# Proteomics Course

## LECTURE-11
## Sample preparation for proteomics applications:
### Bacterial & Plant Proteome, Quantification

Dr. Sanjeeva Srivastava
IIT Bombay

## Previous lecture

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

# Today's lecture

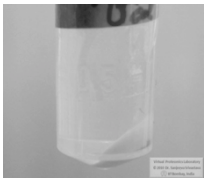
- Specific examples:
  - Sample preparation for bacterial proteome analysis
  - Sample preparation for plant proteome analysis
- Protein quantification

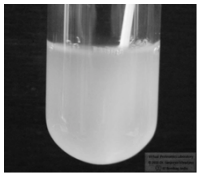


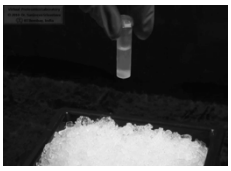
# II. Bacterial proteome analysis

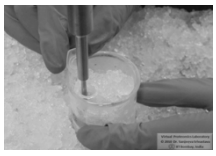
# Bacterial Protein Sample Preparation

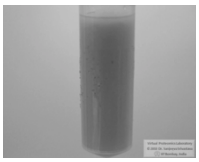

Bacterial master culture

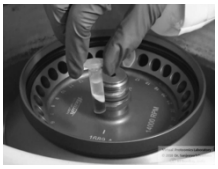

Precipitated pellet of bacteria

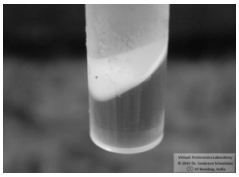

Bacteria grown in broth

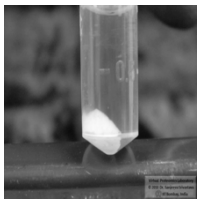

Pelleted, washed bacterial

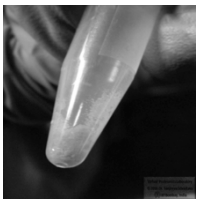

Sonication of bacteria

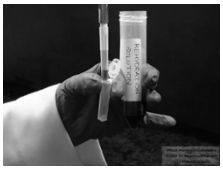

addition of trizol


Centrifuged mixture


Mixture showing separation of proteins, DNA and RNA


Protein pellet


Dried protein pellet


Reconstitution of Pellet

# Bacterial sample preparation

- 2% SDS and heat treatment
- Lyzozyme and acetone precipitation method
- TCA-acetone precipitation method
- Direct extraction with solubilization buffer
- Trizol method

## Bacterial protein extraction: Trizol method

- Able to recover DNA, RNA and Protein
- Trizol having Guanidinium isothiocyanate is inhibitor of RNAase and gives good quality RNA
- No nucleic acid contamination
- No need of desalting
- No lipid contamination (since chloroform dissolves lipids)
- Proteins are easy to resolubilize



## Bacterial protein extraction: Trizol method

- Procedure:
- Add 1 ml trizol reagent to the bacterial suspension
- Add 200 ul chloroform to the mixture
- Vortex vigorously & incubate for 15 min at RT
- Centrifugation at 12000 g for 15 min

## Bacterial protein extraction (contd..)

- Carefully remove upper layer containing RNA using a micropipette
- To bottom layer, add 300 ul ethanol
- Centrifuge at 5000 g for 5 min to remove DNA
- Remove supernatant containing protein collect into a fresh tube
- In supernatant, add 4 volumes of chilled acetone and incubate for ~ 4 hrs at – $20^0$C



## Bacterial protein extraction (contd..)

- After incubation centrifuge at 12,000 g for 5 min
- Discard supernatant and retain pellet
- Wash protein pellet with 95% ethanol (4 times)
- Dry pellet at room temperature
- Reconstitute dried pellet in lysis buffer

# III. Plant proteome analysis

## Plant proteome analysis

- Plants are very important because they are food source for humans and animals
- A thorough understanding of plant proteome is crucial
  - to reveal molecular mechanisms underlying plant growth, development and interactions with environment

# Plant proteome analysis

- Analysis of plant proteome provides information
  - Protein abundance
  - Protein modification
  - Subcellular localization
  - Three-dimensional structure
  - Interaction with other biomolecules



# Leaf protein extraction

- Take weight of 300 mg of leaves and homogenize using a clean mortar pestle with liquid nitrogen
- Add 1500 uL of TCA, acetone to ground tissue
- Incubate homogenous solution at -20$^0$C for 1 hour
- Centrifuge mixture at 14,000 rpm for 30 min at 4$^0$C
- Remove supernatant and wash pellet 3-4 times with chilled acetone containing 0.07% DTT

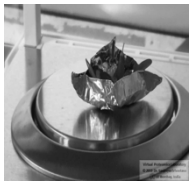
# Leaf protein extraction

- Dry pellet at room temperature
- Reconstitute dried pellet in lysis buffer
- Centrifuge contents at 14,000 rpm for 15 min at $4^0$C
- Collect the supernatant



