

Upstream and Downstream Processing

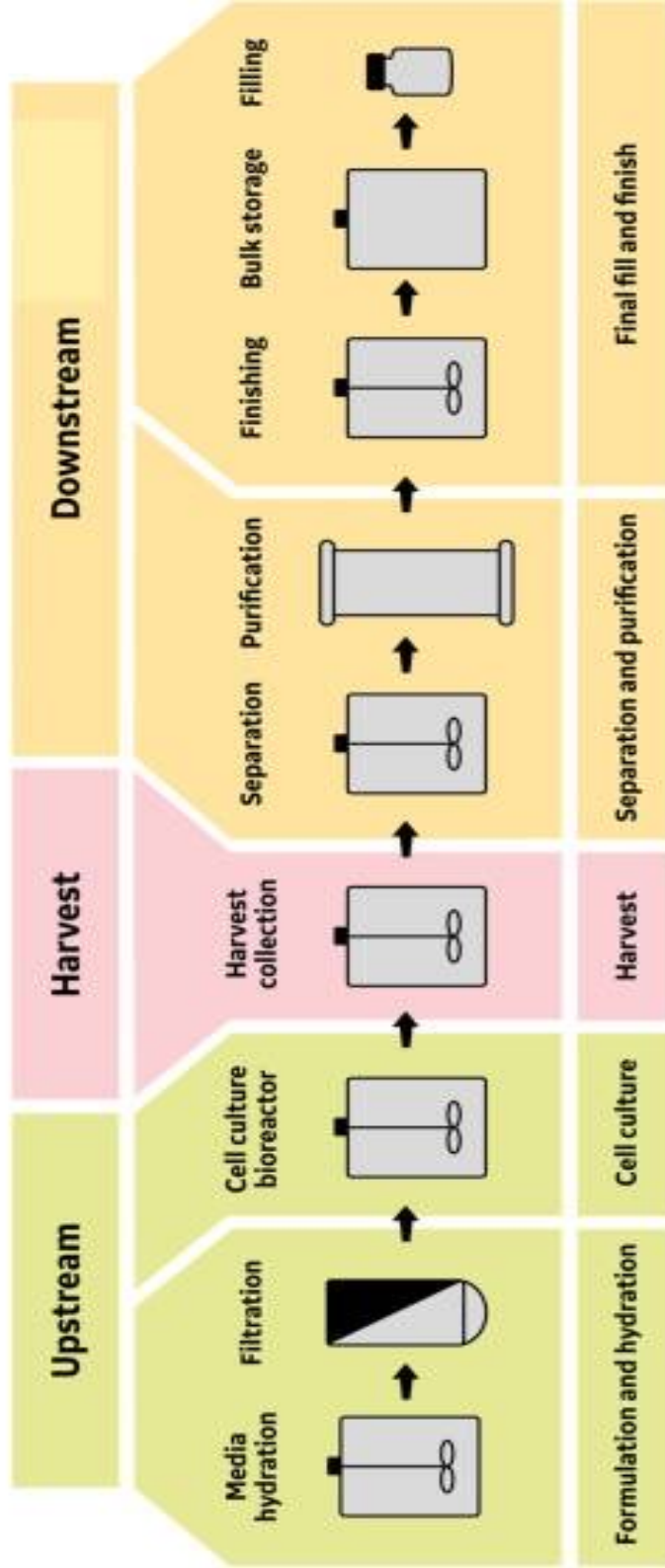




Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

- a) **Upstream processes** include selection of microbial strain characterized by ability to synthesize specific product having desired commercial value.
- b) Strain is subjected to improvement to maximize ability to synthesize economic amounts of product.
- c) Included in upstream phase is fermentation process itself which usually is carried out in large tanks known as fermenters/bioreactors.
- d) In addition to mechanical parts which provide proper conditions inside tank such as aeration, cooling, agitation, etc., tank is usually equipped with complex sets of monitors and control devices to run microbial growth and product synthesis under optimized conditions.
- e) Common fermenter: Stirred-tank fermenter which utilizes mechanical agitation principles, mainly using radial-flow impellers, during fermentation process.

