

## Activity #2 - Molecular Structure and Function Analysis

### Learning Goals:

- To become familiar with the Jmol software tool for analysis of molecular structure.
- To understand the content of .pdb molecular structure files and how it is rendered by the Jmol software.
- To understand the differences between saturated and unsaturated fatty acids.
- To understand the structure of phospholipids and their role in membrane structure.
- To understand nucleotide structure, base pairing and the antiparallel nature of DNA.
- To understand the differences between and the basis for primary, secondary, tertiary, and quaternary structure of proteins
- To perform common calculations used in analyzing molecular structures

### Lab Background:

It is often difficult to visualize molecules and processes that occur at the subcellular level. Illustrations in textbooks and laboratory manuals or physical models of small molecules have historically helped students understand the nature of biomolecules. However, modern tools such as 3D graphical rendering software allow students to interact with molecules by rotating, zooming in, and highlighting different features of complex molecules. More advanced software is used by pharmaceutical companies to construct 3D models of proteins for use in designing drugs to inhibit or activate the protein.

In the 1990's, the stand alone program RasMol was developed (Roger Sayle and E. James Milner-White. "RasMol: Biomolecular graphics for all", Trends in Biochemical Sciences (TIBS), September 1995, Vol. 20, No. 9, p. 374). MDL Chime is a web-browser plug-in that allows one to visualize and interact with molecules on a web page. As web browsers and operating systems continued to be updated, difficulty with maintaining compatibility led to a discontinuation of development. Jmol is a Java-based program that maintains most of the functionality of Chime, but because it is written in Java, can be displayed in any browser in any PC-based operating system (unfortunately, not yet available for smartphones!). All of these programs use PDB files. **PDB is an acronym for Protein Data Bank and is one extension used for 3D structure files. These files define the XYZ coordinates for each atom in the molecule.** The coordinates are used to draw or "render" the molecule on your computer screen. While the software and operating systems used to view 3D structures continues to change and add new features, the data files remain the same.

When studying the molecules found in living organisms, it is important to have a sense of the scale of the molecules relative to each other and the cell as a whole (which will be studied next week). Therefore, you should review and be comfortable with the scientific units for measuring size, volume, mass, molecular weight, amount, and concentration that are described in the next section.

## Review of Metric Measurement.

The metric system operates using powers of ten. For fluid measures, the metric standard is one liter (a little more than an English system quart). For solid measures, the metric standard is a meter (a little more than an English system yard). For mass, the metric standard is a gram (the mass of one milliliter or one cubic centimeter of water).

1 Liter (L) = 1,000 milliliters (mL) = 1,000 cubic centimeters (cm<sup>3</sup> or cc)

1 meter (m) = 100 centimeters (cm) = 1,000 millimeters (mm) = 1,000,000 micrometers (um)

1 gram (g) = 1,000 milligrams (mg) = 1,000,000 micrograms (ug)

<b>Property</b>	<b>Unit</b>	<b>Abbrev.</b>	<b>note</b>
Distance	meter	m	
	Angstrom	Å	10 Å = 1 nm
Volume	liter (preferred)	L	1L = 10cm x 10cm x 10cm
	cubic centimeter	cc, cm <sup>3</sup>	1cc = 1 mL
mass	gram	g	1g = mass of 1 mL of H <sub>2</sub> O
Temperature	degrees Celsius	°C	
	Kelvin	K	0°C = 273K
Amount	mole	mol	1 mol = 6.02 x 10 <sup>23</sup> molecule
molecular weight	Dalton, gram/mole	Da, g/mol	add atomic masses
concentration	molar	M, mol/L	
	weight/volume (w/v)	g/L	
	percent	g/100 mL	

<b>Metric scale</b>	<b>Prefix</b>	<b>Abbrev</b>
10 <sup>12</sup> (trillions)	Tera	T
10 <sup>9</sup> (billions)	Giga	G
10 <sup>6</sup> (millions)	Mega	M
10 <sup>3</sup> (thousands)	kilo	k
10 <sup>-2</sup> (hundredths)	centi	c
10 <sup>-3</sup> (thousandths)	milli	m
10 <sup>-6</sup>	micro	μ or u or mc
10 <sup>-9</sup>	nano	n
10 <sup>-12</sup>	pico	p
10 <sup>-15</sup>	femto	f
10 <sup>-18</sup>	atto	a

## **Common molecular calculations.**

You may also remember these from high school chemistry, but just to refresh, and for those who have not yet taken chemistry:

**Moles:** a mole of a molecule is  $6.022 \times 10^{23}$  copies of that molecule. (For the history buffs among us: this was originally defined as the amount of material that contains as many elementary units as there are atoms in 12 g of carbon-12 isotope.)

