Streamline Manufacturing of Antibody-Based Therapeutics with Novel Purification Approaches

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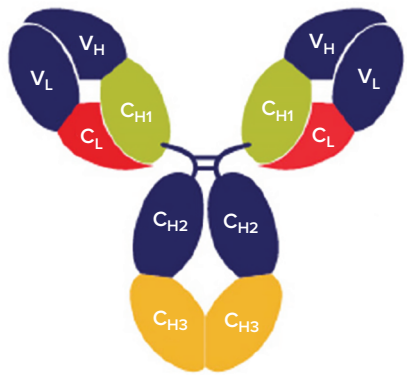
Using affinity capture in purification of antibodies enables developers to reduce the overall number of chromatography steps in their downstream processes. It can increase product yield, reduce the time required for bioprocess development, and ultimately reduce time to market and the overall cost of goods (CoG). Because of its ability to bind to the Fc region of monoclonal antibodies (MAbs), protein A has been used as the platform for affinity capture of IgG-based therapeutics for decades.

In addition to standard IgG MAbs, many antibody formats are in development: e.g., bispecifics, fusion proteins based on the crystallizable fraction (Fc) of antibodies, proteins made up of just the antigen-binding (Fab) fragments of antibodies, and antibody–drug conjugates (ADCs). Such engineered antibodies can pose a challenge to protein A affinity capture if they have altered or absent protein A binding sites. These novel formats also can be difficult to purify because some tend to aggregate, form dimers, and can come with elevated levels of high– and low–molecular-weight (HMW, LMW) species. Light-chain dimers can present alongside some antibody fragments, and overexpressed light chains can complicate their downstream processing further. In addition, some engineered antibodies are sensitive to low-pH/acidic conditions, which can limit a developer's choice of elution buffer.

New Options for Affinity Capture

Novel antibody modalities have driven development of new options for purification such as CaptureSelect technology. CaptureSelect ligands offer a unique affinity purification solution based on camelid-derived single domain (VHH) antibody fragments. The small, 14-kD affinity ligands provide a platform solution to many biopharmaceutical purification challenges and have been proven to deliver increased yields and purities for proteins of interest in many applications. VHH affinity ligands are produced by recombinant yeast culture in a process that is free of animal-origin materials. The molecules are highly stable and screened for high specificity, binding capacity, and desired elution conditions for a target therapeutic of interest. Thermo Fisher Scientific has developed a number of unique CaptureSelect affinity matrices to target different binding sites on an array of human therapeutic proteins (Figure 1).

CaptureSelect CH1-XL ligands bind to the constant heavy domain (CH1) of all human IgG subclasses and purify 100% of kappa and lambda Fabs, providing an excellent platform for Fab purification. Because of its high selectivity, the CH1-XL ligand precludes copurification of free light chains and light-chain dimers. The resin has a high dynamic binding capacity (DBC) of ~19 mg/mL for a polyclonal Fab with efficient elution at relatively mild pH. Fab purity of 98%



