Protein Microarray

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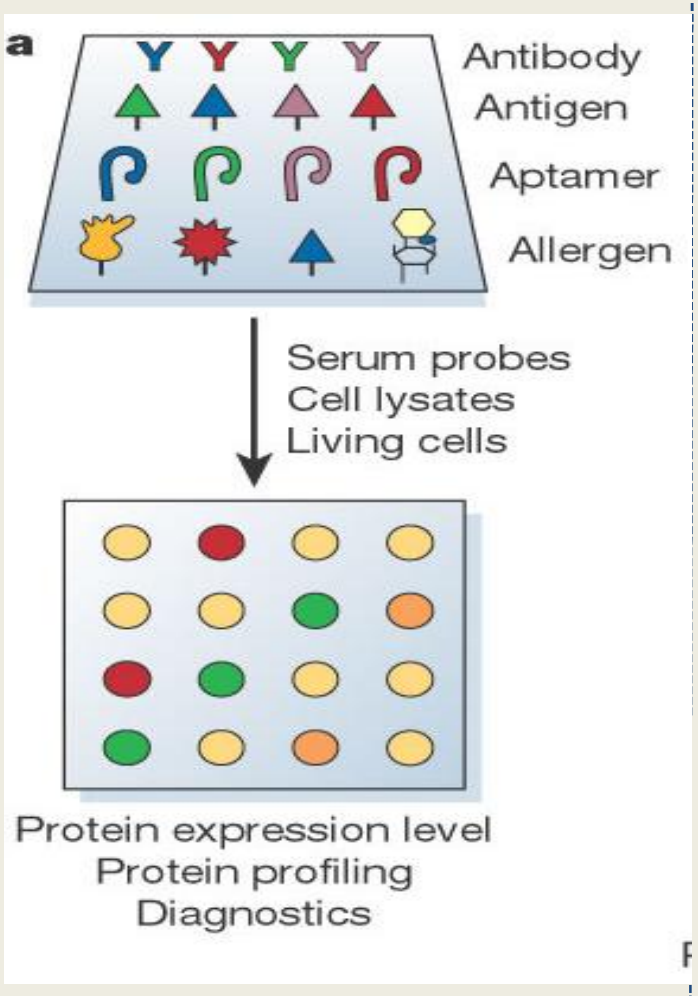
Protein microarray

- A protein microarray (or protein chip) is a high-throughput method used to track the interactions and activities of proteins, and to determine their function, and determining function on a large scale.
- The chip consists of a support surface such as a glass slide, nitrocellulose membrane, bead, or microtitre plate, to which an array of capture proteins is bound.
- Protein microarrays are rapid, automated, economical, and highly sensitive, consuming small quantities of samples and reagents.
- The high-throughput technology behind the protein microarray was relatively easy to develop since it is based on the previously-developed DNA microarray technology.

Types of array

- Analytical microarrays (also known as capture arrays)
- Functional protein microarrays (also known as target protein arrays)
- Reverse phase protein microarray (RPA)

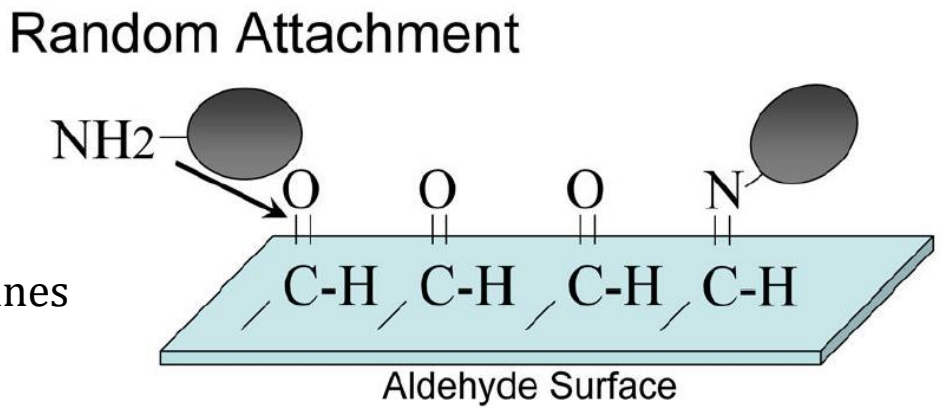
Analytical Microarrays

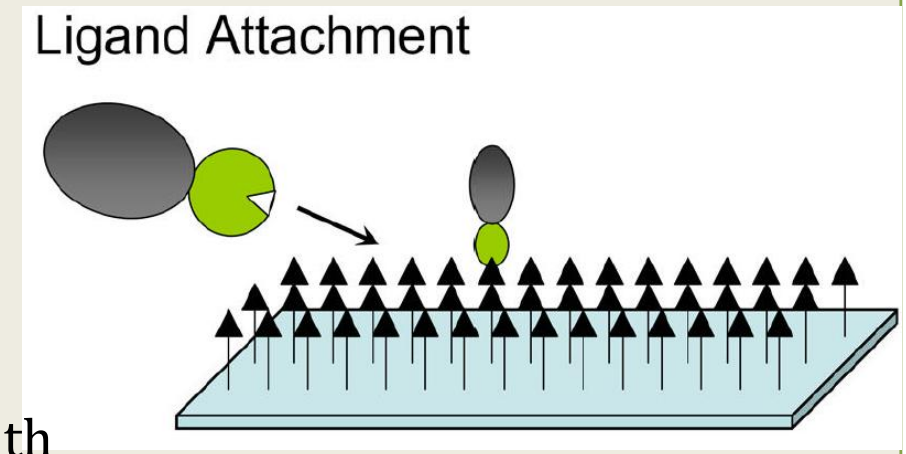


- Profiles Mixture of Proteins
  - Measure Binding Affinity
  - Specificity
  - Protein Expression Levels
- Most Common
- 3 main probe types
  - Antibodies
  - Affibodies
  - Aptamers

