

PURIFYING PROTEINS

PURIFICATION AND
CHARACTERIZATION OF PROTEINS

- **NB.**
- **Whereas this lecture was developed to indicate how proteins are extracted isolated and indentified, some of these same methods were employed in the research under review as can be seen from reading the paper.**

INTRODUCTION

- Goals of purification vary with the intended use of the protein.
- Purity is defined by the general level of protein contaminants and also by the absence of contaminants of special interest such as endotoxin, viruses etc.
- Protein purification can be divided into 5 stages.
 - a) Preparation of the source
 - b) Knowledge of protein properties
 - c) Development of an Assay
 - d) Primary Isolation
 - e) Final Purification

A-- PREPARATION OF SOURCE

- **Selection of raw materials from which proteins will be isolated (microbial or cultured metazoan cell line).**
- **Protein supplies can be increased by increasing the cultivation volume (by growing more cells per unit volume).**

B--KNOWLEDGE OF PROTEIN PROPERTIES

- **Source:** cell type, intra/ extra cellular location, folding state, presence of proteases / glycosidases
- **Stability:** to temperature range, pH range, ionic strength, hydrophobic surfaces, aggregation tendency, cofactor of metal ion loss / requirement.
- **Size :** molecular weight, peptide chain (s), hydrodynamic radius.
- **Size may be determined by**
- dialysis and ultrafiltration
- gel electrophoresis
- gel filtration chromatography
- ultracentrifugation
- **Charge:** isoelectric point, isoelectric focusing titration curve, electrophoretic mobility.
- (electrophoresis) ion exchange chromatography
- **Binding partners:** substrates and cofactors, screening-derived binding agents, metal affinity,
- **Specificity of binding may be determined by -affinity chromatography**

- **Polarity**
- adsorption chromatography
- paper chromatography
- reverse-phase chromatography
- hydrophobic interaction chromatography

Selection of a Protein

- **Source-often one can obtain the same or similar protein from several different sources . Chose a source**
- 1. which can be obtained in large amounts and has..
- 2. a high concentration of the protein
- 3. molecular cloning techniques allow production and purification of proteins from *E. coli*, yeast or other cells.

- **SOURCE**
- **Tissue and cell cultures (bacteria, yeast, mammalian)**
 - Glucose 6-phosphatase is an enzyme required for gluconeogenesis (formation of glucose from noncarbohydrate precursors).
 - Major site of gluconeogenesis is the liver.
- **Heterologous expression**
 - Genetically engineer bacteria, yeast, or insect cells to produce protein.
 - Add tags to protein to facilitate purification.

Stabilization of proteins-- proteins are delicate

- 1. may be denatured by high temperature
- keep solution at appropriate temperature, usually fairly cold.
- can use denaturation of some proteins to help purify a protein which is stable at high temperature
- 2. proteases are enzymes which break peptide bonds
- maintain conditions which inhibit proteases ==> low temperature or change in pH or addition of chemical inhibitors
- may use proteases to digest labile proteins if the protein of interest is stable to proteases
- 3. many proteins are unstable at air-water interfaces or at low concentration
- keep protein solutions concentrated
- keep solution from "frothing" ==> don't mix vigorously

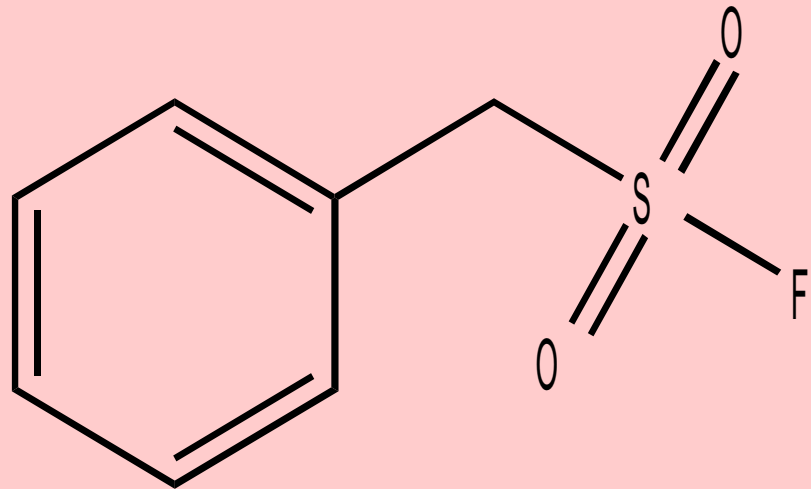
Stabilizing the protein sample involves

- **Controlling pH**
 - Use appropriate buffer
- **Controlling temperature**
 - Keep samples on ice or work in cold room
 - Prechill instruments
- **Preventing frothing/foaming**
 - Handle gently.
- **Maintaining concentrated sample**

Stabilize Sample

- **Protease inhibitors**

- Phenylmethylsulfonyl fluoride (PMSF)
- Leupeptin
- Aprotinin
- Chymostatin
- Pepstatin A



PMSF

Method of solubilization

- 1. serum proteins or secreted proteins are already soluble
- 2. otherwise cells must be broken up
- osmotic lysis; perhaps aided by enzymes or chemicals to weaken cell membranes (detergents, solvents, or lysozyme for bacteria)
- mechanical disruption by grinding, blending, homogenizing or ultrasonic disruption
- 3. filter or centrifuge crude lysate to remove cell debris (membranes, cell walls etc.)
- protein may be in soluble fraction
- protein may be in particulate fraction which may be purified by centrifugation techniques and then solubilized by treatment with detergents or other chemicals

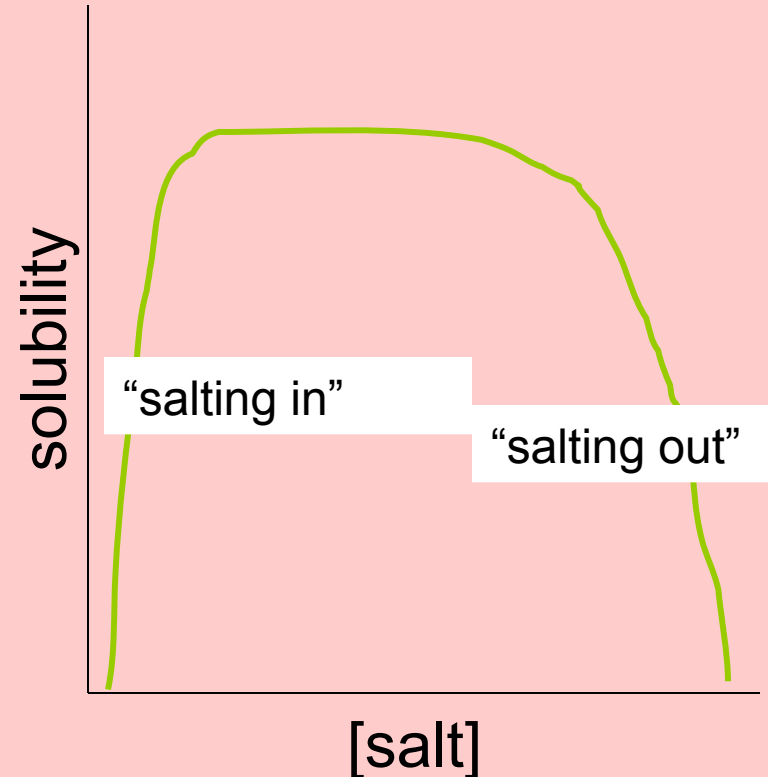
Protein Solubility

- **Salting in**

- Ions shield charges and allow proteins to fold.

- **Salting out**

- Ions compete with water to interact with side groups. When [salt] is high enough, salt wins causing protein to precipitate.
- Generally use ammonium sulfate to precipitate proteins in the lab.



- **Solubilities of Proteins**
- **A. Effects of Salt Concentration**
- **1. salting in** - proteins are less soluble at very low salt concentrations (ionic strength); salt ions helps shield protein's multiple charged groups
- **2. salting out** - proteins are also less soluble at salt concentrations (high ionic strength) because the salt ions bind most of the water molecules
- **3.** These two effects are different for different proteins ==> fractionate by raising the [salt] just below point where protein to be purified becomes insoluble, removed other precipitated proteins by centrifugation, then raise the [salt] to precipitate protein and collect it by centrifugation.

- **B. Effects of organic solvent**
- can selective precipitate some proteins by increasing the concentration of water miscible organic solvents
- **C. Effects of pH**
- All proteins have an **isoelectric point, pI**, a pH at which they have no net charge, and they are least soluble at their pI because there are no net electrostatic repulsions between protein molecules:
- Different proteins have different **pI**'s, so one can manipulate the relative solubilities of a mixture of proteins by changing the pH.
- **D. Crystallization**
- Once a protein is reasonably pure, one may try to crystallize it which is the ultimate criterion of purity.

Solubility of a protein

- Depends strongly on the composition of the lysis buffer.
- Salt concentration

Freeze-thaw protocol

- * Freeze quickly on dry ice and leave for 3 min.
- * Thaw immediately at 42 °C. Vortex vigorously to mix well.
- * Repeat the two previous steps three more times (4 cycles in all).

Membrane proteins

1. Removal of unbroken cells from the cell lysate by low speed centrifugation (20 min at 10,000 g).
2. Isolation of the membrane particles from the supernatant by ultracentrifugation (60 min at >100 000 g).
3. Washing of the membrane particle to remove all soluble proteins.
4. Solubilization of protein from the membrane particles by a mild detergent. (detergent: protein ratio = 1:10)
5. Phosphate buffers(0.1M-0.5M), 5-50% glycerol helps.

(<http://www.ls.huji.ac.il/~purification>)

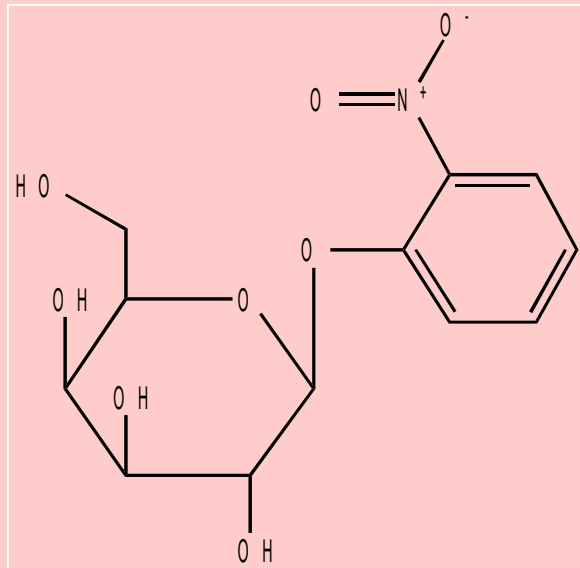
C- DEVELOPMENT OF AN ASSAY

- **An assay for the desired activity or protein is required.**
- **They must be convenient , rapid and extremely precise.**

Assay of Proteins

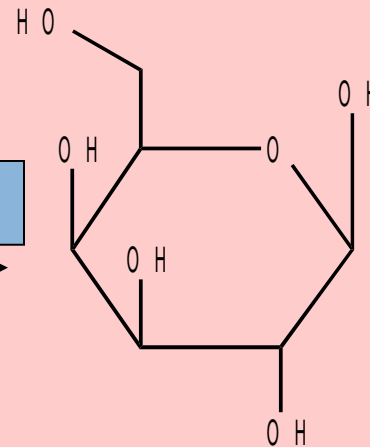
- **Some way of measuring the concentration of a specific protein is needed so that we know when we're doing something right**
- **1. enzymes can be measured by the reactions they catalyze**--either measure products produced or reactants used up.
- **2. other proteins may be measured by their biological effects:** ability to bind specific molecules or the effect of a hormone on cells, tissue or organism.
- **3. Immunochemical techniques-can produce antibodies which bind specifically to particular proteins**--for enzyme linked immunosorbent assay.

