Proteomics Course

LECTURE-9
Sample preparation for proteomics applications

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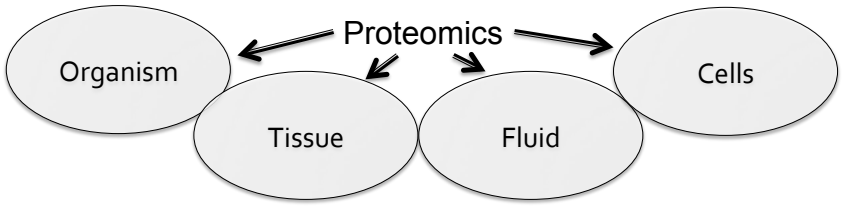
Today's lecture

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

Protein sample preparation for proteomics applications

Sample preparation in proteomics



- Highly reproducible sample preparation essential for any proteomic application

Sample preparation in proteomics (2)

Global Proteomic Analysis	Expression Proteomic Analysis	Targeted Proteomic Analysis

- Different proteomic applications require different approaches for sample preparation

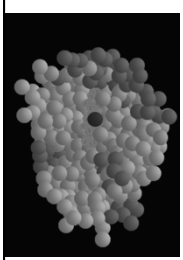
Sample preparation in proteomics (3)

- Process typically involves solubilization, denaturation, reduction and treatment of sample proteins
- Additional steps improve quality of protein extracts but may lead to loss of selective protein species

Protein sample preparation

- Protein extraction protocols should ensure that most, if not all, proteins in a cell or its organelles are extracted
- Presence of interfering compounds are minimized
- Good protein extract can ensure success of further proteomic experiments

Ideal protein preparation



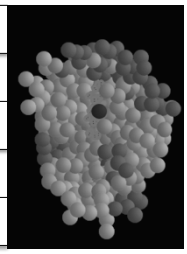
Solubilize all proteins

Prevent protein aggregation

Denature and reduce all proteins

Remove nucleic acid contamination

Remove other contaminants



Guidelines for sample preparation

- Starting point - literature search for good sample preparation strategy
- Remove nucleic acids, salts and particulates
- Prepare sample freshly and store at -20°C in aliquots
- Avoid repetitive freezing and thawing of samples

Importance of good protein sample preparation

Good sample preparation includes, most, in not all, proteins and provides good quality data

Good sample provides reproducible results

Once protocol is optimized, large number of samples can be processed for uniform quality

Contaminants removal can increase the data quality (good signal to noise ratio)

Work-flow:
Protein sample preparation

Sample preparation

- Protein extraction from source material
- Solubilization of proteins before analysis
- Ideal sample preparation disrupts all non-covalently bound proteins and removes interfering components

Work-flow of sample preparation

1. Cell disruption/ lysis
2. Protection from proteolysis
3. Sample fractionation
4. Protein extraction and solubilization
5. Contaminant removal
6. Quantification

(1) Cell disruption/ lysis

Why need cell lysis?

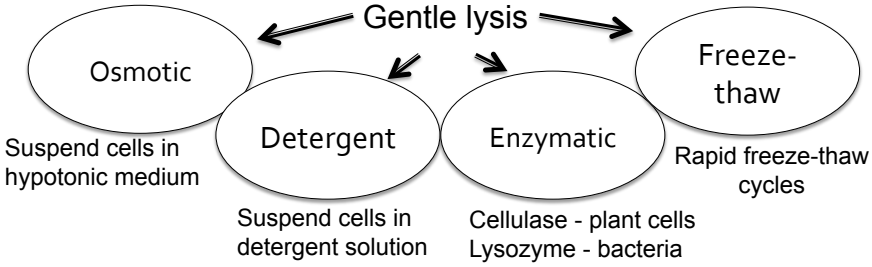
- To facilitate effective disruption of cells or tissues
- To isolate proteins from intact cells and tissues while avoiding loss or modification of proteins
- To obtain all proteins in sample
- Helps to maximize the sample recovery, retain structural integrity

Cell lysis: steps

- Cell disruption
- Protection from proteolysis
- Homogenization and sample solubilization

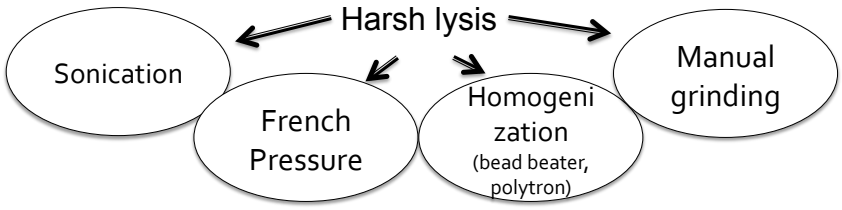
Cell lysis: gentle disruption

- Employed when sample consists of easily lysed cells
  - E.g. tissue culture cells, blood cells
- Employed when particular sub-cellular fraction is to be analyzed



