SCIE5508
Synthetic Biology – Solving global challenges

Lecture 3 – Designing synthetic circuits I: Theoretical concepts

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We are learning on Noongar land

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Designing synthetic circuits I – Theoretical concepts

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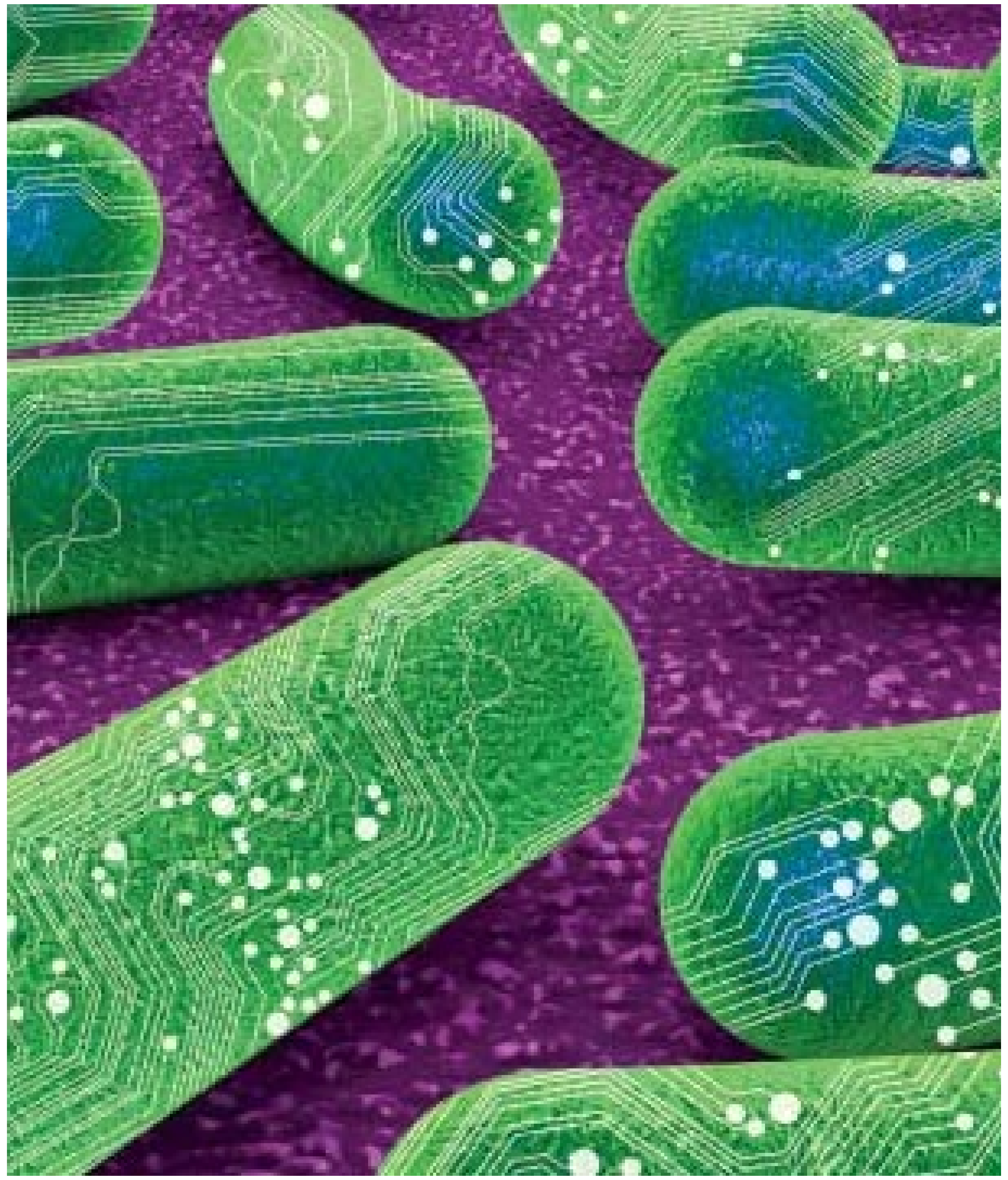
Learning outcomes

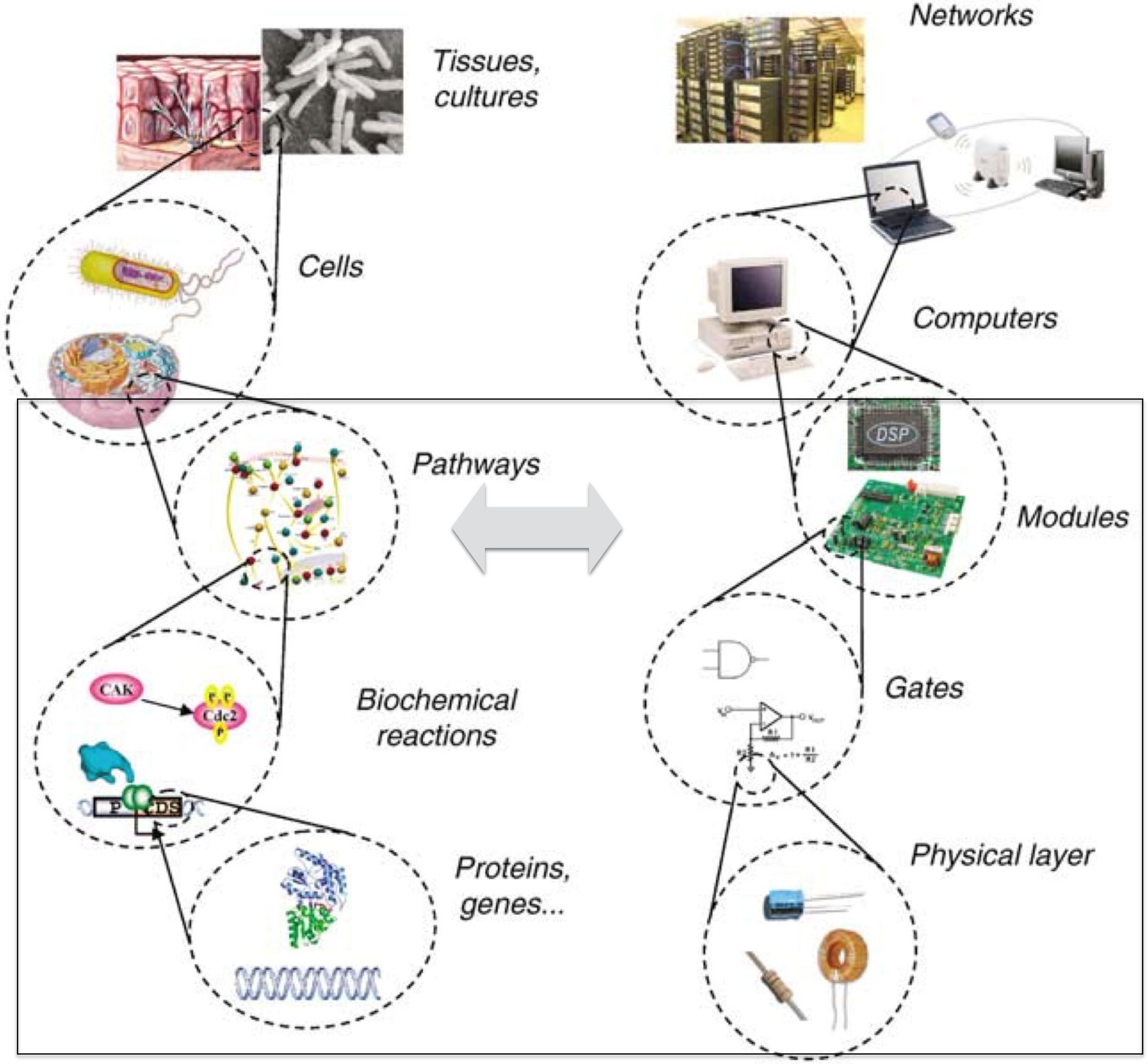
After this lecture, students should understand and be able to describe:

- The Synthetic Biology Open Language as a standard to define biological designs
- How promoter logic is used to build information-processing logic gates and multi-layered circuits
- The role of feedback in regulatory circuits for the emergence of complex behavior, such as memory and oscillatory patterns
- Some of the limitations of genetic circuit design, and how to overcome them



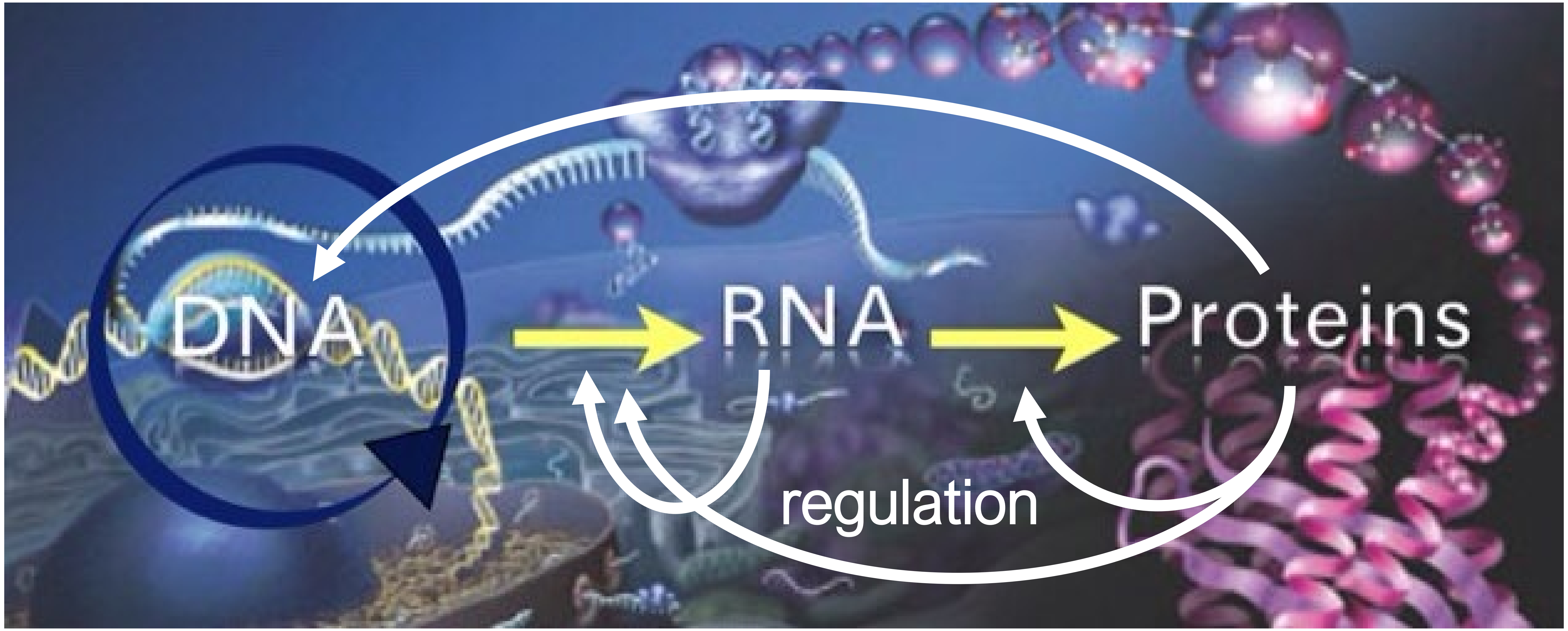
Recap:
Key engineering principles in Synthetic Biology

- Abstraction
- Modularity
- Standardisation
- Modelling & design



Andrianantoandro et al., Mol Syst Biol, 2006

Abstraction of the (extended) central dogma of molecular biology



Here: Focus on bacterial transcriptional regulation via transcription factors

The Synthetic Biology Open Language (SBOL)

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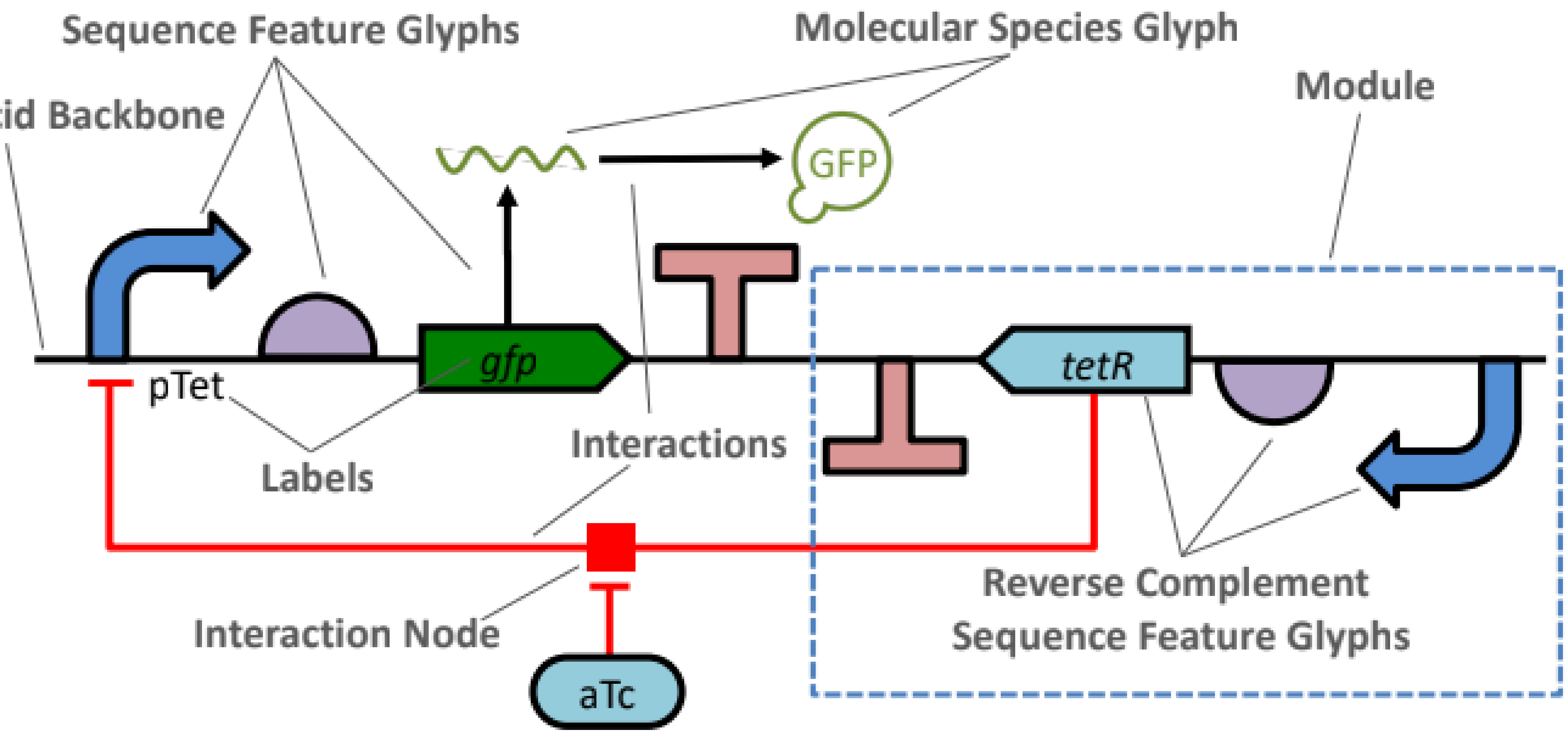
- SBOL is an **open standard** for the representation of *in silico* biological designs.
- Provides schematic glyphs to graphically depict genetic designs called **SBOL Visual**.
- Standardises data used by synthetic biology practitioners, from users to software developers to wet lab biologists

https://sbolstandard.org



Grey text and lines (including this) are annotations

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The Synthetic Biology Open Language (SBOL)

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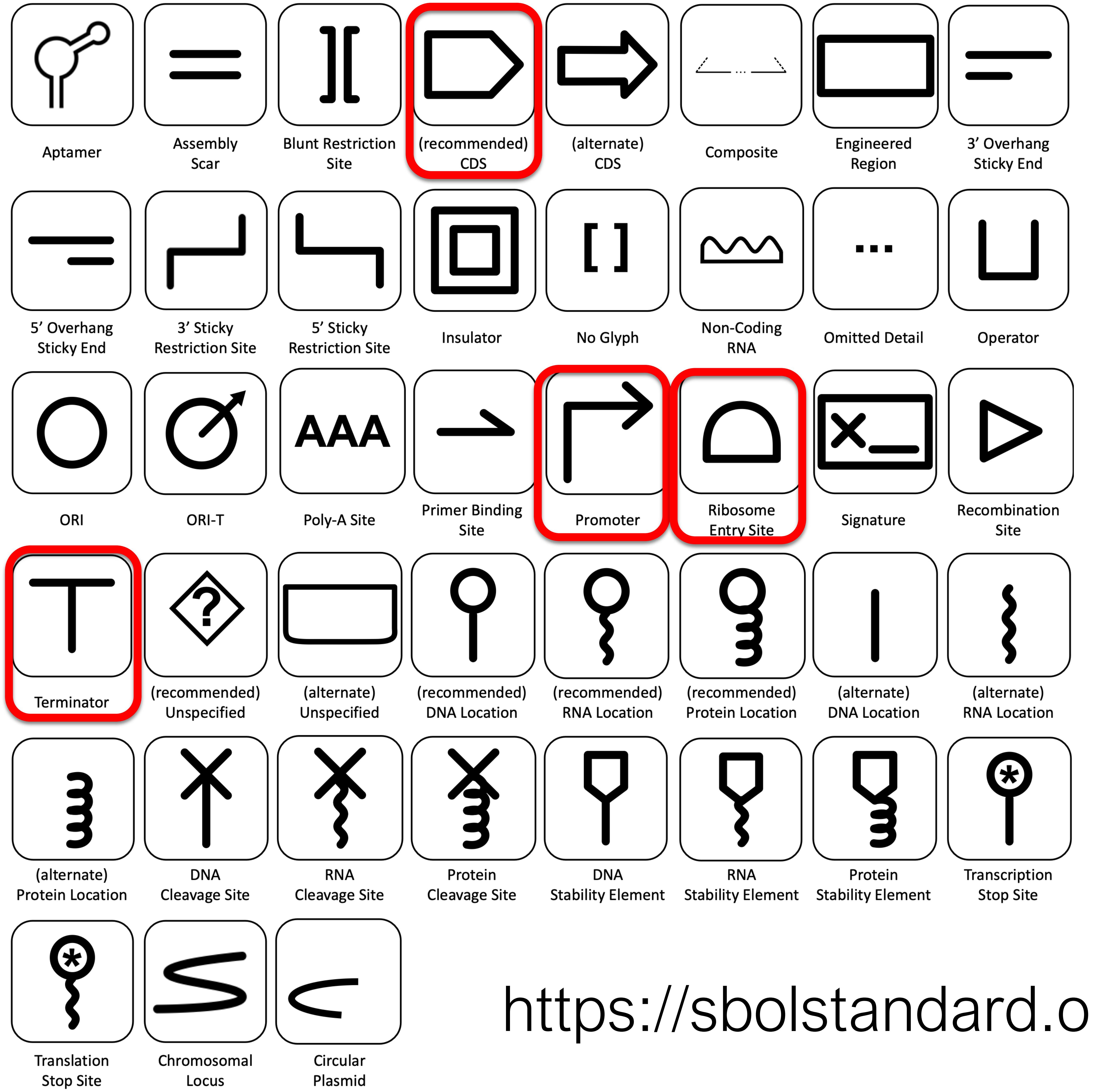
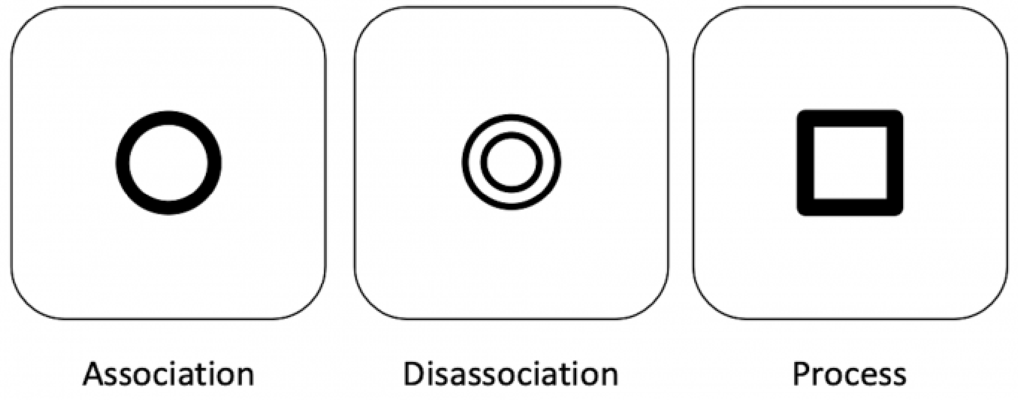
Sequence Feature Glyphs

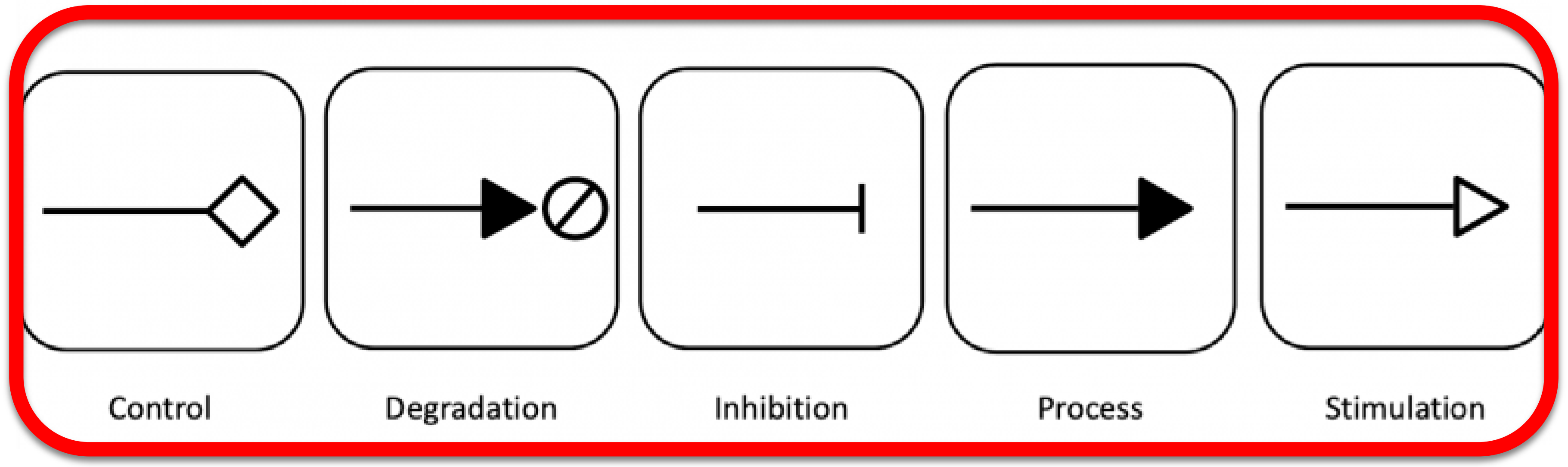


Interaction Node Glyphs

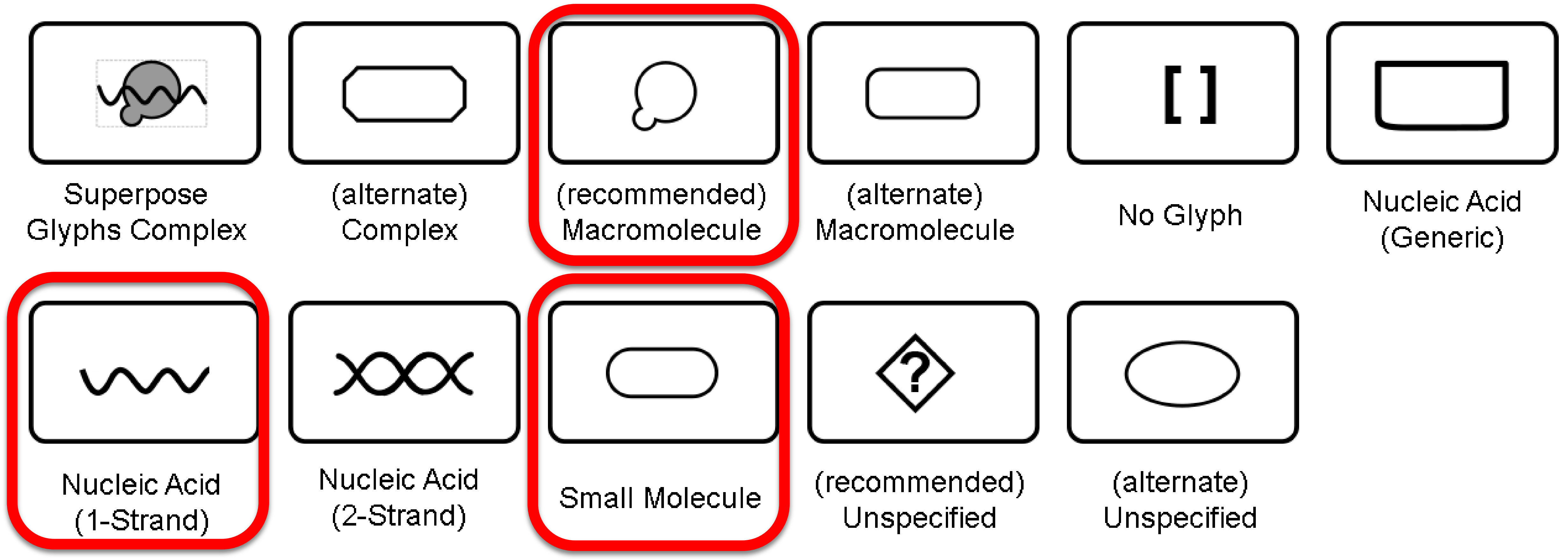


Red = frequently used glyphs

Interaction Glyphs



Molecular Species Glyphs



https://sbolstandard.org

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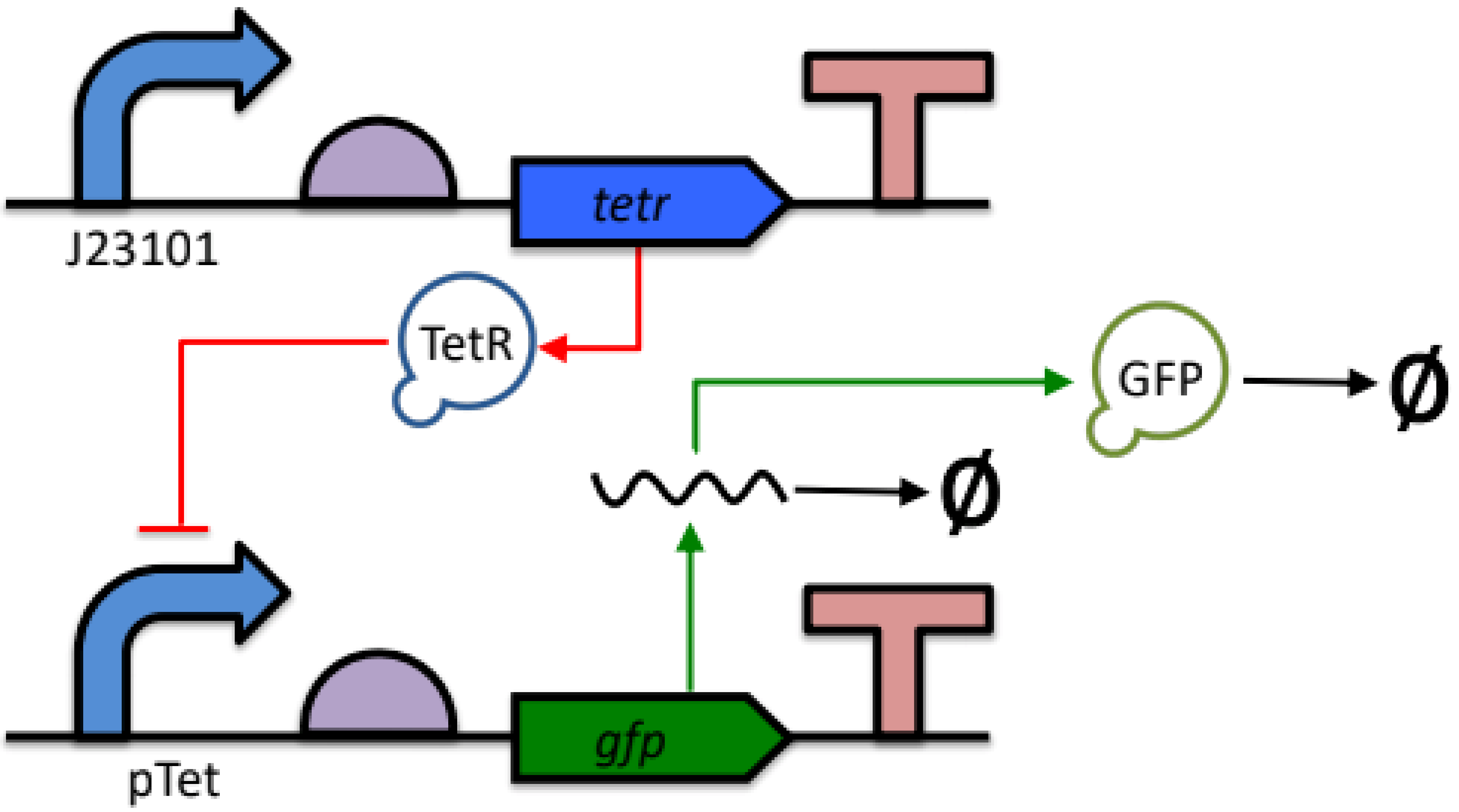
Complex example:



The top functional unit produces the TetR protein constitutively, under control of promoter J23101. TetR represses the pTet promoter, which is regulating production of GFP. The diagram of GFP production explicitly includes the intermediate mRNA and the degradation of both the mRNA and protein products.

https://sbolstandard.org

The Synthetic Biology Open Language (SBOL)

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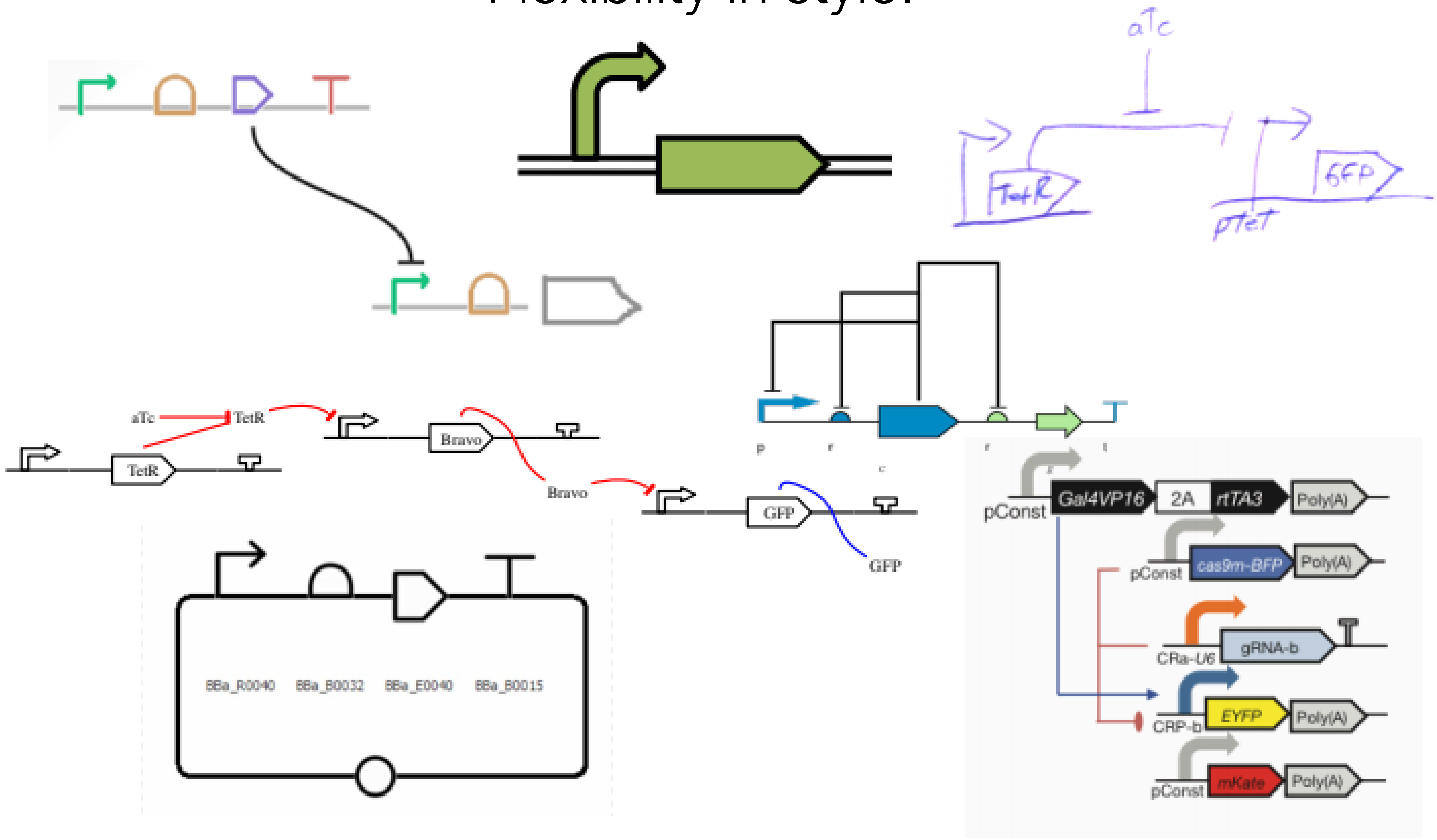
Flexibility in style:



Color, Text, Scaling, Strands, Styling: all your choice

What you know:
Bacterial transcription regulation via "regulated recruitment" of RNA polymerase

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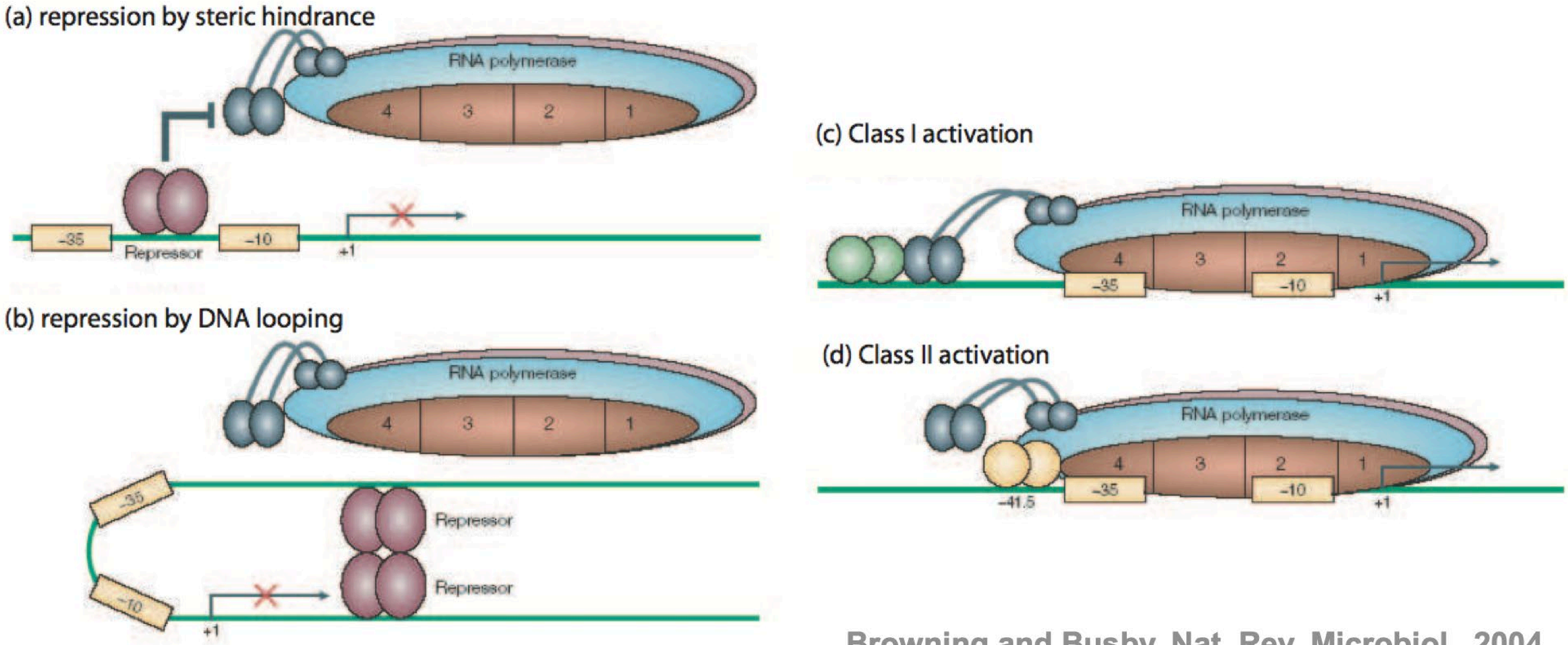
Transcription factors (TF) modify local concentration of RNA polymerase (RNAP), modulating the rate of transcription initiation



Browning and Busby, Nat. Rev. Microbiol., 2004

Promoters = logic gates of genetic circuits

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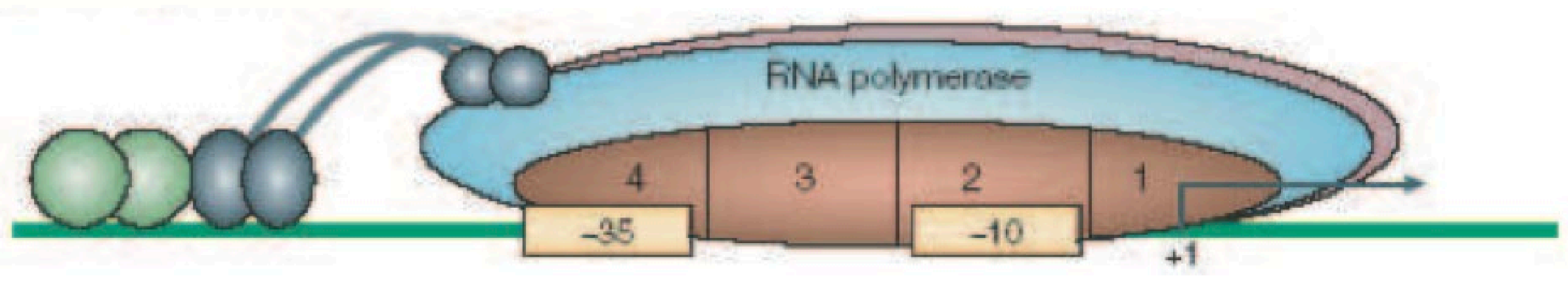
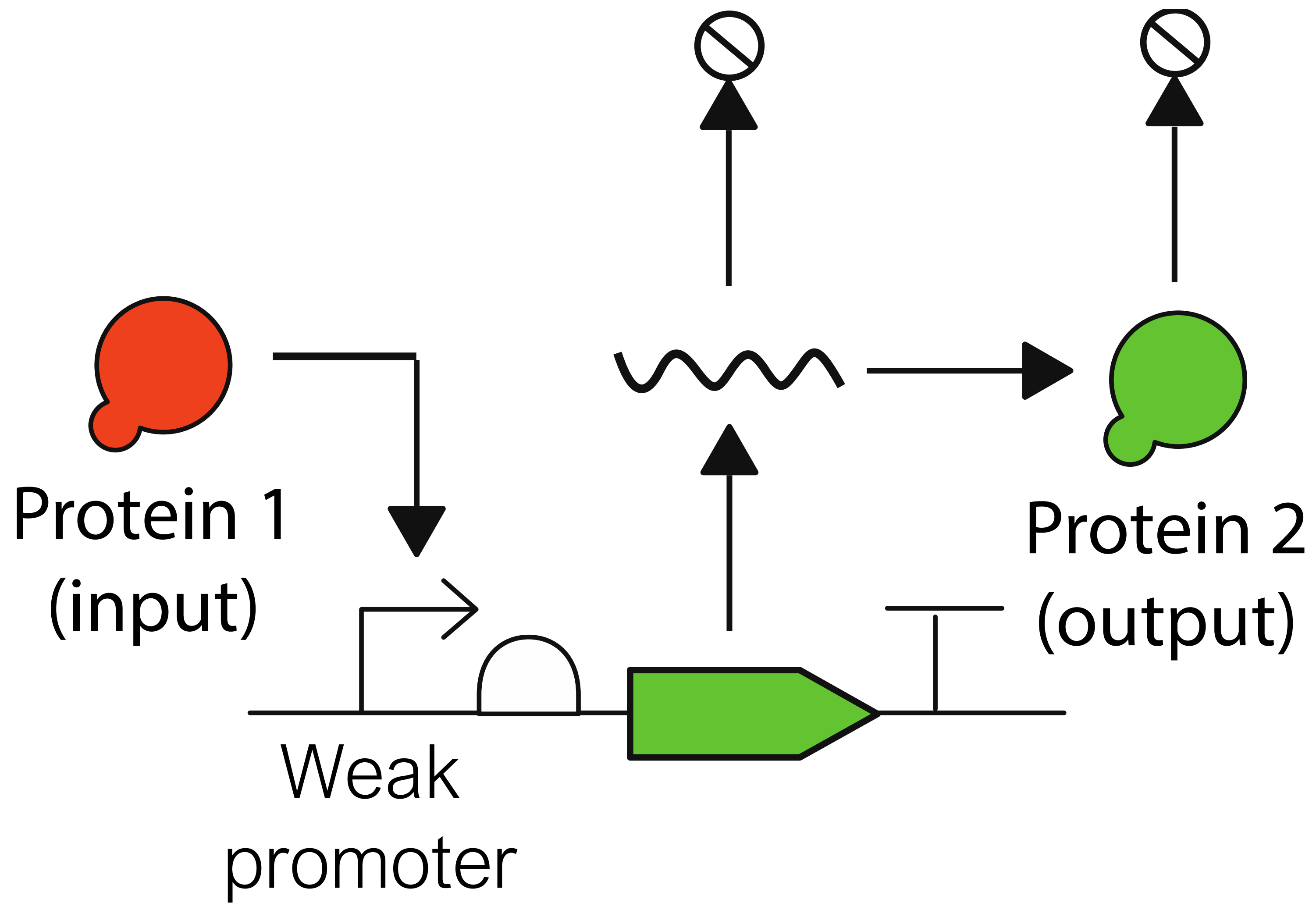
Transcriptional activation

(c) Class I activation



Promoters = logic gates of genetic circuits

Transcriptional activation



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Promoters = logic gates of genetic circuits

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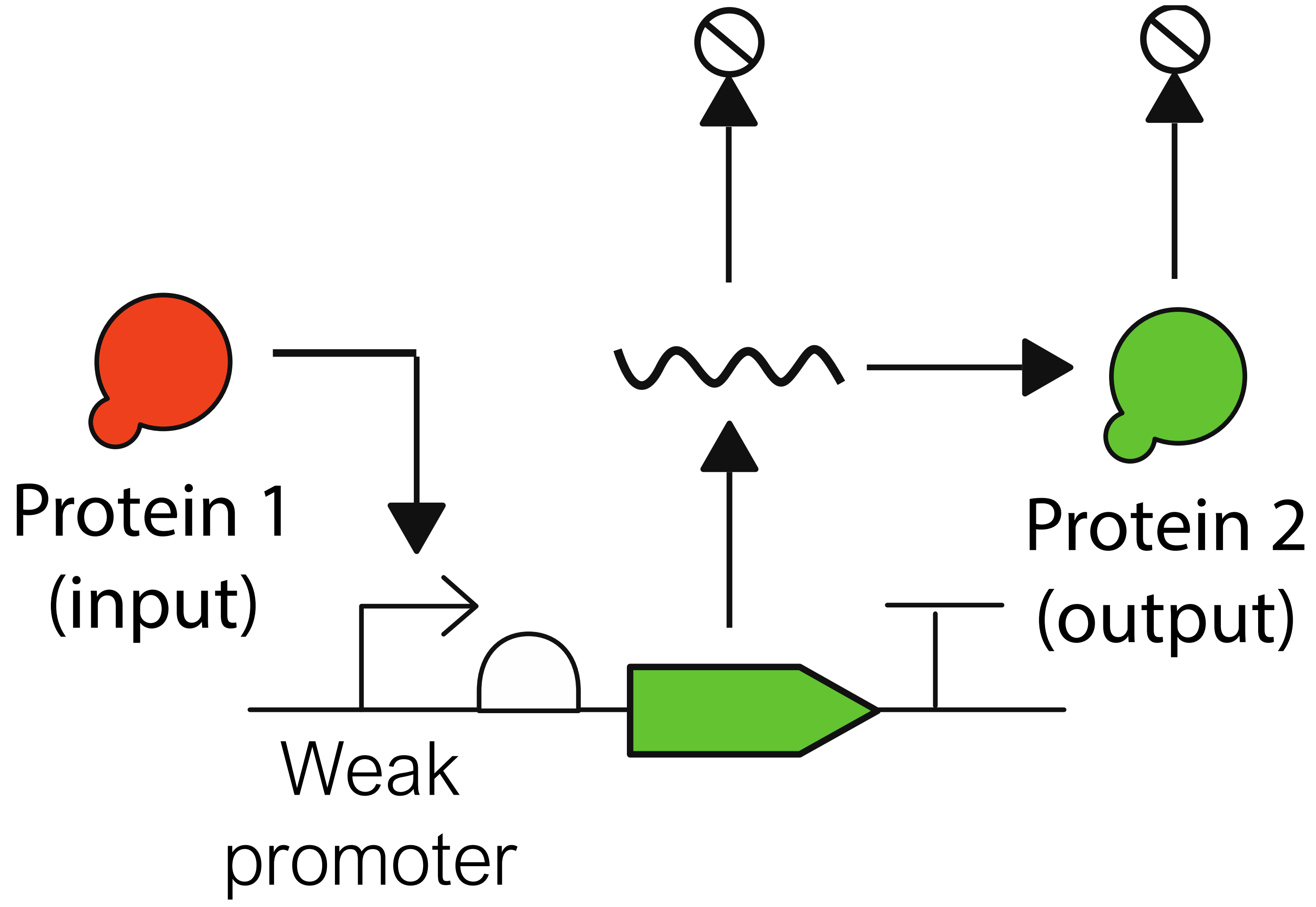
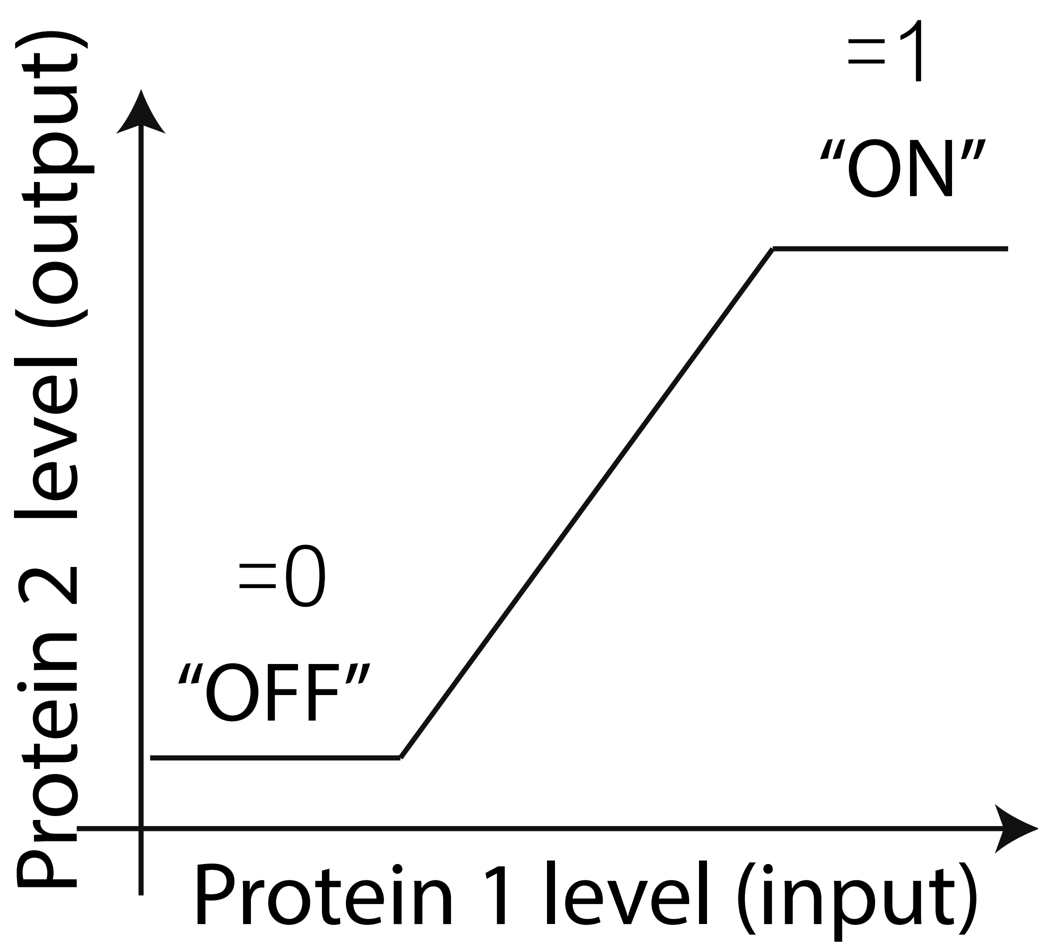
Transcriptional activation



