Rapid Implementation of Novel Affinity Purification

Manufacture of Commercial-Scale Next-Generation Antibody Therapies

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The rapid and cost-effective production of conventional monoclonal antibodies (MAbs) for clinical trials and commercial supply has contributed toward their wide adoption. Production processes have become more efficient because common purification processes are being used across structurally similar MAbs during key steps of process development and manufacturing. Such successful platform approaches can remove unwanted impurities and are stable across processing conditions, irrespective of the MAb being purified. In addition, they are readily available at the required volume to support large-scale current good manufacturing practice (CGMP) production and can be validated easily. Platform approaches also minimize both the duration and number of steps in downstream processing operations, which contributes significantly to maximizing efficient batch production.

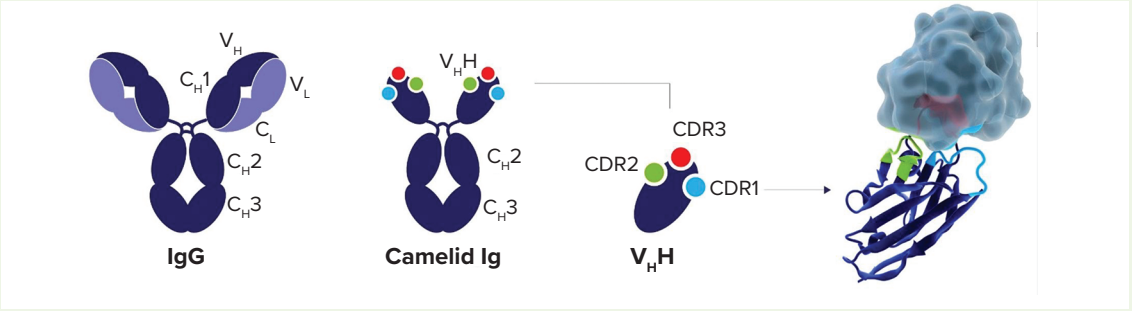
With its ability to bind to Fc regions, protein A has long dominated as the platform of choice for MAb affinity purification. However, novel structures of many next-generation antibody-based therapeutics mean that the binding site on the Fc region is masked or absent, thus making protein A no longer an option.

As an alternative approach, cation-exchange or mixed-mode chromatography can be used for next-generation MAbs without accessible Fc domains (**1**). Unfortunately, nonaffinity-based primary capture demands significant process development efforts for each individual MAb. In addition, this method often provides low product purity and low yield. A nonplatform approach requires development of bespoke purification workflows to be developed, which increases process development times, time to the clinic, and costs.

Designed to be more specific and potent, such next-generation formats include engineered antibodies, antibody–drug conjugates (ADCs), bispecific and multispecific antibodies, antibody fragments, single-domain antibodies, and antibody-like proteins. Many of these exciting therapeutics have received regulatory approval, and hundreds more are progressing through preclinical and clinical development pipelines around the globe.

To address this critical industry need, a suite of affinity ligands based on single domain antibody fragments (V_HH) — the smallest antigen binding molecules — was developed (CaptureSelect™ technology). These ligands, which have only one variable heavy domain, target specific human antibody subdomains such as the C_H1, C_H3 or kappa light chains. These ligands are combined with resin

Figure 1: Regular IgG antibody compared with a Camelid single domain Ig antibody and V_HH antibody fragment; the image on the far right shows V_HH bound to its target protein.



substrates to create new economically viable and scalable affinity resins that are specifically designed to provide high purity of novel antibody formats in a single affinity-capture step. In addition to addressing the obvious technical challenges, biomanufacturers must validate new purification resins as new materials and perform other crucial processes before implementing the resins into a CGMP manufacturing environment. Below, the technical benefits of these new resins are highlighted and accompanied by a real-world case study of rapid validation and implementation into a CGMP manufacturing facility

Sourcing Suitable Affinity Ligands

Camelidae (camels and llamas) produce a unique type of antibody that lacks the light chains and the entire C_H1 domain present in human immunoglobulins. *Camelidae* antibodies thus have only one V_HH domain by which antigens are recognized and to which they bind.

