6.07 Evolution of mAb Downstream Process Platforms

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6.07.1 Introduction to mAb Platform Processes

Monoclonal antibodies (mAbs) are the most successful class of biopharmaceuticals today. More than 50 mAbs have been approved and sales of mAbs are expected to cross $125 billion by 2020.[1] The ability to bind to specific targets with high specificity and affinity and the ease of developing human or humanized sequences to a target have been behind the explosive growth of this class of pharmaceutical products. The last 20 years have seen the rapid growth of this class of therapeutics with over 300 mAbs in clinical development today. Today mAbs are approved for a wide range of indications covering oncology,[2] autoimmune disorders, and rare disease indications.

While the ability to target cell surface targets with great specificity has been behind the rise of mAbs,[3] one must not lose sight of the development of process platform technologies that have enabled rapid production of mAbs for clinical trials as well as at commercial scale. The ease and speed of producing mAbs has enabled rapid entry of these product candidates into clinical trials and the scalability and robustness of these processes has hugely facilitated large-scale commercial supply.

Development of a manufacturing process for a protein requires the consideration of many different factors, including removal of impurities, robustness, scalability, and ready availability of raw materials for large-scale production. Consideration has to be given not only to the scale needed for early clinical supply, but also the ability of the process to support long-term supply needs and scales. As a result, utilizing well-established unit operations is a key aspect of developing manufacturing processes. The aspects of robustness, scalability, and reproducibility mean that manufacturing processes often look quite different from those that can be employed in the laboratory for purifying small quantities of proteins. Process development can be a time-consuming activity and require significant amounts of experimentation. As a result, when possible the industry has gravitated toward platform approaches.

A platform approach has distinct advantages from a business standpoint. Speed to clinic is often a key determinant of a company's success. mAb platforms have enabled progression from gene to Investigational New Drug (IND) in less than a year, which is a significant improvement over molecules that require involved development efforts that can extend up to 2 years. This reduced experimentation also implies a reduction in the cost of the development effort. The predictability of a process platform enables organizations such as manufacturing and quality control to adopt a templated set of documents which also reduces the time and resources spent on production and release testing.

mAb therapeutics particularly lend themselves toward the application of platform approaches. Use of a well-developed mammalian cell culture expression system enables development of stable cell lines in a very rapid and templated fashion for mAbs. Several expression vectors have been optimized specifically for mAb production.[4,5] Robust fed-batch cell culture processes have been developed for mAbs. Several of these have been scaled to large-scale production and characterized extensively giving a good idea of the operating parameters that influence these processes.[6,7] Cell line development and upstream cell culture processes lend themselves to a templated approach very well. However, for most proteins the greatest area of divergence comes in the form of the downstream purification process that has to be customized for each protein based on its properties as well as that of key impurities. The Fc region of mAbs binds very specifically to immobilized Protein A which is a cell wall component of *Staphylococcus aureus*. Protein A affinity chromatography has been shown to be widely applicable for mAbs and can achieve >95% purity with very little development on this mode of chromatography.[8] The chief challenge after Protein A chromatography is to remove residual host cell protein (HCP) impurities, high molecular weight (HMW) aggregate, DNA, and have the capability of removing adventitious viruses.

A number of downstream process platforms for mAbs have been developed at leading biopharmaceutical companies.[9–13] The ability to use a generic approach across molecules and starting from a template decrease the amount of experimentation needed compared with a protein that cannot enable the inclusion of an affinity step in the downstream process. These downstream process platforms have successfully enabled the progression of a large number of mAb products into the clinic and commercial space.

