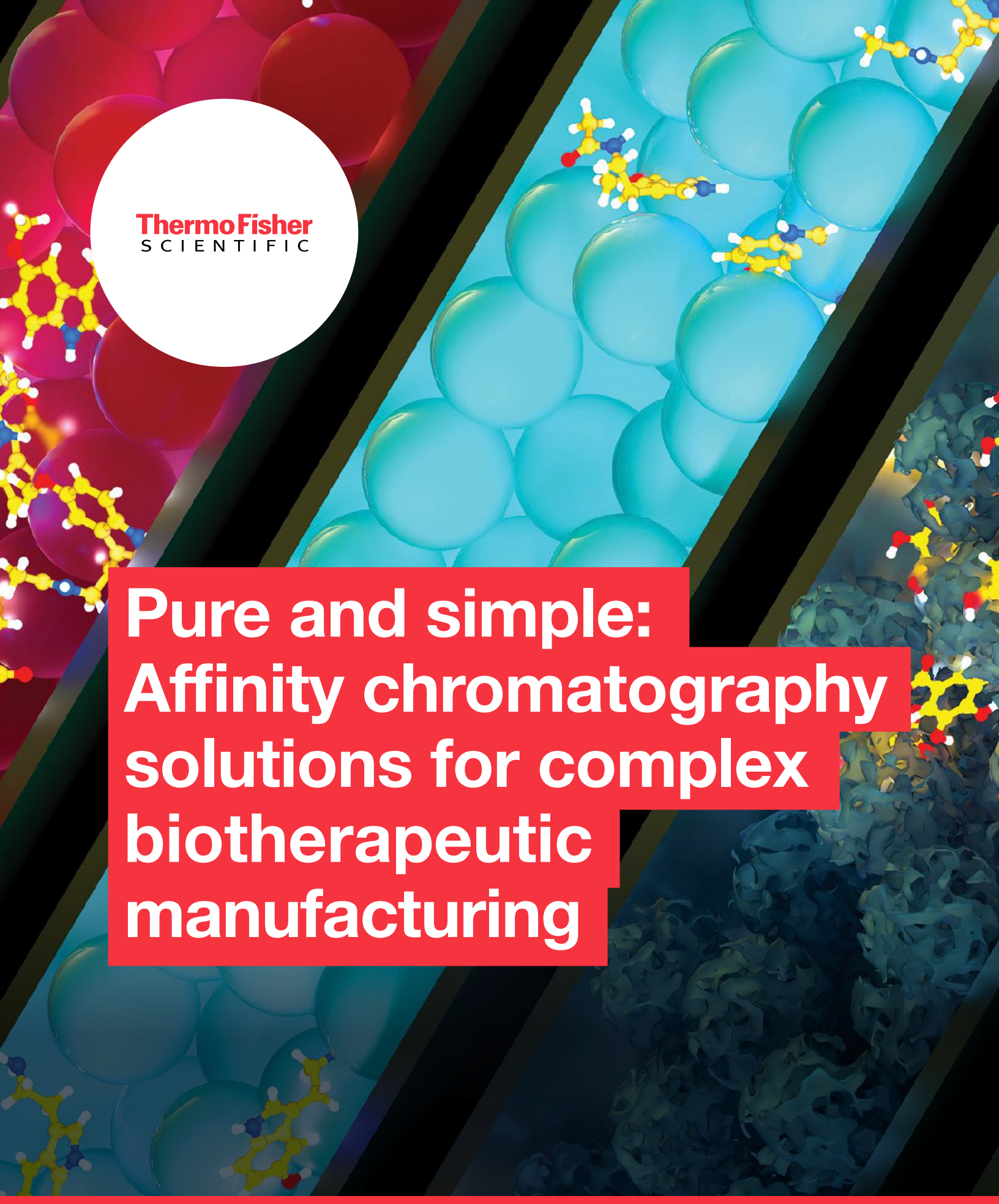

Pure and simple: Affinity chromatography solutions for complex biotherapeutic manufacturing

c&en BRANDLAB

Table of Contents

Introduction

Biotherapeutics are a powerful and expansive class of drugs with diverse applications. These therapeutics are proteins, messenger RNA (mRNA), or other technologies such as viral vectors produced from biological sources that can be used to help the body fight infection, cancer, or other diseases (figure 1).[1]

***Figure 1**. Biotherapeutics have expanded to include an increasingly diverse class of molecules, including viral vectors used in gene therapy, nucleic acids such as mRNA, and fragment antigen-binding (Fab) fragments.[2]*

The global biotherapeutic industry was valued at $285.5 billion in 2020, and analysts estimate global demand will increase by an average of 23% over the next decade.[3,4] In the US, biotherapeutics account for almost half of new drug approvals annually.[1]

The rise of biotherapeutics, and their expanding complexity, has prompted a revolution in pharmaceutical manufacturing. Demand for these treatments exceeds companies' manufacturing capabilities, driving the need for effective production processes.[4] Along the manufacturing pipeline, efficient purification strategies are one of the many things that can help improve the processes.[4]

Biotherapeutic substances need to be isolated from the complex biological systems in which they're made, which can take multiple steps.[4] Yet yield losses accumulate with each process step, highlighting the need for quick, efficient purification schemes (figure 2). Furthermore, purification strategies should be adaptable and sufficiently nimble to meet the manufacturing needs of each biotherapeutic.

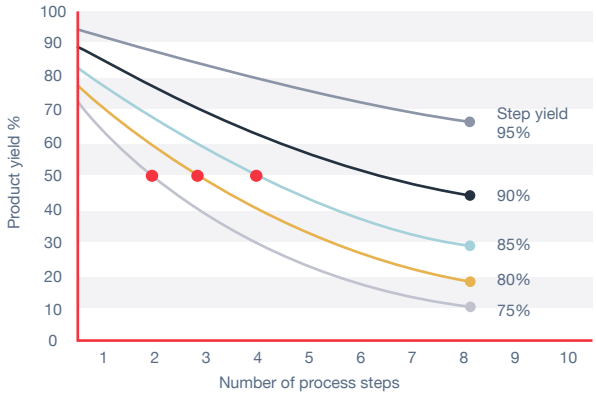

***Figure 2.** Each step in purification results in product yield losses, with yields rapidly decreasing as the number of steps increases. Minimizing the number of process steps needed to achieve pure product is critical to increase efficiency and yield.[5]*

Affinity chromatography is a powerful solution providing high-purity purification. By taking advantage of specific interactions between a ligand and the target molecule, affinity purification captures the target at high purity in a single step, which helps reduce the total number of steps in the downstream process (figure 3).

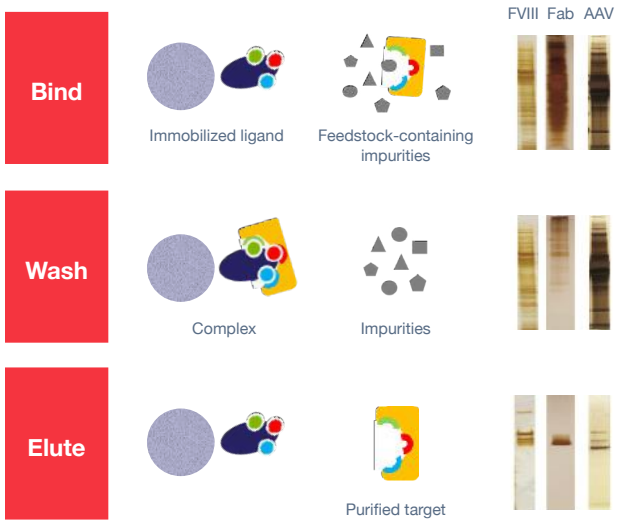

Figure 3: *Affinity ligands are designed to specifically capture target molecules from the complex biological systems in which they are made. After affinity ligands are captured, impurities are washed away and the target can be eluted, enriching the target molecule in a single step. Protein gels, on the right, demonstrate the effectiveness of using affinity captures for varied therapeutic targets: from left, an antibody, an antibody fragment (Fab), and an AAV.*[1]

Many biotherapeutics lack established affinity purification options, however. Tunable affinity ligands, such as those used with Thermo Fisher Scientific's CaptureSelect™ affinity resin technology, open the door to new purification possibilities for complex biotherapeutics.

