Ctrl & Comp in Bio Sys - Westlake Univ., Fall 2025

Lecture 06: Noise in Biology and Equilibrium Physics of Bioregulation

20251016

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Contents

1 Course Overview and Review

CCBS Lecture 06 Noise in Bio & Equilibrium Physics of Bioregulation.

1.1 Review from Last Time

- Noise: Intro.
- Chemical master equation.

1.2 This Time's Content

- Gillespie algorithm.
- Noise analysis (simple, at steady state).
- Some stochastic phenomena.

References: Kardar's book; Erban, Chapman, Maini (2007), "Practical guide to stoch. sim of reaction diffusion processes"; Phillips, PBOC, etc.; Molecular Switch.

2 Equilibrium Physics of Bioregulation

2.1 Energy and Equilibrium: A Physics Perspective

Systems tend towards equilibrium.

- Equilibrium in physics – entropy, etc.
- Microscopic world – Boltzmann distr.
- Detailed balance.

This is the fundamental idea of equilibrium.

2.2 Applied to Enzymatic and Gene Regulation

Focus on single molecule's states.

- Michaelis-Menten.
- Allostery (MWC).
- Lac operon.

2.3 Beyond Equilibrium: Markov Chains

For molecular state transitions.

- e.g. Metabolism, phosphorylation cascades.

2.4 Steady State Distr. and Hitting Times

Steady state distr. and hitting times.

3 Gillespie Algorithm (or SSA – Stochastic Simulation Algorithm)

3.1 Simulation First, Because...

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.
3. e.g.

   [Sketch: Smooth distribution over time vs fluctuating trajectory]

4. 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.

3.2 Distribution vs Trajectory

Can't directly simulate the distribution $\rightarrow$ inf. dim ODE. $P_n(t)$. We can simulate trajectories, then distr. can be obtained from averages over lots of traj.

3.3 Simplest Idea: Just Like ODE Sim.

$\frac{dx}{dt} = f(x)$ (Euler integration)$\rightarrow X(t+dt) = X(t) + f(X(t))dt$.

For $X \xrightarrow{\nu=kX} X-1$,

$$N_X(t+dt) = \begin{cases} N_X(t)-1 & \text{w/ prob } \alpha dt = kN_X(t)dt \\ N_X(t) & \text{w/ prob } 1-\alpha dt \end{cases}$$

3.4 But Need dt Small to Have a Good Approx...

Could be very costly. Could we simulate $N_X(t)$ exactly? Yes, by transforming randomness from whether an event happens in an interval to when does an event happen.

3.5 Start at t, Want τ. s.t. $t+\tau$ is Next Reaction.

Let $f(N_X(t), s)ds \leftarrow$ an infinitesimal pdf for τ.

pdf for τ:

$$f(N_X(t), s)ds = P\{N_X(t)$$

molecules at time t, and the next reaction occurs in time interval [t+s, t+s+ds) } (1)

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

Denote reaction with rate $\nu(N_X)$. (e.g. $\nu = kN_X$)

$$\Rightarrow f(N_X(t), s)ds = g(N_X(t), s) \cdot \nu(N_X(t+s))ds = g(N_X(t), s) \cdot \nu(N_X(t))ds.$$

(Since no reaction $\rightarrow N_X(t+s) = N_X(t)$).

3.6 Let's Solve for $g(N_X(t), s)$.

(Memoryless or independent)

$$g(N_X(t), s + ds) = g(N_X(t), s)[1 - \nu(N_X(t))ds] \tag{2}$$

$$\frac{dg}{ds} = -\nu g \quad \Rightarrow \quad g = e^{-\nu s} \tag{3}$$

$\Rightarrow$

$$f(N_X(t), s)ds = \nu e^{-\nu s} ds \tag{4}$$

$\Leftarrow$ pdf (prob density function) for exponential distr. $\tau \sim \text{Exp}(\nu)$. pdf: $\nu e^{-\nu\tau}$. cdf: $1 - e^{-\nu\tau}$.

We want τ s.t. $t + \tau$ is time for next reaction. For a reaction with rate ν. Then $\tau \in [0, \infty)$ is a random number $\tau \sim \text{Exp}(\nu)$.