Based on the V_HH domain, CaptureSelect ligands are a single protein of 12–15 kDa with three highly variable complementarity-determining regions (CDRs) that determine specificity and affinity. This V_HH domain is the smallest intact and functional antigen-binding fragment derived from a fully functional antibody (Figure 1). These ligands are expressed by recombinant yeast as recombinant V_HH antibodies without animal-derived components and are stable proteins produced readily in the quantities required for industrial-scale bioprocessing applications.

Figure 2: Human IgG and antibody subdomain targets for CaptureSelect affinity ligands

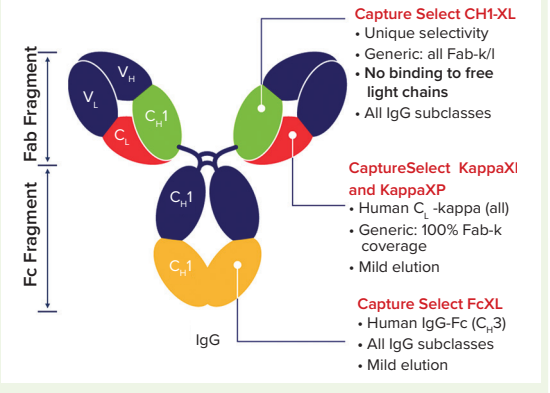


Figure 3: Chromatograms resulting from the purification of Ranibizumab with (A) CaptureSelect KappaXL resin and (B) CaptureSelect CH1-XL resin

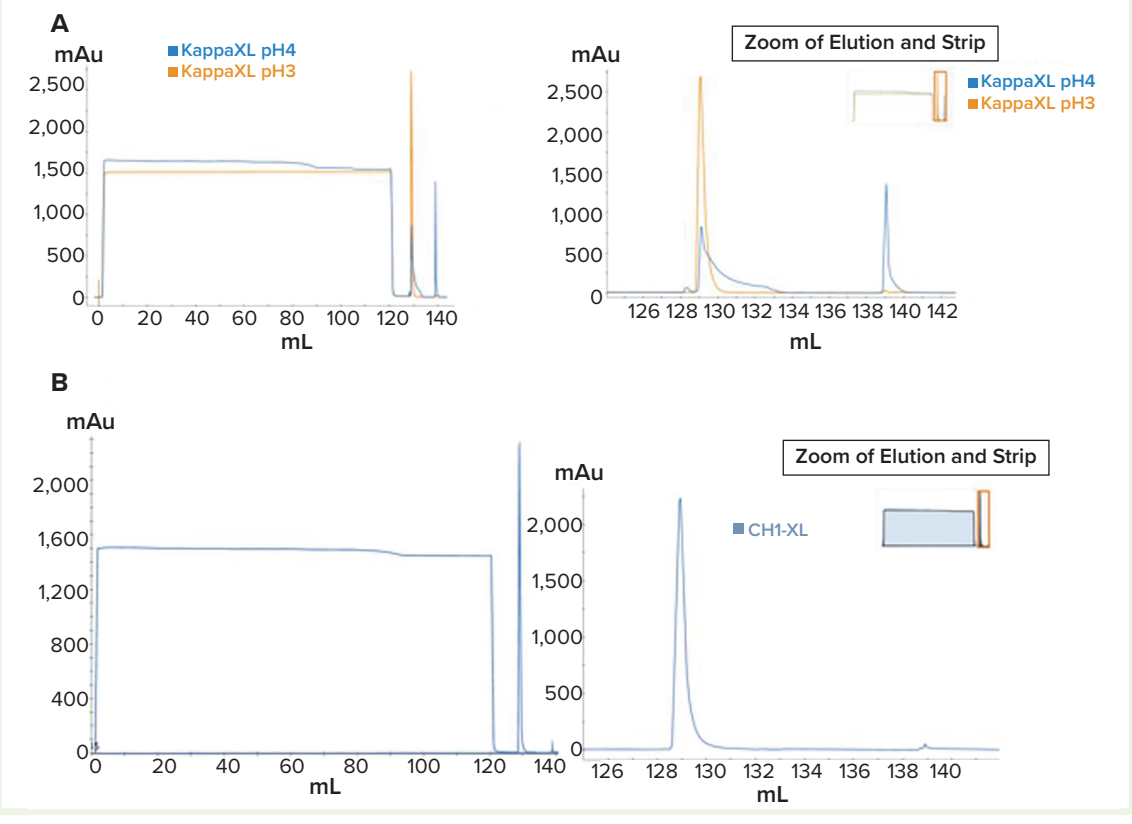


Figure 4: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis shows the elution fractions of ranibizumab purified using CaptureSelect KappaXL resin. Lanes 1 and 6 are molecular weight markers, lanes 2 and 7 are starting material, lane 3 and 8 are flow through, lane 4 is elution at pH 4.0, lane 5 is strip after elution at pH 4.0, lane 9 is elution at pH 3.0, and lane 10 is strip after elution at pH 3.0. Red ovals are elution fractions.

