

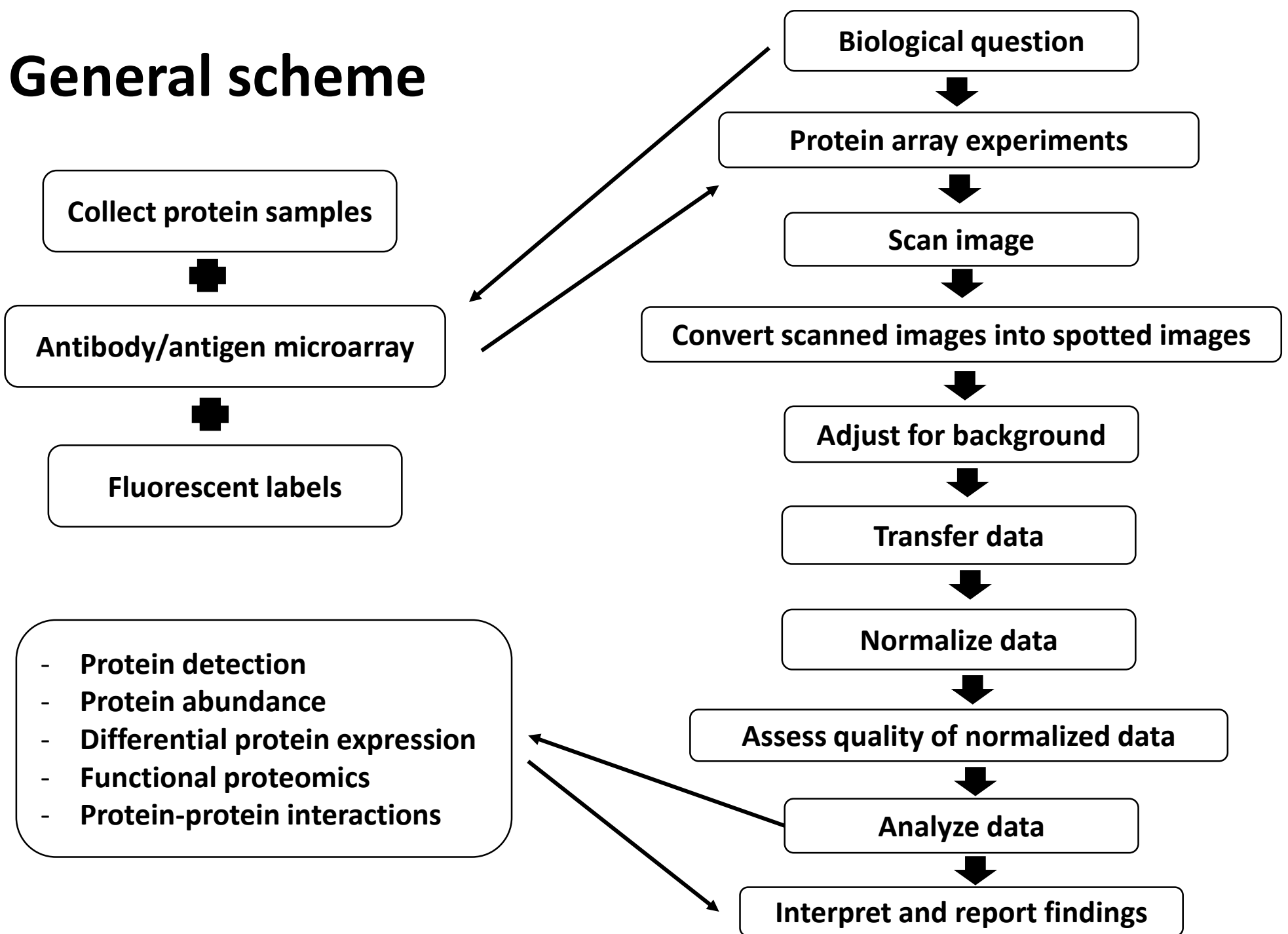
# Protein Array

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# What Are Protein Arrays?

