IMMUNO-ONCOLOGY INSIGHTS

INNOVATOR INSIGHT

Overcoming purification challenges in antibody therapeutics manufacturing with subdomain-specific affinity solutions

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Novel biotherapeutic antibody formats, such as bispecific monoclonal antibodies, Fab fragments, and Fc-fusion proteins have created new purification challenges in downstream processing. The use of bacterial surface proteins, such as Protein A, is not always the most efficient solution for the purification of these novel modalities. Affinity chromatography resins, specifically developed to bind antibody subdomain regions, can provide an alternative solution for a variety of antibody formats.

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The antibody therapeutics arena has undergone a dramatic shift over the past few years, with clinical trials moving from standard monoclonal antibodies (mAbs) to a range of antibody fragments, Fc-fusions, bispecifics, and antibody–drug conjugates. To ensure the successful manufacturing of these next-generation modalities, additional tools are needed, especially in the downstream process of these molecules. In this article, we will focus on the special challenges of purifying novel antibody products.

For standard mAbs, purification is typically carried out by protein A affinity chromatography, but this technique is not always effective for novel antibody-based therapeutics. This has led manufacturers to resort to less efficient multi-step purification processes using

non-affinity capture. Every additional process step reduces the amount of product recovered – even with 85% yields at each step, half of the product produced in the upstream process may be lost over four or five process steps.

To improve product yields, decrease the cost of goods, and accelerate time to market, Thermo Fisher Scientific has developed a portfolio of products combining subdomain-specific affinity capture resins with extremely effective polish steps. Reducing the number of purification steps in the overall process increases total product yield and reduces bioprocess development time, leading to a faster time to market and a decrease in the overall cost of goods.

CLEVER CAPTURE

The CaptureSelect™ product range is the largest portfolio of affinity resins available from any supplier, suitable for purification of a large variety of antibody formats, as well as biosimilars, biobetters, recombinant proteins, and viral vectors. CaptureSelect products feature tunable affinity ligands, which can be designed for virtually any protein or virus particle, giving you high purity in a single step.

Developed in 2002, CaptureSelect technology is based on the variable domain of Camelid heavy-chain-only antibodies, which as the smallest antigen-binding fragments (~15kD), allow binding to difficult to reach epitopes, leading to a unique affinity for the target molecule. Due to their compact structure, these domains are very robust and can withstand the various conditions found in chromatography. The structure and features of CaptureSelect resins are summarized in Figure 1.

CaptureSelect screening technology can generate products to bind to different regions of the antibody molecule so that whatever type of antibody format is being manufactured there is an associated affinity capture step. During the ligand discovery stage, ligands are screened for target specificity, mild elution, and stability (for cleaning and storage). This creates a final resin that has a high affinity to the target, resulting in high yield and purity in a single step. The resins also have mild elution properties and can be cycled multiple times. The advantage of the ligand manufacturing system is that they are produced in Baker's yeast, making it an animal-origin-free

FIGURE 1

Key features of CaptureSelect Affinity Resin.