Dose-response curve
(=transfer-function)



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Promoters = logic gates of genetic circuits

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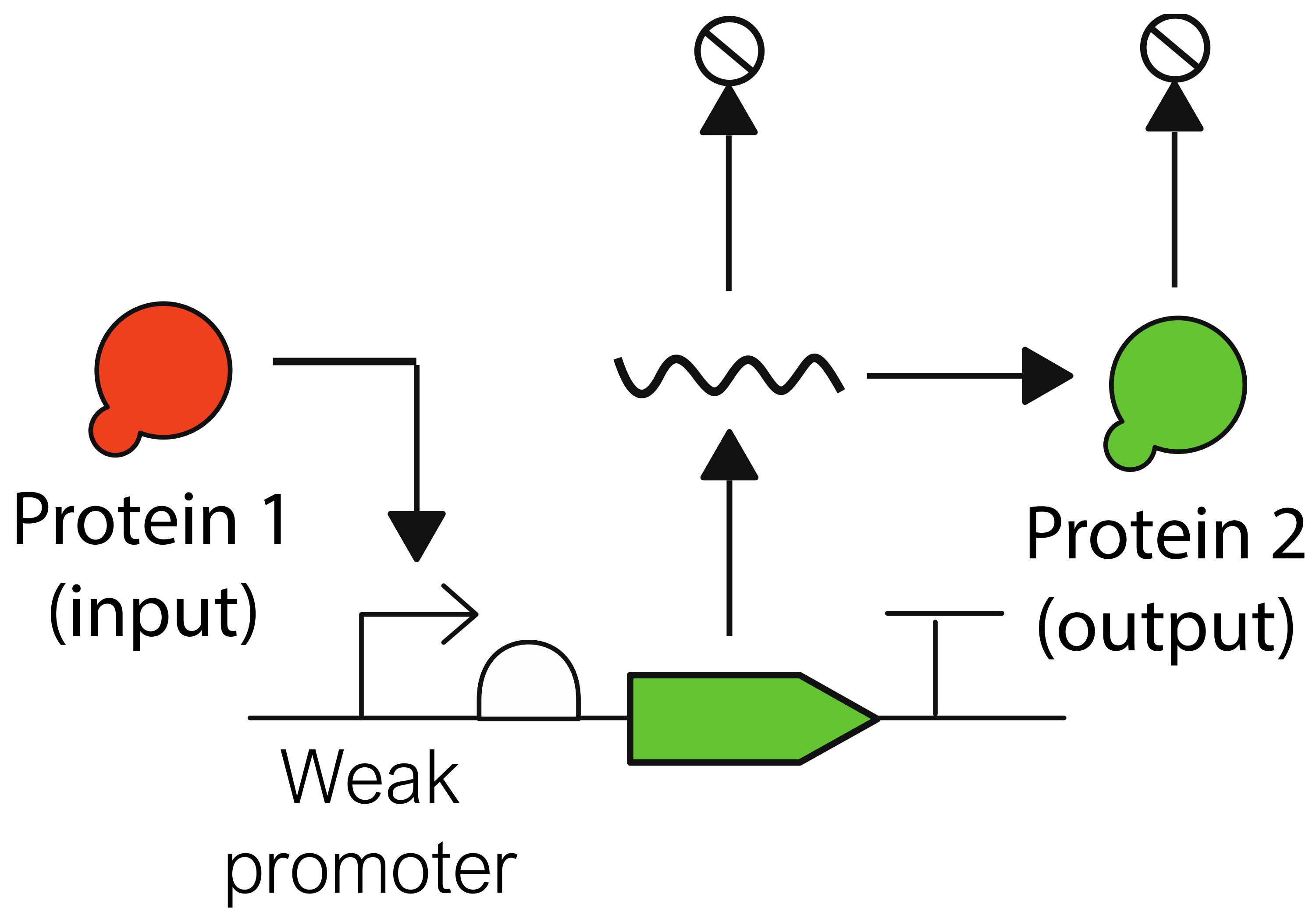
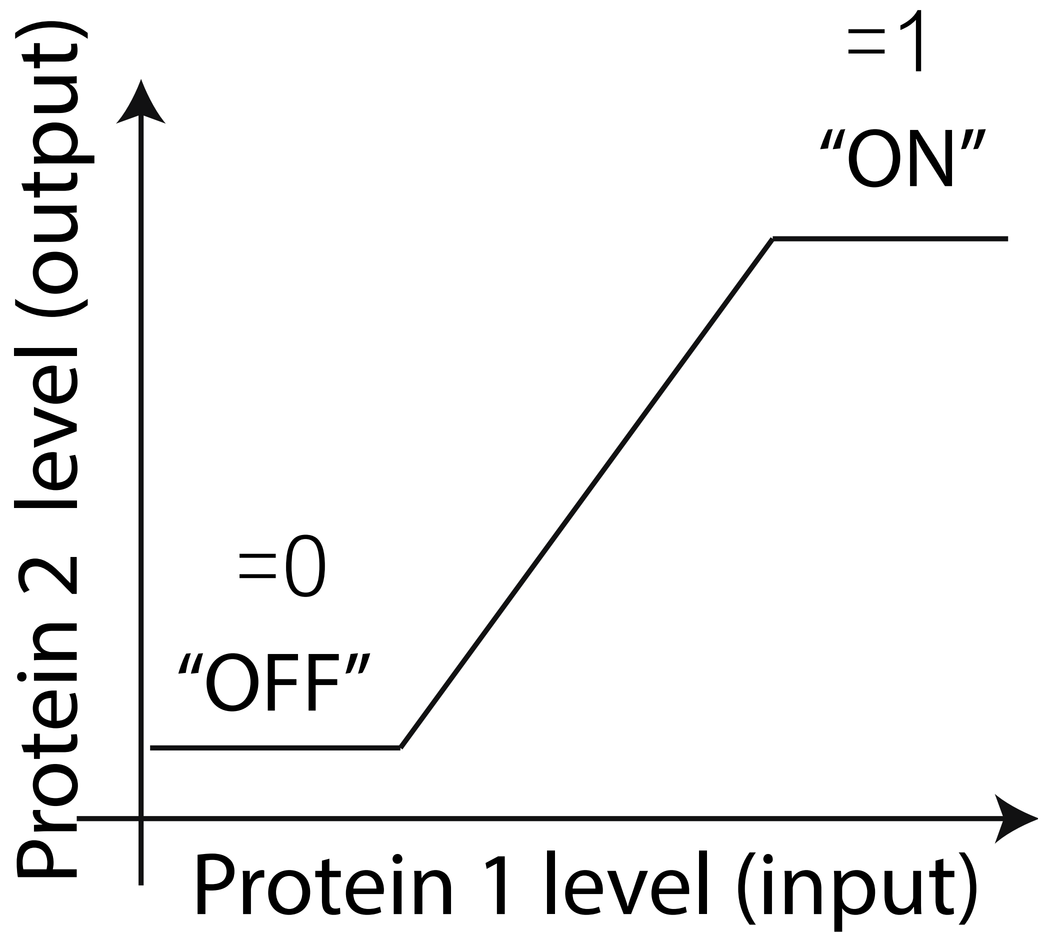
Transcriptional activation

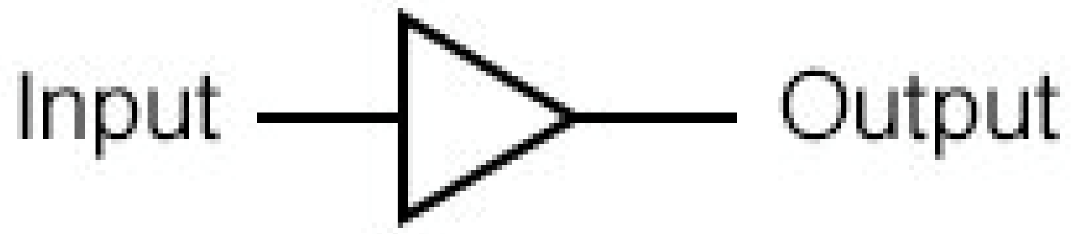


Dose-response curve (=transfer-function)



Logic gate

"Buffer" gate



Input	Output
0	0
1	1

Truth table

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Promoters = logic gates of genetic circuits

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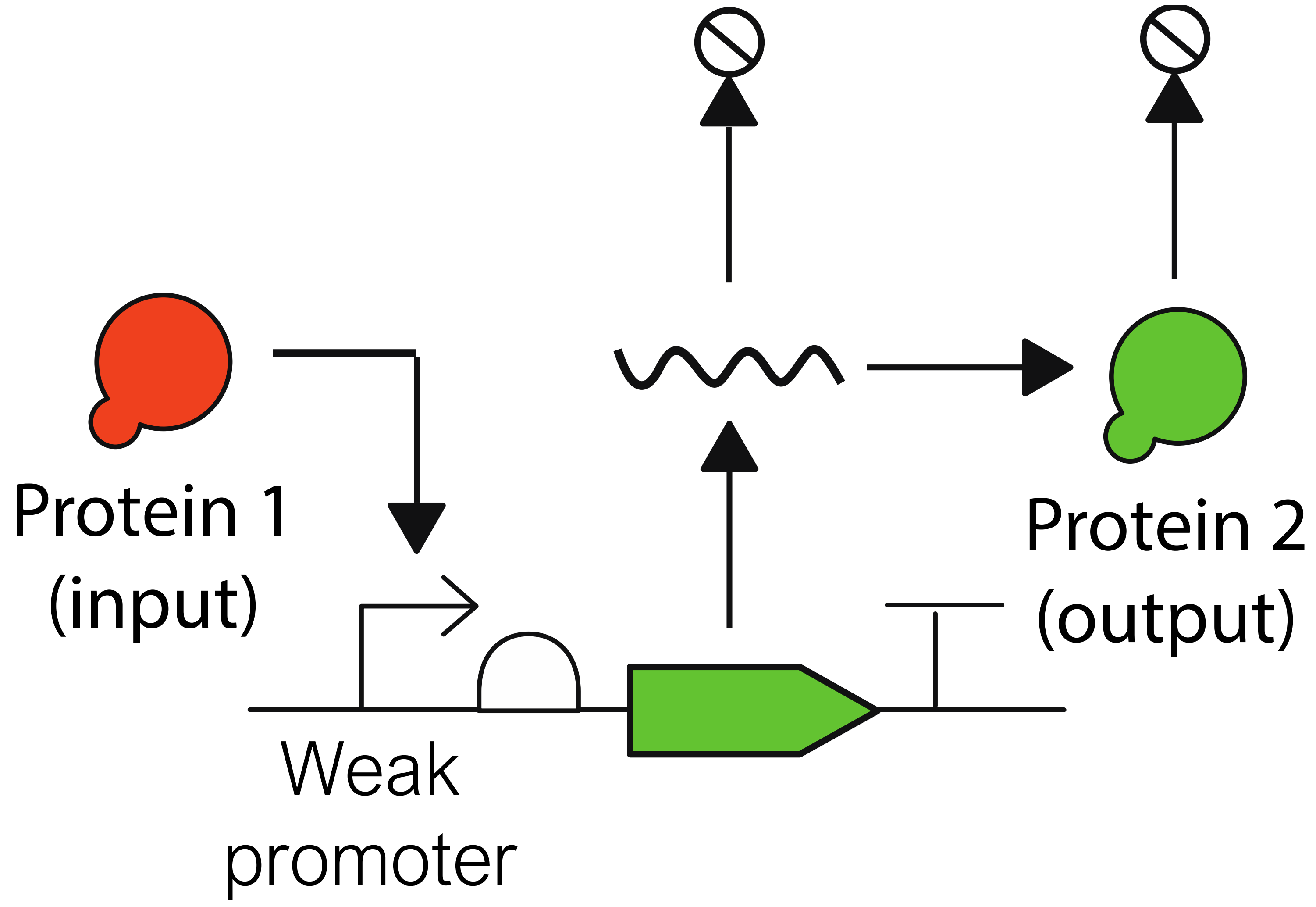
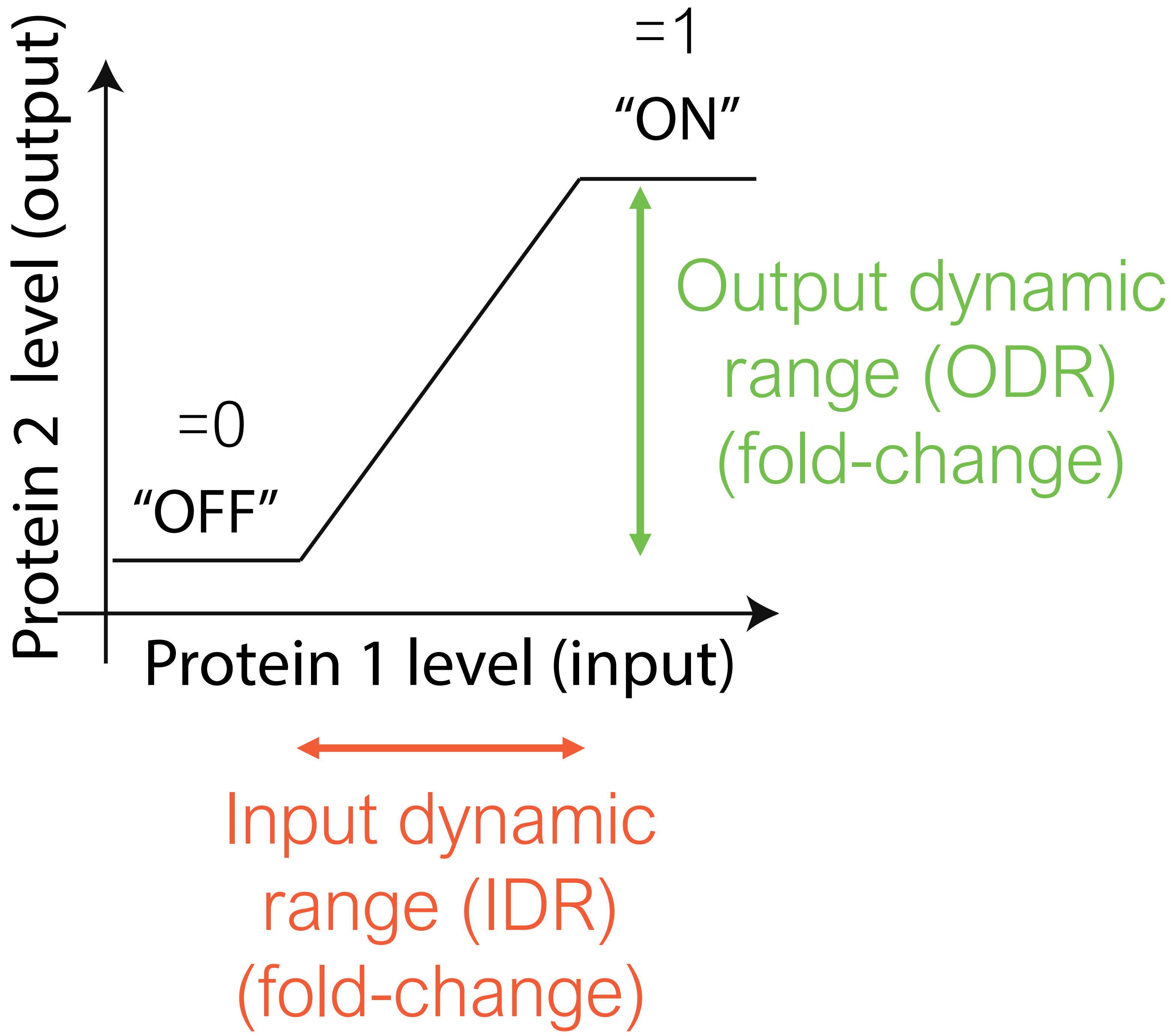
Transcriptional activation



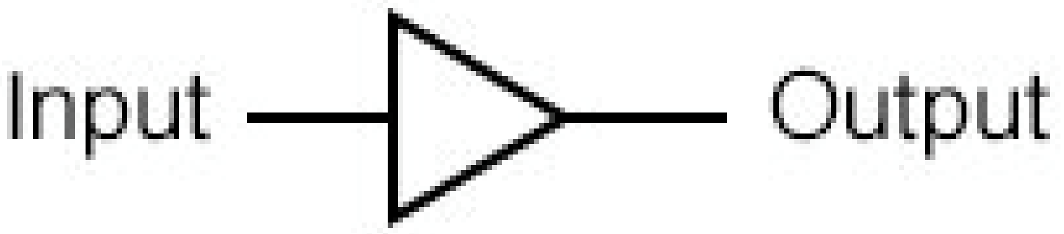
Dose-response curve (=transfer-function)



Signal **amplification**, if ODR>IDR
Signal **dampening**, if ODR<IDR

Logic gate

"Buffer" gate



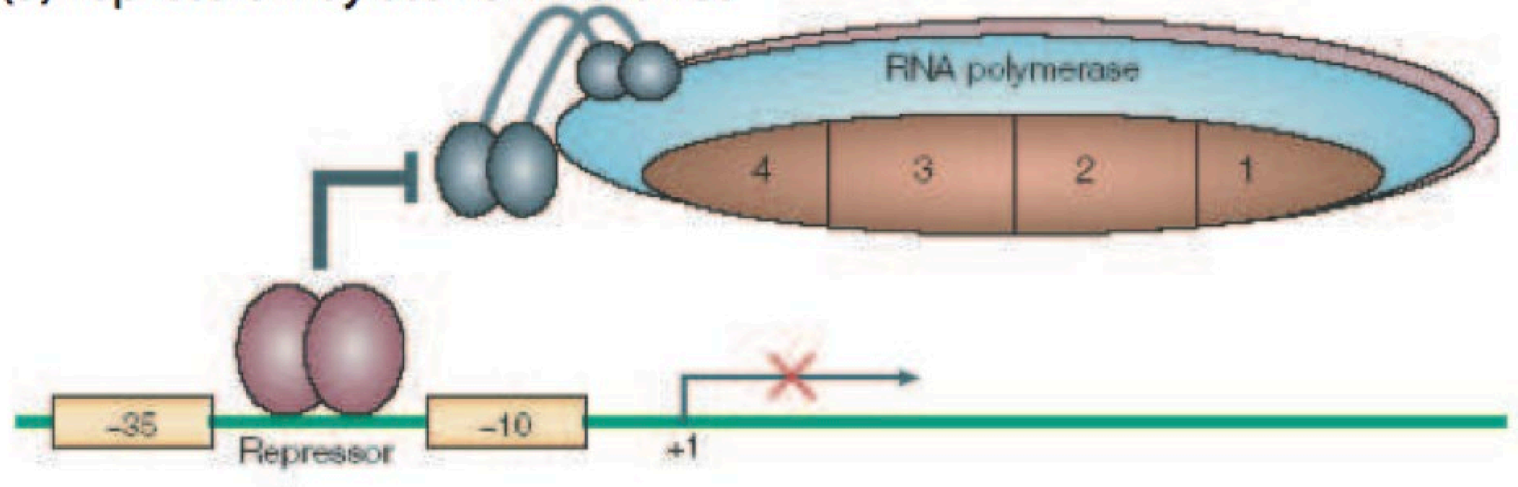
Input	Output
0	0
1	1

Truth table

Promoters = logic gates of genetic circuits

Transcriptional repression

(a) repression by steric hindrance



Promoters = logic gates of genetic circuits

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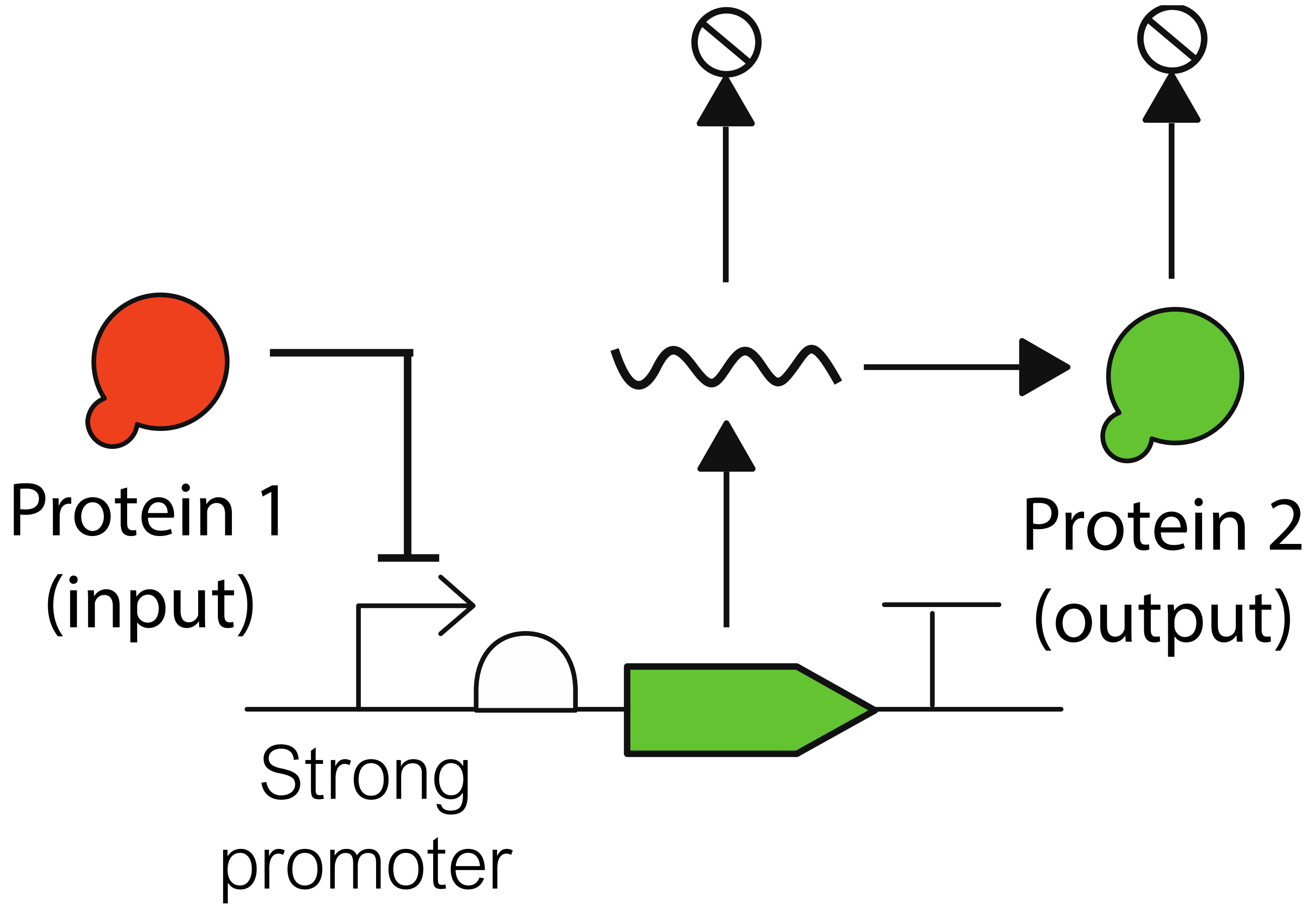
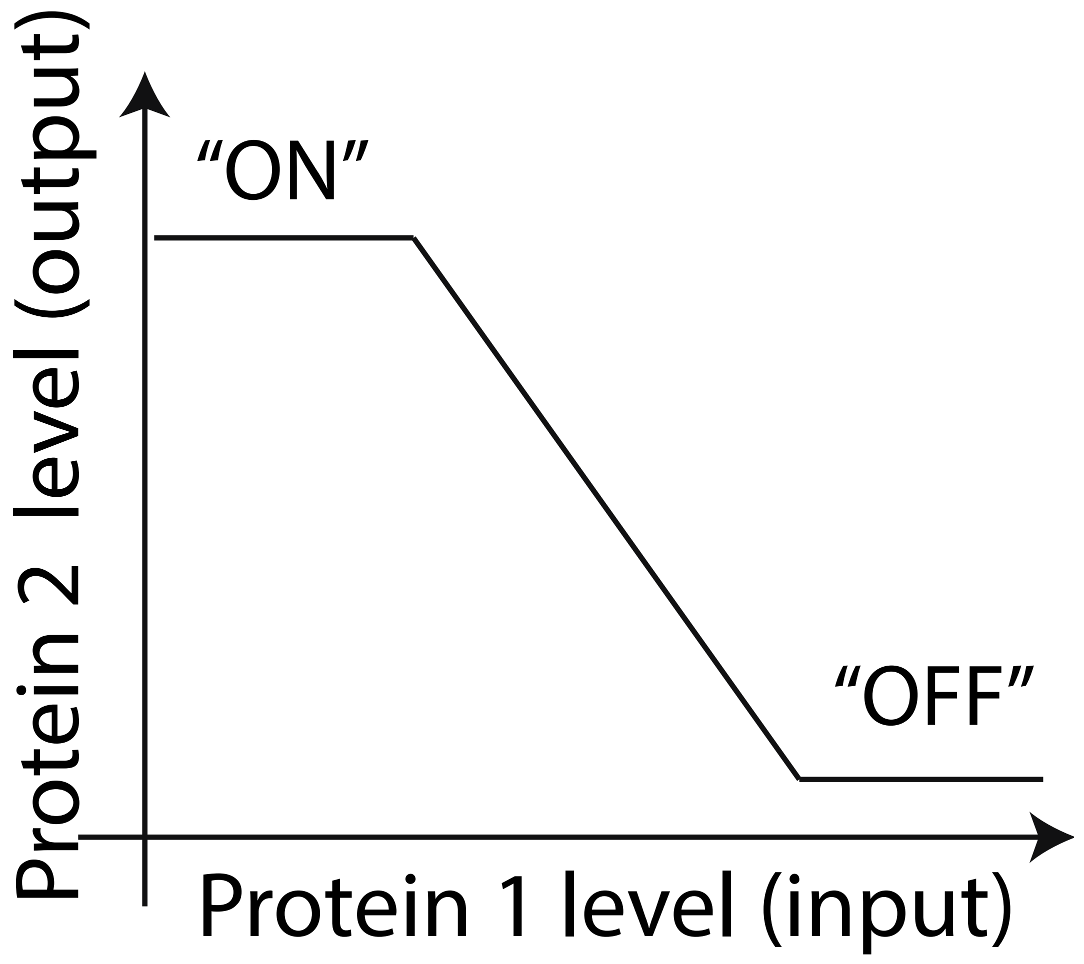
Transcriptional repression



Dose-response curve
(=transfer-function)



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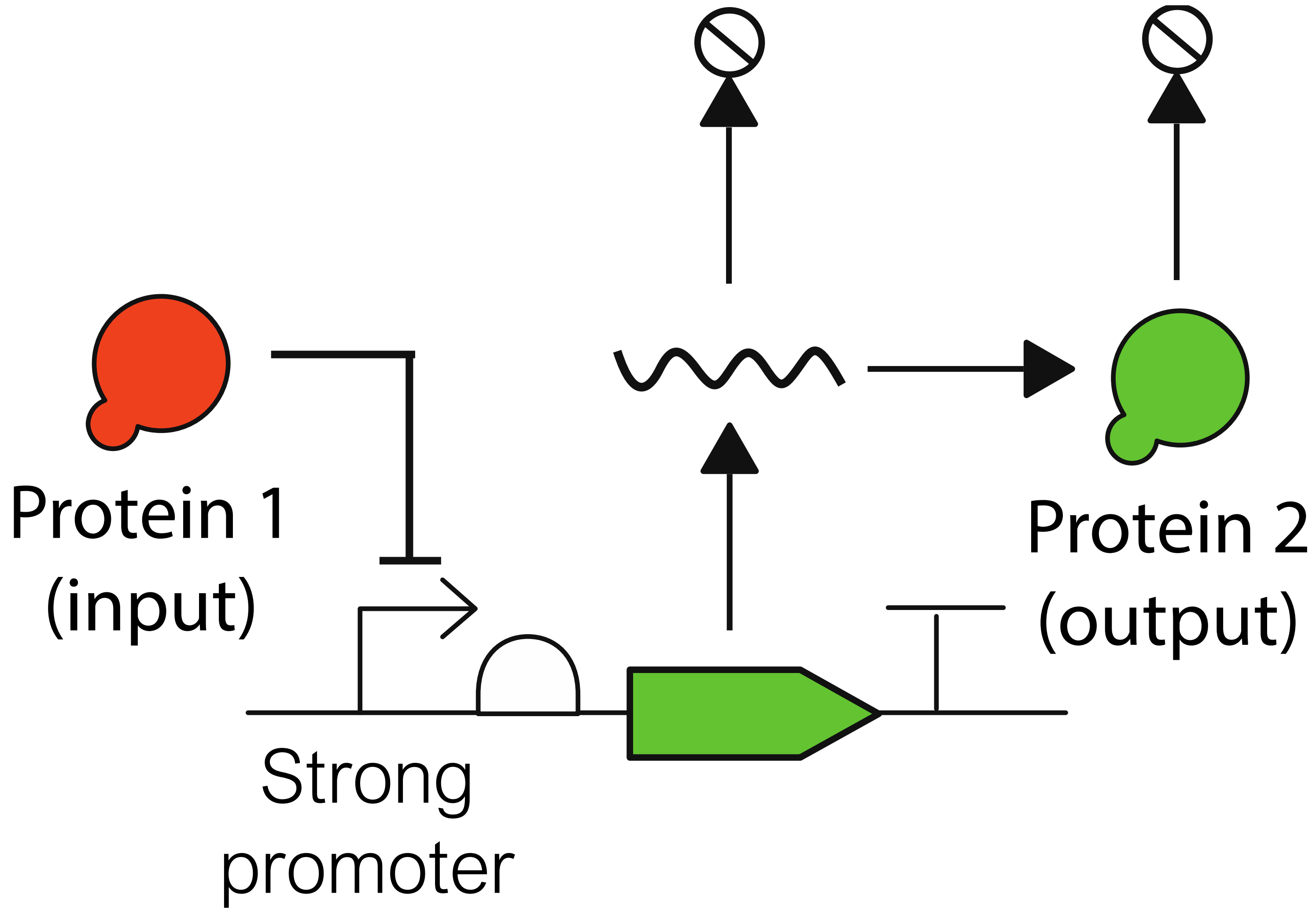
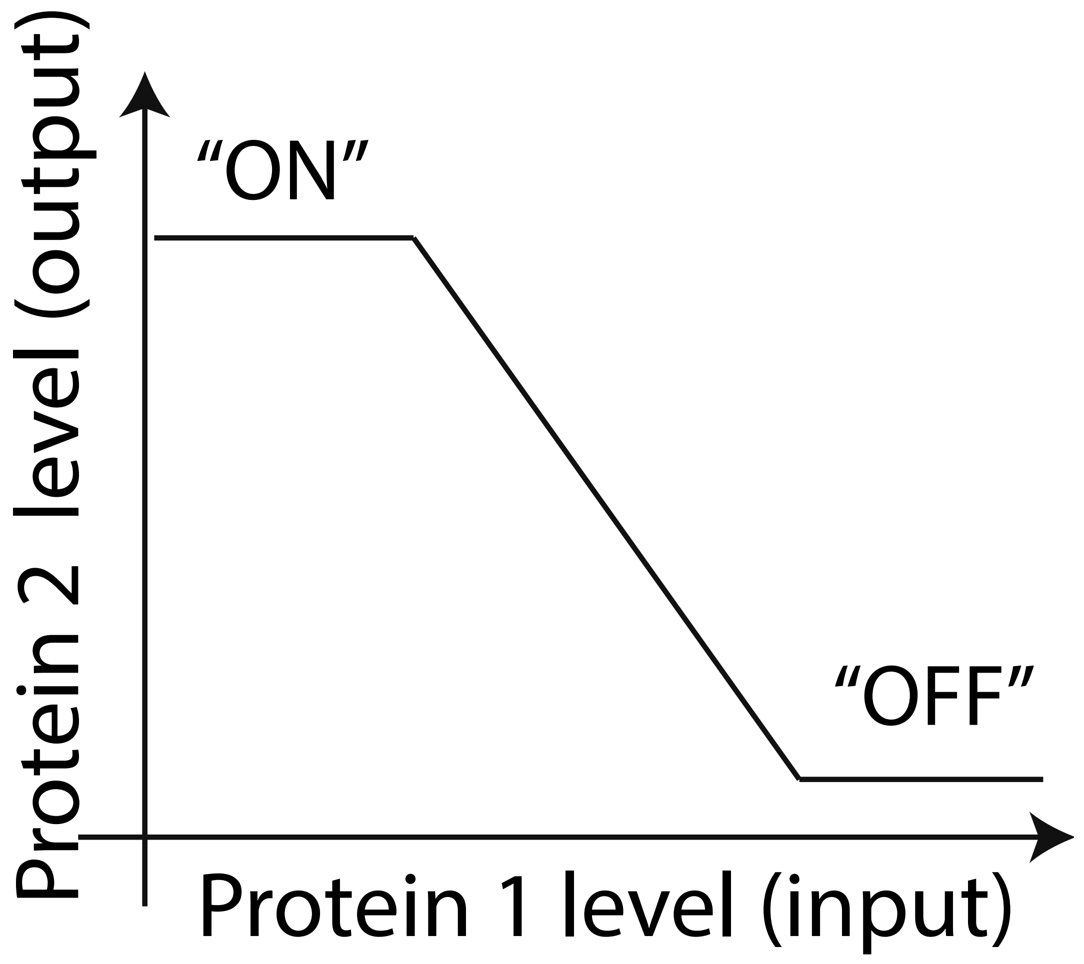
Transcriptional repression

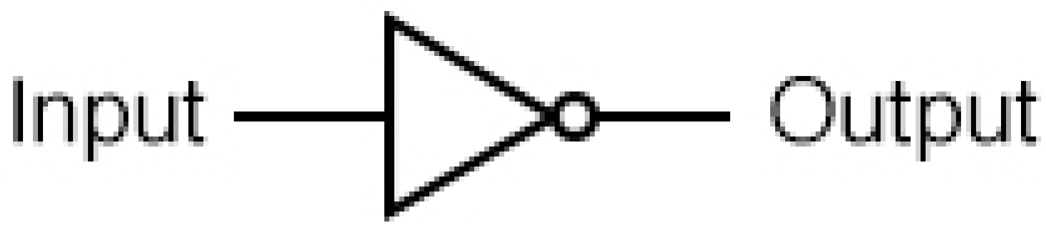


Dose-response curve
(=transfer-function)



Logic gate

Inverter, or NOT gate

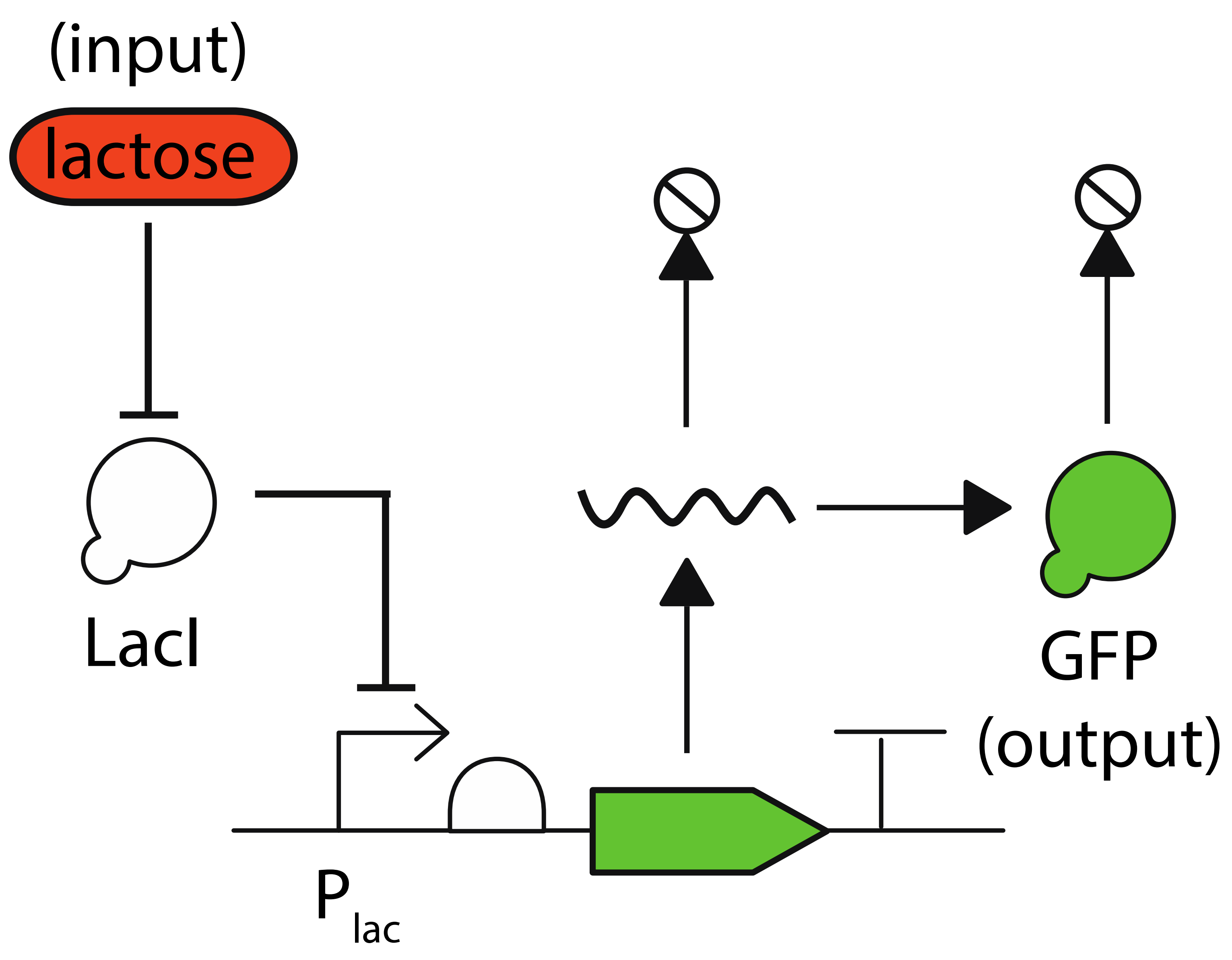


Input	Output
0	1
1	0

Truth table

Promoters = logic gates of genetic circuits

Double-repression



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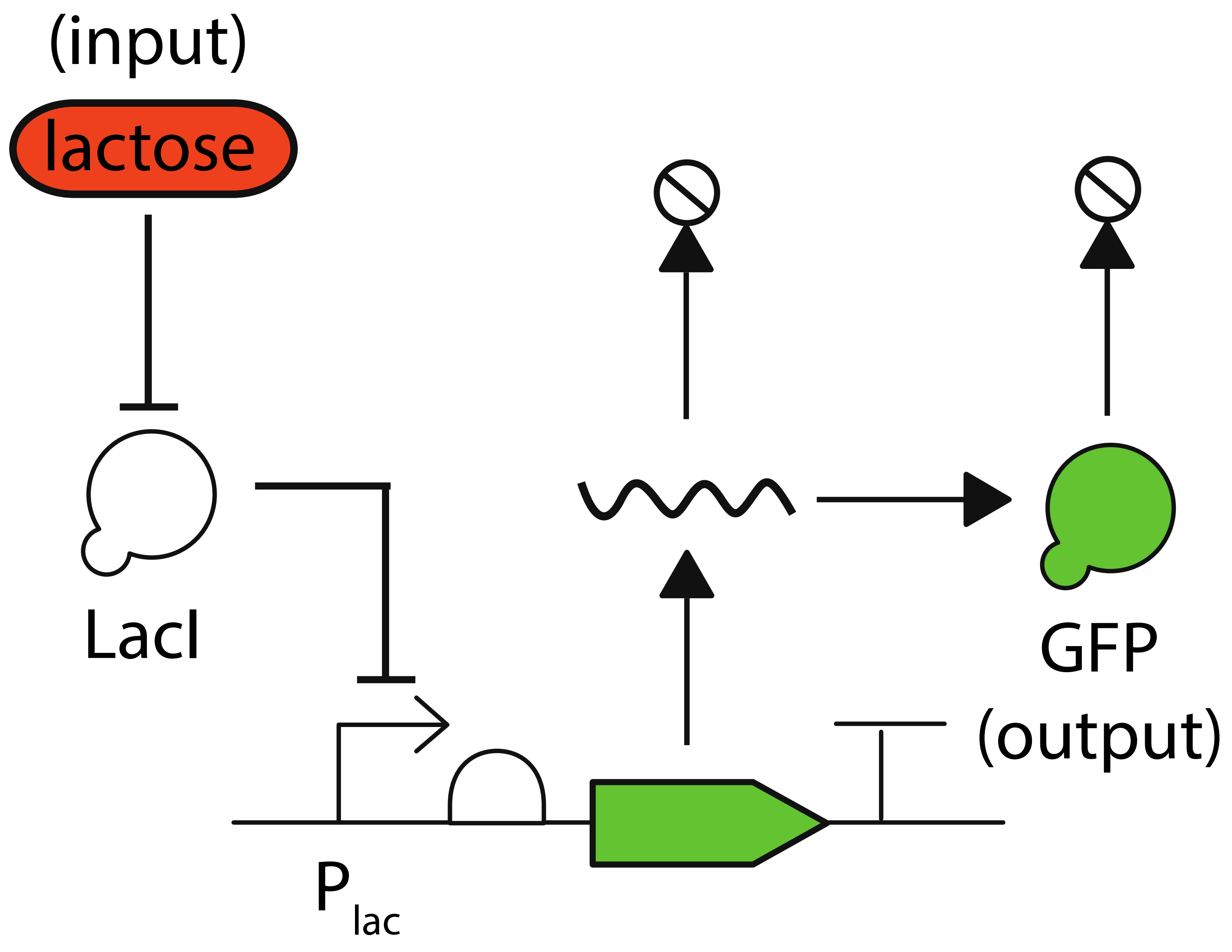
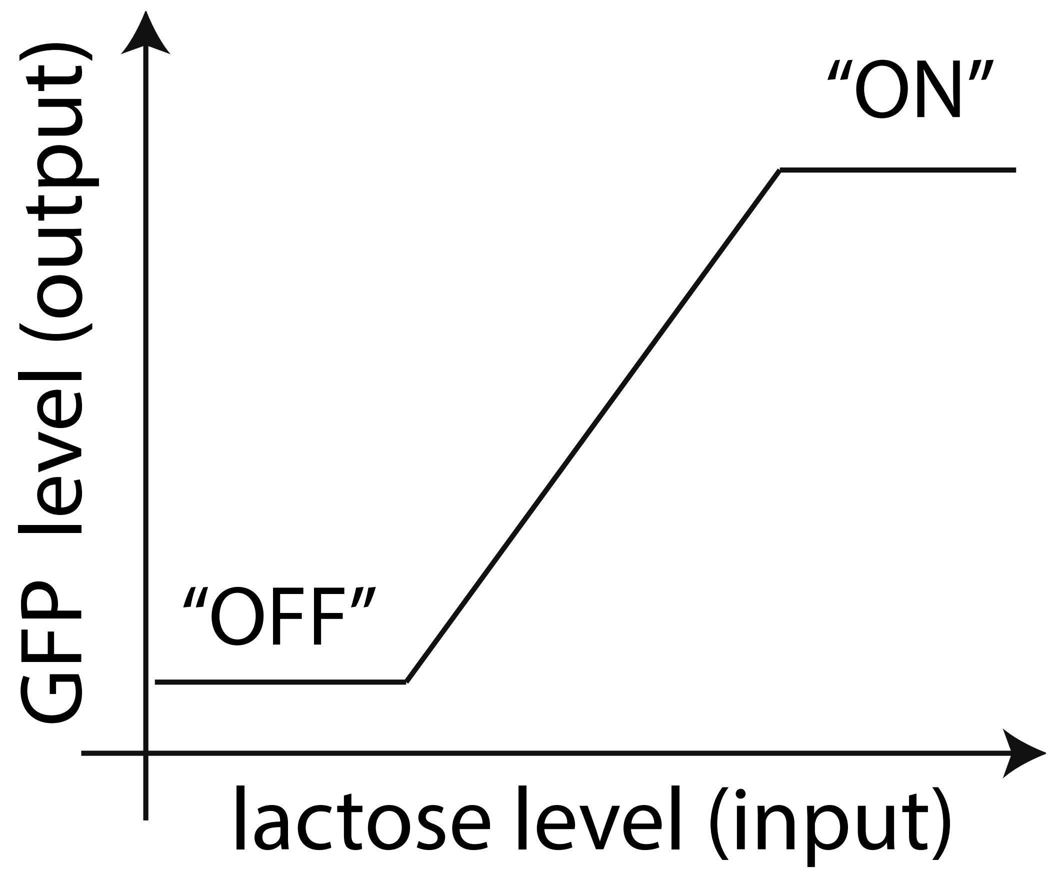
Double-repression

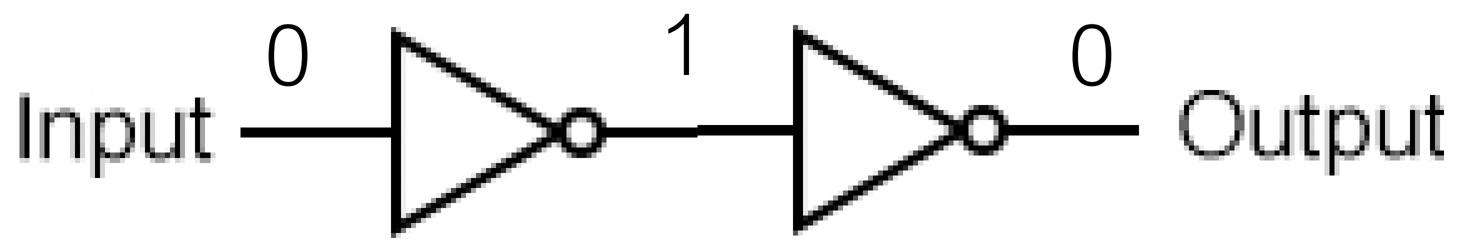


Dose-response curve (=transfer-function)



Logic gate

Double-inverter = Buffer gate



Input	Output
0	0
1	1

Truth table

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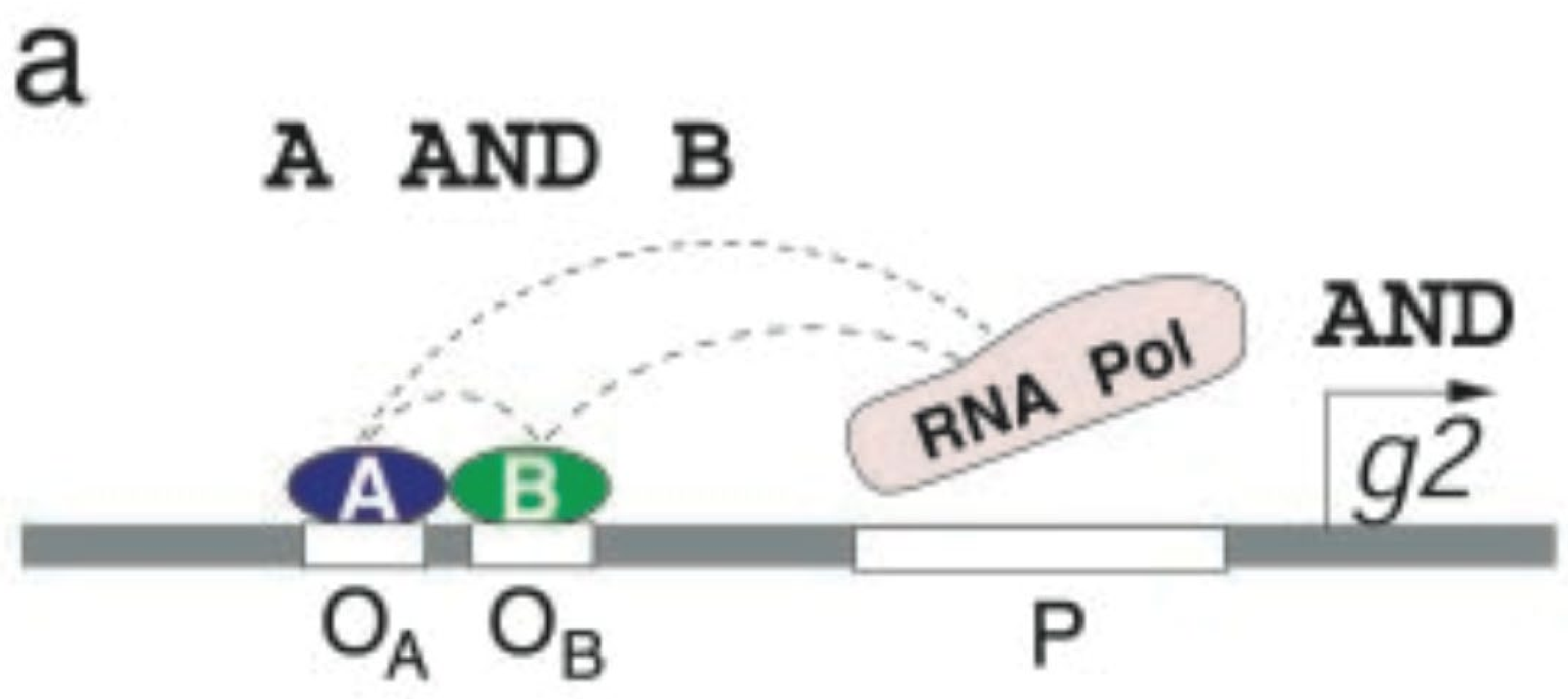
2-input logic gates

Cooperative activation



Weak operator sites for activator binding

Weak promoter

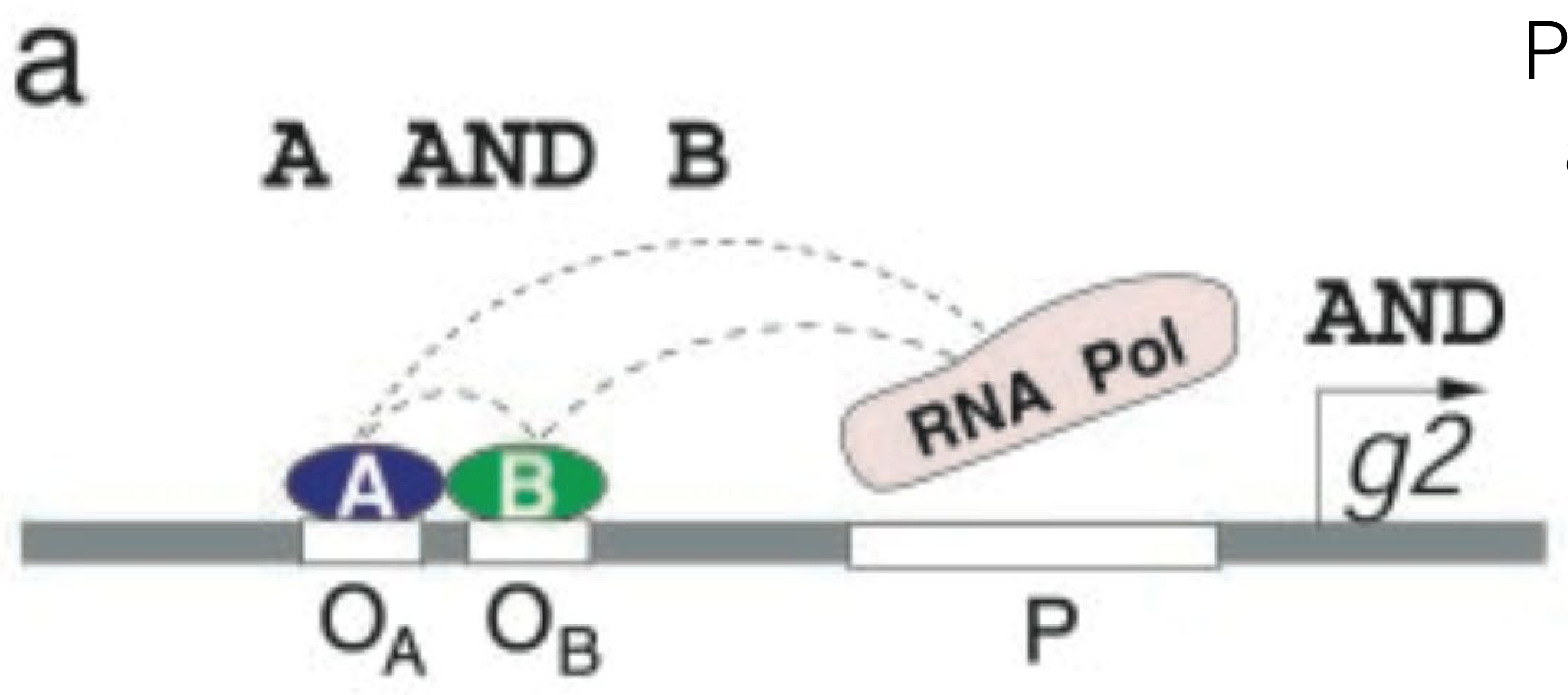
Dashed lines = Cooperative interactions!

