CCBS. Lecture 07 Equilibrium physics of bioregulation

From last time — Noise.

Last bit on noise — Ergodicity.

☆. Equilibrium physics of bioregulation.

① Energy and equilibrium. a physics perspective.

(Reference: KenDill Book)

— Systems tend towards equilibrium.

— Equilibrium in physics — entropy, etc.

Microscopic world — Boltzmann distr.

— detailed balance

The powerful idea of equilibrium.

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. phophorylation cascades.

④ Steady state. distr. and hitting times

- Last bit. on noise. — Ergodicity.

Review — LoM on Noise.
— CME.
— Gillespie algorithm.
— Moment analysis
— Stochastic phenomena

- 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 $X_1^{(t)}, \ldots, X_N^{(t)}$

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

— 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.

  equilibrium steady state
  steady state

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

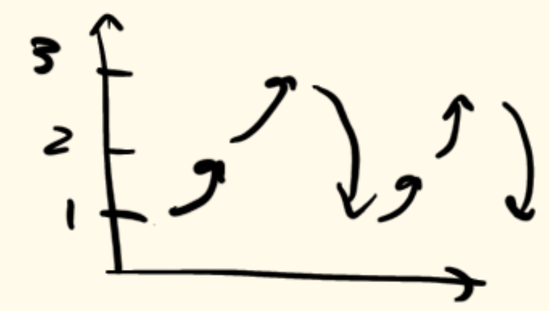
$X_1 \underset{v_{21}}{\overset{v_{12}}{\rightleftharpoons}} X_2$, v_{13} / v_{31} / X_3

- Steady state condition for A:

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

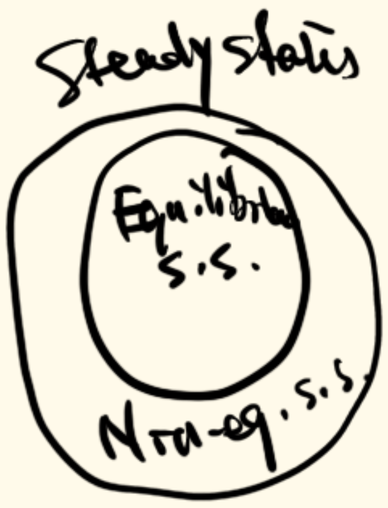
- But dist. reaches steady state doesn't mean trajectories are "not moving"

e.g. 1 ⇄ 2, 1 ⇄ 3, 2 ⇄ 3 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 "not changing".

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.

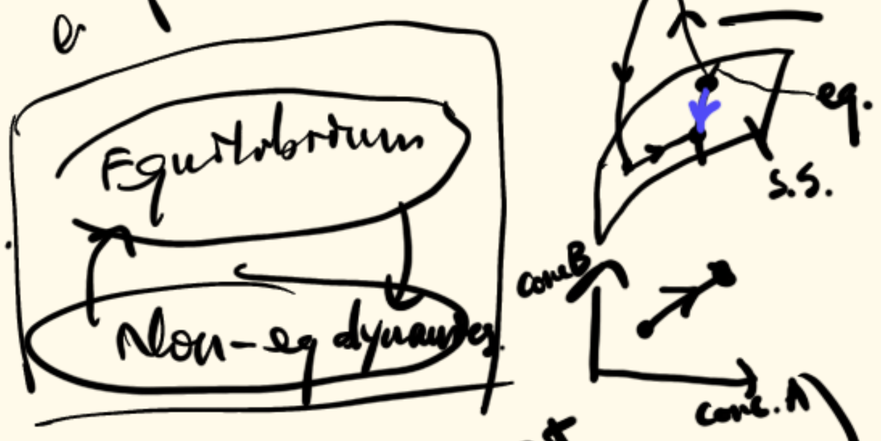
- Side note: But Bio is not closed, so not at equilibrium!

  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

e.g. 