**Molarity:** A 1 Molar (1M) solution contains one mole in one liter (1 mole/liter, or 1M/L).

**Molecular weight:** Each chemical compound has a characteristic molecular weight: the number of grams in one mole of the compound. For example, sodium chloride (NaCl) has a molecular weight of 58.44 grams/mole. This is determined by adding the atomic masses of each atom in the molecule. This means that one mole, or  $6.023 \times 10^{23}$  molecules of NaCl, would weight 58.44 grams. Water, H<sub>2</sub>O, has a molecular weight of 18 grams/mole. Molecular oxygen, O<sub>2</sub>, has a molecular weight of 32 grams/mole.

**Molecular weight of nucleic acids:** The average molecular weight for a nucleotide in DNA is 330 grams/mole. Because DNA is double stranded, one base pair (bp) would therefore have a MW of 660 g/mol.

**Molecular weight of proteins:** The molecular weight and abundance of the amino acids in proteins can vary considerably. However, based on the greater abundance of small amino acids, a value of 110 g/mole aa generally provides a good estimate for the molecular weight of a protein. Thus, a protein composed of 100 amino acids would have an estimated MW of 11,000 g/mol, or 11,000 Da, or 11 kDa.



### Biological Math Practice – metric conversions

1. 200 nm = \_\_\_\_\_  $\mu\text{m}$  = \_\_\_\_\_ mm
2. 5  $\mu\text{m}$  = \_\_\_\_\_ nm = \_\_\_\_\_ mm
3. 25  $\mu\text{m}$  = \_\_\_\_\_ mm = \_\_\_\_\_ nm
4. 0.05 mL = \_\_\_\_\_  $\mu\text{L}$  = \_\_\_\_\_ L
5. 0.25 L = \_\_\_\_\_ mL = \_\_\_\_\_  $\mu\text{L}$
6. 0.025 L = \_\_\_\_\_ mL = \_\_\_\_\_  $\mu\text{L}$
7. 800  $\mu\text{L}$  = \_\_\_\_\_ mL
8. 80  $\mu\text{L}$  = \_\_\_\_\_ mL
9. 2.5 mL = \_\_\_\_\_  $\mu\text{L}$
10. 50 mg = \_\_\_\_\_ g
11. 1 g = 1000 \_\_\_\_\_
12. 1 g =  $1 \times 10^6$  \_\_\_\_\_
13. 1 g =  $1 \times 10^9$  \_\_\_\_\_
14. 1 g =  $1 \times 10^{12}$  \_\_\_\_\_
15. 1g =  $1 \times 10^{15}$  \_\_\_\_\_
16. 1  $\text{cm}^3$  = \_\_\_\_\_ mL
17. 1  $\text{mm}^3$  = \_\_\_\_\_ mL = \_\_\_\_\_  $\mu\text{L}$
18. 1  $\mu\text{m}^3$  = \_\_\_\_\_  $\mu\text{L}$

write the following in scientific notation with 3 significant digits

19. 125,638,000 = \_\_\_\_\_
20. .00038542 = \_\_\_\_\_

Links to the websites below can be most easily accessed from the class website. The addresses are provided for your future reference.

## A.Lipids

### 1. Palmitic Acid

Fatty acids are the building blocks of triglycerides, phospholipids and glycolipids, important components of biological membranes. Fatty acids can be found in every cell in your body.

- a. **Opening the .pdb structure file.** Visit the web site indicated below.  
(<http://biomodel.uah.es/en/model3/index.htm> )



Click on the link for Fatty Acids, then click the X in front of the Palmitic Acid link. The fatty acid, palmitic acid, should now appear as a “sticks” model in your browser window. Sticks shows thick lines to represent the bonds between the atoms, which are not shown.

- b. **Manipulating the view of the molecule.**

**Left click** on the molecule and move your mouse to move the molecule.

**Right click** on the molecule and a pop-up menu should appear. Select *style*, then *sticks* - this displays the bonds as thicker lines.

**Right click** on the molecule, select *style* on the pop-up menu, then *ball and stick*. In this display mode, the atoms are shown as balls with the bonds shown as sticks.

The identity of the atoms can be determined by their color. **In the color scheme called “CPK”:**

- carbon is gray**
- hydrogen is white**
- oxygen is red**
- nitrogen is blue**
- sulfur is yellow**
- phosphorous is orange**

Move the molecule around to view it from different angles. In particular, be sure to view it end on and fully extended. **To move the molecule without rotating it, hold down the ctrl key and the right mouse button while moving the mouse. To zoom in and out, hold down the shift key and the left mouse button while moving the mouse.**

### Analysis of Palmitic Acid Structure

- How many carbon atoms does this molecule contain? \_\_\_\_\_
- How many oxygen atoms? \_\_\_\_\_



## 2. Oleic Acid, a fatty acid

a. Click the button for “Oleic acid” to retrieve the oleic acid structure into your browser. Right click on the molecule and change the *style* to *ball and stick*. Count the number of carbon, hydrogen and oxygen atoms and record them below.

