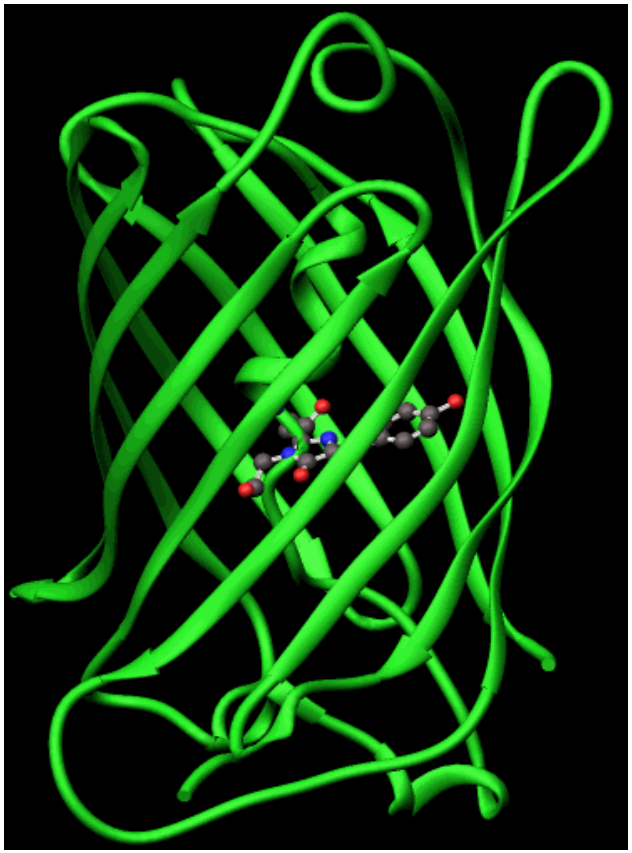
Downstream Processing

Know the Characteristics of Your Protein
Green Fluorescent Protein (GFP)

Sequence of Amino Acids

MSKGEELFTGVVPVLVELDGDV
NGQKFSVSGEGEGDATYGK
LTLNFICTTGKLPVPWPTLVT
TFSYGVQCFSRYPDHMKQH
DFFKSAMPEGYVQERTIFYK
DDGNYKTRAEVKFEGDTLV
NRIELKGIDFKEDGNILGHK
MEYNYNSHNVYIMGDKPK
NGIKVNFKIRHNIKDGSVQL
ADHYQQNTPIGDGPVLLPD
NHYLSTQSALSKDPNEKRD
HMILLEFVTAARITHGMDEL
YK

