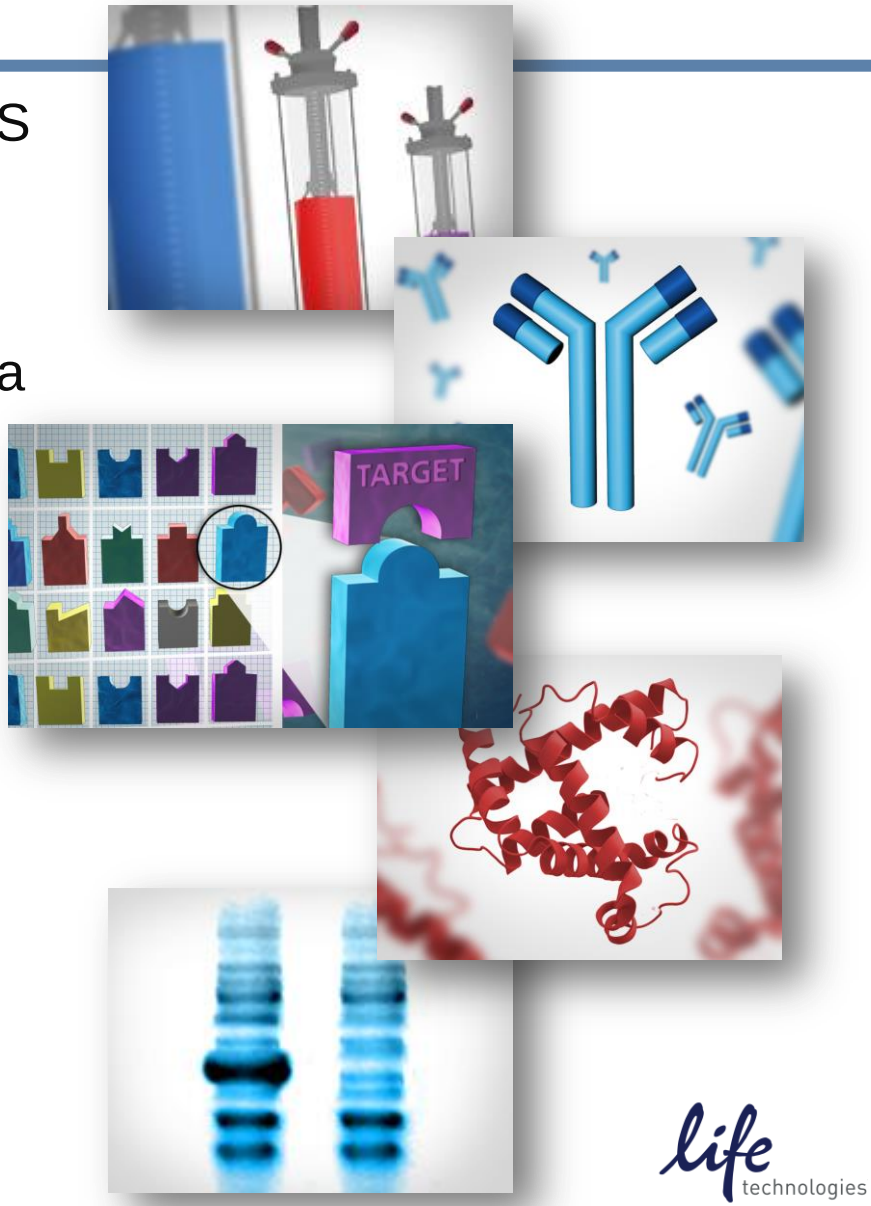
POROS® CEX Slides: HS and XS

April 2014

Overview

- CEX introduction: POROS® HS and XS
  - **There are lots of slides that have duplicate information. You will need to chose the ones you would like to present.**
- Applications and Characterization Data
  - Applications
  - Viral clearance
  - Pressure vs flow curves
  - Particle size analysis
- How to optimize
- Case Studies:
  - POROS® HS process redesign
  - POROS® HS in HTP screening and scale up study
  - Rethinking polish applications: CEX in flow through/overload mode
- Summary



life technologies

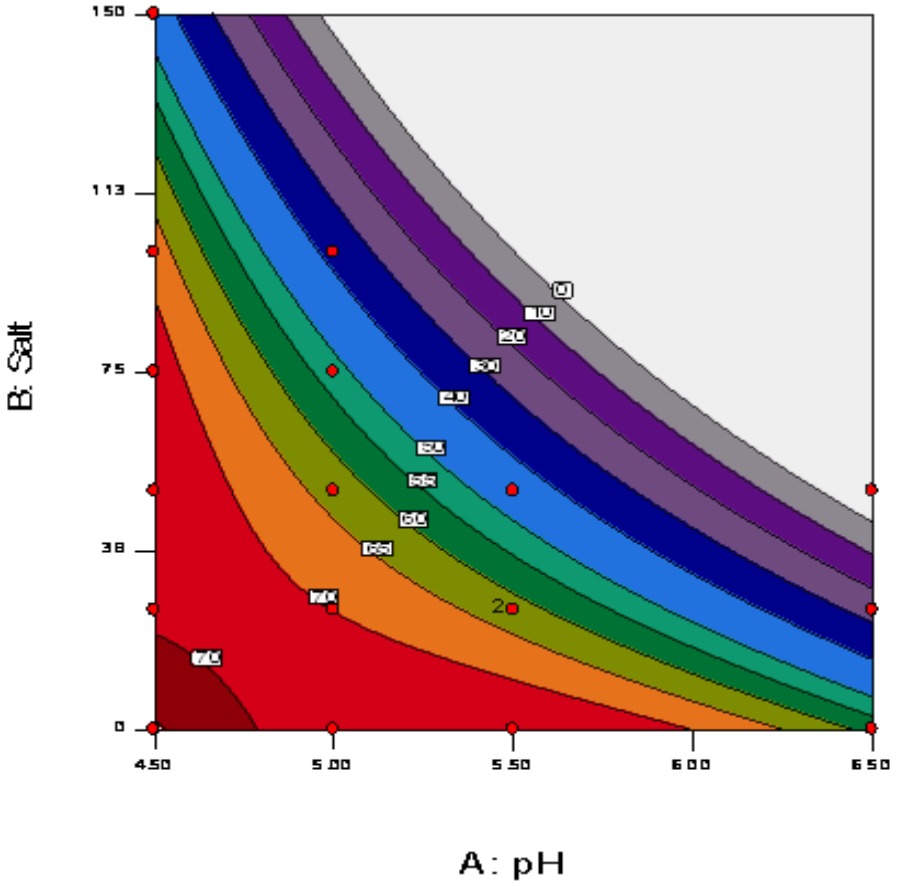
POROS® Process Chromatography Products

- **Cation Exchange**
  - HS 50 — Strong CEX
  - XS — Strong CEX
- **Anion Exchange**
  - HQ 50 — Strong AEX
  - PI 50 — Weak AEX
  - D 50 — Weak AEX
- **Affinity**
  - MabCapture A — rProtein A
  - A 50 — rProtein A (legacy)
  - Heparin — Heparin
  - OH50 — Activated Affinity

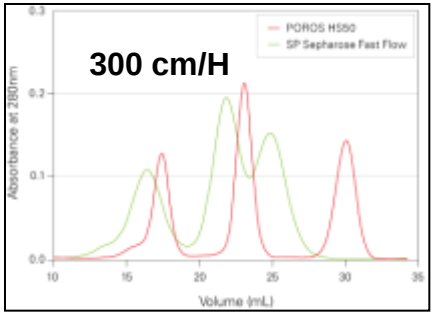
life technologies

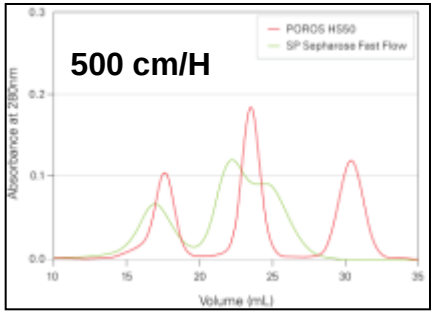
POROS® HS delivers optimal dynamic binding capacity with high resolution

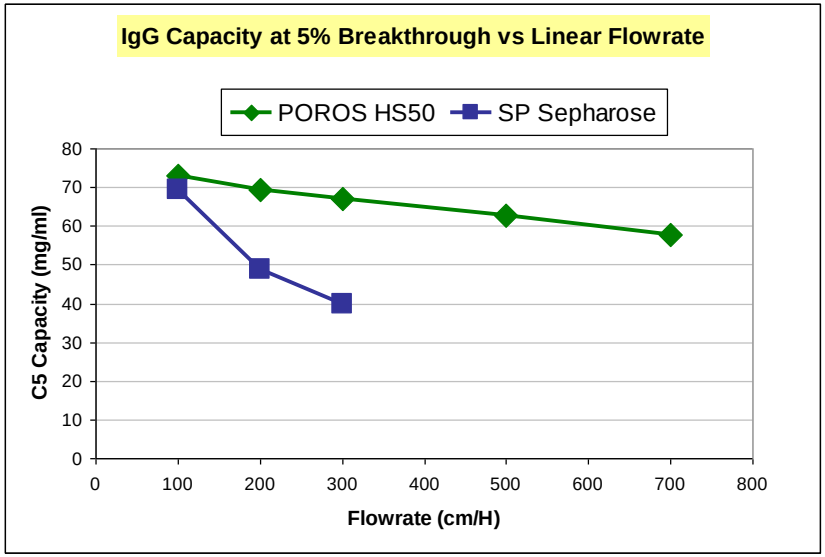
High dynamic binding capacity over a wide range of conditions



High resolution maintained as linear flow rate increases



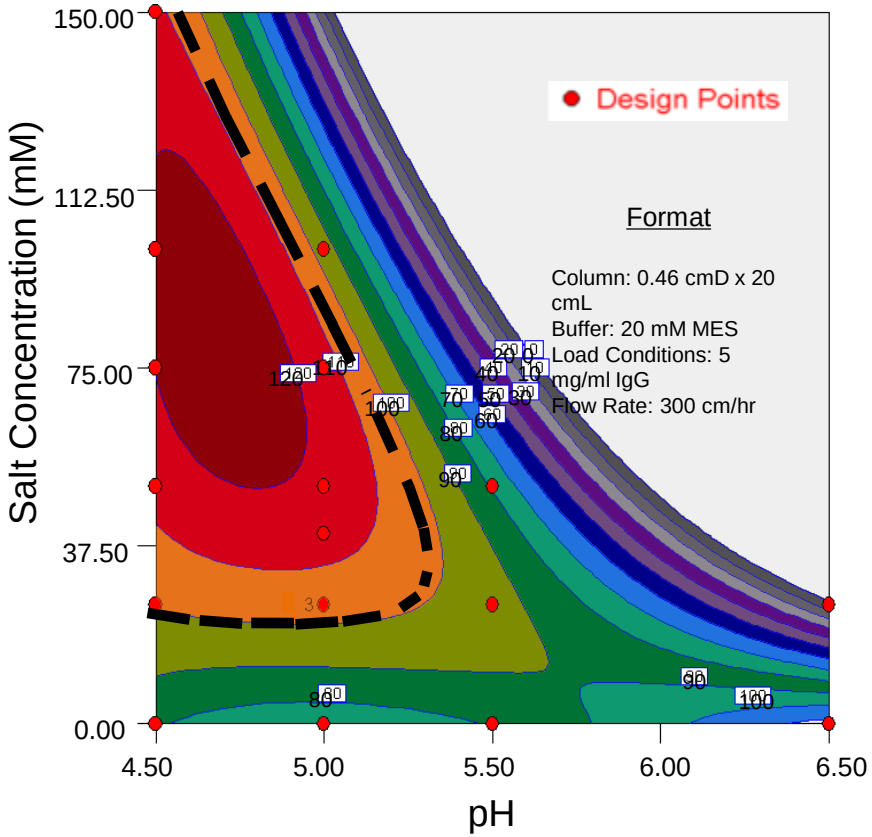




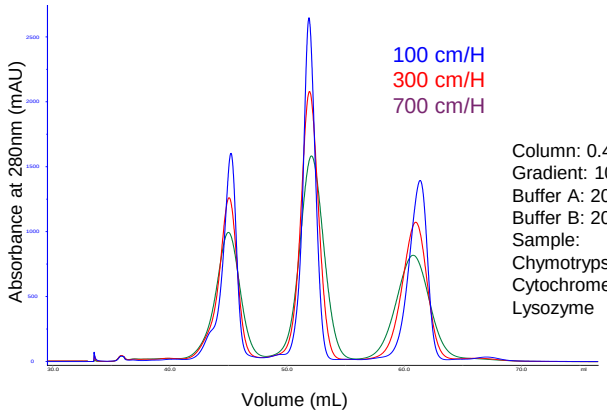
POROS® XS delivers optimal dynamic binding capacity with high resolution

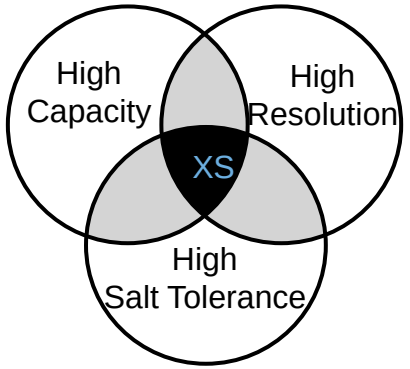
High capacity over a broad range of process conditions

IgG Binding Capacity vs. Salt Concentration and pH
Capacity at 5% Breakthrough



High resolution maintained as linear flow rate increases

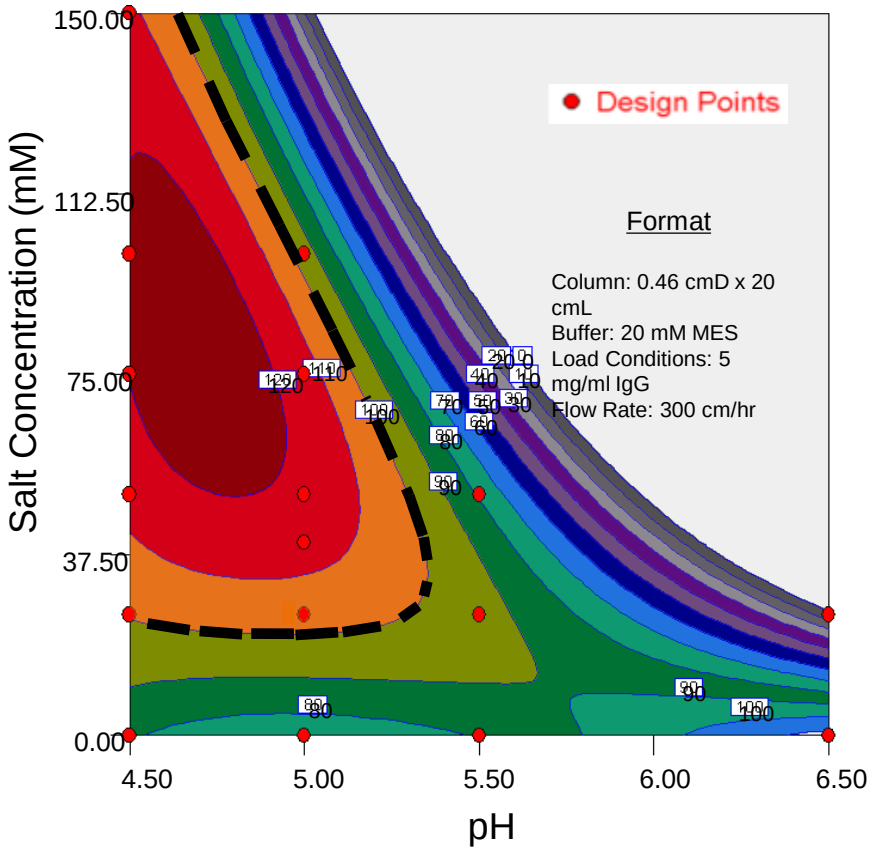




POROS® XS delivers optimal dynamic binding capacity with high resolution

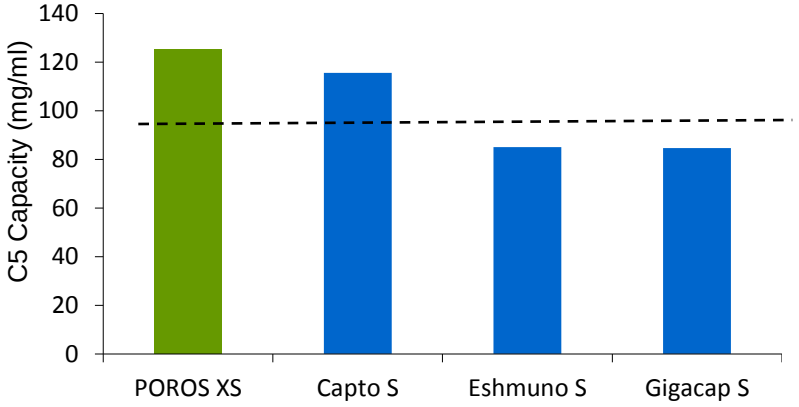
High capacity over a broad range of process conditions

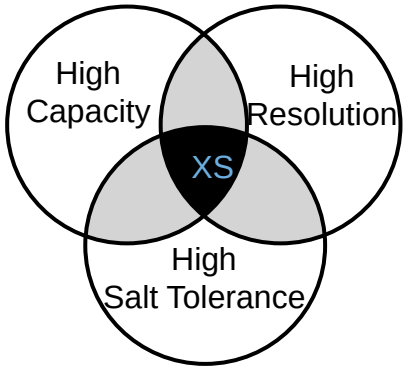
IgG Binding Capacity vs. Salt Concentration and pH
Capacity at 5% Breakthrough



High resolution maintained as linear flow rate increases

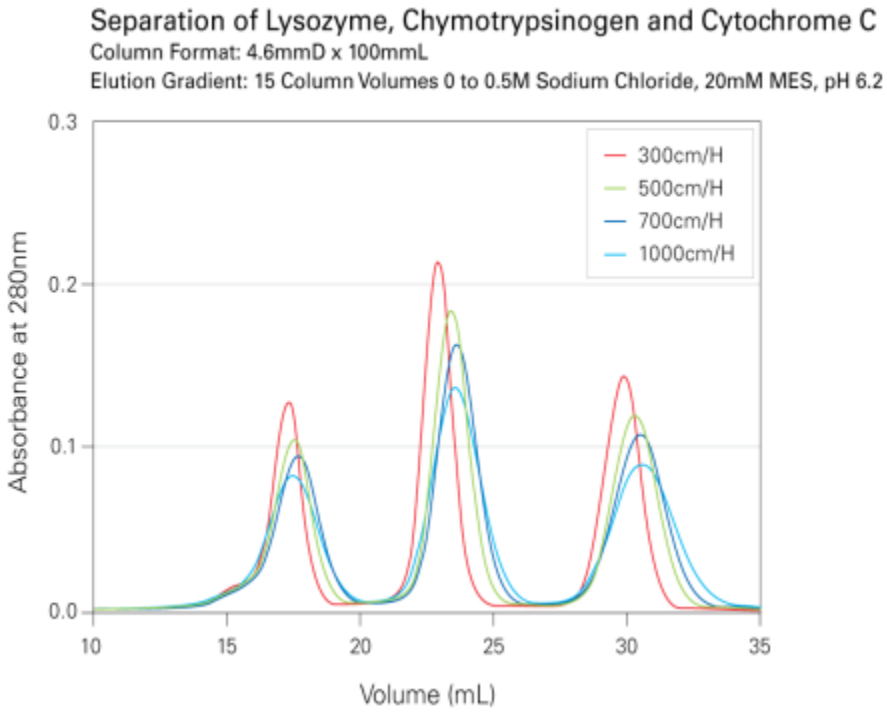
Capacity of Cation Exchange Resins:
100 mM NaCl, pH 4.5, 300 cm/H



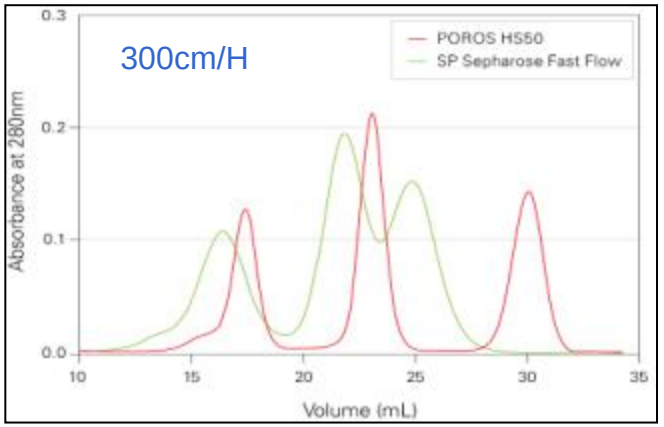


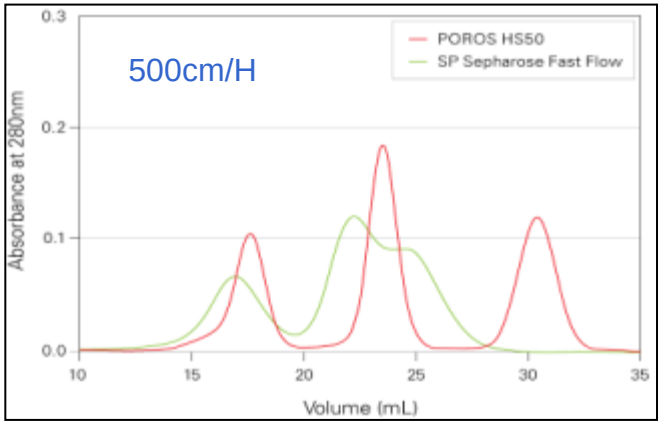
Superior Resolution Independent of Flow Rate

POROS® resins maintain resolution as flow rate increases



Conventional resins lose resolution as flow rate increases





Ion Exchange Chromatography

The workhorse of the purification process

IEX Resin Type	Mode of Operation	Key Applications
Cation Exchange	Bind/elute	Polish of many biomolecules (Mabs, VLP/viruses, fusion proteins, high pI rProteins)
	Overload/ Flow through	Polish for Mabs by binding impurities under normal B/E conditions: impurity removal (aggregates, HCP, DNA, viruses)
Anion Exchange	Bind/elute	Polish of many biomolecules (low pI rProteins, DNA, viruses, plasmids)
	Flow through	Polish for Mabs: binds impurities (DNA, viruses, HCP, aggregates, endotoxin)

POROS® XS
A High Performance Strong Cation Exchanger

- Design Goals:
  - Increase dynamic binding capacity of POROS HS
  - Retain all the beneficial performance attributes of POROS HS
    - resolution, pressure v flow, chemical stability and physical stability
  - Co-developed with industry based biopharma
    - Target flow rate 100-400 cm/hr
    - Increased salt tolerance
- Optimized base bead characteristics
  - Increased total pore volume
  - Decrease average pore size
  - Increased ligand density

POROS® XS vs HS

- The material of construction for the POROS XS and HS base beads is polystyrene-divinylbenzene. The sulphopropyl surface charge for both products is derived from the same negatively charged polymer. In addition, POROS XS and HS both have a 50um average particle size and very similar particle size distributions.
- In the development of POROS XS the based bead morphology and surface functionalization was optimized to drive higher IgG dynamic binding capacity under higher conductivity conditions (salt tolerance). In relation to HS, the average pore size for XS is lower (HS = 1600A and XS = 1100A) and the pore volume is higher (HS = x and XS = x + 18%). Using DOE, the sulphopropyl functionalization process was optimized to drive higher ligand density onto the bead surface and the pore morphology was optimized to yield the highest accessibility to the surface charge. Ligand density is assessed with an ionic capacity measurement. Having high accessible ionic capacity leads to high dynamic binding capacity and improved salt tolerance. The ionic capacity for POROS XS is 40-50% higher than the ionic capacity for POROS HS.

