Beyond 90% Full: Expanding the Role of Anion Exchange Chromatography in AAV Product Quality Control

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Unique competitive positioning

- **Best-in-class capabilities** across AAV, lentivirus & other vector types
- **Trusted by global industry leaders** – successful collaborations with big pharma, established biotech and emerging biotech
- **State-of-the-art facilities & scalable production capabilities** designed to meet the growing demand for C&GTs
- **Deep scientific know-how** – a team of world-leading specialists in viral vector optimisation
- **Cutting-edge technology** – leveraging 30 years of insights to enhance speed, efficacy, quality and safety in new therapies
- **Global reach & strategic positioning** with manufacturing facilities located in key biotech hubs

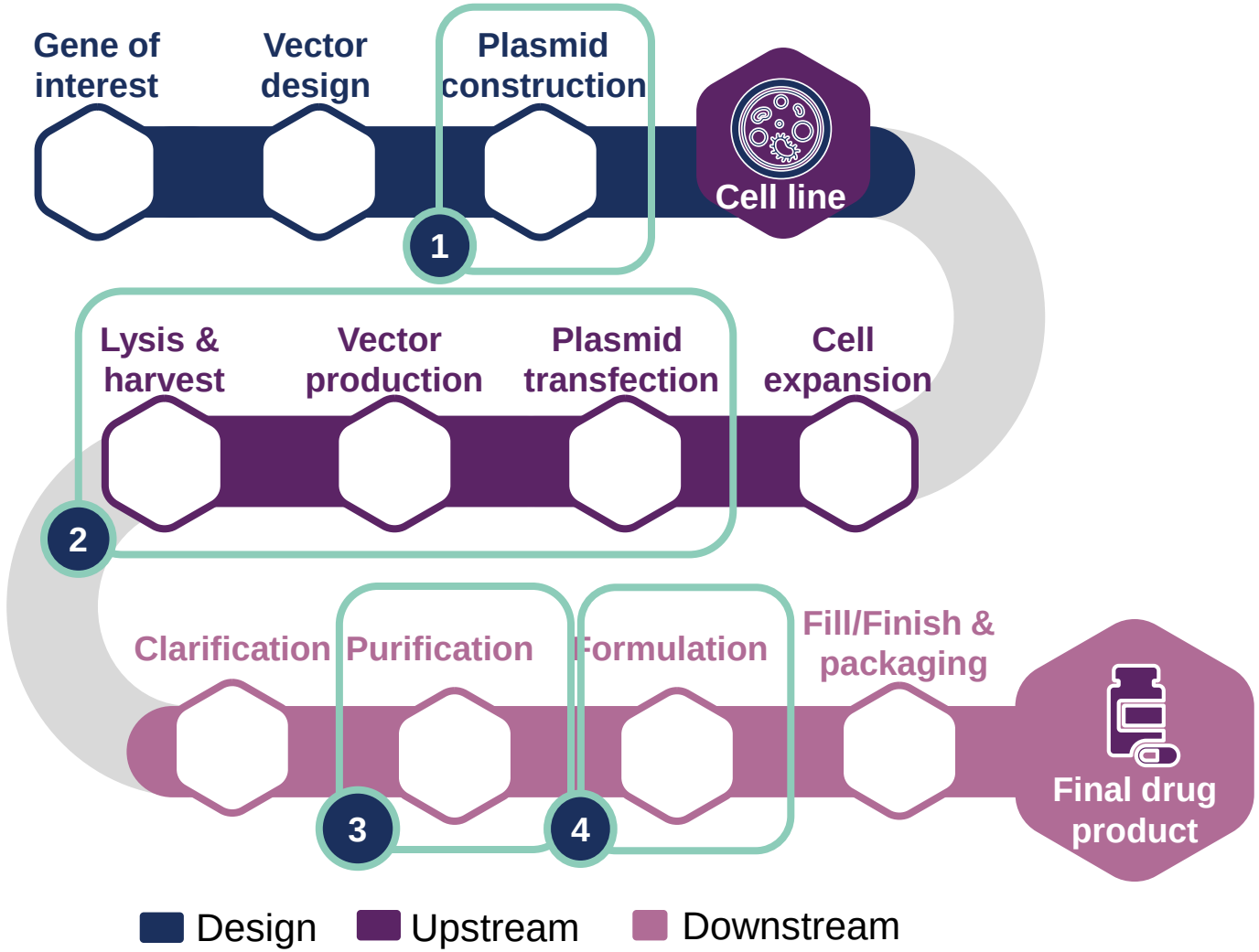
30+	~1,000	40+	45+	100+
Years of manufacturing experience	Successful GMP batches since 2014	Current client programmes	Regulatory submissions	Successful audits

