BCHM 270: Module 2

PROTEIN STRUCTURE AND FUNCTION

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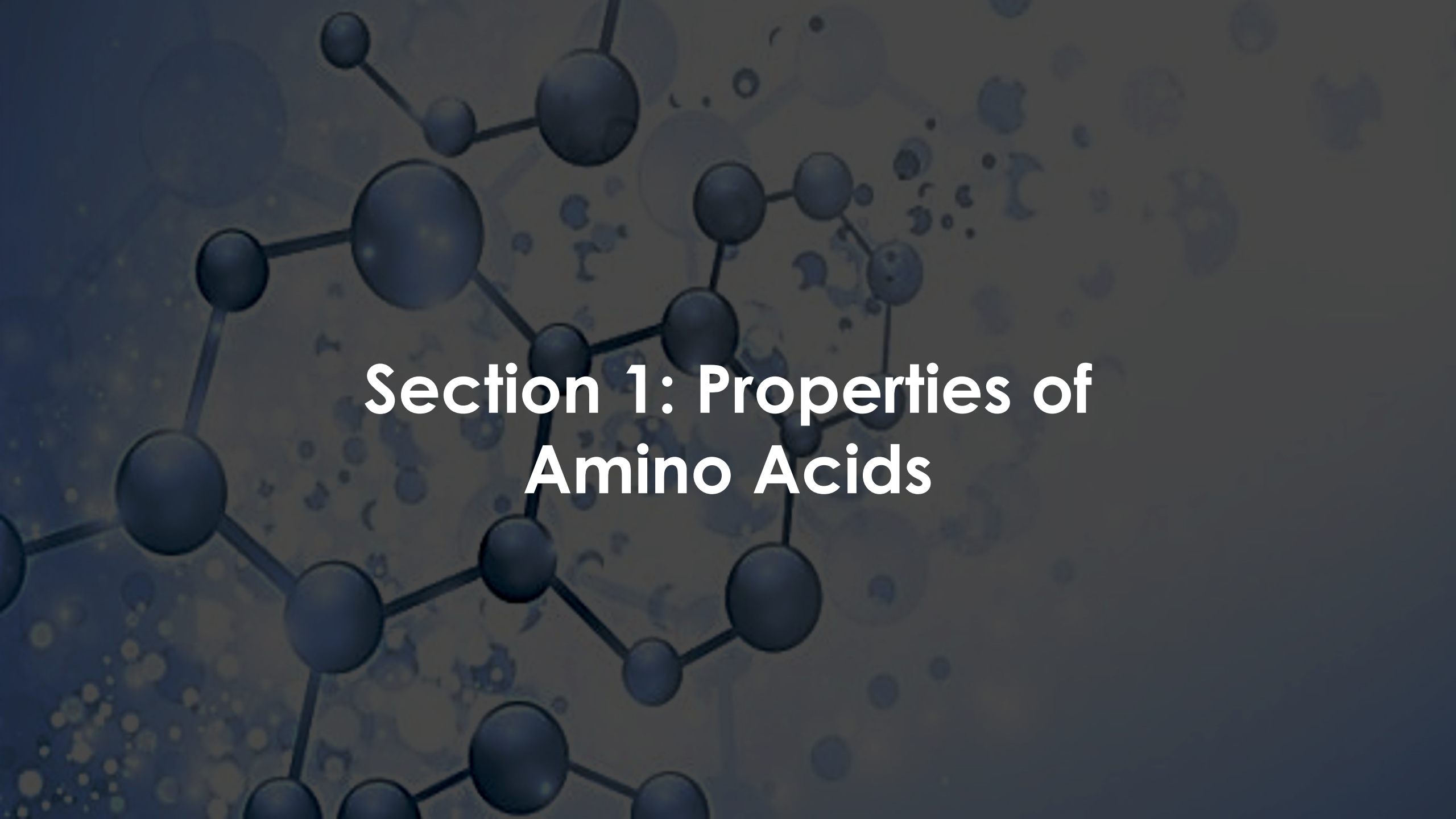
Section 3..... Categories of Protein Structure

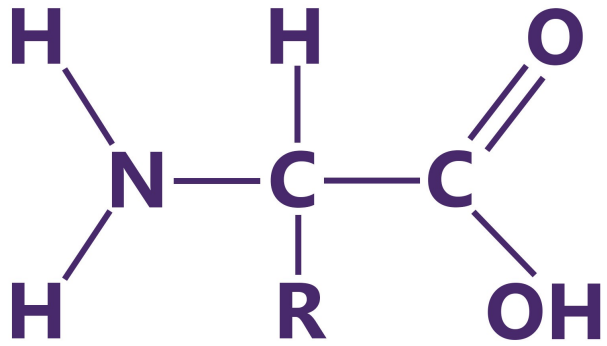
Section 4..... Protein Structure and Function



Section 1: Properties of Amino Acids

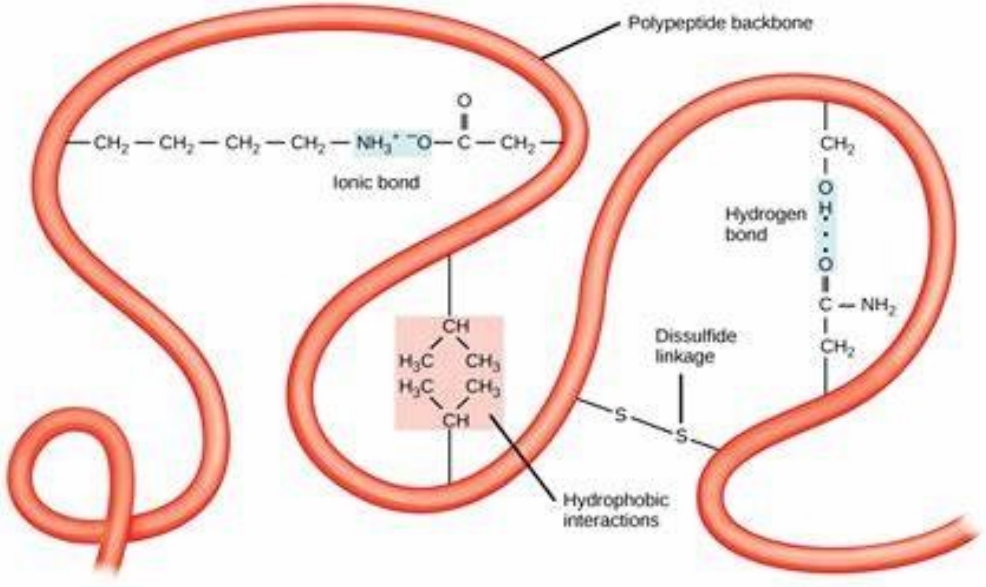
Concept 1.1: Amino Acid Structure

- Amino acids are the building blocks of proteins.
- They consist of an amino group (-NH2), a carboxyl group (-COOH), and a side chain (R group).
- There are 20 different types of amino acids.
- The R group determines the unique chemical properties of each amino acid.
- Proteins are essential for many biological functions.
- Amino acid sequence determines protein structure and function.
- Alterations in the amino acid sequence can impact protein function.



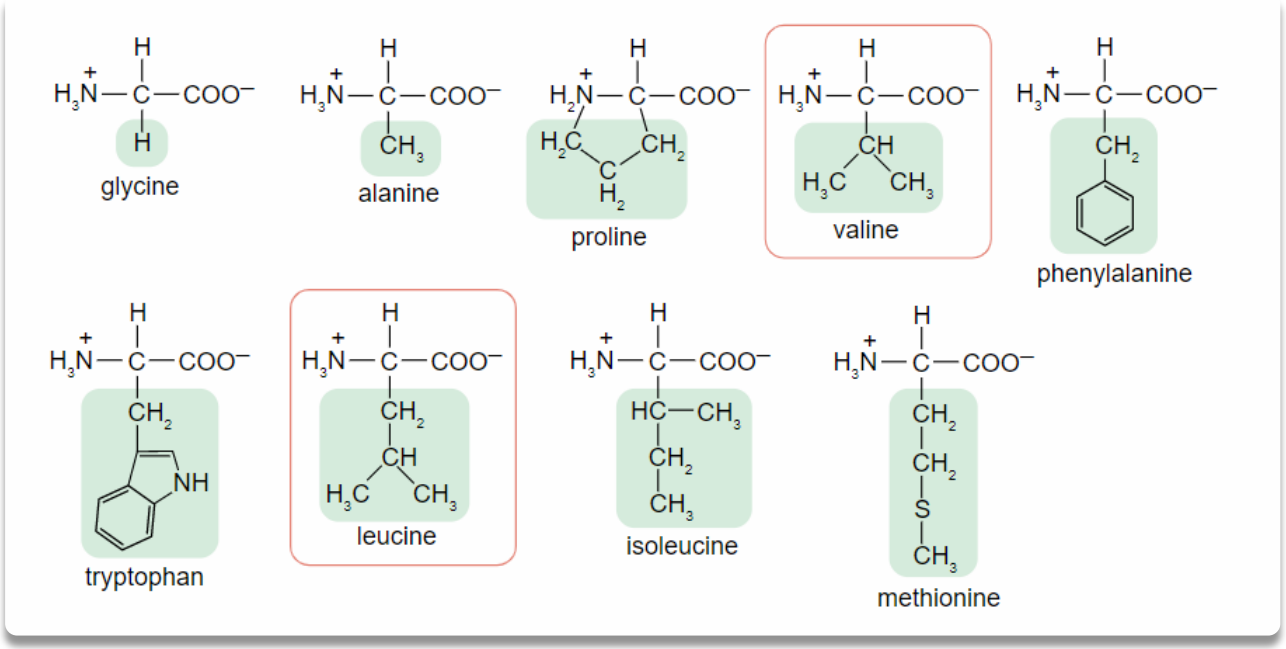
Concept 1.2: Noncovalent Interactions

- Four main types of noncovalent interactions essential to biochemistry
- Weak individually, but strong when many are combined
- Electrostatic (charge-charge) interactions - between oppositely charged molecules
- Molecular dipoles - uncharged molecules with an asymmetrical distribution of charge
- Hydrogen bonds - between a hydrogen donor and a hydrogen acceptor with a lone pair of electrons
- Hydrophobic/hydrophilic interactions - determined by hydrogen bonds and affect the structure and function of molecules, including proteins and DNA
- Hundreds to thousands of noncovalent interactions can exist within a single protein, allowing it to maintain its 3-D structure and resist denaturation.



