Student Seminar
Synthetic and Systems Biology

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Who claims to be synthetic biologists?

- Metabolic engineering:

  Advanced “fermentation”. Optimization of the production of certain metabolites by usually microorganisms. (Jay D. Keasling)

- Synthetic genome:

  Chemically synthesize the complete, usually minimized, genome from a digital record. (Craig Venter)

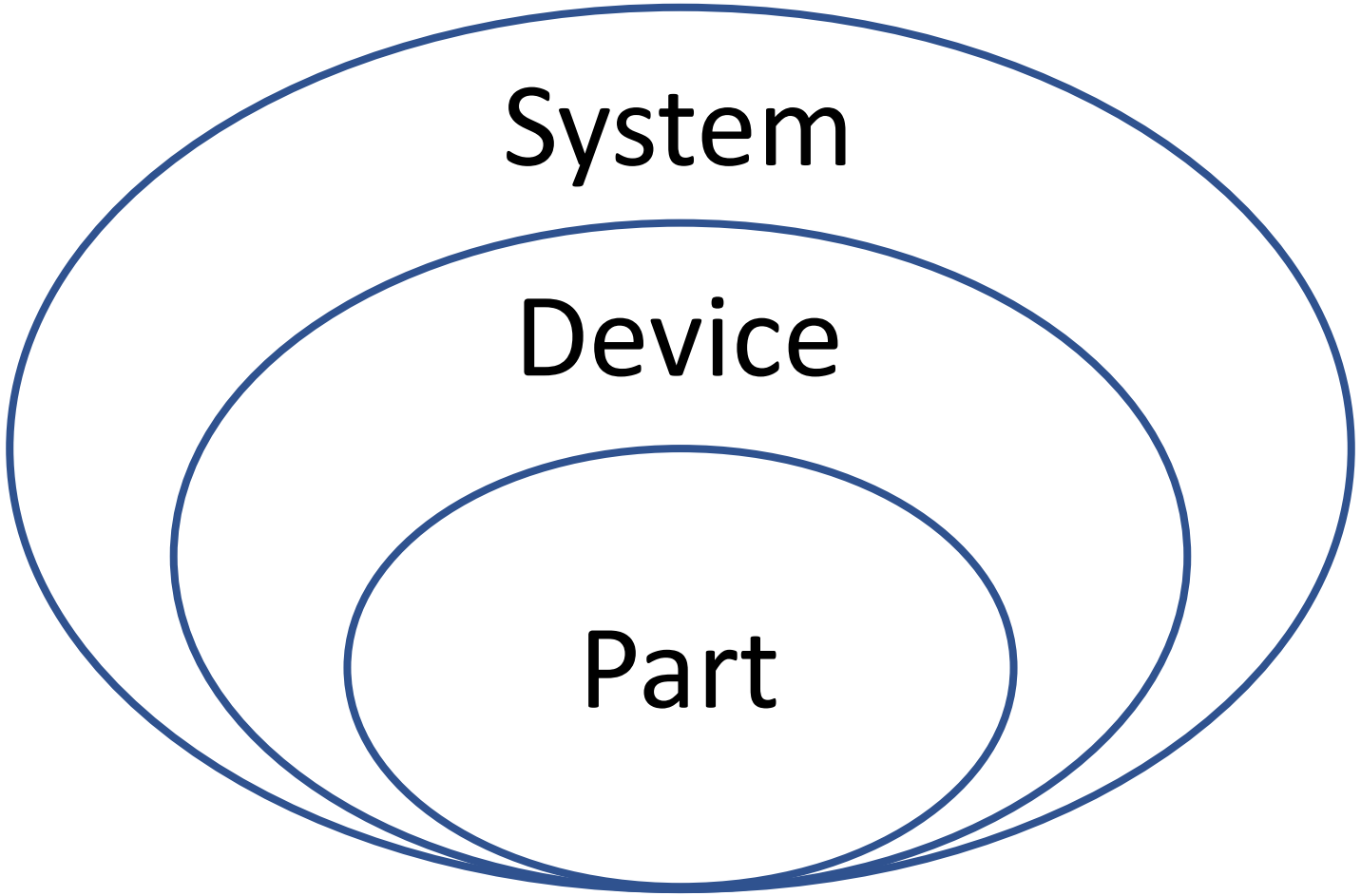
- Various advanced genetic engineering:

  Genome editing (Feng Zhang), Directed evolution (David Liu), Xenobiology, etc.

