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QUALITY INNOVATIONS IN MONOCLONAL ANTIBODY MANUFACTURING

Advances in mAb Purification & Analytical Technologies

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INTRODUCTION

THE STATE OF MONOCLONAL ANTIBODY MANUFACTURING

Progress in antibody therapeutics in recent years has been revolutionary. Although the US Food and Drug Administration approved the first monoclonal antibody (mAb) therapeutic in 1986, only 13 more had been approved by the end of 2002. Over the next 20 years, more than 100 mAbs would be approved (figure 1).[1] This growing interest in antibody therapeutics has propelled the growth of the global market, which had a value of about $200 billion in 2022.[2]

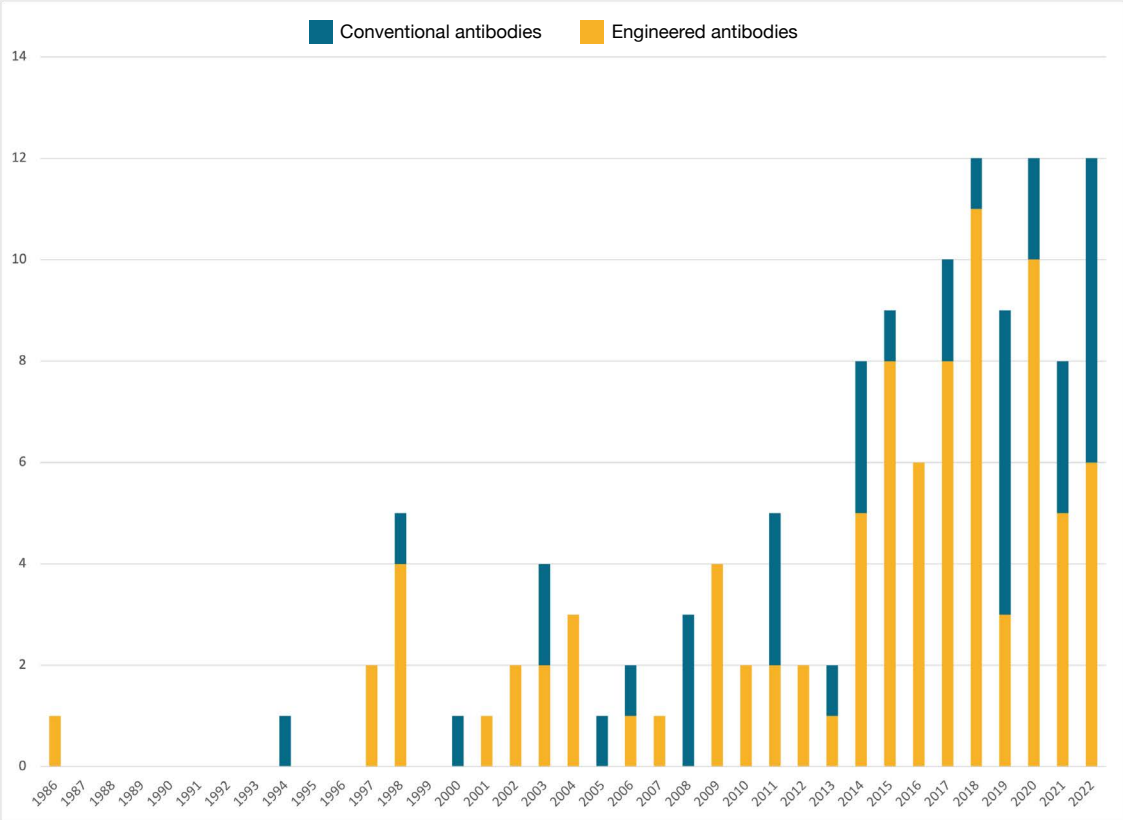

Figure 1: Antibody therapies approved by the US Food and Drug Administration by year. Gray indicates conventional antibodies and blue indicates engineered antibodies, including antibody-drug conjugates, bispecific antibodies, and Fc-fusion scaffolds.
Source: Reference 1.