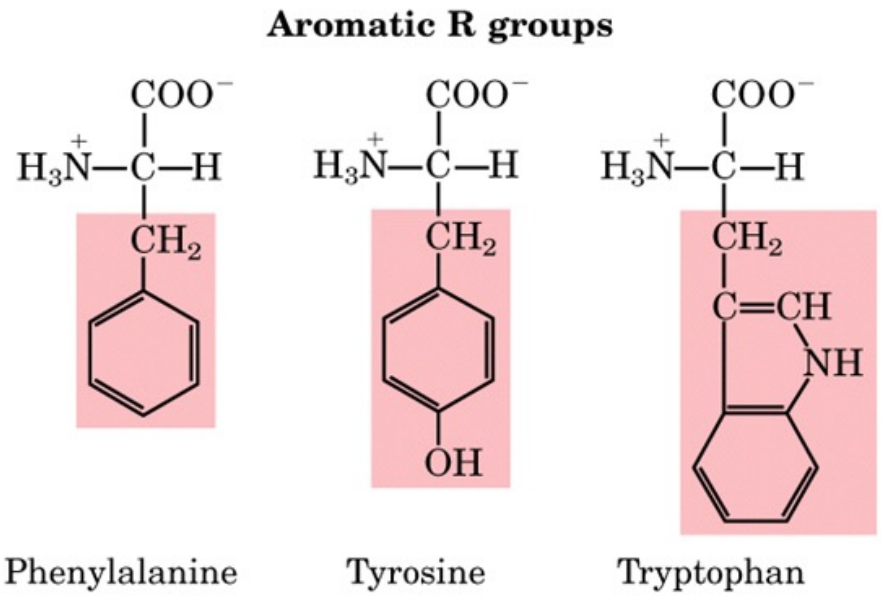
Concept 1.3: Nonpolar amino acids

- Nonpolar amino acid side chains do not gain or lose protons and cannot form hydrogen or ionic bonds
- Nonpolar amino acids are hydrophobic and interact with each other to avoid hydrophilic environments
- This is the major driving force behind protein folding
- In soluble proteins, nonpolar amino acids are clustered together in the core of the protein
- In membrane proteins, nonpolar amino acids interact with the hydrophobic tails of lipids
- 9 amino acids have nonpolar side chains (aliphatic and aromatic), some are linear, some are branched, and others contain ring structures



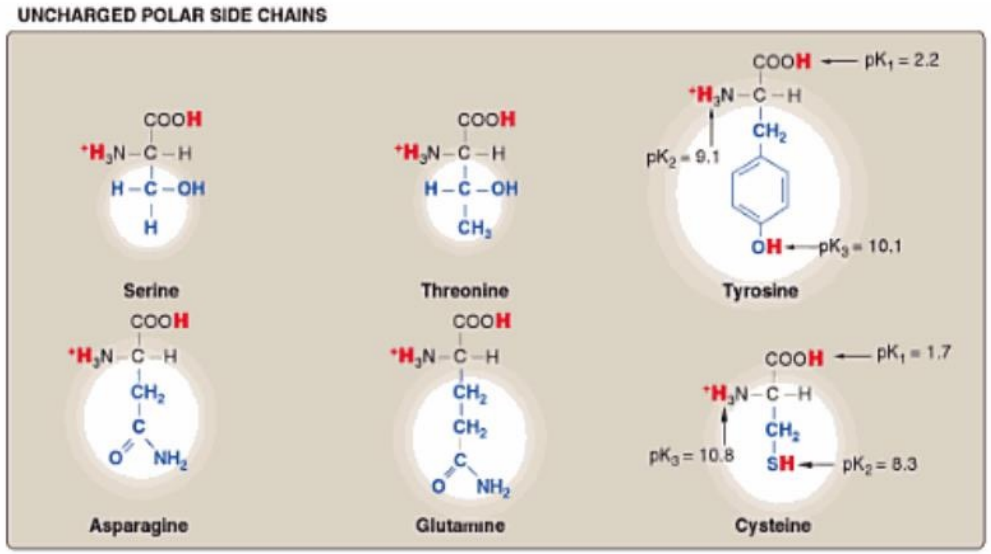
Concept 1.4: Aromatic amino acids

- Amino acids with ring structure side chains are classified as aromatic amino acids
- Aromatic side chains are large and can cause steric hindrance due to repulsion between atoms and the amount of space required
- Larger R-groups need more space and can affect chemical bond formation/structure of the resulting peptide bond
- The physical size of aromatic R-groups can prevent other molecules from interacting with it
- Gain or loss of aromatic amino acids can cause deformities in the protein structure



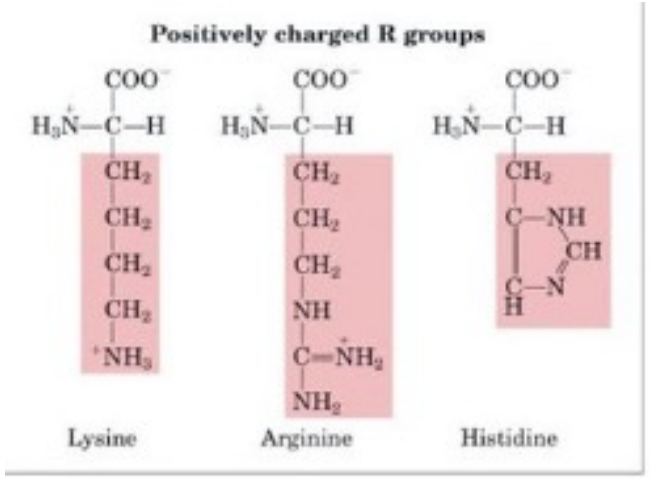
Concept 1.5: Uncharged Polar Amino Acids

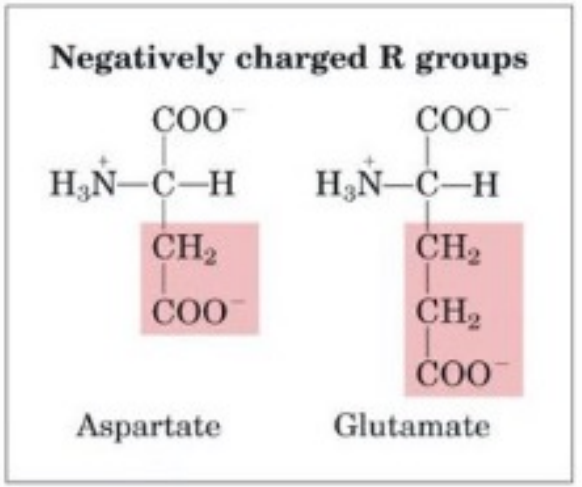
- Uncharged polar amino acids have no net charge at physiological pH and do not form salt bridges
- Polar side chains tend to be found on the outside of soluble proteins
- Serine and Threonine have a hydroxyl group capable of forming H-bonds
- Tyrosine has a hydroxyl group capable of forming a hydrogen bond, a benzene ring, and can lose a proton at alkaline pH
- Asparagine and Glutamine have a carbonyl and amine that can participate in H-bonds and dipole interactions
- Cysteine has a thiol group and can form disulfide bonds with other cysteines, and can lose a proton at alkaline pH.



Concept 1.6: Charged Amino Acids

- **Negatively** charged amino acids Asp (D) and Glu (E) can form salt bridges with positively charged molecules, providing stability to the protein structure.
- These **acidic amino acids** are typically found on the protein surface, where they can interact with the surrounding environment.
- **Positively** charged amino acids His (H), Lys (K), and Arg (R) can interact with negatively charged molecules, stabilizing the protein structure.
- These **basic amino acids** are often buried inside the protein, where they can interact with other amino acids and stabilize the protein structure.
- The structure of charged amino acids enables them to interact with other molecules through electrostatic interactions.





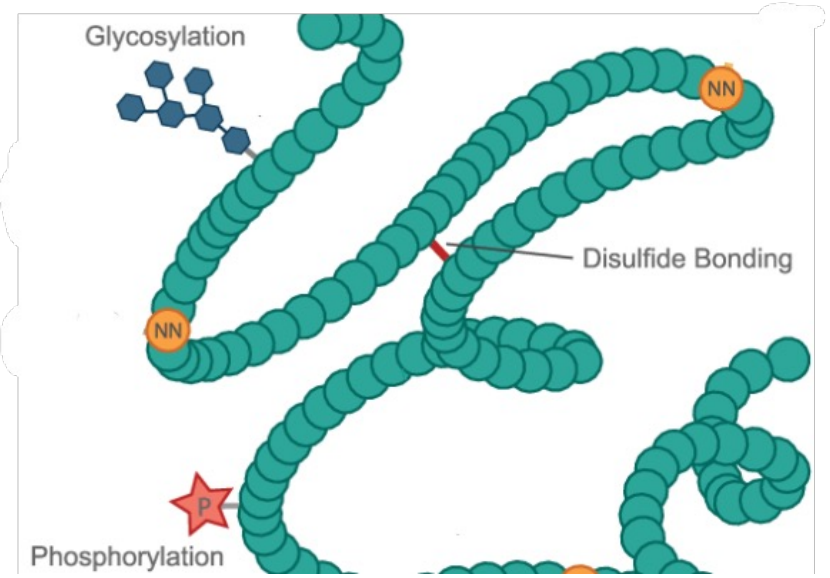
Concept 1.7: Amino Acid Review

- Amino acid properties that students need to know for the exam:
  - R group structures
  - One and three letter codes
  - Classification
  - Knowledge of the 20 standard amino acids and their properties is essential
  - Important properties include: polarity, charge, size, hydrophobicity, and reactivity
  - Understanding the properties of amino acids is crucial for understanding protein structure and function
  - Function follows form, form follows function

Amino Acid	Three letter	One letter
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

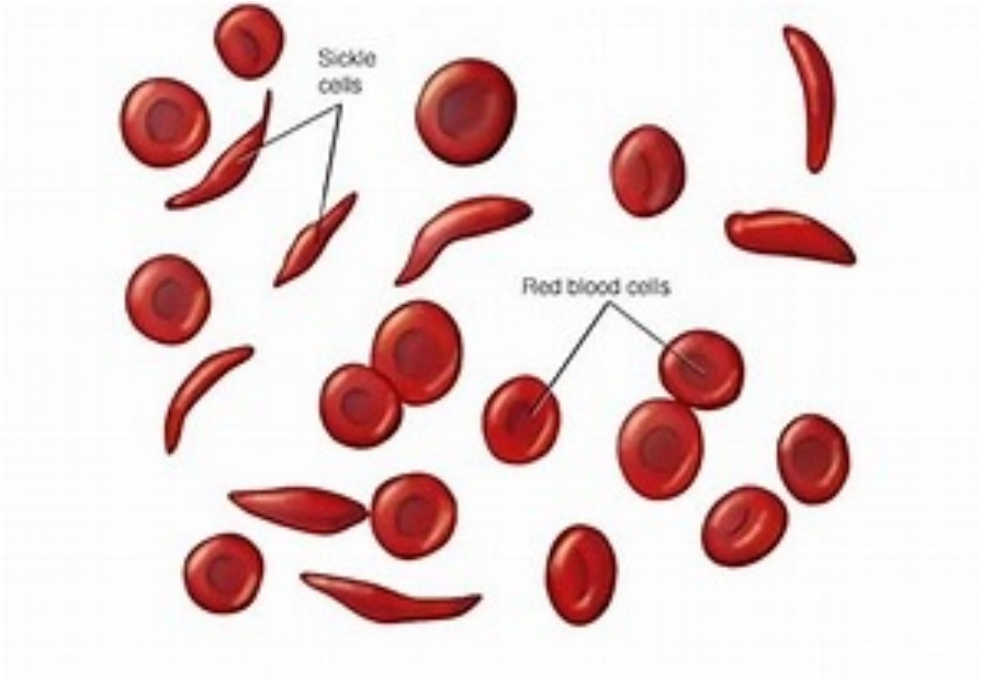
Concept 1.8: Post-translational Modifications

- Disulfide bonds are covalent bonds formed between two cysteine residues in a protein.
- Glycosylation is the process of adding carbohydrate molecules to a protein.
- Phosphorylation is the addition of a phosphate group to a protein.