Cell lysis: vigorous disruption

- Employed when sample consists of cells which can't be easily lysed
  - E.g. Bacterial and plant cells



Overview: Lysis methods

Lysis methods	Target samples	Lysis severity
Detergent	Tissue culture cells	gentle
Enzymatic lysis	Plant tissue, bacterial cells, fungal cells	gentle
Freeze-thaw	Bacterial cells, tissue culture cells	gentle
French pressure	Bacteria, algae, yeasts	vigorous
Glass bead	Cell suspensions, organisms with cell walls	vigorous
Grinding	Solid tissues, microorganisms	vigorous
Mechanical homogenization	Solid tissues	vigorous
Osmotic lysis	Blood cells, tissue culture cells	gentle
Sonication	Cell suspensions	vigorous

(2) Protection from proteolysis

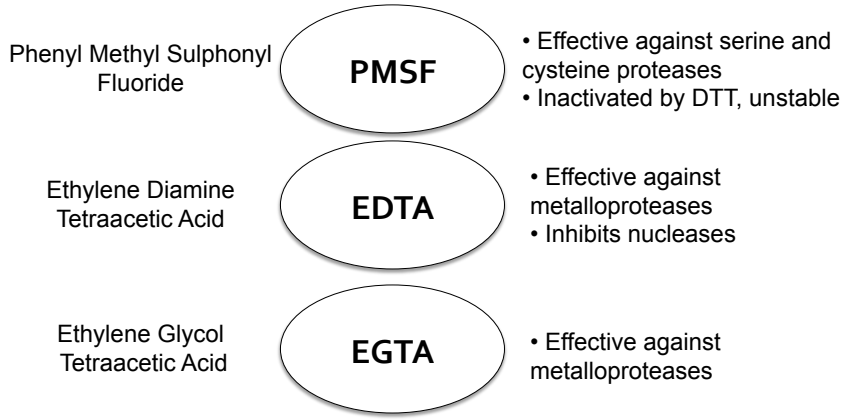
Protection from proteolysis

- Cell lysis releases proteases which may result into proteolysis
- During the sample preparation protease inhibitors are added to minimize artifactual proteolysis

Protease inhibitors (2)

- Effective protease inhibitors contains a mixture of irreversible and reversible protease inhibitors that inhibit serine, cysteine and metalloproteases
- Specifically, useful for gel-based proteomics

Protease inhibitors: examples



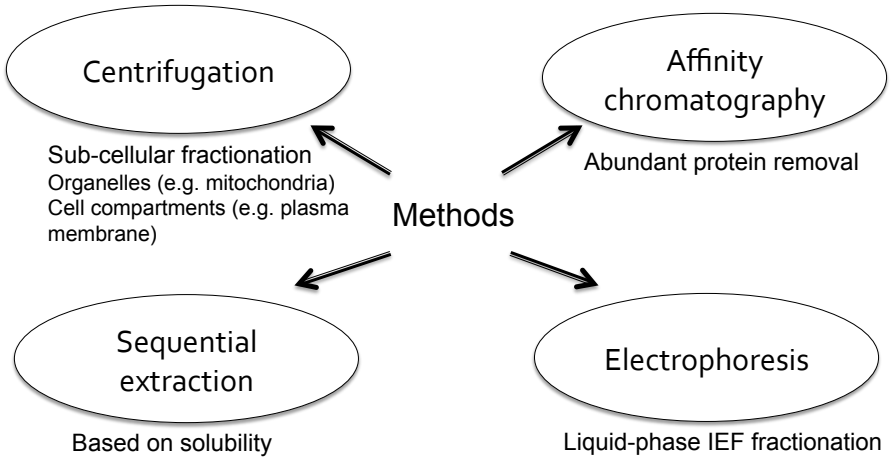
- Sample processing in cold condition also reduce proteolysis

(3) Sample fractionation

Why fractionation?