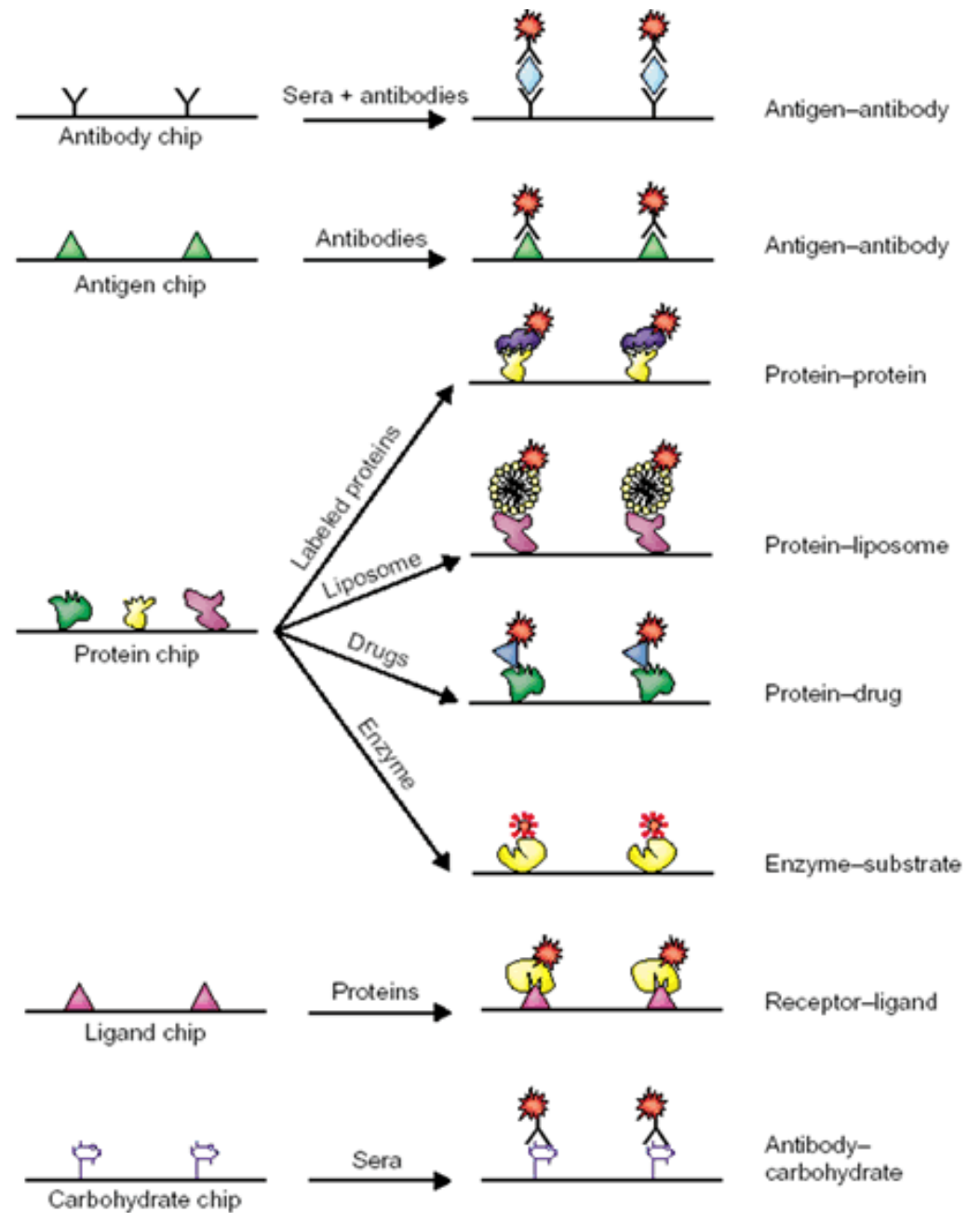
- Similar to DNA microarrays
  - Plate, Probe, Attachment
- Advantage
  - Poor correlation between mRNA and protein expression
- Study protein interactions
  - Protein-Protein
  - Protein-Ligand
  - Protein-DNA
- Monitor Disease States
- Clinical Diagnostics

# General scheme

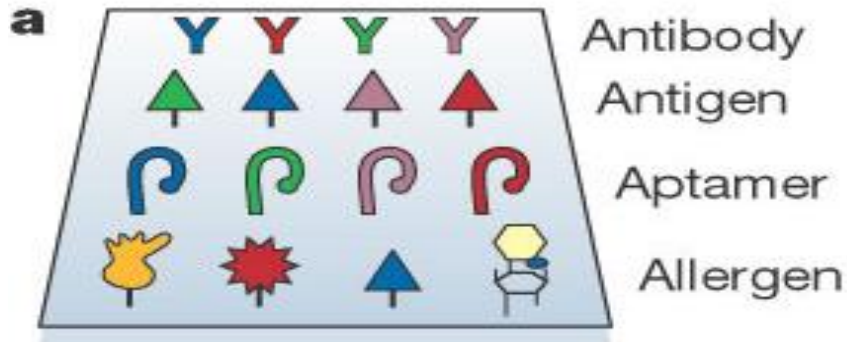


# Types of Arrays

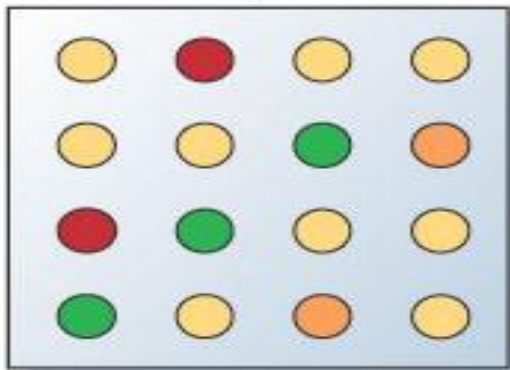
1. Analytical microarrays
2. Functional microarrays
3. Reverse phase microarrays



