HIGH PRODUCTIVITY PROTEIN A MEMBRANE DEVICES COMPLEMENT DISPOSABLE UPSTREAM TECHNOLOGY FOR A FULLY SINGLE-USE PROCESS

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GORE

Key Trends Impacting Bioprocessing Today

SINGLE-USE SYSTEMS

- Smaller footprint, less capital
- Sustainability: less water, chemicals and energy
- Scalable & flexible
- Less downtime & faster turnover
- Lower risk of contamination, bioburden, and operator error

INTENSIFIED PROCESSING

- Smaller footprint producing higher productivity
- Consistent scalability & facility fit to speed tech transfer

SUPPLY CHAIN

- Dual source options
- Short lead times

NEW, COMPLEX MODALITIES

- Multispecifics, ADCs, Fc fusions, etc.

The Downstream Bottleneck: Resin Chromatography

- Protein A resin columns are **oversized/underutilized** to gain flow and speed processing times to keep pace with upstream productivity
- **Need to optimize chromatography** media performance to enable high flow rate and high binding capacities
- **Facility fit challenges** for multiple drugs, scales and potencies
- **Downtime** for packing, qualifying, storing, validating resin columns
- **Bioburden** that can impact your bottom line and your project timeline

Proprietary Membrane, Immobilized Protein A

IMPROVES PRODUCTIVITY

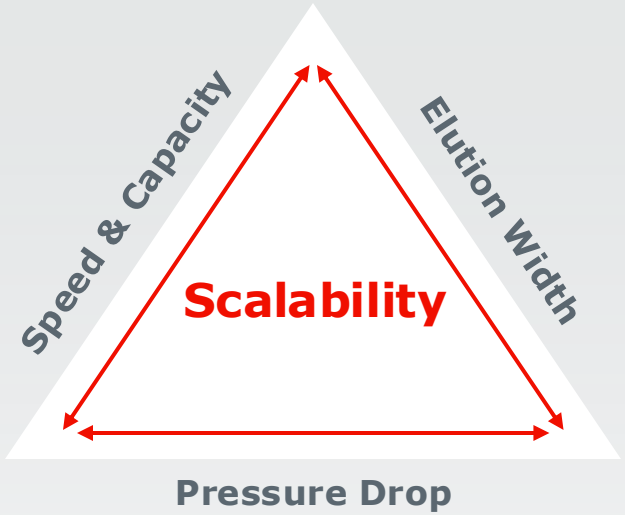
- Consistent scaling, speed, capacity, elution width and pressure drop

RAPID CYCLE AND FLEXIBLE PROTOCOLS

COMMERCIALLY AVAILABLE PREPACKAGED COLUMN SIZES

- Lab scale: 1.0 mL and 3.5 mL
- GMP supported: 9.0 mL, 58 mL, 116 mL, 250 mL, 500 mL, and 1L (GMP)

Focus for Scaling Proprietary Membrane



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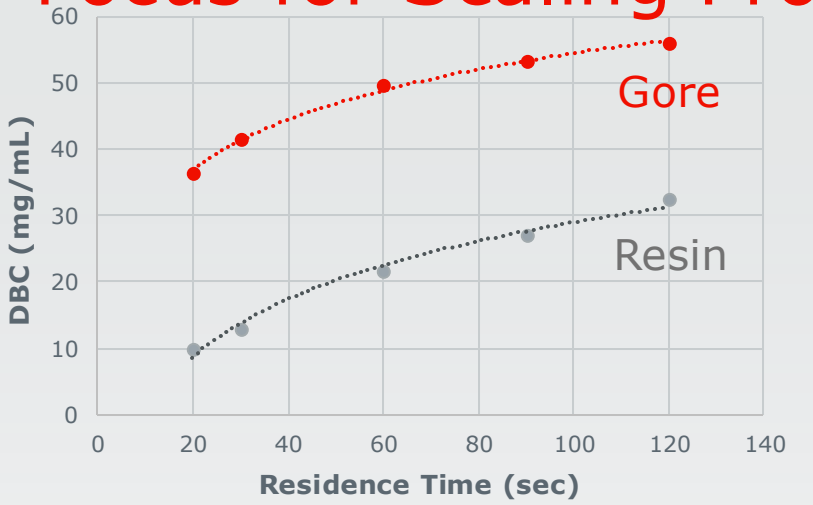

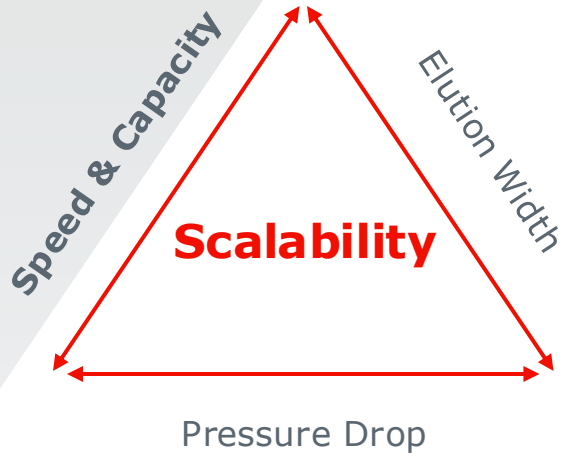

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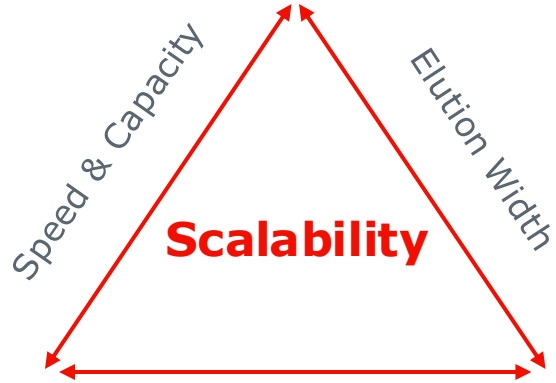

