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# **The Continuing Evolution of Synthetic Ligand Affinity Adsorbents**

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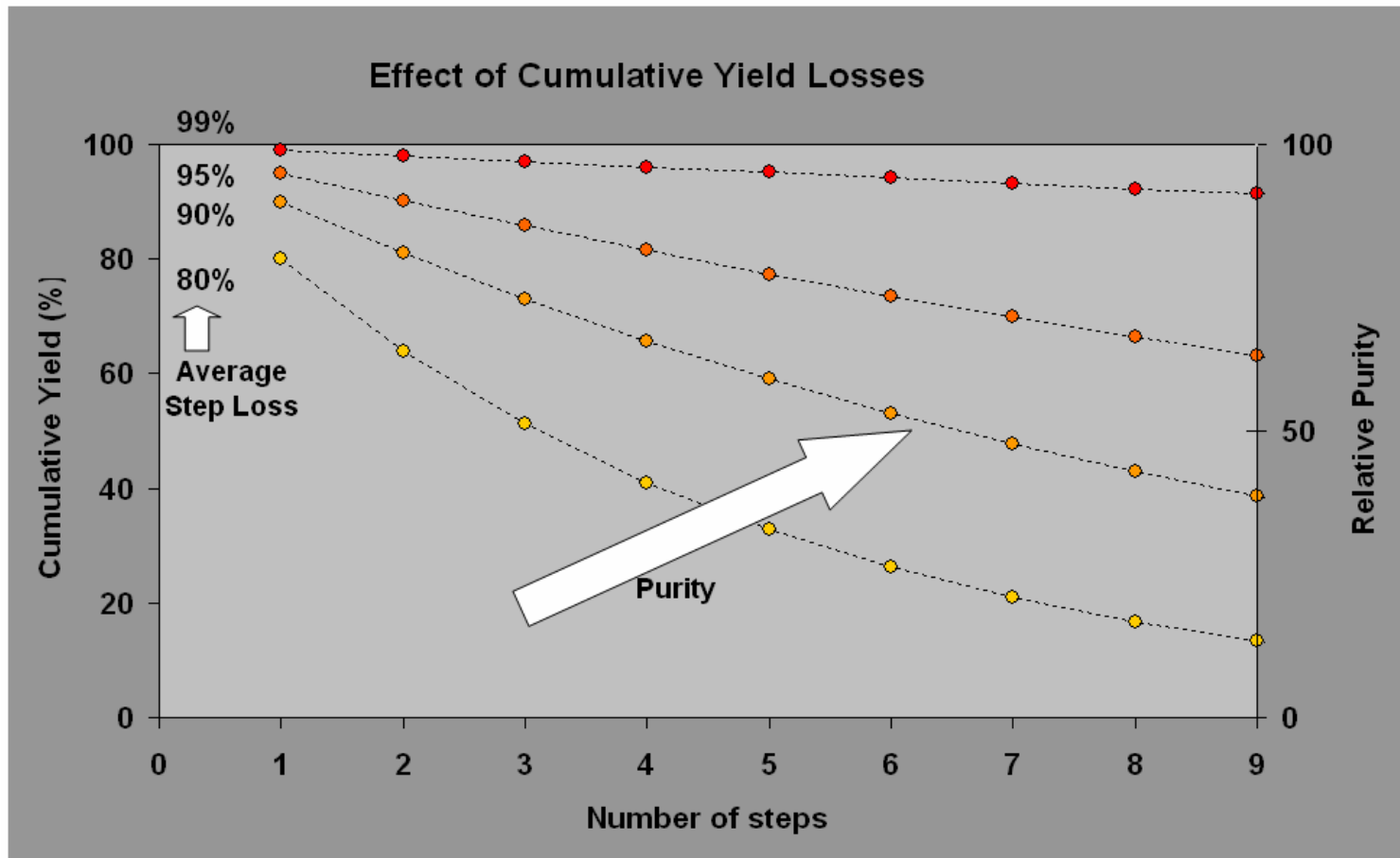


# Evolving Synthetic Affinity Resins - Overview

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- Principles governing selection of Affinity Materials
- Protein-A affinity chromatography for antibody purification
- Synthetic Approaches
- Developing 2<sup>nd</sup> and 3<sup>rd</sup> Generation Products
- Current Progress on Development Products

# Evolving Synthetic Affinity Resins: Yield



- Standard purification methods reflect a compromise between high purity and minimum number of process steps



# Why Protein A?

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- Reliable for most conventional monoclonals
  - Offers “platform technology” for direct capture following clarification
- High capacity
- High flow rates
- Re-useable
- Compatible with multitude of cell culture components
- Well established in regulated processes
  - No shocks in regulatory submissions
- Simplified process development

