Basics of Biopharmaceutics and Biosimilars

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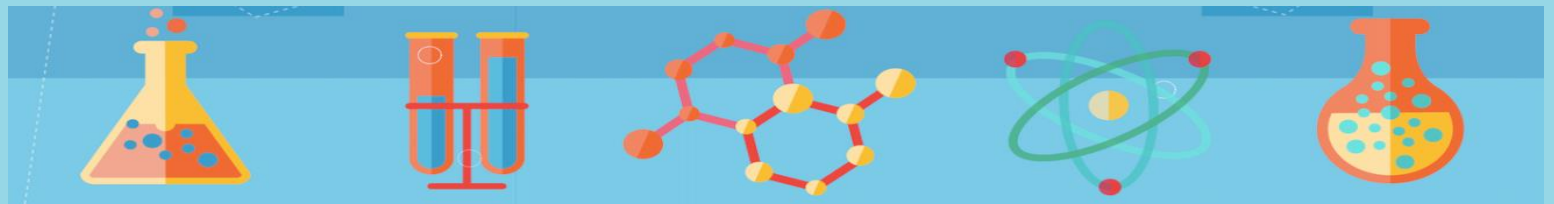
National Vaccine Institute

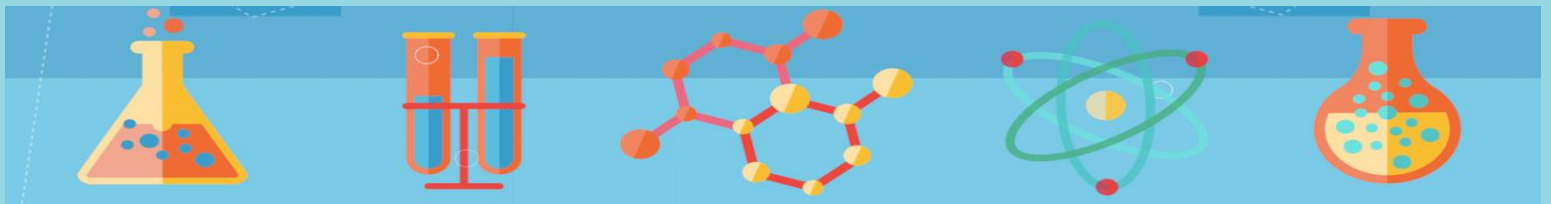


AGENDA

Fundamental of Biosimilar and Regulatory Requirement

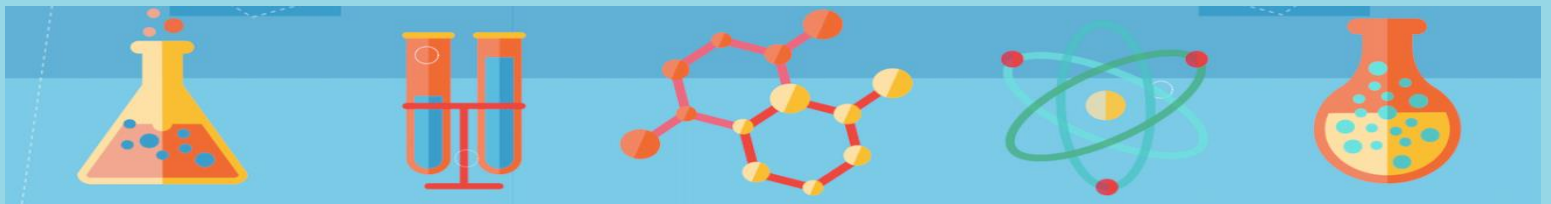
Totality of Evidence and Comparability Exercise

Extrapolation, Switching and Interchangeability Concept

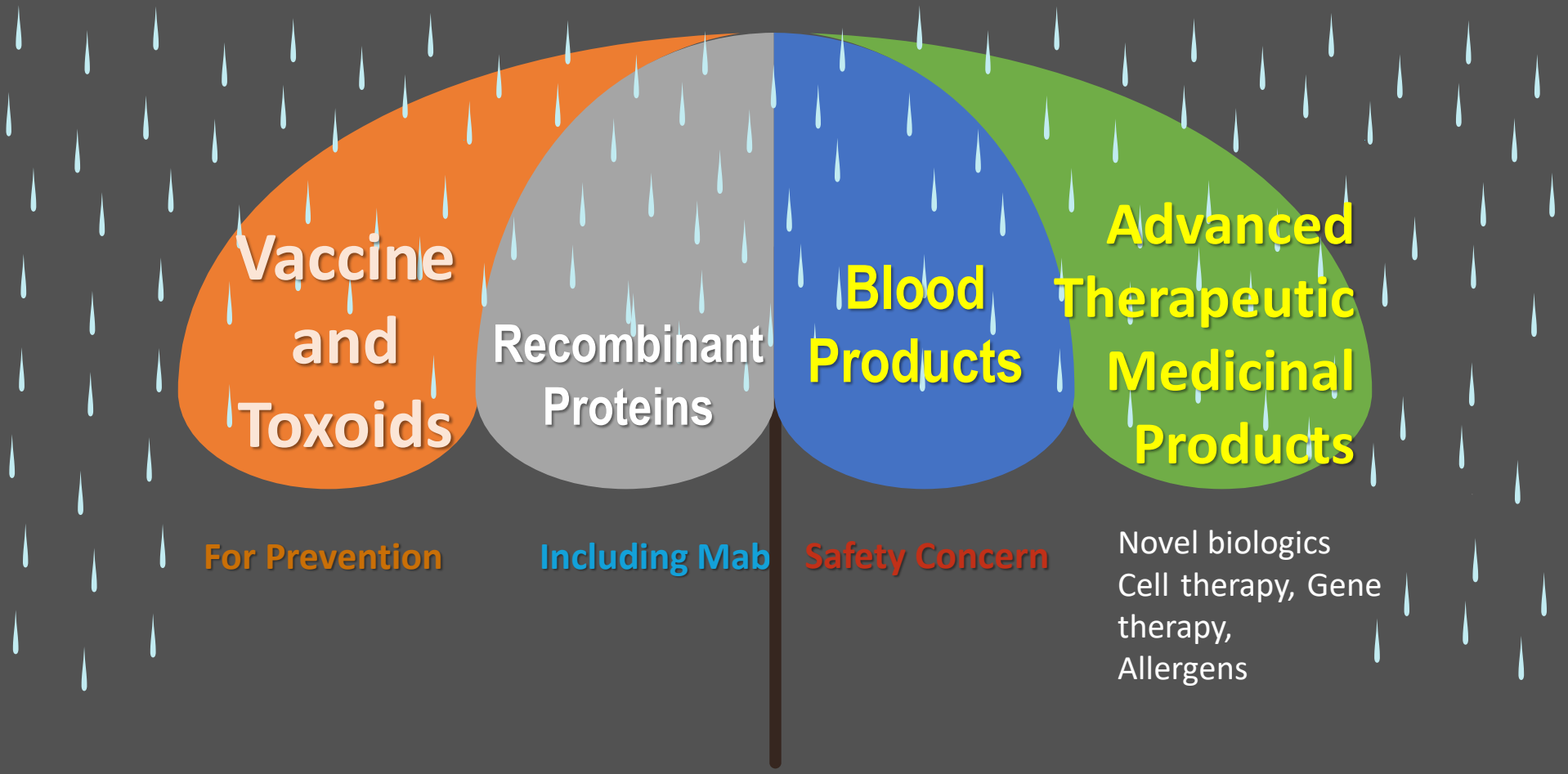


Disclaimers/Disclosures

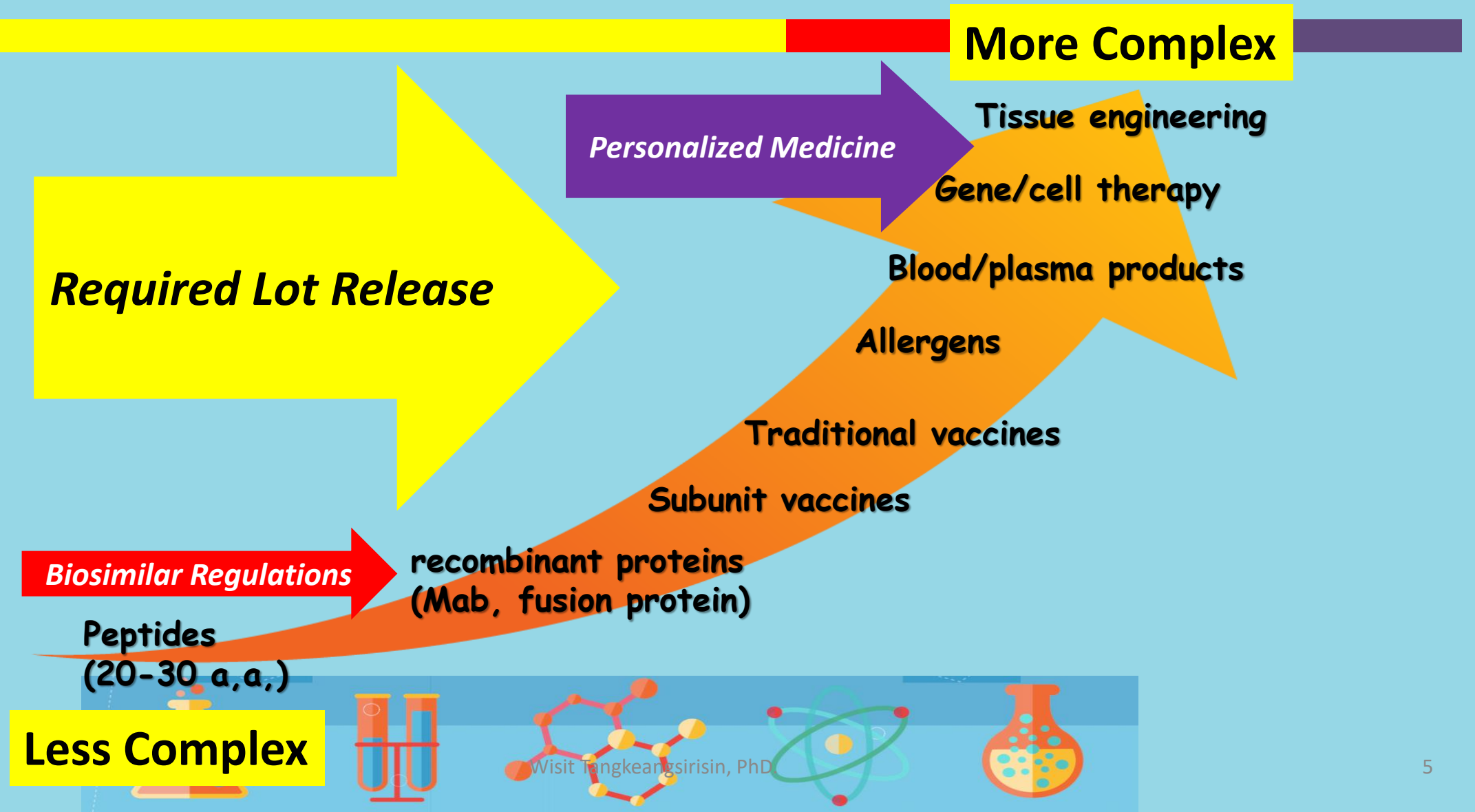
- The views expressed here are entirely my personal views and should not be constructed as those representing the view of the Thai FDA or National Vaccine Institute.
- The information is accurate at the time of presentation.



Biologics


Vaccine and Toxoids
Recombinant Proteins
Blood Products
Advanced Therapeutic Medicinal Products
For Prevention
Including Mab
Safety Concern
Novel biologics
Cell therapy, Gene therapy,
Allergens

Heterogeneity of Biologics



Evolution of Biopharmaceuticals

Biopharmaceuticals	First Generation	Second Generations (Biobetter)
Insulin	Regular Insulin	Glargine, Aspart, Glulisine, Detrimir, Degludec,
Erythropoietin	Epoetin	Darbepoetin
Filgrastim	Filgrastim	PEG-filgrastim
Monoclonal Antibody	-momab, -ximab, -zumab, -umab	Drug conjugated mab Bispecific antibody Nanobodies

Biopharmaceuticals

First generation

- Native Proteins, Unengineered
- murin antibodies, simple replacement proteins
  - Frequent Injection

Second generation

- Engineered, modified, alteration of amino acid sequence, alteration of glycosylation, PEGylation
  - Suitable PK

Pharmaceutical Products

Traditional Pharmaceutical Sectors

Chemical-based drugs

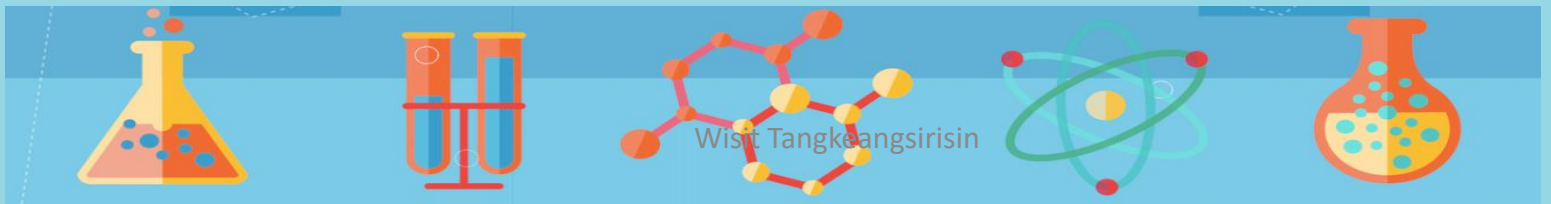
: chemical synthesis

Biopharmaceuticals Sectors

Therapeutic proteins :
modern biotechnological techniques, like recombinant DNA, protein engineering, and hybridoma technologies etc.

General Properties of Biopharmaceuticals

- High molecular weight *MW*
- (Glyco) Proteins / DNA / RNA
- Activity based on secondary, tertiary and quaternary structures
- Species-specific activity
- Chemical synthesis is not possible
- Control over variability in production required
- Complex analytics for Quality Assurance needed



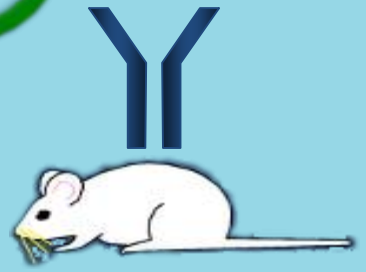
HISTORY OF BIOPHARMACEUTICALS

Evolution of Biotechnology

DNA double helix structure revealed

Genetic code elucidated

DNA cloned



Monoclonal antibodies produced

First human protein synthesized (growth hormone)

Recombinant human insulin approved

Biologicals approved for clinical use

Polymerase chain reactions

First therapeutic MAb approved

Human genome mapped

1953 | 1961-1965 | 1973 | 1975 | 1977 | 1982 | 1983 | 1986 | 2000+

(1962)–Medicine
Watson, Crick, Wilkins

(1968)–Medicine
Holley, Khorana, Nirenberg

(1980)–Chemistry
Berg, Gilbert, Sanger

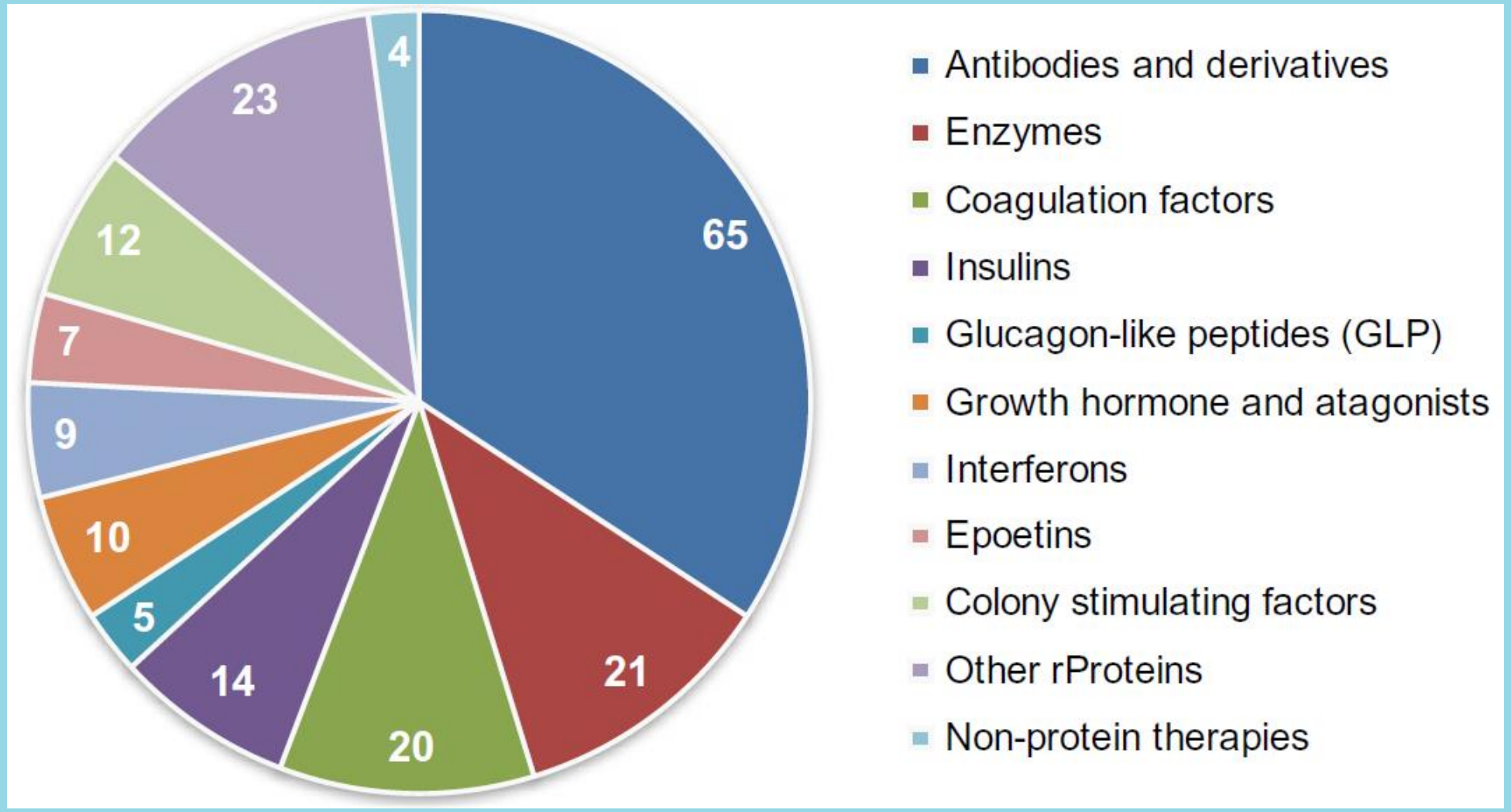
(1984)–Medicine
Jerne, Köhler, Milstein

(1993)–Chemistry
Mullis

Wisit Tangkaangsirisin

Historical Events in Biotechnology. Available at: www.biotechinstitute.org/what_is/timeline.html. Accessed August 10, 2007.
All Nobel Laureates. Available at: www./nobelprize.org. Accessed August 10, 2007

Nobel Prizes (Year Awarded)

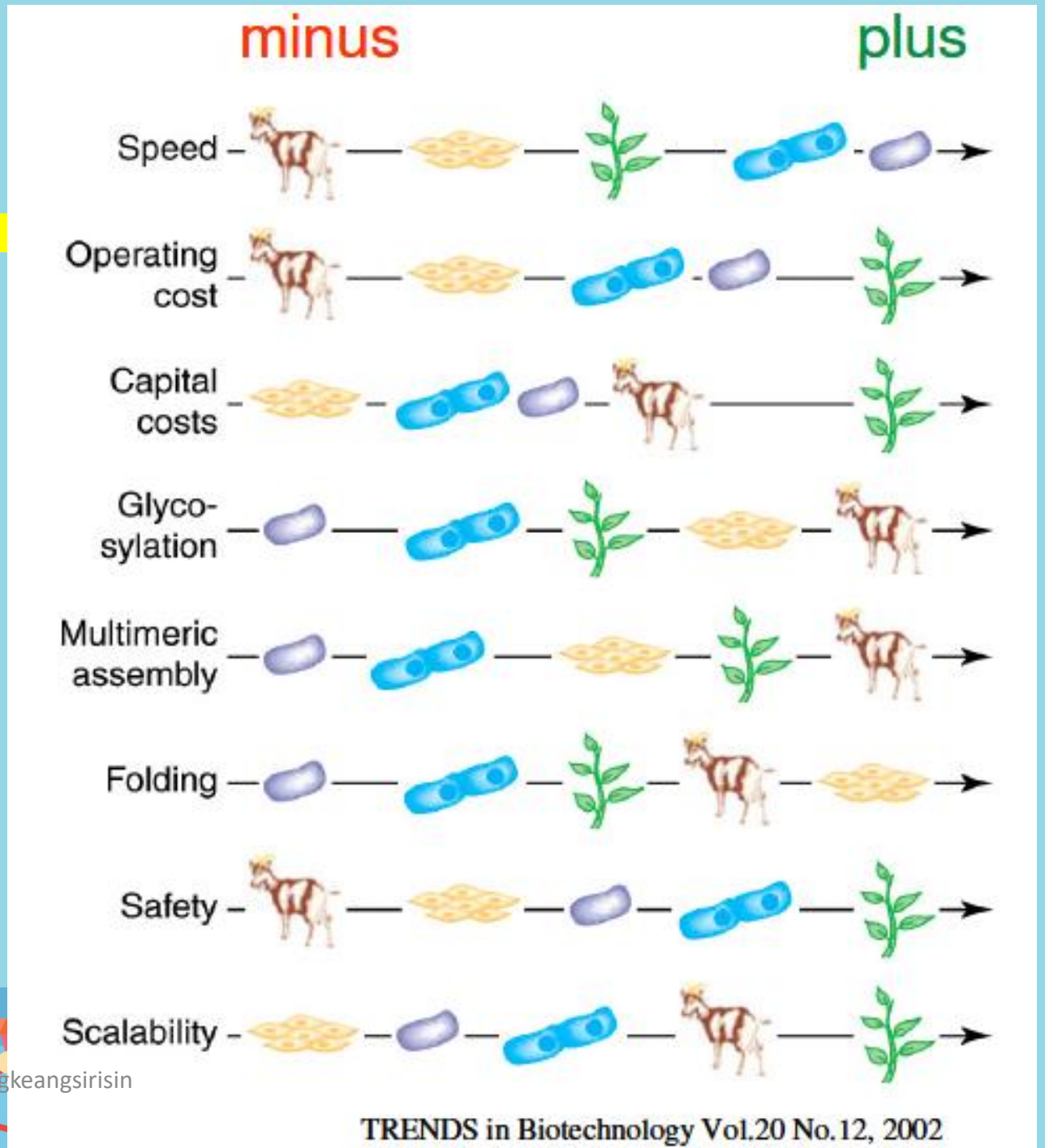


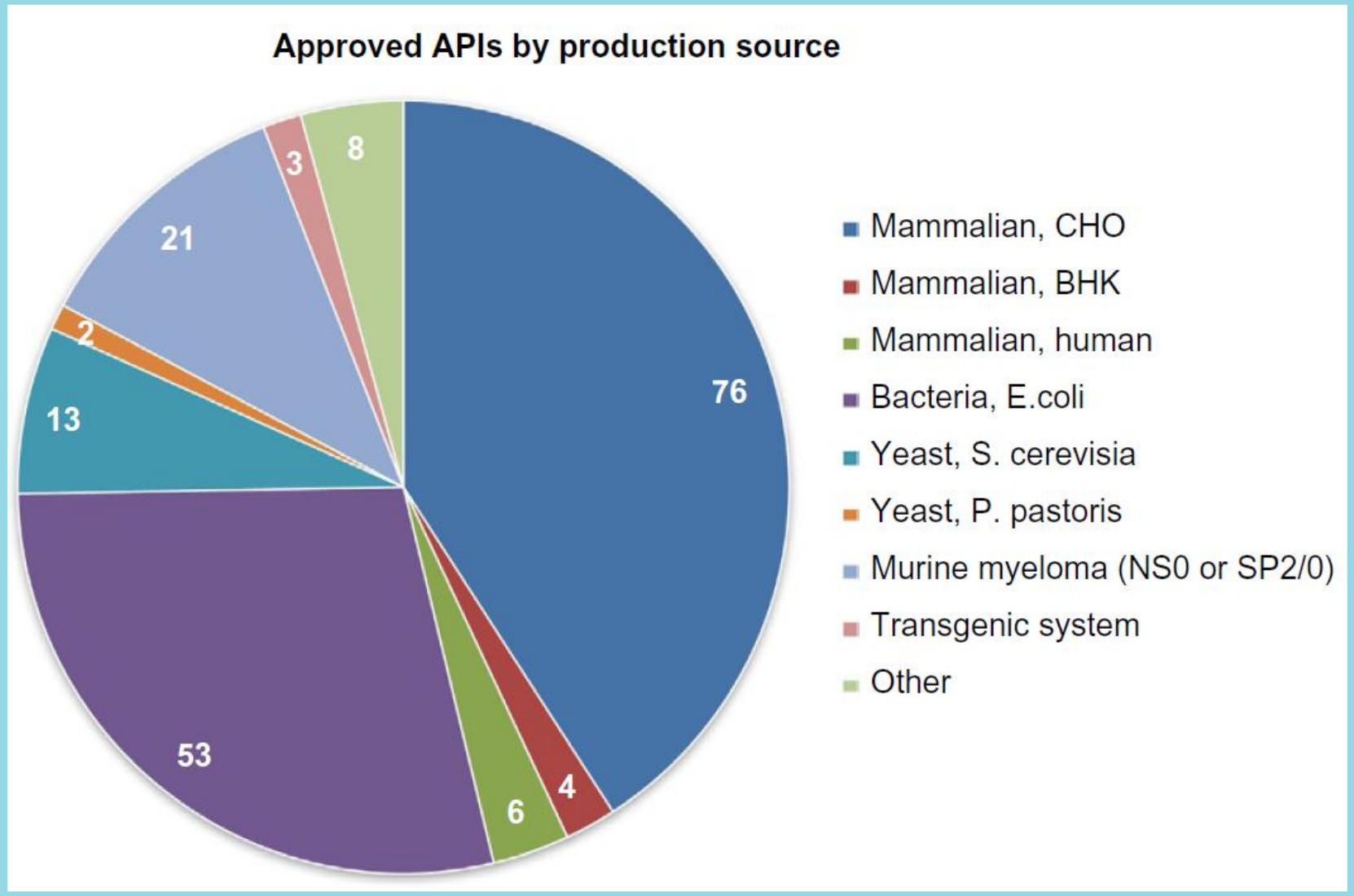
Overview of classes of molecules approved as active pharmaceutical ingredients in injectable biotherapeutics (1982–2016). *Data from FDA and EMA websites*

PRODUCTION PROCESS, ADVANTAGE AND CHALLENGING ISSUES

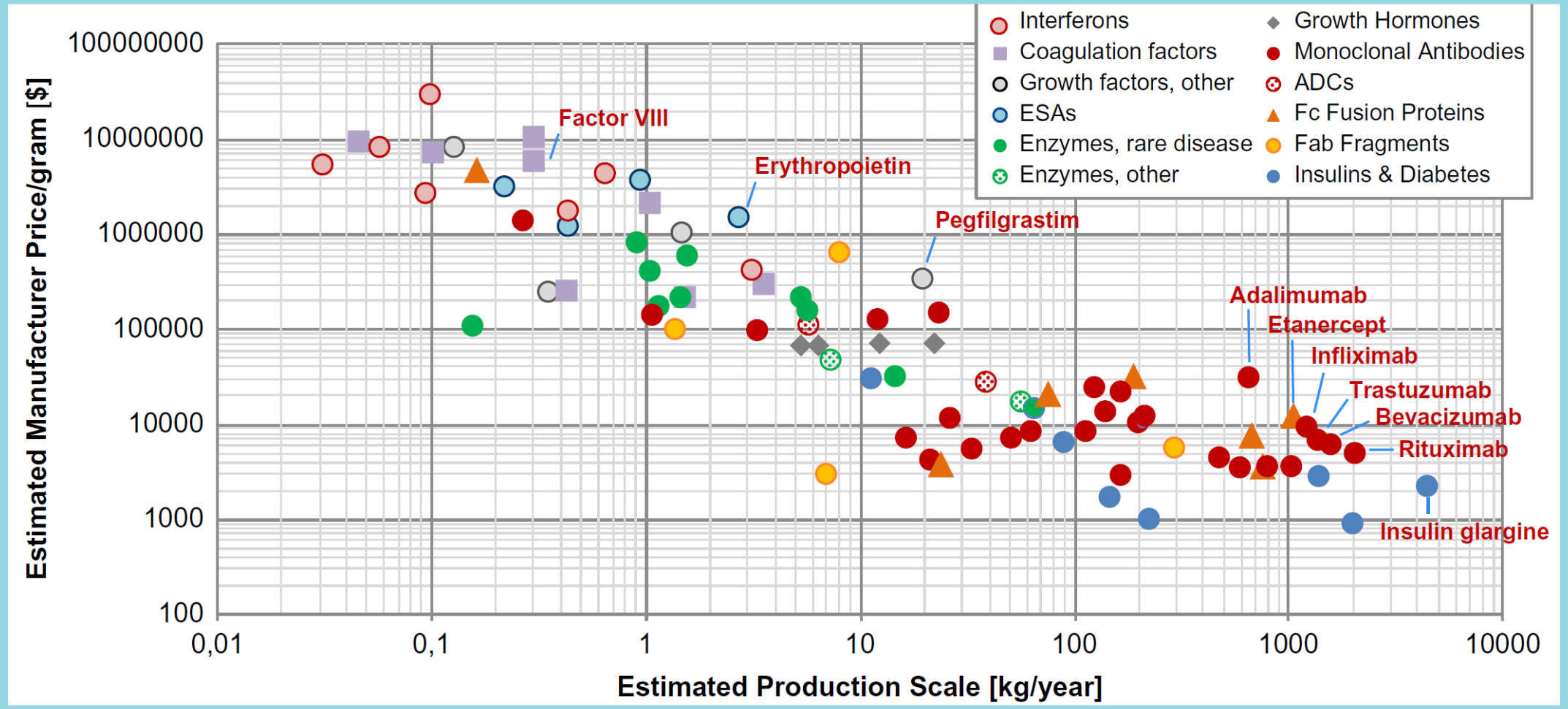
Expression system : Host

1. Prokaryotic: bacterial (recombinant *Escherichia coli*, *Bacilli*, *Actinomycetes* and others)
2. Fungi and Yeasts (recombinant Saccharomyces, Pichia and others)
3. Higher eukaryotic cell lines:
    - Mammalian (CHO, BHK...)
    - Plant
    - Insect
4. Transgenic Plants
5. Transgenic Animals



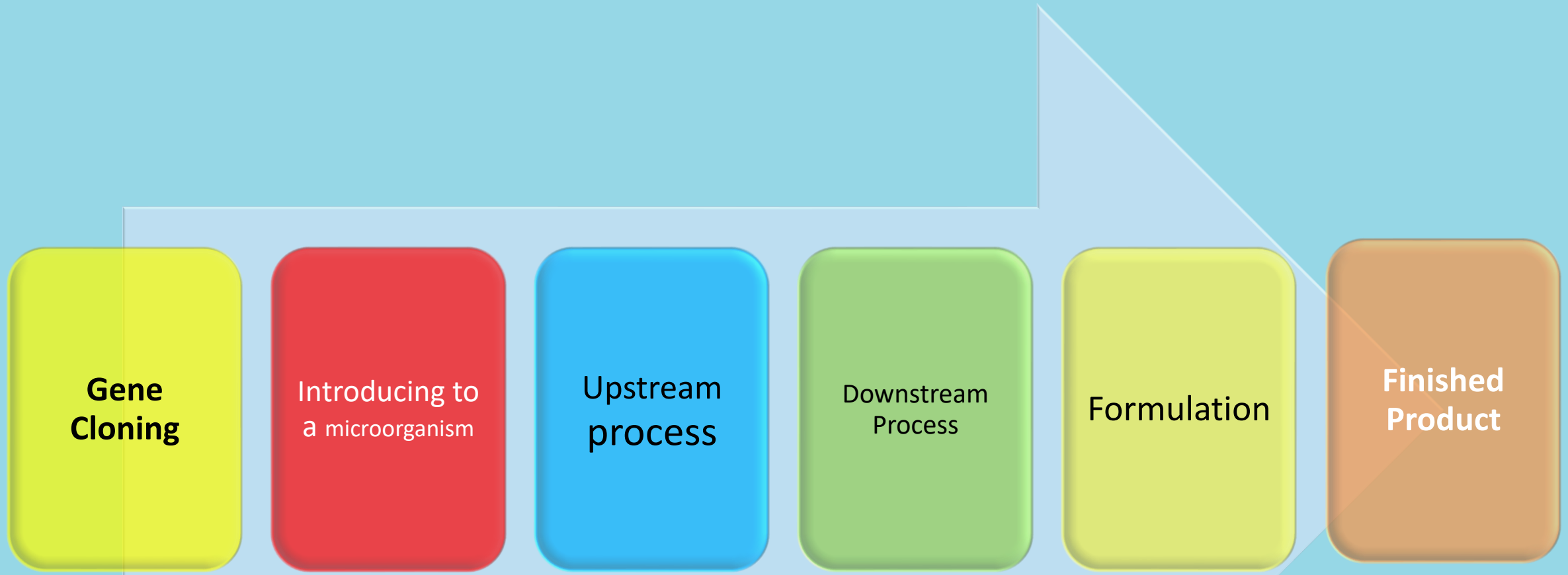


Use of different cell types in biomanufacturing of approved injectable therapeutic proteins (1982–2016).

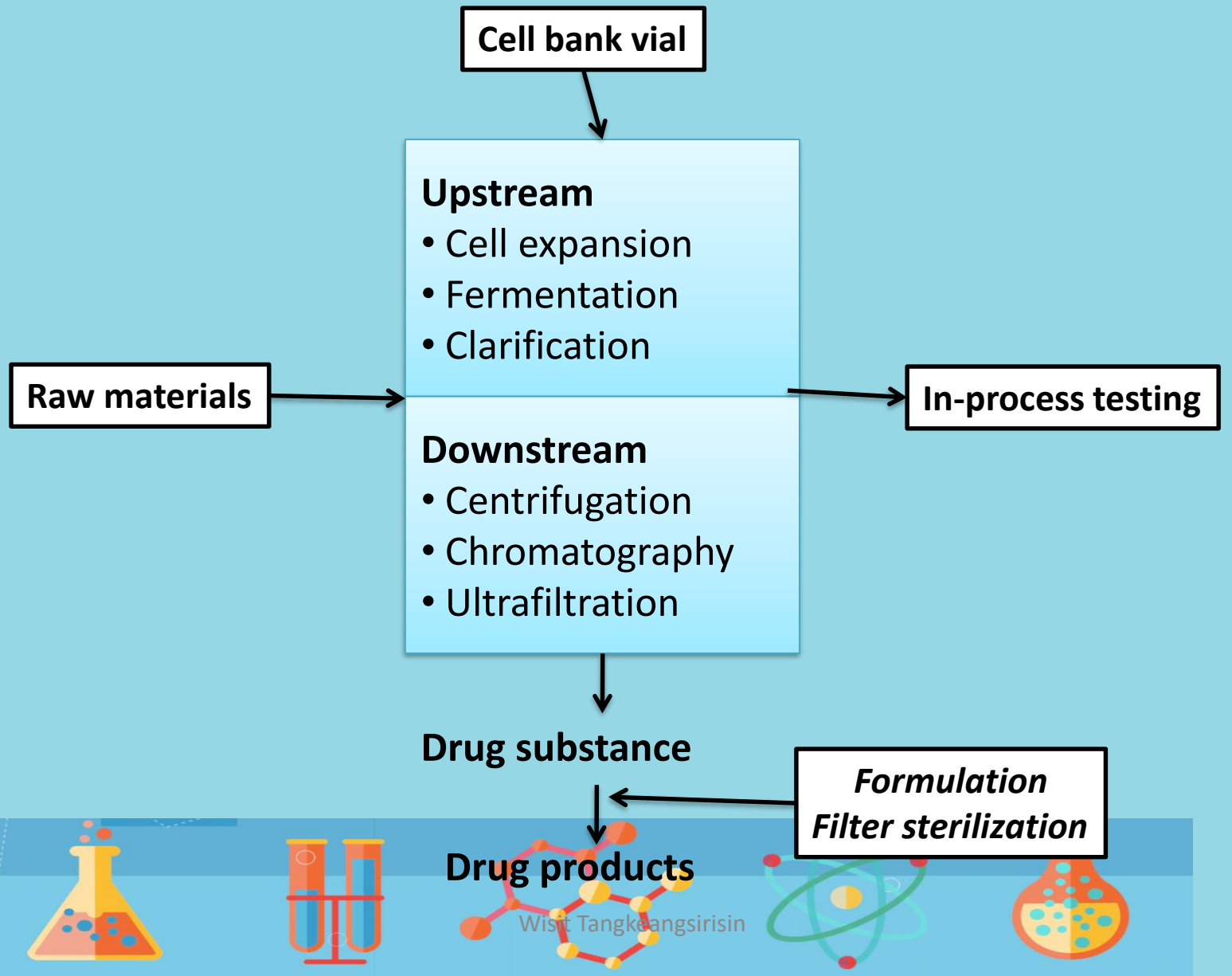


Estimated global manufacturing quantities (kg/year) and manufacturer revenue per gram ($) for marketed biotherapeutic proteins, and biosimilar candidates. Estimate based on published manufacturer revenue (Source: company annual reports), Medicare, US and “Rote Liste”, Germany pricing info, and package leaflet information; assuming Medicare/“Rote Liste” include a 20% markup on manufacturer pricing; assuming manufacturer has to scrap 15% of produced material. Increase of price markup and scrap percentage moves data points to the right (higher production quantity required for the published revenue. The data points labelled with generic biotherapeutic names indicate drugs that are currently subject to intense biosimilar development and market approval activities or other intense competition (for Factor VIII)

Recombinant Protein Production



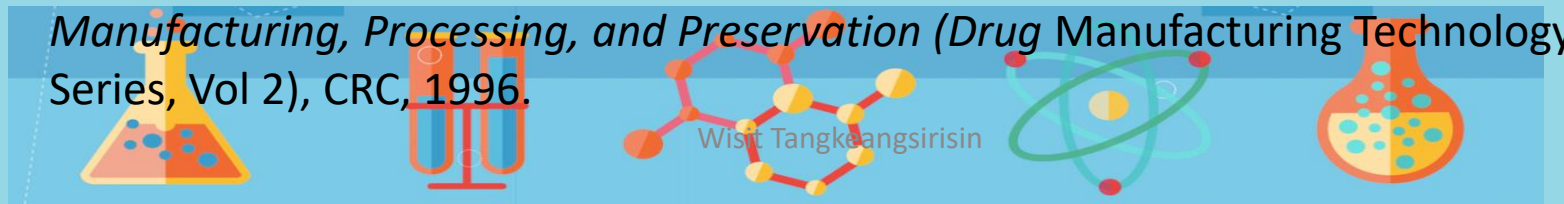
Biopharmaceutical Manufacturing: Overviews



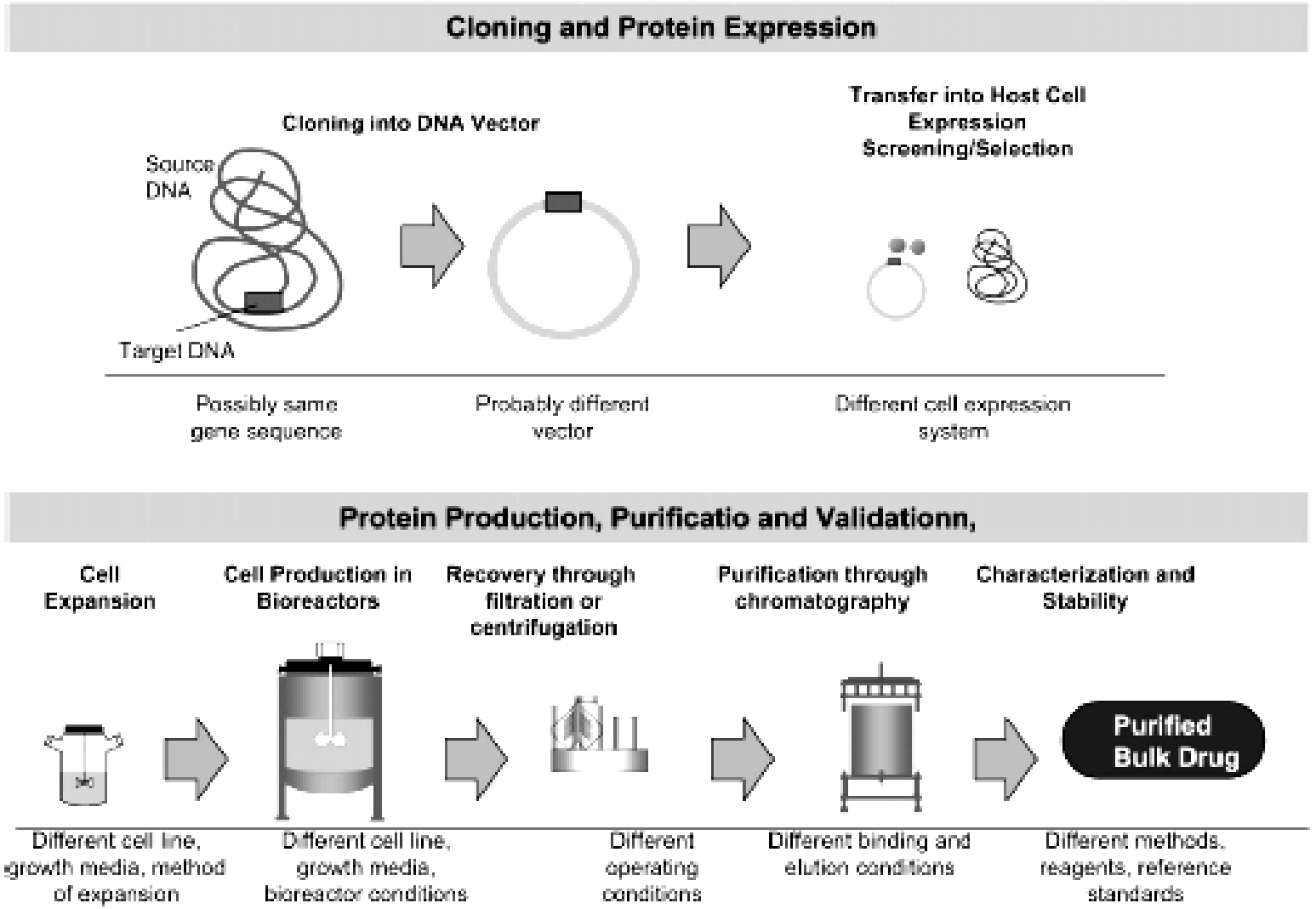
Biomanufacturing costs

- **Process development (30%)**
- **Upstream process (20%)**
- **Downstream process (40%)**

K. E. Avis, and V. L. Wu (eds). *Biotechnology and Biopharmaceutical Manufacturing, Processing, and Preservation (Drug* Manufacturing Technology Series, Vol 2), CRC, 1996.

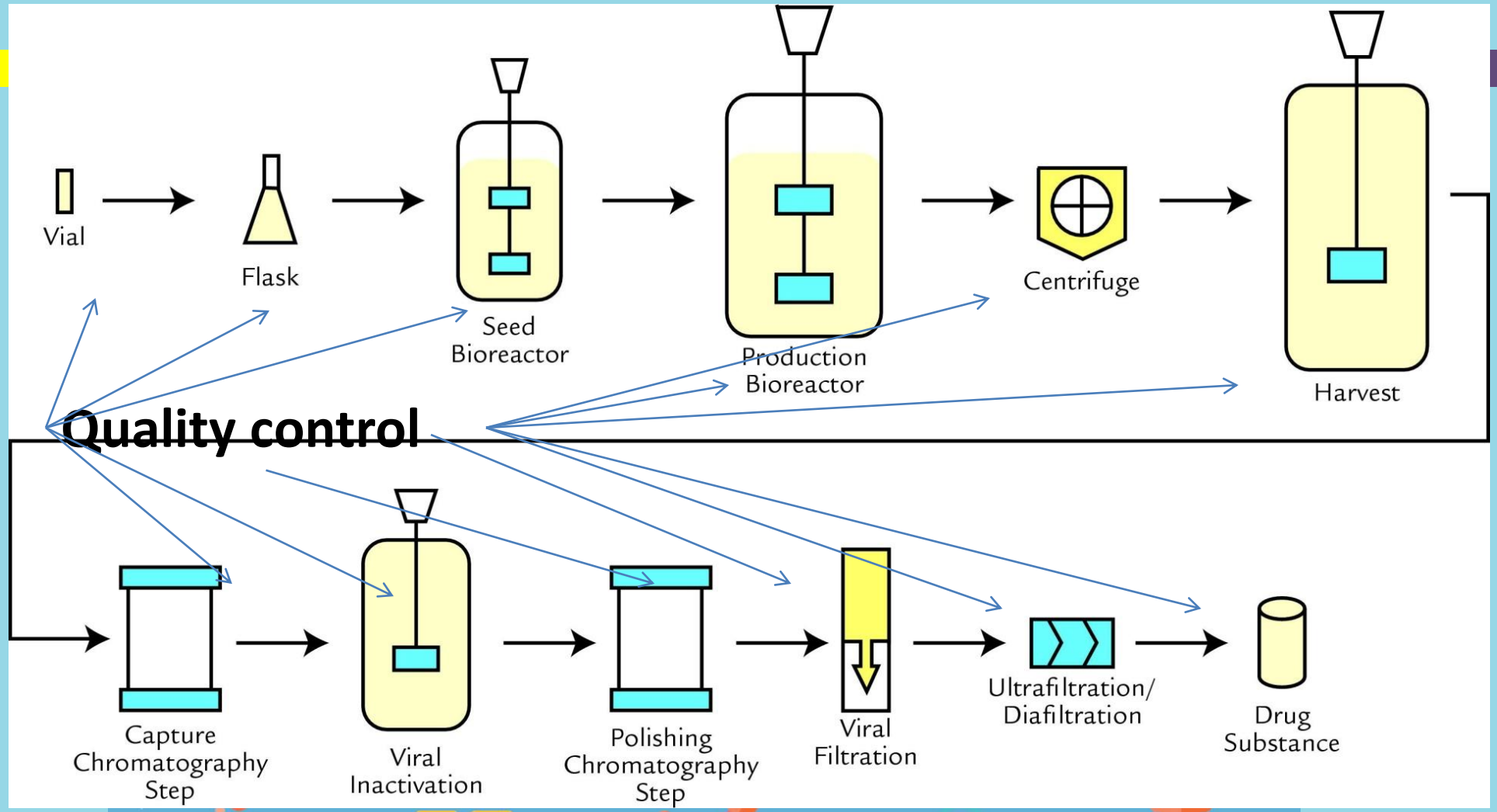


Recombinant protein production: sources of variation between manufacturers



Mellstedt, H. et al. Ann Oncol 2008 19:411-419; doi:10.1093/annonc/mdm345

Manufacturing and Production Processes of a recombinant protein in mammalian cell system: Overview



Clinical Therapeutics 34(2):2012, 400-415

Biosimilars: Impact of Biologic Product Life Cycle and European Experience on the Regulatory Trajectory in the United States

Advantages of Recombinant DNA Technology

- *Overcome the problem of **Source Availability***
  - Interferon, Interleukin, CSF
- *Overcome the problem of **Product Safety***
  - Blood borned pathogen, CJD, HIV
- ***Alternative** to direct extraction from inappropriate/dangerous **source materials***
  - FSH, HCG (from urine)
- *Generation of **engineered version** of native protein*
  - Insulin analogs (fast, basal)
  - Mab (chimeric, humanized)
  - Fusion proteins

Challenge Issues about Therapeutic Proteins

- Contain intrinsic infectious agents (Biological origins)
- Unstable molecules
- Aseptic techniques required during production (Parenteral drugs)
- Some products (esp. vaccines) are given to babies
- Usually have heterogeneous composition
  - Numerous process and product-related impurities
  - Change in the manufacturing process can cause change in product composition
- Exact structure may be unknown (e.g., all possible variants often not fully characterized)
- Large molecules in nature → Potential risk in immunogenicity

Requirement in bioprocessing

- GMP & ICH guidelines
- Special Building Capacity
- Clean room & Biosafety environments
- Well trained staffs
- Aseptic Techniques in almost all process
- Extreme in-process testing
- Robustness & Validation

RELEVANT QUALITY GUIDELINES

Q1 A-F	Stability
Q2	Analytical Validation
Q3 A-E	Drug Substances Impurities
	Drug Product Impurities
	Solvents
	Elementals
	Extractables & Leachables
Q4 A-B	Pharmacopieas
Q5 A-E	Quality of Biotechnology Products
Q6 A-B	Specifications
Q7	Good Manufacturing Practice
Q8	Pharmaceutical Development

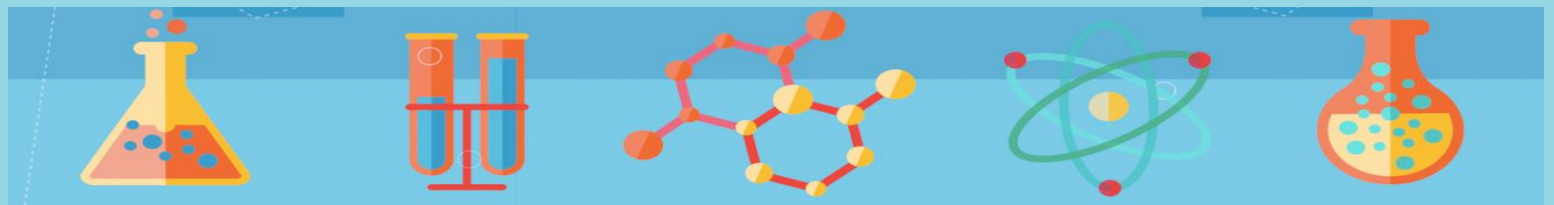
Q9	Quality Risk Management
Q10	Pharmaceutical Quality System
Q11	Development & Manufacture of Drug Substance
Q12	Lifecycle Management
Q13	Continuous Manufacturing of Drug Substances & Drug Products
Q14	Analytical Procedure Development
M4 Q	Common Technical Document
M7	Mutagenic Impurities
M9	BCS Based Biowaivers
M13	Bioequivalence for IR SOD Forms

New ICH Topics in Progress

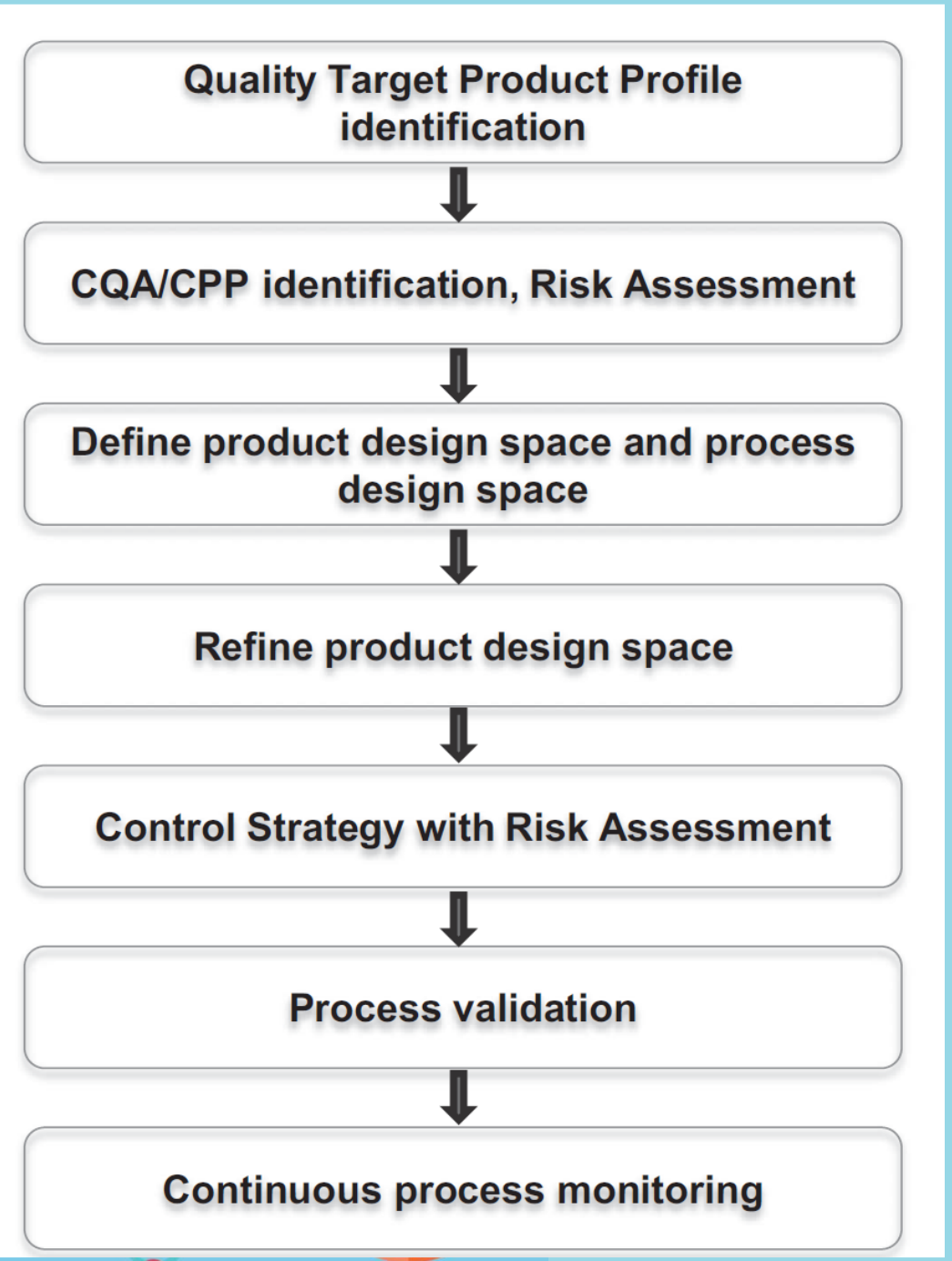
Proposed for Revision

ICH Topics in Revision

Quality by design



A quality by design approach to product and process development



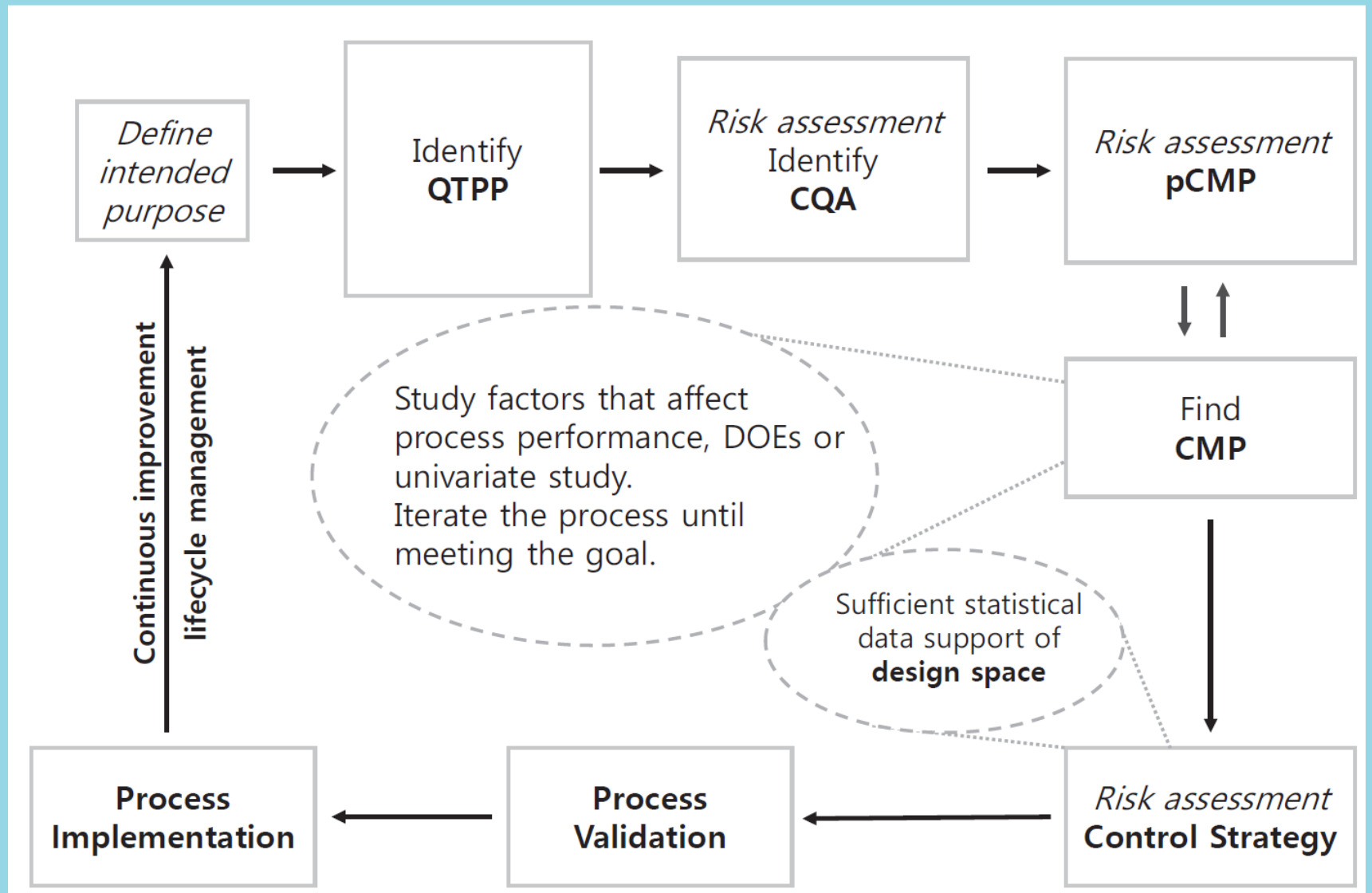


FIGURE 3.5
Steps for process development based on elements of QbD.

TABLE 3.4

Example of QTPP

Attribute	Target
Dosage form	Lyophilized powder
Nominal dose	100 mg/vial, reconstituted to 10 mg/mL in WFI
Administration	Intravenous, diluted with Saline solution.
Potency	$0.8\times10^4 - 1.2\times10^4$ U/mg
Ph. Eur. compliance with monograph for Monoclonal Antibodies for Human Use	Appearance, Solubility, pH, Osmolality, Extractable volume, Total protein, Molecular-size distribution, Molecular identity, and structural integrity, Purity, Stabilizer, Water, Sterility, Bacterial Endotoxin.
Stability	≥2 years at 2 – 8°C in type I borosilicate glass vial with a double vent butyl rubber stopper and flip-off seal

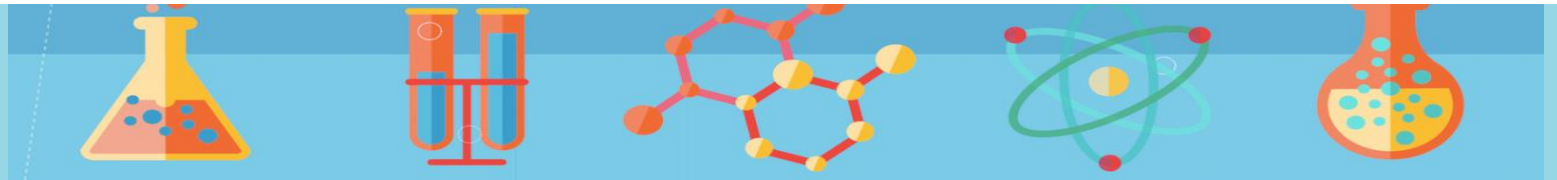


TABLE 3.3

Method Performance Characteristics as Defined in ICH Q2 (R1)

Performance Characteristics	Definition	Categorization
1. Accuracy	The closeness of test results to the true value	Systematic variability(bias)
2. Specificity	The ability to assess unequivocally the analyte in the presence of other components that may be expected to be present	
3. Linearity	Ability to elicit test results that are directly, or by well–defined mathematical transformation, proportional to the concentration of analyte in samples within the given range	
4. Precision	The degree of agreement among individual test results	Inherent random variability
5. Detection limit	A characteristic of limit tests: the lowest amount of analyte in a sample that can be detected	
6. Quantification limit	The lowest amount of analyte in a sample can be determined with acceptable precision and accuracy	
7. Range	The interval between the upper and lower levels of analyte that have been demonstrated to be determined with a suitable level of precision, accuracy, and linearity	N/A
8. Robustness	Capacity to remain unaffected by small but deliberate variations in procedural parameters listed in procedure documentation and indicates its suitability during normal usage	N/A

EXAMPLES OF BIOPHARMACEUTICALS

Haemophilia A

One of the most important genetic disorders 5 to 6 for 100 000 live births per annum

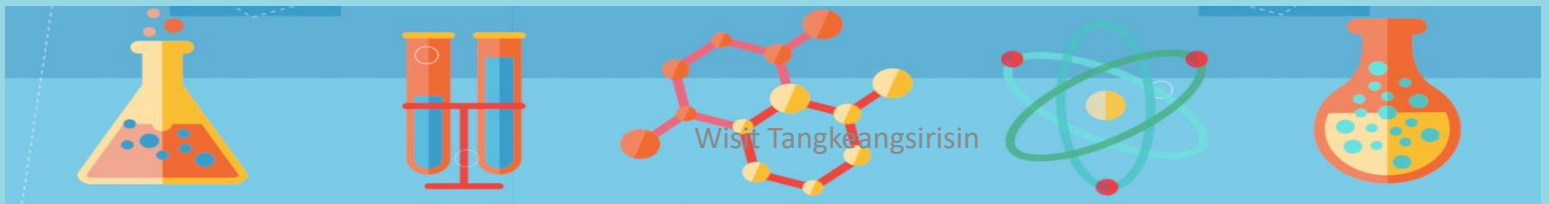
	TREATMENT	SURVIVAL
Until 1920s	None	11y
Until 1970s	Fresh frozen plasma	mid 20s
1970s	Plasma concentrate	68y
1980s	Plasma concentrate	AIDS, Hep B & C
1990s	Recombinant F VIII	normal if no inhibitor
2000s	Preventive treatment	

Growth hormone

- Very short stature is the most serious effect of childhood growth hormone (GH) deficiency
- Since the 1960s, replacement treatment using hGH extracted from pituitary glands from human cadavers was introduced
- That lead to the prion contamination of number of children (Creutzfeld-Jacob's disease)
- The first recombinant human GH devoid of infectious risk was marketed in the late 1980s

Human insulins

- Diabetes is a disease that affects more than 150 millions people worldwide with serious and irreversible complications
- Early therapy (cow/swine insulin) → hypersensitivity
- Human insulin from pancreas → limit supplies
- The first human recombinant insulin was launched in 1982
- New analogues or delivery systems are developed (rapid- or long-acting insulin, inhaled insulin) → PK modifications



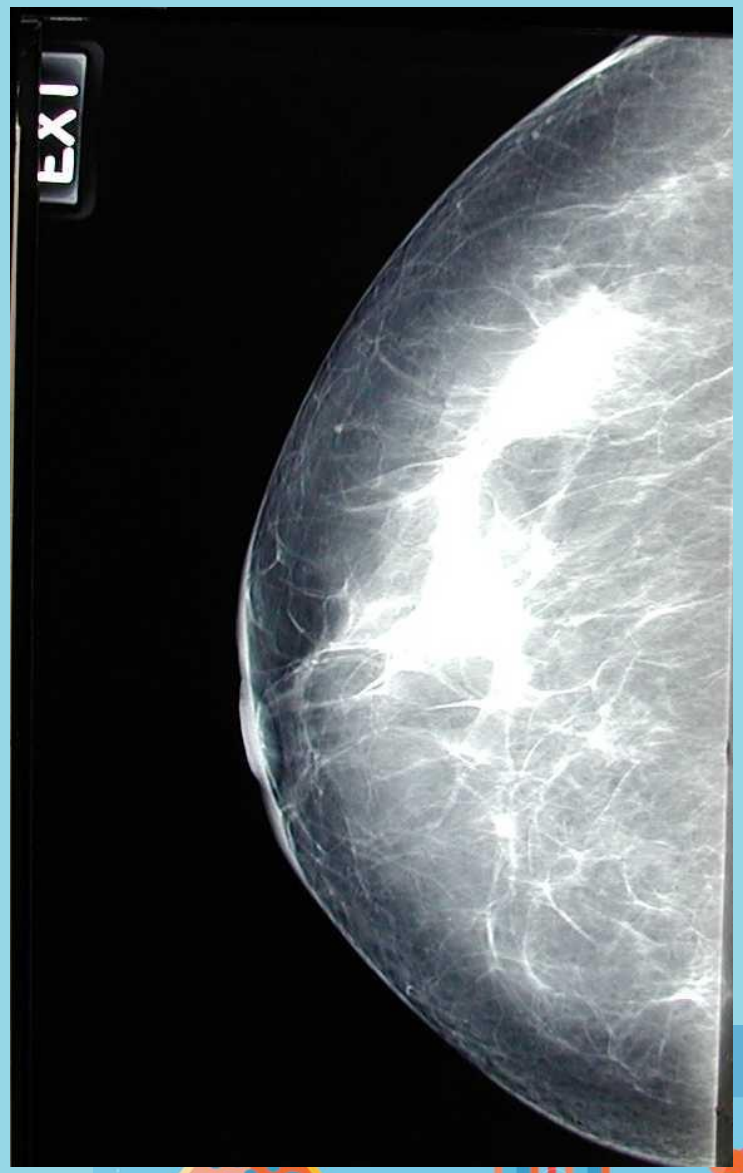
Erythropoietin (EPO)

- Diabetes and high blood pressure, two of the most common diseases of the developed world, are the main causes of renal insufficiency (RI) that lead to severe anaemia
- Recombinant EPO, developed in the 1980s, revolutionized the treatment of anaemia linked to RI
- The EPO market is considerable, about €10bn per annum

Monoclonal antibodies

- Trastuzumab (Herceptin®) for breast cancer
- Rituximab (Mabthera®/Rituxan®)and other anti-CD20 for B lymphoma
- Infliximab (Remicade®) Anti-TNF for Rheumatoid arthritis and Crohn disease

Trastuzumab (Herceptin®)



- Anti-HER 2
- About 25% BC p/t express HER-2
- Trastuzumab increases survival of patients with advanced and metastatic breast cancer

Rituximab and other anti-CD20

- Lymphomas are diseases of the blood that rank 7th as cause of death by cancer in France
- Relapses are frequent and some patients are resistant to chemotherapy
- With monoclonal antibodies, alone or associate with radioactive particles, positive response of up to 75% can be obtained
- Due to its specificity monoclonal antibody therapies have in general fewer side effect than classical chemotherapy

Infliximab (Ramicade®)

- Anti-TNF-alpha antibody for treatment of RA
- Chimeric mouse/human Mab
- $900 for a 100 mg dose! Responsible for $2.1 billion in sales 2009
- Produced in 1,000 liter production reactors

Outlook :

1. Biopharmaceuticals are a key driver for strong growth of the biopharma
2. Biopharmaceuticals provide new therapeutic opportunities in chronic disease
3. DNA, RNA drugs and cell based therapy – Gene therapy and antisense drugs are emerging
4. Biopharmaceuticals are still very expensive
5. Genomics + biopharmaceuticals – pave the way to personalized medicine

Large Scale Production of Recombinant Proteins

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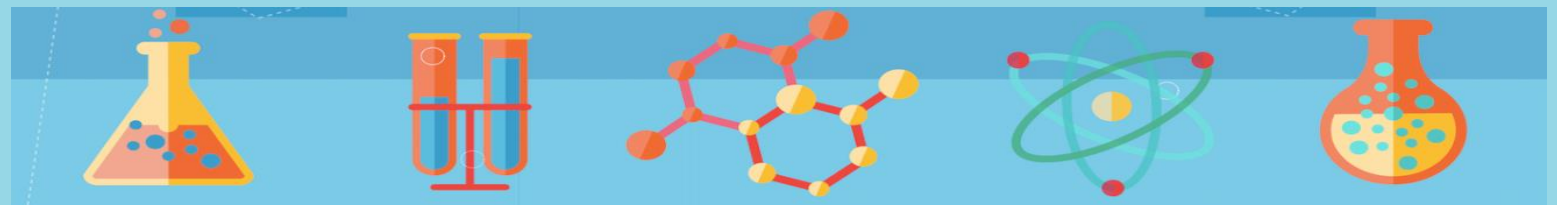
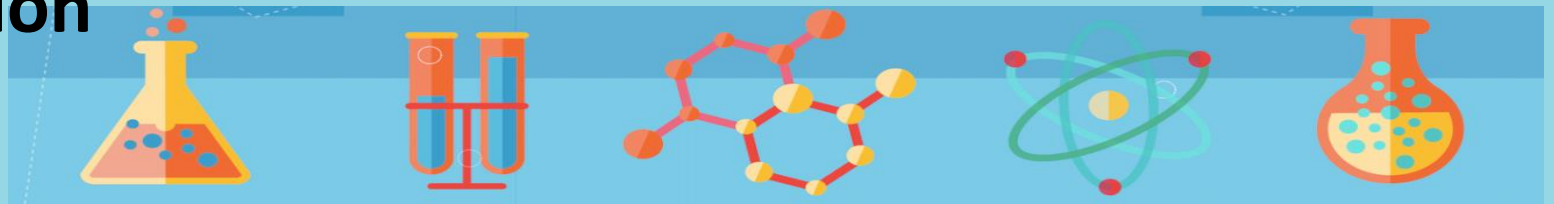


Lecture Outlines

- Introduction
  - Recombinant Proteins significance in Pharmacy
  - Feature of Recombinant Protein
- How to Scaling up
  - Upstream Process technology
    - Fermentation
    - Cell Disruption/ Lysate Preparation
    - Filtration/Concentration
  - Downstream Process technology
    - Column chromatography
  - Formulations/Filling
- Conclusion



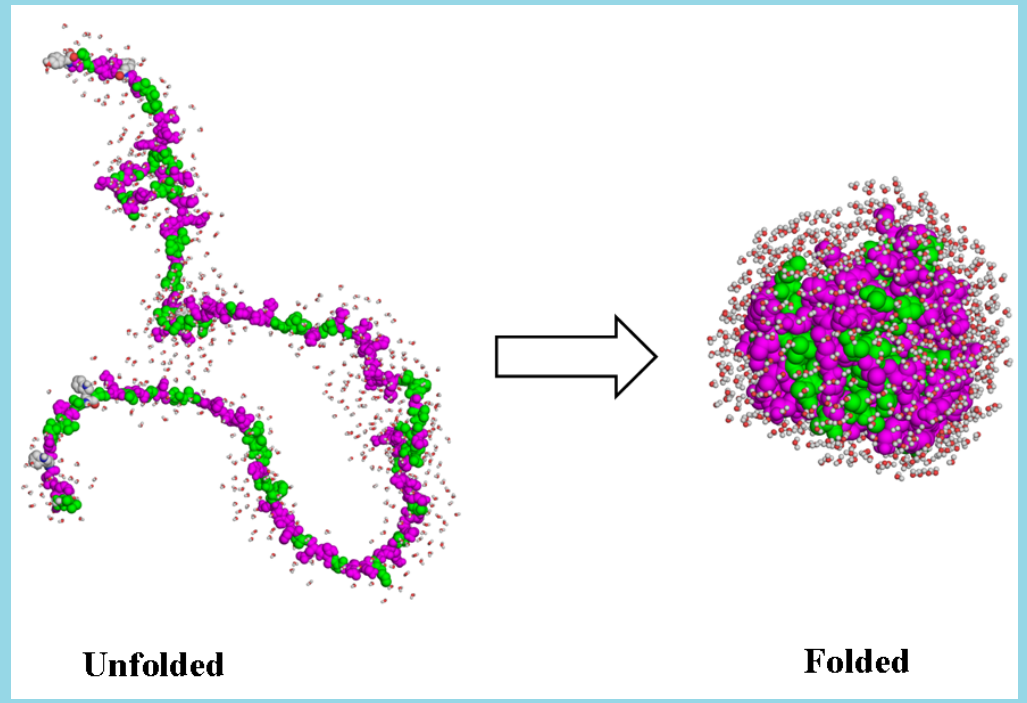
Biopharmaceuticals

- Produced in genetically engineered host cells
- Chinese Hamster Ovary (CHO) cells are most popular culture (>70%)
- Eg. Recombinant proteins, Vaccine, Monoclonal Antibody (Mab)
- Complex, heterogeneous mixture
  - 3D Structure
  - Post-translational modifications
  - Slight process changes affect potency
- Highly regulated processes
  - Process is the product

