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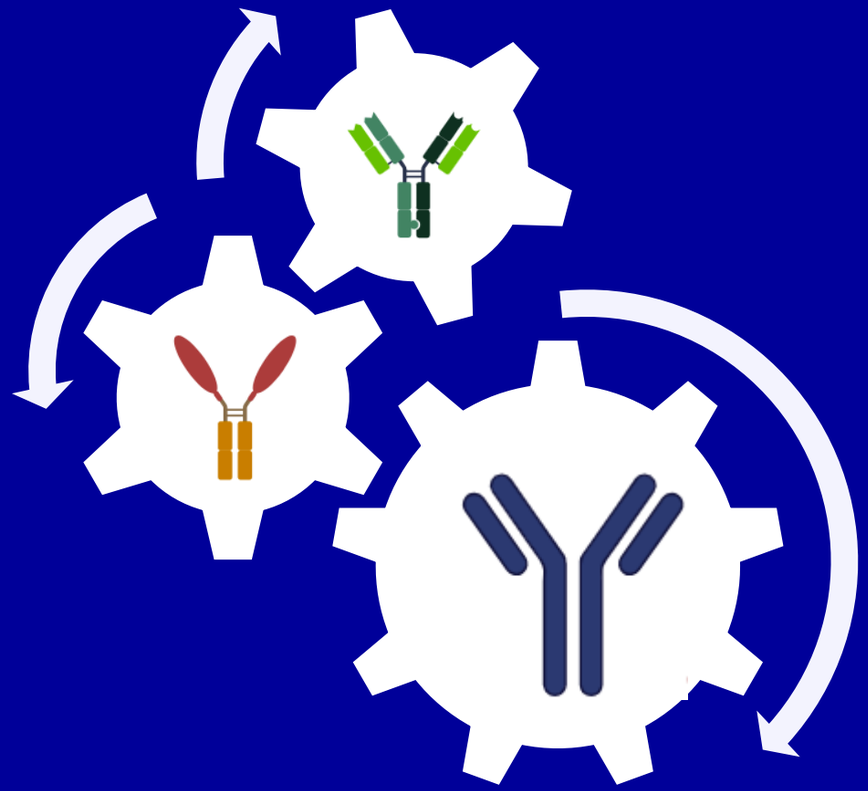
#RESEARCHNEVERSTOPS

Turning the crank using a hybrid continuous purification platform

Michelle Najera, Megan McClure, Shahbaz Gardezi, Beth Larimore



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Process intensification for pre-clinical supplies

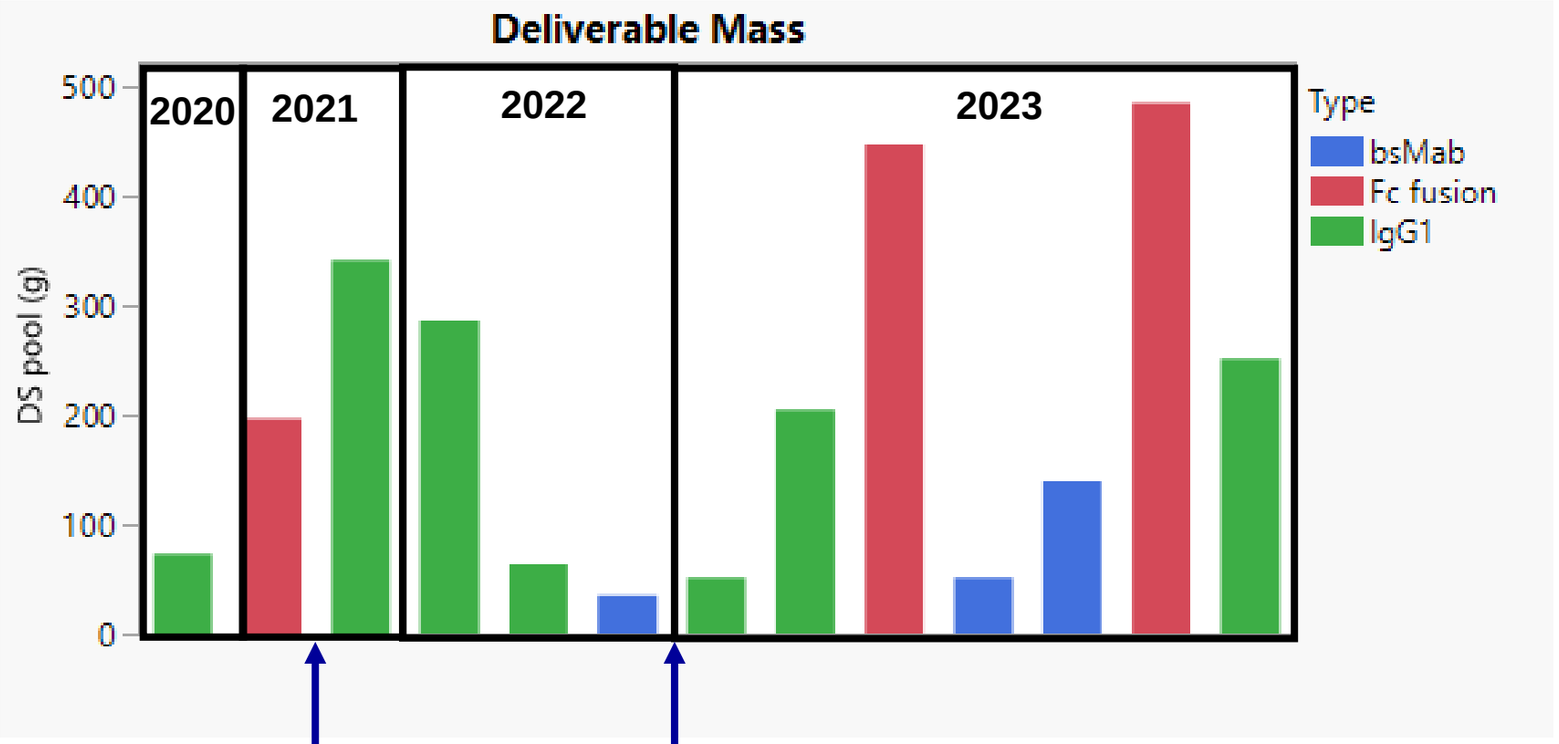
More with less

- Large mass demands for pre-clinical material
- Small footprint in 2 development labs

Seattle, WA
90 ft²

Redmond, WA
150 ft²

- Standard Mabs, Fc fusion, bsMabs
- Alignment with GMP manufacturing
- Hybrid-continuous and end-to-end continuous capable processes



Redmond site brought on-line

50 L SUB added

Hybrid-continuous process

Intensified Perfusion

Continuous harvest & capture

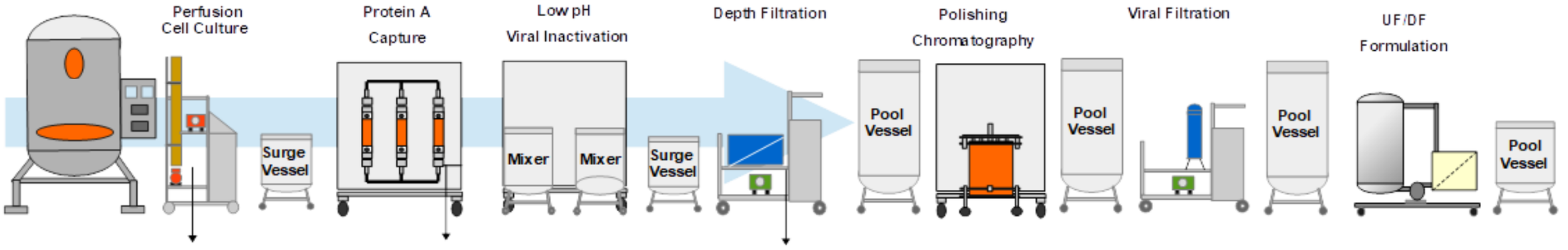
Continuous VI

Continuous DF

Conventional Batch Processing

1 → 2 → 3 → 4 →

Hybrid Process



Flexible format:

- *50L*
- *1, 2, or 3 x 15L*
- *3L and 7L*

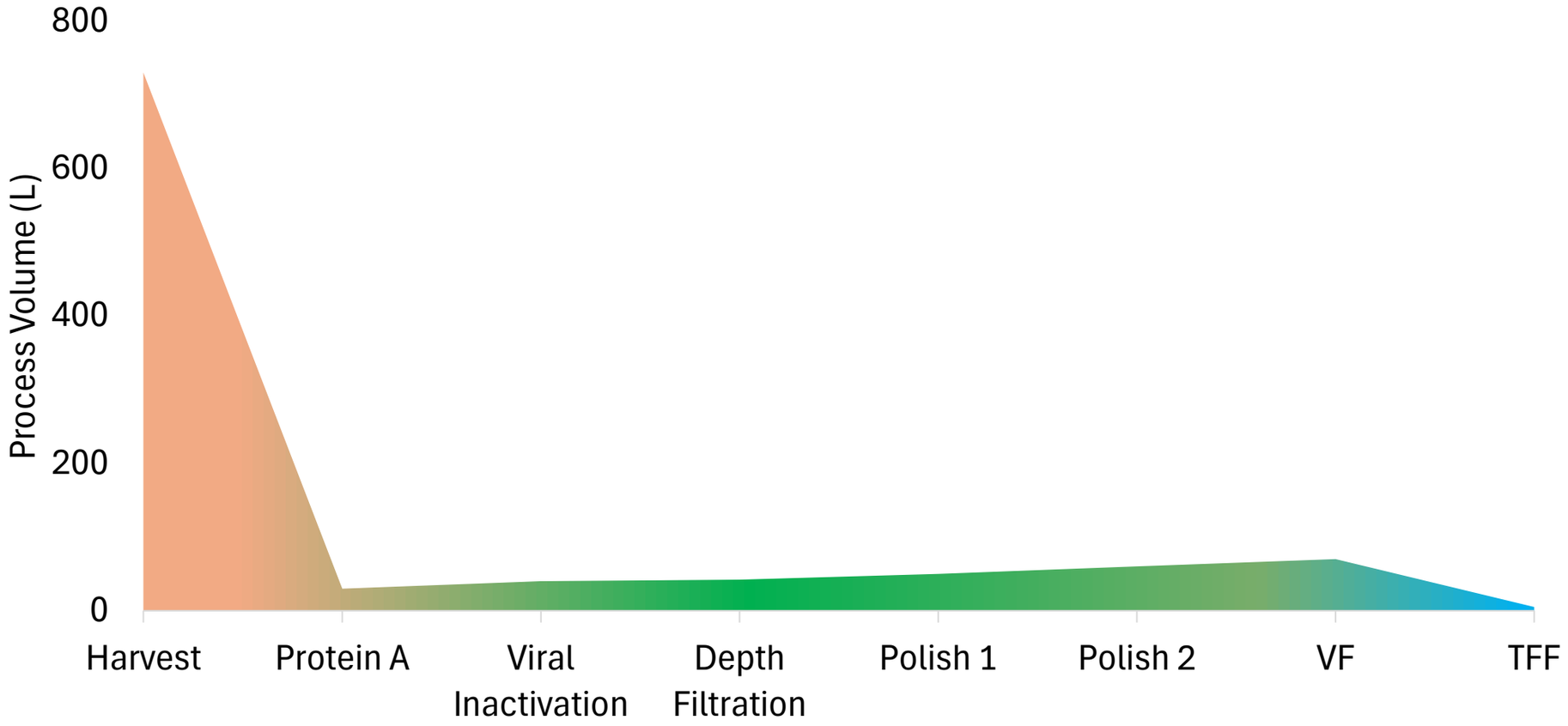
Multi-column capture chromatography

Custom lab solution for viral inactivation and depth filtration

Processing strategy for continuous

Streamlined liquid handling

- **Capture step** cycling with multiple columns to maximize resin utilization and reduce volume
- Minimize low pH exposure for **viral inactivation**
- Flow-through **polishing steps** with minimal adjustment

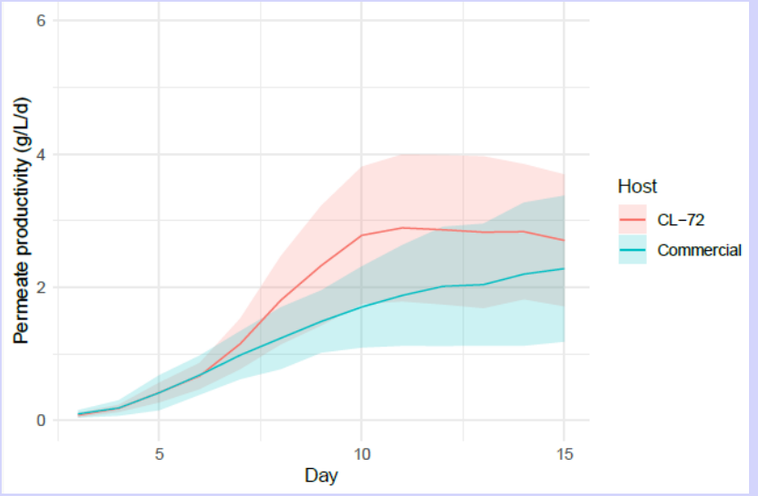


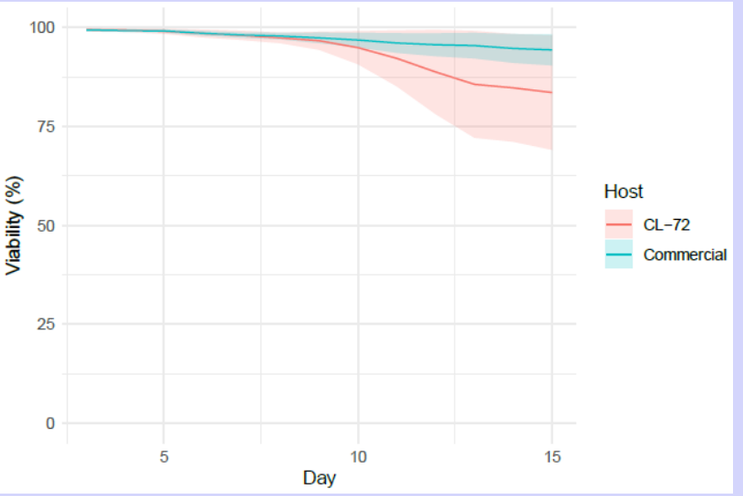
Process inputs

In-house versus commercial cell line

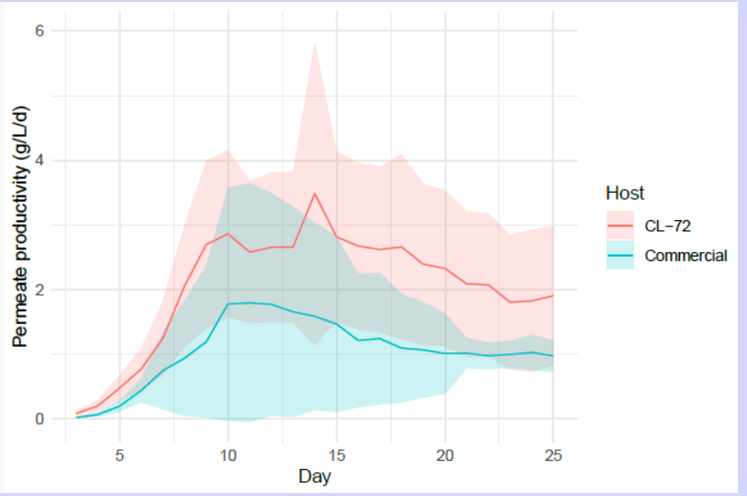
- Permeate titers can reach 2 g/L
- Ability to extend to 25 days

