Affinity Chromatography: An Enabling Technology for Large-Scale Bioprocessing

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Affinity chromatography (AC) has been used in large-scale bioprocessing for almost 40 years and is considered the preferred method for primary capture in downstream processing of various types of biopharmaceuticals. The objective of this mini-review is to provide an overview of a) the history of bioprocess AC, b) the current state of platform processes based on affinity capture steps, c) the maturing field of custom developed bioprocess affinity resins, d) the advantages of affinity capture-based downstream processing in comparison to other forms of chromatography, and e) the future direction for bioprocess scale AC. The use of AC can result in economic advantages by enabling the standardization of process development and the manufacturing processes and the use of continuous operations in flexible multiproduct production suites. These concepts are discussed from a growing field of custom affinity bioprocess resin perspective. The custom affinity resins not only address the need for a capture resin for non-platformable processes, but also can be employed in polishing applications, where they are used to define and control drug substance composition by separating specific product variants from the desired product form.

1. Introduction

The biotechnology industry has experienced unprecedented success over the past few decades. The branch of biotechnology that focuses on the development and commercialization of medicines for several of the civilized world’s severe diseases, such as cancer, immune disorders, and vaccines for preventive or pandemic applications, the so-called red biotechnology, has grown tremendously since 1979 when the first biologic was approved. In 2018, revenues from commercialized biologics reached 210 billion dollars. The market is expected to grow with CAGR estimated at 8.6% for next 10 years. Therefore, it is not surprising that despite an ongoing discussion on the business sustainability of the biopharma industry business model,[3,4] due to high development costs and a relatively low number of newly approved biologics,[5] investors are still willing to invest and support the development of new biologics-based therapies. This investment trend is not surprising considering that out of 221 biological products approved up to 2017, the ten best-selling molecules had a net worth of over 65 billion dollars last year alone (BioProcess Technology Consultants BioTrak Database).

At the same time, the industry has now entered a new phase characterized by increasing pricing pressure due to i) reimbursement policies; ii) a more competitive market where multiple companies are developing products for the same indications that are based on a similar mechanism of action; iii) price erosion due to approval of biosimilars; and iv) the introduction of new expensive personalized therapies. As a result, the industry needs to look for means that assure better investment returns.[5] Better investment returns can come from improvements in the clinical success rate, a shorter time to the clinic, and the reduction of manufacturing costs. The two former activities will result in a significant reduction of research and development cost per program, which at the moment is estimated to average at 1.4 billion dollars.[6] With respect to manufacturing costs, it can be argued that over the lifetime of an approved drug, the cumulative costs of manufacturing might prove as high as the cost of bringing the molecule to the market. Therefore, a reduction of a manufacturing cost can bring significant financial benefits to the parent company, especially that there are reports indicating that even up to 25% of a drug’s price is associated with its cost of goods.[4] At the same time for a highly optimized platform process, based on best of class technologies, the cost of goods can be as low as 1–5% of the selling price.[7] This point clearly indicates that employing the right manufacturing technologies can, over the product lifecycle, bring significant financial gains for the parent company and for their investors, and improve patient access to more affordable treatments.

From the perspective of identifying potential improvements in process economy, it is well understood that the cost of downstream operations contributes a major part of the overall manufacturing cost.[8–12] This higher contribution is because...
biologics must be produced with a very high level of purity following stringent requirements of process quality. A typical downstream process consists of several unit operations, each introduced with a very specific purpose of improving product quality and safety. However, each of these unit operations contributes to a decrease in an overall process yield. Since, for a given facility utilization level, the yield losses are the primary economic drivers of increased manufacturing cost, any technology that reduces the number of processing steps while maintaining or increasing the purity of the drug substance will be of interest. One such technology is affinity chromatography (AC). AC has been used in large-scale bioprocessing for almost 40 years, and is considered the preferred method for primary capture in downstream processing of various types of biopharmaceuticals.

The importance of AC in bioprocessing can be emphasized by examining the downstream process template for the purification of monoclonal antibodies (MAbs). The heart and soul of this process is a highly selective, high yield, capture step relying on AC on Protein A resin. This capture step reduces the overall number of processing steps needed to produce a highly purified drug substance, hence increasing the overall process yield and reducing the processing time. Overall, the use of Protein A chromatography early in the process increases the downstream processing production rates, and thus reduces manufacturing cost. Actually, it can be argued that the commercial success of MAbs is not only related to their mechanism of action, but also due to a Protein A enabled very robust purification process enabling large-scale production of these molecules. In fact, the above mentioned case of COGs being in the range of 1–5% of the average selling price for biotherapeutic refers to a highly optimized manufacturing process for a MAb characterized by a high yield and high facility utilization.

In this review, we will briefly discuss the past, current, and future state of bioprocess scale AC, with special emphasis on suitable ligands and resins. We will discuss the general advantages associated with AC from the bioprocess perspective and also discuss recent development in a growing field of custom bioprocess affinity resins. The review does not discuss the principles of affinity resin optimization (e.g., particle and pore sizes, ligand size and density), nor process approaches leading to more optimum use of these resins (e.g., cycling, optimization of residence time, or continuous operations). Those topics have been covered in several publications and textbooks including a recent review of 12 commercial Protein A resins, which highlights the complexity of stationary phase and ligand designs relationship to achieve optimal performance.

