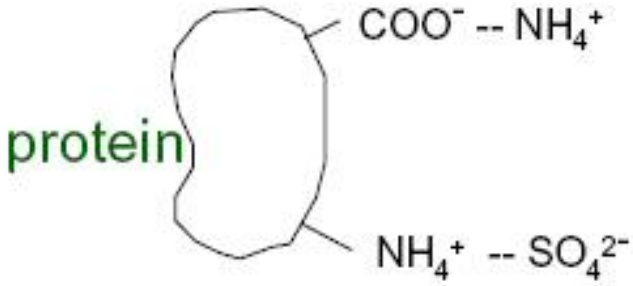
Concentration of product ; Precipitation

A very common first step after cell disruption for recovery of intracellular proteins.

Water-protein interactions are key to understanding protein precipitation / solubility in water.

Salting-Out

addition of $(NH_4)_2SO_4$ or Na_2SO_4 up to high concentrations → 1 to 3 Molar!



salts exclude water from the surface leading to protein-protein interactions and precipitation

Precipitation

Organic Solvent Addition

can also reduce protein-water interactions and promote protein-protein interactions leading to precipitation.

Isoelectric Precipitation

at the pH of the isoelectric point, a protein is uncharged, reducing protein-water interactions which leads to precipitation. Warning: extremes in pH may denature the protein product.

Protein precipitation

- **Factors affecting protein solubility**
  - Protein structure and size
    - Larger size → lower solubility
  - Protein charge
    - Higher charge → higher solubility

Protein solubility is a function of ionic strength (salt concentration).

$$\log\left(\frac{S}{S_o}\right) = -K_S' I$$

S = protein solubility (g/L)

S_o = protein solubility at 0 ionic sstrength, (g/L)

K_S' = a salting out constant (moles/L)

(a function of pH and temperature)

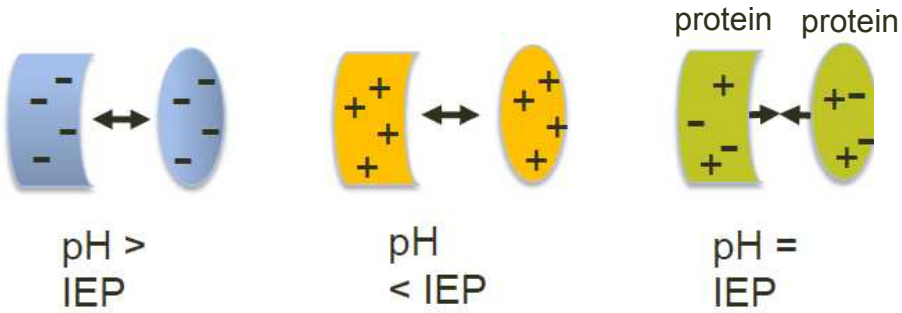
$$I = \text{ionic stsrength} = \frac{1}{2}\sum C_i Z_i^2 \text{ (mole/L)}$$

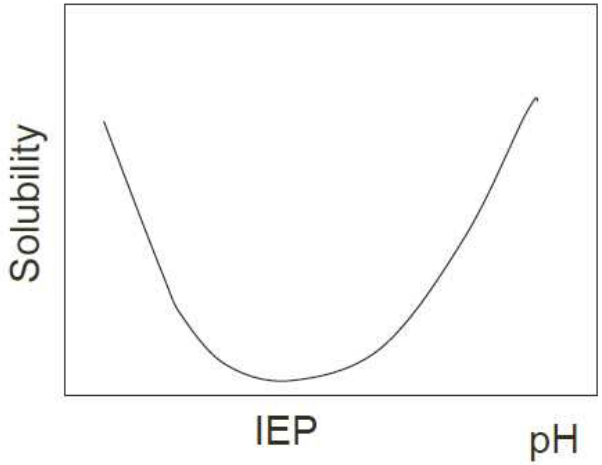
C_i = molar concentration of salt ion (mole/L)

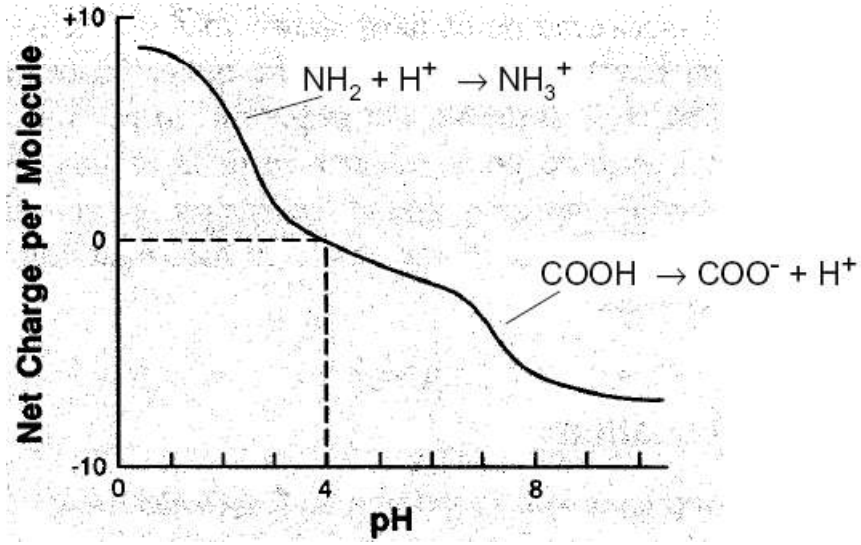
Z_i = charge on salt ion

Concentration of product ; Isoelectric precipitation

- Minimized electrostatic repulsion at pI→ hydrophobic interaction
- Proteins have a minimum solubility around their isoelectric point







Product purification : Chromatography

Contaminants often remain with product after primary isolation.

Chromatography: is the most important separation method for biochemical products.