- Fractionation makes it possible to isolate groups of proteins, or fractions from total proteome
- Simplifies analysis of complex protein mixtures
- Allows for improved resolution when an individual fraction is analyzed
- Provides less crowded 2-D maps

Fractionation methods



Importance of Fractionation

- Increases proteome coverage
- Separates highly abundant proteins from low-abundant proteins of interest and bring them into dynamic detection range
- Increases chances of identifying low abundance proteins of diagnostic or therapeutic interest

(4) Protein extraction and solubilization

Protein extraction

- Protein extraction preceded by sub-cellular fractionation to enrich proteins of interest
- Protein extraction in aqueous buffer
  - Tris-hydrochloric acid followed by desalting
  - Protein precipitation by trichloroacetic acid (TCA)
  - Acetone
  - TCA and acetone
- Protein extract should be soluble, free from protein–DNA/RNA/protein interactions, metabolites

Sample solubilization

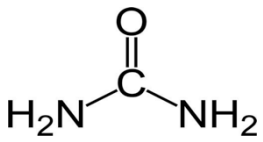
- Proteins
  - naturally form complexes with membranes, nucleic acids or other proteins
  - form various nonspecific aggregates
  - precipitate when removed from their normal environment

Sample solubilization

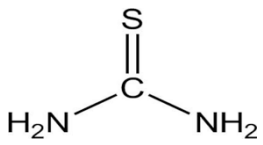
- Effectiveness of solubilization depends on
  - choice of cell disruption
  - protein concentration and dissolution method
  - choice of detergents
  - sample composition
- Solubilization methods differ from sample to sample

Solubilization components: chaotrops

- Urea – used as denaturant to solubilize and unfold most proteins to fully random conformation
- Urea – a chaotropic agent
  - helps in stabilization
  - unfolding of proteins
  - all ionizable groups exposed to solution

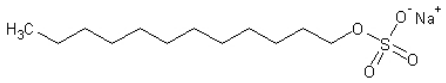


- Thiourea – improves solubilization of membrane proteins



Solubilization components: Detergents

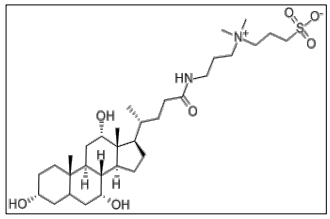
- Sodium dodecyl sulfate (SDS) - extremely efficient in solubilizing hydrophobic proteins



- However, anionic nature limits its effectiveness for conventional proteomic analyses
  - SDS is not compatible with IEF
- Therefore, zwitterionic and nonionic detergents are also used for proteomic techniques (e.g. 2-DE)

Solubilization components: Detergents

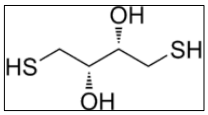
- CHAPS - (3-[(3-cholamidopropyl)dimethylamino]-1-propanesulfonate)
  - zwitterionic detergent
  - prevents non-specific aggregation
    - through hydrophobic interactions
  - help sample solubilization



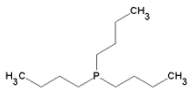
- In few cases sulfobetaine detergents are better solubilizing agents
- Neutral detergents (NP-40) less commonly used
- No single zwitterionic or nonionic detergent can completely solubilize all proteins

Solubilization components: Reductants

- Reducing agents cleaves disulfide bonds between and within protein chains and prevents disulfide bonds formation
- Dithiothreitol (DTT)
  - most common reductant
  - used for reduction of disulphide bonds in proteins



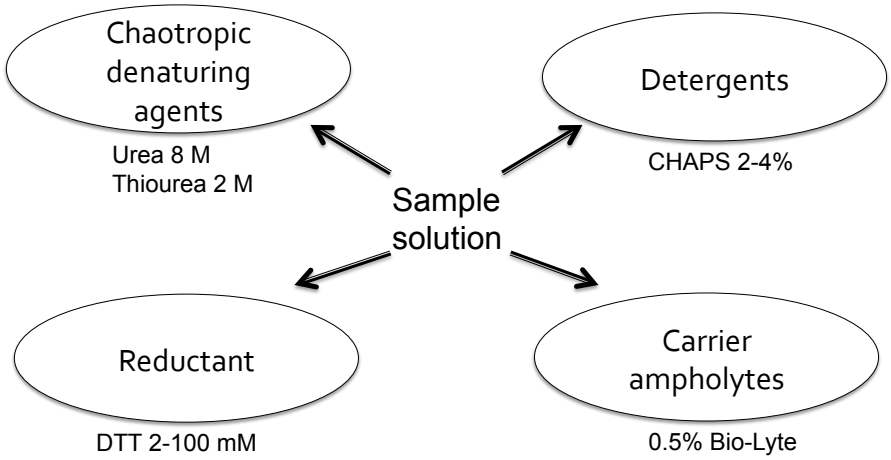
- Tributylphosphine (TBP)
  - non-ionic reducing agent
  - increases solubility of protein



Solubilizing agent

- Carrier ampholytes/IPG Buffer
  - added to sample solution prior to IEF
- Ampholytes possess charge-charge interactions
  - minimize protein aggregation
  - enhancing protein solubility
- Buffers or bases are added sometimes to minimize proteolysis or help full solubilization