POROS® XS Chromatography Resin
Overview

POROS® XS Just Eclipsed All Other Cation Exchange Resins

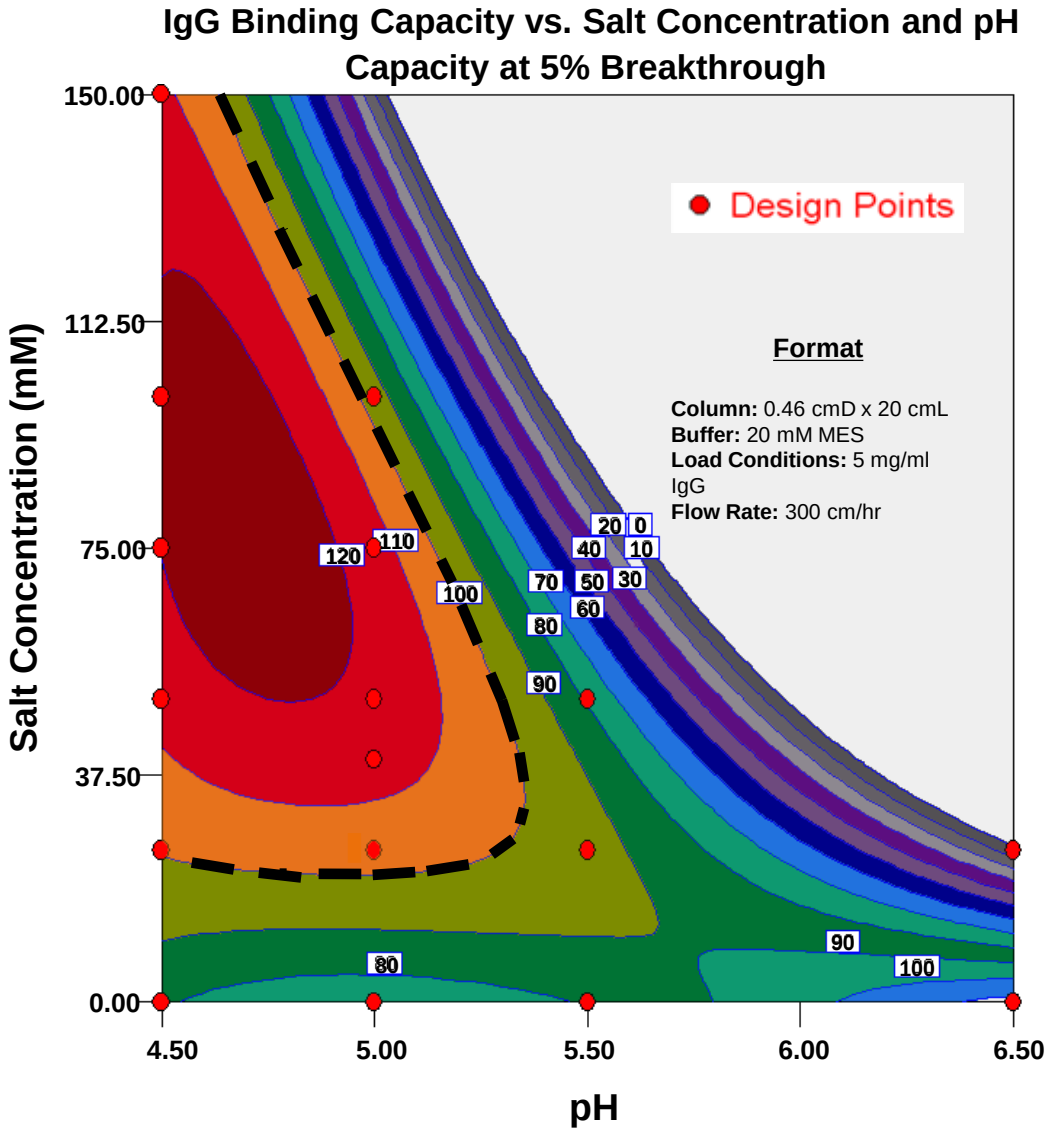
- Increased Process Flexibility and Throughput
- Improved Yield
- Smaller Column Sizes
- Linear and Predictable Scalability
- Easier Handling and Packing

Customer Requirements	XS Delivers
High capacity	>100 mg/mL 5% breakthrough capacity for monoclonal antibodies and recombinant proteins under a wide range of process conditions
High resolution	Optimized 50 µm particle size for improved impurity clearance and better yield
High salt-tolerant protein capacity	>100 mg/mL dynamic binding capacity at 15 mS/cm NaCl concentration
Low back pressure	2.0 bar at 800 cm/H in 20 cm length column
Rigid polymeric bead	Incompressible bead with robust physical stability
A high performance CEX resin that combines all of the above	New industry standard – major improvement on industry proven chromatography platform that provides:

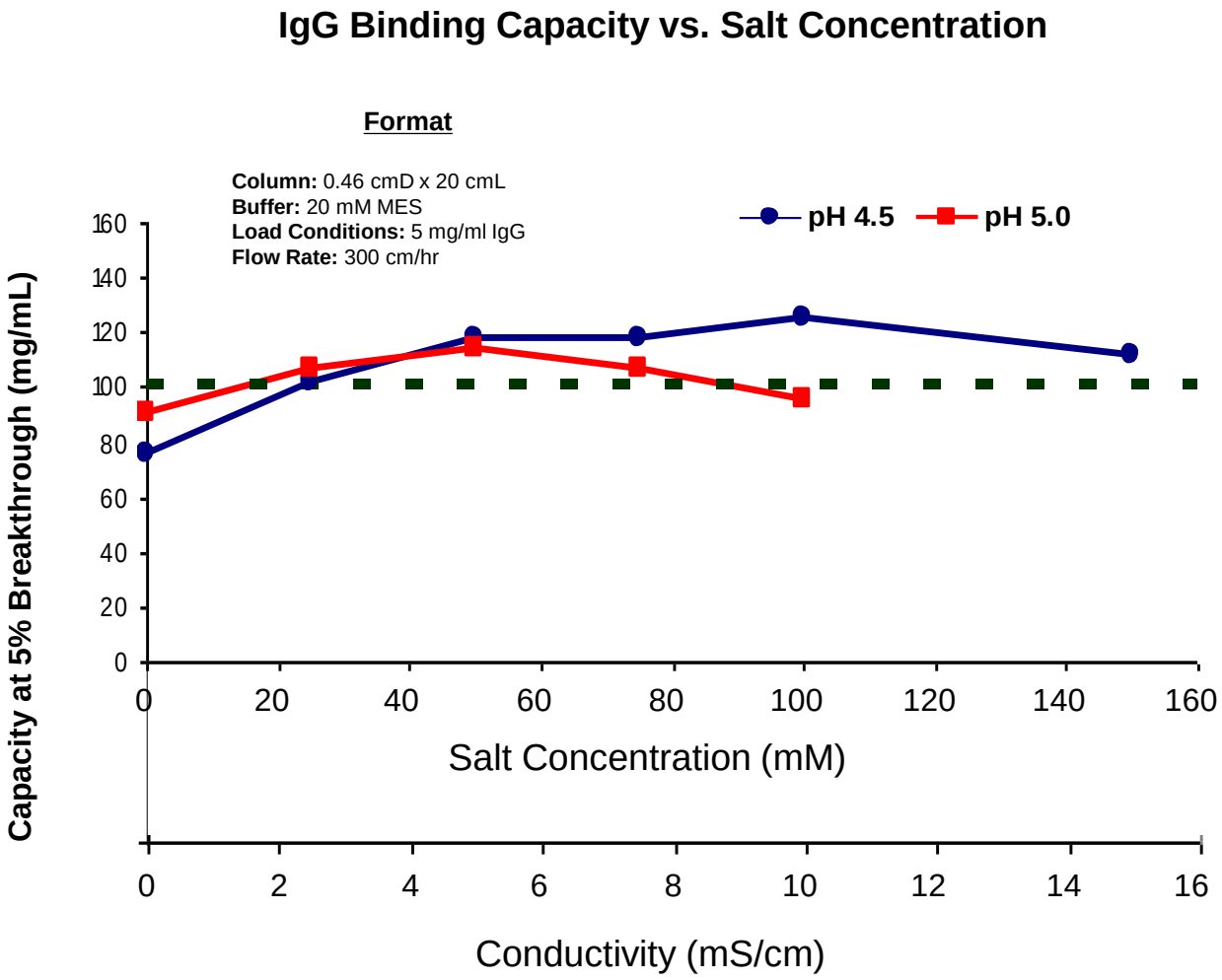
POROS® XS Chromatography Resin

Design space for optimal dynamic binding capacity

POROS® XS delivers high capacity over a broad range of process conditions



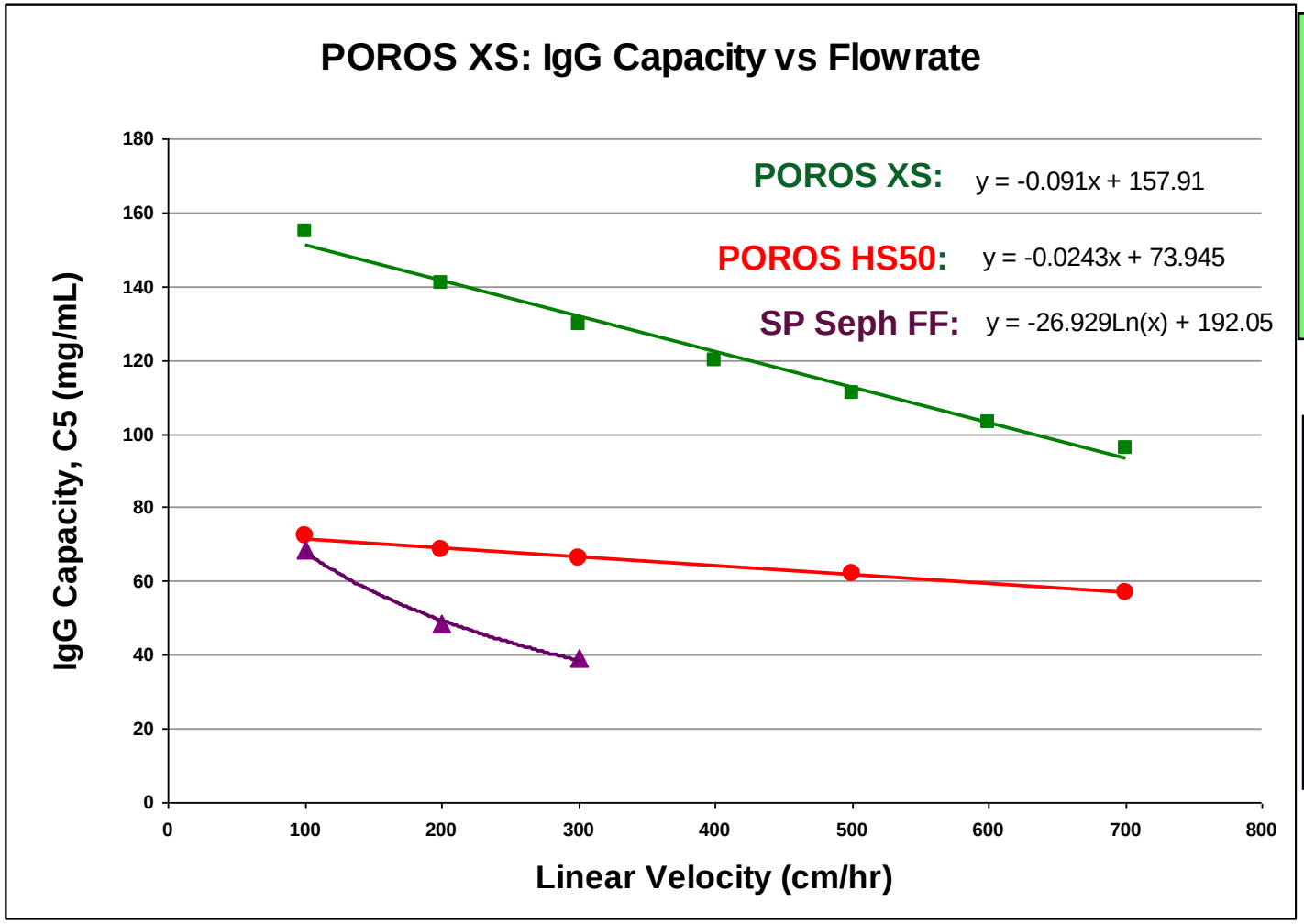
POROS® XS Chromatography Resin
Protein Capacity



- Achieved greater than 100 mg/ml capacity over a broad range of salt concentrations
- Increased salt tolerance for improved process flexibility

life technologies

POROS® XS IgG Binding Capacity with Increased Flow Rate



Format

Column: 0.46cmDx 20cmL

Buffer: 20mM MES, 40mM NaCl pH 5.0

Load Condition: 5 mg/ml Polyclonal IgG in Equil

Flow Rate: 300 cm/hr

Obtain high linear binding capacity over a large flow rate range

life technologies

POROS® XS Lysozyme Binding Capacity

POROS® XS has >100 mg/ml binding with different proteins with increased salt

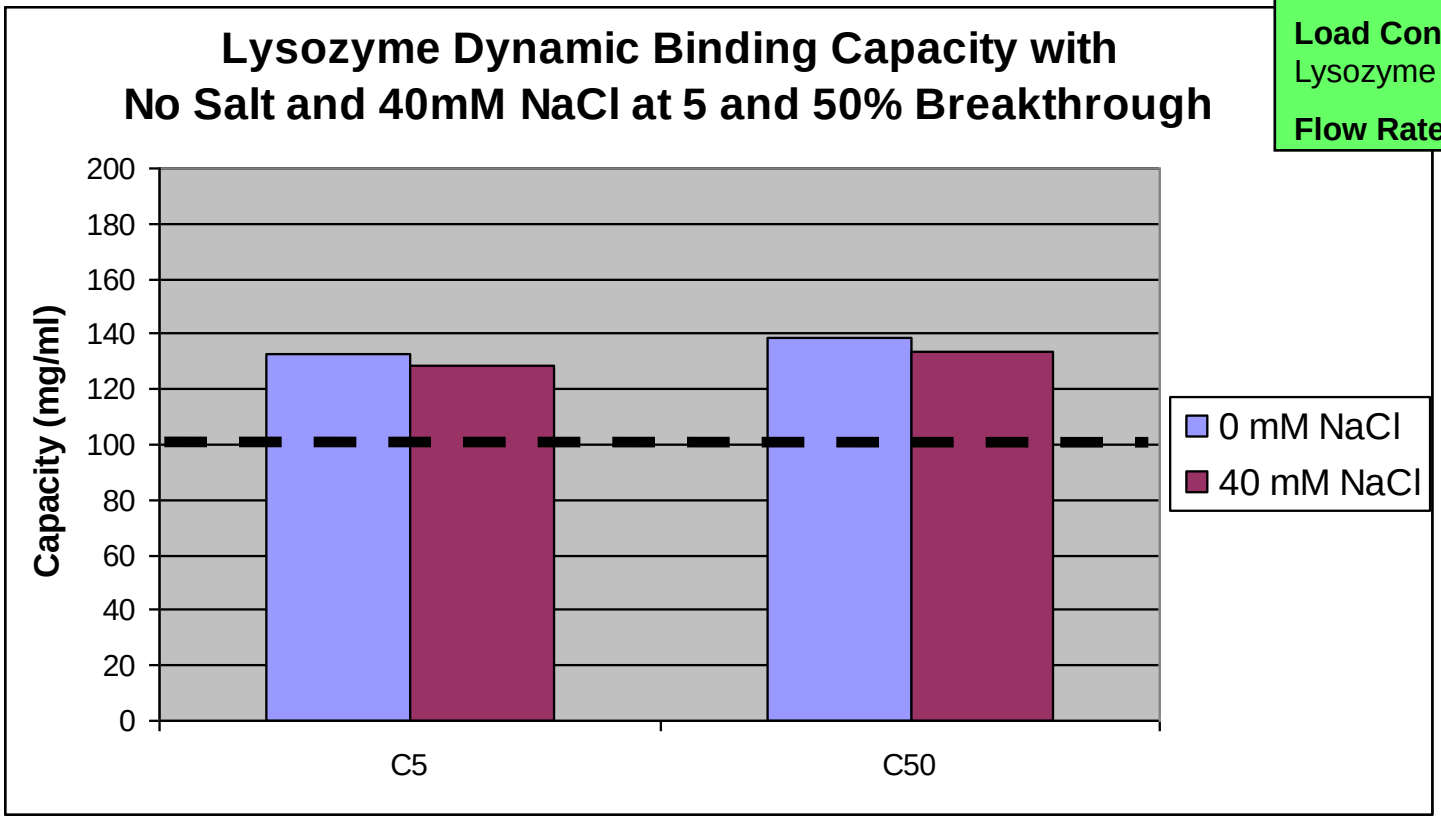
Format

Column: 0.46cmDx 20cmL

Buffer: 20mM MES pH 6.2

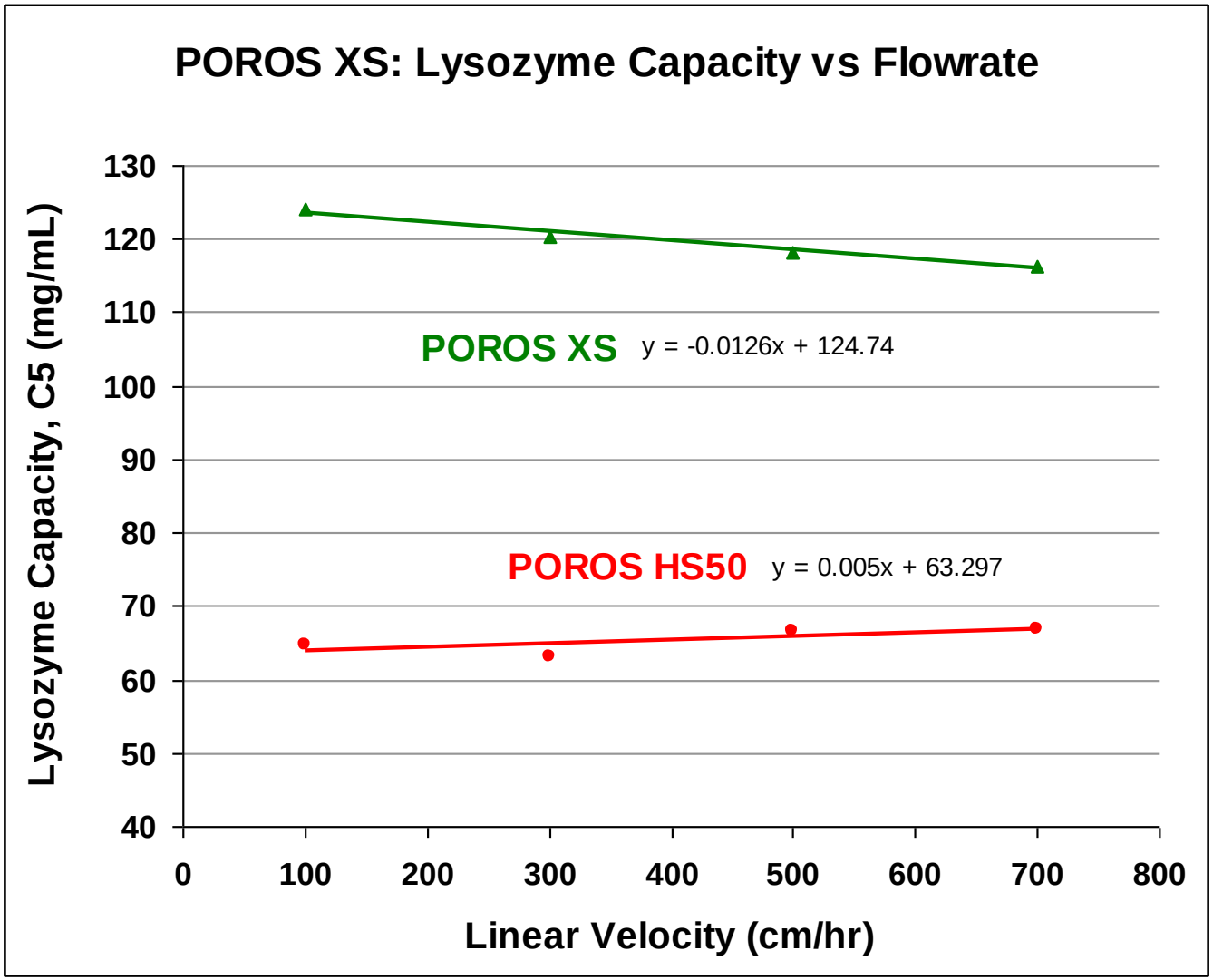
Load Condition: 7-8 mg/ml Lysozyme in Equil

Flow Rate: 300 cm/hr



life technologies

POROS® XS Lysozyme Binding Capacity with Increased Flow Rate



Format

Column: 0.46cmDx 20cmL

Buffer: 20mM MES pH 6.2

Conductivity: 1.1 ms/cm

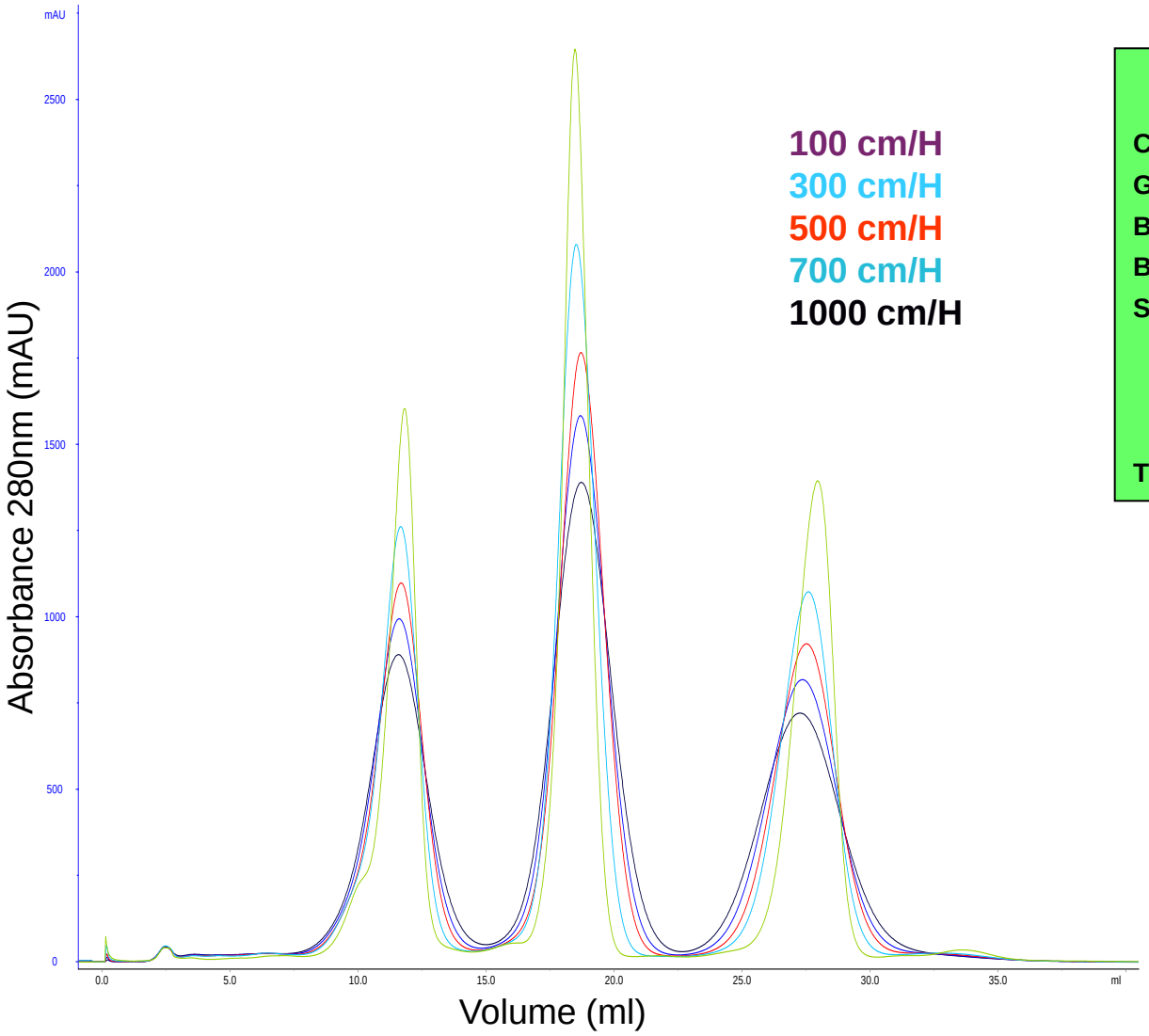
Load Condition: 3 mg/ml Lysozyme in Equil

