# Recitation #6

## Contact Information

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**Recitation:** Friday, 3-4pm, 2-132
**Office Hours:** Friday, 4-5pm, 2-132

## Unit 2 Schedule

| *Recitation/Exam* | *Date* | *Lectures covered* |
|---|---|---|
| Recitation #6 | Tuesday, March 30, 5pm, 2-132 | 15, 16, 17, 18 |
| Exam 2 Review | Wednesday, March 31, 7pm, 10-250 | 10-18 |
| **Exam 2** | **Friday, April 2, 9:30-11am, Walker** | **10-18** |

## Recitation Overview

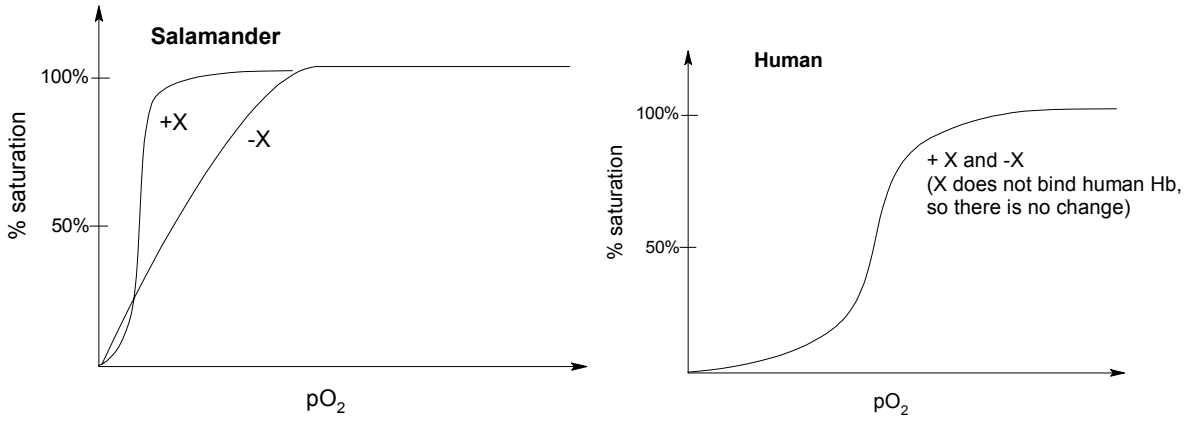
| *Topic* | *Problems* |
|---|---|
| 1. Allostery | 1, 2 |
| 2. Hemoglobin and the Bohr Effect Action | 3-5, [7-9] |
| 3. Hormone Action | 6 |

## Problems

**1. (1994 Exam 4 Question 2, 15 points)**
**Suppose you were able to solve the X-ray crystal structure of hemoglobin with one oxygen bound. Why would you be excited (i.e., what would be the significance) if you found that the subunit with oxygen bound had a different 3º structure from the remaining subunits that did not have oxygen bound?**

If the subunit with oxygen bound had a different tertiary structure from the remaining subunits, the sequential model would be supported.

**2. (2000 Exam 4 Question 7, 15 points)**
**Suppose that you discovered that salamander red blood cells (RBC) lacked BPG, but contained another chemical (substance X). Otherwise, the RBC from salamanders had the same chemical composition as human RBC. You find that substance X binds to salamander oxyhemoglobin much better than it binds to salamander deoxyhemoglobin. You also find that substance X does not bind to human hemoglobin (oxy or deoxy). Draw the oxygen binding curves (as a function of $pO_2$) for the salamander hemoglobin in the presence and absence of substance X. On a separate graph, draw the corresponding curves for human hemoglobin (in the presence of normal levels of BPG) in the presence and absence of substance X.**




**3. The crocodile can remain under water without breathing for up to one hour. An adaptation that aids the crocodile in doing so is that it can utilize virtually 100% of the oxygen in its blood. Whereas humans, for example, can extract only approximately 65% of the oxygen in their blood. Crocodile Hb does not bind BPG. However, crocodile deoxy Hb preferentially binds bicarbonate ions. How does this help the crocodile remain under water for so long?**

