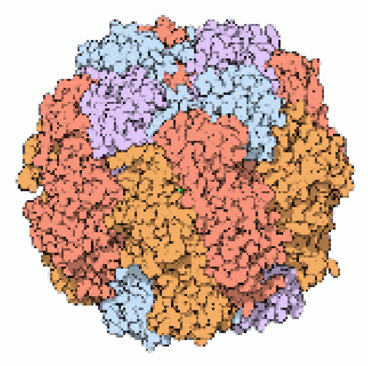
BCM 201
BY
DR. AGUNBIADE

STRUCTURE OF PROTEINS AND BASIC PRINCIPLES OF TESTS FOR PROTEINS

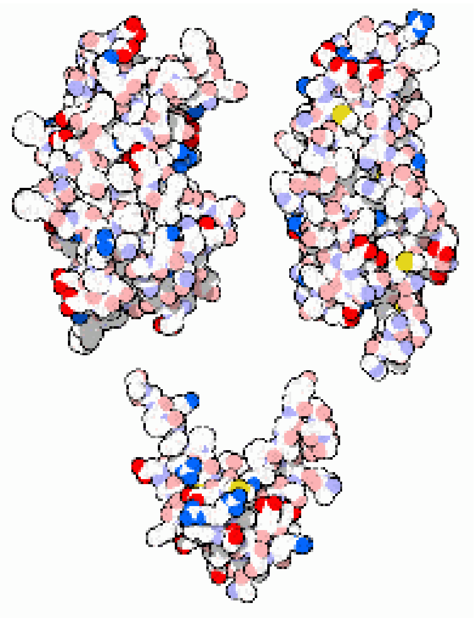
INTRODUCTION

- Proteins are complex biological macromolecules composed of **amino acids linked by peptide bonds**. They perform diverse functions such as enzymatic catalysis, structural support, transport, regulation, and defence.

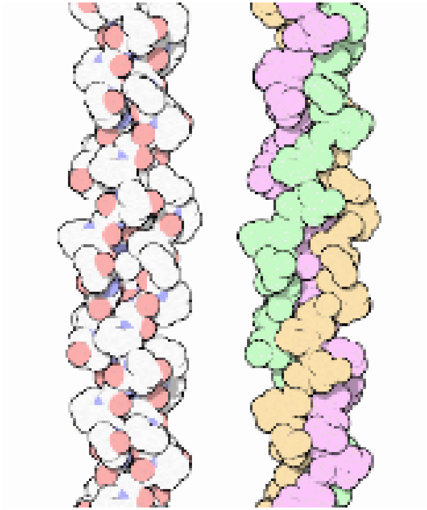
- The biological activity of a protein depends largely on its **structure**, which is organized at four hierarchical levels: **primary, secondary, tertiary, and quaternary structures**. Proteins can also be identified in the laboratory using **qualitative chemical tests** based on their characteristic chemical groups.



PROTEIN



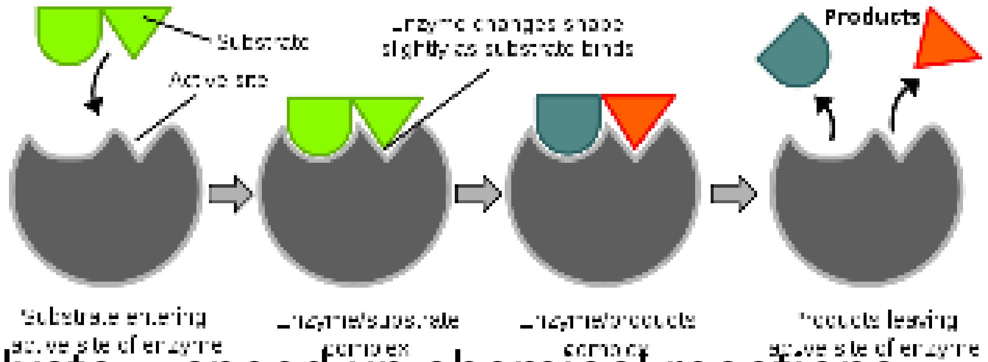
Multipurpose molecules



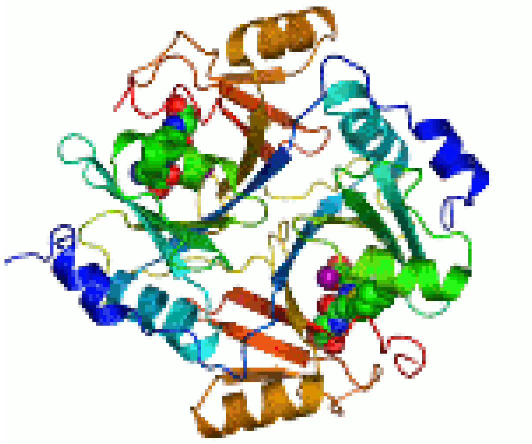
Proteins

- Most structurally & functionally diverse group of biomolecules
- Function:
  - involved in almost everything
    - Metabolism
    - Support
    - Transport
    - Regulation
    - Motion

Metabolism

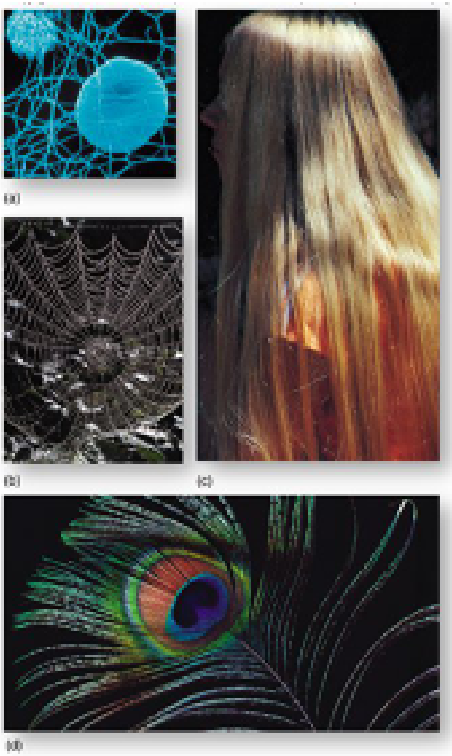


- Enzymes
  - Biological catalysts – speed up chemical reactions
    - Digestive enzymes aid in hydrolysis
      - Lipase
      - Amylase
      - Lactase
      - Protease
    - Molecular Biology
      - Polymerase
      - Ligase
    - Industry
      - Dairy, baby food, rubber, beer, photography, contact lense cleaner



