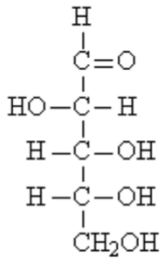
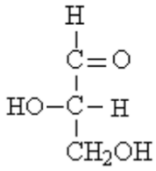
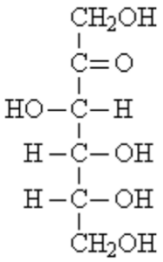
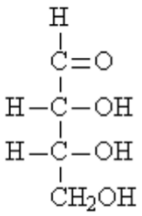
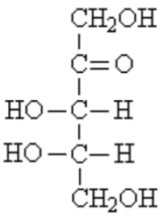
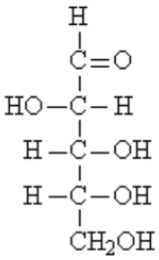
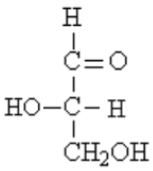
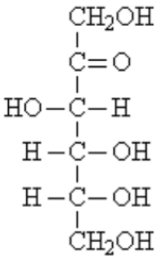
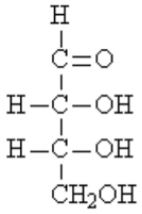
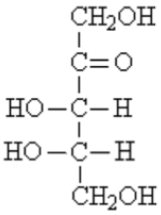
Lecture 3:
Exercise Carbohydrates and Proteins

Question 1:

Below you will find linear structures of several monosaccharides. Classify them based on the (1) length of carbon chain, (2) functional group and (3) stereochemistry (handedness).

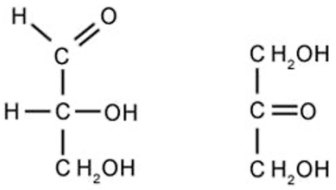
Answer

Pentose	Triose	Hexose	Tetrose	Pentose
Aldose	Aldose	Ketose	Aldose	Ketose
D	L	D	D	L

Question 2:

The smallest monosaccharides are trioses, glyceraldehyde and dihydroxyacetone shown below:



Glyceraldehyde

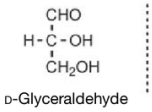
Dihydroxyacetone

a) Can you draw the stereoisomeric versions of each monosaccharide? Include the D- or L-annotation.

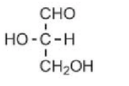
b) Can these monosaccharides assemble into cyclic forms in aqeuous solutions? Explain.

Answers:

a)



D-Glyceraldehyde



L-Glyceraldehyde

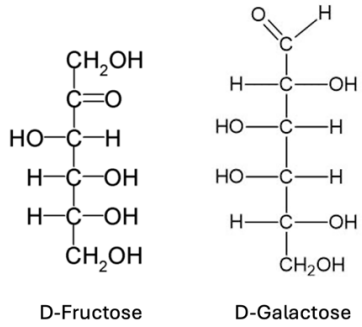
Dihydroxyacetone

Dixydroxyacetone does not have an asymetric chiral center which is why there are no stereoisomers.

b) They cannot assemble spontaneously as pentose and hexose sugars. The length of the carbon backbone does not allow sufficient flexibility for the conversion to cyclic form to take place. To readily convert to cyclic forms the sugars need to have minimally 5 carbons.

Question 3:

Below are the linear structures of D-fructose and D-galactose.



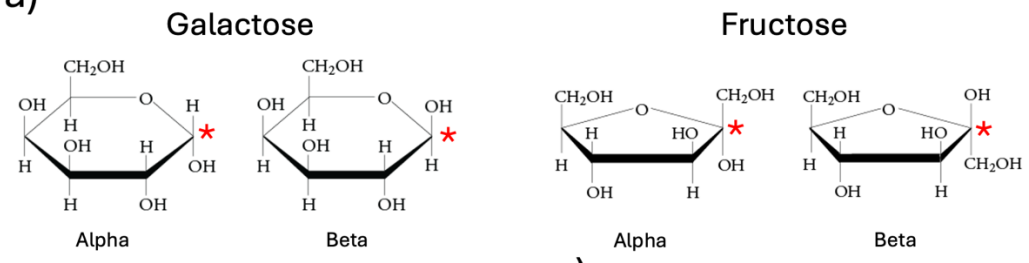
a) Draw the cyclic structures of each sugar in alpha (α) stereoisomeric form and indicate the location of anomeric carbon. How does the beta (β) form differ from the alpha?

b) Lactulose (galatose-β(1→4)-fructose) is a disaccharide used in the treatment of constipation and hepatic encephalopathy. It is assembled from **1 D-galactose** and **1 D-fructose** building block through **β-1,4 O-glycosidic linkage**. Draw the structure of this disaccharide based on the cyclic forms of each monosaccharide building block.

