

Scaling-up rAAV downstream process from 2L to 200L for Engineering Run

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Introduction

The increasing demand for recombinant adeno-associated virus (rAAV) vectors supporting pre-clinical and clinical programmes requires downstream processes that are scalable, robust, and compatible with industrial manufacturing constraints. While bench-scale optimisation is well established, predictable translation to pilot and engineering scale remains a key challenge, particularly for yield and purity. This work describes the scale-up of a rAAV8 downstream process from 2L process development to 200L engineering scale.

The core objective was to demonstrate scalability across downstream unit operations, maintain product quality attributes, and establish process understanding and readiness for GMP manufacturing. A second objective was to enhance drug-product purity by enriching in full AAV capsids the drug product, evaluating an intensified polishing technique to support the quality requirements of clinical-grade rAAV gene therapy products.

Results and Discussion

1 Scaling-up Downstream Process for rAAV8 from 2L to 200L

Scaling-up rAAV8 downstream processing from 2L to 200L for an engineering run in a cleanroom required coordinated cross functional process design and control. The scale up rationale was based on prior process development activities, including screening and optimisation scale-down studies. Comparable performance was observed across all unit operations when transitioning from 2L to larger scales, confirming process scalability. The downstream process delivered an overall vg recovery exceeding 30% with drug substance purity consistently ranging between 60% and 70% full capsid measured by mass photometry.

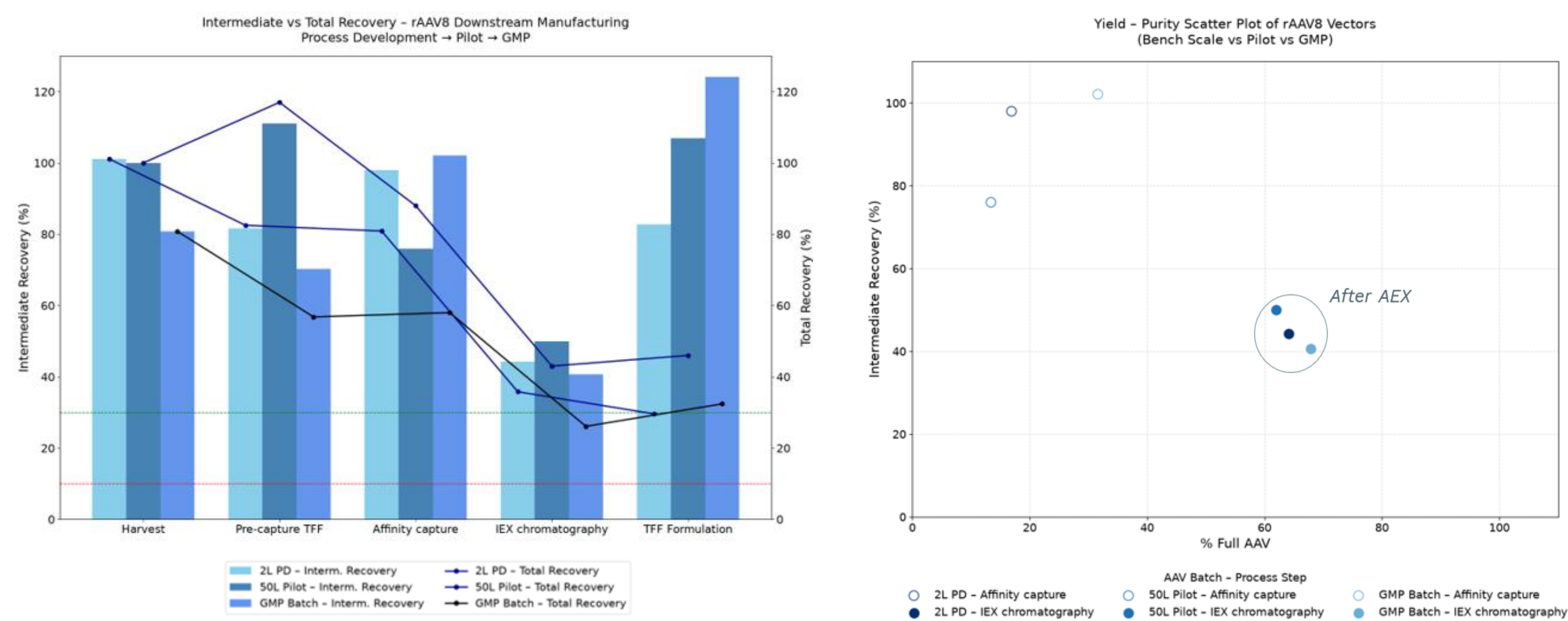


Figure 2: (A) Intermediate and Total Vector Recoveries for rAAV8 Downstream Manufacturing at 2L, 50L and 200L. (B) Recovery – Purity Scatter Plot before/after AEX chromatography polishing across scales showing consistent drug substance purity measured by mass photometry