Buchler, Gerland and Hwa, Proc. Natl. Acad. Sci., 2003

2-input logic gates

Cooperative activation

Dose-response curve
(=transfer-function)

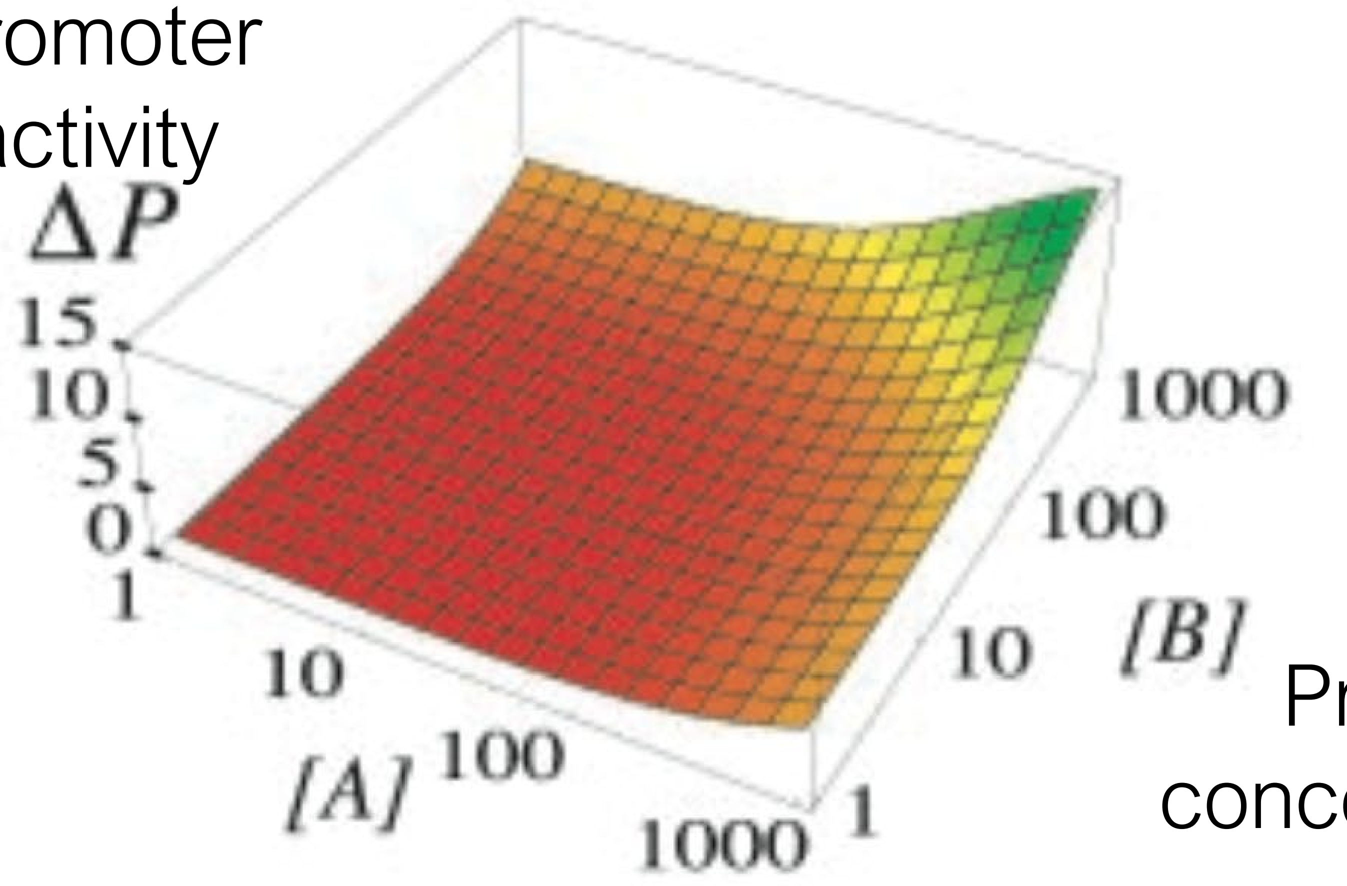


Weak operator sites for activator binding

Weak promoter

Dashed lines = Cooperative interactions!

Promoter activity



Protein concentration

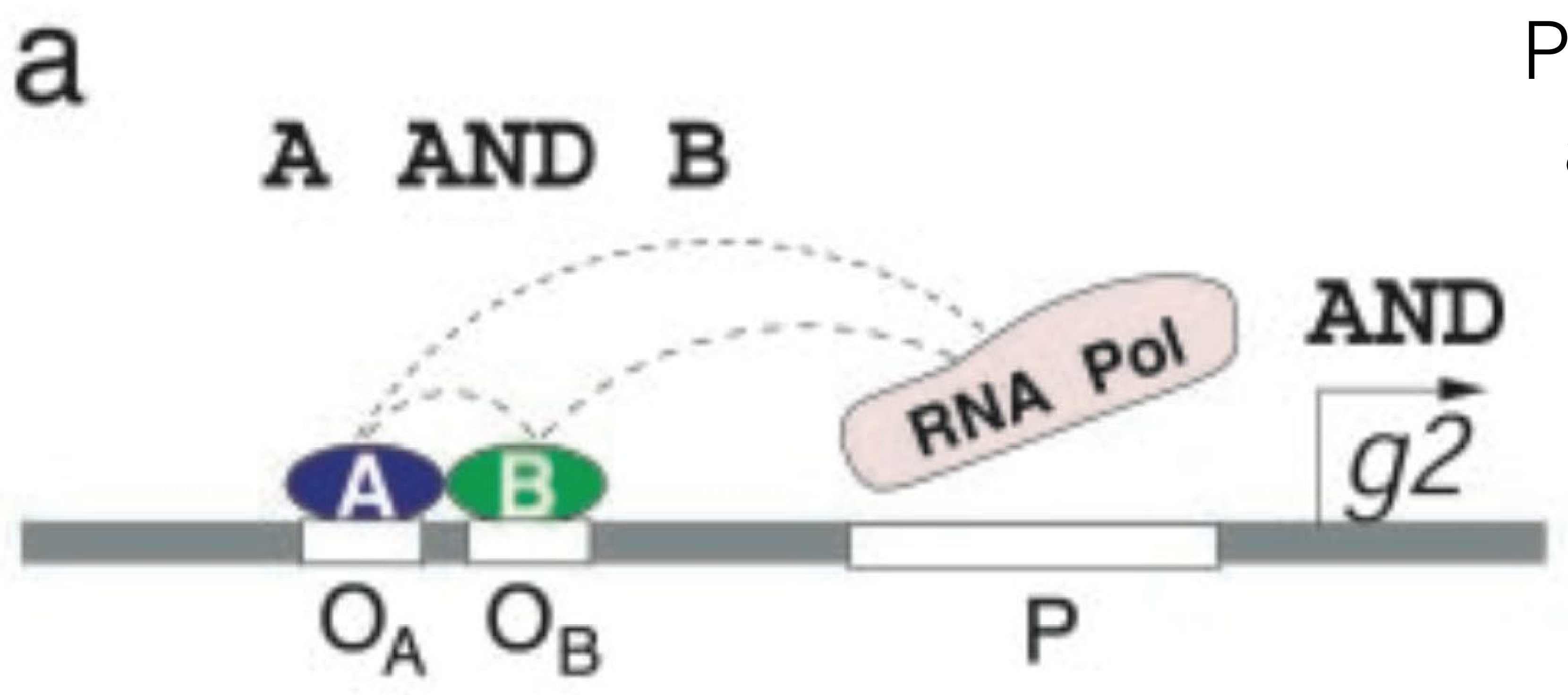
Protein concentration

Promoter is **active**, if
A **AND** B are HIGH

Buchler, Gerland and Hwa, Proc. Natl. Acad. Sci., 2003

2-input logic gates

Cooperative activation



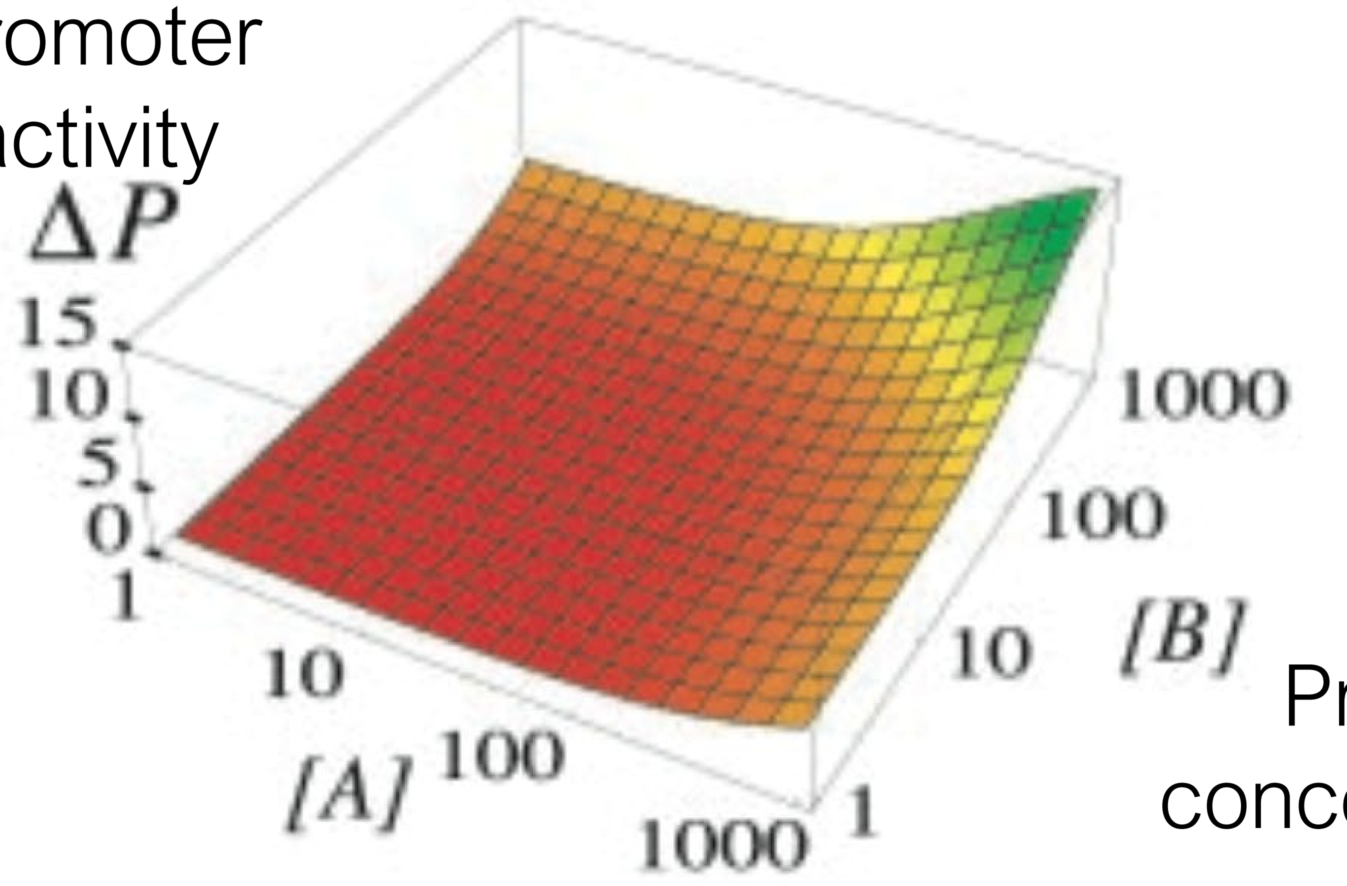
Weak operator sites for activator binding

Weak promoter

Dashed lines = Cooperative interactions!

Dose-response curve (=transfer-function)

Promoter activity



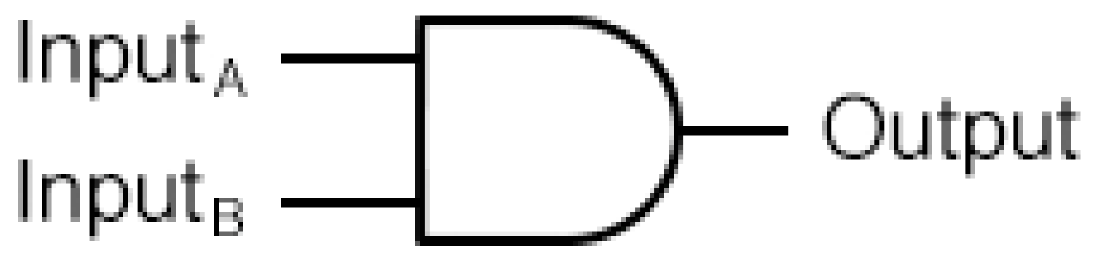
Protein concentration

Protein concentration

Promoter is **active**, if
A **AND** B are HIGH

Logic gate

2 - input AND gate



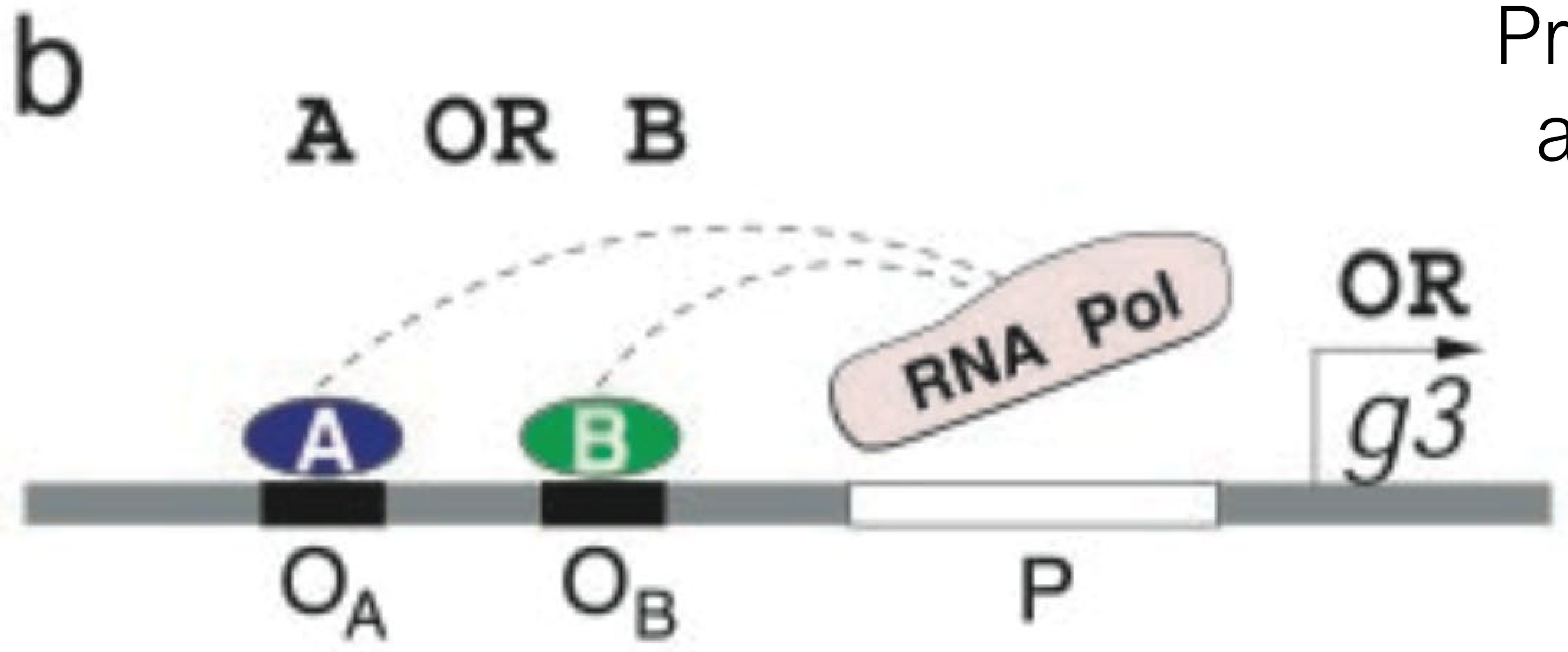
A	B	Output
0	0	0
0	1	0
1	0	0
1	1	1

Truth table

Buchler, Gerland and Hwa, Proc. Natl. Acad. Sci., 2003

2-input logic gates

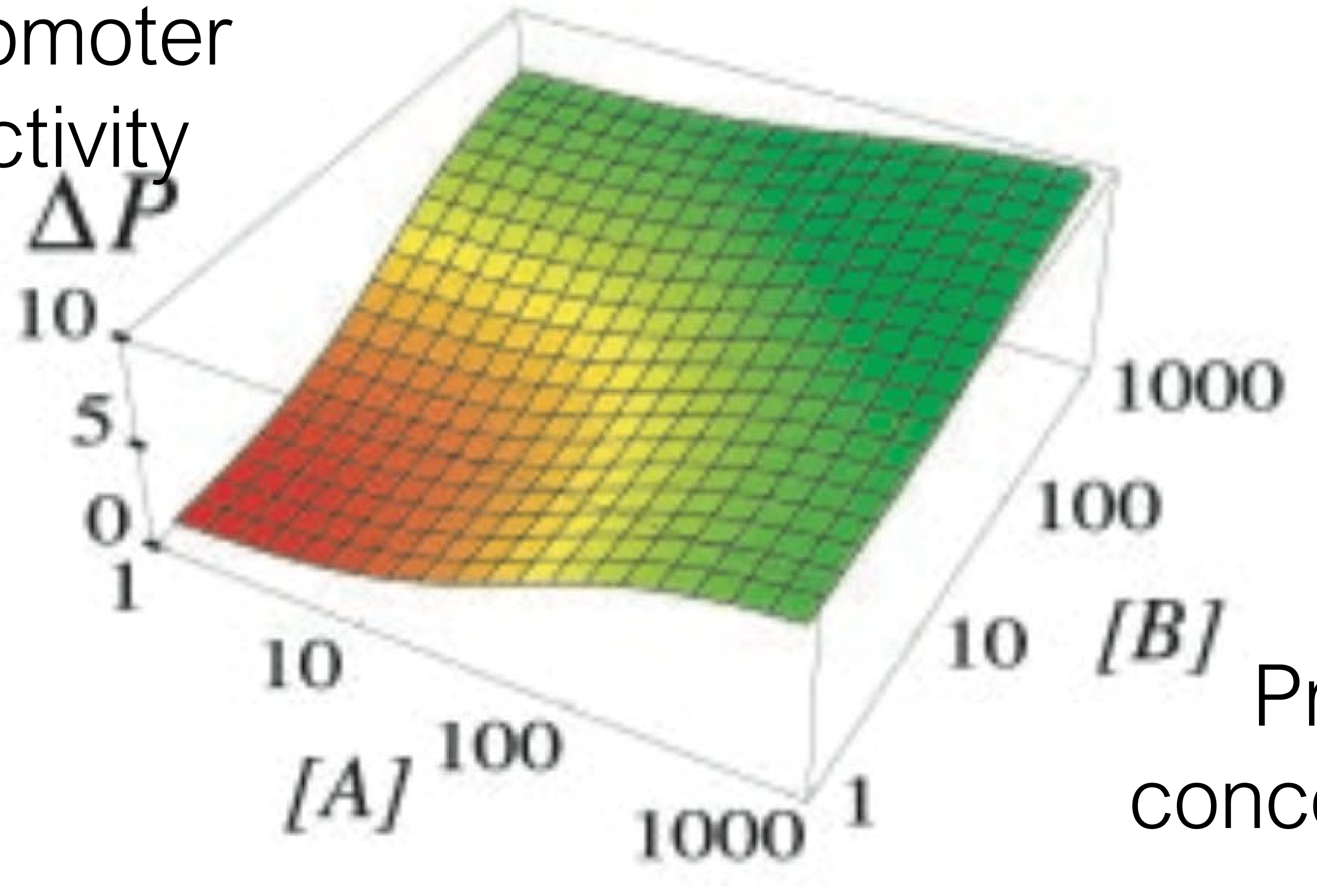
Non-cooperative activation



Strong operator sites for independent activator binding

Weak promoter

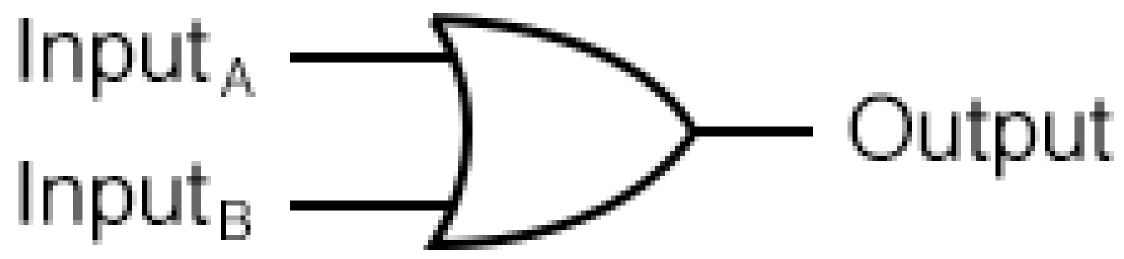
Dose-response curve (=transfer-function)



Promoter is **active**, if either A **OR** B are HIGH

Logic gate

2 - input OR gate



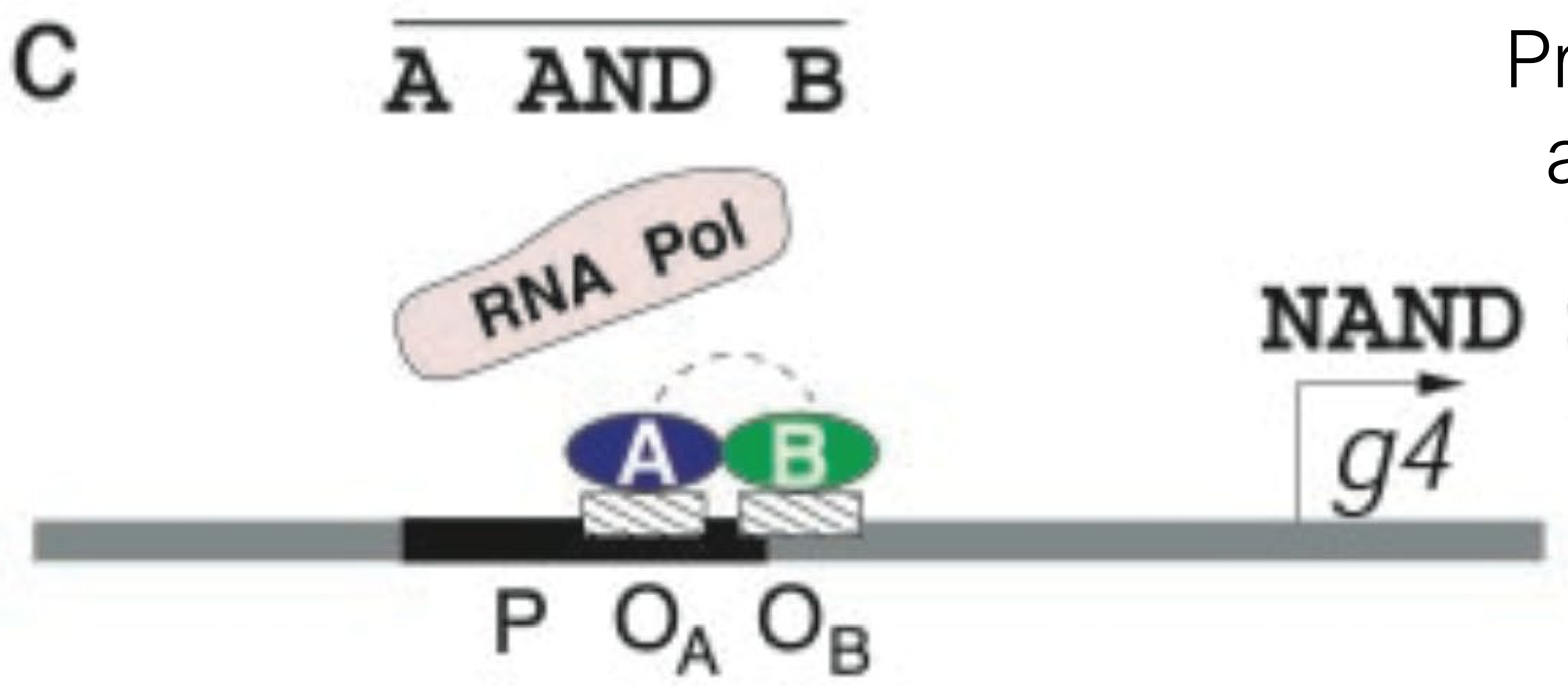
A	B	Output
0	0	0
0	1	1
1	0	1
1	1	1

Truth table

Buchler, Gerland and Hwa, Proc. Natl. Acad. Sci., 2003

2-input logic gates

Cooperative repression

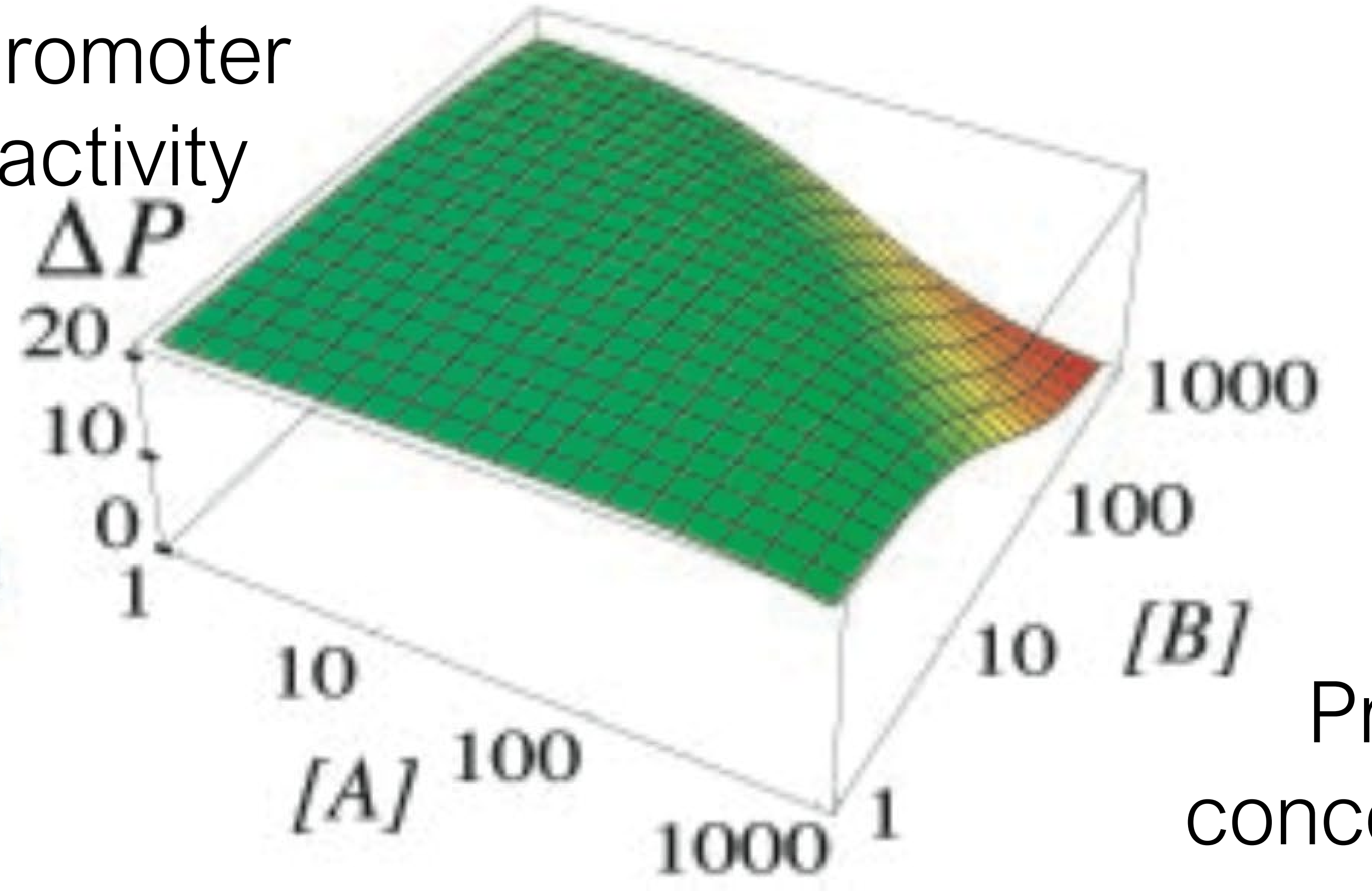


Strong promoter

Weak, cooperative operator sites for repressor binding

Dose-response curve (=transfer-function)

Promoter activity



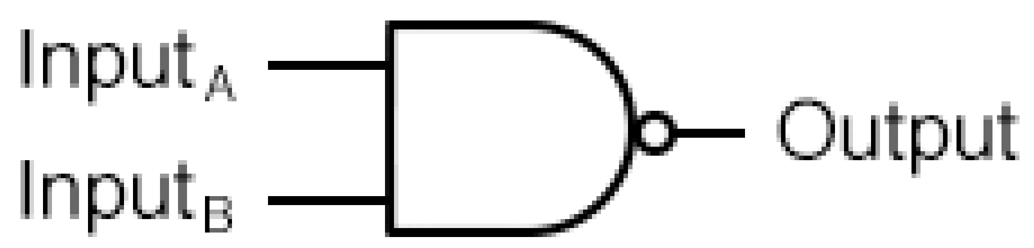
Protein concentration

Protein concentration

Promoter is **inactive**, if
A **AND** B are HIGH

Logic gate

2-input NAND gate



A	B	Output
0	0	1
0	1	1
1	0	1
1	1	0

Truth table

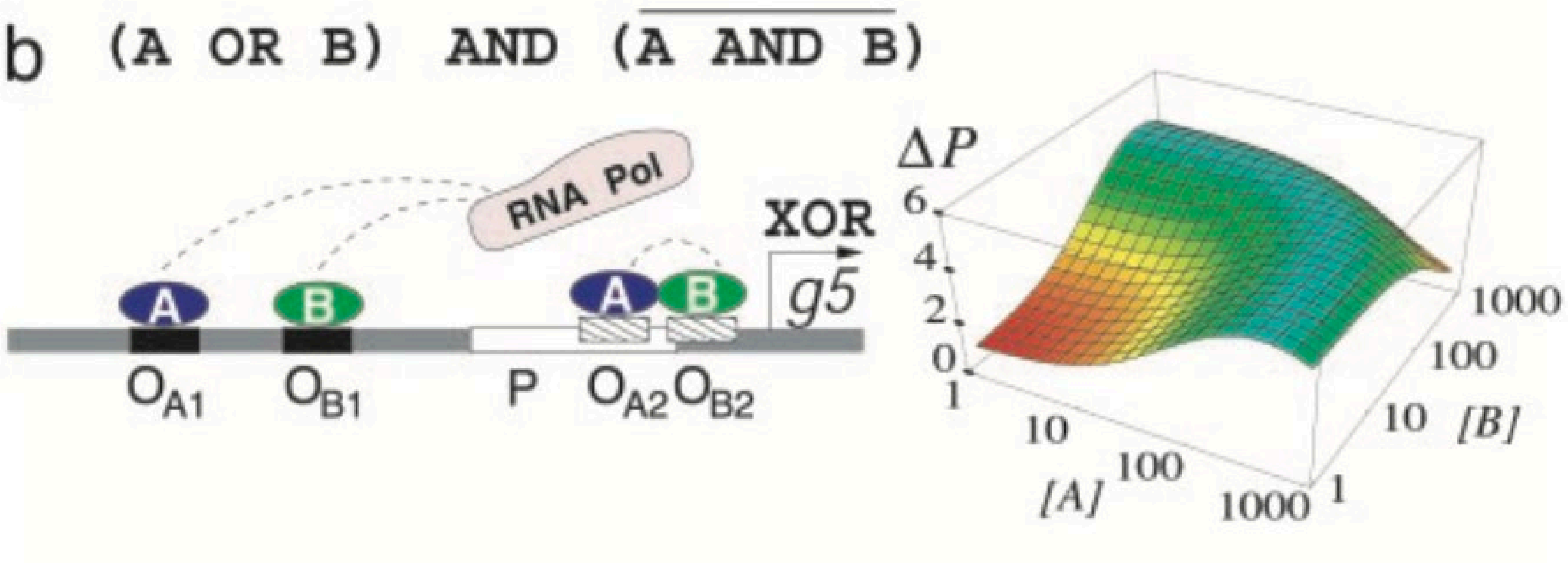
Buchler, Gerland and Hwa, Proc. Natl. Acad. Sci., 2003

2-input logic gates

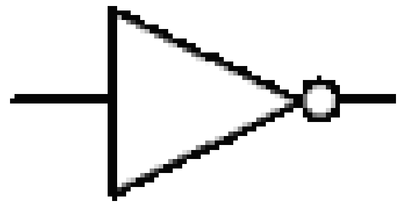
Many more logic gates can be envisaged by assembling various combinations of

- ✓ promoters of different strengths with
- ✓ transcription factors (activators & repressors) with different binding affinities & cooperativities

Example: Exclusive OR gate (XOR):

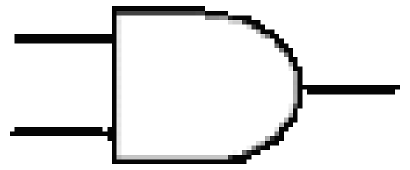


Buchler, Gerland and Hwa, Proc. Natl. Acad. Sci., 2003



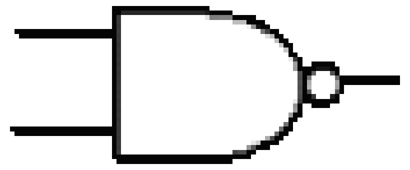
NOT

Input	Output
I	F
0	1
1	0



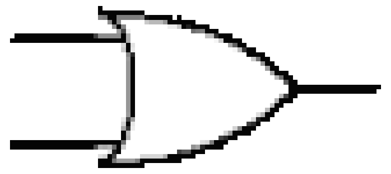
AND

Inputs		Output
A	B	F
0	0	0
1	0	0
0	1	0
1	1	1



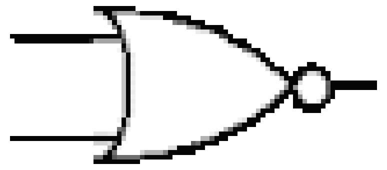
NAND

Inputs		Output
A	B	F
0	0	1
1	0	1
0	1	1
1	1	0



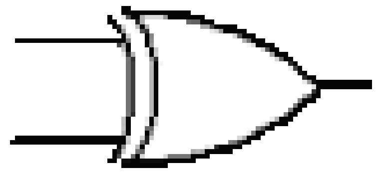
OR

Inputs		Output
A	B	F
0	0	0
1	0	1
0	1	1
1	1	1



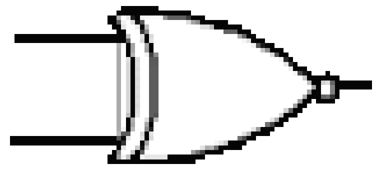
NOR

Inputs		Output
A	B	F
0	0	1
1	0	0
0	1	0
1	1	0



Inputs		Output
A	B	F
0	0	0
0	1	1
1	0	1
1	1	0

EXCLUSIVE OR



EXCLUSIVE NOR

Inputs		Output
A	B	F
0	0	1
0	1	0
1	0	0
1	1	1

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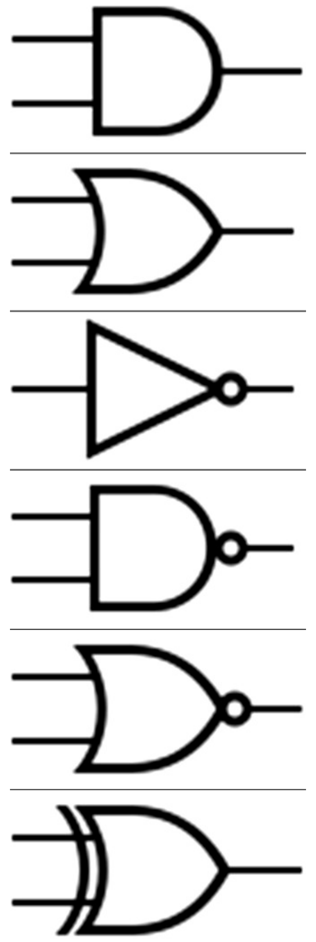
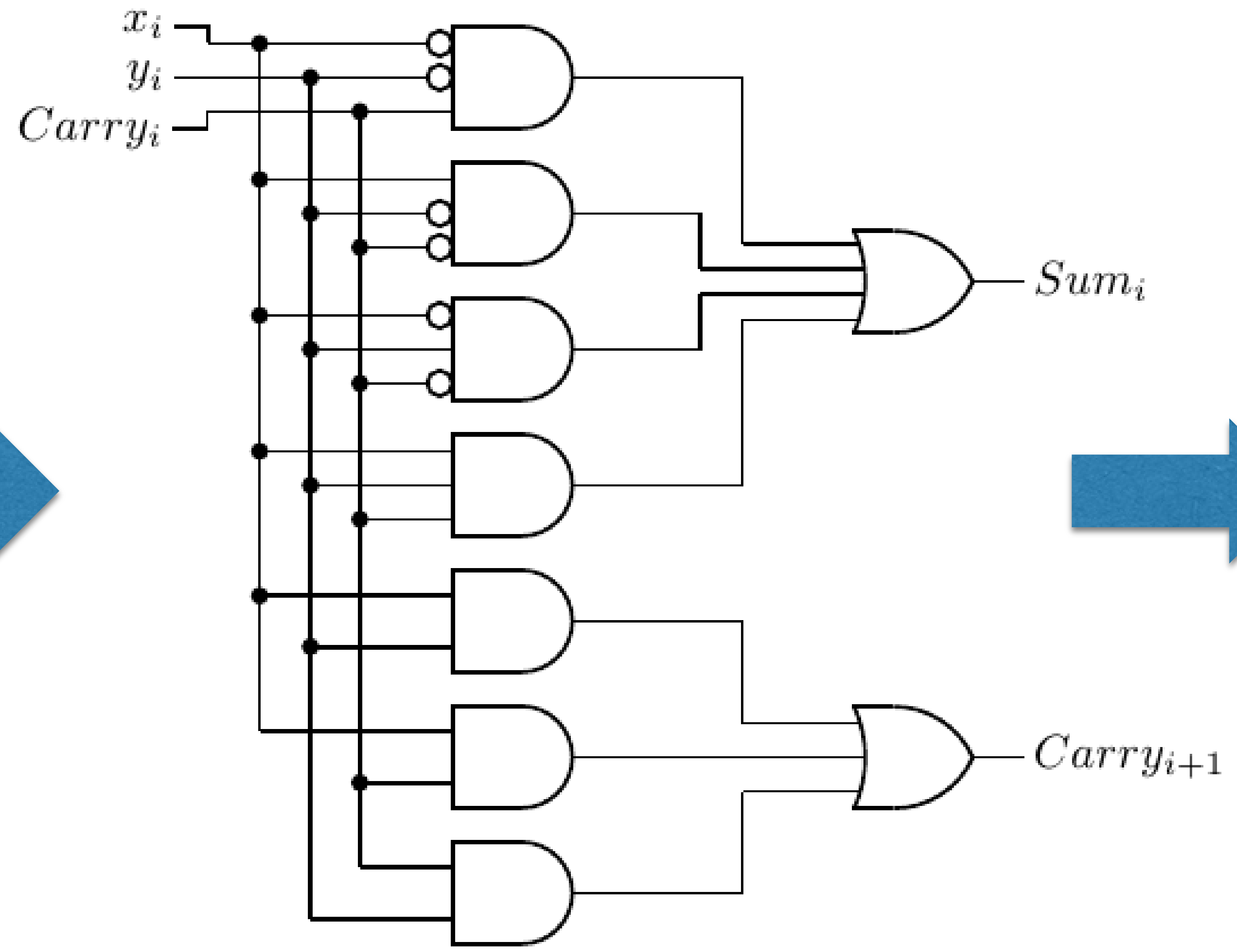
From logic gates to logic circuits: How to build a biological "computer"?