B. Leader et al., Nat. Rev. Drug Discov 2008, 7:21-39

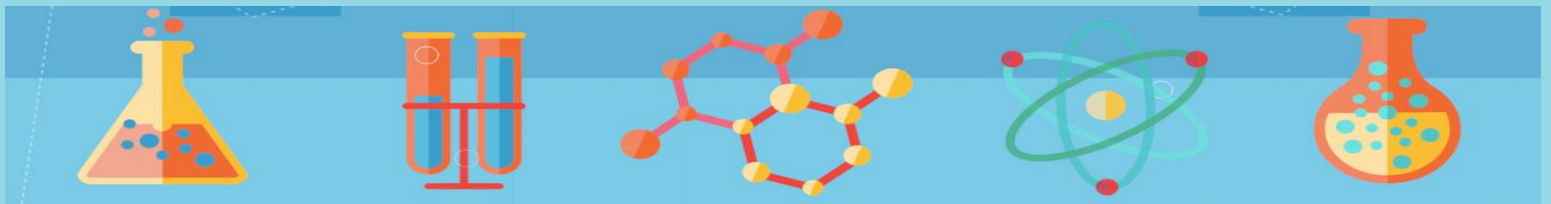
Protein Stability and Folding

- a polypeptide folds into its characteristic and functional three-dimensional structure from random coil
- The correct three-dimensional structure is essential to function



Protein Engineering:

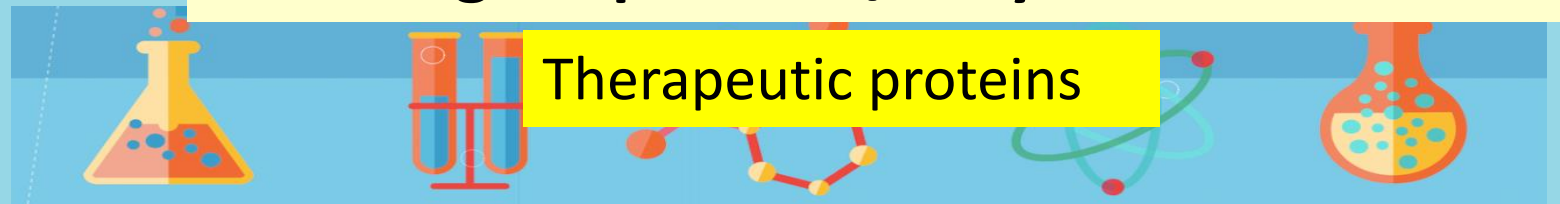
- Alteration of a single amino acid residues at specific site
- Insertion or deletion of a single amino acid residue
- Alteration or deletion of an entire domain
- Generation of a novel fusion protein



Why Protein Engineering

- **Protein/Enzyme :** Evolved for original host itself, not for human
  Most proficient catalysts with high specificity
- **Need further improvement :**
  Substrate specificity
  Binding affinity
  Stability
  Catalytic activity
  Folding/Expression level
  Pharmacokinetic alteration etc..
- **Goal in protein engineering :**Design of protein/enzyme with desired function and property for practical applications

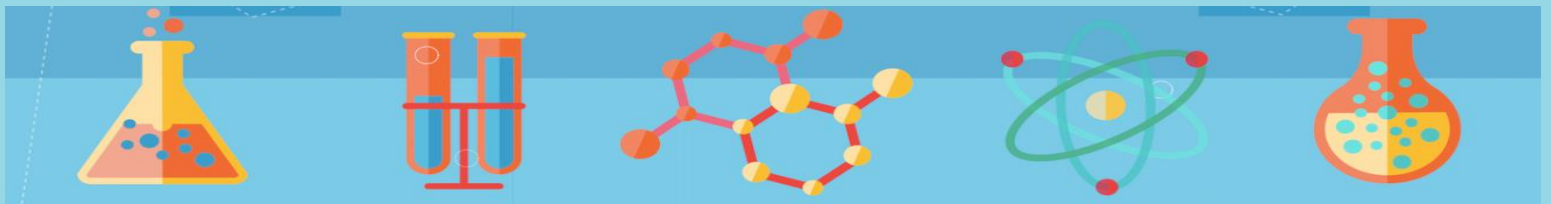
Designer proteins/Enzymes



Therapeutic proteins

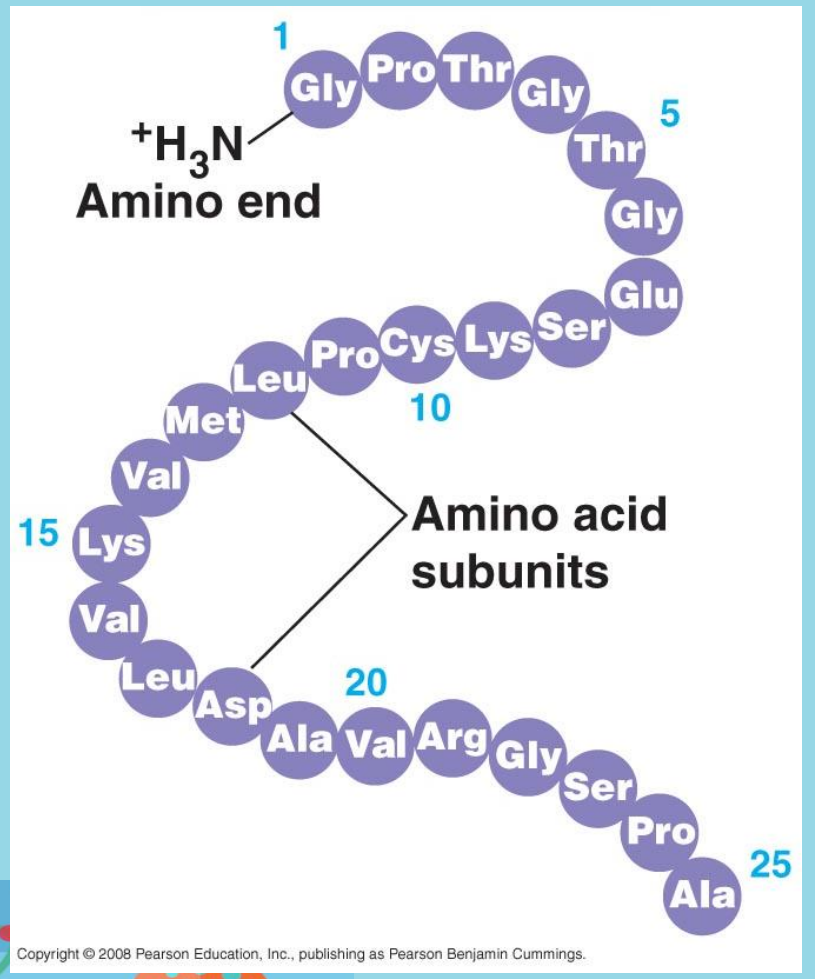
Protein Structures

- Do you remember each level of Protein Structures?
  - Primary Structure
  - Secondary Structure
  - Tertiary Structure
  - Quarternary Structure



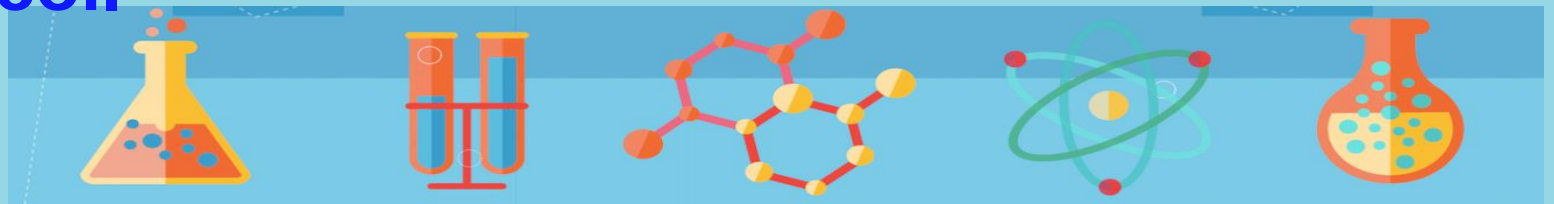
Protein Primary Structures

- Amino Acid Composition
- Amino Acid Sequence
- Molecular mass



Protein Secondary Structures

- Secondary structure refers to a local spatial arrangement of the polypeptide chain
- Two regular arrangements are common:
- The α helix
  - stabilized by hydrogen bonds between nearby residues
- The β sheet
  - stabilized by hydrogen bonds between adjacent segments that may not be nearby
- Irregular arrangement of the polypeptide chain is called the random coil



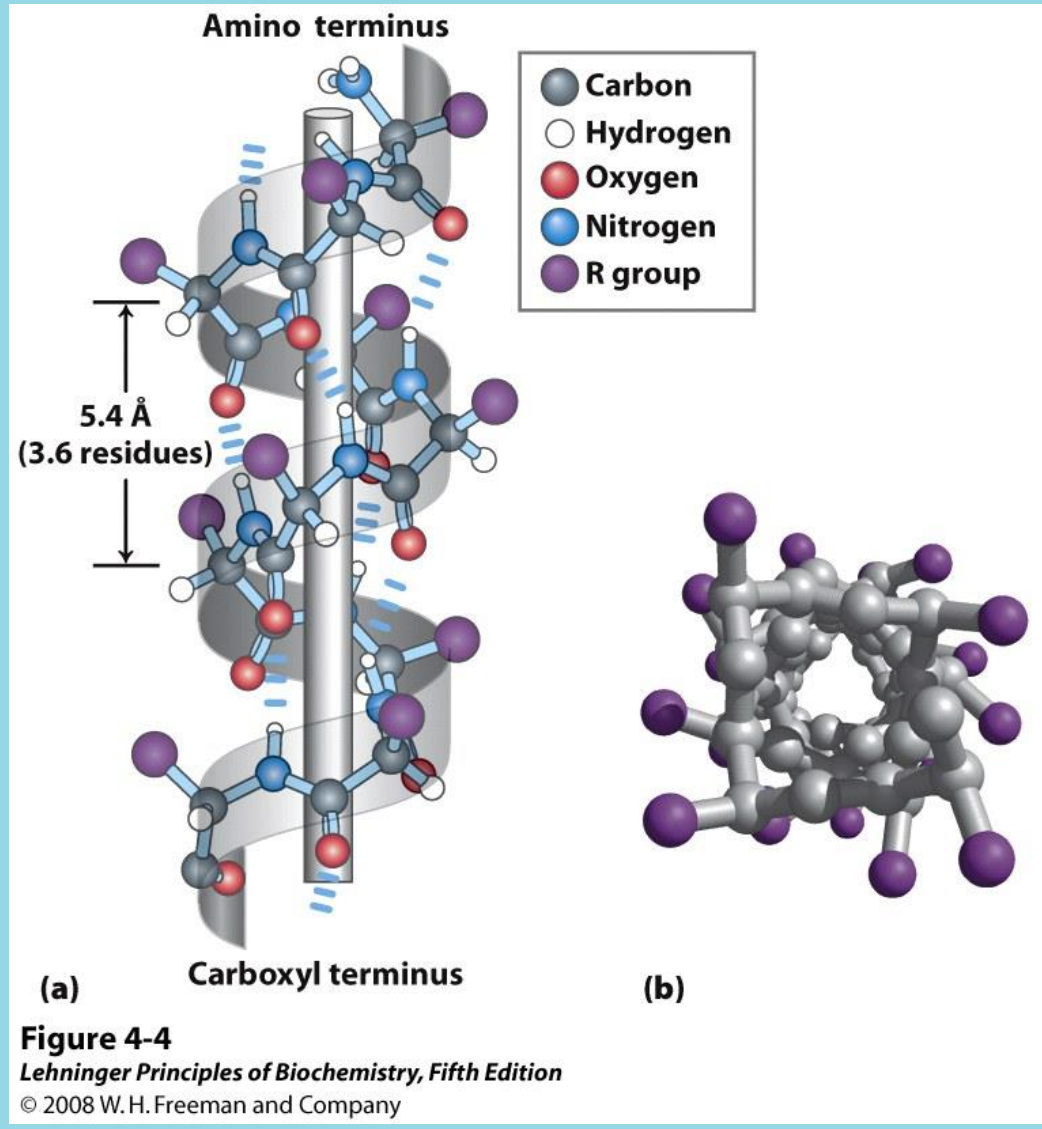


Figure 4-4
Lehninger Principles of Biochemistry, Fifth Edition

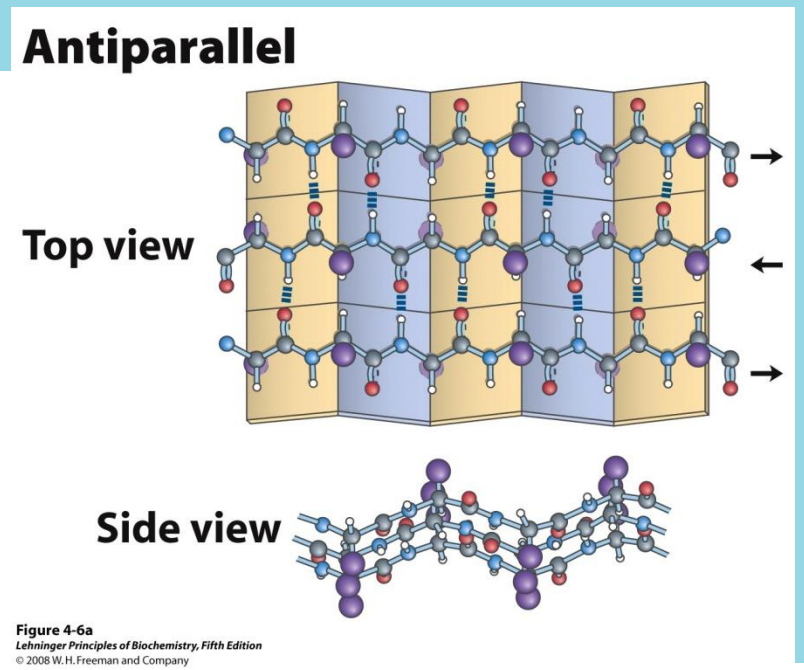


Figure 4-6a
Lehninger Principles of Biochemistry, Fifth Edition

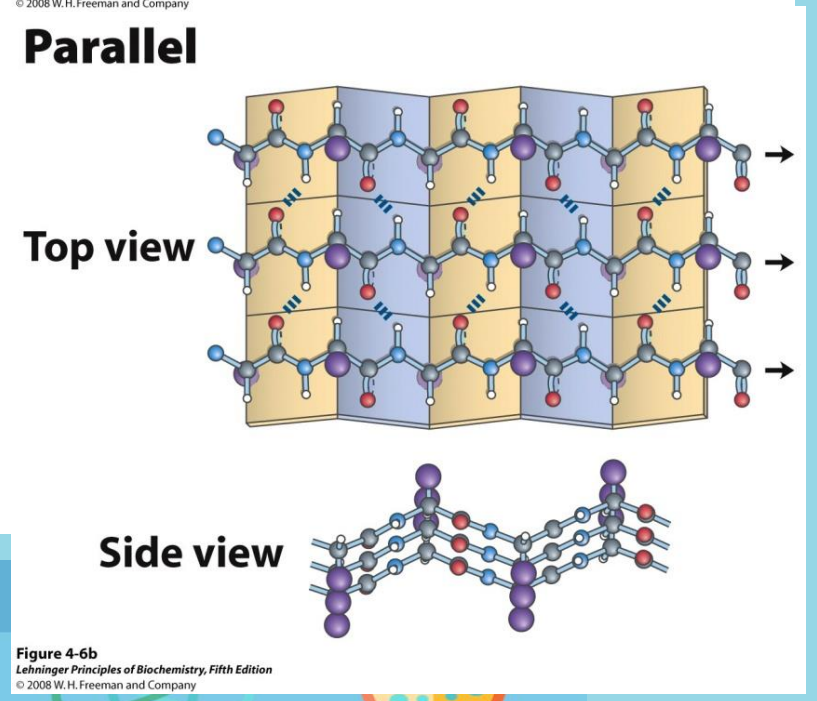


Figure 4-6b
Lehninger Principles of Biochemistry, Fifth Edition

Protein Tertiary Structures

- ***the overall spatial arrangement*** of atoms in a protein
- two major classes
  - fibrous proteins
  - globular proteins
    - water-soluble globular proteins
    - lipid-soluble membraneous proteins

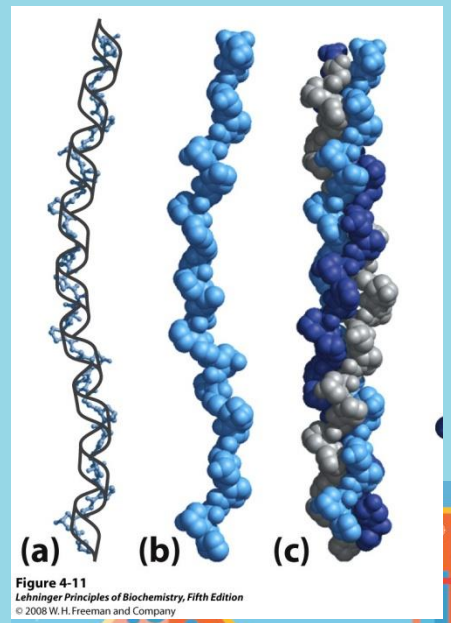


Figure 4-11
Lehninger Principles of Biochemistry, Fifth Edition

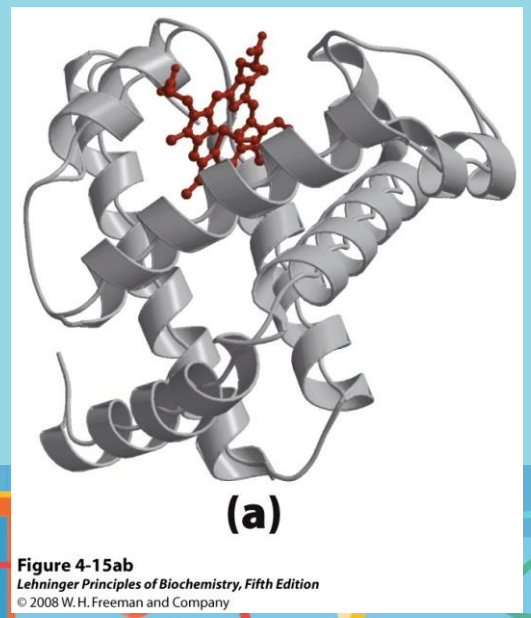


Figure 4-15ab
Lehninger Principles of Biochemistry, Fifth Edition

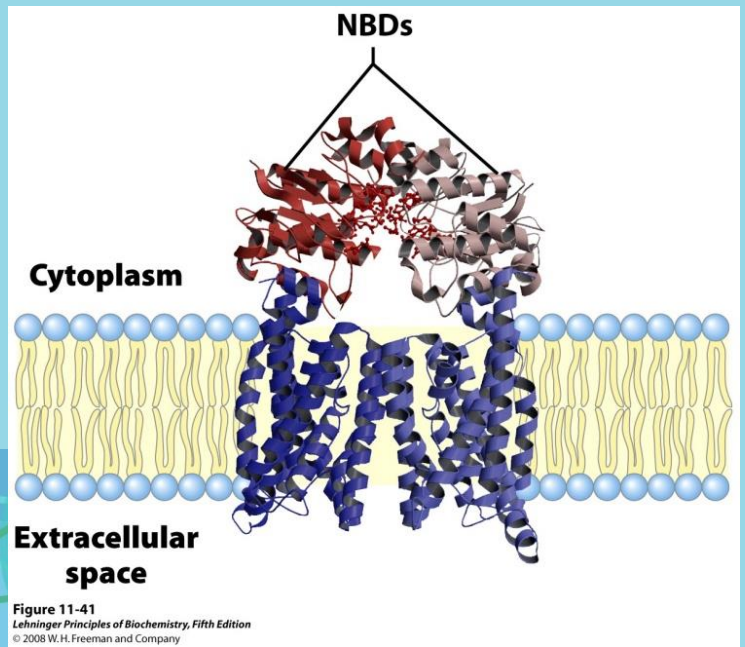


Figure 11-41
Lehninger Principles of Biochemistry, Fifth Edition

Protein Quaternary Structures

- **Quaternary structure** is formed by spontaneous assembly of individual polypeptides into a larger functional cluster

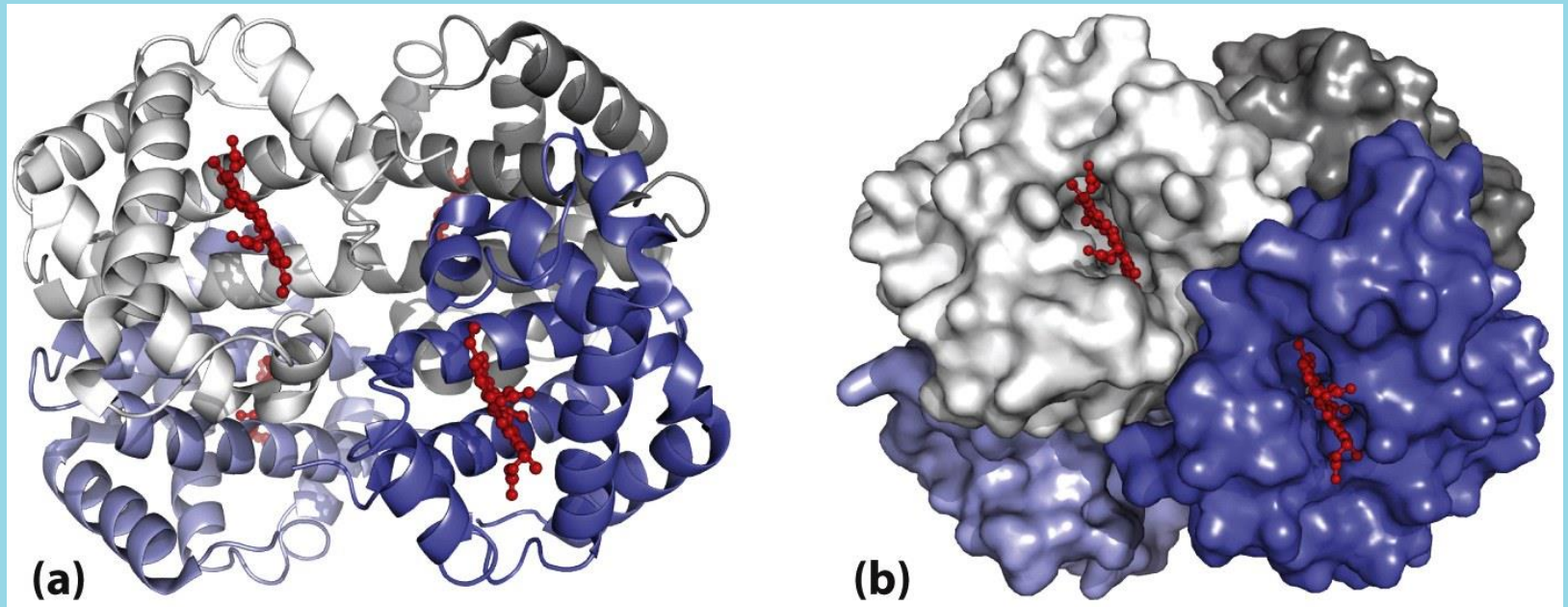
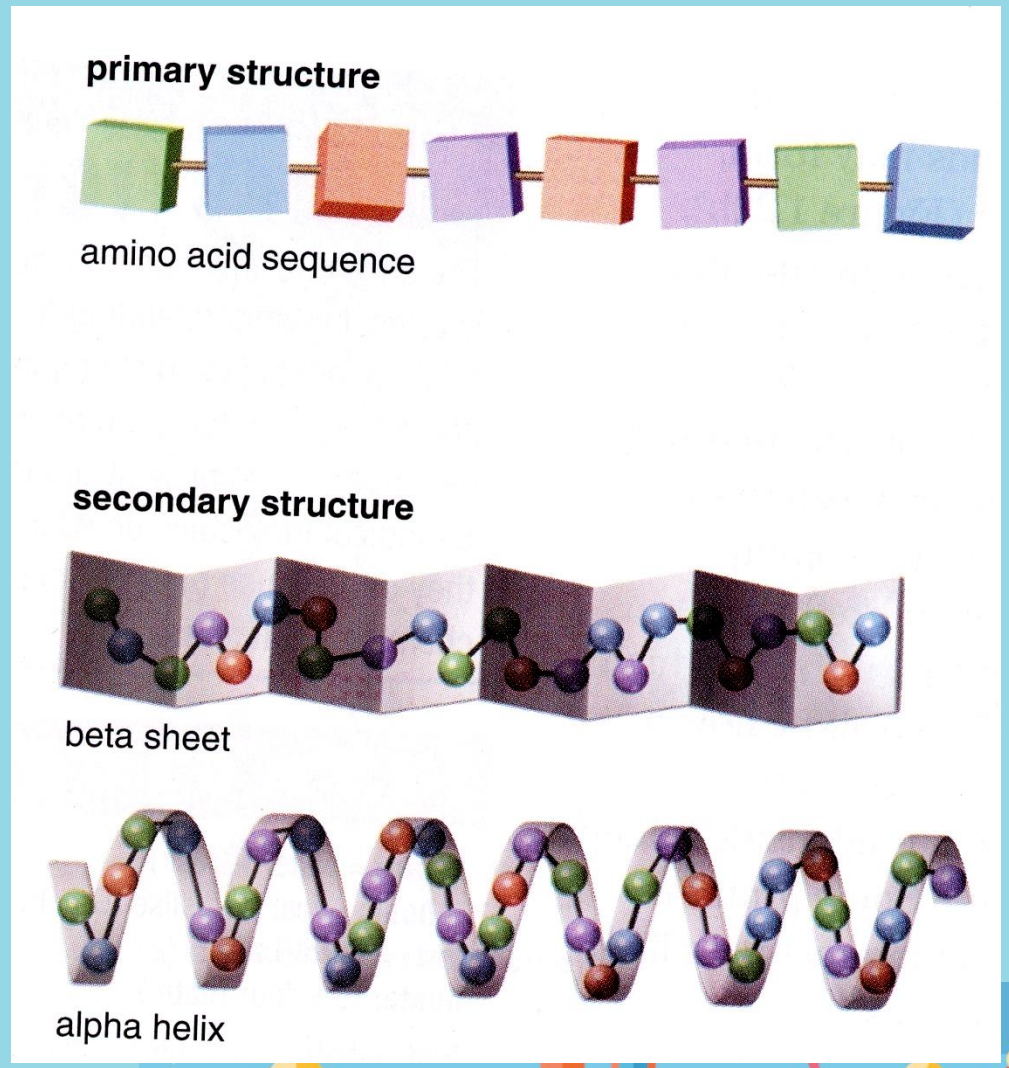
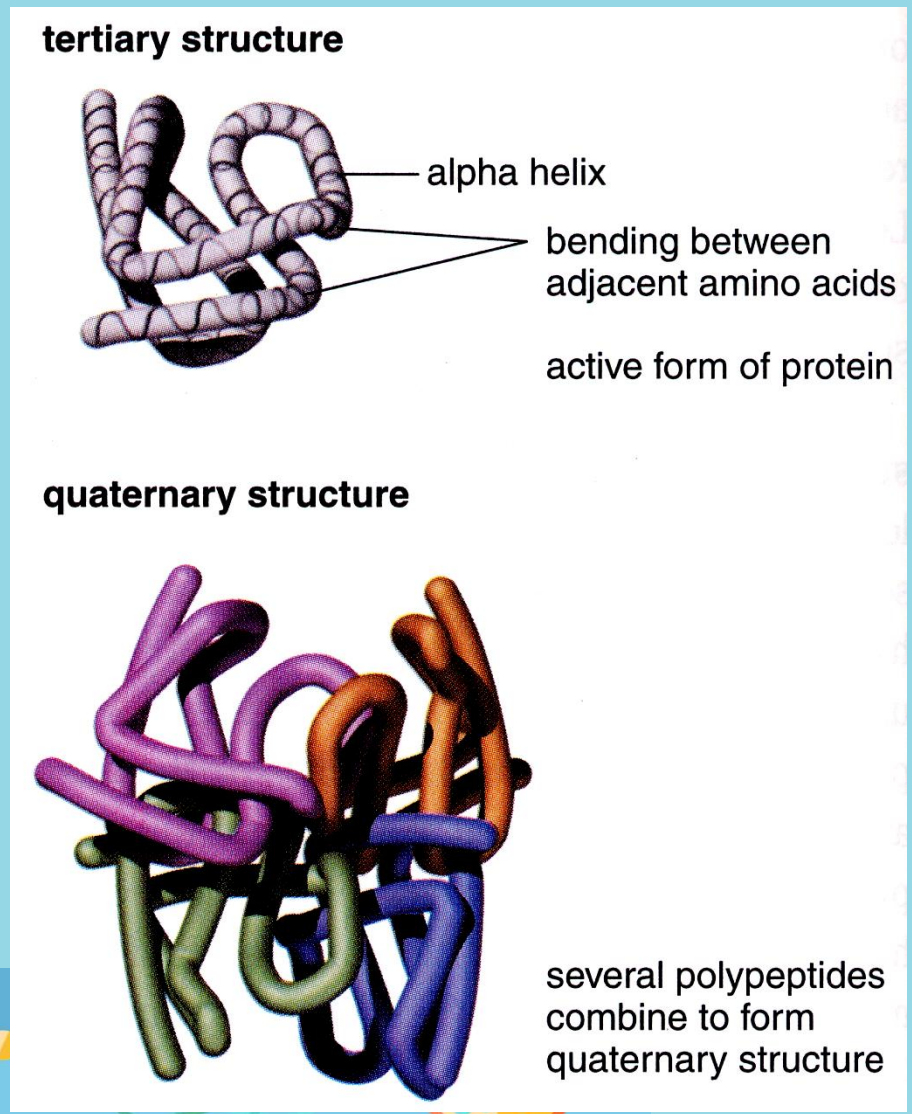


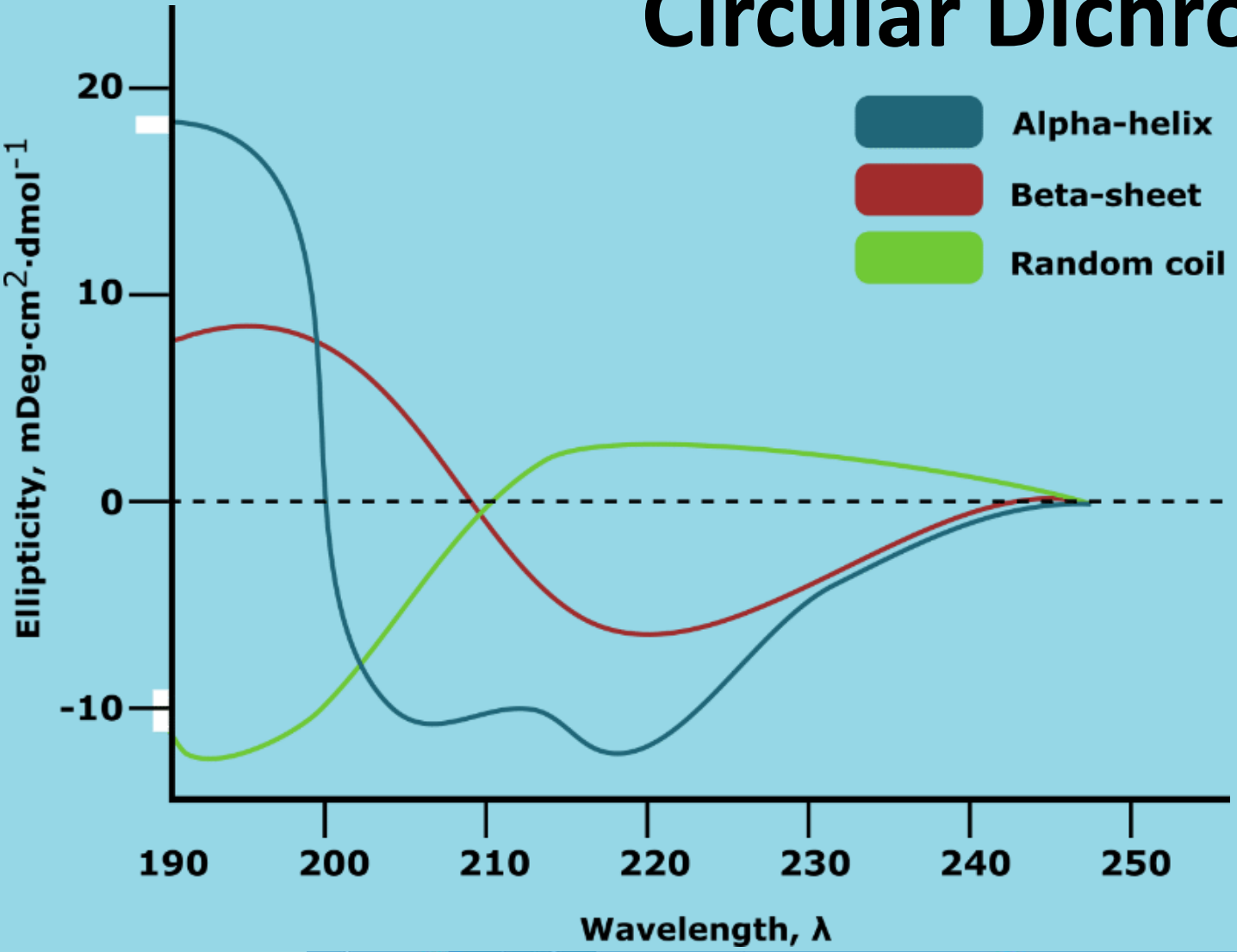
Figure 4-22
Lehninger Principles of Biochemistry, Fifth Edition

Four Level of Protein Structures



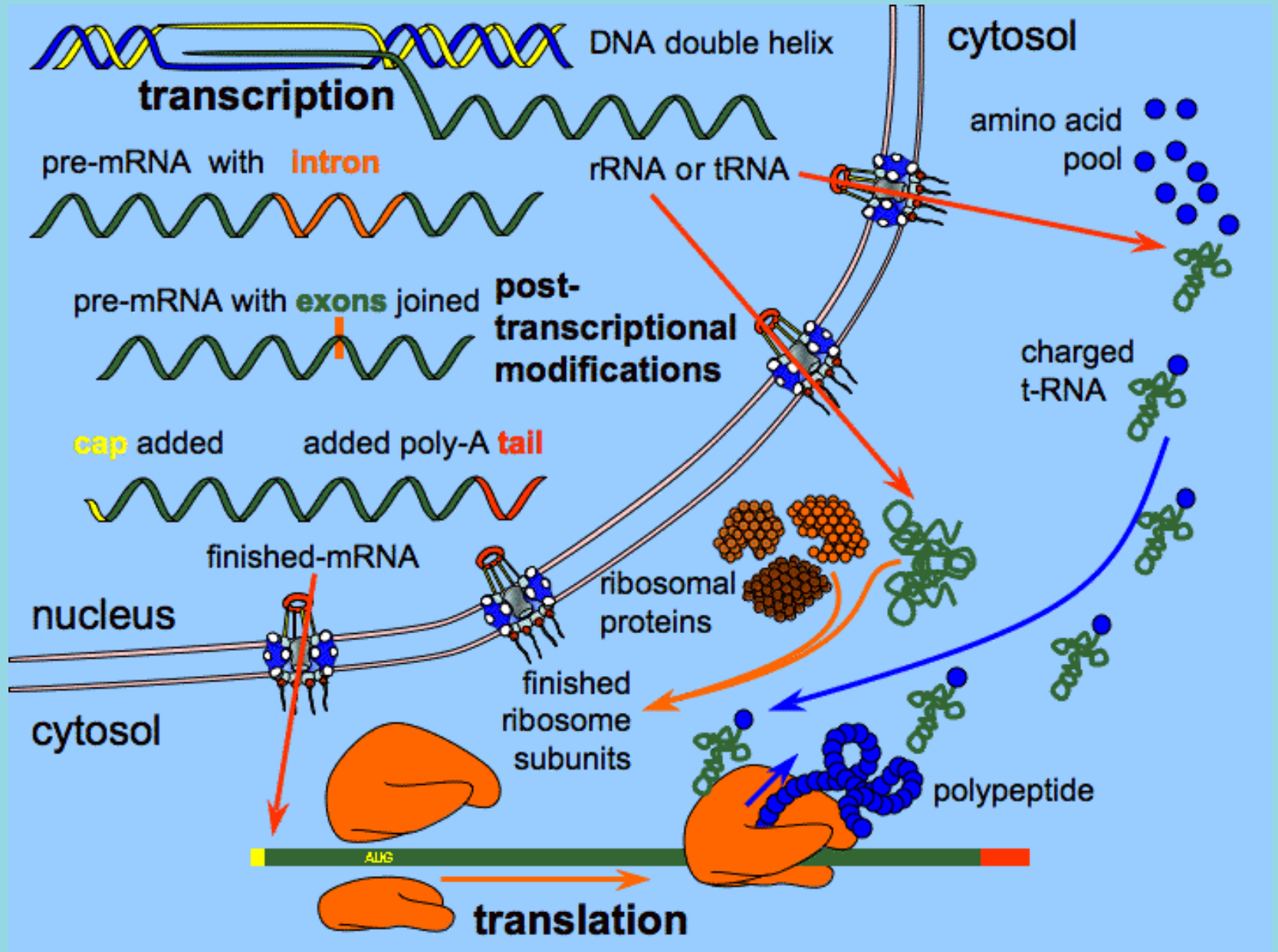


Circular Dichroism

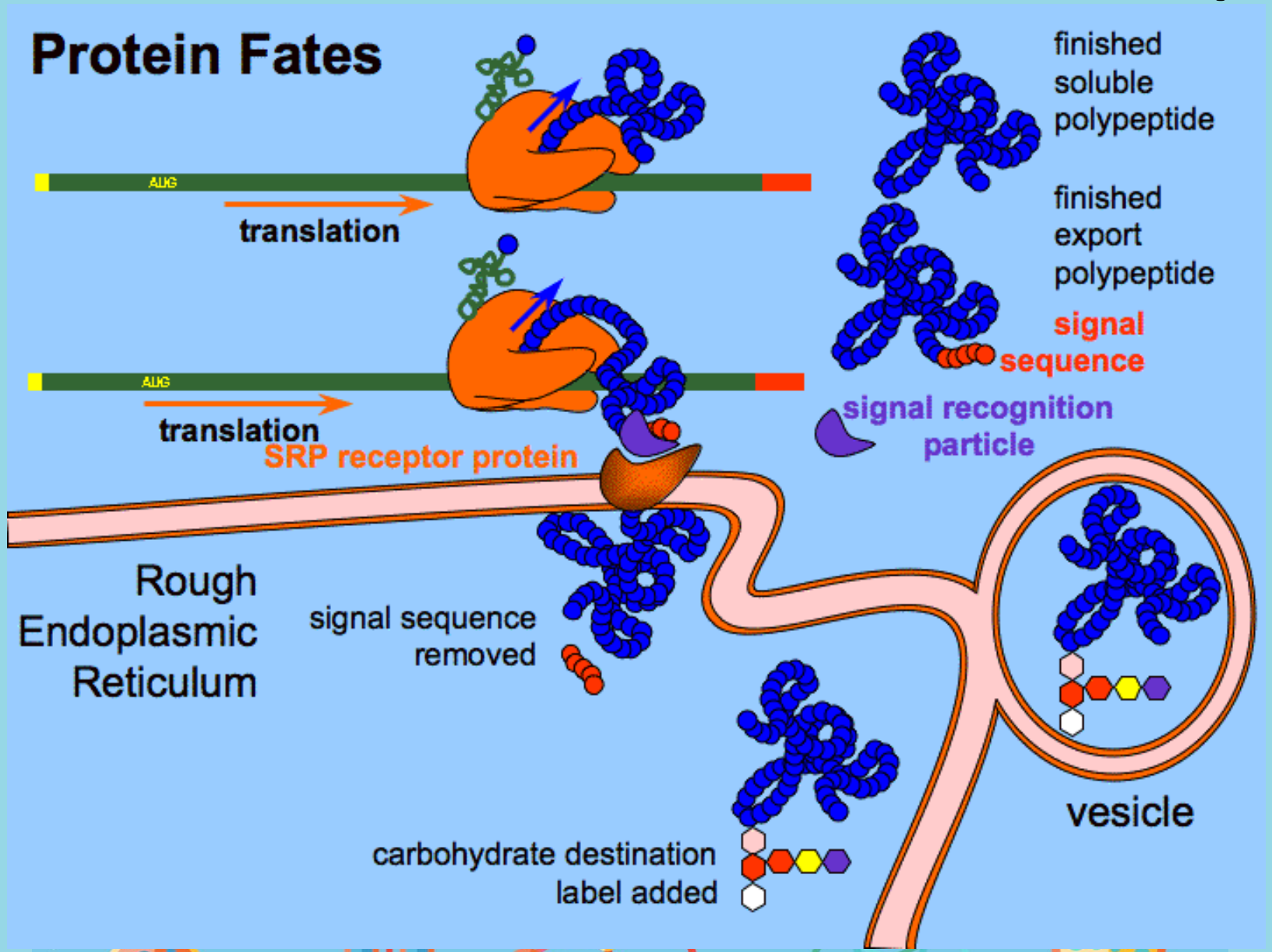


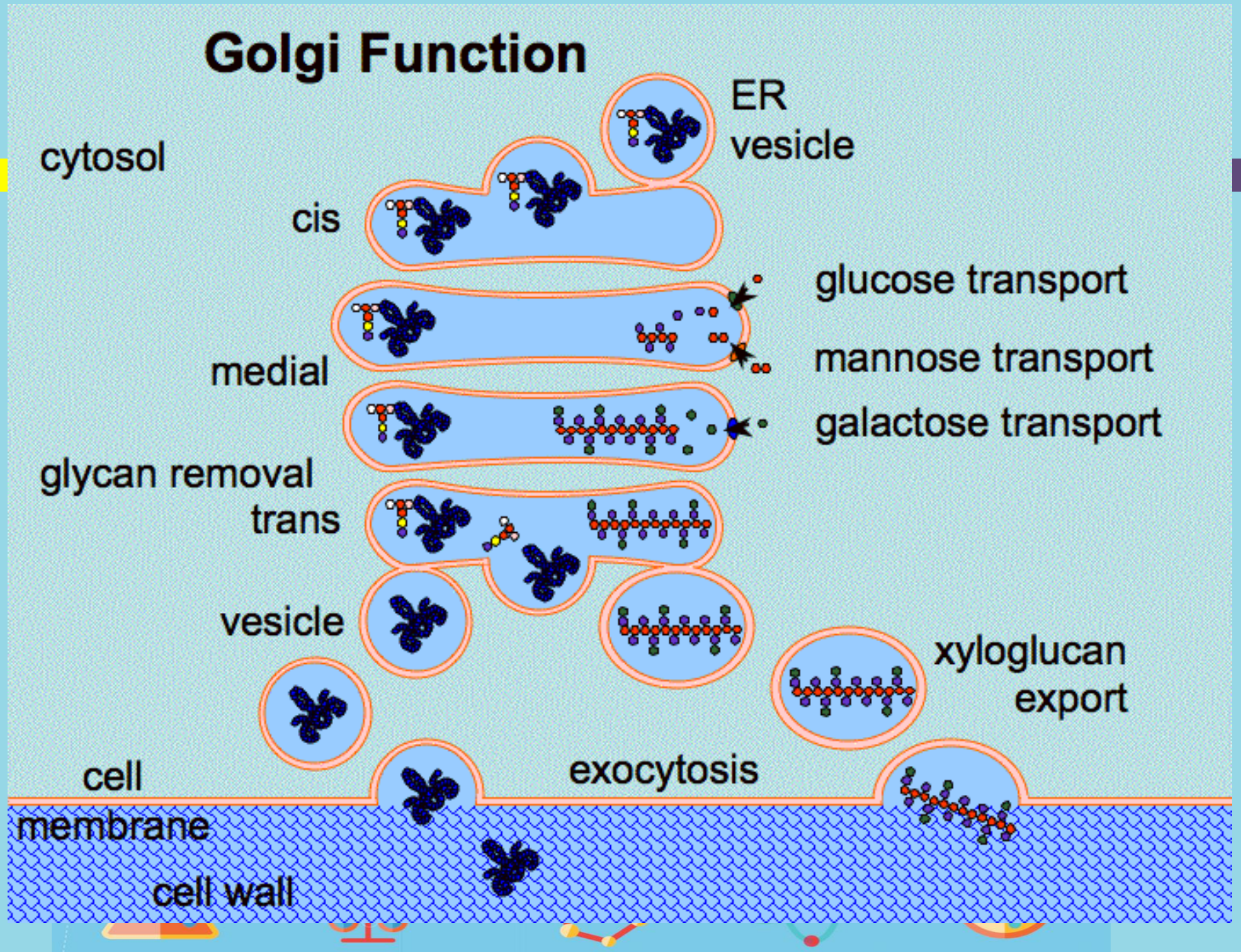
A simplified graph showing the CD spectra for the extreme cases of 100% alpha-helix (blue), 100% beta-sheet (red), and 100% random coil (green). (Image credit: Thomas Warwick.)

From Gene to Functional Protein (1)



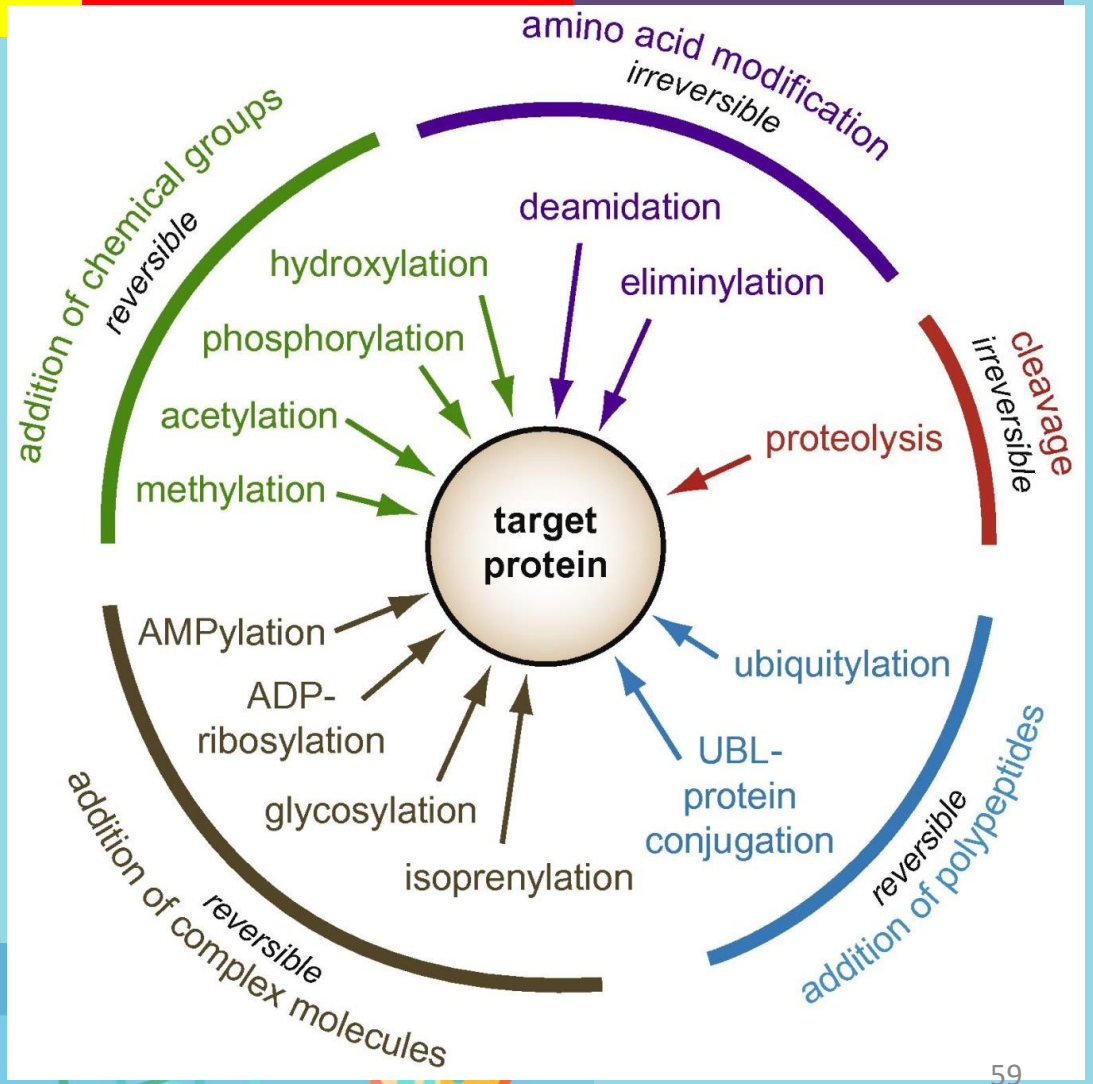
From Gene to Functional Protein (2)



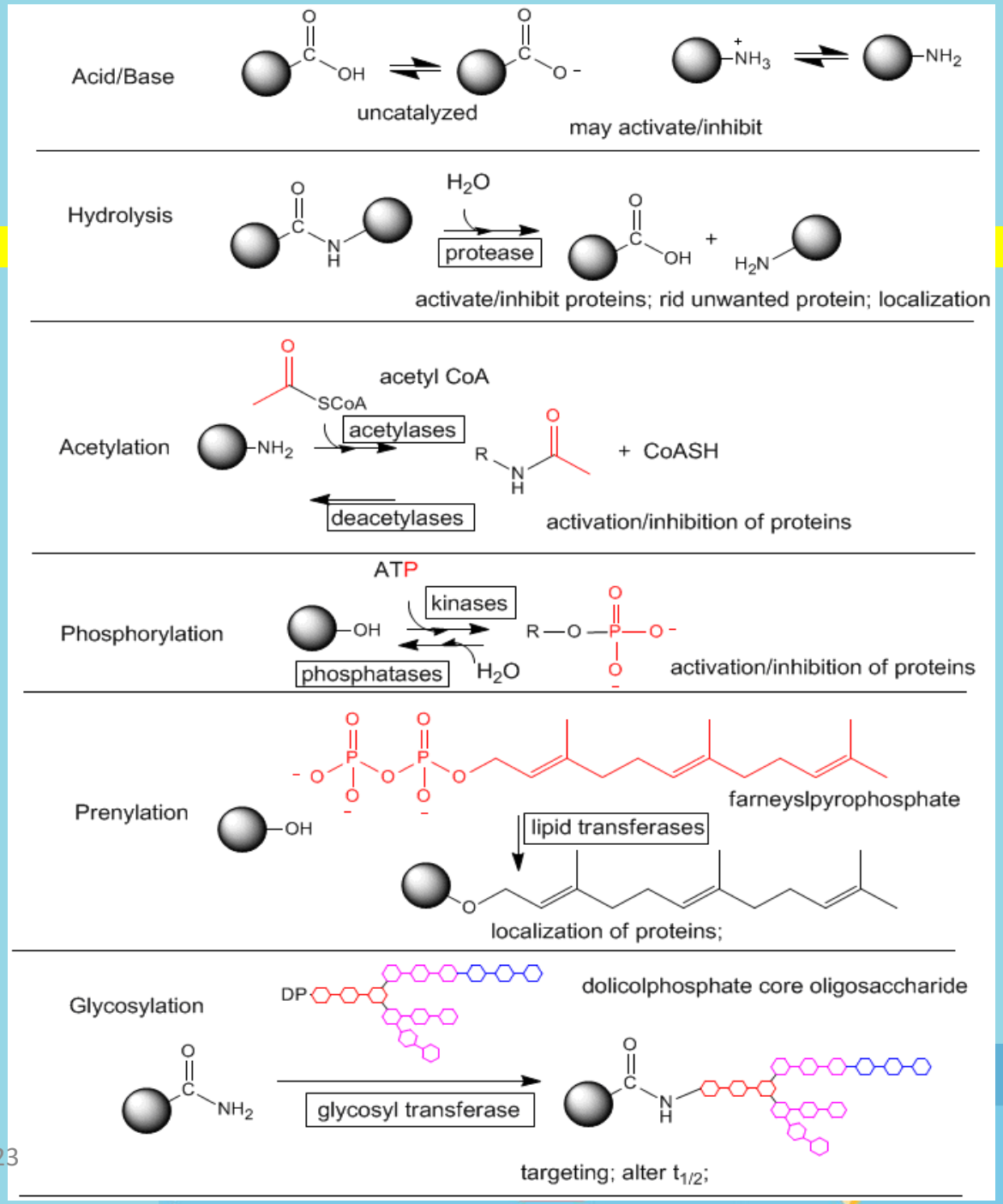

Golgi Function
ER
vesicle
cytosol
cis
glucose transport
mannose transport
galactose transport
medial
glycan removal
trans
vesicle
xyloglucan
export
cell
membrane
exocytosis
cell wall

Post-Translational Modifications

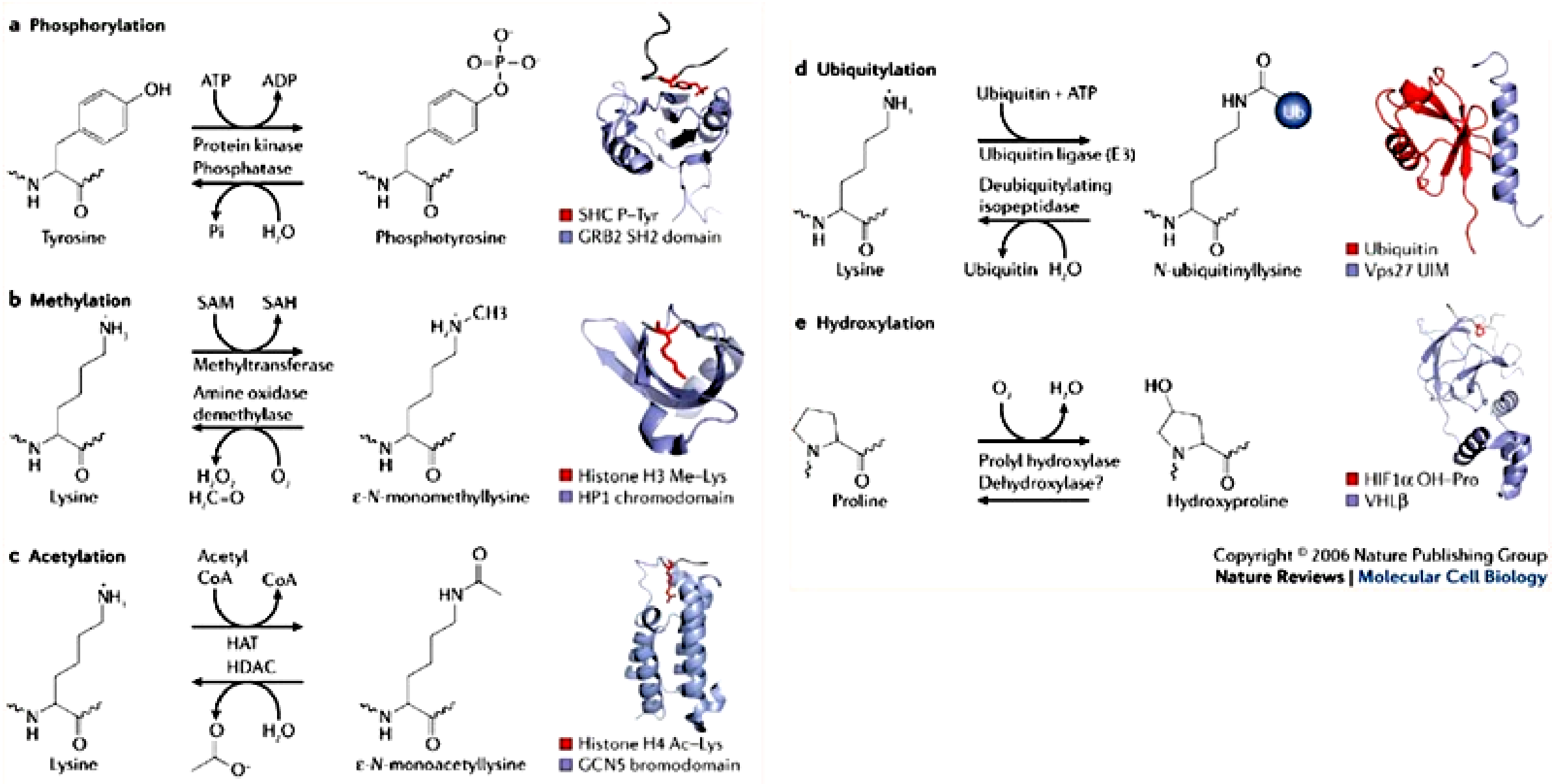
- **Controling Protein Function and Diversity through Enzymatic Chemistry**
- **Essential in full activity**
- **Affect stability, safety (immunogenicity)**
- **Production Processes affect PTM**



Post-Translational Modifications

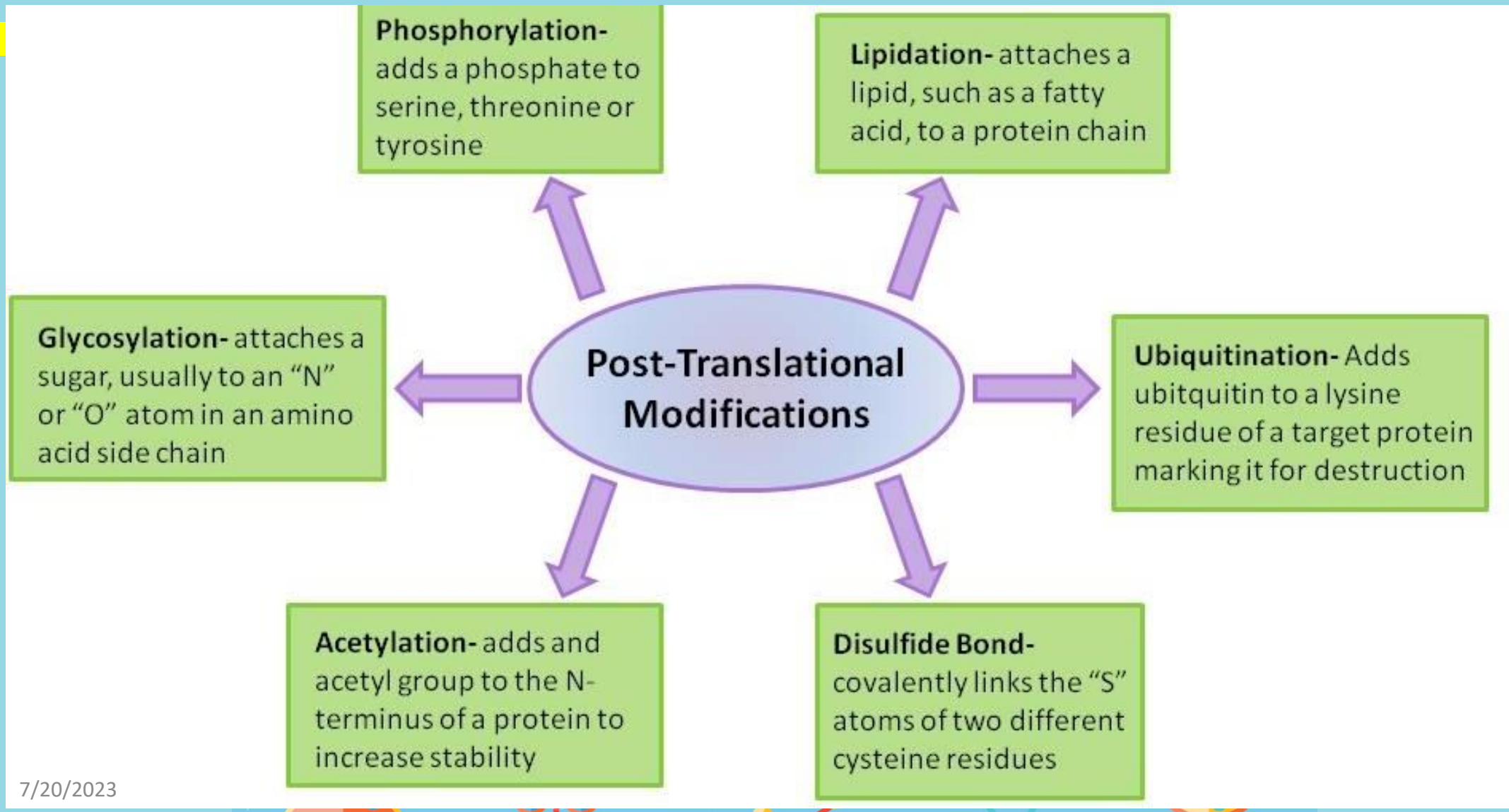


Some Post-translational Modification Reactions



Seet *et al. Nature Reviews Molecular Cell Biology* **7**, 473–483 (July 2006) | doi:10.1038/nrm1960

Post-translational Modifications



Stages of Production : PTM

Producer Cell Line/ Expression System

Major Effect
Dictates ability to nature of PTM

Upstream Processing

Significant potential effect
Media Composition/culture condition

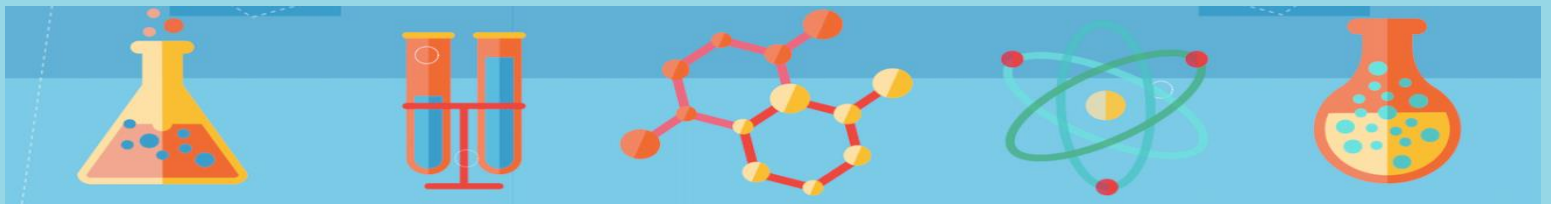
Downstream Processing

Potential effect
Chemical/biological-based PTM modification (oxidation or enzymatic)

Formulation

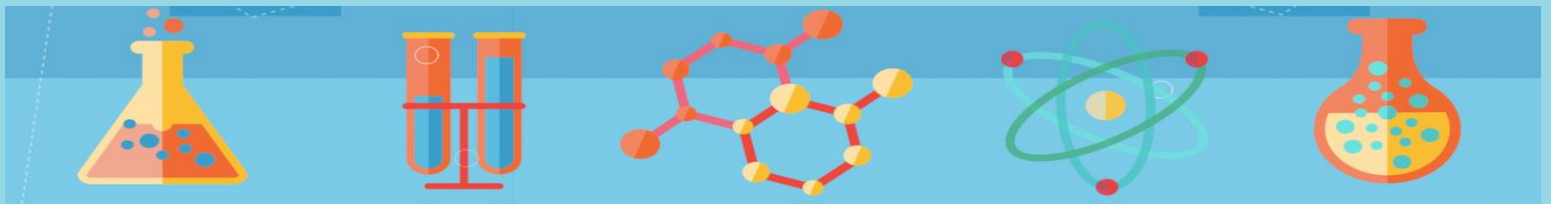
Potential effect

PRODUCTION CELL LINE



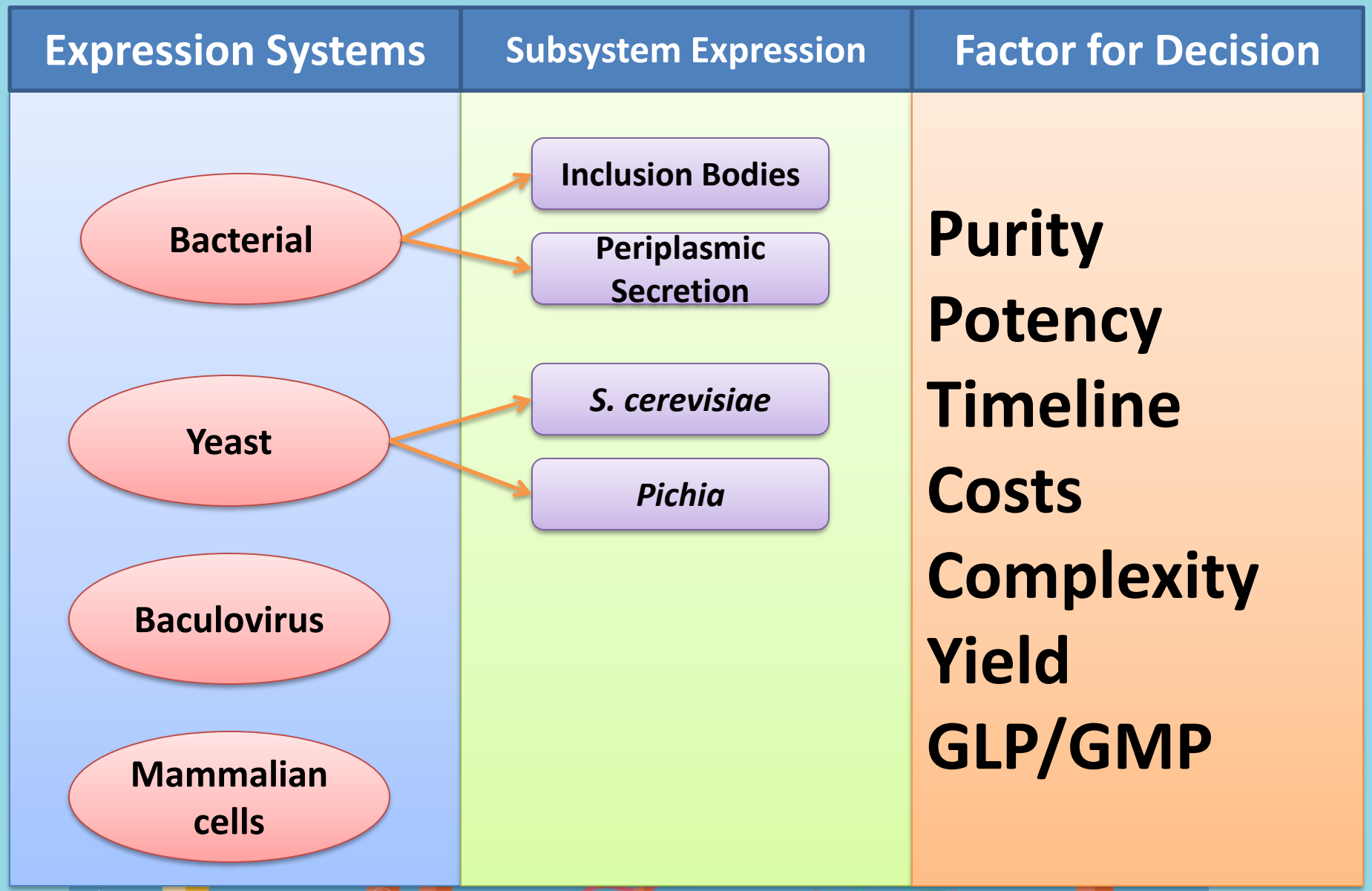
Host & Expression Systems

- Prokaryotes
  - E. coli
- Eukaryotes
  - Yeast
    - Pichea spp.
    - Saccharomyces
  - Insect Cells
  - Chinese Hamster Ovary cell
  - Baby Hamster Kidney cell
  - Human cell lines



Characteristics	E. coli	Yeast	Insect cells	Mammalian cells
Cell Growth	Rapid (30 Min)	Rapid (90 Min)	Slow (18-24 H)	Slow (24 H)
Complexity of Growth Medium	Minimum	Minimum	Complex	Complex
Cost of Growth Medium	Low	Low	High	High
Expression Level	High	Low - High	Low - High	Low - Moderate
Extracellular Expression	Secretion to Periplasm	Secretion to Medium	Secretion to Medium	Secretion to Medium
Protein Folding	Refolding Usually Required	Refolding May Be Required	Proper Folding	Proper Folding
N-linked Glycosylation	None	High Mannose	Simple, No Sialic Acid	Complex
O-linked Glycosylation	No	Yes	Yes	Yes
Phosphorylation	No	Yes	Yes	Yes
Acetylation	No	Yes	Yes	Yes
Acylation	No	Yes	Yes	Yes
gamma-Carboxylation	No	No	No	Yes
Yield (mg) (per liter culture)	50-500	10-200	10-200	0.1-100
Success Rate (%) (soluble or functional)	40-60	50-70	50-70	80-95
Project Cost	Low	Low	Middle	High
Recommended Use	Antigen protein, Protein standards, Functional proteins	Proteins glycosylation, Vaccine, Secreted form, Alternative to insect cell system	Proteins glycosylation, Assay standards, Secreted form, Alternative to yeast system	Functional study, PTM study, Assay standards, Characterization
Advantage	Simple, robust, lowest cost, highest yield	Simple, low cost, good for certain proteins	Relatively higher yield, better PTM	Natural protein configuration, best PTM
Disadvantage	Least PTM[a]	Longer time, less PTM	Longer time, higher cost	Highest cost, lower yield

a PTM = Post-Translational Modification such as glycosylation. *(Top)*


Expression Systems
Subsystem Expression
Factor for Decision
Bacterial
Yeast
Baculovirus
Mammalian cells
Inclusion Bodies
Periplasmic Secretion
S. cerevisiae
Pichia
Purity
Potency
Timeline
Costs
Complexity
Yield
GLP/GMP

Comparison of some key considerations in choosing host cells for recombinant protein expression in pharmaceutical scale

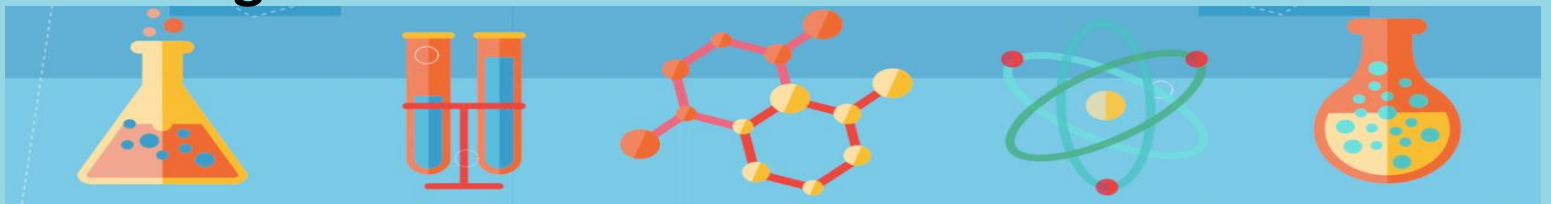
	Prokaryote	Eukaryote	
Consideration	*E. coli*	Yeast	Mammalian Cells (CHO, BHK)
DNA size and characteristics	4.6 Mbp, circular DNA	12.1 Mbp, chromosomal DNA	2000–3000 Mbp chromosomal DNA
Post-translational modification	None	Capable; but different from humans	Capable; similar or identical to humans
growth rate (cycles per hour)	3.33/h	0.25/h	0.02/h[a]
Cultivation method	Fermentation	Fermentation	Fermentation (suspension cells) Roller bottle (adherence cells)
Cost	Less expensive	Intermediate	>$1 million/kg

[a]Based on estimate of antibody producing hybridoma cells.

