Protein Microarrays for Cancer Biomarkers

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Course: Bioanalytical Chemistry

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Instructor: Dr. James Rusling

Overview

- Protein Arrays
  - What are arrays?
  - Significance
  - Market trend

- Manufacturing of Microarrays
  - Microarray Chip Fabrication
  - Protein Immobilization
  - Microarray Signal Detection

- Types of Protein Microarrays
  - Functional Microarrays
  - Analytical Microarrays

Overview

- Applications of Protein microarrays
- Improved Biomarkers for Prostate Cancer: A Definite Need
- Summary

Microarray Development

Relatively Young Technology

Widely Adopted

Mainly used for Gene discovery

What are Protein Arrays

- Microarrays
  - Glass or silicon slides
  - Thousands of spots
- Complicated Technology [ligands, folding, drying…]
- “High throughput”

Significance of Protein Microarrays

- Multiplexing- “the simultaneous transmission of several messages along a single channel of communication”.
  - False Positives
  - False Negatives

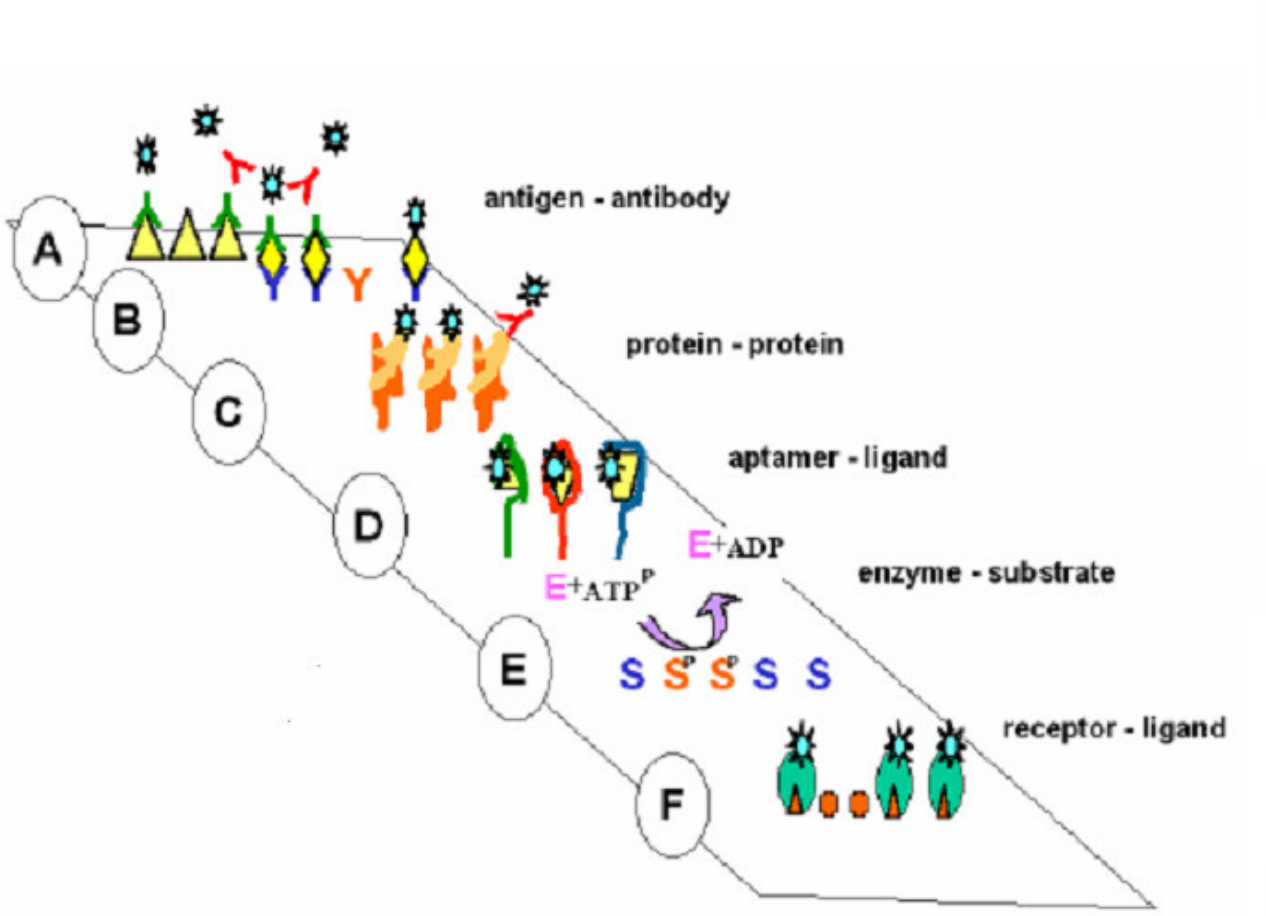
- Miniaturization and Parallelized Immunoassays
  - Superior sensitivity