Flow Rate: variable

Obtain high binding capacity over a large flow rate range

life technologies

POROS® Chromatography Resin
Product Attributes



Format

Column: 0.46cmDx 20cmL, 3.32 ml

Gradient: 10 – 100%B, 16 CV

Buffer A: 20 mM MES, 25mM NaCl pH 6.2

Buffer B: 20 mM MES/ 1.0M NaCl, pH 6.2

Sample:

Chymotrypsinogen A 5mg/mL, pI 9.4

Cytochrome C 8mg/mL, pI 10.4

Lysozyme 4mg/ml, pI 11.3

Total Protein Loaded: 5.175mg, 1.56mg/ml

Resolution is maintained on POROS® resin as linear flow rate increases

life technologies

POROS® XS Characterization

Protein separation compared to POROS® HS

Separation Conditions

Column = 1cmD x 20cmL

Buffer A = 20mM MES, 25mM NaCl pH 6.2

Buffer B = 20mM MES, 1M NaCl pH 6.2

Elution = 10% B – 50% B 7.5CV

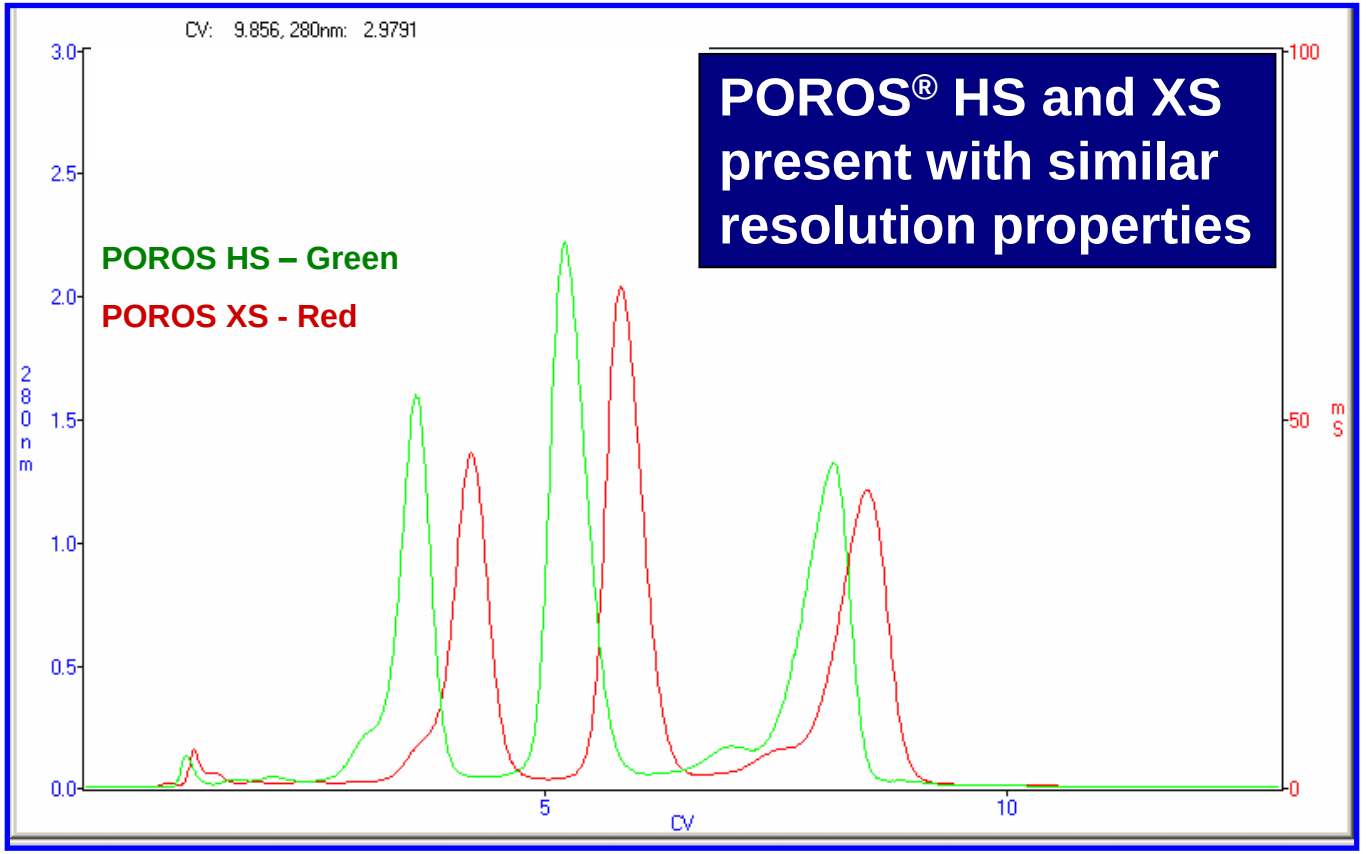
Flow Rate = 300cm/H

Protein Mix =

Chymotrypsinogen A

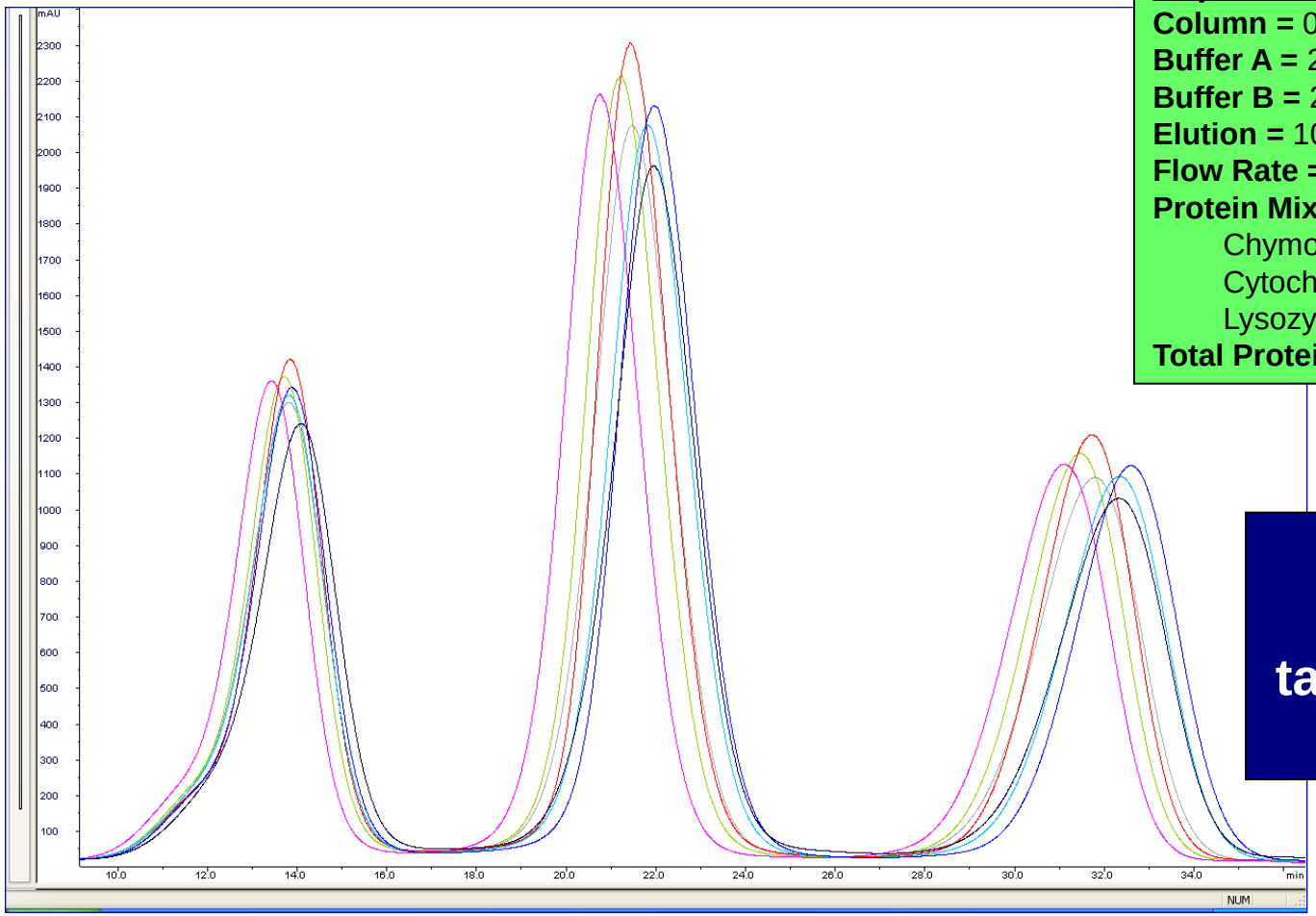
Cytochrome C

Lysozyme



POROS® XS Characterization

Protein separation across the Ionic Capacity range



Separation Conditions

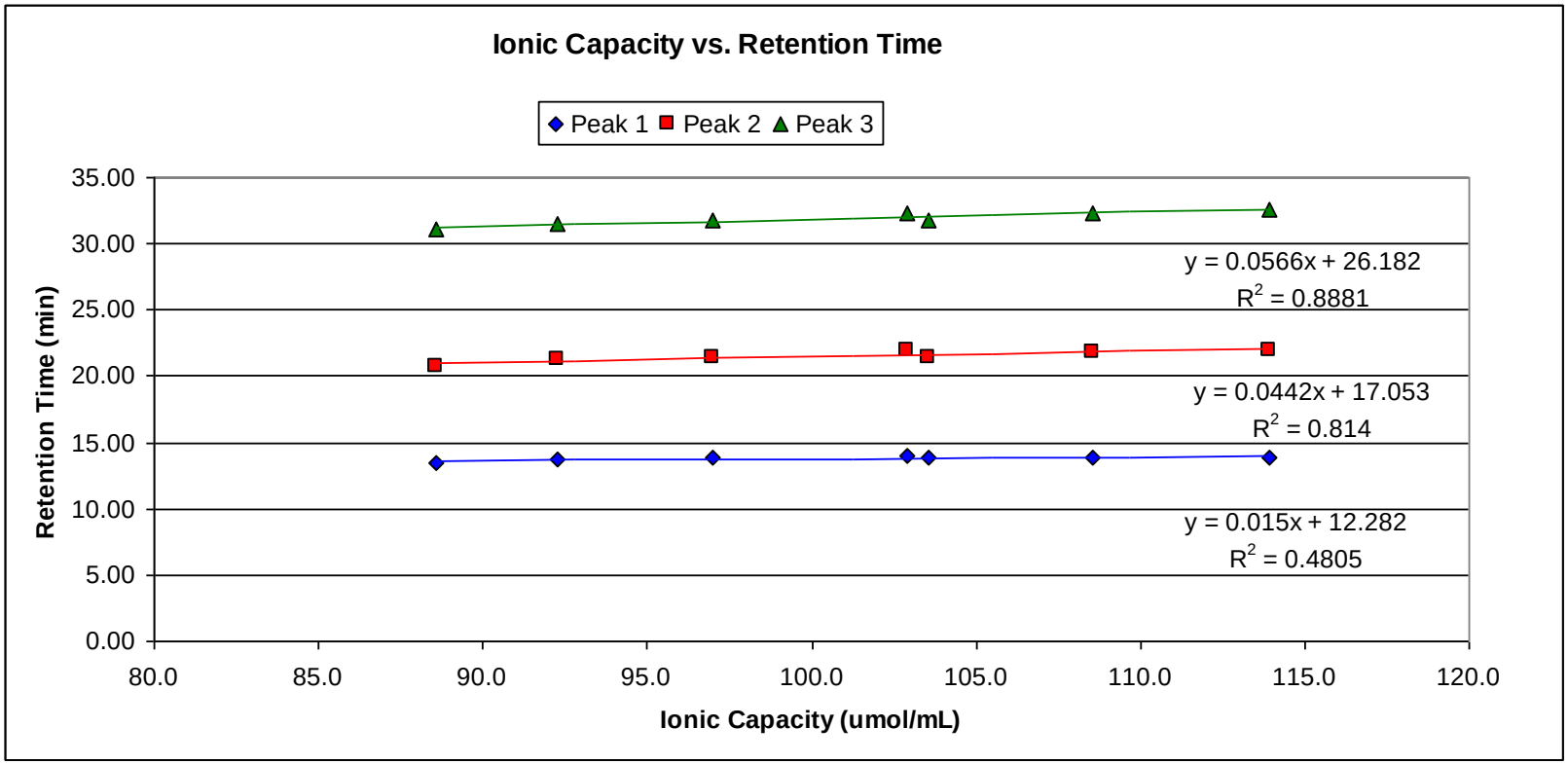
Column = 0.46cmD x 20cmL
Buffer A = 20mM MES, 25mM NaCl pH 6.2
Buffer B = 20mM MES, 1M NaCl pH 6.2
Elution = 10% B – 50% B 7.5CV
Flow Rate = 300cm/H
Protein Mix =
Chymotrypsinogen A 5mg/mL, pI 9.4
Cytochrome C 8mg/mL, pI 10.4
Lysozyme 4mg/ml, pI 11.3
Total Protein Loaded: 5.175mg

Reproducible separation over target ionic capacity range

life technologies

POROS® XS Characterization

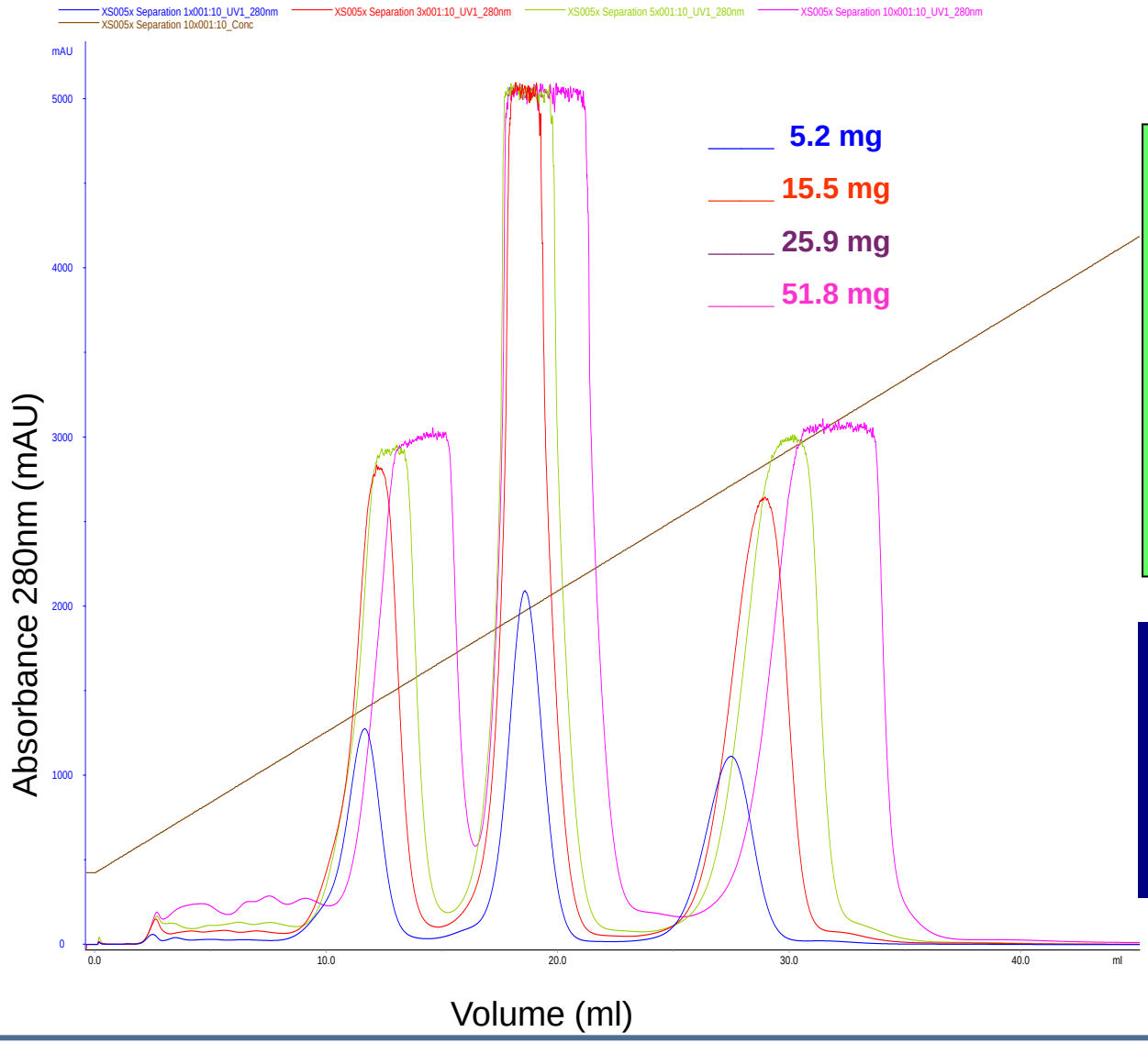
Protein separation across the Ionic Capacity range



Minimal change in retention time over target ionic capacity range

life technologies

POROS® XS Characterization
Separation vs Protein Load



Format

Instrument: AKTA Purifier 10

Column: 0.46cmDx 20cmL, 3.32 ml

Gradient: 10 – 100%B, 16 CV

Buffer A: 20 mM MES, 25mM NaCl pH 6.2

Buffer B: 20 mM MES/ 1.0M NaCl, pH 6.2

Sample:

Chymotrypsinogen A 5mg/mL, pI 9.4

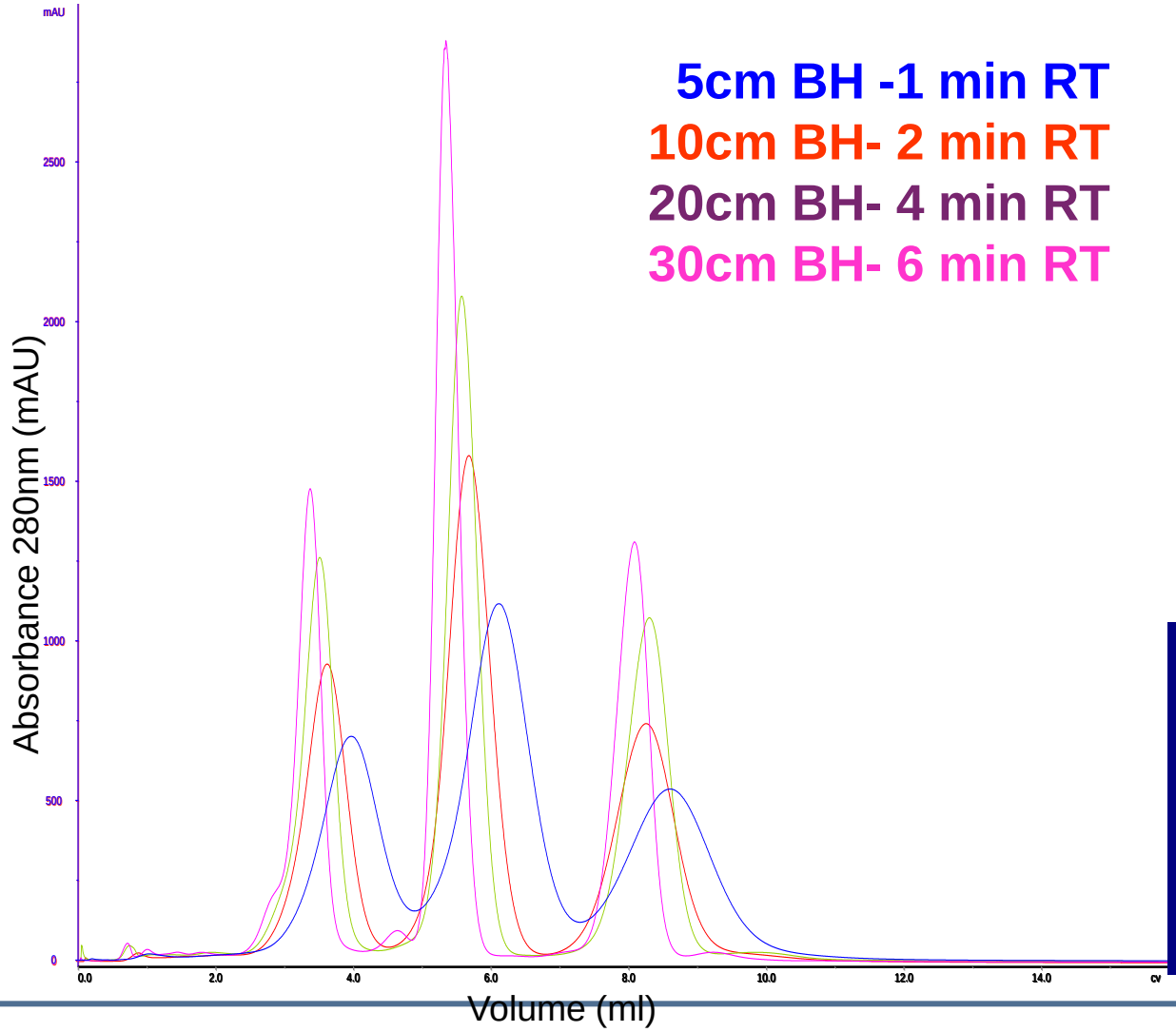
Cytochrome C 8mg/mL, pI 10.4

Lysozyme 4.25mg/ml, pI 11.3

Excellent resolution maintained even as load concentration is increased 10X

life technologies

POROS® XS Characterization
Separation vs Bed Height/Residence Time



5cm BH -1 min RT
10cm BH- 2 min RT
20cm BH- 4 min RT
30cm BH- 6 min RT

Format

Instrument: AKTA Purifier 10

Column: 0.46cmDx 20cmL, 3.32 ml

Gradient: 10 – 100%B, 16 CV

Buffer A: 20 mM MES, 25mM NaCl pH 6.2

Buffer B: 20 mM MES/ 1.0M NaCl, pH 6.2

Sample:

Chymotrypsinogen A 5mg/mL, pI 9.4

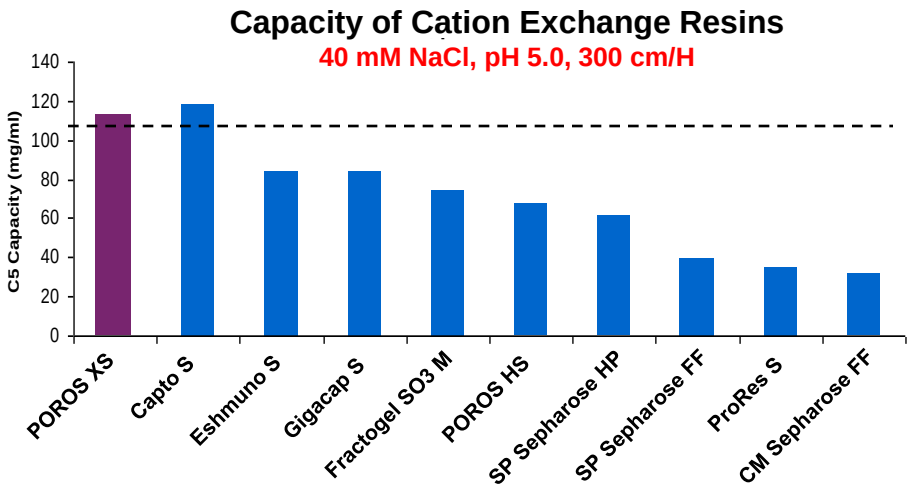
Cytochrome C 8mg/mL, pI 10.4

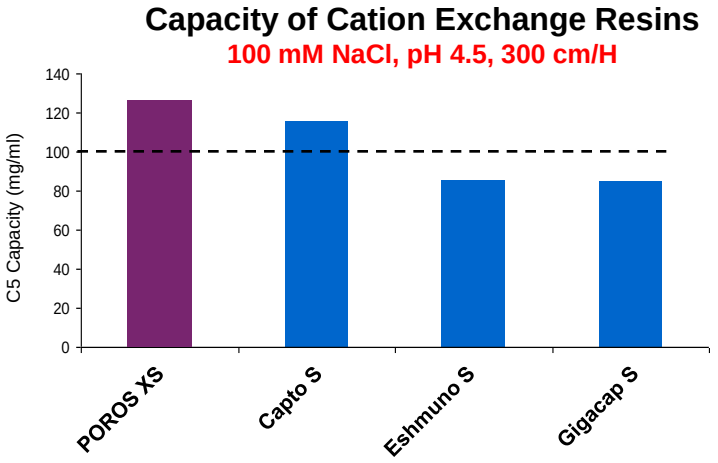
Lysozyme 4.25mg/ml, pI 11.3

Small scale results predict larger scale allowing for flexible scalability during design

