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A COMPARISON OF MULTIMODAL CHROMATOGRAPHIC RESINS: PROTEIN BINDING AND SELECTIVITY

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Abstract

The use of multimodal chromatographic resins in large-scale protein production processes has been steadily increasing due to their ability to provide enhanced selectivity as compared to ion exchangers and hydrophobic interaction chromatography resins. With the increased use of multimodal chromatographic resins, several new stationary phases have been developed, which could each potentially provide a unique, specific selectivity.

A formalism describing protein retention under isocratic conditions (k') as a function of salt concentration was used to combine the influence of hydrophobic and electrostatic interactions for multimodal chromatography.

Given the interplay of ionic and hydrophobic interactions on this mode of chromatography, both pH and conductivity are key variables that influence protein binding. Here we describe a high throughput method for identifying optimal operating conditions in terms of pH and salt concentration for multiple mixed mode resins in a single set of experiments using low material volumes.

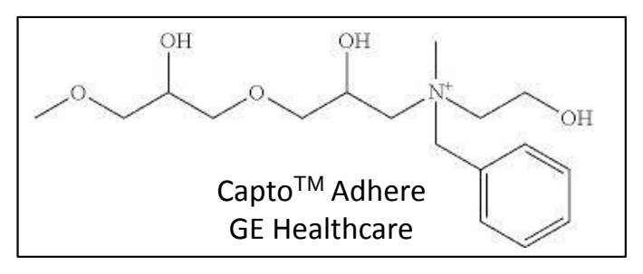
The insights gained from this work are then used to compare the selectivity of multimodal resins to one another. In addition to understanding the relative selectivity of each resin for a target protein, the assessment of multiple proteins enables the identification of the best mixed mode resin for a particular class of proteins.

Mixed Mode Chromatography

- Takes advantage of more than one type of interaction
  - i.e. ionic, hydrophobic, hydrogen bonding
- Provides enhanced selectivity, "pseudo-affinity" over conventional single mechanism based stationary phases such as ion-exchange or hydrophobic interaction chromatography
- Can potentially reduce process steps
- Proteins typically eluted with pH change or with salt increase
- Several mixed mode resins have recently been developed with:
  - Increased loading capacities
  - Higher ionic strength tolerance

Resin	Type
Capto MMC	Multimodal weak cation exchanger
Capto Adhere	Multimodal strong anion exchanger
Nuvia cPrime	Hydrophobic cation exchanger
Eshmuno HCX	Multi-mode cation exchanger
Toyopearl MX-Trp-650M	Multimodal weak cation exchanger
Pall MEP, HEA and PPA Hypercel	Electrostatic and hydrophobic exchanger

Mixed Mode Ligands



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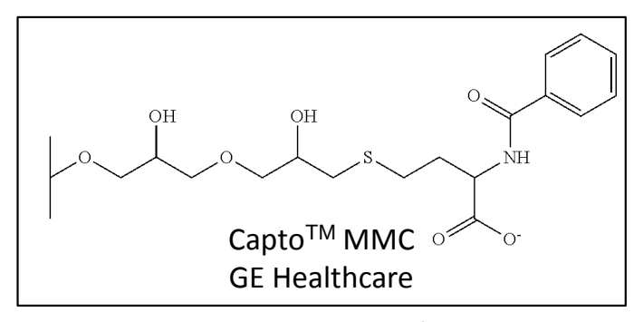

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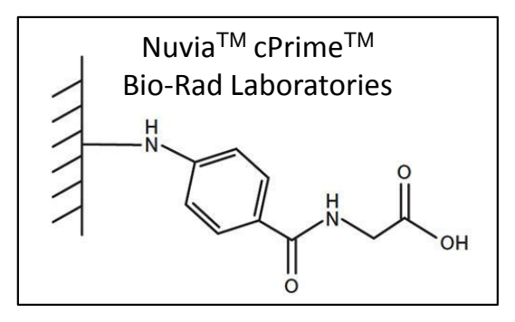
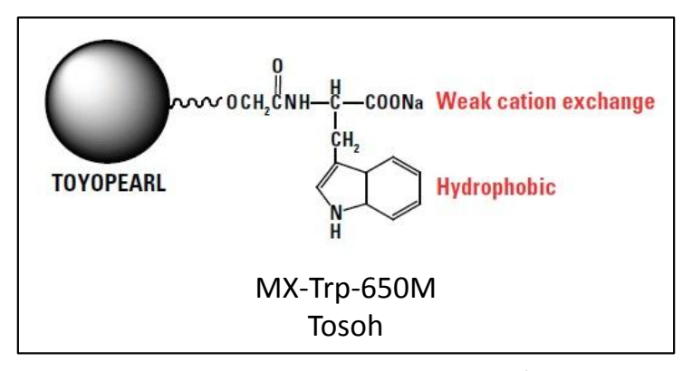