This is exact! e.g. $X \xrightarrow{\nu = kX} X - 1$ At t: draw $\tau \sim \text{Exp}(kN_X(t))$. Then $N_X(t+\tau) = N_X(t) - 1$.

3.7 What if Multiple Reactions?

The reactions are independent, each with rate $\nu_1, \ldots, \nu_m$.

Let τ_0 be time til' any reaction happens. $\Rightarrow \tau_0 \sim \text{Exp}(\nu_1 + \cdots + \nu_m)$.

Which reaction? $P\{\text{it's reaction } j\} = \frac{\nu_j}{\nu_1 + \cdots + \nu_m}$.

This completes the Gillespie algorithm, or SSA. Exact simulation of stochastic trajectories by sampling event times.

4 Analysis of Steady State Distributions

4.1 Simulations Can't Give the Full Picture, Over All Parameters

$\Rightarrow$ Analysis via moments, from CME. Mean, Var.

4.2 Exact Analysis. Example. of Moments.

$X \xrightarrow{\mu} X + 1$ $X \xrightarrow{kx} X - 1$

$$\frac{dP_n}{dt} = k(n+1)P_{n+1} + \mu P_{n-1} - (kn + \mu)P_n$$

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

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

$$\begin{aligned}
\frac{dM}{dt} &= \frac{d}{dt}\sum_{n=0}^{\infty} nP_n \\
&= k\sum_{n=0}^{\infty} n(n+1)P_{n+1} + \mu\sum_{n=0}^{\infty} nP_{n-1} - k\sum_{n=0}^{\infty} n^2P_n - \mu\sum_{n=0}^{\infty} nP_n \\
&= k\sum_{n=1}^{\infty}(n-1)nP_n + \mu\sum_{n=1}^{\infty} n(n-1)P_{n-1} - k\sum n^2P_n - \mu\sum nP_n \quad \text{(reindex)}
\end{aligned}$$

After calculation: $= \mu - kM$. (This is just like deterministic rate eqn. $\frac{dx}{dt} = \mu - kx$. Warning: Not always so.)

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

$$\begin{aligned}
\frac{d}{dt}(V+M^2) &= \frac{d}{dt}\sum n^2P_n \\
&= k\sum n^2(n+1)P_{n+1} + \mu\sum n^2P_{n-1} - k\sum n^3P_n - \mu\sum n^2P_n \\
&= k\sum (n-1)^2nP_n + \mu\sum (n+1)^2P_{n+1} - k\sum n^3P_n - \mu\sum n^2P_n \\
&= \sum [k((n^2-2n+1)n)P_n + \mu(n^2+2n+1)P_n - kn^3P_n - \mu n^2P_n] \quad \text{(reindex for sums)} \\
&= \sum [k(n^3-2n^2+n)P_n + \mu(n^2+2n+1)P_n - kn^3P_n - \mu n^2P_n] \\
&= \sum [kn^3 - 2kn^2 + kn + \mu n^2 + 2\mu n + \mu - kn^3 - \mu n^2]P_n \\
&= \sum [-2kn^2 + kn + 2\mu n + \mu]P_n \\
&= -2k\sum n^2P_n + (k+2\mu)\sum nP_n + \mu \\
&= -2k(V+M^2) + (k+2\mu)M + \mu
\end{aligned}$$

Then

$$\frac{d(V+M^2)}{dt} = -2k(V+M^2) + (k+2\mu)M + \mu$$

$$\frac{dV}{dt} + 2M\frac{dM}{dt} = -2kV - 2kM^2 + kM + 2\mu M + \mu$$

But $\frac{dM}{dt} = \mu - kM$, so $2M\frac{dM}{dt} = 2M(\mu - kM) = 2\mu M - 2kM^2$

$$\begin{aligned}
\Rightarrow \frac{dV}{dt} &= -2kV - 2kM^2 + kM + 2\mu M + \mu - 2\mu M + 2kM^2 \\
&= -2kV + kM + \mu \\
&= \mu + kM - 2kV
\end{aligned}$$

$\frac{dM}{dt} = \mu - kM$

At s.s. $M = \frac{\mu}{k}$, from $\mu + kM - 2kV = 0 \Rightarrow V = \frac{\mu+kM}{2k}$, and with $M = \frac{\mu}{k}$, $V = \frac{\mu+\mu}{2k} = \frac{2\mu}{2k} = \frac{\mu}{k} = M$.