POROS® XS Chromatography Resin

Setting Standards in Resolution and Capacity

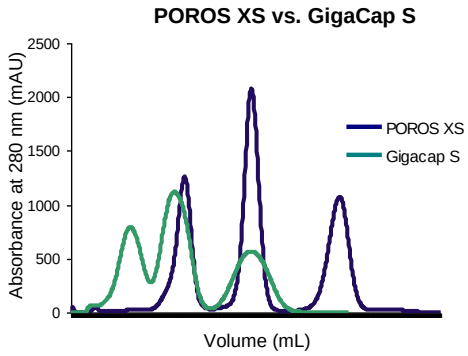


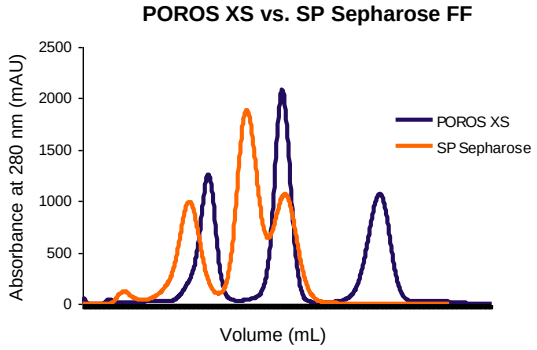


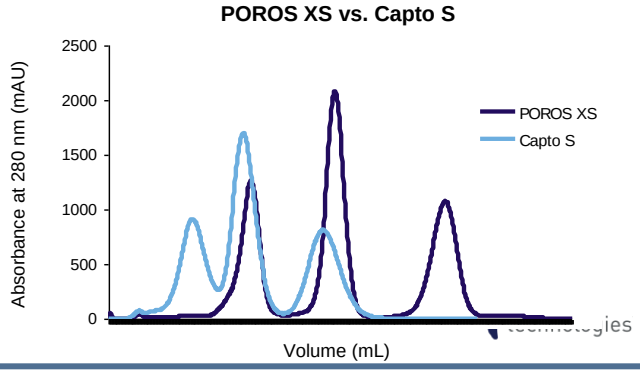
POROS® XS is the only CEX resin to successfully combine superior capacity, salt tolerance & resolution

Format

Column: 1cmD x 20cmL; **Buffer A**: 20mM MES, 25mM NaCl pH 6.2; **Buffer B**: 20mM MES, 1M NaCl pH 6.2;
Gradient: 10% B – 50% B 7.5CV; **Flow Rate:** 300cm/H; **Sample**: Chymotrypsinogen; Cytochrome C; Lysozyme







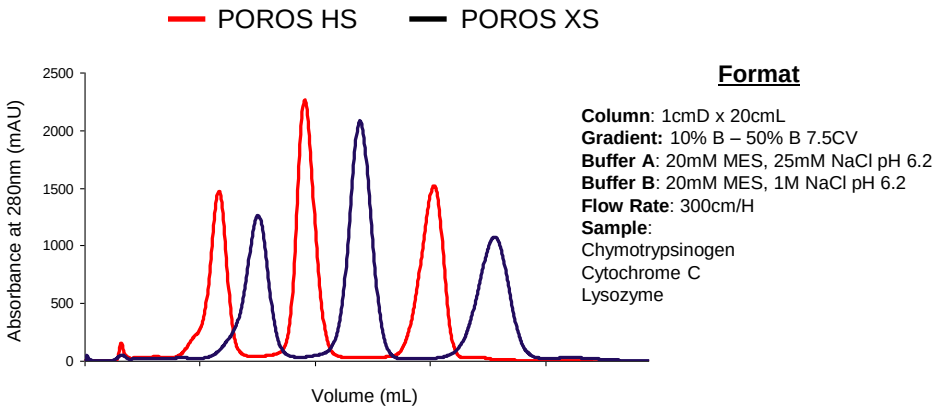
POROS® XS Chromatography Resin

Resolution

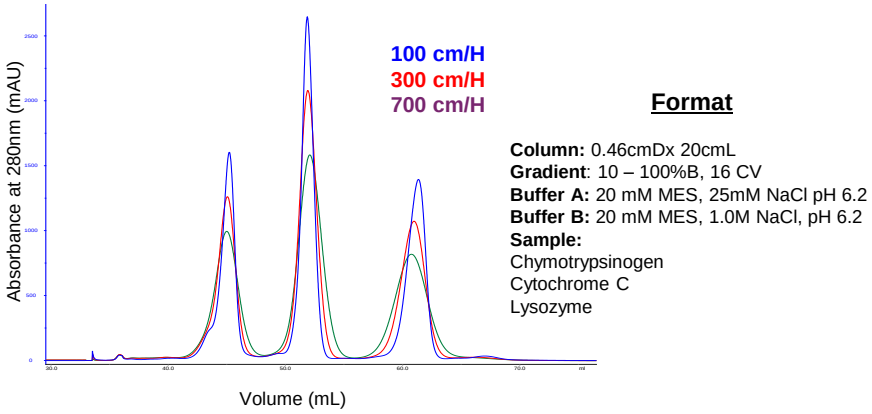
Superior resolution capabilities include:

- Maintaining the superior resolution of POROS® HS
- Providing consistent resolution across a wide range of flow rates
- Retaining excellent resolution through a ten-fold increase in load

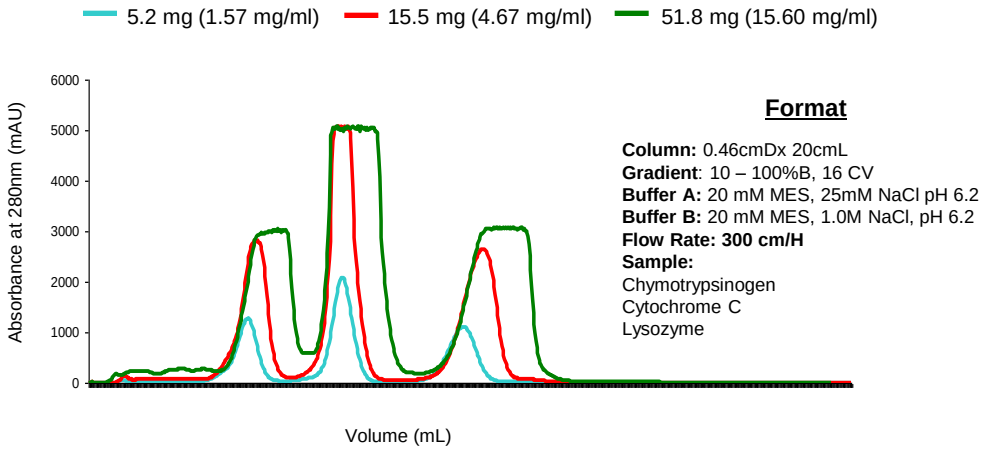
POROS® XS and POROS® HS deliver equivalent resolution performance



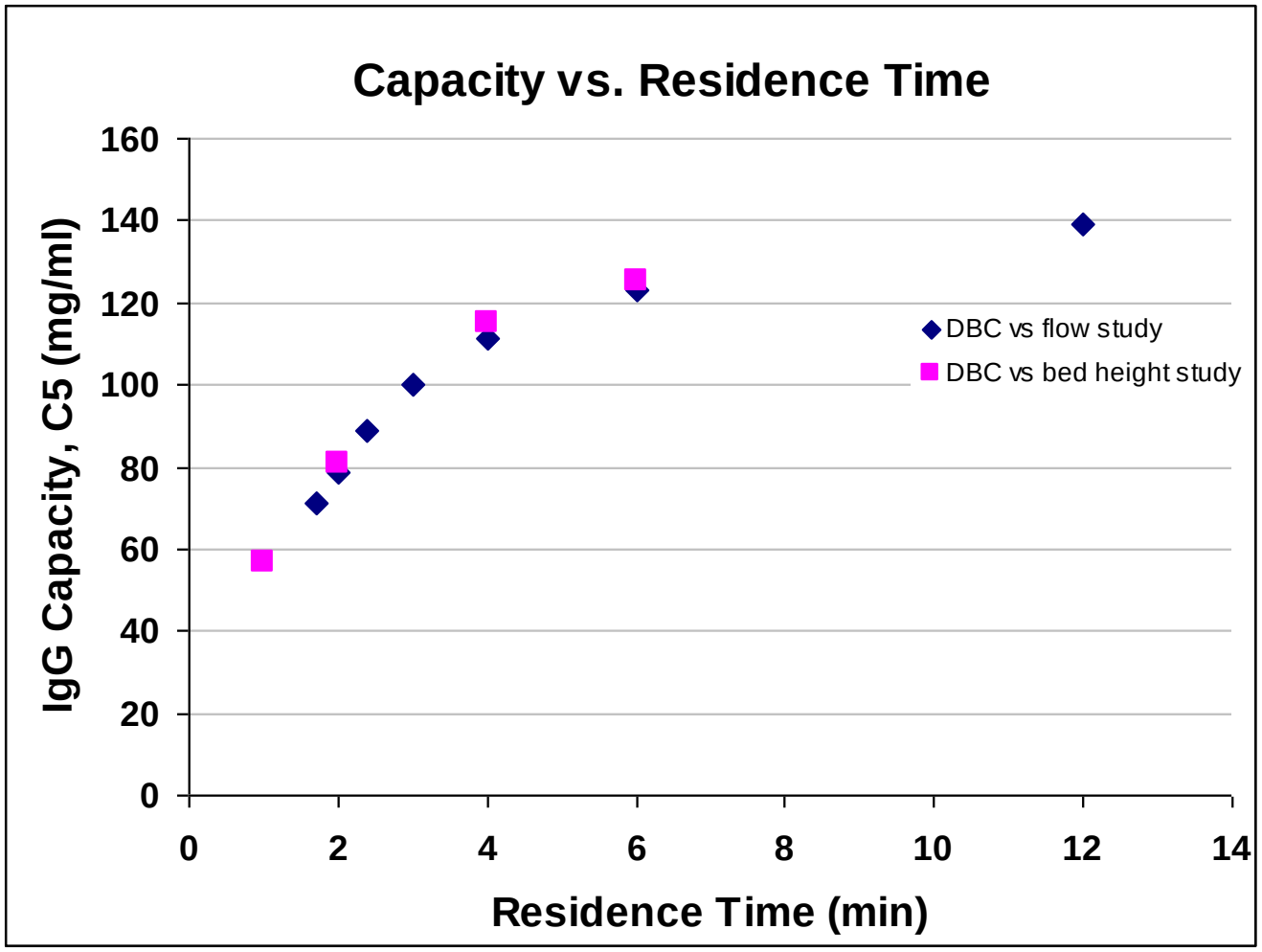
Excellent resolution is maintained as linear flow rate is increased.



Resolution is maintained even as load concentration is increased 10x.



POROS® XS IgG binding capacity vs residence time



Format

Column Diameter: 0.46cmD

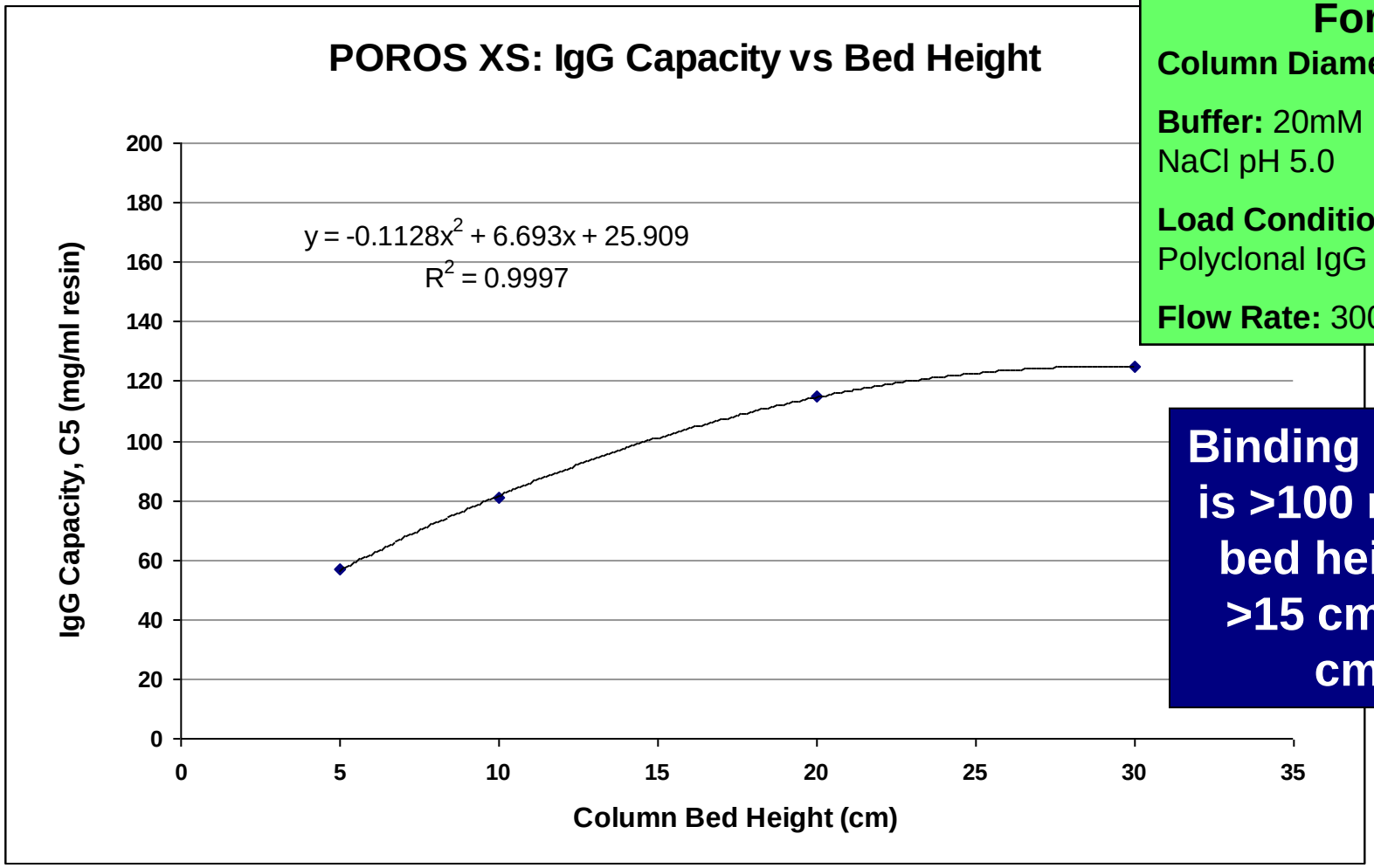
Buffer: 20mM MES, 40mM NaCl pH 5.0

Load Condition: 5 mg/ml Polyclonal IgG in Equil

Binding capacity is ≥100 mg/ml at a residence time of 3 minutes and greater

life technologies

POROS® XS IgG binding capacity vs bed height



Format

Column Diameter: 0.46cmD

Buffer: 20mM MES, 40mM NaCl pH 5.0

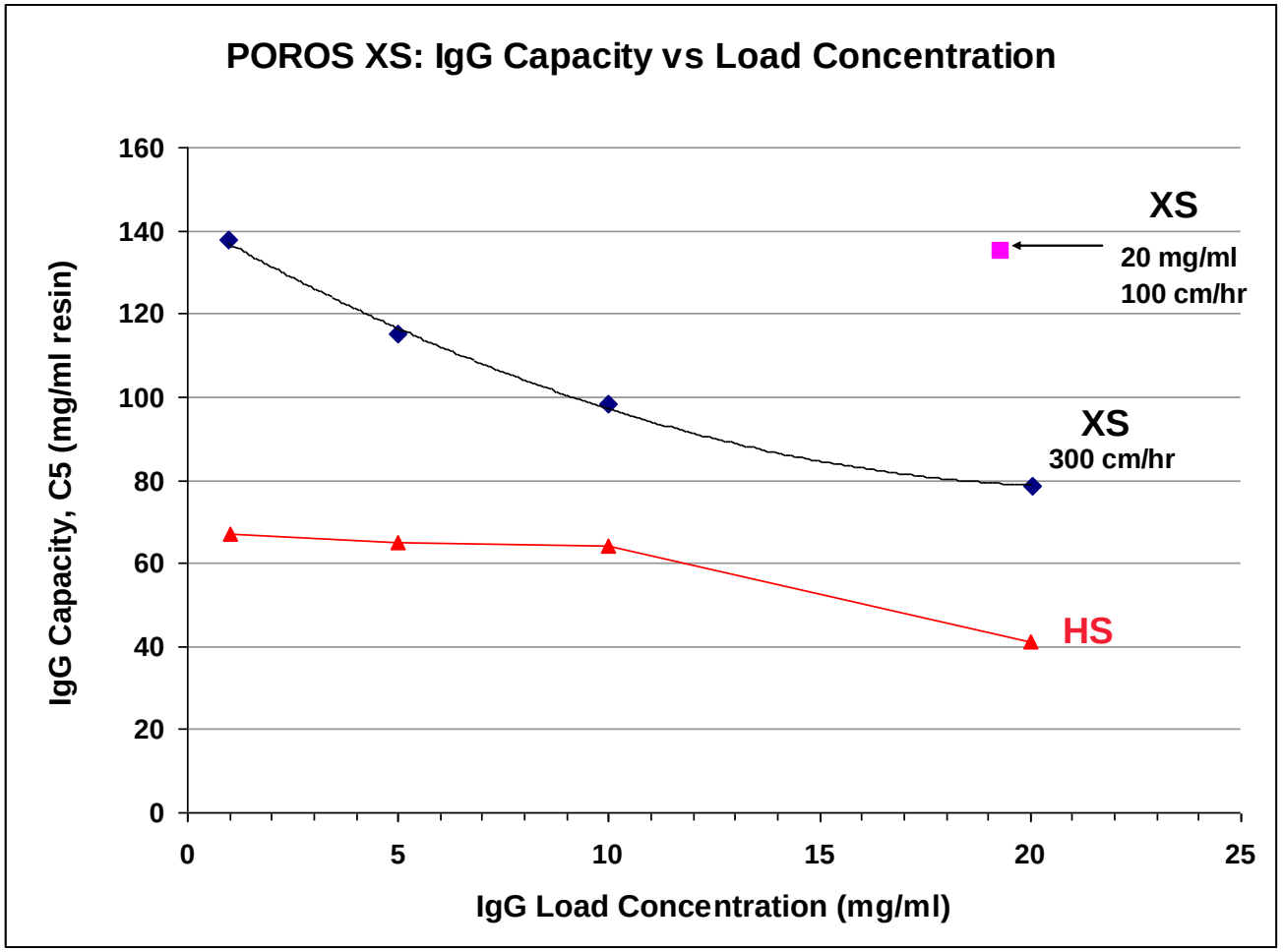
Load Condition: 5 mg/ml Polyclonal IgG in Equil

Flow Rate: 300 cm/hr

Binding Capacity is >100 mg/ml at bed heights of >15 cm at 300 cm/hr

life technologies

POROS® XS IgG capacity vs load concentration



Format

Column: 0.46cmDx 20cmL

Buffer: 20mM MES, 40mM NaCl pH 5.0

Load Condition: Polyclonal IgG in Equil

Flow Rate: 300 cm/hr

Binding Capacity is >80 mg/ml even at concentrations >20 mg/ml

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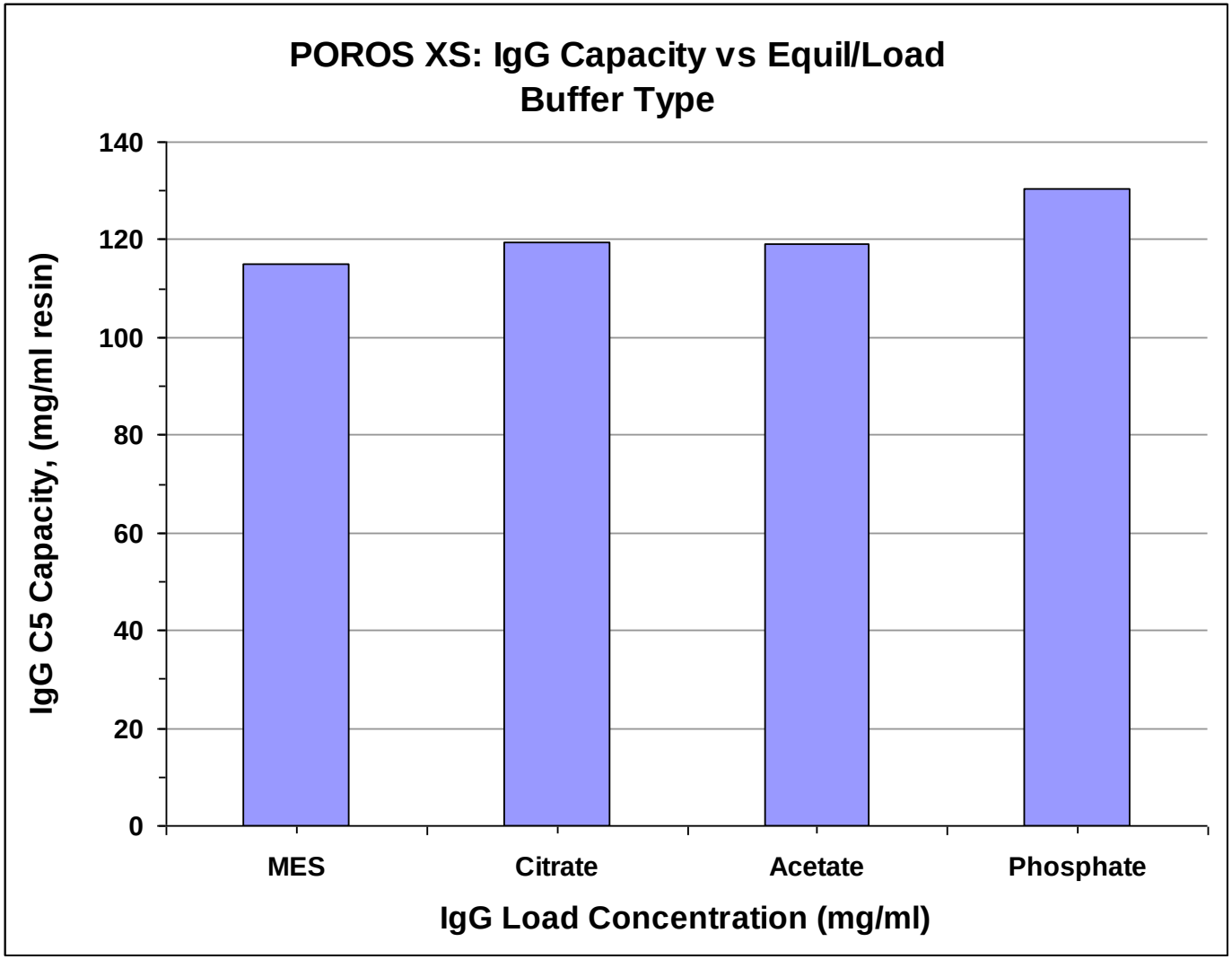
POROS® XS IgG Binding Capacity vs Buffer System



