Lecture 5:
Introduction to Proteins

Question 1:

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

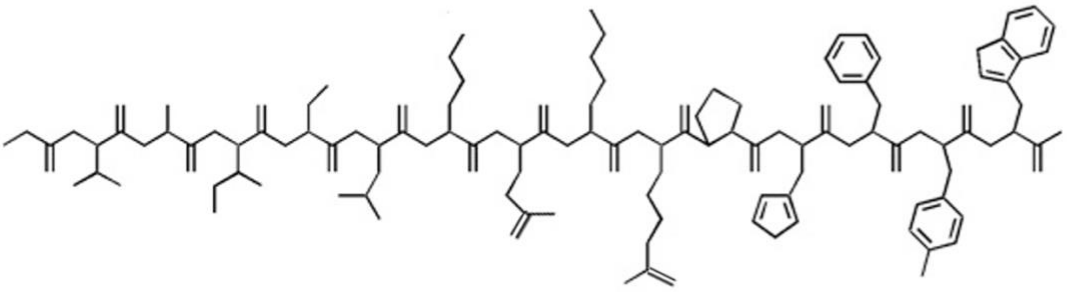
a. Protein domains are units of secondary protein structure.
b. Glycine is less-restricted in terms of possible ϕ and ψ angles, which is why it is often found in loop elements of proteins.
c. Glutamine (Gln) is more hydrophobic compared to Asparagine (Asn).
d. The net charge of hydrophobic amino acids (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.

Answers:

a. **FALSE**: In fact, the opposite is true. Secondary structure elements assemble into protein domains.
b. **TRUE**: The φ and ψ angles for glycine are less restricted due to having just hydrogen as a side chain (see Ramachandran plot). So this amino-acid is enriched in loops and turns as it can more readily accommodate sharp twists of the polypeptide chain or flexibility.
c. **TRUE**: The relative hydrophobicity value for Gln is 3.3 and for Asn is 3.8. This is likely due to the extra CH2 group in the side chain.
d. **FALSE**: At pH 1 the carboxylic acid moiety would get fully protonated and loose its negative charge, making the net charge of this amino acid to be +1, due to the amino group.
e. **FALSE**: The cis bond is disfavored compared to trans in all amino-acids including Pro. Only ~3% of proline residues in existing structures in the PDB are cis. However, due to the imino-nature of proline, the cis state is relatively more common compared to other amino-acids.
f. **FALSE**. Beta sheets can be made of both antiparallel and parallel strands; antiparallel is more common, but parallel arrangements also occur.
g. **TRUE**. Glycine's α carbon is bonded to two hydrogens, so it is not chiral; all other standard amino acids have four different groups attached, making them chiral.

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.



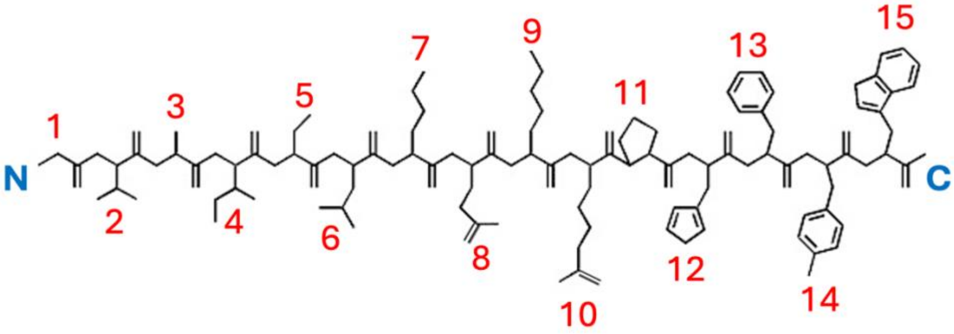
a) Indicate the N- and C-terminus of the polypeptide.

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.

