Natrix HD-Sb Membrane Adsorber

A New High-Performance Purification Tool for mAb Purification in Flowthrough Mode

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Natrix Separations Inc.
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Project Goals

- **Develop a fully single-use mAb purification platform**
  - Implement a single use CEX step
  - Includes replacing ProA resin column by ProA membrane

- **Improve process productivity**
  - Two FT steps:
    - CEX in FT mode
    - Complements/enables high load AEX FT step
    - Tandem CEX_AEX possible

Intensification & Simplification

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Industry Needs For High Productivity Affinity Purification Platforms (mAbs & other biologics)

- **High productivity while maintaining purification efficacy**
  - Rapid processing with high selectivity
  - High yield capture with minimal feed preparation - simplicity
  - Robustness: consistent performance even in changing conditions
- **Simplified and reliable manufacturing process**
  - Very high purity in single step, minimal polishing needed
  - Fewer process steps to achieve target purity
  - Increase in process reliability for fewer OOS batches
- **Increased flexibility**
  - Flex platforms, widely applicable to most processes
  - Dynamic, faster process development & easy technology transfer
    - Responsive to process and product needs

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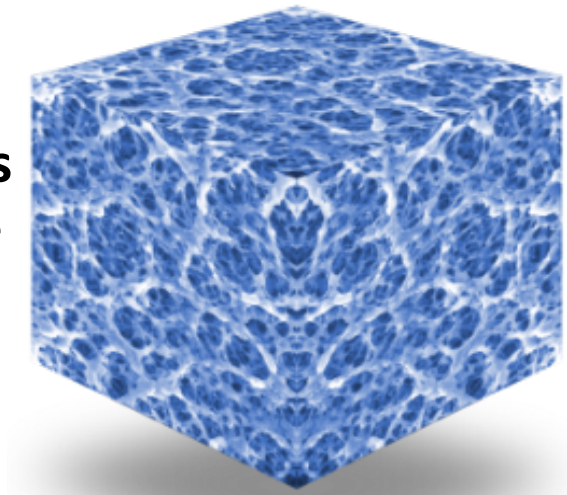
Natrix HD Technology

- **Natrix HD Membranes**
  - Reinforced, porous polymer hydrogels containing a high density of binding groups
  - Unique, interconnected pore structure provides large surface area for protein binding and high membrane permeability
  - Dominated by Advective Flow

  → **High Binding Capacity for Proteins, Virus and DNA**
  → **High Flow Rates**

- **Pre-packed in ready to use, disposable columns**
  - Flexibility and economic benefits of single-use
  - Easy to use from lab scale to GMP



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Goal 1

- **Develop a fully single-use process**
  - Increase flexibility and productivity to minimize cost of goods
  - Replace the ProA resin column with ProA membrane
  - HD-Sb in Bind/Elute mode
  - HD-Q: AEX with extended buffer compatibility

- **Affinity membranes**
  - Natrix has developed libraries of 100's of membranes
    - contain reactive pendant groups for attaching affinity ligands

  - The ProA ligand is coupled to optimized reactive membranes
    - maximize binding capacity and impurity clearance

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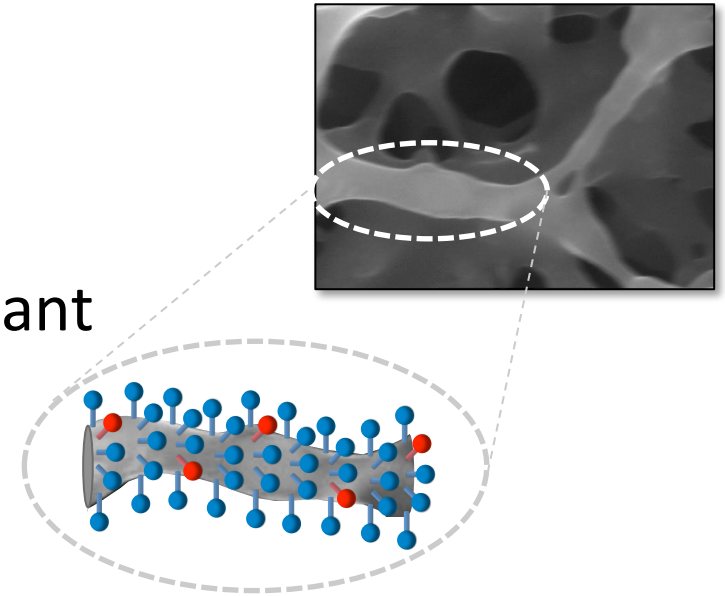
HD-Sb Salt Tolerant CEX w HIC modality

