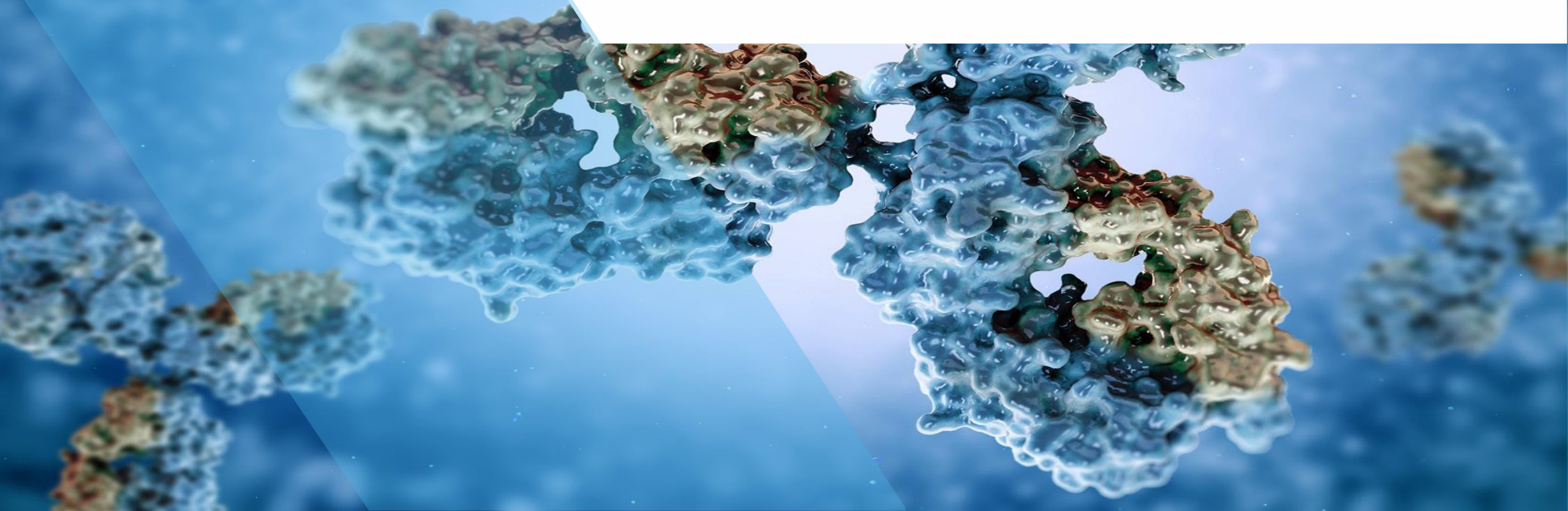
Scale-up of Chromatography



Learning Objectives

Outline the commonly used chromatographic techniques

Describe affinity, ion exchange and hydrophobic interaction chromatography

Know why different chromatographic techniques are used in a typical bioprocess

Learning Objectives

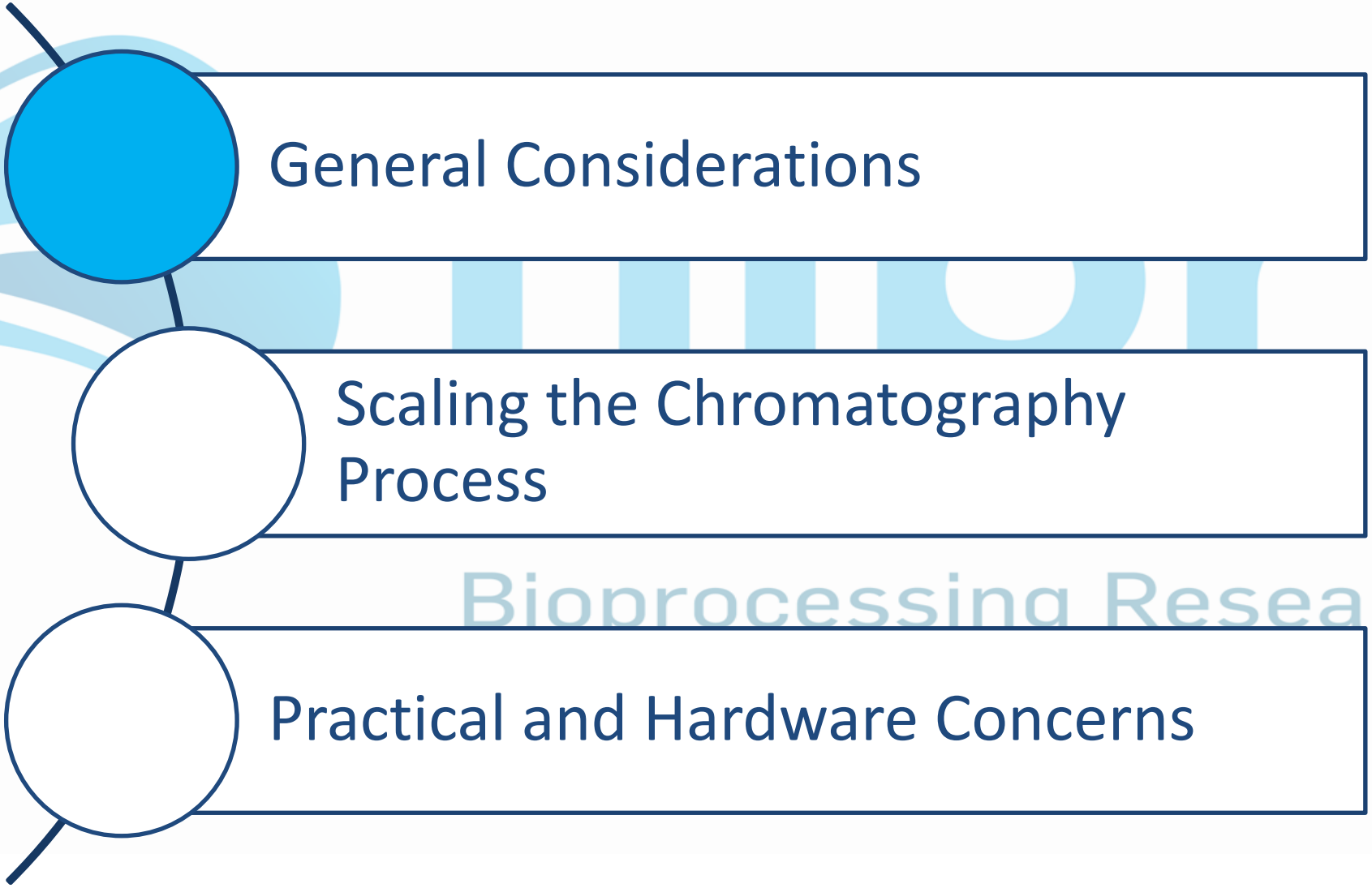
Outline the basic steps in a strategy for scaling-up chromatography parameters for large-scale manufacturing.

Understand the practical concerns in chromatography scale-up.

Discuss why process design must address manufacturing and compliance demands.

Understand the requirements for successful transfer of chromatographic unit operations.

