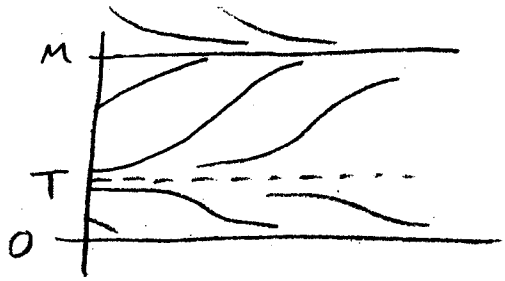
4 A differential equation model of the lac operon

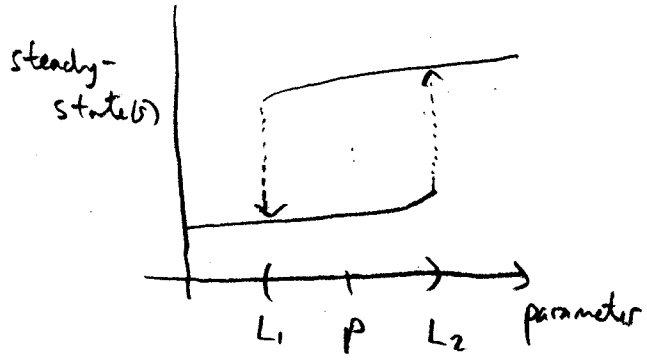
A system is <u>bistable</u> if it is capable of resting in two stable steady-states separated by an unstable steady state.

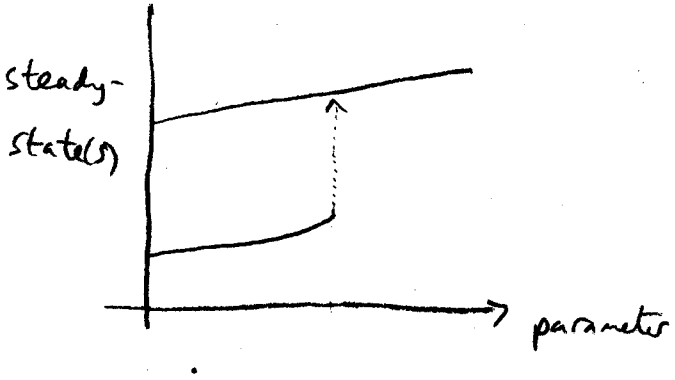
Ex: Recall the threshold population model

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



Bistability can be <u>reversible</u> or <u>irreversible</u>:





One can plot the steady-states as a function of (tuning) some parameter (see above).

Note that the "up-switch" L_2 is higher than the "down-switch" L_1. This is <u>hysteresis</u> – a dependence of a state on its current state <u>and</u> past state.

[2]

The lac operon exhibits a hysteresis effect & bistability:

If lactose concentration: $[L] < L_1$, operon is off

$[L] > L_2$, operon is on.

$L_1 < [L] < L_2$, operon could be on or off.

This "region of bistability", (L_1, L_2), has both induced and uninduced cells.

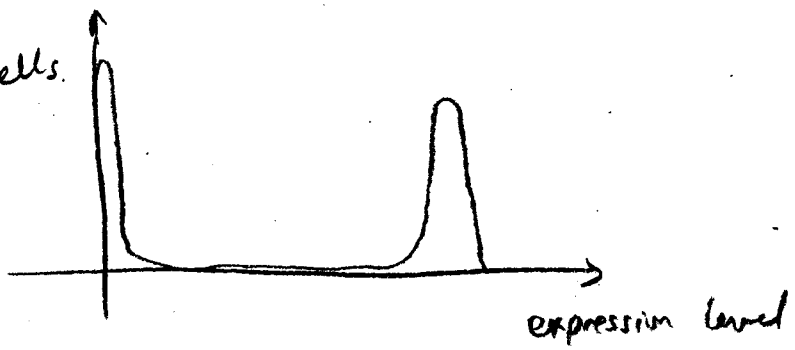
Cells that were grown in a lactose environment likely "on"

Cells grown in an environment with low levels of lactose likely to be "off."

Analogy: Thermostat turns heater: on if temp < 67
off if temp > 72

* If temp is 70°, it's impossible to know if heater is on or off without knowing past data.

Distribution of gene expression levels of cells for some $[L] \in (L_1, L_2)$:



The simple Boolean network models we've seen can't capture bistability.

