Topic 4: Equilibrium binding and chemical kinetics

Outline:

Applications, applications, applications …

- use Boltzmann to look at receptor-ligand binding
- use Boltzmann to look at PolII-DNA binding and gene transcription
- law of mass action and chemical equilibrium
- revisit receptor-ligand binding using law of mass action
- cooperative binding and switch like response

Ligand binding to a receptor

Consider the problem of a ligand binding to a receptor.

Q: What is the probability that the receptor is bound by ligand?

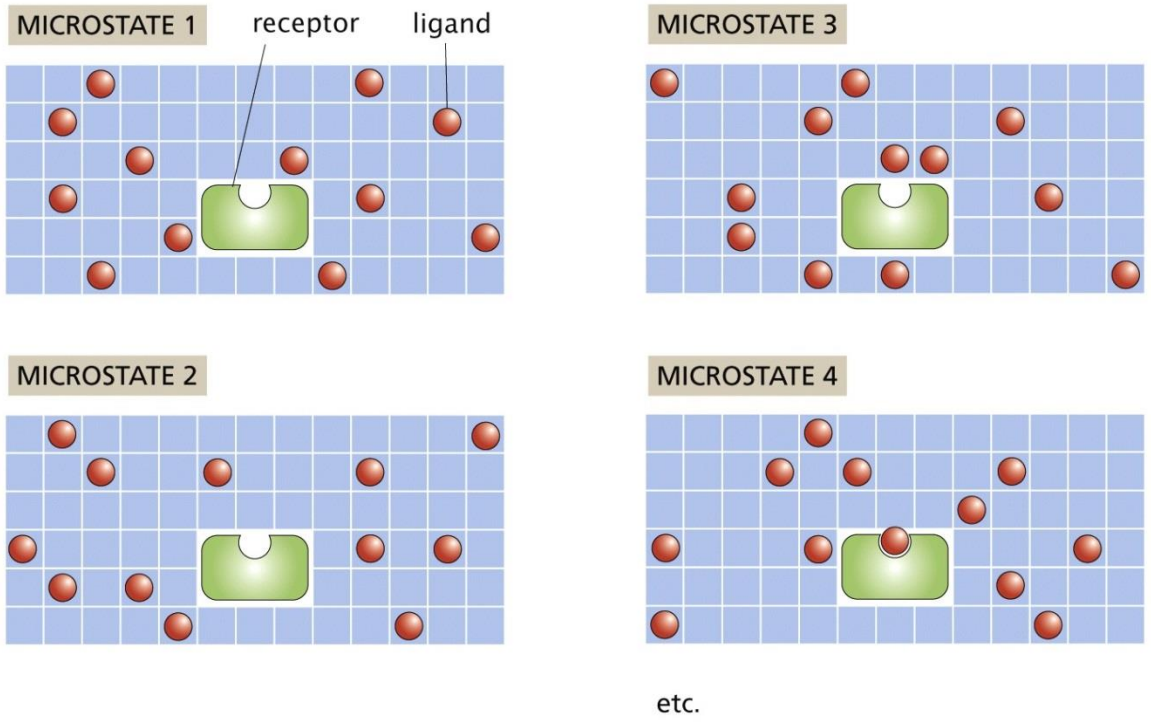


Figure 6.1 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

The answer will depend on the ligand concentration and on the energy differnce for a ligand to be bound or unbound to the receptor

Definining the problem

Free ligand in solution has energy, ε_{sol} and when bound to receptor, ε_b

Use the Boltzmann distribution to calculate P(bound)

Need to enumerate states - like what you did in the assignment

Ω boxes

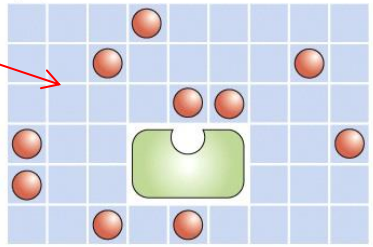
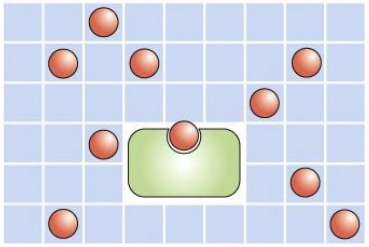
STATE	ENERGY	MULTIPLICITY (# of states)	WEIGHT
(A) 	$L\varepsilon_{sol}$	$\frac{\Omega!}{L!(\Omega-L)!} \approx \frac{\Omega^L}{L!}$	$\frac{\Omega^L}{L!} e^{-\beta L\varepsilon_{sol}}$
(B) 	$(L-1)\varepsilon_{sol} + \varepsilon_b$	$\frac{\Omega!}{(L-1)!(\Omega-L+1)!} \approx \frac{\Omega^{L-1}}{(L-1)!}$	$\frac{\Omega^{L-1}}{(L-1)!} e^{-\beta[(L-1)\varepsilon_{sol} + \varepsilon_b]}$

Figure 6.4 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Using Boltzmann:

Boltzmann says that the probability of a state is: (add up all the way that state can occur x Boltzmann weight) / (all states x Boltzmann weight)

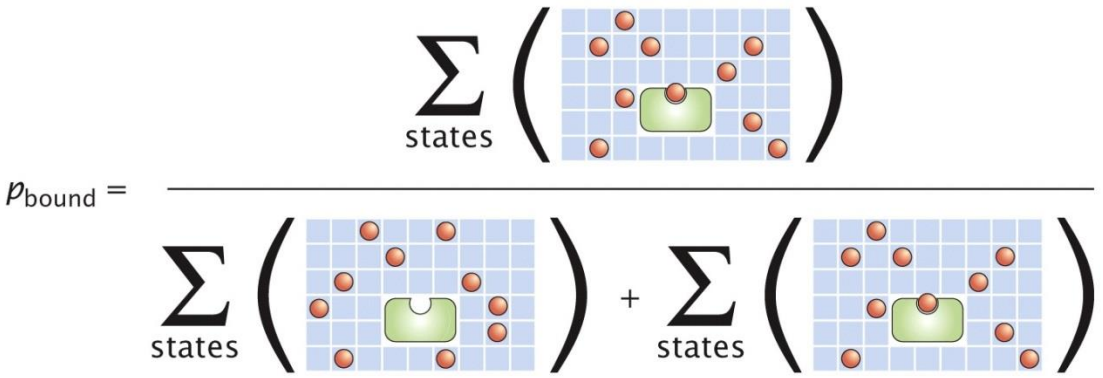


Figure 6.5 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Using results from previous slide:

