Methods to study proteins

Methods

- Isolation and purification of proteins
- Identification of proteins
- Protein sequencing
- Studying protein functions

Isolation and purification of proteins

- There is no known methods to amplify protein molecule.
- In nature proteins usually occur in complex mixes with other proteins and other biological molecules.
- Therefore in order to study an individual protein the protein should be purified from the complex source.

- Protein purification procedure usually consists of several steps.
- On each step complex mixture of proteins is separated into fractions. Fractions containing the protein of interest are enriched with it. They are collected and used as starting material for the next purification step.

How do we know if the fraction contains the protein of interest?

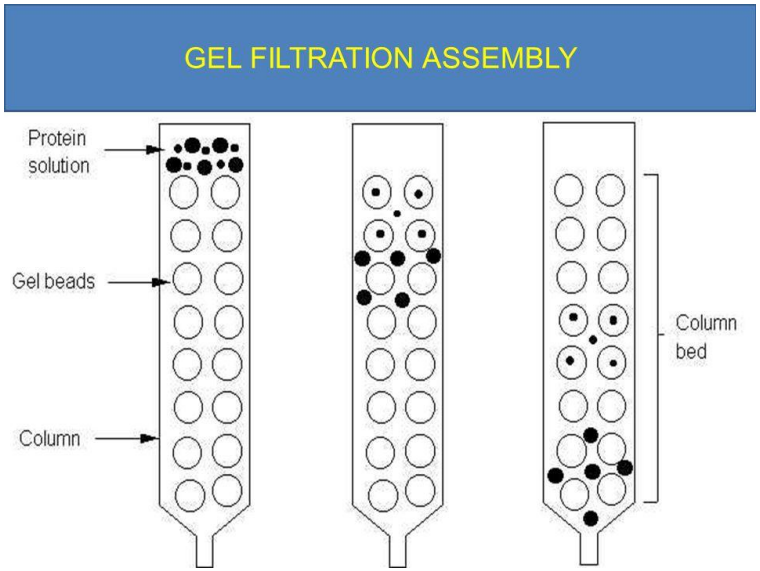
- By registering protein activity – enzymatic activity, binding to specific molecular partners (ligands) including antibodies
- By detecting a protein of specific size (Molecular weight)

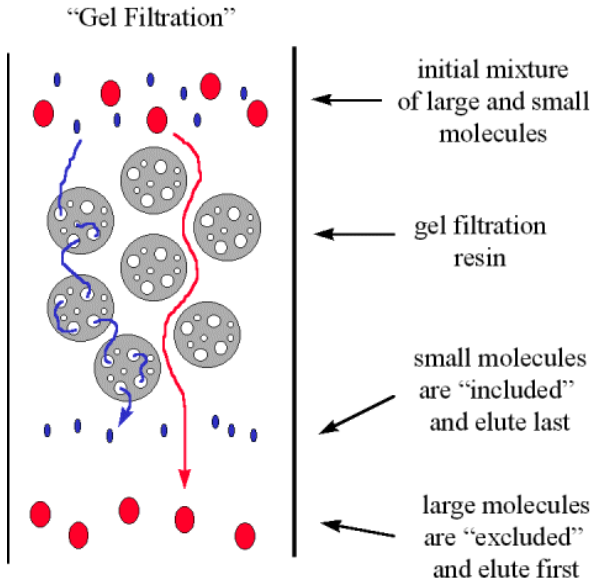
Methods of separation

- Physical methods – centrifugation, heat treatment etc.
- Adsorption – desorption
- Chromatography
- Electrophoresis

Chromatography
Gel filtration (molecular sieve)

