CCBS. Lecture 06. Noise in bio
& Equilibrium physics of bioregulation

From last time — noise. ① Intro
② chemical master equation.

This time — ③ Gillespie.
④ Noise analysis (simple). @t S.S.
⑤ Some stochastic phenomena.

☆. Equilibrium physics of bioregulation.

① Energy and equilibrium. a physics perspective.

(Reference: KenDill Book)

— Systems tend towards equilibrium.

— Equilibrium in physics — entropy, etc.

The powerful idea of equilibrium.

Microscopic world — Boltzmann distr.

— detailed balance

② Applied to enzymatic and gene regulation

(Reference: Phillips PBoL or Molecular Switch.)

— Focus on single molecule's states.

— Michaelis Menten.

— Allostery (MWC)

— Lac operon

③ Beyond equilibrium: Markov chains
for molecular state transitions.

e.g. Metabolism. phosphorylation cascades.

④ Steady state. distr. and hitting times

(3) Gillespie algorithm. (or. SSA — stochastic simulation algorithm.)

- Simulation first. because. (Reference: Erban, Chapman, Maini. 2007 Practical guide to stoch. sim. of reaction diffusion processes)

(1). Deterministic. already hard to analyze for general case.

This is much harder for stochastic case.

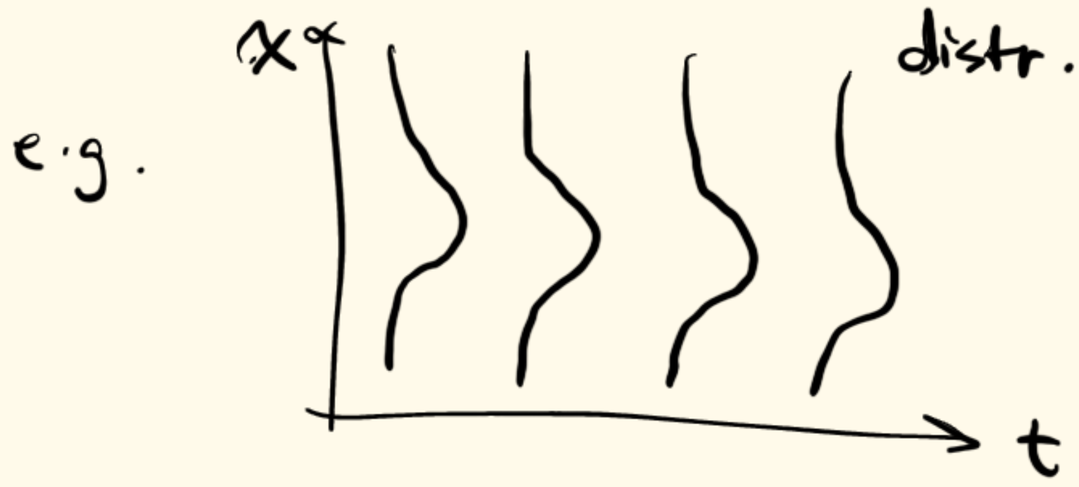
(2). Distribution vs Trajectory.

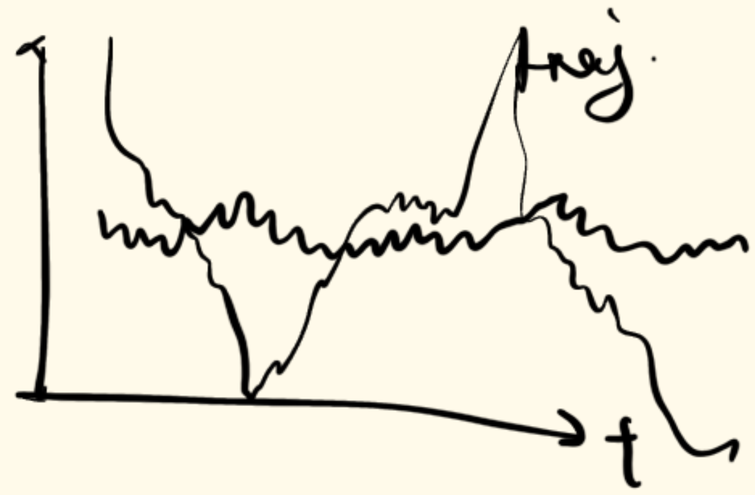
Exact analysis, even when doable, is often only possible for steady state distribution.

or. distribution dynamics.

But that's different from trajectory dynamics.

e.g.





(3). Usually analysis can be done after approximation. such as linearization.

Analysis can give the full picture. but approximate.

so. always helpful to check with simulations.

- Can't directly simulate the distribution. → inf. dim ODE. $P_n(t)$.

We can simulate trajectories. then distr. can be obtained from averages over lots of traj.

- Simplest idea. just like ODE sim. $\frac{dx}{dt} = f(x)$

(Euler integration) ⇒. $x(t+\Delta t) = x(t) + f(x(t))\Delta t$.

$X \xrightarrow{v_2} X-1$ $\quad v_2 = kx$

$$N_x(t+\Delta t) = \begin{cases} N_x(t) - 1 & \text{with prob } a_2\Delta t \\ & a_2 = k N_x(t) \\ N_x(t) & \text{with prob } 1 - a_2\Delta t. \end{cases}$$

But need Δt small to have a good approx...

Could be very costly.

Could we simulate $N_x(t)$ exactly?

Yes. by transforming randomness from

coho other an event happens in an interval

to when does an event happen.

- Start at t, want τ s.t. $t+\tau$ is next reaction.
- Let $f(N_x(t), s)\,ds$ ← an infinitesimal time interval

pdf for τ.

$= \mathbb{P}\{$ $N_x(t)$ molecules at time t, and the next reaction occurs in time interval $[t+s, t+s+ds)$ $\}$

$g(N_x(t), s) = \mathbb{P}\{$ No reaction in interval $[t, t+s)\}$

Denote reaction with rate $v_\cdot(N_x)$.

