Comparison of standard and new generation HIC technologies in the monoclonal antibody purification process

Jie Chen, Jen Tetrault, Art Ley

Process Sciences Department, DYAX CORP.

Outline

1. **HIC Chromatography in mAb purification process**

2. **HIC step development and comparison study**
   - **HIC Purification Block Study;**
   - **Process Flow/Platform Study**

3. **Conclusion.**

Current Industry trends in mAb Product Development

Upstream-Downstream Bottleneck Shift

High throughput, Low cost and High purity DSP Driving Wave

Risk Assessment Philosophy Evolving

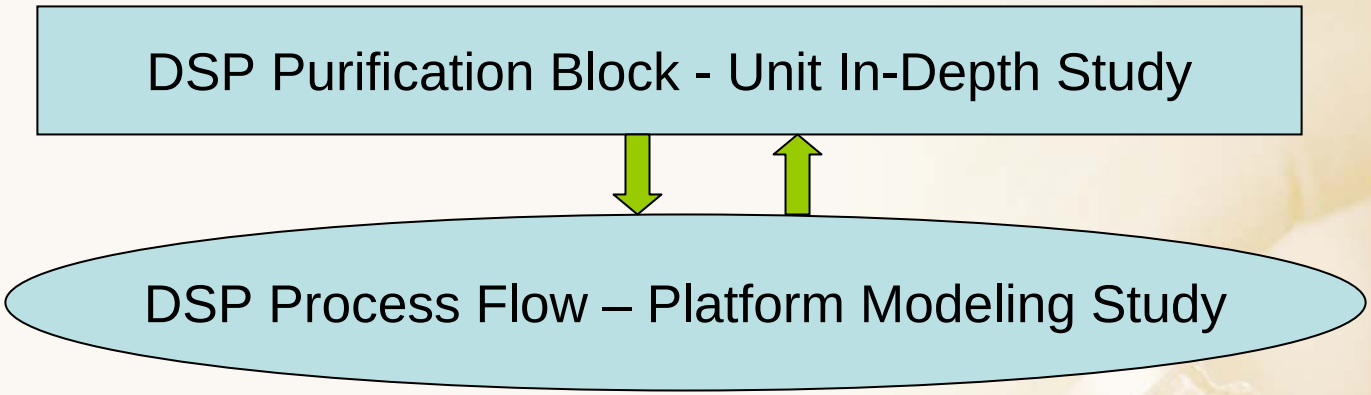
Recent movements in chromatography fields for improving mAb purification

1. **ProA Resin:** **Mabselect SuRe – Improve CIP for resin-reuse,**
   Mabselect Xtra – Improve binding capacity.
2. **Ion Exchange Resin:**
   Capto S, Capto Q,
   TMAE Hicap,
   Toyopearl GigaCap S
   } **Improve binding capacity**
3. **HIC Resin:**

   1970s HIC development → 1995 US patent#5429746 → Present

   - **Toyopearl PPG600M, Butyl-600M, Phenyl-600M**
     Optimization of HIC resin pore size for mAb molecules
   - **Hydrophobic Charge Induction Chromatography (HCIC) - "mixed-mode" of HIC-IEX separation changes standard**
     HIC resin-IgG-salt relationship

Dyax Process Development Strategy



Optimal Process

Powerful Individual technology block

Optimal process flow platform

Product stable condition throughout the process

Main Variables in HIC Purification Block Study

HIC Resins

Toyopearl Ether-650M

Phenyl-650M

Butyl-650M

Hexyl-650C

Toyopearl PPG-600M

Butyl-600M

MEP

Buffer

Salt NaCl

NaCitrate

Na_2HPO_4/NaH_2PO_4

NaAcetate

$(NH_4)_2SO_4$

Na_2SO_4

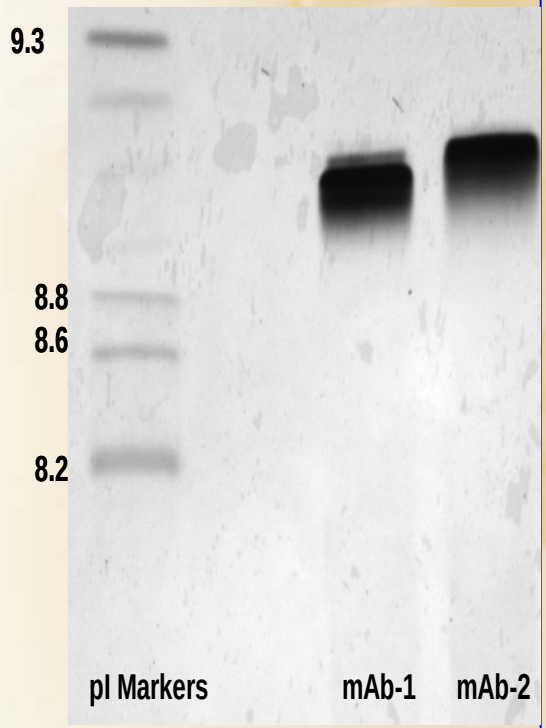
pH pH6 (ProA E^N)

pH7.2 (CEX E)

pH8.5 (AEX FT)

