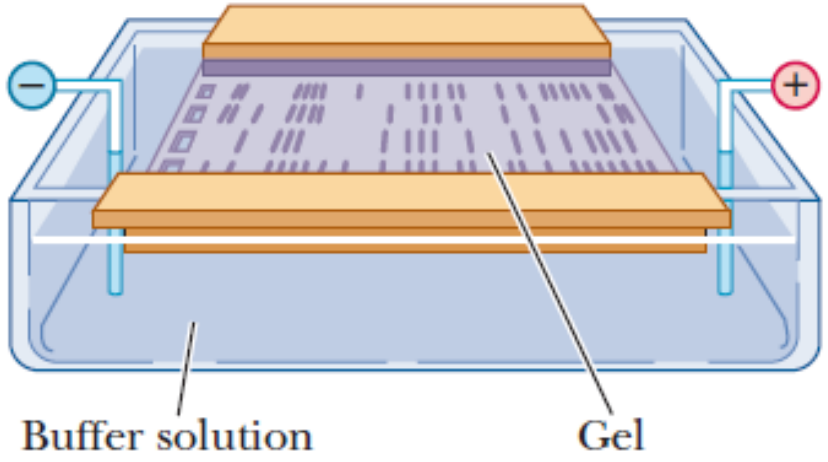
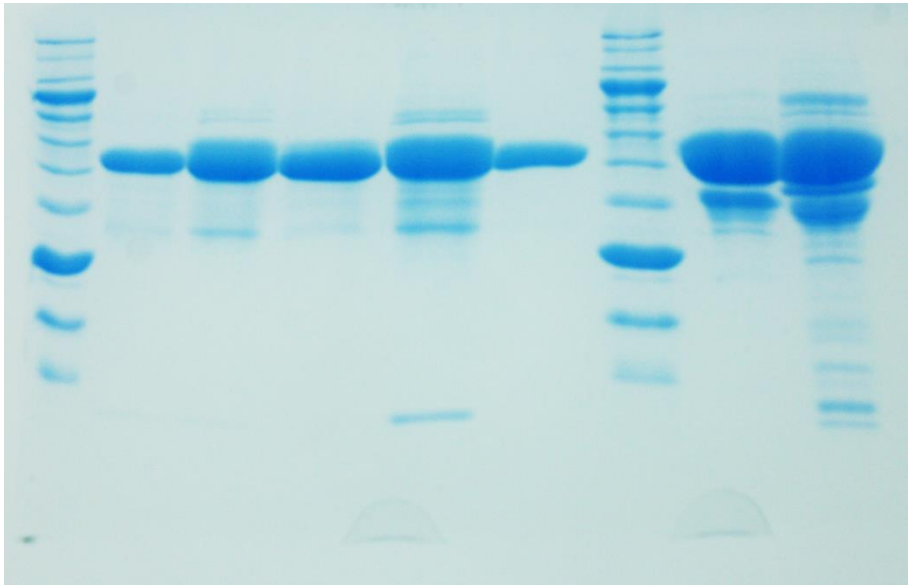
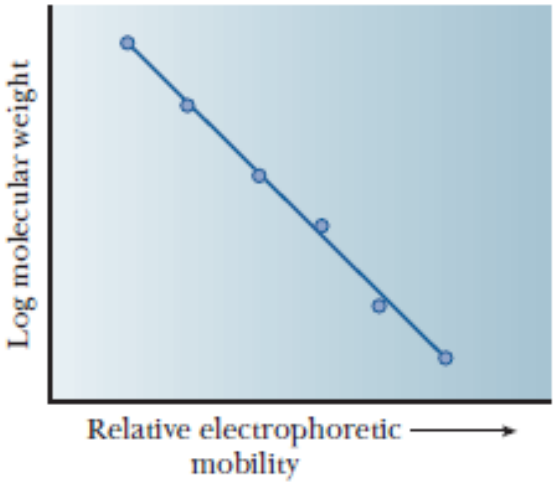
Electrophoresis

- Based on the motion of charged particles in an electric field
- Macromolecules have differing mobilities based on their charge, shape, & size
- The most common medium is a polymer of agarose or acrylamide