Microstates —Multiplicity→ Macro states. Observation.
(All particle states) (lots of microstates are the "same" for our purpose) (System states) Statistical. <x>.
Multiplicity W(x) & Energy E(x) } distr p(x)
$p(x) \propto W(x) e^{-E(x)}$.
X
E(X). energy.

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

namely. the following are equivalent characterizations.

① Boltzmann distr. (over MicroStates)

- 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)

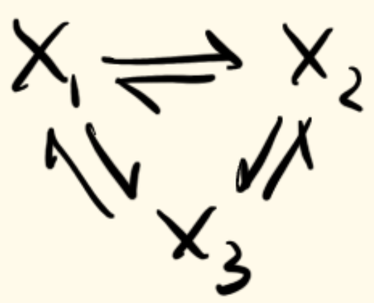
$$\Rightarrow \frac{p_A}{p_B} = \frac{k_2}{k_1}.$$

①↔②: 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!

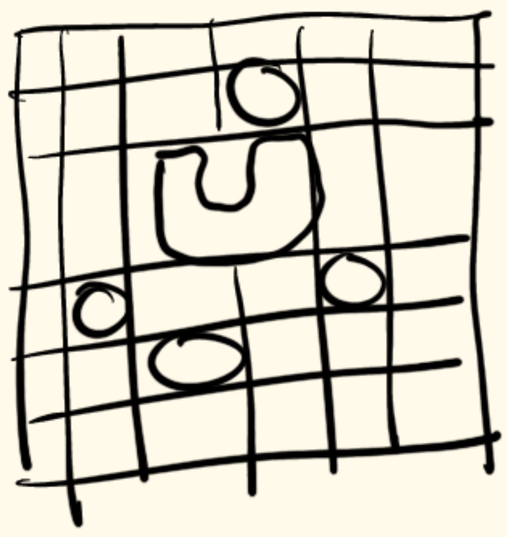
Example 1. Enzymatic reaction. $S \xrightarrow{v} P$ catalyzed by enzyme E.

$E + S \rightleftharpoons C_{ES}$

Consider just 1 Enzyme. S_{tot} substrates.

Microstates:

(Lattice Model)



O = Substrate

U = Enzyme.

Ω = total # of Lattice sites.

In terms of energy, only two macrostates.

		Energy	Multiplicity
Bound	$N_S = N_{S,tot} - 1$. $N_E = 0$. $N_C = 1$	$(N_{S,tot}-1)\varepsilon_f + \varepsilon_b$. ↑ free (substrate) ↑ bound	$\binom{\Omega}{N_{S,tot}-1}$
Free	$N_S = N_{S,tot}$. $N_E = 1$ $N_C = 0$	$N_{S,tot}\,\varepsilon_f$	$\binom{\Omega}{N_{S,tot}}$

$$\binom{\Omega}{N_S} = \frac{\Omega!}{N_S!(\Omega - N_S)!} \approx \frac{\Omega^{N_S}}{N_S!} \quad \text{(when } \Omega \text{ is large)}.$$

$$\Rightarrow \text{Free state weight} \cdot \binom{\Omega}{N_{S,tot}} e^{-N_{S,tot}\varepsilon_f} \approx \frac{\Omega^{N_{S,tot}}}{N_{S,tot}!} e^{-N_{S,tot}\varepsilon_f}$$

$$\text{Bound state weight} \approx \frac{\Omega^{N_{S,tot}-1}}{(N_{S,tot}-1)!} e^{-(N_{S,tot}-1)\varepsilon_f - \varepsilon_b}$$

$$\Rightarrow p_{bound} = \frac{\frac{\Omega^{N_{S,tot}-1}}{(N_{S,tot}-1)!} e^{-(N_{S,tot}-1)-\varepsilon_b}}{\frac{\Omega^{N_{S,tot}}}{S_{tot}!} e^{-N_{S,tot}\varepsilon_f} + \frac{\Omega^{N_{S,tot}-1}}{(N_{S,tot}-1)!} e^{-(N_{S,tot}-1)\varepsilon_f - \varepsilon_b}}$$

