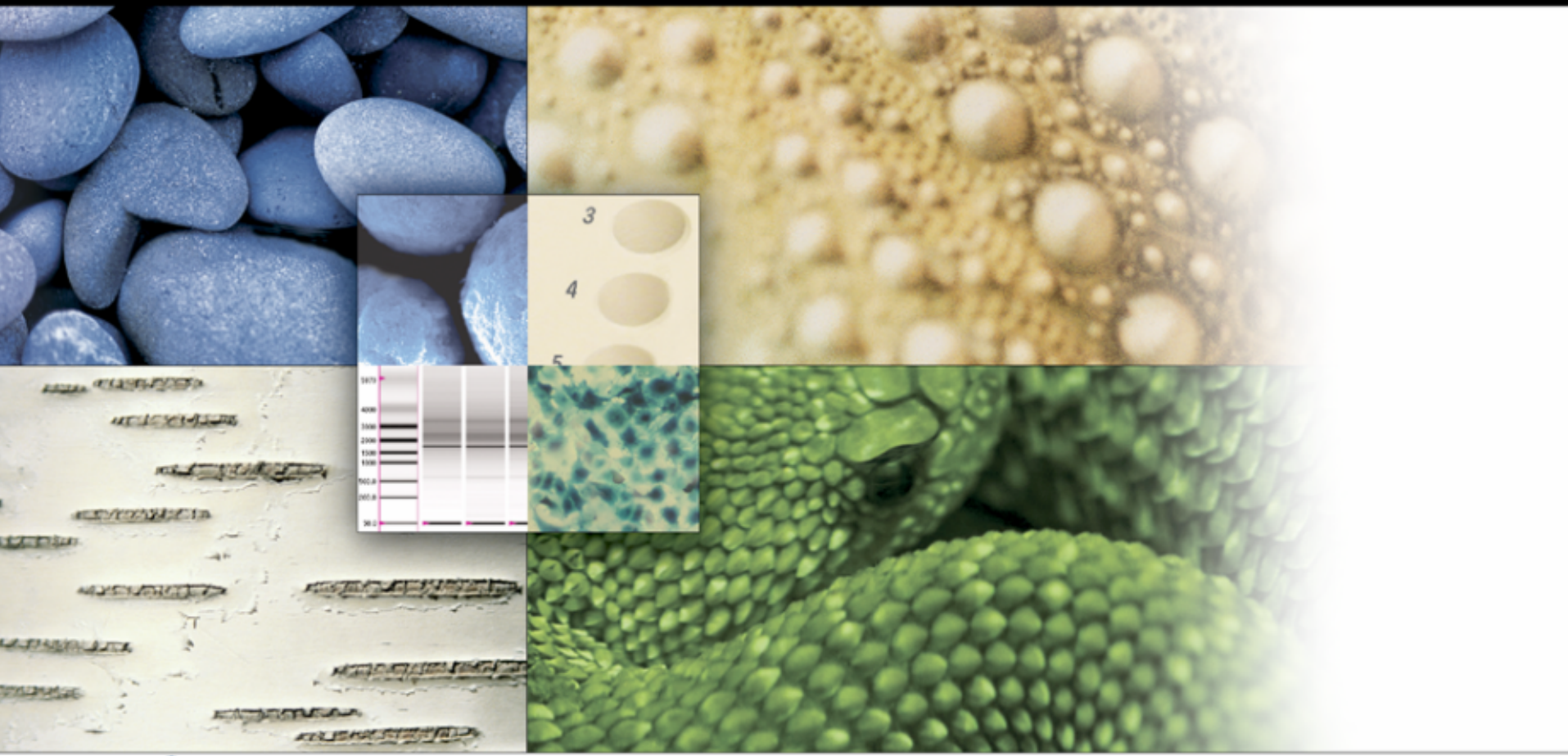
Protein purification



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Chromatography

- 어원 : Chroma (color) + Graphein (write)
- 시료의 성분을 정지상과 이동상에 분포시켜 분리
- GC (Gas chromatography)
- LC (Liquid chromatography)

  - HPLC : 5,000 ~ 6,000psi
  - MPLC(FPLC) : 150 ~ 3,600psi
  - LPLC : 150psi 이하

Why liquid chromatography?

- No heat generation
- No shearing force
- High sample recovery
- High resolution
- Easy to scale-up
- Easy to automation
- History of success

DuoFlow BIO-RAD

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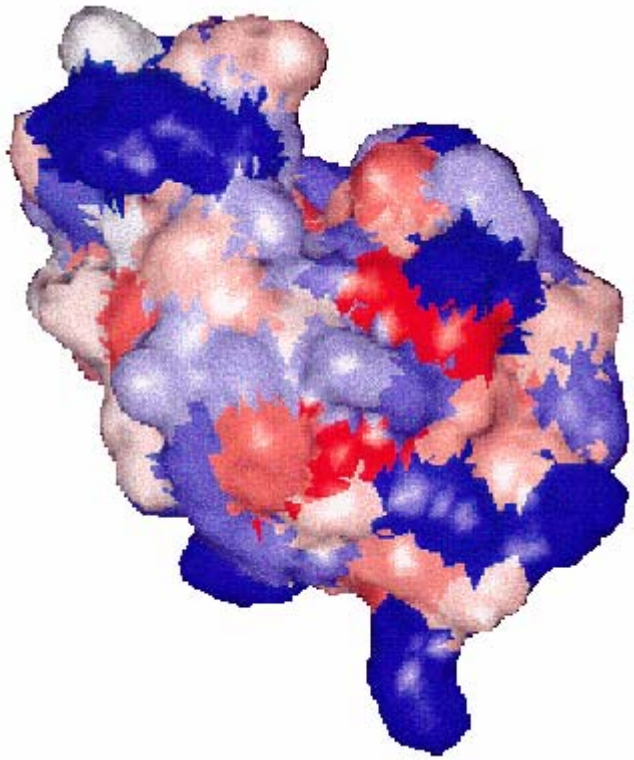
Protein



- Specific binding site
- Charged group
- Hydrophobic patch
  Phe, Trp, Ile, Leu, Val
- Size, shape