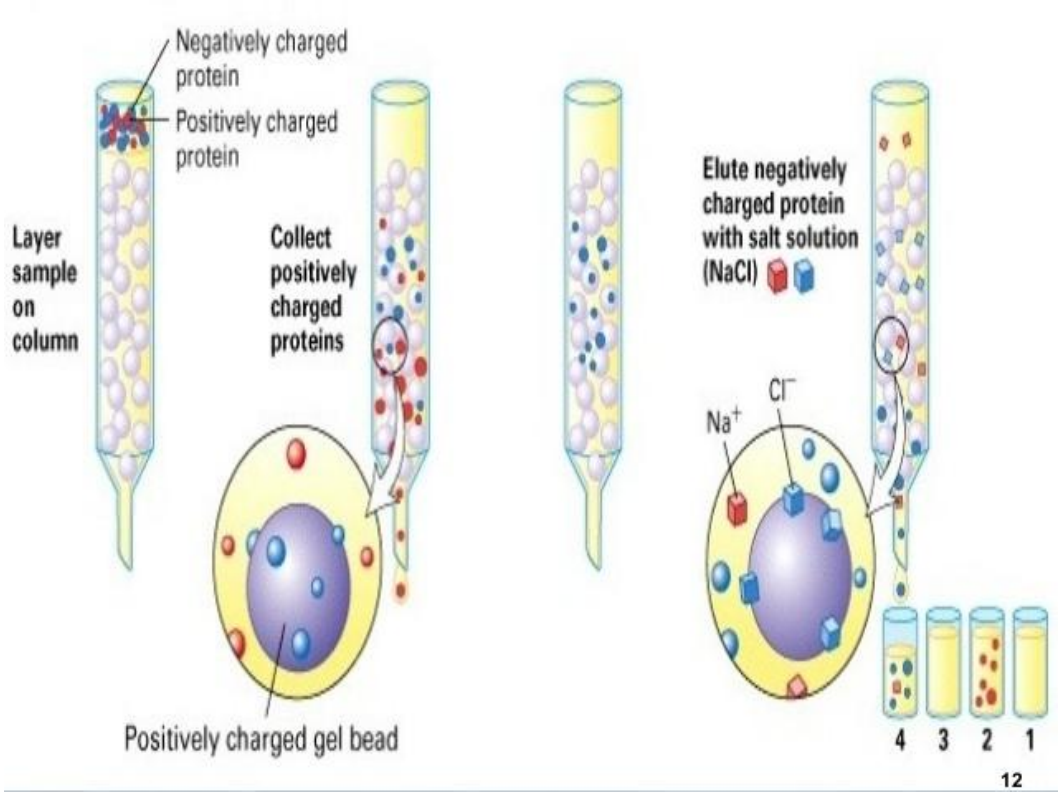
Proteins are separated by their size (Molecular weight)