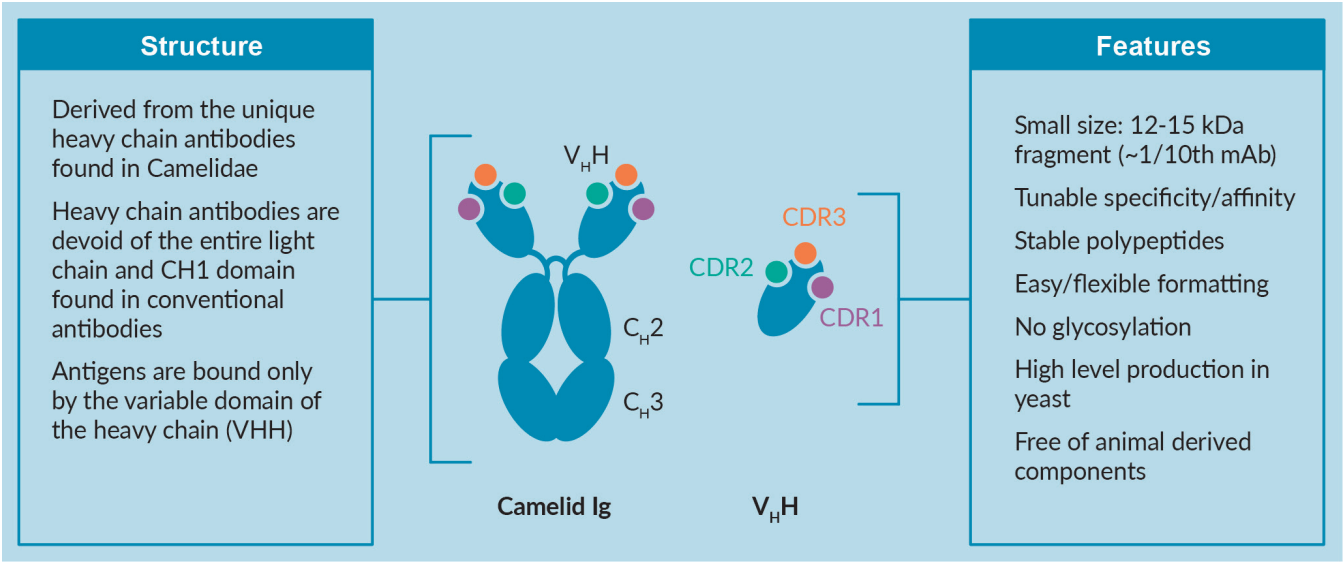

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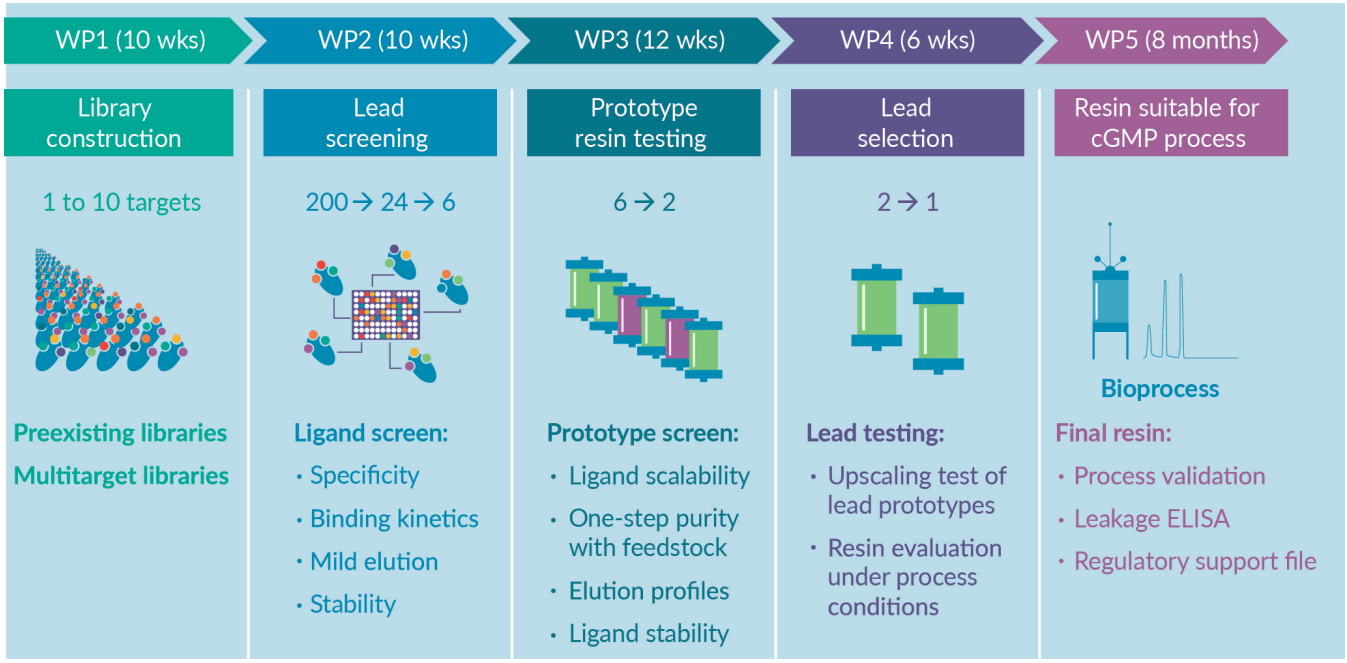
FIGURE 2

