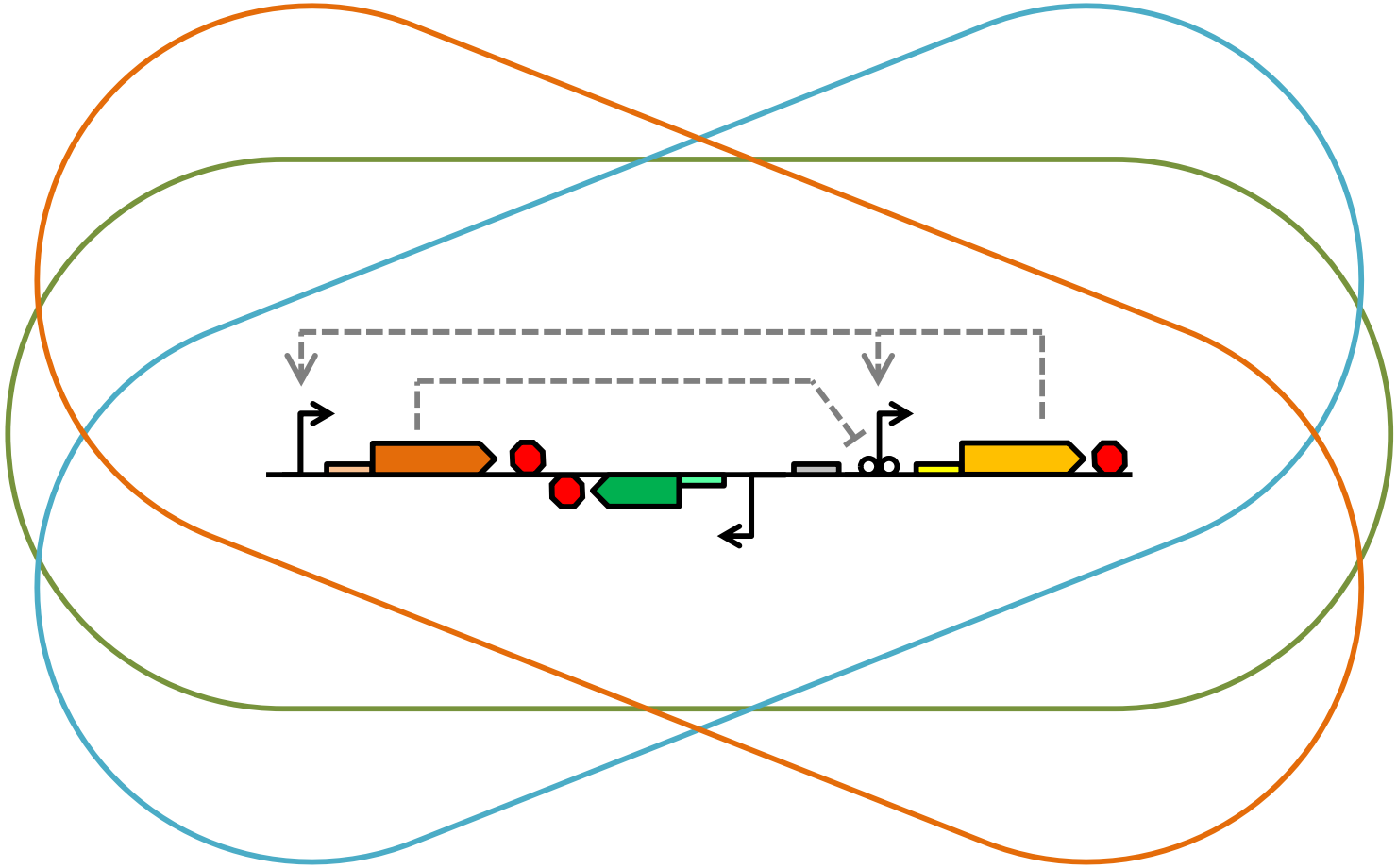
Synthetic Circuits in Prokaryotes

UE2.1 Biological Parts and Devices



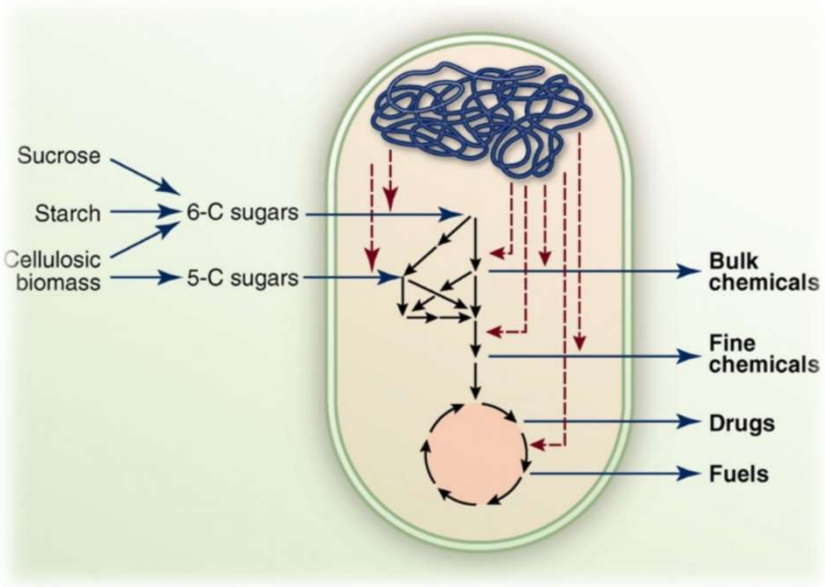
Manish Kushwaha

08 October, 2024

Cells can produce useful products and behaviors

Microbial cell factory

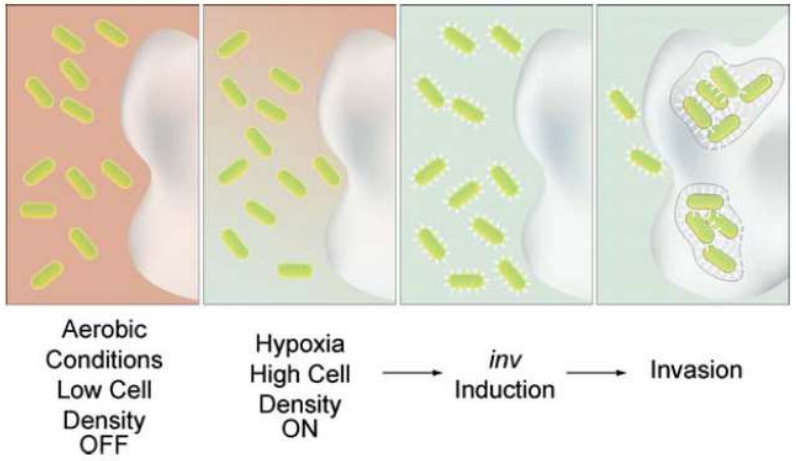
(production)



(Keasling JD, 2010. Science)

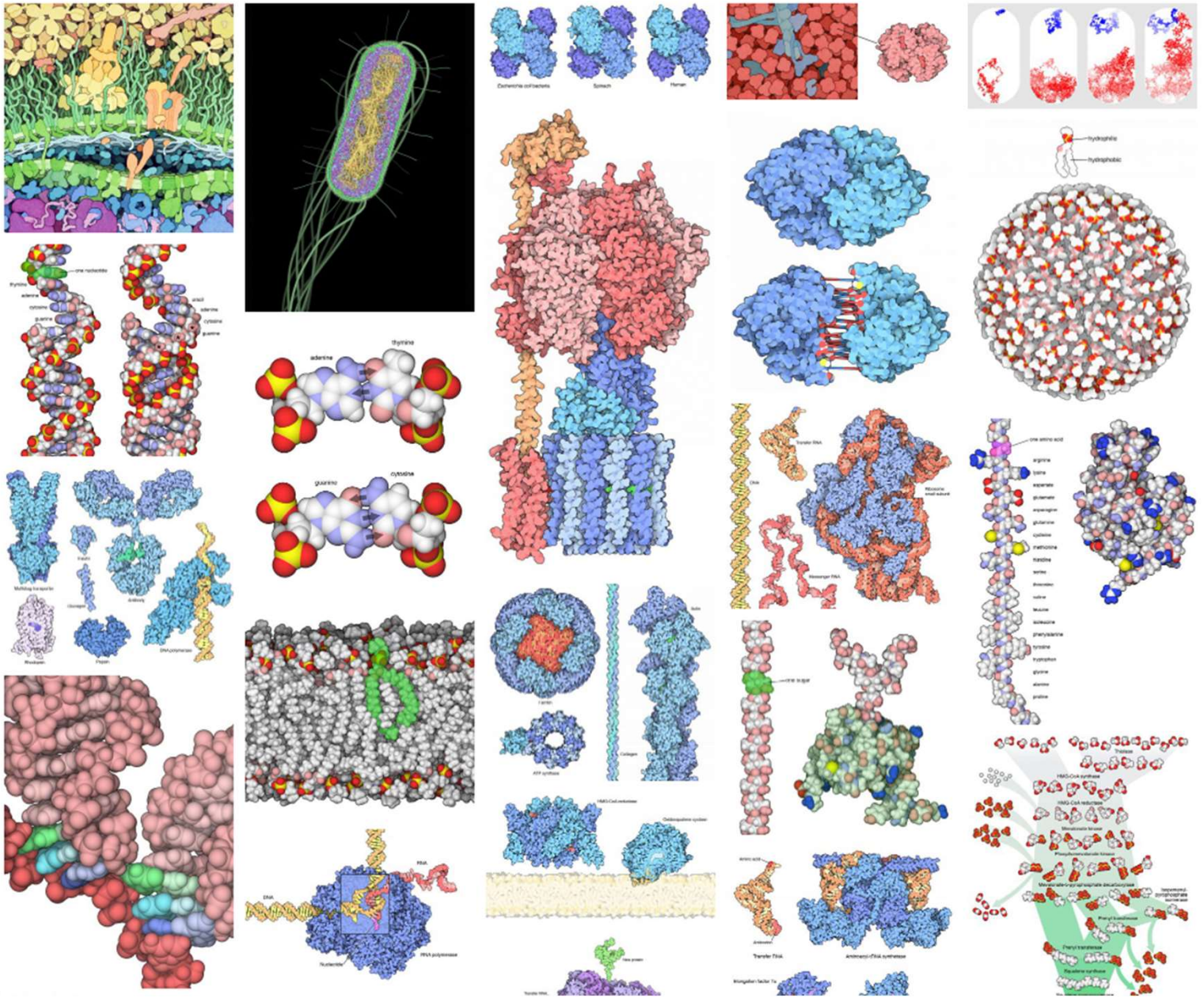
Differential targeting of Cancer cells

(logic + delivery)



(Anderson *et al.*, 2006. JMB)

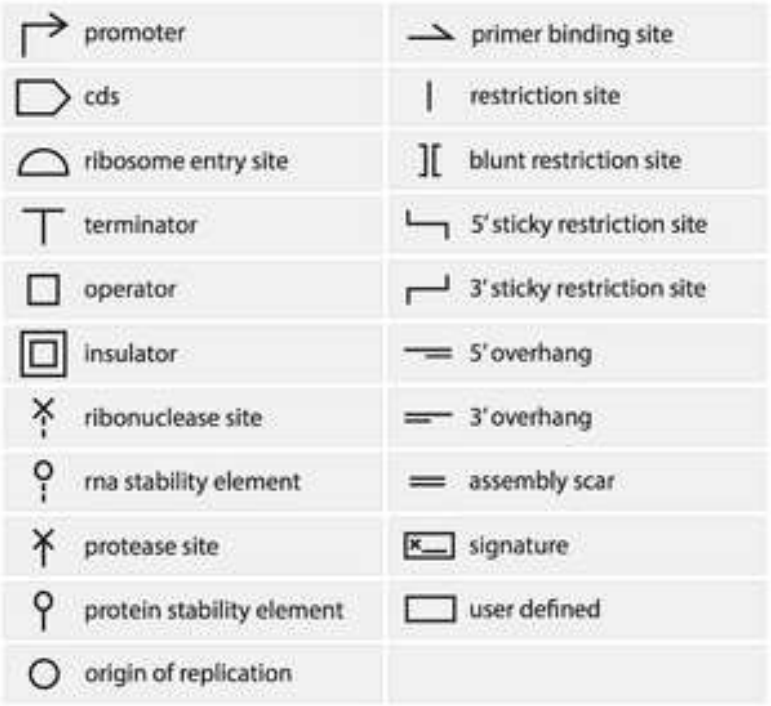
“The Machinery of Life” has many Parts



David S. Goodsell

The Standardised Parts approach of SynBio

- Well-characterised biological parts, like parts of a machine, can be re-used in novel contexts with similar functionality.

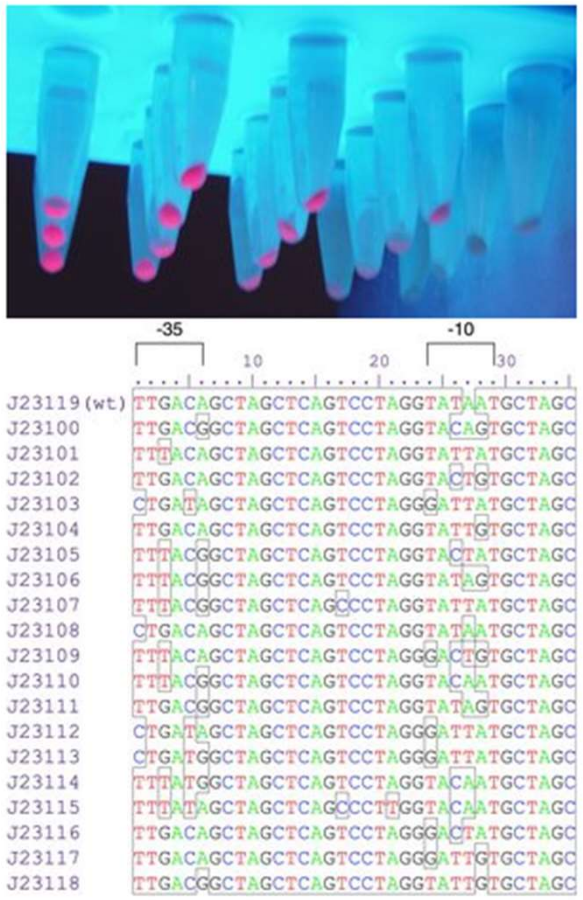


Synthetic Biology Open Language (SBOL) standard visual symbols

Parts Registry

OpenPlant

- Desired behaviours can be picked out of a catalog of Biological Parts.