When the crocodile has its prey underwater and is using lots of energy to keep its jaws clamped on the prey, $O_2$ is consumed and $CO_2$ is produced. $CO_2$ is converted to protons and bicarbonate ions as follows: $CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$
$H^+$ is an allosteric inhibitor that specifically binds the deoxy or T state of hemoglobin, shifting the equilibrium from the R state to the T state. This is the pH Bohr Effect. In addition, the problem states that $HCO_3^-$ also specifically binds the deoxy or T state of crocodile hemoglobin. The shift to the deoxy or T state by $H^+$ and $HCO_3^-$ lowers Hb affinity for $O_2$, allowing for nearly complete $O_2$ unloading and utilization in the crocodile's blood.

**4. (2002 Exam 2 Question 1, 10 points)**
**You decide to construct "mango-globin", a new type of hemoglobin in which you have substituted the smaller Mg atoms (atomic number 12) for the larger Fe atoms (atomic number 26) in the porphyrin ring of all the subunits. Although you are able to find experimental conditions where mango-globin will bind oxygen, mango-globin does not show any cooperativity. Devise an explanation for why this might be.**

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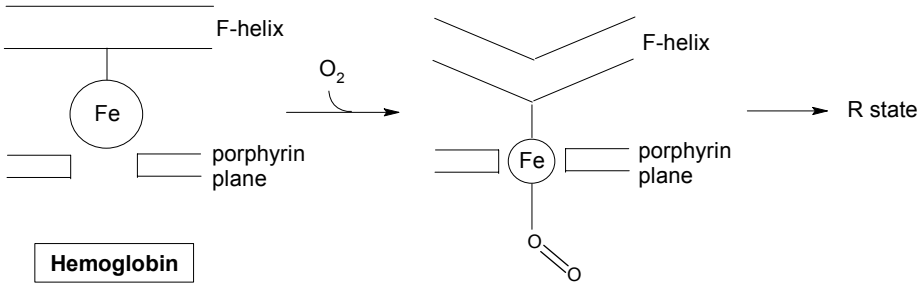
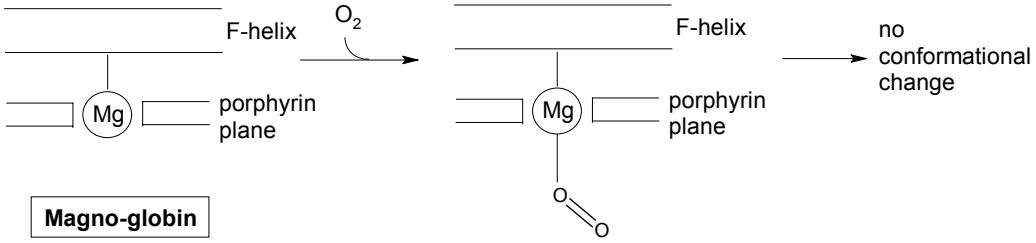
In hemoglobin, the size of the iron atom is normally too big to enter the porphyrin plane. Upon binding of oxygen, the iron becomes smaller, allowing it to enter the porphyrin plane. This pulls the F-helix and causes a conformational change of the entire hemoglobin molecule. The conformational change increases the binding affinity of the other subunits for oxygen, thereby resulting in cooperativity.




In mango-globin, the magnesium atom is small enough to fit into the porphyrin plane even when it is not bound to oxygen. Therefore the binding of oxygen does not cause any further conformational change in the molecule. Without a conformational change, the affinity for binding further oxygen remains the same and it therefore does not exhibit cooperativity.




**5. (2001 Exam 4 Question 5, 20 points)**
**Predict whether each of the following mutations will increase or decrease the tendency of HbS to polymerize in vitro. Provide a brief rationale for each answer.**

**(a) (5 pts) Substituting Tyr-42α with Phe. (Tyr-42 α, also known as C7 Tyr of the α chain. *See attached Figure 7-30* from Stryer, 4th edition)**

Substituting Tyr-42α stabilizes the deoxy form of hemoglobin by forming a hydrogen bond with an Asp on the β chain. Substituting Tyr-42α with Phe removes the hydroxyl group required for this hydrogen bonding, which shifts the equilibrium toward the oxygenated form. Since the oxygenated form does not polymerize, this will <u>decrease</u> polymerization.