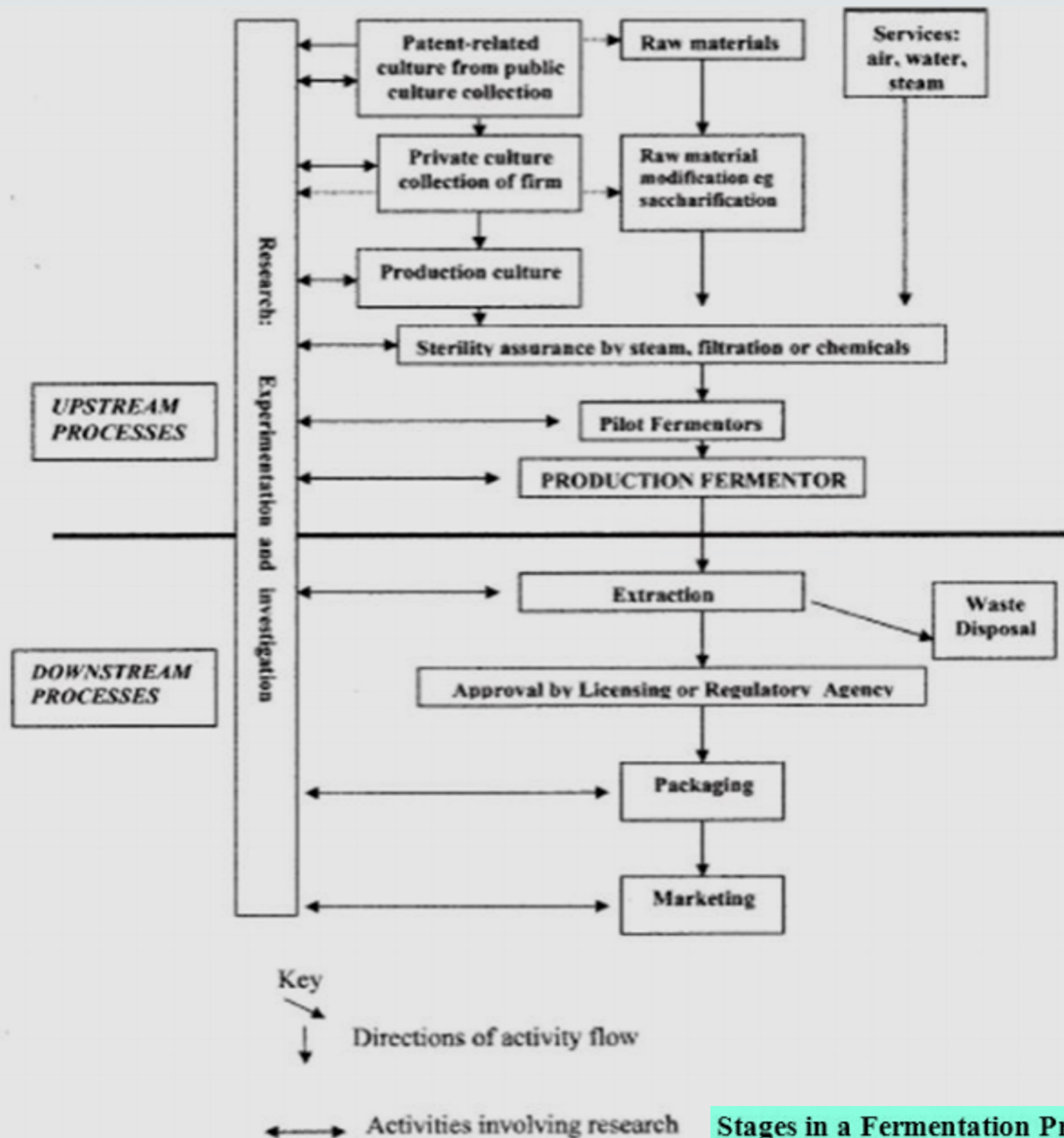


- **Downstream processing**, various stages that follow fermentation process, involves suitable techniques and methods for recovery, purification, and characterization of desired fermentation product.
- Vast array of methods for downstream processing, such as centrifugation, filtration, and chromatography, may be applied.