Nuvia and cPrime are registered trademarks of Bio-Rad



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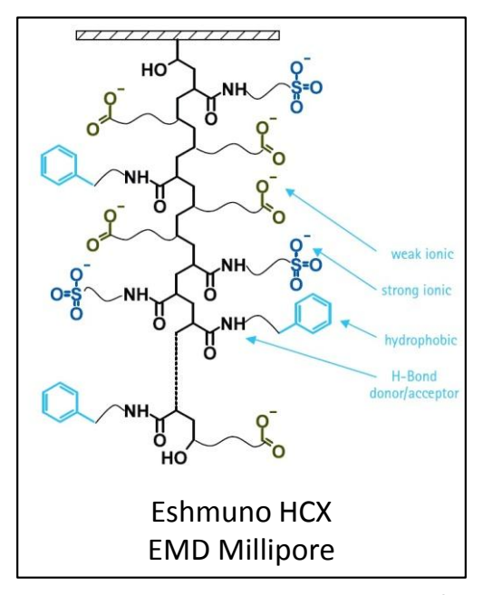
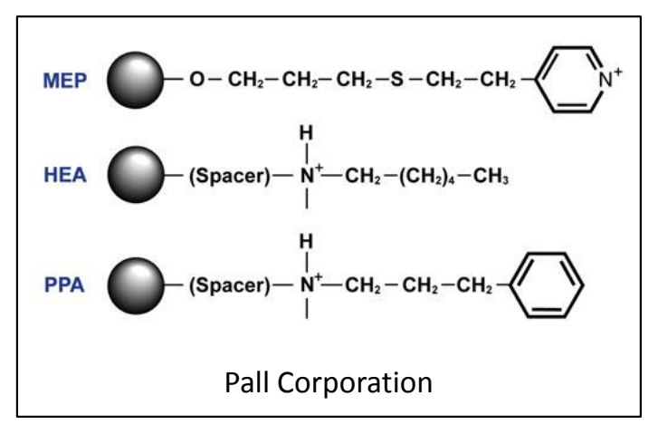


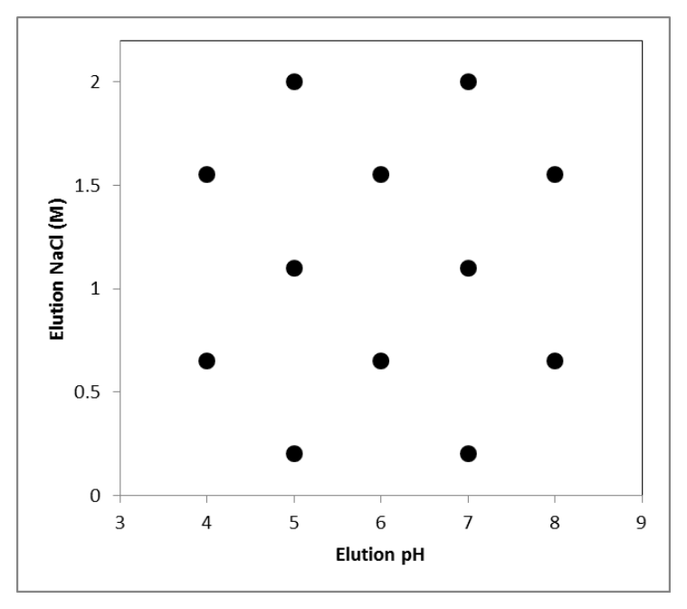
Eshmuno is a registered trademark of Merck KGaA

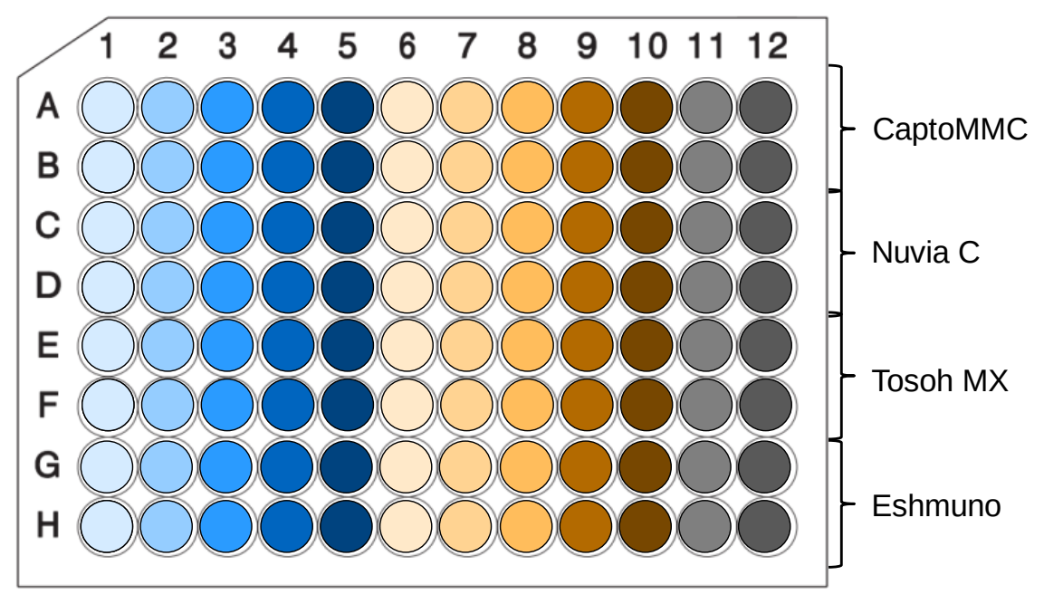


- Capto MMC, Nuvia cPrime, MX-Trp 650M and Eshmuno HCX were selected for comparison in high throughput plate screening studies as well as linear gradient experiments to assess capability for HCP and HMW removal in a mAb unit operation

