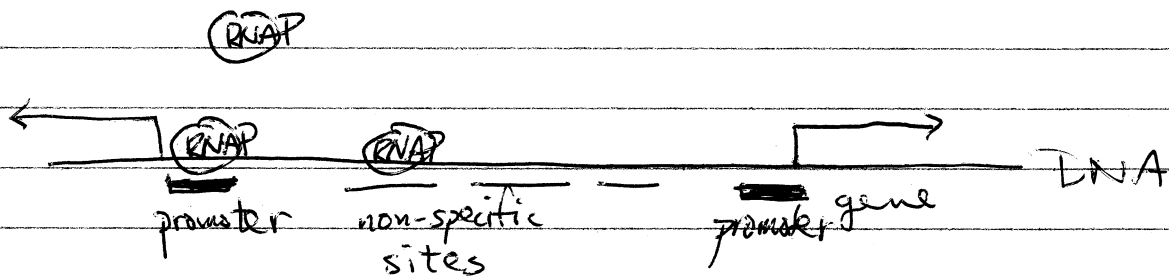


Stat Mech & Biology: Applications

RNA Polymerase Binding to Promoters:

- Previously we looked @ the entropy of DNA binding proteins to N sites on DNA.
- We now revisit this problem in the context of RNA polymerase binding to DNA



- RNAP binding to DNA is competing between the specific sites located @ promoters and non-specific sites
- The specific site has binding energy E_s and the non-specific has energy E_{ns}
- Let's compute the partition function for P RNAP molecules binding to N_{ns} non-specific sites

$$Z_{N_{ns}}(P, N_{ns}) = \frac{N_{ns}!}{P!(N_{ns}-P)!} e^{-\beta P E_{ns}}$$

(2)

When one RNAP binds to a promoter, there are $P-1$ bound to the N_{NS} sites, so the partition function for specific binding is

$$Z_S(P, N_{NS}) = Z_{NS}(P-1, N_{NS}) e^{-\beta E_S}$$

so

$$Z_{TOT} = Z_{NS}(P, N_{NS}) + Z_S(P, N_{NS})$$

The probability of the promoter being bound is,

$$P_{bound} = \frac{Z_S(P, N_{NS})}{Z_{NS}(P, N_{NS}) + Z_S(P, N_{NS})}$$

in full

$$P_{bound} = \frac{\frac{N_{NS}!}{(P-1)!(N_{NS}-P+1)!} e^{-\beta E_S} e^{-\beta E_{NS}(P-1)}}{\frac{N_{NS}!}{P!(N_{NS}-P)!} e^{-\beta E_{NS}P} + \frac{N_{NS}!}{(P-1)!(N_{NS}-P+1)!} e^{-\beta E_S} e^{-\beta E_{NS}(P-1)}}$$

Now we need to simplify: use $\frac{N_{NS}!}{(N_{NS}-P)!} \approx N_{NS}^P$ for $N_{NS} \gg P$

