Molecular Basis for Sickle Cell Disease

Adapted from Nicholas' Story by Didem Vardar-Ulu for CH373-S20

IN-CLASS WORK: This in-class activity is designed to guide you through a molecular exploration of Nicholas's disease using experimentally determined protein structures in the protein data bank (PDB). You are expected to carry out your individual explorations, discuss your findings with a group of four students, and then summarize them on a shared google doc. The outlined work is to be completed in the first 1.5 hrs. of your exam block time that will be followed by a 20 min. TopHat Test session.

WARM UP: (10 min) Compare your answers to the pre-work Part I with your group and discuss the answers to the following questions:

1. What is the composition and overall structure of hemoglobin? Why is it so important for us?
2. How does sickle cell hemoglobin (HbS) in red blood cells cause them to sickle?
3. Based on your background reading how does the sickling of red blood cells cause anemia, a shortage of red blood cells and acute pain?

In the video Nicholas describes his pain crisis as "it feels like someone is squeezing you, thumping – ba boom, ba boom". Nicholas' mom also talks about how he would have to be hospitalized frequently to treat his pain crisis. During this activity you will be exploring the structure of hemoglobin to answer the question "**What is the molecular basis of Nicholas' pain crisis?**"

Exploring the Molecular Basis of Nichola's Pain Crises:

Before you start this section please use the provided google doc link for your group to enter the document that will constitute the "group" part of your in-class assessment. Each student in the group is expected to follow every step of this guided tutorial INDIVIDUALLY, discuss their findings with their group, and record a joint answer to each of the questions indicated in blue on their google doc. Each answer should reflect the summary of the group discussion and ideas and you should take turns in recording the answers.

PART 1: (30 min) Developing a molecular understanding of oxygen binding to hemoglobin:

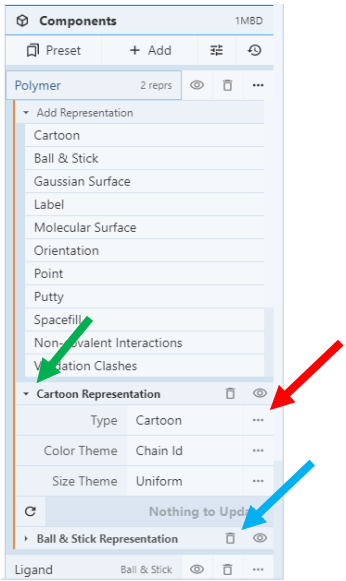
1. Go to the RCSB Protein Data Bank (https://www.rcsb.org/) and input the PDB ID 2DN2 into the PDB ID or Text search bars in the top menu.

Q1. Based on the information on the structure summary page that opens up, which structure does the PDB ID: 2DN2 represent? **Human deoxyhemoglobin**

2. Explore the 2DN2 structure using the same steps you did for 1MBD for your pre-class work. As a reminder the instructions are repeated below.

   a. Click on the "3D View" tab or the hyperlinked word "Structure", below the image. This should open an interactive view that shows the molecule.

   b. **Explore** the molecular structure of myoglobin molecule trying out different representations Different representations are activated by clicking on the "…" (red arrow) which allows you to "Add representation". To remove an existing representation, use the garbage can symbol (blue arrow) next to the corresponding representation from the growing list below. For each representation you can access further options by clicking on the small arrow on the left of the representation name (green arrow).



As you go through each representation note down what kind of molecular information about the protein or its ligand is best in emphasized by each representation.

A. View the "polymer" (in this case your myoglobin molecule" using the different "style" options: Cartoon, Ball & Stick, Molecular Surface, Spacefill, etc.)

B. Repeat the same exercise with the ligand

C. Keeping the polymer "representation" as "cartoon" and "ligand" as "ball & stick" try the different color options from the "set coloring" pull down activated by clicking on the "…" (red arrow). You can further narrow your selection using the small arrows on the left of each new menu that opens up. Ex: Set coloring→ Residue property → Residue name
Bring your cursor over each colored region and read the residue information displayed. What color corresponds to what class of amino acids?

3. Open **a new browser tab** and go to RCSB Protein Data Bank (https://www.rcsb.org/). Input the PDB ID 2DN1 into the PDB ID or Text search bars in the top menu.

Q2. Based on the information on the structure summary page that opens up, which structure does the PDB ID: 2DN1 represent? Human oxyhemoglobin

4. Explore this structure as you did for 2DN2 noting any similarities and differences you observe.

Q3. Based on the two structures you explored (2DN2 and 2DN1) what is the quaternary structure of hemoglobin? Make sure to use specific biochemical terminology in your answer. Include a screenshot of the molecule and annotate it to substantiate the features you summarized. In your annotation make sure to use the conventional names you identified for the subunits in your pre-work.

