

Proteomics Course

LECTURE-40 Proteomics: Advances and Challenges



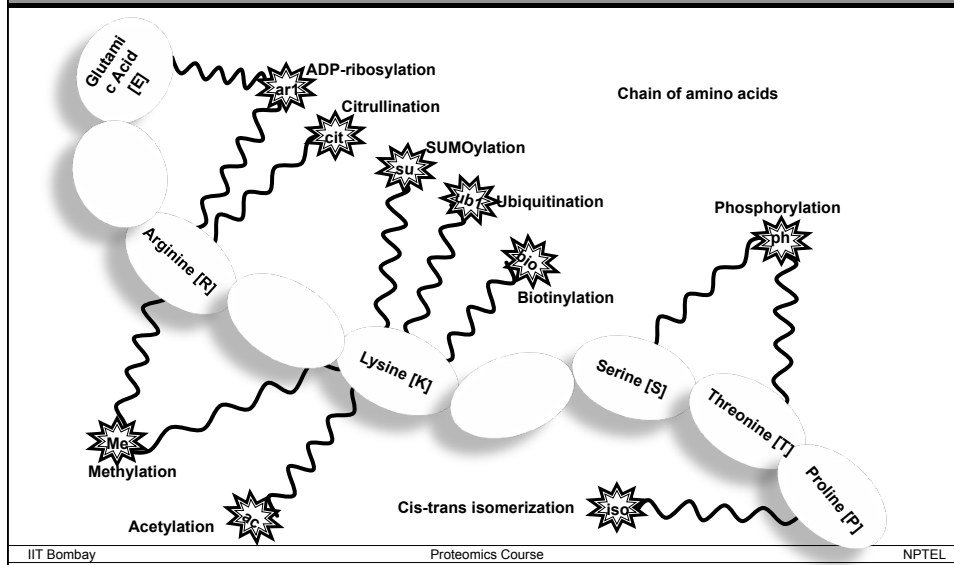
Dr. Sanjeeva Srivastava
IIT Bombay



Lecture outline

- Post translational modifications
- Structural proteomics
- Role of bioinformatics
- Challenges of proteomics
- Future directions of proteomics

Known PTMs and amino acid residues they modify



Post-translational modifications

- PTMs generate tremendous diversity, complexity and heterogeneity of gene products
- PTMs are of extreme biological importance
- Many documented effects of PTMs

Phosphorylation

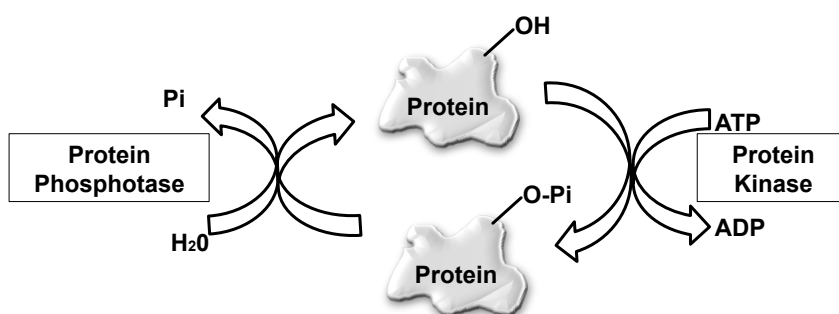
- Primary role is to act as a switch
 - turn "on" or "off" a protein activity
- All processes are regulated by protein phosphorylation
- Reversible, controlled by combined action of kinases & phosphatases

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Phosphorylation



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Glycosylation

- Glycosylation play variety of roles
 - inter- and intracellular activities
 - coordination of immune functions
 - cell division
 - protein regulations and interactions

Detection techniques for PTMs

Detection techniques for PTMs: Gel-based techniques

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PTM identification by 2DE: drawbacks

- Limits of detection of protein staining techniques
- Exclusion of integral membrane proteins due to high hydrophobicity
- Difficulty in localizing the site of modification
- Time required to carry out entire analysis at a proteomic level?