Spotlight on Disease: Sickle Cell Anemia

- The chemistry of each amino acid in a protein is important.
- Amino acid substitutions have dramatic effects, not just on the protein but on the entire organism.
- **Sickle cell anemia** is a disease caused by an abnormal sickle shape of red blood cells that stick together and cause blockages in small vessels.
- The sickle cell shape is a result of protein **hemoglobin aggregation** within the red blood cells.
- The **aggregation** is the result of a single amino acid substitution from **glutamic acid to valine** (E → V).
- This single amino acid substitution can have a significant impact on an organism's phenotype.



Section 1 Quiz: 1 MC

Which of the following amino acids would be **least** soluble in aqueous media?

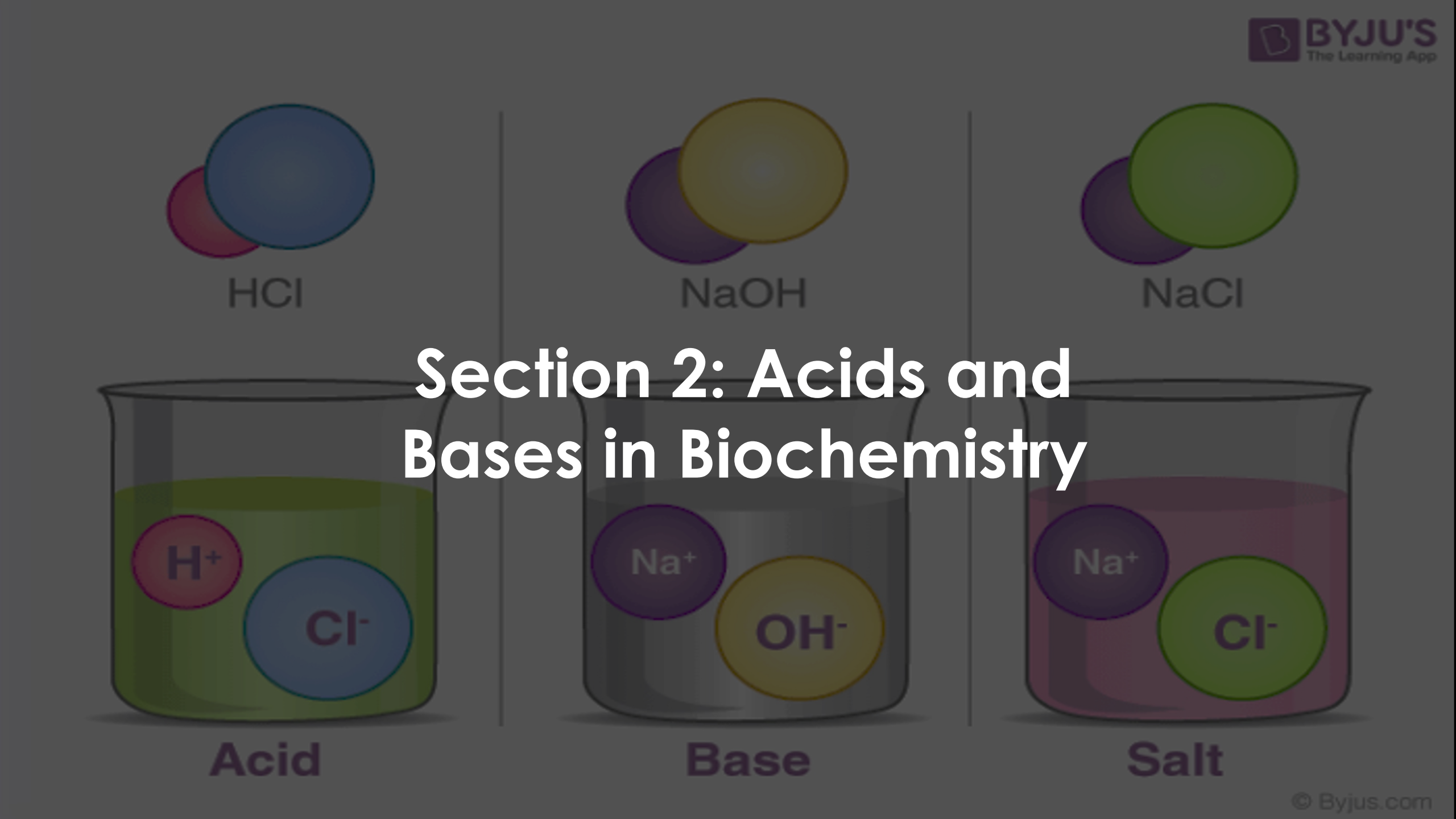
a. D
b. K
c. T
d. F

Answer: D

Section 1 Quiz: 1 Short Answer

What causes sickle cell anemia AND how does this effect the red blood cells?

Answer: Sickle cell anemia is caused by a single amino acid substitution in the protein hemoglobin, where g**lutamic acid is replaced by valine (E → V)**. This substitution causes the hemoglobin protein to **aggregate** and deform red blood cells into a sickle shape. The abnormal shape of red blood cells in sickle cell anemia causes them to stick together and obstruct small blood vessels, leading to pain, organ damage, and an increased risk of infection.



Section 2: Acids and Bases in Biochemistry

Concept 2.1: Amino Acids are Weak Acids and Weak Bases

- All amino acids have two ionizable side chains (N-terminal NH3+ and C-terminal COO-)
- Each side chain has different pKa
- Alpha-carboxylic acid will lose proton first
- Alpha-amide will lose proton next

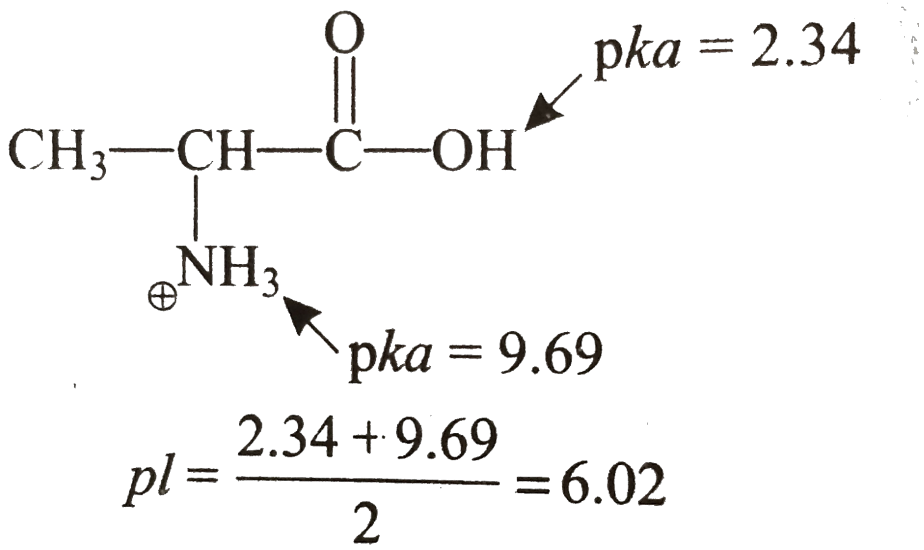


Figure 1. Two ionizable side chains on any amino acid

Concept 2.2: Titration of Amino Acids- Example 1

- Alanine is titrated with base
  - Alpha-carboxylic acid will lose proton at pH of 2.3
  - Alpha-amide will lose proton at pH of 9.1

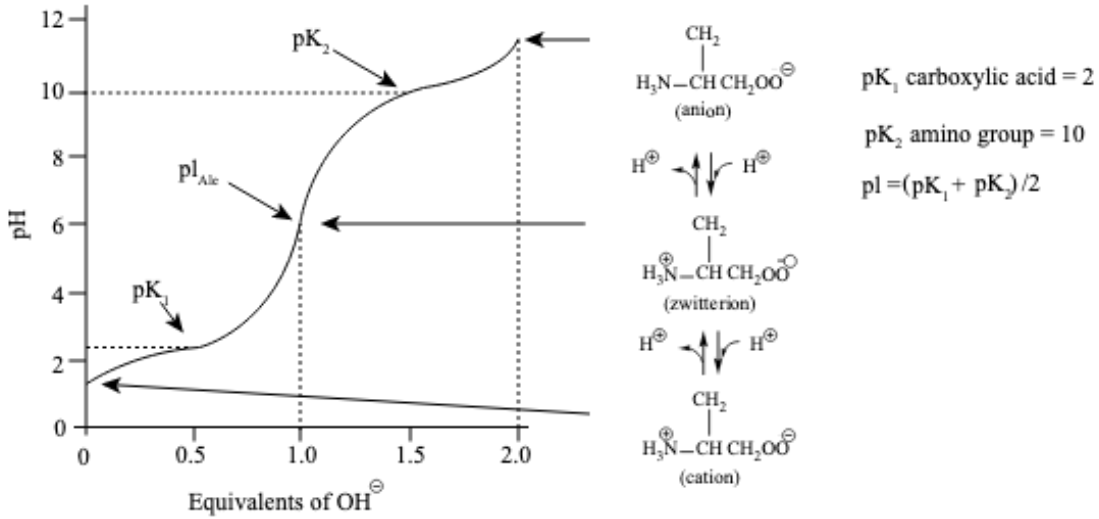


Figure 2. Titration curve for alanine

Concept 2.3: Titration of Amino Acids- Example 2

- Histidine is titrated with a base
  - At pH of 1.82, its alpha-carboxyl group loses proton
  - At pH of 6,0, its ionizable side chain will lose a proton
  - At pH of 9.17, its alpha-amide will lose a proton

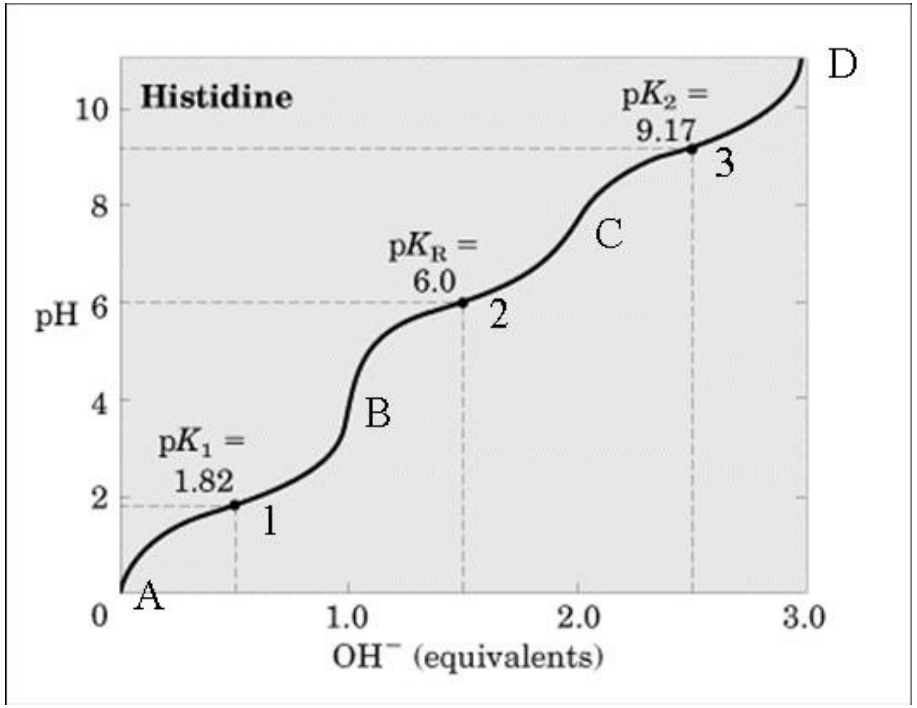


Figure 3. Titration of histidine

Concept 2.4: Bicarbonate Buffer System

- Maintains blood pH
- Chemical reaction: CO2 + H2O <--> H2CO3 <--> H+ + HCO3-
- PH decrease --> bicarbonate ion binds with H+
- PH increase --> carbonic acid releases H+