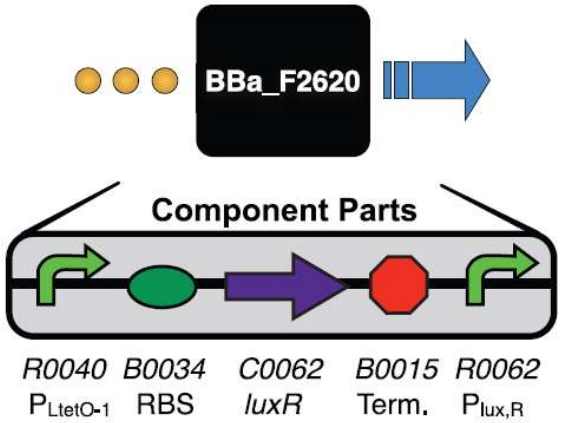


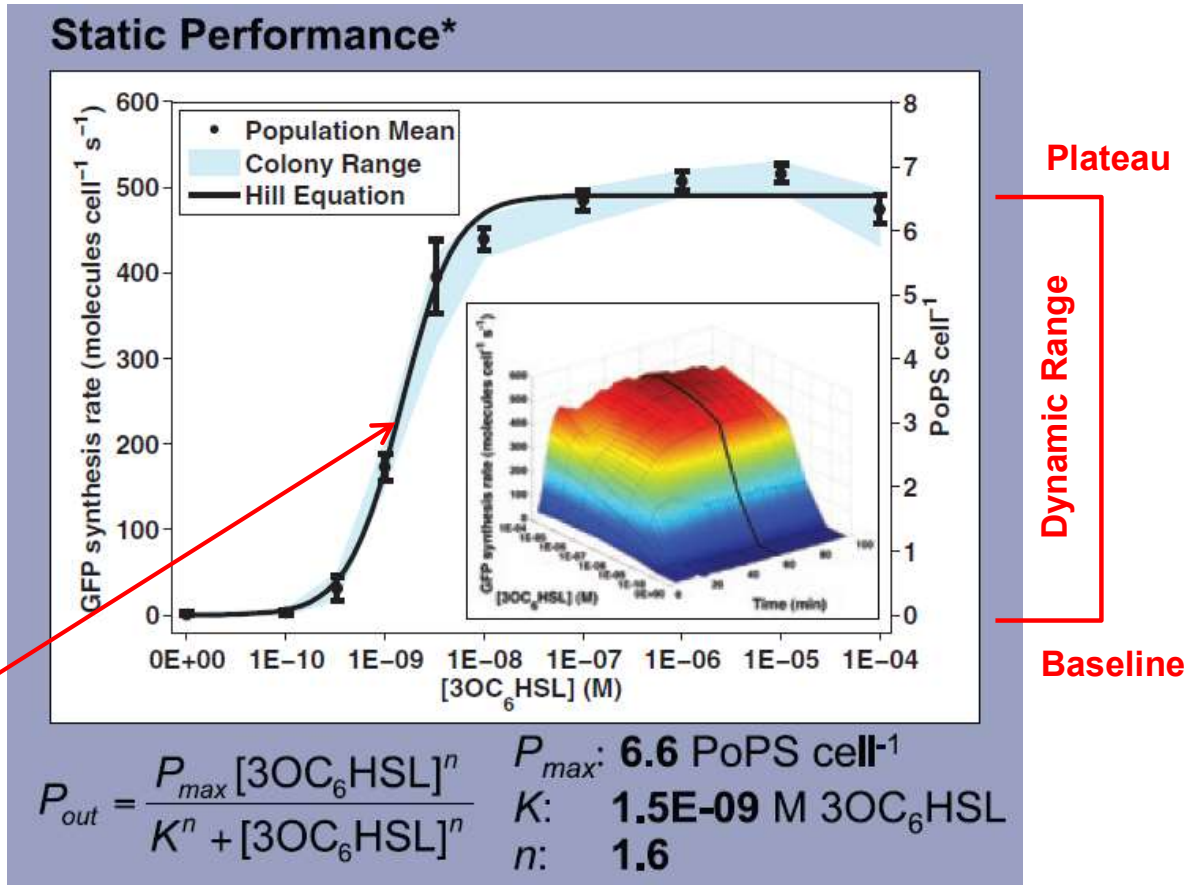
Anderson promoter library
http://parts.igem.org/Part:BBa_J23100

Information Transfer Functions

- More detailed quantitative documentation of Part-associated data

The ‘datasheet’ for genetic parts



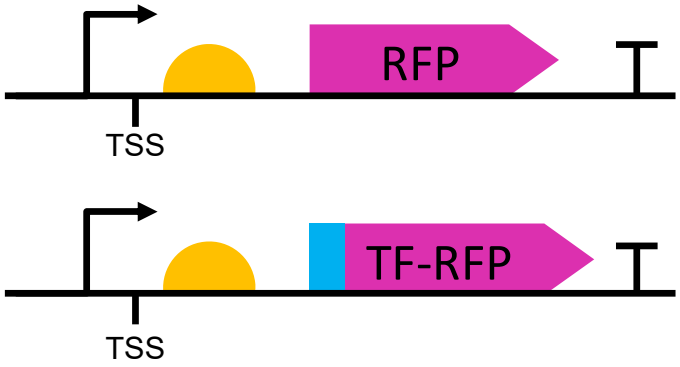


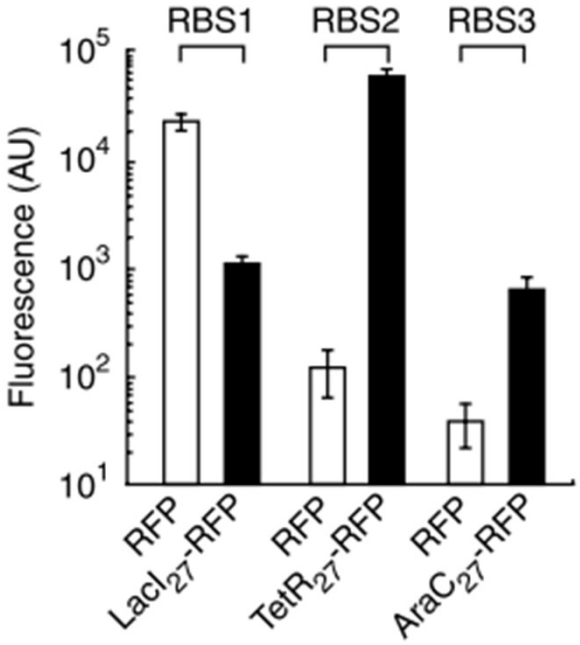
Functional Units
Transcription: PoPS cell^{-1}
Translation: RibS cell^{-1}

(Canton *et al.*, 2008. Nat. Biotech.)

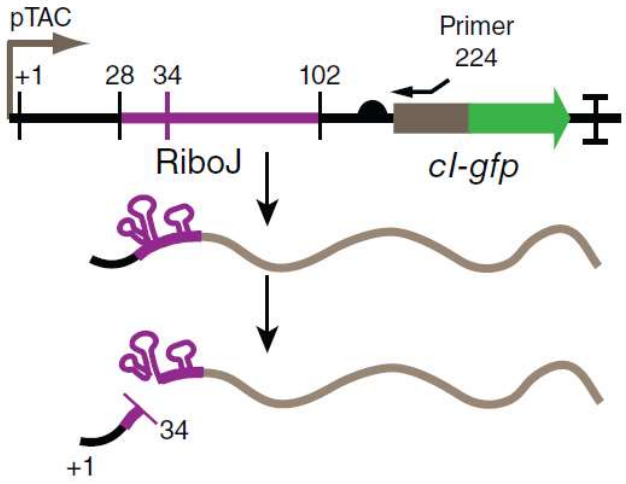
The limits of the Standardised Parts approach (Context Dependence)

- There are limits to the Parts based abstraction of biological function.
- Functions of parts are context dependent!





(Salis *et al.*, 2009)



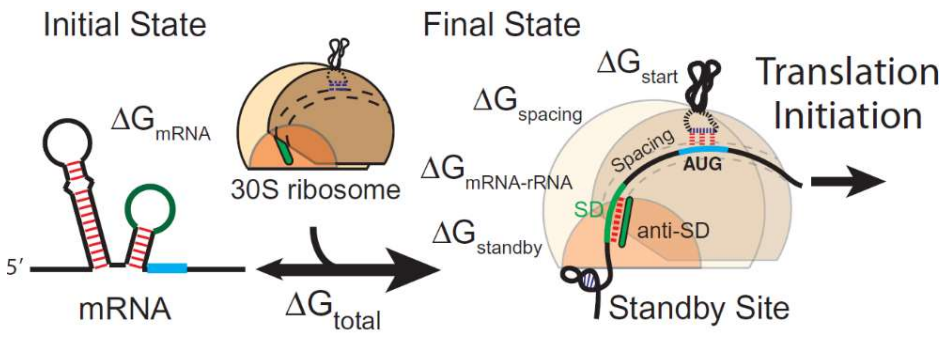
Insulating Ribozymes

(Lou *et al.*, 2012)

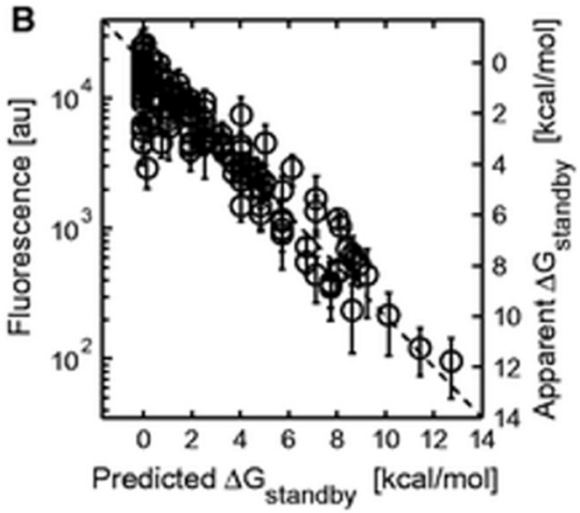
The Functional Modelling approach

- Contextual effects can alter functions of biological parts.
- Quantitative models that comprehensively describe the biological system could better predict system output.

(Salis *et al.*, 2009, Borujeni *et al.*, 2013)



$$\Delta G_{\text{total}} = \Delta G_{\text{mRNA-rRNA}} + \Delta G_{\text{spacing}} + \Delta G_{\text{start}} + \Delta G_{\text{standby}} - \Delta G_{\text{mRNA}}.$$



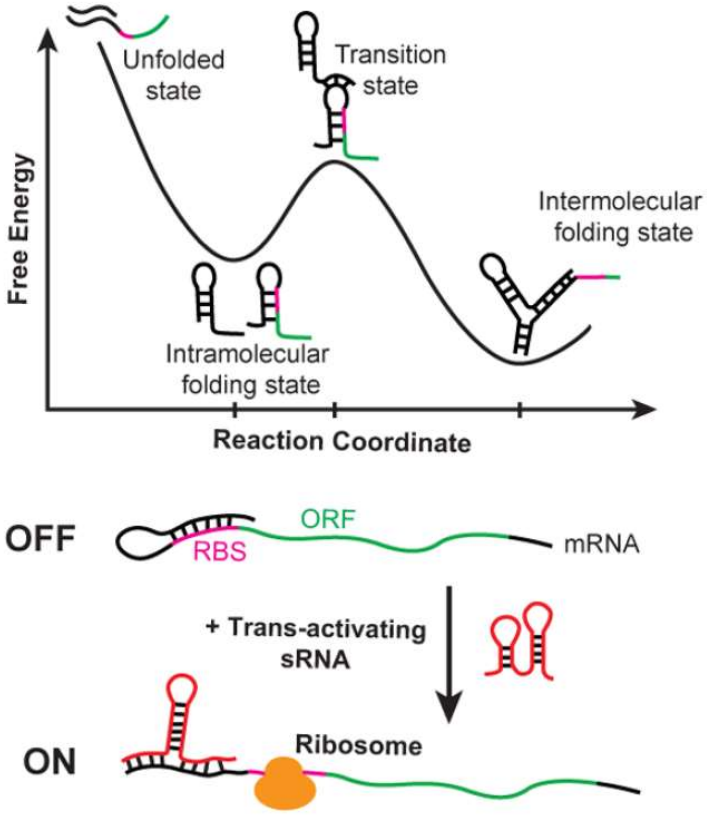
- The RBS Calculator Biophysical Model can predict Translation Initiation rates using the total binding free energy between the ribosome and the mRNA

RBS TIR= K * exp(-β ΔG_{total})

https://salislab.net/software/predict_rbs_calculator

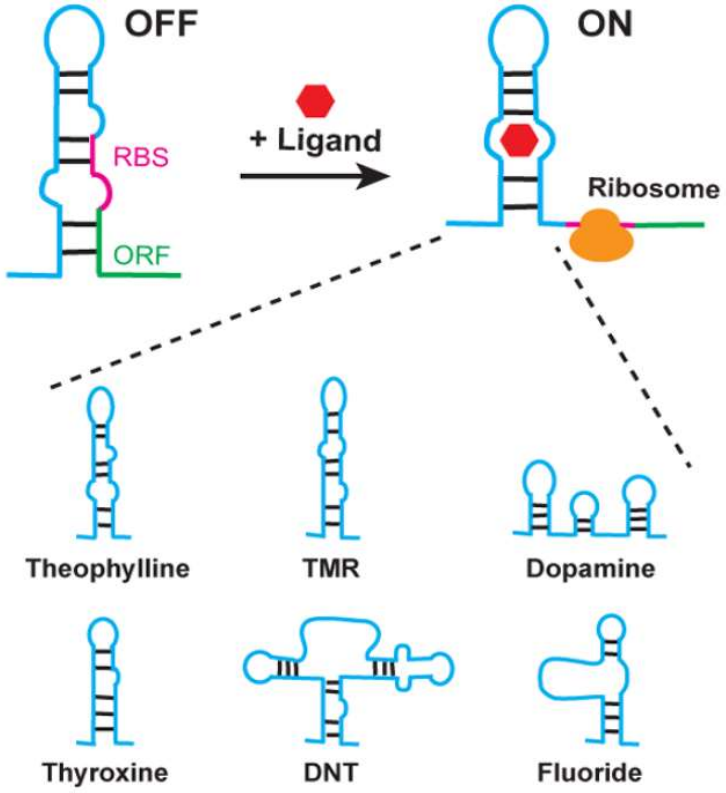
The Functional Modelling approach

- Contextual effects can alter functions of biological parts.
- Quantitative models that comprehensively describe the biological system could better predict system output.