2 Scaling-down Downstream Process Development: Optimisation of AEX for rAAV8

Following industrial scale-up, process development focused on improving product purity using scalable AEX polishing. Feed stream quality guided the strategy: challenging feeds with high vector heterogeneity and/or low packaging may require upstream improvements, whereas suitable feeds can undergo two-pass AEX polishing

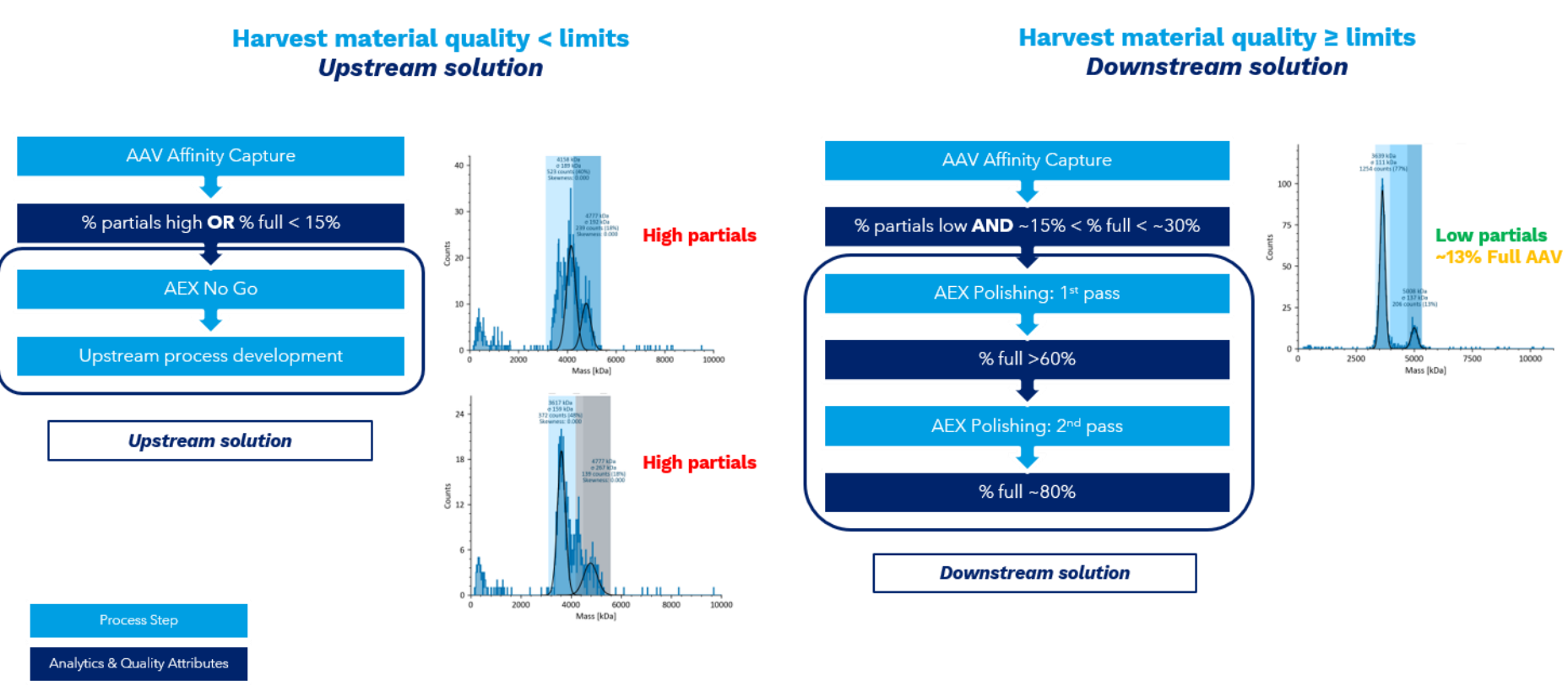


Figure 5: Feed Quality guides the AAV Polishing Strategy

Preliminary screening study supported the identification of AEX purification conditions delivering ~60% full capsids, based on mass photometry analysis.