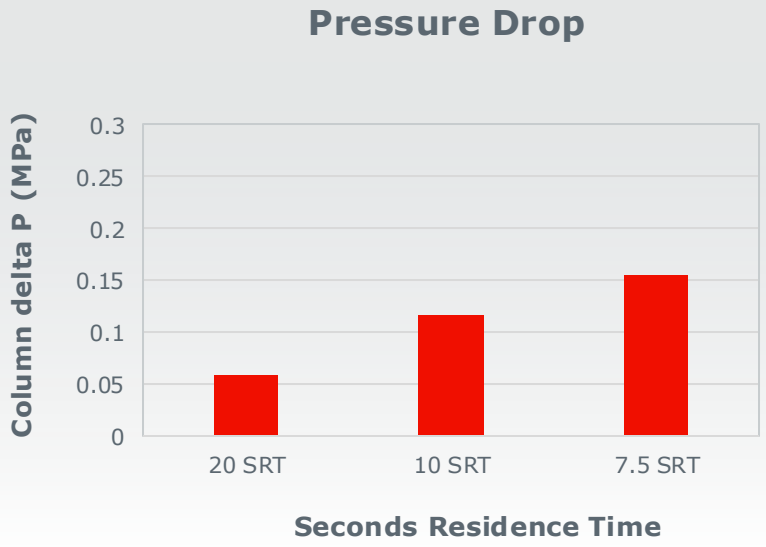

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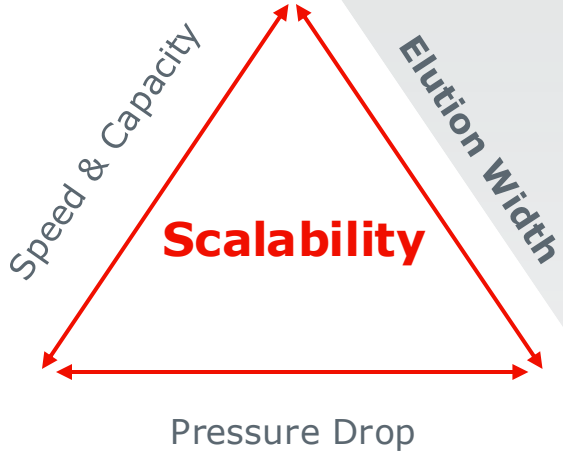
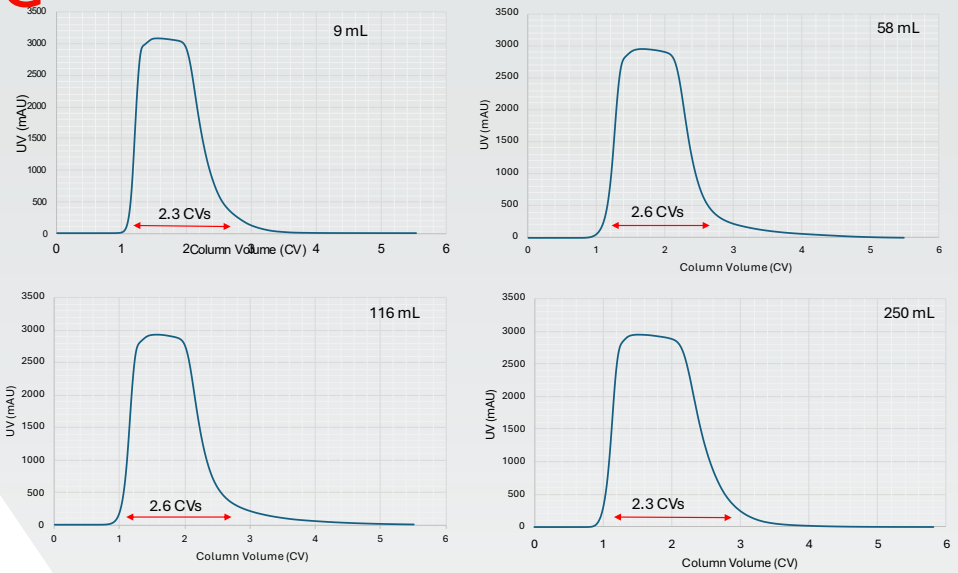
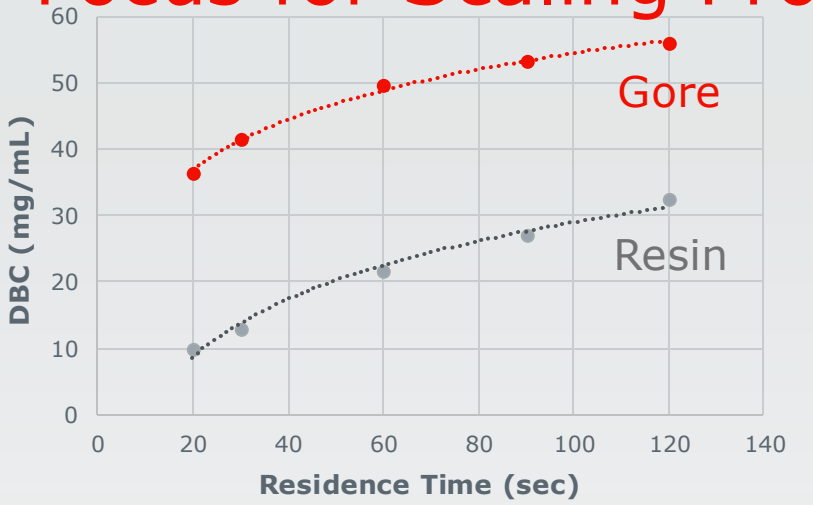
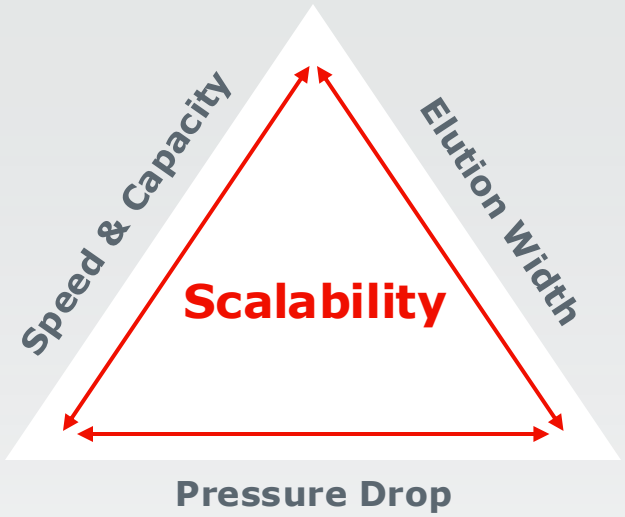