(e.g. $v = kN_x$)

$\Rightarrow f(N_x(t), s)\,ds = g(N_x(t), s)\; v(N_x(t+s))\,ds$

$= N_x(t)$ since no reaction

$= g(N_x(t), s)\, v(N_x(t))\,ds.$

Let's solve for $g(N_x(t), s)$.

$g(N_x(t), \sigma+d\sigma) = \text{Prob}\{$ no reaction $[t, t+\sigma)$ and, no reaction $[t+\sigma, t+\sigma+d\sigma)$ $\}$

(memoryless or. independence) $= g(N_x(t), \sigma)\,[1 - v(N_x(t+\sigma))\,d\sigma]$.

$N_x(t+\sigma) = N_x(t)$

since no reactions occured.

$$\Rightarrow \quad \frac{g(N_x(t), \tau+d\tau) - g(N_x(t),\tau)}{d\tau} = -v(N_x(t))\, g(N_x(t),\tau)$$

$$\Rightarrow \quad d\tau \to 0 \qquad \frac{\partial}{\partial \tau} g(N_x(t),\tau) = -v(N_x(t))\, g(N_x(t),\tau)$$

$$\Rightarrow \quad g(N_x(t),\tau) = e^{-v(N_x(t))\tau}$$

$$\text{So} \quad f(N_x(t), s)\,ds = v(N_x(t))\, e^{-v(N_x(t))s}\, ds$$

↳ pdf (prob density function) for exponential distr.

$\tau \sim Exp(v)$. pdf. $ve^{-v\tau}$

cdf. $1 - e^{-v\tau}$.

- We want τ. s.t. $t+\tau$ is time for next reaction.
  for a reaction with rate v.

Then $\tau \in (0, +\infty)$ is a random number.

$$\tau \sim Exp(v).$$

This is exact! e.g. $X \xrightarrow{v_2} X-1 \quad v_2 = kx$.

At t. draw $\tau \sim Exp(kN_x(t))$.

Then $N_x(t+\tau) = N_x(t) - 1$

- What if multiple reactions?.

The reactions are independent. each with rate $v_1, \dots, v_m$.

Let τ_0 be time $t+\tau_0$ that any reaction happens.

$$\Rightarrow \quad \tau_0 \sim Exp(v_1 + \cdots + v_m).$$

Which reaction? $\mathbb{P}\{\text{it's reaction } j\} = \dfrac{v_j}{v_1 + \cdots + v_m}$

- This completes the Gillespie algorithm, or SSA.

~~Exact~~ simulation of stochastic trajectories by sampling event times.

④ Analysis of steady state distributions.

- Simulations can't give the full picture. over all parameters.

  ⇒ Analysis via moments. from CME.
  (Mean. Var.)

- Exact analysis. of Moments. example. $X \xrightarrow{\mu} X+1$, $X \xrightarrow{k} X-1$

$$\frac{dp_n}{dt} = k(n+1)p_{n+1} + \mu p_{n-1} - (kn+\mu)p_n.$$

Mean: $M(t) = \sum_{n=0}^{\infty} n p_n(t)$

Variance: $V(t) = \sum_{n=0}^{\infty} (n - M(t))^2 p_n(t)$

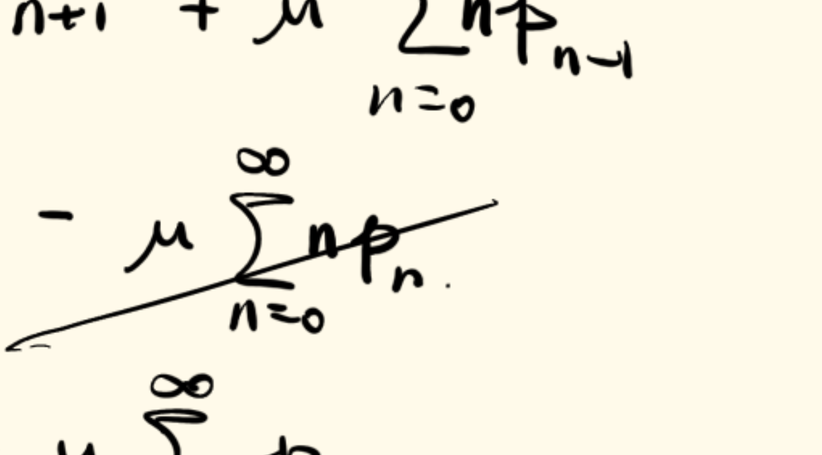
$$\frac{dM}{dt} = \frac{d}{dt}\sum_{n=0}^{\infty} n p_n(t) = k\sum_{n=0}^{\infty} (n-1) n p_n + \mu \sum_{n=0}^{\infty} (n+1) p_n$$

$$= k\sum_{n=0}^{\infty} n(n+1)p_{n+1} + \mu \sum_{n=0}^{\infty} n p_{n-1} - k\sum_{n=0}^{\infty} n^2 p_n - \mu \sum_{n=0}^{\infty} n p_n.$$

$$= -k \underbrace{\sum_{n=0}^{\infty} n p_n}_{M} + \mu \underbrace{\sum_{n=0}^{\infty} p_n}_{1}$$

$$= \mu - kM.$$

(This is just like. $\frac{d}{dt}x = \mu - kx$. deterministic rate eqn. WARNING: Not always so.)