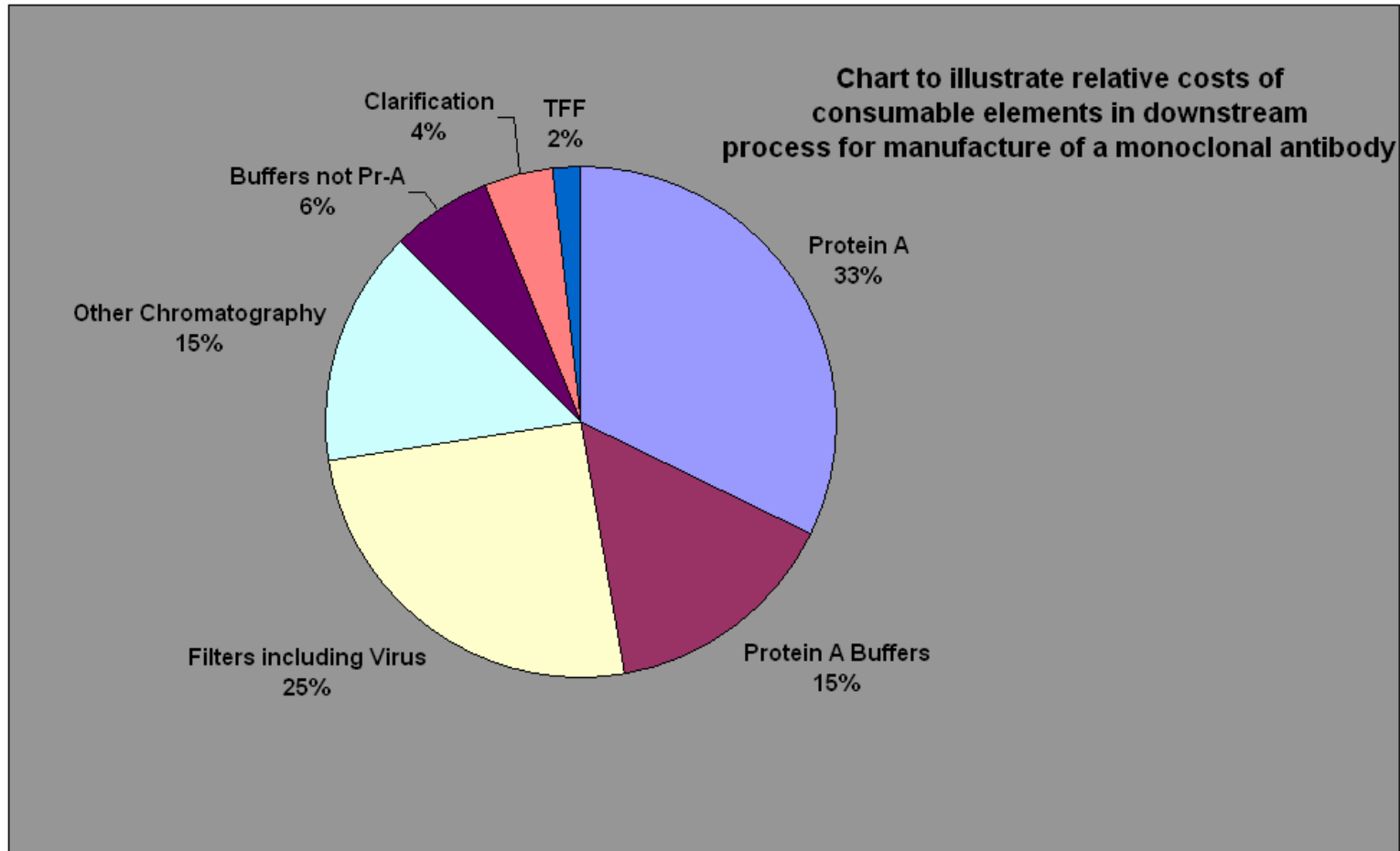


# Protein A: Financial Elements

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- High purchase costs
  - Up to 10x more expensive than ion-exchange media
- High running costs
  - Requires exotic clean-in-place procedures to avoid use of NaOH
  - Increases financial risk
    - Project management
  - Increases likelihood of column cycling
    - Increases labour costs and process time

# Protein A Financials



- Relative to other consumables, may represent as much as 50% of a downstream process

# Application of Protein A

- Requires development of secondary chromatography step to remove Protein A leachates
- End product QC testing for absence of Protein A
- Loss of large-scale columns that have become “infected”
- NaOH “resistant” variants:

Virus	✓	
Bacteria	✓	x
Bacterial Spores	x	
Endotoxin	x	

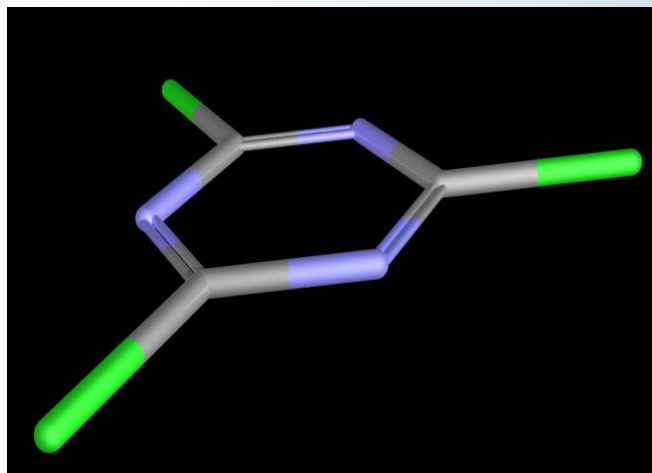
# Synthetic Alternatives

## ■ What if?

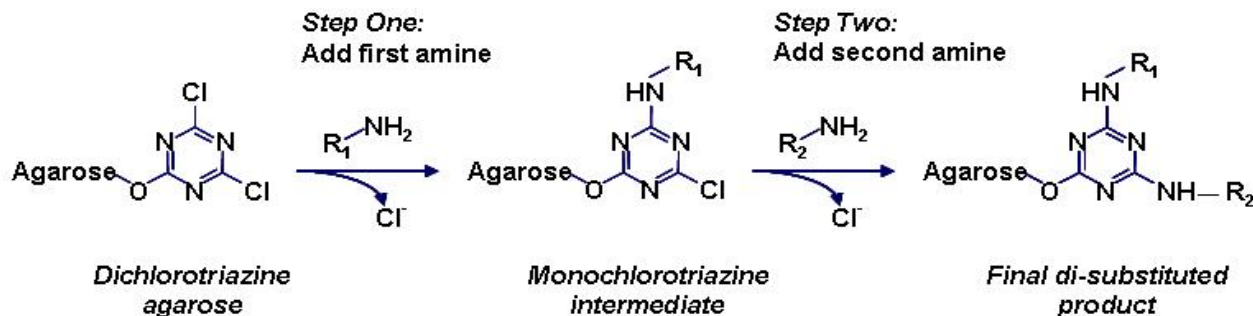
- A product was available that retained all the benefits of a Protein-A ligand...
- and also with improved performance based on
  - real NaOH compatibility
  - ligand stability
  - purchase and running costs
  - toxicity
  - immunogenicity?

Operating Performance	
Linear Velocity (non-binding)	<600 cm hr <sup>-1</sup> , up to >1000 cm hr <sup>-1</sup>
Linear Velocity (binding)	150 – 600 cm hr <sup>-1</sup>
Bed Performance	Scalability, no bed re-arrangements. Or, repeatable pack/unpack.
Cycling	100 – 250 cycles
Others	
Antibody Yield	>95%
IgG sub-classes	Human, humanised.
Binding environment	Clarified Cell Culture. Ionic strength: ~120 mM pH: 6.5 – 8.0
Pluronic	F68 Compatible
pH Stability	2-14 2-10

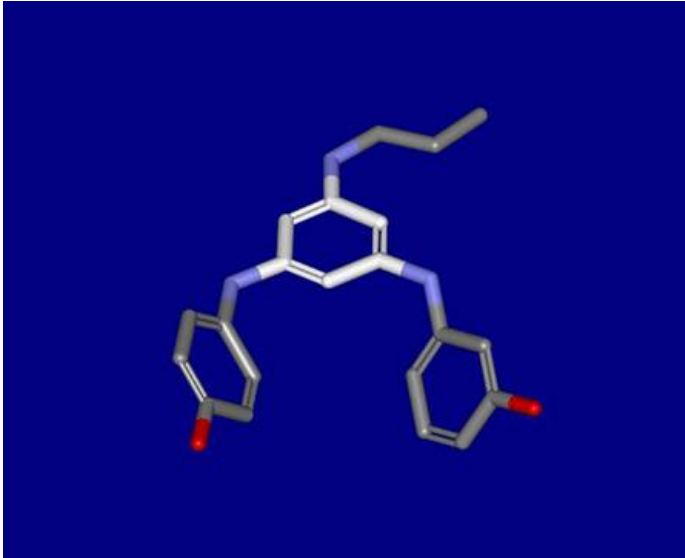
# Synthetic Affinity Ligands – Triazine Structures



- Tri-chloro-triazine
  - 6-atom ring of alternating N & C groups
  - Each C atom has a reactive Chlorine
- Non-toxic
- Chloride groups readily substituted
- Facile synthesis of triazine structure
- Huge number of amine based derivatives
- Base stable secondary-amino bond to functional groups



# Protein A Mimetic: MAbsorbent A2P

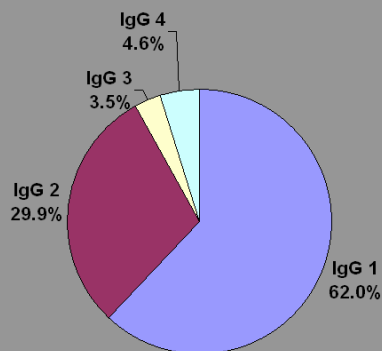


- Closely resembles Phe-Tyr dipeptide binding site in Protein-A
- Two C atoms bound via amine bond to functional groups
- These provide affinity functionality
- Third Cl is substituted for spacer arm linked to agarose bead
- Substituted groups are always:
  - Non-toxic
  - Non-mutagenic
  - Non-teratogenic