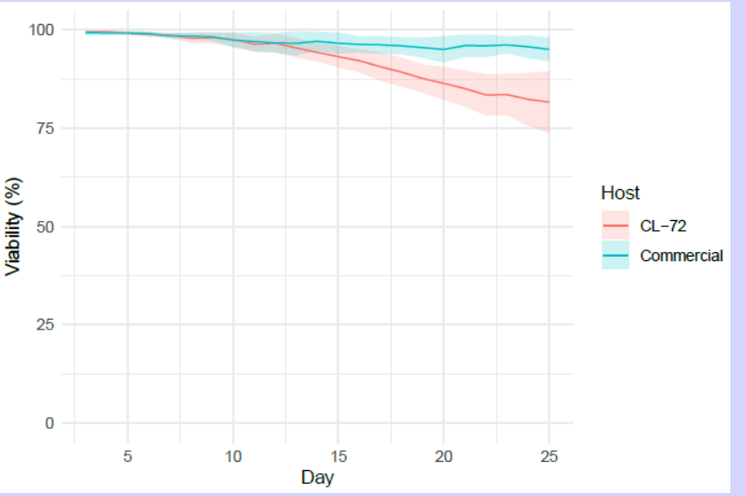
15-day duration





25-day duration





Continuous capture chromatography

Flexibility for purpose

- 2, 3, and 4-column formats all used various purposes
- Respect required at-scale residence times (2 - 4 min) to select column size
- Load CV and equipment flow capabilities determine selected format

$$Load\ CV = \frac{Target\ loading\ \left(\frac{g}{Lr}\right)}{Titer\ \left(\frac{g}{L}\right)}$$

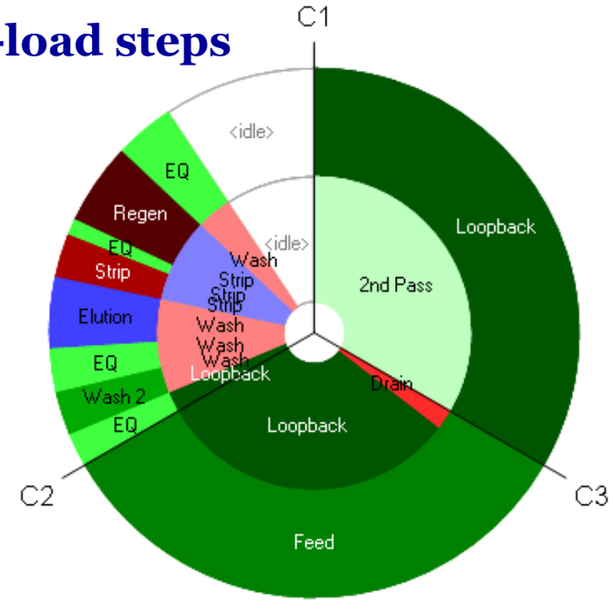
← Lower for Fc fusions and bsMabs

← >2 g/L can require high-capacity resin or 4-column process

Column diameter (cm)	Column height (cm)	CV (L)	Residence times (min) for selected bioreactor working volume	
			400 L 	900 L 
14	7	1.1	1.9	
	9	1.4	2.5	
	10	1.5	2.8	
20	7	2.2		1.8
	9	2.8		2.3
	10	3.1		2.5

Sartorius BioSMB Chronogram

Non-load steps



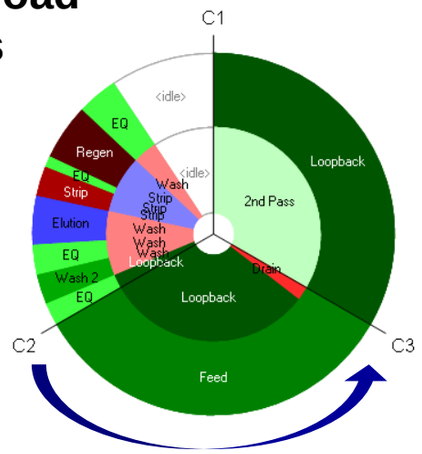
Primary load
(defines time available for non-load steps)

Beyond platform capture resin

- Any capture resin can be used
- Resin loading on platform resin is often sufficient for most molecules
- High-capacity (other) resins have trade-offs

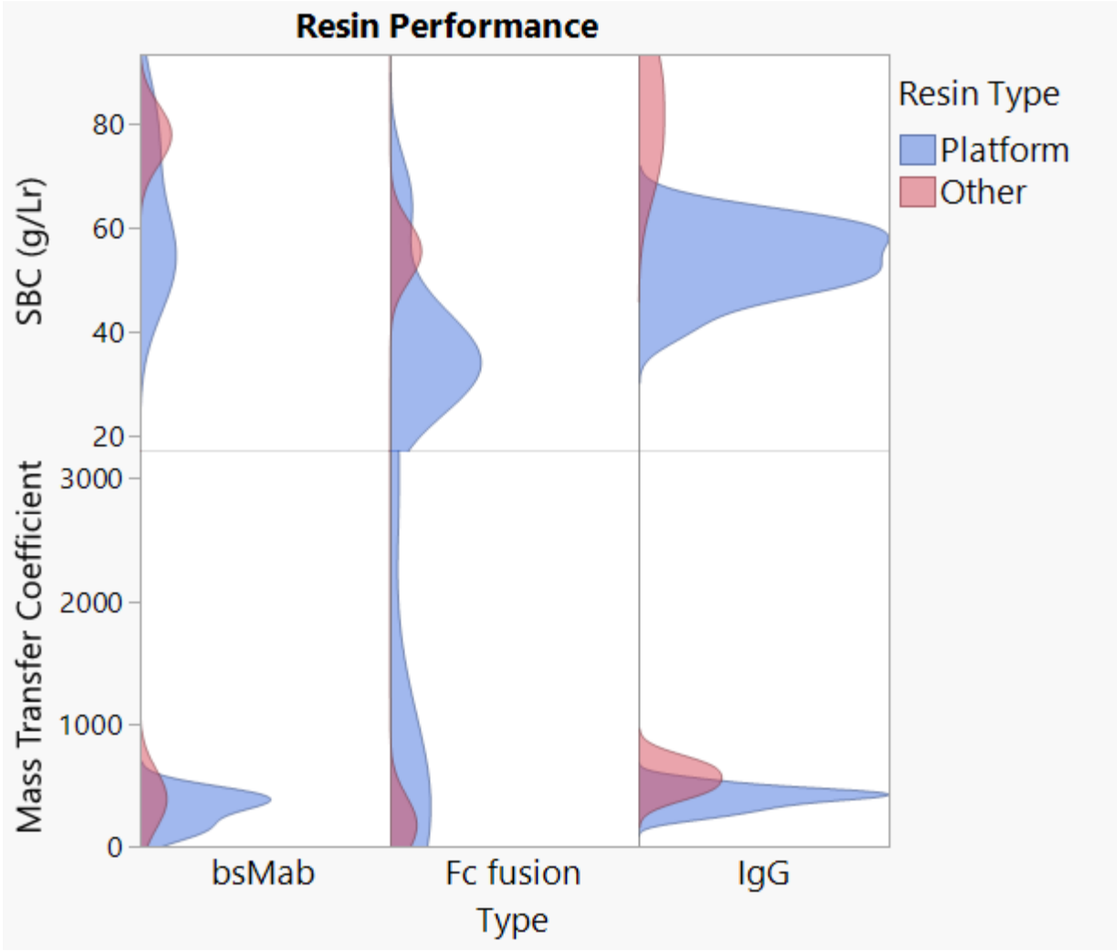
3-column process for typical loading and titer