mAb

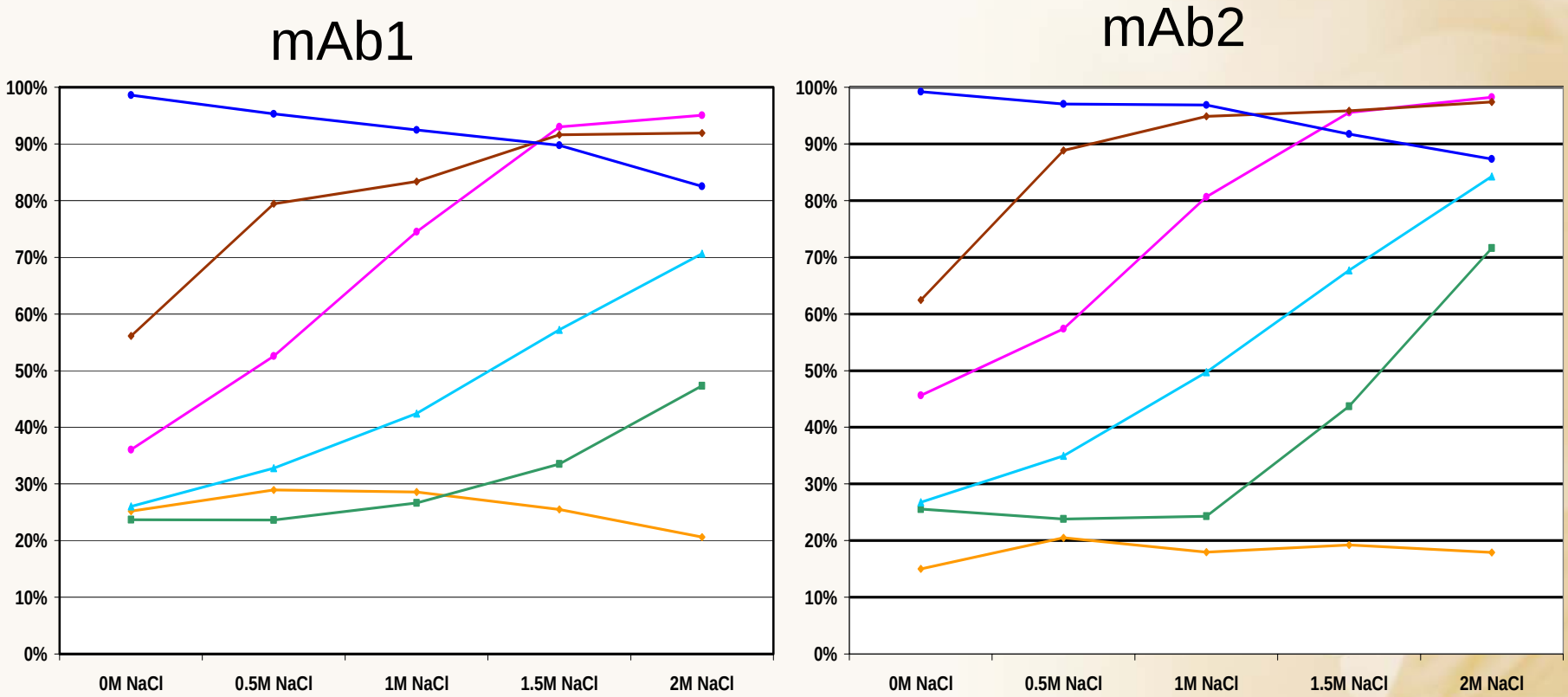
mAb 1 and mAb 2



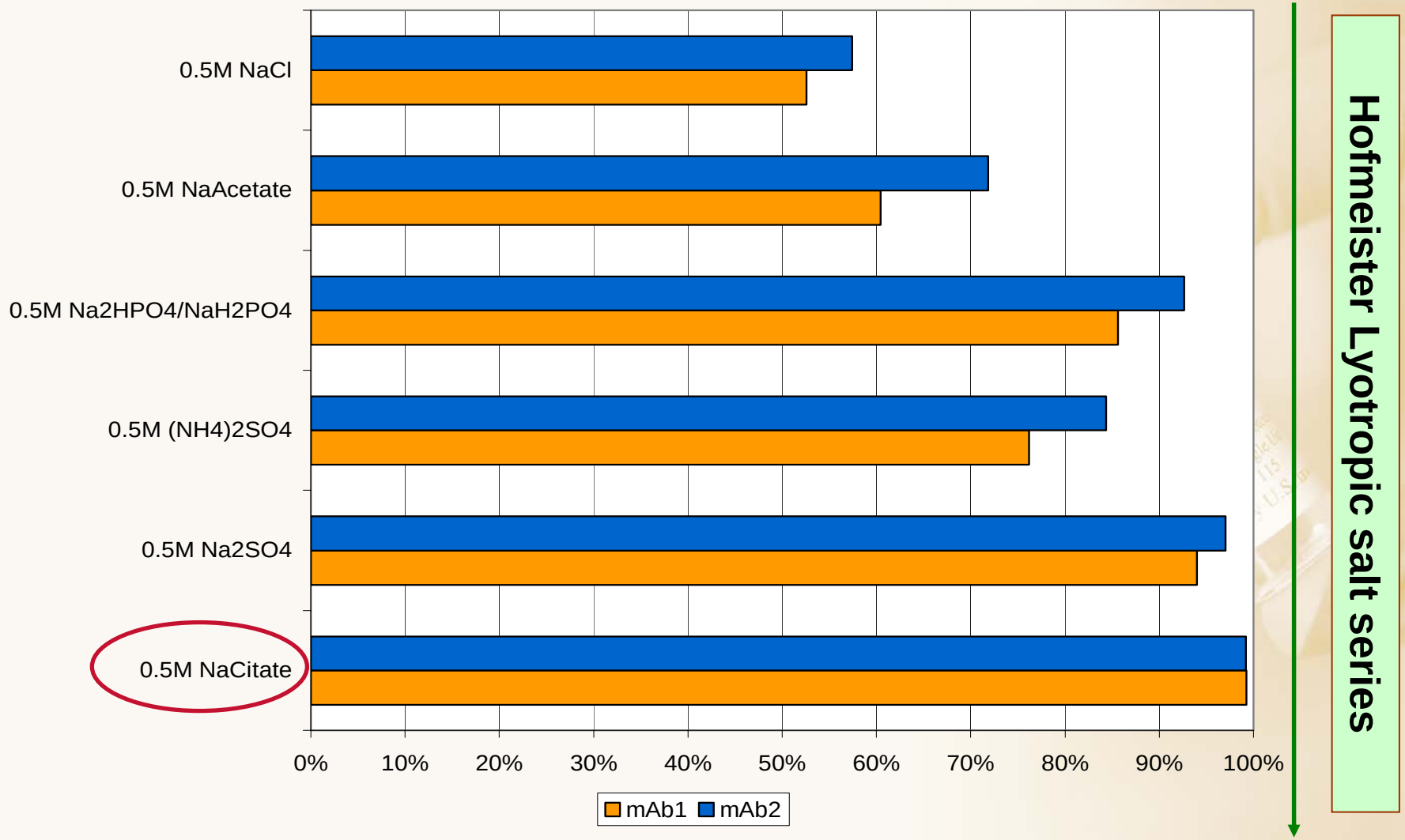
HIC resin screen:
mAb solubility in various salt / pH conditions

Salt	Salt concentration	Neutralized ProA E condition		CEX-E condition		AEX FT condition	
		pH 6		pH7.2		pH8.5	
		mAb1	mAb2	mAb1	mAb2	mAb1	mAb2
No salt added	0 M						
NaCl	0.5M						
	1M						
	1.5M						
	2M						
Na_2SO_4	0.5M						
	1M						
NH_4SO_4	0.5M						
	1M						
	1.5M						
NaCitrate	0.25M						
	0.5M						
	0.75M						
NaAcetate	0.25M						
	0.5M						
	0.75M						
Na_2HPO_4/NaH_2PO_4	0.25M						
	0.5M						
	0.75M						

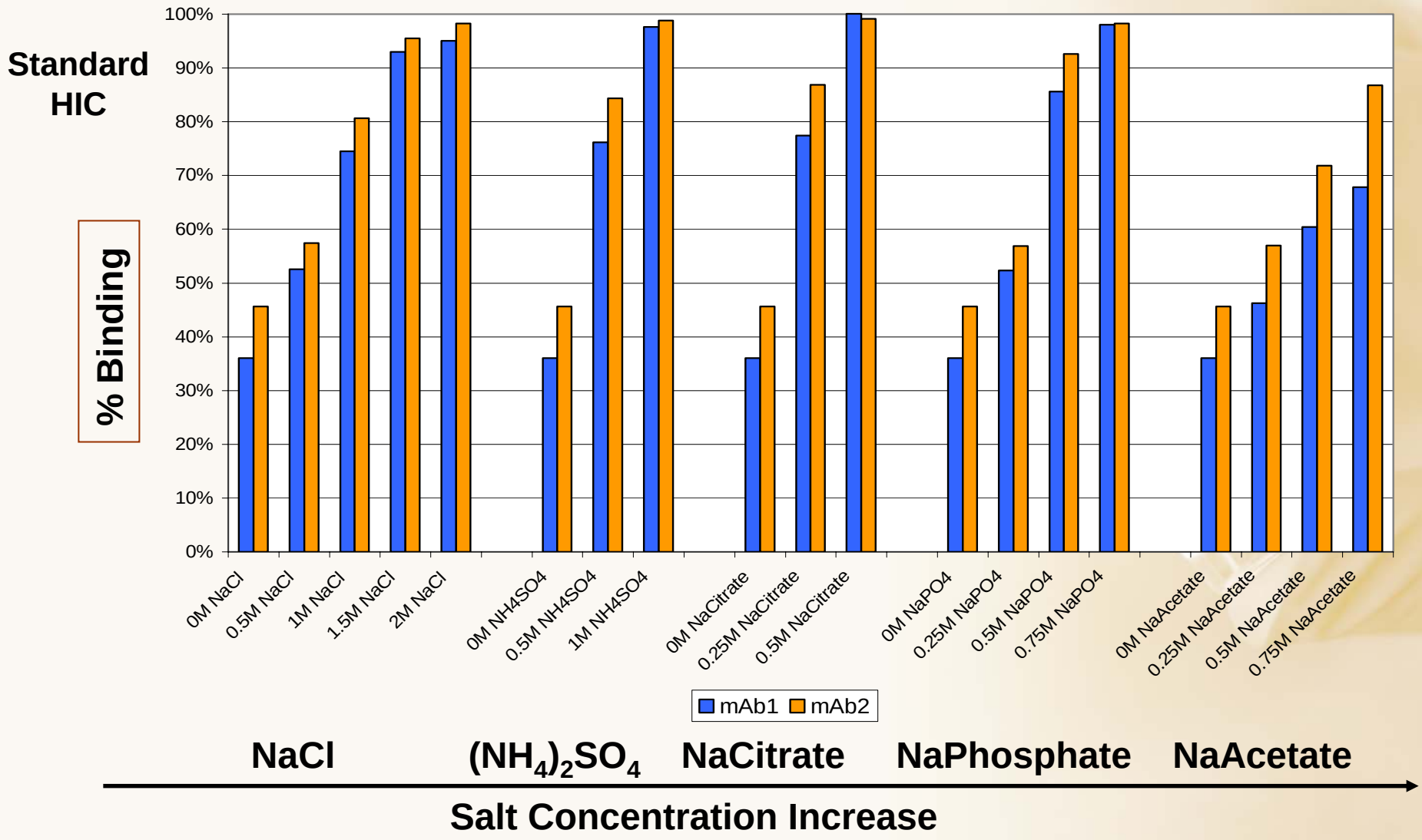
Salt Influence
HIC / MEP resin screen-mAb binding example



