Bistability and a differential equation model of the *lac* operon

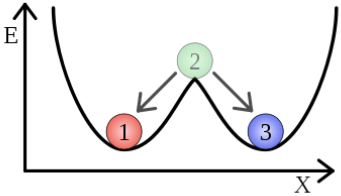
Matthew Macauley

Department of Mathematical Sciences
Clemson University
`http://www.math.clemson.edu/~macaule/`

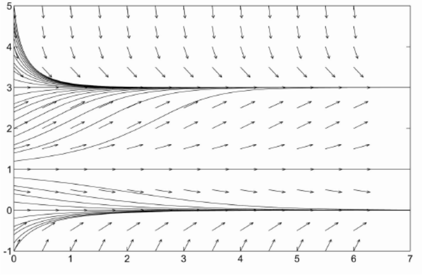
Math 4500, Fall 2016

Bistability

A system is bistable if it is capable of resting in two stable steady-states separated by an unstable state.



From Wikipedia.



The *threshold ODE*: $y' = -ry\left(1 - \frac{y}{M}\right)\left(1 - \frac{y}{T}\right)$.

In the threshold model for population growth, there are three steady-states, $0 < T < M$:

- M = carrying capacity (stable),
- T = extinction threshold (unstable),
- 0 = extinct (stable).

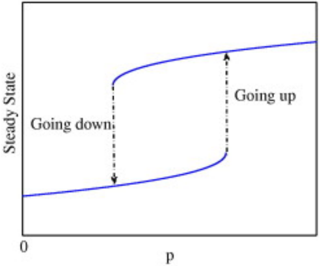
Types of bistability

For an example of bistability, consider the *lac* operon.

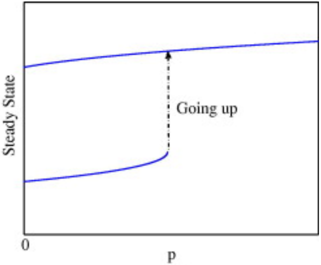
The expression level of the *lac* operon genes are either almost zero ("basal levels"), or very high (thousands of times higher). There's no "inbetween" state.

The precise expression level depends on the concentration level of intracellular lactose. *Let's denote this parameter by p.*

Now, let's "tune" this parameter. The result might look like the graph on the left.



(a)

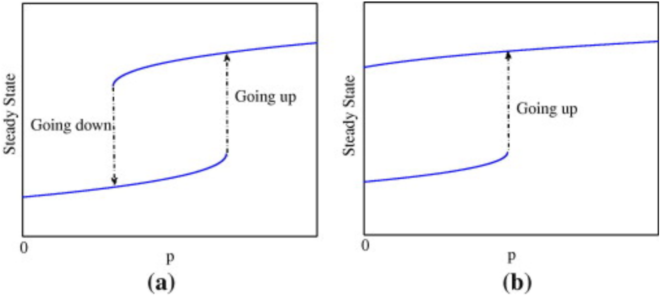


(b)

This is reversible bistability. In other situations, it may be irreversible (at right).

Hysteresis

In the case of reversible bistability, note that the *up-threshold* L_2 *of* p is higher than the *down-threshold* L_1 *of* p.



This is hysteresis: a dependence of a state on its current state *and* past state.

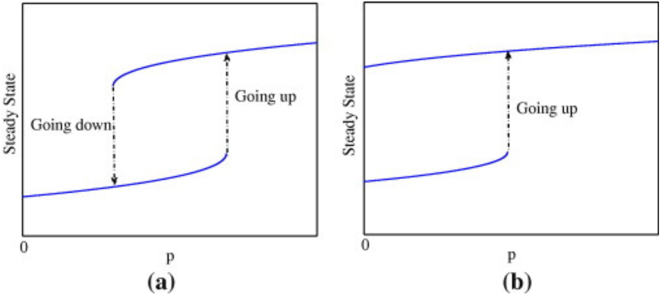
Thermostat example

Consider a home thermostat set for 72°.

- If the temperature is $T < 71$, then the heat kicks on.
- If the temperature is $T > 73$, then the AC kicks on.
- If $71 < T < 73$, then we don't know whether the heat or AC was on last.

Hysteresis and the *lac* operon

If lactose levels are medium, then the state of the operon depends on whether or not a cell was grown in a lactose-rich environment.



Lac operon example

Let $[L]$ denote the concentration of intracellular lactose.

- If $[L] < L_1$, then the operon is OFF.
- If $[L] > L_2$, then the operon is ON.
- If $L_1 < [L] < L_2$, then the operon could be ON or OFF.

