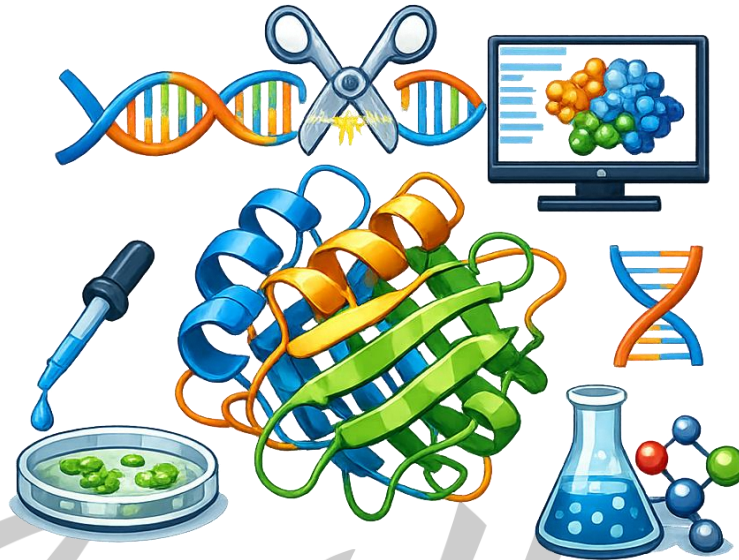


Protein Engineering

Concepts for Protein Engineering

Definition of Protein Engineering

- **Protein engineering** is the process of designing or modifying proteins to improve their properties or to create new functions.



- It mainly involves changing the amino acid sequence so that the protein behaves differently in terms of structure, stability, or activity.

Basic Structure of Proteins

- Proteins are made up of amino acids joined together by peptide bonds.
- The sequence of amino acids is called the primary structure.
- **This sequence folds into:**
 - Secondary structure (alpha-helix and beta-sheet)
 - Tertiary structure (3D folding)
 - Quaternary structure (interaction of multiple protein chains)
- The final 3D shape determines the function of the protein.

Relationship Between Structure and Function

- The function of a protein depends on its shape.
- **Even a small change in amino acid sequence can:**
 - Change folding
 - Effect of binding with other molecules
 - Increase or decrease activity
- Misfolding can lead to loss of function or instability.

Levels of Protein Structure (Important Concept)

- **Primary structure:** Sequence of amino acids.
- **Secondary structure:** Local folding (alpha-helix, beta-sheet).
- **Tertiary structure:** 3D shape of a single protein molecule.
- **Quaternary structure:** Assembly of multiple protein subunits.
- Any modification at the primary level can affect higher levels.

Goals of Protein Engineering

- To increase enzyme efficiency and speed of reaction
- To improve stability against heat, pH, and chemicals
- To change substrate specificity
- To reduce immune response in therapeutic proteins
- To improve solubility and shelf life in formulations

Types of Protein Engineering Approaches

A. Rational Design

- Based on knowledge of protein structure and function
- Specific changes are made intentionally

B. Directed Evolution

- Random mutations are introduced
- Best-performing proteins are selected

C. Semi-Rational Approach

- Combination of both methods
- Uses some structural knowledge with random variation

Key Tools Used

- DNA sequencing
- Recombinant DNA technology
- Computer modeling (bioinformatics tools)
- Site-directed mutagenesis

Applications

- Used in making drugs like insulin and monoclonal antibodies
- Used in industrial enzymes for detergents and food industry
- Used in diagnostic tools like biosensors

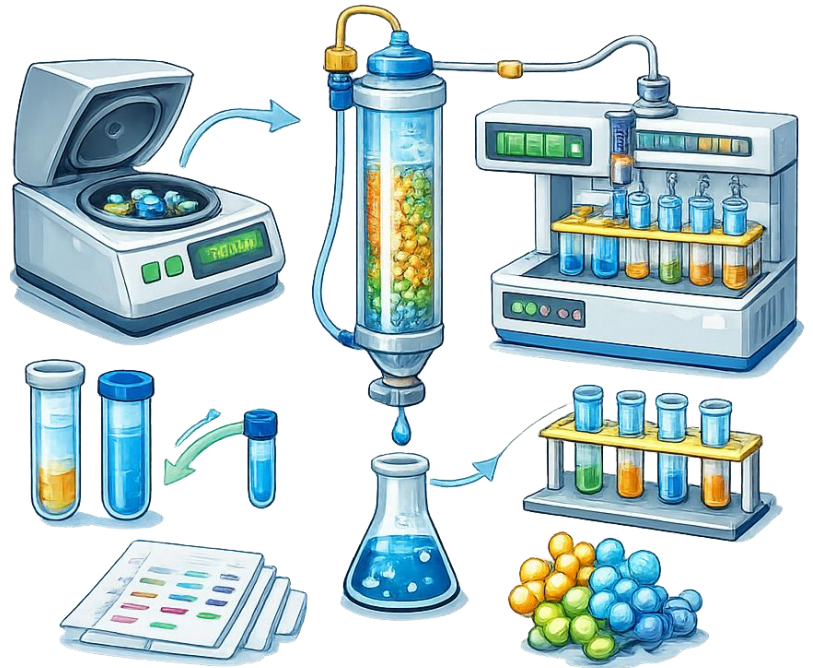
Isolation and Purification of Proteins

Definition

- **Isolation:** Separation of a specific protein from a complex mixture (cells or tissues).
- **Purification:** Removal of all unwanted substances to obtain protein in pure form.