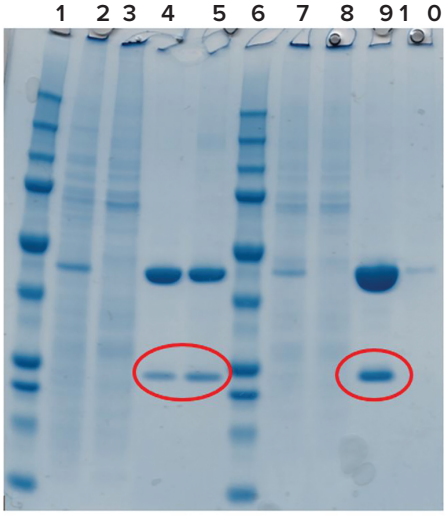
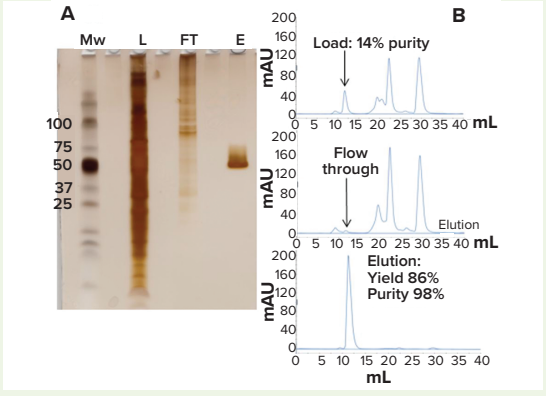


Figure 5: Analysis of the fractions from the purification of ranibizumab with CaptureSelect CH1-XL resin with pH 4.0 buffer (Mw = molecular weight, L = starting material, FT = flow through, E = elution)



Thermo Fisher Scientific has developed a unique set of resins with ligands directed against different human antibody subdomains on either the light or heavy chain. These resins enable the design of affinity purification platforms for a broad range of antibody formats (Figure 2). Such resins include

- CH1-XL affinity matrix, which binds specifically to the CH1 domain and thus can be used to purify Fab fragments of human IgG irrespective of subclass (e.g., IgG 1, 2, 3, or 4)
- FcXL affinity matrix, which binds to the C_H3 domain present on all human IgG subclasses and is suitable for the purification of MAbs, bispecific antibodies, and Fc fusion proteins
- KappaXL and KappaXP affinity matrices, which bind to the constant domain of all kappa antibody light chains, giving generic coverage across all immunoglobulin classes (e.g., IgG, IgM, IgA, IgE, and IgD).

Assessing the Platform Potential of Specific CaptureSelect Resins for Biosimilars

To assess the ability of these new resins to deliver as designed, ranibizumab (Lucentis, Roche) was identified as an ideal representation of a current commercially available therapeutic antibody fragment. Ranibizumab is a humanized Fab fragment with mouse variable chain sequences grafted onto a human IgG1 kappa isotype. It is approved for treating wet age-related macular degeneration, a common form of vision loss in elderly people. Several biosimilars of ranibizumab are in development. Cation-exchange and mixed-mode chromatography in bind–elute mode are the purification methods routinely used for ranibizumab; however, both methods typically result in complex downstream processes that deliver low yields.

Both CaptureSelect KappaXL and CaptureSelect CH1-XL resins were evaluated as potential affinity chromatography purification options for ranibizumab. Ranibizumab was transiently expressed in HEK 293 cells, and 120 mL of feed was loaded onto 5 × 20 mm columns (0.4 mL), which contained KappaXL resin (Figure 3A) or CH1-XL resin (Figure 3B). Columns then were washed with phosphate-buffered saline (PBS) at pH 7.4 and eluted with 50 mM acetic acid at pH 4.0. This step was followed by an acidic strip with 0.1 M glycine pH 2.0.