The region of bistability (L_1, L_2) has both induced and un-induced cells.

Hysteresis and the *lac* operon

The Boolean network models we've seen are too simple to capture bistability.

We'll see two different ODE models of the *lac* operon that exhibit bistability.

These ODE models were designed using Michaelis–Menten equations from mass-action kinetics which we learned about earlier.

In a later lecture, we'll see how bistability can indeed be captured in a Boolean network system.

In general, bistable systems tend to have positive feedback loops (in their "wiring diagrams") or double-negative feedback loops (=positive feedback).

Modeling dilution in protein concentration due to bacterial growth

E. coli grows fast! It can double in 20 minutes. Thus, reasonable ODE models involving concentration shouldn't assume that volume is constant.

Let's define:

- $V =$ average volume of an *E. coli* bacterial cell.
- Let $x =$ number of molecules of protein X in that cell.

Assumptions about these derivatives:

- cell volume increases exponentially in time: $\frac{dV}{dt} = \mu V$.
- degradation of X is exponential: $\frac{dx}{dt} = -\beta x$.

The concentration of x is $[x] = \frac{x}{V}$, and the derivative of this is (by the quotient rule):

$$\frac{d[x]}{dt} = \big(x'V - V'x\big)\frac{1}{V^2} = \big(-\beta xV - \mu Vx\big)\frac{1}{V^2} = -\big(\beta+\mu\big)\frac{x}{V} = -(\beta+\mu)[x].$$

Modeling of lactose repressor dynamics

Assumptions

- *Lac* repressor protein is produced at a constant rate.
- Laws of mass-action kinetics.

- *Repressor binds to allolactose*:

$$R + nA \underset{1}{\overset{K_1}{\rightleftharpoons}} RA_n \qquad \frac{d[RA_n]}{dt} = K_1[R][A]^n - [RA_n]$$

Assume the reaction is at equilibrium: $\frac{d[RA_n]}{dt} = 0$, and so $K_1 = \frac{[RA_n]}{[R][A]^n}$.

- *The repressor protein binds to the operator region if there is no allolactose*:

$$O + R \underset{1}{\overset{K_2}{\rightleftharpoons}} OR \qquad \frac{d[OR]}{dt} = K_2[O][R] - [OR].$$

Assume the reaction is at equilibrium: $\frac{d[OR]}{dt} = 0$, and so $K_2 = \frac{[OR]}{[O][R]}$.

Modeling of lactose repressor dynamics

Let O_{tot} = total operator concentration (a constant). Then, using $K_2 = \frac{[OR]}{[O][R]}$,

$$O_{tot} = [O] + [OR] = [O] + K_2[O][R] = [O](1 + K_2)[R]\,.$$

Therefore, $\frac{[O]}{O_{tot}} = \frac{1}{1+K_2[R]}$. *"Proportion of free (unbounded) operator sites."*

Let R_{tot} be total concentration of the repressor protein (constant):

$$R_{tot} = [R] + [OR] + [RA_n]$$

Assume only a few molecules of operator sites per cell: $[OR] \ll \max\big\{[R], [RA_n]\big\}$:

$$R_{tot} \approx [R] + [RA_n] = [R] + K_1[R][A]^n$$

Eliminating $[RA_n]$, we get $[R] = \dfrac{R_{tot}}{1 + K_1[A]^n}$.

Now, the proportion of free operator sites is:

$$\frac{[O]}{O_{tot}} = \frac{1}{1 + K_2[R]} = \frac{1}{1 + K_2\left(\frac{R_{tot}}{1+K_1[A]^n}\right)} \cdot \frac{1 + K_1[A]^n}{1 + K_1[A]^n} = \underbrace{\frac{1 + K_1[A]^n}{K + K_1[A]^n}}_{:=f([A])},$$

where $K = 1 + K_2 R_{tot}$.

Modeling of lactose repressor dynamics

Summary

The proportion of free operator sites is

$$\frac{[O]}{O_{tot}} = \underbrace{\frac{1+K_1[A]^n}{K+K_1[A]^n}}_{:=f([A])}, \qquad \text{where } K = 1 + K_2 R_{tot}.$$

Remarks

- The function $f([A])$ is (almost) a Hill function of coefficient n.
- $f([A]=0) = \frac{1}{K} > 0$ "minimal basal level of gene expression."
- f is *increasing* in $[A]$, when $[A] \geqslant 0$.
- $\lim_{[A]\to\infty} f([A]) = 1$ "with lots of allolactose, gene expression level is max'ed."