# Analytical Microarrays



Serum probes  
Cell lysates  
Living cells



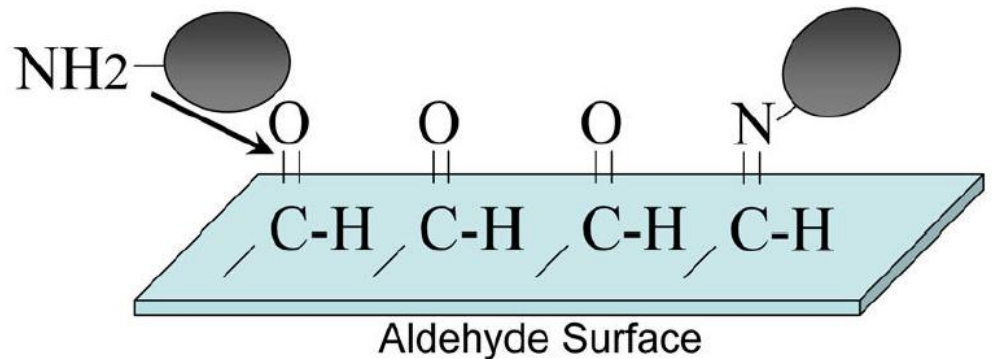
Protein expression level  
Protein profiling  
Diagnostics

- Profiles mixture of proteins
  - Measure binding affinity
  - Specificity
  - Protein Expression Levels
- Most common
- 3 main probe types
  - Antibodies
  - Affibodies
  - Aptamers