High Throughput Identification of Optimal Operating Conditions

- Experimental aim: Assess the impact of load pH, elution pH and elution conductivity on the interaction of three different mAbs with four mixed mode resins
- Variables tested:
  - Load pH: 4, 5, 6, 7, 8
  - Elution pH: 4, 5, 6, 7, 8
  - Elution [NaCl] (M): 0.20, 0.65, 1.10, 1.55, 2.00

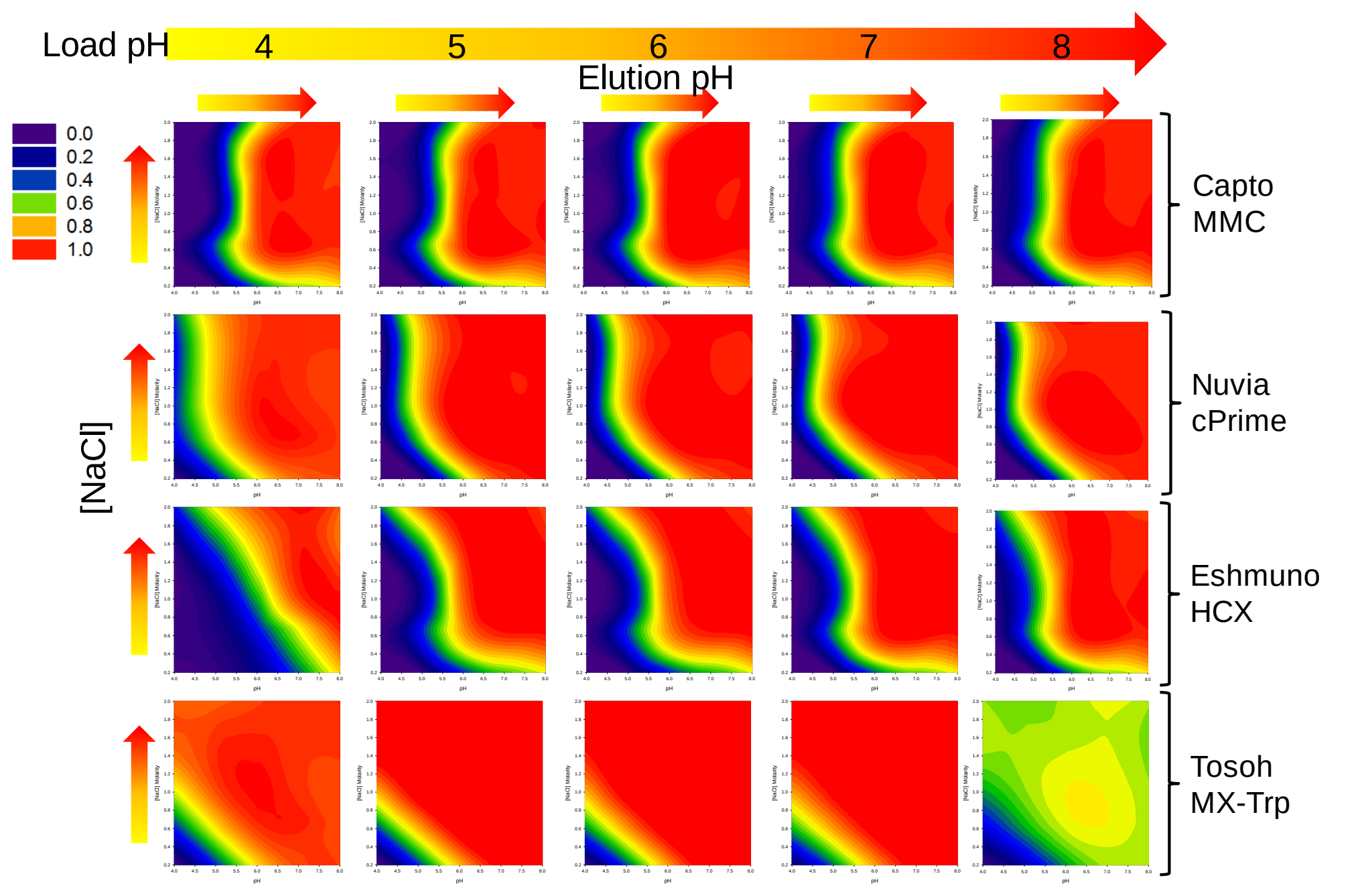




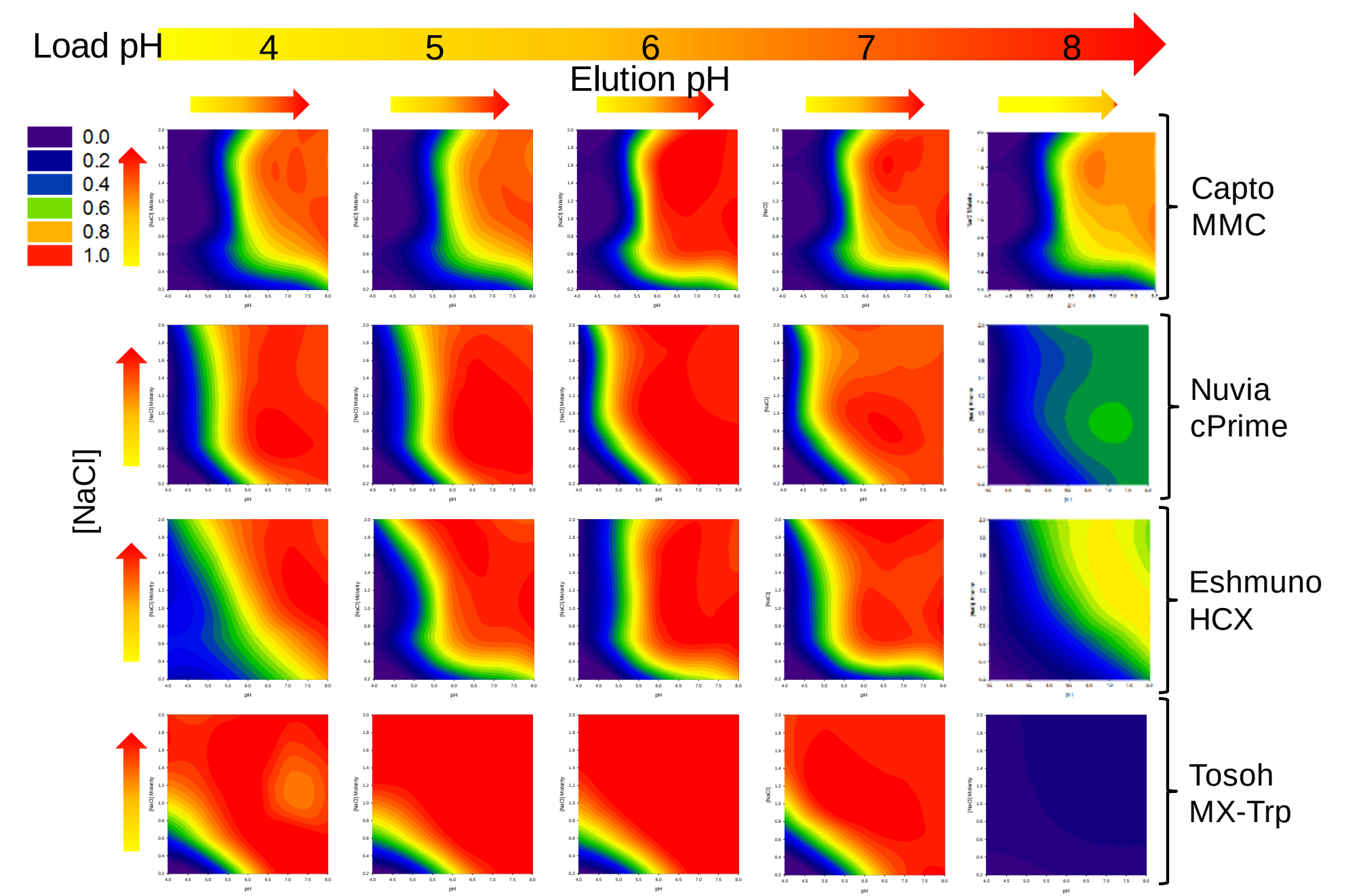
- For each mAb tested at each load pH, a single 96-well plate was used
- In total, 15 plates were used to execute 720 experiments in duplicate

