

# Proteomics Course

## LECTURE-33 Applications of protein microarrays

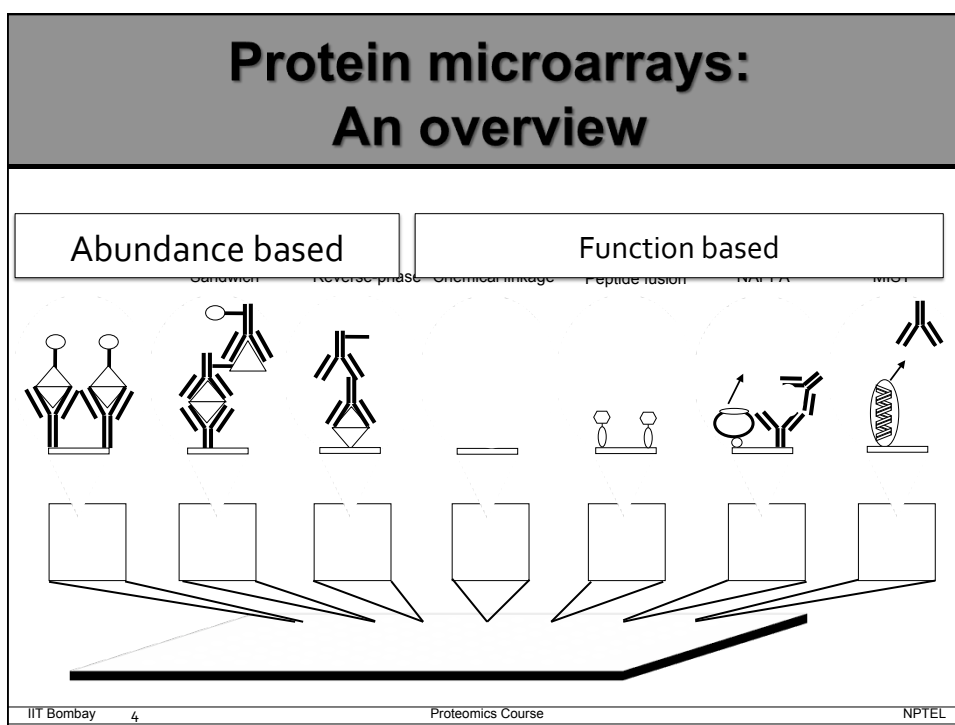
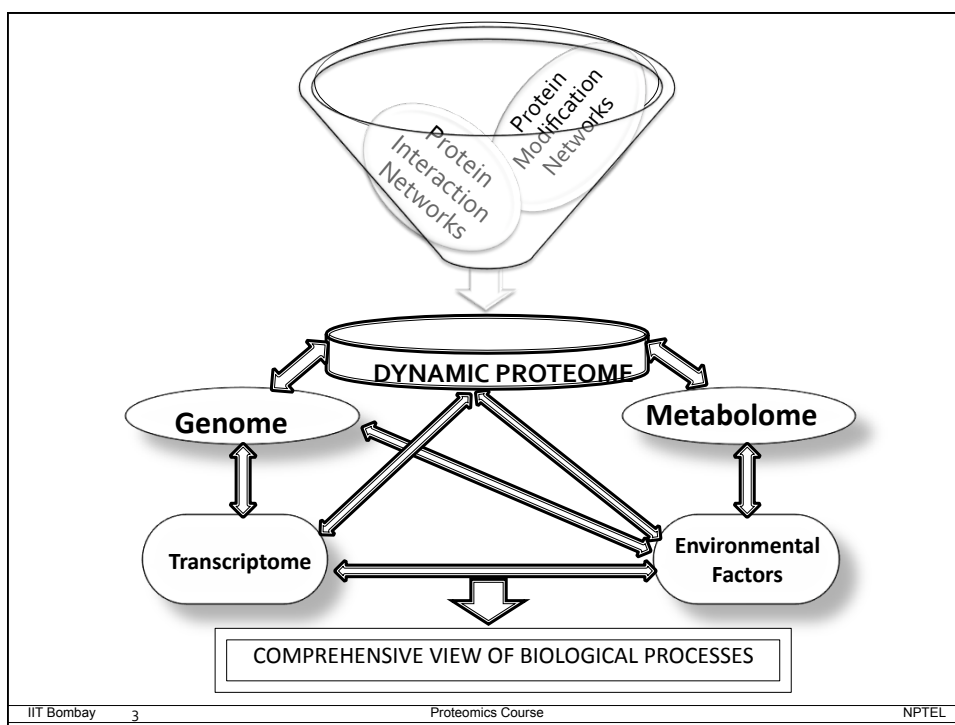


Dr. Sanjeeva Srivastava  
IIT Bombay



## Lecture outline

- Protein microarrays – an overview
  - Protein microarray experiment
- Applications
  - Biomarker screening
  - Protein-protein interactions



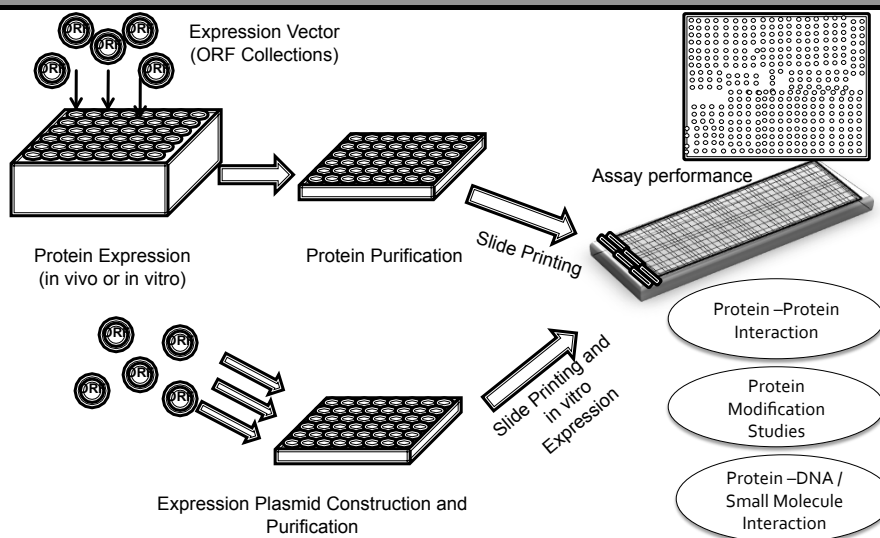
# An overview of protein microarray experiment

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## An overview of protein microarray experiment