- How is oleic acid different from palmitic acid in terms of the number of atoms?
- Which of the two fatty acids is saturated (with hydrogens)?
- Which is unsaturated?

# of	palmitic acid	oleic acid
carbon atoms		
hydrogen atoms		
oxygen atoms		
Total MW		

b. Rotate the molecule to observe its shape.

- How is the shape of oleic acid different from that of palmitic acid?
- What is the basis for this difference? What effect does being unsaturated have on the shape of a lipid molecule?

c. When a lipid’s hydrophobic tails can pack tightly against one another, that lipid will be a solid at room temperature, like bacon fat or lard. When a lipid’s hydrophobic tails are “kinky,” they cannot pack tightly against one another, and that lipid will remain liquid at room temperature, like olive oil.

- What kind of tails (saturated or unsaturated) would you expect in bacon fat?
- What kind of tails (saturated or unsaturated) would you expect in olive oil?

## 3. linoleic acid and linolenic acid

a. Review the structures of linoleic acid and linolenic acid, which have multiple double bonds. What possibly familiar term could be given to such fatty acids?

b. Click the book icon to proceed to the next page

#### 4. Triacylglycerols = glycerol + 3 fatty acids

a. Click the first button in the right hand panel, right click on the image and change the *style* to *ball and stick*. Zoom in to observe the area with the red oxygen atoms.

Glycerol (glycer = 3 carbon; ol = alcohol group = OH) has 3 OH groups, each of which can be condensed with a carboxylic acid (COOH) to create an ester linkage (-COO-) and H<sub>2</sub>O. The fatty acids linked via an ester bond are now referred to as acyl groups, because they no longer have a free carboxylic acid group.

b. Click the second button to observe the different components of the triacylglycerol. Is the non-double-bonded oxygen in the ester bond derived from the carboxylic acid or the hydroxyl group?

c. Click the book icon in the lower right of the panel to proceed to the next page

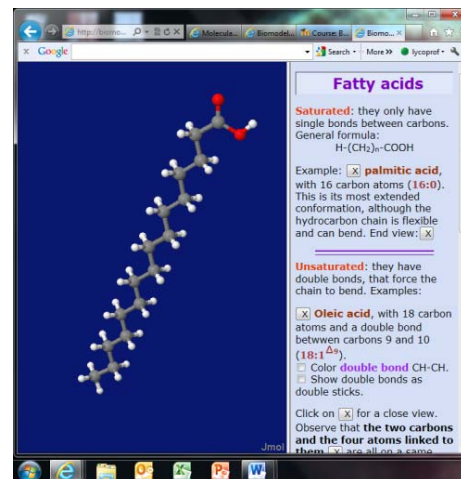
#### 5. Phospholipid

a. Click the first button in the right hand panel, right click on the image and change the *style* to *ball and stick*. Zoom in to observe the area with the red oxygen atoms.  
List the components of a phospholipid below and identify each of these components in the structural model while the atoms are still displayed in CPK colors.

- 1.
- 2.
- 3.
- 4.
- Which parts are hydrophilic, which ones are hydrophobic.

b. The fatty acid components of this phospholipid are called lauric acid.

- How many carbons do the fatty acids have? \_\_\_\_\_



- Are the fatty acids saturated or unsaturated? \_\_\_\_\_
- How might the shape of the molecule differ if the level of saturation of the fatty acids were changed?
- The molecular formula of this molecule could be represented as:  $C_{29}H_{57}O_8NP$  or as  $NH_2CH_2CH_2PO_4CH_2CH[OOC(CH_2)_{10}CH_3]CH_2OOC(CH_2)_{10}CH_3$   
What is the molecular weight of this phospholipid? \_\_\_\_\_

c. Click the book icon in the lower right of the panel to proceed to the next page

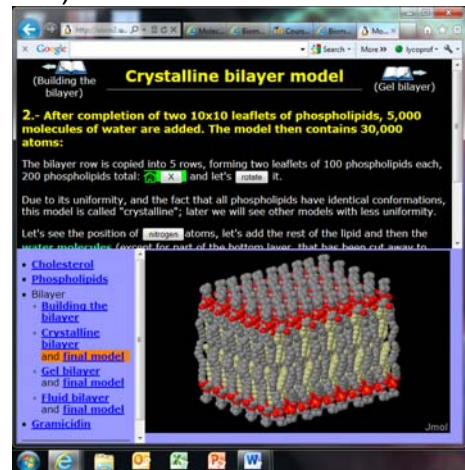
## 6. Cholesterol ( a sterol)