• Simplifying leads to

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

where $\Delta E = E_S - E_{NS}$

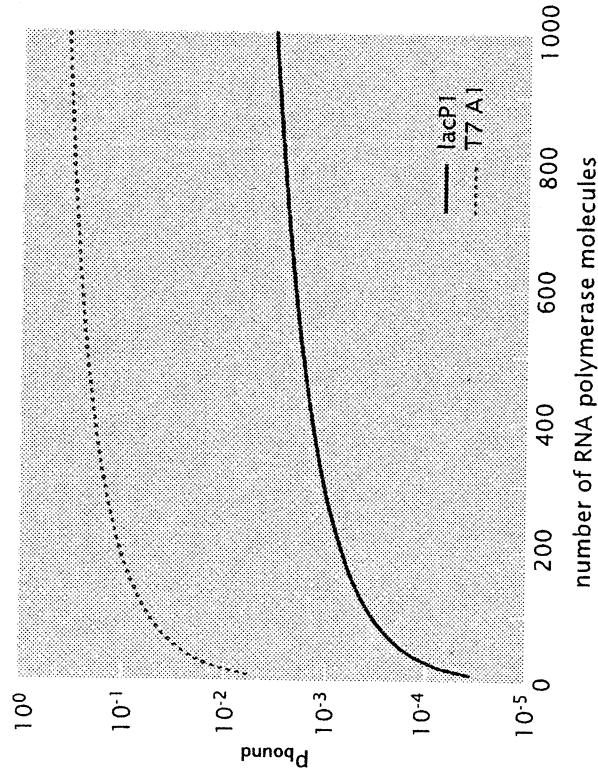


Figure 6.13: Probability of promoter occupancy as a function of the number of RNA polymerase molecules. p_{bound} is computed using values for the specific and nonspecific binding obtained *in vitro* and corresponding to the lac promoter (solid line), and the A1 promoter from the phage T7.

molecules in our hypothetical bacterial cell. Note that in order to make ex-

- Thus the probability of binding only depends on the energy difference between sites
- Fig 1 shows the probability of RNAP binding to a promoter as a function of the # of RNAP. Two different promoters are considered:
 - 1) weak promoter \equiv lac $\rightarrow \Delta E = -2.9 k_B T$
 - 2) Strong promoter \equiv T7 $\rightarrow \Delta E = -8.1 k_B T$.
 (N.B. use $N_{NS} \approx 5 \times 10^6 \sim$ size of *E. coli* genome)
- We will look at how transcription factors affect this probability later

Law of Mass Action:

- Chemical reactions drive most biological processes
- let's consider the reaction $A + B \xrightleftharpoons[k_-]{k_+} AB$
- At equilibrium, the law of mass action says

$$\frac{[A][B]}{[AB]} = K_d \equiv \text{dissociation constant}$$

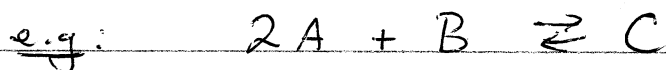
and $K_d = \frac{k_-}{k_+}$ in terms of kinetic rates

• we'll see shortly that K_d is related to the difference in free energy between the bound and unbound states

• More generally for N reacting species,

$$\prod_i [c_i]^{-\nu_i} = K_d^{-\mu} \quad \text{where } \mu = \sum_i \nu_i$$

where $\nu_i \equiv$ stoichiometric coefficients



$$\underbrace{\nu_A = -2}_{\text{lose 2}} \quad ; \quad \underbrace{\nu_B = -1}_{\text{lose 1}} \quad ; \quad \underbrace{\nu_C = 1}_{\text{gain 1}}$$

so $[A]^{+2} [B] [C]^{-1} = K_d^2$

A look @ Ligand Receptor Binding:

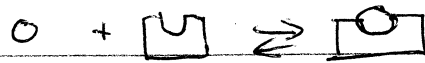
- Consider the problem of a ligand @ concentration $[L]$ binding to a receptor and gaining energy ΔE
- Analogously to the RNAP problem the probability of ligand binding to receptor is

$$P_{\text{bound}} = \frac{\frac{[L]}{[L_0]} e^{-\beta \Delta E}}{1 + \frac{[L]}{[L_0]} e^{-\beta \Delta E}}$$

where $[L_0]$ is a reference concentration (typically 1M)

• Now let's look @ the same problem from the point of view of equilibrium chemistry

• Consider the reaction $L + R \rightleftharpoons LR$



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

• Now the probability of a receptor being bound is

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

so

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

• Thus K_d can be interpreted as the concentration $[L]$ @ which $1/2$ the receptor will be bound.

• Comparing to our stat mech result: $K_d = [L_0] e^{+\beta \Delta E}$

• Usually K_d is determined experimentally.

• see Fig 2 for some experimentally measured binding curves.

