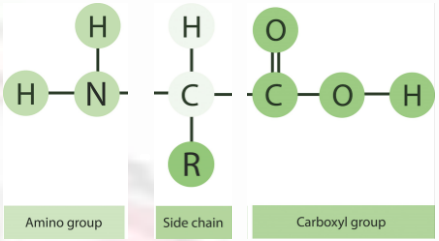
STRUCTURAL BIOLOGY

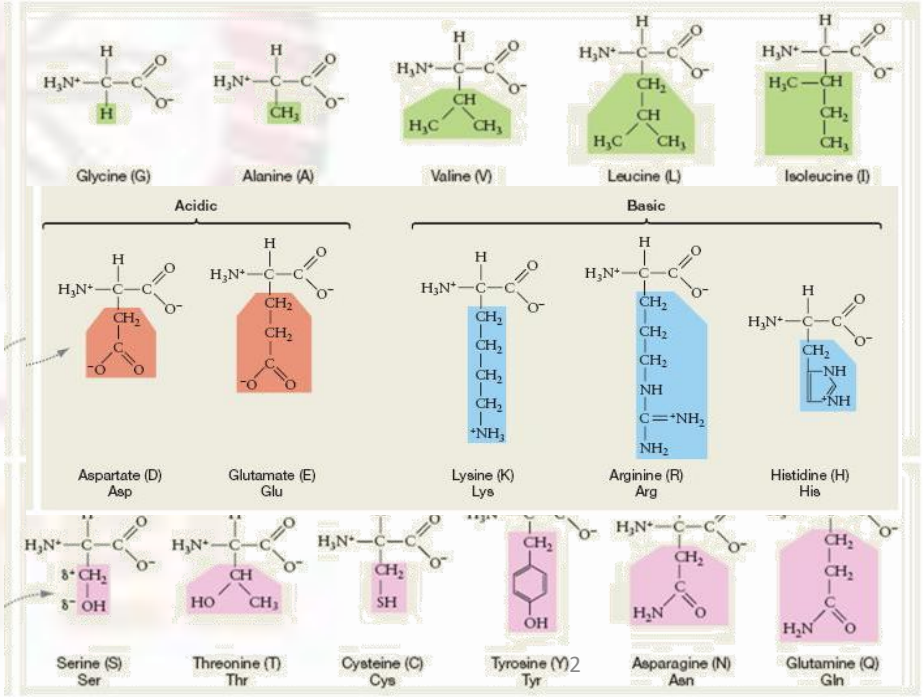
Live Session- Week 2
8 Feb, 2022

Summary

Week_2_Lecture_1: About Amino acids and their properties.

1. Amino acids are the organic compounds containing carbon, hydrogen, oxygen and nitrogen and they serve as monomers of proteins.
2. As the name implies, they contain an amino group and a carboxylic acid group. The various amino acids differ with respect to the side chain R attached to it.
3. Among standard amino acids, nine amino acids contain non-polar side chain. These are *Glycine, Alanine, Valine, Leucine, Isoleucine, Proline, Methionine, Phenylalanine and Tryptophan*.
4. Six amino acids contain uncharged polar side chain, they are *Serine, Threonine, Cysteine, Asparagine, Glutamine and Tyrosine*.
5. *Lysine & Arginine* have side chains that contain positively charged groups and amino acids *Aspartate & Glutamate* contain acidic side chains.

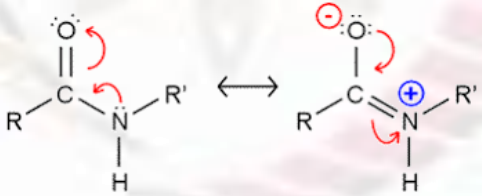




Summary

Week_2_Lecture_2: This lecture is about Peptide bond and levels of protein structure.

1. A peptide is a compound consisting of two or more amino acids. The average molecular weight of a standard amino acid is nearer to 128 Da.
2. During the formation of a polypeptide, one water molecule is removed, so the average molecular weight of an amino acid in a polypeptide is about 110 Da (128-18 (water weight)).
3. The peptide bond has a partial double bond character, where the oxygen has a partial negative charge and the nitrogen has a partial positive charge, setting up a small electric dipole.