Detection Method: Enzyme Assay



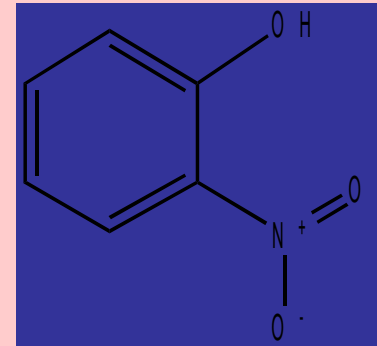
ONPG

β galactosidase



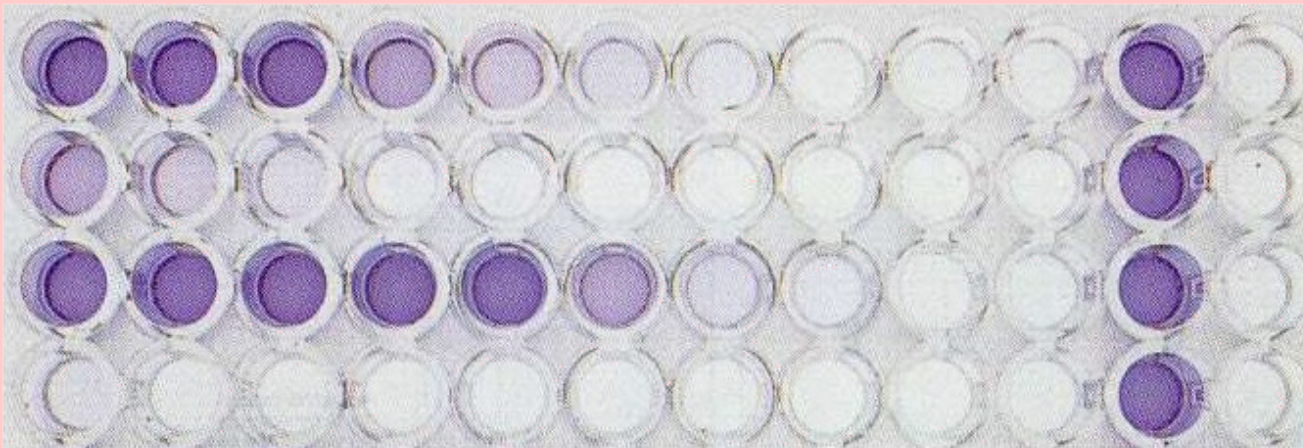
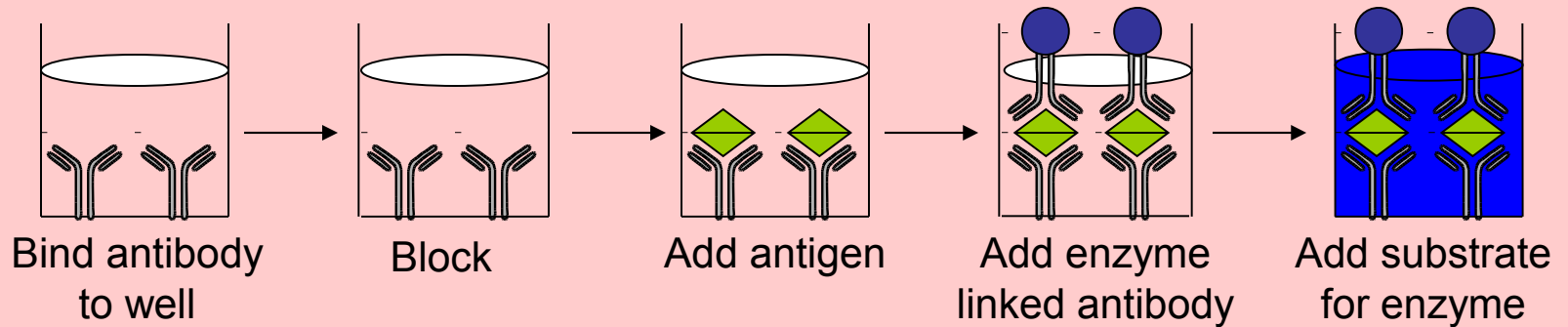
Galactose

+



ONP

Detection Method: ELISA

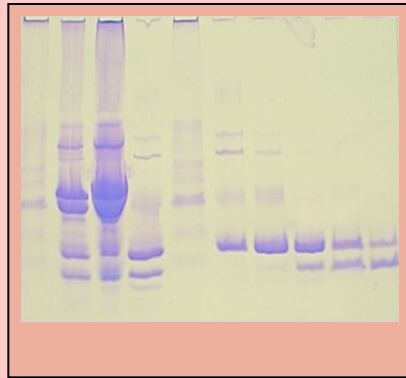


Detection Method: Western Blot

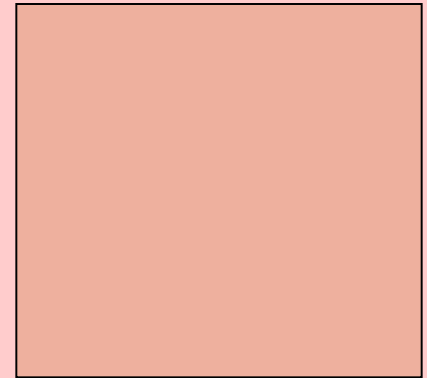
<http://www.austinc.edu/~emeyert/protein%20gel%20sum05.jpg>



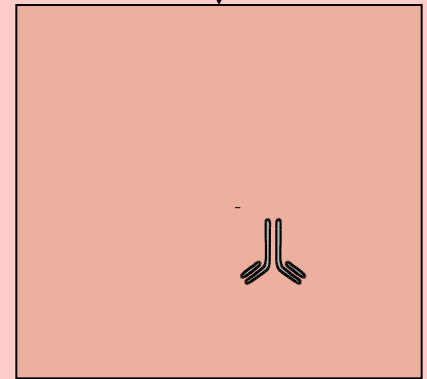
Separate proteins on gel



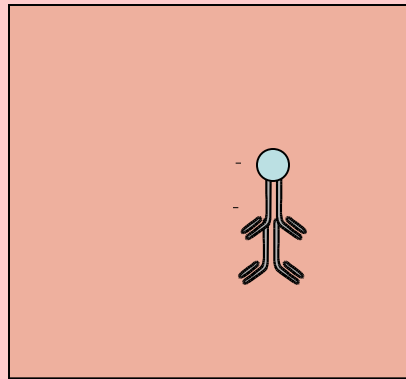
Transfer proteins to membrane



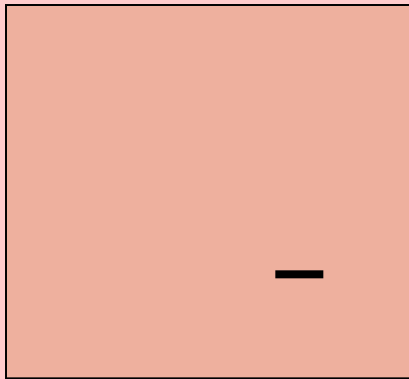
Block



Add 1° antibody



Add 2° antibody



Add substrate for enzyme

D INITIAL ISOLATION

This mainly consists of separation of proteins from water and other cell components.

a) Concentration

b) Cell lysis

c) Refolding

CONCENTRATION

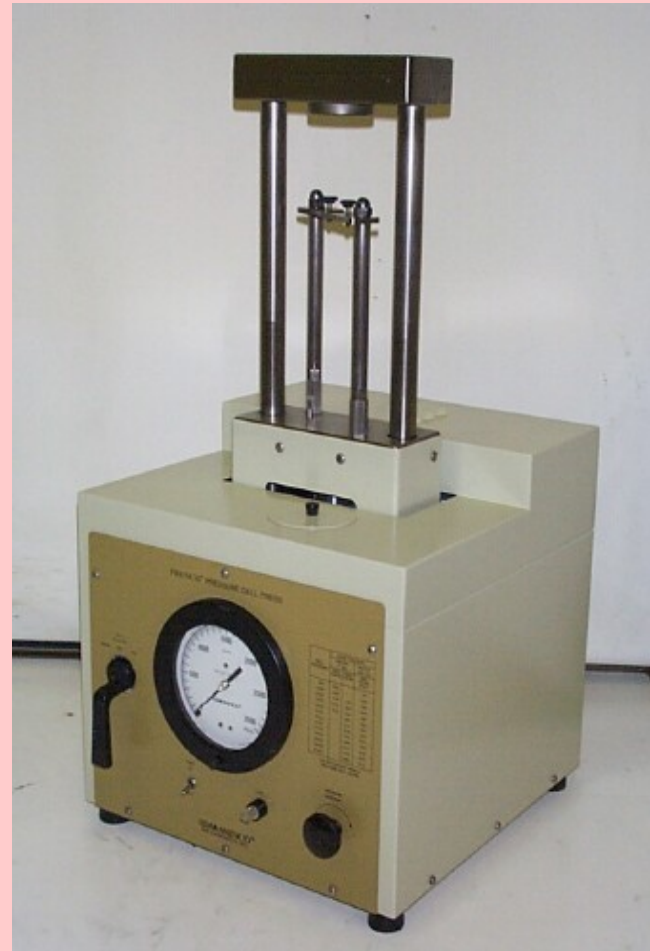
- Extra cellular proteins are usually concentrated from the cell by ultra filtration or adsorption.
- Secreted protein adsorbed to the outside of the cell and can be concentrated along with them and then liberated by washing, often with a high salt buffer.

CELL LYSIS

- Intra cellular proteins are liberated by cell lysis.
- Cell lysis is the process of disintegration of a cell (French Press – forcing cell through an orifice at high pressure).
- Soluble proteins are often recovered from cell lysates by precipitation with ammonium sulfate or polyethylene glycol.

CELL LYSIS

- **Physical**
 - French pressure cell
 - Sonication
 - Glass beads
- **Chemical**
 - Detergents
 - Enzymes
 - Hypotonic buffer



Eventful Protein Purification

- **Grow cells in media (vector+tag)**
- **Centrifuge, Collect the pellet**
- **Lyse the cells (appropriate buffer)**

Pilot Expression
SDS PAGE, Assay

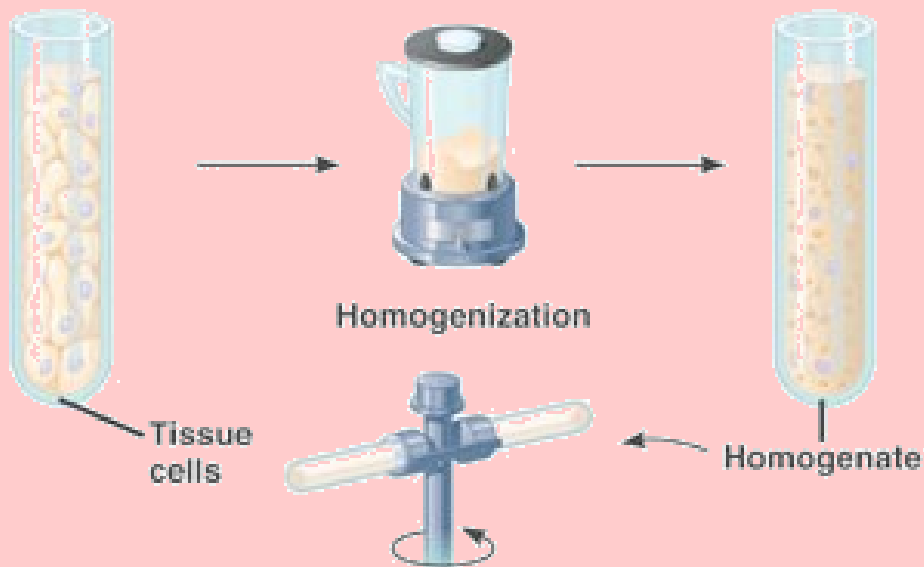
Purification Strategy

Characterization
Mass Spectroscopy
X-ray Crystallography
Functional Assay

Solubility
Aggregation
Recombination



Separate Cell Debris



- Centrifugation
 - Supernate
 - Pellet
- Filter

REFOLDING

- Recombinant proteins often misfold to form dense, insoluble aggregates of inactive protein.
- The first step in the renaturation process is the dissolution of the inclusion bodies in a strong chaotrope solution with 6M urea. Dissolution in denaturant is rapid and reliable.
- The denatured protein is then allowed to renature to its native confirmation by removing the denaturant through dialysis, dilution or chromatographic separation. Allow the refolding process for 7 to 10 days.

Solubilization of Aggregated Proteins

Denaturation and Renaturation

Variables	Good starting point
Buffer composition (pH, ionic strength)	50 mM Tris-HCl, pH 7.5
Incubation temperature	30°C
Incubation time	60 min
Concentration of solubilizing agent	6 M guanidine-HCl or 8 M urea
Total protein concentration	1-2 mg/ml

Re-folding of Proteins

The addition of a mixture of reduced and oxidized forms of low molecular weight thiol reagent usually provides the appropriate redox potential to allow formation and **reshuffling of disulfide bonds**

(1-3 mM reduced thiol and a 5:1 to 1:1 ratio of reduced to oxidized thiol)

The most commonly used are **glutathione, cysteine and cysteamine**.

Reagents used for Re-folding of proteins (Continued)

n-Penthanol	1.0-10.0 mM	Lauryl Maltoside	0.06 mg/ml
n-Hexanol	0.1-10.0 mM	CETAB	0.6 mg/ml
Cyclohexanol	0.01-10.0 mM	CHAPS	10-60 mM
Tris	> 0.4 M	Triton X-100	10 mM
Na ₂ SO ₄ or K ₂ SO ₄	0.4-0.6 M	Dodecyl Maltoside	2.0-5.0 mM
Cyclodextrin	20-100 mM	Sarkosyl	0.05-0.5 %

(<http://www.ls.huji.ac.il/~purification>)

HIGH RESOLUTION PURIFICATION

- Chromatography is the usual method of preparing highly purified active proteins.
- Chromatographic operations are classified as low-pressure, medium-pressure, high-pressure depending on the pressure used to force liquid through the packed bed.

Feature	Low-Pressure	Medium – Pressure	High-Pressure(HPLC)
Particle Size	40-150 μ m	10-75 μ m	2-15 μ m
Flow Driver	Gravity, peristaltic	Piston or syringe	Positive displacement
Run Time	40-100min	15-60min	0.5-30min
Apparatus Cost	Low	Medium	High
Resolving Power	Lowest	Intermediate	Highest
Particulate tolerance	Low	Very low	Lowest

Separating Proteins

- Chromatography

- Mobile phase

- Phase that carries sample throughout procedure.
 - Liquid
 - Gas

- Stationary phase

- Matrix that retards the movement of sample being carried by the mobile phase.