2. Bioprocess Affinity Chromatography

While the term affinity chromatography was initially reserved for chromatography techniques based on functional biological interactions only (e.g., antibody–antigen; lectin–glycoprotein; enzyme–inhibitor) its current definition, at least from the bioprocess perspective, is less restrictive and includes any interaction between specific groups on the target (ligate) surface and an immobilized ligand (e.g., the interaction between an oligo-histidine motif and a chelated metal ion, so-called immobilized metal ion affinity chromatography, IMAC).

2.1. Historical Perspective

The term affinity chromatography was first used in the late 1960s in a seminal article, which described the desirable feature of an affinity resin and described the principles and applications of this technique including the importance of the resin structure and coupling technology. In the mid 1950s through 1960s, the first small-ligand selective chromatography steps were developed for the purification of several enzymes using azo-dye and flavin ligands. Immunoaffinity chromatography preceded the development of small specific affinity ligands. In 1951, Campbell and co-workers used bovine serum albumin to capture rabbit anti-bovine serum albumin antibodies. The 1970s
brought about the introduction of ligands capable of interacting with several targets. These molecules included the coenzymes, lectins, nucleic acids, metal chelates, triazine dyes, Protein A, and heparin.[28] Around that same time, the development of hybridoma technology to produce MAbs enabled a wider use of MABs in protein purification. The first applications of affinity (immunoaffinity) chromatography for large-scale bioprocessing application was the use of MAB capture columns in the 1980s for the purification of commercial proteins such as interferon alpha 2A,[29] and Factor VIII.[30] The production of MAB columns was a complex process in which the antibody cell line was grown in media (often with serum), the MAB was purified from supernatant using conventional chromatography, or Protein A chromatography, and the MAB was then chemically coupled to the resin. The resins were highly effective but very expensive to produce and often used animal-derived components in MAB production. Furthermore, the resins were difficult to clean, and there were concerns with the leaching of anti-protein antibodies into the product. Due to these challenges, the use of MAB affinity columns is not the preferred means to purify commercial biopharmaceuticals.

The modern history of bioprocess AC is associated with the development and commercialization of Protein A resins, which followed development of MAbs as the most abundant class of biologics to date. Significant improvements in the design of Protein A resins resulted from increasing MAB titers and the large number of antibody-based therapeutics in the biologic pipeline. These improvements included advances in immobilization chemistry, ligand engineering, and base matrix design. For instance, improvements involved using recombinant, rather than Staphylococcus aureus, Protein A,[31] and ligand coupling oriented through a C-terminal cysteine. Further improvements resulted in lower ligand leakage and improved ligand accessibility, which in turn resulted in higher binding capacity. Further commoditization of MAbs led to additional developments in Protein A ligand chemistry resulting in significantly improved chemical stability and in subsequent development of Protein A resins with much higher binding capacities. Some other bioprocess affinity resins developed over the years include resins based on Protein G, Protein L,[32] and single domain camelid antibodies ligands.[33]

### 2.2. Bioprocess Affinity Ligands

Currently, ligands used for bioprocessing applications can be either of biological or synthetic origins. However, a classification dividing the ligands into biospecific and pseudo-biospecific, respectively, has been proposed.[34] Regardless of their origin, ligands compatible with bioprocess AC must exhibit high selectivity, relatively high affinity, and the reversible binding to the target yielding target elution under relatively mild conditions. They also need to be chemically stable for maintaining their binding properties following an exposure to various agents during chromatography operation. In addition to the above, the ligands need to be easily conjugated to surfaces of support matrices. Properties of bioprocess suitable affinity ligands are listed in Table 1.

Among the ligands of biological origin, antibodies and antibody fragments are used for immunoaffinity chromatography.[35,36] However, antibodies are not chemically stable especially at high pH conditions (e.g., base cleaning) and also show relatively low binding capacities. As compared to full antibodies, antibody fragments have the same selectivity, but are smaller and can be immobilized at higher ligand density yielding higher binding capacities.[33,37] They are not glycosylated, and because they can be made in microbial expression systems they are potentially cheaper to produce.

Other biological ligands include the so-called scaffold proteins that are relatively stable, small (single chain), and can be produced by recombinant technology. Scaffold proteins have been engineered to improve their chemical robustness and other chromatography relevant properties. Examples of scaffold proteins include affibodies, DARPin, affilins, knottin, monobodies, and anticalins.[38–40]

Among synthetic ligands, synthetic oligonucleotides, with unique secondary structures, such as aptamers have been used for bioprocess scale AC to purify several proteins, including: Vascular Endothelial Growth Factor (VEGF121),[41] Factor VII, Factor H, and Factor IX from serum,[42] Human IgG from serum have also been purified using an Fc aptamer resin.[43]

Other synthetic bioprocess ligands include small peptide affinity ligands and polysaccharides. The former are gaining more attention because of their relative simplicity associated with defined composition and 3D structures, ease of large-scale production, and nontoxic character.[44,45] The latter include ligands such as dextran sulfate used for purification of influenza virus, and heparin used for the purification of some plasma proteins.