- Click the first button in the right hand panel, right click on the image and change the *style to ball and stick*. Zoom in to observe the area with the red oxygen atoms. Do you think this area is hydrophilic or hydrophobic?
- click the bottom button in the panel to see if you are correct
- Click the book icon in the lower right of the panel to proceed to the next page

## 7. Membrane fragment containing many phospholipids.

Go to the following URL: <http://www2.uah.es/biomodel/en/model2/bilayer/inicio.htm>  
(or click on the Lipid Bilayer link on the class Moodle site)

- READ THE INSTRUCTIONS on the first page!  
Then click *Start the Presentation*  
In the window to the bottom left, click the *Phospholipids* link
- In the top window, click through the buttons IN ORDER as the Professor guides you through this brief review of phospholipid structure.  
*The main component of your cell membranes is phospholipids!*



- In the window to the bottom left, click the *Building the Bilayer* link  
In the top window, click through the buttons IN ORDER with the class.

*How are the polar head groups of the lipid layer arranged relative to one another? (are they all mixed up or all on one side?)*

*How are the nonpolar/hydrophobic fatty acid tails arranged relative to one*

*another?(are they all mixed up or all on one side?)*

- d. In the window to the bottom left, click the *Crystalline Bilayer* link  
In the top window, click through the buttons IN ORDER with the class.

*Where are the nitrogens from the polar head groups located in this bilayer?*

*Where are the hydrophobic fatty acid tails in this bilayer?*

*Where are the water molecules located?*

*What real-world analogy could you use to help remember this arrangement?*

- e. In the window to the bottom left, click on the *Fluid Bilayer* link (we are going to skip the Gel Bilayer part for today)  
In the top window, click through the buttons IN ORDER with the class.

*Make your own sketch and/or notes here to help you remember what the lipid Bilayer structure looks like. Make sure you label the phospholipid head groups, fatty acid tail groups, and which parts are hydrophilic or hydrophobic.*

## **B. Nucleic acid (DNA)**

Deoxyribonucleic acid (DNA) is the genetic material contained in our cells' nuclei.  
(<http://molvis.sdsc.edu/dna/index.htm> )

1. Use Mozilla Firefox to go to the web site indicated above, click on "DNA Structure in Jmol," then on the link for "*Double helix by element: base pairs, hydrogen bonding*". (the A. green box)
  - a. Examine the initial wireframe model by rotating the molecule. Note that the hydrogens are not shown.
  - b. After clicking on the *backbone* button, identify the major and minor grooves on the DNA.
  - c. Turn the molecule to view it from the end. Where is the backbone relative to the overall molecule?
  - d. Click on the *Bases* button. Where are the bases relative to the overall molecule?
  - e. Recall that the four bases found in DNA are adenine (A), thymine (T), guanine (G), and cytosine (C). Click the "DNA→AT pair" button to examine how the adenine and thymine bases pair with each other .. After the java script has completed, click the "H bonds" check box to see the position of the

hydrogens.

- f. Identify the 3 parts of each nucleotide
- phosphate ( $\text{PO}_4$ )
  - 5 carbon sugar – either ribose (in RNA) or deoxyribose (in DNA)
  - nitrogenous base – The purines A & G have 2 rings while the pyrimidines T & C have 1 ring
  - Which parts of a nucleotide form the backbone?

- g. Purines always pair up with pyrimidines. In this AT base pair, note how the H-Bond donors line up with the H-bond acceptors.  
What is the main characteristic of an H-bond donor? \_\_\_\_\_

What is the main characteristic of an H-bond acceptor? \_\_\_\_\_

Why can't a third H-bond form between the A & T bases?

- h. Click the "AT pair→DNA" button to send the AT base pair back into the molecule. Then click the "DNA→ GC pair" button to view a GC base pair, after the javascript has completed, click the "H bonds" button to view the hydrogen atoms. Note the pattern of H-bond donors and acceptors. How many H-bonds hold a GC base pair together? \_\_\_\_\_

Click the back arrow when finished examining this base pair.

2. Click on the link for "*Ends, antiparallelism*" (the D. box)

- a. On the subsequent page, click on the button for the 5' end.

- Which part of the nucleotide has atoms numbered with plain numbers?  
\_\_\_\_\_
- Which part is numbered with primes? \_\_\_\_\_
- What functional group is present at the 5' end? \_\_\_\_\_

- b. Click on the link for the 3' end.

