

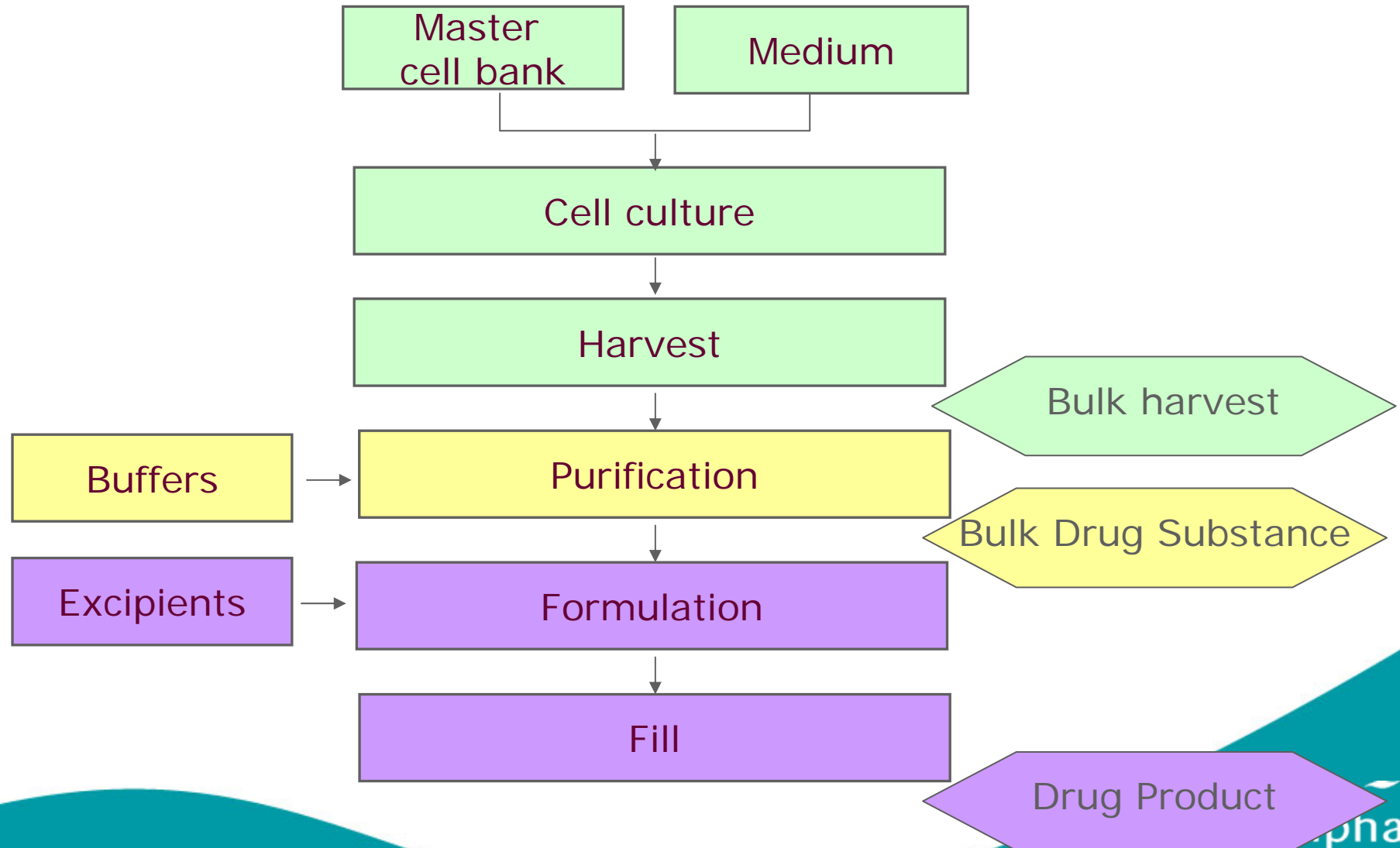
Process Development and clinical supply of biopharmaceuticals

May 2010
Craig Hart

Outline

- General introduction to process development
- Generic project plan and activities
- Cell culture process design and optimization
- Considerations for purification process development

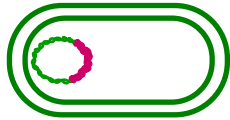
Typical process flow scheme for production of a recombinant therapeutic protein