Relative Effectiveness of Various Salts on Standard HIC Binding

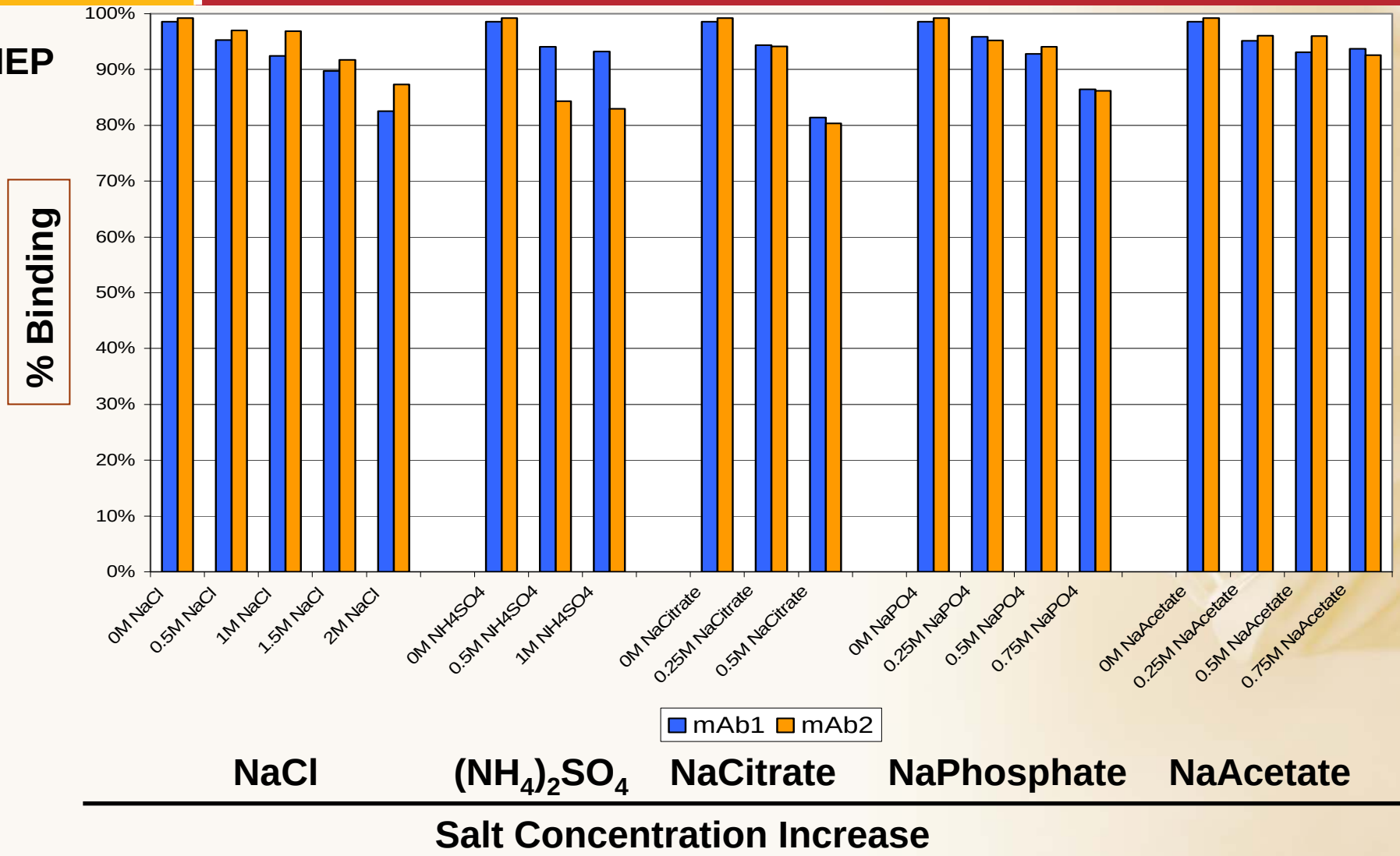


The Difference of salt effect on mAb binding
Standard HIC vs MEP resins

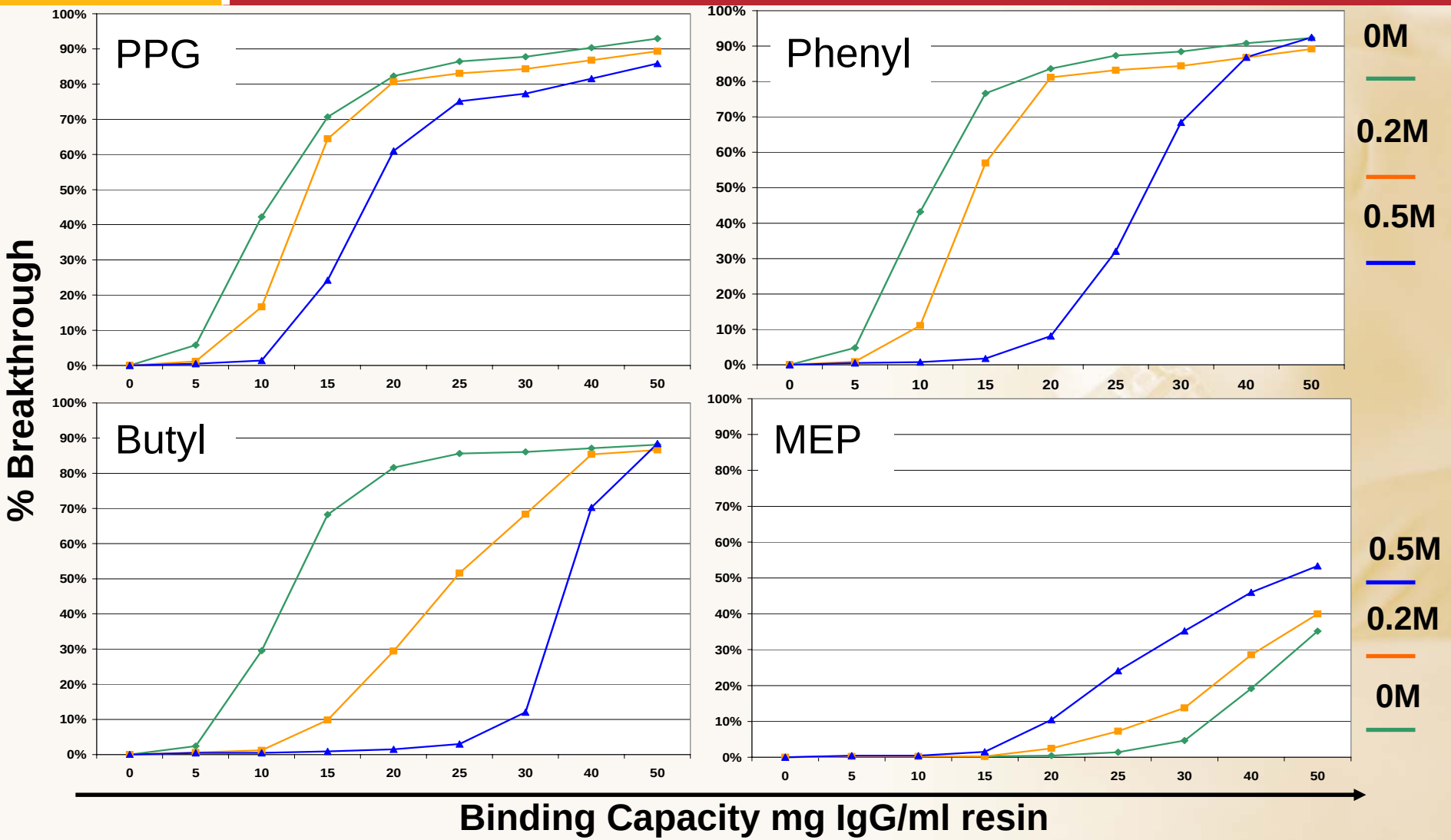


The Difference of salt effect on mAb binding
Standard HIC vs MEP resins

MEP



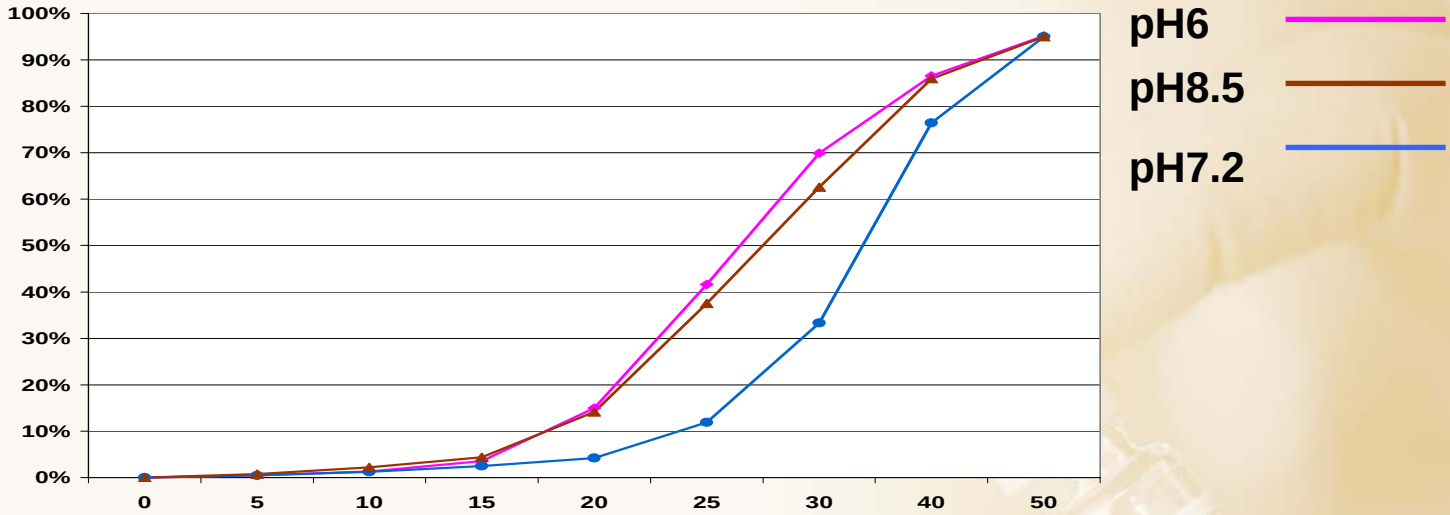
The Difference of salt effect on mAb binding Standard HIC vs MEP resins