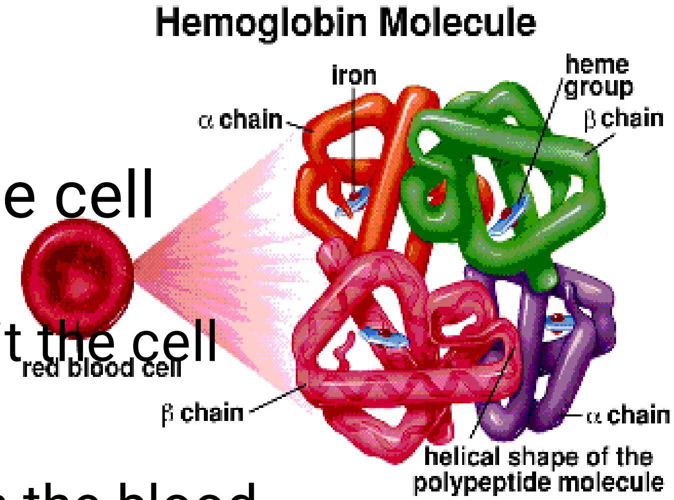
Support

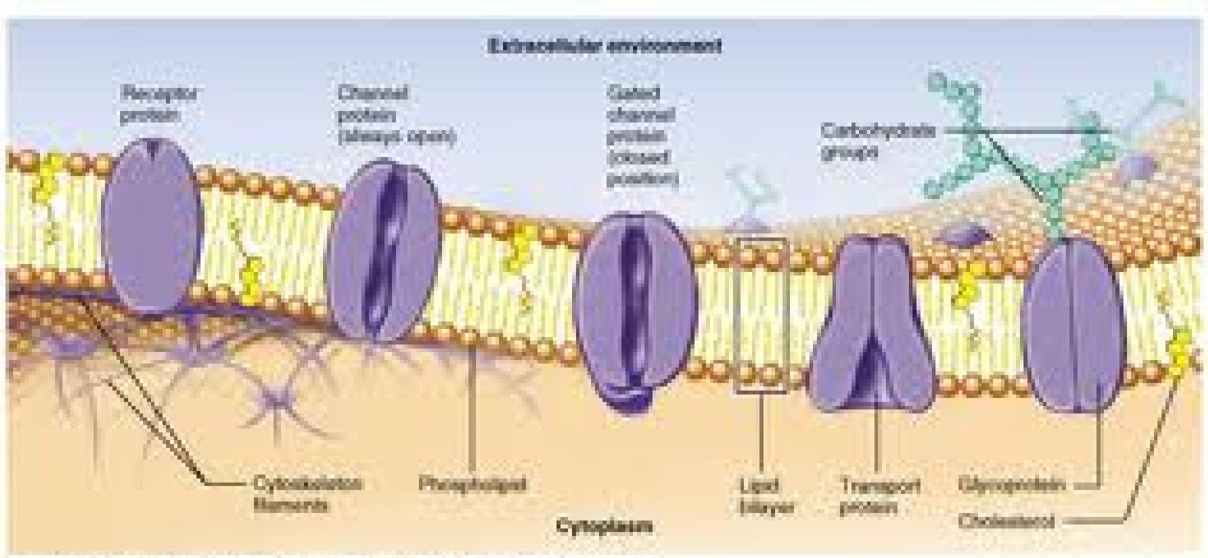
- Structural proteins
  - Keratin – hair and nails
  - Collagen – supports ligaments, tendons, and ski
  - Silk – cocoons and spider webs



Transport

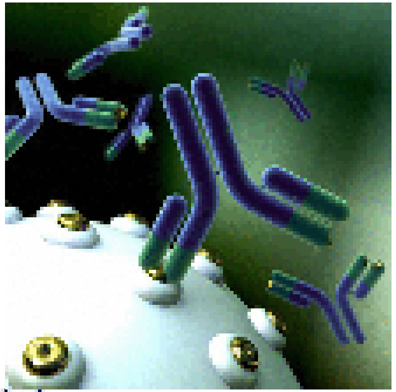
- Channel and carrier proteins in the cell membrane
  - Allows substances to enter and exit the cell
- Transport molecules in blood
  - Hemoglobin – transports oxygen in the blood