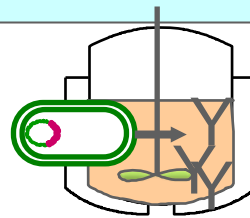
Key elements of process development

Analytical methods for quantity, quality and functionality

Cell line & vector



Cell culture process



Purification process

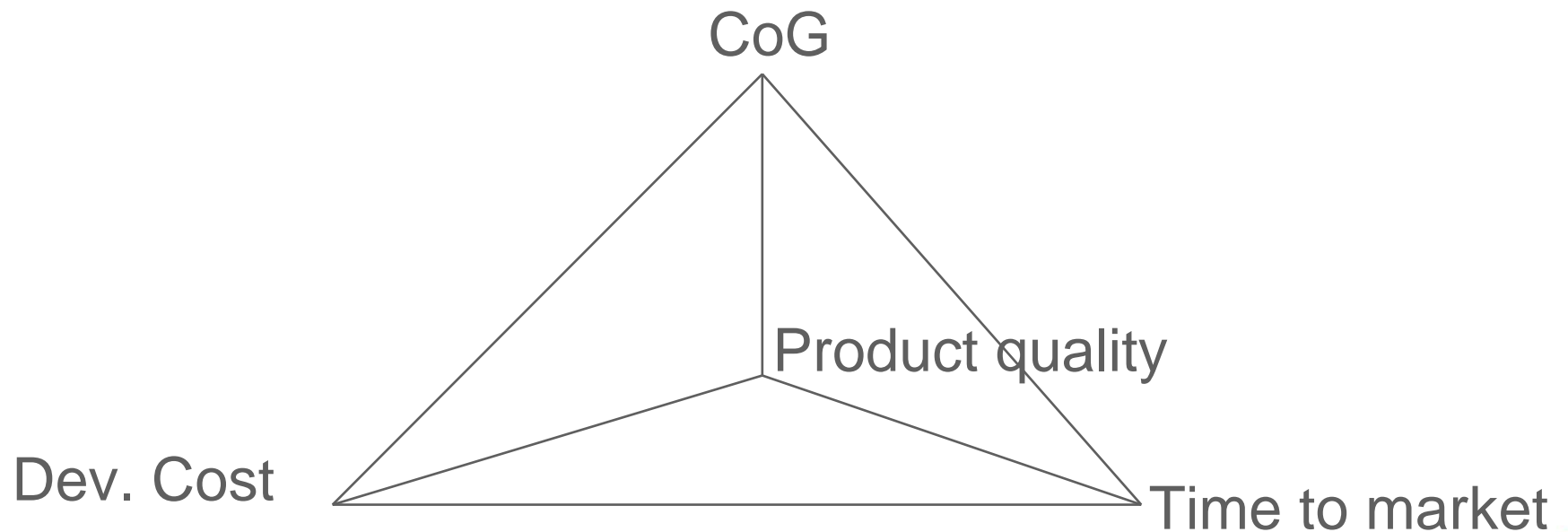


Process demands

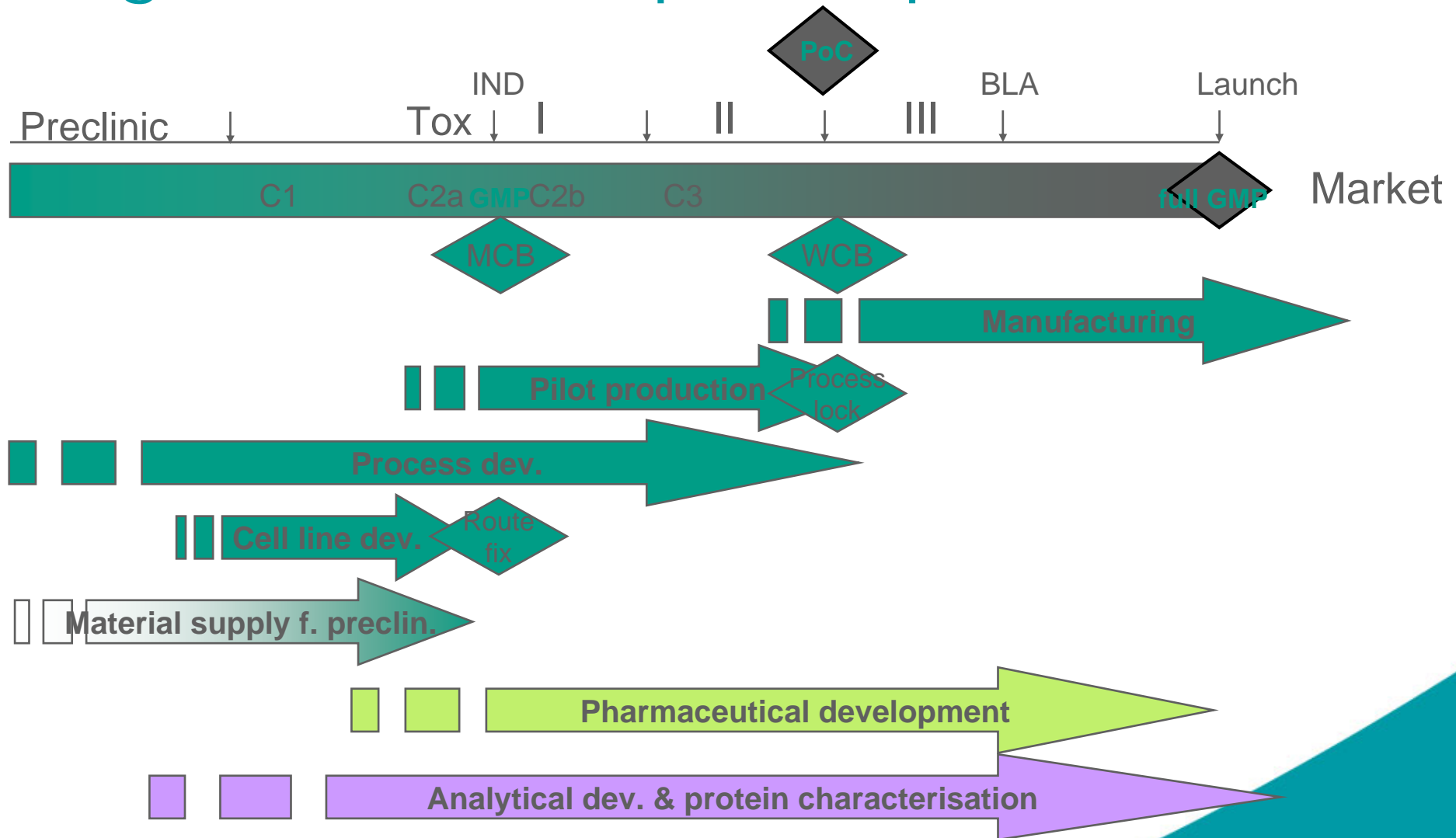
- Scalable process
 - Give a consistent product and titer at various scales
 - DSP able to accommodate volume and titer
- Robust process
 - Give a consistent product and titer within limits of process parameters
 - Need to understand critical process parameters ideally early on
- High productivity process
 - Give a consistent product at high titer
 - DSP shows a “high” yield over the process