CaptureSelect Affinity Resin development.



WP: work package.

production process. When an off-the-shelve resin is not available, we have a proven custom ligand development program. Once a lead has been selected in the screening program, the process for developing a new resin suitable for use in a cGMP process takes around 8 months in total (Figure 2).

CaptureSelect affinity resins also come as ready-to-use prepacked GMP bioprocess columns – CaptureSelect EvolveD™ columns (EvolveD is a registered trademark from Delta Precision Ltd)– which can be directly connected to standard chromatography systems. They have non-metallic flow paths, which eliminates corrosion risk and ensures containment of the process. The columns come with full regulatory support documents.

There are four different CaptureSelect resins for antibody purification which are shown in Figure 3. More details are given in the following sections.

CH1-XL

CH1-XL was specifically developed for Fab fragment purification. It is targeted to the first constant domain (CH1) of the IgG heavy chain and will not bind to free light chains, allowing a one-step purification of intact and correctly assembled antibody fragments. It has a high dynamic binding capacity for Fab fragments and, when needed, elution can be done under mild conditions (pH 4.0–4.5 with 50 mM acetate buffer).

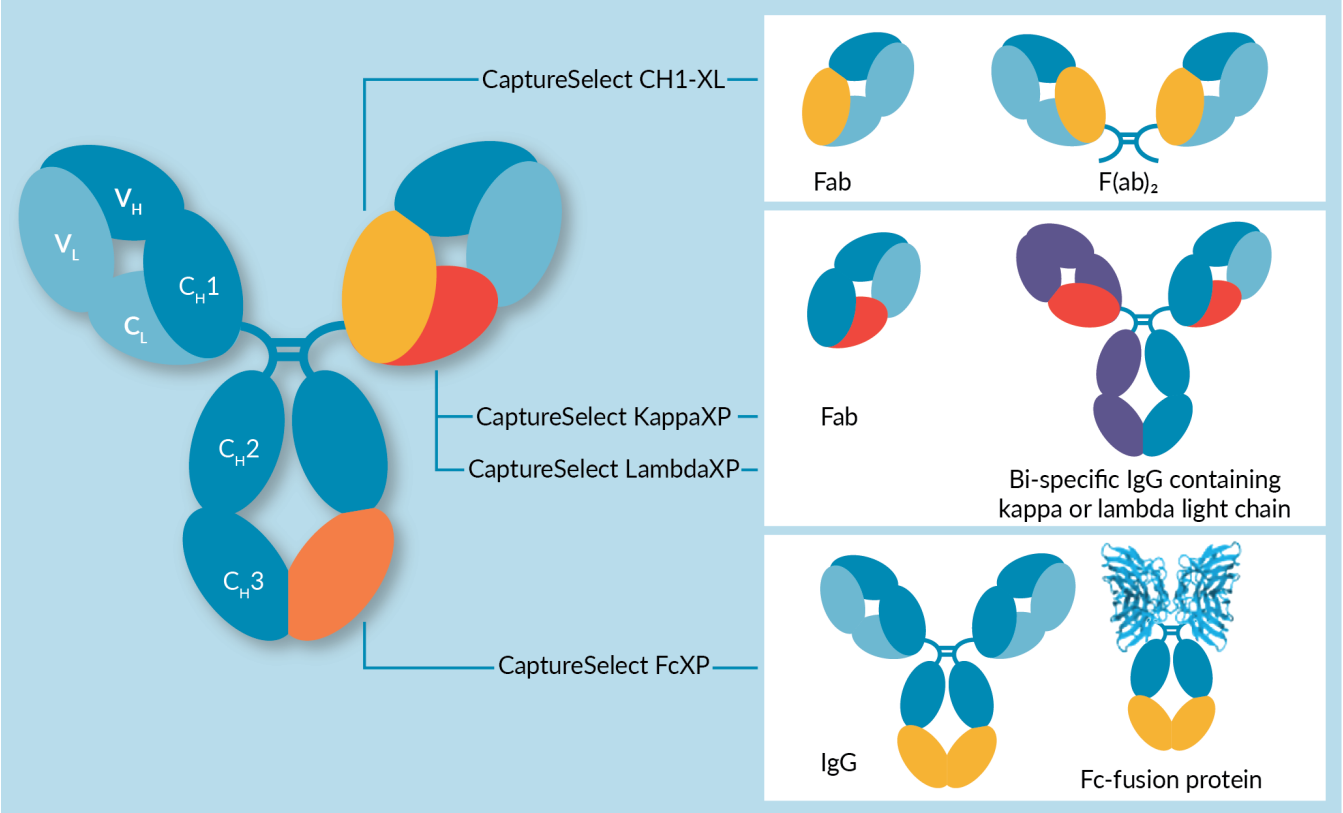
An example of a customer using CH1-XL to purify the Fab fragment of ranibizumab in a single-step process can be seen in Figure 4. Recently, CH1-XL has been made available on magnetic beads – an ideal automated high throughput screening tool for fragment content.

