Affinity Membrane Chromatography Platform to Optimize Purification Process Architecture

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ABSTRACT

As the only purification technique that enables biomolecule separation based on individual biological and chemical structure, affinity chromatography can easily achieve a high degree of purity that would otherwise require a complicated process combining other techniques. The proven success of the widely accepted Protein A affinity resin columns in monoclonal antibody (mAb) manufacturing has demonstrated the ease of method development and the potential for a generic purification approach using affinity chromatography. Unlike mAb manufacturing many biotherapeutics segments still use traditional purification schemes that suffer from low yield and lengthy processing time because multiple unit operations have to be employed together to achieve the desired purity due to the lack of suitable affinity media. This work presents an emerging technology platform that combines high selectivity with the high productivity of Natrix hydrogel membranes, while leveraging all the proven benefits of single-use technologies. This poster presents three proof of concept studies demonstrating optimized process architectures with ligands specifically designed for mAb and vaccine purifications. The host cell protein clearance performance of the Protein A membrane is comparable to the reference resin column (>3 LRV), while the novel Affilin membrane shows excellent impurity reduction for both yeast and HEK293 harvests (4.6 LRV and 3.3 LRV, respectively). As a result, the number of steps is reduced as compared to the reference processes (from 3 to 2 chromatography steps using the Protein A membrane and down to one chromatography step using the Affilin membrane).

NATRIX HD MEMBRANE TECHNOLOGY

Membrane Generation

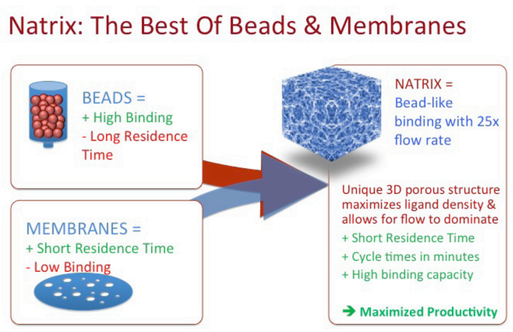
- Flexible, reinforced fiber mesh provides strength and structure
- Mesh is filled with functionalized porous hydrogel
- Functionalized, durable composite membrane is created in a single step

Membrane Characteristics

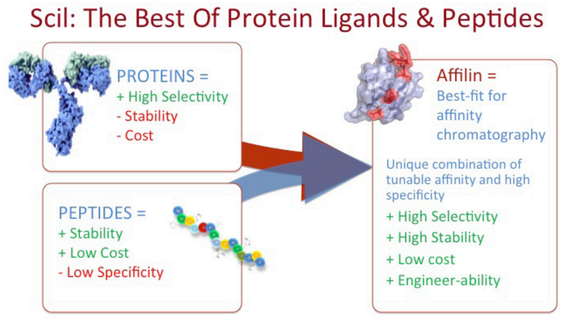
- Hydrogel provides binding groups and final pore structure
- Identical functional binding group chemistry as resins – C, Q & S

Advantages

- High ligand density provides high binding capacity
- No diffusional limitation allows for residence time in seconds
- Fully disposable and scalable for GMP manufacturing



AFFILIN® LIGANDS FOR AFFINITY CHROMATOGRAPHY



Ligand Generation

- Derived from ubiquitin protein
- Engineered from large libraries to generate affinity ligands towards customer targets
- Isolation of binders based on display and high-throughput screening technologies

Ligand Properties

- High affinity, specificity and stability against proteases, chemicals, and high pH
- Low production cost (E. coli expression)

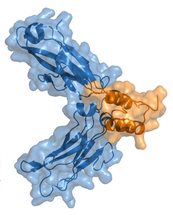
High Engineerability of Affilin® Ligands

- Small, compact molecule enables broad engineering opportunities
- Multimerization allows increased capacity
- Specific and oriented coupling to solid phase

PROTEIN A MEMBRANE

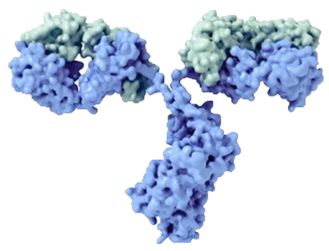
Protein A ligand has been widely used in mAb purification