**Centurion
UNIVERSITY**

*Shaping Lives...
Empowering Communities...*



Stages in a Fermentation Process



Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

Overview of Upstream Processing

- ✓ **Fermentation media, selection of suitable cost effective carbon and energy sources, along with other essential nutrients. Media optimization is vital aspect of process development to ensure maximization of yield and profit.**
- ✓ **Producer microorganism, include strategy for initially obtaining suitable microorganism, industrial strain improvement to enhance productivity and yield, maintenance of strain purity, preparation of suitable inoculum and development of selected strains to increase economic efficiency of process.**
- ✓ **Rigorously controlled conditions developed to optimize growth of organism/production of a target microbial product.**



What is **FERMENTATION** *Medium*

- **Defined, complex or technical medium.**
- **Defined medium consists of chemically defined substrates.**
- **Complex medium is composed of substrates with undefined composition, such as extracts or hydrolysates from waste products, which are cheap substrates used in industrial production. Expensive substrates (yeast extract, brain heart infusion, peptone and tryptone are used for complex medium).**
- **Technical media are used on an industrial scale and cheaper.**
- **Substrate sources derived from industrial waste require pretreatment before fermentation. eg., soy meal, whey, fishmeal, malt extract and sulfite waste liquor.**



Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

- **Wastewater from monosodium glutamate production, which contains high levels of chemical oxygen demand (COD), sulphate, and ammonical nitrogen at low pH, has been used as nitrogen and water source, with sugar beet pulp as carbon source, for production of pectinase.**
- **Media sterilization is necessary to ensure that desired microorganism is present to carry out fermentation, with products of predicted quality.**
- **Sterilization by high temperature achieved by direct or indirect steam/electric heating, membrane filtration, microwave irradiation, high voltage pulses and photo semiconductor powders which involve rupture of cell membrane by increasing transmembrane electric field strength beyond certain threshold.**

