Proteomics

- The study of all proteins, including their relative abundance, distribution, post-translational modifications, functions, and interactions with other macromolecules, in a given cell or organism within a given environment and at a specific stage in the cell cycle.

Areas of Interest

1. Abundance proteomics:
   - Relative abundance of specific proteins in a given tissue under different conditions of health and disease.
2. Cell-mapping:
   - Intracellular signaling pathways and regulatory networks
3. Structural proteomics:
   - Study of active sites and functional domains.
Proteome

- The entire collection of proteins, estimated to be more than 100,000.
- More proteins comprise a proteome than genes a genome.
  - Alternative gene splicing of mRNA,
  - Posttranslational modification (PTM).
- There is neither a one to one correlation of gene to protein, nor mRNA levels to proteins levels.
- PTM and signal transduction play a major role in cell transformation, such as tumor cells.

Post-translational Modification

- Post-translational modification (PTM):
  - Phosphorylation, glycosylation, acetylation, ubiquitination, methylation etc.
  - PTM of proteins, not detected through RNA analysis, may occur at different stages of tumor development indicative of early or late events of transformation.
  - High throughput techniques may useful for screening and surveillance.
**Amino Acids to Proteins**

Steven S. Saliterman  

---

**Tertiary Structure**

Steven S. Saliterman  

---

**Identification of Proteins**

Universal Blot

- (A) Protein Nucleus is denatured
- proteins are run in gel
- detection with secondary antibody

ELISA

- (B) Antibody is added to plate
- Adding antigenic sample
- Detection with a secondary antibody

Bead-based Method

- (C) Antibody is added to beads
- Adding antigenic sample
- Detection with a secondary antibody

Abel, L. et al., Autoimmune profiling with protein microarrays in clinic applications. Biochimica et Biophysica Acta 1846 (2014) 977–987

Steven S. Saliterman
Using Mass Spectrometry

Mass Spectrometers

Principle
Protein Microarrays

- Useful for study of studying protein expression, interaction, function and post-translational modifications.
- High-throughput, high sensitivity, low sample volumes, and efficient sample-to-result time.
- Forward-phase microarrays:
  - Proteins and peptides are immobilized for capturing antibodies.
  - Antibodies, sugars or aptamers are immobilized and labeled proteins are captured.
  - Sandwich mode – a labeled secondary antibody is used for detection.
- Reverse phase microarrays:
  - Complex samples such as serum, plasma, or even tissues are immobilized in an array format and probed with antibodies to determine the differential amount of protein molecules in the screened samples.

Additional Classification

- Differential profiling and screening protein arrays.
- Functional protein arrays.
- Arrays from biological samples.
- Cell-based protein arrays.
- Cell-free protein arrays.
- Antibody arrays.

Microarray Analysis Concept

**Array Formats**

- Analytical protein (detecting) microarray
  - Hybridization of dual-labeled analyte and probe
  - Antibody labeling of analyte and probe
  - Immobilized analyte and probe on glass
  - Analytical array microarray

---

**Experimental Workflow**

- Protein arrays
  - Antibody arrays
  - Protein-DNA interactions using microarrays: applications in biomedicine. Advances in Protein Chemistry and Structural Biology, Volume 95, 2014

---

**Cretich, M. et al.** Protein microarray technology: how far off is routine diagnostics? Analyst, 2014, 139, 520.

Protein, Peptide and Small Molecule Array


Capture Molecules


Fabrication Technologies

Protein In-Situ Array

Technology Comparison

Microarray Immobilization
**Selected Immobilization Strategies**

Selected Examples of Immobilization Strategy in Microarray Fabrication

Steven S. Saliterman


---

**Immobilization Strategy Continued**

Steven S. Saliterman


---

**Protein Microarray Uses**

- Protein expression profiling,
- Studying posttranslational modifications,
- Protein-protein binding,
- Drug interaction,
- Protein folding,
- Substrate specificity,
- Enzymatic activity and
- Interaction between proteins and nucleic acids.

Steven S. Saliterman
Recent Application Examples


Applications Continued

Antigen Array

To Study Autoimmune disease

Steven S. Saliterman
Factors Affecting Performance

- Appropriate surface for the immobilization of either protein or antibody samples.
- Microarray patterning technique.
- Protein conformational changes with expression, purification or immobilization may alter their function or render them inactive.
- Charged surfaces, temperature, pH and solvents may denature some proteins, and therefore surfaces must be biocompatible to minimize denaturation.
- Protein instability may lessen shelf-life.

Summary

- Proteomics - The study of all proteins, including their relative abundance, distribution, post-translational modifications, functions, and interactions with other macromolecules, in a given cell or organism within a given environment and at a specific stage in the cell cycle.
- Proteome - The entire collection of proteins, estimated to be more than 100,000.
- Gene Expression and Regulation.
- Identification of Proteins
  - Western Blot, ELISA, and Bead Methods.
  - Mass Spectrometry

- Protein Microarrays
  - Array Formats
  - Capture Molecules
  - Fabrication Technologies
  - Immobilization Methods
  - Applications
  - Factors Affecting Performance