Presentation Overview

1. OXB's AAV Platform
2. AEX Development at OXB
3. AEX can do more than just remove empty capsids

The inAAVate™ Platform

End-to-end AAV manufacturing with built-in innovation

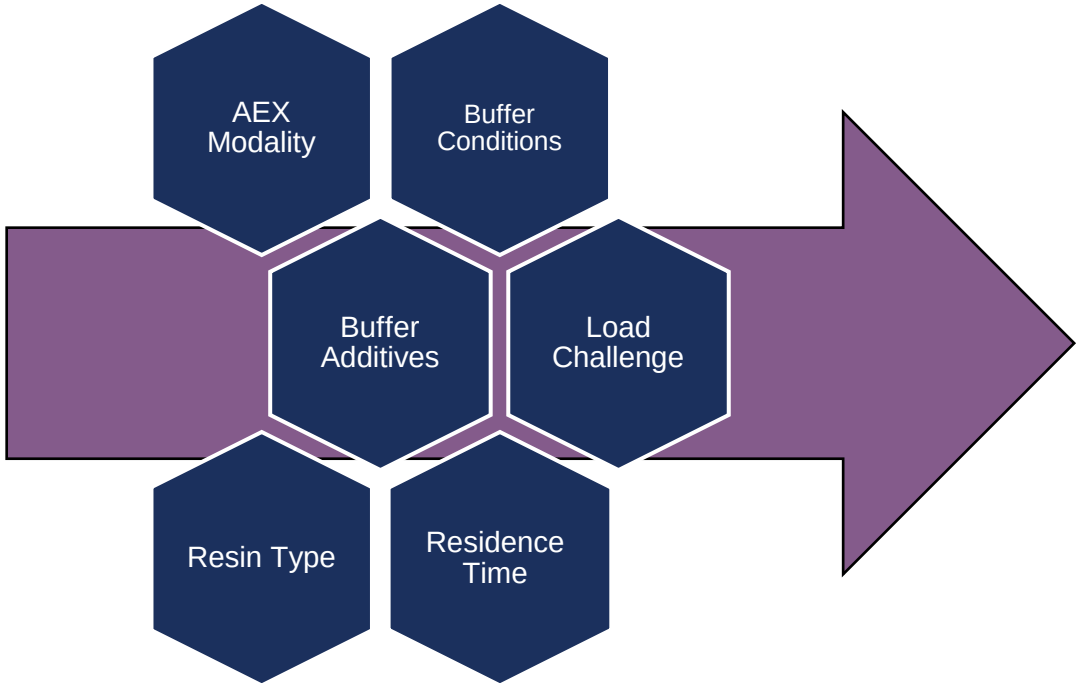


Our platform technologies

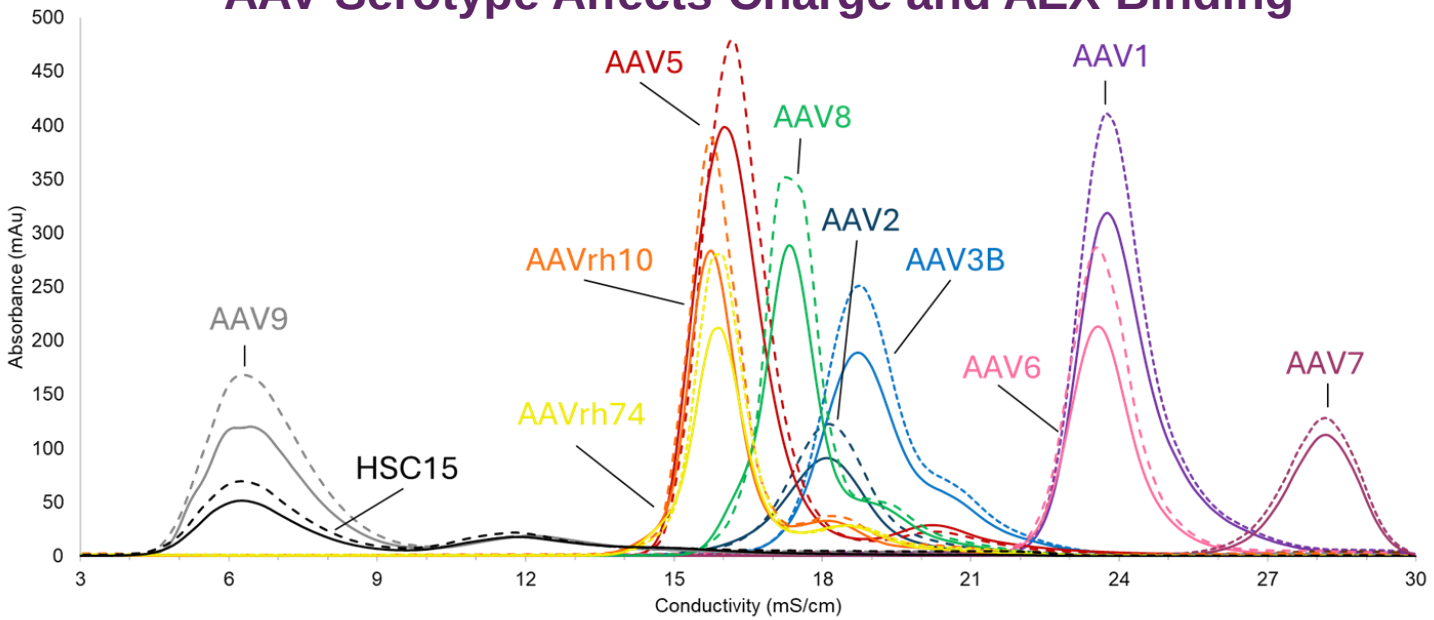
1. **Dual plasmid and pHelper:** improved productivity and packaging efficiency
2. **Vector production and Lysis:** optimized and scalable process for improved productivity and packaging
3. **Purification:** robust and scalable AF and AEX process for high % full capsid and control of PTMs for improved potency
4. **Formulation:** broad applicability to multiple serotypes. Demonstrated stability for 18 months at 2 – 8^{O}C