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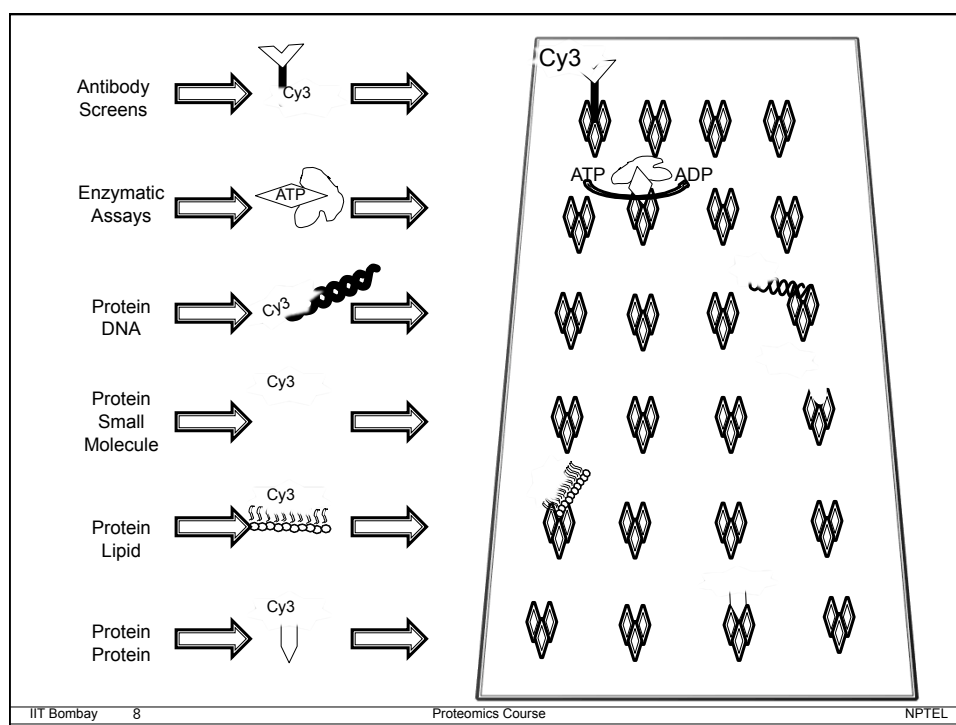
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# Protein microarrays applications

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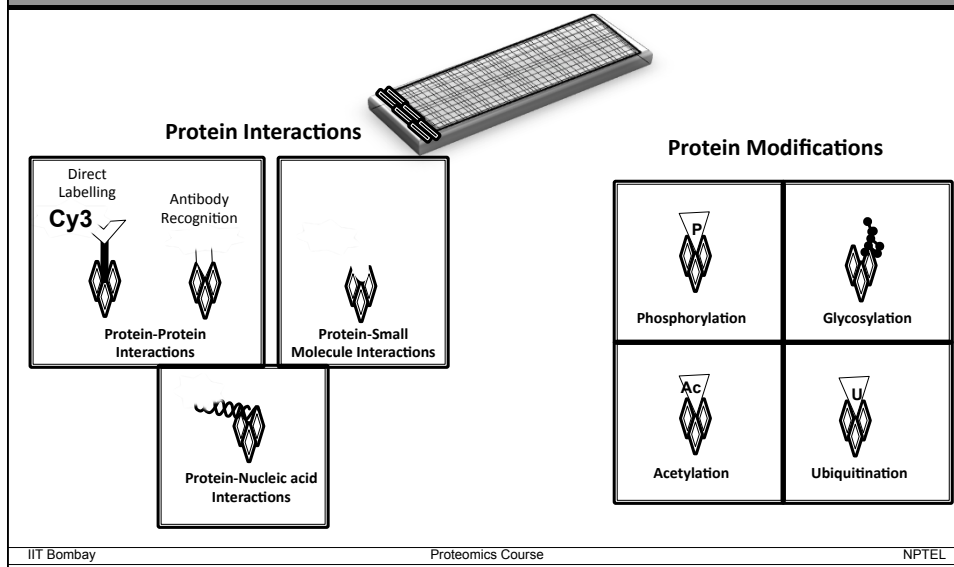
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# Protein microarray applications



## I. Biomarker detection

## Case study-1

### Identification of differentially expressed proteins in ovarian cancer using high-density protein microarrays

*Hudson et al. Identification of differentially expressed proteins in ovarian cancer using high-density protein microarrays. Proc Natl Acad Sci USA. 2007, 104, 17494-9*

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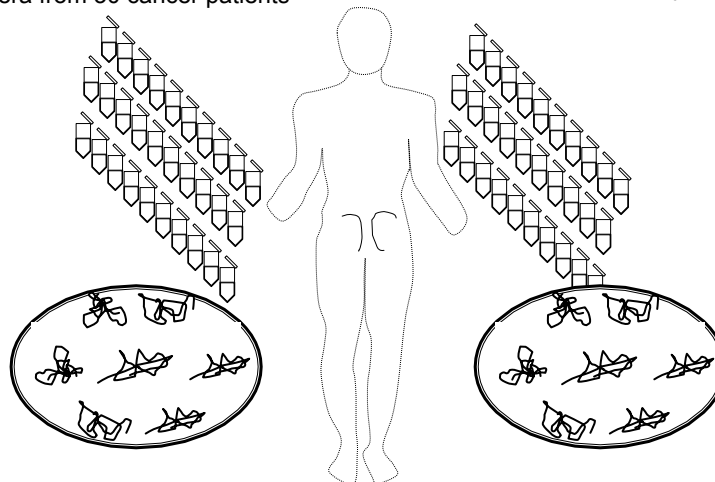
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## Serum sample collection from ovarian cancer patients