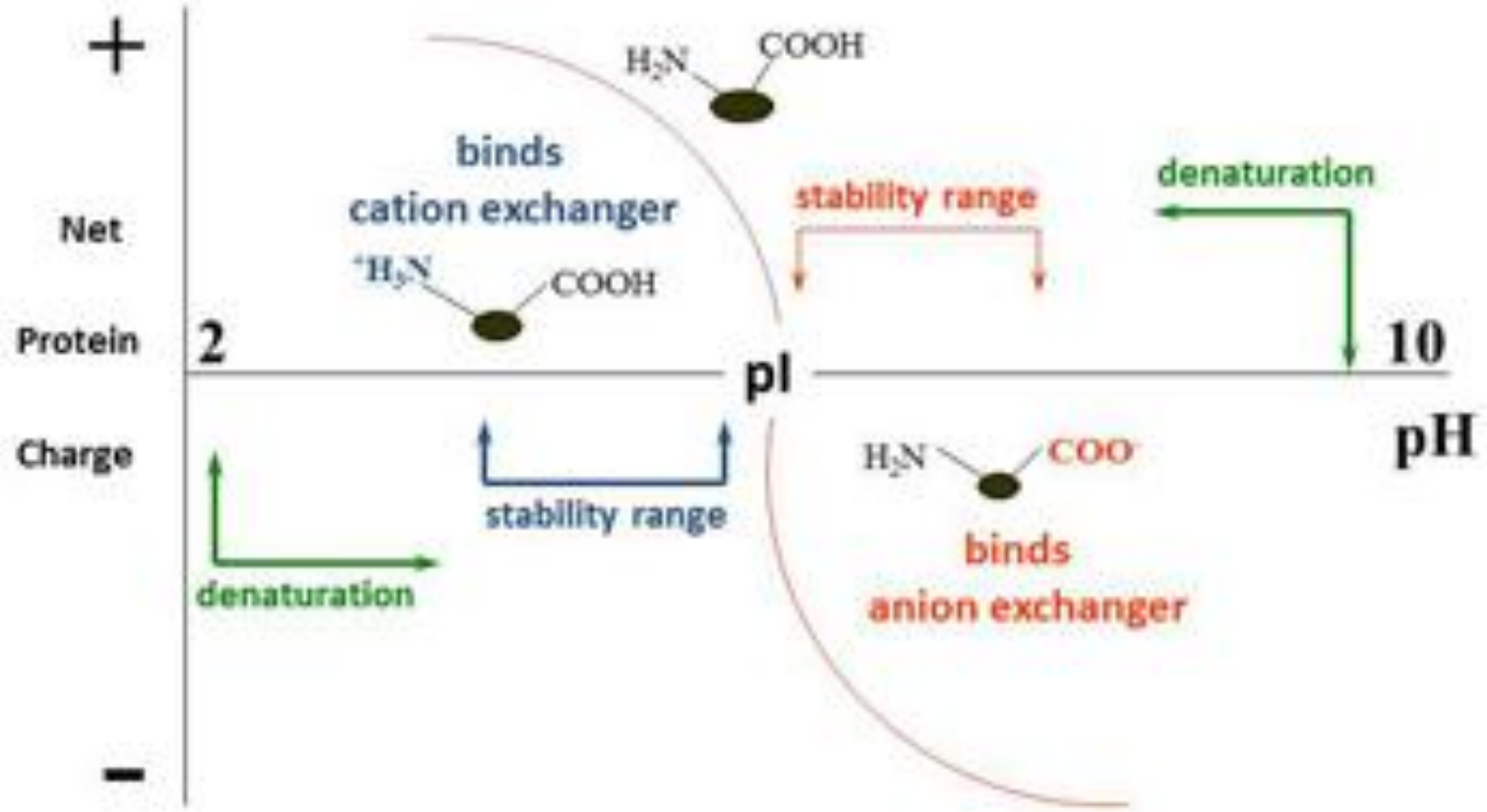


Chromatography
Ion exchange

Proteins are separated by their charge