Figure 3A shows partial elution with KappaXL resin at pH 4.0. However, >25% of the protein was present in the strip fraction. By contrast, Figure 3B clearly shows that at pH 4.0, efficient elution was achieved using CH1-XL resin.

Figures 3A and 4 show that with KappaXL resin, efficient elution is achieved at pH 3.0. However, copurification of the light chains also is observed in the elution fractions (red ovals). Often during Fab production, light chains are overexpressed, resulting in both excessive light chains and the formation of light-chain dimers. Light-chain binding resins such as KappaXL affinity matrix cannot discriminate between

correctly assembled Fabs and light-chain–based impurities, so such contaminants are present in elution pools.

Figure 5A shows the starting material (L), flowthrough (FT), and elution fractions (E) of the purification run of ranibizumab feedstock using CaptureSelect CH1-XL resin assessed using SDS-PAGE. Further analysis with gel filtration on a Superdex (GE Healthcare) column of the starting material, flowthrough, and the elution fraction confirmed 98% purity of the Fab fragment with a yield of 86% with CH1-XL resin. These data clearly show that efficient purification is achieved using CH1-XL resin at pH 4.0.

CH1-XL resin binds only to heavy-chain domains, thus facilitating purification of intact Fab with no copurification of overexpressed free light chains and light-chain dimers. In general, CH1-XL resin is the best affinity ligand option because it can be used as a platform to purify all IgG subclasses and all human Fab fragments independent of class and directly from complex source materials. It can be used in a single step to provide both high purity and yield.

Unlike CH1-XL resin, KappaXL resin is not limited to IgG but also can bind all human immunoglobulins (e.g., IgG, IgA, IgM, IgD, and IgE). KappaXL resin offers slightly more capacity than the CH1-XL resin, and if the problem of overexpressed light chains can be resolved upstream, then KappaXL resin offers a broader purification platform solution. To conclude, CaptureSelect CH1-XL resin shows excellent potential as a platform affinity purification tool for therapeutic antibody fragments such as ranibizumab and its biosimilars.

Rapid Implementation into a Commercial Next-Generation Therapeutic Manufacturing Process

Roche developed a novel-format biopharmaceutical molecule for which the standard purification protocol involving protein A capture was not an option. An early stage process used a light-chain binding affinity resin and three subsequent chromatography steps (Figure 6). For later stages of development and commercial manufacturing, the objective was to develop a process that offers increased robustness, efficiency, and yield and is a better fit to the company's standard production facilities.

To improve the purification process at the initial affinity/capture step, a scale-up screen with four commercially available resins (including CaptureSelect FcXL resin) was carried out after they had been tested initially at laboratory scale. These resins were compared directly with the company's existing light-chain affinity resin.

Table 1 shows that a very high purity profile with low levels of high-molecular–weight (HMW) impurities and very low levels of low-molecular–weight (LMW) impurities were obtained with FcXL resin compared with the other resins tested (three were affinity based and one nonaffinity). In addition, primary capture using FcXL resin supported a reasonable load density while generating a yield as good as the previous capture resin.

The initial light-chain binding resin exhibited lower binding capacity than the FcXL resin, a significantly lower purity profile, and a relatively high proportion of low-molecular–weight contaminants in

Figure 6: Existing manufacturing process (HCCF= harvest cell culture fluid, DS = drug substance)

Table 1: Summary of up-scaled data from capture resin screening without optimization

	Existing Affinity	Affinity B	Affinity C	Nonaffinity	CaptureSelect FcXL
Load density	Medium	Low	Medium	Very high	High
CHOP reduction	High	High	Very high	Low	Medium
Yield %	High	High	Low	Low	High
SumHMWs rel%	Medium	Medium	Medium	Medium	Medium
Main peak rel%	Medium	Medium	Medium	High	High
SumLMWs rel%	High	High	High	Medium	Low

the eluate. These results illustrate clearly how, in this case, the light-chain affinity resin cannot discern among the correctly assembled light chains on the antibody and the single light chains or light-chain dimers that form as result of overexpression in Chinese hamster ovary (CHO) host cells.