These ligands are structurally derived from a heavy-chain-only antibody found in camelids. Camelid antibodies lack the light chains found in typical antibody heavy- and light-chain dimers but still robustly and specifically bind antigens (figure 4).[4]

To develop CaptureSelect™ technology, Thermo Fisher bioengineered the camelid antibody to contain just the variable antigen-binding region, called the V_HH fragment (figure 4). The fragment's variable antigen-binding region can be modified, enabling researchers to tune the ligand to target virtually any protein, antibody, or viral vector (figure 4). Additionally, the CaptureSelect™ ligands are produced in an animal-free system that relies on baker's yeast; all resin materials come with regulatory support documents, allowing for their use in clinical and commercial manufacturing.[2]

Affinity chromatography can also benefit large-scale manufacturing of synthetic genetic material, including mRNA-based biotherapeutics.

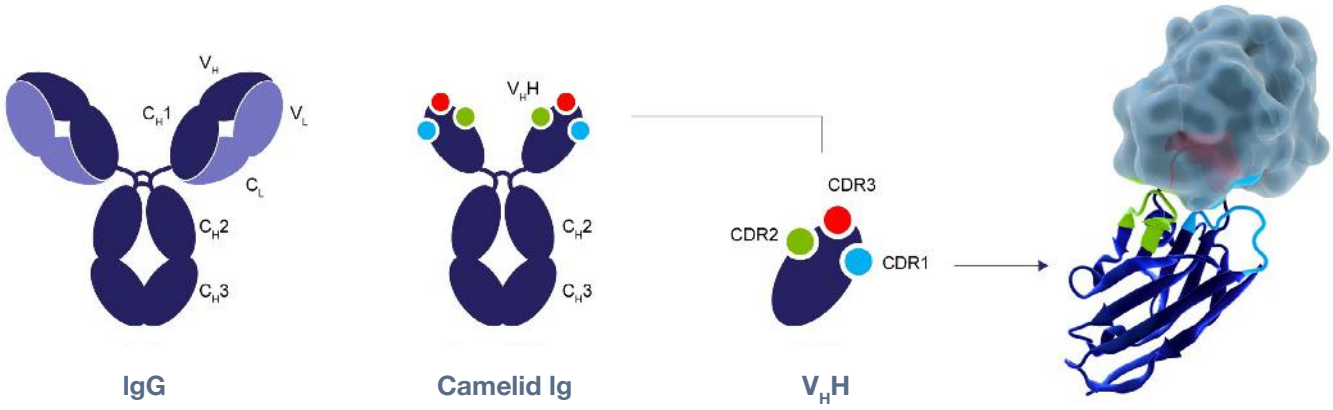

Figure 4*. Conventional antibodies, represented by immunoglobin G (IgG), comprise a heavy chain (dark blue) and a light chain (light blue). Certain antibodies from camelid species are composed solely of the heavy chain, yielding heavy-chain-only camelid Ig. V_HH ligands are a pared down derivative of camelid antibodies which contain only the structures important for antigen binding. These include the complimentary-determining regions (CDR1–3, colored). The V_HH ligands are a 10th the size of traditional antibodies but maintain high affinity and specific antigen binding.*[1]

Conserved features are exploited to develop specific and broadly applicable affinity resins, such as Thermo Fisher Scientific's POROS™ Oligo (dT)25 affinity resin.[6]

Affinity chromatography resins can be reused, increase overall product yields, and streamline processes—all contributing to a good return of investment when comparing price per resin to gram of purified drug product.[7]

Affinity chromatography has and will continue to provide a robust means of simplifying downstream large-scale manufacturing processes and help a biotherapeutic production meet demand. This e-book will explore using affinity purification as a way of improving biotherapeutic manufacturing with specific focus on antibody-based therapeutics (chapter 1), mRNA and vaccines (chapter 2), viral vectors (chapter 3), and custom affinity solutions (chapter 4).

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Chapter 1

Affinity solutions to accelerate antibody therapeutic development

Monoclonal antibodies have become the dominant class of biotherapeutics since the first treatment was approved by the US Food and Drug Administration over 3 decades ago.[1] These biologics, which are lab-grown versions of our body's immune system defense mechanism, can be used to treat disorders ranging from cancer to infection.[2]

They now account for almost a fifth of the FDA's annual new drug approvals, and the global monoclonal antibody segment is expected to generate $300 billion in revenue by 2025.[2,3]

Antibody engineering has evolved dramatically in recent years.[1] Complex alternative antibody formats such as Fab fragments, bispecific antibodies, and antibody-drug conjugates have been developed as promising next-generation biologics (figure 1).[2] Yet these novel formats face manufacturing and processing challenges that could limit their impact.

Historically, monoclonal antibody production has benefited from a robust and scalable purification approach based on protein A, a bacteria-derived affinity ligand. Protein A binds to the invariable fragment crystallization (Fc) region of monoclonal antibodies at the interface of constant heavy chains 2 and 3 (CH2 and CH3), making this purification scheme broadly applicable for this class of biologic.[2]

However, next-generation antibody-based therapeutics rely on novel structures in which the CH2–CH3 interface of the Fc region may be masked or absent, rendering protein A ineffective (figure 1).[2] Non-affinity purification approaches are possible but must be customized to individual antibody formats, increasing process development times and costs.[4,5]

Affinity ligands that instead target specific human antibody subdomains are viable alternatives. Ligands coupled to resins, such as Thermo Fisher's CaptureSelect™ suite, can be used to capture advanced antibody therapies by targeting the CH1 or CH3 constant domains or the kappa and lambda light chains (figure 2).

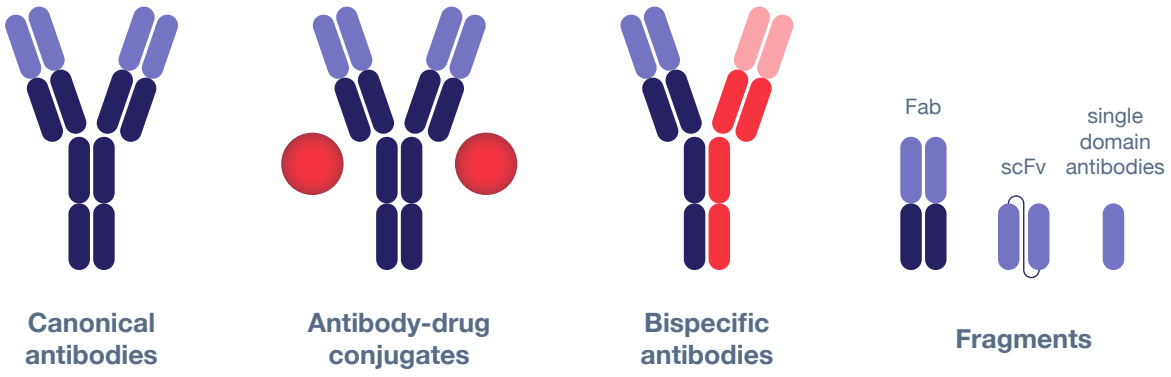


***Figure 1**. Canonical antibodies have dominated the biotherapeutic industry, but novel antibody formats including antibody-drug conjugates, bispecifics, and fragments have emerged and are being developed rapidly. Fragments have a variety of structures, including Fabs, single-chain variable fragment (scFv) constructs, and single domain antibodies.[3]*

