Protein Function: Reversible Ligand Binding

- Proteins often have other structures, known as ligands, which bind to the protein and help the protein to carry out its function. For example, the lac repressor protein binds to DNA, and so does the HIV Tat protein.

How is single-site (non-cooperative) protein-ligand binding described?

$$P + L \rightleftharpoons P \cdot L$$

"Protein" "Ligand" "Protein-Ligand Complex"

$$K_{eq} = \frac{[P \cdot L]}{[P][L]} \qquad K_d = \frac{1}{K_{eq}} = \frac{[P][L]}{[P \cdot L]}$$

Biochemists use K_d, the dissociation constant

Note that the free energy equations for K_d and K_{eq} are different!

→ $\Delta G^{o\prime} = -RT\ln(K_{eq})$

→ $\Delta G^{o\prime} = RT\ln(K_d)$

Binding Affinity:

$$P + L \rightleftharpoons P \cdot L$$

How tightly does a ligand bind to a protein?

Recall the mathematical relationship:

$$K_d = \frac{1}{K_{eq}} = \frac{[P][L]}{[P \cdot L]} \quad \text{and} \quad \Delta G^{o\prime} = RT\ln(K_d)$$

A high affinity (strong binding) → means small K_d value ($K_d << 1$) and a large, very negative $\Delta G^{o\prime}$ value.

A low affinity (weak binding) → means large K_d value ($K_d >> 1$) and a large, very positive $\Delta G^{o\prime}$ value.

Affinity scale:

Low: $K_d = 0.001$, $\Delta G^{o\prime} = -17$ kJ/mol

Moderate: $K_d = 1 \times 10^{-6}$, $\Delta G^{o\prime} = -34$ kJ/mol

High: $K_d = 1 \times 10^{-9}$, $\Delta G^{o\prime} = -51$ kJ/mol

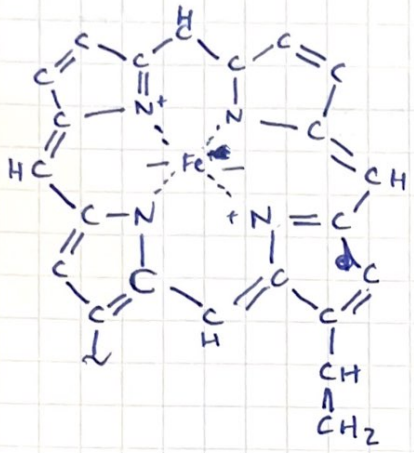
B. Single-Site Protein-Ligand Binding

Some terminology:

Heme - A "prosthetic" group for myoglobin and hemoglobin. Enables the protein to perform its function.

Apo-protein - a protein lacking its prosthetic group. For example, a myoglobin without its prosthetic heme group is known as apo-myoglobin.

Hemes:



The structure to the left is one known as a porphyrin ring. This is a conjugated double bond system, and this is why the color of blood is red.

The shorthand of this structure is drawn as: 

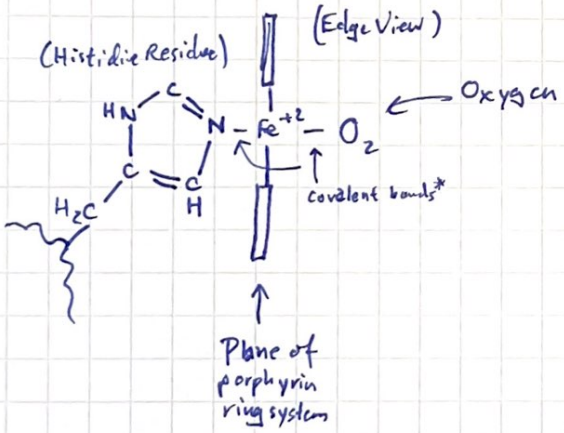
Iron has 6 bonds total, so 2 are available for interaction.

Note that this structure is planar.

Apomyoglobin + heme $\rightleftharpoons$ Myoglobin·heme + O_2 $\rightleftharpoons$ Myoglobin·heme·O_2

(+ heme; + O_2)

Ligands bound to Fe^{+2} in Oxy-Mb and Oxy-Hb



* Myoglobin is a strange case of ligand binding which actually involves a covalent bond. Usually, ligands bind via ionic bonds, or other non-covalent bonds.

Specificity of Protein Ligand Binding

→ Proteins bind tightly (with high affinity) to the biologically-relevant ligands.