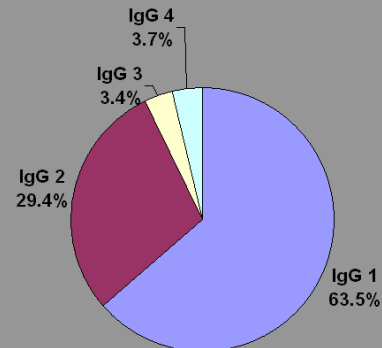
- Bind IgG at neutral pH; salt tolerant
- No requirement for addition of salt or detergents
- Mild elution of bound antibody (acidic pH and/or glycol)
- Good purity and recovery
- Easy to regenerate, resistant to protease/microbial degradation
- Can be sanitised with 1M NaOH
- Can be steam autoclaved
- Contaminant Removal
  - 4.9 LRV of MVM
  - >3.7 LRV of X-MuLV (physical partitioning)
  - 2.0 LRV X-MuLV (PEG Elution)
- DNA concentration reduced from  $\mu\text{g}$  to  $\text{pg}$  quantities – below the limit of detection of Pico Green
- CHO HCP reduced from 10,000 ppm to <100 ppm

# MAbsorbent A2P IgG Capture

Relative Fractions of IgG Sub-Types in Plasma



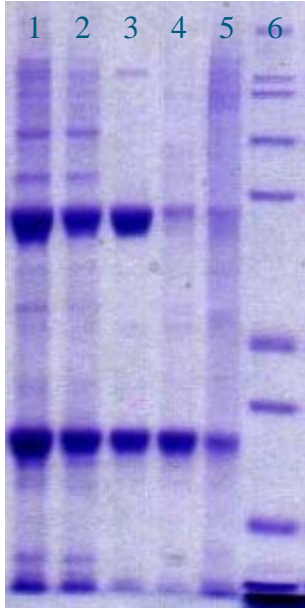
Relative Fractions of IgG Sub-Types in A2P HF Elution



- Illustrative of sub-type IgG capture from human plasma
- Very closely mimics Protein A Fc binding
- Both ligands bind to IgG at Fc region
- Bind human and murine IgG's
- Binding at neutral pH, elution at acidic pH, or by addition of chaotropes at neutral pH

- Binding capacity similar to Protein A: 30 – 40 mg mL<sup>-1</sup> typically encountered, including affinity for IgG3
- Difference in subclass selectivity – MAbsorbent A2P binds all human IgG subclasses including IgG3 and can be used for gamma globulin purification

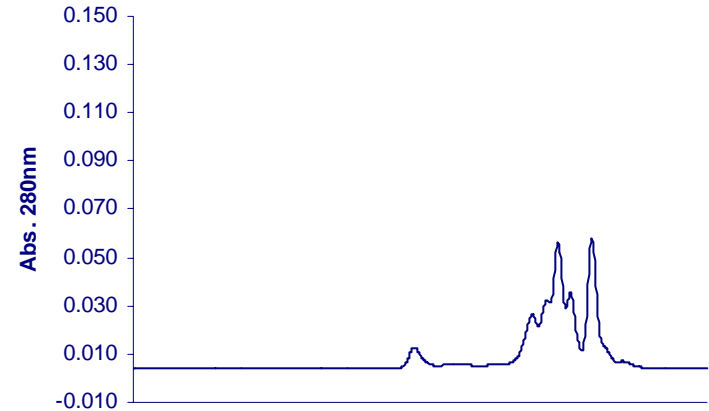
# Monoclonal Antibody Purification on MAbsorbent A2P



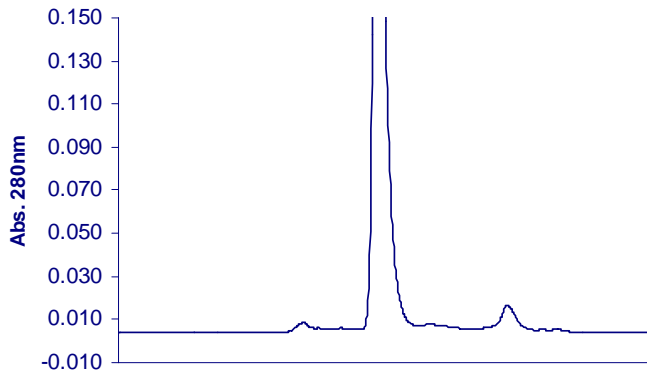
- 1: Culture Supernatant
- 2: Post CEX capture pool
- 3: pH 3.0 elution
- 4: Citric acid strip
- 5: 1M NaOH sanitization
- 6: Mol.wt. markers

■ A2P Eluate < 4% aggregate, significantly lower than Protein A eluate from same material

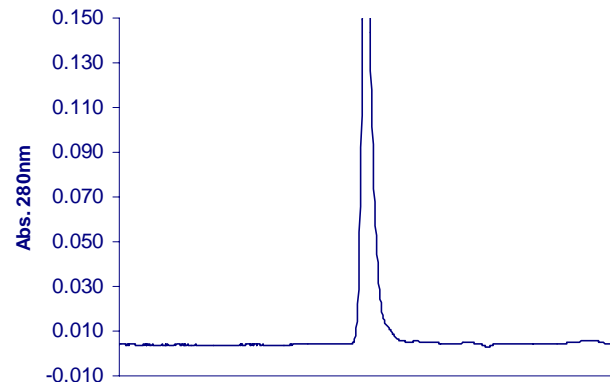
Cell Culture Supernatant



Post Cation Exchange Capture

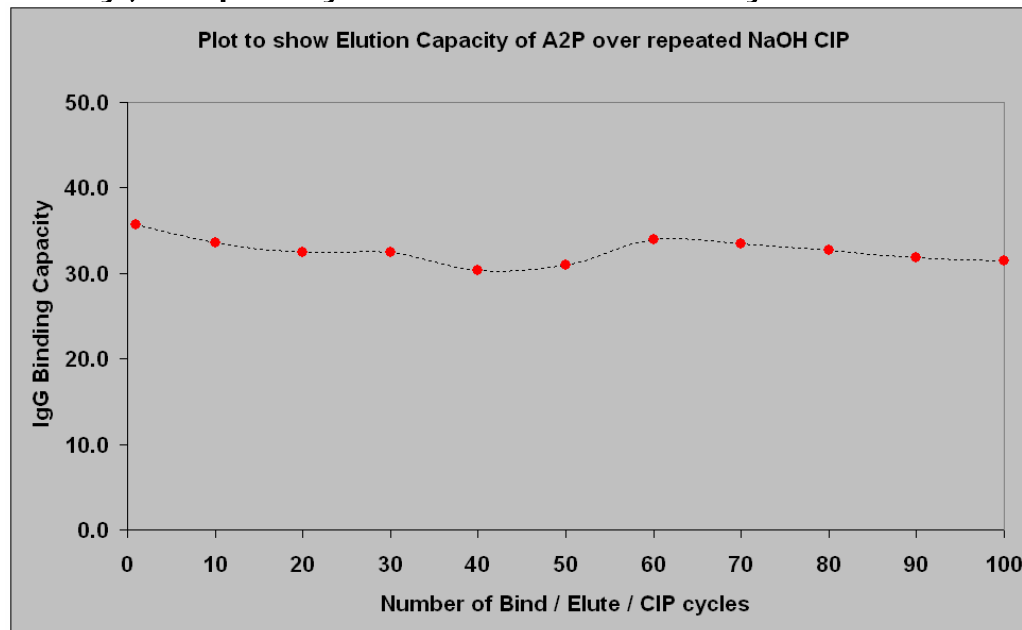


Elution MAbsorbent A2P



# MAbsorbent A2P: Regulatory Support

- NaOH Stability: After 5 days exposure to 1M NaOH at 40°C, 250 ng ligand detected, per mL of adsorbent.
- Toxicology: exposure to 60,000 fold “worst case scenario”- based on the 5 day NaOH study - no toxicological effects.
- Re-use: 100 cycles through 1M NaOH, no loss of binding or elution capacity, or purity as determined by GPC and SDS-PAGE





