Selected Applications of Chemical Equilibrium to Biochemistry: Ligand Binding, Bioenergetics
Chang, Sections 6.4-6.5

Biological Standard State

Discussed on whiteboard

Binding of ligands and metal ions to macromolecules:
A. One binding site per macromolecule

Myoglobin: 1 binding site for O₂
Binding equilibrium

Association
\[ P + L \rightarrow PL \quad K_a = \frac{[PL]}{[P][L]} \]

Dissociation
\[ PL \rightarrow P + L \quad K_d = \frac{[P][L]}{[PL]} \]

Dissociation Constant

Determining \( K_d \)

Numerator: concentration of L bound to P
Denominator: concentration of all forms of P

Define: \[ Y = \frac{[PL]}{[P] + [PL]} \]
\[ \frac{Y}{[L]} = \frac{1}{K_d} - \frac{Y}{K_d} \]

Obtain \( K_d \) by plotting \( Y/[L] \) vs \( Y \):
\[ \text{slope} = -\frac{1}{K_d} \]

B. \( n \) equivalent binding sites

Hemoglobin: 4 binding sites for \( O_2 \)
Caveat: Hemoglobin’s bind sites not really equivalent
2 equivalent sites

\[ P \xrightarrow{K_1} PL \xrightarrow{K_2} PL_2 \]

\[ P + L \rightarrow PL \quad K_1 = \frac{[PL]}{[PL]} \]

\[ PL + L \rightarrow PL_2 \quad K_2 = \frac{[PL][L]}{[PL_2]} \]

Express \( K_1 \) in terms of \( K \)

\( K \) = dissociation constant from any one site

\( K = 2K_1 \)

\[ Y \text{ in terms of } [L] \text{ and } K \]

\[ Y = \frac{[PL] + 2[PL_2]}{[P] + [PL] + [PL_2]} = \ldots = \frac{2[L]}{[L] + K} \]
Generalize to $n$ equivalent sites

$$K_i = \left(\frac{i}{n-i+1}\right)K$$

$$Y = \frac{n[L]}{[L] + K}$$

Determining $n$ and $K$: Plotting method 1 (Direct plot)

$$Y = \frac{n[L]}{[L] + K}$$

Plotting method 2 (double reciprocal plot)

$$\frac{1}{Y} = \frac{1}{n} + \frac{K}{n[L]}$$
Plotting method 3 (Scatchard plot)

\[ \frac{Y}{[L]} = \frac{n}{K} \left( \frac{Y}{K} \right) \]

$n$ non-equivalent sites

Possible but harder → for the expert
We won’t cover

Equilibrium dialysis (to remove bound ligand)

Semi-permeable membrane
Can remove all ligand with repeated ‘rinsings’
**Equilibrium dialysis**
(to determine $n$ and $K$)

At equilibrium:
Interior $[L] =$ Exterior $[L]$ (known or measurable)
Total P concentration (all forms) known
⇒ Can determine $Y$, $n$, $K$ as before

**Isothermal titration calorimetry (ITC)**

Make repeated injections:
Measure power needed to keep sample at same temperature as buffer

$q = V \Delta H_{\text{bound}}$

$P = nL \Rightarrow P_L$

**ITC output**

Integrate peak above to get point below

$\Delta_r G = \Delta_r H - \Delta_r S$

Get $\Delta_r^0$

$\Delta_r^0 = \Delta H^0 - RT \ln K_a$

Curve fitting

Get $\Delta_r G$

$\Delta_r G = \Delta H^0 - RT \ln K_a$
Bioenergetics

OVERVIEW
• Reactions must be exergonic ($\Delta_r G < 0$) to be spontaneous.
• Building a protein (and many other biomolecules) is highly endergonic.
• Must be coupled with other reactions that are highly exergonic, to give a net reaction that is also exergonic.
• Chemical reactions in biological systems are made efficient (rates are increased) by catalysis.

Catalysis

- Reaction barrier ($\Delta G^\ddagger$) reduced $\rightarrow$ Rate increased
- Gibbs energy of reaction ($\Delta_r G$) not affected $\rightarrow$ Equilibrium concentrations unchanged

Coupled reactions

1. Outline effect of stoichiometry and summer reactions on board.

2. Question: How make the following reaction – extraction of copper from ore - “go”?

$$\text{Cu}_2\text{S}(s) \rightarrow 2\text{Cu}(s) + \text{S}(s) \quad \Delta_r G^\ddagger = 86.2 \text{ kJ/mol}$$
Couple reactions (cont’d)

Answer: Couple to an exergonic reaction.

\[
\begin{align*}
\text{Cu}_2S(s) & \rightarrow 2\text{Cu}(s) + S(s) \quad \Delta G^\circ = 86.2 \text{ kJ/mol} \\
S(s) + \text{O}_2(g) & \rightarrow \text{SO}_2(g) \quad \Delta G^\circ = -300.1 \ldots
\end{align*}
\]

\[
\text{Cu}_2S(s) + \text{O}_2(g) \rightarrow \text{SO}_2(g) \quad \Delta G^\circ = -213.9 \ldots
\]

ATP, ADP (adenosine triphosphate)

\[
\begin{align*}
\text{ATP} & \rightarrow \text{ADP} \\
\text{ATP}^4 + \text{H}_2\text{O} & \rightarrow \text{ADP}^3 + \text{H}^+ + \text{HPO}_4^{2-} \\
\text{or (biochemist’s shorthand)} & \\
\text{ATP} + \text{H}_2\text{O} & \rightarrow \text{ADP} + \text{P}_i \\
\Delta G^\text{m} & = -30.5 \text{ kJ/mol (pH=7, T=310 K)}
\end{align*}
\]

Exergonic \rightarrow Can be coupled with endergonic reactions to drive them
Glycolytic pathway

An aerobic pathway is less exergonic compared to the anaerobic pathway. The net production of ATP in the anaerobic pathway is 2 moles, while in the aerobic pathway, it is 38 moles. The aerobic pathway adds more steps, leading to complete combustion products of glucose (CO$_2$, H$_2$O).

Net production of 2 moles ATP

Aerobic pathway

Combustion of glucose:

\[ \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} \]

\[ \Delta G^0 = -2879 \text{ kJ/mol} \]

\[ \text{ADP} + \text{H}^+ + \text{HPO}_4^{2-} \rightarrow \text{ATP} + \text{H}_2\text{O} \]

\[ \Delta G^0 = 30.5 \text{ kJ/mol} \]

Efficiency = \( \frac{38 \times 30.5}{2879} \) = 40%

Energetics of building proteins

Gibbs energy

ATP → Protein

ADP → Amino acids