Antibody therapeutics provide cell-specific recognition customized for a patient and the potential to modulate an immune response. They offer the ability to treat cancers and autoimmune diseases with fewer side effects than traditional treatments such as chemotherapy. Many mAb therapeutics are made from so-called conventional antibodies, Y-shaped proteins with specific binding sites at the end of each arm of the Y.

As the scientific understanding of traditional mAb therapeutics has advanced, researchers have moved into engineering proteins to create next-generation antibody modalities. Antibody fragments, bispecific antibodies, antibody-drug conjugates (ADCs), and Fc-fusion scaffolds have all received FDA approval since 2019.[1]

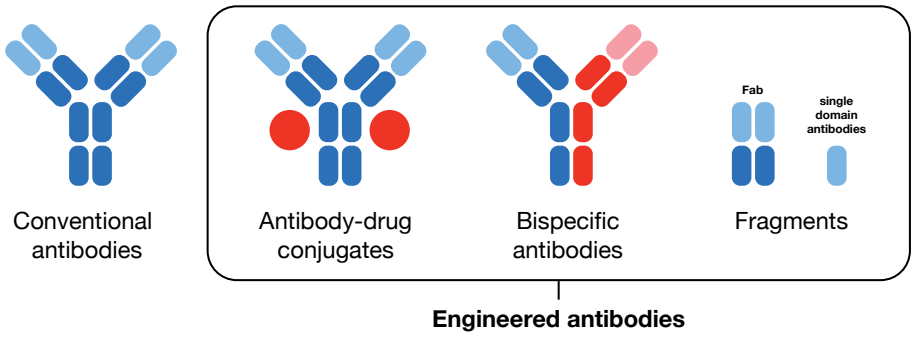

Figure 2: While most antibody-based therapeutics are conventional antibodies (left), an increasing number of engineered antibodies have received regulatory approval. Engineered antibodies include antibody-drug conjugates, bispecific antibodies that have two different recognition sites, and various antibody fragments.
Credit: C&EN BrandLab

Innovations in the field of mAb therapeutics are accompanied by advances in the biotechnologies designed to develop and manufacture them. Engineered antibodies are produced in cultured cells. Research and development teams must show that their entire biomanufacturing process is reliable before receiving approval (figure 3). This effort includes instituting measures to ensure purity and confirming that analytical techniques effectively test for contamination, activity, and other quality concerns. As the saying goes: the process is the product.

Figure 3: The process of antibody manufacturing involves cell culture, separation, purification, and filling.
Credit: Thermo Fisher Scientific

The purification step of antibody manufacturing provides an example of the need for technology evolution. A common purification technique involves a bacterial protein called protein A. Protein A binds to the stem, or Fc region, of immunoglobulin G (IgG) antibodies, which are the antibody type most used for therapeutics. By preparing a chromatographic resin with protein A, it is possible to separate mAbs from other cellular materials. Protein A affinity chromatography remains a popular and effective tool for purifying conventional mAbs. But protein A is ineffective at purifying newer antibody-derived therapies, such as fragments or bispecifics.[3] For this increasingly diverse class of biotherapeutics, scientists must find alternative methods to meet the purity requirements without losing yield.

Now, pharmaceutical manufacturers that produce mAb-based therapeutics face a complex set of scientific and business challenges. Part of the commercial success of antibody-derived therapeutics depends on efficient purification and analytical techniques. Companies that implement state-of-the-art technology can improve their time to market, reduce the chance of quality issues, and lower manufacturing costs. This e-book addresses common purification, analysis, and quality evaluation challenges when manufacturing modern antibody-derived therapies. Tools such as affinity chromatography, quantitative polymerase chain reaction (qPCR), and single-domain antibodies provide effective and flexible solutions for improving the efficiency and reliability of antibody biomanufacturing practices.

