We will use statistical mechanics tools to attack a set of biological problems where the state of the system is described by two-state variables ($\sigma$)
Example 1: Voltage Gated Ion Channels
Example 2: Mechanosensitive Ion Channels

- Functions as a safety valve protect against membrane rupture due to osmotic pressure.
- Under osmotic shock, membrane tension ($\tau$) increases.
- Channels reply by opening.
- Ions flow outside the cell, osmotic pressure decreases.
• Assume a circular “loading device” applying tension on the channel.
• When the channel opens, its radius increases by $\Delta R$.
• The weights are lowered by $\Delta R_{out}$.
• Assuming the total area of the membrane is fixed.

\[
A_{\text{membrane}} = \pi \left( R_{out}^2 - R^2 \right) = \pi \left( (R_{out} + \Delta R_{out})^2 - (R + \Delta R)^2 \right)
\]

\[
2R_{out} \Delta R_{out} + \Delta R_{out}^2 = 2R \Delta R + \Delta R^2
\]

\[
\Delta R_{out} = \Delta R \frac{2R + \Delta R}{2R_{out} + \Delta R_{out}} \approx \Delta R \frac{R}{R_{out}}
\]

\[
\Delta G_{\text{tension}} = \tau \Delta s \times \left( -\frac{R}{R_{out}} \right) \times \frac{2\pi R_{out}}{\Delta s}
\]

\[
\Delta G_{\text{tension}} = -\tau 2\pi R \Delta R = -\tau \Delta A_{\text{channel}}
\]
• Free energy landscape of the ion channel.
• External parameter changes the energy landscape.
• Open state of the channel has lower energy than the closed state at high load.
• The channel opens at high load, and lets the ions flow outside the cell.
What is the open probability of the channel?

In the absence of external forces:

\[ E(\sigma) = \sigma \varepsilon_{open} + (1 - \sigma) \varepsilon_{closed} \]

In the presence of external forces:

\[ E(\sigma) = \sigma \varepsilon_{open} + (1 - \sigma) \varepsilon_{closed} - \sigma \tau \Delta A \]
\[ P(E) = \frac{1}{Z} e^{-\beta E(\sigma)} , \quad \text{and} \quad Z = \sum_{\sigma=0}^{1} e^{-\beta E(\sigma)} \]

\[ E(\sigma) = \sigma \varepsilon_{\text{open}} + (1 - \sigma) \varepsilon_{\text{closed}} - \sigma \tau \Delta A \]

\[ E(1) = \varepsilon_{\text{open}} - \tau \Delta A \quad \text{and} \quad E(0) = \varepsilon_{\text{closed}} \]

\[ Z = e^{-\beta \varepsilon_{\text{closed}}} + e^{-\beta (\varepsilon_{\text{open}} - \tau \Delta A)} \]

\[ p_{\text{open}} = \frac{e^{-\beta (\varepsilon_{\text{open}} - \tau \Delta A)}}{e^{-\beta \varepsilon_{\text{closed}}} + e^{-\beta (\varepsilon_{\text{open}} - \tau \Delta A)}} \]

\[ < \sigma > = \sum_{\sigma=0}^{1} \sigma p(\sigma) = p(1) = p_{\text{open}} \]

Ion channel opening probability as a function of tension.

\[ \varepsilon_{\text{open}} - \varepsilon_{\text{closed}} = 5 \ kT \]

\[ \Delta A = 10 \ \text{nm}^2 \]
Example 3: RNA Folding/Unfolding under Force

- Cell division and protein synthesis depends on cell’s ability to unfold RNA.
- Mechanical forces may be generated by biophysical techniques, e.g. optical trap.

a) Handles are made out of DNA to hold RNA between two polystyrene beads.

b) When RNA is pulled, the DNA handles act like a spring and extend under force.

c) At $f = 14.5$ pN, there is a discontinuity ($\Delta z = 20$ nm).

