The Impact of Empty Capsids on AAV Manufacturing and Strategies for Enhancing Yield, Purity, and Stability in the Production of a Novel Blood-Brain Barrier Penetrant AAV Capsid

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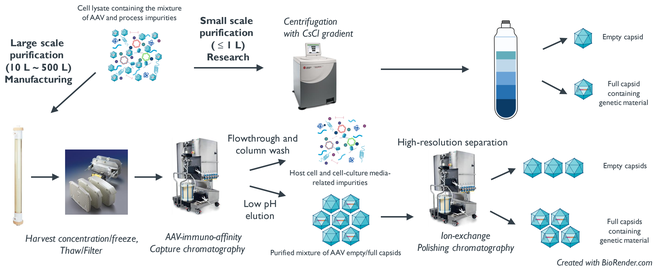
Introduction

- Successful AAV manufacturing is essential to advancing genomic medicines for the treatment of neurological disorders.
- Here we report process optimizations and discuss strategies that enhance the yield, purity, and stability of STAC-BBB, a blood-brain barrier penetrant AAV capsid.
- Column chromatography steps were optimized to enrich full capsid particles containing the therapeutic genome.
- In parallel, an optimized formulation buffer was identified in which STAC-BBB maintains stable vector genome titers under a range of stress conditions.
- Together, these advances support a robust and scalable AAV production process for STAC-BBB

AAV Purification: Lab scale vs. Manufacturing scale



Column loading density: a Critical Parameter for AAV Chromatography Purification

- Column loading density, measured as capsid particles per mL of chromatography resin, is a key factor in purification efficiency.
- Column loading density affects dynamic binding capacity, separation performance, and overall yield. While higher loading densities can enhance product capture, overloading the column compromises recovery and purity - particularly when empty capsids are abundant.
- We investigated whether full and empty capsids exhibit different binding preferences on the affinity column, which could potentially influence loading strategies.

AAV Empty and Full capsid Separation using Ion Exchange Chromatography

- Separation of empty and full AAV capsids can improve product purity and potency.
- Ion exchange chromatography (IEX) separates molecules based on charge differences.
- Full capsids (containing DNA) have a different surface charge than empty capsids (no DNA), allowing separation.
- Buffer pH and salt gradient are critical for optimal separation.
- Careful column selection (anion vs. cation exchange) and gradient design improve resolution.
- We evaluated whether ion exchange chromatography (IEX) can efficiently enrich full capsids and reduce empty capsid content to support regulatory compliance and ensure therapeutic quality.

Methods

Clarified Harvest

AAV	Production Platform	Transfection	Full Capsid
STAC-BBB	HEK293 Suspension	3 plasmids	40 ~ 50%

Capture Chromatography

Affinty Resin	Wash	Elution	Quality Attributes Measured
POROS CaptureSelect	Tris, NaCl, pH 8.0	Buffer (pH 3.0) + Additives	Binding Capacity, Vg titer, Cp titer in the Flowthrough (FT)

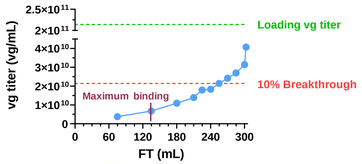
Polish Chromatography

Polish Column	E/F Separation	Quality Attributes Measured
Column Screening (AEX, CEX, Monolith)	Linear Gradient Isocratic Gradient (Large Step → Small Step)	Vg titer, Cp titer, aggregate, E/F in the Elution

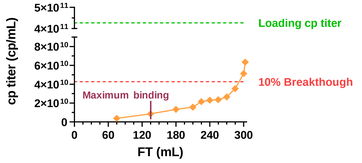
Results

Capture Resin Binding Capacity

Vector genome in the FT



Capsid in the FT



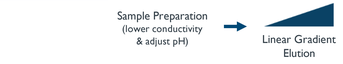
- 300 mL Clarified Harvest / 1 mL affinity column

Dynamic Binding Capacity	Maximum Binding Capacity
5.5E+13 vg / mL-resin	2.9E+13 vg / mL-resin
7.7E+13 cp / mL-resin	5.8E+13 cp / mL-resin
10% breakthrough	1.3 x overload

- At the maximum binding capacity of the affinity column, the eluted AAV contains approximately 50% full capsids (by AUC, SEC-MAL).
- When the AAV load exceeds the column's binding capacity, a greater proportion of vector genome (vg)-containing capsids are found in the flow-through.
- Therefore, optimizing the loading density is essential to improving the full capsid content in the affinity-purified elution.

Polish Chromatography Optimization

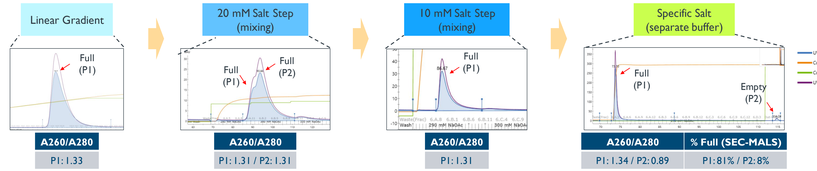
Column Screening



Columns	Binding	Elution	Full capsid Enrichment (A260/A280)
POROS 50 HQ	OK	OK	No
POROS XS	OK	OK	No
CIMmultus QA HR	OK	OK	Yes
CIMmultus SO3	OK	OK	No

Salt Optimization for Full capsid enrichment

- Capture eluate (~ 50% full capsid by SEC-MAL, A260/A280: 1.18) loaded onto CIMmultus QA HR

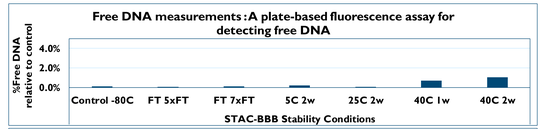


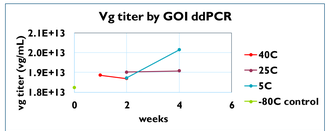
Critical Quality Attributes

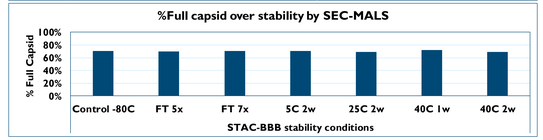
CQAs	Assay	STAC-BBB
Purity	SDS-PAGE	> 99%
	SEC	< 1% Aggregation
Empty / Full capsid	AUC / SEC-MALS	> 70% Full capsid
Potency	ZFR Expression & Target Gene Repression	≥ Shake flask material

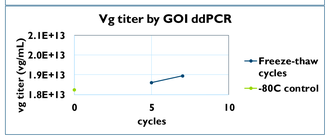
Capsid Stability Assessment

The optimized formulation provided improved stability of AAVs, as demonstrated by selected critical quality attributes (CQAs) shown below









Conclusion

- We evaluated the performance of the Capture and Polish columns using STAC-BBB.
- The affinity resin showed different binding preferences for full versus empty capsids under overload conditions. Therefore, optimizing the loading density is recommended to maximize full capsid recovery during affinity chromatography.
- Polishing column screening and full capsid enrichment were evaluated, achieving over 70% full capsid content using CIMmultus QA HR along with optimization of salt condition.
- The identified formulation meets the target product profile and supports the desired stability budget

Acknowledgments

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