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Table 1. Properties of bioprocess relevant affinity ligands.

| Bioprocess purification performance | Specificity | Avidity | Stability | Size and composition | Safety and manufacturability |
|------------------------------------|-------------|---------|-----------|----------------------|-----------------------------|
|                                    | High purity obtained through interactions between the ligand and naturally occurring domains on the surface of the target | High step yields obtained under permissible elution conditions | Stable across the operational conditions (pH, salt, feed components, etc.), including CIP operations | Large enough to provide specificity but small enough to yield ligands accessibility after the ligand is coupled to the surface of the pores of support material. | Ligands chosen from a family of compounds with clinically proven non-toxic character |
|                                    | Nanomolar affinities similar to industry standards such as Protein A and antibodies, e.g., 10–50 nm range | | | | Manufacturing process does not use mammalian cells and animal-based components |
|                                    | | | | | Scalable technologies supporting requirements for cGMP manufacturing processes with secured secondary production sites, transparency of supply chain |
|                                    | | | | | Acceptable production cost |

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such as antithrombin III. Other type of ligands used in bio-processing include chelated metal ions, amino acids, dyes (e.g., Cibacon Blue for purification of interferon, albumin, and hormones) and small organic molecules (e.g., benzamidine; thiopropyl; nucleoside phosphates; triazine).

Some examples of biopharma relevant target proteins and proposed corresponding affinity ligands have been reviewed. Other biological agents, such as receptors have also been used as ligands for protein purification. For example, recombinant IL-2 receptor was used to purify active IL-2 exclusively.

2.3. Bioprocess Affinity Resins

Bioprocess affinity resins can be divided into two categories: i) resins that are generic and can be used for purification of biologics sharing specific similarities in their structure, e.g., MAb, antibody fragments (FAbs), Adeno-associated viruses (AAVs), and ii) resins that are based on a specific interaction between a pair ligand—protein target that is unique to the pair (single product resins). Both categories of resins deliver the same bioprocess advantages, i.e., smaller number of purification steps and thus a higher overall process yield and a shorter processing time.

2.3.1. Generic Resins

As discussed above, today’s therapeutic MAb s are predominantly purified using Protein A resin as the capture step, followed by few orthogonal chromatography steps such as ion exchange, hydrophobic interaction, or multimodal chromatography. The Protein A benefit is apparent in its widespread use in MAb purification. Actually, the overall Protein A market is estimated to reach a value of $800 million by the year 2023, which corresponds to an annual production of at least 40,000 L.

The race to evolve Protein A resin performance resulted in a fairly well-understood list of required technical attributes that are now considered a foundational requirement for any type of modern AC resin used in bioprocessing applications. These attributes include: high binding capacities, stability in typical bioprocess cleaning conditions containing NaOH; low ligand leaching, and a lifetime of several hundred column cycles. In addition to the technical requirements, a security of affinity resin supply is also seen as an important attribute when the resin is considered for commercial manufacturing operations. In such a case, the resin supplier must prove that the resin can be consistently produced at the required scale, and with the required quality to secure an uninterrupted supply of the resin for the scheduled manufacturing campaigns.

Another example of a class of biotherapeutic drugs that benefit from a bioprocessing affinity capture step are antibody fragments. Antibody fragments are therapeutically desired in situations where full length MAb would not be effective due to their size. This includes some tissues and tumors, and targets that are intracellular or cryptic. These fragments are antibody-like molecules without an Fc portion, and include FAb, FAb2, BiTEs, ScFv, di-ScFv, and sdAb. The lack of an Fc portion prevents their binding to Protein A. However, these fragments, contain kappa light chain that shows affinity toward Protein L. Several bioprocess suitable Protein L-based affinity resins are already commercially available, and it is expected that next-generation Protein L resins will benefit from similar recombinant technology used to improve the properties of Protein A ligands.

Adeno-associated viruses (AAV) and adenovirus are two of several viruses used for in vivo and ex vivo gene therapy (e.g., CAR-T) and for cancer vaccines. Affinity resins are not only available for AAV and adenovirus but also for several other viruses. In a recent review on the use of AC for purification of viral vaccines, it has been suggested that there are currently four traditional affinity technologies that are available for the cGMP purification of virus. These include 1) Capto DeVirS resin that uses a dextran sulfate ligand exhibiting a heparin affinity-like behavior for various viruses including different strains of influenza virus, yellow fever virus, Japanese encephalitis virus, Dengue virus, and West Nile virus; 2) cellufine resin that uses sulphated cellulose, which also mimics the heparin affinity behavior and have been used for purification of human and avian influenza viruses, Moloney Murine Leukemia virus, Dengue virus, and West Nile VLPs; 3) AVB Sepharose High Performance resin, which is an immunoaffinity resin developed by BAC BV (now ThermoFisher Life Sciences) using a 14 kDa Cameldil-derived, heavy chain, single domain antibody fragment with a broad specificity for purification of AAV-1, -2, -3, -5, and -6; 4) CaptureSelect AAV8 and AAV9, which uses a 13 kDa single-domain fragment comprising three complementarity-determining regions (CDRs) that form the antigen-binding domain for AAV-8 and AAV-9 serotypes. These resins all suffer from a relatively low capacity due to a low accessible surface area for very large molecules such as viruses. The limitations of low surface area can be mitigated to some degree by using matrices with larger pores such as those used for preparation of CaptureSelect resins. The successful large-scale purification of AAV9 using CaptureSelect AAV9 at the 10 and 50 L scale, with >80% recovery, has been reported (M. Habben, BioInnovation Leaders Summit, London, 2015).

Recently, a new bioprocess resin (Poros CaptureSelect AAVX) has been specifically developed to purify a wide range of AAV serotypes including AAV1-AAV9 as well as recombinant and chimeric vectors.