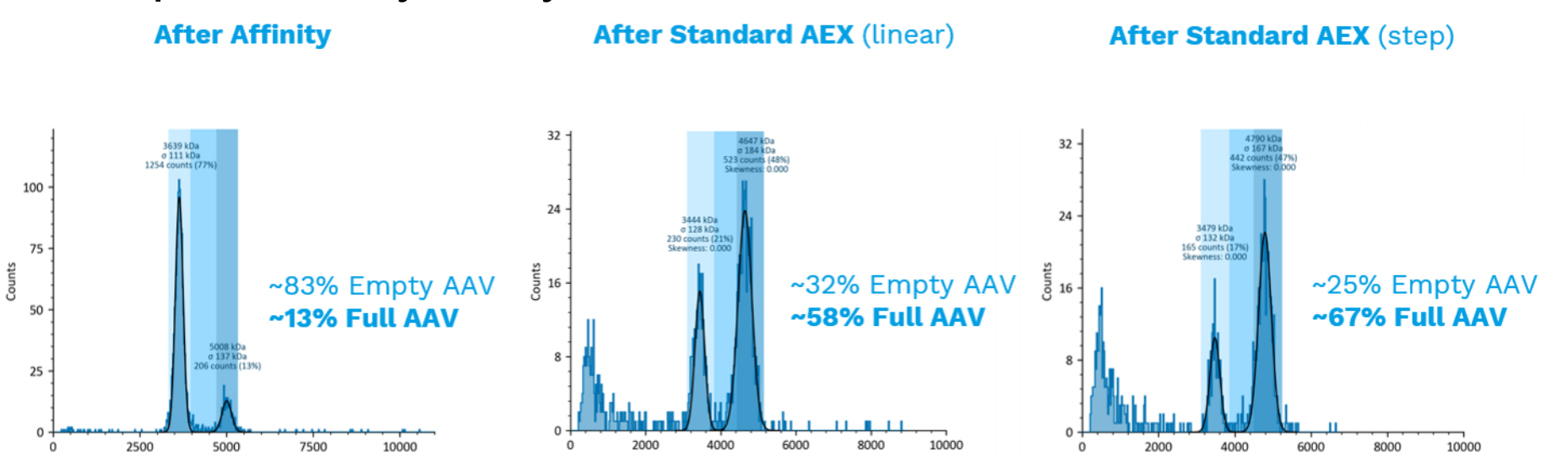


Figure 6: Preliminary screening using AEX polishing showing up to 5-fold enrichment in full AAV capsids

A two-pass AEX polishing strategy using CIM QA monolith chromatography was evaluated with challenging rAAV8 starting material (~13% full capsids). Inspired by continuous chromatography principles, sequential AEX passes progressively enriched full capsids to ≥ 65% after Pass 1 and ≥ 80% after Pass 2.