Sera from 30 cancer patients

Sera from 30 healthy patients

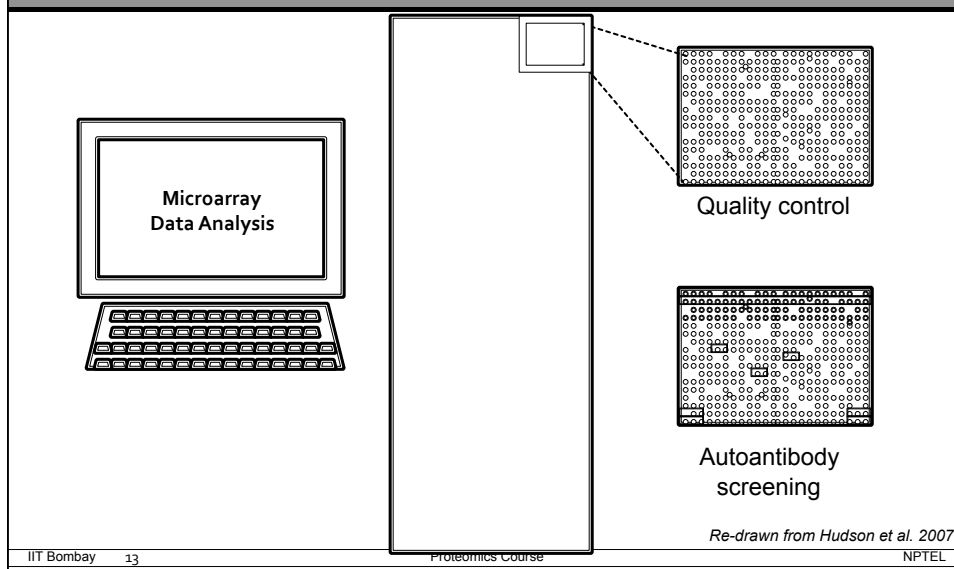


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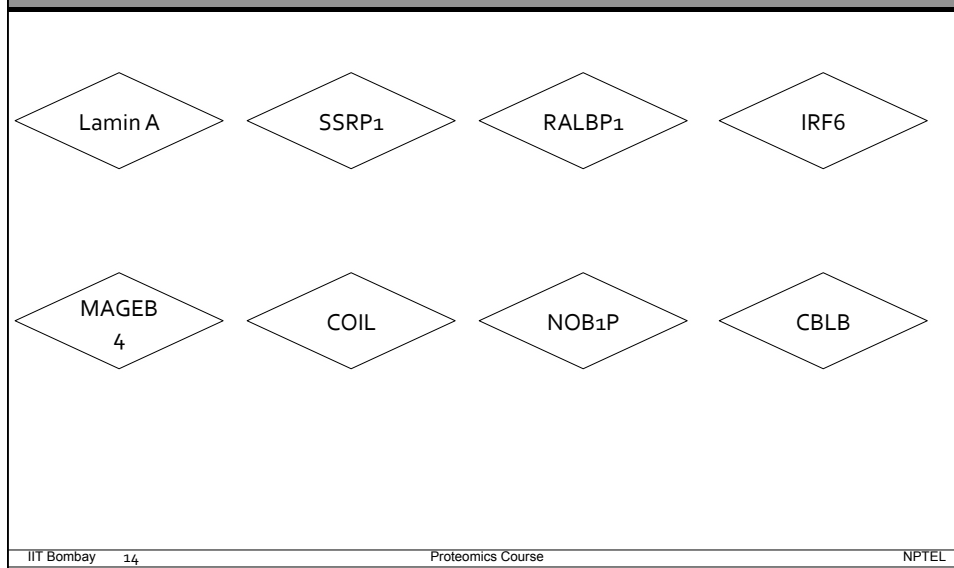
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## Tumor-associated autoantibodies and targeted protein antigens identification



## Differentially expressed proteins



## Validation: Immunoblot analysis

The diagram illustrates the immunoblot analysis process. On the left, a microarray chip with tissue spots is shown. A pipette adds serum and chromogen to the spots. A blocking reagent is also added. The resulting spots are analyzed for Lamin A, Lamin C, and p53. On the right, a gel image shows bands for Lamin A and Lamin C in the 'Healthy Tumor' lane, and a band for p53 in the 'Tumor' lane.

*Re-drawn from Hudson et al. 2007*

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## Tissue microarray analysis

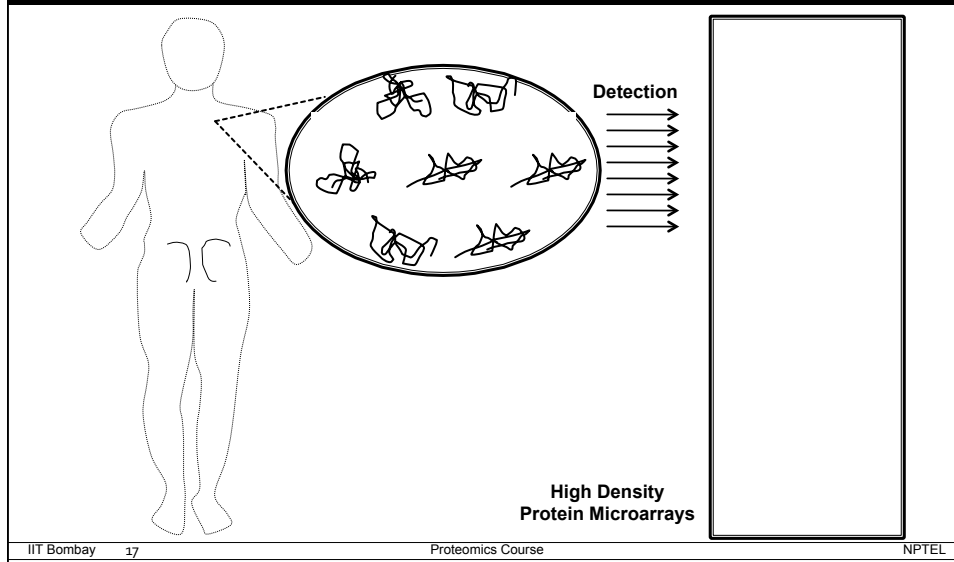
Lamin A/C	Stage 2 EOC	Stage 3 EOC	Stage 4 EOC	Positive Controls	Negative Controls
	Stage 2 EOC	Stage 3 EOC	Stage 4 EOC	Positive Controls	Negative Controls
	Stage 2 EOC	Stage 3 EOC	Stage 4 EOC	Positive Controls	Negative Controls

*Re-drawn from Hudson et al. 2007*

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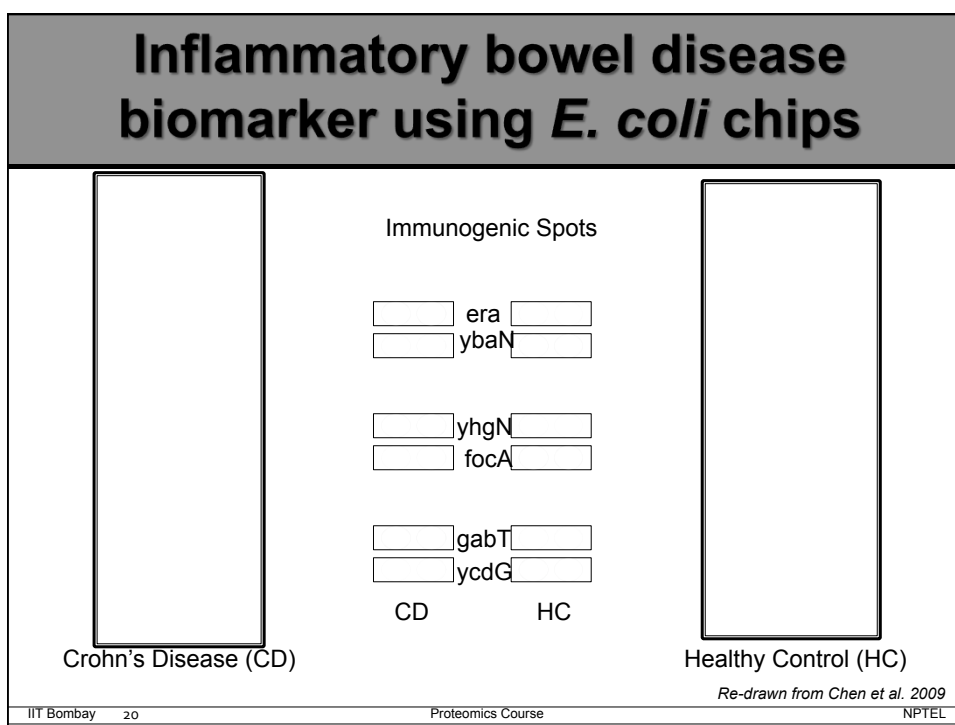
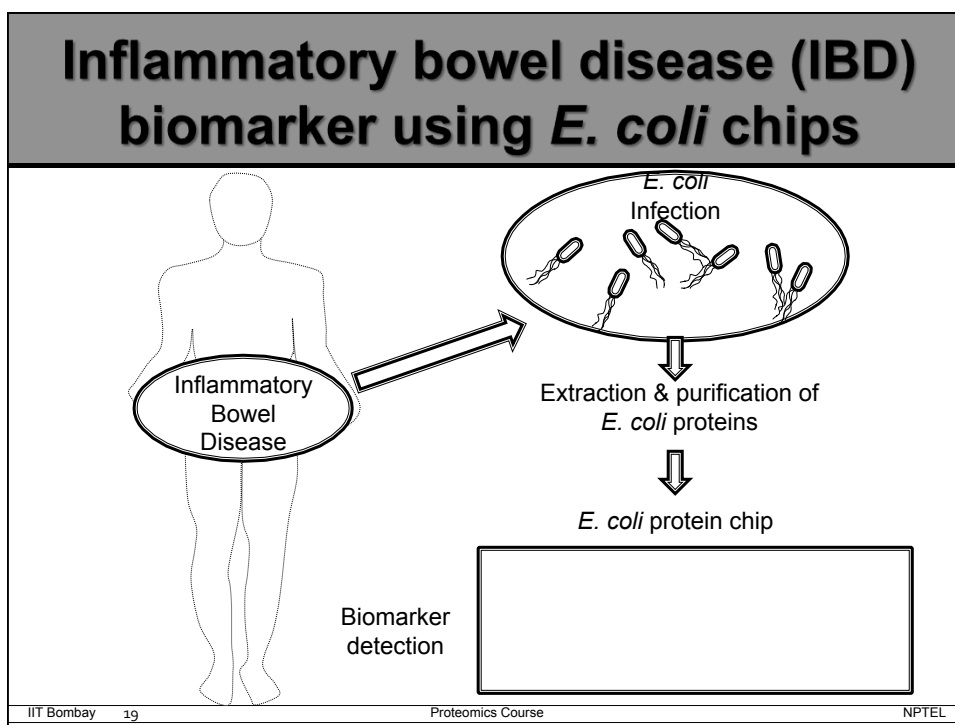
## Ovarian cancer signature markers

