

NAME: \_\_\_\_\_

## Experiment 6 Central Dogma

### Part 3 Mutant Hemoglobin Structure/Function

Today, we will be exploring protein structure using the URL above to allow you access to the National archive of structural coordinates, the Protein Data Bank, or PDB.

<http://www.pdb.org/pdb/home/home.do>

On this site, there is an excellent online tutorial about hemoglobin that I would urge you to look through. Read through quickly now, but I'd suggest you read it through in detail at another time. For now, copy this URL into the browser and see the information provided here for hemoglobin, which has been featured on this site as a molecule of the month. There are four pages to this tutorial, the first page with an intro, the second page with a wonderful animation of hemoglobin cooperativity, a third page highlighting some troubled hemoglobins, i.e. sickle cell hemoglobin, and a last page on exploring the structure.

[http://www.pdb.org/pdb/static.do?p=education\\_discussion/molecule\\_of\\_the\\_month/pdb41\\_1.html](http://www.pdb.org/pdb/static.do?p=education_discussion/molecule_of_the_month/pdb41_1.html)

To check out these structures for yourself, find the search box next to the words "pdb id or keyword" at the top of the screen and type in the code for hemoglobin "2hnb" and then clicking on **SEARCH**. After a few seconds, a window will appear with a cartoon image of hemoglobin in the right side. There will be the option to view the structure using jmol – which is a JAVA application. Select the **"view in jmol"** option, and be patient. It will take a few minutes for Java to start and the application to load. When it finally displays, you will see a tetramer displayed on your screen, with subunits shown in blue (A chain), green (B chain), pink (C chain) and yellow (D chain). You are looking at a backbone representation, so you don't see all the side groups and hydrogen bonds and so forth. Notice that you can use the mouse to spin the structure around and view it from several angles. Play a bit with the structure.

1. What type of secondary structure do you see predominates in hemoglobin?  
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2. The four heme groups are displayed as small balls and sticks. These atoms are color coded by element (black is carbon, blue is nitrogen, red is oxygen). Also notice that you can zoom in and out on the structure by **holding down the shift key AND the left button while moving the mouse forward and back**. This will allow you to find the iron atom at the center of the heme. What color is the iron atom? Is this oxy or deoxy hemoglobin?  
Fe color: \_\_\_\_\_, oxy or deoxy \_\_\_\_\_

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3. Two sets of chains make little to no contact. What are they? \_\_\_\_\_  
\_\_\_\_\_
4. Also notice that if you let the mouse linger over a position, an atom identifier will pop up with the name and number of the amino acid. Find one amino acid from each chain. Write their identifiers here.  
\_\_\_\_\_, \_\_\_\_\_  
\_\_\_\_\_, \_\_\_\_\_
5. Notice that there is a Jmol Script box underneath the picture. You can use this box to type in commands. To select a particular residue, say, amino acid 15 on chain B, type "select 15:B" and hit enter. Once you have done this, this amino acid will be selected. But you still won't be able to see it until you type in "color red" and hit enter and then "spacefill" and hit enter one final time. Now, the amino acid you have selected will be displayed with large red balls (hard to miss). What is the identity of this amino acid? What side of the helix is it on (inside or outside) and does that make sense with what you know about the polarity of this residue?  
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6. Notice that there is a sequence tab button on the top of the page. Use the information on this tab to identify which chains (A, B, C, or D) are the two alpha subunits and which chains (A, B, C, D) are the two beta subunits.  
A: \_\_\_\_\_, B: \_\_\_\_\_, C: \_\_\_\_\_, D: \_\_\_\_\_  
\_\_\_\_\_
7. Notice also that you can access a whole host of commands by clicking on the red "Jmol\_S" at the bottom right corner of the image. While its tons of fun to explore all these options, we will leave that for you to explore on your own and in the future. Just confirm that you do see the box and that you have clicked it and been impressed by the hundreds of commands that are hidden underneath this little icon.

