Production and downstream processing of biopharmaceuticals

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Learning outcomes

- Know the expression systems used for the production of biopharmaceuticals
- Know common unit operations in production and downstream processing
- Insight into how expression system and downstream processing can affect product characteristics

Expression systems for proteins

- Prokaryotic
  - Bacteria
- Eukaryotic
  - Yeast
  - Insect cells
  - Mammalian cells
  - Plant cells
  - Transgenic animals and plants

Any protein can be produced using genetically engineered organisms, but not every type of protein can be produced by every type of cell

Factors important in choosing an expression system

- Product characteristics
  - Protein source (human versus foreign)
  - Post-translational modifications
  - Protein size
  - Protein solubility
  - Refolding behaviour
- Economics
- Available expertise and infrastructure

Features of proteins of different biological origin

Protein feature	Prokaryotic Bacteria	Eukaryotic Yeast	Eukaryotic Mammalian cells
Concentration	High	High	Low
Molecular weight	Low	High	High
S-S bridges	Limitation	No limitation	No limitation
Secretion	No	Yes/no	Yes
Aggregation state	Inclusion body	Singular, native	Singular, native
Folding	Misfolding	Correct folding	Correct folding
Glycosylation	No	Possible	Possible
Retrovirus	No	No	Possible
Pyrogen	Possible	No	No

