

# Proteomics Course

## **LECTURE-26** **Interactomics:** **Yeast Two-Hybrid** **Immunoprecipitation** **Protein microarrays**



Dr. Sanjeeva Srivastava  
IIT Bombay



## **Lecture outline**

- Interactomics
- Yeast Two-Hybrid
- Immunoprecipitation
- Protein microarrays

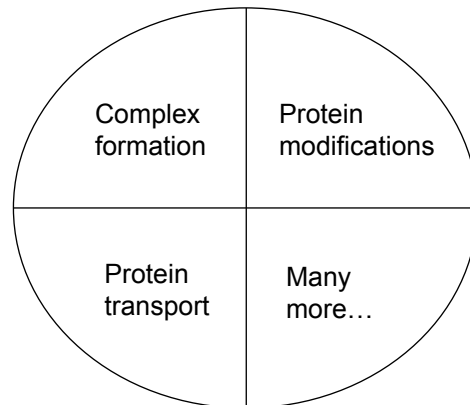
# Interactomics

## Impact of interactions

Tremendous effect of interactions



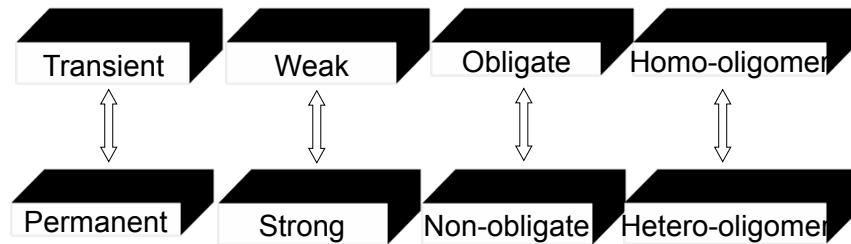
## Protein interactions with biomolecules



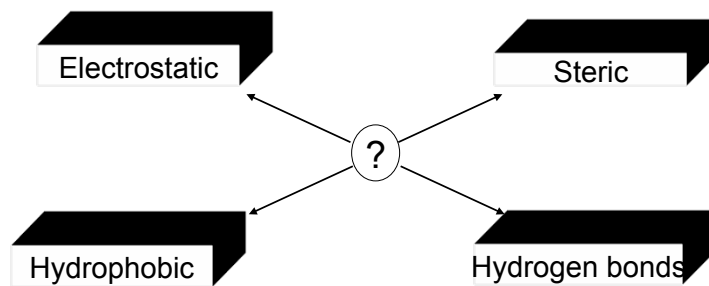
## Interactomics to identify

- Function of uncharacterized proteins
  - New roles for characterized proteins
- Mechanisms to regulate protein activity
- Networks of protein interactions