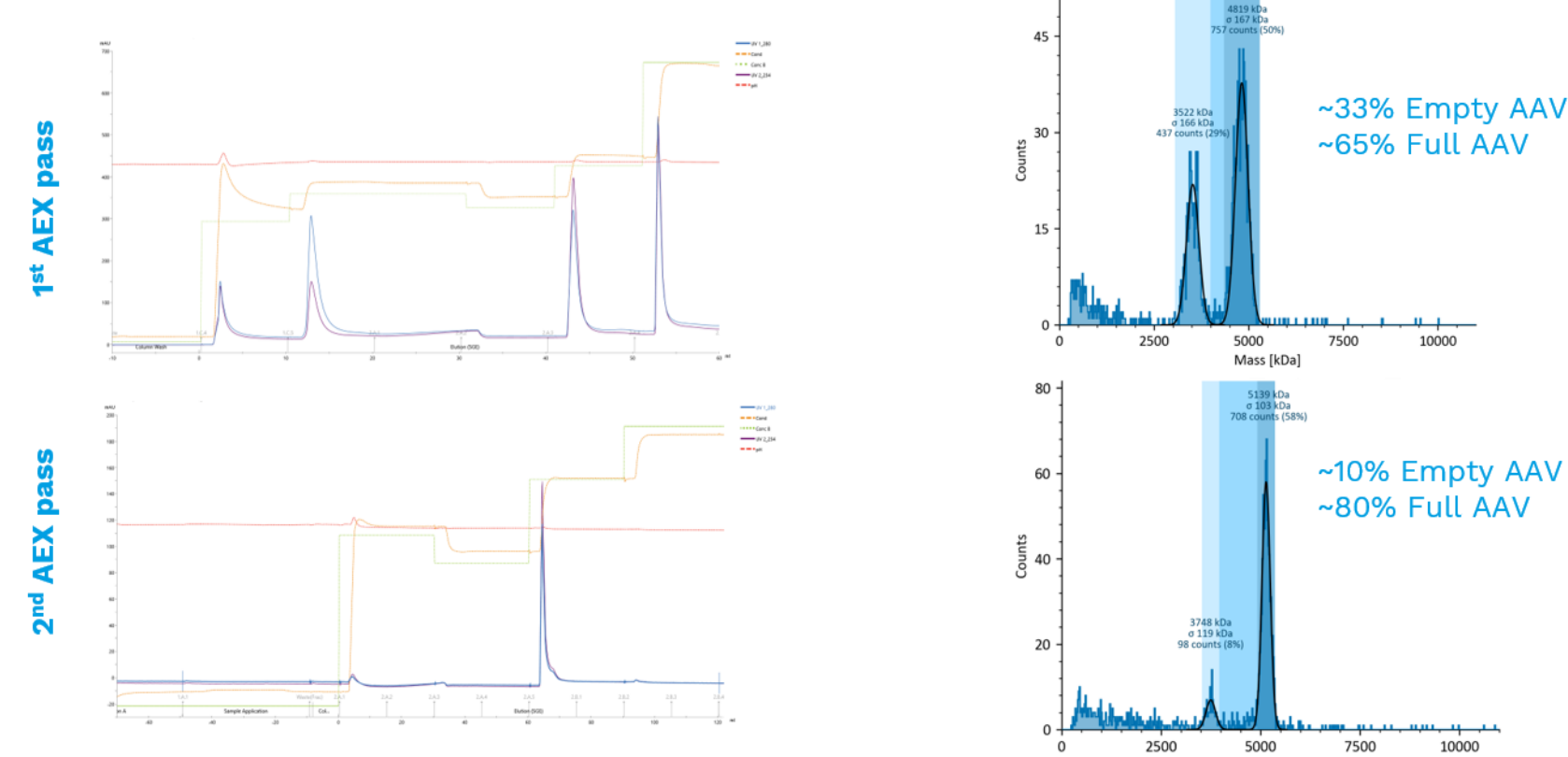


Figure 7: Two-pass AEX polishing delivering ~80% full AAV capsids

Mass photometry enables rapid single-particle characterization of AAV capsid populations. Analysis settings influences reported empty, partial and full distributions, impacting the purity assessment.