Building a Multi-Serotype AEX Toolkit for High Purity

Multiple Parameters Influence AEX Resolution



AAV Serotype Affects Charge and AEX Binding



- Charge profiles of AAV serotypes are spread across a broad spectrum
- Packaging efficiency varies for different serotypes, GOI, and process conditions
- Identifying critical parameters that impact empty/full separation can significantly increase development success rate
- OXB platform conditions have been optimized to achieve high % full for commonly used wildtype AAVs
- A toolbox for rapid process fine tuning has been created to achieve 90%+ full within 1-2 weeks including engineered/novel capsids

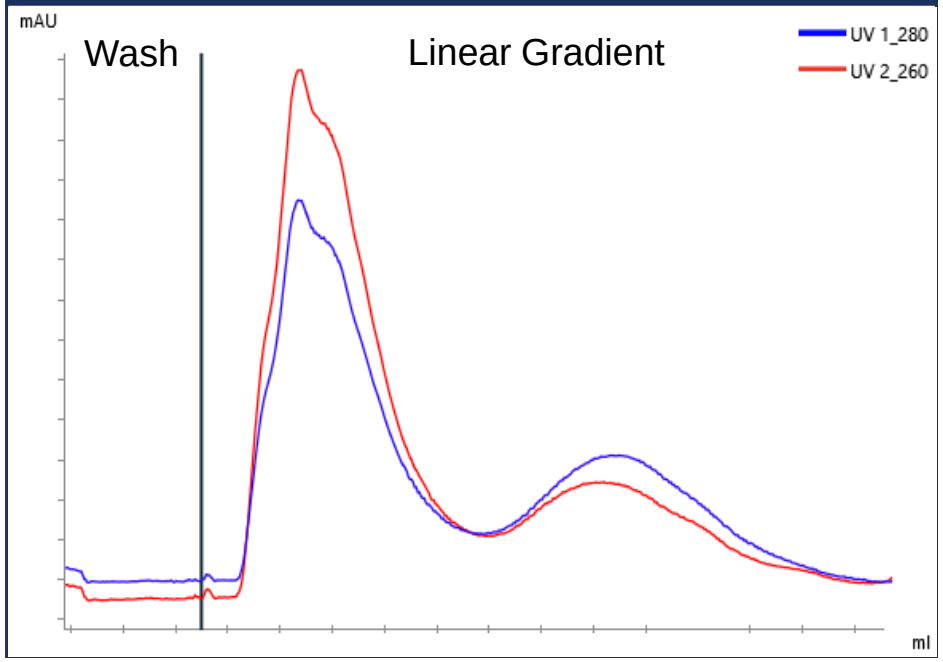
Development in Action with a Difficult Separation