We'll see an ODE model, & a Boolean model that can.

Bistable systems often have positive feedback loops, or a double-negative feedback loop.

Model by Yildirim & Mackey, 2004

3 variables: M = mRNA
B = β-galactosidase
A = allolactose

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

Preliminaries

- Modeling dilution in protein concentration due to bacterial growth

E-coli population grows fast! (Can double in 20 minutes!)

Let V = ave. volume of bacterial cell

x = # molecules of protein X in that cell.

Assume cell volume increases exponentially in time: $\frac{dV}{dt} = \mu V$

(4)

Assume degradation of X is exponential: $\frac{dx}{dt} = -\beta x$

Concentration $[x] = \frac{x}{V}$

$$\Rightarrow \frac{d[x]}{dt} = \left(x'V + V'x\right)\frac{1}{V^2} = \left(-\beta x V - \mu V x\right)\frac{1}{V^2}$$

$$= -(\beta+\mu)\frac{x}{V} = -(\beta+\mu)[x].$$

- Modeling of lactose repressor dynamics

Assume: lac repressor protein is produced at const. rate (regardless of external lactose).

* Repressor binds to allolactose: $R + nA \underset{1}{\overset{K_1}{\rightleftharpoons}} RA_n$

$$\frac{d[RA_n]}{dt} = K_1[R][A]^n - [RA_n]$$

Assume reaction is at equilibrium: $\frac{d[RA_n]}{dt} = 0 \Rightarrow K_1 = \frac{[RA_n]}{[R][A]^n}$

* Repressor binds to operator region (O) if no allolactose:

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

At equilibrium: $K_2 = \frac{[OR]}{[O][R]}$.

Let O_{tot} = total operator concent. (const),

eliminate ↓

Then $O_{tot} = [O] + [OR] = [O] + K_2[O][R]$

$= [O](1 + K_2)[R]$.

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

Let R_{tot} be total concentration of repressor protein.

Assume const. (since not regulated by extracellular lactose)

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

Assume only a few molecules of operator site/cell

$\Rightarrow [OR] \ll \max\{[R], [RA_n]\}$

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

eliminate ↑

$\Rightarrow [R] = \frac{R_{tot}}{1 + K_1[A]^n}$

Plug into $\frac{[O]}{O_{tot}} = \frac{1}{1 + K_2\left(\frac{R_{tot}}{1+K_1[A]^n}\right)} \cdot \frac{1 + K_1[A]^n}{1 + K_1[A]^n}$

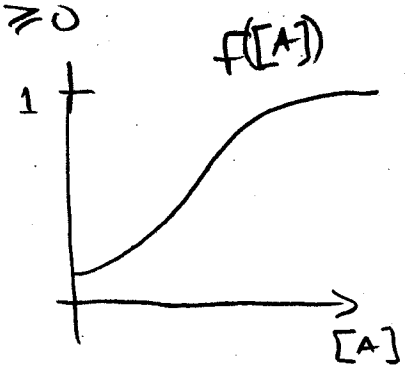
$= \underbrace{\frac{1 + K_1[A]^n}{K + K_1[A]^n}}_{:= f([A])}$ where $K = 1 + K_2 R_{tot}$.

Remarks: • $f([A]=0) = \frac{1}{K} > 0$ "minimal basal level of gene expression"

• f is increasing in $[A]$, when $[A] \geq 0$

• $\lim_{[A]\to\infty} f([A]) = 1$

"With abundance of allolactose, gene expression level is max."



• How to incorporate $f'(t) = -\mu\, p(t-\tau_M)$ [exp. decay w/ time delay]

Suppose Protein P decays exponentially, Concent. = p.

$$\frac{dp}{dt} = -\mu p \Rightarrow \int_{t-\tau_M}^{t} \frac{dp}{p} = -\mu \int_{t-\tau_m}^{t} dt$$

$$\Rightarrow \ln p(t) \Big|_{t-\tau_m}^{t} = -\mu t \Big|_{t-\tau_m}^{t} \, dt$$

$$= \ln \frac{p(t)}{p(t-\tau_m)} = -\mu[t-(t-\tau_m)] = -\mu\tau_M$$

$$\frac{p(t)}{p(t-\tau_m)} = e^{-\mu\tau_M}$$

$$\Rightarrow p(t) = e^{-\mu\tau_M}\, p(t-\tau_M)$$

↑ accounts for time delay τ_m

Yildirim-Mackey model for lac operon (variables M, B, A).