$$p_{bound} = \frac{e^{-\varepsilon_b/kT}\frac{\Omega^{L-1}}{(L-1)!}e^{-(L-1)\varepsilon_{sol}/kT}}{\frac{\Omega^L}{L!}e^{-L\varepsilon_{sol}/kT} + e^{-\varepsilon_b/kT}\frac{\Omega^{L-1}}{(L-1)!}e^{-(L-1)\varepsilon_{sol}/kT}} = \frac{(\frac{L}{\Omega})e^{-\Delta\varepsilon/kT}}{1+\left(\frac{L}{\Omega}\right)e^{-\Delta\varepsilon/kT}}$$

where $\Delta\varepsilon = \varepsilon_b - \varepsilon_{sol} < 0$

Equilibrium binding constant, K

We can express this result in terms of concentration: $l = L/V$ and $l_0 = \Omega/V$

so

$$p_{bound} = \frac{\left(\frac{l}{l_0}\right)e^{-\frac{\Delta\varepsilon}{kT}}}{1+\left(\frac{l}{l_0}\right)e^{-\frac{\Delta\varepsilon}{kT}}}$$

or

$$p_{bound} = \frac{\left(\frac{l}{K_D}\right)}{1+\left(\frac{l}{K_D}\right)}$$

where we have defined the equilibrium dissociation constant, K_D

$K_D = l_0\, e^{\Delta\varepsilon/kT}$ and has units of concentration,

where if the boxes are 1 nm^3, l_0= 0.6 M

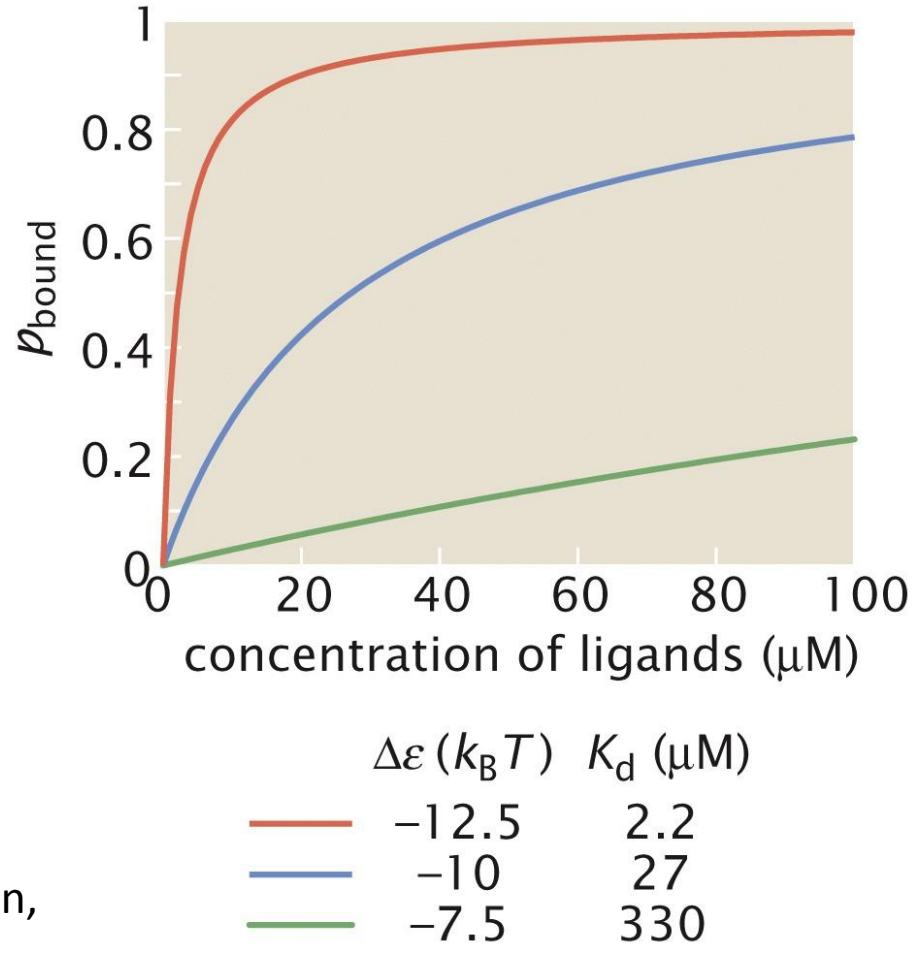


Figure 6.6 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Regulating Gene Expression

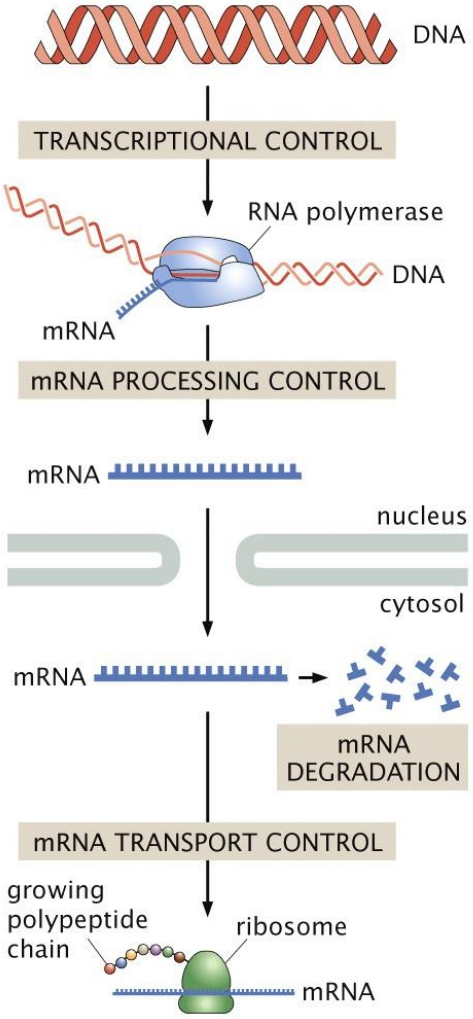


Figure 6.7 (part 1 of 2) Physical Biology of the Cell, 2ed. (© Garland Science 2013)