KAPPAXP

Binding the constant region of the kappa domain, KappaXP is suitable for all immunoglobulins containing a kappa light chain. Its high binding capacity for both Fab and IgG (40g/L at 2 min residence time) makes it a good choice for Fab fragment purification when there is no issue with light chains in upstream development. In addition, it is ideal

FIGURE 3

Key features of the CaptureSelect CH1-XL, KappaXP, LambdaXP, and FcXP resins.



for the purification of bispecifics containing a kappa light chain as this resin allows operation under very mild elution conditions. The KappaXP affinity matrix has a large elution operating space when co-solvents are used, allowing elution up to pH 5-6 when needed (Figure 5).

FCXP

FcXP affinity matrix is the next-generation Fc-binder and is an improved version of CaptureSelect FcXL, sharing the same specificity but with improved binding capacity (Figure 6), elution, and cleaning capabilities. FcXP binds whole IgG molecules via the CH3 domain, in comparison to Protein A, which binds at the interface of CH2 and CH3. The advantage of using FcXP over Protein A is that it can be used with milder elution conditions (pH 4-4.5) – good for IgG-fusion proteins or antibodies which cannot withstand or aggregate during low pH elution. Also, modified therapeutic antibodies can lose the protein A binding site when the interface of CH2 and CH3 domain, to which protein A binds, is altered. Since FcXP only binds CH3, a region less often modified, it can serve as an alternative for purifying antibodies with an altered protein A binding site.

LAMBDAXP

A recent addition to the product portfolio, CaptureSelect LambdaXP binds to the lambda light chain, covering all lambda subtypes with no cross-reactivity to kappa. It has a high dynamic binding capacity of over 35 g/L and a small elution pool volume when eluting at pH 3.5–4. Figure 7 shows the binding capacity for a bispecific