Ribomaker Model

(Rodrigo *et al.*, 2012)

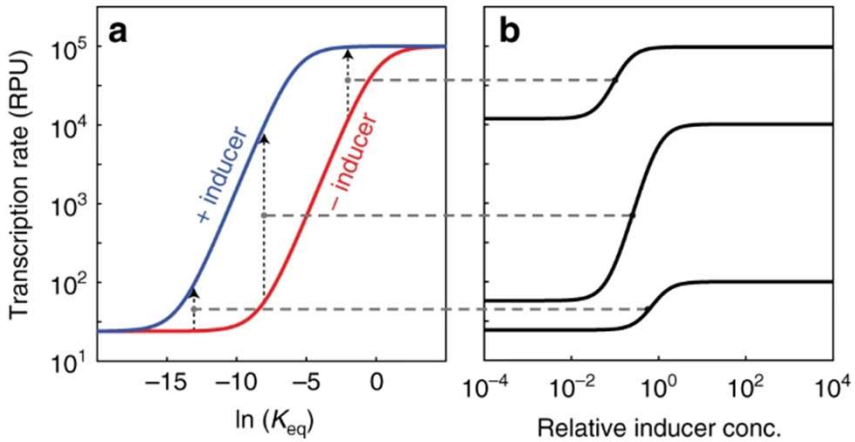


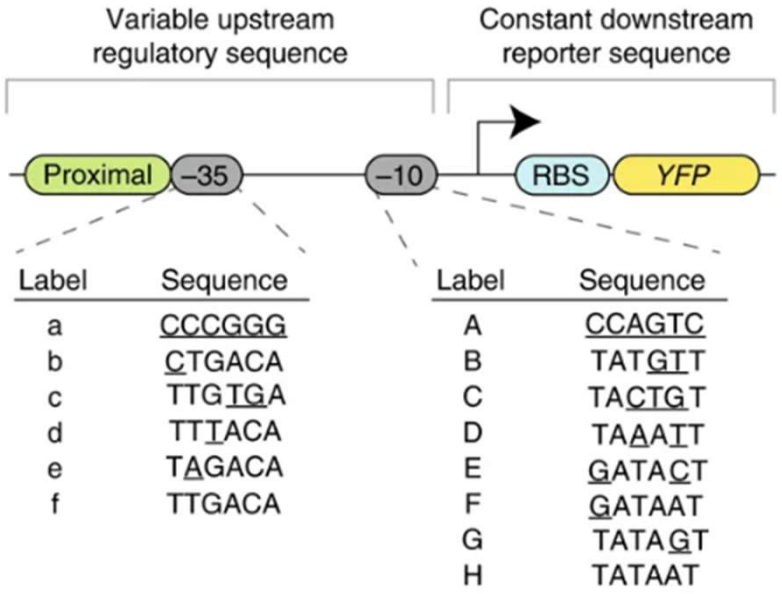
Riboswitch Calculator

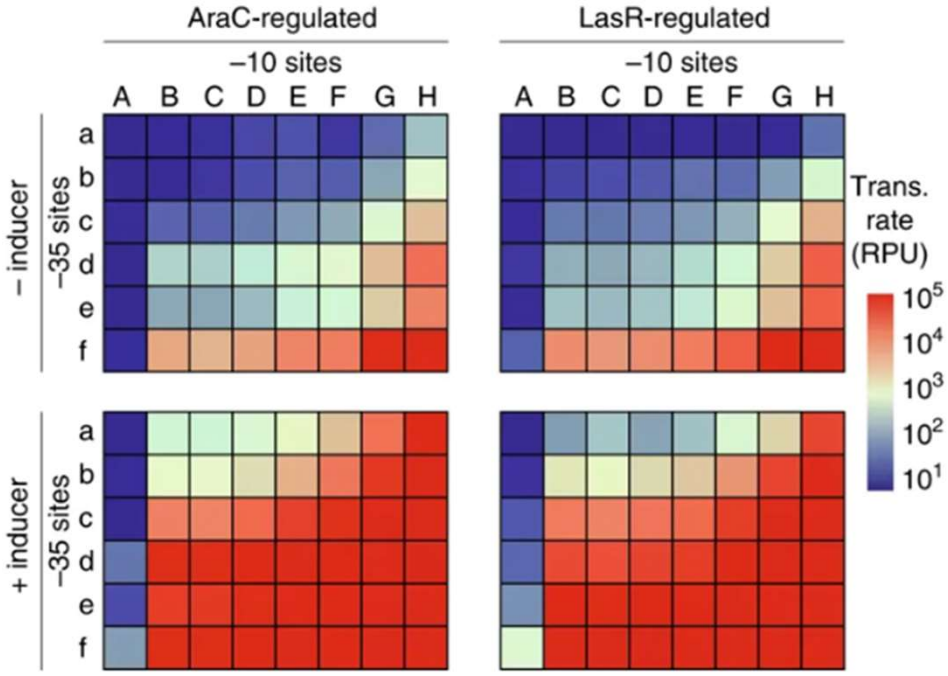
(Borujeni *et al.*, 2016)

The Functional Modelling approach

- Biophysical modelling of ligand-inducible transcription factors binding to their promoters.







(Chen *et al.*, 2018. Nat Comm)

Information Processing: Digital Logic Gates

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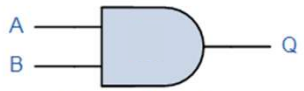
Symbol	Truth Table		
 2-input AND Gate	A	B	Q
	0	0	0
	0	1	0
	1	0	0
	1	1	1
Boolean Expression Q = A.B	Read as A AND B gives Q		

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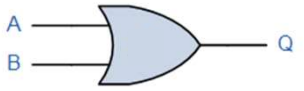
Symbol	Truth Table		
 2-input OR Gate	A	B	Q
	0	0	0
	0	1	1
	1	0	1
	1	1	1
Boolean Expression Q = A+B	Read as A OR B gives Q		

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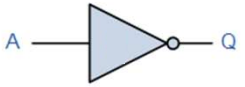
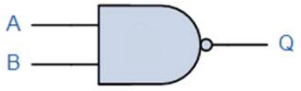
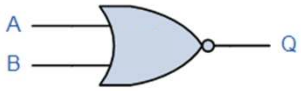
Symbol	Truth Table	
 Inverter or NOT Gate	A	Q
	0	1
	1	0
Boolean Expression Q = NOT A or $\overline{A}$	Read as inversion of A gives Q	

Symbol	Truth Table		
 2-input NAND Gate	A	B	Q
	0	0	1
	0	1	1
	1	0	1
	1	1	0
Boolean Expression Q = $\overline{A.B}$	Read as A AND B gives NOT-Q		

Symbol	Truth Table		
 2-input NOR Gate	A	B	Q
	0	0	1
	0	1	0
	1	0	0
	1	1	0
Boolean Expression Q = $\overline{A+B}$	Read as A OR B gives NOT-Q		

https://www.electronics-tutorials.ws/boolean/bool_7.html

Information Processing: Digital Logic Gates

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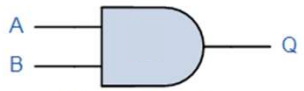
Symbol	Truth Table		
 2-input AND Gate	A	B	Q
	0	0	0
	0	1	0
	1	0	0
	1	1	1
Boolean Expression Q = A.B	Read as A AND B gives Q		

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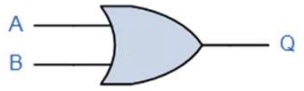
Symbol	Truth Table		
 2-input OR Gate	A	B	Q
	0	0	0
	0	1	1
	1	0	1
	1	1	1
Boolean Expression Q = A+B	Read as A OR B gives Q		

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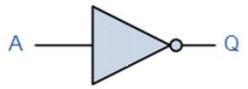
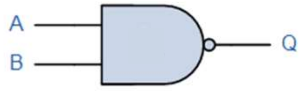
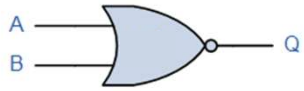
Symbol	Truth Table	
 Inverter or NOT Gate	A	Q
	0	1
	1	0
Boolean Expression Q = NOT A or $\overline{A}$	Read as inversion of A gives Q	

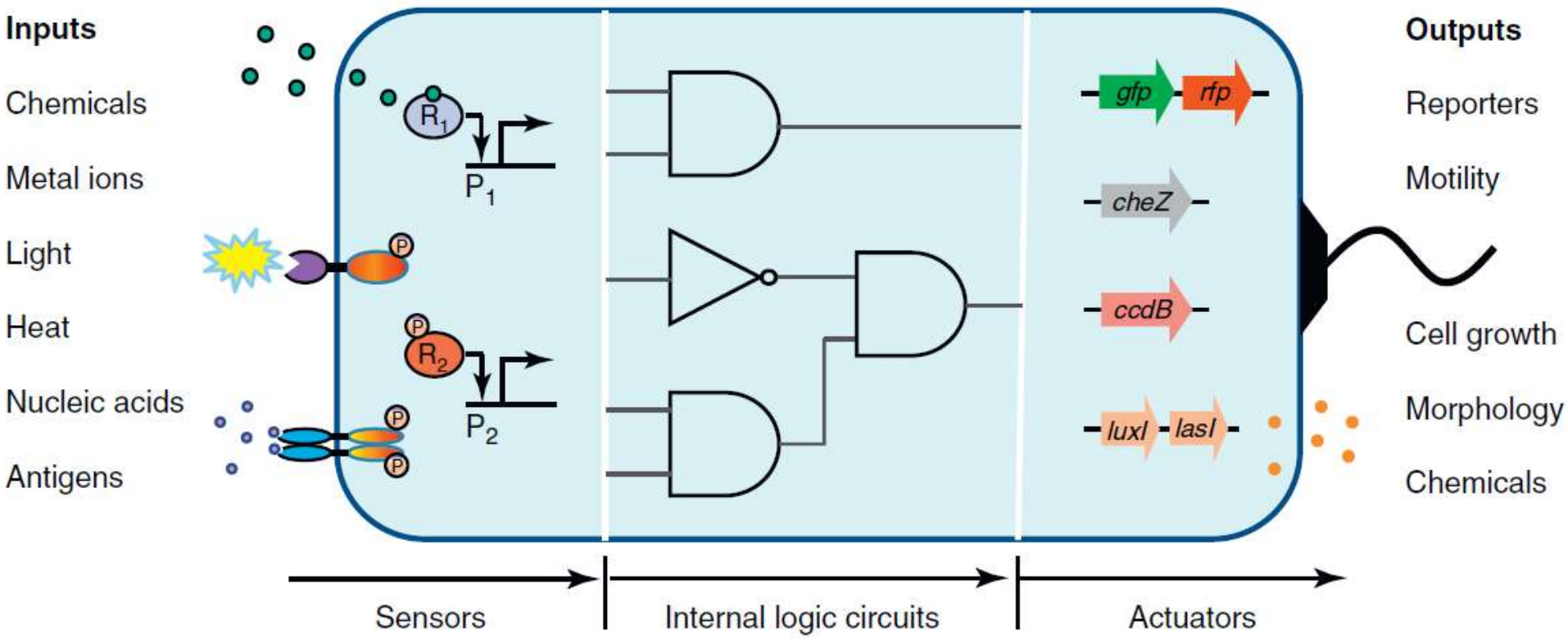
Symbol	Truth Table		
 2-input NAND Gate	A	B	Q
	0	0	1
	0	1	1
	1	0	1
	1	1	0
Boolean Expression Q = $\overline{A.B}$	Read as A AND B gives NOT-Q		

Symbol	Truth Table		
 2-input NOR Gate	A	B	Q
	0	0	1
	0	1	0
	1	0	0
	1	1	0
Boolean Expression Q = $\overline{A+B}$	Read as A OR B gives NOT-Q		

Functionally complete/ Universal gates **How?**

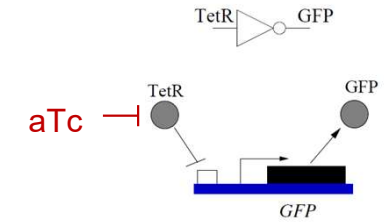
https://www.electronics-tutorials.ws/boolean/bool_7.html

Engineering a Synthetic Genetic Network: Functional Hierarchy



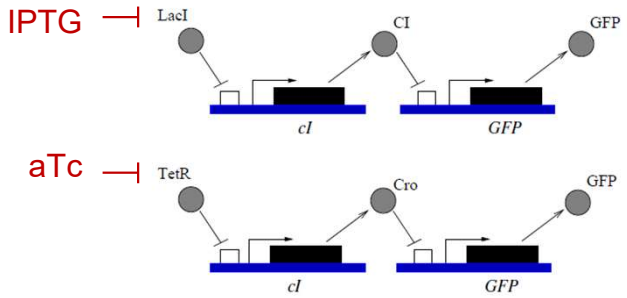
(Wang & Buck, 2012. Trends in Microbiology)