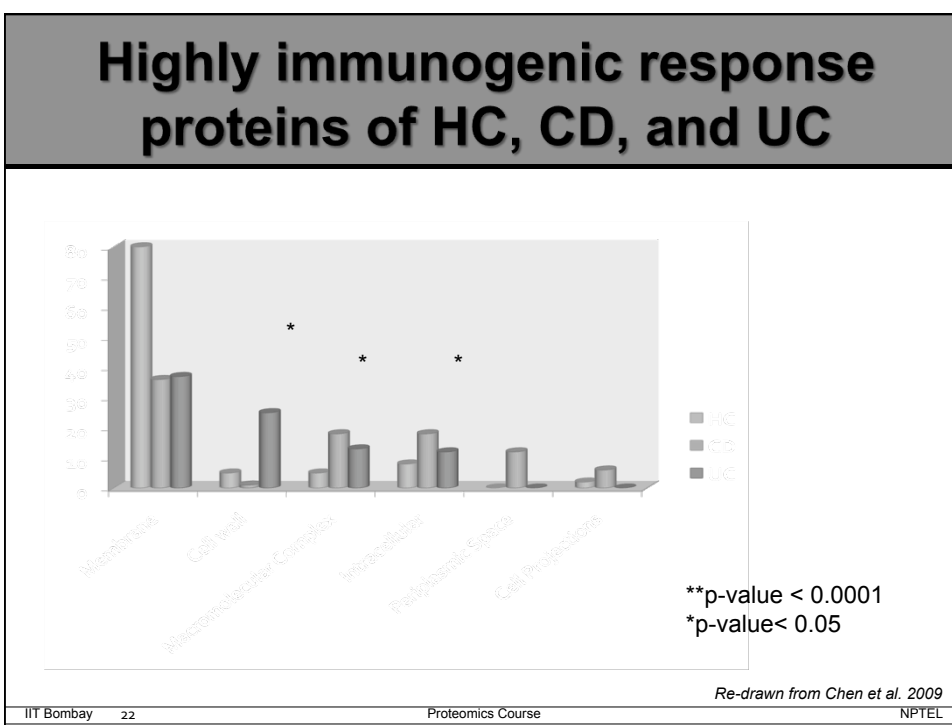
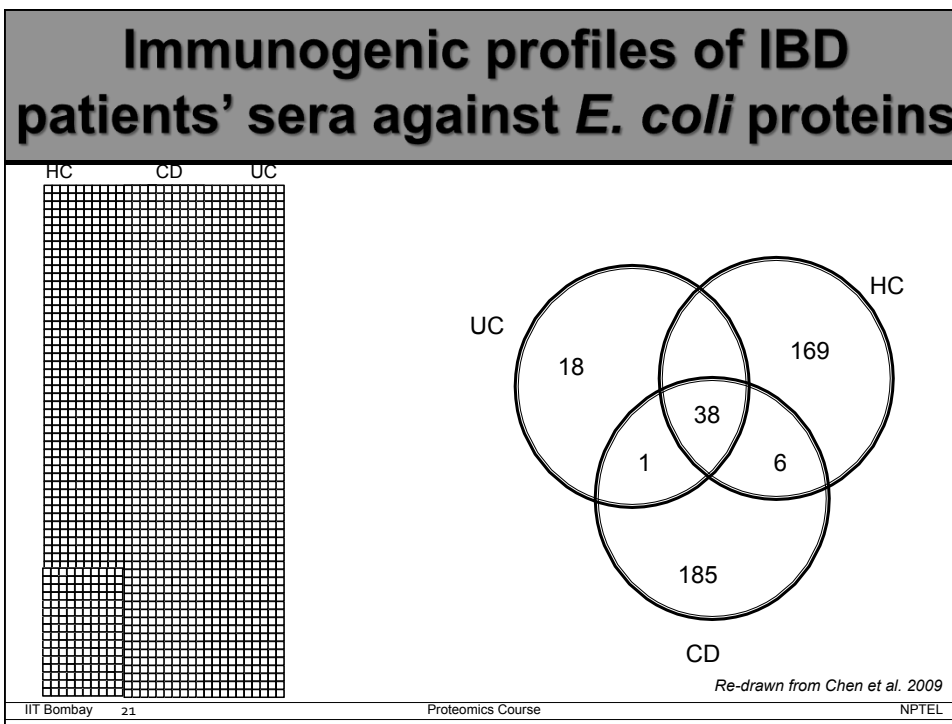


## Case study-2

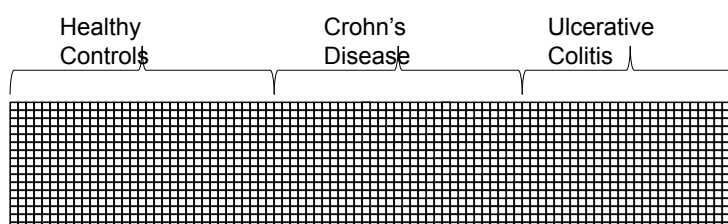
### Identification of novel serological biomarkers for inflammatory bowel disease using *Escherichia coli* proteome chip

Chen et al. Identification of novel serological biomarkers for inflammatory bowel disease using *Escherichia coli* proteome chip  
*Mol Cell Proteomics*. 2009, 8, 1765