8. Working in groups of two, we invite each of you to explore two Hb mutations at position 15\* that you have highlighted on the previous page. These two mutants are Hb Belfast (Trp → Arg) and Hb Randwick. (Trp → Gly). See the accompanying page for the structures of these amino acids. Go back to the window with the jmol structure for the reference hemoglobin. In the Jmol script box, once again locate position 15 in beta globin (if you've clicked away from that screen) by typing in the select commands as previously directed. Remember, if your mutation is at position 15 in the beta chain, you should type "select 15:B" then hit enter, then type "color red" then hit enter, and finally "spacefill" and enter. This will highlight the position of the mutation. Once you have done this, you should consider how each of these mutations might affect the structure or function of this protein. Things to consider are how similar the mutant amino acids are to the wild type amino acid. An acidic residue like a glutamate in the reference sequence being changed for an aspartate won't be too bad because they are both acidic. But if you change to a radically different amino acid, how might that change the function? Also, if your side group is exposed to the solvent, that might be important if you change from a polar residue to a non-polar residue. Similarly, an amino acid side group that is buried and nonpolar that is mutated to another nonpolar group isn't too bad, but changing it to a charged group is horrible! A comment about each of these mutations should be part of the powerpoint presentation you make next week. If you wish to illustrate the slide with a screen capture of the image, make it as big as possible on the computer screen, and then simultaneously press the keys Ctrl, ALT, Print Screen, and then Paste (Ctrl V or right click paste) into your powerpoint document and crop with the picture edit tool. Also include on your power point slide the name and ID of the mutation is and what you might suggest would be the impact of these changes on the structure or function of this protein.
- Normally, in most proteins, you would need to make sure that the sequence of your mutant protein aligns with the reference protein. That is not necessary here because hemoglobin is such a well studied protein, everyone automatically aligns their mutants to the reference sequence. But for the future, if, for example, your mutation is in amino acid 53 in chain A, make sure that position 53 in your mutant sequence is also position 53 in the reference sequence. The numbers don't always line up exactly if there have been some amino acid insertions or deletions. How do you do this? Use the adjacent two or the amino acids on either side of your mutation to make sure your sequence is in register with this reference sequence. If it is, then the same numbering applies, and if it doesn't, keep searching along the reference sequence until you find the amino acids that match on either side of your mutation site. Then when you will model your mutation on the reference protein, you will use that number and not the number given in your mutated hemoglobin. Most of the time, these numbers will be identical so don't fret.

9. Maybe it didn't seem as though the mutation would have a great effect on this deoxy form of the hemoglobin but it might have a large effect on the oxy form. So go back to the search box at the top of the page, and next to the pdbID or keywords, insert **1hho** and hit the **SEARCH** button. Again, in a matter of seconds, the oxyhemoglobin structure will appear. Select **View in Jmol** button and wait as Java starts this application. While you are here, pause to find the bound oxygen, and see if you can appreciate that this structure is more "relaxed", more compact than the deoxy "Tense" state was.

Repeat the series of jmol entries that will let you select, color and spacefill your mutation site, and comment on if there are any effects this mutation will have on the oxy state that were not apparent in the deoxy state. Write any observations or questions in your powerpoint presentation. Again, an image can be captured using the screen capture mode and pasted and cropped into powerpoint.

10. Each group should pick one of the following 11 mutants: Hb M-Saskatoon, Hb Hana, Hb Zurich, Hb Bicetre, Hb J. Altgedt Gardens, Hb Redondo, Hb Milwaukee-2, Hb Duino, Hb Mozhaik, Hb Newcastle, or Hb Sainte Etienne. Sign up for your mutation on the class list and use Jmol to explore the implications of this mutation on the structure and function of the protein. This info should go into your class presentation.
11. Each group should pick one of the following mutants and provide answers to the questions as per 10 in your presentation.
12. Each group should pick two of the following mutants and provide answers to the questions as per 10 in your presentation.