2.3.2. Single Product (Custom) Resins

Over the past 30 years AC, as a capture step, has been identified as a technique enabling cost-effective bioprocessing. A suitable affinity resin toolbox that can help to achieve the purification of targets where a platform purification process could not be applied, is a desirable scenario. Indeed, some bioprocess affinity resins, for non-platform processes, have been successfully developed and used in large-scale commercial production. Some important examples include the already mentioned purification of antithrombin III using heparin-based resin, purification of influenza virus using dextran sulfate, and the purification of interferons and TSH using Cibacon Blue resin.

Commercial affinity resins with antibody fragments as ligands are available for human growth hormone, Factor VIII,
α1-antitrypsin and adeno-associated virus\(^{[33,70]}\) and affibody binders have been developed for Factor VIII.\(^{[71]}\) In 2004, Kelley et al., reported development of a custom affinity resin for purification of recombinant B-Domain Deleted Factor VIII (BDDrFVIII) using a peptide ligand selected from a phage display library.\(^{[72]}\) The resin provided excellent recovery of BDDrFVIII from a complex feed stream and reduced host cell protein and DNA by three to four logs. It eventually replaced an immunoaffinity step in the original ReFacto Antihemophilic Factor process,\(^{[73]}\) which allowed for higher column loading capacities, better resin cleanliness, and the elimination of animal derived materials (from the immunoaffinity ligand production).\(^{[74]}\)

Despite the above mentioned very successful examples of development and the subsequent large-scale implementation of affinity resins for non-antibody products, AC resins for targets other than MAbs are still uncommon.

In the BPOG 2020 Technology Road Map,\(^{[69]}\) it has been suggested that one potential reason for the slow adoption of bespoke affinity resins concept is that “Current approaches to providing affinity resins for novel targets have long development timelines that are difficult to integrate into the desired product development timelines, and have uncertain success rates...”. This statement can be challenged as several companies claim that they can discover bioprocess relevant ligands within 6–12 weeks from the start of a screening program. The subsequent development of prototype affinity resins for process development activities will add to these timelines, but programs with as few as 6 weeks development times have been reported (W. Kett, at the 9th Annual Bioprocessing Summit, Boston 2017). Hence, custom affinity resins might be developed in 3–5 months. Furthermore, these programs can be conducted in parallel to the product development activities such as cell line and/or upstream development (which might take 3–6 months), thus making the whole process of implementing a custom affinity resin for capture of product fairly compatible with the product development timelines. From a success of finding a ligand perspective, while it cannot be guaranteed a priori that a ligand will be found, the odds of finding an appropriate bioprocess ligand (Table 1) improve with the availability of diverse bioprocess proven ligand scaffolds.

The custom AC resins concept need not to be considered only for new drug candidates. Another area of its applications is the development of second-generation processes for legacy products, or development of purification steps for manufacturing of biosimilars. In the case of legacy processes, with introduction of an affinity capture step, process complexity will be reduced, and if significant gains in the product yields and production rates will also be achieved, the new process will be characterized by decreased COGS, while maintaining product quality. In the case of biosimilars, custom AC could be seen as an enabling technology, providing the intellectual freedom to operate while maintaining all of the economic benefits above. In both cases, timelines may not play as important a role as in developing an affinity ligand for a new process/product.

After the successful evaluation of a newly developed custom affinity resin in scale-down studies (acceptable product recovery and purity), a resin manufacturer needs to be contracted to determine the appropriate ligand density range and coupling technology, to scale-up resin production and to supply the resin for the cGMP manufacture of the drug. That manufacturer should have a history and reputation for producing cGMP resin, be capable of controlling each aspect of the resin production process, and capable of generating a Regulatory Support File (RSF), or an equivalent document. A dedicated program that can deliver cGMP resins as quickly as within 4–5 months has been reported (Avitide, personal communication).

Lastly, the resin manufacturer should develop an assay for the detection and quantification of the leached affinity ligand. Drug manufacturers must show that the ligand is not present in the drug substance, or that it is present at a very low level that is not considered a patient safety issue. The development of an assay for the affinity ligand should start when the affinity resin has shown success in scale-down studies. Some companies developing custom affinity resins develop residual ligand assays as part of the cGMP resin supply program (Avitide, personal communication).

The acceptable ligand levels in the final product are based on molecular structure, toxicology studies, and expected dosage regimes. It should also be emphasized that any leached ligand is removed, or its levels substantially reduced, by the subsequent downstream process unit operations such as UF/DF, ion exchange, or hydrophobic chromatography. A comparison of leached ligands levels post capture step and in the drug substance is presented for three commercially used affinity resins in Table 2.

Considering all the advantages of AC, a time frame for its development and production that fits into a Phase I development program, and that new, non-Mab modalities are increasing, it can be expected that the field of custom AC resins will experience a substantial growth. The relevant technologies, i.e., ligand discovery, ligand production, specific solid supports such as porous beads or convective matrices, are all well developed and understood. In fact, now several companies already offer commercial services focused on large-scale custom affinity resin development and supply. Table 3 lists the companies that provide such a service either in ligand discovery, or resin development and supply, or both (note: partnerships between companies listed in Table 3 resulting in a wider service offering have been reported).

### Table 2. Examples of measured levels of leached affinity ligands in product pools post capture step and in drug substances for three bioprocess affinity chromatography resins.

| Resin             | Ligand      | Level [ppm] | Reference |
|-------------------|-------------|-------------|-----------|
| MabSelect Prisma  | Protein A-derived | 10–20       | <1        | \([10]^{[6]}\) |
| Capto L           | Protein L   | <13         | <LOD      | \([12]^{[7]}\) |
| TN8.2 Sepharose   | TN8.2 peptide | 8–66        | <LOD      | \([12]^{[7]}\) |

\(^{[1]}\) Higher level are typically observed during first few cycles.