$ $ $ $ Market Trend $ $ $ $

- $3 billion 2004
- $10 billion 2010

- 2000 – 100 companies - 33% a year

Capture Molecules in Microarray Assays



Label

Antibody

Secondary labeled Antibody

Radioactive (^{32}P) Phosphate

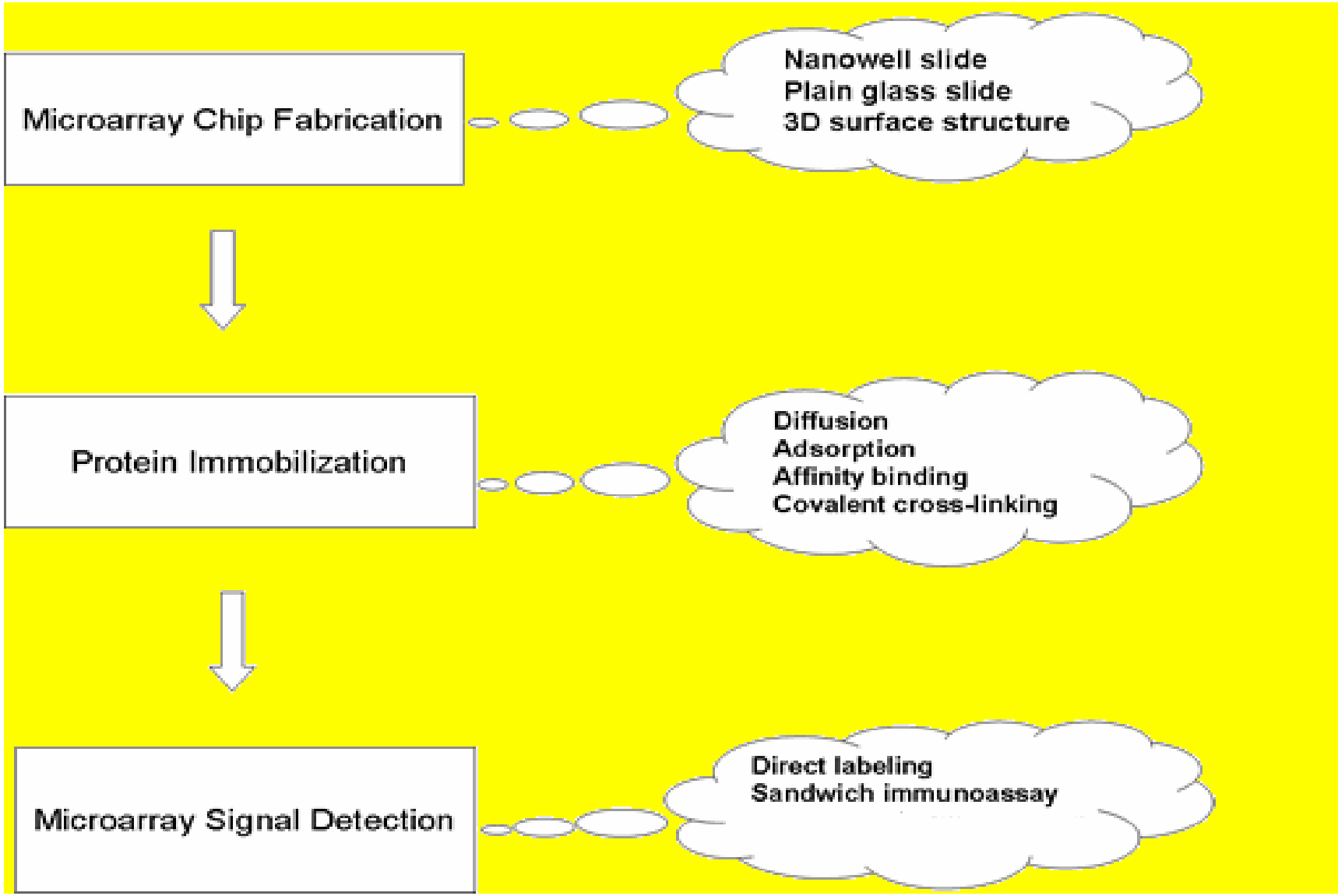
ATP

Adenosin triphosphate

ADP

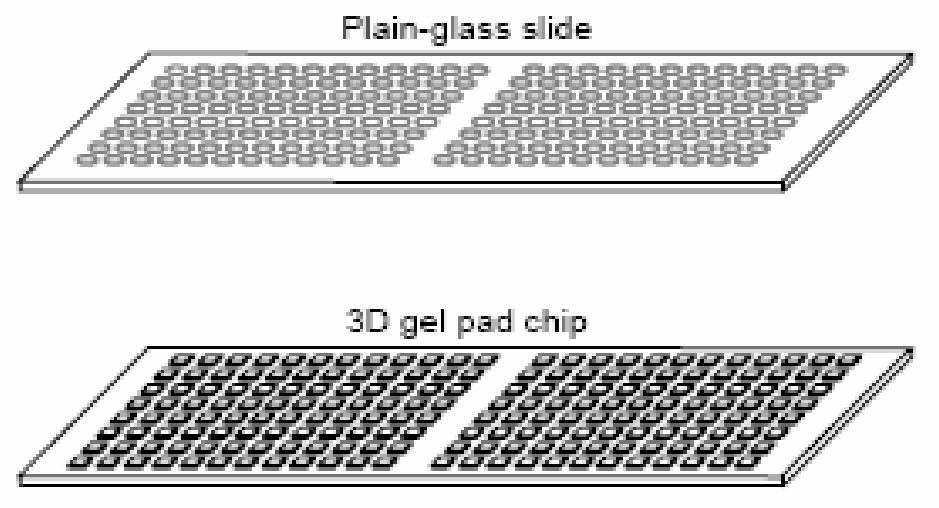
Adenosin diphosphate

Manufacturing of Microarrays

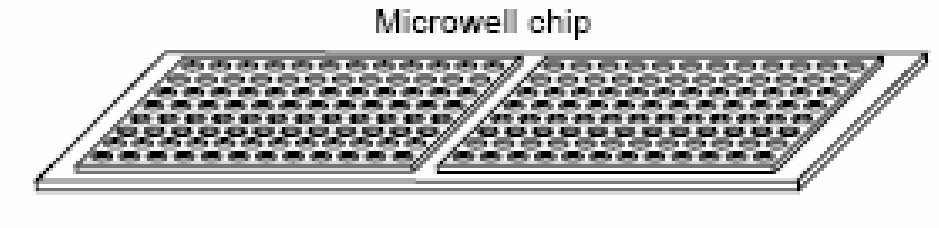


Microarray Chip Fabrication

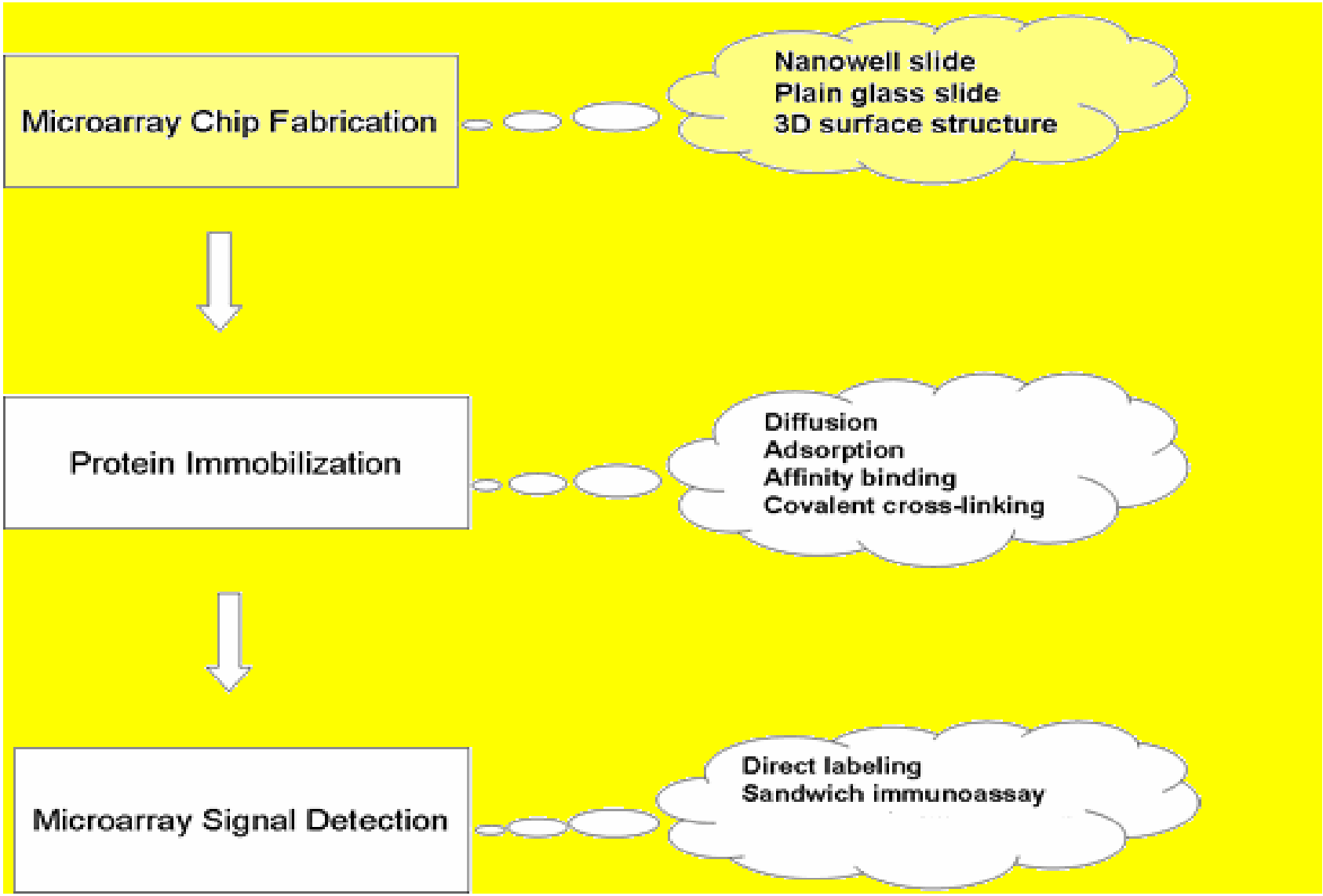
- Acrylamide and agarose – capture antibodies and proteins
- Plain Glass Slides
- 3D Gel Pad chip
  - Water environment
  - Reduce evaporation
  - Minimizes cross-contamination
  - Change of buffer
  - Recovery of trapped molecules



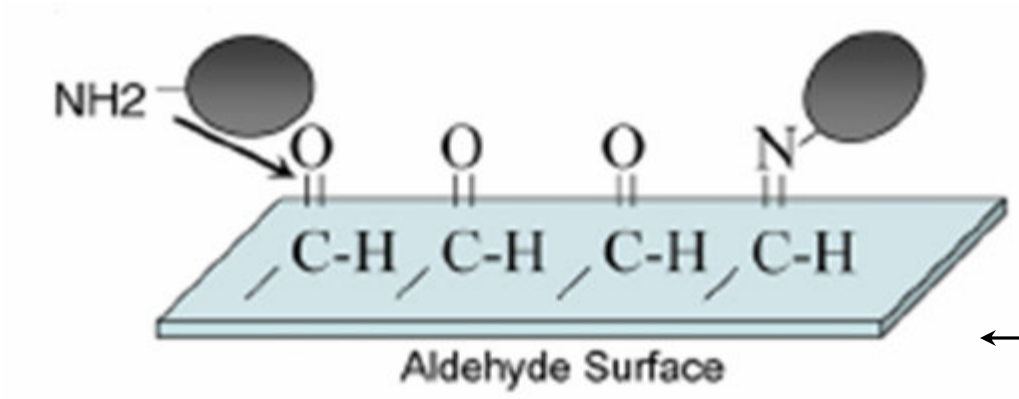
- Nanowells - polydimethylsiloxane surface (PDMS)
  - Multiplexing
  - Easy removal of captured molecules
  - Specialized equipments required