HIGH RESOLUTION PURIFICATION

- Chromatographic operations are broadly classified as
 - a) Ion – exchange Chromatography
 - b) Hydrophobic Chromatography
 - c) Affinity Chromatography
 - d) Size exclusion Chromatography

Chromatographic Modes of Protein Purification

Chromatographic Mode	Acronym	Separation Principle
<i>Non-interactive modes of liquid chromatography</i>		
Size-exclusion chromatography	SEC	Differences in molecular size
Slalom chromatography (for DNA)	-	Diff. in length and flexibility
<i>Interactive modes of liquid chromatography</i>		
Ion-exchange chromatography	IEC	Electrostatic interactions
Normal-phase chromatography	NPC	Polar interactions
Reversed-phase chromatography	RPC	Dispersive interactions
Hydrophobic interaction chromatography	HIC	Dispersive interactions
Affinity chromatography	AC	Biospecific interaction
Metal interaction chromatography	MIC	Complex w/ an immobilized metal

(Christian G. Huber, Biopolymer Chromatography, Encyclopedia in analytical chemistry, 2000)

Ion Exchange Chromatography

In this case, a cation (or alternatively an anion) is attached to the resin beads. Depending upon the electrical properties of the proteins, they may attach to the column. For example, positively charged proteins will stick to a negatively charged column. These proteins can then be removed by washing the column with either a strong salt solution or changing the pH of the wash buffer.

- Anion exchangers such as DEAE (Diethyl amino ethyl) are used. Attraction of proteins at a pH above the isoelectric point of the protein.
- Cation exchangers such as CM (Carboxy methyl) are used. Attraction of protein at a pH below the isoelectric point of the protein.

Ion Exchange Chromatography

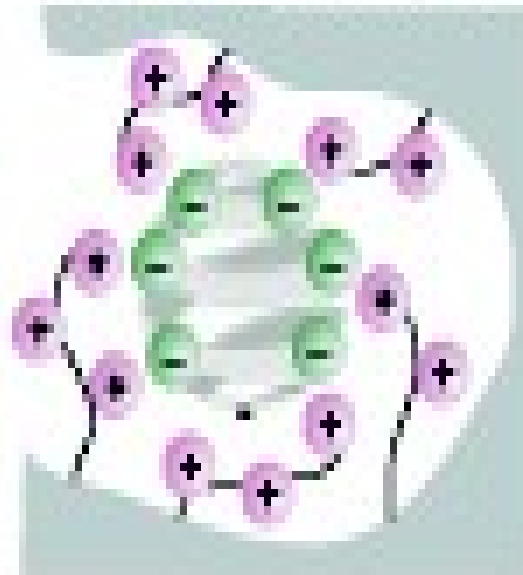
Fractogel matrix is a methacrylate resin upon which polyelectrolyte Chains (or tentacles) have been grafted. (Novagen)

Globular
Protein

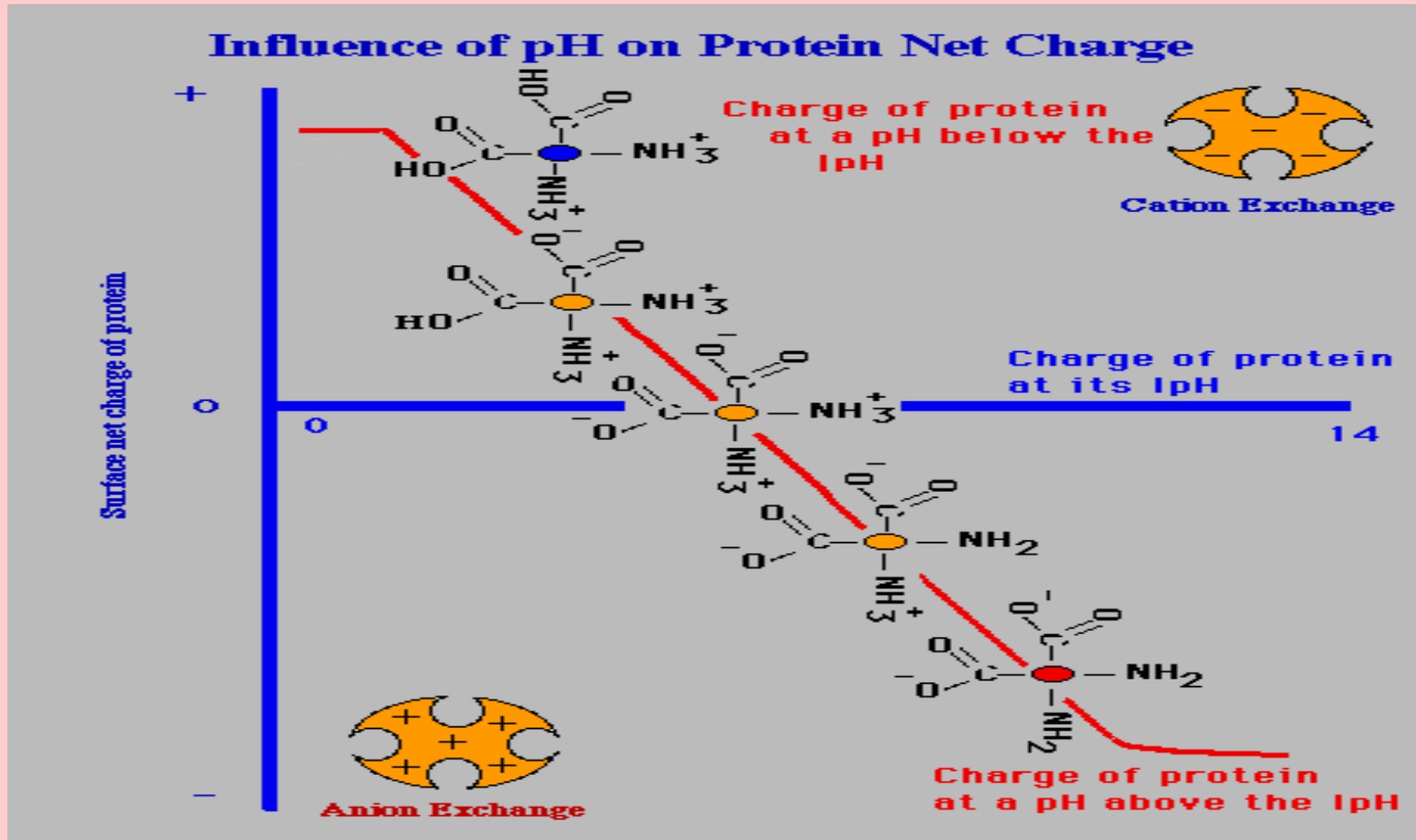


Maintenance of conformation while interacting with tentacle ion exchanger

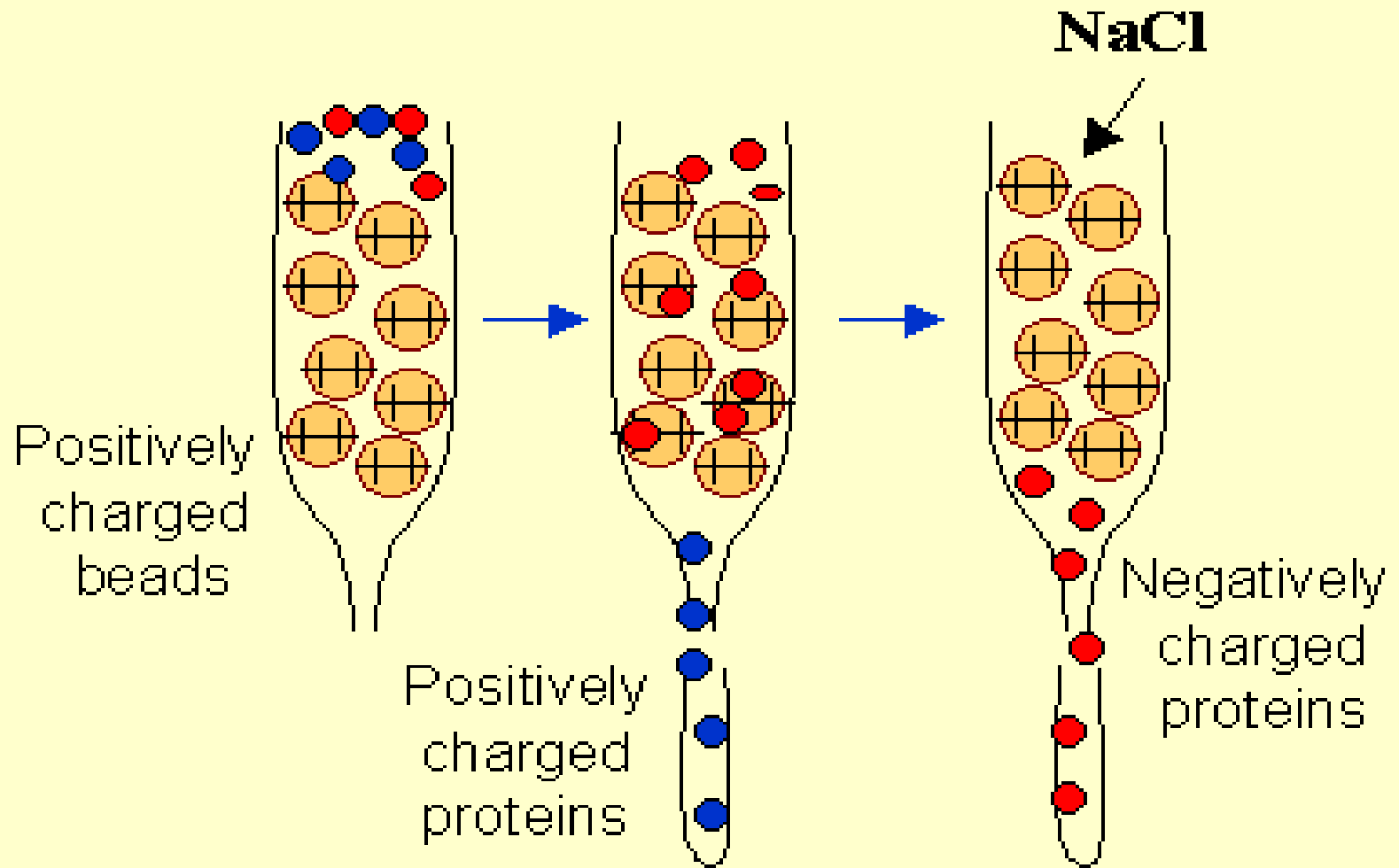
Deformation due to interaction with conventional ion exchanger



Ion – exchange Chromatography

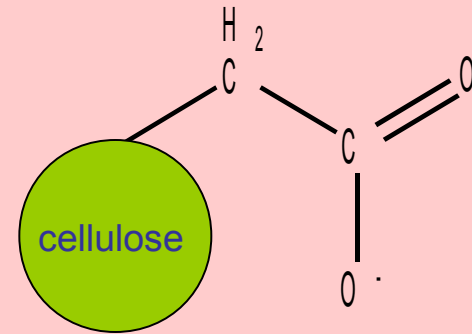


Ion – exchange Chromatography



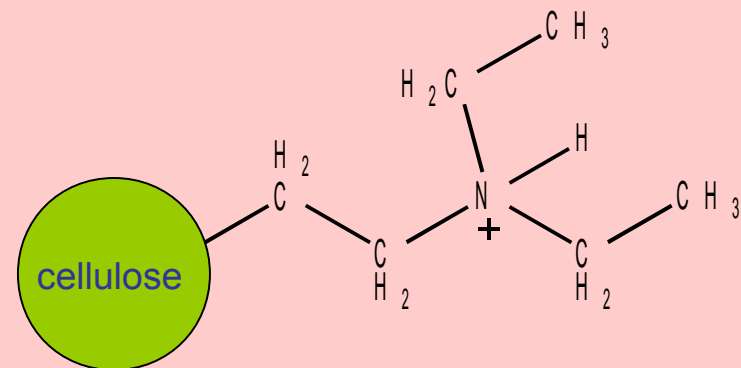
Ion Exchange Chromatography

- Separates molecules based on charge.
- Mobile phase
 - Generally liquid
- Stationary phase
 - Electrostatically charged ions bound to insoluble, chemically inert matrix.
- Elution of protein
 - Add salt to compete with binding of sample to stationary phase.
 - Change pH (alters charge of protein).