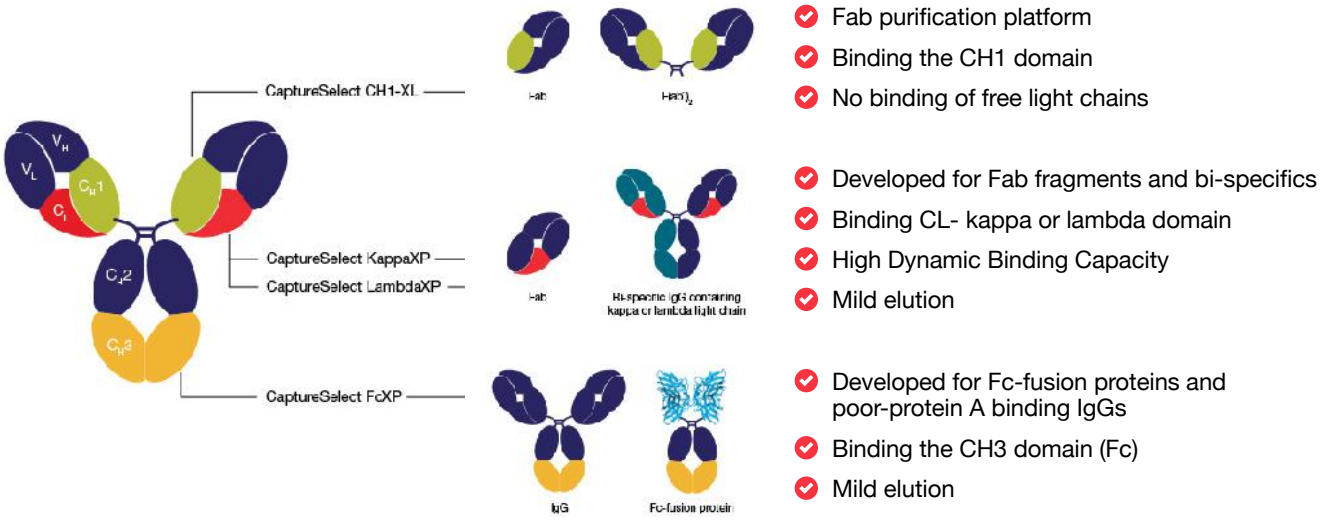


***Figure 2**. The CaptureSelect™ antibody resins have their own unique binding sites on the antibody molecule, allowing for targeted purification of various antibody formats.[4,6]*

Versatile affinity ligands can improve manufacturing and processing of novel antibody formats by increasing the purification efficiency and yield for therapeutic development and clinical trials, and ultimately to meet the needs of patients. These specific examples highlight how the ligands can aid bioprocessing:

- Fabs, antibody fragments that bind antigens, have both therapeutic and diagnostic applications, but there's no universal affinity approach to the purification of Fabs, since they cannot be purified using protein A. In addition, correctly assembled Fabs often need to be isolated from light-chain dimer impurities, which is a challenging impurity to remove. Researchers from a large biopharmaceutical company wanted to find an affinity matrix to isolate correctly assembled Fab biologics contaminating free light chains. They tried Thermo Fisher's CaptureSelect™ CH1-XL Affinity Matrix, which binds to the CH1 domain from correctly assembled Fab fragments but not to free light chains. The approach efficiently purified Fab fragments in a single step (figure 3).[7]
- Health-care company Roche developed a novel antibody format therapeutic, which didn't bind well to its standard antibody

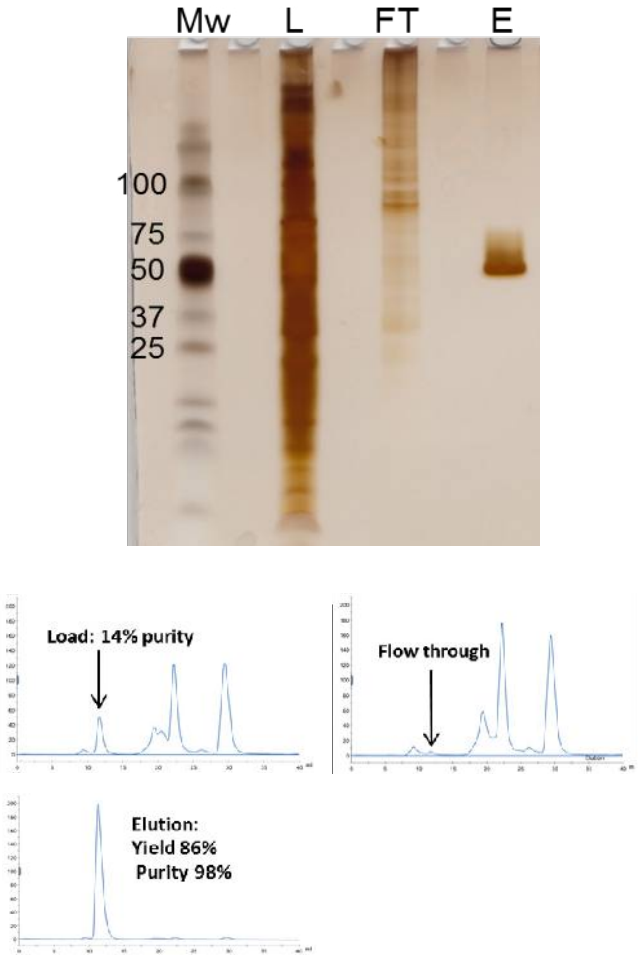
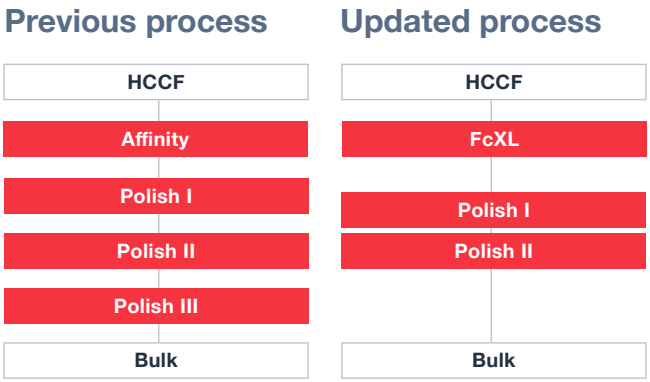
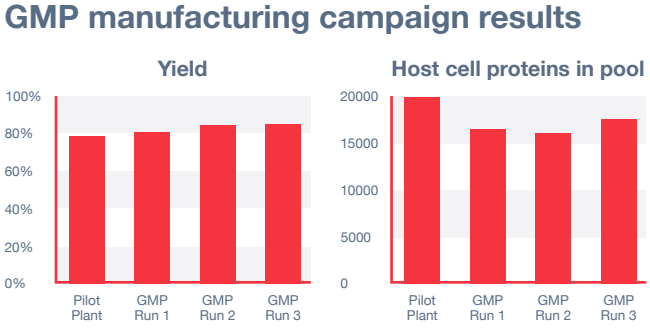


***Figure 3**. CaptureSelect™ CH1-XL Affinity Matrix was used to develop a Fab fragment purification process that delivered high purity and high yield in a first capture step. Mw (Molecular weight), Load (L), Flow through (FT), E (Elution).*

purification protocol using protein A. In early-stage development, the company used a separation strategy that involved a light-chain affinity resin for the initial capture, followed by three additional chromatography steps.[2] To improve the efficiency and yield of the purification scheme for late-stage development and commercial manufacturing, researchers investigated four alternative capture resins (three affinity resins and one nonaffinity resin), including the CaptureSelect™ FcXL Affinity Matrix. Compared with the light-chain affinity resin and the other resins, FcXL resulted in an eluate with higher purity and lower levels of both high- and low-molecular-weight impurities.[2] Furthermore, the FcXL resin was compatible with a higher load capacity. Clinical and commercial manufacturing requires scaling up the purification process. Results of column scaling were highly compatible with pilot-scale experiments, which confirmed that the resin could be easily scaled and implemented in the manufacturing process. In addition, further optimization of the capture step resulted in a reduction of chromatographic steps from four to three (figure 4).[2]





Advantages

- Moved from a four-step to a three-step chromatography process
- Higher binding capacity (improve facility fit)
- Improved impurity profile (no LMWs)
- Better pool stability (pH 4.2)
- Excellent scalability
- Environmentally friendly regeneration solution

Figure 4. *The CaptureSelect™ FcXL Affinity Matrix helped reduce overall processing times of the monoclonal antibody biotherapeutic ranibizumab downstream process with consistent yield and high purity.*[2, 4]

Reliable purification processes help ensure consistent production of antibody-based biologics, driving their viability as more cost-effective therapeutics. Next-generation antibody-based biotherapeutics require novel solutions, such as those provided by the CaptureSelect™ affinity resins, for efficient biomanufacturing processes and accelerated commercial release.

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Chapter 2

Pushing mRNA therapeutics and vaccine development forward with creative affinity solutions

A new therapeutic paradigm was ushered in when mRNA technology first demonstrated success 3 decades ago.[1] Today, mRNA-based therapeutics are rapidly emerging as effective immunotherapies, cancer treatments, and vaccine components. The global mRNA vaccine and therapeutic industry was estimated to be $9.41 billion by the end of 2021 and is forecast to rise to $15.49 billion by 2026.[2]

In particular, the vaccines made headlines after they were developed to fight SARS-CoV-2 infection and put the spotlight on large-scale mRNA and vaccine manufacturing.[1] Traditional methods of synthetic mRNA purification may not be suited to producing the quantities of material needed for large-scale manufacturing (figure 1).[1]

Rapid commercialization of mRNA therapeutics, including vaccines against SARS-CoV-2 infection, has put the spotlight on large-scale commercial mRNA manufacturing. Clinical-grade purification solutions are needed to maximize workflow efficiency and meet production demands.