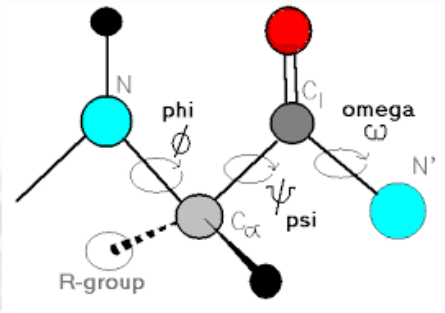


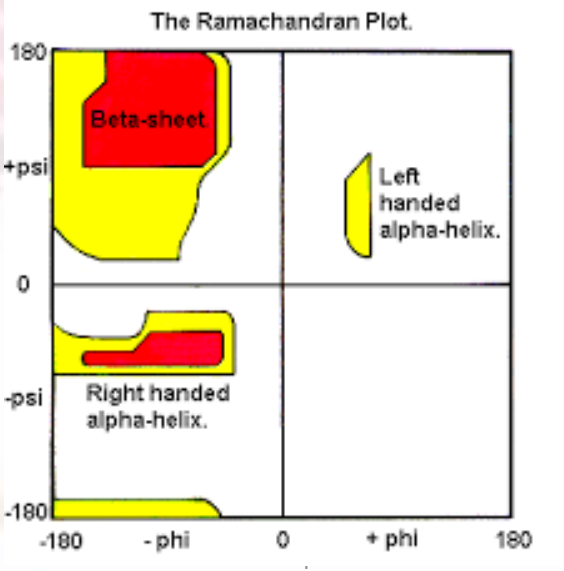
4. Proteins have four levels of the structural organization. *Primary structure* is the amino acid sequence specified by genetic information. As the polypeptide chain folds, it forms certain localized arrangements of adjacent amino acids that constitute secondary structure.
5. The overall three-dimensional shape that a polypeptide assumes is called the tertiary structure. Proteins that's consist of two or more polypeptide chains are said to have a quaternary structure

Summary

Week_2_Lecture_3: Focuses on protein dihedrals and Ramachandran Plot.

1. The torsion angle about the bond between the amino nitrogen and the α-carbon atoms is called φ whereas the torsion angle about the bond between the α-carbon and the carbonyl carbon atoms is ψ.
2. The restricted rotation about C—N bond can be specified by torsional angle omega.
3. Phi and Psi can have any values between +180° to -180°. Most of the values are not allowed due to the steric interference between backbone and side chain atoms.
4. The combinations of phi and psi values that are permitted in a peptide backbone or that are not permitted due to steric constraints were first determined by ***G N Ramachandra***.
5. These permitted values can be visualized on a two dimensional plot called a Ramachandran Plot.
6. The Ramachandran plot provides a way to view the distribution of torsion angles in a protein structure and shows that the torsion angles corresponding to the two major secondary structure elements (α-helices and β-sheets) are clearly clustered within separate regions.





Summary

Week_2_Lecture_4: Lecture 4 is about super secondary structures (motifs) and domains.

1. The exact meaning of terms motif and domain is not easy to define because both are used in several overlapping contexts. Super secondary structures are intermediates between secondary and tertiary structures of protein.
2. Certain combinations of secondary structures with a specific geometric arrangement are called motifs or super secondary structures.
3. Domain is an independently folded stable tertiary structure and shows a certain level of evolutionary conservation. It is a polypeptide chain or part of polypeptide chain that can fold independently into a stable three-dimensional structure.
4. The unique three-dimensional conformations are due to the consequences of the interactions between the side chains.
5. Different types of covalent and non-covalent interactions stabilize the tertiary structure. The covalent bond present in a tertiary structure is the intrachain disulfide bond between two cysteine residues.
6. Non-covalent interactions in aqueous environment include, ionic bonds, hydrogen bonds, van der Waals forces and hydrophobic interactions. These are weak interactions and their bond strengths are much less as compared to covalent bonds.

Summary

Week_1_Lecture_5: Lecture 5 is about Protein folding

1. **Protein folding** is the physical process by which a protein chain is translated to its native three-dimensional structure, typically a "folded" conformation by which the protein becomes biologically functional.
2. Folding is a spontaneous process that is mainly guided by hydrophobic interactions, formation of intramolecular hydrogen bonds, van der Waals forces.
3. Proteins will have limitations on their folding based on the restricted bending angles or conformations that are possible. These allowable angles of protein folding are described with a two-dimensional plot known as the Ramachandran plot, depicted with psi and phi angles of allowable rotation.
4. The folding process also depends on nature of the solvent, the concentration of salts, the temperature, and the presence of molecular chaperones.
5. According to one folding model, folding is initiated by a spontaneous collapse of the unfolded polypeptide chain into a partly organized globular state, mediated by hydrophobic interactions among non-polar residues (Hydrophobic collapse). The collapsed state is referred to as a molten globule.
6. Molecular chaperones are a class of proteins which bind to incompletely folded or unfolded proteins in order to assist their folding or prevent them from aggregating.