From Biopharmaceutical Technologies and Process es in Drug Development, 2004

Start up

1. Clone selections
2. Master cell Bank
3. Working Cell bank
4. Cell Expansion (Seed Train)
5. Production Fermentation Scale
6. Harvest
7. Centrifugation / Filtration
8. Column Chromatography (s)
9. Concentration
10. Sterile Filtration
11. Drug Substances
12. Formulations / Filling
13. Drug Products



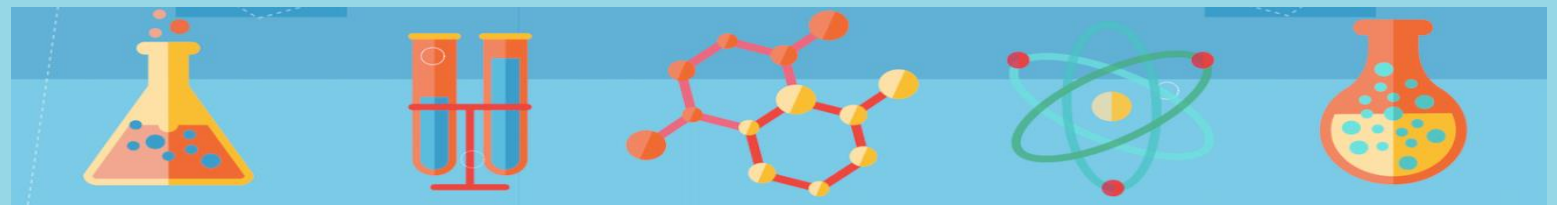
R&D

Clone selection

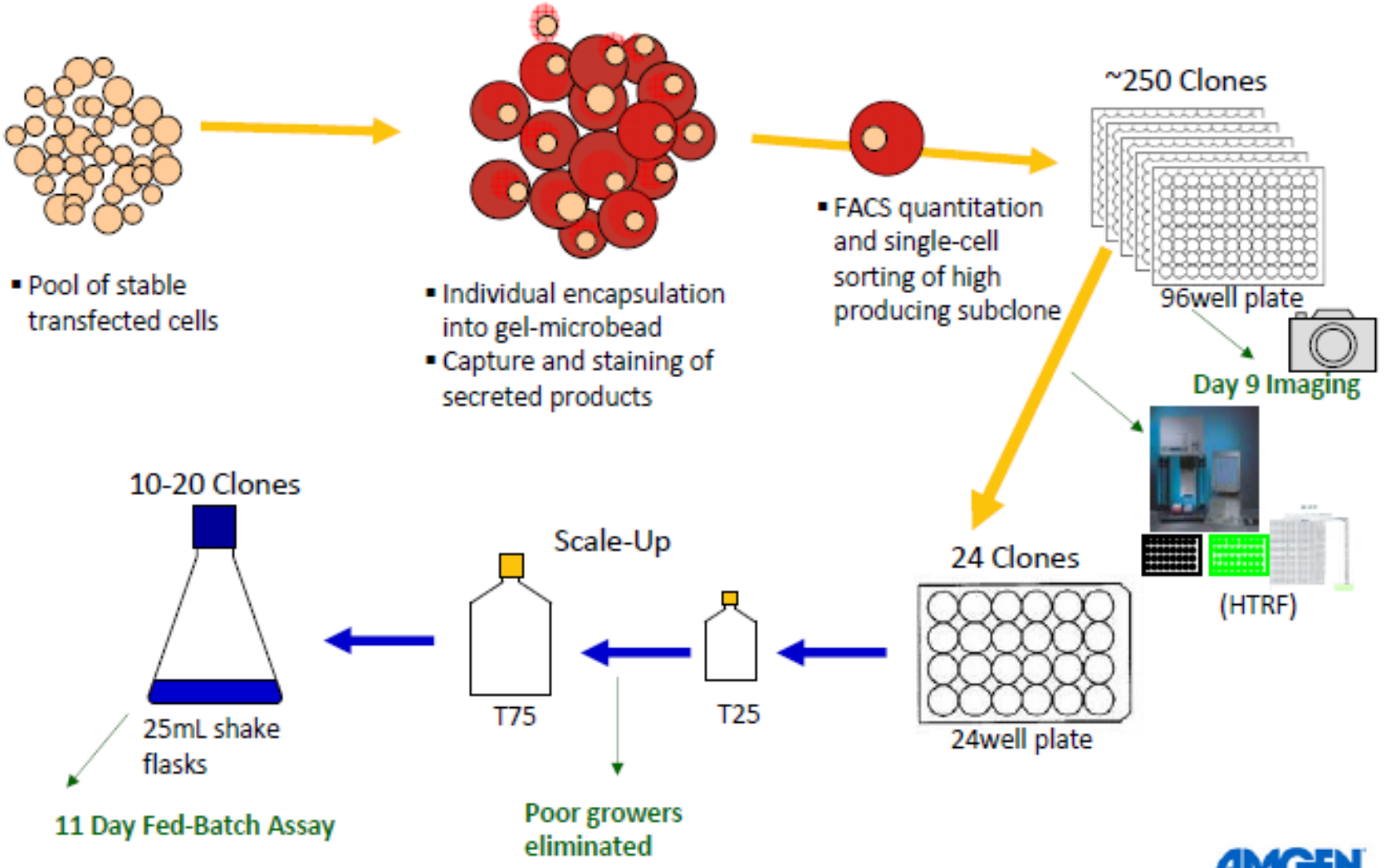
Master Cell Bank

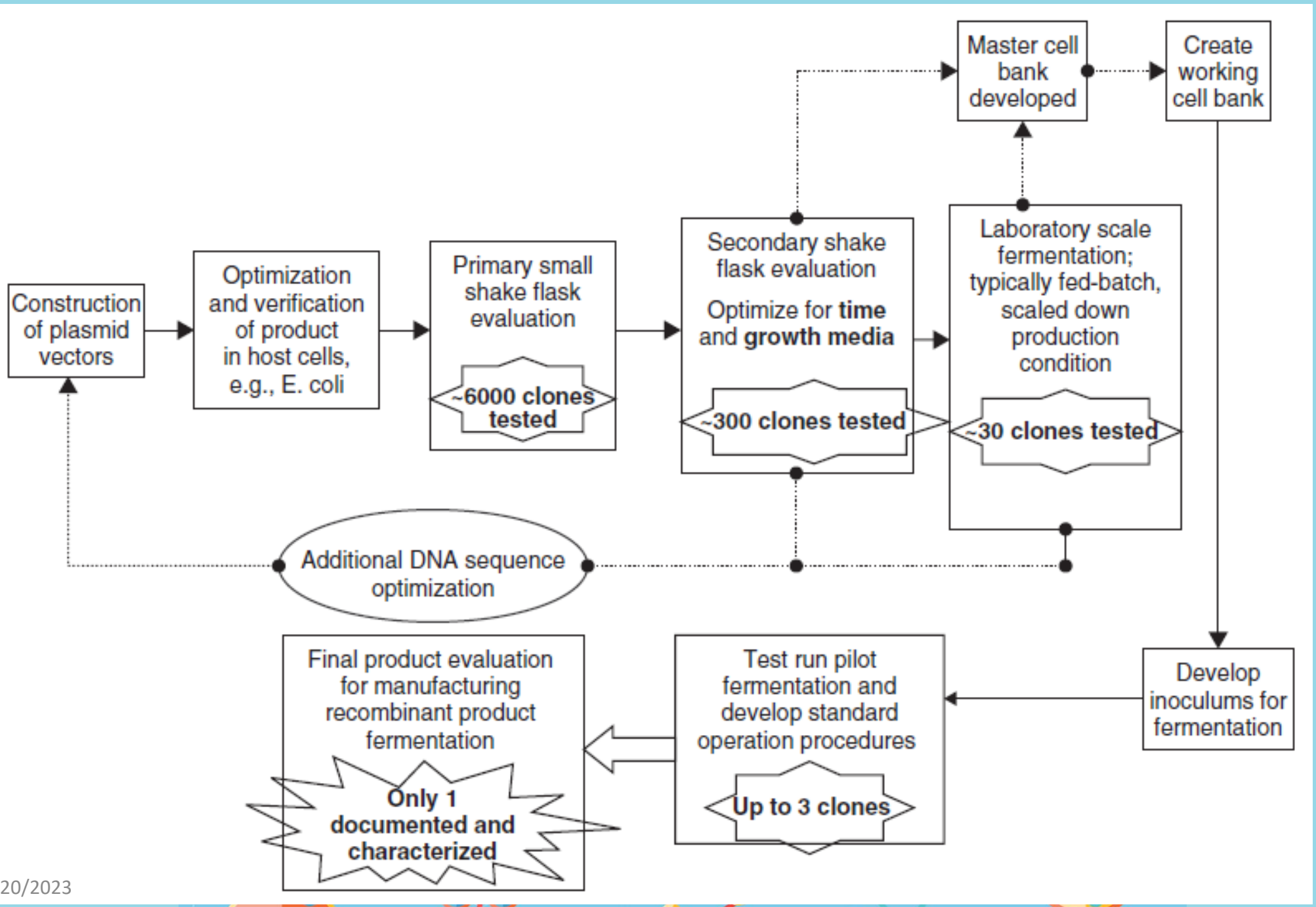
Working Cell Bank

PRODUCTION CELL LINE

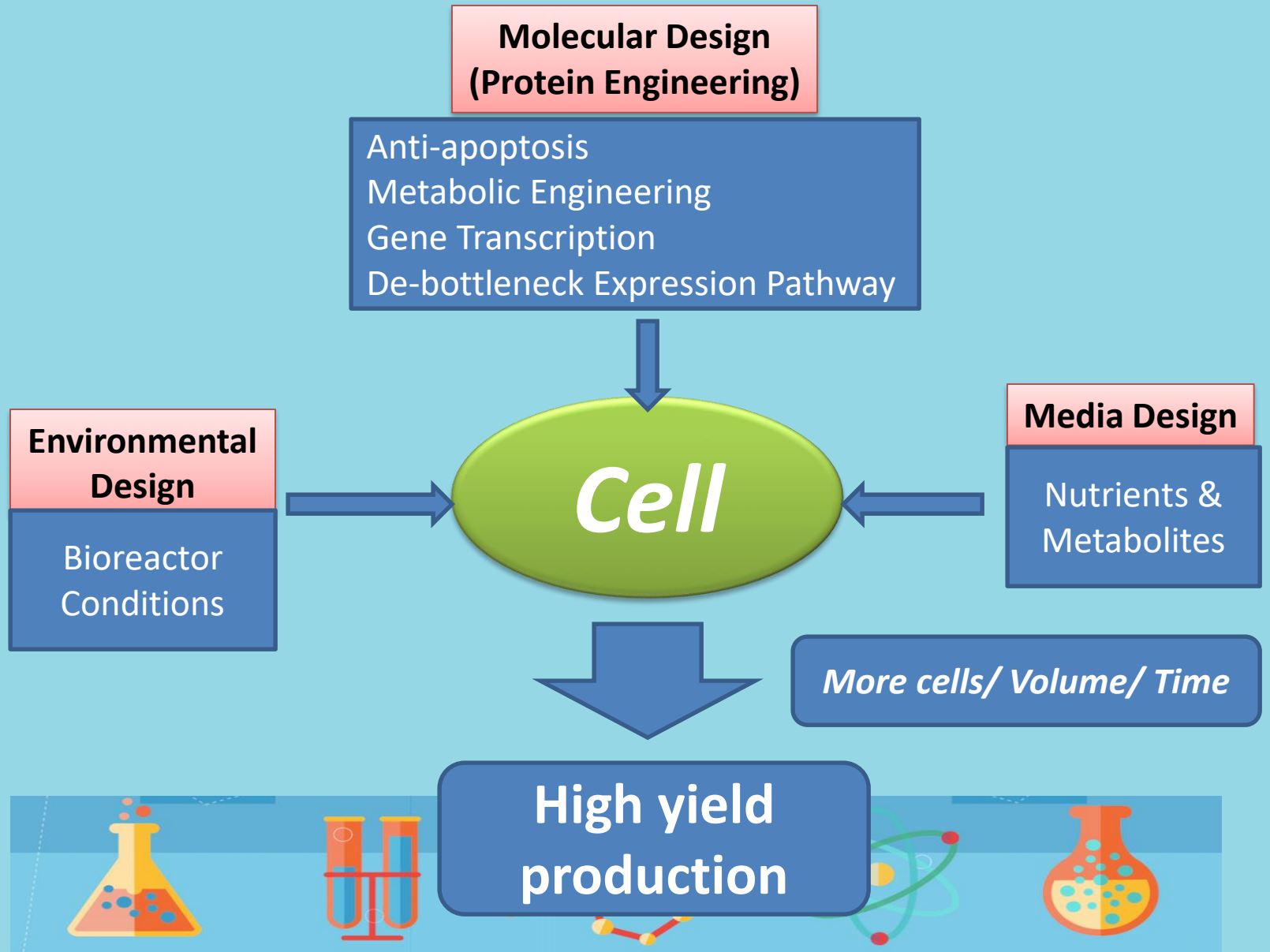


Isolating the best cell clones is challenging and time consuming




Master cell bank developed
Create working cell bank
Construction of plasmid vectors
Optimization and verification of product in host cells, e.g., E. coli
Primary small shake flask evaluation
~6000 clones tested
Secondary shake flask evaluation
Optimize for time and growth media
~300 clones tested
Laboratory scale fermentation; typically fed-batch, scaled down production condition
~30 clones tested
Additional DNA sequence optimization
Final product evaluation for manufacturing recombinant product fermentation
Only 1 documented and characterized
Test run pilot fermentation and develop standard operation procedures
Up to 3 clones
Develop inoculums for fermentation

For Great Productivity, Expression System Designs are IMPORTANT



Holistic Design and Optimization

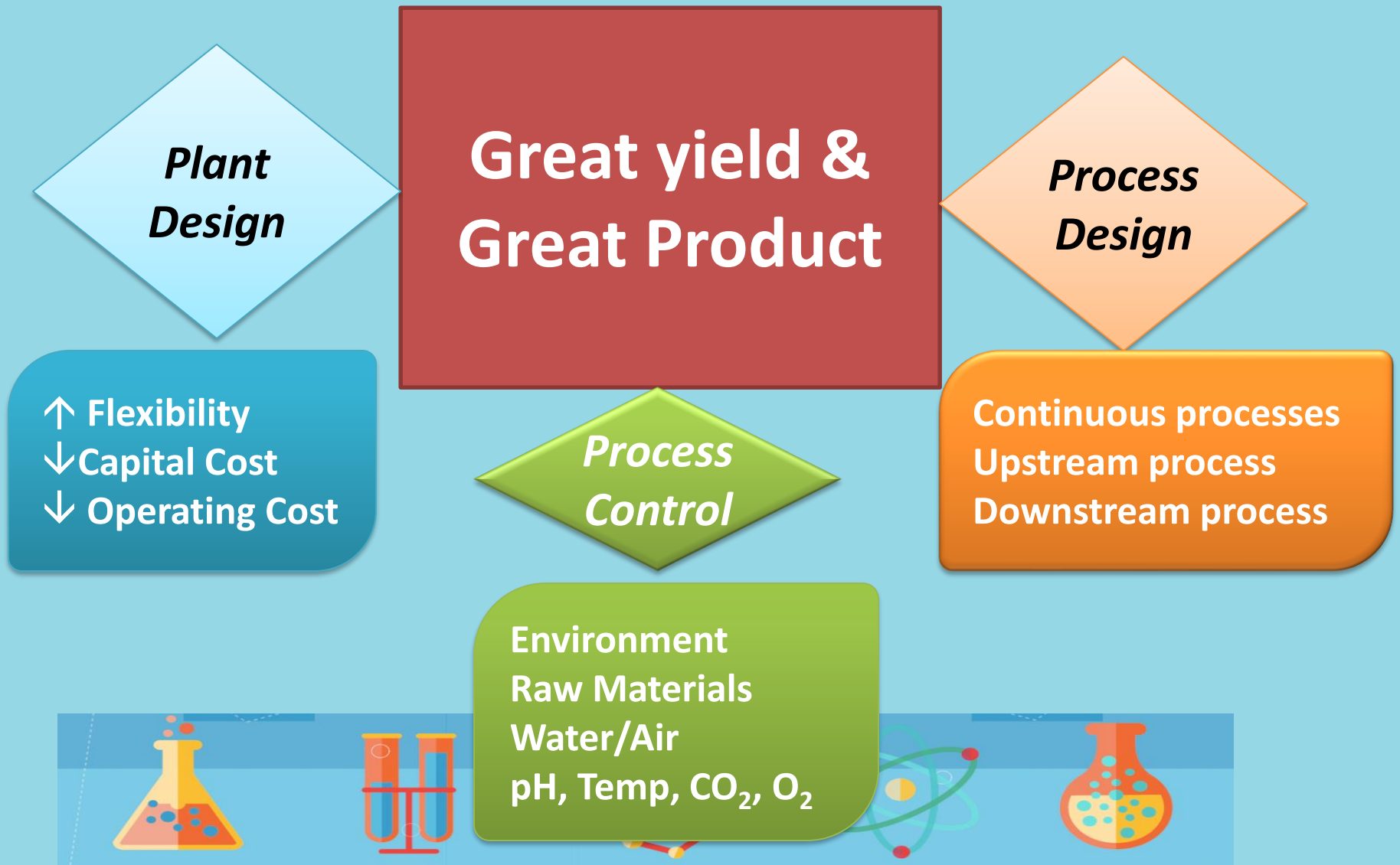


Table I: Summary of the regulatory testing expectations for each type of cell bank. MCB=master cell bank, EOP=end-of-production cell bank, WCB=working cell bank.

Assay	Seed cells	Non-production	MCB/EOP	WCB
Sterility	+	+	+	+
Mycoplasma	+	+	+	+
Adventitious viruses	-	-	+	+
Bovine/porcine viruses	-	-	+	-
Antibody production	-	-	+	-
Species-specific viruses	-	-	+	-
Retroviruses	-	-	+	-

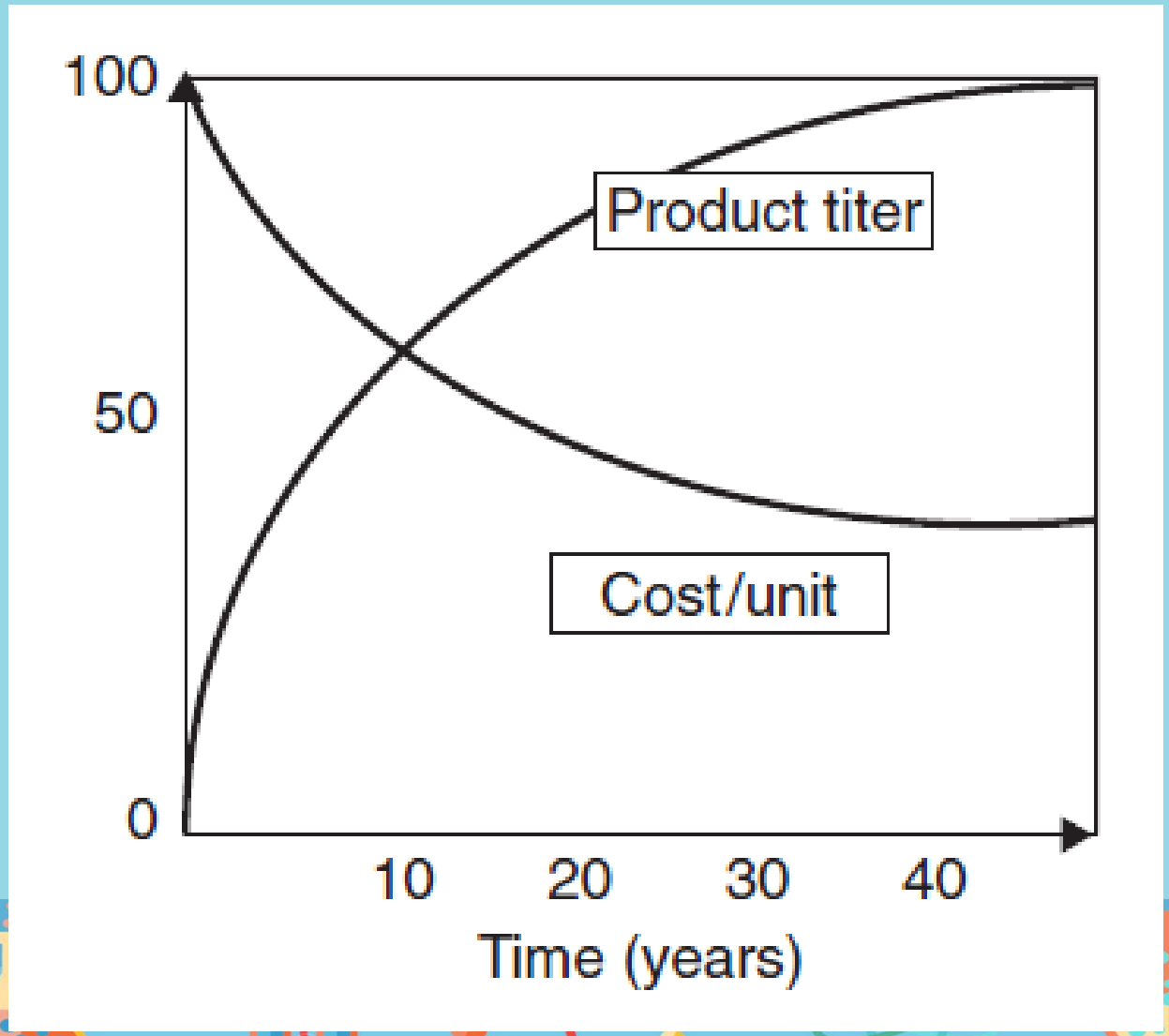
August 2015 www.biopharminternational.com BioPharm International

Lana Mogilyanskiy, Heather Byer, and Weihong Wang

Table II: Summary of standard industry timelines for various cell-banking tests. qPCR=real-time polymerase chain reaction, StandardTAT=standard turnaround time.

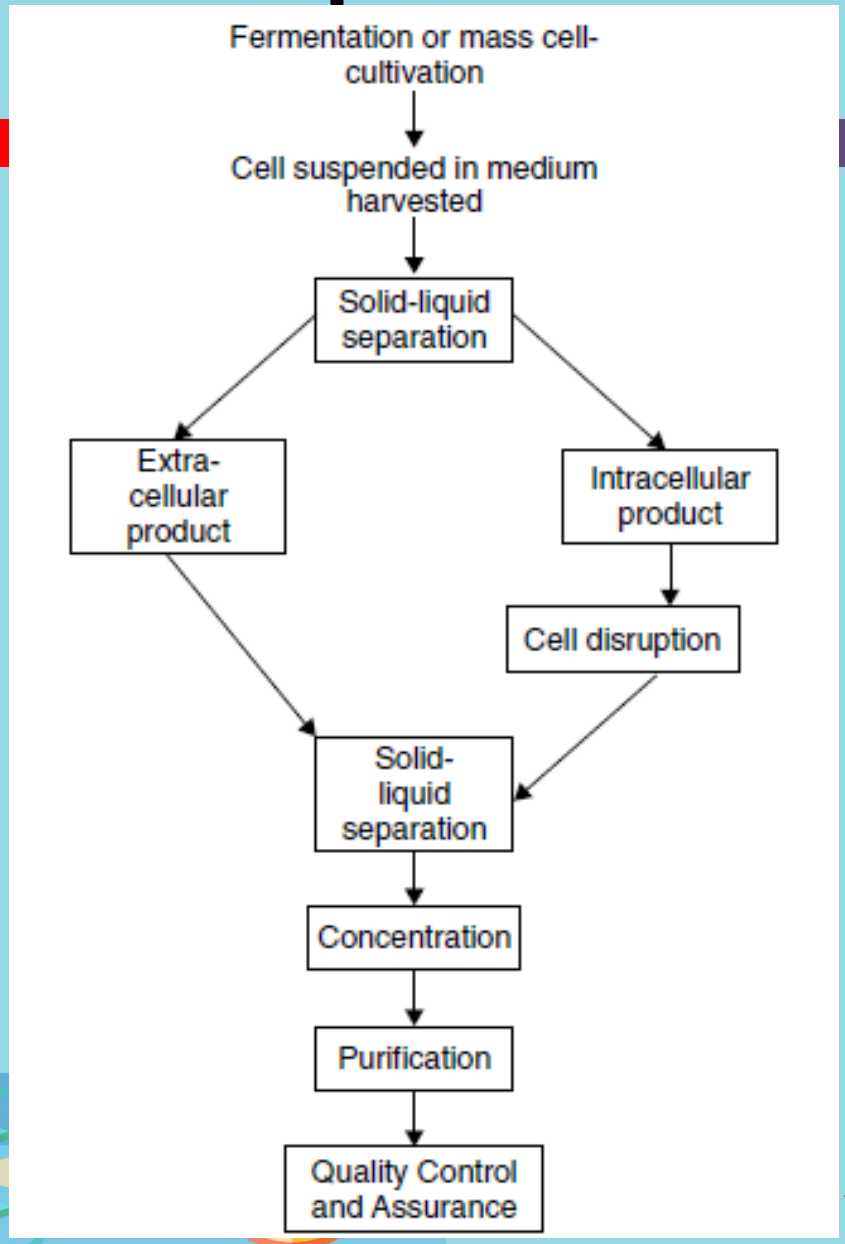
Assay	StandardTAT
Sterility (bacteriostasis/fungistasis)	17 days* 17 days*
Mycoplasma (mycoplasmastasis)	25 days* 20 days*
Adventitious viruses	*In vitro:* 6 weeks *In vivo:* 7 weeks
Bovine/porcine viruses	5 weeks
Antibody production	7 weeks
Species-specific viruses (qPCR)	2 weeks
Retroviruses	5 weeks

Relationship between Unit cost and increased product yield/titre



General Bioprocessing of recombinant protein

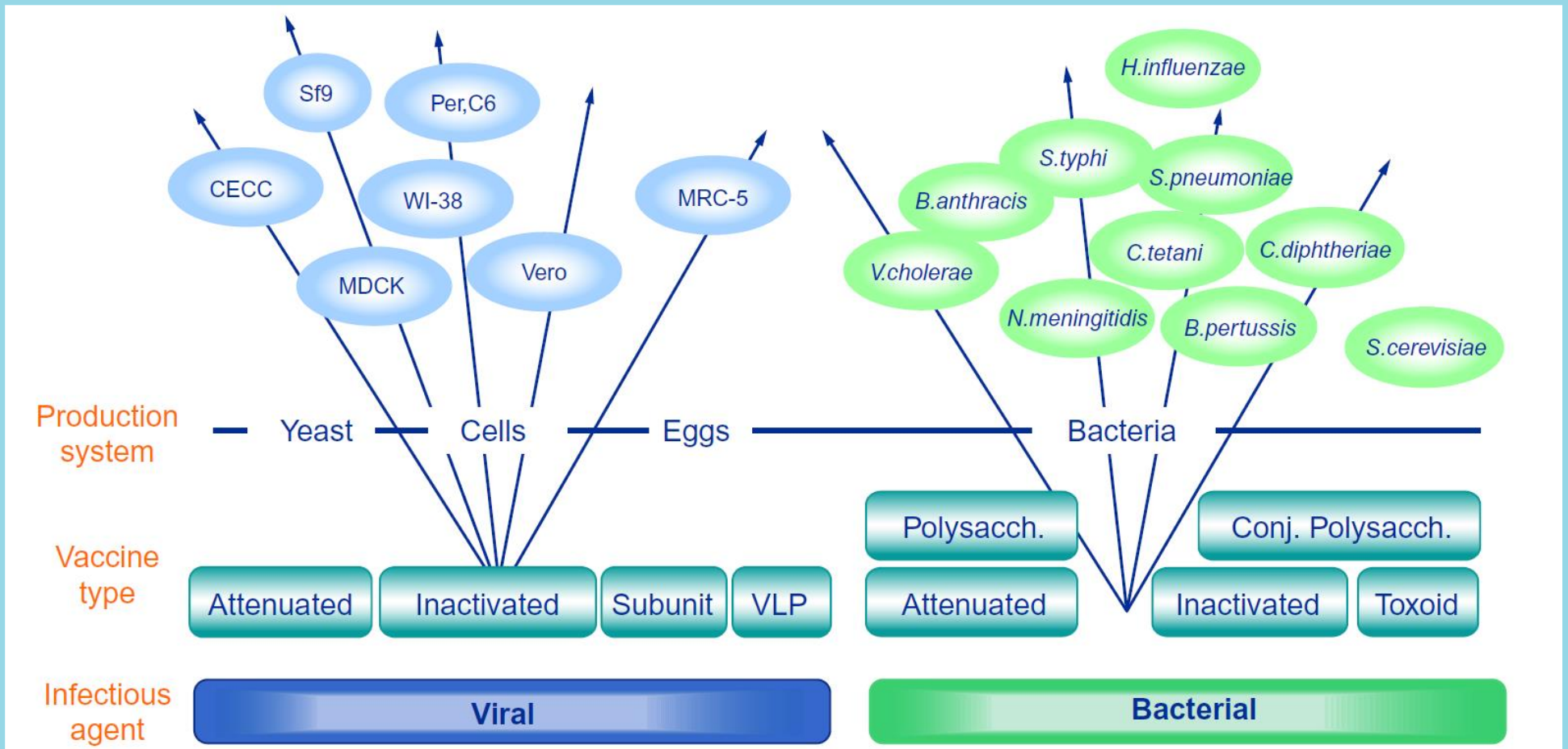
- **Schematic presentation of process stream to purify a recombinant protein, starting from cell suspension harvested from fermenter or cell-cultivation vessels.**



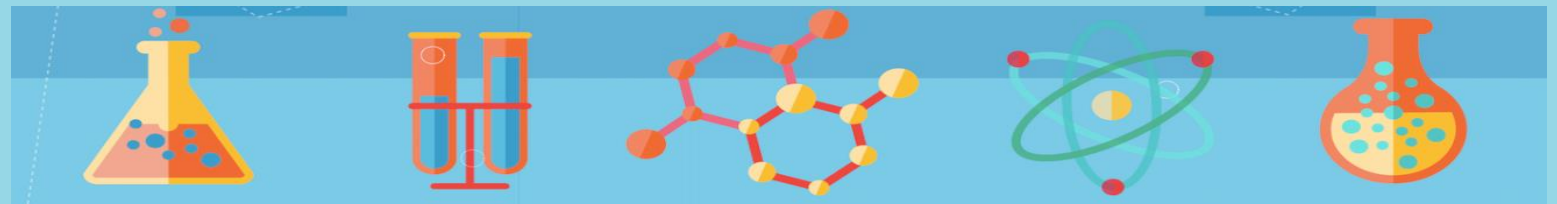
Rule of Thumbs (Heuristics) in Purification Process

- Remove the most plentiful impurities first
- Remove the easiest-to-remove impurities first
- Make the most difficult and expensive separation last
- Select processes that make use of the greatest differences in the properties of the product and its impurities
- Select and sequence process that exploit different separation driving forces

Figure 23.7 (a, b) Piping complexity for biopharmaceutical production.



Production systems and cell substrates used in vaccine manufacturing.



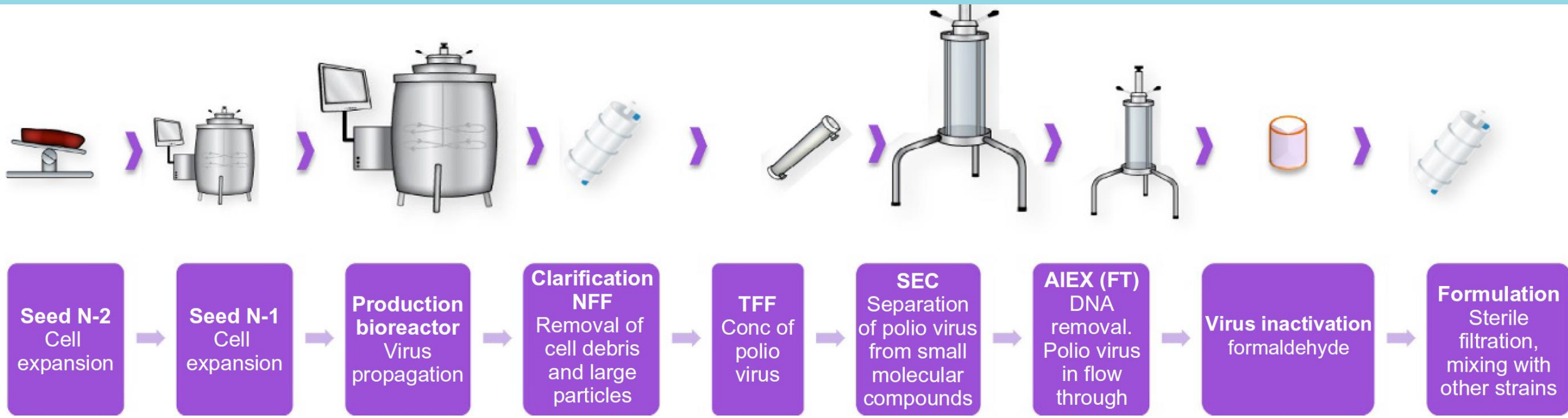
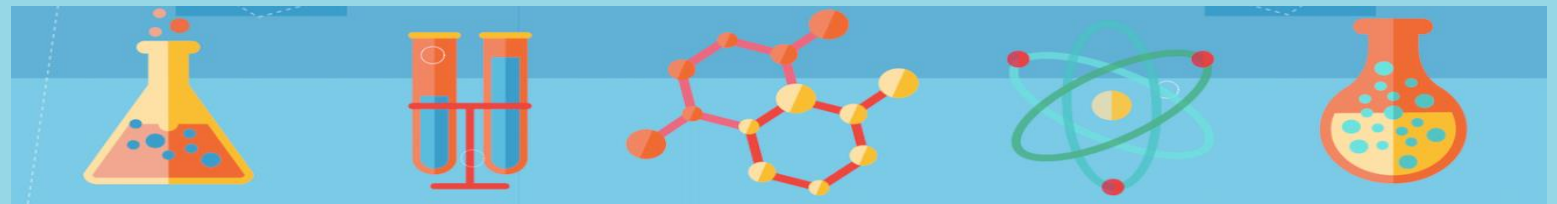
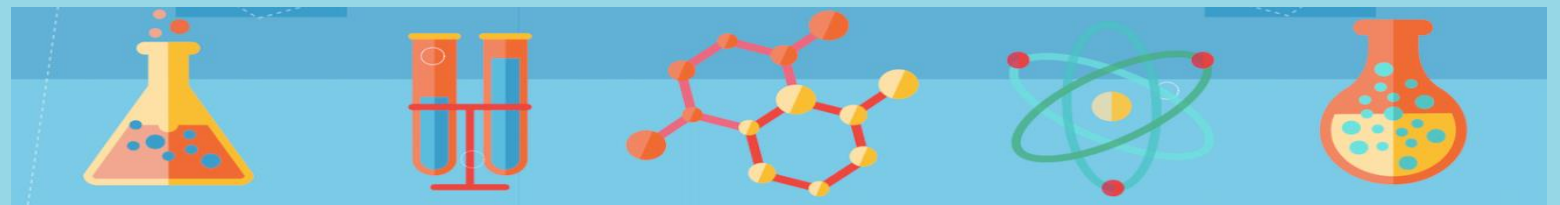


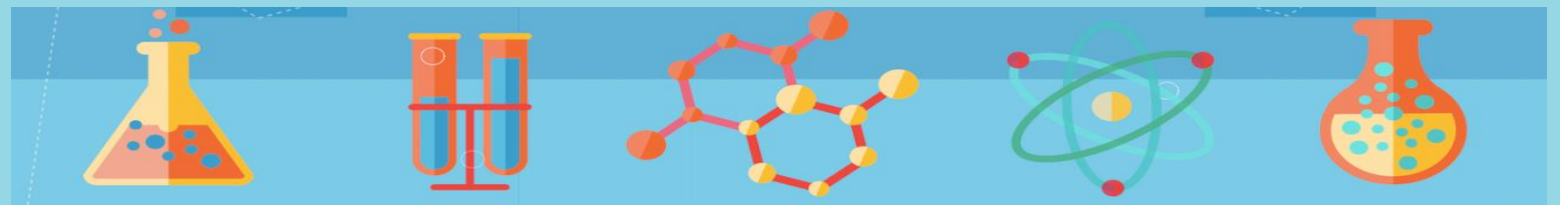
FIG. 43.4 Example of a typical viral vaccine production process.



UPSTREAM PROCESSING

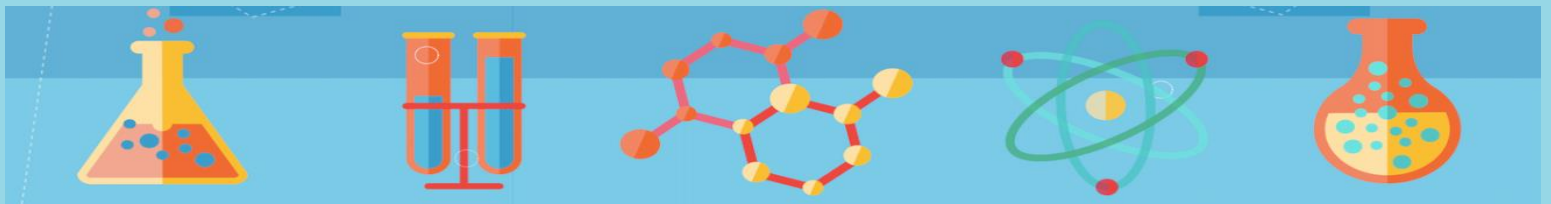


FERMENTATION



Fermentors & Bioreactors

- Larger scale, sustained growth requires bioreactors & fermenters
- Fermenters have been used for centuries – primarily for brewing alcohol and making vinegar
- Modern technology and chemical engineering principles continue to improve fermenter design
- Fermenter – strictly used for anaerobic process



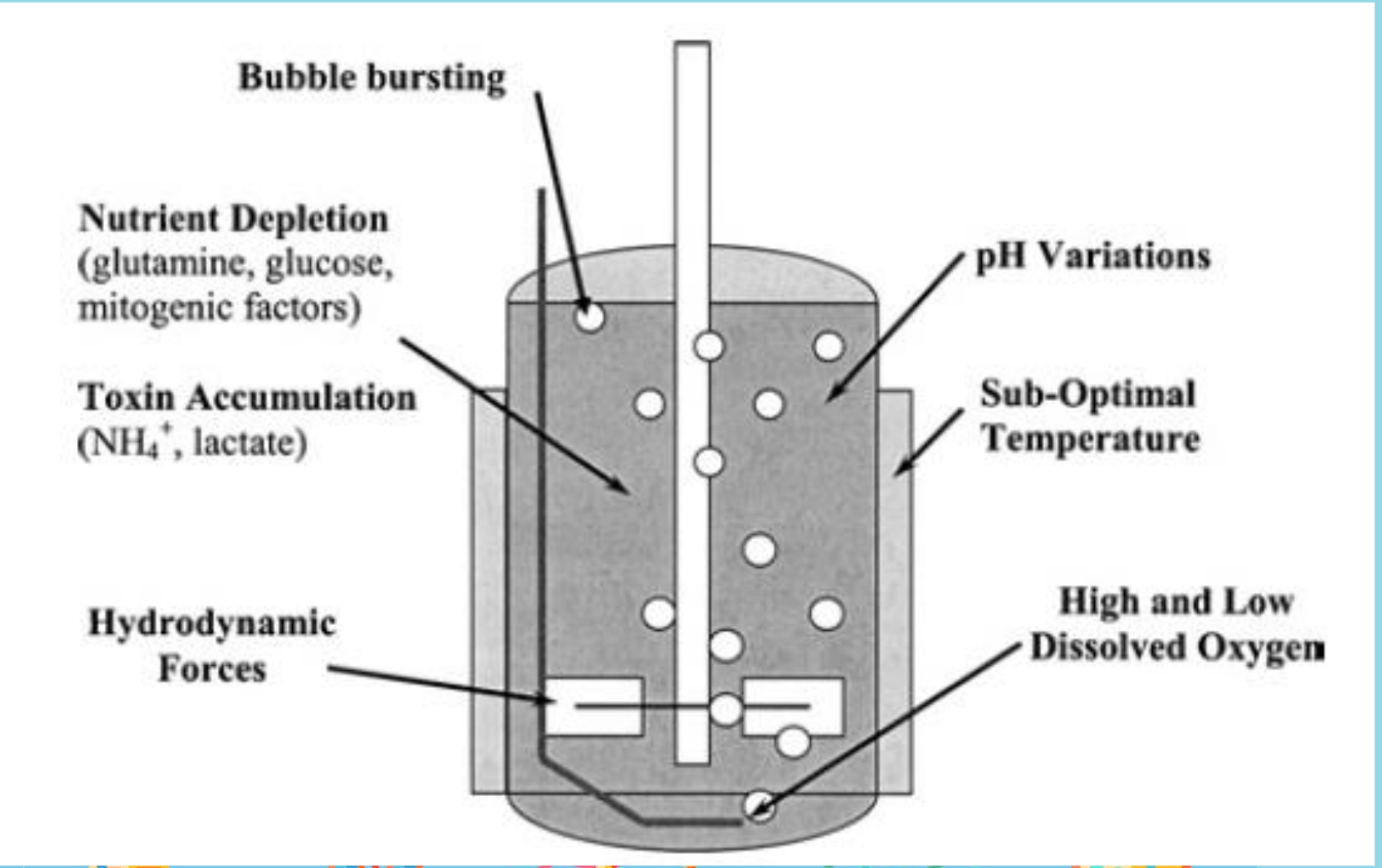
Time Factor in Fermentation

TABLE 4.9. Batch size of cell cultures and estimated time required for fermentation

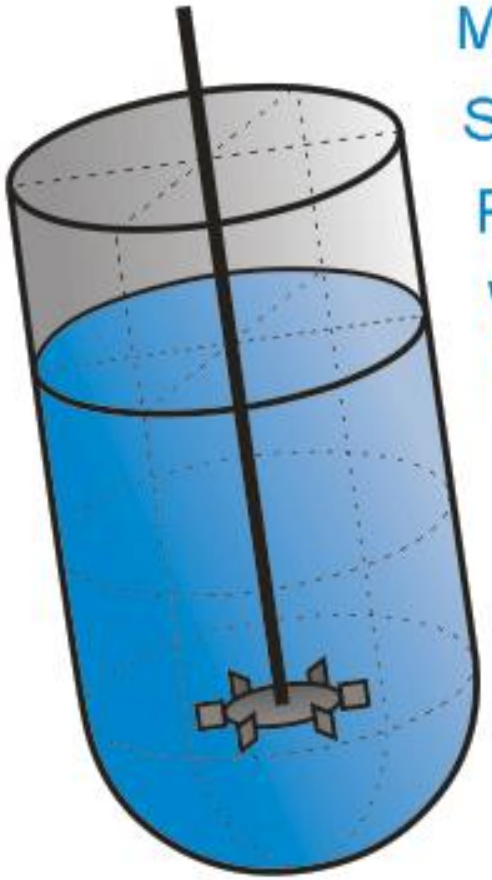
Description	Batch Size (liters)	Time (days)[a]
Laboratory shake flask	0.1	1–2
Bottles or large flask	1–2	2–4
Batch fermenter	50	4–6
Batch fermenter	2500	6–8
Batch fermenter	25,000 to 100,000	10–16

[a]Estimated based on using *E. coli* as host cells for producing recombinant proteins.

Factors that cause cell death in large-scale animal cell culture



Factors to concern in Fermentor design



Hold up

Mixing Time

Stirrer Speed

Power Number

Working Volume

Reynolds number

Maximum Shear Rate

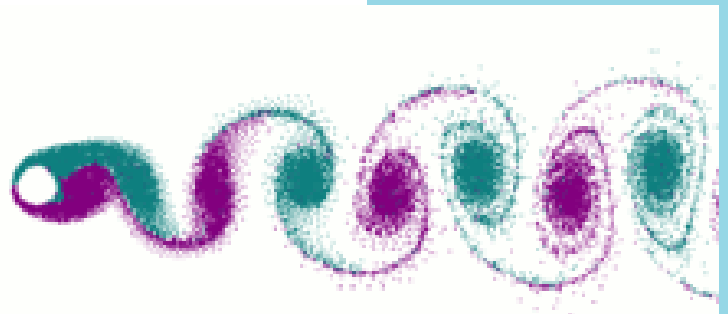
Liquid Dynamic Viscosity

Time Average Shear Rate

Stirrer Power Consumption

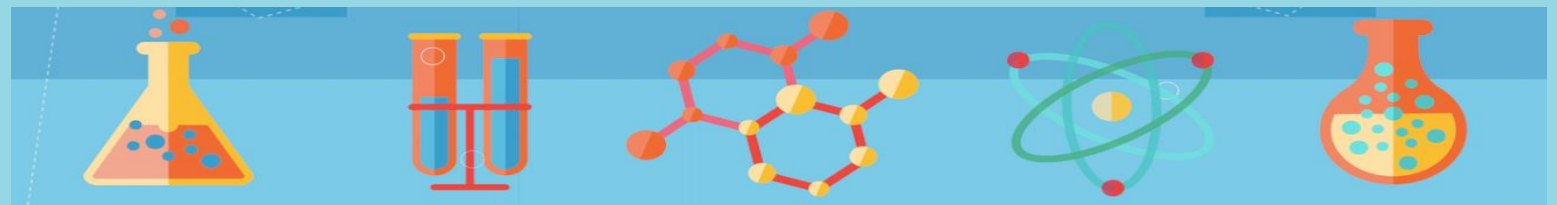
Oxygen Transfer Coefficient

Smallest Turbulent Eddy Length



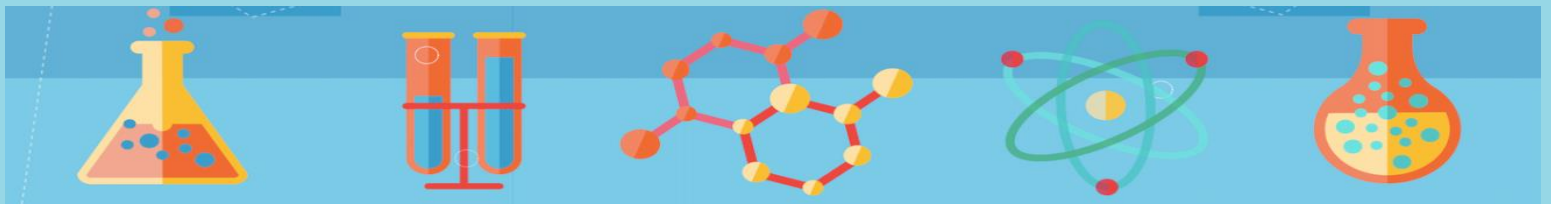
Criteria relevant for selection of cultivation systems for mammalian cells

Characteristics	Criteria
Cells	morphology, shear sensitivity, doubling time, adherent or growth in suspension, process parameters (pH, temp., oxygen, CO2), genetic stability, medium
Product	stability, quantity, production kinetics
Process	automation, scale, operation mode (*batch, fed-batch,* perfusion), cleaning, inoculum
Administrative	regulatory affairs and GMP requirements



Type of Cell Determined suitable Bioreactors

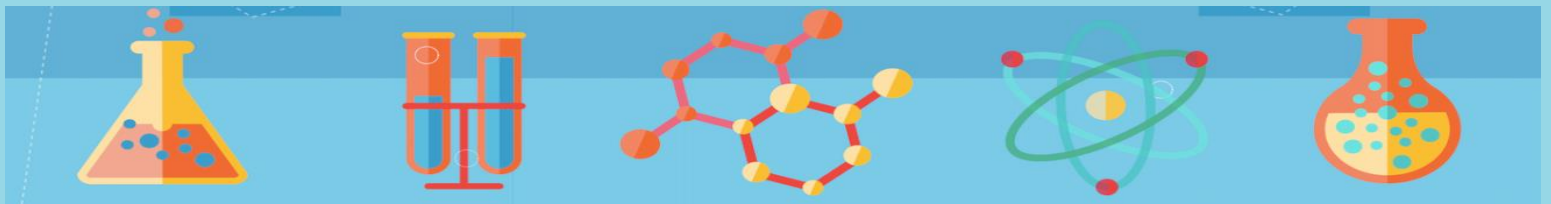
- Anchorage-independent cells - can grow in suspension
  - All Prokaryotes
  - Some Eukaryotes
- Anchorage-dependent cells – adherent cells
  - Need surface to attach



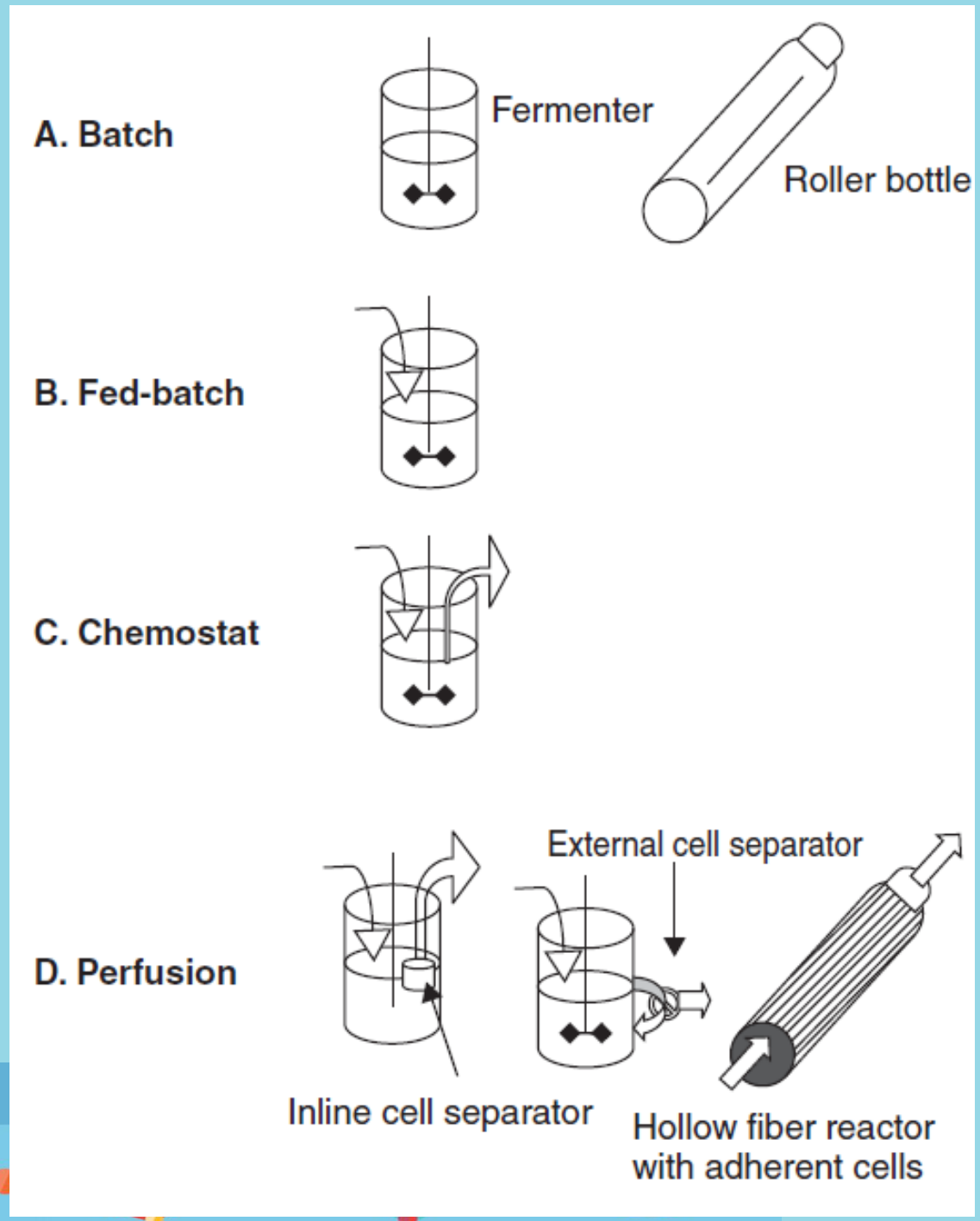
Attached Cell can adapt to grow in Suspension

- Using
  - Solid Microcarrier
  - Macroporous microcarrier

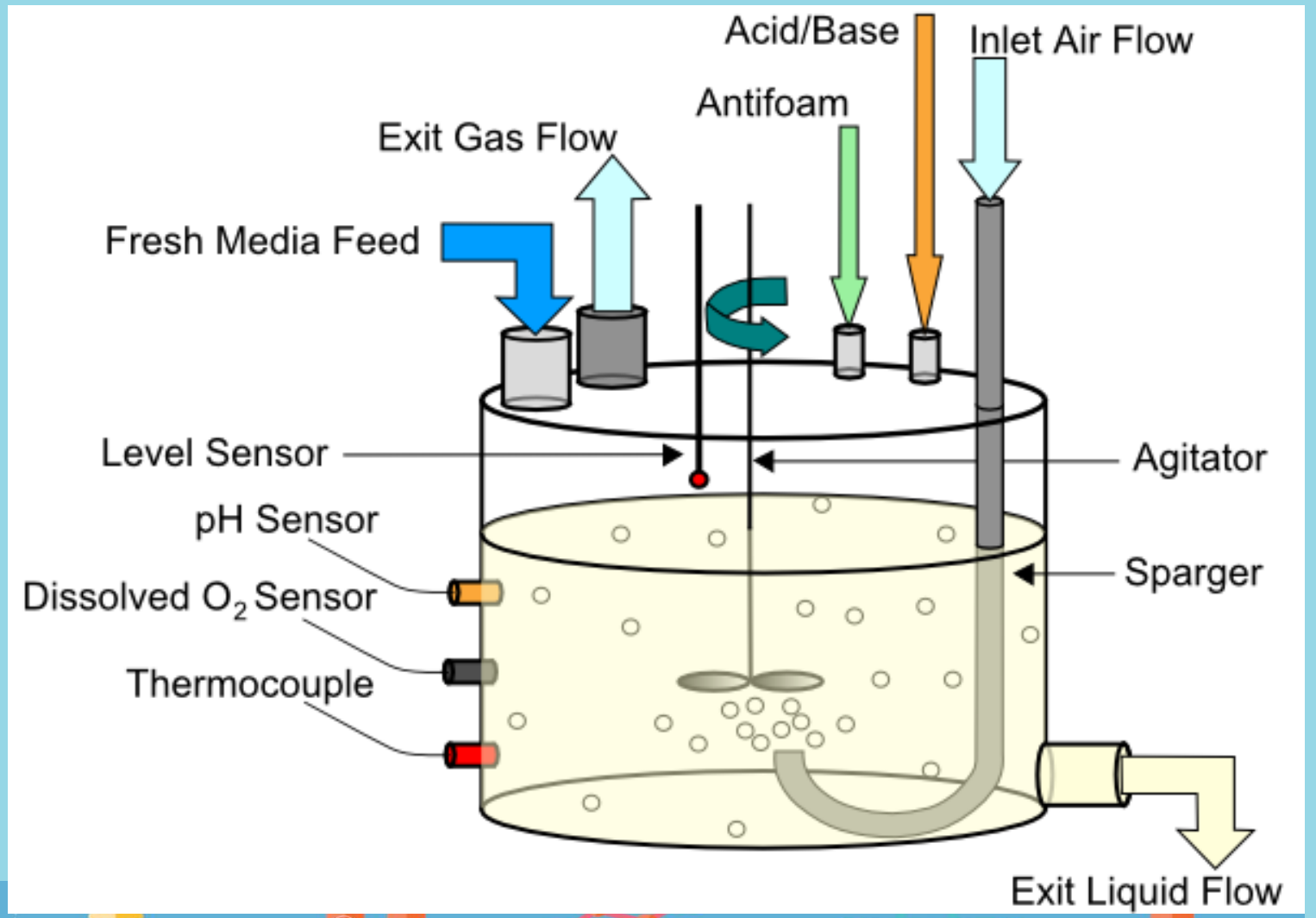
Grow as Fermenter for Suspension cell



Type of Fermentation



Fed-Batch Stirred Tank Bioreactor



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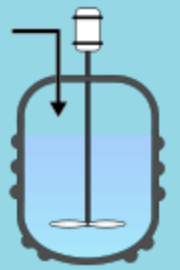
Design of Bioreactors

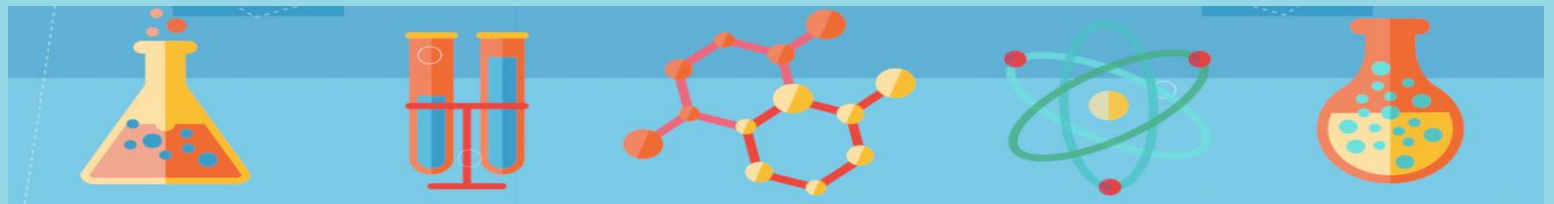


For suspension cell

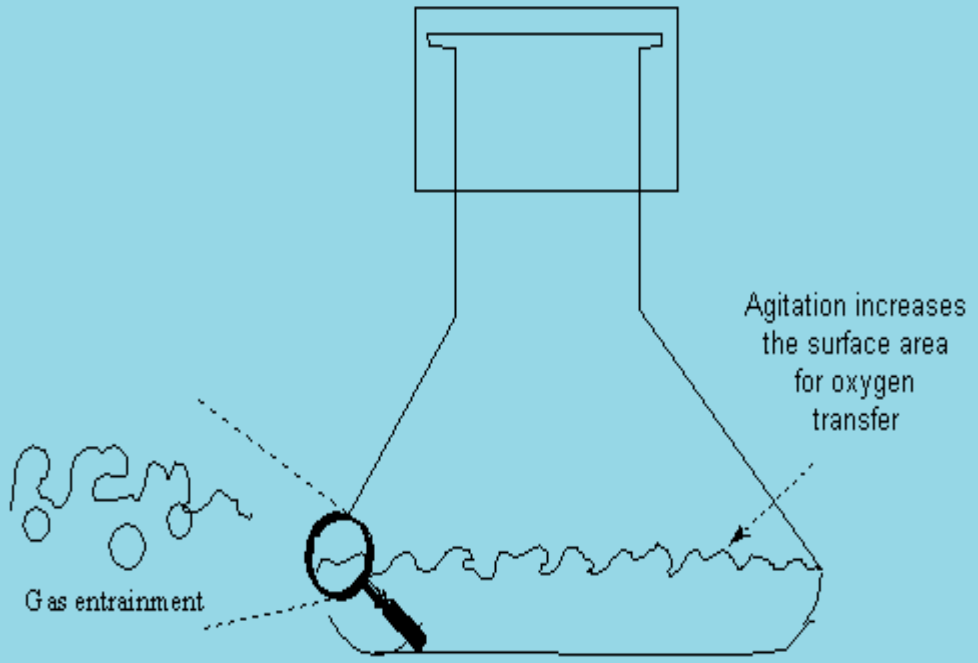
- **Stirred tank bioreactors**
- **Air-lift bioreactor**
- **Bubble-column bioreactors**

For attached cell

- **Rotating wall bioreactor**
- **Packed bed bioreactor**
- **Fluid bed bioreactor**
- **Hollow fiber bioreactor**

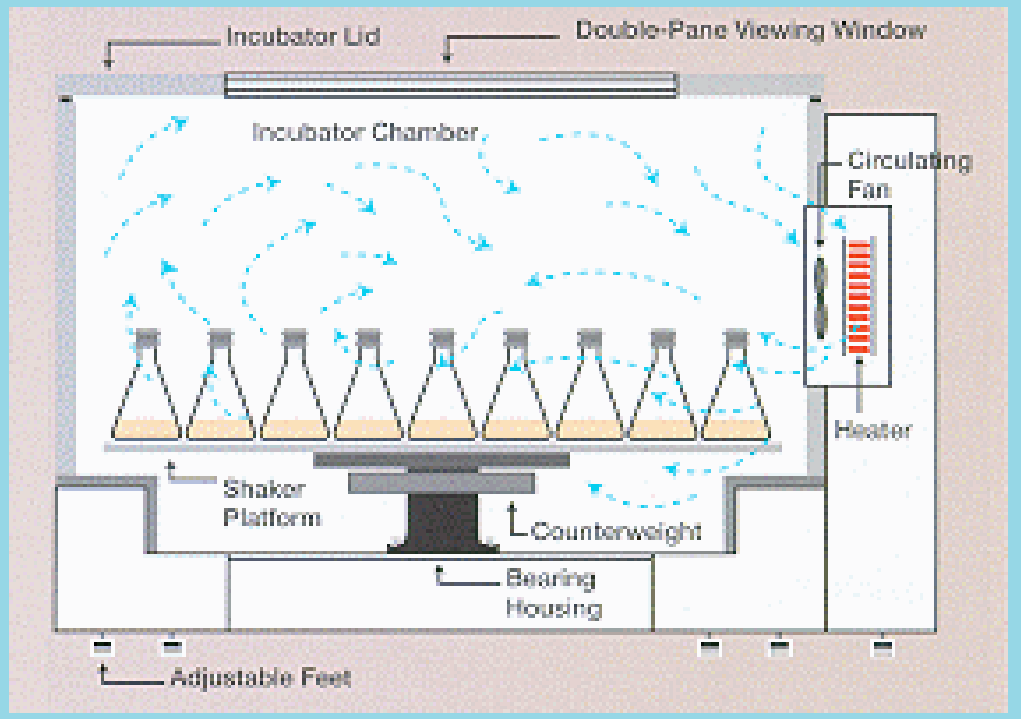


Shake Flask Incubator



Shake Flask Incubator

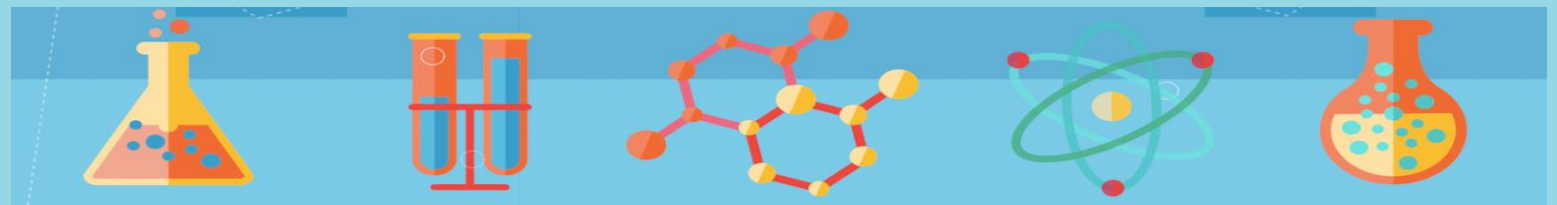
G25 New Brunswick
Floor Model Incubator



Cutaway Model Incubator

Shake Flask Incubators

- Sometimes called environmental chambers
- Heavily insulated, heated with thermoregulation to keep temperature within 0.5 °C of set-pt.
- Rotatable platform to spin up to 500 rpm to facilitate aeration (dissolves N_2 and O_2 needed for growth)
- Designed for small-scale growth



Wave Bioreactor

- For suspension cells
- Up to half their capacity (2-1000 L)
- Rocking motion
  - Good nutrient distribution
  - Off-bottom suspension
  - Increase oxygen transfer without shear damage

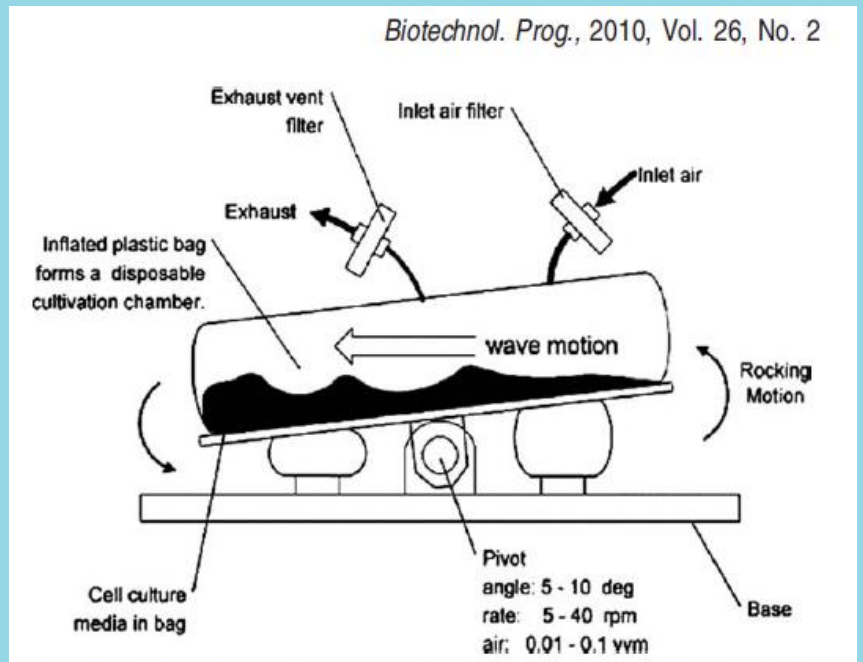
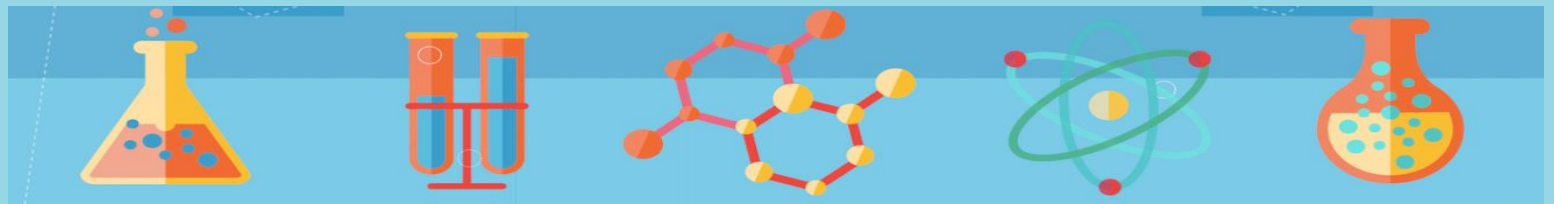
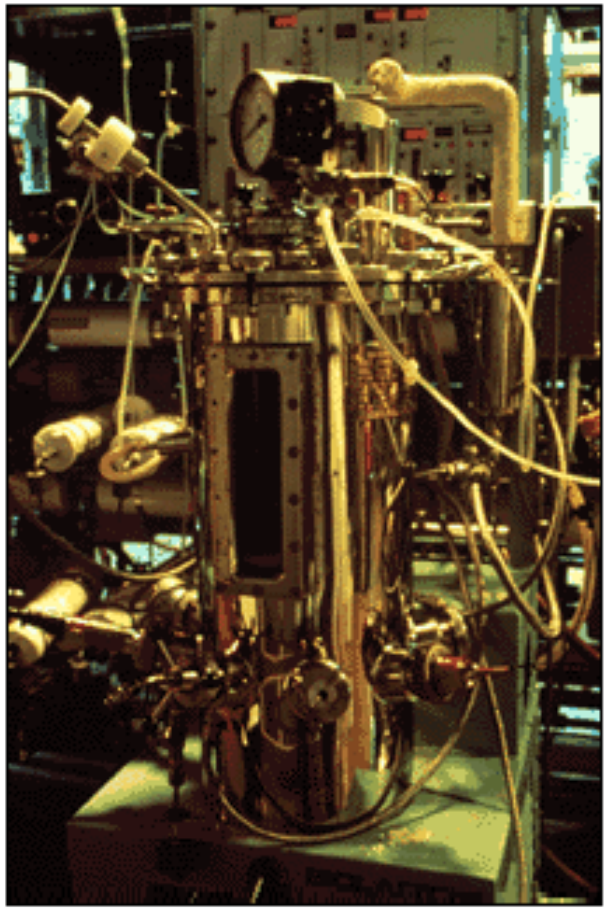


Figure 5. Schematic diagram of the disposable wave bioreactor.[28]

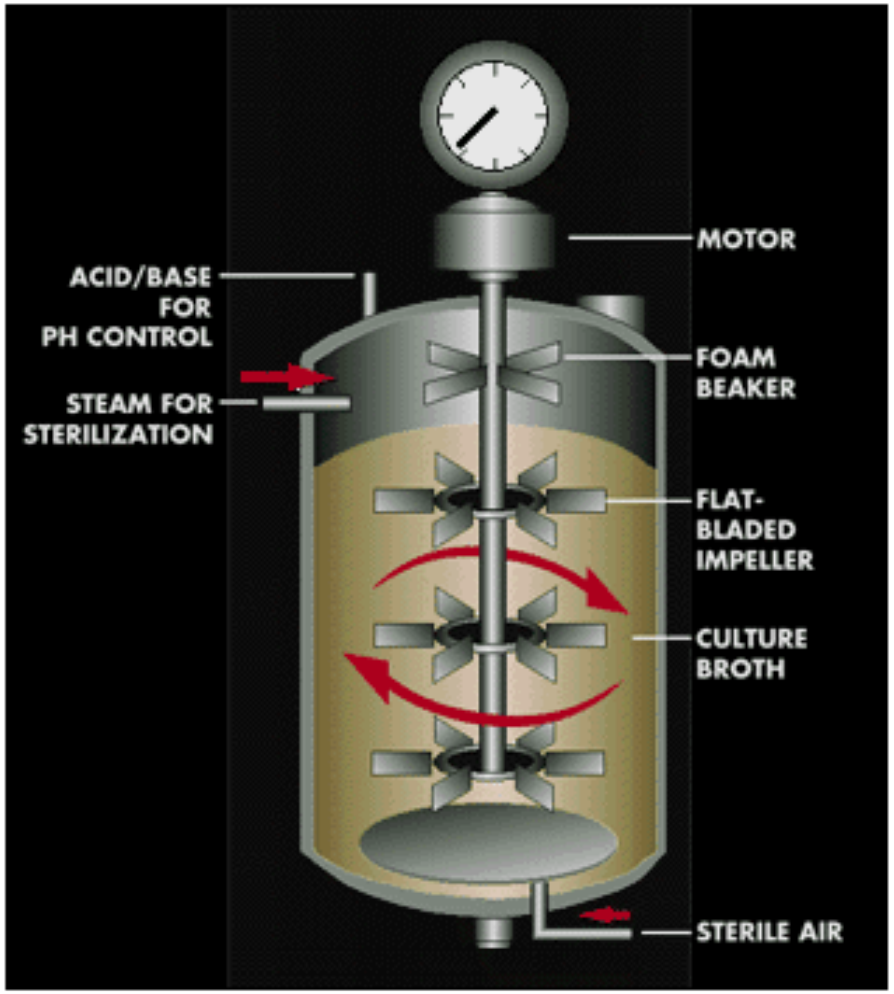



Twave
BIOREACTOR

Stirred Tank Fermenter/Bioreactor

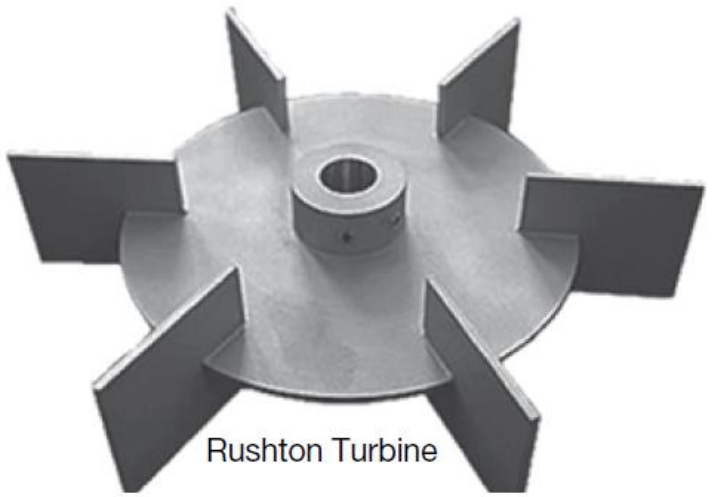


by Genentech, Corporate Communication

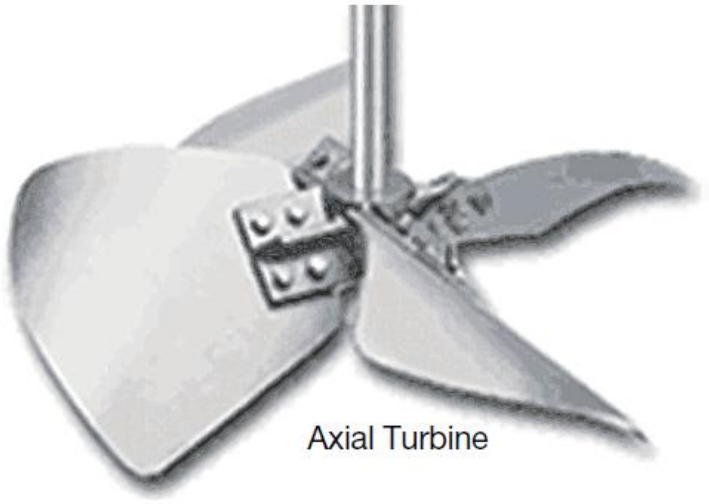


by Genentech, Graphics Department

Some types of Turbine



▲ **Figure 3.** Rushton turbines, which have six flat blades mounted vertically on a disk, were used in early bioreactors.



▲ **Figure 4.** Axial impellers improve mixing in fermenters. They are often combined with radial impellers on a single shaft, which typically has the axial blades above the radial ones.