## Biomarkers that can discriminate HC from CD patients



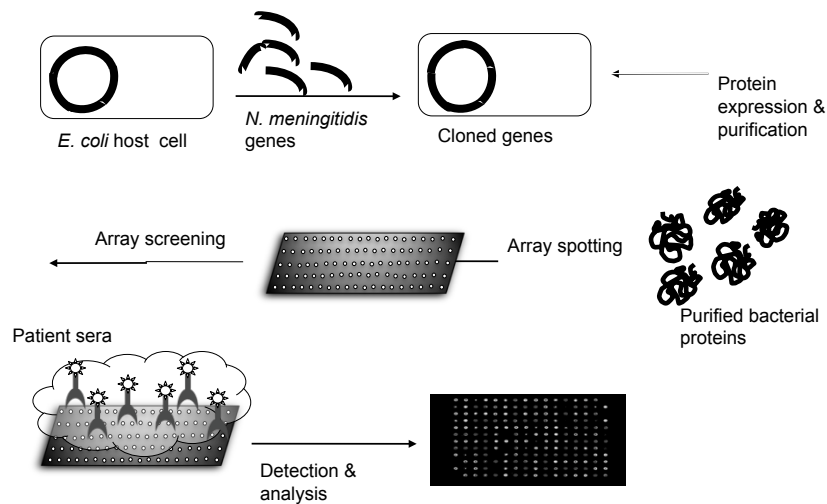
Re-drawn from Chen et al. 2009

## Case study-3

### Identification of potential diagnostic markers for infection from *Neisseria meningitidis*

Steller, S. et al. Bacterial protein microarrays for identification of new potential diagnostic markers for *Neisseria meningitidis* infections. *Proteomics* 2005, 5, 2048-2055.

## Identification of potential diagnostic markers for infection from *Neisseria meningitidis*



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## Case study-4

### Human prostate cancer screening for the identification of potential biomarkers

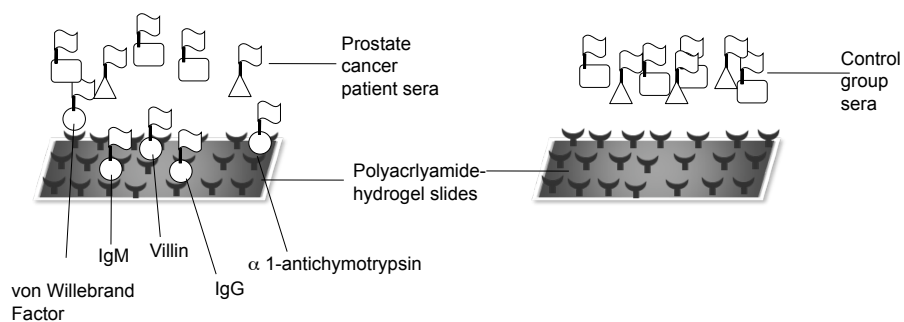
Miller JC, Zhou H, Kwekel J, Cavallo R, Burke J, Butler EB, Teh BS, Haab BB: Antibody microarray profiling of human prostate cancer sera: antibody screening and identification of potential biomarkers. *Proteomics* 2003, 3:56-63.

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## Biomarker prostate cancer



## II. Protein Interaction with biomolecules (proteins, DNA etc.)

## Case study-5

### The development of protein microarrays and their applications in DNA-Protein and Protein-Protein interaction analyses of Arabidopsis transcription factors

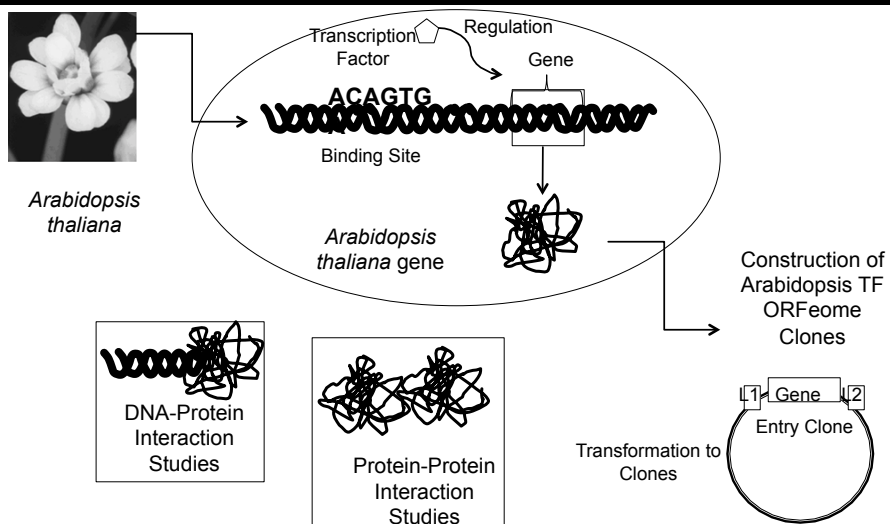
Gong W et al The development of protein microarrays and their applications in DNA-protein and protein-protein interaction analyses of Arabidopsis transcription factors. *Mol Plant*. 2008, 1, :27-41.

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## Protein microarrays for protein-protein and protein-DNA interactions

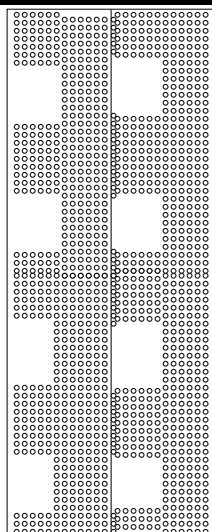


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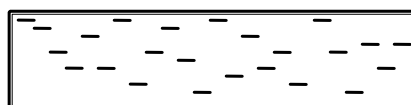
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## Arabidopsis TF Protein microarrays: QC work and generation of microarrays