What proteins get made by a cell is determined by what genes get expressed

this is governed by transcription, which depends on RNA polymerase binding to the promoter regions of genes

The first level of regulating which proteins get made and how much is by controlling transcription

the transcriptome is the collection of every RNA molecule that has been transcribed from the genome

A gene can be transcribed into RNA only if the RNA polymerase (Pol II)binds to its promoter

Thus the probability that a promoter will be bound by Pol II is essential for cellular function

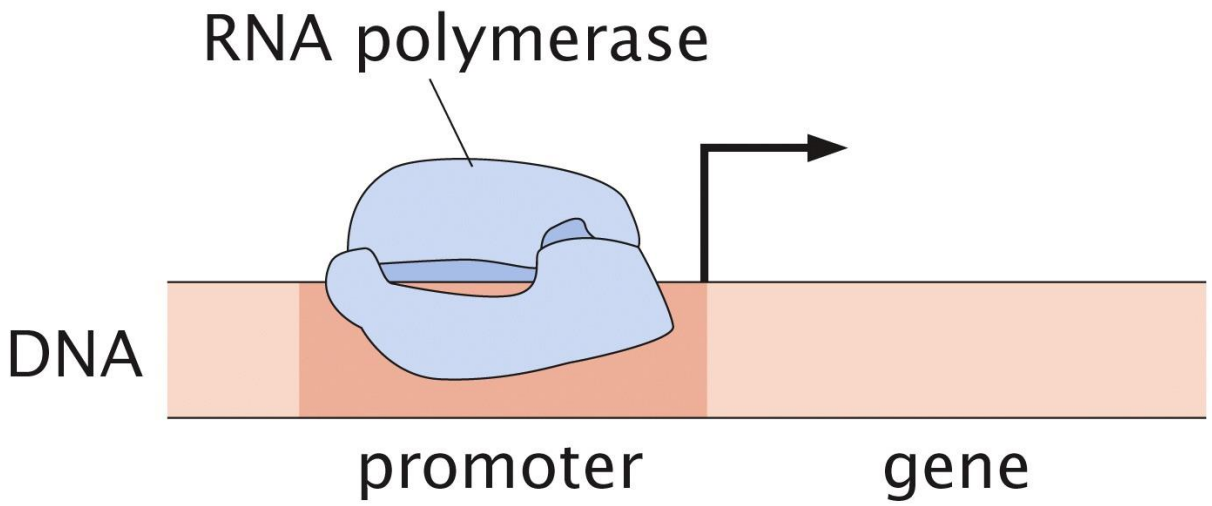


Figure 6.8 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Controlling the probability of PolII binding to promoters (via sequence changes) is one of the key regulatory mechanisms governing gene expression – as we will see in our calculation that follows

The states of PolII binding to DNA

Let's consider that we have, P, Pol II polymerases, and they are all bound to the DNA (this is not a horrible assumption, as there is very little free polymerase in the cytoplasm)