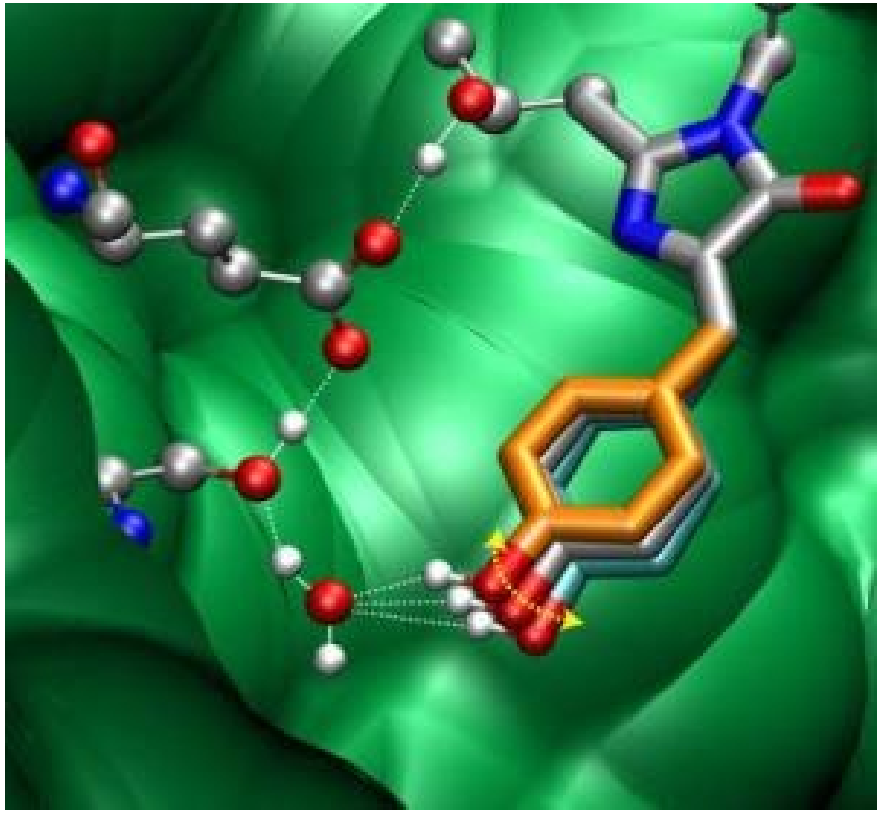
Tertiary Structure



Green Fluorescent Protein (GFP)

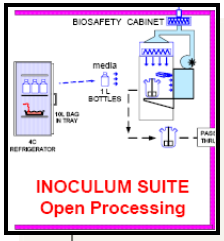
- MW (molecular weight = 27,000 Daltons (27 kD)
- pI (isoelectric point) - 4.8
- Hydropathicity (hydrophobicity) - hydrophobic amino acids make up GFP's fluorophore; amino acids associated with the fluorophore are also hydrophobic

GFP Chromatophore - Hydrophobic



Downstream Processing in Biopharmaceutical Manufacturing

- Protein Purification
- Harvest by Centrifugation
- Clarification by Depth Filtration
- Sterile Filtration (MF)
- Tangential Flow Filtration (UF/DF)
- Low Pressure Liquid Column Chromatography



Working Cell Bank

Media Prep

Large Scale Bioreactor

1 day

Seed Bioreactors

150L Bioreactor

750L Bioreactor

5,000L Bioreactor

26,000L Bioreactor

Centrifuge

Depth Filtration

Collection

Sub-Culture

Sub-Culture

Sub-Culture

Sub-Culture

Sub-Culture

Wave Bag

Inoculum

24 days

Fermentation

31 days

Harvest/Recovery

Viral Inactivation

Harvest Collection Tank 1,500L

Filter

Chromatography Skid

Column

Eluate Hold Tank 8,000L

Chromatography Skid

Column

Eluate Hold Tank 20,000L

Anion Exchange Chromatography (QXL)

Hydrophobic Interaction Chromatography (HIC)

Purification

Filter

Chromatography Skid

Column

Eluate Hold Tank 6,000L

Viral Filtering

Post-viral Hold Vessel 3,000L

Filter

Chromatography Skid

Column

Eluate Hold Tank 5,000L

Ultra Filtration Diafiltration

Bulk Fill

Protein A Chromatography

8 days

Anion Exchange Chromatography (QFF - Fast Flow)

Protein Purification - Filtration

Separate protein using pores in solid media - small pore excludes large proteins (and vice versa):

- Depth Filtration
- Tangential Flow Filtration
- Ultrafiltration
- Sterile Filtration
- Defiltration

Protein Purification – Liquid Chromatography

Separate protein using different affinities for a solid media (matrix or bead) vs. liquid buffer:

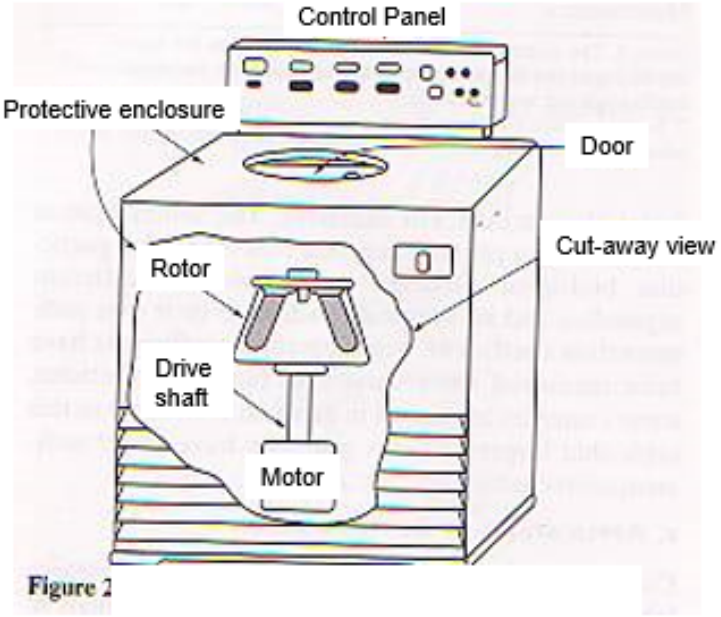
- Hydrophobic Interaction Chromatography (HIC)
- Ion Exchange Chromatography (IEX):
  - Anion Exchange Chromatography
  - Cation Exchange Chromatography
- Affinity Chromatography
- Gel Filtration or Size Exclusion Chromatography