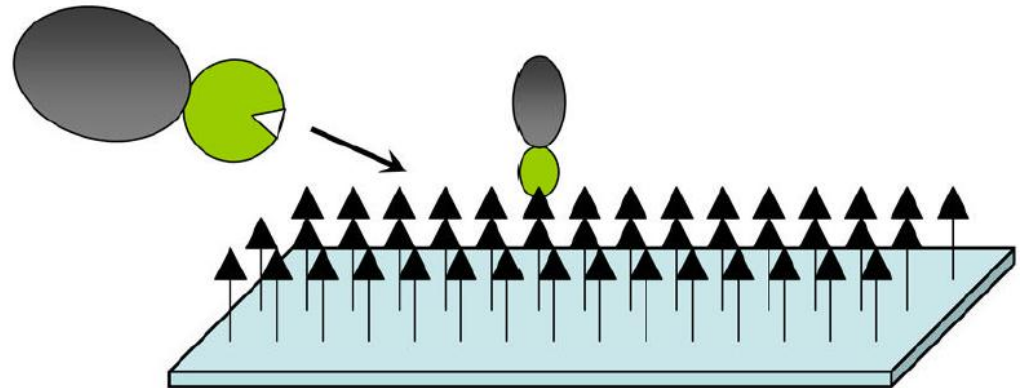
# Plate Set Up

- 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

## Random Attachment

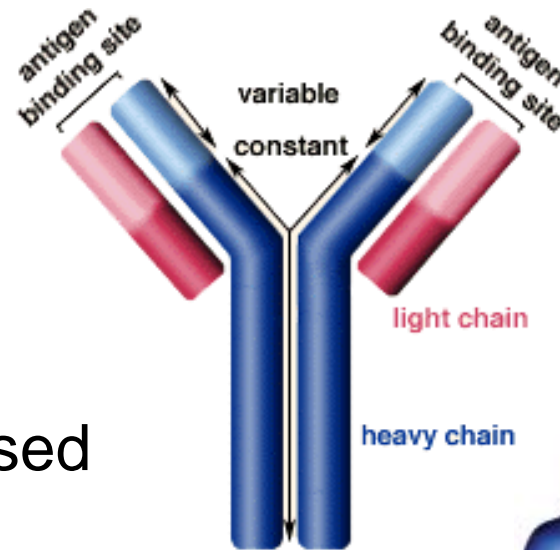


## Ligand Attachment



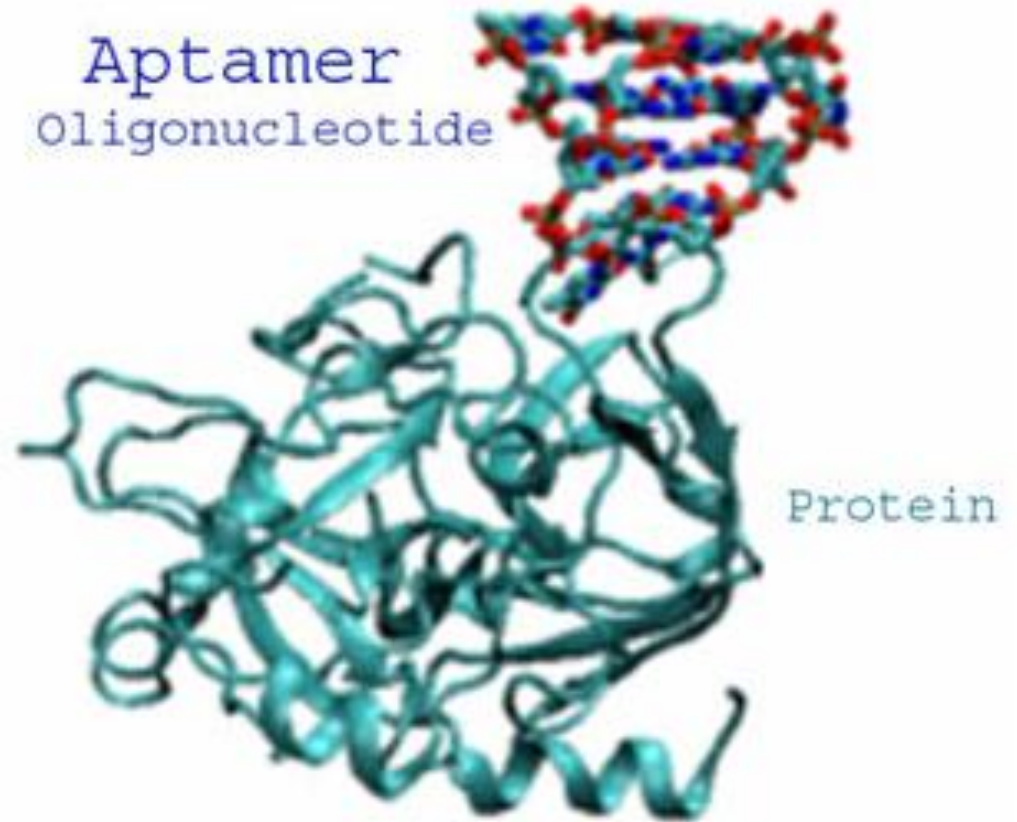
# Analytical Protein Microarray Plates

- 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