- Circuit biology:

  Assemble genetic parts into a whole component, performing usually quantitatively predictable behavior (James Collins)

Hierarchy of genetic circuits

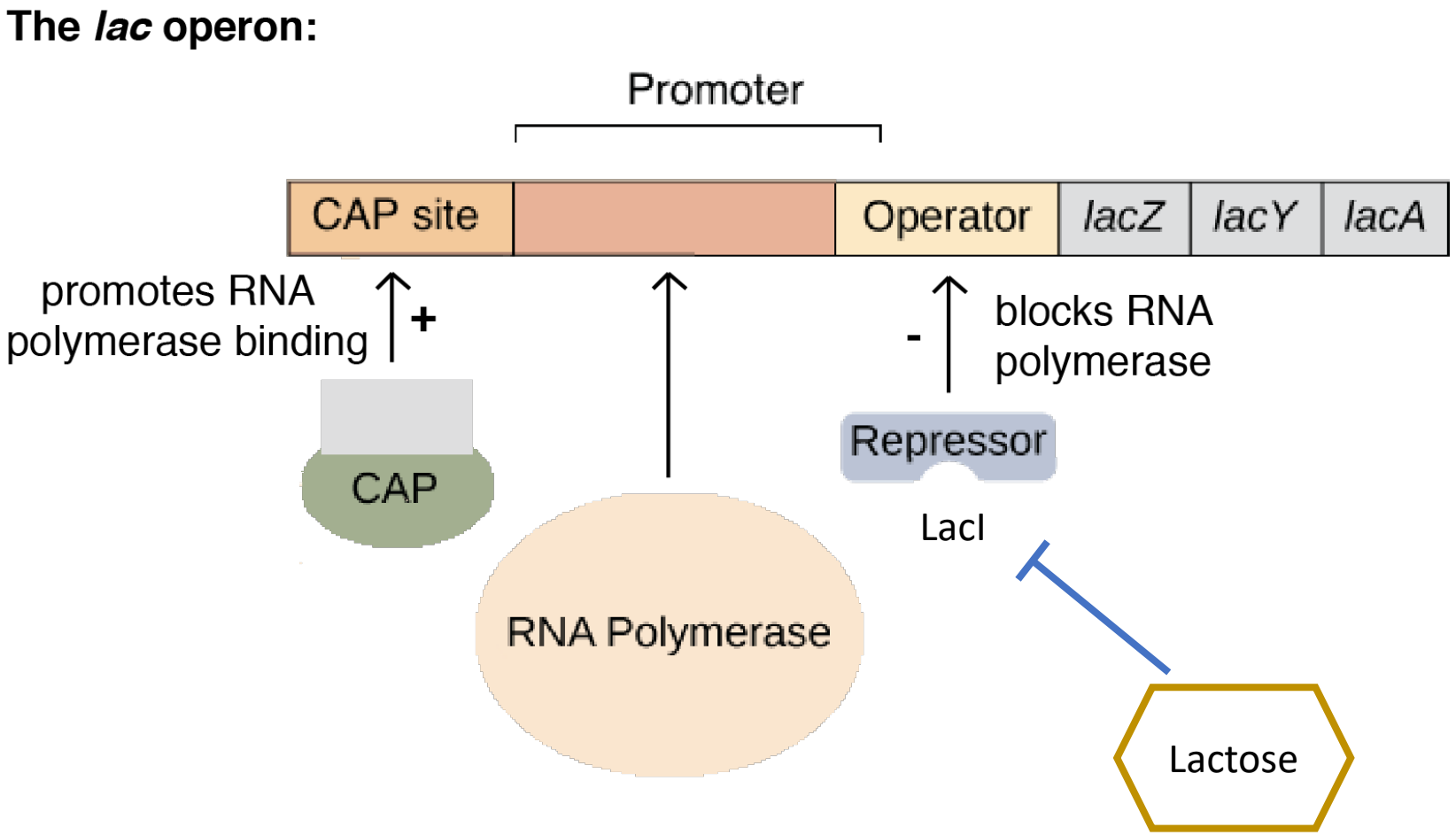


The engineered *E. coli*

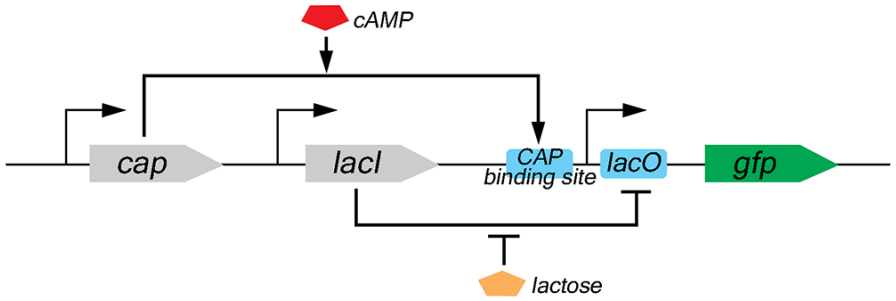
Lactose sensing circuit

GFP (Green Fluorescence Protein)

