Lecture 13: Allosteric Effects and Cooperative Binding

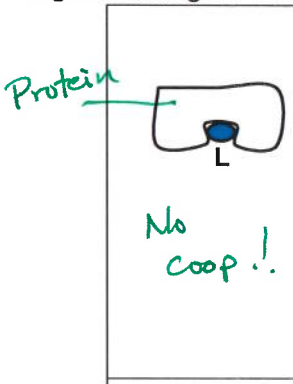
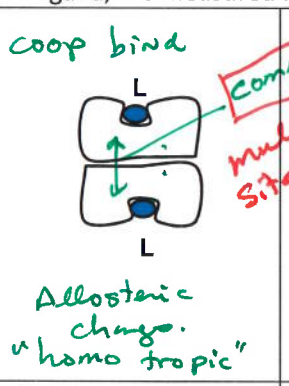
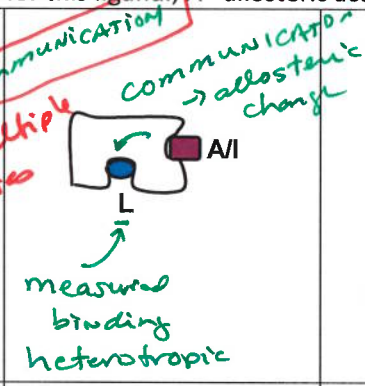
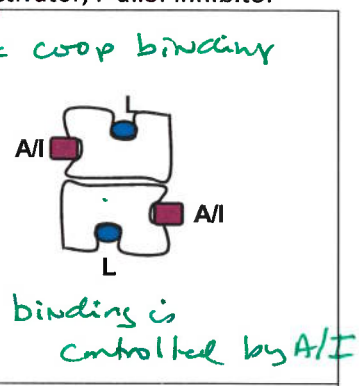
Goals:

- Distinguish between allosteric effects and cooperative binding
- Predict how homotropic/heterotropic activators/inhibitors affect binding.
- Understand pH effects in O_2 transport and altitude adaptation.

Summary of Allosteric Effects and Cooperativity:

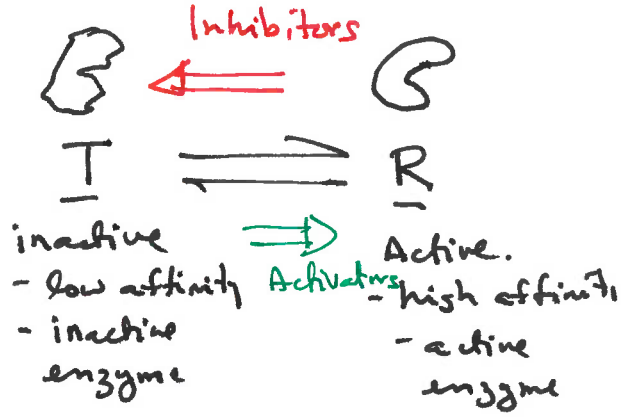
- Allosteric effects are important in the regulation of enzymatic reactions.
- Allosteric effects are the change in the conformation of a protein due to binding of a ligand.
- Allosteric changes affect the binding properties of a second ligand to the protein. Thus, allosteric effects require at least 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

			
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 Activator or Inhibitor. Binding must be non-cooperative and is controlled altered by allosteric compound.*	*Multiple sites one ligand plus site(s) for allosteric control. Possible cooperative binding that is also under 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.

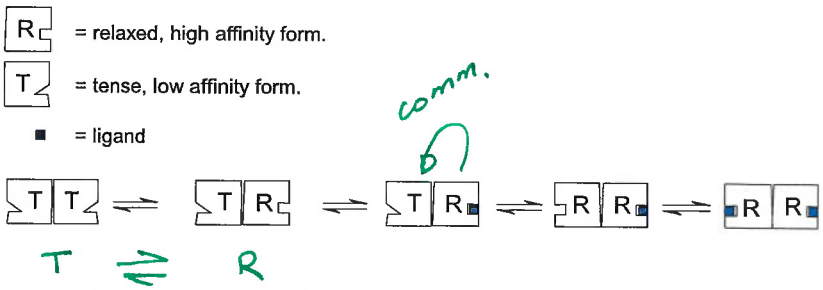


- In the case of allosteric activation, the binding of ligand increases the amount of R state, thus increases the ease of ligand binding.

- 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.