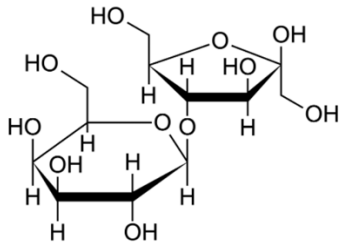
c) Melibiulose (galactose-α(1→6)-fructose) is another disaccharide assembled from the same buidling blocks using the **α(1→6) O-glycosidic bond**. Draw the structure of this disaccharide based on the cyclic forms of each monosaccharide building block.

Answers:

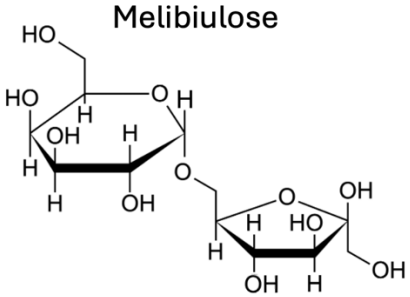
a)



b)



c)



* - anomeric carbon

Question 4:

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

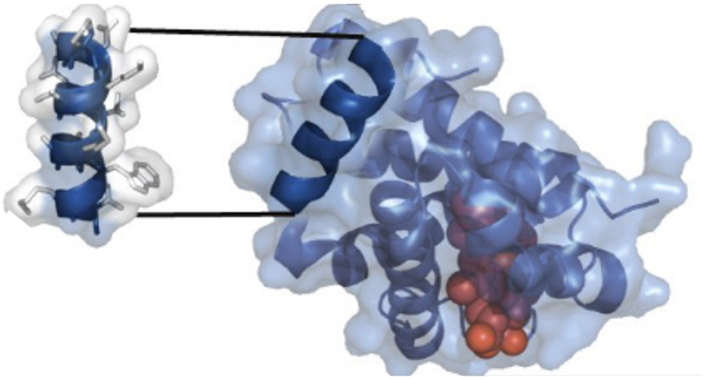
a. Protein domains are units of secondary protein structure.
b. Glycine is less-restricted in terms of possible φ and ψ angles, which is why it is often found in loop structures in proteins.
c. Glutamine (Gln) is more hydrophobic compared to Asparagine (Asn).
d. The net charge of hydrophobic amino acids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

Answers:

a. **FALSE**: In fact, the opposite is true. Secondary structure elements assemble into protein domains. Protein domains are distinct structural and functional units within a protein's three-dimensional structure. These domains often fold independently and contribute to the overall tertiary structure of the protein. They can perform specific functions, such as binding to other molecules or catalyzing reactions.
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**: Due to the imino-nature of proline, the cis bond is heavily disfavored compared to trans. Only ~3% of proline residues in existing structures in the PDB are cis.

Question 5:

Why are isolated secondary structural elements typically not stable in isolation, even though all backbone hydrogen bonds are satisfied?



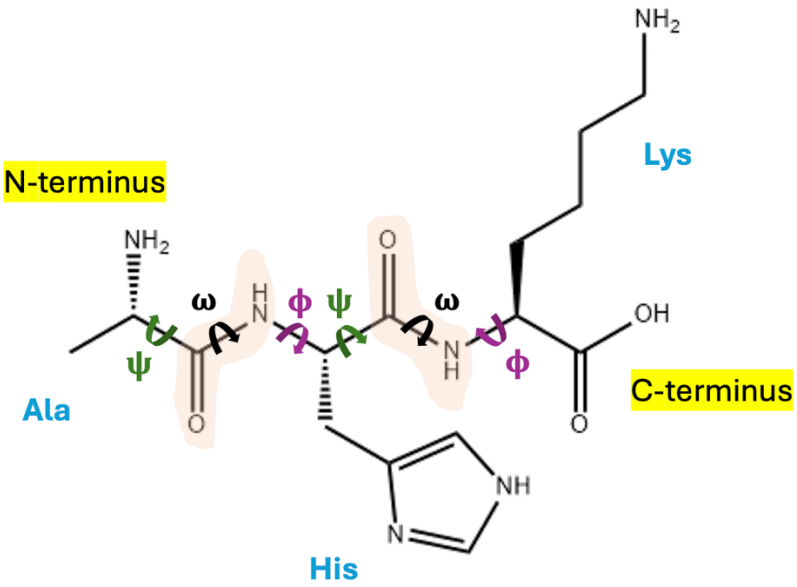
Answer:

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 6:

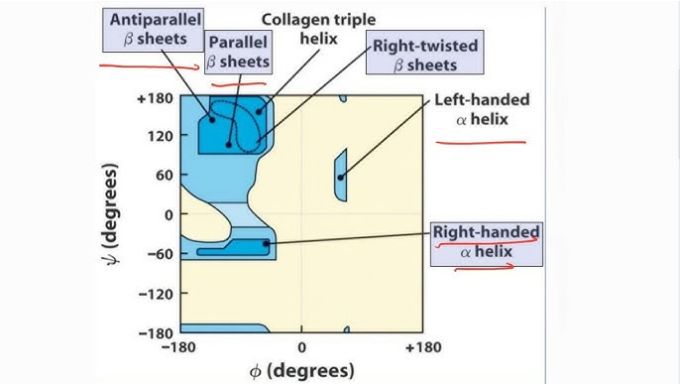
Draw the structure of a tripeptide: **Ala-His-Lys**. Identify and label the N-terminus, the C-terminus and the two peptide bonds. Indicate the backbone rotation angles (**ω**, **ϕ**, **ψ**) around both peptide bonds.
a) How much rotational movement is allowed for the **ω** angle? Why?
b) How much rotational movement is allowed for **ϕ**, **ψ** angles? What chemical diagram defines the most favorable **ϕ**, **ψ** angles for amino-acids?

Answer:



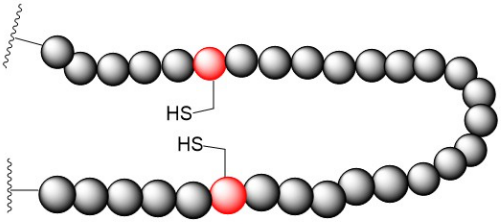
a) The rotation around the peptide bond is restricted to only minor deviations (+/- 10˚) from the default angle of 180˚ (in trans) or 0˚ (in cis), which is imposed by the double-bond character of the peptide bond.

b) The angular space for the possible **ϕ** and **ψ** angles is significantly less restricted, but still has limitations. The allowed and disallowed angles for each amino-acid are defined by the Ramachandran plot. This is an example for alanine:



Question 7:

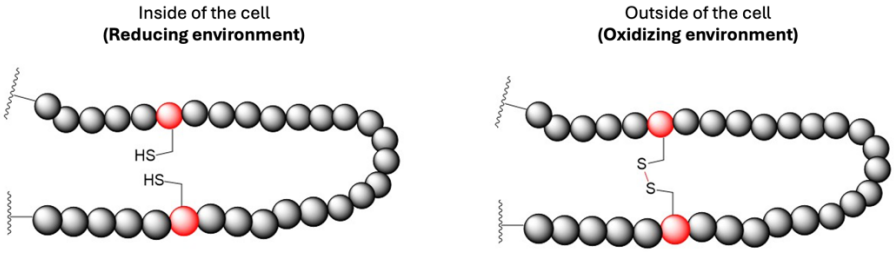
See below structure of a polypeptide chain with 2 cysteine residues that directly face each other.



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?

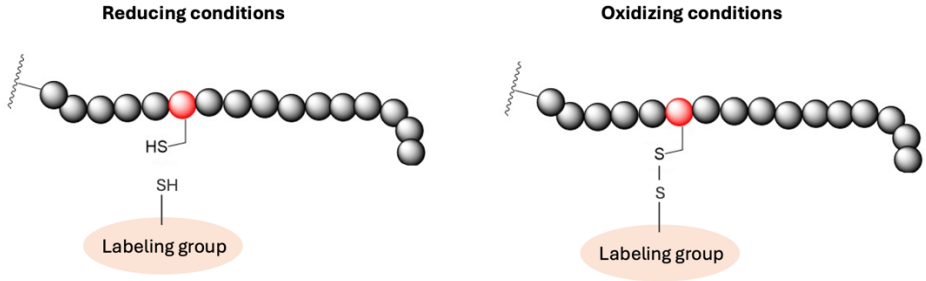
Answers:

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.



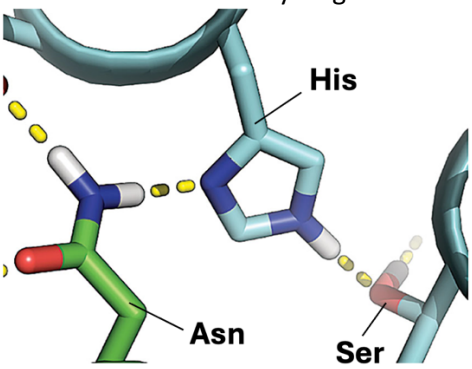
b) Disulfide bond can be broken *in vitro* by the addition of reducing agents such as glutathione (GSH), dithiothreitol (DTT), b-mercaptoethanol. Conversely, the addition of oxidizing agents, or simply removal of reducing agents will create an environment that favors the disulfide formation.