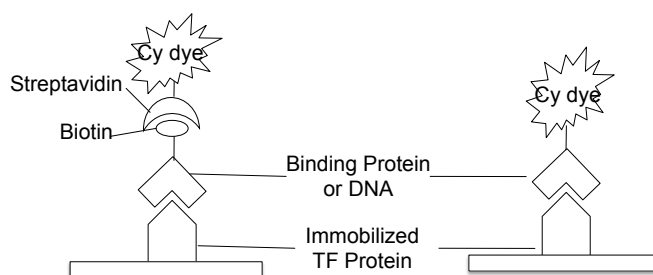


Western blot



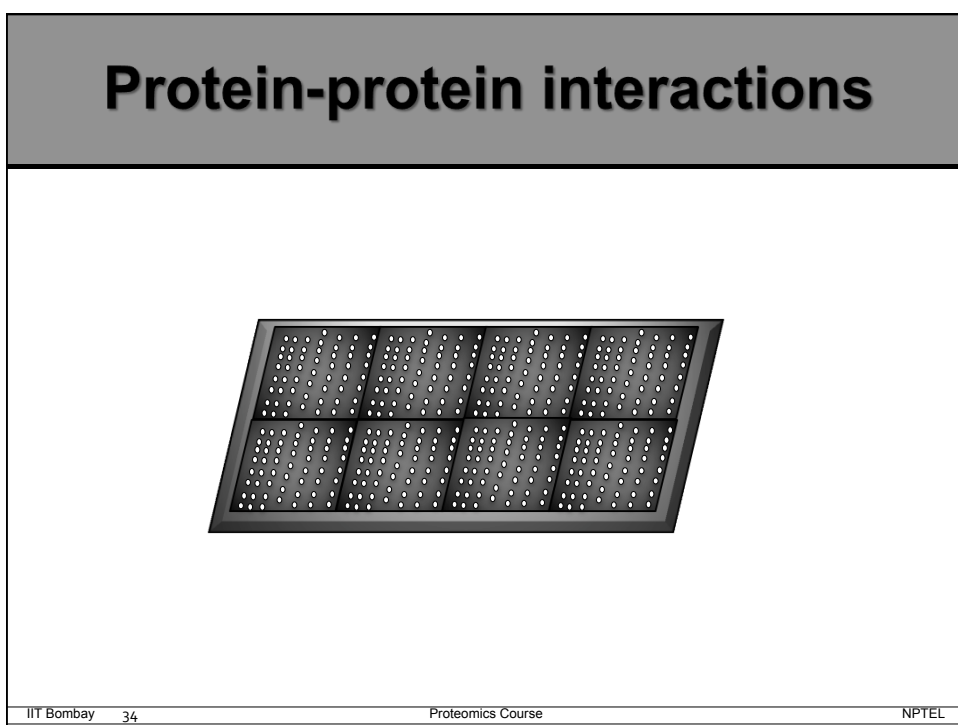
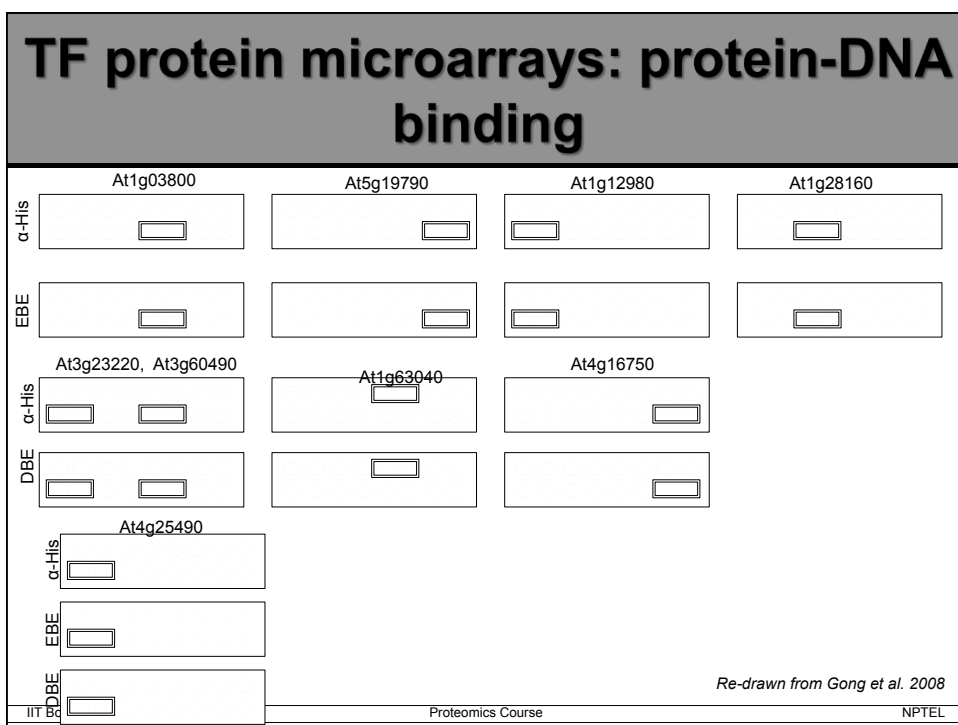
*Re-drawn from Gong et al. 2008*

## Strategy for detection of Protein- DNA and Protein-protein interactions



*Re-drawn from Gong et al. 2008*





## Case study-5

### Analysis of yeast protein activities using proteome chips

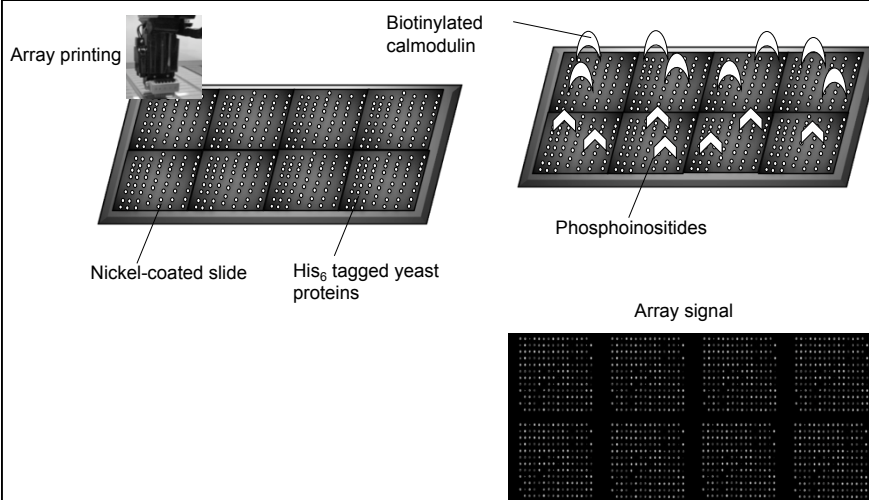
Zhu, H. et al. *Global analysis of protein activities using proteome chips. Science* 2001, 293:2101-2105.

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## Protein interaction studies



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## III. Other applications

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### Case study-6

**Detection of antigen-antibody interactions at various concentrations using antigen and antibody microarrays**

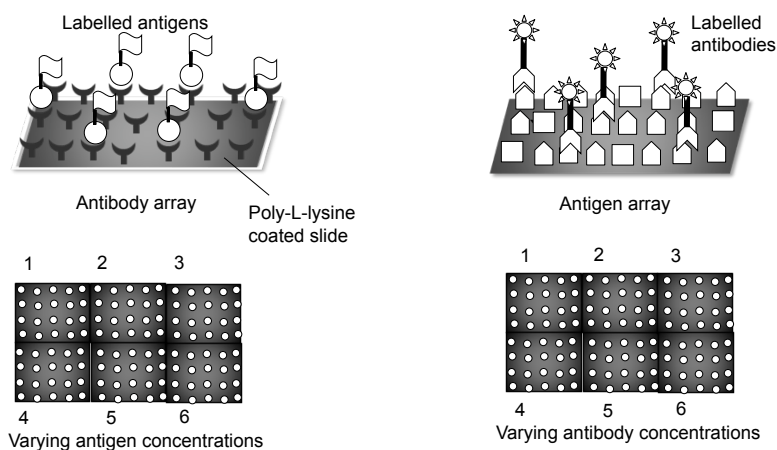
*Haab B, Dunham M, Brown P: Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions. Genome Biol 2001, 2 (2).*

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## Detection of antigen-antibody interactions using protein microarrays



