Stat Mech & Biology: Applications

- RNA Polymerase Binding to Promoters:
  - Previously we looked at 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.

\[ \text{RNA-P} \]

\[ \text{promoter} \quad \text{non-specific sites} \quad \text{promoter gene} \quad \text{DNA} \]

- RNA binding to DNA is competing between the specific sites located at 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 $P$ RNA molecules binding to $N_{ns}$ non-specific sites.

\[
Z_{\text{RNA}}(N_{\text{ns}}, N_{s}) = \frac{N_{ns}^! e^{-\beta P E_{ns}}}{P! (N_{\text{ns}} - P)!}
\]
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

\[
Z_S(P, N_{NS}) = Z_{NS}(P - 1, N_{NS}) e^{-\beta \varepsilon_S}
\]

so

\[
Z_{\text{tot}} = Z_{NS}(P, N_{NS}) + Z_S(P, N_{NS})
\]

The probability of the promoter being bound is,

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

In full,

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

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

Simplifying leads to

\[
P_{\text{bound}} = \frac{P e^{-\beta \varepsilon_S}}{1 + P e^{-\beta \varepsilon_S}} = \frac{1}{1 + \frac{N_{NS}}{P} e^\beta \varepsilon_S}
\]

where \( \Delta \varepsilon = \varepsilon_S - \varepsilon_{NS} \).
Figure 6.13: Probability of promoter occupancy as a function of the number of RNA polymerase molecules. $p_{\text{bound}}$ is computed using values for the specific and nonspecific binding obtained \textit{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 = lac → ΔE = 2.9 kcal 2) strong promoter = T7 → ΔE = -8.1 kcal
(N.B. used Nₜₜ = 5 × 10⁶ as 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 \( A + B \overset{k_+}{\rightleftharpoons} AB \)

At equilibrium, the law of mass action says

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

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,

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

where \( \nu_i \) = stoichiometric coefficients

\[\text{e.g.:} \quad 2A + B \rightleftharpoons C\]

\[
\frac{\nu_A}{\text{lose 2}} = -2 \quad \frac{\nu_B}{\text{lose 1}} = -1 \quad \frac{\nu_C}{\text{gain 1}} = 1
\]

so \( [A]^{-2} [B] [C]^{-1} = K_d^2 \)

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A look @ ligand Receptor Binding:

- Consider the problem of a ligand @ concentration \([L]\) binding to a receptor and gaining energy \(\Delta E\)

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

  where \([L_0]\) is a reference concentration (typically 1M)
Now let's look at the same problem from the point of view of equilibrium chemistry.

Consider the reaction \( L + R \rightleftharpoons LR \)

\[ 0 + [\text{U}] \rightleftharpoons [\text{U}] \]

@ 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 50% of 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.
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 $\Delta \varepsilon \approx -7.04 \ k_BT$ 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 $\Delta \varepsilon \approx -19.84 \ k_BT$ or $K_d = 1.4578 \ \text{nM}$ with $c_0 = 0.6 \ \text{M}$. (C) Binding of NtrC to DNA giving $\Delta \varepsilon = -17.47 \ k_BT$ 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.

![Diagram showing cooperative binding with multiple sites]

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

Let's consider: \( L + L + R \rightarrow 2 LR \)

Now \( \frac{[L]^2[R]}{[2LR]} = K_d \Rightarrow [2LR] = \frac{[L]^2[R]}{K_d} \)

Again \( P_{bound} = \frac{[\text{bound receptor}]}{[\text{total receptor}]} = \frac{[2LR]}{[R] + [2LR]} \)

\( P_{bound} = \frac{[L]^2/K_d^2}{1 + [L]^2/K_d^2} \)
and more generally, for higher cooperative binding we have

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

and $n = \text{Hill coefficient, measures cooperativity}$

$\text{higher cooperativity leads to a "switch"-like response}$

For hemoglobin, $n = 3.0$
Figure 6.27: Family of binding curves with different Hill coefficients. The graph compares binding curves with different choices of Hill coefficients. The graph shows the ligand concentration (K) on the x-axis and bound (Pbound) on the y-axis. The curves are labeled with different Hill coefficients: n = 1, 2, 4.