BIOS 11141 **Lab-6**

**Name:**

Please insert all answers and figures into this Word document, and then save the completed lab as “Lastname\_Lab6.docx”. When you have completed the lab, submit it as an attachment to Canvas.

**Objectives:**

1. An introduction to the Protein data bank (PDB)
2. An introduction to a software package called **Visual Molecular Dynamics**1 **(VMD).** We will use VMD to analyze basic three-dimensional characteristics of a protein.
3. Multiple Sequence Alignment using ClustalOmega website

**\*If you have a Windows computer**, you need to change directories in VMD in order to access any of the files VMD creates or saves. To do so, you should add a cd “C:/Path” command to the end of VMD’s resource script (look in the folder where VMD is installed). You can use the “pwd” command to see the path of your current directory, and the “ls” command to list the files and directories within your current directory. Additionally, typing “cd ..” will back up a step in your path. Your target directory should be the one where VMD is installed. Ask your TA for assistance.

You can download VMD from the following link: <https://www.ks.uiuc.edu/Development/Download/download.cgi?PackageName=VMD>

Also, check the VMD tutorial:

<http://www.ks.uiuc.edu/Training/Tutorials/vmd/vmd-tutorial.pdf>

**Part 1: Protein Data Bank (PDB)**

Proteins are vital biological molecules, which consist of smaller molecules called amino acids or residues, connected by peptide bonds. The resulting chain of amino acids is non-functional until it “folds” into a three-dimensional arrangement called the protein’s tertiary structure. The structure of the protein determines how it performs its function in the cell.

For a brief description of proteins, please see:

<https://cdn.rcsb.org/pdb101/learn/resources/what-is-a-protein/what-is-a-protein.pdf>

PDB 101 page contains a lot of material about proteins that I strongly encourage you to explore.

The Protein Data Bank (PDB, http://www.pdb.org/) archive contains information about experimentally determined (“resolved”) structures of proteins, nucleic acids, and complex biological assemblies. Each structure is identified by a four-character code like “1BCC”, and contains important information about the protein, such as spatial coordinates about individual atoms in the molecule, experimental procedure to obtain the structure, etc.

Navigate to the PDB website, located at <https://www.rcsb.org/>. Enter the code **2ZT9** into the search bar of PDB. This will take you to the specific page for this molecule that contains lots of information about this protein. First you see the authors who obtained the structure and the abstract for their paper. Below this, you will see the “Molecular Description”. This molecule consists of multiple protein subunits, which are separate chains of amino acids (“chains”), and each one is made of a specific number of amino acid residues (“length”). Answer the following questions:

1. What is the biological function of this protein? From what species was this protein taken?
2. What is the reference paper for this structure? Briefly describe the findings of the paper.
3. How many protein chains form the structure? How many amino acid residues are in each chain? (4 points)
4. How many ligands does this structure have? Name three of the ligands. (4 points)
5. At left corner of the window, you see a protein structure; the first structure displayed is called "Biological Assembly 1". Under the picture click the tab “3D View: structure”.
6. A new page should open with a structure of the protein in 3D that you can manipulate; play with the options to the right of the picture and take and insert a screenshot of the protein.

**b)** Go back to the main page and repeat the above by choosing the "Asymmetric Unit" option. What is the difference between the Biological Assembly and the Asymmetric Unit?

Hint, check:

<http://pdb101.rcsb.org/learn/guide-to-understanding-pdb-data/biological-assemblies>

X-ray crystallography is the chief method for determining the 3-dimensional structures of proteins: about 88% of all structures deposited in the PDB were determined (“resolved”) using this technique. This first protein structure using the technique was the oxygen carrying protein myoglobin (isolated from a sperm whale!), and was performed in the 1950s, **winning the 1962 Nobel Prize in Chemistry.**

X-ray crystallography involves creating a highly regular and pure crystal of the protein in question, and then subjecting it to an x-ray beam and measuring the diffraction of the beam, from which the positions of atoms in the molecule can be calculated. An introduction to the process is available here:

<https://stfc.ukri.org/news-events-and-publications/features/crystallography/>

<https://www.youtube.com/watch?v=gLsC4wlrR2A>

1. What was the experimental method used to obtain the 2ZT9 structure? What is the “resolution” of this structure? What is the meaning of the “resolution”? What protein structure has higher quality: One with “high resolution” or “low resolution”? (6 points).