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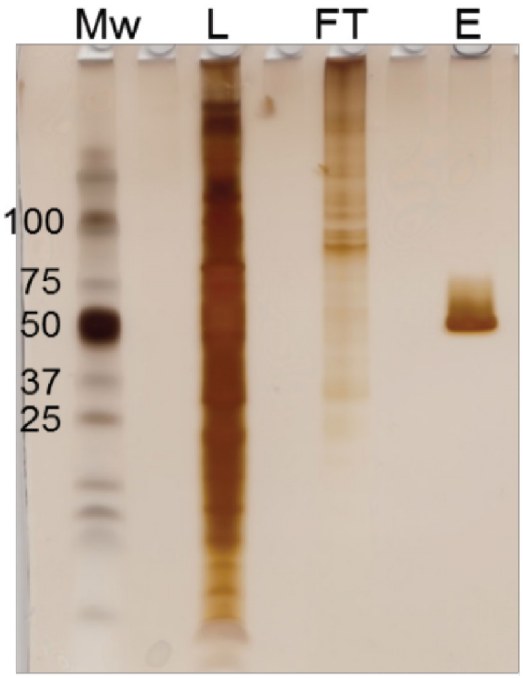
FIGURE 4

Single-step Fab fragment purification.



Purification of ranibizumab, a therapeutic monoclonal IgG Fab fragment produced in HEK293 cells, shows high yield and purity in one step [2].

antibody product. Similar to KappaXP, the LambdaXP resin is highly suitable for bispecific antibodies and Fab fragments, in this case containing a lambda light chain. The resin can be used with milder elution conditions than Protein A, which protects the target molecule during purification.

CASE STUDY: FCXL FOR CAPTURE OF A NOVEL ANTIBODY FORMAT

We worked with a large pharmaceutical customer on a rapid implementation of our CaptureSelect™ FcXL resin into a commercial next-generation therapeutic manufacturing process [1]. Their novel antibody format has modifications to the hinge region, meaning that it no longer binds effectively to protein A. There was no suitable alternative affinity resin available internally as a qualified GMP-compliant raw material, and they were unable to use their platform three-step chromatography process.

We worked with the customer to provide them with the FcXL asset. Like FcXP, FcXL binds to the CH3 domain of IgG and allows efficient elution at a mild pH – something not possible with the original process. FcXL performed well in comparison with the existing affinity capture process and a range of possible alternatives in terms of low density, host cell protein reduction yield, sum of high molecular weights, the main peaks, and the sum of the low molecular weights (Table 1).

The process continued to give a very high consistency in terms of yield and host cell protein in pool when scaled up for GMP manufacture. The introduction of FcXL removed a step from the purification process, resulted in higher binding capacity, improved the impurity profile and pool stability, and added an environmentally friendly regeneration solution.

PERFECT POLISH

The long-established POROS™ line of ion exchange affinity and hydrophobic interaction chromatography (HIC) resins feature high capacity, salt tolerance, and high resolution, enabling the robust separation of product from process- and product related impurities. They offer unique solutions for polish

FIGURE 5

Resin elution efficiency with CaptureSelect KappaXP affinity matrix, showing large elution operating space using magnesium chloride co-solvents.

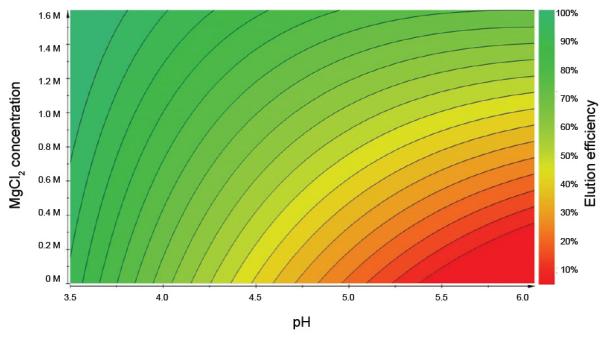


FIGURE 6

Dynamic binding capacity at 10% breakthrough for monoclonal and polyclonal IgGs.