Example for prokaryote transcription regulation

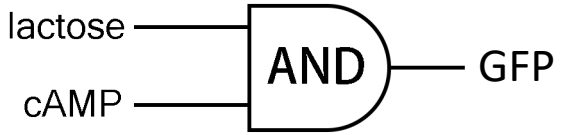


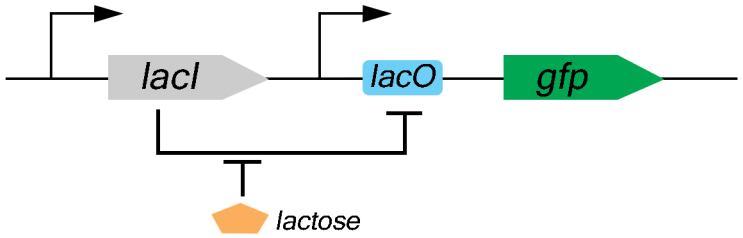
The logic gate representation



In1 (cAMP)	In2 (lactose)	output (GFP)
0	0	0
0	1	0
1	0	0
1	1	1

AND gate



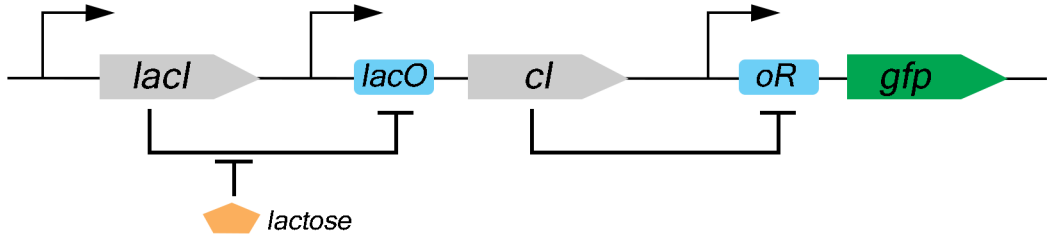


Input (lactose)	output (GFP)
0	0
1	1

IS gate

In1 (lactose)	In2 (LacI)	output (GFP)
0	0	1
0	1	0
1	0	1
1	1	1

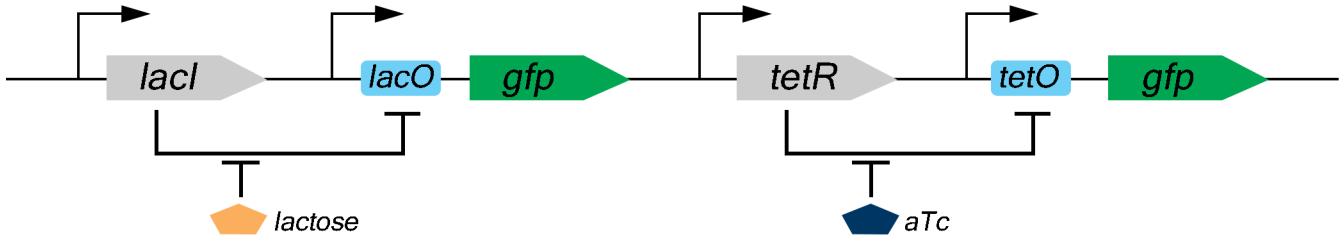
IMPLY gate



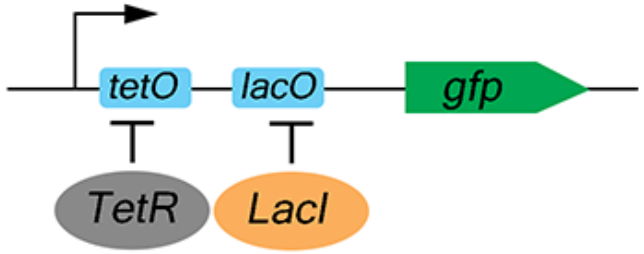
Input (lactose)	output (GFP)
0	1
1	0

NOT gate

The logic gate representation cont.