Hint: check

<http://pdb101.rcsb.org/learn/guide-to-understanding-pdb-data/resolution>

1. Can you think of a reason (there are multiple valid answers) as to why the resolution-value increases as protein size increases? (Do not forget that the smaller the resolution-value, the better the power of resolving two atoms from each other). 3 points.

**Part 2: Introduction to VMD**

The following section is an introduction to basic operations in VMD. You need to begin by downloading the required proteins from the [Protein Data Bank](https://www.rcsb.org/), or PDB. The main unit of information deposited to the PDB is a text file (imaginatively called a .**pdb** file), which contains basic information about the protein: its origin, sequence, the experimental parameters (like its resolution), **the atomic coordinates,** etc.

The bulk of a PDB file is taken up by a list of the protein’s atoms and their respective coordinates—these coordinates describe the three-dimensional arrangement of all the atoms in the molecules, or the *structure of the protein*. These structures are typically obtained via X-ray crystallography or NMR spectroscopy techniques (we will learn about these techniques later in class).

For this lab, we will first look at the crystal structure of the COVID-19 main protease. The particular structure we will be looking at is the one with a PDB ID of “6LU7.” You have two options for importing this structure into VMD:

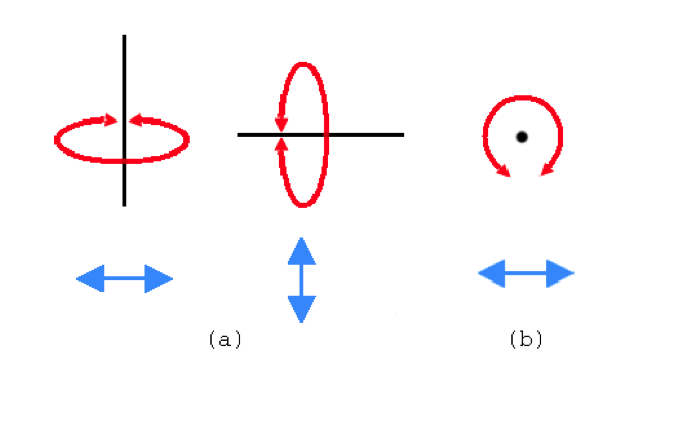
**1)** using your web browser, you can navigate to this protein’s PDB page, click **Download Files** in the top right, and then click **PDB Format**. A drop-down window will open; save this .pdb file to somewhere you will be able to locate later in the lab. Then, launch the program **VMD.** Three windows should open: A display window with a rotating “VMD” icon, a window with menu bars, and a terminal window. Minimize the terminal window. Select the “VMD Main” window and click **File 🡪 New Molecule**. In the new dialog box, select browse, and navigate to the folder where you saved the 6LU7 structure. Click on the item **6lu7.pdb**, select **Load**, and then exit from the window.This will load the 306-residue coronavirus protein *and* a synthetic “N3 inhibitor” into VMD, where the latter is a potential therapeutic agent for the disease. For more information please see: <https://pdb101.rcsb.org/motm/242>

**2) In the easier method,** you may simply **open VMD** as described above**, select File 🡪New Molecule**, and then type in the PDB accession code **6LU7** under **Filename** andclick **Load**. This will automatically retrieve the structure from the PDB for use in VMD. Note: this method currently only works for VMD version 1.9.4 or higher. You should be able to view your version at the top of the display window.

Select the Display window. You should see a wire-frame model of the COVID main protease. In the structure, **teal** corresponds to **carbon** atoms, **red** to **oxygen** atoms, **blue** to **nitrogen** atoms, and **yellow** to **sulfur** atoms. You can change the background color to see the protein representation more clearly. Go to **Graphics** **🡪** **Colors 🡪** **Display 🡪** **Background** **🡪** **8 White**. All of the images you take should be done with a white background.