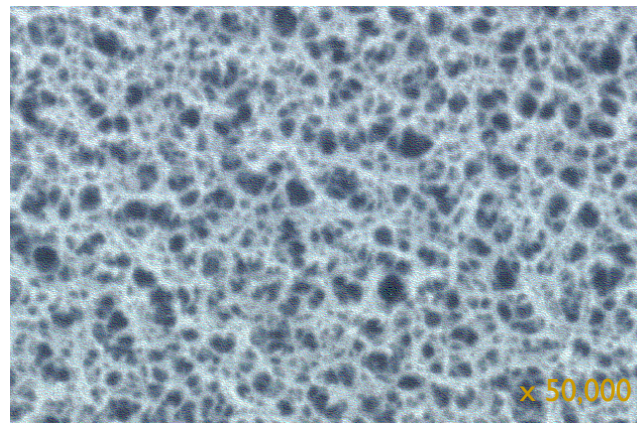
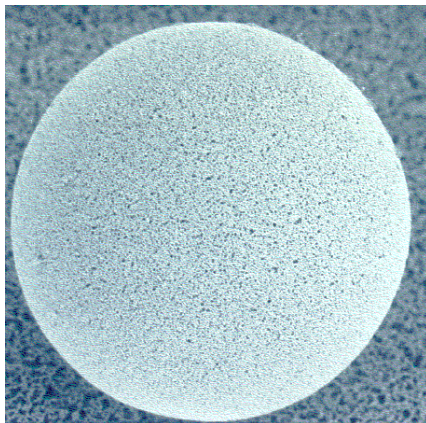
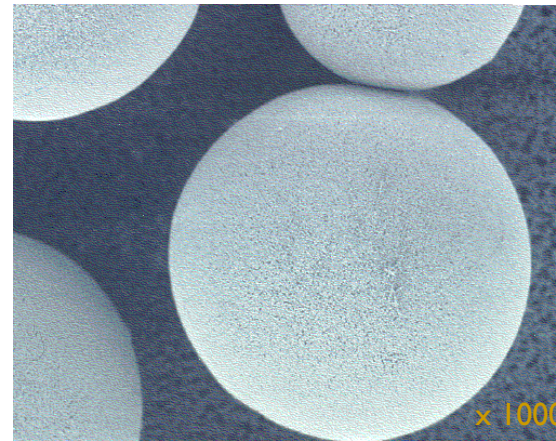
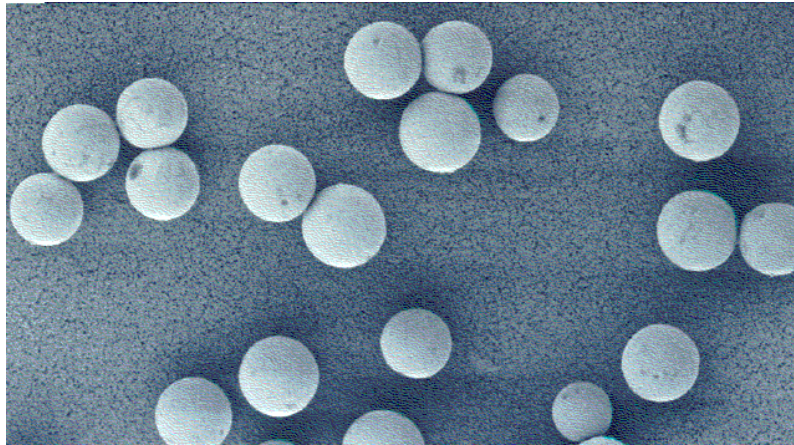
# MAbsorbent A2P High Flow

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- Same A2P ligand – specificity for IgG
  - PuraBead HF – base matrix – 3-fold area of development
- 1) Improved cross-linking chemistry
    - More robust base matrix
    - Resists shrinking and swelling on buffer changes
  - 2) Ligand Density
    - Improved dynamic binding capacity
  - 3) Spacer Chemistry
    - Hydrophilic spacer arm
    - Enhanced ligand accessibility
    - Improved product purity