$$= \frac{\left(\frac{N_{S,tot}}{\Omega}\right) e^{-(\varepsilon_b - \varepsilon_f)}}{1 + \left(\frac{N_{S,tot}}{\Omega}\right) e^{-(\varepsilon_b - \varepsilon_f)}} = \frac{\left(\frac{N_{S,tot}}{\Omega}\right) e^{-\Delta\varepsilon}}{1 + \left(\frac{N_{S,tot}}{\Omega}\right) e^{-\Delta\varepsilon}}.$$

What's Ω in more physical terms?

v = volume of each site.

V = volume of solution

$\Rightarrow V = \Omega v \Rightarrow$ concentration $C_{S,tot} = \frac{N_{S,tot}}{V} = \frac{N_{S,tot}}{\Omega}\left(\frac{\Omega}{V}\right)$ Co.

C_0 is Standard or reference concentration

such that all sites can be considered "occupied".

$\Rightarrow$. C_0 can be arbitrary "concentration unit".

e.g. $C_0 \approx 1M$.

$$\Rightarrow. \quad P_{bound} = \frac{\left(\frac{C_{S,tot}}{C_0}\right) e^{-\Delta\varepsilon}}{1 + \left(\frac{C_{S,tot}}{C_0}\right) e^{-\Delta\varepsilon}}$$

} Langmuir binding curve. Hill function. Michaelis-Menten etc.

(- 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 $K_d = C_0 e^{\Delta\varepsilon}$ $S_{tot} = C_{S,tot}$)

- Free Energy, Entropy, and Chemical Potential.

  - Energy (binding) vs Entropy (multiplicity).

    e.g. K_d = concentration C_S s.t. energy gain of one binding is equal to entropy lost by removing one ligand from solution.

  - $F = E - TS$ free energy

    e.g. $E(N_S) = N_S \varepsilon_p$. for N_S # of substrates in solution.

    $S = k_B \log W(N_S)$. — multiplicity.

    $W(N_S) = \binom{\Omega}{N_S}$.

  - Chemical potential — focus on Free energy change when molecules change.

    $\mu = F(N_S) - F(N_S - 1)$.

    L Free energy change when removing one molecule from solution.

$$\Rightarrow \mu = N_S \varepsilon_f - k_B T \log \frac{\Omega^{N_S}}{N_S!} - (N_S - 1)\varepsilon_f + k_B T \log \frac{\Omega^{N_S - 1}}{(N_S - 1)!}$$

$$= \varepsilon_f + k_B T \log \frac{N_S}{\Omega}$$

$$= \varepsilon_f + k_B T \log \frac{c_S}{c_0}.$$

- $E + S \rightleftharpoons C$ example in terms of chemical potential

$$\Rightarrow p_{bound}^{(c_{S,tot})} = \frac{e^{-(\varepsilon_b - \mu(c_{S,tot}))}}{1 + e^{-(\varepsilon_b - \mu(c_{S,tot}))}}.$$

Review of procedure:

Assume E has two states:

	macro free state	bound state
	E	C_{ES}
energy	1	$\Delta\varepsilon_b$
(multiplicity) i.e. how many micro states correspond to one macro state. → Multiplicity	1	S_{tot}/c_0
weight	1	$S_{tot} e^{-\Delta\varepsilon_b}$

$$\Rightarrow p_{bound} = \frac{S_{tot}/c_0 \, e^{-\Delta\varepsilon_b}}{1 + S_{tot}/c_0 \, e^{-\Delta\varepsilon_b}}.$$

Example 1 Side Note:

- Note that we simplified the problem by

Considering only one enzyme molecule.

So the system has just 2 macro states.