Fig 2

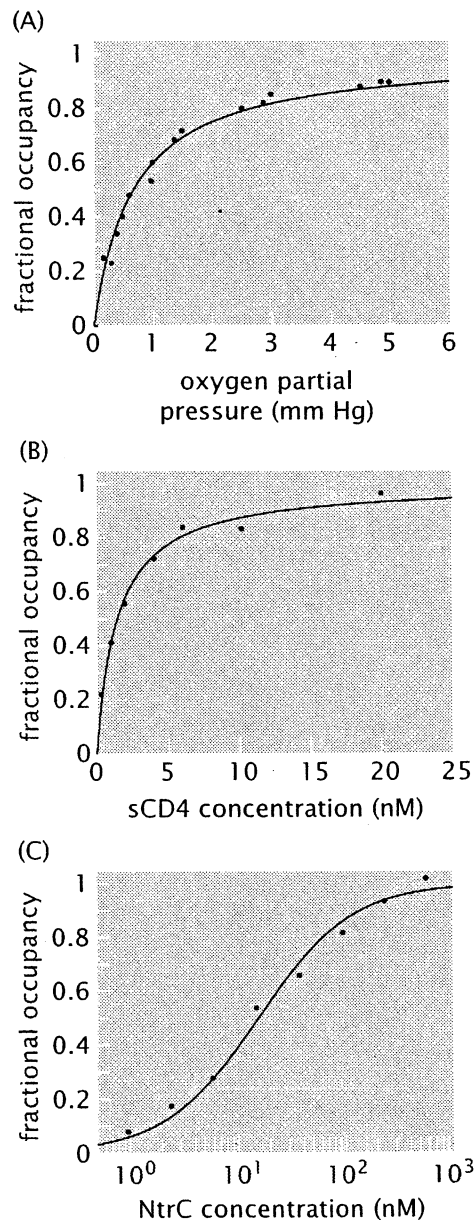
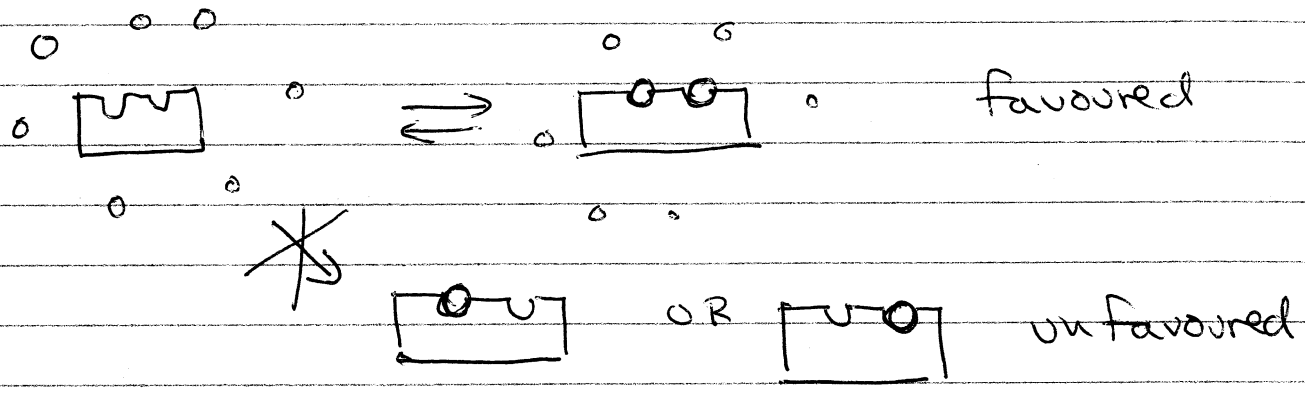


Figure 6.26: Examples of ligand-receptor binding. (A) The binding of oxygen to myoglobin as a function of the oxygen partial pressure. The points correspond to the measured occupancy of myoglobin as a function of the oxygen partial pressure and the curve is a fit to eqn. 6.110. The fit yields $\Delta\varepsilon \approx -7.04 k_B T$ using a standard state $c_0 = 760 \text{ mmHg} = 1 \text{ atm}$, which also corresponds to a dissociation constant $K_d = 0.666 \text{ mmHg}$. (B) Binding of HIV protein gp120 to cell surface receptor sCD4 giving $\Delta\varepsilon \approx -19.84 k_B T$ or $K_d = 1.4578 \text{ nM}$ with $c_0 = 0.6 \text{ M}$. (C) Binding of NtrC to DNA giving $\Delta\varepsilon = -17.47 k_B T$ or $K_d = 15.5 \text{ nM}$ with $c_0 = 0.6 \text{ M}$. (A, data from A. Rossi-Fanelli and E. Antonini,

Cooperative Binding:

- many biological binding events are cooperative, meaning that there are multiple binding sites, and that having all sites occupied is much better than having only a single site occupied



- this happens when there is an interaction between the binding sites that favours full occupancy over partial occupancy.