- CEX group – sulfonic acid
- HIC group – t-butyl
- High binding capacity and salt tolerant
- Target application
  - Capture or polishing of mAbs
  - Removal of mAb aggregates and HCP
  - Bind/elute or flowthrough
  - Flexible binding & elution conditions
  - Base stable (1M NaOH)



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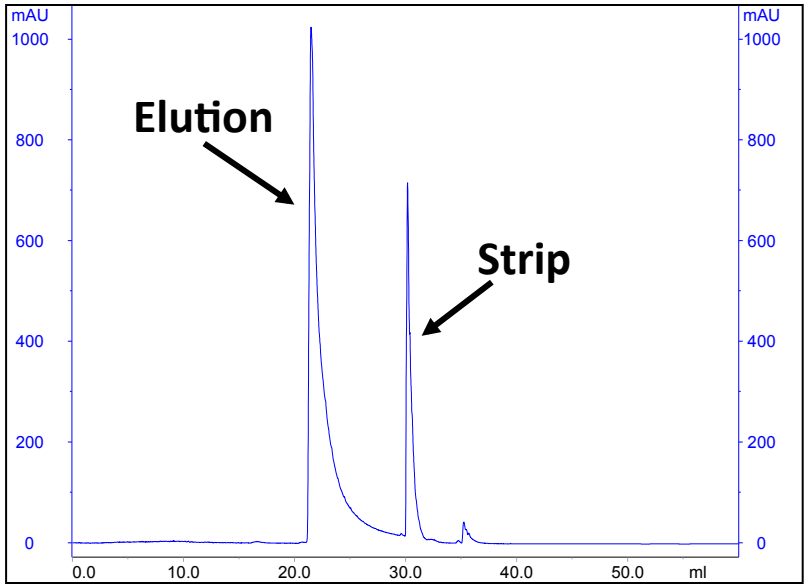
Implementation of Single-Use CEX: HD-Sb

Bind & Elute Performance (2 layer screening device)

Parameter	Performance
Load	50 mg of mAb/mL of membrane
Aggregate Clearance	≈100%
Load	8.1%
Eluate	Not Detectable
Monomer Yield	92%



Excellent separation at high productivity

Flow rate: 10 MV/min
Equilibration: 45 mM Na-acetate + 130 mM NaCl, pH 4.5 & 16.3 mS/cm
Sample: 1 g/L mAb2 in equilibration buffer
Wash: 20 mM phosphate, pH 6.3
Elution: 20 mM phosphate + NaCl, pH 6.3 & 6 mS/cm

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Evaluating New ProA Membranes

Do ProA membranes have good capacity and impurity reduction for different mAbs & buffers?

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HD-A Capture: Robust Impurity Clearance Across Multiple Buffer Systems

	mAb 2	mAb 3	mAb 4
	Eluate HCP (ppm)		
Feed HCP (ppm)	89,667	285,948	1,417,391
PBS buffer	307	527	1,171
Bis-Tris Acetate buffer	382	710	1,782
Phosphate buffer	2,597	294	3,098

Load: 30 mg/mL
Residence time: HD-A Membrane = 6 seconds
Test Cell: 1 layer of membrane (0.1 mL)
Yield: 85-95%

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HD-A Capture: Excellent Impurity Clearance For Multiple Mabs (competes with ProA resin)