Ligand: Protein A Ligand
- MW = 42 kDa



Fragment B of Protein A complexed with IgG

Target: IgG
- MW: 150 kDa



Protein A membrane - Performance

Table 1: Best-in-class Impurity Clearance For Multiple MAbs

		HD-A Membrane		Protein A Resin	
	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

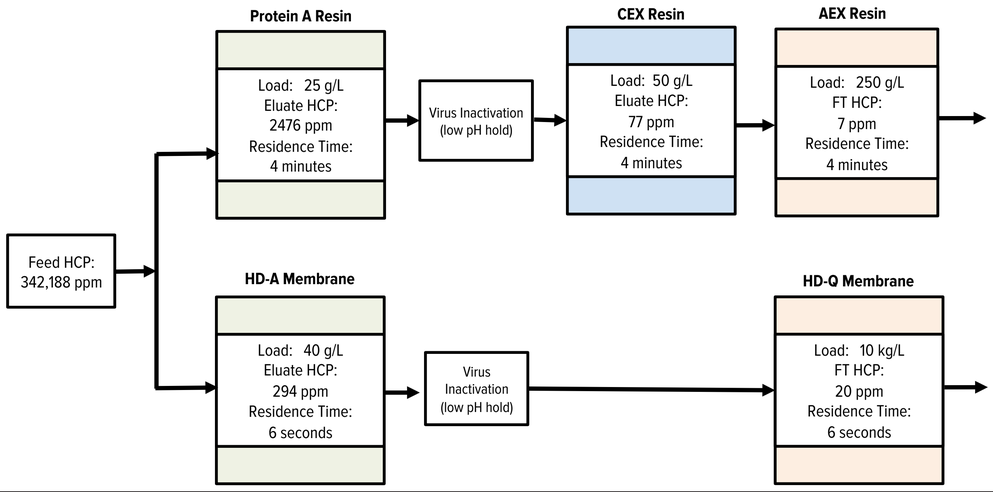
- Residence time: Membrane = 6 seconds; Resin = 4 minutes
- * Pre-treated feed

Table 2: Robust Impurity Clearance Across Multiple Buffer Systems

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

- Typical leached ProA ~10 ppm for both resin and membrane
- Non-optimized buffer and method condition for all HD-A membrane run

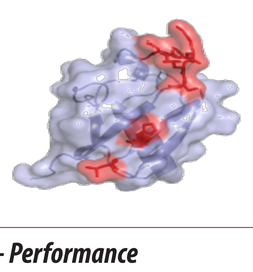
***Process Comparison:** Simplified process architecture reduces 3 chromatography steps to 2*



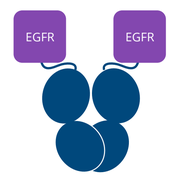
AFFILIN MEMBRANE

EGFR presented as proof-of-concept example for vaccine purification

Ligand: EGFR Affilin
- MW = 11 kDa
- K_d = 1 nM
- T_m = 72°C



Target: EGFR-Fc
- MW = 100 kDa
- ~25 kDa EGFR extracellular domain



Affilin membrane - Performance

Table 3: Capture >99.9% Pure EGFR in a Single Step

HEK-293 Cell Line	
Feed HCP	28.5 µg/ml 444,918 ppm
Eluate HCP	<52 ng/ml 213 ppm
HCP LRV	3.3
Binding Capacity	12mg/mL

- HEK-293 transiently transfected, expressing EGFR
- Lysate concentration is 64 ug/mL EGFR