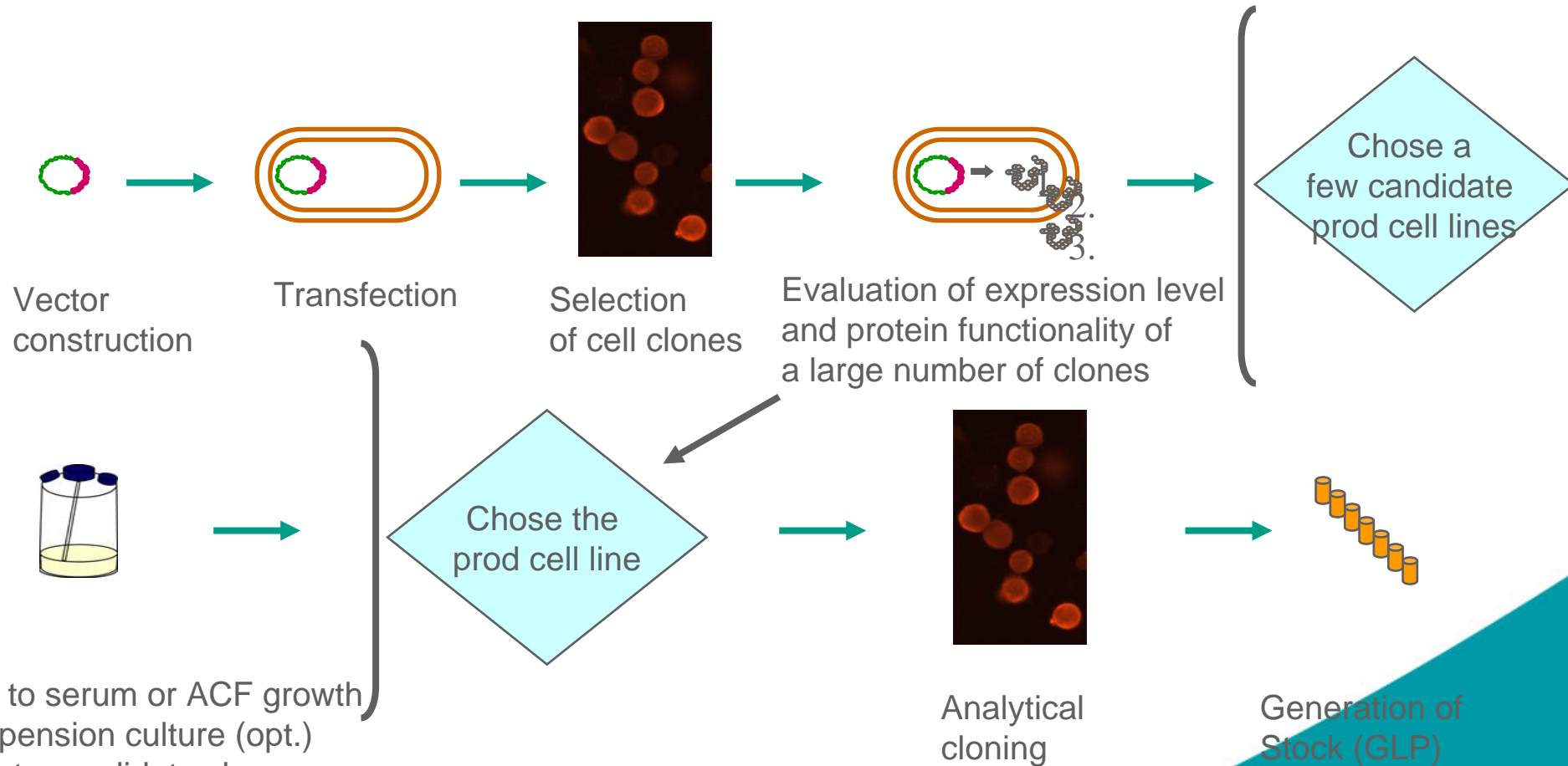
Key parameters in drug development



High level development plan

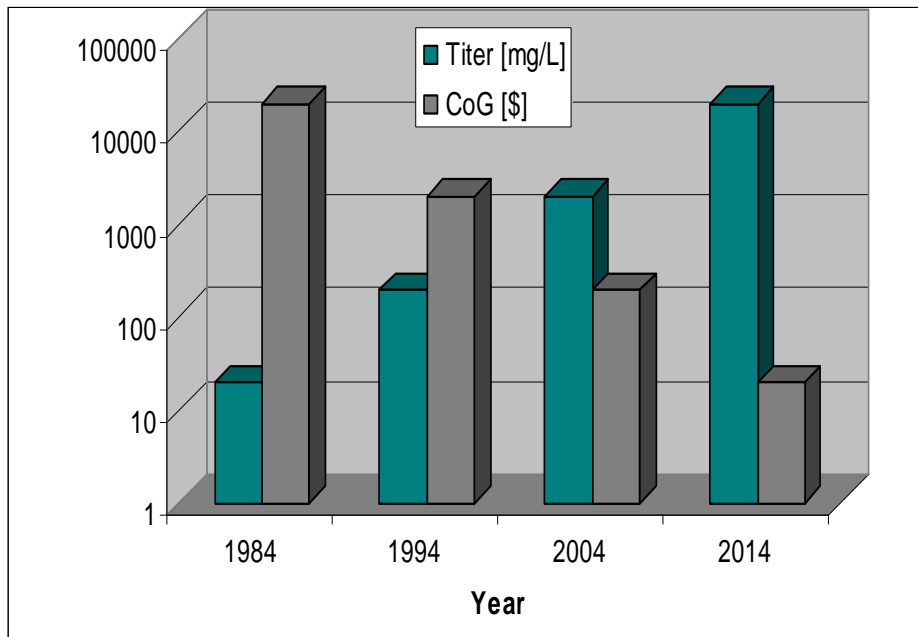


Development of a rec. therapeutic protein in mammalian cells



Adapt to serum or ACF growth in suspension culture (opt.)
Evaluate candidate clones in down-scale production system for expression level and stability
www.recipharmacobrabio.com

Platform systems for mAb expression



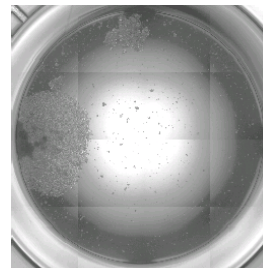
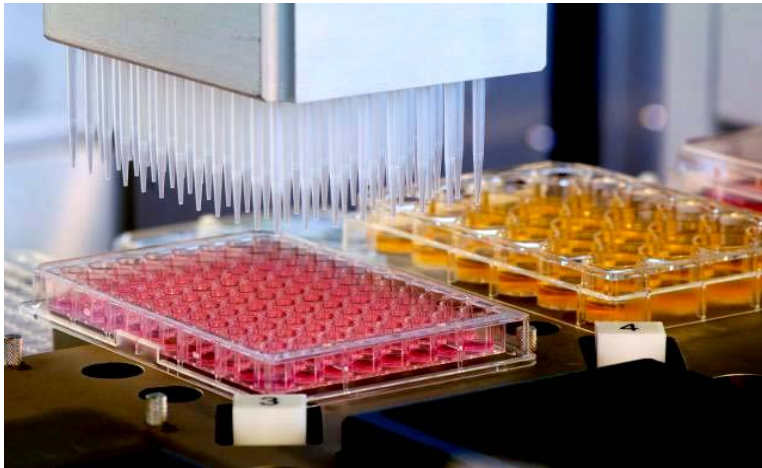
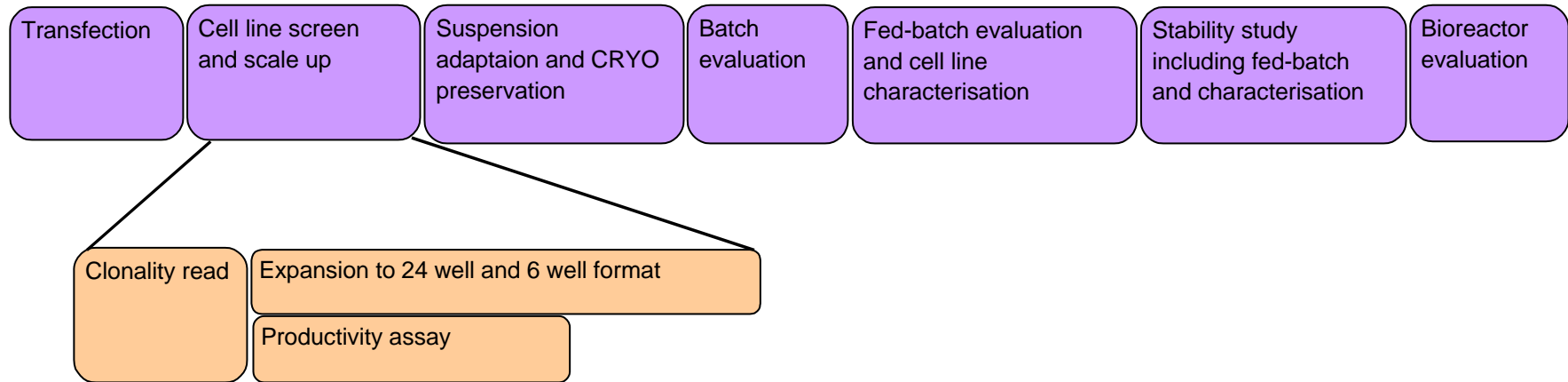
Industry experience

ACE	18.4%
BI Hex	4.4%
DHFR	29.8%
GPex	14.9%
GS	22.8%
MAR	3.5%
OSCAR	11.4%
PER.C6	11.4%
STAR	7.0%
UCOE	4.4%
Other	35.1%

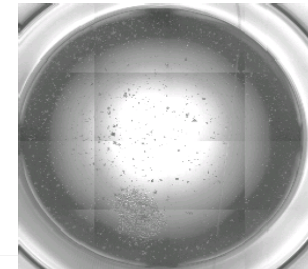
Bioprocess International April 2008

Expected trend of mAb expression titres

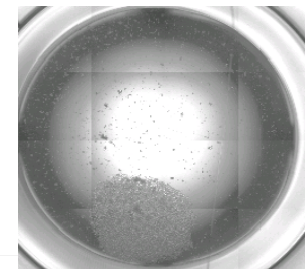
Automated cell line development approach



Polyclonal



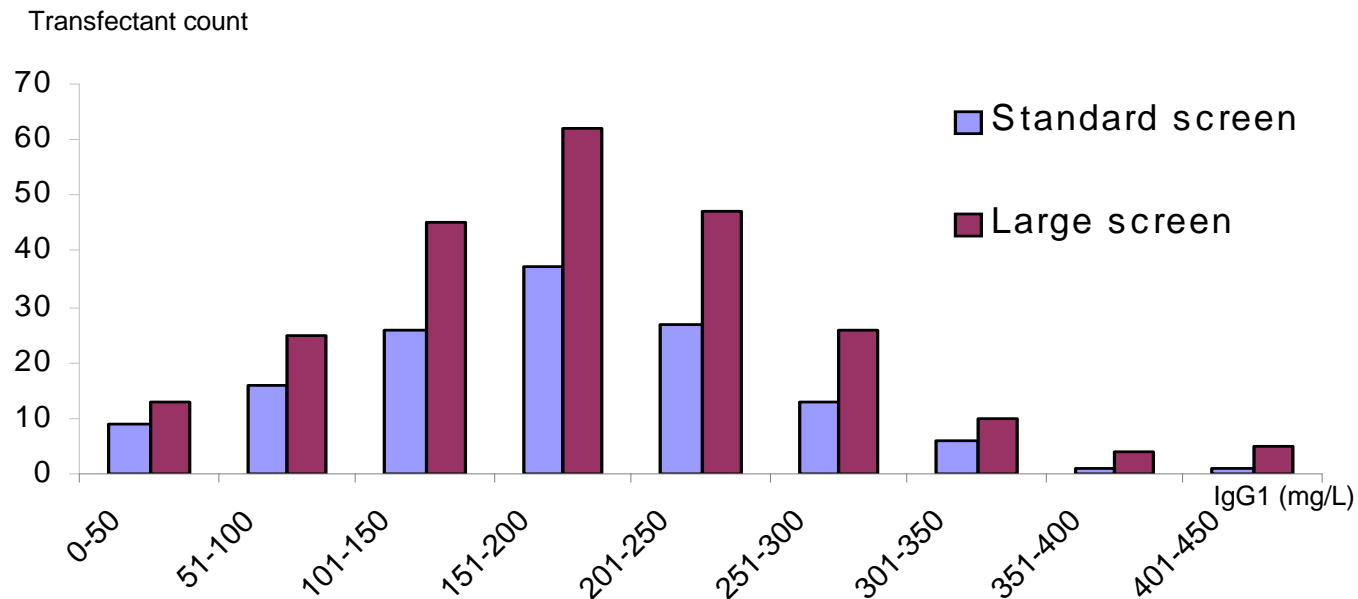
Monoclonal (small)