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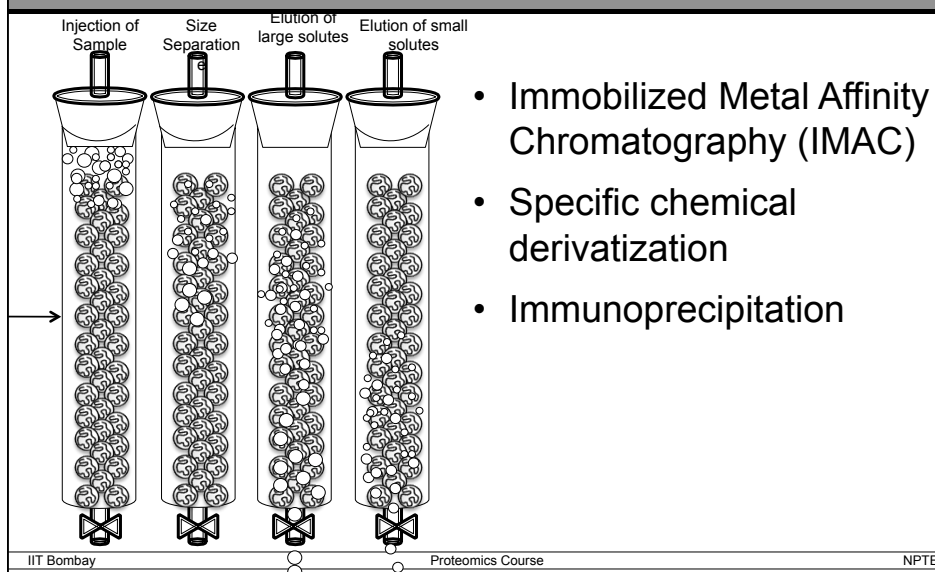
Detection techniques for PTMs: Mass Spectrometry

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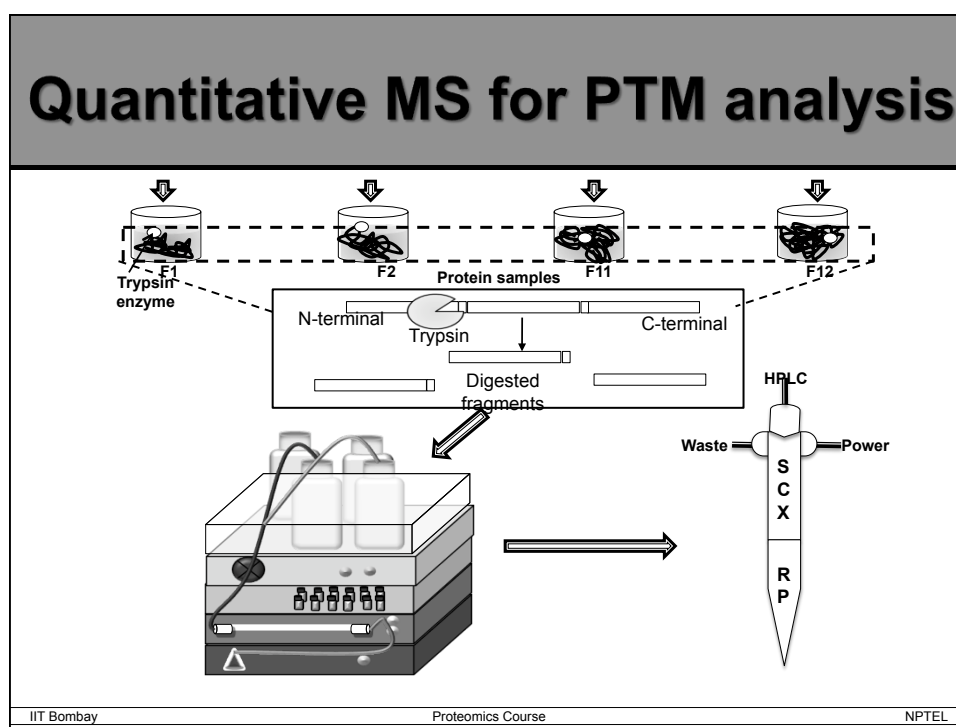
Phosphoproteome enrichment



