Lecture 6: Proteins; entropy rules

Goal: Introduce Boltzmann distribution, probability of microstate

- Ligand-receptor binding
- Gene regulation
- Cooperativity

PBOC Chapter 6.1.1, 6.1.2, 6.4
(except 6.4.4)

Statistical mechanics for biophysics

(implies "large system")

Numbers:

RNA polymerase molecules in the nucleus 3-5,000 (E. coli) ~80,000 (mammalian)

Ligands near cell surface 0.3 - 300 mM acetylcholine neurotransmitter

What can we answer with these models?

Determine the probability of finding the system in a particular (energy) state.

Calculate the average values of observables.

Statistical mechanics for biophysics

Previously (Lecture 3):

Microstates

Definition: a ***microstate*** is a microscopic arrangement of the constituents of a system

Example: Ligand binding to a receptor protein

Lattice model

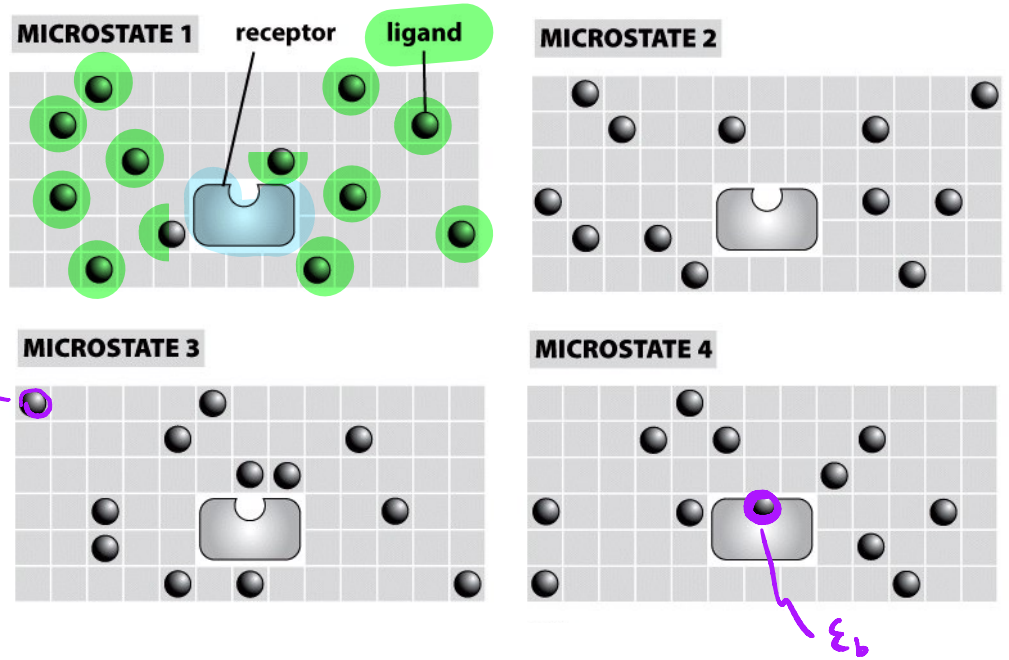
L ligands

Ω boxes

max. one ligand per box

energy ε_b of a bound ligand

energy ε_{sol} of a ligand in solution



Statistical mechanics for biophysics

Microstates

Suppose a system can exist in states with energies E_i.
What is the probability of finding the system in a given state?

Boltzmann distribution, probability of finding the system in a microstate with energy E_i *(derivation, Section 6.1.3)*

$$p(E_i) = \frac{1}{Z} e^{-E_i/k_B T}$$

Partition function, normalization factor so that $\sum_{i=1}^{N} p(E_i) = 1$

$$Z = \sum_{i=1}^{N} e^{-E_i/k_B T}$$

Statistical mechanics for biophysics

Microstates

Suppose a system can exist in states with energies E_i.
What is the average energy of the system?

$\beta = \frac{1}{k_B T}$

The average energy is the (probability) weighted mean of the energies of the states:

$$\langle E \rangle = \sum_{i=1}^{N} E_i p(E_i) = \frac{1}{Z} \sum_{i=1}^{N} E_i e^{-\beta E_i}$$

Note: $\frac{\partial Z}{\partial \beta} = \sum_{i=1}^{N} \frac{\partial}{\partial \beta} \left(e^{-\beta E_i} \right)$

chain rule: $(f(g(x)))' = g'(x) f'(g(x))$

$= \sum_{i=1}^{N} -E_i e^{-\beta E_i}$

$$= -\frac{1}{Z} \frac{\partial Z}{\partial \beta} = -\frac{\partial}{\partial \beta} (\ln Z)$$

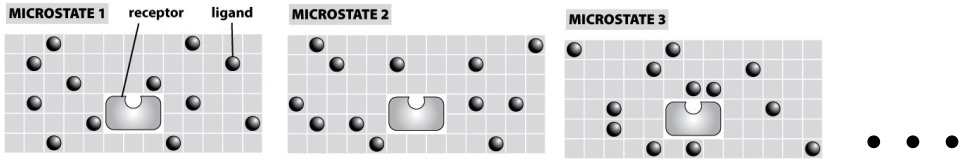
chain rule

Statistical mechanics for biophysics

Example: Ligand binding
Lattice model
L ligands
Ω boxes
max. one ligand per box

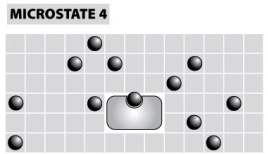
Microstates: multiplicity (Lecture 3)

