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DIVERSITY OF SINGLE-DOMAIN ANTIBODIES FOR PURIFICATION OF BIOTHERAPEUTICS AND ANALYTICAL ASSAYS

Biotherapeutics are on the rise, which has prompted a revolution in pharmaceutical manufacturing. These therapeutics are proteins or other molecules produced from a biological source. They can help the body fight infection, cancer, or other diseases and are a rapidly growing drug class.[1] Biotherapeutics now account for almost half of new drug approvals.[2]

The biological systems from which biotherapeutics are produced present challenges for large-scale manufacturing, purification, and testing. The biotherapeutic must be purified from a complex mixture, unlike small-molecule drugs that are chemically synthesized step by step in the laboratory. Additional challenges stem from the growing diversity of biotherapeutics. For example, researchers have developed viral vectors that deliver therapeutic genes into specific cells and tissues in the body to treat cancer, cystic fibrosis, and other diseases.[3] Antibody therapy has emerged as a particularly promising branch of biotherapy, including a spectrum of antibody types. Therefore, purification and assay methods that specifically capture diverse biomolecules are critical for developing new biotherapeutics.

To achieve this diversity, some researchers have honed in on V_HH antibodies, which are camelid-derived single domain antibodies that can be tuned to specifically bind to an enormous variety of biomolecules. This tunable specificity makes the V_HH antibody an ideal ligand to capture an ever-widening array of biotherapeutic types either as part of a purification protocol or as part of the greater manufacturing process in biotherapeutic-specific assays.

TUNABLE SPECIFICITY

Antibodies are typically composed of two heavy and two light chains that form a complex dimeric molecule, explains Mehdi Arbabi-Ghahroudi, a senior research scientist at Carleton University. Antibodies bind molecular antigens

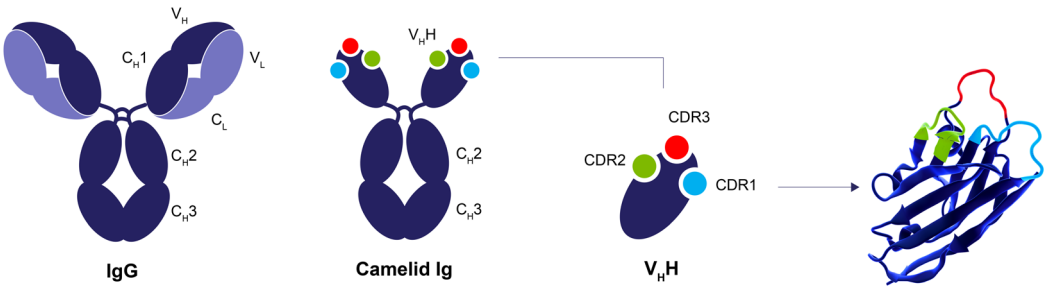

Figure 1: Conventional antibodies, such as immunoglobin G (IgG), are composed of a heavy chain (dark blue) and a light chain (light blue). Certain antibodies from camelid species lack the light chain and are composed solely of the heavy chain, yielding heavy-chain-only IgG. To generate the V_HH ligand, the camelid antibodies are pared down to focus on only those parts that are important for binding an antigen, which includes complementarity-determining regions (CDR1–3, colored). The V_HH ligands maintain high affinity and specific antigen binding while being one-tenth the size of traditional antibodies.

Source: Thermo Fisher Scientific

with a specificity that is mediated by variable regions of the heavy and light chains of the antibody dimer.

But a novel class of antibodies was discovered in the 1990s in camelids, a family of mammals that includes camels, llamas, and alpacas.[4] These camelid antibodies are composed of only two heavy chains, yet they maintain the ability to specifically and robustly bind antigens (Figure 1). Researchers pared the camelid antibody down to just the variable antigen-binding region to produce the V_HH fragment. Arbabi-Ghahroudi says V_HH antibodies are about one-tenth the size of traditional antibodies. Their small size and specificity allow V_HH antibodies to be used for multiple applications, including as ligands for biotherapeutic purification and assays.