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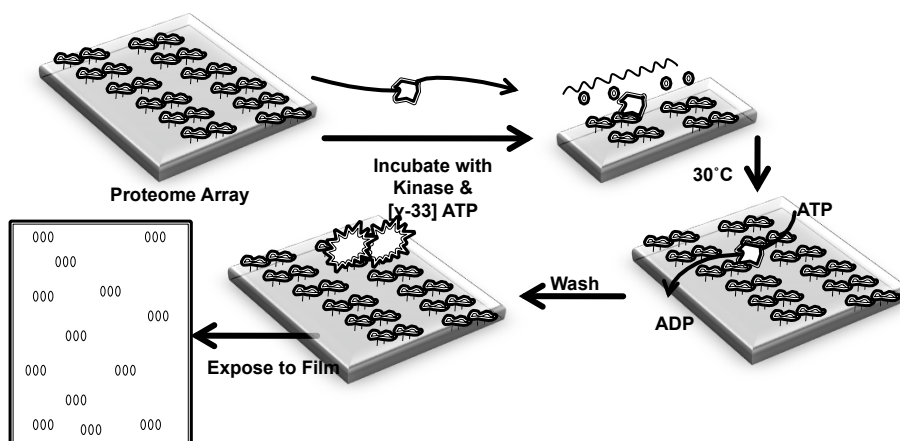
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Detection techniques for PTMs: Microarrays

Microarray for PTM analysis

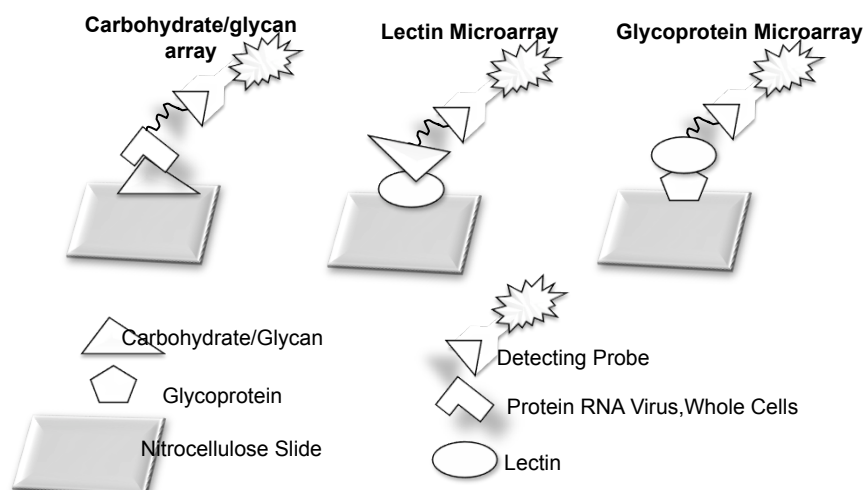


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Different Array Formats for Glycosylation Studies

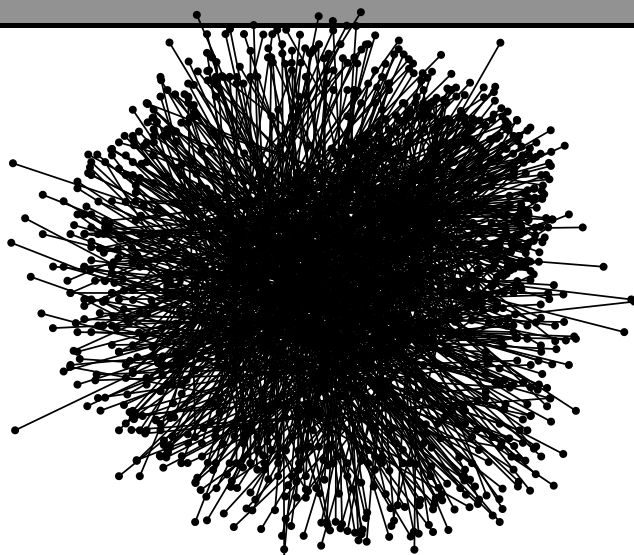


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PTM Studies Very Challenging