Microstates with receptor unoccupied



$$\text{number of microstates} = \frac{\Omega!}{L!(\Omega-L)!}$$

Microstates with receptor occupied



$L \rightarrow L-1$

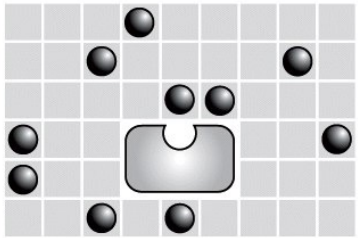
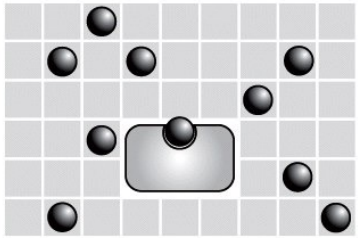
number of microstates?

$$\frac{\Omega!}{(L-1)!\,(\Omega-(L-1))!}$$

Statistical mechanics for biophysics

Example: Ligand binding to receptor protein

Microstates: energy, weight

STATE	ENERGY	MULTIPLICITY	WEIGHT
receptor unbound 	(total energy of system) $L\varepsilon_{sol}$	$\frac{\Omega!}{L!(\Omega-L)!} \approx \frac{\Omega^L}{L!}$ for $\Omega \gg L$	multiplicity x Boltzmann weight "partial" partition function $\frac{\Omega^L}{L!} e^{-\beta L \varepsilon_{sol}}$
receptor bound 	$(L-1)\varepsilon_{sol} + \varepsilon_b$	$\frac{\Omega!}{(L-1)!(\Omega-L+1)!} \approx \frac{\Omega^{L-1}}{(L-1)!}$	$\frac{\Omega^{L-1}}{(L-1)!} e^{-\beta[(L-1)\varepsilon_{sol} + \varepsilon_b]}$

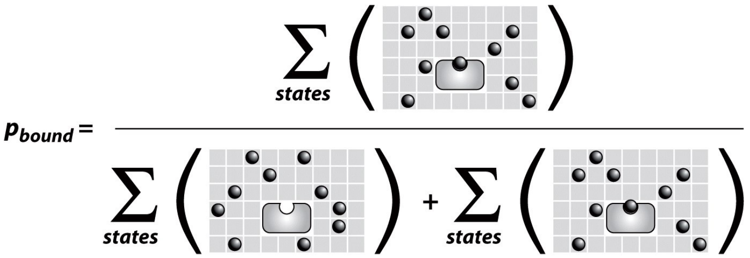
Note: approximation of $\frac{N!}{(N-M)!}$ for $N \gg M$

$= \overbrace{N \cdot (N-1) \cdot \ldots (N-(M+1))}^{M \text{ terms} \approx N \text{ in value}} = N^M$

Statistical mechanics for biophysics

Example: Ligand binding to receptor protein

Microstates: probability



$$= \frac{\frac{\Omega^{L-1}}{(L-1)!} e^{-\beta[(L-1)\varepsilon_{sol}+\varepsilon_b]}}{\frac{\Omega^L}{L!} e^{-\beta L \varepsilon_{sol}} + \frac{\Omega^{L-1}}{(L-1)!} e^{-\beta[(L-1)\varepsilon_{sol}+\varepsilon_b]}}$$

$$= \frac{\frac{L}{\Omega} e^{-\beta[\varepsilon_b-\varepsilon_{sol}]}}{1+\frac{L}{\Omega} e^{-\beta[\varepsilon_b-\varepsilon_{sol}]}}$$

Define: $\Delta\varepsilon =$ ~~$\varepsilon_b - \varepsilon_{sol}$~~ $\varepsilon_{sol} - \varepsilon_b$

$c(L) = L/V_{box}$ (# ligands / system size)

saturation: $c(L=\Omega) = c_0 = \Omega/V_{box}$

$$P_{bound} = \frac{\frac{c}{c_0} e^{-\beta\Delta\varepsilon}}{1+\frac{c}{c_0} e^{-\beta\Delta\varepsilon}}$$

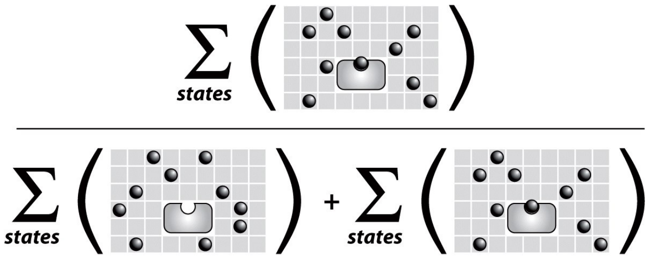
Langmuir isotherm
Hill function of coefficient 1

Statistical mechanics for biophysics

Example: Ligand binding to receptor protein

Microstates: probability (PBoC 6.4.1)

p_{bound} =



$$= \frac{\frac{c}{c_0} e^{-\beta \Delta \varepsilon}}{1 + \frac{c}{c_0} e^{-\beta \Delta \varepsilon}}$$