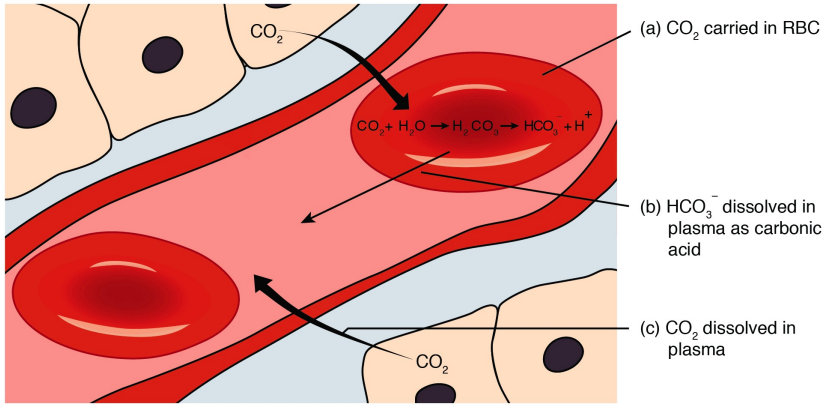


Figure 4. Bicarbonate buffer system in the body

Spotlight on Disease: Respiratory Acidosis

- Sharp increase in [CO2]
  - Increase occurs due to pulmonary obstruction or emphysema
- Greater production of H2CO3
- Leads to greater release of protons, lowering blood pH (respiratory acidosis)

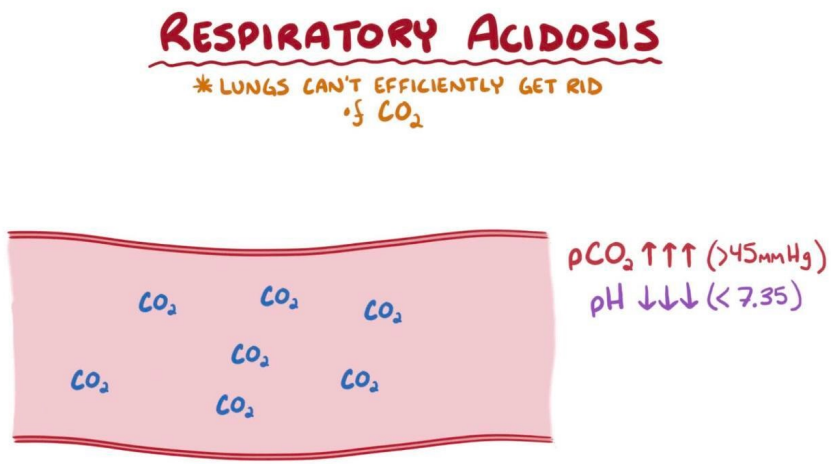


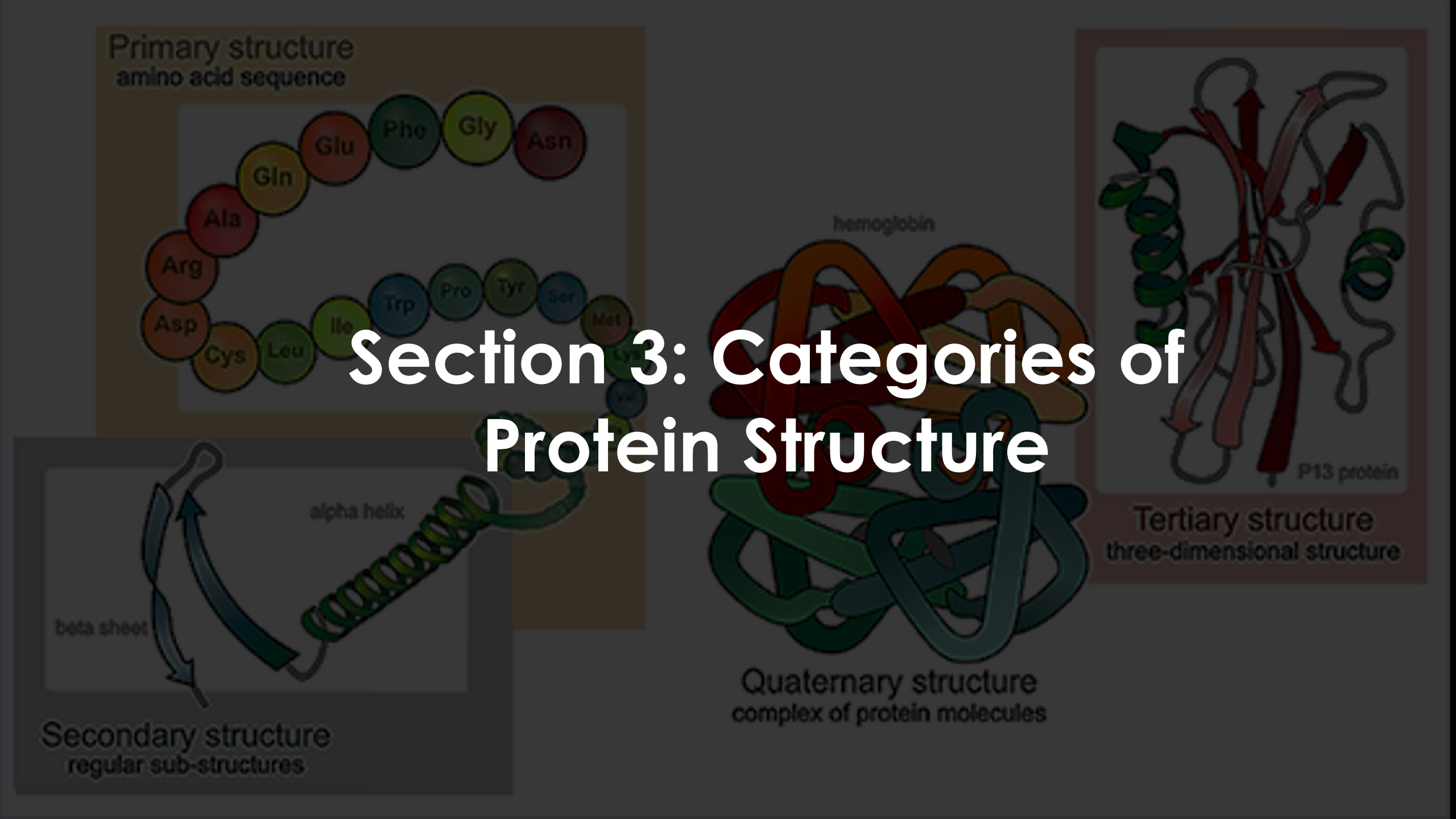
Figure 5. Respiratory acidosis

Section 2 Quiz: 1 MC

Which of group in an amino acid loses its proton first during a titration with a base?

a. Alpha-amide group
b. Alpha-carboxyl group
c. Both lose at the same time
d. None of the above

Answer: B



Section 3: Categories of Protein Structure

Concept 3.1: Hierarchy of Protein Structure

There are four levels of protein structure

Each levels contain different unique structures that help form the final protein

Inter- and intra-protein interaction are associated with the protein's function

Four protein levels:

Primary

Secondary

Tertiary

Quaternary

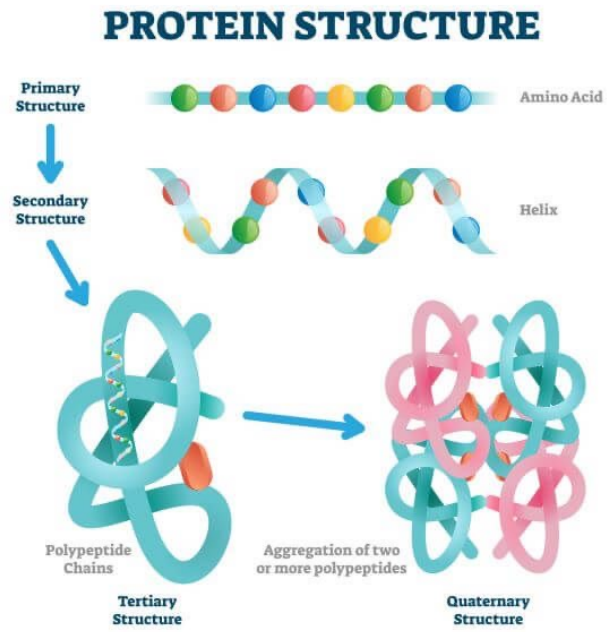


Figure X. Levels of Protein Organization

Concept 3.2: Primary Structure

Directly based on the mRNA use to create the protein.

Peptide bonds: The bonds that keep the amino acids in the primary structure together.

Formed through a condensation reaction the a-carboxyl and a-amide of neighbouring amino acids.

Due to the bond having a resonance structure, it develops notable characteristics:

- Shorter than single bonds
- Stronger than single bonds
- Planar shape with no rotation around the bond.

Almost always in the trans conformation due to steric hinderance.

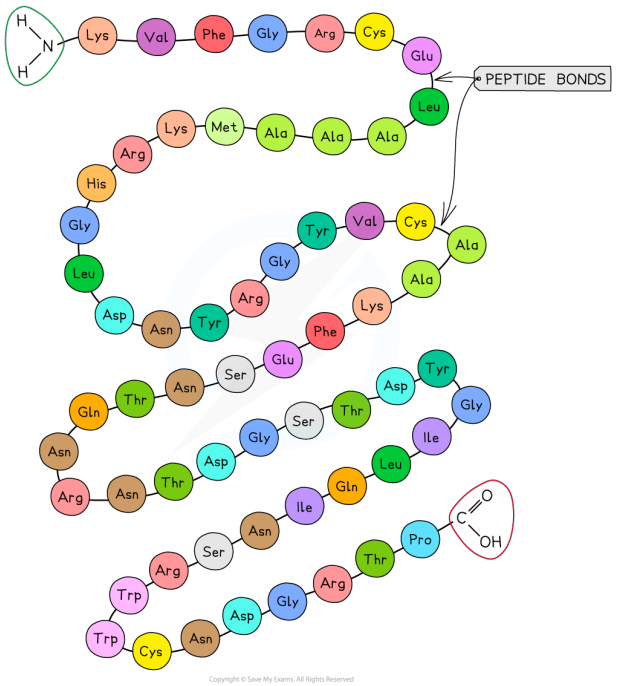


Figure X. Primary structure of a protein.

Concept 3.3: Secondary Structure- Alpha Helices

a-helices are the most common protein structural element.

The carbonyl oxygen of the 1st amino acid creates a hydrogen bond with the amide hydrogen on the amino acid 4 places away.

Notable characteristics:

- Right-handed spiral
- Coiled backbone core
- Side chains point out away from the inside of the spiral.