Information Processing: Digital Logic Gates



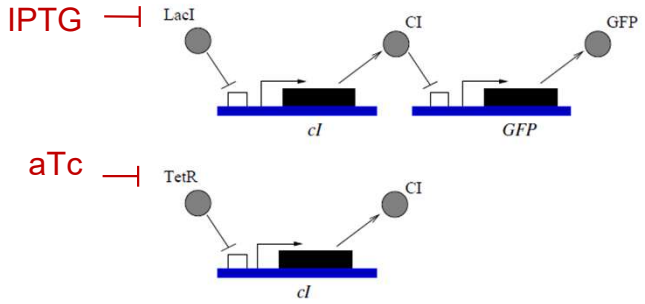
aTc	GFP
0	0
1	1

YES



IPTG	aTc	GFP
0	0	1
0	1	1
1	0	1
1	1	0

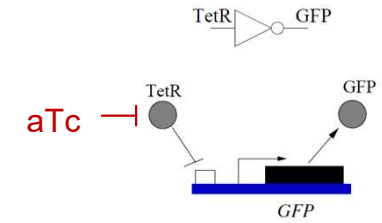
NAND



IPTG	aTc	GFP
0	0	1
0	1	0
1	0	0
1	1	0

NOR

Information Processing: Digital Logic Gates

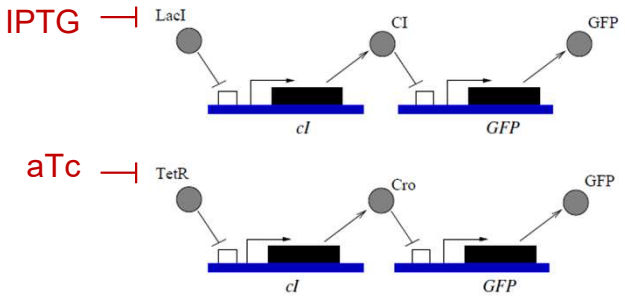


aTc	GFP
0	0
1	1

YES

TetR	GFP
0	1
1	0

NOT gate

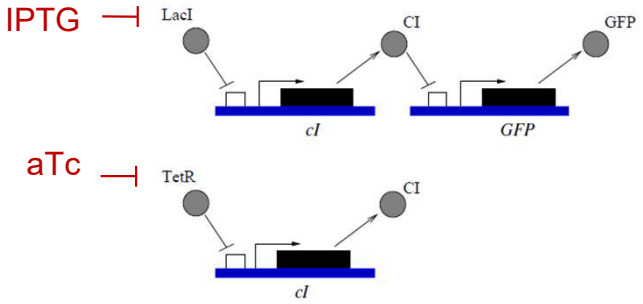


IPTG	aTc	GFP
0	0	1
0	1	1
1	0	1
1	1	0

NAND

LacI	TetR	GFP
0	0	0
0	1	1
1	0	1
1	1	1

OR gate



IPTG	aTc	GFP
0	0	1
0	1	0
1	0	0
1	1	0

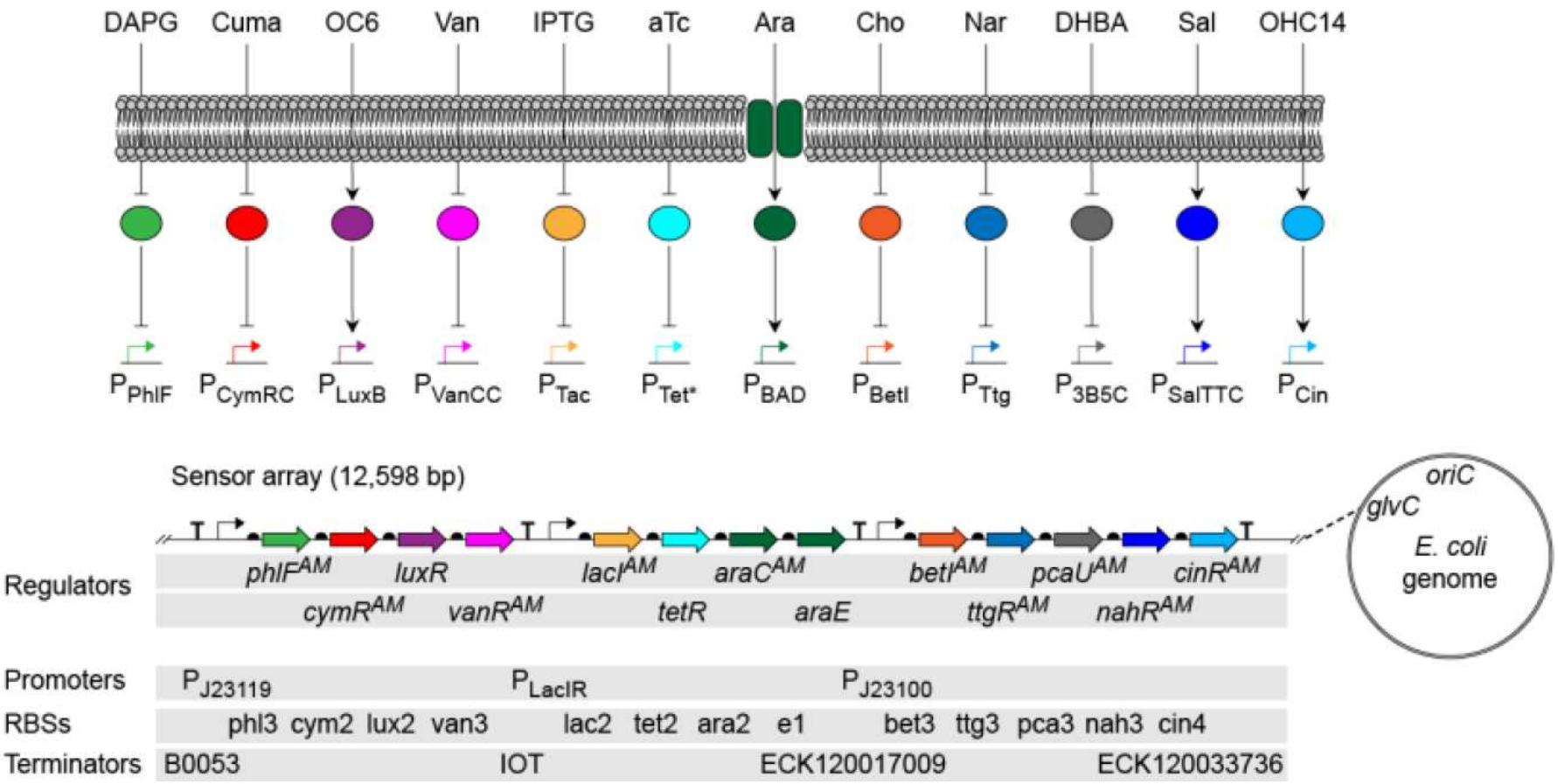
NOR

LacI	TetR	GFP
0	0	0
0	1	0
1	0	0
1	1	1

AND gate

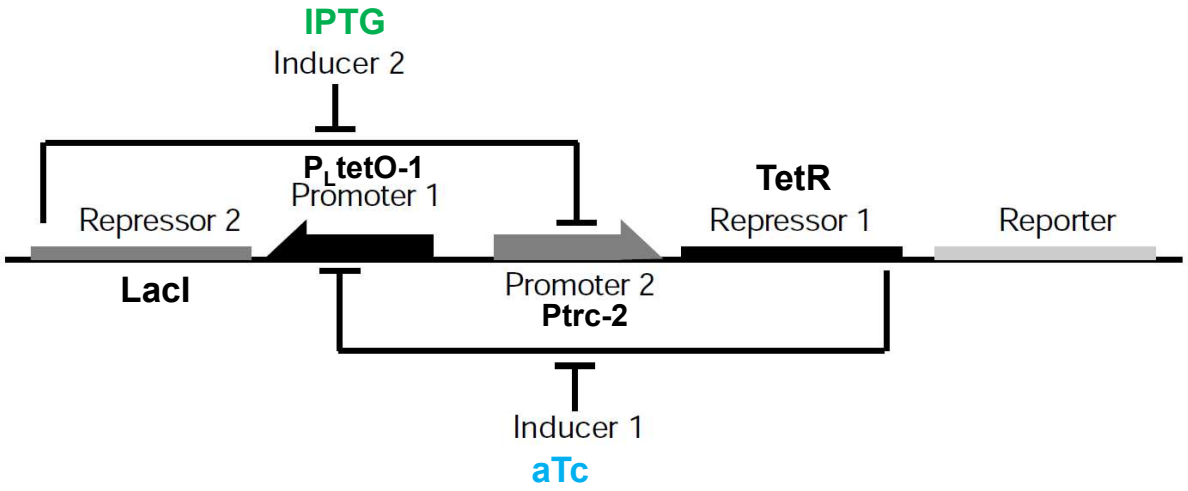
Transcription Regulators for Sensing

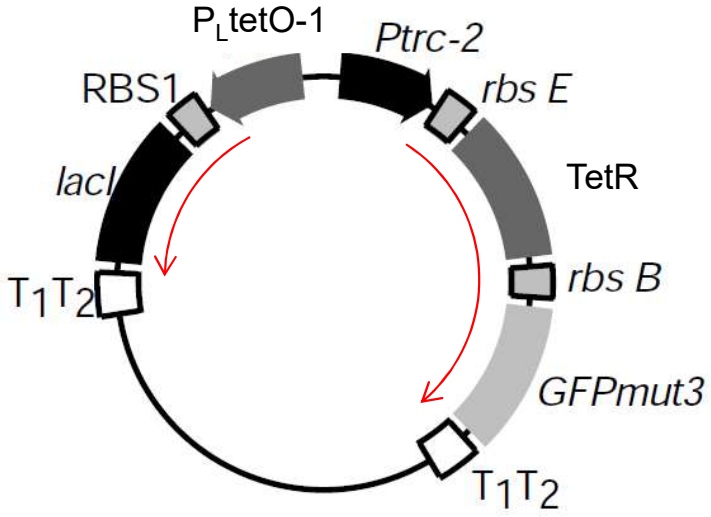
- Recent work has built strains with 12 different “sensor” modules for transcriptional control



(Meyer *et al.*, 2019. Nat Chem Biol)

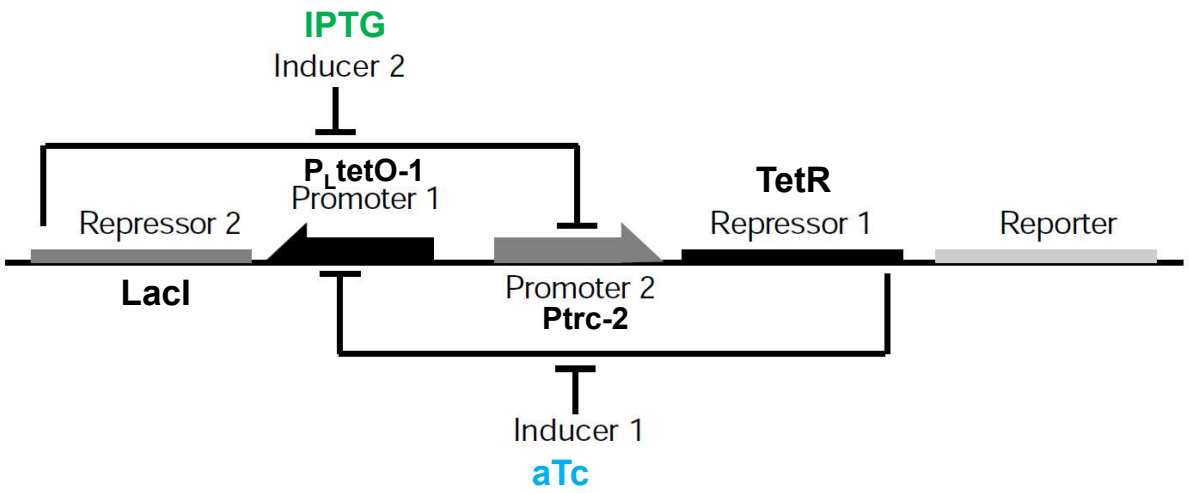
Early Devices: Toggle Switch system

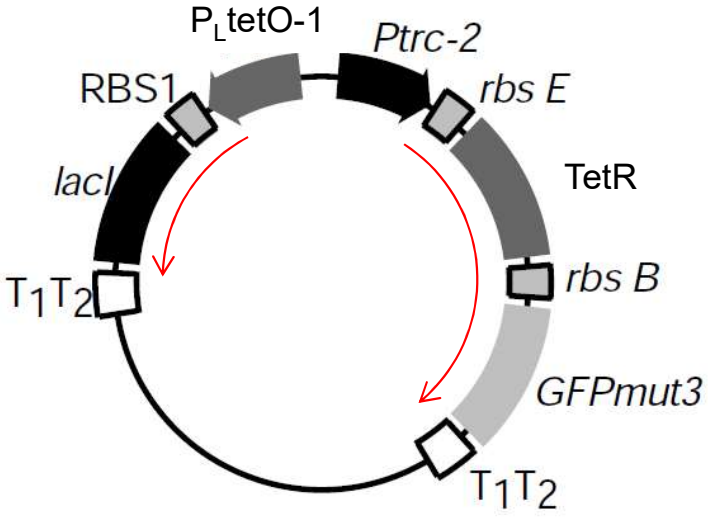


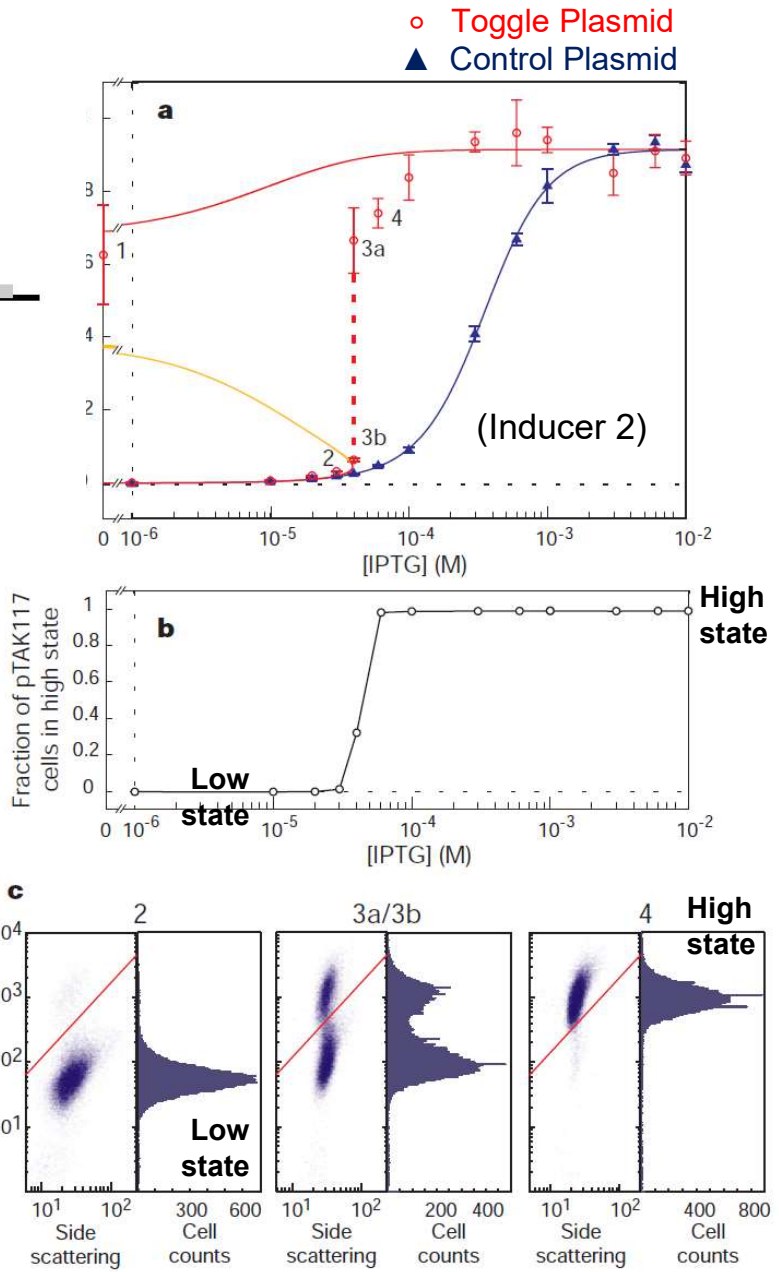


(Gardner *et al.*, 2000. Nature)