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CHAPTER 1

AFFINITY PURIFICATION IN MONOCLONAL ANTIBODY MANUFACTURING

The first step in antibody manufacturing involves producing antibodies in cells using large-scale bioreactors. Cell culture media is a complex mixture of nutrients, salts, and proteins. Antibodies must be purified from this mixture and cellular contents before they can be safely used for therapeutic applications. When developing a purification method, maximizing yield is a priority so that as much antibody is recovered from given culture volume as possible. Development teams also attempt to minimize purification steps to preserve yield and reduce capital and operational expenses (figure 1).

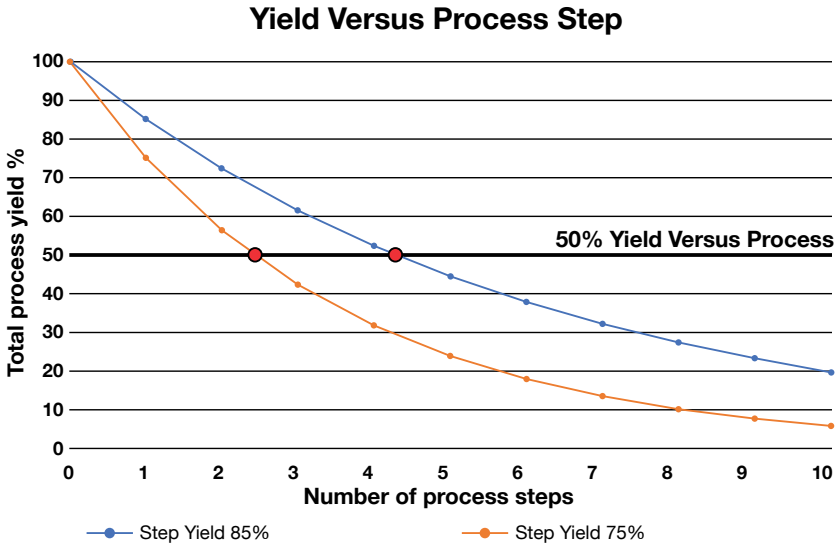

Figure 1: Even with 85% yield for each purification step, overall product yield declines rapidly with an increasing number of process steps.
Credit: C&EN BrandLab

Affinity chromatography is one of the most effective methods for purifying mAbs. This technique uses a resin designed to precisely capture a desired molecule, enabling high purity and recovery after one process step. Pim Hermans, head of ligand discovery at Thermo Fisher Scientific, says that a key advantage of affinity chromatography is that “you can reduce the number of purification steps and increase your product yield.”

NOVEL ANTIBODY RESIN TECHNOLOGY

Protein A affinity chromatography, described in the introduction of this e-book, has been the standard for mAb affinity purification for several years. Thermo Scientific™'s MabCaptureC™ Affinity Matrix is a high-performance protein A resin. "It's designed for high productivity and increased efficiency in the monoclonal antibody purification workflow," says Laurens Sierkstra, senior R&D director in the Purification and Pharma Analytics group at Thermo Fisher Scientific.

The MabCaptureC resin has a uniform bead size to achieve consistent separation resolution, is stable for over 100 cycles in 0.2 M sodium hydroxide, and has an antibody binding capacity of more than 50 g/L. These features make it an excellent tool for a range of antibody purification workflows.[1]

But protein A affinity resins do not capture engineered antibodies that lack a stem, or Fc region. To address the purification challenges for engineered antibodies, scientists at Thermo Fisher Scientific have used CaptureSelect™ technology to develop four antibody affinity resins that each bind to a specific region of an antibody (figure 2). This range of options allows research teams to select the resin that best meets the needs of their product.