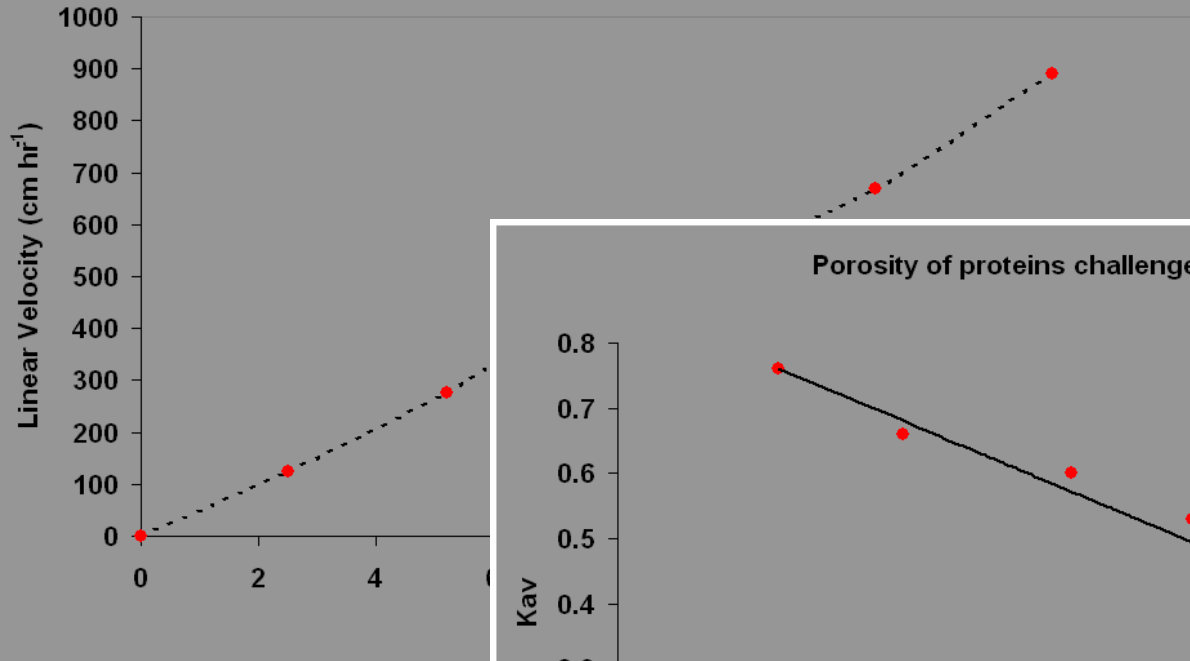


# PuraBead<sup>®</sup>

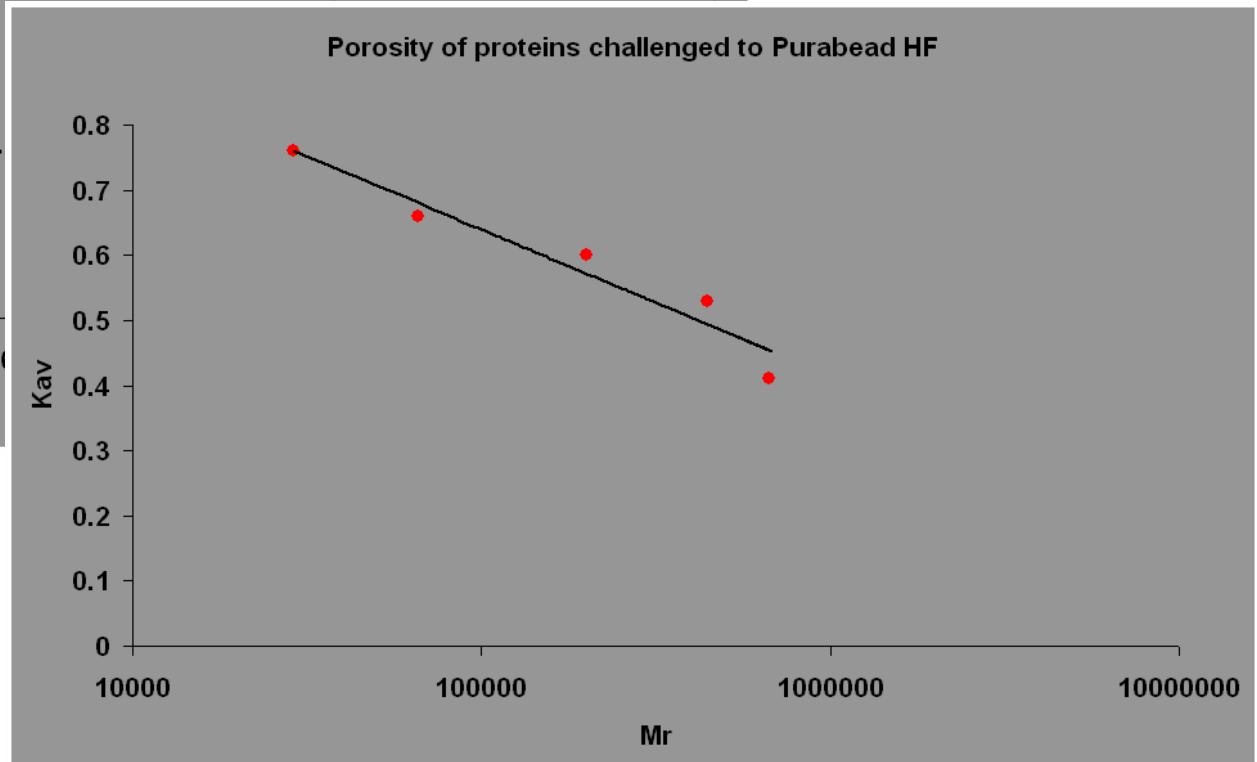


# PuraBead 6HF - Properties

Plot to show linear velocity as a function of pressure drop for Purabead HF



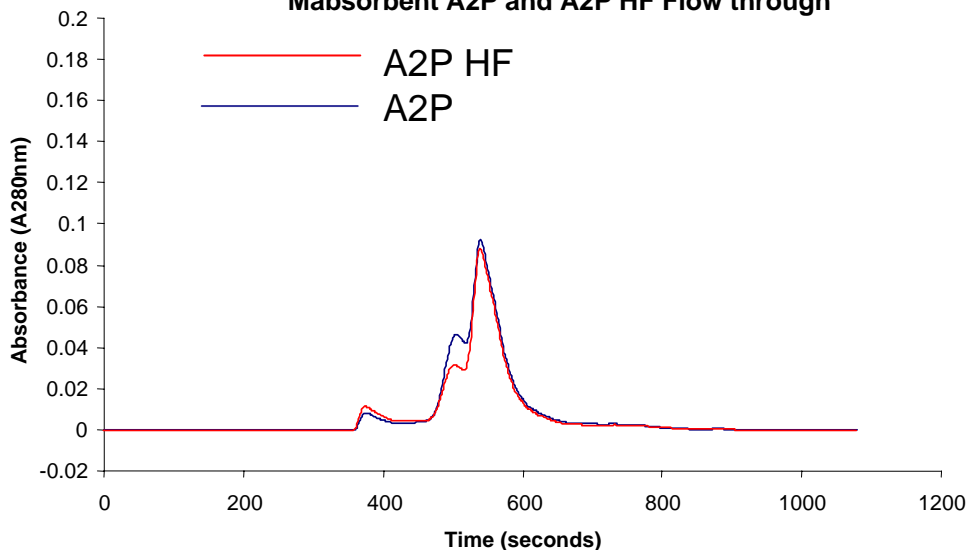
Porosity of proteins challenged to Purabead HF



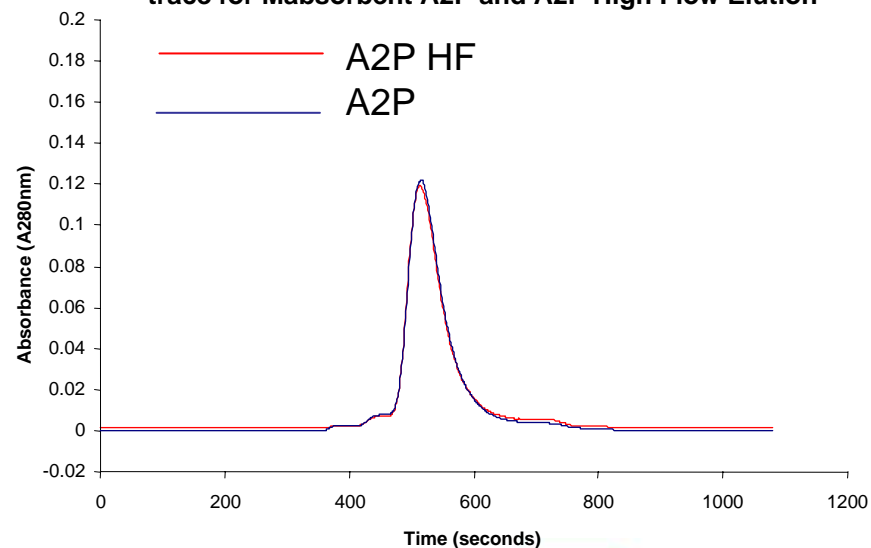
# Gel Permeation Chromatography of key samples challenged to A2P and A2P HF