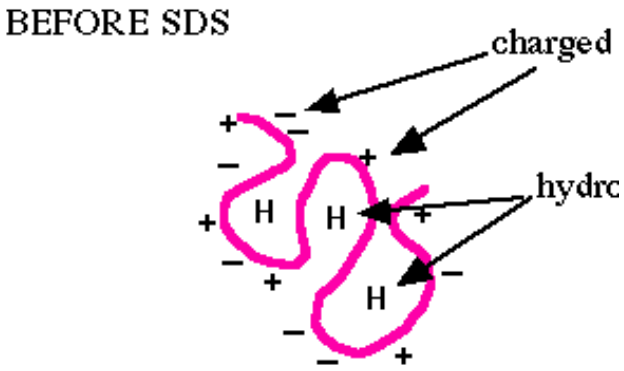
Agarose vs. PAGE

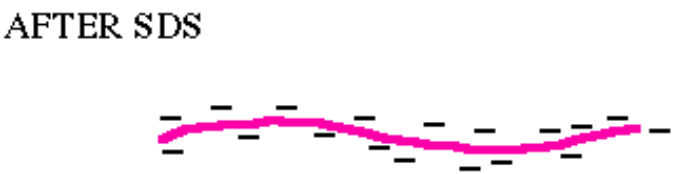


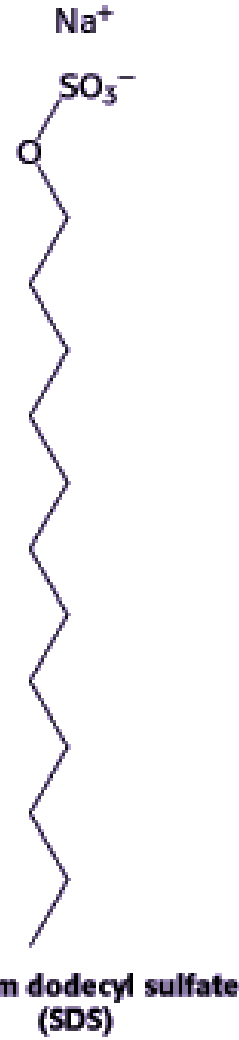
- Agarose (nucleic acids), PAGE (proteins)
- In PAGE: SDS or NO-SDS $\{CH_3(CH_2)_{10}CH_2OSO_3Na^+\}$
- SDS completely denatures proteins (multi-subunit proteins)
- Acrylamide offers higher resistance to large molecules
- Shape and charge are approximately the same (sizes is the determining factor)
- Acrylamide without the SDS (**native gel):** study proteins in their native conformation (mobility is not an indication of size)

SDS (sodium dodecyl sulfate)

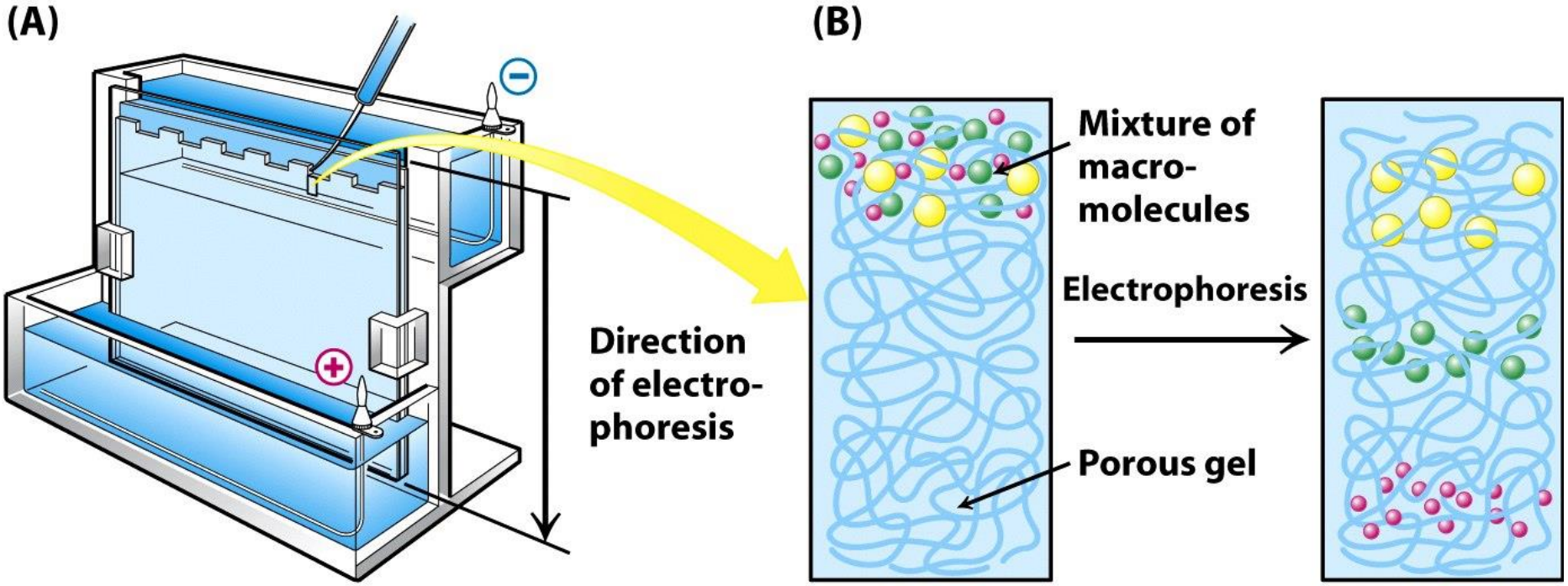
- This technique utilizes a negatively charged detergent (SDS) to denature and solubilize proteins
- SDS makes proteins have a uniform (-) charge
- The mixture of proteins is also treated with reducing agents like β-mercaptoethanol or DTT to reduce disulfide bonds (denaturing condition)
- Otherwise, non-denaturing condition