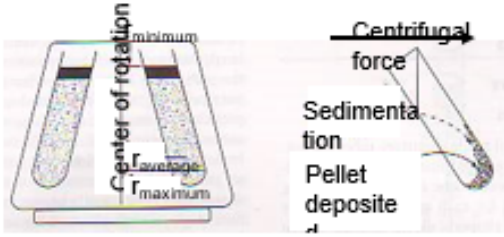
Methods to Remove Cells and Cellular Debris

- Centrifugation
- Depth Filtration

Centrifuge

- **An instrument that generates centrifugal force**
- **Commonly used to separate particles in a liquid from the liquid**

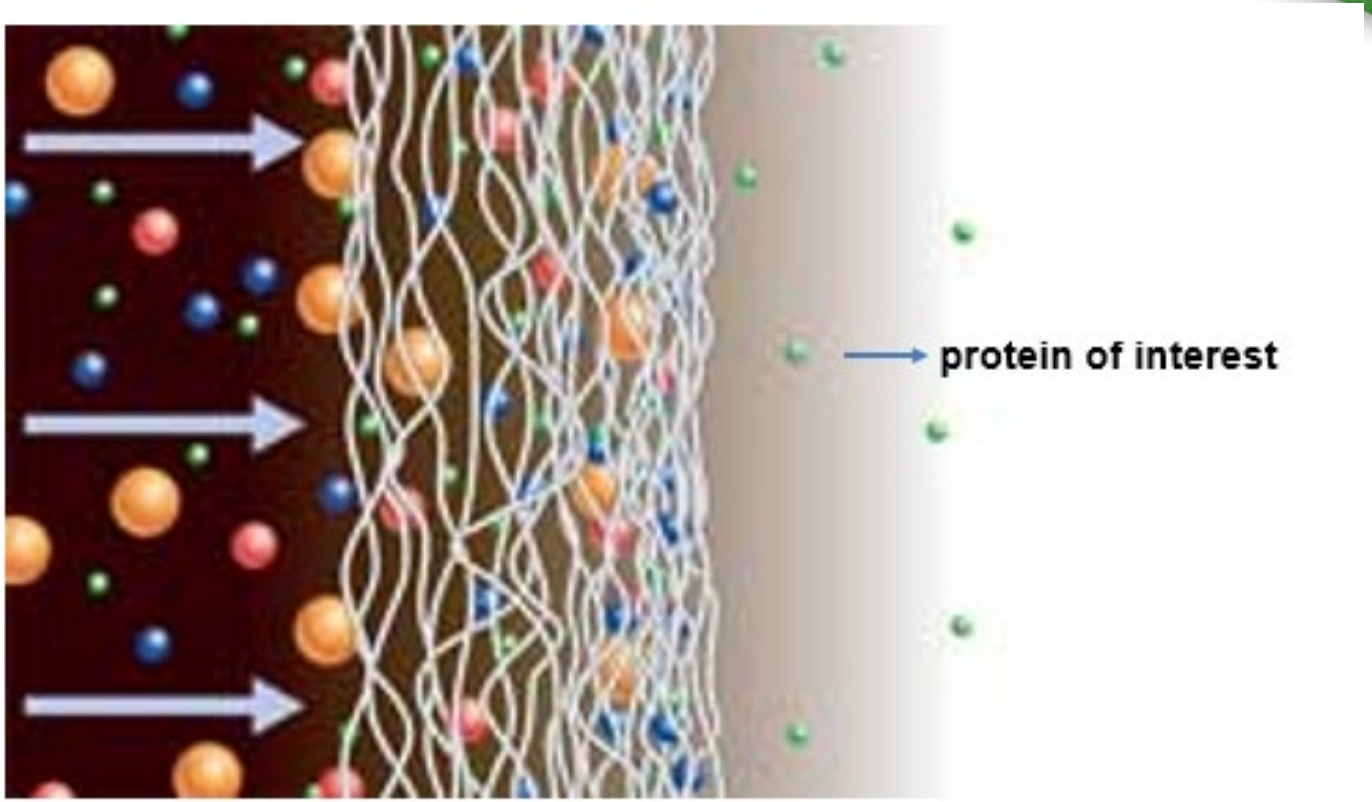




Basic components of a centrifuge

Depth Filtration

Cells and cellular debris stick to ceramic encrusted fibers in pads

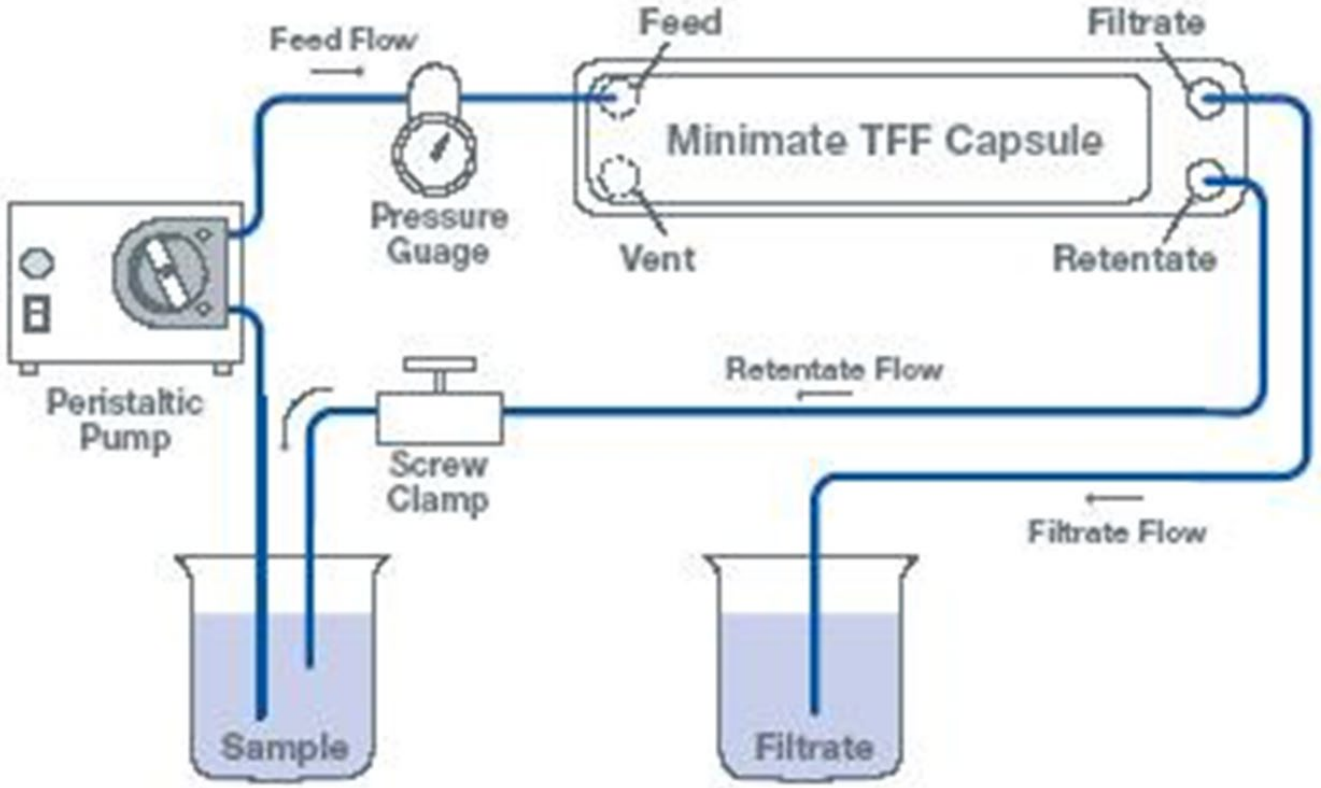


Tangential Flow Filtration- TFF

Used to separate of protein of interest

- Using TFF with the right cut off filters, the protein of interest can be separated from other proteins and molecules in the sterile filtered, clarified medium