[L] ligand concentration

$$L + R \rightleftharpoons LR$$

ligand, receptor, ligand + receptor

stoichiometric equation

$$K_d = \frac{[L][R]}{[LR]}$$

dissociation constant

$$p_{bound} = \frac{[LR]}{[R] + [LR]}$$

substitute: $[LR] = \frac{[L][R]}{K_d}$ (★)

$$p_{bound} = \frac{\frac{[L]}{K_d}}{1 + \frac{[L]}{K_d}}$$

$p_{bound} \to 0.5$

⇒ $K_d = c_0 e^{\beta \Delta \varepsilon}$

chemistry, stat mech

$p_{bound} = 0.5$, $c = K_d$

entropy loss = energy gain

Statistical mechanics for biophysics

Example: Ligand binding to receptor protein

Estimate: $V_{\blacksquare} = 1\ \text{nm}^3$; $c_0 = \frac{1\ \text{molecule}}{\text{nm}^3}$

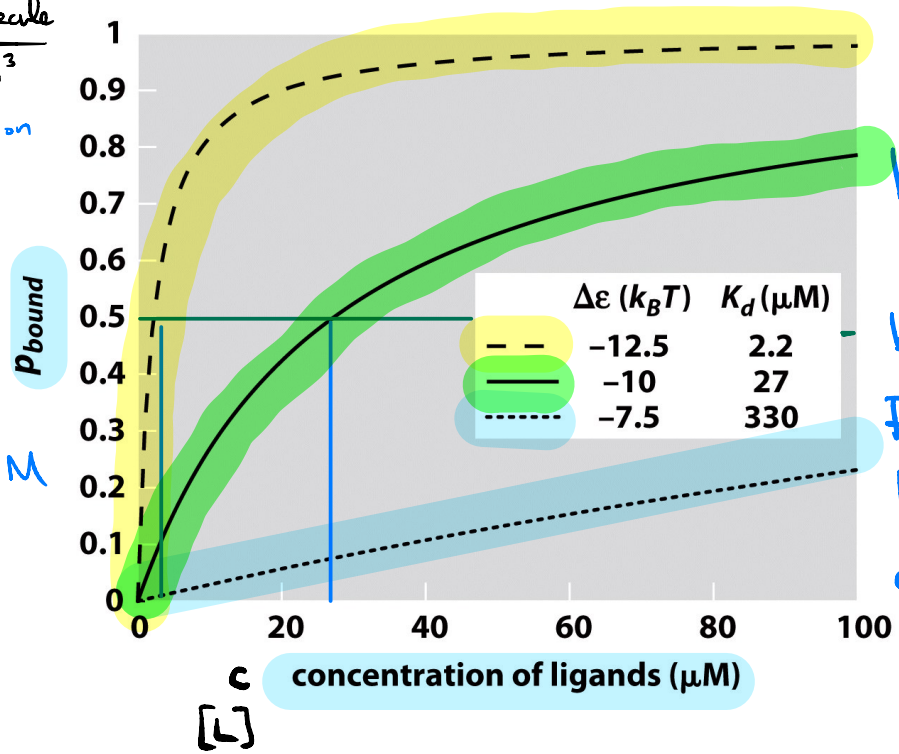
then $c_0 = \frac{\Omega}{V_{box}} = \left(\frac{6\times10^{23}}{10^{24}}\right)$ ⊛ conversion to M

$= 0.6\ M$

(Note: 1 M = $\frac{6\times10^{23}\ \text{molecules}}{1000\ \text{cm}^3}$ ⊛ "molar")

"saturation" in biochemistry texts: ~ 1 M

Receptor occupancy



As Δε increases, exponential dependence leads to strong selection of binding partners. Point mutations can lead to major changes in cell behaviors.