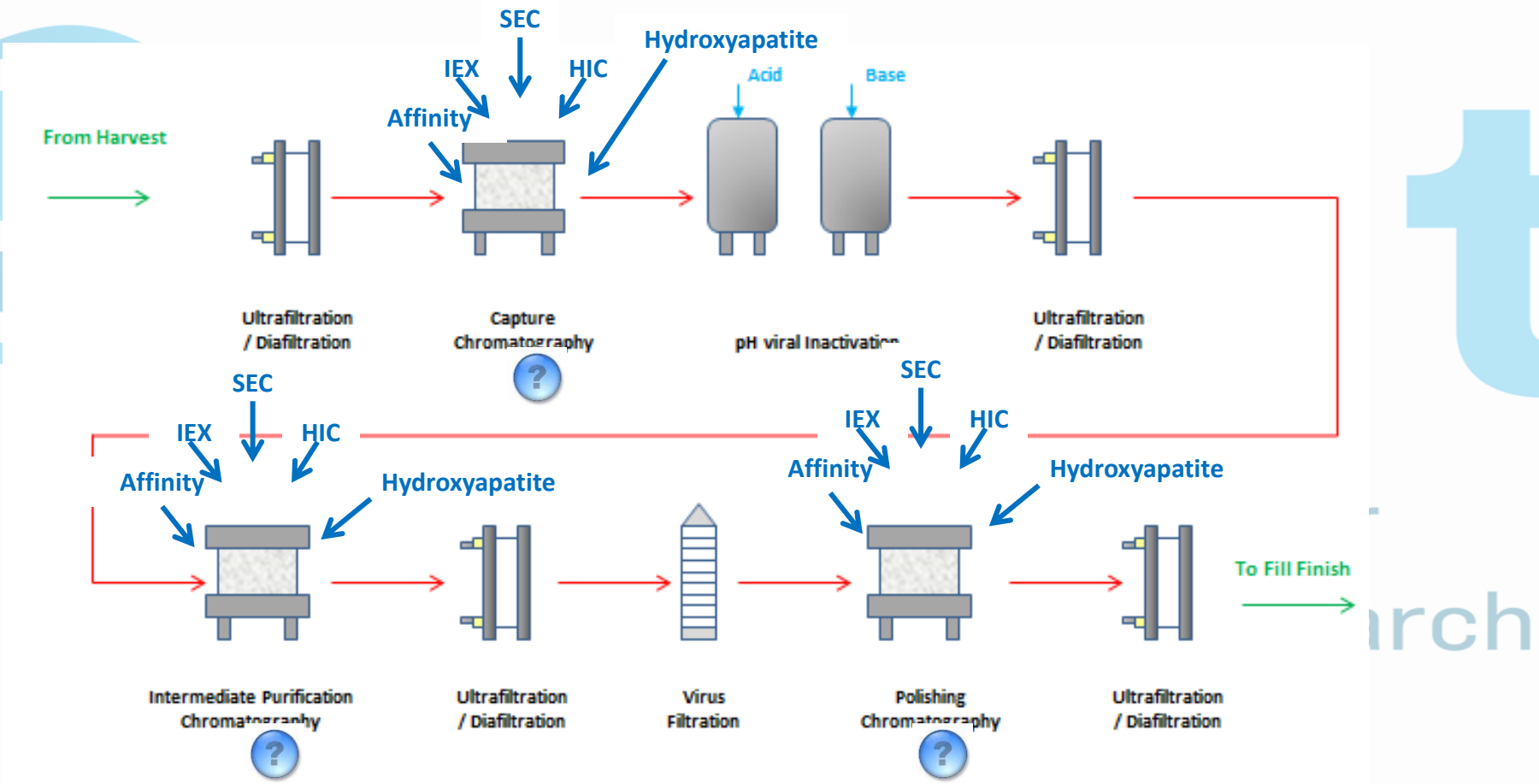
Topics



Scale-up of Chromatography

https://www.repligen.com/technologies/opus

Downstream Process

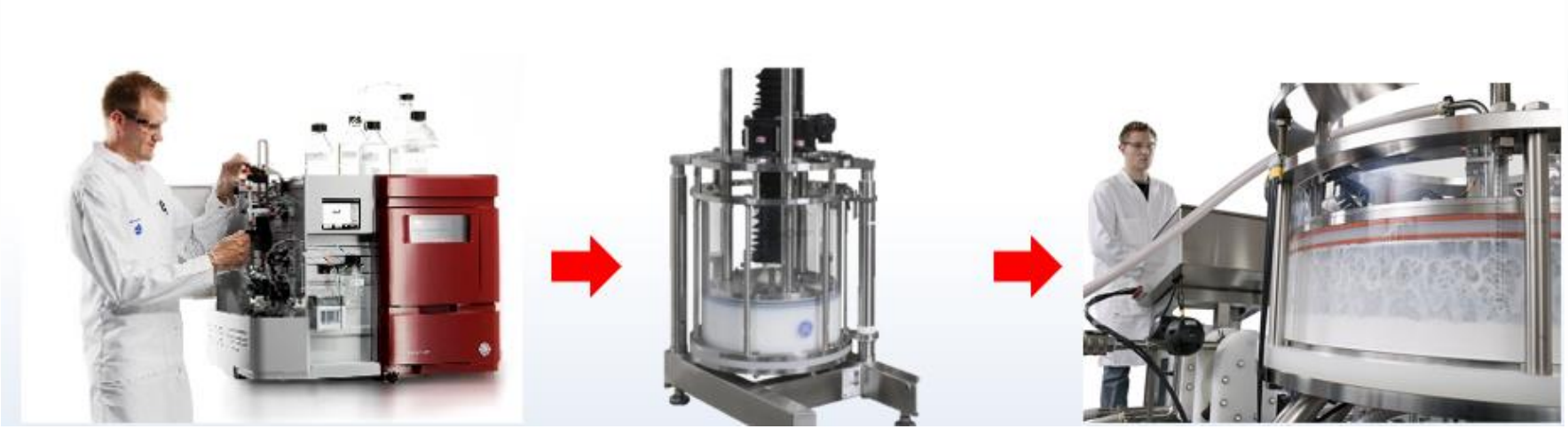


Scale-up: Robustness

- Producing **biologic** molecules is subject to much **variation.**
- The design of a process to manufacture products with **consistent quality** is the greatest challenge for manufacturers.
- The design must allow for certain variability in:
  - Critical processing parameters (e.g., pH, conductivity, temperature)
  - Raw materials (water, buffers, chromatography media)
  - Equipment
  - Personnel
  - Holding times
  - Working routines

Scale-up: Robustness

- Most scale-up **failures** are due to the scope of process development not being broad enough to address the **sources of variation.**
- **Safety margins** must be built into the various process control parameters, based on challenge tests performed at upper and lower limits of **“normal” variations.**



Scale-up: Compliance

- Process design must ensure **compliance** with both **manufacturing and regulatory** requirements.
- The **final planned production** scale must be defined as a **scale-up model** during the research phase.
- It is usual to involve **personnel with production experience** in the evaluation of the **scale-up model.**
- This will ensure that **specifying equipment** and unit operations that are very **difficult** to operate at largescale will be **prevented**.

Chromatography Development

- During **small-scale scouting trials**, a variety of chromatography media will have been screened, to select a suitable resin that can provide a basis for separation and purification.
- Once a medium has been defined, the next stage in the process is to design specific chromatographic procedures that can optimise target product **recovery** and **purity.**
- This is accomplished by defining the buffer solutions/conditions used during:
  - Sample conditioning
  - Media equilibration
  - Product elution

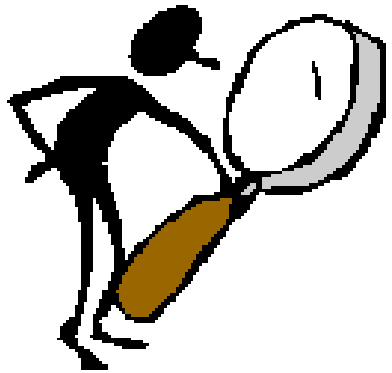
Chromatography Development

- The main result of the **process optimisation** phase is that **product information** and process **parameters** will have been considered in detail and where possible defined.
- This will ensure **smooth technical** transfer as the process is scaled-up to the production scale.
- Full and proper consideration of **scalability** as early as possible during development will make **technical transfer** straightforward.