Question : 1

Which of the following amino acids **NOT** involved in salt bridge formation?

1. Arginine
2. Glycine
3. Lysine
4. Glutamate

Answer the question on Google meet poll

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3. Lysine
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Solution:

1. **A salt bridge** is a combination of two non-covalent interactions namely hydrogen bonding and ionic bonding.
2. Ionic bond is formed by the electrostatic attraction between positive and negative ions. Hydrogen bond exists between a hydrogen atom covalently bonded to a high electronegative atom and an electronegative atom with a lone pair of electrons.
3. The salt bridge arises from the anionic carboxylate of either aspartic acid or glutamic acid and the cationic ammonium from lysine or arginine.
4. Other residues with ionizable side chains such as histidine, tyrosine, and serine can also participate.
5. The distance between the residues participating in the salt bridge is also cited as being important. The distance required is less than 4 Å (400 pm). Amino acids greater than this distance apart do not qualify as forming a salt bridge.

Final answer: **Glycine**

Question : 2

Helix loop Helix is a ?

1. DNA binding motif
2. Protein binding motif
3. Metal binding motif

Answer the question on Google meet poll

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2. Protein binding motif
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Solution:

A **helix–loop–helix** (**HLH**) is a protein structural motif that characterizes one of the largest families of dimerizing transcription factors.

The motif is characterized by two α-helices connected by a loop, and transcription factors based on HLH are dimeric, each with one helix containing basic amino acid residues that facilitate DNA binding.

The helix-loop-helix is a DNA-binding motif commonly found in transcription factors, such as the EF-hand, a calcium-binding domain common to calcium-binding proteins.

In general, one helix is smaller, and due to the flexibility of this loop, allows dimerization by folding and packing against another helix. The larger helix typically contains the DNA-binding regions.

Final Answer **: DNA Binding motif**

Question : 3

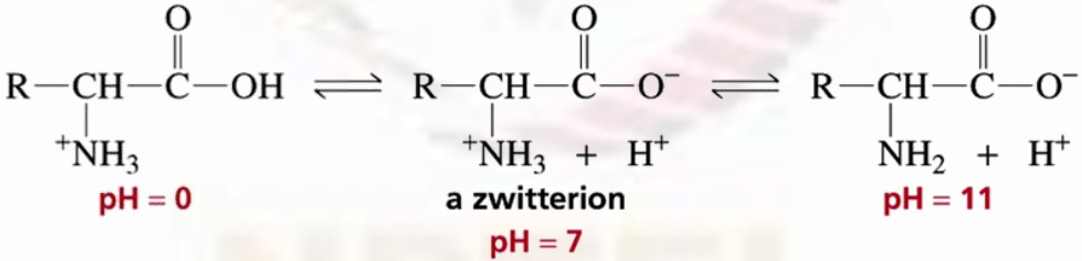
2D gel electrophoresis, the separation is based on?

1. Charge
2. Size
3. Size and Charge
4. None of the above

Answer the question on Google meet poll

Solution:

- In the first dimension, molecules are separated linearly according to their isoelectric point. In the second dimension, the molecules are then separated according to molecular mass.
- Since it is unlikely that two molecules will be similar in two distinct properties, molecules are more effectively separated in 2-D electrophoresis than in 1-D electrophoresis.
- Separation of the proteins by isoelectric point is called isoelectric focusing (IEF). The **isoelectric point** (**pI**), is the pH at which a molecule carries no net electrical charge or is electrically neutral in the statistical mean.
- At pI, the amino groups are protonated (NH_3^+)and carboxyl groups are deprotonated (COO^-), forming a zwitter ion.
- At very low pH, carboxyl and amino group are protonated and the molecule is positively charged. Similarly at very high pH, deprotonation happens and the molecule is negatively charged.



- The proteins applied in the first dimension will move along the gel and will accumulate at their isoelectric point; that is, the point at which the overall charge on the protein is 0 (a neutral charge).

2D gel electrophoresis, the separation is based on?

1. Charge
2. Size
3. Size and Charge
4. None of the above

Solution:

- Before separating the proteins by mass, they are treated with sodium dodecyl sulfate (SDS) along with other reagents. This denatures the proteins and binds a number of SDS molecules roughly proportional to the protein's length.
- The SDS molecules are negatively charged, the result of this is that all of the proteins will have negative charge and allows migration of the proteins in the second dimension.
- In the second dimension, an electric potential is again applied, but at a 90 degree angle from the first field. The proteins will be attracted to the more positive side of the gel (because SDS is negatively charged) proportionally to their mass-to-charge ratio.