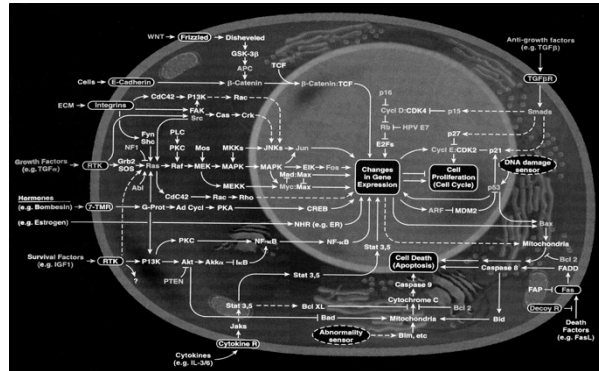
## Different methods of interactions



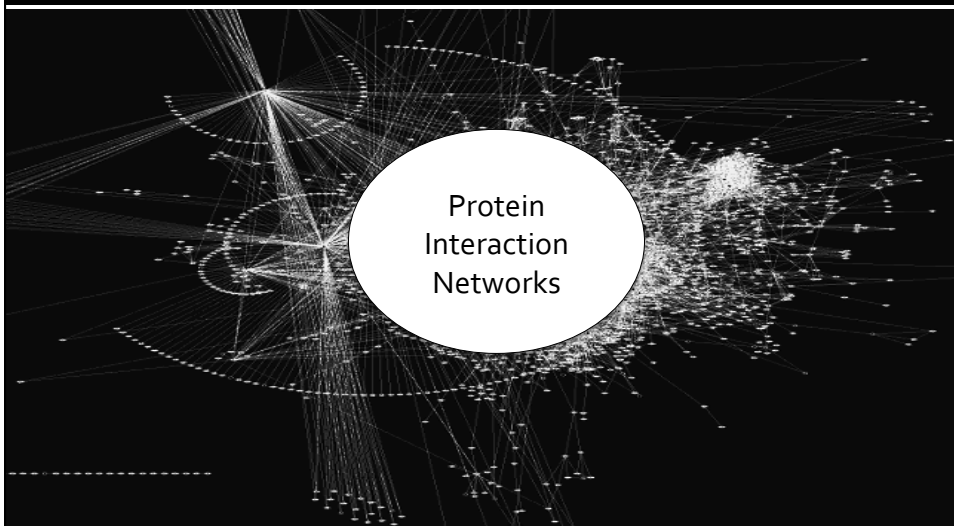
## Physical reasons for interactions



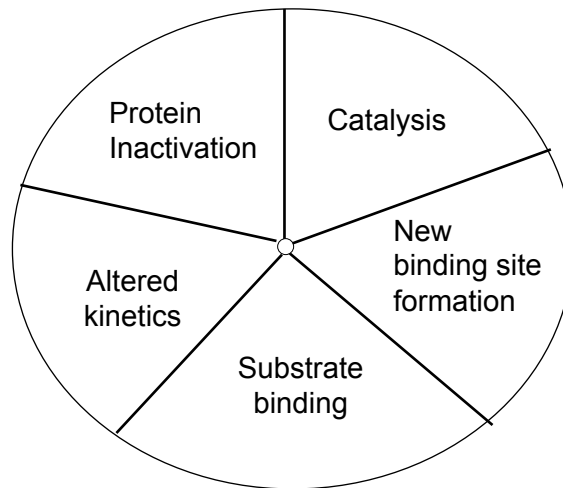
## Several multi-protein complexes in biological pathways



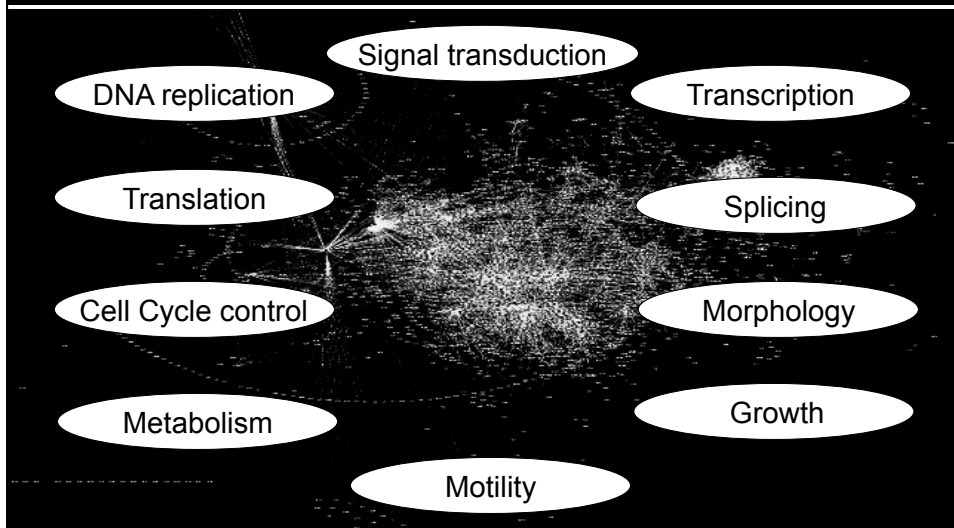
## Proteins involved in many binary interaction