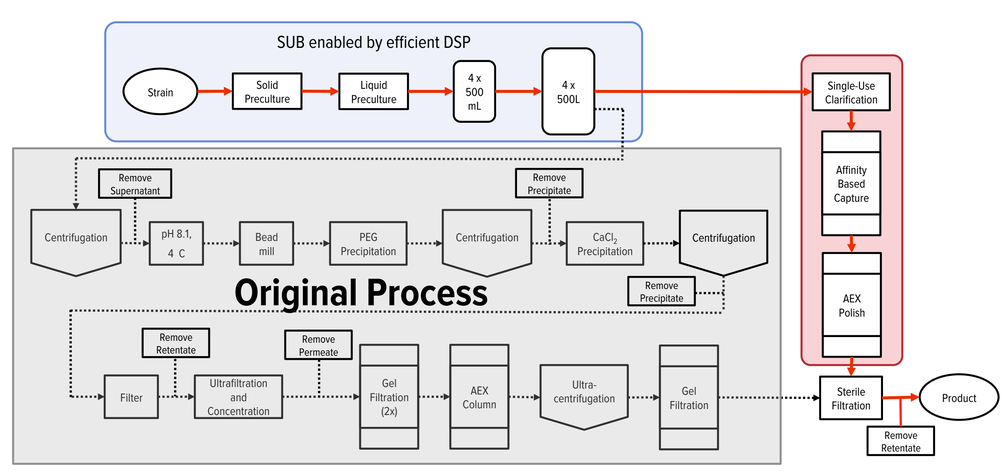
Table 4: Capture >99.9% Pure EGFR in Most Difficult Feed

Yeast Lysate	
Feed HCP	121 µg/ml 2,050,847 ppm
Eluate HCP	< 1.25 ng/ml < 52 ppm
HCP LRV	4.6
Binding Capacity	8 mg/mL

- Lysate produced by high pressure homogenization
- Lysate concentration is 59 ug/mL EGFR

Residence time: 6 to 12 seconds | One layer of Affilin® prototype membrane in 25 mm SS holder
Non-optimized buffer and method condition for all runs | Supernatant clarified using 2-stage depth filtration

***Process Comparison:** Optimized vaccine purification scheme reduces process steps from 20+ to 11*



ABOUT NATRIX SEPARATIONS

Natrix Separations is the developer and manufacturer of Natrix HD membrane technology, an advanced chromatography material that enables significant speed and capacity improvements for the capture and purification of biologics. Natrix products utilize established industry-standard chemistries in a single-use format to provide a low cost manufacturing advantage for drug developers. The Natrix team is comprised of industry leaders in downstream processing, as well as engineering, design, quality and manufacturing. Natrix is privately-held and based in Burlington, Ontario, Canada.

About Natrix Technology: Natrix HD Membranes offer a breakthrough in membrane architecture that will change downstream purification. With a three-dimensional macroporous hydrogel structure that provides a High Density of binding sites and rapid mass transfer, Natrix HD Membranes deliver binding capacity that exceeds resin-based columns with the fast flow rates typical of membrane adsorbers. Additionally, Natrix HD Membrane technology is highly versatile, and can be deployed in flow-through or bind-elute mode, with nearly any ion exchange, affinity or customized chemistry.

CONCLUSIONS

Combining the benefits of specifically engineered ligands with single-use membrane adsorbers provides numerous advantages for optimizing mAb and vaccine purification. The high specificity of the ligand increases the product purity which reduces the need for multiple purification steps. As demonstrated with the Natrix HD-A affinity membrane, the CEX step following Protein A can be removed and mAb purification is reduced from a 3 chromatography step process to a 2 chromatography steps. In the vaccine production case study, incorporating affinity membranes enables a combined upstream and downstream process of only 11 steps compared to 20+ steps in the original manufacturing process. This simplified process architecture increases yield as well as lowers the cost of goods. When the engineered ligands are coupled with the Natrix-HD membrane technology an even more efficient process is obtained. This new affinity material provides better or equal impurity clearance with the increased advantages of higher productivity (6 second residence time) and process robustness. Natrix hydrogel membranes add flexibility and reduce the cost of goods in mAb and vaccine purifications. The attributes of this emerging technology platform are a better fit towards being competitive in biosimilar markets as well as competing with global markets.

Natrix wishes to thank its industrial collaborators for testing NatriPur™ and NatriFlo™ products

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