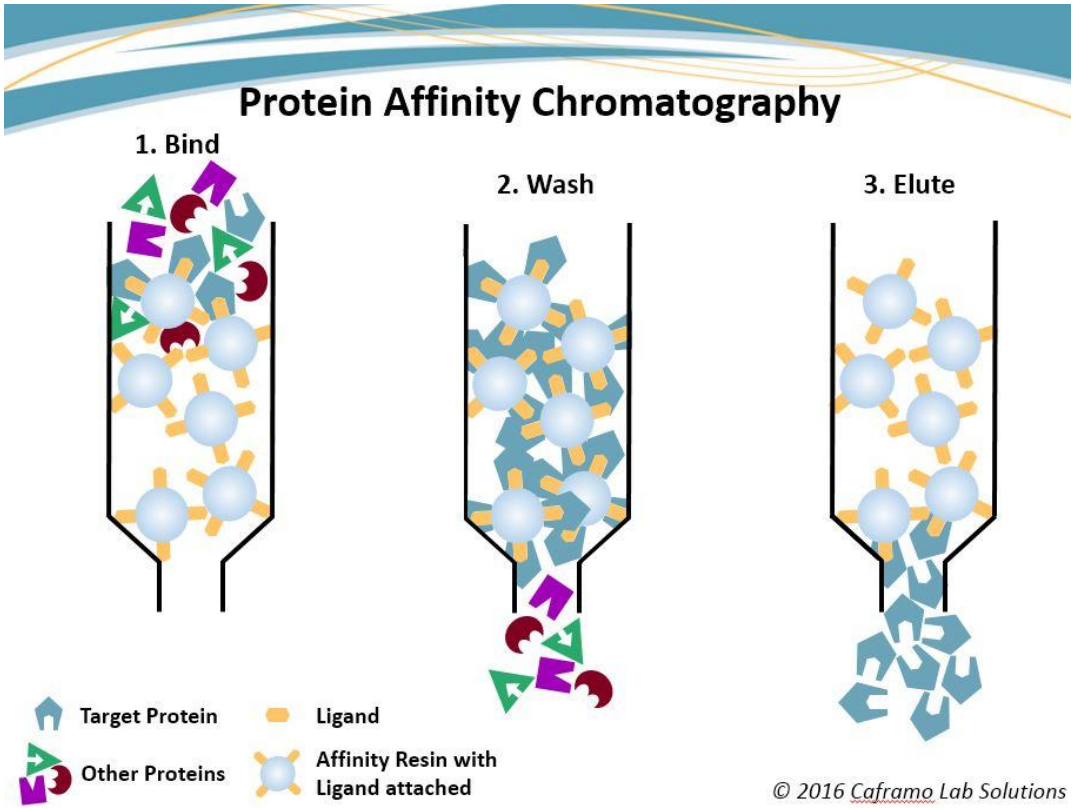


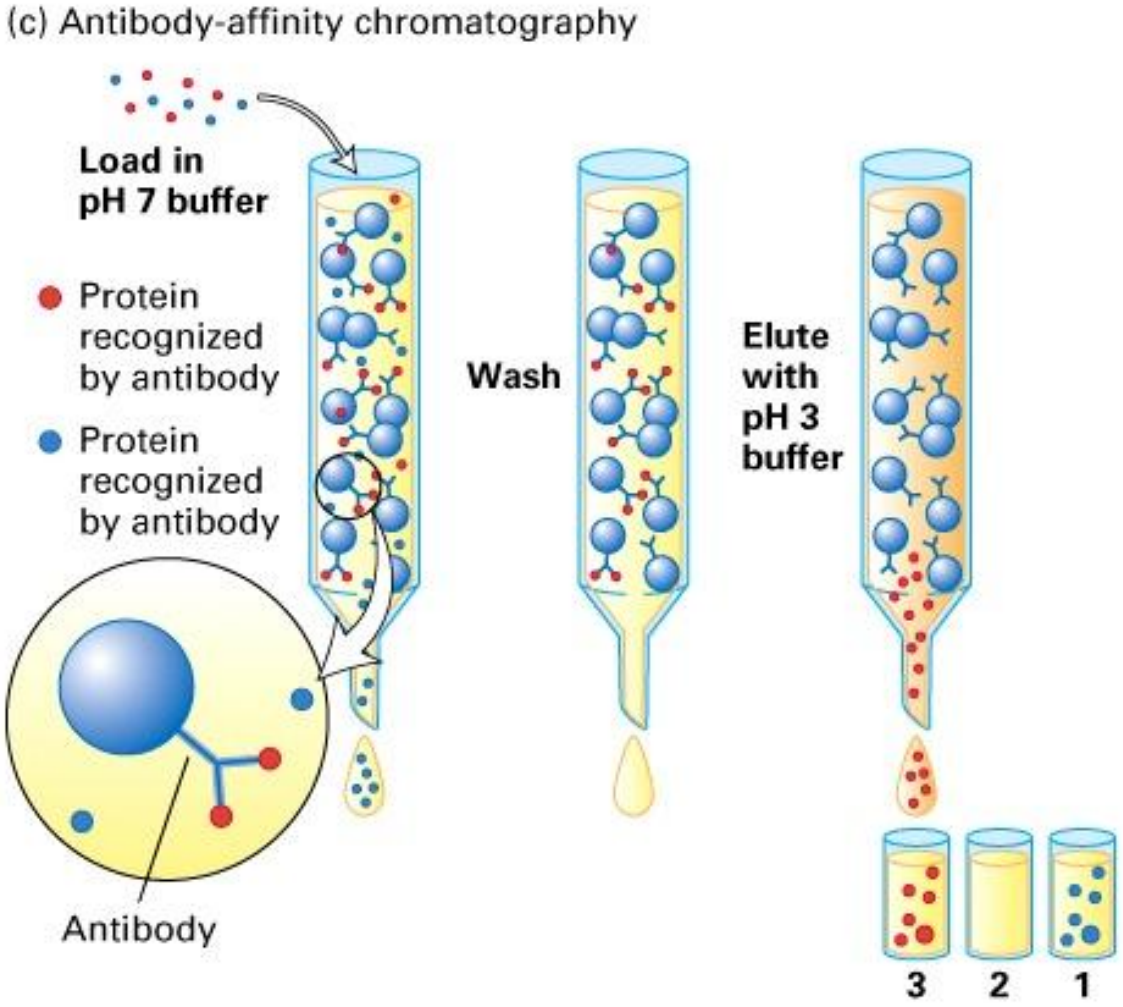
Protein charge depends on the Ph of the solution