Challenge

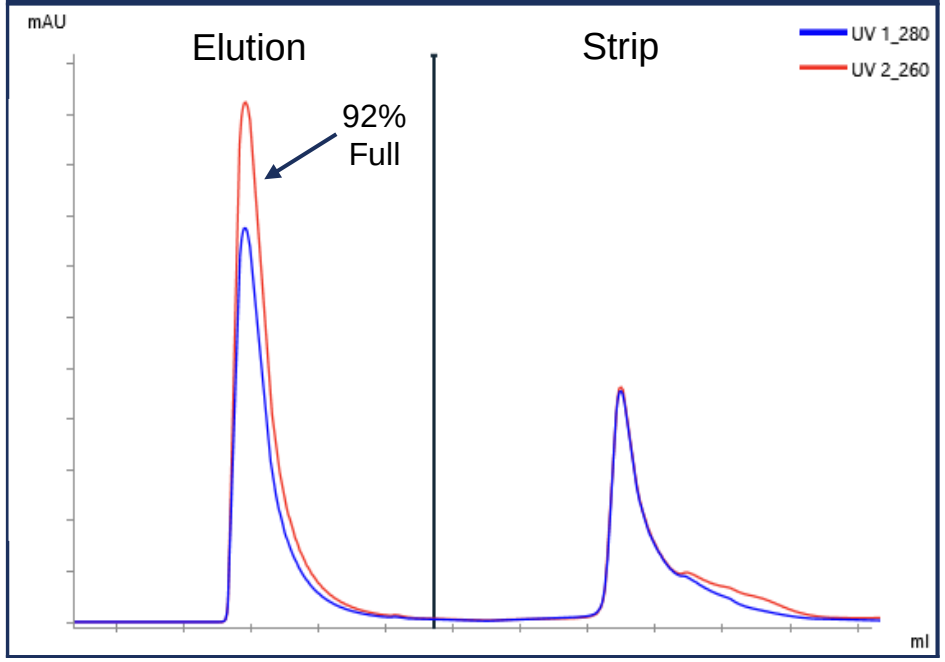
- Novel capsid with modifications that lead to a weak negative charge
- Poor packaging (<20% full) out of the bioreactor results in multiple populations of difficult to remove empty and partial capsids

Toolkit	
AEX Resins with different physiochemical properties	Platform conditions for each resin designed for success with WT vectors

