# Lecture 13: Allosteric Effects and Cooperative Binding

Assigned reading in Campbell: Chapter 4.7, 7.2

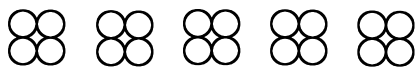
*Key Terms:*

- **Homotropic Allosteric effects**
- **Heterotropic Allosteric effects**
- **T and R states of cooperative systems**
- **Role of proximal His residue in cooperativity of $O_2$ binding by Hb**
- **Regulation of $O_2$ binding to Hb by bis-phosphoglycerate (BPG)**

**Oxygen Binding to Myoglobin and Hemolobin:**

Myoglobin binds one $O_2$:

Hemoglobin binds four $O_2$:



**Allosteric Effects and Cooperativity:**

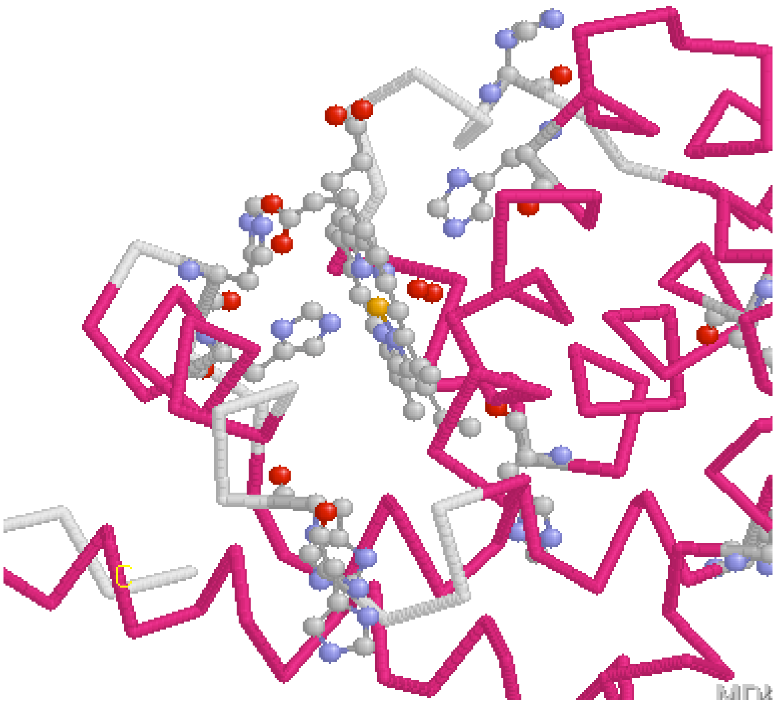
Allosteric effects occur when the binding properties of a macromolecule change as a consequence of a second ligand binding to the macromolecule and altering its affinity towards the first, or primary, ligand. There need not be a direct connection between the two ligands (i.e. they may bind to opposite sides of the protein, or even to different subunits)

- If the two ligands are the same (*e.g.* oxygen) then this is called a **homo-tropic allosteric** effect.
- If the two ligands are different (*e.g.* oxygen and BPG), then this is called a **hetero-tropic allosteric** effect.

In the case of macromolecules that have multiple ligand binding sites (*e.g.* Hb), allosteric effects can generate cooperative behavior. Allosteric effects are important in the regulation of enzymatic reactions. Both allosteric activators (which enhance activity) and allosteric inhibitors (which reduce activity) are utilized to control enzyme reactions.

Allosteric effects require the presence of two forms of the macromolecule. One form, usually called the **T or tense state, binds the primary ligand (*e.g.* oxygen) with low affinity.** The other form, usually called the **R or relaxed state, binds ligand with high affinity**. The T and R states are in equilibrium with each other.

- In the case of **positive cooperativity** the fraction of T states exceeds that of the R state and the binding of ligand increases the amount of R state, thus increases the ease of ligand binding.
- In the case of **negative cooperativity**, the fraction of the T state is smaller than that of the R state. Thus, the initial binding affinity is high. However, the binding of ligand increases the amount of T state, thus reducing the binding affinity.