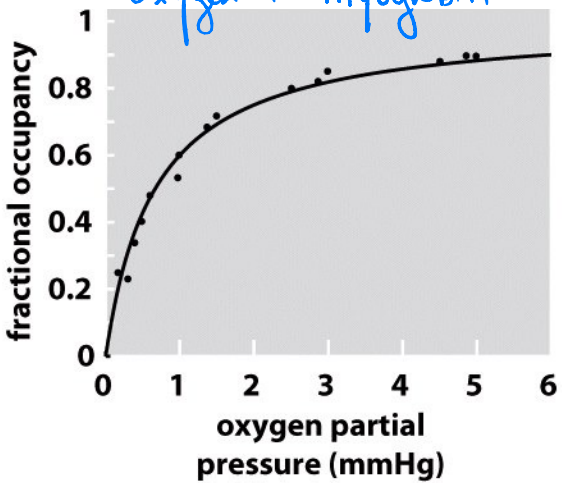
Statistical mechanics for biophysics

Example: Ligand binding to receptor protein

Receptor occupancy

Experimental data

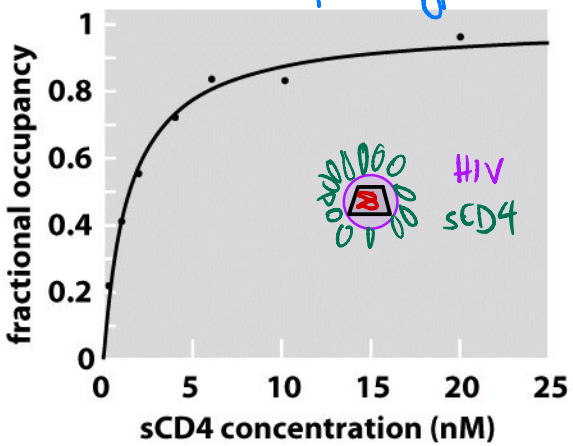
oxygen to myoglobin



$c_0 = 1\ \text{atm} = 760\ \text{mmHg}$

$\Rightarrow \varepsilon \approx -7.04\ k_B T$

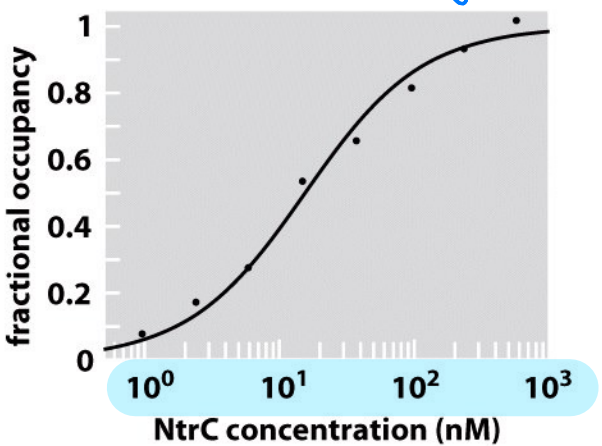
soluble receptor sCD4 to HIV protein gp120



$c_0 = 0.6\ \text{M}$

$\Rightarrow \varepsilon \approx -19.84\ k_B T$

Nitrogen regulatory protein C to DNA (looping)



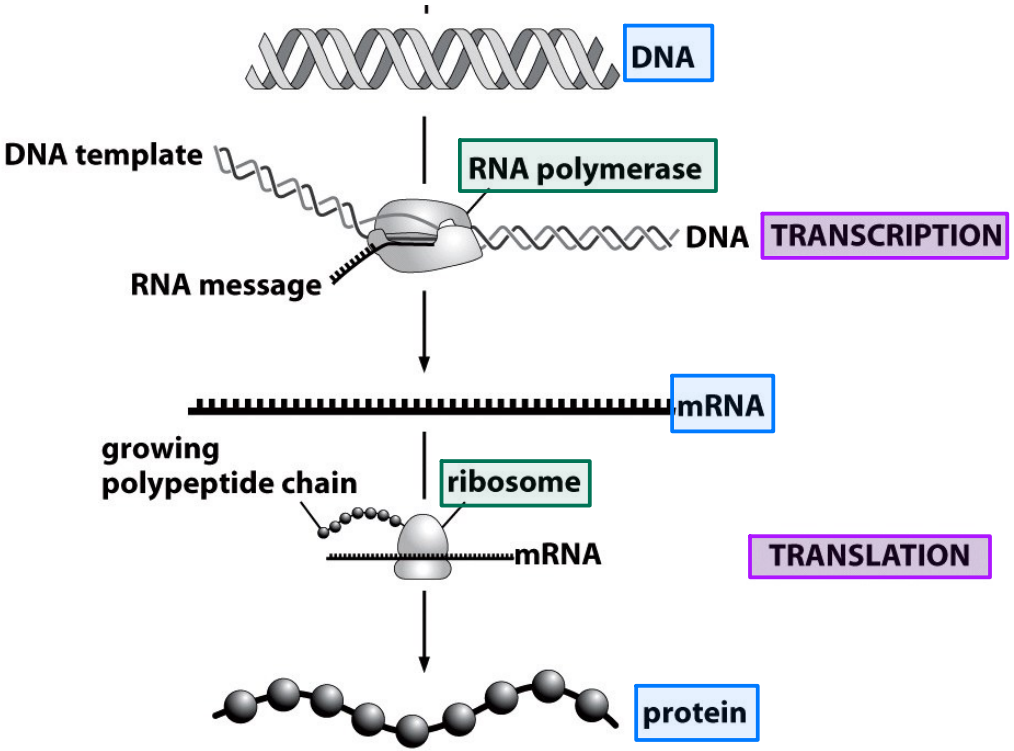
$c_0 = 0.6\ \text{M}$

$\varepsilon \approx -17.47\ k_B T$

Statistical mechanics for biophysics

Previously:

Central dogma



polymer languages

processes

molecular machines

Statistical mechanics for biophysics

How do cells make decisions?

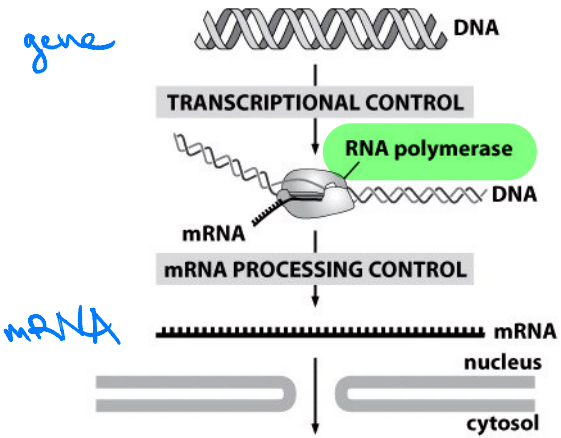
How can different cells in an organism maintain different protein concentrations?

