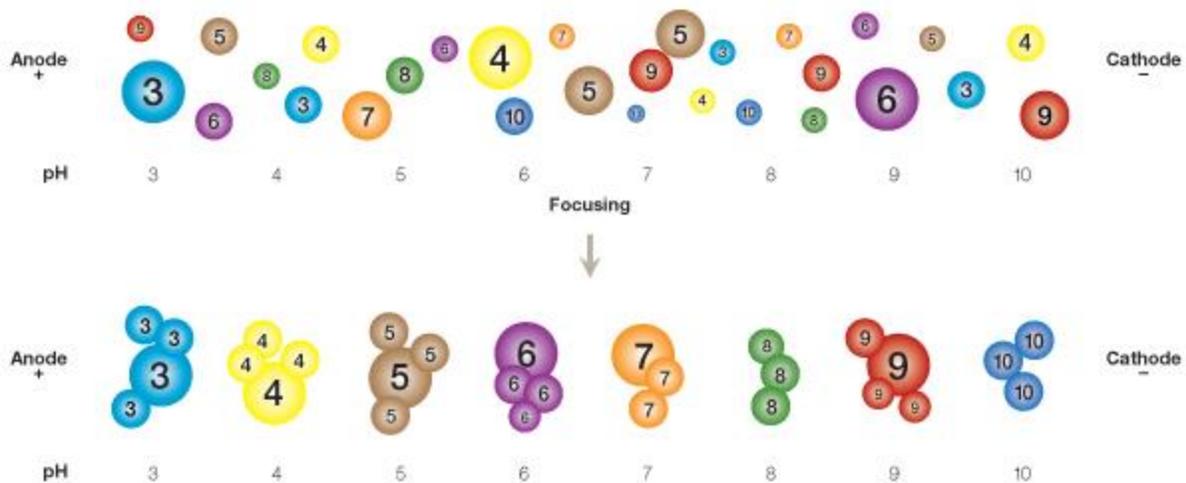
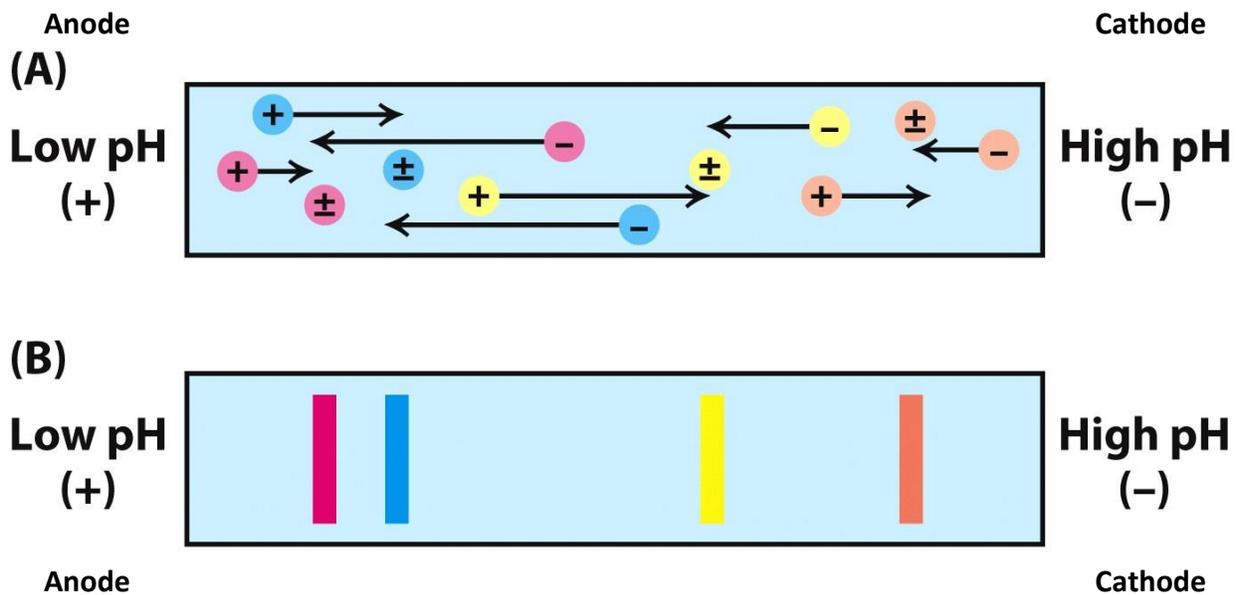


## Isoelectric focusing (IEF)

- An electrophoresis technique that separates proteins based on their isoelectric point (pI).
- A pH gradient is established, such that one end has a high pH (– charge, cathode) and the other has a low pH (+ charge, anode)
  - Recall: for IEF and electrophoresis, the labeling of cathode and anode are *opposite* to what you would think (since they are electrolytic cells)
- Once a protein reaches its pI (the place in the pH field whereby the protein has no net charge), it will no longer move in the electric field.