However, use of FcXL resin resulted in a CHO protein (CHOP) reduction that was lower than the reductions from use of other affinity resins. So before implementation of the FcXL resin, a series of additional experiments had to be performed to optimize purification and enhance removal of CHO impurities. First, a FcXL resin wash screen involving 96 different buffers was carried out using an automated process. The optimum buffer was chosen by assessing relative yield and CHOP reduction. The effect concentration and pH of the acetic acid elution buffer on product yield and impurity removal (including CHO host-cell protein reduction) was further evaluated and optimized. Results showed that the lower acetic acid concentration tested (30 mM) and higher pH 4.2 produced optimal results. Elution at mild pH also supports increased product pool stability.

Figure 7 shows results of pressure-drop experiments. They were performed as a function of resin-bed height, which was scaled-up in pilot studies from 10 cm to 20 cm and then 30 cm. Results show that even with a 30-cm bed height, the maximum operating pressure of 2 bars can support desirable flow rates of >250 cm/h.

Important for platform purification approaches, further analysis showed excellent performance and consistency in the static and dynamic binding capacities of the FcXL resin among all the lots tested (Figure 8).

When the FcXL resin column was scaled up for clinical manufacturing, results showed high consistency with development and pilot-scale columns such that product yield, CHOP, and all other process-

Figure 7: Results of pressure-drop experiments performed as a function of resin-bed height (scaled-up in pilot studies from 10 cm to 20 cm and then 30 cm); even with a 30-cm bed height, the maximum operating pressure of 2 bars can support desirable flow rates of >250 cm/h.

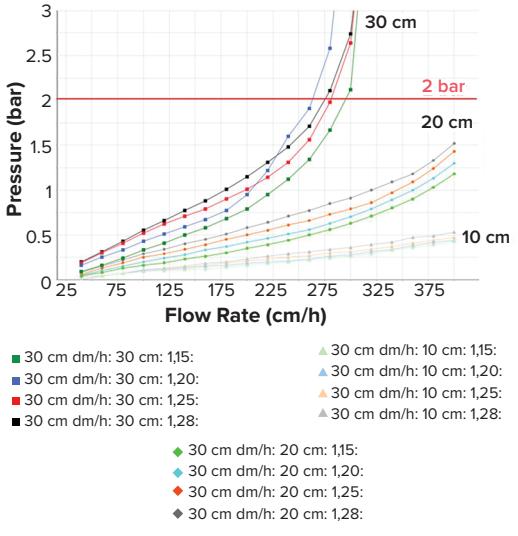
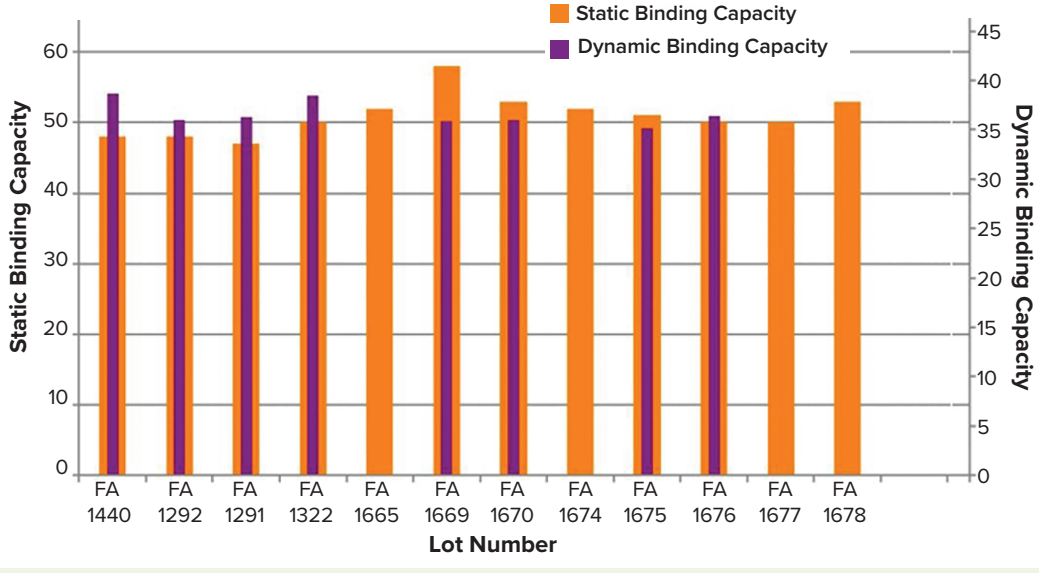


Figure 8: Evaluation of CaptureSelect FcXL resin lot-to-lot consistency



related impurities were directly comparable. This confirmed that implementing the FcXL resin capture step into the manufacturing workflow gave a scalable process.