**(b) (5 pts) Substituting His-146β with Arg. (*See attached Figure 7-36*)**

At low pH, binding of $H^+$ to His-146β stabilizes the deoxy form of hemoglobin by forming a salt bridge with Asp-94β. Arg has a much higher pK than His and will be mostly protonated at physiological pH. Substituting His-146β with Arg will make this position always positively charged and capable of forming a salt bridge with Asp-94β. This will stabilize the deoxy form and <u>increase</u> polymerization.

**(c) (5 pts) Substituting Lys-82β with Ala. (*See attached Figure 7-34*)**

Lys-82β and other positively charged groups are important for binding negatively charged BPG, which stabilizes the deoxy form of hemoglobin. Substituting Lys-82β with Ala, an uncharged amino acid, will cause BPG to bind more weakly to hemoglobin. This will shift the equilibrium to the oxygenated form and <u>decrease</u> polymerization.

**(d) (5 pts) Substituting Val-6β with Ser.**

Val-6β of HbS is responsible for the sickling phenotype since this creates a nonpolar side chain that can interact with a hydrophobic patch in the deoxygenated form of hemoglobin, formed by Phe-85β and Leu-88β. Replacing the hydrophobic Val-6β with a hydrophilic Ser will reduce the interaction with the hydrophobic patch and decrease polymerization.

**6. (2002 Exam 2 Question 4, 20 points)**

**A person is born with a temperature-sensitive mutation (i.e., a mutation that only gives a phenotypic effect at elevated temperatures) in the $G_s\alpha$ subunit of her heterotrimeric G-protein involved in the normal response to epinephrine. Whenever she gets a fever, her heart rate and blood pressure drop. You find that these effects result from decreased stimulation of adenylate cyclase and decreased activation of Protein Kinase A (PKA).**

**(a) (10 pts) Devise at least 2 possible reasons for what this mutation might be doing and explain them in detail (there are multiple possibilities, so there are many correct answers).**

Two possible mutations in the $G_s\alpha$ subunit which might lead to the observed phenotype are:
i) The mutant $G_s\alpha$ subunit has incorrectly folded at the restrictive temperature, making it incapable of binding to adenylate cyclase, even when bound to GTP. Once the $G_s\alpha$ subunit is activated by binding to GTP (a process activated by the β-adrenergic receptor), which frees it from the βγ subunits and allows it to activate cyclase. This, in turn, catalyzes the formation of cAMP, which activates PKA and further signaling. Were the $G_s\alpha$ subunit incapable of binding adenylate cyclase, then even when GTP is bound, no signal transduction would take place, as cAMP is critical in its activation of PKA.
ii) Misfolding of the protein at higher temperatures has increased the intrinsic GTPase activity of $G_s\alpha$. The deactivation of the signal transduction pathway is in part regulated by the intrinsic GTPase activity of the $G_s\alpha$ subunit. This cleaves the GTP bound within the subunit into GDP and $P_i$, and abrogates its binding to adenylate cyclase, promoting reassociation with $G_s\beta\gamma$. Were the $G_s\alpha$ subunit hydrolyzing GTP at an accelerated rate, the pathway would be stopped more rapidly than in a wild-type, and PKA activation would be significantly lowered at elevated temperatures.
Other possible answers were

- The subunit cannot dissociate from the transmembrane receptor.
- The subunit cannot dissociate from $G_s\beta\gamma$.
- The subunit now binds too tightly to GDP to dissociate from it
- The subunit cannot diffuse through the membrane, as it incorrectly binds a fixed protein.
- Transcription of the $G_s\alpha$ gene is somehow diminished at higher temperatures.
- Degradation of the $G_s\alpha$ protein is accelerated at higher temperatures.