To get a better look at the molecule, there are three different styles of mouse movement that you should familiarize yourself with. They are summarized in the table and corresponding figure below. Hold down the indicated mouse button and move the mouse in the prescribed fashion; you should see the molecule follow your input.

|  |  |  |
| --- | --- | --- |
| **Mouse Button** | **Mouse Movement** | **Molecule Movement** |
| Left | Left/Right | Circle about vertical axis |
| Left | Up/Down | Circle about horizontal axis |
| Right | Left/Right | Spin CW/CCW |

Try all three movements now. Additionally, there are two other mouse modes. Pressing the letter **T** on the keyboard and then clicking anywhere on the screen will allow you to shift (or **translate**) the origin of the molecule on the screen. Pressing the letter **S** (for **scale**) and then clicking on the screen will let you zoom in and out on the molecule.

You can also zoom in and out by scrolling. **Try both translating and scaling now, too.**

To return to the regular mouse mode, press **R** (for **rotate**) on the keyboard. If things get too out-of-order, pressing the = (equals) sign on the keyboard will move the molecule back to the default view.

There are several different ways to view a protein in VMD. In the VMD main window, click **Graphics** **🡪** **Representations**. Ensure that your single representation (Lines Name all) is selected; it will be highlighted green if it is. Next, navigate to the “**Drawing Method**” dropdown box towards the bottom left of the Graphical Representations window, select “**VDW,**” and observe its output on the screen. This gives you the Van Der Waals model, which is the space-filling representation of the protein atoms. Also, try the methods “**Licorice**” (which is a useful representation of the atoms and their bonds) and “**New Cartoon**” (which will help you identify the presence of α-helices and β-sheets).

To get rid of any extra atoms that you do not want to include in your visualization, you can make use of the **Selected Atoms** box. For example, after making sure the representation is selected (highlighted green), try typing “**protein**” in the box; you will notice that you can now only see the protein. **Some other commonly used “Selected Atoms” entries are:**

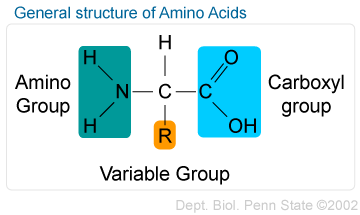
* “resname XXX” – Replacing XXX with the three-letter abbreviation for the residue will display all residues of this type (all letters in upper case). *Example: resname MET will select all Methionine residues in the protein.*
* “resid <number>” or “resid <number1> to <number2>” – Displays the listed residue number or all residues from <number1> to <number2>. The residues are indexed according to the numbering provided by the PDB file; note that multiple residues may be returned if the protein has multiple chains. *Example: resid 20 – will select the 20th residue in the protein; resid 20 to 40 – will select all residues between 20th and 40th residues in the protein, inclusively.*
* “helix” – Displays all atoms that are part of an alpha helix.
* “betasheet” - Displays all atoms that are part of a beta sheet.
* “backbone” - Displays only atoms in the backbone of the protein, i.e. only α-carbon, carbonyl carbon, oxygens in carboxyl group, and nitrogen in amino group will be displayed (see the picture below).

You can add representations by clicking **Create Rep,** and you can change the drawing method, color, and selected atoms it displays without affecting any of your other representations. You can delete drawings you have created by clicking **Delete Rep**. If you want to temporarily hide your drawing without deleting it, double-click on the representation under “**Style Color Selection”** until it turns red. You can double-click it again to unhide it at a later time. You can also increase the resolution of a representation for higher quality drawings. Note: for any changes to make effect, you MUST select the representation you intend to modify. The selected representation is indicated by the highlighting.

**Try all of the above options before moving to the next section.**

**SARS-CoV-2 Analysis in VMD**

**Note:** for any questions for which you must take measurements, please take a screenshot of the measurement (shift+command+4 in Mac) and paste the photo into your lab report. Make sure the picture is large enough to be clear (think page-width).