Then, this can be applied to several enzyme molecule

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

So. $E = p_b E_{tot} = E_{tot} \frac{\frac{S_{tot}}{c_0} e^{-\Delta\varepsilon_b}}{\frac{S_{tot}}{c_0} e^{-\Delta\varepsilon_b} + 1}$ (independent and identically distributed)

- If we don't make that assumption.

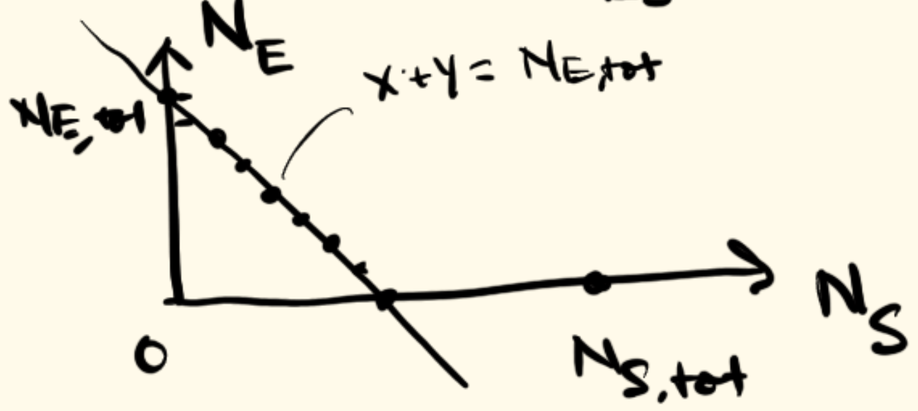
the # of macrostates can be very large or infinite.

e.g. Enzymatic reaction example above.

(Homework to work this out)

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

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

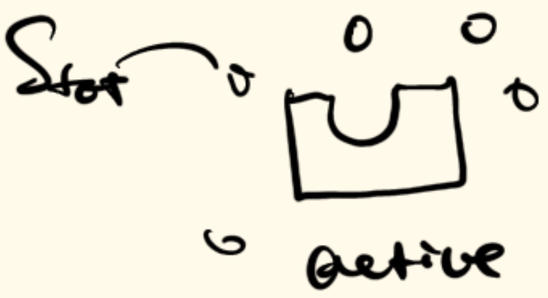
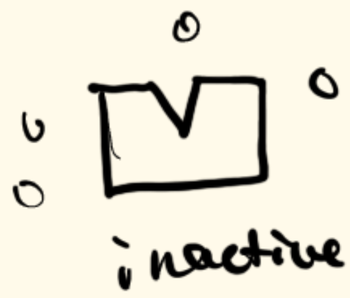


Example 2. MWC. — the second secret of life. Allostery

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

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

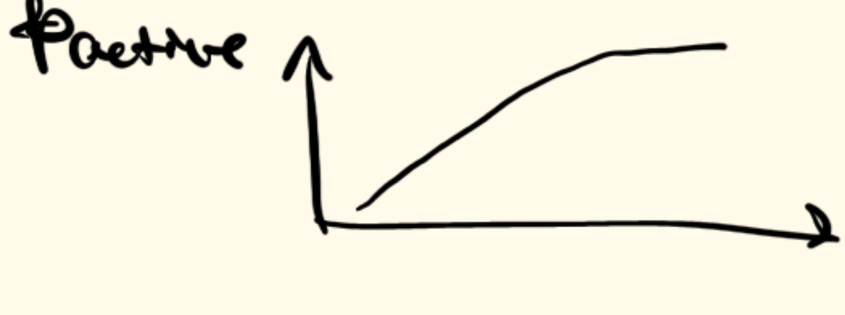
that's 10 fold.

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

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

If $K_A < K_I$ binds tighter when active.

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



(non-allosteric.)