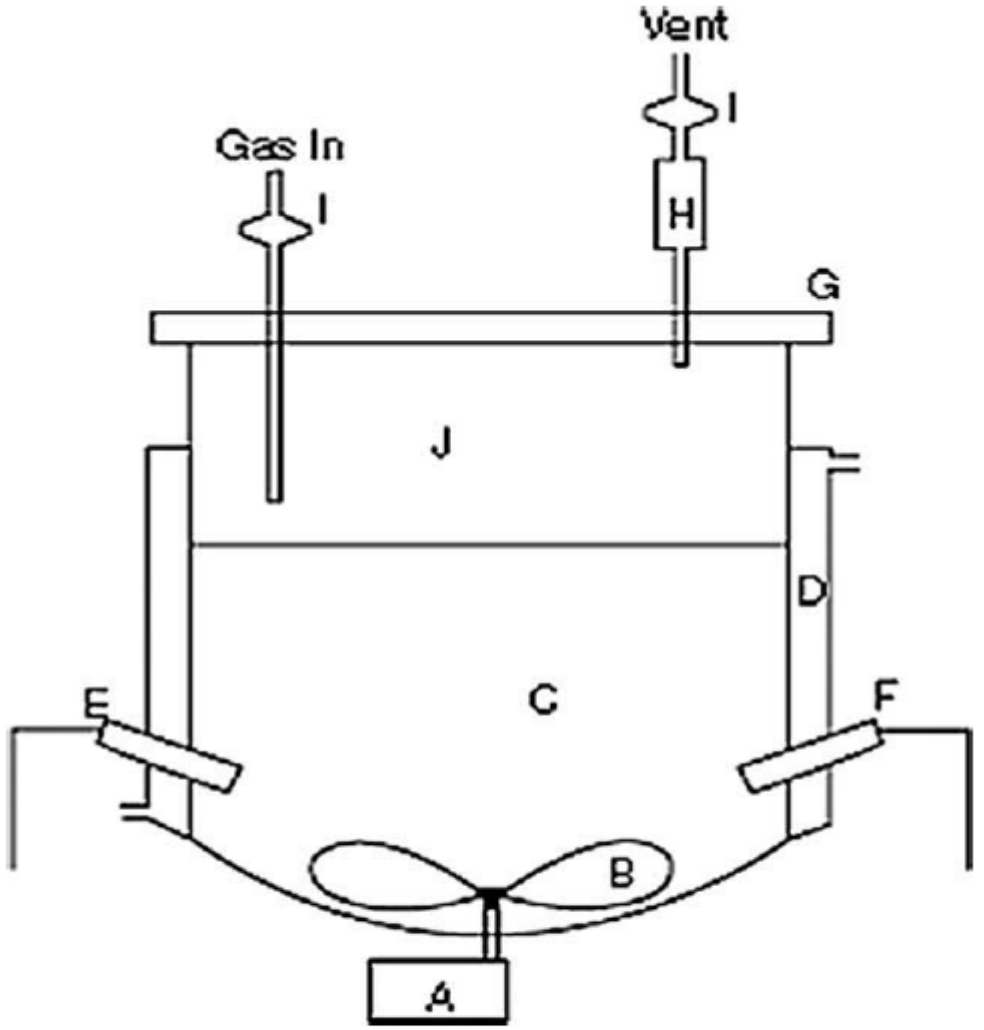
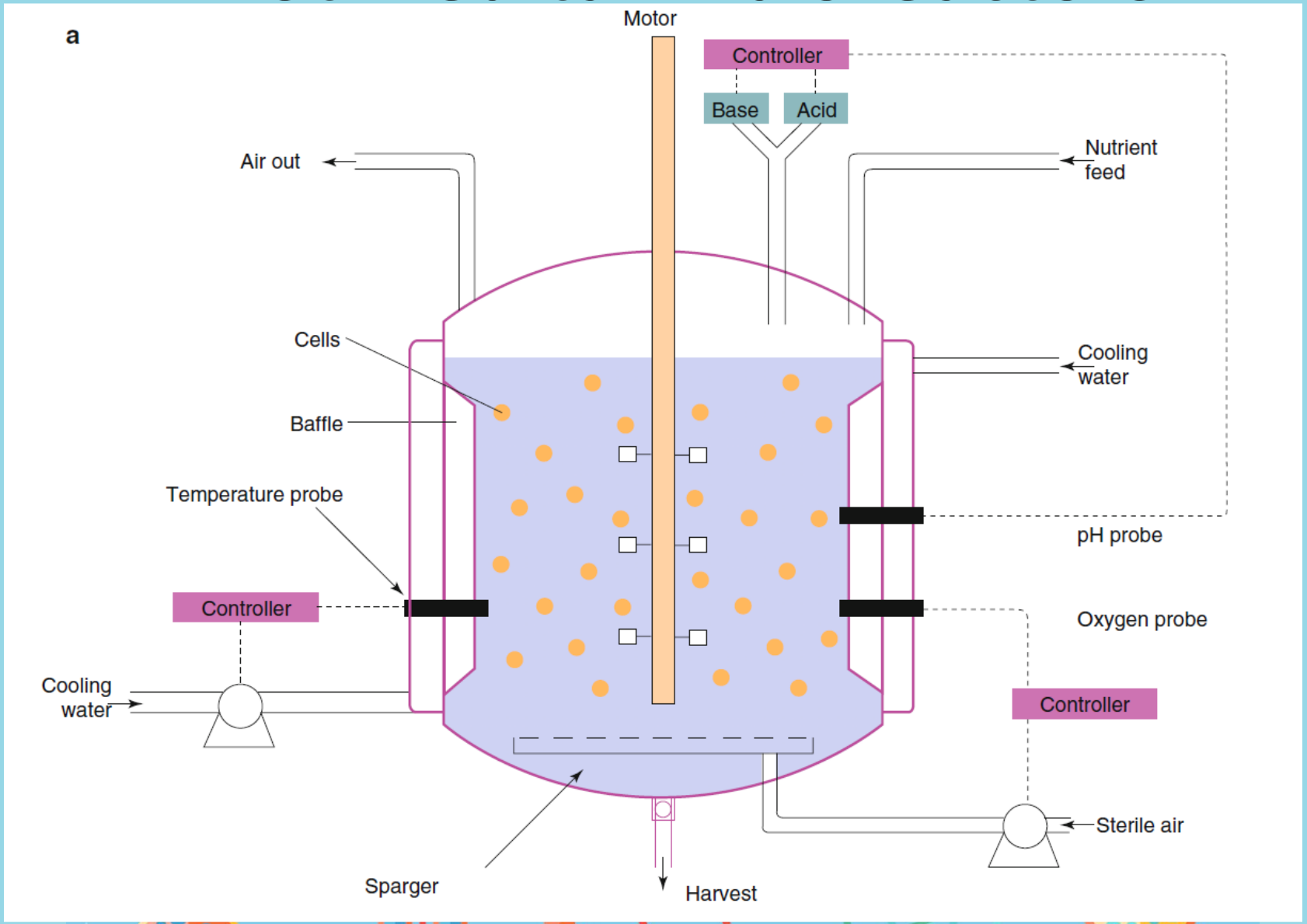


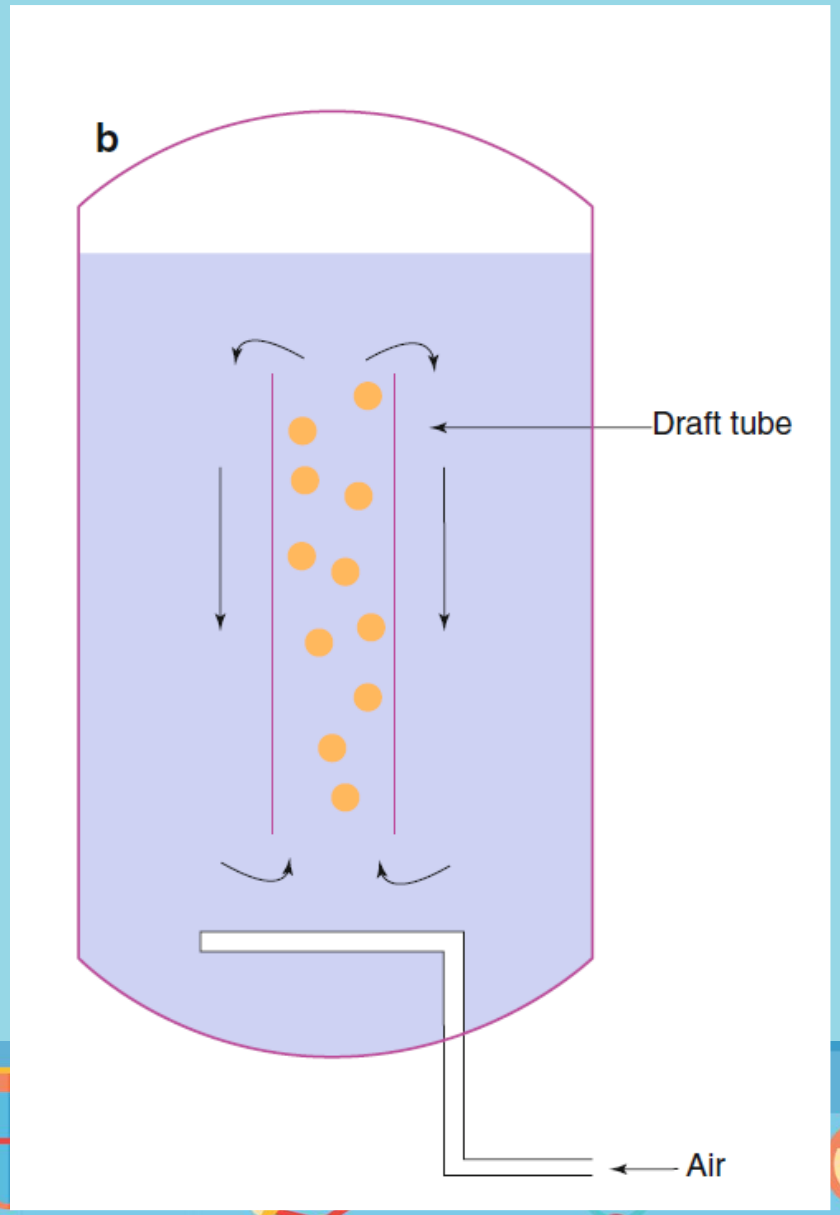
Figure 3. Simplified diagram of a stainless steel stirred tank bioreactor.

(A) Impeller drive, (B) marine impeller, (C) cell suspension, (D) water jacket, (E) pH probe, (F) DO probe, (G) removable headplate, (H) condenser, (I) gas filter, and (J) headspace.[36]

Stirred tank bioreactors



Fixed bed stirred tank bioreactor



Airlift bioreactors

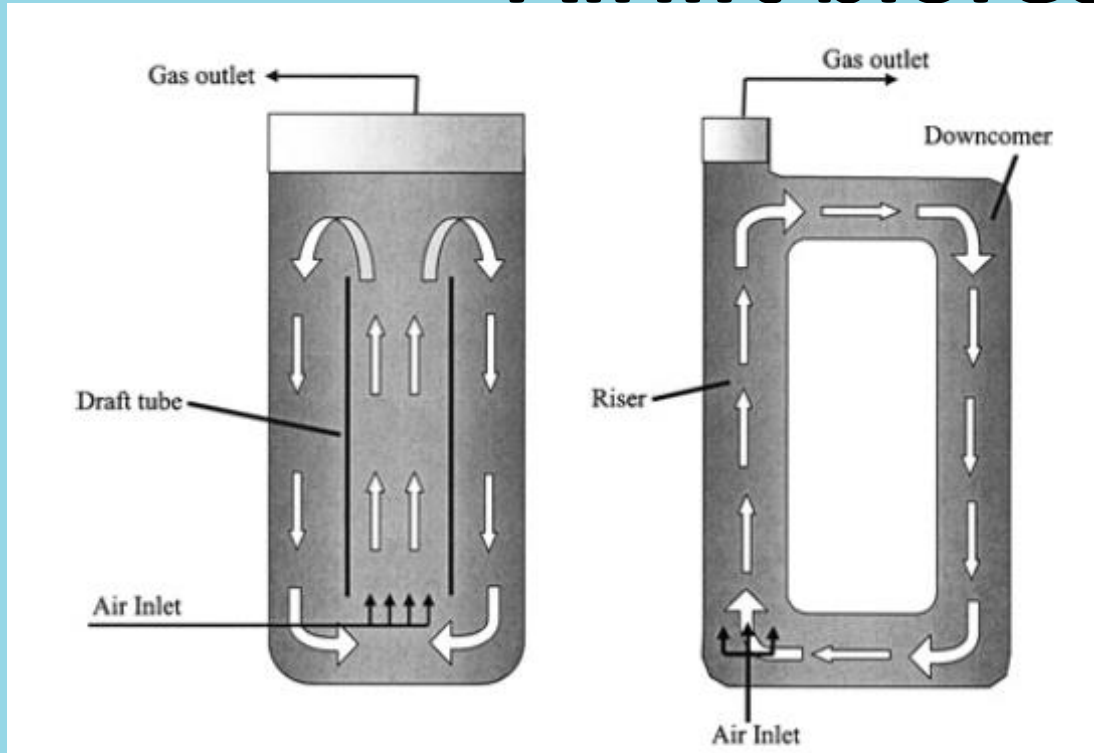


Figure 3 Schematic representation of an airlift bioreactor with internal recirculation (left panel) and external recirculation (right panel)

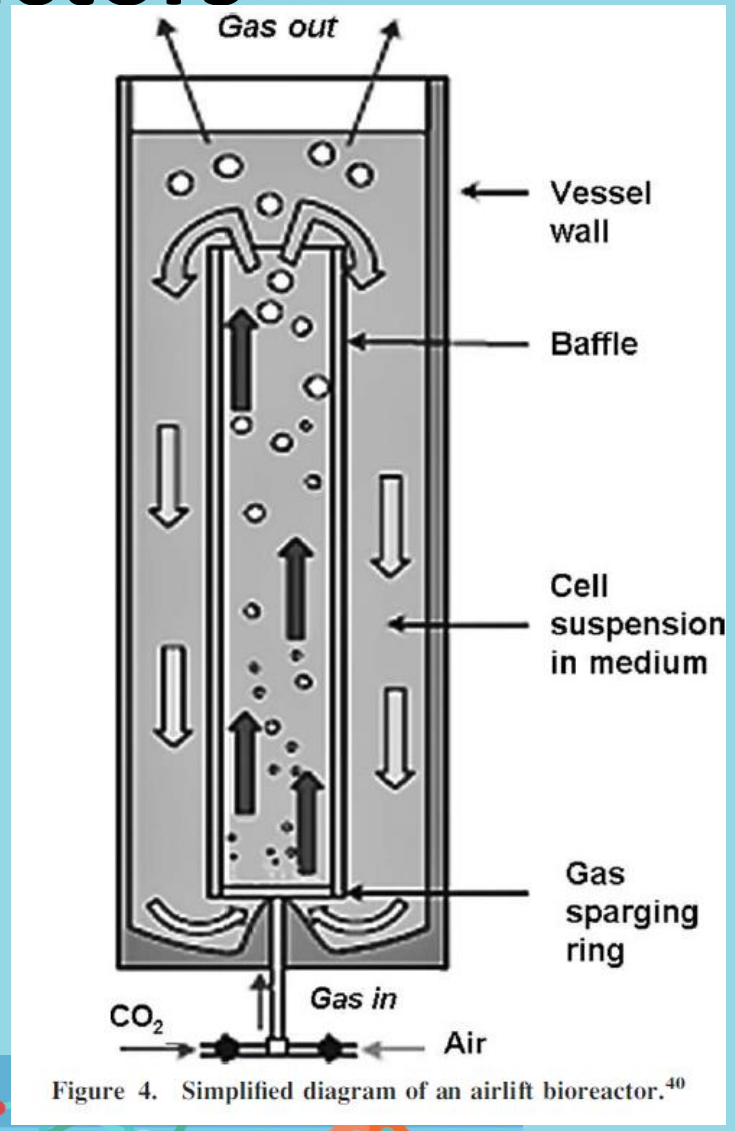


Figure 4. Simplified diagram of an airlift bioreactor.[40]

gentler mixing action and suitability for shear-sensitive cells than STR

Easy to scale up

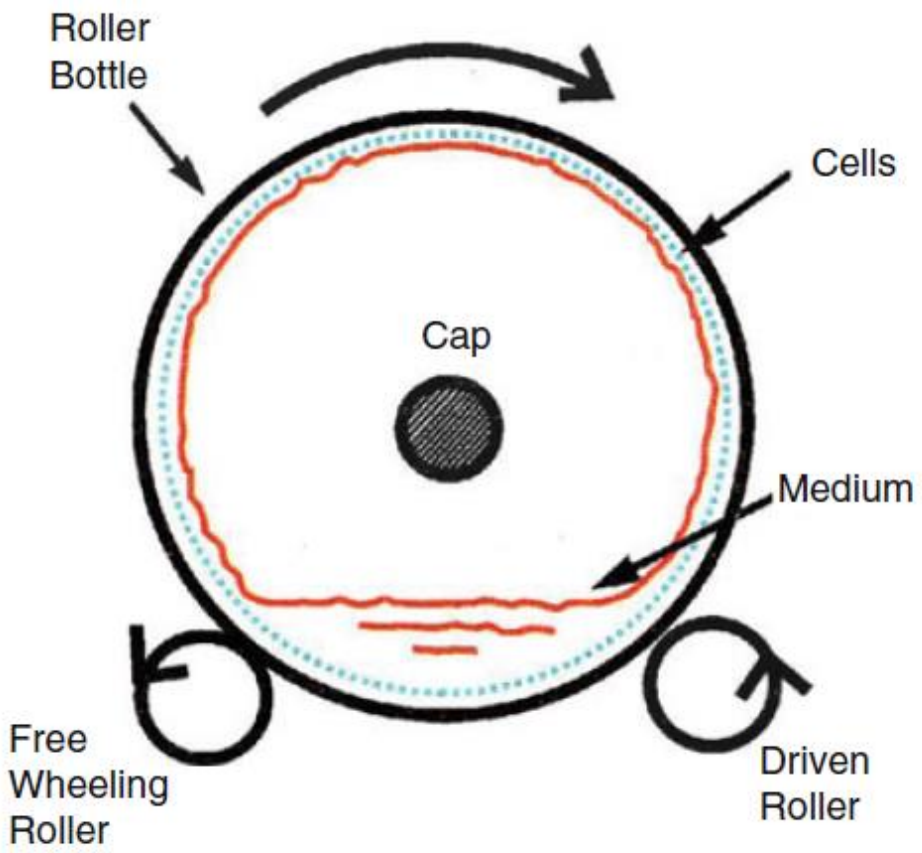
No moving part and mechanical seal

Not widely used as STR

Roller Bottle Fermenter

The Roller bottles provide total curved surface area of the micro carrier beads for growth. The continuous rotation of the bottles in the CO2 incubators helps to provide medium to the entire cell monolayer in culture

Roller Bottle Fermenter



Batch

Continuous, chemostat

V/V_B = 18 %

Perfusion rate q = 0,4/d

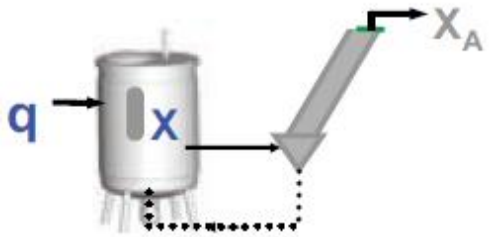
Residence time t = 2,5 d

Continuous with cell retention

V/V_B = 0.7 %

Perfusion rate q = 12/d

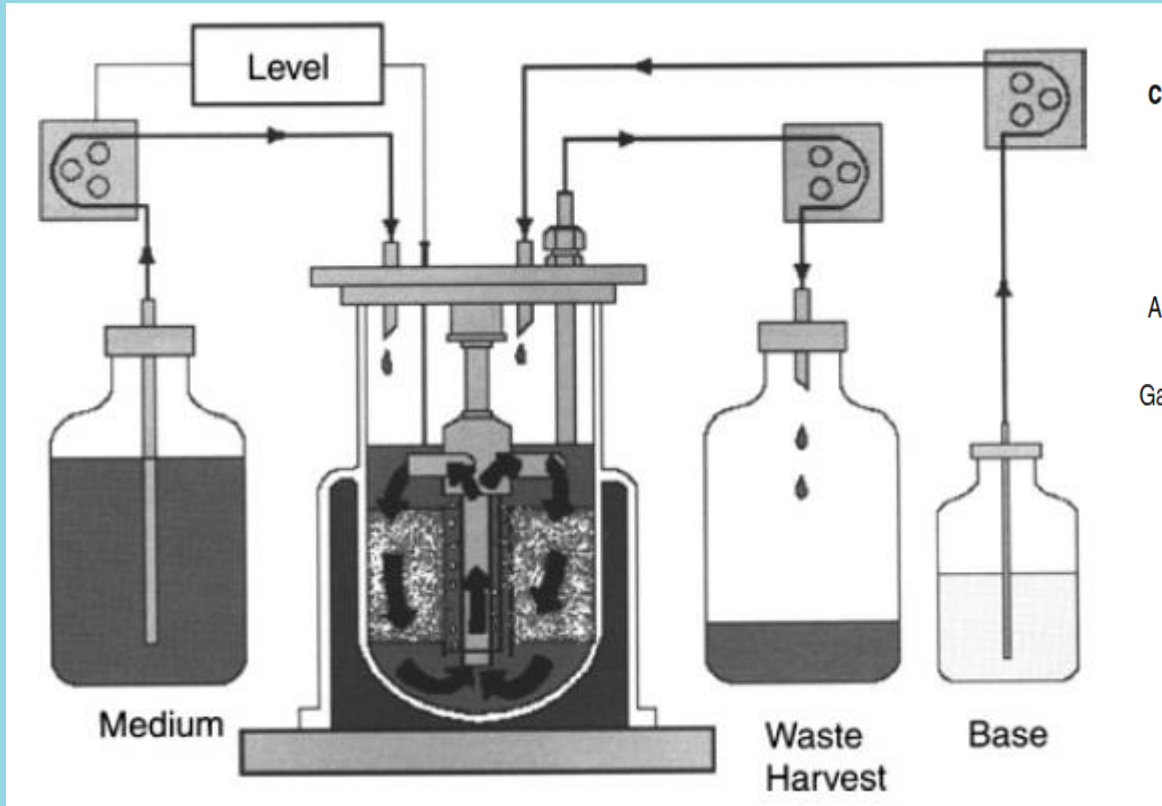
t ≈ 2,4 h

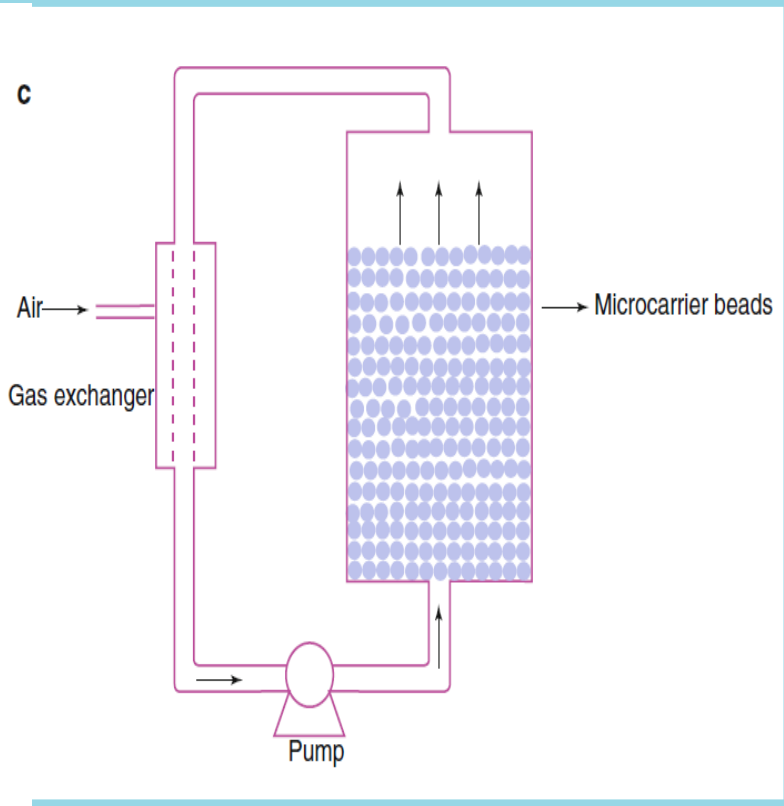


t_P : Fermentation time
V_B : Fermenter volume
q : in fermenter volume per day

Figure 19.7 Reduction of fermenter size using continuous operation and cell retention by increasing volumetric productivity.

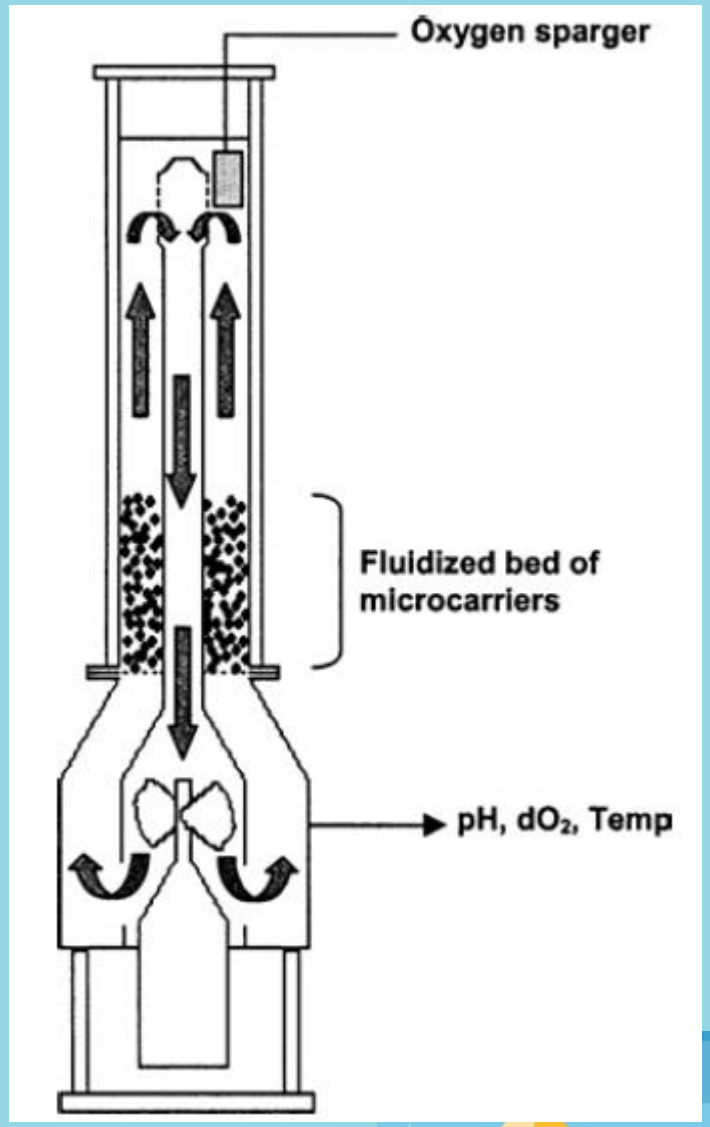
Packed-bed Bioreactor





Cells are immobilized within porous carriers that may be porous ceramic beads porous glass beads or polyester discs, which are packed and retained in a cylindrical vessel through which culture medium is recirculated.

Cytopilot mini fluidized-bed bioreactor

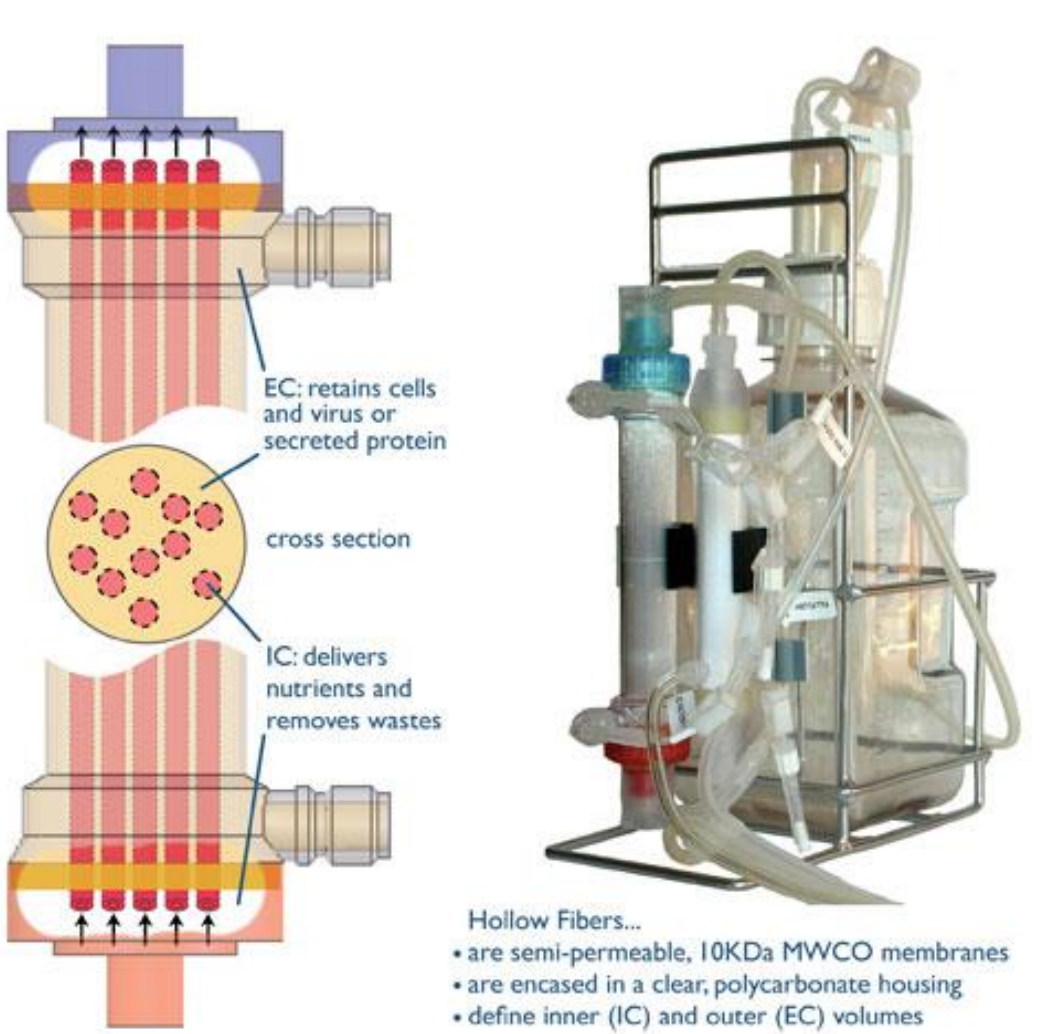


widely used culture system for porous microcarriers

microcarriers of higher density than the culture medium are suspended by the upward flow of the medium, which is circulated through the bed

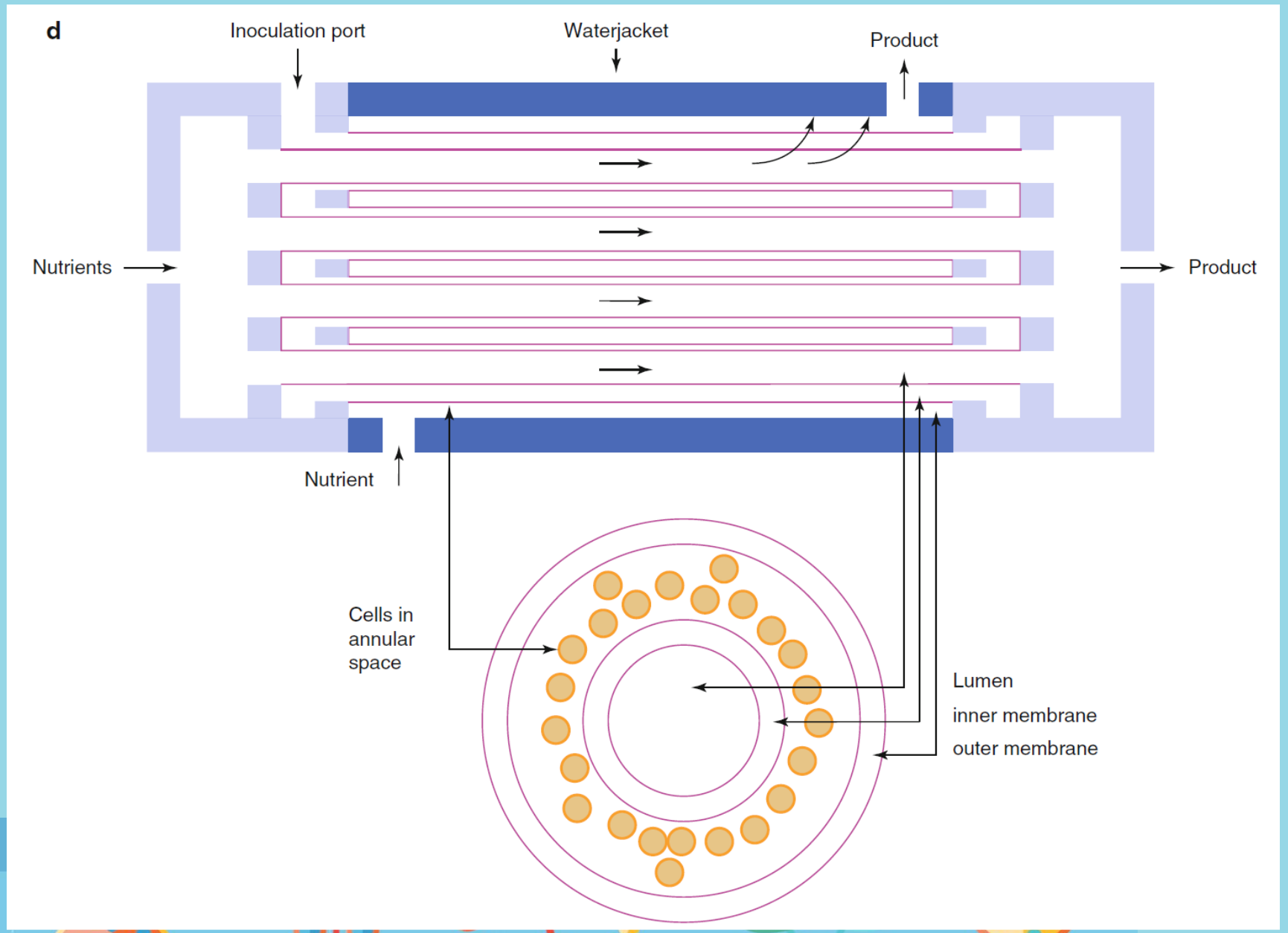
height of the bed will increase as fluid flow increases

Hollow Fiber Bioreactor

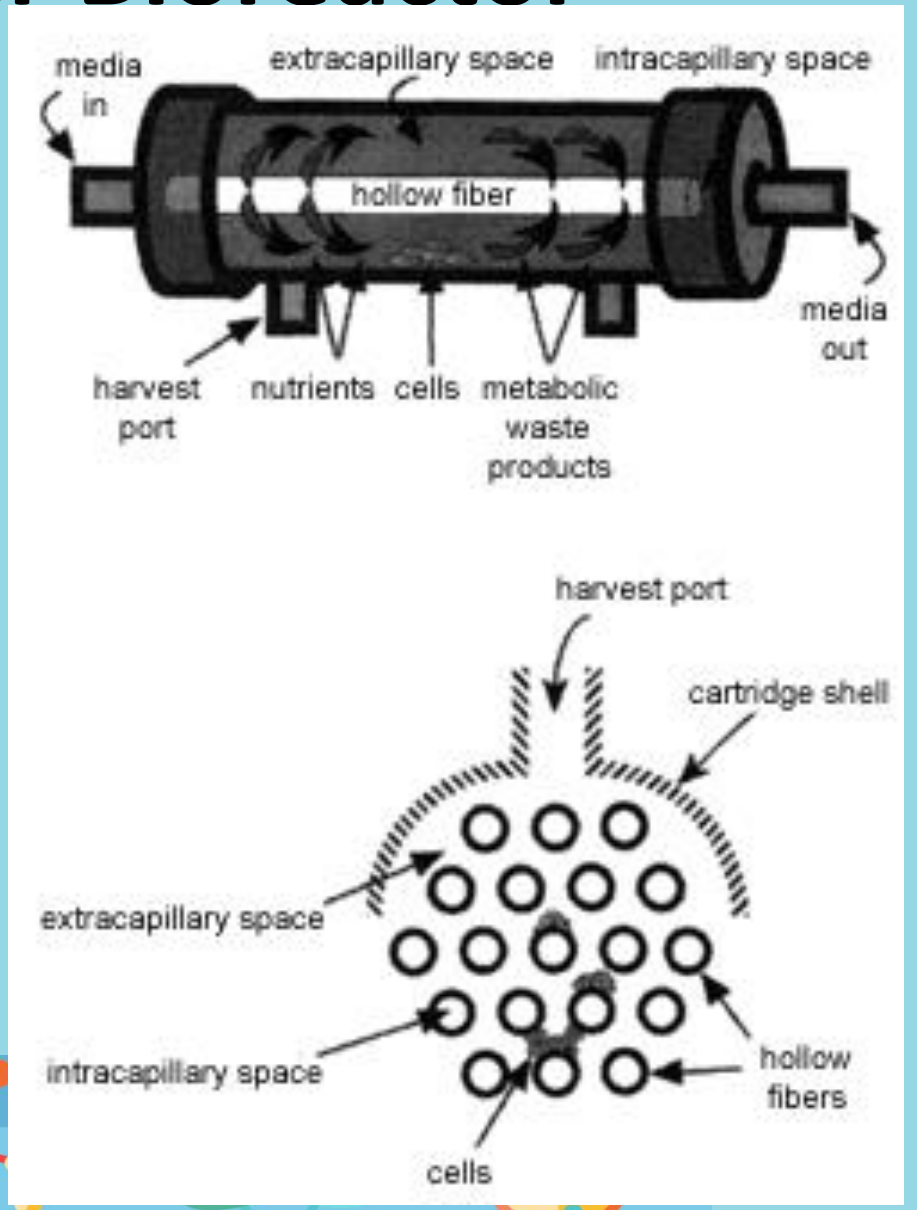


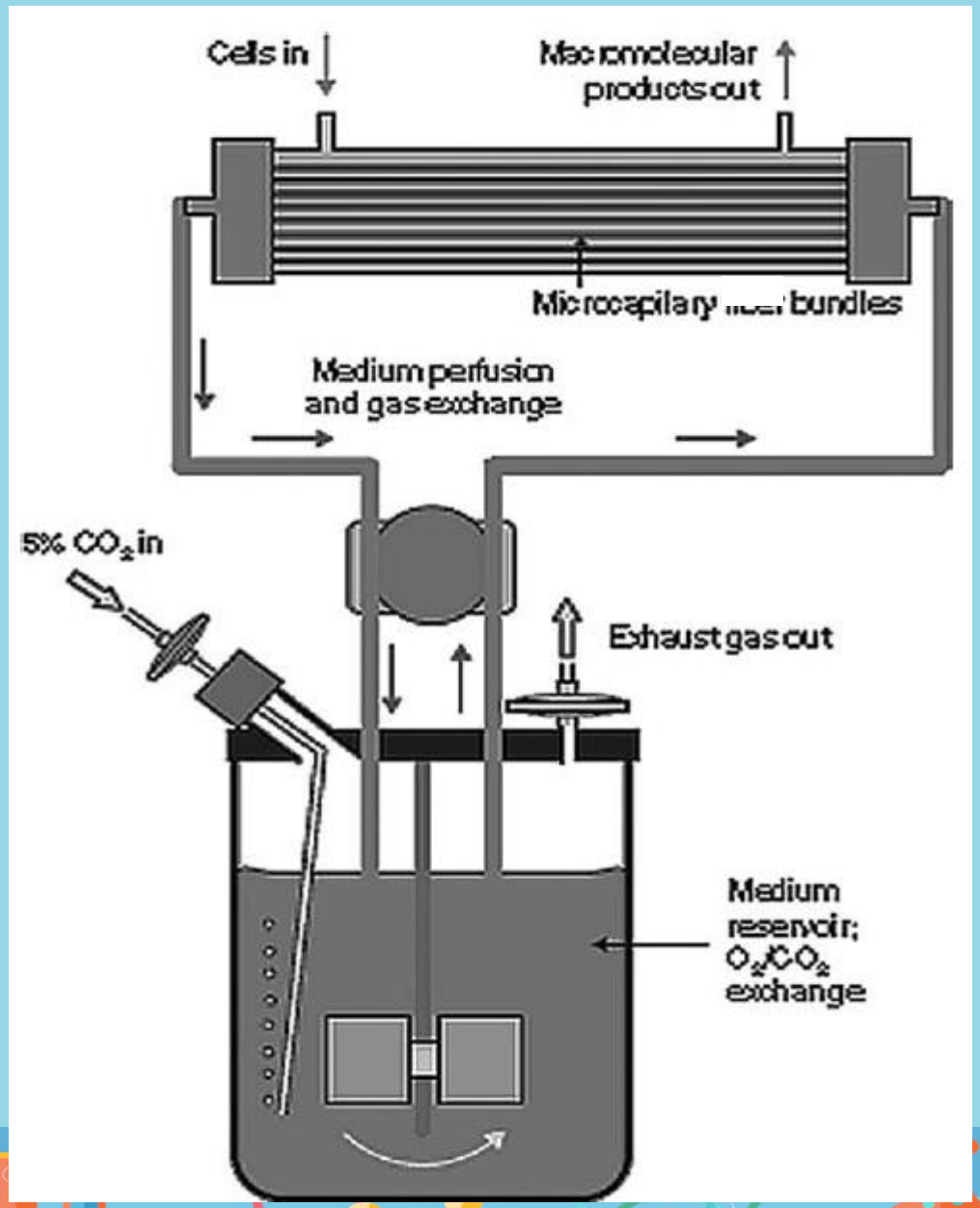
cells attach to the outer surface of semi-permeable fibres, growing in the ECS (extracapillary space) while medium is circulated through the ICS (intracapillary space) or lumen. Nutrients diffuse through the fibres, usually made of cellulose acetate, while toxic metabolites diffuse into the ECS and are carried away from the cells

Hollow fiber perfusion bioreactor



Hollow Fiber Bioreactor




Cells in
Macromolecular products out
Microcapilary bundles
Medium perfusion and gas exchange
5% CO_2 in
Exhaust gas out
Medium reservoir; O_2/CO_2 exchange

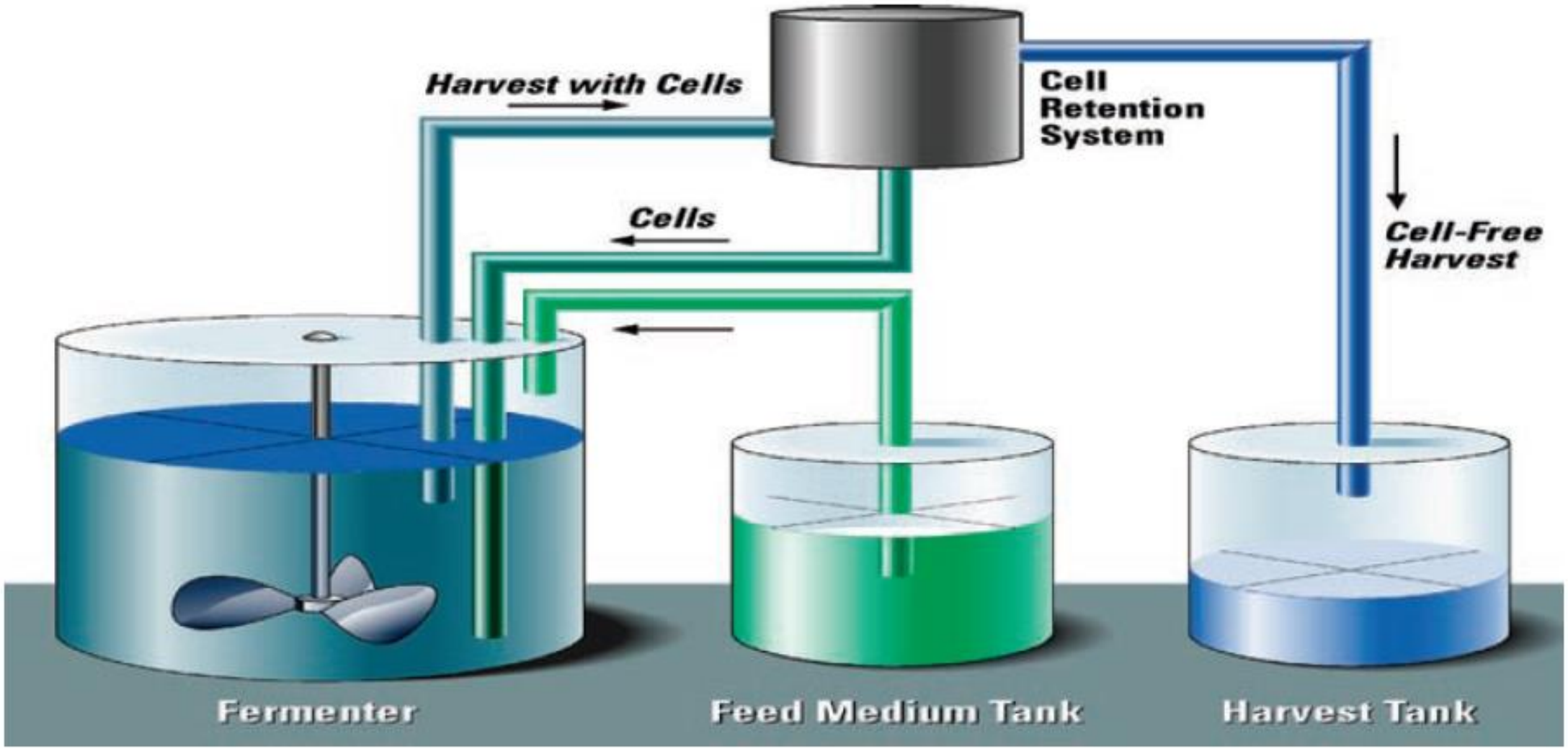
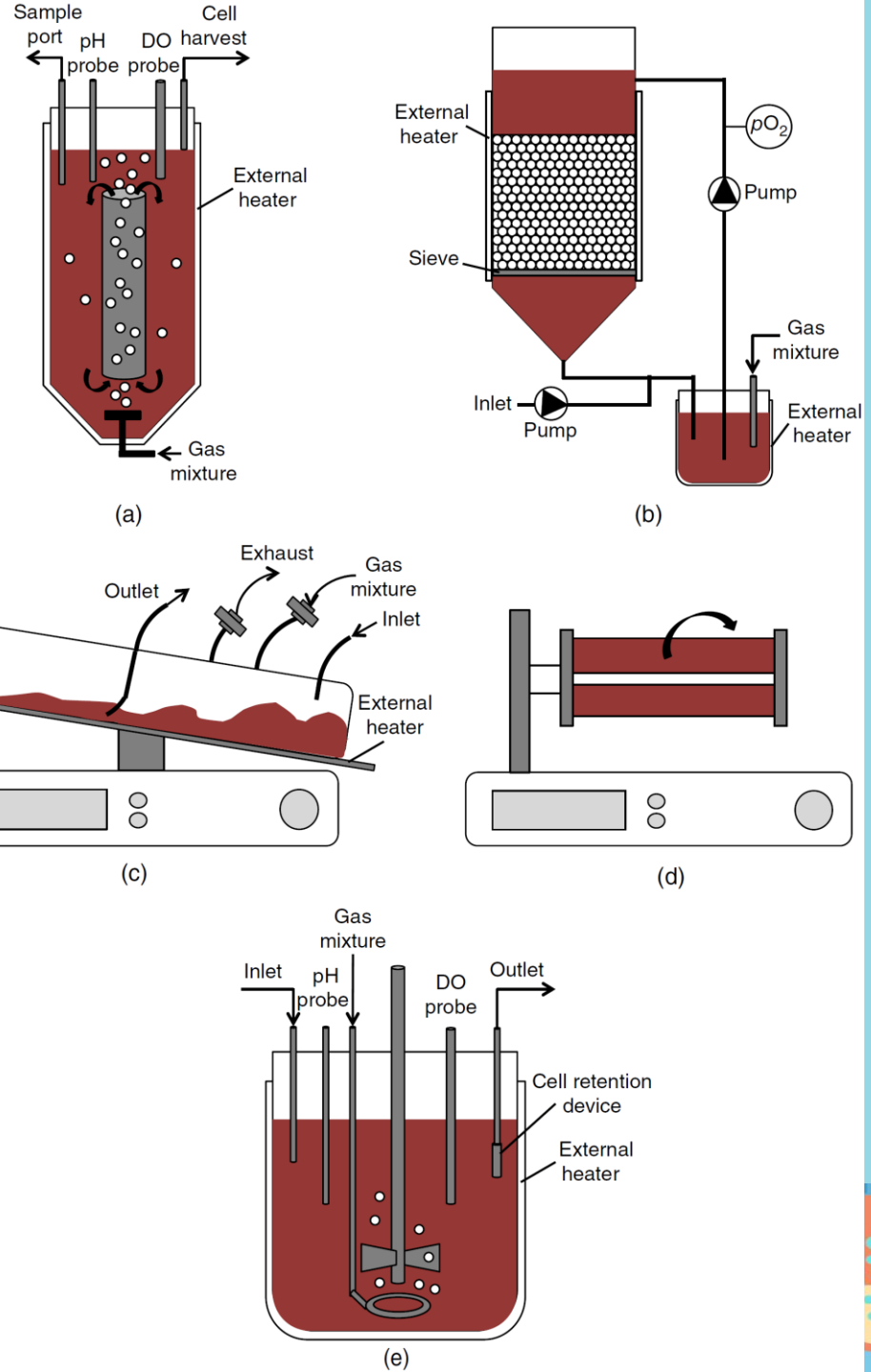


Figure 19.4 Principle of the continuous perfusion culture with cell retention.

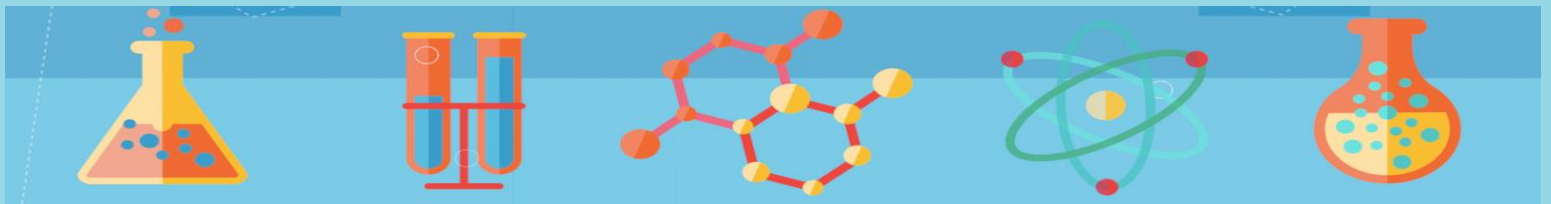

Sample port
pH probe
DO probe
Cell harvest
External heater
Gas mixture
(a)
External heater
pO_2
Pump
Sieve
Gas mixture
Inlet
Pump
External heater
(b)
Exhaust
Gas mixture
Outlet
Inlet
External heater
(c)
(d)
Gas mixture
Inlet
pH probe
DO probe
Outlet
Cell retention device
External heater
(e)

Novel Technology : Disposable Bioreactors

a non-instrumented cultivation container and hence requires an external device (e.g., CO_2 incubator, shaker) to provide the optimal for cell growth and/or product formation

easy handling, reduced incidence of cross contamination, and savings in time and costs

Increase solid waste



Fermentor Scale Up

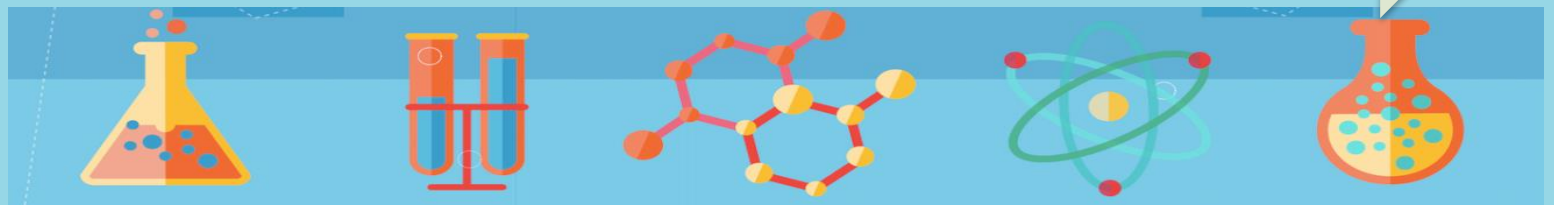
Can’t start cell culture in 100000 L, must do repeated, scaled inoculations

Start with stock culture (5-10 mL)

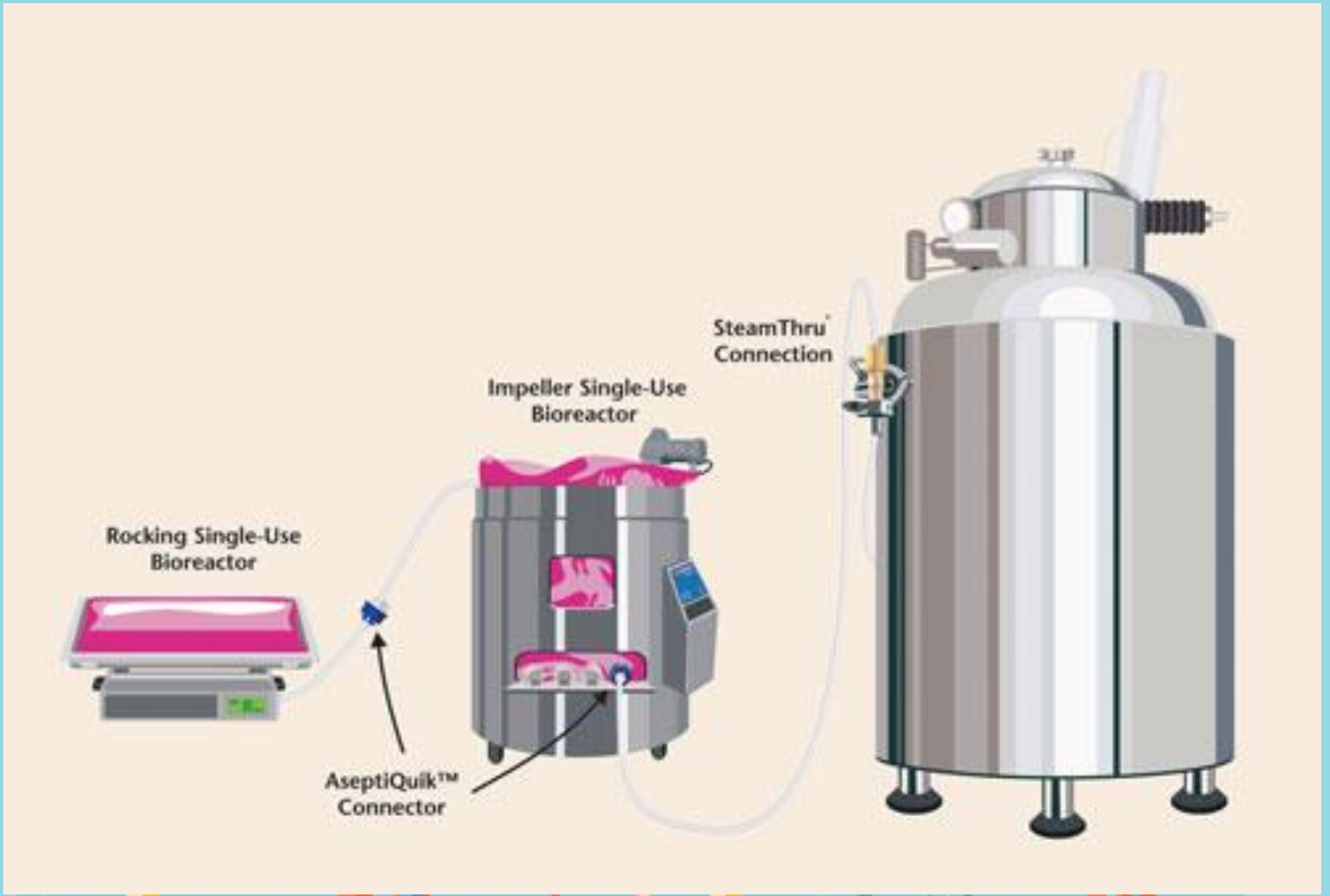
Then shaker flask (200-500 mL)

Then seed fermentor (10L to 100 L)

Then production fermentor (1000 L to 100,000 L)



Seed Train



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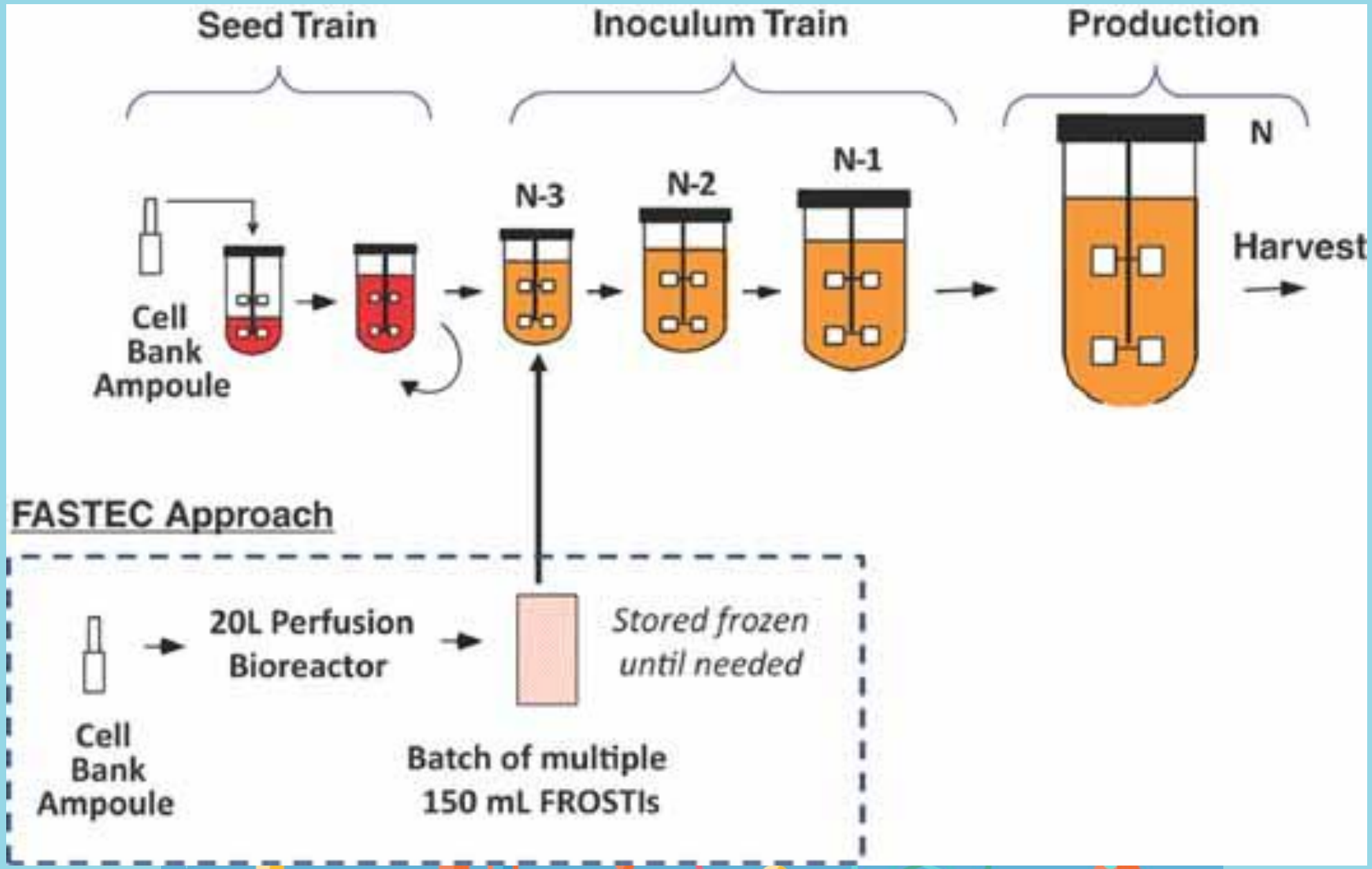
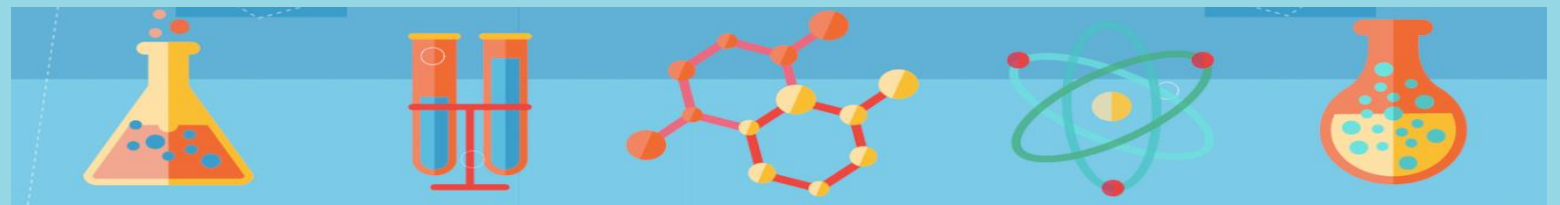


http://onlinelibrary.wiley.com/store/10.1002/bit.24808/asset/image_m/mgra001.jpg?v=1&s=96113027498ebdda530088cf4426617de57c0573

DOWNSTREAM PROCESSING



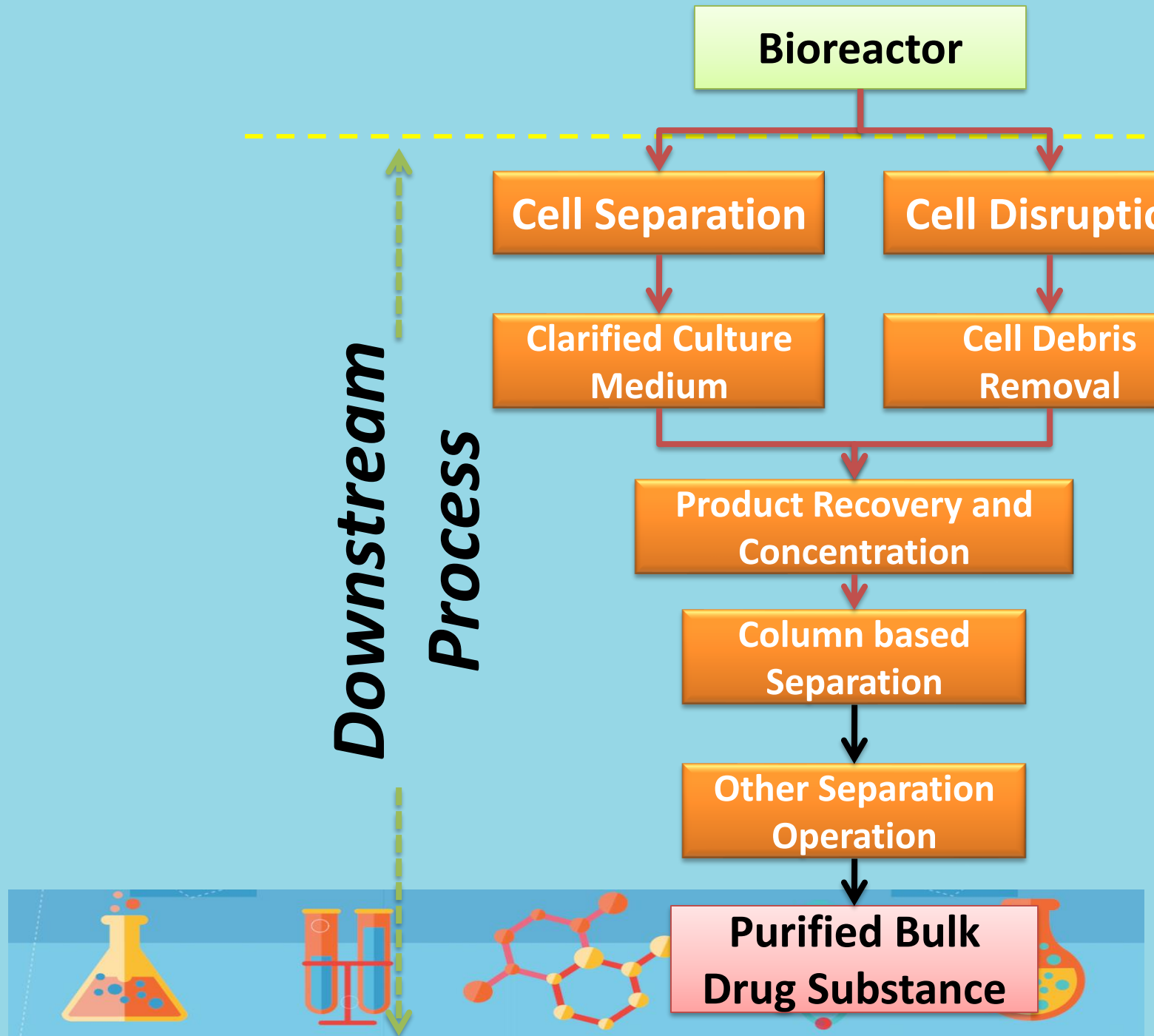
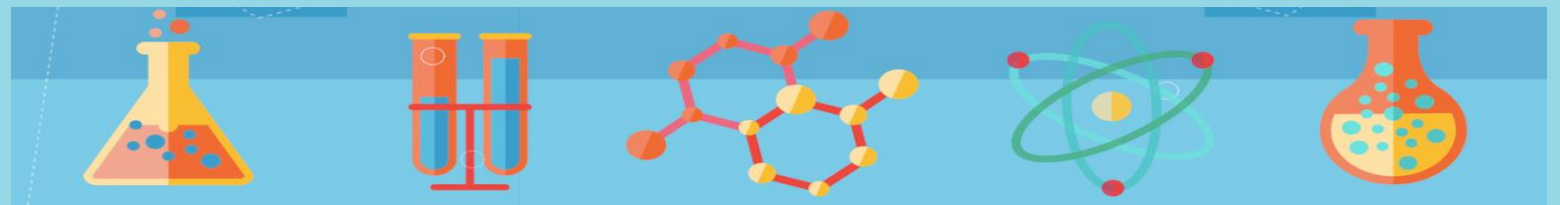

Bioreactor
Cell Separation
Cell Disruption
Clarified Culture Medium
Cell Debris Removal
Product Recovery and Concentration
Column based Separation
Other Separation Operation
Purified Bulk Drug Substance
Downstream Process

TABLE 1.10 Objectives and Typical Unit Operations of the Four Stages in Bioseparations

Stage	Objective(s)	Typical unit operations
Separation of insolubles	Remove or collect cells, cell debris, or other particulates Reduce volume (depends on unit operation)	Filtration, sedimentation, extraction, adsorption
Isolation of product	Remove materials having properties widely different from those desired in product Reduce volume (depends on unit operation)	Extraction, adsorption, ultrafiltration, precipitation
Purification	Remove remaining impurities, which typically are similar to the desired product in chemical functionality and physical properties	Chromatography, affinity methods, crystallization, fractional precipitation
Polishing	Remove liquids Convert the product to crystalline form (not always possible)	Drying, crystallization

CELL DISRUPTION



Cell Disruption Methods

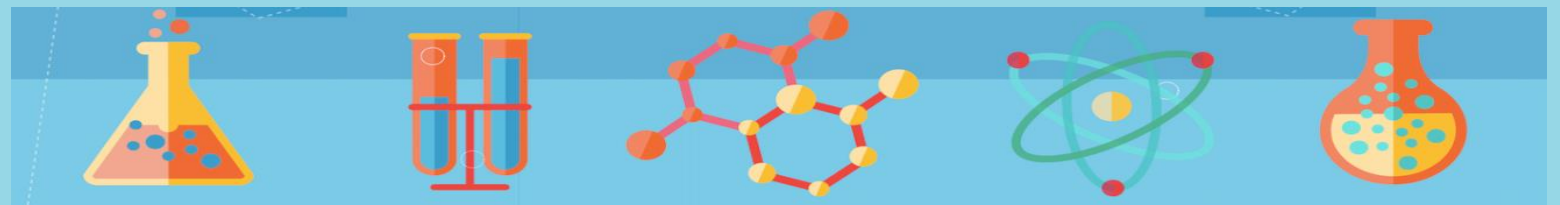
TABLE 4.11. Some methods designed to disrupt cells

Mechanical Methods	Other Methods
Ultrasonic	Drying
Homogenization	Heat or osmotic shock
Agitation with glass beads or abrasive materials	Freeze–thaw
	Organic solvent
	Chaotropic agents
	Enzymes
	Surfactant

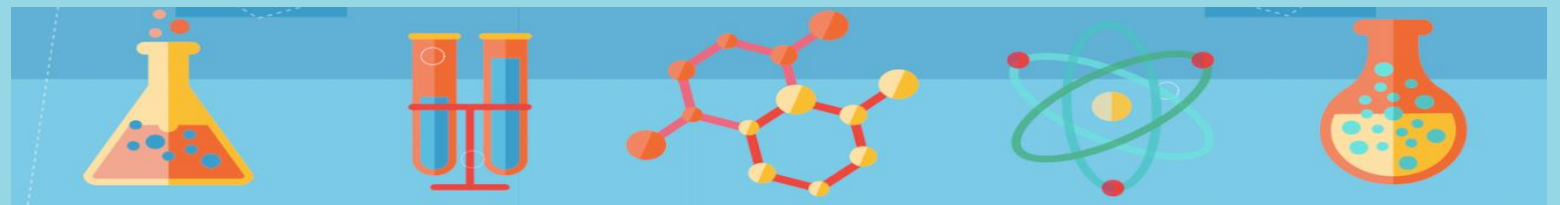
Centrifugation

Filtration

CLARIFICATION : SOLID-LIQUID SEPARATION

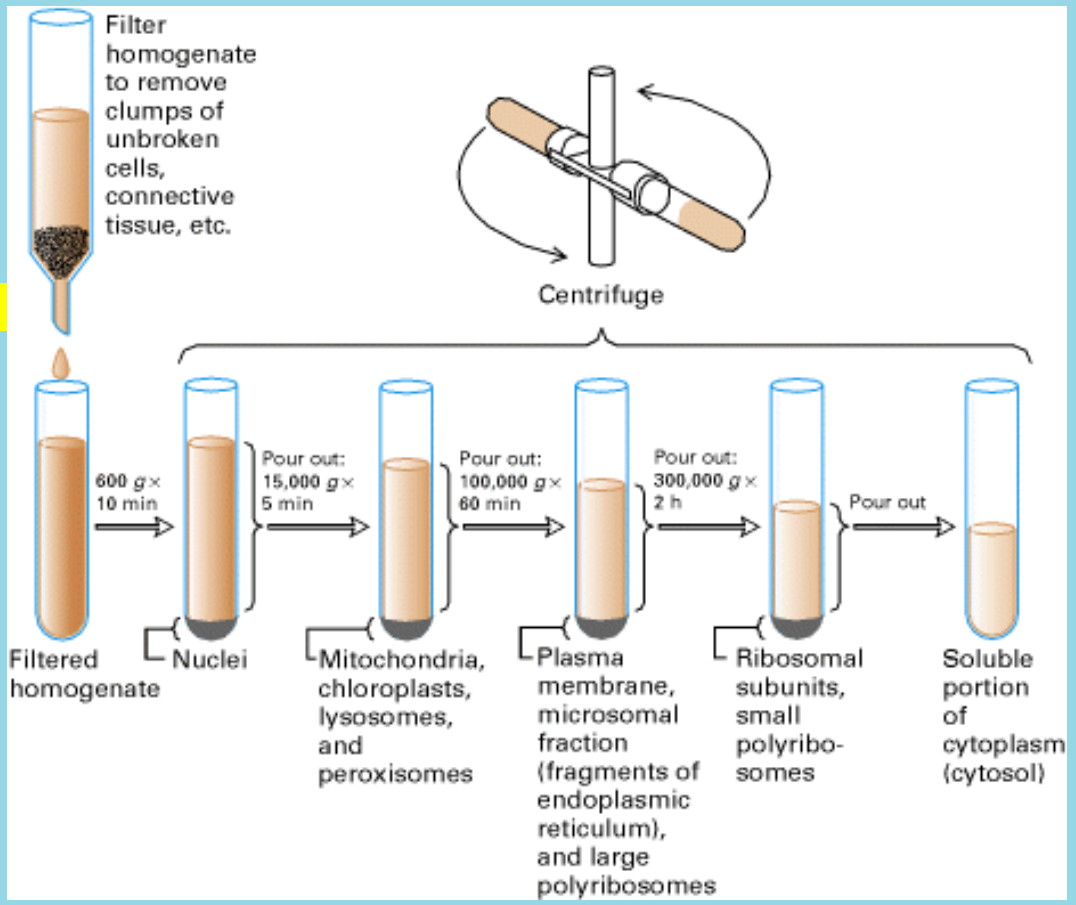


CENTRIFUGATION

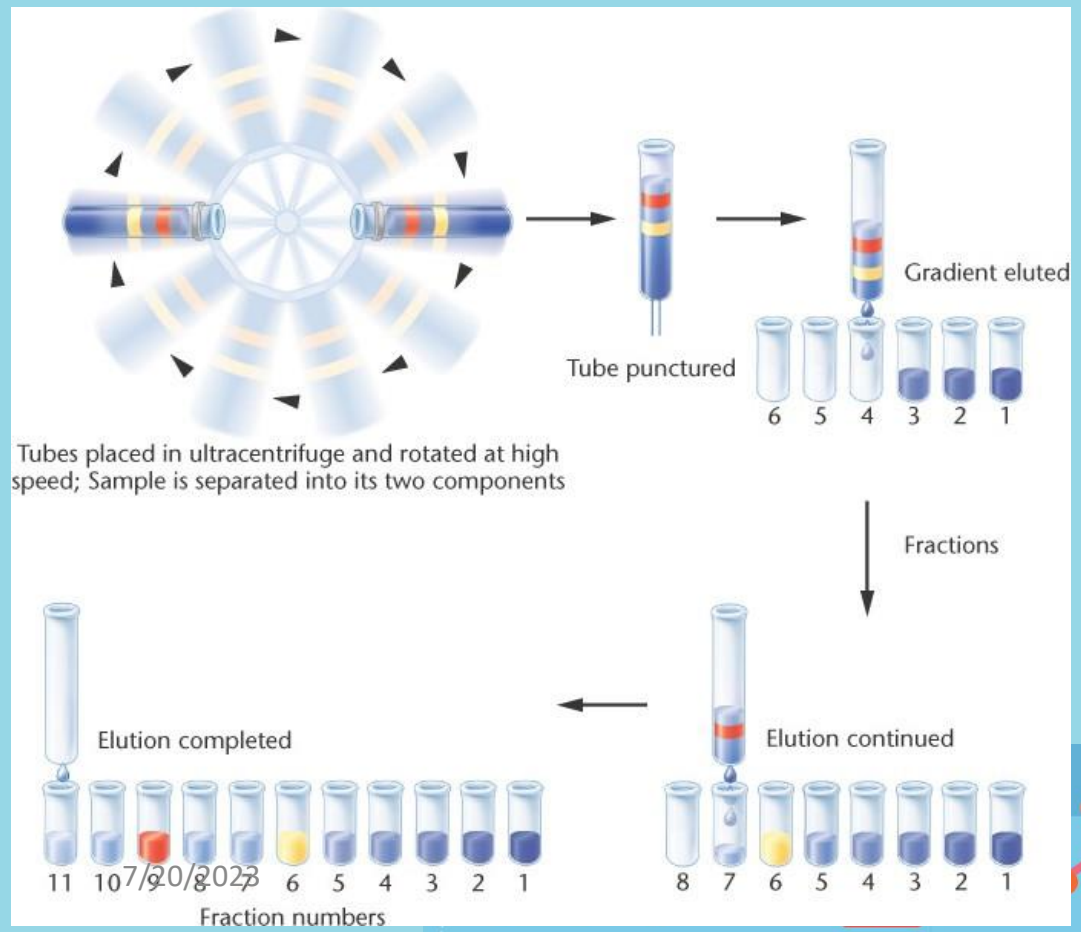


Centrifugations

Simple

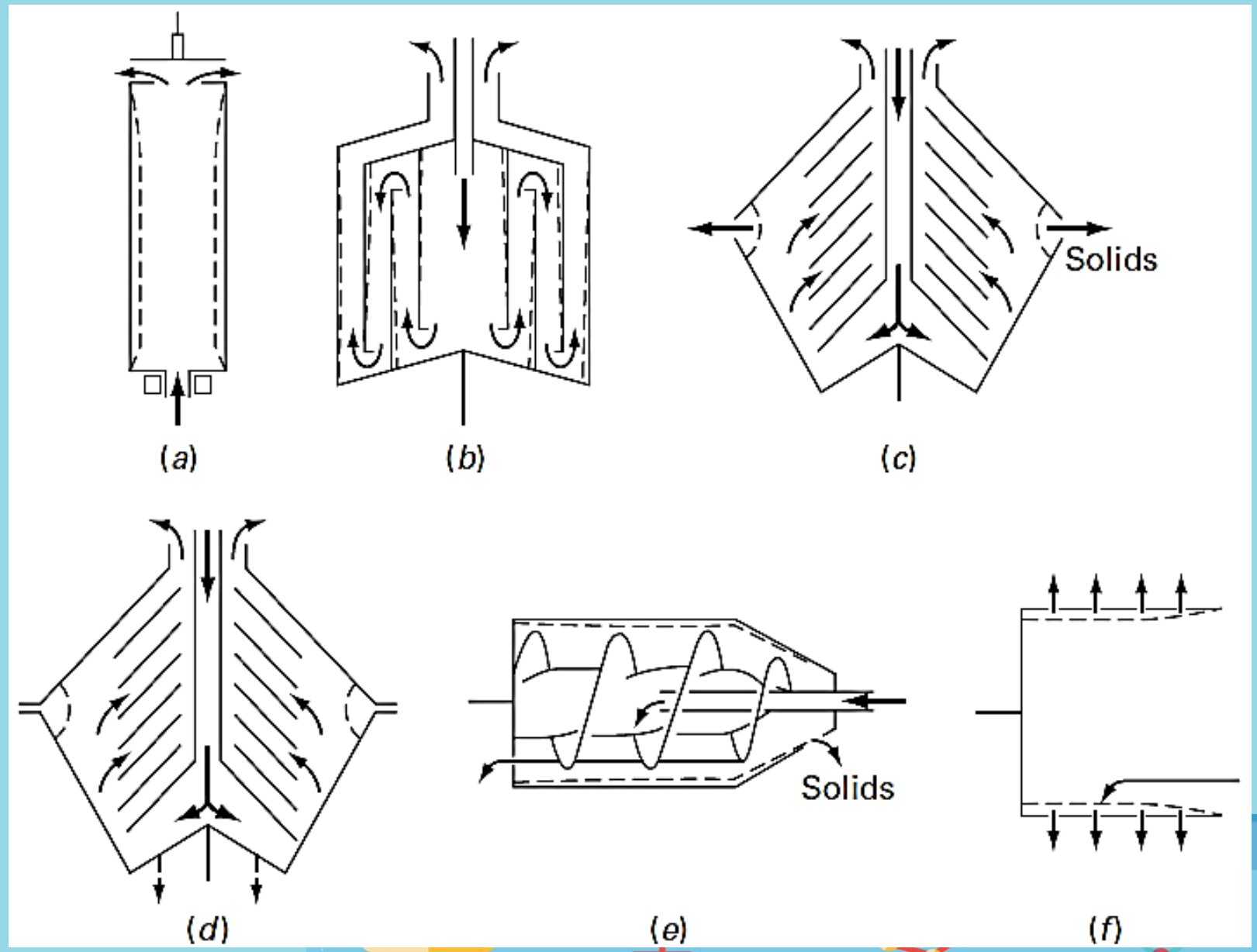


Differential



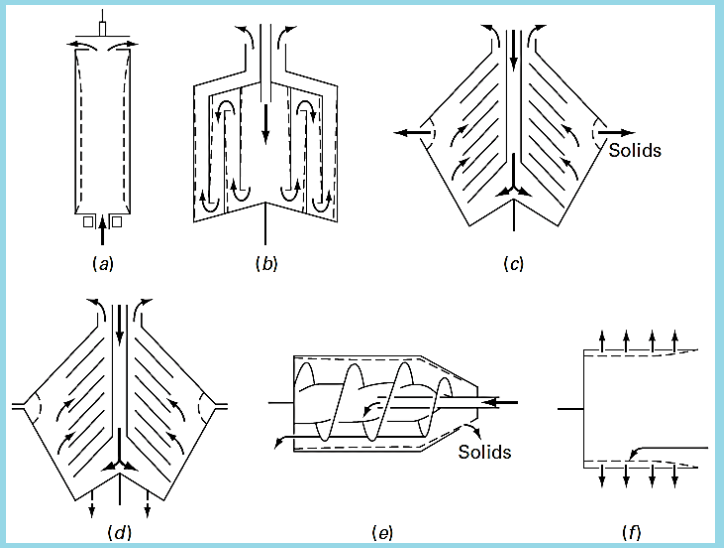
Gradient

Common Type of Production Centrifuges



(a) Tubular bowl,
(b) multichamber,
(c) disk, nozzle,
(d) intermittent discharge,
(e) scroll, and
(f) basket
Arrows indicate the path of the liquid phase; dashed lines show where the solids accumulate.