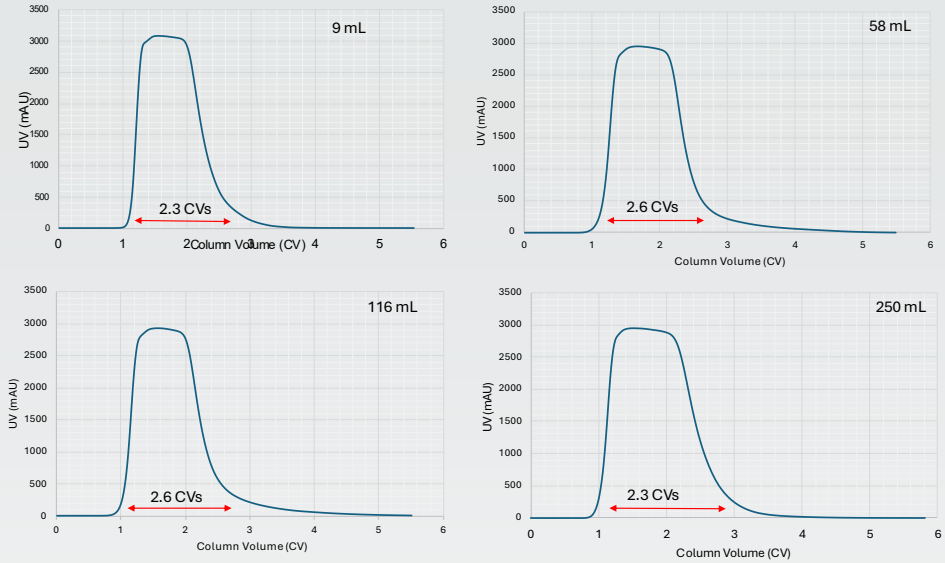


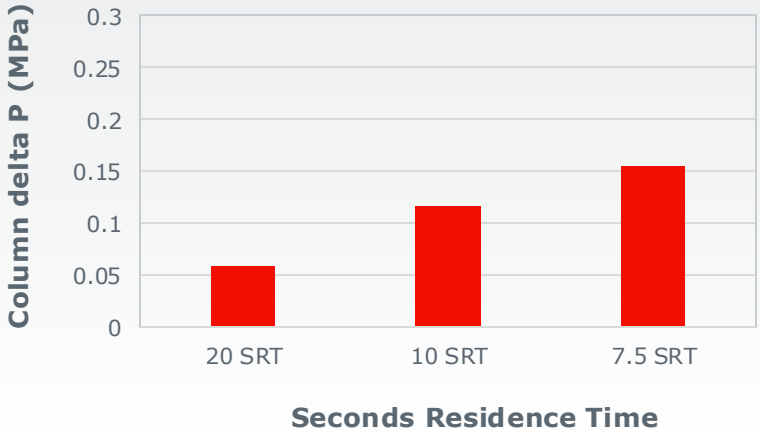
Elution Width on average ~ 2.5 MV

Focus for Scaling Proprietary Membrane





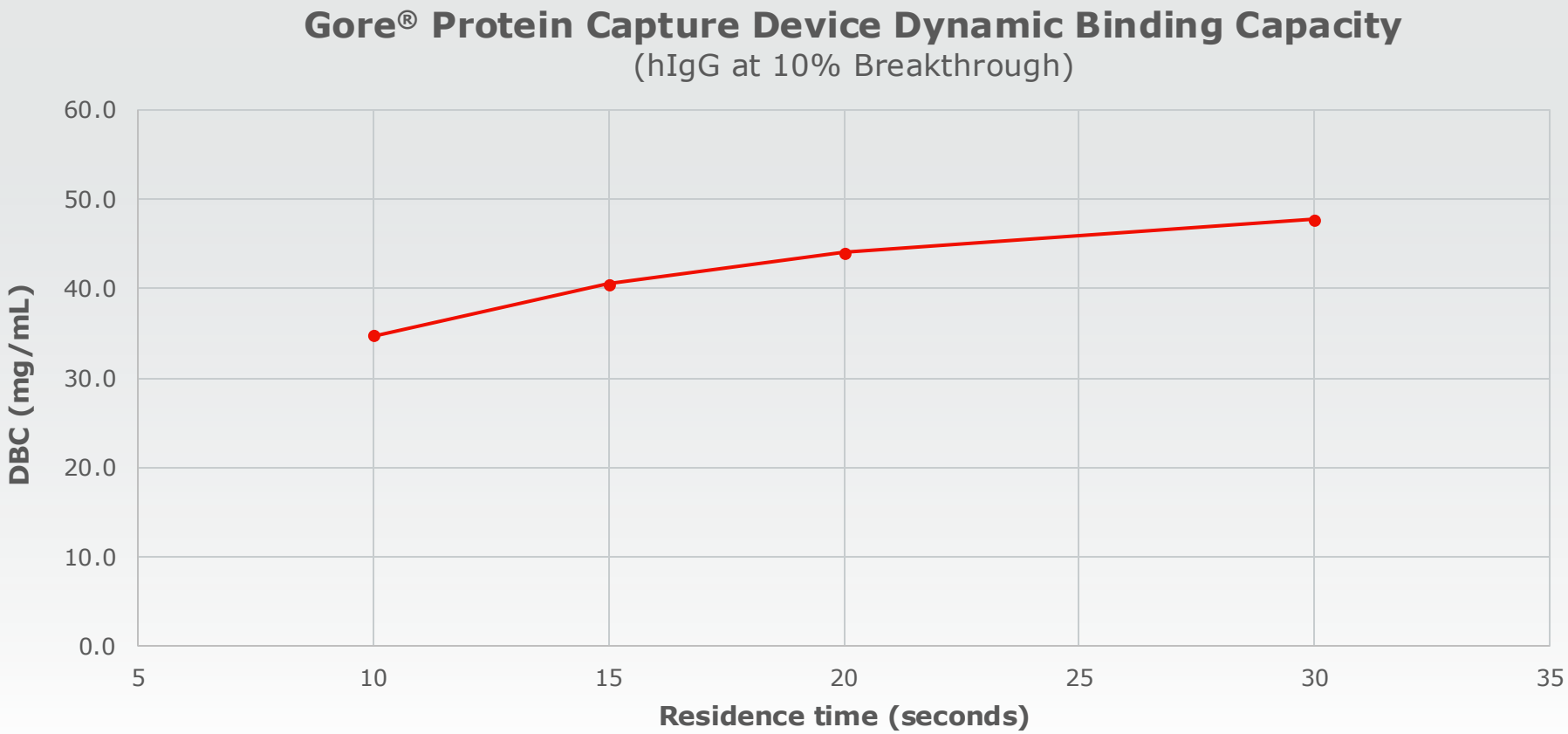




Elution Width on average ~ 2.5 MV

Focus on Productivity Optimization

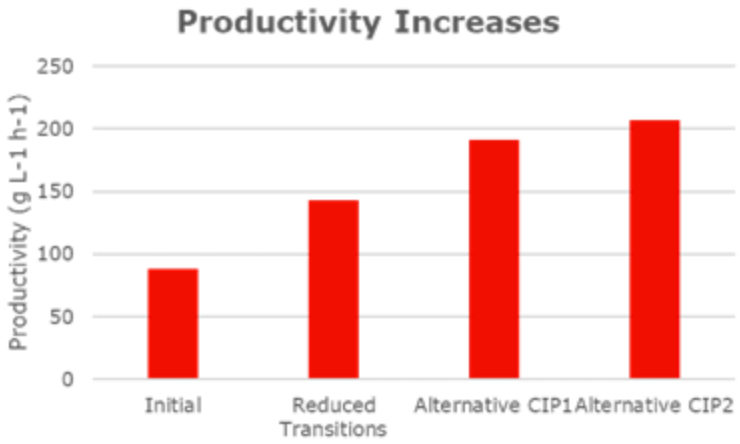
Faster than 20srt



Improved Productivity with Flexible Protocols

GORE® Protein Capture Devices with Protein A — 58 mL: 4g/L CHO cell harvest

Initial Study: 88 g L^{-1} h^{-1}	Reduce Transitions: 143 g L^{-1} h^{-1}	Alternative CIP1: 191 g L^{-1} h^{-1}	Alternative CIP2: 207 g L^{-1} h^{-1}
• Loading: 30srt • Operational: 10srt • Conservative transitions • 3 minute CIP per cycle • 16.8 minutes/per cycle	• Loading: 30srt • Operational: 10srt • Reduced transitions • 3 minute CIP per cycle • 12.6 minutes/per cycle	• Loading: 15srt • Operational: 7srt • Reduced transitions • 2 minute CIP per cycle • 9.5 minutes/per cycle	• Loading: 15srt • Operational: 7srt • Reduced transitions • "Pulse CIP" per cycle • 6.9 minutes/per cycle