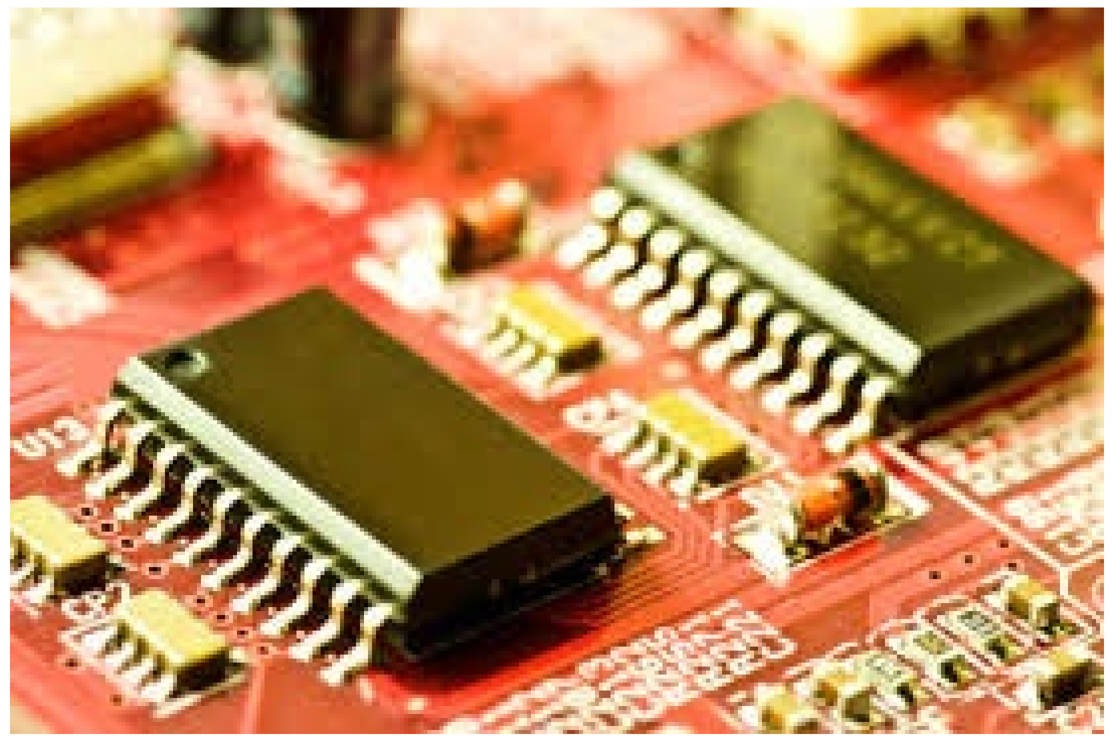
In digital electronics, cascades of logic gates perform advanced logic computation



Logic gates



Circuit



Chip

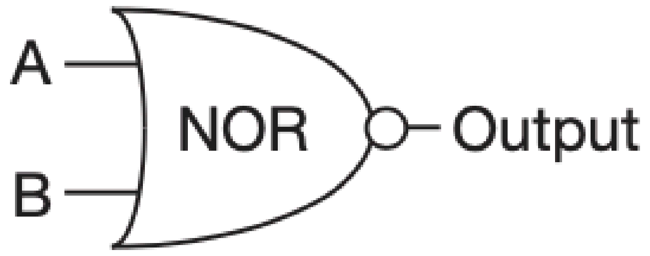
Computer

Increasing complexity by cascading logic gates

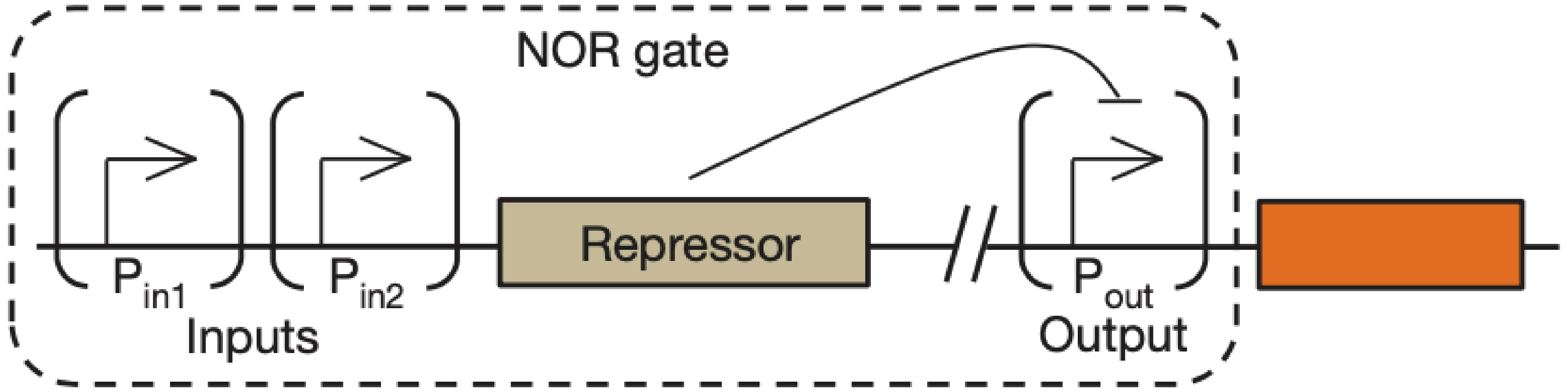
212 | NATURE | VOL 469 | 13 JANUARY 2011

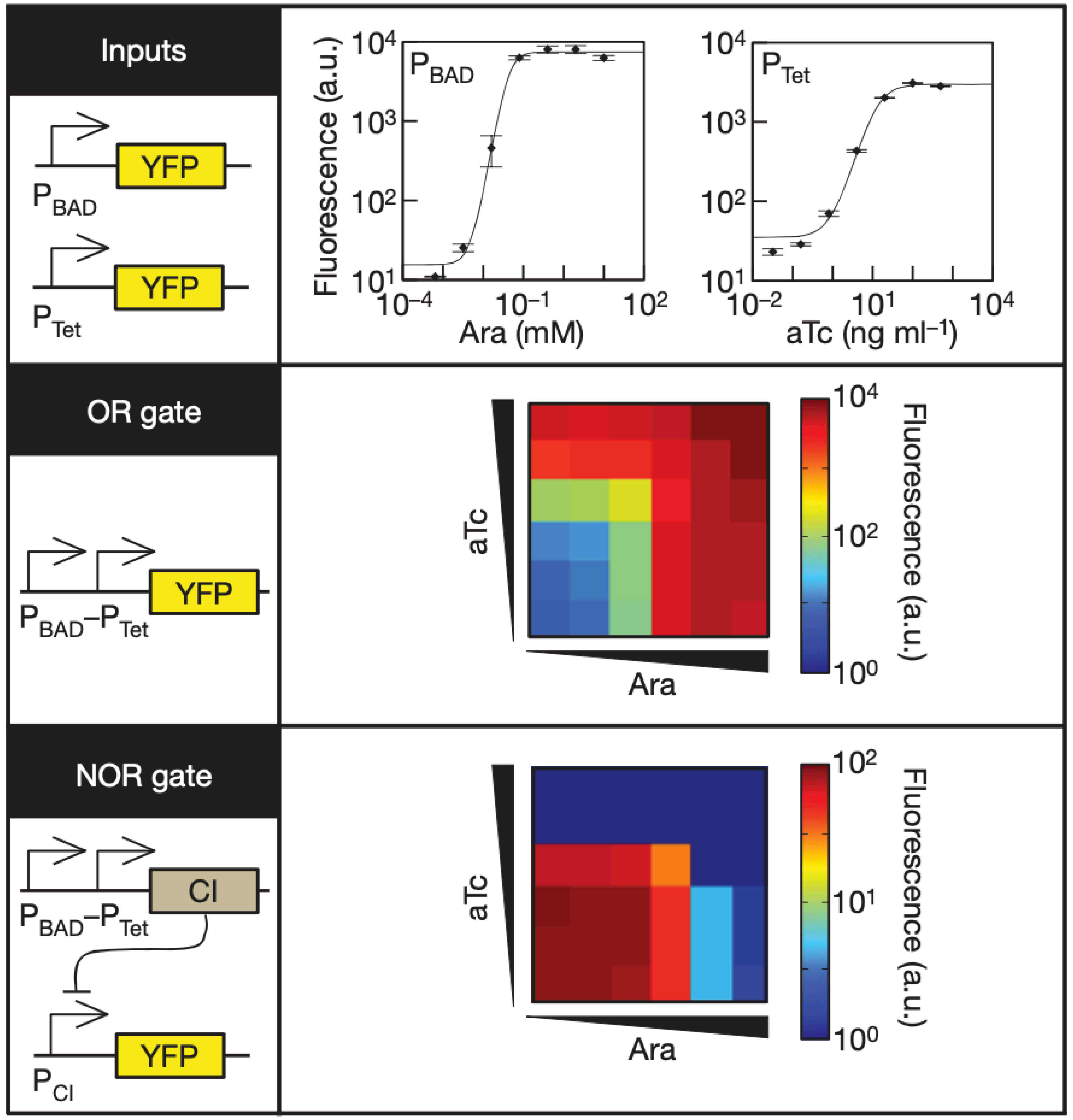
Robust multicellular computing using genetically encoded NOR gates and chemical ‘wires’

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



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





Increasing complexity by cascading logic gates

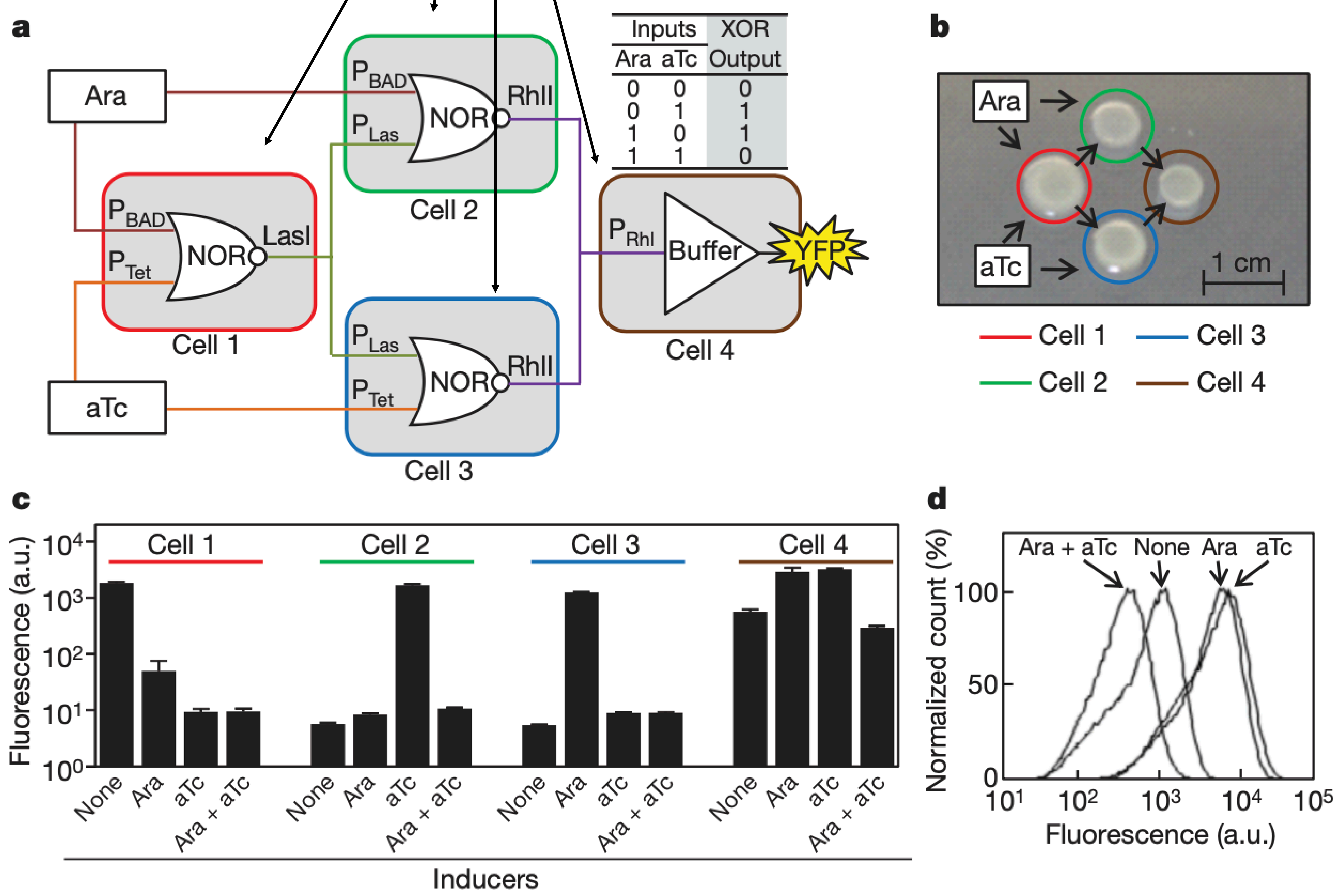
212 | NATURE | VOL 469 | 13 JANUARY 2011

Robust multicellular computing using genetically encoded NOR gates and chemical 'wires'

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

Construction of an XOR gate by using 4 cell types with different NOR gates, connected by quorum sensing signals.

4 different strains



SCIE5508

An automated design framework for logic computation in bacterial cells

SCIENCE 1 APRIL 2016 • VOL 352 ISSUE 6281

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]*

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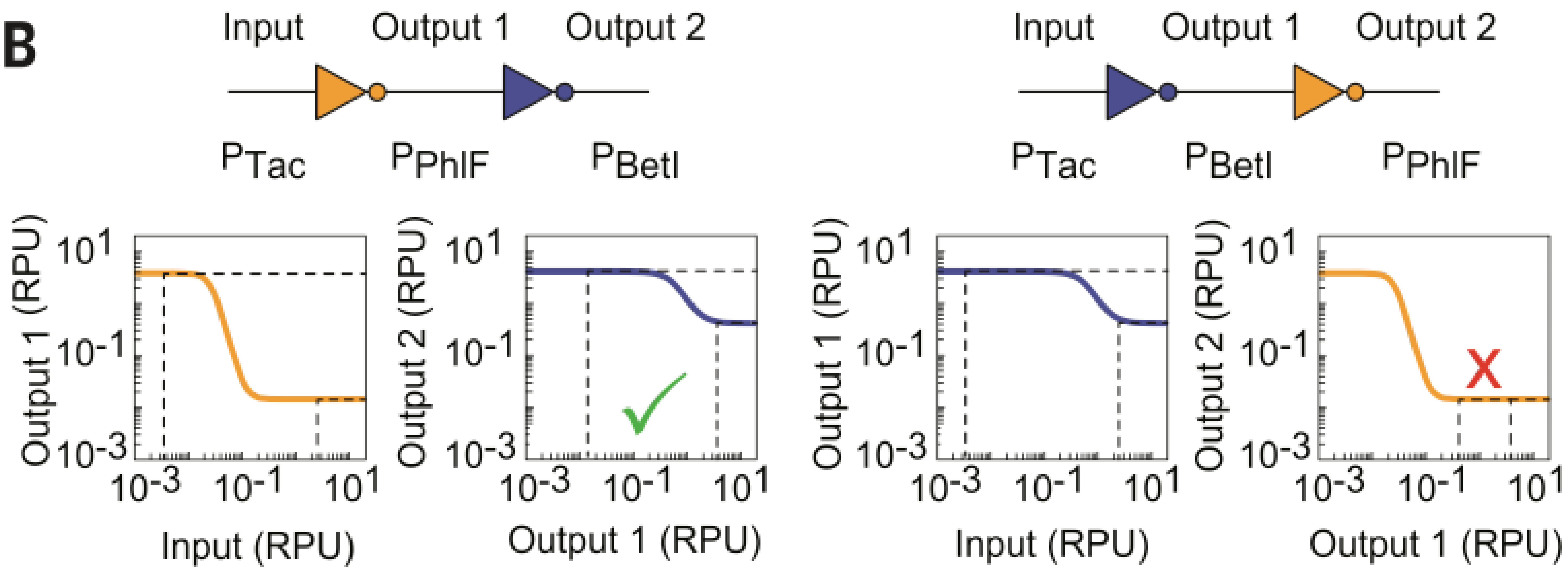
Constraint:

Output dynamic range has to match input dynamic range of downstream gate!

B



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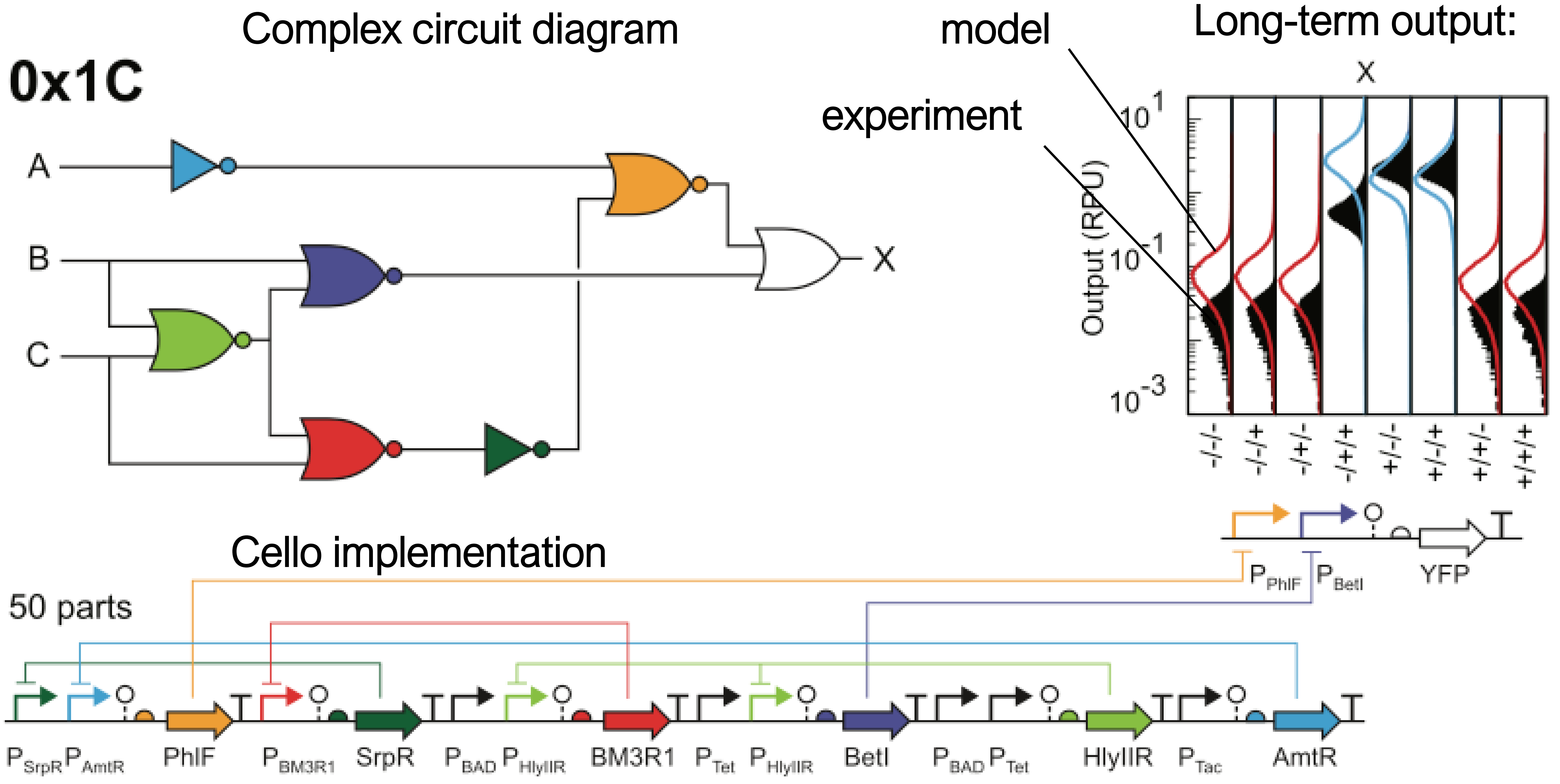

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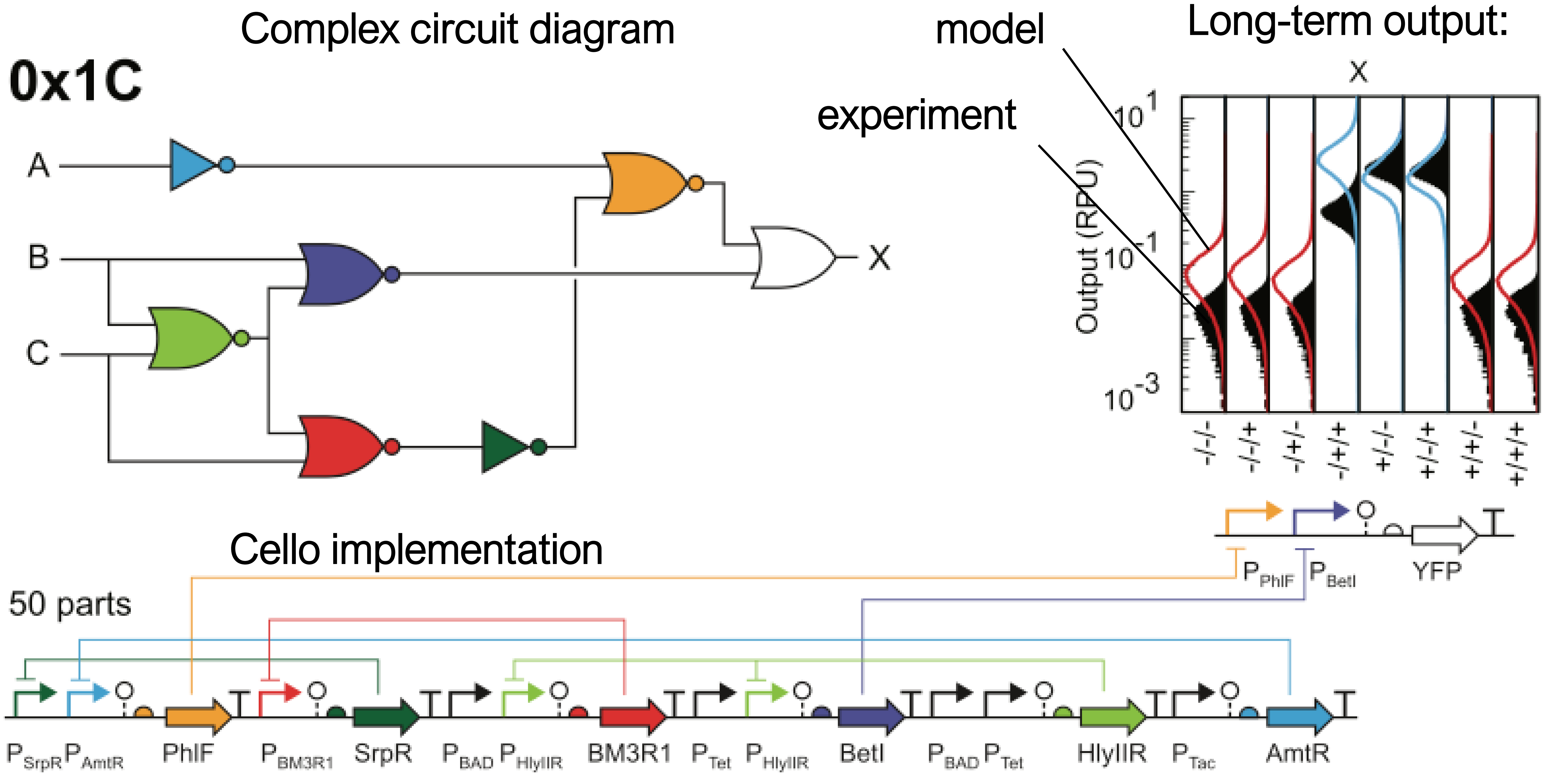
Caveat:

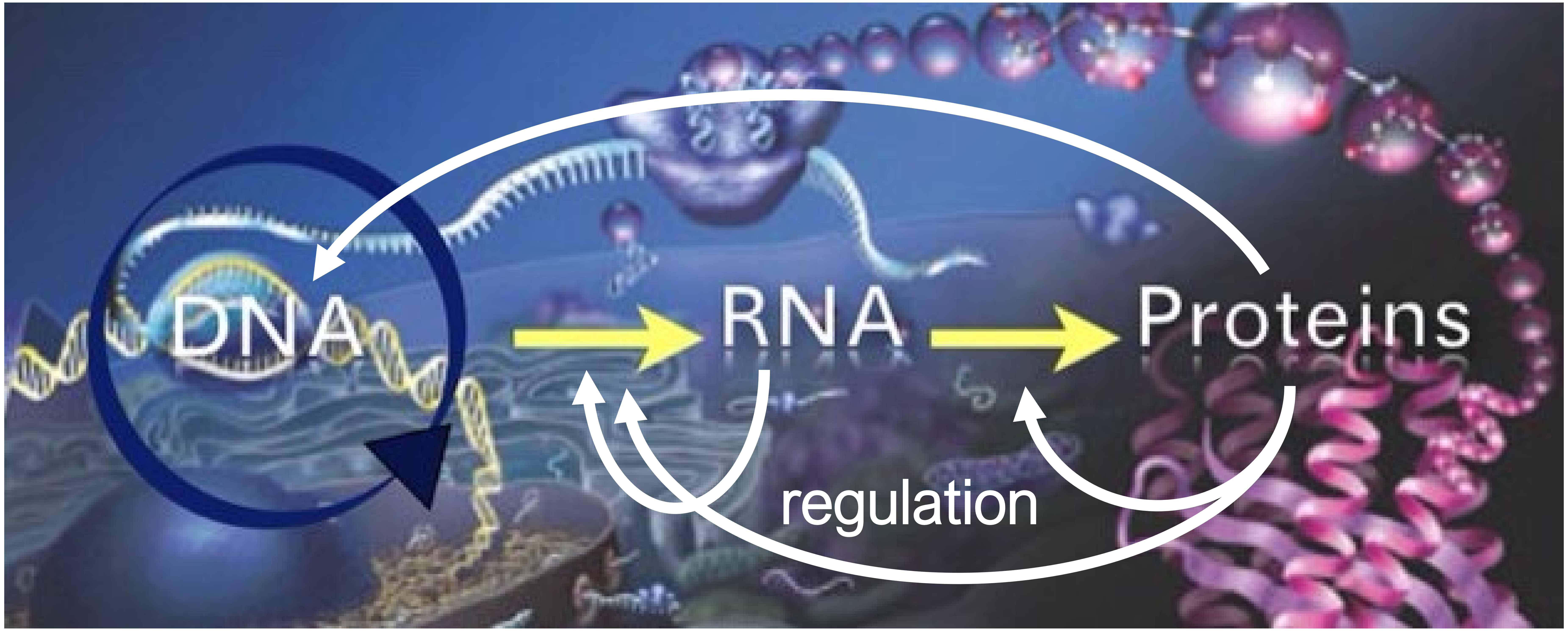
Due to sequential processing of information in this long cascade of logic gates, the final output state (YFP gene expression) is only settled after a very long time (8-10 hours)

Learning:

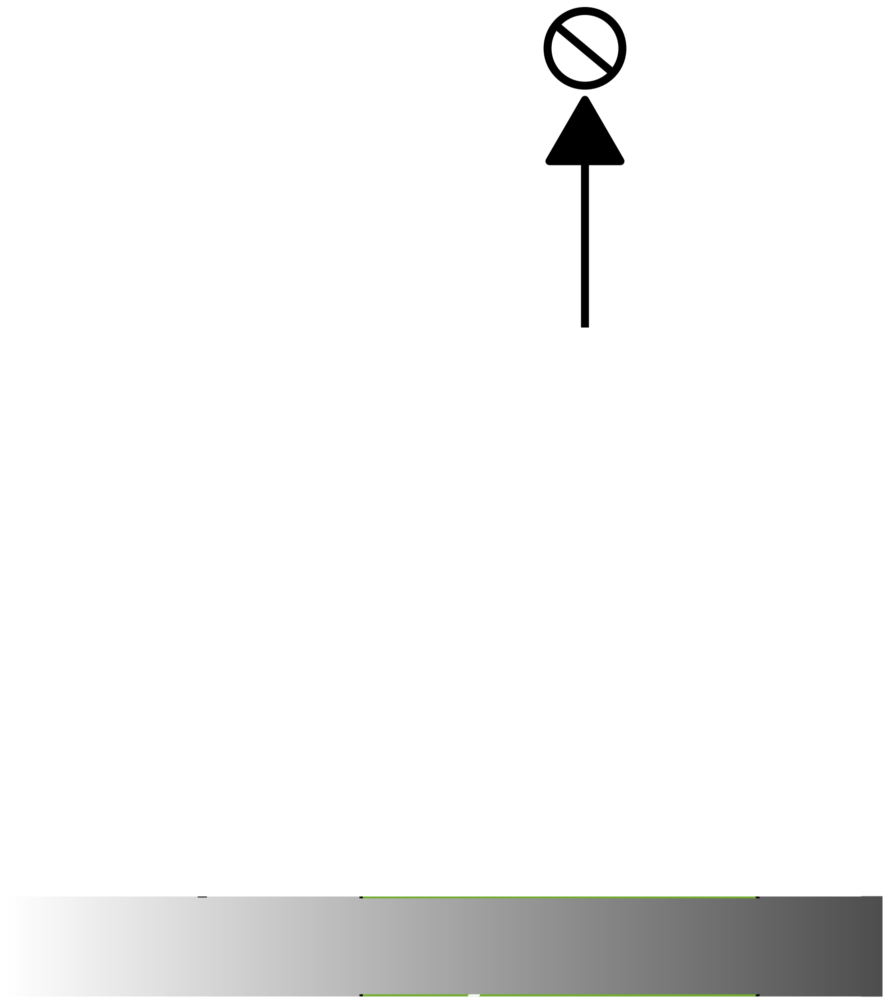
Genetic circuits are slow and asynchronous; can be problematic if timing is important, e.g. when feedback is involved



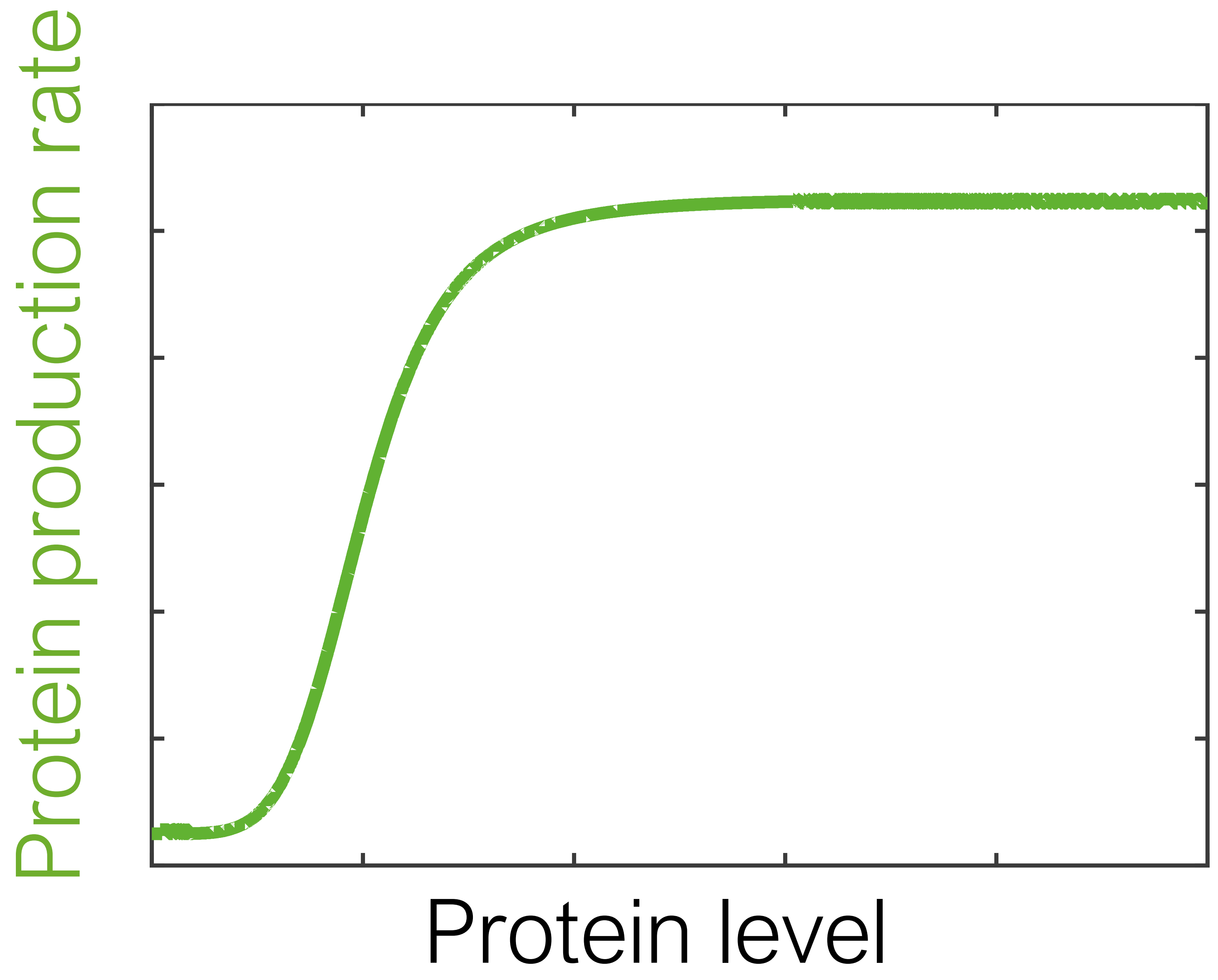
The role of feedback in genetic circuits



Positive feedback regulation can trigger bistability



Positive feedback



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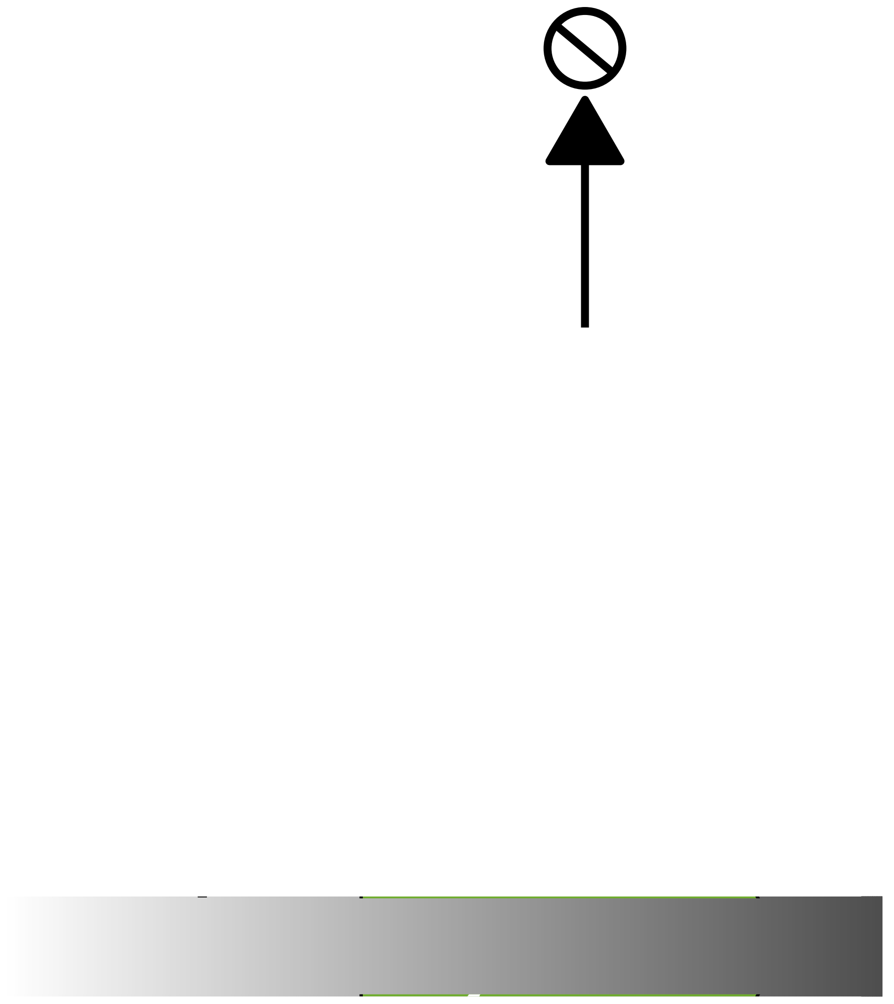

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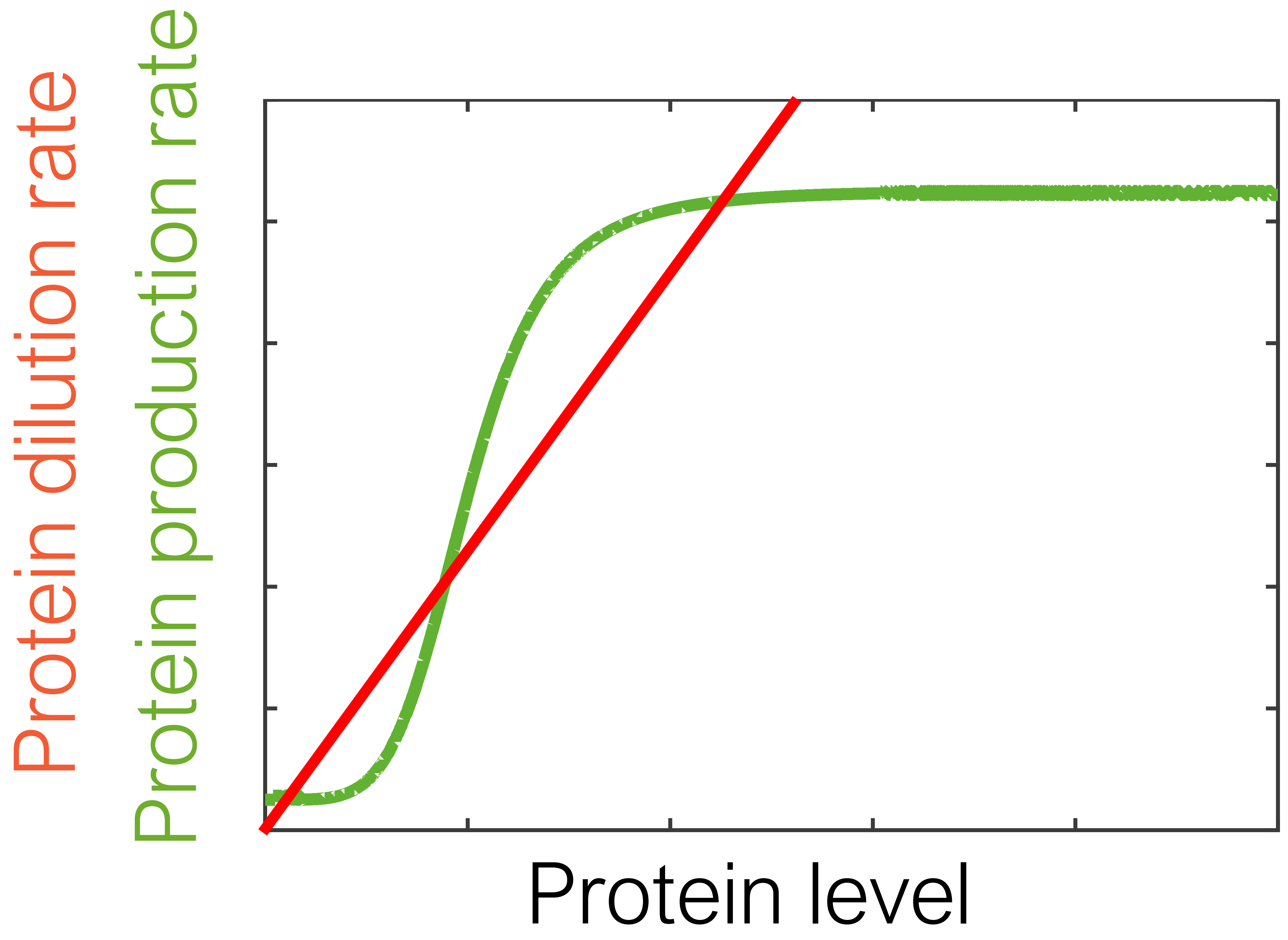

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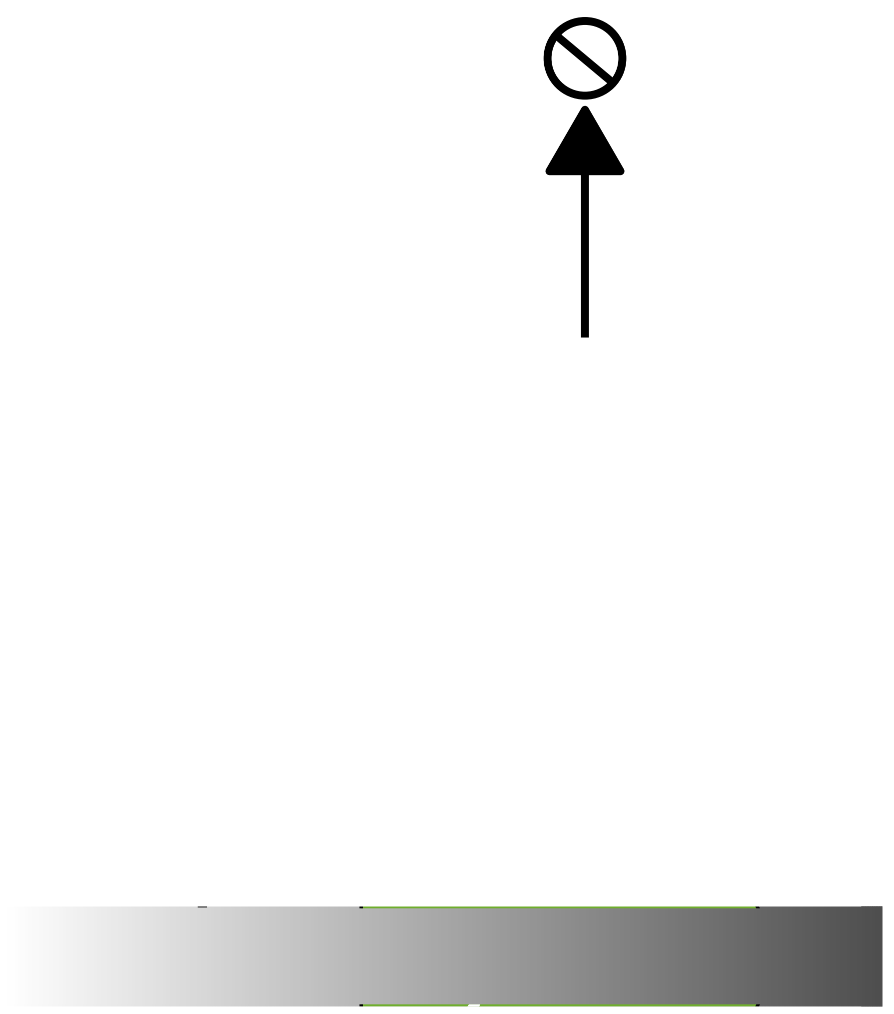
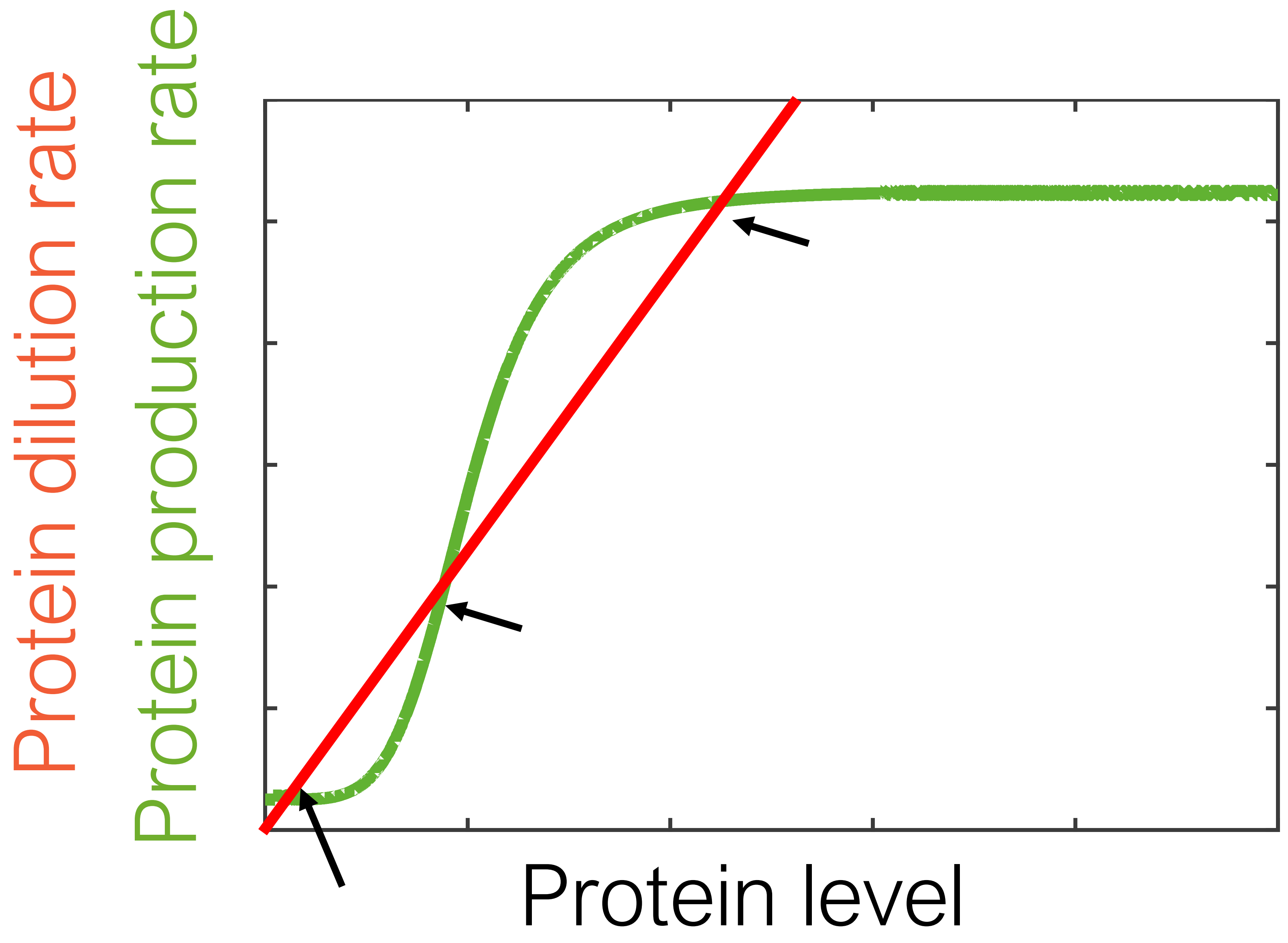
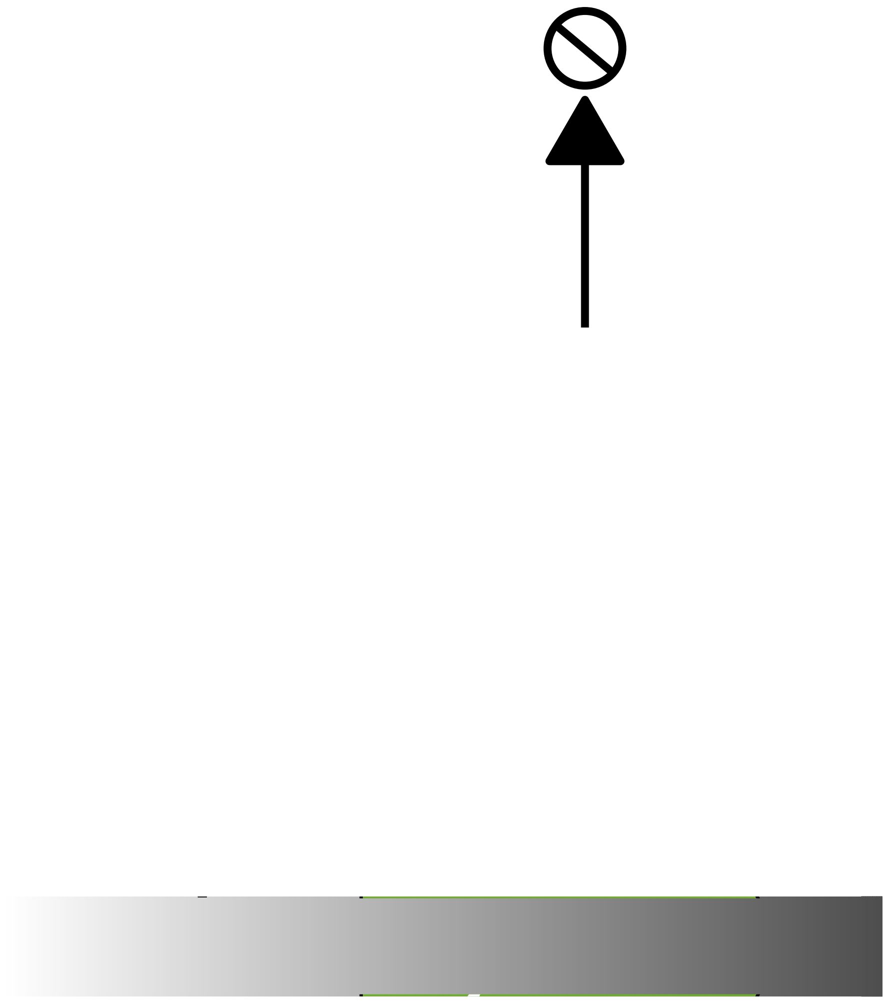


Positive feedback



Equilibrium, if
protein production rate =
protein dilution rate

Positive feedback regulation can trigger bistability



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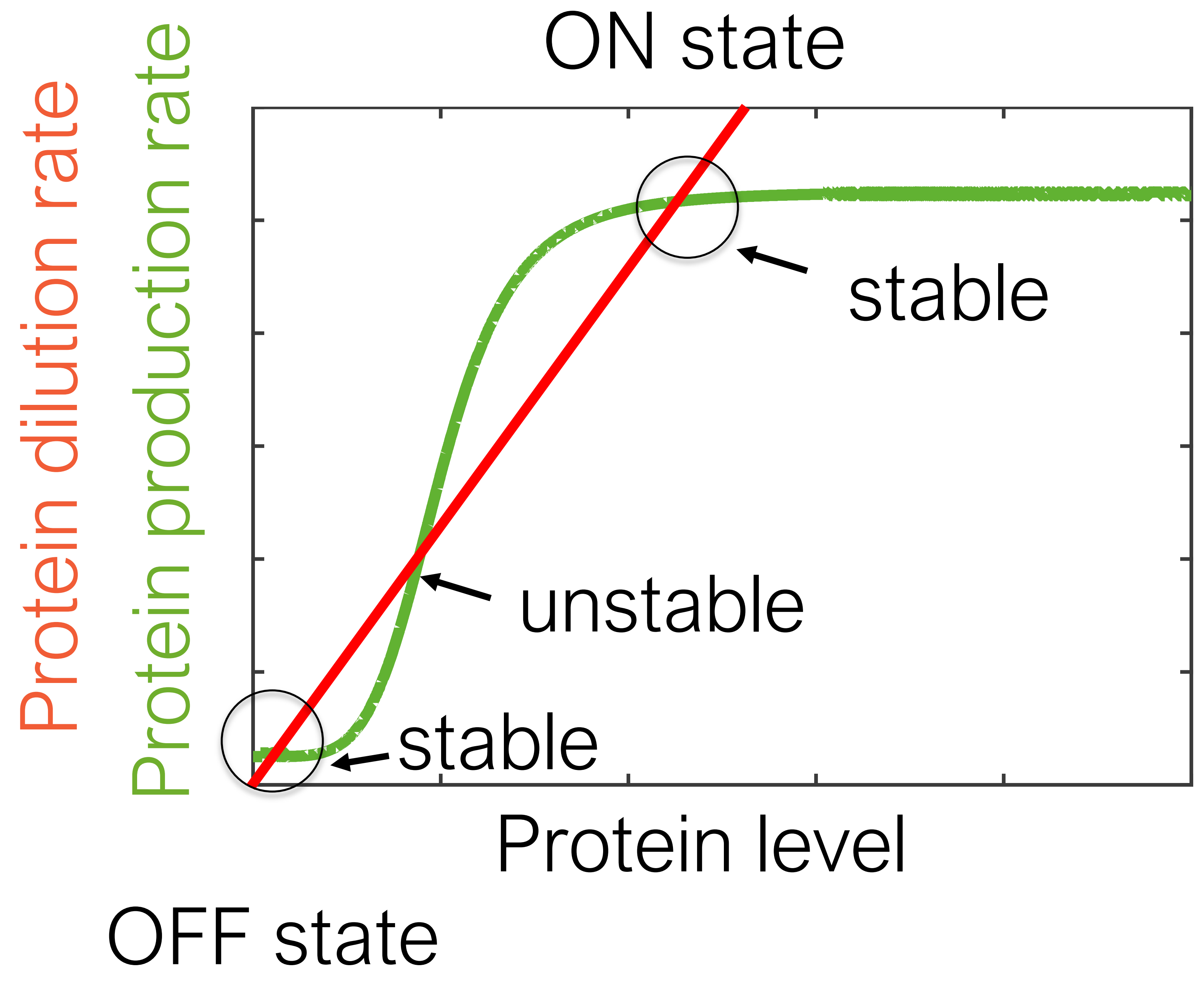