Plate Set Up

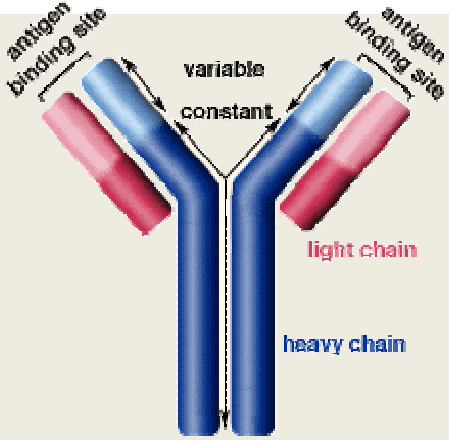
- Choose plate surface
  - Glass, Silicon
- Attachment Method
  - Random Attachment
    - Covalent attachment by amines
      - Aldehyde
      - Epoxy
    - Adsorption
      - Nitrocellulose
      - Poly-L-Lysine
      - Acrylamide Gel Pads
  - Uniform Attachment
    - Affinity Tag
      - Nickel Coat & His tag
      - Streptavidin & Biotin
- Spots vs. Wells
- Sample incubated on plate with probes





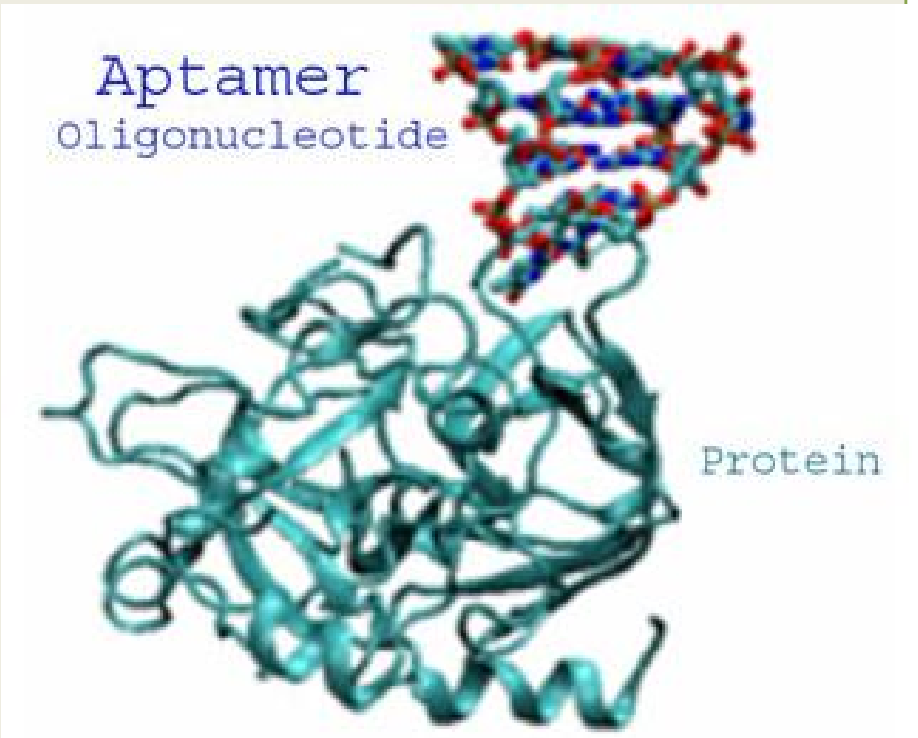
Analytical Microarray Plates

- Antibodies
  - 150 kDa
  - Standard
- Affibodies
  - non-immunoglobulin-based affinity reagents
  - Based on *Staphylococcus aureus* protein A
    - Alpha-helices
    - No Disulfide
    - 6 kDa
  - Randomization of 13 AA in binding domain