Manufacturing of Microarrays



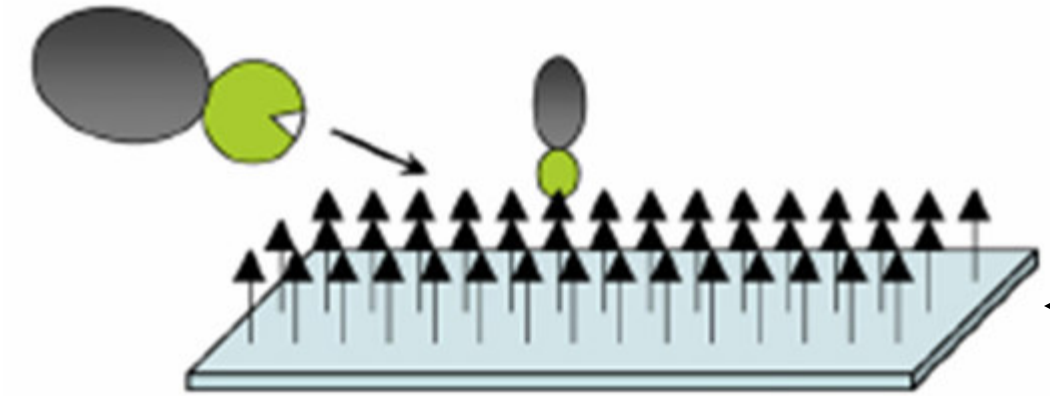
Random and Affinity Attachment



Random Attachment

Adsorption

Covalent cross linking

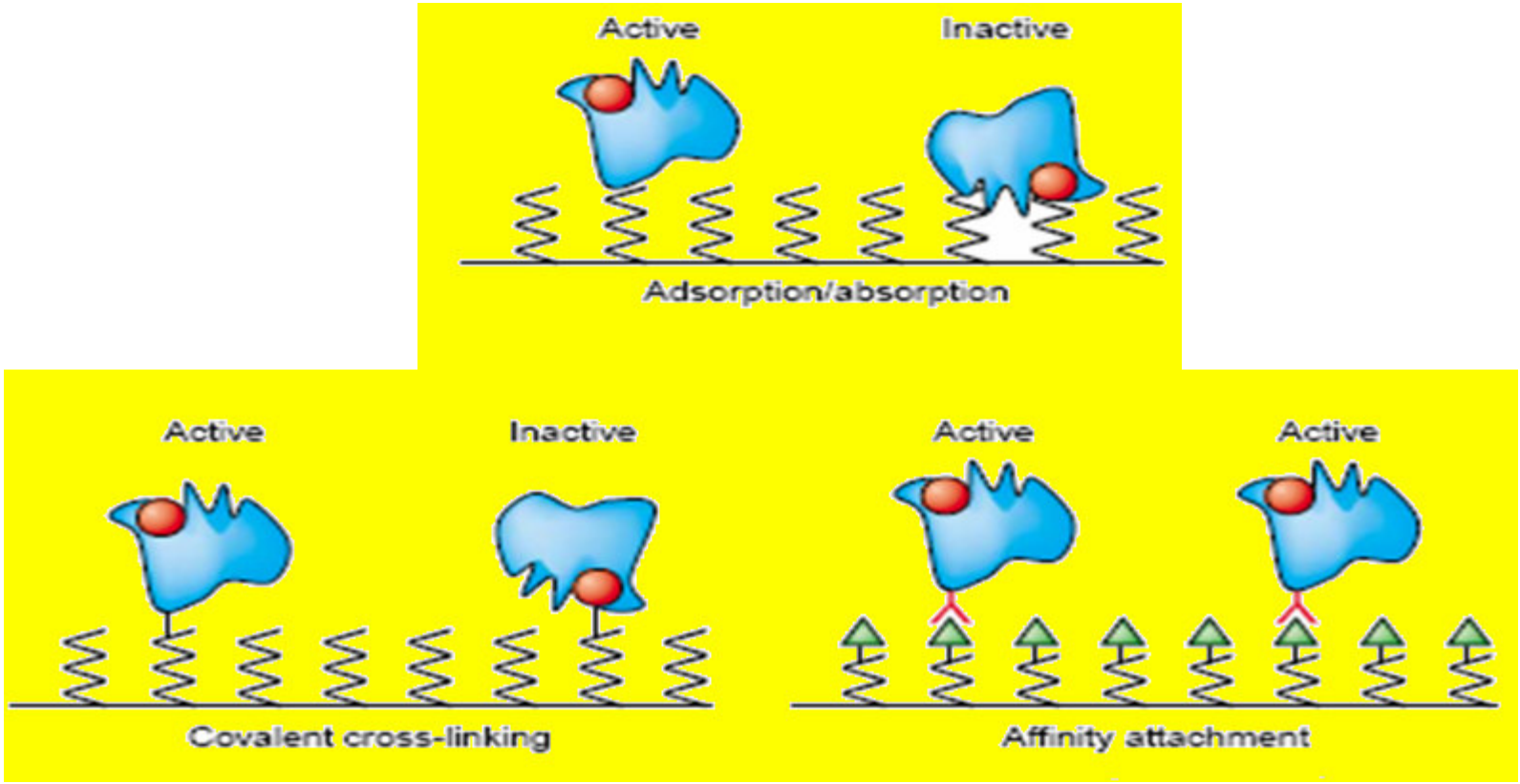


Uniform Attachment

Affinity binding

Zhu, H; Snyder, M: *Current Opinion in Chemical Biology*, **2003**, *7*, 55-63

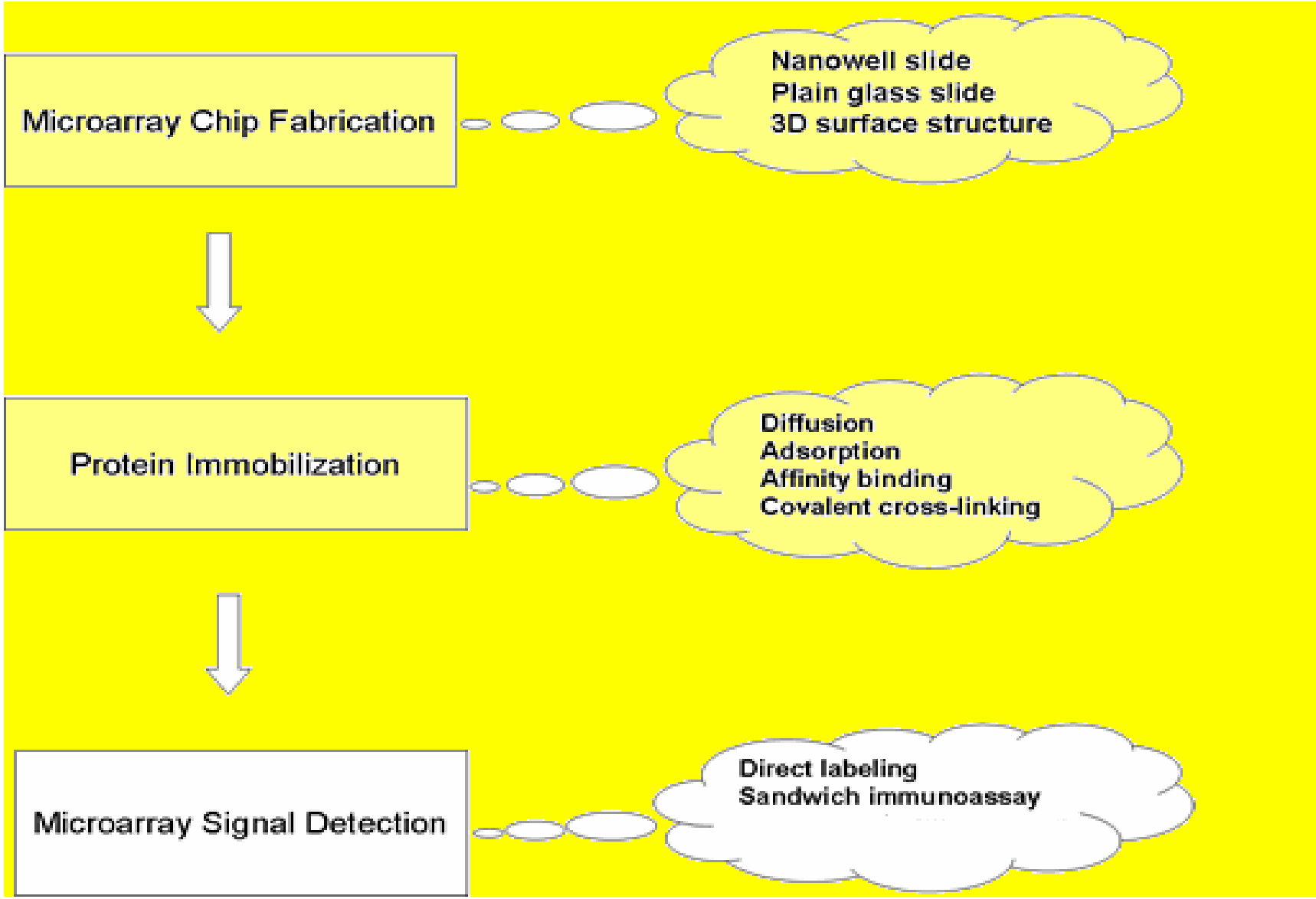
Protein Immobilization



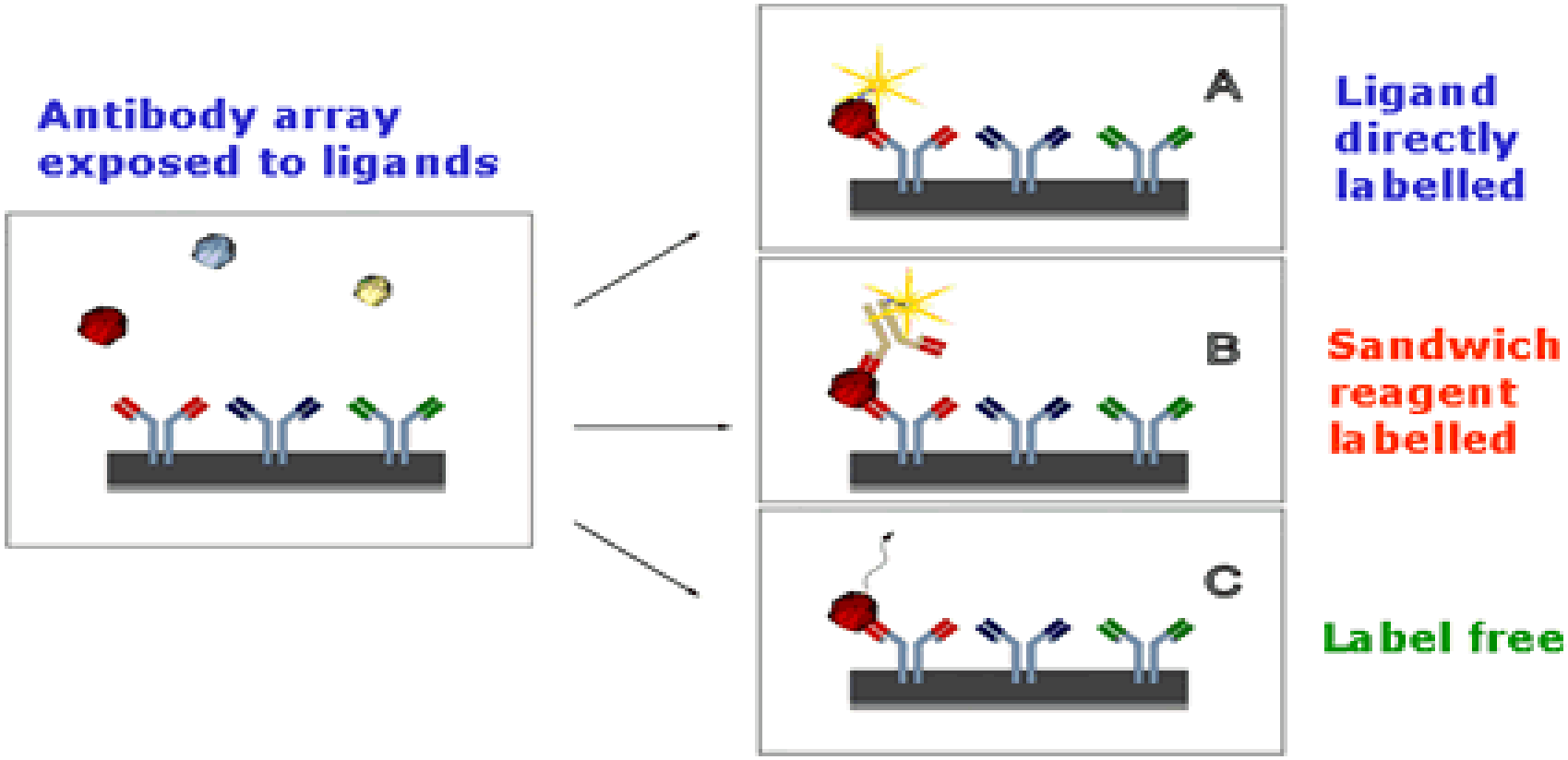
Red dots indicates active sites on proteins

Zhu, H; Snyder, M: *Current Opinion in Chemical Biology*, **2003**, *7*, 55-63

Manufacturing of Microarrays

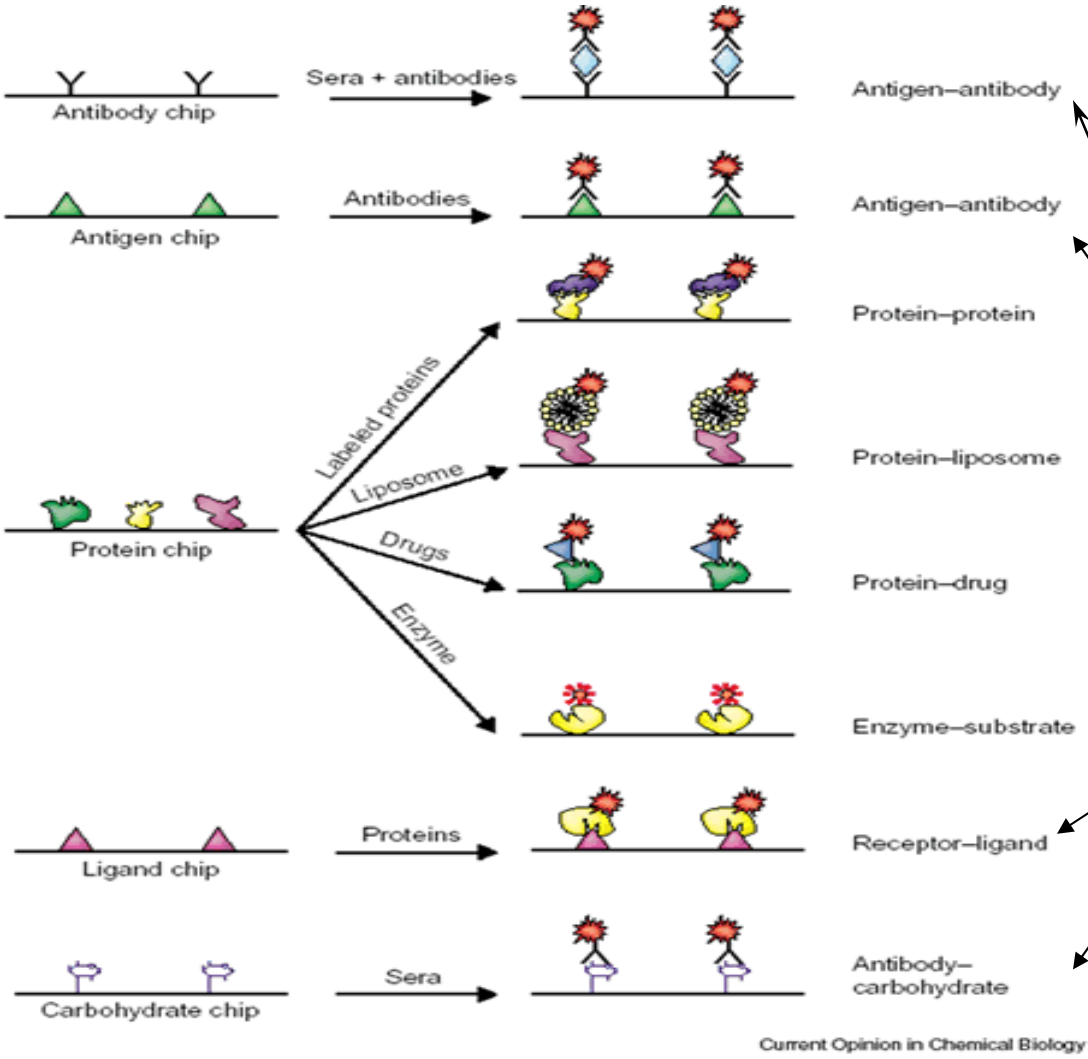


Microarray Signal Detection



www.functionalgenomics.org.uk

Types of Protein Microarrays



Analytical protein microarrays

❖high-density array of affinity reagents

Zhu, H; Snyder, M: *Current Opinion in Chemical Biology*, **2003**, *7*, 55-63

Applications of Protein Microarrays

- Diagnostics
- Proteomics

Improved Biomarkers for Prostate Cancer: A Definite Need

- AMACR (α-methylacyl-CoA racemase) is over-expressed in prostate cancer epithelium
- Expression is increased in premalignant lesions
- Higher dietary intake - of branched-chain fatty acids
- AMACR is detected in the urine of men with prostate cancer after prostatic biopsy

