Introduction to Bioengineering
BIOE/ENGR.80
Stanford University

Spring 2020 Class Slides

Day 11
29 April 2020

Brief Communication | Published: 27 April 2020

Plants with genetically encoded autoluminescence

Tatiana Mitiouchkina, Alexander S. Mishin, […] Karen S. Sarkisyan

Nature Biotechnology (2020) | Cite this article

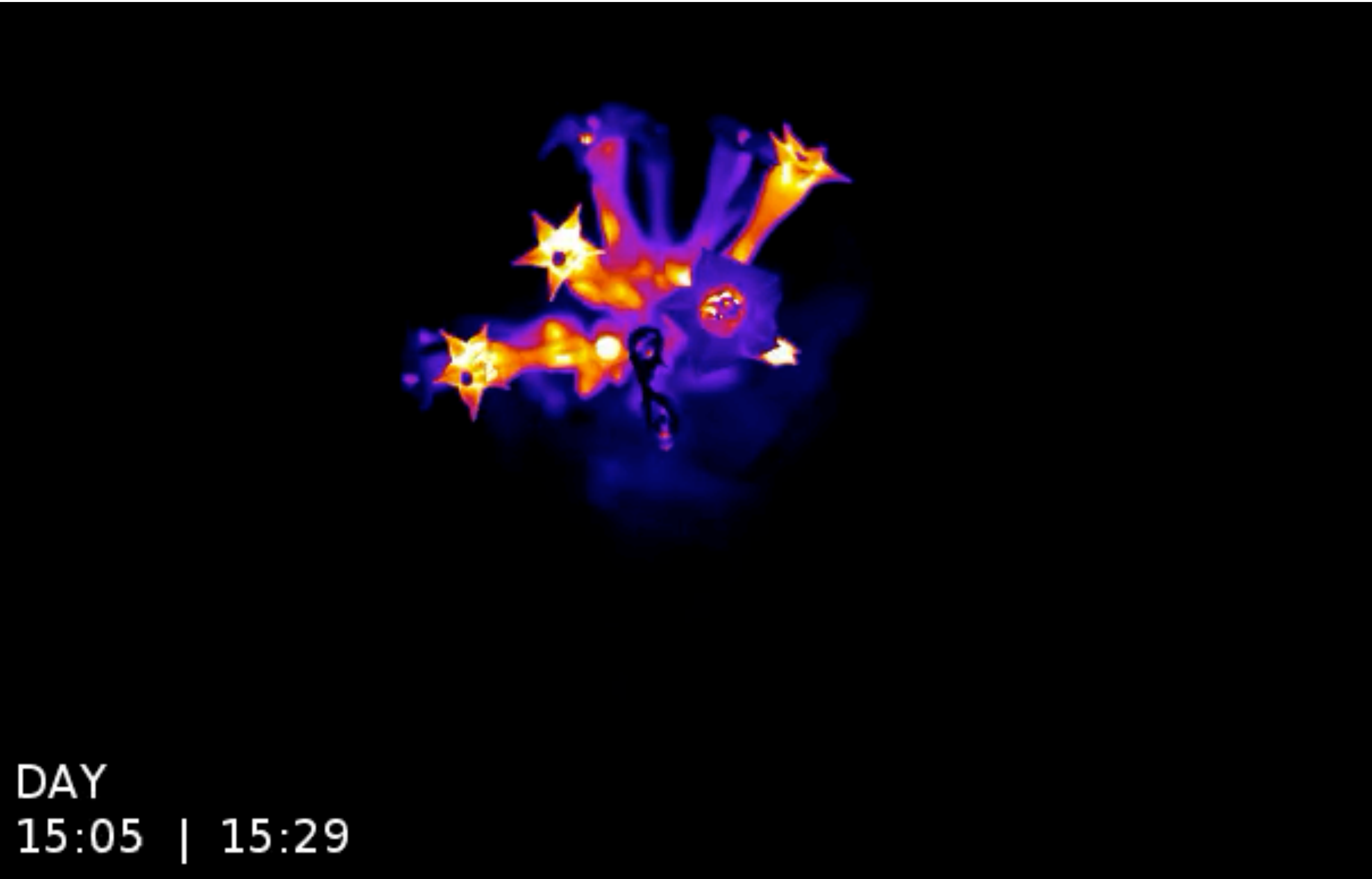
Abstract

Autoluminescent plants engineered to express a bacterial bioluminescence gene cluster in plastids have not been widely adopted because of low light output. We engineered tobacco plants with a fungal bioluminescence system that converts caffeic acid (present in all plants) into luciferin and report self-sustained luminescence that is visible to the naked eye. Our findings could underpin development of a suite of imaging tools for plants.

Plants that make their own light?!

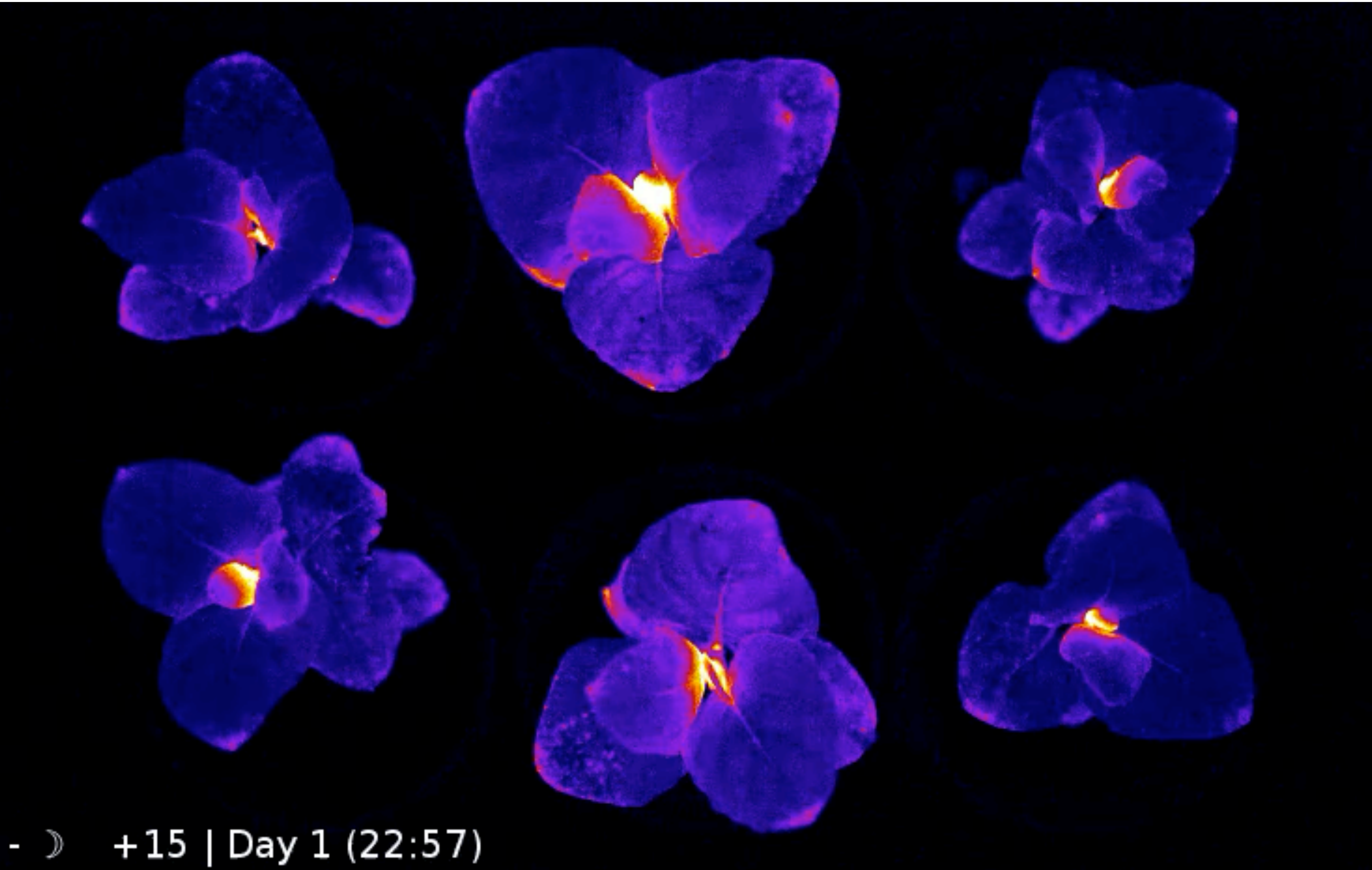
https://www.nature.com/articles/s41587-020-0500-9