pH Influence
HIC / MEP resin screen-mAb binding example

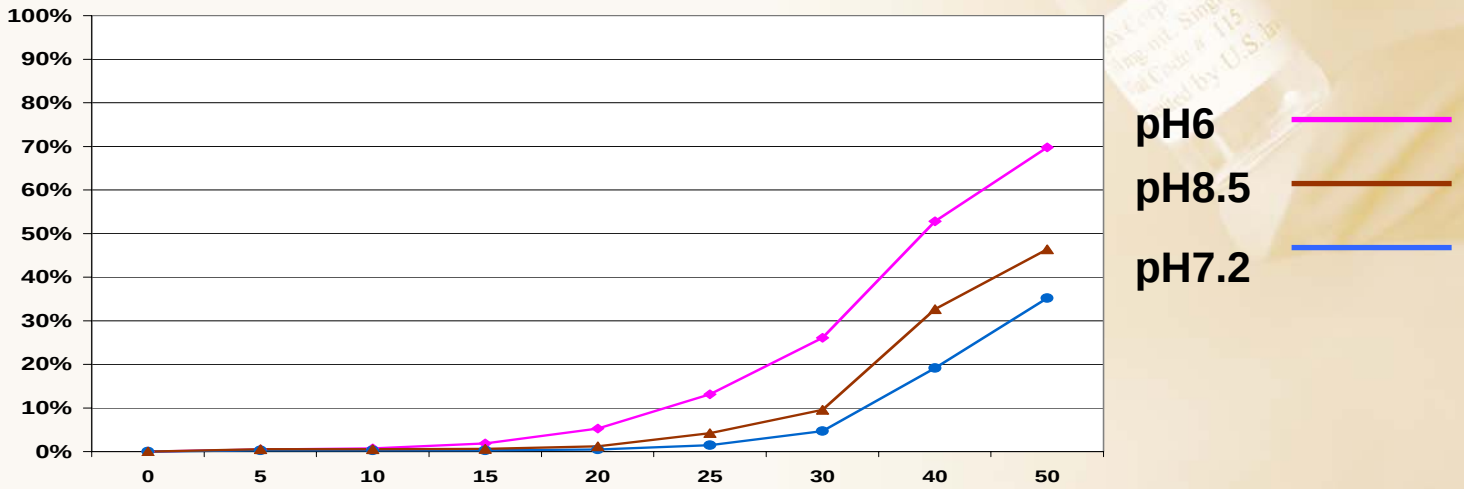
Butyl

Optimal salt concentration Added

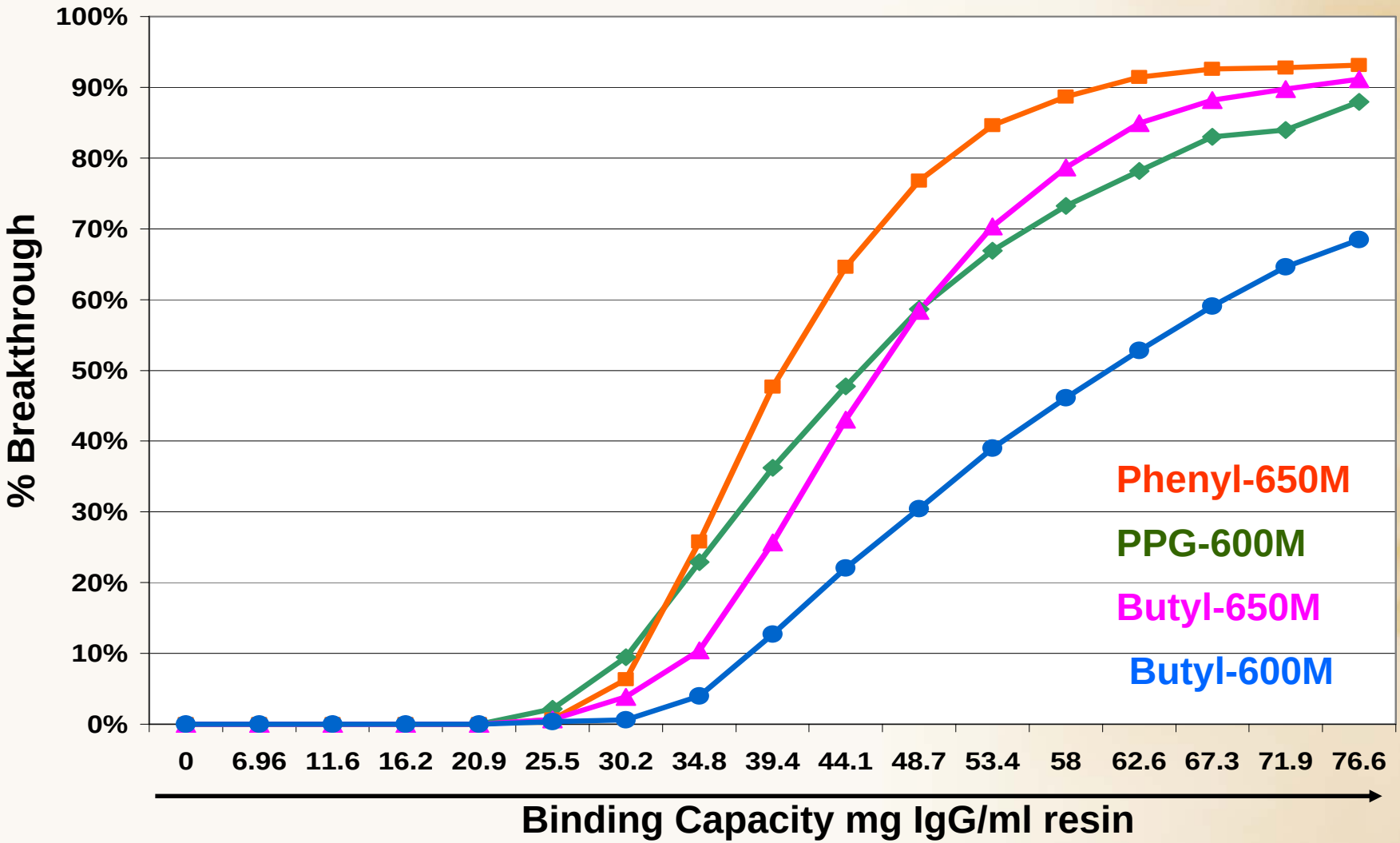


MEP

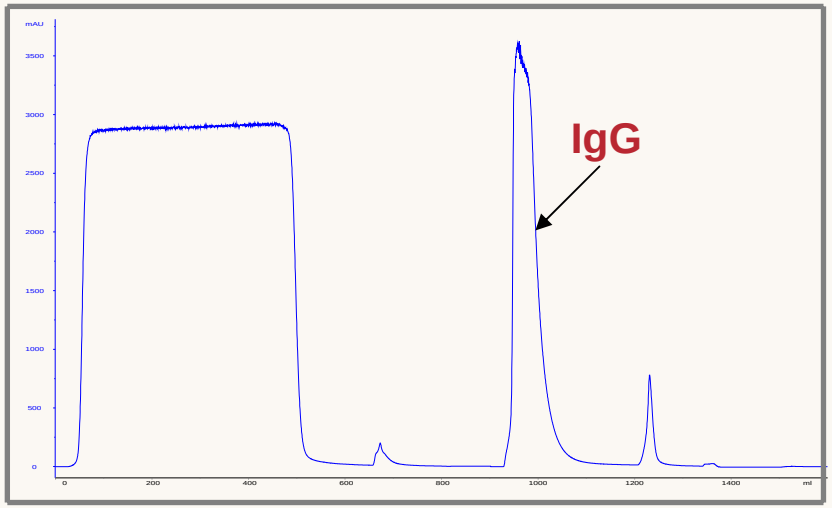
No additional salt added

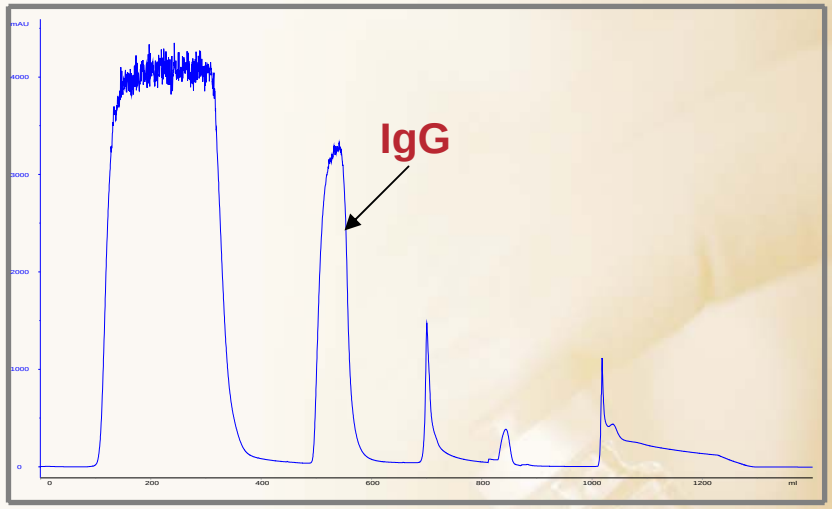


Ligand Hydrophobicity, Resin Pore Size Effect on mAb Binding at maximum mAb1 soluble salt concentration

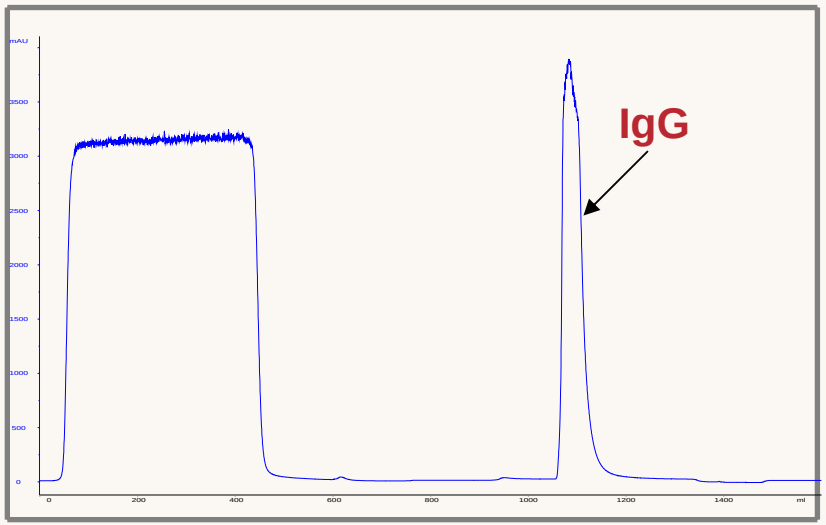


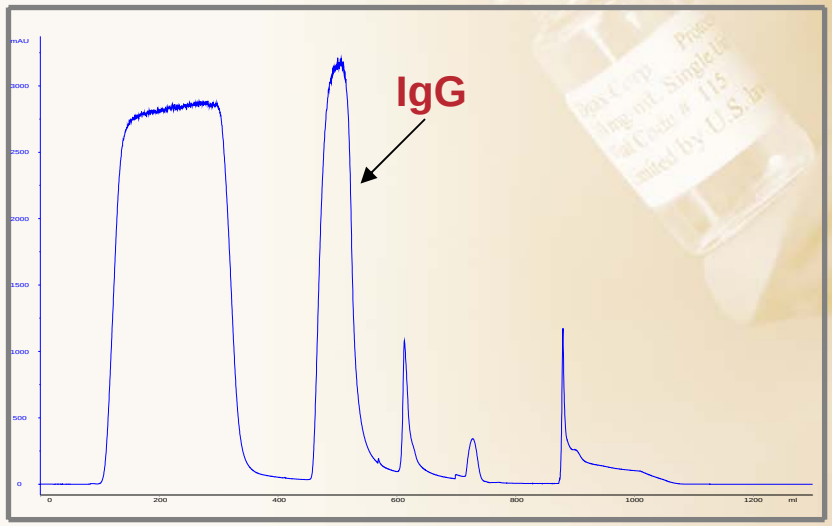
mAb Capture Step Purification
MEP vs ProA