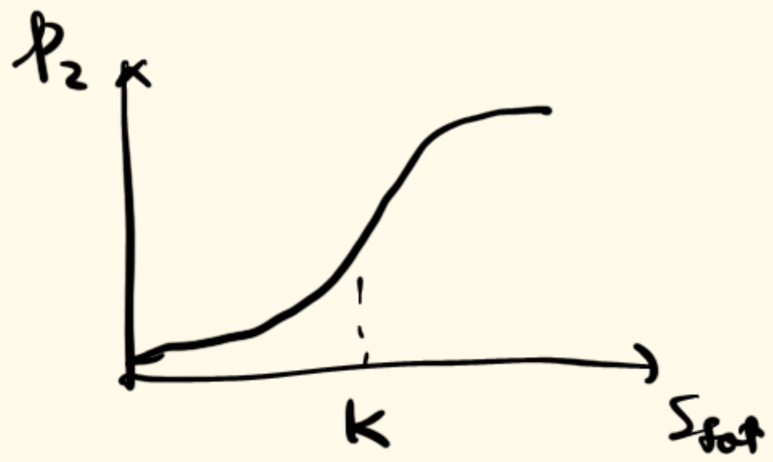
- Cooperativity. (— Sensitivity. fold-change of activity to conc.

$$\text{Sens.} = \frac{\frac{\Delta p}{p}}{\frac{\Delta c}{c}} = \frac{\Delta c}{\Delta p}\frac{p}{c} = \frac{d\log p}{d\log c}.$$)

Energy	Weight
0	1
$\varepsilon_b - \mu$	$\frac{S_{tot}}{K}$
$\varepsilon_b - \mu$	$\frac{S_{tot}}{K}$
$2\varepsilon_b - 2\mu + \varepsilon_{int}$	$\left(\frac{S_{tot}}{K}\right)^2 e^{-\varepsilon_{int}}$

interaction between two binding sites

$$\Rightarrow p_2 = \frac{\left(\frac{S_{tot}}{K}\right)^2 e^{-\varepsilon_{int}}}{1+2\left(\frac{S_{tot}}{K}\right) + \left(\frac{S_{tot}}{K}\right)^2 e^{-\varepsilon_{int}}}$$



If both p_1 and p_2 are equally

$$\text{activity} = p_1 + p_2 = \frac{\frac{S_{tot}}{K} + \left(\frac{S_{tot}}{K}\right)^2 e^{-\varepsilon_{int}}}{1 + 2\left(\frac{S_{tot}}{K}\right) + \left(\frac{S_{tot}}{K}\right)^2 e^{-\varepsilon_{int}}}$$

$\varepsilon_{int} = 0 \Rightarrow = \dfrac{\frac{S_{tot}}{K}\left(\frac{S_{tot}}{K}+1\right)}{\left(1+\frac{S_{tot}}{K}\right)^2} = \dfrac{S_{tot}/K}{1+S_{tot}/K}$. Back to previous case.

So. $\varepsilon_{int} < 0$. Cooperative.

$\varepsilon_{int} = 0$. Non-cooperative.

$\varepsilon_{int} > 0$ Negative cooperativity.

But ... (1). This is phenomenological.

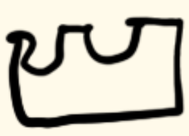
(2). $p_2(\infty) = 1$. which doesn't make statistical sense..

(3). For sensitivity > 1. requires.

additional mechanism. $\varepsilon_{int} < 0$.

Could we have a more realistic model based on allostery?

- Cooperativity. by Allostery. — Hemoglobin.

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.

Example 3. Gene expression

Consider the states of the gene under repression

States	Energy	Weight
	0	1
	$\Delta\varepsilon_p$	$e^{-\Delta\varepsilon_p}\frac{P}{N_{NS}}$
	$\Delta\varepsilon_r$	$e^{-\Delta\varepsilon_r}\frac{R}{N_{NS}}$

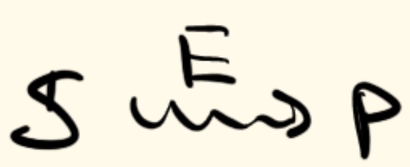
P: RNAP #.
N_{NS}: # non specific binding sites
R: repressor #.