Early Devices: Toggle Switch system

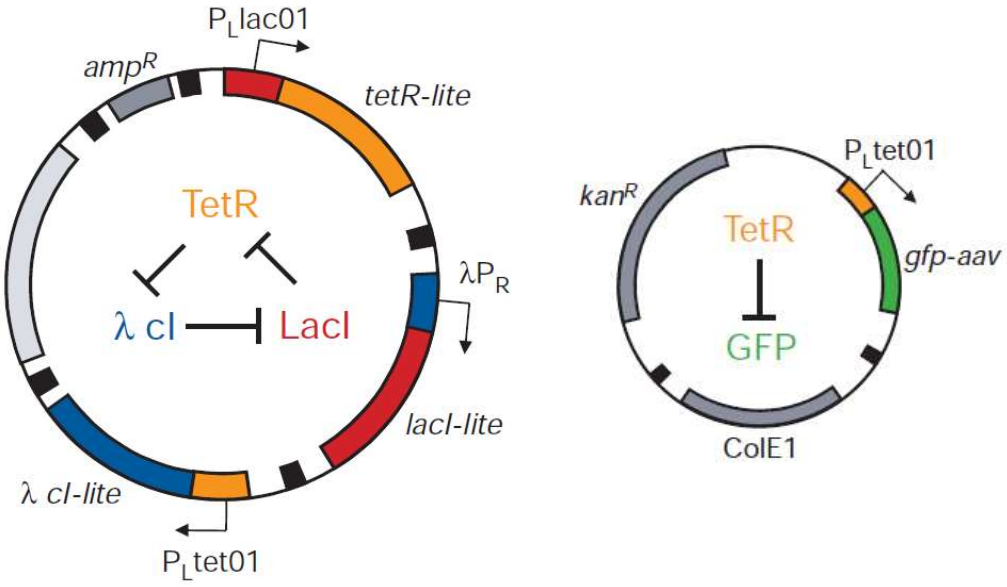






(Gardner *et al.*, 2000. Nature)

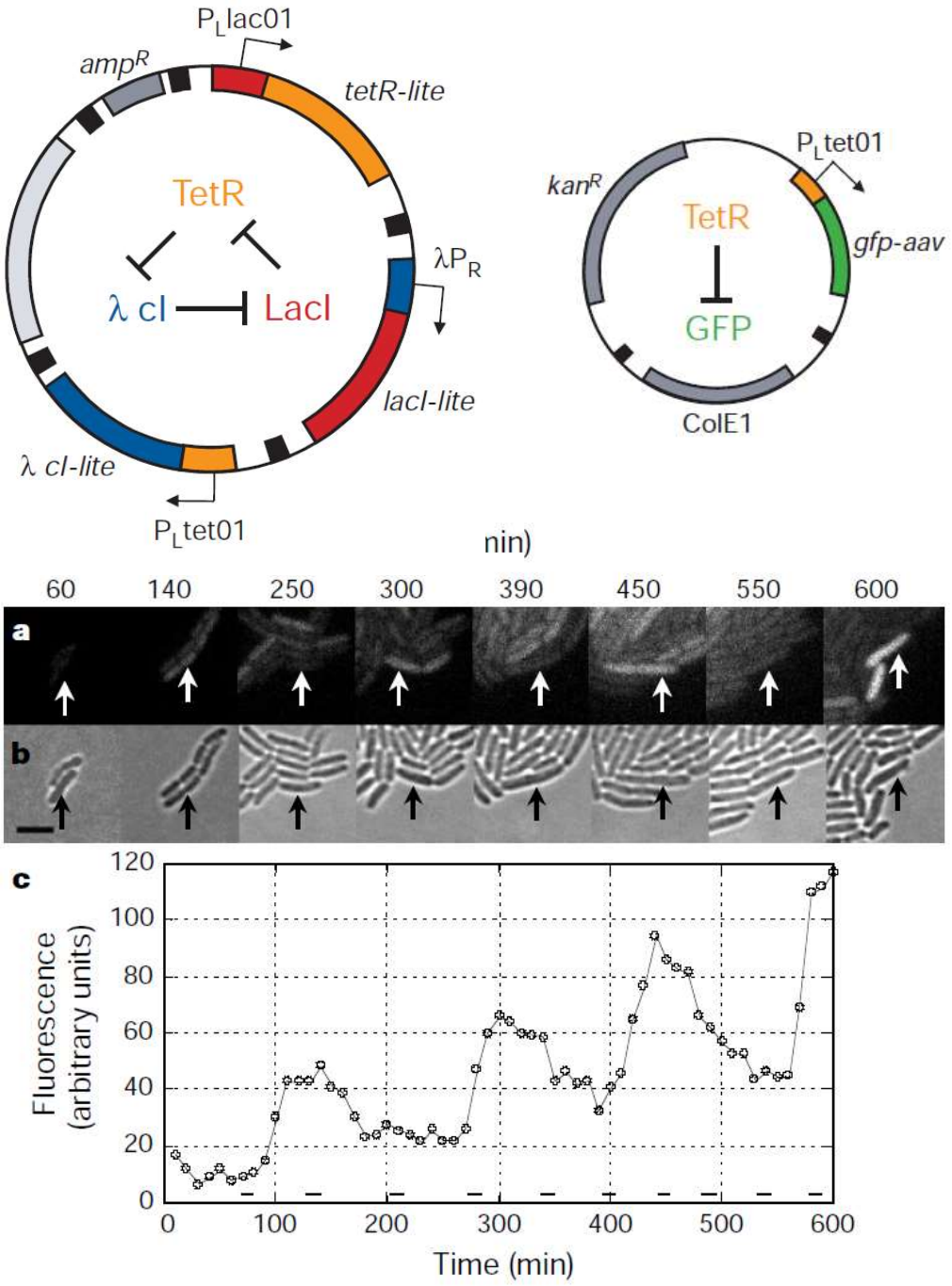
Early Devices: Repressilator system



lite= protein degradation tag
See http://parts.igem.org/Protein_domains/Degradation

(Elowitz *et al.*, 2000. Nature)

Early Devices: Repressilator system



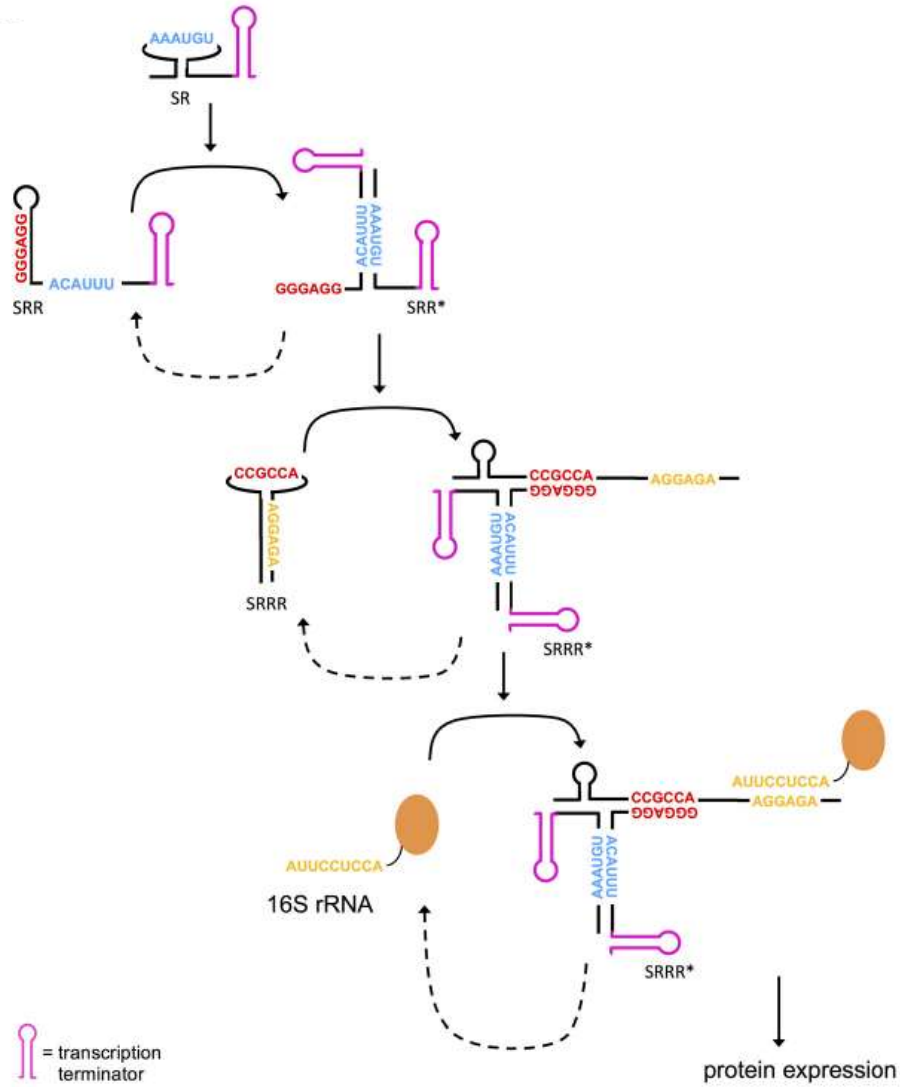
Fluorescence image

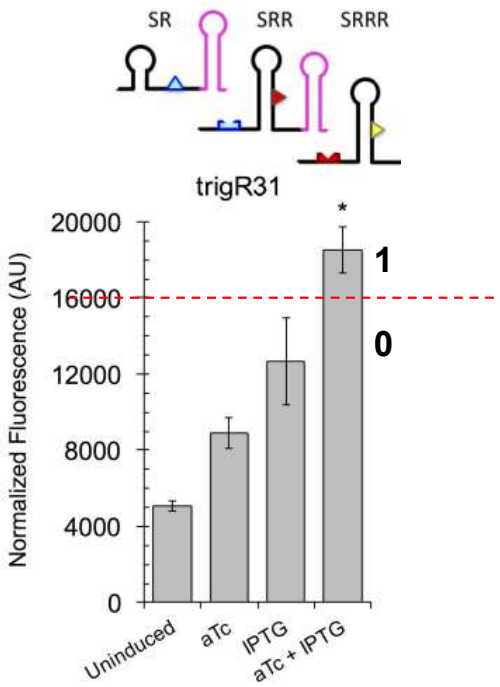
Bright Field image

lite= protein degradation tag
See http://parts.igem.org/Protein_domains/Degradation

(Elowitz *et al.*, 2000. Nature)

Non-TF logic: RNA Logic Gates

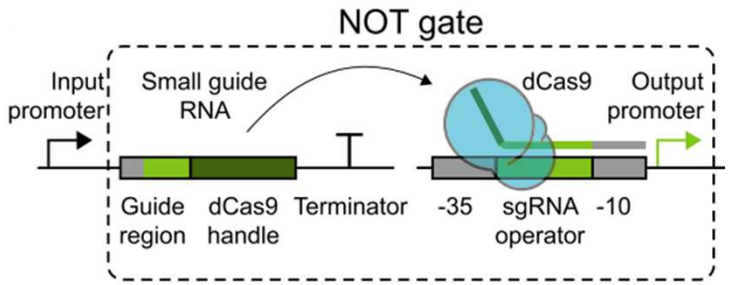


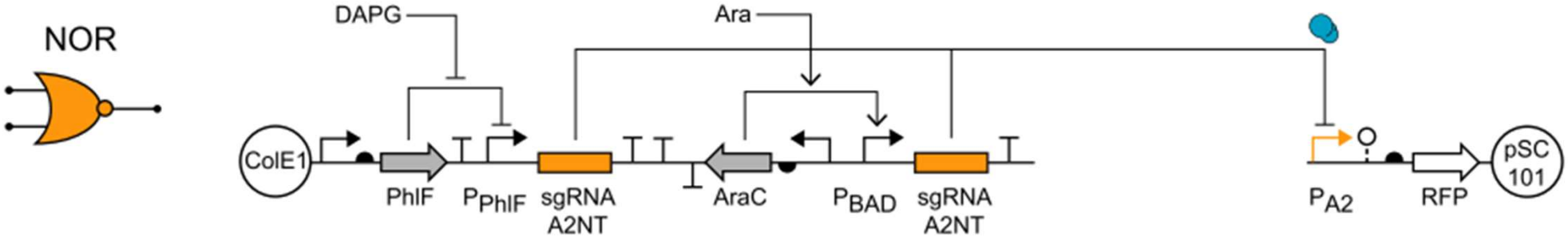


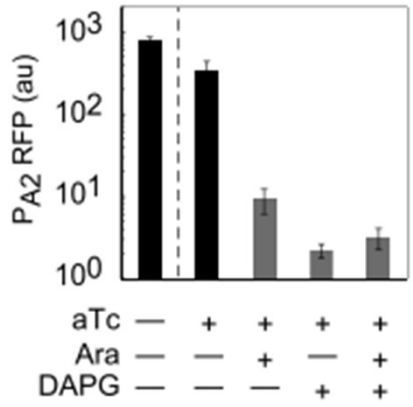
- RNA logic gates allow better predictability of design