Chromatography
Affinity

Proteins are separated by their specific interaction with other molecules

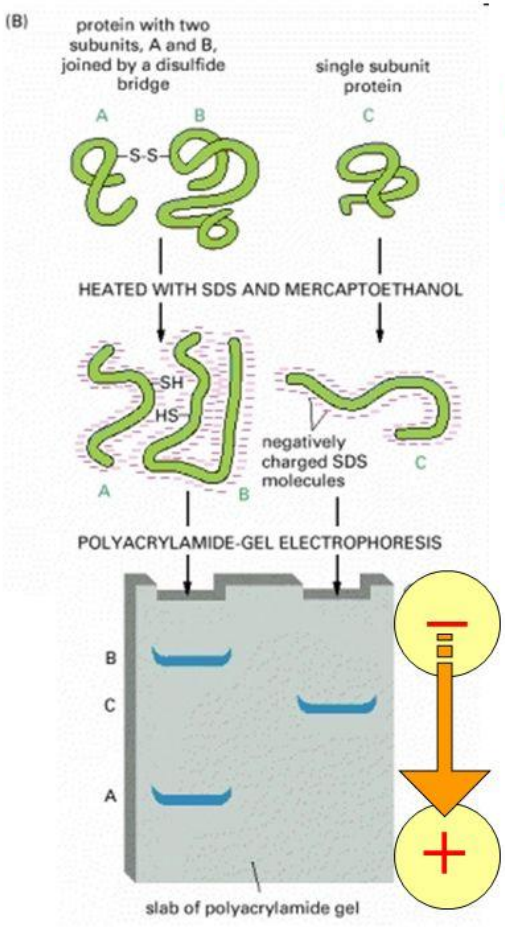



(c) Antibody-affinity chromatography
Load in pH 7 buffer
Protein recognized by antibody
Protein recognized by antibody
Antibody
Wash
Elute with pH 3 buffer
3
2
1

Protein electrophoresis

- Native
- Denaturing

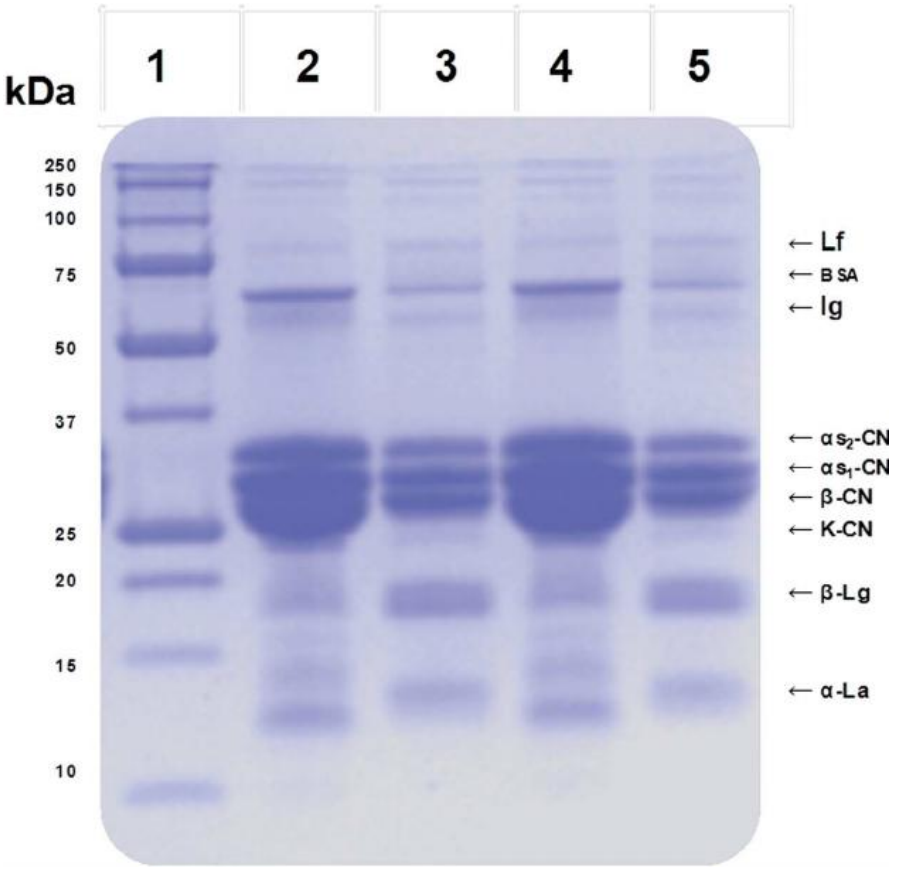
Denaturing electrophoresis (SDS-PAGE)