mAb1



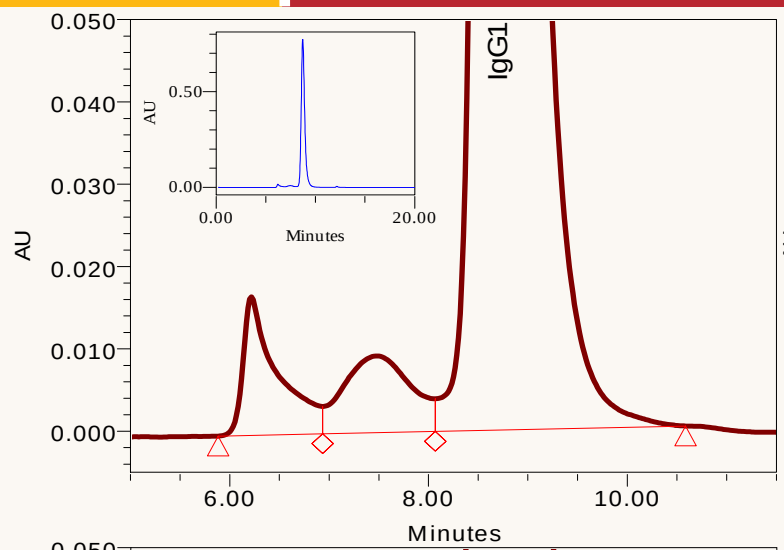


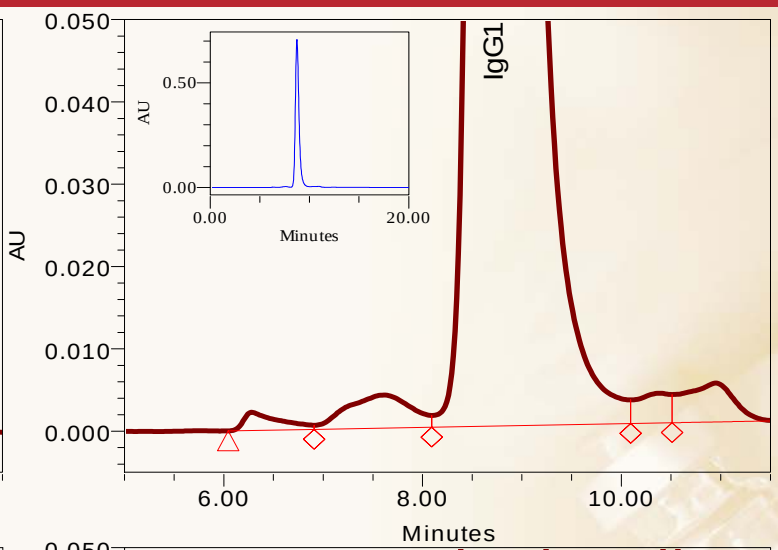
mAb2

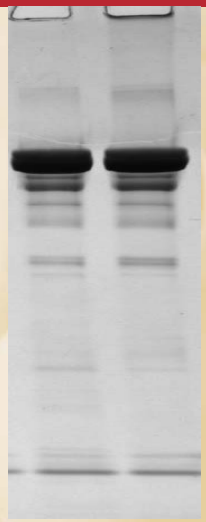
ProA capture

MEP capture

Product Quality:
MEP vs ProA as capture step

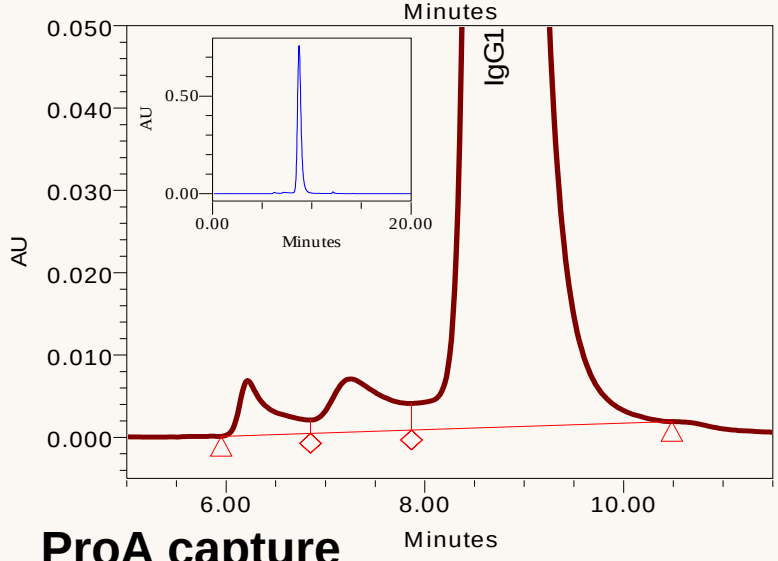




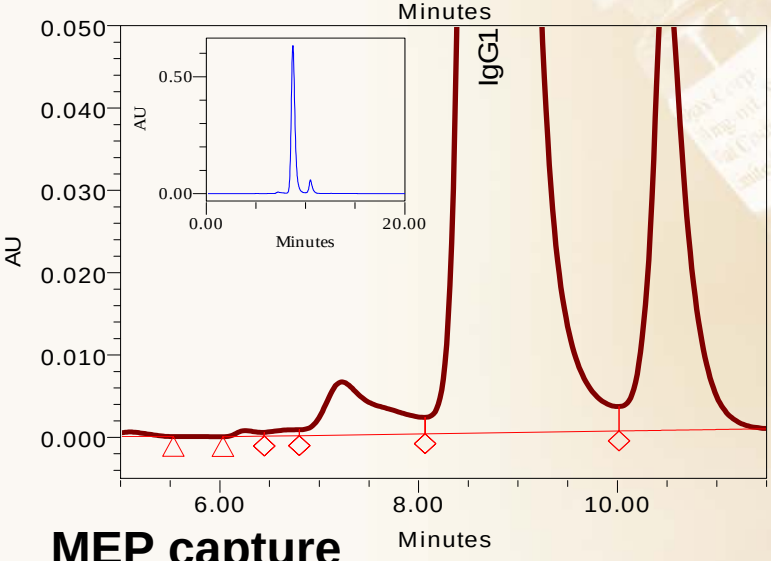


mAb1

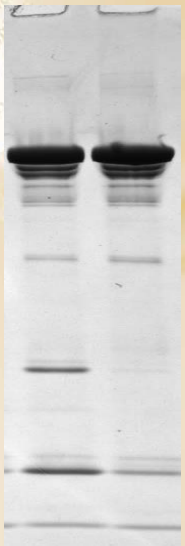
MEP **ProA**



ProA capture



MEP capture

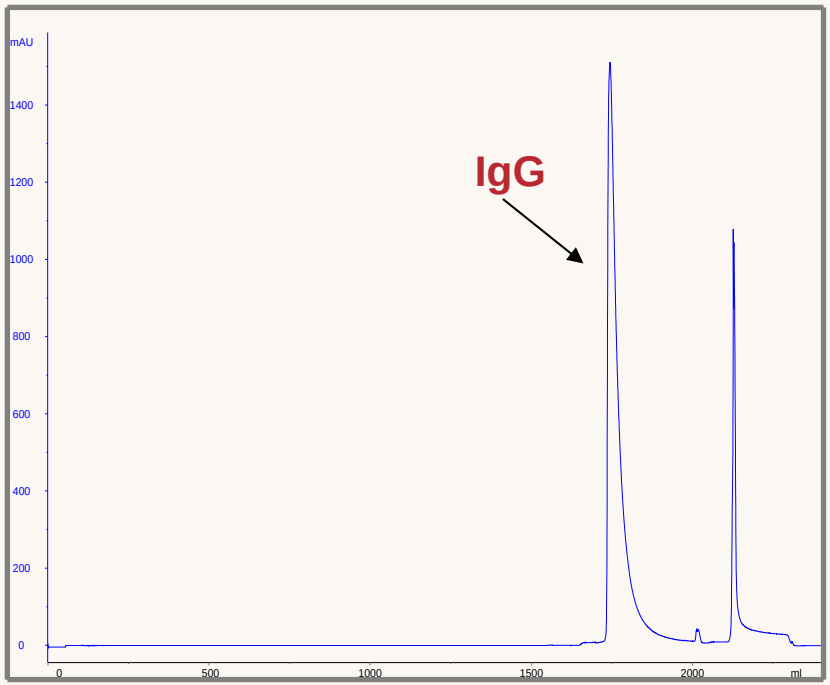


mAb2

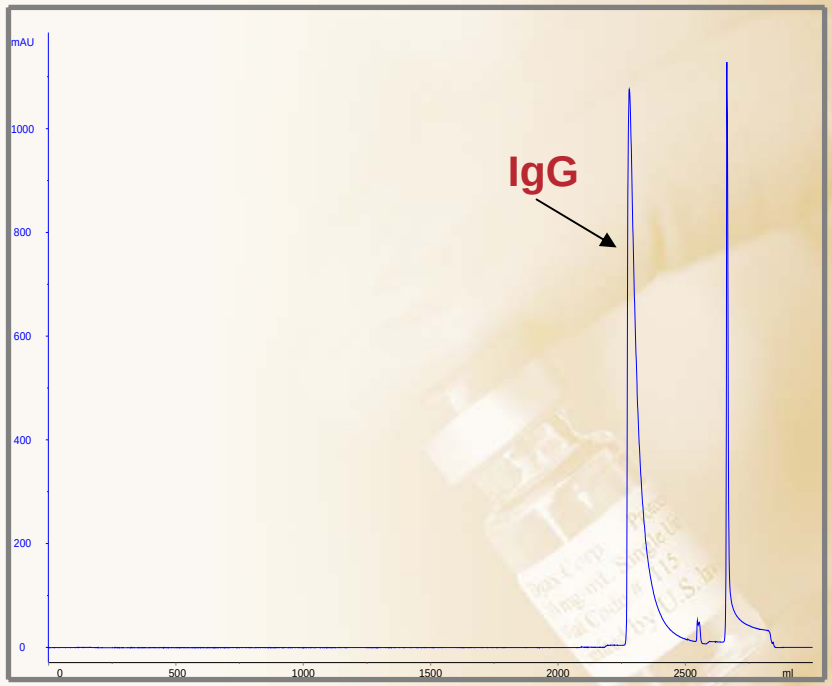
Summary MEP vs ProA as capture step for mAb purification

	ProA capture Step	MEP capture Step
Loading Condition	Clarified mammalian cell culture pH >7, Direct load	Clarified mammalian cell culture pH >7, Add 10mM EDTA, Direct load
Loading capacity	~ 50mg IgG/ml resin at 5% BT	~ 30mg IgG/ml resin at 5% BT
Resin Cost	>$8000/L Multiple Cycle Run reduce cost	$2000/L, Half price or less of ProA for same amount IgG purification
Resin Cleaning/reuse	Industrial Demonstrated CIP up to 200 cycles	Resin discoloration Needs demonstration of reuse robustness
Elution pH	Generally < 3.8 suitable for viral inactivation	Generally > 4.5 Not suitable for viral inactivation
Product quality	Low pH elution, more chance of aggregation	Mild pH elution, less chance of aggregation
Process yield	85-90%	80-85% (with proper wash step)
Host HCP removal	ProA has better HCP removal capability than MEP	
ProA Ligand Leaching	Yes	No