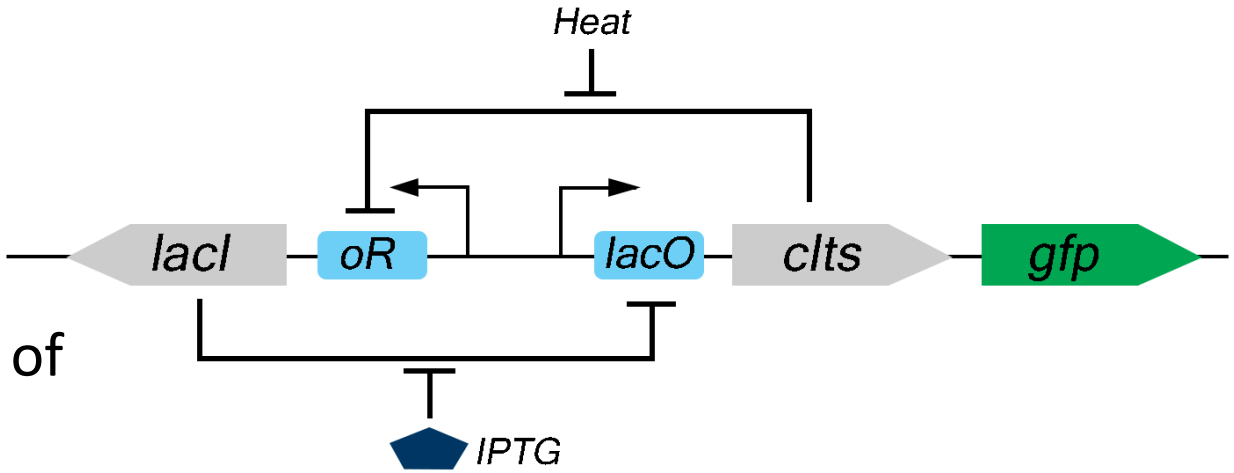
OR gate



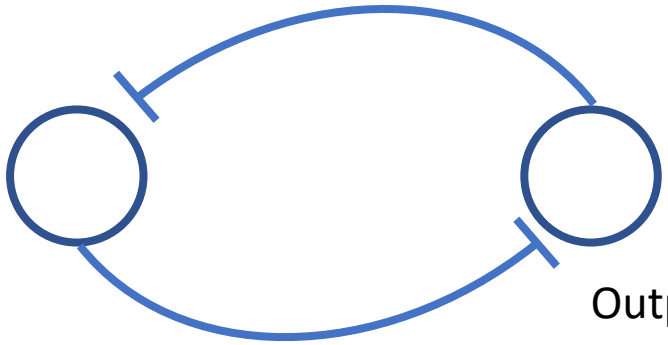
NOR gate

How to build a biological XOR gate?

The inception: bistable toggle-switch

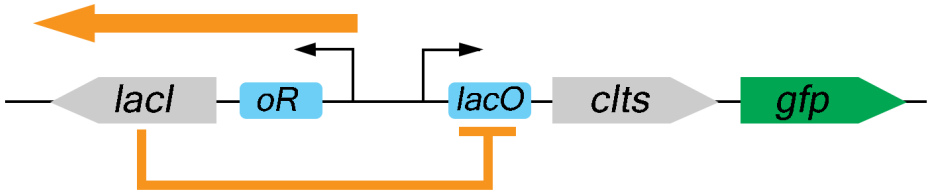


What happens if three of these nodes suppress each other?



Output node

The OFF state (*vise versa*)



Gardner, T., Cantor, C. & Collins, J. Construction of a genetic toggle switch in *Escherichia coli*. *Nature* **403,** 339–342 (2000). https://doi.org/10.1038/35002131

Bistable toggle-switch cont.

$$\frac{dU}{dt} = \frac{\alpha_U}{1 + \left(\frac{V}{K_V}\right)^{n_V}} - U$$

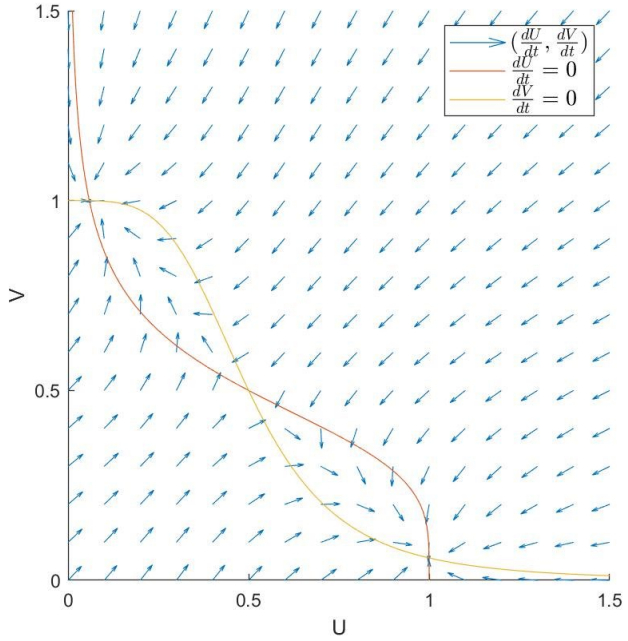
$$\frac{dV}{dt} = \frac{\alpha_V}{1 + \left(\frac{U}{K_U}\right)^{n_U}} - V$$

What is the rationale behind these equations?