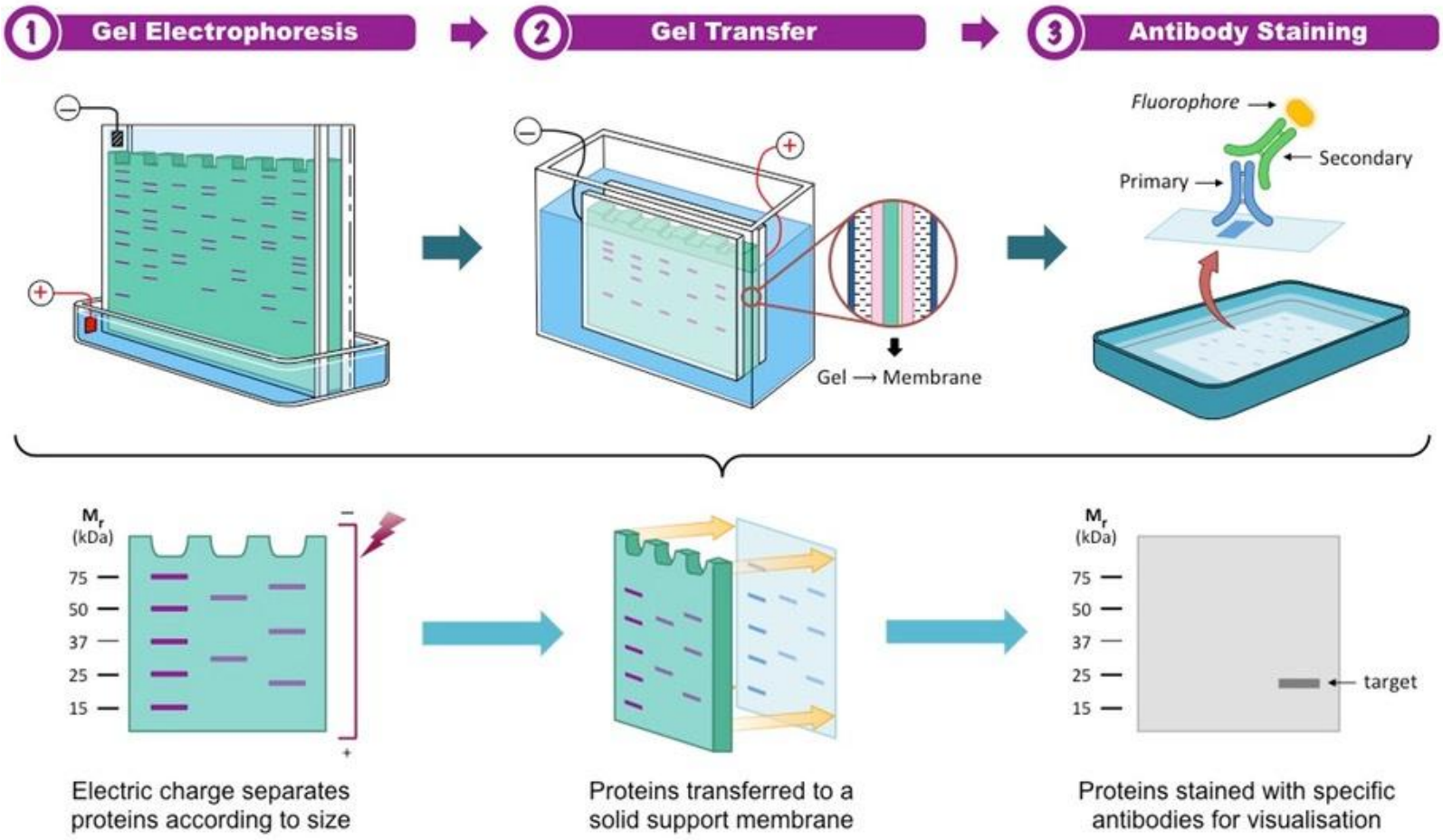
How does an SDS-PAGE separation work?

- **negatively charged proteins move to positive electrode**
- **smaller proteins move faster**
- **proteins are separated by their size (molecular weight)**