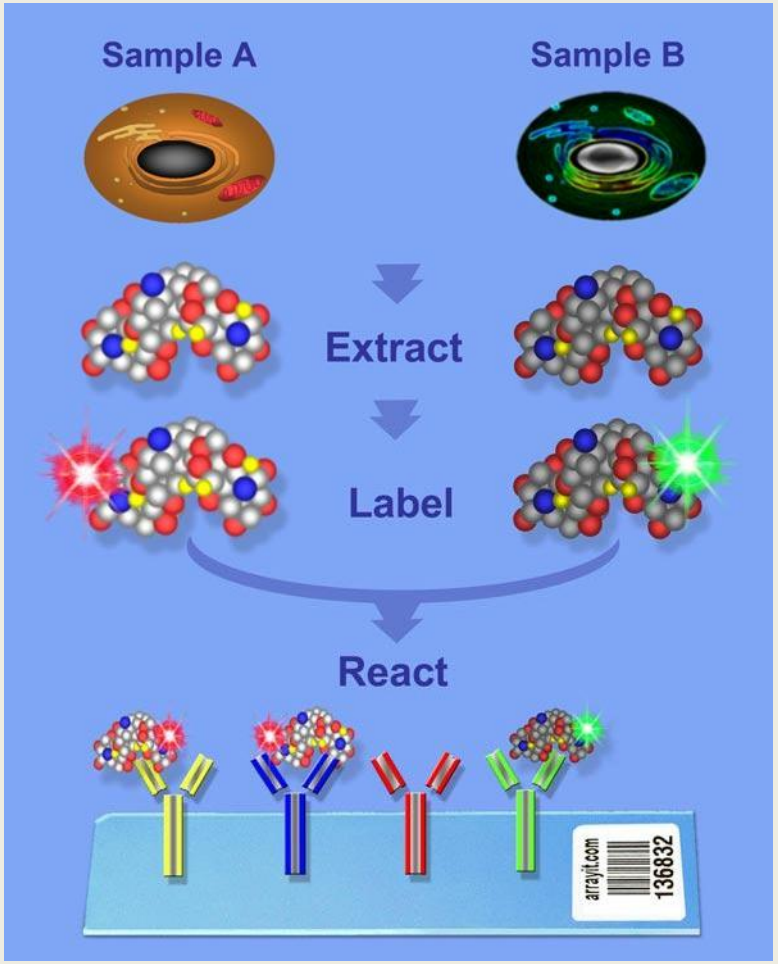
Plates continued

- Aptamers
  - Nucleic Acids
    - DNA, RNA, etc.
  - Peptides
    - Variable loop (10-20 AA)
    - Protein Scaffold
  - Bind Protein
    - Van der Waals Forces, H bonding, Electrostatic Interaction
  - Highly Specific
  - Engineered completely in test tube
    - In vitro selection

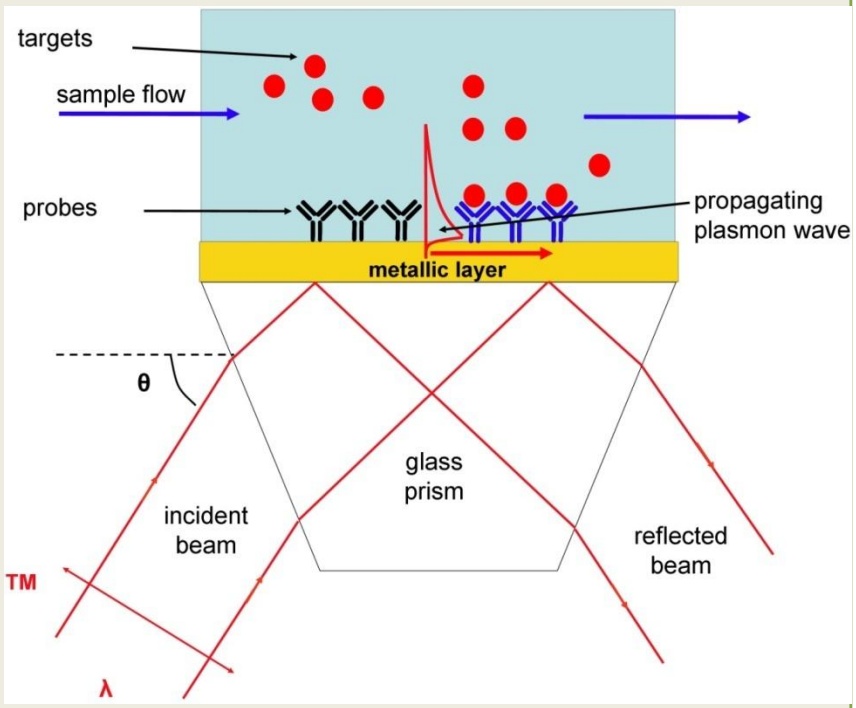


Sample Preparation

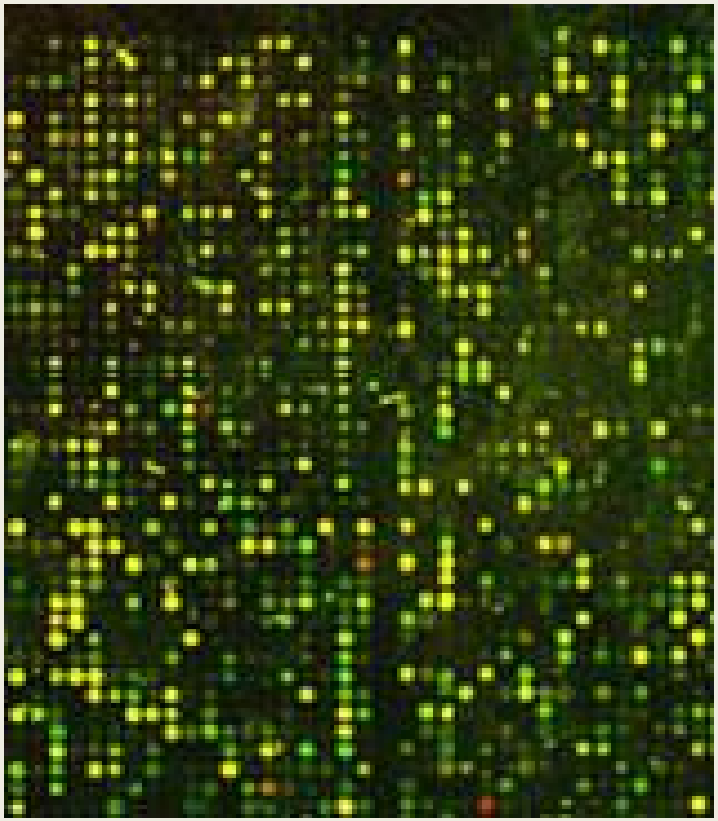
- Sample extracted from cells or tissues
- Bio-Rad assay
- Labeled
  - Fluorescent Dye
    - Cy3/Cy5 via Lysines
  - Photochemical
  - Radioisotope
  - May interfere