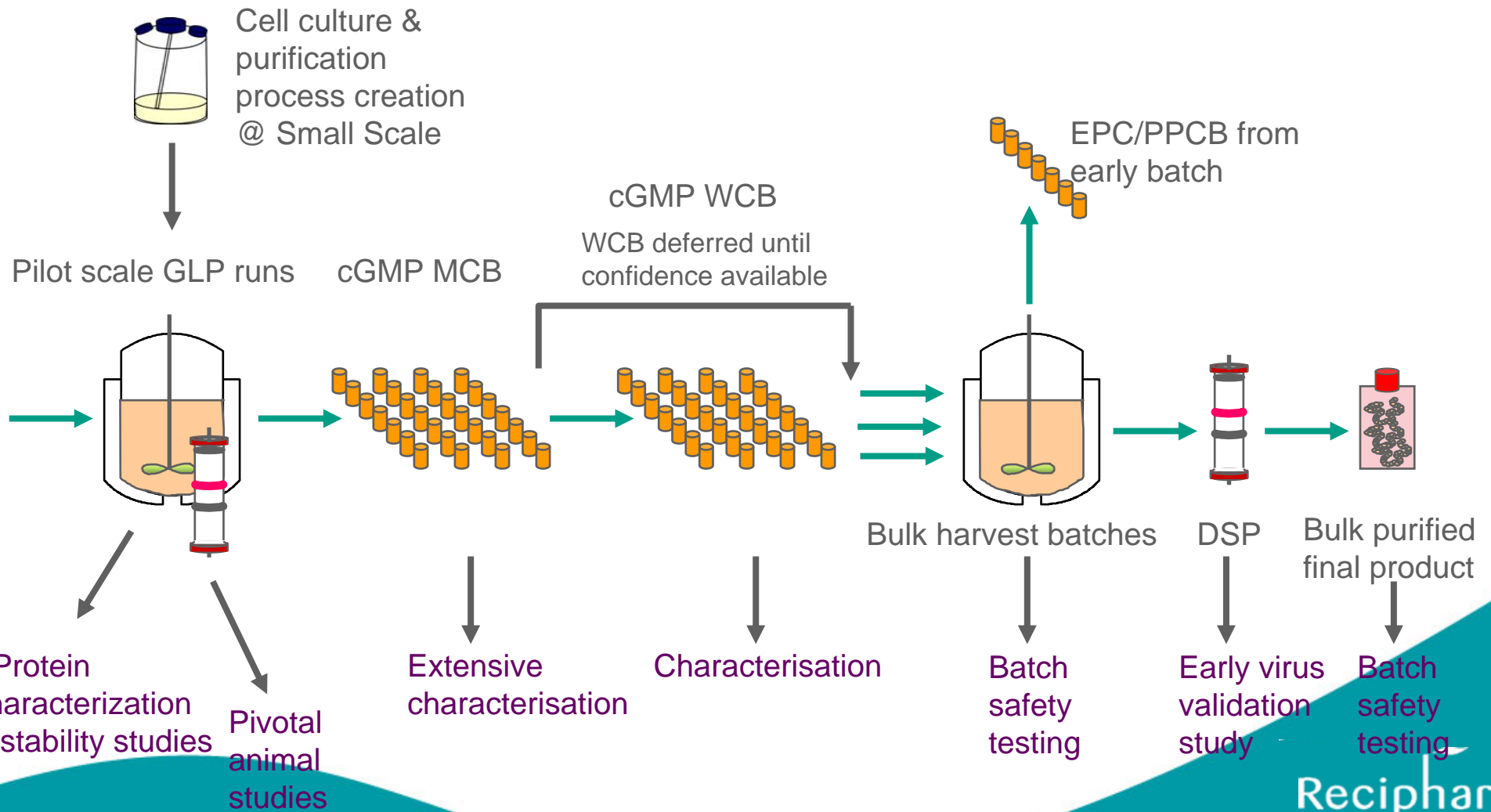
Monoclonal (ready for expansion)

Automated cell line development approach

- Costs are reduced compared to manual process due to automation of labor-intensive manual procedures
- Improved traceability & reproducibility due to automated handling & monitoring
- Larger screens increase probability to find high titer cell lines



Development of a rec. therapeutic protein in mammalian cells



Technical considerations for cell culture production platform

➤ Cell line

- Mammalian cell expressing the desired antibody at *high titer* and *desired quality* (glycosylation, aggregation)

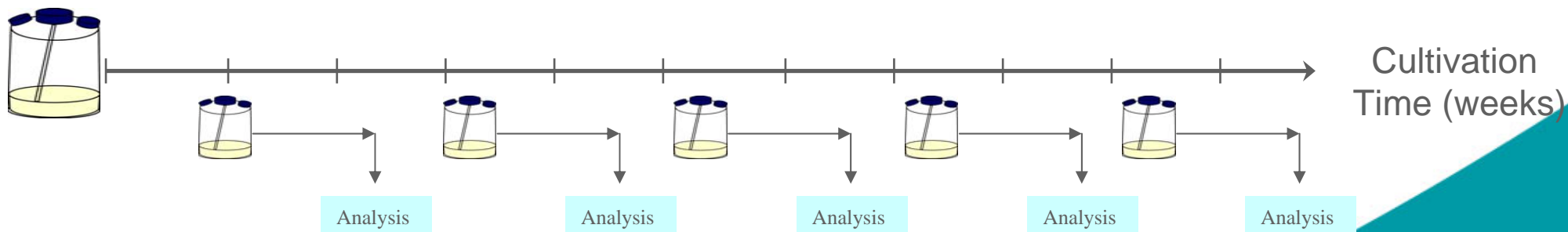
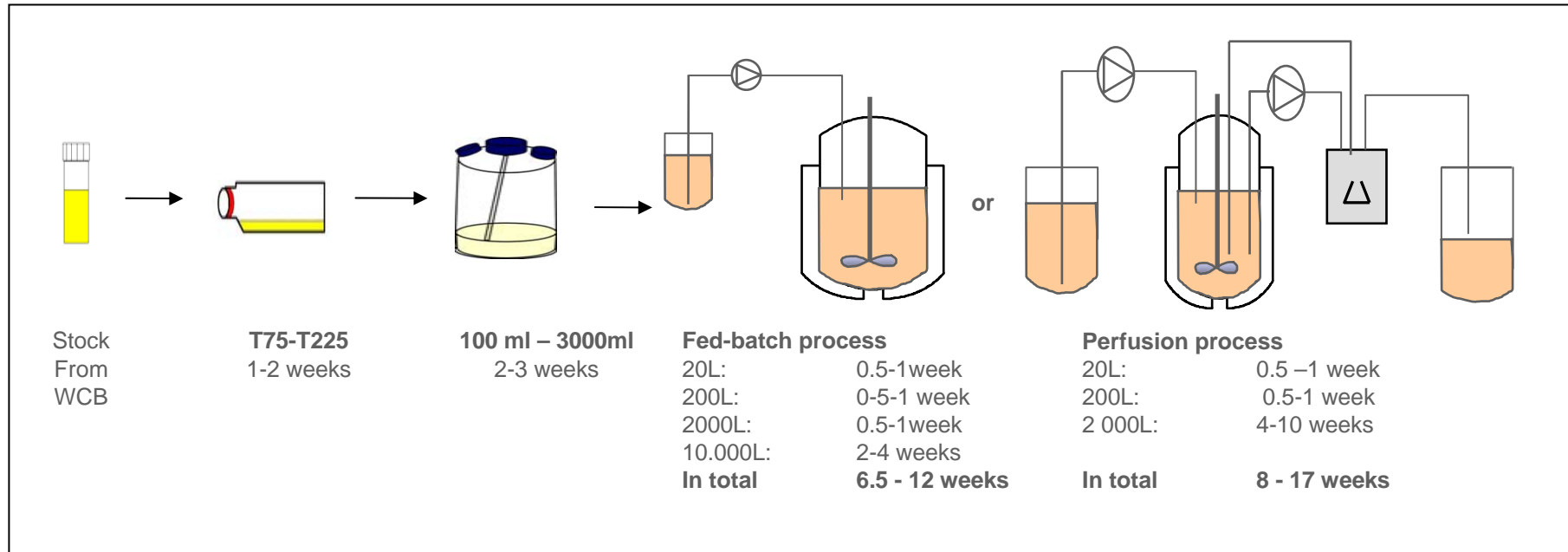
➤ Cell culture process