Basic Concepts:

1. Separation is based on differential affinities of solutes toward a solid adsorbent material.

Chromatography

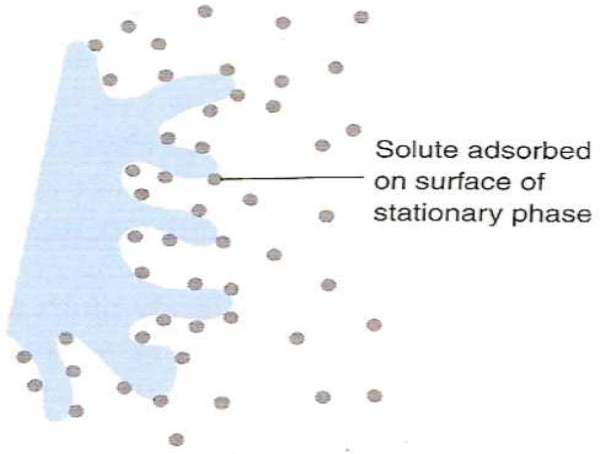
2. Different kinds of affinity

* → electric charge ··· ion exchange chromatography
  → van der Waals force ··· adsorption chromatography
  → solubility in liquid ··· liquid-liquid partitioning chromatog.
  → solute size/diffusion ··· gel filtration chromatography

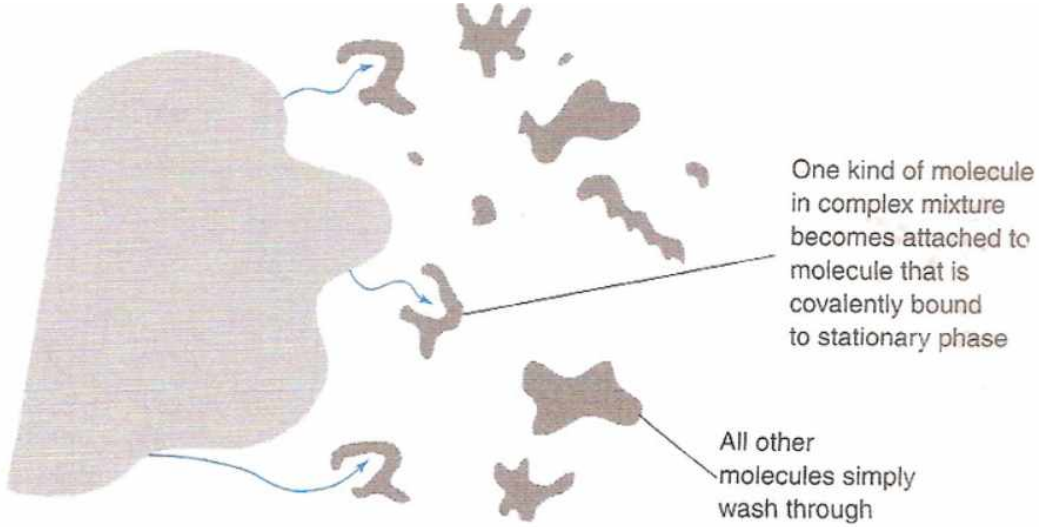
* → receptor - ligand ··· affinity chromatography
  → hydrophobic interactions ··· hydrophobic chromatography

* most common usage

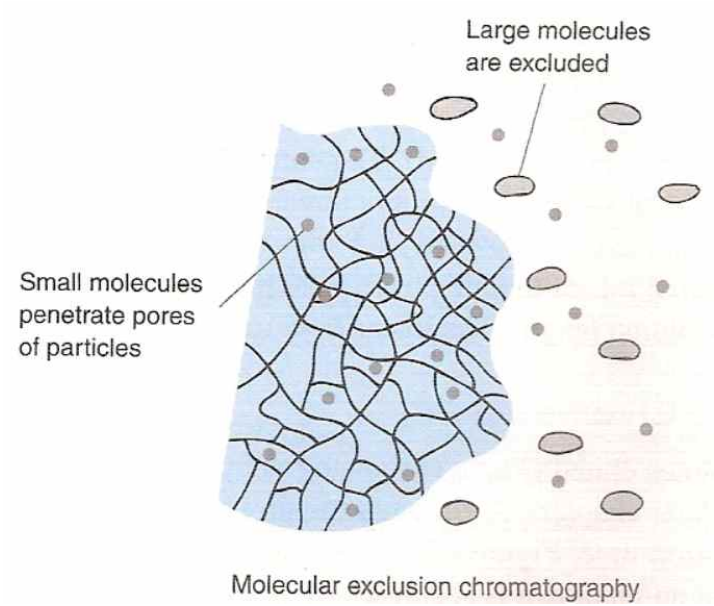
Chromatography



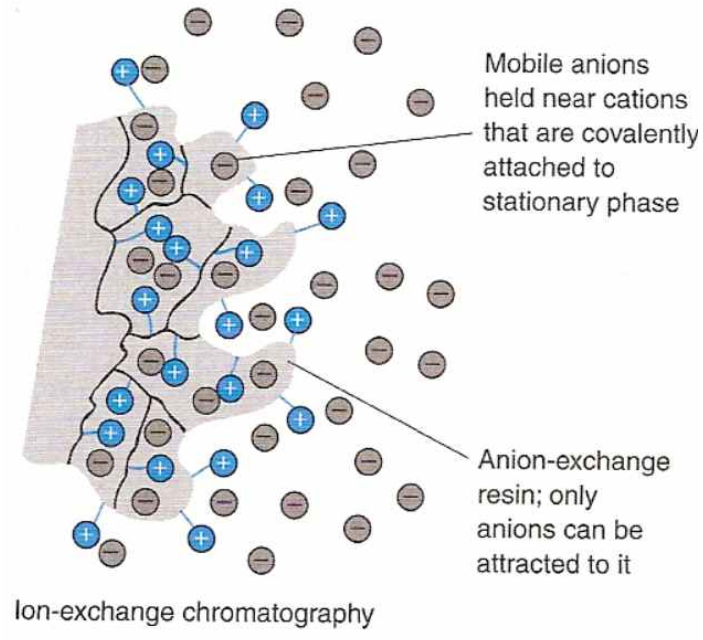
Adsorption chromatography



Affinity chromatography



Molecular exclusion chromatography



Ion-exchange chromatography

Adsorption chromatography

Definition: the removal of selected chemicals from a mobile fluid phase into an immobile solid phase.

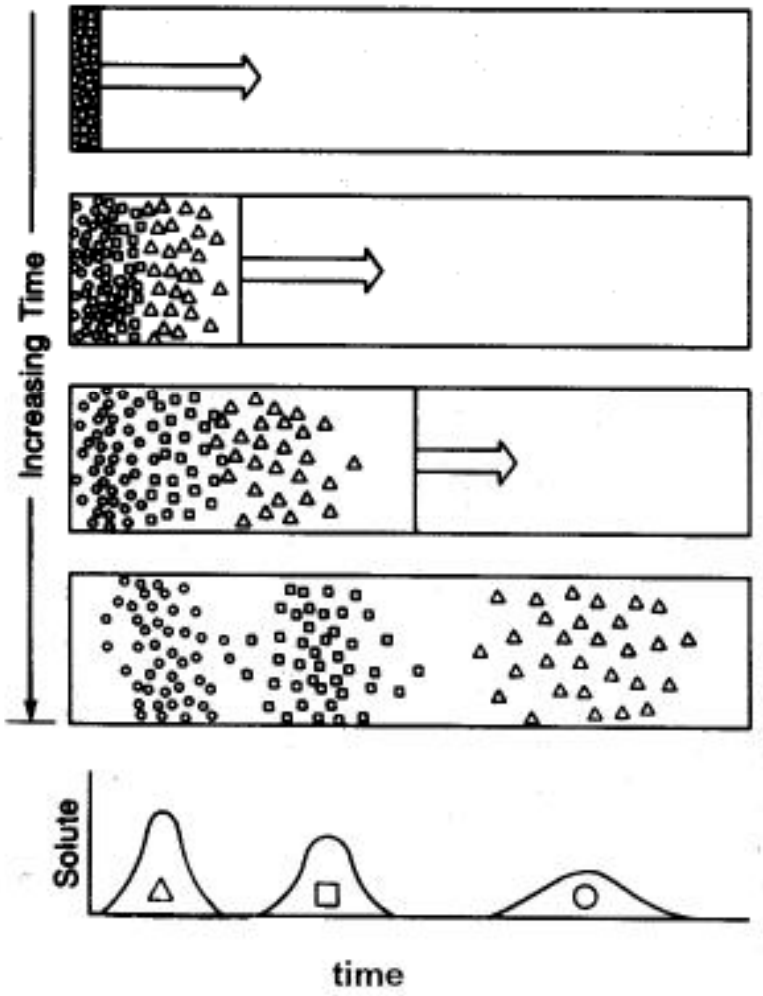
Adsorbents: solid materials to which the chemicals (solutes, adsorbates) adhere. These are the immobile phase.
(= stationary phase)