→ Specificity is achieved by:

- Ligand binding in a specific binding site in the protein
- Chemical and shape complementarity in shape between the protein site and ligand.

Determining K_d from equations and graphs.

Given:

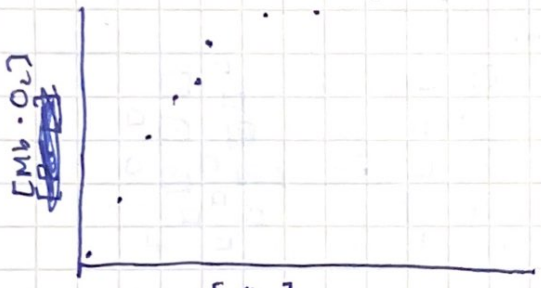
$$Mb + O_2 \rightleftharpoons Mb \cdot O_2$$

What is the affinity of myoglobin for O_2? How do we measure binding affinity?

Experiment:

1) Mix Mb and O_2 → for $[Mb]_{total}$ and $[O_2]_{total}$, $[Mb]_{total} << [O_2]_{total}$
2) Measure $[Mb \cdot O_2]$ as a result of the mixture.
   - The absorbance of Mb and $Mb \cdot O_2$ have different λ_{max} values, so absorbance spectroscopy may be used to determine concentrations.
3) Repeat steps 1 & 2 with different $[O_2]$ concentrations, plotting each data point on a graph with $[Mb \cdot O_2]$ as the y axis and $[O_2]$ as the x.

Typically the graph looks like this:



→ The graph may also be plotted in terms of Y, where $Y = \frac{[P \cdot L]}{[P]_{total}}$. The shape of the graph, however, is mostly the same.

→ Note that the graph data is hyperbolic. What are the maximum y-values (asymptotes) of these graphs?

As $[L]_t \to \infty$, $[P \cdot L] \to$?

- In the graph of $[P \cdot L]$ vs $[L]_{total}$, the asymptote is the original $[P]_{total}$ in the mixture.
- In the graph of Y vs $[L]_{total}$, where $Y = \frac{[P \cdot L]}{[P]_{total}}$, the asymptote is 1. Therefore $0 \leq Y \leq 1$

Fractional saturation:

$$Y = \frac{[P \cdot L]}{[P]_T}, \quad 0 \leq Y \leq 1$$

Answers the question: of all ligand binding sites present in solution, what fraction of those sites are actually occupied with ligand?

If Y is ≈ 1, the ligand concentration is high.
If Y is ≈ 0, the ligand concentration is low.

Important derivations:

$$[P \cdot L] = \frac{[P]_t [L]_t}{K_d + [L]_t} \quad \text{and} \quad Y = \frac{[L]_t}{K_d + [L]_t}$$

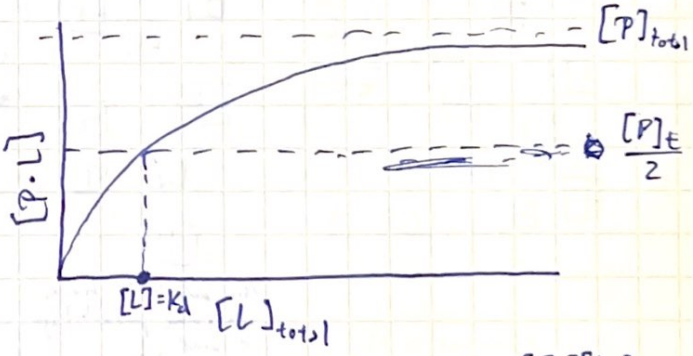
Note: Units of K_d

- Depends on other units in equation: For example:

$$[P \cdot L] = \frac{[P][L]}{K_d + [L]} \quad \text{(M, M, M)}, \quad \text{or} \quad [P \cdot L] = \frac{[P][L]}{K_d + [L]} \quad (\mu M, \mu M, \mu M)$$

K_d tells us what is a low or high concentration of L for P.
If $[L] > K_d$ is high [L], and Y is close to 1; if $[L] < K_d$, is low [L], and Y is low.

The K_d value may be estimated by using the assumption that $[L] = K_d$:



If $[L] = K_d$, then in $[P \cdot L] = \frac{[P_t][L_t]}{K_d + [L_t]}$,

$$[P \cdot L] = \frac{[P_t][L_t]}{2[L_t]} = \frac{P_t}{2}$$

Therefore the halfway point of this asymptote is where the $[L]_t = K_d$.