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

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

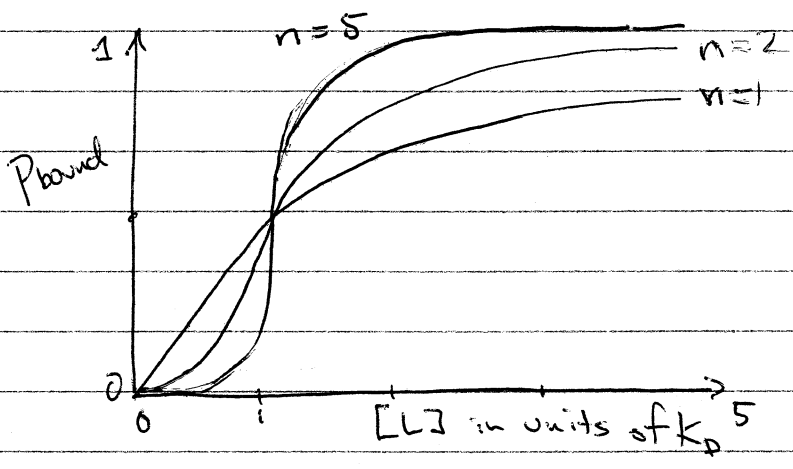
again
$$P_{bound} = \frac{[bound\ receptor]}{[total\ receptor]} = \frac{[2LR]}{[R] + [2LR]}$$

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

and more generally, for higher cooperative binding we have

$$P_{\text{bound}} = \frac{([L]/K_d)^n}{1 + ([L]/K_d)^n} \equiv \text{Hill function}$$

and $n \equiv$ Hill coefficient, measures cooperativity



- higher cooperativity leads to a more "switch"-like response
- For hemoglobin, $n=3.0$

Fig 3

6.4. APPLICATIONS OF THE CALCULUS OF EQUILIBRIUM 491

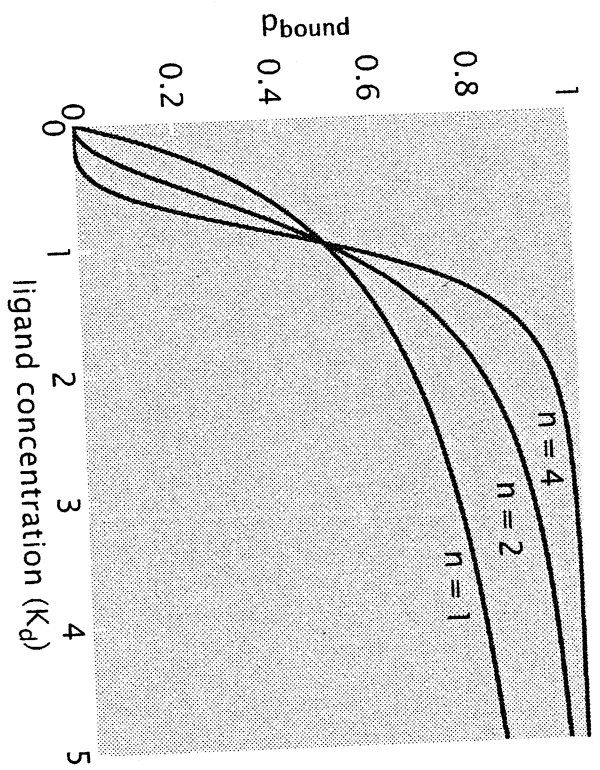


Figure 6.27: Family of binding curves with different Hill coefficients. The graph compares binding curves with different choices of Hill coefficient labeled by $n = 1, 2, 4$.