Separated proteins could be stained in the gel



Western blotting – detection of proteins using antibodies



Isoelectric phocusing

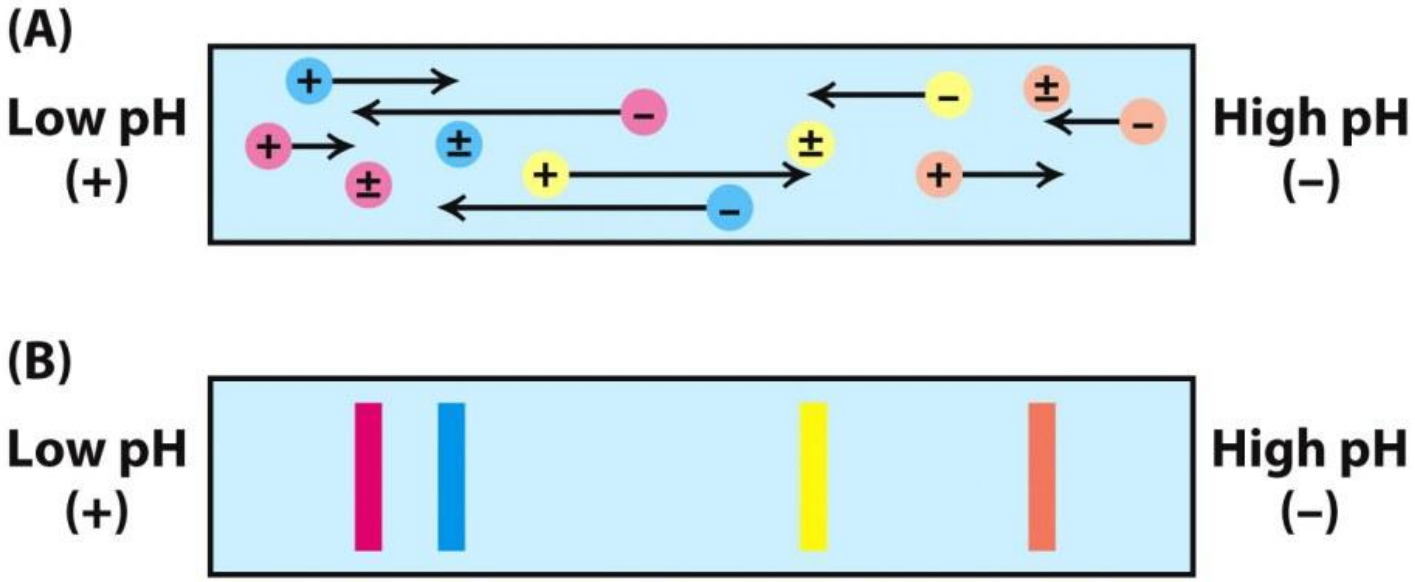