Where U and V are proteins, most likely transcriptional factors, whose expression are down regulated by each other. α is the promoter strength; K is the dissociation coefficient, or the repression sensitivity; n is the cooperative coefficient.

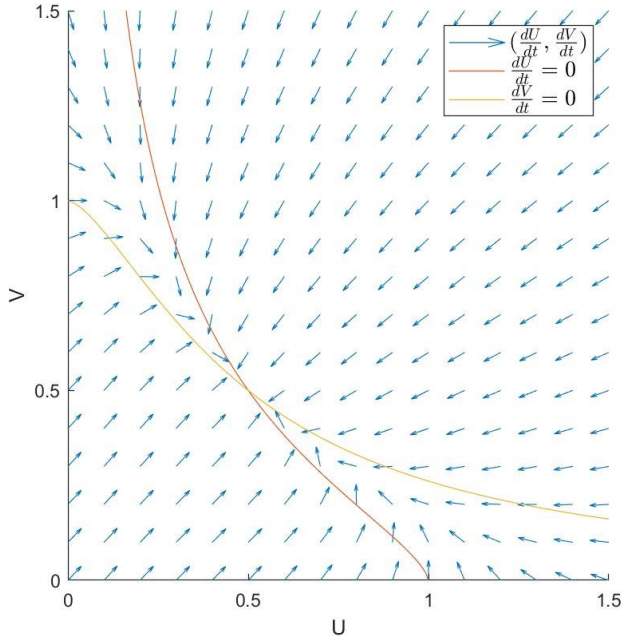
Bistable toggle-switch cont.

$K_U = K_V = 0.5$
$\alpha_U = \alpha_V = 1.0$
$n_U = n_V = 4$



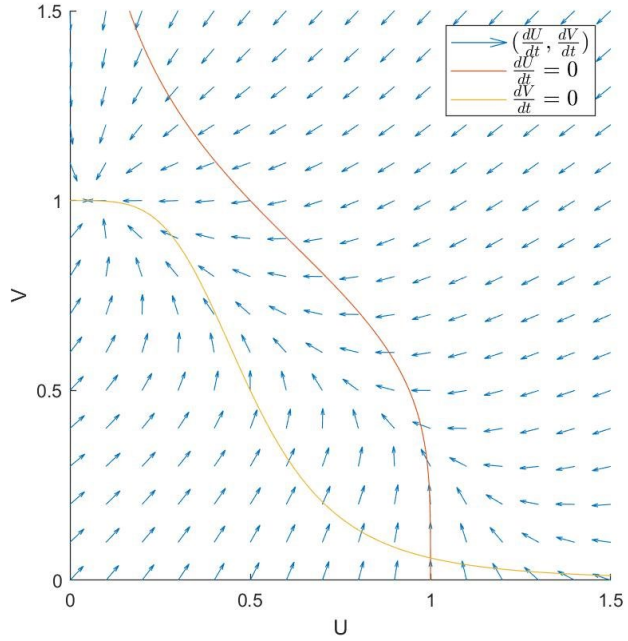
bistable (with one unstable equilibrium point)