**(b) (10 pts) A drug analogue of GTP (GTP-gammaS) has the property that when it is bound to the G protein it cannot be hydrolyzed to GDP. You find that treatment of this person's cells with GTP-gammaS restores the activation of PKA at elevated temperature. With this new data, can you now figure out what the mutation does?**

Yes. It is likely that option ii), listed above, is the mutation. Namely, the signaling function is rescued when a non-cleavable GTP analogue is fed to them, which implies that the initial problem lies in GTP being cleaved too rapidly. Adding the uncleavable GTP variant now constitutively activates the pathway, rescuing function.

## Practice Problems

**7. (1999 Exam 4 Question 6, 10 points)**
**Some biotechnology companies are trying to develop recombinant hemoglobin for use as a blood substitute. Why has it been important to introduce mutations that reduce the oxygen affinity of hemoglobin for this purpose?**

Without red blood cells, there is no BPG, which increases the oxygen affinity of the recombinant hemoglobin, making it unable to unload the oxygen fully when necessary.

**8. Who would have a higher level of BPG: a person at low altitude or one at high altitude? Why?**

A person at low altitude should have a lower level or BPG. BPG decreases the $O_2$ affinity of hemoglobin. At high altitudes, the decreased oxygen pressure is most significant in the tissues, because the binding affinity of hemoglobin for $O_2$ is such that even a reduced level of oxygen can saturate hemoglobin in the lungs. The same reduced level, however, would prevent release of oxygen in the tissues. To compensate, BPG levels will rise at higher altitudes.

**9. (1999 Exam 4 Question 7, 15 points)**
**Predict whether each of the following manipulations will increase or decrease the tendency of HbS to polymerize in vitro. Give a brief rationale for each answer.**
**(a) an increase in the partial pressure of oxygen.**

An increase in the partial pressure of oxygen will inhibit polymerization because only deoxy HbS polymerizes.

**(b) Stripping the HbS molecule of BPG**

BPG stabilizes deoxy HbS, and since only the deoxy form polymerizes, the removal of BPG from HbS would inhibit polymer formation.

**(c) an increase in pH**

Increasing the pH stabilizes oxyhemoglobin S. Since only the deoxy form polymerizes, this would inhibit polymer formation.

Victor Sai
7.05 Spring 2004

# Allostery, Hemoglobin, and the Bohr Effect

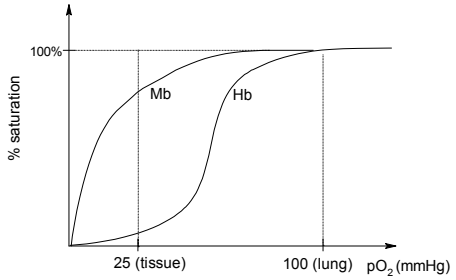
## 1. Allostery

a) Allosteric Interactions: Interactions between spatially distinct sites in a protein

b) Hemoglobin (Hb) vs. Myoglobin (Mb):

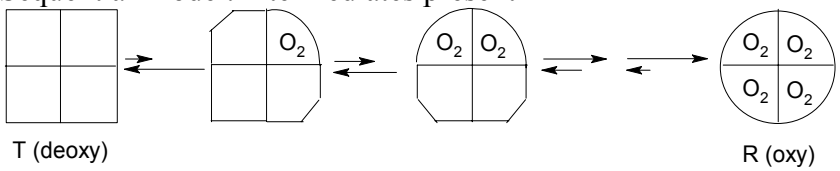
| **Similarities** |
|---|
| Both bind oxygen |
| Both use a prosthetic group, heme, to bind oxygen |

| **Differences** | |
|---|---|
| *Hemoglobin* | *Myoglobin* |
| Oxygen carrier in blood | Oxygen reservoir in muscle |
| High affinity for $O_2$ in lung, low affinity for $O_2$ in tissue | High affinity for $O_2$ in tissue |
| Four subunits ($\alpha_2\beta_2$) - each subunit can bind one $O_2$ | One subunit – binds one $O_2$ |
| Allosteric (cooperative binding) | Not allosteric (non-cooperative) |