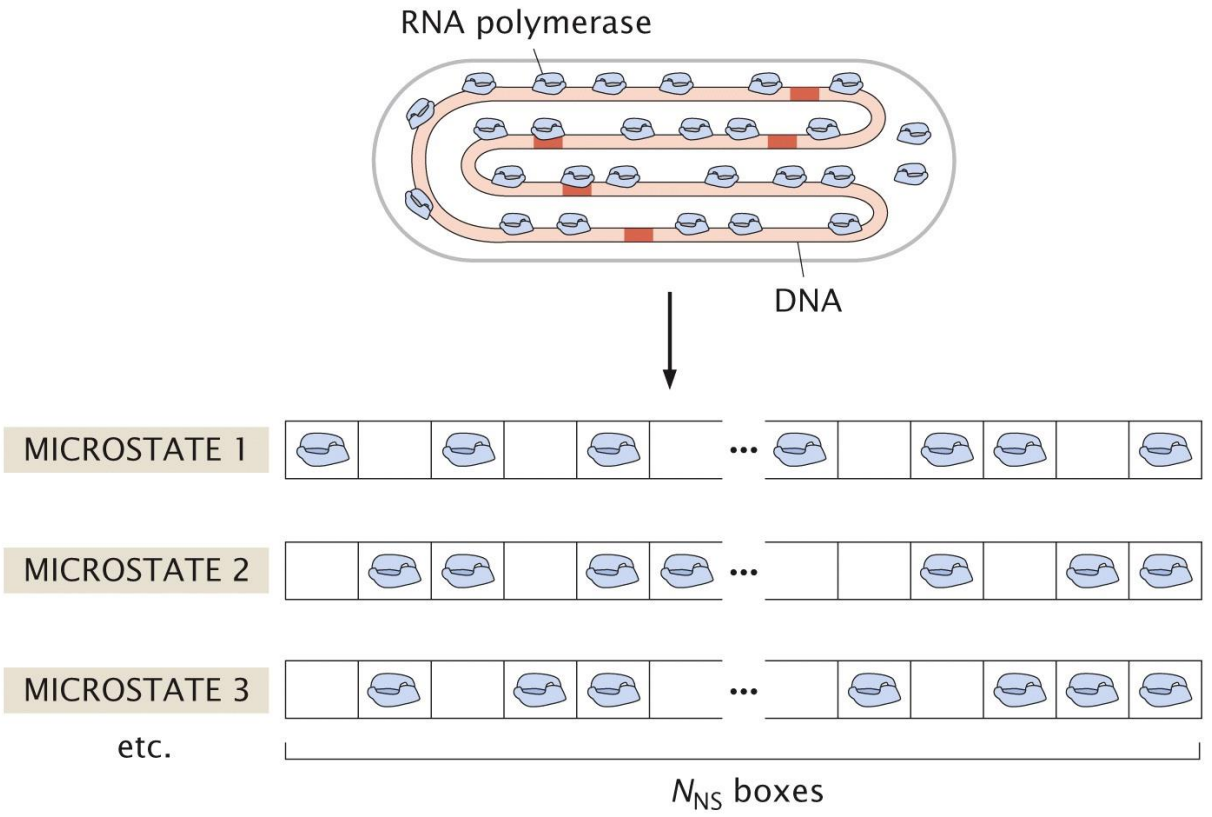


Figure 6.9 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

The DNA can be thought of as consisting of N_{NS}, non-specific binding sites

Binding to a promoter

The non-specific binding of PolII to DNA comes mostly from attractive electrostatic energy between the +ve charged PolII and the –ve charged DNA. This non-specific interaction does not depend on DNA sequence.

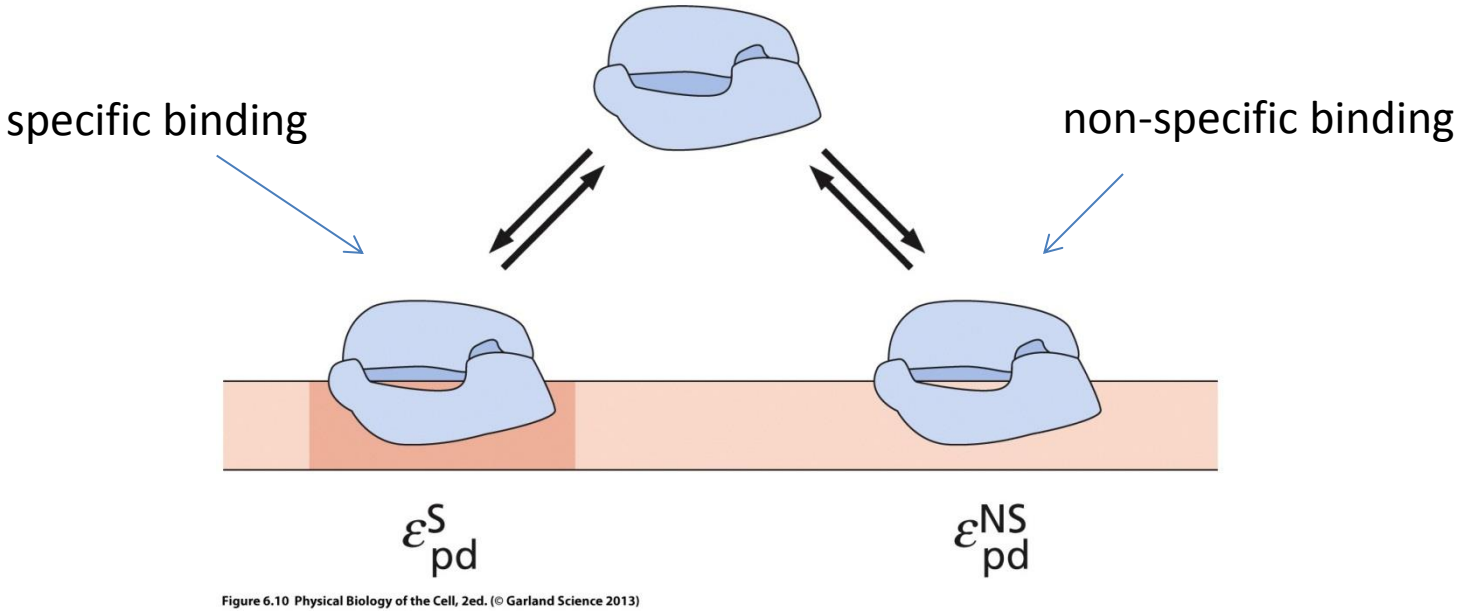
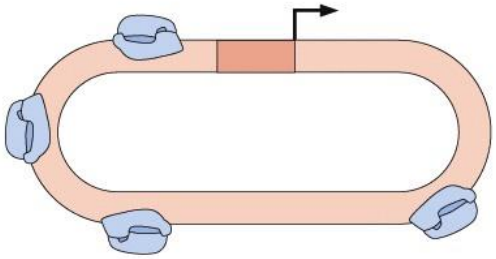
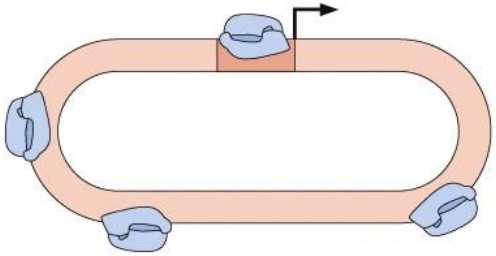


Figure 6.10 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Unlike non-specific binding, specific binding depends very much on the underlying DNA sequence. Promoters have specific DNA sequences that form hydrogen bonds and other specific interactions with regions of PolII leading to lower binding energy

Writing down all the terms

We will focus on the probability to bind to one specific promoter, all other sites will be considered non-specific

STATE	ENERGY	MULTIPLICITY	WEIGHT (MULTIPLICITY x BOLTZMANN WEIGHT)
	$P\varepsilon_{pd}^{NS}$	$\frac{N_{NS}!}{P!\,(N_{NS}-P)!} \approx \frac{(N_{NS})^P}{P!}$	$\frac{(N_{NS})^P}{P!}\, e^{-P\varepsilon_{pd}^{NS}/k_BT}$
	$(P-1)\,\varepsilon_{pd}^{NS} + \varepsilon_{pd}^{S}$	$\frac{N_{NS}!}{(P-1)!\,[N_{NS}-(P-1)]!} \approx \frac{(N_{NS})^{P-1}}{(P-1)!}$	$\frac{(N_{NS})^{P-1}}{(P-1)!}\, e^{-(P-1)\varepsilon_{pd}^{NS}/k_BT}\, e^{-\varepsilon_{pd}^{S}/k_BT}$

Ω

L

Figure 6.11 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

This is essentially the same as the ligand – receptor problem.