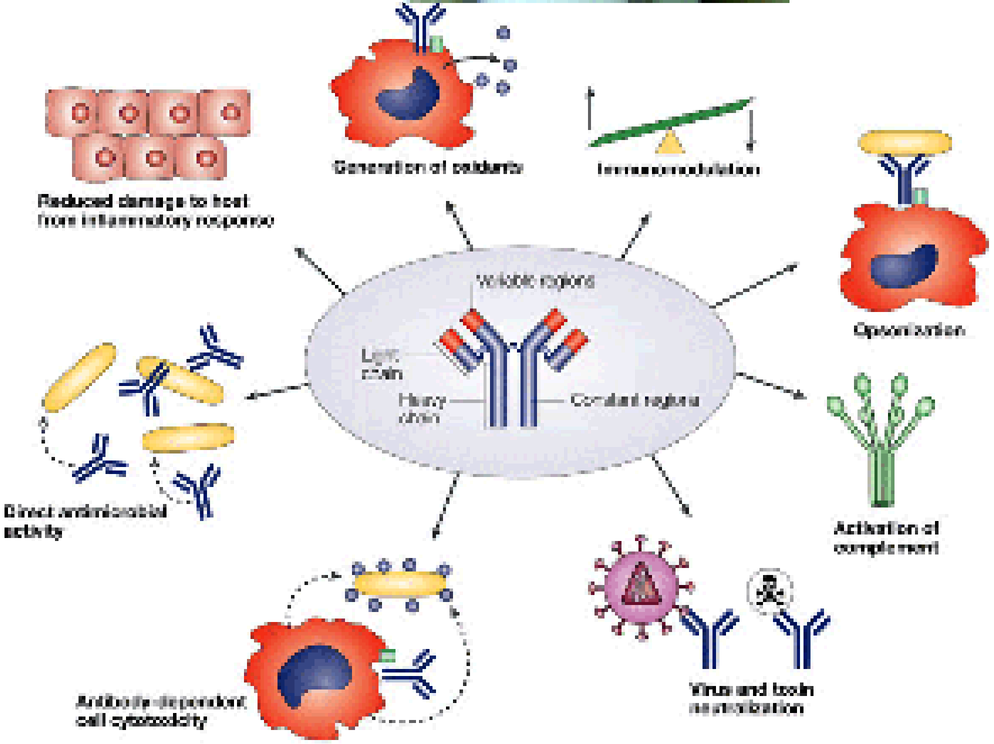




Defense

- Antibodies
  - Combat bacteria and viruses

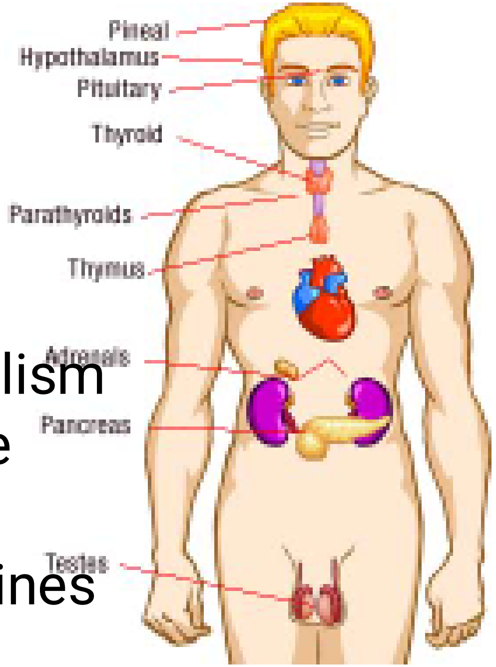




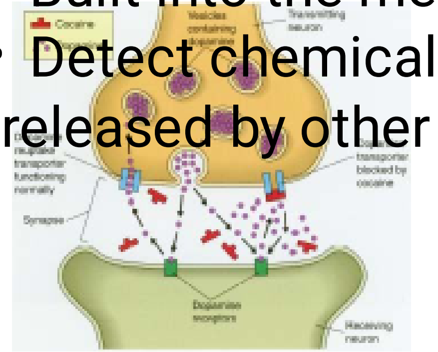
Nature Reviews | Microbiology

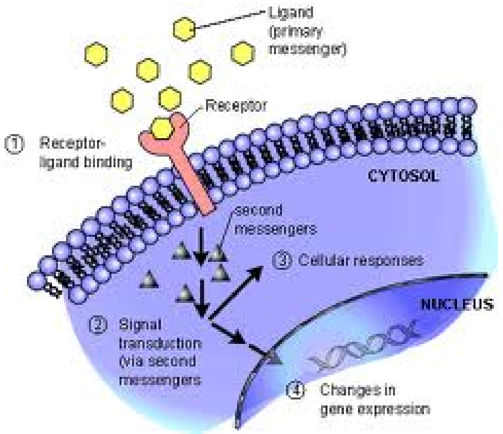
Regulation

- Hormones
  - Intercellular messengers that influence metabolism
  - Insulin – regulates the amount of glucose in the blood and in cells
  - Human growth hormone – its presence determines the height of an individual



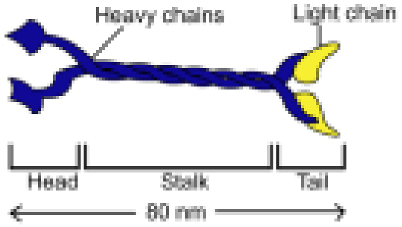
- Receptor Proteins
  - Built into the membranes of nerve cells
  - Detect chemical signals (neurotransmitters) released by other nerve cells