### 3. Bioprocess-Related Advantages of Affinity Chromatography

In the text below, we further discuss the advantages of AC from a bioprocess perspective. The topics include: process economy and process robustness, a platform facility concept, process
development and process design, and integrated continuous operation.

3.1. Process Economy

Undoubtedly, affinity resins are more expensive than conventional resins such as ion exchange or hydrophobic interaction chromatography resins. Their price per liter is three- to tenfold higher, because of i) the manufacturing of affinity ligands for bioprocess applications is more complex and thus costlier as compared to manufacturing of other types of ligands, and ii) the resin price captures the economic benefits that AC provides from a company operation perspective, including reduced process development times and higher yielding manufacturing operations.

From a manufacturing process perspective, a common approach to determine the cost of a resin is to calculate its cost per gram of purified target. This cost is inversely proportional to the number of cycles a resin can be used and the effective binding capacity (load × step yield), and directly proportional to the resin and buffer costs. Because the cost of AC resins is high, its contribution to the overall cost of goods can be significant, e.g., as high as 60% in the case of Protein A. However, if instead of focusing on the relative contribution of AC to the overall cost of goods for a purification process, analysis is focused on the actual cost of manufacturing, then it can be shown that the use of AC in downstream processing typically results in a reduced overall cost of goods. AC reduces the number of purification steps, which in turn increases the overall process yield and reduces processing time. This point is exemplified with Figure 1 that shows comparison of COGs analysis for a non-affinity (four-step) and affinity-based (three-step) purification processes that both produce the same purity drug substance (in both processes, the last two chromatography steps are the same). The three-step process, incorporating AC as the first step, can reduce the overall cost of goods 1.5-fold as compared to the non-affinity process, even if the two first non-affinity steps have much higher loading capacities. Since the first non-affinity step has a much lower step yield as compared with the AC step, the overall process yield process after the two non-affinity steps is significantly lower (30%) as compared to a single AC step. The lower the overall yield, the lower the mass of product produced, which will drive the COGs up even if the consumables cost for the non-affinity process is much lower as compared to the affinity-based process case. Similar results have been reported by others. Obviously, the replacement of multiple nonspecific steps with AC steps should only be considered if the change leads to a substantial increase in the overall process yield.

The COGs with AC can be further reduced by increasing resin cycling and improving target loading. With respect to the former, when a resin is used 33 times its price per liter is effectively reduced to a cost below that of a square meter of single use viral filter (e.g., Protein A case: $15k L–1, load 30 g L–1, 33 cycles → $15 g–1. Planova 15N: $5k m–2, 50 L m–2 at 5 g L–1 → $20 g–1). Additional resin cycles reduce the resin cost further, making resin lifetime an important process optimization parameter. For instance, Protein A resins are now routinely cycled 100–200 times, thus bestowing increased process economic benefits. Binding more target protein will also reduce the apparent resin cost per gram of purified target. This can be achieved for instance by increasing load residence time, or flow programming approaches as presented by K. Łacki and H. Johansson at PREP 2003 in San Francisco and later analyzed by other groups or by periodic counter-current operation. Principles of process optimization developed for Protein A-based capture steps are invariant to the type of affinity resin at hand and can be used in other processes.

![Image](48x737 to 137x758)

**Table 3.** Alphabetical list of companies offering custom designed affinity resins related service for bioprocess applications.

| Vendor                  | Ligands                      | Library size | Service type | Base matrix          |
|-------------------------|------------------------------|--------------|--------------|----------------------|
| Affilogics              | r-Proteins: nanofitins (F)   | 10^14 ligands| Yes          | No                   | N/A                   |
| Apatmer Group           | r-Proteins: aptamers         |              | Yes          | Yes                  | No                    |
| Avacta                  | r-Proteins: Affimers (Lo)    | 10^10 ligands| Yes          | No                   | No                    | N/A                   |
| Avtidie                 | Linear peptides (L) Cyclic peptides (Lo) | >40 different scaffolds, > 10^14 unique ligands | Yes          | Yes                  | Yes                   | Polyethylacrylate, Hardened agarose, Polystyrene |
| EMD Sigma               | N/A                          |              | No           | Yes                  | Yes                   | Methacrylate, Glass   |
| GE Healthcare Lifesciences | N/A                        |              | No           | Yes                  | Yes                   | Hardened agarose      |
| Prometics               | Small molecules              | 96 ligands   | Yes          | Yes                  | Yes                   | Agarose               |
| Navigo proteins         | r-Protein: Affilins (F)      | 10^12 ligands| Yes          | Yes                  | No                    | N/A                   |
| Purolite                | N/A                          |              | No           | Yes                  | Yes                   | Hardened agarose      |
| Repligen                | N/A                          |              | No           | Yes                  | Yes                   | Agarose               |
| Thermo Fisher           | r-Proteins: Camelids (xLo)   | 10^4 ligands | Yes          | Yes                  | Yes                   | Polystyrene Agarose   |

(A) Abbreviation in the bracket represents a general type of primary interactions between ligand and the target: P, pockets; Lo, loops; L, linear; xLo, extended loop; GF, grooved face; F, flat surface; CC, convex/concave.
While reducing COGs should be a process development objective, it needs to be remembered that a highly optimized process must be robust; for even a single failed batch may prove so costly that any savings achieved through process optimization might be lost. From the process robustness perspective, AC may prove a better choice for a capture step than other chromatography methods as it relies on a very specific interaction between the target and the ligand. The interaction that is typically not as sensitive to variations in feed stream composition as the interactions between a target and ion exchangers, mixed mode, or hydrophobic interaction resins. With these resins, displacement phenomena related to both nonspecific interaction and the concentration of different components can occur, which in turn may affect drug substance purity and/or process yields. Furthermore, AC steps tend to have wider operating ranges in comparison to conventional chromatography resins. This wider design space improves process robustness and minimize product investigations and batch failure thereby leading to an improved COGs.