$K_U = K_V = 0.5$
$\alpha_U = \alpha_V = 1.0$
$n_U = n_V = 1.5$



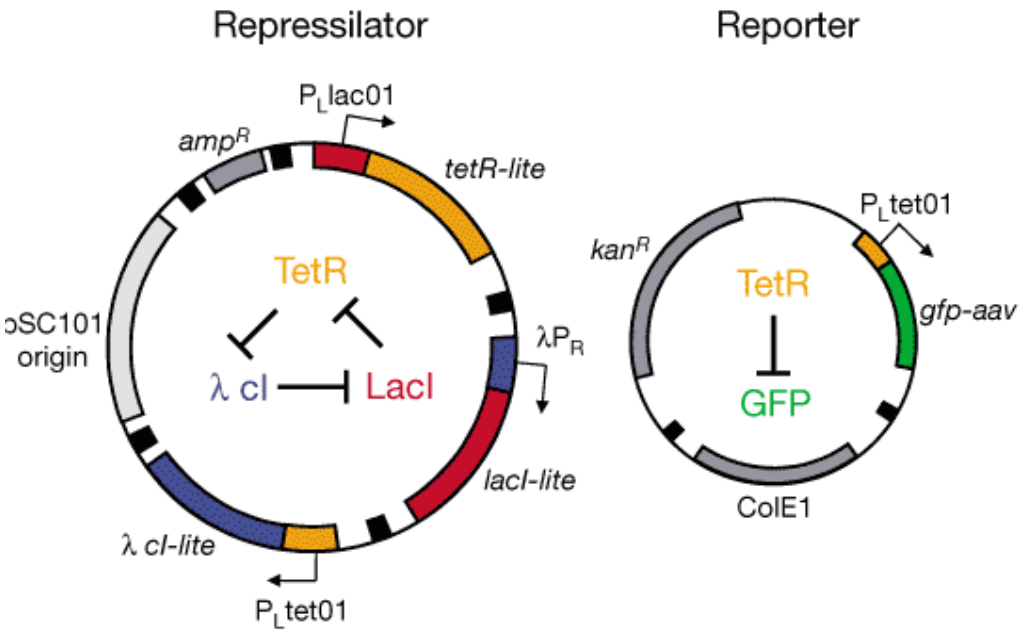
monostable

$K_U = 0.5, K_V = 1.0$
$\alpha_U = \alpha_V = 1.0$
$n_U = n_V = 4$



monostable

The inception: the repressilator



Elowitz, M., Leibler, S. A synthetic oscillatory network of transcriptional regulators. *Nature* **403,** 335–338 (2000). https://doi.org/10.1038/35002125

The math behind input nodes

Hill equation lays the foundation of how a single “node” in the genetic circuit behaviors.

Consider that repressor R binds to DNA. The *dissociation coefficient:*

$$K_d = \frac{[DNA_{free}]\cdot[R]}{[DNA{::}R]}$$

K_d represents the sensitivity of this ligand binding event

Thus, the fraction of UNBOUND DNA is

(Michaelis-Menten)

$$f = \frac{[DNA_{free}]}{[DNA]} = \frac{[DNA_{free}]}{[DNA_{free}]+[DNA{::}R]} = \frac{[DNA_{free}]}{[DNA_{free}]+\frac{[DNA_{free}]\cdot[R]}{K_d}} = \frac{K_d}{K_d+[R]}$$

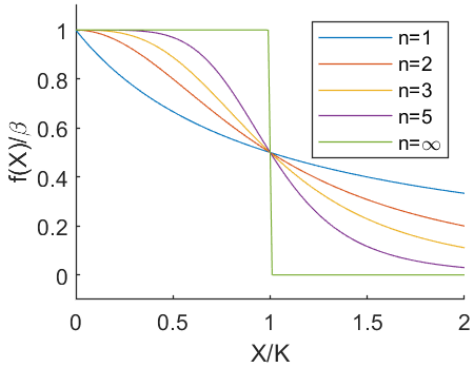
Since the target gene only exist one or a few copies (or a few hundred copies, if on a plasmid) in the cell, the “fraction” here can be understood as “probability”

The math behind input nodes cont.

If the transcription factor forms a homo-polymer (e.g. dimer/tetramer), there will be a constant on the exponent called the *Hill coefficient* (remember the physical meaning of the Hudgkin-Huxley exponent).

$$f = \frac{[A]^n}{[A]^n + K_d^n} (activational)$$

$$f = \frac{K_d^n}{[R]^n + K_d^n} (repressional)$$



Finally, the expression rate of a promoter is proportional to the fraction bound to an activator, or unbound to a repressor:

$$\frac{d[P]}{dt} = \alpha \frac{[A]^n}{[A]^n + K_d^n} - \beta[P]$$

subtract the degradation term

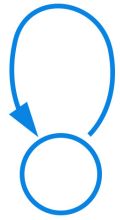
The simplest circuit: autoregulation

Negative feedback accelerates the time to reach equilibrium.