Plate Experimental Results

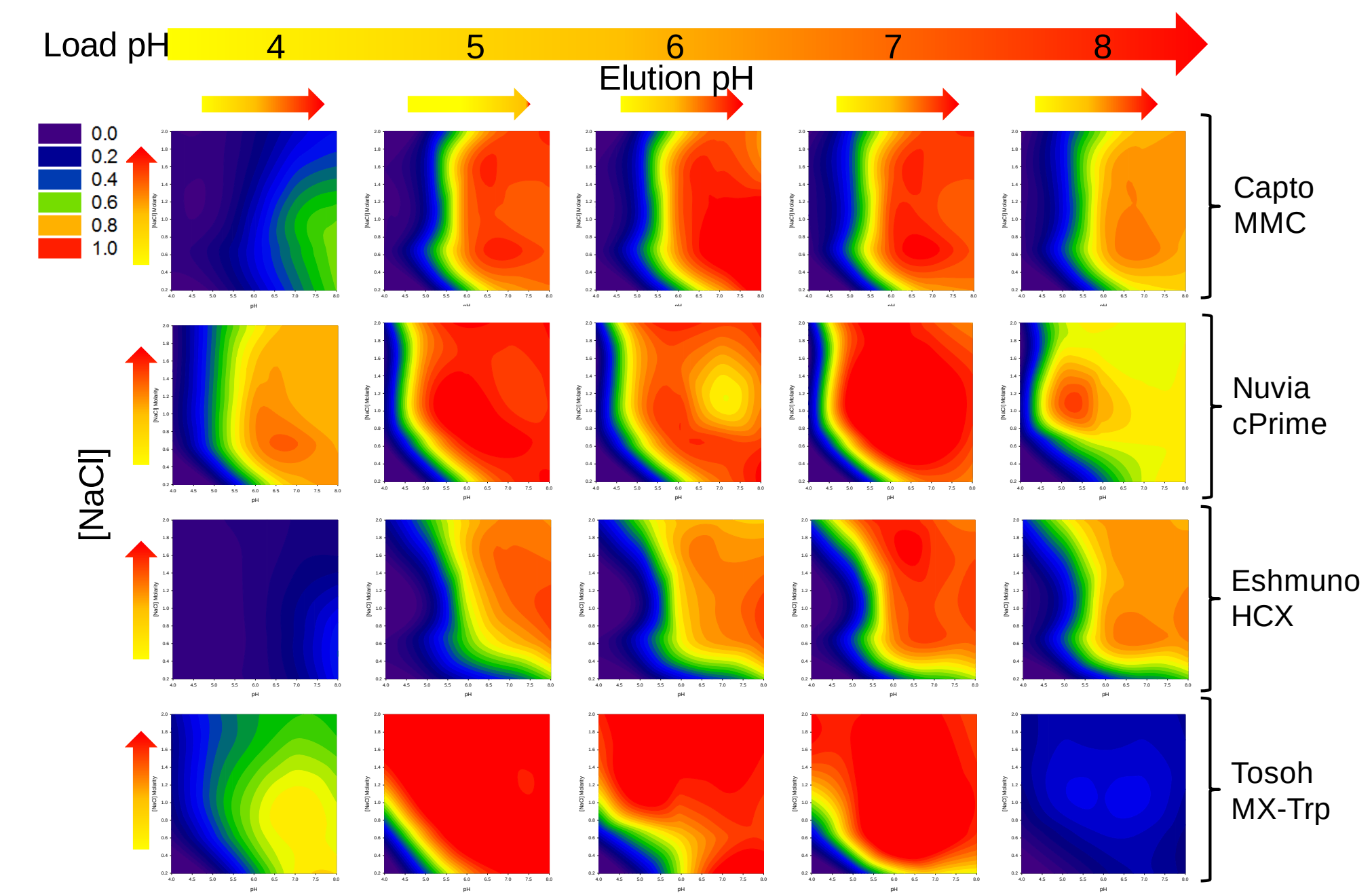
mAb 1



mAb 2



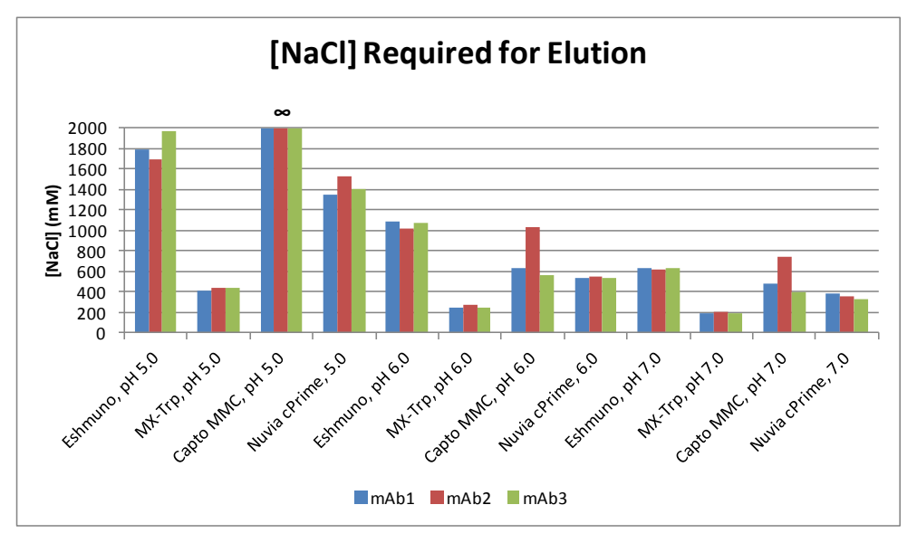
mAb 3

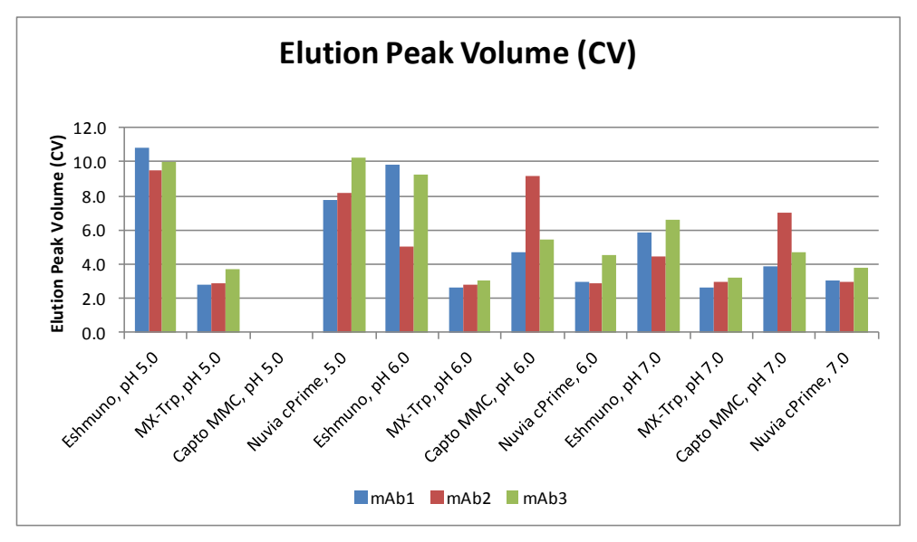


- Conditions that are not amenable to acceptable yields are easily identifiable .
- Each mAb has a unique profile among conditions tested.