V_HH ligands have characteristics, other than their small size, that make them well suited for affinity capture, according to Arbabi-Ghahroudi. “They can resist harsh environmental conditions, such as high or low pH, high temperature, detergents, and proteases,” he notes. The biggest advantage of V_HH ligands is their ability to bind diverse molecules. By modifying the variable region that comprises the antigen-binding domain, the binding specificity of a V_HH ligand can be selectively modified and “tuned” for different targets.

Pim Hermans, the director of ligand discovery at Thermo Fisher says the most important difference between conventional antibodies and V_HH ligands is that the latter can reach the same level of specific and high-affinity binding as the former, but with greater stability and robustness due to their small size and rigid structure. Their tunability and intrinsic stability can enable many purification and analytical applications that aren’t possible using conventional antibodies.

AFFINITY PURIFICATION

Manufacturing processes for biotherapeutics need to generate products of sufficient yield and purity. The yield and purity are typically achieved through

a series of chromatographic steps. These chromatographic steps may rely on the general physiochemical properties of the target molecule—such as its size, charge, and hydrophobicity—or may specifically capture the target molecule.

The specific-capture approach, called affinity chromatography, uses a ligand to specifically bind to the target molecule. The technique allows for the isolation of the biotherapeutic from the complex biological mixture used to produce it.

"A good affinity ligand will only capture your molecule of interest and the rest will wash away," says Hermans. That means that after just one step, researchers can have a sample that contains their target molecule in higher yield and purity than occurs with nonaffinity chromatography. This higher yield and purity can reduce the total number of chromatographic steps needed to achieve sufficient product purity.

Aled Charles, R&D manager for AstraZeneca in Cambridge, England, says diverse affinity ligands could help expand the portfolio of biotherapeutics being developed. Instead of focusing on molecules that have established purification protocols, researchers could use the ligands to better target and test more classes of biotherapeutics. Novel and diverse ligands based on camelid V_HH antibodies have expanded the repertoire of ligands available for affinity chromatography and helped researchers access more types of biotherapeutics (Figure 2).

Commercially available V_HH affinity ligands, some of which Thermo Fisher has developed, can be used for V_HH-based affinity purification. The firm's CaptureSelect™ V_HH ligands "provide simple and efficient purification processes," says Charles.

Another advantage of V_HH affinity ligands is they can be designed to target specific molecular forms out of a mixture of closely related conformational isoforms.[5] "It can be very difficult to separate molecular product forms that are identical sequence-wise, but that may have small differences in their structural conformation," Hermans says.

An example of V_HH ligands' targeting bioactive molecules is the purification of specific antibody derivatives. Traditional antibodies can be broken into various fragments, such as antigen-binding fragments (Fabs). These antibody derivatives are used as reagents, diagnostics, and therapeutics.

Non-V_HH affinity chromatography is commonly used to separate Fabs from undigested antibodies and the unwanted non-Fab fragments. The process requires optimization for each Fab but often leads to the capture of closely related nontarget impurities that result from the derivatization process, Charles says. His team uses V_HH affinity ligands to overcome these challenges.[6]

"We typically use CaptureSelect ligands for preparations of Fabs," Charles says. The ligands the group uses "ensures we do not capture contaminating non-