Similarly. observe. $\sum_{n=0}^{\infty} n^2 p_n = V + M^2$

$$\frac{d}{dt}(V+M^2) = \frac{d}{dt}\sum_{n=0}^{\infty} n^2 p_n = \underbrace{k\sum_{n=0}^{\infty} (n-1)^2 n\, p_n}_{k\sum_{n=0}^{\infty} n^2(n+1)\,p_{n+1}} + \underbrace{\mu\sum_{n=0}^{\infty}(n+1)^2 p_n}_{\mu\sum_{n=0}^{\infty} n^2 p_{n-1}}$$

$(n^2-2n+1)n$ $\qquad$ n^2+2n+1

$$- k\sum_{n=0}^{\infty} n^2 p_n - \mu\sum_{n=0}^{\infty} n^2 p_n$$

$$= \sum_{n=0}^{\infty}\left[k(-2n^2+n) + \mu(2n+1)\right]p_n$$

$$= -2k(V+M^2) + kM + 2\mu M + \mu.$$

$$\frac{d}{dt}(V+M^2) = \frac{dV}{dt} + 2M\frac{dM}{dt} = \frac{dV}{dt} + 2M(\mu - kM).$$

$$\Rightarrow \begin{cases} \frac{dV}{dt} = \mu + kM - 2kV. \\ \frac{dM}{dt} = \mu - kM. \end{cases}$$

At s.s. $M = \frac{\mu}{k}$. $V = \frac{\mu+kM}{2k} = \frac{\mu}{k}$.

So. Mean = Variance. (Poisson! In fact. it is...)

Yeh can solve p_n at s.s. explicitly...

- But. this doesn't always work.

That the moments form a finite number of equations

is called Moment closure.

Not closed if. e.g. $\mathbb{E}(X)$ depends on $\mathbb{E}(X^2)$ depends on $\mathbb{E}(X^3)$...

this happens when e.g. $X \xrightarrow{v} X-1 \quad v = x^2$

- For example.

— Write reactions in net change form

$$X \xrightarrow{\nu_i(x)} X + \nu_i$$

x is a vector of species molecular counts.

$i = 1, \ldots, m$

$x = (x_j)$. $j = 1, \ldots, n$.

— Example: $X \xrightarrow{f^+(x)} x+1$ $\qquad$ $X \xrightarrow{f^-(x)} x-1$

$$\Rightarrow \frac{d}{dt} p(x,t) = f^+(x-1)\, p(x-1,t) - f^+(x)\, p(x,t)$$
$$+ f^-(x+1)\, p(x+1,t) - f^-(x)\, p(x,t)$$

$\langle x \rangle = \mathbb{E}(x)$.

Just another notation...

$$\Rightarrow \frac{d}{dt}\langle x \rangle = \frac{d}{dt} \sum_x x\, p(x,t)$$

$$= \sum_x x f^+(x-1)\, p(x-1,t) - \sum_x x f^+(x)\, p(x,t)$$
$$+ \sum_x x f^-(x+1)\, p(x+1,t) - \sum_x x f^-(x)\, p(x,t)$$

$$= \sum_x (x+1) f^+(x) p(x,t) - \sum_x x f^+(x)\, p(x,t)$$
$$+ \sum_x (x-1) f^-(x) p(x,t) - \sum_x f^-(x)\, p(x,t)$$

$$= \langle f^+(x) \rangle - \langle f^-(x) \rangle.$$

e.g. $f^+(x) = \mu$. $f^-(x) = x^2$. then $\frac{d}{dt}\langle x \rangle = \mu - \langle x^2 \rangle$.

- Cases with moment closure:

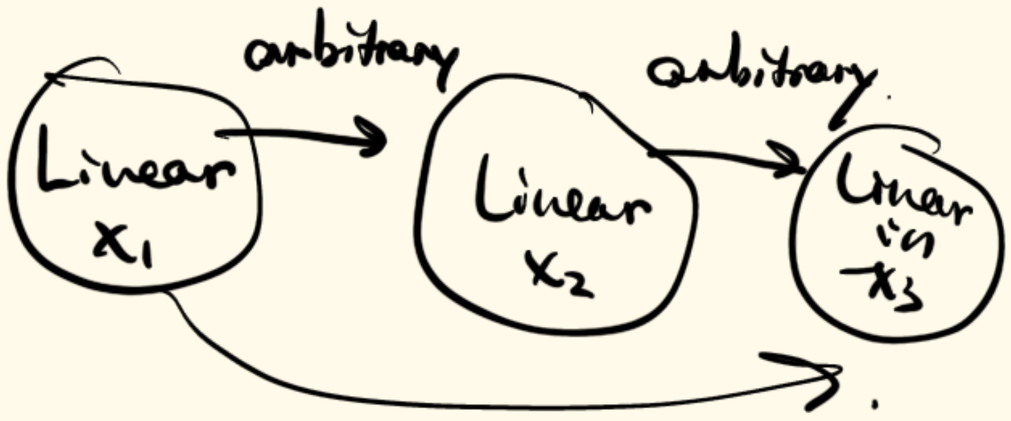
  - Linear. i.e. 1st order or 0th order reactions.

    v_j are all degree 1 polynomials of x.

    e.g. c, $c+x_1$, x_2. but not x_1x_2, x_1^2.

  - Feedforward structure

$$\dot{x}_1 = \mu - x_1$$
$$\dot{x}_2 = x_1^2 - x_2$$
$$\dot{x}_3 = x_1x_2 - x_3$$



This excludes many interesting cases though..

- More generally, how to analyze steady state moments?

  ⇒. Linear noise approximation (LNA).

  - Just like using linearization to analyze nonlinear dynamical systems.

    We can also do linearization for stochastic processes

  - approximate, $v_i(x) \approx v_i(x^*) + \frac{\partial v_i}{\partial x}(x^*)(x - x^*)$.
  - for the example. but with $x^* = \langle x \rangle$.

$$\frac{d}{dt}\langle x \rangle = \langle f^+(x) \rangle - \langle f^-(x) \rangle$$
$$\approx \left\langle f^+(\langle x \rangle) + \frac{\partial f^+}{\partial x}(\langle x \rangle)(x - \langle x \rangle) \right\rangle$$
$$- \left\langle f^-(\langle x \rangle) + \frac{\partial f^-}{\partial x}(\langle x \rangle)(x - \langle x \rangle) \right\rangle$$
$$= f^+(\langle x \rangle) - f^-(\langle x \rangle).$$

  - General solution and application of LNA, see homework.