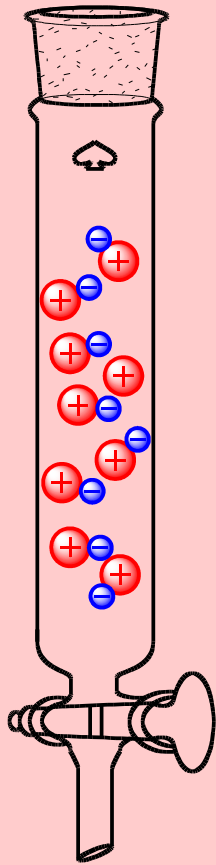
carboxymethyl (CM)

(Cation exchange)

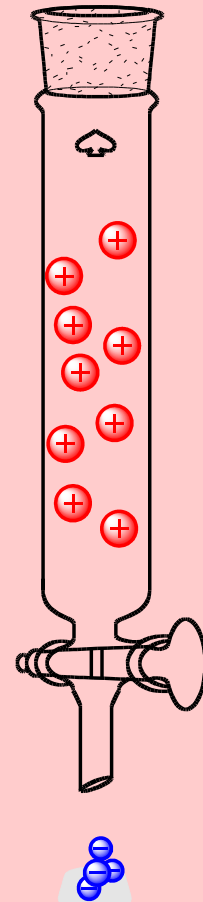
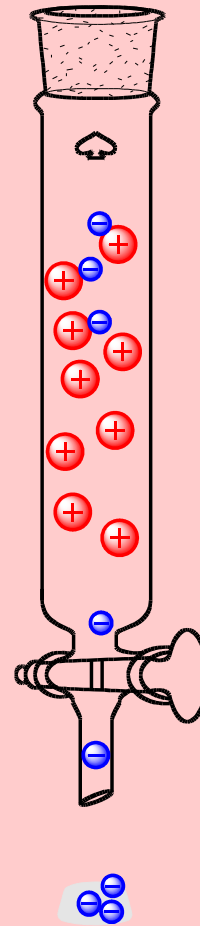
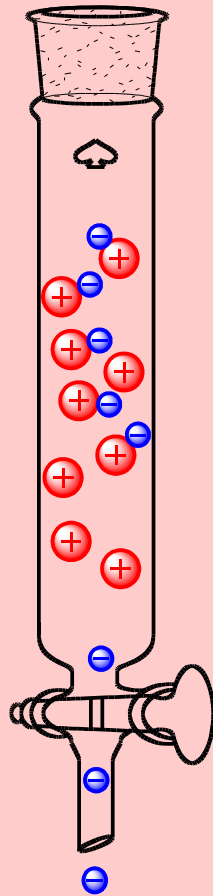


diethylaminoethyl (DEAE)
(Anion exchange)

Ion Exchange Chromatography



Low salt



High salt

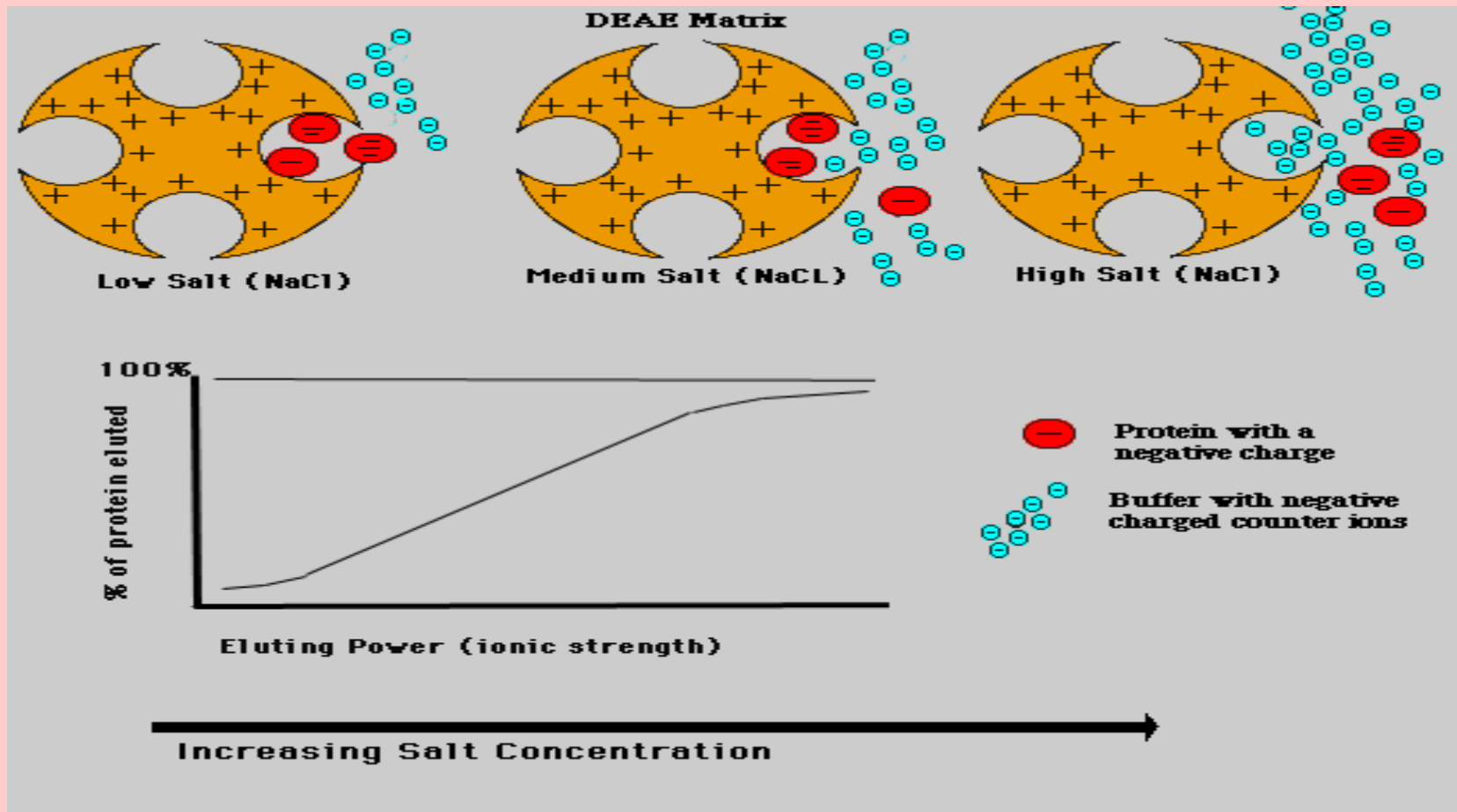


Examples

Name	Ionizable group	Type
DEAE-Sephadex	Diethylaminoethyl	Weakly basic
SP-Sepharose	Methylsulfonate	Strongly acidic
Bio-Rex 70	Carboxylic acid	Weakly acidic
P cellulose	Phosphate	Strongly & weakly acidic

ELUTION

- Done by washing the column with a strong salt solution (NaCl) which increases the ionic strength thereby pushing out the proteins.



HYDROPHOBIC CHROMATOGRAPHY

- **Principle**

Proteins are separated by hydrophobic interaction on columns with hydrophobic groups attached (e.g. phenyl-, octyl groups)

- **Surface hydrophobicity**

Hydrophobicity of amino acid sidechains

Tryptofan > Isoleucine, Phenylalanine > Tyrosine > Leucine > Valine
> Methionine

Most hydrophobic sidechains are buried in interior of protein, but some (clusters of) hydrophobic groups occur at surface of protein.

Surface hydrophobic sidechains can interact with hydrophobic groups for example attached to a column.

HYDROPHOBIC CHROMATOGRAPHY

- **Temperature**

Increasing temperature --> stronger hydrophobic interactions

- **Sample (application)**

Column having high concentration of a salt promotes binding (for example ammonium sulfate just below the concentration that starts to precipitate protein).

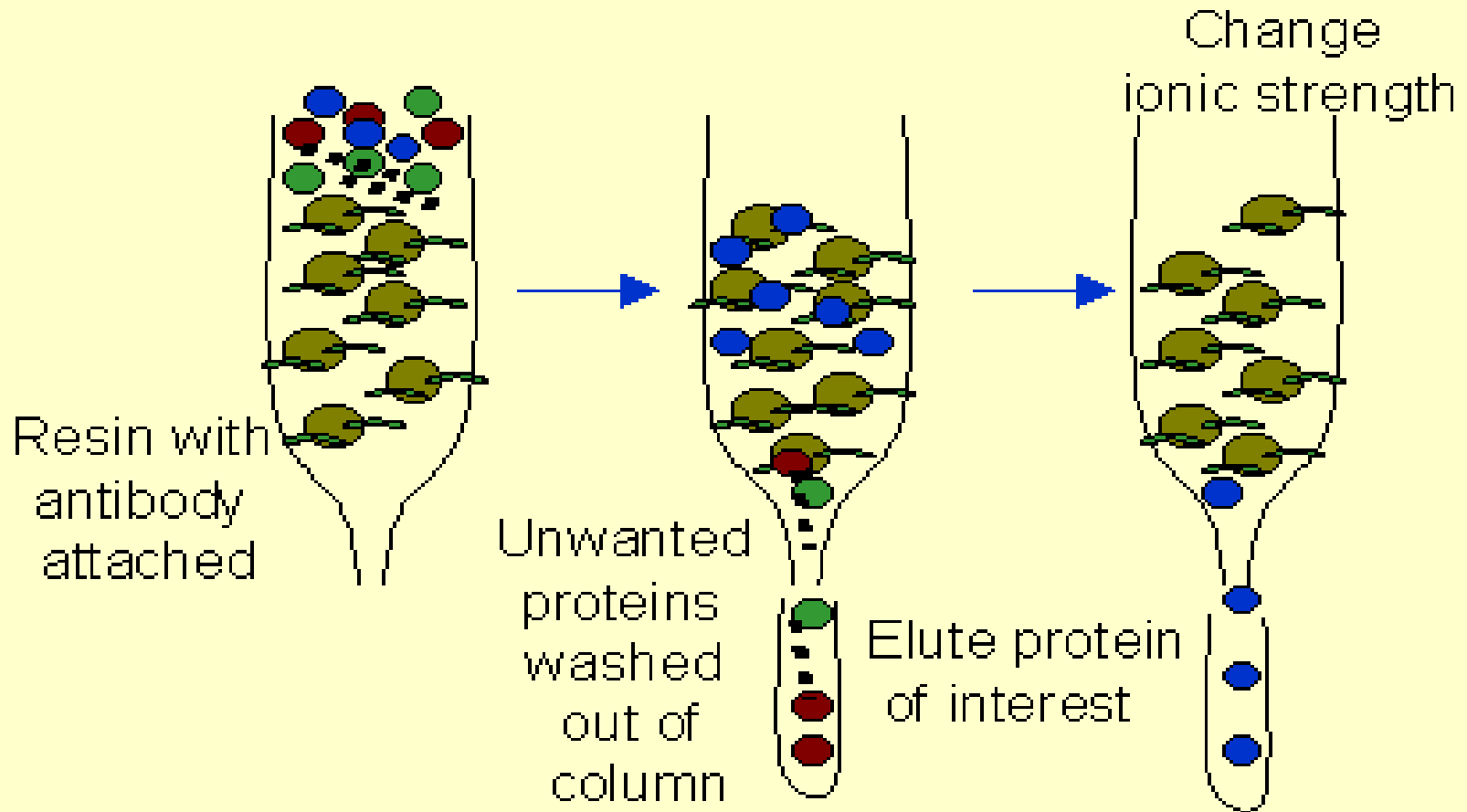
- **Elution of bound proteins**

Negative gradient of salting-out ions (from high to low concentration).

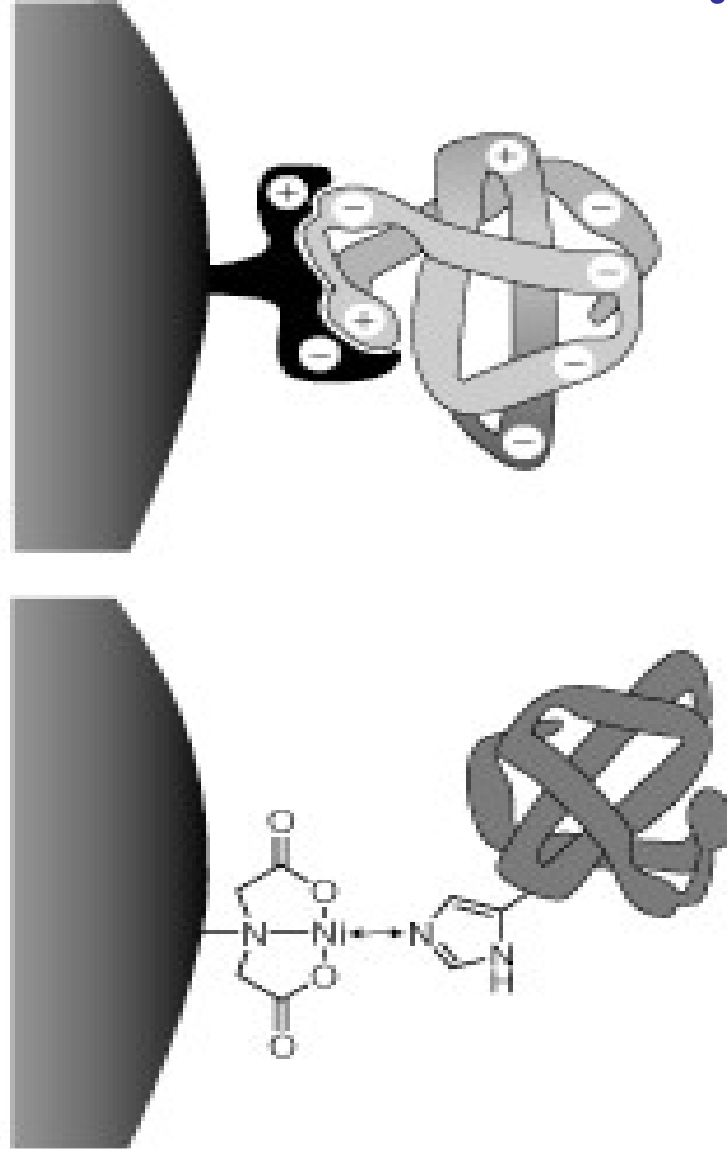
AFFINITY CHROMATOGRAPHY

- In this type of chromatography, a compound with a special affinity for the protein of interest is attached to the resin. For example, in immunoaffinity chromatography **antibodies** to a specific protein (or its domain) are used as the specialised compound.
- The resin is then packed into a column. When a mixture of proteins is passed through the column, only those proteins with special affinity for the compound will stick to the column. All the other proteins will pass through the column. Once the non-specific proteins are eluted, proteins of interest that have stuck to the column can be eluted. These proteins can be removed by changing the ionic strength of the solution (so affecting the strength of binding of the protein to the column). Alternatively the special compound can be added to the elution solution and the equilibrium will change so that the protein will no longer stick to the column.