- Final Answer **: Size and Charge**

Question : 4

Christian Anfinsen experiment describes protein folding is ?

1. Depending on biological systems
2. Inherited from protein sequence
3. Depending on mechanical systems
4. None of the above

Answer the question on Google meet poll

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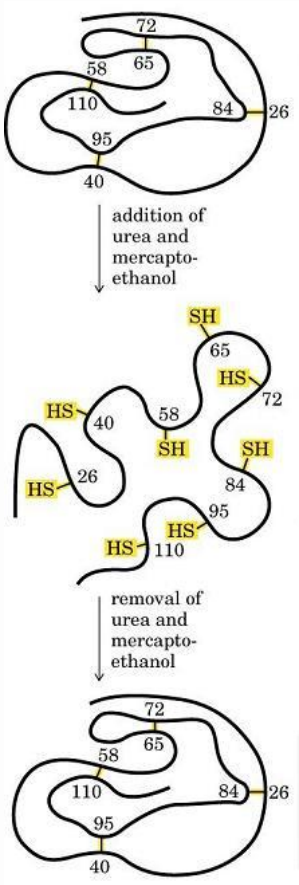
Solution:

- **Protein folding** is the physical process by which a polypeptide folds into its characteristic and functional three-dimensional conformation.
- One of the most important experiment, which helped in understanding the process of protein folding was carried out by Christian Anfinsen.
- Christian Anfinsen studied the refolding of protein ribonuclease A. In the presence of urea, a denaturant and b-mercaptoethanol, a reducing agent, ribonuclease is denatured and the disulfide bonds are broken.
- When the protein is allowed to renature by removing the denaturant and reducing agent, the protein regains its native conformation, including disulfide bonds.
- This finding provided the first evidence that the amino acid sequence of a polypeptide chain contains all the information required to fold the chains into its native three dimensional structure.



- Final Answer **: Inherited from protein sequence**

Question : 5

When a protein is transferred to a solution with a lower pH than its optimal range, which of the following levels of protein structure can be affected?

1. Primary only
2. Secondary only
3. Secondary and tertiary only
4. Secondary, tertiary, and quaternary

Answer the question on Google meet poll

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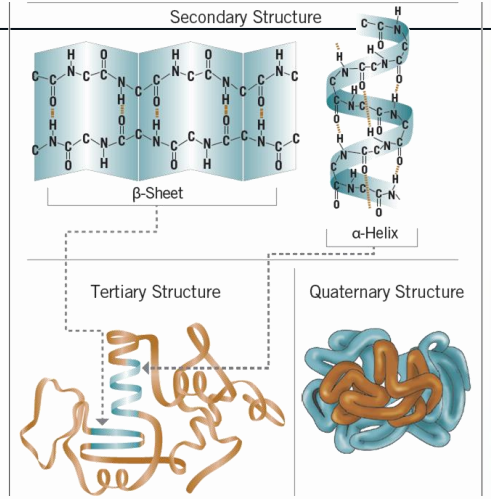

Solution:

- This question is about a protein is being transferred to a highly acidic solution that is outside of the protein's optimal pH range. In such a situation, we would expect the protein to undergo conformational changes that would alter its function.
- The primary structure upon being transferred to an acidic solution, the protein does indeed unfold, but it doesn't break apart into individual amino acids. Thus, there is no change in primary structure.
- The secondary structure of a protein refers to local conformations and are held together by various intramolecular bonds between the amino acid residues. When transferred to an acidic solution, these intramolecular forces are disrupted and, as a result, cause a disruption in the protein's secondary structure.
- The overall three-dimensional conformation of a polypeptide is held together by same intramolecular forces. Because a highly acidic solution interferes with these interactions, the tertiary level of protein structure is indeed affected by pH changes.
- Just as with secondary and tertiary structures, the introduction of a highly acidic solution can disrupt these intermolecular interactions, thus causing a disruption in the quaternary structure of a protein composed of two or more polypeptide chains.

Final Answer **:Secondary, tertiary, and quaternary**

Question : 6

The formation of a peptide bond is an example of what type of reaction?

