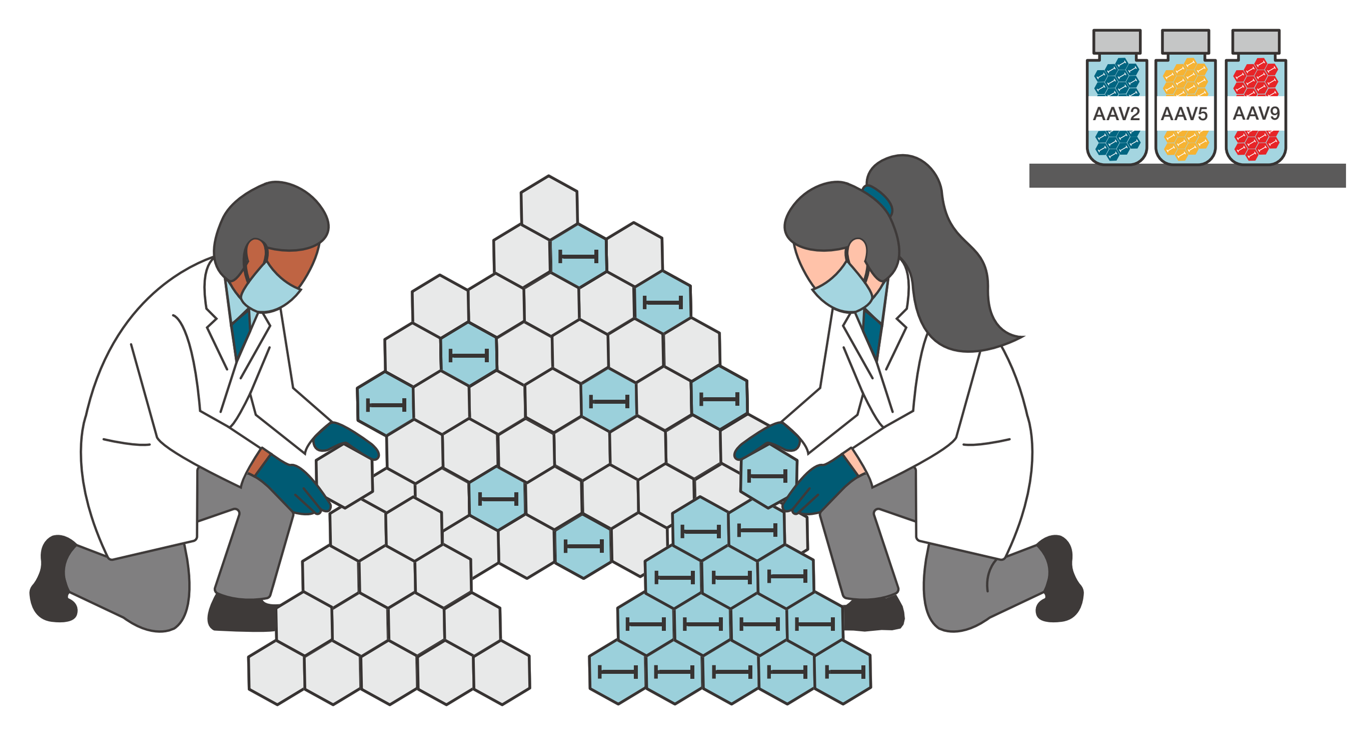
Scale Without Bottlenecks: AAV Polishing for Full Capsid Enrichment