Improved Biomarkers for Prostate Cancer: A Definite Need

- PSA-detected tumors show false-negative rate
- Protein microarrays - AMACR immunoreactivity was statistically significantly higher in the sera from cancer case subjects than from control subjects
- Greater sensitivity and specificity than that of the PSA test
- AMACR is also found in non-prostate normal tissues and other malignant tissues

Carter,B; Isaacs, W: Journal of the National Cancer Institute, **2004**, *11*, 96.

Summary

- Applications
  - Diagnostics
  - Proteomics
- "High throughput"
- Multiplexing and Miniaturization
- Market Trend
- Biomarker discovery to patients

References

- Price, C: *Clinical Chemistry,* **2001**, *8*, 47.
- Sinskey, A; Finkelstein, S; Cooper, S: *PharmaGenomics,* **2002**, 20-24.
- MacBeath, G; Schreiber, S: *Science,* **2000**, 289, 1760-1763.
- Angenent, P; Hoheisel, J; Wanker, E; Kersten, B: *Proteomics* **2005**, *2(4)*, 499-510.
- Miller, J; Zhou, H; Kwekel, J; Cavallo, R; Burke, J; Butler, E; Teh, B; Haab, B: *Proteomics*, **2003**, *3*, 56-63.
- Zhu, H; Snyder, M: *Current Opinion in Chemical Biology*, **2003**, *7*, 55-63.
- *Carter,B; Isaacs, W: Journal of the National Cancer Institute*, **2004**, *11*, 96.