Plants that make their own light?!



https://www.nature.com/articles/s41587-020-0500-9

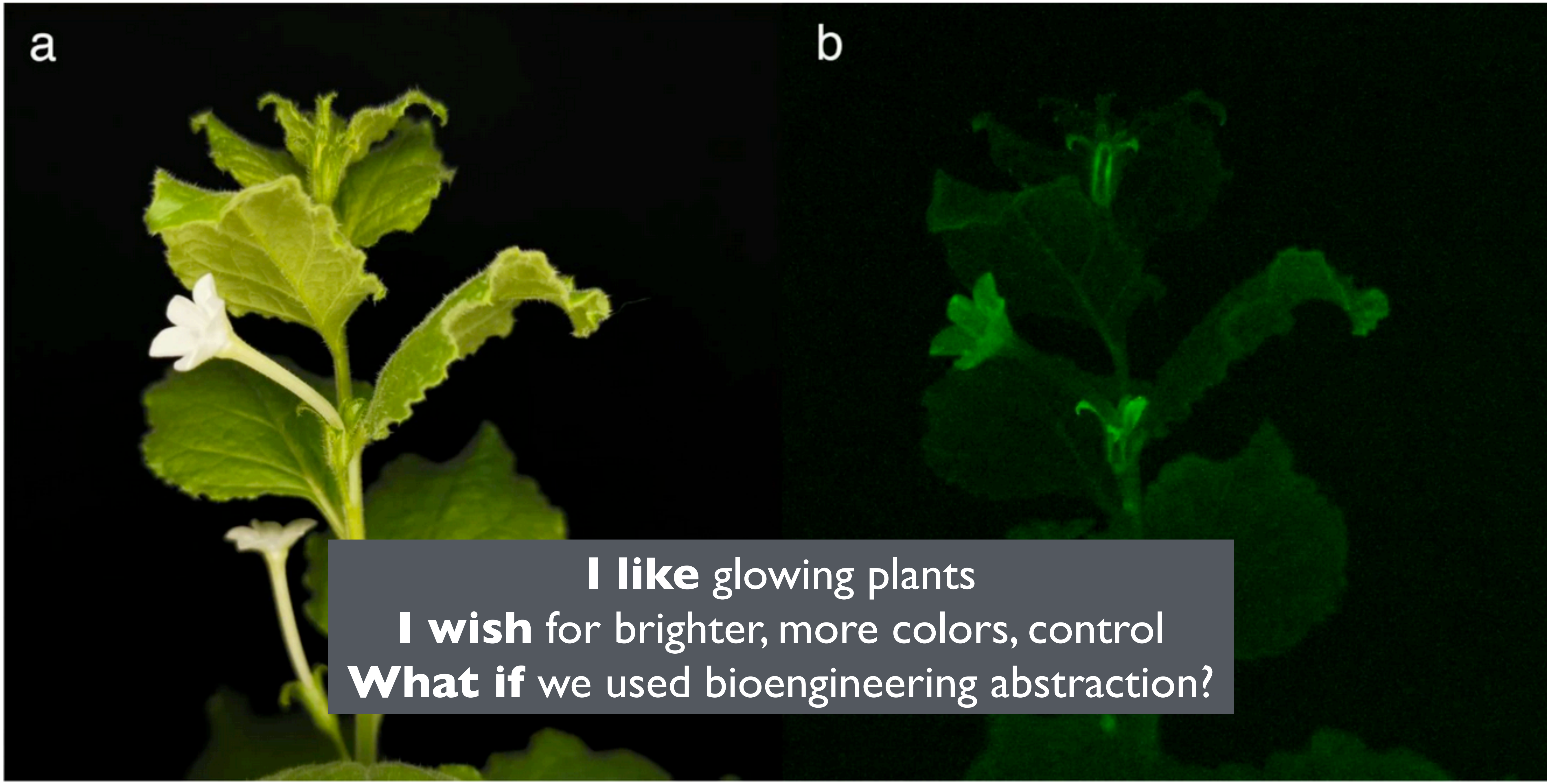
Plants that make their own light?!



https://www.nature.com/articles/s41587-020-0500-9

Trillion-fold less bright than 100w bulb?

From: Plants with genetically encoded autoluminescence



(**A**) Taken on a smartphone in ambient light. (**B**) Taken in the dark with 30-second exposure. Images are the result of a single experiment.

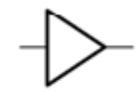
https://www.nature.com/articles/s41587-020-0500-9

Dr. Jennifer Brophy
(previous class)

Turn light on/off?
At specific locations?

Different logic "gates" set their output values depending on their input values

YES



INPUT	OUTPUT
A	
0	0
1	1

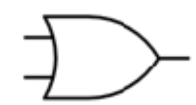
NOT

INPUT	OUTPUT
A	
0	1
1	0

AND

INPUT		OUTPUT
A	B	
0	0	0
1	0	0
0	1	0
1	1	1

OR

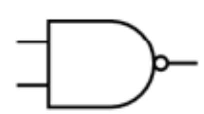


INPUT		OUTPUT
A	B	
0	0	0
1	0	1
0	1	1
1	1	1

XOR

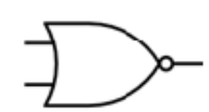
INPUT		OUTPUT
A	B	
0	0	0
1	0	1
0	1	1
1	1	0