6.07.2 Current and Evolving Downstream Process Platforms for mAbs

6.07.2.1 Drivers for mAb Platform Evolution

This section describes several downstream process schemes that have been developed at leading biopharmaceutical companies and successfully employed for mAb manufacturing at large scale. Several aspects are in common across these process schemes. mAbs are extracellularly secreted into the cell culture medium during mammalian cell culture. Harvest and recovery schemes typically utilize centrifugation followed by depth filtration and a series of membrane filters.[14,15] At smaller scales, centrifugation may be dispensed with and a series of depth filters with the coarser pore size filters first may often be utilized. The platform process almost always commences with Protein A chromatographic capture.[16] In some cases, selective washes can be built into the Protein A step operation to further enhance HCP clearance.[17] Non-Protein A schemes have been developed and employed for large-scale manufacturing[18] as for any therapeutic protein but have not caught on given the absence of a generic approach and issues with process robustness. The process will also include two dedicated orthogonal viral clearance steps as per International Committee on Harmonization (ICH) guidelines. Low pH viral inactivation and viral filtration through a parvoviral grade filter are widely employed for mAbs.[19] The low pH incubation step is typically placed immediately after Protein A chromatography since the Protein A column elutes in a low pH buffer. Following Protein A chromatography and viral inactivation, one or two polishing chromatographic steps are employed to clear HMW aggregate,[20] HCPs,[21] DNA, and to provide viral clearance potential. Chief differences among platform approaches lie in the nature of the polishing chromatographic steps. Given the high titer cell culture processes that are increasingly common in industry today (5–10 g L^{-1}), there is a significant need for high column loadings on polishing chromatographic steps.

Each company ends up customizing its mAb downstream platform approach based upon its own expression system and cell culture process as well as the mAb type and subclass they predominantly employ in discovery research. The primary criterion is that the platform approach needs to be robust and applicable across a wide range of IgG molecules without significant modification. Another key criterion is the ability of the platform to fit in the manufacturing schedule and minimize the amount of time spent in downstream processing for any one batch. As a result, another key driver is the loading capacity that is possible for any of the polishing steps.

6.07.2.2 Recent Downstream Platforms for mAbs

Amgen was one of the first companies to disclose its approach to a downstream process platform.[9] A completely templated approach was shown not to be possible, but a small number of development experiments should lead to answers about process parameters that need to be customized.[10] Examples of such process parameters include examples such as the Protein A elution pH and the choice of polishing chromatographic steps depending on the chief set of impurities that need clearance. **Fig. 1** shows the platform downstream scheme in use at Amgen at the time. The polishing steps typically employed were cation-exchange chromatography (CEX) in a bind and elute mode followed by either hydrophobic interaction chromatography (HIC) in a flow-through mode if HMW aggregates needed to be cleared or anion-exchange chromatography (AEX) in a flow-through mode if only HCP

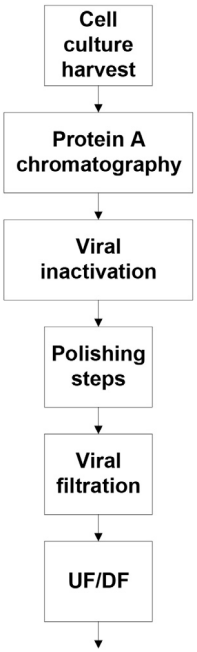

Fig. 1 Platform approach to mAb downstream processing at Amgen.

clearance was needed. In a few cases, hydroxyapatite was employed if specific product-related impurities needed to be removed. In situations in which adequate HCP and HMW clearance were obtained after the first polishing CEX step, anion-exchange membrane chromatography was employed to provide for another step with viral clearance potential.[22]

Another leading company to develop a pipeline of mAbs using a platform approach was Genentech.[13,23,24] Genentech historically employed CEX and AEX as part of its downstream platform (Fig. 2). The trend appeared to initially be to utilize CEX in a bind and elute mode as the first polishing step followed by AEX in a flow-through mode of operation. mAbs usually have a basic p*I* and hence bind tightly to CEX resins and tend to flow-through readily on AEX resins. The CEX step clears both HMW and HCP. The AEX step, however, is noted to require a low load conductivity in order to successfully remove HCPs. This can lead to a bottleneck during plant operation due to the need for large volume in-process hold tanks to hold the AEX load. The possible use of mixed-mode Captoadhere resin in place of more typical AEX resins is mentioned to help reduce the need for significant dilution.

One of the key drawbacks of using AEX is that it only provides clearance for HCPs and not for HMW aggregates. This particular drawback has been mitigated by the development of an AEX step operated in a weak partitioning mode[25] in which the product actually has some measure of retention on the resin. In this mode of operation, the AEX step has actually been shown to be capable of reducing HMW content in addition to HCP species. Mechanistically, we speculate that interactions with the somewhat hydrophobic parts of the resin backbone set in at very low conductivities, enabling some degree of HMW removal to occur.