- Animal component free suspension culture in fed-batch mode to reach *high cell densities and viability*

➤ Integrated platform approach to cell line and process development

- Cell line and vector
- Generic culture medium and process
- Scale-up

Typically clone should be stable over approx. 50-80 PDN*

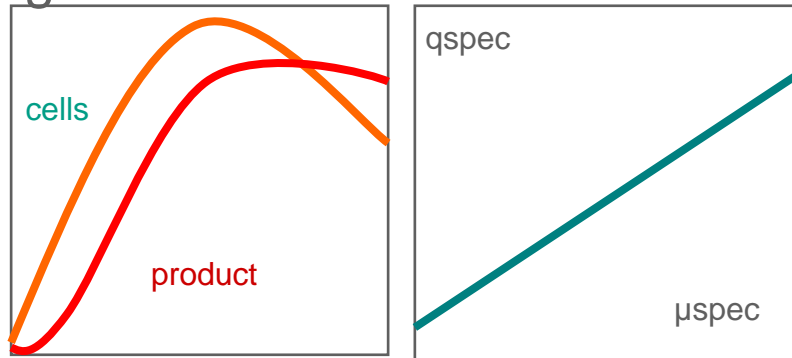


*PDN: production doubling number, quantitative description of number of cell divisions. formula: $\ln(N/N_0)/\ln 2$

Cell culture process design

- ⇒ Growth and product formation and degradation characteristics
- determine C_v , μ , V , $[P]$, q_p , cell cycle distribution
 - is protein stable at culture conditions?

growth associated



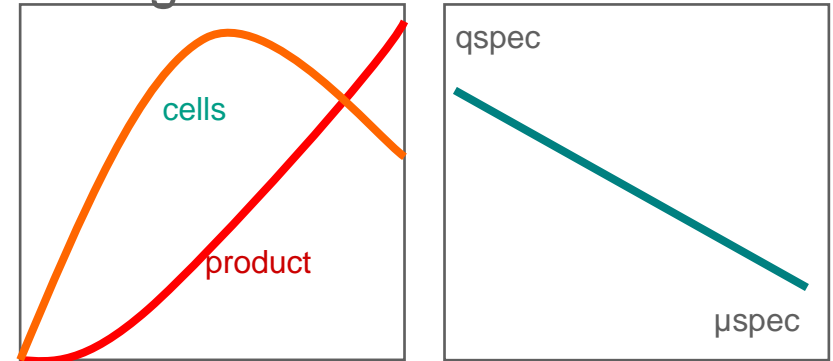
- ⇒ keep culture growing, maximize cell density

- batch, fed batch
- perfusion to push cell density
- cyostat at controlled μ

- ⇒ Antibody production typically function of area under growth curve, i.e. maximize cell density and survival

- ⇒ Rec. proteins can be labile and might need to be stabilized

non-growth associated



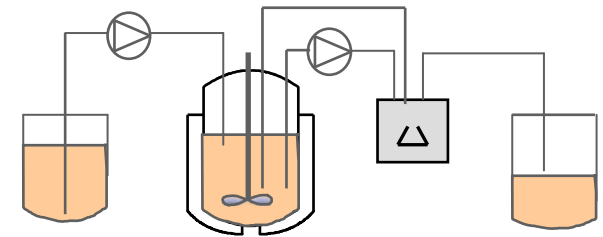
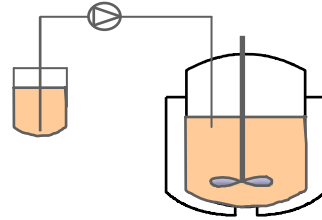
- ⇒ increase biomass in phase I

- fed batch, perfusion

- ⇒ arrest culture in phase II

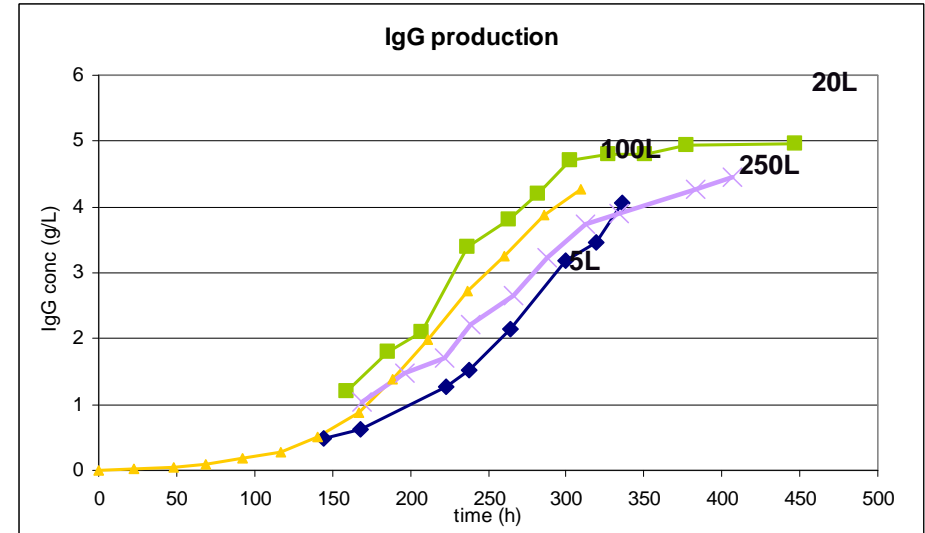
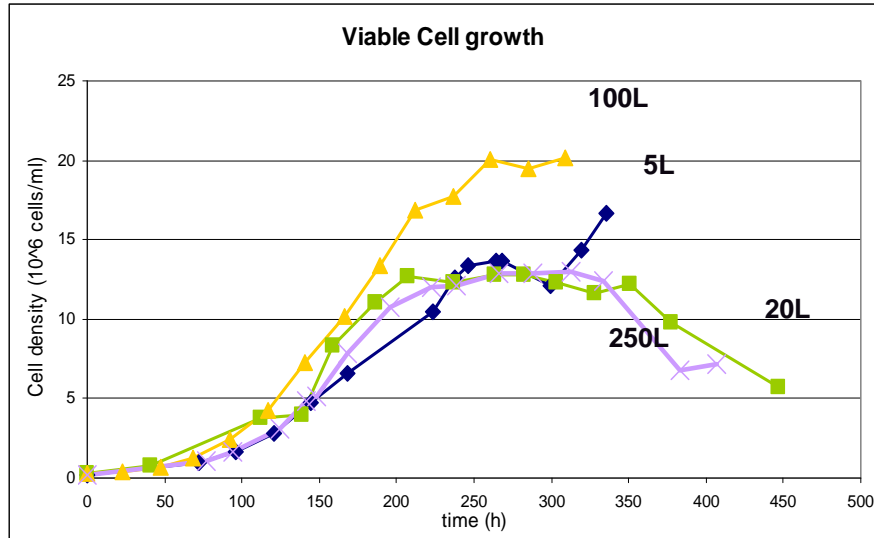
- inducers, temperature decrease
- supply nutrients