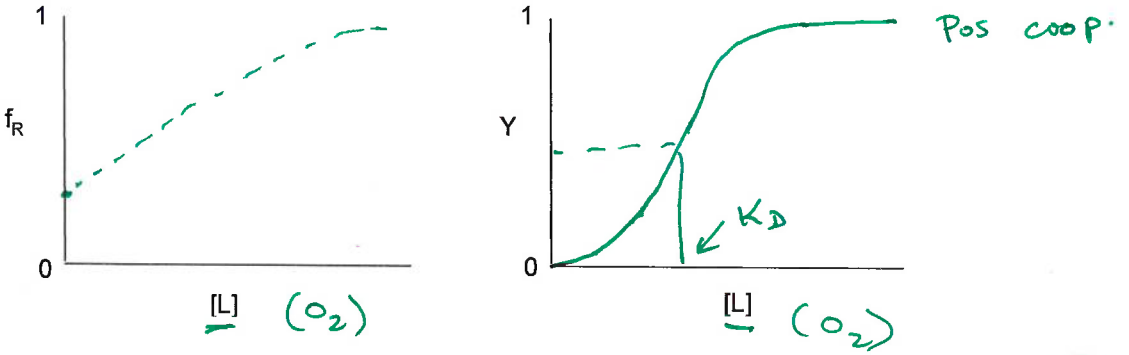
Homotropic Allosteric Moderators = Cooperative Binding.

- 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.



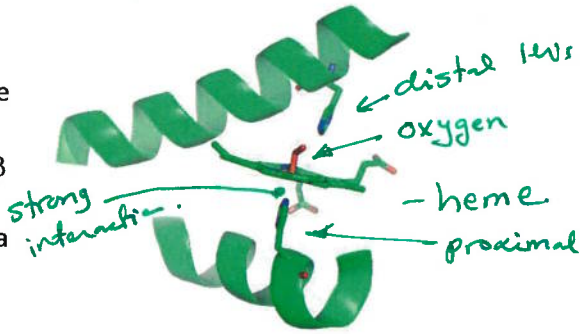
- The interaction between the two (or more) ligands will result in cooperative binding. The cooperativity can either be:
  - ➢ Positive – the binding of one ligand increases the affinity for subsequent ligands.
  - ➢ Negative – the binding of one ligand decreases the affinity for subsequent ligands.

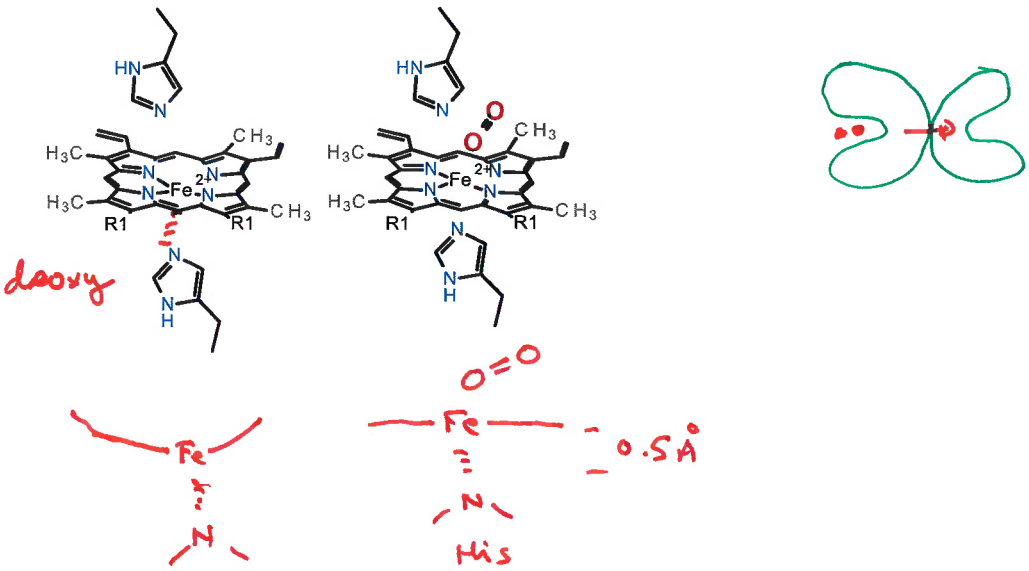
Example – Positive cooperativity: As ligand is bound the fraction of the molecule in the relaxed form f_R increases, as does the affinity.