  - e.g HSA has a molecular weight of 69KD . To make sure that the protein of interest is retained, a 10KD cut-off filter is used
- After ultrafiltration perform defiltration with buffer used to prepare for affinity chromatography of HSA

How TFF Concentrates and Purifies a Protein of Interest



Low Pressure Liquid (Production) Chromatography

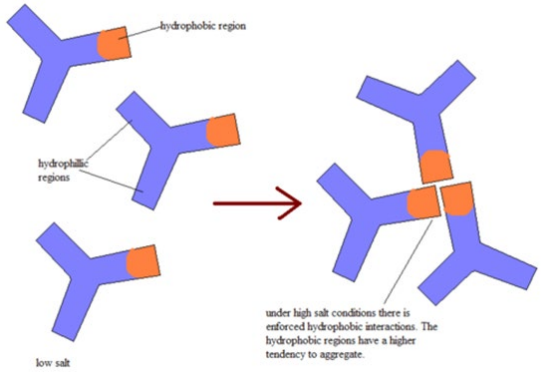
The Media:

- Hydrophobic Interaction (HIC)
- Ion Exchange (Anion AEX and Cation CEX Exchange)
- Gel Filtration (=Size Exclusion)
- Affinity

The System: Components and Processes

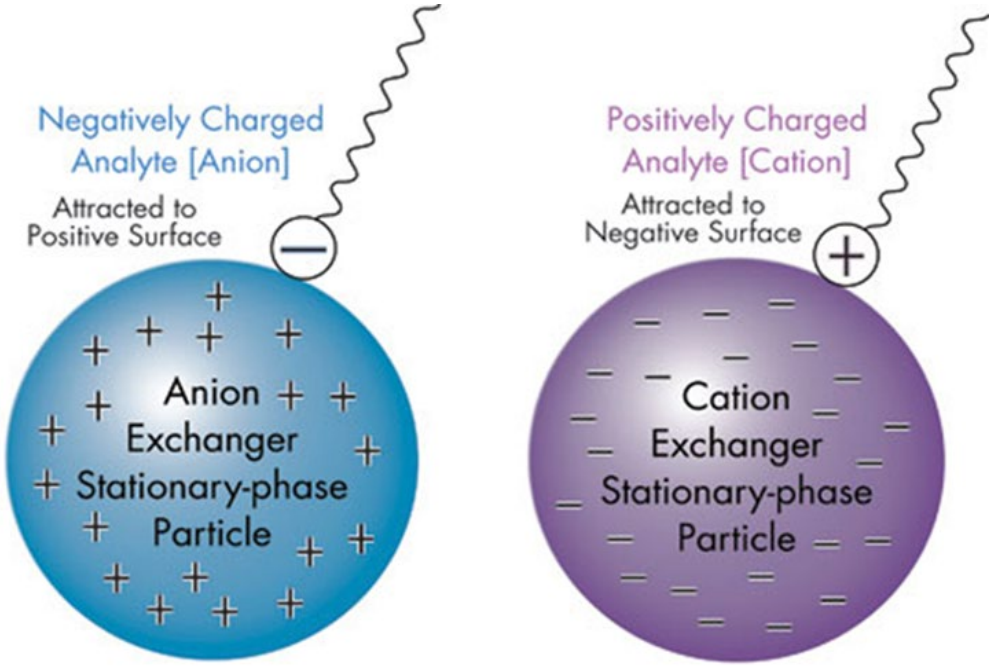
Hydrophobic Interaction Chromatography (HIC

- HIC media have high capacity and are economical and stable; capable of powerful resolution
- used in therapeutic antibody purification because part antibodies are found in membranes, are lipid soluble and therefore hydrophobic
- molecular mechanism relies on unique structural features- serves as an orthogonal method to ion exchange and affinity chromatography
- **adsorption takes place in high salt and elution in low salt concentrations** These special properties make HIC very useful in whole processes for bridging or transitioning between other steps in addition to the separation which is effected