Linear Gradient Studies to Compare Resin Selectivity

- Experimental aim: Determine [NaCl] required to elute model mAbs from Eshmuno HCX, MX-Trp, Capto MMC and Nuvia cPrime
- [NaCl] determined by the %B buffer at peak maxima
- Product eluted with increasing NaCl gradient
- Eluate fractions collected in 1/8th CV fractions from 100mAu/cm to 100mAu/cm and analyzed by SEC-HPLC
- Eluate fractions to achieve step yield of 80% pooled and analyzed by HCP ELISA



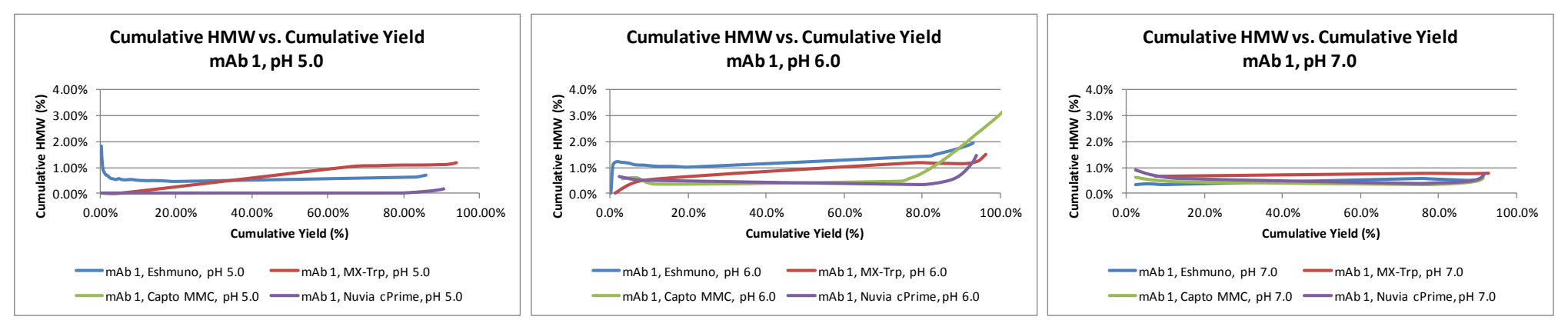


- Experiments performed at a pH farther from the protein isoelectric point resulted in tighter protein: resin interactions
- Irreversible binding with 2M NaCl observed on Capto MMC at pH 5.0
- The weakest binding and sharpest elution peak observed on MX-Trp at all pHs evaluated
- NaCl at peak maxima similar across mAbs at a given condition on each resin; however, elution volume varied among molecules conditions assessed

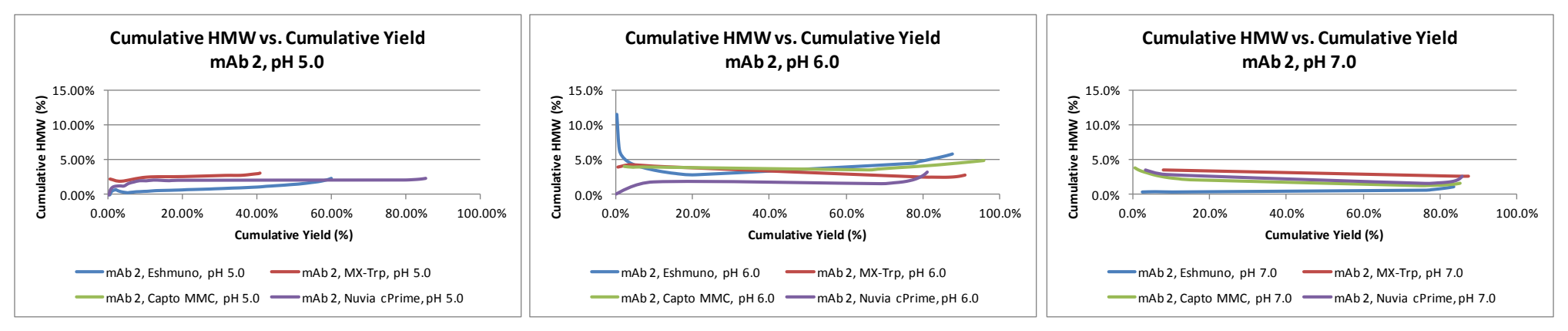
Selectivity Analysis – SEC and HCP Results