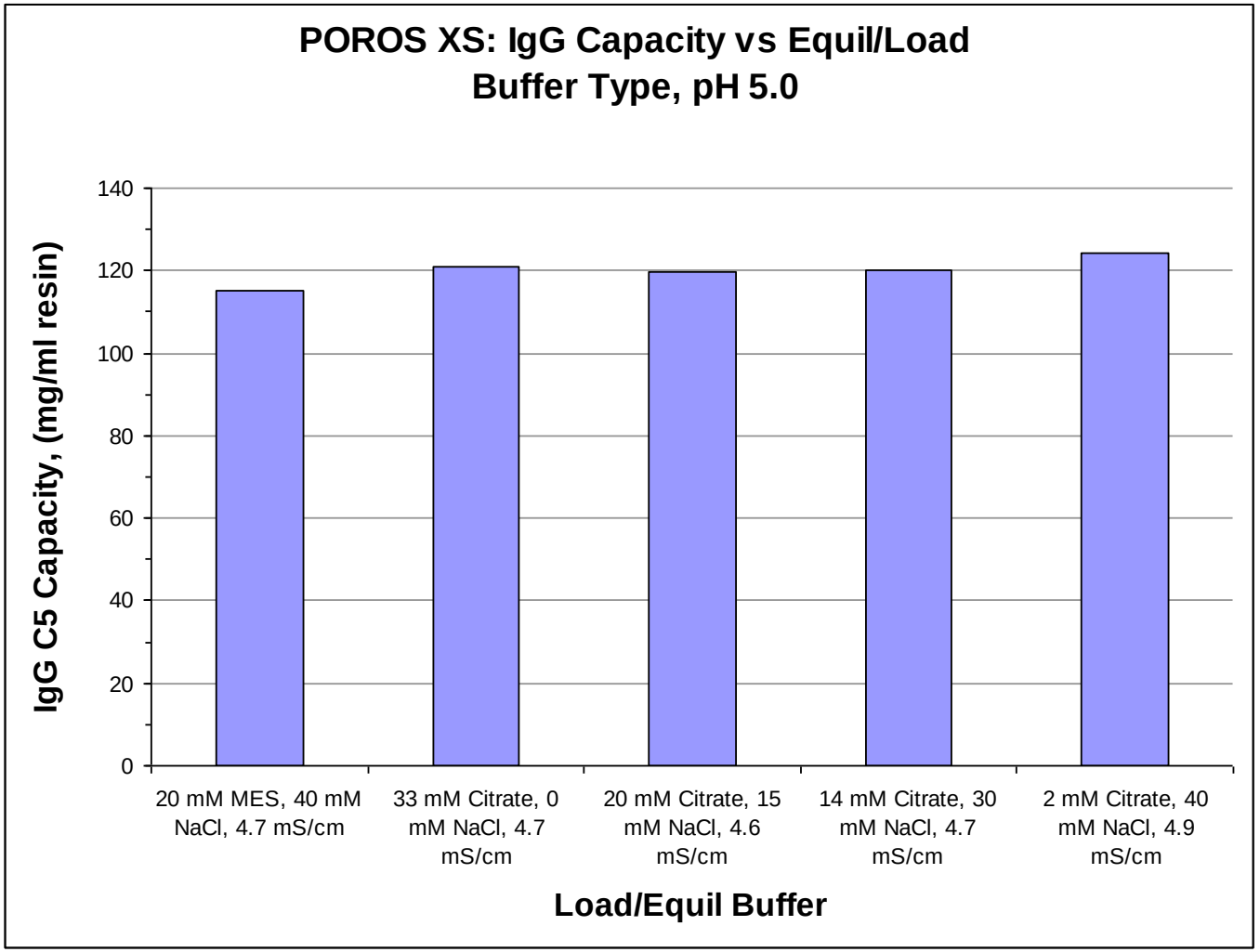
Format

Column: 0.46cmDx 20cmL
Buffer: 20mM MES, citrate, acetate, phosphate
Cond: 4.7ms/cm
pH: 5.0
Load Condition: 5 mg/ml Polyclonal IgG in Equil
Flow Rate: 300 cm/hr

The buffer system used does not impact binding capacity

life technologies

POROS® XS IgG Binding Capacity vs Salt Concentration



Format

Column: 0.46cmDx 20cmL
Buffer: citrate/NaCl in varying ratios
Cond: 4.7ms/cm
pH: 5.0
Load Condition: 5 mg/ml Polyclonal IgG in Equil
Flow Rate: 300 cm/hr

Binding capacity not affected by NaCl concentration at a given conductivity

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POROS® XS Protein Separation vs. Buffer System

Separation Control Conditions

Column = 0.46 cmD x 20 cmL

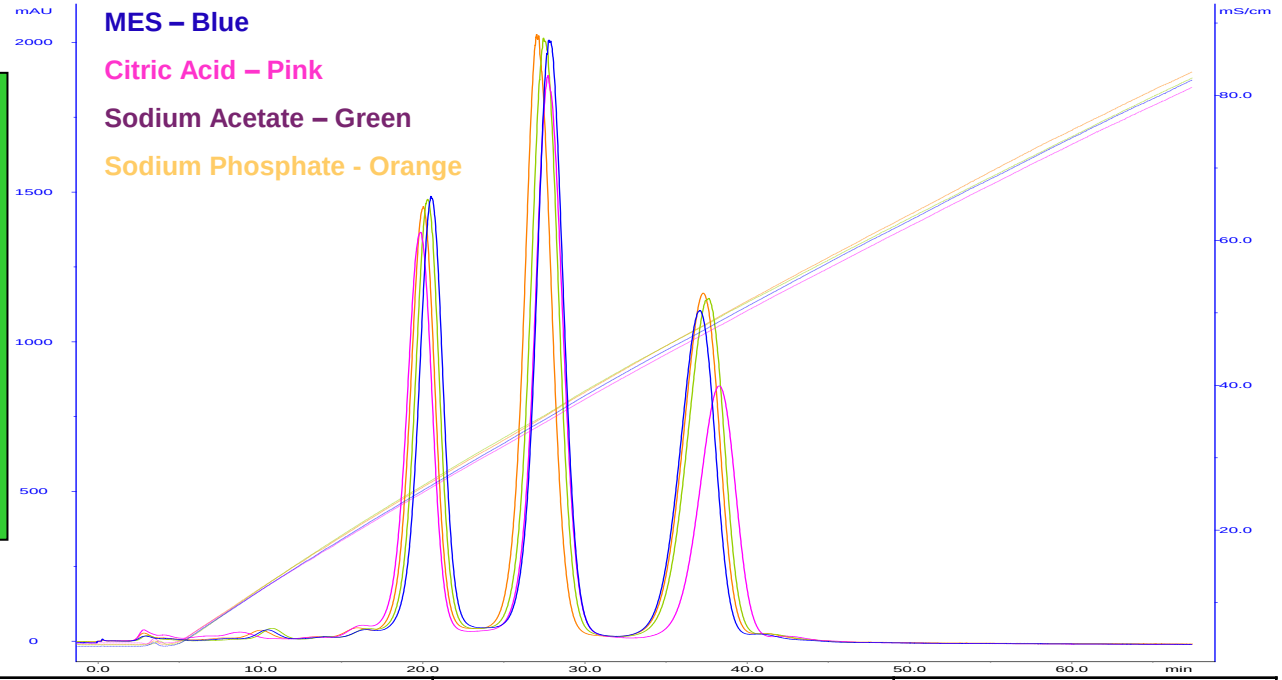
Buffer A = 20mM MES, 25mM NaCl pH 6.2 C = 4.02 mS/cm

Buffer B = 20mM MES, 1M NaCl pH 6.2 C = 89.7 mS/cm

Elution = 0% B – 100% B 16.88 CV

Flow Rate = 300cm/H

Protein Mix = Chymotrypsinogen A
Cytochrome C
Lysozyme



	Retention Time (min)			Elution Conductivity (mS/cm)			Resolution	
Sample	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3	Peak 1 - 2	Peak 2 - 3
MES	20.50	27.77	37.10	26.120	35.589	47.299	2.45	2.55
Citric Acid	19.89	27.73	38.28	25.004	35.112	48.178	2.52	2.80
Sodium Acetate	20.29	27.43	37.63	26.517	35.742	48.568	2.36	2.68
Sodium Phosphate	20.03	27.01	37.27	25.981	35.044	48.261	2.33	2.76

POROS® XS maintains excellent resolution with multiple buffer conditions

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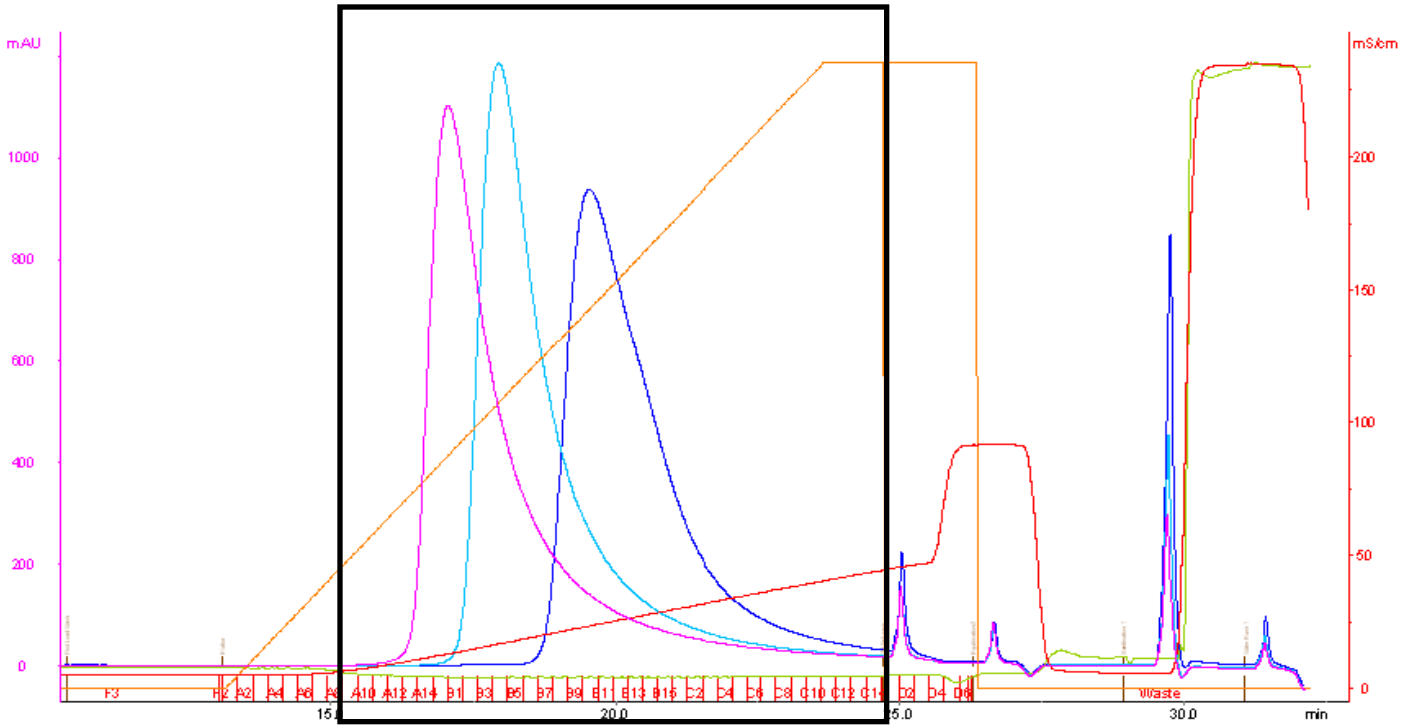
Optimization of a Conventional Soft Gel CEX Resin

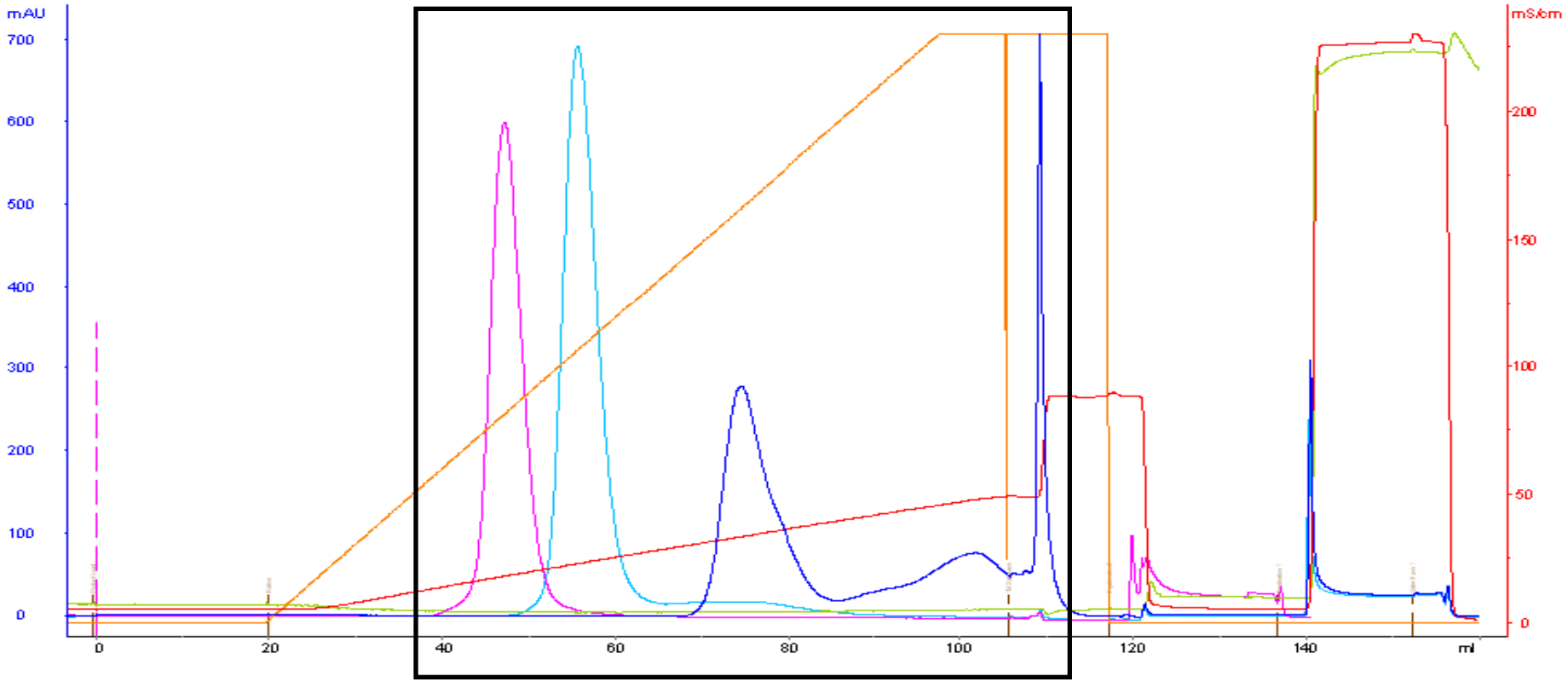
Run 1 – pH 5.5 **Run 2 – pH 5.0** **Run 3 – pH 4.5**



- Large particle size resin lacks resolution capability
- Peak cutting required to separate monomer and aggregate
- Decreased product yield

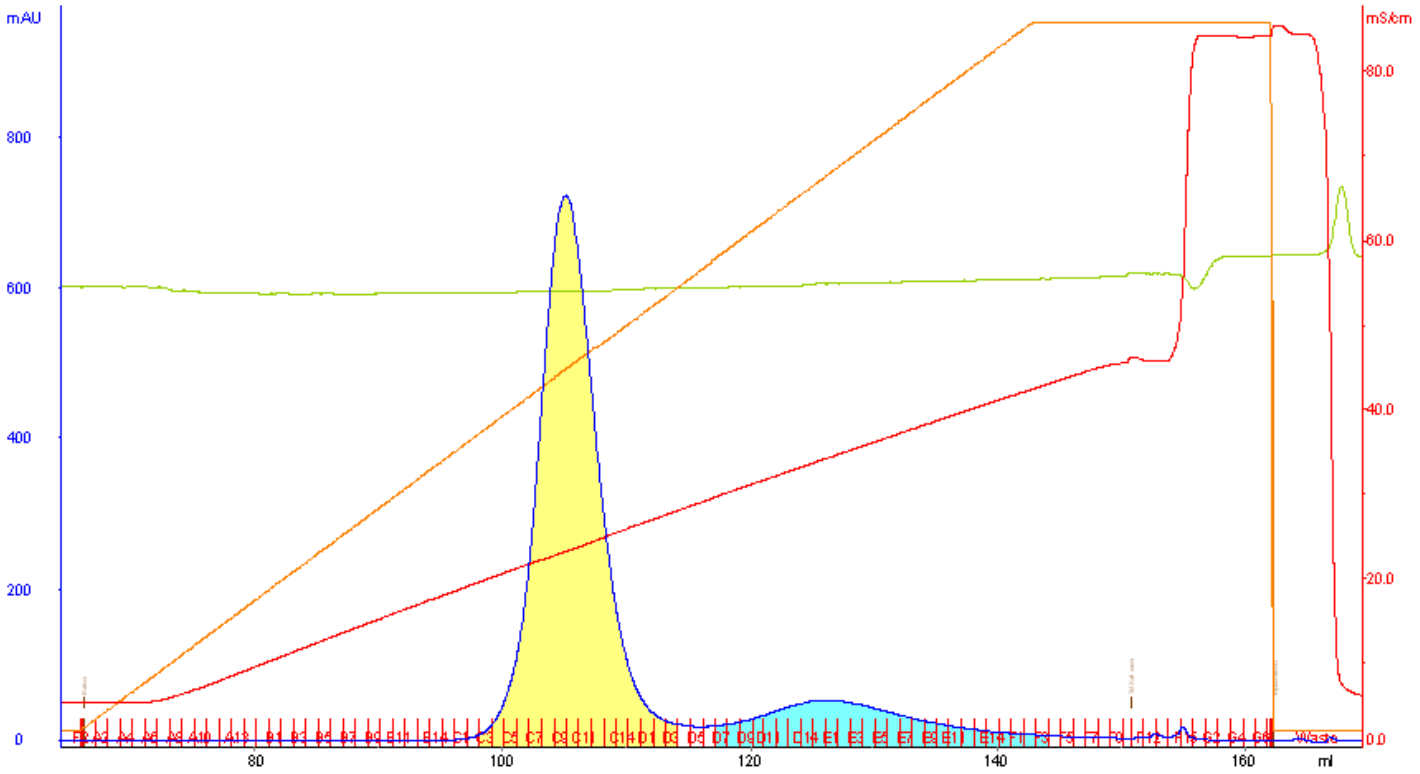
Optimization on POROS® XS

Run 1 – pH 5.5 **Run 2 – pH 5.0** **Run 3 – pH 4.5**



High resolution POROS® Resin provides improved separation

Optimization on POROS® XS



- Optimization of baseline separation
- Gradient can be optimized to step elution
- Improved product yield due to efficient separation of monomer and aggregate

POROS® XS Elution Peak: Customer Application

Goal: Ability to remove leached protein A below 10 ppm and maintain high process yield and product purity

Format

Column = 0.46cmD x 20cmL

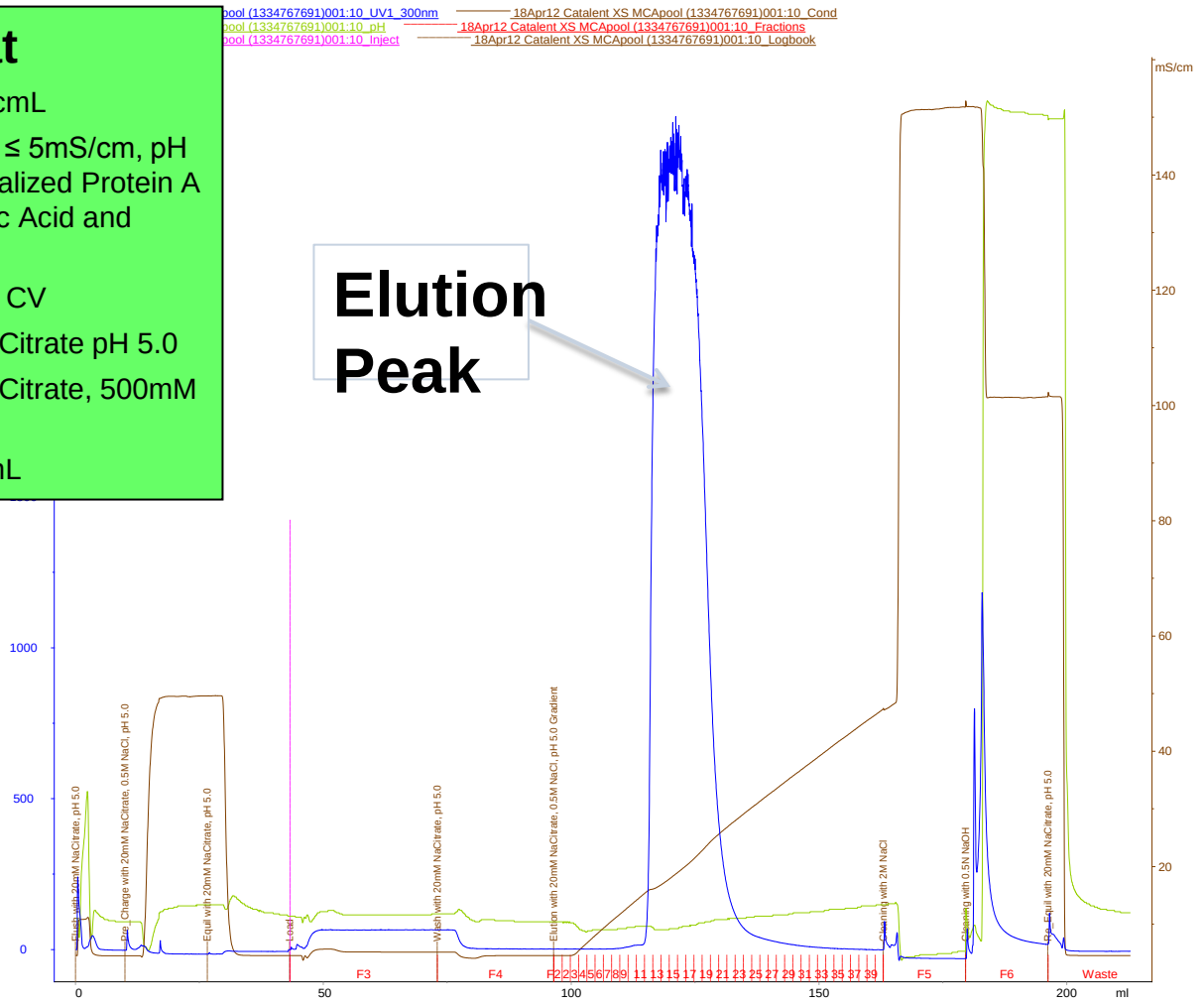
Load : 70 mg/ml of resin, ≤ 5mS/cm, pH 5.0 by adjusting the neutralized Protein A elution peak with 1M Citric Acid and water