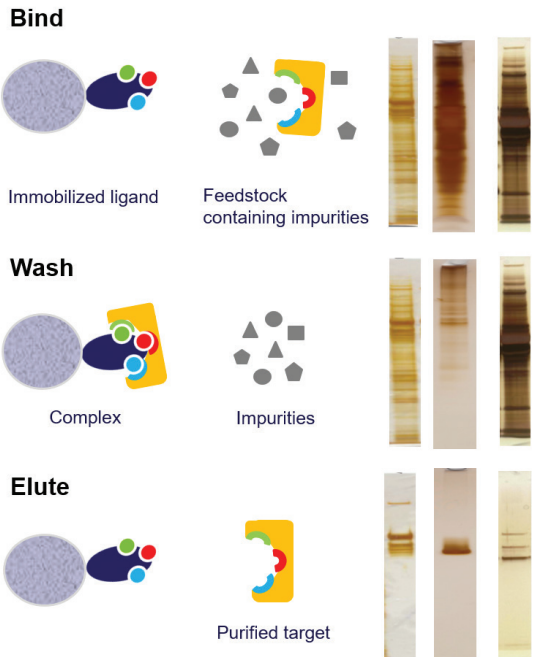

Figure 2: V_HH affinity ligands can be tuned to specifically capture a target biotherapeutic molecule from the complex biological systems in which it is produced. After affinity capture, impurities are washed away, and the target molecule can be eluted. This enriches the target biotherapeutic molecule in a single step. On the right, protein gel images show the effectiveness of using affinity capture for varied biotherapeutic targets: [From left to far right] FVIII (a blood-clotting protein), Fab (an antibody fragment), and AAV (a viral vector).

Source: Thermo Fisher Scientific

Fab antibody derivatives, such as light-chain dimers, and therefore simplifies our workflow." This efficient capture helps improve product purity and saves the time and labor needed with multiple processing steps, Charles adds.

AFFINITY CAPTURE ENABLES ANALYTICAL METHODS

V_HH ligands are also suited to high-throughput analytical methods because of their specificity, selectivity, small size, and stability. In recent years, these ligands have been used to develop better analytical techniques for biotherapeutics.

One way to expand the analytical application of V_HH ligands is by directly conjugating them to the vitamin biotin. The biotin moiety interacts with its binding partner streptavidin and this interaction can be used to either capture or detect the molecules selected for by the V_HH ligand (Figure 3). The biotinylated V_HH ligand can be immobilized to capture target molecules or it can detect target molecules already immobilized. Examples of both the capture and detection applications of biotinylated V_HH ligands include:

Testing biotherapeutic stability in serum

Antibody-drug conjugates (ADCs) are a class of biotherapeutics that have shown promise treating cancer, among other diseases.[7] ADCs are made by attaching

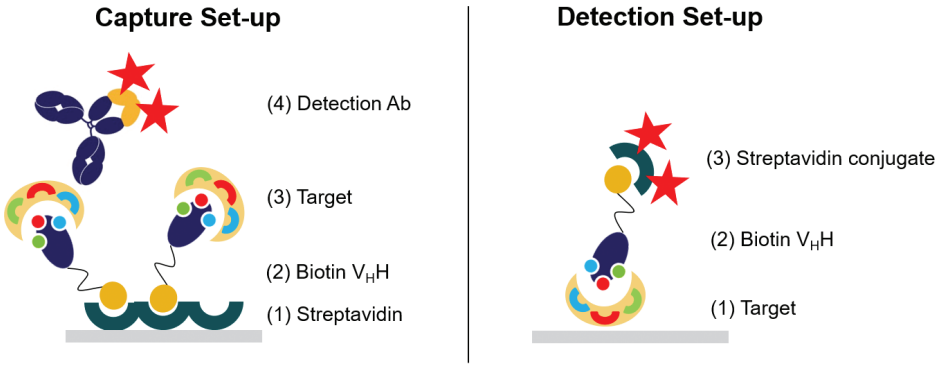

Figure 3: V_HH ligands can be biotinylated and used in a variety of analytical assays aimed at capture or detection of molecular targets. Improved assays and expanded selectivities can aid the development of diverse biotherapeutics.

Source: Thermo Fisher Scientific

a small therapeutic agent to a monoclonal antibody, which then targets the complex to a specific cell type.

However, the monoclonal antibody can detach from the drug prior to reaching the target cell, limiting efficacy and potentially triggering side effects. Therefore, researchers need reliable assays to monitor ADC stability during preclinical development.