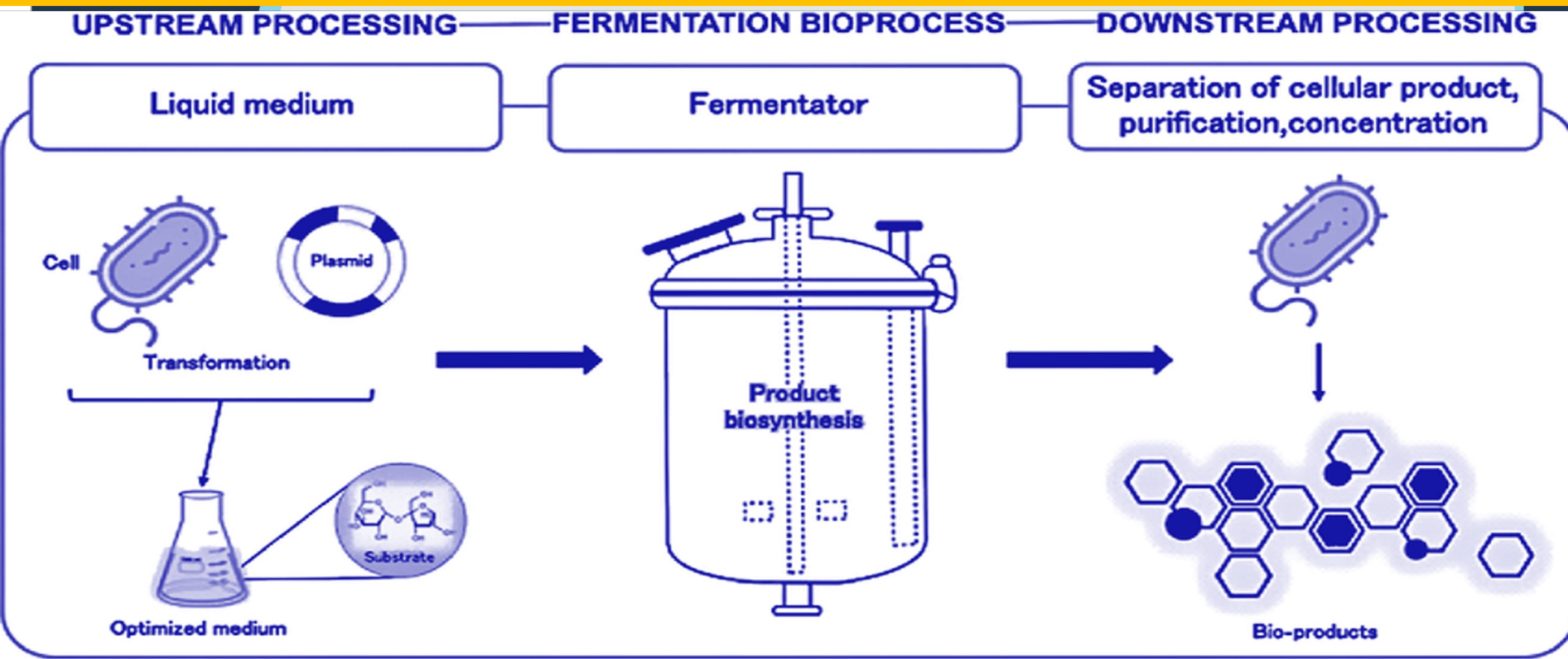


Centurion
UNIVERSITY
*Shaping Lives...
Empowering Communities...*

- **Inoculation is transfer of seed material/inoculum into the fermentor.**
- **Inoculation of laboratory fermentor is done using pre-sterilized tubing and peristaltic pump.**
- **Inoculum transfer is done by applying positive pressure on inoculum fermentor and connecting it aseptically to production fermentor.**
- **Connecting lines are sterilized before used for transfer of inoculum.**
- **Heat susceptible substances such as amino acids and some vitamins must be dissolved in small volumes of water, sterilized by filtration and added separately to the final medium aseptically.**

Fermentation system:

- Usually operated through batch, fed batch or continuous.
- Choice of fermentation mode is dependent on relation of consumption of substrate to biomass and products.
- Common type of upstream processing of proteins utilizes two tools: bioreactors and suspension (or attached) cells transformed with expression vectors genetically engineered to contain one (or more) human genes that produce copious amounts of their protein(s).





Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

Inoculum:

- Upstream processing of proteins using bioreactors and cells usually begins with preparation of inoculum which proceeds in scale-up steps until enough inoculum is made to aseptically inoculate final, sterile, media filled bioreactor.
- During culture period, samples are removed, and various parameters are measured by fermentation technicians including optical density (OD) and live cell count.
- Upstream processing are initial purification steps which include centrifugation and/or filtration to separate cells from media. Cells/media would be discarded to the kill tank, depending on where protein was located.



Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities...

- Glass bioreactors are representative of three types of cells used in upstream processing of human protein pharmaceuticals: bacterial, animal, and fungal cells.
- In *E. coli*, proteins are secreted into media so media is saved for later isolation and purification of protein of interest in downstream processing.
- Proteins remain inside cell so, cells are separated from media and media is discarded to kill tank.



Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

Overview of Downstream Processing

- ✓ **Primary aim of efficiently, reproducibly and safely recovering target product to required specifications (biological activity, purity) while maximizing recovery yield and minimizing costs.**
- ✓ **Target product may be recovered by processing cells or spent medium depending upon whether it is intracellular/extracellular product.**
- ✓ **Level of purity that must be achieved is usually determined by specific use of product.**
- ✓ **Fermentation factors affecting downstream processing include properties of microorganisms, particularly morphology, flocculation characteristics, size and cell wall rigidity.**
- ✓ **Presence of fermentation by products, media impurities and fermentations additives such as antifoams may interfere with downstream processing steps.**



Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

1.

- **Cell disruption (high pressure homogenization, wet milling, and lysis)**

2.

- **Clarification of extract (centrifugation, extraction, dead end filtration, and cross flow filtration)**

3.

- **Enrichment (precipitation, batch adsorption, ultrafiltration, and partition)**

