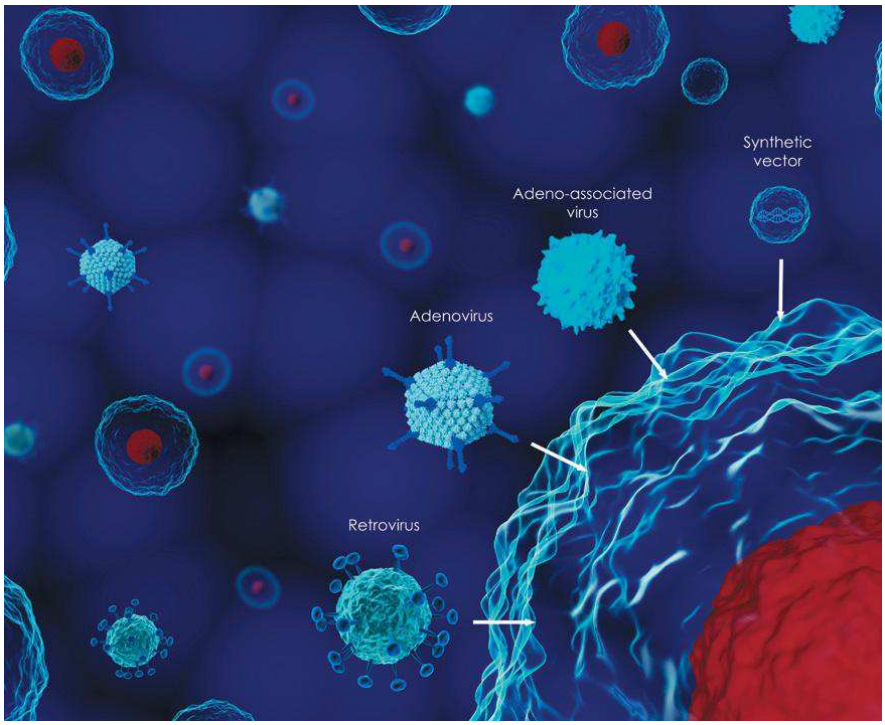


Meletios/Shutterstock.com

Affinity Chromatography Accelerates Viral Vector Purification for Gene Therapies

A case study demonstrates that affinity chromatography can offer efficiency and scalability for gene therapy manufacturing using viral vectors.

ORJANA TEROVA, PIM HERMANS, STEPHEN SOLTYS, AND FRANK DETMERS

On Nov. 2, 2012, UniQure's Glybera, an AAV-1 based drug, was approved in Europe for use in adult patients diagnosed with familial lipoprotein lipase deficiency (LPLD) (1). Since then, interest in gene therapies has skyrocketed, with a fourfold increase in the number of gene therapies being developed between 2012 and 2015 (2). With the power to overcome genetic disorders, these therapies could prove revolutionary to the field of medicine.

The technology aims to treat diseases by delivering genetic material encoding a protein with a therapeutic effect into a patient's cells (1). In most gene-therapy studies, a carrier molecule or a vector must be used to deliver the therapeutic gene to the target cells. There are a number of molecular vectors and techniques available for use; however, most frequently this is achieved with viruses. Viral

ORJANA TEROVA is global product manager for POROS chromatography products, Bioproduction at Thermo Fisher Scientific (Bedford MA, USA). **PIM HERMANS** is Director of Ligand Discovery, Bioproduction at Thermo Fisher Scientific (Leiden, The Netherlands). **STEPHEN SOLTYS** is Senior Field Application Scientist for POROS and CaptureSelect chromatography products, Bioproduction at Thermo Fisher Scientific (NC, USA). **FRANK DETMERS** is Director of Ligand Applications, Bioproduction at Thermo Fisher Scientific (Leiden, The Netherlands).

vectors can be administered *in vivo* to patients either locally or systemically, and upon infection, begin to generate the desired protein (1). Several types of viruses can be used as delivery vehicles to infect and transfer a functional gene into a patient's cells, including retrovirus, lentivirus, adenovirus, and herpes simplex virus. The adeno associated virus (AAV) sub-classes have emerged as the vector of choice for many therapies. AAV vectors can infect nondividing cells and mediate long-term tissue-specific gene expression, and they have a low immunogenicity. Twelve distinct subtypes of AAV have been identified, each varying in their tissue tropism.