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2. Structural Proteomics

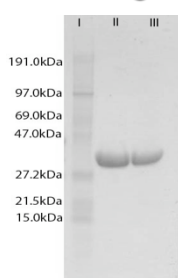
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Protein Purification

- Cloning and expression of proteins in heterologous systems
- **Different tags used for protein purification using affinity chromatography**

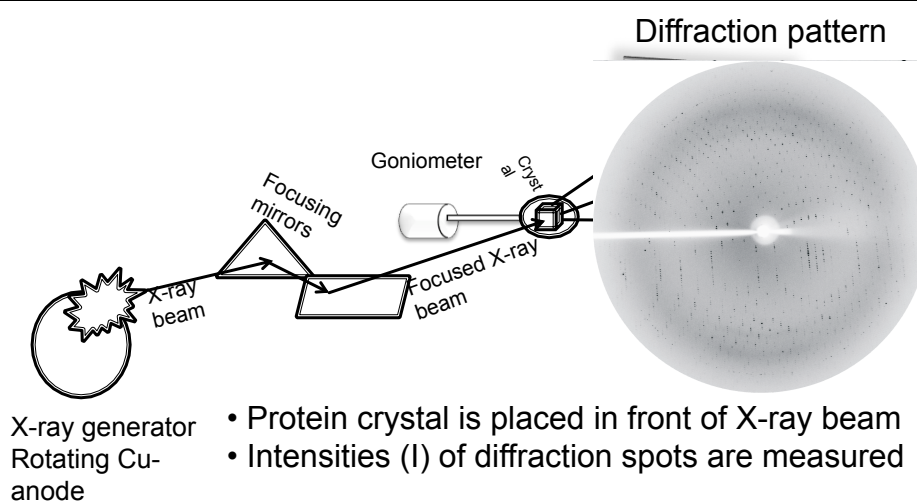


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X-ray Crystallography

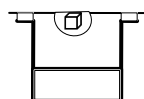


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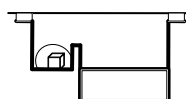
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Protein crystallization (Vapor diffusion method)

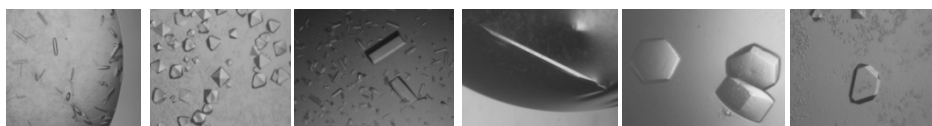


Hanging drop



Sitting drop

Protein Crystals

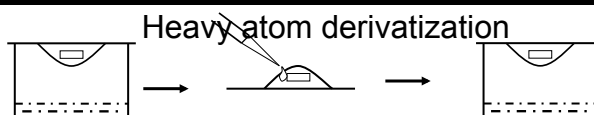


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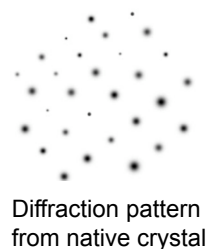
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MIR and MAD

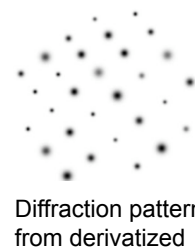
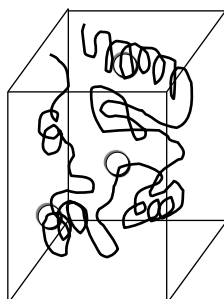