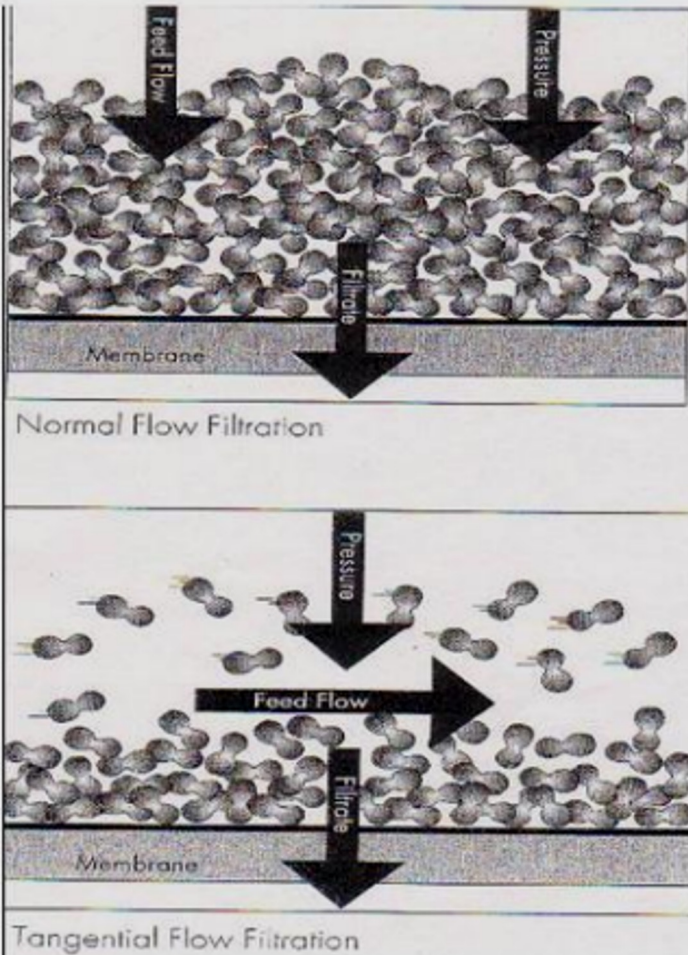
4.

- **High resolution techniques (ion exchange, affinity, hydrophobic, gelfiltration, adsorption chromatography, and electrophoresis)**

5.

- **Concentration (sterile filtration, diafiltration, ultrafiltration, freeze drying, spray drying, and precipitation)**

**Typical downstream operations and unit operations
involved in processing of fermentation broth**



Continuous centrifugation wherein the media and cells get in & clarified media go out

Fig. 26.3: Filtration methods wherein separation of particles from liquid occur by applying a pressure to the solution to force the solution through a filter.

