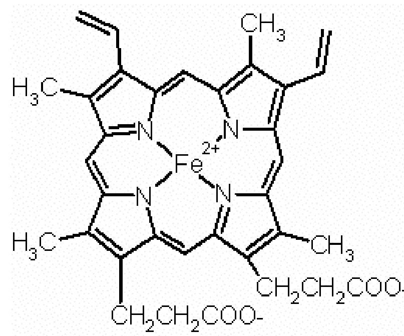
Lecture 12: O_2 Binding by Myoglobin & Hemoglobin

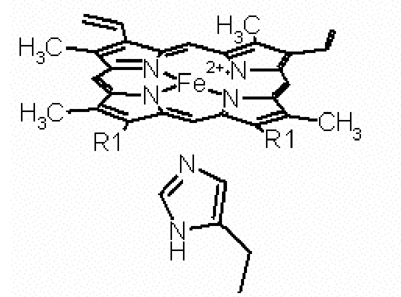
Assigned reading in Campbell: Chapter 4.5



Key Terms:

- **Prosthetic Group: heme**
- **Tertiary structure of myoglobin.**
- **Quaternary Structure of hemoglobin**
- **Role of myoglobin and hemoglobin in O_2 transport**
- **O_2 (ligand) binding curves of myoglobin and hemoglobin**

General features of oxygen transport:
Oxygen is absolutely required for life in most organisms. All tissues need oxygen. Oxygen is usually taken up in the lungs by the protein **hemoglobin** and carried throughout the body in the circulatory system. In some cases, there is a need to store large quantities of oxygen in the tissue itself. In this case a specialized oxygen storage protein, **myoglobin**, is used to store the oxygen and to facilitate its diffusion within cells.



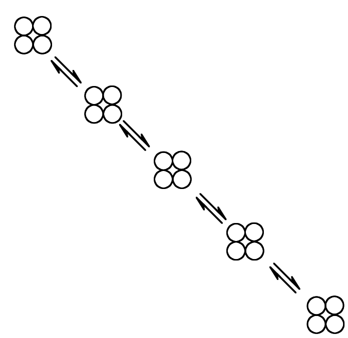
Structural Features of Myoglobin and Hemoglobin

Properties of heme group

- Example of a **prosthetic group** in proteins. A prosthetic group is usually an organic compound or a metal ion what is tightly bound to the protein and plays an essential role in the function of that protein.
- Heterocylic ring containing 4 pyrrole rings
- Central atom is Fe^{2+} (usual oxidation state) in Myo and Hemoglobin
- Proximal histidine is important in transducing the binding event to protein.
- O_2 binding induces a change in the electronic state of Fe^{2+} that changes its absorbance spectrum. This change can be used to monitor oxygen binding in diagnostic instruments, called pulse oximeters.

Myoglobin (Mb)

- Monomeric (tertiary structure)
- Contains a single heme group with a bound Fe^{2+}
- Binds 1 oxygen molecule per molecule of protein..
- Carries O_2 from capillaries to sites of usage in cells. (i.e. mitrochondria)
- <u>Non-cooperative</u> binding of O_2.



Hemoglogin (Hb)

- Tetrametric, two alpha chains and two beta chains (Quaternary Structure)
- Each chain is structurally similar to myoglobin
- Each chain contains a bound heme-Fe^{2+}
- Binds a total of 4 oxygen molecules to its four heme groups.
- Carries O_2 from lungs to tissues, increasing the solubility of O_2 in blood
- <u>Positive cooperativity in binding of O_2</u>; the binding affinity increases as more O_2 are bound.

Oxygen Binding and Delivery:
The efficient delivery of oxygen to the tissues presents a difficult problem. How can a protein that will bind oxygen well in the lungs also *efficiently* release that oxygen in the tissues where it can be bound by myoglobin! A comparison of the oxygen binding curves of myoglobin and hemoglobin shows how this works.

