**Lecture 13: Allosteric Effects and Cooperative Binding**



**Goals:**

* Distinguish between allosteric effects and cooperative binding
* Predict how homotropic/heterotropic activators/inhibitors affect binding.
* Understand allosteric effects in O2 transport.

**Warm-up:**

a)Sketch, on the same graph, the binding curves for a ligand that binds to a protein with a KD of 1 uM and 10 uM. Which ligand binds with higher affinity?



b) The binding curve for oxygen to Myoglobin and hemoglobin is shown to the right. Which curve is hemoglobin and how does O2 affect its own binding affinity to hemoglobin?

c) Determine the amount of oxygen released as Hb goes from the lungs ([O2]=12 kPa) to the tissues ([O2]=3.5 kPa).

**Summary of Allosteric Effects and Cooperativity:**

* Allosteric effects are important in the regulation of enzymatic reactions.
* Allosteric effects are the change in the conformation of a protein due to binding of a ligand.
* Allosteric changes affect the binding properties of a second ligand to the protein. Thus allosteric effects require at least two **interacting** binding sites.
* The allosteric compound and the ligand may be the same (**homotropic**), leading to **cooperative** **binding**. The binding of the first affects the second, etc. The cooperativity can be pos. or neg.
* The allosteric compound and the ligand may be different (**heterotropic**).

**Ligand binding Scenarios** (L = ligand, Y is measured for this ligand., A= allosteric activator, I=allo. inhibitor

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| *Single binding site for ligand (L)*  ***Must be non-cooperative*** | *Multiple sites for*  *Same ligand(L)*  *Possibly cooperative, but could be non-coop.* | *Two sites: Ligand (L) and A or I.Binding must be non-cooperative.*  *Possible allosteric control* | *Multiple sites one ligand plus site(s) for allosteric control. Possible cooperative binding and allosteric control.* |

**General Model for Allosteric Effects:** 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 allosteric activation the binding of ligand increases the amount of R state, thus increases the ease of ligand binding.
* In the case of allosteric inhibition, the amount of the T state is increased. Thus, the initial binding affinity is high. However, the binding of ligand increases the amount of T state, thus reducing the binding affinity.

**Homotropic Allosteric Moderators = Cooperative Binding.**

* If the two ligands are the same (e.g. oxygen affecting its own binding) then this is called a **homotropic** allosteric effect. Example shown below is a *positive* homotropic modulator, it increases the affinity of the system. As ligand is bound fR increases.



**Mechanism of positive homotropic cooperativity in Hb:**



* Binding of O2 to Fe+2 in heme moves the proximal His residue and its attached helix (F)
* Helix F adjusts its conformation by movement of the α and β subunits.
* Change in interaction between the α and β subunits causes a conformational change of the other subunits to the R-state, increasing their affinity for oxygen.

**Heterotropic Allosteric Effects.**



* If the two ligands are different, then this is called a **heterotropic** allosteric effect. The example shows both a heterotropic *negative* allosteric modulator (I), and a *positive* allosteric activator (A) affecting a single ligand binding site.

**Heterotropic Activators/Inhibitors – Effect on Non-cooperative binding:**

* Activators decrease the KD for the other ligand, increasing the amount of the relaxed state (fR increases).
* Inhibitors increase the KD for the other ligand, decreasing the amount of the relaxed state (fR decreases)



* The binding curve shape (hyperbolic) remains the same, because only one ligand can bind.



**Heterotropic Allosteric Activators/inhibitors - Cooperative systems:**

The activator/inhibitor will change the affinity **and** cooperatively for the ligand.

* Activators – increase affinity, curve shifts to the left, decreasing KD.
* Inhibitors – decrease affinity, curve shifts to the right, increasing KD.
* Shape of the curve (cooperativity) can also change.



**Hemoglobin:** There are many heterotropic allosteric effectors of oxygen binding in Hemoglobin, 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 when the pH drops or BPG increases. The allosteric effector stabilizes the tense state. Consequently, the oxygen affinity is reduced and the binding curve is shifted to the right. Note that the system, with respect to oxygen binding is still positively cooperative, and eventually high levels of O2 will shift the equilibrium to the R state, and Hb will eventually become saturated with oxygen.



**Molecular nature of the action of BPG:**



* In deoxy hemoglobin, a positively charged binding pocket exists between two of the four subunits. 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.

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|  | **pO2 (kPa)** | **5mM BPG** |  | **5mM BPG** |  | **8 mM BPG** |  |
| **Sea level** | 12 | Y=0.96 |  |  |  |  |  |
| **Rockies** | 8 |  |  | Y=0.90 |  | Y=0.87 |  |
| **Muscle** | 4 | Y=0.56 |  | Y=0.56 |  | Y=0.47 |  |

**Effect of BPG on O2 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 ~5mM. After adaptation to high altitudes in 2-4 days the BPG level rises to about 8 mM. Although the affinity has decreased (the KD at 8 mM BPG is higher), the cooperativity has increased, so the overall oxygen delivery improves.