Large scale tangential flow filtration methods



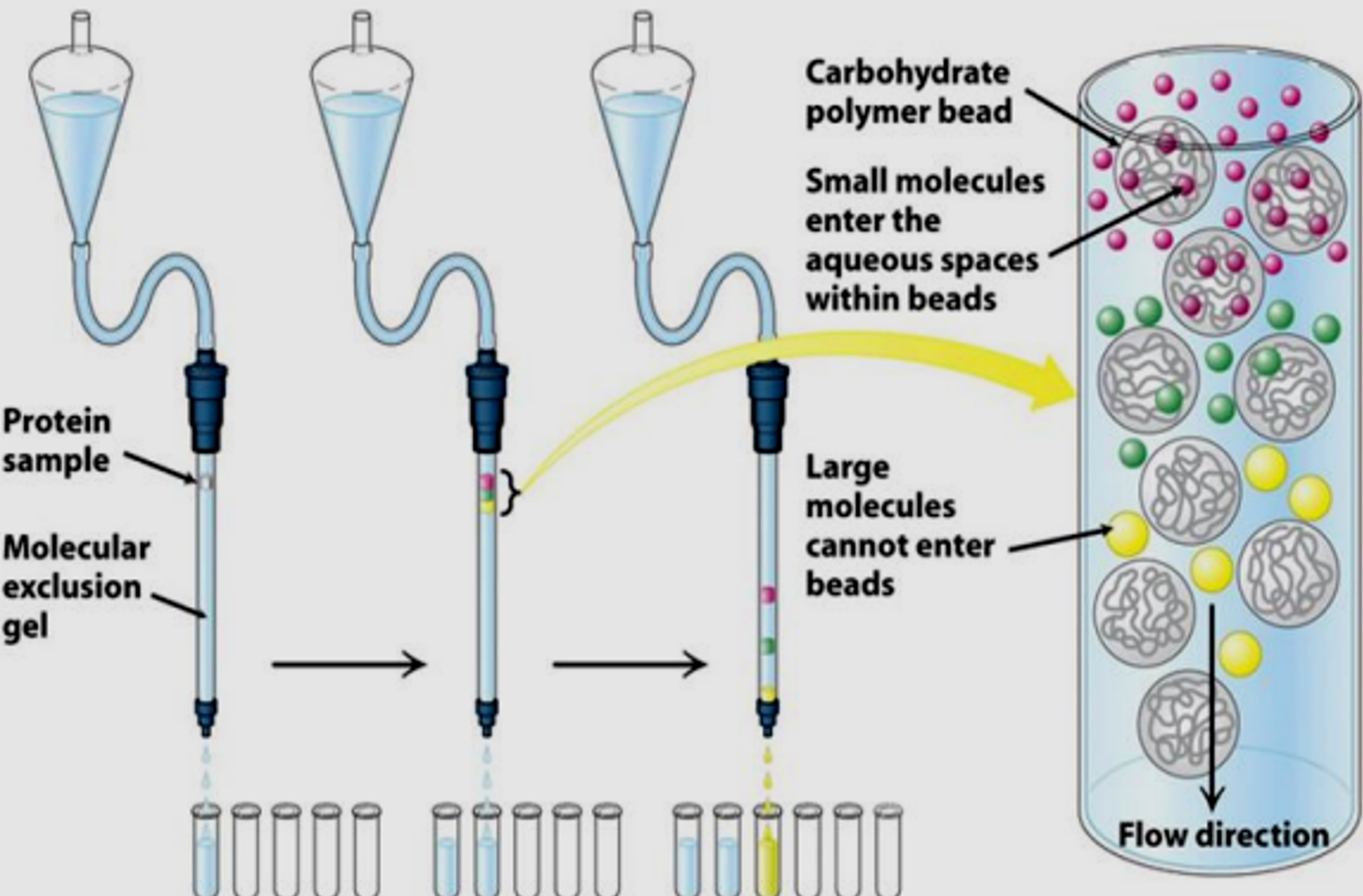


**Centurion
UNIVERSITY**

*Shaping Lives...
Empowering Communities...*

High resolution techniques like Chromatography

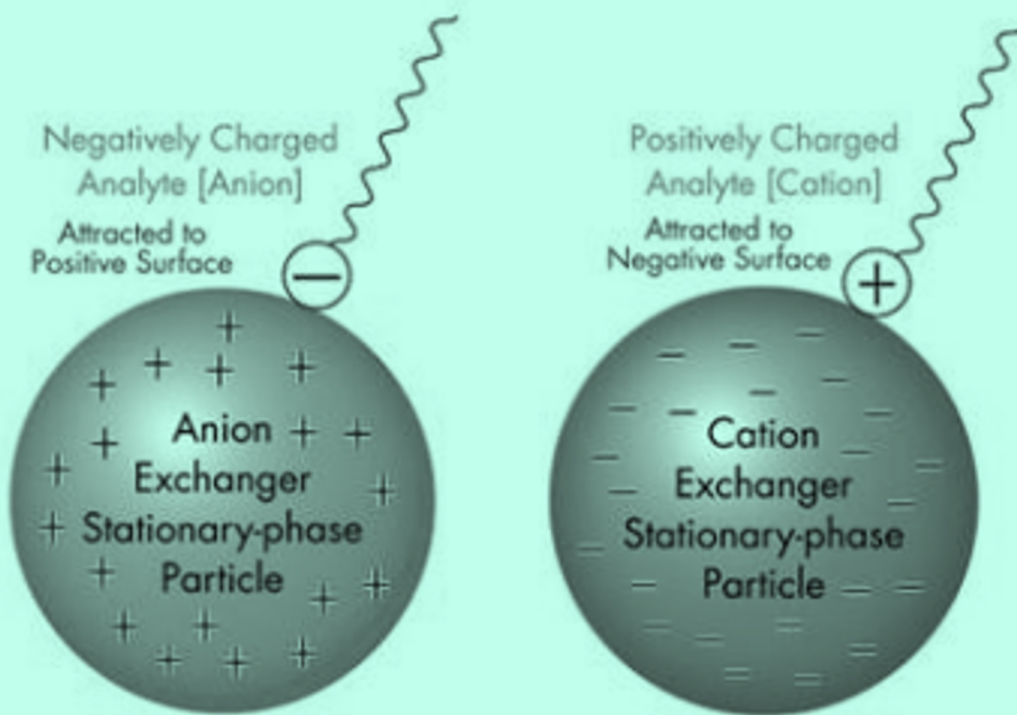
- ✓ **Molecules of interest are adsorbed or stuck to beads packed in column.**
- ✓ **Higher the affinity of molecule (protein) for the bead, the more will be bound to column at any given time.**
- ✓ **Proteins with a high affinity travel slowly through column because they are stuck a significant portion of time.**
- ✓ **Molecules with lower affinity will not stick and will elute more quickly.**
- ✓ **Common type of adsorption chromatography: ion exchange chromatography.**
- ✓ **Column chromatography: Separates molecules by their chemical and physical differences**
- ✓ **Most common types: Size exclusion (Gel filtration): separates by molecular weight, Ion exchange: separates by charge, Affinity chromatography: specific binding Hydrophobic Interaction: separates by hydrophobic/hydrophilic characteristics.**