- **Four distinct polypeptide chains: two alpha-subunits and two beta-subunits.**
- **One alpha subunit pairs with one beta subunit to form an alpha-beta dimer.**
- **The two alpha-beta dimers then associate primarily by ionic interactions (salt bridges) to form the hemoglobin tetramer.**

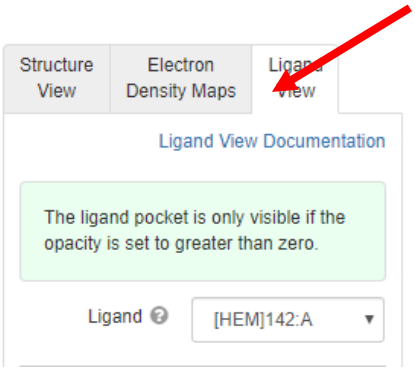
Q4. How does each of the subunits of hemoglobin (2DN2 & 2DN1) compare to the myoglobin structure you explored (1MBD) in your pre-class work?

- **Each chain is very similar secondary and tertiary structures**
- **Made up of 8 helices and (this unique fold is called the globin fold)**

5. Go back to the "Structure view" for both the deoxy- and oxy- hemoglobin structures. Set style in structure view tab to "cartoon" and color by "chain". Orient the two molecules as similarly to each other as possible, then switch the style to "spacefill"

Q5. Which of the two structures represent the "T state" and which one represents the "R state" of the hemoglobin molecule? The Deoxyhemoglobin state is the T-state and the Oxyhemoglobin state is the R-State

To explore the various kinds of non-covalent interactions stabilizing the bound heme groups in the **deoxyhemoglobin molecule** (2DN2), set the "Ligand" to "Ball & Stick" and then click on the "Ligand view tab" on the right of the screen and select a specific heme group (HEM 142 in chain A) to explore its interactions with the hemoglobin A chain.



Note: Atoms are displayed in using CPK coloring system (in the above diagram). The protein polymer chain is not shown here for clarity. The atoms are color coded so that: Red = oxygen; Blue = nitrogen; Gray = carbon; Yellow = sulfur; Orange = iron. Hydrogen atoms are not typically shown in a protein structure, instead they are assumed to be present.

A. Dial the Opacity and Clipping options under the "Pocket" section to get a better feel for how the heme sits in the protein.
B. Rotate the molecular display to examine the interactions.
C. Turn on/off the various types of interactions shown here to explore them closely

D. Once you get comfortable with the different visualization options, use the following settings for the pocket on a white background: opacity 15, near clipping 0, radius clipping 100, color by hydrophobicity.

Q6. Identify four different interactions (one of each: H-bond, hydrophobic contact, pi-pi interaction, metal interaction) through which the heme group is bound to the deoxy-hemoglobin molecule. Have each group member pick one to study in detail and create an annotated screenshot of their interaction(s) to describe the interaction. Make sure to indicate the identity of the interacting atoms and the type of interaction in your annotation. You should be including four separate figures as an answer to this question on your google doc.

6. Repeat your exploration for the oxyhemoglobin (2DN1) structure.

Discuss with your group the similarities and differences you noted between the deoxy- and oxy- hemoglobin around the oxygen binding site focusing on the following questions. Note this is a discussion exercise that does not require you to write down your answers to the google doc.

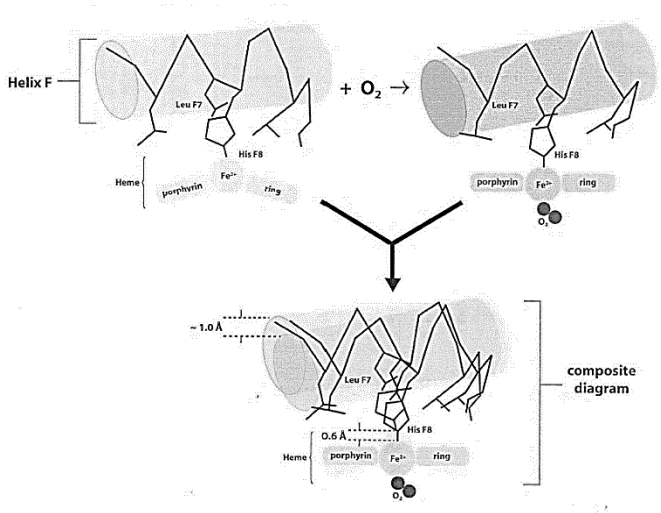
- **Where and how does oxygen bind to hemoglobin?**
- **How do the interactions you identified in Q6 change upon oxygen binding?**

7. Go to *Online Macromolecular Museum* exhibit: http://earth.callutheran.edu/Academic_Programs/Departments/BioDev/omm/jsmolnew/hemo/hemoglobina.html#II.
8. Go through each guided step in sections I (Introduction) and part (a) of section II (Tertiary and Quaternary Structure). Then read the following information and study the accompanying model.

By convention the α-helical segments of each hemoglobin subunit are labeled A through H. The nonhelical segments that connect between helices are labeled AB, BC, CD etc., which refer to the α-helical segments being connected. The histidine labeled F8 in the figure below is the proximal histidine involved in coordination with the Fe^{2+} of the heme ring. In the α-chain this is H87 in the sequence, whereas in the β-chain it is H92 in the sequence. Both histidines are in the F Helix of their respective chain and therefore labeled F8.

