Protein Module Student Handout

Name_____

1. What are some of the functions that proteins have in a living organism.

2. Define the following and list two amino acids that fit each description.

Term	Definition	Example Amino Acids
Hydrophobic		
Hydrophilic		
Acidic		
Basic		

3. Go to https://concord.org/stem-resources/protein- and play with the simulation. Focus on how the protein folds in water. Where do the hydrophobic residues tend to move? Where do the hydrophilic residues tend to move?

4. If the following primary sequence of amino acids is placed in water, describe where each of the amino acids would go (inside/outside) when the protein folds.
Ala-His-Phe-Trp-Tyr-Gly-Lys

5. In the process of protein folding, once the primary sequence has undergone hydrophobic collapse, hydrogen bonds begin to form between the backbones of the amino acids. Describe and sketch the two main types of secondary structures.

6. Two other important structures that for during protein folding are **β** -turns and disulfide bonds. Sketch both of these structures below showing the amino acids involved in each structure.

7. You will now use computational chemistry in order to explore the connection between the primary sequence of a protein of your choice and its overall structure. Describe three reasons why it would be important to know how a mutation (change in amino acid) would affect a protein.

8. Describe three advantages of using computational chemistry over traditional lab techniques to explore chemical processes.

9. Briefly describe how a computer simulates chemical processes.

10. Go to www.rcsb.org You will use the protein data bank in order to select a protein which you would like to explore in more detail. It is best to choose a protein with a short primary sequence (<25 amino acids). Each protein is given a four character identifier. These can be typed into the search bar on the website. Some examples of small proteins are listed below. You can use one of these or search for your own. (You can limit the size of the proteins if you use advanced search —>search by molecular mass—> between 1 and 3000 Daltons).

2N0E   1GJE   2MFX   2EQH   PDB Code of Protein Chosen_____
2N2S   2N2S   2N07
11. Briefly describe the function of the protein that you chose.

12. Open (Download if needed) the program VMD. Click "Extensions" → "Simulations" → "qwikMD" There is a blank bar at the top of the qwikMD window. Enter your 4-character PDB code into this bar and click "load". Your protein should appear in the viewer window.

You may get error messages during this process. Follow these steps to check for and fix these errors.
• For any pop-up window that appears, click "ok"
• Click the button that says "Structure Manipulation" on the upper right of the
  qwikMD window.
• In the Structure Manipulation window you will see a list of amino acids (residues). If any molecules are highlighted in red, you must delete them. To do this, click the circle next to delete in the upper right of the window. Click on item to be deleted and then click "apply." Repeat this process for all items highlighted in red.
• On the lower right of the Structure Manipulation window, click "ignore" under structure check.

13. Now that your protein is loaded into qwikMD, we will explore its primary and secondary structure. Use the viewer window to rotate and move the protein until you have a good view of its entire structure. The left mouse button allows you to rotate the view. You can use the scroll wheel to zoom and the right mouse button to adjust the orientation of the window. Pressing "t" on your keyboard will change the left mouse button to translate the view of the protein. Pressing "r" on your keyboard will return the mouse to its default behavior. Capture and save a screen shot of your protein in its native (not mutated) state. Go to the VMD Main window and click "File"→ "Render"→ "Render the current scene using"→ "Tachyon (internal, in-memory rendering)"→ "Browse." Navigate to where you want to save your image and enter a name. Be sure to use the .tga file extension. Click "OK" and then click "Start Rendering." The image should immediately open.

You can change the representation of the protein by clicking "NewCartoon" under "Representation" and selecting a representation from the drop-down menu. NewCartoon is the best for viewing structural changes to the protein, while "Licorice" is good for viewing the structure of the amino acid side chains. You can change the representation at any point during the simulation.

14. List the types and number of the secondary structures, and turns in your protein. For **β**-sheets, record the number or rows that are in the structure. To view the secondary structures by color code, click on the color of the protein under "Color" in the qwikMD window, scroll up in the drop-down menu, and select "Structure." The key to the colors can be found in the Structure Manipulation window.

15. Return to the Structure manipulation window and select the circle next to "View". If you now click on an amino acid, it will be highlighted in the viewer window. You will now choose 3 amino acids to mutate and explore the effects of these mutations on the overall structure of the protein. For this simulation we will be doing a substitution, switching one amino acid for another. When selecting these amino acids and what they will be replaced with, you should consider the following in order to produce the largest structural changes:
• Hydrophilicity/hydrophobicity
• Physical size of the amino acid side chain
• Shape of the amino acid side chain
•Charge of the amino acid side chain
• Position of the amino acid relative to secondary structures
• Intermolecular forces between the amino acid side chains

In the chart below, list the amino acids that you have selected to mutate, number in the primary sequence, the amino acid that you will substitute in, and why you decided to make this change.

****Note: Avoid mutating cysteine residues as changing residues in disulfide bonds can crash the program.

Residue Number	Original Amino Acid	Substituted Amino Acid	Describe why you made this change.

16. Predict how the secondary structures and overall structure of your protein because of the mutations you chose. Be sure to explain why you think these changes will occur based on the factors described in question #15.

17. You will now use qwikMD to make these mutations in your protein. In the Structure Manipulation window, click the circle next to "Mutate." You may choose whether you want to try all of the mutations at once or run them one at a time. Click on the name of the amino acid that you want to change and then select the amino acid which you would like to substitute in from the drop-down menu. Repeat for up to three amino acids.

18. To run the simulation, return to the qwikMD window and click "Advanced Run". Click the drop-down menu next to "Solvent" and change it to "Implicit." Click the arrow next to "Protocol." In the chart under "Protocol" select "Annealing" and then click the minus sign. It should disappear from the chart. Repeat this process for "Equilibration." Under "Simulation Setup" check the box next to "Live View" and then click "Prepare." The program will ask you to save the files (make sure that there are no spaces in the file name you choose), then click "Start Minimization Run" at the bottom of the screen and select no more than 3 processors when asked. If you get a message that "namd2 could not be found," click "Yes" to locate it, navigate to the location that you downloaded NAMD to, and choose the file "namd2."

19. When the progress bar at the bottom of the qwikMD window is filled, click "finish." Next, click "Start MD Simulation at the bottom of the screen and select no more than 3 processors when asked. Now return to the viewer window and watch as the software calculates the new positions of all of the atoms in your protein. If the protein is moving, the program is working. You can use the "view" function of the Structure Manipulation window to highlight specific amino acids as they move.

20. There is a progress bar at the bottom of the qwikMD window. This tells you how much "time" has been simulated for the protein. We will only be looking at how the protein moves within a nanosecond. Once the simulation has run for about 0.3 nanoseconds, you can update the color coding of the secondary structures. To do this, go to the VMD Main window and click "Extensions"→ "Tk Console." Type `mol ssrecalc top` into the Tk Console window and press enter. The colors in the viewer window will update to represent the secondary structure in the mutated protein.

21. Click "Finish" at the bottom of the qwikMD window to stop the simulation and save a screen capture of the protein as described in step #13. Carefully compare the structure of your protein before and after the mutations. Note: Do not close VMD without clicking "Finish" in qwikMD, otherwise your simulation will continue to run in the background!

You will now summarize the results of your simulation in three paragraphs as follows:

 Paragraph 1: Describe, in detail, how the overall structure and secondary structures of
  your protein changed or did not change during the simulation.
 Paragraph 2: Compare these changes to your predictions in #16.
 Paragraph 3: Explain, using what you have learned about amino acids and intermolecular
  forces, why or why not your result matches your hypothesis.
   ***Be sure to include the before and after images of your protein in your summary and refer to
   them in your summary.