Gel filtration chromatography

Ion Exchange Chromatography

- ✓ Relies on charge-charge interactions between protein of interest and charges on a resin (bead).
- ✓ Ion exchange chromatography can be subdivided into cation exchange chromatography in which a positively charged protein of interest binds to negatively charged resin; and anion exchange chromatography, in which a negatively charged protein of interest binds to positively charged resin.





Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

Isoelectric Focusing

- ✓ Once the pI of protein is known (or the pH at which your protein is neutral), it can be placed it in a buffer at a lower or higher pH to alter its charge.
- ✓ If the pH of buffer is less than pI , protein of interest will become positively charged.
- ✓ If the pH of buffer is greater than pI , the protein of interest will become negatively charged.



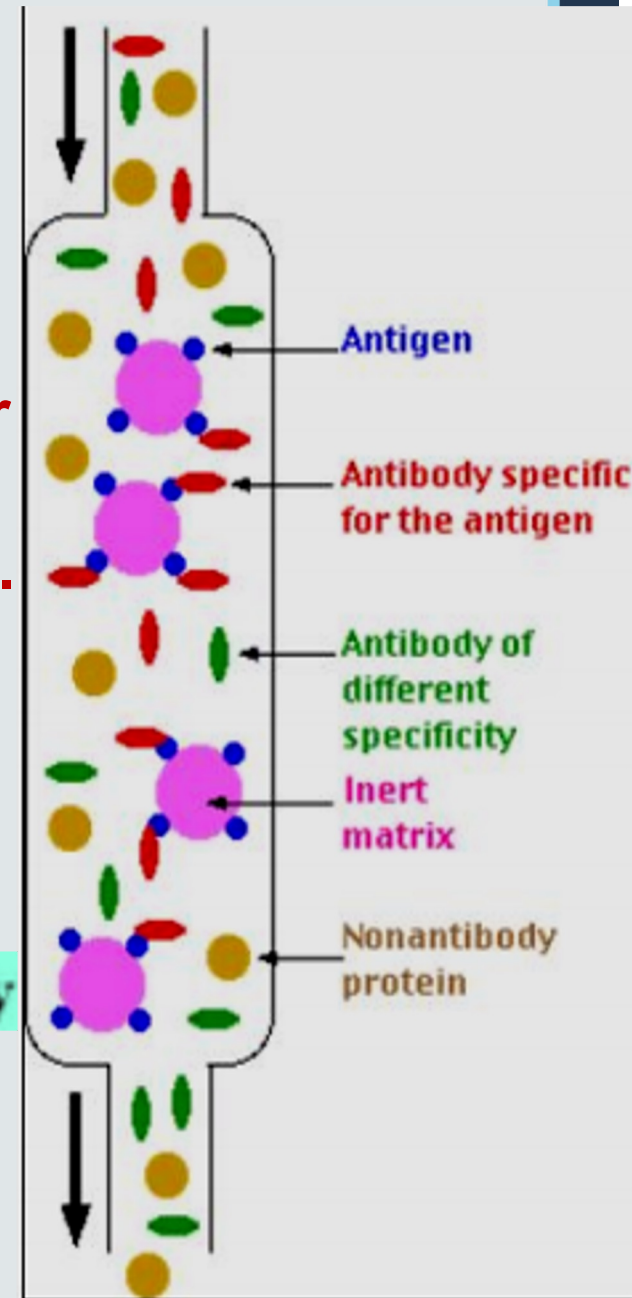
**Centurion
UNIVERSITY**

*Shaping Lives...
Empowering Communities...*

Affinity chromatography

- ✓ Separates protein of interest on basis of a reversible interaction between it and its antibody coupled to a chromatography bead (here labeled antigen).
- ✓ With high selectivity, high resolution, and high capacity for protein of interest, purification levels are several thousand-fold.