Other sources

Transgenic animals…

ATryn® – recombinant human antithrombin from transgenic goat milk

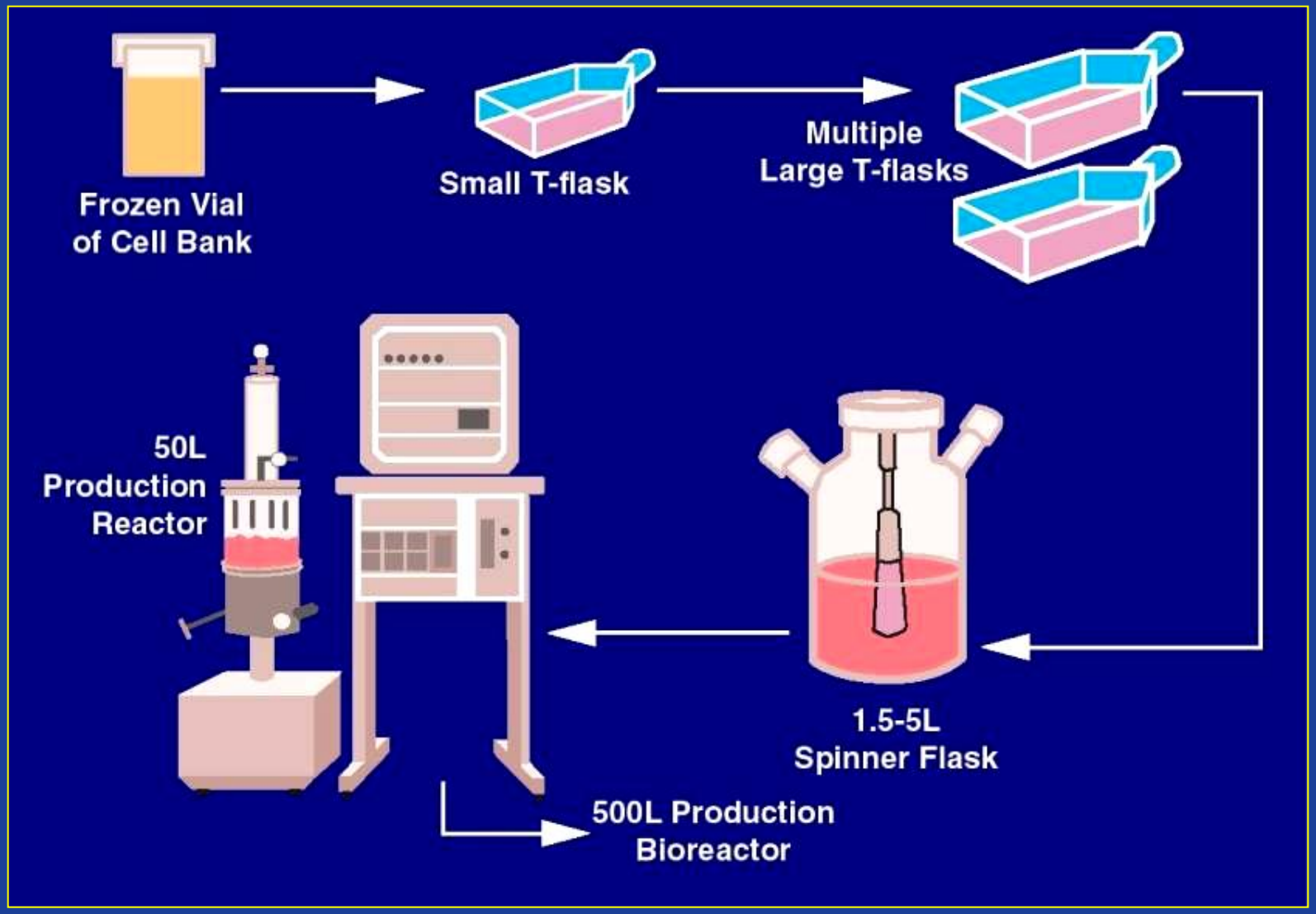
Approved

…and plants

Locteron® – recombinant human interferon-alfa from *Lemna* (duckweed)