1. Double displacement reaction
2. Decomposition reaction
3. Combustion reaction
4. Condensation reaction
5. Hydration reaction

Answer the question on Google meet poll

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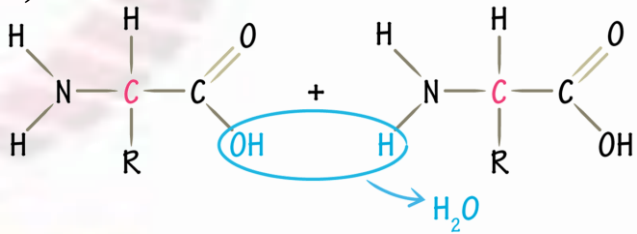
5. Hydration reaction

Solution:

- A **peptide bond** is an amide type of covalent chemical bond linking two consecutive alpha-amino acids.
- In this reaction, two amino acids approach each other, with the non-side chain carboxylic acid moiety of one coming near the non-side chain amino moiety of the other..
- One loses a hydrogen and oxygen from its carboxyl group (COOH) and the other loses a hydrogen from its amino group (NH_2). This reaction produces a molecule of water (H_2O) and two amino acids joined by a peptide bond (-CO-NH-).



- This is a dehydration synthesis reaction (also known as a condensation reaction where a two molecules are combined to form a single molecule, usually with the loss of a small molecule such as water).
- Final Answer **: Condensation reaction**

Question : 7

Why are antiparallel beta sheets more stable than parallel beta sheets?

1. More covalent interactions between its amino acids
2. The antiparallel sheets are composed of more stable amino acids
3. There are more hydrophobic interactions between its amino acids
4. The hydrogen bonding angle is optimized by antiparallel sheets
5. The hydrogen bond angle is 150 degrees

Answer the question on Google meet poll

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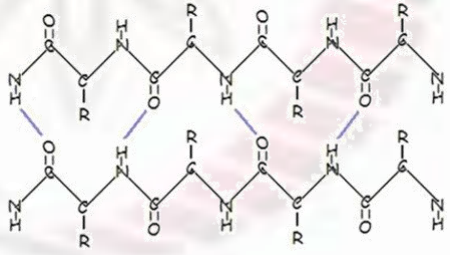
5. The hydrogen bond angle is 150 degrees

Solution:

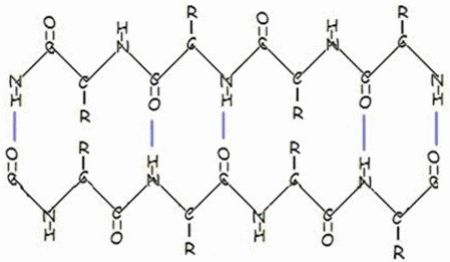
The parallel arrangement is less stable because the geometry of the individual amino acid molecules forces the hydrogen bonds to occur at an angle, making them longer and thus weaker.

Contrarily, in the anti-parallel arrangement the hydrogen bonds are aligned directly opposite each other, making for stronger and more stable bonds.

In an antiparallel beta sheet, the hydrogen bonding angle is 180 degrees and optimal; this is the most stable angle. In parallel sheets, it is a less stable 150 degrees.



Parallel



Anti-Parallel

Final Answer **:The hydrogen bonding angle is optimized by antiparallel sheets**

Question : 8

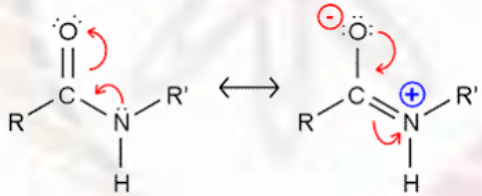
In a sequence of amino acids within an alpha helix, between which amino acids in the sequence does hydrogen bonding occur?

1. 1 and 3
2. 1 and 5
3. 1 and 4
4. 1 and 6
5. 1 and 6

Answer the question on Google meet poll

Question : 9

What unique feature of peptide bond makes it stronger than that of esters?



Solution:

The peptide bond is more highly resonance-stabilized than the ester bond. This stabilization strengthens the peptide bond.

Answer the question on Google meet poll

Question : 10

Most of the cytosolic proteins lack disulfide bonds, whereas extracellular proteins usually contain them. Why?

Solution:

The cytosol is a reducing environment, whereas the extracellular milieu is an oxidizing environment. Disulfide bond forms as a result of oxidation of thiol group and it requires an oxidizing environment.

Answer the question on Google meet poll

Question : 11

Answer the following regarding the alpha-helix?

1. Rise per residue:
2. Residues per turn:
3. Rise per turn:

Alpha helix with 9 amino acids, what is the height of the helix?

Answer the question on Google meet poll