NAND



INPUT		OUTPUT
A	B	
0	0	1
1	0	1
0	1	1
1	1	0

NOR

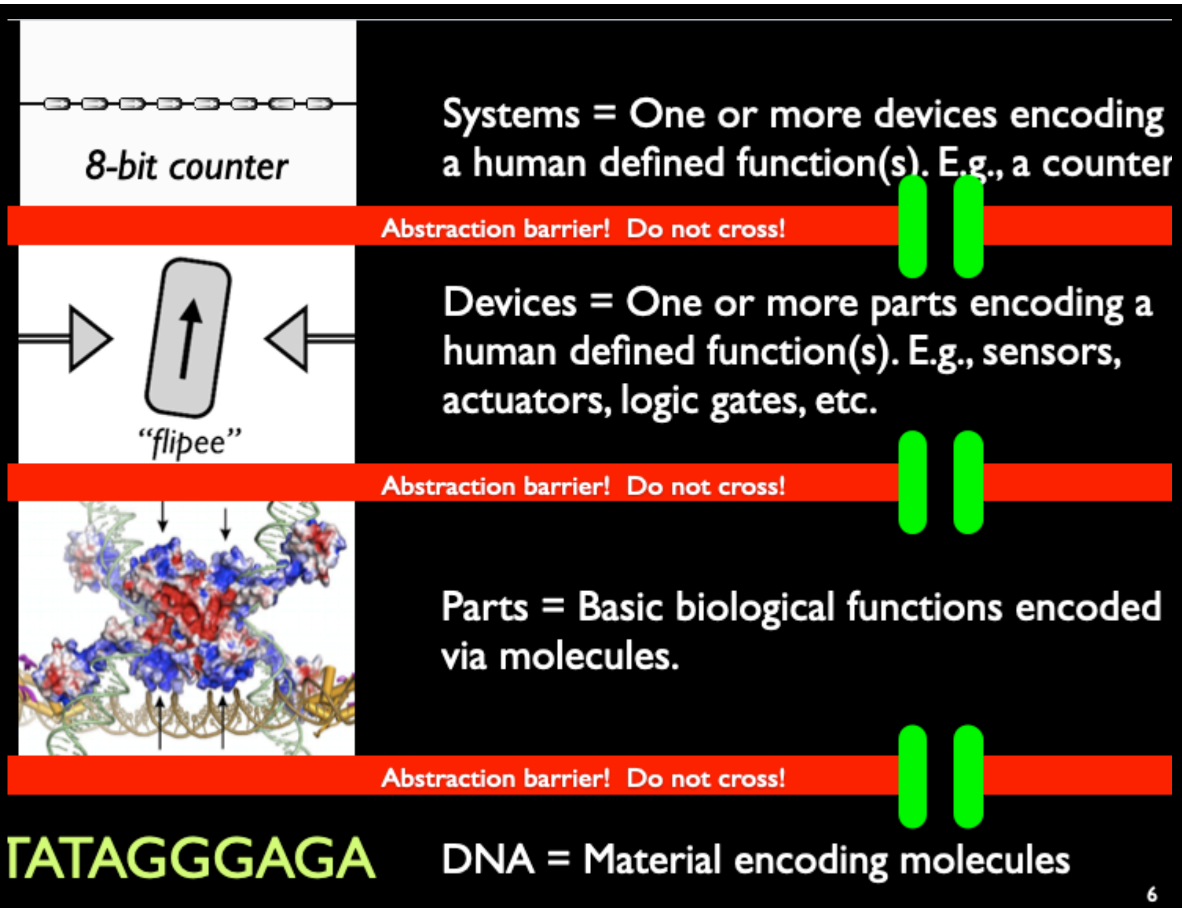


INPUT		OUTPUT
A	B	
0	0	1
1	0	0
0	1	0
1	1	0

XNOR

INPUT		OUTPUT
A	B	
0	0	1
1	0	0
0	1	0
1	1	1

The challenge of abstraction…

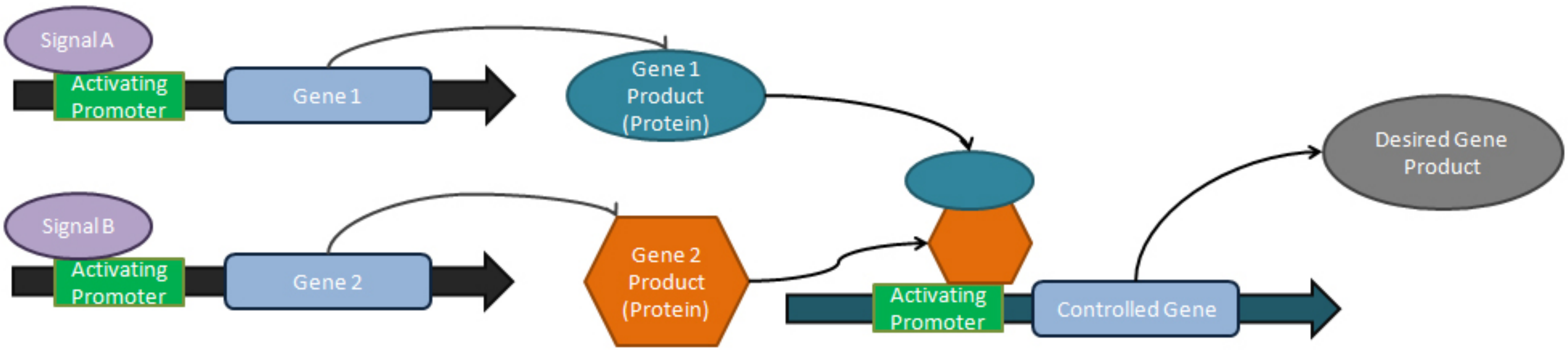


How do we bioengineer genetic logic gates so that they are easy for others to use*?

*if we can do so for logic gates then we can likely do so for any sort of genetically-encoded device

AND gate

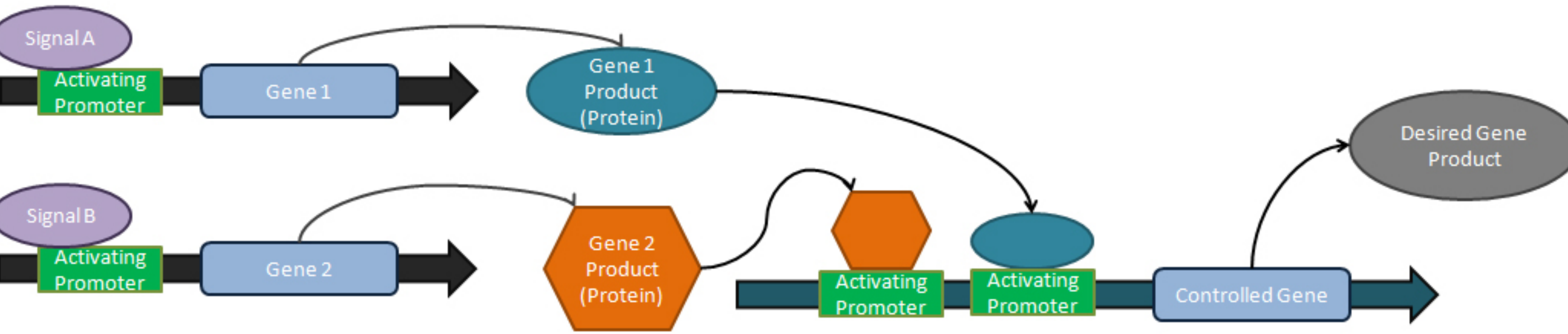
(transcription-initiation based)



https://en.wikipedia.org/wiki/Synthetic_biological_circuit

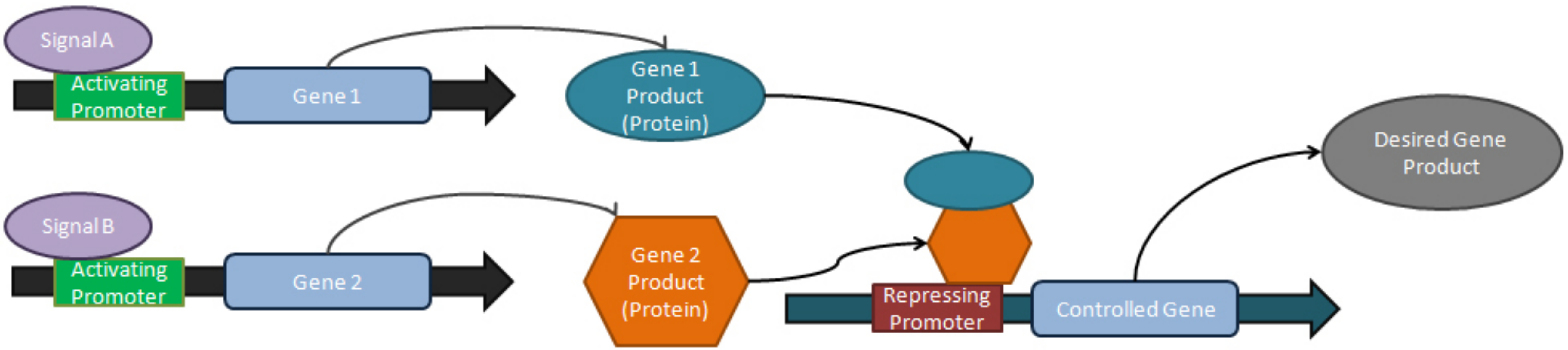
OR gate

(transcription-initiation based)