Examples:
- activated carbon
- ion exchange resins
- alumina
- silica gel
- other gels: dextran or agarose

Chromatography

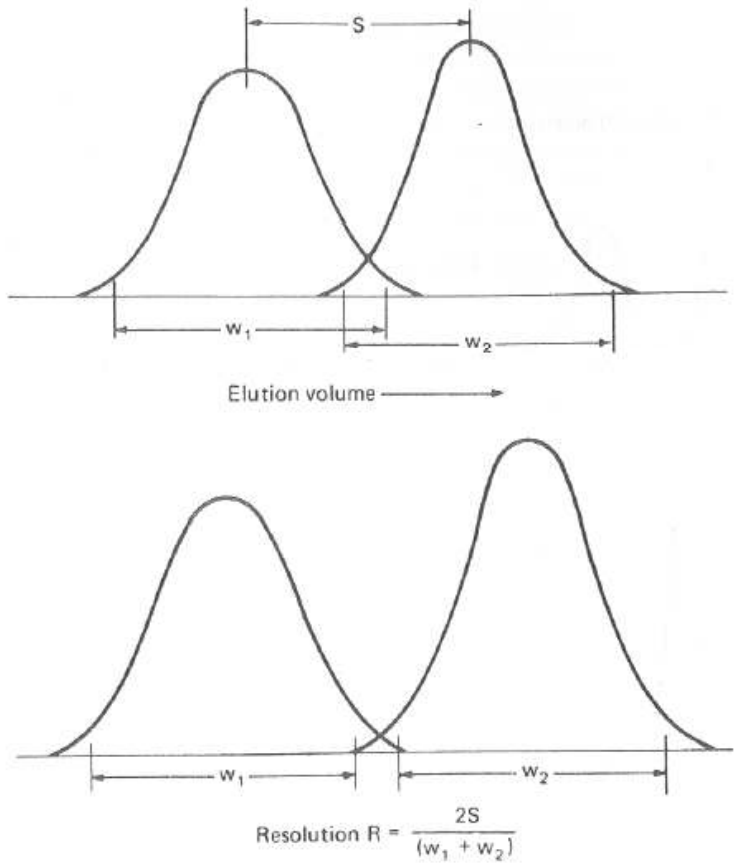
- each solute is carried along at a different apparent velocity, depending upon the strength of interaction with the column packing.
  (stationary phase)
- ideally, each solute exits the column as a discrete band of material.

"Bioprocess Engineering: Basic Concepts"
Shuler and Kargi,
Prentice Hall, 2002



Chromatography resolution

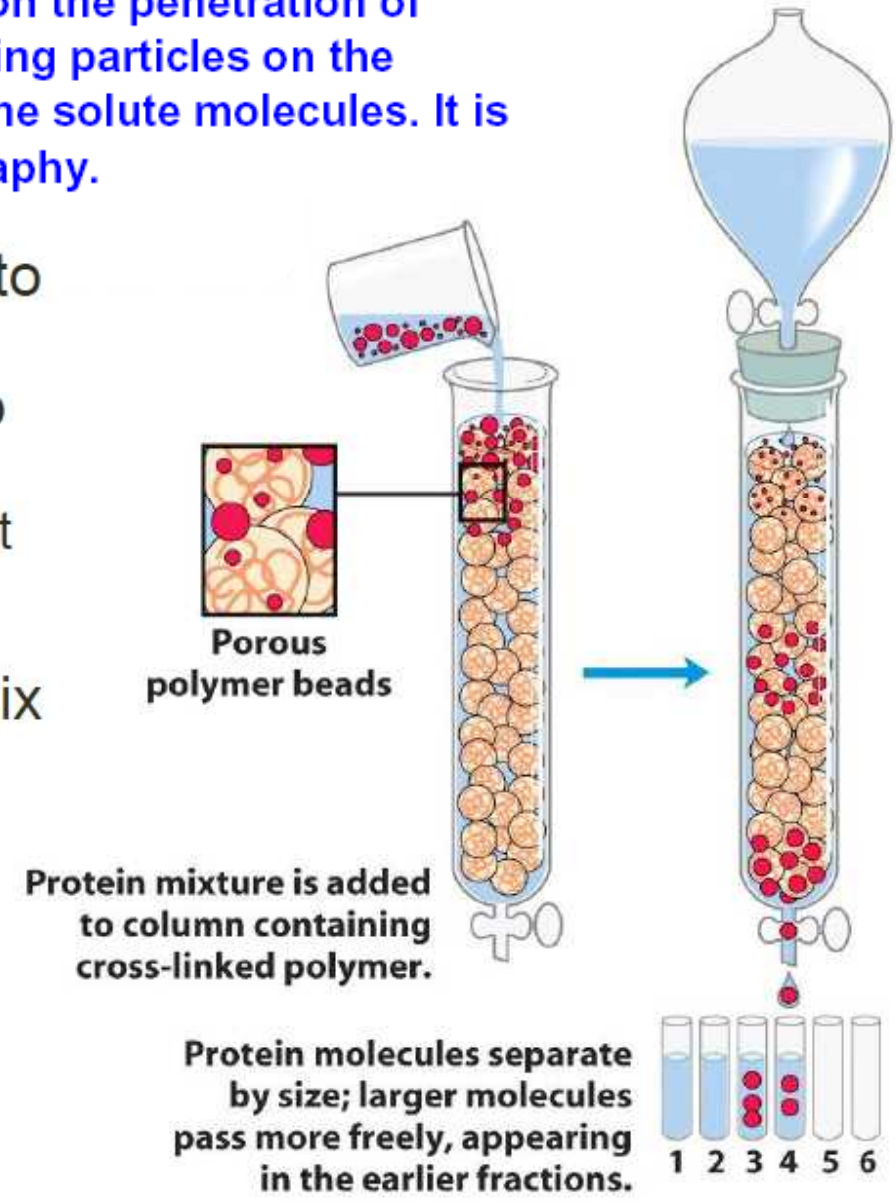
- **R = 2S/(w1 + w2)**
  - S : Separation
    - The distance between two neighboring peak maxima
  - W : Peak width
    - Defined according to requirements of allowable cross-contamination
    - Cannot defined based on total width because of trailing edges