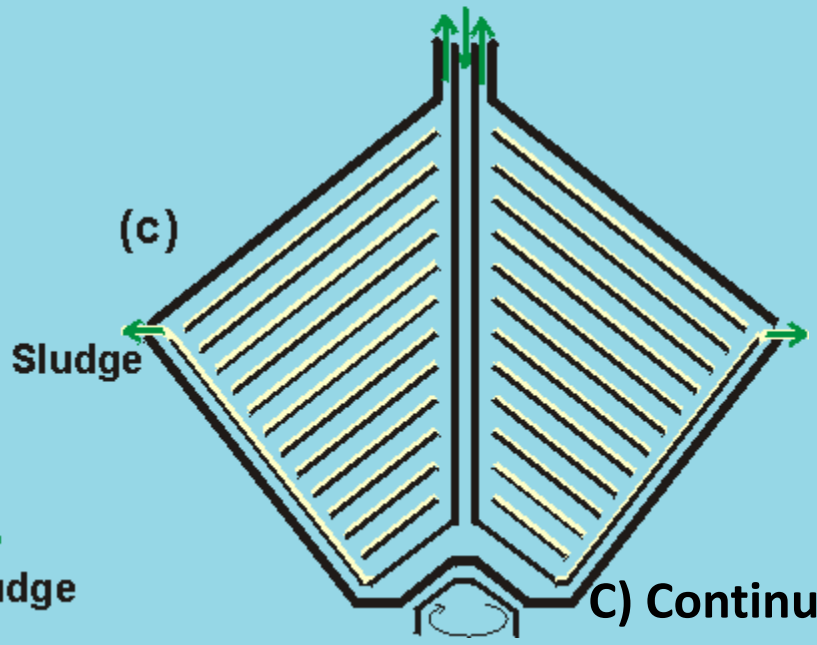
System	Advantages	Disadvantages
Tubular bowl	(a) High centrifugal force	(a) Limited solids capacity
	(b) Good dewatering	(b) Foaming unless special skimming or centripetal pump used
	(c) Easy to clean	
	(d) Simple dismantling of bowl	(c) Recovery of solids difficult
Chamber bowl	(a) Clarification efficiency remains constant until sludge space full	(a) No solids discharge
	(b) Large solids holding capacity	(b) Cleaning more difficult than tubular bowl
	(c) Good dewatering	(c) Solids recovery difficult
	(d) Bowl cooling possible	
Disk centrifuge	(a) Solids discharge possible	(a) Poor dewatering
	(b) Liquid discharge under pressure eliminates foaming	(b) Difficult to clean
	(c) Bowl cooling possible	
Scroll or decanter centrifuge	(a) Continuous solids discharge	(a) Low centrifugal force
	(b) High feed solids concentration	(b) Turbulence created by scroll
Basket centrifuge	(a) Solids can be washed well	(a) Not suitable for soft biological solids
	(b) Good dewatering	(b) No solids discharge
	(c) Large solids holding capacity	(c) Recovery of solids difficult



Industrial Centrifuge

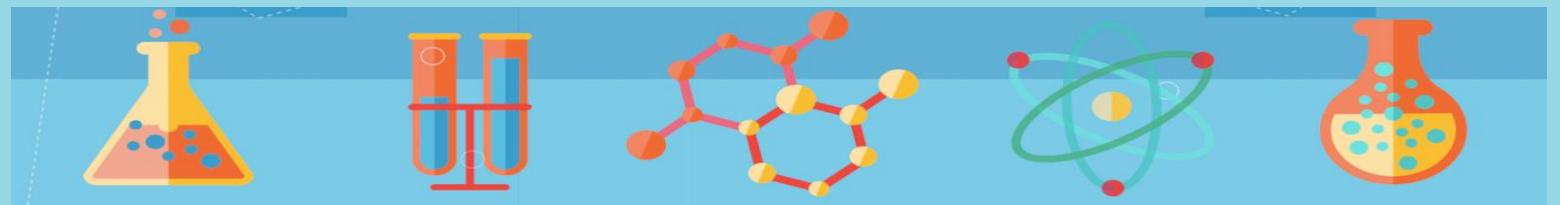
A) Tubular Bowl

B) Continuous Scroll



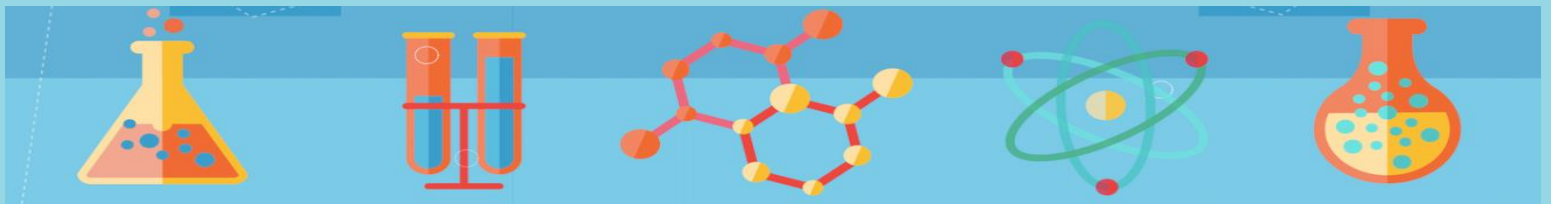
C) Continuous multichamber disc-stack

FILTRATION



Filtration

- A mechanical or physical operation which is used for separation of solids from liquids
- Two main types of Filter Medias:
  - Surface Filtration (Membrane Filtration) (eg. Buchner Funnel, Cross Flow Filter)
  - Depth Filtration (eg. Sand Filter)



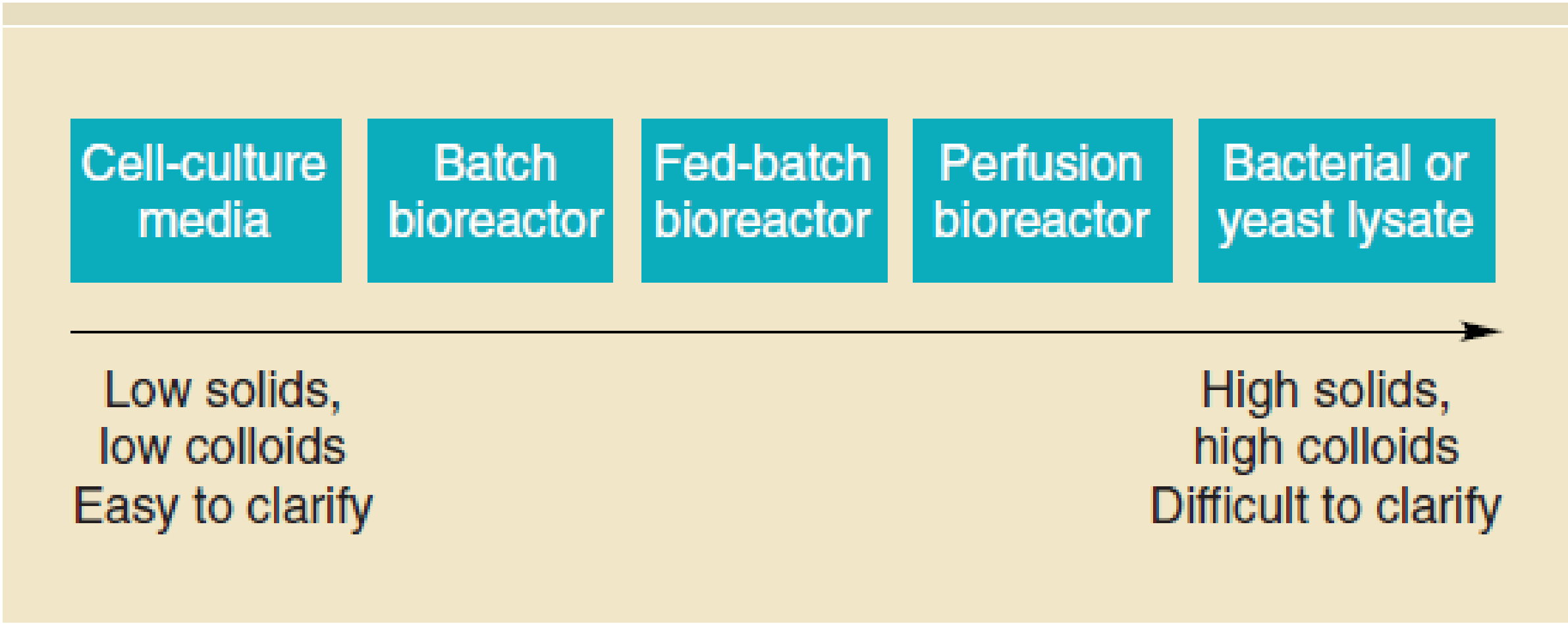
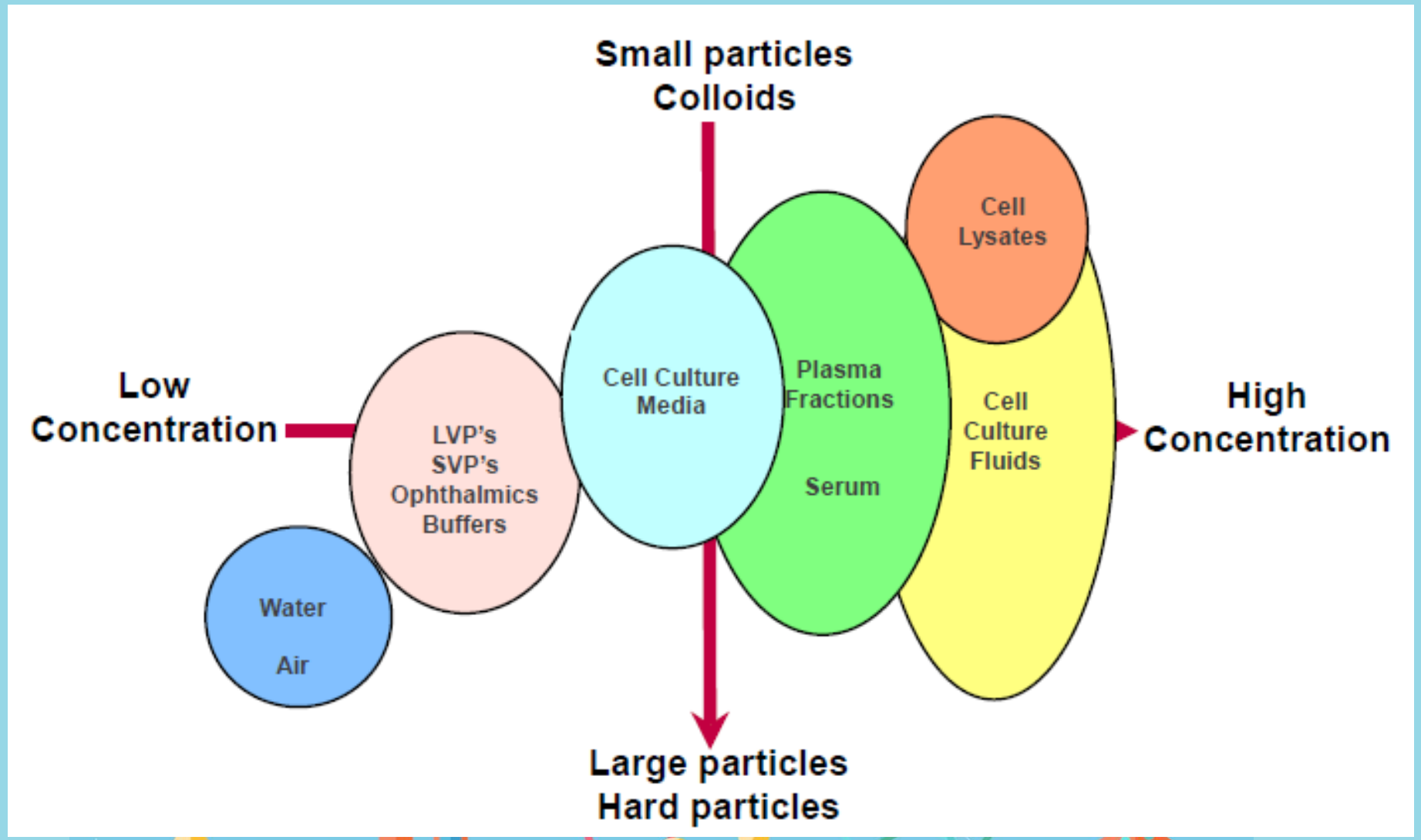
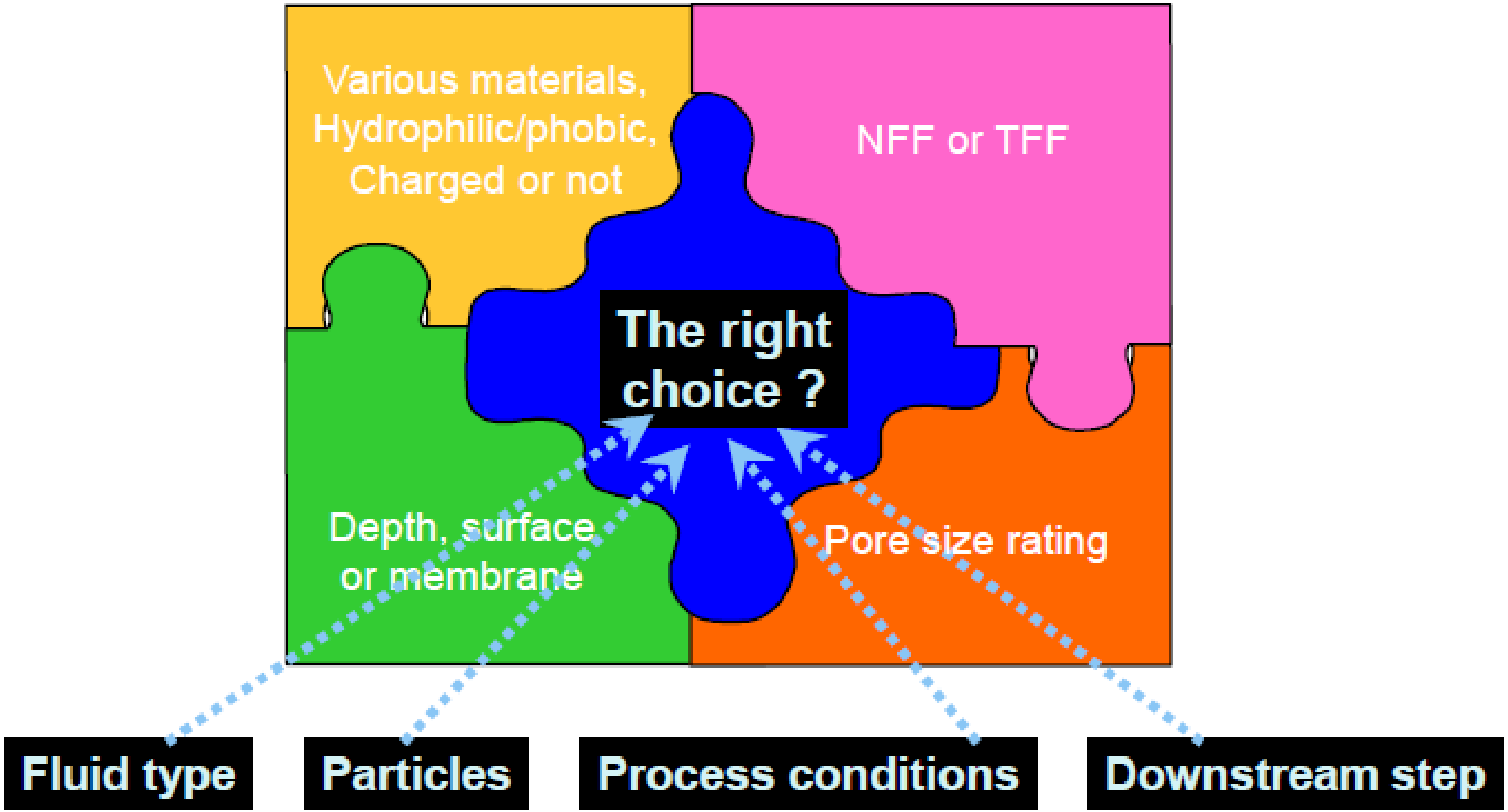


Figure 3: Cell-culture characteristics from various bioreactor types.

Contaminants and Application Mapping

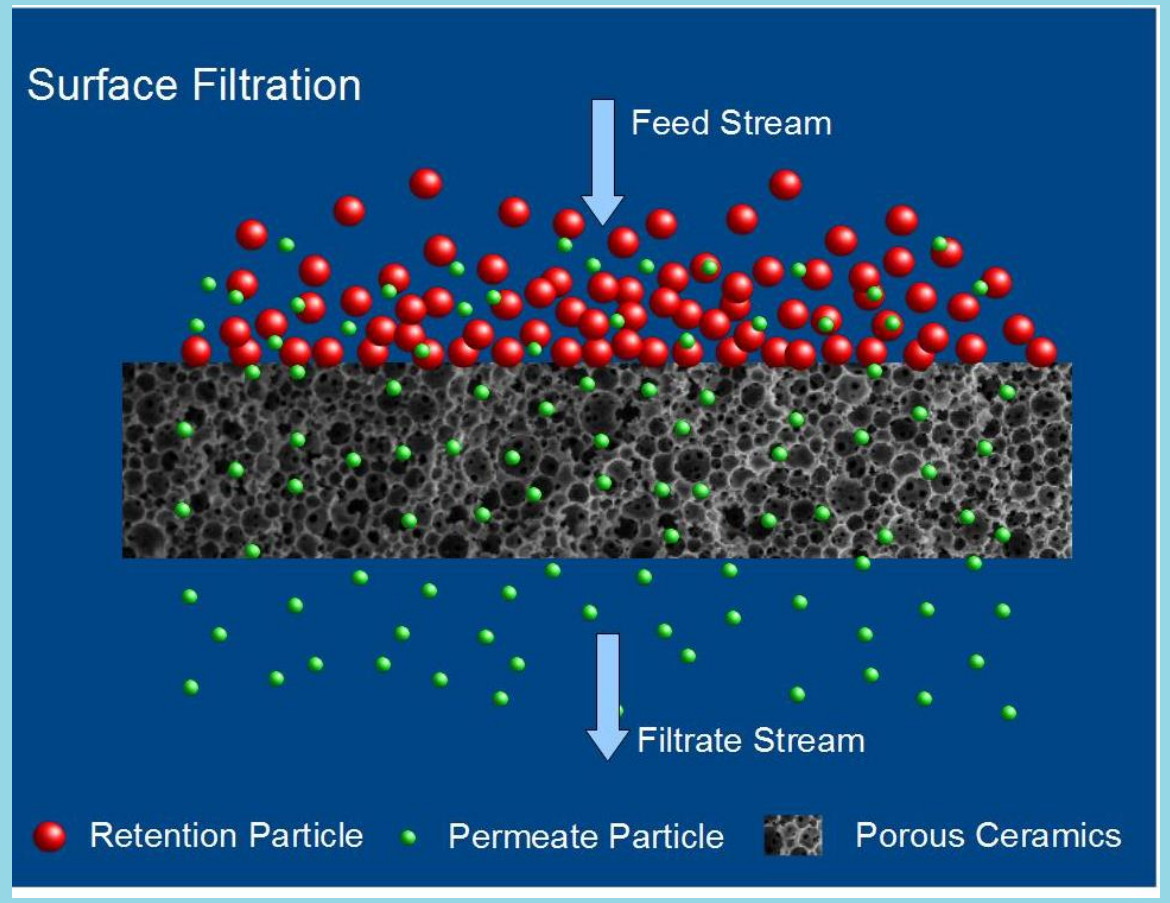


Filtration Puzzles

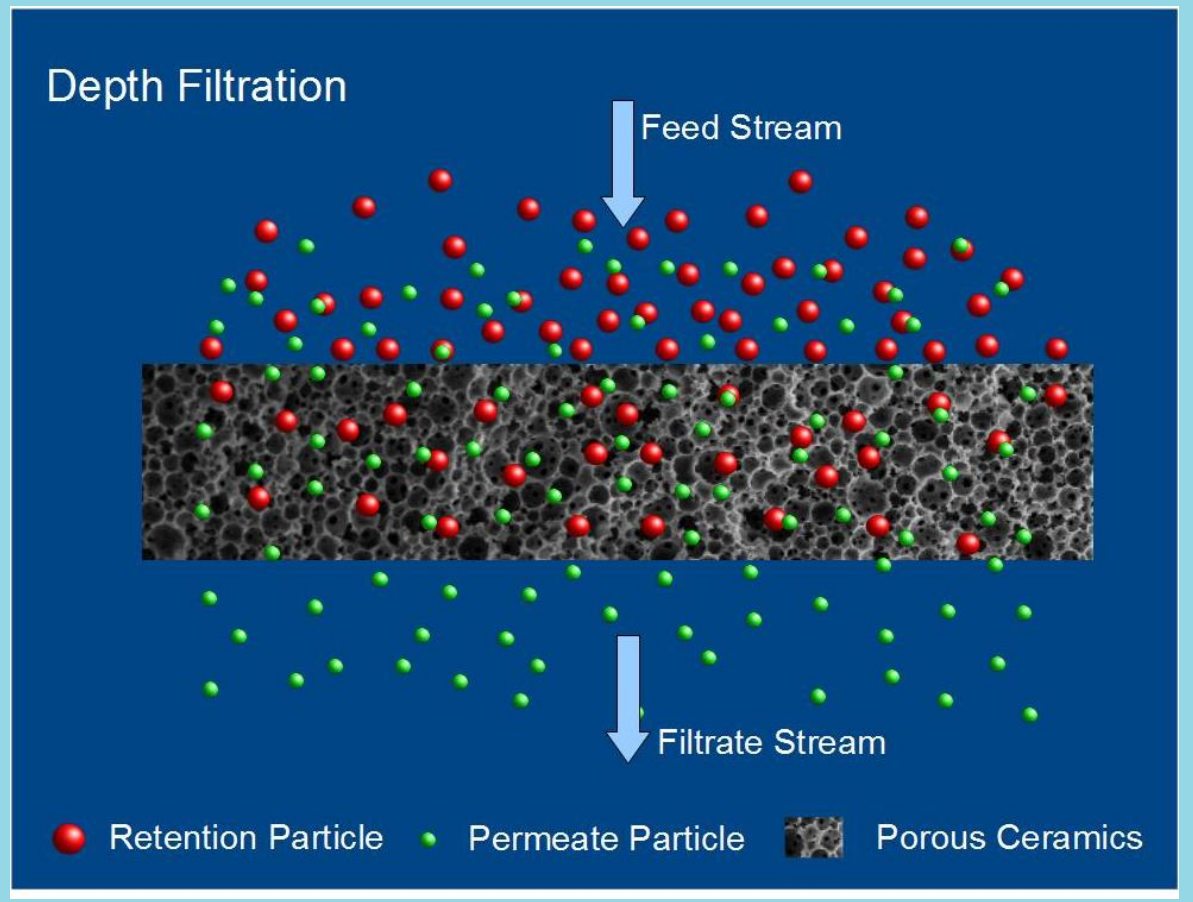


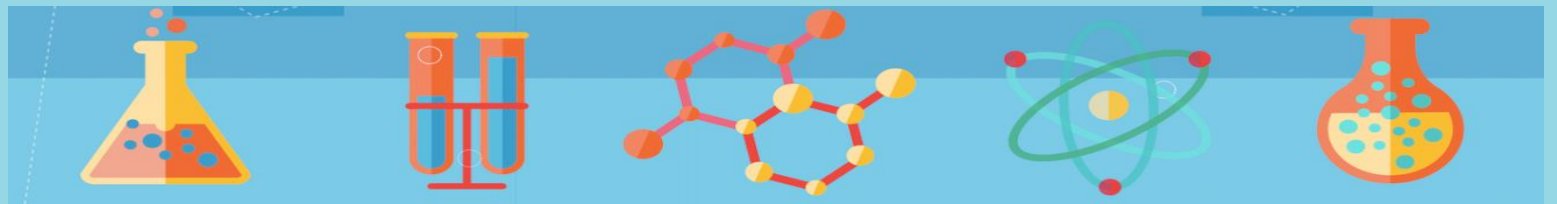
Filtration: Classification

Surface Filtration



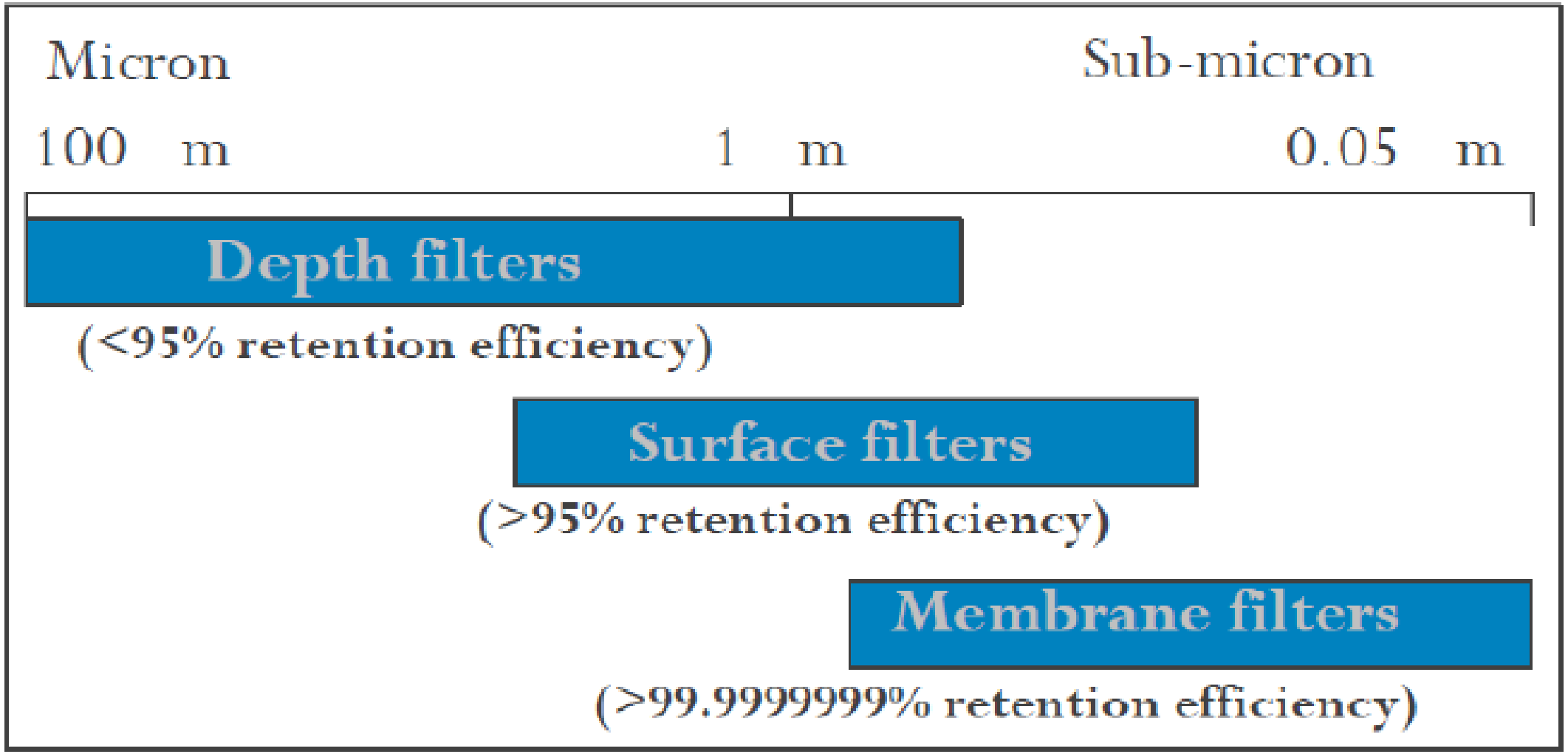
Depth Filtration





Parameter	Surface Filters	Depth Filters
Deformable Particles	May blind off pleats	Recommended - adsorptive retention
Non deformable Particles	Removes narrow range	Removes broader range of particles
Rating	Absolute or nominal	Absolute or nominal
Classification/Clarification	Classification	Clarification
Economic - Particle Retention < 10 Micron	Holds more dirt than depth, handles higher flow rate	More economical than pleated at greater than 10 microns
Cartridge Cost *	More expensive initially than depth, fewer replacements, holds more dirt	More economical initially than pleated, holds less dirt
Housing Cost *	Fewer cartridges - smaller housing	More cartridges-bigger housing

Relative Retention Efficiency



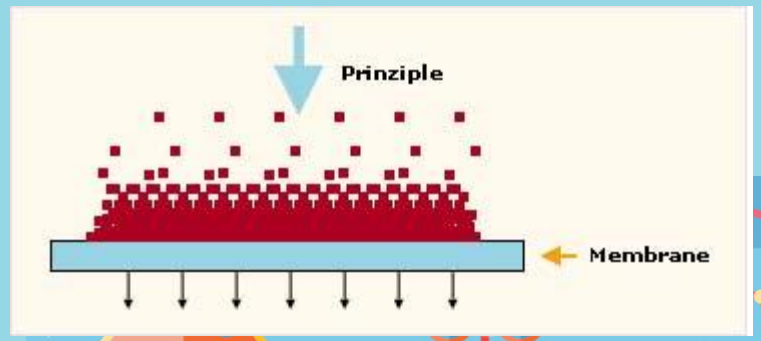
Filtration Techniques

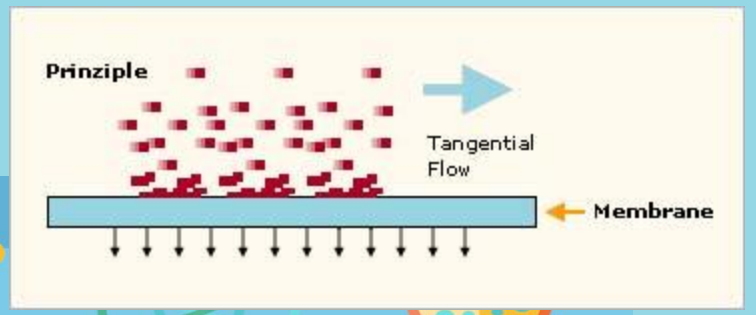
Dead-End Filtration

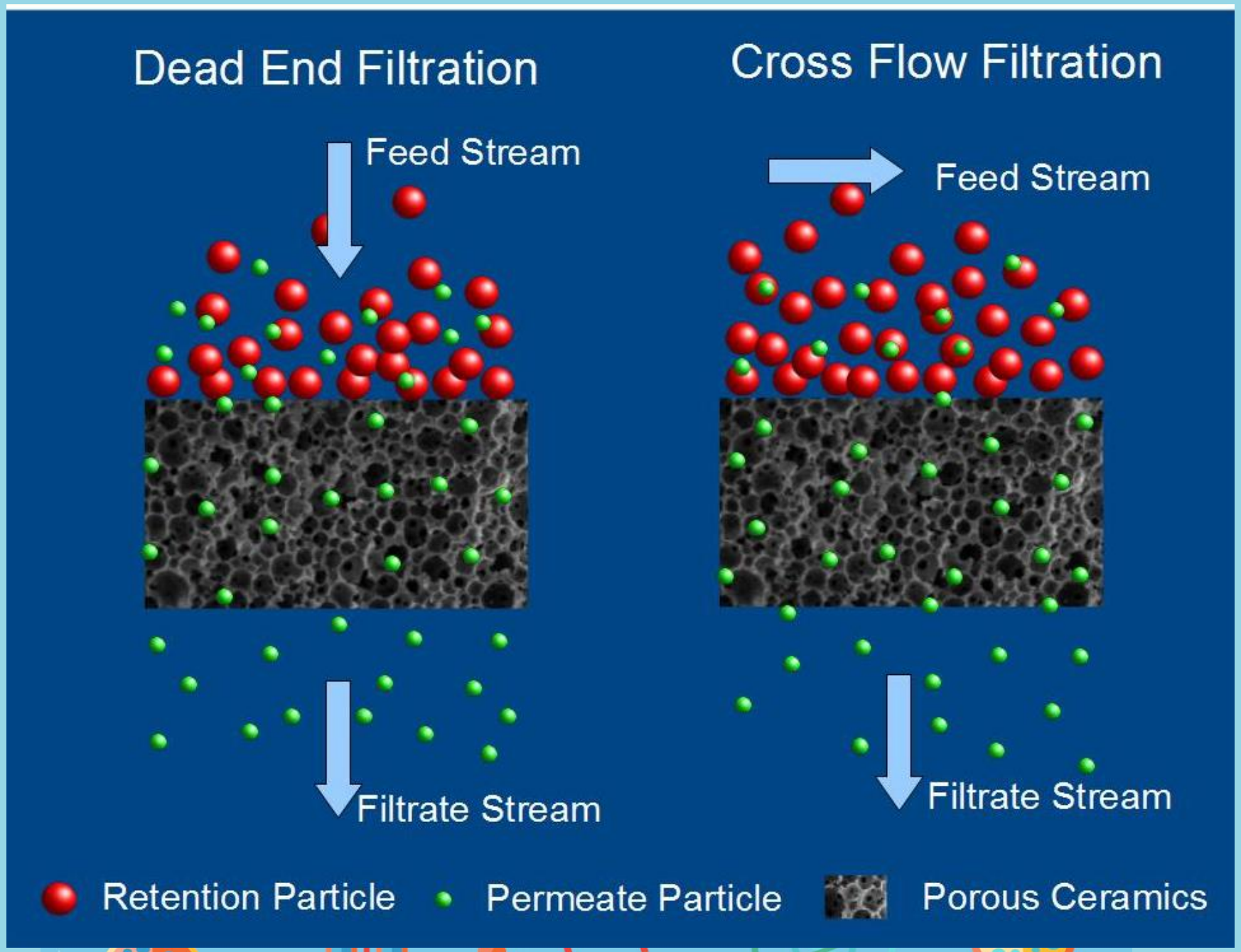
- All fluid passes through membrane
- Larger Particles stop on the membrane
- Form “Filter Cake”
- Batch operation

Crossflow Filtration

- Fluid feed stream run tangential to the membrane
- Some particles stop, other flow across membrane
- Prevent “Filter Cake”
- Continuously operation



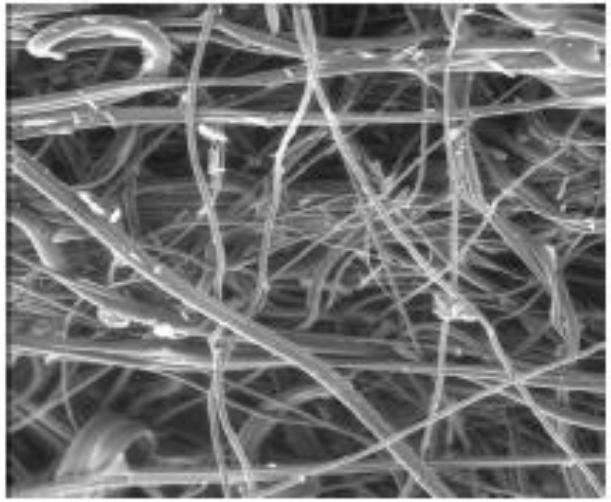




http://www.induceramic.com/porous-ceramics-application/filtration-separation-application

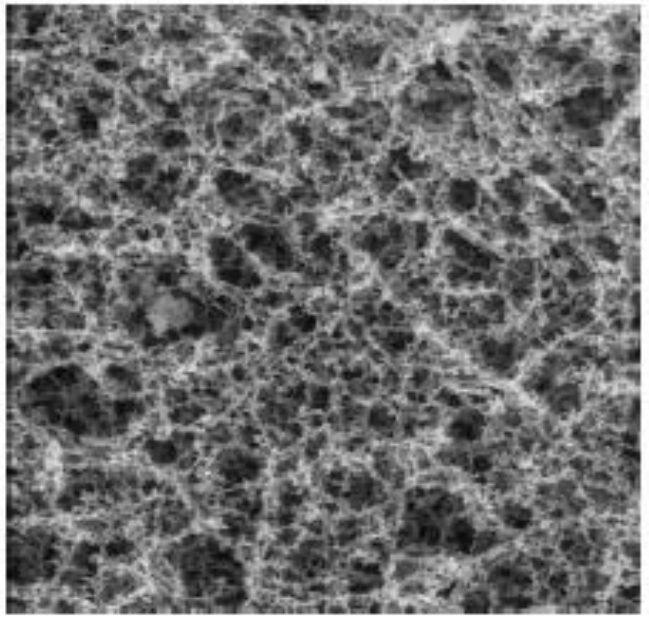
What do Depth Filters look like?

- Fibrous (can shed fibers)
- Difficult to give an accurate pore size rating
- Thick (3 - 30 mm) & often adsorptive
- Give a typical percentage (i.e. 30 - 70%) particle reduction
- Have the greatest capacity
- Examples
  - Microfiberglass
  - String-wound filters
  - Sheet / pad filters



What do Surface Filters look like?

- Fibers locked together by heat or membrane coating
- Given a nominal rating or rated by the filter it protects
- Thin (1 mm or less) & Slightly Adsorptive
- Give a typical percentage (90 - 99.9%) particle reduction
- Examples
  - Cellulose ester coated cellulose
  - Heat-treated polypropylene filters



Filter Selection Process - Update

Compatibility ✓

- Materials of construction
  - Philic or phobic
  - Specific testing

Retention ✓

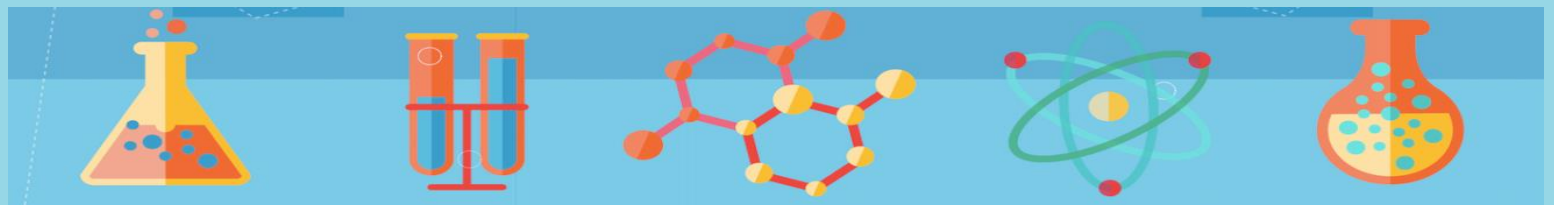
- Media structure
  - Depth, surface, membrane
- Filter configuration
  - Depth or pleated cartridges, pads

Ease of use ✓

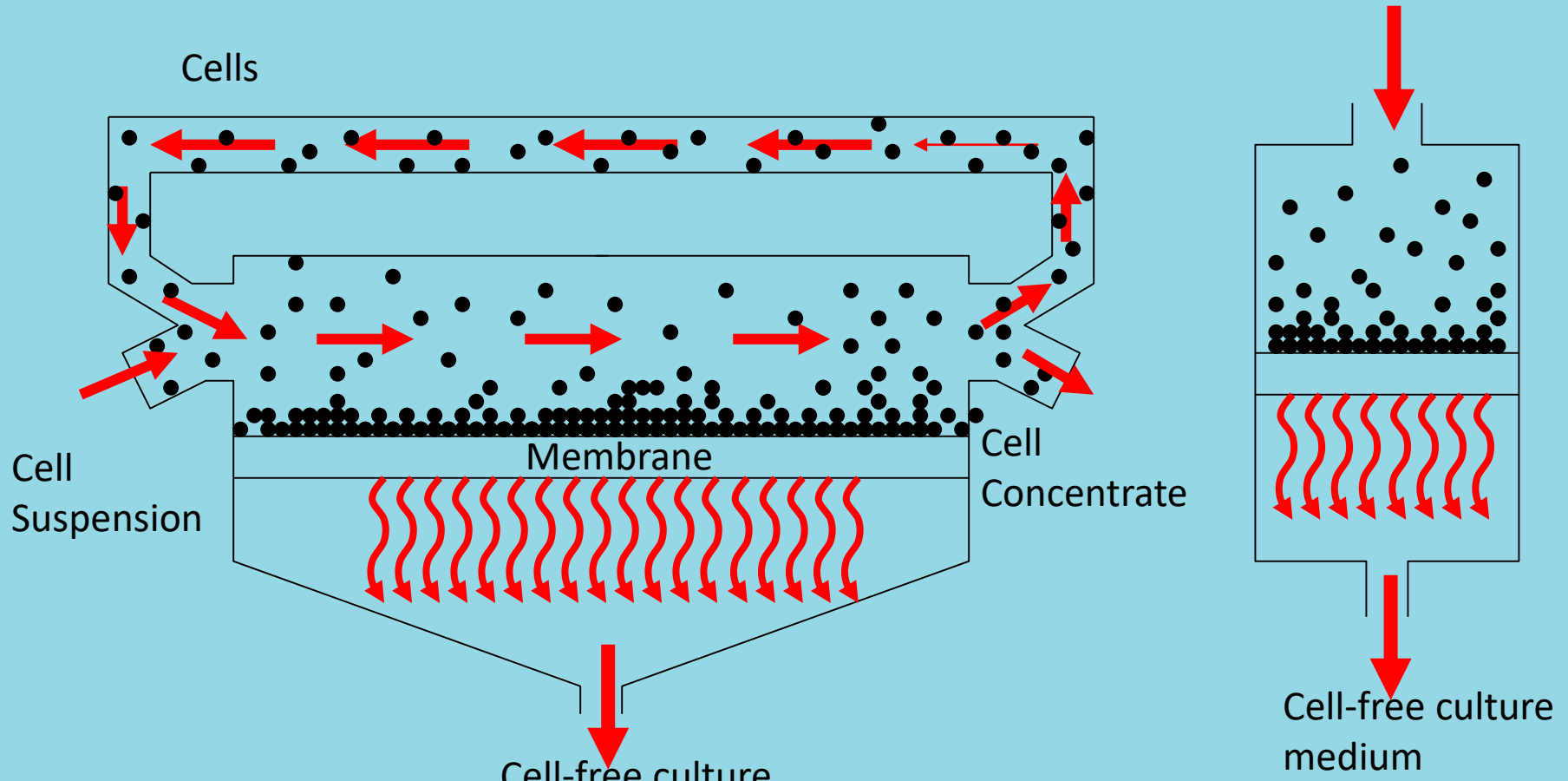
Filter configuration
Cartridges, pads, capsules

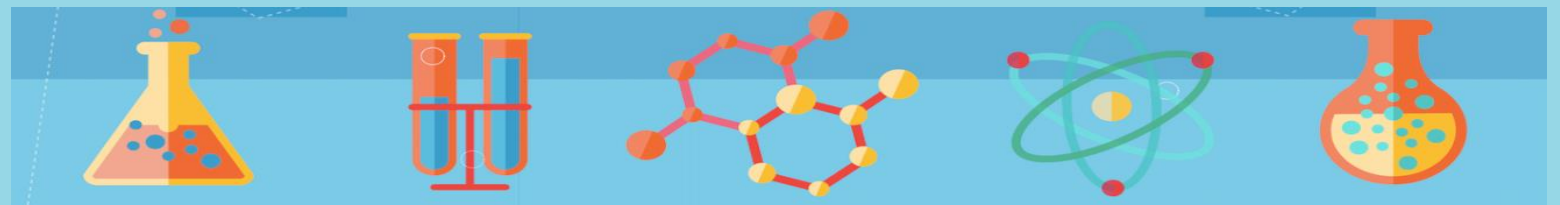
Filtration cost

Filter sizing
Flow rate
Capacity
Price/L



Cell Isolation/Harvesting





Schematic Representation of Filter Modules

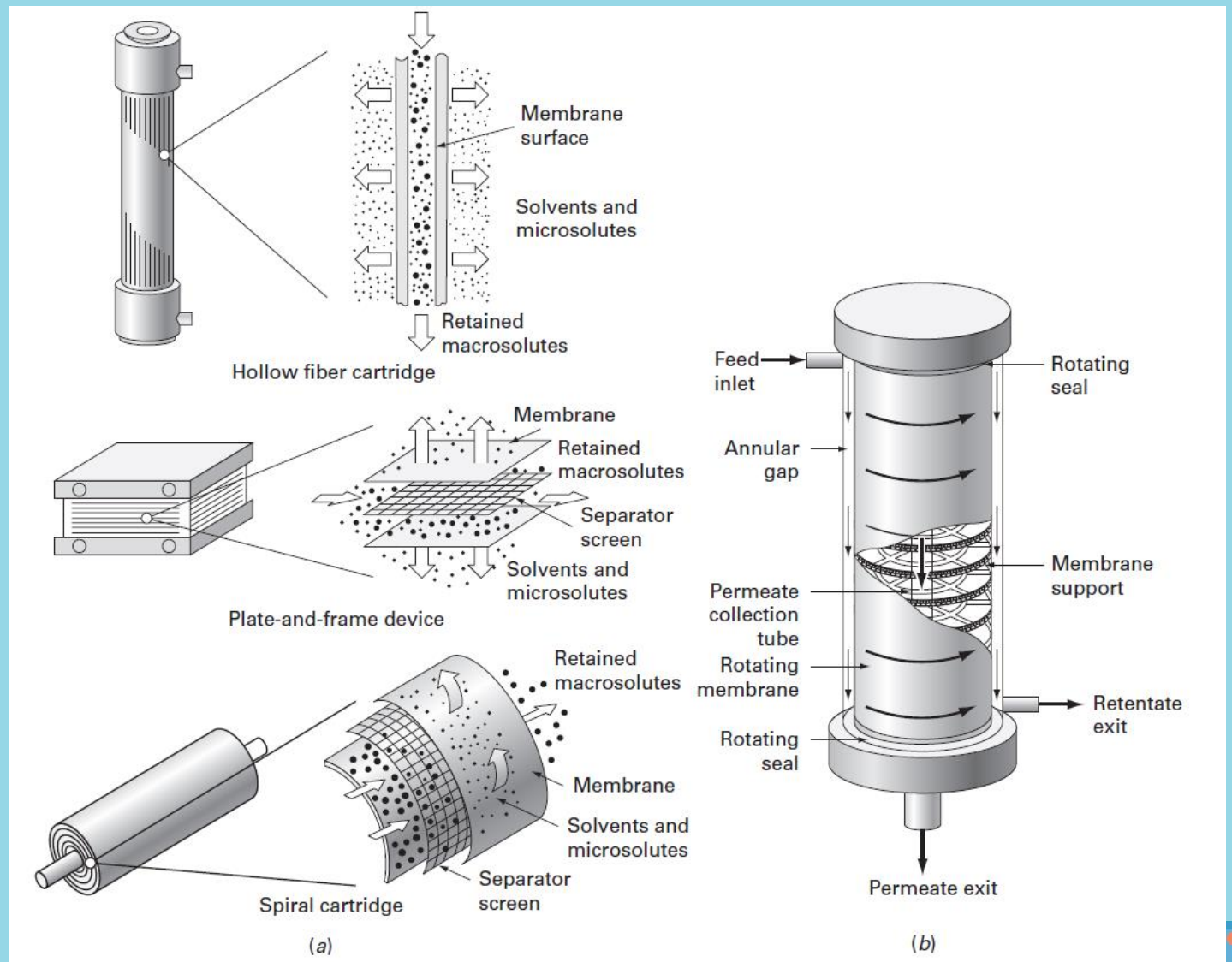
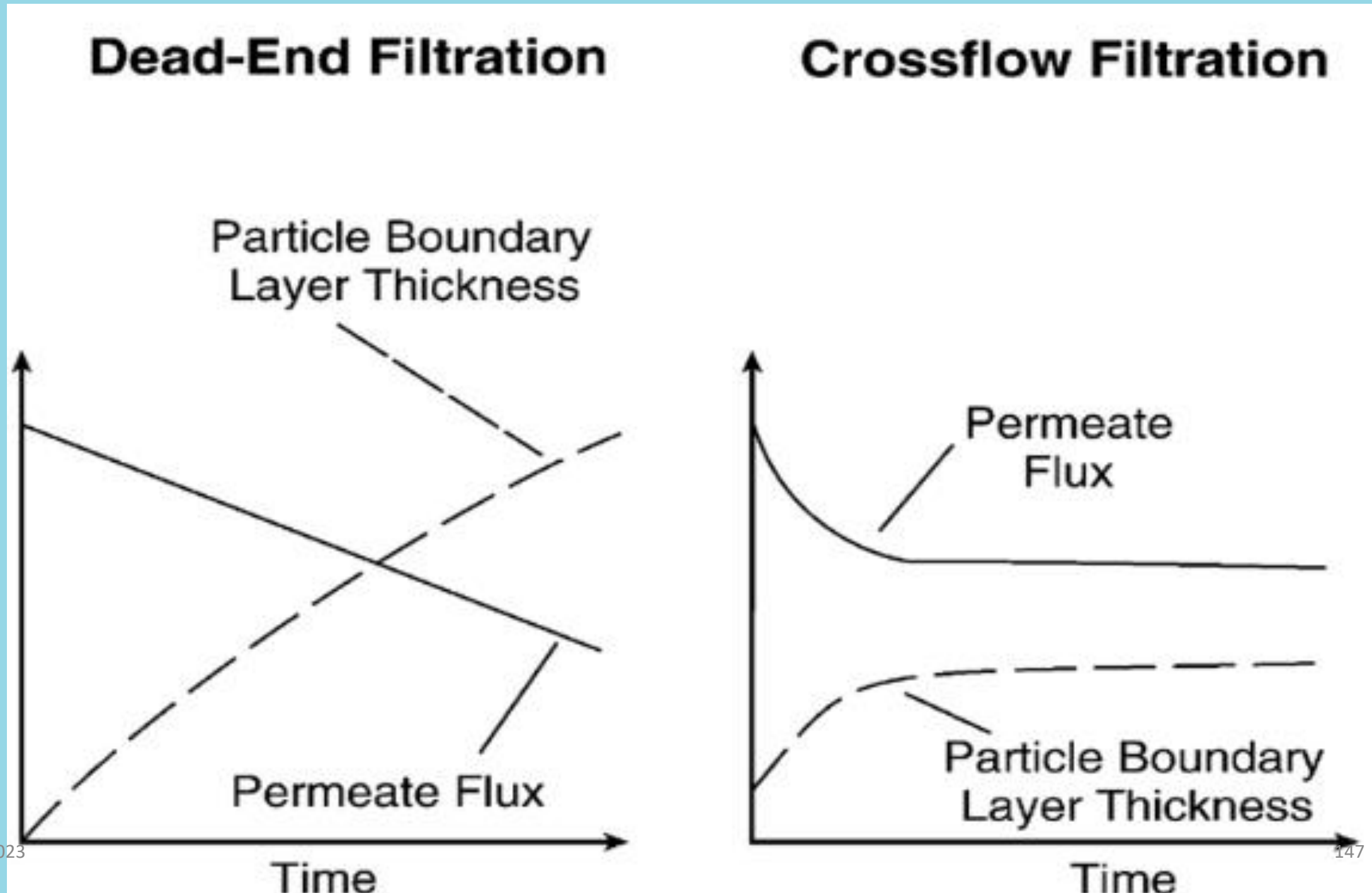


FIGURE 4.11 Schematic representations of filter modules. (*a*) Hollow-fiber, plate-and-frame, and spiral-wound membrane modules. (*b*) A rotating cylinder module.


Dead-End Filtration
Particle Boundary Layer Thickness
Permeate Flux
Time
Crossflow Filtration
Permeate Flux
Particle Boundary Layer Thickness
Time

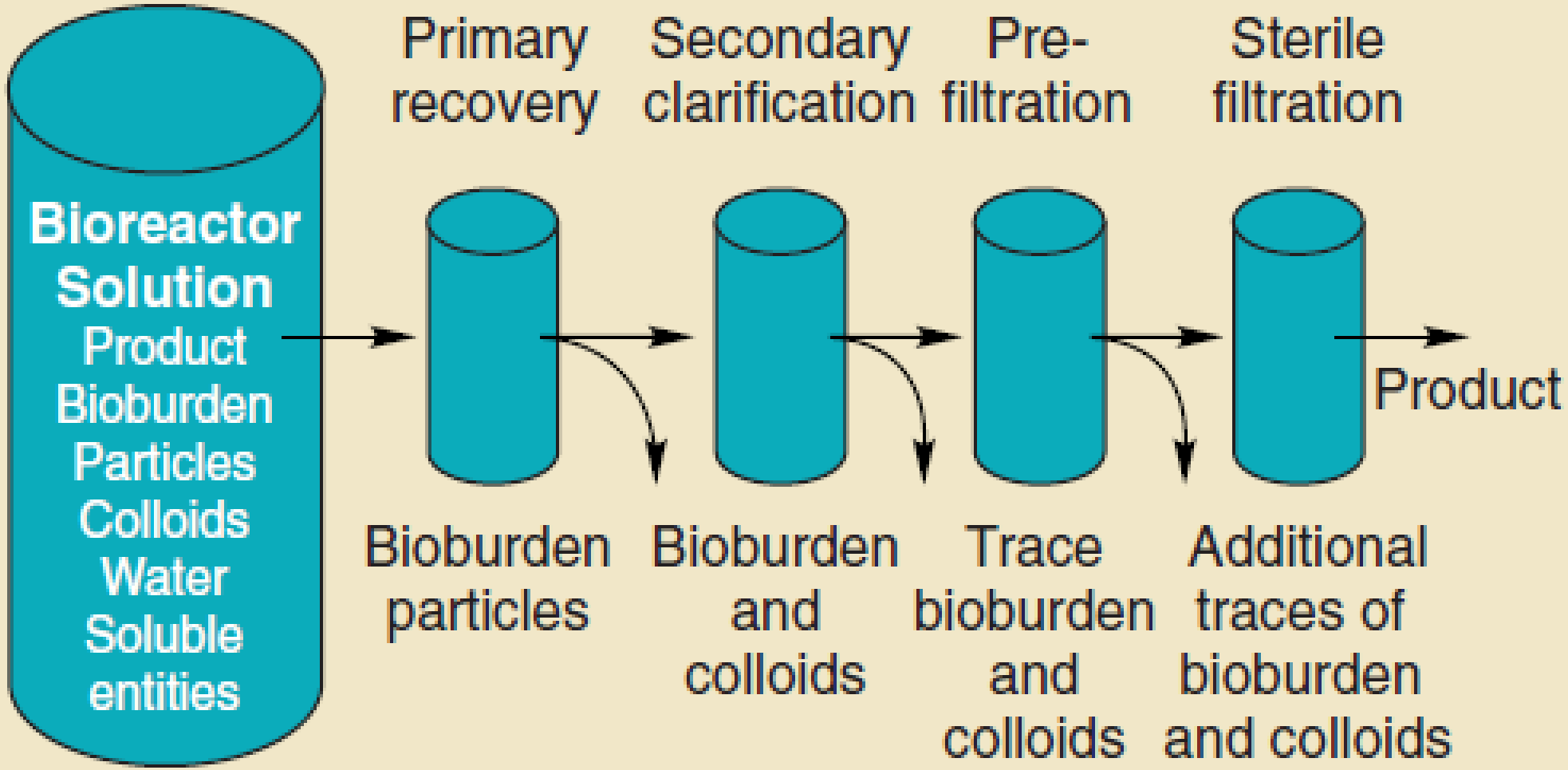


Figure 1: Typical filtration of a biological product.

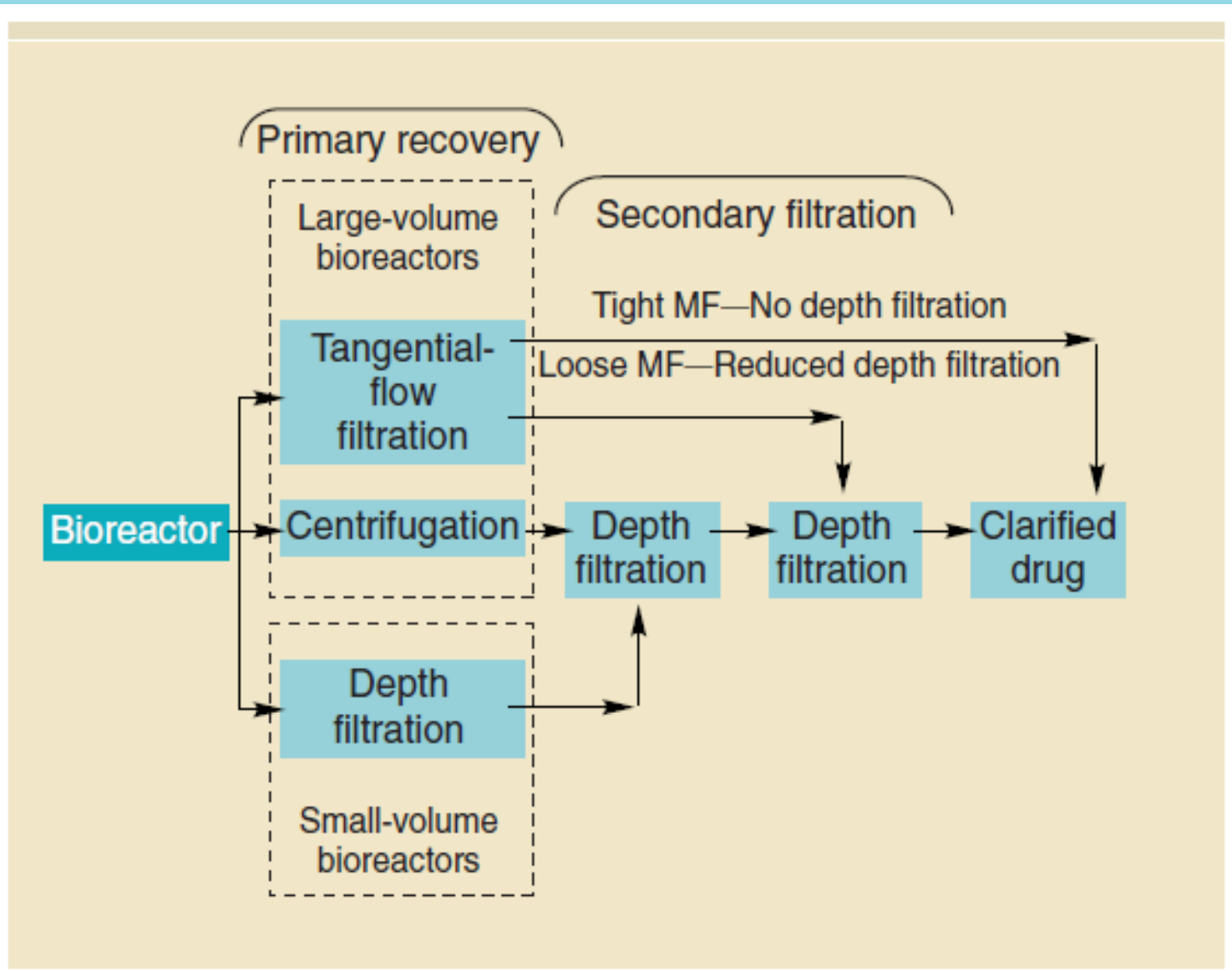
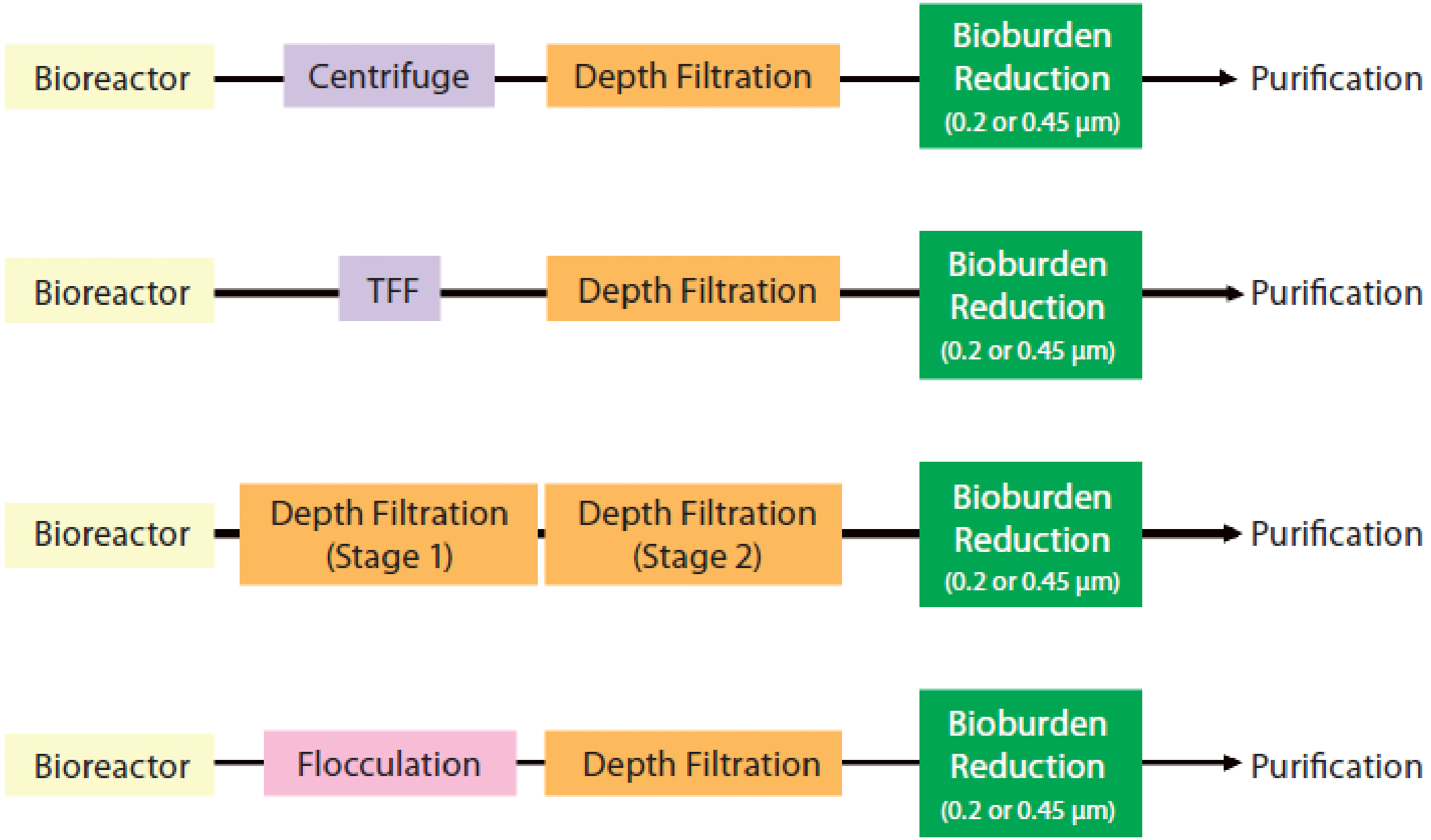
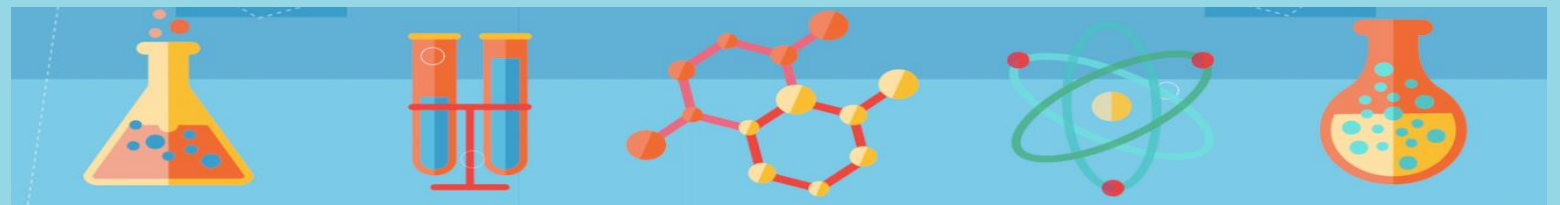


Figure 2: Primary recovery and secondary clarification scenarios for removal of cells, cell debris, and contaminants from a bioreactor process stream.