Equilibrium, if
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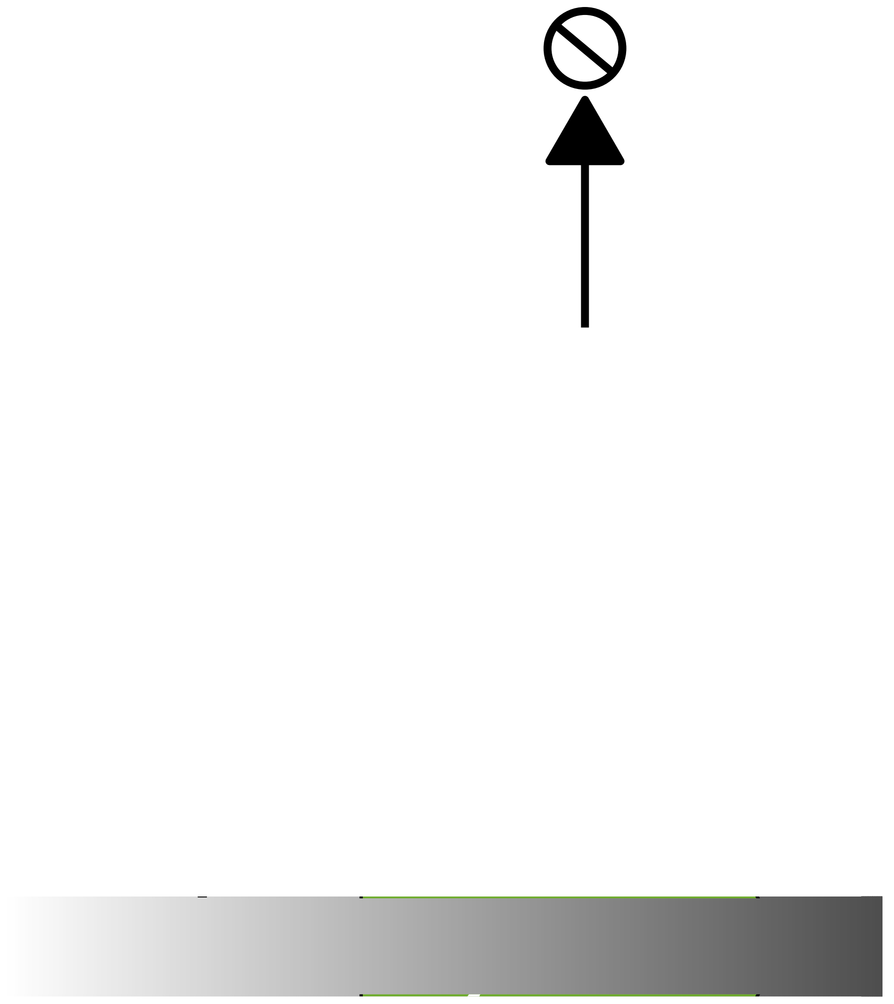
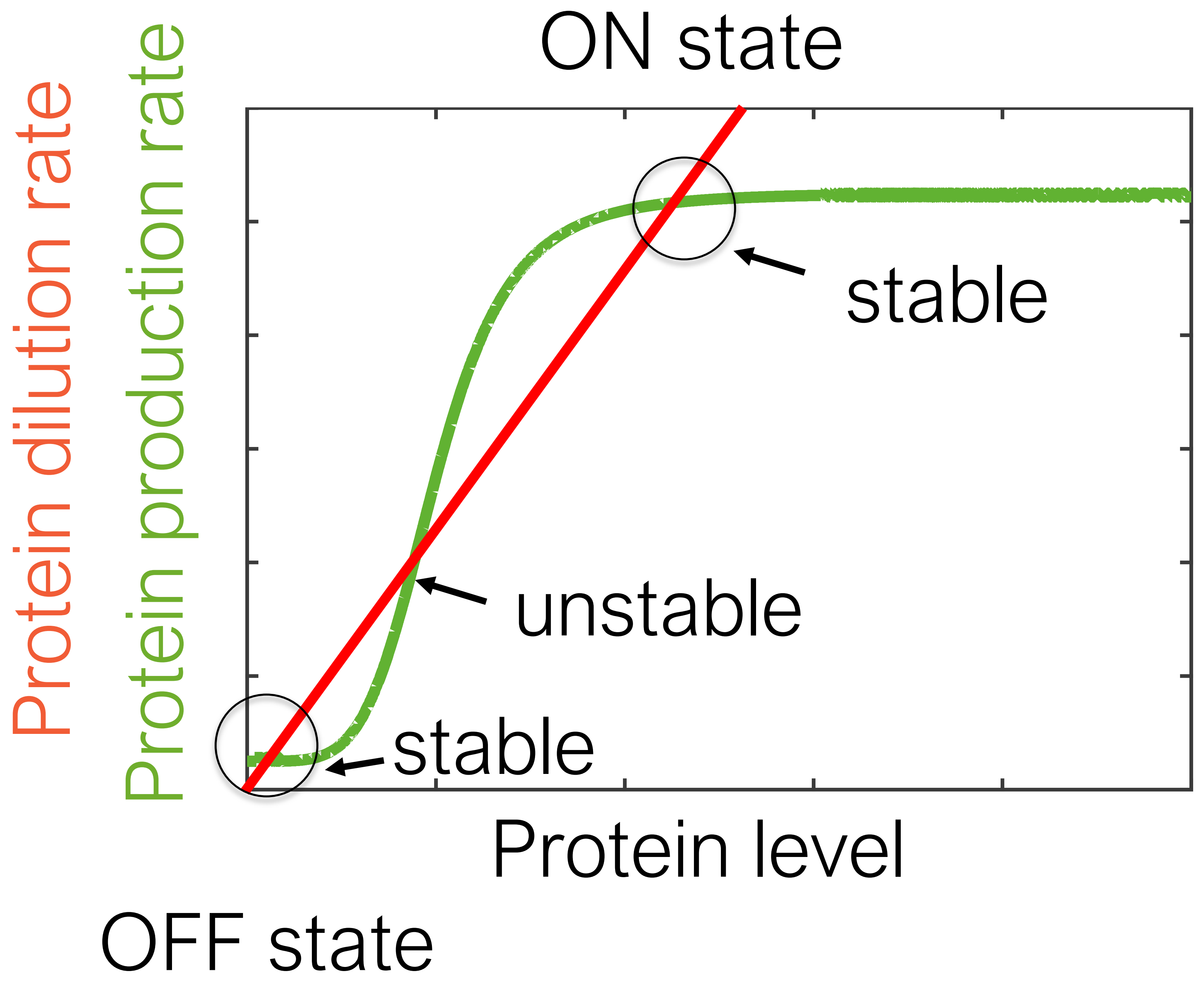
protein dilution rate

Two stable states = bistability

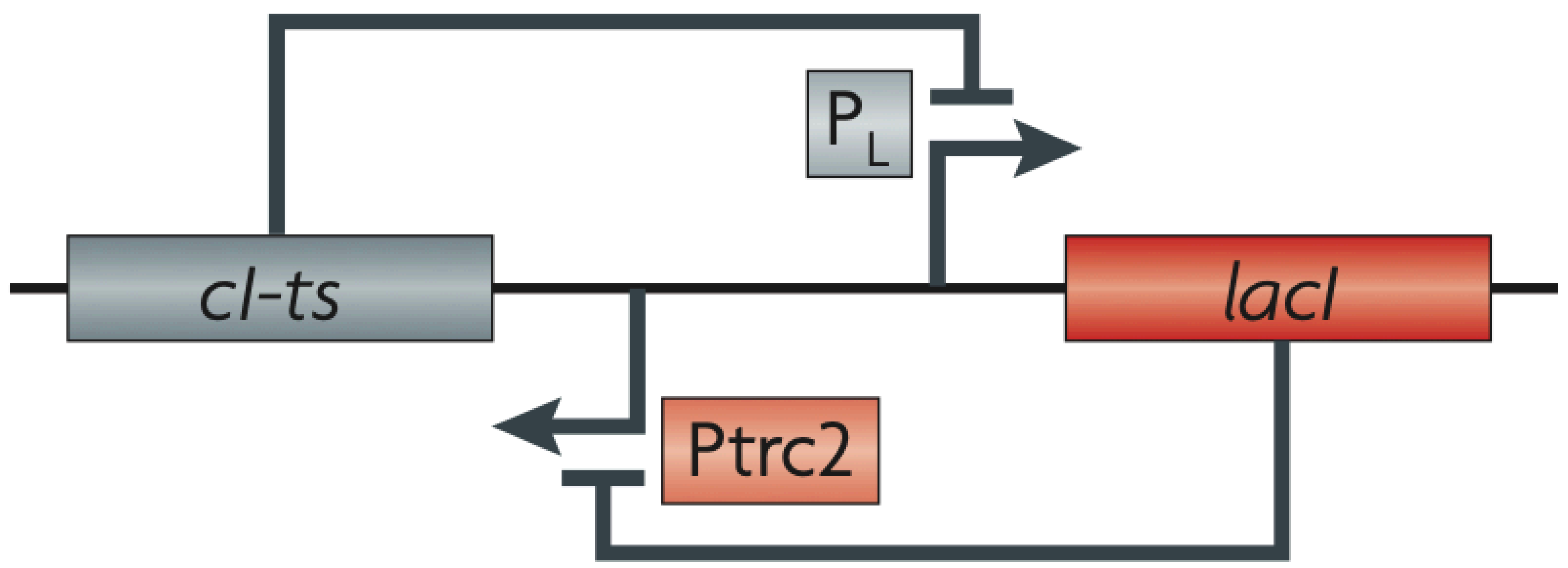
Positive feedback regulation can trigger bistability



Positive feedback



Genetic toggle switch



Two stable states = bistability

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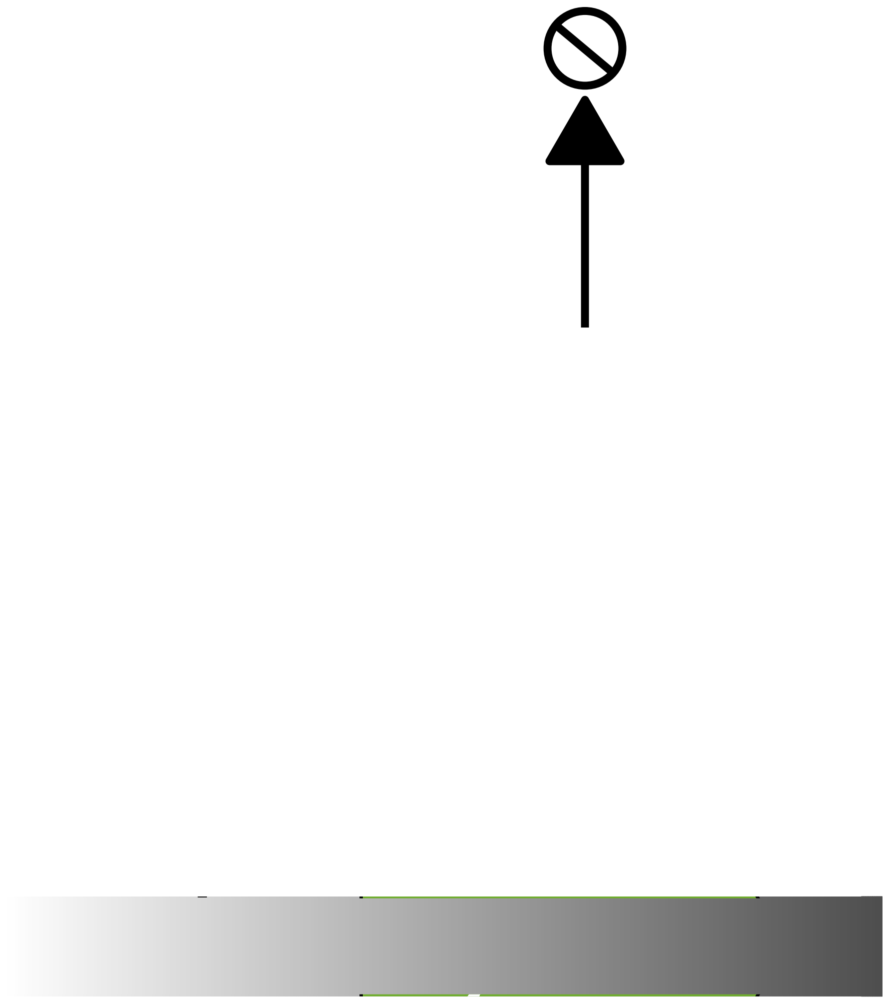
Positive feedback regulation can trigger bistability



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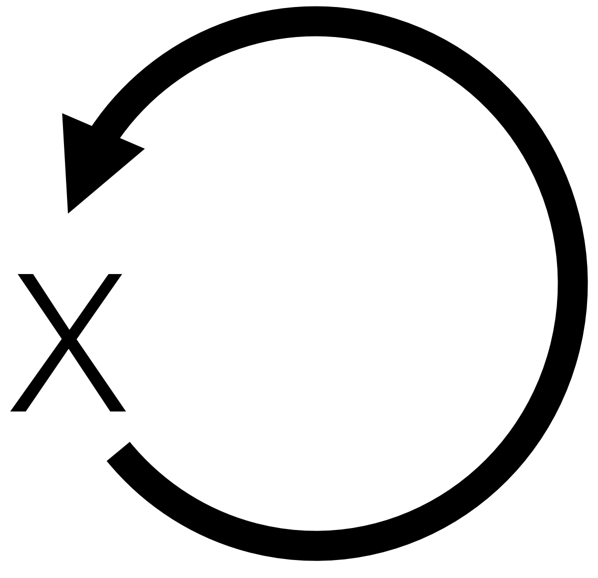
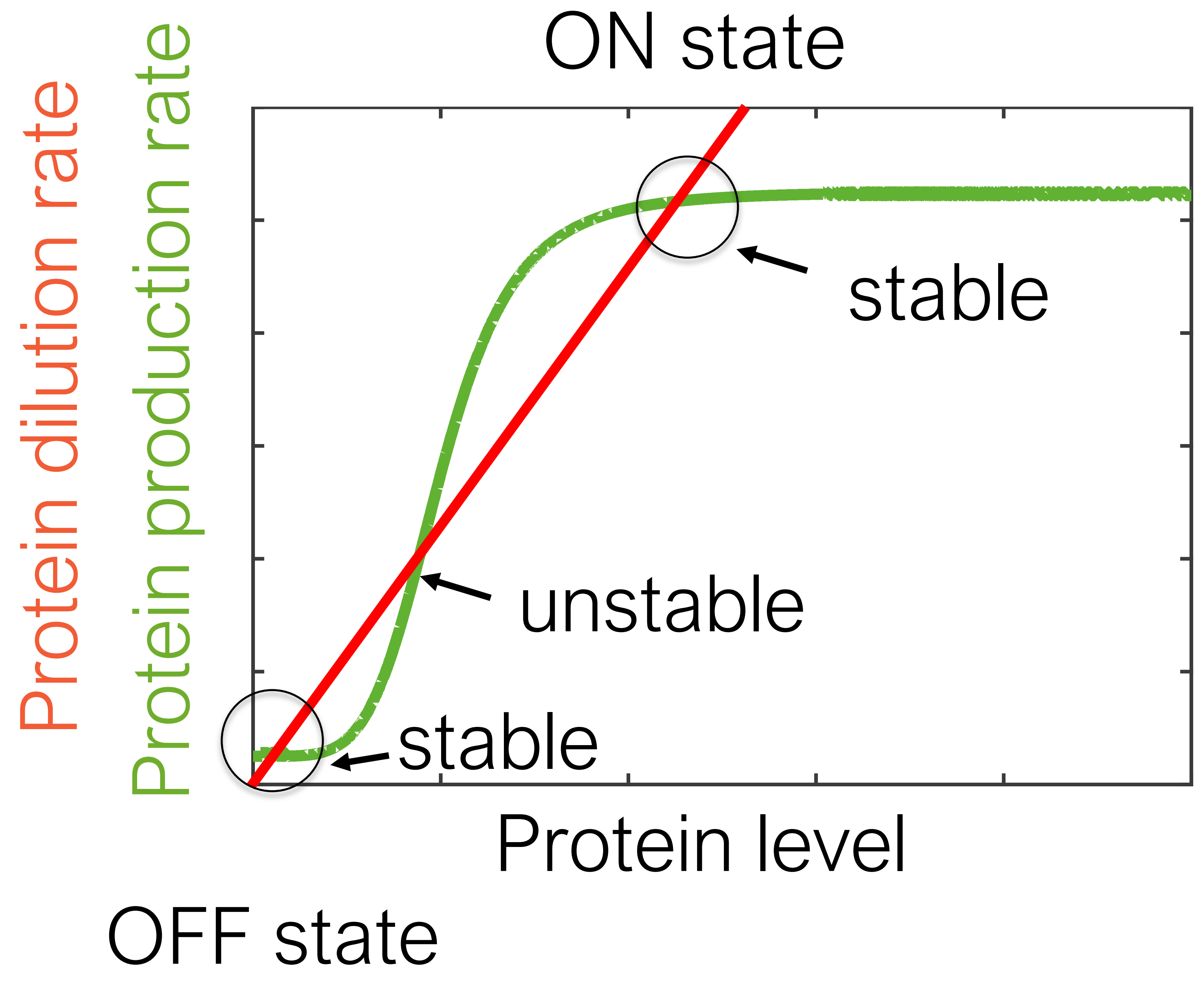
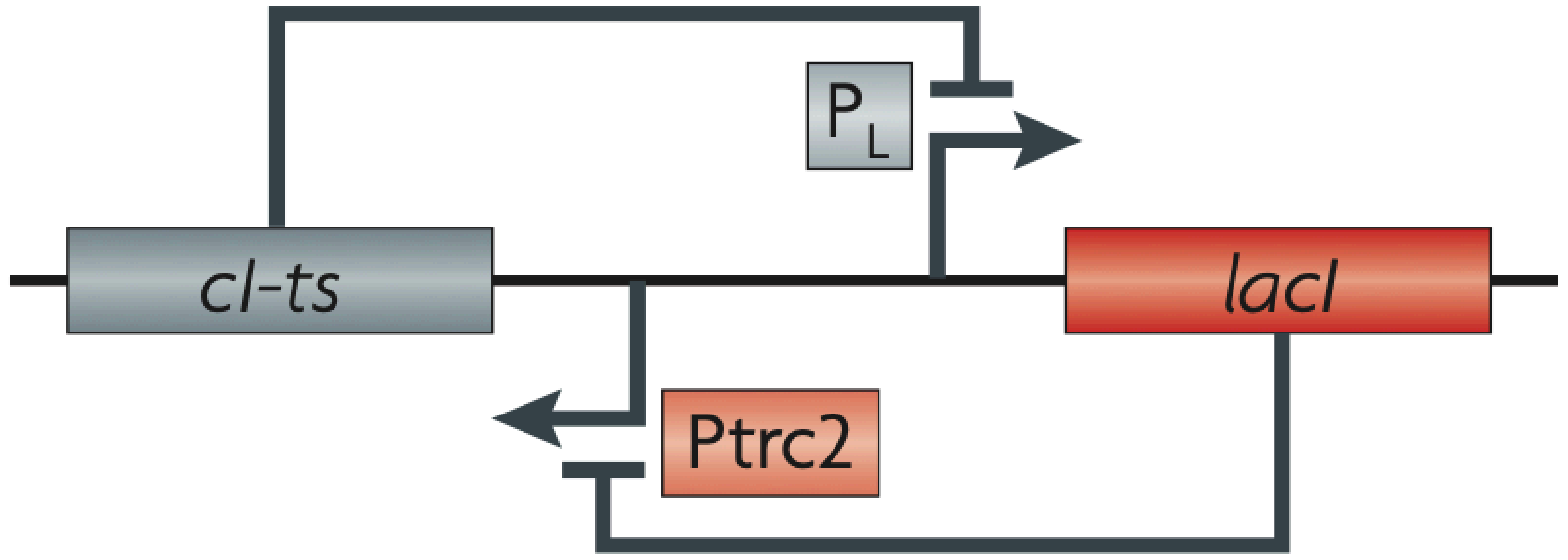


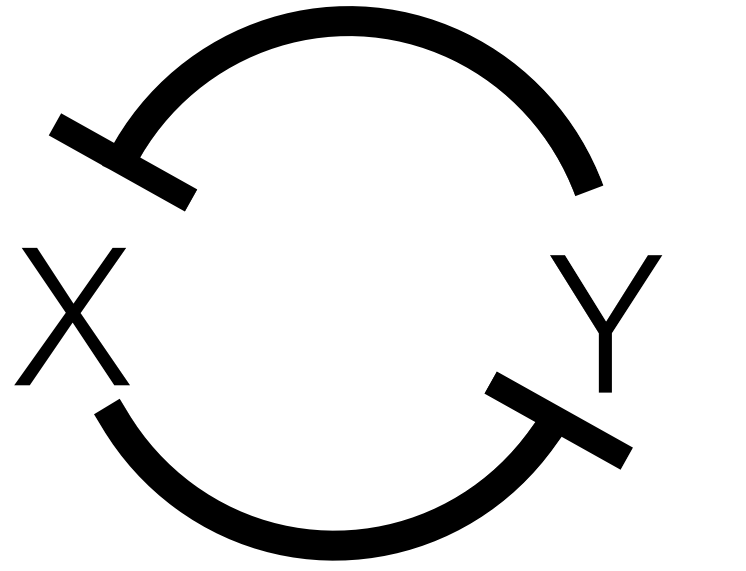


Two stable states = bistability

Genetic toggle switch



Double negative feedback



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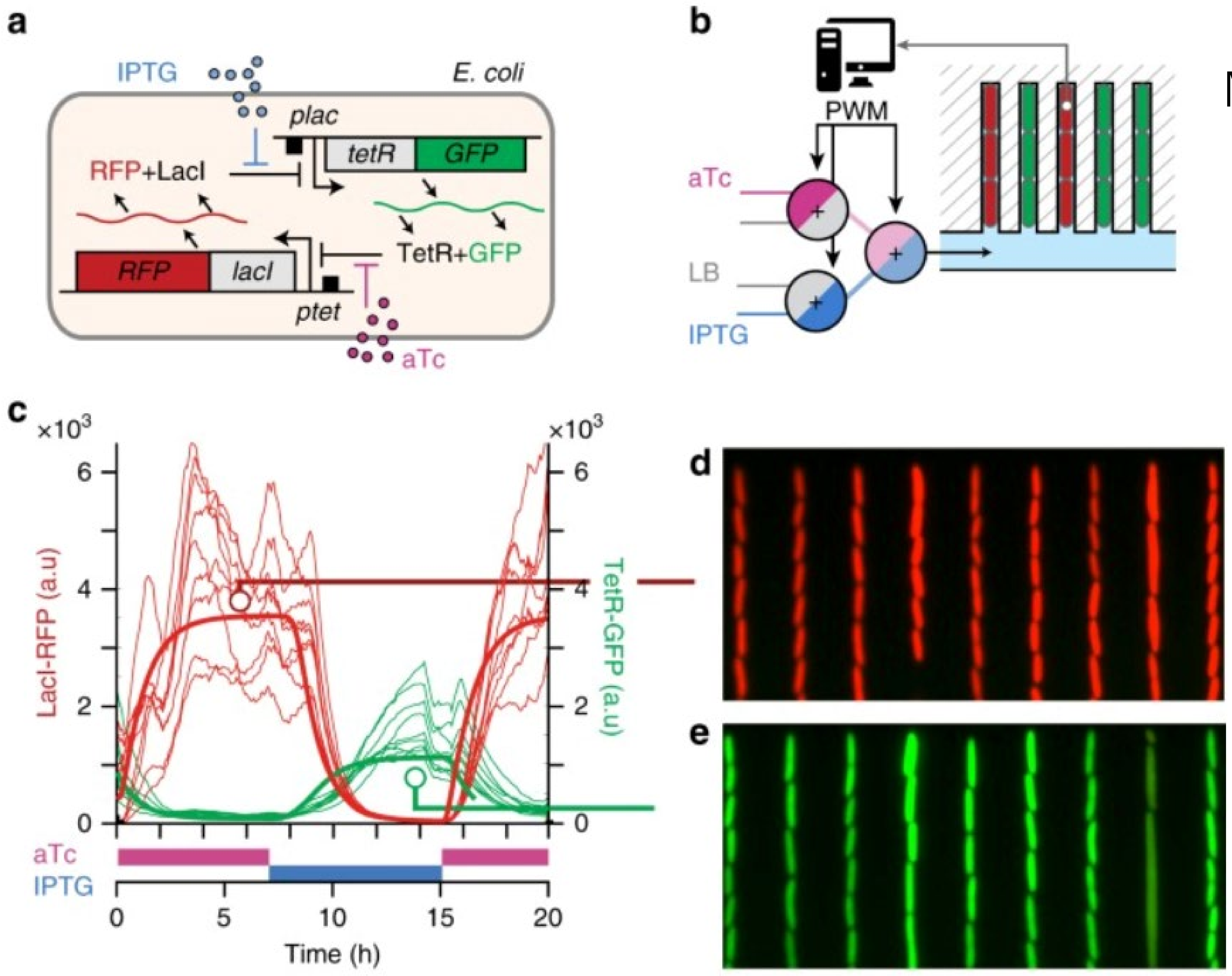
Positive feedback regulation can trigger bistability



Microfluidic device: “mother machine”

Lugagne et al. (2017), *Nat. Commun*, 8(1):1671

Negative feedback can trigger oscillations

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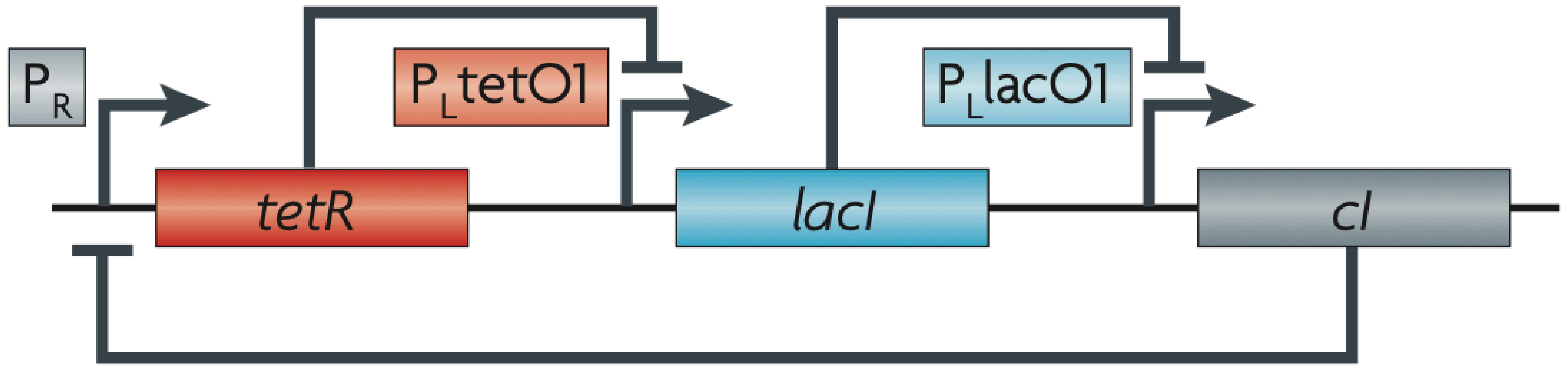
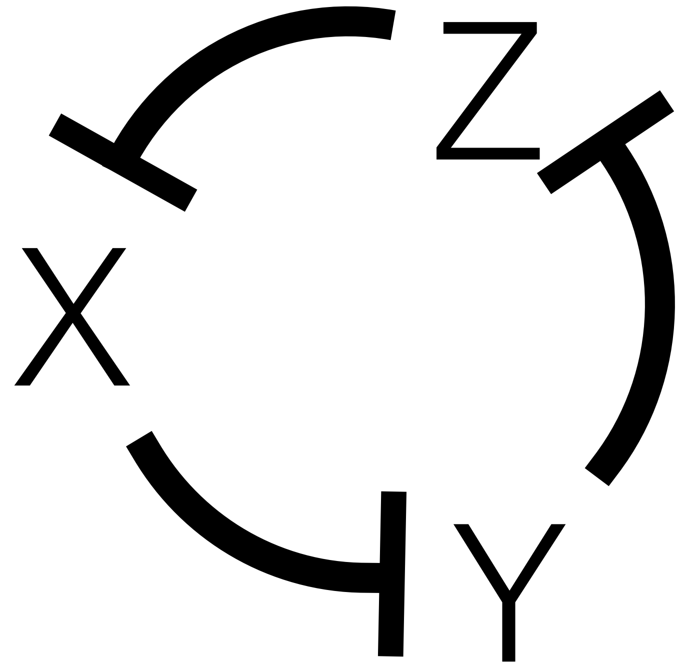
Repressilator circuit

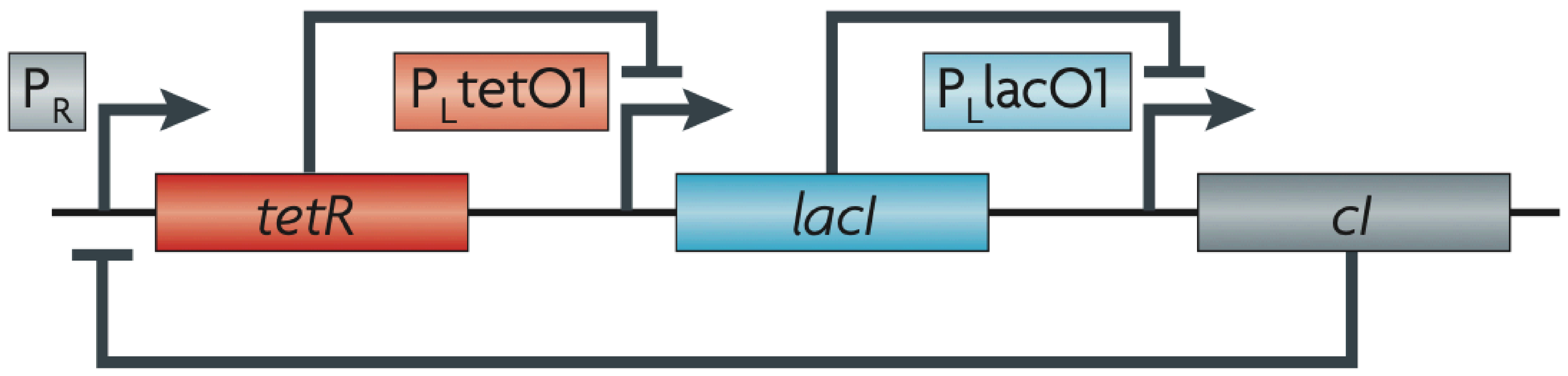


Triple negative feedback



Negative feedback can trigger oscillations

Repressilator circuit



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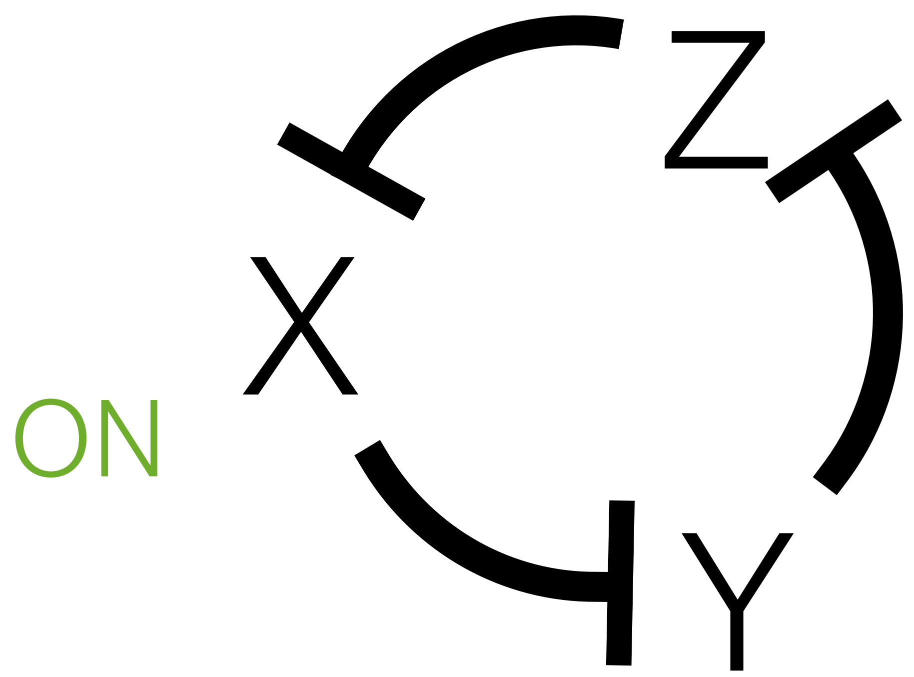

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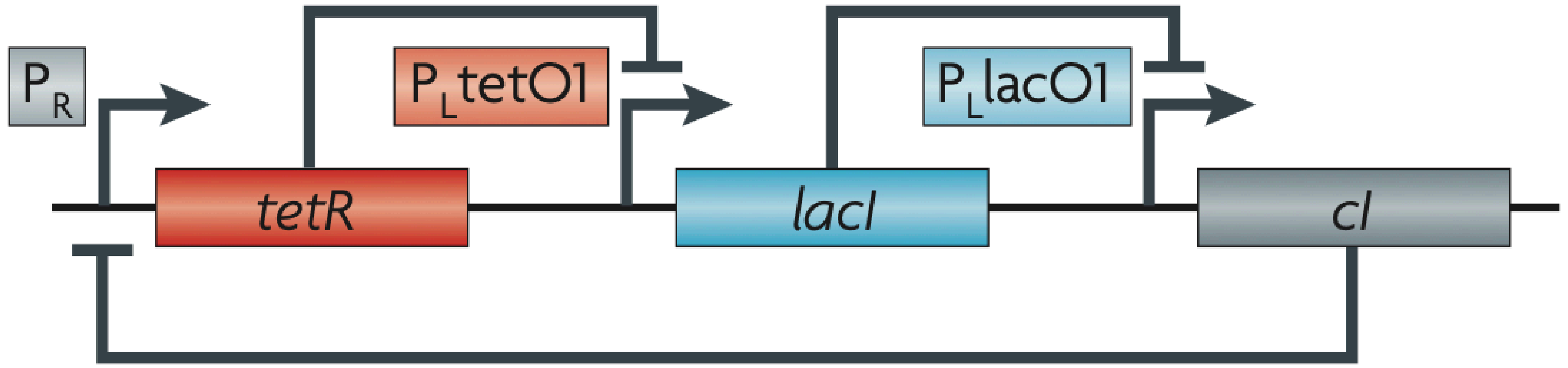

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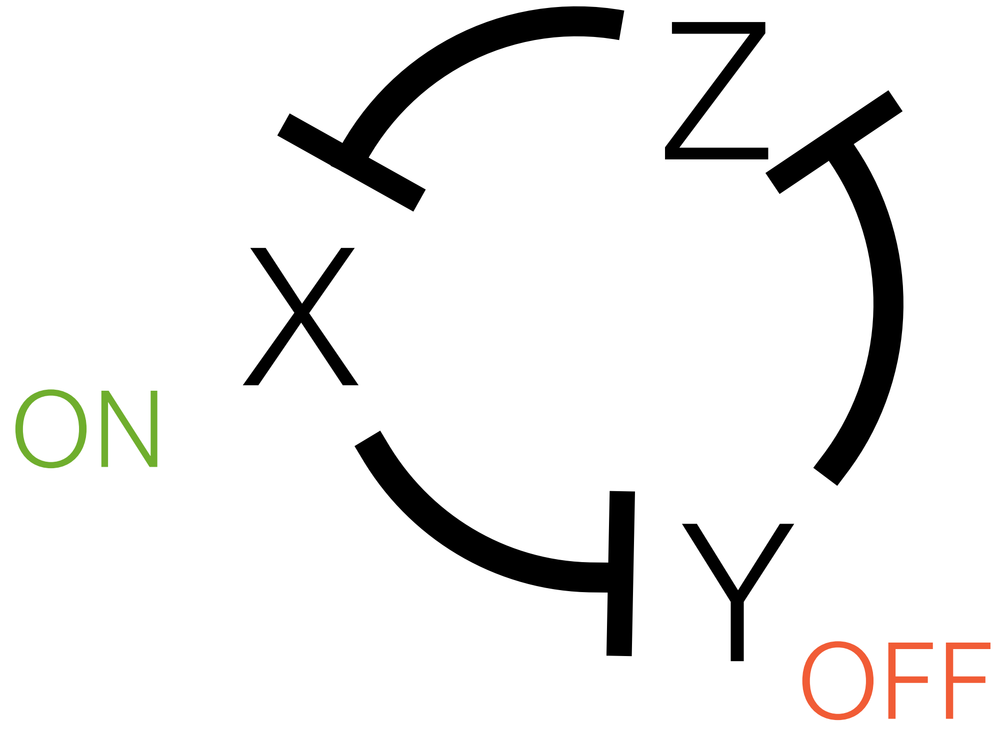

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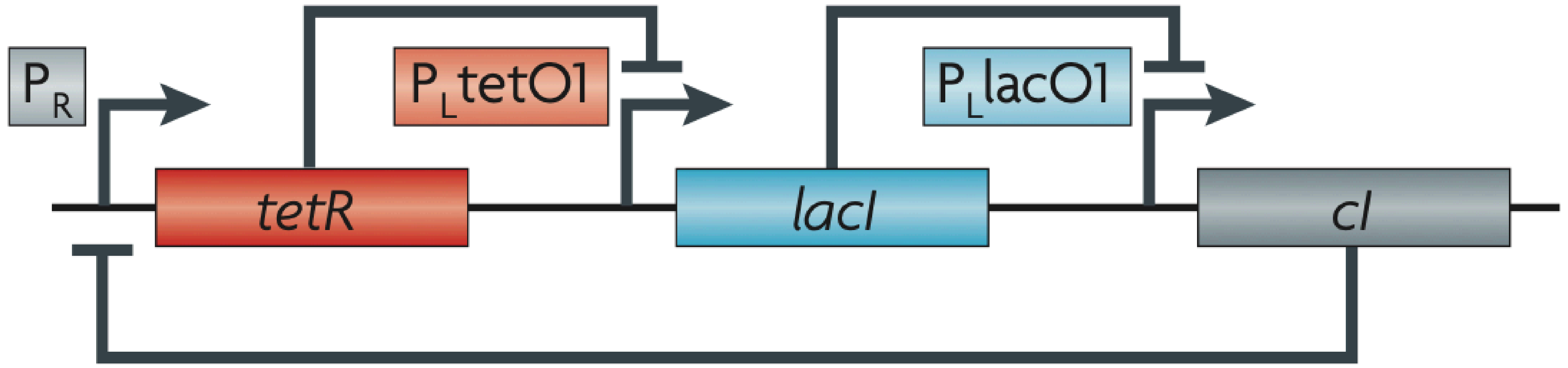

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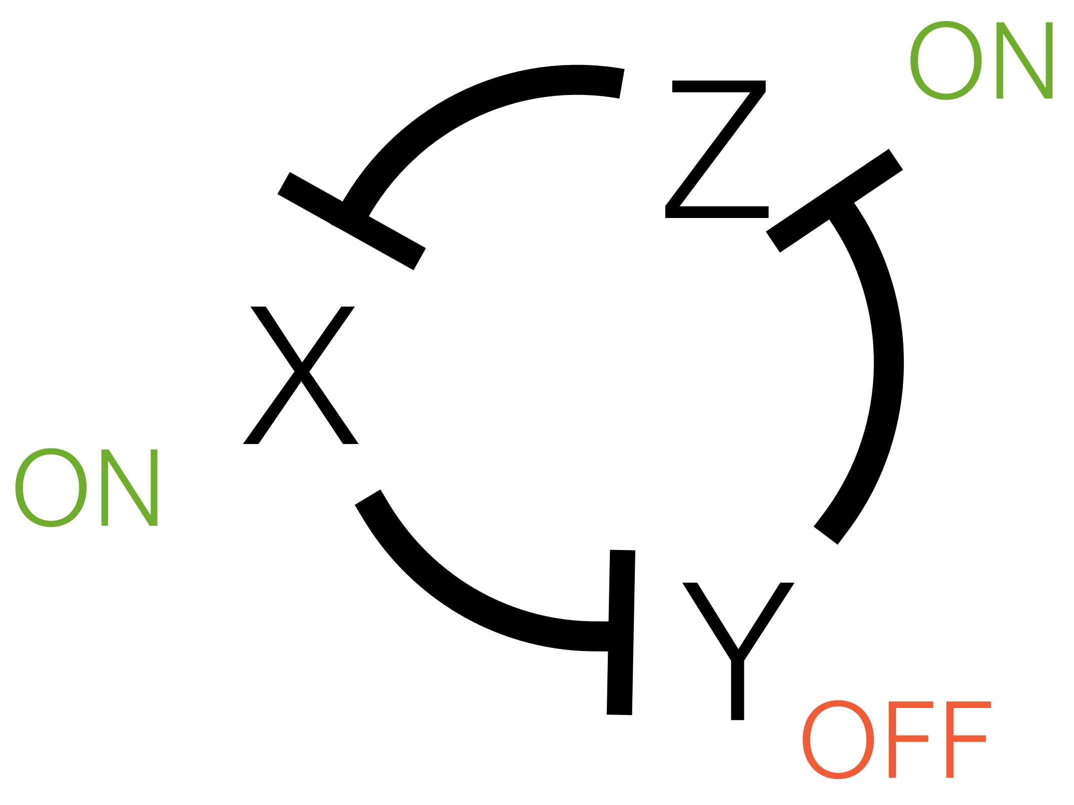

Negative feedback can trigger oscillations

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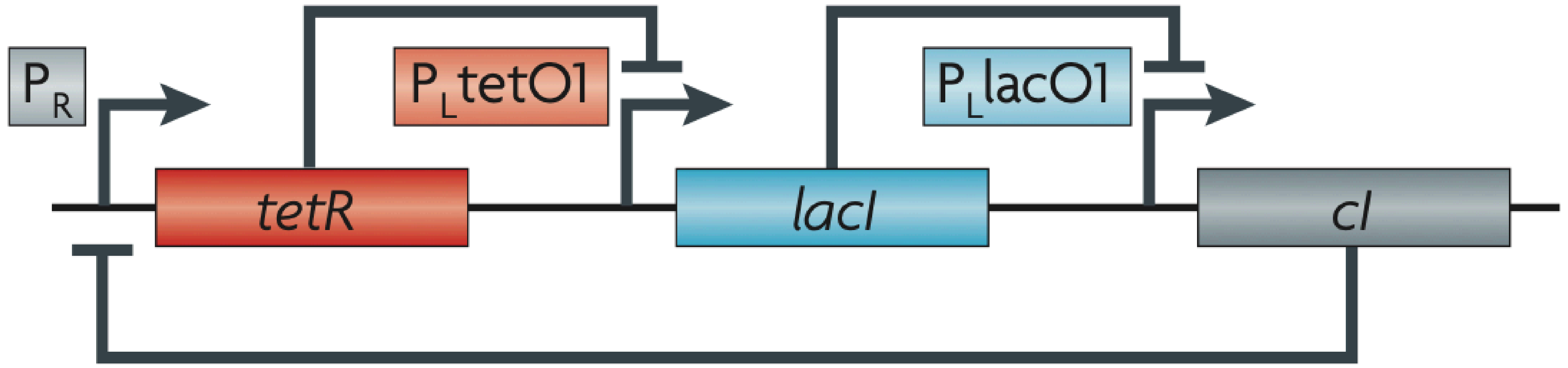
Repressilator circuit



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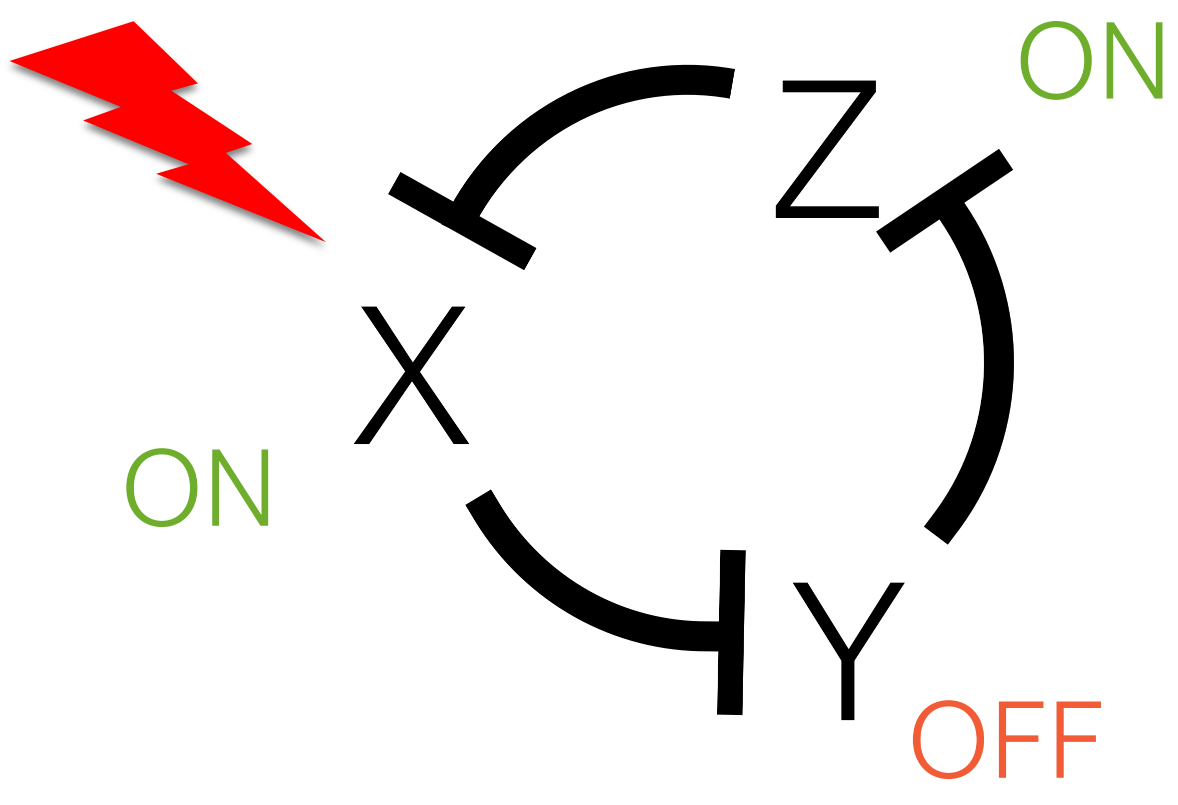

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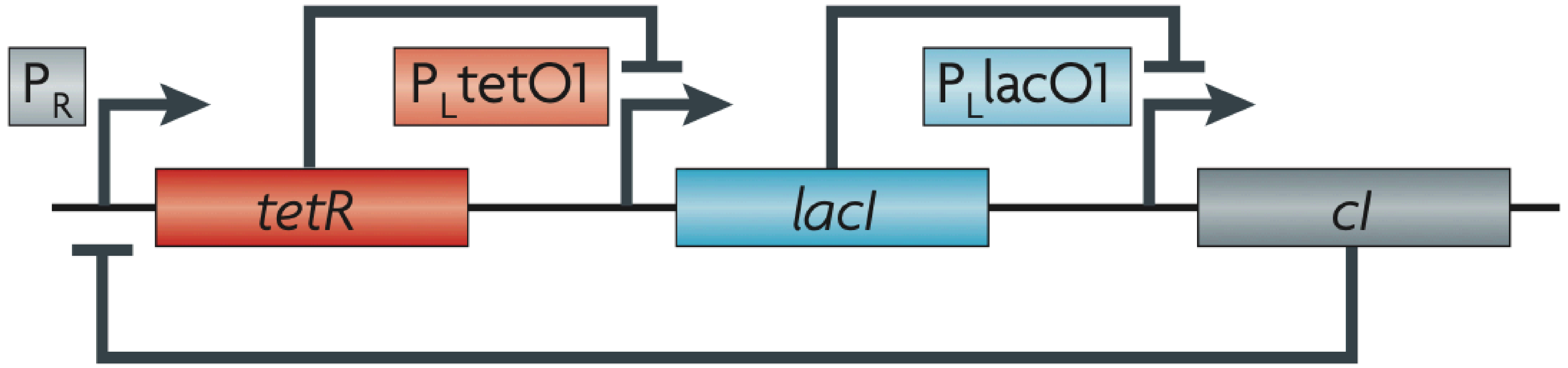