Finally, from a company process portfolio perspective use of AC can deliver additional financial benefits that are associated with a reduction in number of full time equivalents (FTEs) needed for process development, scale-up, and tech transfer. For a single program, these benefits can total several million dollars over the various phases of product development (see below).

### 3.2. Standardization of Downstream Processing

AC allows for standardization of various stages of product development starting from research activities (e.g., clone screening), through streamlined process development including easier tech transfer until pilot- and large-scale manufacturing. In the case of manufacturing operations, when the same type of affinity resin is used as a capture step for multiple target within the same family of molecules, e.g., MAbs or AAVs, the whole downstream process is referred to as a platform process. Bioprocess community adopted this term in early 2000s as it very elegantly captured the industry-wide trend where Protein A was used as a capture step for purification of all MAbs produced or being developed at that time. In addition to standardizing manufacturing operations, a platform process concept plays a vital role in accelerated process development, scale-up, and technology transfer as well as in defining layouts of manufacturing facilities.

#### 3.2.1. Platform Processes

A platform process consists of a highly selective AC-based capture step that is followed by few polishing steps to reach the final purity and, in some cases a specific composition of drug substance. Typically, each company develops a few variants of a platform process that vary in the sequence of unit operations post the capture step to ensure that all the types of impurities, either process or product related, can be dealt with regardless of variant of target molecule and a purification challenge at hand.

To date, purification platforms exist for only a few classes of therapeutic molecules, such as MAbs, some antibody fragments, and viruses. For other type of molecules that already have, or maybe engineered to have, a conserved motif in their structure, a company can consider introducing an internal downstream processing platform based on a custom affinity resin developed toward the conserved motifs, which would allow the company to quickly move the several drug candidates considered for clinical development. Such a purification platform can be, and typically is, exclusive to the company, which provides additional competitive advantage in an already very competitive field of bioprocessing.
3.2.2. Process Development

One of major drawbacks to non-affinity-based primary capture steps is a significant process development effort must be applied to all unit operations for each new molecule considered for further development.[53] To reduce the development effort and time, an ideal solution would be to use an existing affinity capture resin that is either generic for a wide range of similar molecules or, if such a resin does not exist, that can be quickly developed without affecting overall process development timelines. In fact, it can be argued that use of AC shortens timelines and reduces cost of process development, because a very robust downstream purification process can be developed, and scale-up with a lower number of FTEs. For instance, the total number of FTEs associated with process development for a recombinant protein can be as high as 36 FTEs totaling a cost of several millions of dollars[93] and, therefore, even a small reduction in number of FTEs needed for process development would result in substantial reduction in the process development cost. However, the biggest gains associated with the reduction of length of process design activities are due to reduced time to market. It has been reported that each day shaved off the time to market can be worth even around half a million dollars in net present value of after-tax net returns.[94]

3.2.3. Platform Downstream Processing Suite

AC can standardize manufacturing facilities. A single product class facility (e.g., MAbs) can be “transformed” into a multiple product (classes) facility through “platformization” of the downstream processing suite. The suite would be designed (e.g., number of dedicated skids and tanks[95]) to accommodate a standard purification process consisting of cell removal step followed by a bind and elute AC capture step followed by one or two chromatography steps, a viral filter, and a UF/DF step.

Since the capture step for each product type would be operated according to the same protocol consisting of cleaning, equilibration, loading, washing, elution, and strip phases, operation standardization would occur. Standardization would result in facility simplification and potentially the automation of manufacturing processes. Furthermore, since all the auxiliary equipment and documentation would also be similar, such a platformized DSP suite would result in the improved process robustness, operational efficiency and facility utilization, and thus reduced COGs.

3.3. Continuous Operations

Another advantage associated with AC is its enabling character for continuous and intensified processing due to the standardization of processes and facilities. Increasingly processes are being developed in which the upstream is run in perfusion mode, or as an N-1 intensified batch, and clarified cell culture fluid (CCCF) is directly (continuously) loaded on to the capture column. However, a potential variability in CCCF composition can lead to variable composition of the capture column elution pools. These variations in the composition of the eluate can prove to be challenging for the unit operations downstream of the capture step. Therefore, it is beneficial that the capture elution pools are comparable irrespective of upstream variation that can be expected with longer perfusion runs[89,96] or with transient fed-batch processes, where the product collection begins when the titer reaches a minimum level and the harvest continues for about 2 weeks (J. Salm at Recovery of Biological Products XVIII, Asheville, 2018). From the above perspective, an affinity-based capture step would act as a process control node that would normalize the process stream post the capture step. This normalization would be observed because as long as the capture column is loaded to the same target density, the variations in feed composition will be mitigated as the elution pools from the capture step would likely have the same compositions and concentrations. Consequently, the remaining steps in purification train will be standardized, what in turn will improve the overall downstream process robustness.