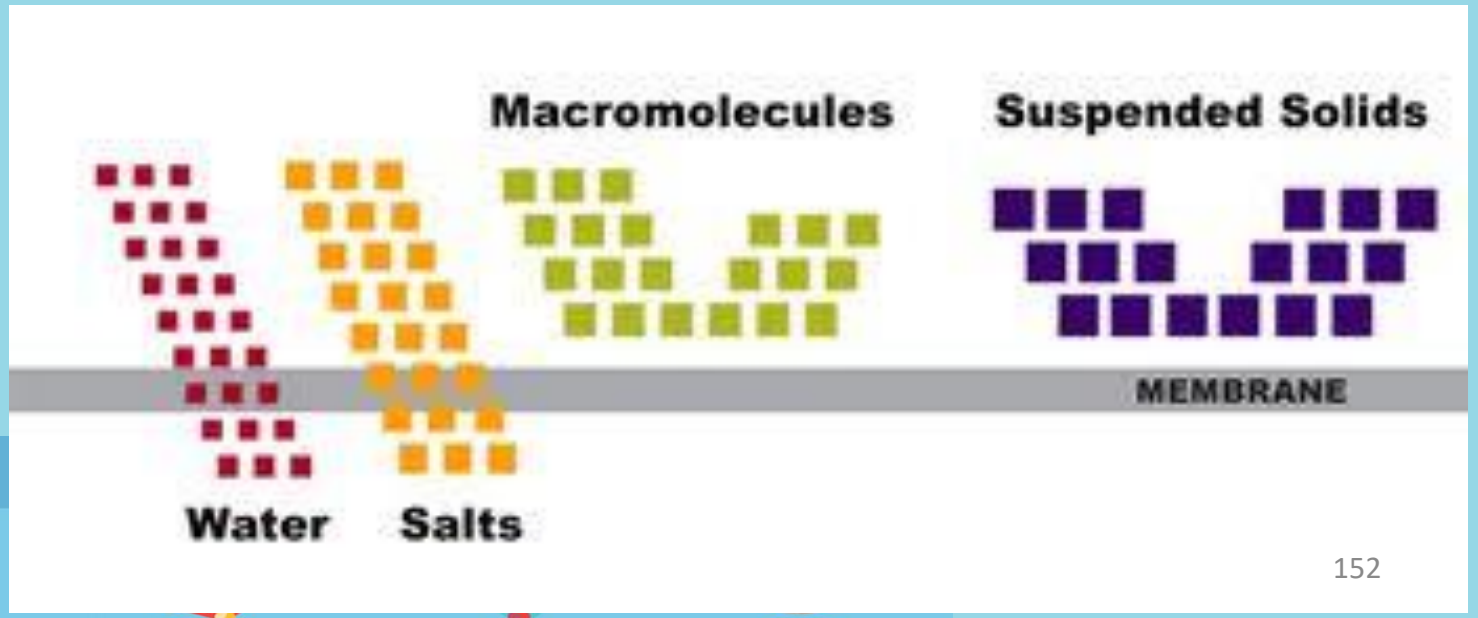

Bioreactor
Centrifuge
Depth Filtration
Bioburden Reduction (0.2 or 0.45 µm)
Purification
Bioreactor
TFF
Depth Filtration
Bioburden Reduction (0.2 or 0.45 µm)
Purification
Bioreactor
Depth Filtration (Stage 1)
Depth Filtration (Stage 2)
Bioburden Reduction (0.2 or 0.45 µm)
Purification
Bioreactor
Flocculation
Depth Filtration
Bioburden Reduction (0.2 or 0.45 µm)
Purification

ULTRAFILTRATION/DIAFILTRATION



Ultrafiltration

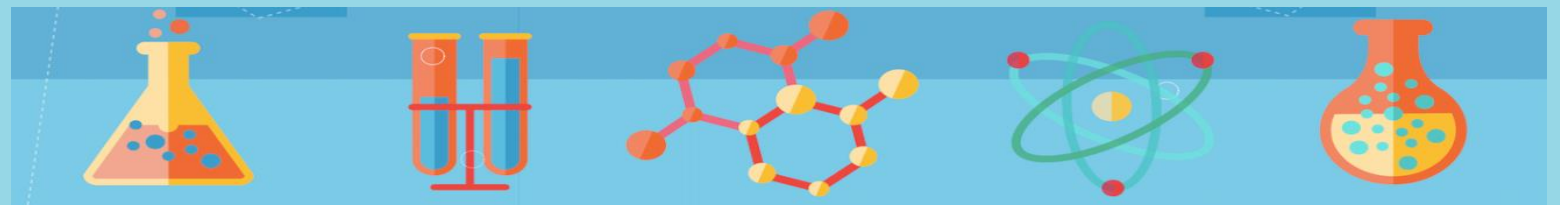
- a variety of membrane filtration in which **hydrostatic pressure forces** a liquid against a **semipermeable membrane**.
- Suspended solids and solutes of high molecular weight are retained, while water and low molecular weight solutes pass through the membrane



Ultrafiltration

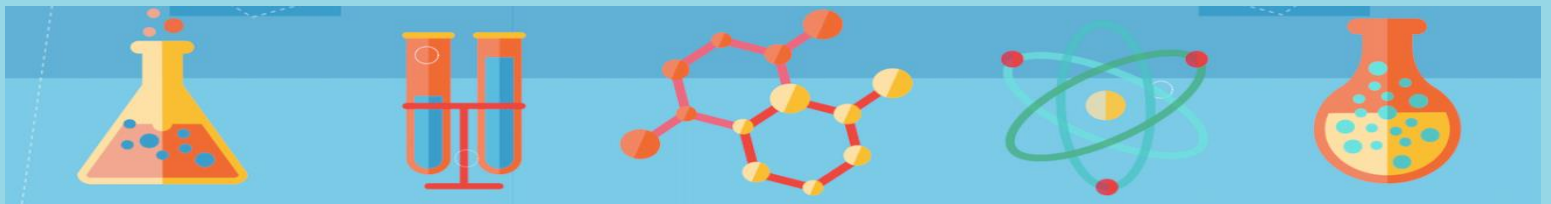
Chromatography

PROTEIN PURIFICATIONS



Protein Isolation & Purification

- After cells (or media) are harvested proteins may be purified/isolated
- Intracellular (inside cell) proteins are harder to purify
  - Require cell disruption, separation, removal of cell debris, DNA, RNA, lipid
- Extracellular (outside cell) proteins are easier to purify
  - No cell disruption needed, just isolate

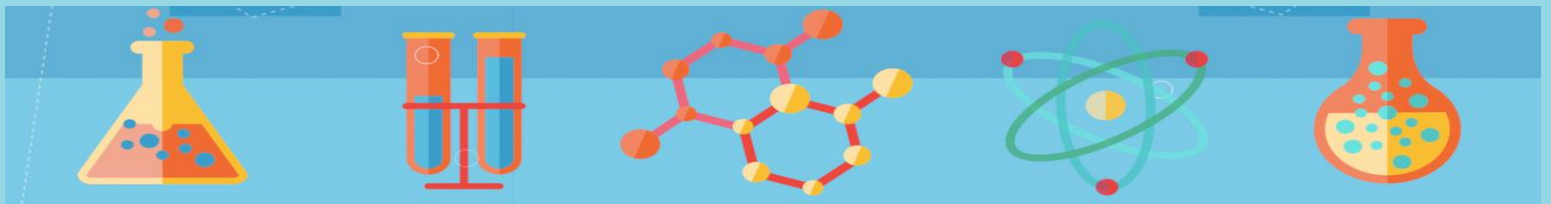


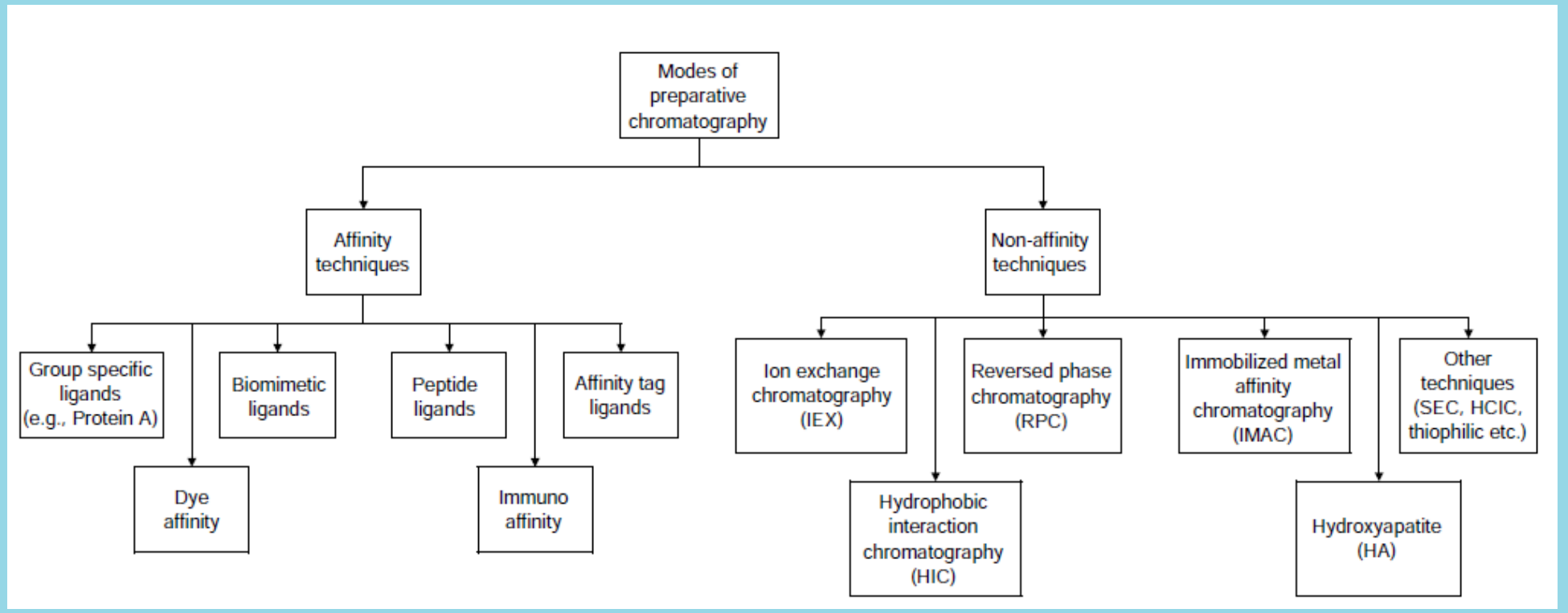
Protein Isolation Methods

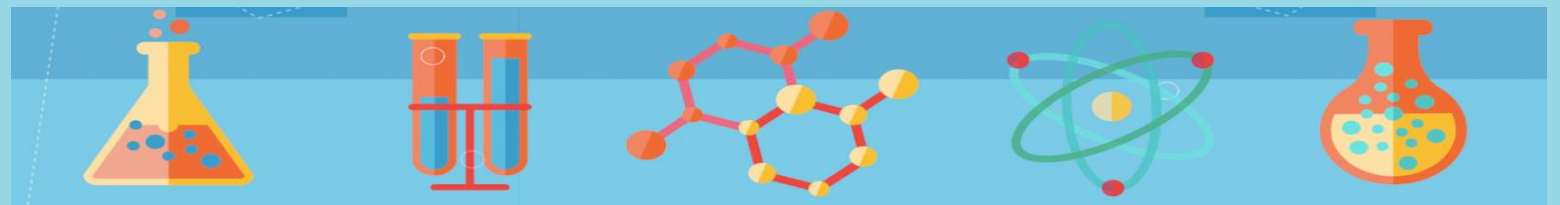
- Differential salt precipitation
- Differential solvent precipitation
- Differential temperature precipitation
- Differential pH precipitation
- Two-phase solvent extraction (PEG)
- Preparative electrophoresis
- ***Column chromatography***

Most purifications require combinations of 2-3 steps

CHROMATOGRAPHY



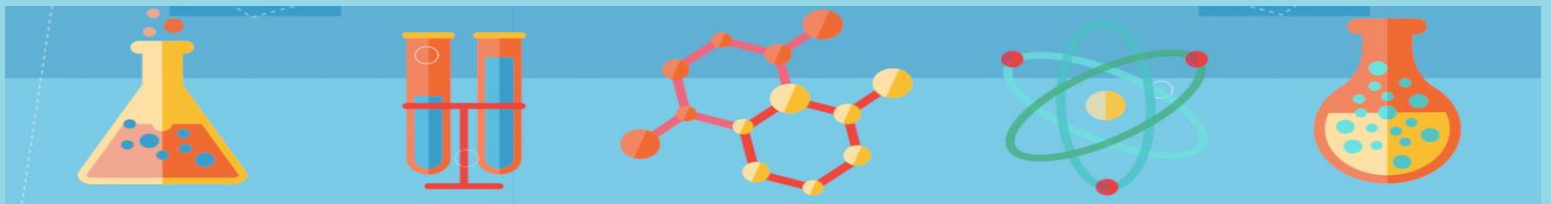

Modes of preparative chromatography
Affinity techniques
Non-affinity techniques
Group specific ligands (e.g., Protein A)
Biomimetic ligands
Peptide ligands
Affinity tag ligands
Dye affinity
Immuno affinity
Ion exchange chromatography (IEX)
Reversed phase chromatography (RPC)
Immobilized metal affinity chromatography (IMAC)
Other techniques (SEC, HCIC, thiophilic etc.)
Hydrophobic interaction chromatography (HIC)
Hydroxyapatite (HA)



Column Chromatography

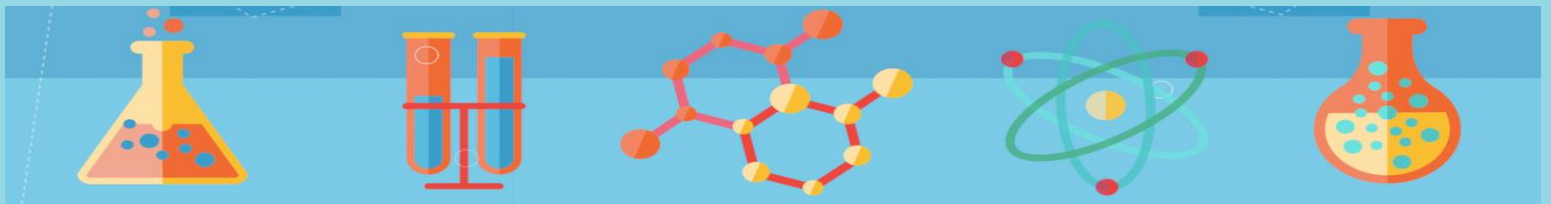
Column Chromatography

- Most common (and best) approach to purifying larger amounts of proteins
- Able to achieve the highest level of purity and largest amount of protein with least amount of effort and the lowest likelihood of damage to the protein product
- Standard method for pharma industry



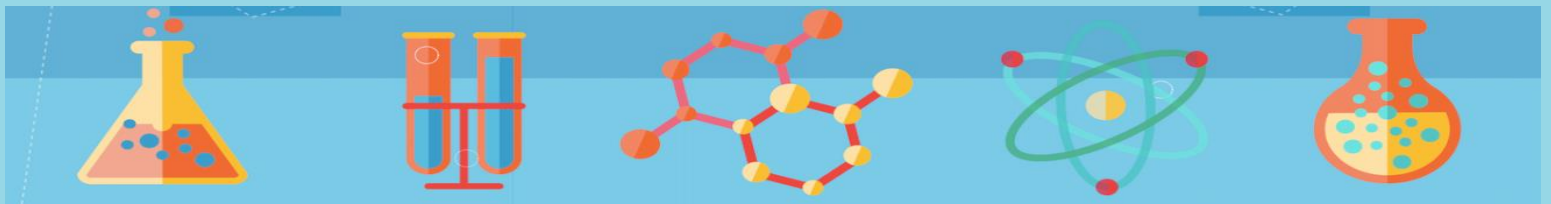
Column Chromatography

- Can be done either at atmospheric pressure (gravity feed) or at high pressure (HPLC, 500-2000 psi)
- Four types of chromatography:
  - Affinity chromatography
  - Gel filtration (size exclusion) chrom.
  - Ion exchange chromatography
  - Hydrophobic (reverse phase) chrom.

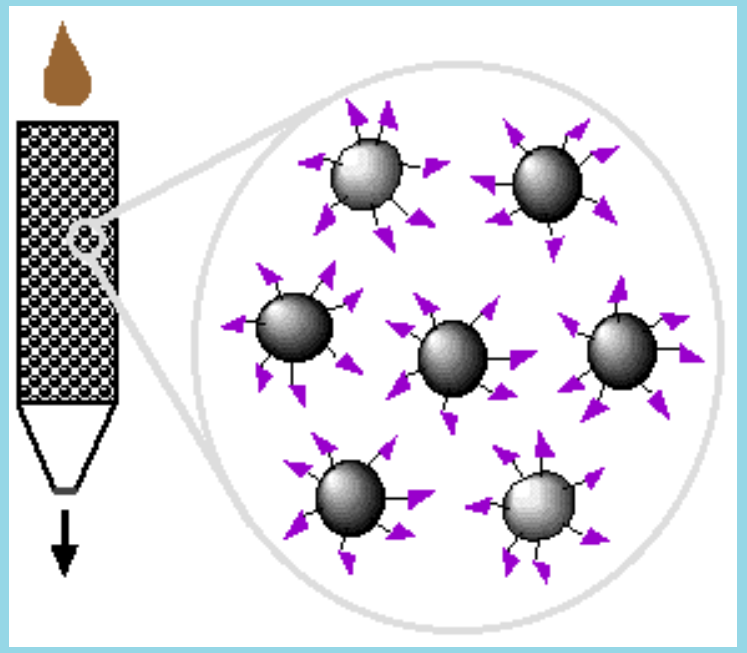


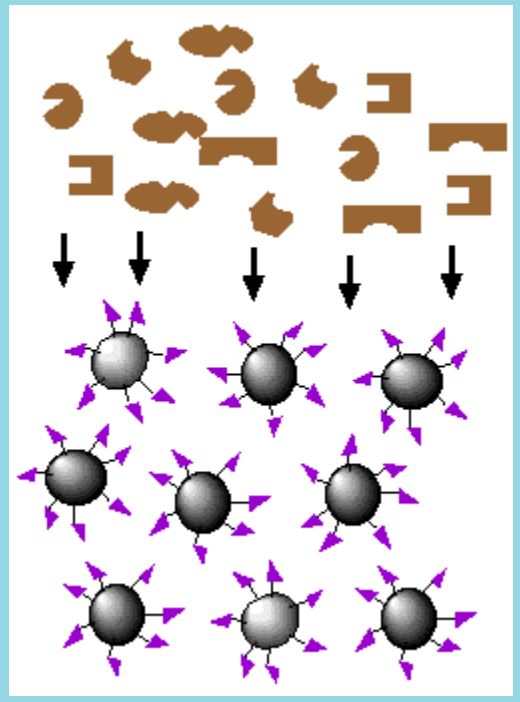
Affinity Chromatography

- Adsorptive separation in which the molecule to be purified specifically and reversibly binds (adsorbs) to a complementary binding substance (a ligand) immobilized on an insoluble support (a matrix or resin)
- Purification is 1000X or better from a single step (highest of all methods)
- Preferred method if possible



Affinity Chromatography

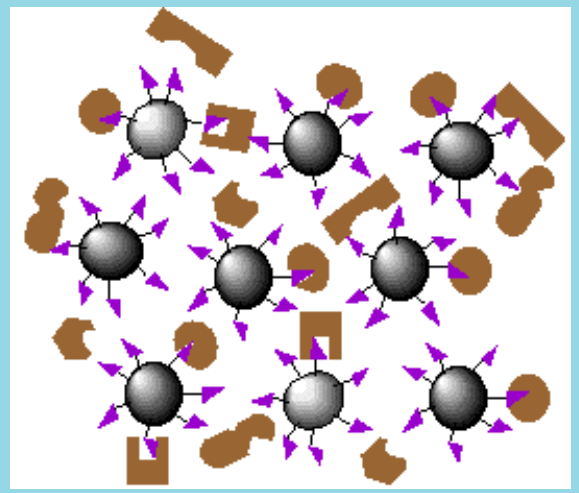




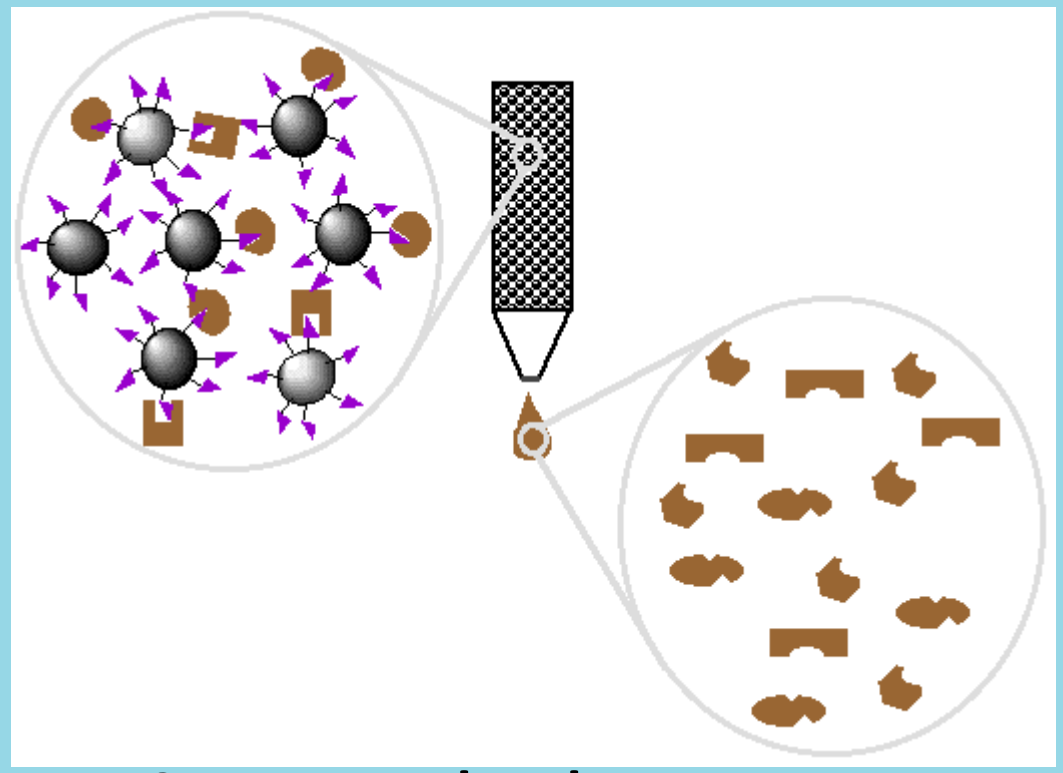
Step 1: Attach ligand to column matrix

Step 2: Load protein mixture onto column

Affinity Chromatography



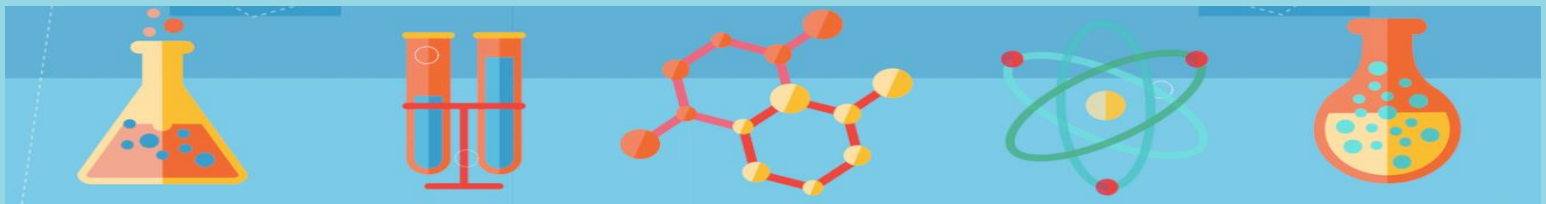
Step 3: Proteins bind to ligands



Step 4: Wash column to remove unwanted material, elute later

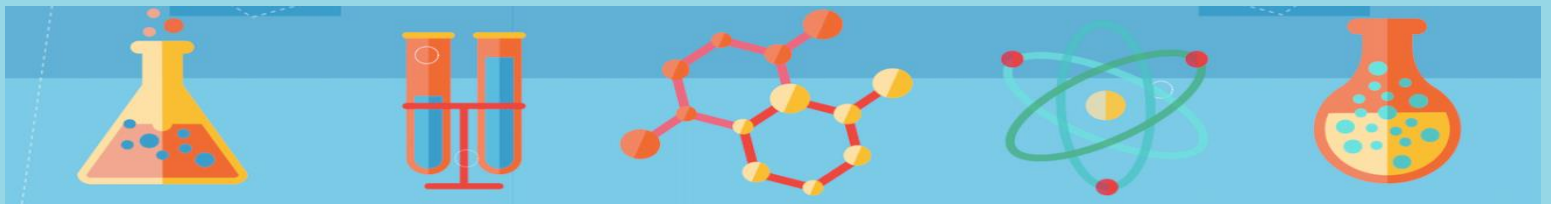
Affinity Chromatography

- Used in many applications
- Purification of substances from complex biological mixtures
- Separation of native from denatured forms of proteins
- Removal of small amounts of biomaterial from large amounts of contaminants

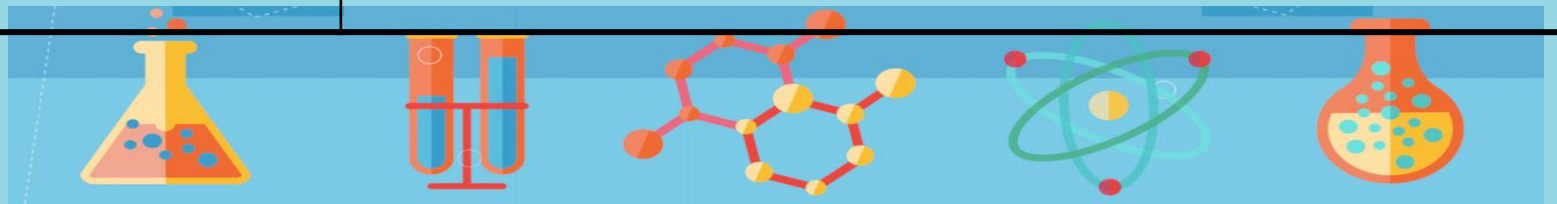


Affinity Chromatography

- The ligand must be readily (and cheaply) available
- Ligand must be attachable (covalently) to the matrix (typically sepharose) such that it still retains affinity for protein
- Binding must not be too strong or weak
- Ideal K_D should be between 10^{-4} & 10^{-8} M
- Elution involves passage of high salt or low pH buffer after binding



Ligand	Specificity
AMP	Enzymes with NAD cofactors an ATP dependent kinases
Arginine	Proteases such as prothrombin, kallikrein, clostripain
Cibacron Blue Dye	Serum Albumin, Preablumin
Heparin	Growth factors, cytokines, coagulation factors
Protein A	**Fc region of immunoglobulins**
Calmodulin	Calmodulin regulated kinases, cylcases and phosphatases
EGTA-copper	Proteins with poly-Histidine tails

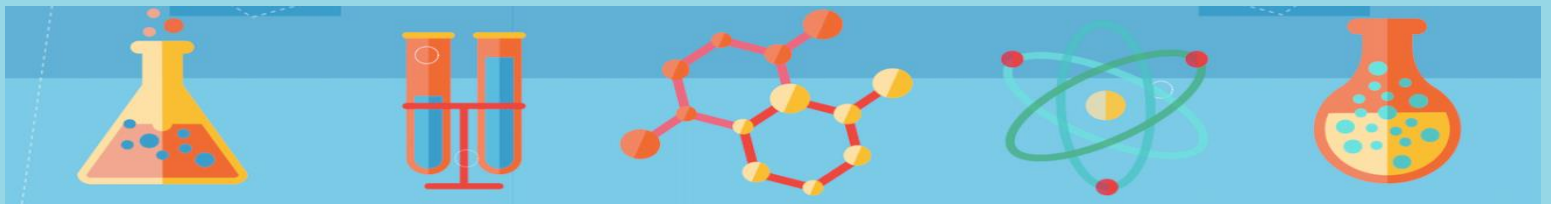


Size Exclusion Chrom.

Molecules are separated according to differences in their size as they pass through a hydrophilic polymer

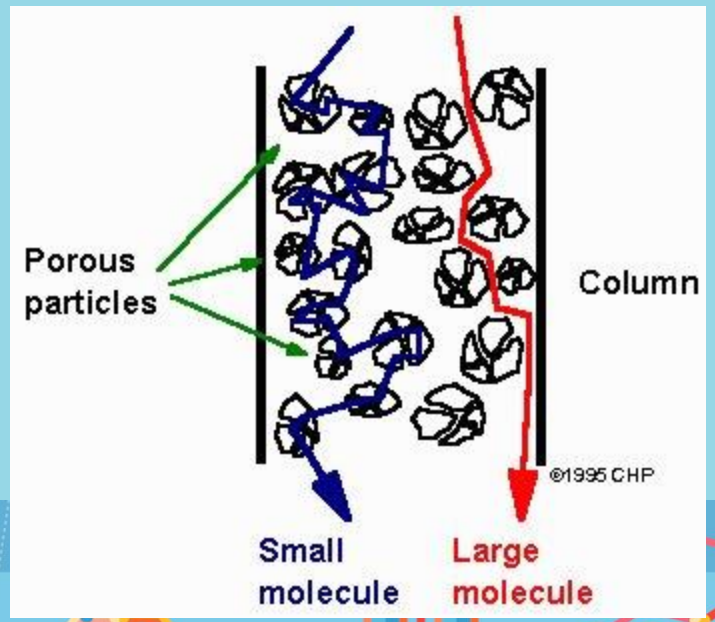
Polymer beads composed of cross-linked dextran (dextrose) which is highly porous (like Swiss cheese)

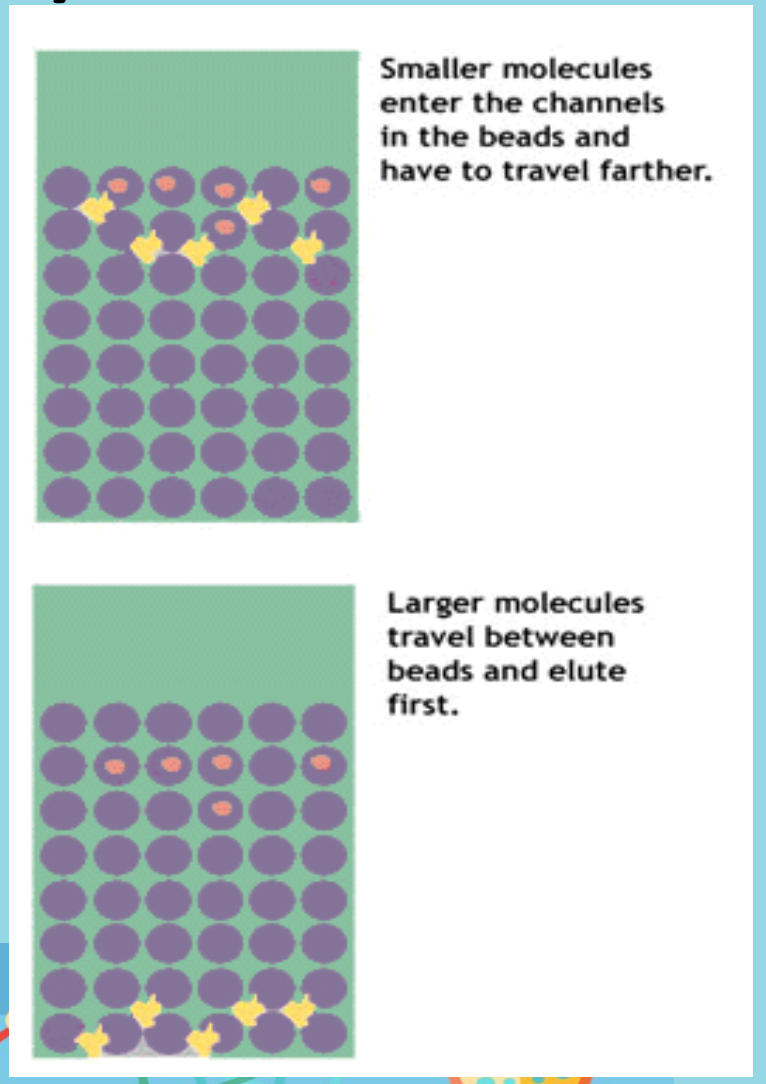
Large proteins come out first (can’t fit in pores), small proteins come out last (get stuck in the pores)



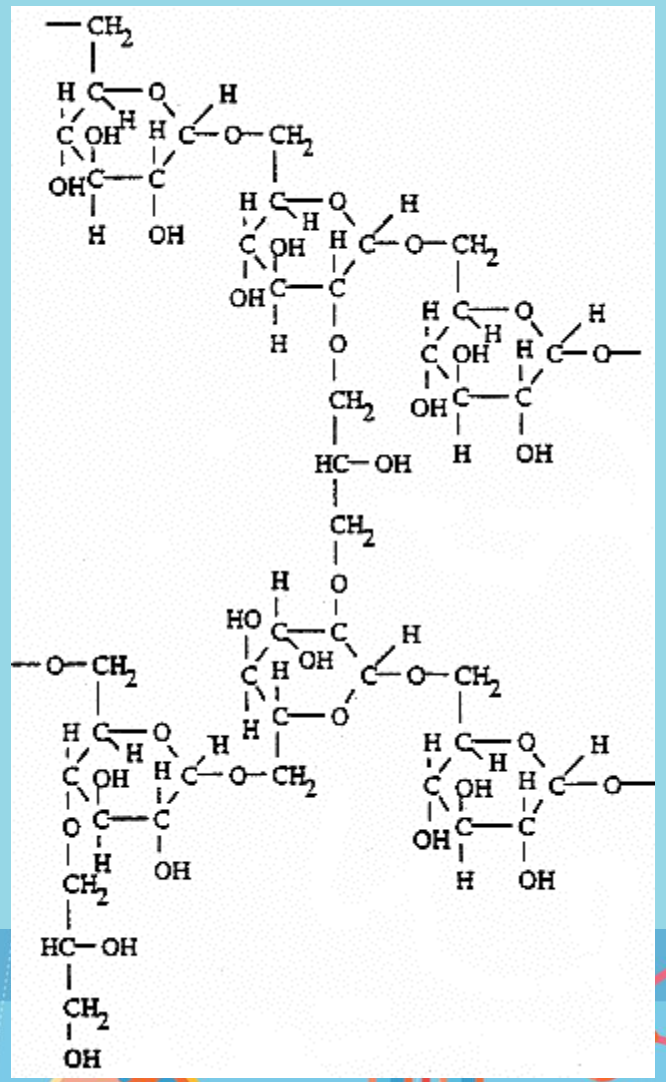
Size Exclusion Chromatography (SEC)

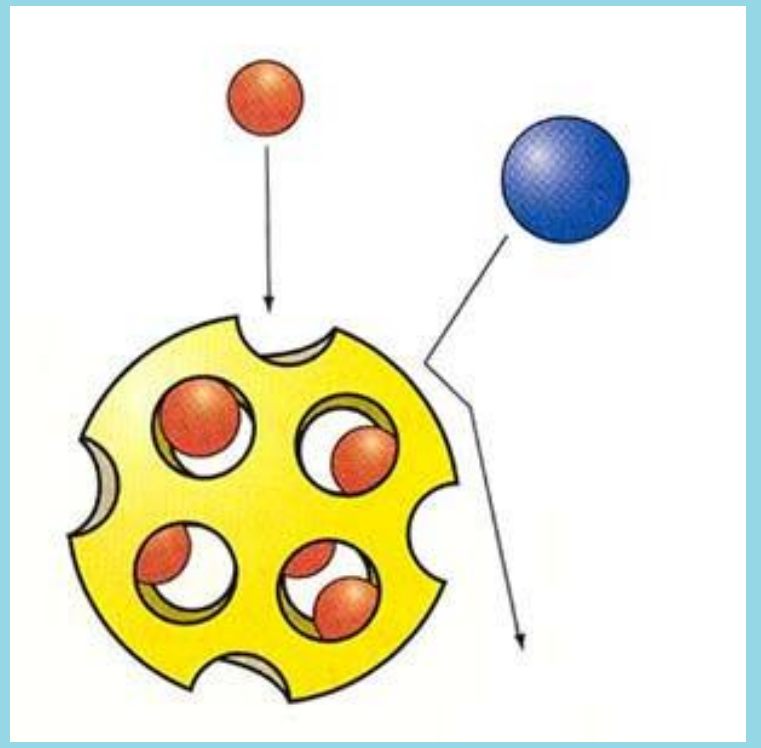
A gel filtration column has beads with channels running through them.





Sephadex Structure



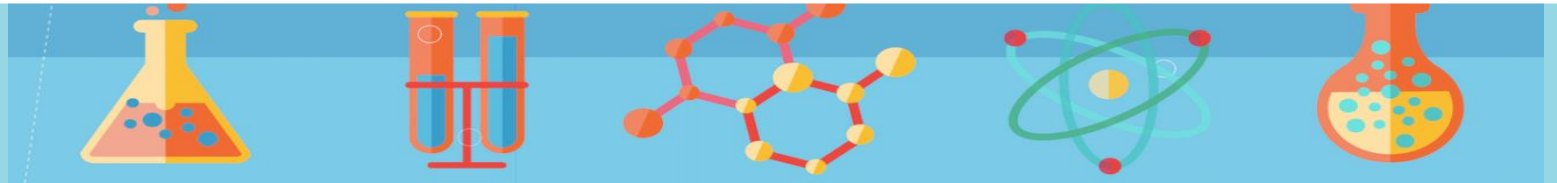


Ion Exchange Chromatography (IEC)

Principle is to separate on basis of charge “adsorption”

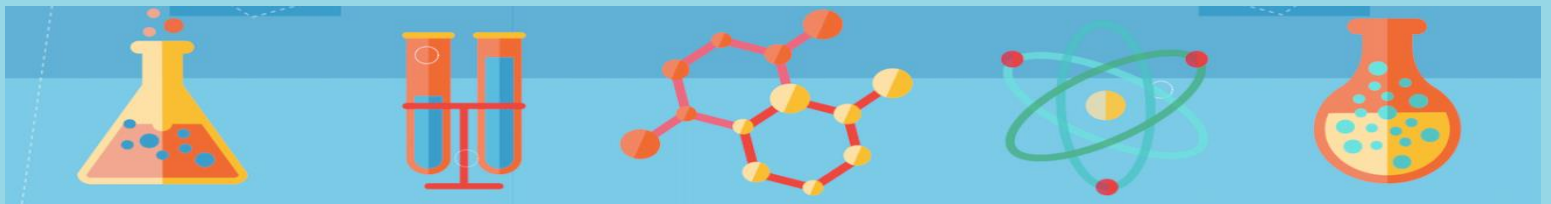
Positively charged proteins are reversibly adsorbed to immobilized negatively charged beads/polymers

Negatively charged proteins are reversibly adsorbed to immobilized positively charged beads/polymers

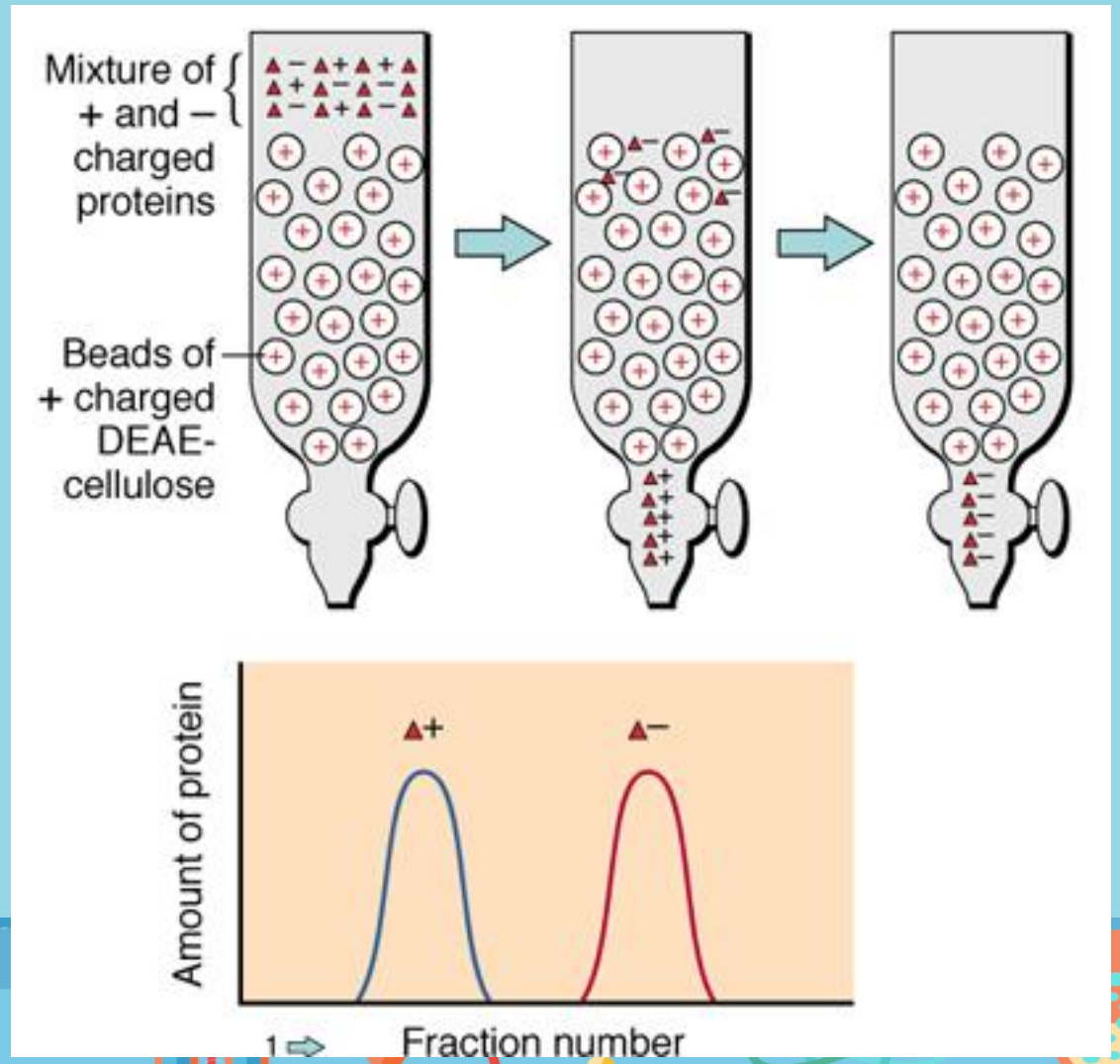


Ion Exchange Chromatography

- Has highest resolving power
- Has highest loading capacity
- Widespread applicability (almost universal)
- Most frequent chromatographic technique for protein purification
- Used in ~75% of all purifications



IEC Principles

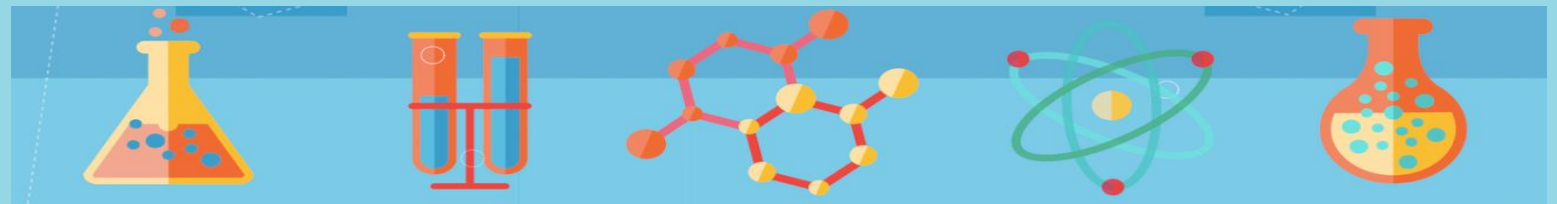


IEC Nomenclature

Matrix is made of porous polymers derivatized with charged chemicals

Diethylaminoethyl (DEAE) or Quaternary aminoethyl (QAE) resins are called anion exchangers because they attract negatively charged proteins

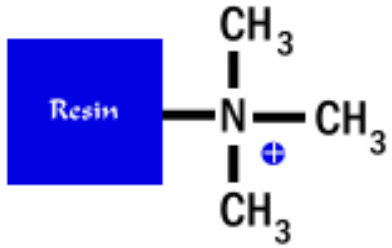
Carboxymethyl (CM) or Sulphopropyl (SP) resins are called cation exchangers because they attract positively charged proteins



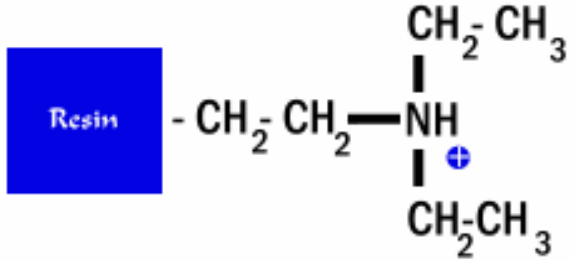
IEC Groups

S-cation exchanger

CM-cation exchanger



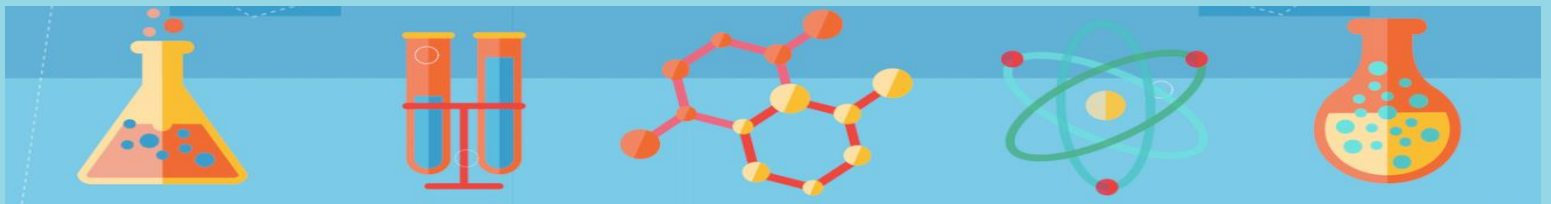
Q-anion exchanger



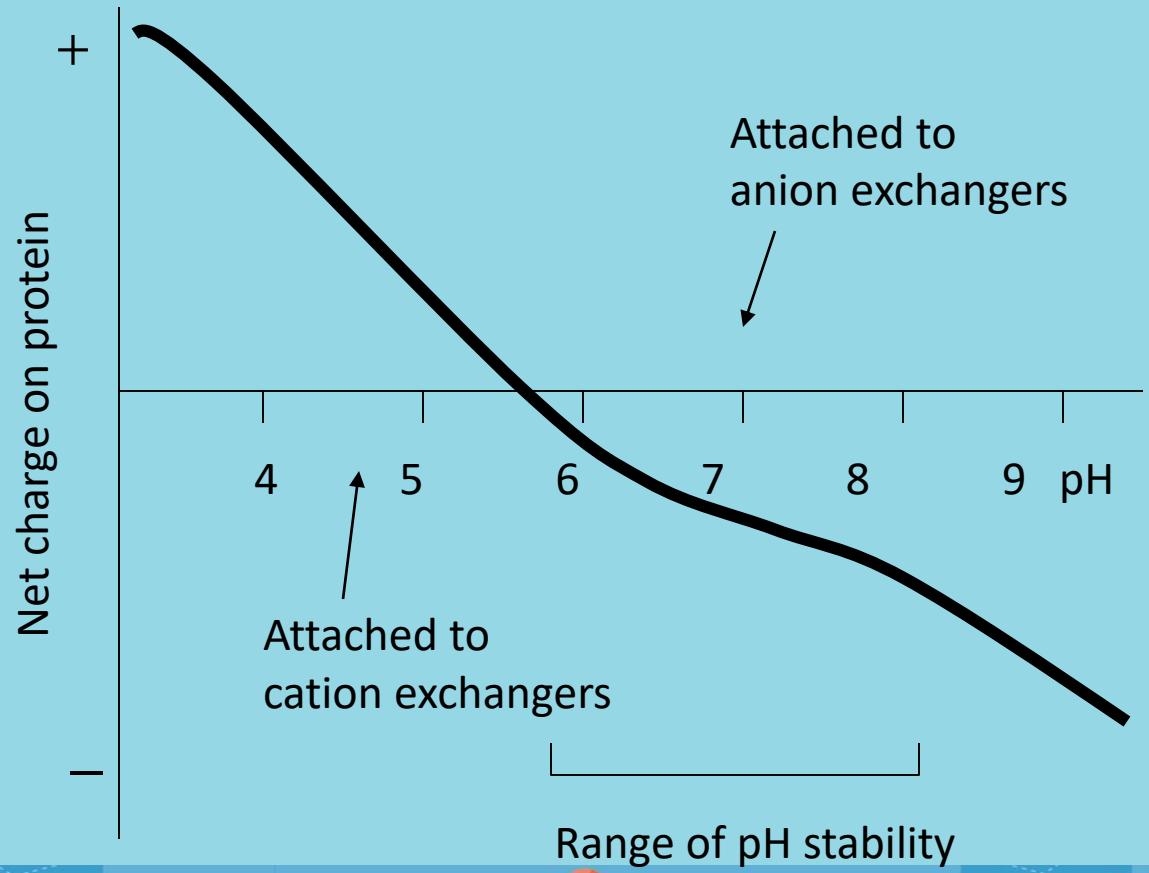
DEAE-anion exchanger

IEC Techniques

- Strong ion exchangers (like SP and QAE) are ionized over a wide pH range
- Weak ion exchangers (like DEAE or CM) are useful over a limited pH range
- Choice of resin/matrix depends on:
  - Scale of separation
  - Molecular size of components
  - Isoelectric point of desired protein
  - pH stability of the protein of interest

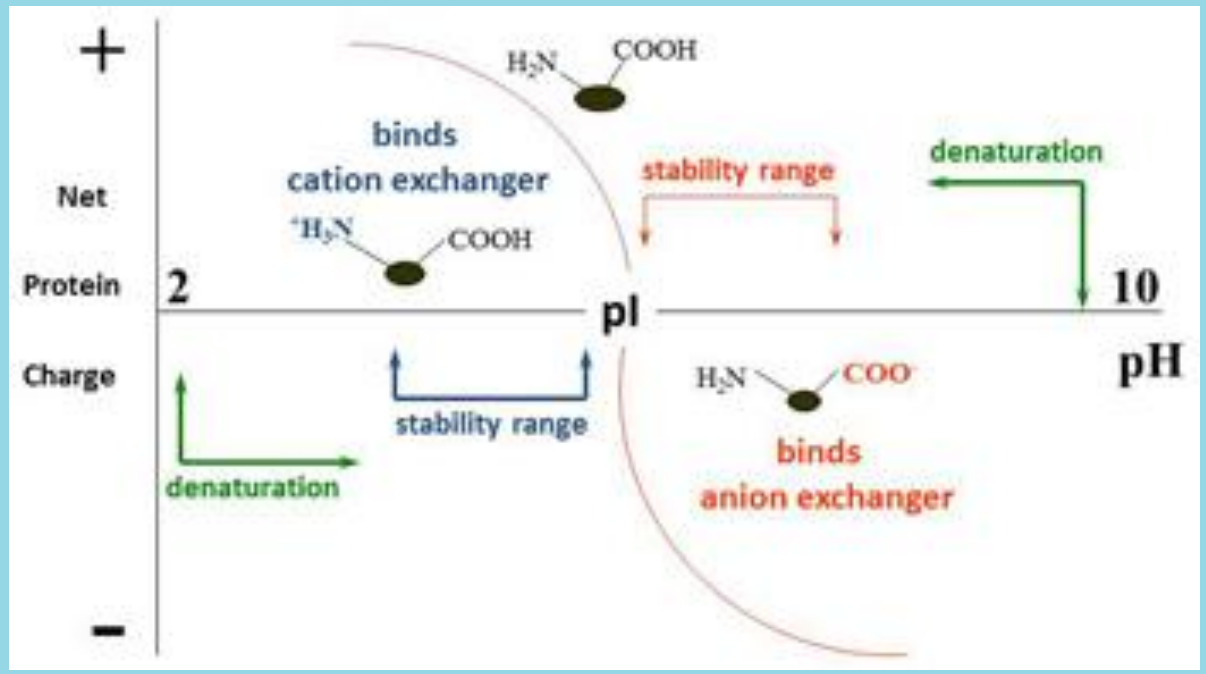


Protein pH Stability Curve



IEC Rules of Thumb

- If a protein is most stable below its pI, a cation exchanger should be used
- If a protein is most stable above its pI, an anion exchanger should be used
- If stability of the protein is known to be good over a wider pH range then either type of ion exchanger can be used



Technical Drivers in Downstream Processing

- **Increased expression levels and yields**
  - **In 2,000, titers of 0.5 g/L were common and 1 g/L was very high**
  - **Currently, titers of 1 g/L are common while titers in the range of 2 – 10 g/L or more are being reported**
- **Increased yields due to increased efficiency**
  - **Downstream yields have risen from 50% to 60-70%**
- **Long downstream processing times**
  - **No economy of scale – Driven by total product mass**
  - **Reduced process space and flexibility**
  - **Increased risk of product degradation, contamination**

U. Gottschalk, PharmTech, Future of Downstream Processing, May 1, 2011

C. Scott, BioProcess International, September 2008, pp. 1-7

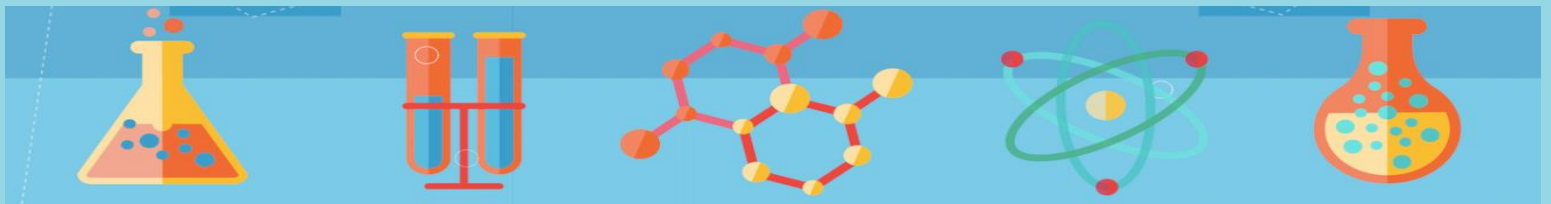
Technical Drivers in Downstream Processing (2)

- **Increased manufacturing costs**
  - **Downstream purification costs have risen to 50 – 80% of total production costs (1/4 of total COG of a biopharmaceutical company)**
- **Increased flexibility, process development**
  - **Larger variety of new products, host cell systems**
- **More extensive validation [Q8(R2)]**
  - **Quality by Design (QbD) – DOE Concepts**
    - **Process design space: Relationship between process parameters and Critical Quality Attributes (CQA)**
  - **Monitoring process parameters and attributes – Use process analytical technology (PAT) if available**

C. Scott, BioProcess International, September 2008, pp. 1-7

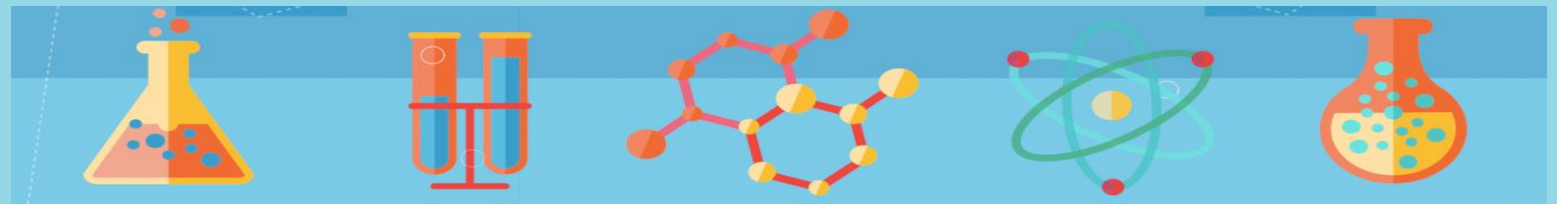
New Downstream Process Approaches (1)

- **Dealing with high titers and increasing speed**
  - **Enhanced capacity adsorption systems**
    - **> 50 mg product/ml of device**
  - **High throughput, low pressure drop, convective mass transfer devices**
    - **Membranes, monoliths**
  - **Non-chromatographic approaches**
    - **Precipitation, crystallization, aqueous two phase (ATP) extraction**
  - **Improved cell removal approaches**
    - **Flocculation step with PEI, $CaCl_2$, etc.**
    - **Enhanced depth filtration, tangential flow filtration (TFF)**



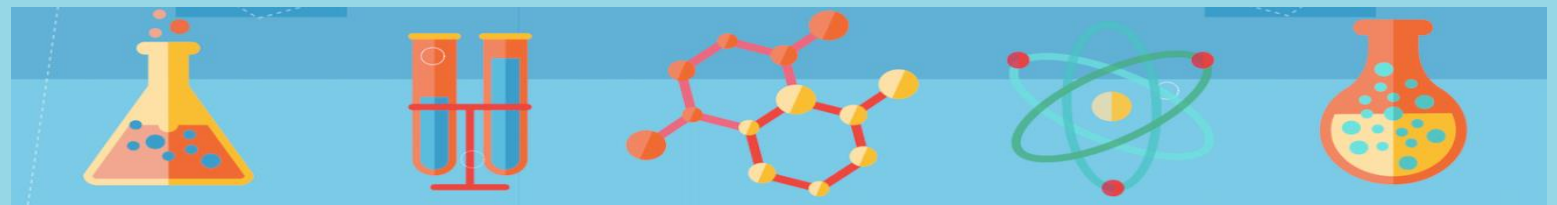
New Downstream Process Approaches (2)

- **Reducing production costs**
  - **Replacement of Protein A in capture step**
    - **Protein A contributes to 35% of total raw materials costs in downstream purification**
    - **Harsh elution, wash conditions**
    - **Camelid antibodies, peptides, organic molecules, mixed-mode adsorbents**
  - **Reduce number of process steps**
    - **Robust, inexpensive affinity ligands for a variety of targets**
    - **Novel adsorption systems for DNA plasmid, viruses, etc.**
    - **Process integration: Expanded Bed Chromatography (EBC), Simulated Moving Bed (SMB) Chromatography**
  - **Disposable separation devices**
    - **Compatible with disposable bioreactors**



New Downstream Process Approaches (3)

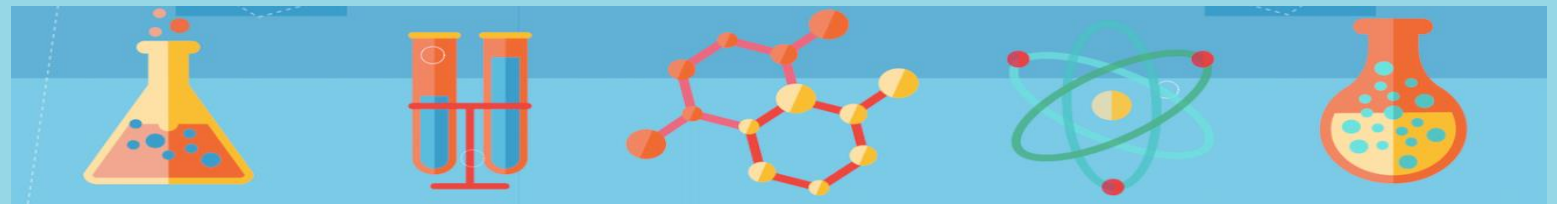
- **Dealing with more extensive validation**
  - **Rapid process analysis and process development strategies**
    - **High throughput screening, expert systems, improved process models**
  - **Implement more in-line process analyzers and PAT**
    - **Develop protein product and impurity sensors for upstream and downstream – Critical Quality Attributes**
    - **Reduce cycle times, process variations**
    - **Process control, potential in-line validation**
- **Increasing flexibility and speed in plant**
  - **Facility of the Future (FoF)**
    - **Disposables, purification platforms, smaller footprint**



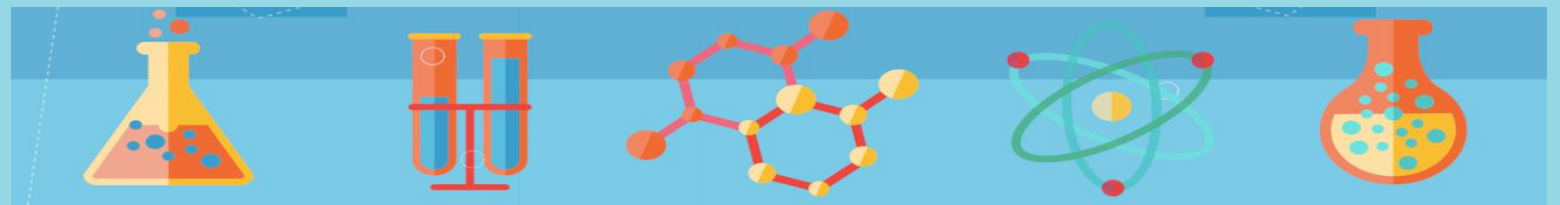
Status of Disposable Downstream Processing

- Single use disposable downstream technologies available at the 1,000 L scale (TFF, UF)
- Capital spending can be reduced by 40% and project timelines by 30%
- Major reductions due to elimination of CIP and SIP utilities
- Disadvantages
  - Volume limitations in TFF, Chromatography
  - Less automation capability
  - Risk of leachables/extractables and leakage
- Disposable sensors are in their infancy

- As with USP, there are key advantages to using SUT versus conventional, re-usable systems in DSP: (1) lower investment costs, (2) reduced development and implementation times, (3) reduced qualifi cation and maintenance expenses and (4) increased fl exibility [Laukel et al. 2011]. However, compared to the rapid development of SUS in USP and its potentially complete application, the situation in DSP has been different. Disposable mixers up to 1000 L and disposable versions of classic microfi ltration (0.1/0.2 µm) and depth fi ltration systems have already become mainstream. The latter have even allowed cell separations in high cell density culture processes (fed batch) with animal cells up to 1000 L scale [Dudziak, 2010]. Alternatively, single-use centrifuges such as the UniFuge (Carr Centritech) are available for cell separation.



FORMULATIONS & FILLING



Why Formulations ?

- Proteins are often sensitive to heat, denaturation from liquid shear, or denaturation at air-liquid interfaces
- Basically, formulation contains
  - salt (tonicity adjustment)
  - optimal pH solution (stability, physiological pH)
  - Aid lyophylization
  - Detergent
- Try to avoid animal and human source excipients

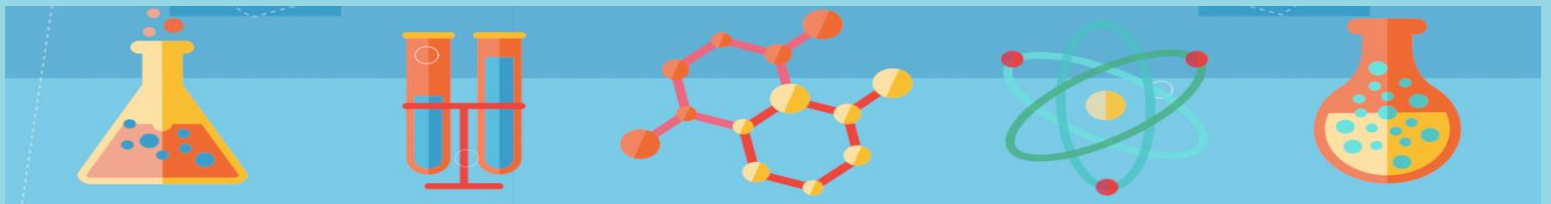
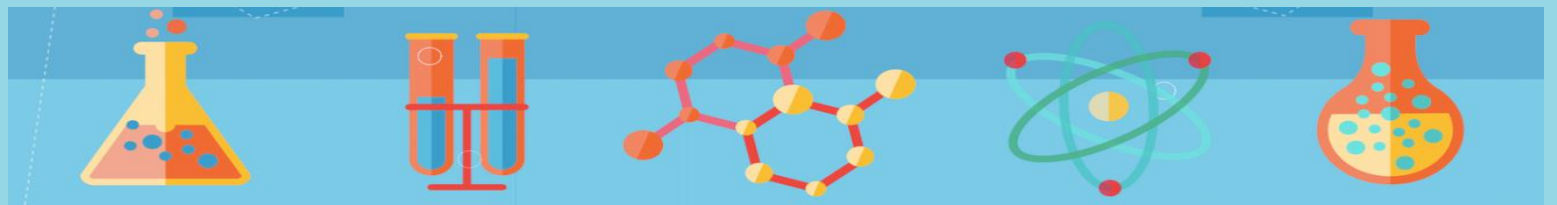
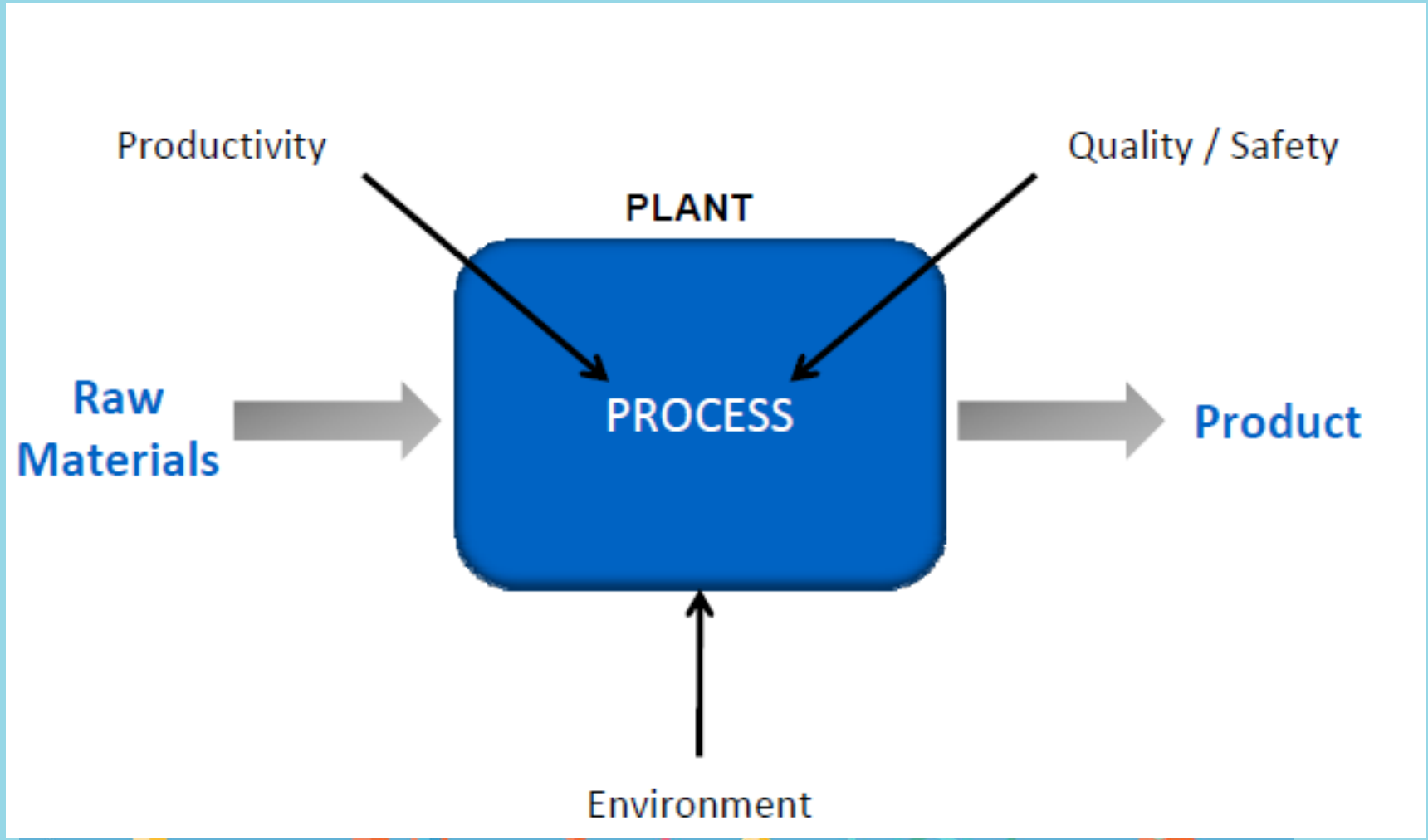


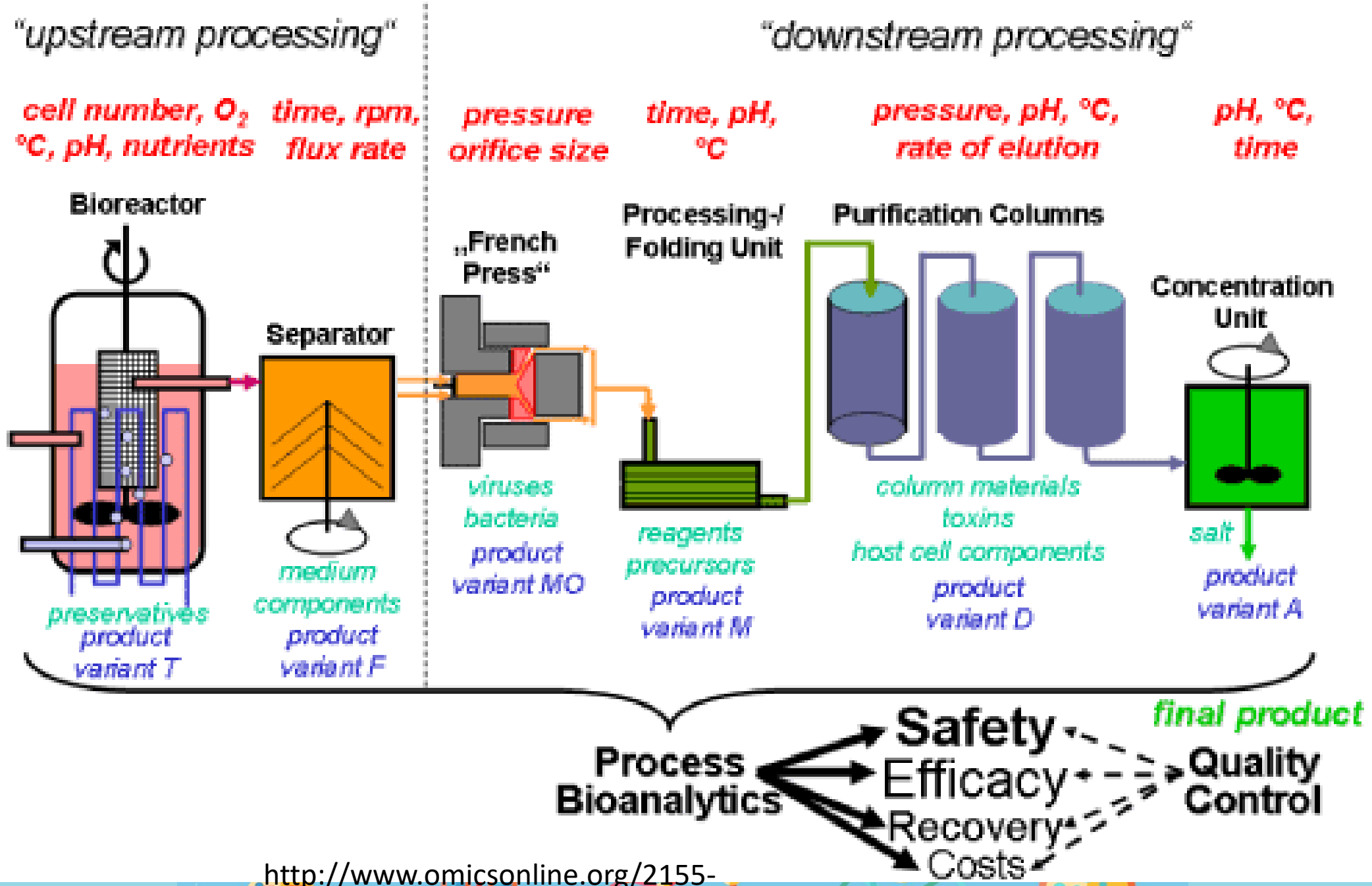
Table 1: Commonly Used Excipients for Biotherapeutics

Sugars	Trehalose	**Amino Acids**	Histidine
	Mannose		Aspartic acid
	Sucrose		Alanine
	Dextrose		Glutamic acid
Polyols	Sorbitol	**Polymers**	Polysorbate
	Mannitol		Albumin
	Glycerol		Gelatin



The Manufacturing operation- opportunities for measurements and standards

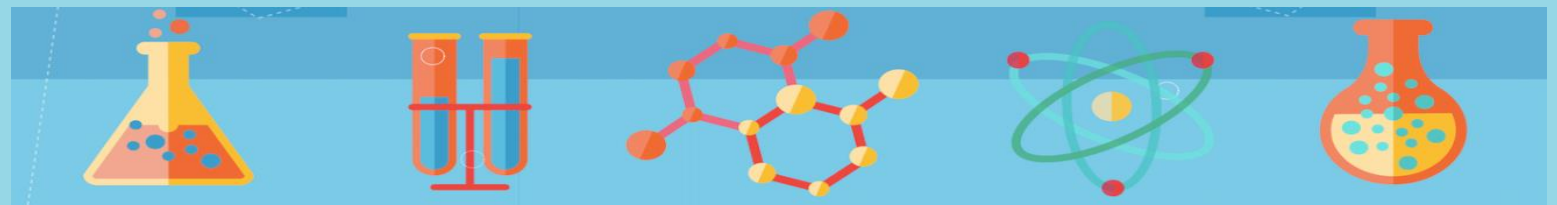




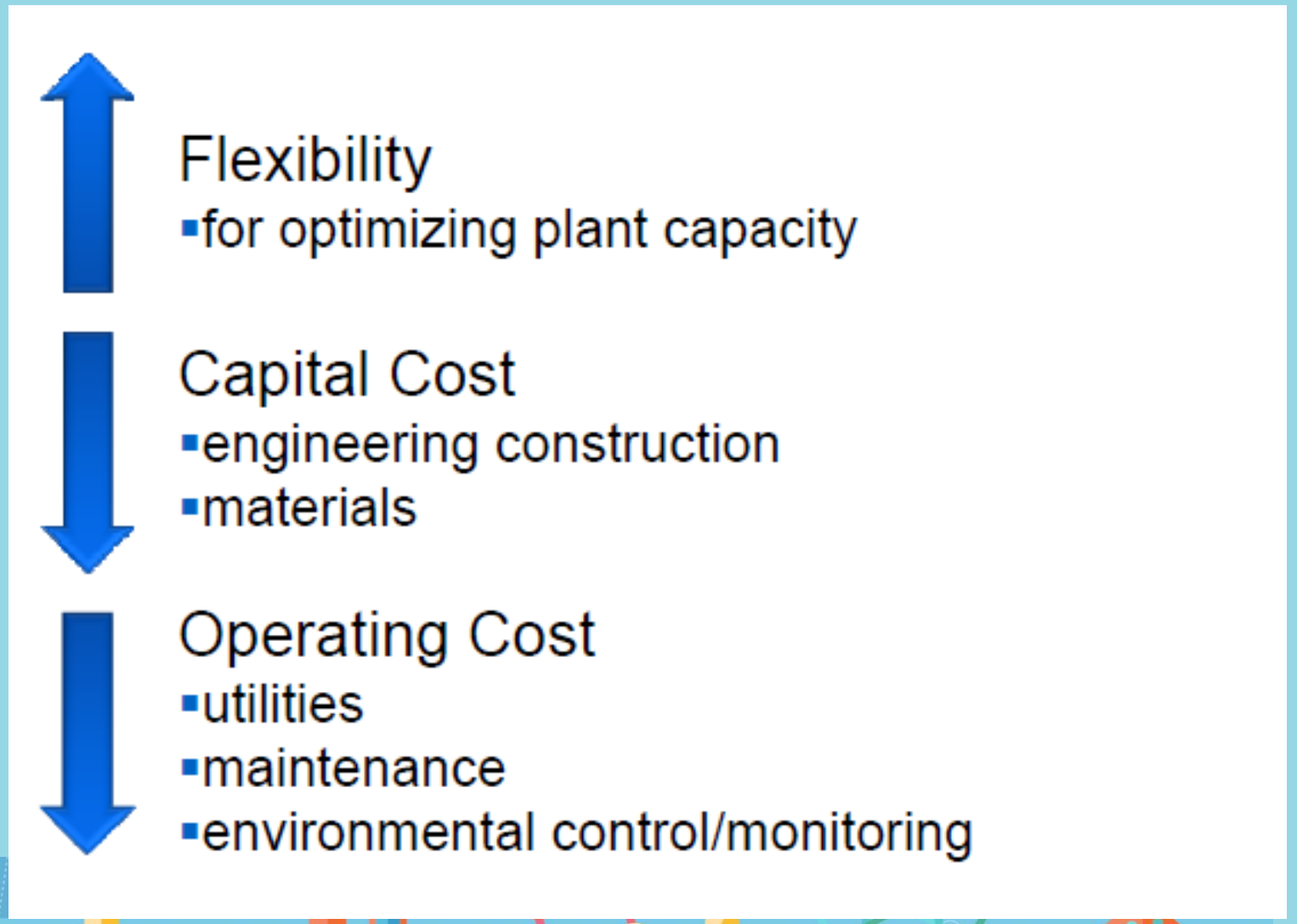
http://www.omicsonline.org/2155-9821/images/2155-9821-2-115-g002.gif

Manufacturing operations will be more efficient in the future

- Higher yielding processes
- Greater plant flexibility
- Better utilization of capital
- Significant reduction in operating costs

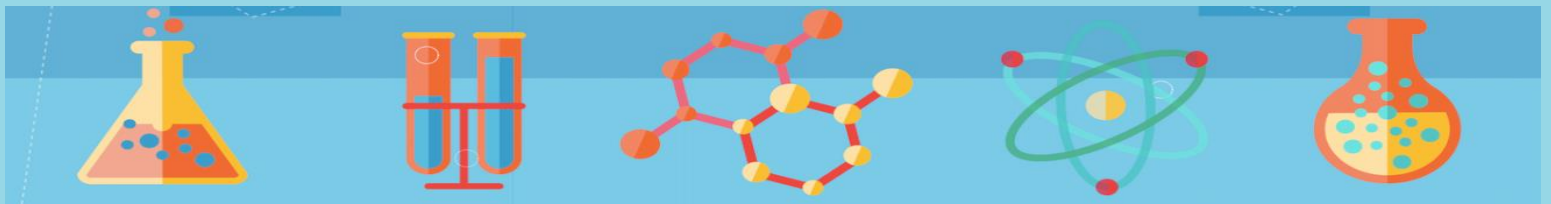


Trends in Manufacturing plant design



Conclusion

- Scale up is the major step of manufacturing
- Lab scale technology may not be adapted well with large scale production
- Cost, Efficiency and plant layout determined scale up technology



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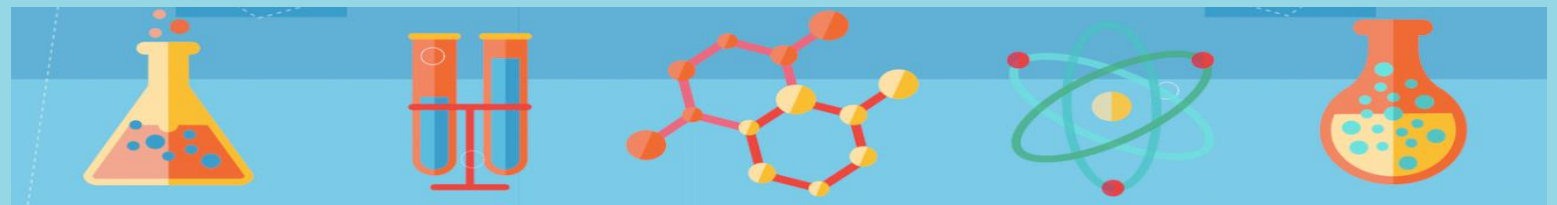
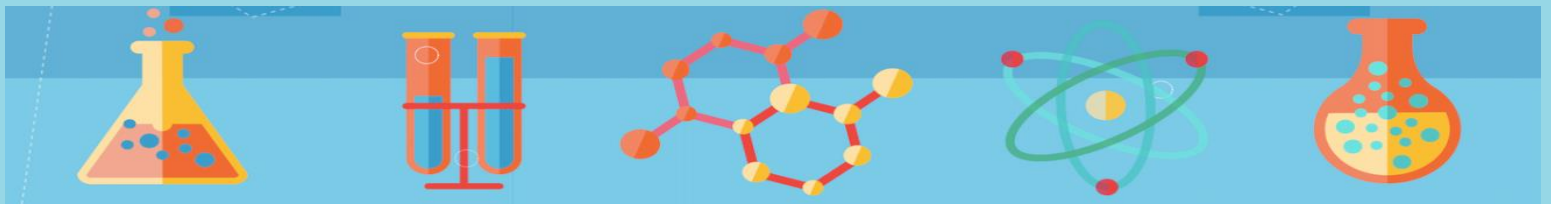
1. Butler, M. and A. Meneses-Acosta, *Recent advances in technology supporting biopharmaceutical production from mammalian cells.* Applied Microbiology and Biotechnology, 2012. 96(4): p. 885-894.
2. Warnock, J.N. and M. Al-Rubeai, *Bioreactor systems for the production of biopharmaceuticals from animal cells.* Biotechnol Appl Biochem, 2006. 45(Pt 1): p. 1-12.
3. Eibl, R., et al., *Disposable bioreactors: the current state-of-the-art and recommended applications in biotechnology.* Appl Microbiol Biotechnol, 2010. 86(1): p. 41-9.
4. Sukhla, A., et al., Process Scale Bioseparations For The Biopharmaceutical Industry, 2007. p.63-79.