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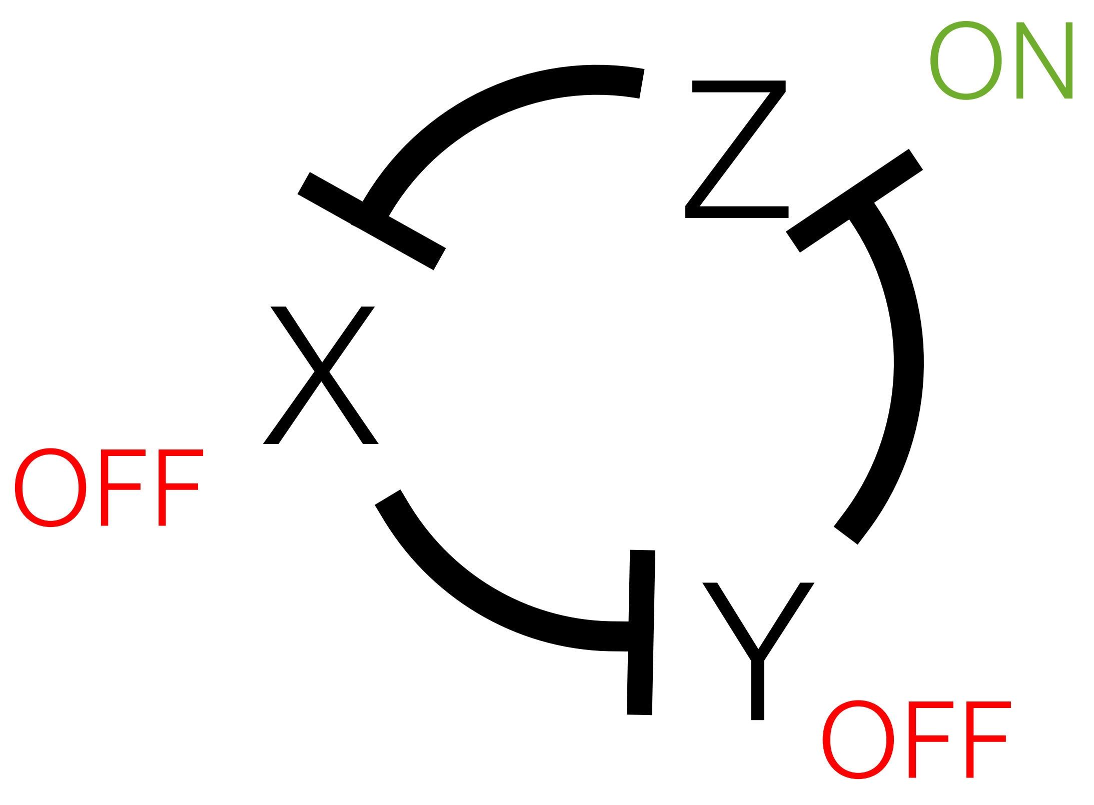

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Negative feedback can trigger oscillations

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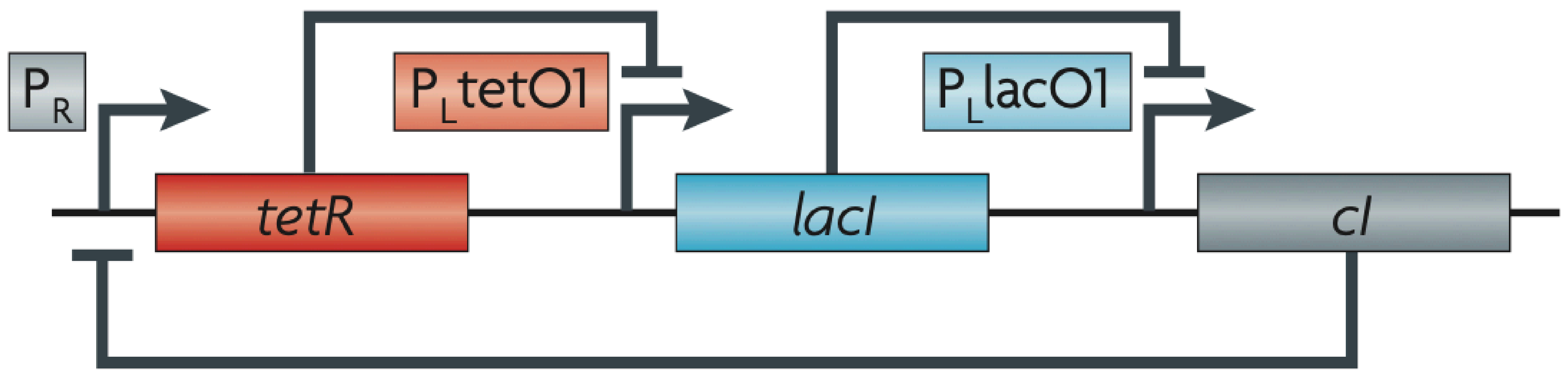

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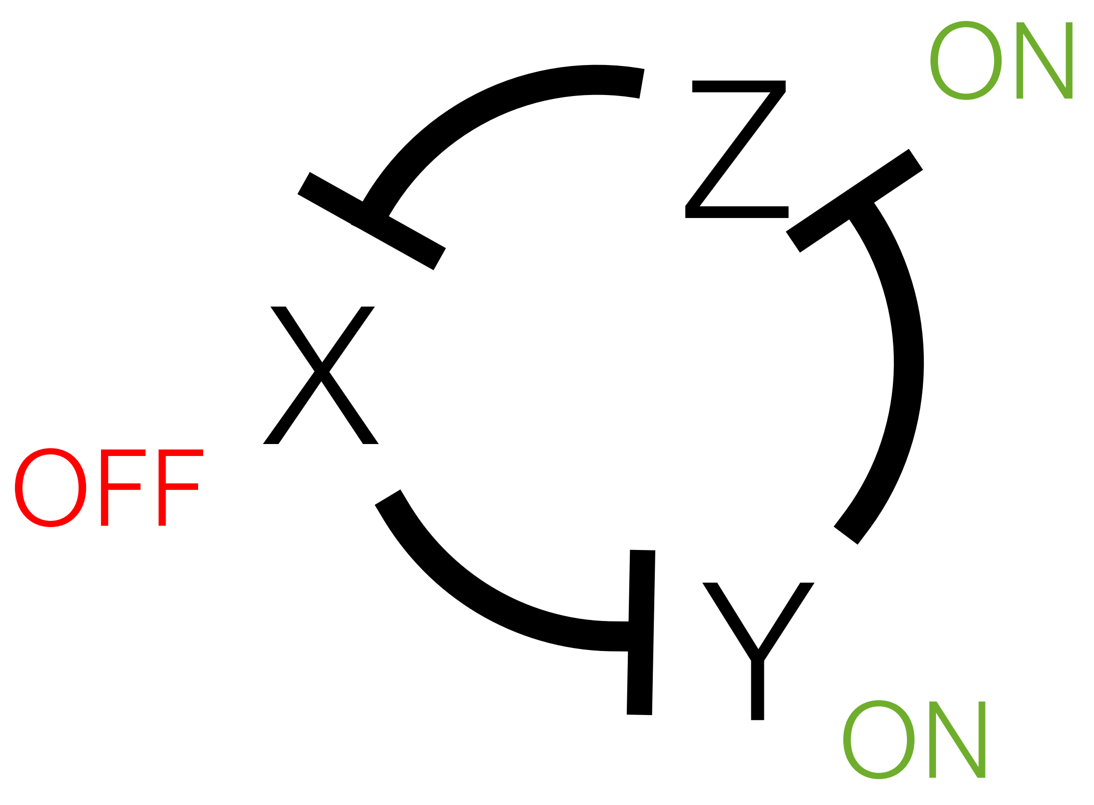

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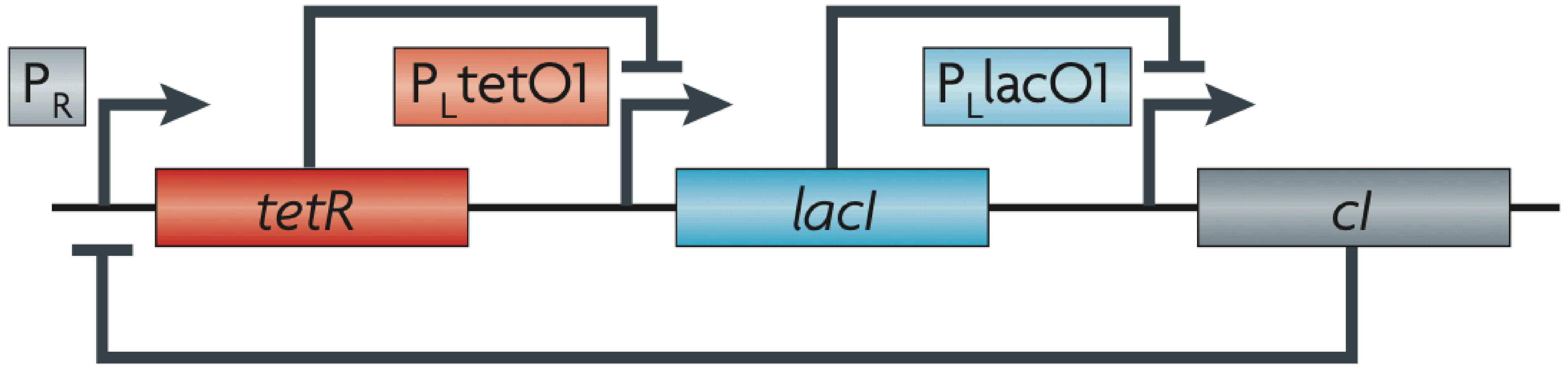
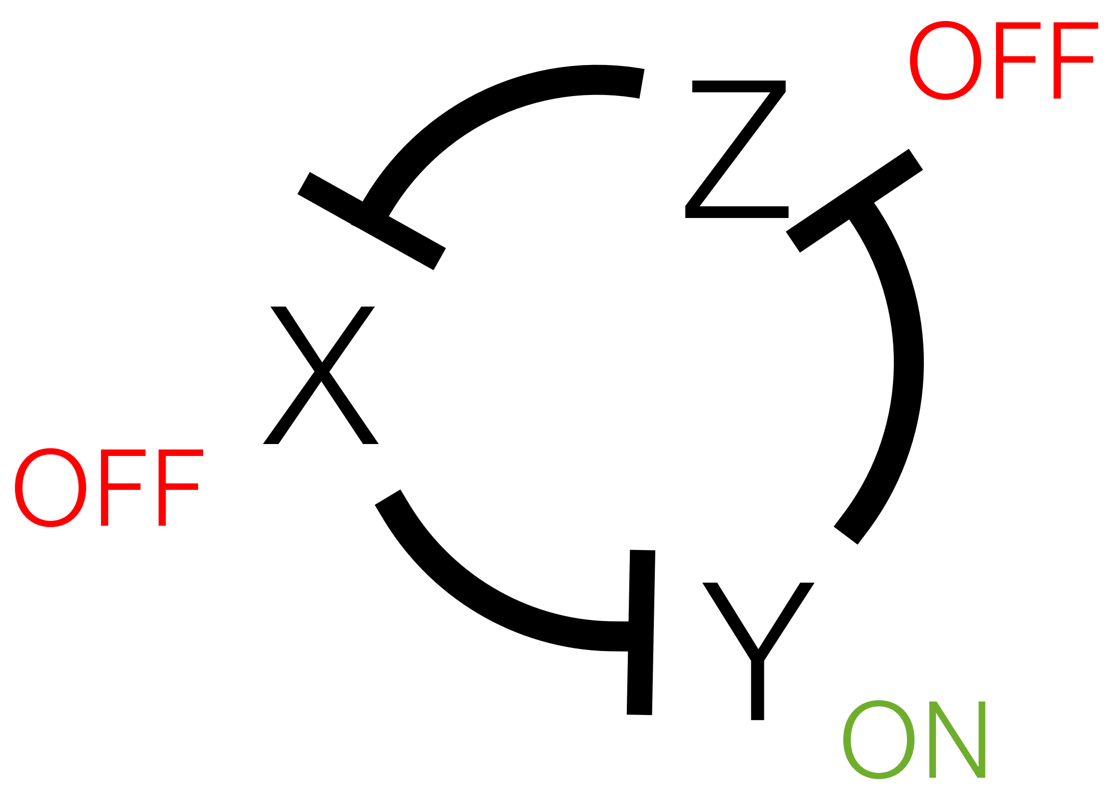


Elowitz & Leibler (2000), *Nature*, 23:689-694

Triple negative feedback



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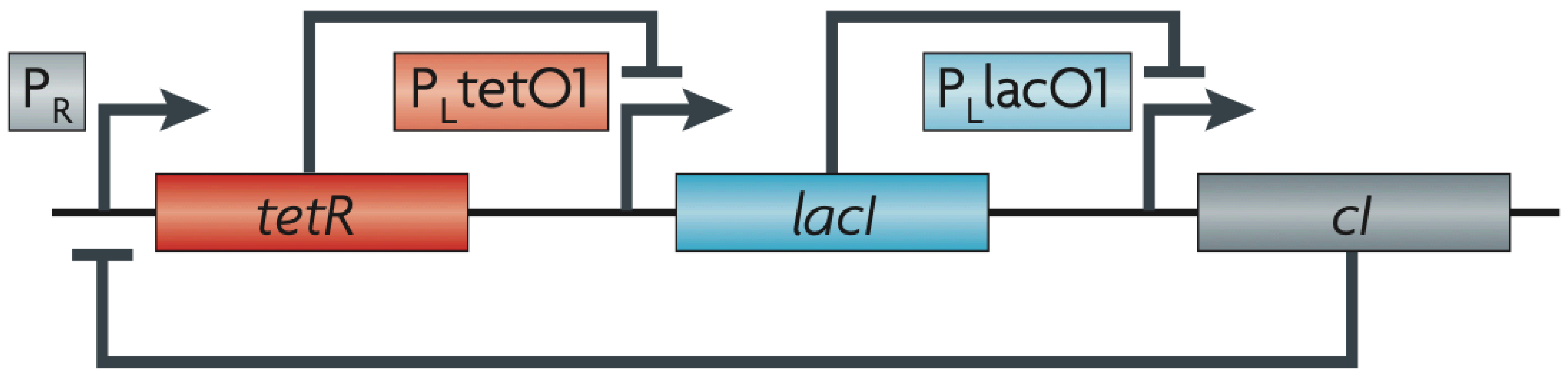

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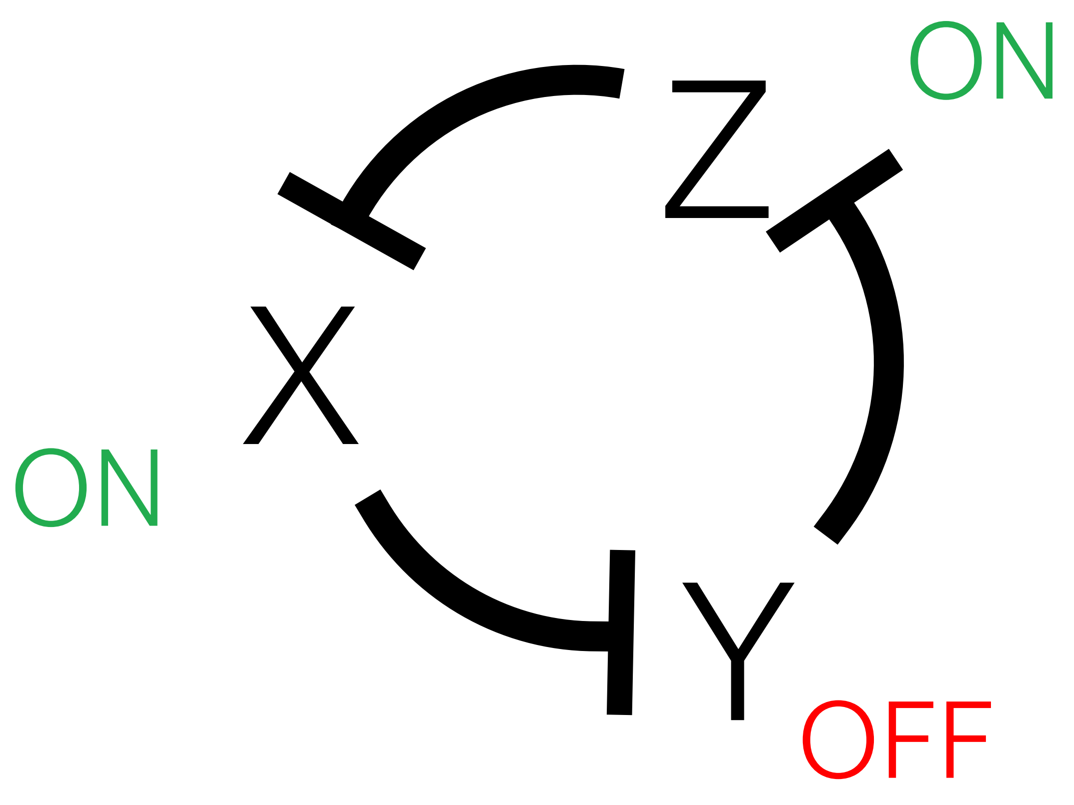
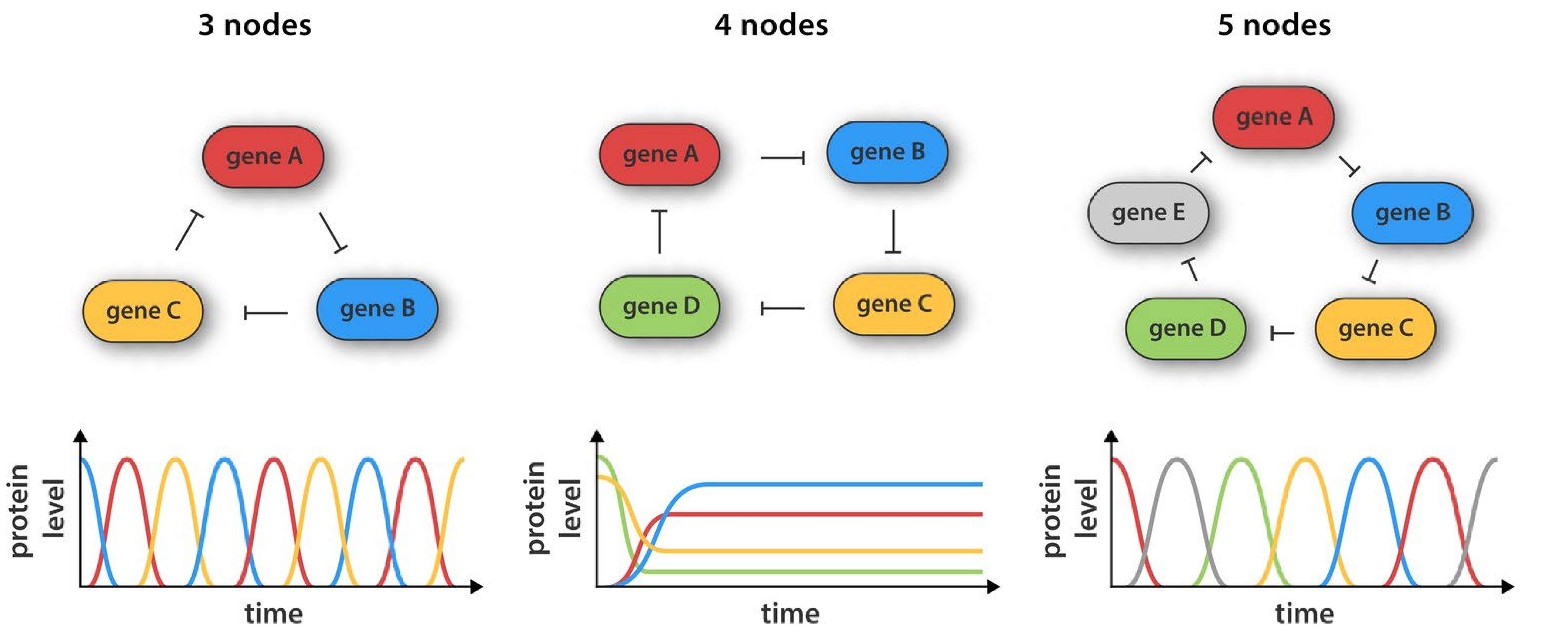
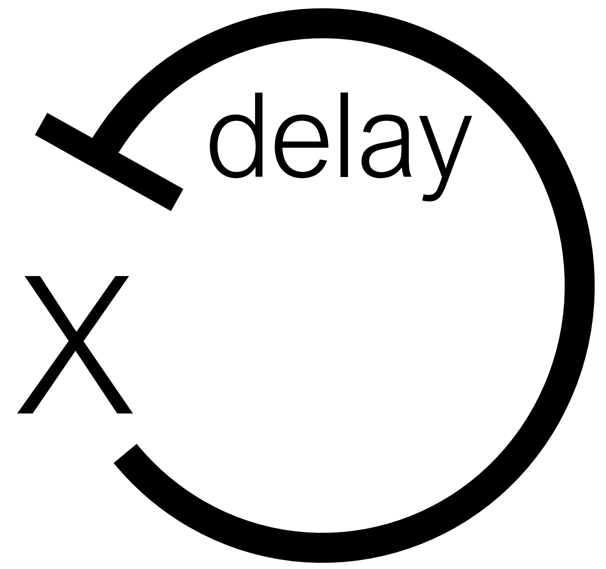


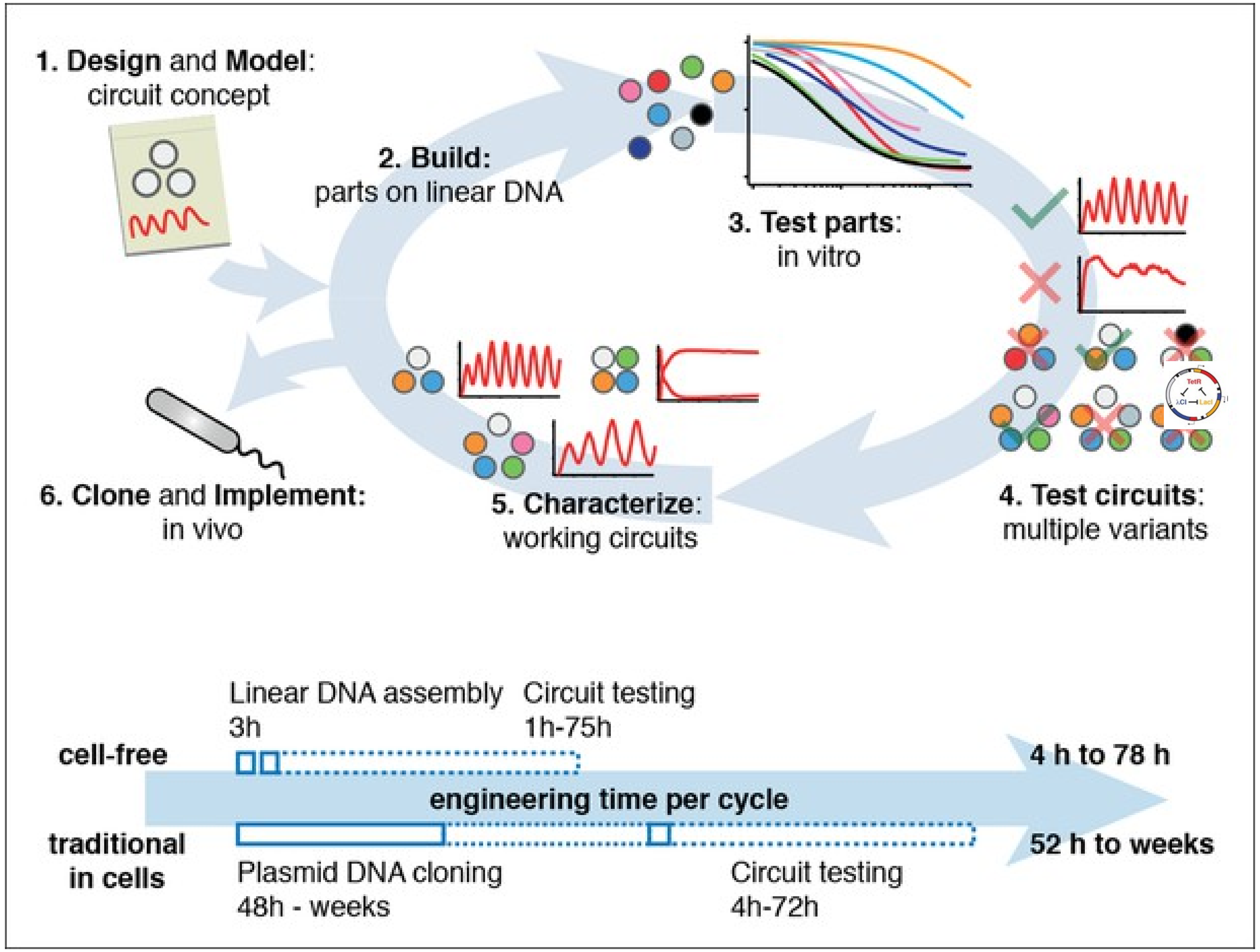


Rosier and de Greef. eLife 2015;4:e12260

Time delayed negative feedback is necessary for oscillations!

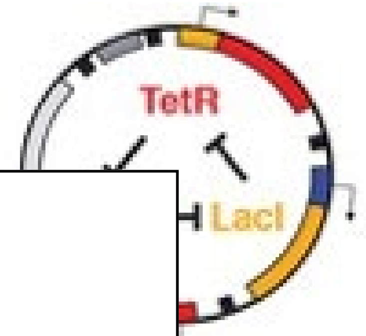


Negative feedback can trigger oscillations

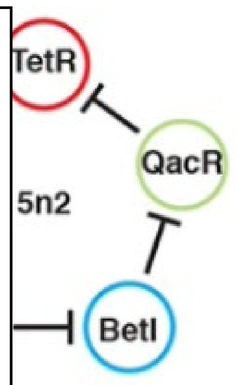


Niederholtmeyer et al. eLife 2015;4:e09771

In vitro gene expression



In vivo gene expression in “mother machine”



Negative feedback can trigger oscillations

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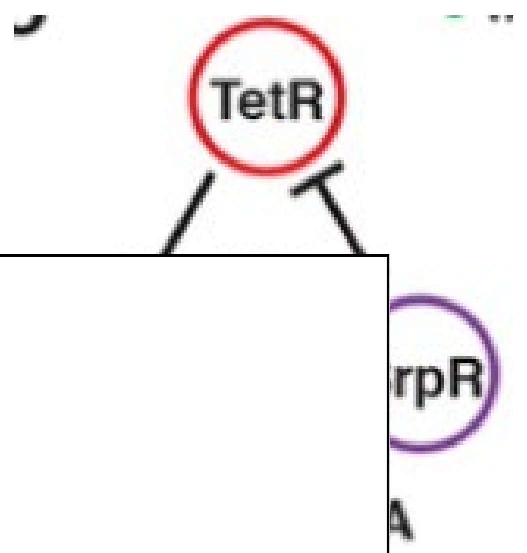
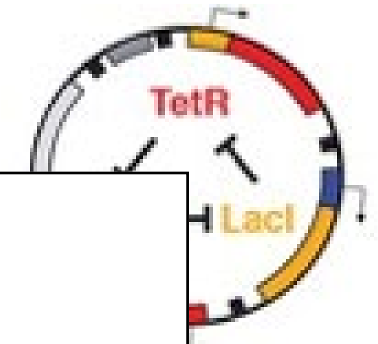
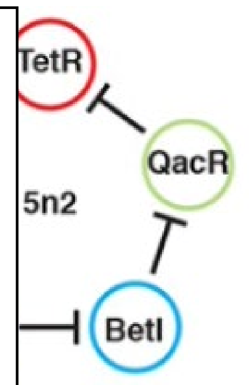
In vivo gene expression in “CellASIC” chamber



In vitro gene expression



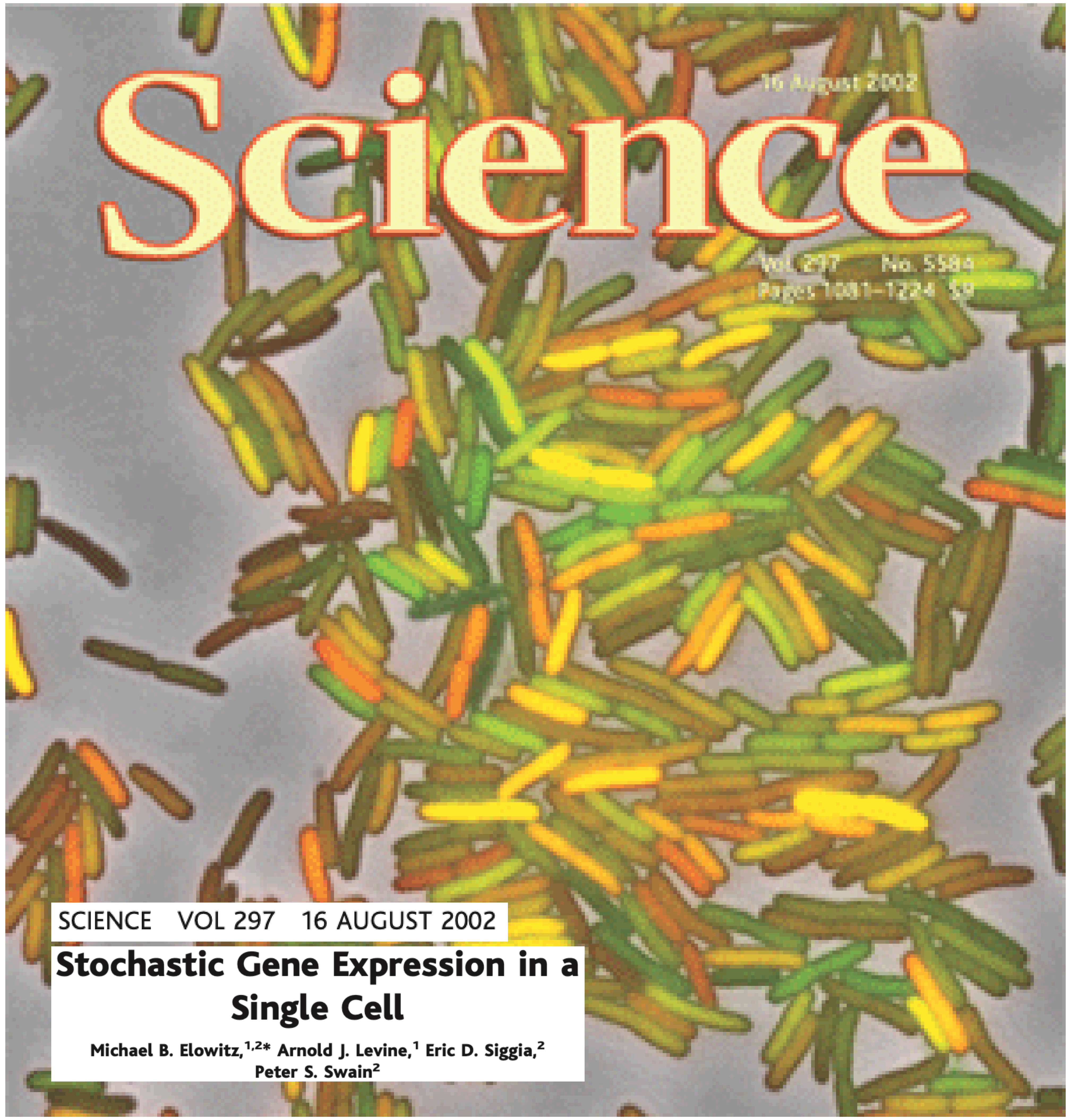
In vivo gene expression in “mother machine”



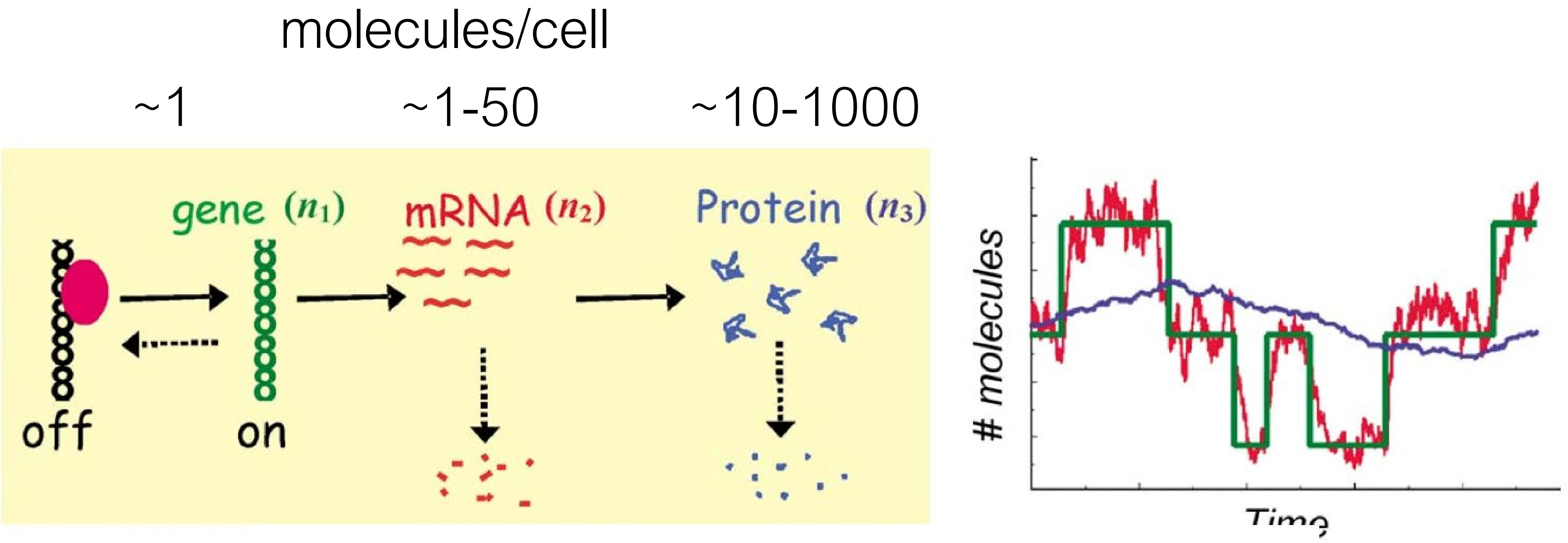
Niederholtmeyer et al. eLife 2015;4:e09771

What limits genetic circuit design?

Limitations to synthetic circuit design: Gene expression noise



At low molecule numbers, biochemical reactions are stochastic events.



J. Paulsson / Physics of Life Reviews 2 (2005) 157–175

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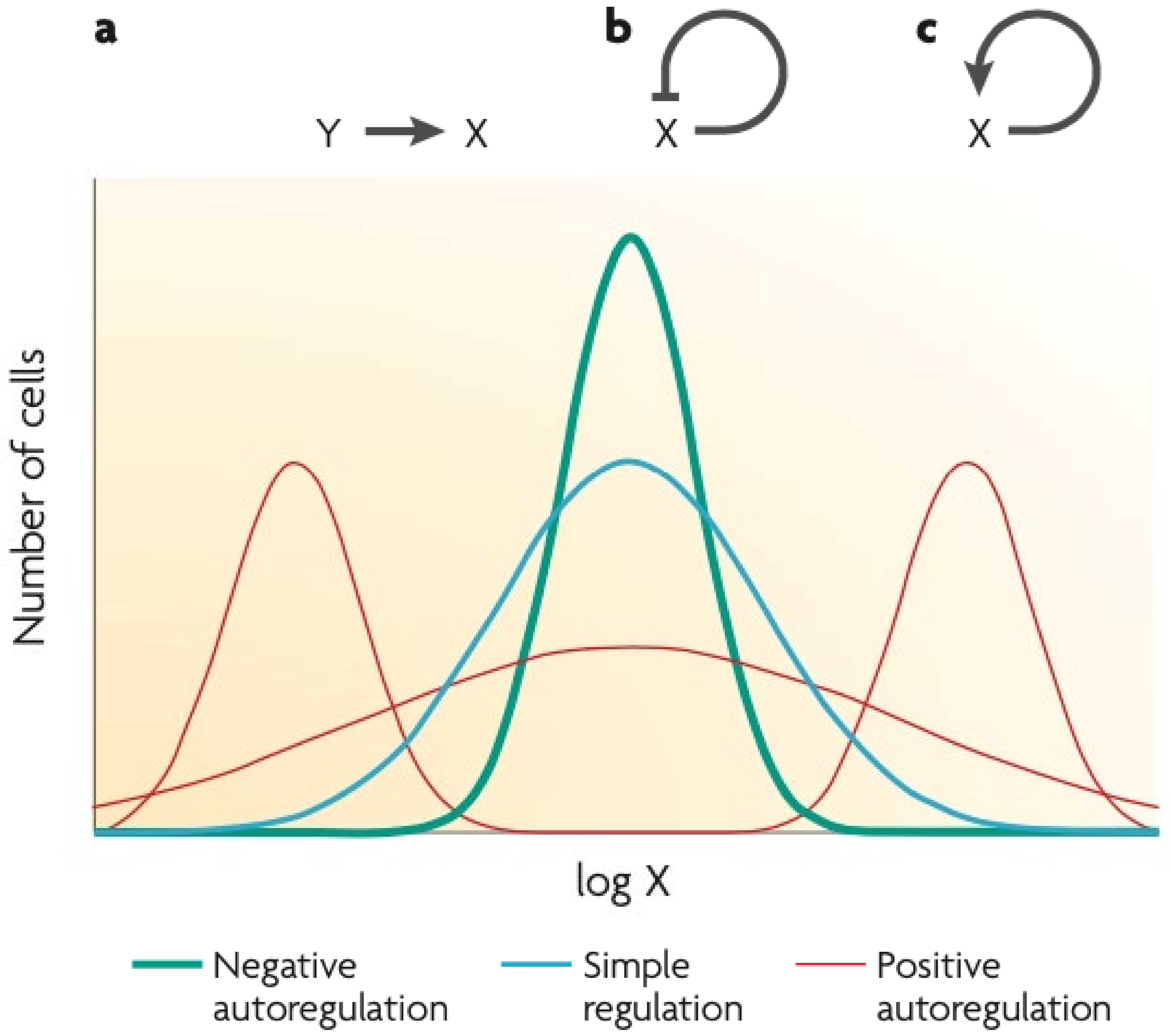
Controlling gene expression noise via feedback regulation

- Schematic cell–cell distribution of protein levels for simple regulation
- Negative autoregulation tends to make this distribution narrower in comparison with simple regulation.
- Positive autoregulation tends to make the distribution wider, and in extreme cases bimodal with two populations of cells.



Alon, Nat. Rev Genet. 2007;8:450

Limitations to synthetic circuit design: Genetic context dependence of gene activity

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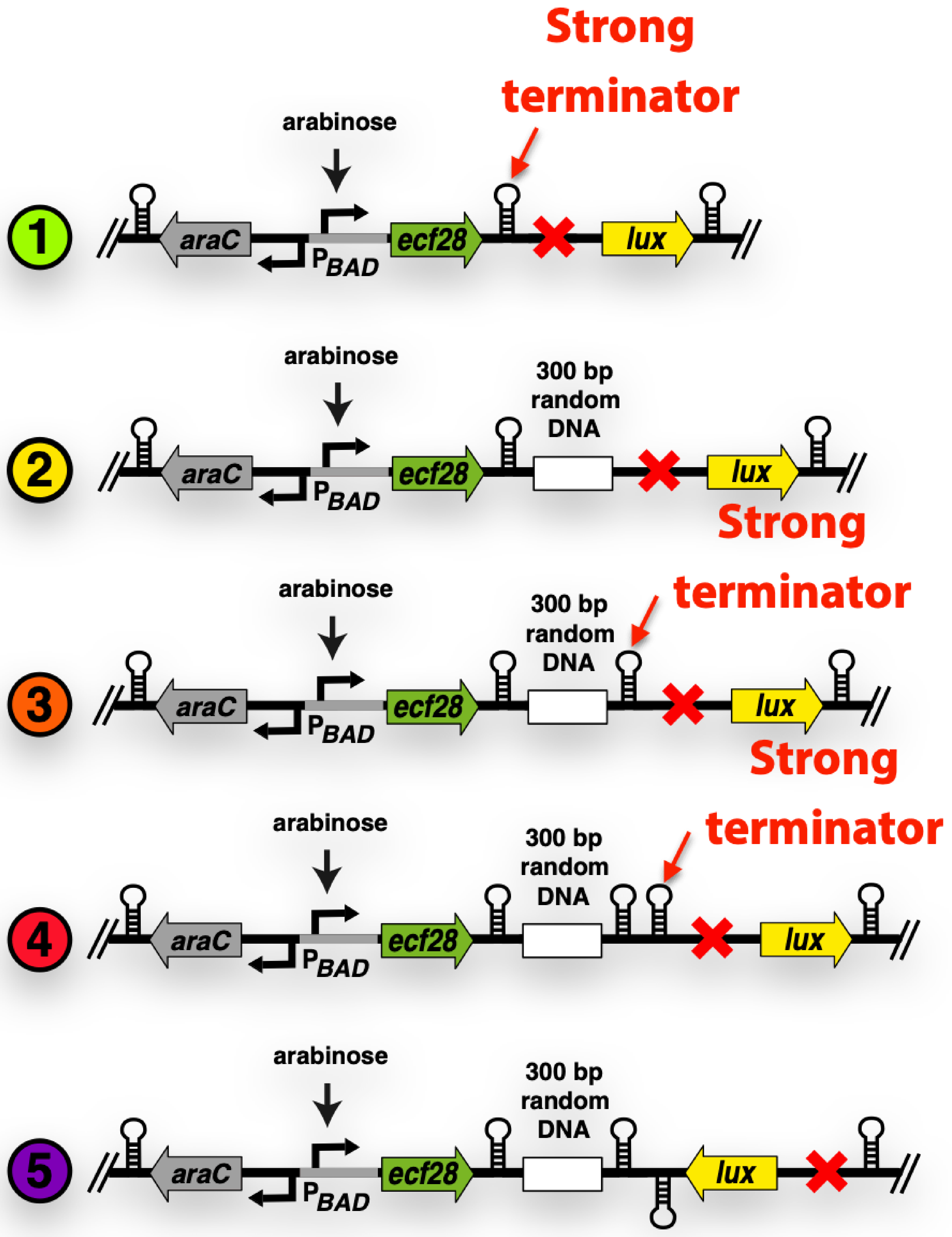

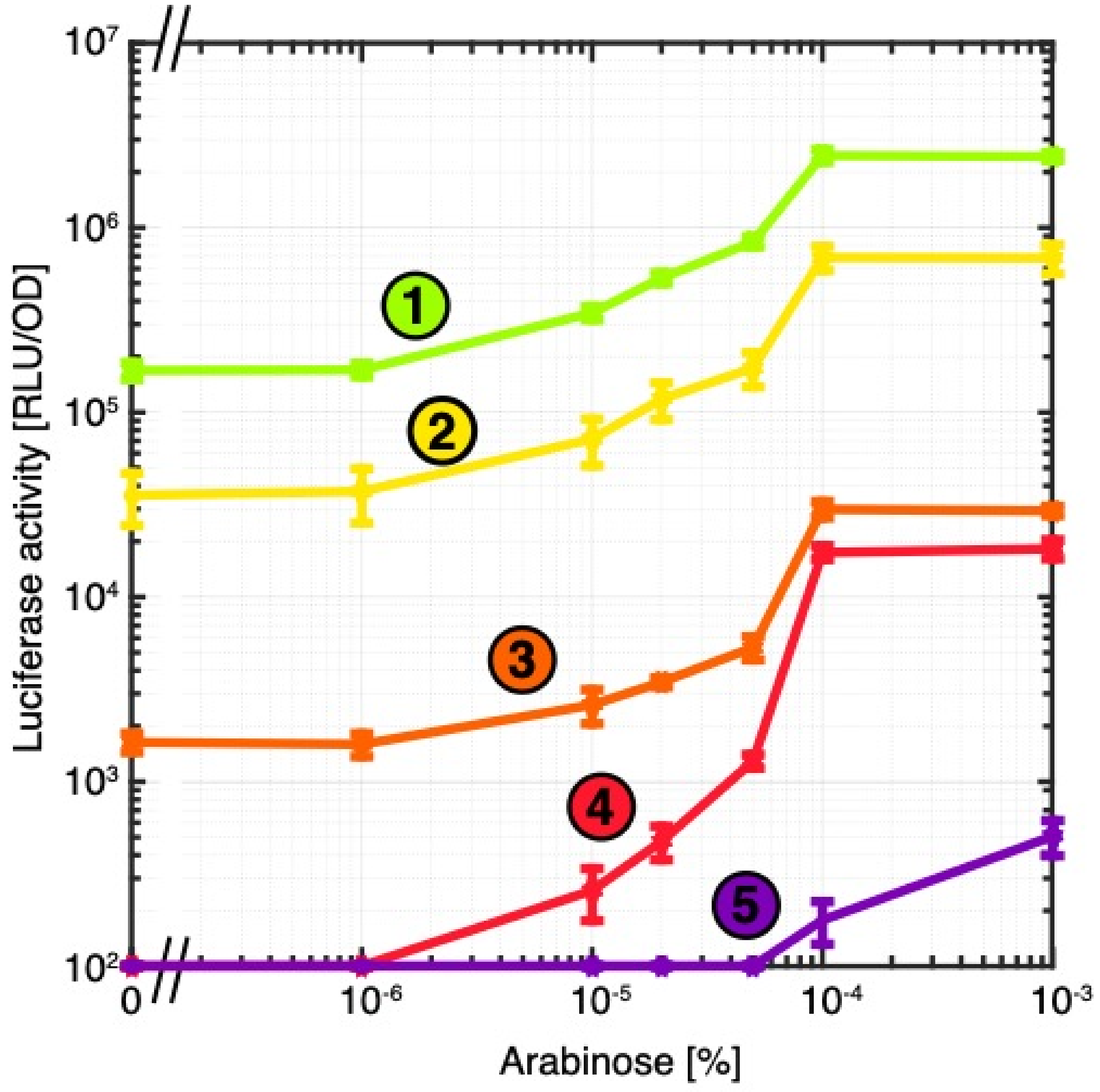

Undesired reporter gene activation

Pinto, Vecchione et al. (2018), *Nucl. Acids Res.*, 46(14): 7450–7464

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Limitations to synthetic circuit design: Genetic context dependence of gene activity