Heavy atom derivatization

Addition of heavy atom solution



Diffraction pattern from native crystal



Diffraction pattern from derivatized crystal

Multiple Isomorphous replacement (MIR)

Multiple Wavelength Anomalous Dispersion (MAD)

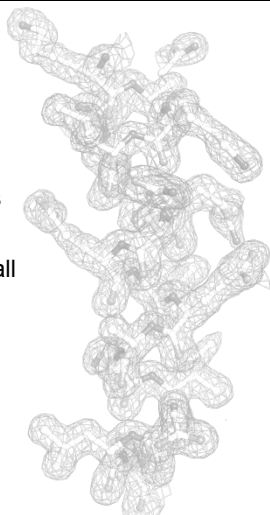
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Electron density and model building

- Electron density is shown as purple mesh
- Protein model is shown in ball and stick representation



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Example: Histo Aspartic Protease (HAP)



Bhaumik *et al.* (2009). *J. Mol. Biol.* **388**(3): 520–540.

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Nuclear Magnetic Resonance (NMR)

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Nuclear Magnetic Resonance (NMR)

- To measure proteins in their native state
- To characterize protein–protein interactions
- To determine protein dynamics

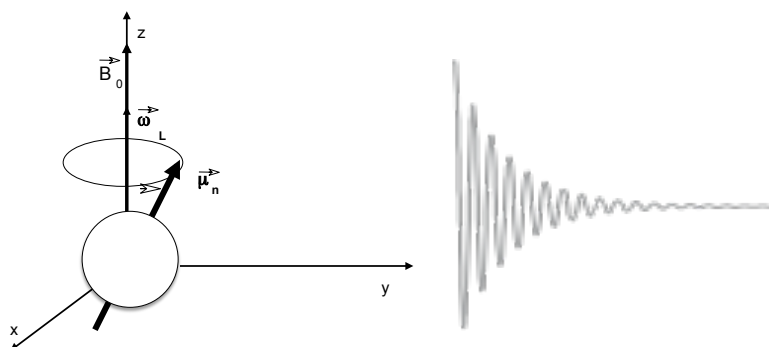
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Elements of NMR

- Elements of NMR:
 - Nuclear, **Magnetic**, Resonance

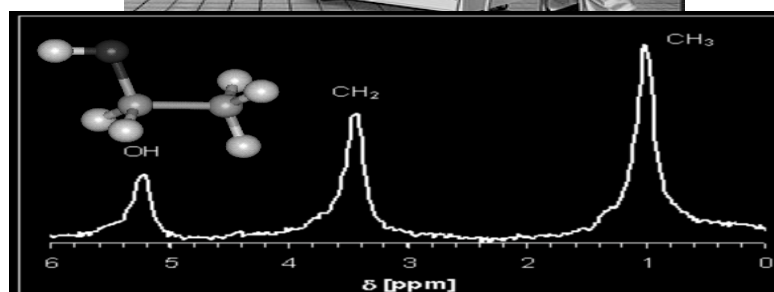


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NMR Spectrometer



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Advancement in NMR

- Transverse Relaxation Optimization Spectroscopy (TROSY) for applications with biological macromolecules
- **Rapid & promising developments at various fronts in progress**

XRD vs. NMR

- NMR is rapid, non-destructive, small amount of sample, no need for crystal formation and large amounts of purified protein samples.
- X-ray crystallography has the advantage of defining ligand-binding sites with more certainty

Imaging Techniques

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Imaging Techniques

- Electron crystallography determines the structure of macromolecular complexes
- Electron tomography technique is capable to provide 3D images at molecular resolution
- Development of hybrid approaches for Electron tomography and maximum resolution will advance the structural proteomics research

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3. Bioinformatics & Proteomics

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Bioinformatics and Proteomics