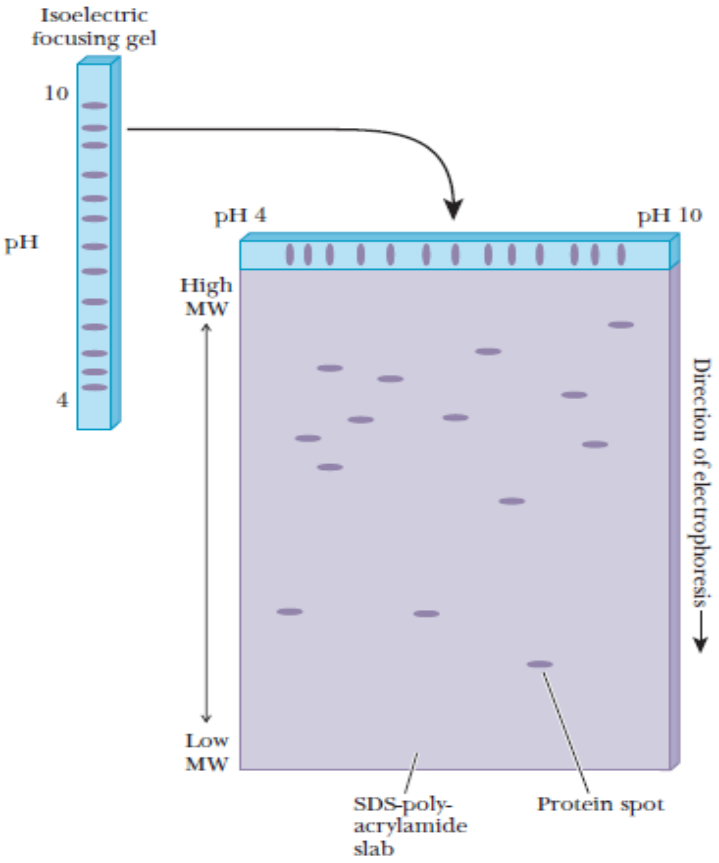
SDS - PAGE

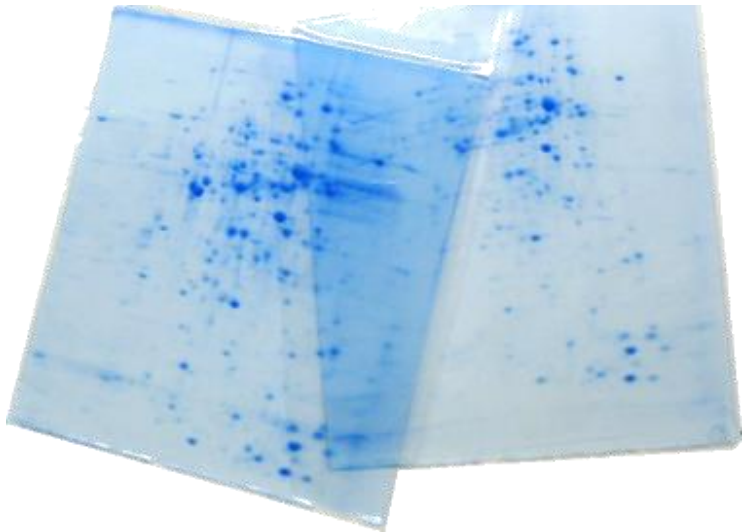

(A)
Direction of electro-phoresis
(B)
Mixture of macro-molecules
Electrophoresis
Porous gel

