Lecture 5: Statistical Mechanics of Biomolecules

Dr Eileen Nugent
Reading List

1. Molecular Biology of the Cell, Alberts et al (Chapter 3 : Proteins) [general info about proteins and section on protein phosphorylation]

2. PBoC2, Phillips et al (Chapter 7) - [Macromolecules with multiple conformational states as multi-state systems - basic]


4. Biological Physics, Nelson (Chapter 9 Cooperative Transitions in Macromolecules)
Phosphorylation

Ligand-Gated Ion Channels

Allosteric Regulation, Cooperativity
The Mynod-Wyman-Changeux (MWC) Model

Theory and Experiments
Phosphorylation of Proteins

• The covalent addition of phosphate groups to proteins (phosphorylation) is one of the most important regulatory mechanisms in biological systems.
• The ability of biological systems to respond to environmental changes often depends on the being able to rapidly change the activity of a protein which functions as an enzyme in the system.
• Phosphorylation is a covalent post-translational modification which modifies the activity of a protein.
• The addition of a phosphate group changes the charge distribution of the protein which leads to structural changes. If the protein is an enzyme the ability of the enzyme to catalyse reactions (activity) will change on phosphorylation.
• We can apply statistical mechanics to calculate the change in activity of a protein on phosphorylation.
• The macro-states of the protein describe it’s structural state which dictates whether it is active or not.
Bio-molecules can exist in Multiple Conformal States

Phosphorylation: Kinase, phosphatase enzymes add/remove phosphate groups

- Proteins are constantly in motion transitioning between their active and inactive forms which are distinct thermodynamic macro states
- It is the probability of being in the active state that is altered by phosphorylation
\[ E(\sigma_s, \sigma_p) = (1-\sigma_p) [(1-\sigma_s)0 + \sigma_s \varepsilon] + \sigma_p [(1-\sigma_s)(-l_2) + \sigma_s (\varepsilon - l_1)] \]
Phosphorylation Modifies the Activity of the Protein

Energy of the protein

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

Active state probability (NOT phosphorylated)

\[ p_{active} = \frac{\exp(-\beta E(\sigma_s = 1, \sigma_p = 0))}{\sum_{\sigma_s = 0,1} \exp(-\beta E(\sigma_s, \sigma_p = 0))} = \frac{\exp(-\beta \varepsilon)}{\exp(-\beta \varepsilon) + 1} \]

Active state probability (phosphorylated)

\[ p_{active}^* = \frac{\exp(-\beta E(\sigma_s = 1, \sigma_p = 1))}{\sum_{\sigma_s = 0,1} \exp(-\beta E(\sigma_s, \sigma_p = 1))} = \frac{\exp(-\beta (\varepsilon - I_1))}{\exp(-\beta (\varepsilon - I_1)) + \exp(\beta I_2)} \]
Effects of Phosphorylation

Change in activity due to

\[
\frac{p^*_{\text{active}}}{p_{\text{active}}} = \frac{1 + \exp(\beta \epsilon)}{1 + \exp\left(\beta \left( \epsilon + l_2 - l_1 \right) \right)}
\]

Example:

\( \epsilon = 5 \ k_B T, \ l_2 - l_1 = -10 \ k_B T \)

\[
\frac{p^*_{\text{active}}}{p_{\text{active}}} = 150
\]

- Increase in activity can be by a factor of up to 1000
- Phosphorylation is fast, Reversible and costs far less costly in terms of energy than synthesising an enzyme from scratch and waiting for it to clear
- Allows implementation of control/circuits that has nothing to do with gene expression - protein circuits rather than genetic circuits.
Phosphorylation

Ligand-Gated Ion Channels

Allosteric Regulation, Cooperativity
The Mynod-Wyman-Changeux (MWC) Model

Theory and Experiments
Ligand-Gated Ion Channels

- Cell Membrane decorated with many different molecular species
- Ion channels (voltage, mechanical, ligand-gated)
- Opening/Closing of ligated-gated ion channel regulated by ligand binding
- Examples of ligand-gated channels:
  - (i) nACh receptors at neuromuscular junction
  - (ii) cGMP-gated ion channels in photoreceptors

