Affinity Membrane Chromatography For Robust And Cost-Efficient Industrial Vaccine Purification

M Zhao[1], E Komkova[1], A Katzschmann[2], G Skarja[1], J Stout[1], U Haupts[2], A Pralong[3] and R Jacquemart (1) Natrix Separations (2) Scil Proteins (3) GSK Vaccines • *For more information: renaud@natrixseparations.com*

ABSTRACT

Due to the outdated manufacturing technologies used for majority of legacy products, many current vaccines processes suffer from a lack of control and robustness; hence, the highest levels of quality are maintained only at the expense of extensive QC release testing and high lot rejection rates.

While manufacturers are engaged in life cycle management projects, the use of process chromatography is gaining its popularity but the implementation is still limited mostly due to the low throughput and vastly capital expense associated with resin columns. This work presents an innovative process architecture strategy taking advantage of the purification power of affinity chromatography and the high throughput of hydrogel membranes, while leveraging all the proven benefits of disposable technologies.

Proof of concept studies combining Scil Affilin ligands and Natrix hydrogel membranes technologies are presented. The potential for a robust, high yield and generic vaccine affinity-based purification platform is discussed. Relying on a full process economic model independently developed by a customer, the impact of this approach on cost of goods of the drug substance for a current vaccine candidate is demonstrated.

AFFILIN® LIGANDS FOR AFFINITY CHROMATOGRAPHY

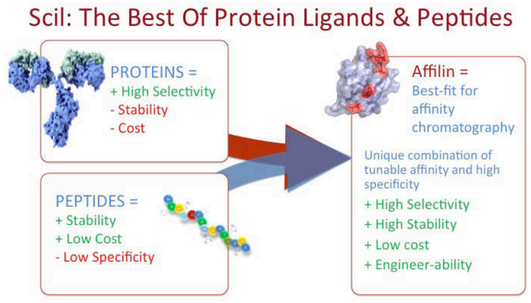
Ligand generation
- Derived from ubiquitin protein
- Engineered and selected from large libraries to generate de-novo binding affinity towards customer targets
- Isolation of binders based on display and high-throughput screening technologies

Ligand properties
- High affinity and specificity
- High stability against proteases, chemicals and high pH treatment
- Small, compact molecules; easy to engineer
- Low production cost (E.coli expression)

High engineerability of Affilin® ligands
- Small and compact nature of molecule enables broad engineering opportunities
- Multimerisation → increased capacity
- Specific and oriented coupling to solid phase
- Linker composition and length

scil proteins

Scil: The Best Of Protein Ligands & Peptides



NATRIX HD MEMBRANE TECHNOLOGY

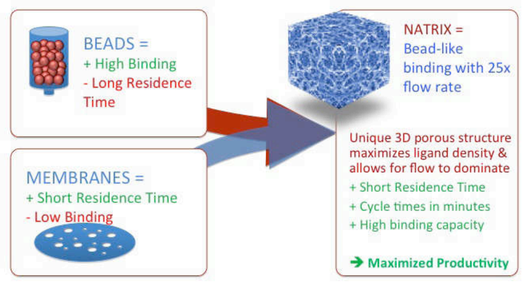
Membrane Generation
- Flexible, reinforcing fiber mesh provides strength and structure
- Mesh is filled with functionalized porous hydrogel
- Functionalized, durable composite membrane is created in a single step

Membrane Characteristics
- Hydrogel provides binding groups and final pore structure
- Identical functional binding group chemistry as resins – C, Q , mixed-mode, affinity

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

Natrix®

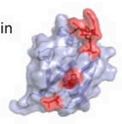
Natrix: The Best Of Beads & Membranes



STUDY DESCRIPTION

Affinity Separation PoC: Affilin Natrix membranes

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



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

Affilin ligand is coupled to Natrix membrane via multi-point attachment

Device: one layer of Affilin prototype membrane in 25 mm SS holder (membrane volume = 0.1 mL, device volume 1.2 mL)

Feed: the study comprises two parts with different feeds
- Purification of EGFR-Fc from HEK 293 clarified supernatant (process stream)
- Purification of EGFR-Fc spiked in homogenized whole yeast lysate

Standard chromatography method and buffer system used, no process development executed

SUMMARY OF RESULTS

- > 3 LRV HCP clearance in a single step
  - Very specific binding of the EGFR target to the Affilin membrane translates in minimum non-specific capture of contaminants
- ~3X concentration factor despite large hold-up volume of the testing device and non-optimized binding capacity
  - Binding capacity of the prototype membrane equivalent or superior to best in class resins, not yet challenged
- Optimized ligand presentation and binding would improve overall performance and recovery profile

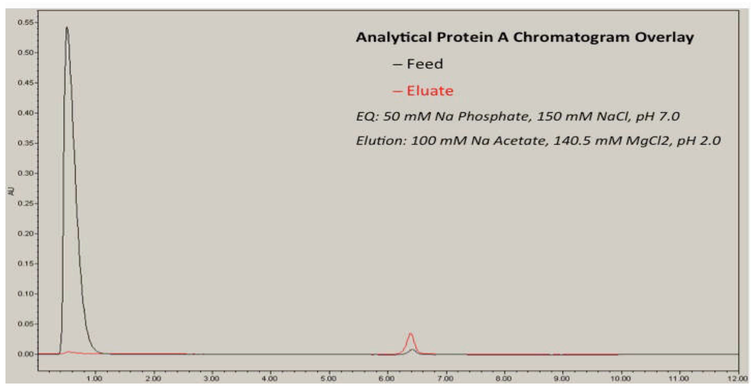
RESULTS: PART 1

Part 1: Purification of EGFR-Fc in HEK 293 Clarified Supernatant

- Supernatant concentration = 64 ug/mL EGFR
- Load: 12 mg/mL
- Residence time = 3-6 seconds
- ~3X concentration factor in eluate