References

- Sreekumar, A; Nyati, M; Varambally, S; Barrette, T; Ghosh, D; Lawrence, S; Chinnaiyan, A: *Cancer Research*, **2001**, *61*, 7585-7593.
- Gavin MacBeath: *Nature Genetics,* **2002**, *32*, 526 – 532.
- http://en.wikipedia.org/wiki/Protein_microarray#Applications

Thank you

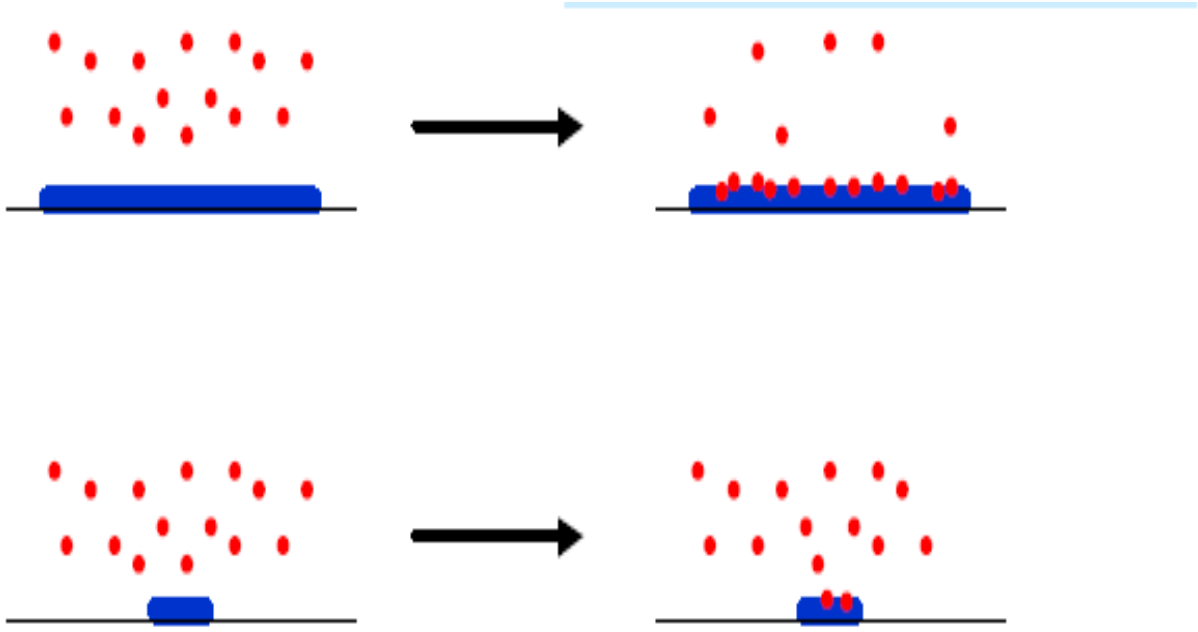
for your patient attentiveness

Questions Please!!!!!!!!

Difference between Proteins and DNA

Properties	DNA	Protein
Structure	Uniform, stable	Individual types, individual strability
functional state	Denatured, no loss of activity => can be stored dry	3D Structure important for activity, => keep hydrated all the time
Activity prediction	Based on primary nucleotide sequence	Hardly possible yet. Bioinformatics is working on prediction models based on sequence homologies, structure prediction etc.
Amplification	Established (PCR)	Not available
Interaction Affinity	high	Dependent on Protein: ver low to high
Interaction Specificity	high	Dependent on Protein: ver low to high
Interaction sites	1 by 1 interaction	Multiple active interaction sites

Microspot



Microarray Fabrication

Glass slides	Matrix slides	Nanowells
Compatible with standard microarrayer and detection equipment	Compatible with standard microarrayer and detection equipment	Compatible with standard microarrayer and detection equipment but requires alignment
High evaporation	Reduce evaporation	Reduce evaporation
Poor for multiple-based reactions	Solution-based reaction can be carried out, but requires longer washing times for material	Versatile for solution-based assays; multiple-component reactions
Inexpensive	Expensive; requires photo lithography to make matrix	Inexpensive
Possible cross-contamination	No cross-contamination	No cross-contamination

Protein Microarray Attachment Methods

Requirement	Strong Binding, Retention of function, High signal:noise ratio, low background
Diffusion e.g polyacrylamide gel (Hydrogel), agarose	No protein modification, High capacity, Low background, Random Orientation
Surface Adsorption e.g PVDF, Nitrocellulose, Poly-L-lysine	Protein Denaturation, High capacity, Variable background, Random Orientation
Covalent e.g Silane (various) Aldehyde	Protein modification, Strong attachment, Low background, Random or Uniform Orientation
Affinity Biotin/streptavidin	Protein modification, Strong attachment, Low background, Uniform Orientation

CHALLENGES TO THE DEVELOPMENT OF PROTEIN ARRAYS

- Monoclonal Antibodies (Capture molecules) - labour intensive and expensive
- Proteins- adsorb non-specifically
- Proteins are complex, and maintaining their native state and orientation during immobilization
- Automate large number of antibody / antigen binding studies directly on the slide surfaces

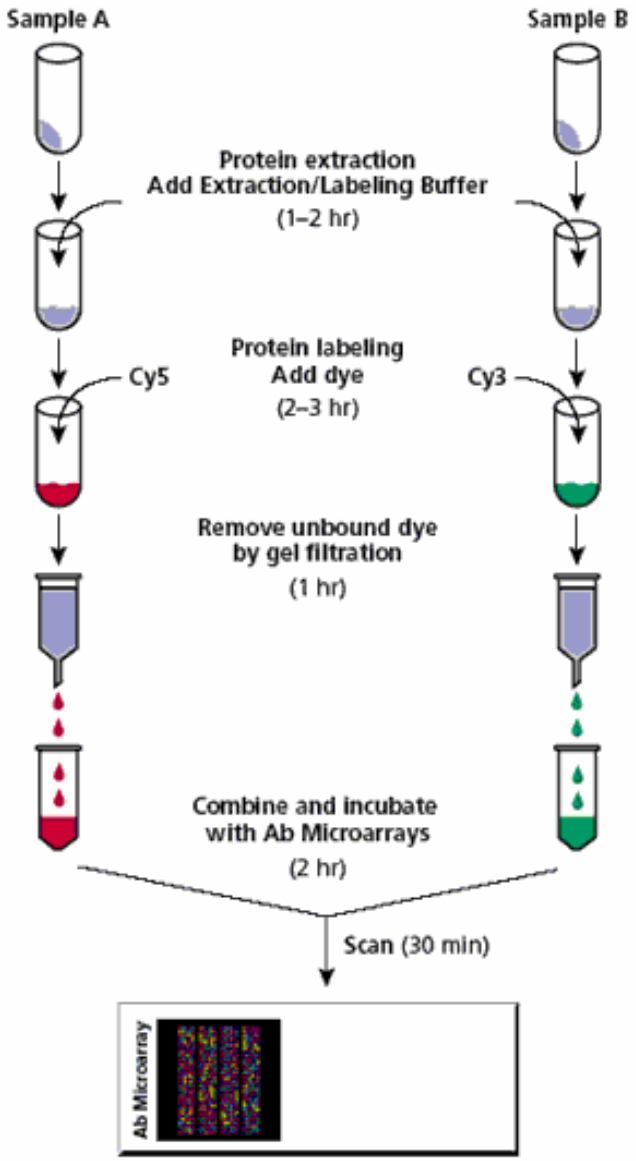
SOLUTIONS THROUGH AUTOMATION

- Antibody Production
  - grow cultures in microtitre plates

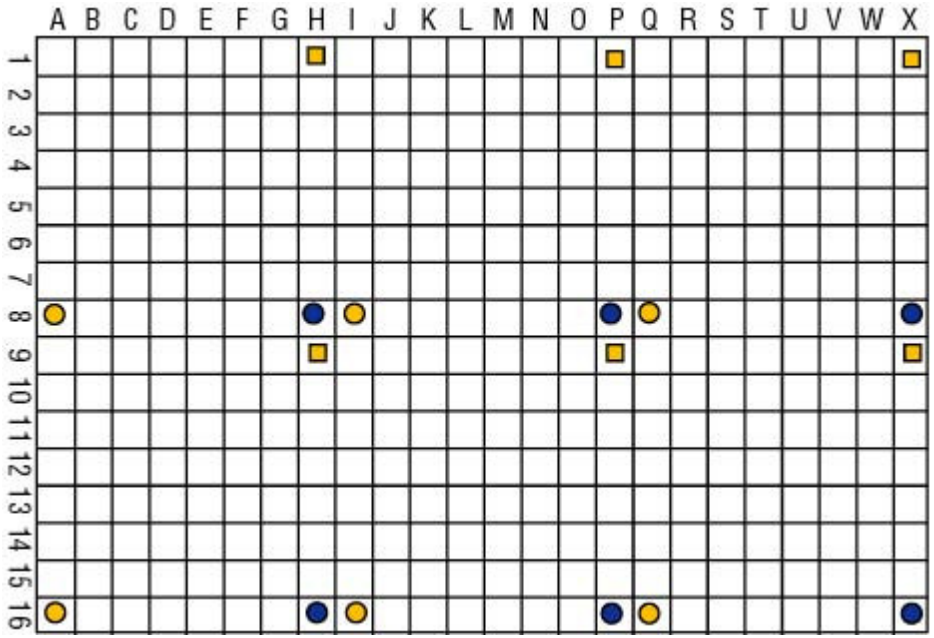
- Protein Adsorption
  - solve the issues of binding and orientation

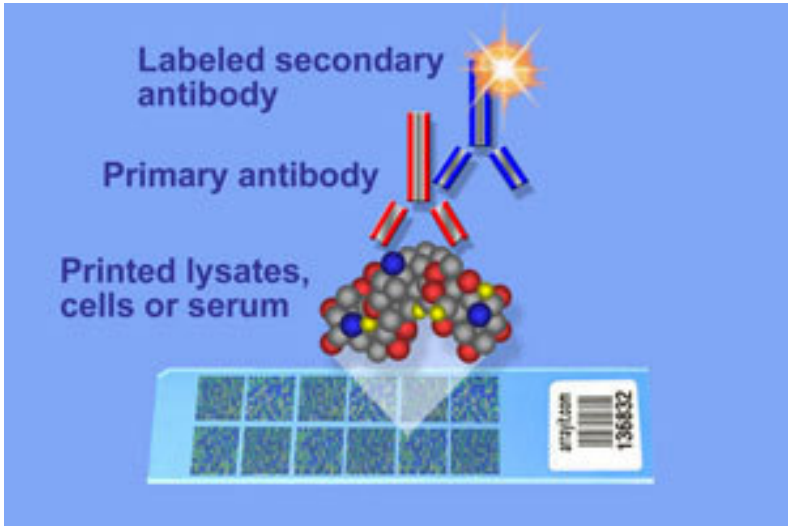
- Protein Microarray
  - choice of slide chemistry
  - spotting solution
  - combining with the environmental considerations