Example: Soil-dwelling amoeba, Dictyostelium discoideum

Vegetative, aggregation, migration, culmination

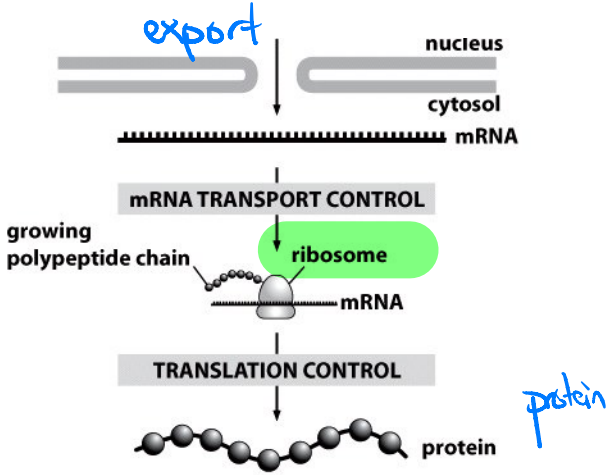
Statistical mechanics for biophysics

Will the cell make a particular mRNA?



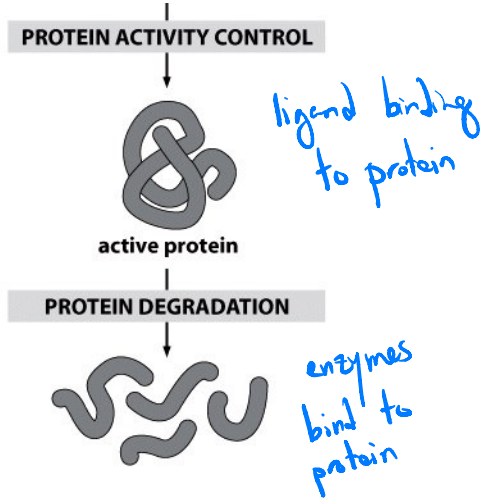
Will an mRNA be processed to become mature?

Will an mRNA be transported to the cytoplasm?



Will an mRNA be translated by the ribosome?

Will a protein exist in an active conformation?



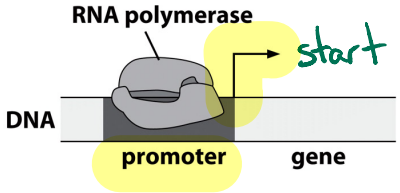
How long will a protein last before being degraded?

Statistical mechanics for biophysics

Will the cell make a particular mRNA?