(5) Some Stochastic Phenomena.

- Gene expression burstyness is an important reason that noise gets large

How to model this?

- Attempt 1 : $G \xrightarrow{k} G + bP$

where b is burst size.

But the burst size itself varies...

- Attempt 2 : burst size b is a random variable.

But it should actually come from binding of the gene with transcription factors, RNAP, etc...

So it should be different for different genes.

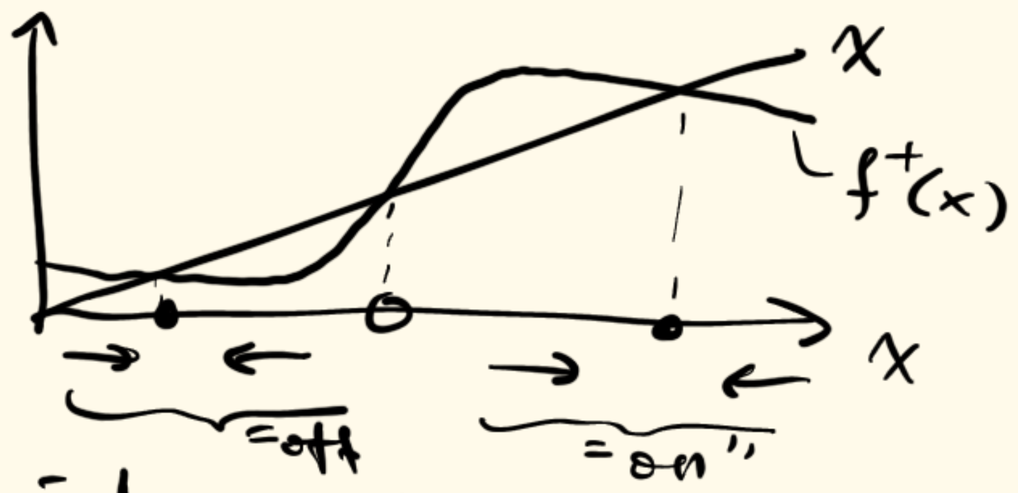
- Attempt 3 : $G_{off} \underset{k^-}{\overset{k^+}{\rightleftharpoons}} G_{on}$. $G_{on} \xrightarrow{k_{tx}} G_{on} + P$.

- This illustrates how features of the variations observed in data is structurally organized into mechanisms + noise (stochasticity).

o Multistability with (fast) noise becomes multi-modality.

- From deterministic model. we know

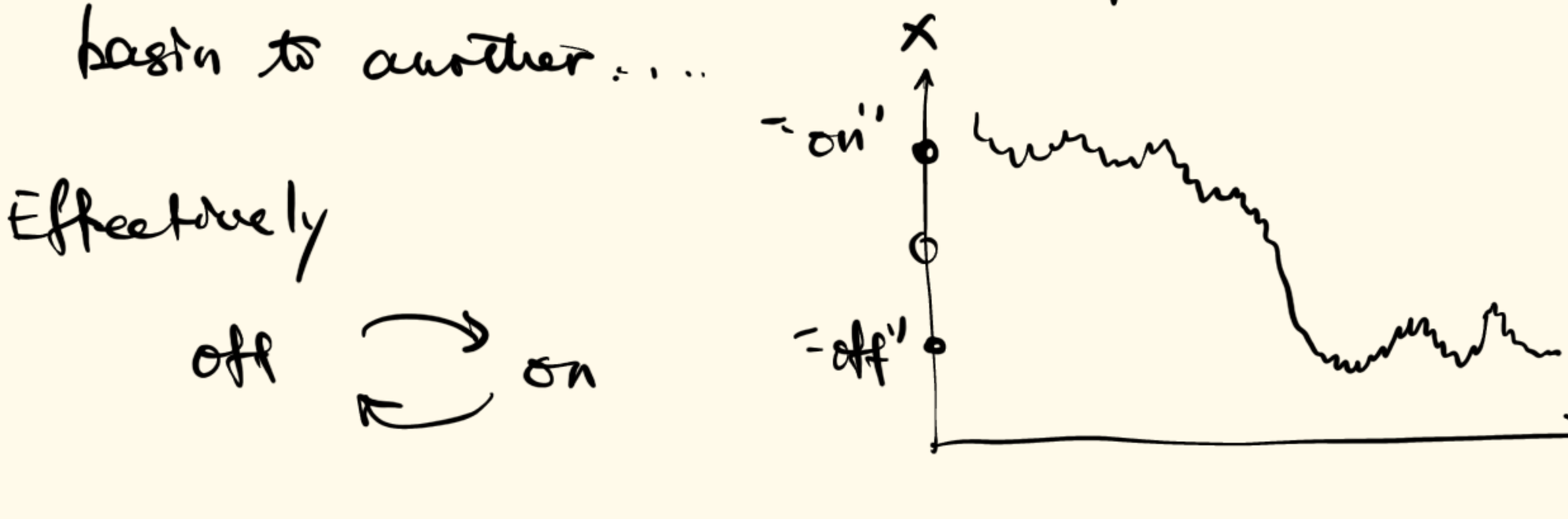
$$\dot{x} = f^+(x) - x.$$



you can get bistability.

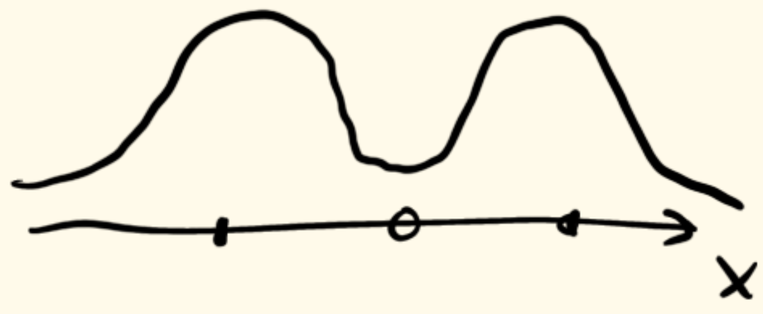
The stable fixed points have "basin of attraction" that partitions the state space.