- Unlabeled
  - Antibody Sandwich
    - 2nd antibody with label incubated on top of sample
  - Surface Plasmon resonance
    - Measure electromagnetic waves
    - Angle changes in the order of 0.1° with 1 nm film adsorption
    - Needs special equipment
  - Don't affect protein structure



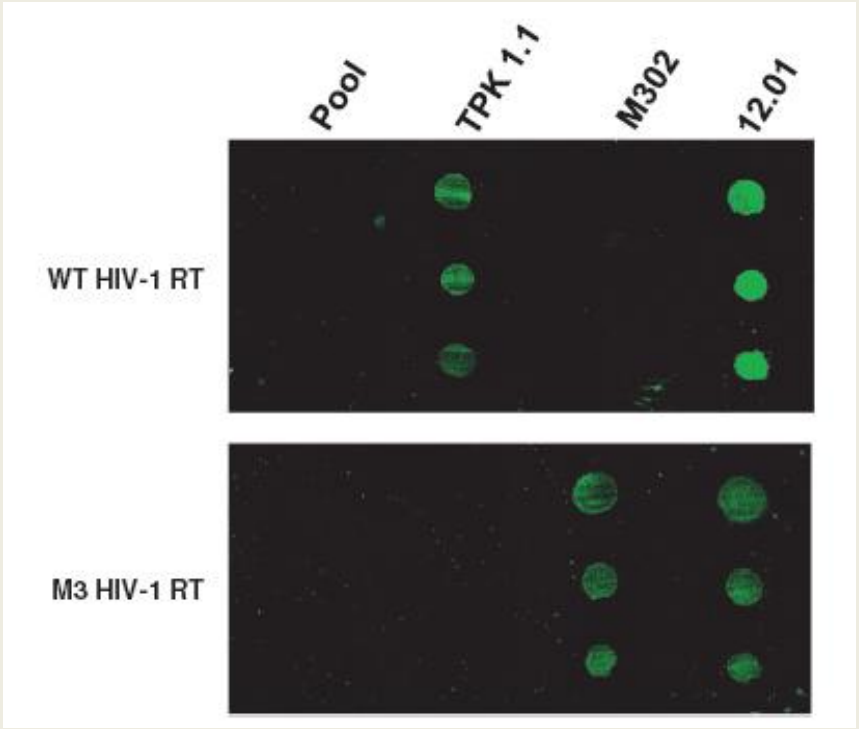
Detection & Quantification



- Scanner
  - Detects dye
  - Adjusts for background
- Reference spots
  - Labeled known concentrations
- Computational Analysis