## Potential effects of protein-protein interactions



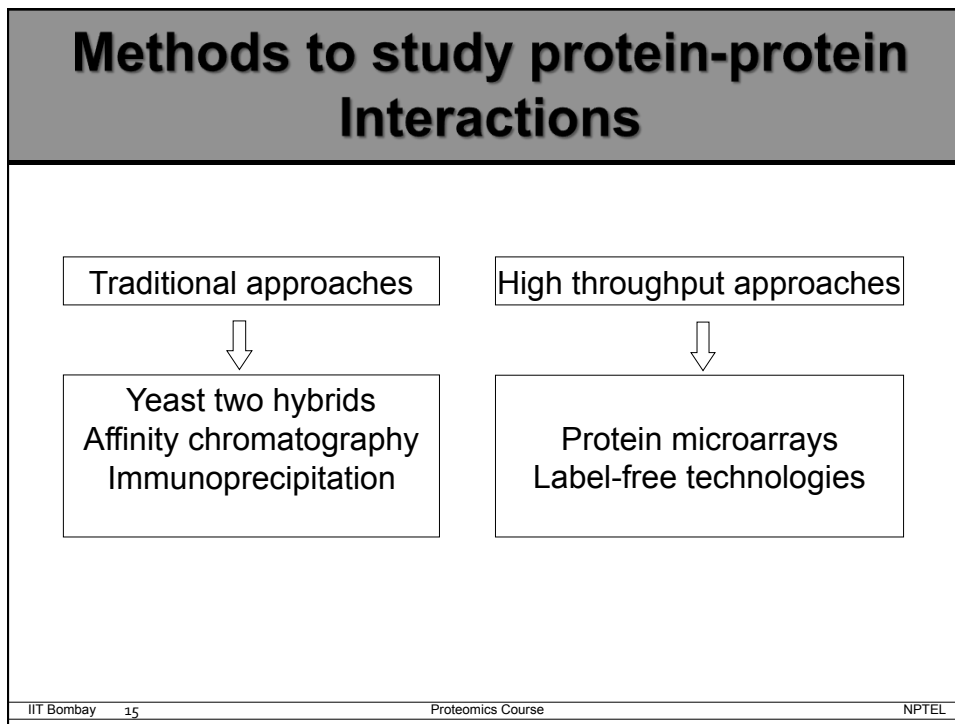
## Protein interactions essential for any event in cell



## Significance of protein interactions

- Dynamic, interact with a wide variety of biomolecules
  - lipids, nucleic acids, small drug inhibitors, and many others
- Proteins also interact with one another
  - form macromolecular complexes to regulate signal transduction & gene regulation

## Methods to study protein-protein interactions



## Methods to study protein-protein interactions: Traditional approaches

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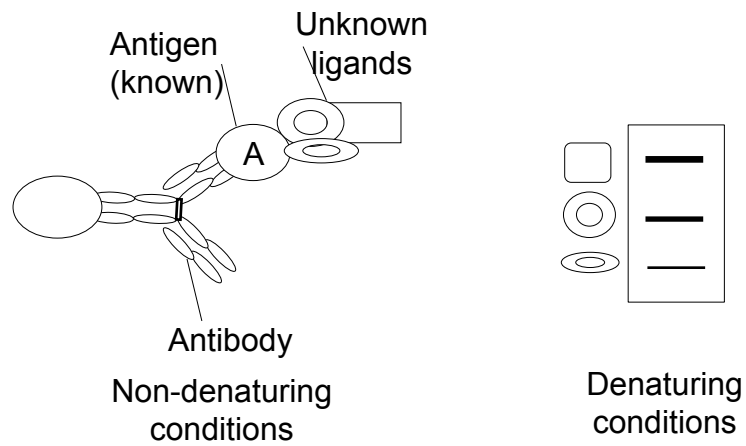


## **(I) Immunoprecipitation**

## **Immunoprecipitation (IP)**

- Purification of protein complexes by Immunoprecipitation (IP) or tandem affinity purification (TAP)
- Target protein and its interacting partner isolated from complex sample

## Immunoprecipitation



## Immunoprecipitation: Merits

- Proteins in native state
- Interactions are natural
- Large order complexes can be observed

## Immunoprecipitation: Demerits

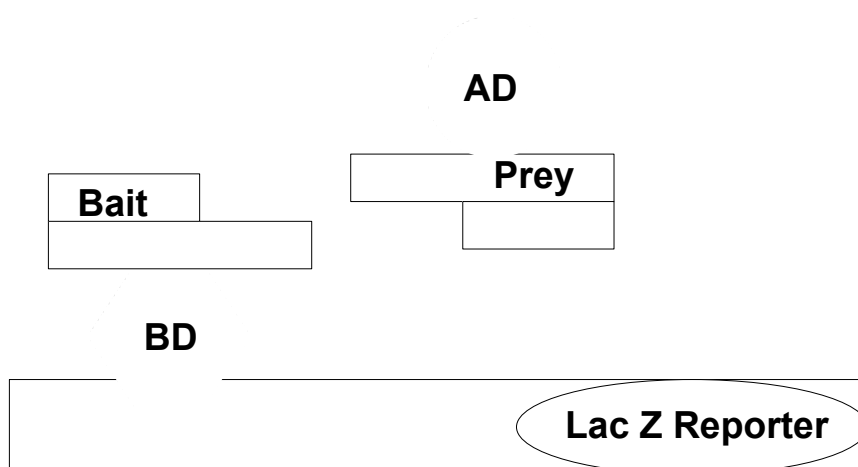
- Sticky proteins appear regularly
- Unclear whether interaction is direct or indirect
- Expensive
- Applications in other organisms challenging