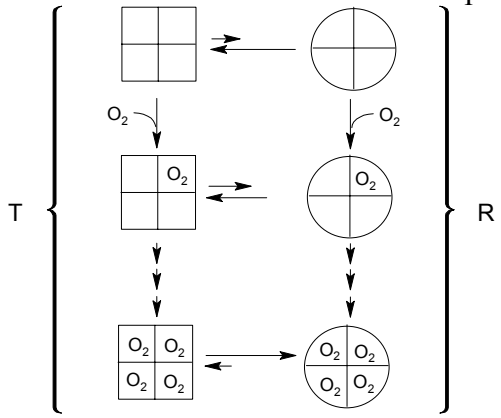



c) Two models of allostery that explain the cooperative binding of hemoglobin:

i) Sequential Model: intermediates present




ii) Concerted Model: no intermediates present

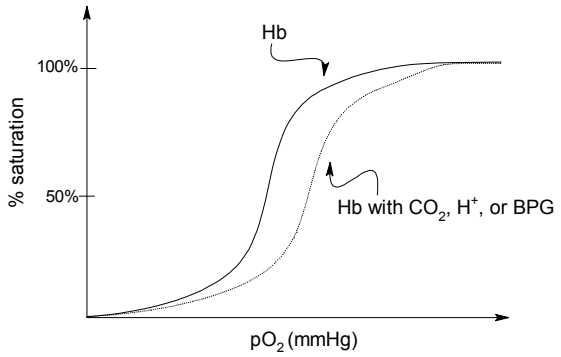


## 2. Bohr Effect

a) **Allosteric inhibitors** bind the T form and stabilize the T form, thereby shifting the equilibrium toward the T form and shifting the oxygen binding curve to the RIGHT

**Allosteric activators** bind the R form and stabilize the R form, thereby shifting the equilibrium toward the R form and shifting the oxygen binding curve to the LEFT

b) BPG, $H^+$, and $CO_2$ are all **allosteric inhibitors** of Hemoglobin: they allow oxygen to be unloaded in the tissue where it is needed




Therefore, **as [BPG]↑ or [$H^+$]↑ or [$CO_2$]↑ , $O_2$ affinity ↓**

c) Production of BPG, $H^+$, and $CO_2$:
   i) BPG is produced in red blood cells; its production varies with environment and need of the individual; for example, when one goes mountain climbing, more BPG is produced in response to the low $O_2$ content in the environment, which allows Hb to unload $O_2$ more readily in the tissue
   ii) $H^+$ and $CO_2$ are produced in tissue; for example, $H^+$ can be produced by lactic acid giving off a protein and $CO_2$ is produced by the TCA cycle

## 3. Fetal Hemoglobin

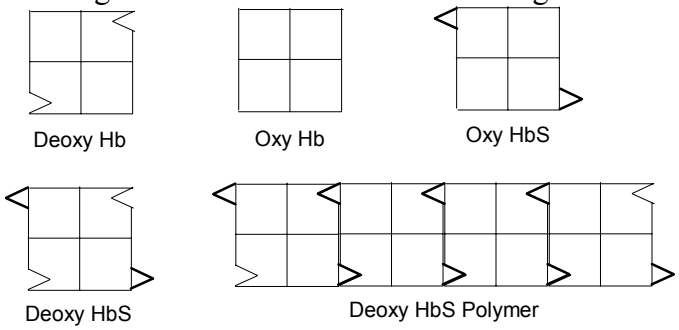
a) Fetal Hb (HbF) differs structurally from adult Hb. HbF has four subunits: $\alpha_2\gamma_2$

b) HbF has a higher $O_2$ affinity than adult Hb *in vivo* because BPG does not bind well to HbF.

## 4. Sickle Cell Anemia

a) Caused by a Glu to Val mutation in the β chains of Hb.

b) Sickling occurs ONLY when there is a high concentration of the deoxy HbS.




c) Sickled cells cannot pass through small blood vessels easily, which causes blockage of vessels and leads to hindered oxygen delivery, which causes more deoxy HbS to polymerize.