Development of a scalable downstream process with AEX polishing for AAV8 vectors delivering 50% vg recovery and 3-fold enrichment of full AAV capsids

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Introduction

Facing the increasing demand for AAV vectors supporting pre-clinical and clinical programs, AAV8 manufacturing process was scaled up to 50L pilot production. The platform is based on HEK293 suspension cell line transiently transfected with three plasmids system, using clinically relevant transgenes, relying on scalable technologies with single-use bioreactor and empty/full AAV capsid separation based on anion-exchange (AEX) chromatography (see Fig. 1).
The core objectives of the purification process development was to characterize and establish scalable and reproducible critical unit operations such as AAV affinity capture and AEX polishing for empty/full AAV capsid separation using scale-down and scale-up demonstration[1-3].

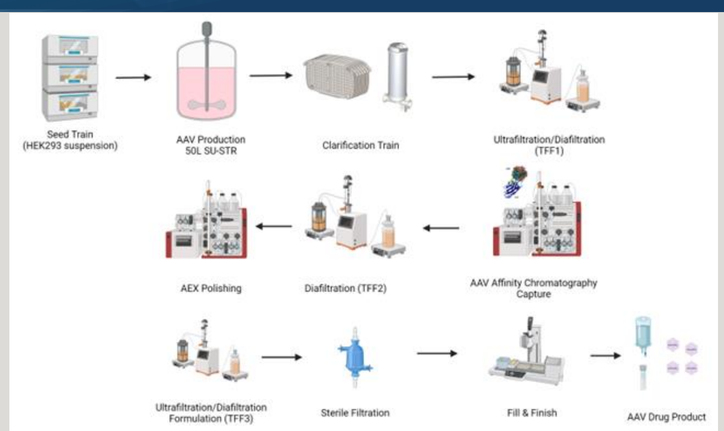

Figure 1: Process Flow Diagram of Pilot AAV Manufacturing Process established at Siegfried DINAMIQS using HEK293 suspension cells.

Results and Discussion

1 Scaling-down Downstream Process Development

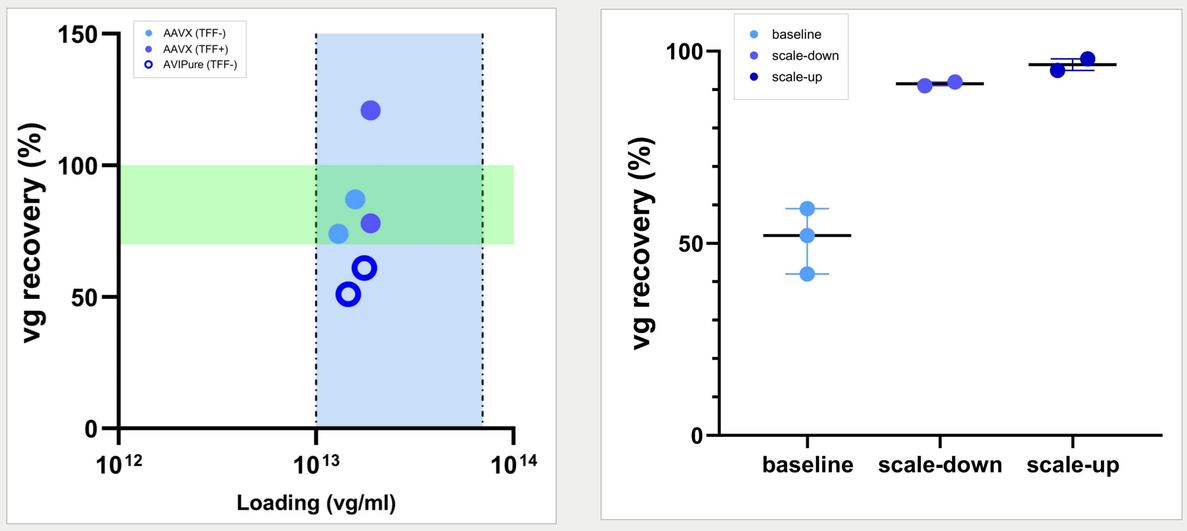

Figure 2: bench-scale AAV Affinity Capture process characterization study assessing effect of loading density, ligand, and loading matrix nature (with and without TFF pre-capture). Optimal conditions found in scale-down were successfully transferred to pilot-scale.

Process development on AAV affinity capture led to 2-fold increase in vector genome (vg) recovery using 0.2ml micro-column. Two critical process parameters (CPP) were identified for AAV8 capsid: loading density and elution conditions with the CaptureSelect™ AAVX affinity technology. Process performance was confirmed from small-scale up to 50L pilot using TFF pre-capture (see Fig. 2).

