Proteomics Course

LECTURE-10
Sample preparation for proteomics applications:
Serum and bacterial proteome

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Previous lecture

- Work-flow: protein sample preparation
  1. Cell disruption/ lysis
  2. Protection from proteolysis
  3. Sample fractionation
  4. Protein extraction and solubilization

Today's lecture

- Precipitation methods
- Removal of interfering substances
- Specific examples:
  - Sample preparation for serum proteome analysis
  - Sample preparation for bacterial proteome analysis

Precipitation procedures

Ammonium Sulfate Precipitation

- In Ammonium sulfate precipitation, due to high salt concentrations, proteins lose water in their hydration shell, aggregates and precipitate out of solution
- Ammonium sulfate is added at > 50% conc. and up to full saturation. Protein precipitate can be recovered by centrifugation.

Acetone Precipitation

- In acetone precipitation method many organic-solvent soluble contaminants, e.g. detergents, lipids are left in solution
- An excess of at least 3 or 4 volume of ice cold acetone add to extract and incubate at −20◦C to allow for protein precipitation
- Proteins can be pelletted by centrifugation and acetone is removed

Trichloroacetic Acid Precipitation

- TCA is very effective protein precipitant
- 10% TCA is added to samples and allowed to precipitate on ice for 30 min
- Protein pellet should be washed with acetone or ethanol
- In this method, 100% sample recovery is expected

Precipitation with TCA and Acetone

- TCA and acetone combination is often used to precipitate proteins
  - More effective than either TCA or acetone alone
- Sample lyse in 10% TCA in acetone containing 15-20 mM DTT
- Proteins allow to precipitate for 1 h at −20◦C
- After centrifugation, wash pellet with cold acetone in presence of 20 mM DTT

Precipitation with Ammonium Acetate in Methanol

- In this precipitation method, proteins are extracted in phenol and subsequently precipitated by adding 0.1M acetate in methanol
  - Pellet is finally washed with acetone
- Used for plant samples containing interfering substances e.g., polyphenols

Removal of Interfering Substances

Need to remove contaminants

- Common contaminants are salts, small ionic compounds, polysaccharides, nucleic acids, lipids
- If aim is to perform 2DE experiment, salt is the most likely reason for bad IEF
- Polysaccharides, lipids, nucleic acids can clog the gel of the strip and may form complexes with proteins by electrostatic interactions

Salts and Buffers

- High amounts of salts are present in biological fluids (urine, plasma)
- Salt removal techniques:
  - Dialysis, spin dialysis, gel filtration, precipitation and resolubilization
- Dialysis is popular method but it is time consuming, increases sample volume, makes it dilute
- Other methods are based on precipitation of proteins with dyes

Nucleic Acids Contamination

- Nucleic acids (DNA/RNA) increase sample viscosity and cause background smears
- High molecular weight nucleic acids can clog gel pores
- Nucleic acids bind to proteins through electrostatic interactions, prevent proper IEF and produce severe streaking
- Nucleic acid also form complexes with carrier ampholytes

Nucleic Acids contamination removal

- For nucleic acid removal, sample should be treated with protease-free DNase/ RNase mixtures
- This can be accomplished by adding 1/10th of sample volume of a solution containing
  - 1 mg/mL DNase
  - 0.25 mg/mL RNase
  - 50 mM MgCl2

Polysaccharides Contamination

- Polysaccharides also cause problems, similar to nucleic acids
  - but severity is less
- Uncharged polysaccharides (starch, glycogen, etc.), being large molecules, could clog the pores of polyacrylamide matrices

Polysaccharides contamination removal

- Polysaccharides removal:
  - TCA
  - Ammonium Sulfate
  - Phenol/ Ammonium acetate precipitation

Lipids

- In membranous material, lipids bind to specific proteins, lipid carriers, and could give rise to artifactual heterogeneity
- If low amount of lipid is present:
- presence of detergents in solubilization solution should disaggregate lipids, delipidate and solubilize proteins

Lipids contamination removal

- Samples such as plant seeds, algae etc. when large amount of lipid is present
- Chemical delipidization prior to sample resolubilization is effective
- Delipidation can be achieved by extraction with organic solvents containing chlorinated solvents or ethanol or acetone

Ionic detergents

- Sodium dodecyl sulfate (SDS), ionic detergent, forms strong complexes with proteins
  - resulting negatively charged complex don't focus unless SDS is removed
- SDS solubilized sample can be diluteded into high conc. of nonionic or zwitterionic detergents, such as CHAPS, Triton X-100
  - to ensure final SDS concentration less than 0.25%

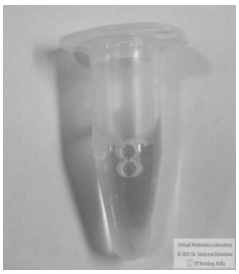
Other interfering compounds

- Other interfering compounds in plants extracts
  - Lignins
  - Polyphenols
  - Tannins
  - Alkaloids
  - Pigments

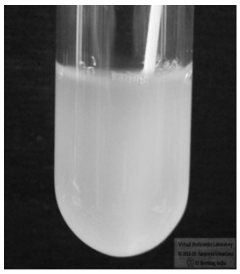
Sample preparation for proteomics applications: specific examples