In phase II clinical trial

Production of biopharmaceuticals: upstream processing



Production of biopharmaceuticals: upstream and downstream processing

Master seedlot → Working seedlot → Small scale culture

(final) bulk

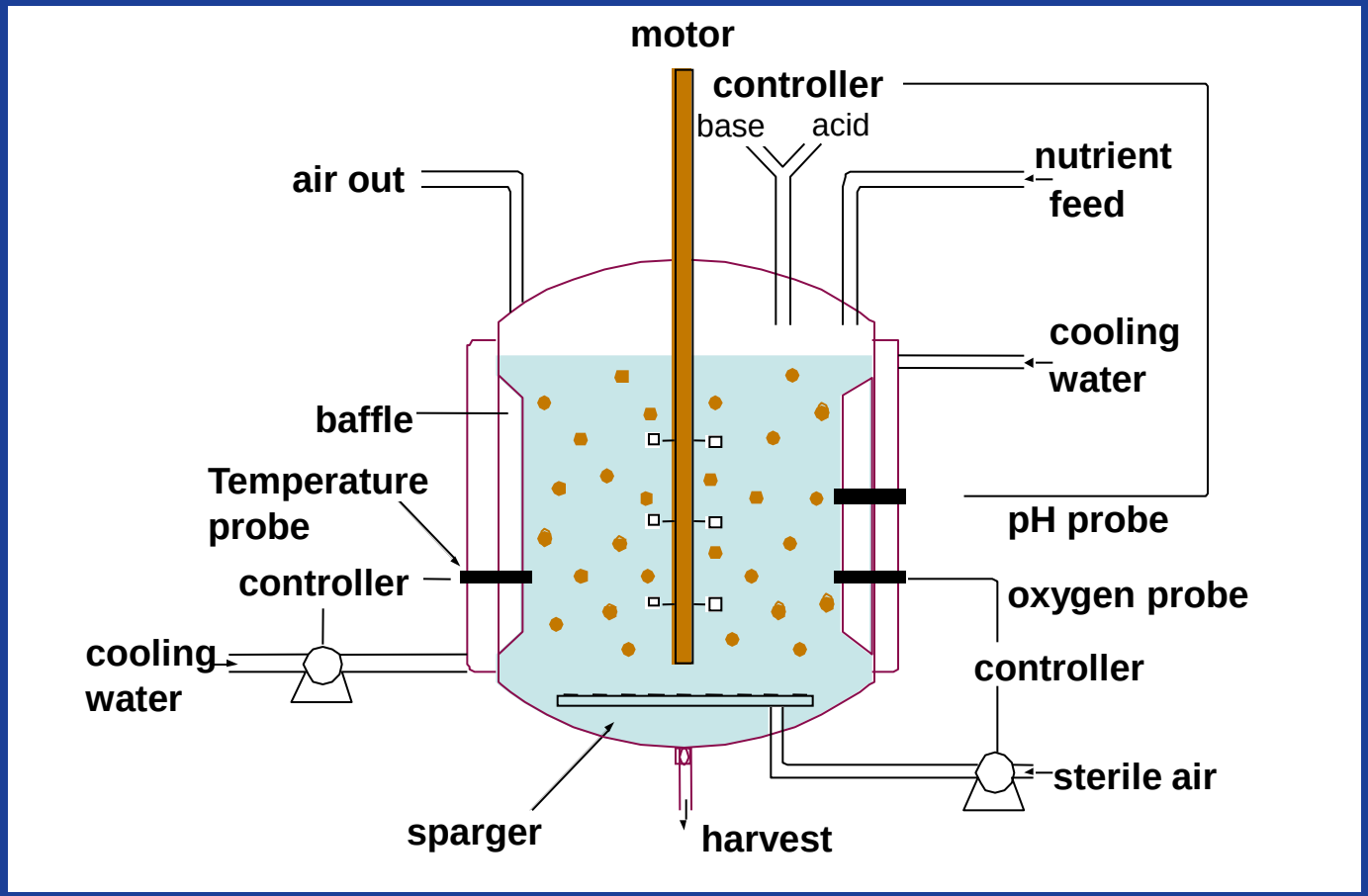
final lot

Large scale cultivation → Purification → Formulation

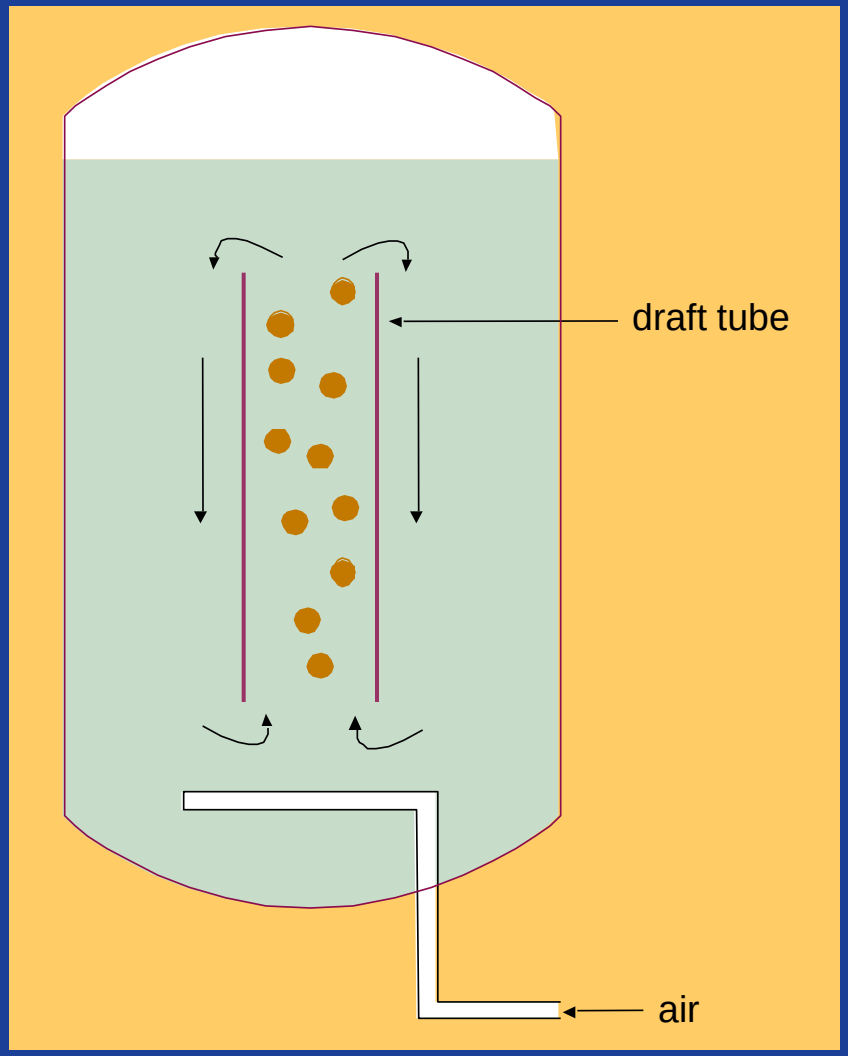
(bioreactor)

(downstream processing; multi-step process)