(Rodrigo & Prakash *et al.*, 2017. NAR)

CRISPRi gates: Protein+RNA regulator

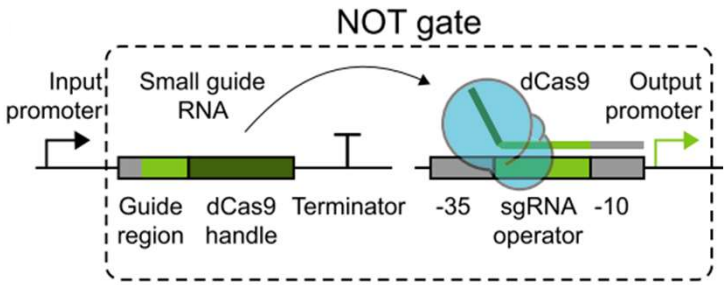


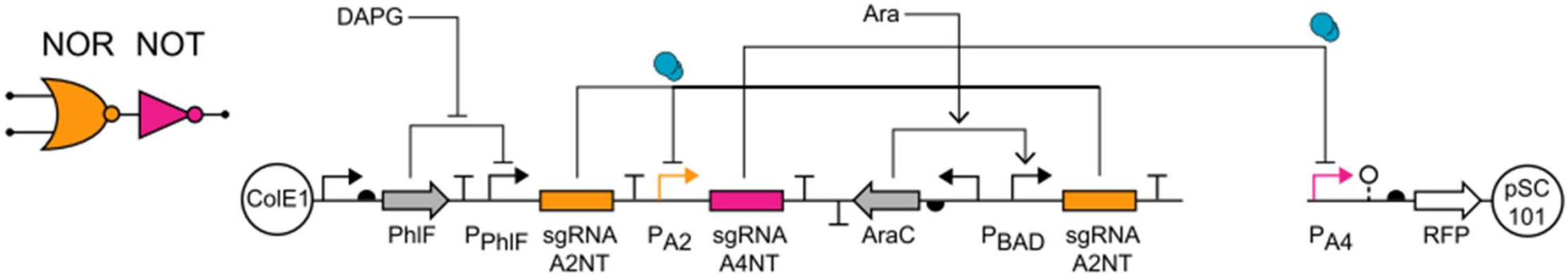


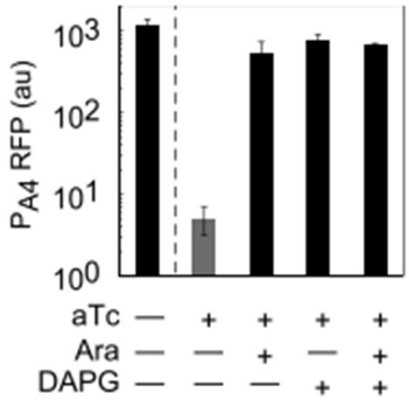


(Nielsen *et al.*, 2014. MSB.)

CRISPRi gates: Protein+RNA regulator

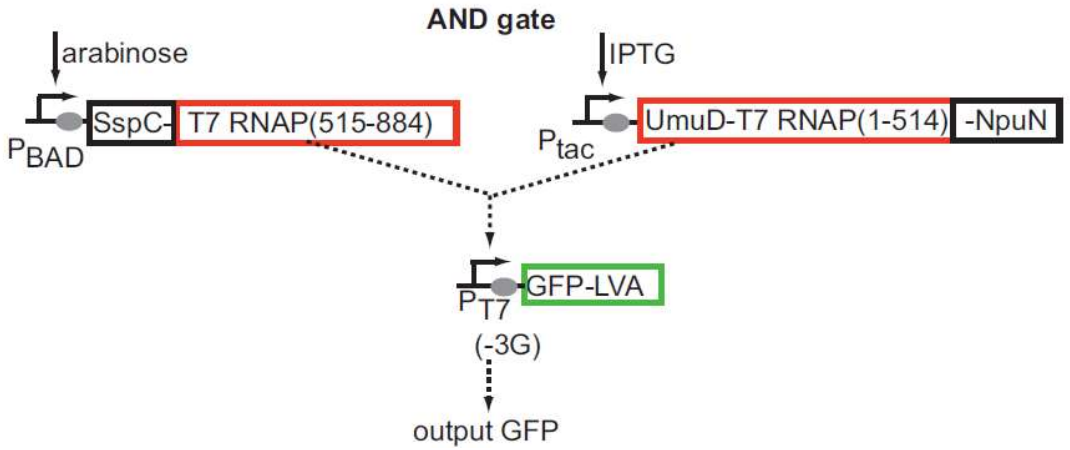


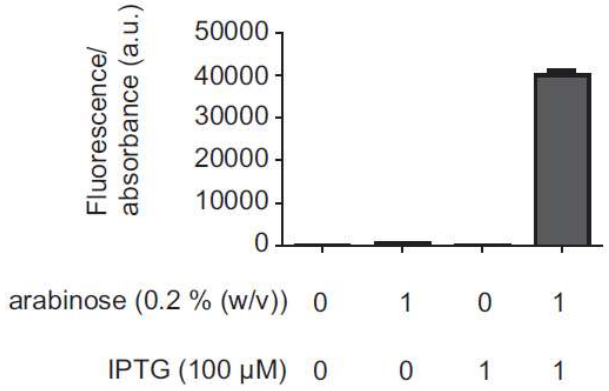




(Nielsen *et al.*, 2014. MSB.)

Non-TF logic: Protein Logic Gates

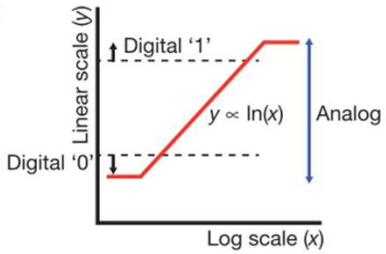


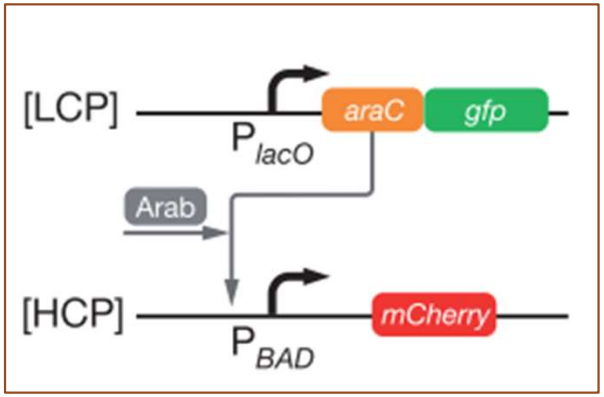


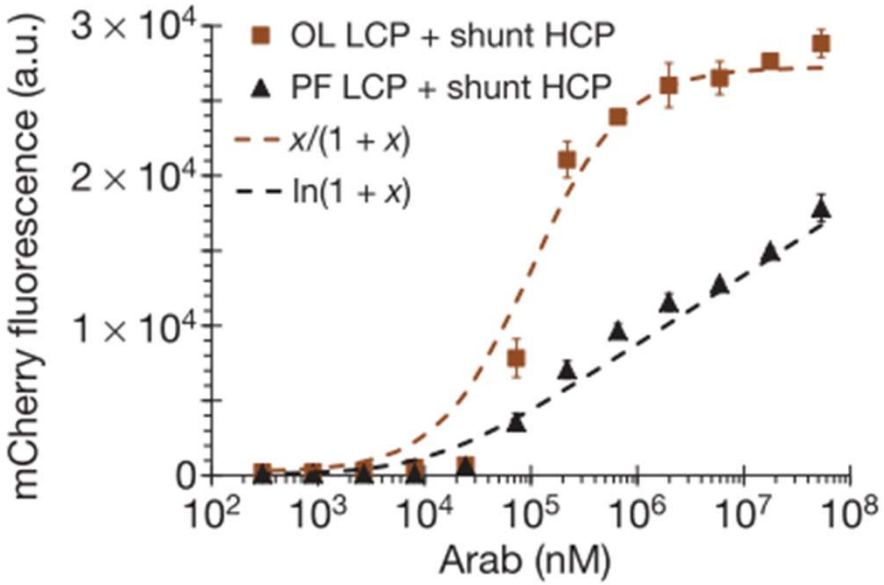
(Schaerli *et al.*, 2014. NAR)

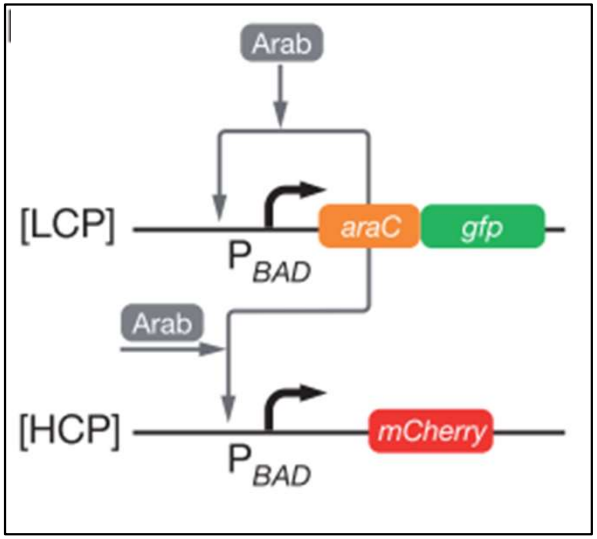
Digital vs Analog Logic in Synthetic Biology

- Is Digital Logic a special case of Analog logic?



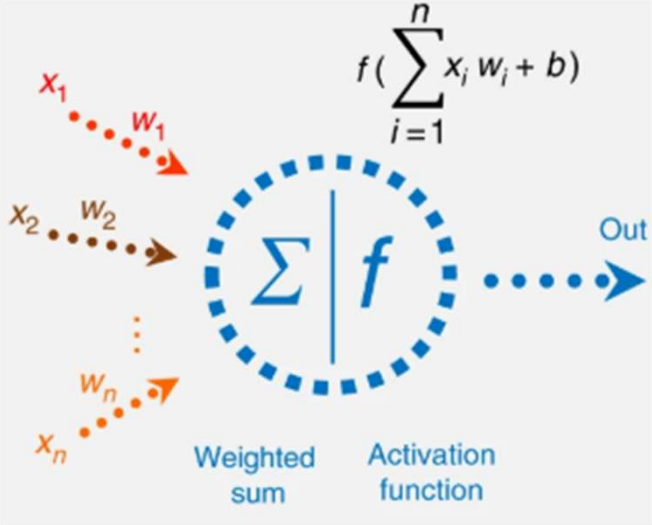


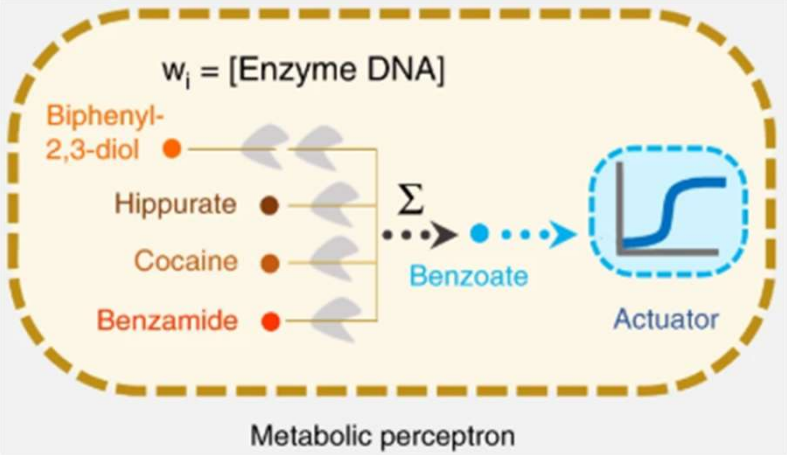




(Daniel *et al.*, 2013. Nature)

A Metabolic Perceptron (Hybrid Logic) Cell-Free System

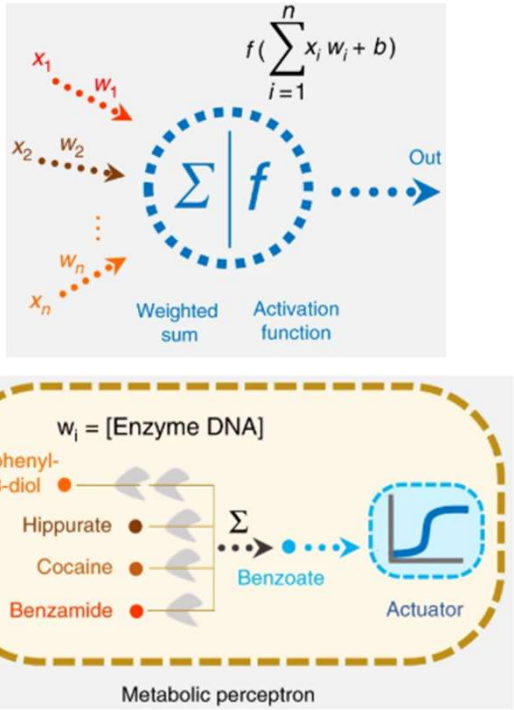


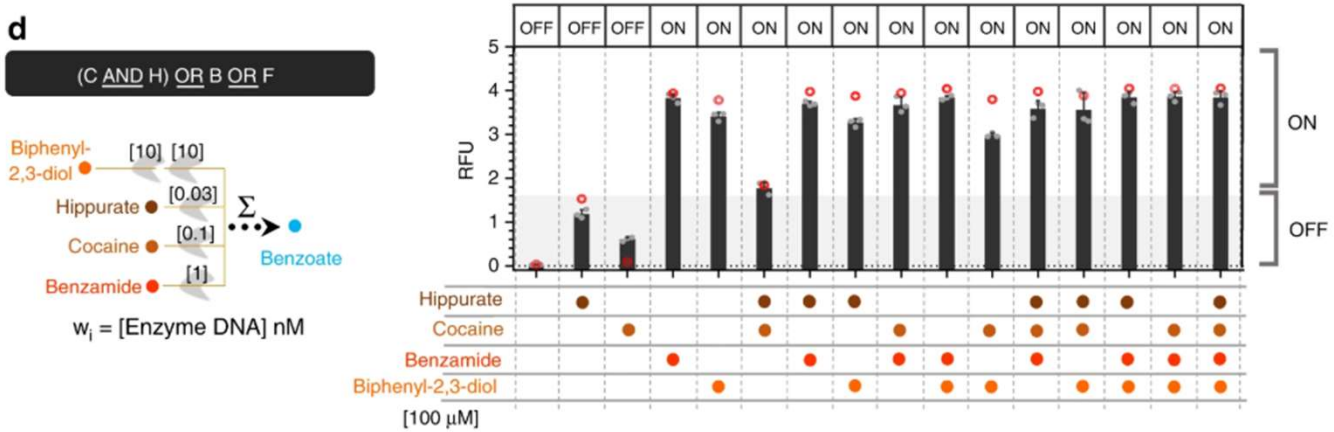


- Different weights applied using the same enzymes can give us different classification/ logic behaviours

(Pandi & Koch *et al*., 2019. Nat. Comm.)