3.4. Polishing Applications

The use of affinity resin in bioprocessing applications is not limited only to capture steps. Affinity resins can also be considered for polishing applications such as removal of process related impurities when used in flow through mode (scavenging affinity resins), or they can be used to separate the desired form of a target molecule from closely related product impurities by, for instance, selective elution. Example of the former application can be the removal of endotoxin[97–99] while an example of the latter can be a purification of bispecific antibody using Protein A affinity chromatography described by Regeneron.[100] The Regeneron bispecific antibody format is based on two heavy chains co-expressed in the same cell culture that leads to the formation of two parental IgG impurities. However, because one of the heavy chains was modified through a dipeptide substitution in the Fc portion, which ablates Protein A binding, a purification of bispecific antibody from a non-substituted parental IgG was accomplished using Protein A resins via selective elution from a second protein column packed with high resolution resin, by exploiting avidity difference during the selective elution of the bound bispecific and a parental non-substituted IgG. A new version of this purification process was later developed using a different Protein A resin that resulted in a single affinity step purification by exploiting differences in the other Protein A ligand binding characteristics.[101]

4. Future State of Bioprocess Affinity Chromatography Field

The history of Protein A resin development shows that significant improvements have been made in resin capacity and alkali stability over time.[51] Especially the improvements in alkaline stability (0.1 M NaOH), first realized with introduction of a Protein A ligand based on modification of Protein-A B-domain (MabSelect SuRe) to the market in 2013, have marked a start of new era in bioprocess Protein A resin market. Since then, several new ligands based on modified versions of the Protein A C-domain have
been introduced. These products offer alkali stability similar to that of the original MabSelect SuRe ligand. Recently, additional development of the B-domain of Protein A has resulted in ligands that are significantly more stable (up to 0.5 M NaOH) than MabSelect SuRe [56] (T. Björkman, 249th ACS Natl. Meeting, Denver, CO, March 2015). Ability to clean these resins under harsher conditions has resulted in better control of bioburden and improved resin lifetime.

Undoubtedly, for affinity resins developed for targets other than MABS, both resin capacity and stability will be as important. However, it can be expected that developing a resin with a higher chemical stability can prove an easier task than developing a resin with a higher capacity. Stability is predominantly determined by ligand composition, concentration, and coupling chemistry, whereas resin capacity is related to properties of the solid support such as pore size, porosity, and even the solid support format. While companies offering custom affinity resin services have access to variety of standard base matrices (Table 3), these matrices may not provide the optimum pore structure for a given target, and the development of an optimal base matrix for a specific target may prove costly, and especially time prohibitive. From that perspective, testing as many commercial base matrices as possible with a ligand with desired affinity will assure that the best bioprocess affinity resin can be developed quickly and at a reasonable cost.

In principle, affinity resins with exquisite selectivity could also be used as polishing resins in the cases where separations with conventional resins, at process yields that would be considered commercially acceptable, can prove very difficult, or even impossible. For instance, separation challenges that could benefit from an affinity-based purification step include separation of isoforms, truncated forms of target molecule, or active and non-active forms of therapeutic enzyme, to name just a few. In fact, recent advances in ligand discovery and subsequent bespoke affinity resin development show that affinity resins can solve these type of separation problems and can be quickly developed. For instance, it has been reported that affinity resins capable of separating active and inactive forms of a therapeutic enzyme, or separating full-length from a truncated form of a growth factor have been developed (W. Kett, at the 9th Annual Bioprocessing Summit, Boston, 2017). Such high-specific selectivity, available on demand, ligands may redefine the development paradigm of future processes for non-platformable molecules. The paradigm envisions designing ligands that can select for the drug and remove process and product-related impurities as defined in the Quality Target Product Profile (QTTP). Additional affinity resins could be designed to yield the desired QTTP in the fewest number of steps.

In Table 4, a resin design example is provided. The purification challenge was to separate Fc-fusion protein from closely related product impurities, such as homodimers, high molecular weight species, and miss-paired variants. The original process was developed based on a Protein A capture step, followed by subsequent polishing steps to remove remaining HCPs and closely related product impurities. The latter accounting for 30% of the Protein A elution pool. A new affinity resin was developed to capture the Fc-fusion protein from the Protein A elution pool, at >95% yield and with 99% purity. Interestingly, when the new resin was used as the capture step, it showed 1.5-fold higher target dynamic binding capacity, 38% increase in purity, and equivalent HCP and DNA clearance in comparison to Protein A.

Affinity ligands that separate closely related forms of target protein may extend the target patent protection, as the final drug composition reflects the ability of the affinity resin to separate the closely related forms. Therefore, it is likely to be extremely difficult for a competitor to match the drug product composition with conventional resins. In other cases, affinity resins that separate product forms may provide freedom to operate from legacy product manufacturing patents employing traditional chromatography approaches.

### Table 4. Comparison of process options for purification of Fc-fusion protein using various affinity resins.

| Purification Process | Target | HMW | LMW | DBC | Effective* DBC | Log reduction |
|----------------------|--------|-----|-----|-----|---------------|---------------|
| Protein A purified   | 69     | 20  | 11  | 22  | 16            | ?             |
| Avitide affinity resinb) | >97   | <3  | 0   | 25  | 24            | –             |
| Avitide affinity resinb) | >95   | <3  | <2  | 25  | 23            | 2.1           |
|                       |        |     |     |     | 3.2           |               |

*a* Protein A purified pool. *b* From clarified feed stream. *c* DBC for Fc-fusion protein.