Samples for proteomic studies

Different samples are used for various proteomic applications



I. Serum sample



II. Bacterial sample

III. Plant leaf sample

I. Serum/Plasma proteome analysis

Why analyze serum or plasma

- Blood proteome is one of the most complex components of human proteome
- Liquid portion of blood is referred as plasma, removal of fibrinogen and other clotting factors from plasma results in serum

Why analyze serum or plasma

- Human serum or plasma proteins mostly originate from a variety of tissue and blood cells as a result of secretion or leakage
- The rapid alteration in expression pattern of various serum proteins in response to an external stimulus

Major challenges in serum/ plasma proteome analysis

1. Dynamic range of protein concentration

- Large diversity of proteins in very dynamic conc.
- Conc. of serum proteins range across more than ten orders of magnitude
- Full spectrum analysis by conventional proteomic techniques challenging
  - Typical dynamic range 10^2 - 10^4

2. High-abundance proteins

- Presence of different high-abundance proteins
- 22 most abundant proteins represent about 99% of total protein mass in plasma
- High-abundance proteins prevent detection of low-abundance proteins

3. High salts and other interfering compounds

- Salts are one of the several components of blood, which are required for various functions such as maintenance of osmotic balance, acid-base balance
- Few salts such as sodium chloride or potassium chloride are further added during sample processing

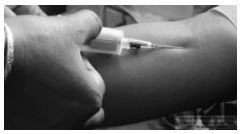
3. High salts and other interfering compounds

- Presence of excessive salts, detergents or other contaminants can tremendously influence the electrophoretic separation of proteins
- It also affects the direct determination of peptides or proteins by MS-based techniques

4. Variations among individuals and lack of reproducibility

- Inter- and intra-individual variation
- Drastic heterogeneity or large biological variations such as gender, age, genetic factors, dietary considerations, environmental factors and drug treatment

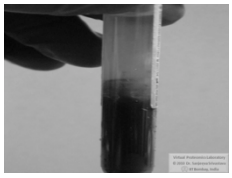
Serum Sample Preparation



Withdrawal of intravenous blood

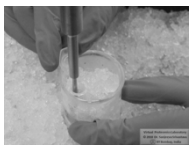
Blood collection tube on ice

Centrifugation of whole blood



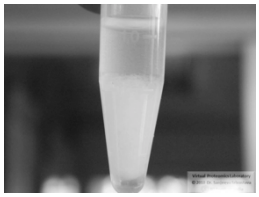
Serum separated from whole blood

Serum

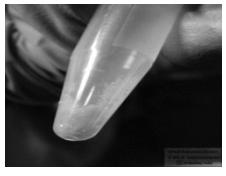


Sonication of serum proteins

Column loaded with sample



Acetone precipitated proteins

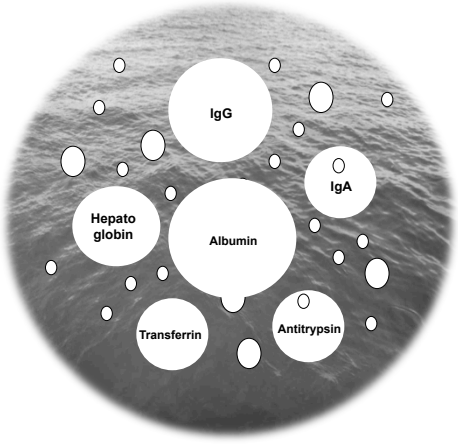


Dried protein pellet

Serum high abundant protein removal

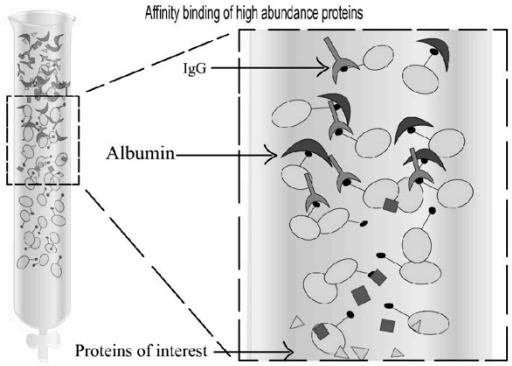
Abundant proteins in serum

- Albumin alone covers approximately 50%
- IgG (15-25%), IgA, Haptoglobin, Transferrin and Anti-trypsin are also major high-abundance