Make sure that the COVID protease protein (in complex with the inhibitor)is loaded and displayed in VMD. A protein consists of the generic amino acid backbone repeated many times. The generic form for an amino acid (one building block in a protein **not connected to other amino acids**) is shown in the right.

Each amino acid in a protein proceeds from an **N-terminal**

(N for amine, or the **–NH2** group), a **Cα**carbon bonded to a characteristic **R-group** (the R stands for the amino-acid **side-chain** and is the only difference between different amino acids), and the C-terminal of the amino acid (C for carboxyl, or **–COOH** group). **Proteins are read from N-terminals to C-terminals.**

We will begin examination of our 6LU7 structure by finding **its first residue**, which is Serine (SER). Check the structure of a Serine residue using the included amino-acid side chain handout at the end of this document. If you are having trouble finding this, remember that proteins are folded from linear chains and thus Serine, as the first amino acid, must be on one of the ends of the chain.

CAUTION: This protein structure was imaged in complex with an inhibitor (N3). Check the Protein Data Bank page (here: <https://www.rcsb.org/structure/6lu7>) to make sure you are looking at the proper chain. You will find that there are two chains (A and C), and that one of them corresponds to the protease and the other corresponds to the N3 inhibitor. Make sure you are considering the first residues from the proper chain.

After finding this Serine, work your way down the protein molecule and identify the next three amino acids in the protease by looking at the amino acid side chains (and using the table provided at the end of the lab), or by clicking in the VMD Main window, selecting **Mouse 🡪 Label 🡪 Atoms,** **OR by pressing “1”** on the keypad. Clicking an atom will display the residue name, number, and which atom is selected. Using the Drawing Method “CPK” will be helpful for making precise residue selections for this and future exercises.

To clear these labels, go to **Graphics 🡪 Labels**. After ensuring that “Atoms” is selected in the drop-down at the top left, you can select one or more labels and either “Hide” them, causing the text to turn red, or you can “Delete” them. You can also go to **Graphics 🡪 Colors** to change the color of the label if you can’t read it well. The colors settings for labels will be in the **Labels** category. Note that you can modify the colors of atom, bond, angles and other labels. This will be useful later in the lab.

**Q1.** Fill in the blanks in the following table by finding the residue names of the next three residues. Note that the R-group may be a hydrogen atom, as in glycine.

|  |  |
| --- | --- |
| **Residue Number** | **Amino Acid** |
| 1 | SER |
| 2 | ? |
| 3 | ? |
| 4 | ? |

Finding amino acids this way can be tedious and slow. A different, more efficient way of accomplishing this in VMD involves the Sequence Viewer, which can be found by clicking on the VMD Main window and going to Extensions **🡪** Analysis **🡪** Sequence Viewer. This tool gives a residue’s number and name, as well as any secondary structure the residue is involved in. The secondary structure letters stand for:

|  |  |  |
| --- | --- | --- |
| **Letter** | **Color** | **Secondary Structure** |
| T | Teal | Turn |
| **E** | **Yellow** | **Extended conformation (beta sheet)** |
| B | Gold | Isolated bridge |
| **H** | **Purple/Magenta** | **Alpha helix** |
| G | Light Pink | 3-10 helix |
| I | Red | Pi helix |
| C | White | Coil |

**Q2.** Use the Sequence Viewer to find the residue numbers and names of the last five amino acids in the COVID protease (NOT the inhibitor).

|  |  |
| --- | --- |
| **Residue Number** | **Amino Acid** |
| ? | ? |
| ? | ? |
| ? | ? |
| ? | ? |
| ? | ? |

**Q3.** The first non-coil secondary structure is an alpha helix.

1. Which residue number does the helix start at, and what amino acid is it?
2. What is the ending residue number, and what amino acid is that residue?
3. Select the alpha helix the Sequence Viewer. Amend the representation of this alpha helix by using “**Secondary Structure**” as the coloring method and “**NewCartoon**” as the drawing method. Include a screenshot of this alpha helix along with the rest of the protease protein.