Both of these schemes suffer from the drawback of requiring significant dilution of the load material for the AEX step. In addition, CEX and AEX alone are often unable to provide adequate HMW clearance. HMW levels can vary for mAbs, but levels of $>5\%$ HMW aggregate after Protein A chromatography are not that uncommon. Such levels often require recourse to a HIC step as has been mentioned earlier.[9] Another innovative approach has been to operate the HIC step under highly overloaded conditions (>200 g L^{-1}) under no salt conditions.[26] This approach entails the use of a highly hydrophobic HIC resin with no additional kosmotropic salt added to the load. pH conditions of the load are modulated to enable HMW removal under these extreme overloaded conditions. The use of such an ultra-high capacity step enables the processing of large production quantities of mAbs and is used as part of the mAb platform at Biogen (Fig. 3) in combination with a flow-through AEX step. The ability to load the polishing steps at very high loads enables a reduction in column cycling and decreases the duration of floor time taken for a batch.

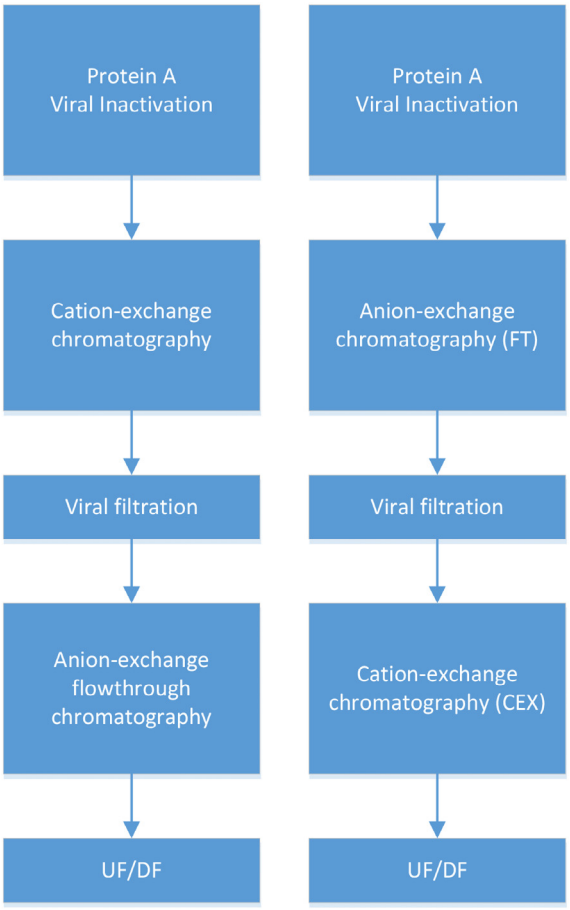

Fig. 2 Genentech mAb downstream process platform.

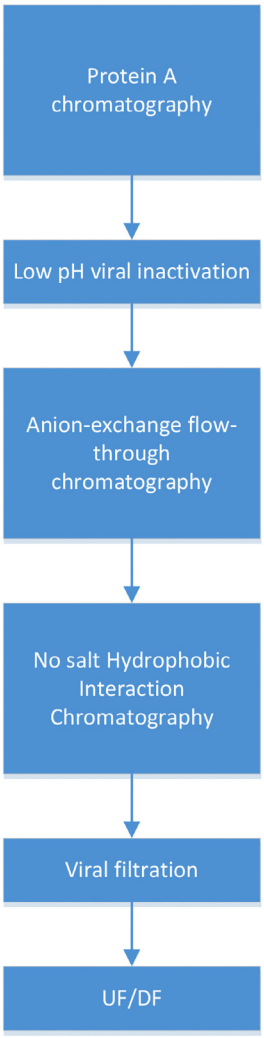

Fig. 3 Platform mAb downstream process at Biogen.

A similar flow-through only polishing scheme has been discussed by Millipore-Sigma.[27] In this proposed format, CEX would be operated under highly overloaded conditions during the loading step. When operated in this fashion, CEX has the ability to clear some level of HCPs. It is again speculated by us that this occurs due to weak hydrophobic interactions with the chromatographic backbone. This polishing step is then combined with AEX flow-through to complete the sequence.