Affinity Chromatography



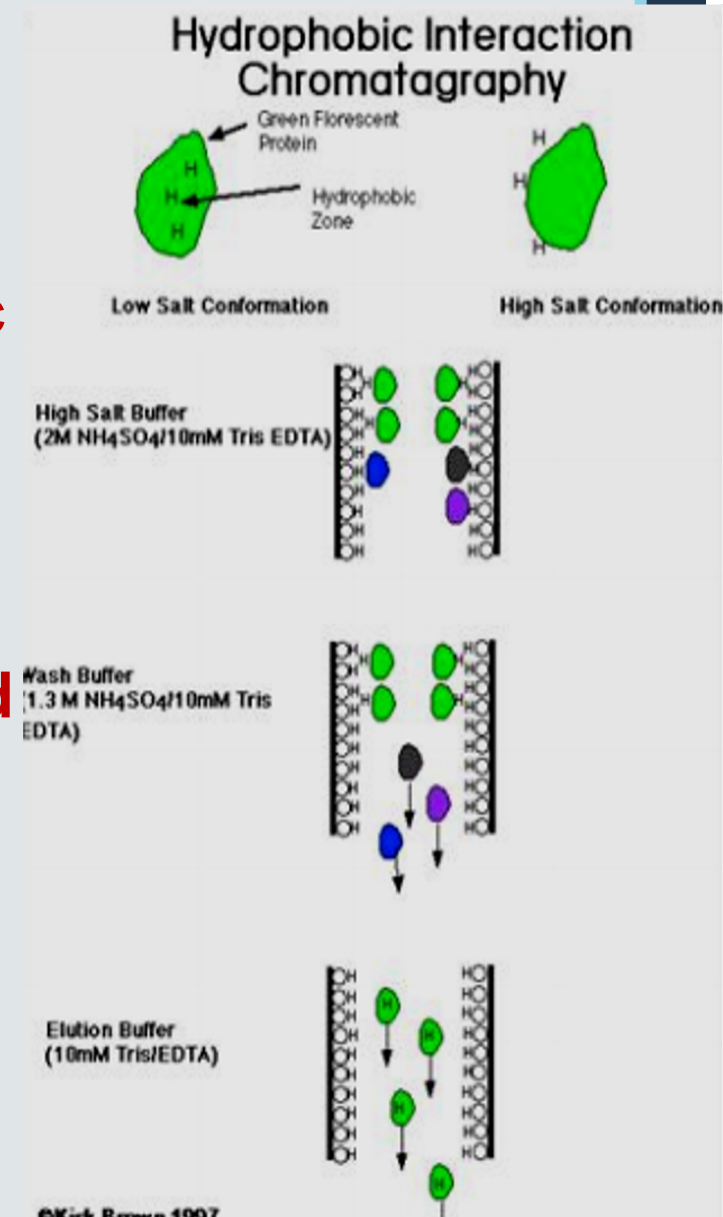


**Centurion
UNIVERSITY**

*Shaping Lives...
Empowering Communities...*

Hydrophobic Interaction Chromatography (HIC)

- ✓ Antibodies are quite hydrophobic and therapeutic antibodies are most important proteins in biopharmaceutical pipeline.
- ✓ HIC media have high capacity and economical and stable.
- ✓ Adsorption takes place in high salt and elution in low salt concentrations.



Component	Culture Harvest Level	Final Product Level	Conventional Method	Common process compounds and methods of purification or removal
Therapeutic Antibody	0.1-1.5 g/l	1-10 g/l	Ultra Filtration (UF)/ Cromatography	
Isoforms	Various	Monomer	Chromatography	
Serum and host proteins	0.1-3.0 g/l	< 0.1-10 mg/l	Chromatography	
Cell debris and colloids	10 ⁶ /ml	None	Membrane Filtration (MF)	
Bacterial pathogens	Various	<10 ⁻⁶ /dose	MF	
Virus pathogens	Various	<10 ⁻⁶ /dose (12 LRV)	virus filtration	
DNA	1 mg/l	10 ng/dose	Chromatography	
Endotoxins	Various	<0.25 EU/ml	Chromatography	
Buffer	Growth media	Stability media	UF	
Extractables/leachables	Various	<0.1-10 mg/l	UF/ Chromatography	
Purification reagents	Various	<0.1-10mg/l	UF	