- CMO and pharma evaluations show no difference in product quality attributes relative to productivity
  - HCP clearance
  - Yield
  - Protein A leaching
- Similar CQAs to resin
- Validated through 100 cycles and demonstrated 200 cycles
- Flexible CIP and durability enable ability to clear 2000-L bioreactor in fully disposable process

Results from Partner Research: Single-Use Upstream + Protein A Purification Devices

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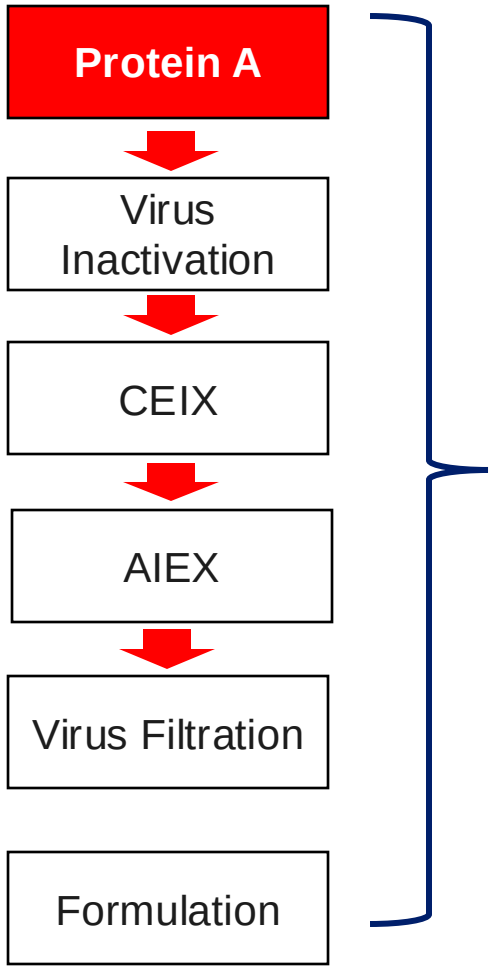
Protein A Resin Limitations in Standard mAb Platforms



- High volumes in harvest
- Limited hold times
- Long residence times during loading
- Large columns required
- Expensive
- Difficult to clean
- Bioburden issues
- Re-use validation

Gore Protein A Membrane | 1 L Manufacturing Scale Device

2 x 1 L Protein A Devices

The Research Execution

- Scale-up run using 2 x 1 L Gore Protein A devices
- Small footprint required (approx., 10 x 10 inch for each column).
- Standard single use chromatography skid used

Protein A Membrane | Short Residence Time and Rapid Mass Transfer

Harvest titer : 1.717 g/L
Membrane volume : 2 L
Load volume: 23.5 L (40 g/cycle)

We performed two method to reduce sanitization volume between cycles

Cycle 1-10

Step	Volume (MV)	Residence Time (min)	Total Time (min)
Equilibration	3.0	0.2	0.6
Load	11.6	0.4	4.66
Wash 1(EQ wash)	1.43	0.4	0.57
Wash 2(Salt wash)	3	0.2	0.60
Pre-elution wash (EQ wash)	3	0.2	0.60
Elution	3.75	0.2	0.75
Sanitization (0.1N NaOH)	5	0.4	2.00
Re-Equilibration	3	0.2	0.60
Total	33.8	-	**10.38**

Cycle 11-19

Step	Volume (MV)	Residence Time (min)	Total Time (min)
Equilibration	3.0	0.2	0.6
Load	11.6	0.4	4.66
Wash 1(EQ wash)	1.43	0.4	0.57
Wash 2(Salt wash)	3	0.2	0.60
Pre-elution wash (EQ wash)	3	0.2	0.60
Elution	3.75	0.2	0.75
Sanitization (0.1N NaOH)	3	0.4	1.20
Re-Equilibration	3	0.2	0.60
Total	31.8	-	9.58

