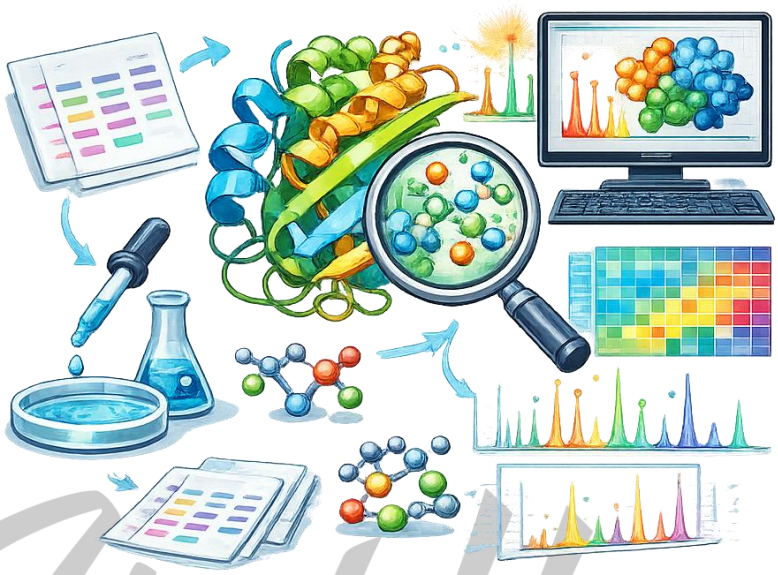
Proteomics

Proteomics

Introduction to Proteomics

- Proteomics is the large-scale study of all proteins present in a cell, tissue, or organism at a given time.



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- **It focuses on understanding:**
  - Protein expression
  - Structure
  - Function
  - Interactions
- **Unlike the genome** (which is constant), the proteome changes depending on conditions **such as:**
  - Environment
  - Disease state
  - Development stage

Importance of Proteomics

- Helps in understanding cellular processes at the protein level
- Identifies disease-related proteins (biomarkers)
- Supports drug discovery and development
- Important in studying post-translational modifications

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Levels of Proteomics Study

A. Expression Proteomics

- Studies change in protein levels under different conditions

B. Structural Proteomics

- Determines 3D structure of proteins

C. Functional Proteomics

- Studies protein interactions and functions

Types of Proteomics Approaches

1. Gel-Based Proteomics

- Uses techniques like 2D gel electrophoresis
- Separates proteins based on physical properties

2. Gel-Free Proteomics

- Uses advanced techniques like mass spectrometry
- Faster and more sensitive

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Key Techniques in Proteomics

- Mass spectrometry (MS)
- Chromatography
- Electrophoresis
- Bioinformatics tools

Applications

- Disease diagnosis (cancer biomarkers)
- Drug target identification
- Vaccine development
- Personalized medicine

Protein Identification and Characterization: Methods/Strategies

Overview of Protein Identification and Characterization

- Protein identification means finding out which protein is present in a sample.
- **Protein characterization means studying it:**

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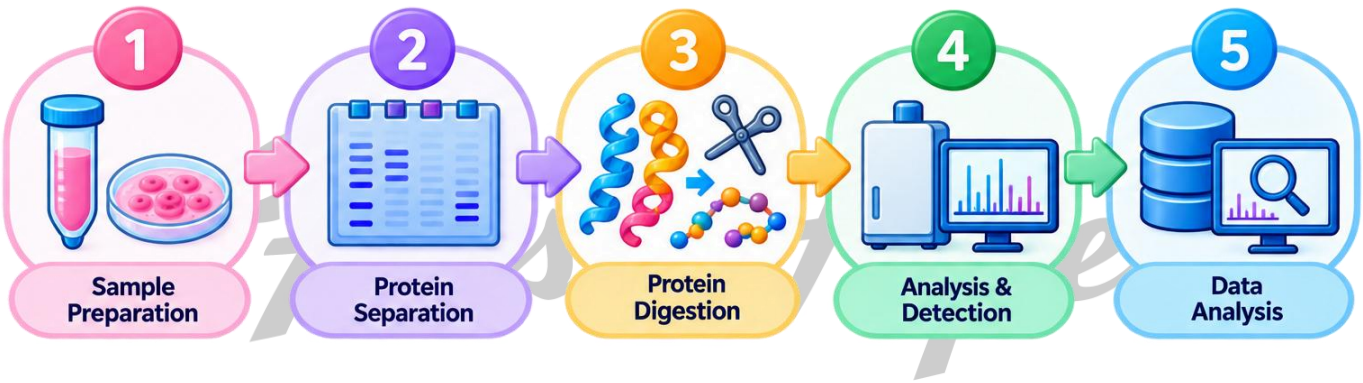
- Structure
- Function
- Physical and chemical properties