So. Mean = Variance. (Poisson! In fact, it is...) You can solve P_n at s.s. explicitly...

4.3 But This Doesn't Always Work.

That the moments form a finite number of equations is called Moment Closure. Not closed if, e.g. $E(X)$ depends on $E(X^2)$ depends on $E(X^3)$... this happens when $X \xrightarrow{\nu=x^2} X - 1$

4.4 For Example.

Write reactions in net change form $x \xrightarrow{\nu(x)} x + \nu$. x is a vector of species, molecular counts. $x = (x^j)_{j=1}^m$. $x = (x^j)$. $j = 1, \ldots, m$.

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

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

$\langle X \rangle = E(X)$.

Just another notation

$$\frac{d\langle X \rangle}{dt} = \langle f^+(x) \rangle - \langle f^-(x) \rangle$$

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

4.5 Cases with Moment Closure

– Linear, i.e. 1st order or 0th order reactions. ν_j are all degree 1 polynomials of x. e.g. C, $c + x_1$, x_2, but not $x_1 x_2$.

– Feedforward structure $\dot{x}_1 = \mu - x_1$, $\dot{x}_2 = x_1^2 - x_2$, $\dot{x}_3 = x_1 x_2 - x_3$.

This excludes many interesting cases though...

4.6 More Generally, How to Analyze Steady State Moments?

$\Rightarrow$ Linear noise approximation (LNA).

– Just like using linearization to analyze nonlinear dynamical systems. We can also do linearization for stochastic processes.

– approximate, $\nu_i(x) \approx \nu_i(x^*) + \sum_j \frac{\partial \nu_i}{\partial x^j}(x - x^*)$.

– For the example, but with $x^* = \langle x \rangle$.

$$\begin{aligned}\frac{d\langle x\rangle}{dt} &= \langle f^+(x)\rangle - \langle f^-(x)\rangle \\ &\approx f^+(\langle x\rangle) + \frac{\partial f^+}{\partial x}\langle x - \langle x\rangle\rangle - f^-(\langle x\rangle) - \frac{\partial f^-}{\partial x}\langle x - \langle x\rangle\rangle \\ &= f^+(\langle x\rangle) - f^-(\langle x\rangle)\end{aligned}$$

– General solution and application of LNA, see homework.

5 Stochasticity in Biological Systems

How biological noise arises from molecular mechanisms and impacts cellular processes like gene expression and cell fate decisions.

5.1 Gene Expression Noise Burstiness

Gene expression is often ″bursty″ or ″noisy,″ meaning protein/mRNA levels fluctuate significantly over time in identical cells. This noise is largely attributed to transcriptional bursting—genes switching between active (ON) and inactive (OFF) states, producing a ″burst″ of mRNA molecules when ON. Understanding this noise is crucial for explaining phenotypic variability in genetically identical cell populations.

5.2 Modeling Transcriptional Bursting

Attempt 1: Simple Burst Model

Reaction:

$$G \xrightarrow{k} G + bP \tag{5}$$

(where b is the average burst size, i.e., number of proteins produced per activation event).

Limitations:

Assumes a constant, fixed burst size `b`.

Attempt 2: Random Burst Size:

Improvement:

Model burst size `b` itself as a random variable (e.g., drawn from a geometric distribution).

Limitation:

Still a phenomenological model; doesn't explain the *origin* of the variability in `b`.

Attempt 3: Mechanistic Two-State (ON/OFF) Model

Mechanism:

Explicitly models the gene's promoter switching.

$$G_{off} \rightleftharpoons G_{on} \tag{6}$$

(stochastic switching between states)

$$G_{on} \xrightarrow{K_{+x}} G_{on} + P \tag{7}$$

(protein production only in the ON state)

The observed "burst size" emerges naturally from the time the gene spends in the ON state and the rate of transcription. Different genes have different switching kinetics, explaining gene-specific noise profiles.