Modeling time-delays

The production of mRNA from DNA via transcription is not an instantaneous process; suppose it takes time $\tau > 0$.

Thus, the production rate of mRNA is not a function of allolactose at time t, but rather at time $t - \tau$.

Suppose protein P decays exponentially, and its concentration is $p(t)$.

$$\frac{dp}{dt} = -\mu p \quad \Longrightarrow \quad \int_{t-\tau}^{t} \frac{dp}{p} = -\mu \int_{t-\tau}^{t} dt\,.$$

Integrating yields

$$\ln p(t)\Big|_{t-\tau}^{t} = -\mu t\Big|_{t-\tau}^{t} dt = \ln \frac{p(t)}{p(t-\tau)} = -\mu[t-(t-\tau)] = -\mu\tau.$$

Exponentiating both sides yields $\frac{p(t)}{p(t-\tau)} = e^{-\mu\tau}$,and so

$$p(t) = e^{-\mu\tau} p(t-\tau).$$

A 3-variable ODE model

Consider the following 3 quantities, which represent *concentrations* of:

- $M(t) =$ mRNA,
- $B(t) = \beta$-galactosidase,
- $A(t) =$ allolactose.

Assumption: Internal lactose (L) is available and is a parameter.

The model (Yildirim and Mackey, 2004)

$$\begin{aligned}
\frac{dM}{dt} &= \alpha_M \frac{1 + K_1 (e^{-\mu\tau_M} A_{\tau_M})^n}{K + K_1 (e^{-\mu\tau_M} A_{\tau_M})^n} - \tilde{\gamma}_M M \\
\frac{dB}{dt} &= \alpha_B e^{-\mu\tau_B} M_{\tau_B} - \tilde{\gamma}_B B \\
\frac{dA}{dt} &= \alpha_A B \frac{L}{K_L + L} - \beta_A B \frac{A}{K_A + A} - \tilde{\gamma}_A A
\end{aligned}$$

These are *delay differential equations*, with discrete time delays due to the transcription and translation processes.

3-variable ODE model

ODE for β-galactosidase (B)

$$\frac{dB}{dt} = \alpha_B e^{-\mu\tau_B} M_{\tau_B} - \tilde{\gamma}_B B,$$

Justification:

- $\tilde{\gamma}_B B = \gamma_B B + \mu B$ represents loss due to β-galactosidase degredation and dilution from bacterial growth.
- Production rate of β-galactosidase, is proportional to mRNA concentration.
- τ_B = time required for translation of β-galactosidase from mRNA, and $M_{\tau_B} := M(t - \tau_B)$.
- $e^{-\mu\tau_B} M_{\tau_B}$ accounts for the time-delay due to translation.

3-variable ODE model

ODE for mRNA (M)

$$\frac{dM}{dt} = \alpha_M \frac{1 + K_1(e^{\mu\tau_M} A_{\tau_M})^n}{K + K_1(e^{-\mu\tau_M} A_{\tau_M})^n} - \widetilde{\gamma}_M M$$

Justification:

- $\widetilde{\gamma}_M M = \gamma_M M + \mu M$ represents loss due to mRNA degradation and dilution from bacterial growth.
- Production rate of mRNA is proportional to fraction of free operator sites,

$$\frac{[O]}{O_{tot}} = \frac{1 + K_1[A]^n}{1 + K_1[A]^n} = f([A]).$$

- The constant $\tau_M > 0$ represents the time-delay due to transcription of mRNA from DNA. Define $A_{\tau_M} := A(t - \tau_M)$.
- The term $e^{-\mu\tau_M} A_{\tau_M}$ accounts for the concentration of A at time $t - \tau_M$, and dilution due to bacterial growth.

3-variable ODE model

ODE for allolactose (A)

$$\frac{dA}{dt} = \alpha_A B \frac{L}{K_L + L} - \beta_A B \frac{A}{K_A + A} - \widetilde{\gamma}_A A$$

Justification:

- $\widetilde{\gamma}_A A = \gamma_A A + \mu A$ represents loss due to allolactose degredation and dilution from bacterial growth.
- The first term models production of allolactose from the chemical reaction $lac \xrightarrow{\beta-gal} allo$.
- The second term models loss of allolactose from the chemical reaction $allo \xrightarrow{\beta-gal} glucose \;\&\; galactose$.