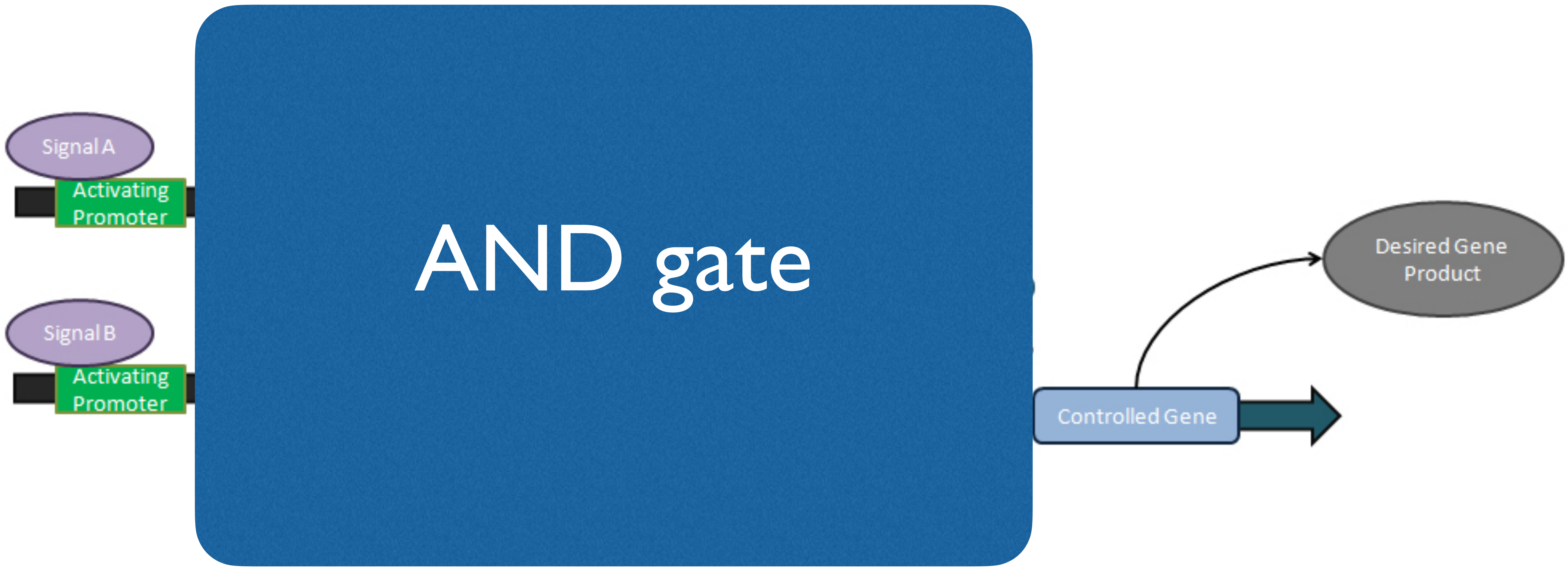
https://en.wikipedia.org/wiki/Synthetic_biological_circuit

NAND gate
(transcription-initiation based)



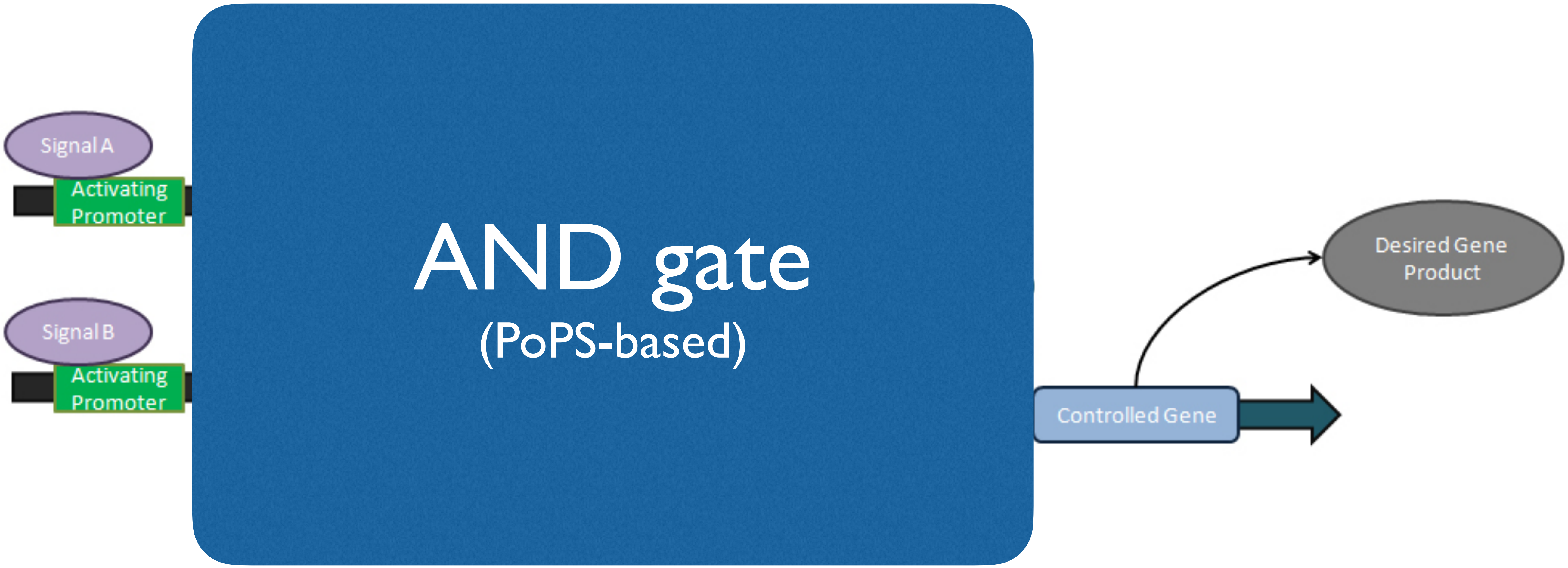
https://en.wikipedia.org/wiki/Synthetic_biological_circuit

Where we draw the “box” determines how easy/hard device will be to reuse



https://en.wikipedia.org/wiki/Synthetic_biological_circuit

A common-signal carrier enables proper abstraction

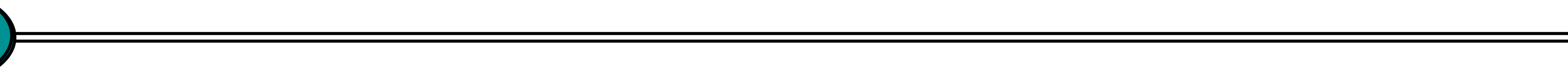


https://en.wikipedia.org/wiki/Synthetic_biological_circuit

Polymerase Per Second

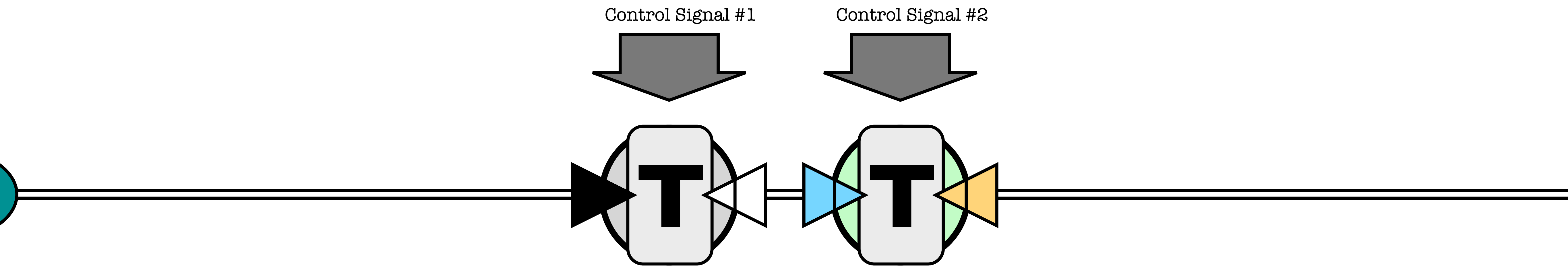
(transcription elongation ‘current’)

(to any destination)



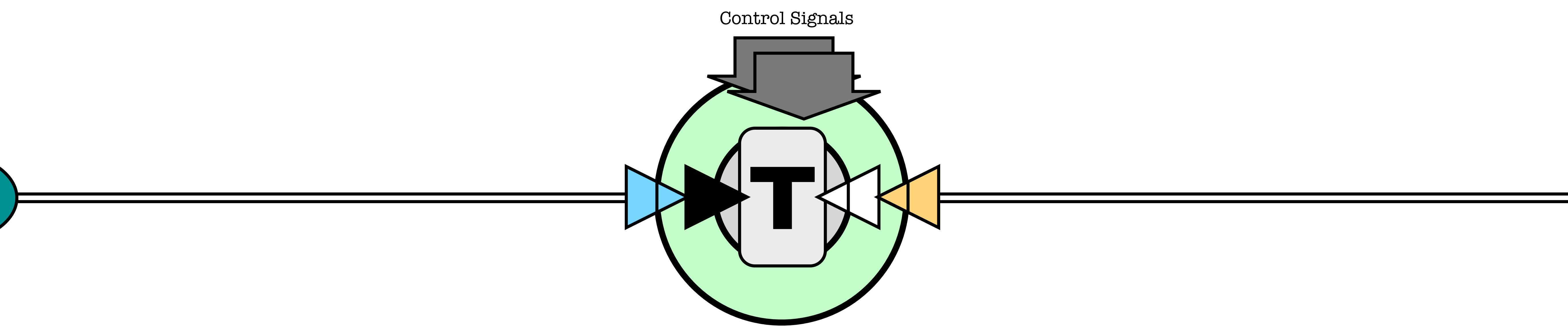
(from any source)