## ■ Flow through

Gel Permeation Chromatography trace for Mabsorbent A2P and A2P HF Flow through

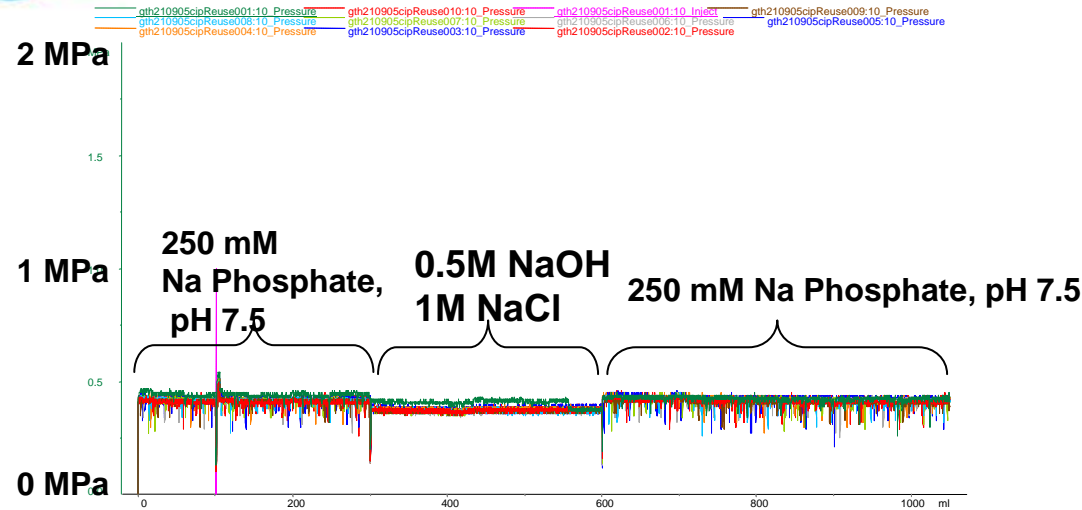


Gel Permeation Chromatography trace for Mabsorbent A2P and A2P High Flow Elution

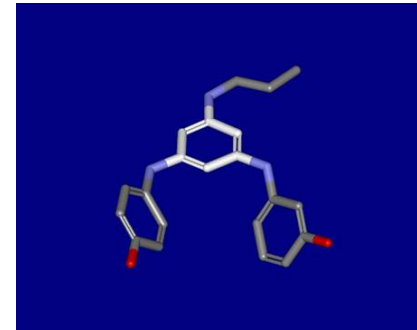


- Elution –difference in profile attributed to elevated capacity
- Specified at  $> 38 \text{ mg mL}^{-1}$

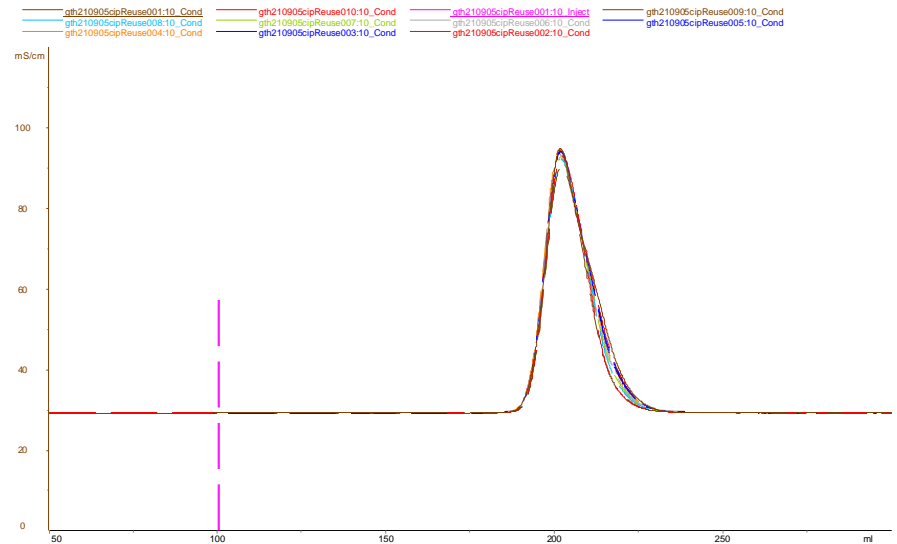
# A2P HF: Pressure response to NaOH cycling



■ 10 cycles through NaOH, at 300 cm hr<sup>-1</sup>



- No significant bed rearrangements over the same 10 cycles
- Consistent and reproducible bed performance



# Summary MAb Absorbent A2P HF

Parameter	Value	
Static Binding Capacity	~70 mg mL <sup>-1</sup>	✓
Dynamic Binding Capacity	~37 mg mL <sup>-1</sup> @ 600 cm hr <sup>-1</sup> ~41 mg mL <sup>-1</sup> @ 500 cm hr <sup>-1</sup>	38 mg mL <sup>-1</sup> @ 150 cm hr <sup>-1</sup>
\$ per g MAb	<< \$316 g <sup>-1</sup>	\$100 – 150 g <sup>-1</sup>
Contaminant and Impurity Removal		
Host Cell Protein	220,000 reduced to 1000 ng HCP mg <sup>-1</sup> MAb	3 LRV
DNA	3 log reduction: 10 µg mL <sup>-1</sup> to < 10 ng mL <sup>-1</sup>	3 LRV
Virus	LRV 4 for MVM, not pH based clearance	4.9 LRV MVM
Albumin	Tolerate 4 mg mL <sup>-1</sup> feed	✓
Cell Culture Components	Tolerate phenol red, cholesterol, surfactants etc.	A3P HF

# Summary of MAbsorbent A2P HF

Operating Performance		
Linear Velocity (non-binding)	<600 cm hr <sup>-1</sup> , up to >1000 cm hr <sup>-1</sup>	✓
Linear Velocity (binding)	150 – 600 cm hr <sup>-1</sup>	✓
Bed Performance	Scalability, no bed re-arrangements. Or, repeatable pack/unpack.	✓
Cycling	100 – 250 cycles	✓
Others		
Antibody Yield	>95%	✓
IgG sub-classes	Human, humanised.	✓
Binding environment	Clarified Cell Culture. Ionic strength: ~120 mM pH: 6.5 – 8.0	✓
Pluronic	F68 Compatible	A3P HF
pH Stability	2-14 2-10	✓