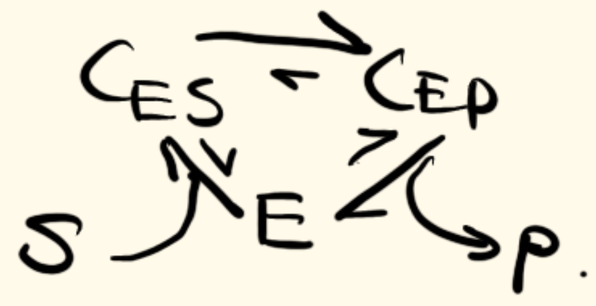
Q: what are assumptions, other than equilibrium in this case?

③. Beyond Equilibrium — Markov chains.

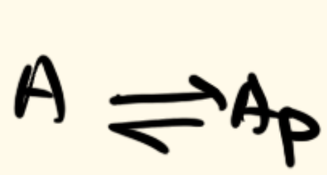
- Equilibrium has the powerful property that we don't need to know the detailed kinetic mechanisms. just the thermodynamics (i.e. interacting energies...)
- But what if I encounter a behavior that is for sure not at equilibrium?
  - my behavior of interest can only be achieved out of equilibrium...
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

e.g. enzymatic reaction





e.g. phosphorylation cascade.



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.)

- Continuous-time Markov Chain
  (from CME of chemical reactions).

  - $A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B$ chemical reactions.

    Two interpretations:

    Concentration change ↔ single molecule state transition.

    Rate equation: $\frac{dA}{dt} = k_2 B - k_1 A$

    Define. $p_A = \frac{A}{A_{tot}}$, $p_B = \frac{B}{A_{tot}}$. $A_{tot} = A + B$.

    ⇒ $\frac{d\,p_A}{dt} = k_2 p_B - k_1 p_A$. State transition!

    Same for CME. (check.)

  - This holds because linearity / first order is special.
    It's the same as scaling with size / number.
    So. 1st order = per molecule

  - All CRNs with 1st and 0th order reactions can be interpreted as state transitions.

    0th order? $\emptyset \to A$ $A \to \emptyset$.
    Can be viewed as $B_0 \to A$, $A \to B_0$
    and B_0 is held constant by external means.
    This also happens when studying certain dynamics of Markov chains.

  - Master equation for continuous-time Markov Chain.

    p. — vector of state probabilities.

    $q_{ij} > 0$ — rate of transition $j \to i$

    $q_{ii} < 0$ — rate of leaving state i.

$$\frac{dp_i}{dt} = \sum_{j \neq i} q_{ij} p_j - q_{ii} p_i$$

$$\frac{dp}{dt} = Q p. \qquad Q - \text{transition rate matrix.}$$

- Conservation of prob.

$1^T p = 1$ always.

So. $\frac{d\, 1^T p}{dt} = 1^T Q p = 0 \quad \forall p.$

So $\underline{1^T Q = 0}$. $\left(\text{So. } q_{ii} = -\sum_{j \neq i} q_{ji} \right)$.

- Steady state distribution.

$\underline{\pi. \text{ s.t. } Q\pi = 0}$

(Solve : Take. Q. ask for "Null space".)

Irreducible $\Leftrightarrow$ Ergodic $\Leftrightarrow$ steady state is unique

State transition graph is strongly connected.

Prob starting in i, reach j. at time t. is positive. $\forall i, j$

Q's nullspace has dimension 1.
All eigenvalues has real part < 0.
except one. has 0.

- Equilibrium? π is an eq. steady state. then

Detailed balance: $q_{ij} \pi_j = q_{ji} \pi_i$.

This can only happen if the <u>rates</u> satisfy conditions.