This model illustrates how the structure of a mechanism (ON/OFF switching) directly organizes the features of observed variation (burstiness).

5.3 From Multistability to Multimodality: The Role of Noise in Dynamical Systems

In dynamical systems theory, particularly in biological and chemical contexts, the relationship between deterministic structure and stochastic behavior is fundamental. This document formalizes the connection: **multistability combined with noise yields multimodal probability distributions**.

Deterministic Model

$$\frac{dx}{dt} = f(x), \quad \text{where } f(x) \text{ has a cubic (N-shaped) form} \tag{8}$$

This model has the following behavior:

- Two stable fixed points: "ON" (x_{on}) and "OFF" (x_{off})
- One unstable saddle point (x_{saddle}) in between
- Basins of attraction partition the state space

Interpretation: Initial condition determines final state $\Rightarrow$ perfect bistability. Cells are permanently in one fate or the other.

Adding Stochasticity (Noise)

The equation becomes:

$$dX = f(X)\,dt + \sigma\,dW \quad \text{(Stochastic Differential Equation)} \tag{9}$$

where W is a Wiener process (Brownian motion) and σ quantifies noise intensity. Noise enables transitions between basins $\Rightarrow$ **stochastic switching** between ON and OFF states.

Steady-state distribution (SSD): At $t \to \infty$, probability distribution $P_{ss}(x)$ becomes **bimodal**, with peaks at the two stable states.

Population view: A heterogeneous population emerges, with fractions in each state corresponding to the peaks of $P_{ss}(x)$.

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5.4 Extinction and Zero

Autocatalysis in Biological Growth

Many biological processes involve autocatalysis:

$$X \xrightarrow{K} X + X \quad \text{or} \quad X \xrightarrow{f(X)} X + 1 \tag{10}$$

Key feature: You need X to make more X.

The Extinction Problem:

- If $X = 0$, the system cannot produce more X
- This creates an **absorbing state** at $X = 0$
- Once the system reaches $X = 0$, it remains there forever

Deterministic Model

For a simple autocatalytic system:

$$\frac{dx}{dt} = kx - rx = (k - r)x \tag{11}$$

Fixed points:

- $x^* = 0$ (unstable if $k > r$)
- Growth occurs for any $x(0) > 0$

With External Disturbances

Adding external mortality/removal:

$$\frac{dx}{dt} = \frac{kx}{K + x} - (r + \mu)x \tag{12}$$

- Can create a **stable** extinction state if μ is large enough
- But still deterministic: either always extinct or never extinct

5.5 Ergodicity

Definition: A system is ergodic if, starting from any point, over time it can visit **every point** in the state space.

For a Markov process, ergodicity requires:

1. **Irreducible:** For any states i and j, there exists $t > 0$ such that:

$$P(X(t) = j \mid X(0) = i) > 0 \tag{13}$$

2. **Positive recurrent:** The expected return time to any state is finite

A stochastic process $\{X(t)\}_{t\geqslant 0}$ is ergodic if:

$$\lim_{T\to\infty} \frac{1}{T}\int_0^T \mathbf{1}_{\{X(s)\in A\}}\, ds = \pi(A) \tag{14}$$

where π is the stationary distribution.

Obtaining Distributions from Trajectories

Ensemble Approach (Many Trajectories)

Take N independent trajectories $\{X_i(t)\}_{i=1}^N$:

$$\hat{p}_t(x) = \frac{1}{N}\sum_{i=1}^{N} \mathbf{1}_{\{X_i(t)=x\}} \tag{15}$$

As $N\to\infty$:

$$\hat{p}_t(x) \to P(X(t)=x) \tag{16}$$

Steady-State Distribution

If the system has a stationary distribution π:

$$\pi(x) = \lim_{t\to\infty} P(X(t)=x) = \lim_{t\to\infty}\lim_{N\to\infty} \frac{1}{N}\sum_{i=1}^{N} \mathbf{1}_{\{X_i(t)=x\}} \tag{17}$$