A 3-variable ODE model

Steady-state analysis

To find the steady states, we must solve the nonlinear system of equations:

$$\begin{aligned}
0 &= \alpha_M \frac{1 + K_1(e^{-\mu\tau_M} A_{\tau_M})^n}{K + K_1(e^{-\mu\tau_M} A_{\tau_M})^n} - \widetilde{\gamma}_M M \\
0 &= \alpha_B e^{-\mu\tau_B} M_{\tau_B} - \widetilde{\gamma}_B B \\
0 &= \alpha_A B \frac{L}{K_L + L} - \beta_A B \frac{A}{K_A + A} - \widetilde{\gamma}_A A
\end{aligned}$$

This was done by Yildirim et al. (2004). They set $L = 50 \times 10^{-3}$ mM, which was in the "bistable range."

They also estimated the parameters through an extensive literature search.

Finally, they estimated $\mu = 3.03 \times 10^{-2}\ \text{min}^{-1}$ by fitting the ODE models to experimental data.

Steady states	A^* (mM)	M^* (mM)	B^* (mM)
I.	4.27×10^{-3}	4.57×10^{-7}	2.29×10^{-7}
II.	1.16×10^{-2}	1.38×10^{-6}	6.94×10^{-7}
III.	6.47×10^{-2}	3.28×10^{-5}	1.65×10^{-5}

3-variable ODE model

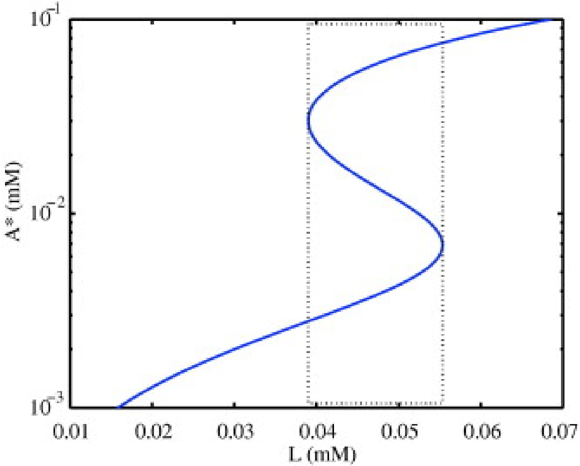


Figure: Bistability is (L, A^*) space. The y-axis is in logarithmic scale. For a range of L concentrations there are three coexisting steady states for the allolactose concentration.

3-variable ODE model

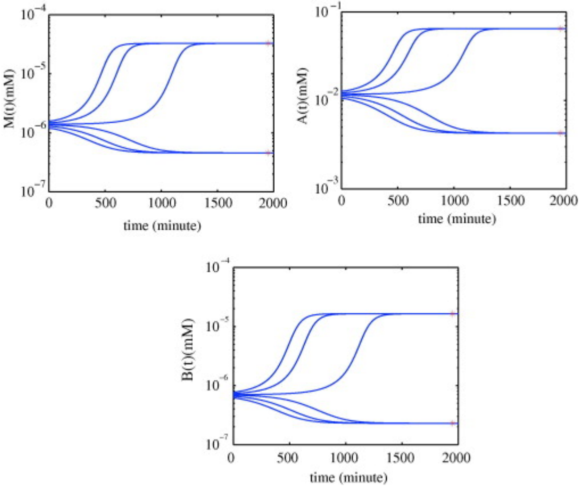


Figure: Time series simulations of mRNA, β-galactosidase and allolactose concentrations. These were produced by numerically solving the 3-variable model using $L = 50 \times 10^{-3}$ mM, which is in the bistable region.

5-variable ODE model

Consider the following 5 variables, which represent *concentrations* of:

- $M(t)$ = mRNA,
- $B(t)$ = β-galactosidase,
- $A(t)$ = allolactose.
- $P(t)$ = lac permease.
- $L(t)$ = intracellular lactose.

The model (Yildirim and Mackey, 2004)

$$\begin{aligned}
\frac{dM}{dt} &= \alpha_M \frac{1 + K_1(e^{-\mu\tau_M} A_{\tau_M})^n}{K + K_1(e^{-\mu\tau_M} A_{\tau_M})^n} + \Gamma_0 - \widetilde{\gamma}_M M \\
\frac{dB}{dt} &= \alpha_B e^{-\mu\tau_B} M_{\tau_B} - \widetilde{\gamma}_B B \\
\frac{dA}{dt} &= \alpha_A B \frac{L}{K_L + L} - \beta_A B \frac{A}{K_A + A} - \widetilde{\gamma}_A A \\
\frac{dP}{dt} &= \alpha_P e^{-\mu(\tau_B + \tau_P)} M_{\tau_B + \tau_P} - \widetilde{\gamma}_P P \\
\frac{dL}{dt} &= \alpha_L P \frac{L_e}{K_{L_e} + L_e} - \beta_{L_e} P \frac{L}{K_{L_e} + L} - \alpha_A B \frac{L}{K_L + L} - \widetilde{\gamma}_L L
\end{aligned}$$