## Plant Protein Sample Preparation

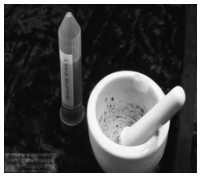
Fresh Leaves



Weigh leaves

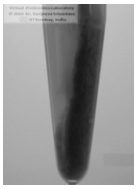
Transfer to mortar

Grinding in liquid nitrogen

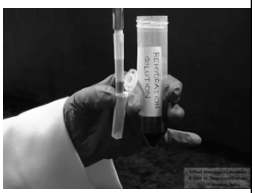


Homogenized Plant Leaves

Removal of supernatant



Protein pellet



Reconstitution of pellet

# Protein quantification

## Protein concentration determination by UV absorption

- Determination of protein concentration by absorbance measurement at 280 nm
  - oldest method
  - based on the absorbance of UV light by aromatic amino acids in protein solutions
  - due to tryptophan and tyrosine residues, to a lesser extent phenylalanine residues

## Protein concentration determination

- $A_{280}$ method requires that protein contains tryptophan and tyrosine
- Due to variability in aromatic amino acid content the absorptivity at 280 nm will be variable
- Higher protein concentrations are necessary



## Protein quantification assays

- Different reagent can be used to determine the concentration of proteins in solution
  - Lowry assay
  - BCA assay
  - Bradford assay

# Colorimetric methods: to determine protein concentration

## Lowry assay

- Common method for quantitation of soluble protein
- 1. Alkaline cupric tartrate forms complex with peptide bond of protein
- 2. A reduction step with Folin and Ciocalteu's reagent
- Reaction yields purple color and absorption is read between 500-800 nm

*Ref: Lowry et al. J Biol Chem. 1951 Nov;193(1):265-75. Protein measurement with the Folin phenol reagent.*

# Lowry assay (2)

- Advantages:
- Sensitivity, simplicity, precision
- Problems:
- Unsuitable for proteins without tyrosine residues,
  - assay depends on reaction of tyrosine residues with reagent
- Sensitive to interference of Tris, EDTA etc.
  - this limitation can be overcome by precipitation methods such as TCA



# Bicinchoninic Acid (BCA) assay

- Proteins form complex with $Cu^{2+}$ ions in alkaline solution
  - $Cu^{2+}$ ions are reduced to Cu+ ions (Biuret reaction)
  - form a violet color complex with Bicinchoninic Acid (BCA)
  - Amount of reduction is proportional to protein present

*Ref: Smith, P. K., et al. (1985). "Measurement of Protein Using Bicinchoninic Acid," Anal. Biochem. 150: 75–85.*

# BCA assay (2)

- Advantages:
- More sensitive than Biuret or Lowry methods
- Color complex is stable, less susceptibility to detergents
- Useful for membrane proteins and detergents
- Problems:
- Disrupted by high concentrations of complex-forming reagents EDTA, ammonium sulfate; reducing materials DTT



# Bradford Assay

- Assay is based on complex formation between Coomassie brilliant blue G-250 dye and protein
- Due to binding absorption max of color shifts
  - 465 nm without protein
  - 595 nm with protein
- Increase of absorption at 595 nm is use to measure protein concentration

*Ref: Bradford, M. (1976). "A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principles of Protein-dye Binding," Anal. Biochem. 72: 248–254.*

# Bradford Assay (2)

- Advantages:
- Compatible with reducing agents and thiols, unlike Lowry, BCA
- It is quick and compatible for microwell plate assay
- Problems:
- Dye binds most readily to arginyl and lysyl residues of proteins
  - this specificity may lead to variation
- Commonly used detergents such as TRITON-X-100, SDS and CHAPS interfere



# Bradford assay to determine protein concentration

- Requirements
  - Standard protein solution (0.5 mg/ml BSA), 0.15 M NaCl, Coomassie brilliant blue solution, cuvette
- Standard preparation
  - Add 0.5 mg/ml BSA 5, 10,15, 20, 25 µl
  - Dilute with 100 µl of 0.15 M NaCl (use NaCl alone as blank)
  - For unknown – take 10 µl of sample and dilute with NaCl

## Bradford assay to determine protein concentration

- Add 1 ml Coomassie brilliant blue solution and vortex
- Incubate reaction for 2 min
- Measure absorbance at 595 nm
- Use standard curve to determine protein concentration of unknown protein sample



## Summary

- Sample preparation: work-flow
- Specific examples
  - Human serum
  - Bacteria
  - Plants
- Protein quantification

# REFERENCES


- Shaw MM and Riederer BM. Sample preparation for two-dimensional gel electrophoresis. Proteomics. 2003, 3, 1408-17.
- Chen S and Harmon AC. Advances in plant proteomics. Proteomics. 2006, 6, 5504-16.
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- Bradford, M. (1976). “A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein. Utilizing the Principles of Protein-dye Binding,” Anal. Biochem. 72: 248–254.
- Michael H. Simonian, John A. Smith. 2001. Current Protocols in Molecular Biology. DOI: 10.1002/0471142727.mb1001as35. UNIT 10.1A Spectrophotometric and Colorimetric Determination of Protein Concentration