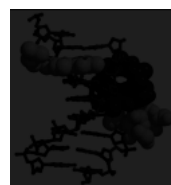
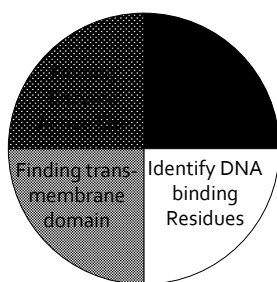
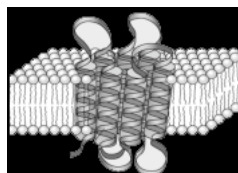
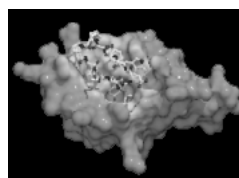
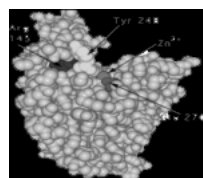
- To predict the structure and biological function of an uncharacterized protein, bioinformatics tools are required
- **Required for structure-function relationship and predict biochemical function of uncharacterized proteins**

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Bioinformatics and Proteomics



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Data Management in Proteomics

DELSA

Other initiatives –
PRIDE, TRANCHE, UniProt

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4. Challenges of Proteomics

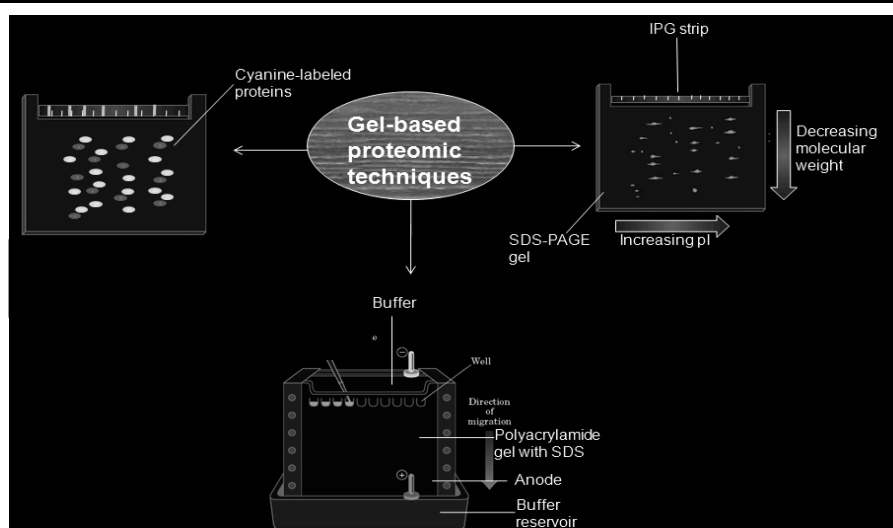
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Challenges in gel-based Proteomics