Contract development and manufacturing (CDMO) requires the ability to incorporate many different cell lines and cell culture processes into the downstream platform approach. This places significant challenges on the downstream platform scheme since both HCP and HMW reduction are simultaneously required. These simultaneous demands on the downstream mAb platform are illustrated in Fig. 4.

6.07.2.3 A Broadly Applicable mAb Downstream Platform

CEX and AEX in their traditional flavors do not fulfill this need. A key modification made in this regard has been the use of multimodal chromatography as part of the mAb downstream platform. Multimodal chromatography involves the incorporation of a hydrophobic moiety into the ligands structure for either AEX or CEX.[28] The increased hydrophobicity of the chromatographic resin now enable improved clearance of HMW on both CEX and AEX modes which are arguably most suited for mAb processing. In addition, both these modes of chromatography are also capable of HCP and DNA clearance as well. mAbs do differ in terms of their own hydrophobicity. As a result, the platform downstream process for mAbs at KBI is defined as AEX (with resin hydrophobicity that can vary from Q Sepharose FF or Capto Q to Fractogel SO3 to multimodal chromatographic resins such as Captoadhere and Nuvia cPrime). This modulation of hydrophobicity enables optimal conditions to be tailored for each mAb. Similarly, the CEX bind and elute step is also operated on a range of hydrophobicities ranging from mild to moderate depending on the resin selected.

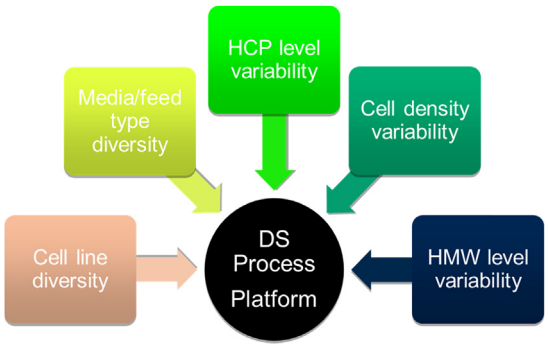

Fig. 4 Range of factors considered in determining KBI Biopharma's platform approach.

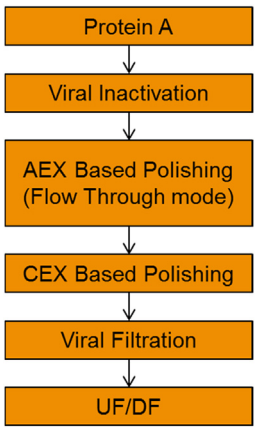

Fig. 5 KBI Biopharma's approach to mAb downstream processing for FIH manufacturing.

While this approach does entail some degree of experimental work, a preferred approach using multimodal chromatography for both AEX and CEX is employed as the primary platform with recourse to less hydrophobic stationary phases should the mAb require it. This approach is illustrated in Fig. 5 and has been useful in terms of its breadth of covering a wide range of mAb constructs, cell lines, and cell culture processes at KBI.

Fig. 6 shows the clearance profiles for HMW aggregates and HCPs through this platform process for a number of mAbs. As can be seen from the figure, HMW aggregate levels of $<1\%$ and low HCP levels <50 ppm are always observed using this platform. The ability to cover a wide range of mAb constructs is a key, especially for a CDMO organization such as KBI.

6.07.3 New Technologies Impacting mAb Downstream Processing

Traditional chromatography is batch process. Steps start from column sanitization, equilibration, and loading through to washes, elution, strip, and column regeneration and storage. A batch operation is limited in terms of its throughput and productivity. First of all chromatographic columns can only be operated at a maximum diameter of 2 m owing to flow distribution limitations. Current resins can only be packed to a maximum of 30 cm bed height (20 cm typically) owing to pressure drop limitations. This inherently limits the amount of product that can be processed per cycle. When multiple cycles are required, product intermediate needs to be held for a longer duration of time. Product holds also end up using valuable tank volume in manufacturing facilities which are also limited. Continuous chromatographic separations can turn this batch operation into a continuous or semicontinuous process[29] as shown in Fig. 7. Combined with a continuous upstream perfusion cell culture process, this can alter the current paradigm of how biopharmaceutical production processes are designed.[30,31] Continuous chromatographic separation can be conducted using one of many formats. Periodic counter current chromatography is one of the formats (from GE Healthcare) and utilizes multiple columns that are continuously operated in different phases of the operation cycle.[32] Other formats include multicolumn countercurrent solvent gradient purification that enables recycling of the front and tail ends of the peaks to boost yield as well as purity via the ContiChrom system from ChromaCon and the BioSMB technology from Tarpon Biosystems (now part of Pall Corporation).