AND gate
(transcription-elongation based)



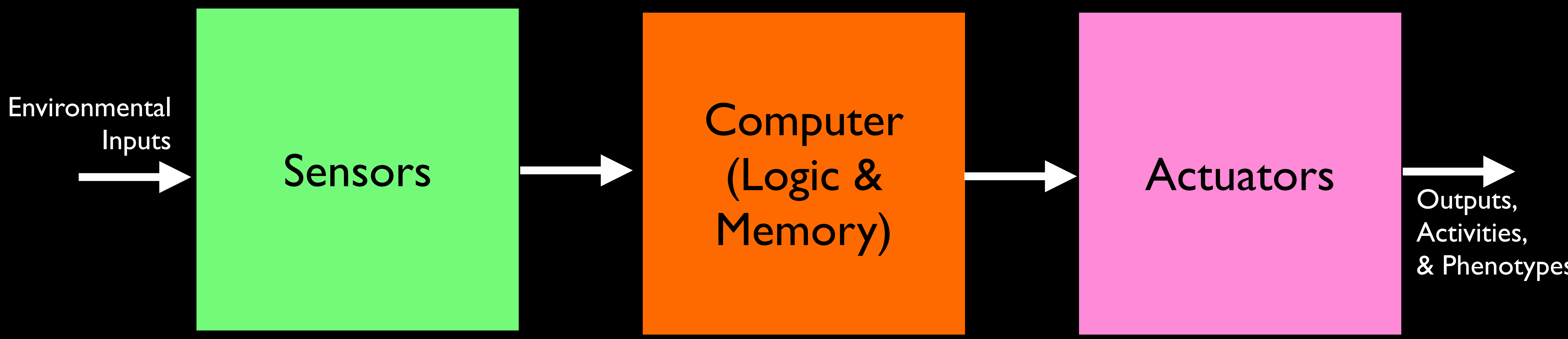
Bonnet et al., *Science*, 2013

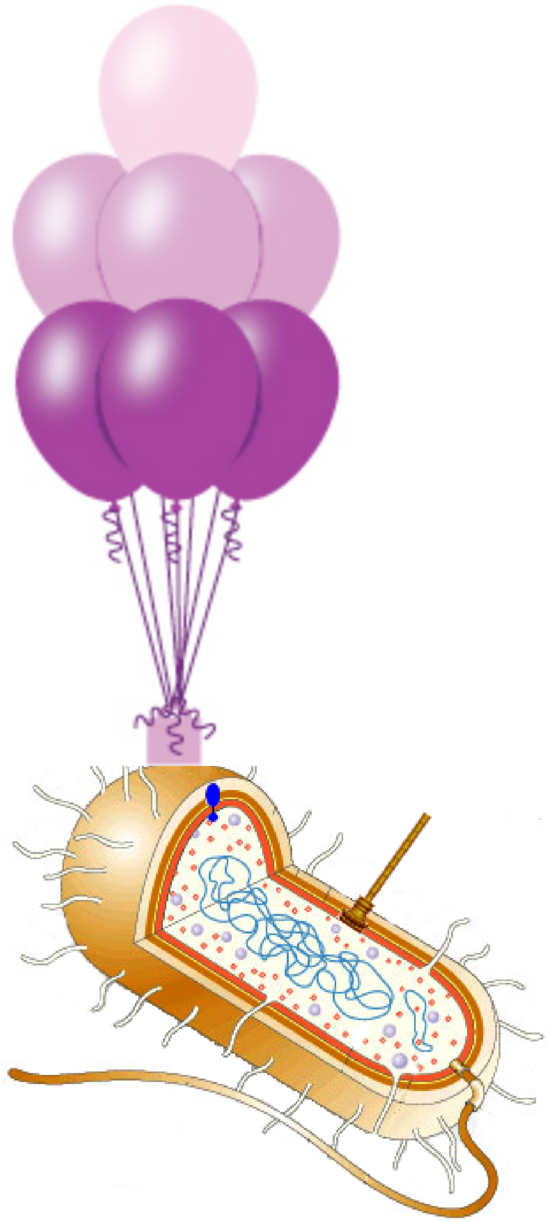
XOR gate
(transcription-elongation based)



Bonnet et al., *Science*, 2013

Can abstract and connect not only logic but sensing and actuation via PoPS





article | discussion | edit | history

Part:BBa_I750016:Design

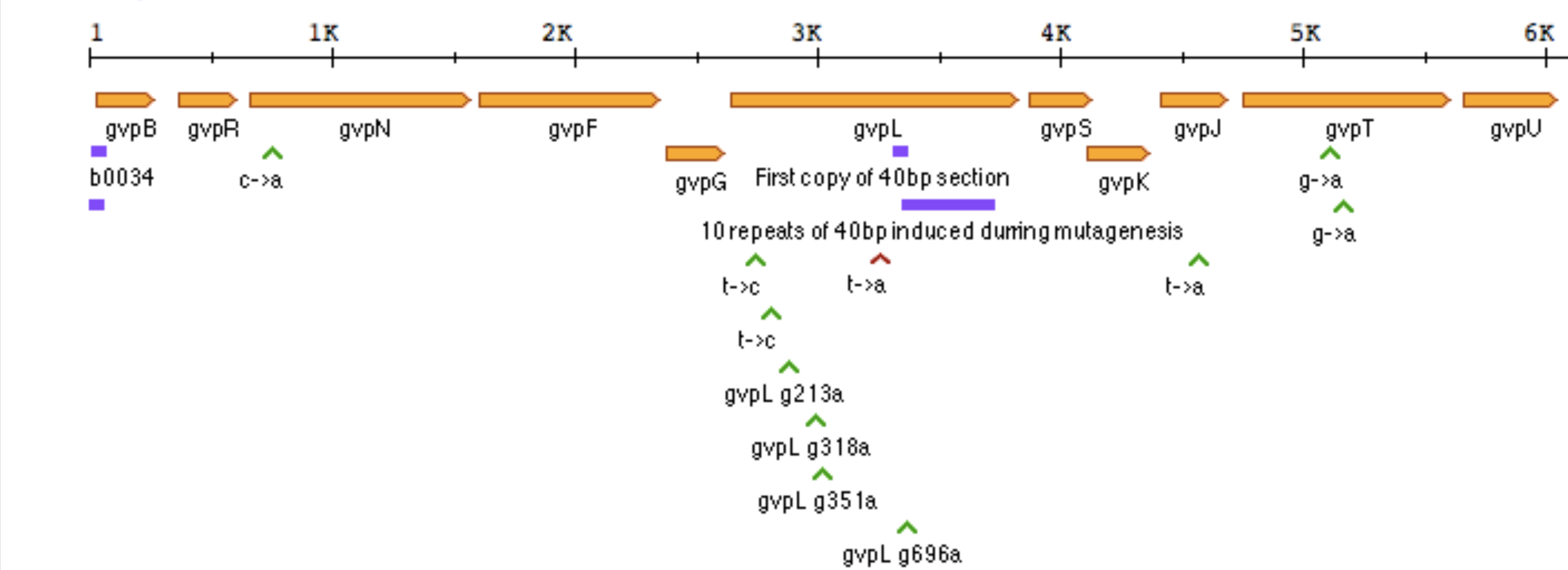
DNA Sent
Experience:

Designed by Phillip Dodson

Entered: 2007-10-21

Gas Vesicle polycistonic gene

Format: Subparts | **Ruler** | SS | DS **Search:** **Length: 6064 bp** **Context: Part only** Get selected sequence



Design Notes

[edit]

Site directed mutagenesis was performed in four rounds to remove 3 PstI sites and one EcoRI site from gvpL in the sequence.