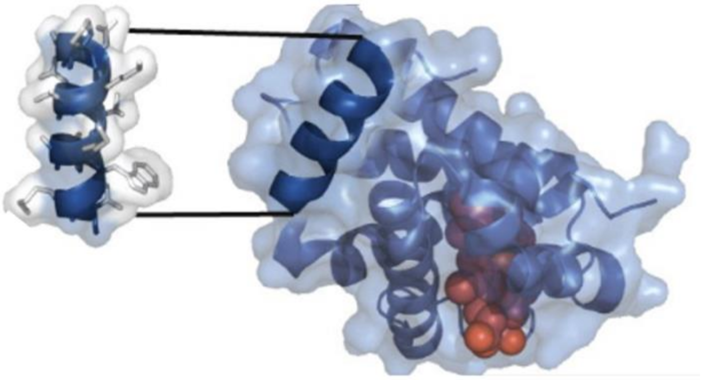
Answer:

Position	Amino acid (option 1)	Category	Amino acid (option 2)	Category
1	G	non-polar aliphatic		
2	V	non-polar aliphatic	T	polar uncharged
3	A	non-polar aliphatic		
4	I	non-polar aliphatic		
5	S	polar uncharged	C	polar uncharged
6	L	non-polar aliphatic		
7	M	non-polar aliphatic		
8	E	negatively charged	Q	polar uncharged
9	K	positively charged		
10	R	positively charged		
11	P	non-polar aliphatic (cyclic)		
12	H	polar uncharged or positively charged		
13	F	aromatic		
14	Y	aromatic		
15	W	aromatic		



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?



Answer:

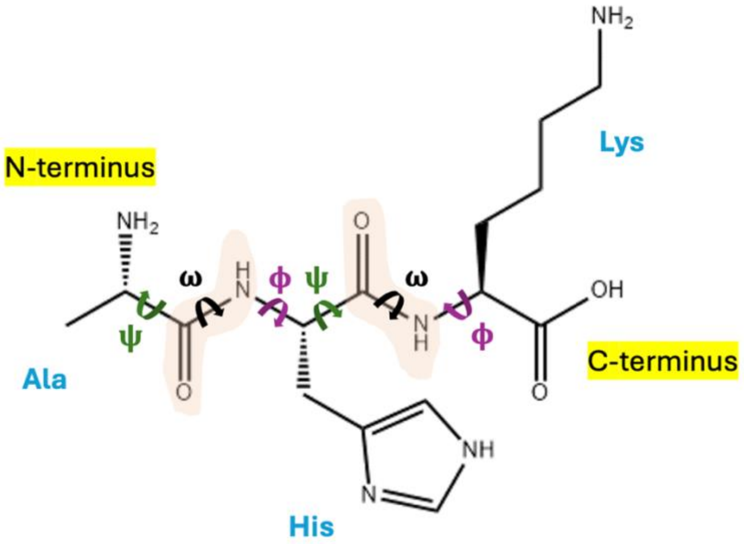
The hydrophobic effect is the major force driving protein folding and governing the stability of globular proteins. Isolated secondary structural elements cannot bury hydrophobic residues away from water and thus do not gain stability from the hydrophobic effect. While backbone hydrogen bonds could contribute stabilizing energy to the folded state, the backbone –NH and –C=O groups of the unfolded polypeptide hydrogen bond to water. Upon folding, formation of secondary structure elements replaces hydrogen bonds to water with hydrogen bonds to other parts of the protein backbone. Thus, the summed energy of hydrogen bonding does not change upon folding.

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.

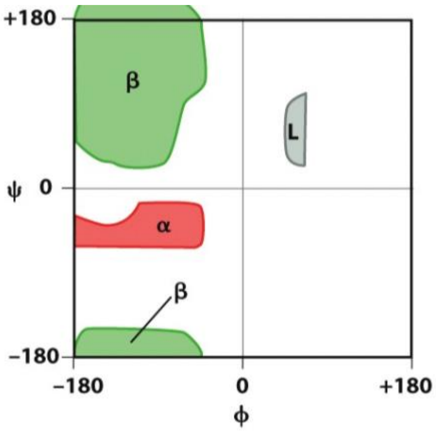
a) How much rotational movement is allowed for the ω angle? Why?
b) Are ϕ and ψ angles more restrained compared to ω? What chemical diagram defines the most favorable ϕ, ψ angles for amino-acids?