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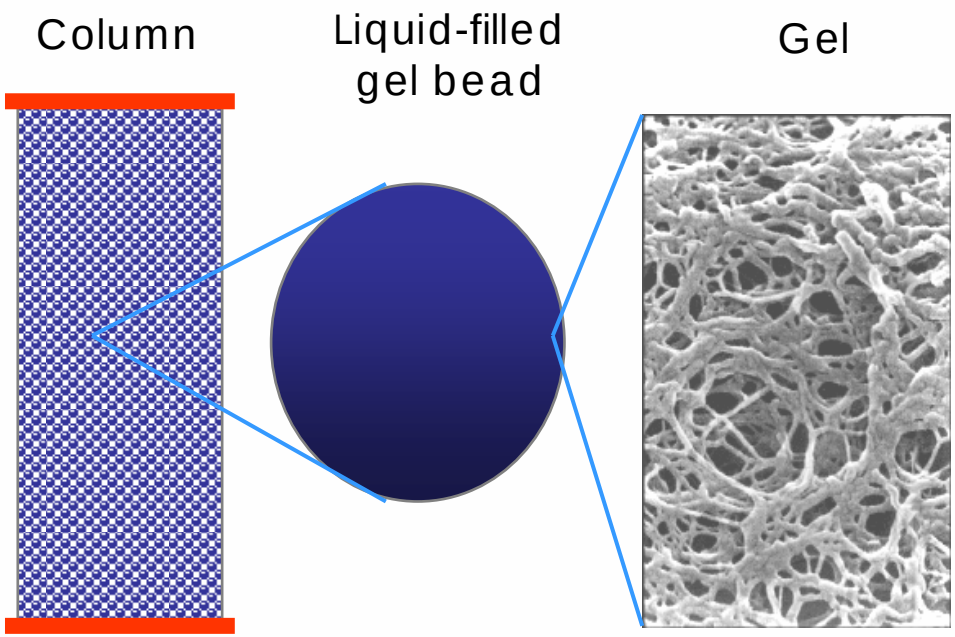
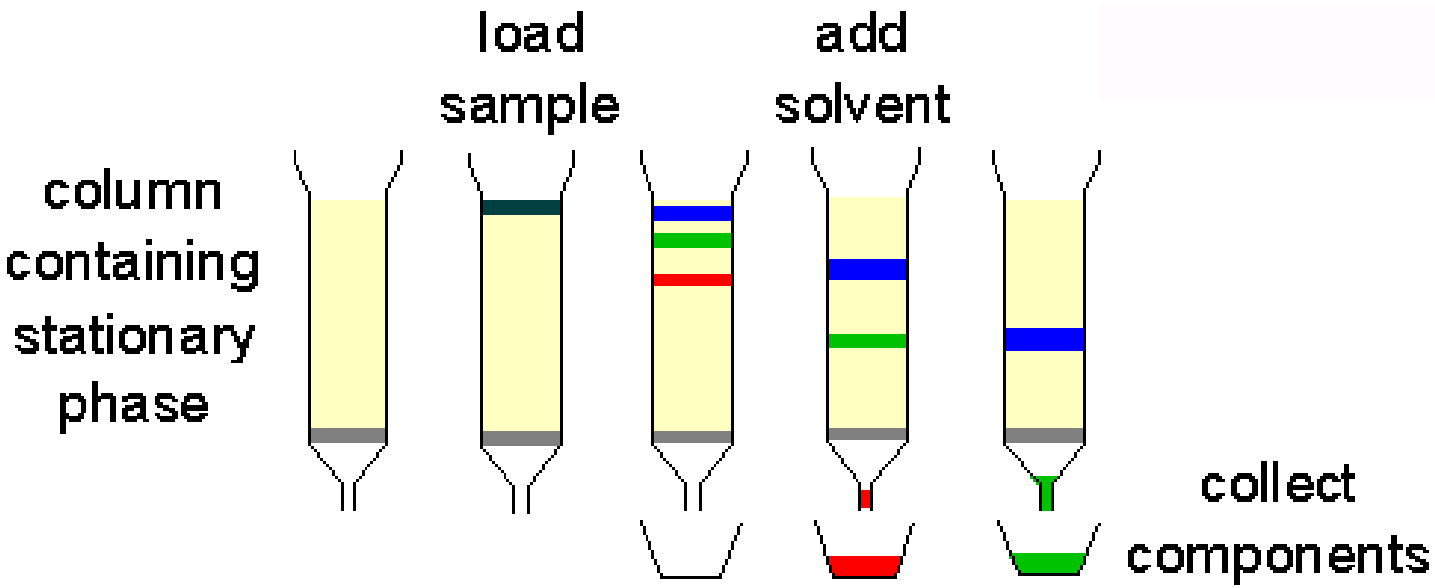
What happens in chromatography?



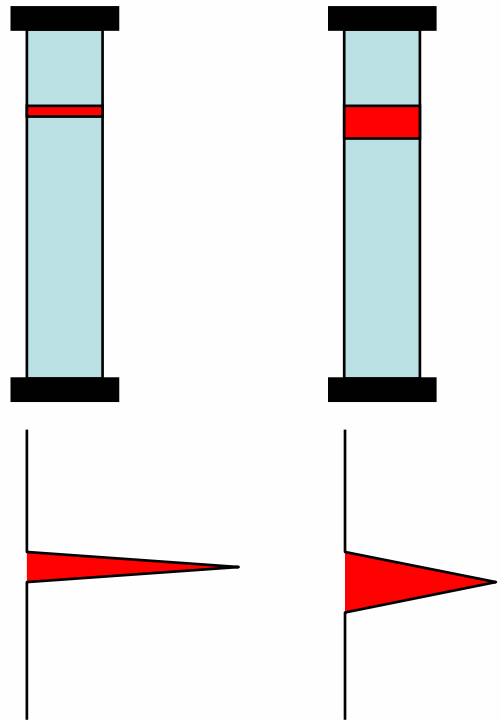
- Molecules to be separated diffuse into the beads
- They bind under one set of conditions and are released under (usually) other conditions
- Different molecules interact differently

Liquid Chromatography



Zone broadening

- Ideally a sample applied as a small, sharp zone elutes as a small sharp zone.
- This does not always happen!
- The zone will spread out on its way through the column.
- In many techniques this is opposed by concentration effects



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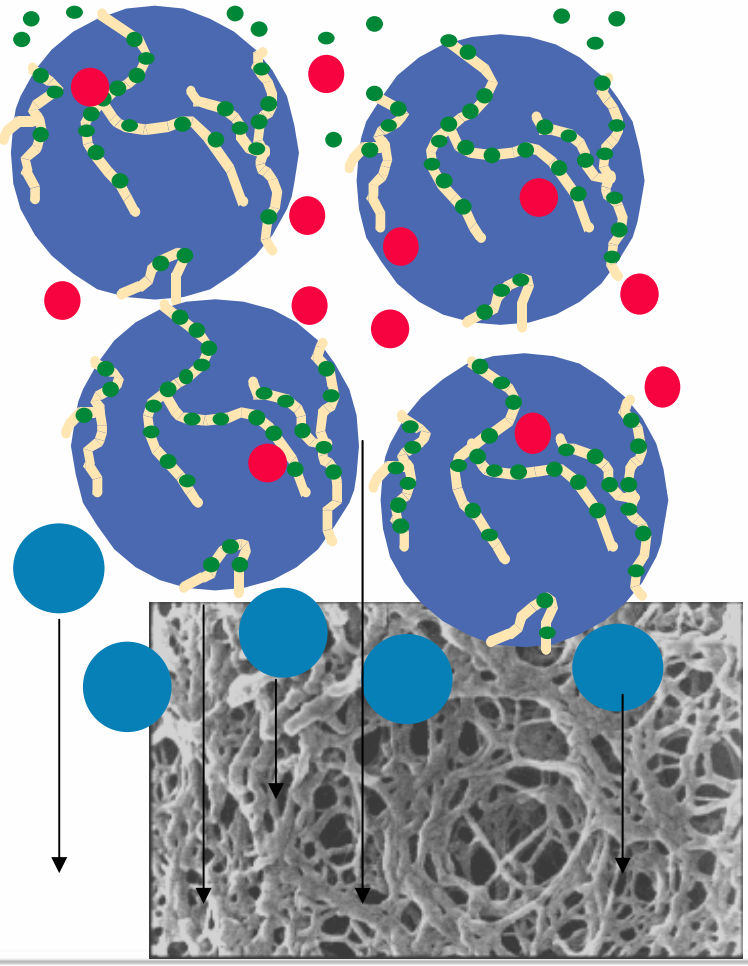
Zone broadening

- Depends on:
  - Particle size of matrix
  - Particle size distribution of matrix
  - Packing quality of the column
  - Sample (volume and viscosity)
  - Flow rate
- Expressed as efficiency

What is Gel filtration?