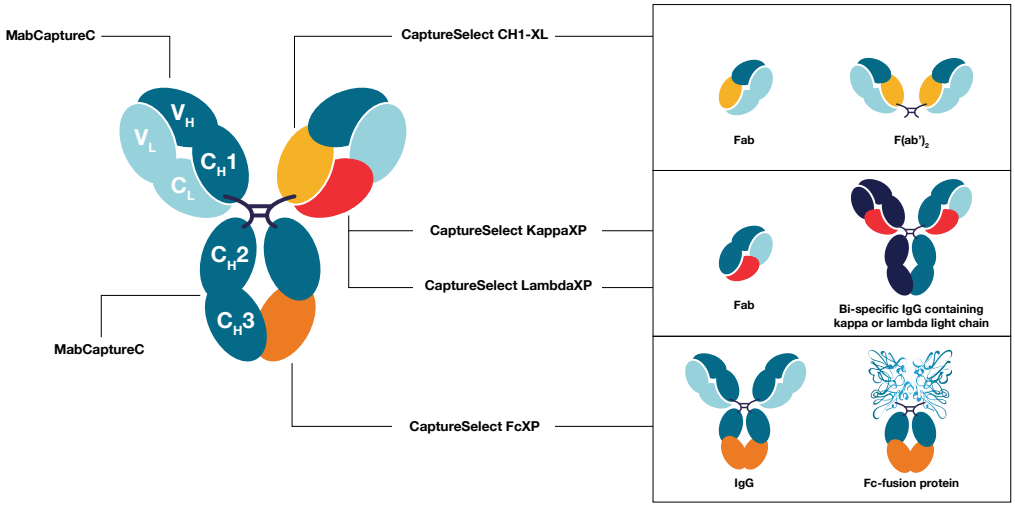

Figure 2: CaptureSelect affinity resins are designed to recognize unique sites shown on this composite antibody. The MabCaptureC resin binds to the heavy chain (dark blue) of a typical antibody. CaptureSelect antibody resins bind to specific sites on an antibody to enable purification of fragments like Fab and F(ab′)$_2$, bispecific antibodies with different combinations of heavy and light chains, and Fc-fusion proteins containing the stem of an immunoglobulin G antibody connected to a protein.
Credit: Thermo Fisher Scientific

CASE STUDY: FAB PURIFICATION

A research team set out to develop a method to isolate a biotherapeutic Fab. Fabs are antibody fragments that include only a single light chain and the C_H1 and V_HH domains of the heavy chain. Antibody light chains are often overexpressed during biomanufacturing—meaning excess light chains must be removed from the final product. Protein A cannot purify these antibody fragments because the Fabs lack the Fc region involved in binding.[2]

The researchers employed the Thermo Scientific™ CaptureSelect™ C_H1-XL Affinity Matrix, which binds specifically to the C_H1 domain of Fab fragments, rather than light chains that lack the C_H1 domain. Using this approach, the team was able to effectively purify the Fab fragments in a single step.

AFFINITY, FLEXIBILITY, AND RELIABILITY

Innovations in engineered antibody therapeutics are likely to continue. Purification platforms that are flexible enough to meet the needs of a particular therapeutic as well as reliable enough to meet operational requirements are key to bringing that innovation to market. “Whatever your product challenge, we will have a product to bind to a certain domain of your antibody-derived therapeutic, which will help you to get through your clinical phases,” Hermans says.

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CHAPTER 2

QUALITY AND SAFETY: ANALYTICS DURING MAB MANUFACTURING

KEY TERMS FOR PROCESS ANALYTICS

Specificity:
Ability to assess an analyte in the presence of other components

Linearity:
Test results proportional to the concentration of the analyte

Range:
Concentration interval for which the method is effective

Accuracy:
Closeness between the value found and the actual value

Limit of quantitation:
Lowest level at which the concentration of an analyte can be accurately determined

Precision:
Closeness of agreement between measurements conducted on the same sample

Contamination from adventitious agents such as viruses and bacteria and process related impurities such as residual DNA (resDNA) threaten the quality of antibody-based therapies. These contaminant and impurities can trigger immune responses or infections in patients. Even low levels of contamination can compromise an entire production batch. Regulatory guidance limits the amount of impurities in the final drug product. Antibody manufacturing facilities maintain sterility and adherence to current good manufacturing practices (CGMPs).