- What functional group is present at the 3' end? \_\_\_\_\_

- c. Click *reset*. Look at both ends of both strands of the whole molecule.

- Why is the DNA described as **antiparallel**? \_\_\_\_\_
- How many base pairs are present in this DNA molecule? \_\_\_\_\_
- What is the molecular weight of this DNA molecule? \_\_\_\_\_

## C. Protein Structure Analysis

Proteins make up half the mass of cell membranes as well as much of the cytoplasm, and do most of the work inside the cell, such as catalyzing chemical reactions (enzymes), moving things around (motor proteins and transporters), providing structure (cytoskeleton), perceiving signals (receptors), activating and inhibiting genes (transcription factors), and many others

### 1. Amino Acids

- a. Without closing the first window, Return to the Biomodel-3 web site indicated below by going back to the index, then clicking on the link for amino acids. (<http://biomodel.uah.es/en/model3/index.htm> )
- b. Read the material in the right side panel and recall that acids such as carboxylic acids donate  $H^+$  ions to solution and acquire a negative charge. On the contrary, amino groups are basic, and they will accept an  $H^+$  ion from solution, and therefore have a positive charge.
- c. The twenty amino acids found in proteins (there are several others that are not found in proteins) can be grouped based on the chemical properties of their R-groups. As you review the five classes of amino acids, choose the ball and stick models and take note of how they are similar and how they are different.
- d. When finished reviewing the amino acids, click the “back to index” button

### 2. Primary Structure – Peptides

- a. On the Biomodel-3 web site indicated below, click on the link for peptides. (<http://biomodel.uah.es/en/model3/index.htm> ), then click the first button in the right side panel. Right click on the image and change the *style* to *ball and stick*
- b. Write the complete structural formula of this tripeptide. Circle and label the three sidechains that correspond to the three amino acid residues (a residue is an amino acid that has been incorporated into a peptide).

- c. Indicate the peptide bonds in the formula you wrote, using arrows and labels as appropriate.
- d. Identify the N-terminus and write the amino acid residue name on your worksheet here \_\_\_\_\_.
- e. Identify the C-terminus. Write its name on your worksheet here \_\_\_\_\_.
- f. Note that each peptide group is coplanar. Where do adjacent planes connect to each other?
- g. Click the “back to index” button

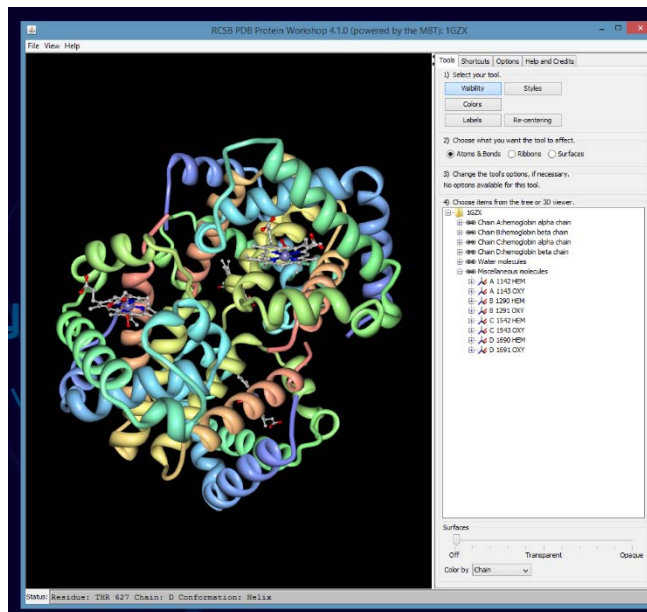
### 3. Hemoglobin Structure (Protein)

Hemoglobin is the protein in your blood that carries oxygen molecules throughout your body. The hemoglobin protein forms a framework that holds 4 heme groups. Each heme group contains an iron atom, and each iron atom can bind to one oxygen (O<sub>2</sub>).

#### a. Loading the hemoglobin molecule PDB file:

1. Go to the following URL: <http://pdb.org/pdb/home/home.do>
2. In the search box near the top of the page, type in “1gzx” and then press enter. In the Biological Assembly box on the right side of the page, click on “protein workshop”. If popup screens appear, select “Open”, “Run”, and/or “Install”

3. In X-ray crystallography, the protein of interest is purified and dissolved at a high concentration. As the solvent evaporates, the solution becomes saturated, and the protein molecules aggregate in a repeating pattern to form a crystal, which comes out of solution. The crystals are placed in a high intensity beam of X-rays. As the X-rays interact with electrons in the molecules of the crystal, they are deflected and then strike a sensor to generate a diffraction pattern. The diffraction pattern is used to generate a three dimensional electron



density map, which, together with the known amino acid sequence of the protein can be used to determine the position of atoms within the molecule.