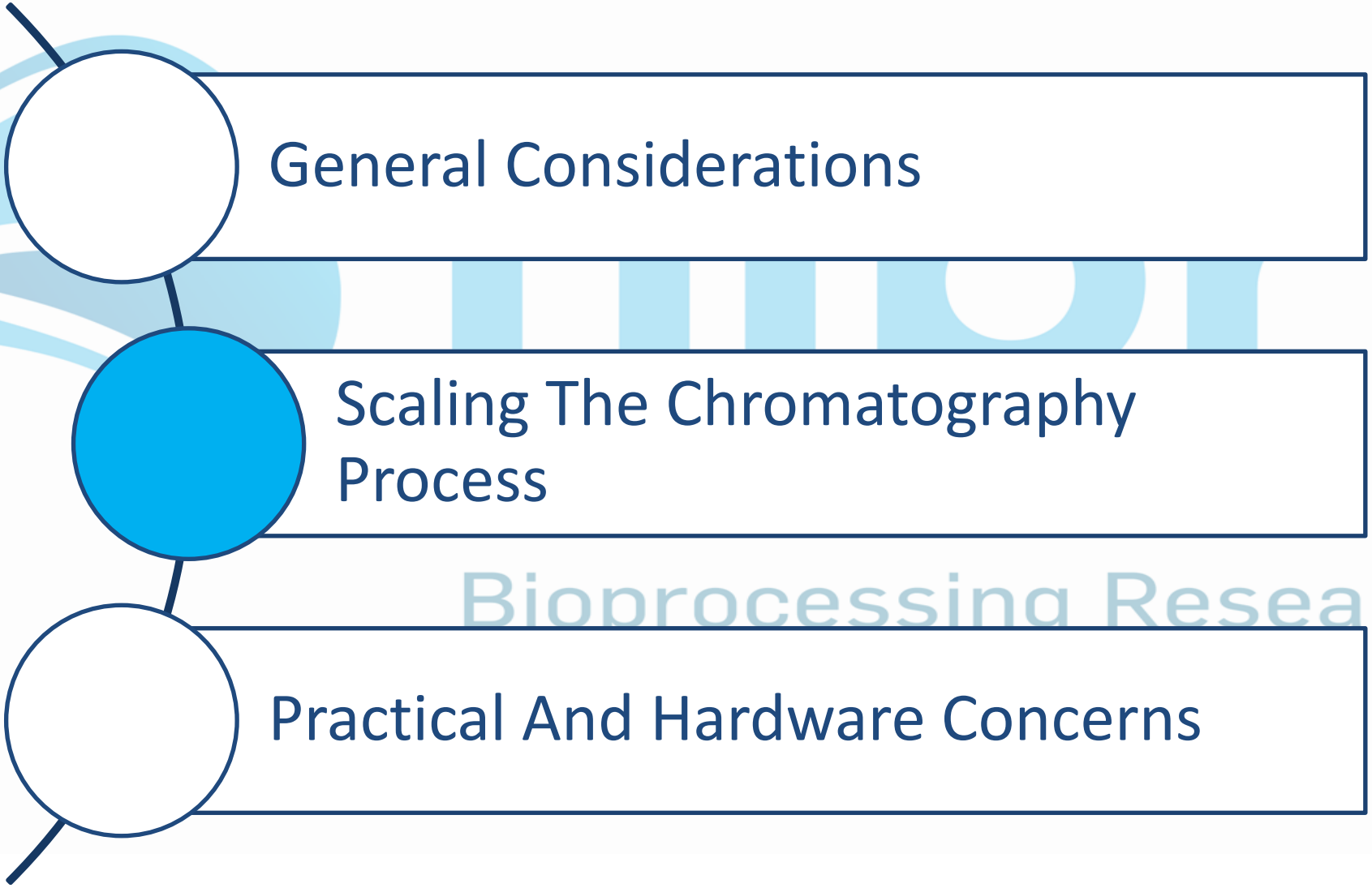
Optimisation Phase of Development

Output: Parameters to be Defined

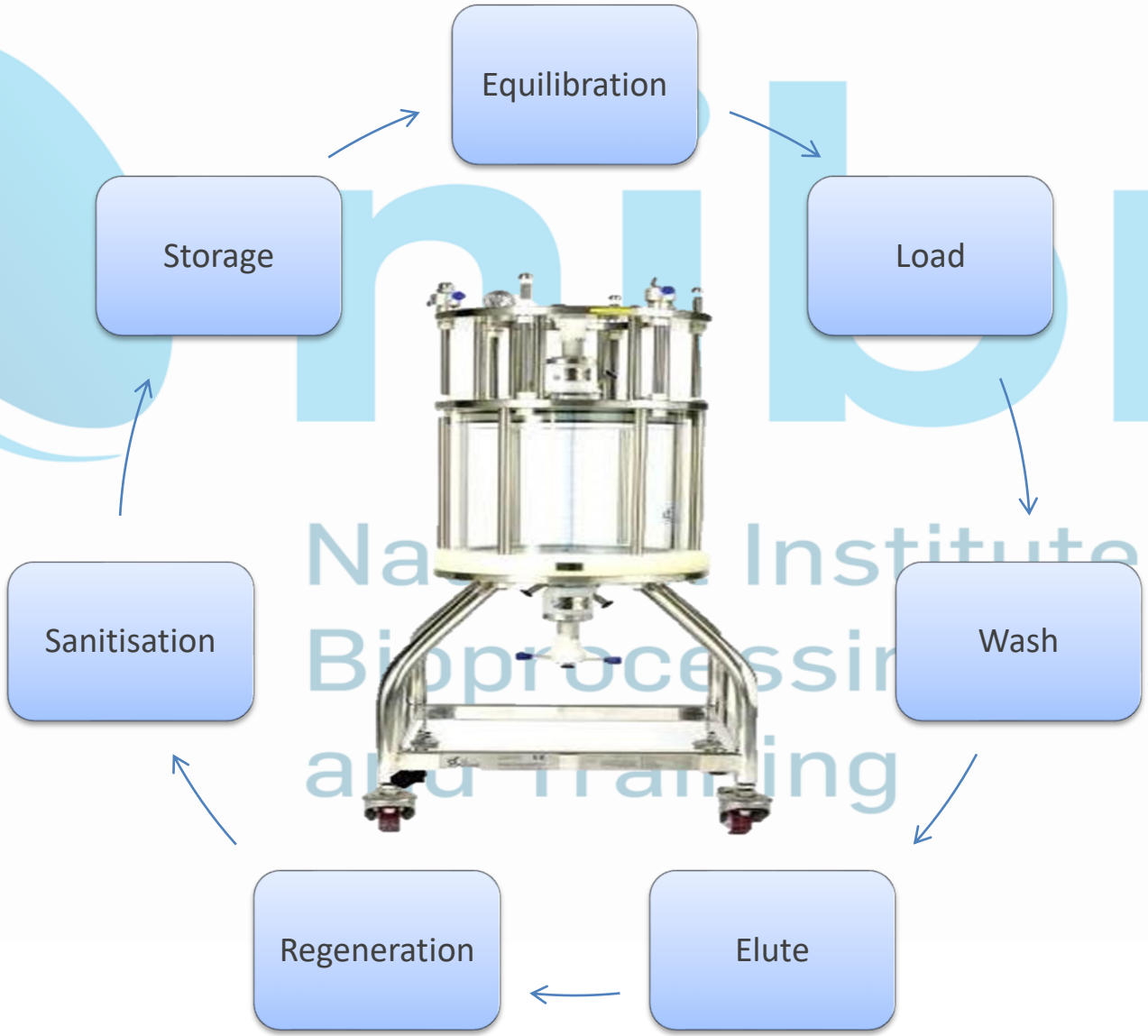
Process Parameters	Product Parameters
Sample pre-conditioning required	Stability
Sample concentration (load/ml resin)	Solubility in buffers
Sample volume (load/ml resin)	Storage conditions
Product concentration (load/ml resin)	Storage time
Resin bed height (cm)	
Linear flow rates (cm/h)	
Process volumes	
Maximum pressure drops	
Packed column qualification	
Buffer conditions (pH, conductivity)	
Fractionation scheme, if used	
Stability of resin to sanitisation agents	