(cycle flux = 0.).

Q: But not all systems are memoryless/Markovian...

- The idea of state
  - Everything is a state space system. / Markovian.
  - Markov chains are just stochastic state space systems.

State: all info about the system you need to know to describe its internal dynamics evolving into the future.
i.e. if isolated. its future only depends on its state. not on its past.

- $\dot{x} = f(x)$. state-space Deterministic system
- $\frac{d}{dt} p(x) = Q\, p(x)$. Stochastic state-space system. !
- All systems can be written as state space systems.
  i.e. All systems can be written as Markovian
  (all systems can be made Memoryless)
  ↳ if the state is large/representative enough!

e.g. $X(t)$ depends on its past 5 time points.
Then make $\hat{X}$ s.t. $X_1 = X(t)$ $X_2 = X(t-1)$...
$X_5 = X(t-5)$.

e.g. Newtonian mechanics. position x's future depends on velocity (past). ⇒. $\hat{X}_1 = x$. $\hat{X}_2 = \dot{x}$.

- Kinetics from a Markov chain.

— How long does it take to transition to an active state?

e.g. gene expression. $G_{off} \rightleftharpoons G_{exposed} \rightleftharpoons G_{active}$.

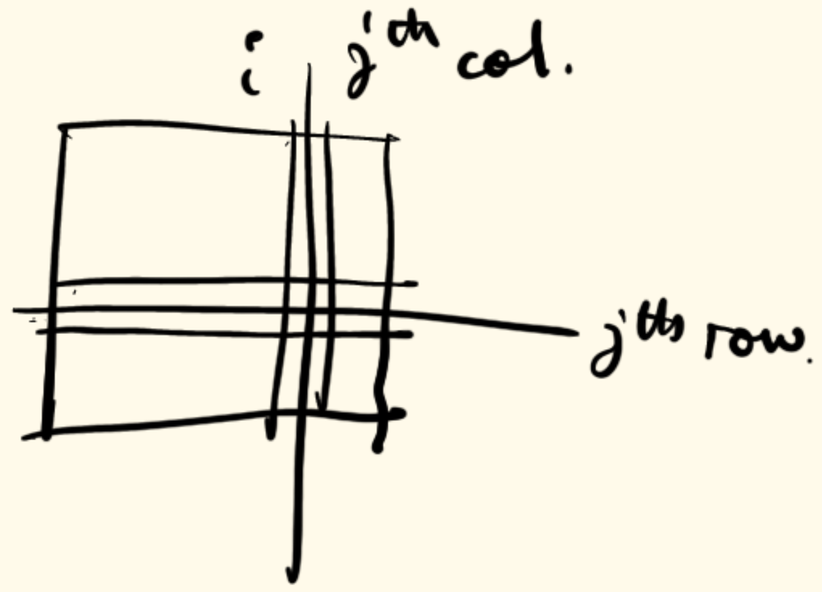
enzymatic reactions.

— This is captured by "Hitting times" or "First Passage Times"

τ_{ji} = expected time it takes to hit i when starting at j

$$\tau_{ji} = \underbrace{\frac{1}{|q_{ii}|}}_{\text{wait}} + \frac{q_{ji}}{|q_{ii}|} \underbrace{\cdot 0}_{\tau_{ij}} + \sum_{i' \neq i,j} \frac{q_{i'i}}{|q_{ii}|} \tau_{ji'}$$

$$-q_{ii}\tau_{ji} = \sum_{i' \neq i,j} q_{i'i}\tau_{ji'}$$



$$\Rightarrow \sum_{i' \neq j} q_{i'i}\tau_{ji'} = 0$$

Hitting time vector $\tau^{(j)}$ — time to hit state j.

$$Q^{(j)}\tau^{(j)} = 0.$$

$Q^{(j)}$ is Q after deleting the j^{th} row and col.

- Finite state projection. — Solving CME by Markov chain methods