Non-load steps



Contact time

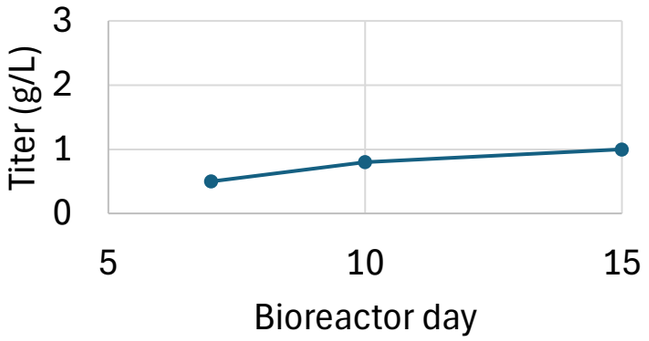
Primary load



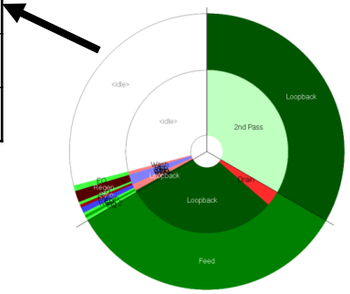
Processing strategy optimization

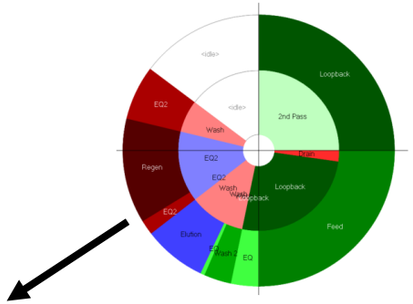
Impact of titer and resin loading

Normal titer, typical binding

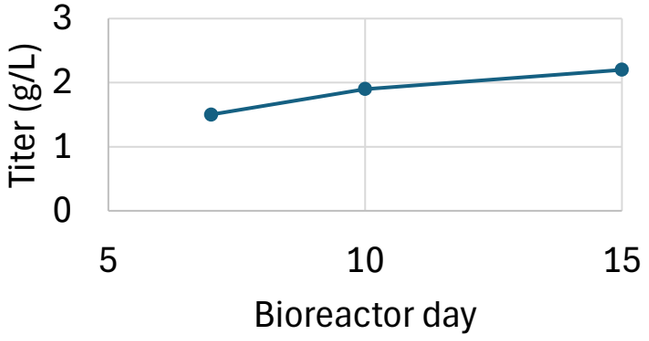


Titer	Platform			High capacity		
	Loading (g/Lr)	Load CV	Contact time (hr)	Loading (g/Lr)	Load CV	Contact time (hr)
0.5	50	100	3.0	80	160	**4.8**
0.8		63	1.9		100	3.0
1		50	1.5		80	2.4





High titer, low binding

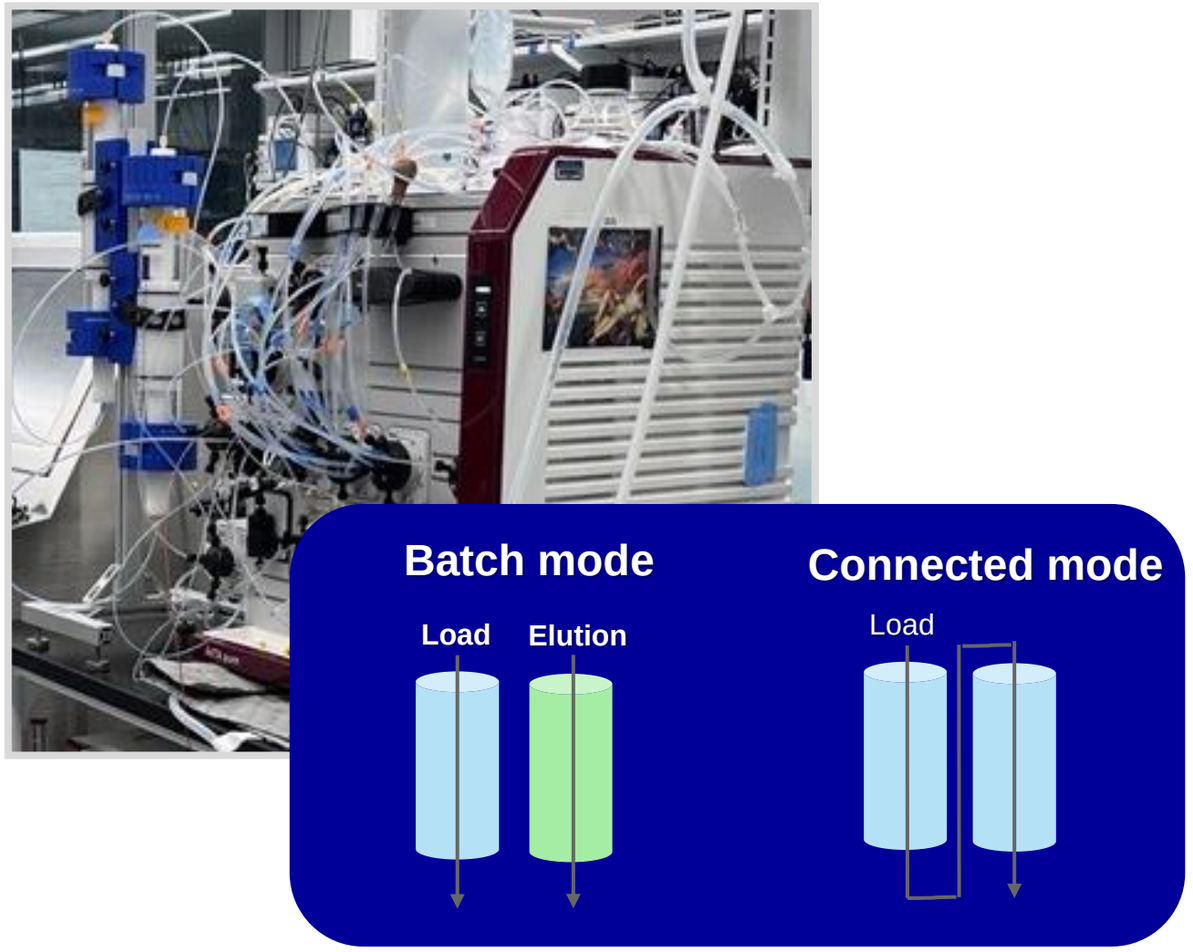


Titer	Platform			High capacity		
	Loading (g/Lr)	Load CV	Contact time (hr)	Loading (g/Lr)	Load CV	Contact time (hr)
1.5	25	17	0.5	40	27	0.8
1.9		**13**	0.4		21	0.6
2.2		**11**	0.3		18	0.5