Time-Average Approach (Single Trajectory)

For an ergodic system, follow one trajectory $X(t)$:

$$\pi(x) = \lim_{T\to\infty} \frac{1}{T}\int_0^T \mathbf{1}_{\{X(t)=x\}}\, dt \tag{18}$$

6 Equilibrium physics of bioregulation

The world is connected, and our "eye" to see such connections are orders of magnitude reasoning. Seemingly unrelated observations could be in fact deeply constraining each other. To practice this "vision", we explore some calculations below, with contexts gradually shifting from the macroscopic world we are more familiar with to the microscopie world of molecules and cells.

6.1 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

- **Energy** is a concept from physics that a closed system (i.e without energy input) would dissipate energy and
- **Side note:** But Bio is not closed, so not in equilibrium!

◦ Answer:

- But energy In other words, equilibrium is **"easier to implement"**.
- Also, driven processes can still have several behaviors that "look like" equilibrium, i.e., they balance 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 particles.

$$\text{Microstate} \quad \big(\text{all particle states}\big) \qquad \xrightarrow{\text{Multiplicity}} \qquad \text{Macrostate} \quad \big(\text{statistical states}\big)$$

$$x \qquad\qquad E(x) \text{ (energy)}$$

$$\text{weight } w(x) \qquad \text{energy } E(x) \qquad \text{distribution } P(x)$$

$$\langle X \rangle \text{ (observation)}$$

- Equilibrium: a distribution over microstates with expected property. equilibrium distribution. Namely, the following are equivalent characterizations:

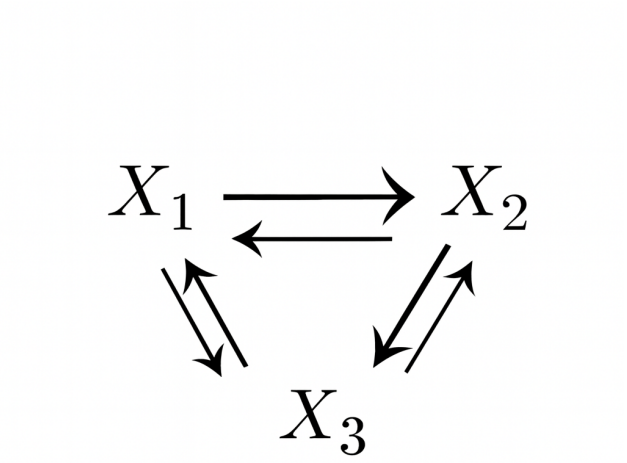
1. **Boltzmann distribution**

   Every (micro) state has an energy $E(x)$, and the equilibrium distribution is

   $$p(x) \propto e^{-\beta E(x)}, \qquad \beta = \frac{1}{kT}.$$

   where $\beta = \frac{1}{k_B T}$, that we often omit it.

2. **Detailed balance**



For every transition between two microstates,

$$p_A k_{A\to B} \rightleftharpoons p_B k_{B\to A}.$$

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

$$k_1 P_A = J_{A\to B} = J_{B\to A} = k_2 P_B$$

$$\Rightarrow \frac{p_A}{P_B} = \frac{k_2}{k_1}$$

So we can define E_A, E_B, s.t. $p_A \propto e^{-E_A}$, $p_B \propto e^{-E_B}$, then $\frac{p_A}{p_B} = e_{-(E_A - E_B)} = \frac{k_2}{k_1}$

- Transition rates and energies are related.

3. **No cyclic flux**

   Detailed balance:

$$k_{12} p_1 = k_{21} p_2$$

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

   Equilibrium constrains transition rates:

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

In a system with states $X_1 \leftrightarrow X_2 \leftrightarrow X_3$, the absence of cyclic flux implies

$$\frac{k_{1\to2}\, k_{2\to3}\, k_{3\to1}}{k_{2\to1}\, k_{3\to2}\, k_{1\to3}} = 1.$$

i.e

$$k_{1\to2}\, k_{2\to3}\, k_{3\to1} = k_{2\to1}\, k_{3\to2}\, k_{1\to3} \tag{19}$$

which is called **"Cycle condition"**.

