Protein Purification Part I:
The Basics

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Presentation Outline

- An introduction to proteins
- Chromatography basics
- Biotechnology chromatography
- From research scale to manufacturing
- Examples of manufacturing scale operations
- Cell Extraction
- Protein Isolation

Some classes of proteins

- Structural (Collagen, Keratin)
- Contractile (Myosin, Actin)
- Transport (Hemoglobin)
- Storage (Casein, Ferritin)
- Growth factors (NGF, IGF-1)
- Hormones (Protropin™, Nutropin™, Humulin™)
- Enzymes (Activase™, Pulmozyme™)
- Antibodies (Herceptin™, Avastin™)

Protein basics

- Proteins are macromolecules that consist of amino acids joined together by peptide bonds (primary structure).
- Proteins contain secondary and tertiary structures that provide structural stability and maintain the biologically active conformation of the protein.
- Some proteins consist of more than one subunit, possessing quaternary structure (hemoglobin).
- Disruption of any structural component may lead to a loss of biological activity, rendering the protein less potent as a biopharmaceutical.

Chromatography Basics

- Chromatography is the science involved in the separation of molecules based on their differing interactions with supports and solvents.
- Chromatography types include:

  Gas, Liquid, Paper, Thin Layer

- Liquid chromatography is the most commonly used method for the purification of proteins.
- Purifications are typically carried out using specialized media (gels) packed in columns (column chromatography).

Chromatography in the classroom

Paper chromatography

- Separation of transparency marker inks on a coffee filter using water and/or vinegar as solvents.

Liquid chromatography

- Separation of blue dextran from phenol red on Pharmacia PD-10 columns (Sephadex G-25).

Thin-layer chromatography

- Separation amino acids on silica thin-layer chromatography plates (ninhydrin visualization).

Types of liquid chromatography used in biotechnology

- Ion-exchange chromatography
- Hydrophobic interaction chromatography
- Gel-filtration chromatography
- Affinity chromatography

From research to manufacturing: Idea → Product

- Is this a novel protein? Will it treat a novel disease? Are there potentially multiple indications for this therapeutic?
- What information is available on the protein?
- What testing methods are available?
- How soon does this need to be done?

From research to manufacturing: chromatography media

- Is the chromatography media commercially available for large-scale application?
- Is the chromatography media affordable?
- Does the manufacturer produce consistent lots of the chromatography media?
- Are there any issues with re-use of the chromatography media for a large number of runs?

From research to manufacturing: equipment considerations

- Are any unit operations not scaleable?
- Are there any specialized unit operations?
- Are sufficient tanks available for the storage of all required buffers, solutions and protein pools?
- Are there chemical incompatibilities that might not be appropriate for use at large scale (corrosives)?

From research to manufacturing: operational goals

- Reproducible steps independent of scale.
- Minimize the number of steps in the process.
- High mass recovery.
- High product purity.
- Biologically active molecule in a stable formulation.

Process Development ⟶ Pilot Plant

1 meter column (Manufacturing)

Cell Extraction Basics

- The purpose of the extraction is to remove the protein from the cell to a more suitable medium for initial purification.
- The protein may be found in the soluble or insoluble phase following cellular extraction (may be host cell dependant).
- The initial extraction of the protein will provide the greatest challenge in the purification of the target protein.
- The extraction should be done as quickly as possible to prevent the protein from being harmed (degraded) by the contents of the cell (enzymes).
- The protein's biological activity must be maintained during the extraction of the protein from the cell.

Host cell: E. coli

- The E. coli expression system can place the protein of interest into the soluble phase or the insoluble phase (refractile bodies).
- Extraction may be done using mechanical and/or enzymatic methods.
- Refractile body extraction is accomplished through the use of a denaturant, and may include a reducing agent (mercaptoethanol, DTT).

Cell Isolation: Centrifugation

- The cells are initially isolated from the fermentation broth by centrifugation.
- The centrifuge speed and g-force is both machine and rotor dependant.
- Microcentrifugation (1 mL samples) to larger-scale centrifugation (1 L samples) are typically used for laboratory scale recoveries.
- The centrifuge may be temperature regulated to provide the optimal separation.

Protein Isolation I: Cell Extraction

- The extraction of the cell should be done at low temperature (2-8 C) in order to avoid protein precipitation as well as to slow down enzymatic degradation.
- The cells are suspended in a buffer that maintains biological activity of the protein, and may include enzyme inhibitors.
- The cells may be mechanically extracted (sonication, French press), chemically extracted (lysozyme) or broken through repeated freeze/thawing. These methods may be combined for increased cell disruption efficiency.

Protein Isolation II: Centrifugation

- The temperature of the centrifugation may be adjusted to fit the purification scheme.
- The viscosity of the extracted cell may make centrifugation problematic, due to protein-DNA interactions.
- Clarification of the lysate may be enhanced through the use of cheesecloth, Celite (diatomaceous earth), or a cell debris remover (Whatman).

Protein Isolation III: Precipitation

- Ammonium sulfate has been historically used for the initial isolation of proteins.
- Ammonium sulfate is a hydrophilic salt that orders the structure of water, causing a "salting out effect" on proteins.
- Ammonium sulfate precipitation maintains the biological activity of the protein.
- The precipitated protein can be recovered by centrifugation, and stored frozen until needed.

Extraction and Isolation Summary

- Cells are centrifuged away from cell media.
- Cells are extracted to provide the first purification of the target protein.
- Cells are centrifuged to separate soluble from insoluble phases.
- The soluble phase is ammonium sulfate precipitated, and the resulting pellet is stored frozen until needed.