Topics



Chromatography Operations



Chromatography Equipment

Column considerations:

a) Wall support
b) Distribution system
c) Handling / packability
d) Chemical resistance
e) Pressure rating
f) Hygienic design

Chromatography Scale-up

- Scaling-up a chromatographic process from laboratory to pilot plant can involve scale-up factors of 50- to 100-fold.
- Scaling up a chromatographic process from pilot plant to commercial production will involve another 10- to 50-fold scale-up factor.

Chromatography Scale-up Steps

Scaling up a chromatographic process includes:

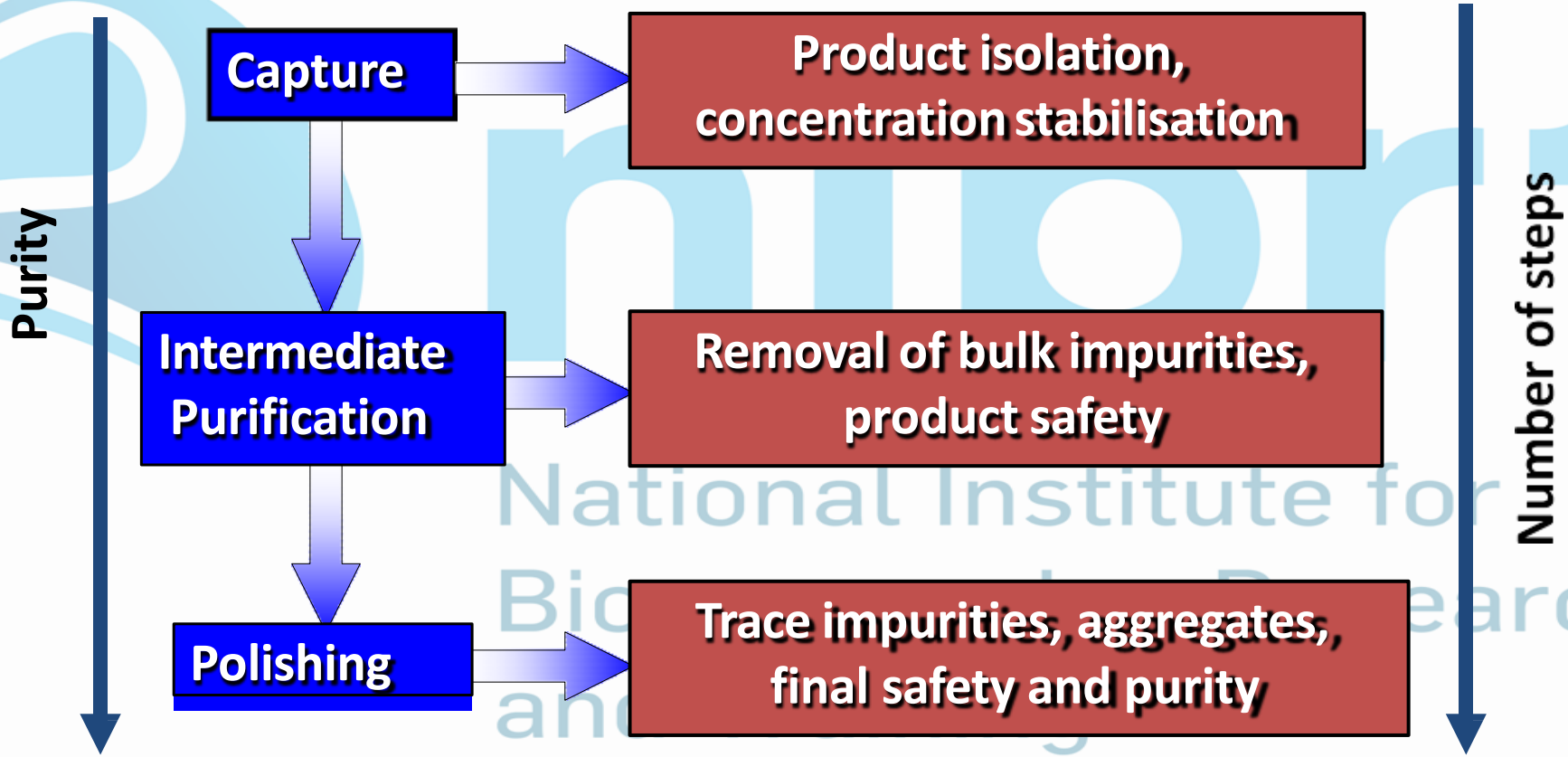
- Scaling up process parameters
- Choosing equipment
- Verifying or fine tuning results
- Defining final operational parameters
- Setting acceptable operational ranges

Proper documentation will facilitate subsequent validation

Scale-up of Process Parameters

Maintain constant	Increase
Packed column bed height (cm)	Column diameter
Linear flow rate (cm/h) at all stages	Volumetric flow rate (ml/min)
Sample attributes (concentration and conditioning)	Sample volume (proportionally)
Ratio of sample volume to media volume	Gradient volume (proportionally)
Ratio of gradient volume to media volume	Buffer volumes (proportionally)
Specifications (pH, conductivity, temperature) for all buffers used in the chromatography step	

Typical Strategy for Protein Purification



Capture Chromatography

Capture: need high capacity: Goal is to capture as much IgG & remove contaminants e.g. proteases; therefore, speed is important & high volumes are loaded (Protein A)