		HD-A Membrane		Protein A Resin Column	
	Feed HCP (ppm)	Load (mg/mL)	Eluate HCP (ppm)	Load (mg/mL)	Eluate HCP (ppm)
mAb 1 (*)	25,600	30	102	25	203
mAb 2 (*)	89,667	30	307	25	247
mAb 3	319,649	25	527	25	2,404
mAb 4	1,417,391	30	1,171	25	1,123

(*) Pre-treated mAb

Residence time: HD-A Membrane = 6 seconds; Protein A Resin Column = 4 minutes
Typical leached ProA <10 ppm for both resin and membrane
Yield: comparable (85-95%)

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Performance Of The Membrane Antibody Process Is Comparable To The Chromatography Resin Process

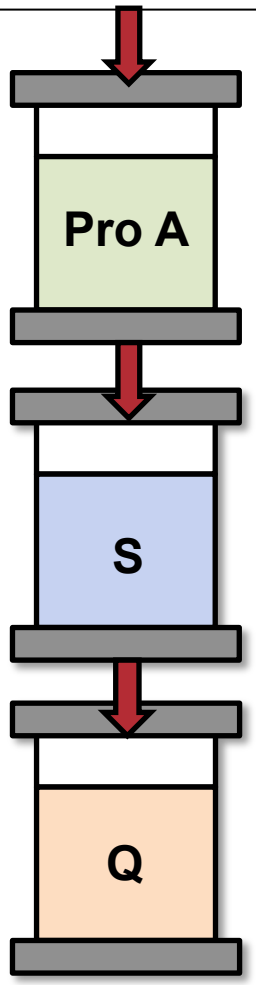
Clarified biosimilar mAb
HCP: 342,188ppm

Resin Process
4 min residence time

B&E Load: 25 g/L
B&E Yield: 95%
Elution HCP = 2,476 ppm

B&E Load: 50 g/L
B&E Yield: 80%
Elution HCP = 77 ppm

FT Load: 250 g/L
FT Yield: 99%
FT HCP = 7 ppm



Membrane Process
6 sec residence time

B&E Load: 40 g/L
B&E Yield: 95%
HD-A Elution HCP = 294 ppm

B&E Load: 55 g/L
B&E Yield: 85%
HD-Sb Elution HCP = 21 ppm

FT Load: **20,000 g/L**
FT Yield: 99%
HD-Q FT HCP = 9 ppm

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Goal 2

- **Improve process productivity**
  - 2 FT steps: CEX in FT mode; AEX FT final polish

- **High productivity IEX membranes (Tune in productivity)**
  - HD-Sb: Great performance in high conductivity
    - Process opportunity & flexibility
  - High loading capabilities at low residence time multiply productivity

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Case Study: Optimized Productivity DSP for an Innovator mAb

- mAb to polish post ProA capture has high HMW content
- High DSP productivity obtained with membranes
  - Further increased by changing CEX Bind/Elute step to FT step
- Productivity of the optimized double-FT membrane DSP
  - Enhanced by directly connecting the 2 FT steps
  - No buffer adjustment between IEX steps