Protein A Membrane | A280 Chromatograms & High Reproducibility

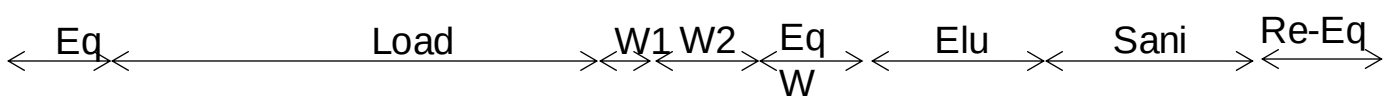
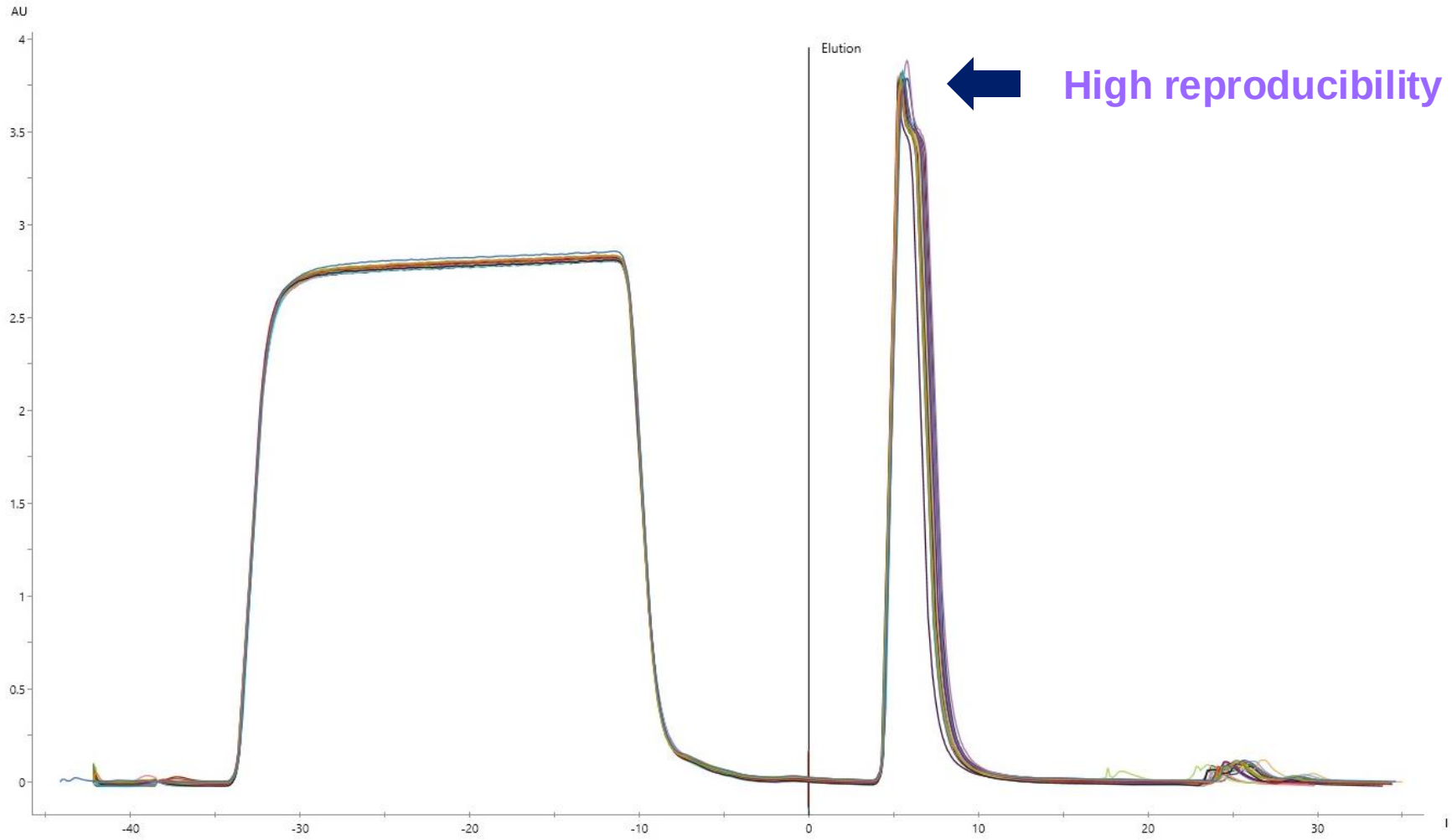
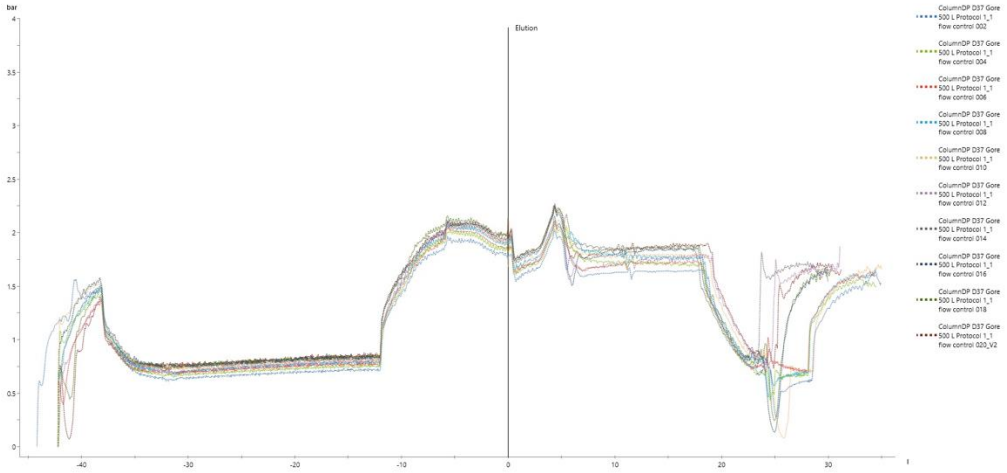


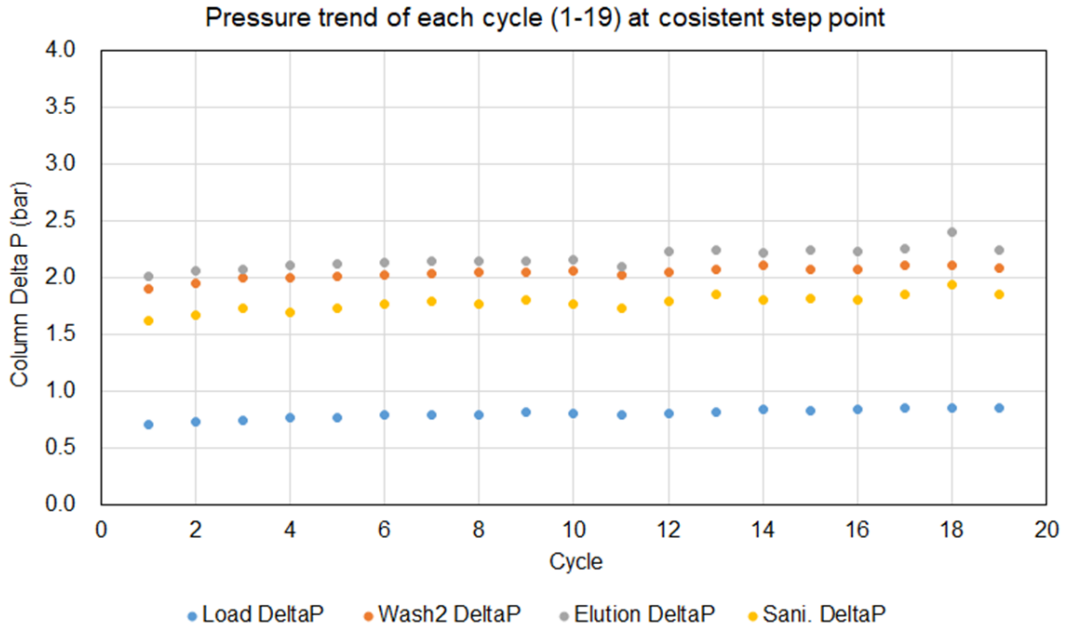
Diagram: *Overlay of A280 Chromatograms for each cycles 1-19*



High reproducibility from cycle to cycle

Protein A Membrane at 500L | Pressure Trends





Maximum operating pressure : 4 bar

Over 19 cycles:

- No clogging of the device occurred and there was no sudden increase in pressure
- There was no increase in pressure when Sani volumes were reduced (after Cycle 11)

Lab Scale Results: Protein A Resin vs. Gore Device Demonstrate Good Performance and Quality

	5 mL Protein A Resin column	3.5 mL Gore Device with Protein A	2 L Gore Device with Protein A
Productivity (g/L/h)	13.8	**960% increase** **132.8**	**132.6**
Average elution volume [100-100 mAU cutoff]	2.72	**1.84**	**2.68**
Elution HCP (LRV)	2.03	**2.12** **Similar performance**	**2.22**
Elution Protein A (ppm)	4.28	**5.25**	**2.49**
Average SEC Product Quality (% main/%HMW)	89.9 / 10.1	**93.3 / 6.7**	**93.9 / 6.1**