Protein	Feed %HMW	Feed HCP (ppm)
mAb1	~1%	4400
mAb2	~4%	135
mAb3	~7%	3700

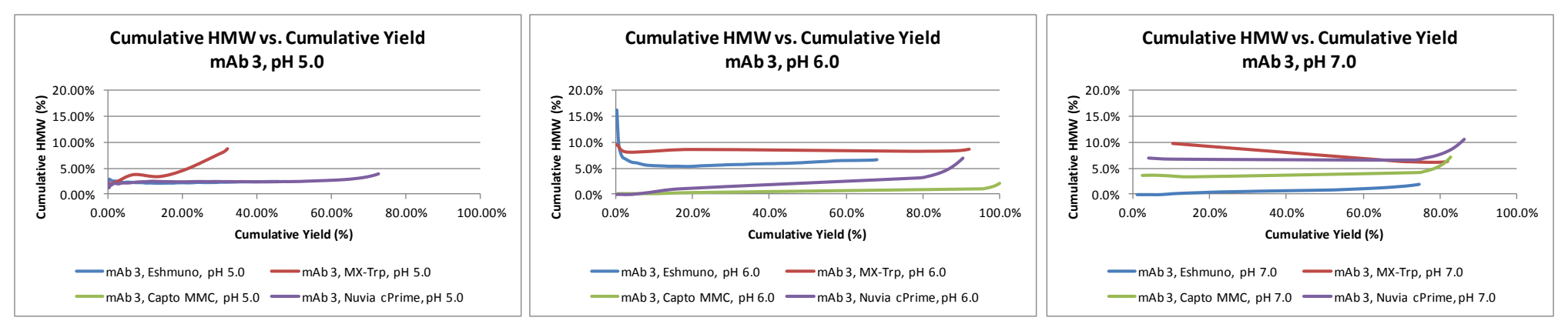
mAb 1



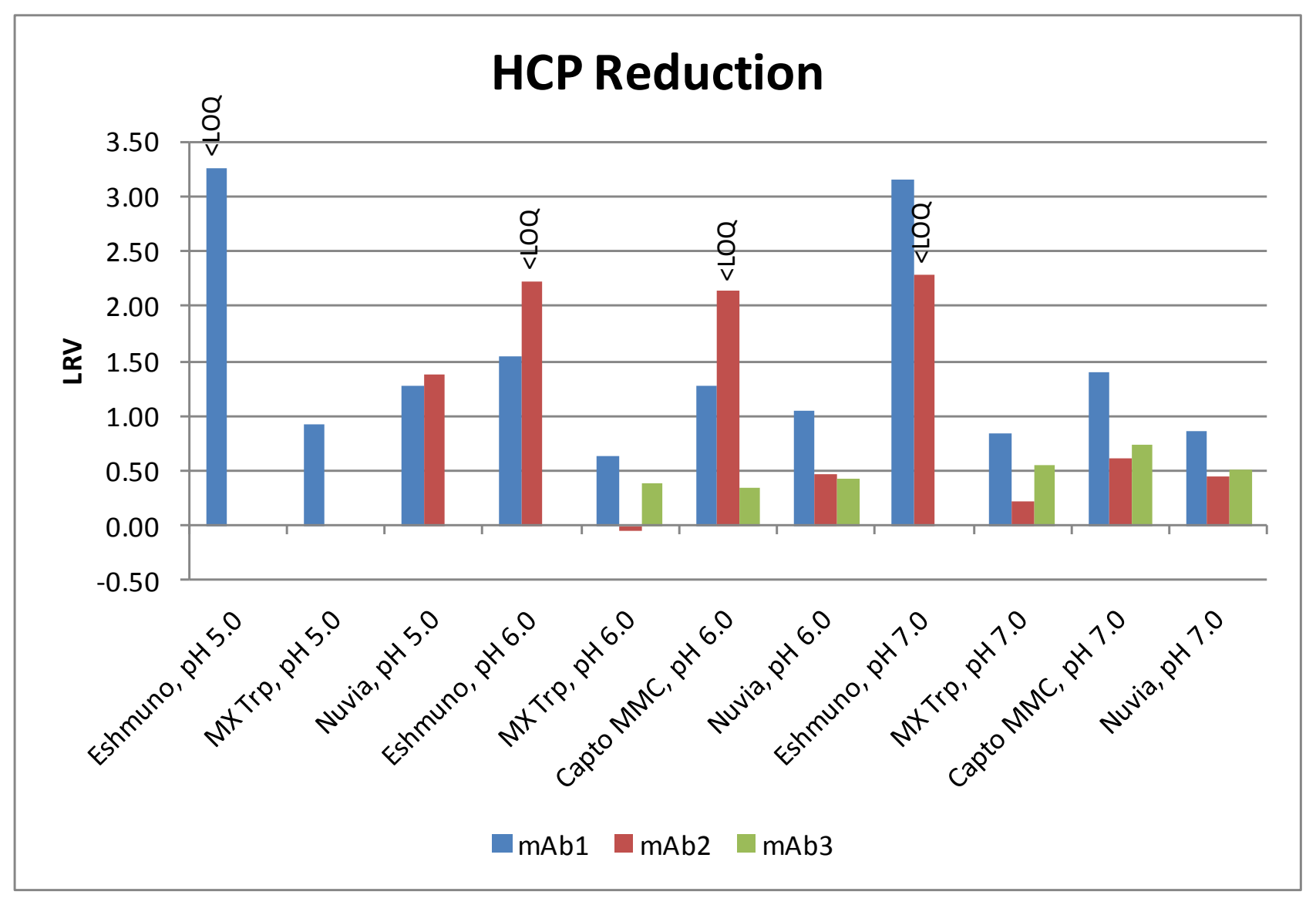
mAb 2



mAb 3



- At pH 5.0, poor yields were observed for mAb 2 and mAb3.
  - Nuvia cPrime performed the best at pH 5.0
- The most variability in selectivity among resins observed at pH 6.0 for all mAbs tested



LOQ ranges from 0.7-2.4ppm for the samples that tested <LOQ.