AFFINITY CHROMATOGRAPHY



Affinity Chromatography



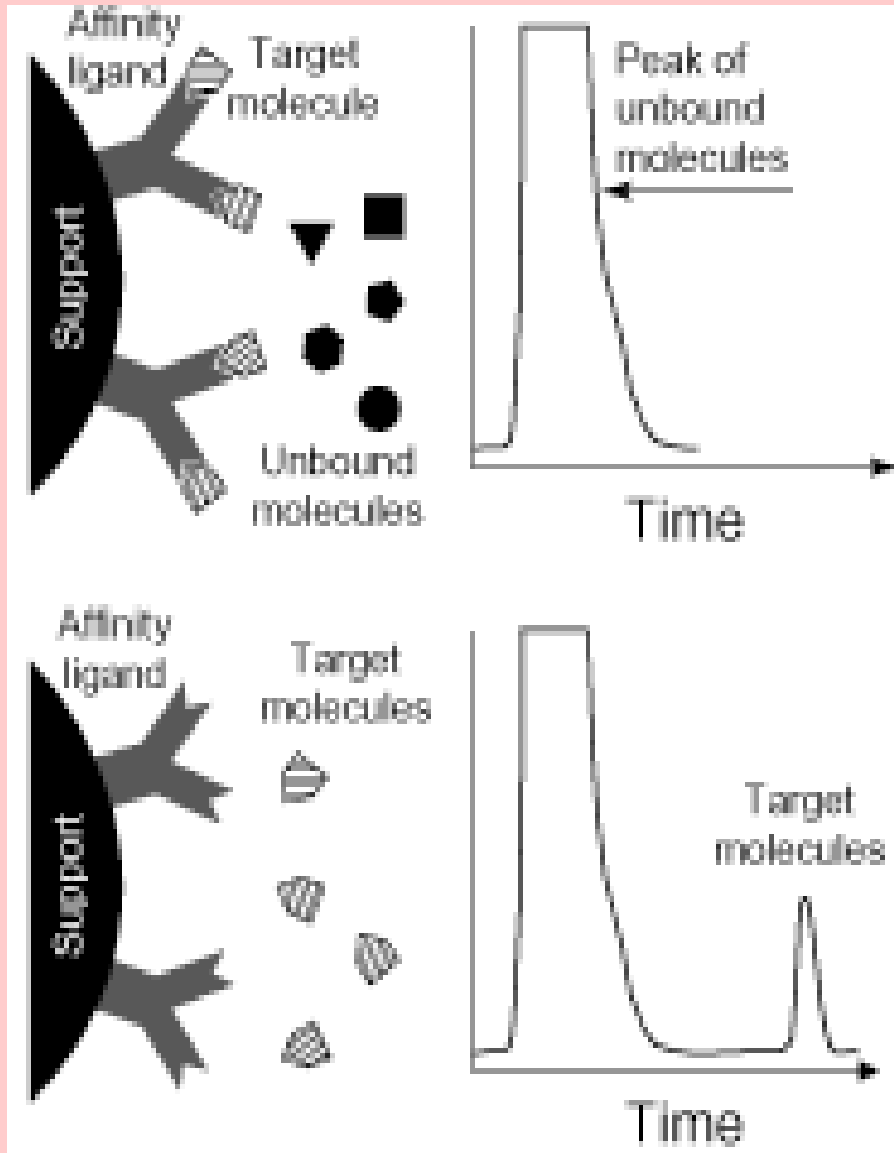
Affinity Chromatography

Surface bound with
Epoxy, aldehyde or aryl ester groups

Metal Interaction Chromatography

Surface bound with
Iminodiacetic acid + $\text{Ni}^{2+}/\text{Zn}^{2+}/\text{Co}^{2+}$

Affinity Chromatography



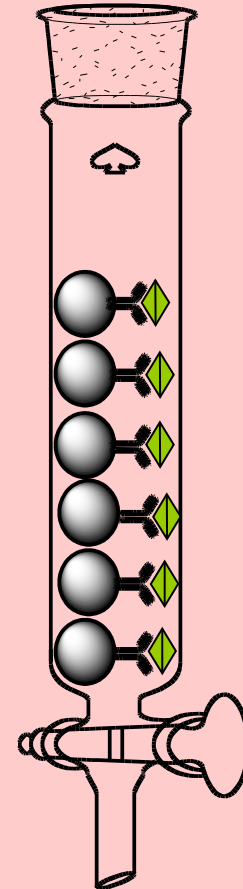
Binding Capacity (mg/ml) medium
12mg of histag proteins (MW= 27kDa)
Depends on Molecular weight

Degree of substitution /ml medium
 $\sim 15\mu\text{mol Ni}^{2+}$

Backpressure $\sim 43\text{psi}$
Change the guard column filter

Affinity Chromatography

- Mobile phase
 - Usually liquid
- Stationary phase
 - Receptor bound to inert bead

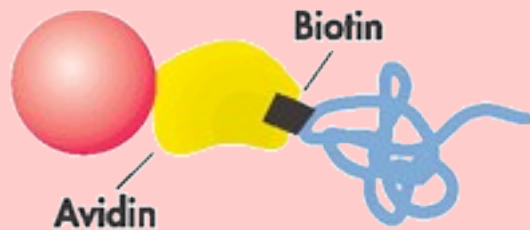


Immunoaffinity column

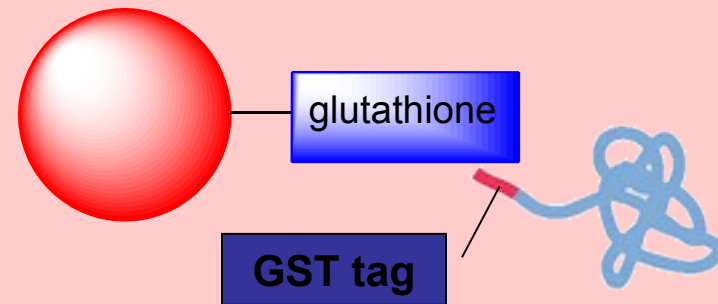
Affinity Chromatography



LiquiChip Ni-NTA Beads



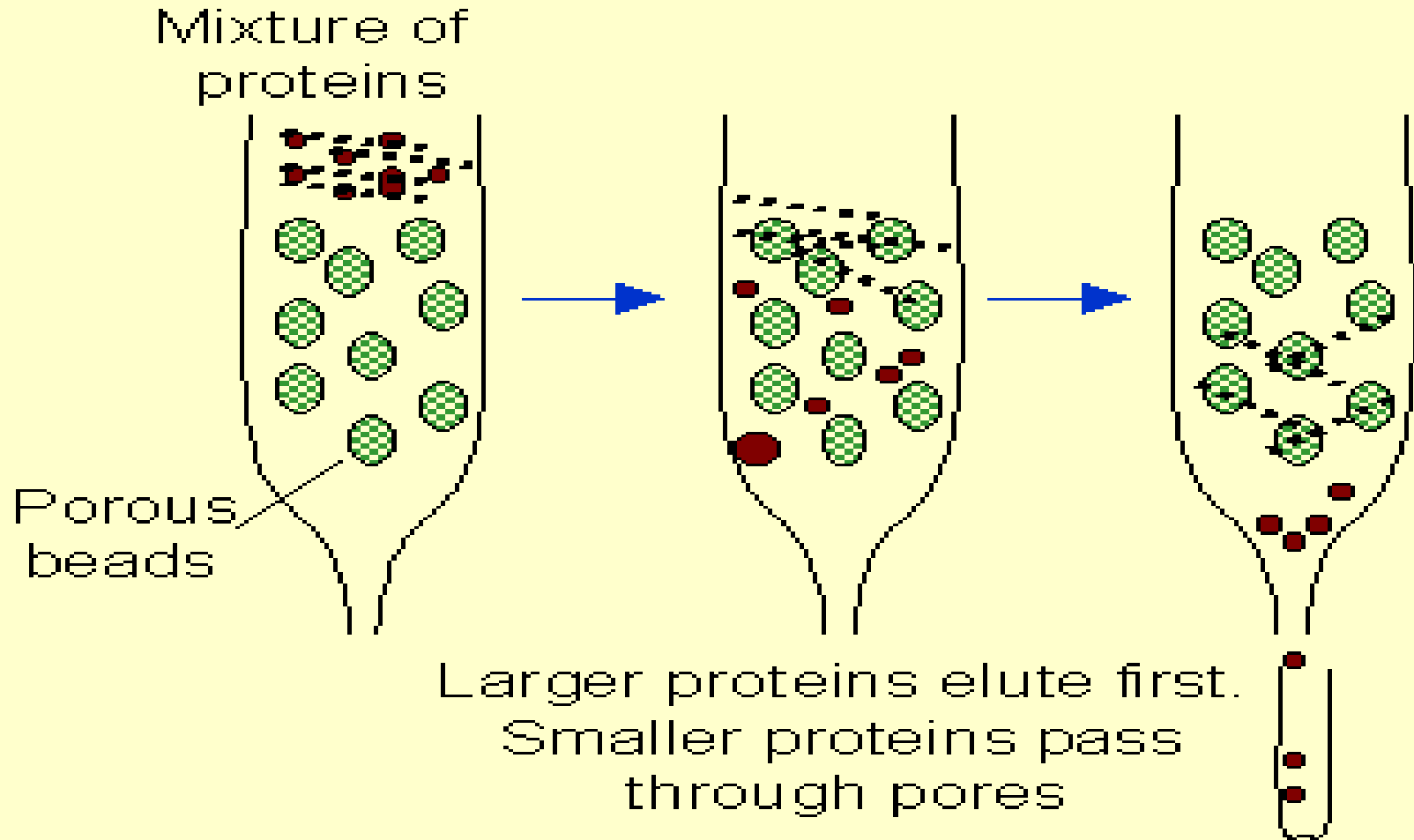
LiquiChip Avidin Beads



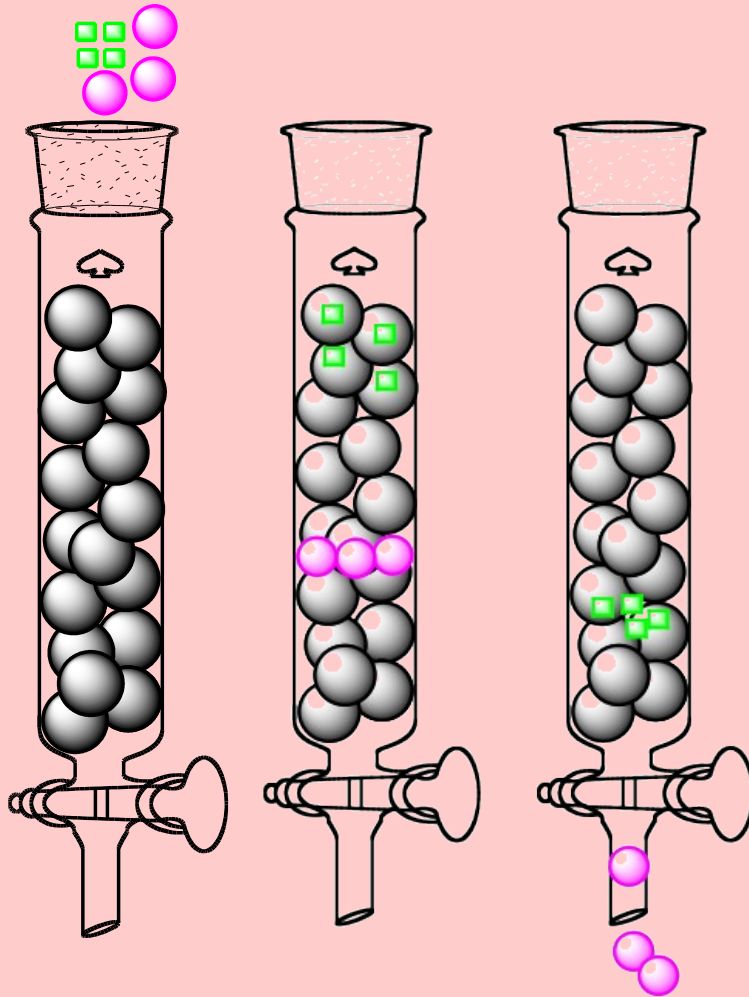
SIZE EXCLUSION CHROMATOGRAPHY

- It is also known as Gel Filtration
- Used to separate proteins on the basis of their molecular weight. The column is packed with a porous resin.
- The matrix retards proteins of different sizes for different periods. The proteins are collected automatically as they flow out of the column in tubes held in a fraction collector.
- Larger proteins will be eluted first since the smaller proteins travel through the pores of the resin.