Isoelectric focusing

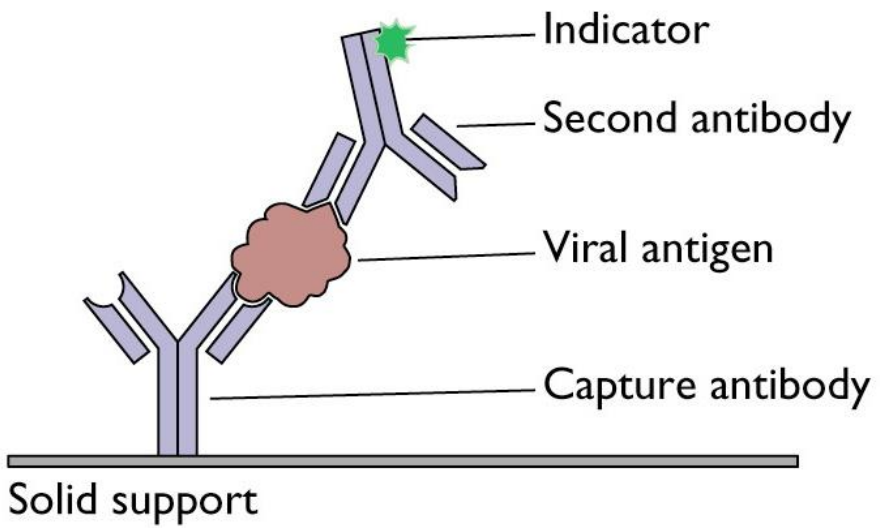
- Proteins have different isoelectric points
- Gel prepared with a pH gradient parallel to electric field gradient