A number of clinical studies involving recombinant AAV-based vectors have reported excellent clinical outcomes. Originally the main focus of gene-therapy clinical applications was on the treatment of diseases caused by single-gene defects such as cystic fibrosis, hemophilia, muscular dystrophy, and sickle cell anemia. Today, the clinical applications of the viral vectors have expanded to include the treatment of cancers and neurological, cardio-vascular, and infectious diseases such as HIV and hepatitis. In 2017, the *Journal of Gene Medicine* reported 2463 gene-therapy clinical trials, with 64.4% being conducted in the United States. The majority of gene therapy clinical trials are in early phase development, primarily focused on cancer, monogenic, infectious, and cardiovascular indications (3).

CHALLENGES IN VECTOR MANUFACTURING

Although extensive methods have been investigated and optimized for upstream production of viral vectors, efficient downstream purification to generate clinical product of high titer, high potency, and high purity remain extremely important. One of the major challenges the field faces is the lack of industrialized purification platform technologies specifically for the purification of viral vectors. Without these technologies, it is difficult for biopharma companies to increase productivity and meet industry needs for these unique therapies. Currently, recombinant AAV vector manufacturing involves using purification methods consisting of multiple steps, for example, cesium chloride (CsCl) density-gradient centrifugation, iodixenol gradients, several chromatography steps (e.g., ion-exchange chromatography, hydrophobic interaction, and heparin or immobilized metal ion affinity chromatography [IMAC]), and a concentration procedure (4–7). In addition to lengthy processing time in manufacturing, such a multi-step process increases process development lead times, is a more expensive process, and generates cumulative yield losses. The poor scalability of this process can limit the commercial feasibility of any promising viral vector. It is crucial that an efficient downstream purification process maintain the biological activity of an AAV vector while removing impurities and contaminants present in a feedstock that originates from host cells or culture media. This is crucial to ensure stable and effective intercellular transgene expression, to prevent transmission of infectious disease, and to comply with strict regulatory guidelines. Many years of research have not resulted in a platform approach for viral vectors that fulfills all of the above criteria.

AFFINITY CHROMATOGRAPHY TO ENABLE VIRAL VECTOR PURIFICATION

A recent development for viral vector purification is the use of affinity chromatography. Affinity chromatography is an essential established part of platform technologies for purification of biomolecules because it provides advantages such as highly specific separation, high fold purification, and robust methodology with less process optimizations. It delivers significant improvement to the downstream processing, by reducing the purification steps and maximizing productivity, offering scalability, and processing consistency. This improvement has been seen in the industry with the use of protein A for purification of monoclonal antibodies, and more recently by having specific affinity purification products for other therapeutic compounds like blood coagulation factors, hormones, and antibody-derived therapeutics (8–11).

Affinity chromatography is based on bio-specific binding interactions between a ligand immobilized to the chromatographic stationary phase and a target biological molecule in the sample. The immobilized ligand/support matrix combination forms a highly selective stationary phase that, in theory, will only bind to the target molecule (usually a protein) of the ligand pair. Immobilized ligands may include either proteins or small molecules that interact specifically with the target of interest (**Figure 1**).

The technique is used almost exclusively to purify proteins and antibodies, although there are some applications with peptides and nucleic acids. Nearly

all biological molecules interact in some selective way with some other molecules through binding at a specific site. In the case of bio-specific or affinity binding, however, the charged and hydrophobic groups are arranged on the pair of binding molecules or ligand's in a unique 3-D orientation where weaker forces such as hydrogen bonding also play an important role. The two ligands thus fit together very much like a lock and key, with a high degree of specificity.