- With stochasticity. a point can jump from one basin to another....



- So. when the stochastic process reaches steady state.

you'd have a bimodal distr.



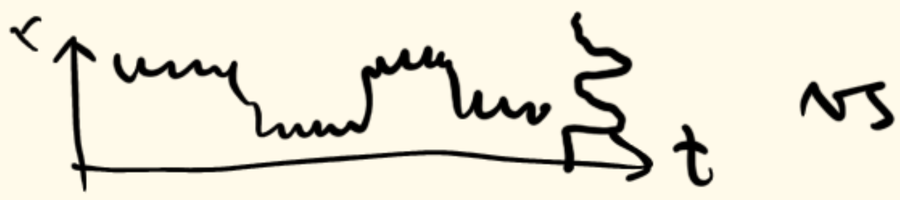
- Can describe cell fate,

cell cycle, cell states, etc...

- Time scale. { • at ∞ time. distribute over all states... i.e. a cell becomes all possible cell types...
• at finite (but long) time. may not spread.

as "differentiation".

So. steady state distr. may not capture the behavior we are interested in....



vs

s.s. same distr. Different traj. dynamics.

- Extinction and Zero

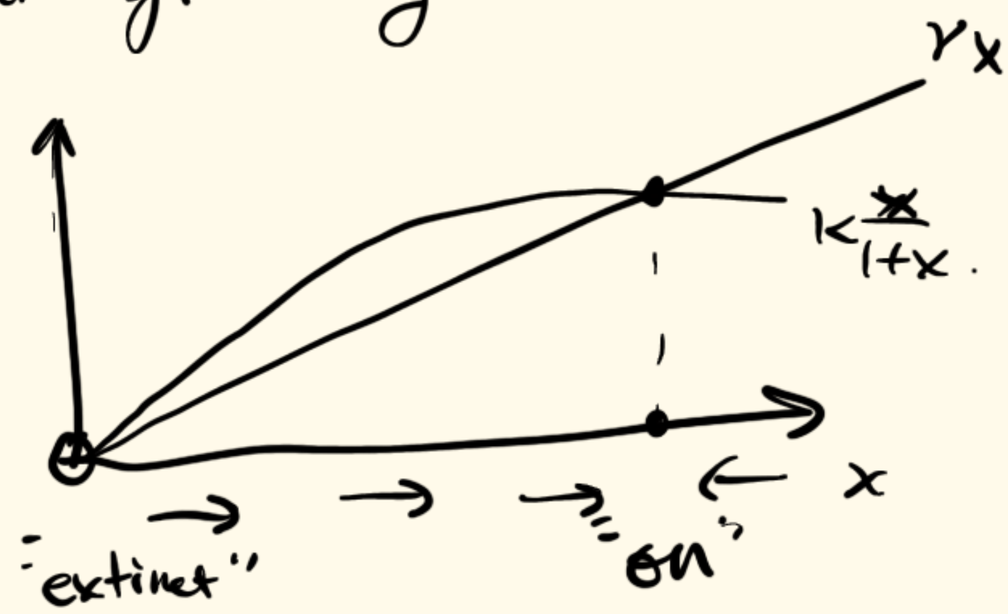
— Biological growth consists of autocatalysis reactions.

$X \xrightarrow{kX} X+1$

Key feature: you need x to make more x.

— So. you could have extinction: perturbation s.t. $x=0$. then can't grow anymore

— This doesn't happen autonomously in deterministic systems.



e.g. $\dot{x} = k\frac{x}{1+x} - rx$

$x=0$ is an unstable fixed pt.

— But it can happen under disturbances.

e.g. $\dot{x} = k\frac{x}{1+x} - (rx+w)$.

— Noise also make extinction happen

In fact. at steady state. with prob. 1.

X extincts ...

- So. in these aspects. noise is like a persistent random disturbance. from a traj. perspective.

But noise offers a distinct perspective from probability / distribution point of view.

- Ergodicity.
  - We see from the extinction example. that whether you can visit state A from state B is important for the steady state distr.

  - Ergodicity: Starts from any point, over time, (intuitive) can visit every point in state space.

    Rigorous: in finite time, start from any state, you have positive prob to visit any other state.

    e.g. binomial distr. example is ergodic.
    Extinction is NOT ergodic.

- Time vs Space average.

How to get distr. from trajectories?

- Take lots of sample trajectories $X_1^{(t)}, \dots, X_N^{(t)}$

$$p(x,t) \approx \frac{|\{ i : X_i(t) = x \}|}{N} \longleftarrow \#\text{ traj. at point } x \text{ at time } t.$$

- steady state. $p_\infty(x) \approx \lim_{t\to\infty} \frac{|\{i : X_i(t) = x\}|}{N}$

But if ergodic, then at steady state,
all trajectories are "the same".
→ They have visited all states according to
the same rules of state transitions!

- So. we can use time average of just one
trajectory to get s.s. distr.

$$\text{Time average} \begin{cases} \lim_{T\to\infty} \frac{\int_0^T \mathbb{1}\{X_i(t) = x\}}{T} = \lim_{T\to\infty} \frac{\text{duration of } X_i \text{ in state } x}{T} \\ = p_\infty(x) = \frac{|\{i : X_i(\infty) = x\}|}{N} \end{cases} \text{Space average.}$$

where $X_1^\infty, \dots, X_N(\infty) \sim p_\infty$
are samples from

- Ergodicity also means **stability**
for the dynamics in distribution space...
(Unique s.s. distr.)

☆. Equilibrium Physics of Bioregulation.