SIZE EXCLUSION CHROMATOGRAPHY



Size Exclusion Chromatography

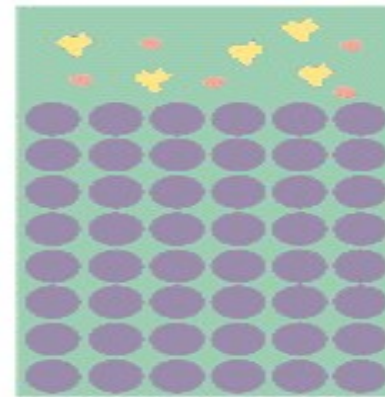
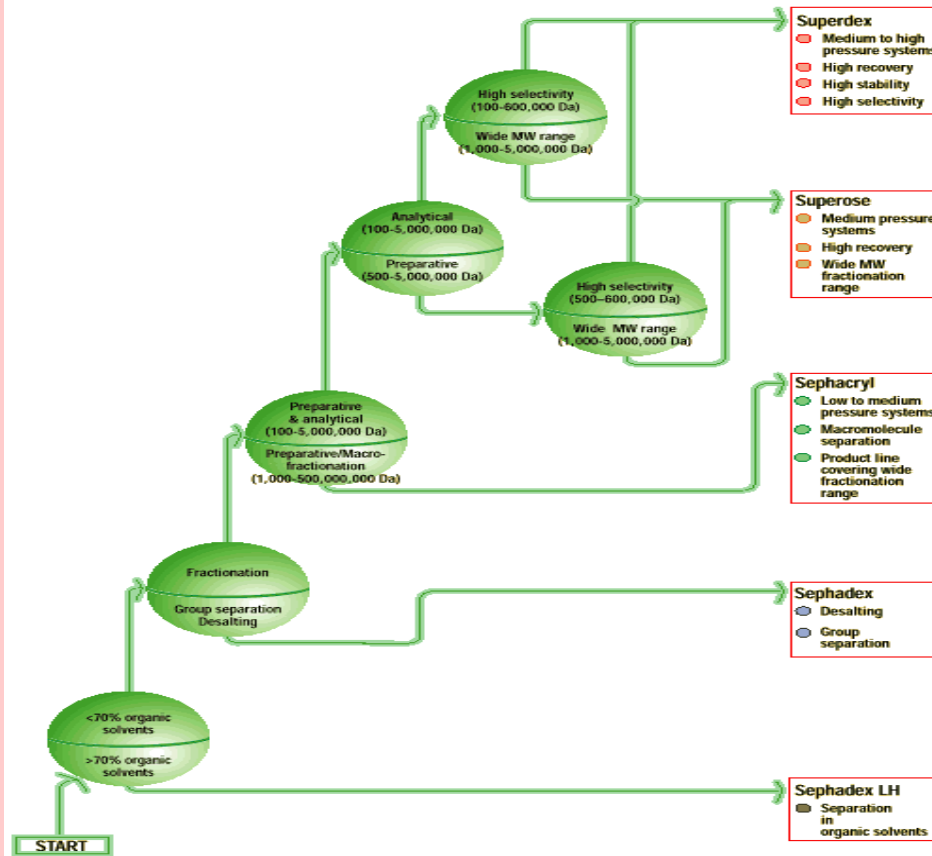


- Separates molecules based on size.
- Large molecules exit first.
- Mobile phase
 - Liquid
- Stationary phase
 - Insoluble, porous carbohydrate beads

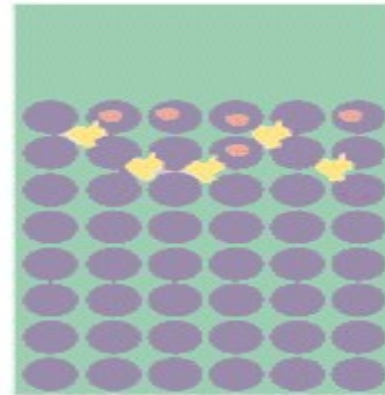
Gel Filtration



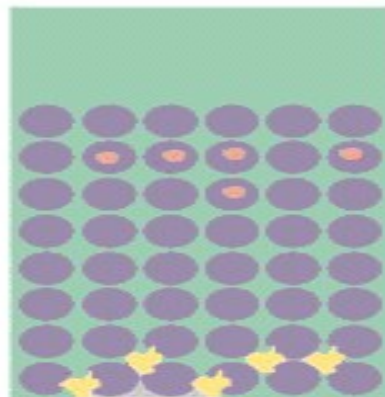
A gel filtration column has beads with channels running through them.



Load proteins into column



Smaller molecules enter the channels in the beads and have to travel farther.



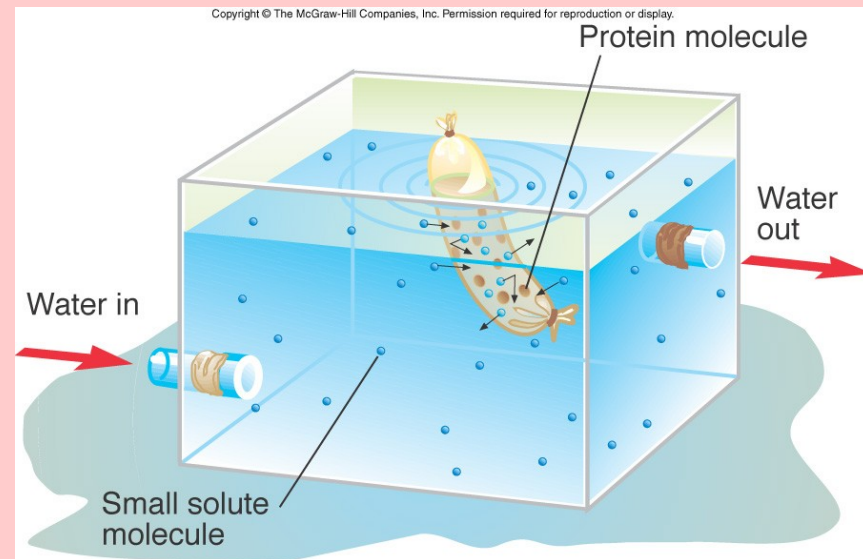
Larger molecules travel between beads and elute first.

Gel Filtration Chromatography

- **1. Column Packing** -- spherical porous beads of defined size
- **crosslinked dextrans** -- Sephadex (Pharmacia)
- **crosslinked polyacrylamide** -- Bio-Gel P (Bio-Rad)
- **crosslinked agarose** -- Sepharose (Pharmacia) or Biogel A (Bio-Rad). Agarose beads have very large pores and are, therefore, good for separating very large molecules
- other materials developed by other companies
- **2.** Gel beads are designed to have a distribution of pore sizes around a mean pore size. The mean pore size and the distribution determines the size range of molecules which can be separated.
- **3.** Dialysis is a form of molecular

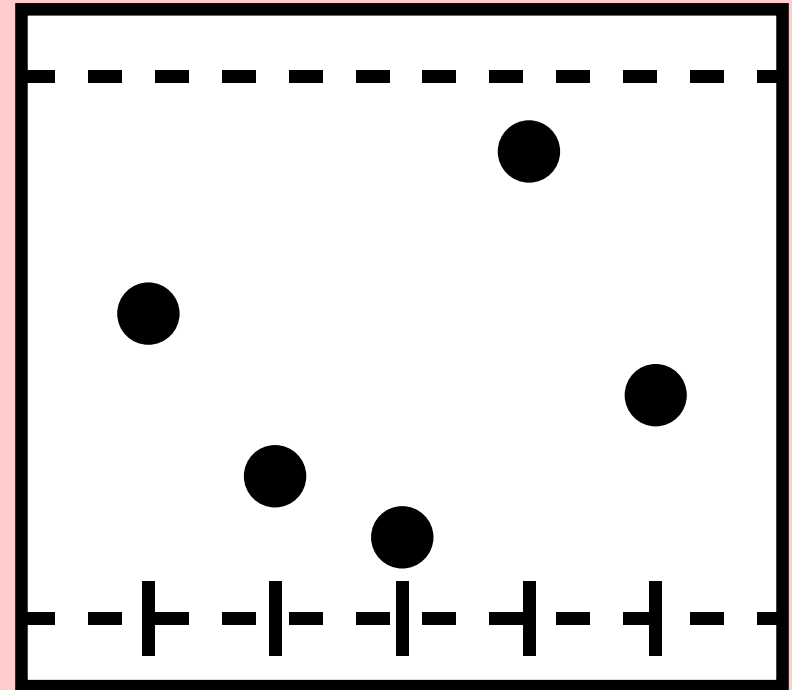
Dialysis

- **A form of size exclusion chromatography.**
- Used to desalt and concentrate protein samples.
- Dialysis tubing has set molecular weight cut off. Only molecules or ions smaller than MWCO will move out of the dialysis bag.



Thin Layer Chromatography

- Mobile phase
 - Nonpolar liquid
- Stationary phase
 - Polar solid material spread on backing (glass or thin sheet of metal)
- Separates molecules based on polarity



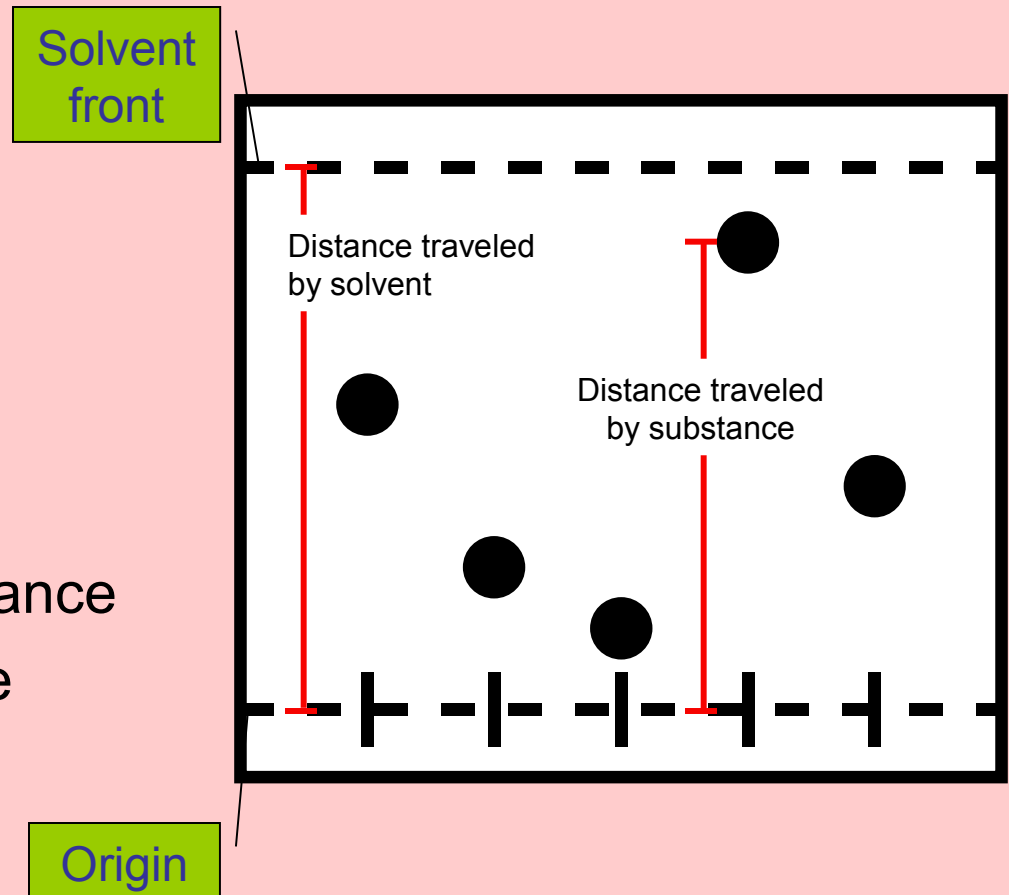
Thin Layer Chromatography

- Relative front value

$$R_f = \frac{\text{distance traveled by substance}}{\text{distance traveled by solvent}}$$