A Metabolic Perceptron (Hybrid Logic)
Cell-Free System





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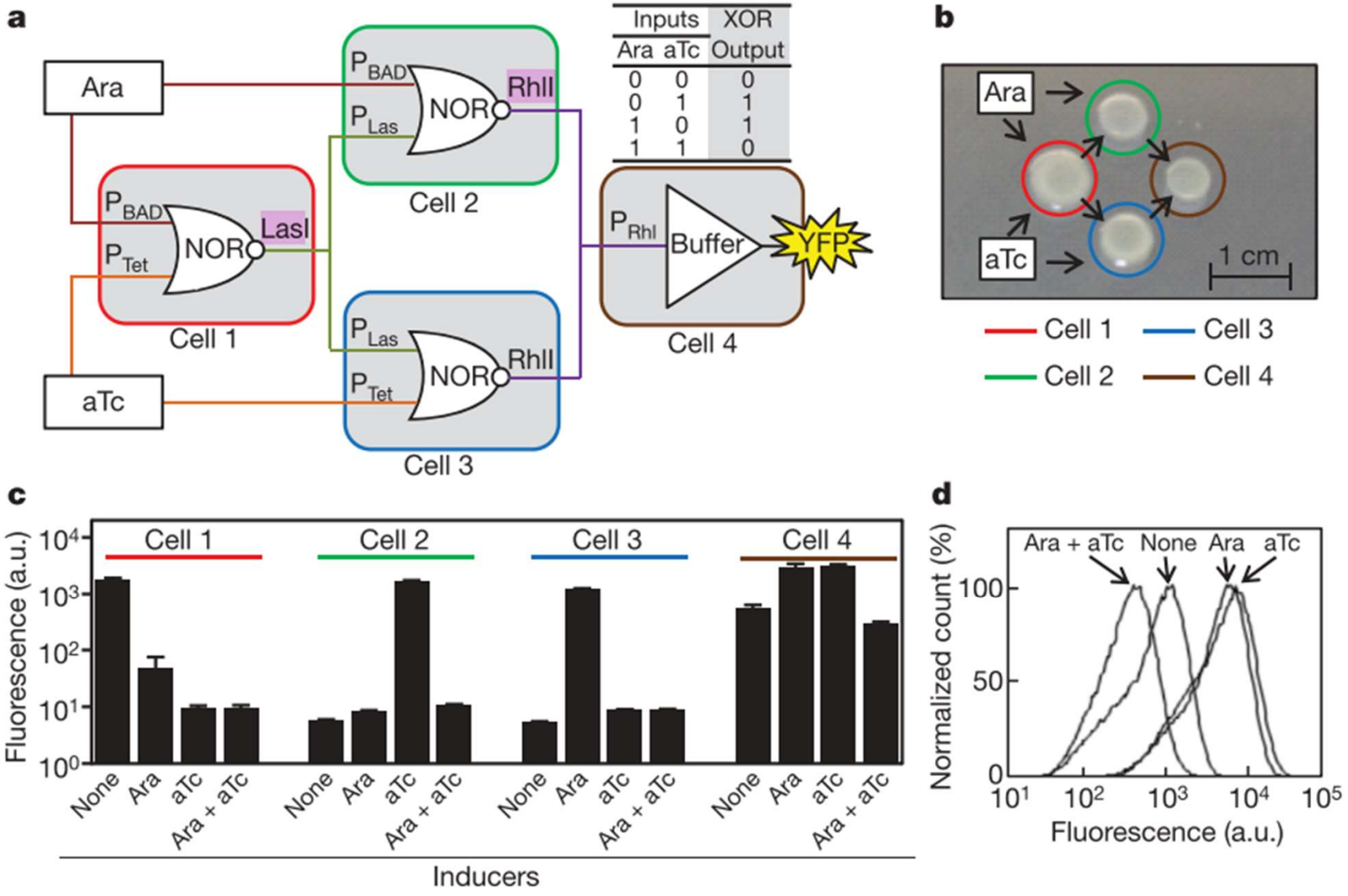
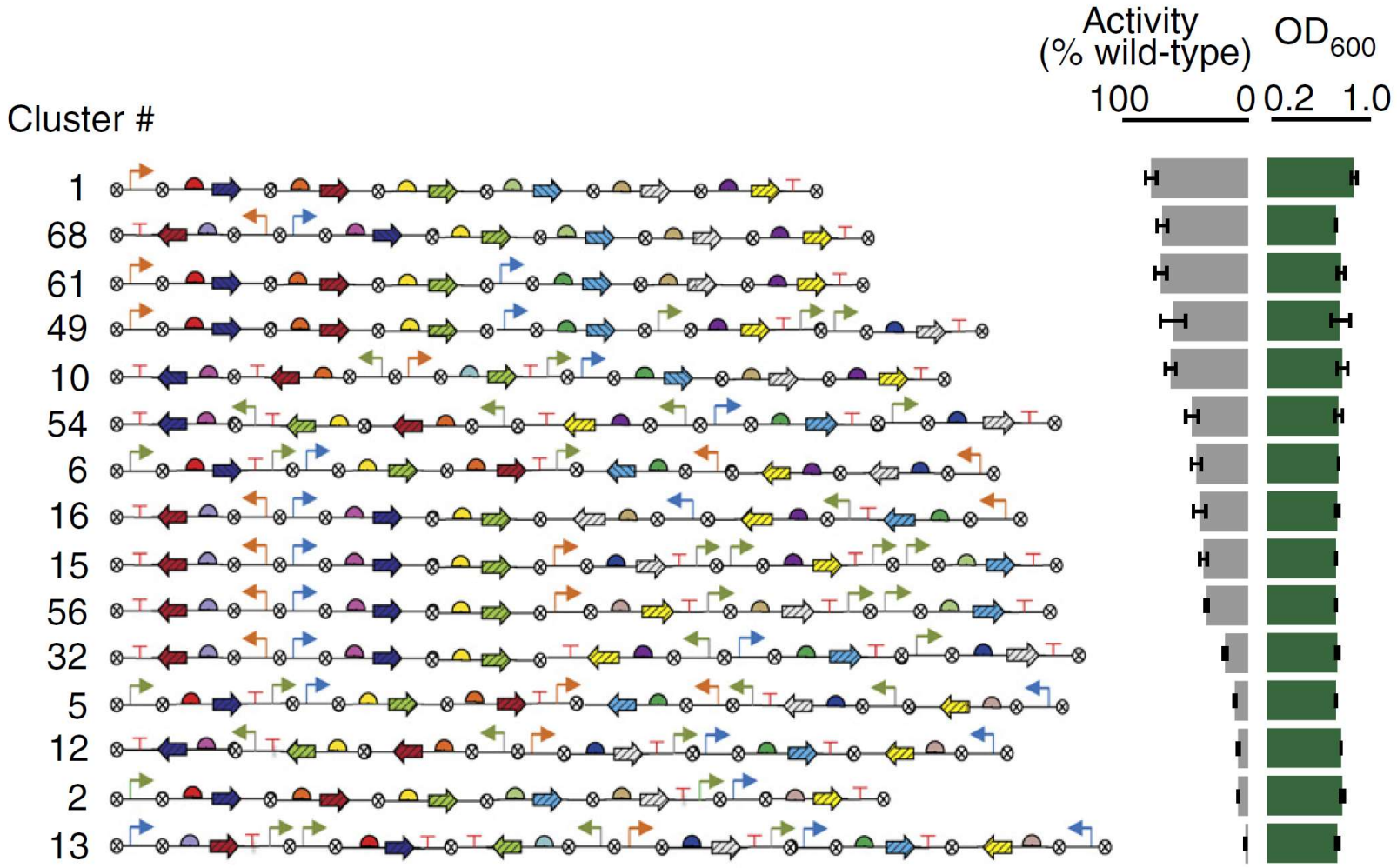
(Pandi & Koch *et al.*, 2019. Nat. Comm.)

External Wires for circuit control



(Tamsir *et al.*, 2011. Nature)

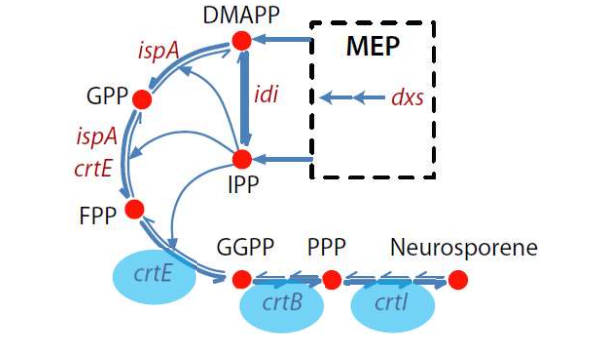
Multiprotein Genetic Systems: Metabolic Pathways



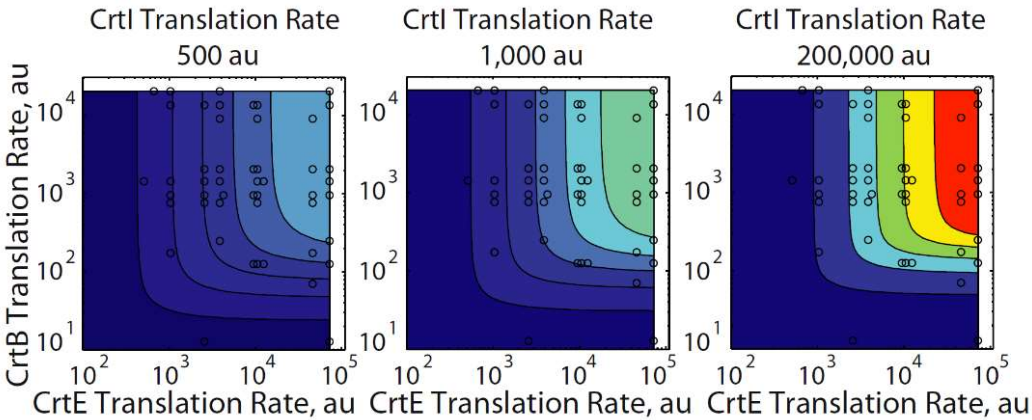
- Refactoring Nitrogen Fixation Cluster

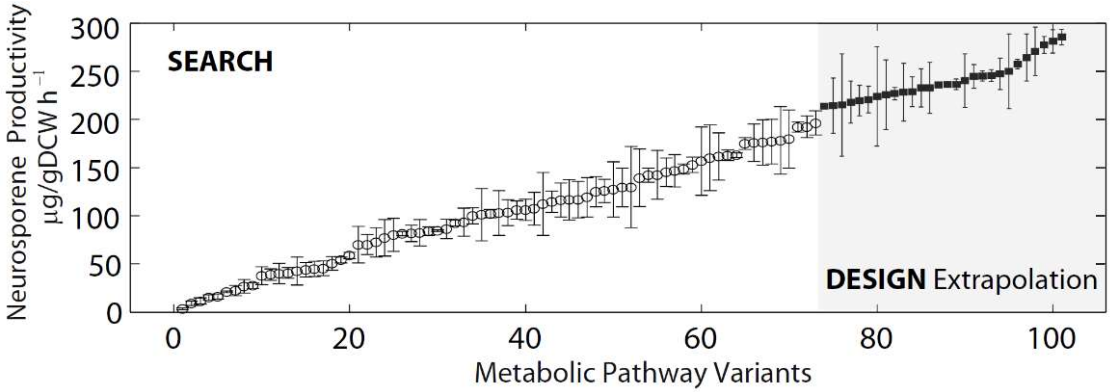
(Smanski *et al.*, 2014. Nat. Biotech.)

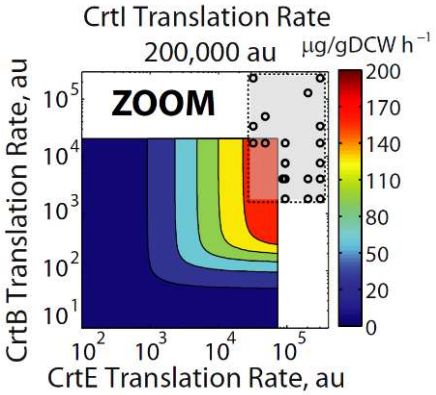
Design of Experiments (DoE) approach in Synthetic Biology



Combinatorial Gibson Assembly







- RBS library engineering allows sampling of a large multi-enzyme expression space with limited number of experiments (73 variants)
- A Kinetic model could then be used to extrapolate (28 variants) pathway design

(Farasat *et al.*, 2014. Mol. Sys. Biol.)