- Gel filtration separates molecules according to their sizes

- Restricted penetration of intraparticle pore volume of gel matrix due to incompatibility of the solvated size of the molecules and the pore dimensions



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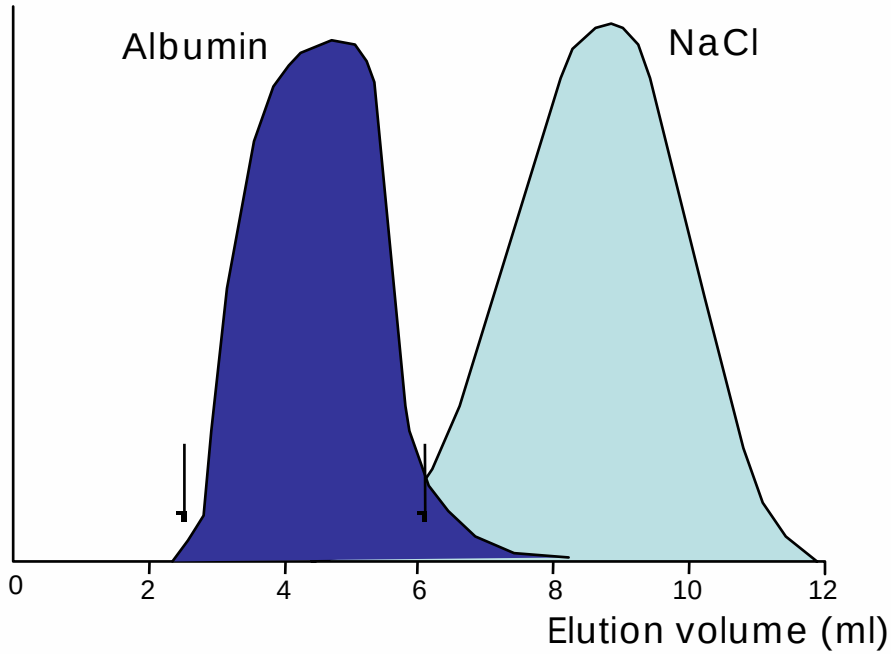
Group separation

- Desalting proteins



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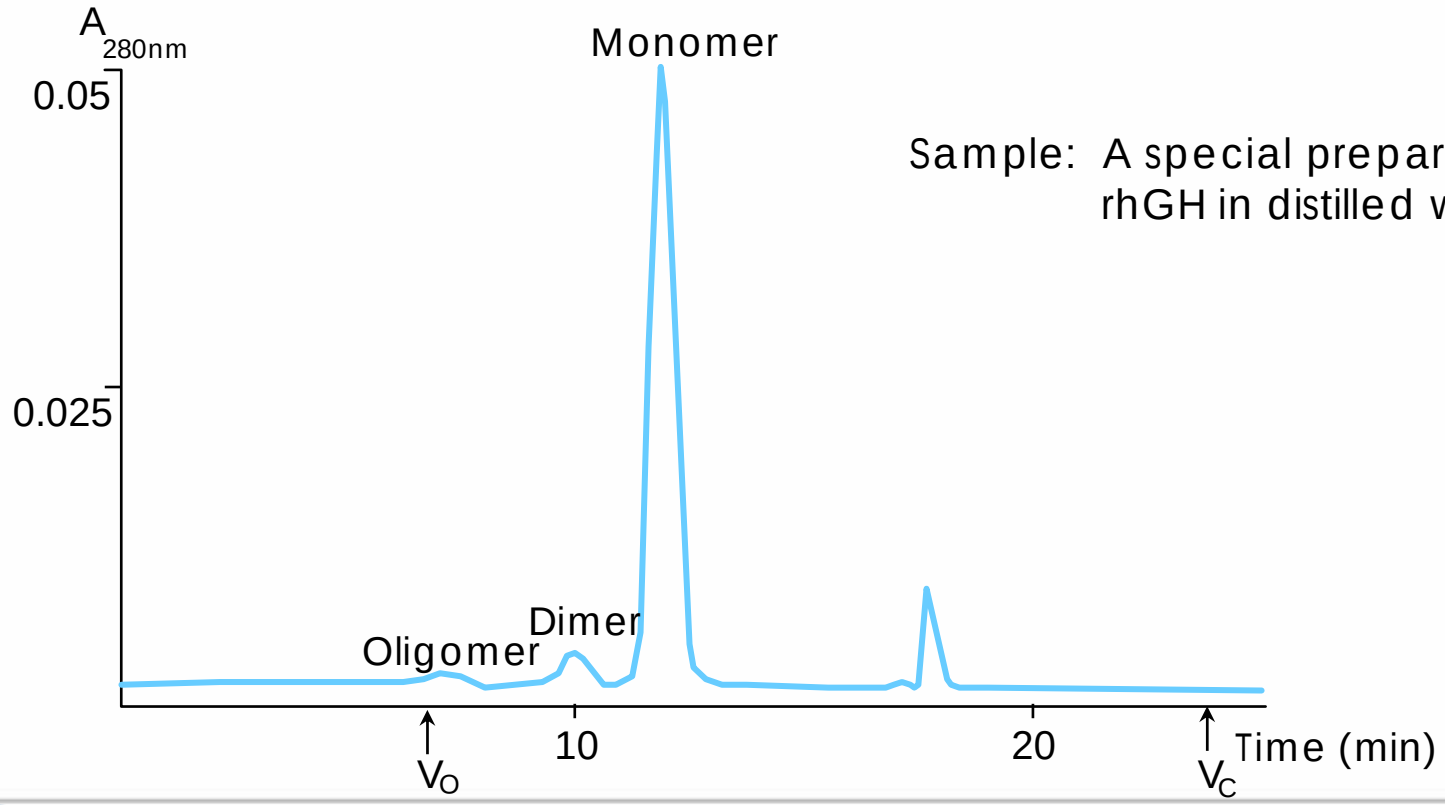
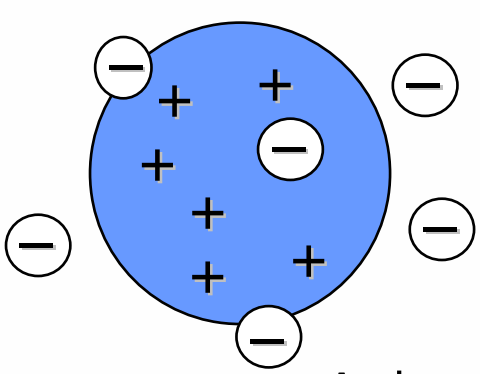
Fine fractionation

- Separation of dimer and oligomers from monomer

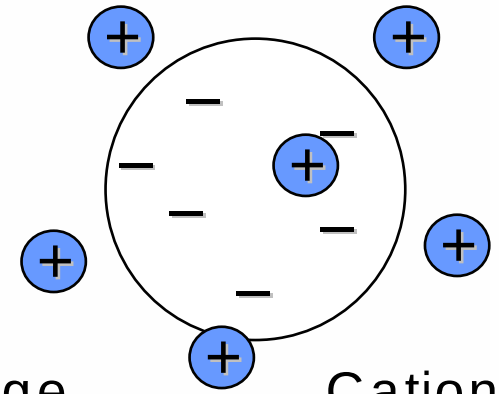


Ion exchange chromatography

- Separation in ion exchange chromatography depends upon the reversible adsorption of charged solute molecules to immobilized ion exchange groups of opposite charge



Anion Exchange



Cation Exchange

Strong and weak ion exchangers

- Weak ion exchangers: DEAE, CM
  capacity varies with pH
- Strong ion exchangers: Q, S
  capacity is constant over a wide range of pH