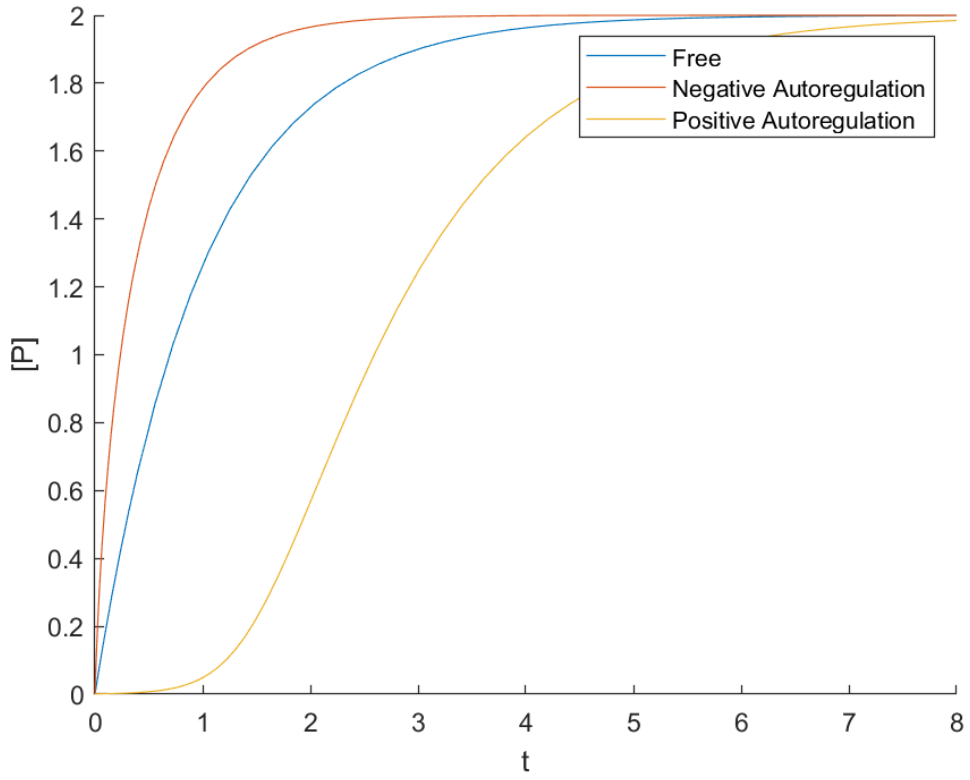
*Hill coefficient = 1 for concise

$$\frac{\mathrm{d[P]}}{\mathrm{dt}} = \alpha' \cdot \frac{\mathrm{K_d}}{\mathrm{K_d} + \mathrm{[P]}} - \beta \cdot \mathrm{[P]}$$

$$\frac{\mathrm{d[P]}}{\mathrm{dt}} = \alpha'' \cdot \frac{[P]}{\mathrm{K_d} + \mathrm{[P]}} - \beta \cdot \mathrm{[P]}$$



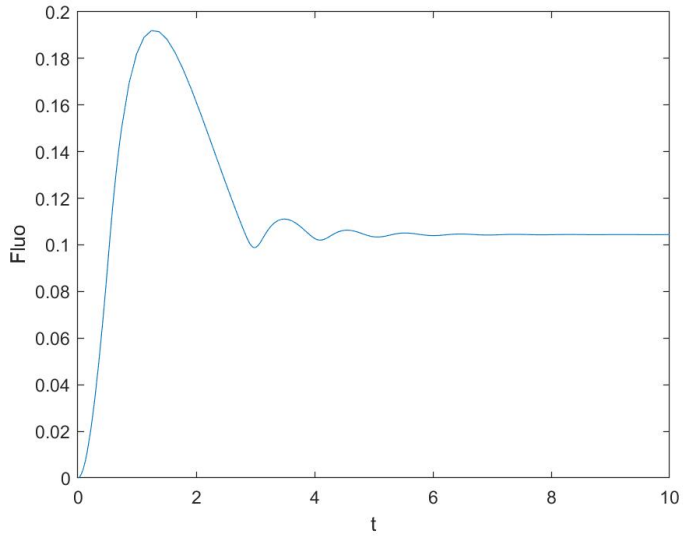
“Move” the equilibrium expression to the same level by varying the promoter strength α but keeping the degradation rate β the same



Autoregulation cont.