- **Two-dimensional gel electrophoresis (2-D gels)**

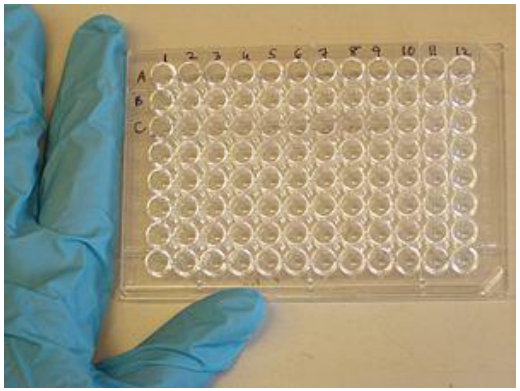




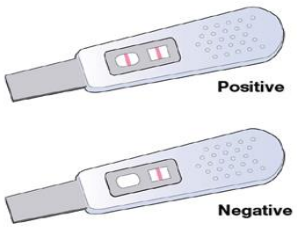
Immunoassays – ELISA

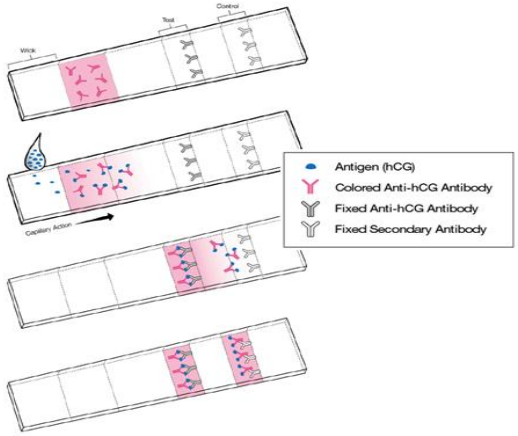


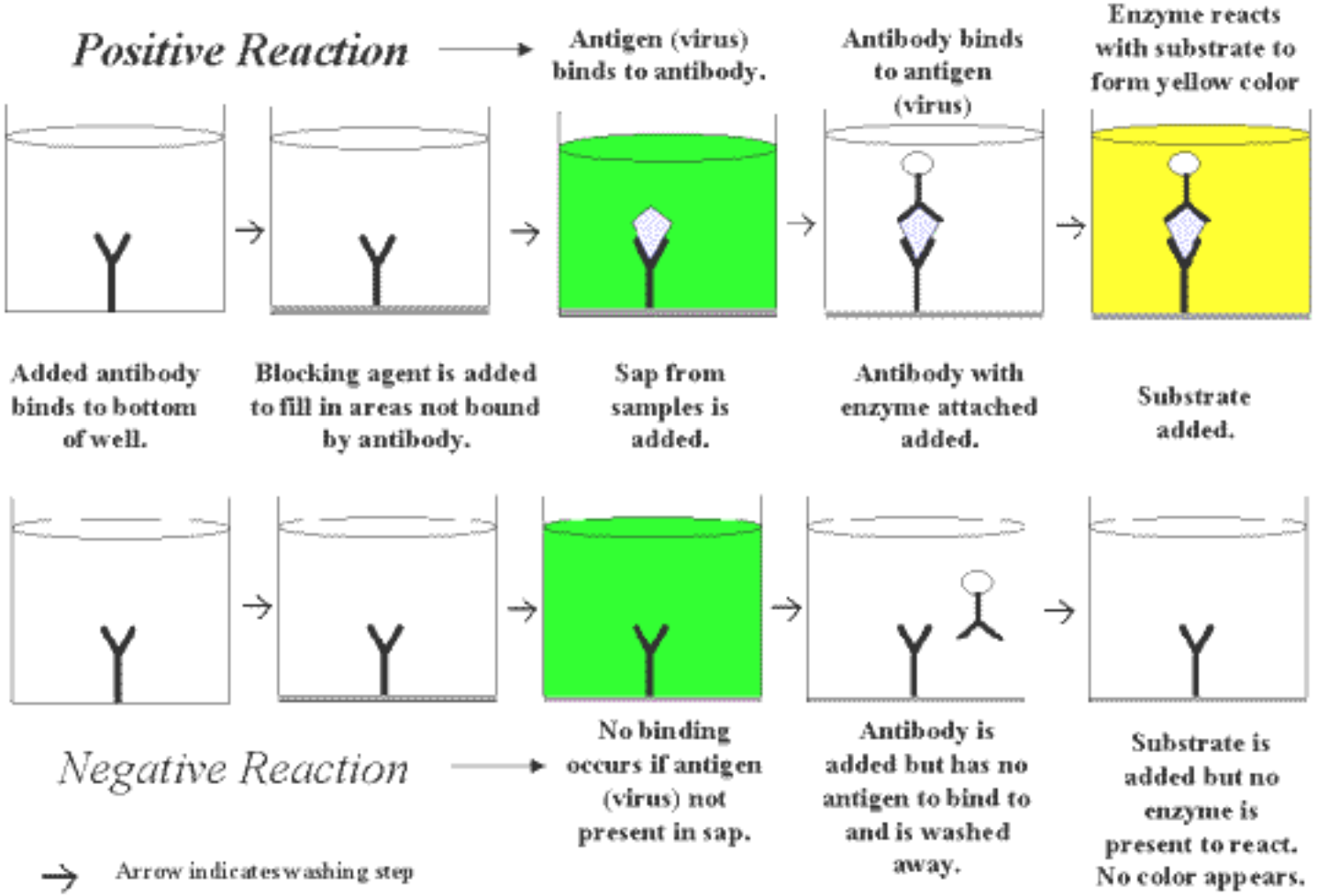
- Enzyme-linked immunosorbent assay
  - Rapid, convenient, and sensitive (less than a nanogram (10^{-9} g) of a protein)
  - Detect & quantify substances (peptides, proteins, antibodies & hormones)
  - Usually done in 96-well polystyrene plates (passively bind antibodies and proteins)
  - Apllication:
  - Screening (HIV, Hepatitis B&C)
  - Hormones (HCG, LH, TSH, T_3, T_4)



