
- Use spectrophotometric or equilibrium dialysis to obtain amount of ligand bound. Calculate fractional saturation from raw data.
- Understand the molecular basis of oxygen binding by myoglobin and hemoglobin
- Understand the relationship between oxygen binding curve and transport.

Fractional Saturation:
\[ Y = \frac{[ML]}{[M] + [ML]} \rightarrow Y = \frac{[L]}{K_D + [L]} \]

Measurement of \( Y \) to obtain \( K_D \):

A. Equilibrium Dialysis:

- A dialysis membrane will allow small ligands to pass through, but will retain proteins (M) as well as protein-ligand complexes (ML).
- A series of different experiments are performed, with the ligand concentration varied. The fractional saturation is obtained for each ligand concentration.
- Experimentally, the ligand concentration inside ([L]_in) and outside ([L]_out) the dialysis membrane is measured. Given that the total amount of macromolecule, \([M]_T\), is also known, it is possible to calculate \( Y \) from these two measurements.

\[ [ML] = [L]_{in} - [L]_{out} \]
\[ [L]_{free} = [L]_{out} \]

The following figures show how the equilibrium dialysis experiment can be used to determine the concentrations of M, L, and ML at binding equilibrium.

Model reaction: \([M] + [L] \leftrightarrow [ML]\)

Initially (A, left panel), the protein (M) is present only in the left cell of the dialysis chamber. The small molecule (L) is present only in the right cell. The semi-permeable membrane only allows ligand to pass; M (and ML) is too large. At equilibrium (B, right panel) the free ligand has the same concentration on both sides of the membrane.

\[ Y = \frac{[ML]}{[M] + [ML]} \]
\[ [L]_{free} = \frac{[ML]}{[M] + [ML]} \]

\[ Y = \frac{7}{5} = \frac{2}{4} \]
\[ Y = \frac{7 - 5}{5} = \frac{2}{5} \]
\[ K_D = 5 \]
B. Measuring fractional Saturation by Spectrophotometry

If the absorption spectra of the free macromolecule (M) is different from the macromolecule-ligand complex (ML), then the absorbance of the solution can be used to measure the fractional saturation.

\[
Y = \frac{A - A_M}{A_{ML} - A_M} = \frac{0.4 - 0.2}{0.8 - 0.2} = \frac{0.2}{0.6} = \frac{1}{3} = 0.333...
\]

**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 (Hb)** and carried throughout the body in the circulatory system. **Myoglobin (myo),** 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 Hb

**Myoglobin (Mb)**
- Monomeric (tertiary structure)
- Contains a single heme group with a bound Fe$^{2+}$
- Binds 1 oxygen molecule per molecule of protein.
- Stores/carryes O$_2$ from cell membrane to sites of usage in cells. (i.e. mitochondria).

**Hemoglobin (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.
- **Proximal histidine** is important in transducing the binding event to other protein subunits in hemoglobin, leading to cooperative binding.
**Oxygen Binding:** The binding equilibrium, using myoglobin (Myo) as an example is:

\[
(Y) + O_2 \leftrightarrow (Y-O_2)
\]

The ligand concentration is often given as \( pO_2 \), or the partial pressure of oxygen. The units are in kPa or in Torr. The fractional saturation is given as shown on the right for the case of myoglobin (single oxygen bound). For oxygen binding proteins the \( K_0 \) is also referred to as the “\( pO_0 \)”, the amount of oxygen required to give a fractional saturation of \( Y=0.5 \). In the case of myoglobin, the \( K_0 \) is about 0.25 kPa.

The degree of oxygen binding can be easily measured using UV absorption because the absorption spectra of the heme changes when oxygen bonds (see graph on right).

**Example:** A sample was made with an oxygen concentration of 4 kPa. What is the fractional saturation of hemoglobin at this oxygen concentration (use the absorption values at 764 nm)?

\[
\frac{A_y - A_M}{A_M - A_M} = \frac{0.22 - 0.5}{0.1 - 0.5} = \frac{-0.28}{-0.4} = 0.7
\]

**Oxygen Delivery – The cooperative binding of Oxygen to Hemoglobin:**

The efficient delivery of oxygen to the tissues presents a difficult problem. How do you design a protein that will bind oxygen well in the lungs and then efficiently release that oxygen in the tissues where it can be bound by myoglobin.

A comparison of the oxygen binding curves of myoglobin (myo) and hemoglobin (hb) shows how this works:

<table>
<thead>
<tr>
<th>Calculating Oxygen Delivery.</th>
<th>Hemoglobin</th>
<th>Myoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount delivered = ( \Delta Y )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In the lungs ([O_2] \sim 12 \text{ kPa})</td>
<td>( Y = 0.98 )</td>
<td>( Y = 0.96 )</td>
</tr>
<tr>
<td>At the tissues ([O_2] \sim 3.5 \text{ kPa})</td>
<td>( Y = 0.60 )</td>
<td>( Y = 0.90 )</td>
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</tbody>
</table>