4. *Left click* on the molecule and move it around to get a feel for the complexity of proteins. Protein Workshop initially shows proteins with the polypeptide as just a backbone ribbon and any non-polypeptide components in ball and stick with CPK coloring.

*What do you think is the large structure shown in ball and stick within hemoglobin?*

*What is the grey atom in the center of this structure? (check the status bar at the bottom of the window)*

*What are the two red atoms attached to one side of the grey atom?*

### **Examination of different levels of protein structure**

Just as written language can be analyzed at the levels of word spelling, sentence structure, paragraph structure, and chapter structure, protein structure has several different levels of complexity. We will explore each of these in turn below.

#### **b. Examination of primary structure**

1. In section 4 of the right side panel, click the “+” next to Chain A:hemoglobin alpha chain to reveal the sequence of amino acids in this polypeptide chain. There are one-letter and three-letter codes for each amino acid. This display uses the three-letter codes in the main box. How many amino acid residues are in this chain? \_\_\_\_\_
2. Click the “+” next to Chain B:hemoglobin beta chain to reveal the sequence of amino acids in this polypeptide chain. Note that the residue numbers start at 144, because 142 and 143 are used by the alpha chain heme group and oxygen molecule. Click the “-” next to Chain A:hemoglobin alpha chain to hide that sequence of amino acids.
3. In sickle cell anemia, a one base change in the  $\beta$ -globin gene changes the residue at position 6 from glutamic acid (GLU — hydrophilic, 1 letter code = E) to valine (VAL – hydrophobic, 1 letter code = V). The annotation used to describe this change is E6V. We will see many more examples of this annotation later in the semester when we study human genetic variation.
4. On the right side panel, click the Visibility button in section 1; then the GLU at the 6<sup>th</sup> position on the beta chain. This should make that amino acid residue appear in ball and stick. Click Styles in section 1; CPK for radius of atoms in section 3, and then the GLU at the 6<sup>th</sup> position on the beta chain again. This should make that amino acid residue appear in spacefill.

- Repeat step 4 above to reveal the GLU at the 6<sup>th</sup> position on beta Chain D.  
*Where on the protein are these residues located? Is this what you would expect? Why?*

*What effect might changing this residue to a hydrophobic valine have on the protein?*

- The two sequences below correspond to the one letter codes for the first 31 residues of the two different types of chain. A gap has been introduced to optimize the alignment.

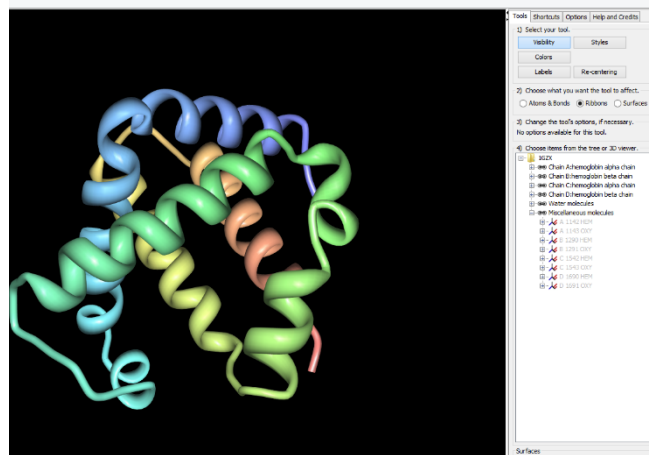
```
V L S P A D K T N V K A A W G K V G A H A G E Y G A E A L E R
V H L T P E E K S A V T A L W G K V -- N V D E V G G E A L G R
```

This part of the two chains is approximately 50% identical. Several of the residues that are not identical, are chemically similar, e.g. both serine (S) and threonine (T) have –OH groups. *What do you think is the significance of this in terms of the structure of each hemoglobin subunit?*

- Click on 1. Visibility 2. Ribbons then click on each chain to make the chains disappear. Click on 2. Atoms and Bonds, then the beta chains to make the GLU residues disappear, and on miscellaneous molecules to make the hemes and oxygens disappear. Your molecule panel should be blank.

- Click on 2. Ribbons, then Chain A. This color scheme colors the protein from blue at the N(amino)-terminus (think blue=nitrogen) to red at the C (carboxy)-terminus (think red=oxygen).

- Follow along the contours of the backbone noting the beginning and end of the chain.