Applying the Boltzmann Distribution

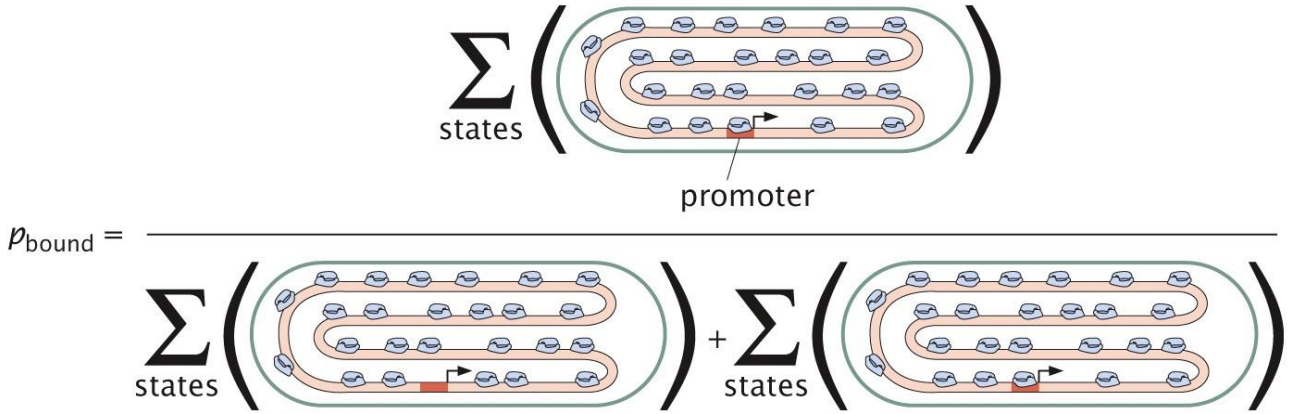


Figure 6.12 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Using results from previous slide:

$$p_{bound} = \frac{e^{-\varepsilon_S/kT}\frac{{N_{NS}}^{P-1}}{(P-1)!}e^{-(P-1)\varepsilon_{NS}/kT}}{\frac{{N_{NS}}^{L}}{P!}e^{-P\varepsilon_{NS}/kT} + e^{-\varepsilon_S/kT}\frac{{N_{NS}}^{P-1}}{(P-1)!}e^{-(P-1)\varepsilon_{NS}/kT}} = \frac{(\frac{P}{N_{NS}})e^{-\Delta\varepsilon/kT}}{1+\left(\frac{P}{N_{NS}}\right)e^{-\Delta\varepsilon/kT}}$$

where $\Delta\varepsilon = \varepsilon_S - \varepsilon_{NS} < 0$

Sequence controls the probability of PolII being bound

The probability of a promoter being bound is

$$p_{bound} = \frac{\left(\frac{P}{N_{NS}}\right)e^{-\frac{\Delta\varepsilon}{kT}}}{1+\left(\frac{P}{N_{NS}}\right)e^{-\frac{\Delta\varepsilon}{kT}}}$$

The number of polymerases, P is roughly a constant across cells, as is the number of non-specific binding sites

A cell controls the probability of a promoter being bound by controlling $\Delta\varepsilon$. It does this by changing the DNA sequence in the promoter.

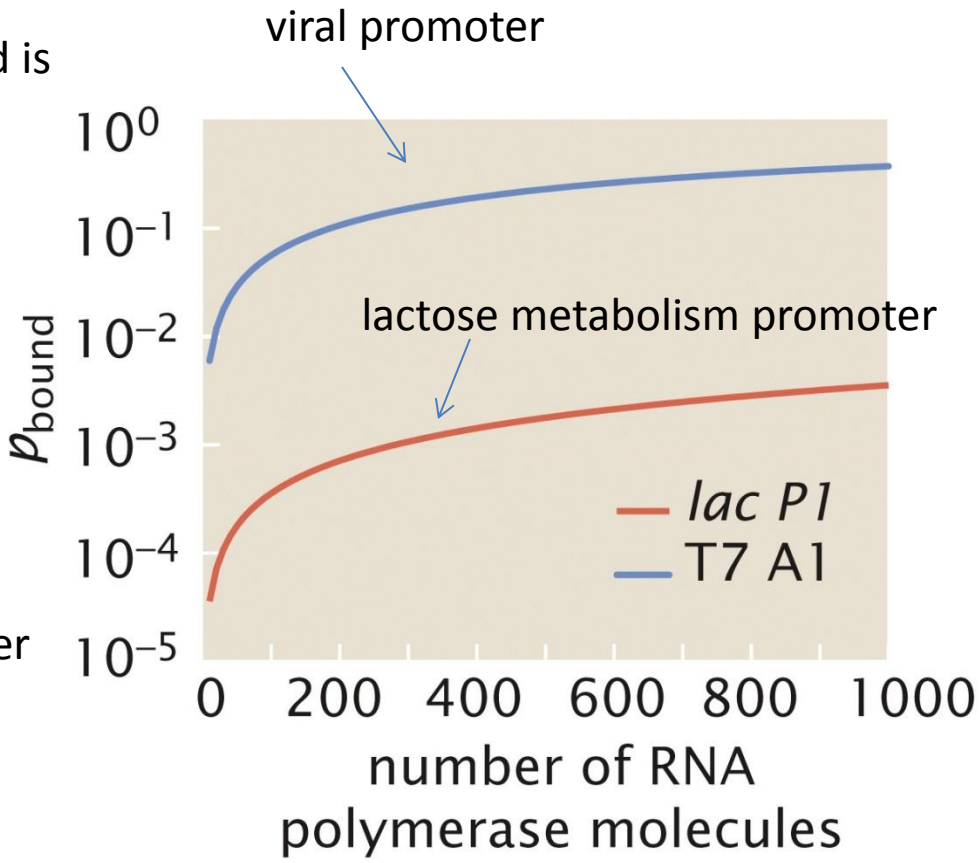


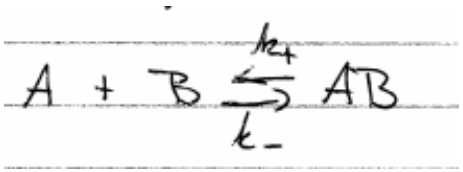
Figure 6.13 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Shown are the probabilities of being bound for the lac promoter in E. coli (this controls the machinery that processes the important sugar source, lactose), as well as the promoter of a gene in a bacterial virus. The virus has a much stronger promoter. Here, $\Delta\varepsilon_{lac} = -2.9\text{kT}$ and $\Delta\varepsilon_{T7} = -8.1\text{kT}$, with $N_{NS} = 5 \times 10^6$