Sequence Viewer also allows you to highlight specific amino acids in the display window by clicking on their names in the Sequence Viewer window. A selected residue will turn yellow, and it will become highlighted in the display window. Multiple residues can be selected by clicking while holding down the **Shift** command. A continuous range of atoms can be selected by clicking and dragging, which will produce a red box that can be used to select atoms. If you want to see more or fewer amino acids, use the Zoom bar on the left side of the Sequence Viewer.

Selecting residues in the Sequence Viewer will produce a new representation of that selection in the Graphical Representations window. This representation will be changed the next time you make a selection, so to duplicate the current representation, you can click on it and click “Create Rep”.

Note that you can go into the “Display” settings and choose between “Perspective” and “Orthographic” display modes. The “Perspective” setting gives the viewer greater depth perception at the expense of distorting scale relationships, while the “Orthographic” setting preserves these scale relationships and maintains the parallelism of lines, but with less depth perception. Try both options. **The “Orthographic” setting is better for creating pictures using VMD. Use this setting to create the pictures in the questions below.**

Before proceeding to the imaging questions, we need to clean up our visualization a bit.Firstly, recall that the 6LU7 structure was resolved via x-ray diffraction. As an artifact of this imaging technique, you will notice that there are extra red atoms (oxygen) indicative of water molecules. In order to exclude water, we can use the selection “not water.” We do not want the water in the image, so it is essential that it be removed.

**Q4.** Try selecting protein residues 21, 48, and 165 at once using the Sequence Viewer. Find the representation created by the Sequence Viewer for these residues.

1. What is typed into the “**Selected Atoms**” box to give you these residues?
2. What is the drawing method that the Sequence Viewer is using?
3. Include a screenshot of the *protein* shown as **new cartoon** with these selected residues shown clearly. Note that, as mentioned before, there is also the *N3 inhibitor* present. Therefore, in this same screenshot, the N3 inhibitor should separately have the style DynamicBonds. Also, set the inhibitor’s coloring method to ColorID, and then pick a color that contrasts clearly against the protease.

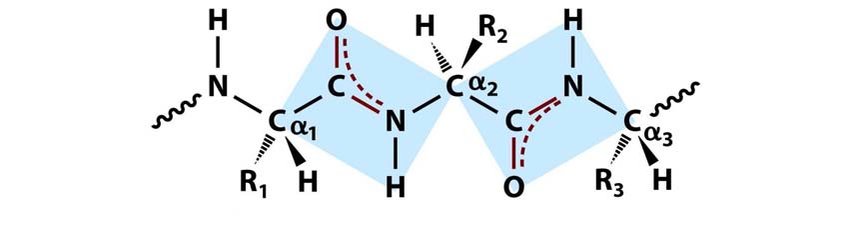
With VMD, you can also measure bond lengths, angles, and dihedral angles of your protein. To measure the distance between two atoms, press **Mouse 🡪 Label 🡪 Bonds** **OR the number “2”** on the keypad, and then choose the two atoms you want to measure; for bond angles, press **Mouse 🡪 Label 🡪 Angles** **OR the number “3”** on the keypad, and then select three atoms (Atom1 🡪 Atom2 🡪 Atom3).

If you are zoomed out, it can be hard to select the center of an atom, so it may be helpful to zoom in. If it seems like it is not working, it is possible that you are not selecting the center of the desired atom. Remember that using the drawing method “CPK” might make it easier to click on a particular atom.

To un-label an atom that is labeled, simply right-click the atom. Alternately, you can go into the VMD Main window and click on Graphics 🡪 Labels to either hide or delete labels.

**Q5.** Measure the distance between any two atoms

1. Insert a picture of the measurement that clearly shows the distance. You might need to modify the color for this label. Refer to prior instructions on how to do it. We recommend using Black as your new color.
2. Drawing on your previous knowledge, what unit do you think these measurements are in? If you are not sure, this information might be found by looking at the PDB page for 6LU7 (think about how the structure was obtained).