Dual Column Chromatography (DCC) for development labs

Scaled-down processing

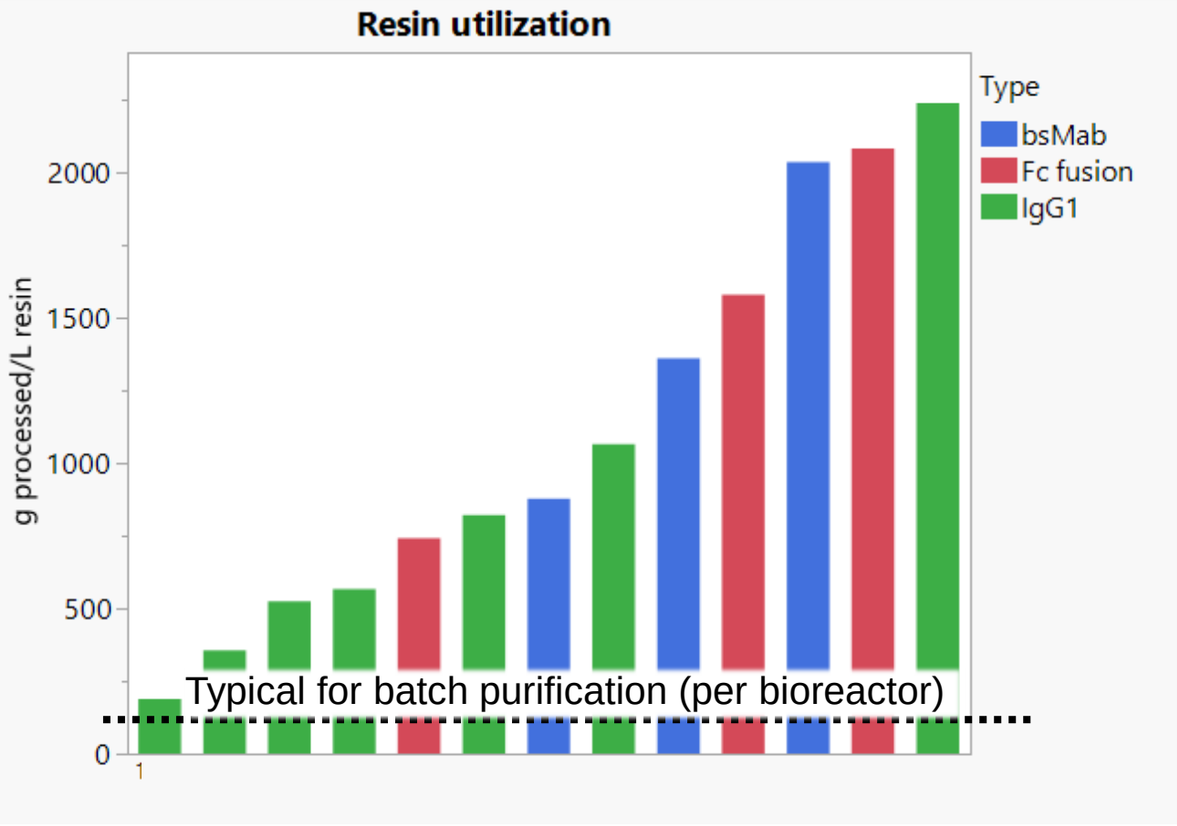
- **Dual-column chromatography**
  - A portion of the load phase is performed without a column in second pass (i.e. risk of breakthrough)
  - Equipment available in development labs
  - Informs at scale performance
- **Multi-column overloaded capture step**
  - A column is in second pass position for the entire load phase
  - Maximizes efficiency of capture resin
  - Keeps pace with bioreactor perfusion
  - Scale-down equipment has limited flow capabilities



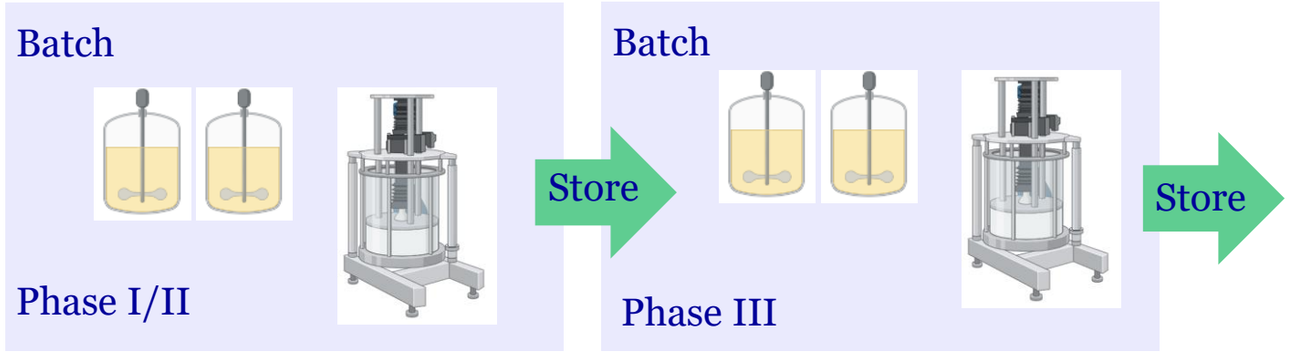
Slide courtesy of Andrew Wagner

High resin utilization with many small columns

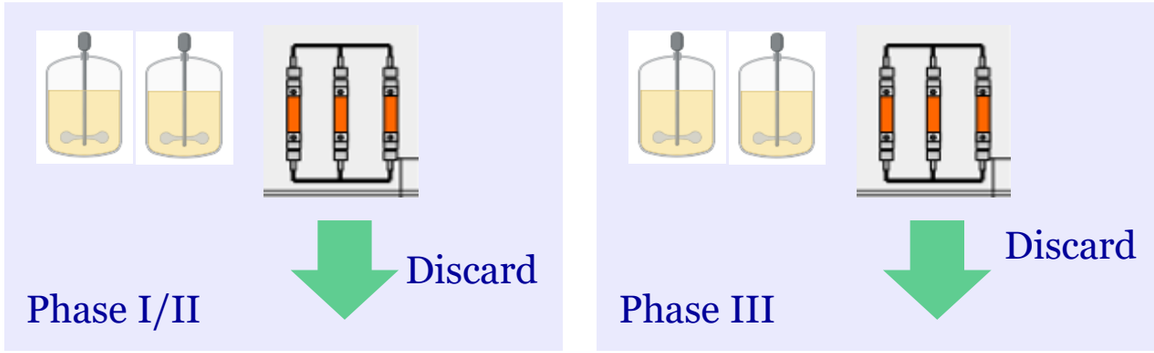
Intensified cycling simplifies resin life cycle



Batch



Continuous capture



Typical Mab assuming 3 cycles at-scale for each bioreactor

Transition Analysis Metrics

Determining root cause

Each parameter provides distinct insight into the performance of the column

Value increases

HETP GHETP

Column efficiency decreases

Possible Column Integrity Breach

Number of inflections

Cumulative error

Possible Mixing Column's headspace

Asymmetry

Cumulative error

Possible channeling

Asymmetry

Value decreases

HETP GHETP

Column efficiency increases

Possible Column Integrity Breach

MROC

Breakthrough volume

Asymmetry

Suggested importance

HETP

↓

Cumulative Error

↓

Maximum Rate of Change (MROC)