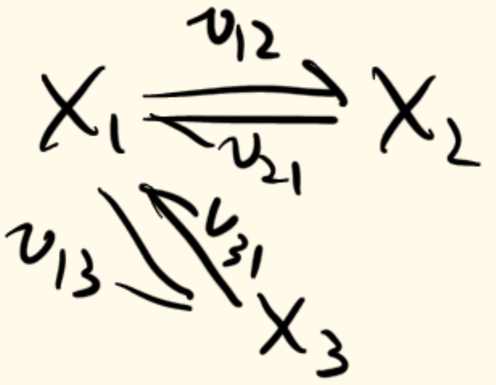
- Previously. we have taken a dynamics-centric perspective.

  However. the world is physical, and governed by a central idea of energy. which we haven't considered.

- In particular. it considers special types of steady state behavior.

Consider. $X_1 \rightleftharpoons X_2$, $X_1 \rightleftharpoons X_3$ Three chemical reactions. A molecule transits among 3 possible states...

- Steady state condition for A:



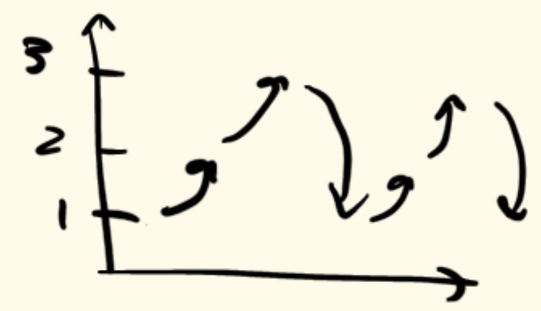
$$\frac{d}{dt}\frac{X_1}{X_{tot}} = \frac{d}{dt}P_1 = (v_{21}+v_{31}) - (v_{12}+v_{13}).$$

- But distr. reaches steady state

doesn't mean trajectories are "not moving"

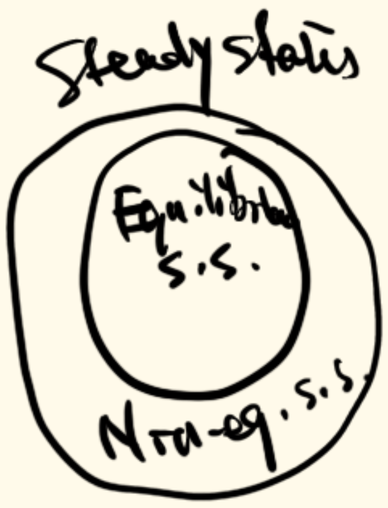
e.g. 

Then. we have a cyclic flux at steady state.



- Physical intuition says. a particle makes cyclic movement means it's "spending energy", can't persist.

- Compare this steady state to the following.



$v_{12} = v_{21}$ $1 \rightleftharpoons 2$ $v_{13} = v_{31}$ $1 \rightleftharpoons 3$ etc.

So. each reaction process "equilibrates":

Forward = Reverse.

Such steady states are more like "not moving".

This distinction is captured by concepts of "Energy and Equilibrium"

① Energy and Equilibrium in Statistical physics.

- Energy is a concept from physics.

  that a closed system (i.e without energy input).

  would dissipate energy and go towards energy

  minimization.

In statistical physics, — physics concerning systems

(e.g. ideal gas. solution of molecules...) with lots of identical components interacting,

the energy-minimized state is called equilibrium

and has lots of characterizations.

- The following are equivalent.
  - The system reaches Equilibrium
  - ~ Energy (of the system) is minimized at Equilibrium.
  - Entropy (of the system) is maximized at Equilibrium.
  - Every state transition process of the system

    satisfies detailed balance i.e. flux forward

    = flux reverse

- Physical law says, for a closed system,

  eventually, the system reaches equilibrium!

  — This is the powerful idea of equilibrium

  Since we can say a lot about equilibrium.

  Much more than steady state.

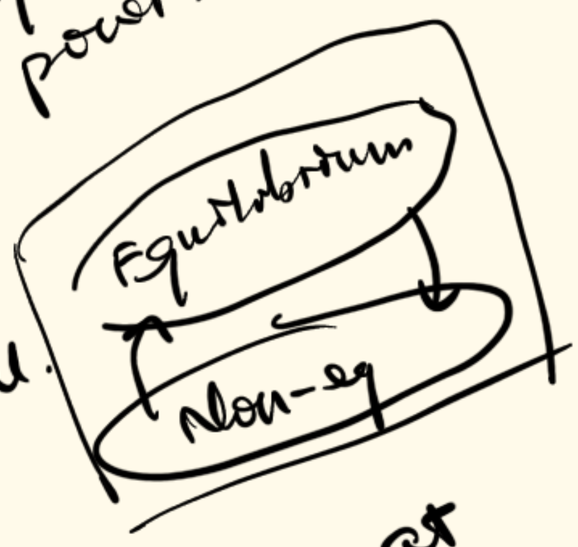
- Side note: But Bio is not closed, so not at equilibrium!

  Answer: — But energy is limited. so only key parts are

  driven out of equilibrium to achieve things that

  can't be done at equilibrium.

Equilibrium is a powerful ~~tool~~!



(= Napoleon is at Equilibrium" —Rob Phillips)

In other words. equilibrium is "easier to
= less cost to use". implement".

— Also. driven processes can still have overall behaviors that "look like" equilibrium.
i.e. they behave just like an equilibrium system due to other constraints.
e.g. network topology (no cycles.)

- How to use equilibrium? — Statistical Mechanics.

A system consists of lots of particles.

So we only need to care about statistics of the particles

Micro states —Multiplicity→ Macro states. Observation.
(All particle states) (System states Statistical.) $\langle x \rangle$.

X
$E(X)$. energy.

weight $W(x)$ } distr $p(x)$
energy $E(x)$ }

- Equilibrium: a distr. over microstates with special property. "equilibrium distribution".

namely. the following are equivalent characterizations.

① Boltzmann distr.