Notes:

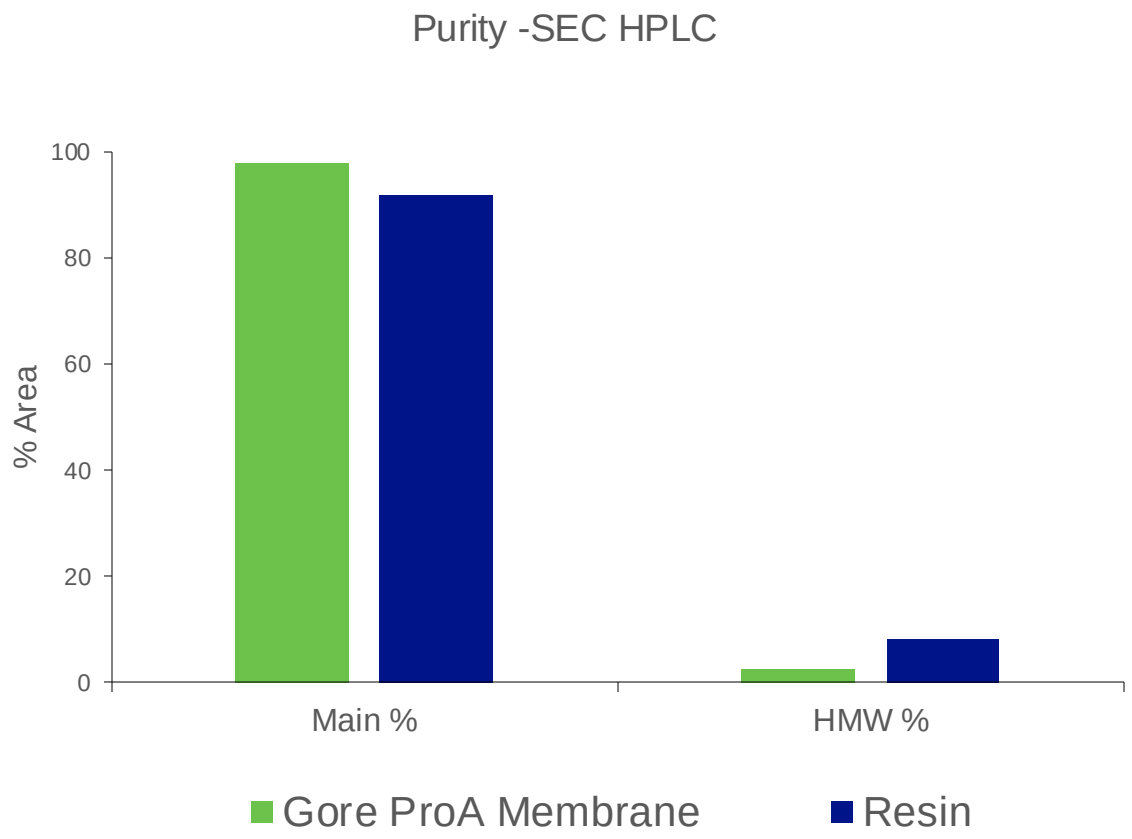
Cell culture harvest purified with laboratory-scale columns to provide benchmark performance targets & scaling reference for the Gore Protein A membrane device.

Productivity calculations indicate a consistent **10X increase in productivity** for the Gore Protein A membrane devices compared to the resin column.

Chromatographic performance and process and product impurity data were comparable between the two Gore Protein A membrane devices and the Protein A resin control.

Consistent scaling in membrane makes the process **easy to transfer across sizes** based on residence time

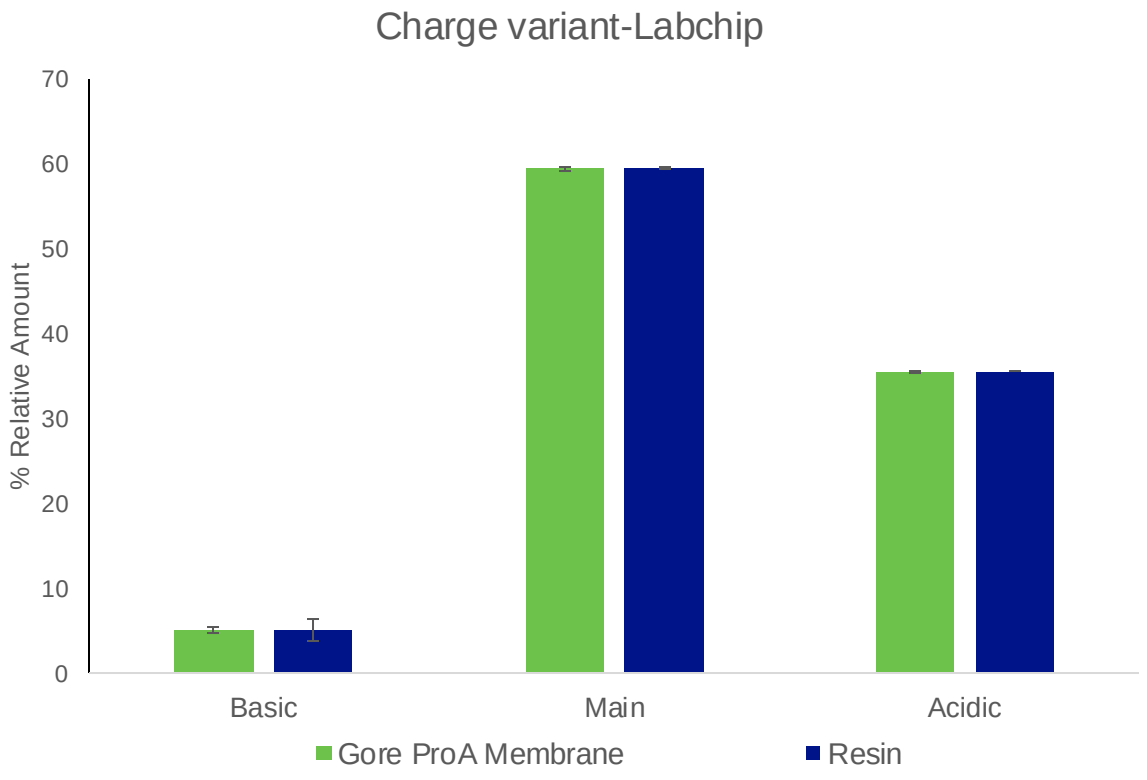
Demonstrating Similarity Membrane to Resin – Lab Scale