- Advantages of strong ion exchangers
  - charged at all pH
  - constant charge at all pH
  - faster and easier to equilibrate

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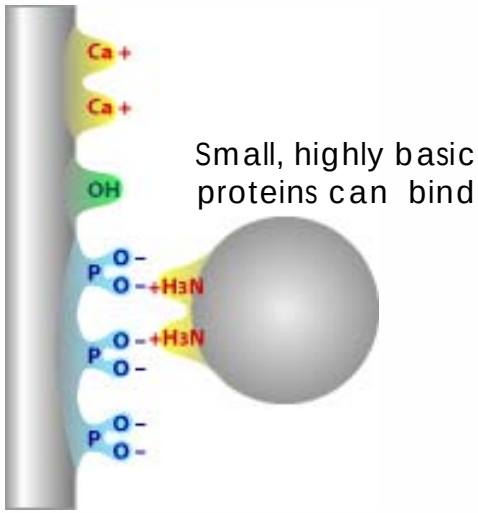
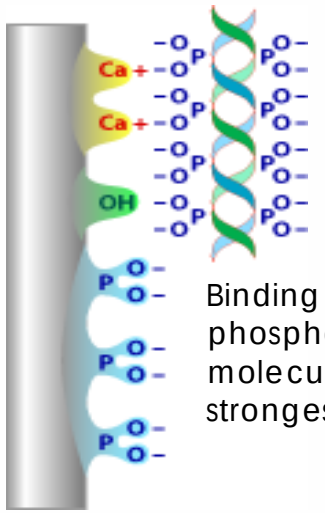
Mixed mode ion exchanger

- Hydroxyapatite chromatography separates molecules according to size, pI and charge
- Contains a calcium (Ca^{2+}) and phosphate (PO^{4-}) backbone

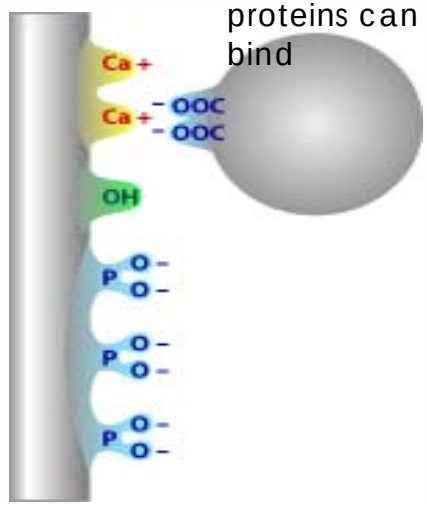


Small, highly basic proteins can bind



Binding of phosphorylated molecules is the strongest

Highly acidic proteins can also bind



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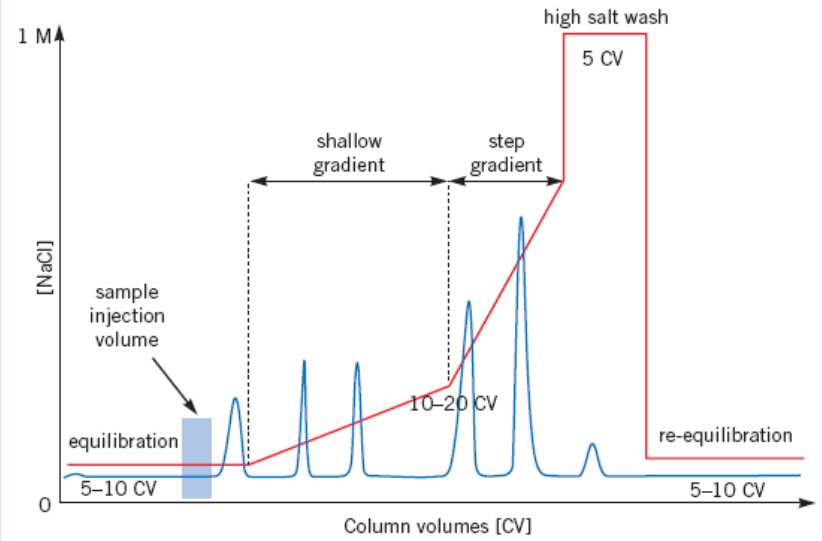
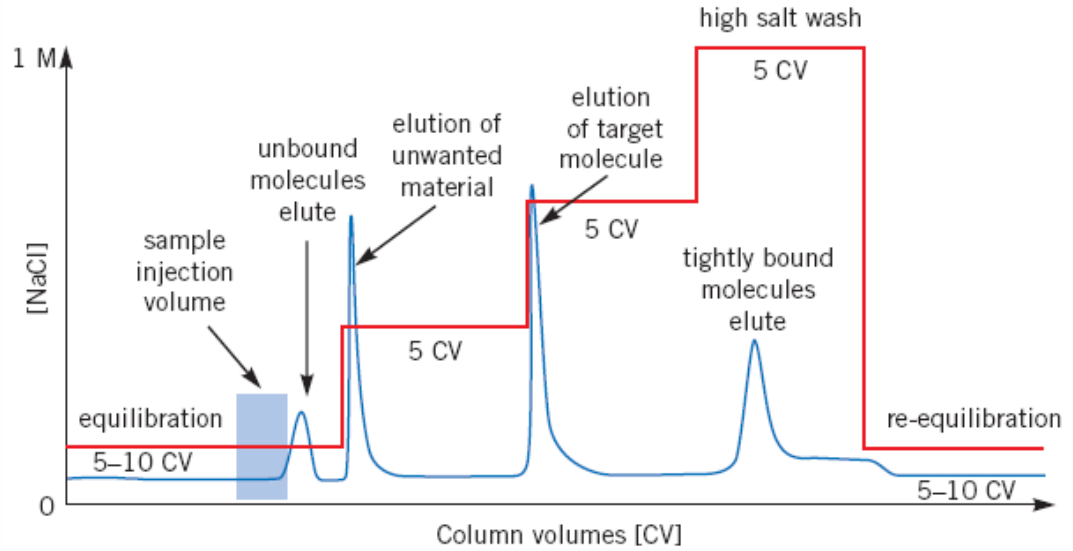
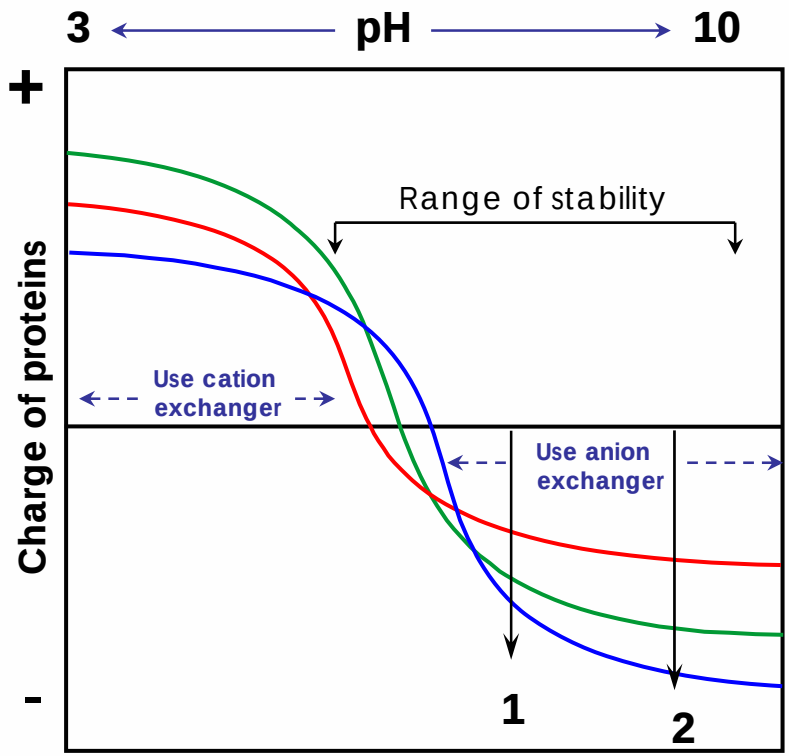
Typical linear gradient & step elution