$$(1)\quad \frac{dM}{dt} = \alpha_M \frac{1 + K_1 (e^{-\mu \tau_m} A_{\tau_m})^n}{K + K_1 (e^{-\mu \tau_m} A_{\tau_m})^n} - \tilde{\gamma}_M M \qquad (\underline{mRNA})$$

where $A_{\tau_m} = A(t - \tau_m)$ (time delay τ_m)

$\tilde{\gamma}_M = \gamma_M + \mu$ (degradation & dilution constants)

Justification:

* Production rate of mRNA, $\frac{dM}{dt}$, proportional to fraction of free operator sites: $\frac{[O]}{O_{tot}} = \frac{K_1 [A]^n}{K + K_1 [A]^n}$

* $e^{-\mu \tau_m} A_{\tau_m}$ accounts for the concentration of A at time $t - \tau_m$, which is proportional to $\frac{dM}{dt}$.

* $\tilde{\gamma}_M M = \gamma_M M + \mu M$
  - $\gamma_M M$: loss due to mRNA degradation
  - μM: loss due to dilution from bacterial growth.

$$(2)\quad \frac{dB}{dt} = \alpha_B e^{-\mu \tau_B} M_{\tau_B} - \tilde{\gamma}_B B \qquad (\underline{\beta\text{-galactosidase}})$$

τ_B = time required for mRNA translation, $\tilde{\gamma}_B = \gamma_B + \mu$.

$M_{\tau_B} = M(t - \tau_B)$

(3) $\frac{dA}{dt} = \alpha_A B \frac{L}{K_L + L} - \beta_A B \frac{A}{K_A + A} - \tilde{\gamma}_A A$ (allolactose)

degradation & dilution.

lac $\xrightarrow{\beta\text{-gal}}$ allo

allo $\xrightarrow{\beta\text{-gal}}$ glucose & galactose

(Michaelis-Menten eqn)

These are "delay differential eqn's with discrete timesteps."

Next: Steady-state analysis & numerical simulations.

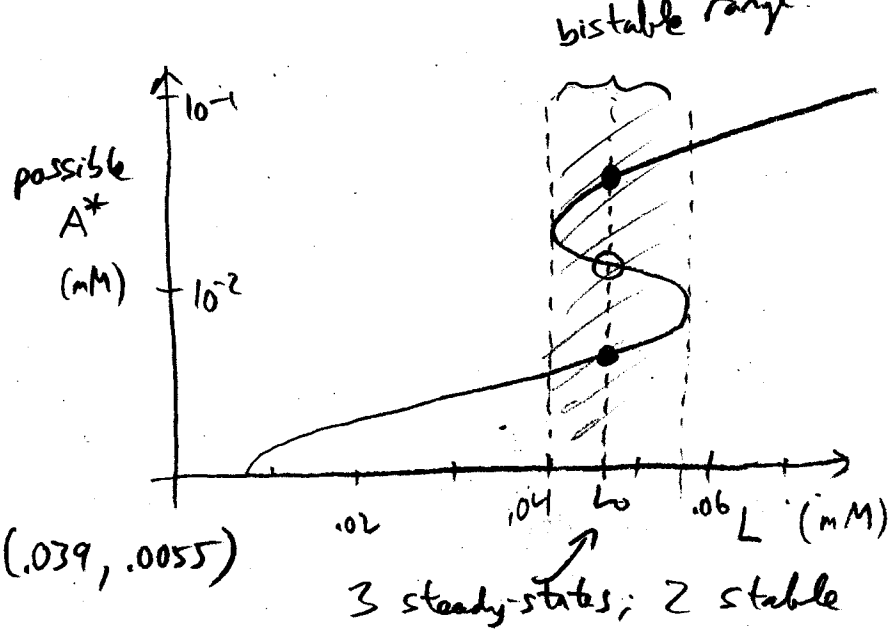
Need to fix unknown parameters, constants.

Approach: Estimate by fitting these models to experimental data.

[e.g., $M = 3.03 \times 10^{-2}$ mM^{-1}, Hill coeff. $n = 2$]

Steady-state analysis:

- Set $M' = B' = A' = 0$
- Solve for range of L concentrations (L is the "tuning parameter").



Result: Bi-stable range: $L \in (.039, .0055)$

3 steady-states; 2 stable