IPG strip

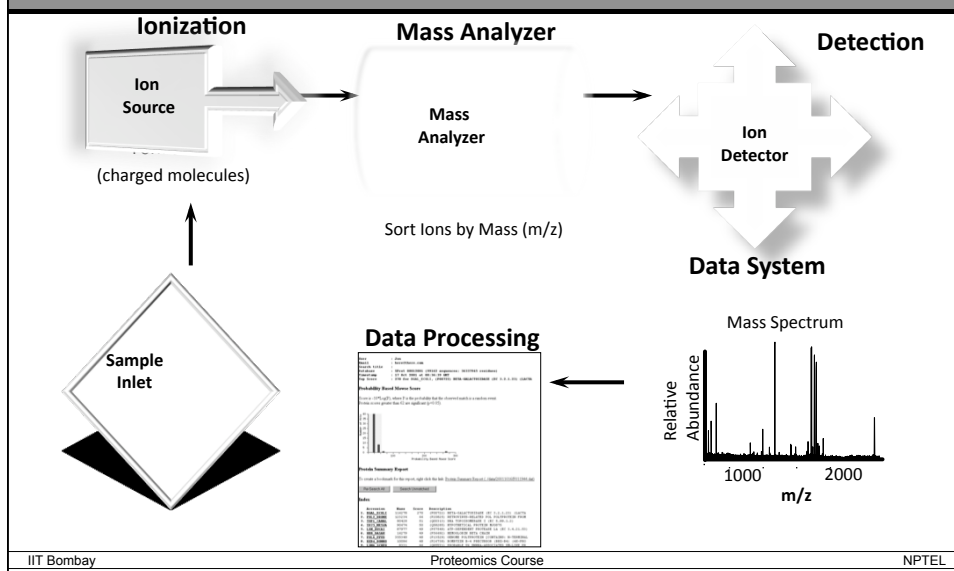


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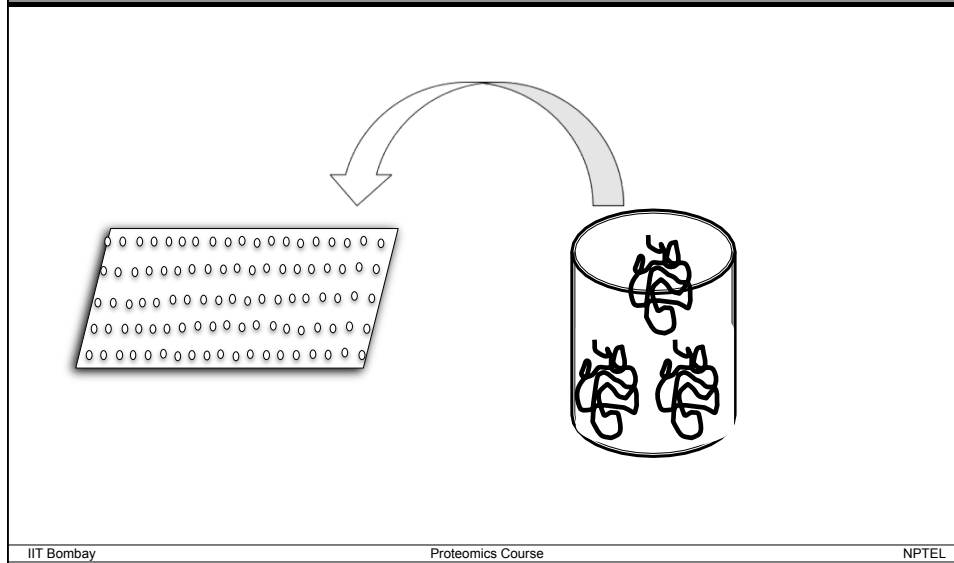
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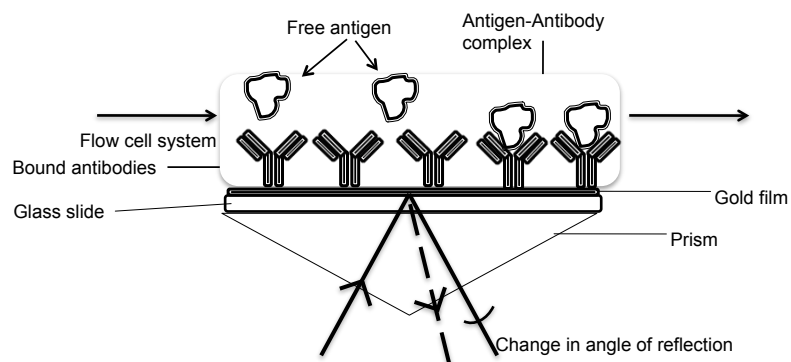
Challenges of Mass Spectrometry