## (II) Yeast two hybrid

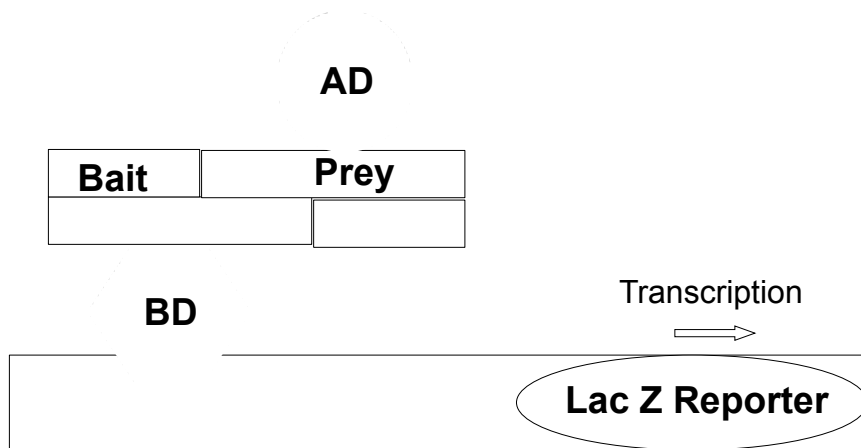
## Yeast two hybrid (YTH or Y2H)

- YTH system demonstrated by Fields and Song (1989)
  - to detect protein interactions
- The bait-BD and prey-AD hybrid proteins are jointly expressed in yeast nucleus
- If protein-protein interaction is established
  - results in activation of reporter gene transcription

## Yeast two hybrid



## Yeast two hybrid



## Yeast two hybrid: advantages

- Used for protein-RNA, protein-DNA interactions, analysis of particular complexes, construction of protein interaction network
- Simple protocol
- No expensive equipment requirement
- Ability to screen large libraries

## Yeast two hybrid: disadvantages

- High false positive/negative rates
- Proteins must localize and interact in nucleus
- Application in a non-yeast environment questionable
- Sensitive to toxic gene
- Limited to pair-wise interaction

## Methods to study protein-protein interactions: Protein microarrays

## (III) Protein microarrays: HT approach

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## Protein microarrays

- Microscopic arrays comprising thousands of discrete proteins
  - High throughput platform
  - biomarker discovery
  - protein-protein interactions
  - functional characterization

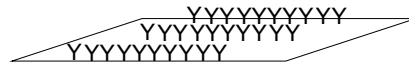
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## Protein microarrays

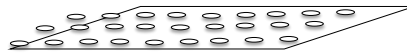
### Antibody Array



Microscopic array of antibodies

Measure levels of proteins or other biomolecules in samples

### Protein Array



Microscopic array of proteins

Assay protein function, Protein interactions, Small molecule interactions,  
Identify substrates

## Protein microarrays

Direct  
Label-based

- Spotting purified proteins (*E. coli*)
- Limited number of proteins



*MacBeath, G., and Schreiber, S. 2000. Science 289:1760*



## Protein microarrays

Direct  
Label-based

• 5800 yeast clones (His-tagged)