*** Li & Cannon, J Bacteriol, May 1998, p. 2450-2458, Vol. 180, No. 9 (U. Mass. Amherst)**

Protein Balloon DNA

aaagaggagaaatactagatgtctattcaaaaaagtactaatagttcaagtttagcagaagtcattgaccgtattttagataaaggaattgttattgatgcttttgcaagagtttctgttgtaggaattgaaattttaacgattgaagcgcgagtggtt
attgccagtgttgatacatggttacgctatgcagaagcagtagggcttcttcgtgacgacgtagaagaaaacggtcttcctgaacgttcaaattcaagtgaagggcagccgcgttttagtatttaatatgaagtggtatggaaccttccttttttgag
ggtggttccagcttttagatctaactattggaggctactaaaaatggaaattaaaaaaattatgcaagccgtgaacgactttttcggtgaacacgtagctcctcctcataaaattacctcggtggaagctactgaagatgaaggttggagagttatt
gttgaagtcattgaagaacgagaatatatgaaaaaatacgccaaagatgaaatgctcggaacgtacgagtgctttgtaaataaagaaaaagaagtcatttcattcaaacgactcgacgtcagatatagaagcgccattggcattgaagcataaac
gataagatggcaggaggaacgtaaaaatgaccgtcttaacagacaaaaggaaaaaaggcagtggagcttttatacaagatgacgagacaaaagaggttctttcaagagcgctgagctatttaaaatccggctattccattcattttacaggtcct
gccggcggaggcaaaacctctttagcgcgagcgcttgctaaaaagagaaagcgtcctgtaatgctgatgcacgggaatcacgagctcaacaacaaagatttaattggcgattttacgggatacacgagcaaaaaagtaatcgaccagtacgttc
gttctgtctataaaaaagatgaacaggtgagtgaaaactggcaggatggccgattgcttgaagctgtaaaaaatggctatacgctgatttacgacgaatttactcgttctaagcctgcgacgaataatatctttctatcgatattagaagaaggcgt
gctgccgctgtatggagtaaaaatgaccgatccttttgtgcgcgtgcatcccgatttccgcgtcatcttcacaagcaatccagctgagtatgccggcgtatatgatacgcaagatgcgcttctcgacaggttaattaccatgtttattgattataaa
gacatcgacagagagacagcgattttaacggagaaaacggacgtagaagaagatgaagcgcgcacaattgtaacgctcgtagcaaacgtgcgaaaccgctctggagacgaaaacagcagcggacttagcctgcgggcttcgcttatgatcgct
acccttgccacgcagcaagacattcctatcgatggaagtgacgaagattttcaaacgttatgtatcgatattttgcatcatccgcttaccaaatgtttggatgaagaaaatgcaaaaagcaaagccgaaaaaatcattttagaagaatgtaagaat
atagacactgaagaaaagtaaaggagcttgaaaacatgagtgaaacaaacgaaacaggtatttatatttttagcgccattcaaacggataaagacgaagaatttggcgcccgtggaagtagaaggaacaaaagctgaaacatttttgattcgctac
aaagacgcggctatggtagcagctgaagtaccgatgaaaatttatcatcctaatcgccaaaatttattaatgcatcaaaacgcagtagcagcgattatggacaagaacgatacggttattccaatcagctttgggaatgtattcaaatcaaaagaa
gacgtaaaagttcttttggaaaacctttatccgcagtttgaaaagctgtttccagcgatcaaaggaaaaaattgaagtcggtttaaaagtaattgggaaaaaagaatggcttgagaaaaaagtaaacgaaaatcctgaacttgagaaagtatcagca
tccgtaaaaggaaaatcagaagcagccggttattatgagcgtattcaacttggaggaatggctcaaaagatgtttacttccctgcaaaaagaagtcaagacagatgtgttttctccgcttgaagaagcagcggaagcagcaaaagcaaatgagcc
aacgggcgaaacgatgcttttaaacgcgtctttcttaattaaccgagaagatgaagcgaagtttgatgaaaaagtaaatgaagcgcatgaaaactggaaagacaaagccgattttcattacagcggtccttggcctgcttataattttgtgaacatt
cgcctaaaagtagaagagaaataacgtgcttcacaaattagtaaccgcacccattaaccttgtagtgaaaatcggcgaaaaagtacaggaagaagctgataaacagctatatgaccttccgacgattcagcaaaagctcattcagcttcaaatg
atgtttgagcttggtgaaattccagaagaagcgtttcaagaaaaagaagatgaattgttaatgaggtacgaaattgcgaaacgcagagaaattgaacaatgggaagagctaacacaaaaaagaaatgaggaatcctagatgggagaattactgta
tttatacggtttaattccaacaaaagaagcagcagccatagagccgtttccatcttataaggggtttgacggagaacattcactgtacccaattgcgtttgatcaggtgacggctgtagtttctaagctggatgctgacacctattcagaaaaagtg
attcaagaaaaaatggagcaggatatgagctggctgcaagaaaaagcatttcatcatcacgaaacggtagccgctttgtacgaagaatttacgatcattccattaaaattttgcaccatttataaaggtgaagaaagtctgcaagcagctattgag
attaacaaagaaaaagatagaaaattcactgacgctgcttcaaggaaatgaagagtggaatgtgaaaatttactgtgatgatacagagcttaaaaaaggaatcagcgaaacgaatgaaagcgtgaaagcgaaaaaacaagaaattagtcacttat
caccaggaagacagtttttttgaaaagaaaaaaatagatcagctgattgaaaaagaattagagcttcacaaaaacaaagtgtgtgaagagatacatgacaagctaaaagaattatcgctttatgactctgttaaaaagaattggagcaaagacgta
actggcgcagctgaacagatggcgtggaacagcgtgtttcttctcccgtctctgcaaattactaagttcgtacgtgtttcttctcccgtctctgcaaattactaagttcgtacgtgtttcttctcccgtctctgcaaattactaagttcgtacgtgttt
cttctcccgtctctgcaaattactaagttcgtacgtgtttcttctcccgtctctgcaaattactaagttcgtacgtgtttcttctcccgtctctgcaaattactaagttcgtacgtgtttcttctcccgtctctgcaaattactaagttcgtacgtgtttc
ttctcccgtctctgcaaattactaagttcgtacgtgtttcttctcccgtctctgcaaattactaagttcgtacgtgtttcttctcccgtctctgcaaattactaagttcgtacgtgtttcttctcccgtctctgcaaattactaagttcgtaaacgaaat
agaagagcttcagcaaaggcttgaaaataaaggctggaagtttgaagtgacgggaccatggccgccctatcatttctcgagctttgcgtaaagtgaggaattaacattatgtctcttaaacaatccatggagaataaagatattgctcttattgata
ttttagatgtcattttagataaaggagtcgccattaaaggagacttaatcatttccatagctggcgtcgatttagtgtatttggatttgcgggtgcttatttcttcggttgaaacgcttgtgcaagcaaaagaaggaaatcacaaaccaatcacttctg
aacaatttgataaacaaaaggaggaattaatggatgcaaccggtcagccaagcaaatggacgaatccacttggatcctgatcaagctgaacaaggcttagcgcagcttgtgatgacagttattgagctattgaggcaaatcgttgaacgtcatgc
catgaggcgggtggagggtggaacgttgacggacgaacaaattgaaaacttaggaattgcactaatgaacttagaagaaaaaatggacgagttgaaagaggtgttcggtctggatgcagaagatttaaatattgatcttggaccgctaggcagcc
tgctttaagcggtcagtaggaggaacagtatggcagtcgaacataatatgcagtcaagtacgattgtagatgtgctcgaaaagattttggataaaggagtcgttatagcgggggacatcaccgtaggaattgcagatgtcgagctattaacgataa
agatccgcttgattgtggcttcggttgataaggcaaaagaaatcggcatggactggtgggaaaatgatccgtatctcagttcaaaaggagccaataacaaagcgctcgaagaagaaaataaaatgctgcatgagcggttaaaaacgcttgaaga
aaaaatagaaacgaaacgttaaaaactgtacgctacttaaaaaatggagggatttacaatggcaactgaaacaaaattagataacacacaggcagaaaacaaggaaaataaaaatgcggaaaacggttcaaaagaaaagaacggttcaaaagc
aagcaaaacaacaagcagcgggccaatcaaacgagcggtagcaggaggcatcatcggtgcaacgattggatatgtatcgactcctgaaaatcgaaaaagtctccttgaccgcattgatacagacgaattaaaaagcaaagcatctgatttagg
aacaaaggtaaaagaaaaatcaaaaagcagcgtggccagcctgaaaacatctgcgggaagcttgtttaaaaaaagataaagataaatcaaaagatgatgaagaaaacgtaaattcttctagtagcgaaacagaagacgataacgttcaagagta
cgacgagttaaaagaagaaaatcaaactcttcaagatcgcttatcacagcttgaagaaaaaatgaacatgcttgttgagcttagcctcaataaaaatcaagacgaagaagcggaagatacagattccgacgaagaagagaacgatgagaacgat
gaaaacgatgaaaacgagcaggacgatgaaaacgaagaagaaacatctaagccacgtaaaaaggataaaaaagaagctgaggaagaagaaagtgaaagtgacgaagacagcgaggaagaagaggaagattctcgctcaaacaaaaaaaata
aaaaagtaaaaacagaagaagaagacgaagatgaaagcgaagaagaaaaaaaggaagcgaaaccaaaaaagtcaacagctaaaaaatcaaaaaatacaaaagcaaagaaaaacacggacgaagaagatgatgaagcaacatctctttctag
tgaagacgatacaacagcctaagacgtaaaggaggaaagaaagacatgagtacaggcccttctttttcaactaaagacaatacgcttgaatactttgtgaaagcttctaataaacacggcttttcacttgatatttcattaaatgtaaacggcgct
gtgatttccggtaccatgatttcagcaaaagaatactttgattacttaagcgaaacgtttgaagaaggcagtgaagtggctcaggcgctaagcgaacaattctctttagcaagcgaagcgagcgaatcaaacggagaagcagaagcccattttat
tcatttgaaaaatacaaagatttactgtggagacagtaaatctactccttctaaaggcaaaatcttttggagaggaaaatagcagaagtagacgggtttttcttaggaaagatttctgatgcaaaatcaacgagtaaaaagagttcataa