- The system hops between two states.
\( \sigma = 1 \) folded state
\( \sigma = 0 \) unfolded state

\[
E(1) = \varepsilon_{\text{folded}} \\
E(0) = \varepsilon_{\text{unfolded}} - f \Delta z
\]

\[
\Delta G = -\Delta W' = -f \Delta z
\]

\( p_{\text{folding}} \) depends on free energy \( \Delta \varepsilon = \varepsilon_{\text{unfolded}} - \varepsilon_{\text{folded}} \) and external force.

\[
E(\sigma) = \sigma \varepsilon_{\text{folded}} + (1 - \sigma) \varepsilon_{\text{unfolded}} - (1 - \sigma) f \Delta z
\]

\[
p_{\text{folding}} = \frac{1}{1 + e^{-\beta (\Delta \varepsilon - f \Delta z)}} \quad \text{and} \quad p_{\text{folding}} = \frac{1}{2} \text{ when } f = 14.5 \text{ pN}
\]

\[
p = \frac{1}{2} \Rightarrow e^{-\beta (\Delta \varepsilon - f \Delta z)} = 1 \Rightarrow \Delta \varepsilon = f \Delta z = 14.5 \text{ pN} \times 20 \text{ nm} = 290 \text{ pNnm} = 70 \text{ kT}
\]
Example 4: Phosphorylation of Proteins (Two Internal State Variables)

- Post-translational modification.
- Regulation of protein activity.
- Humans have more than 500 kinases.
- Kinases transfer the terminal phosphate from ATP to an amino acid side chain (e.g. serine or threonine) of a protein.
- Phosphatases use water to cleave the phosphate bond.
- Pi group carries two negative charges, leads to conformational change and alters association with binding partners.
- The state of phosphorylation determines the active state of the protein.
Phosphorylation alters relative free energies, shifting the equilibrium from inactive to active state.

\[ \sigma_s = 0 \text{ inactive} \quad \sigma_p = 0 \text{ dephosphorylated} \]
\[ \sigma_s = 1 \text{ active} \quad \sigma_p = 1 \text{ phosphorylated} \]

\[ \text{Phosphorylation alters relative free energies, shifting the equilibrium from inactive to active state.} \]
How Phosphorylation Alter Enzymatic Activity

\[ G = \left(1 - \sigma_p\right)\left((1 - \sigma_s) \cdot 0 + \sigma_s \varepsilon\right) + \sigma_p \left((1 - \sigma_s)(-I_2) + \sigma_s(\varepsilon - I_1)\right) \]

\[ G = \sigma_s \varepsilon - I_2 \sigma_p + (I_2 - I_1) \sigma_s \sigma_p \]

to find \( p_{\text{active}} \) if it is not phosphorylated \((\sigma_p = 0)\)

\[ p_{\text{active}} = \frac{e^{-\beta G(\sigma_s=1,\sigma_p=0)}}{e^{-\beta G(\sigma_s=0,\sigma_p=0)} + e^{-\beta G(\sigma_s=1,\sigma_p=0)}} = \frac{e^{-\beta \varepsilon}}{1 + e^{-\beta \varepsilon}} \]

to find \( p^*_{\text{active}} \) if it is phosphorylated \((\sigma_p = 1)\)

\[ p^*_{\text{active}} = \frac{e^{-\beta G(\sigma_s=1,\sigma_p=1)}}{e^{-\beta G(\sigma_s=0,\sigma_p=1)} + e^{-\beta G(\sigma_s=1,\sigma_p=1)}} = \frac{e^{-\beta(\varepsilon - I_1)}}{e^{-\beta(\varepsilon - I_1)} + e^{\beta I_2}} \]

\[ \frac{p^*_{\text{active}}}{p_{\text{active}}} = \frac{1 + e^{\beta \varepsilon}}{1 + e^{\beta (\varepsilon + I_2 - I_1)}} \]

If \( \varepsilon = 5kT \) and \( I_2 - I_1 = -10kT \), \( \frac{p^*_{\text{active}}}{p_{\text{active}}} = 150 \)
Hemoglobin as a Case Study in Cooperativity

- Hemoglobin is a tetramer of a 16 kDa protein.
- There are roughly $3 \times 10^8$ hemoglobin per red blood cell.
- Hemoglobin is responsible for transporting oxygen from lungs to cells.
- The heme group consists of a porphyrin ring that can bind an iron ion (red color).
- Iron can bind to $O_2$ or CO gas molecules.
Hemoglobin and Sickle Cell Anemia

- The structures of oxygen-bound and no oxygen-bound hemoglobin are different.
- Mutation of a glutamic acid residue to a valine in one of the $\beta$ chains leads to significant changes in protein function.
- Mutant form is more hydrophobic and hemoglobin molecules aggregate to form fibers.
- Banana-shaped red blood cells form, instead of disc-shaped native cells.
- Such cells are rigid (due to the hemoglobin fibers).
- The cells are trapped in capillaries and cutting off oxygen to some tissues.
What is the fractional occupancy of hemoglobin?