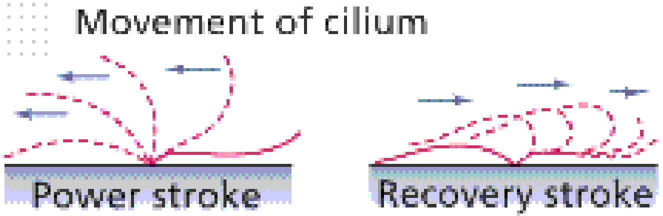


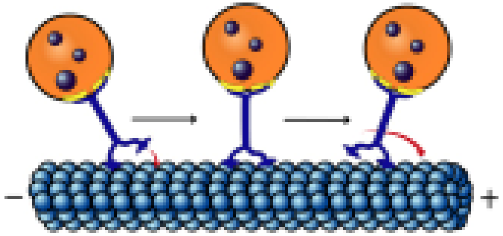


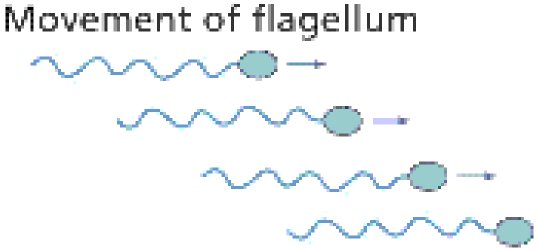
Motion

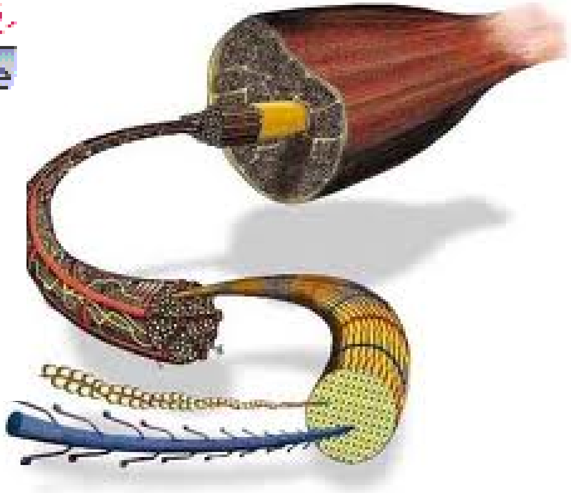
- Muscle contraction
  - Actin and myosin – make up muscle fibers
- Motor proteins within the cell
  - Allow cell components to move from place to place
  - Flagella- move the cell
  - Cilia- move contents around the cell



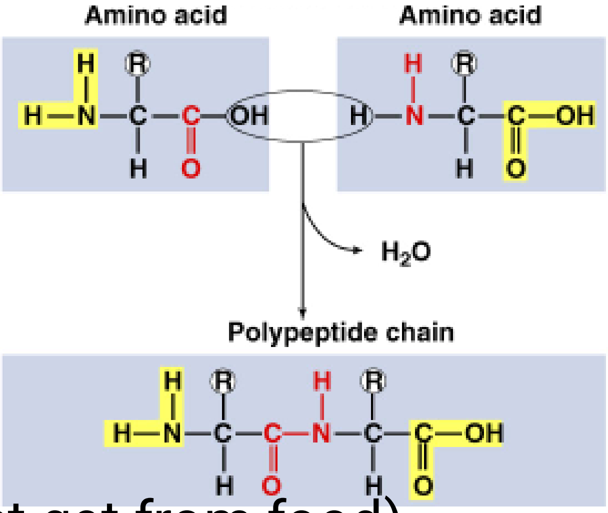




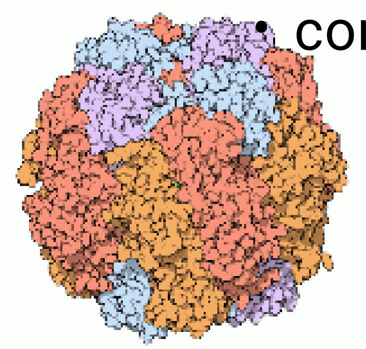




Proteins

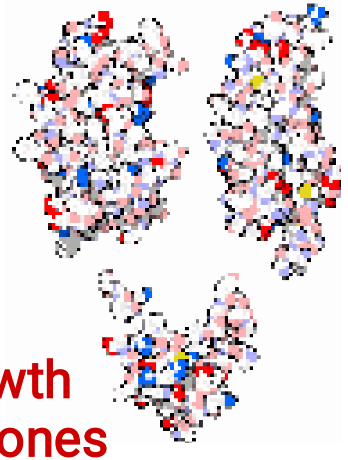


- Structure:
  - monomer = amino acids
    - 20 different amino acids
      - 12 made by body
      - 8 essential amino acids (must get from food)
  - polymer = polypeptide
    - protein can be one or more polypeptide chains folded & bonded together
    - large & complex molecules
    - complex 3-D shape



Rubisco

hemoglobin

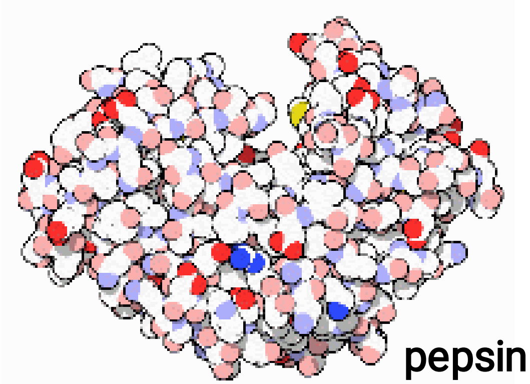


growth hormones

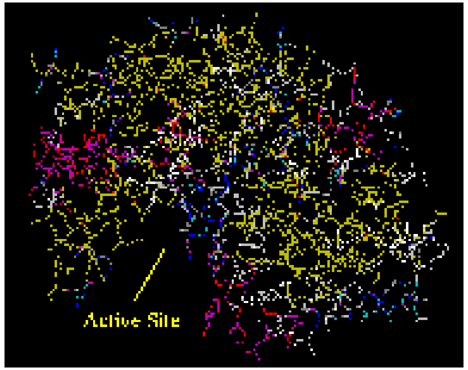
Protein structure & function

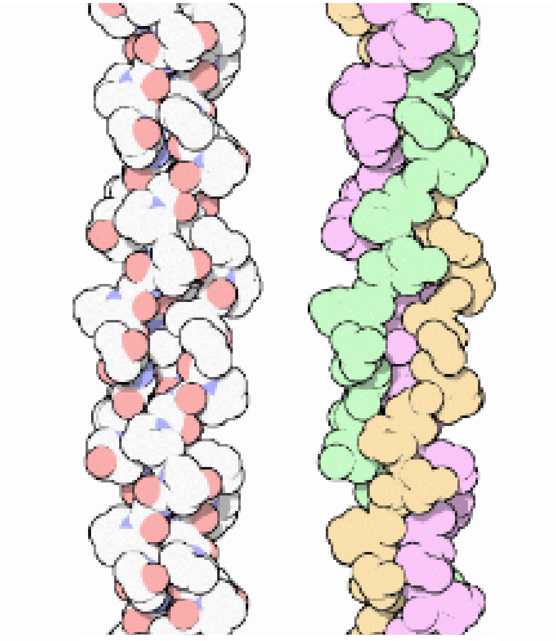
- Function depends on structure
  - 3-D structure
    - twisted, folded, coiled into unique shape

hemoglobin



pepsin





collagen

LEVELS OF PROTEIN STRUCTURE
Primary Structure of Proteins

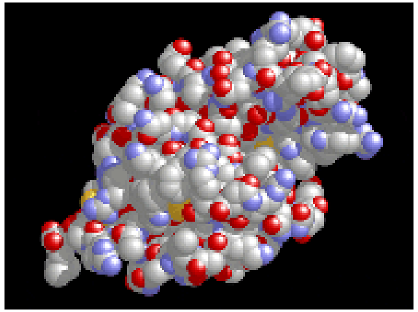
The primary structure refers to the **linear sequence of amino acids** in a polypeptide chain, joined together by **peptide bonds**.