The AEX purification study aimed at developing scalable and reproducible empty/full AAV8 capsid separation using CIM QA 1mL as scale-down device. Starting material vg/cp ratio was estimated between 31% and 41%.The results showed conflicting recovery and purity responses. The optimal response was achieved by minimizing vector loading density and operating towards an optimal alkaline pH region delivering up to 3-fold full AAV capsid enrichment (see Fig. 3).

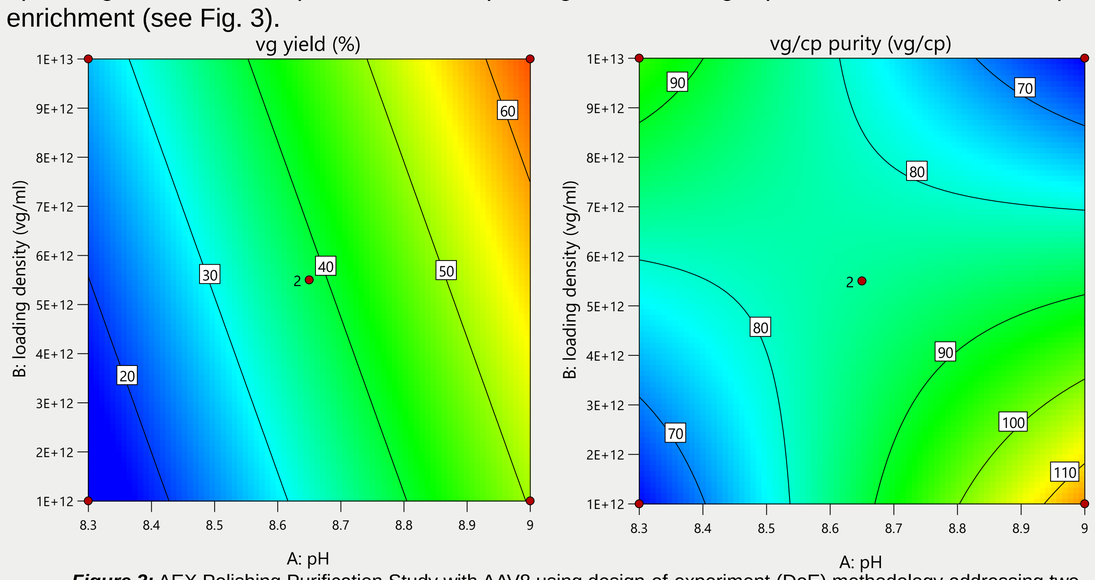

Figure 3: AEX Polishing Purification Study with AAV8 using design-of-experiment (DoE) methodology addressing two conflicting goals: yield and purity.

2 Scaling-up Downstream Process Development

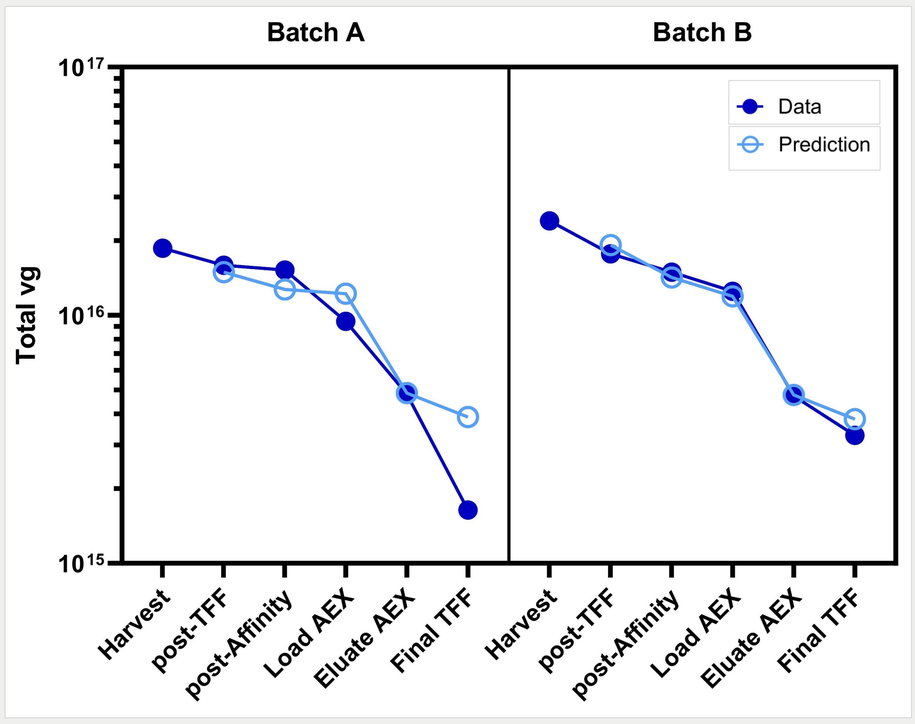

Figure 4: Scale-up to 50L AAV8 downstream process for two AAV8 batches. A good fit was found between predicted vg mass balance obtained from scale-down studies and pilot manufacturing runs.