- (Left) This question is physiologically relevant, because hemoglobin tends to bind O\textsubscript{2} when it is abundant (lungs) and it has a higher propensity to drop bound oxygen in O\textsubscript{2} starved environment (muscle).

- (Right) **Bohr effect**: Hemoglobin’s binding affinity changes as a function of pH. The competition between H\textsuperscript{+} ion and O\textsubscript{2} gas makes Hemoglobin to release bound O\textsubscript{2} in an acidic environment (e.g. muscle). a (pH = 7.4), e (pH = 6.8).
Cooperativity

• **Cooperativity**: Binding of ligands on different sites on the same receptor is not independent.

• For hemoglobin, binding of one oxygen to any of the four binding sites increases the likelihood of binding of a second oxygen molecule and so on ([*allostery*]).

• In the **Hill Equation**, we assume that a solution of hemoglobin with limited amount of oxygen will contain a mixture of hemoglobin with no bound oxygen at all, and hemoglobin with four bound oxygen.

• The idea of cooperativity suggests that when more than one sites are bound to the ligand, the energy is not just the sum of the individual binding energies to the receptor.

\[
E(\sigma) = \varepsilon(\sigma_1 + \sigma_2) + J\sigma_1\sigma_2
\]

J is the cooperativity factor.
The Average Occupancy of Dimoglobin

\[ Z = 1 + e^{-\beta(\varepsilon-\mu)} + e^{-\beta(\varepsilon-\mu)} + e^{-\beta(2\varepsilon-2\mu+J)} \]

- no occupancy
- single occupancy
- double occupancy

\[ < N > = \frac{2e^{-\beta(\varepsilon-\mu)} + 2e^{-\beta(2\varepsilon-2\mu+J)}}{1 + 2e^{-\beta(\varepsilon-\mu)} + e^{-\beta(2\varepsilon-2\mu+J)}} \]
\[ \mu = \mu^0 + kT \ln \left( \frac{c}{c_0} \right) \text{ and } \Delta \varepsilon = \varepsilon - \mu^0 \]

\[ < N > = 2 \frac{\left( \frac{c}{c_0} \right) e^{-\beta \Delta \varepsilon} + \left( \frac{c}{c_0} \right)^2 e^{-\beta (2\Delta \varepsilon + J)}}{1 + 2 \left( \frac{c}{c_0} \right) e^{-\beta \Delta \varepsilon} + \left( \frac{c}{c_0} \right)^2 e^{-\beta (2\Delta \varepsilon + J)}} \]

\[ \Delta \varepsilon = -5kT, c_0 = 760 \text{ mmHg} \]

\[ J = 0 \text{ (no cooperativity)}, -2.5kT, \text{ and } -5kT \]
Statistical Models of the Occupancy of Hemoglobin

**noninteracting model**

weights 1
4e\(^{-\beta(e-\mu)}\)
6e\(^{-2\beta(e-\mu)}\)
4e\(^{-\beta(e-\mu)}\)
6e\(^{-3\beta(e-\mu)}\)
e\(^{-4\beta(e-\mu)}\)

**Pauling model**

weights 1
4e\(^{-\beta(e-\mu)}\)
6e\(^{-2\beta(e-\mu)}\) - \(\beta J\)
4e\(^{-3\beta(e-\mu)}\) - 3\(\beta J\)
e\(^{-4\beta(e-\mu)}\) - 6\(\beta J\)

**Adair model**

weights 1
4e\(^{-\beta(e-\mu)}\)
6e\(^{-2\beta(e-\mu)}\) - \(\beta J\)
4e\(^{-3\beta(e-\mu)}\) - 3\(\beta J\) - \(\beta K\)
e\(^{-4\beta(e-\mu)}\) - 6\(\beta J\) - 4\(\beta K\) - \(\beta L\)