Affinity chromatography offers a solution. Resins designed to effectively capture mRNA based on conserved features, such as the polyadenylated mRNA tail (poly-A tail), can be broadly applied as large-scale purification approaches for mRNA-based therapeutics.[3] For example, Thermo Fisher's POROS™ Oligo (dT)25 affinity resin allows for simple capture of mRNA based on A:T base pairing with the poly-A mRNA tail and poly-T affinity ligand (figure 2).

Methods of RNA purification

Method	Advantages	Disadvantages
Reversed Phase	• High resolution • Some selectivity for product impurities	• Limited column capacity • Use of expensive/ flammable/toxic chemicals • Column fouling impacts resolution
Ion Exchange Chromatography	• Native purification possible • Scalable	• Column capacity and recovery (HPLC) • May need toxic chemicals for denaturation • Purified product can contain traces of elution salts
Size Exclusion Chromatography	• Native purification possible	• Separation efficiency affected by alternative folding • Flow limited
HIC	• Native purification possible • Scalable • Replacement for Reversed Phase	• Non selective
Affinity Chromatography	• Native purification possible • Scalable • Platform solution for wide range mRNA molecule sizes – selective to polyA	• Requires additional polishing step to remove product-related impurities

***Figure 1**. Synthetic mRNA can be purified using a variety of methods, many of which introduce challenges when scaling up to large-batch processing. Affinity chromatography can provide a solution to help researchers meet manufacturing demands.[1]*

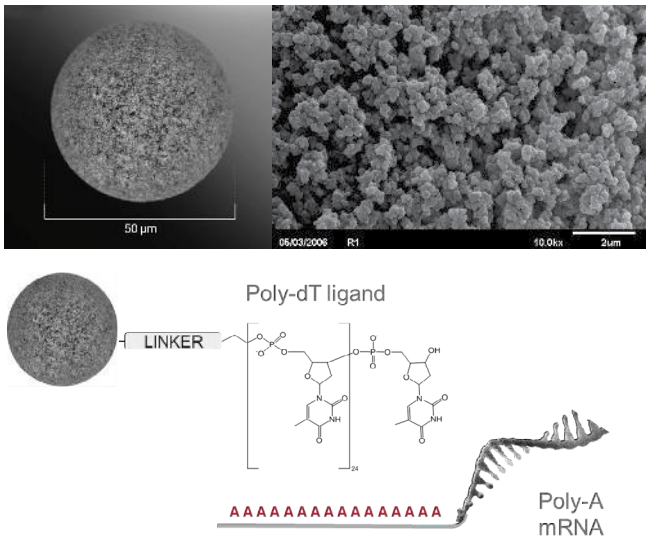


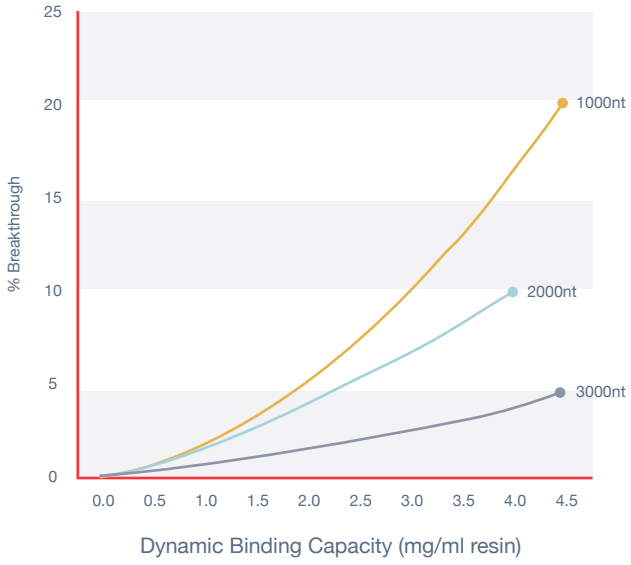
Figure 2. *Thermo Fisher Scientific POROS™ Oligo (dT)25 affinity resin is composed of a 50 µm poly(styrene-co-divinylbenzene) porous base bead with a (poly-T) 25-mer conjugated to the surface for efficient capture of poly-A mRNA. The top panel shows scanning electron microscope images of the POROS™ resin bead, with a magnified image showing the through-pores on the top right. The bead size and through-pores increase surface area and increase binding capacity while also separating out the target biomolecule from impurities. The bottom panel depicts the A:T base binding interaction used to capture mRNA.[1,4]*

Resins based on rigid beads, such as the POROS™ beads from Thermo Fisher Scientific, provide a linear scalable solution from bench top purification all the way through commercial manufacturing. Furthermore, resin bead through-pore and particle size can influence purification profiles and separation from impurities. The POROS™ bead has large through-pores and a small size, which enhances elution and separation profiles (figure 2).[3]

The size of a target mRNA molecule will vary based on the biotherapeutic application. Researchers analyzing the POROS™ Oligo (dT)25 affinity resin found that it provided suitable binding capacities, regardless of mRNA size, and consistent recoveries above 90% (figure 3).[1]

Affinity resins designed with mRNA in mind can improve processing scale and efficiency, accelerating clinical development. As highlighted by the rapid development and deployment of SARS-CoV-2 vaccines, large-scale mRNA and vaccine production is essential to protecting against disease.[1,4]

mRNA Binding Capacity



Recovery at different mRNA molecule sizes

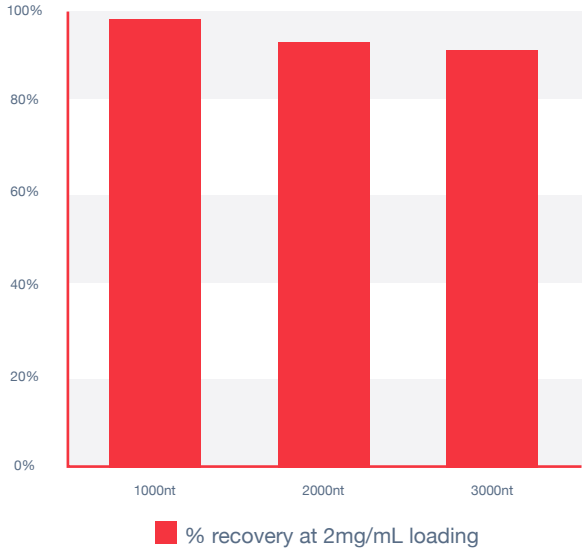
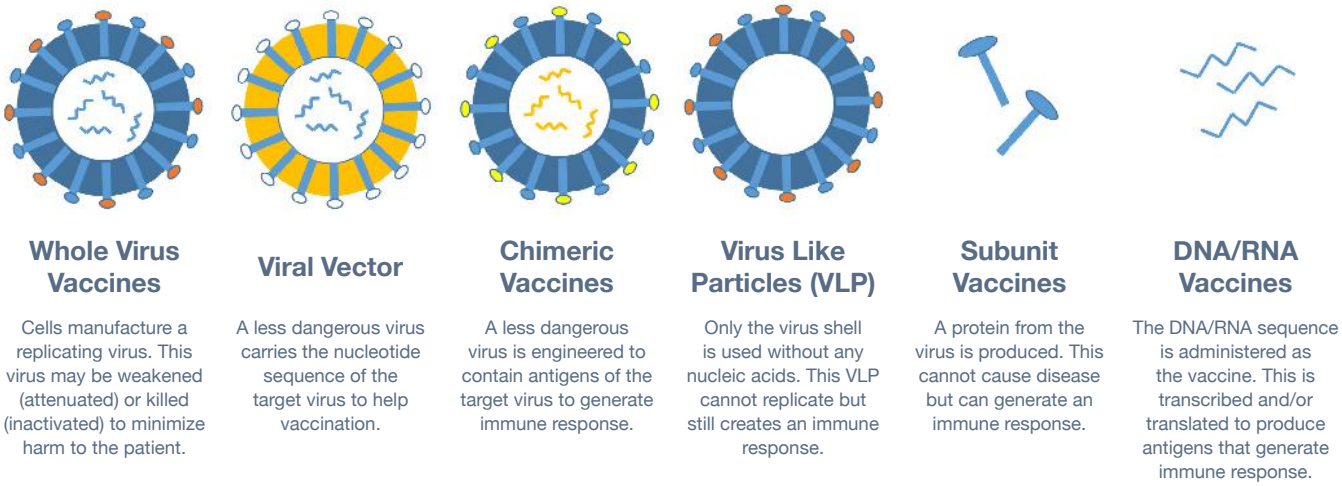


Figure 3. *Smaller mRNA molecules have higher binding capacity (left), partially because of size limitations and steric hindrance on the resin bead surface with larger molecules. Size does not impact final recovery (right), however, with recovery rates of over 95% for all three mRNA molecular sizes tested.[1]*



Whole Virus Vaccines

Cells manufacture a replicating virus. This virus may be weakened (attenuated) or killed (inactivated) to minimize harm to the patient.