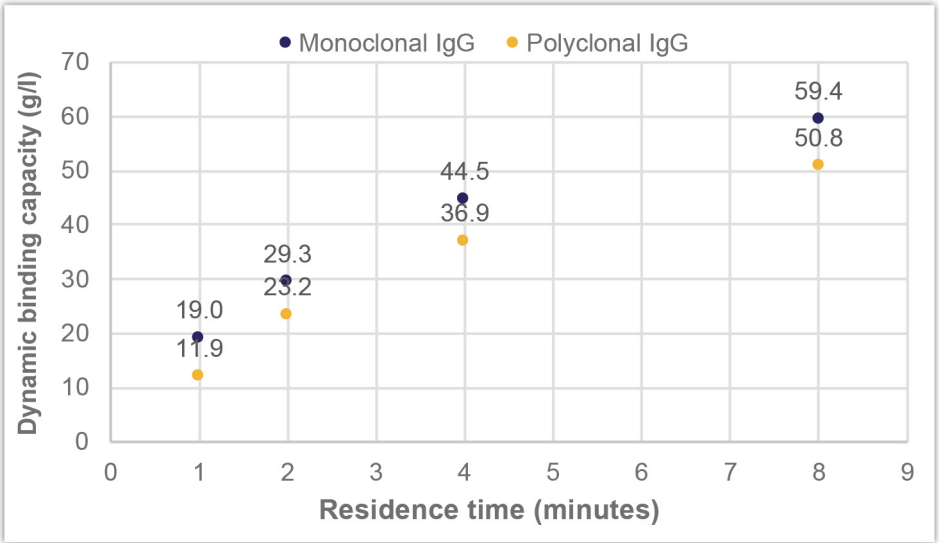
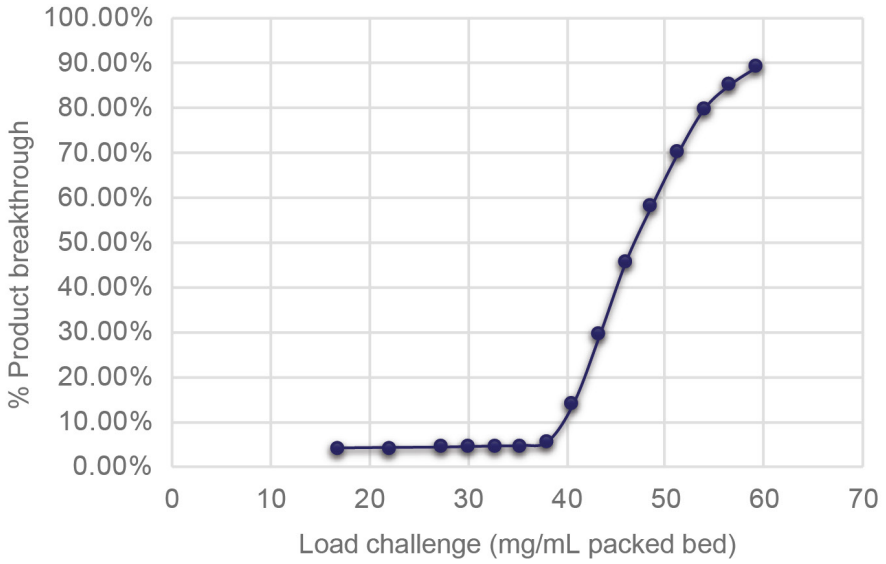


FIGURE 7

Dynamic binding capacity of a bispecific antibody using LambdaXP affinity resin at 4 min residence time.



chromatography in both bind/elute and flow-through modes.

Thermo Fisher Scientific has a full range of POROS polish solutions for novel antibody formats, including ion exchange, cation exchange, and hydrophobic interaction (Table 2).

A unique feature of POROS resins is that performance is independent of the flow rate – resolution and binding capacity are

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TABLE 1

Comparison of purification techniques for a novel antibody format.

	Existing affinity	Affinity B	Affinity C	Non-affinity	Capture Select FcXL
Load density	Med	Low	Med	Very high	High
CHOP reduction	High	High	Very high	Low	Med
Yield %	High	High	Low	Low	High
Sum HMWs rel%	Med	Med	Med	Med	Med
Main peak rel%	Med	Med	Med	High	High
Sum LMWs rel %	High	High	High	Med	Low

maintained as the linear flow rate increases. The linear flow rate and linear pressure curve associated with POROS resins mean that scalability is linear and predictable.