6.2 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)**.** $S \overset{\nu}{\rightsquigarrow} P$, catalyzed by enzyme E. Consider the reversible reaction

$$E + S \rightleftharpoons ES.$$

We focus on a *single enzyme molecule*. Here S denotes the substrate. Assume the enzyme has two possible macrostates:

- free state: E
- bound state: ES

Energy.

$$E(E) = 1, \qquad E(ES) = \Delta G_b.$$

Multiplicity (number of microstates). Multiplicity is the number of microstates corresponding to each macrostate:

$$\Omega_E = 1, \qquad \Omega_{ES} = \frac{S_{tot}}{C_0},$$

where S_{tot}/C_0 is the dimensionless concentration factor.

Statistical weight.

$$w_E = 1, \qquad w_{ES} = \frac{S_{tot}}{C_0}\, e^{-\Delta G_b}.$$

Probability of being in a bound state. From equilibrium statistical mechanics:

$$p_{bound} = \frac{w_{ES}}{w_E + w_{ES}} = \frac{(S_{tot}/C_0)\, e^{-\Delta G_b}}{1 + (S_{tot}/C_0)\, e^{-\Delta G_b}}.$$

This matches the Michaelis–Menten occupancy formula. If $S_{tot} = E_{tot}$, one may define

$$C = \frac{S_{tot}}{E_{tot}}, \qquad K = Ce^{-\Delta G_b}.$$

Note that. This simplified derivation considers only *one enzyme molecule*. Therefore, the system only has 2 macro states while a full system with many enzymes has many possible microstates.

Then, this can be applied to **several** enzyme molecules by **assuming each enzyme is *i.i.d*(independent and identically distribution)**, so $E = p_b E_{tot} = E_{tot} \frac{S_{tot} e^{-\Delta G_b}}{S_{tot} e^{-\Delta G_b} + 1}$

For example, if

$$N_E, \quad N_S, \quad N_{ES}$$

denote molecular counts, then they satisfy the constraint

$$N_E + N_S + N_{ES} = N_{E,tot},$$

and the number of microstates can become very large.

△

Example 2 (MWC Model: The Second Secret of Life — **Allostery**)**.** Enzyme activities can be regulated by ligands or substrates. The MWC (Monod–Wyman–Changeux) model describes allostery: enzymes have **multiple conformations** and **binding states**.

- **Allostery:** an enzyme may have multiple conformations.
- **Multiple conformations + binding:** an enzyme can bind ligands in different conformational states.
- **Independent contributions:** conformational state and ligand-binding state contribute multiplicatively to statistical weight.

We consider two conformations:

$$\text{active (A)} \qquad \text{and} \qquad \text{inactive (I)}.$$

Each conformation can be either ligand-free or ligand-bound.

State Energies and Weights The four macrostates are:

$$\text{A}, \ \text{A} + \text{bound}, \ \text{I}, \ \text{I} + \text{bound}.$$

Their energies and statistical weights are:

State	Energy	Weight	Bio Notation
Active	E_A	e^{-E_A}	
Active + bound	$E_A + E_{A,b}$	$e^{-(E_A+E_{A,b})}\,\frac{S_{tot}}{C_0}$	$e^{-\epsilon_A}\,\frac{S_tot}{K_A}$
Inactive	E_I	e^{-E_I}	
Inactive + bound	$E_I + E_{I,b}$	$e^{-(E_I+E_{I,b})}\,\frac{S_{tot}}{C_0}$	$e^{-\epsilon_I}\,\frac{S_tot}{K_I}$

Here S_{tot}/C_0 is the multiplicity factor (number of accessible microstates of ligand binding).

Microstate Counting When the "Single-enzyme" Assumption is Removed If the assumption of a single enzyme molecule is removed, the number of microstates can become very large or even infinite.

For example, the system may include:

$$N_E, \quad N_S, \quad N_{ES},$$

with the constraint

$$N_E + N_S + N_{ES} = N_{E,tot}.$$

The combinatorial number of microstates grows rapidly with molecule counts. This is why equilibrium statistical mechanics is useful — it allows us to compute distributions without enumerating all microstates.