- These steps are very important in proteomics and protein formulations.

General Strategy for Protein Identification

- **Protein identification follows a step-by-step workflow:**
  - Extract proteins from a biological source (cells or tissues)
  - Separate proteins into individual components
  - Break proteins into smaller peptides
  - Analyze peptides using advanced techniques
  - Compare results with known protein databases

Steps Involved in Protein Identification



1. Sample Preparation / Protein Extraction

- Proteins are extracted using suitable buffers
- Conditions (pH, temperature) are controlled to prevent degradation

2. Protein Separation

- Complex protein mixtures are separated into simpler fractions
- **Techniques used:**
  - Gel electrophoresis (SDS-PAGE, 2D-PAGE)
  - Chromatography (based on size, charge, or affinity)

3. Protein Digestion

- Proteins are broken into smaller peptides for easier analysis
- **Common enzyme used:**
  - Trypsin (cuts at specific amino acids)

4. Analysis and Detection

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Mass Spectrometry (MS)

- Measures mass-to-charge ratio (m/z) of peptides
- Produces a peptide mass fingerprint
- Highly sensitive and accurate

Other Methods

a) **Edman Degradation**

- Determines amino acid sequence step by step
- Mainly used for small peptides

b) **Western Blotting**

- Uses antibodies to detect specific proteins
- Highly specific technique

5. Data Analysis (Database Search)

- Experimental data is compared with known protein sequences
- Software tools are used to identify proteins accurately

Key Methods Used

A. Mass Spectrometry (MS)

- Measures mass-to-charge ratio of peptides
- Highly sensitive and accurate

B. Edman Degradation

- Determines amino acid sequence step by step
- Mainly used for small peptides

C. Western Blotting

- Uses antibodies to detect specific proteins
- Highly specific method

D. Chromatography

- **Separates proteins based on:**
  - Size
  - Charge
  - Binding affinity

Factors Affecting Identification

- Sample purity

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- Protein concentration
- Presence of contaminants
- Instrument sensitivity

Importance in Protein Formulations

- Ensures correct protein is used
- Confirms identity and purity
- Helps maintain quality and safety of protein-based drugs

Protein Identification Techniques

Introduction

- Protein identification is the process of determining the **exact protein present in a sample**.
- It is a key step in proteomics and involves analyzing proteins after breaking them into smaller peptides.

General Approach

- Protein is digested into smaller peptides (commonly using trypsin)
- Peptides are analyzed using advanced techniques
- Data obtained is compared with known protein sequences in databases

Major Protein Identification Techniques

1. Mass Spectrometry (MS)

- The **most important and widely used method** for protein identification
- Measures the **mass-to-charge ratio (m/z)** of peptides
- **Types of Mass Spectrometry**
  - MALDI-TOF (Matrix-Assisted Laser Desorption Ionization – Time of Flight)
  - ESI-MS (Electrospray Ionization Mass Spectrometry)
- **Information Provided**
  - Molecular weight of proteins/peptides
  - Peptide sequence information

2. Peptide Mass Fingerprinting (PMF)

- Protein is digested into peptides
- Mass of peptides is measured using MS
- Each protein produces a **unique pattern (fingerprint)**

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