Status of Non-Chromatographic Methods

- Aqueous Two-Phase (ATP) Extraction
  - Low selectivity
  - Dilute product concentrations
  - Difficult removal of phase-separation media (salts, PEG, Dextran)
- Precipitation
  - Can work well in small volume systems
  - Difficult to screen conditions, difficult scale up
  - Low yield at lower product concentrations
  - Difficult removal of residual precipitating salts (caprylate), charged polymers
  - Cost of polymers, additives (recycle)



Status of New Chromatography Approaches

- **Expanded Bed Chromatography**
  - Eliminates need for clarification
  - Process integration
  - MAb capture of 10-20 mg IgG/mL
  - High dispersion, low product concentrations
  - Expensive support, difficult operation
- **Simulated Moving Bed (SMB) Chromatography**
  - Multi-column systems
  - Complex operation and control
  - Continuous process – Reduces time
  - Potential savings in resin and media volumes

UpFront Chromatography

Tarpon Systems, Silo System

Status of Novel Adsorptive Membranes

- Convective mass transfer
  - No diffusion limitations
- Low pressure drop
- Best suited for flow-through applications
  - Impurity removal, polishing
- Much higher capacity than porous particles for large targets
  - Virus, DNA plasmids
- Not enough capacity for product capture
- Large elution volumes, high dispersion, low product concentration

Pall Mustang

Sartorius

Status of Monolith Technologies

- Convective mass transfer – no diffusion limitations
- Good dispersion properties, low pressure drops
- Larger channels (~2 µm) optimum for large product capture (virus, DNA plasmids, IgM)
  - IgM capacities reported in the range of 40-50 mg/mL
- Cast as a single unit – Size limitations

BIA Separations

Overview of KogenateFS/ Bayer Manufacturing Process

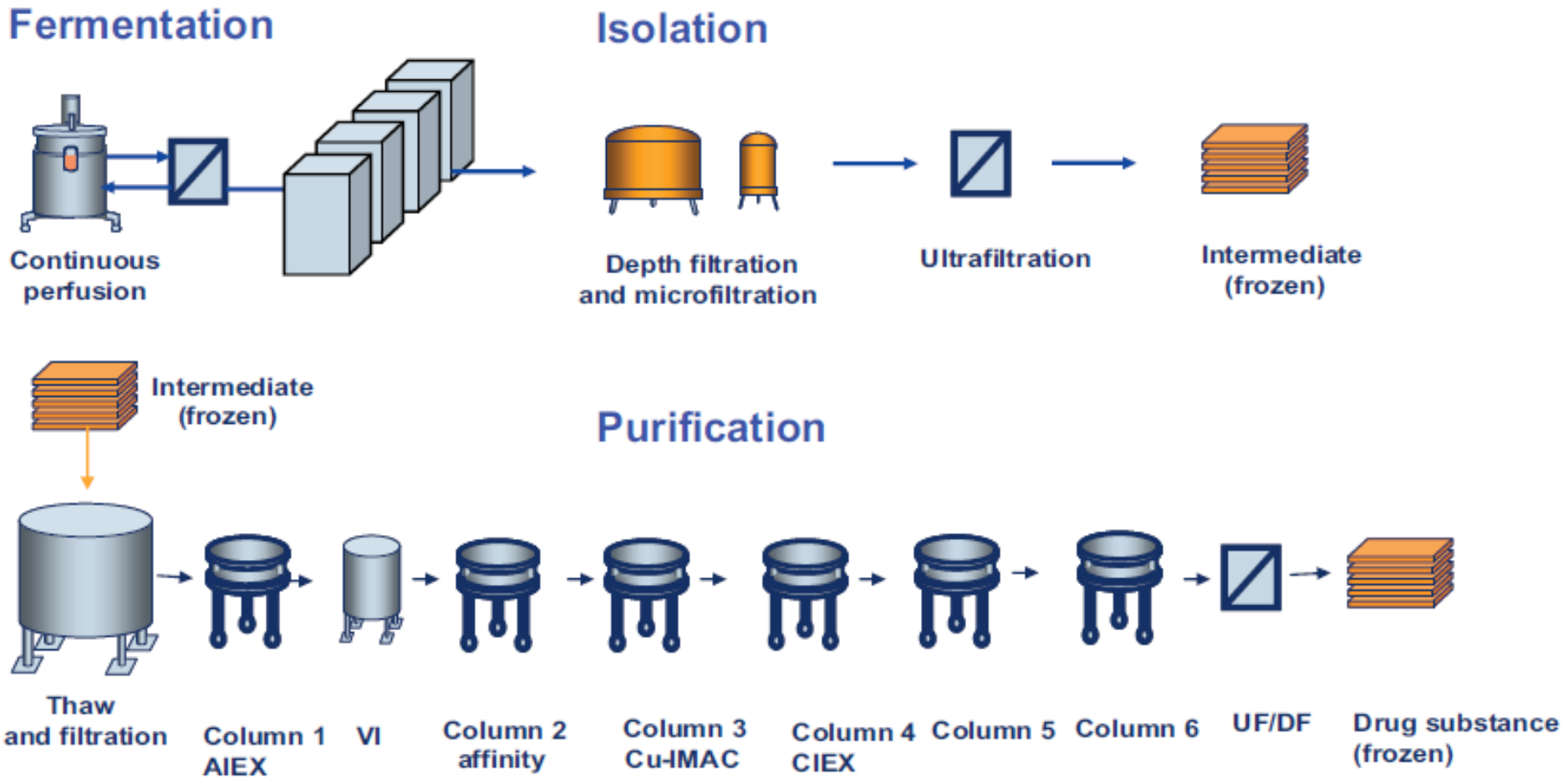
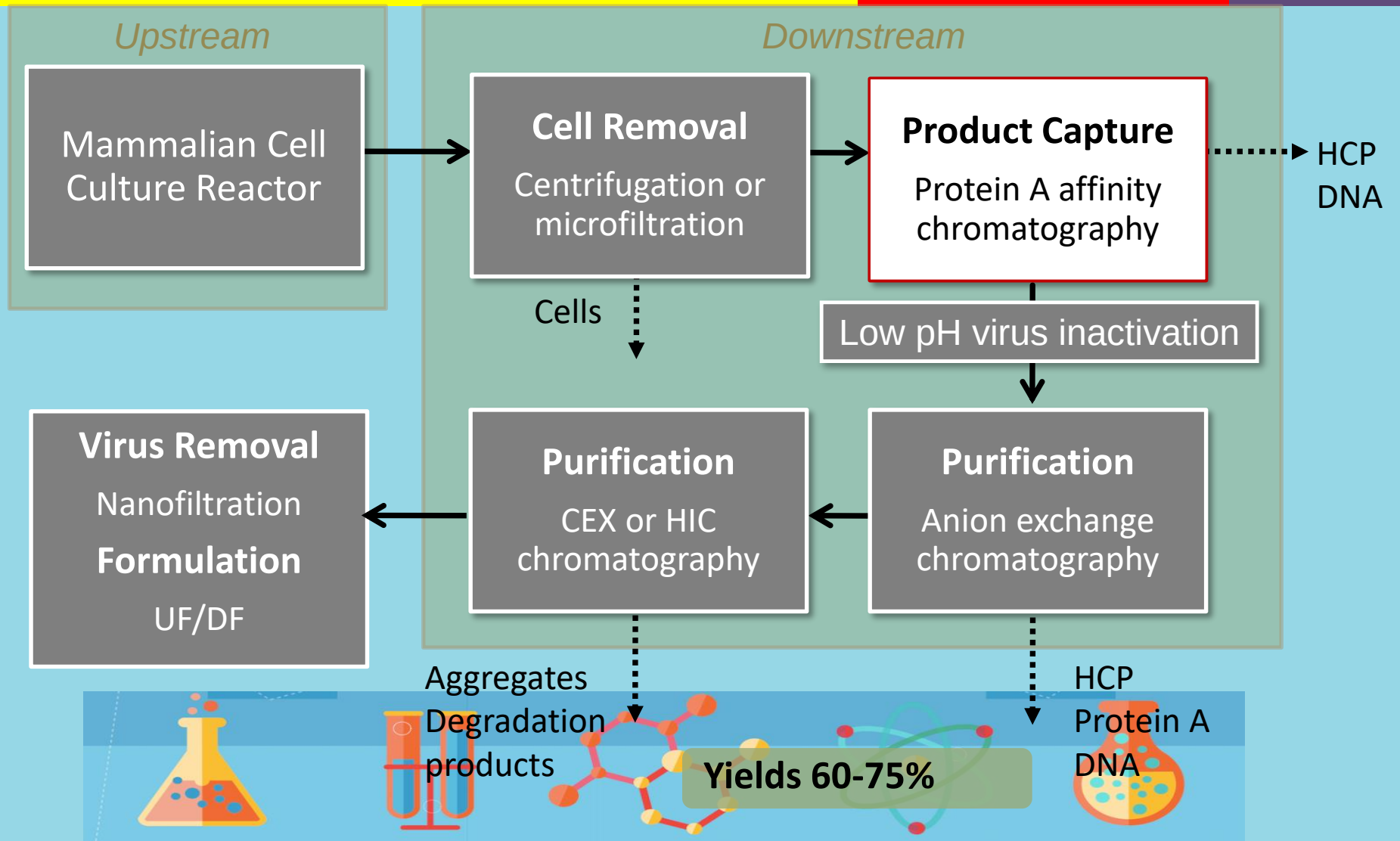


Figure 19.3 Overview of the KogenateFS/Bayer manufacturing process.

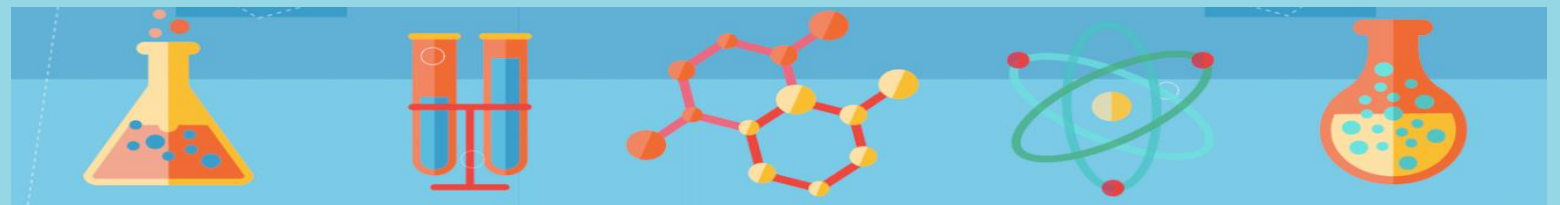
Generic Platform Process for Purification of MAbs



BOX 4.5. POINTS TO CONSIDER IN QUALITY CONTROL AND ASSURANCE OF PROTEIN AND PEPTIDE PHARMACEUTICALS

- Drug versus biologic (consider which branch, CBER or CDER of FDA will review the final product)
- Quality assurance and quality control
  - Documentation of process and raw materials
  - Validation
  - cGMP compliance
- Certificate of analysis or lot release
  - Sterility
  - Endotoxin
  - Identity
  - Purity
  - Concentration
  - Activity
  - Composition (pH, salts, buffers, excipients)
  - Stability
- Purity assays (chromatographic, electrophoretic, immunochemical)
  - Reverse phase HPLC
  - Ion exchange HPLC
  - Hydrophobic interaction HPLC
  - Gel filtration HPLC
- Validation (prior to license)
  - Sterilization procedures
  - Assays
  - Cleaning (especially for multiple-use facilites)
  - Viral clearance (prior to IND)
  - Installation qualification (IQ), operation qualification (OQ), performance qualification (PQ): types of validation to show that equipment, ancillary systems, and process function as intended

Biosimilar



Biosimilars Deliver on Their Promise of Access and Savings

The U.S. Generic & Biosimilar Medicines Savings Report

SEPTEMBER 2022

39
APPROVED

22
MARKETED

BIOSIMILAR SAVINGS SINCE 2015
$13.3 BILLION

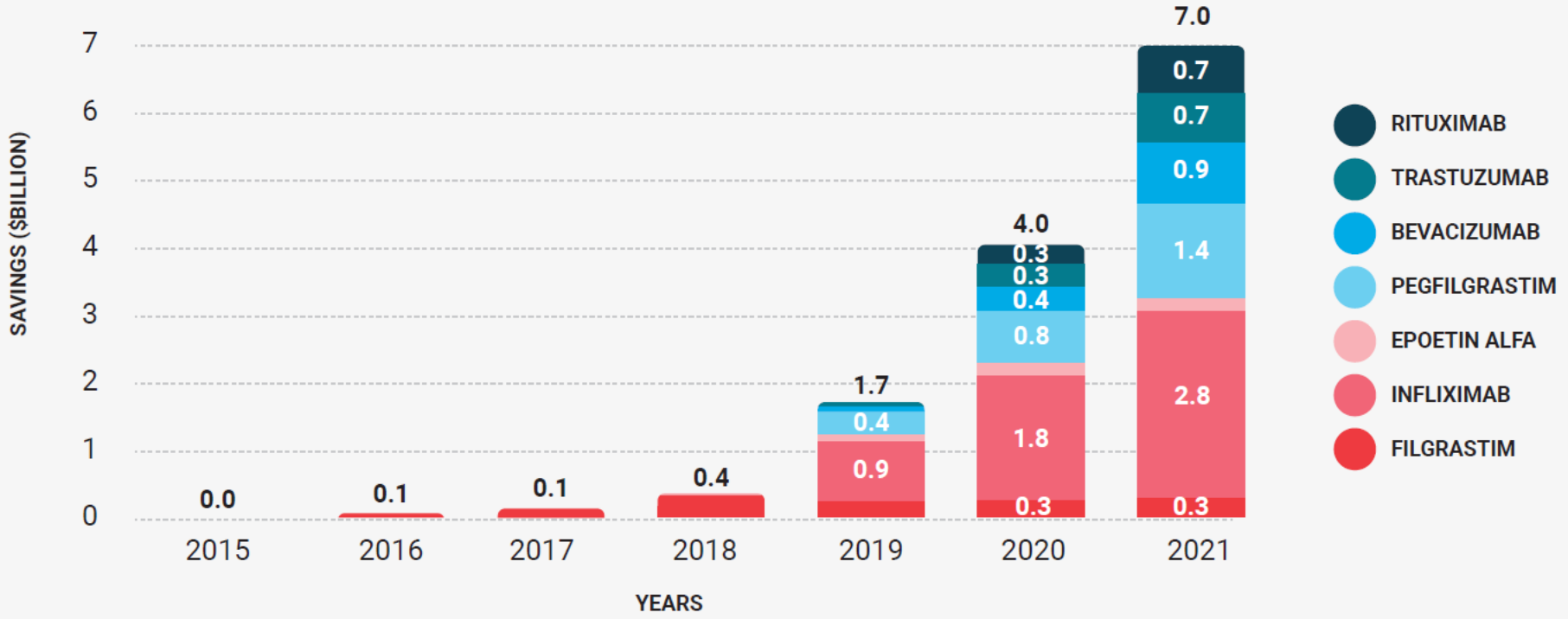
BIOSIMILARS HAVE BEEN USED IN MORE THAN **364 MILLION DAYS** OF PATIENT THERAPY AND HAVE RESULTED IN MORE THAN **150 MILLION INCREMENTAL DAYS** OF THERAPY

BIOSIMILAR COMPETITION IS DRIVING LOWER PRICES AMONG BIOSIMILARS AND THEIR REFERENCE PRODUCTS

Biosimilar Savings Totaled $7 Billion in 2021

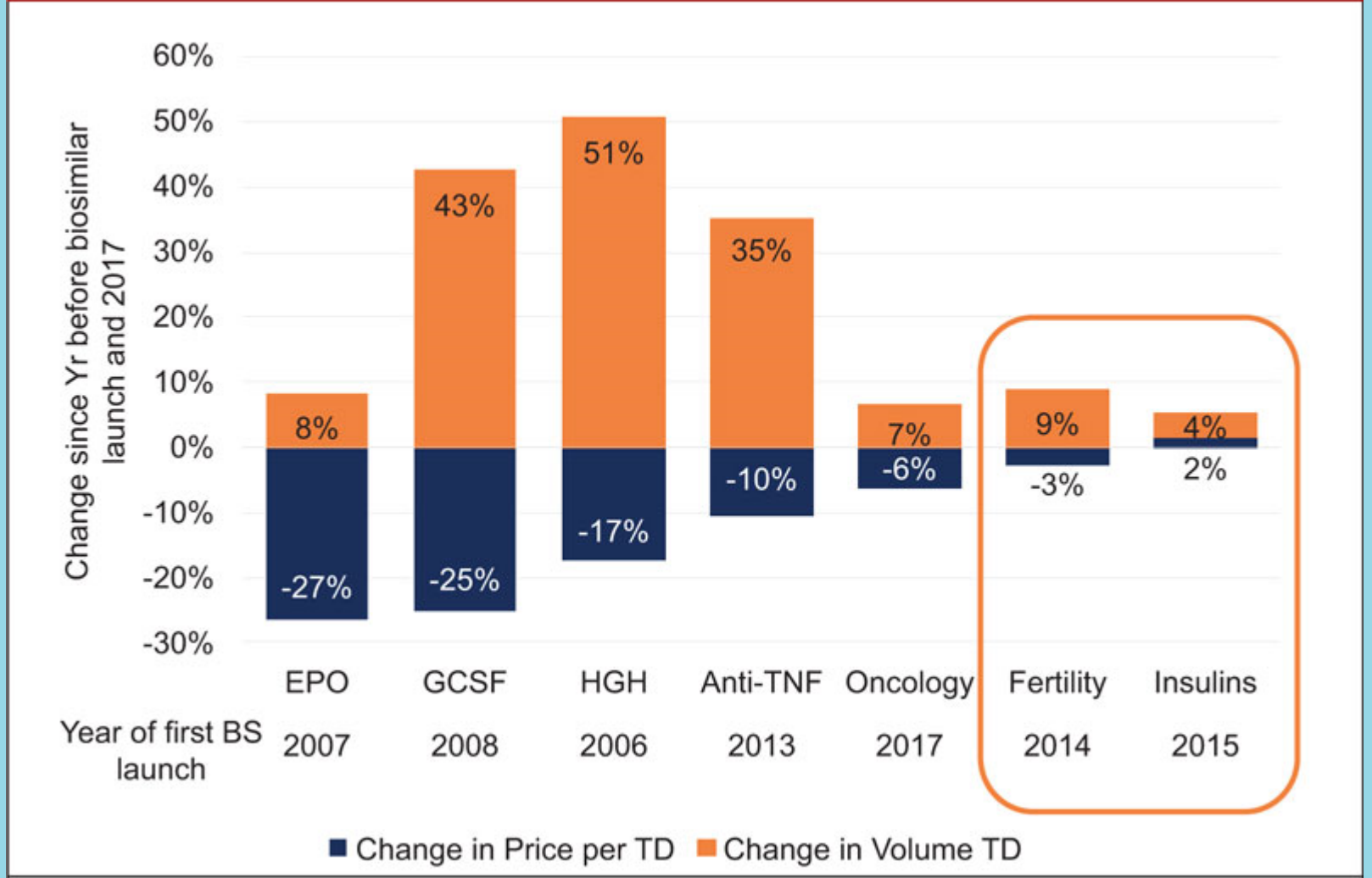
SINCE 2015, BIOSIMILARS HAVE GENERATED $13.3 BILLION IN SAVINGS

BIOSIMILAR SAVINGS BY MOLECULE 2015 – 2021



Source: IQVIA, National Sales Perspectives, Dec 2021.

Figure 2: Change in price and volume treatment days of total market between year before biosimilar launch and 2017



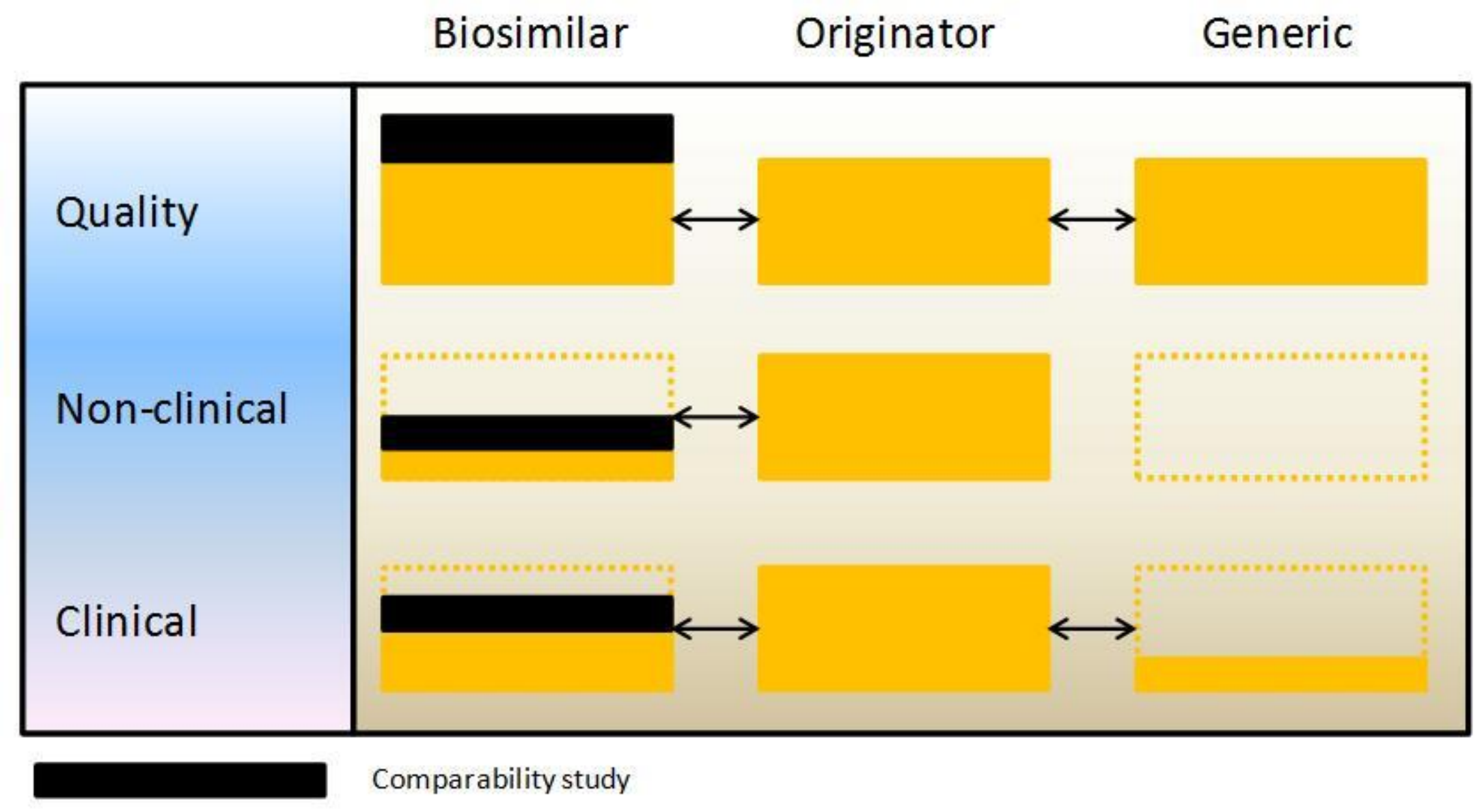
Anti-TNF: anti-tumour necrosis factor; BS: biosimilar; TD: treatment days; Yr: year.

Source: IQVIA.

Biosimilar เพิ่มโอกาสการเข้าถึงยา

Biosimilar ทำให้ราคายาลดลง

Dossier Landscape of Different Type Biologics



Biosimilar Assessment
“Totality of Evidence Approach”

Key Steps in Biosimilar Development & Marketing

Quality Target Product Profiling

Extensive Comparability Studies in Analytical

(a) pivotal clinical comparability study

Extrapolation of Indications

During Development

Approval

Interchangeability

Market Perception and Concern

Post Approval Challenge

Market Challenge Issues

Biosimilar is not built through traditional clinical training (Educational issues)

Perceptions and Concerns brings to unsuccessful communication to patients (nocebo effect)

Interchangeability

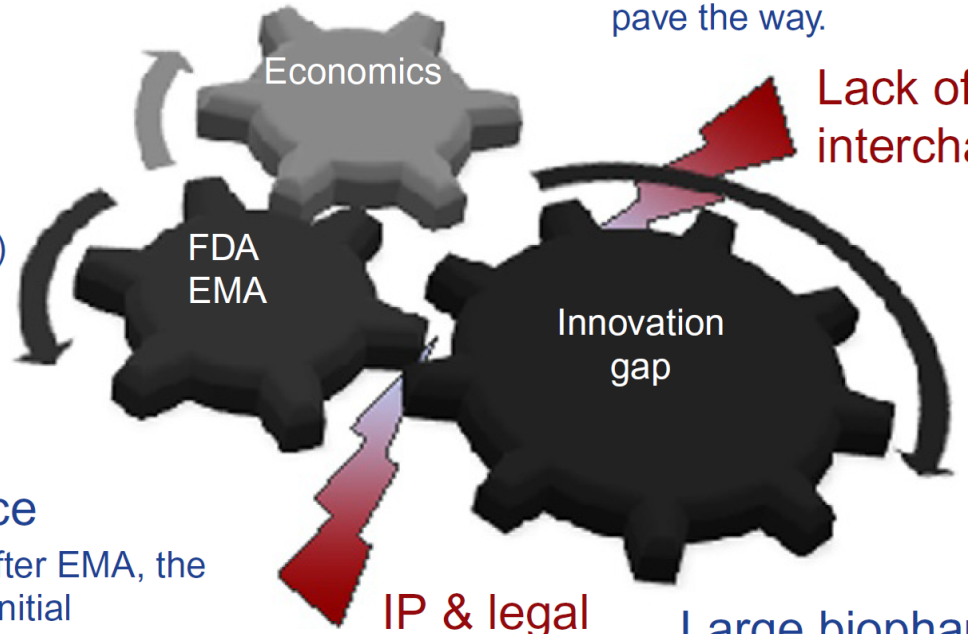




Treatment cost

With financial crisis still lurking, healthcare systems around the globe will not let go of any cost reduction option and legislation will (have to) pave the way.

Despite of cost reductions, quality demands will not slip and patients will honor the brands that come with quality facts and reputation.



Lack of interchangeability

Totality of evidence approach (FDA) requires advanced development capabilities and effectively excludes weaker players from the market.....

Beyond the regulatory challenge, smaller biosimilar players just took another hit to their business cases by multinational players' initiatives for biosimilars....

Regulatory guidance

With seven years delay after EMA, the FDA has launched their initial guidance for biosimilar developers. Many of the large markets are getting predictability on expectations for biosimilars.

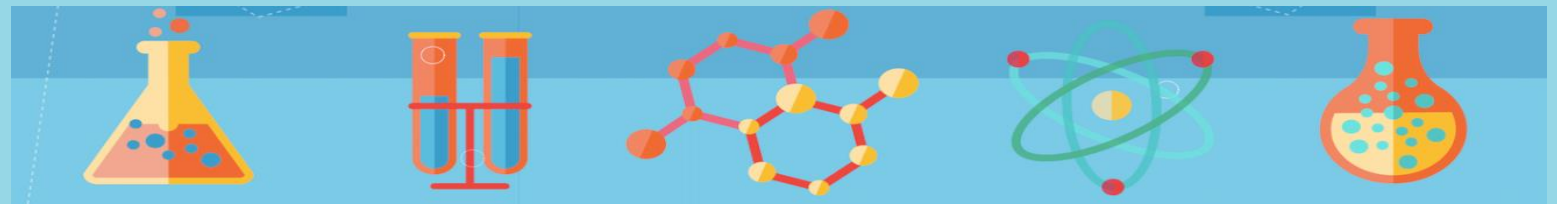
IP & legal delays

Large biopharma players strategy

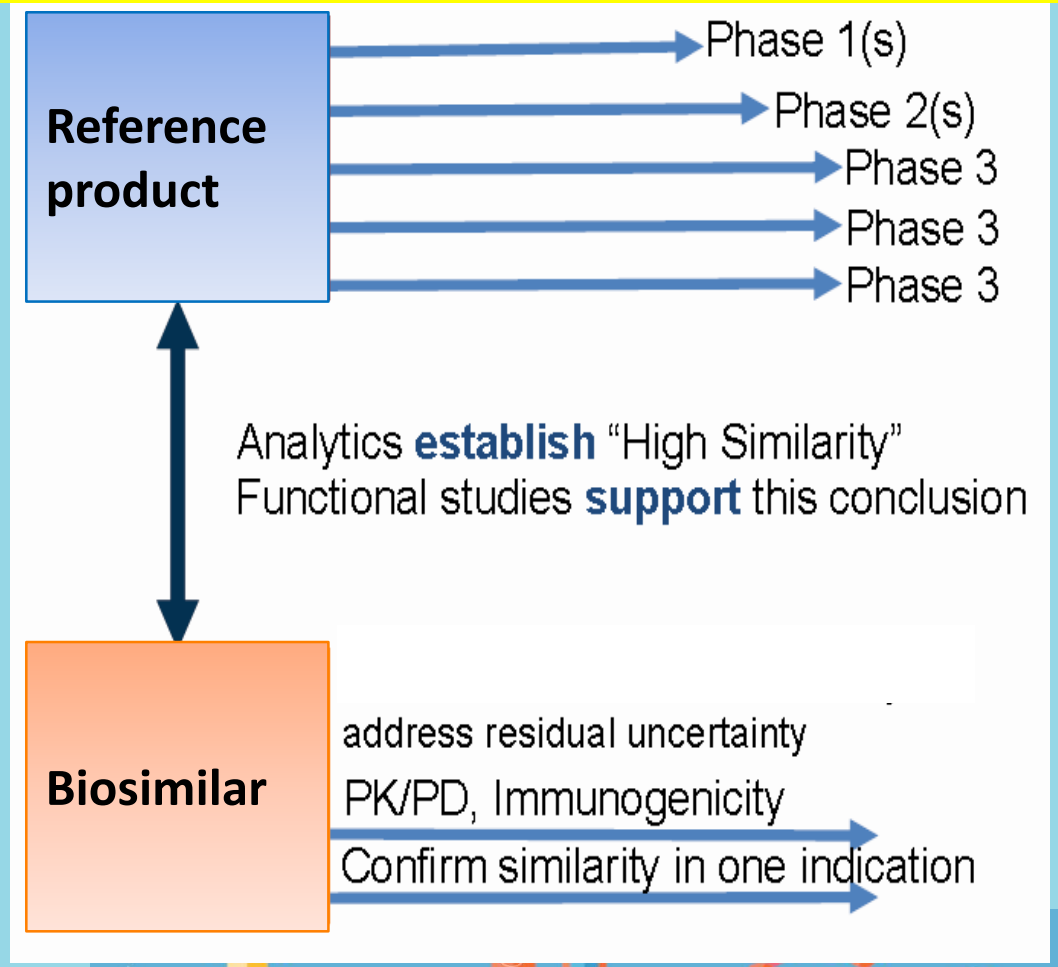
With Novartis (Sandoz) leading since 2006, several multinational players have more recently started initiatives to fill R&D pipelines and manufacturing sites with biosimilars hoping for a low attrition rate.

FIG. 2.11 Drivers and hurdles for the successful introduction of biosimilars.

KEY CONSIDERATIONS REGARDING BIOSIMILAR CLINICAL TRIAL DESIGN



Clinical development : Biosimilar vs. Reference bioproducts

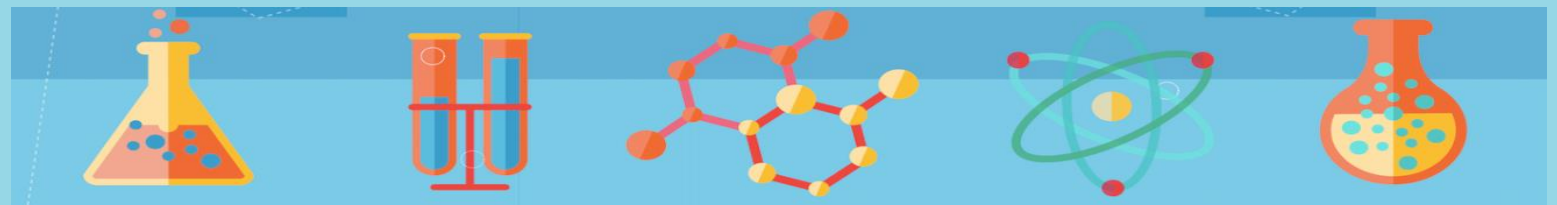


Multiple Indications:
No extrapolation → clinical trials in each indication

Multiple Indications:
Extrapolation possible if scientifically justified → clinical trials not required in each indication

Clinical Trial for Biosimilarity

- **The extent of the clinical program depends on the degree of similarity demonstrated in preclinical testing, including structural, functional, and animal studies.**
  - **Clinical Efficacy**
  - **Clinical Safety**
  - **Immunogenicity**



Assessment of potential residual risks – Trial design considerations

- **Key elements in the trial design:**
  1. **Selection of appropriate and sensitive populations and endpoints.**
  2. **Definition of equivalence margins for the selected endpoint (case-by-case assessment based on clinical & statistical considerations).**
- **Considerations regarding equivalence margins:**
  - **Equivalence margins define the required sample size.**
  - **Equivalence margins need to be optimized:**

Sensitive human models and study conditions" are required to evaluate biosimilarity

> Comparability should be demonstrated in scientifically appropriately sensitive clinical models and study conditions (whether licensed or not), and the applicant should justify that the model is relevant as regards efficacy and safety, and sensitive to demonstrate comparability in the indication(s) applied for.

- **"Sensitive" study populations are patient (sub)groups who are most likely to benefit from the treatment and show potential differences between the biosimilar and reference product:**
  - **e.g., HER2+ population for anti-HER2 mAbs**
  - **e.g., trastuzumab in early breast cancer/neoadjuvant/adjuvant setting**

- **Appropriately "sensitive" and homogenous study populations are necessary to increase the chances of detecting potential differences between a biosimilar candidate and the reference product.**

EMA. Guideline on mAb biosimilars 2012. EMA/CHMP/BMWP/403543/2010.

Human Pharmacology Study to Support Biosimilarity

Pharmacokinetics Studies

in appropriate populations
(90% CI; 80-125% range (AUC, Cmax)

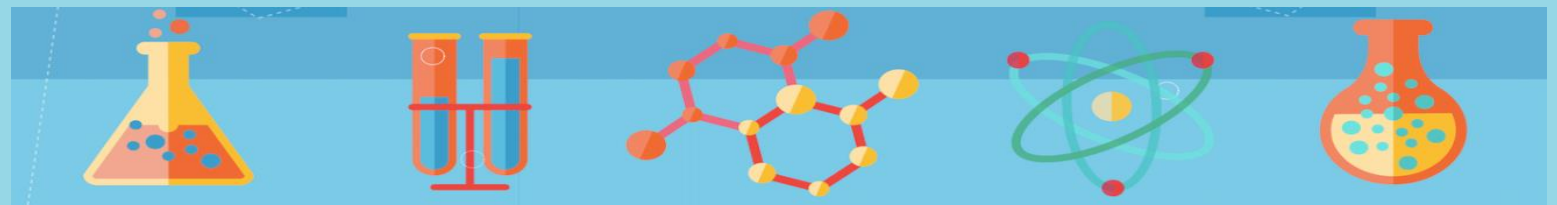
Healthy Volunteers

or

Patients

Pharmacodynamic Studies

in case relevant marker to MOA is available and provide info. regarding clinical efficacy



Sensitivity is key for detection of potential differences

Endpoints and study population determine sensitivity

The idea is to study the biosimilar in the population of patients in whom *– if there is a difference between biosimilar and reference product –* that difference will most easily be detected

Sensitive endpoints

- Differentiate effective from less effective treatments with high likelihood
  - Large treatment effect size
- Strongly correlated with clinical outcomes
  - E.g. correlation of response rates with event-free or overall survival

Sensitive population

- Homogenous population allows “clean” comparison
- Heterogeneity may confound comparison and decreases sensitivity, for example:
  - Prognostic baseline characteristics affect efficacy
  - Co-morbidities affect safety
  - Chemotherapies affect immunogenicity

Clinical Endpoints: Oncology cases

×	Overall survival (OS)	• the gold standard, providing clinical benefit. • <u>Not practical for biosimilarity demonstration</u>
√	Overall response rate (ORR) and complete response (CR)	• Suitable endpoints
√	Pathologic complete response (pCR)	• can be used as a good surrogate marker in neoadjuvant breast cancer

What does “sensitive indication” mean?
A Rituximab Case

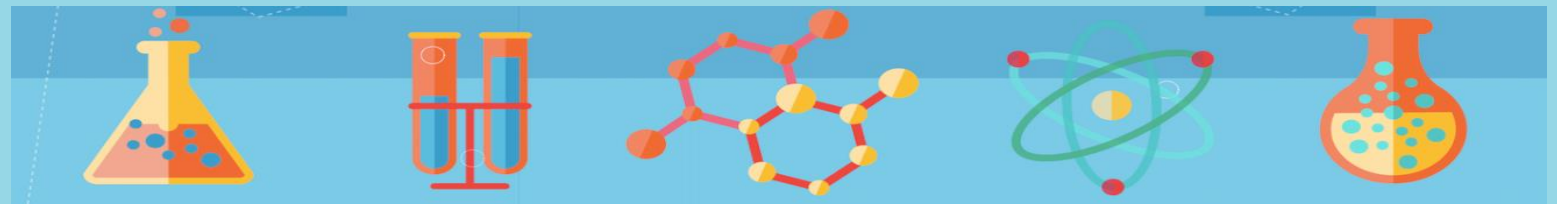
Indications approved for Rituximab	ORR Control	ORR Active	Effect Size
NHL Follicular Induction (CHOP)	90%	96%	6%
NHL Follicular Induction (CVP) (CR)	10%	41%	31%
NHL Follicular Relapsed (CHOP)	74%	87%	13%
NHL DLBCL Induction (CHOP) (CR)	76%	84%	8%
Chronic Lymphocytic Leukemia	72%	86%	14%
Rheumatoid Arthritis (ACR20)	18%	51%	33%

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3949011/
Product information Rituxan (SPC)

Sensitive Populations and Endpoint in Biosimilar Clinical Comparability

Mab	Indication	Sensitive Population	Sensitive Endpoint
Rituximab	Oncology-Lymphoma Autoimmune	NHL-Follicular Induction (CVP)	ORR
Trastuzumab	Metastatic Neoadjuvant/Adjuvant Breast/Gastric	MBC (heterogeneous; less sensitive) Neoadjuvant EBC (Homogeneous; more sensitive)	PFS ORR/tpCR
Anti-TNF-alpha	RA	RA (plus MTX) Psoriasis (monotherapy)	ACR20 PASI75
Bevacizumab	Oncology-Adjuvant	Previously Untreated Advanced NSCLC (Pac/Carbo)	ORR

EXTRAPOLATION OF INDICATION, SWITCHING AND INTERCHANGEABILITY CONCEPT

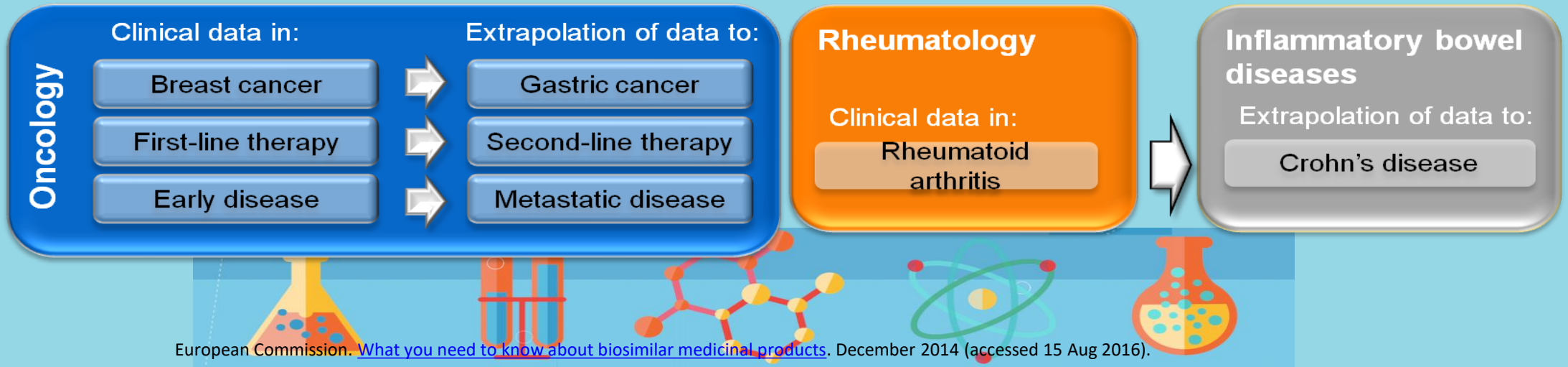


What is extrapolation of indication?

Definition of extrapolation:

- The decision whether to extend the efficacy and safety data from an indication (i.e. a medical condition, disorder or disease) for which the biosimilar has been clinically tested to other conditions for which the branded product is approved, is known as “extrapolation”.

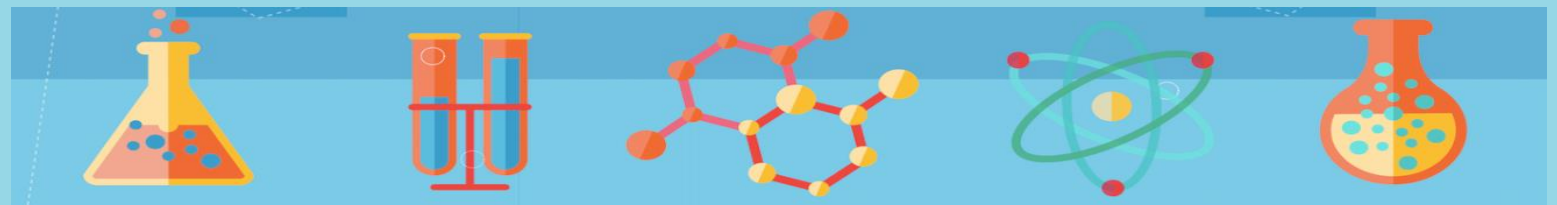
Examples of extrapolation (within the same therapeutic area or to a different one)



European Commission. What you need to know about biosimilar medicinal products. December 2014 (accessed 15 Aug 2016).

Interchangeability, Substitution and Switching

- **Interchangeability** - *Health Regulatory Authority* Designation
- **Substitution** – *Pharmacist Action*
  - If without the prescribing physician's permission or knowledge, it is considered "automatic" or "involuntary" substitution
- **Switching** - *Physician Decision*



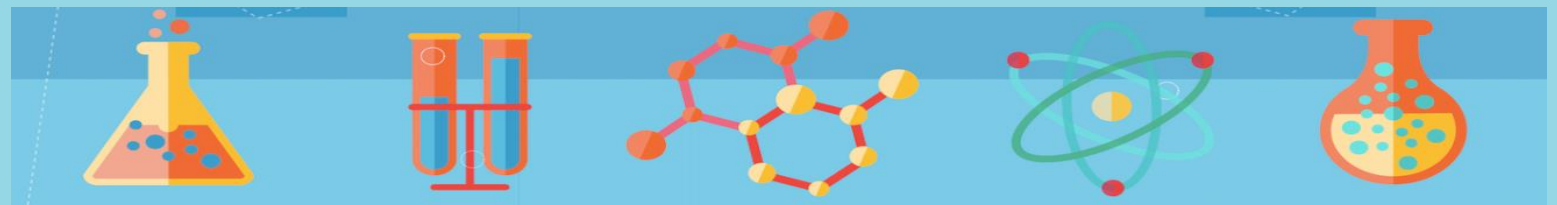
Drugs (2018) 78:463–478
https://doi.org/10.1007/s40265-018-0881-y

SYSTEMATIC REVIEW

Switching Reference Medicines to Biosimilars: A Systematic Literature Review of Clinical Outcomes

Hillel P. Cohen[1] · Andrew Blauvelt[2] · Robert M. Rifkin[3] · Silvio Danese[4] · Sameer B. Gokhale[5] · Gillian Woollett[6]

Conclusions While use of each biologic must be assessed individually, these results provide reassurance to healthcare professionals and the public that the risk of immunogenicity-related safety concerns or diminished efficacy is unchanged after switching from a reference biologic to a biosimilar medicine.



EXPERT OPINION ON BIOLOGICAL THERAPY, 2017
https://doi.org/10.1080/14712598.2017.1341486

Taylor & Francis
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REVIEW

Is there a reason for concern or is it just hype? – A systematic literature review of the clinical consequences of switching from originator biologics to biosimilars

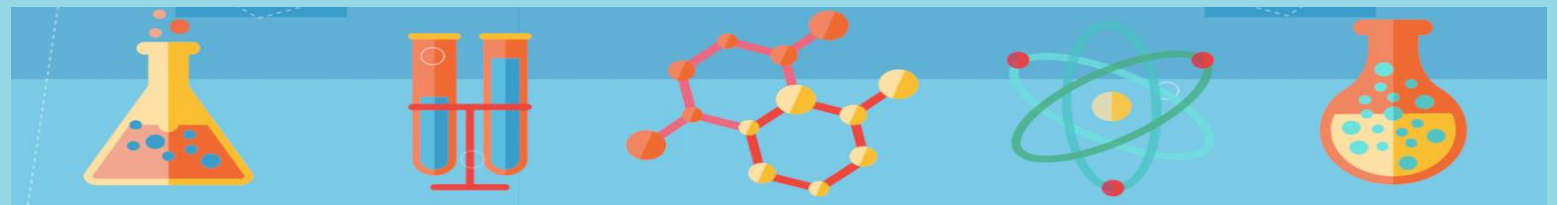
András Inotai[a,b], Christiaan P.J Prins[c], Marcell Csanádi[a], Dinko Vitezic[d], Catalin Codreanu[e] and Zoltán Kaló[a,b]

[a]Syreon Research Institute, Budapest, Hungary; [b]Department of Health Policy & Health Economics, Faculty of Social Sciences, Eötvös Loránd University (ELTE) Budapest, Hungary; [c]Department of Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands; [d]University of Rijeka School of Medicine and University Hospital Centre Rijeka, Rijeka, Croatia; [e]Center for Rheumatic Diseases, University of Medicine and Pharmacy, Bucharest, Romania

- **In countries with more limited patient access to biologics, biosimilars can increase the number of patients on biologic medicines without a need for additional resources**
- **While prescribing a biosimilar drug for patients naive to biologic treatment is a well-accepted option, switching clinically stable patients from an originator product to a biosimilar alternative is a concern for clinicians**
- **Until the final data are published from ongoing phase 4 clinical trials specifically designed to evaluate the outcomes of switching to biosimilars, a systematic review of relevant publications can provide the most comprehensive evidence**
- **Altogether, neither systematic reviews, nor empirical papers identified by our review reported that switching from an originator biologic to a biosimilar treatment is associated with an increased risk**
- **Preventing patients on biologic medicines from switching to biosimilars due to anticipated risks seems to be disproportional compared to the expected cost savings and/or improved patient access as societal benefits**

Interchangeability

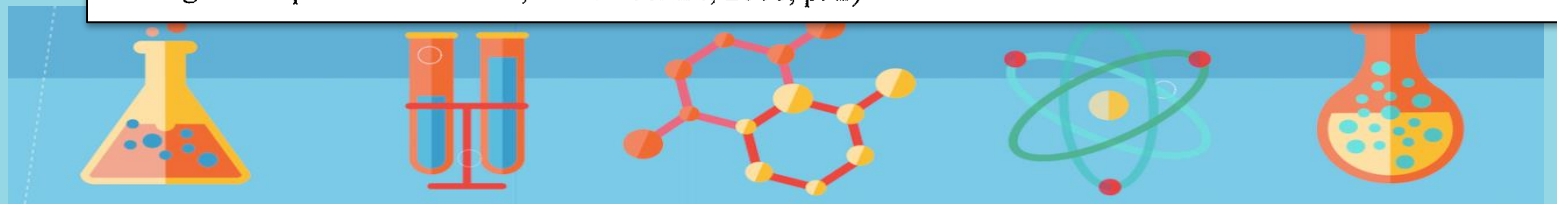
- **In EU**, the biosimilar approval **do not automatically allow interchangeability**
- ***Interchangeability/switching remains a national decision.***
- After 10 yrs experience of biosimilar in the market, several EU countries change regulations to ***less stringency on Interchangeability/switching/substitution*****.**



EU Countries Forbidding Substitution (2008)

Country	Ruling
Austria	Physicians obliged to prescribe by brand name
Czech Republic	Physicians obliged to prescribe by brand name
Denmark	Guidelines against substitution
Finland	No injectable drug may be substituted
France	Automatic substitution prohibited without consent of physician
Germany	No automatic substitution
Greece	Physicians obliged to prescribe by brand name
Hungary	No automatic substitution
Italy	No automatic substitution
The Netherlands	No automatic substitution
Norway	No automatic substitution
Slovakia	Official list stating which products cannot be substituted
Slovenia	No automatic substitution
Spain	No automatic substitution
Sweden	No automatic substitution
UK	No automatic substitution

Source: Hogan & Hartson, Morgan Stanley Research (as published in the Morgan Stanley Report "Follow-On Biologics: Expect a Slow Start", November 24, 2008, p. 2)



Finnish drug regulator recommends interchangeability of biosimilars

Posted 29/05/2015

The Finnish Medicines Agency, Fimea, announced on 22 May 2015 that it was recommending the interchangeability of biosimilars for their reference biologicals.

Fimea is of the position that biosimilars licensed in the European Union are interchangeable, and it is therefore making this recommendation to the healthcare system in Finland.

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Home / Policies & Legislation / France to allow biosimilars substitution

France to allow biosimilars substitution

Posted 21/02/2014

Pharmacists in France will now be allowed to substitute a biosimilar for the prescribed (reference) biological under certain conditions, including only when initiating a course of treatment and that the prescribing physician has not marked the prescription as 'non-substitutable'.

Interchangeability of Biosimilars – Position of Finnish Medicines Agency FIMEA

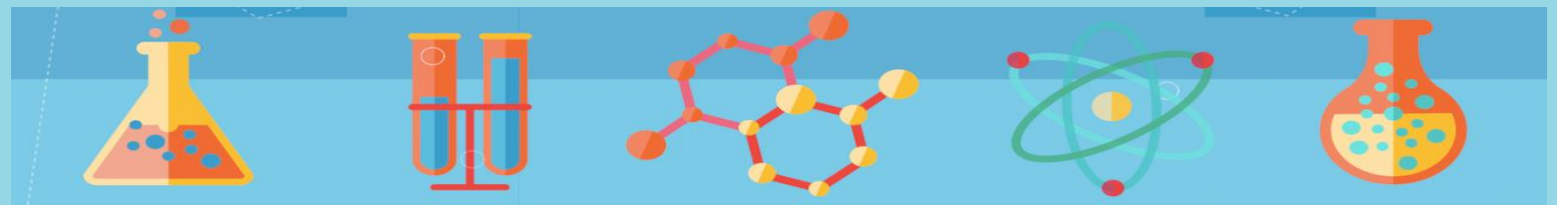
Switches between biological products are common and usually not problematic, e.g. in the context of hospital tendering processes.

For time being, there is no evidence for adverse effects due to the switch from a reference product to a biosimilar

The theoretical basis of such adverse effects is weak.

Risk of adverse effects can be expected to be similar to the risk associated with changes in the manufacturing process of any biological product.

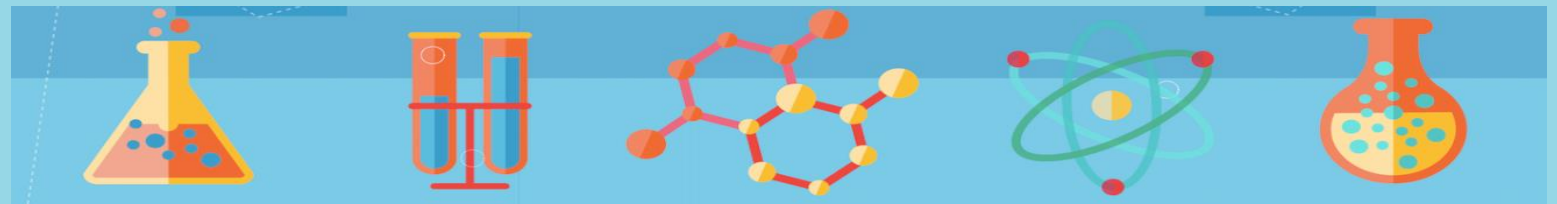
Automatic substitution at the pharmacy level is not within the scope of this recommendation.



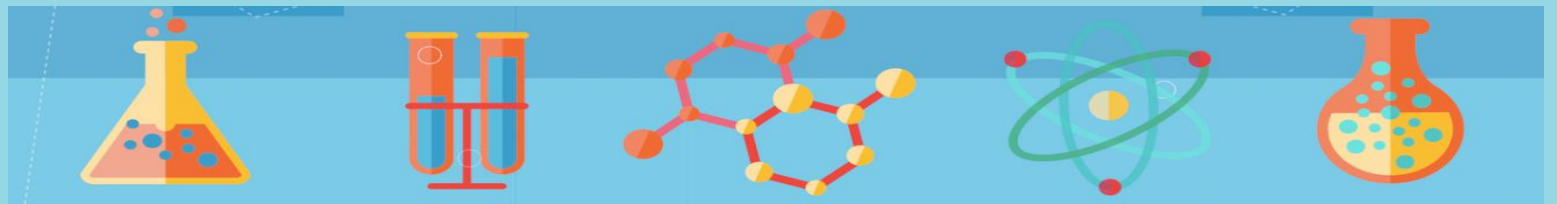
Interchangeability of Biosimilars – Position of Finnish Medicines Agency FIMEA

Therefore, the current position of Fimea is that

biosimilars are <u>interchangeable</u> with their reference products under the supervision of a health care person.



Do we really need biosimilar interchangeability study?



NOR-SWITCH

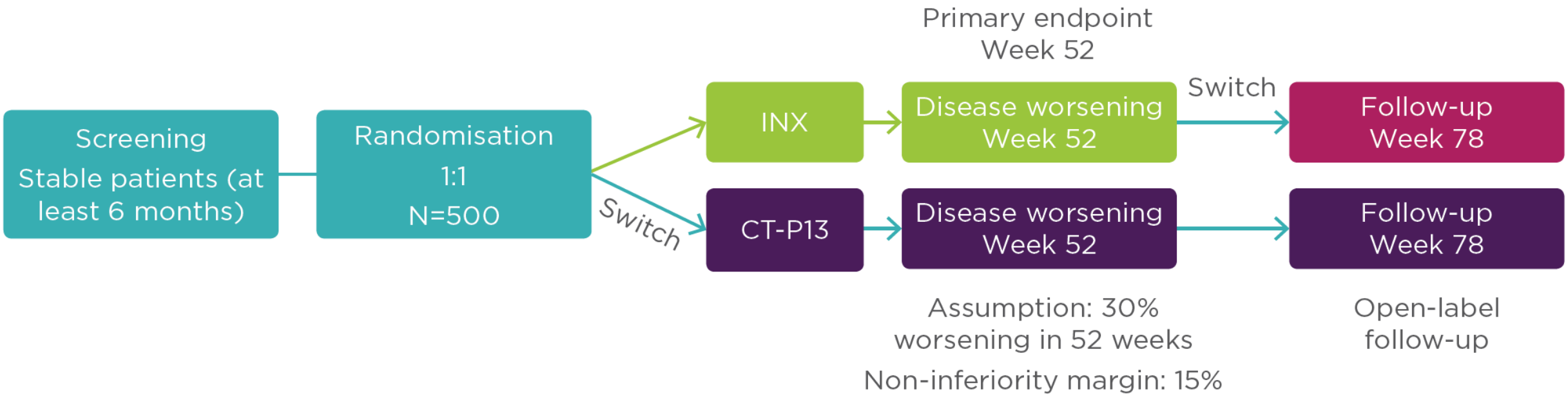
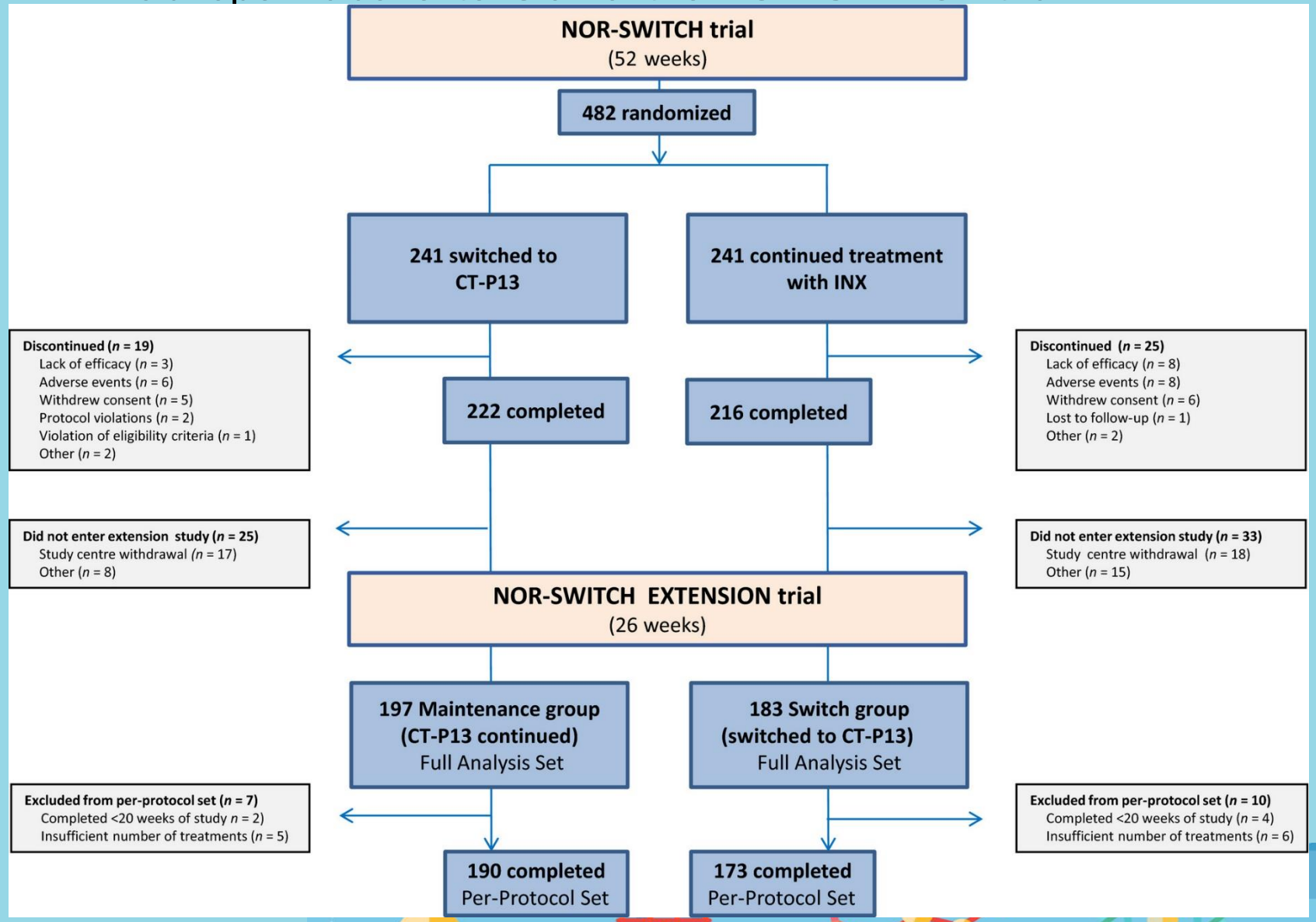


Figure 2: NOR-SWITCH study design.[12]

A randomised, double-blind, parallel-group study to evaluate the safety and efficacy of switching from innovator infliximab to biosimilar infliximab compared with continued treatment with innovator infliximab in patients with rheumatoid arthritis, spondyloarthritis, psoriatic arthritis, ulcerative colitis, Crohn's disease, and chronic plaque psoriasis.

INX: infliximab.

