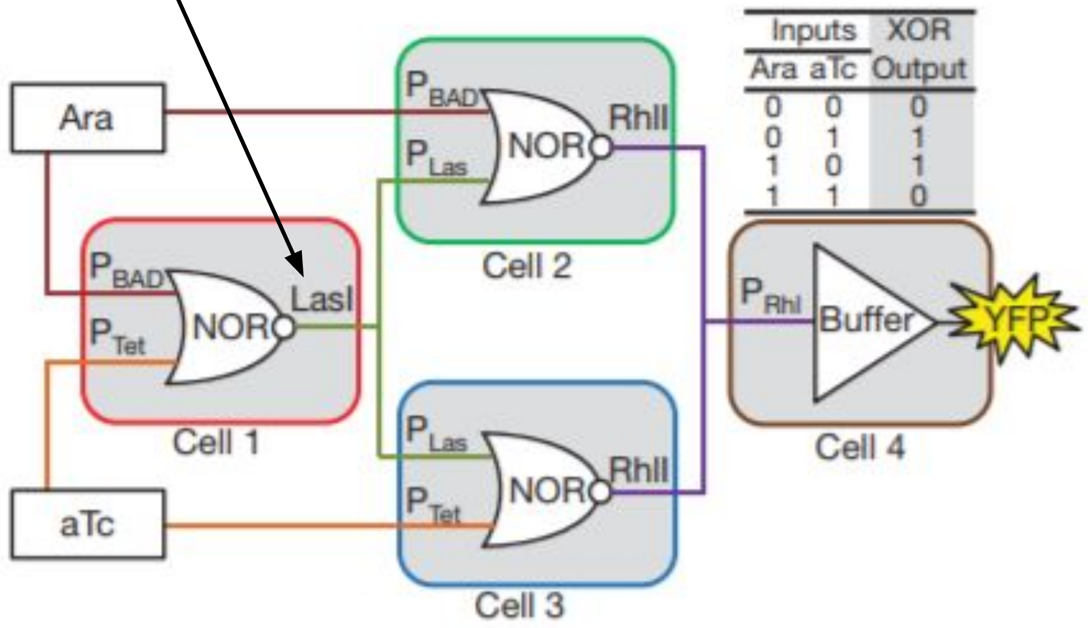
Inputs		XOR
Ara	aTc	Output
0	0	0
0	1	1
1	0	1
1	1	0

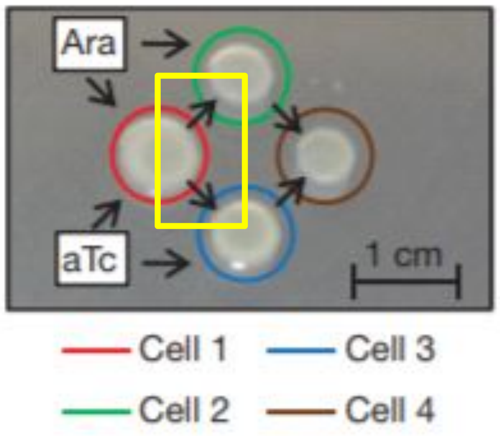


AHL (acyl homoserine lactone) acts as the “wires” to connect the colonies as they communicate left to right.

Output of first gate produces AHL synthase (LasI or RhlI)

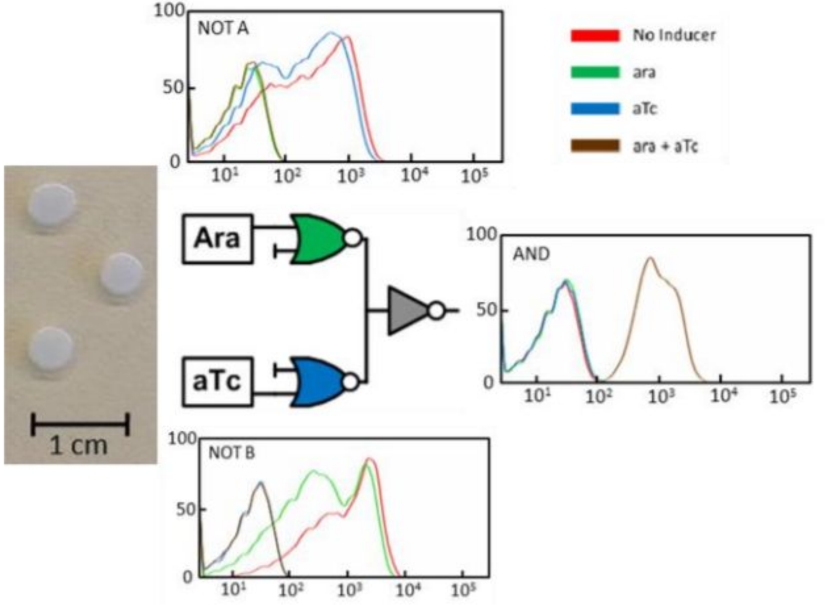
AHL diffuses through the cell membrane, binds to a transcription factor, and turns on the promoter of the next gate.