Challenges of Array based technologies



Challenges of Label-free detection techniques

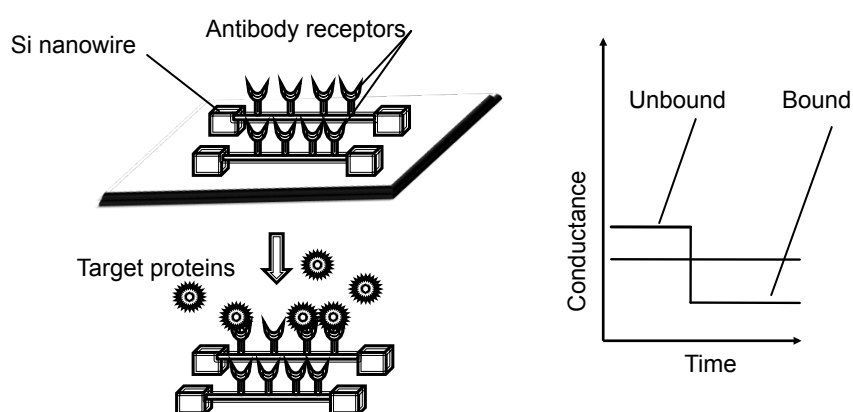


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Challenges of nanoproteomics

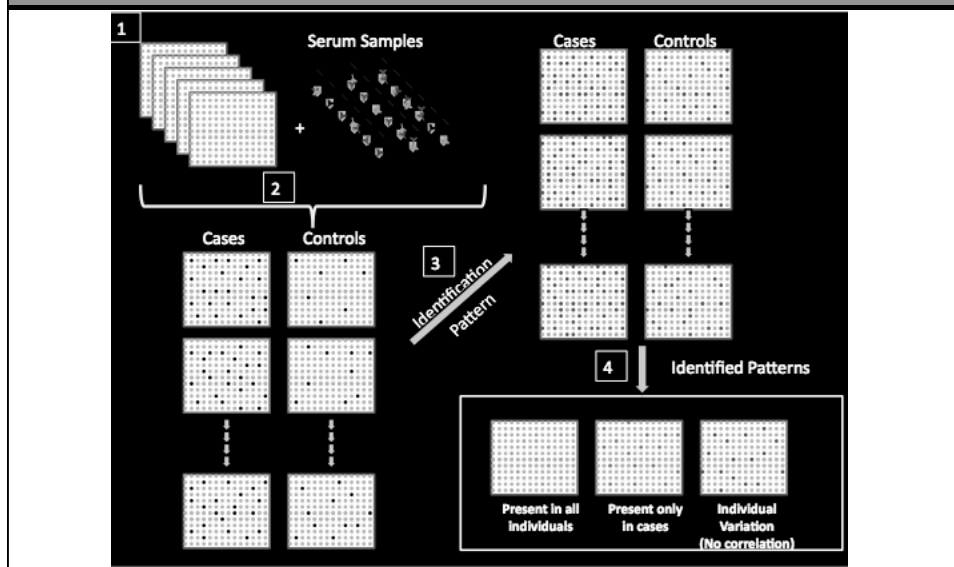


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Challenges of biomarker discovery



5. Future of Proteomics

Future of Proteomics

- Human Proteome Project
- Human Liver Proteome Project
- Human Plasma Proteome Project
- Brain Proteome Project
- Human Kidney and Urine Proteome Project
- **Proteomics and India**

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Proteomics and India



Summary

- Proteomic technologies are advancing rapidly
- It is anticipated that existing limitations of proteomic technologies will be resolved
- Proteomics discipline will become robust, sensitive, reliable, rapid, cost-effective and user-friendly platform for resolving real-life biological problems

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Acknowledgements

- Profs. Prasenjit Bhaumik and Ashutosh Kumar, IIT Bombay for discussion and reference material on X-ray crystallography and NMR, respectively.
- Prof. Cynthia Goh, University of Toronto for discussion on entrepreneurship.
- E-learning projects from MHRD – OSCAR and Virtual Laboratories
- Students of proteomics course at the IIT Bombay and researchers & staff in my laboratory for help, contributions and stimulating discussions
- CDEEP team – Mr. Tushar, Ms. Sangeeta and Mr. Arun