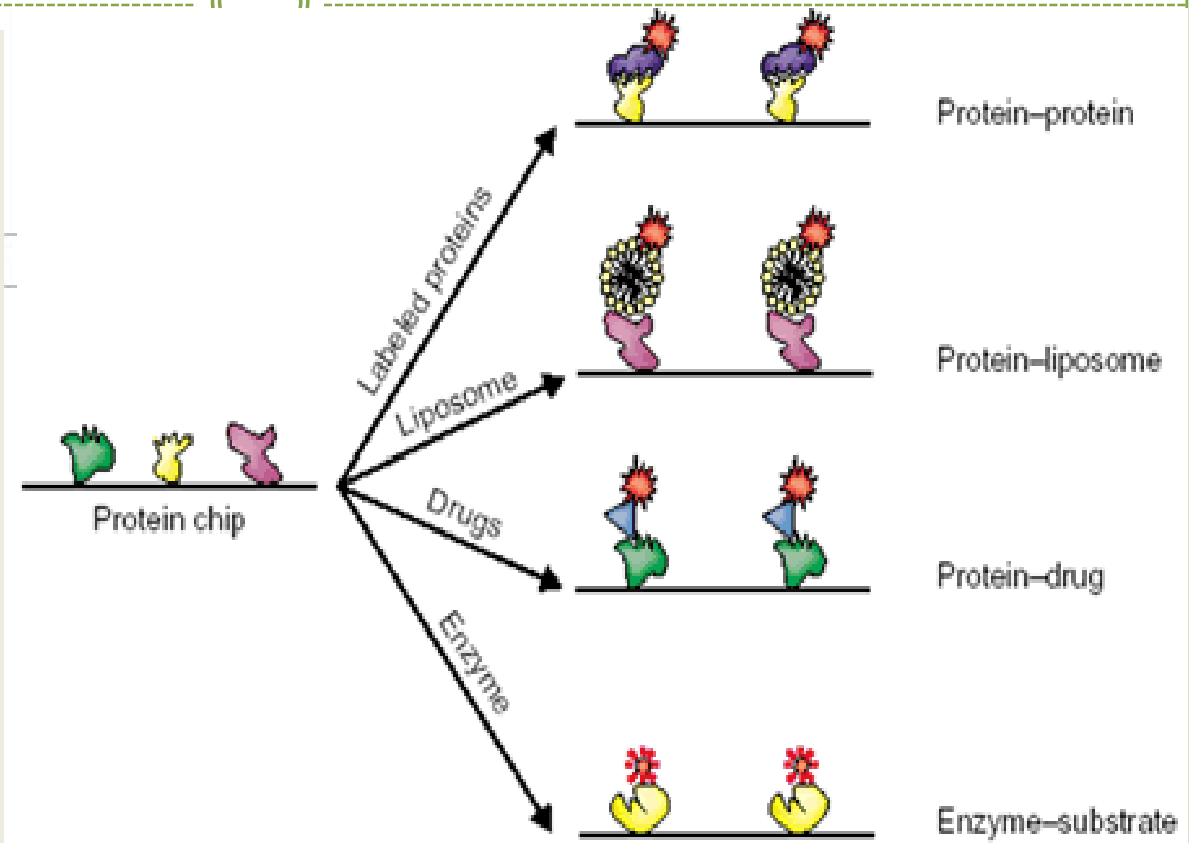
Analytical Microarray Example

- Individualized Medicine
  - Aptamers that recognize drug-resistant HIV-1 reverse transcriptase
    - In vitro selection
    - Attached with neutravidin & biotin
    - Incubated w/ M3 or WT HIV-1 RT
    - 1° Antibody
      - Rabbit Anti-HIV-1
    - 2°Antibody
      - Goat Anti-rabbit Cy-3 label
    - Read by microarray scanner



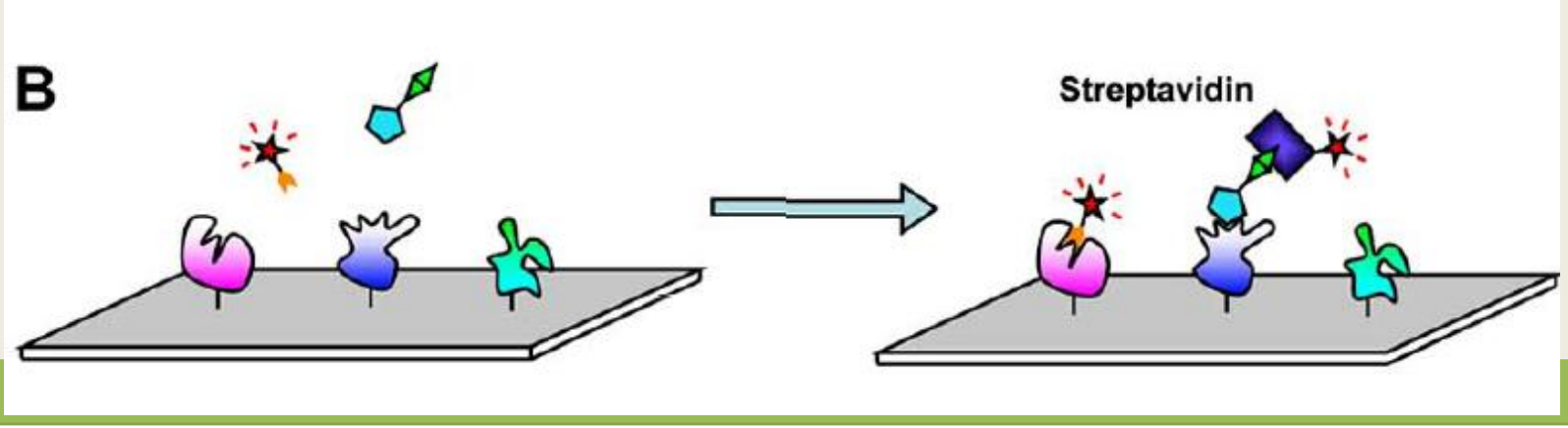
Functional Microarrays

- Plates
  - Full length proteins & protein domains
    - Functional
- Samples
  - Purified & Labeled
    - Nucleic Acids
    - Proteins
    - Lipids
    - Small Molecules