Polishing Step in mAb Purification
HIC vs MEP



HIC Polish Step for mAb1 post ProA



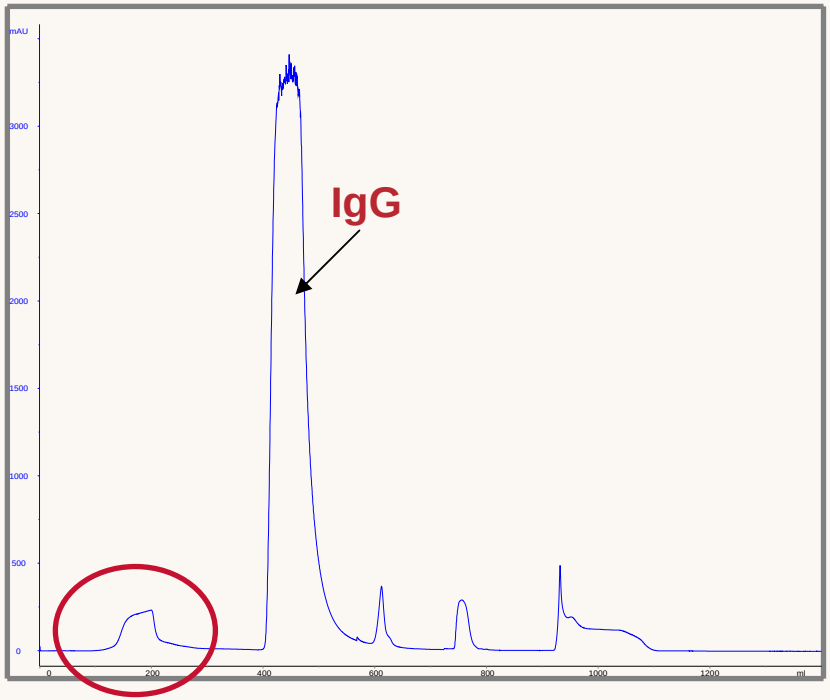
HIC Polish Step for mAb2 post ProA

Load requires to add substantial amount of salt

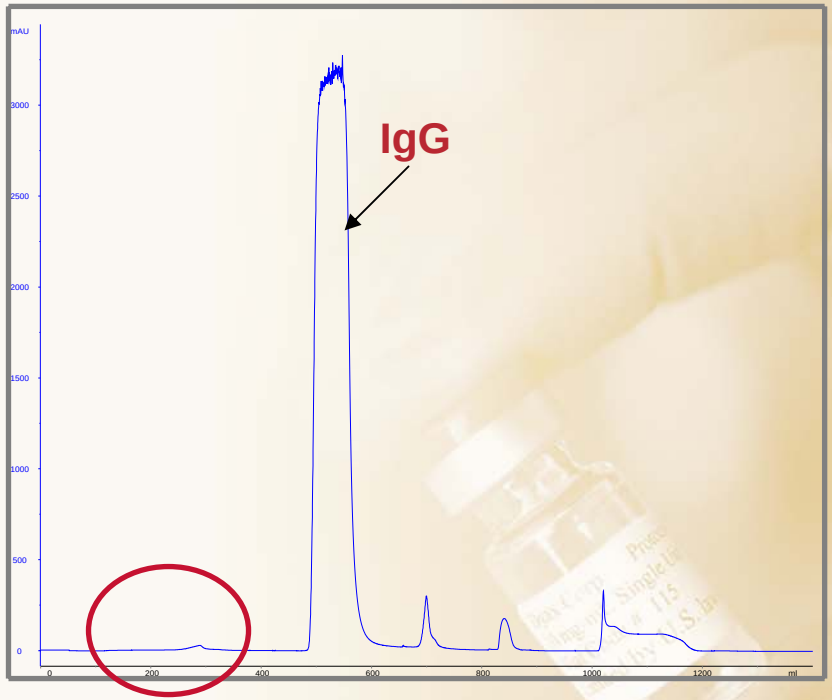
Elution with decreased salt concentration,

but optimal elution buffer still contains fair amount of salt.

Polishing Step in mAb Purification
HIC vs MEP



MEP Polish Step for mAb1 post ProA



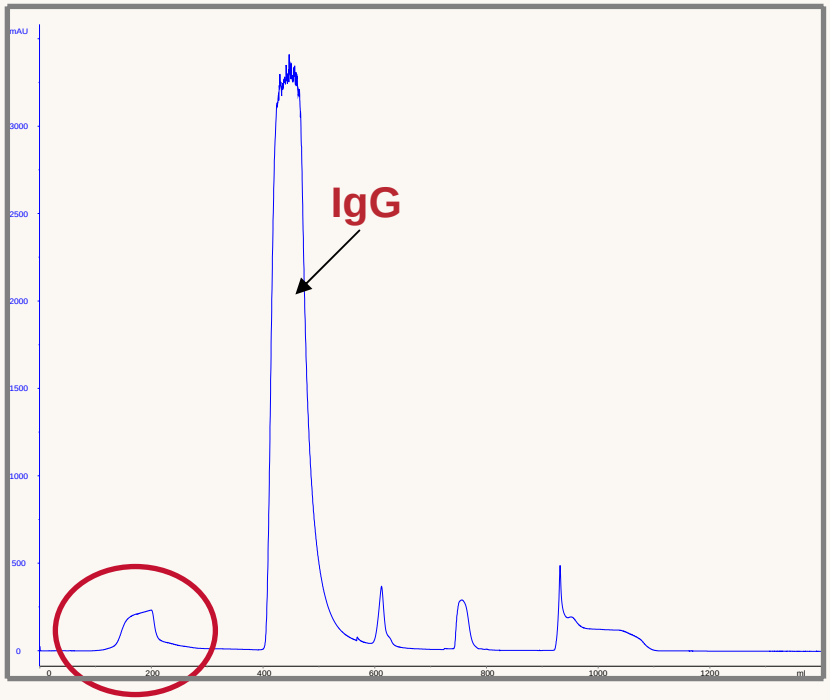
MEP Polish Step for mAb2 post ProA

Load requires no adjustment

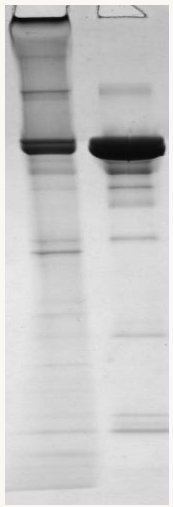
Elution with low/mild pH - low conductivity buffer

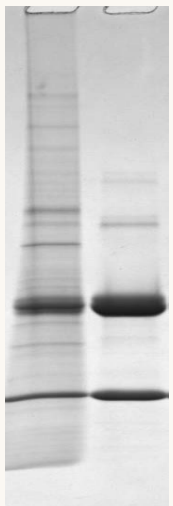
Problem !! ?? **Small amount of breakthrough during loading, mAb1 > mAb2**