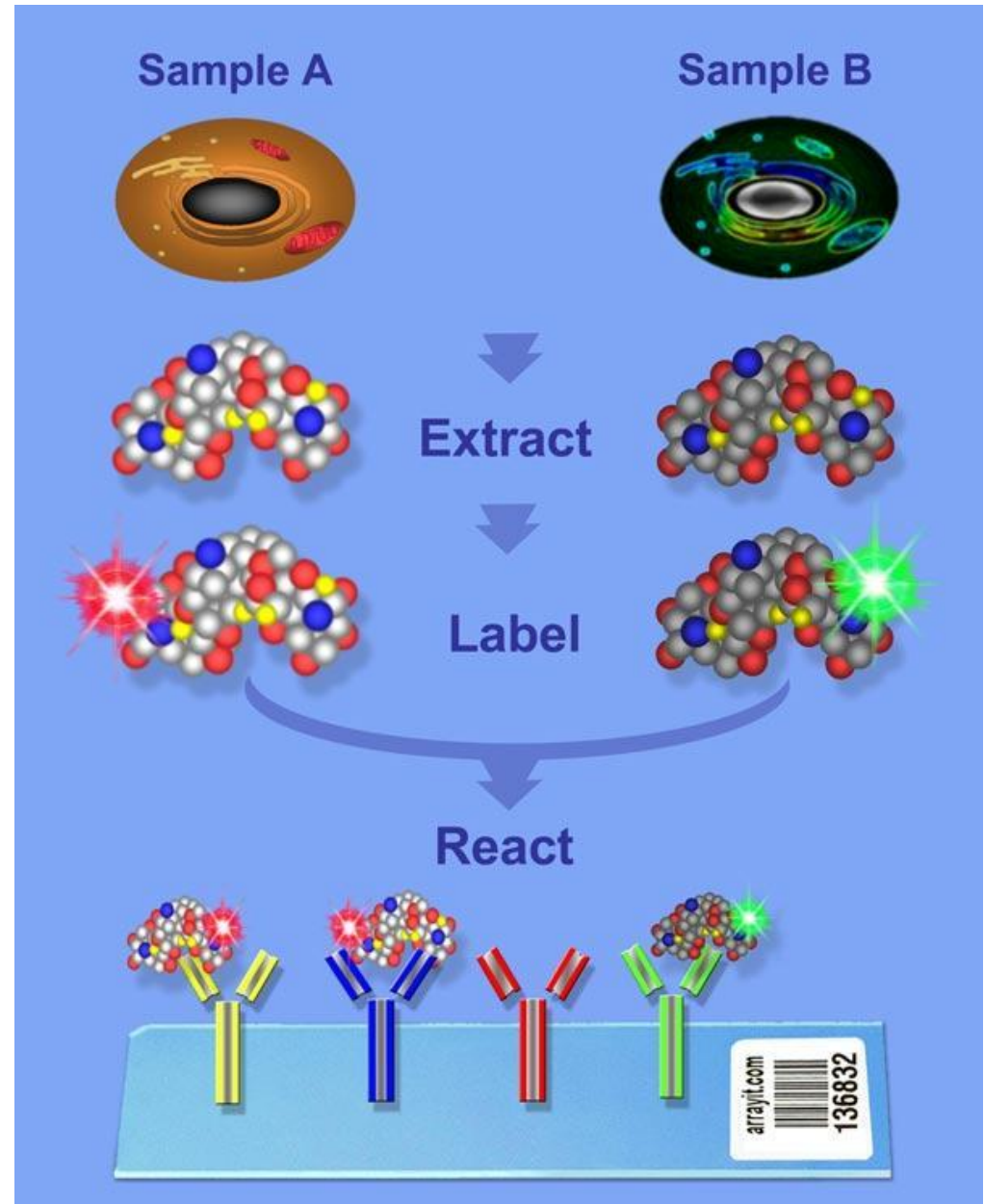
- Aptamers
  - Nucleic Acids
    - DNA, RNA, etc.
  - Peptides
    - Variable loop (10-20 AA)
    - Protein Scaffold
  - Bind Protein
    - Van der Waals Forces, H bonding, Electrostatic Interaction
  - Highly Specific
  - Engineered completely in test tube
    - In vitro selection