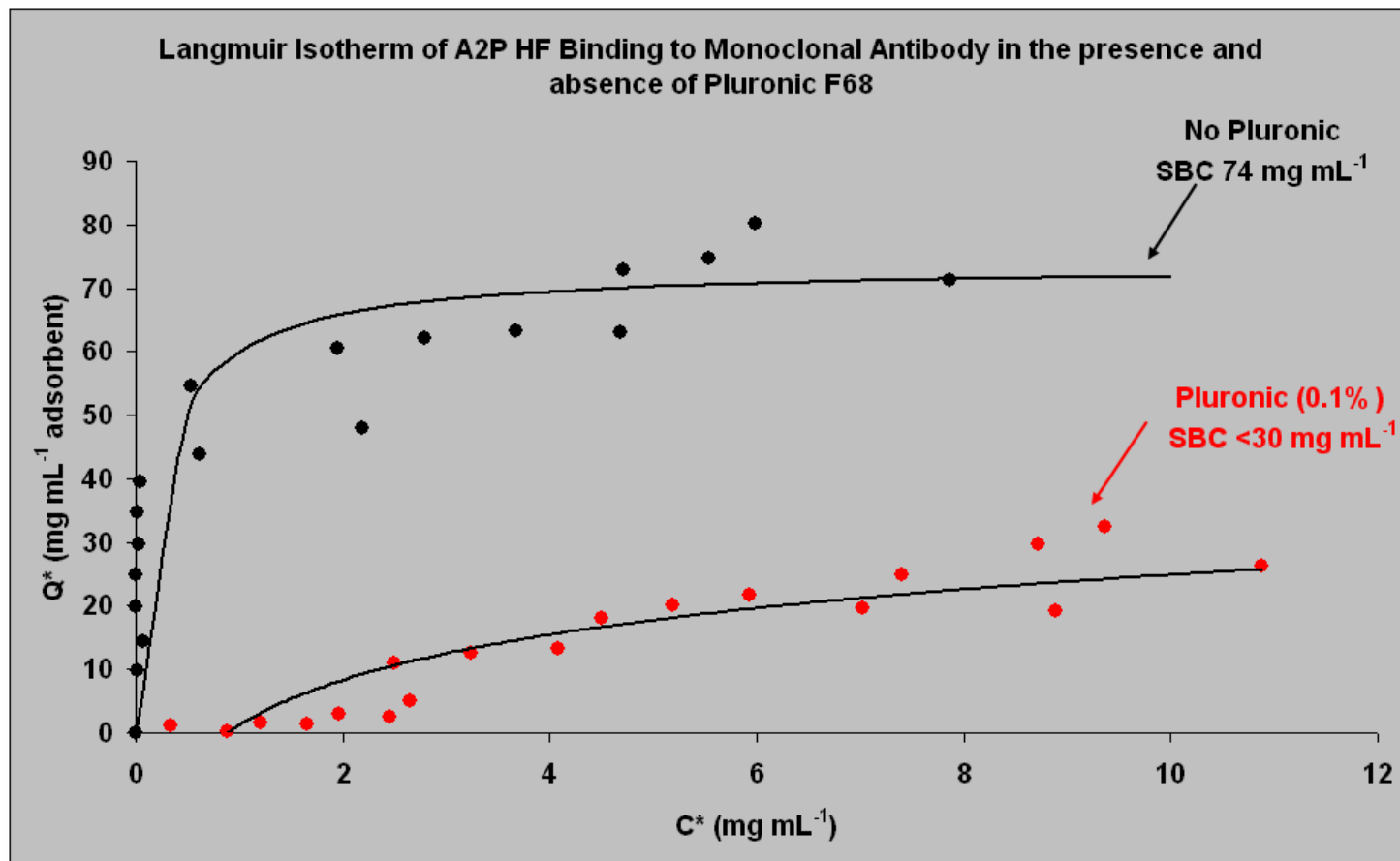


# Summary of MAbsorbent A2P HF

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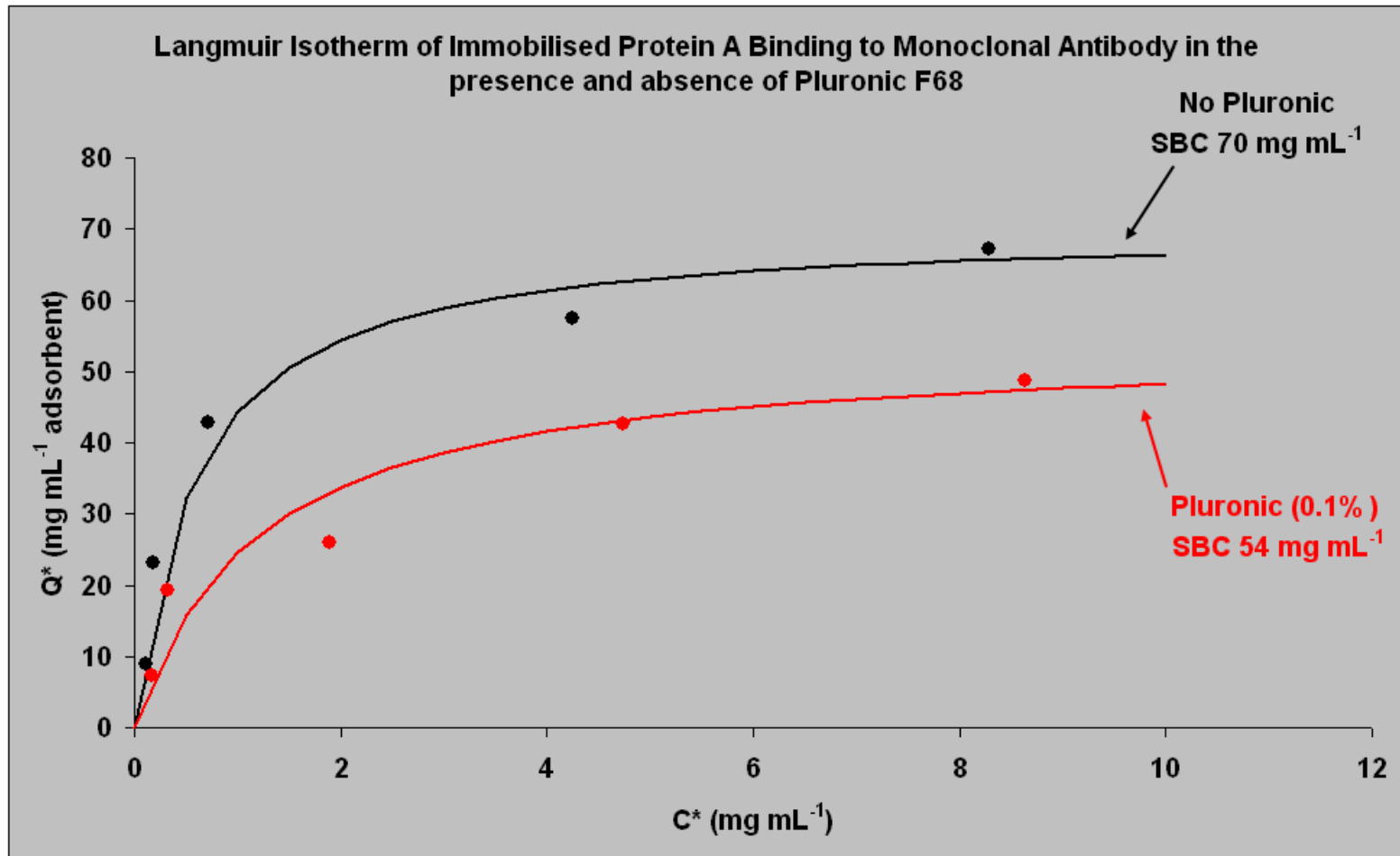
- Through development of key parameters, and understanding chemistry behind the ligand, a robust adsorbent has been developed
- Capable of use in monoclonal manufacturing
- Limited to polyclonal serum-based products or cell cultures without Pluronic F68
  - Pluronic sensitivity can be overcome with ion exchange as an initial capture step
  - Application of a pre-treatment column to remove Pluronic F68 can also enable A2P HF application
- But pre-treatments conflict with process simplification and cost reduction demands of regulated processes
- Which has prompted research into Pluronic F68 effects and its incompatibility with synthetic ligands