- **Key Features:**
- Specifies the exact order of amino acids from the N-terminal to the C-terminal end

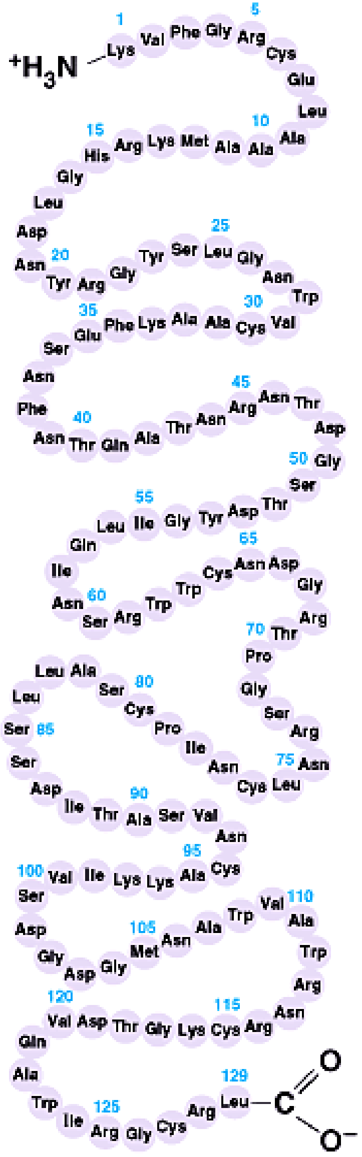
- Even a single amino acid change can alter protein function (e.g. , sickle cell anemia)

Primary (1°) structure

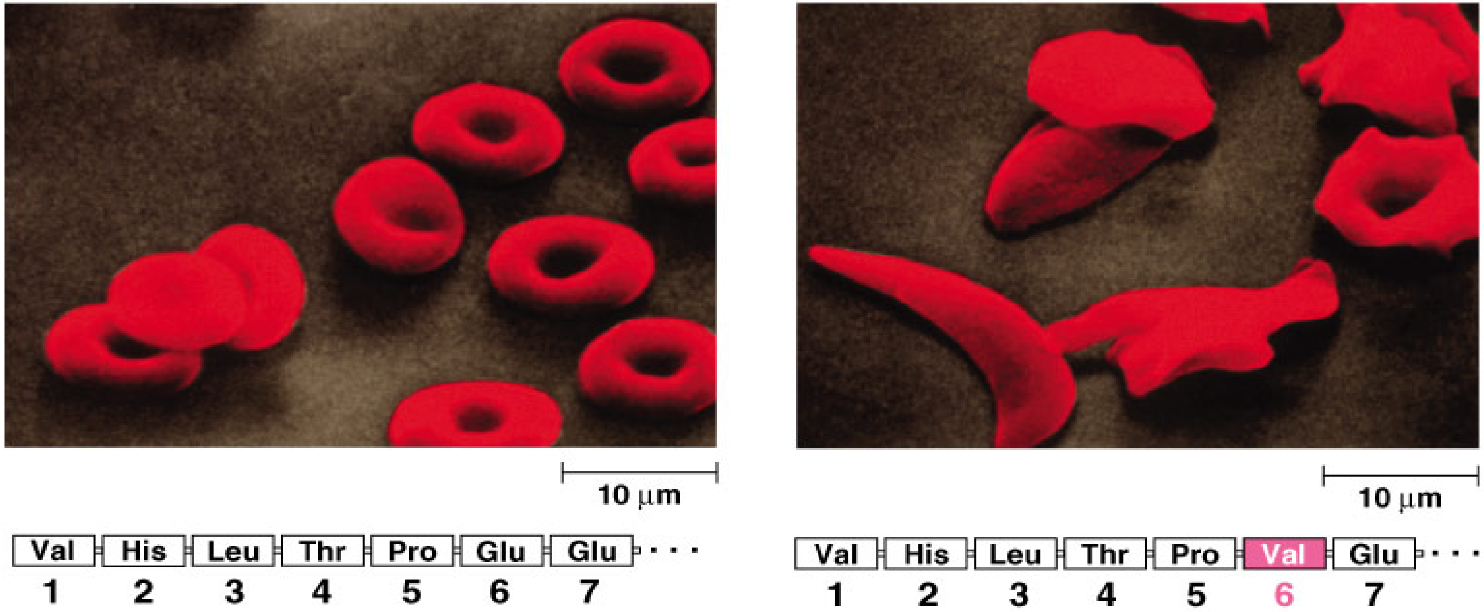
- Order of amino acids in chain
  - amino acid sequence determined by gene (DNA)
  - slight change in amino acid sequence can affect protein's structure & it's function
    - even just one amino acid change can make all the difference!

lysozyme: enzyme in tears & mucus that kills bacteria



Sickle cell anemia



(a) Normal red blood cells and the primary structure of normal hemoglobin

(b) Sickled red blood cells and the primary structure of sickle-cell hemoglobin

Bond Involved

- Peptide bond (covalent bond)
- **Significance:**
- Determines all higher levels of protein structure
- Essential for correct folding and biological activity

Secondary Structure of Proteins

- **Definition:**
  The secondary structure refers to the **local folding of the polypeptide chain** into regular patterns stabilized by **hydrogen bonds** between peptide backbone atoms.
- **Common Types:**