High R_f value = nonpolar substance

Low R_f value = polar substance

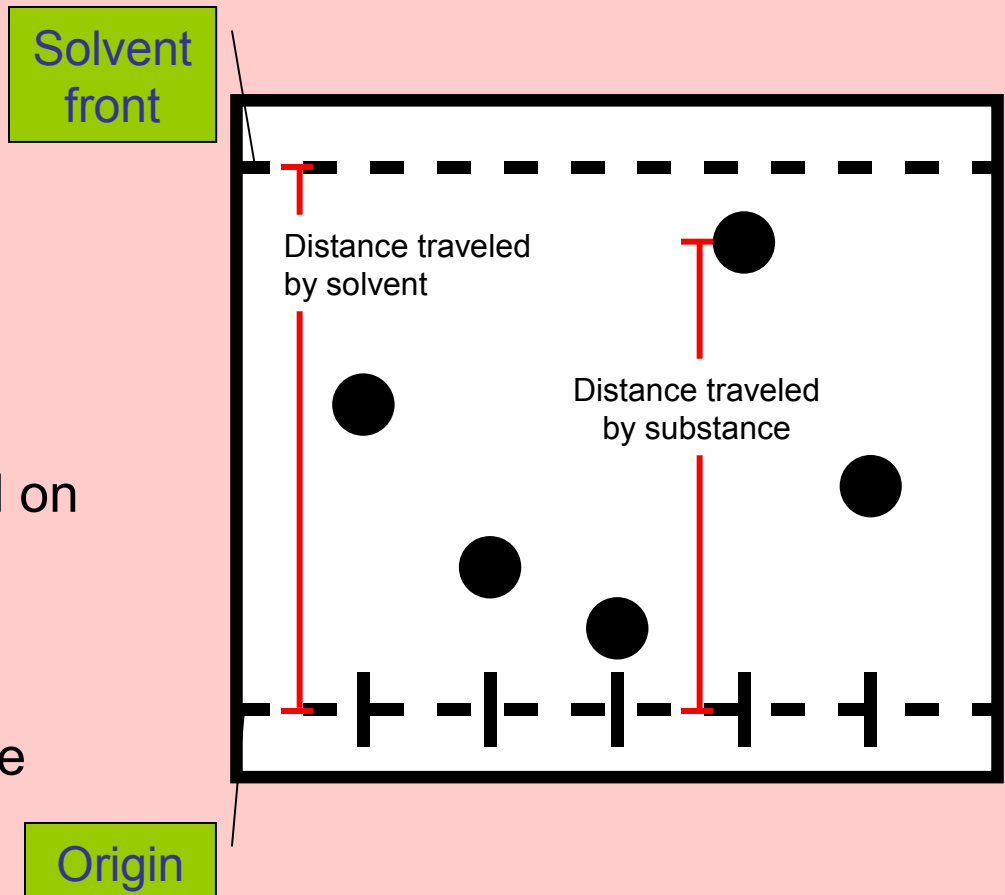


Reverse Phase Chromatography

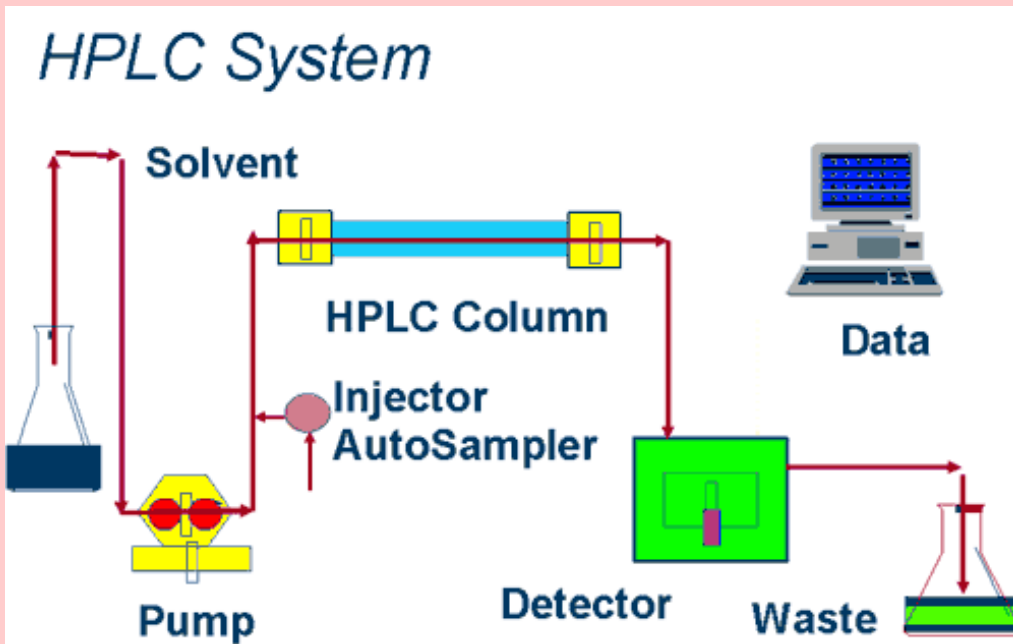
- Mobile phase
 - Polar liquid
- Stationary phase
 - Nonpolar liquid immobilized on inert solid

High R_f value = polar substance

Low R_f value = nonpolar substance



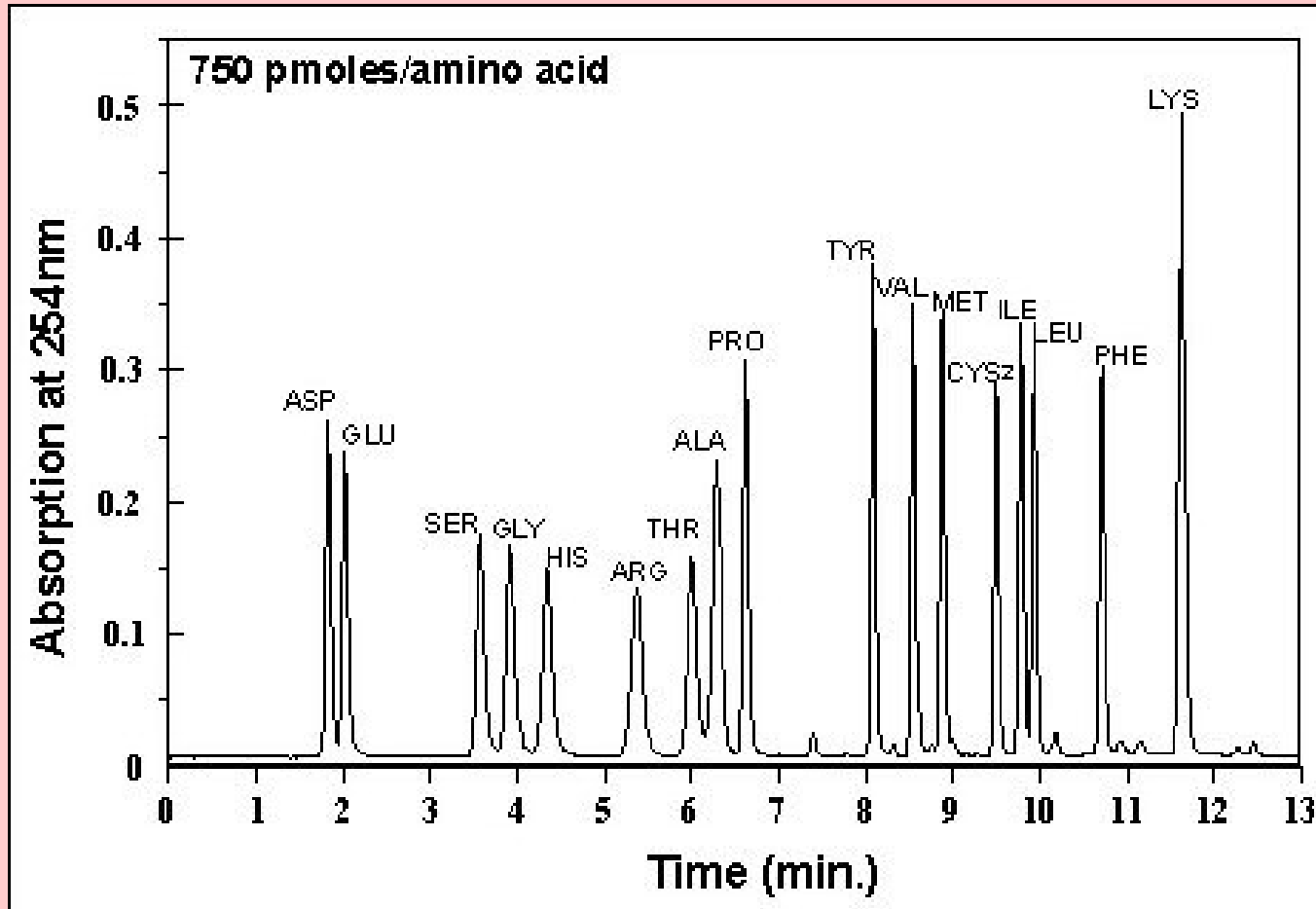
High Performance Liquid Chromatography



<http://www.waters.com/watersdivision/ContentD.asp?watersit=JDRS-5LTGBH>

- Mobile phase
 - Liquid
- Stationary phase
 - Small diameter particles packed into column.
- Pressure is required to push liquid through column.
- Advantages
 - Better resolving power
 - Faster

High Performance Liquid Chromatography



CHARACTERIZATION

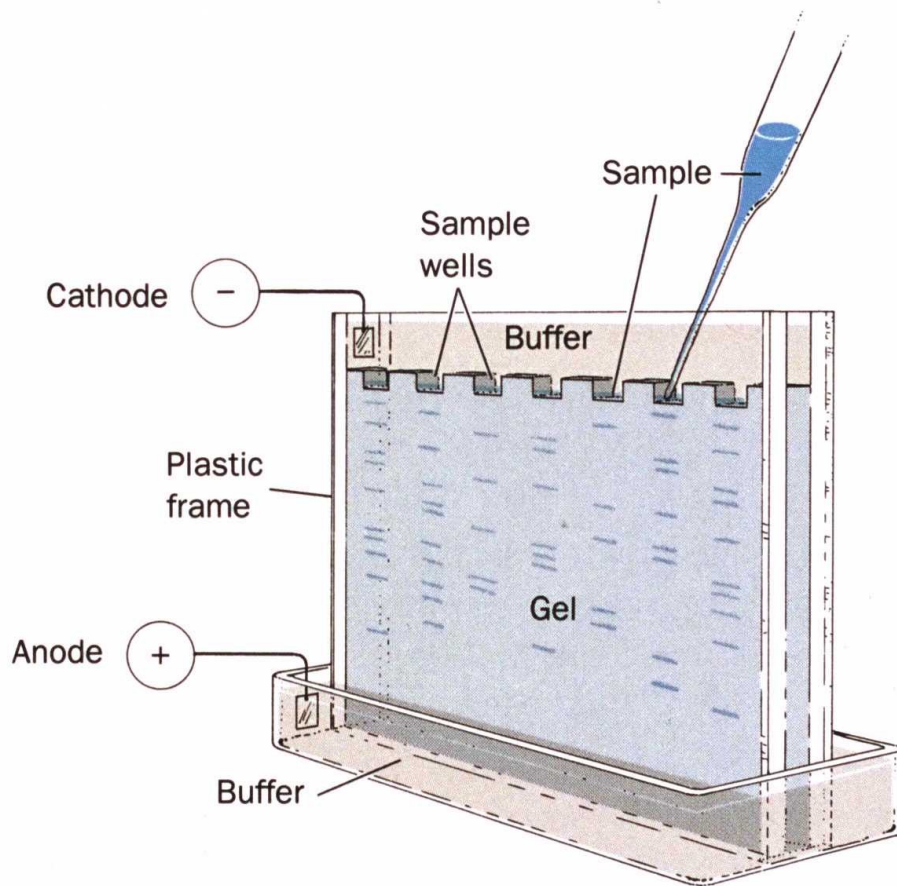
The method of protein characterization are as follows

- **Electrophoresis**
- **Peptide Sequencing**
- **Tryptic Mapping**
- **Analytical Ultracentrifugation**
- **Spectroscopy**
- **Biosensors**
- **Mass Spectrometry**

Characterization of Protein

- **Molecular mass**
 - **Electrophoresis**
 - **Matrix-assisted laser desorption-ionization time of flight (MALDI-TOF)**
- **Isoelectric point**
 - **Isoelectric focusing**
- **3-D Structure**
 - **X-ray crystallography**

Electrophoresis



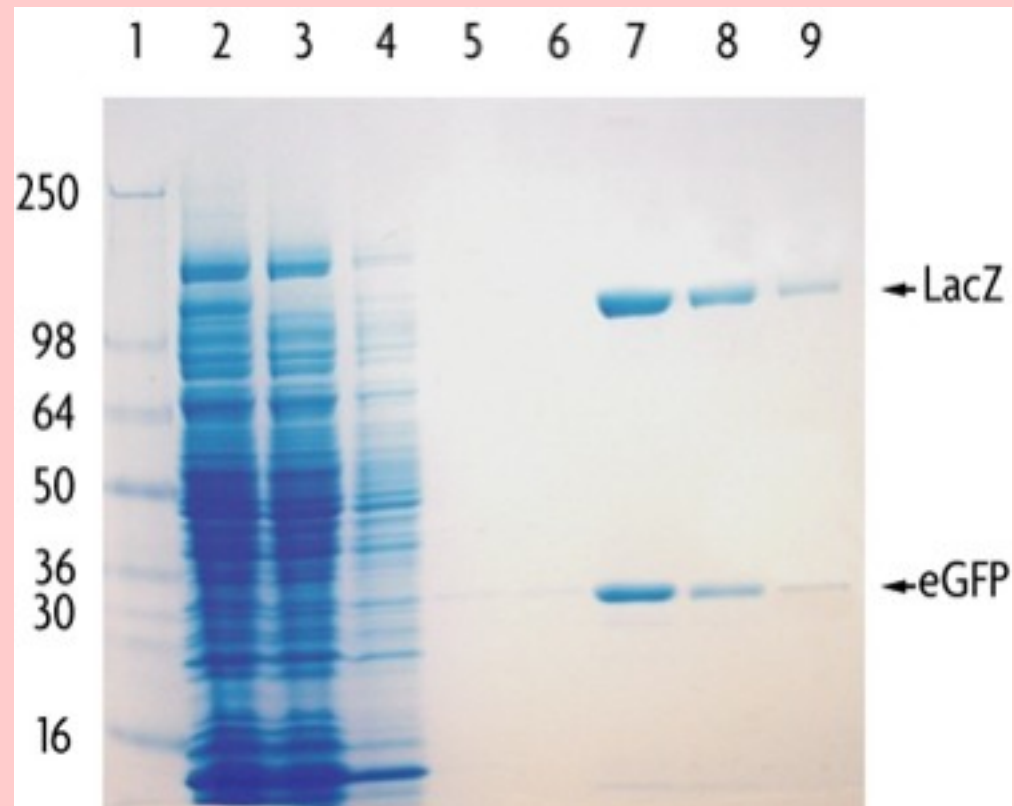
- **Separates molecules based on molecular mass and/or charge.**

ELECTROPHORESIS

- **Proteins are separated on the basis of their molecular mass using sodium dodecyl sulfate polyacrylamine (SDS-PAGE).**
- **It reduces proteins into regular rod like forms of constant charge density per unit mass.**
- **SDS breaks all hydrogen bonds and partially unfolds the protein structure.**
- **The other method coming up is Capillary electrophoresis.**
- **Capillary electrophoresis**
 - a) Conducted in a tubing of very small diameter using high voltage.
 - b) Takes very less time.
 - c) Good separation is achieved.