Antibody Array Labeling Scheme



Types of Protein Arrays - Cellular Lysate Protein Arrays





Pierce Protein Array Kits for lysis of cellular or bacterial samples

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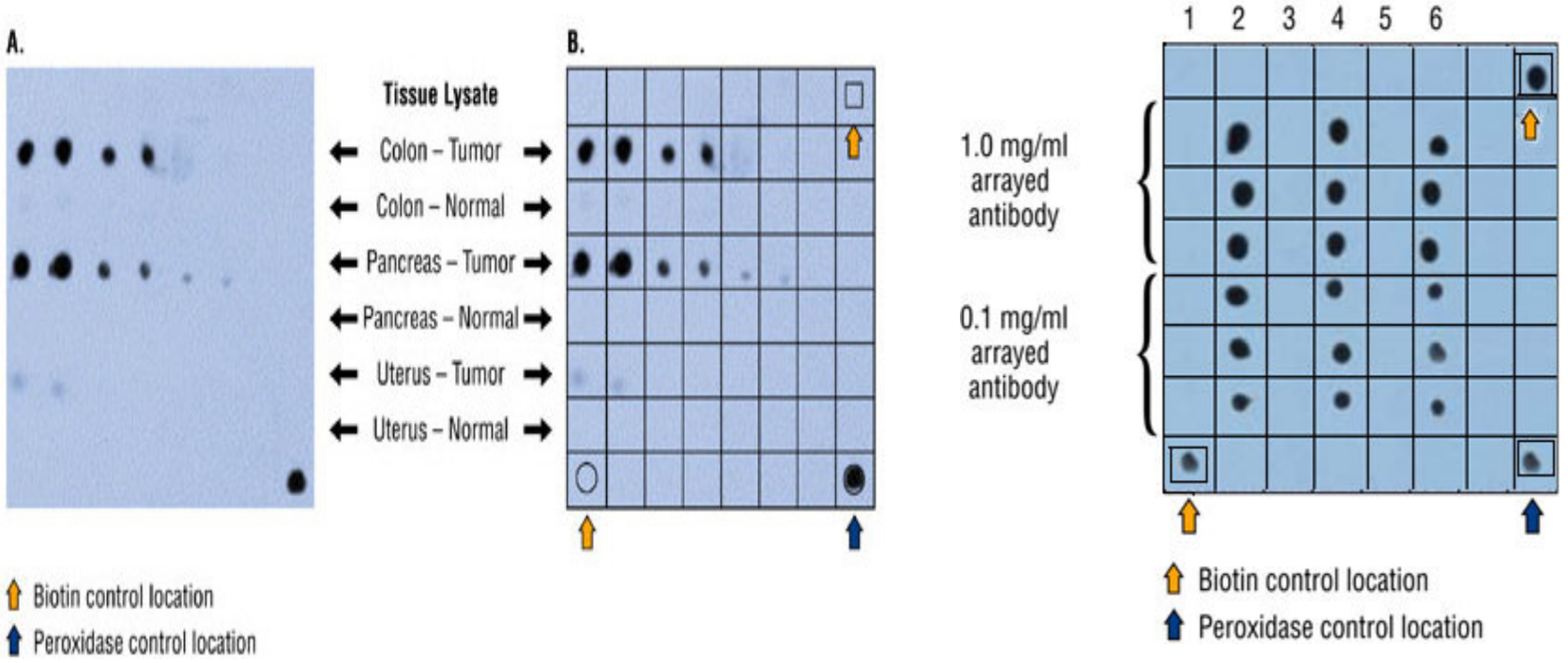

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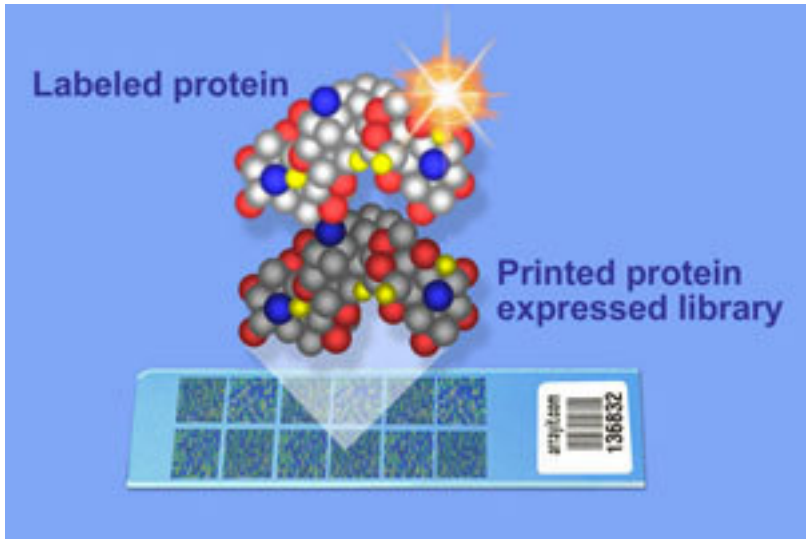
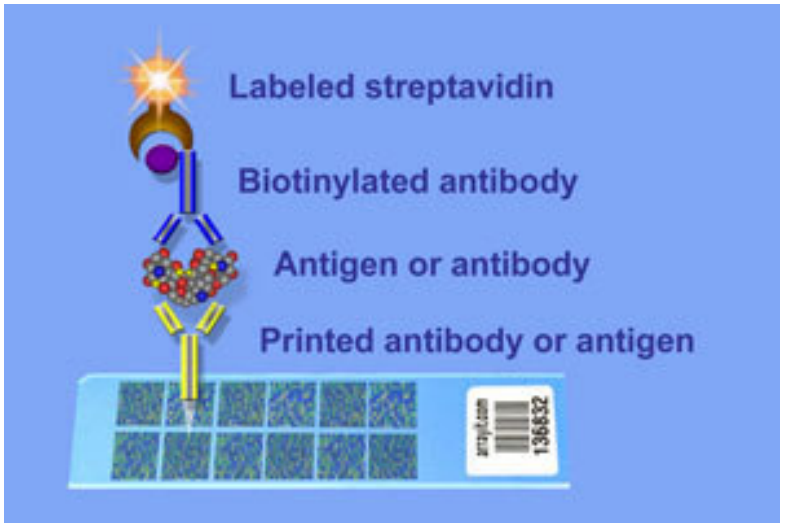
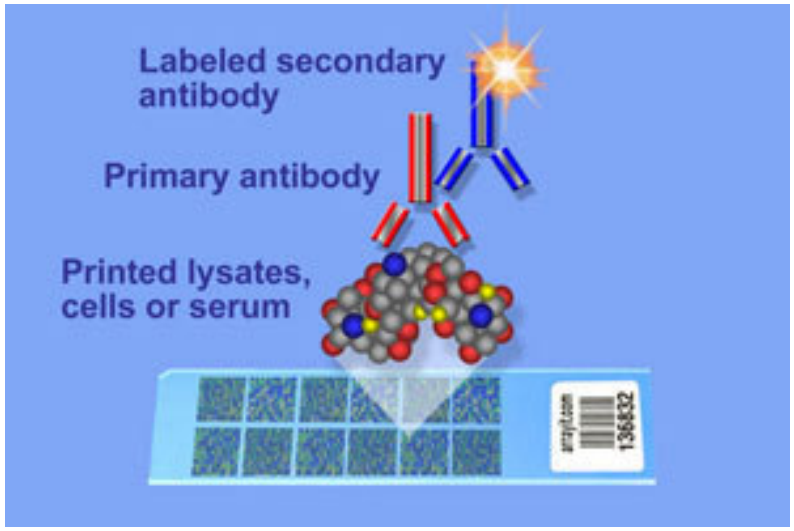
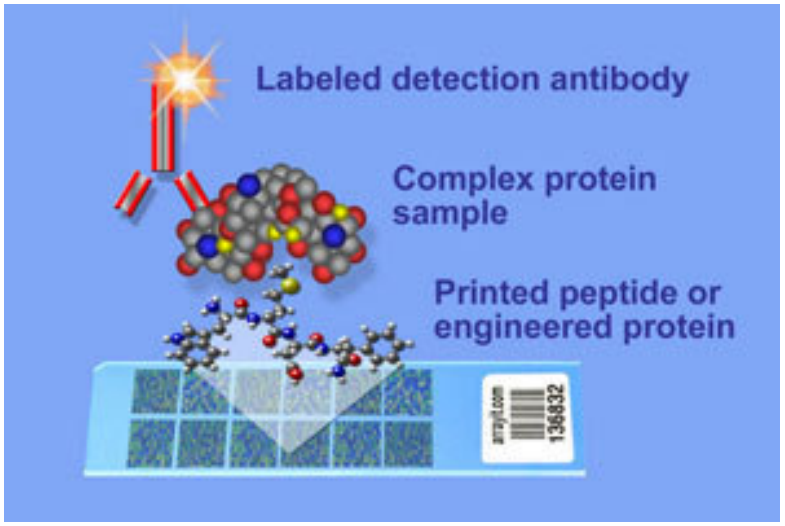
Types of protein arrays