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## Case study-7

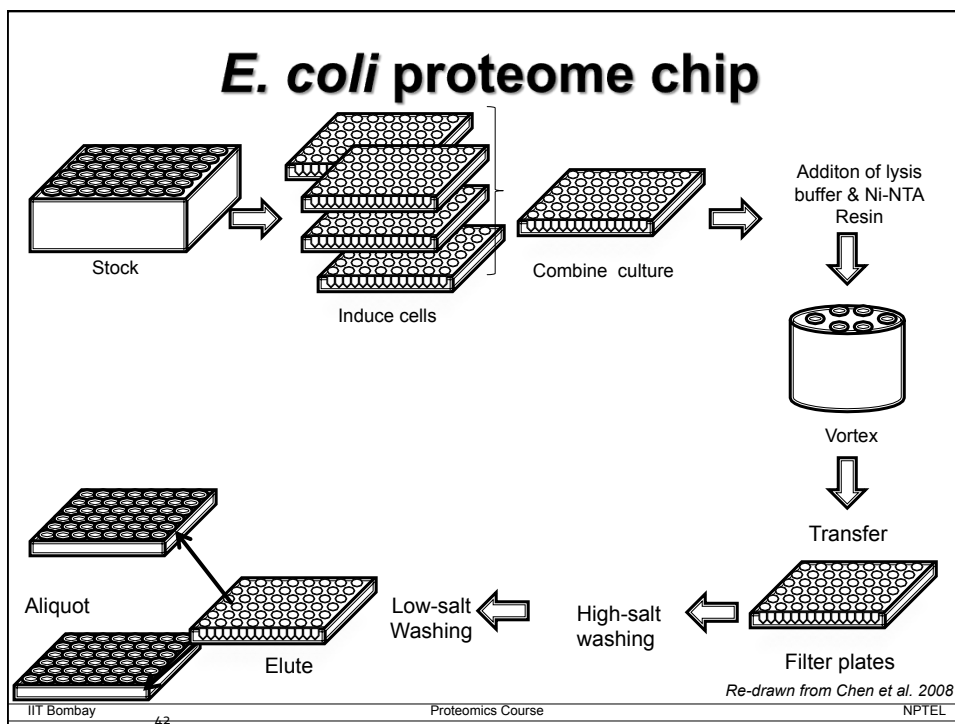
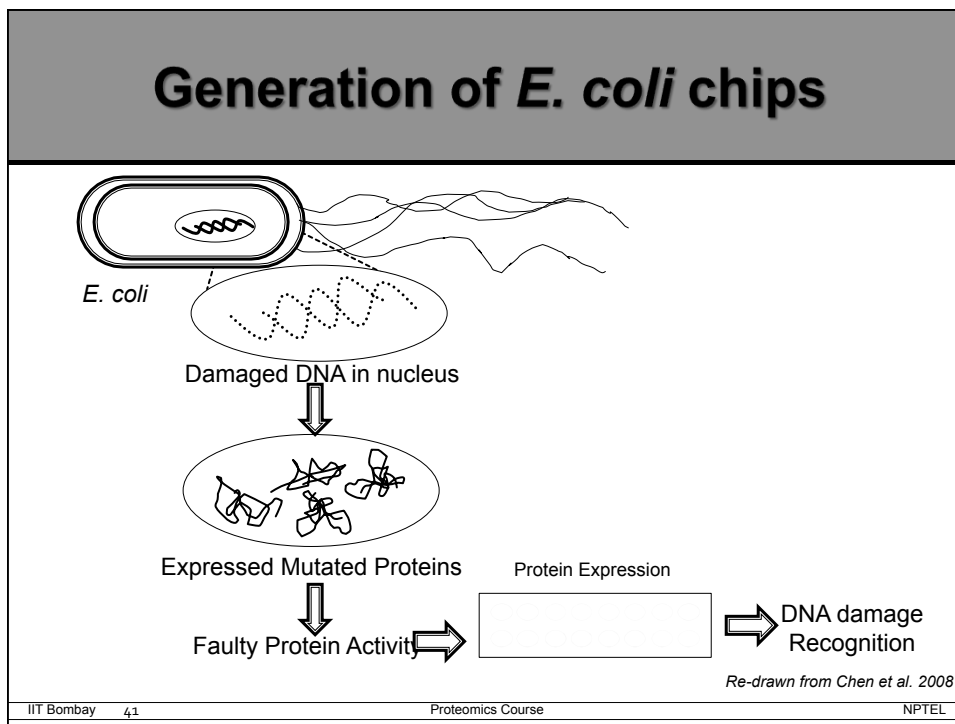
### A proteome chip approach reveals new DNA damage recognition activities in *Escherichia coli*

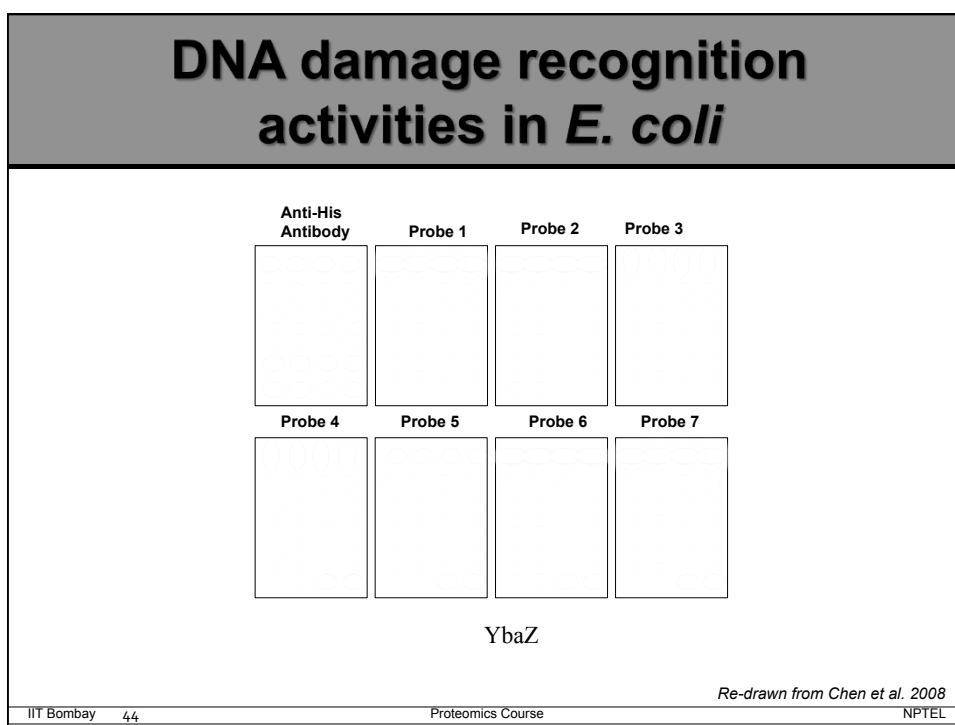
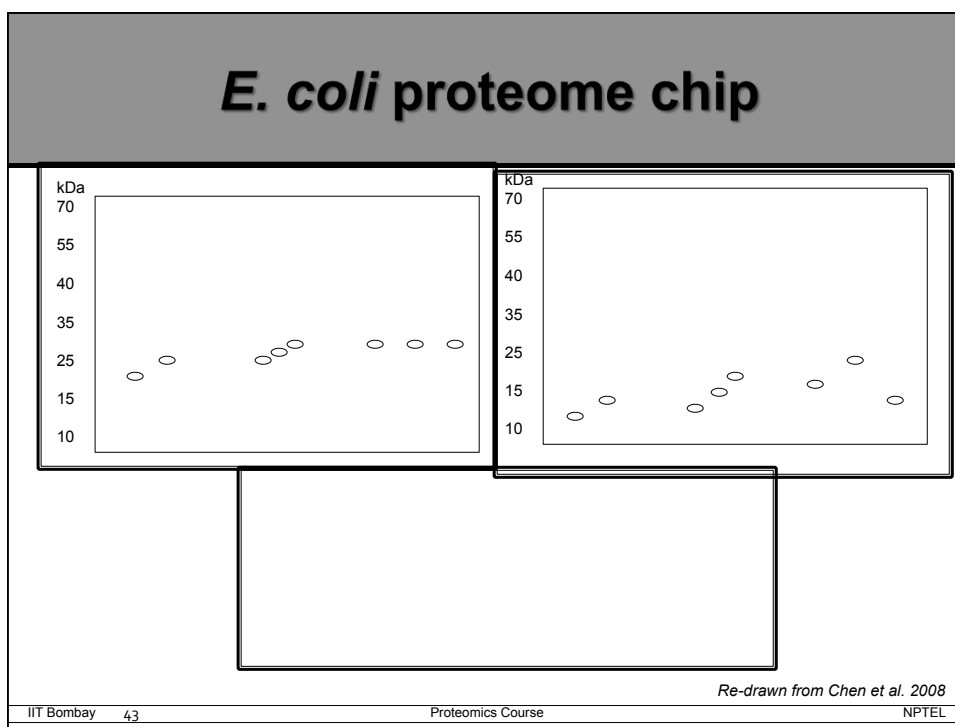
Chen et al. A proteome chip approach reveals new DNA damage recognition activities in *Escherichia coli*. *Nat Methods* 2008, 5, 69-74