Example: Transcriptional control

Single case, direct binding



Lattice model

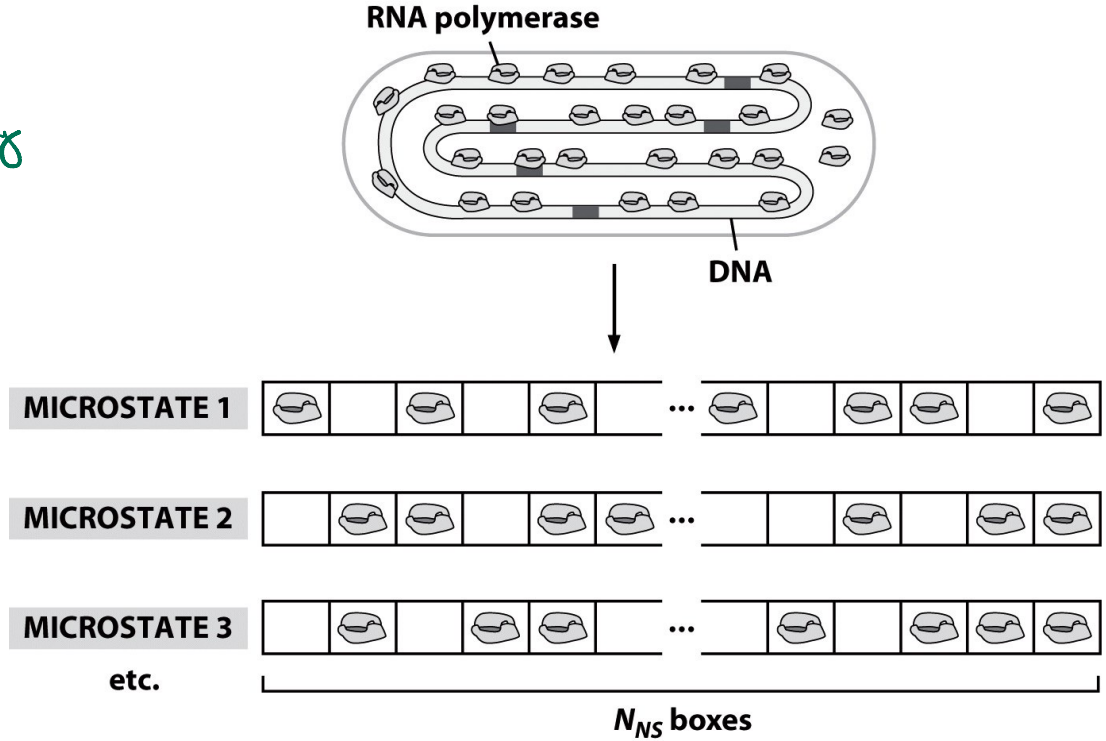
P RNA polymerases

N_{NS} boxes

max. one RNAp per box

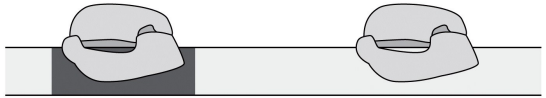
assume all are bound

Microstates

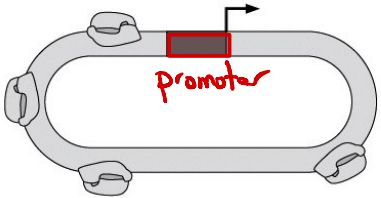
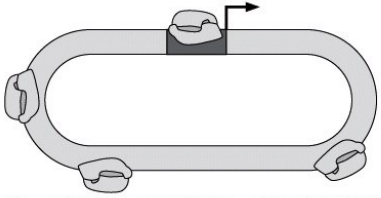


Statistical mechanics for biophysics

Example: Transcriptional control



Microstates

STATE	ENERGY	MULTIPLICITY	WEIGHT (MULTIPLICITY x BOLTZMANN WEIGHT)
promoter not bound 	$P\varepsilon_{pd}^{NS}$	$\frac{N_{NS}!}{P!\,(N_{NS}-P)!} \approx \frac{(N_{NS})^P}{P!}$	$\frac{(N_{NS})^P}{P!}\, e^{-P\varepsilon_{pd}^{NS}/k_BT}$
promoter bound 	$(P-1)\varepsilon_{pd}^{NS} + \varepsilon_{pd}^{S}$	$\frac{N_{NS}!}{(P-1)!\,[N_{NS}-(P-1)]!} \approx \frac{(N_{NS})^{P-1}}{(P-1)!}$	$\frac{(N_{NS})^{P-1}}{(P-1)!}\, e^{-(P-1)\varepsilon_{pd}^{NS}/k_BT}\, e^{-\varepsilon_{pd}^{S}/k_BT}$

Statistical mechanics for biophysics

Example: Transcriptional control

Microstates: probability

$$p_{bound} = \frac{\sum_{states}\left(\text{promoter}\right)}{\sum_{states}\left(\right)+\sum_{states}\left(\right)}$$

$$= \frac{\frac{N_{NS}{}^{P-1}}{(P-1)!}e^{-\beta[(P-1)\varepsilon_{NS}+\varepsilon_S]}}{\frac{N_{NS}{}^{P}}{P!}e^{-\beta P\varepsilon_{NS}}+\frac{N_{NS}{}^{P-1}}{(P-1)!}e^{-\beta[(P-1)\varepsilon_{NS}+\varepsilon_S]}}$$

$$= \frac{\frac{P}{N_{NS}}e^{-\beta\Delta\varepsilon}}{1+\frac{P}{N_{NS}}e^{-\beta\Delta\varepsilon}}$$

Define: $\Delta\varepsilon = \varepsilon_{NS} - \varepsilon_S$

$$= \frac{1}{\frac{N_{NS}}{P}e^{\beta\Delta\varepsilon}+1}$$

Langmuir isotherm
Hill function of coefficient 1

Statistical mechanics for biophysics

Example: Transcriptional control

RNA polymerase occupancy

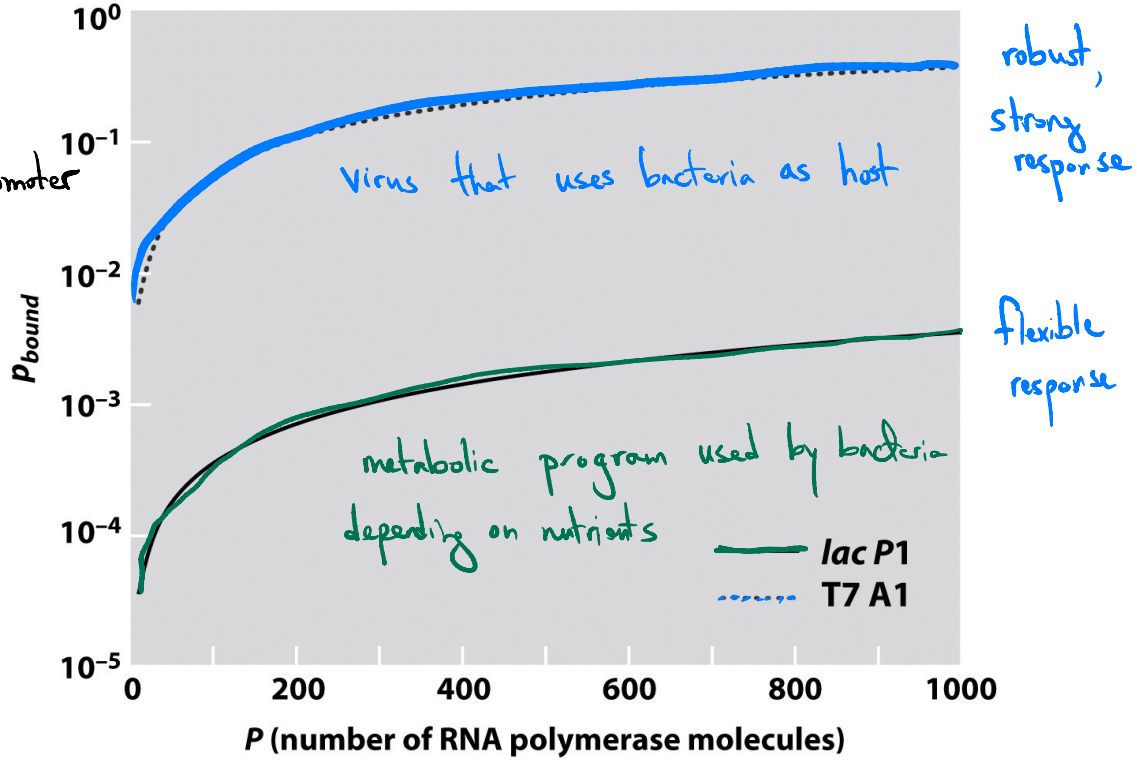
Estimate:

$\Delta\varepsilon = -2.9\ k_BT$ E. coli lac P1 promoter

$\Delta\varepsilon = -8.1\ k_BT$ bacteriophage T7 promoter

~5000 RNA polymerase in E. coli

"hijacking" resources of host make it much more likely to transcribe pathogen genes than own genes.



Statistical mechanics for biophysics

Cooperativity (PB.C 6.4.3)

concentration → on/off

Analog signal → digital output

reaction

$$L + L + R \rightleftharpoons L_2R$$

equilibrium dissociation constant

$$K_d = \frac{[L]^2[R]}{[L_2R]}$$

$$\Rightarrow [L_2R] = \frac{[L]^2[R]}{K_d}$$

Statistical mechanics for biophysics

Cooperativity (PB.C 6.4.3)

concentration → on/off

Analog signal → digital output

reaction

$$L + L + R \rightleftharpoons L_2R$$

equilibrium dissociation constant

$$K_d = \frac{[L]^2[R]}{[L_2R]}$$

$$\Rightarrow [L_2R] = \frac{[L]^2[R]}{K_d} \quad (*)$$

probability of binding

$$p_{bound} = \frac{[L_2R]}{[R] + [L_2R]} \quad (*) = \frac{[L]^2}{K_d^2 + [L]^2}$$

$$= \frac{\left([L]/K_d\right)^2}{1 + \left([L]/K_d\right)^2}$$

Hill function with coefficient $n = 2$

Statistical mechanics for biophysics

Cooperativity (PB.C 6.4.3)

concentration → on/off

Analog signal → digital output

probability of binding

$$p_{bound} = \frac{[L_2R]}{[R]+[L_2R]} = \frac{[L]^2}{K_d^2+[L]^2}$$

$$= \frac{\left([L]/K_D\right)^2}{1+\left([L]/K_D\right)^2}$$

Hill function with coefficient $n = 2$

In general, for n^{th} degree cooperativity

$$p_{bound} = \frac{\left([L]/K_d\right)^n}{1+\left([L]/K_d\right)^n} = \frac{\left(\frac{c}{c_0}e^{-\beta\Delta\varepsilon}\right)^n}{1+\left(\frac{c}{c_0}e^{-\beta\Delta\varepsilon}\right)^n}$$

Hill function with coefficient n

Statistical mechanics for biophysics

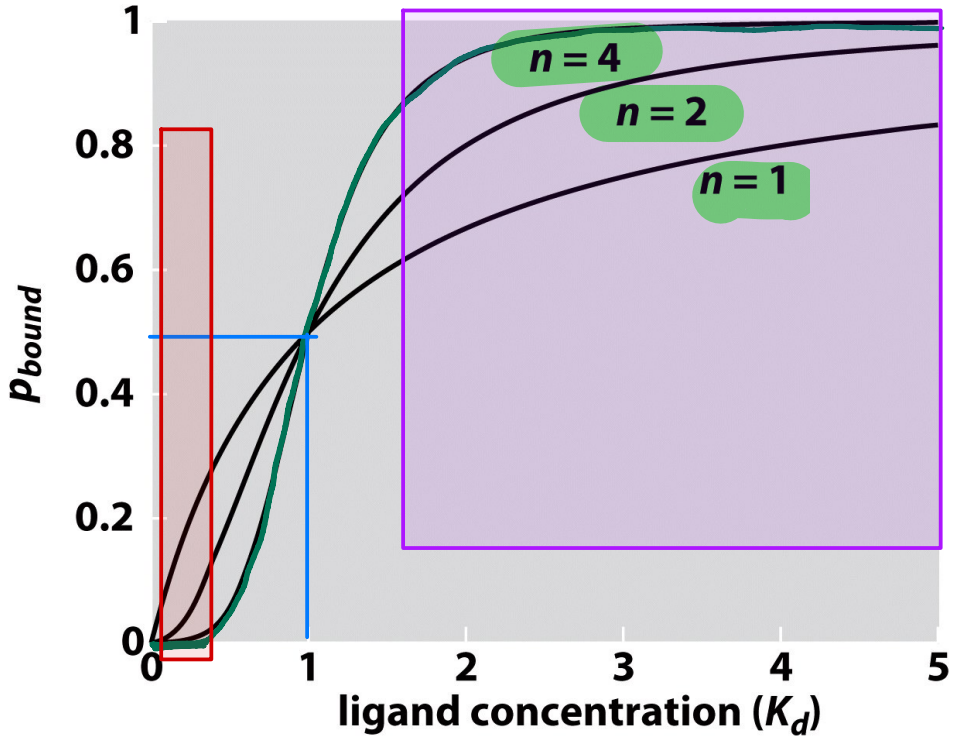
Binding curves with different Hill coefficients