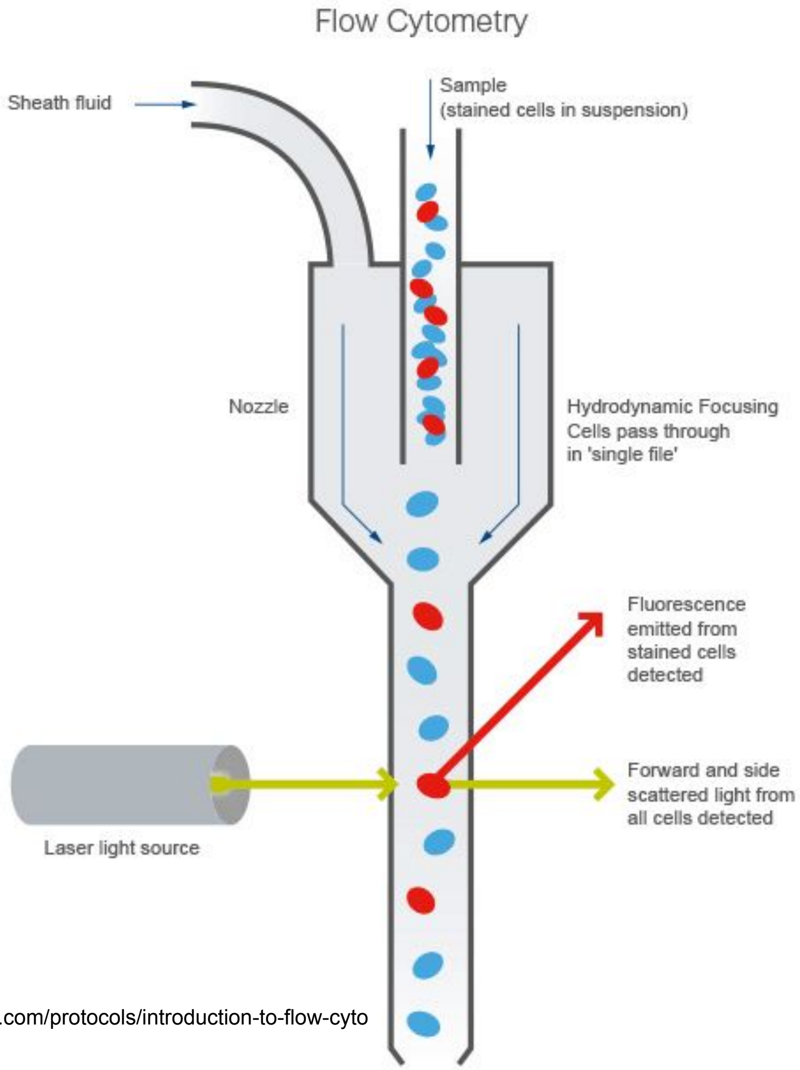
Population averaging was exploited by the authors to mitigate "leaky" expression.

- Ensures that the desired output is delivered as the input to the next system.
  - Broad/overlapping distributions from intermediates are negated
- This also increases the distance between the output and input cells such that cell-cell communication is required to further prevent false positive responses.
- Another method of solving this problem would be to decrease sensitivity of the operator site for the input, or increasing sensitivity of the operator site for the output.



Population averaging prevents the variability within a colony from propagating in downstream layers.

Flow cytometry was used to find the fluorescence distributions of each logic gate system.