↓

Breakthrough volume

↓

Number of Inflections

↓

Asymmetry

↓

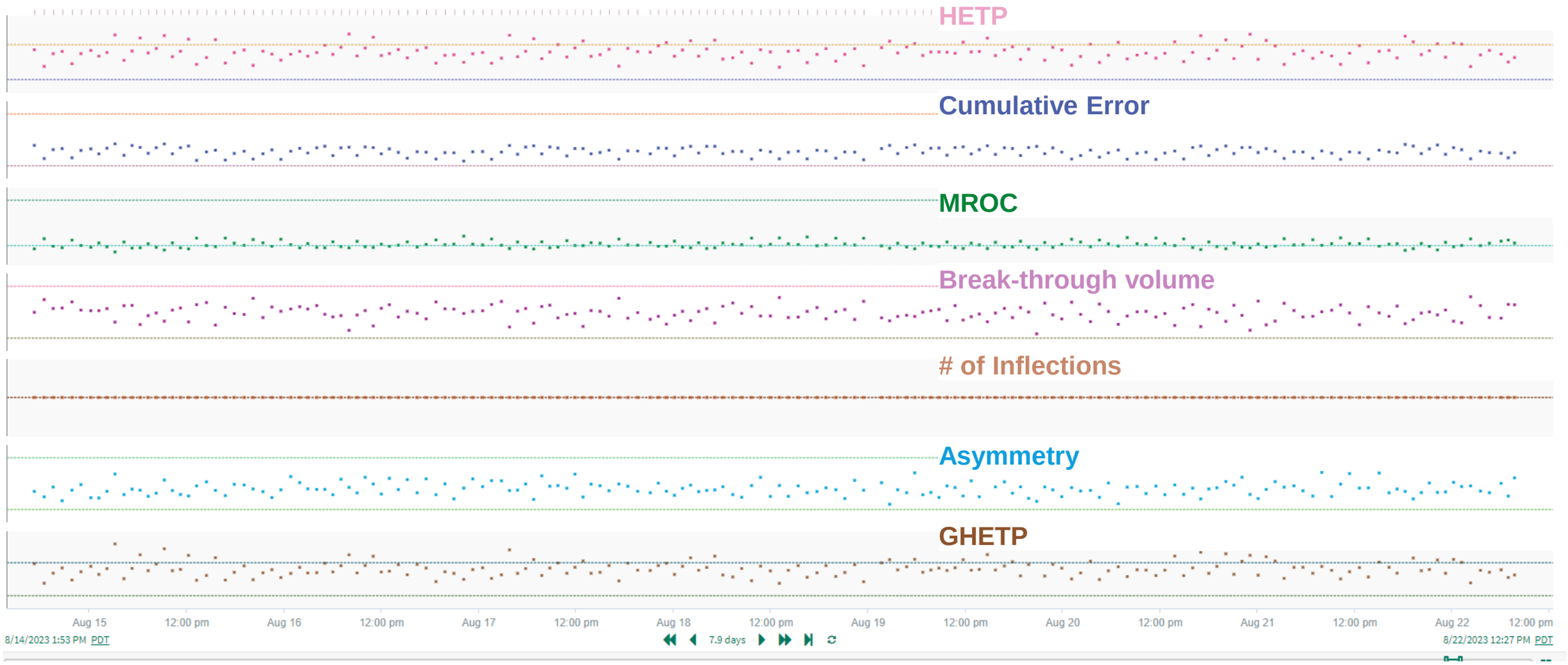
Gaussian HETP

Decreases

Larson, TM; Davis, J; Lam, H; Cacia, J. Use of Process Data to Assess Chromatographic Performance in Production-scale Protein Purification Columns. Biotechnology Progress. 2003. doi: 10.1021/bp025639g

Column health monitoring

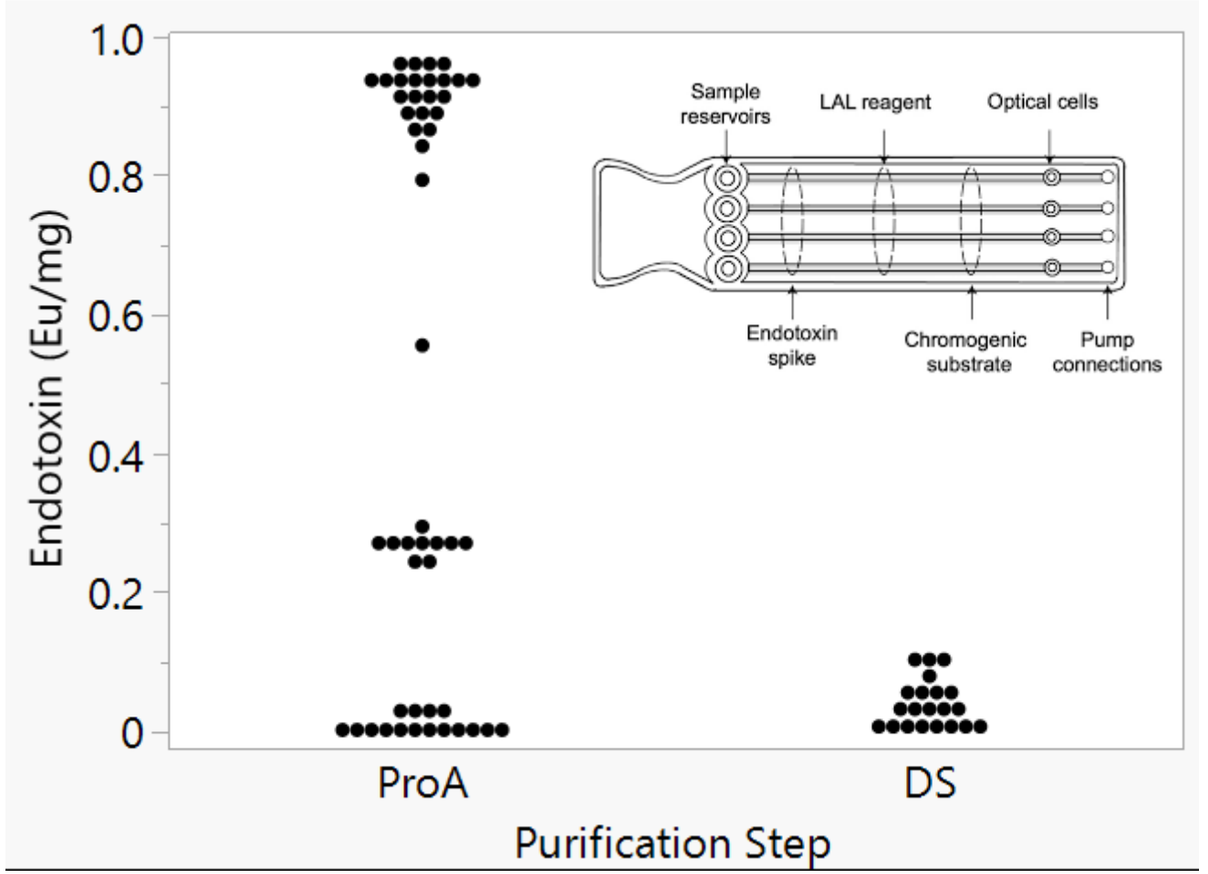
Transition analysis in real time



Keeping it clean

Endotoxin

- Platform targets met for all molecule types
- Tested with turbidimetric method (Charles River Endosafe® Next gen-PTS)
- No environmental controls in development labs
- Operations closed or functionally closed
- Sanitized equipment, single use components, multi-compendial materials for final process formulation