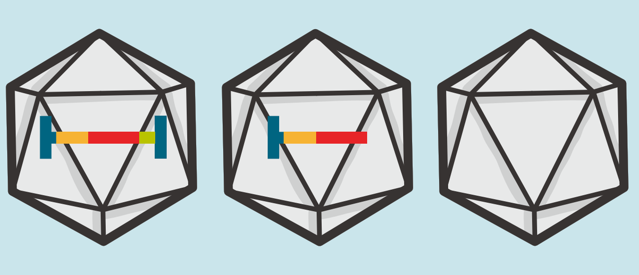


Adeno-associated virus (AAV) is a leading vector for gene therapy, with several approved therapies and many more in clinical development.[1] While full capsid enrichment is vital for therapeutic potency, separating them from empty or partial capsids is challenging due to their similar physicochemical properties. Anion exchange chromatography (AEX) offers a scalable solution for multiple AAV serotypes.[2]

This infographic outlines key downstream challenges, process development considerations, and AEX-based strategies for full capsid enrichment across AAV serotypes.

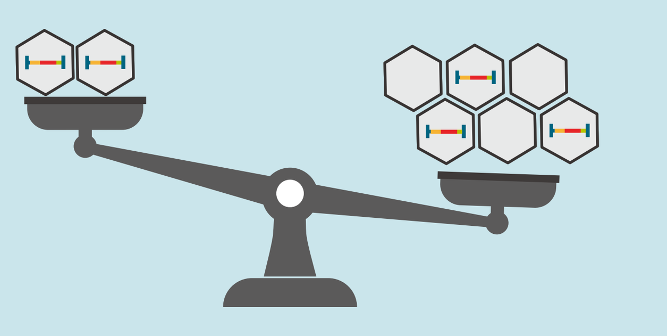
Challenges in AAV downstream processes

Purifying AAV vectors for clinical use requires balancing yield, purity, and scalability. Understanding the core challenges of downstream processing is the first step toward optimizing your polishing strategy.



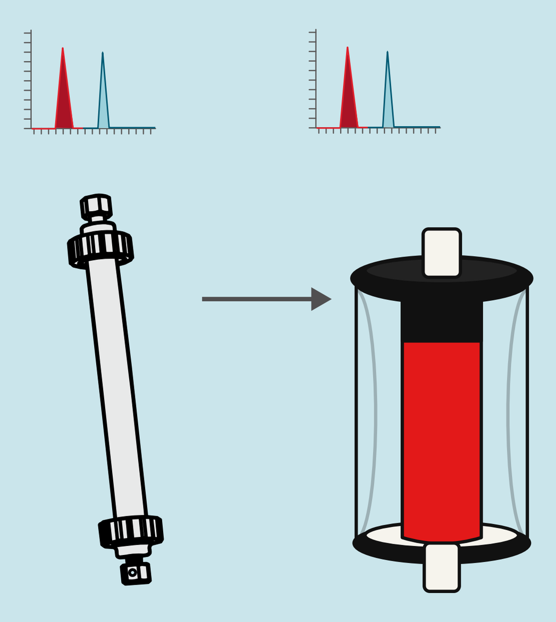
Full capsid enrichment

The structural similarity between empty, partial, and full capsids presents a significant hurdle for high-resolution separation.[3]



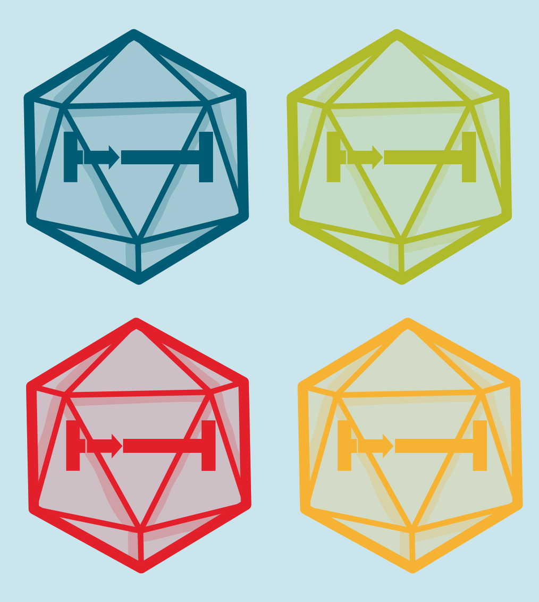
Recovery vs. purity

Strict purity requirements often lead to substantial product loss, forcing a trade-off between final yield and product quality.[4]