Overcoming Adoption Barriers to Enable Implementation into CGMP Manufacturing

Before implementation into a CGMP manufacturing facility, a resin must be qualified as a new raw material. Because CaptureSelect ligands and resins have been validated for use in multiple FDA- and EMA-approved manufacturing processes, they come with all necessary technical support, information, and documentation.

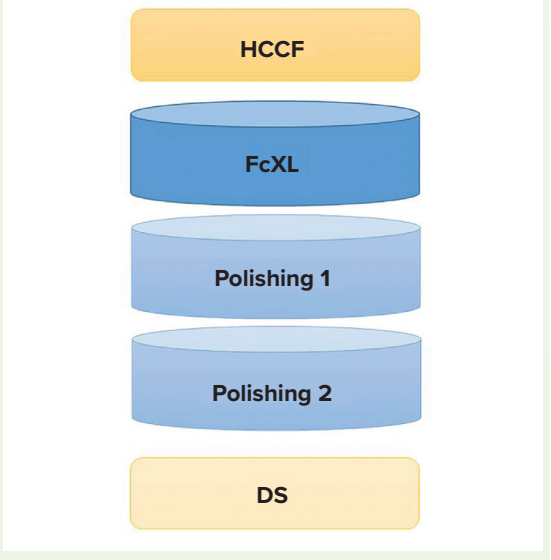
However, normal timelines for adopting and implementing new materials in any phase of a CGMP manufacturing process can be quite long. In the case study above, both resin developer and end user worked together efficiently to qualify the new FcXL resin material. Several sequential workflows and approval processes had to be carried out in parallel. Although this approach was atypical, it did enable the user to meet accelerated timelines. Key to the success of this approach and the on-schedule implementation of resin into the manufacturing workflow was exceptional collaboration between all parties involved internally within both organizations.

Adoption and rapid implementation of the FcXL resin enabled Roche to switch from a four-step chromatography process to a more efficient three-step procedure (Figure 9). Use of the FcXL resin resulted in higher binding capacity than with a light-chain affinity resin at the scale required. It also enabled an improved impurity profile, with no low-molecular–weight impurities. The company's novel format clinical candidate could be eluted readily using a mild acetic acid buffer, thus supporting better product pool stability.

Solutions for Unmet Purification Needs

Consistency and predictability of purification platforms have been key drivers in the significant production of MAbs and other biologics as viable and cost-effective therapeutics. However, as the number of next-generation antibody-based candidates with modified or absent Fc domains increases, biomanufacturers face a need to establish additional affinity platform purification technologies. Such technologies then can confer the efficient, robust, cost-effective, and scalable process benefits of affinity chromatography and keep production efficient and economical.

Figure 9: Revised manufacturing workflow reduced to industry-standard three-step purification process (HCCF= harvest cell culture fluid, DS = drug substance)



CaptureSelect antibody subdomain-specific affinity ligands and their resulting resins are addressing the purification challenges that are arising with many of today's next-generation novel antibody formats and fragments currently in therapeutic development. Such alternative affinity ligands enable creation of scalable purification platform solutions that provide the required high purity and yield that readily fit into downstream processing workflows of standard bioprocess industry facilities. In addition, use of mild pH buffers and achievement of effective elution not only protect vital biological activities, but also enhance product stability and help prevent aggregate formation of novel antibody formats.

The human antibody subdomain-specific resins described herein offer practical platform purification options that have been proven in a commercial CGMP setting. They can support efficient, cost-effective manufacturing and help the biomanufacturing industry in its drive to increase speed to market and enhance patient access.

Reference

1 Scanlan C, et al. Downstream Purification and Formulation of Fab Antibody Fragments. *BioPharm Int.* 27(1) 2014.

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