Helix breakers: Large amino acids too big for a-helices (ex. tyrosine

Hydrophobic and hydrophilic amino acids group on different sides of the helix

Amphipathic Helix: an a-helix with a hydrophobic and hydrophilic sides

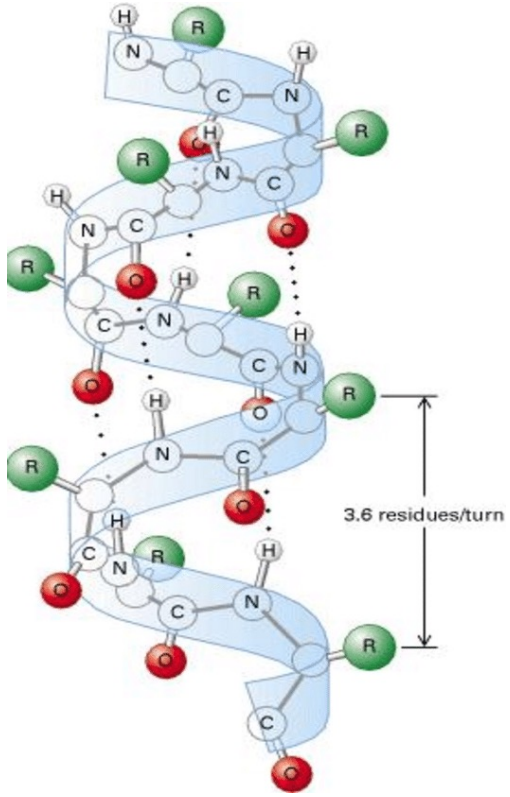


Figure X. Diagram showing the alpha-helix structure.

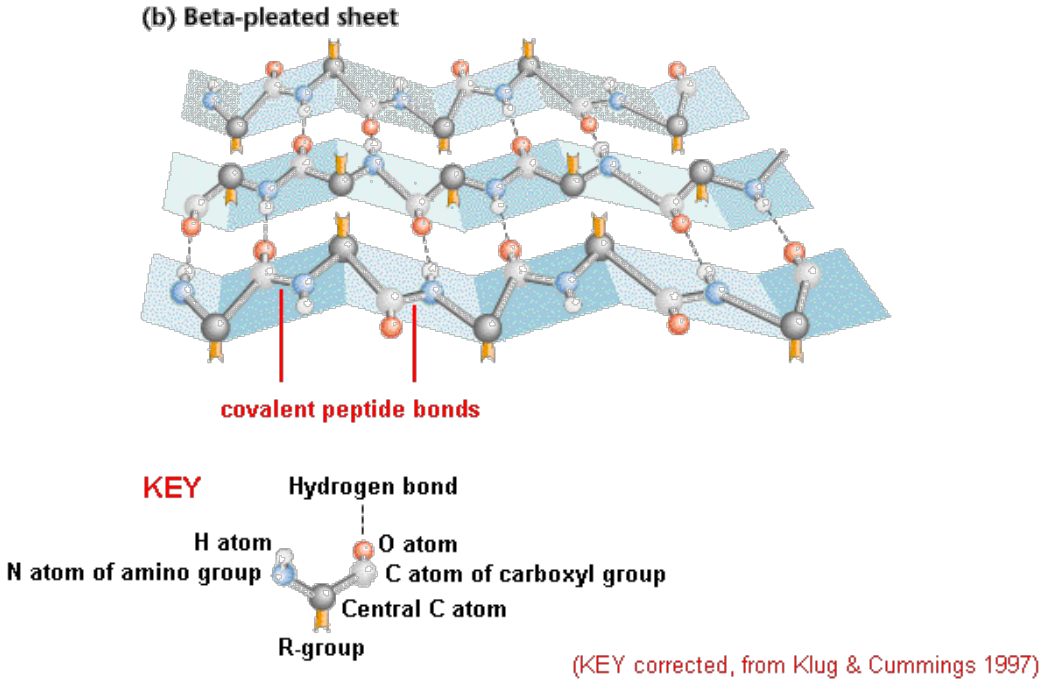
Concept 3.4: Secondary Structure- Beta Sheets

β-sheets are composed of sections of the primary amino acid sequence that are fully extended (called β-strands)

β-strands form hydrogen bonds with one another, between the carbonyl oxygen and amide hydrogen in the amino acid backbone.

Similar to a-helices, hydrophilic and hydrophobic faces are formed on β-sheets.

Secondary Structure Motifs: When adjacent a-helices and β-sheets interact via non-covalent bonds to form supersecondary structures.



(KEY corrected, from Klug & Cummings 1997)

Figure X. Diagram of the structure of β-sheets.

Concept 3.5: Tertiary Structure

Tertiary Structure: The overall 3D structure of the polypeptide.

Comprised of domains formed by the secondary structures (α-helices and β-sheets) and unfolded sections.

Hydrophobic amino acids remain on the inside of the protein, while hydrophilic amino acids stay on the outside.

Four important stabilizing interactions:

- Disulfide bonds
- Hydrophobic Interactions
- Hydrogen Bonds
- Salt Bridges

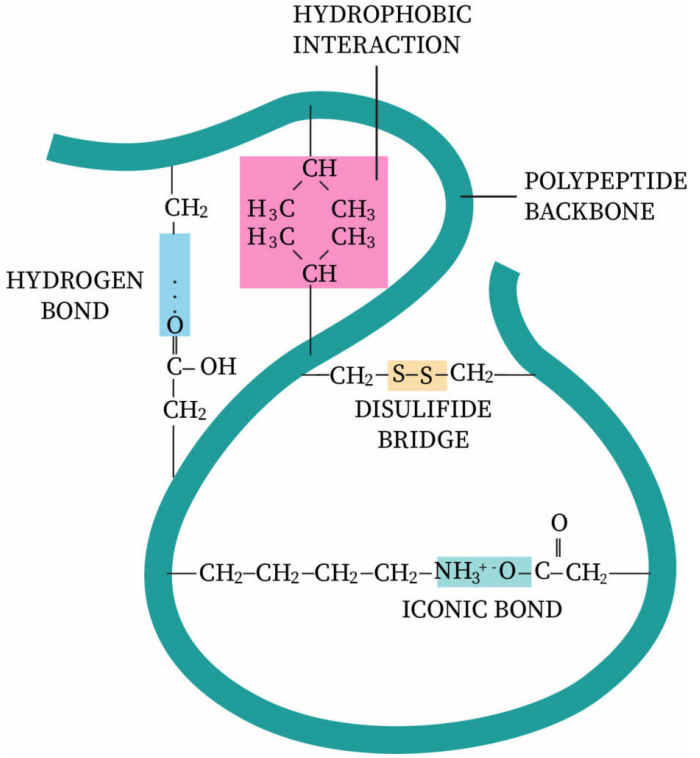


Figure X. Diagram of the stabilizing interactions in the tertiary structure of proteins.

Concept 3.6: Quaternary Structures

Quaternary Structure: When multiple individual protein chains bind together.

Not all proteins have quaternary structures.

Quaternary structures are formed through non-covalent and disulfide bonds between tertiary structures.

Ex. Hemoglobin has a quaternary structure; 2 a-subunits and 2 β-subunits.

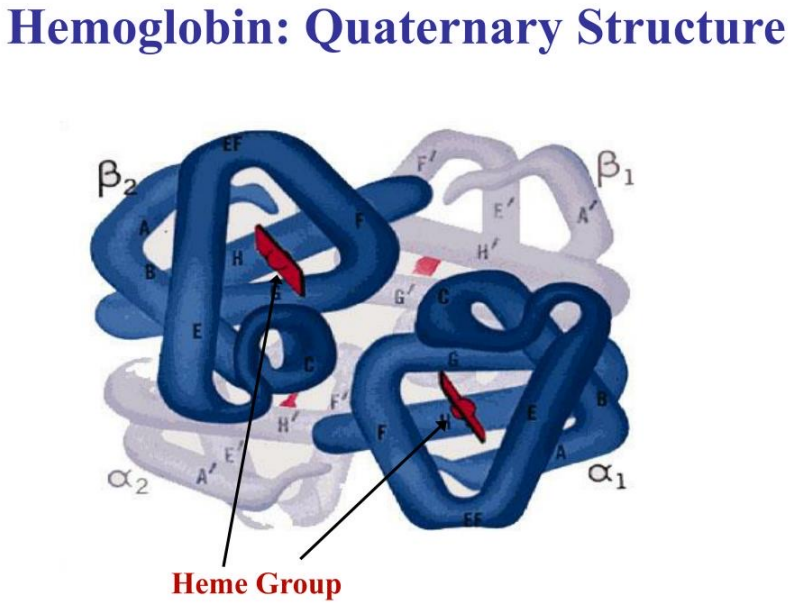


Figure X. Diagram of the quaternary structure of hemoglobin.

Spotlight on Disease: Alzhiemer's

Errors in protein folding can result in several diseases

Amyloid Precursor protein are found on cell surface in neurons

Improper cleavage of the protein causes a hydrophobic section to be released (β-amyloid protein)

β-amyloid proteins aggregate to form plaques, which are toxic to the brain

Results in symptoms: Brain atrophy, dementia, etc.

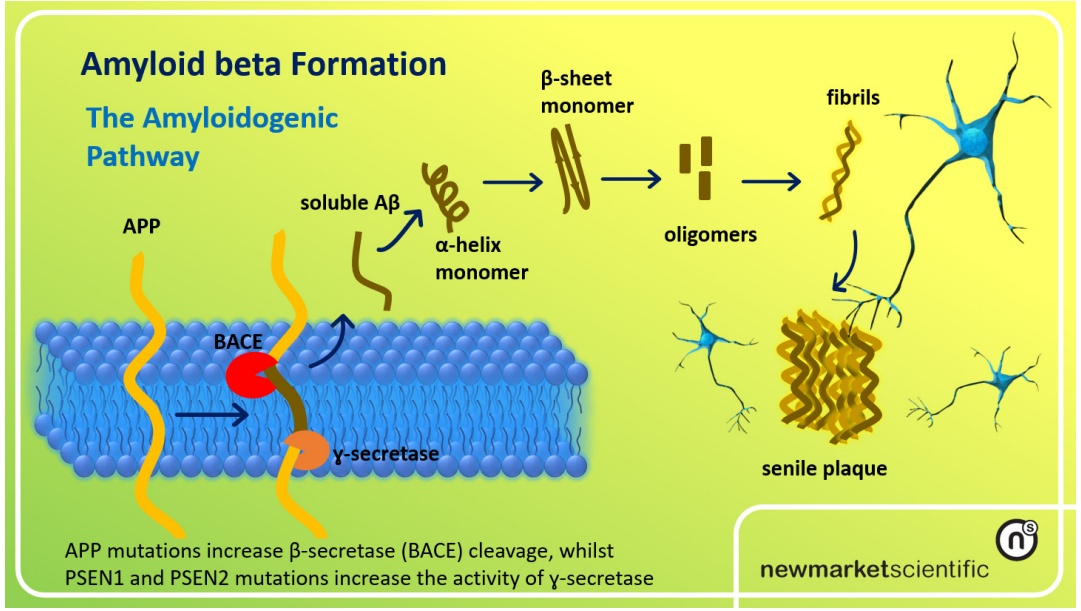


Figure X. Diagram of beta-amyloid proteins aggregating in neurons.

Spotlight on Disease: Prion Disease

Cellular prion proteins (PrP^C) are found in brain tissue, and have a-helix rich structure

The protein can develop into a pathogenic form, where the structure becomes most β-sheets.

Note: This can either occur randomly or be acquired

The infectious form causes normal PrP^C to also take on the

β-sheet rich structure

Similar to Alzheimer's Disease; PrP^{SC} is insoluble, protease-resistant and accumulates causing nerve damage.