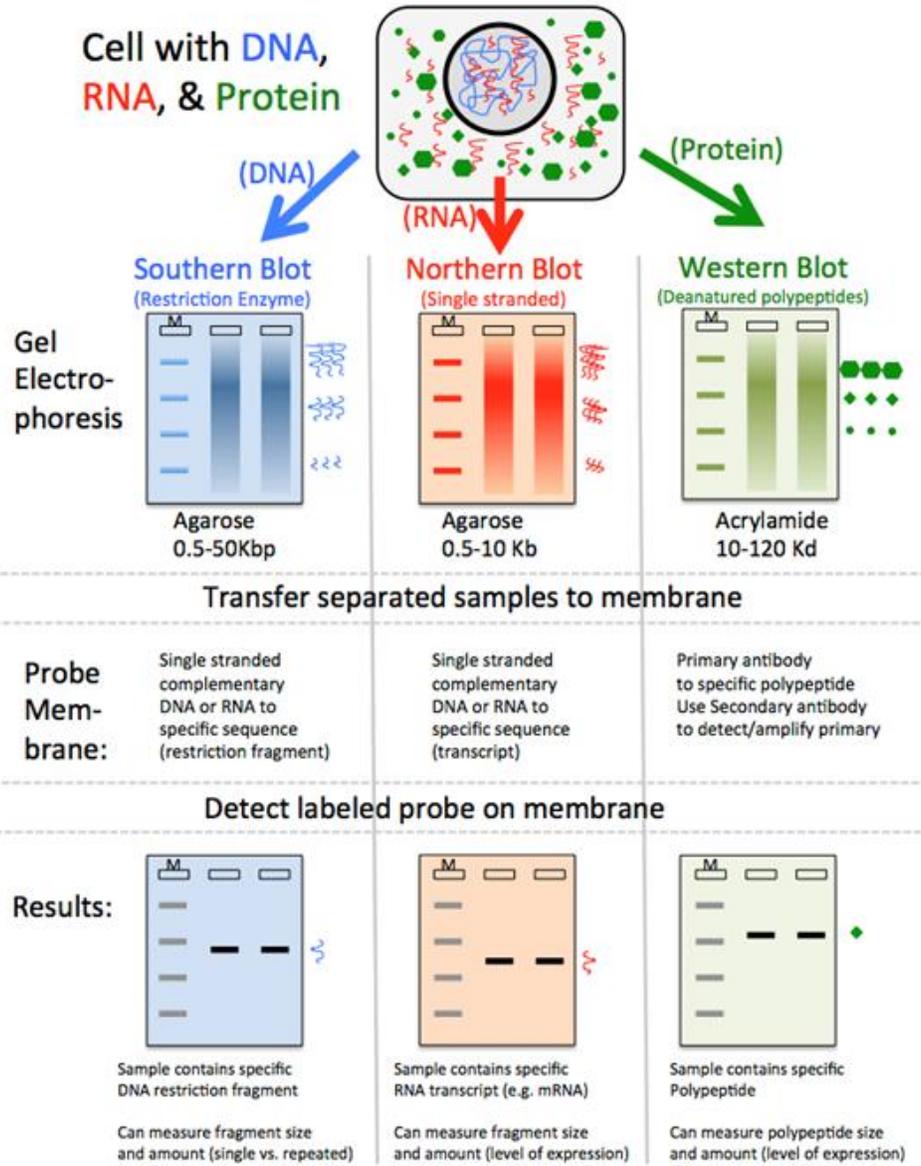
## Polyacrylamide Agarose Gel Electrophoresis (PAGE)

- Mobility of a protein or nucleic acid is a function of the **length, conformation, and charge** of the molecule.
- **Native PAGE**
  - Preserves the molecules' higher order structure
  - Separates based on both size *and* charge
- **SDS PAGE**
  - SDS (sodium dodecyl sulfate) is a negatively charged chemical that binds to proteins in a set ratio, approximately one molecule of SDS for every 2 amino acids.
  - In this way, the detergent provides all proteins with a uniform charge-to- mass ratio, independently of their original charge (therefore charge is no longer a factor)
  - By binding to the proteins, the detergent denatures proteins by destroying their 2°, 3°, and/or 4° structure, turning them into negatively charged linear poly peptide chains.
  - Thus, **SDS-Page** separates based exclusively on size
  - Two types:
    - Non-reducing
      - Can separate out subunits, *provided they are not covalently linked by disulfide bonds*
    - Reducing
      - Removes disulfide bonds ( $R-S-S-R \rightarrow R-SH + SH-R$ ), further denaturing the protein
      - Can separate out subunits, even if they are covalently linked by disulfide bonds.

### Summary:

<b>Native-PAGE</b>	Separates based on <b>size and charge</b>
<b>SDS-PAGE (non-reducing)</b>	Separates based on <b>size</b> only Separates out SUs, provided they're not covalently linked by S-S bonds
<b>SDS-PAGE (reducing)</b>	Separates based on <b>size</b> only Separates out SUs, even if they are covalently linked by S-S bonds
<b>Isoelectric focusing (IEF)</b>	Separates based on <b>charge</b> only

# Blots



Mnemonic:

**SNOW**  
**DR P**

S - SOUTHERN - DNA - D  
 N - NORTHERN - RNA - R  
 O - ○○○○○○○○ - ○○○○ - O  
 W - WESTERN - PROTEIN - P