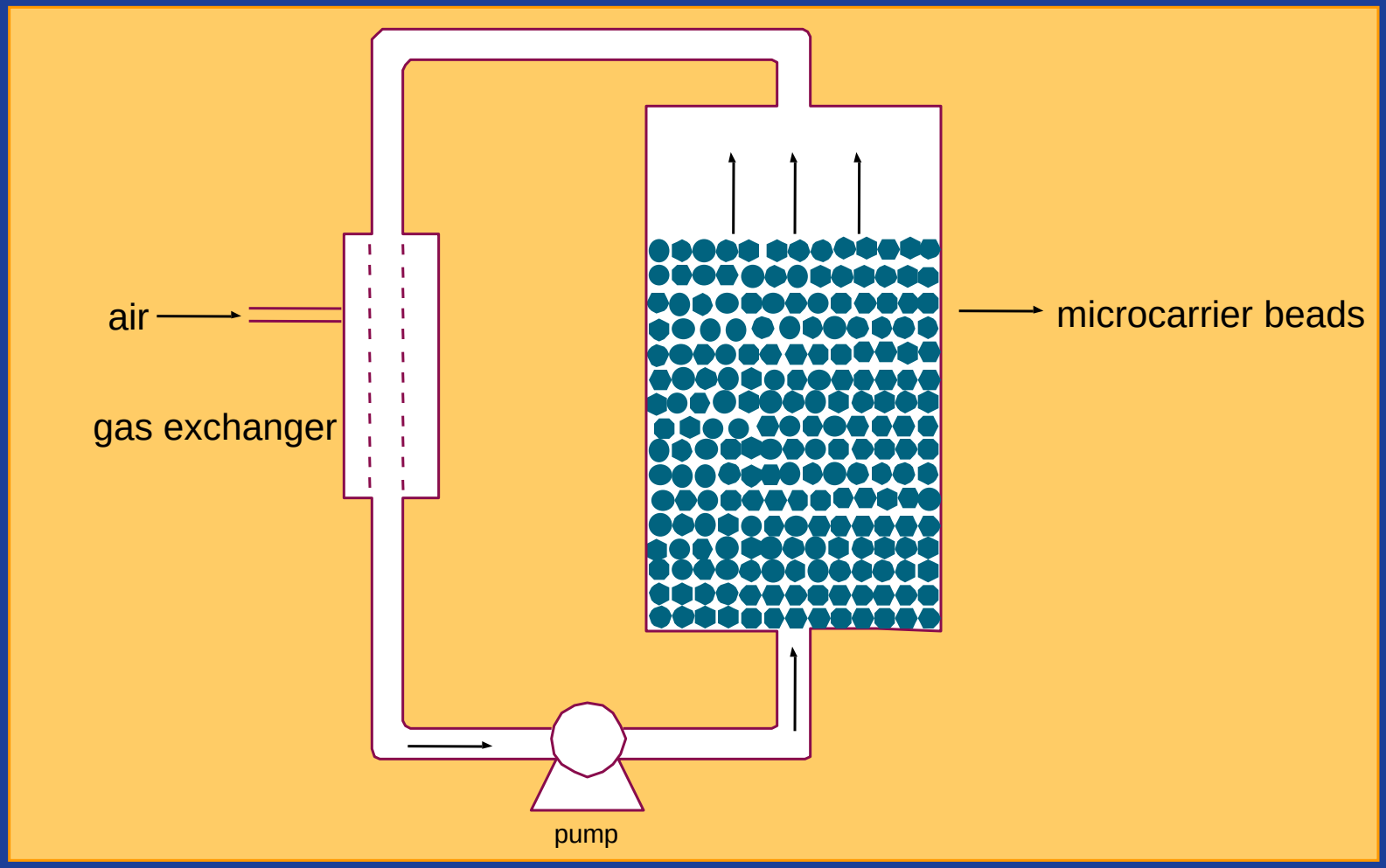
Schematic representation of a stirred-tank bioreactor