Example antibody subdomain structure

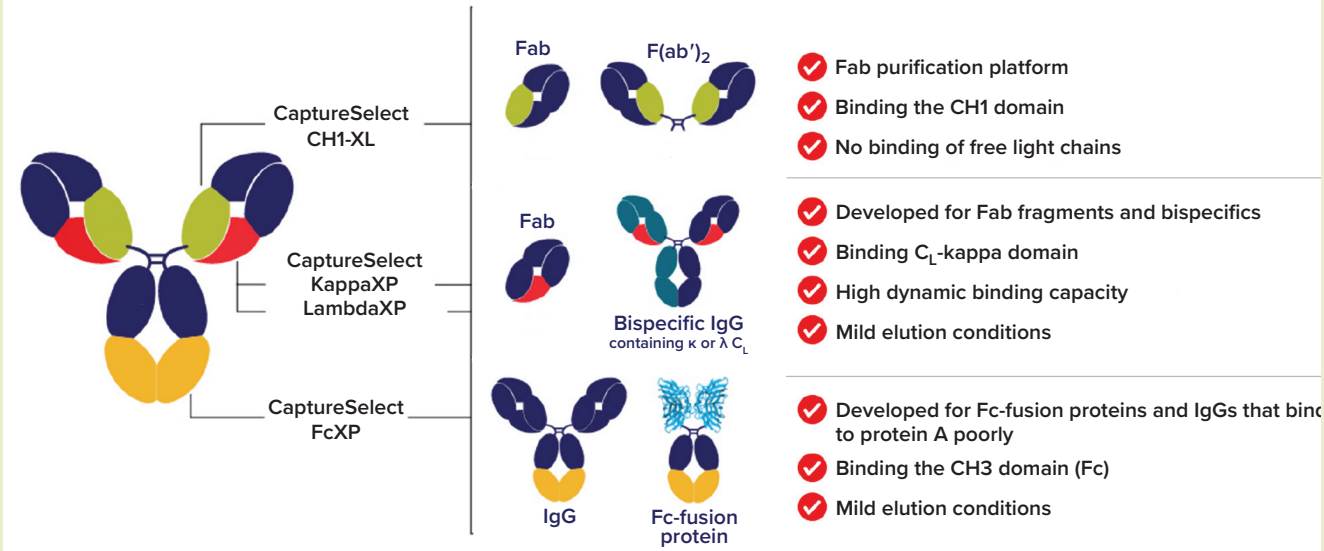
has been achieved using CaptureSelect CH1-XL resin, with a nearly 90% yield.

CaptureSelect KappaXP resin was designed for purification of Fab fragments and bispecific antibodies. The ligand provides 100% kappa subtype coverage for all immunoglobulins that contain a kappa light chain. This resin has a high DBC of 20–30 g/L for kappa Fabs and has a DBC of 30-45 g/L IgG. Efficient elution can be achieved at relatively mild acidity (up to pH 6).

CaptureSelect LambdaXP ligands offer 100% lambda-subtype coverage and can be used to purify IgGs that contain a lambda light chain, including bispecifics and Fab fragments. The resin has a high DBC of >35 g/L for IgGs and can be eluted at pH 3.5–4.0.

CaptureSelect FcXP ligands bind only the constant heavy domain CH3. These were developed for Fc-fusion proteins, chimeric antibodies, and molecular formats in which the protein

Figure 1: CaptureSelect ligands offer a range of highly specific affinity-capture options.



A binding site is either removed or blocked. The resin also has a high DBC — >40 g/L with 10% breakthrough (BT) and 5-minute residence time — and elutes efficiently at pH 4.0–4.5.

Case Study in Streamlining Purification

This case study exemplifies rapid implementation of CaptureSelect FcXL resin, a predecessor of the FcXP resin mentioned above, into a commercial next-generation therapeutic manufacturing process. A purification process had been established for a novel-format antibody based on an affinity capture step followed by three polishing steps. The process had a low DBC because the antibody did not bind well to protein A, and lower-pH elution limited product stability. Other problems included elevated fragment levels and an environmentally unfriendly regeneration solution. The developer wanted to remove one process step to enable a better fit within a particular manufacturing facility.

Several capture options were screened for load density, yield, and reduction of host-cell protein (HCP), HMW, and LMW impurities. CaptureSelect FcXL resin scored the best on most attributes, including the sum of LMW (Table 1). Following implementation this resin for affinity capture, the following results were achieved:

Table 1: Comparing affinity-capture options for a novel-format antibody that had suboptimal binding to protein A

	(Existing) Affinity A	Affinity B	Affinity C	Nonaffinity	CaptureSelect FcXL
Load density	Medium	Low	Medium	Very High	High
HCP reduction	High	High	Very High	Low	Medium
Yield	High	High	Low	Low	High
HMW	Medium	Medium	Medium	Medium	Medium
Main peak	Medium	Medium	Medium	High	High
LMW	High	High	High	Medium	Low

- reduction of a four-step chromatography process to three steps, removing the need for one of the polish steps
- consistent yields of ~80% in one pilot and three good manufacturing practice (GMP) runs
- increased binding capacity to improve facility fit
- an improved impurity profile
- increased pool stability due to elution at mild pH
- excellent scalability
- use of a more environmentally friendly regeneration solution.