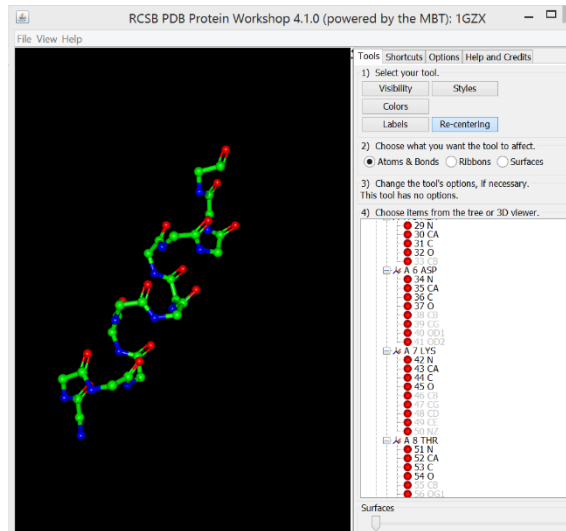


### c. Examination of secondary structure

Just as words can be strung together in common patterns (subject-verb-object) to form sentences, The polypeptide backbone can fold up in common patterns to form secondary structures.

1. To see the arrangement of backbone atoms in an alpha helix, first we must hide the whole molecule and show only the parts we are interested in.

- Select 1. Visibility, 2. Atoms and Bonds and in section 4, click the miscellaneous molecules group.
- Select 2. Ribbons then in section 4 click on each of the polypeptide chains to make them disappear.
- Select 2. Atoms and Bonds and in section 4, click the plus next to residues 5-17 and select the N, CA, C and O atoms for each of those residues.
- When finished select 1. Re-centering then click an atom in the middle of the helix.



2. Rotate the molecule to view it from different perspectives.
  - *How would you describe the shape of the backbone?*
  - *How would you describe the orientation and position of the nitrogens (which have a Hydrogen attached that is not visible) and oxygens?*
  - *How are the nitrogens and oxygens positioned relative to each other?*
  - *What type of interaction/bond can form between a hydrogen (with a polar covalent bond to nitrogen) and an oxygen?*

**The defining characteristic of secondary structural elements is that they are stabilized by \_\_\_\_\_ bonds between backbone atoms.**

**Hey, that's important:** Let's review it again: \_\_\_\_\_ structural elements are stabilized by hydrogen bonds between \_\_\_\_\_ atoms!

This means that secondary structures like the alpha helix do NOT depend on the R group/side chain atoms! Wow, that sounds like a lab quiz question, doesn't it?

3. To view the packing of atoms in the alpha helix, select 1. Styles; 2 Atoms and Bonds; 3. CPK; and in section 4, select the N, CA, C and O atoms for each of those residues from 5-17.
4. To view the location of sidechains (R-groups),
  - a. Select 1. Styles; 2 Atoms and Bonds; 3. small;
  - b. Select 1. Visibility and in section 4, select the atoms from residues 5-17 that are NOT the N, CA, C and O atoms.

*Where are the sidechains located relative to the backbone forming the helix?*

*The chemical properties of these atoms are the primary determinants of the next level of structure – tertiary.*

#### **d. Examination of tertiary structure**

Just as sentences can be strung together to compose a cohesive paragraph, the secondary structural elements can further fold on themselves to form tertiary structures. We will now examine the arrangement of several alpha-helical secondary structures into a compact tertiary structure in the hemoglobin molecule.

1. On the Options tab, click the Reset Button.  
This should re-load the original view of the hemoglobin molecule into your browser.
2. To view just one of the four subunits, click on the Tools tab, select 1. Visibility; 2. Ribbons; and in section 4 click on chains B, C & D to make them disappear. select 2. Atoms and Bonds, then in section 4, click on the Oxygen and Heme molecules in Chains A, B & C to make them disappear. select 1. Re-centering, then in section 4 click on the oxygen for chain A

Examine the way the alpha helices are arranged, making particular note of the short loops between alpha helices.

3. What types of side chains are located on the surface of the protein?  
In the shortcuts tab, select hydrophobicity and click enact.

In this view, hydrophilic residues are colored blue, intermediate residues are purple, hydrophobic sidechains are shown in red.

*Which type of sidechains are most common on the surface of the protein? Why?*

4. How are the heme groups associated with the hemoglobin protein structure?  
On the tools tab, select 1. Visibility; 2. Surfaces; then in section 4, select chain A

The heme group is still shown in ball and stick inserted into the space filling model of the hemoglobin protein. Note that the heme group fits into a slot in the protein.

5. Select 1. Visibility; 2. Atoms and Bonds; then in section 4, select the Heme and Oxygen molecules for chain A to make them disappear, note the empty slot.