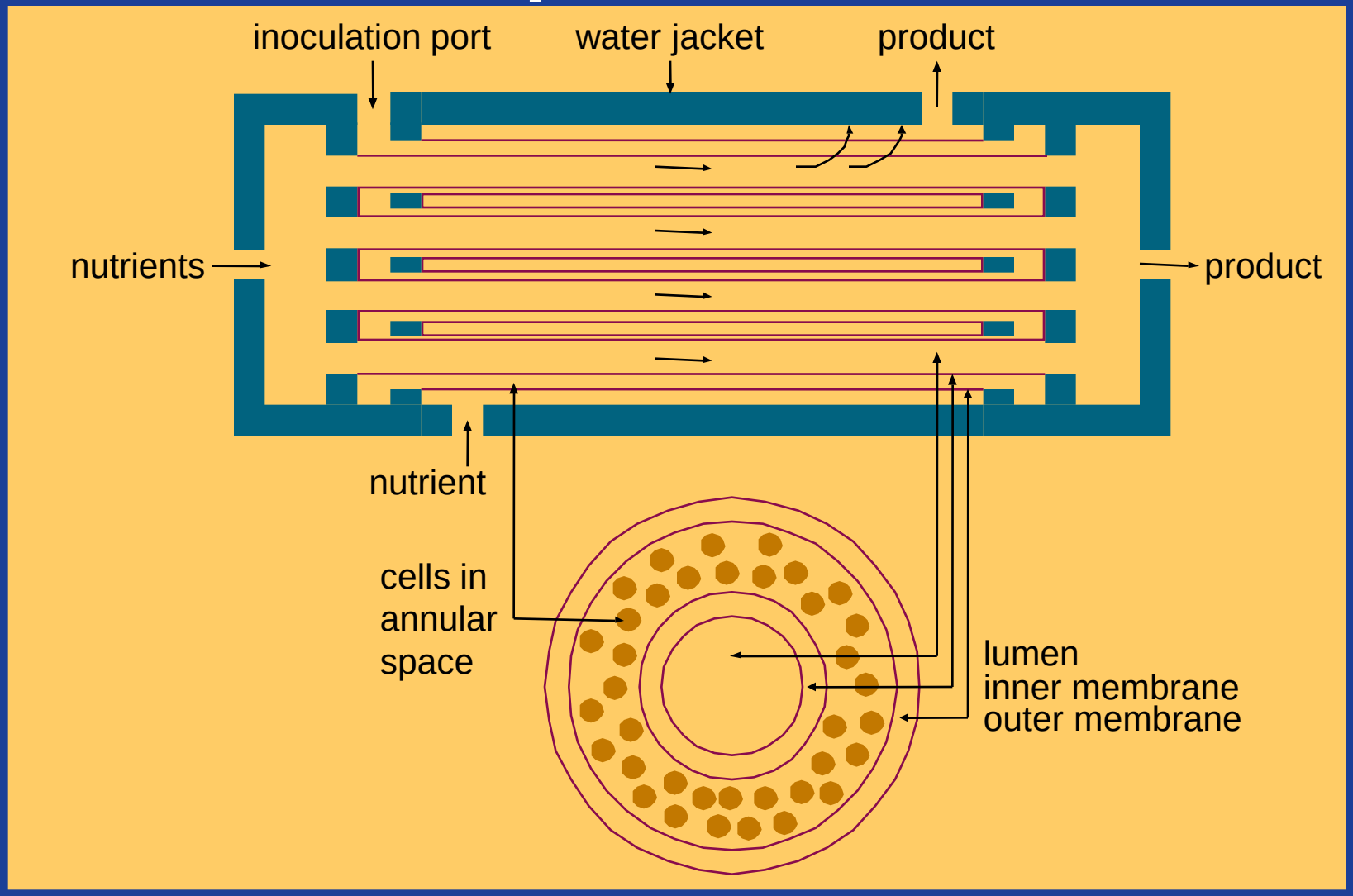
Schematic representation of an air-lift bioreactor



Schematic representation of a fixed-bed bioreactor



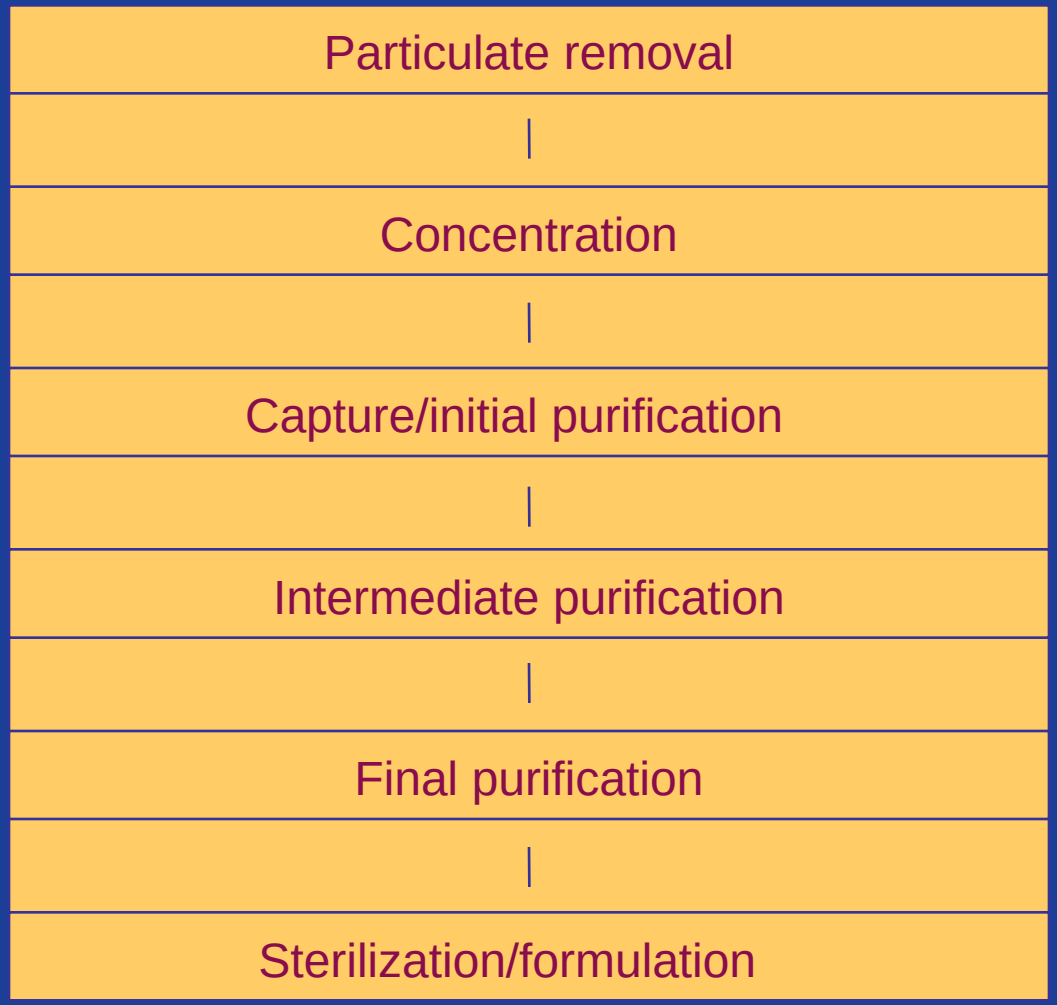
Schematic representation of a hollow-fibre perfusion bioreactor