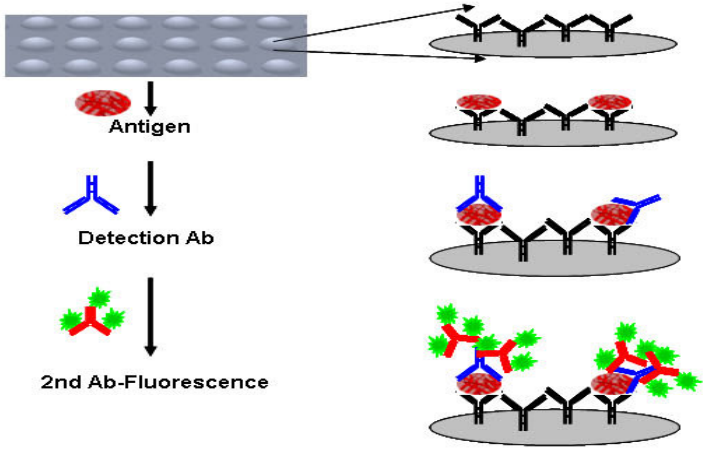




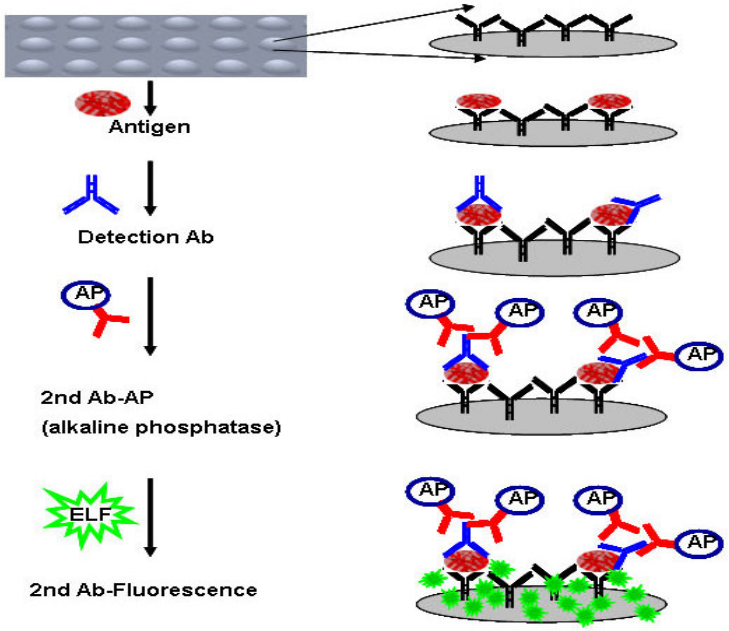


A few ways to create and detect antibody microarrays

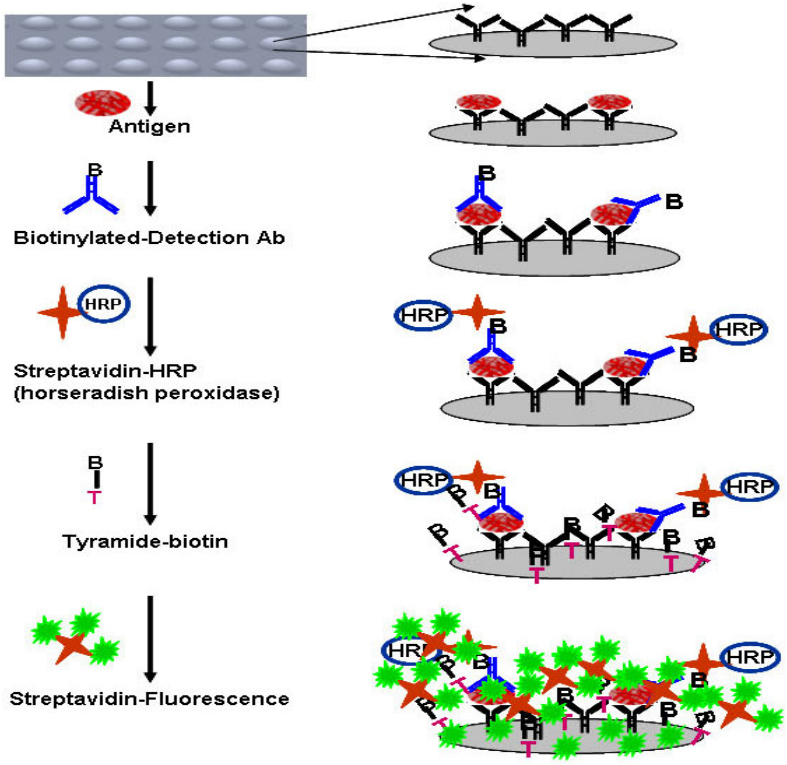
Fluorescence-linked Imminosorbent Assay



Enzyme-linked Immunosorbent Assay (ELISA)
Using insoluable fluorescence dye



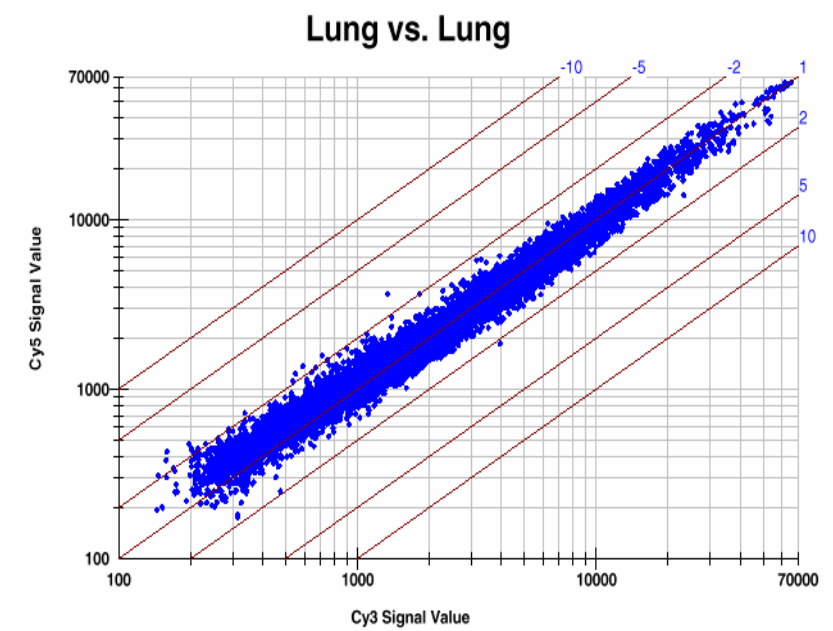
Enzyme-linked Immunosorbent Assay (ELISA)
Using Tyramide Signal Amplification (TSA)
technology



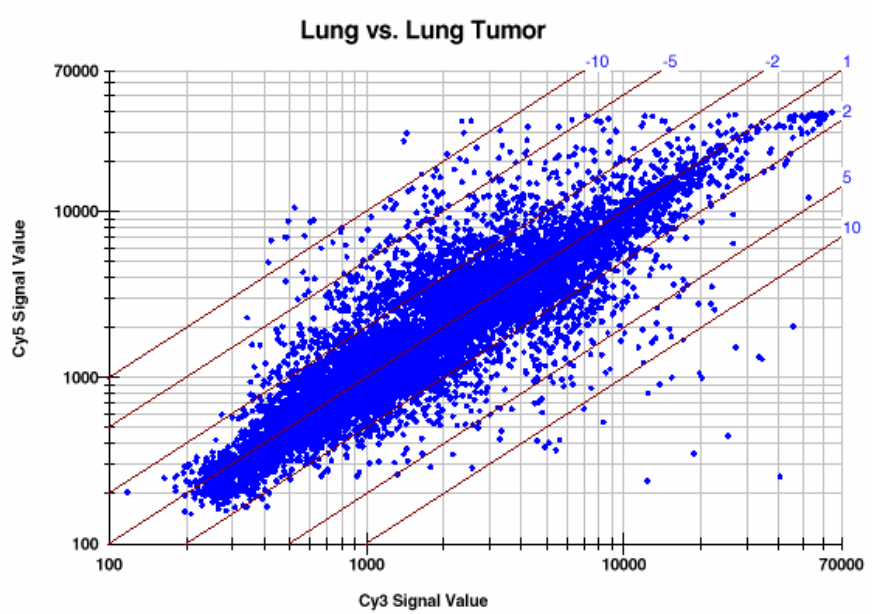
Component	Description	Size
PATH® Slide	Thin-Film Nitrocellulose Slide	10 PATH Slides
GenTel® Array	Print Buffer (5X)	10 mL
GenTel® Block	Block Buffer (5X)	30 mL
GenTel® Wash	Wash Buffer (10X)	2 x 250 mL
GenTel® Rinse	Rinse Buffer (10X)	250 mL
User Protocol	Sandwich Immunoassay Protocol	