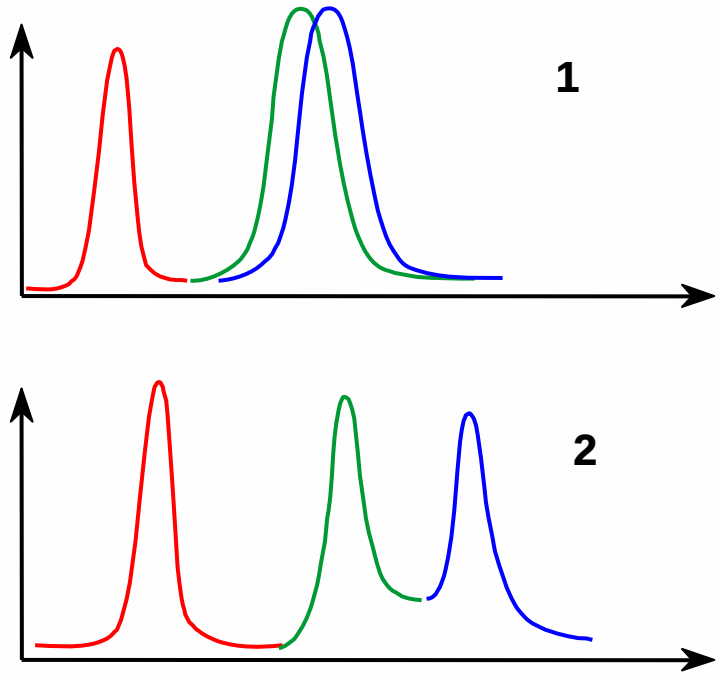


Importance of pH

Titration curves



Chromatograms at the marked values of pH



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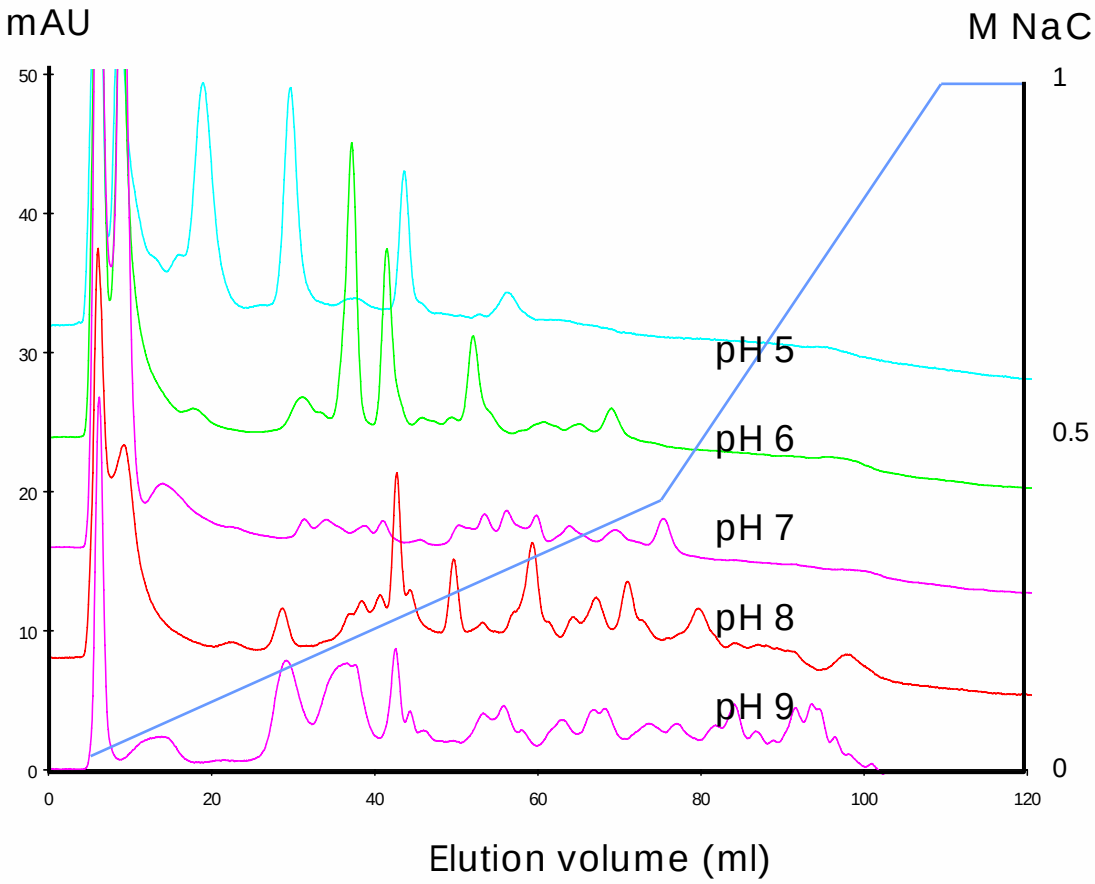
Determining optimal pH by scouting



pH scouting for the separation of pancreatin

Column: AIEX
Sample: 2 mg crude pancreatin

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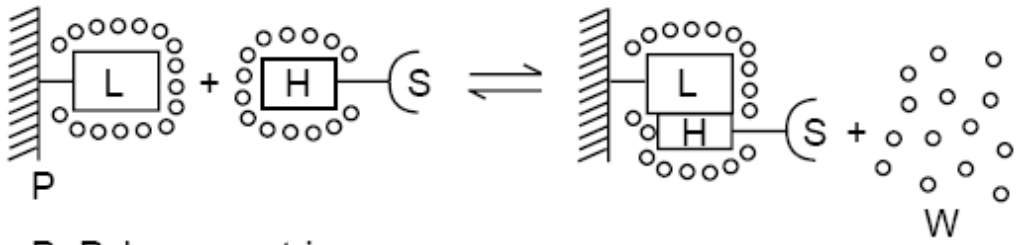
Hydrophobic interaction chromatography

- HIC separates proteins with differences in hydrophobicity on the surface
- The separation is based on the reversible interaction between hydrophobic patch on the protein surface and hydrophobic group of a chromatographic medium



P=Polymer matrix
S=Solute molecule
L=Ligand attached to polymer matrix
H=Hydrophobic patch on surface of solute molecule
W=Water molecules in the bulk solution