Regulators expect manufacturers to verify the purity of their therapeutics before they are distributed to patients. These testing methods must be validated to demonstrate sufficient specificity, linearity, range, accuracy, limit of quantitation, and precision (see box).

Pharmaceutical development teams traditionally relied on bespoke analytical methods to test for contaminants and impurities associated with a specific product. Developing and validating these methods is time intensive and expensive. Tests for adventitious agents are slow and potential bottlenecks for batch release. Advances in testing methods can simplify development and validation while accelerating manufacturing timelines. This chapter explores the latest analytical techniques to detect viruses, mycoplasma, and resDNA.

VIRAL CONTAMINATION

"Viruses are likely the most difficult biological contaminant to detect," says Paul Barone, director of the Consortium on Adventitious Agent Contamination in Biomanufacturing (CAACB) at the Massachusetts Institute of Technology. "They are not visible microscopically and may not lead to obvious changes in cell culture. A viral particle in a raw material will be essentially undetectable."

Members of the CAACB include many of the largest biopharmaceutical companies in the world. This group focuses on identifying and sharing best practices around mitigating the risk of adventitious agent contaminations. The CAACB suggests using a "three pillar" approach to protecting against virus contamination that is based on recommendations from the International Council for Harmonisation.[1-3]

Selecting materials with a low risk of containing adventitious virus

Testing cell banks and in-process materials to ensure that they are free from detectable viruses

Incorporating steps to remove and inactivate potential undetected viral contaminants during product purification

While there are no known cases of virus-contaminated biotherapeutics causing a viral infection in patients since the HIV epidemic, a 2020 study from the CAACB reported 18 known instances of virus contamination over the past 36 years.[1] The researchers found that these viral contamination events can cost millions of dollars in investigation expenses and lost production time.

Conventional methods of checking for viral contamination rely on animal testing or cell cultures. These tests take up to a month to complete, providing results long after the fastest part of a viral growth cycle in a cell culture. As such, these tests are impractical for in-process testing and taking preventative or corrective action.

Detecting specific sequences of DNA or RNA isolated from a culture sample can identify nucleic acid signatures of specific viruses. According to Barone, many companies use quantitative polymerase chain reaction (qPCR) methods to test bioreactor samples for viruses. These qPCR-based methods are sensitive and can be completed in 4–5 h. The Applied Biosystems™ ViralSEQ™ Real-Time PCR System generates accurate results and includes solutions for sample preparation, reverse transcription, and qPCR. Kits are available for viruses commonly associated with Chinese hamster ovary (CHO) and other rodent cell culture, including mouse minute virus (MMV) and vesivirus.

MYCOPLASMA MONITORING

Bacterial contamination is significantly more common than viral contamination. “Half of the companies that responded to one of our surveys have not had a viral contamination event, while bacterial contaminations appear to be more common, based on informal discussions with biomanufacturers,” Barone says. Fortunately, most bacterial contamination is relatively easy to detect because of the size of the cells and their faster growth rates.

The main exceptions to this are bacteria in the genus *Mycoplasma*, which are the smallest known free-living organisms. These tiny microbes can infect humans, causing pneumonia and other diseases.[4] As a common contaminant of mammalian cell cultures, mycoplasma can disrupt cellular processes, resulting in lower productivity, malformed drug product, and direct toxicity to patients.

Biopharmaceutical manufacturers use two forms of testing to avoid mycoplasma contamination: lot release and in-process testing. Lot release testing ensures that the final product is free of contamination and is required by regulatory agencies.[5] In-process testing detects the presence of mycoplasma during the manufacturing process. This form of testing helps protect the final medical product while also avoiding the contamination of equipment and materials. Multiple test methods may have to be developed to meet the needs of both lot release and in-process testing.