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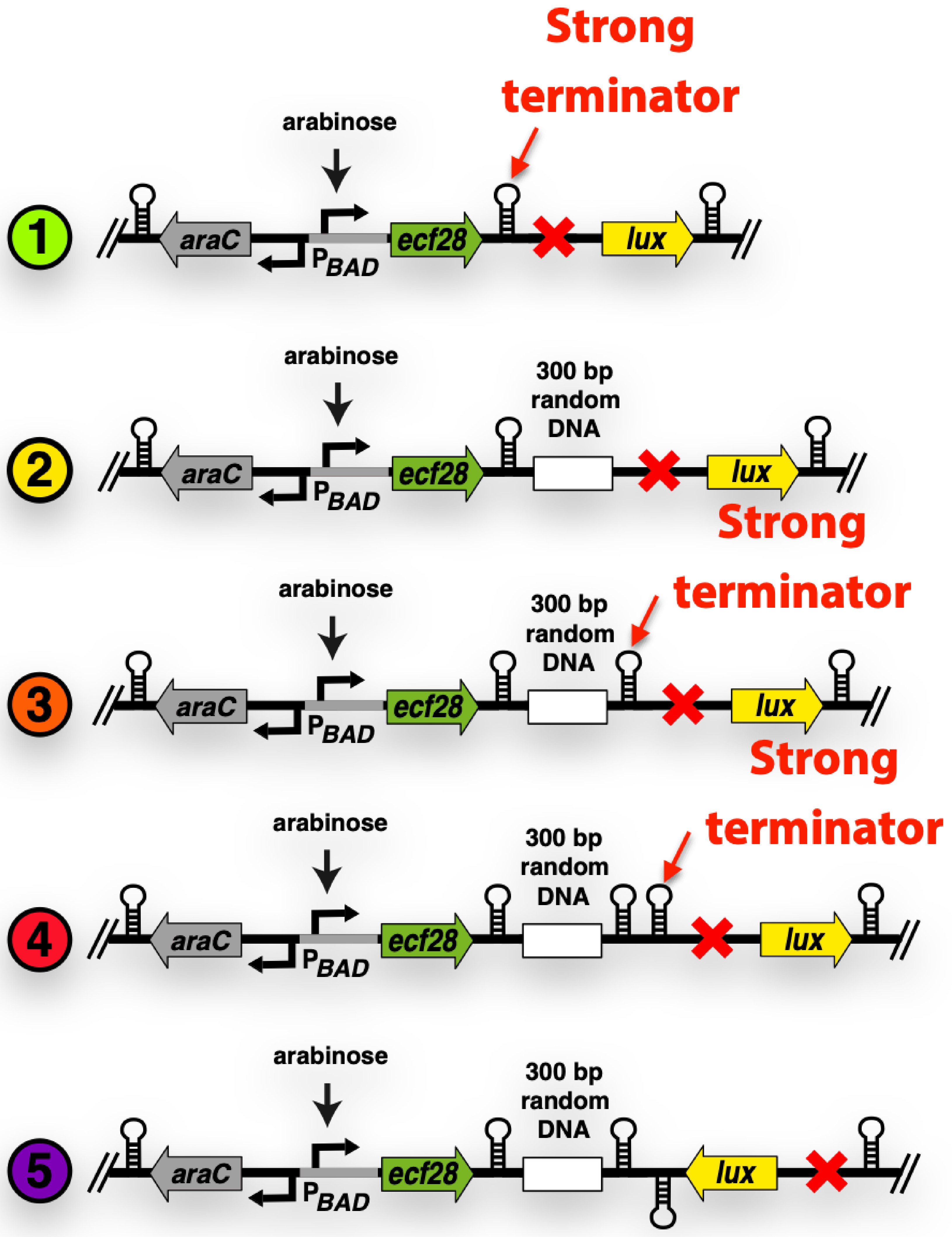
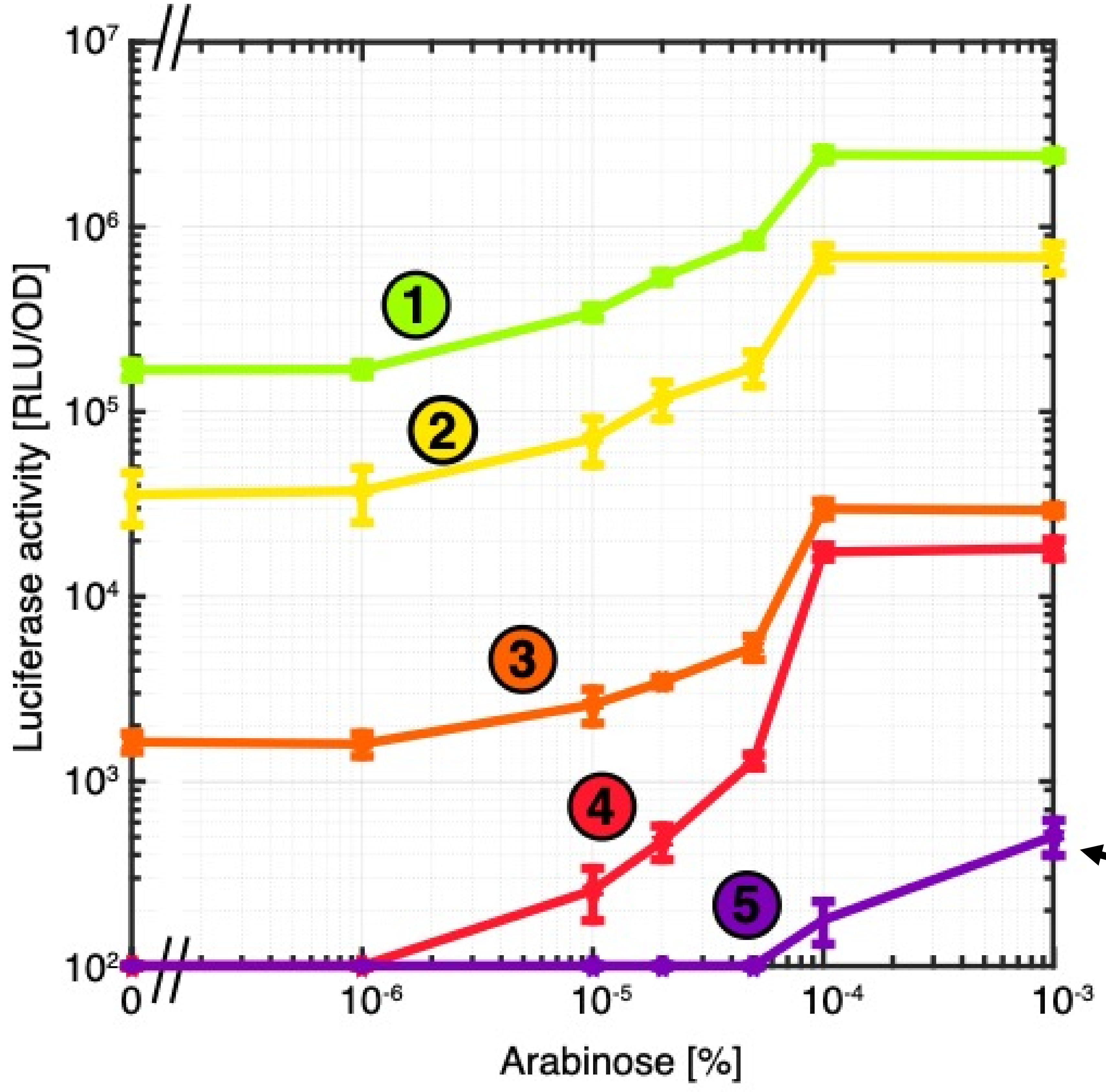




Pinto, Vecchione et al. (2018), *Nucl. Acids Res.*, 46(14): 7450–7464

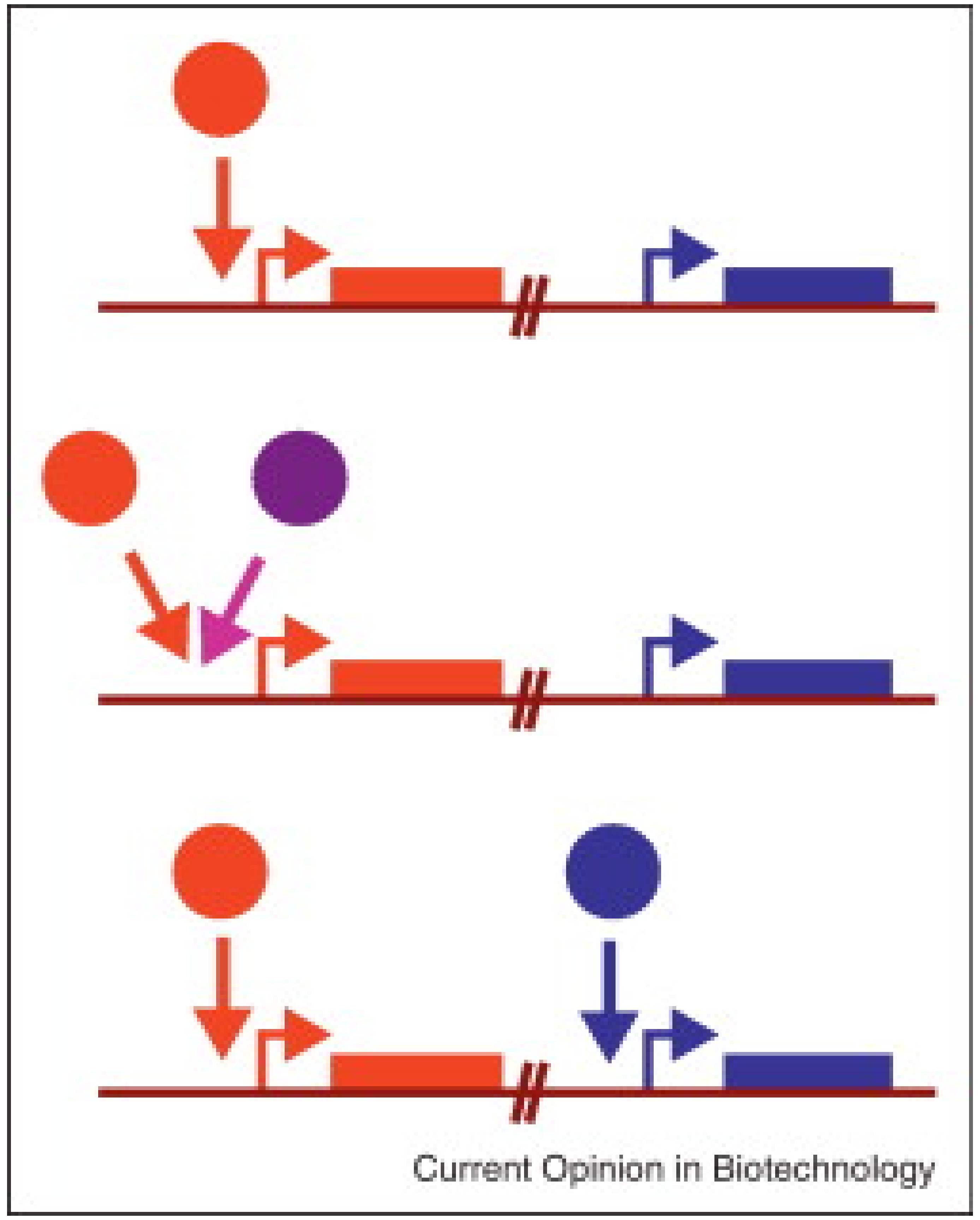
Undesired reporter gene activation

Prevent transcriptional read-through by insulators + alternating orientation of transcription units

Limitations to synthetic circuit design: Regulatory cross-talk

Gene duplication of regulator leads to redundant regulation

One gene regulator freely evolves to recognise another operator sequence

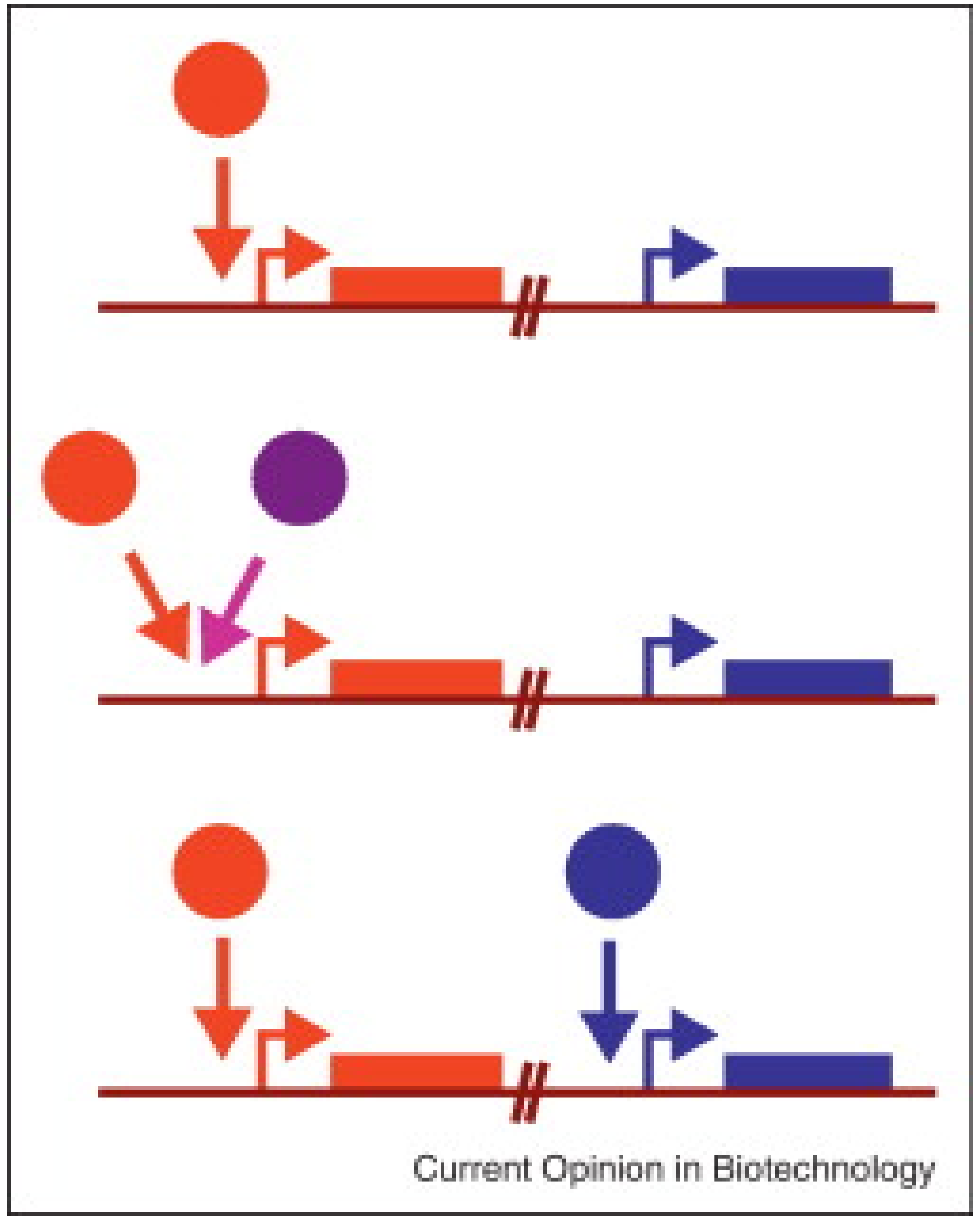


Rao (2012), *Curr. Opin. Biotech.*, 23:689-694

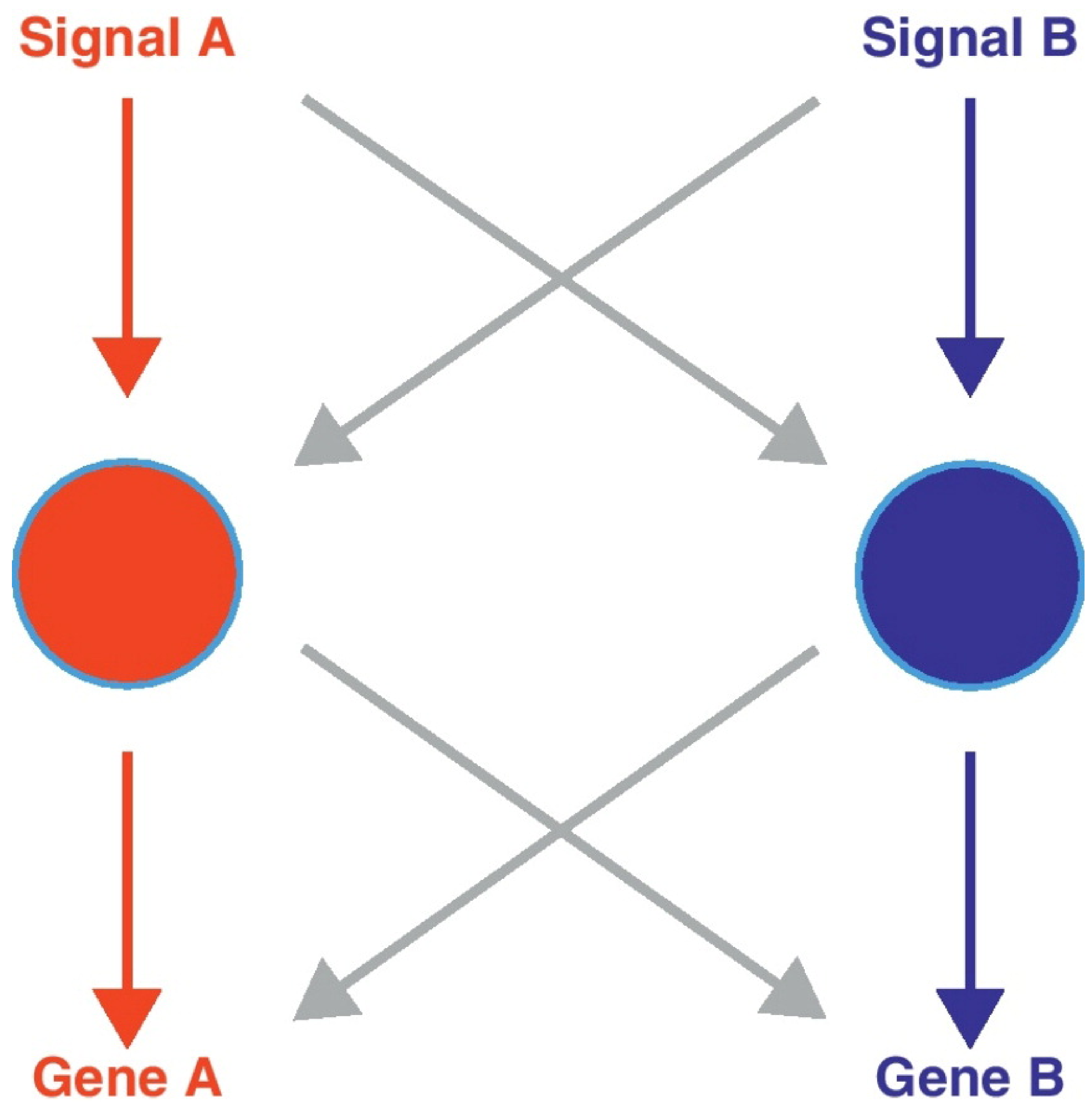
Limitations to synthetic circuit design: Regulatory cross-talk

Gene duplication of regulator leads to redundant regulation

One gene regulator freely evolves to recognise another operator sequence



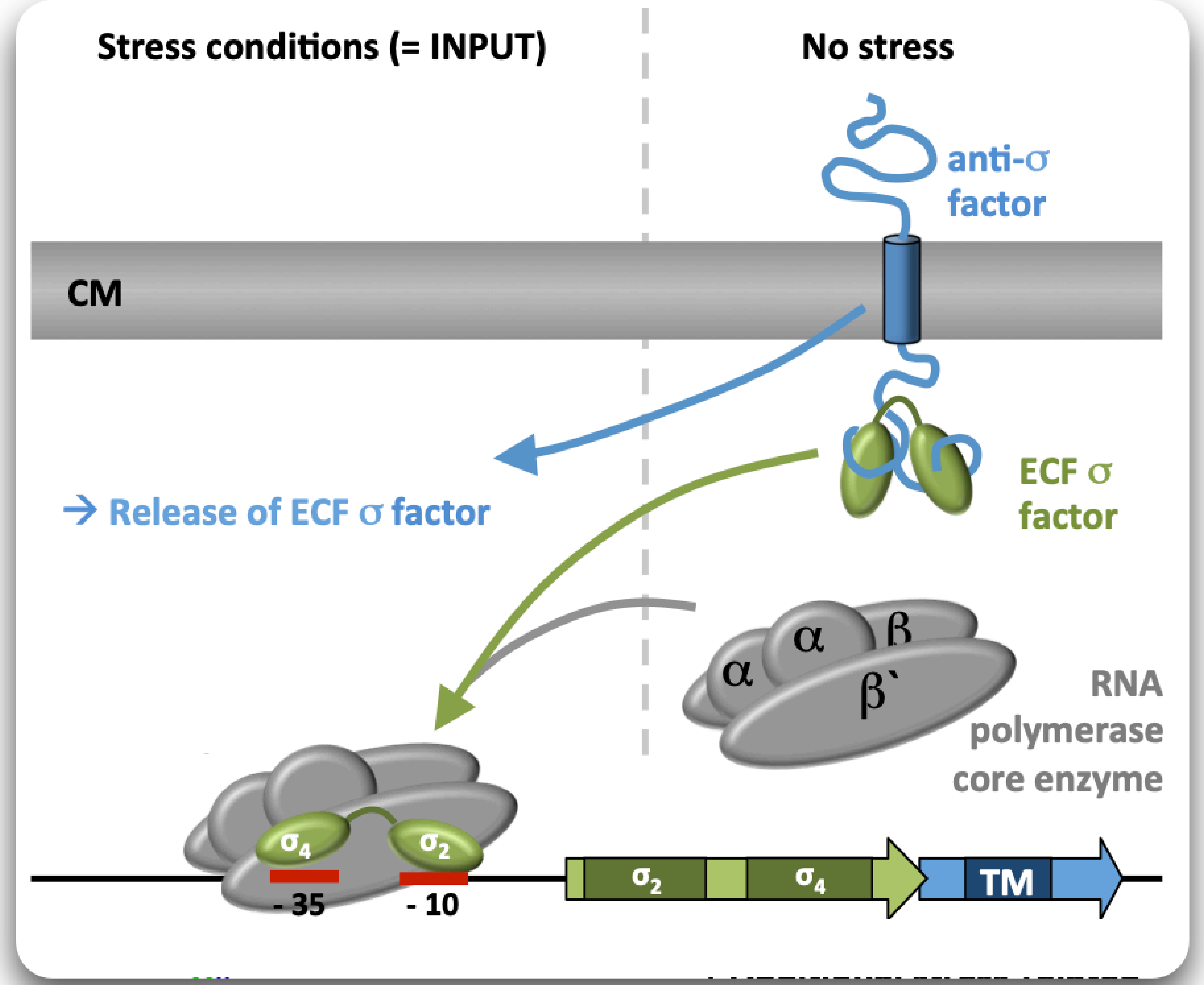
Incomplete divergence leads to cross-talk



Rao (2012), *Curr. Opin. Biotech.*, 23:689-694

Circumventing regulatory cross-talk through orthogonal systems

Extracytoplasmic function (ECF)
σ factors control orthogonal regulons
under stress conditions



Mascher, *Curr. Opin. Microbiol.* (2013)

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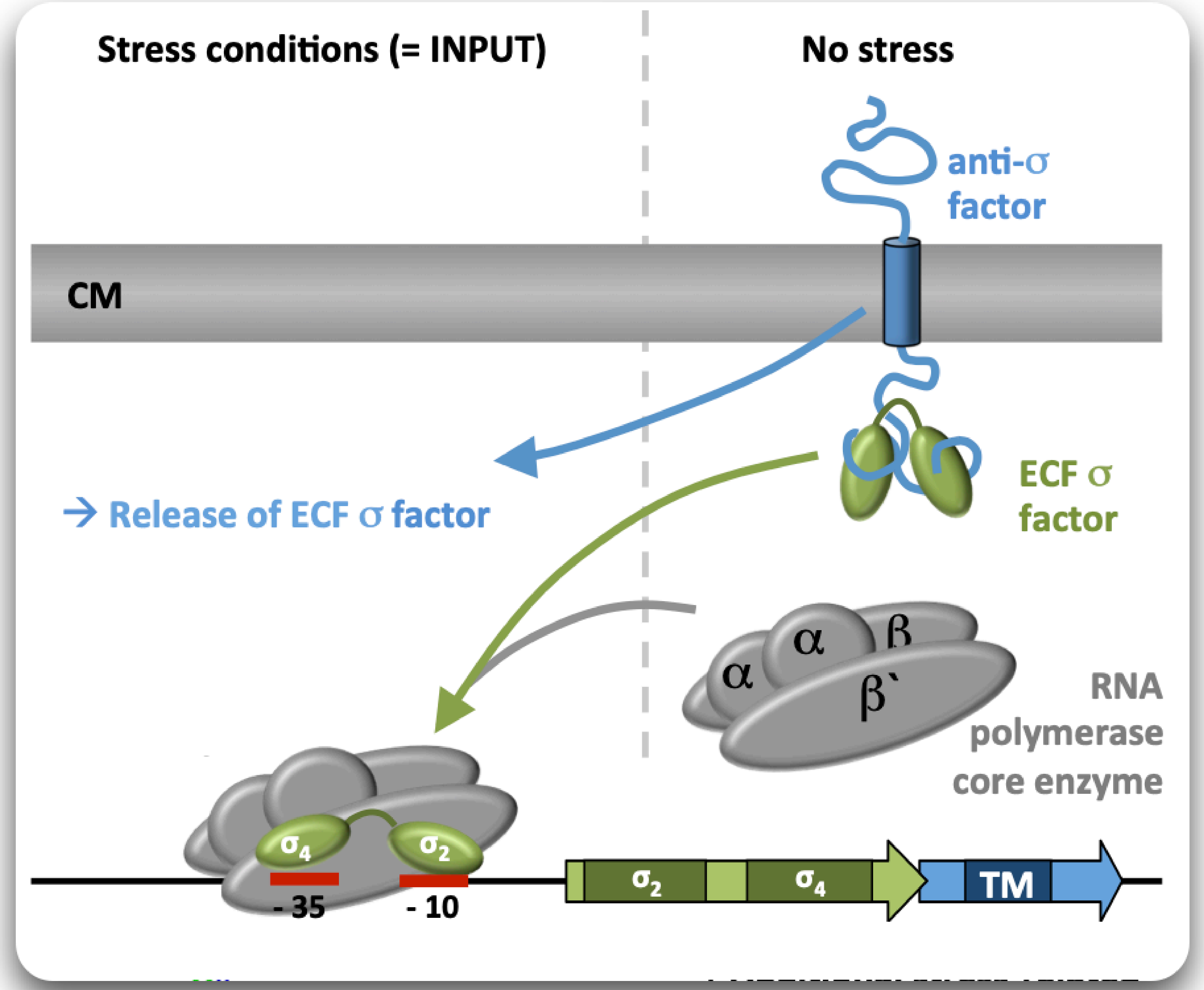
Circumventing regulatory cross-talk through orthogonal systems

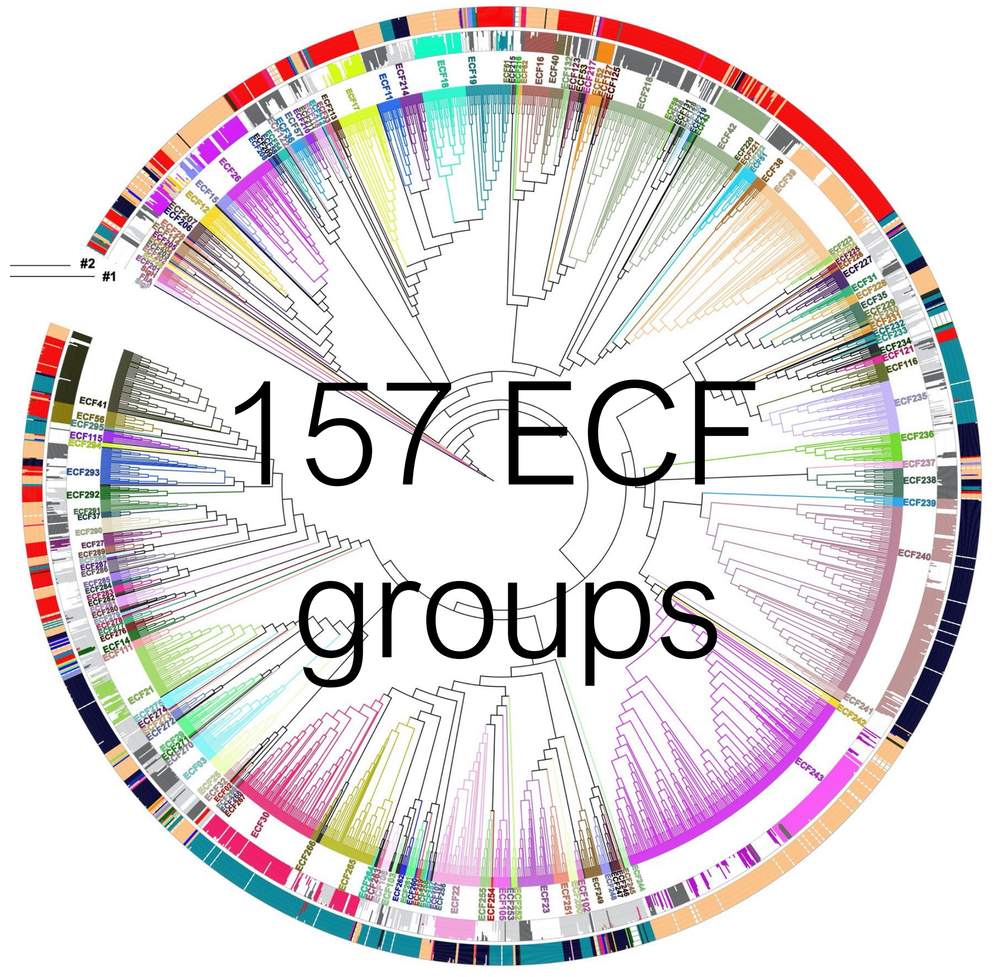
THE UNIVERSITY OF WESTERN AUSTRALIA

Extracytoplasmic function (ECF) σ factors control orthogonal regulons under stress conditions

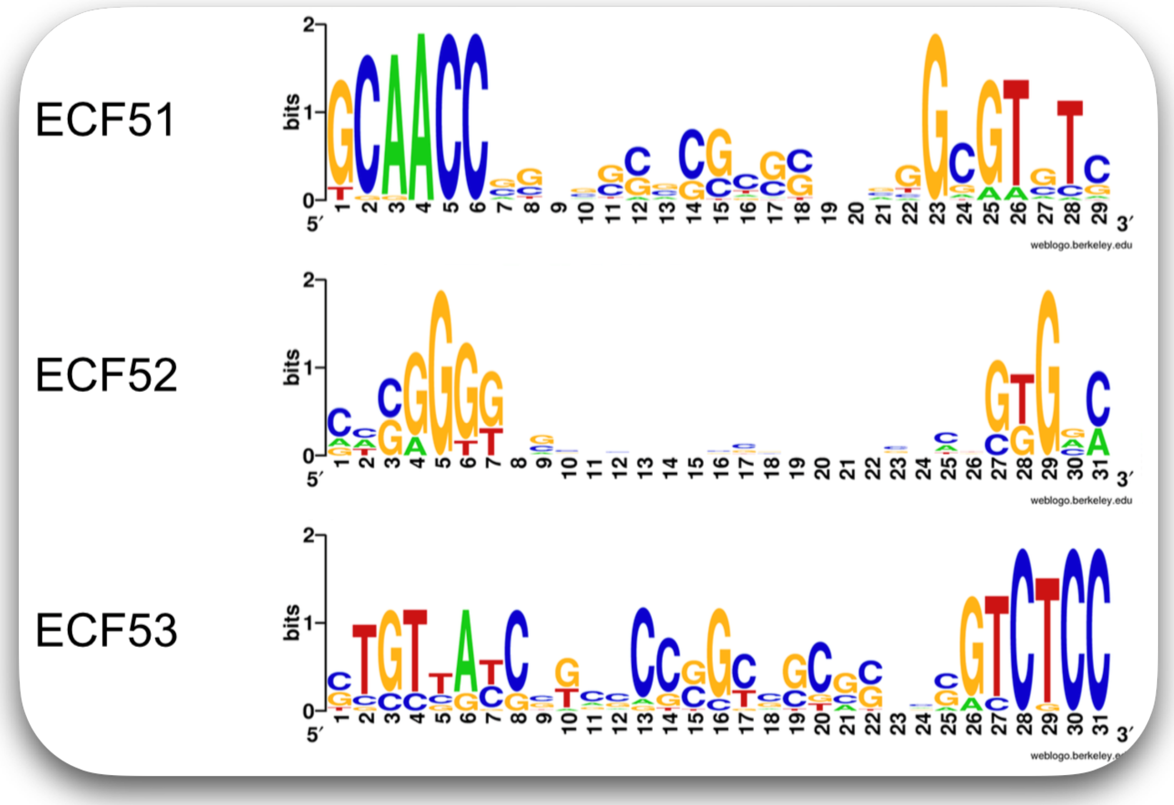


Mascher, *Curr. Opin. Microbiol.* (2013)

Diverse ECF groups feature different promoter preference



Casas-Pastor et al, *bioRxiv* (2019)
https://doi.org/10.1101/2019.12.11.873521



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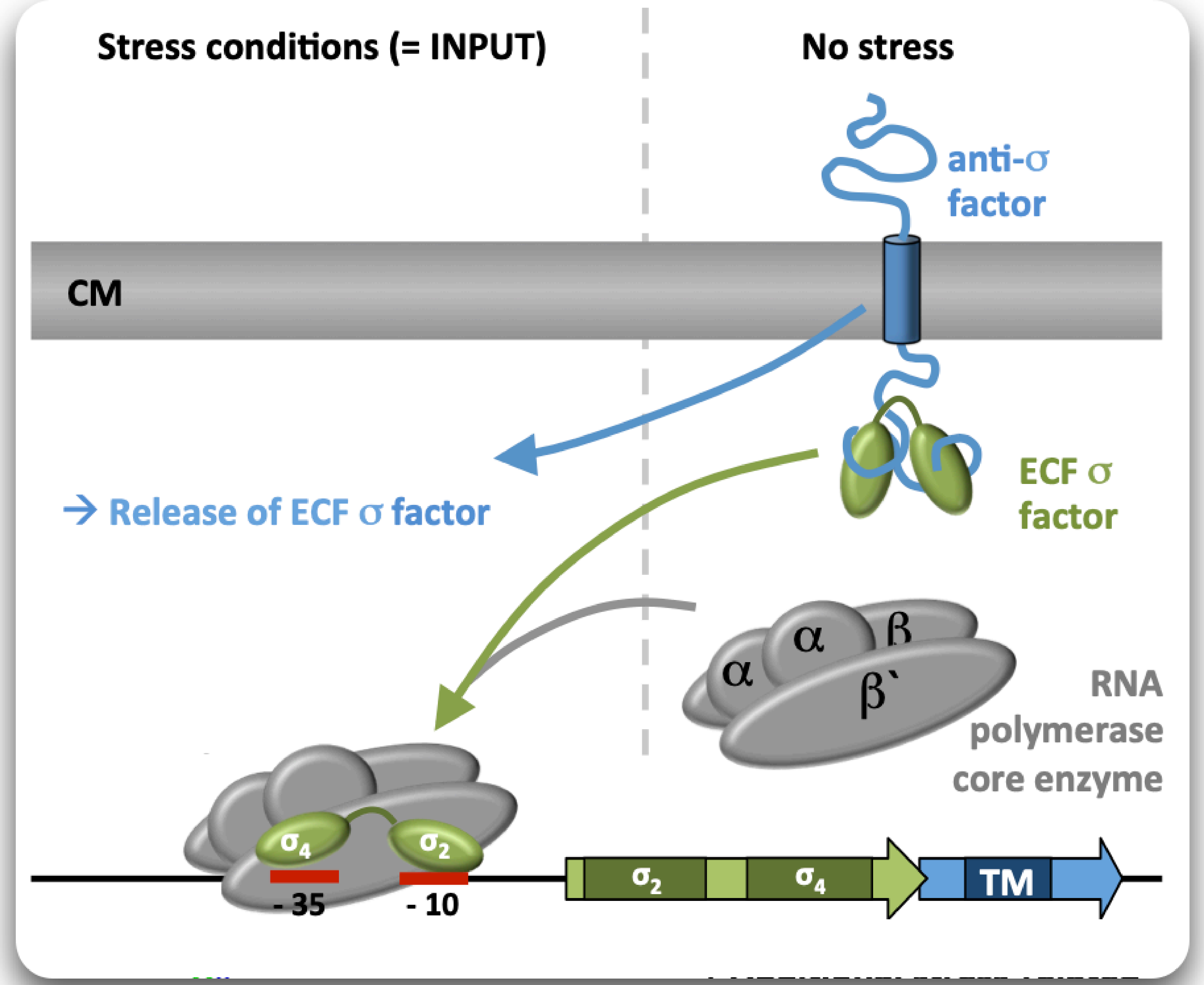

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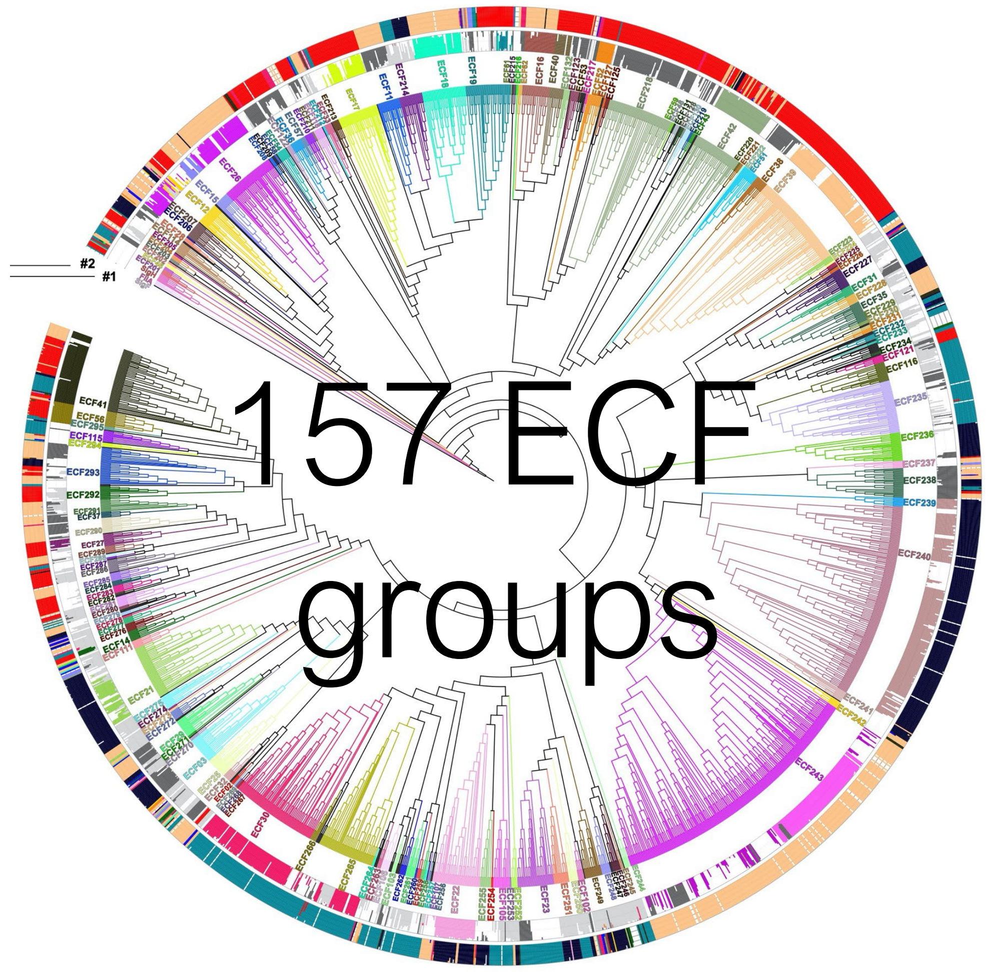

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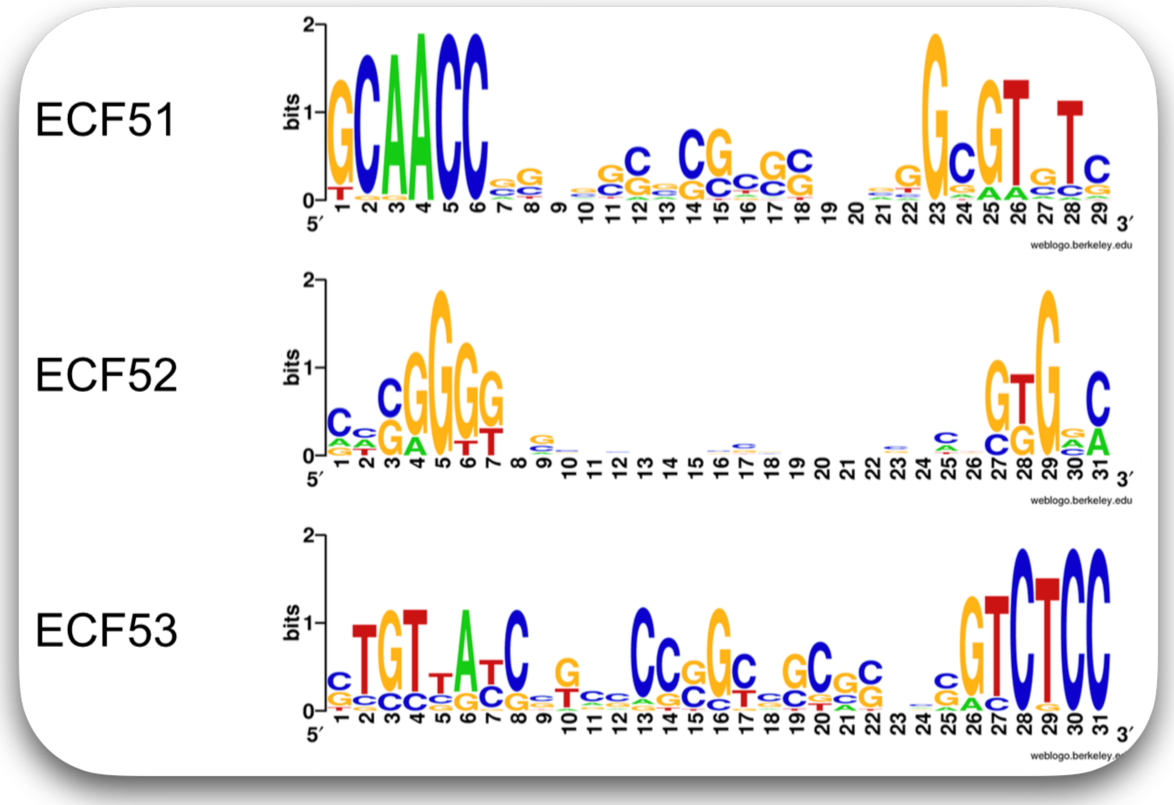

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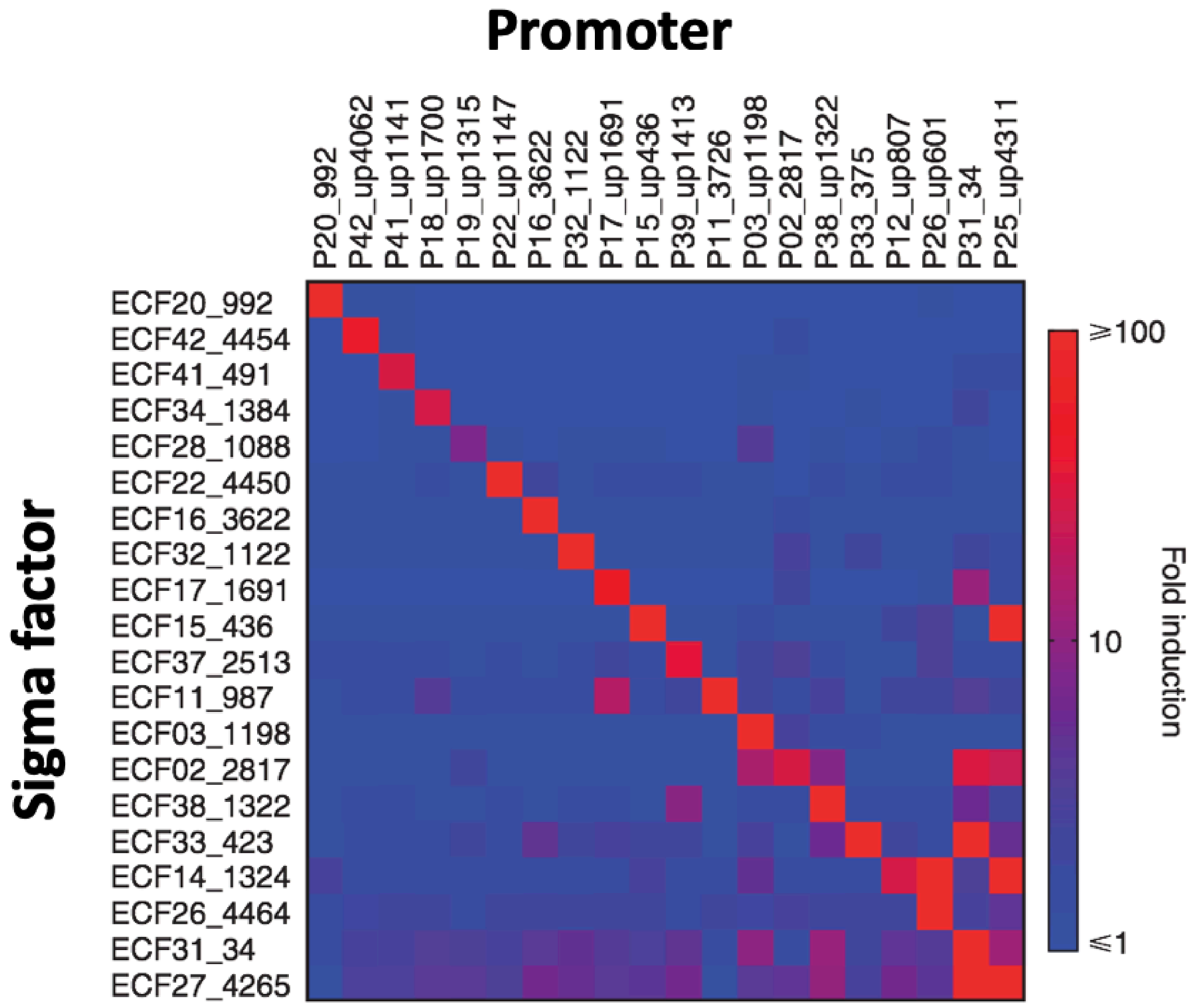
Heterologous expression of ECFs from diverse species in *E. coli* leads to recognition of cognate promoters



Rhodius, et al., *Mol. Syst. Biol* (2013)

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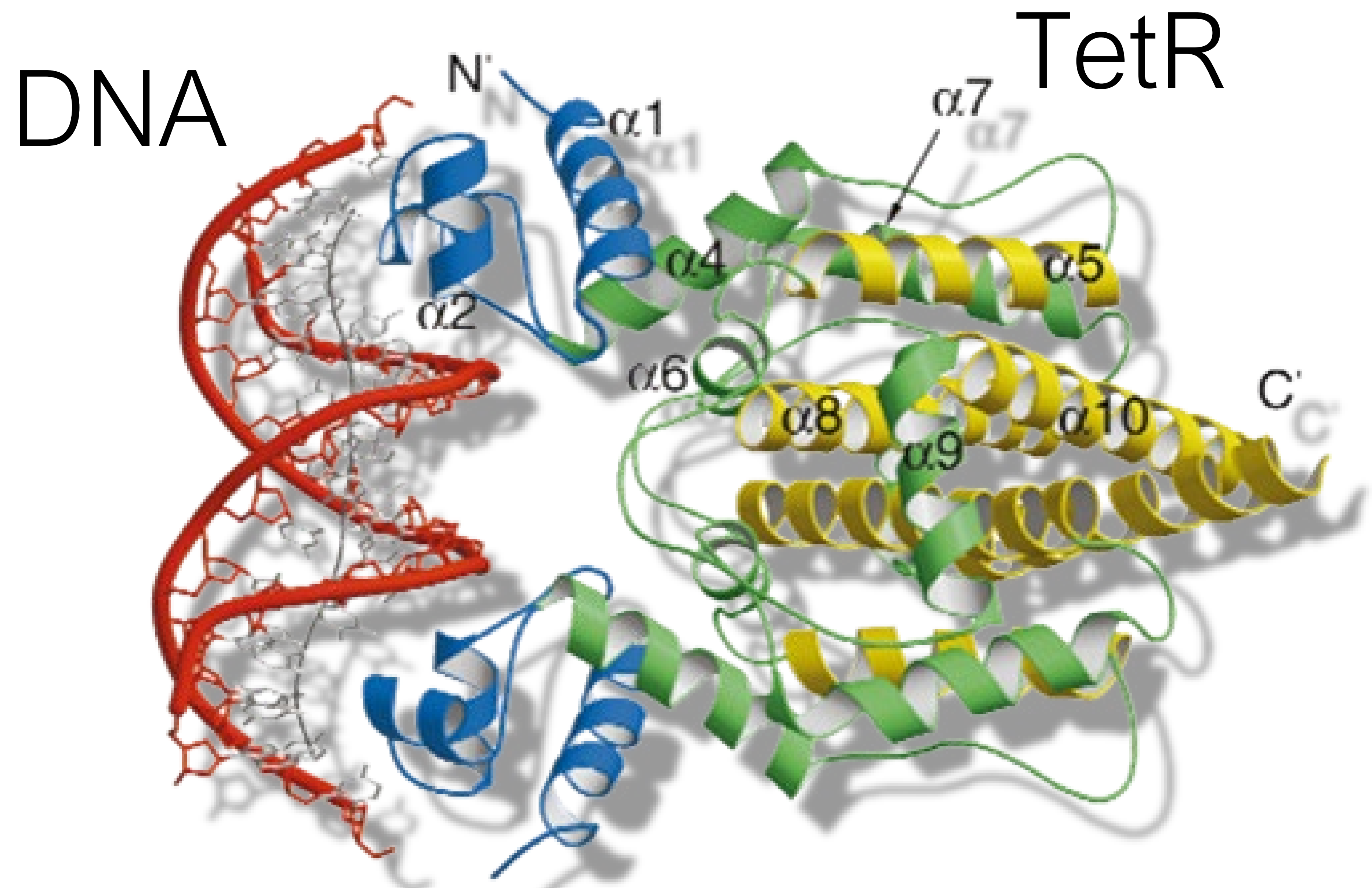
NATURE CHEMICAL BIOLOGY | VOL 10 | FEBRUARY 2014