Cello: Automated Design of Biological Circuits

Genetic circuit design automation

Alec A. K. Nielsen,[1] Bryan S. Der,[1,2] Jonghyeon Shin,[1] Prashant Vaidyanathan,[2]
Vanya Paralanov,[3] Elizabeth A. Strychalski,[3] David Ross,[3]
Douglas Densmore,[2] Christopher A. Voigt[1]*

Computation can be performed in living cells by DNA-encoded circuits that process sensory information and control biological functions. Their construction is time-intensive, requiring manual part assembly and balancing of regulator expression. We describe a design environment, Cello, in which a user writes Verilog code that is automatically transformed into a DNA sequence. Algorithms build a circuit diagram, assign and connect gates, and simulate performance. Reliable circuit design requires the insulation of gates from genetic context, so that they function identically when used in different circuits. We used Cello to design 60 circuits for *Escherichia coli* (880,000 base pairs of DNA), for which each DNA sequence was built as predicted by the software with no additional tuning. Of these, 45 circuits performed correctly in every output state (up to 10 regulators and 55 parts), and across all circuits 92% of the output states functioned as predicted. Design automation simplifies the incorporation of genetic circuits into biotechnology projects that require decision-making, control, sensing, or spatial organization.

(Nielsen *et al.*, 2016. Science)

Cello: Automated Design of Biological Circuits

Cello design specification

Sensors

name	low	high	promoter sequence
A	0.003	2.8	AACGATCGTTGGCTGTGTTGACAATT
B	0.001	4.4	TACTCCACCGTTGGCTTTTTTCCCTA
C	0.008	2.5	ACTTTTCATACTCCCGCCATTCAGAG

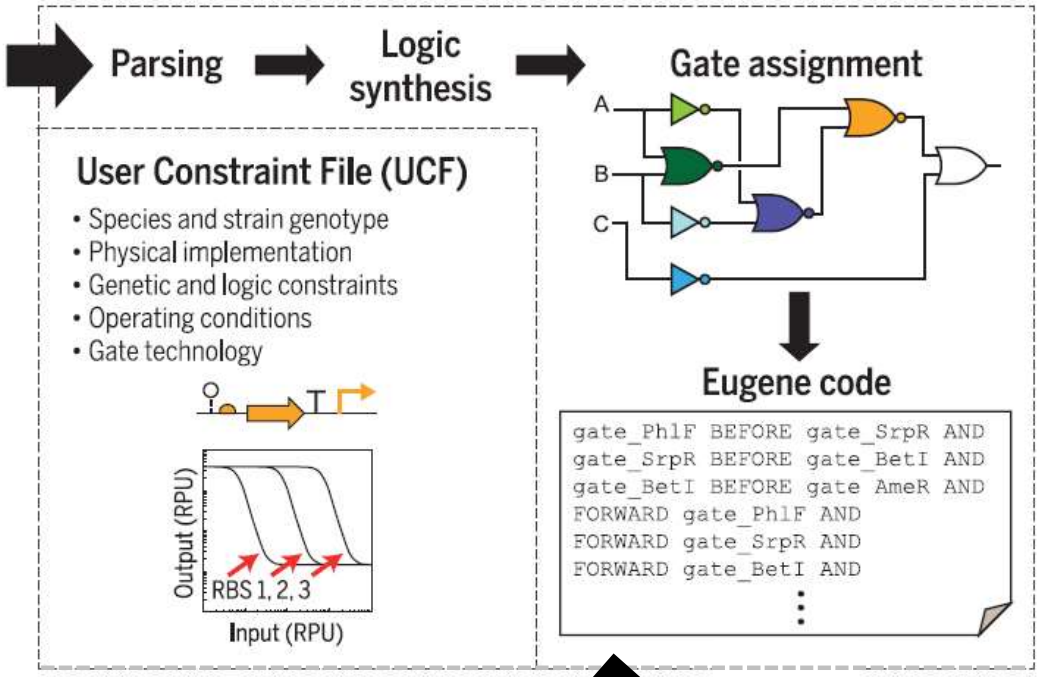
Verilog

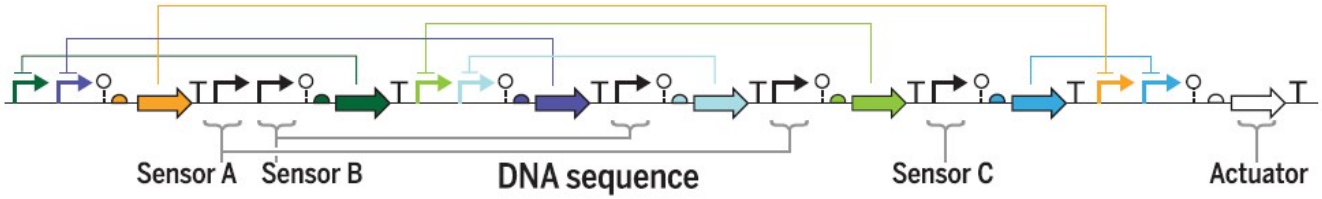
```
module 0xF6(output out, input A,B,C);

always@(C,B,A)
   begin
      case({C,B,A})
        3'b000: {out} = 1'b1;
        3'b001: {out} = 1'b1;
        3'b010: {out} = 1'b1;
        3'b011: {out} = 1'b1;
        3'b100: {out} = 1'b0;
        3'b101: {out} = 1'b1;
        3'b110: {out} = 1'b1;
        3'b111: {out} = 1'b0;
      endcase
   end
endmodule
```

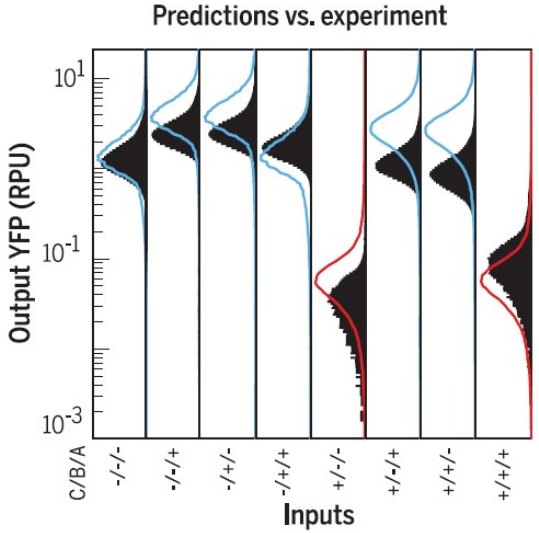
Actuators

name	sequence
YFP	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGT

Run

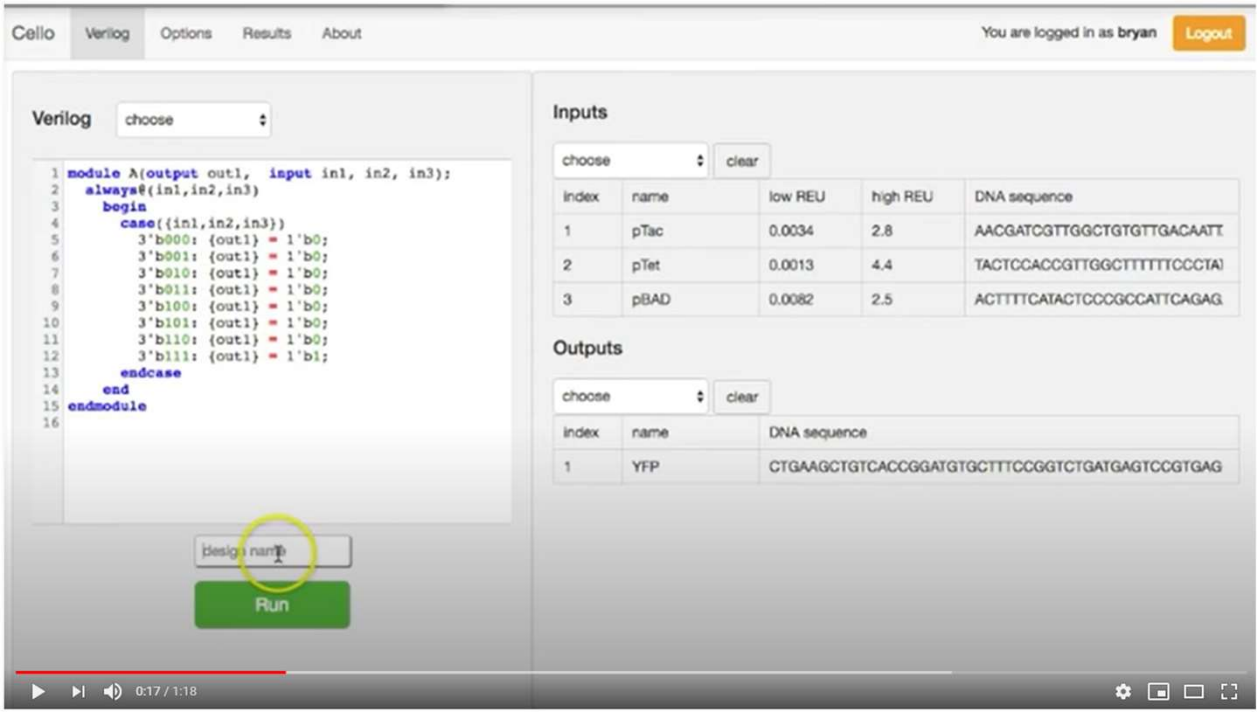






(Nielsen *et al.*, 2016. Science)

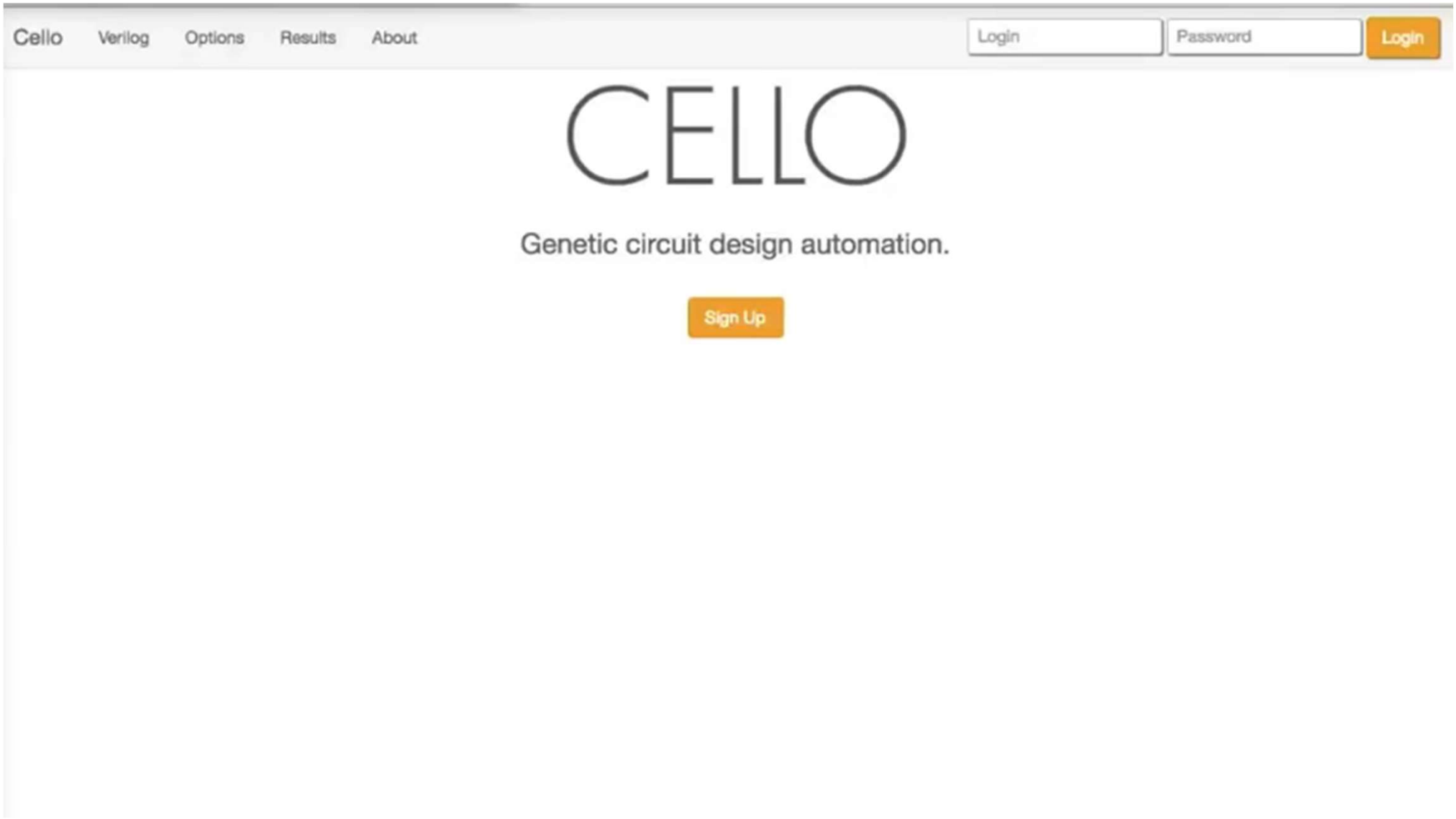
Cello: Automated Design of Biological Circuits



- Youtube (Cello demo): https://www.youtube.com/watch?v=SLn_SkL7vkQ
- http://www.cellocad.org/ (Voigt Lab, MIT)
- Youtube (Programming Living Bacteria, by Voigt): https://www.youtube.com/watch?v=lNttxYdGHs4
- Takes in Verilog specification of circuit design
- Takes more specifications: organism type, gate technology, output type
- Calculates genetic design, using data from previously characterised parts and outputs DNA sequence

(Nielsen *et al.*, 2016. Science)

Cello: Automated Design of Biological Circuits



- Youtube (Cello demo): https://www.youtube.com/watch?v=SLn_SkL7vkQ

(Nielsen *et al.*, 2016. Science)

Questions welcome.

manish.kushwaha@inrae.fr