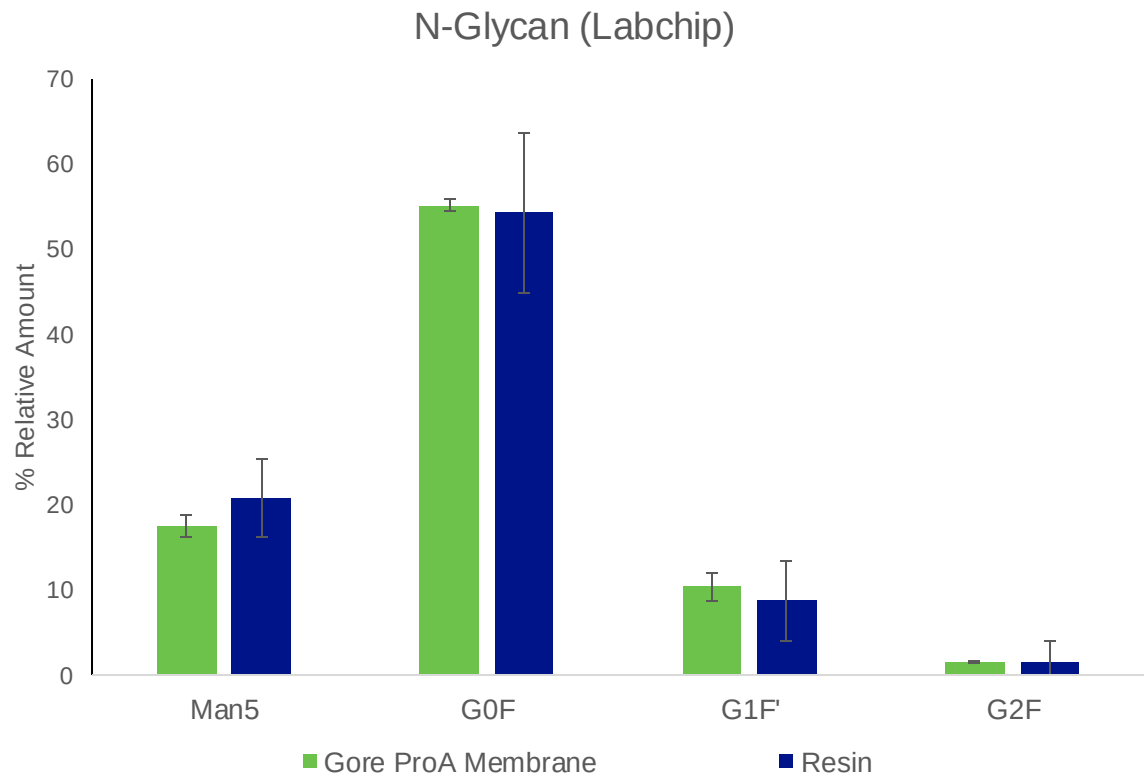
- Eluent Average SEC Product Quality distributions for laboratory scale Protein A resin column and Gore Protein A membrane device
- LMW species, indicative of protein fragmentation, were typically < 0.04 area percent in all cases

Demonstrating Similarity Membrane to Resin – Lab Scale

Eluent Charge Variant distributions for Laboratory scale Protein A resin column and Gore Protein A membrane device*.



Eluent n-glycan distributions for Laboratory scale Protein A resin column and Gore Protein A membrane device*.



*Error bars represent relative standard deviation on replicate preparations

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Single-Use Downstream Performance Characterization Summary

Step	Step Yield (%)	Step Productivity	HCP		Residual Protein A [ppm]	Host Cell DNA	SEC-HPLC (%)	
			ppm	Step LRV		Step LRV	Main	HMW
Harvest (Filtration)	-	-	160,187	-	-	-	-	-
Protein A Affinity (Gore Protein A)	103.8	132.6 $g\ L^{-1}\ h^{-1}$	830	2.29	2.49	3.37	93.9	6.11
Viral Inactivation (Single-use Mixer)	-	-	915	-	2.36	-	92.6	7.37
Anion Exchange (Sartobind Q)	88.9	189.2 $g\ L^{-1}\ h^{-1}$	72	1.10	2.20	1.70	92.1	7.89
TFF: UF/DF (Pellicon Capsule)	104.4	72.0 $g\ m^{-2}\ h^{-1}$	99	-	2.42	-	93.9	6.05

Table summary:

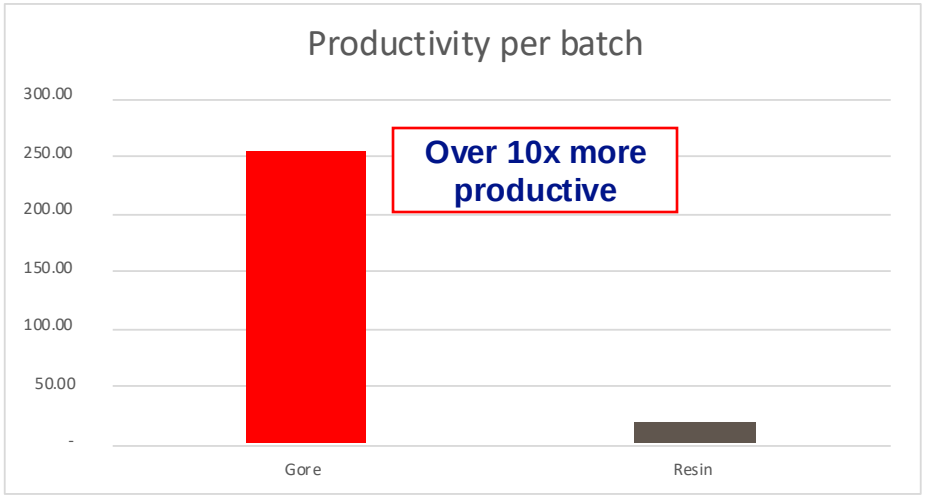
1. Step yields, step productivities, and pooled HCP, Protein A and SEC characterization data for the affinity capture, VI, AEX and TFF steps.
2. Comparison of step yield, productivity, and process/product quality data for the downstream unit operations. Purity data were analyzed from the final pool at each operation.

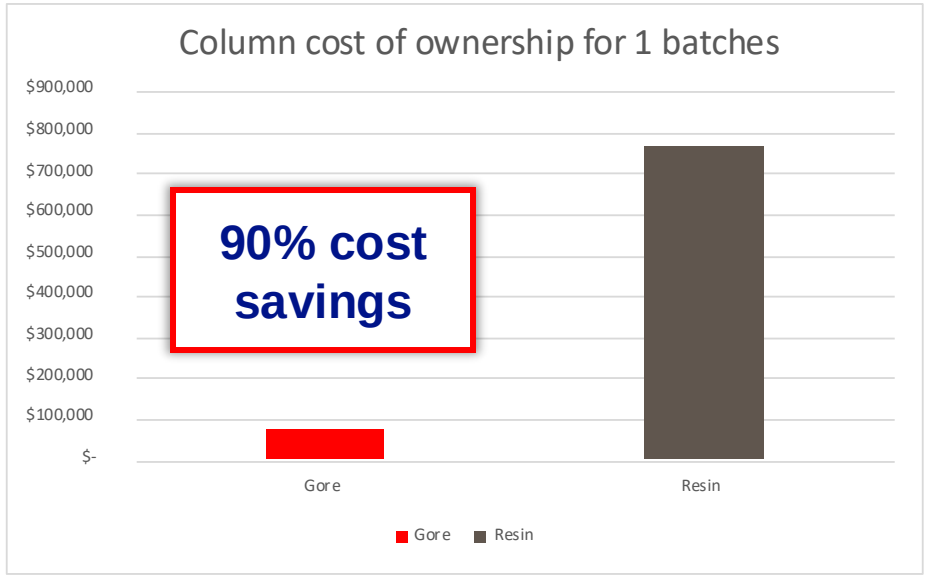
Potential for 90% Cost Savings, More Productivity and Faster Preparation