- Every (micro) state has an energy $E(x)$

and the distr. is $p(x) \propto e^{-E(x)\beta}$ or $\beta = \frac{1}{k_B T}$.
(we often omit β.) unit of energy.

② Detailed balance.

$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B$. transition between two micro states.

Detailed balance says forward flux = reverse flux
for every state transition.

$$k_1 p_A = J_{A\to B} = J_{B\to A} = k_2 p_B$$
(flux)

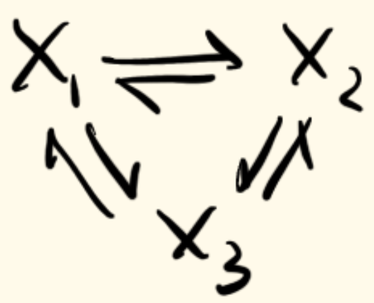
$$\Rightarrow \frac{p_A}{p_B} = \frac{k_2}{k_1}.$$

So we can define E_A, E_B s.t. $p_A \propto e^{-E_A}$, $p_B \propto e^{-E_B}$

then $\frac{p_A}{p_B} = e^{-(E_A - E_B)} = e^{\Delta E_{A\to B}} = \frac{k_2}{k_1}$.

- Transition rates and energies are related.

③ No cyclic flux.



Detailed balance: $k_{12} p_1 = k_{21} p_2$

$$\Rightarrow J_{\circlearrowright} = k_{12} p_1 + k_{23} p_2 + k_{31} p_3 - k_{21} p_2 - k_{13} p_1 - k_{32} p_3 = 0.$$

Equilibrium constrain transition rates

$$\Rightarrow p_1 = \frac{k_{21}}{k_{12}} p_2 = \frac{k_{21}}{k_{12}} \frac{k_{32}}{k_{23}} p_3 = \frac{k_{21}}{k_{12}} \frac{k_{32}}{k_{23}} \frac{k_{13}}{k_{31}} p_1$$

$$\Rightarrow k_{21} k_{32} k_{13} = k_{12} k_{23} k_{31}. \quad = \text{Cycle condition}$$

② Equilibrium in bioregulation.

- Equilibrium is very powerful.
  - Distribution directly obtained.
    Only need to know the states.
    no need to know reaction mechanisms!

Example 1. Enzymatic reaction. $S \xrightarrow{v} P$
catalyzed by enzyme E.

Focus on the enzyme molecule. (one enzyme. S substrates)

Assume E has two macro states: free state bound state

	E	ES.
energy	1	ΔG_b
(multiplicity i.e. how many micro states correspond to one macro state.) → Multiplicity	1	S_{tot}/C_0 ↑ conc. of one molecule per vol
weight	1	$\frac{S_{tot}}{C_0} e^{-\Delta G_b}$

$$\Rightarrow \quad p_{bound} = \frac{S_{tot}/C_0\, e^{-\Delta G_b}}{1 + S_{tot}/C_0\, e^{-\Delta G_b}}$$

- This is just like Michaelis-Menten formula we derived from kinetic mechanism $E + S \rightleftarrows C$.

under $S_{tot} \gg E_{tot}$. we have. $\frac{C}{E_{tot}} = \frac{S_{tot}/K}{1 + S_{tot}/K}$

and $K = C_0 e^{\Delta G_b}$

- Note that. we simplified the problem by

Considering only <u>one</u> enzyme molecule.

So the system has just 2 macro states.

Then. this can be applied to <u>several</u> enzyme molecule

by assuming each enzyme molecule is i.i.d.

So. $E = p_b E_{tot} = E_{tot} \frac{S_{tot} e^{-\Delta G_b}}{S_{tot} e^{-\Delta G_b} + 1}$ (independent and identically distributed)

- If we don't make that assumption.

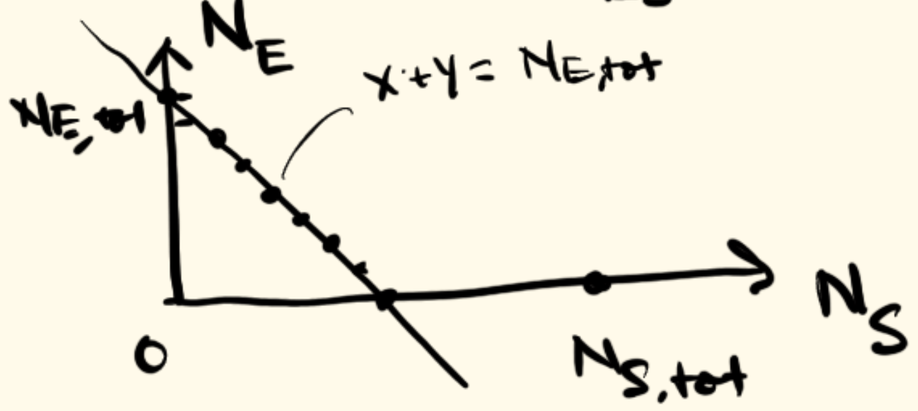
the # of macrostates can be very large or infinite.

e.g. Enzymatic reaction example above.

(Homework to work this out)

(N_E, N_S, N_{CES}). for $N_E + N_{CES} = N_{E,tot}$

$N_S + N_{CES} = N_{S,tot}$.

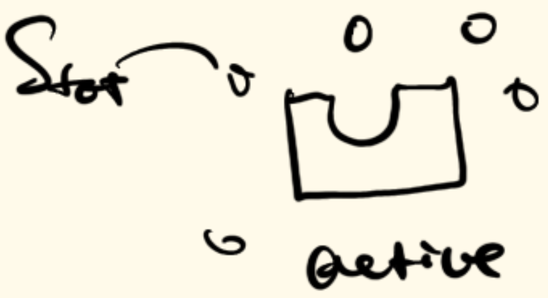
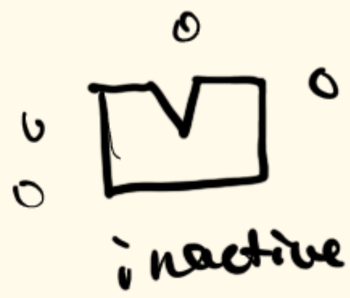


Example 2. MWC. — the second secret of life. Allostery

- Enzymes' activities can be regulated by ligands/substrates
  - via allostery. i.e. an enzyme having multiple configurations.