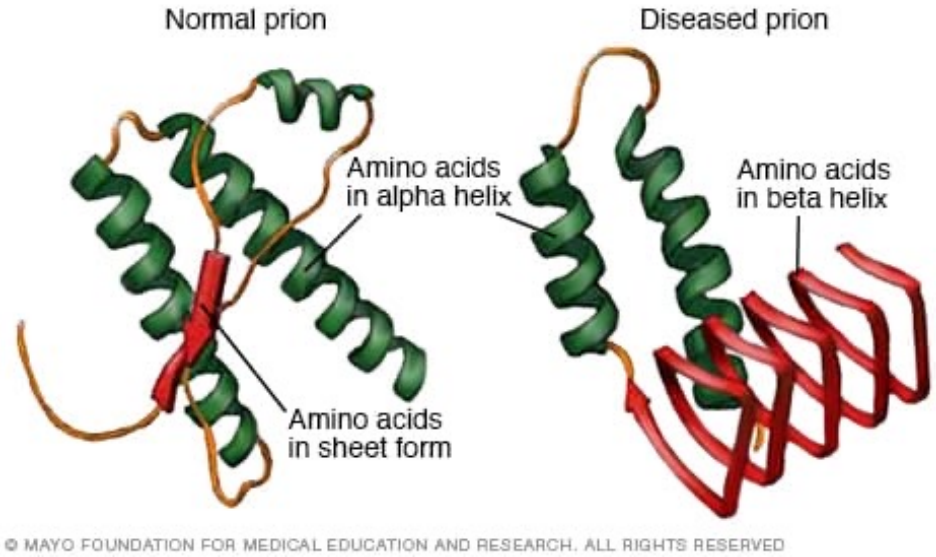


Figure X. Protein structure change seen from PrP^C to PrP^{SC}

Section 3 Quiz: 1 MC

Oxytocin is a mammalian hormone with the primary amino acid sequence CYIQNCPLG. The tertiary structure of oxytocin is primarily stabilized by:

a. Hydrophobic interactions
b. Hydrogen bonds
c. Salt bridges
d. Disulfide bonds

Answer: D

Two cysteine residues in close proximity would make the formation of a disulfide bond highly likely and would greatly stabilize tertiary structure

Section 3 Quiz: 1 SA

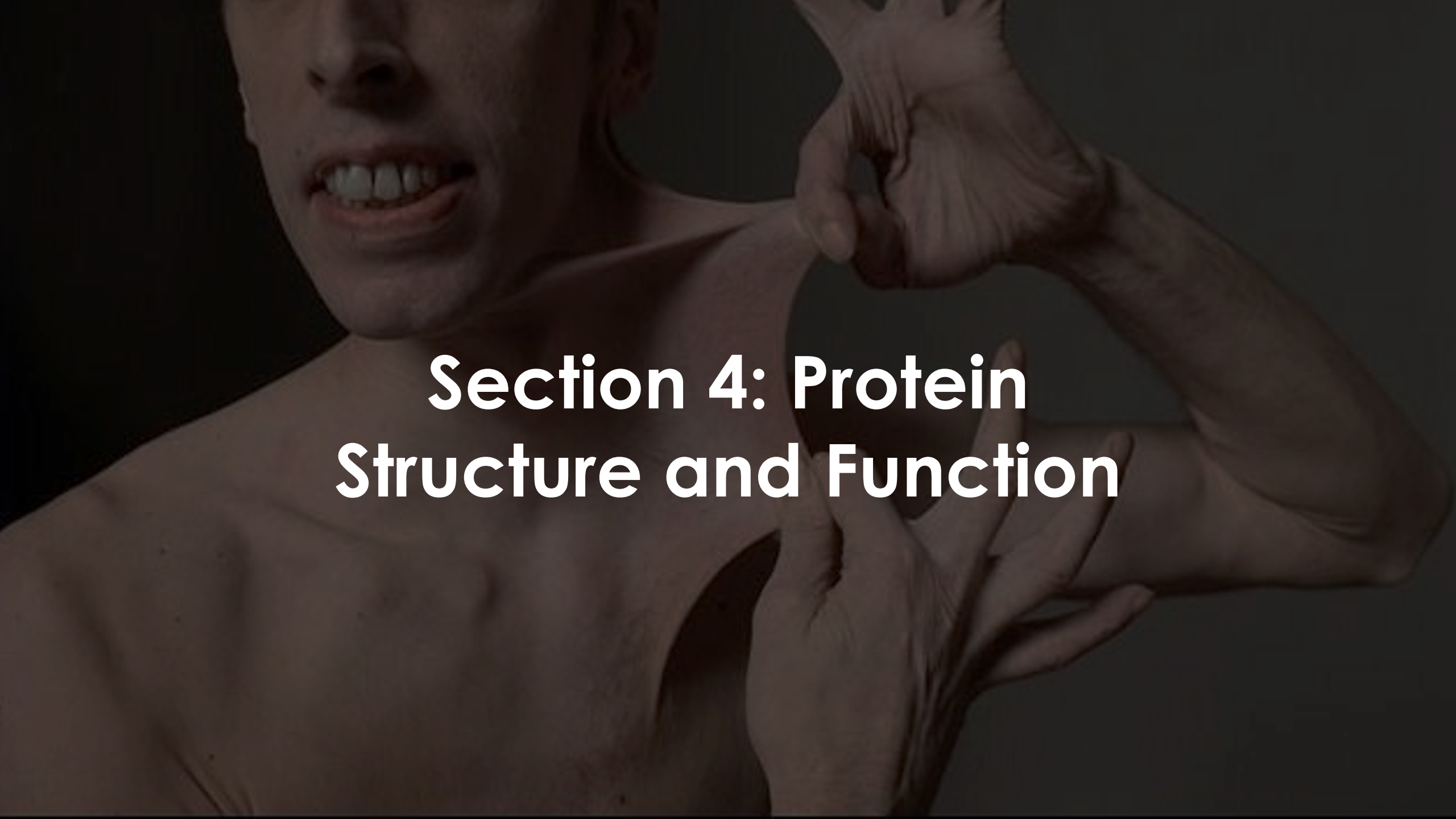
List and describe two of the stabilizing interactions seen in the tertiary structure.

Disulfide Bonds: Covalent bond formed between two cysteine. Help form stability in the tertiary structure

Hydrophobic Interactions: Main driving force of protein folding. Causes hydrophobic amino acids to migrate to the protein core.

Hydrogen Bonds: Oxygen and nitrogen on side chains form bonds with hydrogen on other residues.

Salt Bridges: Contribute to the tertiary structure stability through interactions between positively and negatively charged side chains.



Section 4: Protein Structure and Function

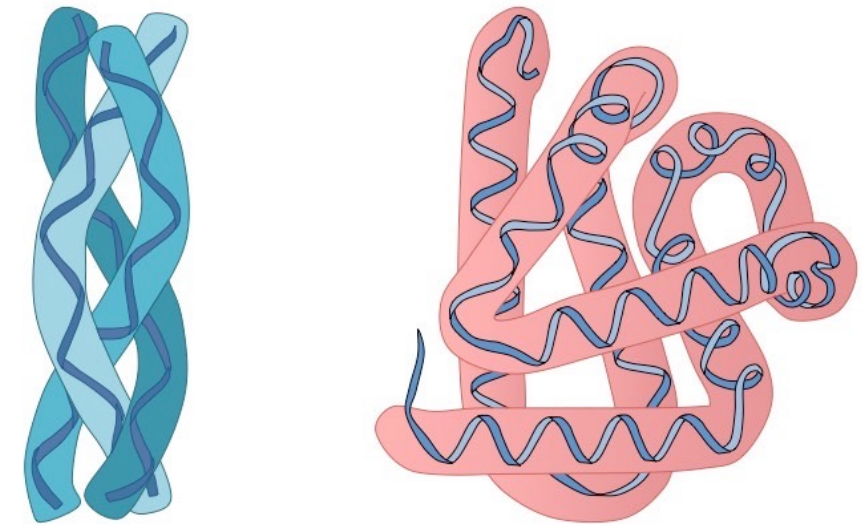
Concept 4.1: Globular and Fibrous Proteins

Globular proteins

- Spherical with hydrophilic exterior surfaces and hydrophobic cores
- Usually made of secondary and tertiary structures
  - Can also have quaternary structure (less common)
- Have a diverse list of functions
- E.g. Hemoglobin

Fibrous proteins

- Made of long multimer chains that are twisted together
- Stabilized by disulfide bonds
- Provide structural support
- E.g Collagen

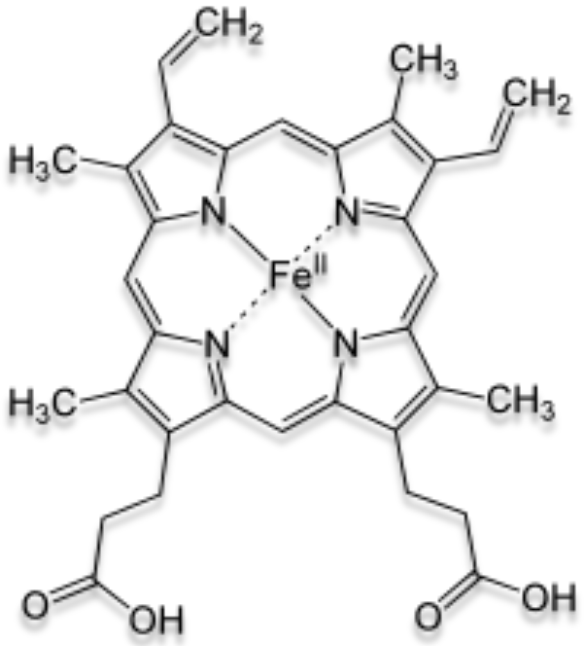


Depiction of Fibrous and Globular Proteins

Heme

Heme

- Prosthetic group
- Examples:
  - Electron carrier in cytochrome proteins
  - Part of enzymes such as catalase
  - Hemoglobin and myoglobin, reversibly binds to Oxygen



Myoglobin

Location

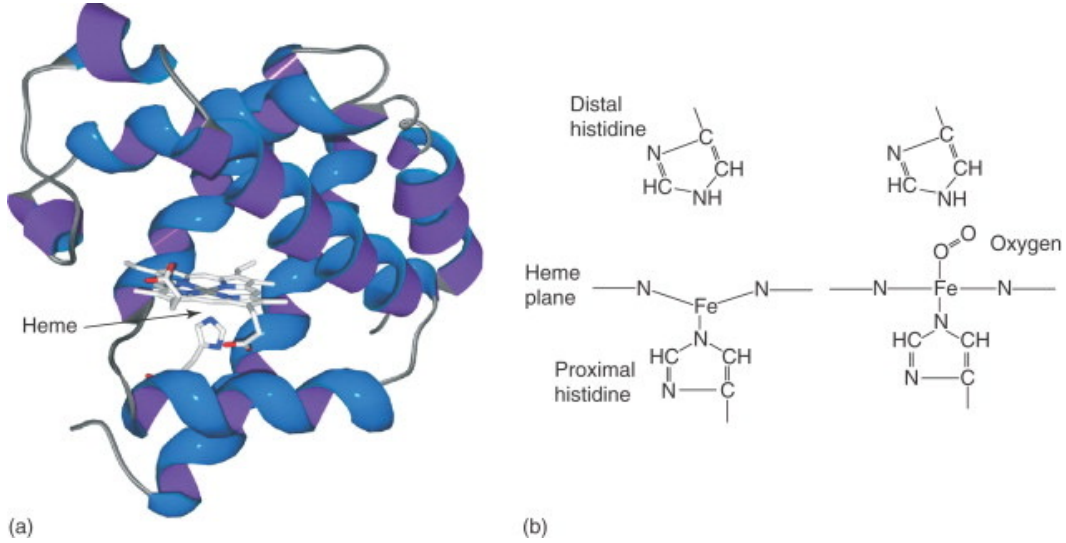
- Heart and skeletal muscle

Function

- Reservoir for oxygen and as an oxygen carrier to increase O_2 delivery to muscles