MEP can distinguish certain product-related contaminants

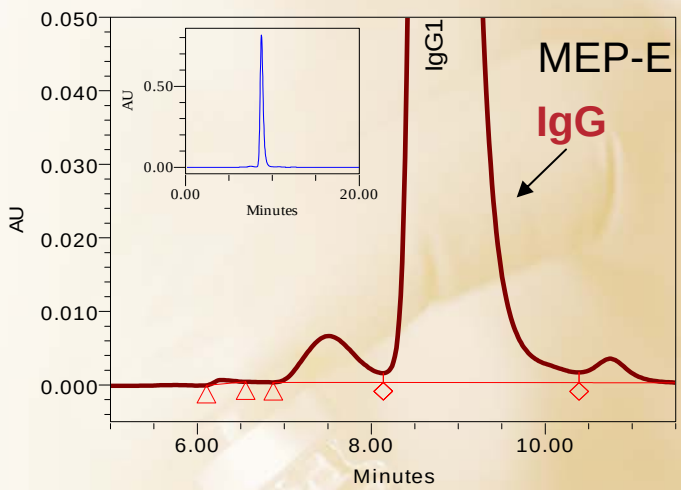


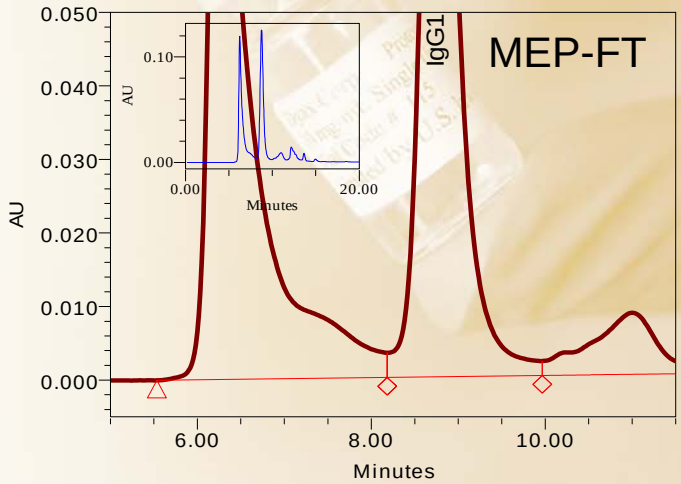
MEP-FT Analysis





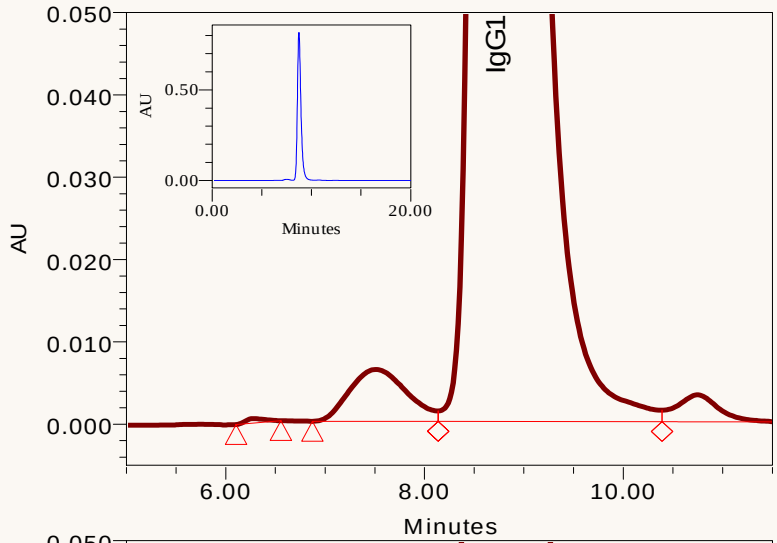
FT E

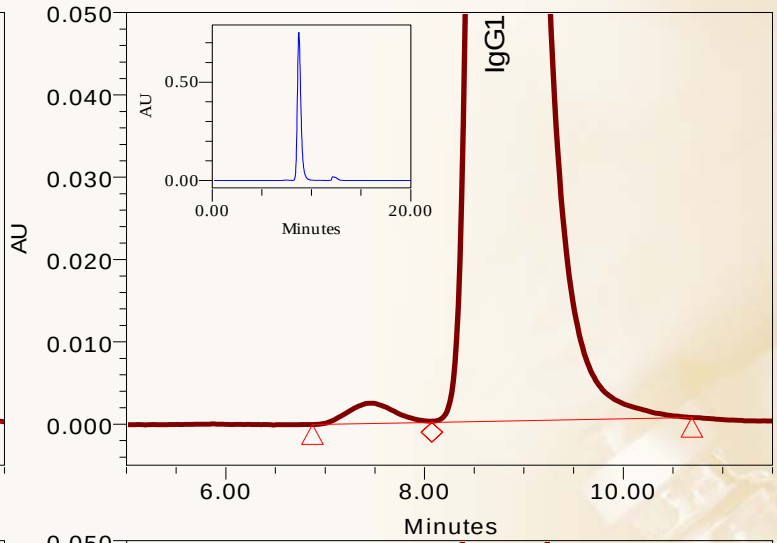


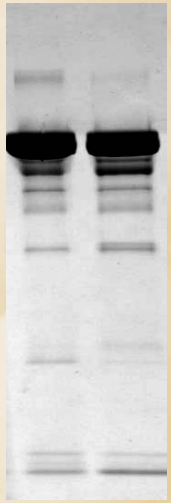


SEC-HPLC

Product Quality from MEP vs HIC as polishing step

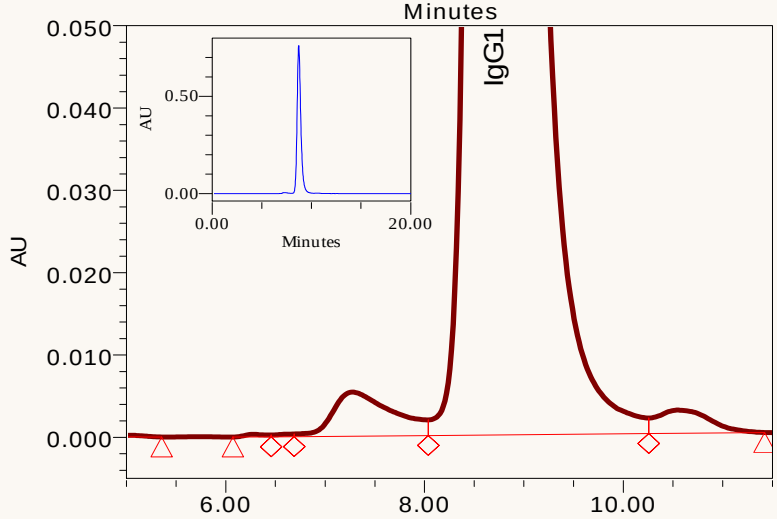




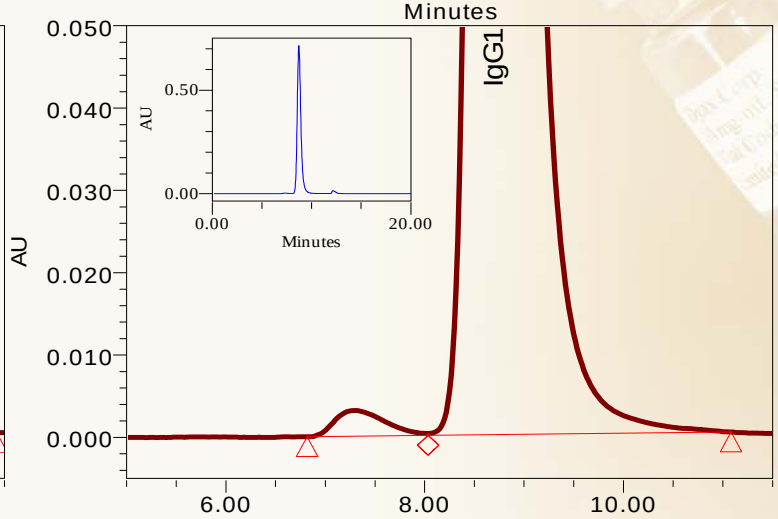


mAb1

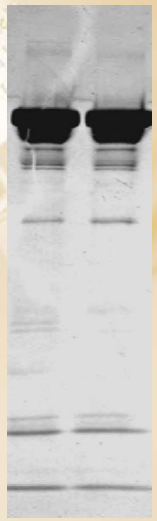
MEP HIC



MEP Polish



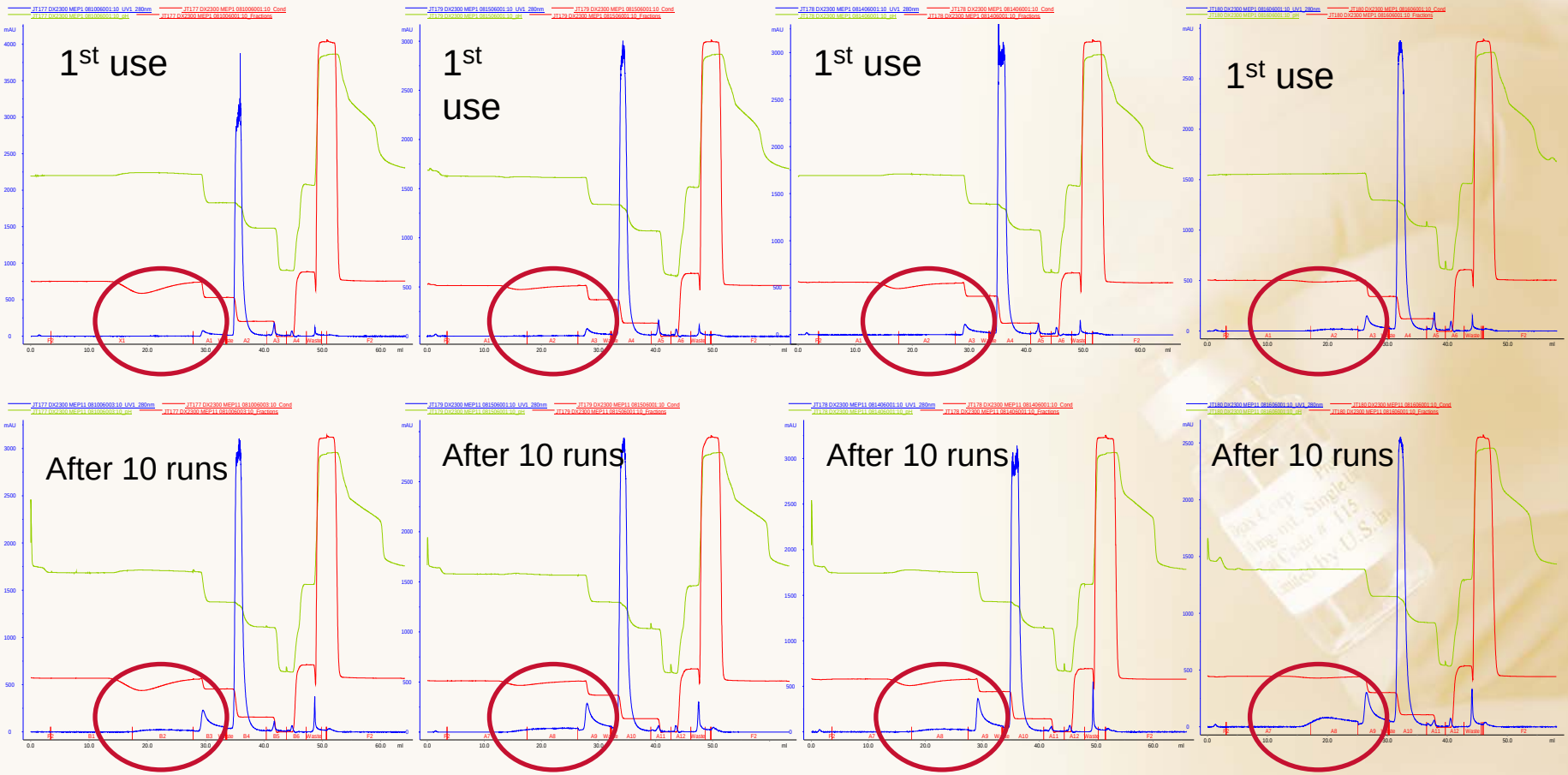
HIC polish



mAb2

MEP resin Cleaning / Reuse Issue

MEP Binding Capacity Decline observed in Polishing Step



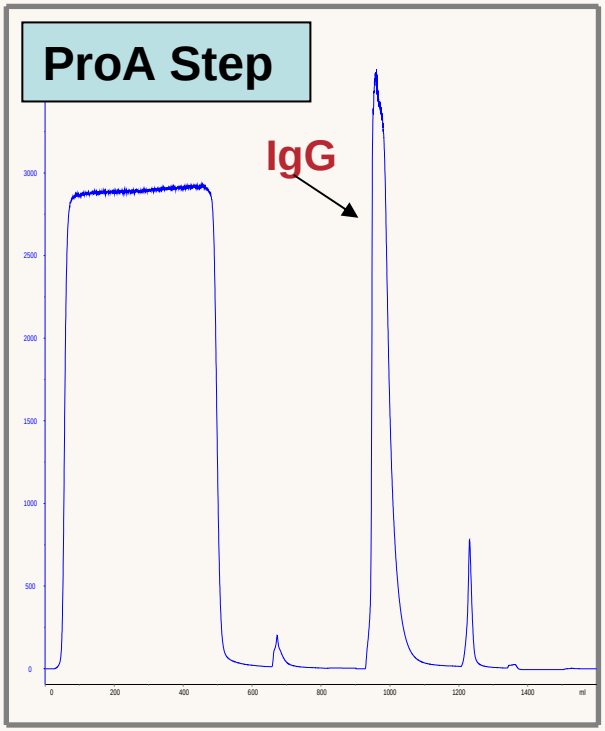
ProA E Acetate Buffer | ProA E Citrate Buffer | ProA E Glycine Buffer | DF to 1X PBS

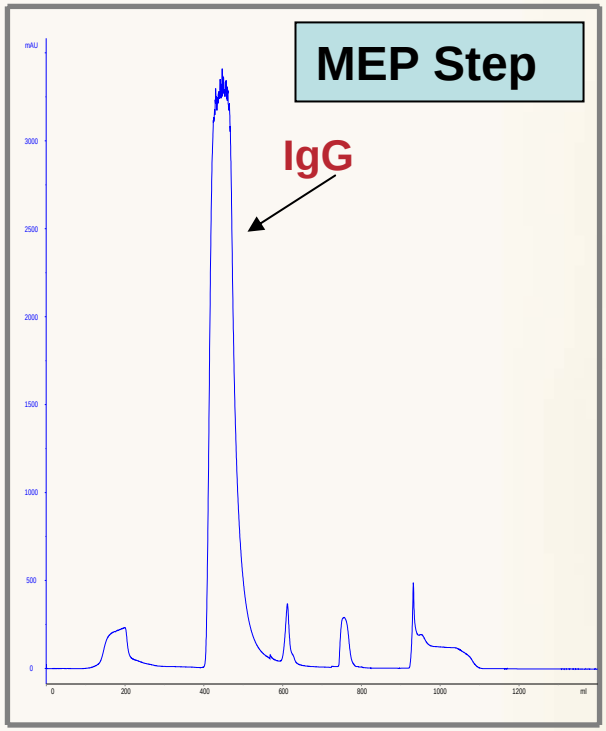
IgG slowly irreversibly binds on the column??

