Laboratory 2 Worksheet

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Q1. What is the secondary structure (alpha helix or beta strand) predicted by AlphaFold based on the primary sequence? **(2 pts)**

Alpha Helix

Q2. What is the amino acid present at the N-terminus of the peptide? **(2 pts)**

Methionine

Q3. Looking at the primary sequence, what four amino acid residues do you think favors the formation of a helix? **(2 pts)**

L (Leucine), E (Glutamate), K (Lysine), A (Alanine)

Q4. How does this new AlphaFold predicted structure compare to the first structure you loaded on PyMOL? How are they similar and different? Briefly explain why there is a difference. **(8 pts)**

The new AlphaFold predicted structure compares to the first structure by having a different structure.

The difference is that the first structure has a full alpha helix, whereas the new structure stops the alpha helix halfway in the protein.

The reason why there is a different structure is because in the new structure, there contains a GPGP repeat of Proline and Glycine, which are helix breakers. As a result, the new structure does not have a full helix.

The similarity between the two AlphaFold structures are that they both contain a section of alpha helixes at one point.

Q5. 2EVQ contains β-strands as its secondary structure. Is the β-sheet parallel or anti-parallel? Briefly explain why. **(4 pts)**

Antiparallel the strands are running in different directions as shown in AlphaFold with both arrows pointing away from each other instead of in the same direction. If it was the same direction, the beta sheet would be parallel. However, because they are different directions the beta sheet is anti-parallel

Q6. Using the **PYMOL measurement wizard**, measure the donor – acceptor hydrogen-bond distances that form the **expected characteristic (see references at back of this lab manual)** hydrogen bonds **for the type of β-sheet identified in Question 5**. **(6 pts)**

Residue and Number (H-acceptor C=O)	Residue and Number (H-donor NH)	Bond distance (Å)
K9	N4	1.8

T2	T10	1.8
T10	T2	2.0
N4	K9	1.8

Q7. Examine the residues of the two β-strands, paying close attention to the relative abundance of polar and apolar groups. Note that a β-strand can separate in space, hydrophilic residues on one side from apolar residues on the opposite side. What structural pattern (*alternating sides, same side)* is evident for the side chains for each amino acid in the N-terminal to C-terminal direction of the β-strands? Which side chains of each β-strand contribute to the polar surface, and which contribute to the apolar surface? **(5 pts)**

The structural pattern that is observed is alternating sides for the side chains for each amino acid in the N-terminal to C-terminal direction of the β-strands. The side chains THR2, ASN4, THR11, and LYS9 contribute to the polar surface. The side chains TRP3 and TRP10 contribute to the apolar surface.

Q8. Measure and record the distance of any hydrogen bonds expected in a turn. Record your data in the table below. **(1.5 pts)**

Residue and Number (H-acceptor C=O)	Residue and Number (H-donor NH)	Bond distance (Å)
P5	G8	3.4

Q9. Measure the φ (i+1), ψ (i+1), φ (i+2), ψ (i+2) dihedral angles in the turn. The i+1 and i+2 angles are those for the second and third amino acid residues in the β turn. Record your data in the table below. **(2 pts)**

Φ(i+1)	ψ(i+1)	Φ(i+2)	ψ(i+2)
-63.3	-40.3	-107.4	-23.2

Q10. According to the dihedral angle recording in **Question 9**, classify the turn (**γ Classical, β-Type I, β-Type II, β-Type III etc)** according to the criteria listed in the table at the back of the lab manual. Note: Angles will differ by as much as ± 30° from the ideal expected values for β-Type turns, and ± 40° for γ-Type turns due to steric hindrance. **(2 pts)**

β-Type I

Q11. Based on the yellow dotted lines, identify the residues on the protein that are interacting with the ligand. To identify the residue, click on the residue and check if it connects with the end of the yellow line. If it is, click on **S (*Show*)** and select **sticks** and **L *(Label)*** followed by **residues**. Record your data in the table below. **(10 pts)**

Amino Acid Name and Number	Bond distance(s) (Å)
A180	3.3, 3.4

R10	3.1
D32	2.7, 3.0, 3.0
S95	3.5
I11	3.2

Q12. What residue did you select to mutate and what did you mutate it to? What is the new hydrogen bond distance between your newly mutated residue and the ligand. Record your data in the table provided below. **(1.5 pts)**

Original Residue and Number	Mutated Residue and Number	Bond distance (Å)
D32	G32	0