Mechanism of positive homotropic cooperativity in Hb:

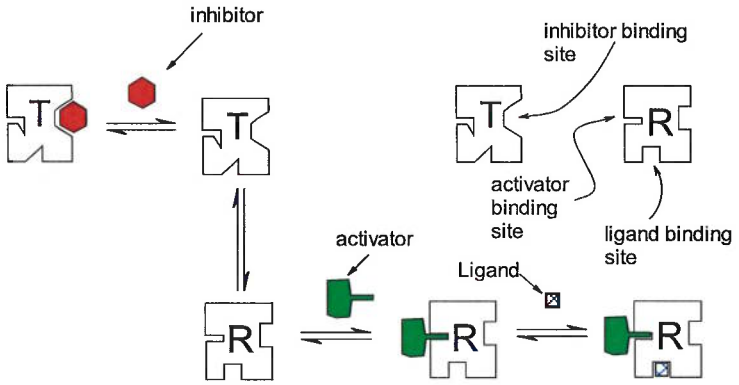
- Binding of O_2 to Fe^{+2} in heme moves the proximal His residue and its attached helix (F)
- Helix F adjusts its conformation by movement of the α and β subunits.
- Change in interaction between the α and β subunits causes a conformational change of the other subunits to the R-state, increasing their affinity for oxygen.





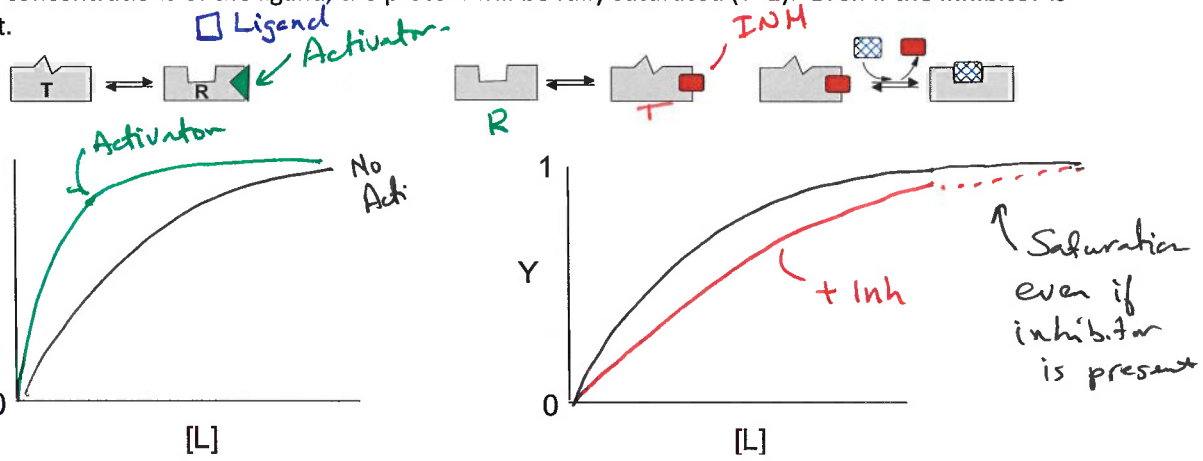
Heterotropic Allosteric Effects – Key concept: Le Chatelier's principle

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.



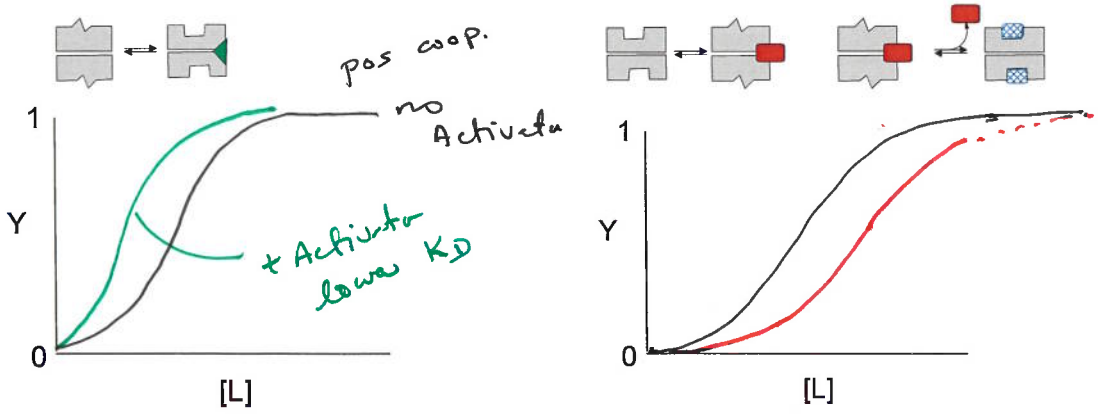
Effect on Non-cooperative binding:

- Activators decrease the K_D for the other ligand, increasing the amount of the relaxed state (f_R increases).
- Inhibitors increase the K_D for the other ligand, decreasing the amount of the relaxed state (f_R decreases)
- The binding curve shape (hyperbolic) remains the same, because only one ligand can bind.
- At high concentrations of the ligand, the protein will be fully saturated (Y=1). Even if the inhibitor is present.