Gradient: 0 – 100%B, 20 CV

Buffer A: 20mM Sodium Citrate pH 5.0

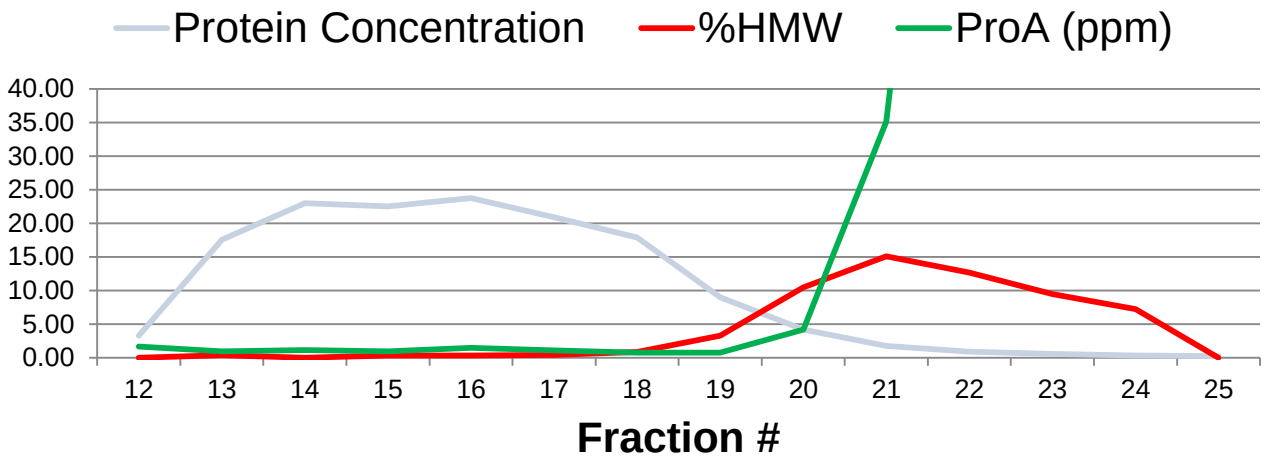
Buffer B: 20mM Sodium Citrate, 500mM Sodium Chloride pH 5.0

Fractions: ½ CV, 1.662mL



POROS® XS Product Pool
Yield & Purity

Sample	% HMW	Protein A (ppm)	% Yield
XS Load	1.007	40.65	
XS Elution Pool	**0.533**	**Below LOD**	**94.5%**



- High resolution yields effective separation of aggregate and leached rPA with high monomer yield
- SEC-HPLC analysis showed aggregate levels were reduced 2-fold
- Leached protein A reduced to below LOD
- Leached protein A was well separated from the MAb peak (salt strip) and final CEX yield was high

Viral Clearance on POROS XS in Bind/Elute Mode

	XMuLV Clearance (Log10)		
Load pH	**pH 6.0**	**pH 5.0**	**pH 4.5**
Load Salt Concentration (mM)	**25**	**25**	**100**
Load Capacity (mg/ml)	**80**	**80**	**100**
FT/Wash	3.5	4.0	2.1
20 mM MES, **50 mM NaCl**		3.0	
20 mM MES, **100 mM NaCl**	1.8	1.8	
20 mM MES, **200 mM NaCl**	1.1	1.9	
20 mM MES, **300 mM NaCl**	1.0	2.0	3.1
20 mM MES, **400 mM NaCl**		1.9	
20 mM MES, **500 mM NaCl**	1.0	1.5	1.7
2M NaCl		1.1	

Study Conditions

Column Format: 0.46cmD x 20 cmL, 3.3 ml

Flow Rate: 300 cm/hr

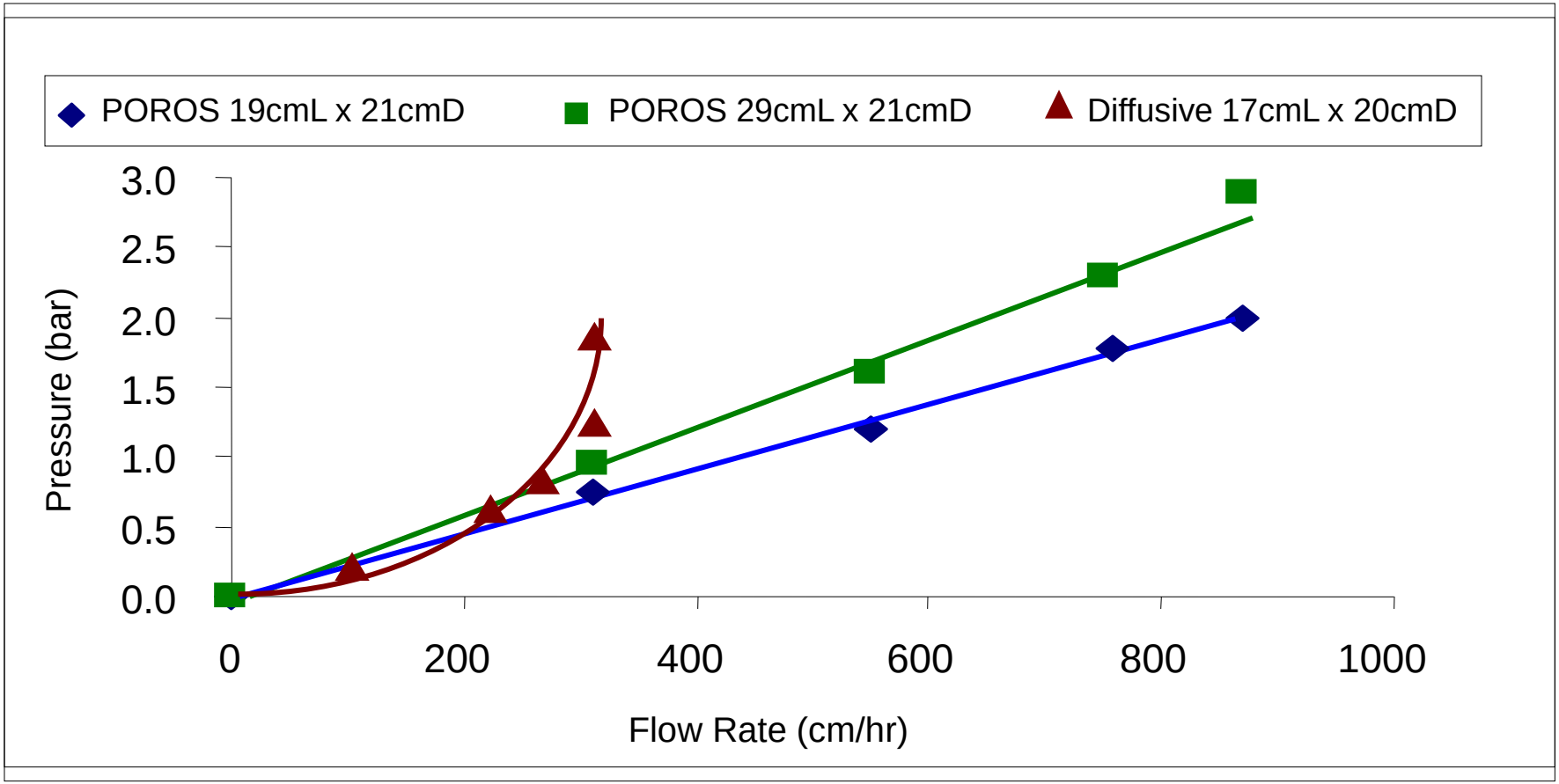
Residence Time: 4 min

Load: 5 mg/ml Human Polyclonal IgG

Virus Spike: 5% Vol

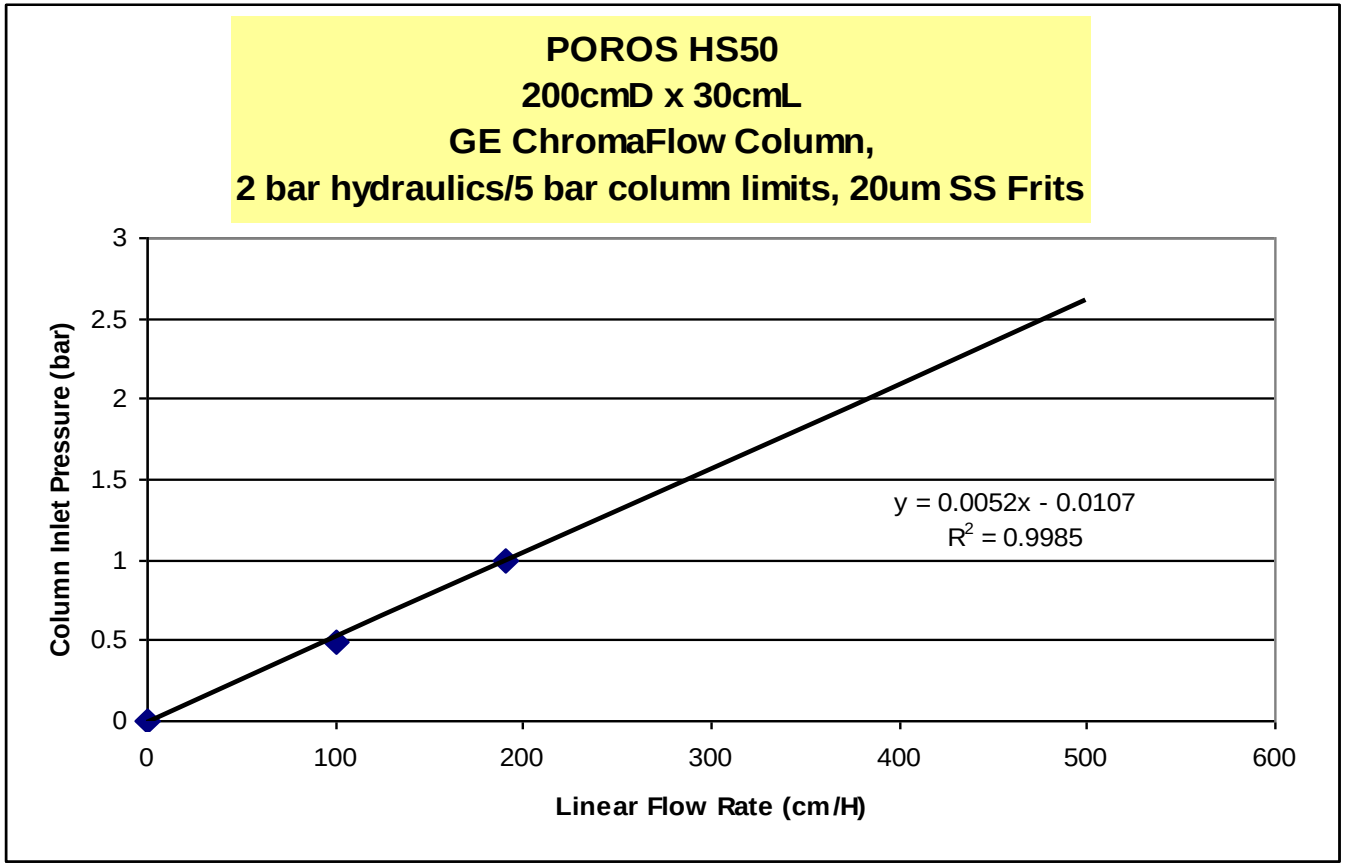
- Salt concentration in the load appears to increase virus flow through on POROS XS (lower clearance in FT/Wash) more than pH in the range tested
- pH 4.5 with 100mM NaCl in load appears to have best clearance when eluting with 300 mM NaCl: 3.1 LRV
- POROS XS is capable of good viral clearance depending on the process conditions as with any cation exchange resin

POROS® 50 um: Pressure vs. Flow Curves



POROS® Resin: Linear pressure response with no bed compression

POROS® HS Chromatography Resin Pressure Flow
200 cm Diameter Column



Note: The system pressure was not subtracted from data

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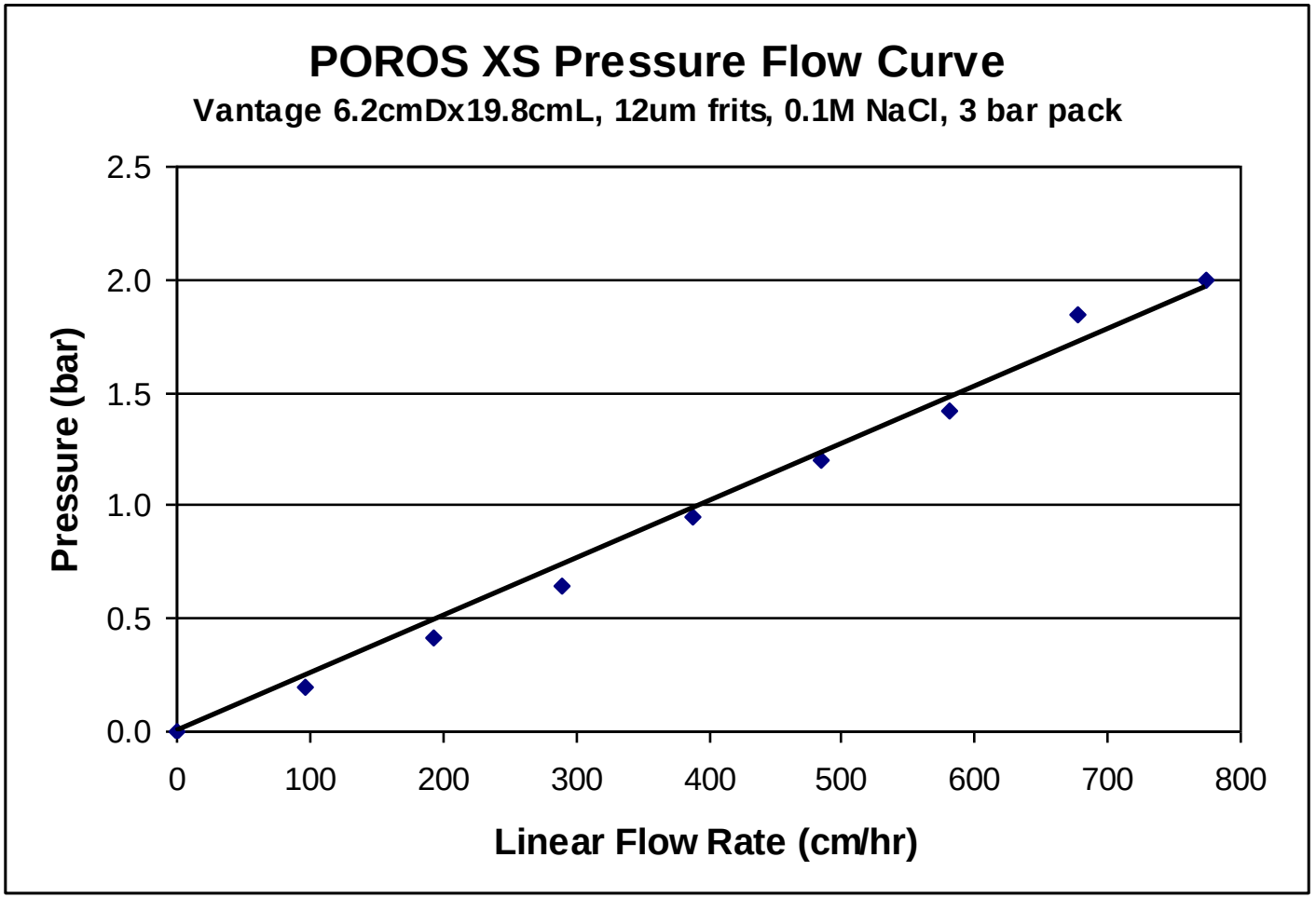
POROS® XS Pressure vs Flow

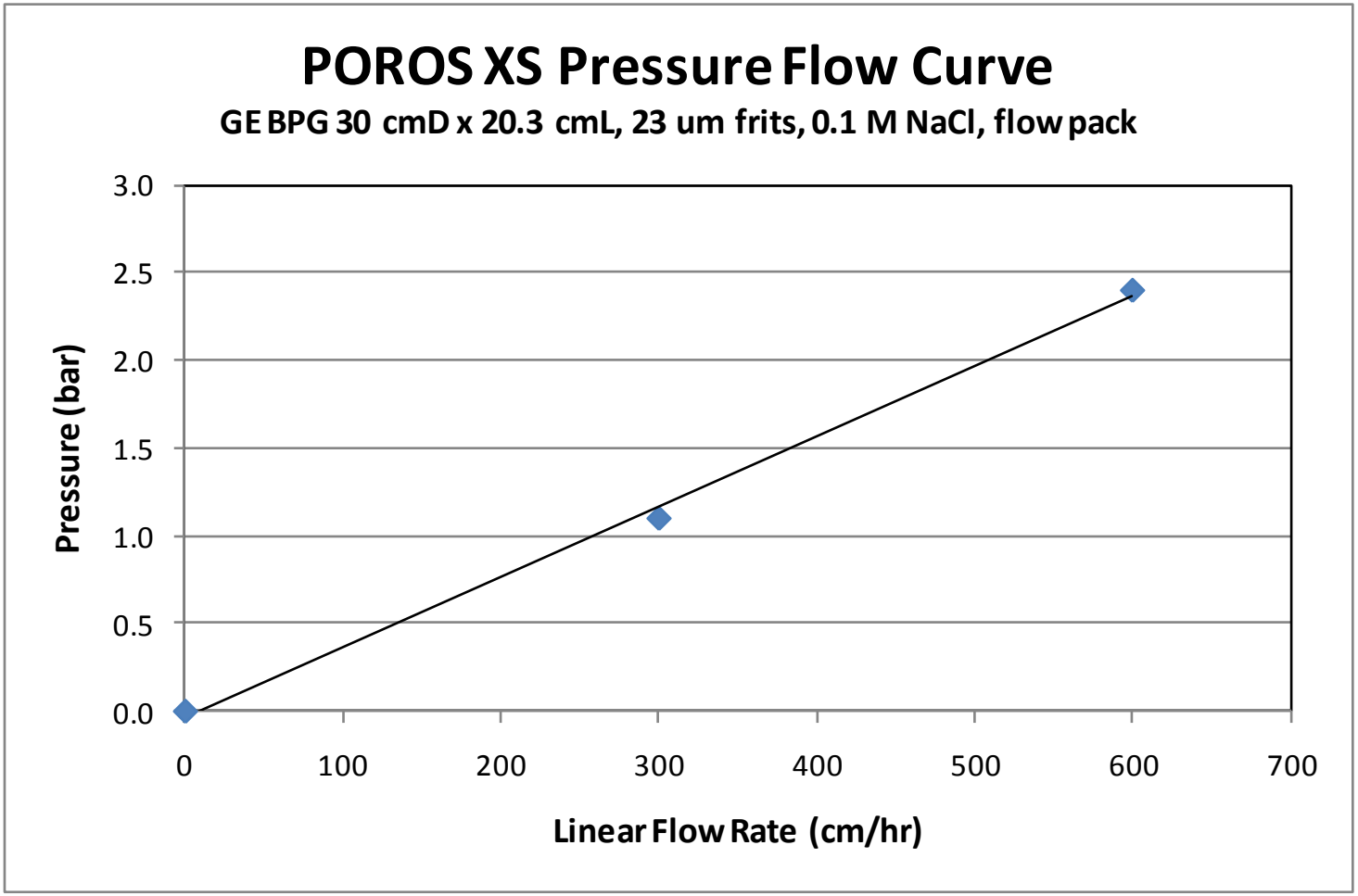
6.2 cm Diameter Column



Linear pressure response

POROS® XS Pressure vs Flow

30 cm Diameter Column

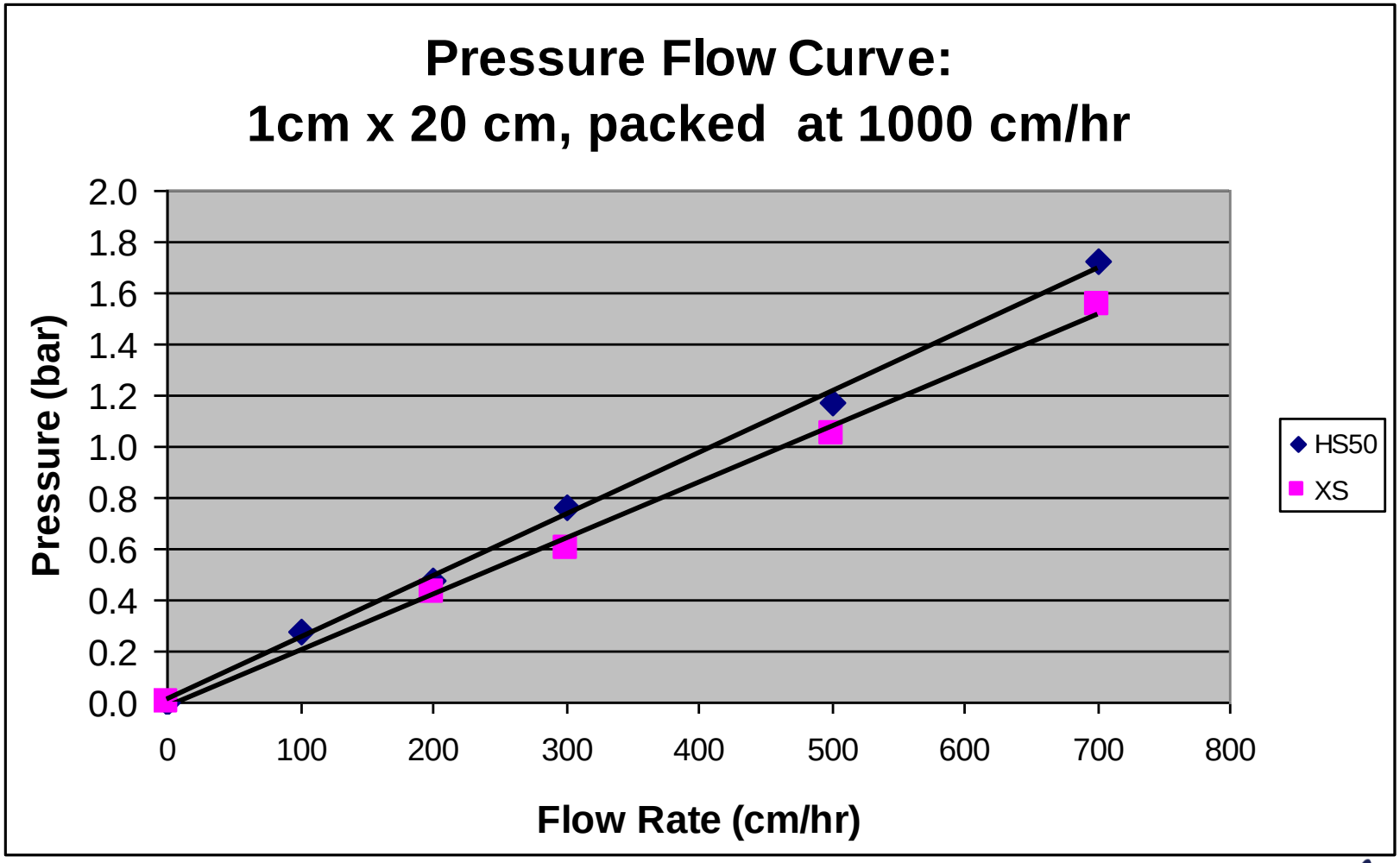


* System pressure not subtracted

Linear Pressure Response

POROS® XS and HS Pressure vs Flow

1 cm Diameter Column



Linear pressure response

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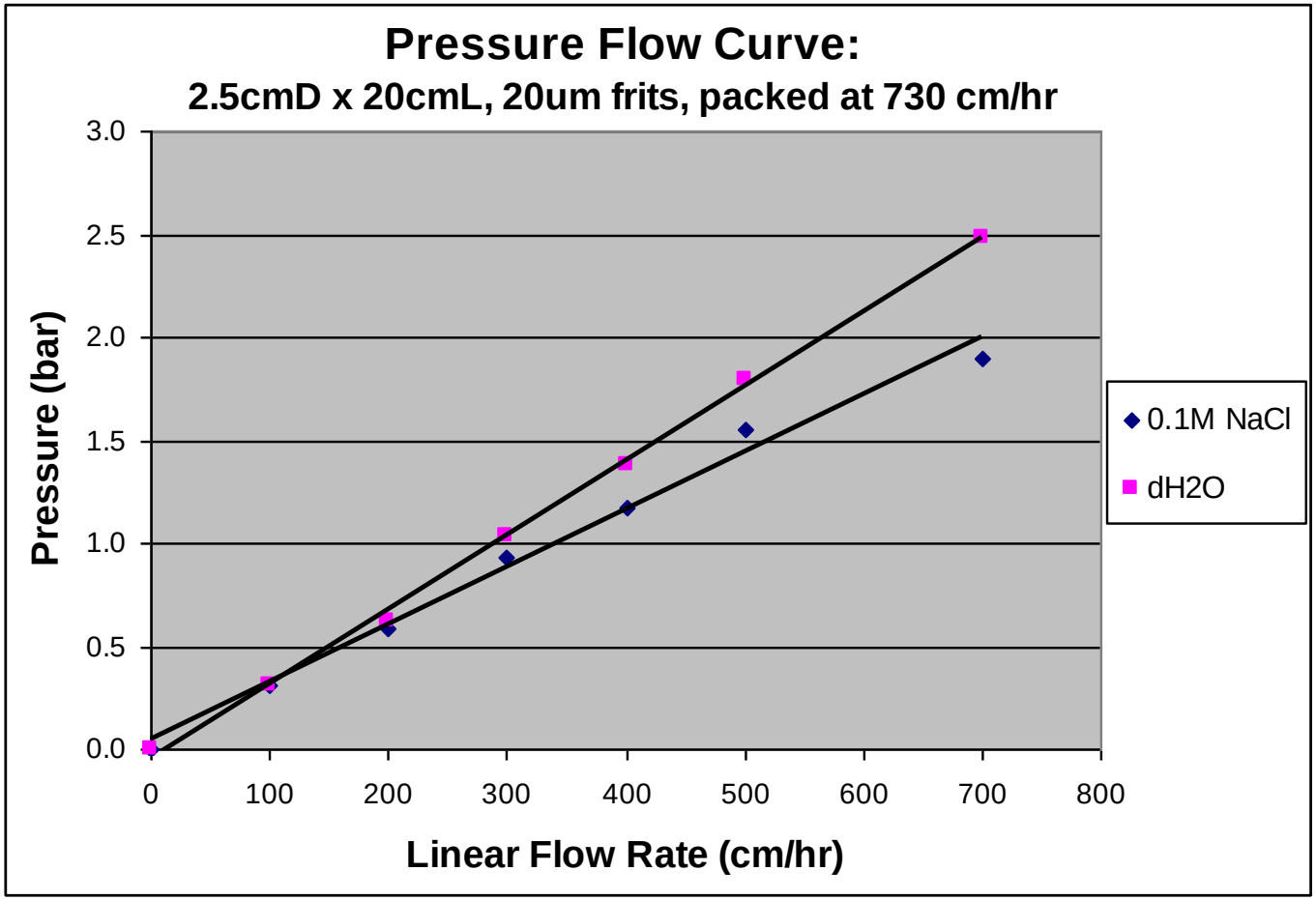
POROS® XS Pressure vs Flow