Example:
Nicotinic acetylcholine Receptor
Possible States

<table>
<thead>
<tr>
<th>STATE</th>
<th>WEIGHT</th>
<th>STATE</th>
<th>WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>closed</td>
<td>$1$</td>
<td>open</td>
<td>$\ e^{-\beta \epsilon} \times e^{-\beta (\epsilon_{\text{open}} - \mu)}$</td>
</tr>
<tr>
<td></td>
<td>$e^{-\beta (\epsilon_{\text{closed}} - \mu)}$</td>
<td></td>
<td>$e^{-\beta \epsilon} \times e^{-\beta (\epsilon_{\text{open}} - \mu)}$</td>
</tr>
<tr>
<td></td>
<td>$e^{-\beta (\epsilon_{\text{closed}} - \mu)}$</td>
<td></td>
<td>$e^{-\beta \epsilon} \times e^{-\beta (2 \epsilon_{\text{open}} - 2 \mu)}$</td>
</tr>
<tr>
<td>closed</td>
<td>$w_{\text{closed}} = (1 + e^{-\beta (2 \epsilon_{\text{closed}} - \mu)})^2$</td>
<td>open</td>
<td>$w_{\text{open}} = e^{-\beta \epsilon} (1 + e^{-\beta (\epsilon_{\text{open}} - \mu)})^2$</td>
</tr>
</tbody>
</table>
Probability of being in the open state

Probability of the nACh receptor being in the open state

\[ p_{\text{open}} = \frac{e^{-\beta \varepsilon} \left( 1 + e^{-\beta (\varepsilon_{\text{open}} - \mu)} \right)^2}{e^{-\beta \varepsilon} \left( 1 + e^{-\beta (\varepsilon_{\text{open}} - \mu)} \right)^2 + \left( 1 + e^{-\beta (\varepsilon_{\text{closed}} - \mu)} \right)^2} \]

In terms of quantities we can measure (using \( e^{\beta \mu} = (c/c_0) e^{\beta \mu_0} \)):

\[ p_{\text{open}} = \frac{e^{-\beta \varepsilon} \left( 1 + \frac{c}{K_{\text{open}}} \right)^2}{e^{-\beta \varepsilon} \left( 1 + \frac{c}{K_{\text{open}}} \right)^2 + \left( 1 + \frac{c}{K_{\text{closed}}} \right)^2} \]

\[ K_{\text{open/closed}} = c_0 e^{-\beta (\varepsilon_{\text{open/closed}} - \mu_0)} \]
Mutants

tryptophan at position 149:
- Trp (wild type)
- 5,6,7-F3-Trp
- 5-F-Trp
- 4,5,6,7-F4-Trp
- 5,7-F2-Trp
Parameter Dependence

\[ p_{\text{open}} = \frac{e^{-\beta \varepsilon} \left( 1 + c K_{d}^{\text{closed}} \frac{K_{d}^{\text{closed}}}{K_{d}^{\text{open}}} \right)^2}{e^{-\beta \varepsilon} \left( 1 + c K_{d}^{\text{closed}} \frac{K_{d}^{\text{closed}}}{K_{d}^{\text{open}}} \right)^2 + \left( 1 + c \right)^2} \]
Phosphorylation

Ligand-Gated Ion Channels

Allosteric Regulation, Cooperativity
The Mynod-Wyman-Changeux (MWC) Model

Theory and Experiments
Cooperativity and Allostery

- Allostery: action at a distance effect of ligand binding on other binding sites of an allosteric receptor molecule

- Ligand binding causes a conformational change of the receptor changing the likelihood of further ligands binding

- Cooperativity: binding of ligands on different sites of the same molecule is not independent

- Binding energy for a ligand depends on number of other ligands already bound

- Classic Example: Haemoglobin

- All-or-nothing binding gives a step change in the activity of the receptor as a function of ligand concentration

- Central role in regulation of biological reactions
L-R binding and cooperativity: Hemoglobin

- Example of cooperative binding
- When oxygen binds to the iron in the α1 monomer at right, it induces a strain on the whole tetramer
- This strain causes a conformational change to the other monomers, allowing better access to the iron groups in them

- Hemoglobin is an oxygen and CO_2 carrier
- 97% of RBC dry content
Myoglobin versus Hemoglobin

- Myoglobin has one binding site
- Hemoglobin has four binding sites (Hill coefficient $N = 3.1$)
Hill Functions