As mentioned above, affinity chromatography offers a number of benefits including highly specific separation, which results in the ability to do one-step purification from crude material to generate a product with high purity and yield. This approach reduces the purification steps and maximizes productivity. This mode of chromatography can be implemented as a scalable platform in the downstream purification of viral vectors, using, for example, camelid heavy-chain-only antibodies (VHH), which as single antibody domains provide full functionality in antigen specific recognition and high affinity binding (**Figure 2**). Due to their compact structure, these domains are robust and can withstand the various and often extreme conditions seen typically in chromatography.

Many biotherapeutics such as AAV are quite large in terms of their weight and size and more complex in their composition, making them challenging to purify. To overcome this challenge, immunoaffinity columns using, for example, affinity resins have been specifically developed to offer a flexible approach, enabling one-step purification of any protein-based biotherapeutic.

Figure 1: Affinity chromatography principle—a particular ligand is chemically immobilized or "coupled" to a solid support. The complex mixture that contains the target molecule with impurities is loaded over the affinity column, and the target molecule that has specific binding affinity to the ligand on the resin will bind. The impurities are washed away, and the bound molecule is eluted from the column, resulting in its purification from the original sample.

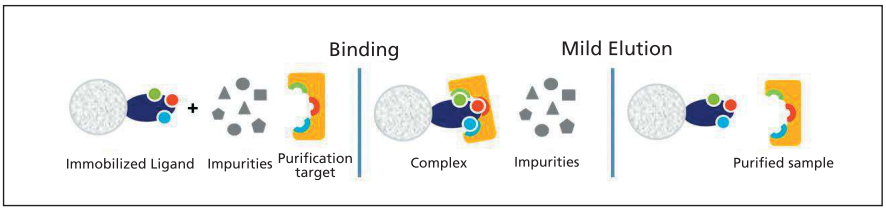


Figure 2: Describes the difference between a classical antibody and a heavy chain only antibody as found in camelid, using the variable domain (VHH). It is the smallest binding domain; it is compact and highly specific but also robust under various chromatographic conditions. Affinity to 3 complementarity defining regions (CDRs) provides unique, tunable specificity.

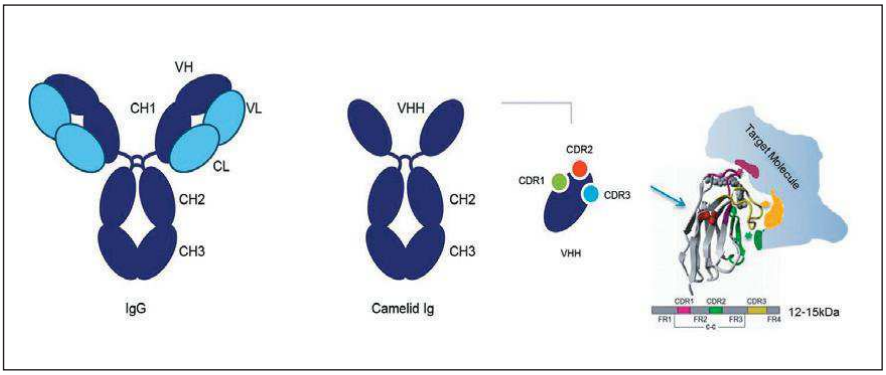
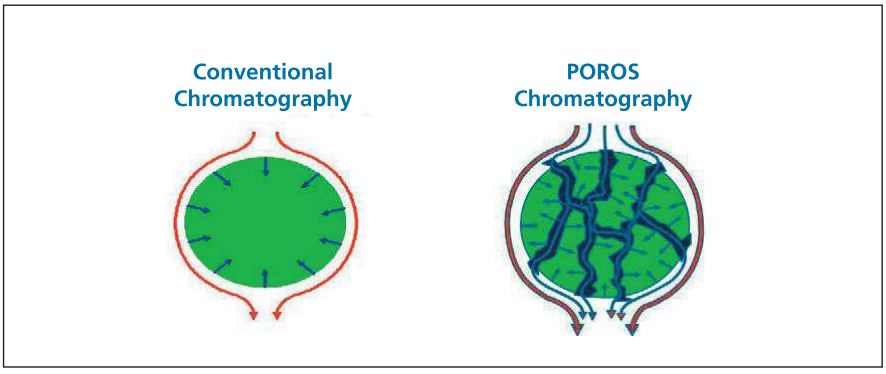