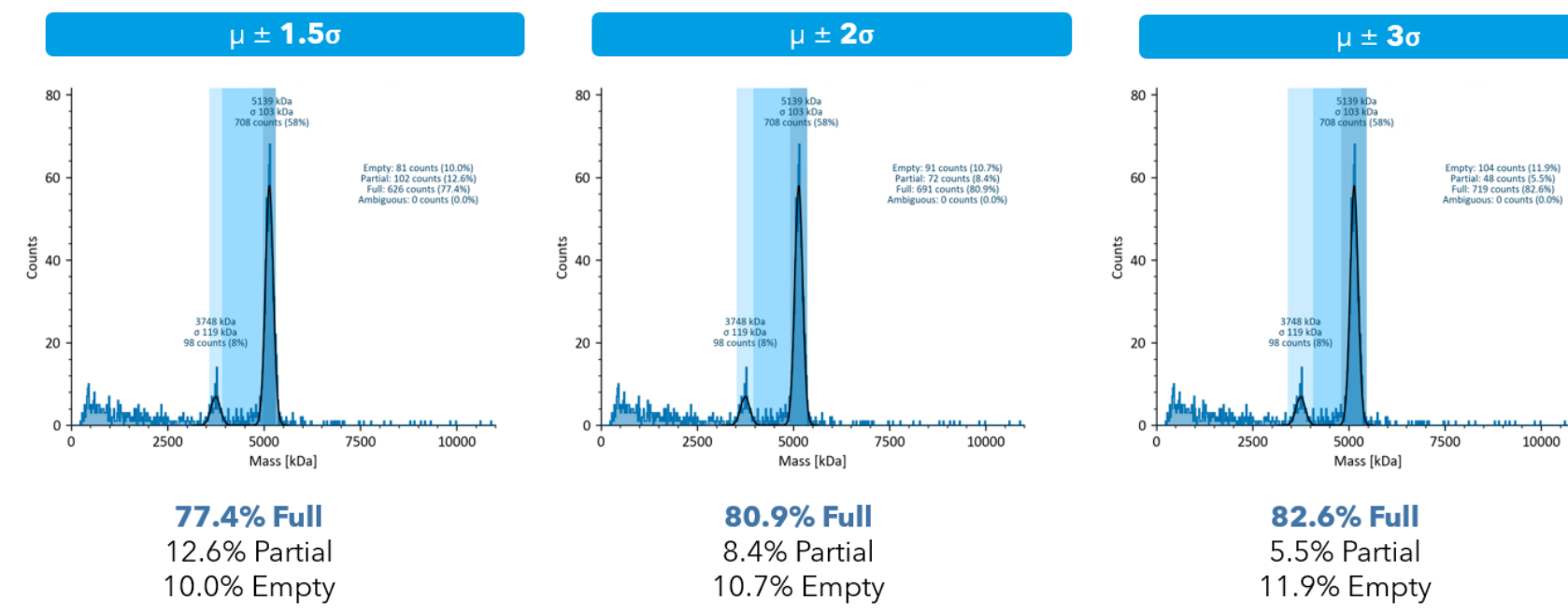


Figure 8: Mass photometry analysis supporting purity assessment

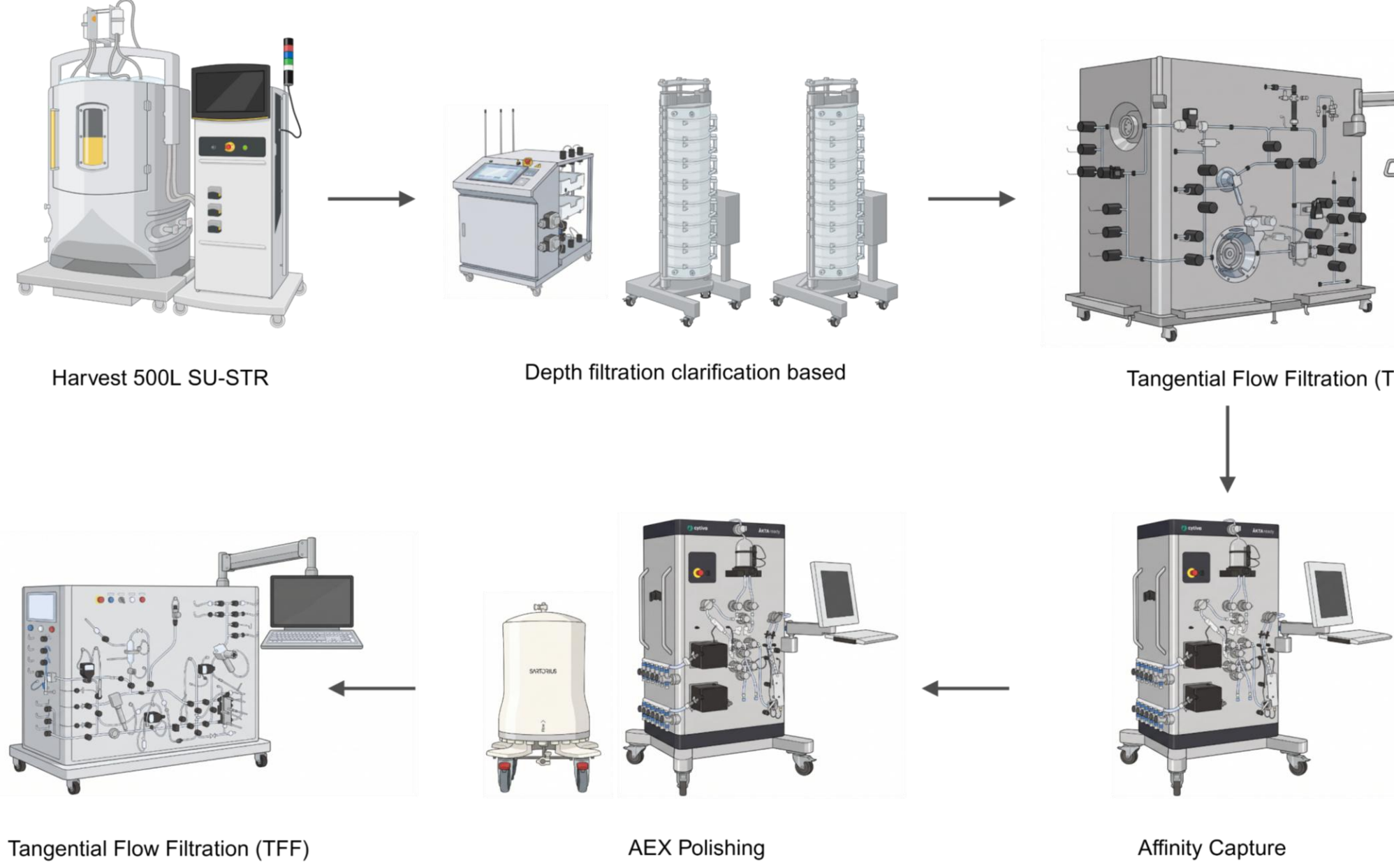


Figure 1: Simplified Process Flow Diagram of the large-scale AAV Downstream Process (DSP) established at Siegfried DINAMIQS using HEK293 suspension cells.

Across pre-clinical and clinical manufacturing scales, a similar control strategy was used where the measure of strength, infectivity and several purity attributes were comparable. The % full AAV capsid measured by mass photometry ranged between 60–70%, with residual host-cell DNA remaining below 30 ng/1E+12 vg by qPCR. Residual HEK293 host-cell proteins were at, or below, the detection limit (<20 ng/mL).

Table 1: Quality control test results of the rAAV8 drug products

	2L production	50L production	200L GMP production
Total Genomes qPCR	1.8E+14 vg	7.8E+15 vg	6.8E+16 vg
Total Genomes dPCR	8.2E+13 vg	4.3E+15 vg	4.8E+16 vg
Infectious titer	6.3E+9 IU/mL	6.3E+9 IU/mL	1.9E+10 IU/mL
vg/IU ratio	1.48E+03 vg/IU	4.13E+03 vg/IU	2.11E+03 vg/IU
% Full by mass photometry	64%	62%	67%
Host cell protein (ELISA)	< 20 ng/mL	< 20 ng/mL	20.6 ng/mL
Host cell DNA per 1E+12 vg	12.5 ng	26.5 ng	21.7 ng