Viral Vector

A less dangerous virus carries the nucleotide sequence of the target virus to help vaccination.

Chimeric Vaccines

A less dangerous virus is engineered to contain antigens of the target virus to generate immune response.

Virus Like Particles (VLP)

Only the virus shell is used without any nucleic acids. This VLP cannot replicate but still creates an immune response.

Subunit Vaccines

A protein from the virus is produced. This cannot cause disease but can generate an immune response.

DNA/RNA Vaccines

The DNA/RNA sequence is administered as the vaccine. This is transcribed and/or translated to produce antigens that generate immune response.

Figure 5. *Although they vary in composition, all vaccines include at least one molecular antigen or nucleic acid sequence encoding an antigen capable of generating a specific immune response. Each vaccine modality comes with different manufacturing and processing challenges.*[4]

When specifically considering vaccines, however, mRNA is only one of many modalities. Although their composition varies, vaccines include at least one molecular antigen or piece of genetic material encoding an antigen that elicits a specific immune response (figure 5).[4]

Each vaccine modality requires a unique manufacturing process—a level of customization that can be time consuming and costly. Innovative solutions that enable high-quality purification of vaccine components are critical to vaccine development, approval, and use.[4]

Affinity purification can be used to develop tailored processes for various vaccine modalities. But some modalities, such as virus like particles and protein subunits, aren't amenable to off-the-shelf options. For these vaccine antigens, a short peptide tag can be added to the C-terminal region of the protein to assist purification and help avoid the need for custom affinity resins. Tag-based affinity capture can streamline vaccine development both during early-stage screening of multiple candidates and at later stages of large-scale purification.

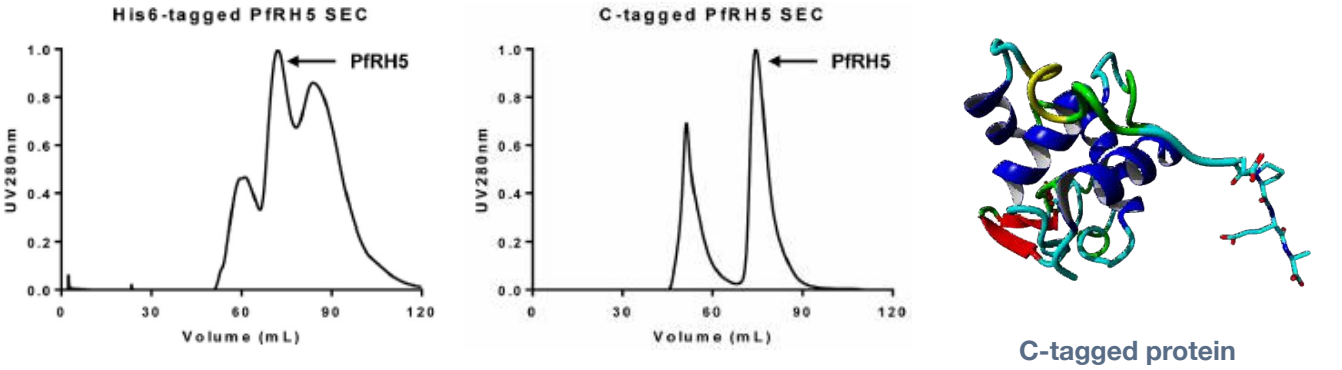


Figure 6. *Comparison of purification of Plasmodium falciparum reticulocyte-binding protein homolog 5 (PfRH5) antigens using a His-tag with nickel-based affinity resin (left) or C-tag with CaptureSelect™ C-tag affinity resin (right). The absorbance chromatograms of size-exclusion chromatography (SEC) eluents were used to assess PfRH5 elution and purity. The use of a C-tag and the corresponding C-tag resin yielded higher purity and an increase in process yield of almost 70% compared with His-tag and nickel-based column purification.*[5]

For example, researchers at the Oxford University's Jenner Institute were working to develop a vaccine against malaria, a potentially deadly infection caused by Plasmodium parasites.[4,5] They were originally using a histidine tag (His-tag)—a string of histidine residues that binds nickel-based affinity resin—to purify their recombinant protein antigen, but they were having issues with product quality and yield.[4,5]

They then switched to a C-tag—a four-amino-acid sequence added to the C-terminus of the recombinant protein—and a corresponding CaptureSelect™ C-tagXL resin designed to capture C-tagged proteins. The change resulted in increased yield and purity (figure 6).[4,5] Regulators approved the malaria vaccine with the short C-tag left on the licensed protein product, which reduced the need for additional cleavage steps.[4] The approval of a tagged vaccine was largely unprecedented, and opens the door for other vaccine developers to pursue a similar regulatory pathway.

Tagging isn't practical for all vaccine modalities, including viral vector and whole virus vaccines. Alternative nonaffinity approaches to purification can lead to lengthy processing times, progressive yield losses, and poor purity. To address these gaps, resin manufacturers such as Thermo Fisher have developed affinity resins. Viral vector affinity ligands will be discussed further in chapter 3.

The success of mRNA-based vaccines against SARS-CoV-2 has highlighted the utility of mRNA technology and spurred development of myriad therapeutics based on it. Effective affinity chromatography solutions can mean biomanufacturing of vaccine components and mRNA therapies occurs with ease and can help get these life-saving therapies to the clinic more quickly.

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Chapter 3

Improving viral vector purification to accelerate gene therapy

Gene therapy is ushering medicine into a new frontier.[1] This promising treatment modality involves strategically delivering a gene or piece of genetic material into patient cells to replace disease-causing genes, help the body fight disease, and more.

The FDA has approved two gene therapy drugs since 2017; that number is expected to increase to 10 to 20 by 2025.[1] Worldwide, gene therapy is projected to grow to a $6.21 billion industry by 2026 from $393.35 million in 2018.[2]

Progress may be hindered by manufacturing bottlenecks, however. Gene therapies rely on vehicles—most commonly noninfectious viral vectors—to deliver genetic material to target cells.[3] Recombinant AAV has become the vector of choice for many gene therapy applications: the number of AAV gene therapy Phase I clinical trials rose from 180 in 2020 to 254 in 2021.[4]

Large-scale production of high-yield, high-quality viral vectors is crucial to meeting industry needs as the gene therapy field continues to expand. Biotherapeutic manufacturers don't have the capacity to produce enough gene therapy solutions to meet current demand.[5]

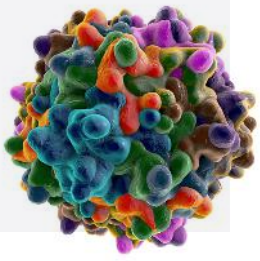
AAV manufacturing is typically labor intensive and time consuming. Traditional purification approaches can create challenges when a producer is scaling up to large-batch manufacturing, including lengthy processing times, higher costs, or culminative yield losses (figure 1).[3]

Affinity chromatography with resins specifically designed to capture AAV vectors, such as Thermo Fisher's POROS™ CaptureSelect™ AAV resins, have enabled a paradigm shift in viral vector purification by reducing the number of process steps, which can improve yield.

Resins may be specific to AAV serotypes or more general. For example, the POROS™ CaptureSelect™ AAVX resin can capture a broad variety of AAV serotypes making it suitable as a scalable purification platform for AAV vectors. (figure 2).