Ion Exchange Chromatography

- Separates by Charge



Isoelectric Focusing or IEF

- If the pI of your protein (or the pH at which your protein is neutral) is known , you can place it in a buffer at a lower or higher pH to alter its charge
- If the pH of the buffer < pI, the protein of interest will become positively charged
- If the pH of the buffer > the pI, the protein of interest will become negatively charged

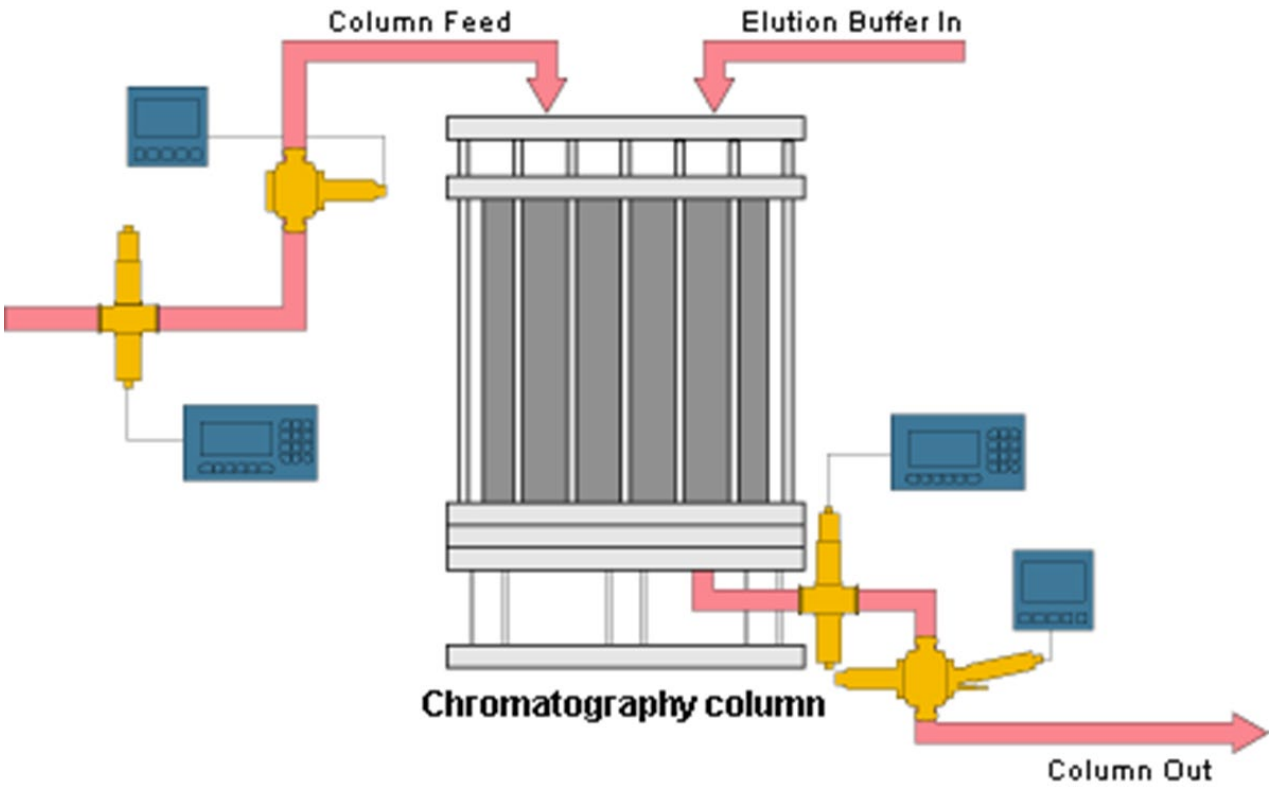
pH < pI < pH

+ 0 -

Liquid Column Chromatography Process

- **PURGE** Air from Column use *Equilibration Buffer*
- **PACK** Column with *Beads* (e.g. ion exchange, HIC, affinity or gel filtration beads/media)
- **EQUILIBRATE** Column with *Equilibration* Buffer
- **LOAD** Column with Protein of Interest *in Equilibration* Buffer
- **WASH** Column with *Equilibration Buffer*
- **ELUTE** Protein of Interest with *Elution Buffer* of High or Low Salt or pH
- REGENERATE Column or Clean and Store (NaOH)