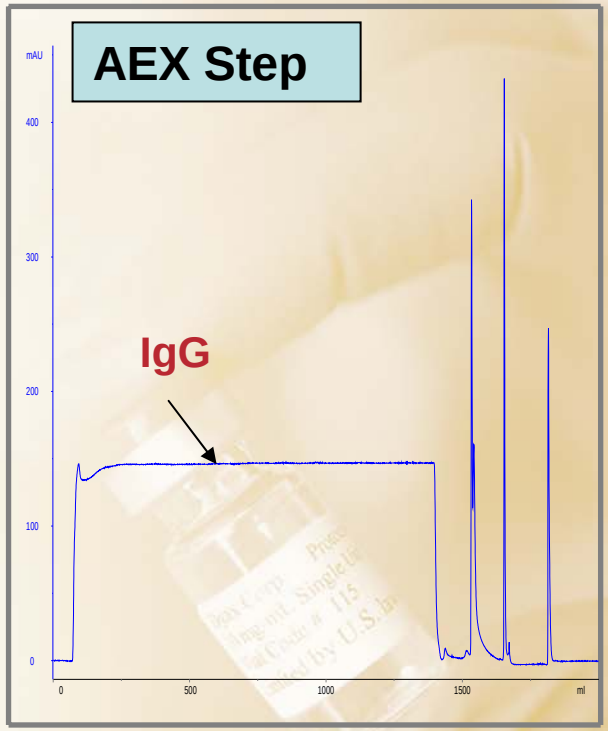
Summary MEP vs HIC as polishing step for mAb purification

	MEP Polishing Step	HIC Polishing Step
Loading Condition	No salt added, pH >7 Direct load	Need add significant amount of salt Product solubility/stability study is required
Loading capacity	~ 30mg IgG/ml resin at 5% BT at optimal loading condition (HIC-600M resin binding capacity > MEP)	
Resin Cost	Similar price	
Resin Cleaning/reuse	Needs demonstration of reuse robustness	Industrial Demonstrated CIP
Elution condition	Generally low but mild pH low conductivity buffer	Generally close to neutral pH decreased conductivity (could be still relatively high depending on process)
Product quality	Removes most of aggregates Monomer/aggregates resolution HIC is slight better than MEP	
Process yield	Removes less active product related impurities Similar yield ~ 80-85%	
Host HCP removal	HIC has slight better HCP removal than MEP under tested condition	
Leached ProA Removal	HIC has slight better ProA removal than MEP under tested condition	

ProA → MEP → AEX Platform



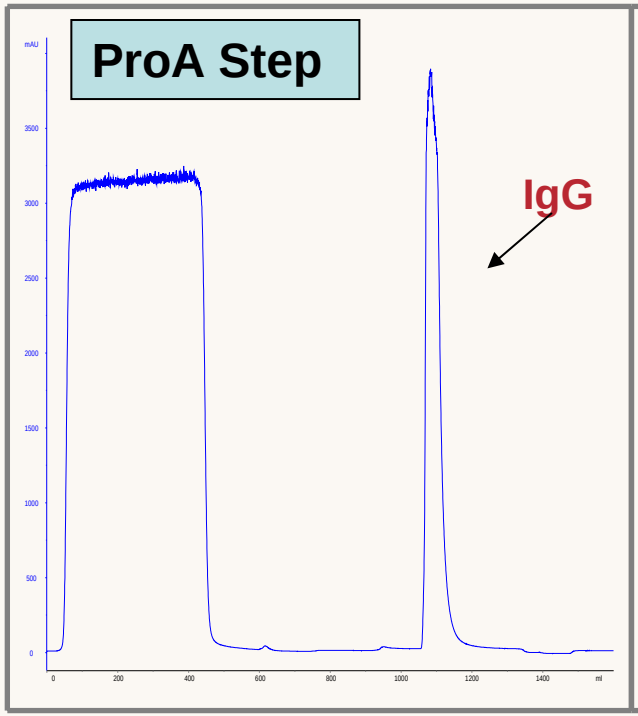


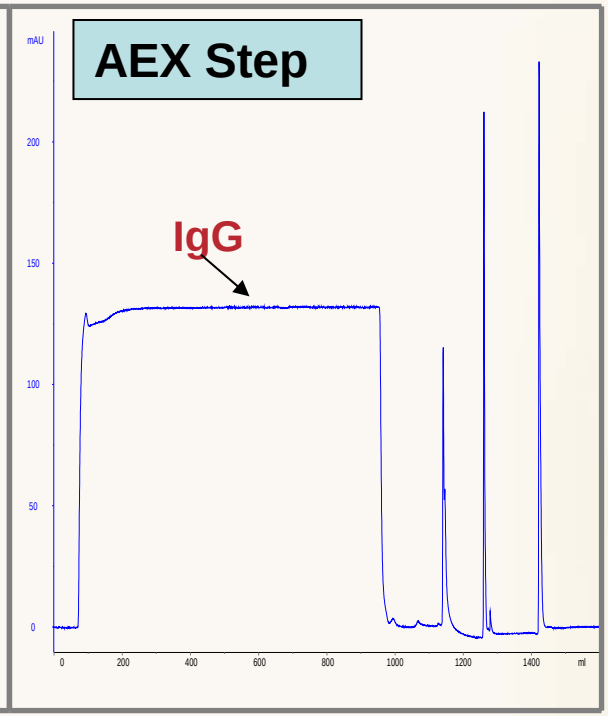


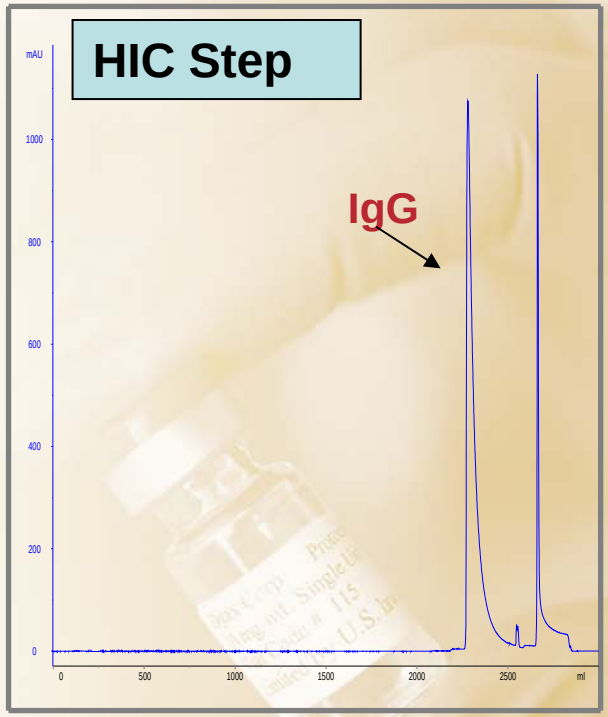
ProA step → MEP step → AEX step

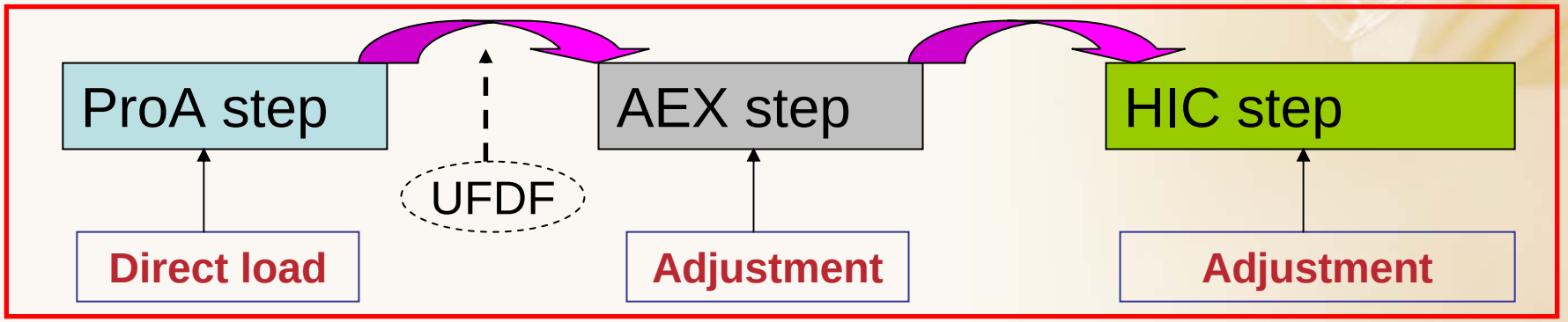
Direct load | Direct load | Minor adjustment

ProA → AEX → HIC Platform









Summary of HIC / MEP platform process study

Process Scheme	MEPcap platform	ProA - MEP - AEX	ProA - HIC - AEX
Perferred use	Proof of Principle Preclinical projects	Proof of Principle Preclinical projects	Development projects
Process Development	Moderate	Simplest	Complex
Sample Manipulation	Moderate	Least	Significant
Process Yield	Fair	Fair	Fair
Product purity by SEC	Good	Good	Good
CHO HCP Level	~ 5ppm	<1ppm	<1ppm
Leached ProA Level	None	Low	Low
Process Issue	MEP CIP	MEP capacity decline	Industrial std process

Note: ProA and CHO HCP measured by commercial ELISA kit.

HIC / MEP Study Conclusion

1. **Optimization of HIC resin pore size for mAb molecules:**
   - Increases the binding capacity by >15% for same ligand resin
     - improve standard HIC process efficiency.
2. **Hydrophobic Charge Induction Chromatography (HCIC) - MEP**
   - Enlarged HIC application areas in the mAb purification process
     – not limited to only polishing step;
   - Predictable process parameters
     – simplify process development work
   - Bind mAb at low salt condition
     - greatly facilitate the process flow optimization and enhance entire process efficiency.
   - Practical issues need to be further resolved with MEP resin.

HIC resins need continue their evolution with low salt binding/high binding capacity/high resolution/robustness to increase mAb downstream purification process efficiency

Acknowledgement

Dyax

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Analytical Development Group

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Kevin O’Donnell

Pall Scientific

Warren Schwartz