(a) Alpha (α) Helix

- Right-handed coiled structure
- Stabilized by hydrogen bonds between C=O of one amino acid and N–N of the fourth amino acid ahead
- Common in fibrous and globular proteins

(b) Beta (β) Pleated Sheet

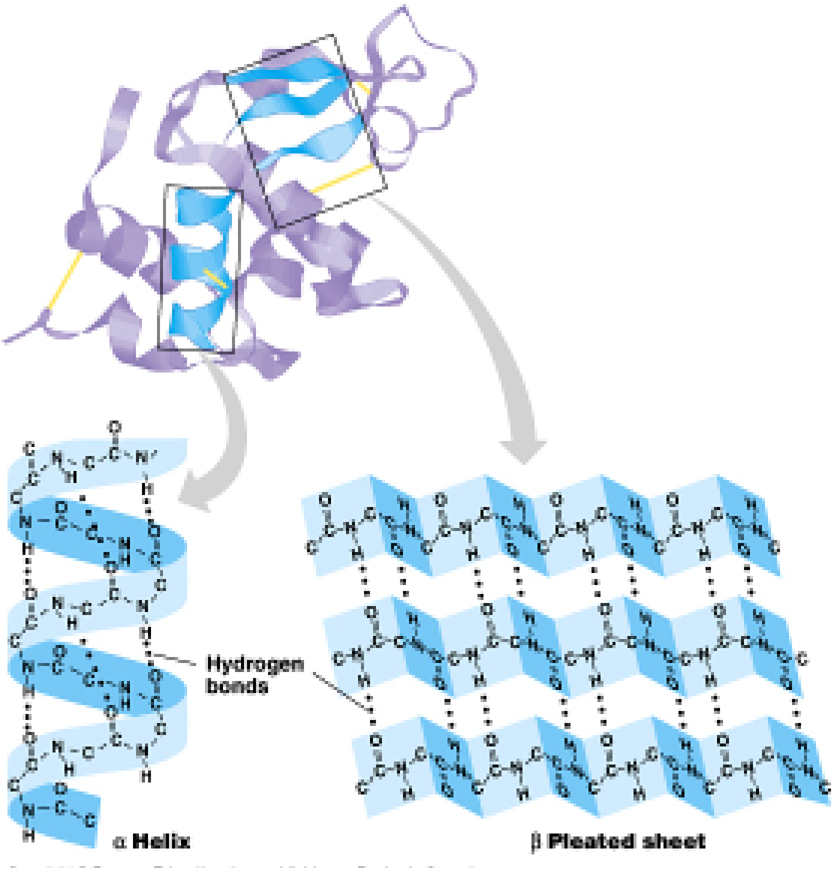
- Polypeptide chains arranged side-by-side
- May be **parallel or antiparallel**
- Stabilized by hydrogen bonds between adjacent chains
- Found in proteins like silk fibroin

Bond Involved:

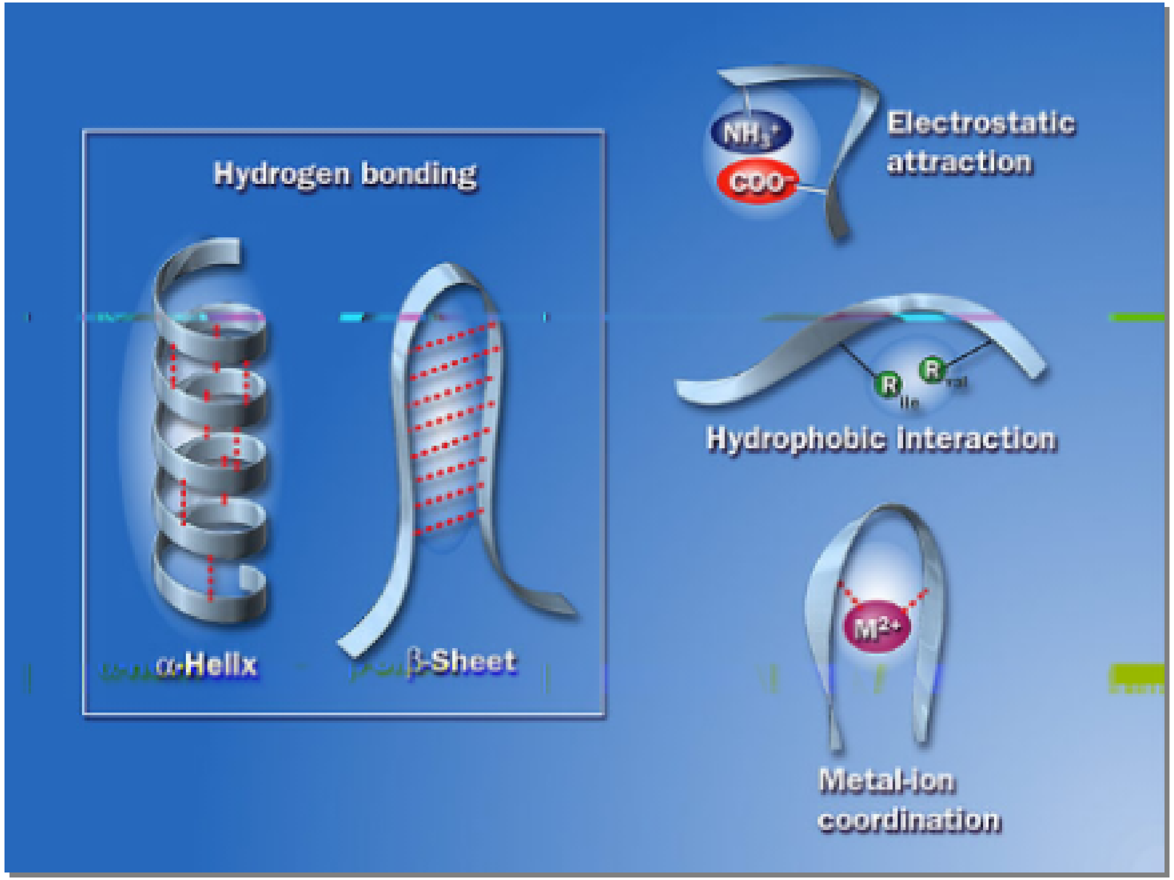
- Hydrogen bonds
- **Significance:**
- Provides structural stability
- Contributes to protein flexibility and strength