Liquid Column Chromatography

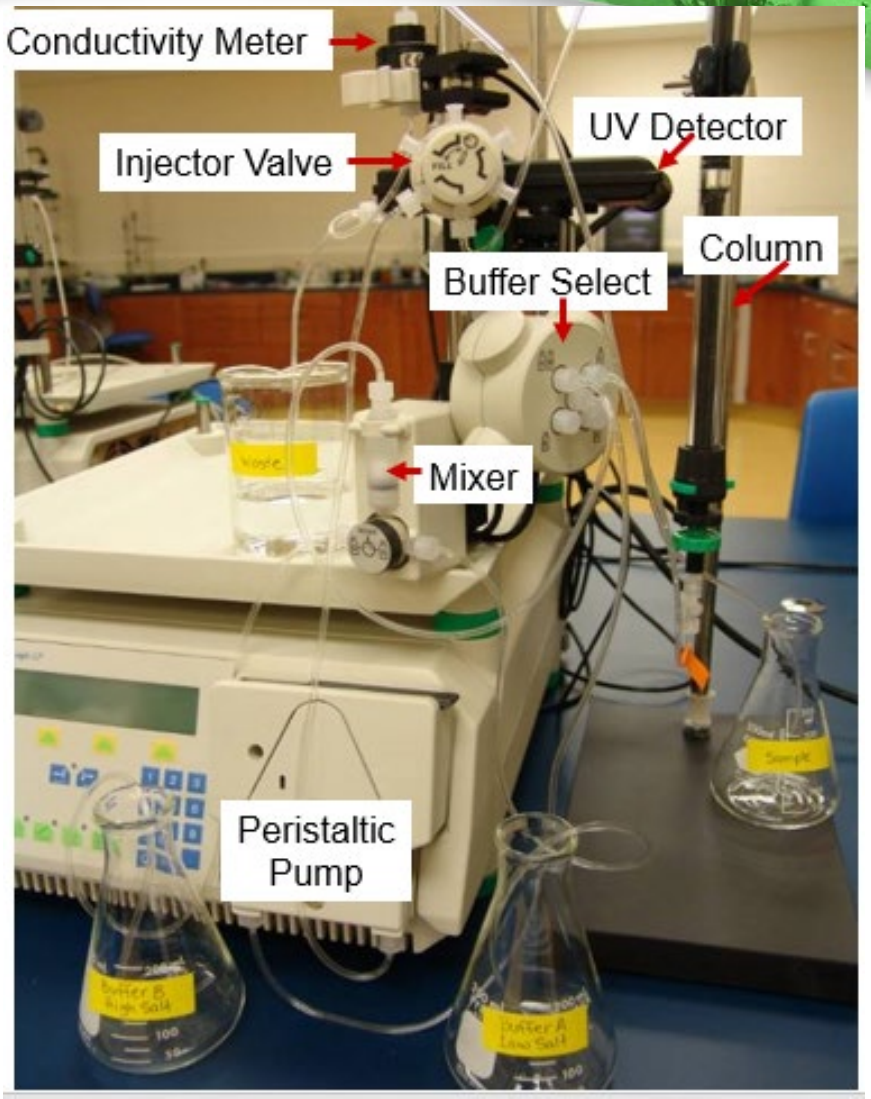


GFP Chromatography (HIC)