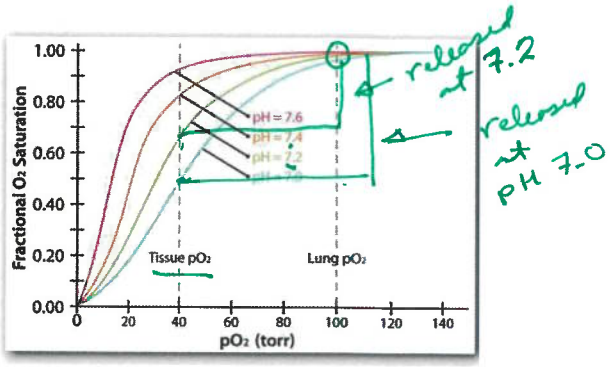
Cooperative systems: The activator/inhibitor will change the affinity and cooperativity.

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



Hemoglobin: There are many heterotropic allosteric effectors of oxygen binding in Hemoglobin:

1. Protons – Bohr effect: 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, increasing O_2release.



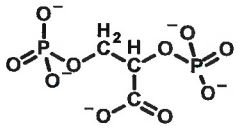
Example: What is the increase in O_2 delivery if the pH is lowered from 7.2 to 7.0?

The efficiency of delivery goes from 32% to 44%

pH 7.2: $\Delta Y = 0.97 - 0.65 = 0.32$

pH 7.0: $\Delta Y = 0.94 - 0.50 = 0.44$

2. BPG: bis-phosphoglycerate binds to the deoxy form of hemoglobin. It reduces oxygen affinity, but its increase in concentration allows better oxygen transportation. This is an adaptive response, requiring several days at high altitude.



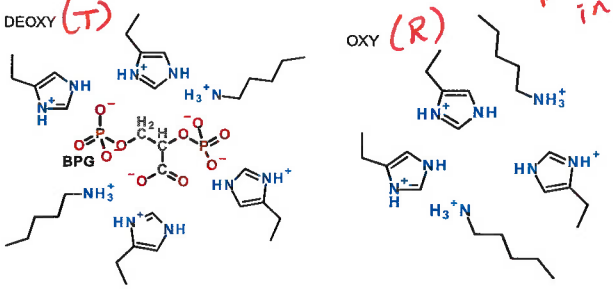
bisPhosphoglycerate - BPG

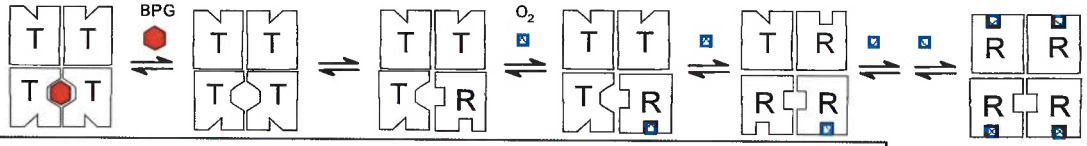
BPG is an allosteric effector that stabilizes the tense state. Consequently, the oxygen affinity is reduced and the binding curve is shifted to the right. Note that the system, with respect to oxygen binding is still positively cooperative, and eventually high levels of O_2 will shift the equilibrium to the R state, and Hb will eventually become saturated with oxygen.

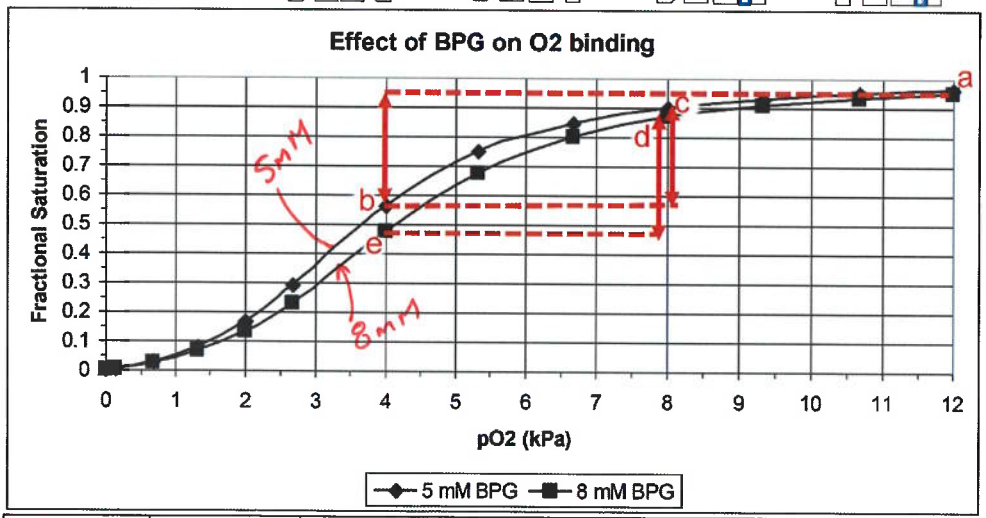
Allosteric inhibitor

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.