Assumptions made in calculations:

- 2,000 L Bioreactor | 5 g/L | **1** batch example | Resin: $18,000/L

	Resin Column	Gore Protein A Membrane Column	
Column size	32 L packed bed	2 x 1 L	**More efficient facility fit**
Cost for 1 batch	$767,900*	$76,400	
Residence/Step time	4 minute residence time	Rapid cycle (30 seconds load, 7 seconds operational, 30 seconds CIP)	
Cycles per batch	8 cycles/batch	157 cycles/batch	
Processing time	17.5 hours	19.6 hours	**Similar unit op. time**
Preparation time	32 hours* *Assumes full Resin packing of empty column (23 hours) + setup (6 hours)	2 hours	**94% faster preparation**
Storage cost	10% Resin cost	None	





*Pricing includes packing & prep costs and 20% additional resin to account for bed compression.

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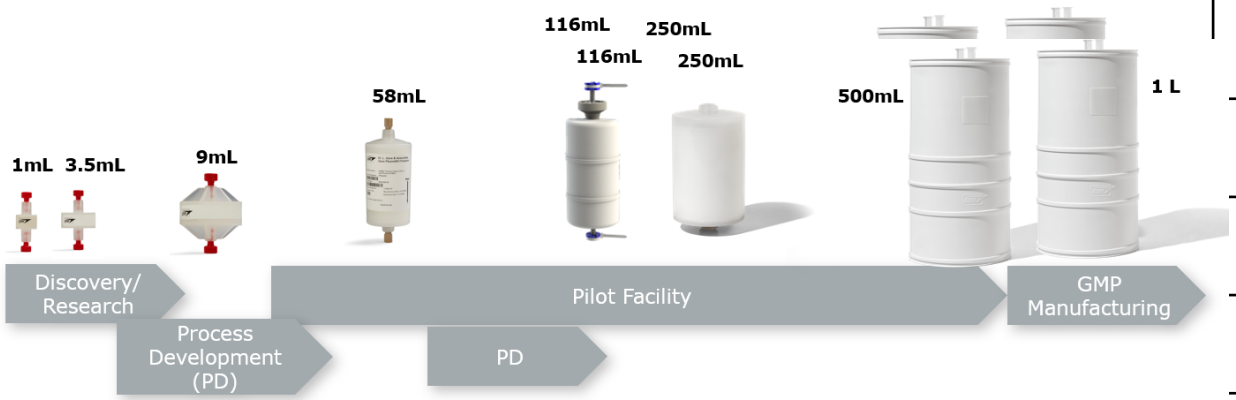
Scalability of GORE Protein Capture Device

Lab Scale to GMP (2000-L focus)



1mL	3.5mL	9mL	58mL	116mL	250mL	500mL	1L
R&D	R&D	PD	Pre-clinical	Pre-clinical & clinical	Pre-clinical & clinical	Pre-clinical & clinical	Pre-clinical & clinical
Early PD	Early PD	Late Phase R&D	Tox	Tox	Tox	Tox	Tox
		GMP	PD	PD	PD	PD	PD
			GMP	GMP	GMP	GMP	GMP

Bioreactor Batch Size (L)	Titer (g/L)	Gore Protein A Membrane Device Configuration	Cycles[2] Needed to Clear Bioreactor	Total Time per Cycle[3] (min)	CIP Time per Cycle (min)	Total Bioreactor Clearance Time (h)	Productivity (g/L/h)[4]
2000	1.5	1 L	94	14.8	0.5	23.2	130
				16.3	2	25.5	118
				17.3	3	27.1	111
2000	3	2 x 1 L parallel manifold	94	9.5	0.5	14.8	203
				11.0	2	17.2	175
				12.0	3	18.7	160
2000	6.4	4 x 1 L parallel manifold	100	6.7	0.5	11.1	289
				8.2	2	13.6	236
				9.2	3	15.3	210
2000	10	4 x 1 L parallel manifold	157	5.8	0.5	15.0	334
				7.3	2	18.9	265
				8.3	3	21.5	233
				10.3	3	26.7	187

[1]Assumes $DBC_{10\%}$ of 40 g/L at 30 SRT, loading to 80% of $DBC_{10\%}$ at 30 SRT, non-loading steps at 7 SRT.

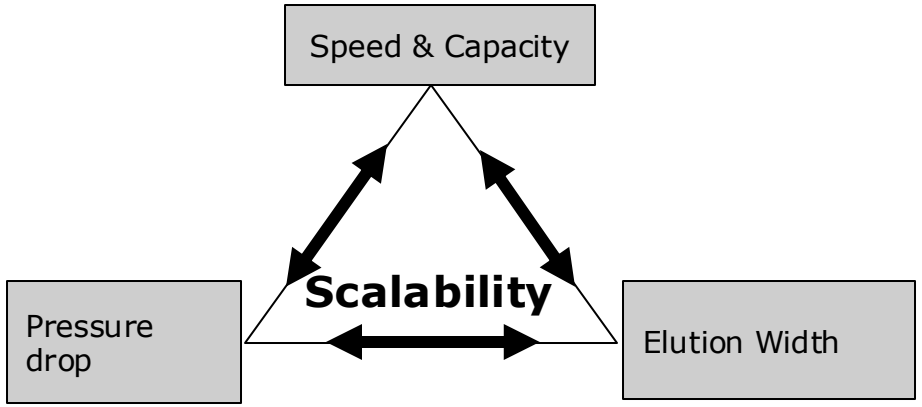
[2]GORE Protein Capture Devices with Protein A are validated to 100 cycles and demonstrated to 200 cycles.

[3]Range of total time per cycle is inherently higher for lower titers due to increased time needed for loading low titer harvest to 80% of $DBC_{10\%}$

[4]The Gore Productivity Calculator (Gore document PB11711) can be used to model a wide range of additional scenarios

Conclusions & Looking Forward

- Consistent device performance from 1mL to 1L
  - ✓ Elution widths
  - ✓ Cycling purity & yield
  - ✓ Elution widths
  - ✓ Low pressure drop



Increase productivity through High binding capacity, short residence time, and low pressure drop.

THANK YOU

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W. L. Gore & Associates, Inc.

For more information:

bbarrett@wlgore.com

- Thanks to AGC Biologics Bothell and Longmont Teams

Together, improving life