AAV vector advantages

- Non-pathogenic / non-toxic
- Ability to infect nondividing and dividing cells
- Persistence of the virus
- Many available seroptypes
- Cost effective to manufacture with a simple vector design
- Highly precise and specific target cell delivery



AAV Downstream Process Challenges

- Multiple upstream platforms with different load and impurity profiles, rapidly improving titers
- Increased impurity burden due to cell lysis
- Cumulative yield losses with each unit operation and complex analytics
- Lack of platform process to enable a quick-to-clinic strategy for multiple serotype and transgene combinations

Figure 1. *AAV vectors have become the vehicle of choice for many gene therapy applications. However, there are significant processing challenges that limit the large-scale manufacturing needed to meet demand.*[3]

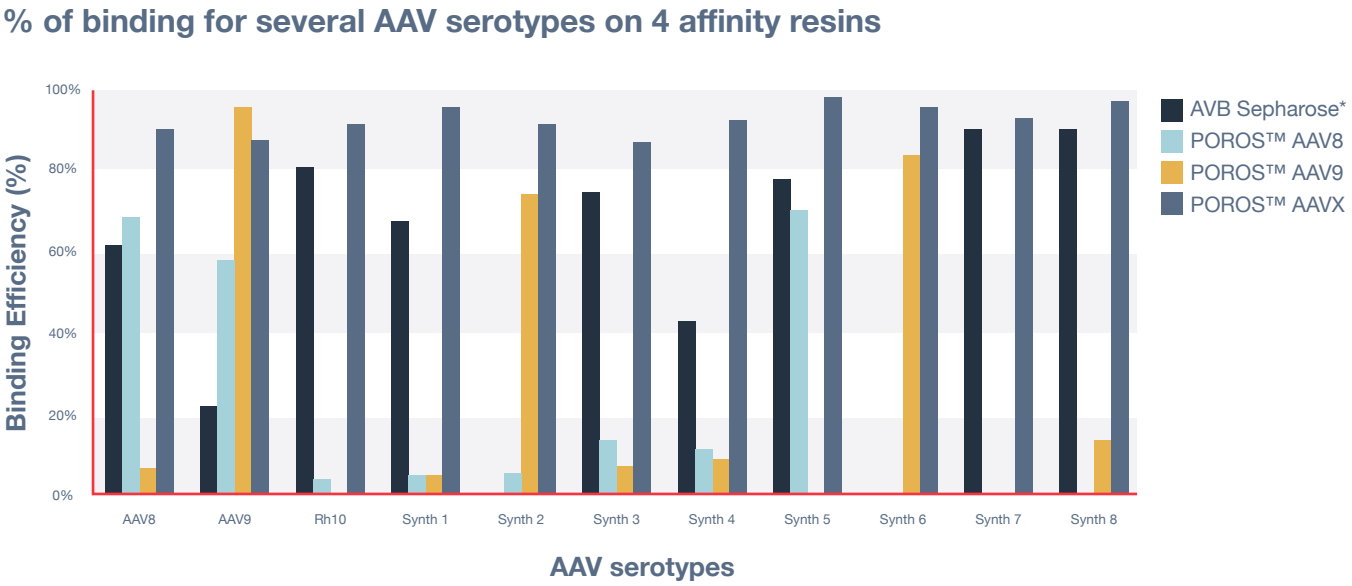
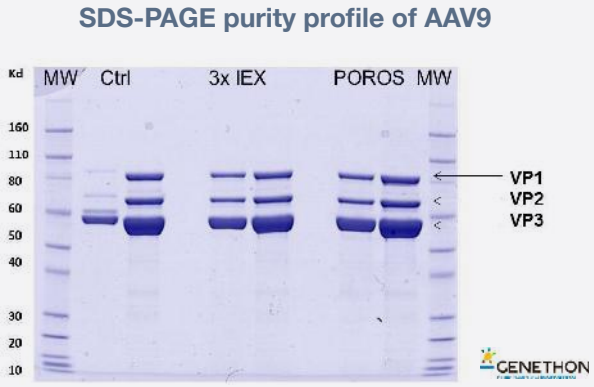


Figure 2. *To date, Thermo Fisher's POROS™ CaptureSelect™ AAVX ligand has shown affinity toward all AAV serotypes tested, as well as equal or better binding efficiency than alternative commercially available AAV ligands.[6] *AVB Sepharose is made by Cytiva.*

Case study:

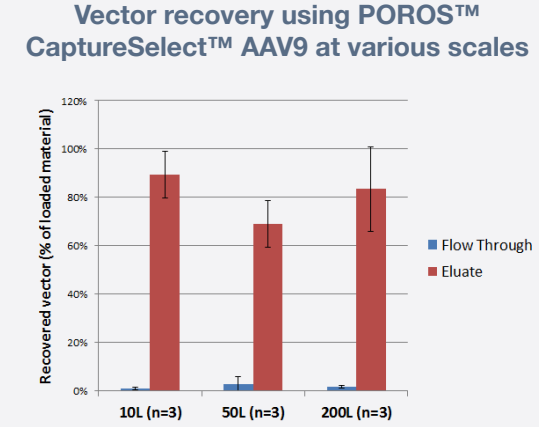
Genethon, a nonprofit organization in France working to develop gene therapies for people with rare diseases, was trying to isolate AAV9 for development in conjunction with treatments. Researchers adopted the POROS™ CaptureSelect™ AAV9 affinity resin to improve their processing times and found that AAV9 purity after a single affinity step equaled that of three steps of ion exchange chromatography (figure 3). Additionally, their vector yield was improved by

Purity



Equivalent purity profile as conducting 3 steps of IEX chromatography

Yield



Improved yield: ≥ 80% vector recovery

Figure 3. *Genethon researchers incorporated POROS™ CaptureSelect™ AAV9 affinity resins into their purification process and found one step of affinity purification to be equally effective as three steps of ion-exchange (IEX) chromatography (left panel). Vector recovery was consistently high regardless of input volume (right panel).[3]*

80% across all starting volumes tested. Purity, yield, and processing times were improved from the previous process.[3]

With robust and reliable AAV-affinity resins, biomanufacturing challenges can be overcome to meet industry needs and to spur further development of revolutionary gene therapies.

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Chapter 4

Unlocking the future of affinity purification with custom resins

The pipeline of global biologics is incredibly diverse (figure 1). AAV-enabled gene therapies, mRNA-based technology, and noncanonical antibodies are rapidly emerging as next-generation biotherapeutics.

The growing variety of therapeutic modalities has exposed gaps in available chromatography products. While affinity solutions for a number of molecules have been developed, off-the-shelf solutions still don't exist for modalities such as enzymes, hormones, and therapeutic proteins. Resins, if available, may lack the specificity or scalability needed to develop high-purity, high-yield, and cost-effective processes.[1,2]

These challenges drive the need for custom affinity chromatography resins designed to match the specific requirements of each biologic, such as those offered by Thermo Fisher (figure 2).[1]

Affinity ligand design is critically important in custom resin development: the ligand must bind the target molecule selectively, specifically, and reversibly. Affinity ligands can be identified by screening the target biomolecule against existing or customized libraries.[1,2]

For example, Thermo Fisher uses single-domain V_HH antibody fragments as part of its CaptureSelect™ affinity ligand suite (figure 3). Molecules can be screened against existing V_HH libraries, or custom libraries can be created for the requested target molecule.[2]

Next, ligands will undergo high-throughput screening to identify leads with appropriate specificity, binding kinetics, and stability. The top leads will be immobilized on a solid support to create affinity resin prototypes, which are tested to see how they perform during a trial purification.