Purpose / Importance

- To obtain a specific protein for study and applications.
- **Required for:**
 - Structural studies
 - Functional analysis
 - Drug formulation
 - Industrial applications
- **High purity ensures:**
 - Safety of pharmaceutical products
 - Consistent biological activity
 - Stability during storage and use



Steps in Protein Isolation and Purification



Cell Disruption



Removal of Cell Debris



Protein Extraction



Protein Precipitation

1. Cell Disruption (Cell Lysis)

- Proteins are located inside cells, so cells must be broken open.
- **Methods:**
 - **Mechanical:** homogenization, sonication

- **Chemical:** detergents
- **Enzymatic:** lysozyme

2. Removal of Cell Debris

- Cell fragments and large particles are removed after lysis.
- Done by **centrifugation**:
 - Supernatant: contains soluble proteins
 - Pellet: contains cell debris

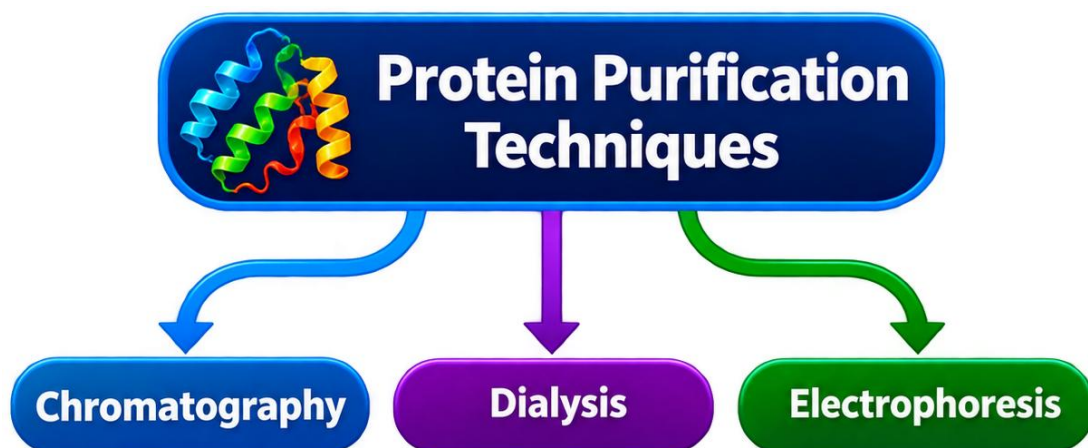
3. Protein Extraction

- Proteins are extracted into a suitable buffer.
- **Buffer maintains**:
 - pH
 - Ionic strength
 - Protein stability

4. Protein Precipitation

- Used to concentrate and partially purify proteins.
- Based on differences in solubility.
- Common method: **ammonium sulfate precipitation**

Protein Purification Techniques



A. Chromatography (Most Important Method)

- **Separates proteins based on specific properties**:
 - Ion-exchange chromatography – based on charge
 - Size-exclusion chromatography – based on size
 - Affinity chromatography – based on specific binding
 - Hydrophobic interaction chromatography – based on hydrophobicity

B. Dialysis

- Remove small molecules such as salts.
- Uses a semi-permeable membrane.

C. Electrophoresis

- Separates proteins based on size and charge.
- **SDS-PAGE** is commonly used to check purity.

Factors Affecting Purification

- **Temperature:** kept low to prevent protein degradation
- **pH:** must be optimal for protein stability
- **Protease inhibitors:** prevent protein breakdown

Assessment of Purity

- SDS-PAGE analysis
- Spectrophotometry
- Activity assays (to confirm functional protein)

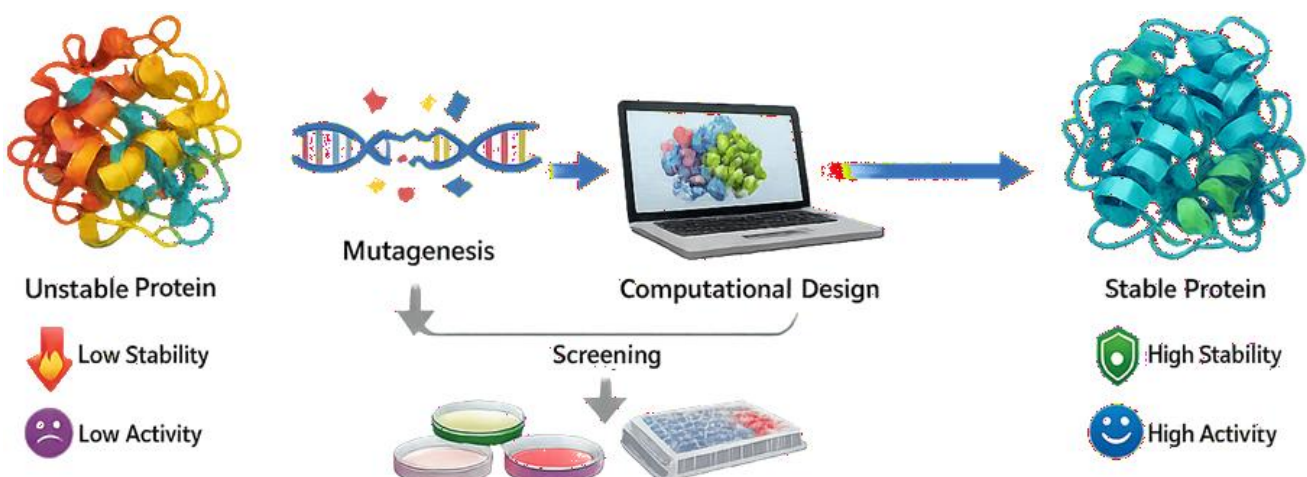
Storage of Purified Proteins

- Store at low temperatures (4°C, -20°C, or -80°C)
- Stabilizers may be added (e.g., glycerol, salts)
- Avoid repeated freeze–thaw cycles

Stability-Based Protein Engineering

Definition

- Stability is the ability of a protein to maintain its structure under different environmental conditions such as temperature, pH, and solvents.





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