**Q6.**  Again, find the first Serine residue on the molecule (**type protein and "resid 1 2" in the VMD selection window; then push the equal sign to center these residues in your screen; remember to hide any other representations to reduce clutter**). From this residue, follow the molecule with your mouse (from N-terminal to C-terminal) until you reach the atom to which an oxygen molecule is bonded. This is the carbonyl carbon, and the preceding carbon is the alpha carbon. Note that in the labels, the carbonyl carbon has “C” after the colon, while the alpha carbon has “CA”. You may find the “lines” or “CPK” drawing methods helpful. Measure the following bond distances (No picture is required but units are):

1. Amide N to alpha C:
2. Carbonyl C to alpha C:
3. Carbonyl C to N in the peptide bond:

**Q7.** Repeat this exercise for the same bonds in residue 25 (hint: in the VMD screen just show residue 24, 25, and 26; no picture is required):

Amide N to alpha C:

Carbonyl C to alpha C:

Carbonyl C to N in the peptide bond:

**Q8.** Of the three types of bonds we measured, which type of bond appears to be the shortest? Can you think of the reason why this is the case?

**Q9.** Describe the function of the COVID-19 main protease and briefly explain the purpose of the synthetic N3 inhibitor. (Hint: check the PDB101 page; you can google the answer!).

**Q10.** Go to the PDB page for the 6LU7 protein, at left corner of the window you see a protein structure; the first structure displayed is called "Biological Assembly 1". If you click on the arrow above this picture you will see another picture called "Asymmetric Unit". What is the difference between the Biological Assembly and the Asymmetric Unit?

Hint, check:

<http://pdb101.rcsb.org/learn/guide-to-understanding-pdb-data/biological-assemblies>

**Part 3: Multiple Sequence Alignment with ClustalOmega**

In your previous labs, you implemented the Center Star Algorithm to create multiple sequence alignments. However, there are many other heuristic approaches to multiple sequence alignment, packaged in many different alignment programs.

The EMBL-European Bioinformatics Institute provides online servers and links to descriptions for several common multiple sequence alignment programs: <http://www.ebi.ac.uk/Tools/msa/>

Let's construct a multiple sequence alignment for a selection of proteins from the globin family of proteins, a widely distributed family of proteins that bind and transport oxygen. Copy the file globins.fasta from Canvas that contains sequences of six globin proteins from diverse eukaryotic organisms. These include the human hemoglobin subunits alpha and beta; sperm whale myoglobin; globin proteins from a lamprey (Chordate) and insect (Arthropod) species; and a plant leghemoglobin. These sequences are stored in the **FASTA file format** discussed in class. Each sequence contains a header line by which the gene is identified, marked with the ">" character. The sequence begins after the header line and includes all characters up until the subsequent ">" character.

***DO NOT USE CHROME FOR THE FOLLOWING EXERCISE***

From the EMBL-EBI Multiple Sequence Alignment page linked above, click "Launch Clustal Omega," which opens a form for web-based submission of a set of sequences to be aligned. All of these programs also contain original source code, which can be downloaded to one's own computer to compute multiple alignments locally.

Copy the contents of globin.fasta to the Input window. Click "Submit" with the default options. The calculation should run relatively quickly given the small number of sequences we are aligning. The results of the alignment will appear in the window after several seconds. Go to "Results Viewer" and under the "Jalview" section, copy and paste the URL with the clipboard next to it into a new tab.

**1.** There are three symbols that appear below alignment columns, which represent three different qualities of the column: sites that are entirely conserved as a single amino acid, sites in which all sequences have amino acids with strongly similar biochemical properties, and sites in which all sequences have amino acids with weakly similar biochemical properties.

a) Which symbol corresponds to which class?

b) How many sites are completely conserved across the alignment?

c) What does this imply about the functional importance of these sites?