Choosing batch or perfusion mode



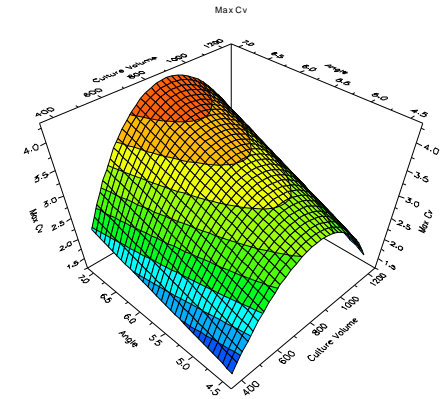
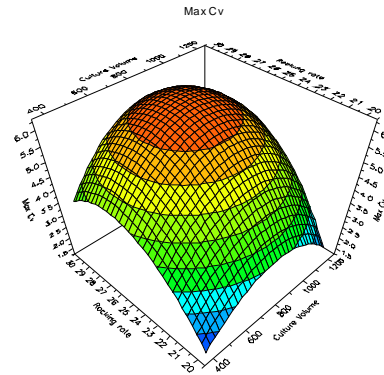
	Batch	Perfusion
Complexity	Low	High
Scale-up	Up to 20.000L and more	2000L
Product quality	Batch to batch variability	Variable over process time Can cope with unstable product due to frequent harvests
Industry standard	Monoclonal antibodies	Blood factors, other unstable or low titer proteins

mAB case: fed-batch culture



Cell culture process parameter optimization

- pH, DOT, temp
- pCO₂
- Osmolarity
- Metabolites and waste products
 - Medium composition
 - Feed composition and timing of addition
 - Perfusion rate
- Cell density, bleed rate
- Inoculum density
- Harvest timing
- Productivity
- Product quality



- Early development
 - Determine general process design
 - Evaluate standard or platform parameters
 - Defer optimization to later phases if titers acceptable
 - Frontload only if success chances high
- Late development
 - Identify critical process parameters
 - Optimize critical parameters and understand ranges
 - Further titer increases if possible
 - Validate process

Technical considerations for purification process development

Harvest

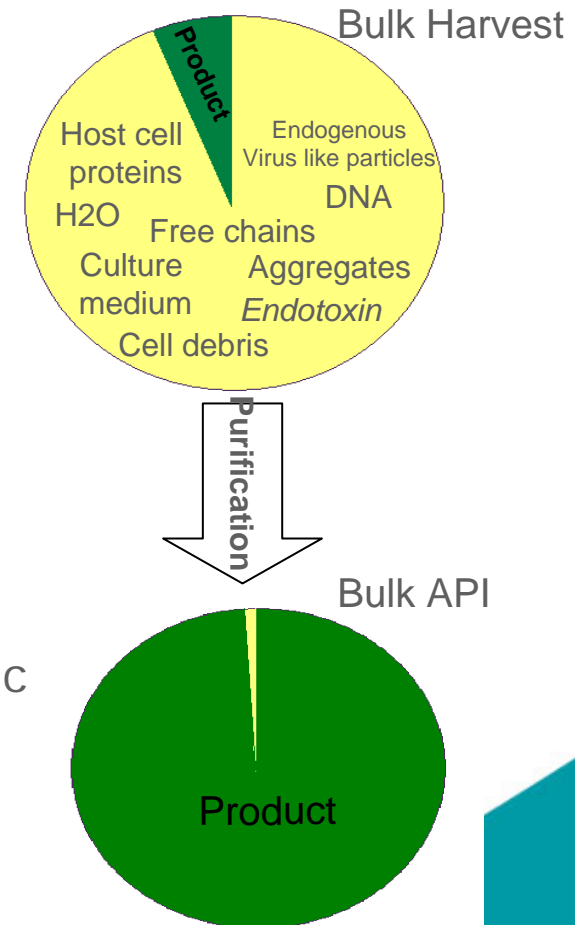
- Removal of cells and debris using filtration or centrifugation

Purification

- Reduction of volumes and impurities via Protein A affinity chromatography step that selectively binds mAb via Fc domain
- Ion exchange step(s) and/or other chromatographic steps to reduce impurities to target levels

Virus reduction steps

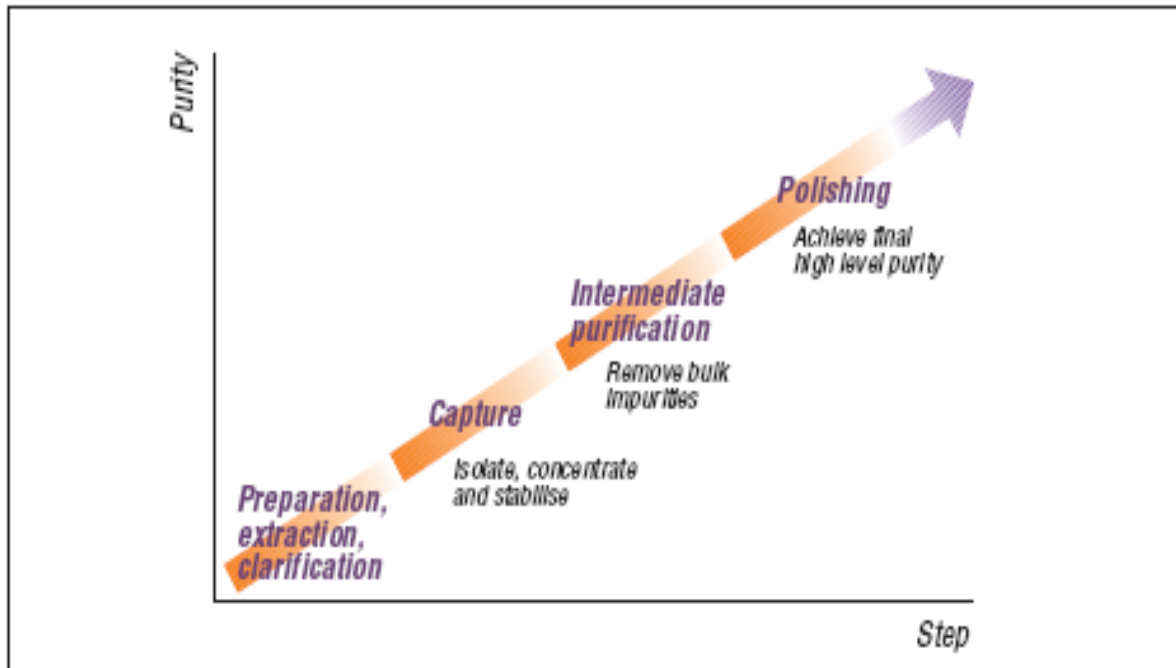
- Mitigate safety risk from viruses introduced from cell line, raw materials or operator



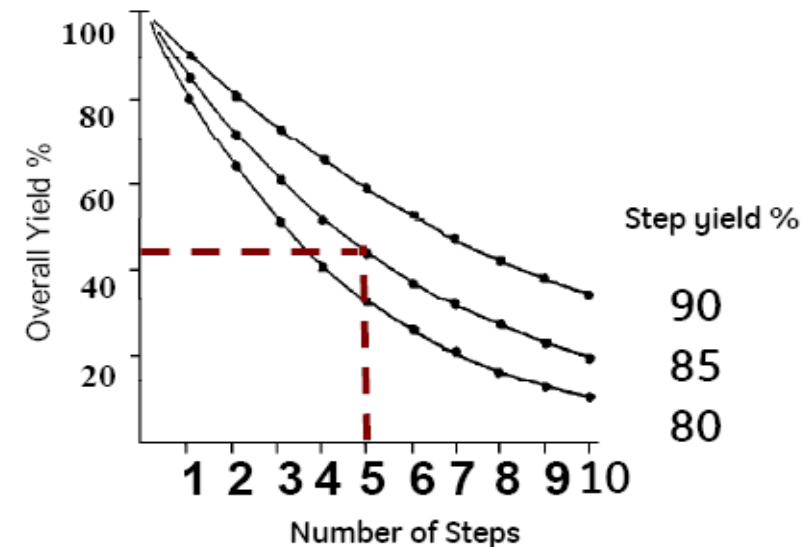
Purification – A phased strategy

Platform process design for monoclonal antibodies

- Minimized number of steps and process time to achieve high yield and purity, not compromising patient safety whilst maintaining activity