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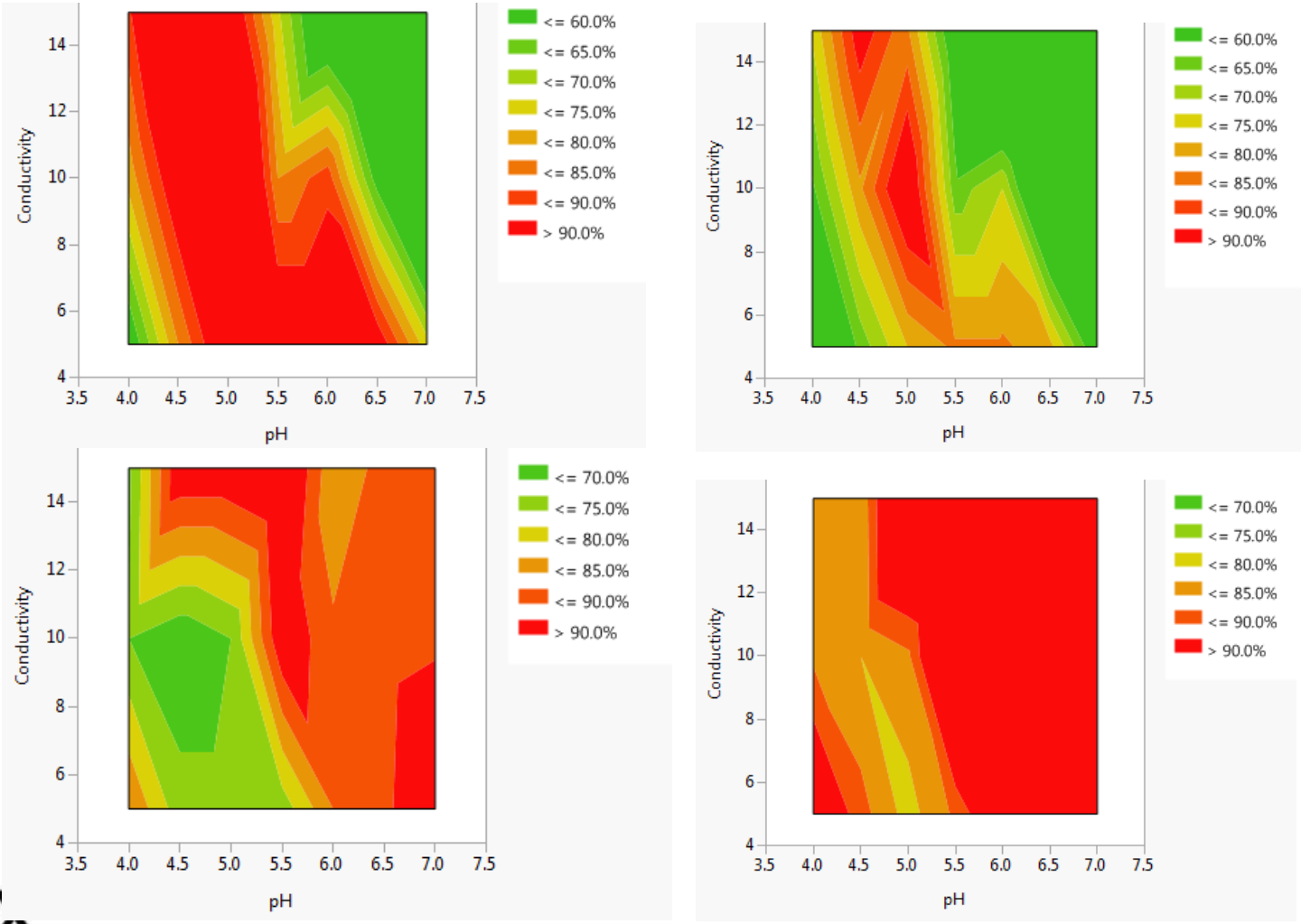
HD-Sb:
Flowthrough Screening Space

300 mg/mL Load

500 mg/mL Load

Aggregate Reduction

Monomer Yield



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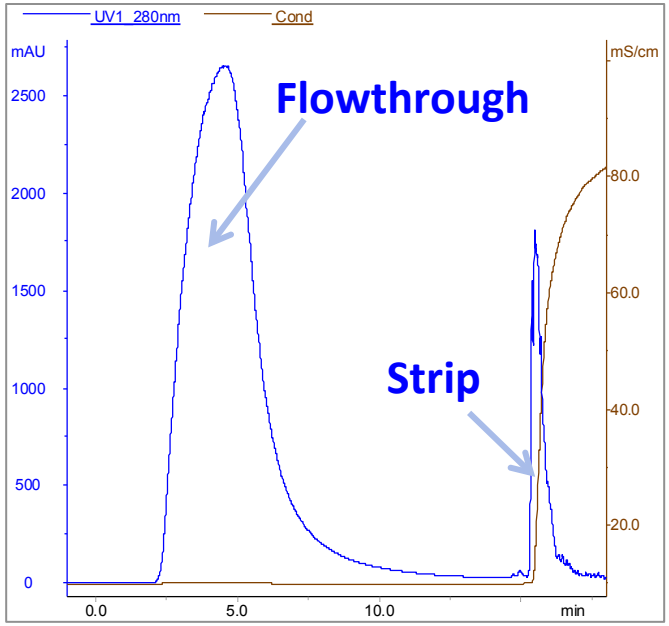
HD-Sb Flowthrough Mode Performance:

High Aggregate Clearance (2 layer screening device)

Parameter	Performance
Load	300 mg of mAb/mL of membrane
Residence Time	0.1 minute
HCP Clearance	81%
Load (ppm)	247
Flowthrough (ppm)	47
Aggregate Clearance	95%
Load	10.4%
Flowthrough	0.5%
Monomer Yield	>90%



Excellent separation at very high productivity

Equilibration: 50 mM Acetate + NaCl, pH 5.5 & 10 mS/cm
Sample: 10.5 g/L Protein A purified mAb2, pH 5.5 & 10 mS/cm
Strip: 25 mM Tris + 1 M NaCl, pH 8.1

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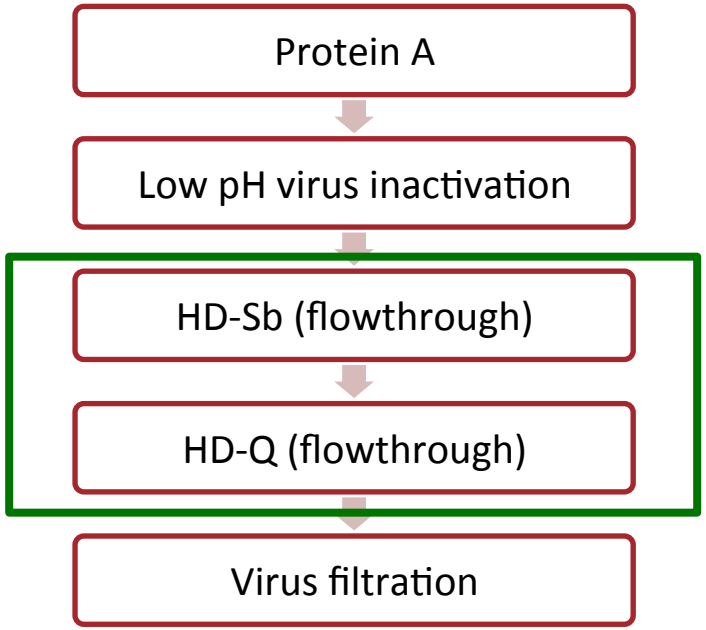
Tandem Process without Sample Adjustment:

HD-Sb → HD-Q

Parameter	Performance
Load	300 mg of mAb/mL of membrane
Residence Time	0.1 minute
HCP Clearance	89%
Load (ppm)	239
Flowthrough (ppm)	26
Aggregate Clearance	94%
Load	9.9%
Flowthrough	0.6%
Monomer Yield	90%



Potential for continuous processing

Equilibration: 25 mm Tris, pH 7.5 & 2 mS/cm
Sample: 3.5 g/L Protein A purified mAb2, pH 7.5 & 2 mS/cm
Strip: 25 mM Tris + 1 M NaCl, pH 8.1

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Conclusions

- Single-use process achieves target quality attributes for mAbs
- Two FT steps provide further optimization of media utilization
  - Reduction of overall DSP time; better productivity; improved economics
- Process productivity is further enhanced by directly connecting the 2 FT steps with no sample/buffer adjustment
- Single-use process multiplies productivity compared to traditional column process
  - Flexibility and productivity improved for enhanced DSP competitiveness

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