Six high-abundance serum proteins

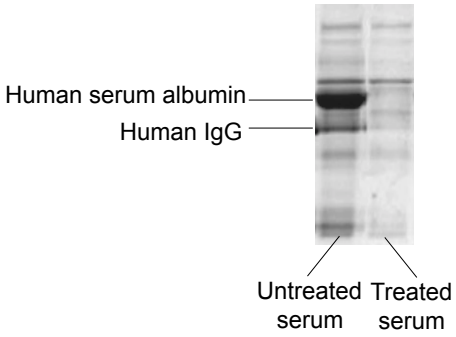
Removal of abundant proteins



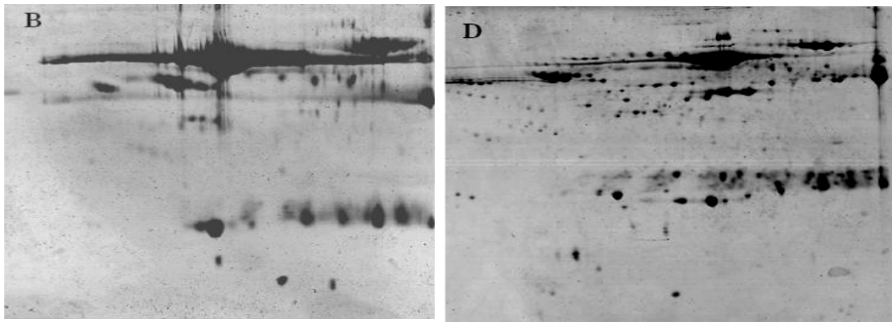
- Antibody affinity ligands for albumin, IgG etc. result in more specific depletion. Resins selectively binds these proteins, unbound protein is eluted

Removal of abundant proteins

- High abundant protein issue can be addressed by affinity resin based fractionation procedures and depletion strategies



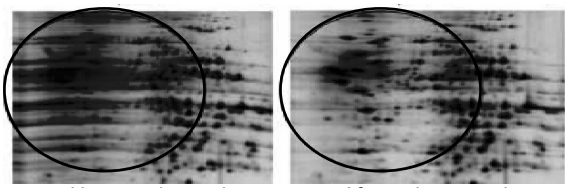
Removal of abundant proteins



Crude serum Depleted serum

Salt removal

- Salts be removed/maintained very low (< 10 mM)
- Removal by dialysis, gel filtration, precipitation
- Sample clean up kits available commercially



Untreated sample After salt removal

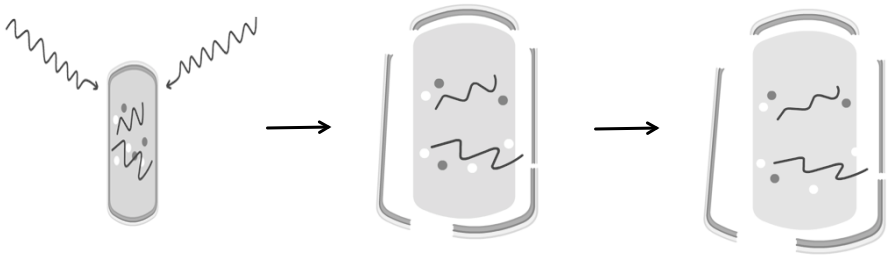
II. Bacterial proteome analysis

Bacterial sample preparation for proteomic applications

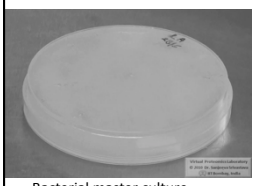
- Aim to solubilize all the proteins to obtain best possible representation of total protein content
- Bacteria can be lysed
  - by constituents of lysis buffer
  - by sonication
  - by enzymatic digestion
  - Or apply a combination of above

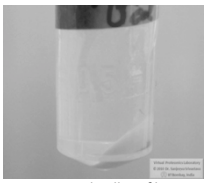
Sonication

- Sonication is a physical method that breaks complex inter- and intra- protein interactions
- Ultrasonic waves generated by a sonicator lyse cells through shear forces
  - Care should be exerted to minimize heating and foaming

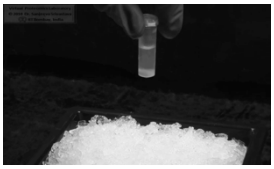


Bacterial Sample Preparation

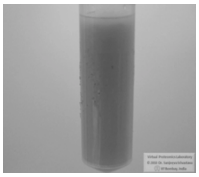

Bacterial master culture

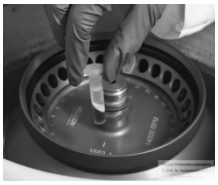

Precipitated pellet of bacteria

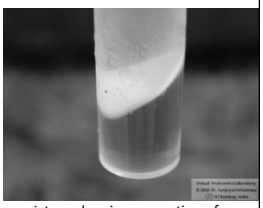

Bacteria grown in broth

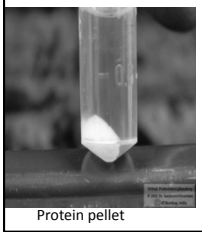

Pelleted, washed bacterial

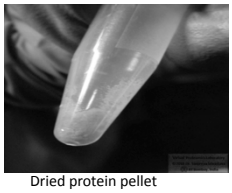

Sonication of bacteria

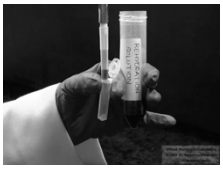

addition of trizol


Centrifuged mixture


mixture showing separation of proteins, DNA and RNA


Protein pellet


Dried protein pellet


Reconstitution of Pellet

Summary

- Sample preparation: work-flow
- Specific examples
  - Human serum
  - Bacteria

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