#### **e. Examination of quaternary structure**

Just as paragraphs may be connected into larger units such as chapters, proteins may interact with other proteins to form quaternary structures.

1. On the options menu, click reset. On the tools tab, move the surfaces slider at the bottom of the right side panel through transparent to opaque in several steps. Be sure that the “color by” option is “chain”. Opaque shows the actual shape of the folded molecule see how the subunits interact with each other.

How would you describe the fit at the interface between the subunits?

2. Move the slider back to transparent. Select 1. Visibility; 2. Ribbons, then sequentially click each chain to identify which subunits are alpha and which are beta.

*How would you describe the arrangement of the 4 subunits relative to each other?*

*What real-world example could you use to help you remember this structure?*

#### f. Examination of non-peptide components.

1. Move the surfaces slider to off. Select 1. Visibility; 2. Atoms and Bonds; then in section 4, click the “+” next to chain A, then click on residues 58 His and 87 His in chain A.
2. Select 1. Styles; 2. Atoms and Bonds; 3. Radius of atoms: CPK then in section 4, click on residues 58 His and 87 His in chain A.

*Where in the protein are these residues located?*

*What real-world example could you use to help remember this?*

3. Carbon monoxide binds normal hemoglobin very tightly, which is how carbon monoxide poisoning can kill you. Histidine #58 prevents carbon monoxide from binding optimally. Without His58, CO would be much more toxic.
4. Notice how His87 interacts with the Fe atom on the side of heme opposite the O<sub>2</sub>. Both His87 and O<sub>2</sub> essentially “pull” on the Fe atom. When the O<sub>2</sub> is released into the tissues, His87 pulls the Fe out of the plane of the heme. This shifts the position of the alpha helix containing His 87.  
*How might this affect the other subunit in contact with the His87 alpha helix?*

This is the mechanism of cooperativity – the characteristic of many multiple subunit proteins in which events (such as binding or release of O<sub>2</sub>) on one subunit increase the likelihood of the same event on another subunit.

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## Summary Questions

1. Explain the structural differences between saturated and unsaturated fatty acids, and their implications for the physical state of a lipid (solid or liquid).

2. Indicate which nutrition facts table corresponds to bacon fat and which corresponds to olive oil.

<b>Nutrition Facts</b>	
Serving Size 1 ounce (28g)	
Amount Per Serving	
Calories 248	Calories from Fat 248
% Daily Value*	
Total Fat 28g	43%
Saturated Fat 4g	19%
Trans Fat	
Cholesterol 0mg	0%
Sodium 1mg	0%
Total Carbohydrate 0g	0%
Dietary Fiber 0g	0%
Sugars 0g	
Protein 0g	
Vitamin A 0%	Vitamin C 0%
Calcium 0%	Iron 1%

\*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

**nutritionData.com**

<b>Nutrition Facts</b>	
Serving Size 1 ounce (28g)	
Amount Per Serving	
Calories 251	Calories from Fat 251
% Daily Value*	
Total Fat 28g	43%
Saturated Fat 11g	55%
Trans Fat	
Cholesterol 27mg	9%
Sodium 42mg	2%
Total Carbohydrate 0g	0%
Dietary Fiber 0g	0%
Sugars 0g	
Protein 0g	
Vitamin A 0%	Vitamin C 0%
Calcium 0%	Iron 0%

\*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

**nutritionData.com**

3. How are individual phospholipids oriented in a biological membrane?
4. What is the chemical basis for A-T and G-C pairing? Why can't T pair with C?
5. What are the components of a nucleotide, and where is each component in the structure of a DNA molecule?
6. If one strand of DNA has the sequence 5'-TACAGTACTGATTACA - 3'  
What is the sequence of the other strand?
7. Explain antiparallelism in DNA structure.
8. If your genome contains  $6 \times 10^9$  bp, what is its molecular weight?  
What is the mass of DNA in one cell?

9. Sketch a diagram of a peptide bond. Identify the location of the bond, and of the amino acid side chains, with labels and arrows.

10. Summarize the interactions that comprise the following levels of hemoglobin structure:

primary

secondary

tertiary

quaternary

11. Briefly explain how the heme group fits into the overall hemoglobin structure.

12. What parts of the hemoglobin/heme structure actually bind oxygen?

13. What is the total molecular weight of hemoglobin?

14. You need to make one liter of a 0.5 M solution of sodium chloride. How many grams of sodium chloride should you dissolve in a final volume of 1 L of water?

15. Which has more molecules, 18 grams of water, 58.44 grams of sodium chloride, or 32 grams of oxygen?

16. Identify the structures below as specifically as possible.