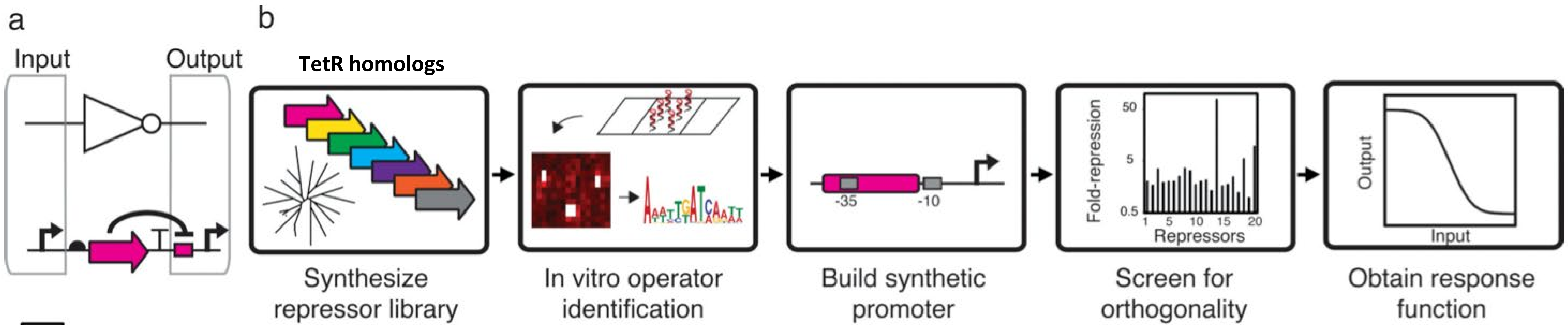
Genomic mining of prokaryotic repressors for orthogonal logic gates

Brynne C Stanton[1], Alec A K Nielsen[1], Alvin Tamsir[2], Kevin Clancy[3], Todd Peterson[3] & Christopher A Voigt[1]*

- Library of synthetic TetR homologs



http://parts.igem.org/File:TetR.png



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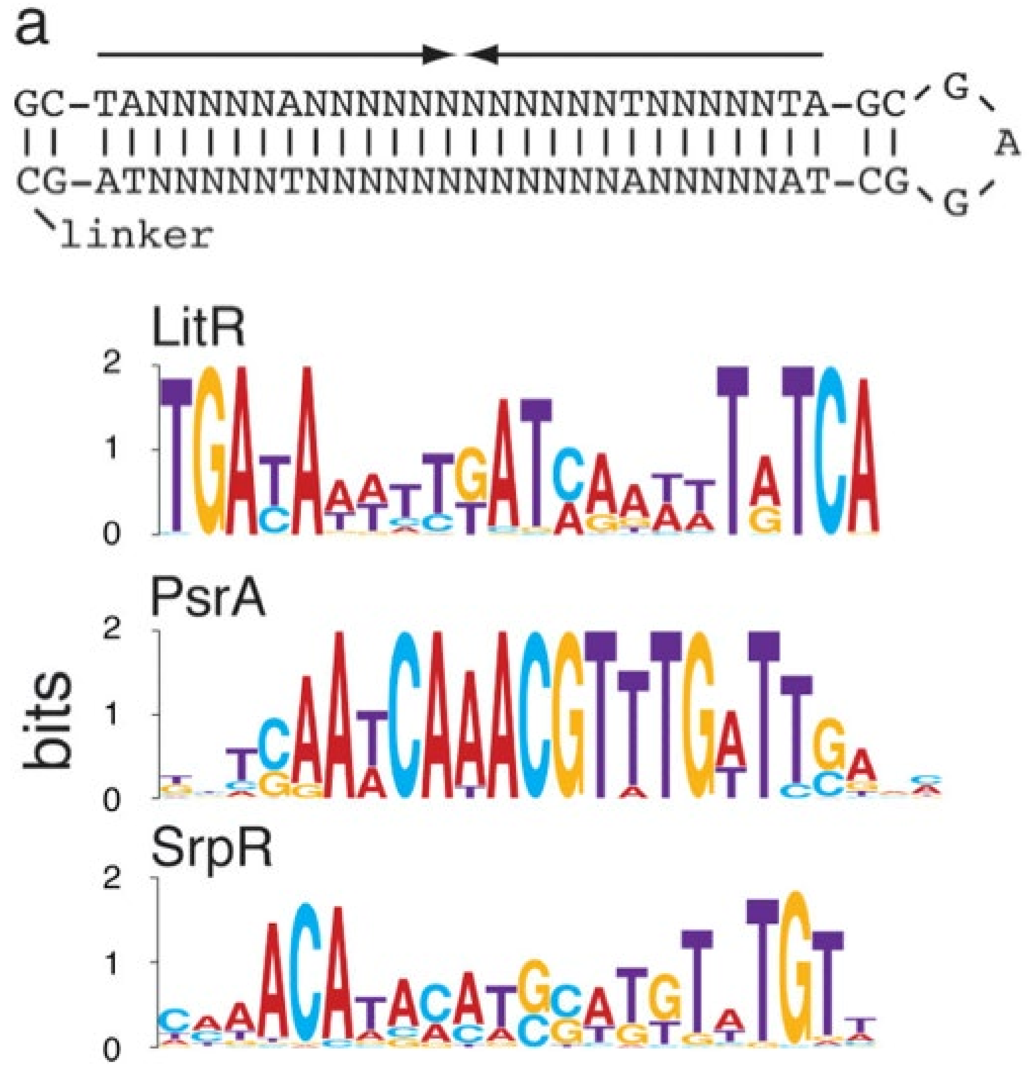
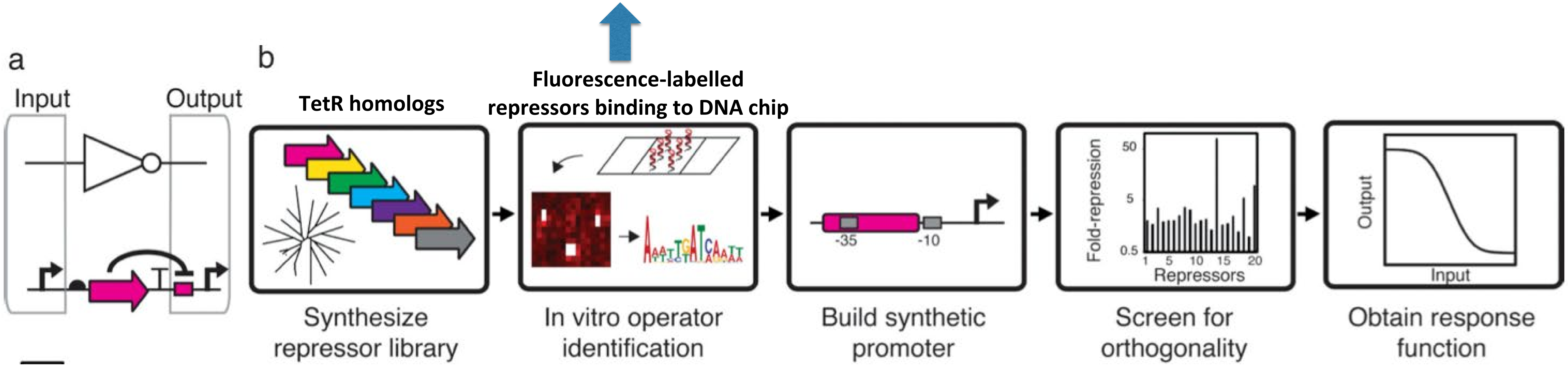
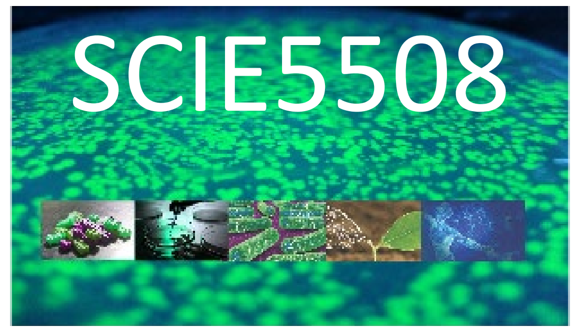


- Library of synthetic TetR homologs
- Bind fluorescence-labelled repressors to DNA chip to identify optimal binding logo





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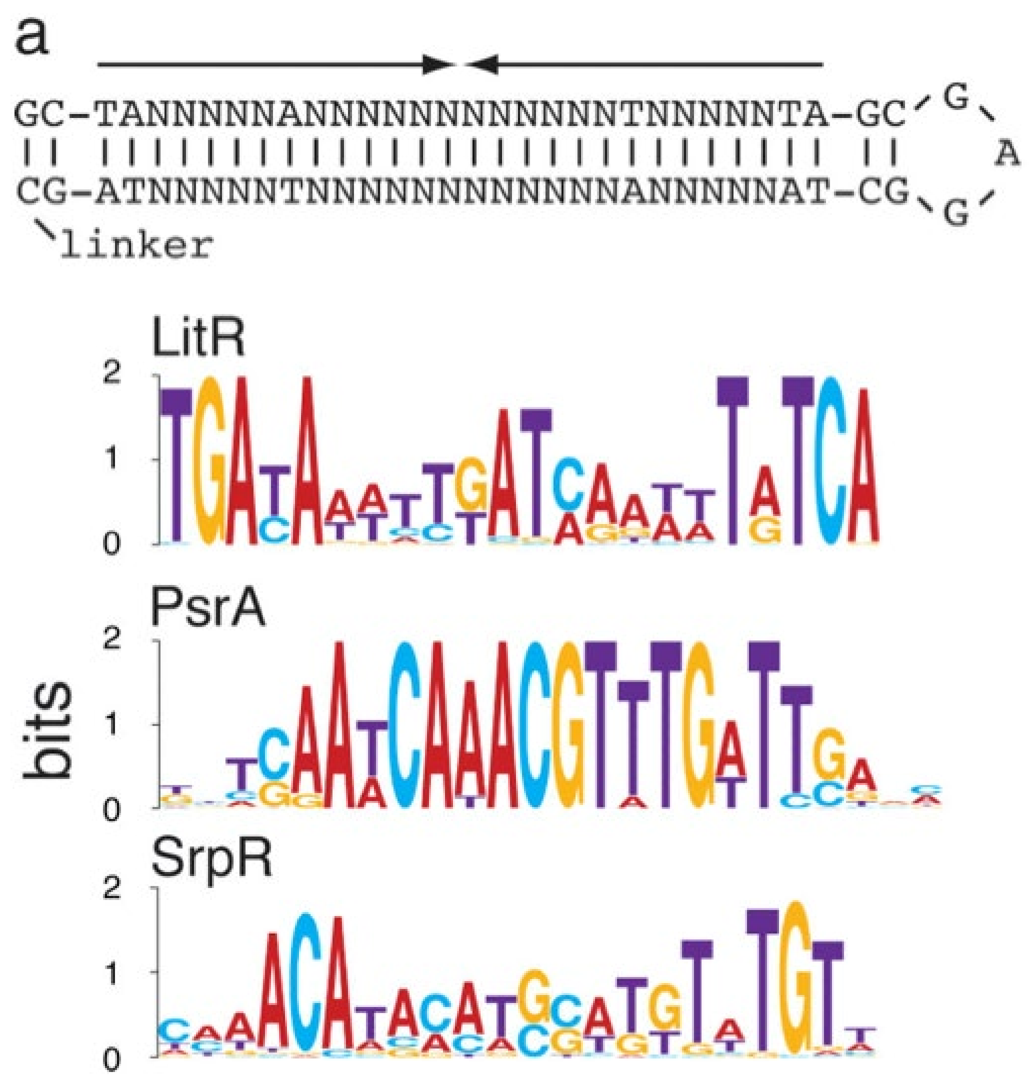
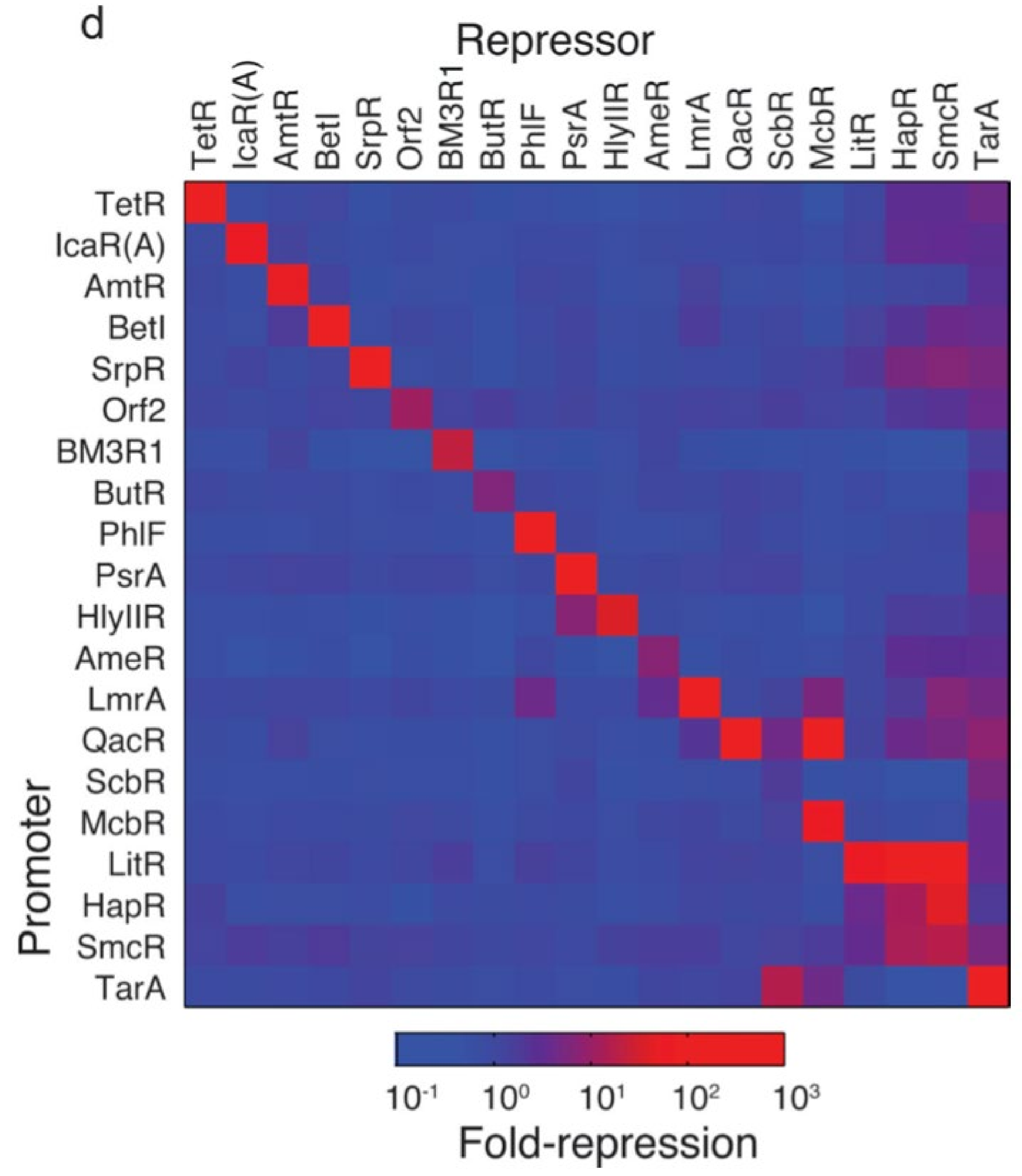
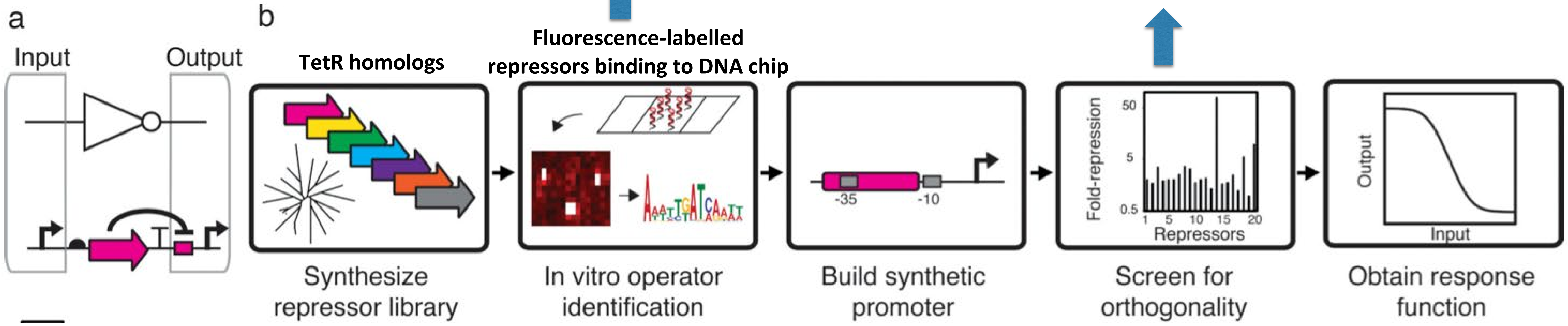




- Library of synthetic TetR homologs
- Bind fluorescence-labelled repressors to DNA chip to identify optimal binding logo
- Build synthetic promoters and test for repressor/promoter orthogonality in *E. coli*



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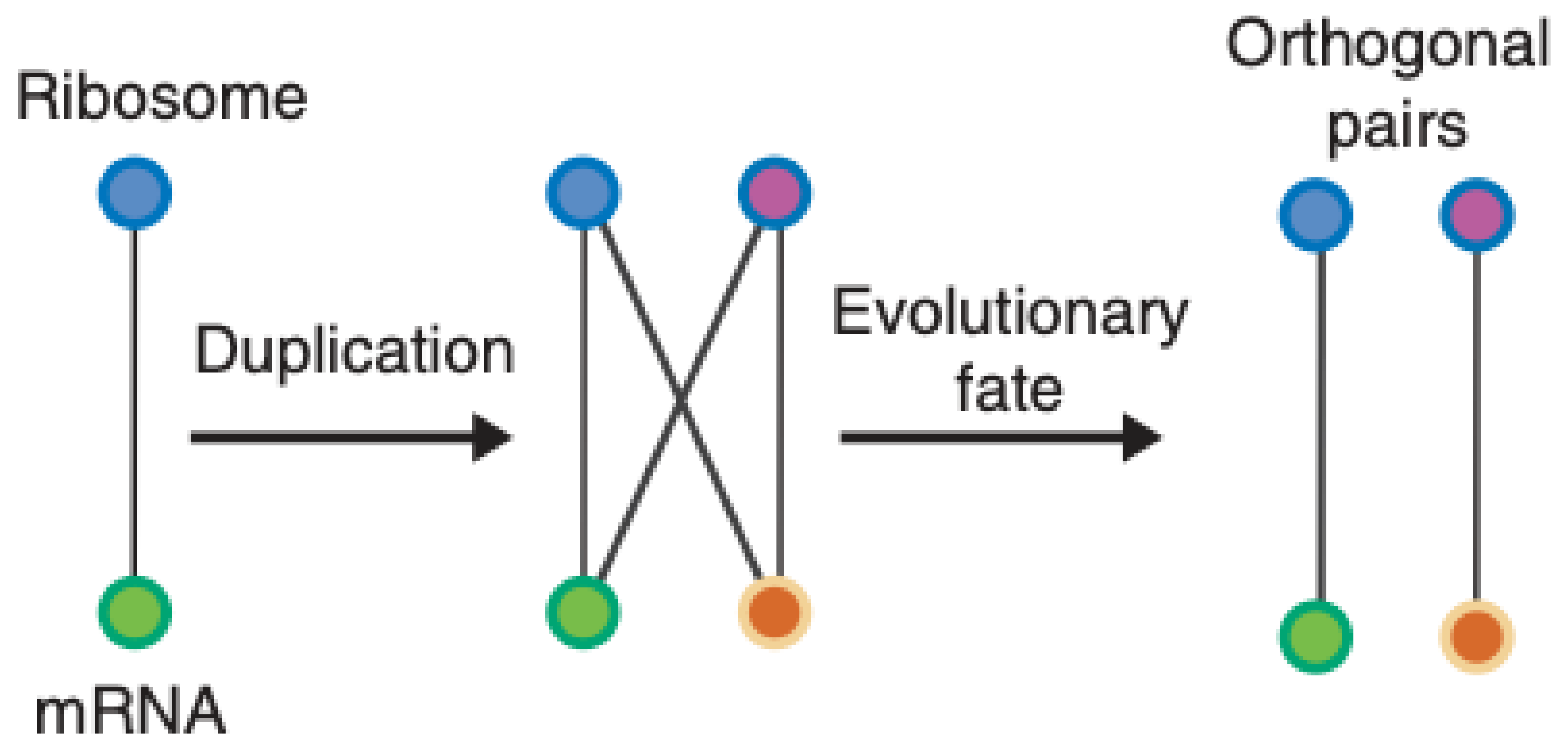
NATURE CHEMICAL BIOLOGY VOLUME 1 NUMBER 3 AUGUST 2005

A network of orthogonal ribosome•mRNA pairs

Oliver Rackham & Jason W Chin



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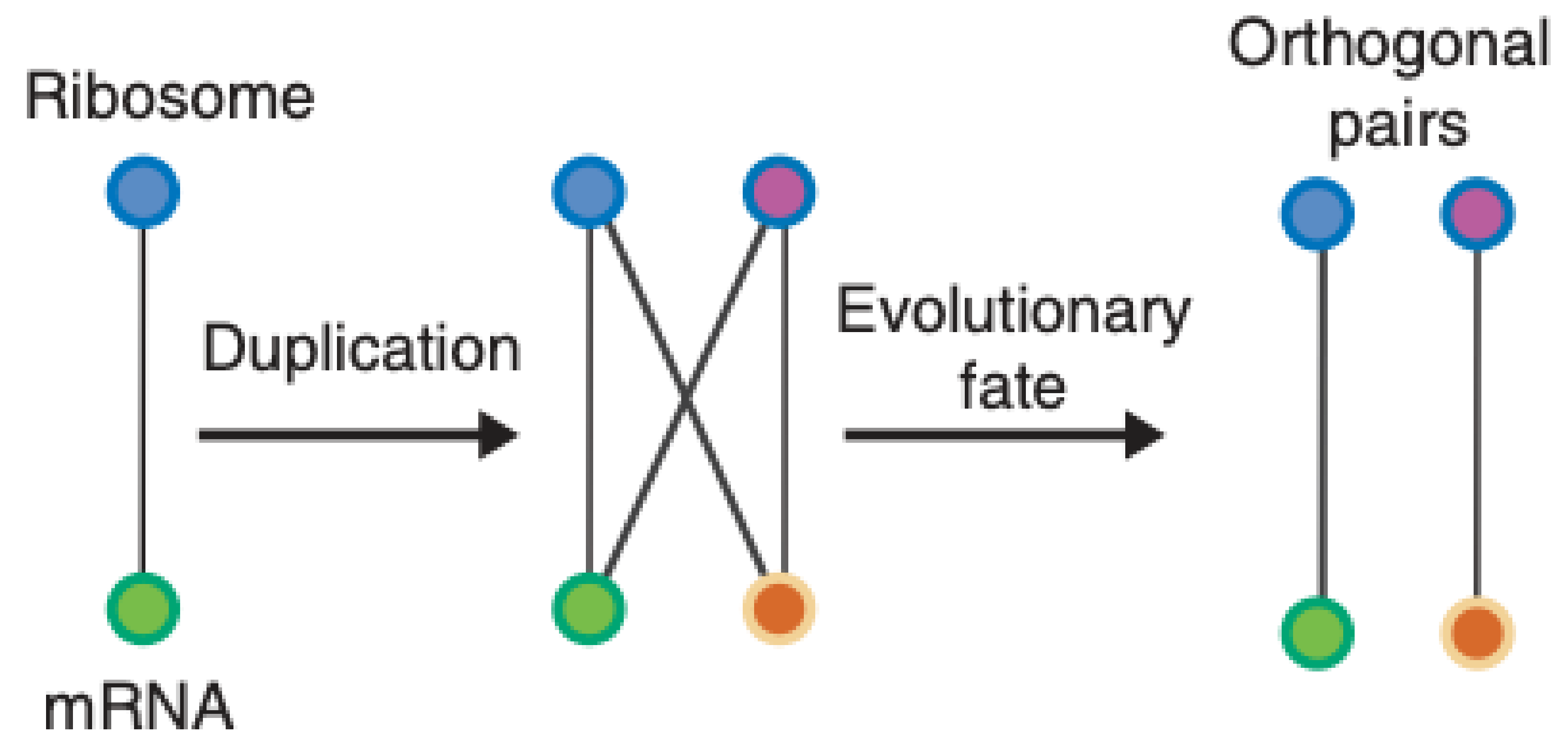
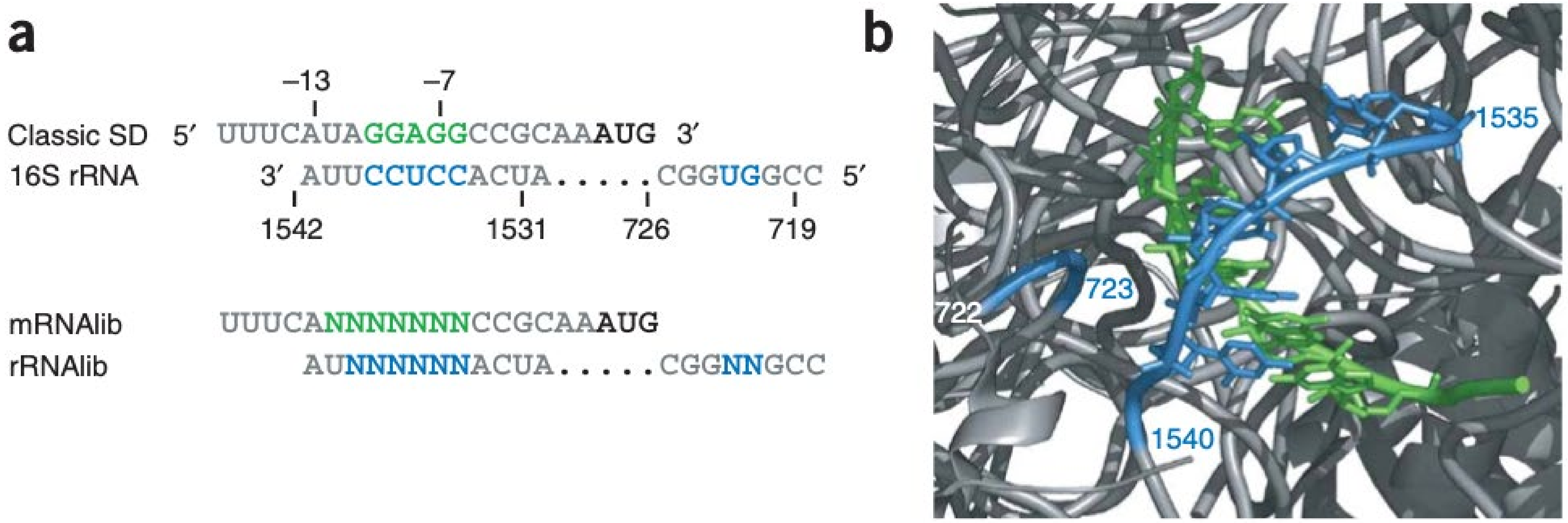
Translation initiation depends on recognition of the Shine-Dalgarno (SD) sequence on mRNA by the ribosome, via complementary base-pairing with the Anti-Shine-Dalgarno sequence (ASD) in 16S rRNA





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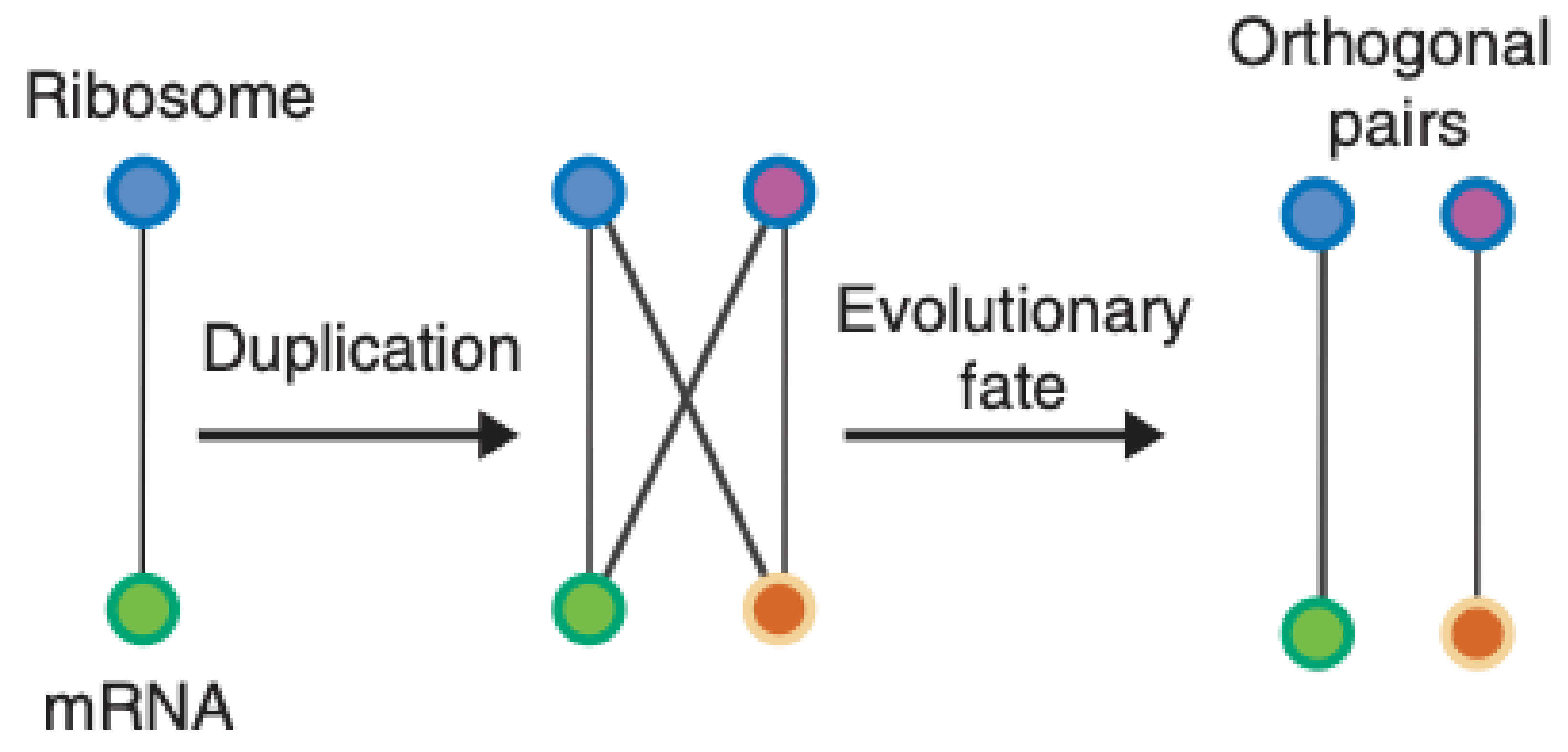
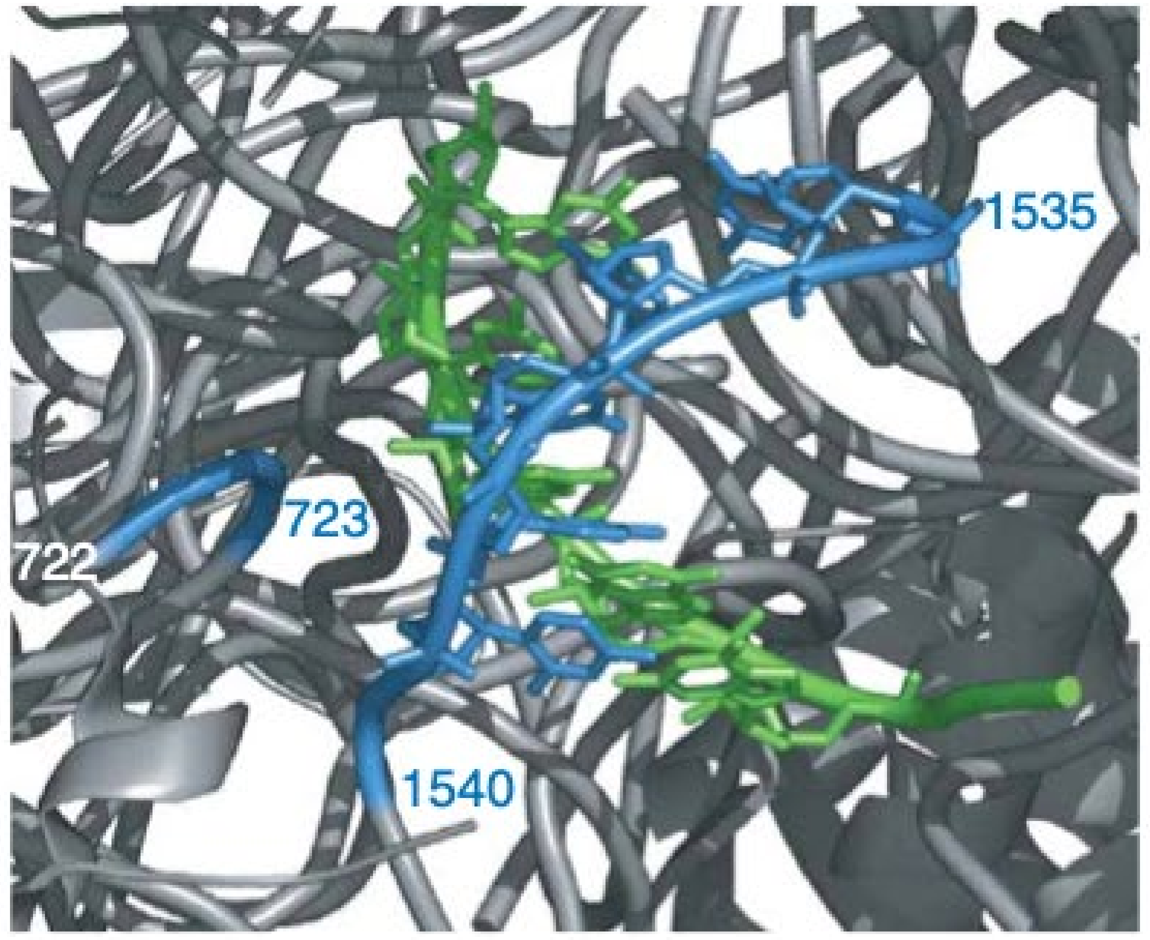
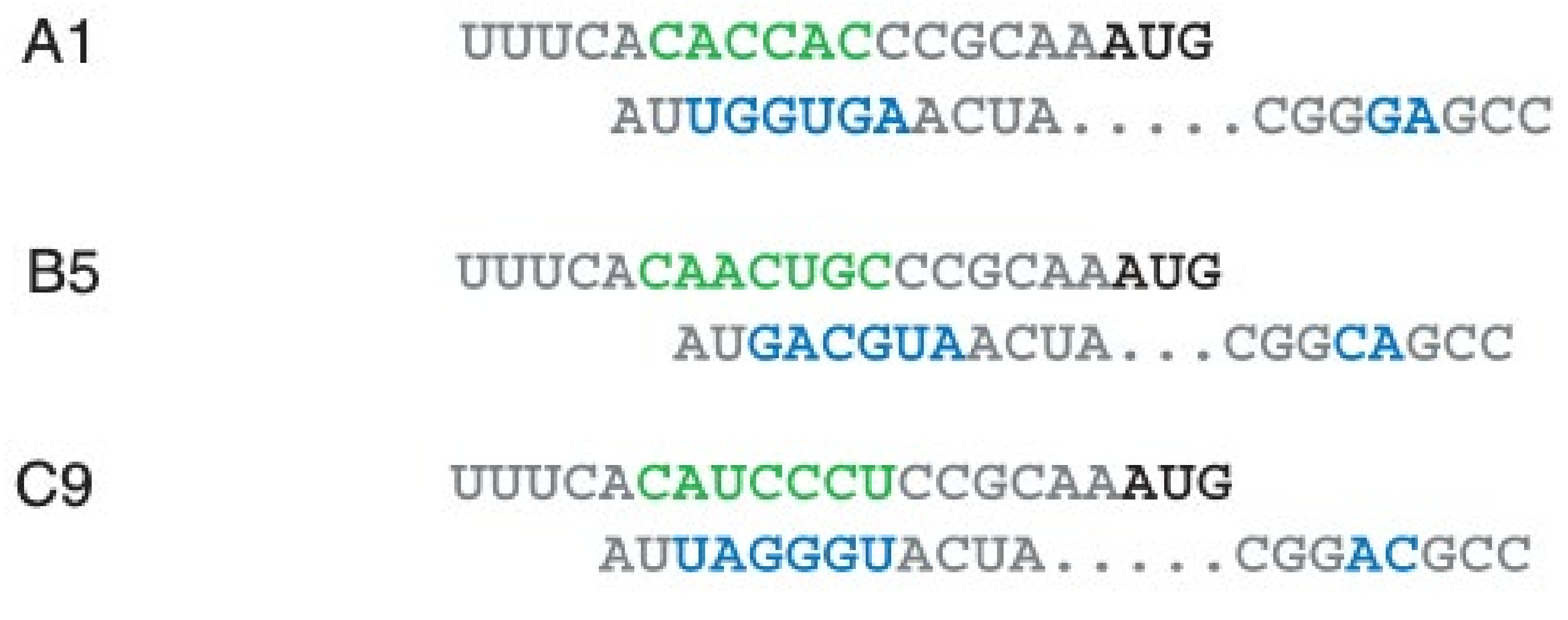
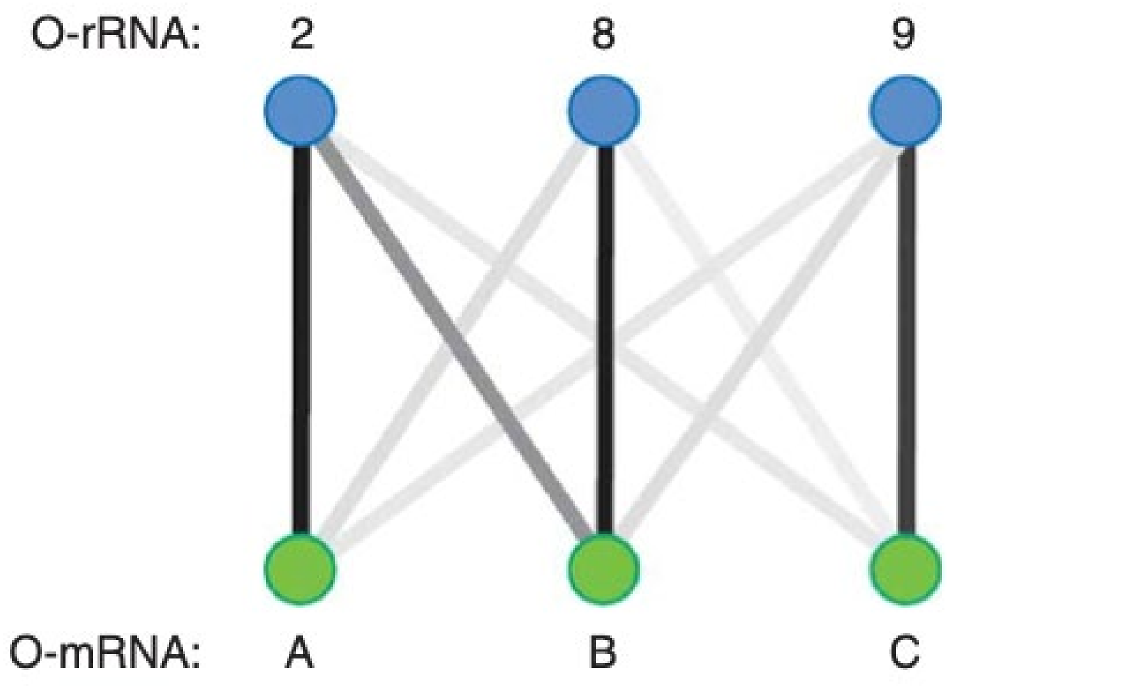




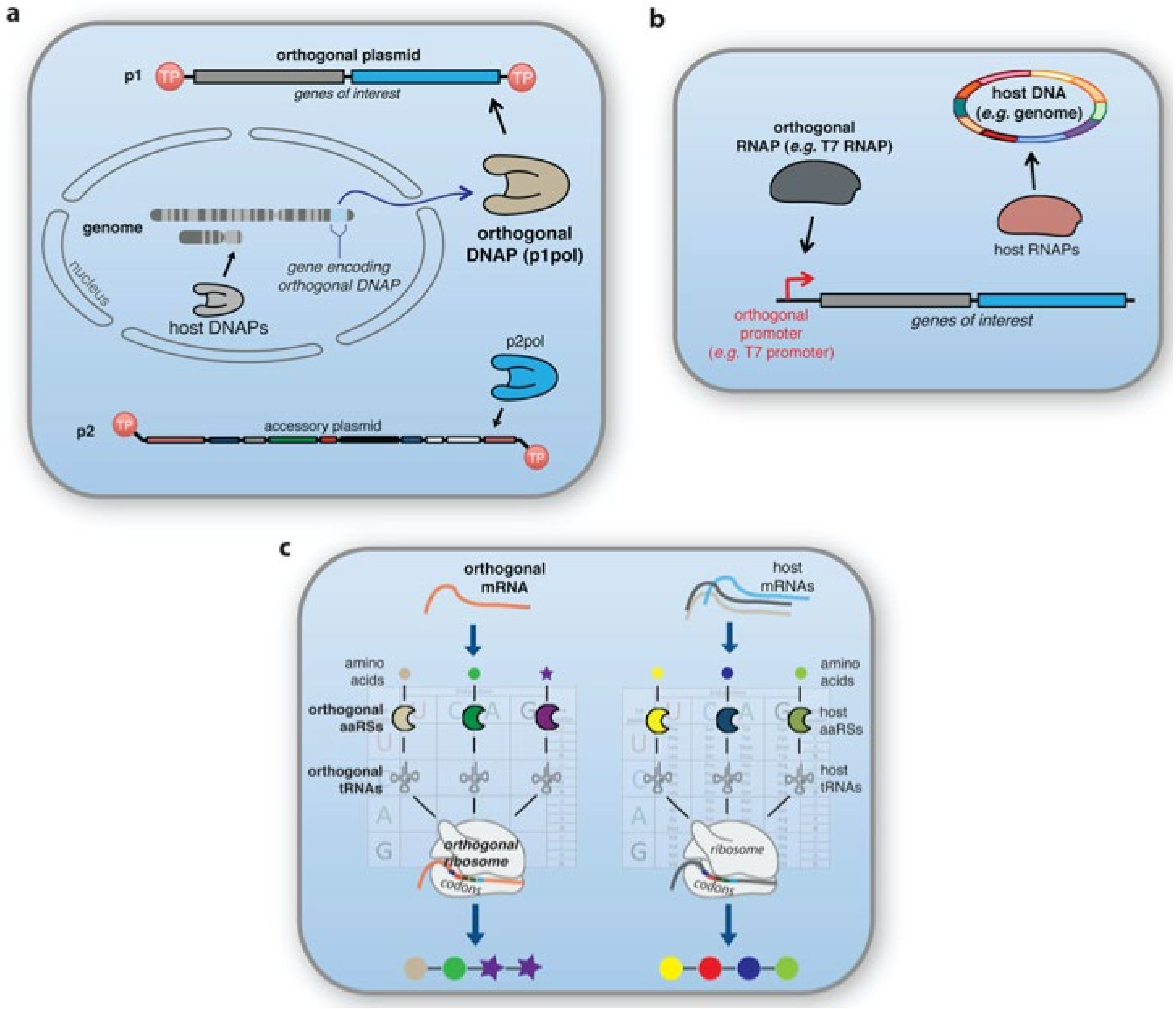
3 orthogonal pairs of O-rRNAs/O-mRNAs

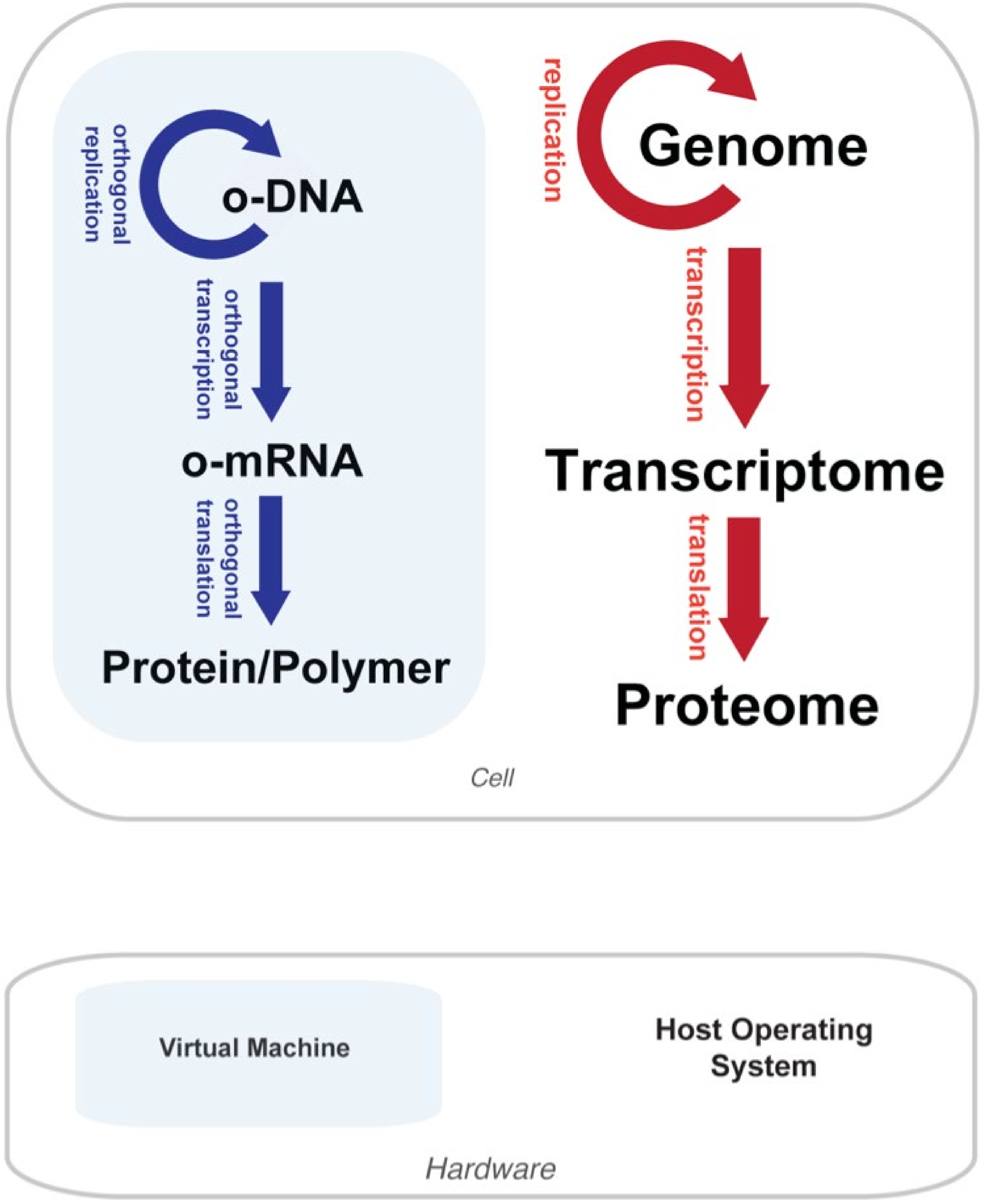


Low cross-talk between non-cognate pairs



Toward an orthogonal central dogma





Liu et al., *Nat. Chem. Biol* (2018), 10.1038/nchembio.2554

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Summary

- Synthetic Biology Open Language is a simple XML standard to define biological designs
- Promoters can act as 1-,2-, and multi-input logic gates; cascades of logic gates (AND, NOT, NOR, etc.) can encode arbitrary computational logic
- Caveat: Genetic designs are slow compared to digital electronics, leading to asynchronous behavior
- Positive feedback can lead to bistability (memory); it amplifies cell-to-cell variations (noise) and may lead to a bimodal distribution of gene expression levels in a population of cells
- Time-delayed negative feedback can lead to oscillations in gene expression activity; negative feedback without time delay generally reduces gene expression noise
- Limitations to synthetic circuit design: Gene expression noise; Genetic context dependence; Regulatory cross-talk
- (Partial) solutions: Neg. feedback regulation to suppress noise; Genetic context insulation; Orthogonal central dogma

Thanks for watching!

Please post your questions on the LMS Discussion board & tune in the Q&A session

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