GFP Chromatography

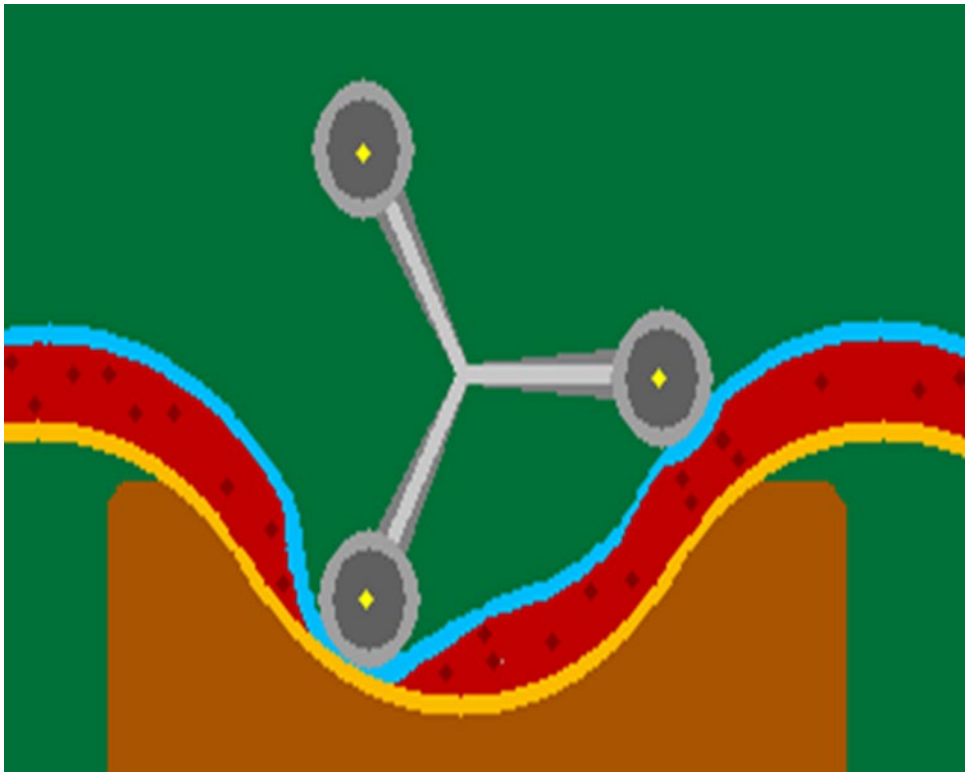
LP LC System Components

- Mixer for Buffers, Filtrate with Protein of Interest, Cleaning Solutions
- **Peristaltic Pump**
- Chromatography Column and Media (Beads)
- **Conductivity Meter**
- **UV Detector**



LP LC System Components- Peristaltic Pump

- Creates a gentle squeezing action to move fluid through flexible tubing



LC System Components

- UV Detector - detects proteins from the column by measuring absorbance at 280nm

- Conductivity Meter - measures the amount of salt in the buffers coming out of the columns – high salt or low salt are often used to elute the protein of interest from the chromatography beads

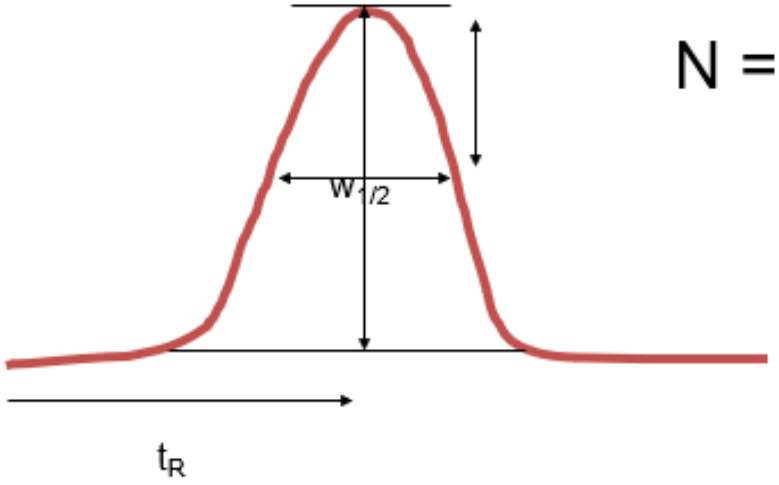
Height Equivalent to Theoretical Plate (HETP)

HETP = L/N

- **L=length of column in mm**
- **N=column efficiency**

- allows comparison of columns of different lengths
- smaller the HETP the better
- shorter the column the better

Calculating Column Efficiency (N)



$$N = 5.54 \left(t_R / w_{1/2}\right)^2$$

A Typical Chromatogram