The binding of oxygen to the heme group results in flattening of the heme group and that event is communicated through the rest of the protein as Helix F of the α and β chains change position relative to the other helices and their respective subunits. This movement results in adjustments in the ion pairs at the interface between the α1 and β2 subunits and between the α2 and β1 subunits. The end result is a narrowing of the central channel of hemoglobin (the area in the center of the molecule). Therefore, structural changes within one subunit result in overall change in the quaternary structure of hemoglobin.

MODEL FOR O_2 BINDING TO HEMOGLOBIN:



9. Go back to

http://earth.callutheran.edu/Academic_Programs/Departments/BioDev/omm/jsmolnew/hemo/hemoglobina.html#II.

and complete part b (Heme-mediated O_2 Binding/Release, and Cooperativity) of section II making sure that you identify the specific structural elements shown in the model above in the 3D structure you are viewing.

Q7. Describe the structural details of the oxygen binding site in hemoglobin by answering the following questions:

a. **What is the geometry of the heme group, (porphyrin-Fe^{2+} -ring) without oxygen bound to Fe^{2+}? Bent**
b. **What is the geometry of the heme group (porphyrin-Fe^{2+} -ring) with oxygen binds to Fe^{2+}? Planar**
c. **Where is the proximal histidine (His that holds the heme in place) located within the hemoglobin subunit? What is the amino acid residue number for this His? On the F8 helix His 87**
d. **Where is the distal histidine (His that binds the O_2) located within the hemoglobin subunit? What is the amino acid residue number for this His? On the E7 helix His 58**

PART 2: (30 min) Developing a molecular understanding of sickle cell disease:
Go back to:
http://earth.callutheran.edu/Academic_Programs/Departments/BioDev/omm/jsmolnew/hemo/hemoglobina.html#

and complete part III **(**Hemoglobin and Sickle Cell Anemia)

10. Take individual notes on your observations and your group discussion points. You will be expected to reference them in the "Take home" component of your exam.

Q8. In a short paragraph, 3-4 sentences and using two figures describe the molecular basis of Sickle cell disease, i.e. how a single amino acid mutation can explain Nicholas's condition? Make sure to include the impact of the specific amino acid mutation on each protein structure level and intermolecular forces that contribute to the stability of these structures. Hint: Consider selecting one screenshot of HbA (regular deoxy-hemoglobin) and one of HbS (SCA Hemoglobin) to highlight critical differences.
Sickle cell hemoglobin has a mutation in the beta hemoglobin chain. The amino acid glutamate, at the 6th position of the chain is changed to valine. There is also a small cluster of hydrophobic residues (Phe85, Ala70, Leu88) that forms a pocket on the surface of both globin β chains. The mutation creates an additional hydrophobic patch on the surface of the β subunit facilitating association of these two hydrophobic surfaces on different hemoglobin molecules to form large polymers or fibers. This in turn distorts the shape of the red blood cell and it sickles.

Q9. If you are given the experimental data that the red blood cells sickle only in low oxygen saturation, propose a molecular explanation for why sickle cell hemoglobin fibers are formed by deoxy hemoglobin and not oxy hemoglobin? There are two requirements for aggregation. E6V mutation that brings a hydrophobic patch onto the surface and a partially exposed hydrophobic cluster formed by (Phe85, Ala70, Leu88). This second cluster is only surface exposed in the deoy form of the molecule. In the oxy (R state) they are much more buried and hence cannot participate in aggregation

PART 3: (15 min) Explaining Nicholas' Pain Crises in Molecular terms
A review article (Hematology Am Soc Hematol Educ Program. 2017 Dec 8; 2017(1): 546–555.), explains ***"A unique feature of SCD is vaso-occlusive crises (VOCs) characterized by episodic, recurrent, and unpredictable episodes of acute pain. Microvascular obstruction during a VOC leads to impaired oxygen supply to the periphery and ischemia reperfusion injury, inflammation, oxidative stress, and endothelial dysfunction, all of which may perpetuate a noxious microenvironment leading to pain."***
A glossary for of the key words/phrases used in the explanation above is included below:

- Microvascular obstruction – small blood vessels are obstructed
- ischemia reperfusion injury - tissue damage caused when blood supply returns to tissue after a period of lack of oxygen

- inflammation - a localized physical condition in which part of the body becomes reddened, swollen, hot, and often painful, especially as a reaction to injury or infection.
- oxidative stress - a state where oxidative forces exceed the antioxidant systems due to loss of the balance between them
- endothelial dysfunction – condition when the inner lining of blood vessels fail to perform their normal functions.
- noxious microenvironment – poisonous local environment

Based on the structural explorations you completed in Part 2 that reveals how hemoglobin with the sickle cell mutation can aggregate and the biological changes associated with pain described in the quote above discuss the relationship between aggregation and pain with your group.

Q10. Based on your understanding of the structural basis of pain, can you suggest two approaches that can avoid the pain crises?

-Oxygen rich environment (avoiding high altitudes and strenuous exercise)

-Any vasodialator that that increases circulation or other chemicals that reduces the adherence of RBC to blood vessels.