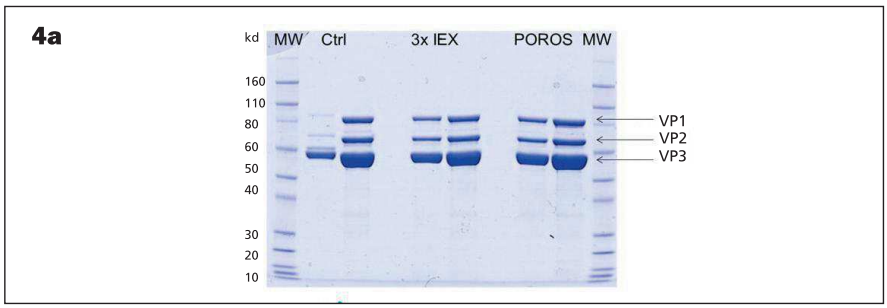
Figure 3: Displays the difference between conventional chromatography and resin that is characterized by large throughpore bead architecture.

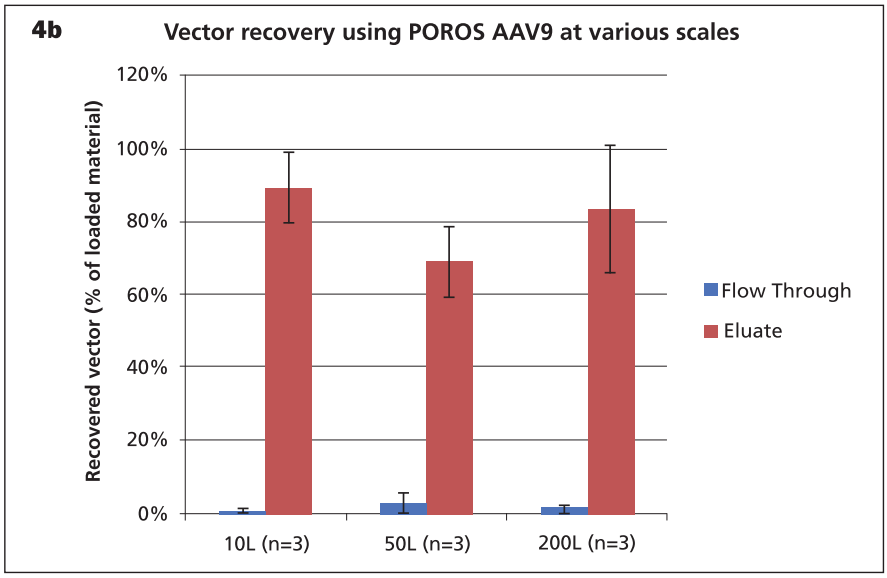


Several VHH ligands (AAV8, AAV9, and AAVX) have been identified and developed against a broad range of AAV subtypes as well as chimeras, to enable a one-step affinity chromatography procedure. During ligand discovery, the final ligand that meets all predefined requirements is selected and produced in an animal-origin-free (AOF) system in Saccharomyces cerevisiae at any scale (7–10). The affinity ligands were then immobilized on a large throughpore matrix