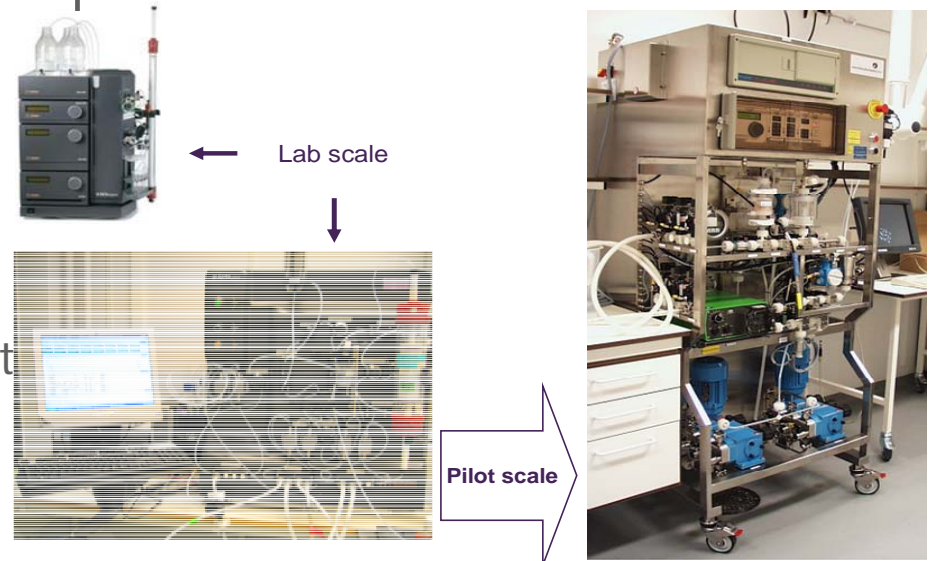
Yield vs. Process Steps



Three phase strategy

- use orthogonal separation techniques

- Capture
isolates, concentrates and stabilizes the target protein
 - capacity important
 - example: affinity chromatography / IEX
- Intermediate purification
removal of process impurities and product variants
 - resolution important
 - example: A IEX/CIEX
- Polishing
achieve final high level purity
 - recovery and resolution important
 - example: CIEX/A IEX/HIC/MMC

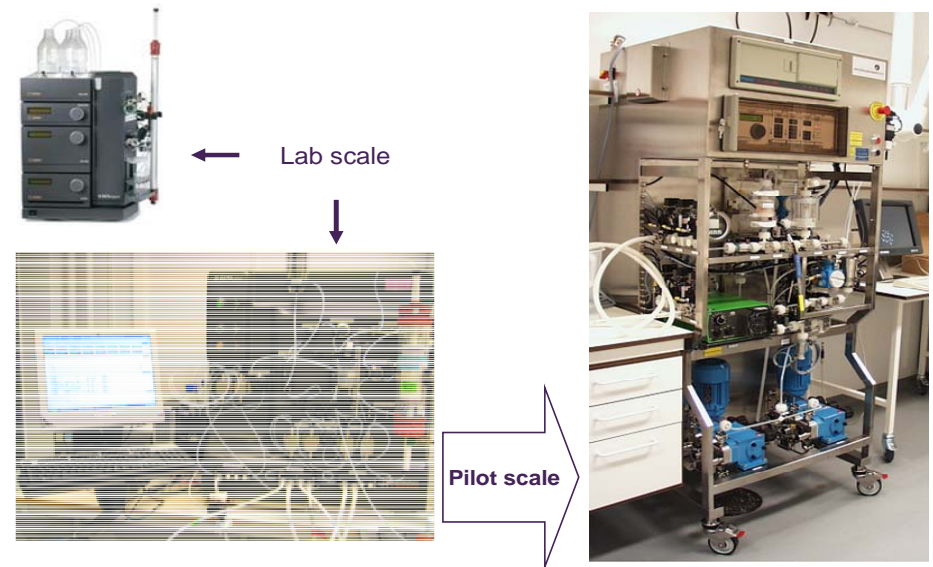


Three phase strategy

- use orthogonal separation techniques

Why orthogonal?

- From a virus reduction/safety perspective, utilise different mechanisms so spreading “risk”
- Different mechanisms to target different impurities via their biochemical properties.
- Anion and Cation exchange are orthogonal, strong or weak exchangers are NOT generally considered orthogonal



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Capture step

- For monoclonals typically a Protein A affinity (but not necessarily so)
- Protein A affinity chromatography shows excellent reduction in ***process impurities*** but often less so for ***product impurities***
- Moderate binding capacity of resin thus perform several cycles on a "small" column to reduce cost. (25-35g/L)

Intermediate step(s)

- For mAb's typically an AIEX column to remove residual DNA and act as a virus reduction step
- Or possibly a CIEX or MMC column to remove product impurities and remaining process impurities
- Can be in bind elute or flow through mode

Polishing step

- Final high resolution high recovery step.
- Deliver final purity required for the product
 - <10ng/dose DNA
 - <200-300ppm HCP's
 - <5% PRODUCT variants (and they should be "characterised")
- Ideally in "low volume" and "high concentration" (but not essential)

Useful Viral reduction steps

➤ Nanofiltration

➤ Inactivation

- Low pH
- Solvent Detergent
- Heat (Wet or Dry)