Structure

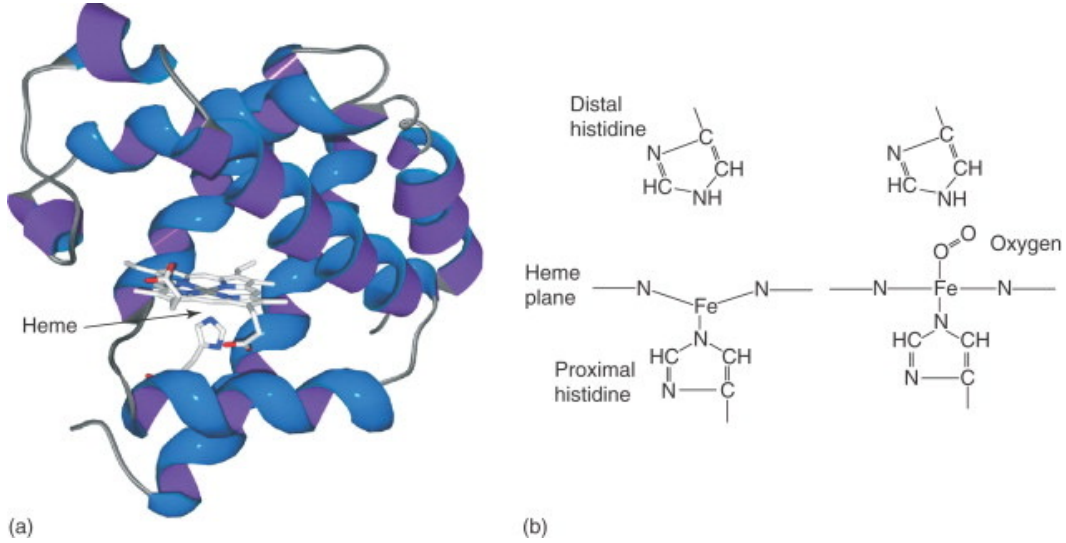
- Single peptide chain, 80% in the form of an alpha-helix
  - Interior almost all non-polar amino acids
  - Exterior almost all polar amino acids



Myoglobin Continued..

Structure

- Right environment for heme binding and reversible binding of oxygen
- The heme group is bound in a pocket lined with nonpolar a.a.
- The proximal His binds to the Fe of heme
- The distal His stabilizes the binding of O_2 to Fe of the heme group



Hemoglobin

Location

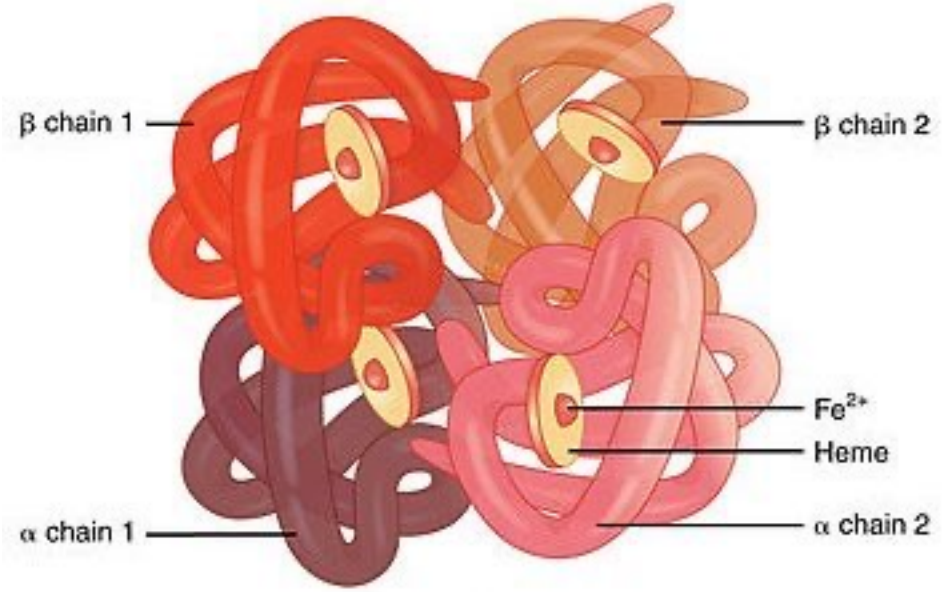
- RBC

Function

- Transport oxygen from lungs to capillaries in tissues

Structure

- Made of 4 peptide chains
  - 2 alpha-chains and 2 beta-chains
- Each chain is similar to myoglobin in structure, however, not the same



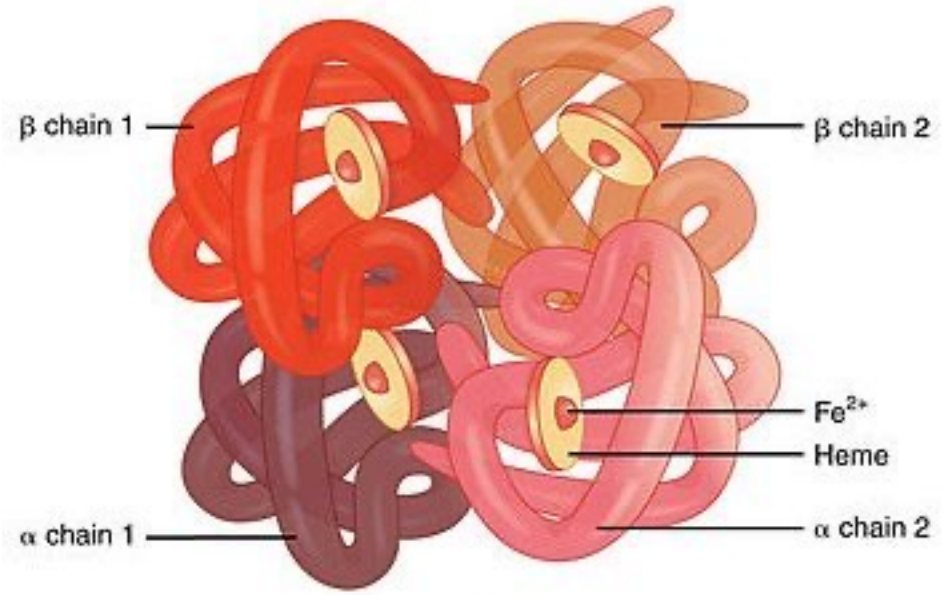
Hemoglobin Continued…

Structure

- Dimer of dimers
  - An alpha chain and beta chain are bound by hydrophobic interactions, forming a dimer
  - So 2 dimers
  - Both dimers are held together by weak H-bonds and ionic interactions

Significance

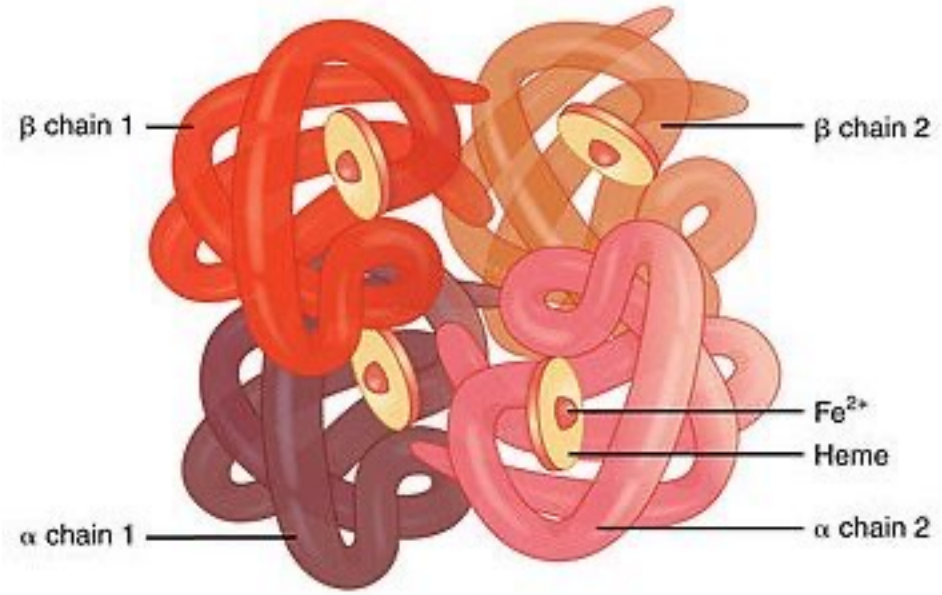
- Subunit cooperativity



Hemoglobin

Significance

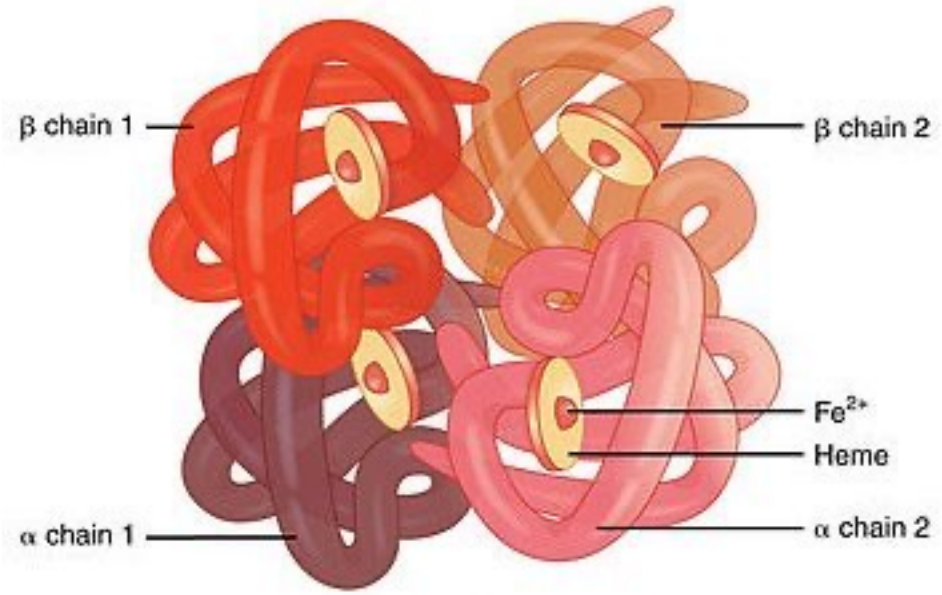
- When measuring the amount of oxygen bound to:
  - Myoglobin
    - Hyperbolic binding curve
  - Hemoglobin
    - Sigmoidal curve
    - Why?
      - Subunit cooperativity



Hemoglobin

Significance

- When 1 oxygen binds, a conformational change occurs
  - This breaks some of the ionic interactions among dimers, changing the structure
  - The structure is more relaxed now, and this increases affinity for oxygen binding, allowing more oxygen to bind