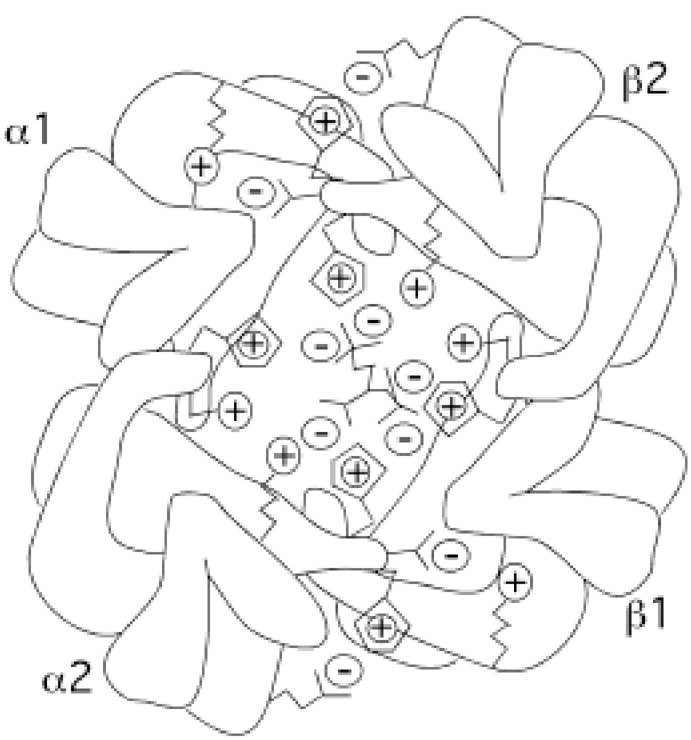
**Mechanism of Positive Cooperativity in Hemoglobin:**

1. Binding of $O_2$ to $Fe^{+2}$ moves $Fe^{+2}$ atom into the plane of the porphyrin ring.

2. Movement of $Fe^{+2}$ pulls the proximal His residue and its attached helix (F) towards the ring.

3. Helix F adjusts conformation by rotation of $\alpha_1\beta_1$ dimer relative to $\alpha_2\beta_2$ dimer.

4. Movement of αβ dimers alters conformation of $Fe^{+2}$ at unliganded sites through the breaking of an extensive network of salt bridges at subunit interfaces.

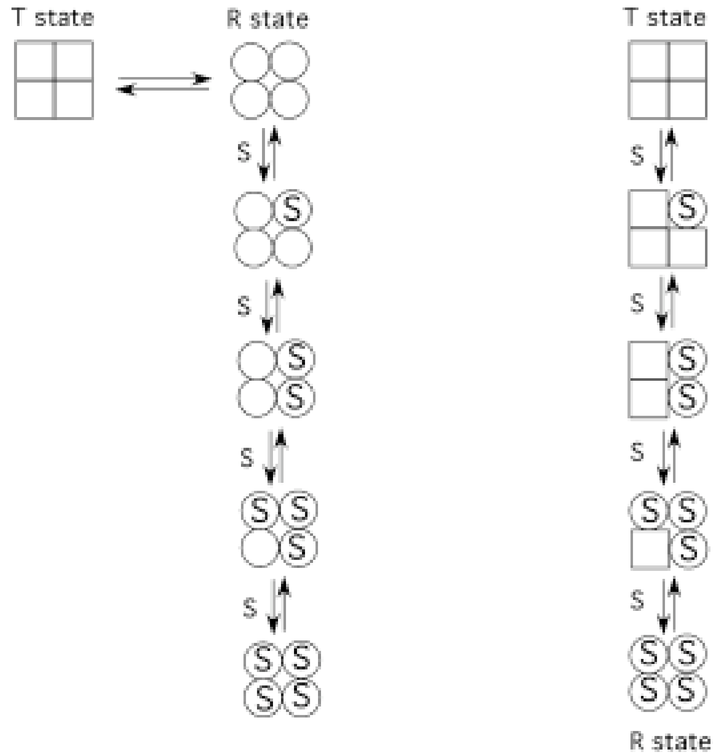


**Models of Cooperativity in Hemoglobin:**
Change from T to R states may occur:

- In unison , or in a concerted manner (MWC model)
- Sequentially (Koshland model)

Either of these models fit the experimental data well but neither of these models is likely to be completely accurate in describing the T to R transition of subunits in hemoglobin.

Recent results suggest that the first part of the Hb oxygenation reaction occurs in unison, and the remainder occurs sequentially.