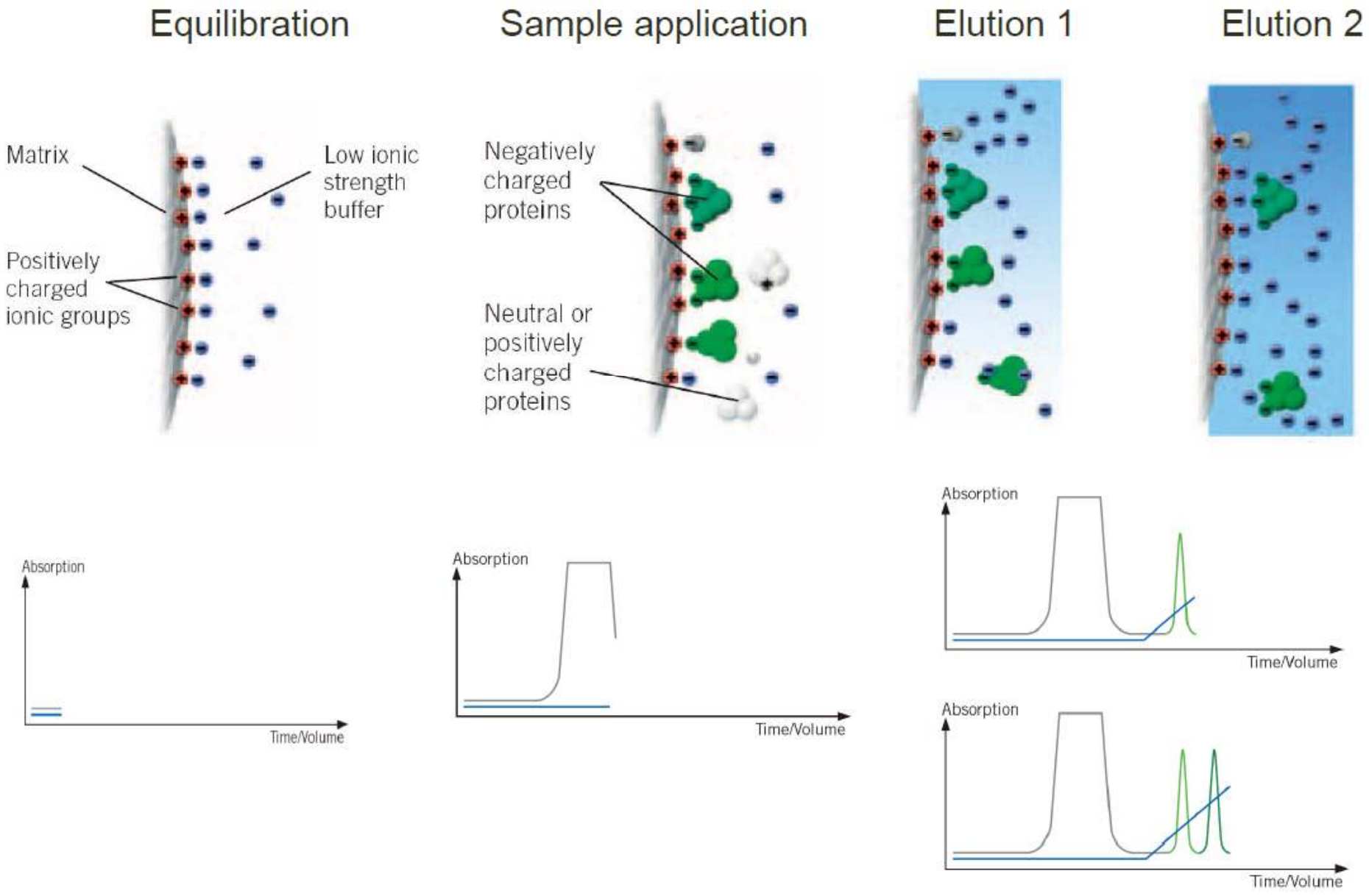
Gel permeation chromatography

Gel Permeation Chromatography: **is based on the penetration of solute molecules into small pores of packing particles on the basis of molecular size and the shape of the solute molecules. It is also known as size exclusion chromatography.**

- Separation of protein according to differences in size
- Limited accessibility of protein to cross-linked porous matrix
  - Small molecules diffuse in and out of the pores → stay longer on the column
- No binding of protein to the matrix
  - Buffer condition does not affect resolution