5-variable ODE model

Remarks

- The only difference in the ODE for M is the extra term Γ_0 which describes the basal transcription rate (in the absence of extracellular lactose).
- The ODEs for B and A are the same as in the 3-variable model.
- The ODE for P is very similar to the one for B:
  - production rate of *lac* permease is proportional to mRNA concentration, with a time-delay.
  - the 2nd term accounts for loss due to degredation and dilution.
- The ODE for lactose,

$$\frac{dL}{dt} = \alpha_L P \frac{L_e}{K_{L_e} + L_e} - \beta_{L_e} P \frac{L}{K_{L_1} + L} - \alpha_A B \frac{L}{K_L + L} - \widetilde{\gamma}_L L,$$

is justified by the following:
  - The 1st term models gain due to transport of external lactose by *lac* permease.
  - The 2nd term accounts for loss due to this process being reversible.
  - The 3rd term describes loss due to $lac \xrightarrow{\beta-gal} allo$.
  - the 4th term accounts for loss due to degredation and dilution.

A 5-variable ODE model

To find the steady states, we set $M' = A' = B' = L' = P' = 0$ and solve the resulting nonlinear system of equations.

This was done by Yildirim et al. (2004). They set $L_e = 50 \times 10^{-3}$ mM, which was in the "bistable range."

They also estimated the parameters through an extensive literature search.

Finally, they estimated $\mu = 2.26 \times 10^{-2}$ min^{-1} by fitting the ODE models to experimental data.

SS's	A^* (nM)	M^* (mM)	B^* (mM)	L^* (mM)	P^* (mM)
I.	7.85×10^{-3}	2.48×10^{-6}	1.68×10^{-6}	1.69×10^{-1}	3.46×10^{-5}
II.	2.64×10^{-2}	7.58×10^{-6}	5.13×10^{-6}	2.06×10^{-1}	1.05×10^{-4}
III.	3.10×10^{-1}	5.80×10^{-4}	3.92×10^{-4}	2.30×10^{-1}	8.09×10^{-3}

5-variable ODE model

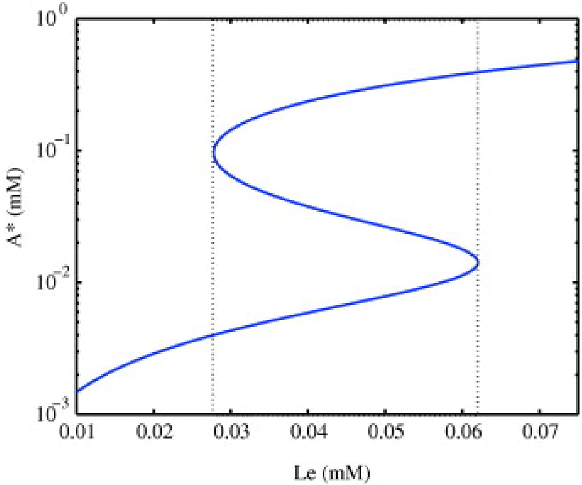


Figure: Bistability is (L, A^*) space. The y-axis is in logarithmic scale. For a range of L_e concentrations there are three coexisting steady states for the allolactose concentration.

5-variable ODE model

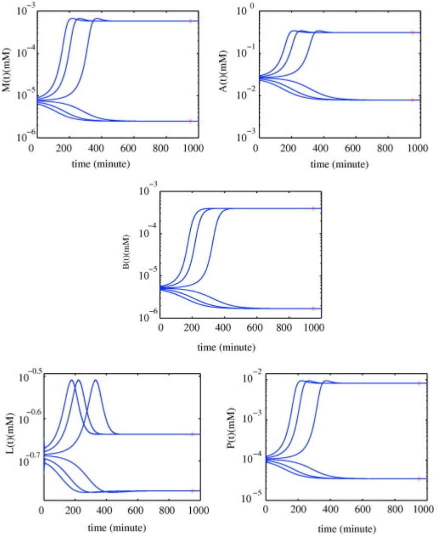


Figure: Time series simulations of mRNA, β-galactosidase and allolactose concentrations. These were produced by numerically solving the 3-variable model using $L_e = 50 \times 10^{-3}$, which is in the bistable region.