higher n → more switch-like binding response

"on" state and "off" state

very narrow in-between range

Cooperativity



(Chapter 7)

Case study in cooperativity: toy model for hemoglobin, "dimoglobin"

N oxygen molecules, Ω boxes, ε_{sol}, ε_b, J cooperativity energy

State	Energy	Multiplicity	Weight
			P_0
			P_1
			P_2

two oxygen binding sites

Write down P_2, probability that both sites are bound.

What is the average occupancy $\langle N \rangle$?

(Chapter 7) Case study in cooperativity: toy model for hemoglobin, "dimoglobin"

two oxygen binding sites

State	Energy	Multiplicity	Weight
	$N\varepsilon_{sol}$	$\Omega^N/N!$	$\frac{\Omega^N}{N!} e^{-\beta N \varepsilon_{sol}}$
	$(N-1)\varepsilon_{sol} + \varepsilon_b$	$\Omega^{N-1}/(N-1)!$	$\Omega^{N-1}/(N-1)!\ e^{-\beta[(N-1)\varepsilon_{sol} + \varepsilon_b]}$
	$(N-2)\varepsilon_{sol} + J + 2\varepsilon_b$	$\Omega^{N-2}/(N-2)!$	$\Omega^{N-2}/(N-2)!\ e^{-\beta[(N-2)\varepsilon_{sol} + J + 2\varepsilon_b]}$

Write down P_2, probability that both sites are bound.

What is the average occupancy $\langle N \rangle$?

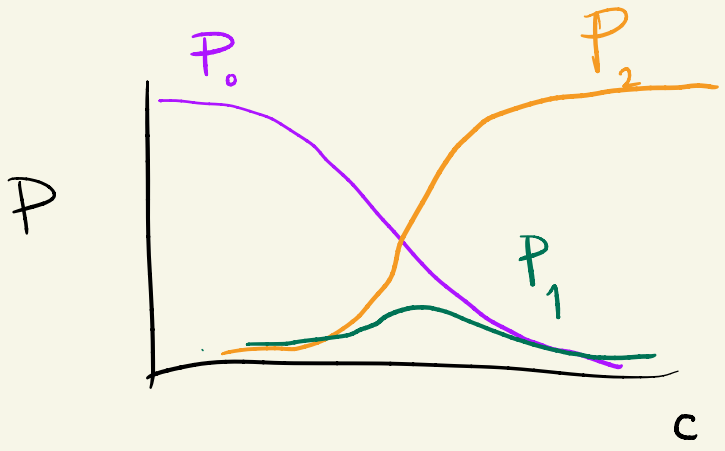
Case study in cooperativity: toy model for hemoglobin, "dimoglobin"

State

- 
- 
- 

$$P_2 = \frac{\frac{N\cdot(N-1)}{\Omega^2}\, e^{-\beta(J+2\varepsilon_b-2\varepsilon_{sol})}}{1+2\frac{N}{\Omega}e^{-\beta(\varepsilon_b-\varepsilon_{sol})}+\frac{N\cdot(N-1)}{\Omega^2}e^{-\beta(J+2\varepsilon_b-2\varepsilon_{sol})}}$$

$$\simeq \frac{\left(\frac{c}{c_0}\right)^2 e^{-\beta(J+2\Delta\varepsilon)}}{1+2\frac{c}{c_0}e^{-\beta\Delta\varepsilon}+\left(\frac{c}{c_0}\right)^2 e^{-\beta(J+2\Delta\varepsilon)}}$$



$$\langle N\rangle = P_1 + 2P_2$$

Lecture 6: Entropy rules

Summary

Models

- Lattice model with microcanonical ensemble requires states, energies, Boltzmann weights
- Lattice model can be used to calculate
  Probability of energy states, Average values
- Cooperativity can be described by a Hill function, with n the degree of cooperativity.

Interpretation

- Strong binding ($|\varepsilon| >> k_B T$, $>10\times$) means higher occupancy at low concentration, long-lived
- Weak binding ($|\varepsilon| > k_B T$) makes for low occupancy, short-lived states
- Cooperativity doesn't change K_d, but changes shape of curve.
- Receptor proteins binding to ligands can start a chain of events (signaling), or block
- Each part of the central dogma relies on binding

Lecture 7: Two-state system, ion channels

Goal: Statistical mechanics modeling. Compute the probability of microstates, including applied forces.

- Two-state system
- Mechanosensitive ion channels

PBOC Chapter 7.1.2, 11.5