Ion exchange chromatography



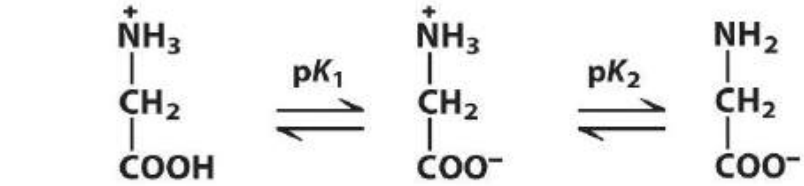
Ion exchange chromatography

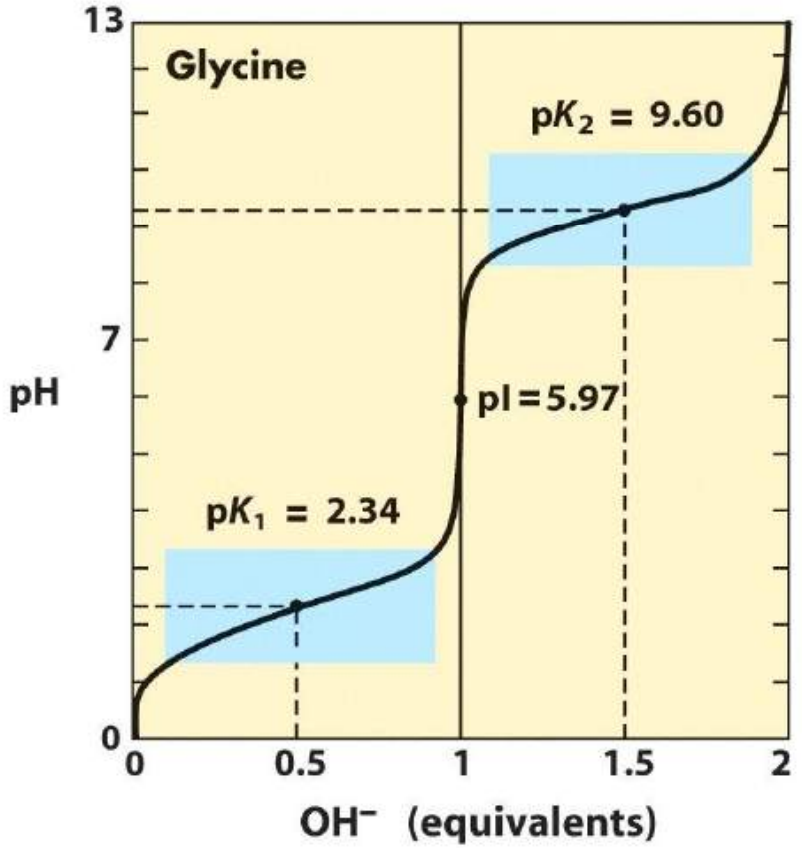
- **Choice of buffer pH**
  - Anion exchanger
    - 0.5 to 1.5 pH unit above the pI of the target protein
  - Cation exchanger
    - 0.5 to 1.5 pH unit below the pI of the target protein

pI	Ion exchange	Buffer pH
8.5	Cation	7~8
7.0	Cation	5.5~6.5
5.5	Anion	6~7

Ion exchange chromatography

- **Amino Acids as Acids and Bases**
  - Zwitterion
    - Acts as either an acid or a base
  - Two p*Ka* and two buffering regions
  - pI: ioselectric point or isoelectric pH
    - The point with zero electric charge
    - Above pI : negative charge
    - Below pI : positive charge
  - pI = (pK_1 + pK_2)/2 = 5.92



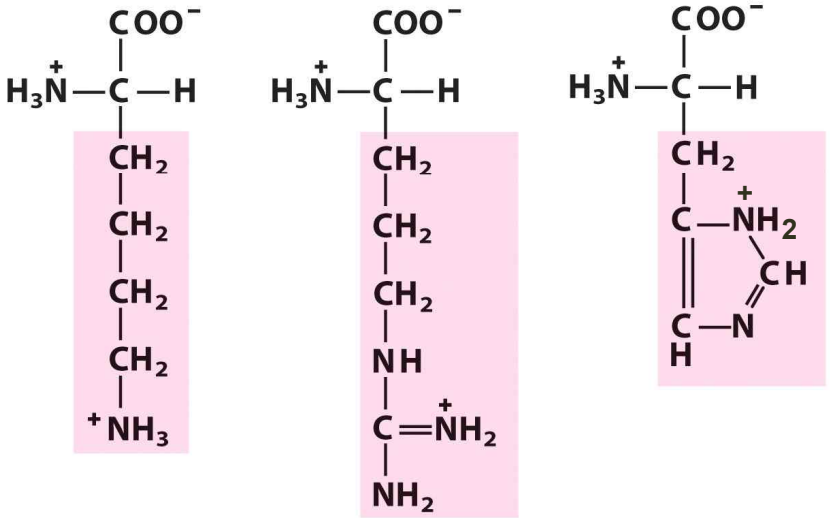


- **Charges in a protein**
  - **Positive charge**
    - N-terminal NH^{3+}
    - Arg, Lys (pH< 8.5)
    - His : pK_R = 6
  - **Negative charge**
    - C-terminal COO^-
    - Asp, Glu
    - Cys (pH>8)

Ion exchange of amino acids

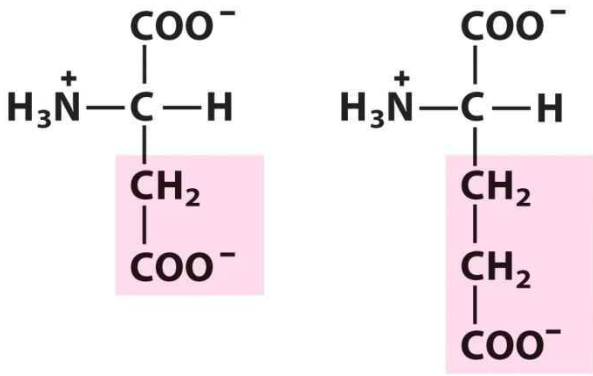
Charged Amino Acid R Groups at Neutral pH

Positively charged R groups



Lysine Arginine Histidine

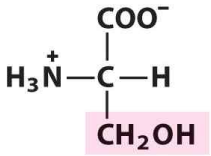
Negatively charged R groups



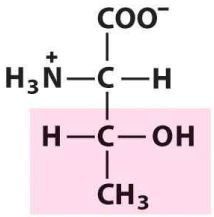
Aspartate Glutamate

Ion exchange of amino acids

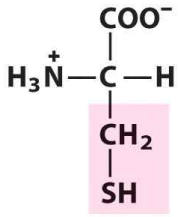
Polar Amino Acid R Groups at Neutral pH



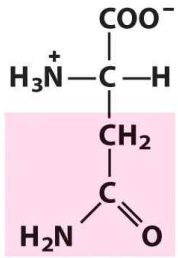
Serine



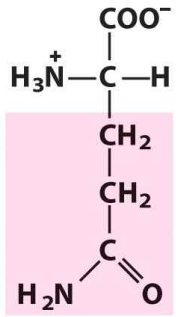
Threonine



Cysteine

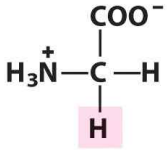


Asparagine

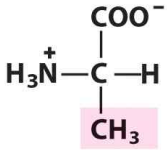


Glutamine

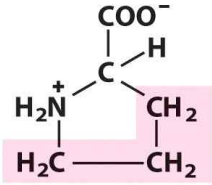
Nonpolar Amino Acid R Groups at Neutral pH