➤ Chromatography

- Typically not classed as robust
- Can achieve good contributory values

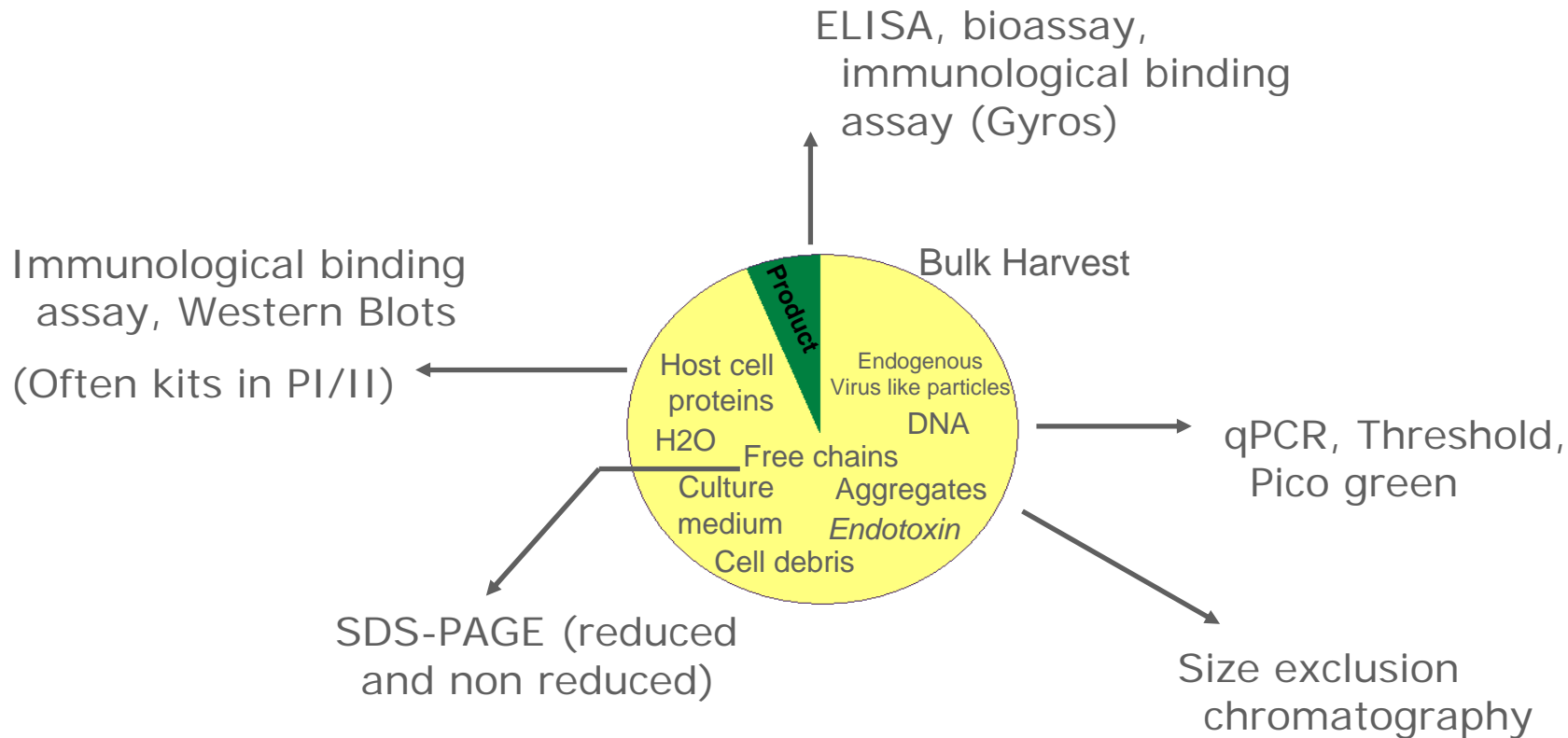
➤ Precipitation

- Typically plasma products

Nanofiltration and inactivation are the two most common specific virus reduction steps in a downstream process.

It is important to think about where in your process you will place them from the outset, not as an "add on" later

Analytical support to process development

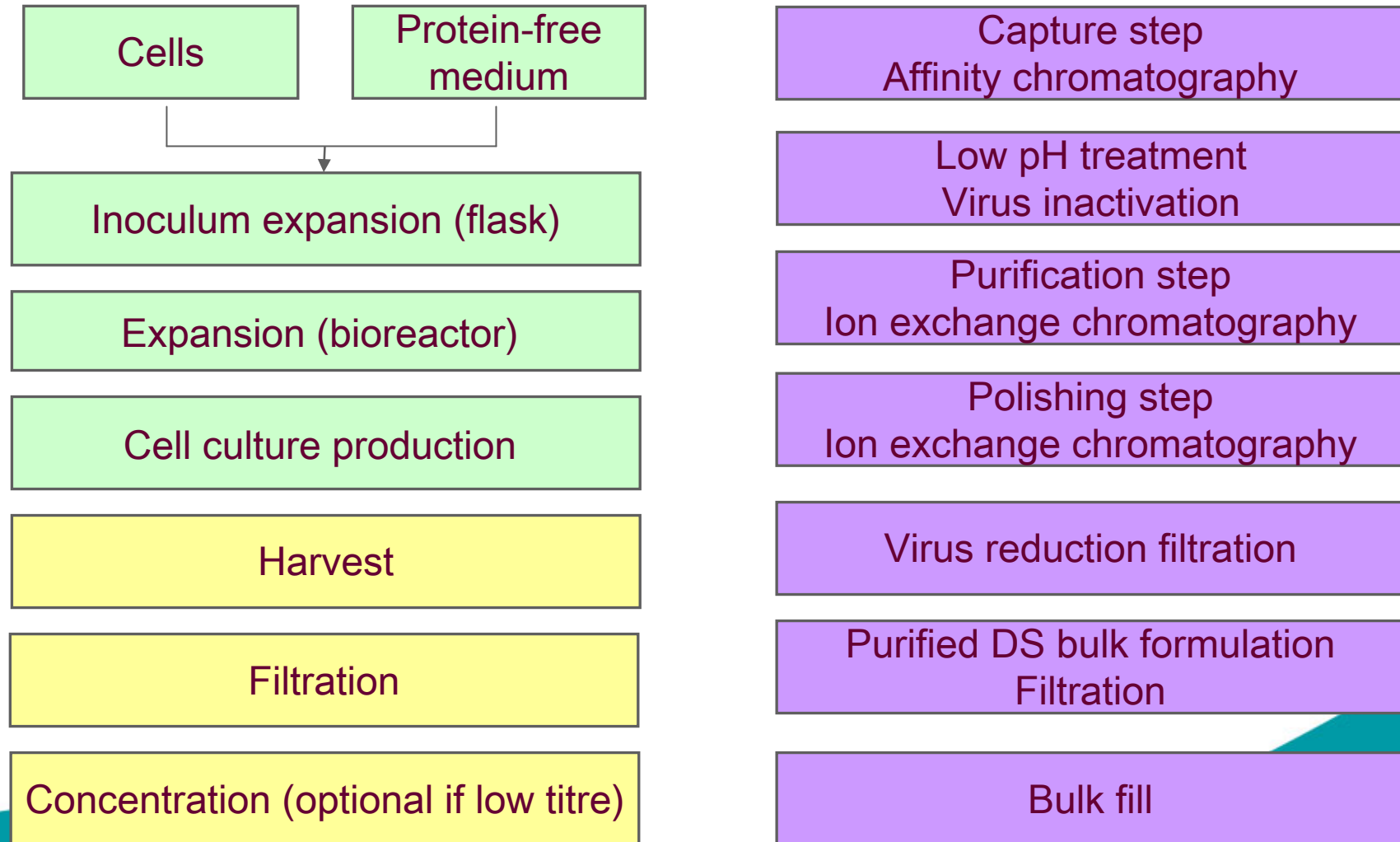


➤ Additionally; IEF, MS, other HPLC, Protein conc,.....etc etc!

Analytical support to process development

- Show where the separation occurs in chromatography
- Give quantitative values for impurity reduction
- Confirm activity of protein
- Allow yield and mass balance determination
- Indicate purity
- Allow stability of intermediates to be investigated

Typical process flow scheme for production of monoclonal antibody



Perfusion versus batch considerations

Perfusion

- Typically low expression
- Large process volumes
- Multiple sub harvests
- "Instabile" product

Batch/ Fed Batch

- Monoclonal antibody for example
- Fixed volume per batch
- Single harvest
- Typically High titre

Consider

- Concentration of sub harvests
- Aliquotation and pooling
- Homogenous column loads
- "Fast" processing time

Consider

- Multiple cycles (especially for capture)
- Column capacity v. plant time
- Removal of process imp. (aggregate) may impact yields heavily.

Scale up to Clinical supply

- What works on the bench might not work at scale!
 - Remember buffer volumes in relation to column size and flow rates
 - Allow tolerance for buffer pH and conductivity
 - Multiple cycles increase plant time, balance this against COG's
 - Equipment compatibility
- Think about what will be required for your commercial product from the beginning.

Clinical supply, purification considerations

- Are there are critical parameters even at early stage?
- What in process analyses or controls are required?
- Are there hold steps, are the intermediates stable?
- For tech transfers, is the downscale representative?
- How well do you know your process, how many times has it been performed?
- Do you have a downscale for viral clearance validation in place?

Clinical supply, purification considerations

- Can the DSP support the proposed specification?
 - Often useful to run the proposed DSP on worst case cell culture feed stock, very low viability at harvest, to simulate highest impurity load.
- Are all raw materials available to "GMP" grade?
- Is the Bulk Drug Substance formulation available and stable?

Future considerations

- Higher titre antibodies moves the "bottleneck" to downstream
- "Disposables" in the downstream area are no longer just virus reduction filters!
- More rigid resins are allowing for higher flow rates and shorter process times.
- Large pharma is looking more and more for "workable" CMC as well good clinical data....(No desire to develop a new purification process – comparability study = cost and time loss)

Acknowledgements

My team at Recipharm Biologics
Ex-colleagues at AstraZeneca



RecipharmCobra Biologics

- Two GMP facilities – Sweden and UK.
- Development and phase I/II clinical supplies.
 - Recombinant proteins.
 - Monoclonal antibodies.
 - DNA.
 - Viruses and cell products.
- Comprehensive service offering.
 - Cell line development – automated and manual.
 - Analytical and process development.
 - Advanced protein characterisation.
 - Cell banking, virus seed stock, pre clinical and clinical supplies.
 - Formulation development and fill/finish.
 - QC/QP release.
- IP expression technologies including ORT-VAC.