Lecture 5:
Introduction to Proteins

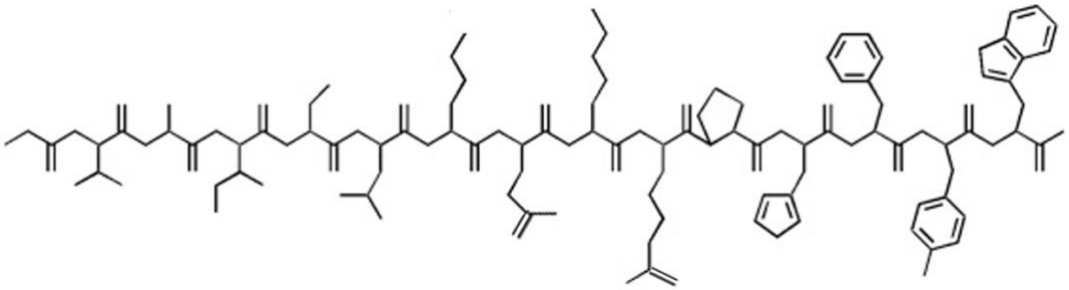
Question 1:

Which of the following statements are TRUE, and which are FALSE?

a. Protein domains are units of secondary protein structure.
b. Glycine is less-restricted in terms of possible ϕ and ψ angles, which is why it is often found in loop elements of proteins.
c. Glutamine (Gln) is more hydrophobic compared to Asparagine (Asn).
d. The net charge of hydrophobic amino acids (e.g., Ala) would not change if the pH was set to 1.
e. Proline residues favor forming *cis* peptide bonds while *trans* is found less frequently.
f. Beta sheets in proteins are exclusively composed of antiparallel strands.
g. All amino acids except glycine are chiral at the α carbon.

Question 2:

This sketch shows the bonding topologies of amino acids in a polypeptide. Only bonds between non-hydrogen atoms are shown but single- and double-bonds are distinguished.



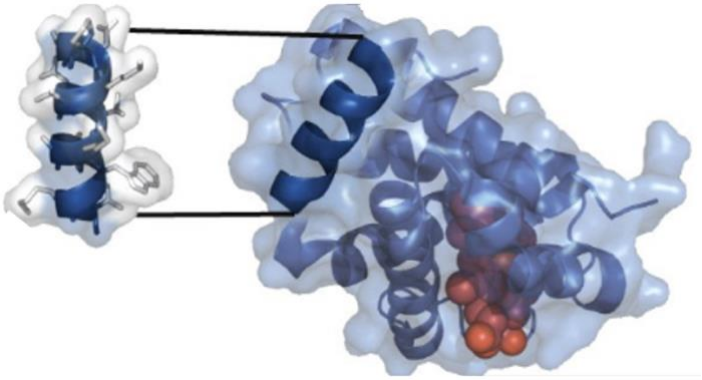
a) Indicate the N- and C-terminus of the polypeptide.

b) Identify the amino-acids at each position in the polypeptide chain and label them using their one-letter code. Note that in some places there can be more than one choice that matches the shape. In that case indicate the alternative (isosteric) amino acid.

c) Categorize each amino acid into the following categories: (i) non-polar aliphatic, (ii) aromatic, (iii) polar uncharged, (iv) positively charged, (v) negatively charged.

Question 3:

Why are isolated secondary structural elements typically not stable in solution, even though all backbone torsion restraints are satisfied, and stabilizing hydrogen bonds can form?



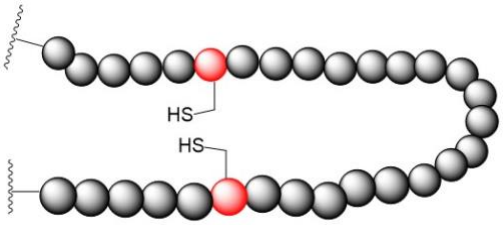
Question 4:

Draw the structure of a tripeptide: **Ala-His-Lys**. Identify and label the N-terminus, the C-terminus and the two peptide bonds. Indicate the backbone rotation angles (ω, ϕ, ψ) around both peptide bonds.

a) How much rotational movement is allowed for the ω angle? Why?
b) Are ϕ and ψ angles more restrained compared to ω? What chemical diagram defines the most favorable ϕ, ψ angles for amino-acids?

Question 5:

See below structure of a polypeptide chain with 2 cysteine residues that directly face each other.



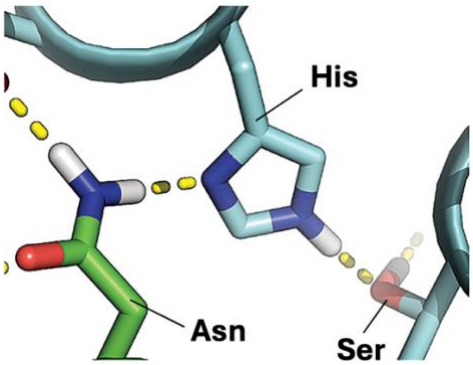
a) Will this peptide assembly differ if the protein was located inside versus outside of a cell? How?

b) Can the assembly of this peptide be influenced by treatment with external chemicals possessing oxidizing or reducing properties? What would be the outcome in each case?

c) Cysteine is an amino-acid that can be used to covalently attach chemical groups, labels or even other proteins. Can you describe how this could work? What would be the necessary chemical group that the binding partner must have in order to attach?