ELISA

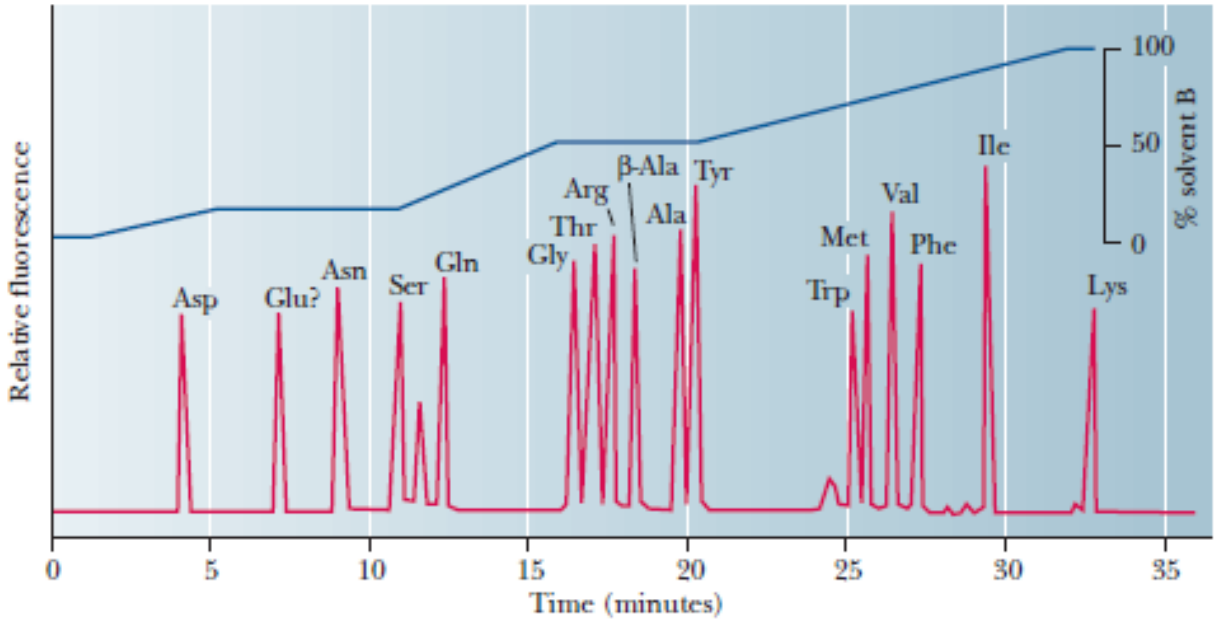

Positive
Negative

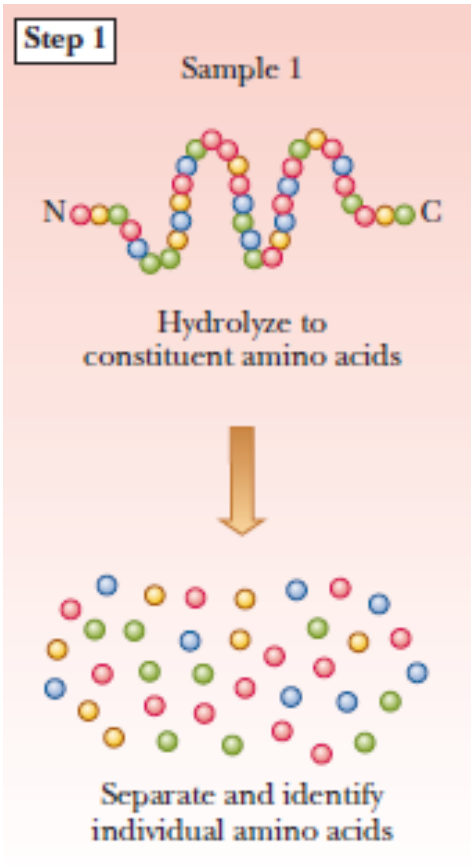

Antigen (hCG)
Colored Anti-hCG Antibody
Fixed Anti-hCG Antibody
Fixed Secondary Antibody


Positive Reaction
Antigen (virus) binds to antibody.
Antibody binds to antigen (virus)
Enzyme reacts with substrate to form yellow color
Added antibody binds to bottom of well.
Blocking agent is added to fill in areas not bound by antibody.
Sap from samples is added.
Antibody with enzyme attached added.
Substrate added.
Negative Reaction
No binding occurs if antigen (virus) not present in sap.
Antibody is added but has no antigen to bind to and is washed away.
Substrate is added but no enzyme is present to react. No color appears.
Arrow indicates washing step

Protein sequencing – Edmans' Method

- How much & which amino acids are involved
- **Hydrolysis & Separation** (ion-exchange chromatography or **high performance liquid chromatography, HPLC)**