Major components of growth media for mammalian cell cultures

Type of nutrient	Example(s)
Sugars	Glucose, lactose, sucrose, maltose, dextrins
Fat	Fatty acids, triglycerids
Water (high quality, sterilized)	Water for injection
Amino acids	Glutamine
Electrolytes	Calcium, sodium, potassium, phosphate
Vitamins	Ascorbic acid, a-tocopherol, thiamine, riboflavine, folic acid, pyridoxin
Serum (fetal calf serum, synthetic serum)	Albumin, transferrin
Trace minerals	Iron, manganese, copper, cobalt, zinc
Hormones	Growth factors

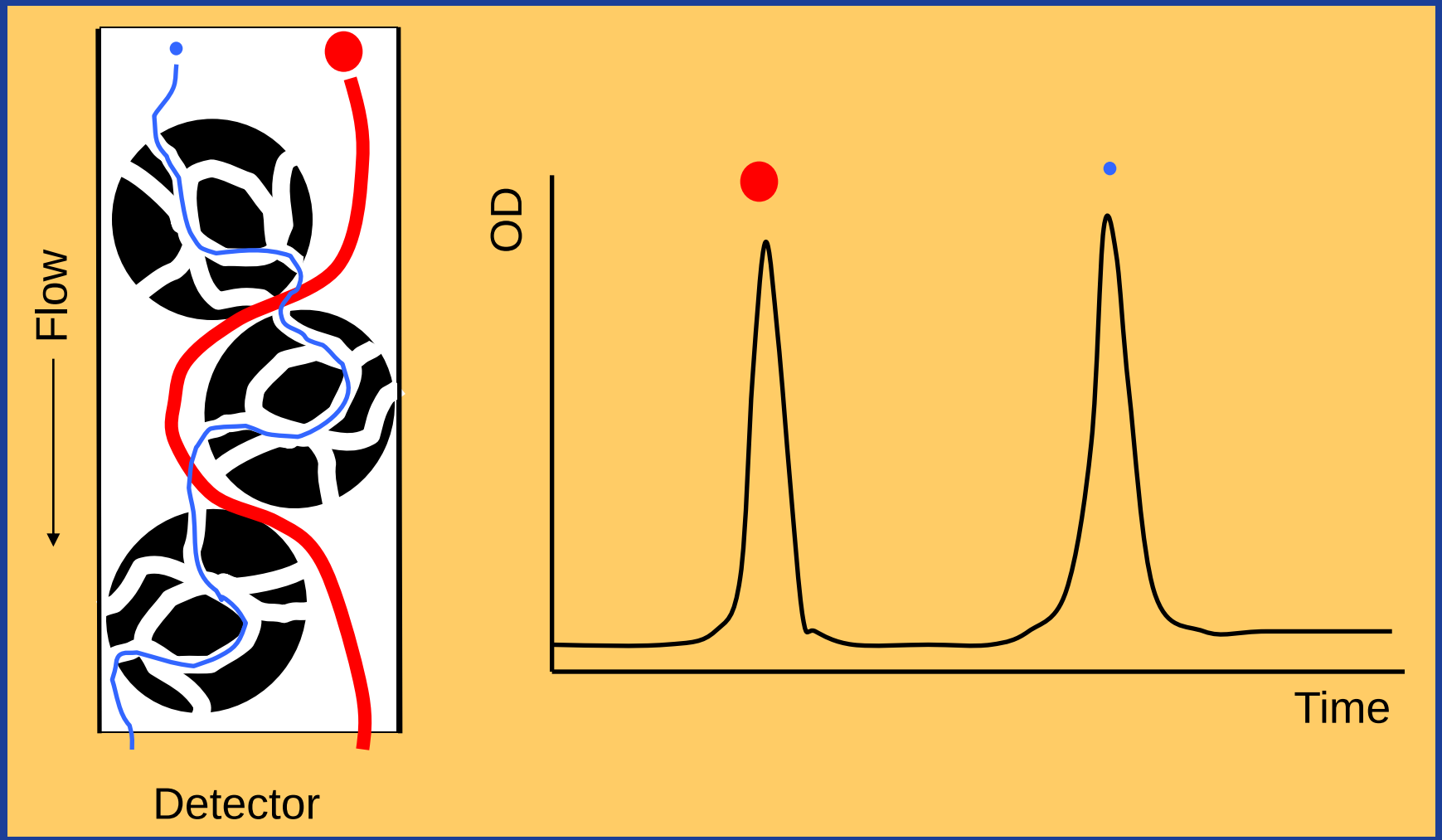
Basic operations required for purification of a biopharmaceutical