Sample solution components: 2-DE analysis

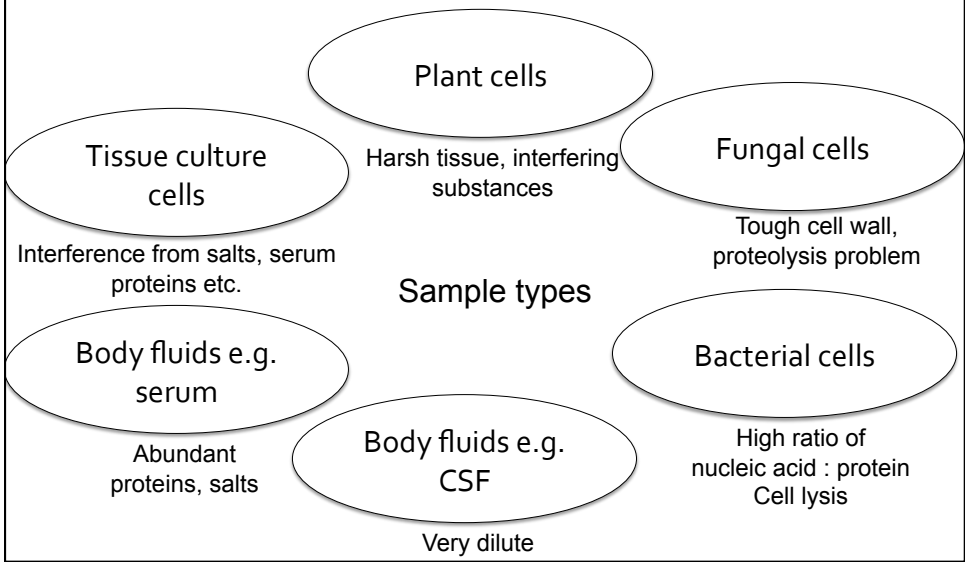


Sample solution components

- Sample solution components ensure protein solubility during extraction and separation
- Typical sample solution example:
- 8 M urea, 2 M thiourea, 4% (w/v) CHAPS, 2% (v/v) IPG buffer (pH 4-7; Linear), 40mM DTT

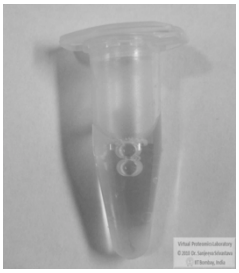
Sample types and challenges

Biological sample types and challenges

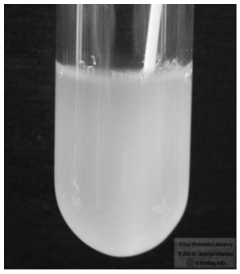


Samples for proteomic studies

Different samples are used for various proteomic applications



I. Serum sample



II. Bacterial sample

III. Plant leaf sample

I. Serum proteome analysis

Clinical Proteomic Studies: Pre-analytical issues

Pre-analytical factors

- Proteomics aims for simultaneous analyses of thousands of proteins
- Impact of pre-analytical factors, which occur prior to the point of actual sample analysis, is very high for proteome-scale clinical studies
- Pre-analytical factors are biological or technical

Pre-analytical factors: biological

Intrinsic influences - gender, age, ethnicity

Extrinsic influences - diet, medication, smoking, alcohol consumption

- In a large cohort of patients, number of nondisease-related biological effects influence the proteome changes induced due to disease
- Study design should aim to match age, gender and minimize other influences without any bias

Pre-analytical factors: technical

- Technical - effects of sample collection, processing, and storage

Sample collection mode

- the gross effects of factors such as patient posture and tourniquet application time

Sample container types

- serum and plasma exhibit differences as a result of coagulation, specifically the removal of fibrinogen

Pre-analytical factors: technical

Sample collection and handling procedure

- Collection and handling procedures of bio-fluids affect sensitivity, selectivity, reproducibility
- Collection tubes, affect serum proteome by shedding components from tube or by adsorption of serum proteins to tube
- CSF measurements of β-amyloid & tau proteins differ when collected in tubes of different materials
  - lowest in polystyrene tubes

Pre-analytical factors: technical

Sample storage

- sample storage conditions, particularly the temperature – 20C, -80C, are influential factors for serum analysis
- Storage in small aliquots
- Avoid multiple freeze–thaw cycles
- Avoid long-term storage or storage at improper temperature
  - progressive degradation of unstable serum proteins

Summary

- Cell lysis
- Protection from proteolysis
- Sample fractionation
- Protein extraction and solubilization
- Specific examples

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