2.5 cm Diameter Column in 0.1M NaCl and water



Linear pressure response

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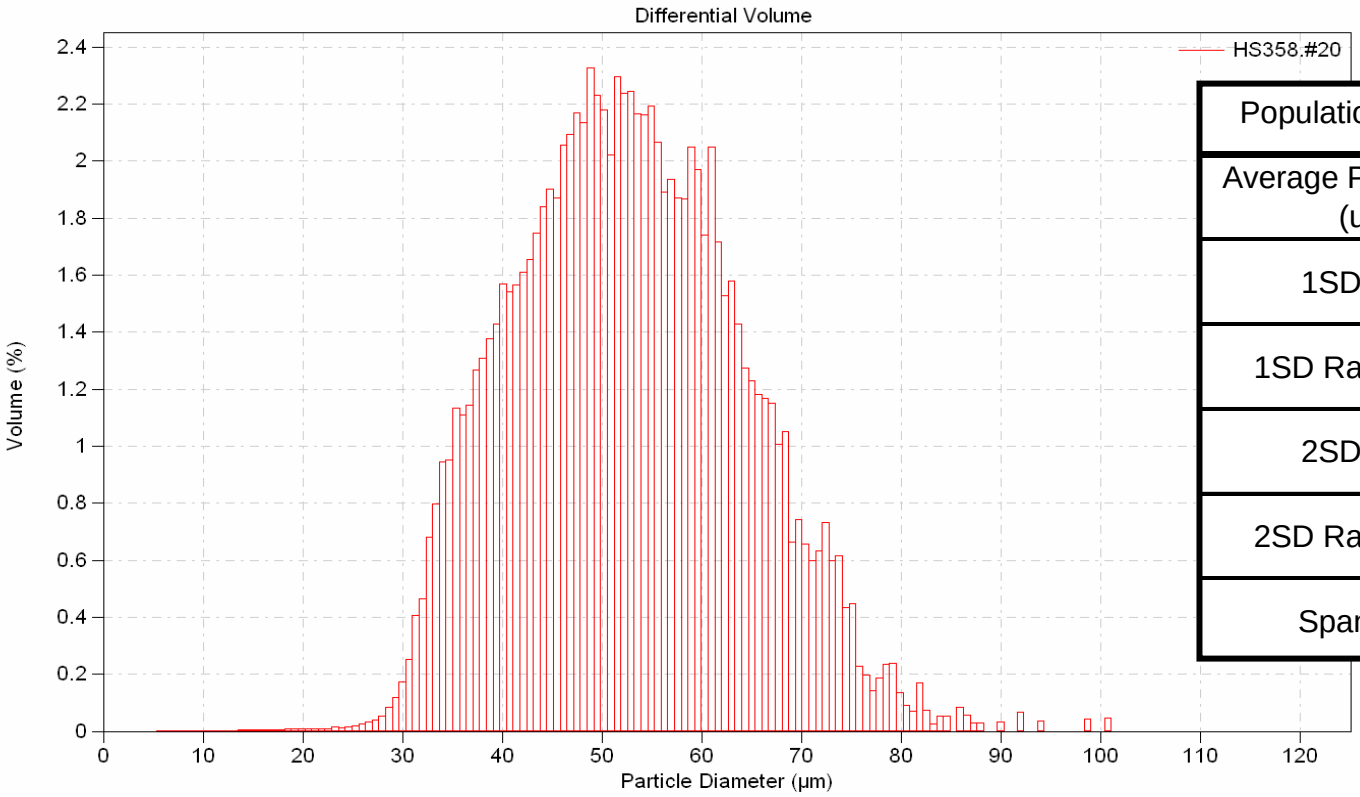
POROS® HS Chromatography Resin
Particle size distribution

- 50um Bulk Resin: Tight Particle Size Distribution



Population Size (n)	32
Average Particle Size (um)	51.6
1SD, 67%	11.33
1SD Range (um)	40 - 63
2SD, 95%	22.66
2SD Range (um)	29 - 74
Span (um)	45

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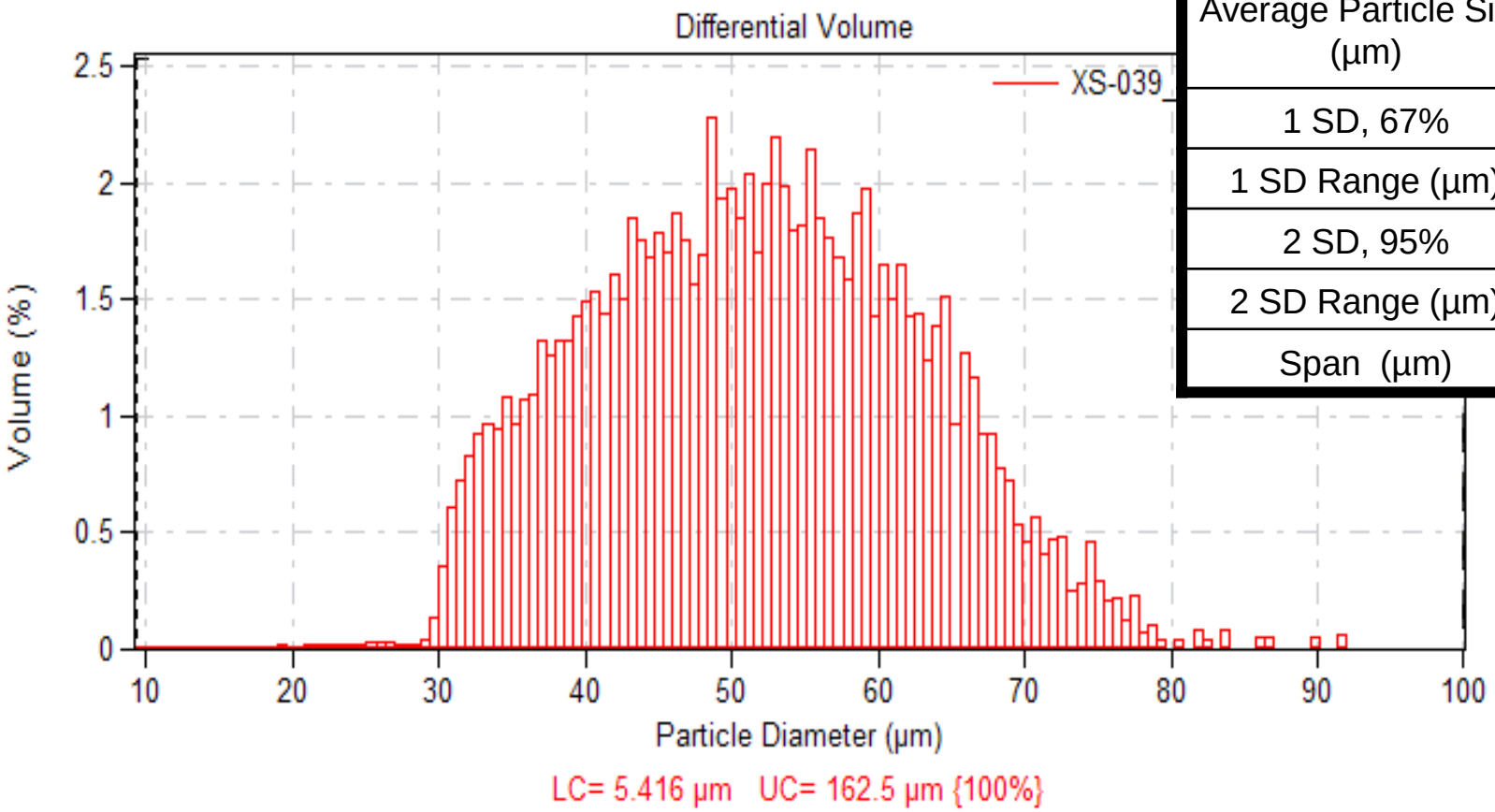
POROS® XS Chromatography Resin
Particle size distribution

- POROS® XS Resin: Tight Particle Size Distribution

Population Size (n)	25
Average Particle Size (µm)	51.8
1 SD, 67%	11.6
1 SD Range (µm)	40-63
2 SD, 95%	23.2
2 SD Range (µm)	29-75
Span (µm)	46



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Optimizing POROS CEX Conditions: Where to start?

- Although similar sulphopropyl functional groups are used on most strong CEX resins, the optimal binding and elution conditions can vary significantly due to a number of resin characteristics
- Different CEX resins operated with the same process conditions will yield variable results; therefore, standardized conditions or platform-type evaluations are not recommended
- It is important to test different loading and elution conditions to optimize capacity, separation and yield based on the target molecule characteristics and process challenges

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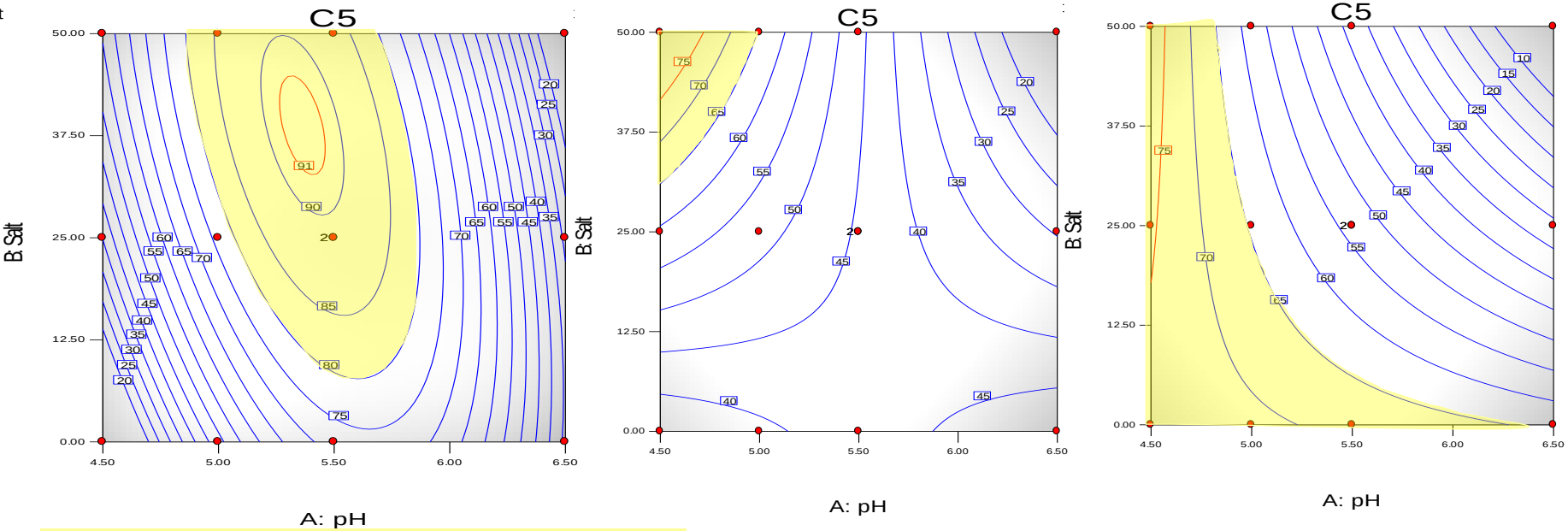
Benchmarking Cation Exchange Performance
hIgG DBC surface response plots



Shaded Area = 10mg/ml within max IgG C5

CEX IgG binding performance is highly variable despite all chemistries being SO_3 derived

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Optimizing CEX Conditions
Binding conditions

- **pH**
  - Use a binding buffer pH 1 to 3 units below the isoelectric point of the target molecule. Dynamic binding capacity typically increases as the loading pH decreases
- **Buffer system**
  - MES, acetate, phosphate, citrate, and citrate-acetate are often used
  - Consider molecule stability, binding optimization, and the ability of the buffer to control pH in the desired operating range
- **Conductivity**
  - The load conductivity should be between 2 and 15 mS/cm
  - The optimum buffer condition depends on the target molecule and buffer pH
- **Flow rate**
  - The target operating flow rate is flexible

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Optimizing CEX Conditions
Elution conditions

- **Salt gradient**
  - Elution optimization should begin with a gradient elution
  - Run a 20 CV gradient from low salt to approximately 500 mM to 1M sodium chloride
  - Once elution performance is understood, a step elution can be implemented
- **pH**
  - Start with the elution pH matched to the binding pH
  - Then optimize the pH of the elution buffer as these can differ
- **Dynamic binding capacity**
  - Separation as a function of DBC should be assessed
  - The max DBC at which a given separation can be obtained depends on a number of factors (i.e. sample solubility, column selectivity, buffer pH, and buffer conductivity)
- **Bed height**
  - Initial screening can be run with shorter bed heights, but development should be conducted at the final desired bed height

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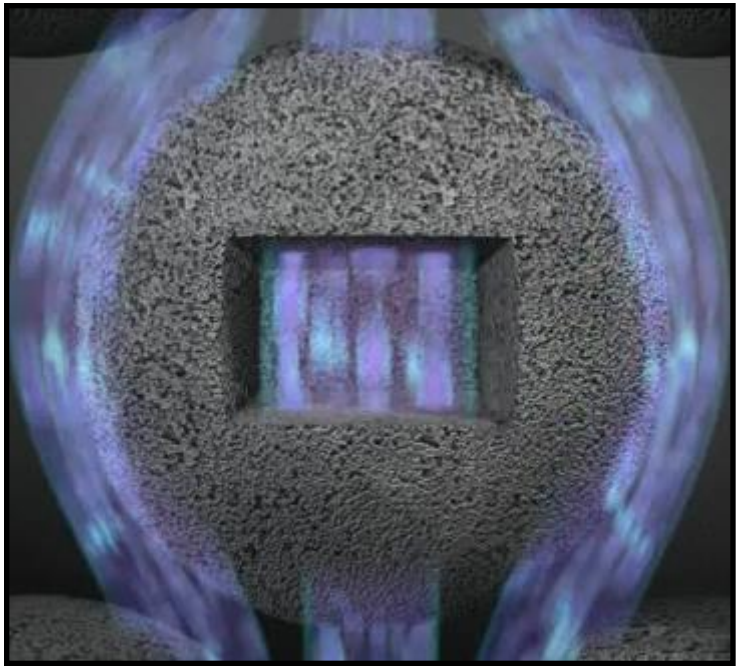
POROS® HS Case Study: Process Redesign

- Goal: To improve process yield and product purity within the manufacturing confines of multiple facilities for Avastin, an approved, commercially-available monoclonal antibody
- Data presented by Debbie O'Connor at May 06 IBC Antibody Development and Manufacturing Conference
  - Summarized the outcome of 2 years of process re-development by Genentech
  - 3 CEX Resins Evaluated
    - SP Sepharose Fast Flow (SCX, GE)
    - CM Sepharose Fast Flow (WCX, GE)
    - POROS HS50 (SCX, AB)
  - Protein MW 149 kDa, pI 8.1
  - Each resin loaded to 40 mg protein per mL resin
- CMSeph FF used in the existing process



- Each resin was optimized individually and the three optimized chromatography unit operations were summarized and compared

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POROS® HS Case Study: Process Redesign

- Evaluated the following for each resin:
  - Ease/Robustness of Elution
  - Yield
  - Elution Pool Volume
  - Impurity Removal (Aggregate, HCP, DNA, Leached rPA, Retrovirus)
- POROS HS50 resulted in the simplest unit operation
  - NaCl step elution
  - Tight elution peak resulted in smallest elution peak volume which contributed to ability to scale into most stringent facility (tank size limited)
  - No temperature effects; Temp 15-25°C, no change in elution peak volume or impurity levels
  - NaCl could be used to elute the SPFF column, but [2x] required and the elution pool volume was very high

Resin	Elution Buffer	Peak Pooling
CMFF	**Sodium Sulfate**	**Equation**
SPFF	**Sodium Acetate**	**High OD Calculation**
HS	**Sodium Chloride**	**OD**

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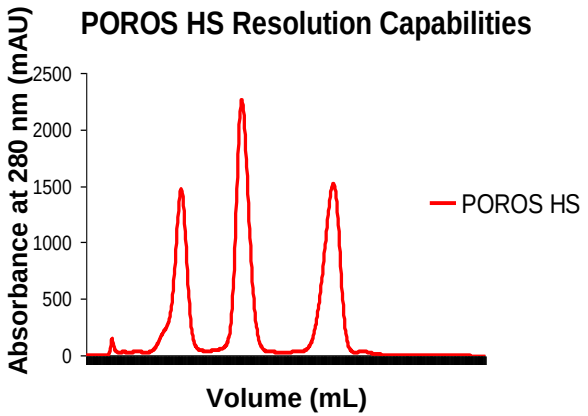
POROS® HS Case Study: Process Redesign

- POROS HS was chosen over SPSephFF and CMSephFF due to the following:
  - 11% increase in product yield
  - Improved resolution of impurities
    - HCP Removal
      - CMSephFF << SPSephFF < POROS HS
    - Aggregate Removal
      - SPSephFF: 0.2-29% increase in [aggregate] across elution pool
      - POROS HS: No increase in [aggregate] across elution pool
    - Leached rPA and DNA both below LOD for POROS HS
  - Efficient binding and eluting characteristics
  - Comparatively, POROS HS could be loaded over a wider range of pH and conductivity without impacting yield or impurity removal (aggregate)
  - POROS HS presented with the same re-use, cleanability and sanitization profile as the current resin
  - Better viral clearance capability
    - 4 Log POROS HS vs. 2 log SPSephFF
  - Resulted in the simplest and most robust step



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Case Study: Boehringer Ingelheim Poster Presentation at Recovery XIII Conference

- This poster was presented in June 2008 by Rathjen, Wenzel, Nothelfer, Eckermann, and Ambrosius from BI.
- RAPPTor technology (Rapid Automated Protein Purification Technology) allows for multiple binding and elution conditions to be screened and evaluated in parallel.
- Although the poster was on the applications of the screening system, the data shows the excellent performance of POROS HS compared to other resin.

BI Case Study: Purification of 2 Monoclonal Antibodies using POROS HS 50

- BI conducted an evaluation of CEX resins for recovery and Host Cell Protein removal for 2 Protein A purified mAbs
  - mAb A: human IgG1, 145 kDa, PI=7.3
  - mAb B: human IgG1, 145 kDa, PI=7.9
- 6 CEX resins were tested and 3 presented: POROS HS, Fractogel S03, and Toyopearl SP 650M
- Evaluated different pH conditions using an acetate/tris buffer system: 4.5, 5.0, 5.5, and 6.0 (Binding/eluting at same pH condition)
- Elution carried out by increasing salt concentration from 50, 100, 200 and 300 mM

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BI Case Study: Purification of 2 Monoclonal Antibodies using POROS HS

- POROS HS received the highest rating for combined HCP clearance and recovery for both antibodies in the RAPPTor screening study
- Overall HS had the highest HCP removal factor of all the resins (pH 4.5: 18.9 fold HCP removal)

CEX resin	cand.	binding pH	Product recovery [%]	HCP removal factor	overall rating
Poros 50 HS	A	4.5	24	5.6	
		5.0	87	13.6	
		5.5	100	8.7	
		6.0	99	7.0	
Fractogel SO3		4.5	21	1.1	
		5.0	87	3.6	
		5.5	101	4.7	
		6.0	100	3.9	
Toyopearl SP		4.5	78	1.7	
		5.0	94	3.8	
		5.5	98	3.9	
		6.0	59	3.7	
Poros 50 HS	B	4.5	12	18.9	
		5.0	82	15.0	
		5.5	104	10.0	
		6.0	109	7.7	
Fractogel SO3		4.5	10	0.9	
		5.0	84	2.9	
		5.5	105	8.7	
		6.0	107	7.8	
Toyopearl SP		4.5	65	1.7	
		5.0	95	7.4	
		5.5	104	7.5	
		6.0	88	4.3	

Summary of CEX Screening: Product recoveries >90% and HCP removal >8 fold are highlighted in green.

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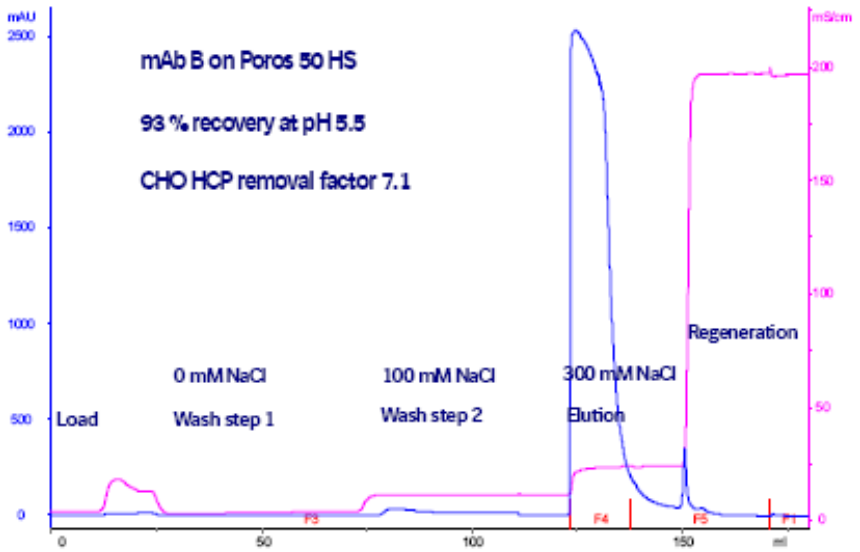
BI Case Study: Purification of 2 Monoclonal Antibodies using POROS HS 50

- The RAPPTor POROS HS process was scaled up 330X by CV and yielded 93% recovery and a 7.1 fold removal of HCP showing the predictability of the model
- Column Dimensions:

1 cmD x 21 cmH, 16.5 ml

- Load, 4.6 mg mAb B/ ml resin
- Wash: 100 mM NaCl, pH 5.5
- Elution: 300 mM NaCl, pH 5.5



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Membrane Adsorbers vs. Traditional Chromatography in Flow Through Mode

- Membrane Advantages
  - Faster flow rates
  - 95% less buffer usage
  - Disposability
  - Ease of use
- Membrane Disadvantages
  - Complicated scale-down models
  - Difficult to compare to other membranes and resin

- Chromatography Advantages
  - Reusable at commercial scale
  - Available for all scales
- Chromatography Disadvantages
  - Very large diameter columns to ↑ flow rate to ↓ bottleneck
  - Packed for throughput and not for binding capacity

So…Where does POROS® resin fit in???