All figures are courtesy of the authors

Figure 4: 4a-b: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) comparing purity of AAV9 viral vector, purified by two downstream processing methods: one utilizing three ion-exchange steps and the POROS CaptureSelect (Thermo Fisher) AAV9 resin as a one-step capture process. The data show the purity profile of viral vector AAV9 is equivalent when comparing both downstream processing approaches. The gel also reveals similar purity and the capsid viral protein (VP) topology for viral vector AAV9 is confirmed showing the bands corresponding to the viral structural proteins VP1, VP2, and VP3. Vector recovery data of three different AAV9 batches were produced to assess reproducibility of the process. The data show ≥70% vector recovery for all three AAV9 batches at each step of Genethon's downstream process. Data source: Genethon.





support (POROS, Thermo Fisher) and used for immunoaffinity chromatography to purify the specific AAV serotype.

CASE STUDY—PURIFICATION OF AAV9 VIRAL VECTOR

In a collaborative study with Genethon, a gene-therapy company developing treatments for rare diseases, the purification of AAV9 viral vector using multiple ion-exchange steps was compared with immunoaffinity chromatography method utilizing a AAV9 affinity resin (POROS CaptureSelect, Thermo Fisher), which contains an immobilized ligand that specifically adsorbs AAV9. The crosslinked poly[styrene divinylbenzene] resin backbone is further derivatized with the AAV9 affinity ligand, a single-domain monospecific antibody fragment. The resin used in this case study (POROS, Thermo Fisher) is of 50-micron average particle size characterized by the addition of a large throughpore structure, which makes it suitable for the capture of large biomolecules such as viral vectors, where dynamic binding capacities of >1 × 1013 viral genome per mL have been obtained.

Figure 3 presents the differences between conventional chromatography and immunoaffinity chromatography. Typically with conventional media, the pores are much smaller; therefore, diffusion in and out of the small pores controls performance. Diffusional processes are also dependent of the flow rate, where in order to maximize capacity, the flowrate needs to be very slow, maximizing residence time or utilizing a very long column. However, the utilization of resins that are characterized with large throughpores allows for increased capacity by unlocking the bead's interior making the process more efficient thereby decreasing cycle times. Also due to the large throughpore structure, the beads have improved mass transfer capability allowing for a more efficient bead; therefore, high capacity and resolution can be maintained independent of the operating flow rate. This leads to efficiency at increased linear flow rates and improves process productivity.

When assessing the results of the case study, it can be concluded that although equivalent AAV9 purity was obtained when utilizing both methods (3 ion exchange steps versus affinity, **Figure 4a**), using the immunoaffinity column reduced processing steps and increased yield.

Contin. on page 35

surface of more than 20 different tumor types, Panacea has interest in further study of this vaccine in Phase I/II clinical trials in breast, colon, ovarian, and bone cancers.

REFERENCES

1. L. Lavaissiere et al., *J Clin Invest*. 98 (6) 1313 (1996).
2. N. Ince, S.M. de la Monte, and J.R. Wands, *Cancer Res*. 60 (5) 1261 (2000).
3. Y.A. Yeung et al., *Hum Antibodies*. 16 (3-4) 163 (2007).
4. T. Xue et al., *Hybridoma (Larchmt)*. 28 (4) 251 (2009).
5. H. Yang et al., *Oncol Rep*. 24 (5) 1257 (2010).
6. E. Revskaya et al., *Cancer Biotherapy and Radiopharmaceuticals*. 32 (2) 57-65 (2017).
7. H.J. Lee, *Arch Pharm Res*. 25 (5) 572-584 (2008).
8. J.P. Williams and W. Lednar, *The American Journal of Managed Care*. 8 (supp 5) S145-S154 (2002).
9. I. Fraser-Moodie, Delivery Mechanisms for Large Molecule Drugs, successes and failures of leading technologies and key drivers for market success. *Business Insights Ltd.* (2008).
10. J.F. Nicolas, B. Guy *Expert Reviews Vaccines*. 7 (8) 1201-1214 (2008).
11. C.S. Godin, S. Stewart, S. Fuller, "An Intradermal Toxicity Study of a Therapeutic Nanoparticle Cancer Vaccine in Sprague Dawley Rats" (Abstract and Poster), American College of Toxicology 37th Annual Meeting, Baltimore, MD (2016). **BP**