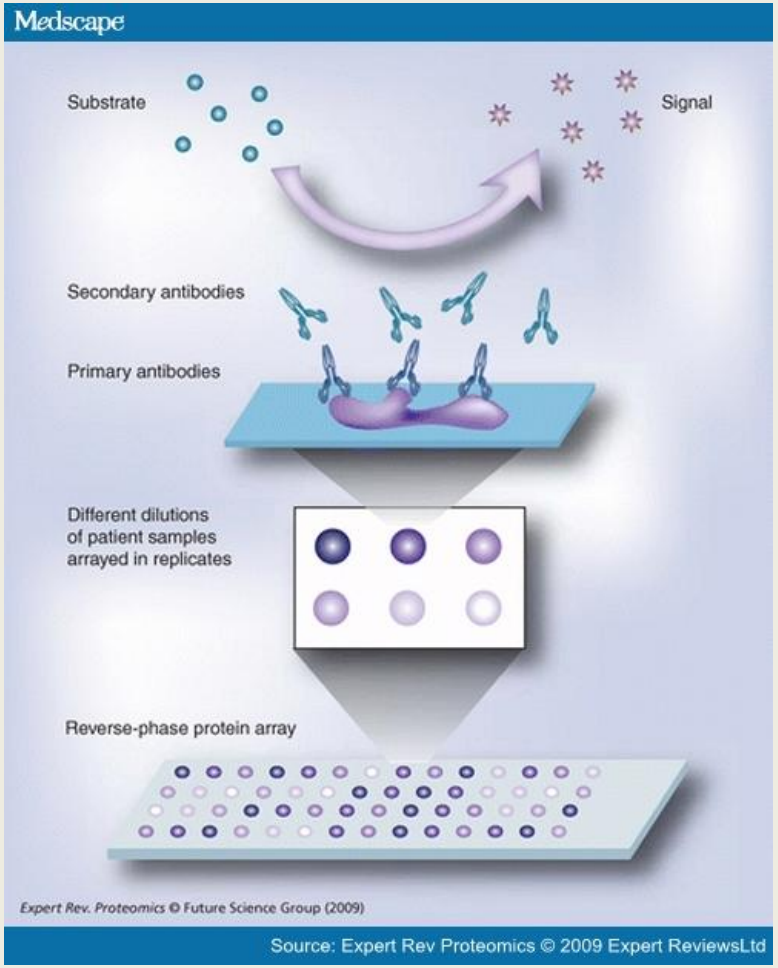
Functional Array Example

- Protein-Small Molecule Interaction
  - Plate has whole proteome
  - Monitor Specificity
  - Off-target effects



Reverse Phase Microarrays

- Plates
  - Cell Lysate
- Sample
  - Antibodies of interest
    - Primary
      - Attach to spots
  - Secondary
    - Attach to primary
    - Labeled
- Detect Altered Proteins
  - Post-translation modification problems
  - Disease



Reverse Phase Example

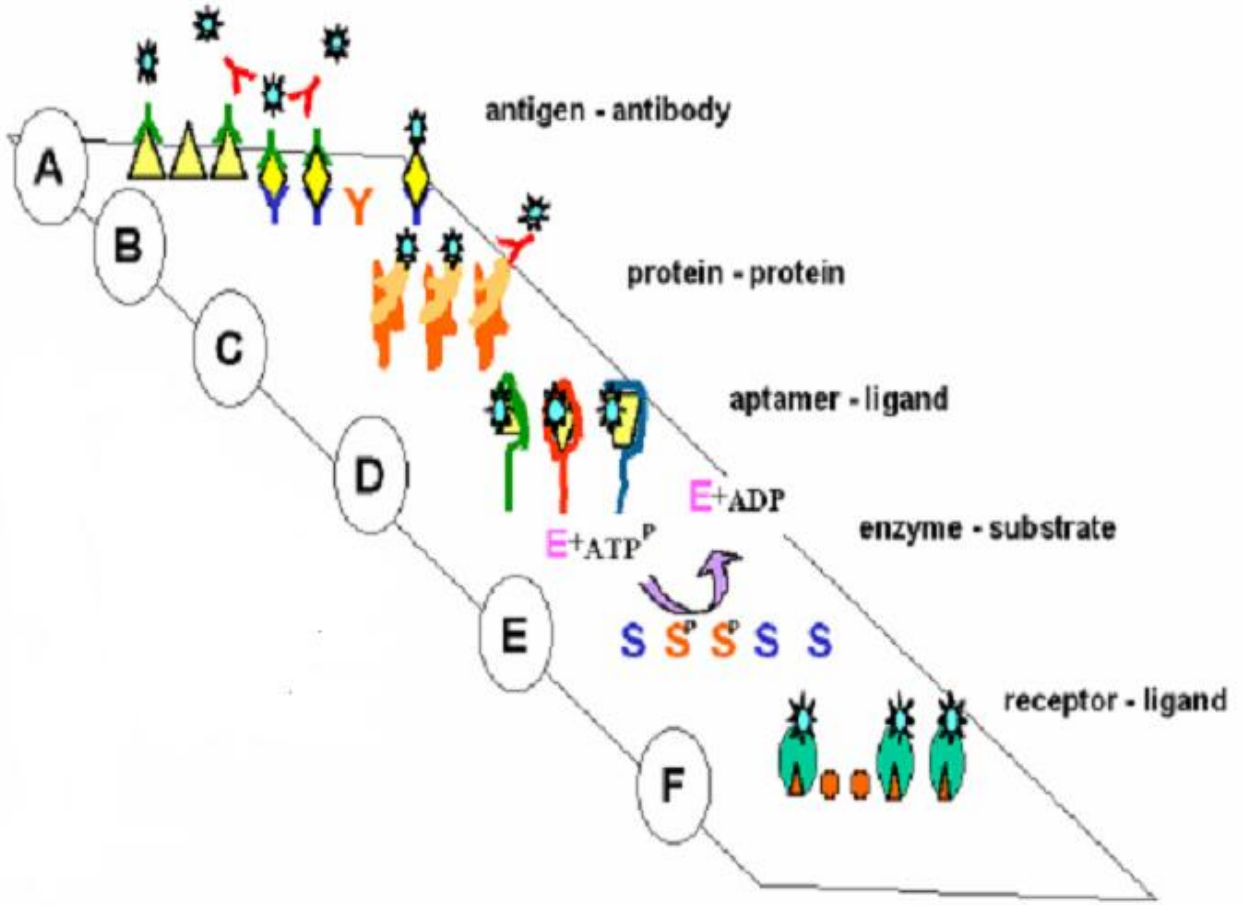
- Quantitative cell signaling analysis reveals down-regulation of MAPK pathway activation in colorectal cancer
  - Mitogen-activated protein kinases
    - Role in colonic cancer
  - Lysates of WT and Cancerous cells attached via nitrocellulose
  - Phospho-specific Rabbit antibodies
  - Staining
- Ras mutations in colorectal cancer
  - Thought to increase MAPK pathway
  - Cautioned against kinase inhibition therapy