CASE STUDY: MAB PURIFICATION

A customer was using standard protein A chromatography to capture their mAb product, followed by anion exchange in flow-through, and due to a high level of aggregates, a generic mixed-mode bind/elute step was used as a final polishing step. This process was achieving a clearance of aggregates <1% and a 90% recovery with loading at 25 g/L at 6 min residence time. To improve the recovery of the polish process, the customer was keen to replace the mixed-mode bind/elute step, provided that a high level of aggregate clearance could be maintained.

POROS Benzyl Ultra was optimized at a low salt concentration to clear aggregates. For buffer, we chose Tris-acetate pH 6.8 with a conductivity below 4mS to allow for a pool-less purification design flowing from the AEX step directly onto HIC for aggregate clearance without any further buffer conditioning.

A confirmation run showed running at 500 cm/h or 1.2 min residence time and loading 80g/L resin. The process showed complete removal of aggregates from 85.5% monomer worst-case scenario in the load feed to 99.3% in the final FT pool. A high-productivity

TABLE 2

POROS anion and cation exchange resins.

POROS Resin	Type of AEX Resin	AEX Applications
D50	Weak	**Bind/elute:** Protein, virus, plasmid DNA purification
PI50	Weak	
HQ50	Strong	**Flow through:** Trace impurity removal by binding impurities (DNA, viruses, HCP, aggregates, endotoxin)
XQ	Strong	
POROS Resin	**Type of CEX Resin**	**CEX Applications**
HS50	Strong	**Bind/elute:** Polish of many biomolecules (Mabs, VLP/viruses, fusion proteins, high pI rProteins)
XS	Strong	**Flow through:** Polish for Mabs by binding impurities under normal B/E conditions: impurity removal (aggregates, HCP, DNA, viruses)
POROS Resin	**Level of hydrophobicity**	**HIC Applications**
Ethyl	Moderate	Bind/elute mode of moderately to considerably hydrophobic molecules
Benzyl	Medium	Bind/elute or flow-through mode depending on molecule
Benzyl Ultra	High	Flow-through mode in lower salt to bind impurities such as aggregates

TABLE 3

Comparison of mixed-mode bind/elute (BE) and hydrophobic interaction chromatography (HIC) for aggregate removal step.

mAb-A process	Mixed-mode BE	HIC FT
Load density (g/L resin)	25	80
Monomer purity FT (%)	99	>99
Mon. recovery (%)	90	98
HCP (ppm)	<LLOQ	<LLOQ
Residence time (min)	6.0	1.2

flow-through process was achieved that can be directly coupled to the upstream anion exchange process without any buffer manipulation in between. The result was increased load and recovery while still obtaining high purity and great aggregate clearance, and the process was run faster for increased throughput (Table 3).

In this case, the customer has reported that they can remove more than 95% of clusterin in a single pass, but also more than 65% of other HCPs. We hypothesize that these other HCPs may piggyback with the clusterin. We are also working on resins targeting two other notoriously hard-to-remove HCPs – LPL and PLBL2.

USING AFFINITY PURIFICATION FOR HCP REMOVAL

There are several notoriously difficult to remove host cell proteins (HCPs) described for CHO-produced mAbs and bispecific Abs, including clusterin. To address this challenge, we recently embarked on a journey together with a customer, to develop an affinity resin to remove clusterin, resulting in the POROS CaptureSelect ClusterinClear affinity matrix. This is currently a research-use-only matrix, but we are working on a GMP version for use with novel antibody formats, where it can be hard to achieve appropriate HCP levels. It is designed as a scavenging resin and can be used in flow-through mode rather than in bind/elute mode.

CONCLUSION

If you have a purification challenge with a novel antibody format, and protein A is not resulting in optimal performance, there are alternatives available in the CaptureSelect range that can provide high purity and yield in a single capture step. Combined with POROS polish resins, there is an opportunity to reduce the processing steps or even switch from a bind/elute to a flow-through process. All of our products allow seamless scale-up for commercial manufacturing purposes and our fully experienced field applications and service team supports customers throughout the process.