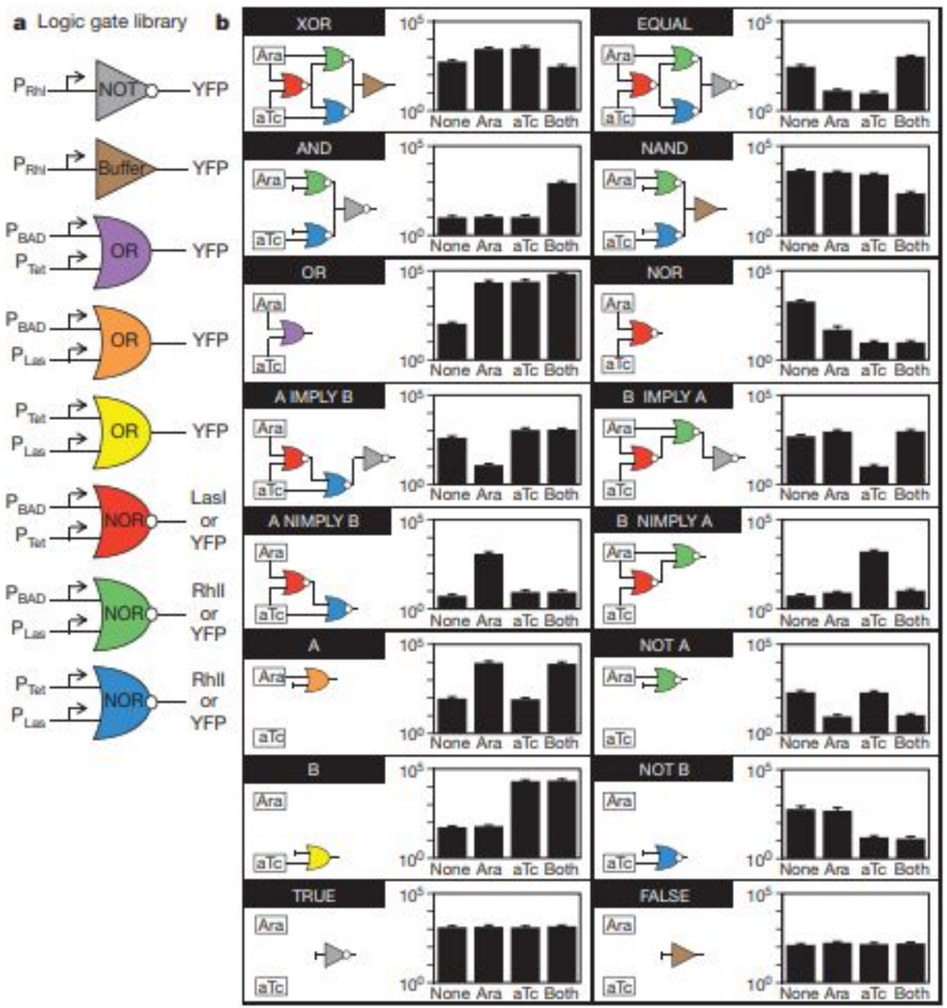
https://www.abcam.com/protocols/introduction-to-flow-cytometry

Conclusions and Big-Picture Concepts:

Construction of complex logic functions
Modularity of logic gates
Robust systems

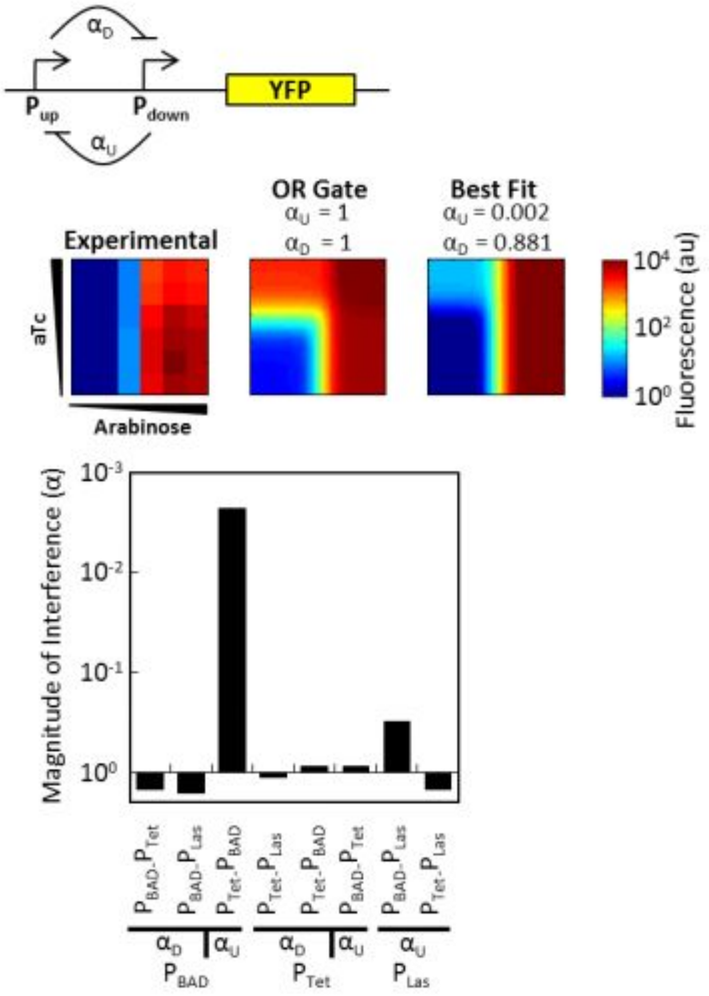
The authors constructed all 16 two-input Boolean logic gates with a library of eight logic gates.

- This produced stronger and more robust responses: between 5 and 300 times greater response between “on” and “off” states.



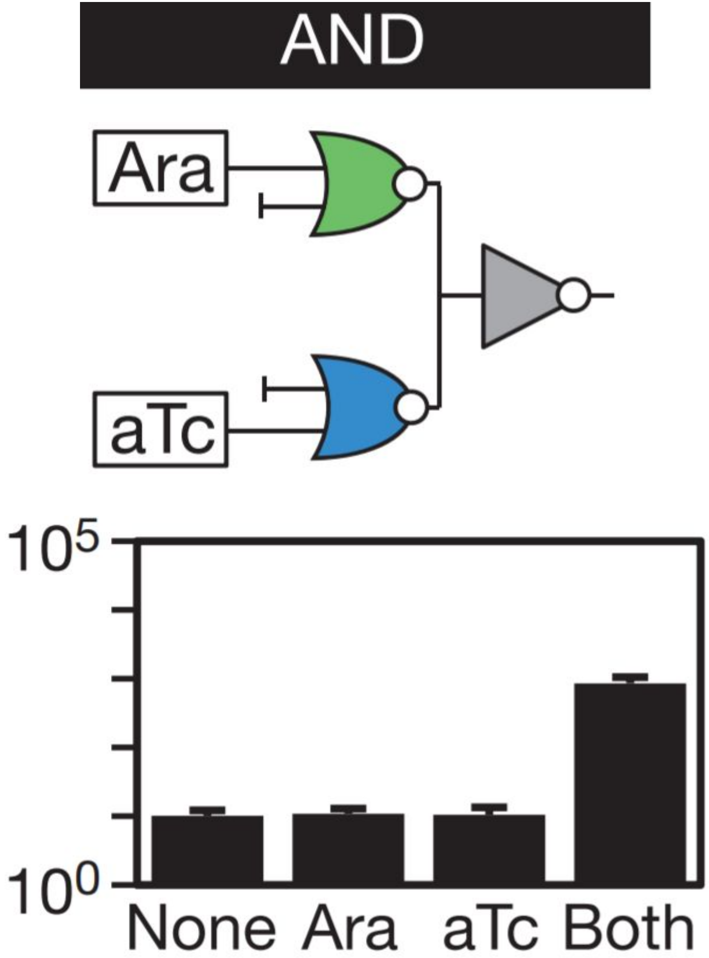
Limitations

- At the time of publication (January 2011) the ability to construct logic gates in a single cell was limited, but they were able to produce these more complex logic systems using cell-cell communication.
- Interference between the tandem promoters can occur



In-Class Concepts Utilized by the Authors

- Developed and applied logic gates for use in systems in synthetic biology.
- Utilized cascade-like interactions between systems to make their overall logic devices more robust and increase the differences between the “on” and “off” states.



In-Class Concepts Utilized by the Authors

- Utilized the analogous concepts between computer science and synthetic biology to develop their ideas.
- Created modular systems in order to produce all of the two-input Boolean logic gates.