Multiple configurations (enzymes) + Binding. (Independent. No explicit activation.)

e.g. active ⇌ inactive

conf.	Energy	Weight	Bio Notation
	ε_A	$e^{-\epsilon_A}$	
	$\varepsilon_A + \varepsilon_{A,b}$	$e^{-(\epsilon_A + \epsilon_{A,b})\frac{S_{tot}}{C_0}}$	$e^{-\epsilon_A}\frac{S_{tot}}{K_A}$
	ε_I	$e^{-\epsilon_I}$	
	$\varepsilon_I + \varepsilon_{I,b}$	$e^{-(\epsilon_I + \epsilon_{I,b})\frac{S_{tot}}{C_0}}$	$e^{-\epsilon_I}\frac{S_{tot}}{K_I}$.

$$\Rightarrow p_{active} = \frac{e^{-\varepsilon_A}\left(1+\frac{S_{tot}}{K_A}\right)}{e^{-\varepsilon_A}\left(1+\frac{S_{tot}}{K_A}\right)+e^{-\varepsilon_I}\left(1+\frac{S_{tot}}{K_I}\right)}$$

as $S_{tot}\to 0$ $\quad p_{active}(0) = \dfrac{e^{-\varepsilon_A}}{e^{-\varepsilon_A}+e^{-\varepsilon_I}} = \dfrac{1}{1+e^{\varepsilon_A-\varepsilon_I}}$.

If $\varepsilon_I < \varepsilon_A$ by 2 to 3 k_BT. (one hydrogen bond.)

that's 10 fold.

$p_{active}(0) \approx \frac{1}{10}$

as $S_{tot}\to +\infty$ $\quad p_{active}(\infty) = \dfrac{e^{-\varepsilon_A}\frac{S_{tot}}{K_A}}{e^{-\varepsilon_A}\frac{S_{tot}}{K_A}+e^{-\varepsilon_I}\frac{S_{tot}}{K_I}} = \dfrac{1}{1+e^{\varepsilon_A-\varepsilon_I}\frac{K_A}{K_I}}$

If $K_A < K_I$ binds tighter when active.

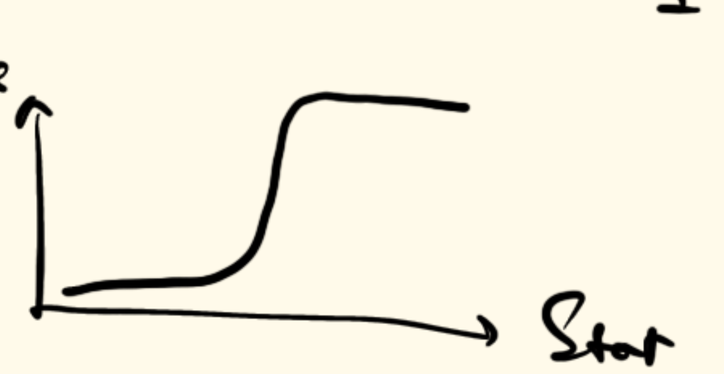
by 100- fold. then $p_{active}(\infty) \approx \dfrac{1}{1+10^{\frac{1}{100}}} \approx 0.9$.

- Cooperativity. — Hemeglobin.

e.g. a dimer.  each site becomes active/inactive bound/free independently.

then $p_{active} = \dfrac{e^{-\varepsilon_A}\left(1+\frac{S_{tot}}{K_A}\right)^2}{e^{-\varepsilon_A}\left(1+\frac{S_{tot}}{K_A}\right)^2+e^{-\varepsilon_I}\left(1+\frac{S_{tot}}{K_I}\right)^2}$

$$p_{active}(\infty) = \frac{e^{-\varepsilon_A}\left(\frac{S_{tot}}{K_A}\right)^2}{e^{-\varepsilon_A}\left(\frac{S_{tot}}{K_A}\right)^2+e^{-\varepsilon_I}\left(\frac{S_{tot}}{K_I}\right)^2} = \frac{1}{1+e^{\varepsilon_A-\varepsilon_I}\left(\frac{K_A}{K_I}\right)^2}$$



sharper transition.

- Another form of cooperativity is by

  Interaction between binding sites.

States	Energy	Weight
	0	1
	ε_b	$e^{-\varepsilon_b} \frac{S_{tot}}{C_0}$
	$2\varepsilon_b + \underbrace{\varepsilon_{int}}_{\text{interaction.}}$	$e^{-(2\varepsilon_b + \varepsilon_{int})} \left(\frac{S_{tot}}{C_0}\right)^2$.

neg : cooperative

pos : anti-coop.

Example 3. Gene expression

Consider the states of the gene under repression

States	Energy	Weight
	0	1
	$\Delta\varepsilon_p$	$e^{-\Delta\varepsilon_p} \frac{P}{N_{NS}}$ — P: RNAP #; N_{NS}: # non specific binding sites
	$\Delta\varepsilon_r$	$e^{-\Delta\varepsilon_r} \frac{R}{N_{NS}}$ — R: repressor #.

③. Beyond Equilibrium — Markov chains.

- Equilibrium has the powerful property
  that we don't need to know the detailed
  kinetic mechanisms just the thermodynamics
  (i.e. interacting energies...)
- But what if I encounter a behavior
  that is for sure not at equilibrium?
  - my behavior of interest can only be achieved
    out of equilibrium...
  - e.g. Kinetic proofreading ... (super precise).

How to analyze that?

- For the special case of finite # of states
  if we know the state transition rates.
  this is a Markov chain.
  (A special case of chemical master equation
  where we can get the full distribution.)