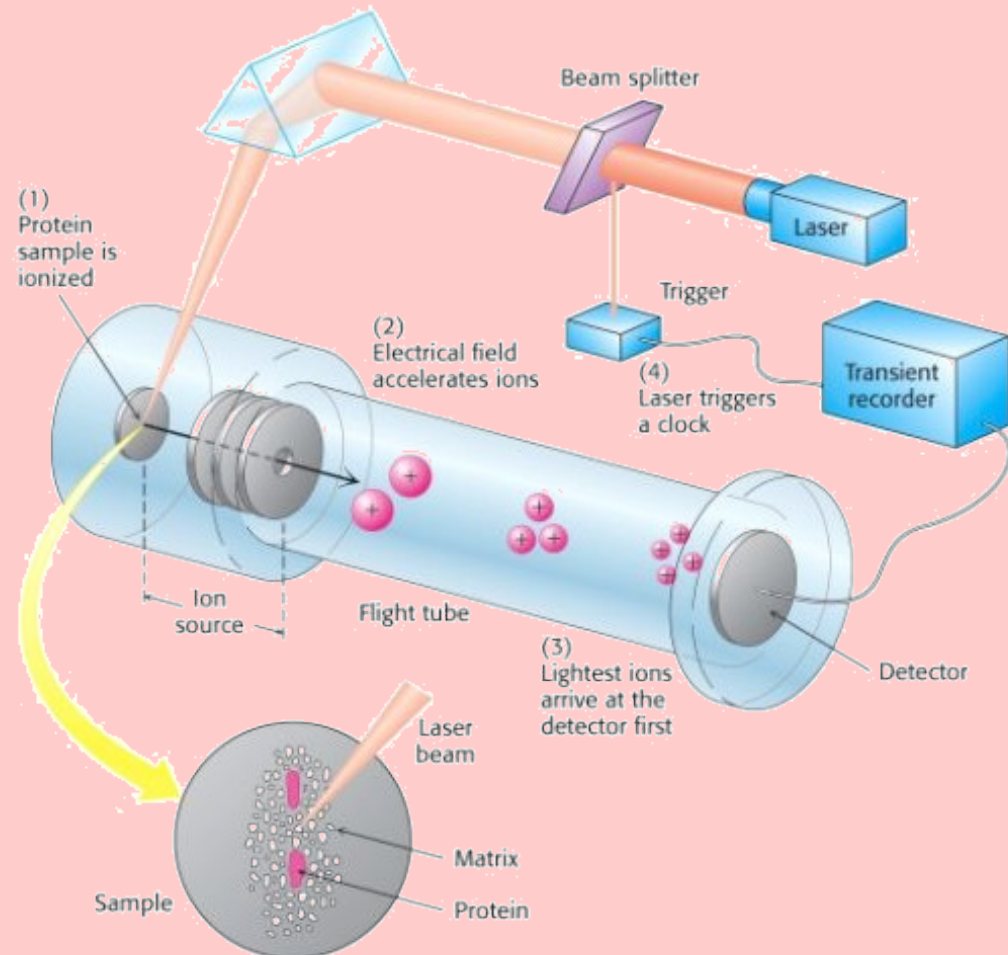
SDS-PAGE

- **Sodium dodecyl sulfate polyacrylamide gel electrophoresis**
- Separation based on molecular mass.
- Coat samples with SDS to give uniform charge to mass ratio.
 - Makes all proteins negatively charged.

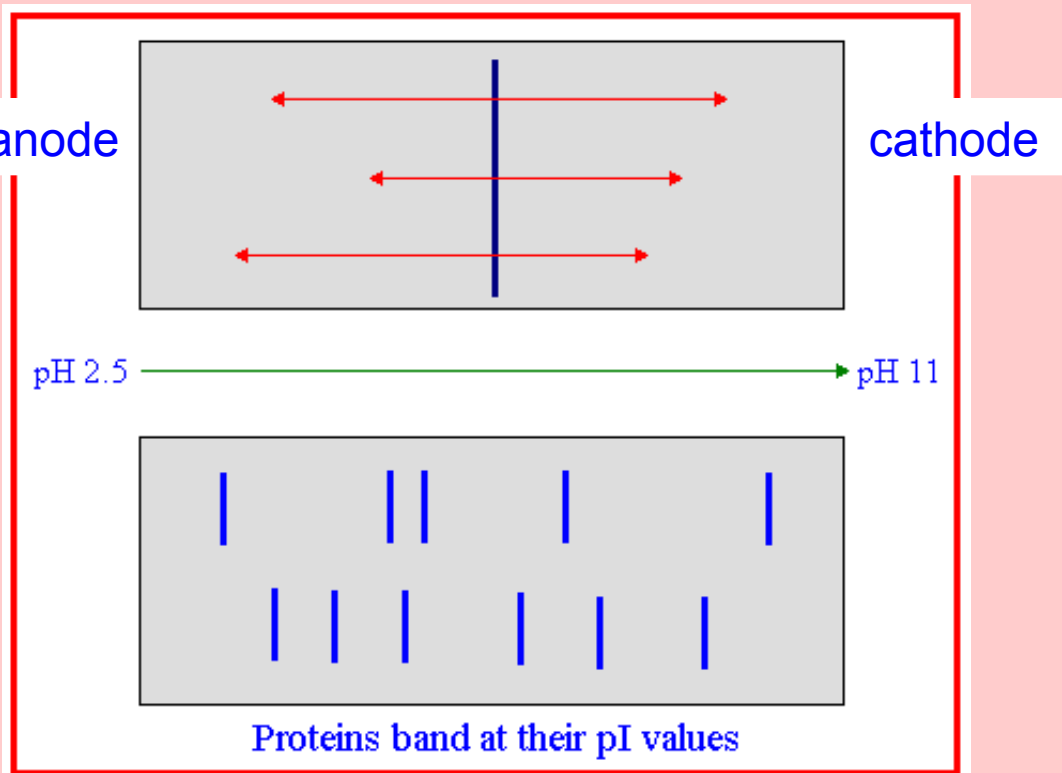


MALDI-TOF

- Laser displaces sample into ionization chamber.
- Ions travel through electrical field.
- Heavier ions travel more slowly.



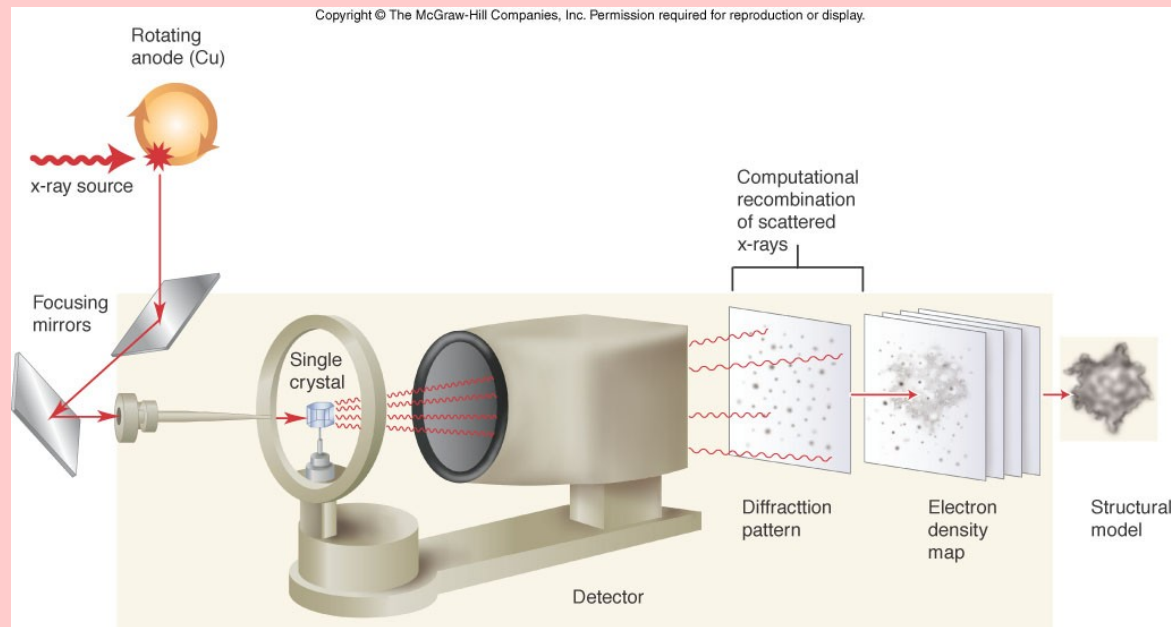
Isoelectric Focusing



- Separation based on charge.
- Can be used to experimentally determine pI.

X-ray Crystallography

- Electrons in crystal scatter x-rays to produce an image.
- Fourier transformation is used to convert raw data into 3-D structure.



PEPTIDE SEQUENCING

- Also known as amino terminal sequencing.
- Used to identify the first few amino acids of the protein.
- This sequence information can be used to confirm the identity of the protein.
- It depends on sequential stepwise removal of N-terminal amino acids by HPLC and then identified by characteristic retention times.

TRYPTIC MAPPING

- Small peptides derived from the protein by endoprotease action are separated by high resolution reverse phase HPLC.
- The individual peptides are then subjected to sequencing to confirm the identity of the given peptide.

ANALYTICAL ULTRACENTRIFUGATION

- This technique allows measurement of a variety of properties of a protein sample, including solution molecular weight, interaction with other molecules and sample homogeneity.

SPECTROSCOPY

- It gives an indication of the fraction of the polypeptide that is composed of specific secondary structural features such as α -helix and β -sheet.
- It is also used for characterizing metal containing protein cofactors.

BIOSENSORS

- Device used for the detection of protein cells.
- An antibody to a particular protein is immobilized on the sensor surface, addition of sample containing that particular protein will produce an immediate signal.

MASS SPECTROMETRY

- A technique by which you can determine the mass of a protein with remarkable precision of the order of a few hydrogen atoms.
- It can detect any important modification (post translational modification) or variation in a protein structure.

EMERGING TRENDS

- Automation
- Capillary Electrophoresis and Mass spectrometry are advancing rapidly.
- Mass Spectrometry will be more widely used, largely through core facilities.
- Capillary Electrophoresis will likely replace SDS-PAGE in a few more years.

Monitoring Progress of Purification Protocol

- **Total protein (mg)**
 - Quantity of protein present in fraction
- **Total activity (units of activity)**
 - Use a portion of sample to determine activity.
 - Multiply activity by total volume to determine total activity.

Monitoring Progress of Purification Protocol

- **Specific activity (units of activity/mg)**

Specific activity = Total activity/Total protein

- **% yield:** measure of activity retained after each step in procedure.

$$\% \text{ yield} = \frac{\text{Total activity at particular step}}{\text{Total activity of initial extract}}$$

Monitoring Progress of Purification Protocol

- **Purification level:** Measure of increase in purity of protein throughout procedure.

$$\text{Purification} = \frac{\text{Specific activity at particular step}}{\text{Specific activity of initial extract}}$$

Monitoring Progress of Purification Protocol

Step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Yield (%)	Purification level
Initial extract	15,000	150,000	10	100	1
(NH ₄) ₂ SO ₄ precipitation	4,600	138,000	30	92	3
Ion-exchange	1,278	115,500	90	77	9
Size exclusion	68.6	75,000	1,100	50	110
Affinity column	1.75	52,500	30,000	35	3,000

- **SUMMARY**

Eventful Protein Purification

- Grow cells in media (vector+tag)
- Centrifuge, Collect the pellet
- Lyse the cells (appropriate buffer)

Pilot Expression
SDS PAGE, Assay

Purification Strategy

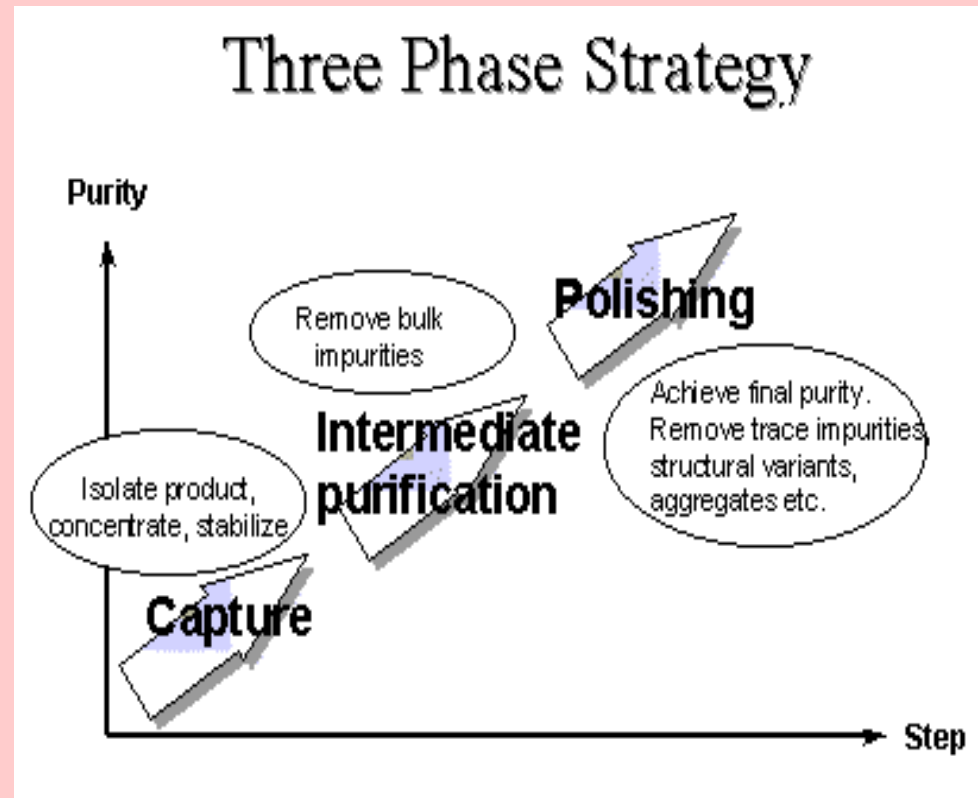
Characterization
Mass Spectroscopy
X-ray Crystallography
Functional Assay

Solubility
Aggregation
Recombination



Protein Purification Strategies

1. Evaluate an assay for the protein of interest
2. Shortlist a method to have a reasonable source for that activity



(http://www5.amershambiosciences.com/aptrix/upp00919.nsf/Content/LabSep_EduC%5CAboutPurBiom%5CHowToCombine)