Conventional mycoplasma testing takes several weeks to complete and requires live mycoplasma cells as a positive control.[5] The long testing time and the need to culture hazardous infectious bacteria are burdensome and undesirable for manufacturing teams. Many companies outsource mycoplasma testing to specialized labs, though this is also time-consuming, expensive, and often acts as a bottleneck in antibody manufacturing.

According to Barone, many companies are now using qPCR to test for many mycoplasma species simultaneously. The Applied Biosystems™ MycoSEQ™ Mycoplasma Detection Kit is designed to meet these mycoplasma testing needs. It has been validated in numerous studies to detect a variety of *Mycoplasma, Acholeplasma,* and *Spiroplasma* species. This test is also highly sensitive, exceeding the recommended regulatory guidance of 10 CFU/mL; its 4–5 h testing time allows for testing in-process material to limit the impact of a contamination event.

RESIDUAL GENETIC MATERIAL

In the antibody biomanufacturing process, antibodies are produced through expression in living cells, such as CHO cells. Genetic material from the cells, known as residual host cell DNA, is a common impurity that needs to be removed from the final drug product.

ResDNA from host cells is potentially hazardous if included in the final drug product because it can provoke a potentially life-threatening immune response in a patient. Beyond immunogenicity are risks of genetic mutations. “If DNA stays in the final product and then is injected into patients, it can cause issues with genetic integration,” explains Ilaria Scarfone, a field application specialist at Thermo Fisher Scientific. Genetic integration events can occur when resDNA insert into a cell’s genome through naturally occurring reverse transcription, which could lead to a cancer-causing mutation. “That’s why, apart from its quantity, you need to check the size of the DNA.”

Regulatory agencies require manufacturers to demonstrate that resDNA is significantly removed to below specified amounts during the purification of biopharmaceutical products.6 Research teams often use custom methods to test for residual genetic material, which are often costly and time intensive to develop. “Current testing methods are basically home brewing your own assay,” Scarfone says. “The biggest drawback is that this requires revalidation of components. You have to make your own standards. Development of this assay requires specialized expertise.”

More recently, scientists are adopting qPCR-based methods for residual genetic testing, complemented by testing kits. Thermo Fisher Scientific offers a series of Applied Biosystems™ resDNASEQ™ kits designed to quantify the presence of resDNA for many host cell species, including commonly used CHO cells. These commercially available tools simplify development and validation and ensure that technical assistance is available once an antibody therapeutic moves into production. “It’s much easier to validate a commercial test, and we can provide validation support for these commercial tests, but not the home-brewed method,” Scarfone says.

TESTING WITH CONFIDENCE

Quality testing is crucial in ensuring the safety and efficacy of mAb pharmaceuticals. Molecular methods are being adopted as an essential component of the testing process, enabling manufacturers to detect and quantify potential contaminants and ensure product consistency. Implementing these modern testing methods can simplify development and manufacturing, saving considerable effort and money over the life cycle of a biopharmaceutical product.

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CHAPTER 3

INNOVATIVE USES OF SINGLE-DOMAIN ANTIBODIES IN MAB THERAPEUTIC RESEARCH AND DEVELOPMENT

Scientists are using engineered antibodies in creative ways to solve challenges in antibody therapeutic research and development. In some cases, they are using V_HH fragments, which are single-domain antibodies that can be tuned to bind to a variety of biomolecules.[1]

Conventional antibodies have two copies of two different protein chains, a heavy chain and a light chain. In the late 1980s, an atypical variety of antibodies was discovered that contained only a heavy chain. The V_HH domain of these heavy-chain-only antibodies recognizes a binding partner. This V_HH domain can be engineered as an antibody fragment, producing single-domain antibodies with tunable binding sites (figure 1).[2]