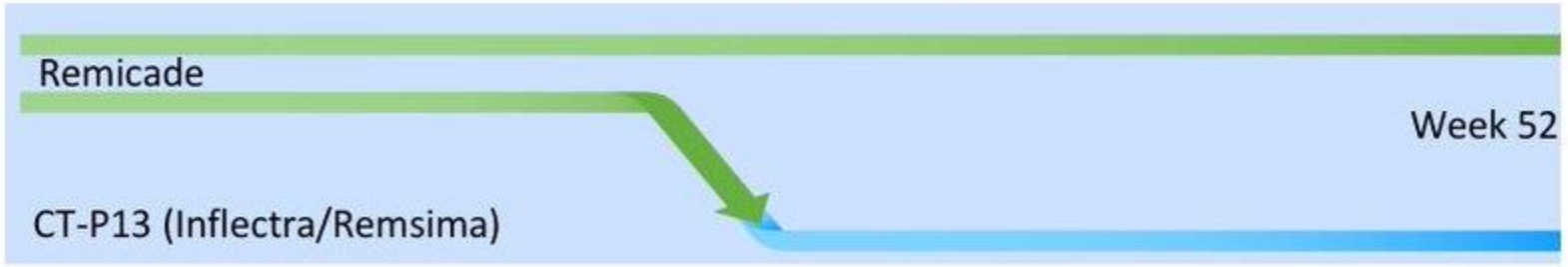
Long-term efficacy and safety of biosimilar infliximab (CT-P13) after switching from originator infliximab: open-label extension of the NOR-SWITCH trial



Journal of Internal Medicine, Volume: 285, Issue: 6, Pages: 653-669, First published: 14 February 2019, DOI: (10.1111/joim.12880)

NOR-SWITCH (Jørgensen, et al. abstract LB15) Presented TODAY at UEGW (Vienna)

- Phase IV **multi-indication** prospective non-medical switch study in Norway by Norwegian govt.
- 52 weeks randomized, double-blind non-inferiority study

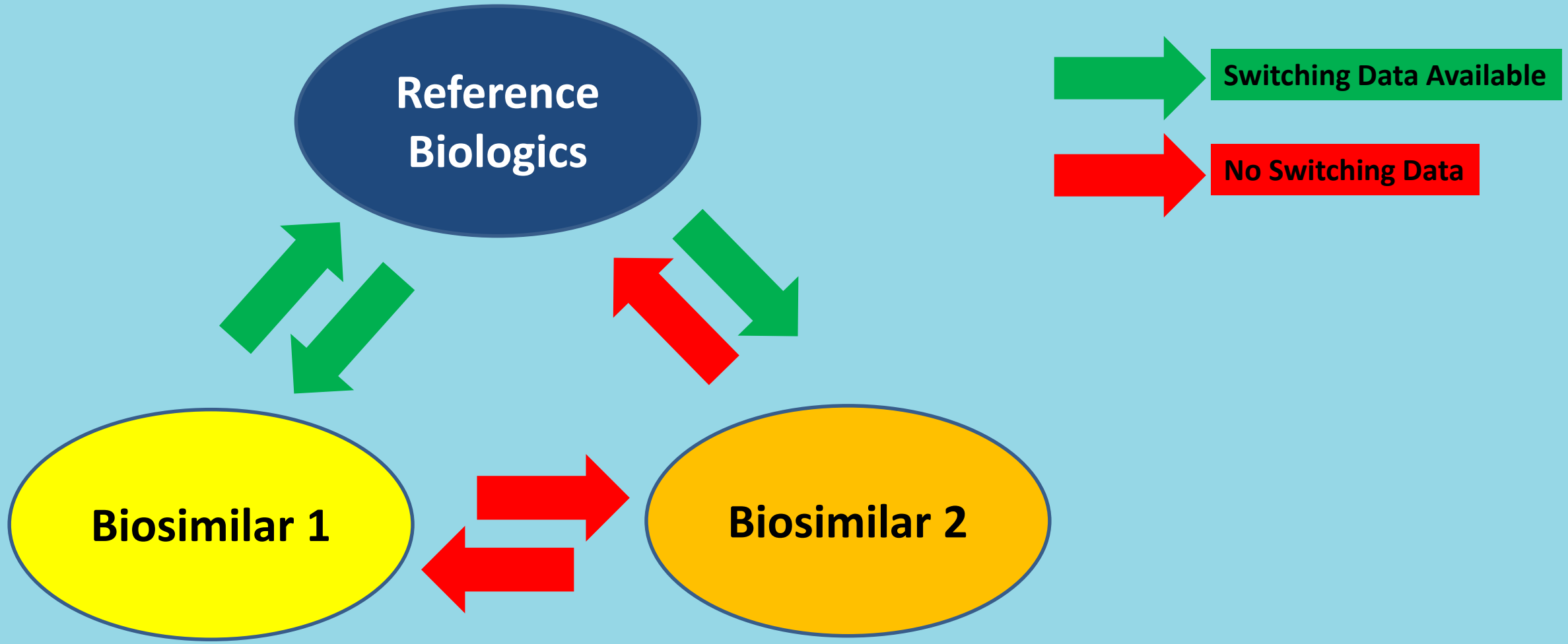


- RESULTS:
  - Primary outcome: disease worsening at 12 months
    - Remicade 53/202 (26.2%) vs. CT-P13 61/206 (29.6%)
  - Anti-drug antibodies:
    - Remicade 7.1%
    - CT-P13 7.9%

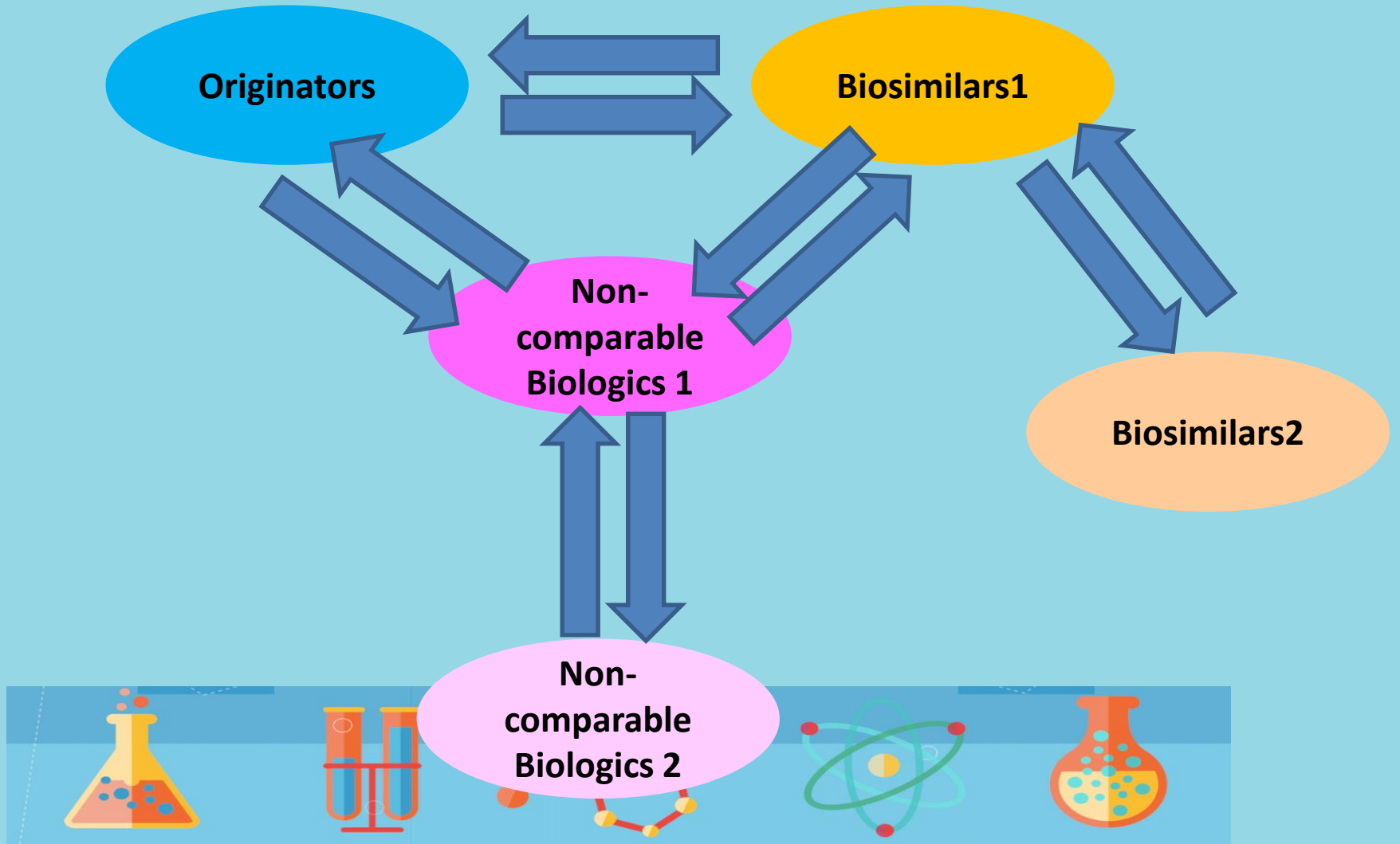
Disease Worsening

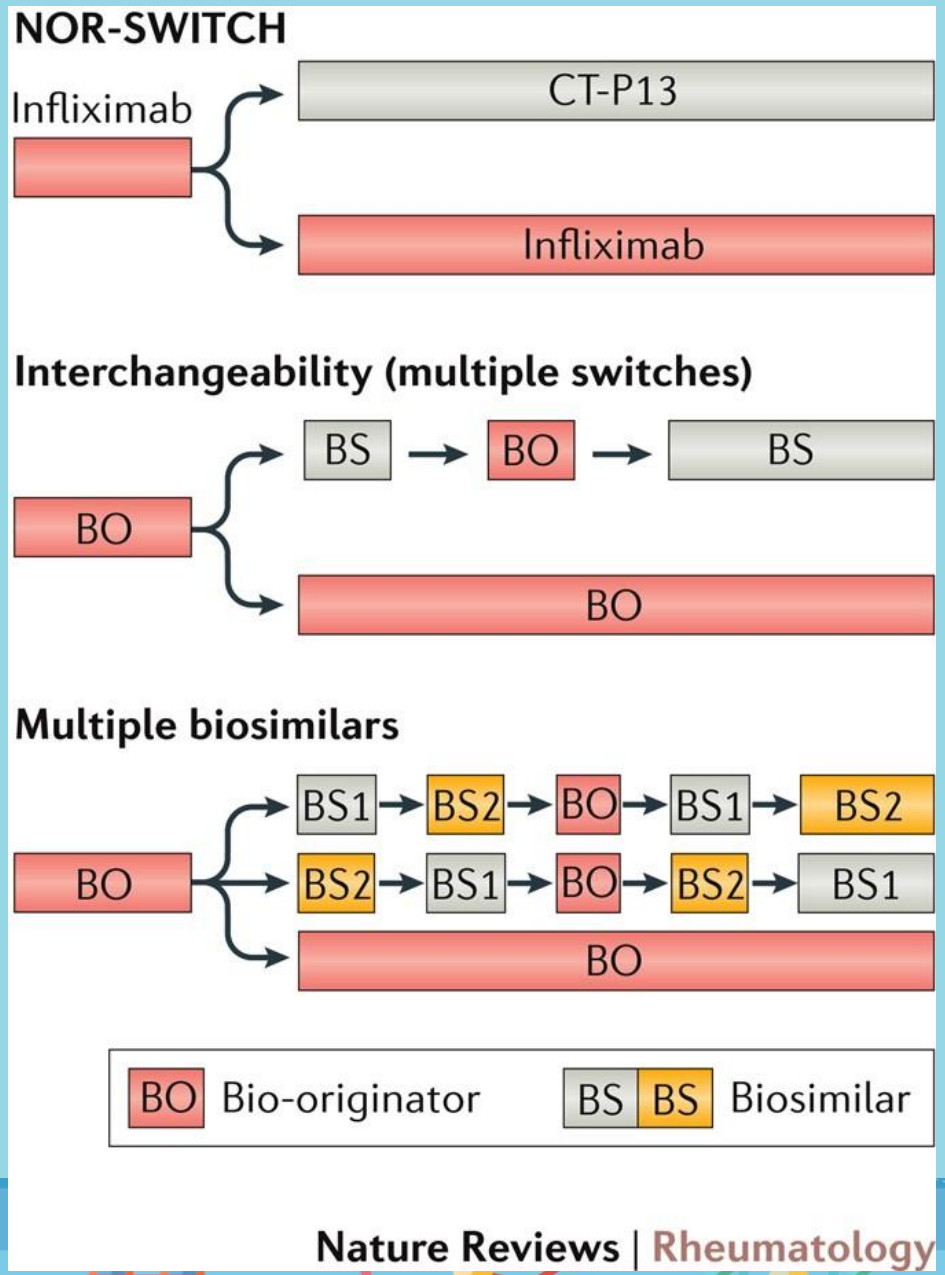
	Remicade	CT-P13
CD (n=155)	14 (21.%)	23 (36.5%)
UC (n=93)	3 (9.1%)	5 (11.9%)

Switching study model in real world situation



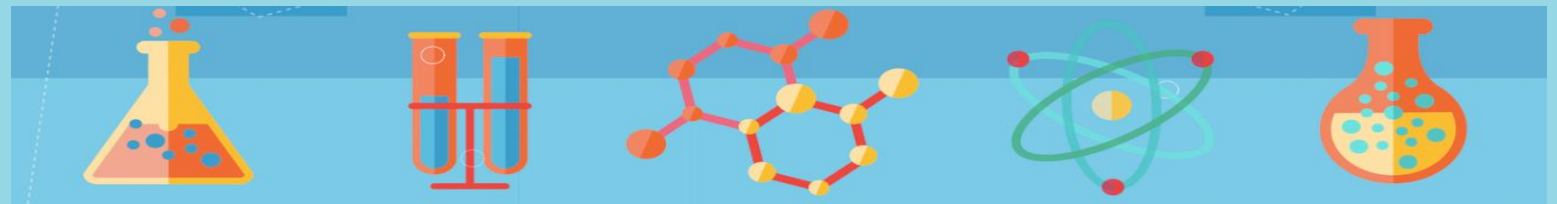
Real World of Switching on Biopharmaceuticals




NOR-SWITCH
Infliximab
CT-P13
Infliximab
Interchangeability (multiple switches)
BO
BS
BO
BS
BO
Multiple biosimilars
BO
BS1
BS2
BO
BS1
BS2
BS2
BS1
BO
BS2
BS1
BO
BO Bio-originator
BS BS Biosimilar
Nature Reviews | Rheumatology

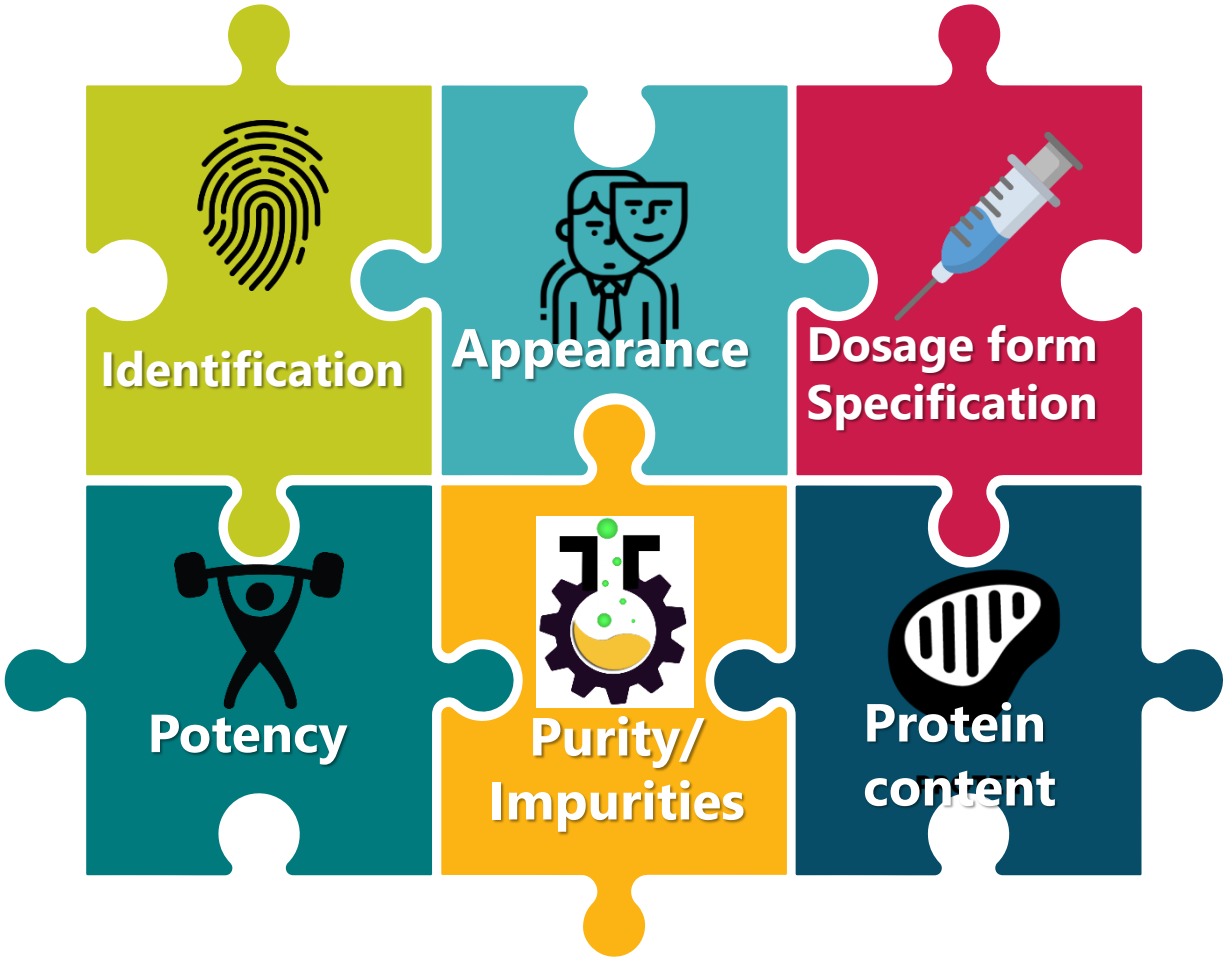
Take Home Message

- Biologics are complex and heterogenous mixture
- Biosimilar registration apply for therapeutic recombinant proteins
- Biosimilar may be interchangeable with awareness
- Non-comparable biologics should not be interchangeable



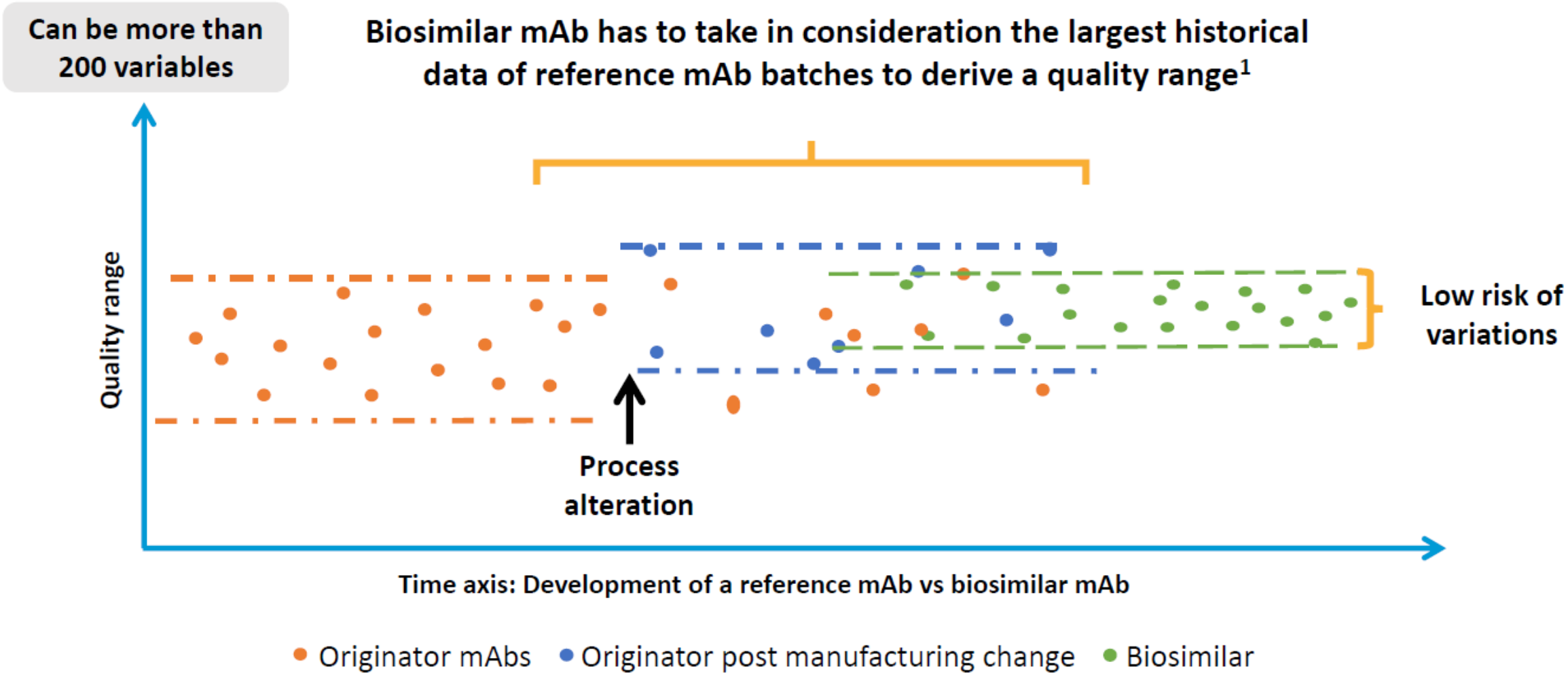
Specification Key Components

PPP AID

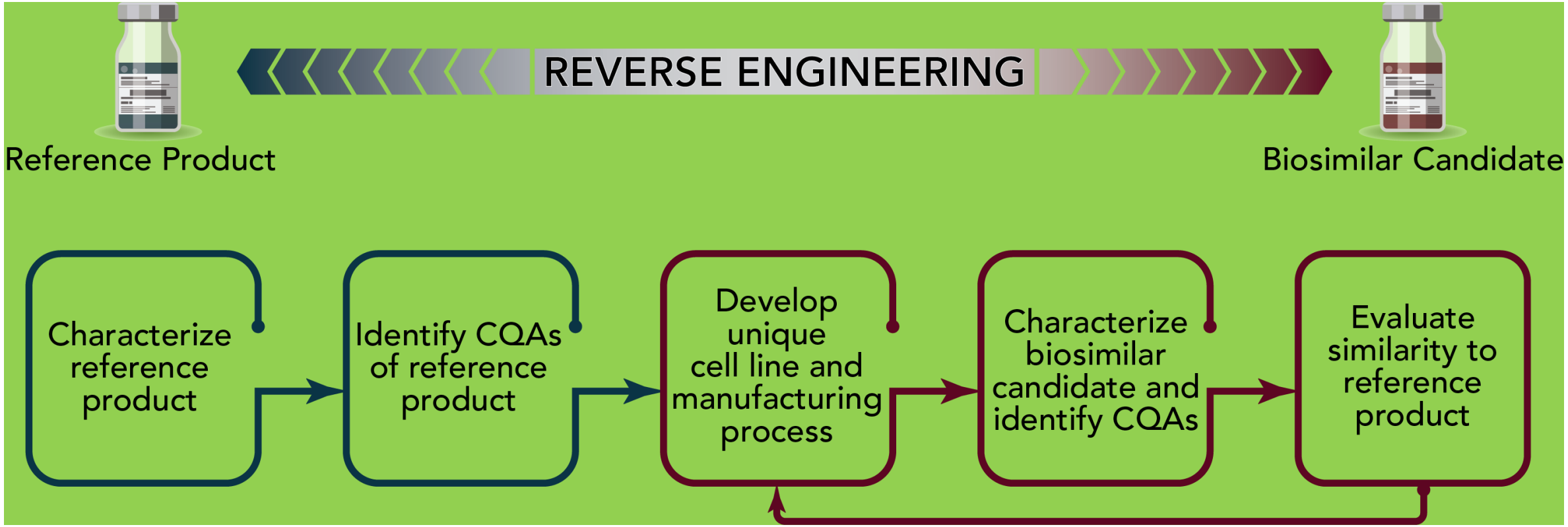


- Potency
- Purity/Impurities
- Dosage form specifications
- Appearance
- Identification
- Protein Content

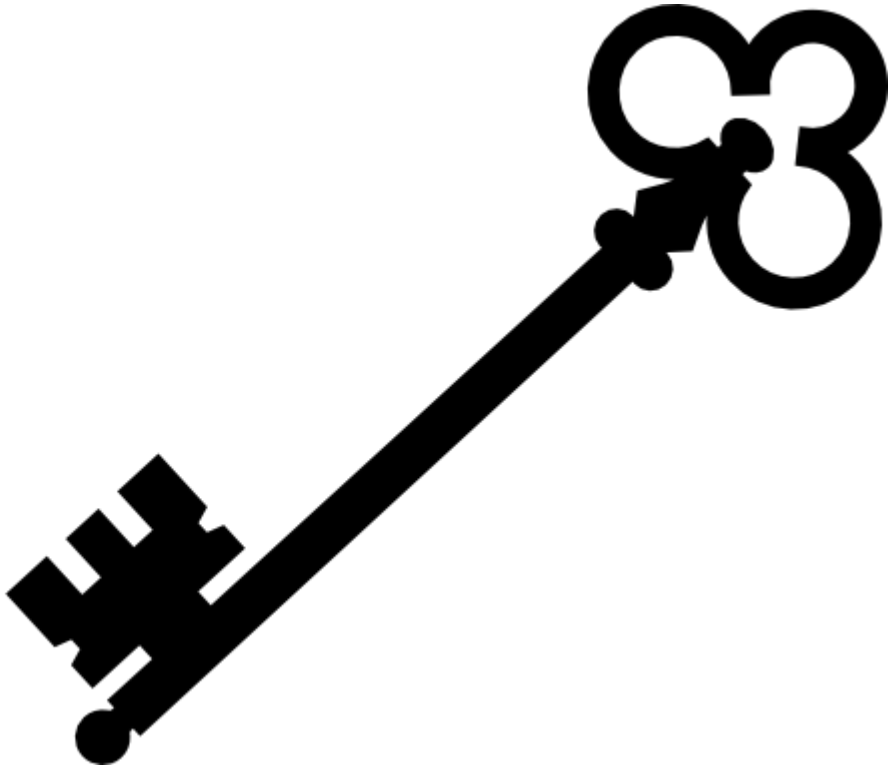
The Originator Sets the Rules for Quality



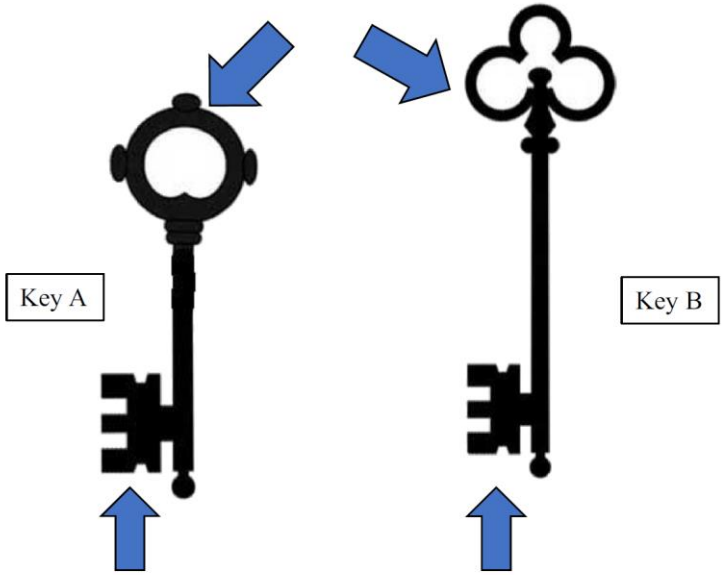
Biosimilars Are Reverse Engineered



Comparison of the shapes of two keys: similarity of function can be concluded from known likeness of composition

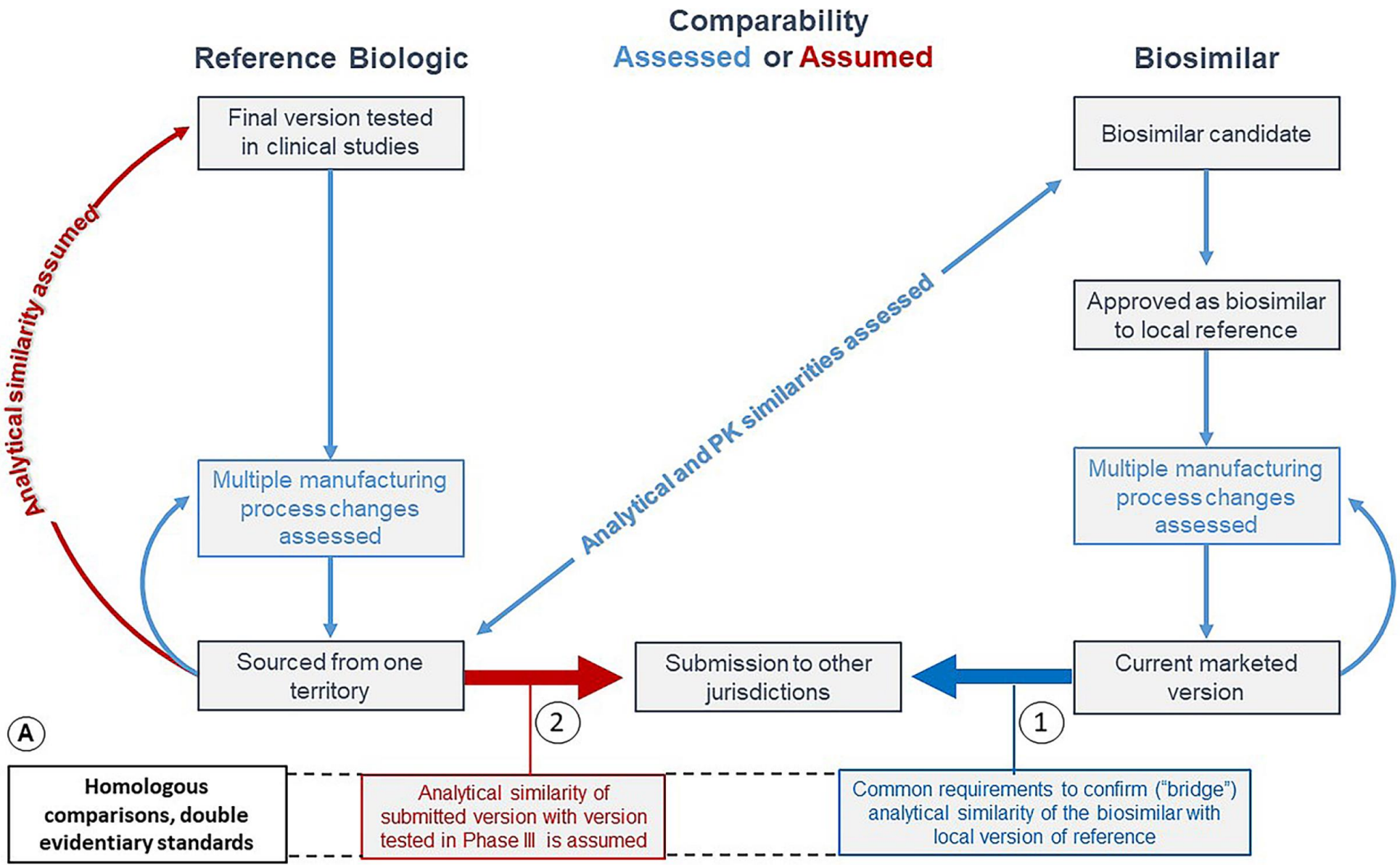


Non-functional parts of the molecule may have some differences in composition and conformation



Biologically active parts of the molecule have same composition and conformation as determined by analytical inspection

Comparability of Biologics and Biosimilars



Timeline on Biosimilar Adoption (EMA, USFDA, WHO, TFDA)

2005

2009 — WHO guidelines on evaluation of similar biotherapeutic products (SBP)

2013 — Thailand Biosimilar Guidelines

2015 — **US FDA biosimilar guidelines**

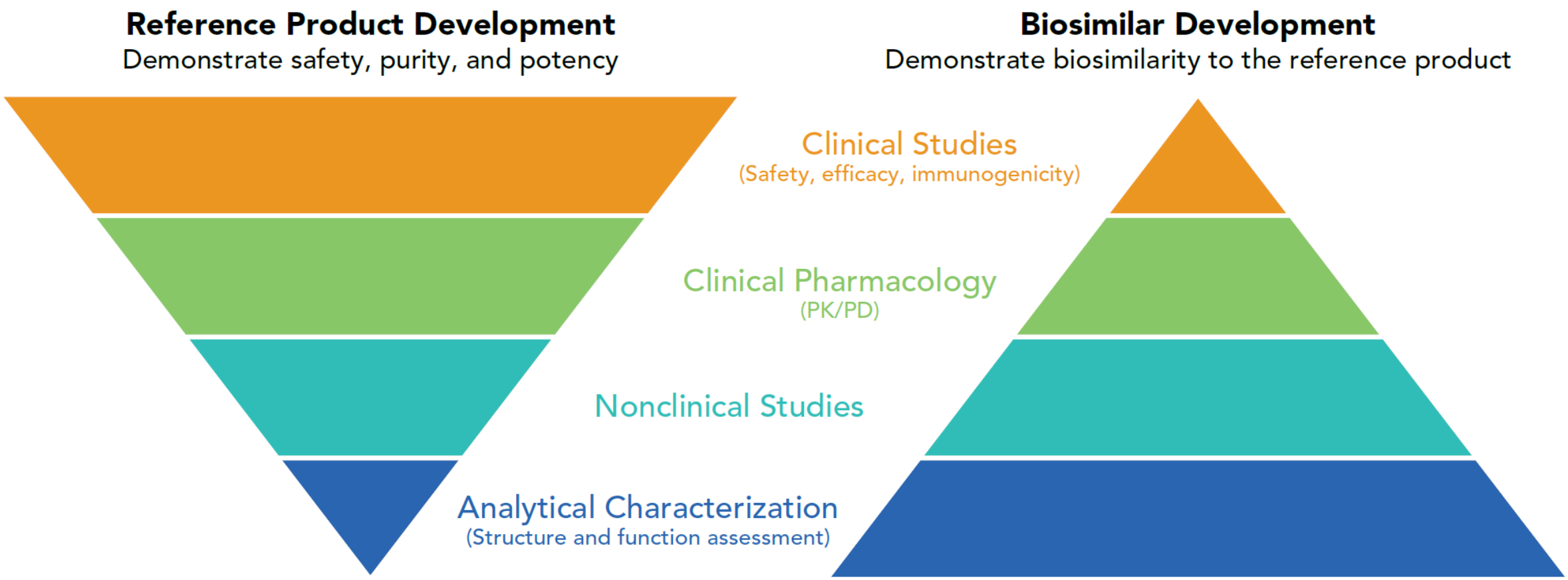
2015

2017 — Thailand Biosimilar Guidelines Revision 1

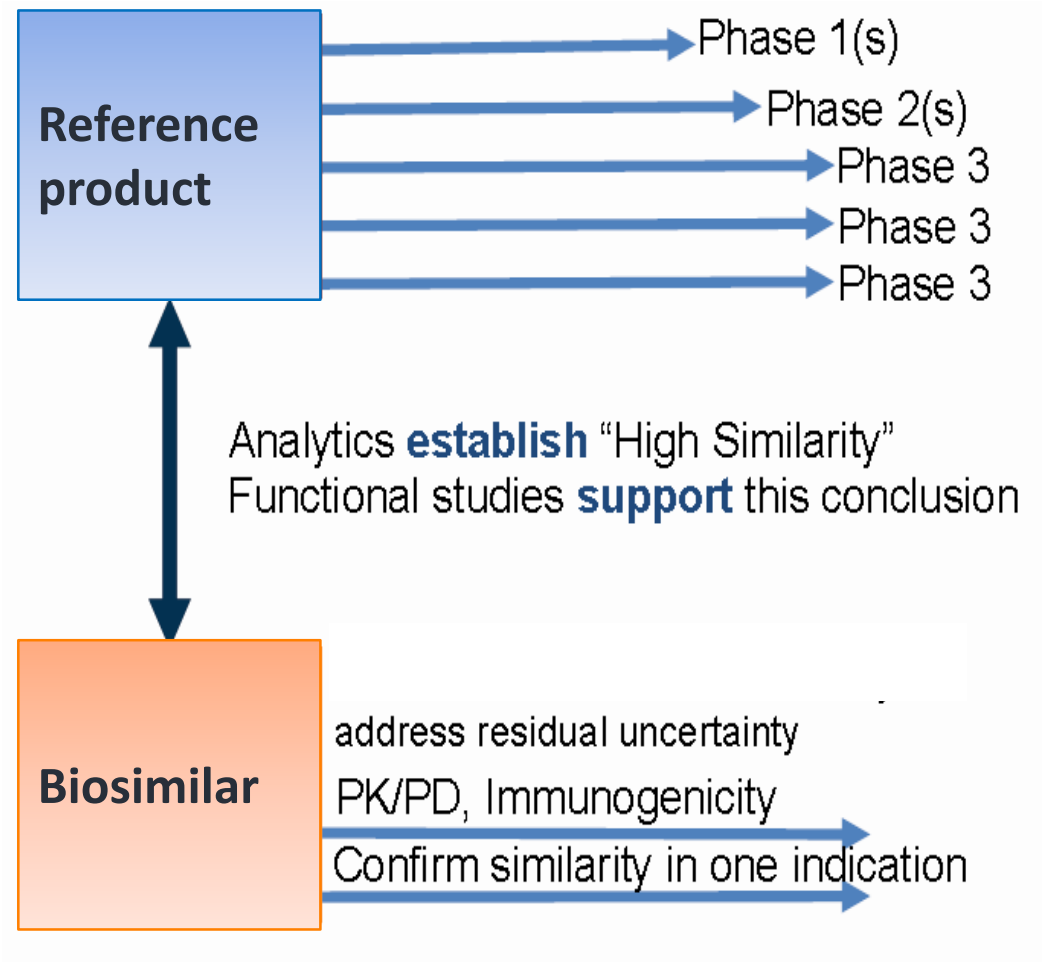
2022 — WHO guidelines on evaluation of Biosimilars

NOW

Reference Product vs. Biosimilar Development



Clinical development : Biosimilar vs. Reference bioproducts



Multiple Indications:

No extrapolation → clinical trials in each indication

Multiple Indications:
Extrapolation possible if scientifically justified → clinical trials not required in each indication

Interchangeability

In EU, the biosimilar approval **did not automatically allow interchangeability**

Interchangeability/switching remains a national decision.

More than 10 yrs experience of biosimilar in the market, several EU countries change regulations to ***less stringency on Interchangeability/switching/substitution***.

WHO position of Biosimilar

NRA could improve access to biosimilars of assured quality, safety and efficacy by improving the efficiency of their regulatory evaluation.

WHO guidelines on Evaluation of similar biotherapeutics products (SBP) adopted by ECBS in 2009

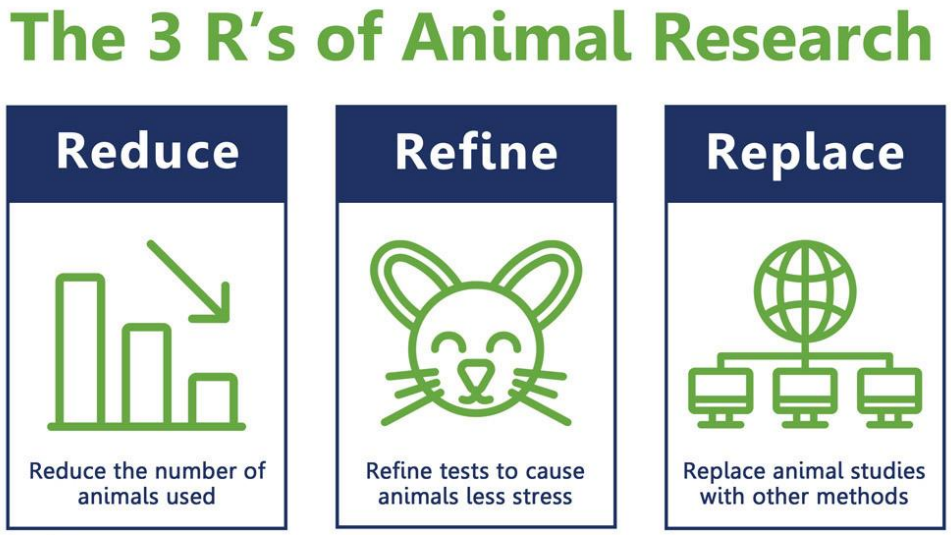
In 2019 – more tailored and potentially reduced clinical data package by the available scientific evidence.

The revised 2022 WHO biosimilars guideline

- Provides a timely opportunity to collectively re-evaluate the way regulatory requirements can be better advance biosimilar access
- Key updates in the revised guideline:
  - Animal Studies - A limited, exception-based approach
  - Clinical Comparability Requirement - A streamlined approach with CMC
  - Sourcing of comparator products – Simplified approach

Key updated: 1 A limited, exception-based approach towards animal studies

- 3Rs Principles
- “The need for additional in vivo animal studies would be expected to represent a rare scenario”
- State-of-the-art analytical and in vitro functional testing and robust PK/PD studies are sufficient to demonstrate biosimilarity



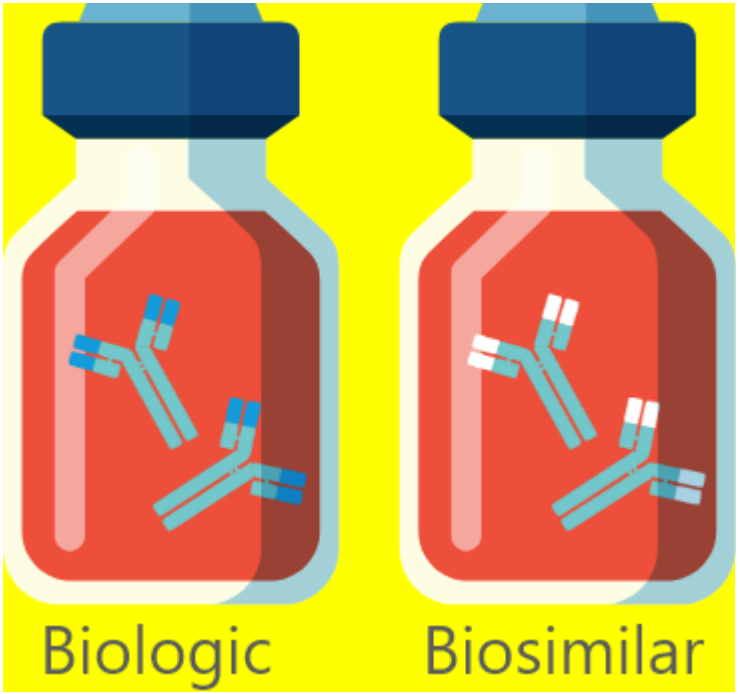
Key updated: 2 Streamlined approach to clinical efficacy & safety comparability requirements

- “Comparative efficacy and safety trial will not be necessary if sufficient evidence of biosimilarity can be drawn from other parts of the comparability exercise”

- ✓ **Quality**
- ✓ **Non-clinical**
- ✓ **PK/PD**
- ✓ **Efficacy**
- ✓ **Safety**

Key updated 3: Simplified approach to the sourcing of comparator products

- The use of a non-local reference product as comparator is acceptable.



Challenging Issues on Biosimilar

Nocebo Effect

Healthcare Providers and Patients Education

Traceability

Communication

Market Access

Switching Concern of Biosimilar

- switching from reference medicines to biosimilars is associated with altered immunologic responses.
- Some (but not all), therapeutic proteins are inherently immunogenic.
- Immunologic responses induced during treatment with therapeutic biologics and their clinical significance may be influenced by a wide variety of factors
  - medicine features
  - patient variables
  - treatment parameters

The Efficacy, Safety, and Immunogenicity of Switching Between Reference Biopharmaceuticals and Biosimilars: A Systematic Review

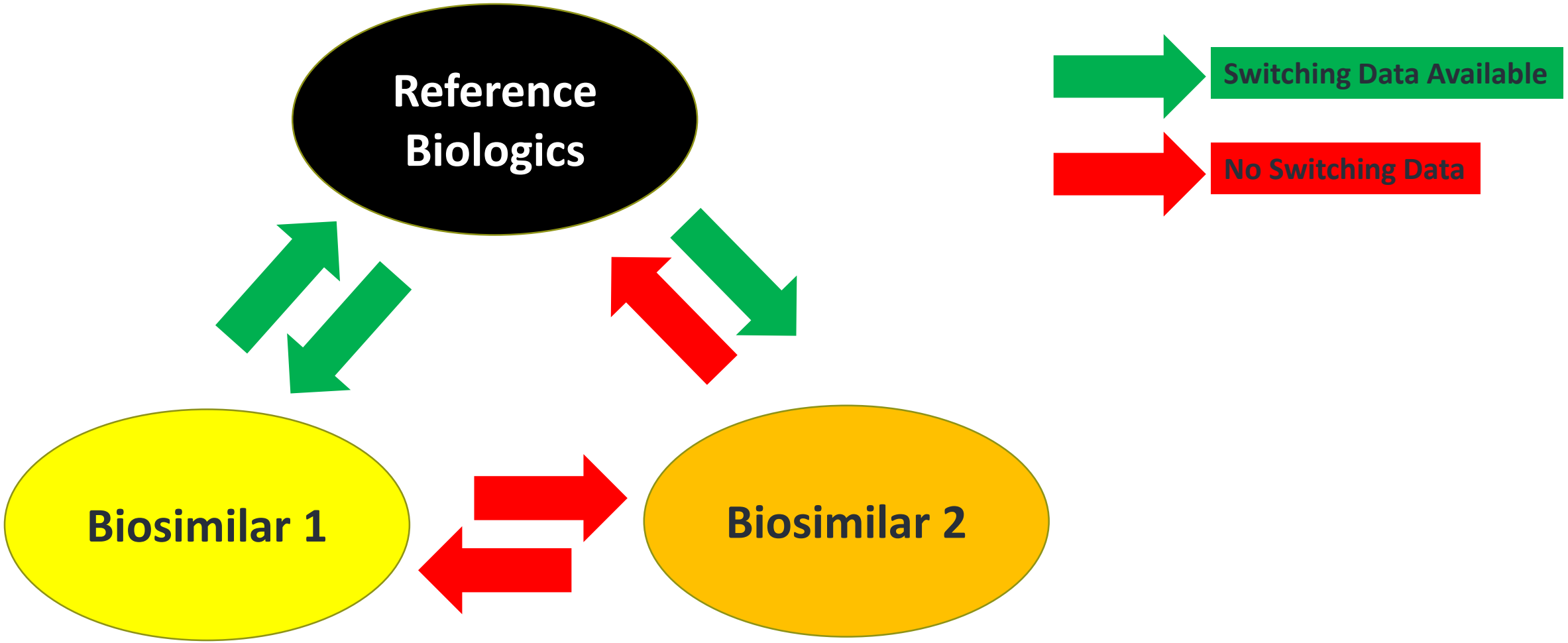
Liese Barbier[1,*], Hans C. Ebbers[2], Paul Declerck[1], Steven Simoens[1], Arnold G. Vulto[1,3,†] and Isabelle Huys[1,†]

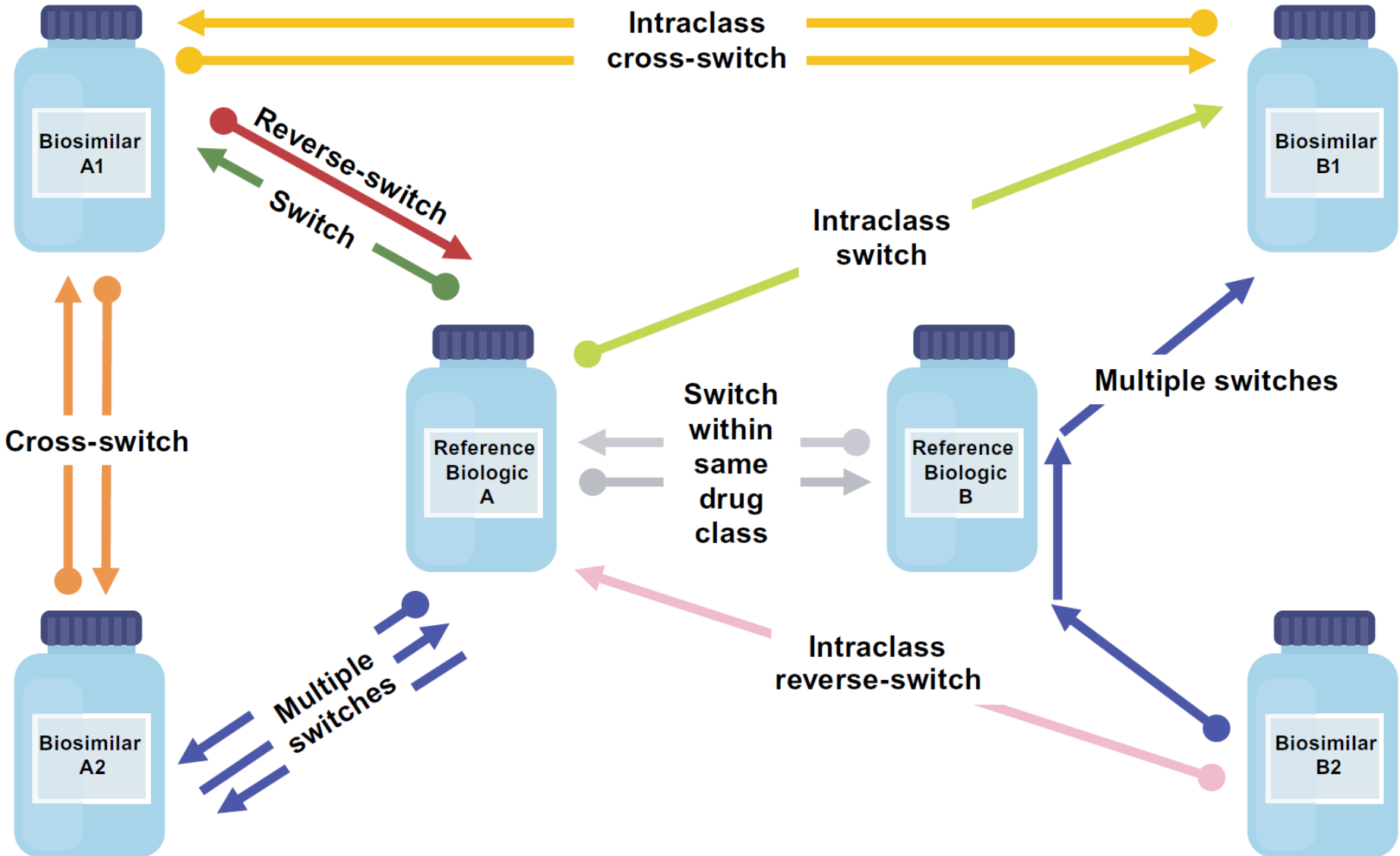
To date, no consensus exists among stakeholders about switching patients between reference biological products (RPs) and biosimilars, which may have been curbing the implementation of biosimilars in clinical practice. This study synthesizes the available data on switching and assesses whether switching patients from a RP to its biosimilar or *vice versa* affects efficacy, safety, or immunogenicity outcomes. A total of 178 studies, in which switch outcomes from a RP to a biosimilar were reported, was identified. Data were derived from both randomized controlled trials and real-world evidence. Despite the limitations stemming from a lack of a robust design for most of the studies, the available switching data do not indicate that switching from a RP to a biosimilar is associated with any major efficacy, safety, or immunogenicity issues. Some open-label and observational studies reported increased discontinuation rates after switching, which were mainly attributed to nocebo effects. Involvement of the prescriber in any decision to switch should remain and attention should be paid to the mitigation of a potential nocebo effect.

Do we really need biosimilar interchangeability study?

Switching study model in real world situation

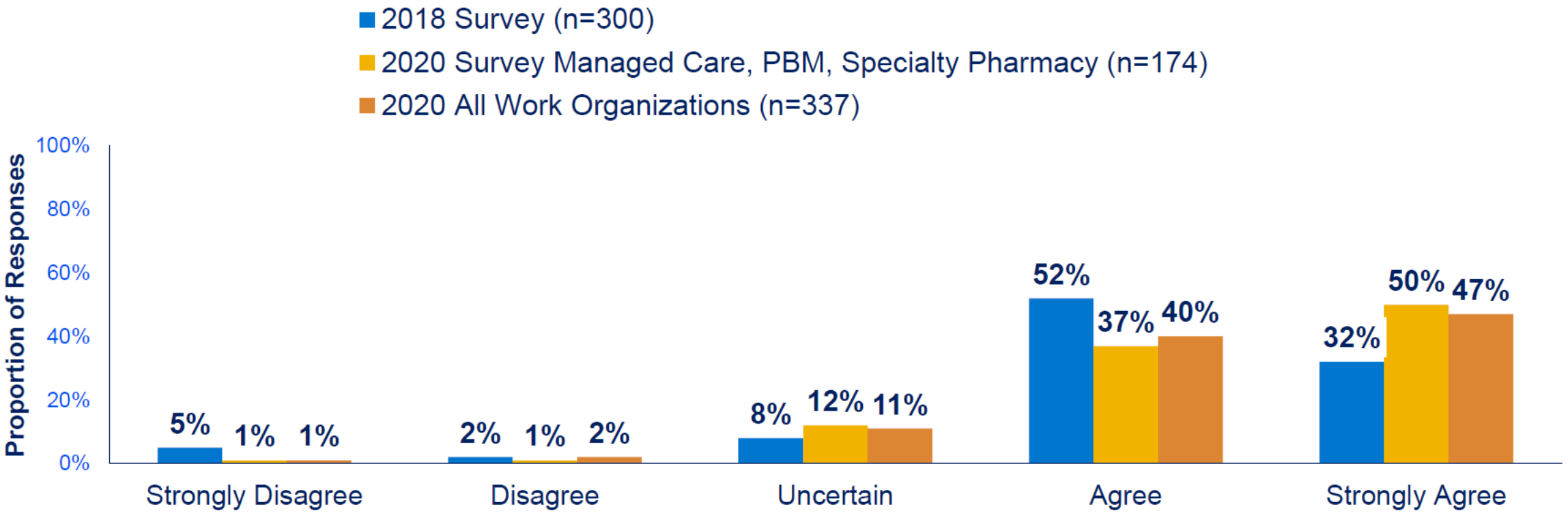
Switching study model in real world situation




Intraclass
cross-switch
Biosimilar
A1
Biosimilar
B1
Reverse-switch
Switch
Intraclass
switch
Cross-switch
Reference
Biologic
A
Switch
within
same
drug
class
Reference
Biologic
B
Multiple switches
Multiple
switches
Intraclass
reverse-switch
Biosimilar
A2
Biosimilar
B2

AMCP Foundation Survey: Higher Levels of Agreement Switching to Biosimilars

Q: For patients whose conditions are treated on reference biologics, switching to a biosimilar product is safe and effective



Survey respondents in 2020 reported higher levels of agreement than survey respondents in 2018.

$U\ (n_{2018} = 300, n_{2020} = 174) = 22{,}601,\ p = 0.019$

$U\ (n_{2018} = 300, n_{2020} = 337) = 42{,}847,\ p = 0.001$

BioDrugs
https://doi.org/10.1007/s40259-022-00571-5

ORIGINAL RESEARCH ARTICLE

Malaysian Hospital Pharmacists' Perspectives and Their Role in Promoting Biosimilar Prescribing: A Nationwide Survey

Noraisyah Mohd Sani[1,2] · Zoriah Aziz[1,3] · Adeeba Kamarulzaman[1]

Accepted: 22 November 2022

Perceived barriers to promote biosimilars in clinical practice

>50%

Product Efficacy Concerns
Prescribers' Preference
Products' quality concerns
Insufficient information resources
Product Safety Concerns

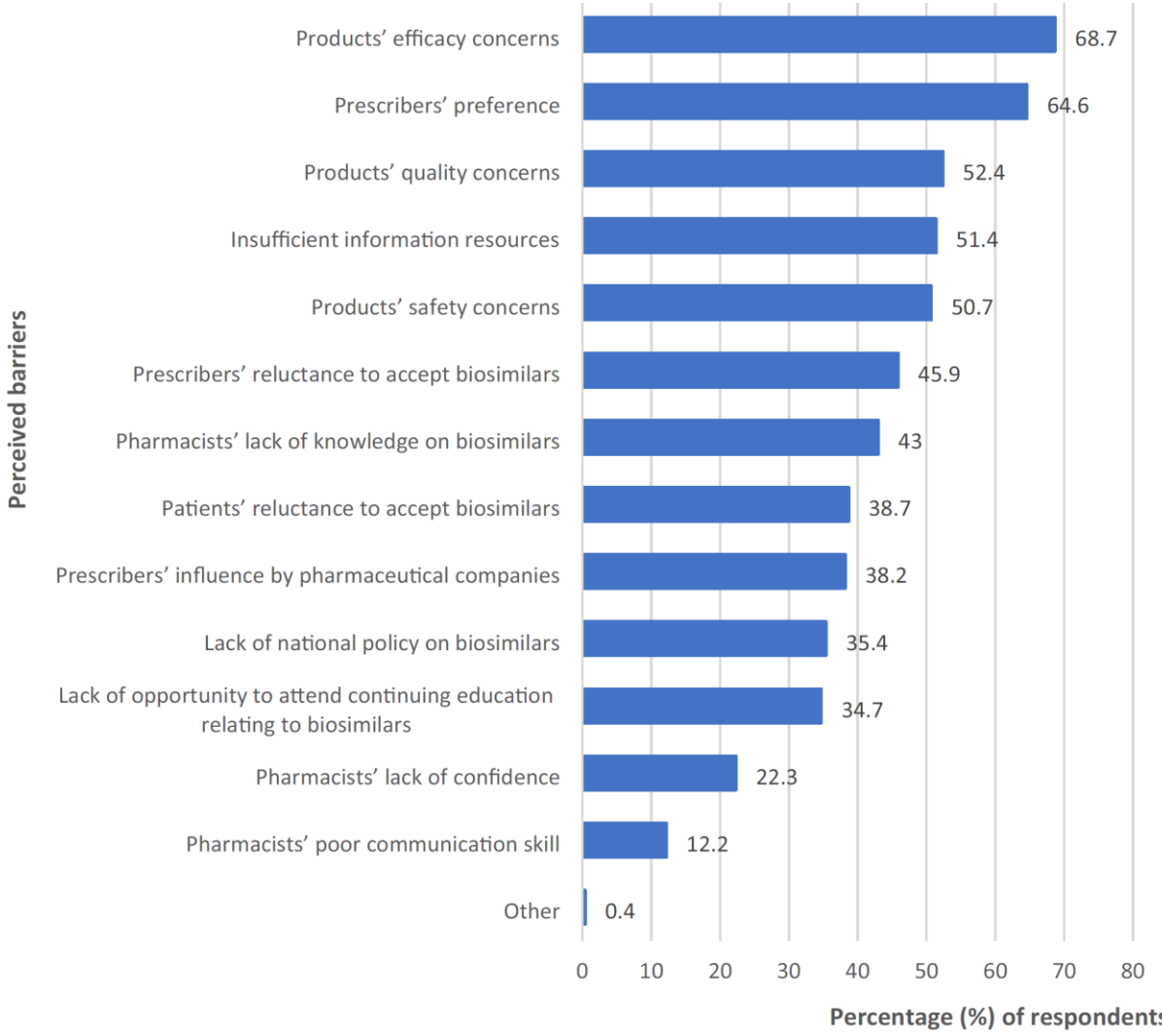
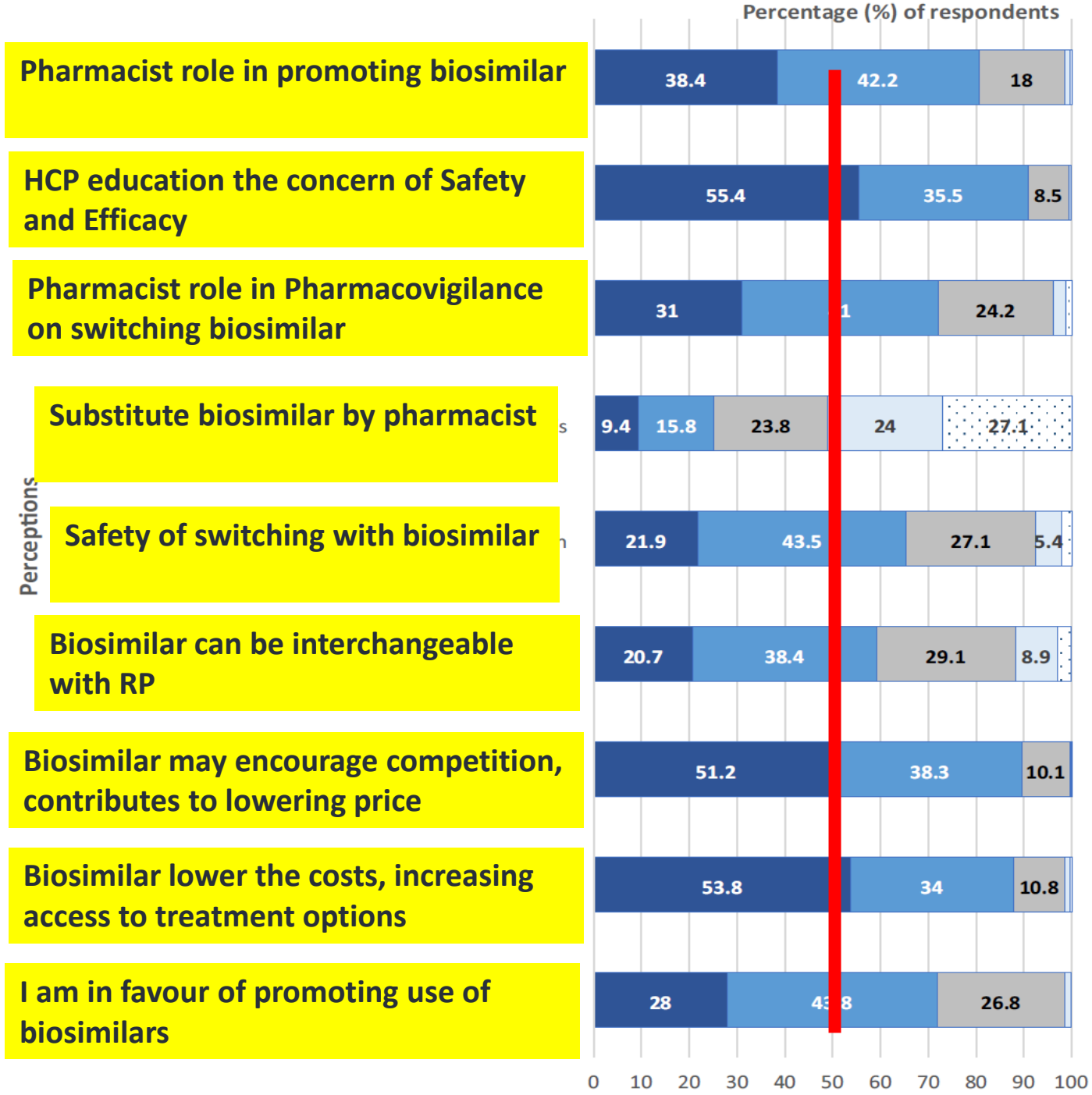


Table 2 Respondents' answers to knowledge statements about biosimilars (N = 913)

No.	Please indicate whether the characteristics stated below are true or false about biosimilars	Correct answer	Correct responses, *n* (%)
Q13	A biosimilar is structurally identical to its originator product	False	195 (21.4)
Q14	A biosimilar is similar to its originator product that has gone off-patent	True	780 (85.4)
Q15	A biosimilar has no meaningful differences from its originator product in terms of QUALITY	True	727 (79.6)
Q16	A biosimilar has no meaningful differences from its originator product in terms of SAFETY	True	753 (82.5)
Q17	A biosimilar has no meaningful differences from its originator product in terms of EFFICACY	True	734 (80.4)
Q18	A biosimilar has the same recommended dosage as its originator product	True	762 (83.5)
Q19	A biosimilar is approved for marketing authorisation by the Malaysian Drug Control Authority solely based on its pharmacokinetic bioequivalence to the originator product	False	195 (21.4)
Q20	A biosimilar requires more comprehensive data to support its marketing authorisation approval compared to a generic drug	True	750 (82.1)
Q21	A biosimilar requires data on comparative PRECLINICAL STUDIES to its originator to support its marketing authorisation approval in Malaysia	True	736 (80.6)
Q22	A biosimilar requires data on comparative CLINICAL STUDIES to its originator to support its marketing authorisation approval in Malaysia	True	798 (87.4)

Attitude on Biosimilar Malaysian Pharmacist Survey



Survey on knowledge and attitude of physicians and pharmacists associated with biosimilars

Thanabodee Thongbai, Jitrin Fongsataporn, Thitiwat Kamolchum, Phanuwat Chaovirakij, Thamonwan Pornpanichjaroen
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Introduction

- Originator products are expensive due to the manufacturing process.
- Patients are difficult to access.
- Biosimilars can produce cost saving.
- Biosimilars can interchange to originator products.
- The way of using biosimilars is importance.
- The factors affect activities regarding biosimilars should be investigated.

Objectives

• To study the current level of knowledge and attitude of biosimilars in physicians and pharmacists.

• To study a relationship between personal information, knowledge and attitude of biosimilars in physicians and pharmacists.

Method

Create surveys
- Personal data
- Knowledge
- Attitude

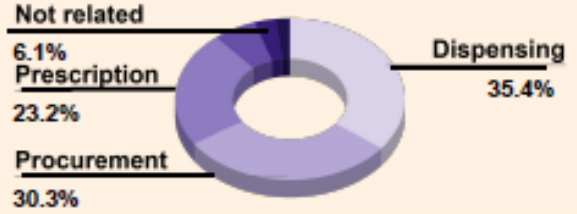
Receive data

Conclusions and discussion

Send surveys to healthcare providers

Analyze the result (Descriptive and

The relationship between healthcare providers and biosimilars



Knowledge

	Correct	Incorrect
Definition	63.24%	36.76%
Registration	43.15%	56.85%
Administration	63.01%	36.99%
Interchangeability	49.77%	50.23%

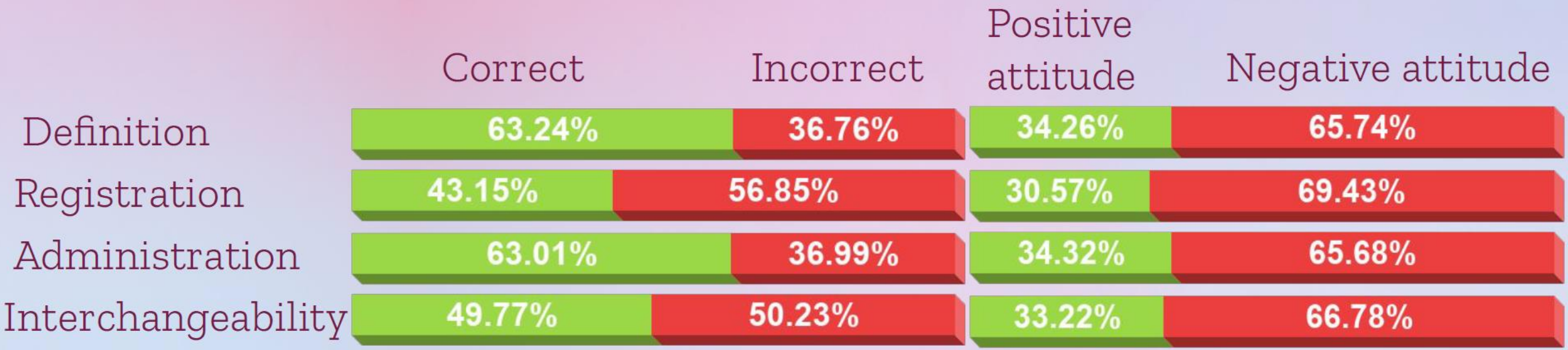
Attitude

	Positive attitude	Negative attitude
Definition	34.26%	65.74%
Registration	30.57%	69.43%
Administration	34.32%	65.68%
Interchangeability	33.22%	66.78%

A relationship between personal information and

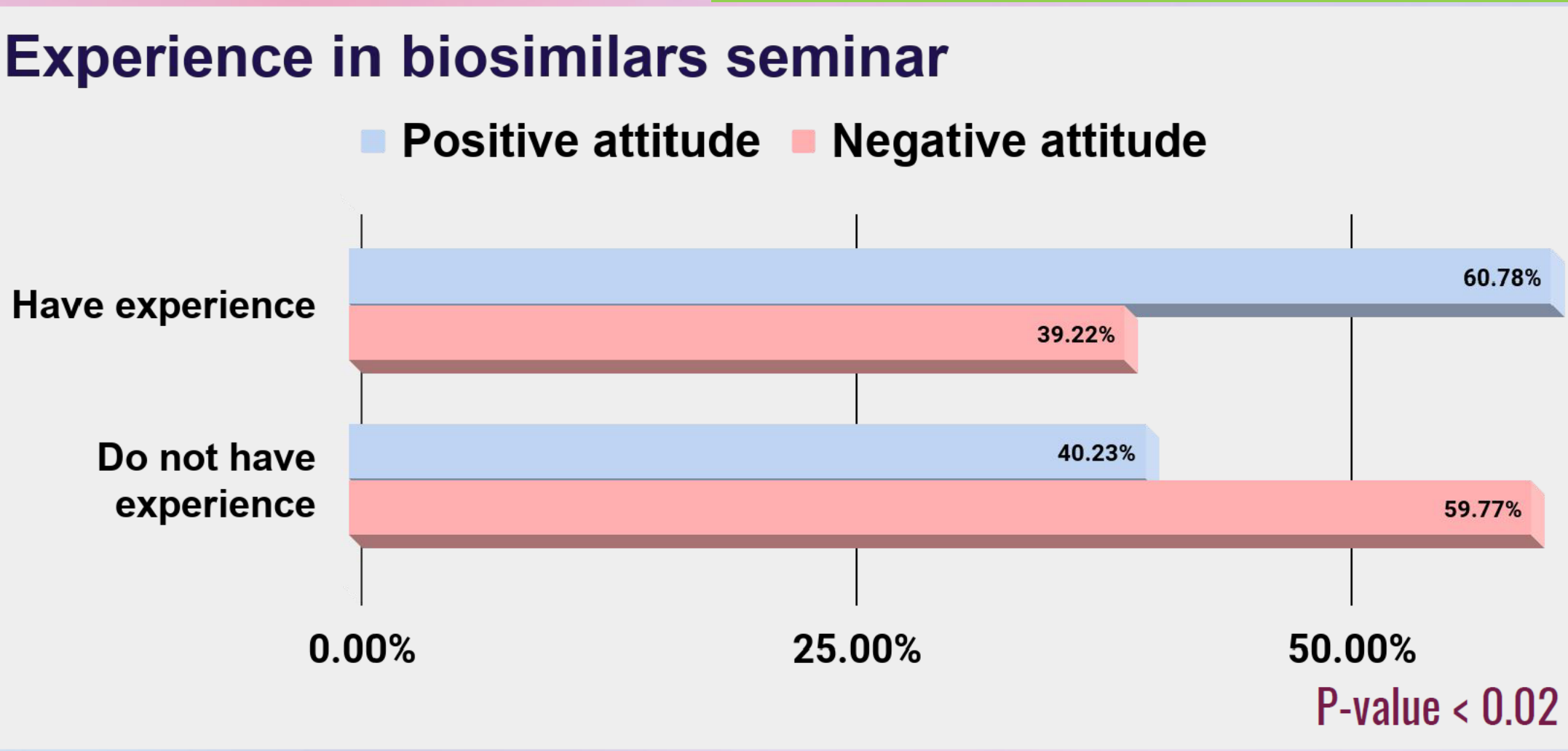
Knowledge

Attitude



Relationship

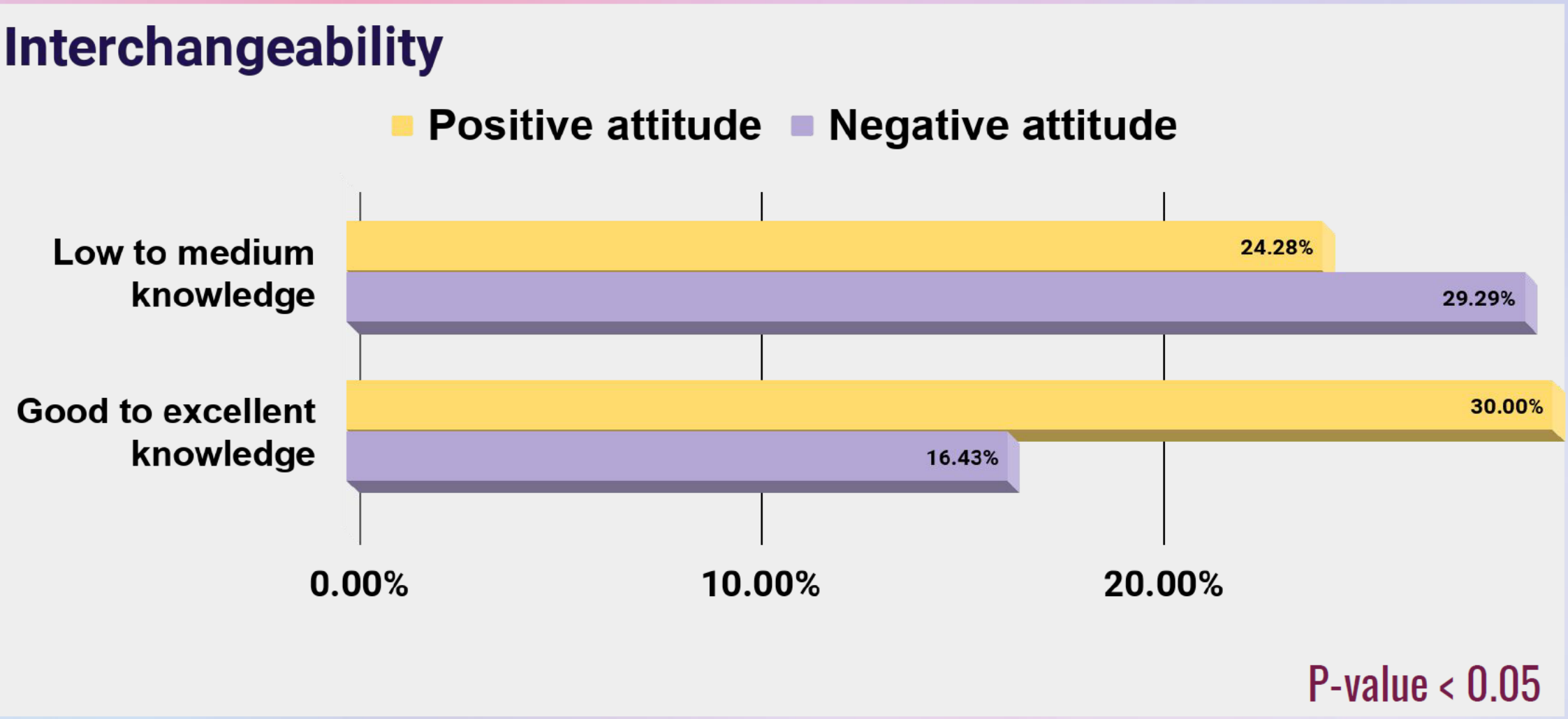
The More experience, the positive attitude



P-value < 0.02

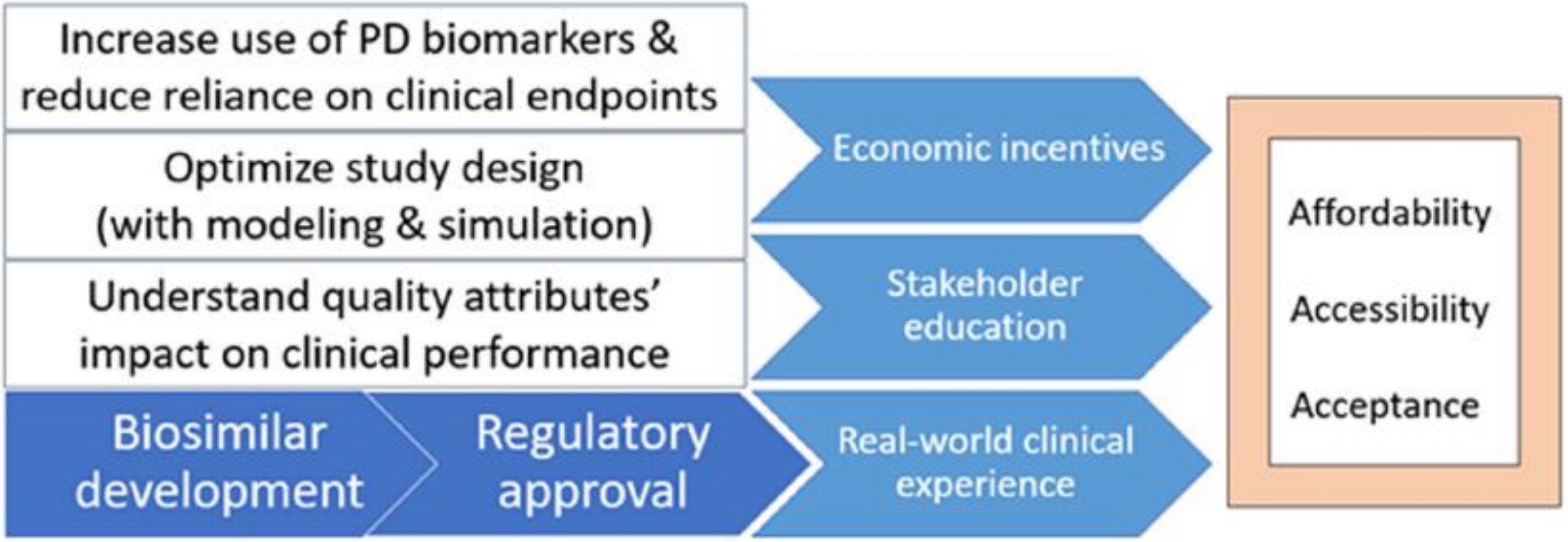
Relationship

The more knowledgeable, the more positive attitude



P-value < 0.05

Leveraging Innovations in Clinical Pharmacology and related disciplines to advance biosimilar development and support broader uptake of biosimilars




Thank you
Any questions, comments and suggestions are welcomes
twisit@gmail.com