# Effect of Pluronic F68 on A2P HF Binding Capacity

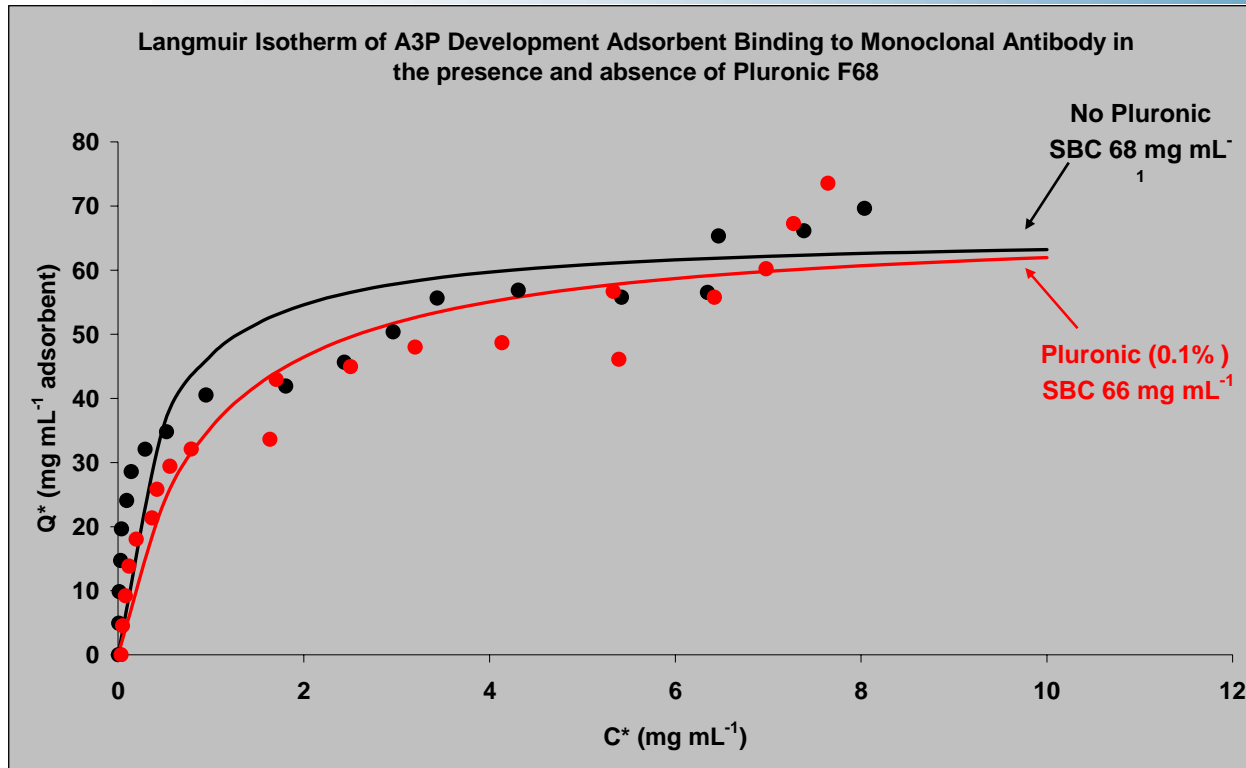


- Isotherms produced over 24 hr incubation
  - Indicates a dynamic between pluronic, adsorbate and antibody

# Immobilised Protein A and Pluronic F68

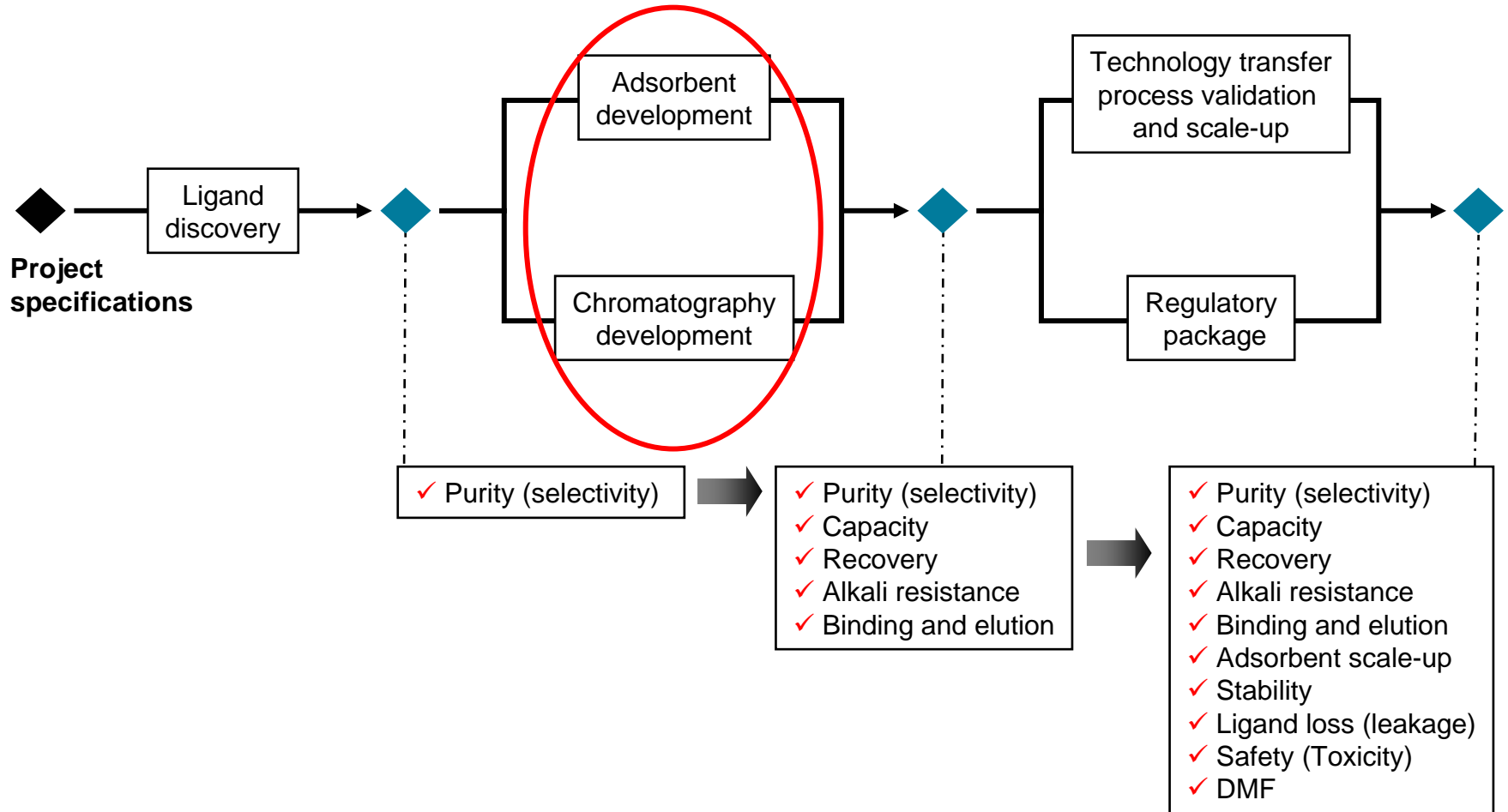


# A Pluronic F68 resistant synthetic ligand: A3P



- In this example, an antibody specific ligand has been engineered to tolerate the presence of Pluronic F68
- Binding capacities are compatible with those demanded for manufacturing processes
- Launch – H2 2006

# Progress of MAbsorbent A3P R&D





# Future Work and Conclusions

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- A2P HF

- Continue to explore scale-up

- A3P

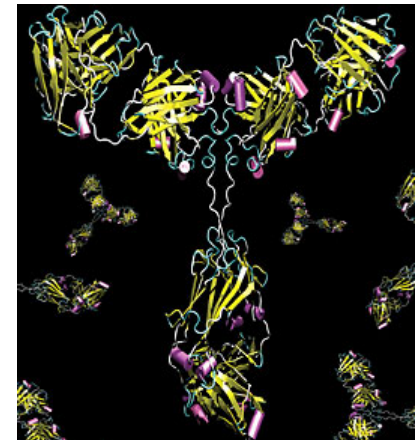
- An R&D project

- Ligand density, orientation, spacer arm, bioprocess performance

- Manufacturing scale-up, tech transfer, regulatory support

Address all antibody affinity purification needs through application of a single platform technology

- A1P Murine antibodies
- A2P Monoclonals & Polyclonals
- A3P Monoclonals
- A4P Antibody fragments
- A5P Antibody fragments
- ...
- Custom Unique engineered antibodies & fragments





# Acknowledgements

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- A2P and A2P HF
  - Guy Harris
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  - Sharon Williams
  - Carina Lobley
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  - Hussan Mamdani