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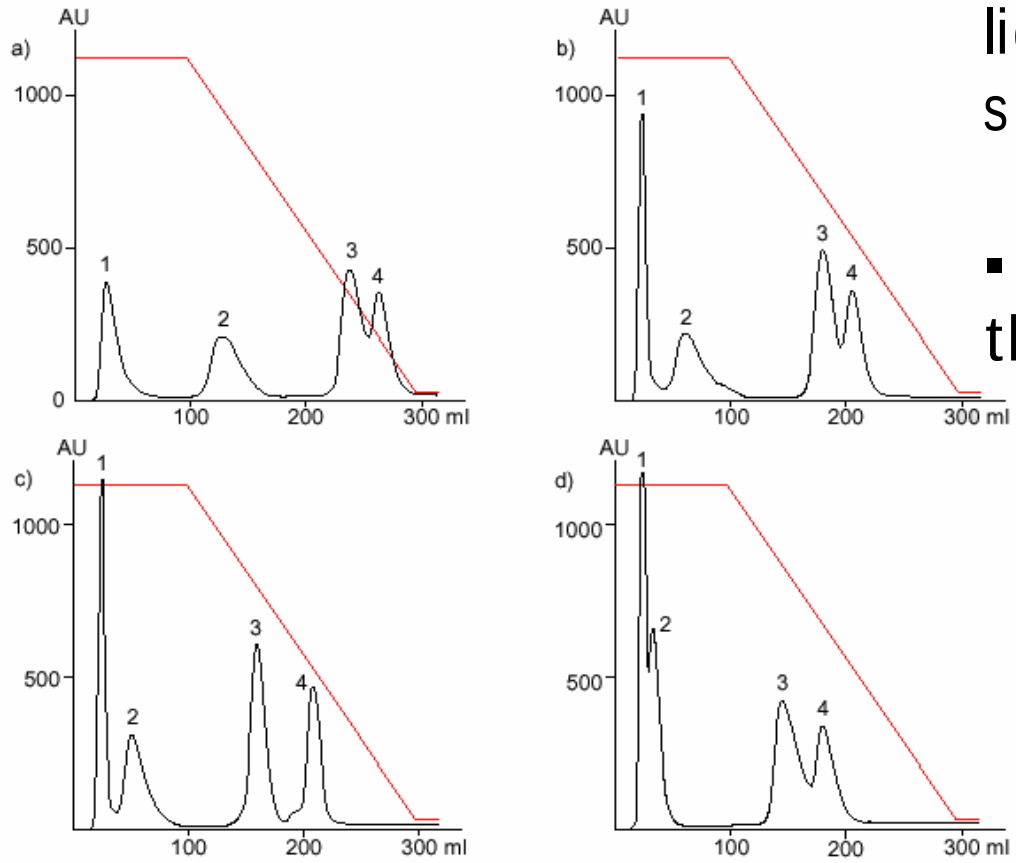
Factors affecting HIC

- Ligand type and degree of substitution
- Type of base matrix
- Type and concentration of salt
- pH
- Temperature
- Additives

Media selection



- There is no general rule to select specific ligand suitable for the specific sample.
- Ligand screening is the unique solution.

(a) Phenyl (high sub)
(b) Phenyl (low sub)
(c) Butyl
(d) Octyl

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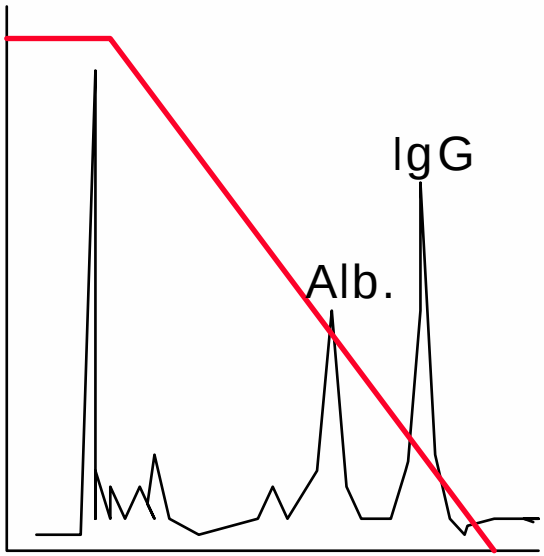
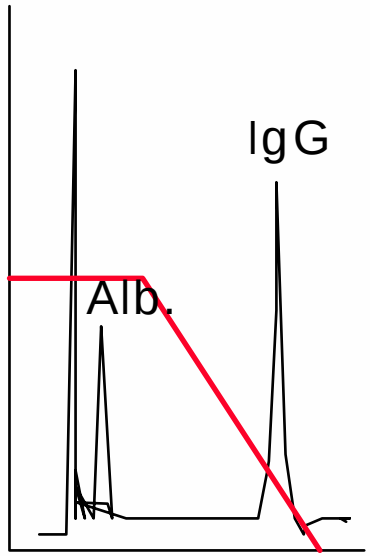
Factors affecting HIC, type and concentration of salt



a) sample applied in 2 M $(NH_4)_2SO_4$



b) sample applied in 1 M $(NH_4)_2SO_4$

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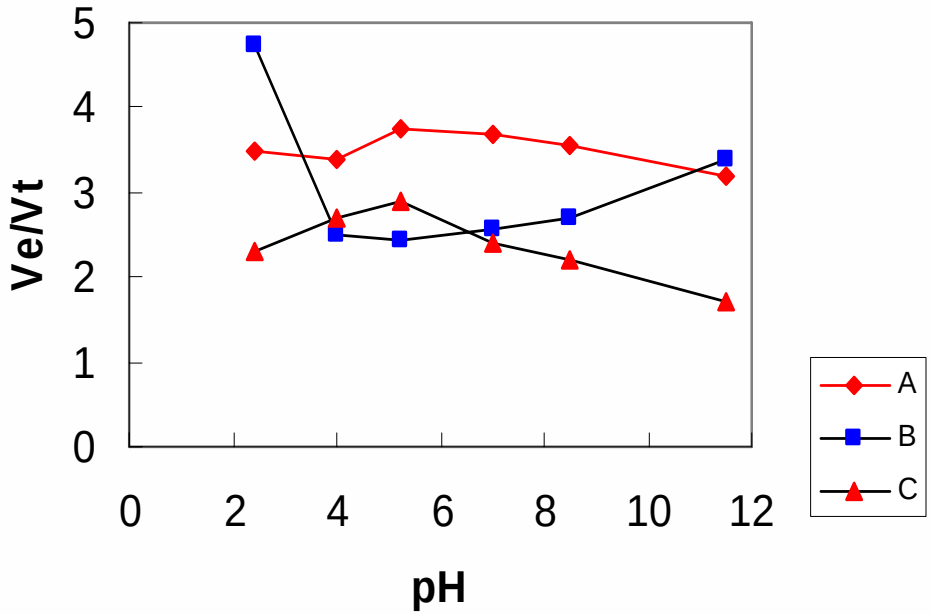
Factors affecting HIC, pH

- Low pH shows increased hydrophobic interaction
- Protein binding to HIC-media is generally not changed much between pH 5 and 8.5



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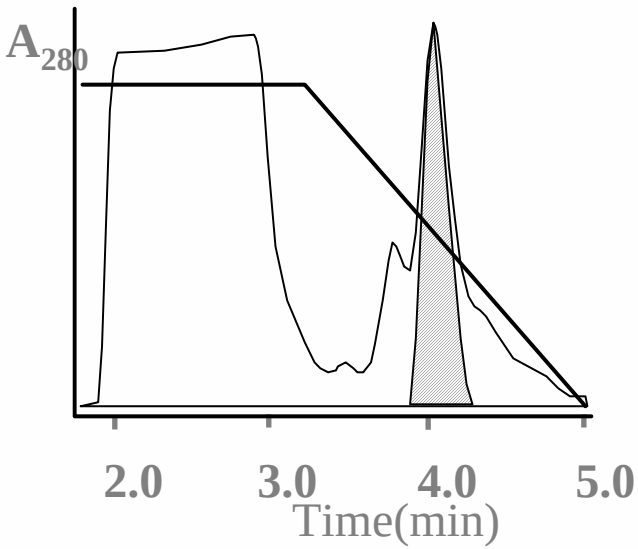
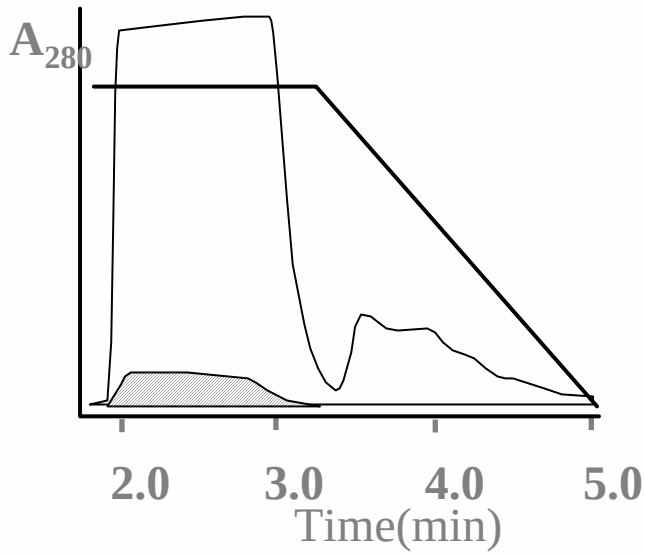
Factors affecting HIC, temperature