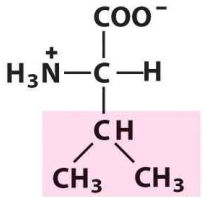
Glycine



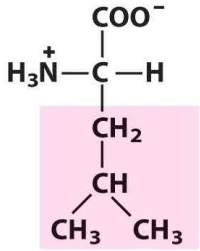
Alanine



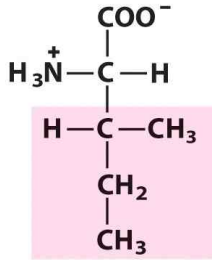
Proline



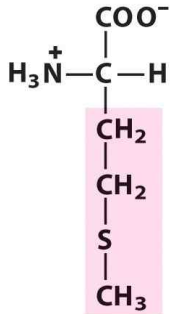
Valine



Leucine



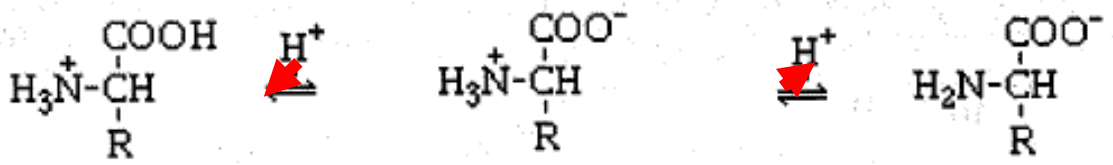
Isoleucine

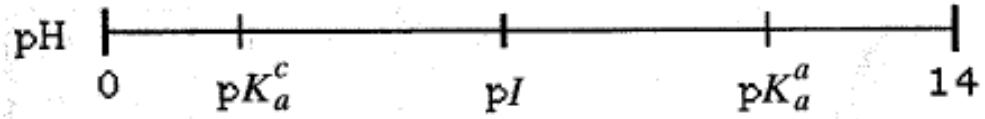


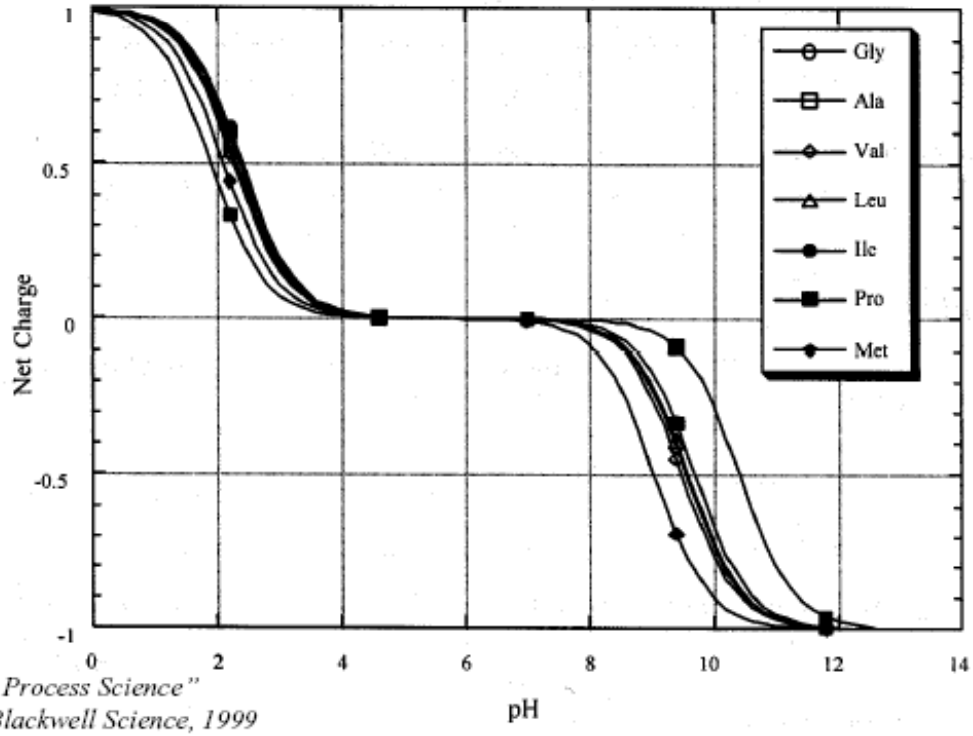
Methionine

Ion exchange of amino acids with nonpolar and polar R groups

← Protonation Deprotonation →

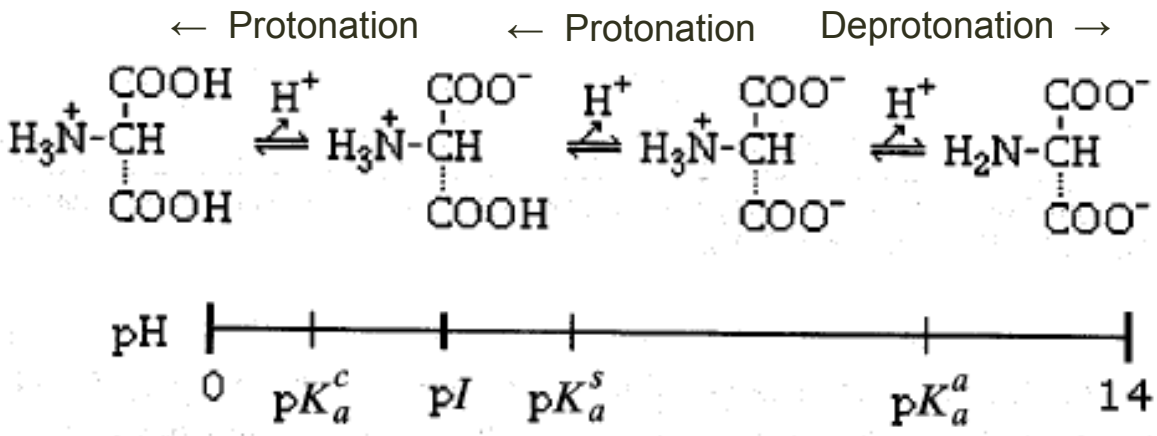


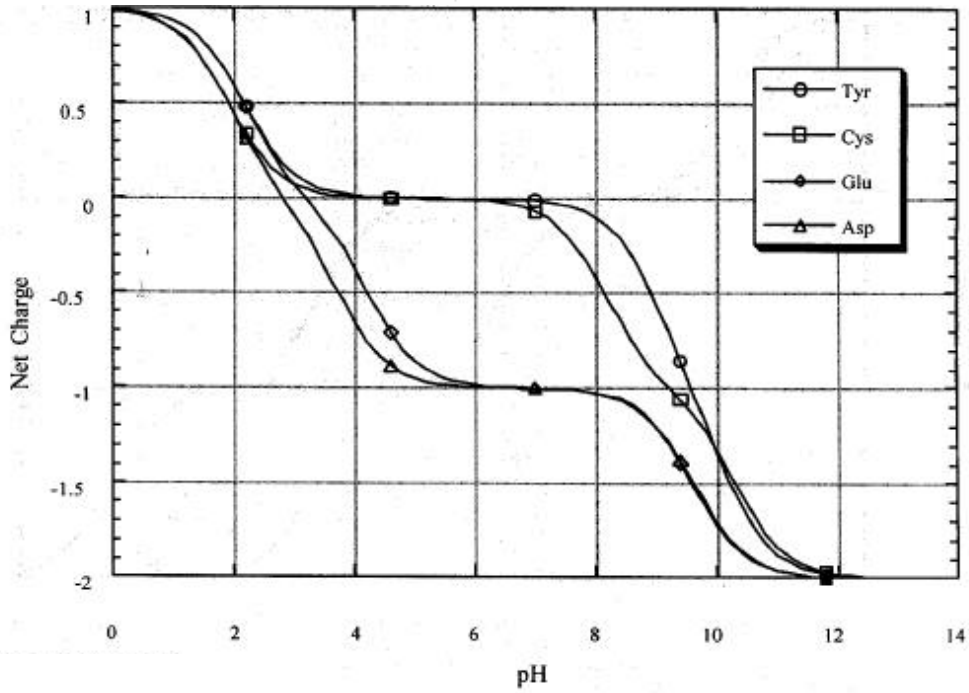