2021 pipeline of global biologics

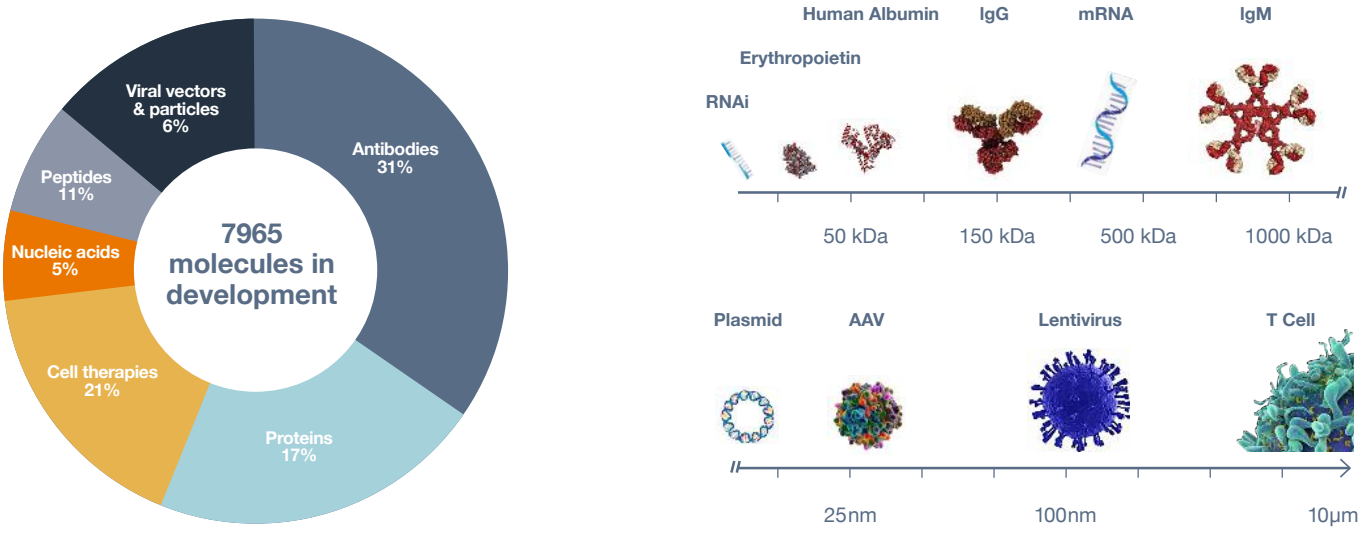


Figure 1. *Biomolecules in clinical development pipelines are increasingly diverse and range in composition and size. These new molecule modalities have created purification challenges that drive the need for customizable tools.[2]*

Why pursue a custom resin?

- No suitable chromatography resin exists
  - Either affinity or polish
- Current resin lacks specificity
  - Available chemistries do not achieve capacity or resolution requirements
- Current process is not scalable
  - The current purification process is not scalable or cost efficient due to low yield or purity
- Molecule requirements
  - Available resin chemistries restrict the design space or limit operation conditions
- Improve process efficiency and reduce COGs
  - Reduce steps in the chromatography process by introducing a high yield and high purity step

Figure 2. *Beyond lack of viable off-the-shelf options, there are many reasons to pursue a custom affinity resin. Custom resins can accelerate development by improving processes and reducing cost of goods (COGs).*[2]

CaptureSelect Affinity Resin Development

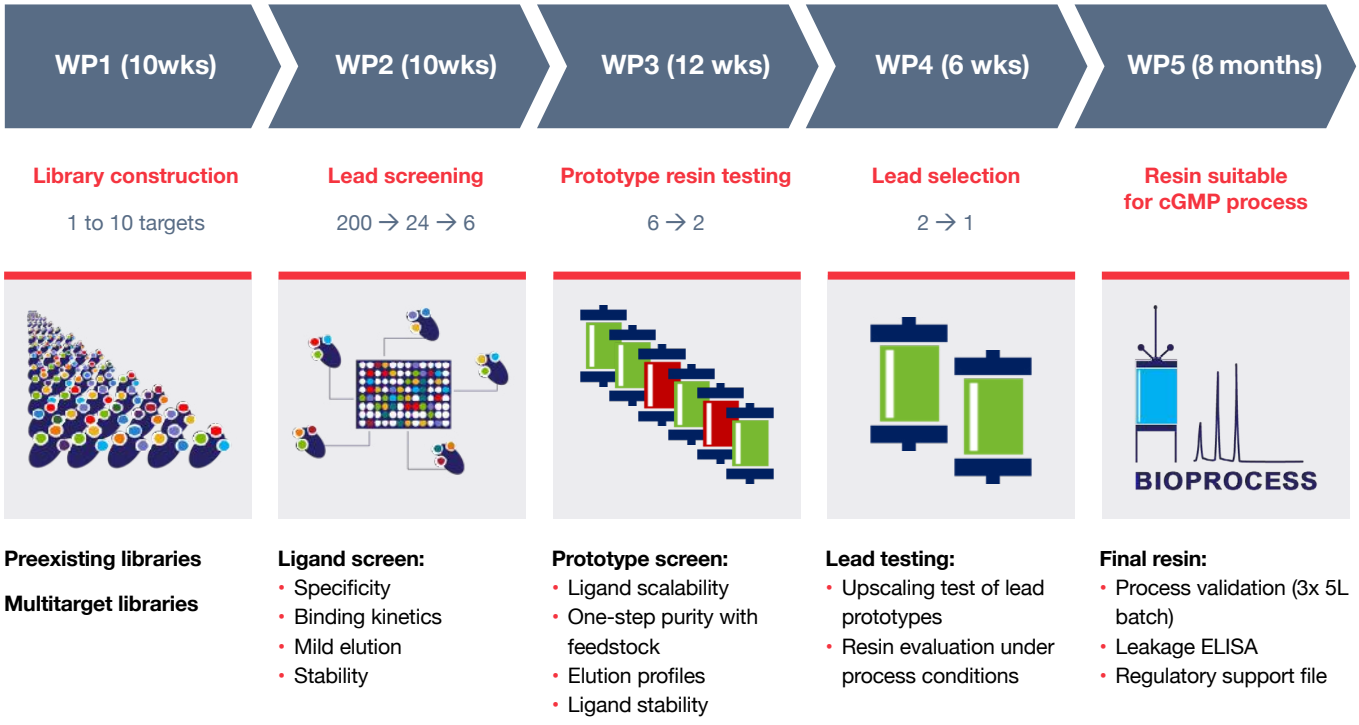


Figure 3. *Thermo Fisher's CaptureSelect™ affinity resin development pipeline relies on sequential work packages (WPs) to create custom ligands specific to a known biomolecular target.*[2]

Ligand scalability, crucial for large-scale manufacturing of biologics, is also assessed (figure 3).[1,2]

The final resin will then be developed for production, including generating regulatory support files to allow use of the resin in clinical and commercial manufacturing. The entire affinity resin customization process takes about a year and can be made simpler by open communication about previous purification problems and goals.

Custom resins can help manufacturers purify hard-to-isolate biomolecules. By picking a vendor with a good track record for custom resin development, manufacturers can accelerate their production process (figure 4).

Case study:

Biotechnology company Genexine worked with with Thermo Fisher to codevelop a custom affinity resin for human thyroid stimulating hormone (TSH) to improve process efficiency and cost. The goal was for the final resin to purify TSH in a single step without cross-binding-related hormones.[2]

Prototype V_HH-based resins were developed and then tested for their ability to purify TSH from pure stocks and cell-based mixtures. In the end, a single resin with clean TSH elution, high yield, and high purity was identified. After optimizing the resin in its pipeline, Genexine reported an almost doubled recovery, 46% to 71%, from the previous purification process (figure 5).[2]

The multitude of biotherapeutic modalities hitting the clinic will continue to drive the need for custom affinity resins. Development of such resins can help biotherapeutic companies stay ahead of the game and introduce new drugs faster.