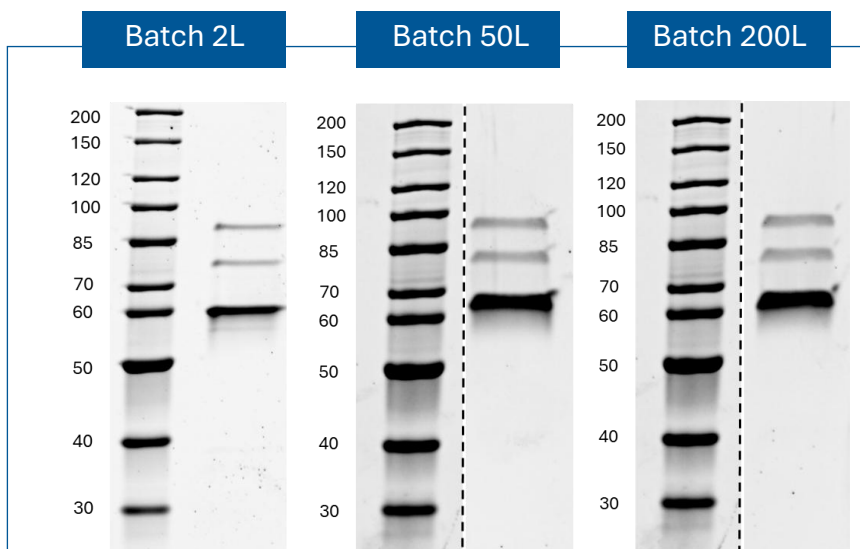


Figure 3: Capsid Protein Purity by SDS-PAGE of drug products

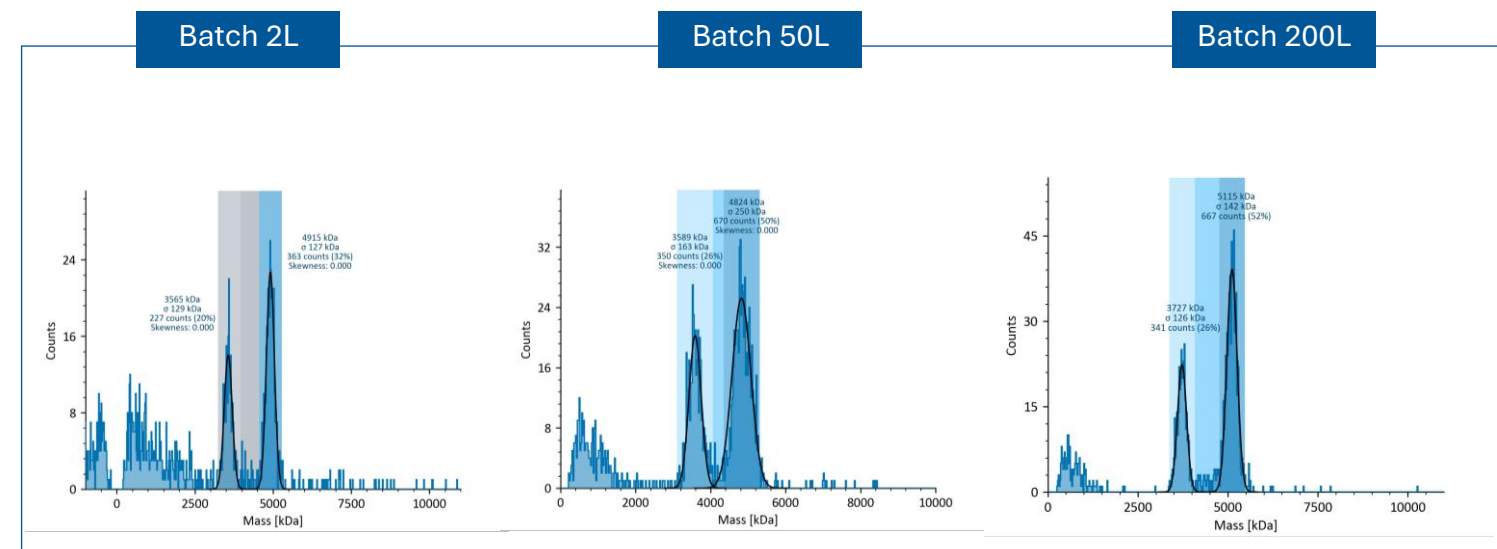


Figure 4: Purity measured on drug substance with mass photometry

3 Development & Manufacturing Service Offering

DINAMIQS offers DINAPEAK® its phase-appropriate flexible platform, design to support projects, progressing from feasibility and manufacturability assessment to 2L process development, up to 50L pilot-scale, and ultimately 200–500L GMP manufacturing. Process and analytical knowledge generated at each stage supports scalability while progressively reducing technical and CMC risk before clinical manufacturing.

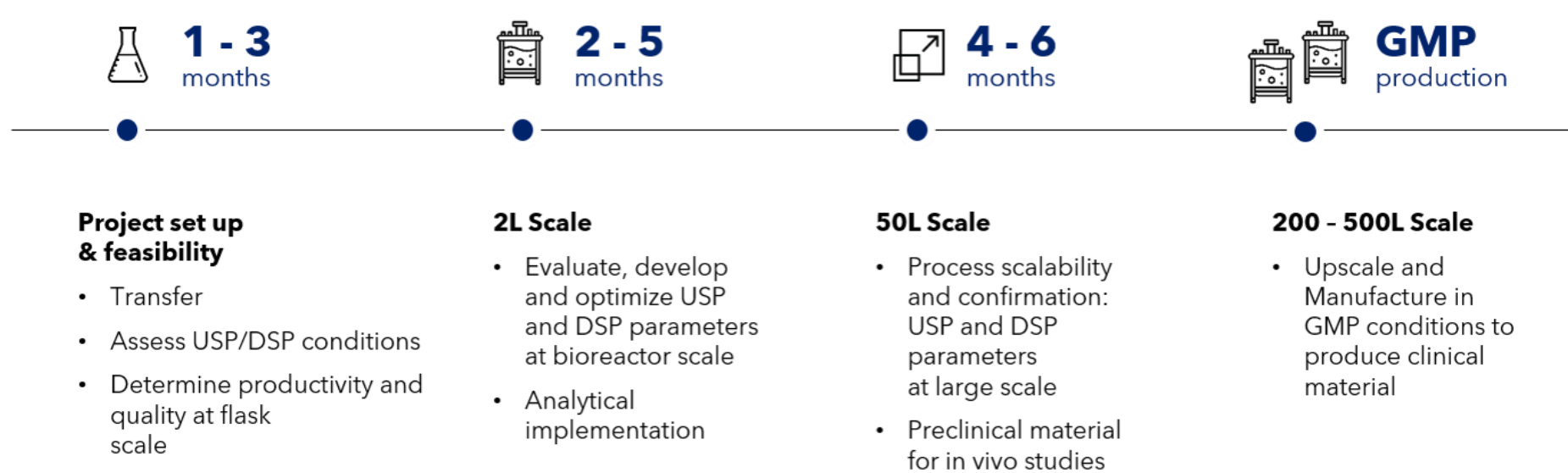


Figure 9: DINAPEAK® offering for phase-appropriate development & manufacturing services

Intensified AEX closes the purity gap with a scalable two-pass strategy reducing residual empty AAV capsids to ~10% and achieving ≥ 80% full capsids, providing a robust polishing solution for gene therapy programs with demanding product-quality requirements.