Another means of boosting productivity is to move from chromatographic operations to nonchromatographic separations. Various systems such as selective precipitation schemes using polymers and aqueous two-phase separations (ATPS) can be utilized

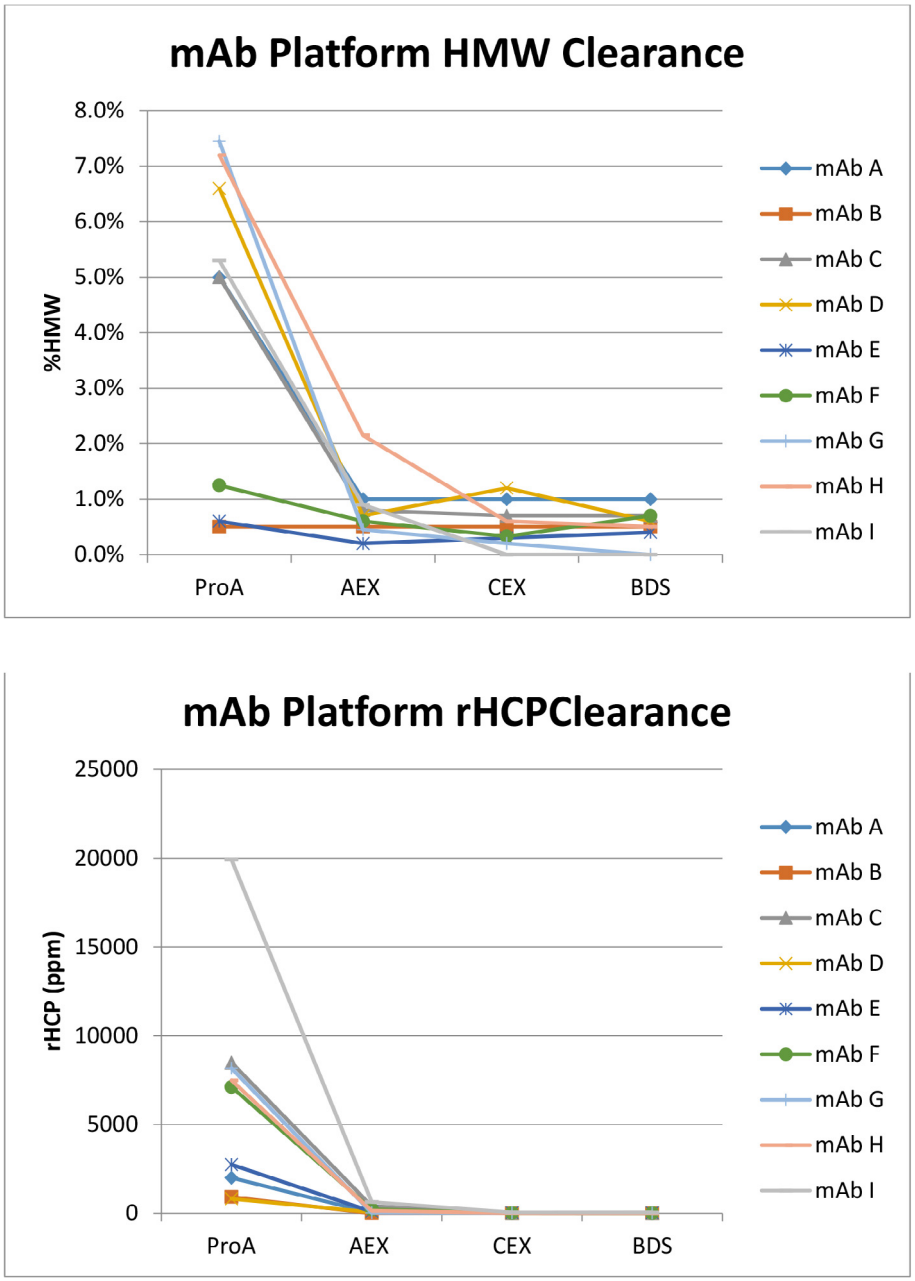

Fig. 6 Performance of the KBI Biopharma platform DSP approach for several mAbs. (A) HMW clearance and (B) HCP clearance.