Viral Vectors —*Contin. from page 30*

Typical affinity chromatography optimizations in terms of binding, washing, and elution conditions can be applied when utilizing AAV9 affinity resin. For example, in terms of binding considerations, for equilibration standard neutral buffers (pH 6–8) such as 10–50 mM sodium phosphate or Tris can be used. Elution conditions differ because the target molecules differ in their binding/elution behavior. When eluting most target molecules, however, reducing the pH to the range of pH 2–3 is generally successful. Other elution buffer components that can be used include phosphate, hydrochloric acid, glycine, acetate, or other components that buffer well at low pH. Other additives such as 2M magnesium chloride ($MgCl_2$) or 50% propylene glycol may be useful.

Lastly, the study results showed that when using the AAV9 affinity resin in the capture step during vector purification, a satisfactory vector recovery of ≥70% is obtained. **Figure 4b** shows that viral vector recovery is reproducible at different scales as process scale-up occurs 20-fold. Genethon also noted that viral vector purification processes are simplified when using affinity chromatography, increasing product yield from 20–60% and reducing cost by a factor of six over alternative methods. The utilization of an immunoaffinity column frequently requires only a single capture step and then a concentration step, significantly simplifying a purification process. Fewer unit operations means higher product yield obtained thereby enabling faster time to market, while helping reduce cost of goods. This is crucial because the industry is focusing on developing industrial capabilities to produce viral vectors in large amounts to meet clinical and future market demand.

CONCLUSION

Gene therapy shows great potential to treat a variety of diseases, and the industry is working to establish efficient commercial manufacturing capabilities for these unique therapies.

The methodology described above offers the following:

- One-step AAV purification from crude material with high purity and yield
- High specificity and capacity, reducing the process volume significantly for subsequent steps and maximizing yield
- Basis of platform purification (reproducible)
- Robust with less process optimization.

Affinity chromatography is set to have a significant impact on increasing process productivity and enabling the industry to meet market needs. The utilization of immunoaffinity columns will be an important improvement to downstream processing of viral vectors. A case study demonstrated that the columns can reduce the purification steps, maximize productivity, and offer the scalability and processing consistency needed for the production of clinical-grade gene therapy molecules.

REFERENCES

1. Advances in Gene Therapy R&D/Drug Discovery, 2012.
2. B. Wilkinson and C. Bennett, Will Reimbursement Prove to be the Biggest Barrier as Three Gene Therapies Gain Regulatory Approval?, DMK C0162772, July 2016.
3. J. Wiley, *Journal of Gene Medicine*, 19 (4) (April 2017).
4. N. Clement and C. Grieger, Molecular Therapy — Methods & Clinical Development (2016) 3, 16002; doi:10.1038/mtm.2016.2.
5. M Lock, M Alvira, LH Vandenberghe, A Samanta, J Toelen, Z Debyser, and J Wilson, *Human Gene Therapy* (October 2010) 21:1259–1271
6. MA Vance, A Mitchell, RJ Samulski, "AAV Biology, Infectivity and Therapeutic Use from Bench to Clinic, Gene Therapy–Principals and Callenges," Dr. D. Hashad, Ed., *INTECH*, 2015; doi: 10.5772/61988.
7. RH Smith, JR Levy, RM Kotin, *Mol. Ther.* 17 (11), 1888–1896 (2009).
8. MM Harmsen and HJ De Haard, *Appl. Microbiol. Biotechnol.* 77, 13–22 (2007).
9. S. Winge, et al., *Protein Expr. Purif.* 115, 165–175 (2015).
10. J. McCue et al. *Biologicals* 43, 213–219 (2015).
11. F. Detmers, F. Mueller, and J. Rohde *BioProcess Int.* 11(6) S36– S40 (2013). **BP**