### 5. Conclusions

AC is now firmly established as a preferred technology in industrial manufacturing of biologics, because of its selectivity, scalability, and ease of process development. While the selectivity offered by natural protein ligands has not been matched by alternative binders such as aptamers or low molecular weight “biomimetics” (i.e., de novo designed affinity ligands mimicking natural biological recognition), other type of ligands can be considered. For the purification tasks for which natural ligands are not available, the trend is to discover or develop ligands based on engineered stable proteins or peptides based on scaffolds, antibody fragments, or domains of a larger protein. The use of display technologies for creating large libraries of protein or peptide binder candidates underpins this development. This emergence of custom affinity ligands and resin technologies will drive for increased adoption and utilization of AC for traditionally non-platformable biologics.

While not covered in this review, AC also plays an important role in other aspects of bioprocessing including quality control analysis. For instance, purification of non-antibody biologics for quality attribute analysis in process development has been achieved using immunoaffinity chromatography. Also, analytical AC with Protein A can be used to determine antibody titers during process development and for other antibody quantification purposes.

The cost of AC resins, although perceived high, is actually fairly modest when expressed per gram of drug product considering that the resins can be used multiple times, increase overall process yield, and that they reduce the number of out of specification batches. Therefore, maturation of the custom AC concept,
both on business and technology platforms, will increase the frequency of utilization of custom-affinity resins, enhancing the efficiency and reduced COGs of biopharmaceutical manufacturing processes.

In summary, large-scale AC has provided, and will continue to provide, robust means of simplifying downstream processing operations. This is not only true for platformable molecules, such as MAb, Fab, or AAV, but it also applies for targets that could not benefit from a purification platform. In the latter case, bioprocess bespoke affinity resins developed for specific molecules will provide all the benefits associated with affinity-based platform processes.

Conflict of Interest
K.M.L. works at Avitide, Inc. F.J.R. declares no conflict of interest.

Keywords
affinity, bioprocess, chromatography, continuous operation, custom resins, facility platform

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[1] BPTC BioTRAK Database. Woburn, MA: BioProcess Technology Group, BDO-USA Life Sciences Practice, https://www.bptc.com/what-we-do/biotrak-database/ (accessed: May 2019).

[2] Biologics Market 2018 Blatant Growth with CAGR of 8.6%—Analysis and Forecast to 2027, https://www.marketwatch.com/investor/press-release/biologics-market-2018-blatant-growth-with-cagr-of-86-analysis-and-forecast-to-2027-2018-11-20 (accessed: May 2019).

[3] J.Royce, Bioprocess Int. 2015, 13, 58.
[4] G. Rodrigo, M. Gruvegård, J. Mol. Biol. 2008, 382, 1211.
[5] M. Lönne, S. Bolten, A. Lavrentieva, F. Stahl, J.-G. Walter, Biotechnol. Rep. 2015, 8, 16.
[6] C. Ferrier, E. Boschetti, M. Ouhammouch, A. Cibiel, F. Ducongé, M. Nogré, M. Tellier, D. Bataille, N. Bihoreau, P. Santambien, S. Chourou, G. Perret, J. Chromatogr. A 2017, 1489, 39.
[7] S. Miyakawa, Y. Nomura, T. Sakamoto, Y. Yamaguchi, K. Kato, S. Yamazaki, Y. Nakamura, RNA 2008, 14, 1154.
[8] M. C. Martinez-Ceron, M. M. Marani, M. Taulés, M. Etcheverrigrayar, F. Albericio, O. Cascone, S. A. Camperi, ACS Comb. Sci. 2011, 13, 251.
[9] S. Menegatti, A. Naik, R. Carbonell, Pharm. Bioprocess. 2013, 1, 467.
[10] M. Miller-Andersson, H. Kerg, L.-O. Andersson, Thromb. Res. 1974, 5, 439.
[11] W. J. Jankowski, W. Von Muenchhausen, E. Sulkowski, W. A. Carter, Biochemistry 1976, 15, 5182.
[12] R. J. Leatherbarrow, P. D. Dean, Biochem. J. 1980, 189, 27.
[13] R. Chaudhary, T. Arora, N. Vashistha, S. Gera, K. Muralidhar, IOSR J. Pharm. 2012, 2, 242.
[14] P. Bailon, D. V. Weber, R. F. Keeney, J. E. Frederick, C. Smith, P. C. Familietti, J. E. Smart, P. C. Familletti, D. V. Weber, R. F. Keeney, P. Bailon, J. Invest. Dermatol. 1990, 94, s158.
[15] F. Detmers, P. Hermans, J.-A. Jiao, J. McCue, BioProcess Int. 2010, 2010, 50.
[16] J. E. Smart, P. C. Familietti, D. V. Weber, R. F. Keeney, P. Bailon, J. Invest. Dermatol. 1990, 94, s158.
[17] F. Detmers, P. Hermans, M. ten Haaf, Mannuf. Chem. 2007, 56.
[18] H. K. Binz, P. Amstutz, A. Plückthun, Nat. Biotechnol. 2005, 23, 1257.
[19] D. Steiner, P. Forrer, A. Plückthun, J. Mol. Biol. 2008, 382, 1211.
[20] B. Mouratou, G. Béhar, P. Santambien, S. Chourou, G. Perret, J. Chromatogr. A 2017, 1489, 39.