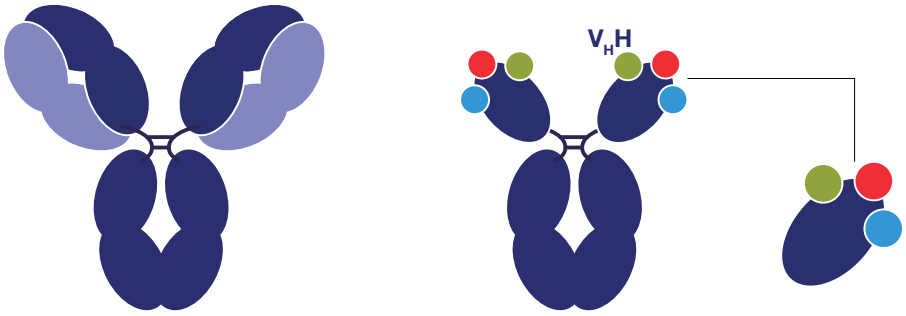


Figure 1: A conventional antibody (left) contains two different protein chains: a heavy chain (dark purple) and a light chain (light purple). There are also antibodies that contain only heavy chains (center). The top part of this antibody (V_HH) recognizes antigens at three sites (red, green, and blue dots). It can be separately engineered to create a single-domain antibody (right).
Credit: Thermo Fisher Scientific

Single-domain antibodies offer several advantages over conventional antibodies when used in research applications[2]:

Conventional antibodies require two heavy chains and two light chains to function, while single-domain antibodies require only a single protein. This simplifies production compared with conventional antibodies, which in turn reduces costs.

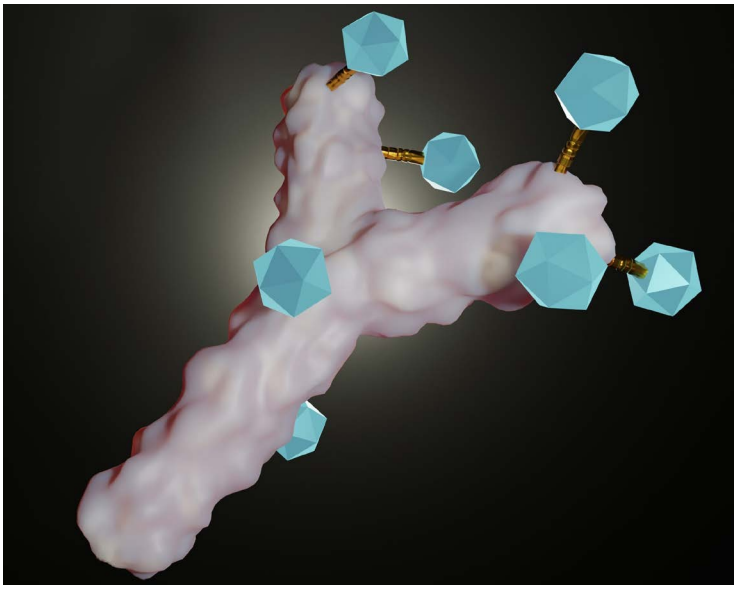
Single-domain antibodies are more pH and heat tolerant, which allows for their use in a wider range of conditions.

The molecular weight of a single-domain antibody is 12–15 kDa, about one-tenth the weight of a standard antibody. Its small size improves the single-domain antibody's ability to bind to antigen sites that are difficult for conventional antibodies to reach.[2]

There is significant interest in adapting single-domain antibodies into biotherapeutics.[3] Thermo Fisher's CaptureSelect affinity resins, described in the first chapter, make use of single-domain antibodies. Other uses for single-domain antibodies include assessing the stability of linkers in antibody-drug conjugates (ADCs), identifying metabolic bottlenecks in biomanufacturing, and measuring antibody-ligand binding interactions.

STABILITY TESTING THE LINKER IN ANTIBODY-DRUG CONJUGATES

ADCs are an emerging class of targeted cancer treatments. They use a mAb to deliver an anticancer drug directly to specific cells. Ideally, this targeted delivery maximizes the therapeutic impact on the cancerous tissue while limiting the effect on healthy tissue.