Law of mass action and chemical equilibrium

We can also arrive at the previous results using results from equilibrium chemistry

Consider the reaction:



chemical equilibrium occurs when the flow from the left equals the flow from the right

or $$k_+[A][B] = k_-[AB]$$

reactants product

or $$\frac{[A][B]}{[AB]} = \frac{k_-}{k_+} = K_D \equiv \text{dissociation constant}$$

so at equilibrium, the ratio of the concentration of the reactants to the product is the dissociation constant – it has units of concentration.

We will identify this with our previous result for the dissociation constant that depended on the energy difference between the reacting and product states

General result for chemical reactions

In general, if we have multiple species involved in the reaction with different numbers of each coming together to bind

$$nA + mB + pC \rightleftharpoons D$$

then equilibrium occurs when

$$\frac{A^n B^m C^p}{D} = K_D^{n+m+p-1}$$

for example consider:

$$2A + B \rightleftharpoons C$$

then

$$\frac{[A]^2[B]}{[C]} = K_D^2$$

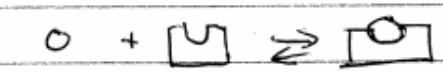
Ligand-Receptor binding redux:

Let's consider the problem of ligand binding to a receptor again. Previously we showed that,

$$p_{bound} = \frac{\left(\frac{l}{l_0}\right)e^{-\frac{\Delta\varepsilon}{kT}}}{1+\left(\frac{l}{l_0}\right)e^{-\frac{\Delta\varepsilon}{kT}}}$$

Using equilibrium chemistry:

- Consider the reaction $L + R \rightleftarrows LR$



- @ equilibrium: $\frac{[L][R]}{[LR]} = K_d$ or $[LR] = \frac{[L][R]}{K_d}$

- Now the probability of a receptor being bound is

$$P_{bound} = \frac{[\text{bound receptor}]}{[\text{total receptor}]} = \frac{[LR]}{[R]+[LR]}$$

So

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

looks familiar

Ligand receptor binding experiments

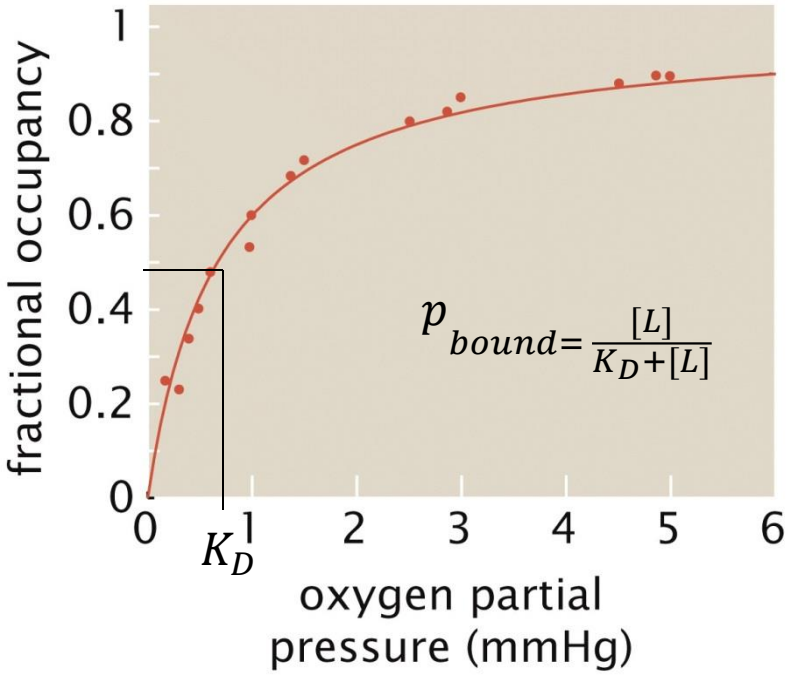


Figure 6.28a Physical Biology of the Cell, 2ed. (© Garland Science 2013)