The actual binding equilibrium, using myoglobin (M) as an example is:

$$M + O_2 \rightleftharpoons MO_2$$

The ligand concentration is given as pO_2, or the partial pressure of oxygen. The units are torr. The fractional saturation is given as the following for the case of myoglobin (single oxygen bound):

$$Y = \frac{pO_2}{K_D + pO_2} = \frac{[L]}{K_D + [L]}$$

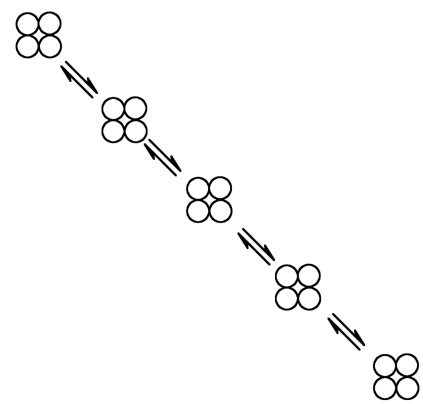
For oxygen binding proteins the K_D is also referred to as the "p_{50}", the amount of oxygen required to give a fractional saturation of Y=0.5. In the case of myoglobin, the K_D is 2-3 torr.

This rather strange binding behavior for the hemoglobin binding curve is due entirely to the fact that it can bind oxygen in a cooperative fashion.

- The affinity increases as more oxygen is bound, favoring loading of O_2 in the lungs.
- The affinity decreases as less oxygen is bound, favoring release of O_2 in the tissues.

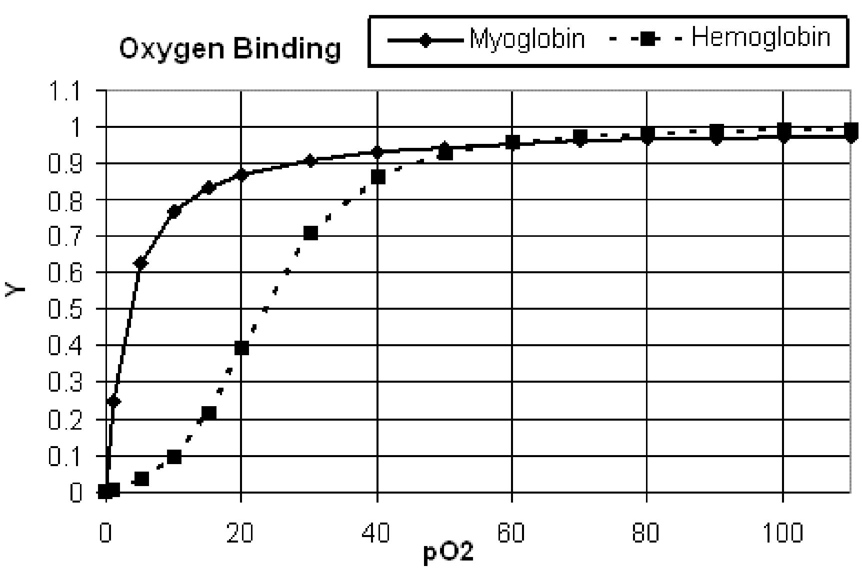
In the case of hemoglobin the binding is to multiple sites and the simple formula for Y does not apply. Instead, a new term is defined, the *partial* saturation, ν. This is the total *amount* of ligand bound/per macromolecule. Consequently it ranges from 0 ([L]=0) to n, when [L] is very high (n is the number of binding sites). In the case of oxygen binding to hemoglobin:

$$v = \frac{[ML] + \mathbf{2}[ML_2] + \mathbf{3}[ML_3] + \mathbf{4}[ML_4]}{[M] + [ML] + [ML_2] + [ML_3] + [ML_4]} = \mathbf{4}Y$$



The fractional saturation, Y, is obtained from ν by dividing by the number of binding sites. Therefore, the fractional saturation goes from 0 to 1, as before.

The K_D values for *each* individual binding oxygen binding step are listed to the right. Does the affinity of hemoglobin increase as more oxygen is added?



Individual K_D values for O_2 binding to Hemoglobin

K_{D1}	180 torr
K_{D2}	140 torr
K_{D3}	100 torr
K_{D4}	0.1 torr