Capture → **Product isolation, concentration stabilisation**

1600 - 2000 mm Diameter
CV = 600 - 950L

The Capture Step

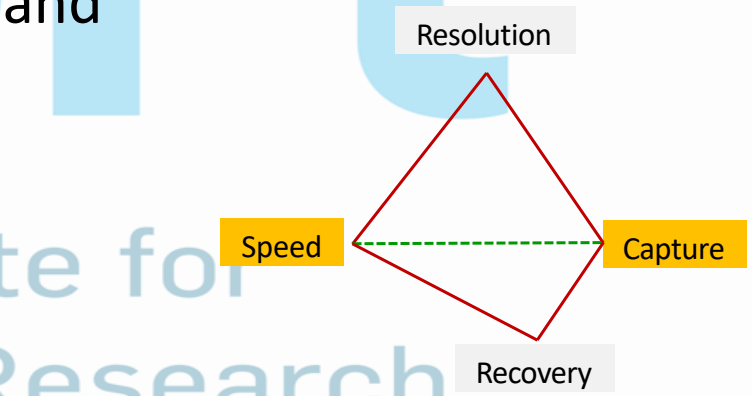
- **Capacity and speed highest priority since large sample volumes and many impurities**
- Primary aim: Concentrate product, remove bulk contaminants and avoid degradation e.g. proteolysis
- **Requirements:**

  a) Shortest possible time cycle

  b) High dynamic binding capacity

  c) High selectivity (to concentrate and remove bulk impurities)

  d) Accept lower recovery and resolution to avoid degradative processes



Purification Chromatography

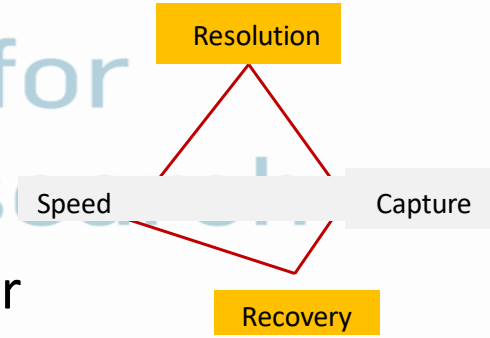
Intermediate purification: Must remove impurities; smaller volumes so speed & capacity less important (IEX)

Intermediate Purification → **Removal of bulk impurities, product safety**

800 - 1200 mm Diameter
CV = 150 - 340L

Intermediate Purification Step

- **Primary aim**: get high resolution and capacity in order to remove contaminating proteins and isotypes whilst getting high productivity (g product per column volume per hour)
- ***Important considerations***
  - High selectivity during binding to get binding capacity and purification
  - High selectivity during elution e.g. by multi-step or gradient elution
  - Involves balance between capacity and resolution
  - Speed less important since smaller volumes material and less danger of product degradation



Polishing Chromatography

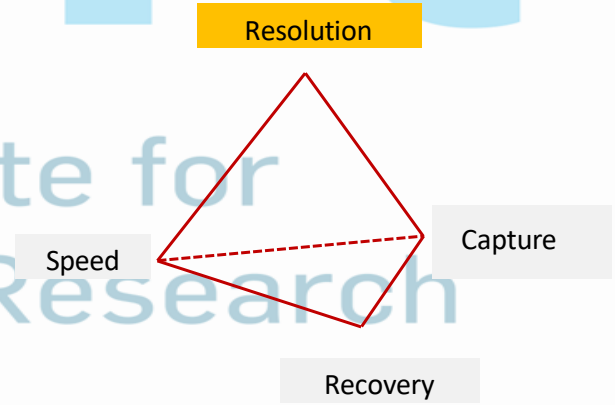
Polishing: prepare product for finishing Usually to remove small impurities from capture stage (Hydroxyapatite or Hydrophobic Interaction)

Polishing → **Trace impurities, aggregates, final safety and purity**

400 - 600 mm Diameter
CV = 25 - 60L

Polishing Step

- **Primary aim: RESOLUTION**
- AIM to remove:
  - Trace contaminants
  - Cellular proteins
  - Product variants e.g. Isotypes, dimers, polymers, reagents
  - Leached chromatic materials (e.g. Protein A)
  - Endotoxins
  - DNA
  - Viruses
  - Must attain final product quality



Polishing

Requirements:

- High selectivity during desorption e.g. gradient shape
- Usually requires smaller gel bead sizes
- Therefore slower, but back-pressure avoided by use of small bead size distribution
- Smaller volumes involved therefore column size smaller

Column Cleaning Requirements

- **Should be able to be cleaned effectively**
  - Packed
  - Unpacked
- **Validated**
  - In design process
  - Dye tests
  - Bacterial challenge
- **In use testing**
  - TOC
  - Swabbing
  - Etc, etc

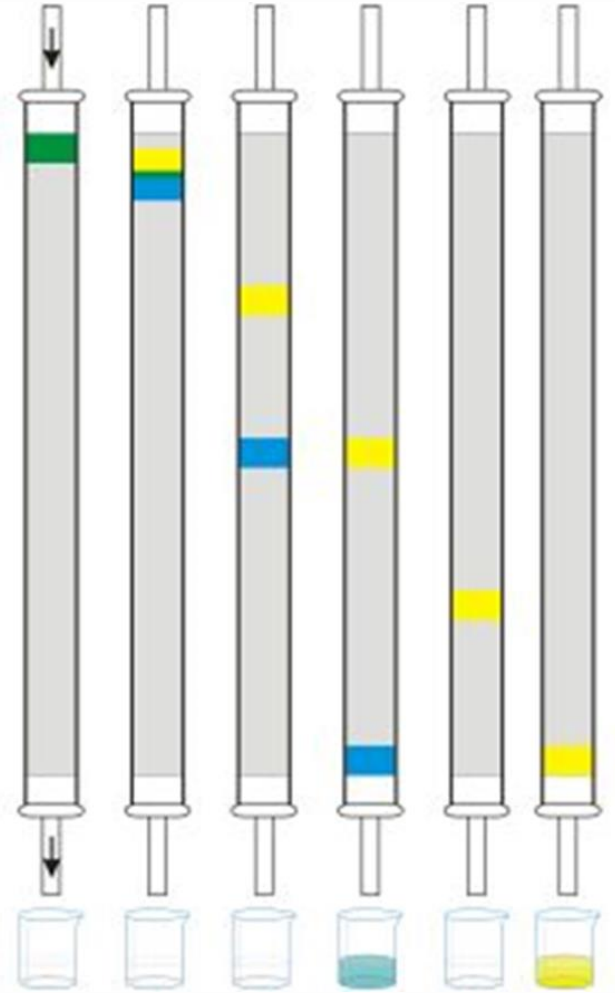
- **Based upon**
  - Good design
  - CIP protocol to deal with particular column design
  - CIP solution
  - NaOH
  - Acetic Acid etc…

Scale-up: Adsorption Techniques

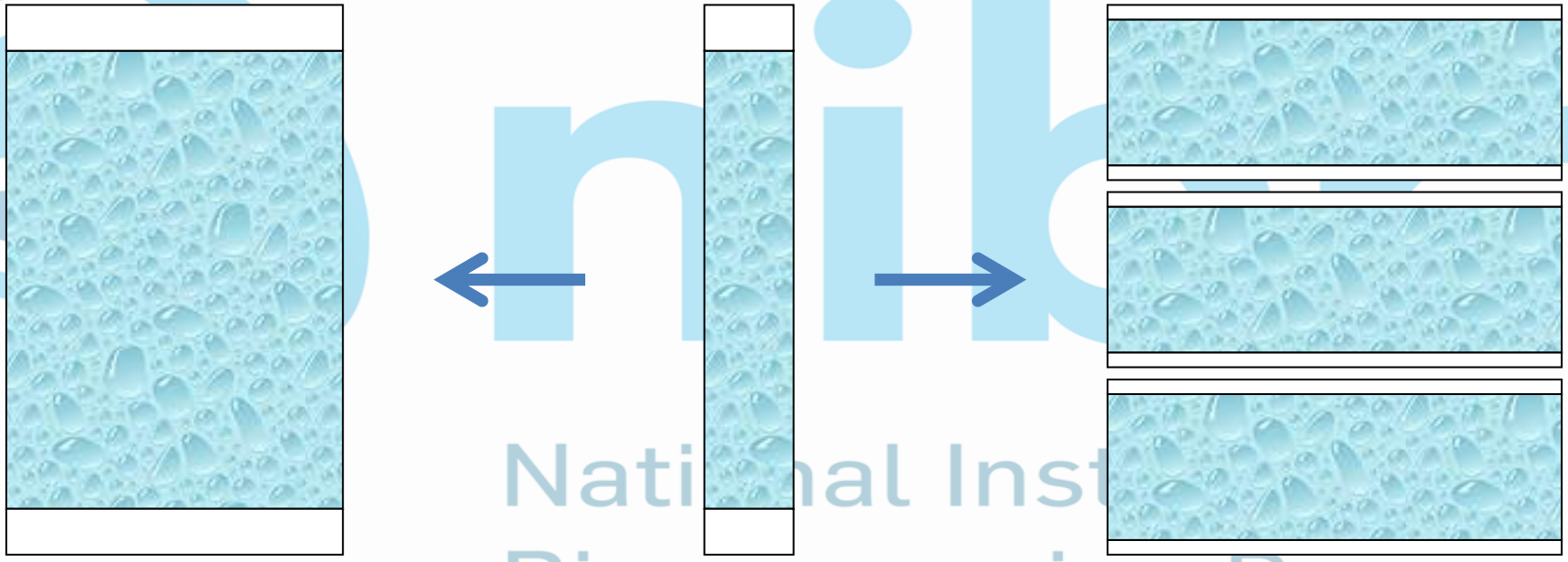
- Increase diameter
- Maintain bed height
- Columns in series