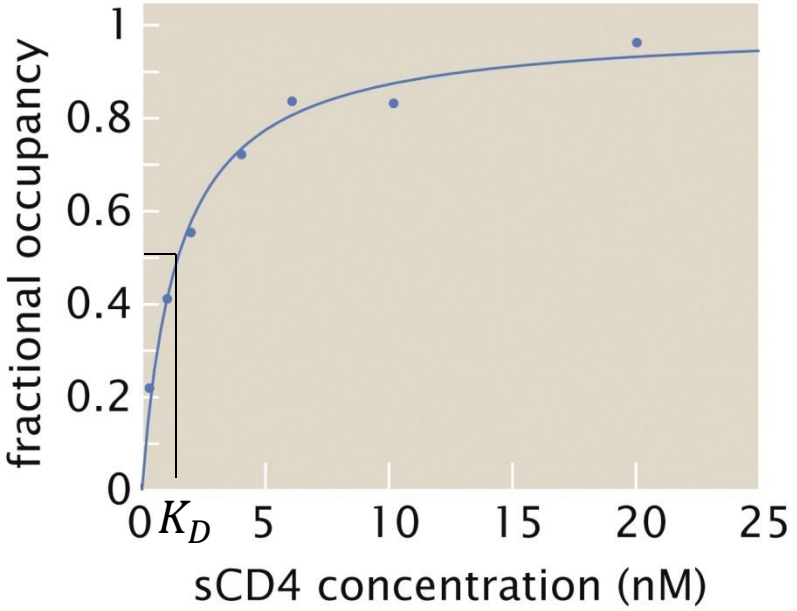


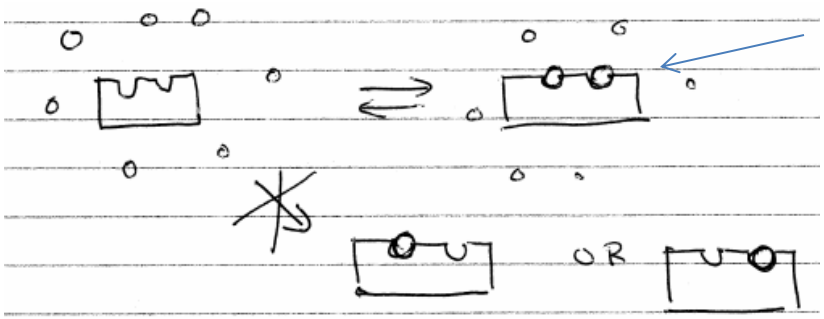
Figure 6.28b Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Comparing to our previous result, we see that as before, $K_D = l_0 e^{\Delta\varepsilon/kT}$.

We also see that the dissociation constant corresponds to the concentration where the receptor is ½ bound. The above plots show how the binding energy can be measured experimentally.

Cooperative binding

Many binding events in molecular biology are cooperative, meaning that the binding of multiple sites is better than having each site bound individually



cooperative interaction between sites

In cooperative binding there is additional binding energy that occurs between the bound ligands

Let's consider: $L + L + R \rightleftarrows 2LR$

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

again $P_{bound} = \frac{[\text{bound receptor}]}{[\text{total receptor}]} = \frac{[2LR]}{[R] + [2LR]}$

$$P_{bound} = \frac{[L]^2/K_d^2}{1 + [L]^2/K_d^2}$$

Cooperative binding: Hill function

More generally, if there is cooperativity amongst, n binding sites:

$$p_{bound} = \frac{[L]^n}{K_D^n + [L]^n}$$

Hill coefficient – measures cooperativity

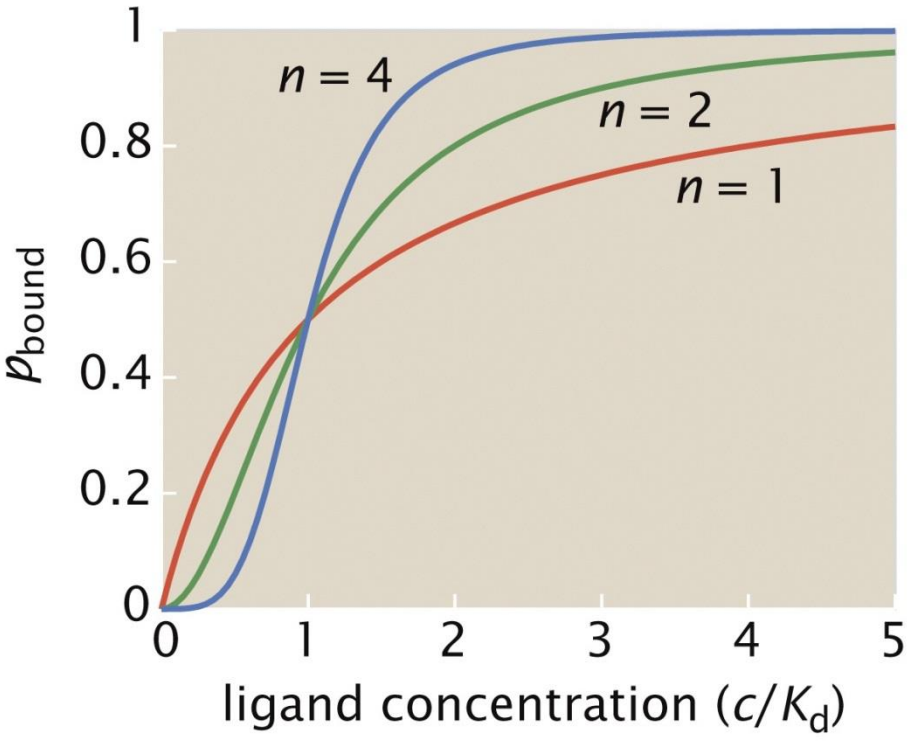
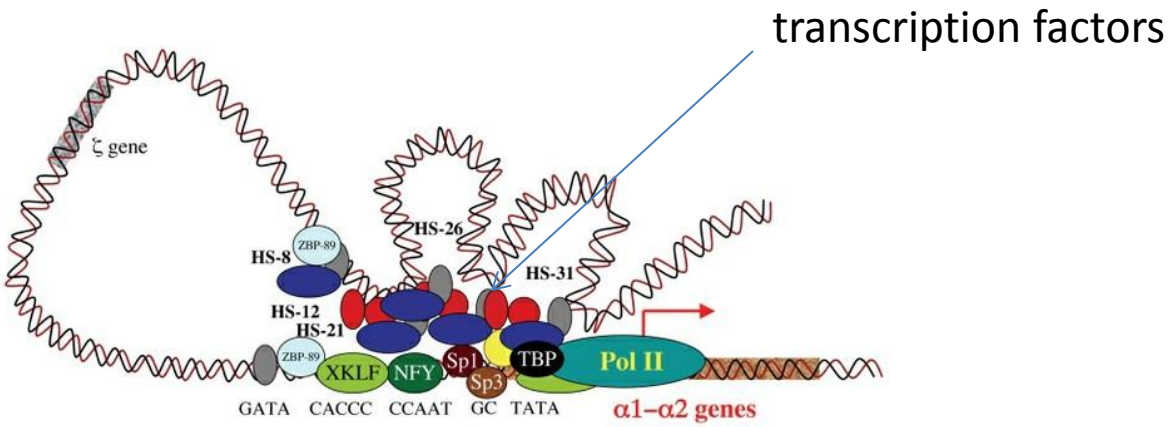


Figure 6.29a Physical Biology of the Cell, 2ed. (© Garland Science 2013)

the higher the cooperativity, the more switch like response to binding as a function of input concentration

this can lead to chemical systems being 'digital'. That is they can be either 'on' or 'off' as a function of input ligand.