Question 6:

Below you will find a structure of a small region inside a random protein, showing Histidine (His) interacting with surrounding residues (Asn and Ser), in a solution that is at neutral pH (=7.0). In the image, the carbons are depicted cyan/green, oxygens are red, nitrogens are blue, and hydrogens are white. Carbon-bound hydrogens and double bonds are intentionally not shown to improve visibility.



a) Can you identify which interaction is formed between His and Asn (dashed line)? What about His and Ser? Identify the role of each group in each interacting pair.

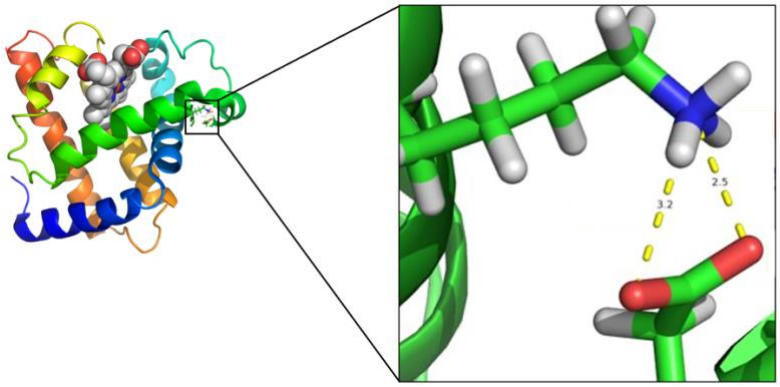
b) Can you describe what will happen with the Histidine residue if the pH of the solution was decreased to 4.0?

c) How will this affect the interaction network?

Question 7:

Mutations are changes in the order of nucleotides in DNA genes that translate into changes in amino-acid sequence of the corresponding gene-encoded proteins. If a mutation occurs at a single amino-acid position, it is called a point-mutation. Biochemists often intentionally introduce point-mutations in their proteins of interest to perturb underlying interaction networks and evaluate how the mutated amino-acid(s) impact protein structure or function.

Below you will find a close-up view of the interface of 2 protein domains highlighting two amino-acids that interact with each other at the interface of two domains. In the close-up panel, carbons are depicted in green, hydrogens in white, nitrogens in blue and oxygens in red.



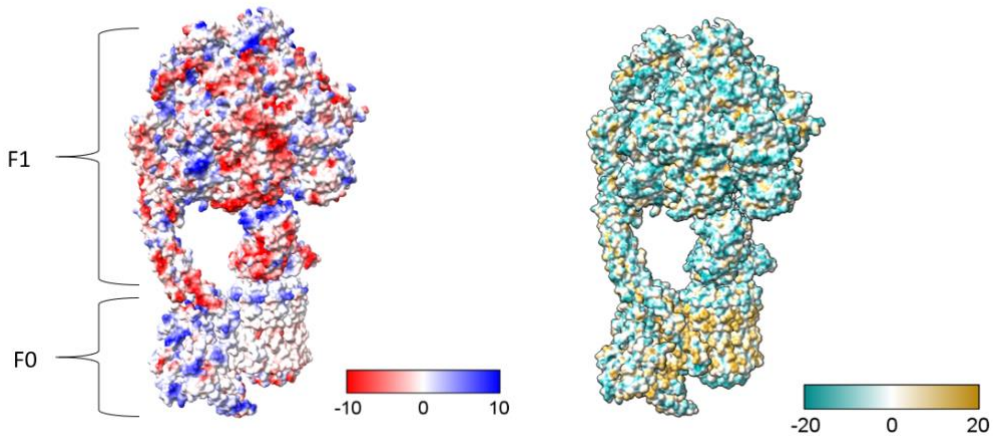
a) Can you identify the two amino-acids based on the side chains groups?

b) Can you identify the type of interaction that is created by these two amino-acids? What equation describes the energy potential of this interaction?

c) The biochemist working on this project hypothesized that this interacting pair is the key to keeping the two helical domains and the entire protein structure stabilized in the current state. The next step is to test that by mutating one of the two amino-acids in this pair to a different type of amino-acid that would completely disrupt this interaction. Given the type of interaction between these amino-acids, what mutations would you propose for testing? Propose a few alternatives if you can and discuss what mutations would have the strongest effect. What would be the effect of simultaneously mutating the two amino-acids to each other (swapping their respective locations)?

Question 8:

Answer the following questions looking at the electrostatic and hydrophobic surface maps of the ATP synthase. Assume all amino acid side chains are in their typical charge states at physiological pH. Electrostatic potential map is shown on the left and colored from negative (red) to positive (blue). Hydrophobicity surface map is shown on the right and colored from least (cyan) to most (yellow) hydrophobic.



a) Which amino acids comprise the red and blue surface patches in the panel on the left? What about the possible amino acids in the white surface patches?

b) Correlate that to the panel on the right (hydrophobicity). Based on these two plots, which amino acids likely comprise the continuous yellow region in F0 subunit?

c) What does this suggest about the function or cellular location of this part of the protein? Propose, what is the likely cell localization of F0 and F1 subunits?

d) Electrostatic forces at the catalytic sites within the F1 domain are important for binding phosphate and facilitating the release of newly synthesized ATP. If a mutation replaced a positively charged arginine in the binding site with a 1) lysine 2) alanine 3) glutamate, hypothesize the possible effect from an amino acid property perspective.