- But the myoglobin curve underlines a problem: saturation occurs quickly.
- What happens if we have a receptor where multiple ligands can bind simultaneously? Sigmoidal function

\[ L + L + R \Leftrightarrow L_2R \Rightarrow K^2_d = \frac{[L]^2[R]}{[L_2R]} \]

\[ p_{\text{bound}} = \frac{[L_2R]}{[R] + [L_2R]} = \frac{([L]/K_d)^2}{1 + ([L]/K_d)^2} \]

General: \( p_{\text{bound}} = \frac{([L]/K_d)^n}{1 + ([L]/K_d)^n} \)
Dimoglobin

\[ \Delta \varepsilon = -5k_B T, \quad J = -2.5k_B T, \quad c_0 = 760 \text{ mmHg} \]

Figure 7.18 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Figure 7.17 Physical Biology of the Cell, 2ed. (© Garland Science 2013)
Two distinct conformational states: T ($\sigma_m = 0$) and R ($\sigma_m = 1$)

In the absence of ligand, T is favoured over R (energy cost = $\varepsilon$)

But ligand binding affinity has higher affinity for R

\[ E = (1 - \sigma_m) \varepsilon_T \sum_{i=1}^{2} \sigma_i + \sigma_m \left( \varepsilon + \varepsilon_R \sum_{i=1}^{2} \sigma_i \right); \]
\[ \Xi = 1 + 2 \exp[-\beta(\varepsilon_T - \mu)] + \exp[-\beta(2\varepsilon_T - 2\mu)] + \exp[-\beta(\varepsilon_R + \mu)] + \exp[-\beta(2\varepsilon_R - 2\mu)] \quad \text{T terms} \]
\[ + \exp[-\beta\varepsilon T] \left[ 1 + 2 \exp[-\beta(\varepsilon_R - \mu)] + \exp[-\beta(2\varepsilon_R - 2\mu)] \right] \quad \text{R terms} \]
Occupancy

Occupancy can be found from the grand partition fn:

\[
\langle N \rangle = k_B T \left( \frac{\partial}{\partial \mu} \right) \ln \Xi \\
= \frac{2}{\Xi} \left[ x + x^2 + \exp(-\beta \varepsilon)(y + y^2) \right] \\
x = (c / c_0) \exp[-\beta(\varepsilon_T - \mu_0)]; \ y = (c / c_0) \exp[-\beta(\varepsilon_R - \mu_0)]
\]
Models of Hemoglobin Binding

noninteracting model

weights

1

4e^{-\beta (e-\mu)}

6e^{-2\beta (e-\mu)}

4e^{-\beta (e-\mu)}

4e^{-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)
Effects of Cooperativity

Figure 7.23 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Figure 7.24 Physical Biology of the Cell, 2ed. (© Garland Science 2013)
Phosphorylation

Ligand-Gated Ion Channels

Allosteric Regulation, Cooperativity
The Mynod-Wyman-Changeux (MWC) Model

Theory and Experiments
Activity curve and key conceptual parameters

(a) Probability of the "active" state

\[ p_{\text{active}} = \frac{1 + \frac{n}{c_0} e^{-\beta (v^{(0)} - \mu_0)}}{1 + \frac{n}{c_0} e^{-\beta (v^{(0)} - \mu_0)}} \]

(b) Minimum and maximum probabilities of being in the active state

\[ p_{\text{active}}^\text{min} = \frac{n}{1 + \frac{n}{c_0} e^{-\beta (v^{(0)} - \mu_0)}} \]
\[ p_{\text{active}}^\text{max} = \frac{1}{1 + \frac{n}{c_0} e^{-\beta (v^{(0)} - \mu_0)}} \]