Besides controlling the promoter sequence to regulate PolII binding, cells use transcription factors to enhance or repress transcription



Transcription factors have cooperative interactions with PolII and themselves

Their binding leads to regulatory logic – i.e. various logic functions on the factors lead to the expression of the gene. This leads to cellular computation.

Transcriptional activators:

Consider a simple system of the PolII plus an additional activator protein, A

The PolII can bind to a promoter, and ther activator to a specific site upstream of the promoter

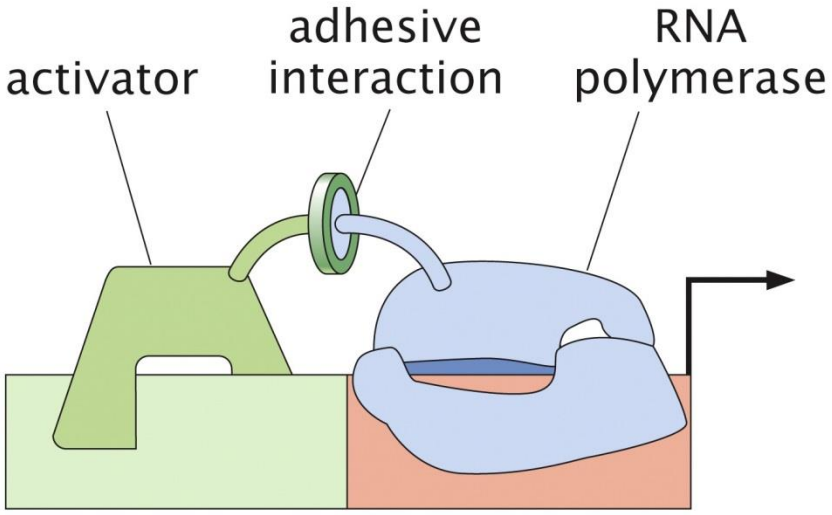


Figure 19.7 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

there is a cooperative interaction between the activator and PolII that helps to stabilize the PolII on the promoter → enhancing the probability of being bound and therefore more transcription

Enumerating states

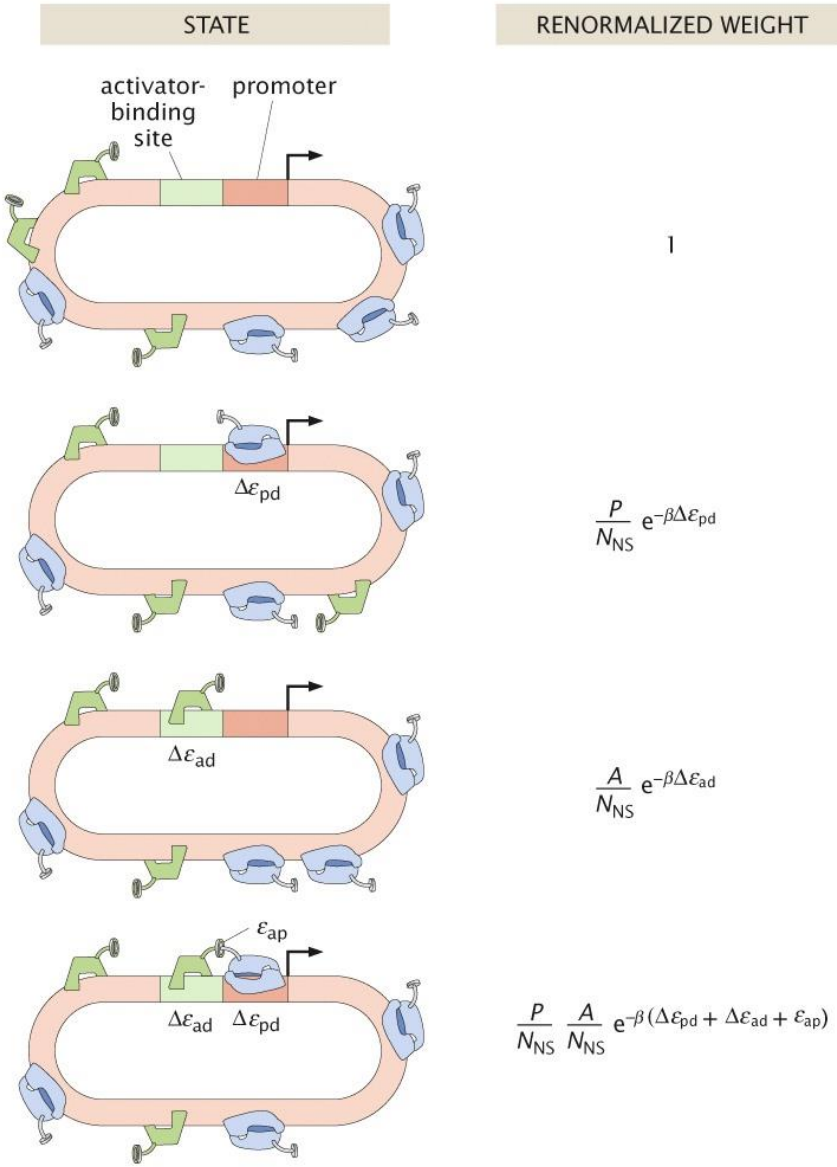


Figure 19.9 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Just as before, you enumerate the possible configurations

there's a lot more to keep track of

fortunately we can also treat this problem using equilibrium chemistry

you'll work this out on an assignment

Summary:

- We've begun to apply the Boltzmann distribution to calculate probabilities
- We saw how we can define the strength of binding in terms of the energy gained in binding
- gene transcription depends critically on the probability of PolII binding to the promoter
- we can treat binding reactions using equilibrium chemistry
- cooperative binding leads to switch like responses as a function of chemical input
- transcriptional logic is implemented by cooperative interactions between transcription factors and PolII