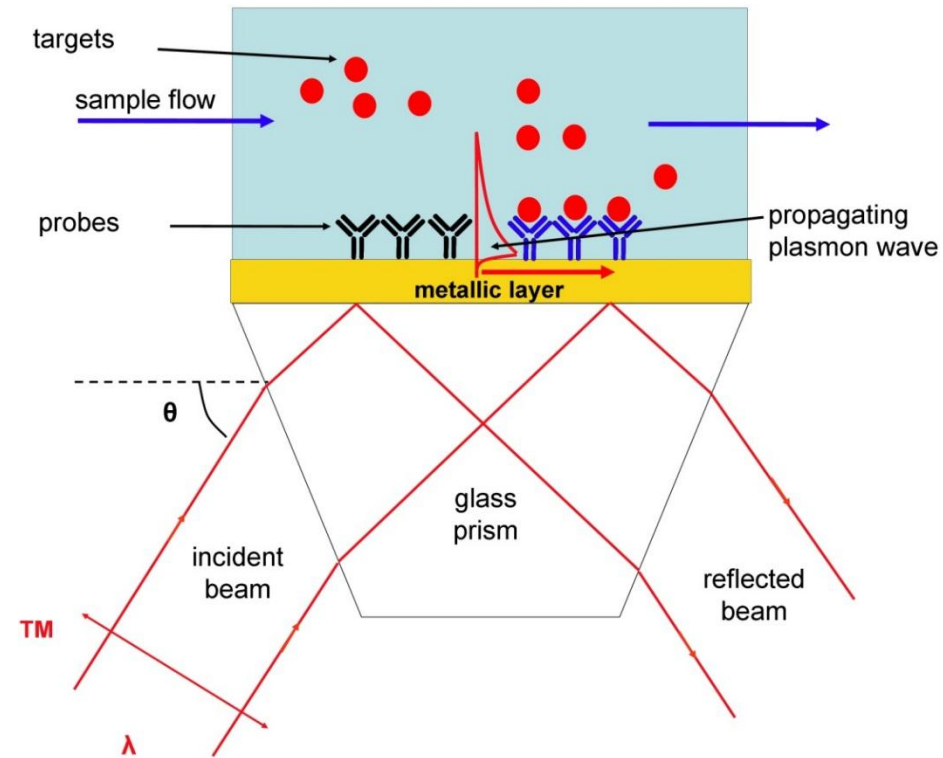


# Sample Preparation

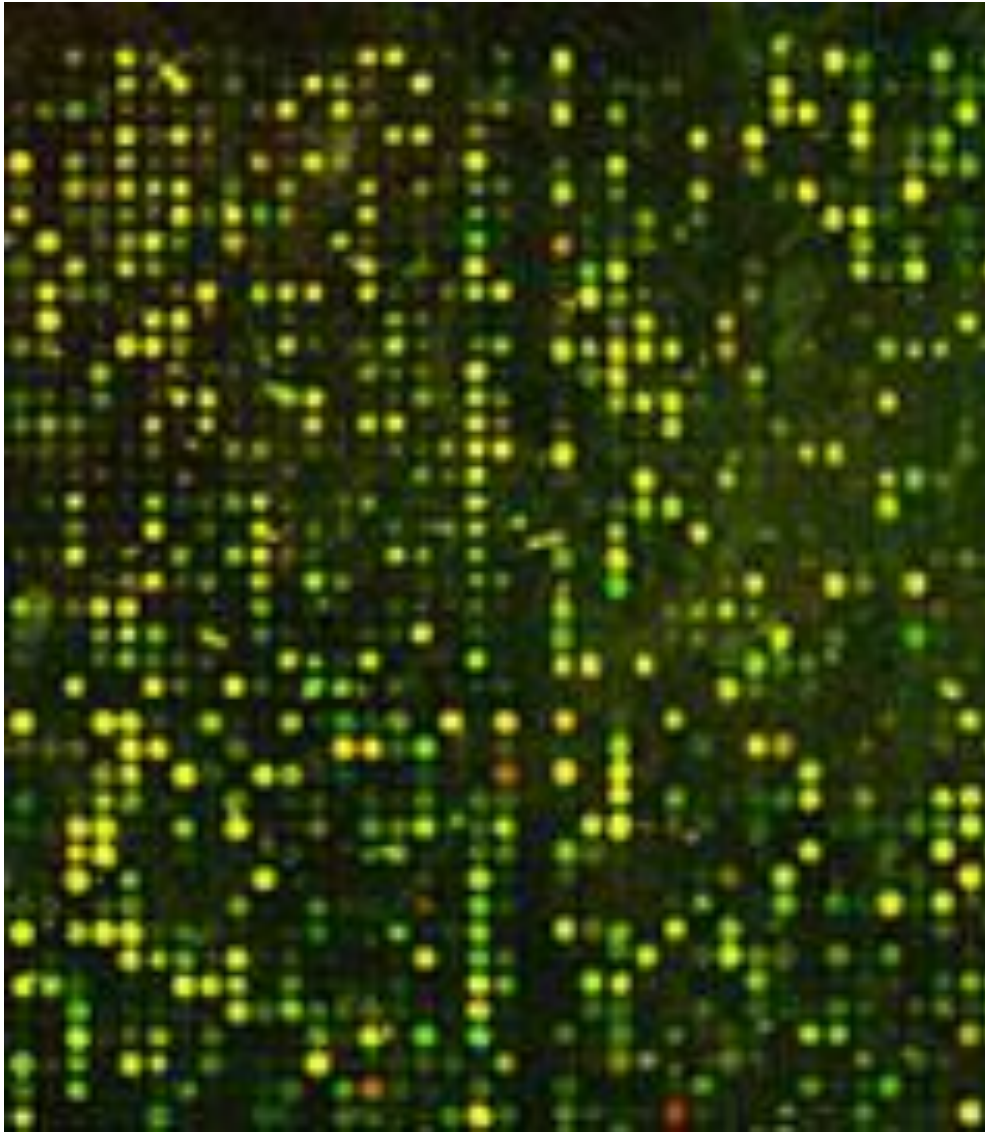
- Sample extracted from cells or tissues
- Labeled
  - Fluorescent Dye
    - Cy3/Cy5 via Lysines
  - Photochemical
  - Radioisotope



- Unlabeled
  - Antibody Sandwich
    - 2<sup>nd</sup> antibody with label incubated on top of sample
  - Surface Plasmon resonance
    - Measure electromagnetic waves
    - Angle changes in the order of  $0.1^\circ$  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