Interpretation The MWC model explains how enzymes can switch activity states depending on ligand concentration, through changes in relative statistical weights of conformational states.

△

Activation Probability in the MWC Model From the statistical weights of the four states (A, A+bound, I, I+bound), the probability of being in the active conformation is

$$P_{active} = \frac{e^{-E_A}\left(1+\frac{S_{tot}}{K_A}\right)}{e^{-E_A}\left(1+\frac{S_{tot}}{K_A}\right) + e^{-E_I}\left(1+\frac{S_{tot}}{K_I}\right)}.$$

Limit as $S_{tot} \to 0$.

$$P_{active}(0) = \frac{e^{-E_A}}{e^{-E_A}+e^{-E_I}} = \frac{1}{1+e^{E_A-E_I}}.$$

If the inactive state is lower in energy (e.g. $E_I < E_A$ by 2–3 $k_B T$, about a hydrogen bond), then

$$P_{active}(0) \approx \tfrac{1}{10}.$$

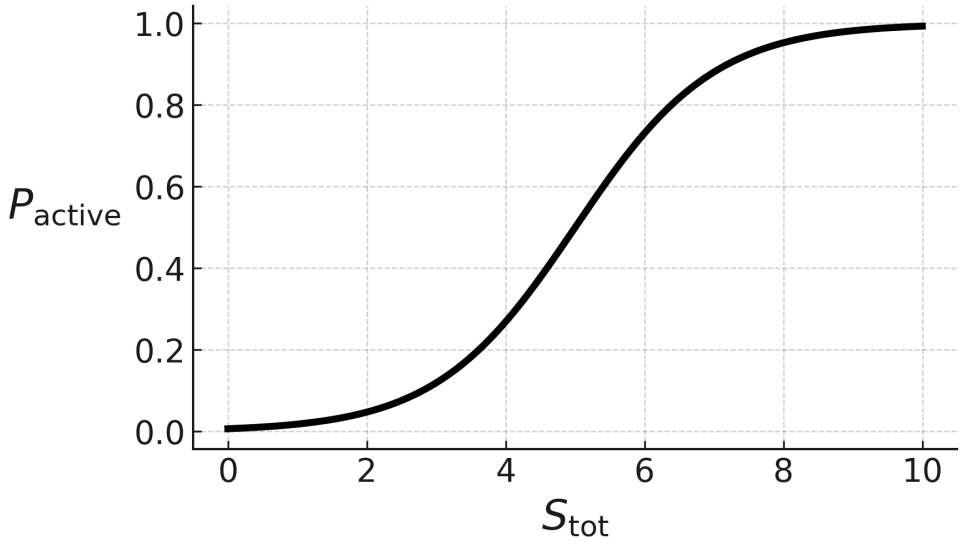


Figure 1 $P_{active} vs S_{tot}$

Limit as $S_{tot} \to \infty$.

$$P_{active}(\infty) = \frac{e^{-E_A}\,\dfrac{S_{tot}}{K_A}}{e^{-E_A}\,\dfrac{S_{tot}}{K_A} + e^{-E_I}\,\dfrac{S_{tot}}{K_I}} = \frac{e^{-E_A}/K_A}{e^{-E_A}/K_A + e^{-E_I}/K_I} = \frac{1}{1 + e^{E_A - E_I}\,\dfrac{K_A}{K_I}}.$$

If $K_A < K_I$, the ligand binds tighter in the active state. For example, if $K_I/K_A \approx 100$, then

$$P_{active}(\infty) \approx \frac{1}{1 + 10^{-2}} \approx 0.9.$$

Cooperativity: Example of a Dimer

Consider a dimeric enzyme where each binding site becomes active/inactive independently and can bind/free ligand independently.