Scale-up: Size-exclusion (SEC)

- When using long beds such as in **size exclusion** (gel permeation) chromatography, it is often recommended to **stack the column in several shorter segments**, connected in series.
- This will **reduce drag forces** on the beads in the column when column wall support decreases at **larger column diameters**.
- Thus **higher flow velocity** at **large scale** can be facilitated.



Scale-up: Size-exclusion (SEC)



- Increase diameter
- Maintain bed height
- Columns in series

Scale-up: Choices

- Normally volumetric scale-up is performed by **increasing** the column diameter.
- However this may not always be feasible due to the fact that required column dimensions **may not** be available commercially.
- Alternative routes can be taken:

1. Endure additional costs of over-dimensioning a process!!
2. Use an empirical guideline (Yamamoto *et al.**).
3. Cycling of an undersized column.

* Yamamoto *et al.*, *Resolution of proteins in linear gradient elution ion exchange and hydrophobic interaction chromatography*. J. Chromatography, 409 101-110 (1987).

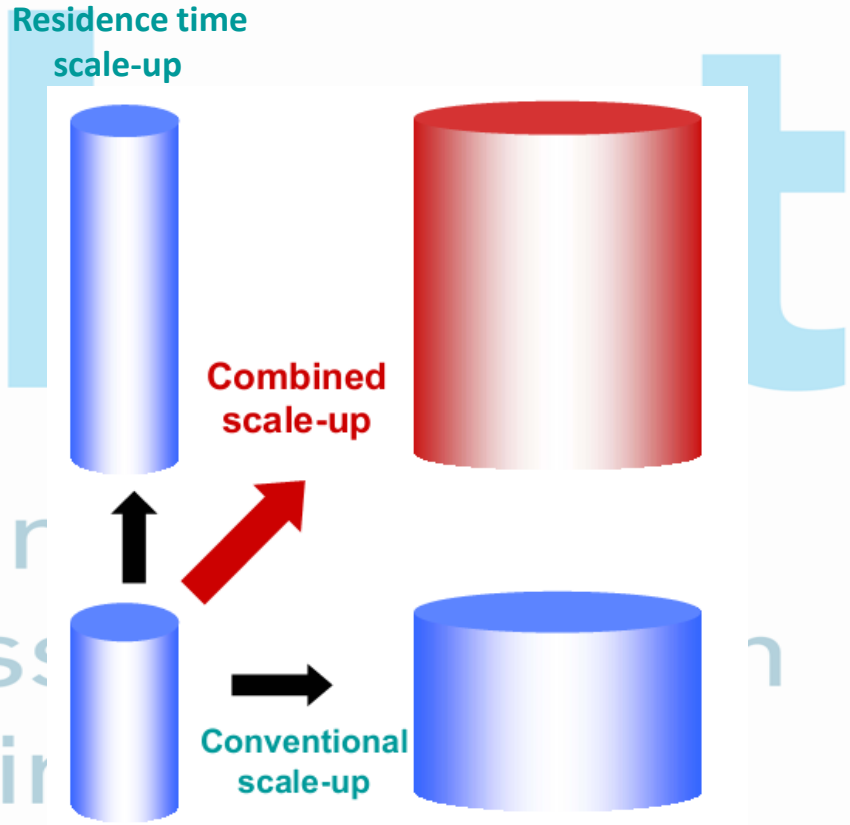
Scale-up: Choices

- **Yamamoto *et al*.,** state that resolution is kept constant provided that the column length increases in proportion to:
  - The eluent velocity times
  - The gradient slope per unit bed volume

- Following this guideline, it is recommended that:
  - Load volume is increased in proportion to bed volume
  - Eluent velocity is kept constant
  - Gradient slope per unit bed volume is increased in proportion to the column length (i.e. gradient time will increase in proportion to the increase in column length)

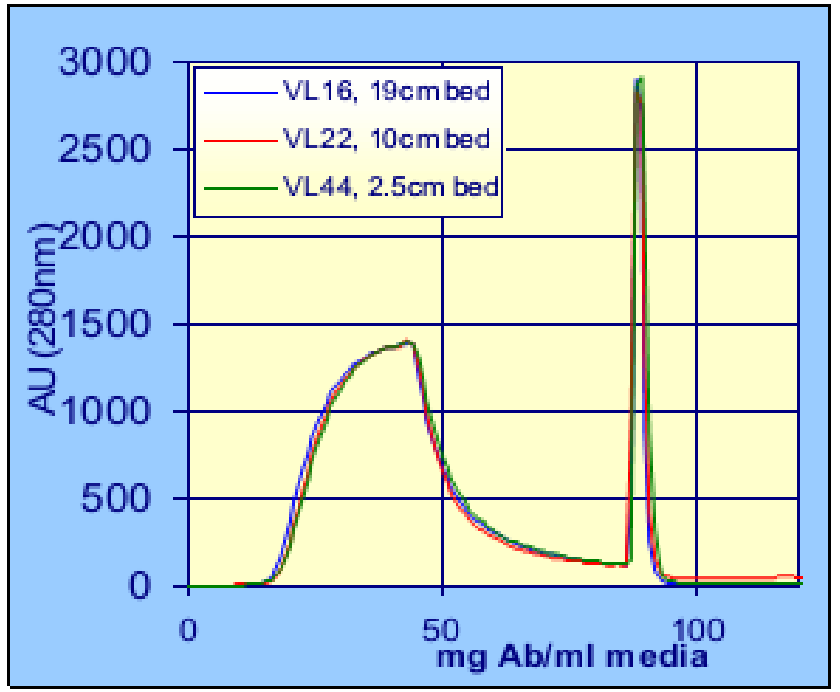
Scale-up: Methods for More Flexibility

- Conventional scale-up
  - Increase diameter
  - Constant bed height and linear flow rate
- Residence time scale-up
  - Linear increase of bed height and flow
  - Pressure flow limitations due to compressibility of conventional media
- Combined scale-up
  - Modern media are incompressible and maintain a low pressure drop
  - Permits scale-up in any dimension at constant residence time
  - Greater flexibility for equipment