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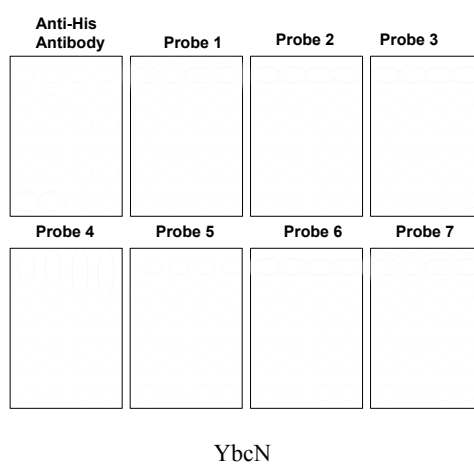
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## DNA damage recognition activities in *E. coli*



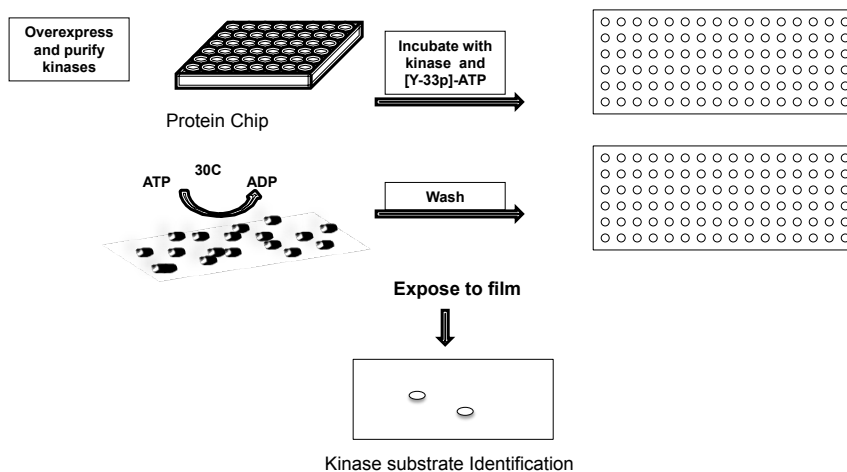
*Re-drawn from Chen et al. 2008*

## Case study-8

### Protein phosphorylation study in yeast

*Ptacek et al. 2005, Global analysis of protein phosphorylation in yeast. Nature 438, 4187*

## Identification of kinase substrates using protein chips

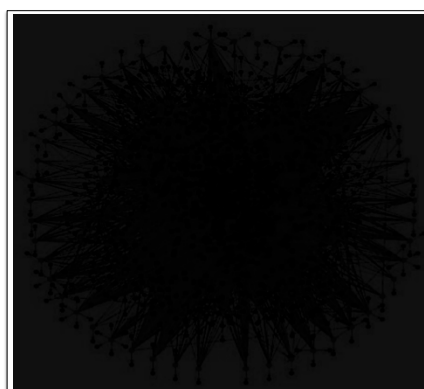


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## *In vitro* phosphorylation map of yeast



Modified from Ptacek et al. 2005

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## Summary

- An overview of protein microarray experiment
- Applications
  - Biomarker screening
  - Protein-protein interactions
  - Protein-DNA interaction
  - DNA damage study
  - Kinase substrate identification

## References

- Hudson et al. Identification of differentially expressed proteins in ovarian cancer using high-density protein microarrays. *Proc Natl Acad Sci USA*. 2007, 104, 17494-9
- Chen et al. Identification of novel serological biomarkers for inflammatory bowel disease using *Escherichia coli* proteome chip *Mol Cell Proteomics*. 2009, 8, 1765
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- Miller JC, Zhou H, Kwekel J, Cavallo R, Burke J, Butler EB, Teh BS, Haab BB: Antibody microarray profiling of human prostate cancer sera: antibody screening and identification of potential biomarkers. *Proteomics* 2003, 3:56-63.
- Gong W et al The development of protein microarrays and their applications in DNA-protein and protein-protein interaction analyses of *Arabidopsis* transcription factors. *Mol Plant*. 2008, 1, :27-41.
- Zhu, H. et al. Global analysis of protein activities using proteome chips. *Science* 2001, 293:2101-2105.

## References

- Haab B, Dunham M, Brown P: Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions. *Genome Biol* 2001, 2 (2).
- Chen et al. A proteome chip approach reveals new DNA damage recognition activities in *Escherichia coli*. *Nat Methods* 2008, 5, 69-74
- Ptacek et al. 2005, Global analysis of protein phosphorylation in yeast. *Nature* 438, 4187
- Harini Chandra, Sanjeeva Srivastava. 2010. Cell-free synthesis-based protein microarrays and their applications. *PROTEOMICS*. Volume 10, Issue 4, pages 717–730, No. 4 February 2010
- Harini Chandra, Panga Jaipal Reddy and Sanjeeva Srivastava. 2011. Expert Review of Proteomics. Protein microarrays and novel detection platforms. February 2011, Vol. 8, No. 1, Pages 61-79 10.1586/epr.10.99
- Joshua LaBaer , Niroshan Ramachandran. 2005. Protein microarrays as tools for functional proteomics. *Current Opinion in Chemical Biology*. Volume 9, Issue 1, February 2005, Pages 14–19.

## References

- Wei Huang, Lin Wu, Guozhen Liu, Siqi Liu. Protein microarray: A key approach of proteomics, .August 2010, Volume 5, Issue 4, pp 331-338
- Wei Huang, Lin Wu, Guozhen Liu, Siqi Liu. Protein microarray: A key approach of proteomics. *Frontiers in Biology*, August 2010, Volume 5, Issue 4, pp 331-338