Microarray Data Analysis

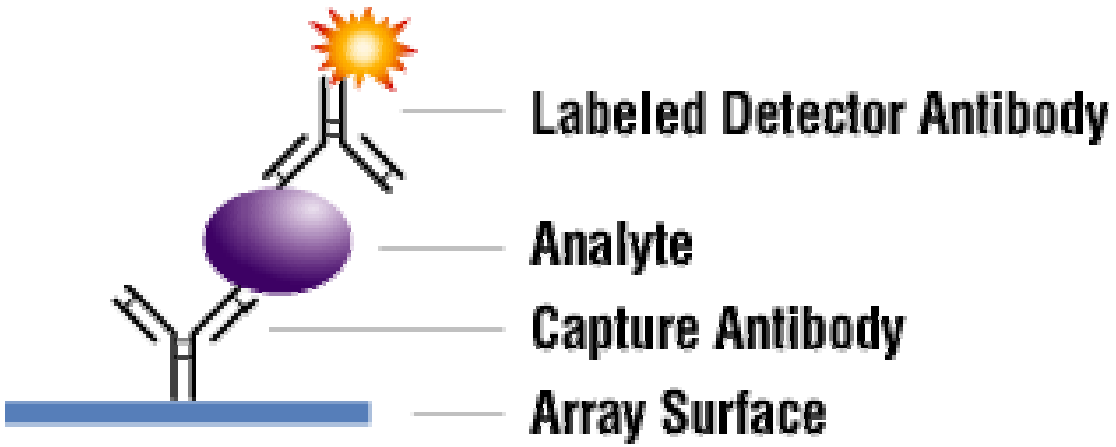
Normal vs Normal



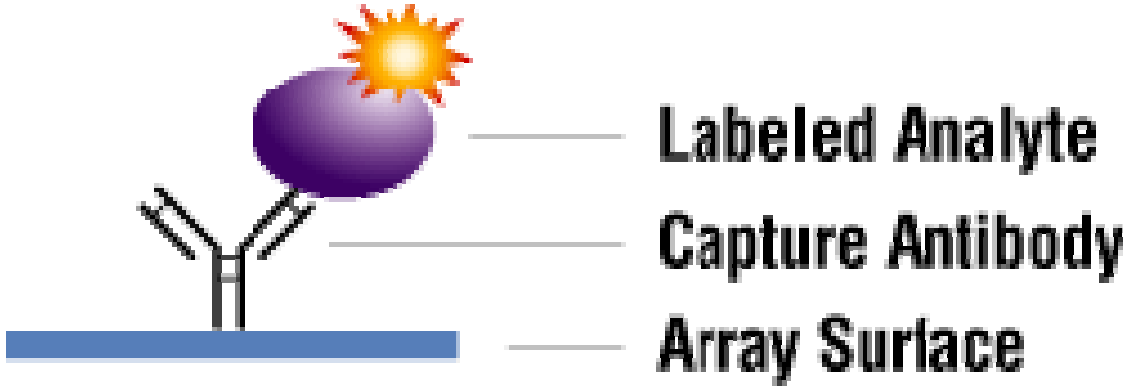
Normal vs Tumor



Microarray Signal Detection



Sandwich-style antibody-pair microarray assays



Single antibody/labeled sample microarray assays

Applications of Protein Microarrays

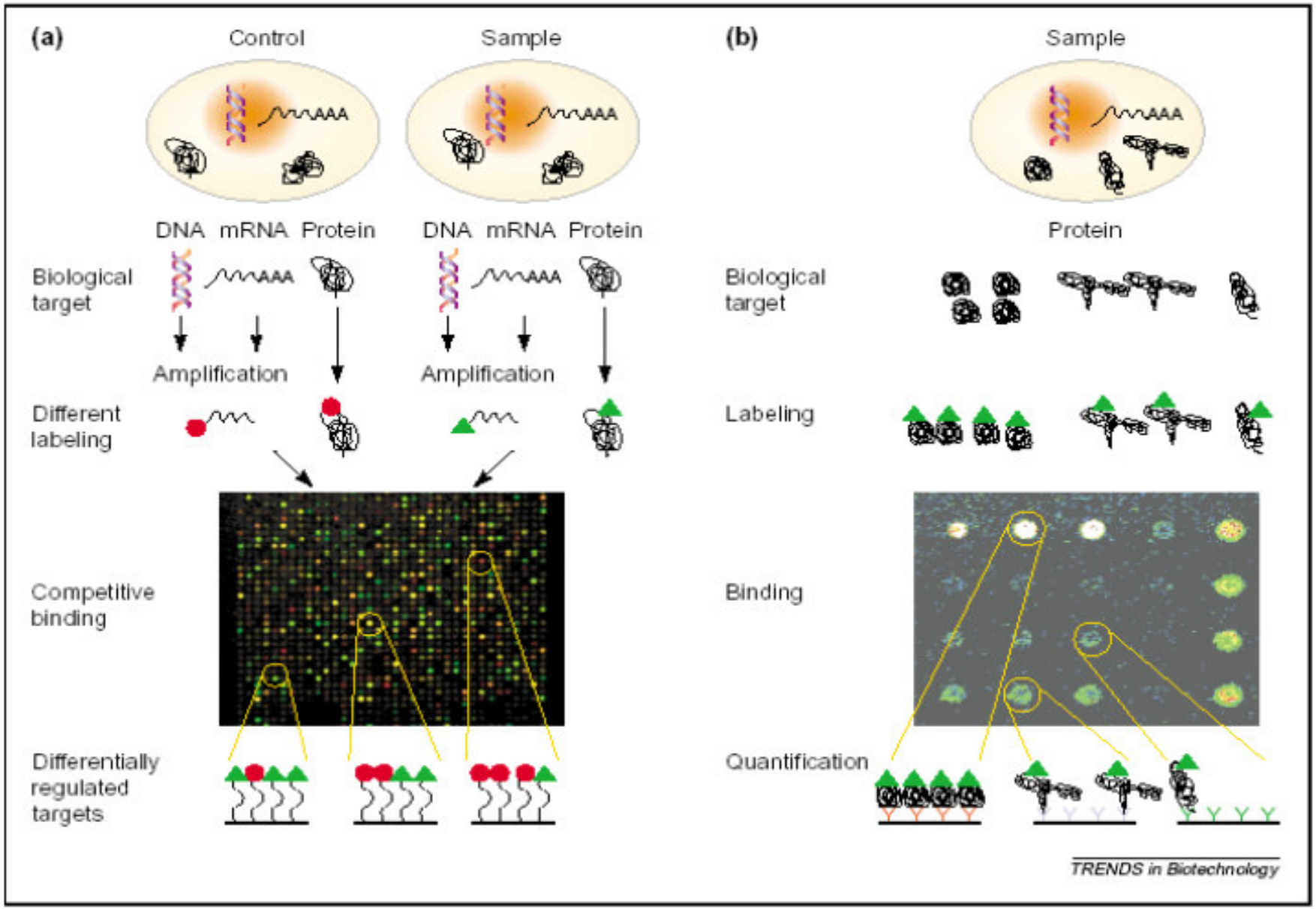
- Immunoassays for diagnostics
- Protein microarrays for proteomics

Immunoassays for Diagnostics

- High-throughput, enzyme-linked immunosorbent assays
  - multiplex detection of arrayed antigens
  - miniaturized and parallelized immunoassays

- Microarray sandwich immunoassays
  - modified polyacrylamide gel
  - eighteen different autoantigens,
  - less than one μl of a patient serum

Protein Microarrays for Proteomics



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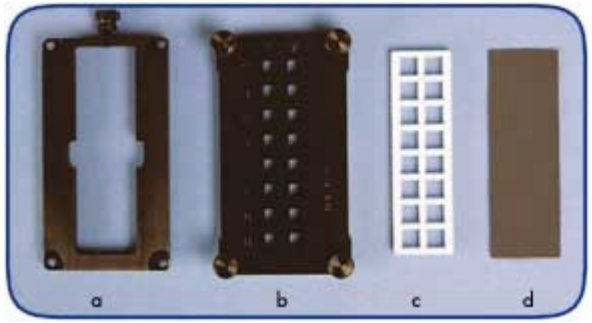

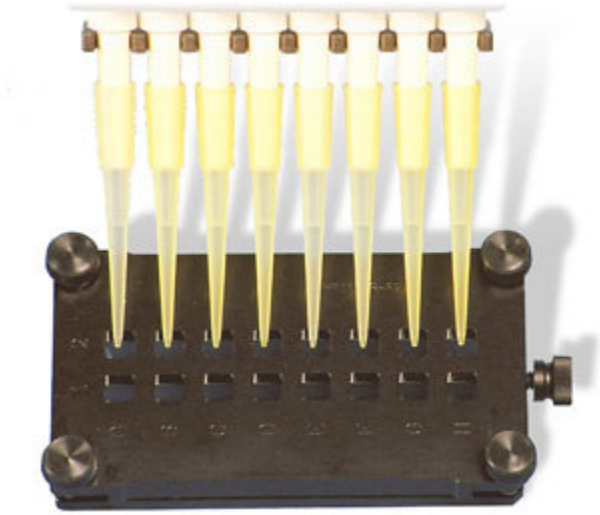

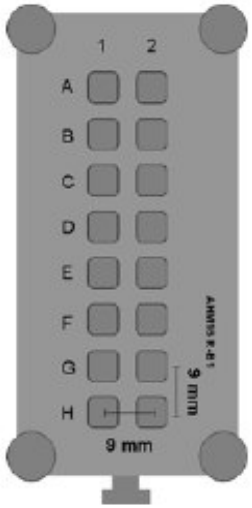

Bottom (a), top (b), gasket (c), PATH plus slides (d) sold separately

www.gentelbio.com

Functional Microarrays