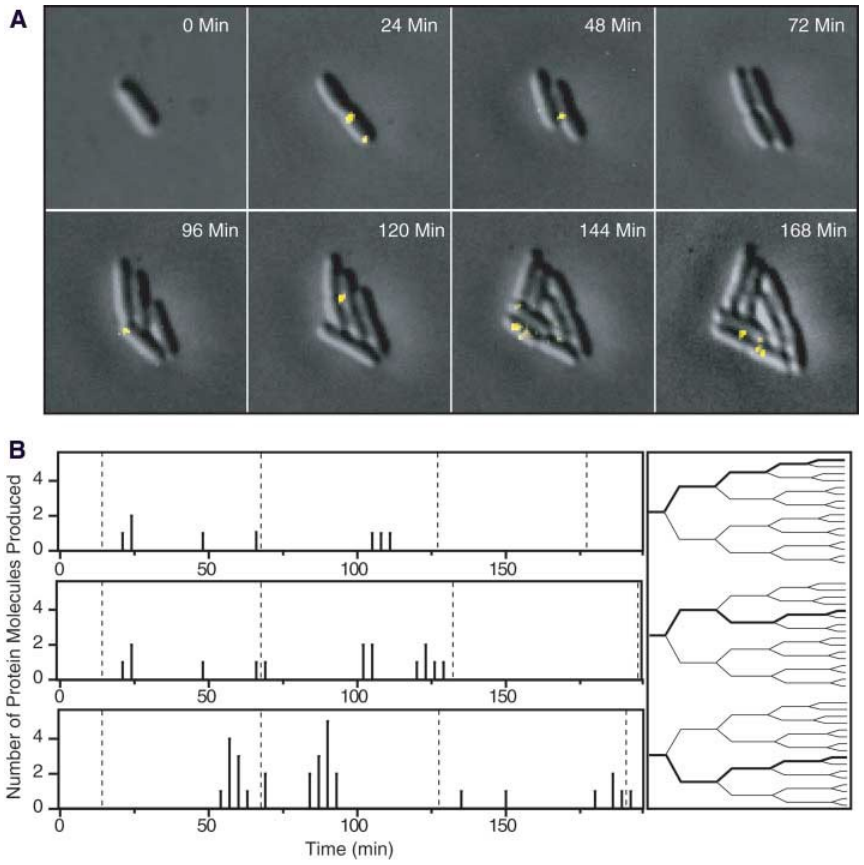
- Negative autoregulation:
  - Speedup the time to plateau
  - Give rise to oscillation
  - Suppress noise
- Positive autoregulation
  - Slow down the time to equilibrium
  - Switch-like behavior
  - Amplify signal

Can single negatively autoregulated node lead to oscillation?



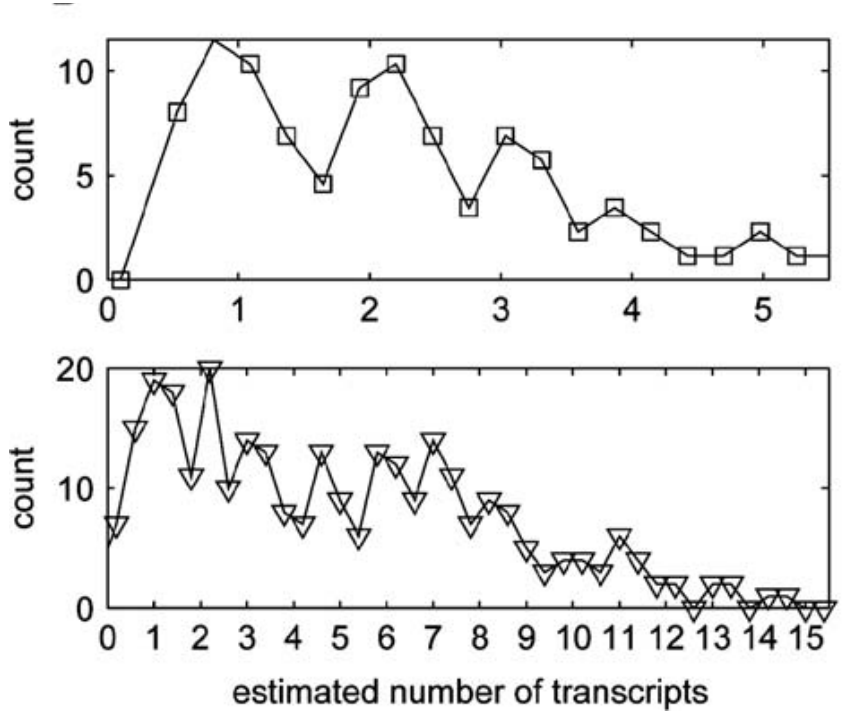
$K_d = 0.1$
$n = 50$

Caveat: the transcriptional burst



J. Yu, 2006

(right) Work done by Dr. Johan Paulsson



I. Golding, 2005

Future direction and challenges

- How to build robust directed evolution platform?
- Therapeutic genetic circuits
- Protein and RNA circuits
- DNA storage
- Single cell genetics

Thank You!