A picture containing graphical user interface

Description automatically generated

Still under the "Result Viewers" tab, download the Jalview app by clicking the "Free installation programs are available". Once you've done this, click "View results with Jalview". **Jalview** is a very common alignment viewer with many useful features. It can also be downloaded as a local program from the Jalview homepage. Once the Jalview applet opens click the "Color" tab and select "Clustalx". You will see a window like this:

The alignment we saw before is now layered with additional information. This coloring scheme colors amino acids by conservation of biochemical properties. This allows us to visualize broader patterns that pertain to protein sequences beyond conservation of amino acid identities. Strict conservation at the level of amino acid identities is reflected in the "Conservation" bar chart below. The Quality score reflects the local Blosum62 alignment score contributed by a given column. Finally, Jalview has a couple of convenient features for indexing. Along the top of the alignment, the multiple sequence alignment is numbered by column. Within each sequence, if you move your pointer over an amino acid, at the bottom of the window you can see the index number that amino acid holds in that particular protein.

**2.** Consult the [UniProt](https://www.uniprot.org/) page for human hemoglobin subunit beta (the UniProt accession number is P68871). Under the "Sites" annotation, this page lists two amino acids that participate in the coordination of an iron ion and binding oxygen (designated "Metal binding"). Determine the residue numbers of these amino acids and find their corresponding columns in the alignment. What are the identities of these amino acids in hemoglobin beta and the other globins in this alignment? What might this imply about functional conservation in this family?

Navigate in the Menu toolbar to Color > Hydrophobicity. In this view, the most hydrophobic residues are colored red while hydrophilic residues are blue.

**3.** Scroll along the entire alignment and look at how well the hydrophobic/hydrophilic character of an amino acid position is maintained within an alignment column. What might this imply about the 3-dimensional structures of these six proteins? (Remember that generally speaking, hydrophobic amino acids are buried in the interior of a protein structure while hydrophilic residues are found on the surface.)

**4.** Do you see any qualitative correlation between hydrophobicity and conservation? What does this say about where amino acid substitutions are best tolerated in a protein?

Return to Color > Clustalx. Now let's annotate the alignment with the known secondary structure elements of human hemoglobin beta. Right click on the "Conservation" label and select "Add New Row." Give this new row the Annotation Label "Secondary Structure." Return to the hemoglobin beta UniProt page and scroll down to "Structure." At the beginning of this section, click the "Show more details" link below the Secondary Structure cartoon. This lists the different secondary structural elements of this protein. The first helix corresponds to residues 6-17. Determine which columns of the alignment these residues correspond to by moving your cursor over the hemoglobin beta amino acids (remember, the hemoglobin beta numbering will be different than the multiple sequence alignment numbering). In the newly made "Secondary Structure" row of the annotations window, drag your mouse across to create a red highlighted region. Right click and label as "Helix," with the sub-designation "a" to mark the first helix of the protein. Be careful. Navigating away from the "Result Summary" tab in your web browser will close the Jalview applet and lose any annotations.

**5.** Repeat this process for the other helices noted on the UniProt page. Paste a screen shot of the Jalview window spanning as much of the alignment as you can.

While sequence alignment developed historically in the context of evolutionary analysis (and was introduced that way in these exercises), many of these algorithms are finding additional uses with modern sequencing technologies. For example, if we sequence an individual's genome and want to see where his or her genome differs from a reference genome, we will conduct pairwise alignments between genomic regions. Similarly, pairwise alignment to a reference genome is used to infer from where reads from assays such as ChIP-Seq or RNA-Seq derived. Furthermore, sequence alignment is used in the initial assembly of genomes from millions of short, overlapping reads. Thus, the algorithms and difficulties in sequence alignment relevant for evolutionary analysis have deep implications for many different fields of biology.

**Works Cited**

1. Humphrey, W., Dalke, A. and Schulten, K., ”VMD - Visual Molecular Dynamics”, J. Molec. Graphics, 1996, vol. 14, pp. 33-38.

2. Vijay-Kumar et al., JMB, 194:531, 1987

3. "Working with a Single Molecule." *Using VMD*. University of Illinois at Urbana-Champaign, 1 Feb. 2012. Web.