An antibody-drug conjugate, shown in this conceptual illustration, has three components: an antibody (white), a molecular linker (yellow), and a cytotoxic drug molecule (blue).
Credit: Love Employee/ Shutterstock

In practice, the stability of the molecular linker joining the mAb and the cancer drug can compromise the effectiveness of an ADC therapeutic. If the linker degrades while the ADC is circulating in the blood, the released cytotoxic drug could cause off-target side effects. Linker stability testing is a fundamental part of ADC development and often involves molecular tools customized for a given experiment's conditions.

A team of researchers wanted to develop a more universal method for analyzing the stability of an ADC linker. First, the researchers gave an ADC made with a human antibody to mice, rats, and cynomolgus monkeys—common animal models used for preclinical testing. They collected a serum sample from the animals. The researchers then separated the ADC from the animal-cell contents using a commercially available CaptureSelect™ single-domain antibody that recognizes a stable stem structure in common human antibodies. Finally, the team analyzed the purified ADCs with mass spectrometry to determine if a drug molecule cleaved from the antibody while circulating through the animal. This procedure was repeated over 11 days to assess the metabolic stability of the ADC. The stability results obtained with the new method were comparable to those from a common affinity assay.[4]

UNBOTTLENECKING ANTIBODY PRODUCTION

Cell culture platforms for mAb production are well established yet sometimes perform below expectations. Low yields compromise the overall performance of an antibody production process. Identifying the source of productivity issues by dissecting cellular function can be time consuming and resource intensive.

Cell cultures for antibody production grow in the controlled environment of a bioreactor.
Credit:
Thermo Fisher Scientific

A research team developed a CHO cell line that produced a bispecific antibody engineered to recognize two different targets. The researchers noticed that cultures of the engineered cell were not generating the expected amount of antibody. They confirmed that the first steps of protein production were happening at acceptable levels so then wanted to see if protein transport within the cell was causing a production bottleneck.[5]

The group added single-domain antibodies carrying a fluorescent tag to the engineered cells. These V_HH antibodies bind to the bispecific antibodies, marking them with the fluorescent label. The researchers used fluorescence microscopy to visualize the labeled antibodies and determine their locations at sites inside the cell. They found that the endoplasmic reticulum had high concentrations of the bispecific antibody, which implies that this organelle was the source of the protein transport bottleneck.[5]

According to the team, the results suggest that this automated microscopy technique could be applied to investigate other intracellular actions and improve the productivity of cell lines used in mAb biotherapeutics.

SURFACE MEASUREMENTS OF FAB-TARGET BINDING

Single-domain antibodies can also be used in surface binding experiments to monitor the binding of an engineered antibody and its target.

Researchers created a bispecific antibody fragment designed to bind two different cellular growth factors. They called this type of engineered antibody a dual-targeting Fab, or DutaFab. It can enable specialized therapeutic mechanisms, such as neutralizing two antigens simultaneously or bringing two proteins close together.

The scientists studying this DutaFab wanted to use surface plasmon resonance (SPR) to show that both growth factors bonded to the antibody at the same time.[6] First, they attached CaptureSelect™ Human Fab-kappa Kinetics Biotin Conjugate to the SPR chip. This bifunctional antibody fragment recognizes a domain of the light chain and a domain of the heavy chain of the engineered human antibody. The result was that the CaptureSelect fragment oriented the DutaFab away from the chip surface so that both growth factor binding sites were available to capture the targets.

The researchers introduced each growth factor and monitored binding to the DutaFab via surface plasmon resonance. The DutaFab bound each growth factor regardless of the order in which factors were introduced, demonstrating that the engineered antibody was binding each growth factor independently.[6]

FUTURE APPLICATIONS FOR SINGLE-DOMAIN ANTIBODIES IN MAB MANUFACTURING

Engineered antibodies are finding creative uses throughout the research, development, and manufacturing process for antibody-based therapeutics. The ability to tune recognition sites and quickly manufacture stable antibody fragments makes single-domain antibodies a promising tool for investigating details of stability, biomanufacturing, and binding of antibody therapeutics.

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