- In practical terms, one should thus be aware that a downstream pu rification process developed at room temperature might not be repr oduced in the cold room, or *vice versa*

23°C Sample & System



4°C Sample, 23°C System



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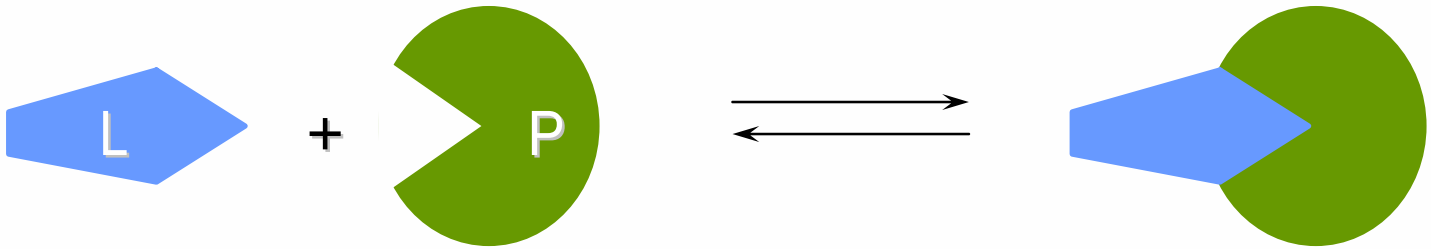
Affinity chromatography

- Affinity chromatography separates proteins on the basis of a reversible interaction between a protein (or group of proteins) and a specific ligand coupled to a chromatography matrix



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Coupling ligand to the matrix

Coupling chemistry

- N-hydroxysuccinimide
- CNBr
- Carbodiimide
- Epoxide
- Thiol exchange

Kinds of group coupled

- NH2
- NH2
- NH2 , - COOH
- SH, - NH2, - OH
- SH

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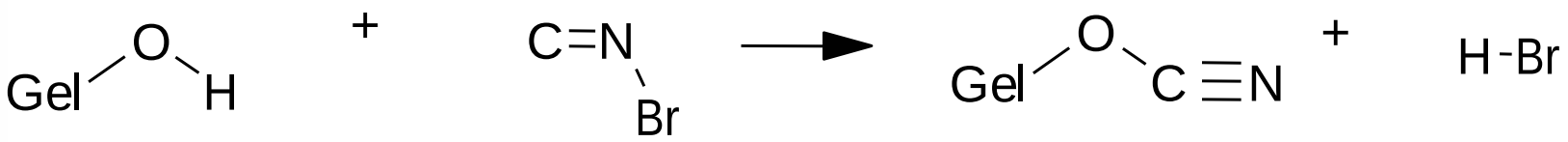
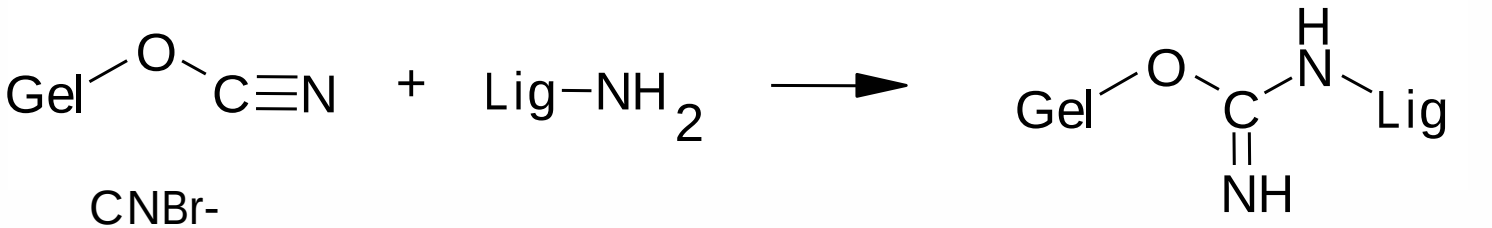
Cyanogen bromide activation



Agarose bead

Cyanogen bromide

Cyanate ester formed



CNBr-activated Agarose

Protein ligand

N-substituted isourea

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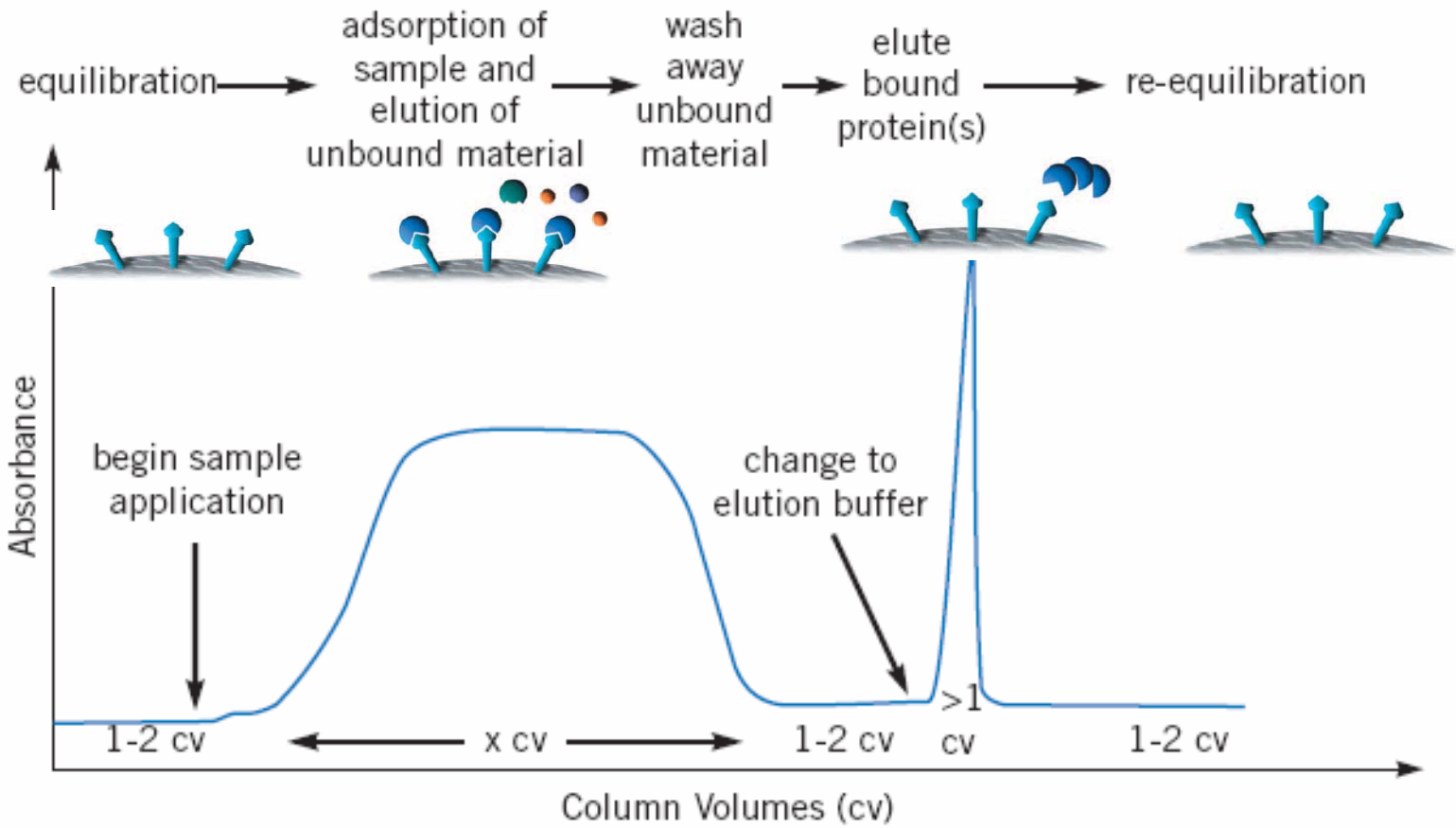
Typical example of affinity chromatography