to capture the entire bioreactor batch in one operation rather than relying on multiple chromatographic cycles. A variety of polymers have been utilized for mAb precipitation including polyanionic polymers to precipitate the product[33] and caprylic acid precipitation of HCP impurities.[34] ATPS rely upon the creation of two separate phases in solution by mixing a polymer and salt or two polymers with each other. Examples include poly-ethylene glycol (PEG)-salt and dextran-PEG ATPS.[35] Both of these separation technologies have interesting possibilities and can significantly raise the bar on throughput that is currently possible from manufacturing facilities. However, both areas need further development to establish themselves as scalable technology that can be applied without significant development for a wide range of mAbs.

6.07.4 Next-Generation Antibody Constructs Impacting the Process Platform

The other major factor that leads to evolution of the platform approach is the specific mAb-like construct that is being developed as a potential therapeutic. The biotechnology industry is rapidly moving beyond conventional mAbs into a variety of constructs

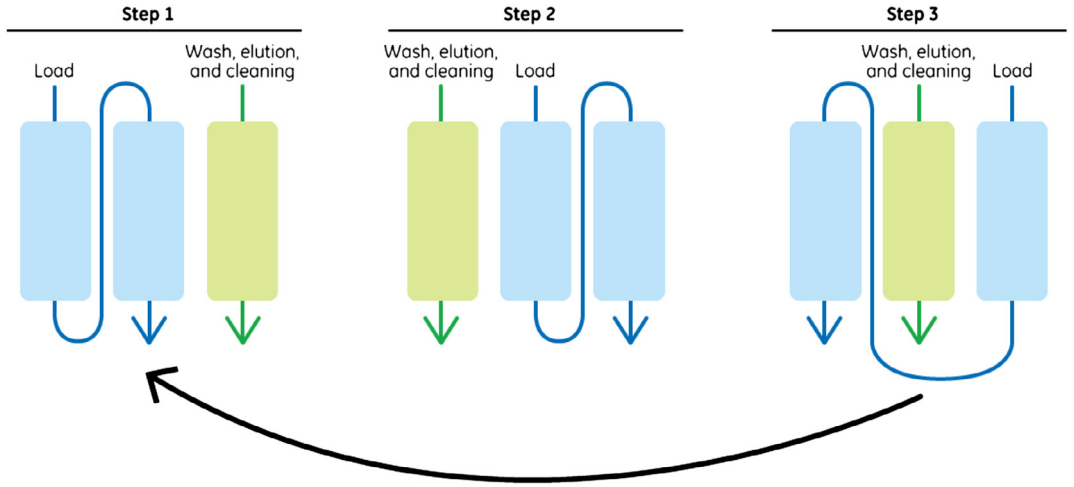

Fig. 7 Principle of continuous chromatography.

including Fc fusion proteins, bispecific antibodies (bsAbs), and antibody-fusion proteins. Each of these new constructs requires modifications of the original mAb platform process to enable their production.

Fc fusion proteins are created by joining the coding sequence for the Fc region of a mAb to the coding sequence for another protein.[36] The Fc region offers several advantages as a fusion partner. Many biologically active peptides and proteins have a short serum half-life reflecting their rapid clearance through the kidneys. The Fc region can bind to the neonatal Fc receptor to extend the half-life of antibodies, and the same benefits are conferred on fusion partners.[37] Seven Fc fusion proteins are commercially available as approved biopharmaceuticals and at least two of them (Enbrel and Orencia) have already achieved blockbuster status with sales of over US$ 1 billion per year. The original platform for mAbs at Amgen included the purification of Fc fusion proteins.[9] However, several key downstream differences do exist for Fc fusion proteins, including the possibility of susceptibility to proteolytic cleavage and the possibility of higher HMW aggregate levels being present than regular mAbs. A typical mAb downstream platform approach usually is effective, with possible adjustments to the polishing steps to account for stability of the molecule and effective HMW clearance.