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POROS® HS Case Study: Overloaded CEX

- Goal: To evaluate CEX in overload mode to increase process efficiency and yield and reduce production costs
- Current Mab platform process: ProA > CEX (B/E) > AEX (FT)
- Although new CEX resins allow for 100 mg/ml with no loss in purity, still want to increase throughput
- Evaluated CEX in flow through type mode at binding conditions for selective displacement of impurities
  - 4 CEX products evaluated
    - POROS HS (Life)
    - SP Sepharose Fast Flow (GE)
    - Mustang S (Pall)
    - Monolith SO_3 (BIA)
  - 3 IgG1 Mabs tested with isoelectric points ranging from 8.2 to 9.2 with varying impurity amounts
  - Evaluated the following for each resin:
    - Dynamic binding capacity
    - Yield
    - Impurity Removal: HMW (dimer and higher order aggregates), CHOP, DNA, Leached rPA, Gentamicin
  - Optimized loading conditions: pH 5.5, conductivity 5 mS/cm, 600 g/L

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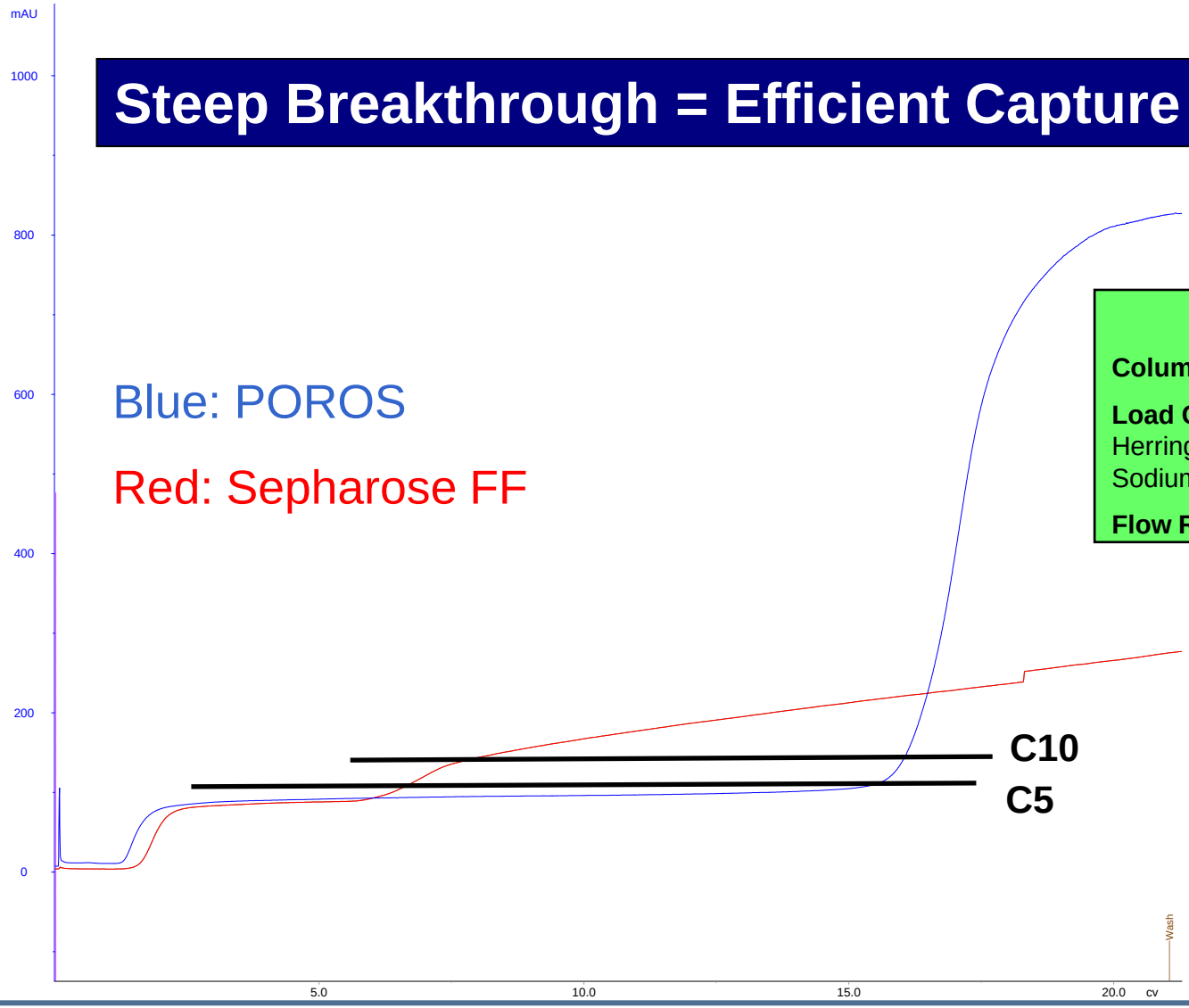
POROS® Chromatography resin
Impurity Breakthrough Curves

Steep Breakthrough = Efficient Capture



Format

Column: 0.46cmDx 20cmL

Load Condition: 1.6 mg/mL DNA from Herring Sperm (Sigma D3159) in 20mM Sodium Phosphate, 50mM NaCl, pH 7.0

Flow Rate: 300cm/hr

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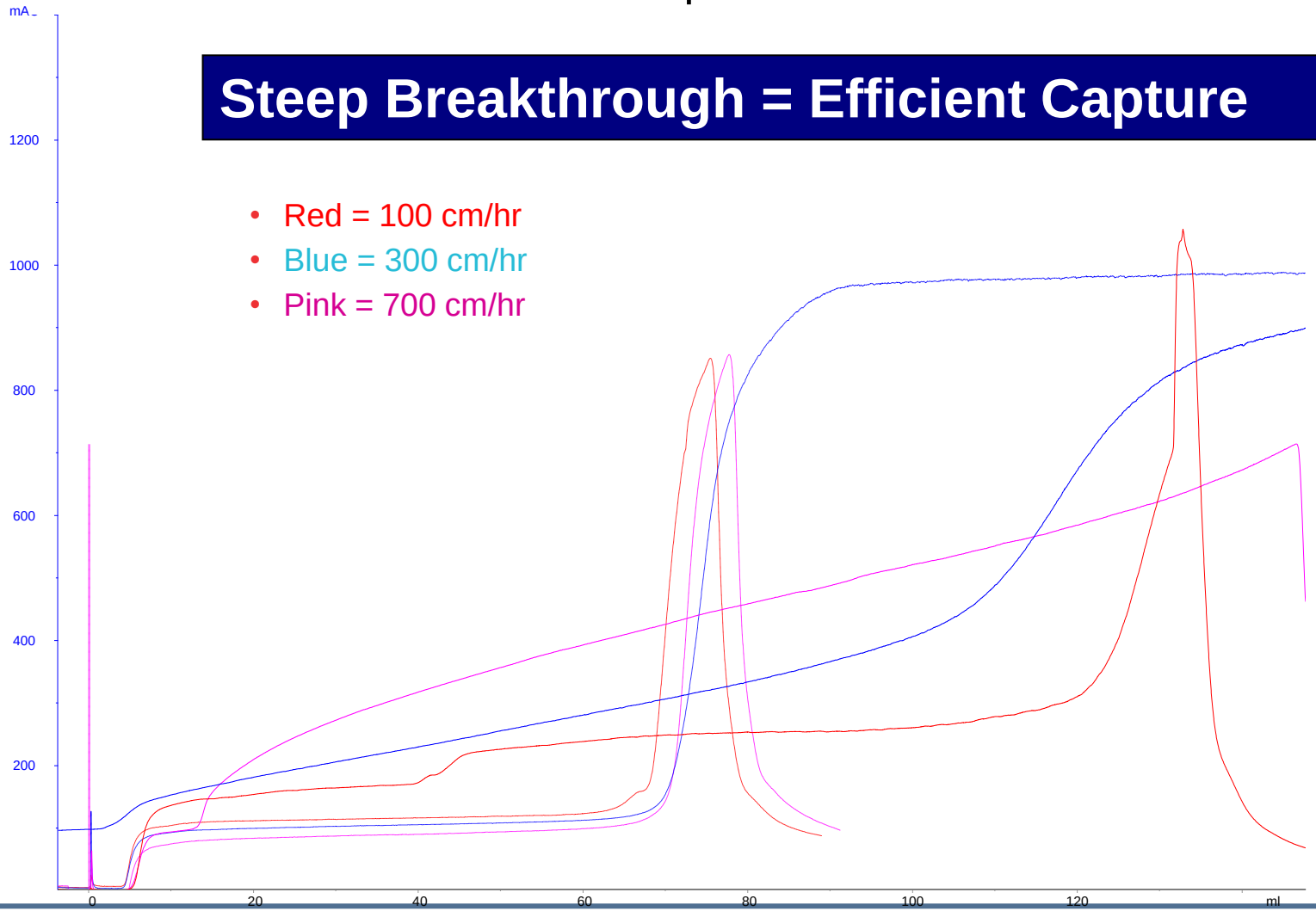
POROS® Chromatography resin: Impurity Breakthrough Curves

POROS® = Dotted Lines Seph FF = Solid Lines

Steep Breakthrough = Efficient Capture

- Red = 100 cm/hr
- Blue = 300 cm/hr
- Pink = 700 cm/hr



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ThermoFisher SCIENTIFIC

POROS® HS Case Study: Overloaded CEX

- POROS® HS50 resulted in the best performance
  - >90% yield when loaded at a capacity of 600 g/L
  - The best impurity removal of all CEX products especially HMW (dimers and higher order aggregates) and DNA
  - Maintained performance up to 300 cm/hr whereas SPFF decreased in performance even as low as 200 cm/hr (higher flow rates than 300 cm/hr not tested)
  - Leached ProA and Gentamicin reduced to below limit of detection
- Data based on two sources:
  - Publication: Liu, Hui F, et al, “Exploration of overloaded cation exchange chromatography for monoclonal antibody purification”, Journal of Chromatography A, V1218, 2011, Pages 6943-6952
  - Data presented by Hui Liu Ph.D., Genentech Process Development Scientist at IBC Antibody Development & Production Conference, Bellevue, Washington, March 17, 2011

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Comparison of Overload vs B/E CEX Process

(13.5 kg, 2700L Process, Life Tech Model Process)

	POROS HS in Overload Format	Conventional Soft Gel Resin in B/E	High Capacity CEX in B/E	% Difference w/ POROS HS	% Difference w/ High Capacity
Column/Membrane	60 x 8 cm	140 x 22 cm	100 x 17 cm		
Column/Membrane Volume	23	339	134	93%	61%
Load Capacity (mg of protein/ml of resin)	600	40	100		
Linear Flow Rate (cm/hr or	300	100	300		
Volumetric Flow Rate (L/min)	14	26	39		
Product Load Process Time	3.2	1.7	1.2		
Total Process Time (hr)*	4.3	6.6	2.8	35%	57%
Buffer Volume (L)	542	8129	3204	93%	61%

- Both POROS HS in overload mode and High Capacity resins, such as POROS XS, in B/E mode could result in significantly smaller columns than the conventional B/E process resulting in smaller columns and equipment and less buffer usage
- Both POROS resins allow for faster flow rates resulting in shorter processing times
- Both POROS processes allow for more efficient and/or cost effective approaches to traditional B/E CEX processes
- POROS XS may offer more process flexibility to the traditional process due to better salt tolerance

Cost Comparison of an Overload vs B/E CEX Process (13.5 kg, 2700L Process, Life Tech Model Process)

	POROS HS in Overload Format	Conventional Soft Gel Resin in B/E	High Capacity CEX in B/E	Fold Savings w/ POROS HS	Fold Savings w/High Capacity CEX
Buffer Cost ($)	1627	24386	9612	15	3
Process Labor Costs ($)	4876	5572	4438	1	1
Cost of Resin/Membrane ($)	45,200	338,700	393,825	7	1
Total Cost of Processing/Cycle ($)					
1 Cycle	51,703	368,658	407,875	7	1
5 Cycles	15,543	97,698	92,815	6	1
10 Cycles	11,023	63,828	53,433	6	1
50 Cycles	7,407	36,732	21,927	5	2
100 Cycles	6,955	33,345	17,989	5	2

- Both POROS processes allow for a significant decrease in buffer costs compared to the traditional CEX process
- The POROS HS overload process could result in major cost savings due to the 93% decrease in column size and buffer usage

CEX in Flow-Through Applications - Conclusions

- Flow-Through Applications
  - High impurity binding capacity and clearance over a wide range of process conditions
  - Reduce resin volume, equipment sizes, processing time and process complexity
  - Membrane performance in a bead
    - > Flow rate-independent performance
    - > Improved throughput potential
    - > Smaller column sizes possible
  - Competitive with performance of membrane adsorbers
    - > Reusable at commercial scale
    - > Easily scalable
      - Design for commercial scale from beginning to reduce redevelopment costs
    - > GoPure™ Pre-Packed Columns for convenience and disposability

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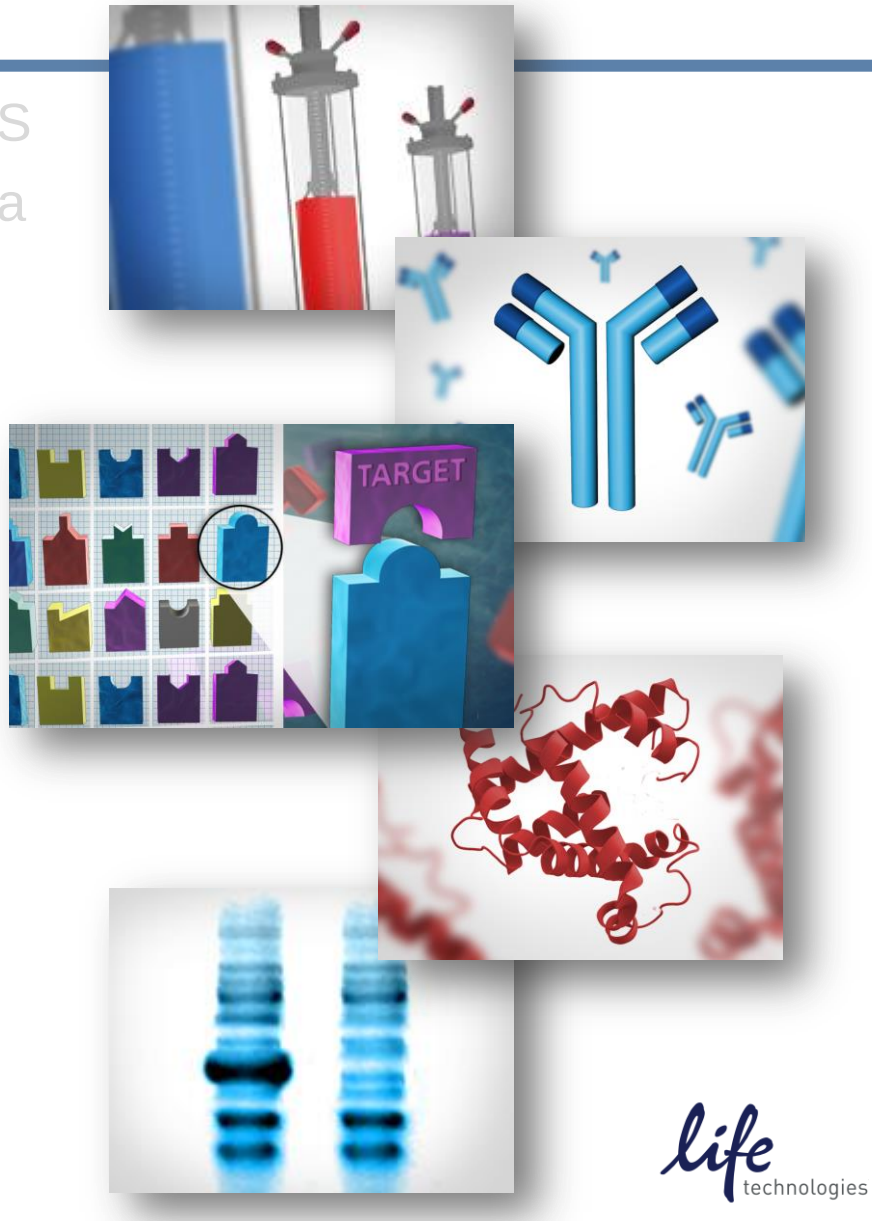
Overview

- CEX introduction: POROS® HS and XS
- Applications and Characterization Data
  - Applications
  - Viral clearance
  - Pressure vs flow curves
  - Particle size analysis
- How to optimize
- Case Studies:
  - POROS® HS process redesign
  - POROS® HS in HTP screening and scale up study
  - Rethinking polish applications: CEX in flow through/overload mode
- Summary



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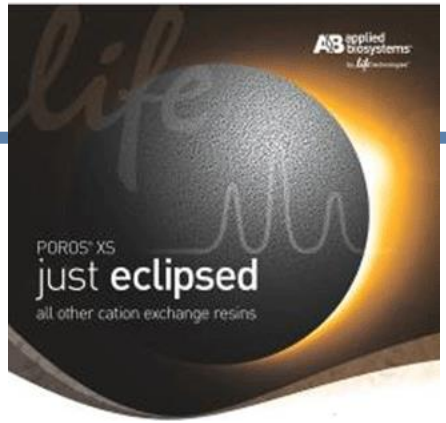
How does POROS® resin improve process performance and productivity?

PRODUCT ATTRIBUTE	PROCESS BENEFIT
• High Capacity	• Reduced Column Size
• High Resolution	• Better Impurity Clearance • Higher Product Yield • Step versus Gradient Elution
• Salt Tolerance	• Reduced or Eliminated Dilution • Elimination of Unit Operations (TFF) • Improved Process Flow
• Efficient Chromatography	• Reduced Pool Volume (Tank Flexibility)

POROS® XS in the Industry



- POROS® XS has been incorporated into 7 Phase 3 processes
- POROS® XS has become a platform CEX polish resin at large biopharma companies in the US and Europe
- POROS® XS is enabling non-protein A capture chromatography of monoclonal antibodies
- Customer Quotes on POROS ® XS…
  - POROS® XS "provides performance consistent with your literature, POROS® XS has a higher salt tolerance, elutes at a higher salt concentration than all resins tested, and has a tight, symmetrical peak shape. Capacity looks very good".
  - POROS® XS "has enabled a 4x reduction in process time due to capacity and throughput capability"
  - "We were able to remove 2 UF/DF steps due to POROS® XS"
  - "We incorporated POROS® XS into a troublesome biosimilar process in the course of 1 week due to the capacity advantage offered"

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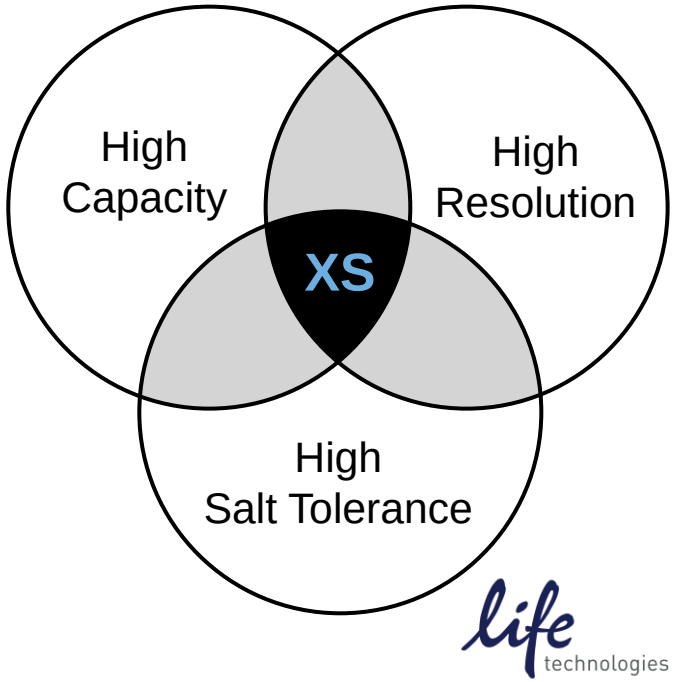
Summary

- POROS® XS sets the bar in fulfilling the most important demands in today's high titer downstream purification processes
- POROS® XS provides the combination of high capacity, high resolution, and high salt tolerance that surpasses all other cation exchange resins
- POROS® XS provides key downstream processing benefits including improved yield and throughput, with added process flexibility



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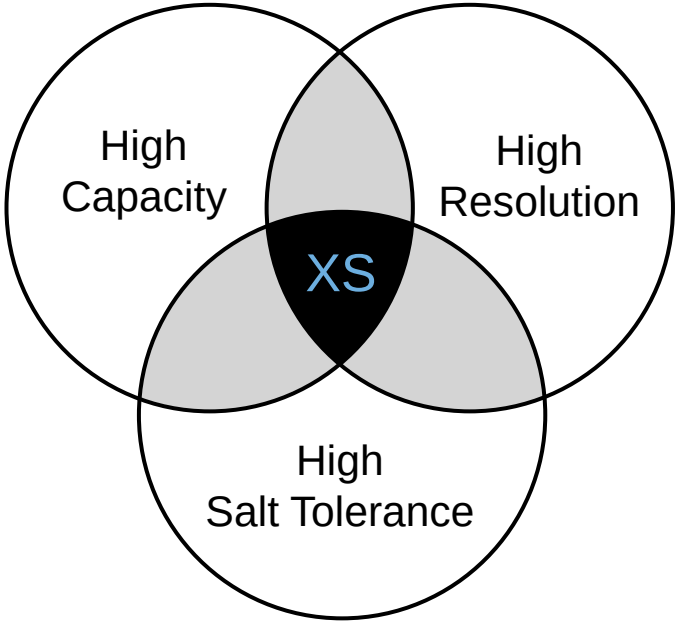
CEX for Intermediate Polish Chromatography

POROS® XS resin is setting standards in resolution and capacity



- **High capacity and resolution needed**
  efficient removal of aggregate, host cell proteins, and other impurities
- **Superior resolution**
  independent of load concentration or flow rate
- **More process flexibility**
  over a broad range of process conditions
- **Provides solutions to today's industry demands**
  POROS® XS successfully combines superior capacity, resolution and salt tolerance improving yield, throughput and increased flexibility

Features	Benefits	Products and Services
• **High 5% breakthrough capacity** >100 mg/mL over a range of process conditions • **Superior resolution** even with varying linear flow rates and load concentrations • **Incompressible 50 µm bead** with robust physical stability • **Validated manufacturing process** • **Worldwide technical support**	• **Improved yield** • **More process flexibility** • **Reduced column sizes** • **Decreased tank sizes** • **Improved throughput** • **Reduced number of unit operations** • **Enables CEX capture chromatography, eliminating need for rPA affinity**	• **Bulk Resin** • **GoPure™ Pre-Packed Chromatography Columns**