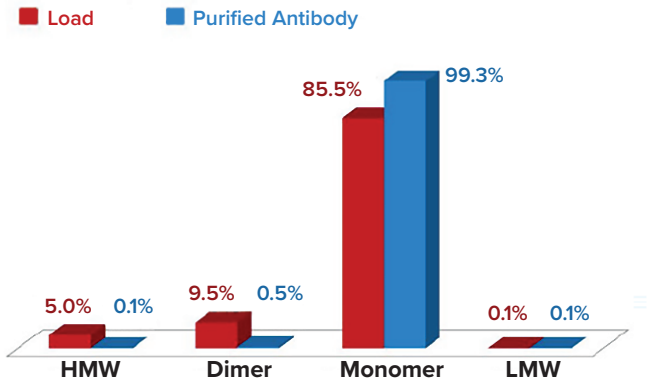
Case Study in Improved Recovery

Using bind–elute polishing steps to remove impurities such as HMW components and dimers can be cumbersome. In this case study, a customer with a three-step purification process step sought to improve product recovery and process efficiency. The original process used protein A capture followed by anion-exchange polish in flow-through (FT) mode and a mixed-mode bind–elute to remove a high percentage of aggregates (12%). Cation exchange had been evaluated for aggregate removal but was not efficient for the MAb in this case. The existing process provided only 90% monomer recovery, with loading at 25 g/L and a 6-minute residence time.

The goal of our collaboration was to replace the bind–elute mixed-mode step with a FT operation to improve recovery, aggregate clearance, and efficiency. Mixed-mode chromatography was replaced with Poros Benzyl Ultra hydrophobic-interaction resin. Three attributes of such resins make them extremely well-suited for manufacturing scale: Product resolution is maintained as linear flow rate increases. High linear binding capacity is possible over a large range of flow rates. And scalability is linear and predictable.

The final process cleared aggregates to <1%, with 90% monomer recovery after 25-g/L resin loading

Figure 2: Results of the new process — final verification



Effective reduction of dimer and HMW aggregates in low-conductivity solutions, with high recovery

MAb-A Process	Mixed-Mode BE	HIC FT
Load density (g/L resin)	25	80
Monomer purity FT (%)	99	>99
Monomer recovery FT (%)	90	98
Host-cell proteins (ppm)	<LLoQ	<LLoQ
Residence time (minutes)	6.0	1.2

A successful MAb polish step for highly efficient aggregate removal and near-complete monomer recovery

and a 6-minute residence time (Figure 2b). The FT process was coupled directly to the upstream AEX process with no need for buffer exchange, which saved both time and money. These results demonstrate the power of HIC as an alternative strategy in MAb-aggregate polishing.

Removing HCPs with Affinity Polishing

Product- and expression-related HCPs can be difficult to remove even by multiple polishing options. HCPs that coelute with proteins of interest are particularly difficult to remove. If they are not addressed sufficiently, a product's advancement from one clinical phase to the next can be delayed. Clusterin, cathepsin D, galectin-3 binding protein, and G-protein coupled receptor 56 are among the many HCPs that can be difficult to remove. To address such challenges, Thermo Fisher Scientific developed an approach called "affinity for polish" that applies CaptureSelect affinity ligands on POROS beads to enable fast and efficient HCP removal in FT applications. POROS CaptureSelect ClusterinClear beads were used to remove >95% of clusterin as well as >65% of other top HCPs in one customer evaluation.

New Solutions for New Antibody Formats

Purifying the next generation of antibody therapeutics requires a diverse set of high-performance chromatography options. The tools described herein can deliver important benefits including high purity and yield in a single capture step, a reduction of the number of necessary steps in a downstream process, and seamless upscaling for biomanufacturing as products move through clinical phases toward commercialization.

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POROS resin is a pharmaceutical grade reagent for manufacturing and laboratory use only. CaptureSelect ligands and affinity matrix are for research use or further manufacturing, not for diagnostic use or direct administration in humans or animals.