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Elution

- There is no generally applicable elution scheme for all affinity media
- Elution methods may be either selective or non-selective
  - pH elution
  - Ionic strength elution
  - Competitive elution
  - Reduced polarity of eluent
  - Chaotropic eluents

Thank you for listening

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BioLogic DuoFlow Pathfinder

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Components

- Pump : F10 (10ml/min, 3,500psi)

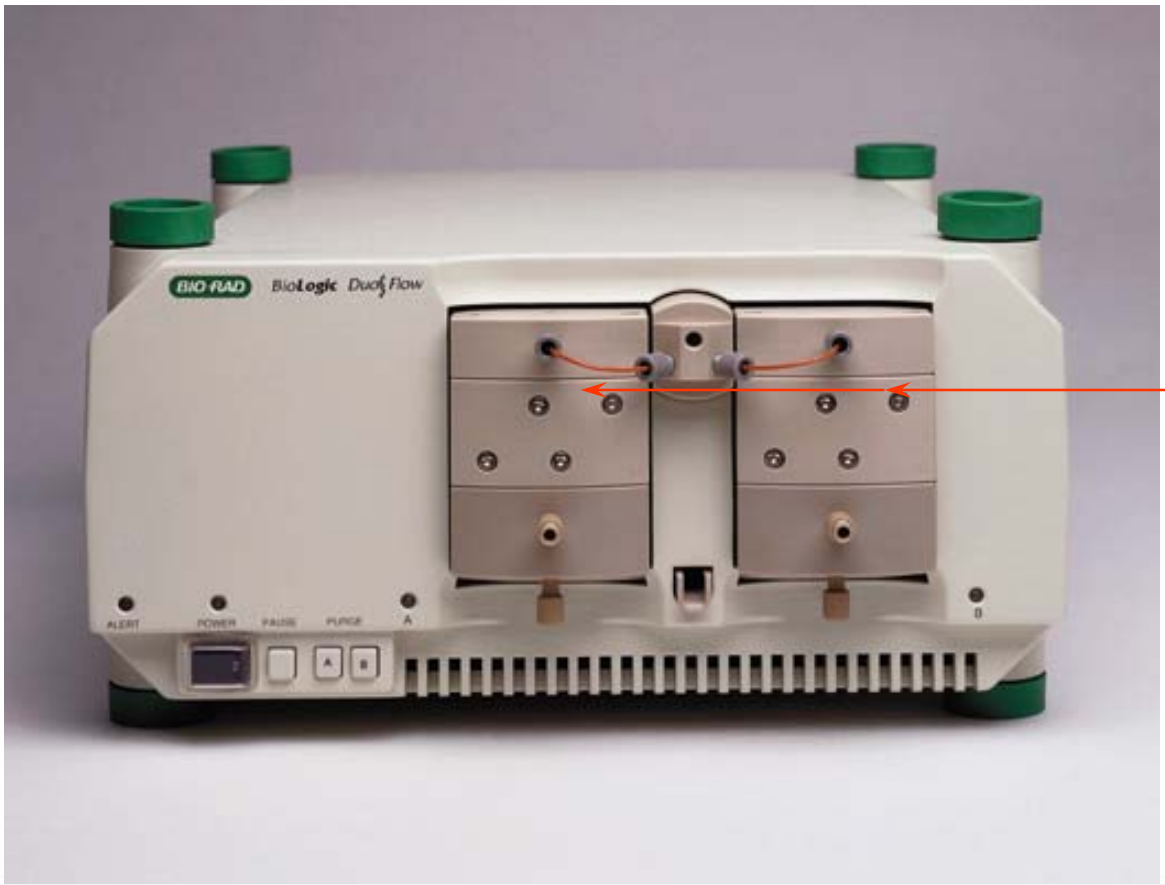
  F40 (40ml/min, 1,000psi)

- Detector : UV, Conductivity

  QuadTec

- BioLogic Duoflow System 4.0
- Fraction collector : 2110, BioFrac

F-10 or F-40 Gradient Pump



Middle section houses the F-10 or the F-40 pumpheads

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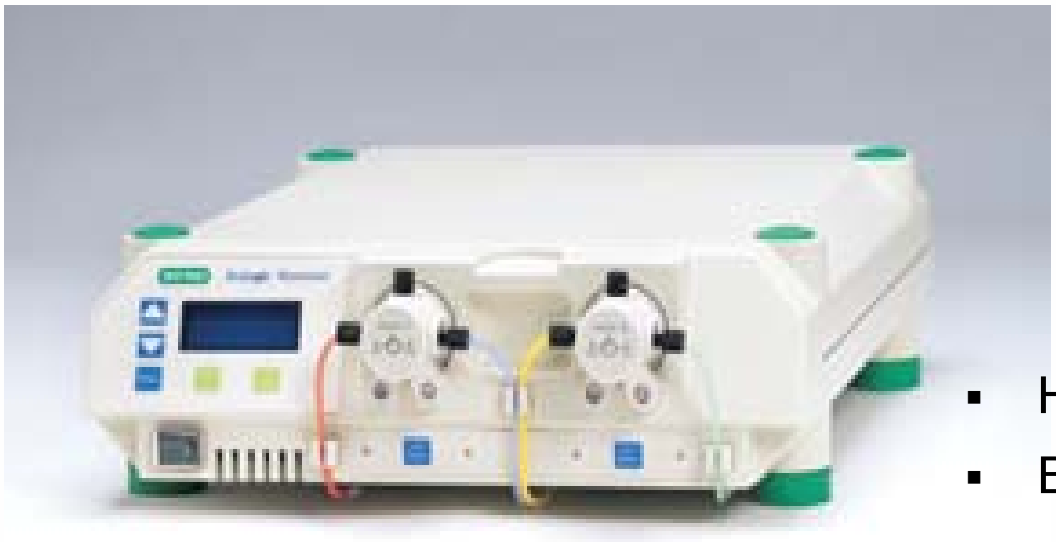
BioLogic Maximizer valve system



- Higher flow rate
- Buffer blending
- pH scouting

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BioFrac - Fraction Collection System

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