Frequently used separation processes and their physical basis

Separation technique	Mode/principle	Separation based on
Membrane separation	Microfiltration Ultrafiltration Dialysis	Size Size Size
Centrifugation	Isopycnic banding Non-equilibrium settling	Density Density
Extraction	Fluid extraction Liquid/liquid extraction	Solubility Partition, change in solubility
Precipitation	Fractional precipitation	Change in solubility
Chromatography	Ion-exchange Gel filtration Affinity Hydrophobic interaction Adsorption	Charge Size Specific ligand-substrate interaction Hydrophobicity Covalent/noncovalent binding

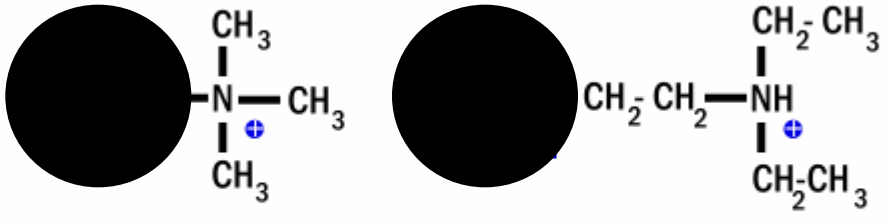
Gel filtration chromatography



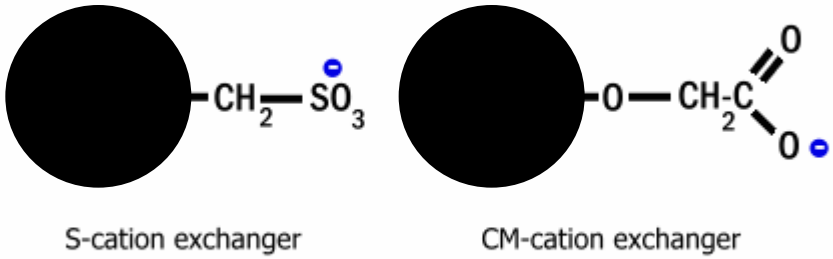
OD, optical density

Ion-exchange chromatography

Anion exchanger



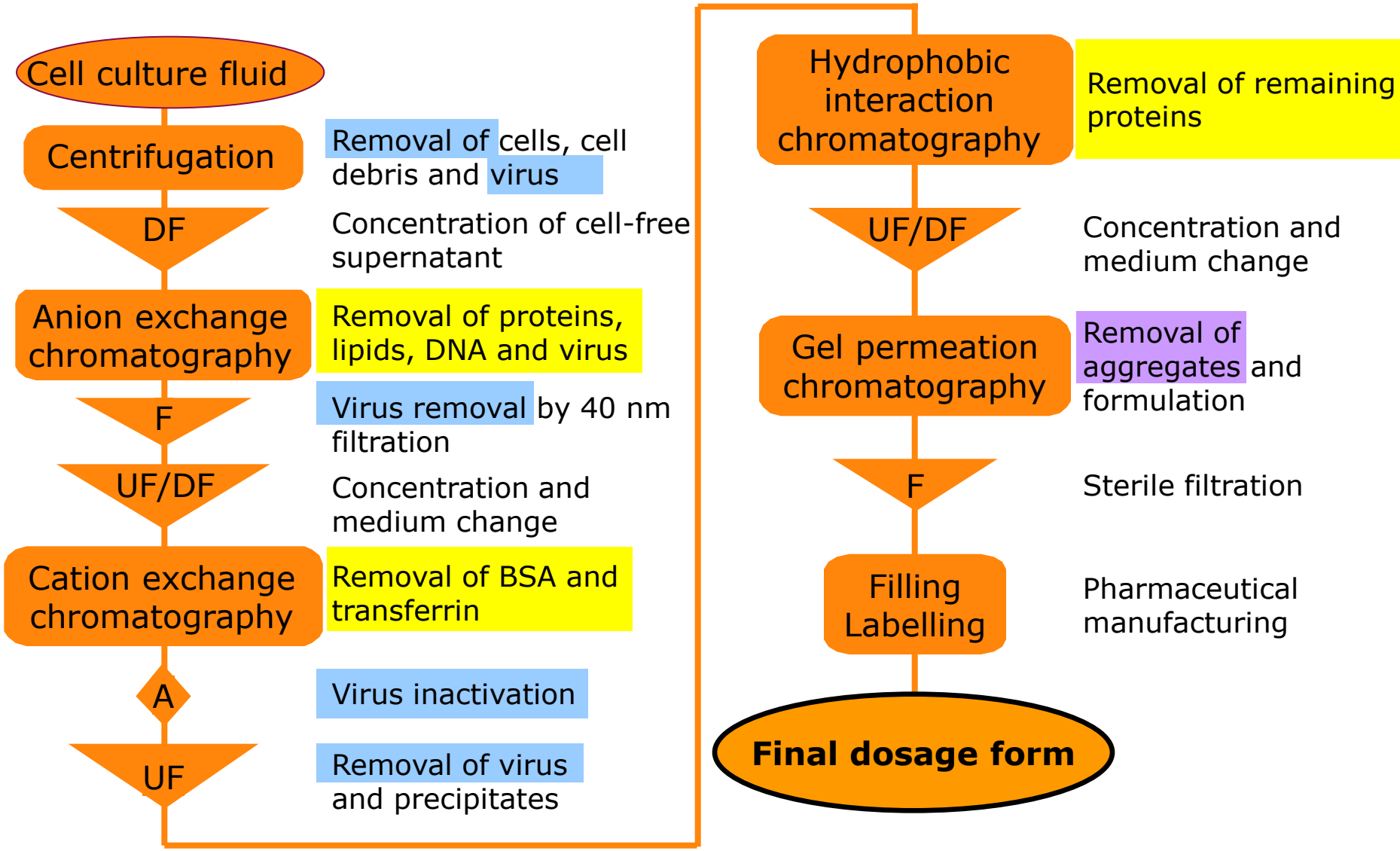
Cation exchanger



Methods for reducing and inactivating viral contaminants

Category	Type	Example
Inactivation	Heat treatment	Pasteurization
	Radiation	UV light
	Dehydration	Lyophilization
	Chemical, cross linking agents chemical denaturating or disrupting agents	b-propiolactone, formaldehyde, NaOH, organic solvents (eg chloroform), detergents (eg Na-cholate)
	Neutralization	Specific, neutralizing antibodies
Removal	Chromatography	Ion exchange, immuno-affinity, chromatography
	Filtration	Ultrafiltration
	Precipitation	Cryoprecipitation

Production flowsheet of a recombinant interferon



Potential impurities and contaminants

Origin	Impurity/contaminant
Host related	Viruses, bacteria
	host-derived proteins and DNA
	Glycosylation variants
	N- and C-terminal variants
	Endotoxins (from gram negative bacterial hosts)
Product related	Amino acids substitution and deletion
	Denatured protein
	Conformational isomers
	Dimers and aggregates
	Disulfide pairing variants
	Deamidated species
	Protein fragments
Process related	Growth medium components
	Purification reagents
	Metals
	Column materials

Issues to consider in production and purification of proteins

- Heterogeneity
  - N- and C-terminal heterogeneity
  - Chemical modification/conformational changes
  - Glycosylation
  - Proteolytic processing
- Protein inclusion body formation
  - High initial purity
  - Inactive, aggregated protein requires refolding steps

Conclusions

- Expression systems include bacterial, yeast and mammalian cells, as well as transgenic organisms
- Production (upstream processing) requires well-controlled conditions and usually involves the use of large-scale bioreactors
- Multi-step purification (downstream processing) processes to remove impurities and contaminants are required to yield highly pure biopharmaceuticals
- Expression system + upstream processing + downstream processing + formulation = final product