Registry of Standard Biological Parts

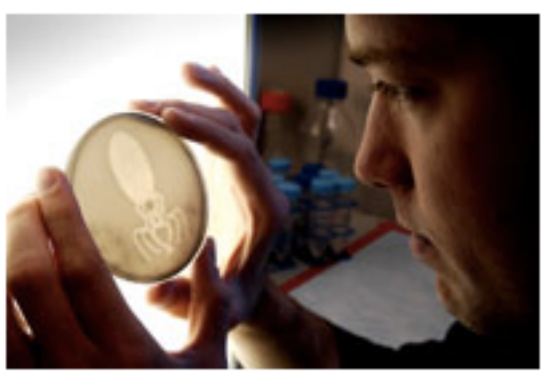
Featured Parts:Light Sensor

From Levskyaya *et al.*

> **"We have designed a bacterial system that is switched between different states by red light. The system consists of a synthetic sensor kinase that allows a lawn of bacteria to function as a biological film, such that the projection of a pattern of light on to the bacteria produces a high-definition (about 100 megapixels per square inch), two-dimensional chemical image."**

Sample photos

Here are a selection of sample **coliroid** taken with the bacterial photography system.

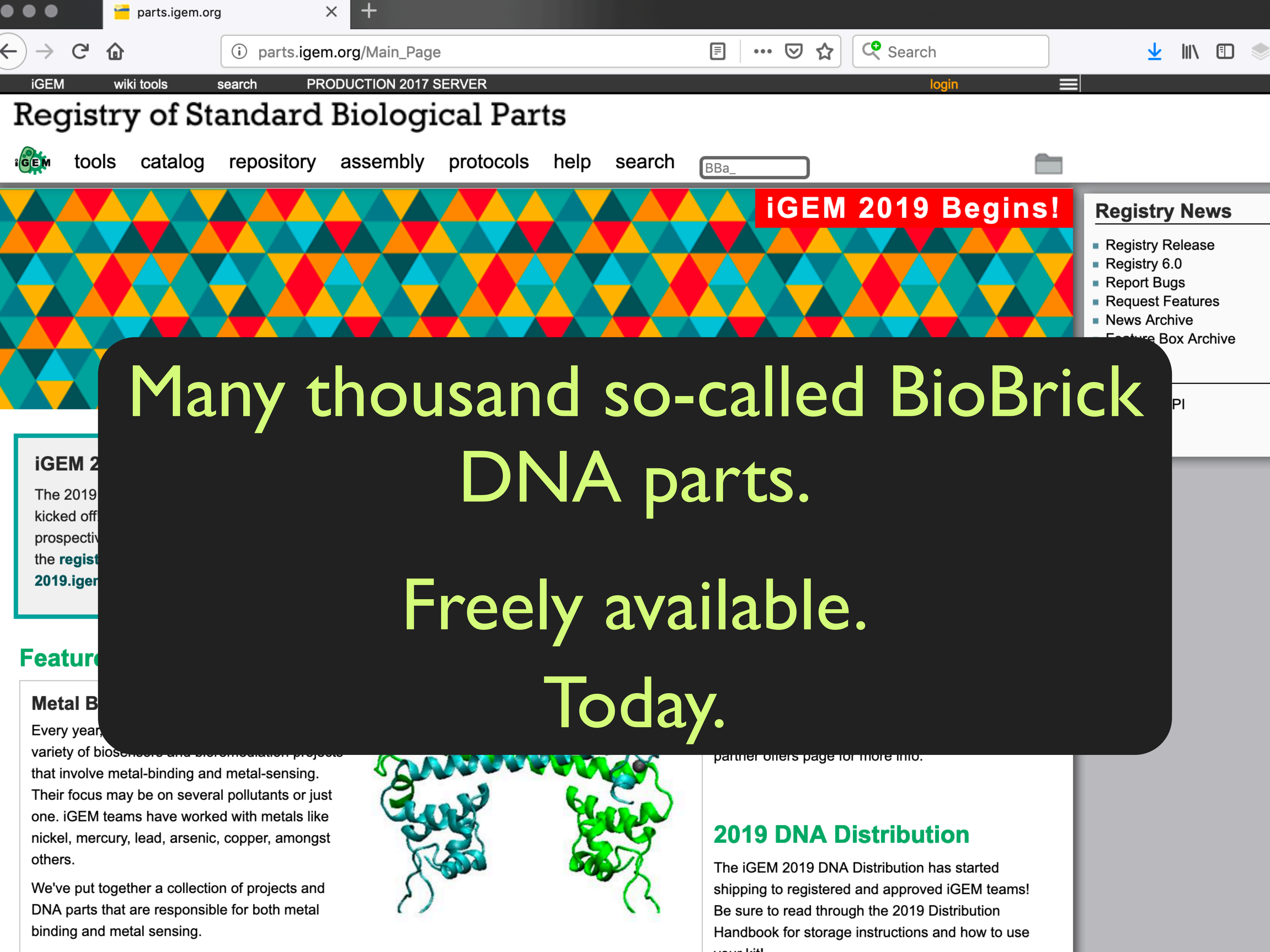


Jeff Tabor holding a **coliroid**.
Photo credit: Marsha Miller, University of Texas at Austin. Image courtesy of UT/UCSF.

Hello World **coliroid** published in Levskaya *et al.*, Nature, 2005.

This is a **coliroid** portait of Andy Ellington. You can compare it with the real Andy. Image courtesy of UT/UCSF.

This is a **coliroid** of the Flying Spaghetti Monster. Image courtes UT/UCSF.