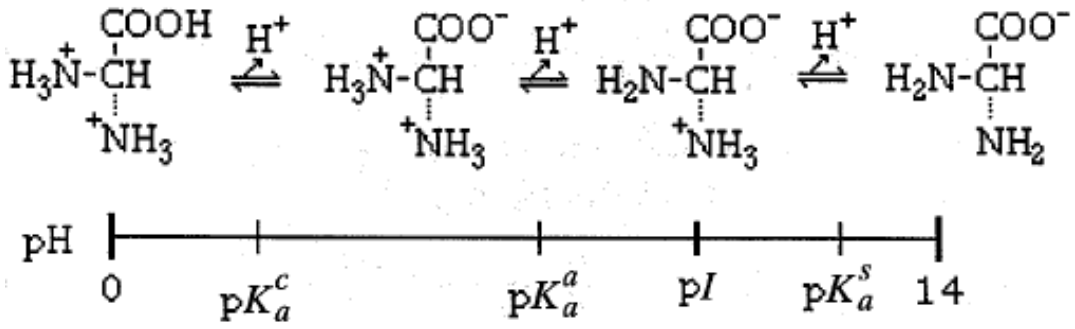
"Bioseparaion Process Science"
Garcia et al., Blackwell Science, 1999

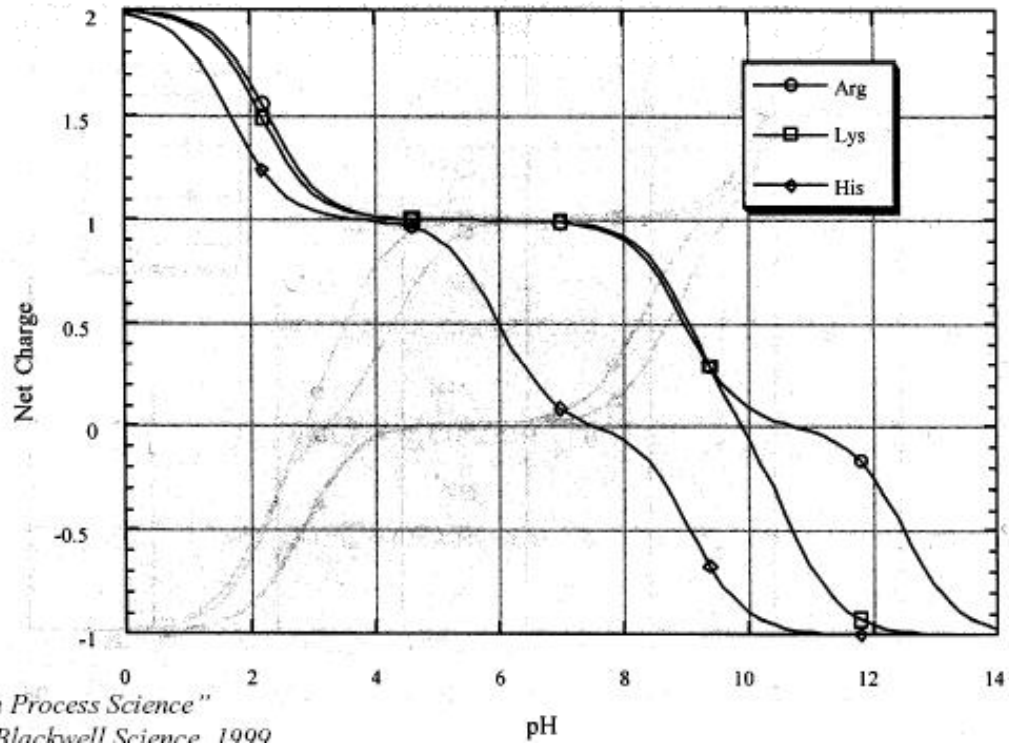
Ion exchange of amino acids with negatively charged R groups





Ion exchange of amino acids with positively charged R groups





"Bioseparaion Process Science"
Garcia et al. Blackwell Science 1999

Product purification : Crystallization

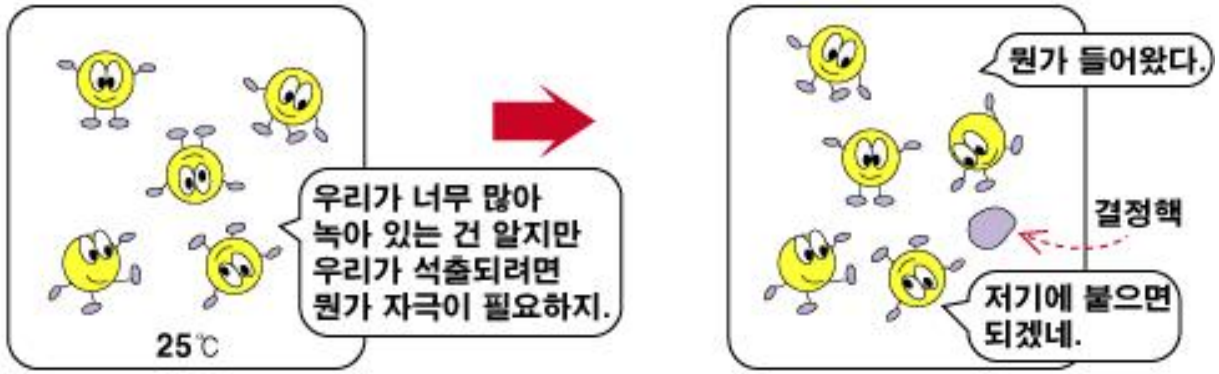
Crystallization is a nucleation process started from a concentrated solution:

1. Occurs when concentration exceeds saturation

2. Crystals have a well-defined morphology, large particle size

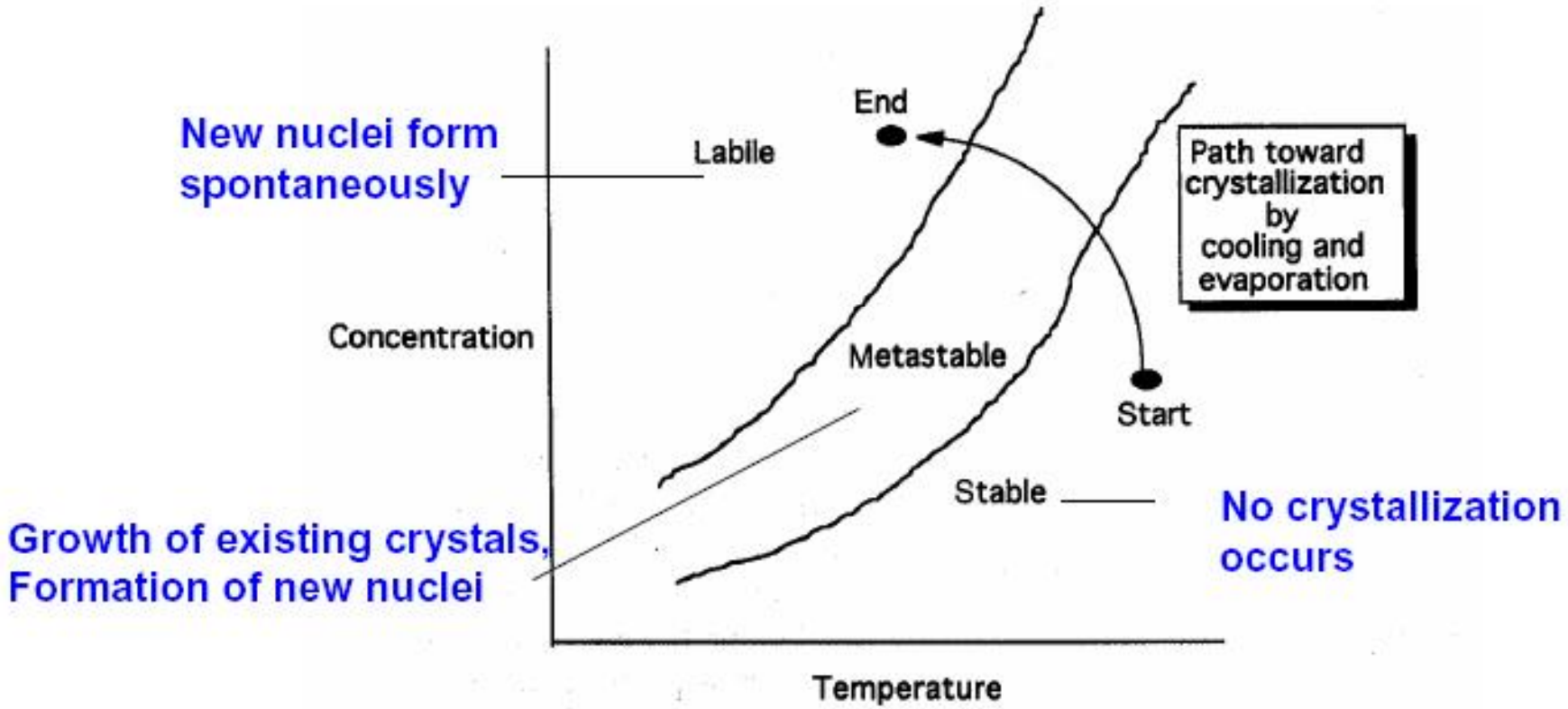
3. Homogeneous nucleation occurs when a solid interface is absent

4. Heterogeneous nucleation occurs when a foreign interface is present.



Crystallization

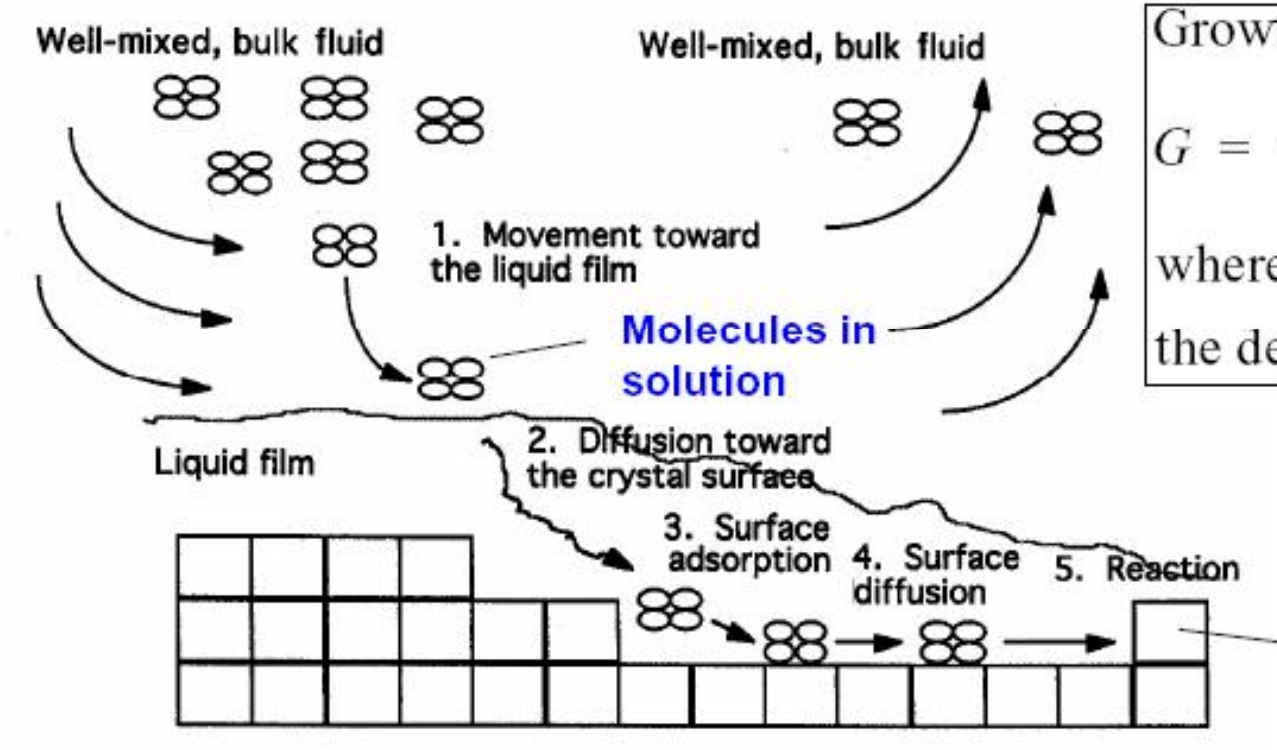
Characteristic zones of crystallization



"Bioseparation Process Science"
Garcia et al., Blackwell Science, 1999

Crystallization

Transport Processes During Crystallization



Growth Rate of Crystals,

$$G = \frac{dL}{dt} = k_g \Delta C$$

where $\Delta C = C^* - C$ is the degree of saturation

"Bioseparation Process Science"
Garcia et al., Blackwell Science, 1999