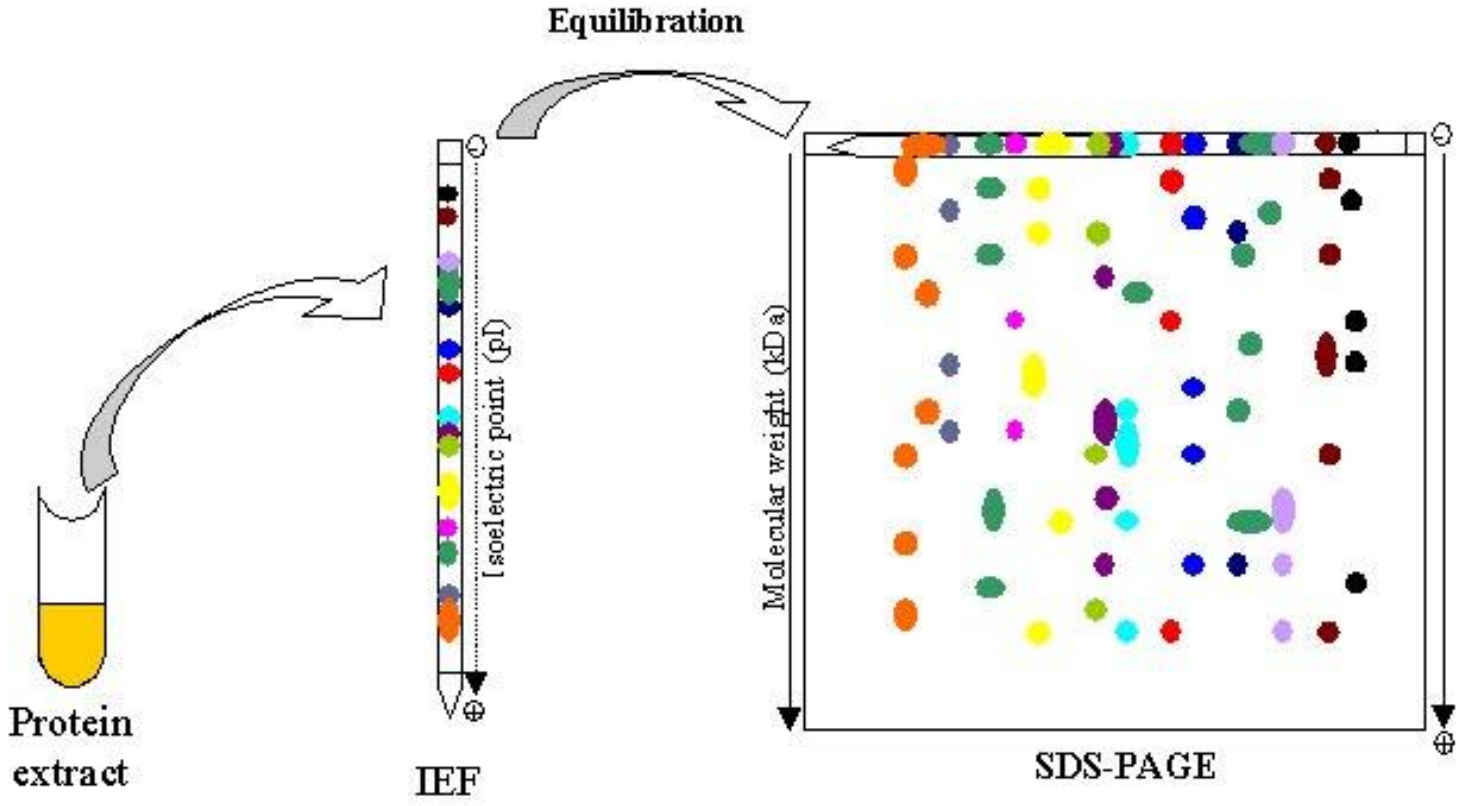
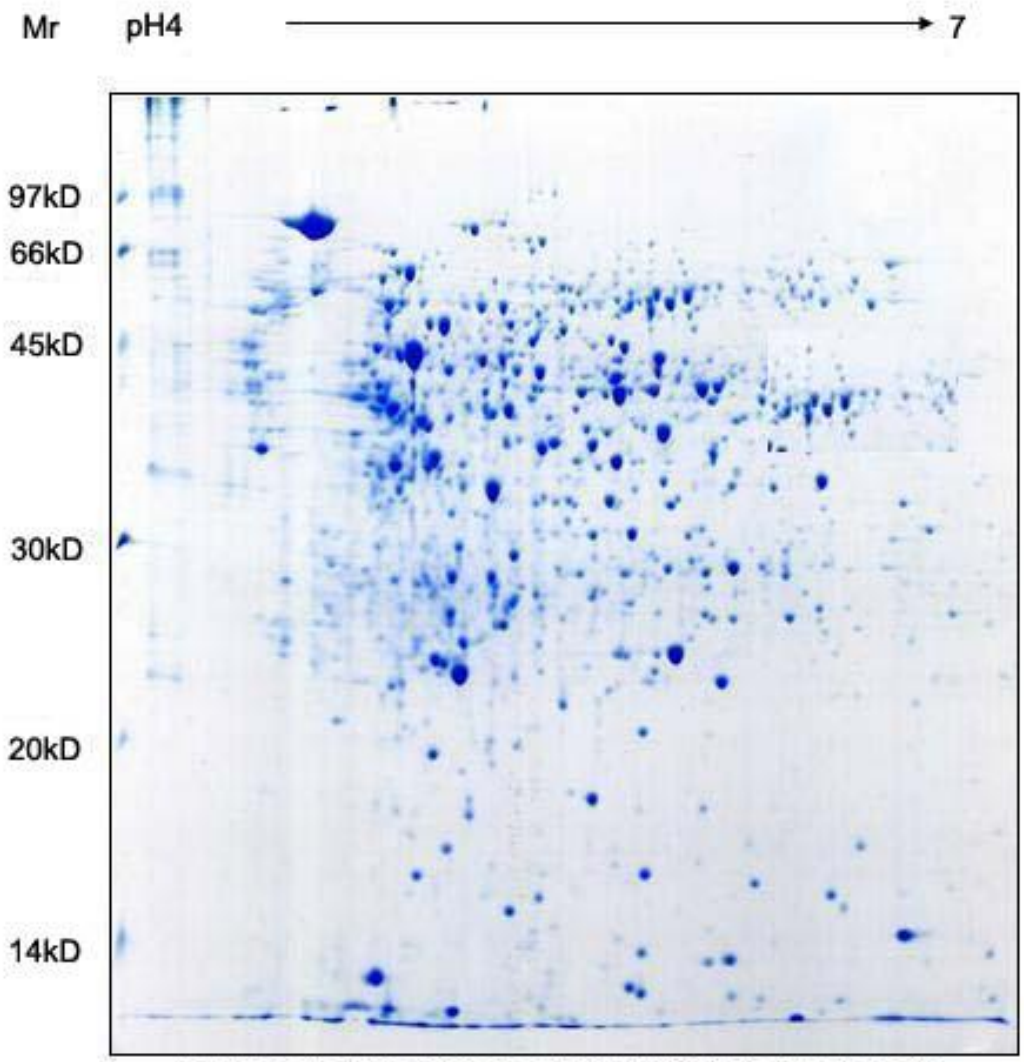


Figure 3.11
***Biochemistry*, Seventh Edition**

2D electrophoresis





Commassie Blue Stain bacteria 2D Gel Electrophoresis