**Hetero-tropic Allosteric Effectors in Hemoglobin.**

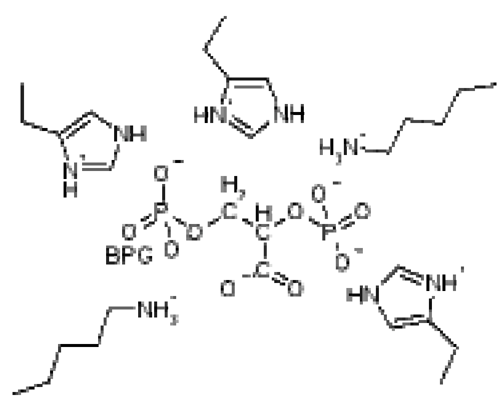
**There are many hetero-tropic allosteric effectors in Hemoglobin; two examples are:**

1. **Protons:** oxygen affinity is decreased at low pH, such as in active muscle that is producing lactic acid. This provides an immediate response to the metabolic state of the tissue.
2. **BPG:** bis-phosphoglycerate binds to the deoxy form of hemoglobin. Therefore it reduces oxygen affinity. This is an adaptive response, requiring several days at high altitude. The production of excess BPG, although it reduces the oxygen affinity, it makes the protein more efficient at delivering oxygen to the tissues.

In the above examples, the tense state of hemoglobin becomes more prevalent than the relaxed state. The allosteric effector stabilizes the tense state, or lowers its energy relative to that of the relaxed state. Consequently, the oxygen affinity is reduced and the binding curve is shifted to the right, which enhances oxygen release.

The molecular nature of the action of BPG is quite clear:

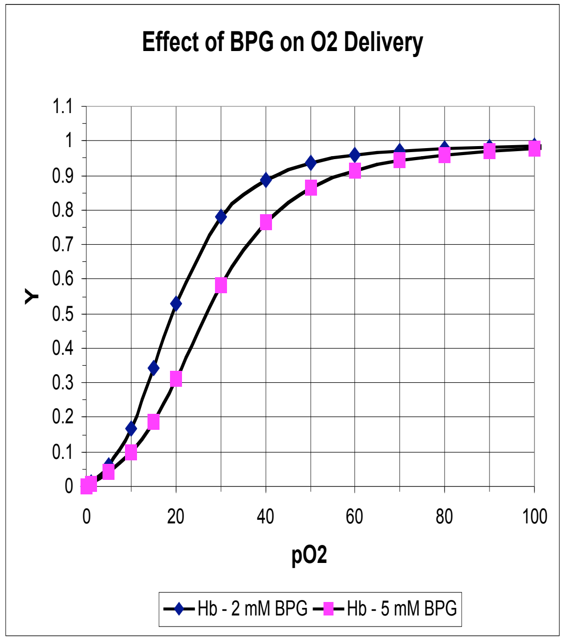
- In deoxy hemoglobin, a positively charged binding pocket exists between two of the four subunits (see Figure 4.26 in Campbell or the Chime page). Thus BPG can easily bind, and when it does so, it stabilizes the deoxy, or tense, form of the protein.




- In oxy-hemoglobin, the relative movement of the chains that occurs during the allosteric transition to the R state closes this pocket, so BPG can no longer fit.

**Effect of BPG on $O_2$ Delivery**

This graph shows the effect of BPG (bisphosphoglycerate) on the oxygen affinity of normal hemoglobin. The level of BPG in the blood at sea level is 2mM. After adaptation to high altitudes in 2-4 days the BPG level rises to about 5 mM.




Since BPG binds to the T-state, the binding affinity of $O_2$ to hemoglobin is reduced as the BPG concentration increases. (Note: this curve is somewhat different than previous lecture notes because it includes the effect of blood pH and salt on the oxygen affinity).

| | $pO_2$ (torr) | Fractional Saturation 2mM BPG | 5 mM BPG |
|---|---|---|---|
| **Sea level** | 100 | 0.99 | 0.98 |
| **Rockies** | 50 | 0.93 | 0.86 |
| **Muscle** | 20 | 0.51 | 0.32 |

What fraction of $O_2$ is delivered to the tissues at sea level? ([BPG]=2 mM)

$Y_{pO2=100}$ = $Y_{pO2=20}$ = Diff =

What fraction of $O_2$ is delivered to the tissues on arrival in Banff? ([BPG]=2 mM).

$Y_{pO2=50}$ = $Y_{pO2=20}$ = Diff =

What fraction of $O_2$ is delivered to the tissues after 5 days on the trails? ([BPG]=5 mM)

$Y_{pO2=50}$ = $Y_{pO2=20}$ = Diff =