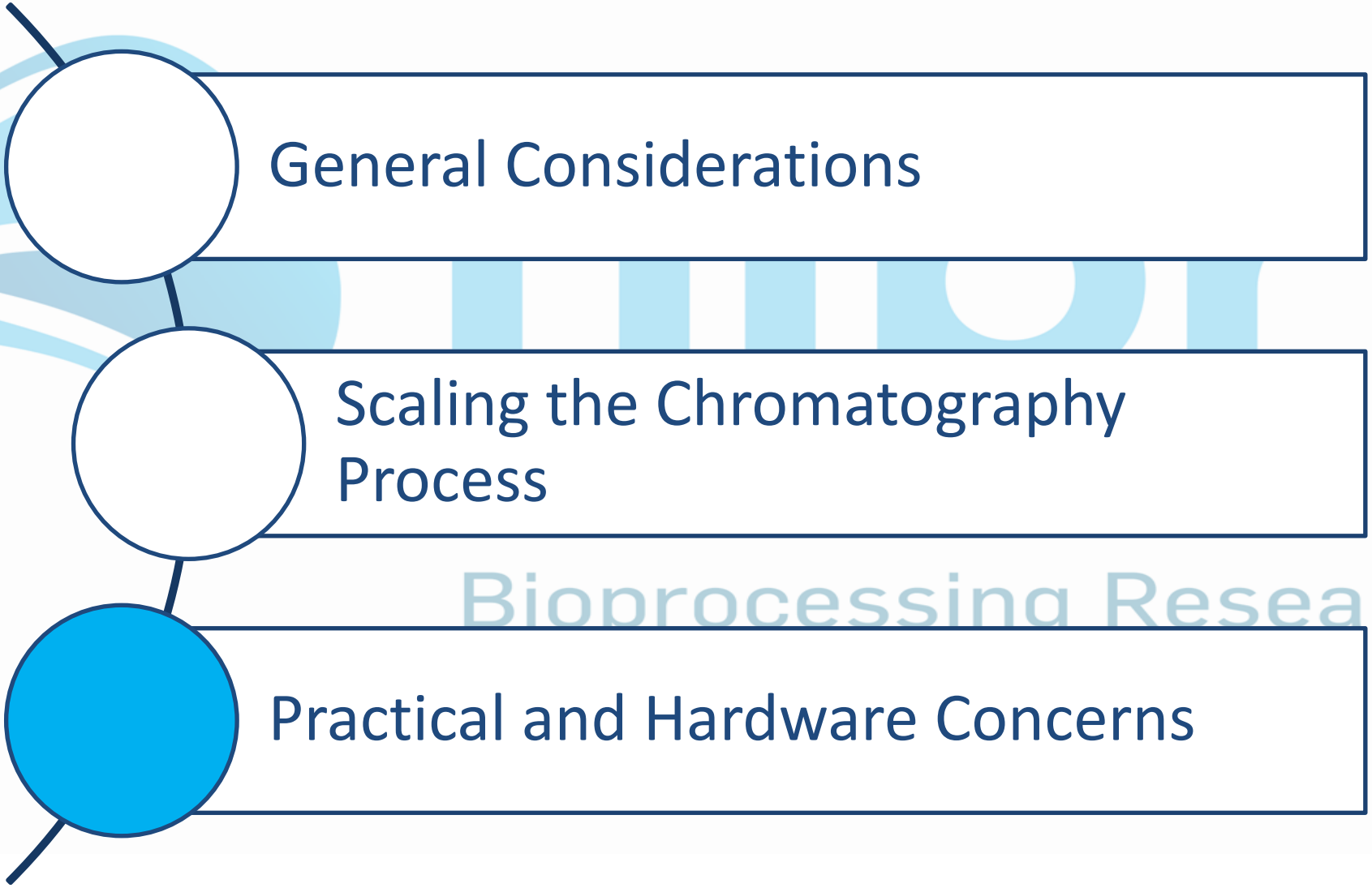
Scale-up: Constant Residence Time

- Columns with same bed **volume** and different **bed dimensions**
  - Same dynamic capacities
  - Overlapping chromatograms



Column diameter	Bed Height (mm)	Bed volume (ml)	Residence time (sec)	Flow velocity ($cm.h^{-1}$)	Dynamic capacity ($mg.ml^{-1}$)
44	25	38	90	100	17.8
22	100	38	91	395	17.7
16	190	38	91	750	16.2

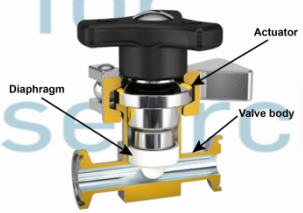
Topics



Scale-up: Equipment Considerations

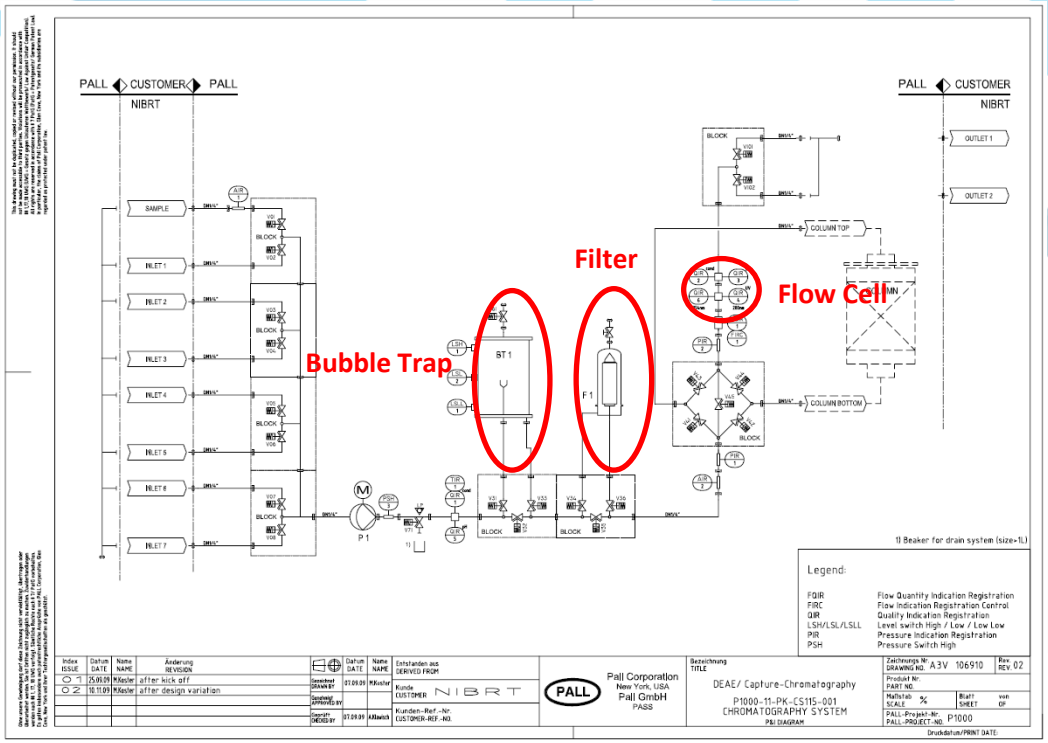
Scaling-up a chromatography step also means converting to hardware components that have a different design compared with small-scale chromatography systems.

- Rotary, diaphragm or peristaltic pumps replace high-precision piston or displacement pumps.
- Simpler 1-way diaphragm valves replace low volume multi-port valves.
- Tubing size and length is optimised to reduce system pressure in large-scale chromatography skids.