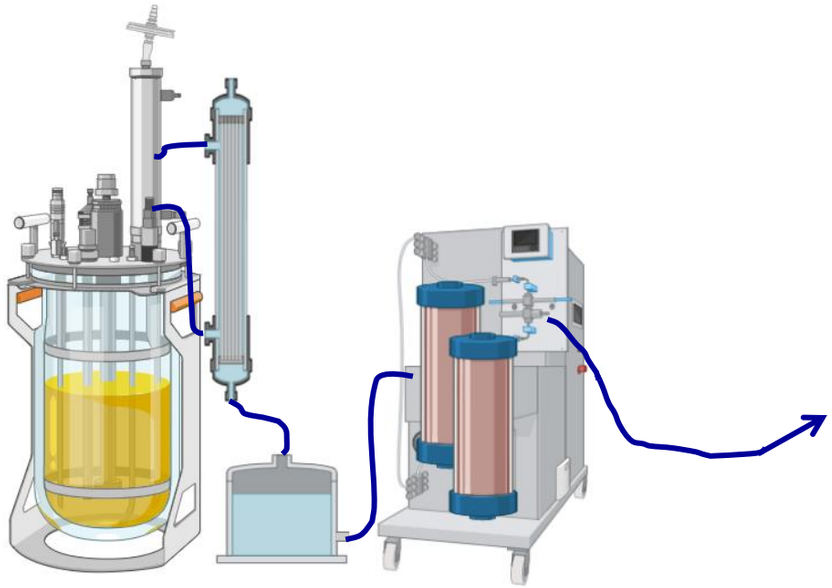


Making friends

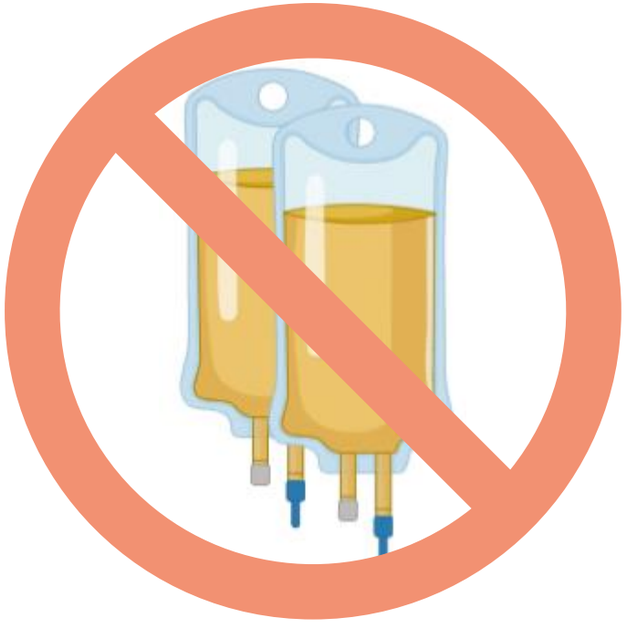
Hybrid continuous process in the labs

- Typical perfusion rate of 2 vessels volumes/day
- 50 L SUB (45 L working volume), 8 days of collection = 720L

More of this….



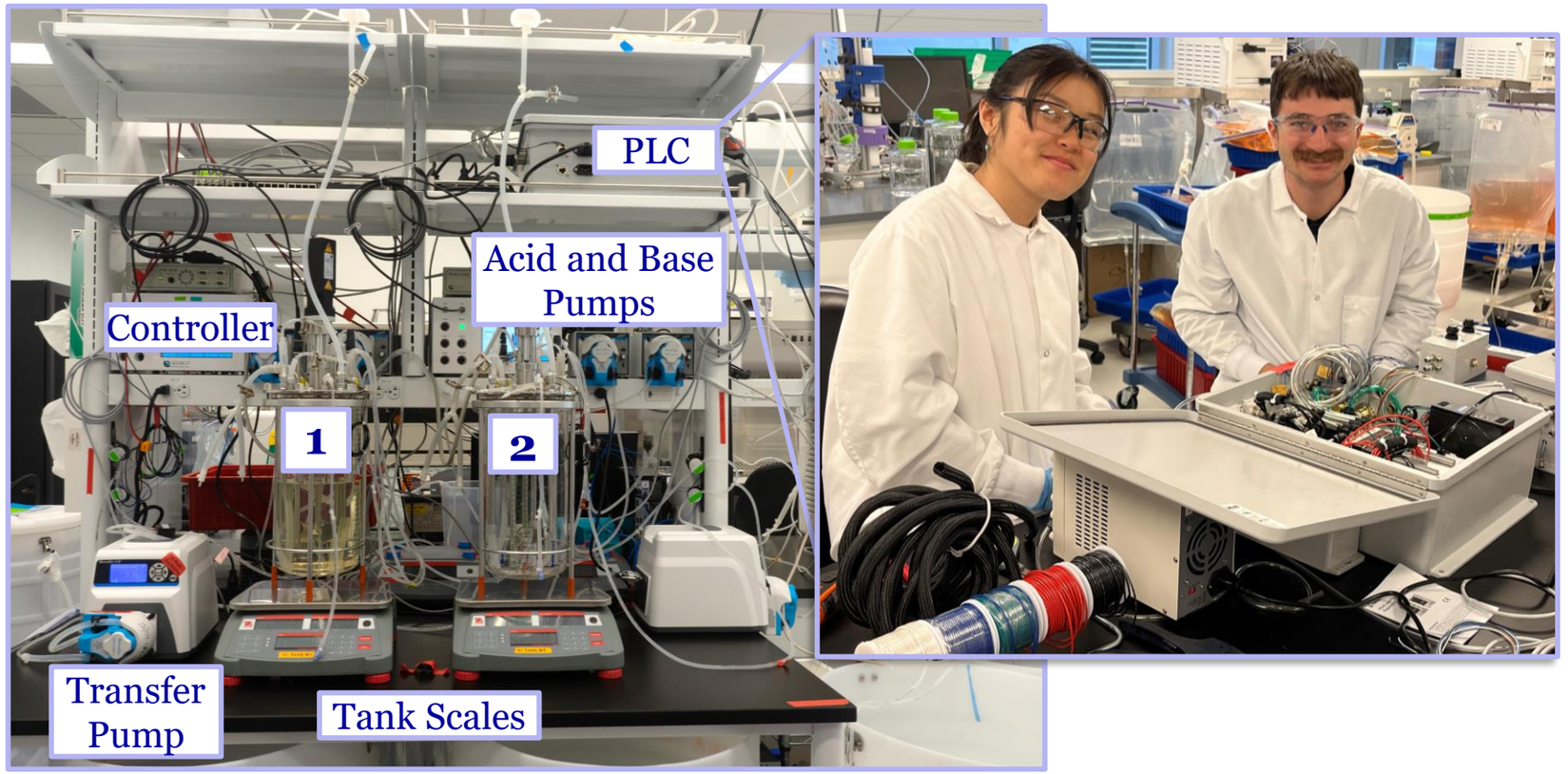
And less of this…



Images created with Biorender.com

Custom Two-Tank Viral Inactivation for Bench -Scale

Hands-free operation



Components: Bioreactor vessels with sensors and controller, in-house assembled PLC, pumps, valves, scales, flow sensors, bubble sensors

