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.
- Heterocyclic 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. mitochondria)
- Non-cooperative binding of O$_2$.

Hemoglobin (Hb)
- Tetrameric, 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
- Positive cooperativity in binding of O$_2$: 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, $\mathbb{Y}$. 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:

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

The fractional saturation, $Y$, is obtained from $\mathbb{Y}$ 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?