Scale-up: Equipment Considerations

- Chromatographic performance can be affected by the difference in hardware design.
- Increased ratio of total system to column volume can affect efficacy of equilibration, washing and elution steps.
- Introducing filters, air traps, flow cells and column distribution systems can also contribute to this effect.



Column Distribution System

Scale-up: Column considerations

The following factors need to be considered in scale-up column:

- Wall support
- Distribution system
- Handling/packability
- Chemical resistance
- Pressure rating
- Hygienic design

Scale-up: Practical Concerns

Fraction Collection

Product Loading

Bed Stability

System Design

Gradient Slope

Flow Distribution

Packing Quality

Media Availability

Scale-up: Practical Concerns

- Bed Stability:

  Physical:

  Lack of support from column wall (>25-30 cm) when packed with compressible media, can restrict maximum bed height.

  Chemical:

  Commercial media typically used 50-200 times which will lead to leaching of ligands and deterioration of reused matrices due to the necessary harsh CIP/regeneration conditions.

Scale-up: Practical Concerns

- Product loading
  - Increasing the product loading (/mg adsorbent) reduces resolution.
- Gradient slope
  - At large scale accurate and reproducible gradients are challenging.
- Flow distribution
  - Achieving uniform flow at large scale is difficult, can lead to peak tailing.
- Packing quality
  - At large scale homogeneously packed columns are difficult to obtain, channelling will lead to peak broadening/peak splitting.

Scale-up: Practical Concerns

- System design
  - Large scale systems employ piping, valves, flow meters, air sensors.
  - Such components may give rise to increased dead volume, which can lead to;
    - Dilution
    - Higher pressure drops
    - Peak broadening

Note: Peak broadening will cause extra dilution of the product fraction or even loss of resolution if the application is sensitive to variations in plate number in the system used.

Optimised Hardware Designs

- Compact modern valve designs
  - Replace 3-way valves with modular valve clusters
- Combined bubble trap/filter
  - Decreases dead volumes & piping and improves residence time and yields

Scale-up: Practical Concerns

- Fraction collection
  - If fraction collection is required, the detector must be close to fraction collector to ensure accuracy and collection scheme should be robust.
- Media availability
  - Long term availability, cost, lot-to-lot consistency, resin lifetime and regulatory need for supporting documentation also important.

Scale-up: Fine Tuning

- Adjustment of gradient volume
- Adjustment of flow rate
- Adjustment of equilibration volume
- Modification of fractionation protocols
- Modification of CIP routines.

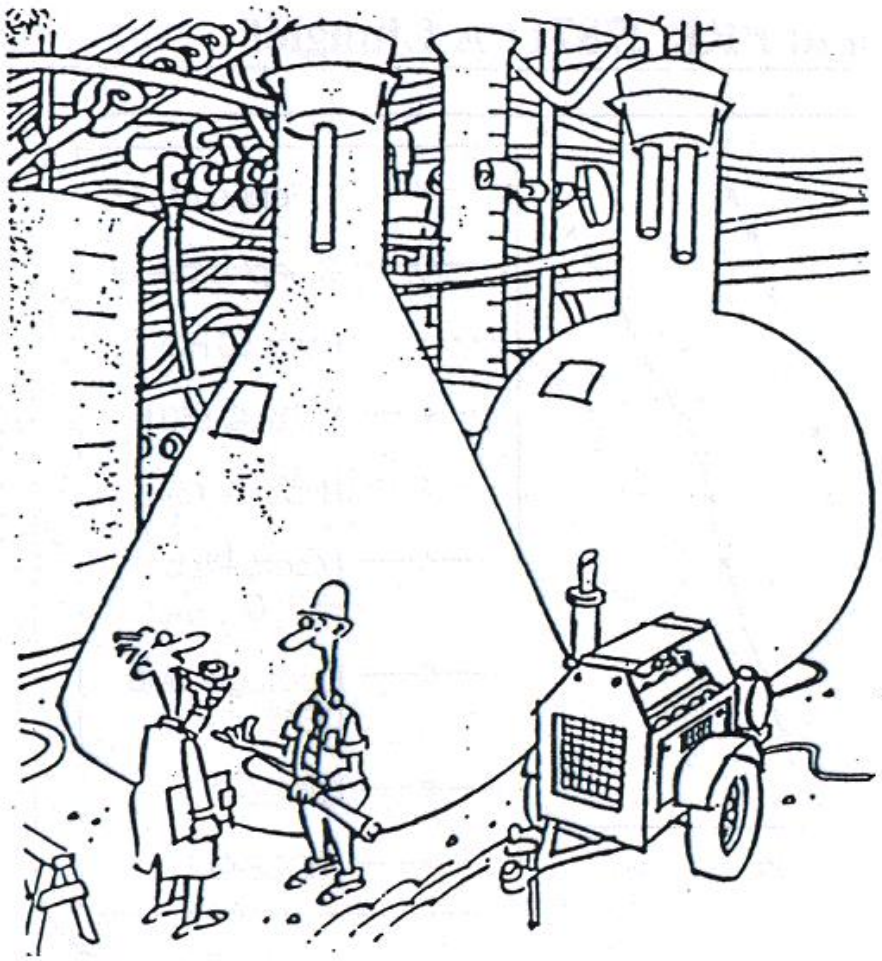
Scale-up: Non-chromatographic Factors

- Non-chromatographic factors can alter the purification result during scale-up of the entire process.
- As fermentation scale increases, changes can occur in
  - Sample composition
  - Sample concentration
- Precipitation in the biological feedstock can occur due to longer hold times when large volumes must be handled.

Scale-up: Non-chromatographic factors

- Non-reproducibility of large-scale buffer preparations.
- Microbial growth in buffers due to increased handling and longer holding times.
- Changes in temperature, pH and conductivity can alter sample properties and hence the chromatographic result.
- Buffer physical properties and temperature are crucial and should be maintained on scale-up.

Scale-up: Can be problematic!!



"Got a few problems with our linear scale up."

Chromatography Scale-up: Summary

- Scale-up of chromatographic procedures is a crucial phase in product development.
- It is difficult to foresee all possible problems during scale-up.
- However, a thorough understanding of the chromatographic process and scale-up guidelines will aid in determining if the cause of the problem is the chromatography step itself or not.

SUMMARY

- **Purification strategy** must ensure that purity requirements of the final product are met while achieving the highest throughput and yield at the lowest cost
- **Have a detailed knowledge of your protein and process**
  - Composition of the starting mixture
  - Starting volume and concentration of target protein
  - Target purity required, the acceptable limits for process and product- related impurities, and the analytical methods for their detection

SUMMARY

- **Product and process related impurities**
  - Aggregates
  - Degradents
- **Contaminants derived from the manufacturing process**
  - Cell substrate-derived impurities
  - Cell culture-derived impurities
  - Downstream-derived impurities
  - Chemical Additives
  - Foreign and viral DNA

- **Scale-up:** simplest way is to maintain the same column height and to increase the cross-sectional area to maintain a constant ratio of unknown to column volume.
  - Keep the ***linear flow rate*** **constant**

Sample Questions

- **SAQ:** In the scale up of DSP chromatography, comment on factors affecting the design and scale up of columns.
- **LAQ:** In the scale-up of process chromatography, what factors will most likely impact on column efficiency?
- **LAQ:** How is efficiency measured and comment on factors that influence efficiency?
  - *Define efficiency*
  - *Equation for HETP*
  - *Explain key elements in HETP*

Topics

- General Considerations
- Scaling The Chromatography Process
- Practical And Hardware Concerns

Thank You