Scalability

Maintaining consistent separation performance is increasingly complex when transitioning from lab to manufacturing scale.[4]



Serotype variability

Different serotypes and payloads can shift binding strength and elution windows, often requiring unique operating conditions specific to each serotype and payload.[5]

AEX polishing workflow for full capsid enrichment

A structured AEX polishing workflow helps separate empty and full AAV capsids after affinity capture. This approach guides sample preparation, resin screening, buffer and elution optimization, and scale-up to support robust full capsid enrichment across process development and manufacturing.

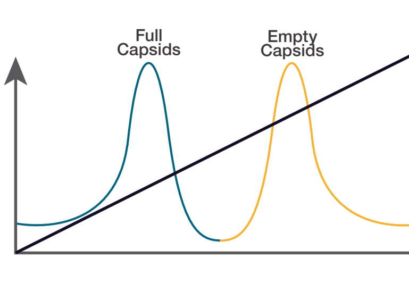
1 Sample preparation

After affinity capture, dilute the AAV eluate ~10x into the AEX starting buffer.

A 10x dilution typically reduces salt and promotes binding to the AEX resin.

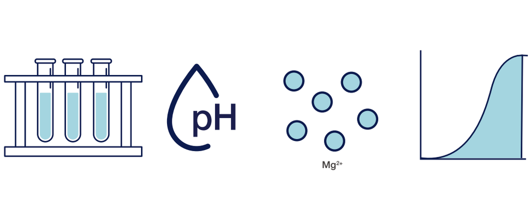
i Typical starting buffer: 20 mM Tris or Bis-Tris propane, pH 8.5–9.0, low conductivity.

2 Resin screen using linear salt gradients



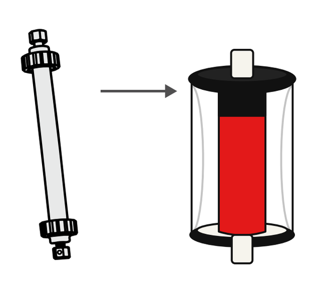
Screen AEX resins with linear conductivity gradients to identify empty/full capsid separation, elution order, and the target conductivity window.

3 Further optimization (buffer, excipients, and elution conditions)



Optimize buffer, excipient, and elution conditions to improve empty/full capsid separation, recovery, and robustness. Define the final elution strategy as an isocratic, gradient, or hybrid AEX polishing method.

4 Method confirmation and scale-up



Confirm the optimized AEX polishing method and transfer it to scalable column formats. Evaluate elution conditions, loading, flow rate, recovery, and empty/full separation to support consistent performance from process development to manufacturing.

Key considerations during AEX polishing

1 **Empty/full capsids elution order**

For most serotypes, empty capsids generally elute first under typical AEX conditions. However, for AAV9-derived serotypes, it is common for full capsids to elute first on AEX when using a conductivity gradient elution. Confirm the elution order during screening for your serotype and resin.

2 **Some serotypes (e.g., AAV2) can aggregate at low conductivity**

Very low conductivity conditions can promote aggregation, especially for AAV2. Determine the lowest conductivity starting buffer that prevents aggregation while providing good binding.

Key parameters to test

Salts/excipients
- Magnesium-containing salts (e.g., $MgCl_2$, $MgSO_4$)
- Sodium chloride
- Arginine (optional)

pH
- Typical screening range: pH 8.0–9.5 (Bis-Tris propane or Tris buffers)

Buffers
- Bis-Tris propane or Tris.
- Evaluate buffer concentration (10–50 mM)

Elution conditions
- **Isocratic:** Determine target conductivity
- **Gradient:** Determine maximum conductivity and slope
- **Hybrid gradient:** An alternative to meet specifications where feasible

Other factors
- Column load density (e.g., vg/mL resin)
- Flow rate/residence time
- Temperature

Note: Optimal conditions are serotype- and resin-specific. Always confirm separation of full vs. empty capside (e.g., by analytical AEX, AUC, or mass photometry) during development.