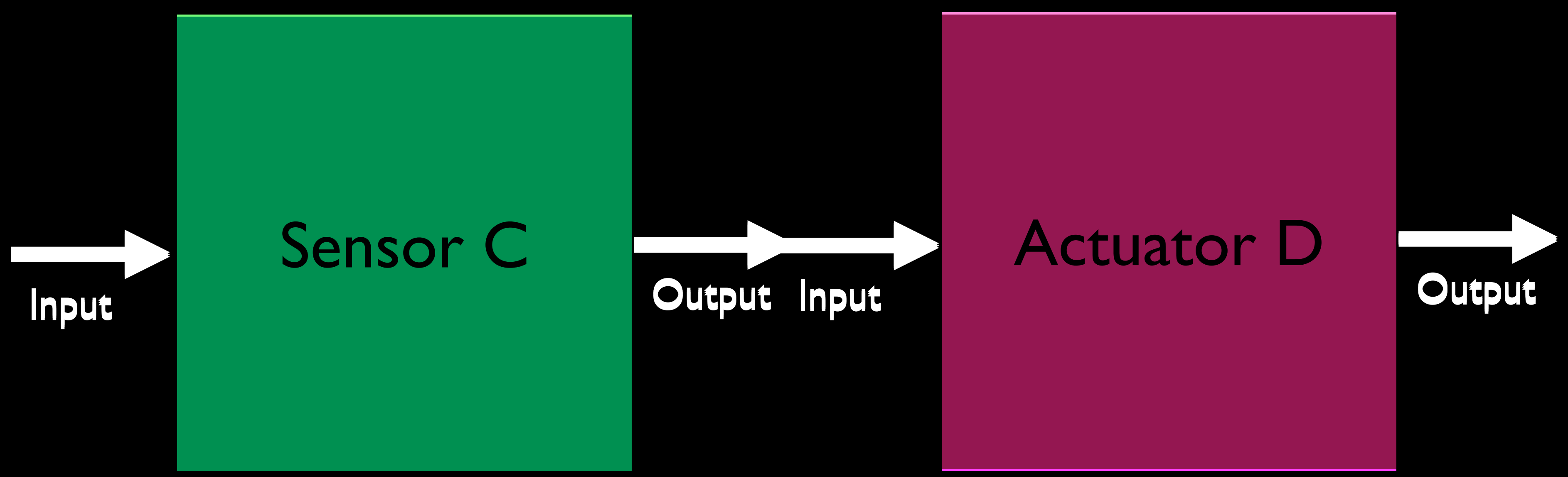
Many thousand so-called BioBrick DNA parts.

Freely available.

Today.

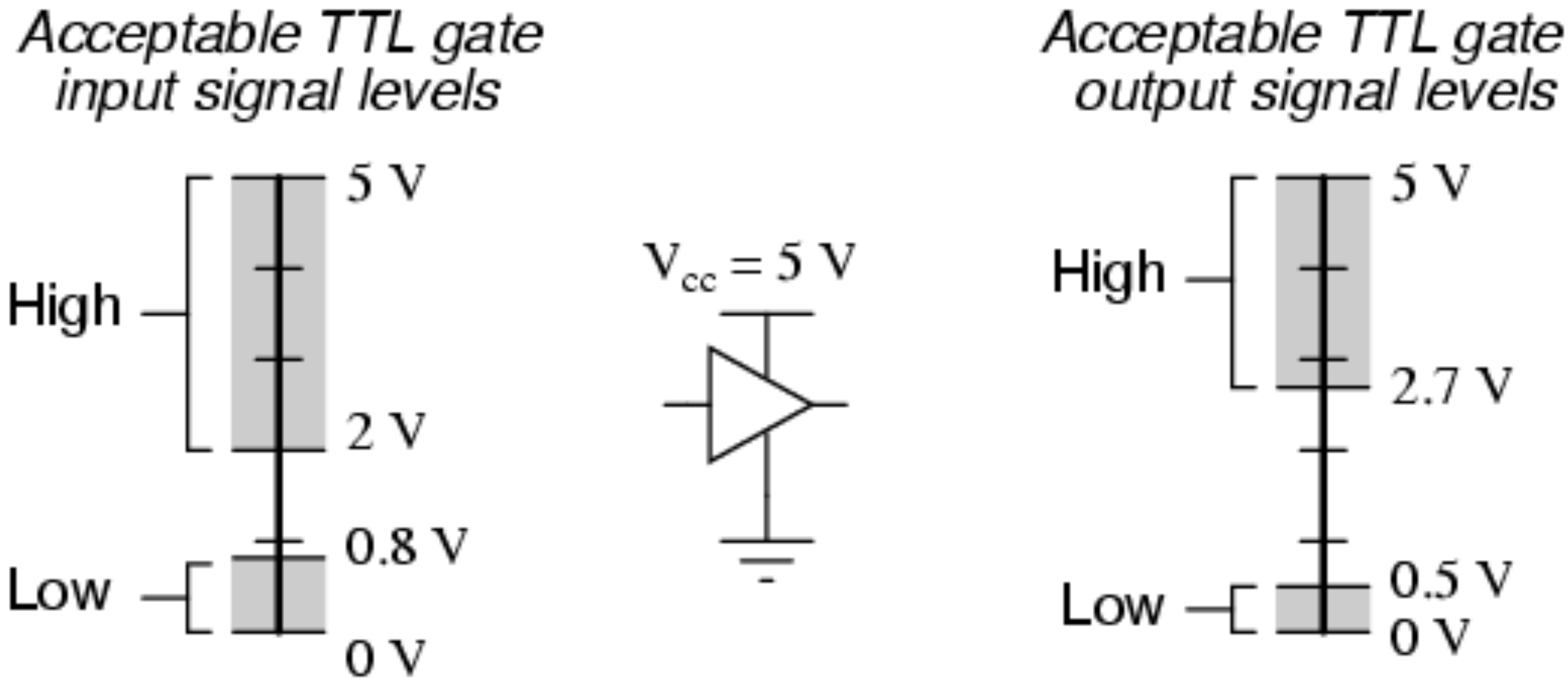
iGEM 2010

Functional composition... what should the “output” of any Sensor be so that it can connect with the “input” of any Actuator?



Polymerase Per Second (PoPS) as common signal carrier for transcription-based devices

Signal levels (standards) & digitization

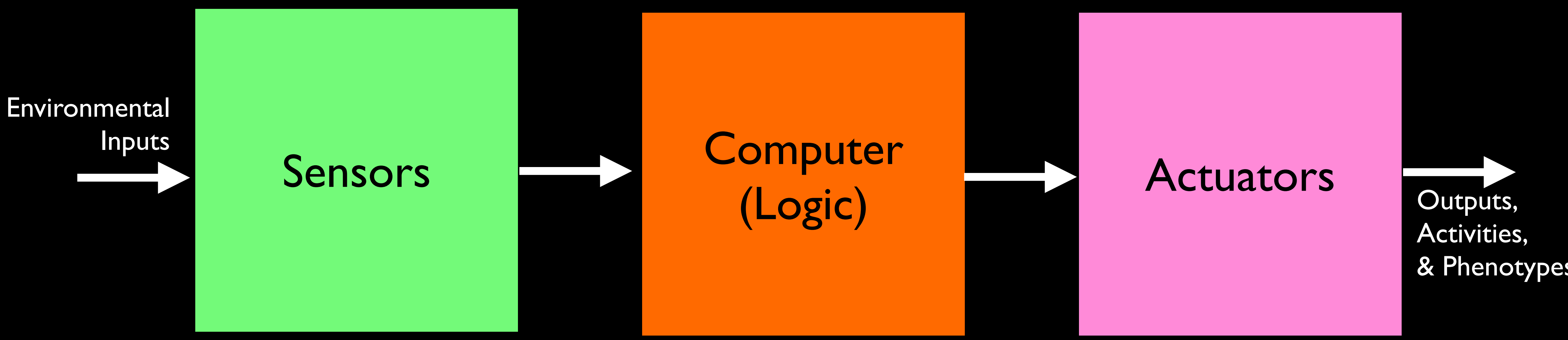


https://www.allaboutcircuits.com/textbook/digital/chpt-3/logic-signal-voltage-levels/

You can make genetically-encoded molecular machines.

Doing so smartly requires going up and down our ***abstraction*** hierarchy (otherwise too complicated).

Most of these engineering approaches are entirely new to biology (i.e., this type of bioengineering is v. new).



Details include:

Identifying and implementing **device boundaries** & **common signal carriers**.

Considering signal **level matching** & **digitization/ amplification**