(c) Quantity

<table>
<thead>
<tr>
<th>Description</th>
<th>Formula (Stat. Mech.)</th>
<th>Formula (Thermo.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probability of the &quot;active&quot; state</td>
<td>( p_{\text{active}} = \frac{1 + \frac{n}{c_0} e^{-\beta (v^{(0)} - \mu_0)}}{1 + \frac{n}{c_0} e^{-\beta (v^{(0)} - \mu_0)}} )</td>
<td>( \frac{1 + \frac{n}{c_0} e^{-\beta (v^{(0)} - \mu_0)}}{1 + \frac{n}{c_0} e^{-\beta (v^{(0)} - \mu_0)}} )</td>
</tr>
<tr>
<td>Average number of bound ligands</td>
<td>( &lt;N_{\text{bound}}&gt; = \frac{1}{1 + \frac{n}{c_0} e^{-\beta (v^{(0)} - \mu_0)}} )</td>
<td>( \frac{1}{1 + \frac{n}{c_0} e^{-\beta (v^{(0)} - \mu_0)}} )</td>
</tr>
<tr>
<td>Minimum probability of being in the active state</td>
<td>( p_{\text{active}}^\text{min} = \frac{n}{1 + \frac{n}{c_0} e^{-\beta (v^{(0)} - \mu_0)}} )</td>
<td>( \frac{1}{1 + \frac{n}{c_0} e^{-\beta (v^{(0)} - \mu_0)}} )</td>
</tr>
<tr>
<td>Maximum probability of being in the active state</td>
<td>( p_{\text{active}}^\text{max} = \frac{1}{1 + \frac{n}{c_0} e^{-\beta (v^{(0)} - \mu_0)}} )</td>
<td>( \frac{1}{1 + \frac{n}{c_0} e^{-\beta (v^{(0)} - \mu_0)}} )</td>
</tr>
<tr>
<td>Ligand concentration at the transition point</td>
<td>( c^* = \frac{1 - \lambda}{\frac{1}{c_0 e^{-\beta (v^{(0)} - \mu_0)}} - \frac{1}{c_0 e^{-\beta (v^{(0)} - \mu_0)}}} )</td>
<td>( c^* = \frac{1 - \lambda}{\frac{1}{c_0 e^{-\beta (v^{(0)} - \mu_0)}} - \frac{1}{c_0 e^{-\beta (v^{(0)} - \mu_0)}}} )</td>
</tr>
<tr>
<td>Effective Hill coefficient</td>
<td>( h_{\text{eff}} = 2 \frac{\log \frac{p_{\text{active}}^\text{max} - p_{\text{active}}^\text{min}}{p_{\text{active}}^\text{min}}}{\log c} )</td>
<td>( c^* = \frac{1 - \lambda}{\frac{1}{c_0 e^{-\beta (v^{(0)} - \mu_0)}} - \frac{1}{c_0 e^{-\beta (v^{(0)} - \mu_0)}}} )</td>
</tr>
<tr>
<td>Static gain</td>
<td>( G(c) = \frac{\partial p_{\text{active}}}{\partial c} )</td>
<td>( \frac{n c^* (c_0 e^{-\beta (v^{(0)} - \mu_0)}) - c_0 e^{-\beta (v^{(0)} - \mu_0)}}{c^* + c_0 e^{-\beta (v^{(0)} - \mu_0)}} )</td>
</tr>
</tbody>
</table>
Measuring Binding Constants

(A) 

- affinity chromatography
- receptors
- immobilized ligands
- light source
- detector

(B) 

- equilibrium dialysis
- receptors
- ligands
dialysis tubing

⇒ (C) Surface Plasmon Resonance
⇒ (D) Isothermal titration calorimetry
Binding Constant Experiments

\[ P_{\text{bound}} = \frac{(c / c_0)e^{-\beta \Delta \varepsilon}}{1 + (c / c_0)e^{-\beta \Delta \varepsilon}} = \frac{[L] / K_d}{1 + [L] / K_d} \quad (1) \]

(a) Myoglobin + O₂
(b) HIV gp120 + sCD4
(c) NtrC + DNA

Arch. Bioche. Biophys. 1958
- \( \Delta \varepsilon \approx -7.04 \ k_B T \)
- \( c_0 = 760 \ \text{mmHg} \)
- \( K_d = 0.666 \ \text{mmHg} \)

PNAS 1991
- \( \Delta \varepsilon \approx -19.84 \ k_B T \)
- \( c_0 = 0.6 \ \text{M} \)
- \( K_d = 1.4578 \ \text{nM} \)

PNAS 1992
- \( \Delta \varepsilon \approx -17.47 \ k_B T \)
- \( c_0 = 0.6 \ \text{M} \)
- \( K_d = 15.5 \ \text{nM} \)