- Not all experiments yielded 80% product in eluate fractions.
  - Capto MMC at pH 5.0 did not yield any product for the 3 mAbs tested
  - mAb 3 only achieved 80% yield in 6 of the 12 conditions tested
- HCP clearance is mAb specific with conditions tested.
  - The greatest HCP reduction consistently achieved across the experiments is with mAb 1

mAb 1

Resin	Max. Yield	Purity at Optimal Yield	HMW at Optimal Yield	%Reduction in HMW	HCP (ppm)	Optimization Factor (Max. Yield * %HMW Red. * %HCP Red.)
pH 5.0						
Capto MMC	N/A*	N/A*	N/A*	N/A*	N/A*	N/A*
Nuvia cPrime	90%	100%	0%	94%	230	0.81
Eshmuno	86%	99%	1%	46%	<LOQ	0.39
MX-Trp	94%	99%	1%	9%	536	0.08
pH 6.0						
Capto MMC	102%	100%	0%	60%	232	0.44
Nuvia cPrime	94%	100%	0%	68%	395	0.51
Eshmuno	93%	99%	1%	10%	127	0.08
MX-Trp	96%	99%	1%	-30%	1019	-0.21
pH 7.0						
Capto MMC	92%	80%	0%	53%	179	0.47
Nuvia cPrime	92%	83%	0%	45%	547	0.36
Eshmuno	89%	88%	1%	33%	3	0.29
MX-Trp	93%	76%	1%	-3%	628	-0.03

*mAb did not elute from Capto MMC at pH 5.0 in up to 2M NaCl

mAb 2

Resin	Max. Yield	Purity at Optimal Yield	HMW at Optimal Yield	%Reduction in HMW	HCP (ppm)	Optimization Factor (Max. Yield * %HMW Red. * %HCP Red.)
pH 5.0						
Capto MMC	N/A*	N/A*	N/A*	N/A*	N/A*	N/A*
Nuvia cPrime	85%	98%	2%	41%	5.8	0.32
Eshmuno	60%	98%	2%	58%	N/A**	N/A**
MX-Trp	41%	97%	3%	25%	N/A**	N/A**
pH 6.0						
Capto MMC	96%	96%	4%	5%	<LOQ	0.04
Nuvia cPrime	81%	88%	2%	60%	46	0.26
Eshmuno	88%	95%	5%	-20%	<LOQ	-0.15
MX-Trp	91%	97%	3%	33%	149	0.11
pH 7.0						
Capto MMC	86%	80%	1%	50%	33	0.33
Nuvia cPrime	84%	76%	2%	41%	48	0.22
Eshmuno	84%	76%	1%	79%	<LOQ	0.65
MX-Trp	88%	87%	3%	3%	82	0.01

*mAb did not elute from Capto MMC at pH 5.0 in up to 2M NaCl
**Not tested as 85% yield was not achieved

mAb 3

Resin	Max. Yield	Purity at Optimal Yield	HMW at Optimal Yield	%Reduction in HMW	HCP (ppm)	Optimization Factor (Max. Yield * %HMW Red. * %HCP Red.)
pH 5.0						
Capto MMC	N/A*	N/A*	N/A*	N/A*	N/A*	N/A*
Nuvia cPrime	73%	97%	3%	66%	N/A**	N/A**
Eshmuno	46%	98%	2%	73%	N/A**	N/A**
MX-Trp	32%	75%	5%	47%	N/A**	N/A**
pH 6.0						
Capto MMC	84%	98%	1%	90%	1668	0.39
Nuvia cPrime	91%	96%	3%	66%	1384	0.33
Eshmuno	68%	86%	7%	31%	N/A**	N/A**
MX-Trp	92%	85%	8%	14%	1531	0.07
pH 7.0						
Capto MMC	83%	74%	4%	47%	693	0.31
Nuvia cPrime	86%	73%	6%	14%	1163	0.08
Eshmuno	75%	73%	2%	78%	N/A**	N/A**
MX-Trp	82%	80%	6%	23%	1008	0.14

*mAb did not elute from Capto MMC at pH 5.0 in up to 2M NaCl
**Not tested as 85% yield was not achieved

Summary Based on Linear Gradient Studies

Molecule	Best resins based on yield	Best resins based on HMW reduction	Best resins based on HCP reduction	Overall best resin based on optimization factor
mAb 1	Capto MMC Tosoh MX-Trp	Nuvia cPrime Capto MMC	Eshmuno HCX Capto MMC	Nuvia cPrime Capto MMC
mAb 2	Capto MMC Tosoh MX-Trp	Eshmuno HCX Nuvia cPrime	Eshmuno HCX Capto MMC	Eshmuno HCX Nuvia cPrime
mAb 3	Tosoh MX-Trp Nuvia cPrime	Capto MMC Eshmuno HCX	Capto MMC Tosoh MX-Trp	Capto MMC Nuvia cPrime

- For each mAb, the best resin for each output category varies
- Across the 3 mAbs examined, Nuvia cPrime consistently rated as a top performing resin

Conclusions

- High throughput filter plate data can be used to quickly limit the focus of a chromatography evaluation using minimal amounts of the target protein
  - pH and NaCl conditions not favorable for high yields are easily identifiable
- Resin selectivity varies based on mAb and condition assessed; however, Nuvia cPrime consistently performs to balance yield with HMW and HCP clearance

Acknowledgements

- KBI Process Development Team
- KBI Analytical Development Team