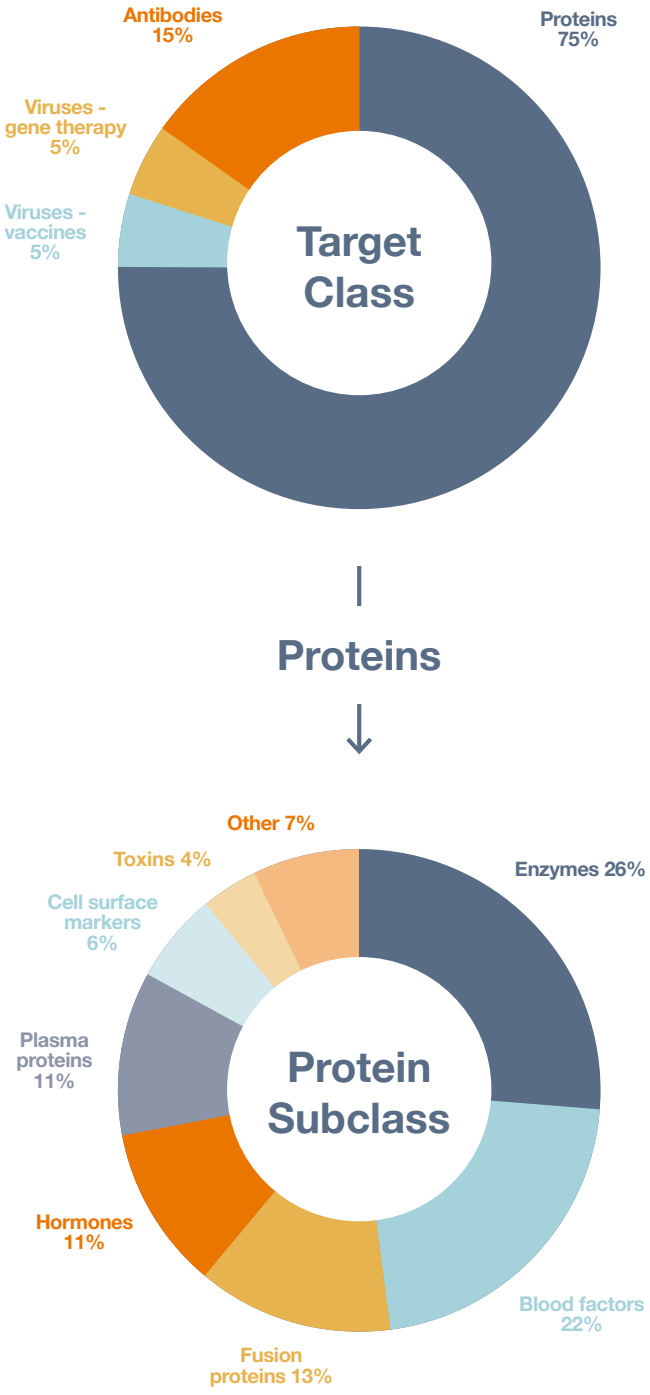
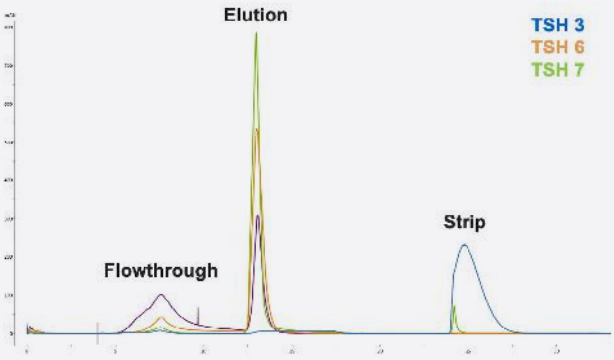


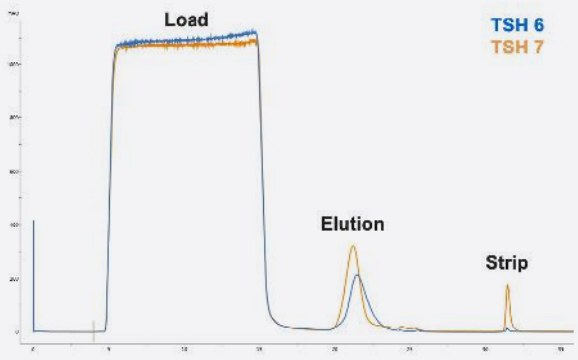
***Figure 4**. Biotherapeutic target molecule classes for which Thermo Fisher has developed custom CaptureSelect resins. Since 2003, Thermo Fisher has developed more than 60 custom resin solutions, with the class type breakdown shown above as a percentage of that total.*

Prototype resin testing: TSH ligand 3, 6 & 7



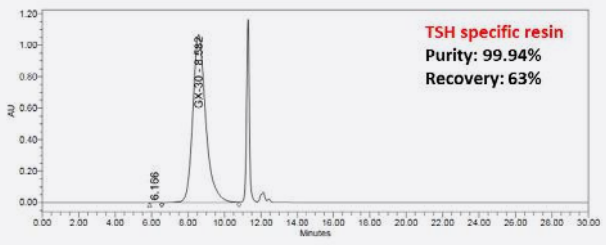
- TSH 6 & 7 show good binding capacity and efficient elution
- TSH-3 does not elute

Feedstock test: TSH ligand 6 & 7



- TSH 6: full elution
- TSH 7: some material in strip

Purity & recovery testing of TSH resin candidate 6



SEC-HPLC analysis revealed a purity of 99.94% with a recovery of 63%

***Figure 5**. Custom TSH affinity resin elution profiles from purification of recombinant TSH (top-left panel) and actual feedstocks (top-right panel) were analyzed to assess their efficiency. Purity and recovery rates were also tested and deemed acceptable for the final selected resin (bottom panel).*[2]

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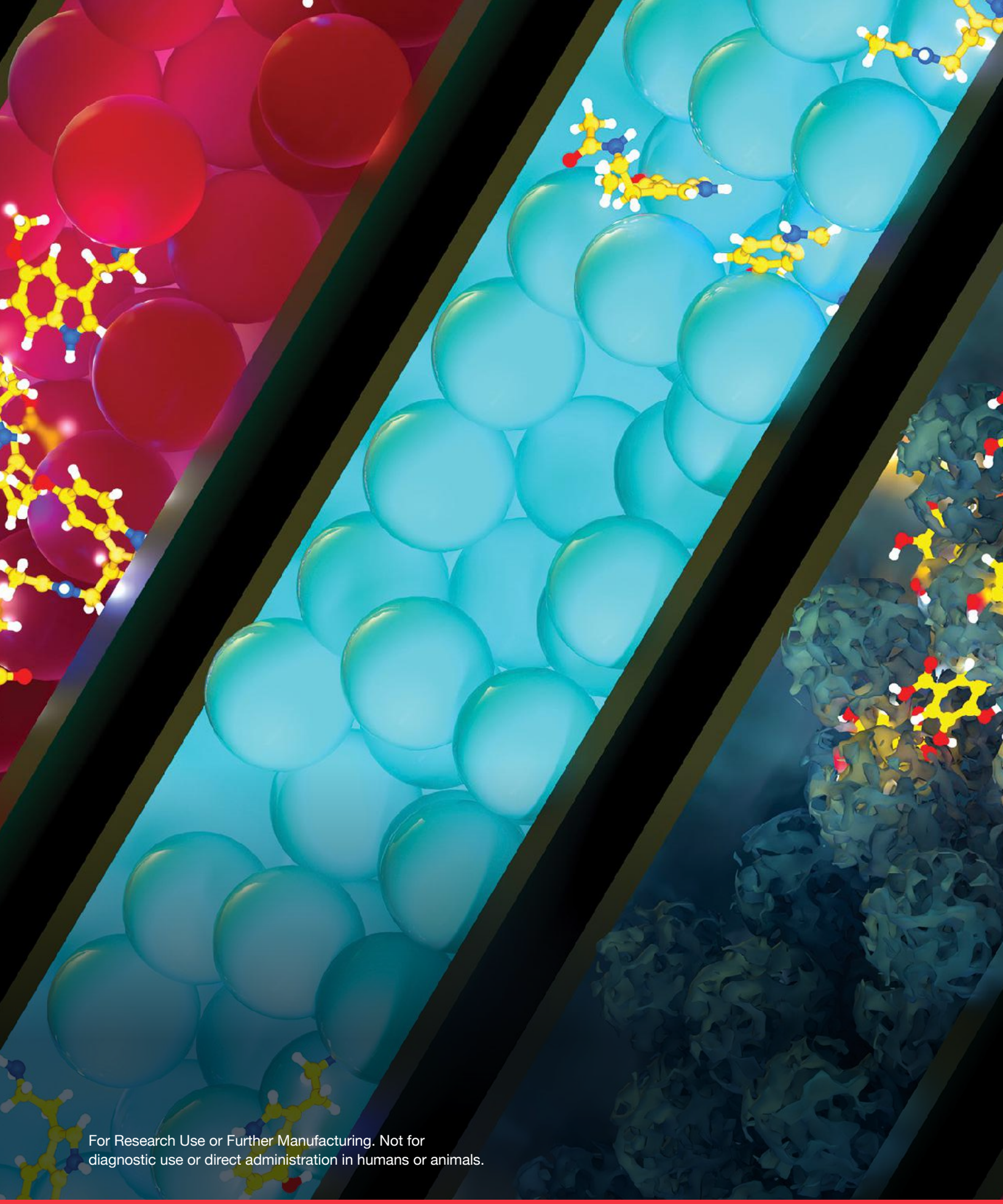

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