	Affilin Membrane	Protein A Membrane
Feed HCP	28.5 µg/mL 444,918 ppm	28.5 µg/mL 444,918 ppm
Eluate HCP	52 ng/mL **213 ppm**	60 ng/mL **313 ppm**
HCP LRV	3.3	3.2
Elution Yield	94% (MB = 97%)	

Analytical method
- HCP: HEK 293 HCP ELISA (Cygnus)
- Mass balance/ Yield: Analytical Protein A column (Fc region)



RESULTS: PART 2

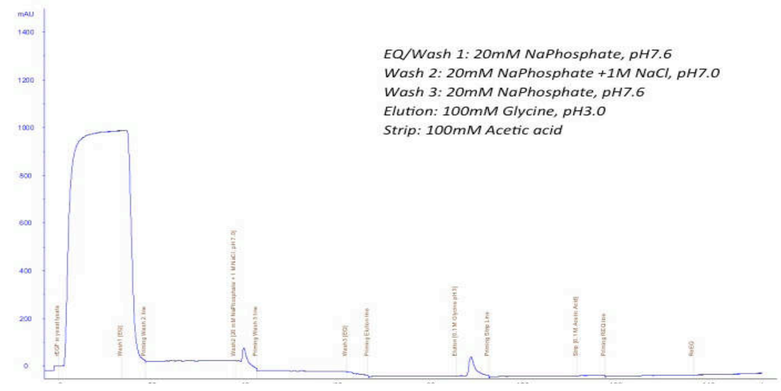
Part 2: Purification of pure EGFR-Fc Spiked in Homogenized Whole yeast lysate

- Supernatant concentration = 59 ug/mL EGFR
- Load: 8 mg/mL
- Residence time = 6-12 seconds
- ~3X concentration factor in eluate

Analytical method
- HCP: S. cerevisiae HCP ELISA (Cygnus)
- Mass balance/ Yield: Analytical Protein A column (Fc region)

	Affilin Membrane
Feed HCP	121 µg/mL 2,050,847 ppm
Eluate HCP	<1.25 ng/mL **<52 ppm**
HCP LRV	4.6
Elution Yield	82% (MB = 75%) (*)

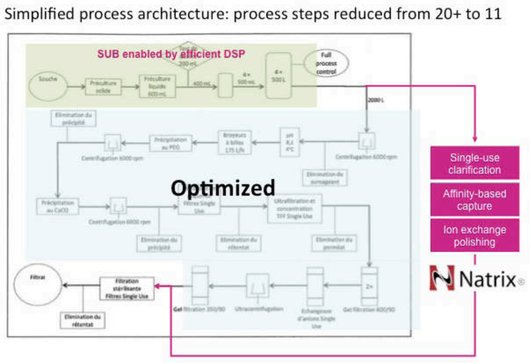
(*) EGFR spiked in the yeast feed is degraded during the run time, explaining MB and recovery lower than on HEK293 feed



ECONOMIC MODEL SUMMARY

Optimized New Process Architecture

Simplified process architecture: process steps reduced from 20+ to 11



Key Outputs From Case Study Modeling

	Current Process	Incremental Improvement Plan	Optimized New Process Architecture
USP Highlights	1600L SSB	3x1600L SSB + 3 clarification trains	2x500L SUB + continuous SU clarification
DSP Highlights	4 UC + multi columns chrom	2 UC multi-cycle + multi columns chrom	No UC + affinity membrane chrom
KEY OUTCOMES			
Throughput per year (MSD)	30	120	180
Cycle time (days)	12.5	13.7	6.2
Cost/dose (€)	~0.75	~0.40	<0.10
CAPEX investment (€)	Sunk	>300M	<50M
Scale-up flexibility	Cannot meet market demand	Limited	Yes, add more SUB to increase further (DSP capacity not limiting)

MSD= Million Sellable Doses; SSB= Stainless Steel Bioreactor; SUB= Single Use Bioreactor; UC= UltraCentrifuge

MODEL CONCLUSIONS

- Develop new specific vaccine processes that assure product quality and consistency
  - Single-use bioreactors enabled by high robustness and high productivity affinity chromatography
  - Fit into highly flexible manufacturing facilities with minimal costs
- Reduce process complexity, improve quality and compliance
  - Eliminate intrinsic compliance issues, 6σ operations yield few enforcement actions
- Increase efficacy, provide sustainable, competitive cost-of-goods for global markets
  - Build facilities in-market; regain/retain market share lost to low cost producers

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

Challenges for the vaccine industry
- Legacy vaccine production processes are outdated and overly complex
- Global health trends require robust, increased supply with lower cost
- "Process=product" mindset impedes process advancements

Upcoming needs for purification platforms
- High productivity while maintaining purification efficacy
- Simplified and reliable manufacturing process
- Increased flexibility

The data presented in this poster indicates that
- Affinity membrane chromatography has the potential to provide high purification factor, high robustness and high yield
- Implementation of affinity membrane chromatography can help to streamline industrial vaccine purification strategy and enable single-use process architecture

Natrix Separations, Inc. 5295 John Lucas Drive - Unit 6, Burlington, ON L7L 6A8 Canada 1.905.319.2682, info@natrixseparations.com

www.natrixseparations.