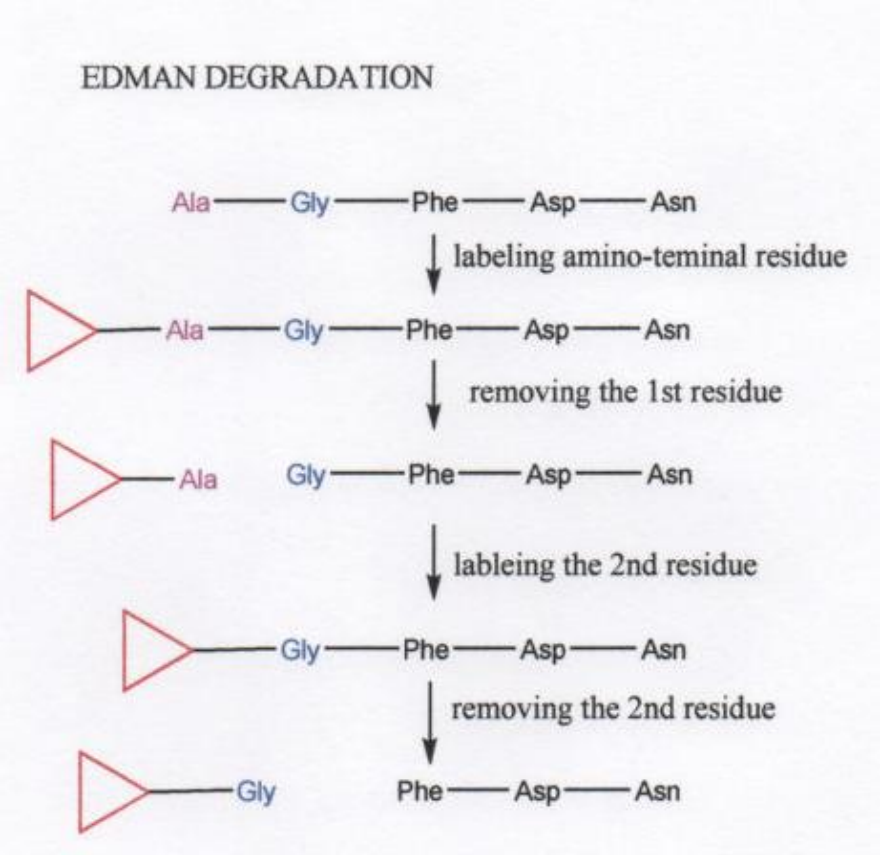




Protein sequencing

- Involves a step-by-step cleavage of the N-terminal residue of a peptide, allowing for the identification of each cleaved residue
- Utilizes phenylisothiocyanate (PITC) to react with the N-terminal residue

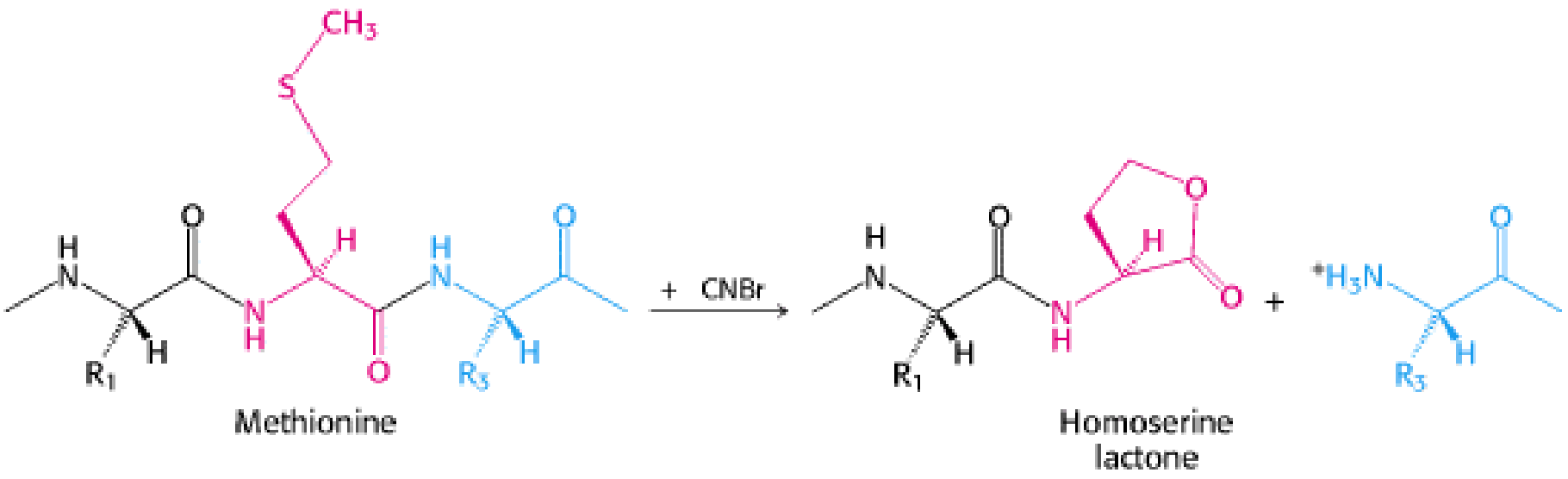
- Does not allow peptides more than **50** residues to be sequenced



Methods?!