bsAbs are designed proteins capable of simultaneously binding and neutralizing two different antigens (ligands, cell receptor, or cytokines) or two distinct epitopes on the same antigen.[38] As a result of this property, bsAbs can serve as mediators in redirecting immune effectors and cytotoxic agents like T-cells to tumors or to infecting organisms such as bacteria. The fact that two arms of the mAb are different leads to several processing challenges that are unique to bsAbs. For example, if the two halves of the mAb are expressed separately, a downstream process that requires disassembly of the two halves followed by reassembly to form the heterodimeric species will have to be conducted.[39] In addition to the recombination steps, even if the formation of homodimeric species is discouraged (by use of a knob-in-hole methodology or a similar technology that encourages heterodimer formation), small quantities of the homodimers will form and will have to be removed by downstream processing. As a result, this format of bsAbs requires a more complex downstream process.

However, use of a common light chain along with the knob-in-hole technology (KiH) enables the formation of a bsAb in cell culture expression.[40] Homodimer formation is discouraged due to the KiH construct. The small quantities of homodimer that are formed can be removed if the sequences selected for those have a biochemical difference. This can be done even in the absence of a common light chain. For example, in the XmAb technology from Xencor, the homodimer and heterodimer have a small difference in charge in their engineered Fc regions that enables their separation on CEX. The other purpose of the engineering approach is the enhance effector function of the bSAb.[41]

An antibody–drug conjugate (ADC) is a mAb conjugated with a cytotoxic agent via a linker with the primary aim of treating cancer.[42] ADCs can be produced in a downstream process identically to conventional mAbs except for a chemical conjugation step at the end of the downstream process. This is typically followed by ultrafiltration/diafiltration (UF/DF) to remove the conjugating chemicals. The chief difference from mAbs is the requirement for more stringent containment and personnel protection due to the toxic nature of the conjugate.

Other next-generation mAb constructs include peptide fusions to mAbs at either the C or N termini to further enhance the ability to bind to more than one target at a time. Examples include anticalins fused to a mAb structure.[43] A whole range of other therapeutic options exist as shown in Fig. 8, including engineered scFvs, diabodies, and tribodies and Fab conjugates in the form of dimers or trimers.[44] Each of these therapeutic modalities can lead to the development of platform process approaches if they become prevalent as a therapeutic modality.

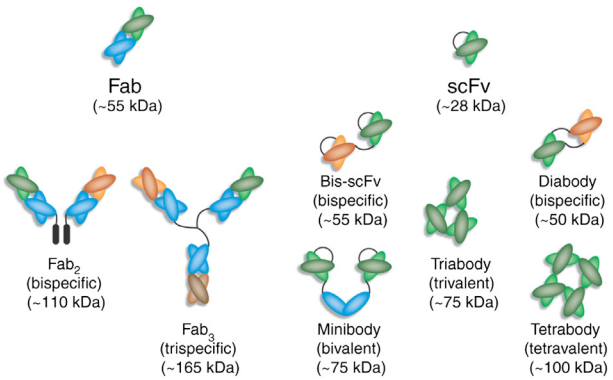

Fig. 8 Possible next-generation antibody formats.

6.07.5 Conclusions

This article has discussed the need for a platform approach for mAbs and its utility in accelerating the progression of many different therapeutics toward the clinic and market. The use of a platform approach has enabled many biopharmaceutical companies to successfully progress mAbs from gene to IND in a year or less. Based upon their internal antibody construct, cell line, and cell culture process, each biopharmaceutical organization has developed its favorite platform approach. Latest trends include the use of multimodal chromatography as part of the process platform and the use of two high loading polishing steps in a flow-through mode of operation. These modifications have enabled even broader applicability of the mAb platform as well as are meaningfully addressing the throughput bottleneck in downstream processing.

As cell culture productivity continues to advance, other alternative formats to help improve the productivity of the downstream process are being advanced. These include the operation of the Protein A chromatographic step in a continuous mode rather than a batch format. Continuous processing could conceivably be extended for the entire downstream process in the future. Nonchromatographic separation steps using precipitation or ATPS are another possible future direction for mAb downstream processing.

More radical alterations of the mAb downstream process platform are being undertaken for next-generation mAb products such as bsAbs and antibody fragments. The next decade will see further evolution of the mAb downstream process platform based on the drivers of productivity and new molecule formats.

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