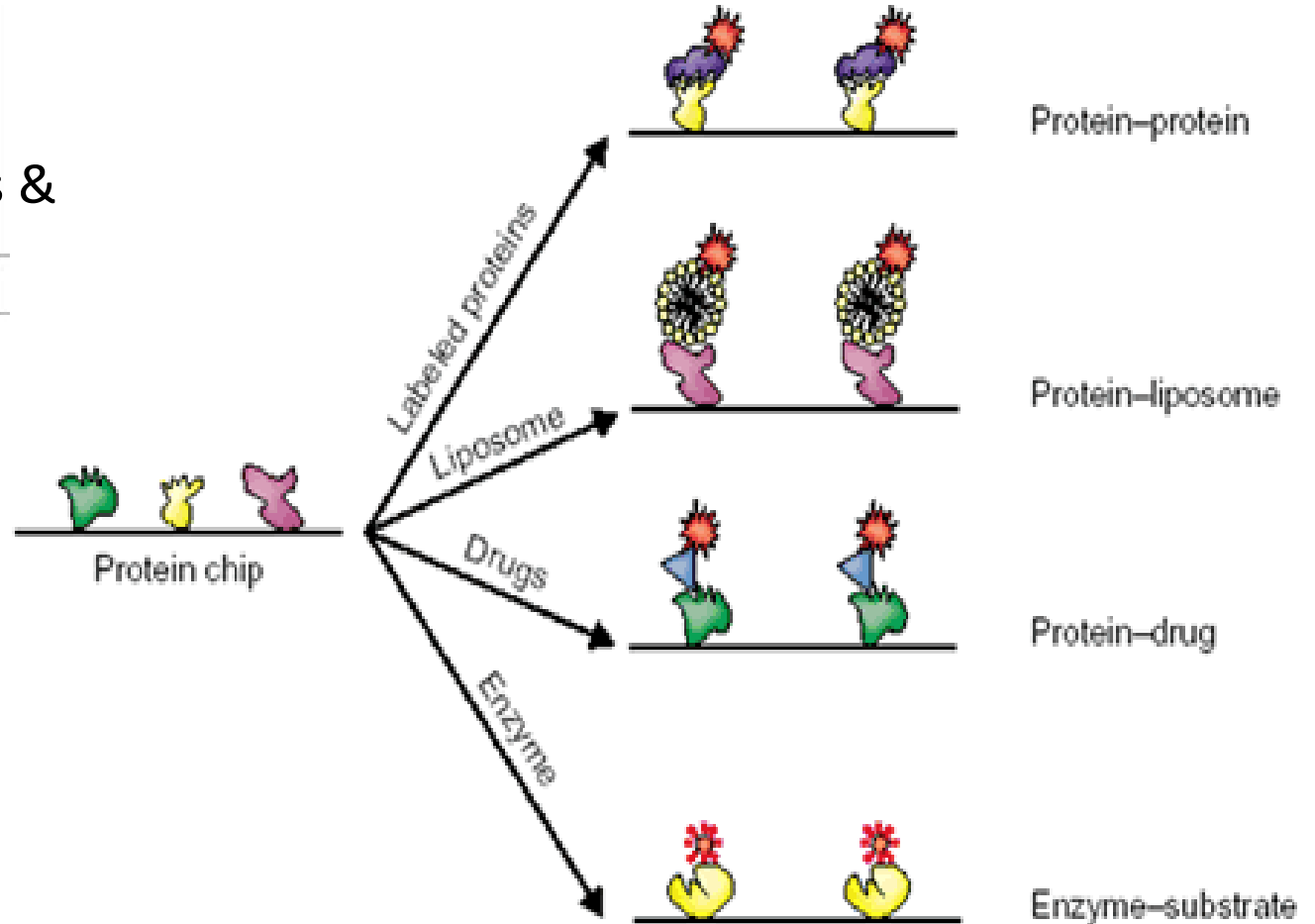
# Functional Microarrays

- Plates

- Full length proteins & protein domains
  - Functional

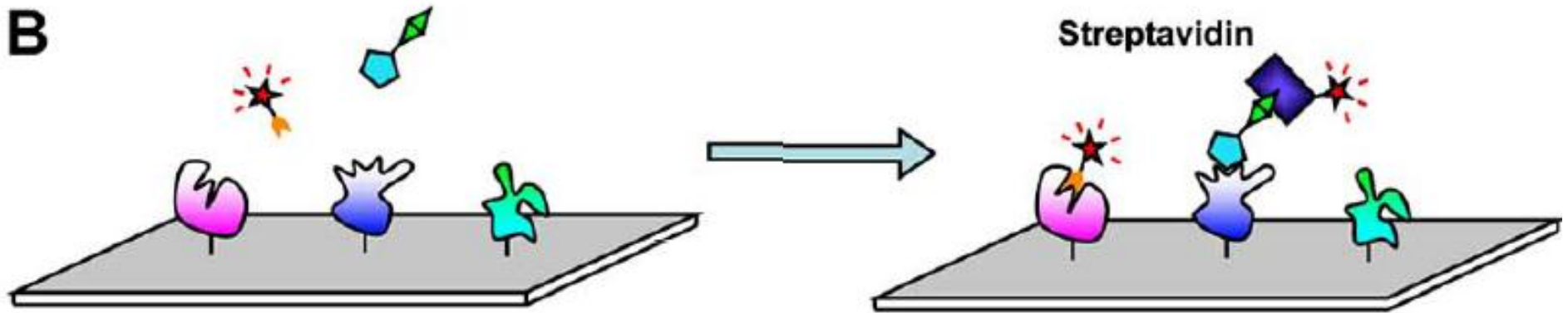
- Samples

- Purified & Labeled
  - Nucleic Acids
  - Proteins
  - Lipids
  - Small Molecules