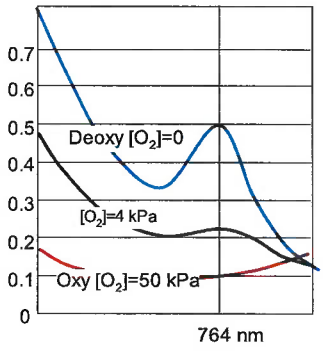




	pO_2 (kPa)	5mM BPG		5mM BPG		8 mM BPG	
Sea level	12	Y=0.96 (a)	40 %		34 %		40 %
Rockies	8			Y=0.90 (c)		Y=0.87 (d)	
Muscle	4	Y=0.56 (b)		Y=0.56 (b)		Y=0.47 (e)	

This graph shows the effect of BPG 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. This changes the affinity and cooperativity.

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

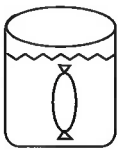


Example: A sample was made with an oxygen concentration of 4 kPa. What is the fractional saturation of hemoglobin at this oxygen concentration (use the absorption values at 764 nm)?

$$Y = \frac{A - A_M}{A_{ML} - A_M}$$

A =.22, A_M = 0.5, A_{ML} = 0.1 Y = (0.22-0.5)/(0.1 - 0.5) = 0.7.

2. In an equilibrium dialysis experiment, 1 uM of protein is placed inside the dialysis membrane, ligand is added to the outside of the bag and when equilibrium is reached the ligand concentration outside the bag was 5 uM and inside the bag it was 5.1 uM. $Y = \frac{[ML]}{[M]+[ML]}$



i) What is the ***free*** ligand concentration inside the bag? 5 uM, same as outside.

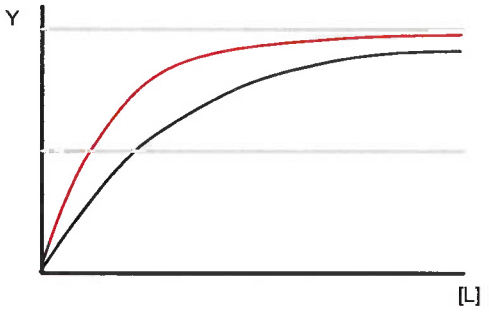
ii) What is the fractional saturation at this ligand concentration?

[ML] = 5.1-5 = 0.1

Y = 0.1/1 = 0.1 (typical error is: Y=0.1/1.1, this is incorrect because no additional protein was added)

3.

i) The binding curve for protein A is shown on the right. Protein B has higher affinity for the ligand. Use the graph on the right to sketch the binding curve for protein B. Label the axis.



ii) Briefly explain why you drew the curves the way that you did.

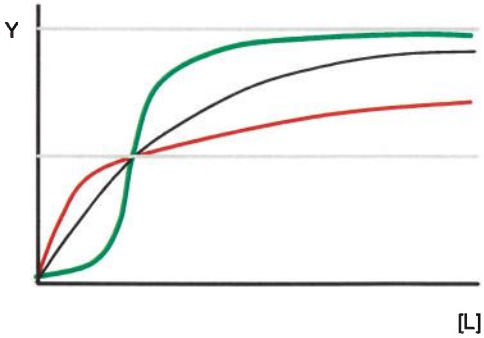
If the affinity is higher, then more should be bound at any concentration.

iii) Which curve has the greater initial slope, the low affinity ligand or the high affinity ligand.

The higher affinity curve.

4.

i) Draw on the graph to the right a binding curve that you would expect to obtain for a protein that binds more than one ligand and the binding of the first ligand ***decreases*** the binding of the subsequent ones. *Your curve should show the same K_D as the curve on the right.*



Shown in Red

ii) Do the same for a protein where the binding of one ligand ***increases*** the affinity of subsequent ones.

Shown in Green

iii) Briefly explain why you drew the two curves in the manner that you did.

The initial slope of the red curve should decrease as more ligand binds, making it difficult to saturate.

The initial slope of the green curve should increase as more ligand binds, making it easier to saturate.