Table 4. Relative protein expression of tumour vs. 'normal'

	TE relative to NE			TS relative to NS		
	Direction	*p*	% of NE	Direction	*p*	% of NS
p38	↑	0.0025	146.7	↑	0.081*	119.5
p-p38	↓	<.0001	51.0	↓	0.0023	66.9
p-ERK	↓	0.0003	26.6	↓	0.005	32.7
p-JNK	—	—	—	↓	0.0008	53.5

TE, tumour epithelium; TS, tumour stroma; NE, 'normal' (i.e. uninvolved) epithelium; NS, 'normal' (i.e. uninvolved) stroma.
Direction ↑ or ↓ -relative protein expression in tumour samples greater than or less than in 'normal' samples (respectively).
* Borderline significance only.

Capture Molecules in Microarray Assays



Label

Antibody

Secondary labeled Antibody

Radioactive (^{32}P) Phosphate

ATP

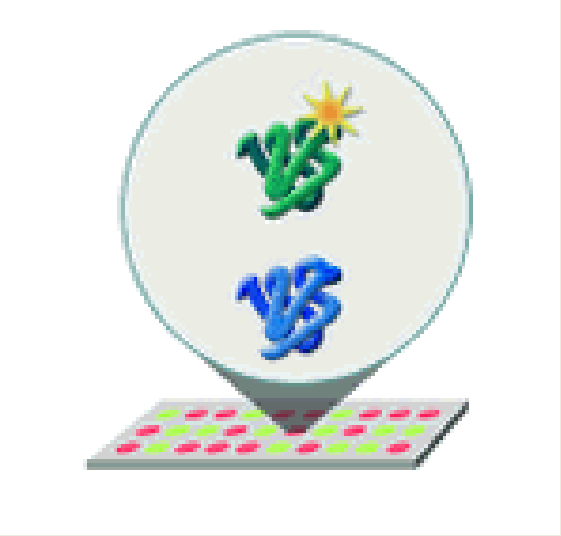
Adenosin triphosphate

ADP

Adenosin diphosphate

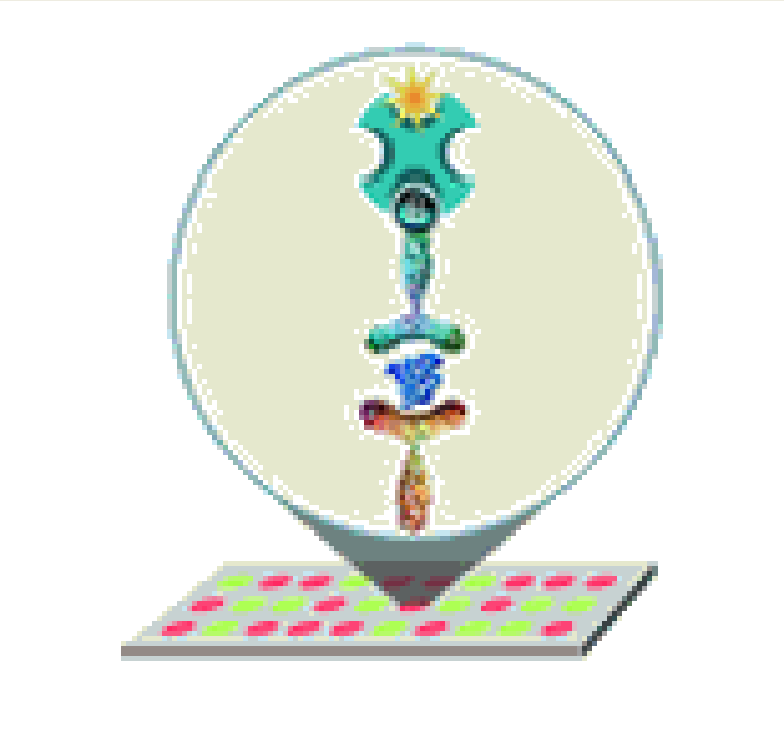
Proteomic Arrays

- Proteomic arrays are typically high-density arrays (> 1000 elements/array) that are used to identify novel proteins or protein-protein interactions. The library that is arrayed can come from many possible sources, including expression libraries, and can contain known, as well as unknown, elements. The sample to probe the array can come from virtually any source