In this case, the active-state weight is squared:

$$P_{active} = \frac{e^{-E_A}\left(1 + \dfrac{S_{tot}}{K_A}\right)^2}{e^{-E_A}\left(1 + \dfrac{S_{tot}}{K_A}\right)^2 + e^{-E_I}\left(1 + \dfrac{S_{tot}}{K_I}\right)^2}.$$

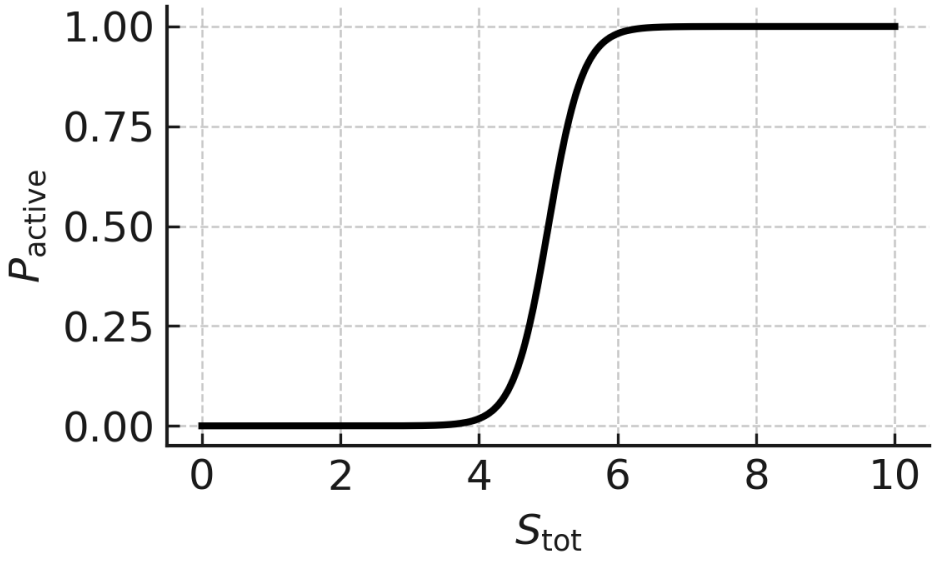


Figure 2 Caption

High-ligand limit.

$$\mathrm{P}_{\text{active}}(\infty) = \frac{e^{-\mathrm{E_A}}\left(\frac{\mathrm{S_{tot}}}{\mathrm{K_A}}\right)^2}{e^{-\mathrm{E_A}}\left(\frac{\mathrm{S_{tot}}}{\mathrm{K_A}}\right)^2 + e^{-\mathrm{E_I}}\left(\frac{\mathrm{S_{tot}}}{\mathrm{K_I}}\right)^2} = \frac{1}{1 + e^{\mathrm{E_A}-\mathrm{E_I}}\left(\frac{\mathrm{K_A}}{\mathrm{K_I}}\right)^2}.$$

This yields a *sharper transition* in $\mathrm{P}_{\text{active}}$ as a function of ligand concentration, characteristic of cooperativity.

Example 3 (Gene Expression)**.** Consider the states pf the **gene** under repression: We enumerate the possible promoter states, their energies, and statistical weights.

State	Energy	Weight
Promoter free	0	1
RNA polymerase (RNAP) bound	$\Delta\varepsilon_{\mathrm{p}}$	$e^{-\Delta\varepsilon_{\mathrm{p}}}\frac{\mathrm{P}}{\mathrm{N_{NS}}}$
Repressor bound	$\Delta\varepsilon_{\mathrm{r}}$	$e^{-\Delta\varepsilon_{\mathrm{r}}}\frac{\mathrm{R}}{\mathrm{N_{NS}}}$

Where:

- P: number of RNA polymerase molecules - R: number of repressor molecules - $\mathrm{N_{NS}}$: number of nonspecific DNA binding sites - $\Delta\varepsilon_{\mathrm{p}}$: RNAP binding energy - $\Delta\varepsilon_{\mathrm{r}}$: repressor binding energy

These statistical weights can be used to compute promoter occupancy and the probability of transcription initiation.

△

6.3 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?
  - my behavior of interest can **only** be achieved out of equilibrium...
  - e.g. Kinetics proofreading... (super precise)

  How to analyze that?
- For the special case of **finite number 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**.)