Secondary (2°) structure

- "Local folding"
  - folding along short sections of polypeptide
    - interaction between adjacent amino acids
    - H bonds between backbones (O:H)
    - α-helix
    - β-pleated sheet
    - Fibrous proteins – only have secondary structure
      - Keratin
      - Silk



Secondary (2°) structure

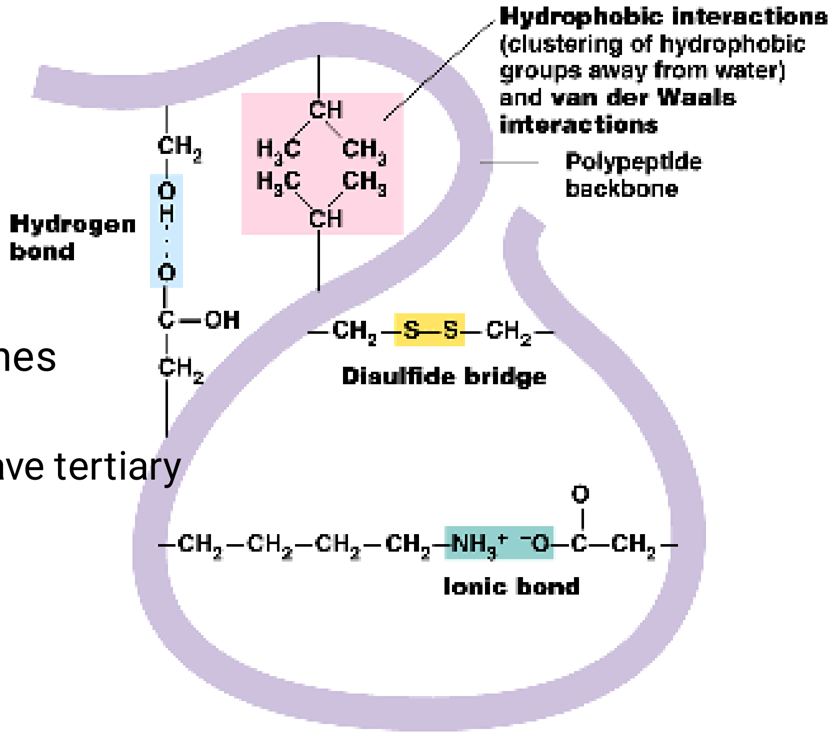


Tertiary Structure of Proteins

- **Definition:**
  The tertiary structure refers to the **three-dimensional folding** of a single polypeptide chain resulting from interactions between side chains (R-groups).
- **Forces Involved:**
- Hydrogen bonds
- Ionic (salt) bonds
- Disulfide bonds (−S−S−)
- Hydrophobic interactions
- Van der Waals forces

Tertiary (3°) structure

- "Whole molecule folding"
  - **created when the secondary structure fold and form bonds to stabilize the structure into a unique shape**
  - determined by interactions between R groups
    - Hydrophobic interactions
    - anchored by disulfide bridges
    - Ionic Bonds between R groups
    - Hydrogen bonds between backbones
    - Van der Waals Force (velcro)
      - Globular (spherical) proteins – have tertiary structure
      - enzymes



- **Key Features of tertiary structure:**
- Gives the protein its specific shape
- Forms active sites of enzymes
- Most globular proteins are functional at this level
- **Significance:**
- Determines biological activity
- Denaturation (by heat, pH, chemicals) disrupts tertiary structure

Quaternary Structure of Proteins

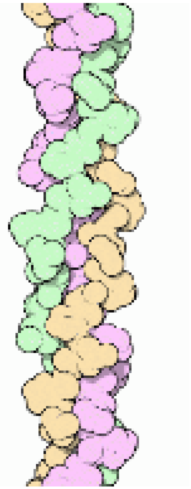
- **Definition:**
  The quaternary structure refers to the **association of two or more polypeptide chains (subunits)** into a functional protein complex.
- **Key Features:**
- Each subunit has its own tertiary structure
- Subunits may be identical or different

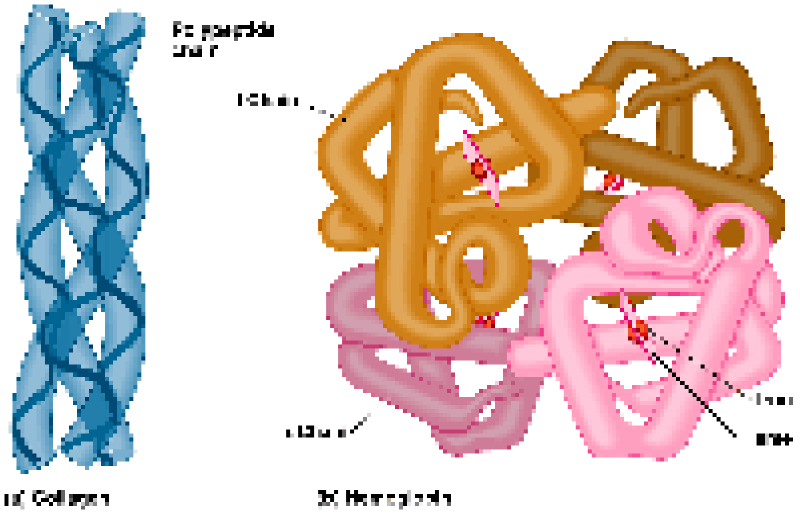
Quaternary (4°) structure

- **two or more tertiary folded peptide subunits bonded together to make a functional protein**
  - Hemoglobin – 4 polypeptides
  - Collagen – 3 polypeptides