Answer:



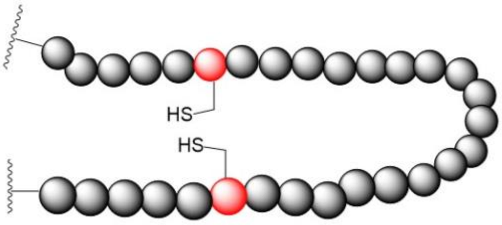
a) The rotation around the peptide bond is restricted to only minor deviations (+/- 10˚) from the default angle of 180˚ (in trans) or 0˚ (in cis), which is imposed by the double-bond character of the peptide bond.

b) The angular space for the possible ϕ and ψ angles is significantly less restricted compared to ω, but still has limitations. The allowed and disallowed angles for each amino-acid are defined by the Ramachandran plot.



Question 5:

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



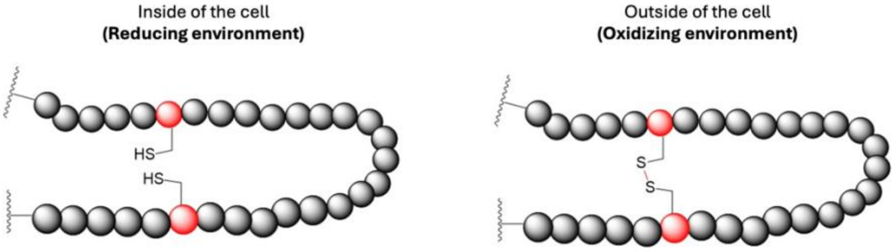
a) Will this peptide assembly differ if the protein was located inside versus outside of a cell? How?

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?

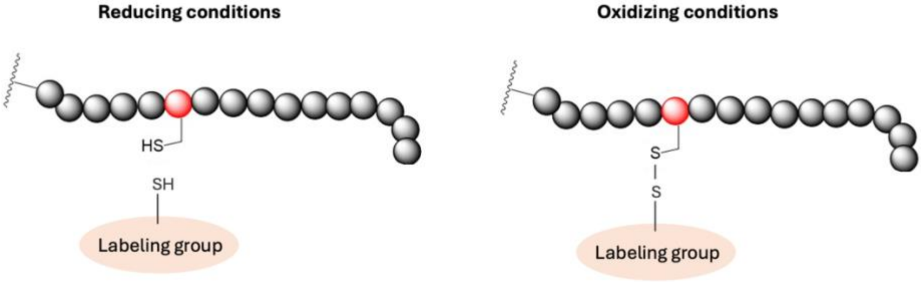
Answers:

a) Cell cytoplasm is a reducing environment and therefore the two cysteines will stay in their reduced form. Outside of the cell, the environment is oxidizing, and the disulfide bond ("cystine") will form between the two cysteines.



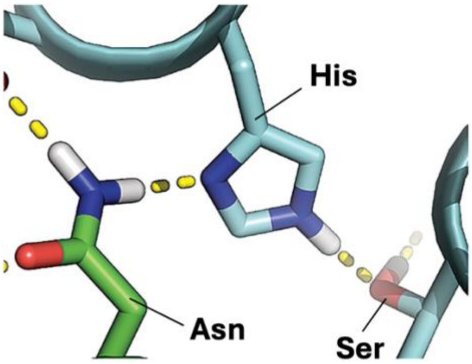
b) Disulfide bond can be broken *in vitro* by the addition of reducing agents such as glutathione (GSH), dithiothreitol (DTT), b-mercaptoethanol. Conversely, the addition of oxidizing agents, or simply removal of reducing agents will create an environment that favors the disulfide formation.

c) Exposed cysteine residues in proteins can be used for attachment of different chemical agents, tags or proteins that must have a free exposed sulfhydryl (-SH) group (e.g., another cysteine). Under reducing conditions the two groups can be mixed and the reaction will be induced by removal of the reducing agent (e.g., by dialysis).



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.