Chemical digestion

- The most commonly utilized: cyanogen bromide (CNBr)
- C-terminaus of methionine residues
- A protein that has 10 methionine residues will usually yield 11 peptides on cleavage with CNBr



Endo & exopeptidases

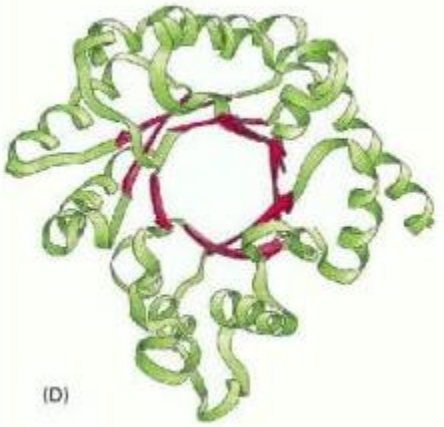
- Endo: cleave at specific sites
- Exo: two types
  - Aminopeptidases: cleave at the N-terminus
  - Carboxypeptidases: cleave at the C-terminus

Enzyme	Specificity
Trypsin	C-terminal to R, K, but not if next to P
Chymotrypsin	C-terminal to F, Y, W but not if next to P
Elastase	C-terminal to A, G, S, V, but not if next to P
Pepsin	N-terminal to L, F, W, Y, but when next to P

Protein sequencing – prediction from DNA & RNA

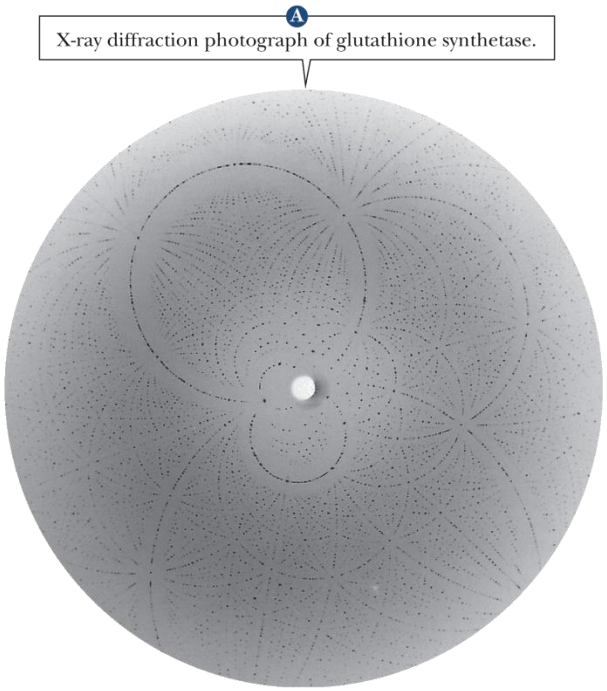
- If the sequence of the gene is known, this is very easy
- If the sequence of the gene is unknown (newly isolated proteins)? Sequence a short segment, complementary RNA, isolate mRNA, PCR, gene sequencing

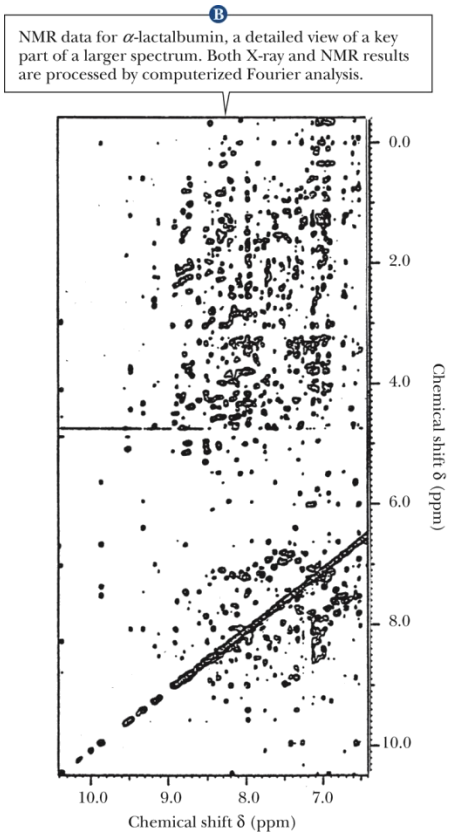
Determination of 3°Structure



- **X-ray crystallography**
  - uses a perfect crystal; that is, one in which all individual protein molecules have the same 3D structure and orientation
  - exposure to a beam of x-rays gives a series diffraction patterns
  - information on molecular coordinates is extracted by a mathematical analysis called a Fourier series
- **2-D Nuclear magnetic resonance**
  - can be done on protein samples in aqueous solution

X-Ray and NMR Data





- High resolution method to determine 3˚ structure of proteins (from crystal)
- Diffraction pattern produced by electrons scattering X-rays
- Series of patterns taken at different angles gives structural information

- Determines solution structure
- Structural info. Gained from determining distances between nuclei that aid in structure determination
- Results are independent of X-ray crystallography