Strategies for scalable full capsid enrichment

Effective full capsid enrichment depends on resins that provide selectivity, resolution, and scalability. Thermo Scientific™ POROS™ AEX resins are designed to support high-resolution separation of closely related capsid populations, with prepacked formats available to simplify use from process development through scale-up.

POROS XQ resin

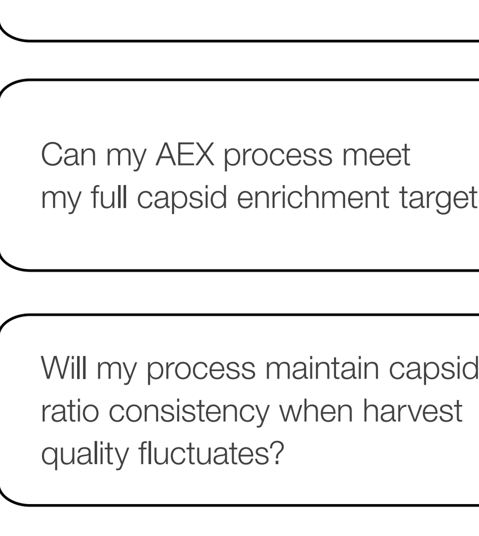
Suitable for most AAV serotypes, POROS XQ is a strong AEX resin with fully quaternized amine surface chemistry.

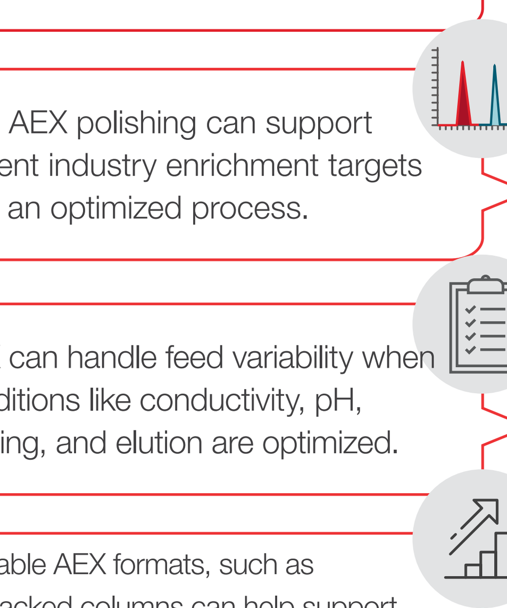
POROS HQ resin

Recommended for specific or challenging serotypes such as AAV9, POROS 50 HQ offers a distinct bind-elute profile containing 60% quaternary amines.

AAV Polishing FAQs: Key questions to guide AEX strategy development

Selecting an AAV polishing solution requires balancing serotype-specific behavior, capsid composition, process robustness, and scale-up needs. Moving beyond labour-intensive traditional methods, AEX chromatography offers the precision and scalability necessary to support consistent performance from process development through manufacturing.





Key questions	AEX polishing approach
Should I change my polishing methods for different AAV serotypes?	Additional optimization may be required across pH, salt type, and elution for different serotypes.[6]
Can my AEX process meet my full capsid enrichment target?	Yes, AEX polishing can support current industry enrichment targets with an optimized process.
Will my process maintain capsid ratio consistency when harvest quality fluctuates?	AEX can handle feed variability when conditions like conductivity, pH, loading, and elution are optimized.
Will the flow dynamics, binding capacities, and resolution hold up at manufacturing scale?	Scalable AEX formats, such as prepacked columns can help support reproducible performance from development through GMP production.

Struggling with AAV purification bottlenecks?

Watch the webinar: Addressing AAV purification bottlenecks

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