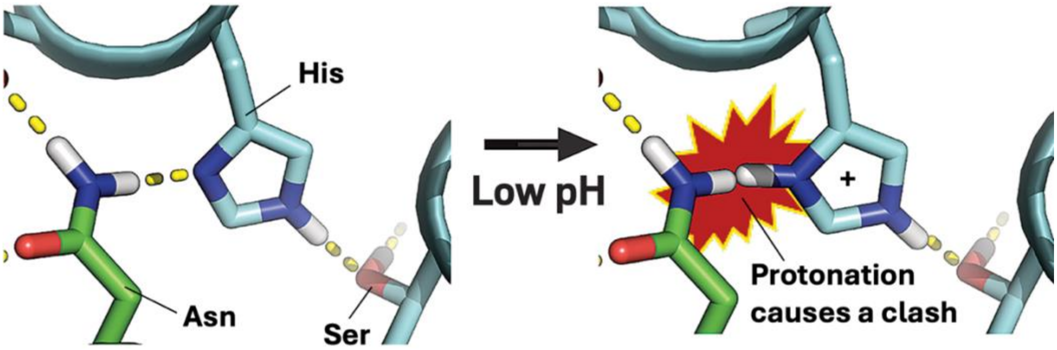
b) Can you describe what will happen with the Histidine residue if the pH of the solution was decreased to 4.0?

c) How will this affect the interaction network?

Answers:

a) These are both hydrogen bonds. In the case of Ser, Histidine acts as a hydrogen bond donor via the NH group. In the case of Asn, Histidine acts as a hydrogen bond acceptor via the non-protonated nitrogen.

b) At low pH, the non-protonated nitrogen atom in the ring facing towards Asn, will receive a hydrogen atom from water giving a net positive charge to the His side chain.

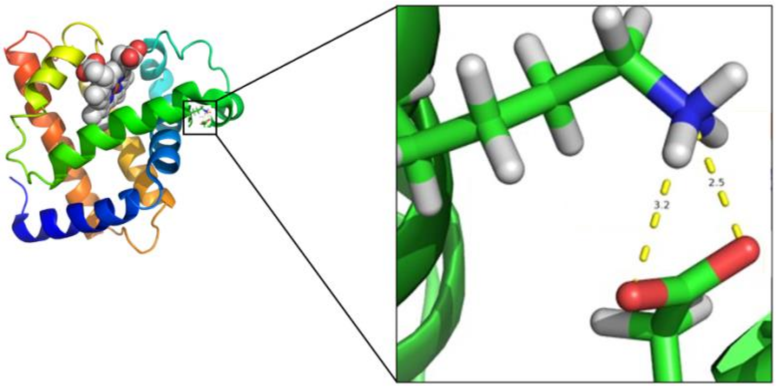


c) This will result in breakage of the existing hydrogen bond with Asn residue, and induce a direct clash with the NH2 group of Asn. As a consequence, one of the two residues will have to adjust its position to accommodate this change. This can sometimes trigger a much larger conformational rearrangement that involves movement of entire protein domains (often used by viral proteins).

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.



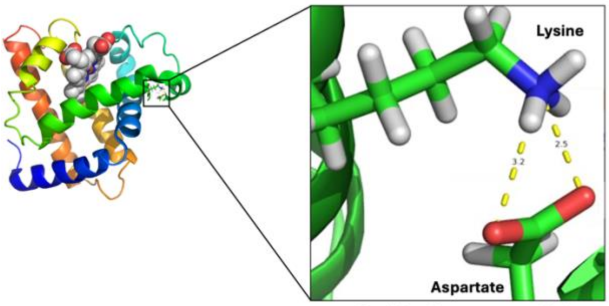
a) Can you identify the two amino-acids based on the side chains groups?

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)?

Answers

a)



Could be Glutamate as well since we don't see the bottom part

b) Lysine is a positively charged amino acid while Aspartate is negatively charged. Therefore, they form attractive electrostatic interaction with each other. To calculate the interaction energy we can use Coulomb’s law:

$$U(r) = \frac{1}{4\pi\epsilon_0}\frac{1}{D}\frac{q_1 q_2}{r}$$

The net charge of Lys at neutral pH is +1 while the net charge of Asp is -1.