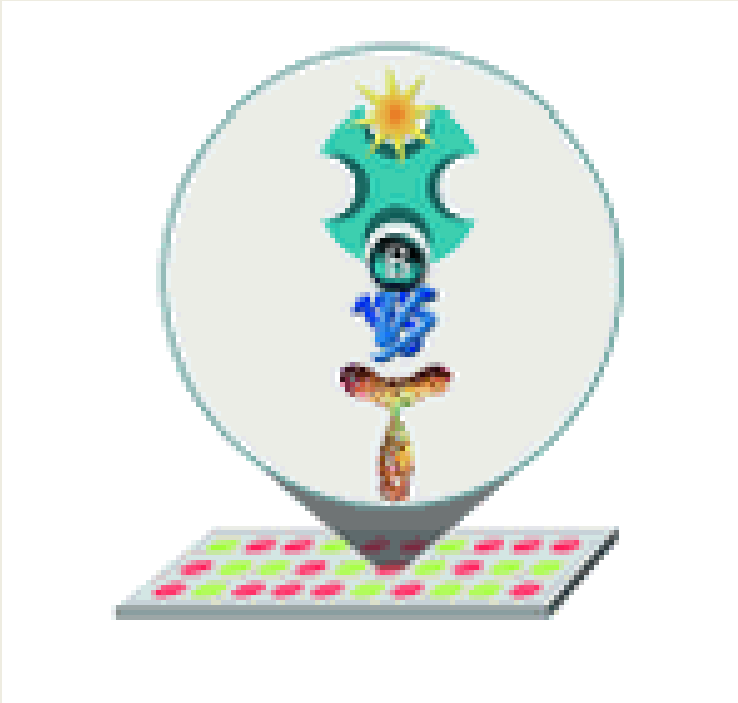
Microspot ELISA and Antibody Arrays

- Microspot ELISA and antibody arrays are used for quantitative profiling of protein expression in cell cultures or clinical specimens. Typically these arrays are low-density (9 to 100 elements/array).



Single-Capture Antibody Arrays

- Single-capture antibody arrays consist of multiple, known antibodies arrayed to a solid surface and used to profile the presence of specific antigens from a pooled sample, usually consisting of both a normal and disease-present sample.

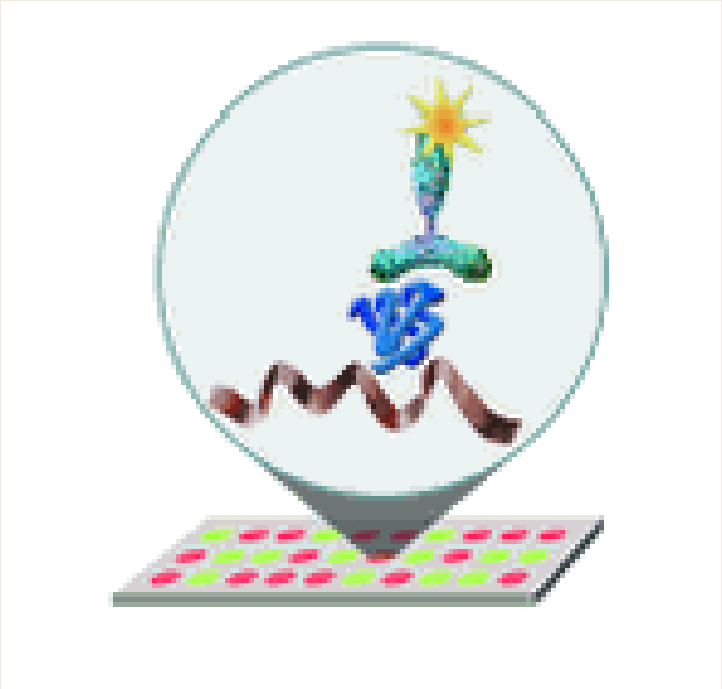


Antigen Arrays or Reverse Arrays

- One application of antigen arrays is to interrogate research or clinical samples for the presence of auto-antibodies.

Protein Binder Arrays

- Protein arrays can be used to identify novel protein binding motifs or protein-protein interactions.



Challenges

- Finding a surface and a method of attachment that allows the proteins to maintain their secondary or tertiary structure and thus their biological activity and their interactions with other molecules.
- Producing an array with a long shelf life so that the proteins on the chip do not denature over a short time.
- Identifying and isolating antibodies or other capture molecules against every protein in the human genome.
- Quantifying the levels of bound protein while assuring sensitivity and avoiding background noise.
- Extracting the detected protein from the chip in order to further analyze it.
- Reducing non-specific binding by the capture agents.
- The capacity of the chip must be sufficient to allow as complete a representation of the proteome to be visualized as possible; abundant proteins overwhelm the detection of less abundant proteins such as signaling molecules and receptors, which are generally of more therapeutic interest

Applications

- **Diagnostics -**

  The detection of antigens and antibodies in blood samples; the profiling of sera to discover new disease biomarkers; the monitoring of disease states and responses to therapy in personalized medicine; the monitoring of environment and food.
- **Proteomics -**

  Protein expression profiling i.e. which proteins are expressed in the lysate of a particular cell.
- **Protein functional analysis -**

  The identification of protein-protein interactions (e.g. identification of members of a protein complex), protein-phospholipid interactions, small molecule targets, enzymatic substrates (particularly the substrates of kinases) and receptor ligands.
- **Antibody characterization -**

  Characterizing cross-reactivity, specificity and mapping epitopes.
- **Treatment development -**

  The development of antigen-specific therapies for autoimmunity, cancer and allergies; the identification of small molecule targets that could potentially be used as new drugs.