Process

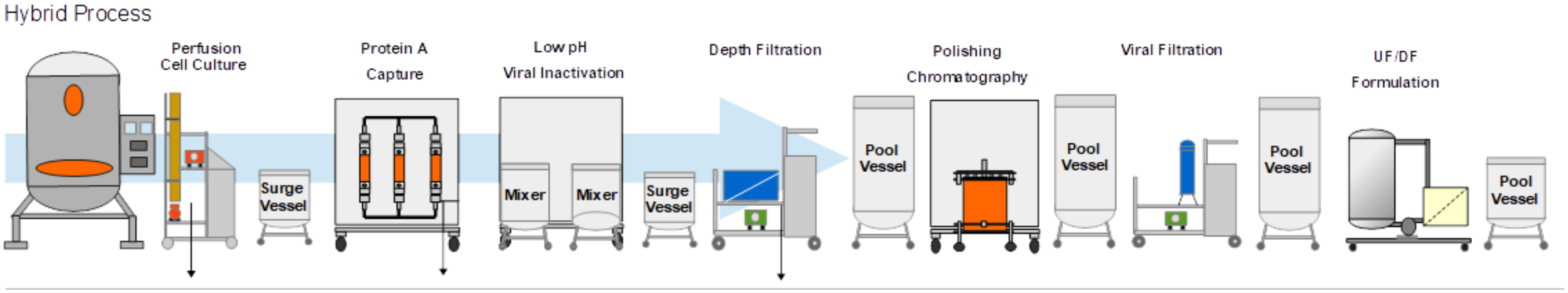
1. Collect several eluates in tank 1
2. Add acid in tank 1 using step-wise proportional control loop
3. Confirm pH and transfer to tank 2 (begin eluate collection tank 1)
4. Hold in tank 2
5. Neutralize in tank 2
6. Transfer to collection vessel

Niall Peters Chris Ford, Yan Brodksy, Caroline Mueller, Alex Zaytsev, Jodi Chien, Philippa Bailey, Anastasia Nicolov, Megan McClure. Turning Connected into Continuous. ACS, Spring, 2023

Extending the continuous envelope

Capability at all scales

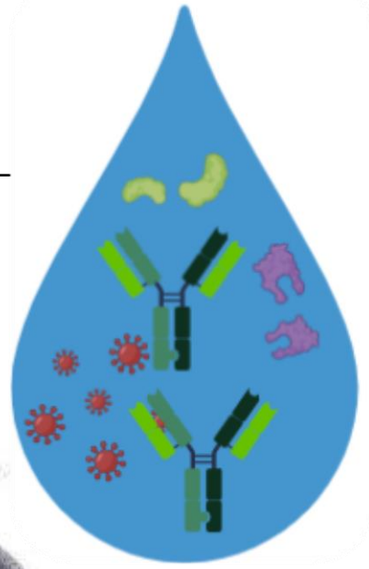
- In-line mixing
- Depth filtration – automated filter swaps
- Polishing steps – intensification through cycling
- Viral filtration – automated filter swaps and bracketed spike viral clearance study design
- Single pass TFF and in-line diafiltration
- PAT, especially for concentration and microbial monitoring

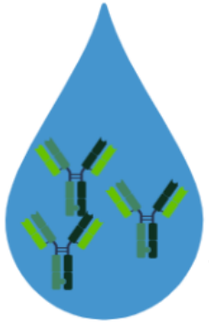


Summary

Continuous processing at all scales

- Molecular optimization and high-quality cell lines
- Continuously perfused bioreactors
- Simple platform processes that are continuous capable
- Continuous processing to ease liquid handling pain points
- Flexible column formats for scale-down processing while maintaining critical process parameters





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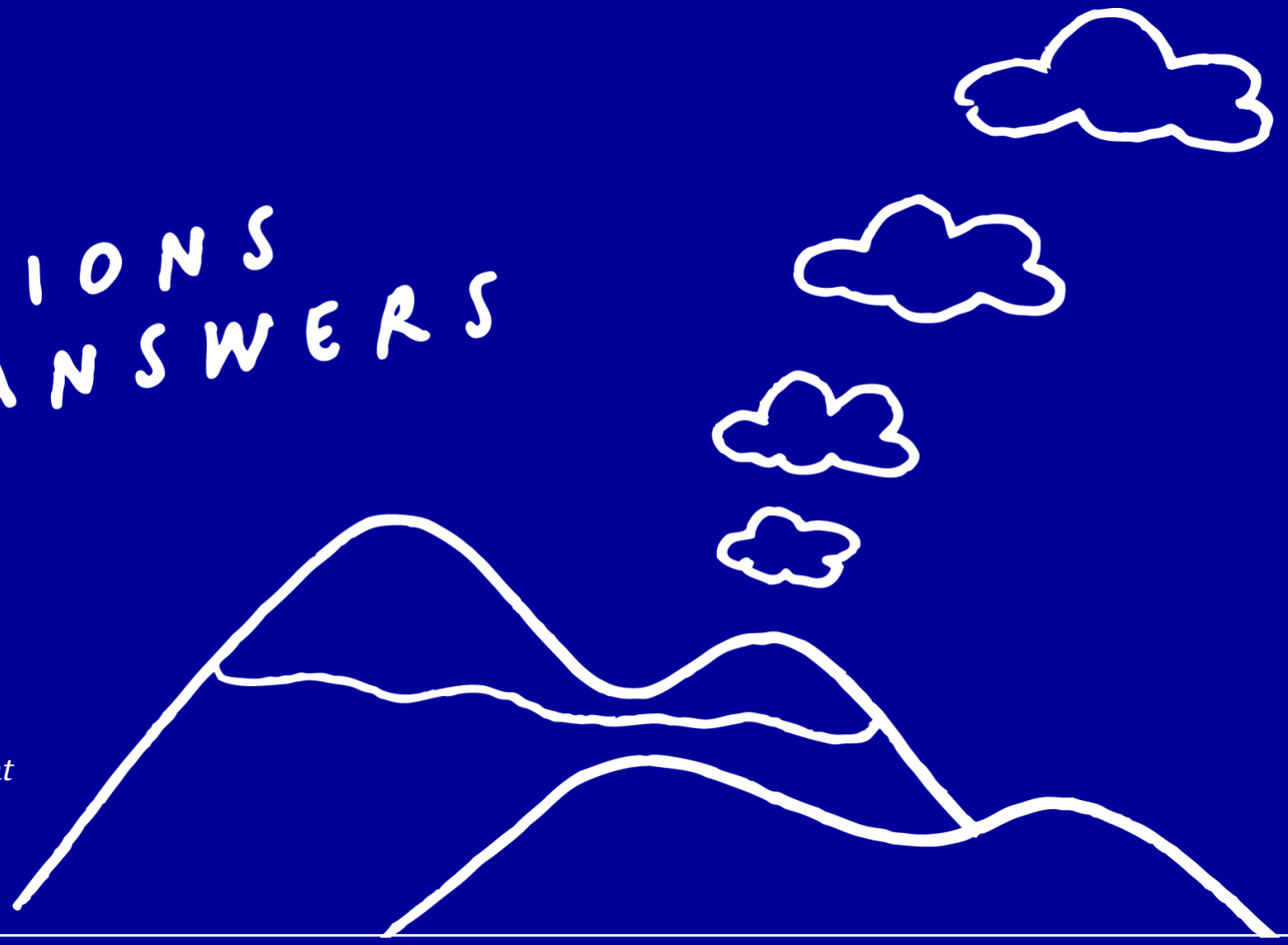
QUESTIONS AND ANSWERS



Michelle Najera
Principal Scientist, Purification Process Development

michelle.najera@evotec.com