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Q & A

Laurens Sierkstra
Senior Director, Business Leader Purification, Thermo Fisher Scientific

Laurens Sierkstra answers your questions on subdomain-specific affinity solutions.

Q Where in the process can you use POROS CaptureSelect ClusterinClear? Can it replace an ion exchange step?

LS: The ClusterinClear resin is an add-on to polish steps. It is not designed to replace all polish steps, but to ensure materials that cannot be removed by conventional methodology are being captured. Whether or not it can be combined with ion exchange polish needs to be determined.

Q A term that seems to cause confusion in the sector is cGMP. You mentioned in your presentation that the resins are cGMP-compliant – what do you mean by that statement in this context?

LS: Only pharmaceutical products can be cGMP. However, while our products cannot be cGMP in themselves, they are made to a standard that allows them to be used in cGMP processes.

Q How do you ensure security of supply for the affinity resins?

LS: From master working cell bank up to the final resin, we've got redundancy in place. Right from the start, our master and working cell bank are always stored at two locations. In terms of fermentation and purification, our products are manufactured in two fully independent facilities in the Netherlands and Lithuania. For resin manufacturing, we have redundancy in our agarose-based production facility, and Thermo Fisher Scientific is investing in a second US facility to achieve full redundancy within the POROS polish product lines.

Q When using the resins, does ligand leakage occur?

LS: A ligand immobilized on a resin will always leak to some extent. The levels for our products are low PPM, similar to protein A. For all of our products that are GMP-suitable,

we have developed a product-specific ligand leakage ELISA, which is purchasable off the shelf and will enable leach ligand determination.

Q Do you have any capture resins for retroviral or lentiviral vectors?

LS: We do have ongoing activities in lentiviral vectors – currently in the screening stage – working towards a lentivirus affinity capture step.

BIOGRAPHY

Laurens Sierkstra
Senior Director, Business Leader Purification, Thermo Fisher Scientific

Laurens received his PhD in biotechnology in 1994 from the University of Utrecht after studying biology at the University of Leiden. He then joined Unilever as Project Manager and Unit Leader. In 2005, after the spinout of BAC BV from Unilever, he became CEO of BAC BV and set up the business in using single- domain antibodies for affinity purification, called CaptureSelect, which was sold in 2013 to Life Technologies. Since the acquisition by Thermo Fisher Scientific, he has been the business leader for the affinity purification business within the Bioproduction Division

AUTHORSHIP & CONFLICT OF INTEREST

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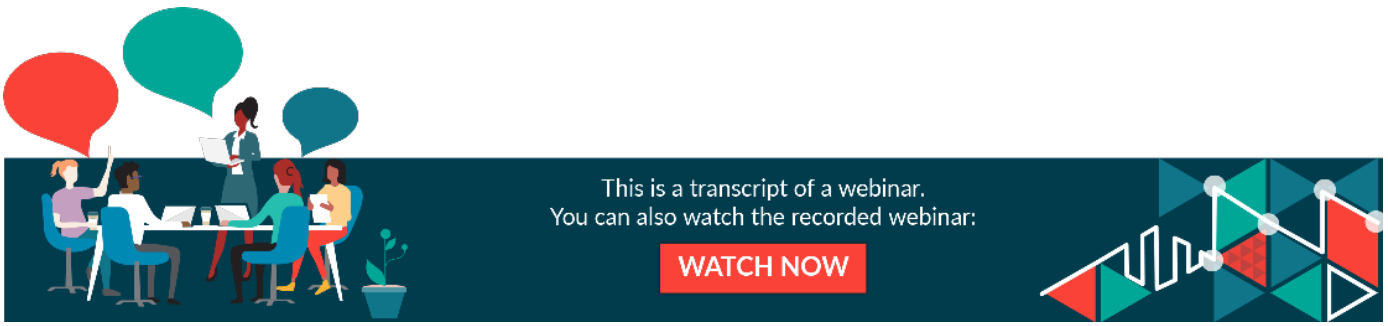

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