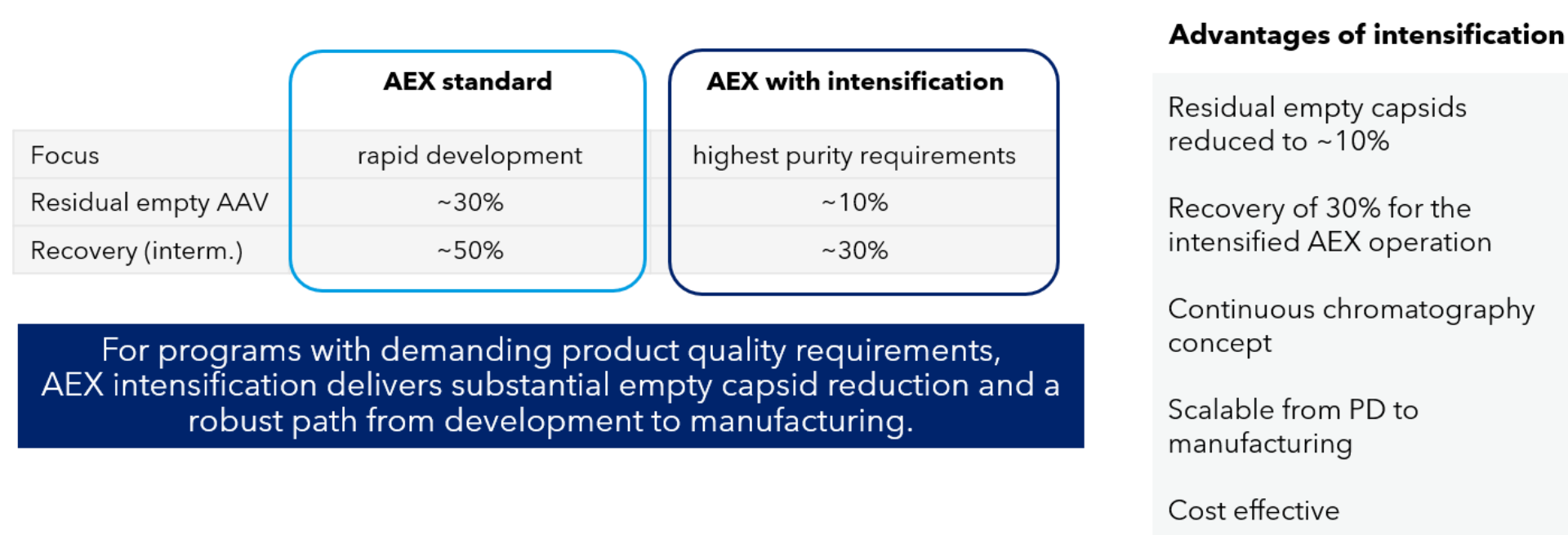


Figure 10: Elevate your AAV purity with our intensification offering

Conclusions

- Demonstration of scale** with consistent downstream performance from 2L to 200L for rAAV8.
- Yield & purity trade-off** — the campaign delivered >30% overall recovery with baseline purity at ~60–70% full AAV capsids.
- Scale-down predicts scale-up** — AEX recovery and purity transferred reliably across an 800× scale factor, de-risking GMP manufacturing.
- Intensified AEX closes the purity gap** — two AEX passes reduced empty capsids ~10% and achieved ≥ 80% full capsids.
- The **monolith column technology** enables purification of AAV at large scale delivering a competitive, cost-effective and fast processing solution
- Holistic approach** – AAV manufacturing relies on robust upstream delivering high titer with high packaging efficiency as a critical pre-requisite to achieve high purity (> 80% full AAV capsids)
- Integrated development accelerates the path to clinic** where fit-for-purpose analytics are required

Successful campaign demonstrating readiness for GMP clinical manufacturing

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