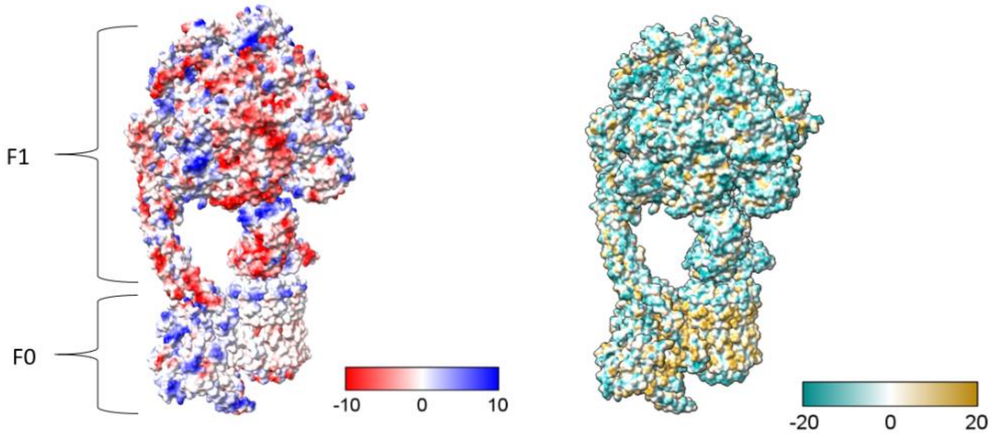
c) In general, you can disrupt this interacting pair by simply replacing any of the two residues with a non-charged amino acid. This would eliminate the electrostatic interaction between them.

However, the strongest effect (at least in theory) would be caused by mutation of either amino-acid (Lys or Asp) to a residue of opposite charge. This would result in a pair of positively or negatively charged amino acids in close proximity which would electrostatically repel each other, thereby strongly disrupting the assembly of this domain. For example, you could mutate the Lys to either Asp or Glu (both negatively charged). On the other side you could mutate the Asp to either Lys or Arg (both positively charged).

If you swapped the locations of two amino acids (i.e., Lys -> Asp and Asp -> Lys) the electrostatic interaction would still form similarly to the original version of the protein. However, the geometry of binding (e.g., group distances) may be slightly different due to swapped residue locations, which could affect the strength of this interaction.

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.

Answers:

a) Acidic amino acids (aspartic acid, glutamic acid) carry negative charges and cluster in the red (negatively charged) areas. Basic amino acids like lysine and arginine are positively charged and cluster in the blue (positively charged) areas. Histidine is classed as basic but is mostly neutral at physiological pH (~7.4), though it can become positively charged in certain environments or active sites to participate in electrostatic interactions. White surface patches comprise all other amino acids including uncharged polar and hydrophobic residues.

b) The hydrophobicity plot provides additional information regarding the locations of hydrophobic residues. It is almost an inverse of the electrostatic plot, but not perfect. The largest continuous yellow surface in F0 likely comprises hydrophobic residues such as: isoleucine, leucine, valine, alanine, phenylalanine, tyrosine, tryptophan, methionine, proline or glycine.

c) The largest continuous hydrophobic area appears in the F0 domain, shown by yellow coloring in the hydrophobicity map. This indicates a region rich in hydrophobic amino acids, consistent with a transmembrane domain, possibly embedded in a lipid bilayer. The F1 subunit has polar (charged and uncharged) segments and does not have any continuous surface-exposed hydrophobic area to serve as transmembrane domain.

Therefore, it is likely exposed to solvent on the external or internal side of the membrane (cannot be sure based on just this data). Indeed, the F0 subunit forms the membrane-embedded channel, whereas the F1 subunit is the catalytic head responsible for ATP synthesis.

d) Effects of mutating a positively charged arginine in the binding site:
1) Lysine: Both are positively charged, so the mutation may retain ionic interactions with phosphate, though slightly different side chain geometry might alter binding somewhat.
2) Alanine: Neutral and hydrophobic, losing the positive charge would weaken electrostatic binding to phosphate, likely reducing phosphate affinity and catalytic efficiency.
3) Glutamate: Negatively charged, this mutation would introduce charge repulsion with phosphate groups, strongly disrupting phosphate binding and ATP synthesis.