Physiological Importance

Myoglobin

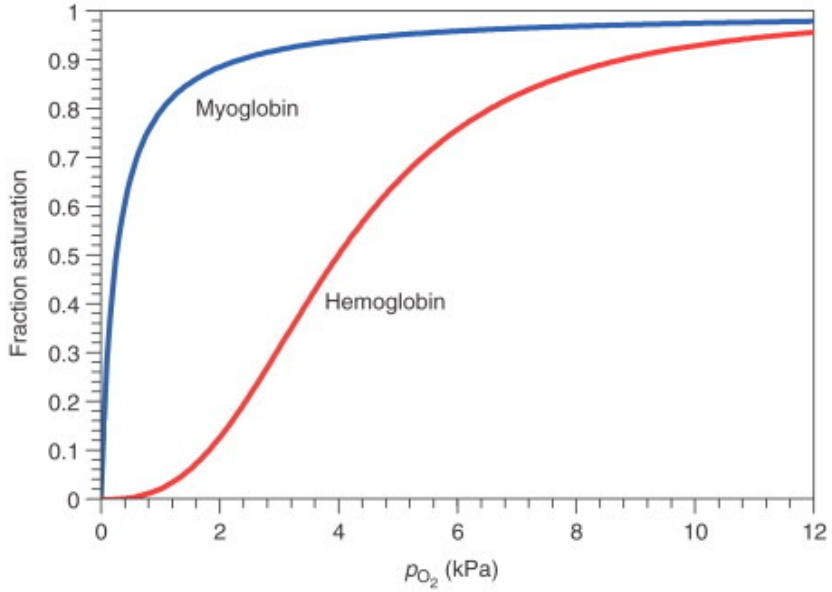
- Binds to oxygen with the same affinity, does not easily release bound oxygen → approximately 90% saturated at levels of oxygen present in the tissues

Hemoglobin

- Cooperative binding leads to complete saturation the lungs (pO2 = around 100 mmHg)
- At lower pO2 levels, due to cooperativity allowing for easier release of oxygen and stronger affinity from myoglobin, oxygen is transferred from hemoglobin to myoglobin

Summary

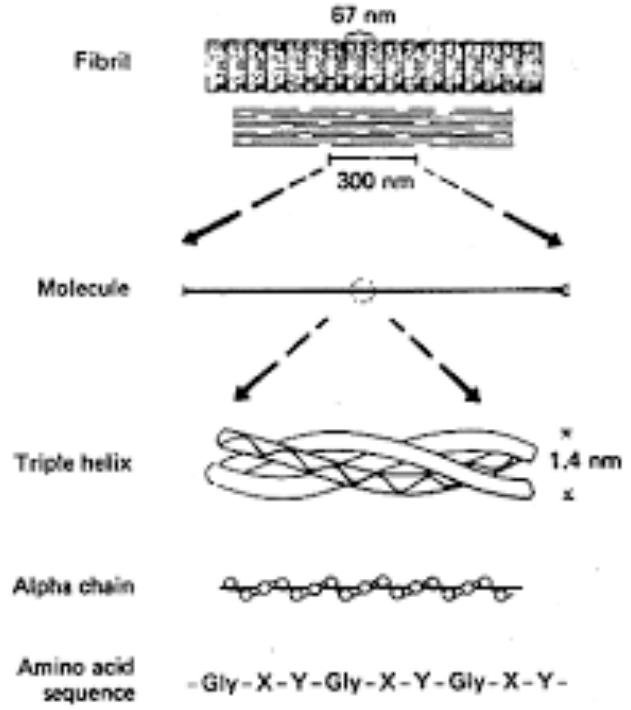
- Hemoglobin → delivery system
- Myoglobin → storage system, releases when needed



Concept 4.2: Collagen Structure

Primary structure

- Has a Gly-X-Y sequence that repeats
  - Glycine is every 3rd amino acid repeat
- Have hydroxyproline and hydroxylysine added as post-translational modifications
  - Proline causes the collagen chain to become a left-handed helix
- 3 left-handed helices come together to form one right-handed helix (collagen polypeptide)
- Collagen polypeptides come together to form a strong collagen fibril



Primary and secondary structure of collagen

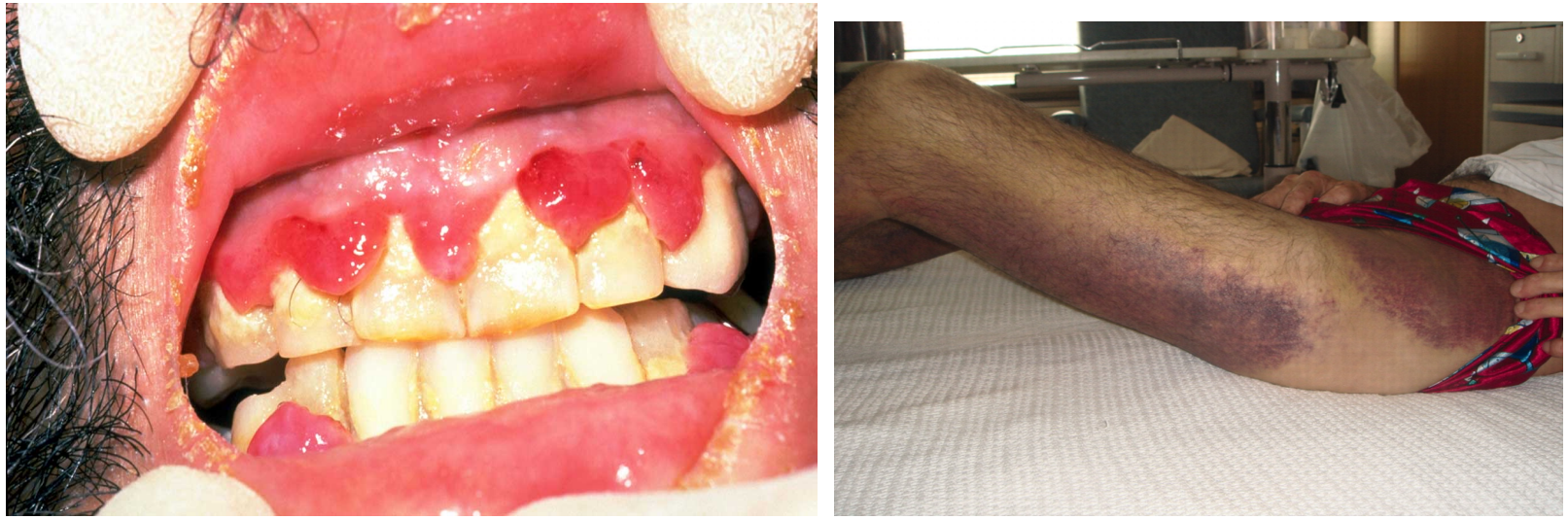
Spotlight on Disease: Scurvy

Causes

- Vitamin C deficiency
  - Necessary for hydroxylation of proline and lysine
- Leads to formation of unstable collagen polypeptides

Symptoms

- Bleeding gums
- Loose teeth
- Limb bruising



Bleeding gums (left) and bruising (right) as a consequence of collagen deficiency due to scurvy

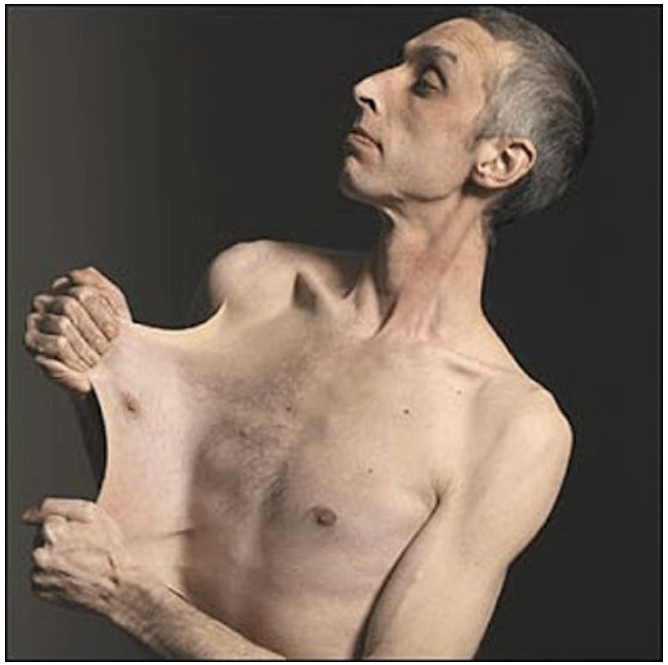
Spotlight on Disease: Ehlers-Danlos Syndrome

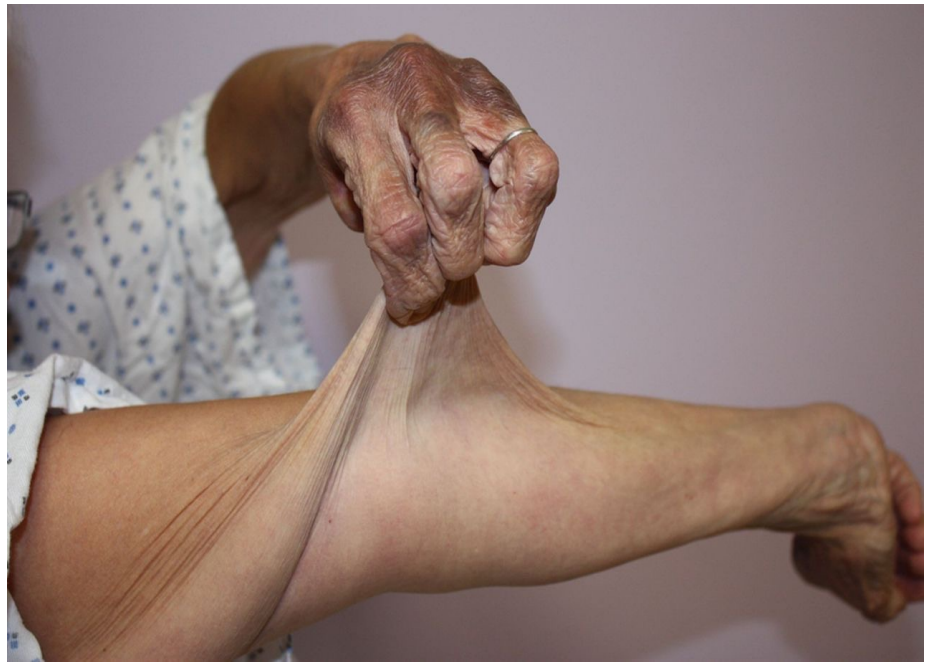
Cause

- Mutations in biosynthetic enzymes or the collagen sequence result in impaired collagen metabolism

Symptoms

- Skin fragility
- Skin extensibility
- Joint hypermobility





Skin extensibility as a consequence of impaired collagen metabolism in two patients with Ehlers Danlos syndrome.

Spotlight on Disease: Osteogenesis Imperfecta

Cause

- Mutations in COL1A1 and COL1A2 are the main causes
    - Provide instruction for forming proteins that are used to assemble type I collagen
- Most common mutation is a change of substitute of the Gly in Gly-X-Y to a bulky amino acid

Healthy bone density vs. osteogenesis imperfecta bone density

Symptoms

- Bones fracture very easily
- Thus it is often referred to as 'brittle bone disease'

Section 4 Quiz: 1 MC

Which amino acid is responsible for making collagen peptides left-handed helices?

a. Glycine

b. Proline

c. Tyrosine

d. Lysine

Answer: B