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 8:

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?

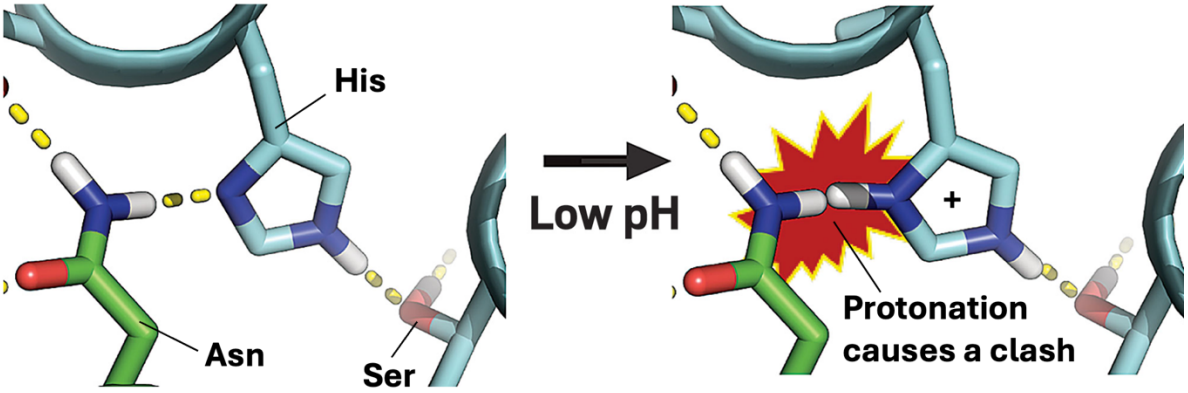
b) Can you describe what will happen with the Histidine residue if the pH of the solution was decreased to 4.0?

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.

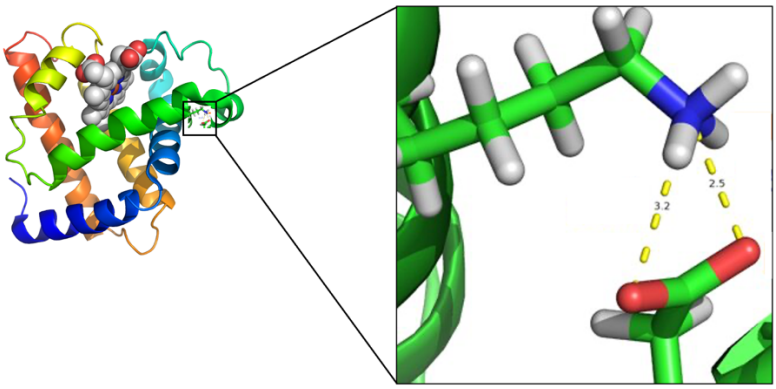


c) This will result in breakage of the existing hydrogen bond with Asn residue, and induce a direct clash with the NH2 group of Asn. As a consequence, one of the two residues will have to adjust its position to accommodate this change. This can sometimes trigger a much larger conformational rearrangement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

Question 9:

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.



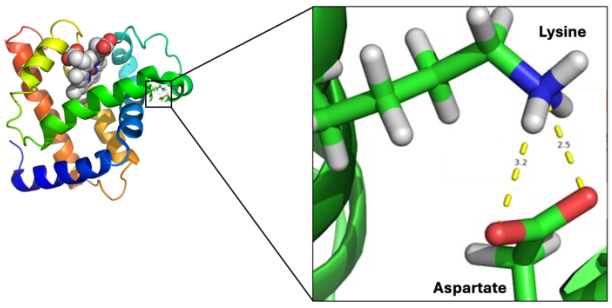
a) Can you identify the two amino-acids based on the side chains groups?

b) Can you identify the type of interaction that is created by these two amino-acids? Calculate the energy associated with this interaction if the average distance between the functional groups is 3.0Å. Assume aqueous environment with the dielectric constant (D) of 80.

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. The biochemist will then measure if the mutation really impacted the 3D assembly of this domain. 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

electrostatic interaction with each other. To calculate the interaction energy we can use Coulomb's law:

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

$$= 1391\ kJ \cdot mol^{-1}\frac{q_i q_j}{r_{ij}[\mathring{A}]}\frac{1}{D}$$ **in water**

The net charge of Lys at neutral pH is +1 while the net charge of Asp is -1. The distance is 3.0Å and the dielectric constant is 80. When you plug in the numbers you get:

U(r) = -5.8 kj/mol

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.