collagen =

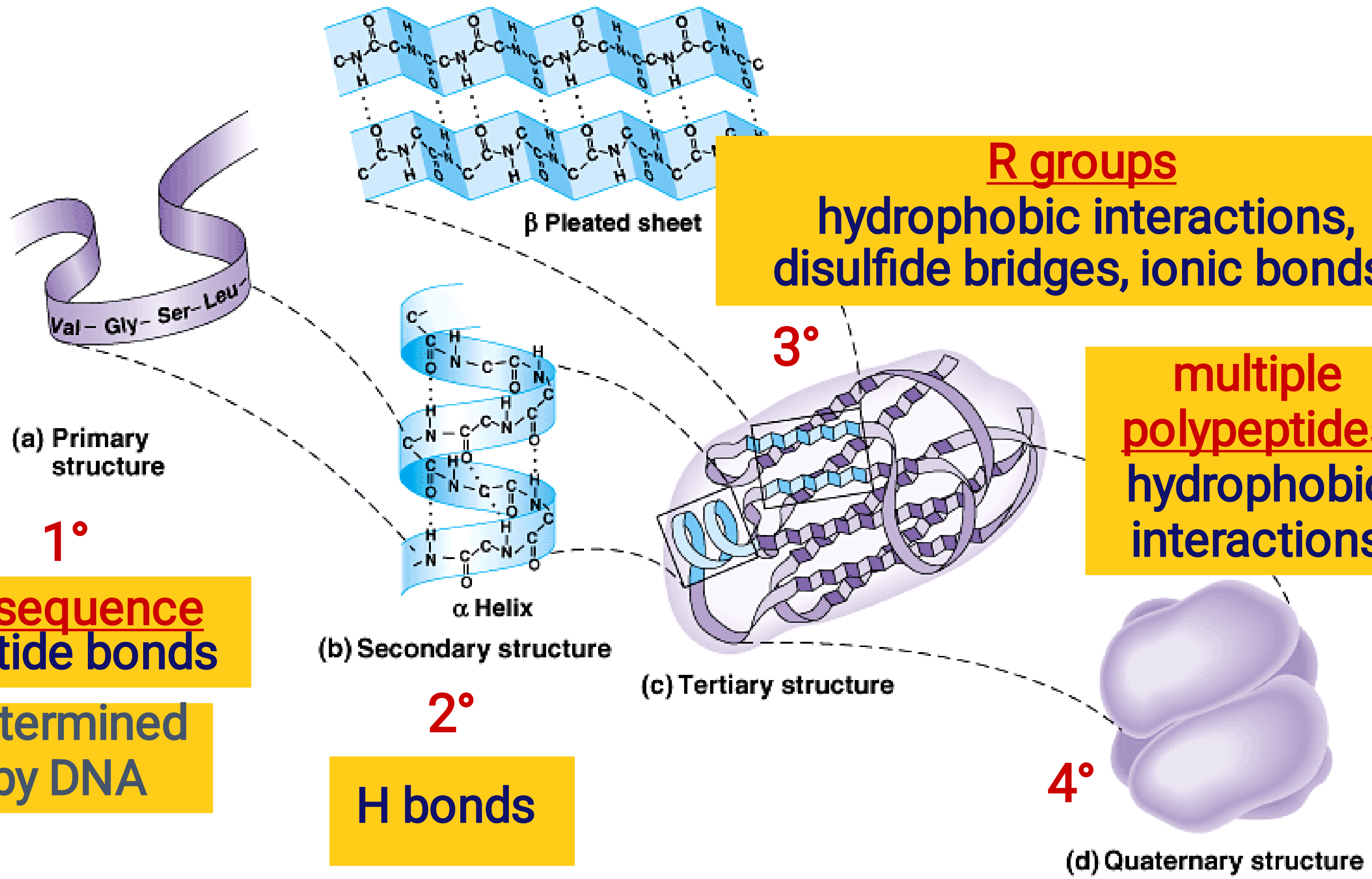
skin & tendons





hemoglobin

Protein structure (review)



1°

aa sequence
peptide bonds

determined by DNA

2°

H bonds

3°

R groups
hydrophobic interactions, disulfide bridges, ionic bonds

4°

multiple polypeptides
hydrophobic interactions

Examples:

- Hemoglobin (4 subunits)
- Immunoglobulins
- Lactate dehydrogenase

Forces Involved:

- Non-covalent interactions (hydrogen bonds, ionic bonds, hydrophobic interactions)
- Sometimes disulfide bonds

Significance:

- Enables cooperative binding (e.g., oxygen binding in hemoglobin
- Enhances functional regulation

BASIC PRINCIPLES OF TESTS FOR PROTEINS

Proteins can be detected using chemical tests that react with **peptide bonds or specific amino acids**

Biuret Test

- **Principle:**
  Peptide bonds react with copper ions (Cu^{2+}) in alkaline solution to form a **violet or purple complex**.

Positive Result:

- Violet or purple color

Significance:

- General test for proteins
- Requires at least two peptide bonds

Ninhydrin Test

- **Principle:**
  Ninhydrin reacts with **free amino groups** to produce a **purple (Ruhemann's purple)** color.
- **Positive Result:**
- Purple color (yellow with proline and hydroxyproline)
- **Significance:**
- Detects amino acids and proteins with free amino groups

Xanthoproteic Test

- **Principle:**
  Concentrated nitric acid reacts with **aromatic amino acids** (tyrosine, tryptophan, phenylalanine) forming yellow nitro derivatives.
- **Positive Result:**
- Yellow color turning orange with alkali
- **Significance:**
- Indicates presence of aromatic amino acids

Millon’s Test

- **Principle:**
  Millon’s reagent reacts with the **phenolic group of tyrosine**.
- **Positive Result:**
- Red or brick-red color
- **Significance:**
- Specific for tyrosine-containing proteins