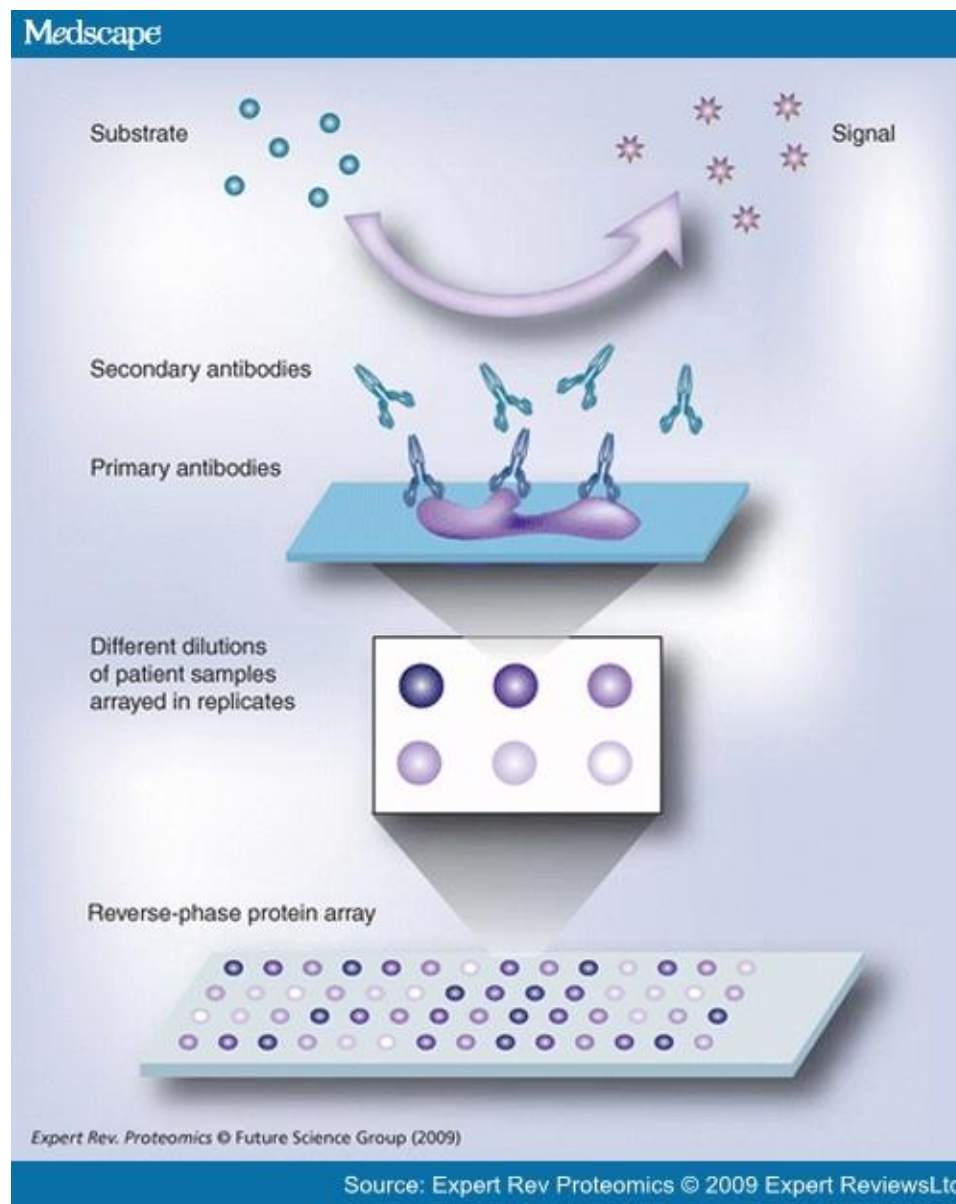
# Functional Array Example

- Protein-small molecule interaction
  - Plate has whole proteome
  - Monitor Specificity



# 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



# References

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