AEX polishing was further scaled-up to 50L pilot operation using 400ml CIM QA column for two independent AAV8 vector batches. Purity after AEX was ranging from 87% to 104% measured by vg/cp ratio and vg recovery measured between 42% and 62% by qPCR targeting ITR showing process reproducibility within the statistical model limits.

The drug substance and drug product quality was further investigated using orthogonal methods, including mass photometry showing % full capsid up to 54% (see Fig. 5).

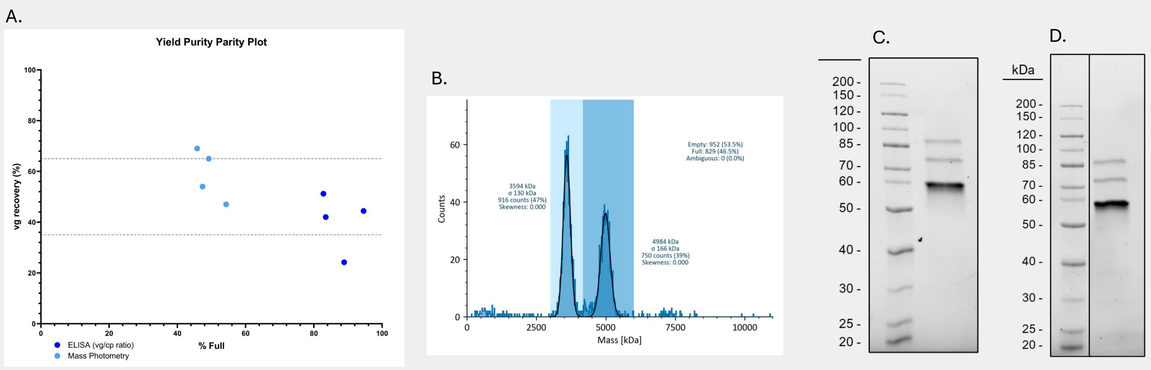

Figure 5: A) Yield/Purity Parity Plot for 4x Pilot AEX Polishing Cycles with drug substance AAV Batch B.
B) Mass Photometry Analysis of the AAV drug product after pooling multiple AEX Polishing Cycles. C/D) SDS-PAGE of batches A and B showing protein purity after downstream processing.

Residual impurities analysis showed HEK293 host cell protein (HCP) below 20 ng/ml in the pilot scale AAV8-based drug products. Furthermore, residual host-cell DNA (hc-DNA) was measured at/or below 50ng per 1E12 vg, with majority being nuclease resistant suggesting encapsidated hc-DNA form (see Table 1).

Table 1: Process-related impurities characterization on AAV drug products after pilot AAV downstream process

Attribute	Test	Batch A	Batch B	Unit
Bacterial endotoxin	LAL-KTA	**1.3**	**1.1**	EU/ml
Residual HCP	ELISA	**< 20**	**< 20**	ng/ml
Residual host-cell DNA (total)	qPCR	**51.0**	**35.2**	ng/1E12 vg
Residual host-cell DNA (nuclease-resistant)	qPCR	**40.2**	**30.5**	ng/1E12 vg

Conclusions

Overall, the accelerated process development project led to establishing and up-scaling an end-to-end AAV8 manufacturing process with compressed timeline while demonstrating reproducibility, consistent quality, predictable yield and competitive productivity.

- Process development focused on critical unit operations driven by Quality-by-Design (QbD).
- AAV affinity capture development led to a significant improvement in recovery from 40% up to 90%, demonstrated from small scale throughout 50L scale.
- AEX polishing process characterization for AAV8 empty/full separation by DoE enabled identification of optimal design space delivering up to 3-fold full AAV8 capsid enrichment
- End-to-end AAV8 pilot Downstream Process delivered ~15% vg recovery with significant clearance of impurities.

References

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Acknowledgements

1. This work is funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or HaDEA. Neither the European Union nor the granting authority can be held responsible for them. **2.** This work is funded by the UK Research and Innovation (UKRI) under the UK government's Horizon Europe funding guarantee grant numbers 10080927 (The Francis Crick Institute Limited), 10079726 (King's College London), 10082354 (Muscular Dystrophy Group of Great Britain and Northern Ireland) and 10078461 (University College London). **3.** This work has received funding from the Swiss state Secretariat for Education, Research and Innovation (SERI).

Project funded by
Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra
Federal Department of Economic Affairs, Education and Research EAER
State Secretariat for Education, Research and Innovation SERI
Swiss Confederation

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Funded by the European Union