One approach being explored to monitor ADCs is to use a ligand to capture the 'carrier' monoclonal antibodies from preclinical animal serum and assess if the therapeutic agent is still attached. In a study, researchers attached a CaptureSelect V_HH ligand—designed to be specific for the monoclonal antibody portion of an ADC—to magnetic beads. The ligand-bearing beads were added to an animal serum containing ADCs. The beads were then isolated from the serum using a magnet and the ADC was eluted from the V_HH ligand to assess whether the ADC was intact.[7]

Most notably, the V_HH ligand targets a region of the monoclonal antibody that stays constant.[7] That means this workflow can assay most ADCs in pre-clinical development regardless of the attached therapeutic agent.

Assessing and improving biotherapeutic production

Mammalian cell culture systems are often used to produce biotherapeutics. However, many of these systems fail to express ample protein, which increase production time and costs. Identifying the factors that contribute to low yield can help researchers design better cell lines for biotherapeutic manufacturing.

In one example, researchers struggling to achieve high yields of a biotherapeutic antibody from a mammalian cell line turned to a V_HH-based assay to help them track the way the antibody was formed and secreted by the cells. They used a CaptureSelect biotinylated V_HH ligand that specifically recognizes the light

chain of the therapeutic antibody. Fluorescent dyes were then used to visualize the biotinylated V_HH ligand location in the cell by microscopy.[8]

By using V_HH ligands to visually track antibody production and trafficking in the cell, the researchers identified the endoplasmic reticulum as a potential bottleneck hampering efficient production of the antibody.[8] These results could help scientists design a cell system to overcome these limitations and improve biotherapeutic yield.

Capturing and detecting viral vectors

Nonreplicating viral vectors have become highly valuable in gene therapy as a means to deliver genetic material to cells. Adenovirus-associated virus (AAV) vectors are widely used to attach to and enter target cells, resulting in stable expression of the therapeutic gene carried by the virus.[9]

Production and purification of AAV vectors are vital steps in manufacturing gene therapies, but production is an expensive and elaborate process with small yields typically. Analytical techniques with enhanced sensitivity are critical to helping prevent product loss.

The Gyrolab® AAVX Titer Kit includes a V_HH ligand to quickly determine AAV concentration data. Helena Nilshans, a senior product manager at Gyros Protein Technologies, says the ready-to-use kit can measure viral concentration throughout the manufacturing process, offering a simple way to monitor production and support process development for AAV vectors.

Nilshans describes the AAVX Titer Kit as a sandwich-style immunoassay in which a biotinylated V_HH ligand captures the AAV vector molecule. A separate V_HH AAVX ligand is then employed to detect the viral vectors that have been captured.

V_HH LIGANDS IN BIOTHERAPEUTIC DEVELOPMENT

The ultimate goal, according to Hermans, is to have established, broadly applicable purification and analytical processes that can be implemented across multiple classes of biotherapeutics rather than a process tailored to each individual molecule. V_HH ligands have already helped advance affinity purification and assay processes for diverse classes of biomolecules.

Hermans doesn't think that the V_HH ligands themselves will inherently change but that the diversity of selectivities will continue to grow and improve the purification of various types of biotherapeutics. He foresees designing V_HH ligands to address challenges with the purification of target molecules that have been elusive and points to viral vectors as an example.

Hermans also hopes that the V_HH ligand technology can be coupled with new methods to help produce faster or more selective assays. For instance, he says, the CaptureSelect ligands are already being used to design enzyme-linked

immunosorbent assays for novel biotherapeutic molecules, next to other platforms like Gyrolab and label-free biosensors. They can also be combined with high-pressure liquid chromatography applications to rapidly detect titers during process development of viral vectors.

"The ligands are already there," Hermans says. The challenge lies in modifying their selectivities and developing new detection assays to address the needs in the biotherapeutics market.

For more information, watch the webinar here.

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AMERICAN CHEMICAL SOCIETY
1155 Sixteenth Street, NW,
Washington, DC 20036