- Screening for known and novel Calmodulin & lipid binding partners

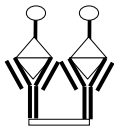


*Zhu et al. 2001 Science, 293, 2101*

## Protein microarrays: Various platforms

## Protein microarrays: Direct labeling

Direct  
Label-based



## Protein microarrays: Sandwich immunoassay

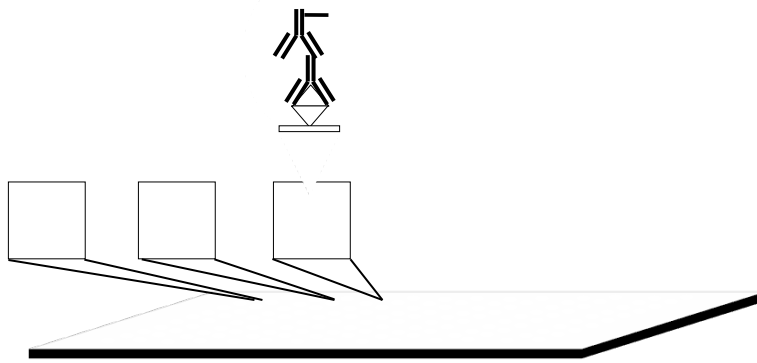
Direct  
Label-based

Sandwich



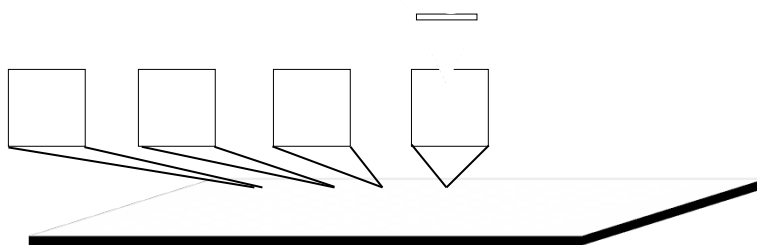
## Protein microarrays: Reverse phase protein blot

Reverse-phase



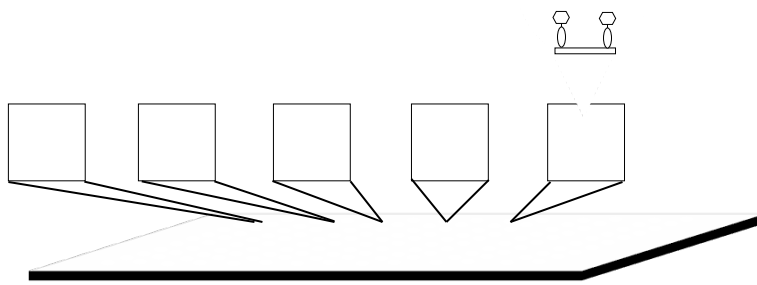
## Protein microarrays: Chemically linked

Chemical linkage



## Protein microarrays: Peptide fusion tags

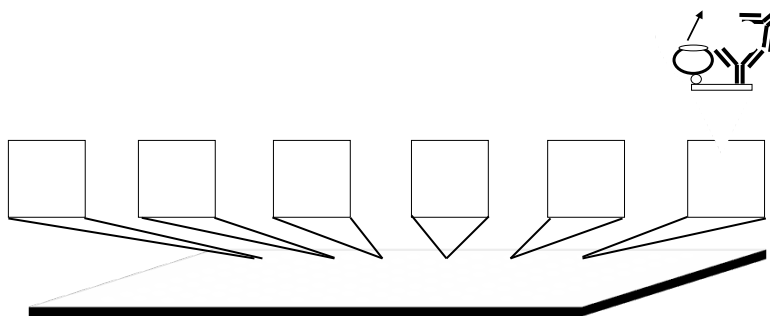
Chemical linkage      Peptide fusion



## Protein microarrays: Nucleic acid programmable protein array

Direct  
Label-based

NAPPA



## Protein microarrays: Multiple spotting technique

The diagram illustrates a protein microarray slide with seven rectangular spots. A callout labeled 'MIST' shows a protein being spotted onto a surface. The slide is shown in perspective, with a thick black line representing the bottom edge.

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## Protein microarrays: An overview

Abundance based	Function based
<p><small>Sandwich</small>   <small>Reverse-phase</small>   <small>Chemical linkage</small></p>	<p><small>Peptide fusion</small>   <small>MAFA</small>   <small>MIST</small></p>

The diagram shows a protein microarray slide with seven rectangular spots, corresponding to the techniques shown in the table above. The slide is shown in perspective, with a thick black line representing the bottom edge.

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## Protein microarrays

### Achievements

High density  
Small volume  
Multifunctional assays

### Challenges

Protein purification  
Protein functionality

## Summary

- Interactomics
- Yeast Two-Hybrid
- Immunoprecipitation
- Protein microarrays

## References

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