*Figure 7.22, Physical Biology of the Cell, 2ed. (© Garland Science 2013)*
Noncooperative Model

\[ E(\sigma) = \varepsilon (\sigma_1 + \sigma_2 + \sigma_3 + \sigma_4) \]

\[ Z = \sum_{\sigma_1=0}^{1} e^{-\beta(\varepsilon-\mu)\sigma_1} \sum_{\sigma_2=0}^{1} e^{-\beta(\varepsilon-\mu)\sigma_2} \sum_{\sigma_3=0}^{1} e^{-\beta(\varepsilon-\mu)\sigma_3} \sum_{\sigma_4=0}^{1} e^{-\beta(\varepsilon-\mu)\sigma_4} \]

\[ Z = \left(1 + e^{-\beta(\varepsilon-\mu)}\right)^4 \]

\[ < N > = \frac{1}{\beta \delta \mu} \frac{\delta}{lnZ} = \frac{4e^{-\beta(\varepsilon-\mu)}}{1 + e^{-\beta(\varepsilon-\mu)}} = \frac{4 \left( \frac{c}{c_0} \right) e^{-\beta(\varepsilon-\mu^0)}}{1 + \left( \frac{c}{c_0} \right) e^{-\beta(\varepsilon-\mu^0)}} \]
Pauling Model

Assume that there is a pairwise interaction between sites

\[ \text{Assume pairwise interaction between sites} \]

\[ s = \sum_{\alpha=0}^{4} \sigma_\alpha = \sigma_1 + \sigma_2 + \sigma_3 + \sigma_4 \]

\[ Z = \sum_{s=0}^{4} \left( \binom{4}{s} e^{-\beta(s(\epsilon-\mu)+\binom{s}{2}J)} \right) = 1 + 4e^{-\beta(\epsilon-\mu)} + 6e^{-2\beta(\epsilon-\mu)-\beta J} + 4e^{-3\beta(\epsilon-\mu)-3\beta J} + e^{-4\beta(\epsilon-\mu)-6\beta J} \]

\[ < N > = \frac{4e^{-\beta(\epsilon-\mu)} + 12e^{-2\beta(\epsilon-\mu)-\beta J} + 12e^{-3\beta(\epsilon-\mu)-3\beta J} + 4e^{-4\beta(\epsilon-\mu)-6\beta J}}{1 + 4e^{-\beta(\epsilon-\mu)} + 6e^{-2\beta(\epsilon-\mu)-\beta J} + 4e^{-3\beta(\epsilon-\mu)-3\beta J} + e^{-4\beta(\epsilon-\mu)-6\beta J}} \]

if \( j = e^{-\beta J} \) and \( x = \left( \frac{c}{c_0} \right) e^{-\beta(\epsilon-\mu^0)} \)

\[ < N > = \frac{4x + 12x^2j + 12x^3j^3 + 4x^4j^6}{1 + 4x + 6x^2j + 4x^3j^3 + x^4j^6} \]
Adair Model

Assume that there are two-, three- and four-body interactions between sites

\[
Z = \sum_{s=0}^{4} \binom{4}{s} e^{-\beta (s(\varepsilon-\mu) + \binom{s}{2} J + \binom{s}{3} K + \binom{s}{4} L)}
\]

\[
Z = 1 + 4e^{-\beta (\varepsilon-\mu)} + 6e^{-2\beta (\varepsilon-\mu)-\beta J} + 4e^{-3\beta (\varepsilon-\mu)-3\beta J-\beta K} + e^{-4\beta (\varepsilon-\mu)-6\beta J-4\beta K-\beta L}
\]

if \( j = e^{-\beta J}, k = e^{-\beta K}, l = e^{-\beta L} \) and \( x = \left( \frac{c}{c_0} \right) e^{-\beta (\varepsilon-\mu^0)} \)

\[
< N > = \frac{4x + 12x^2 j + 12x^3 j^3 k + 4x^4 j^6 k^4 l}{1 + 4x + 6x^2 j + 4x^3 j^3 k + x^4 j^6 k^4 l}
\]

if \( j = k = l = 1 \), we recover the same result in the noncooperative model.
• **Cooperativity**: Binding of ligands on different sites on the same receptor is not independent.

• The average occupancy and partial filling probability of hemoglobin can be calculated from the partition function (Gibbs Distribution).

• In the **case of cooperativity**, partial filling of the hemoglobin molecule becomes negligible (major assumption of the Hill Equation).

• Without cooperativity, partial filling of hemoglobin becomes significant in limited oxygen concentration.