Resin #2

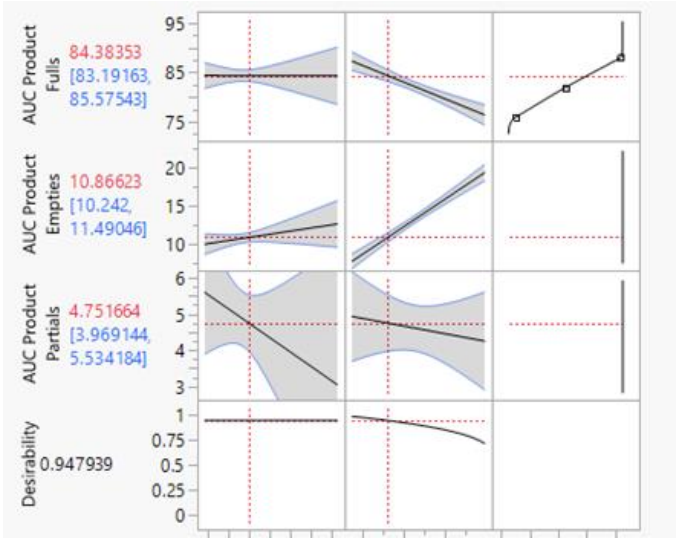


Scale-up Isocratic



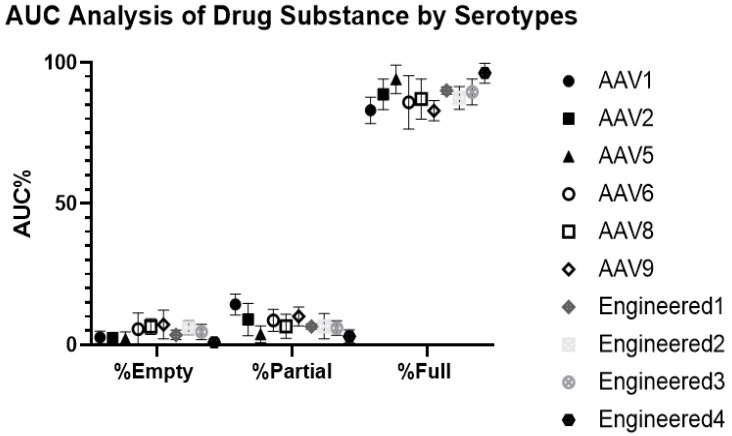
Delivering High Purity without the Need for Extended Development

Defined design space



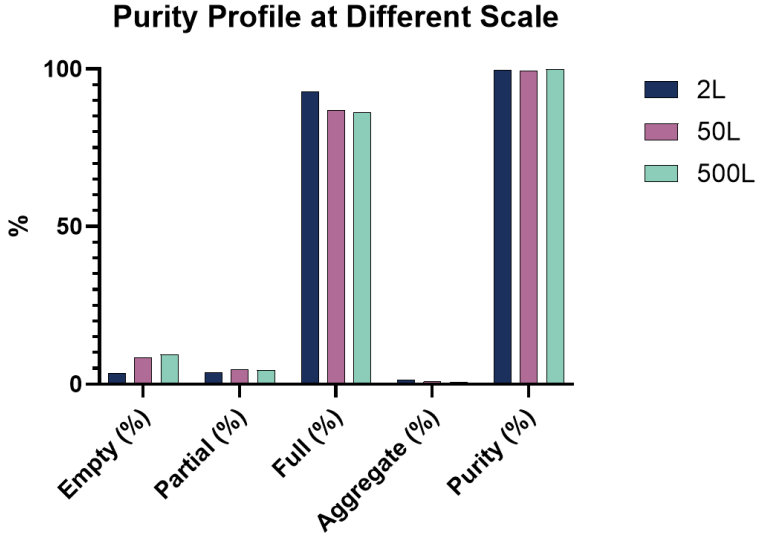
Leverage DoE results, AEX operation space is narrowed down to optimum range for multiple serotypes

%Full enrichment over AEX (dual vs. triple transfection)



Most AAV serotypes tested with the optimum process range demonstrated up to 90%+ full capsids

Scalable product quality



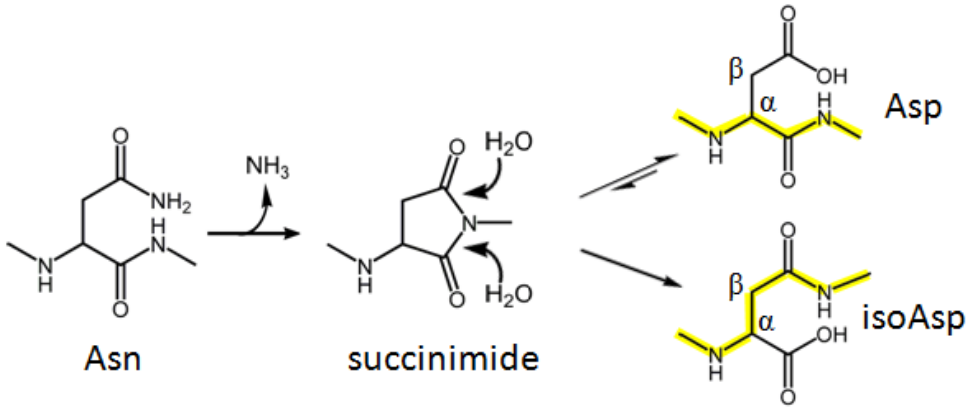
Scalability is maintained from PD bench scale to GMP full scale

What other product related impurities can AEX remove besides empty capsids?

Deamidation and How it can Occur in the Process

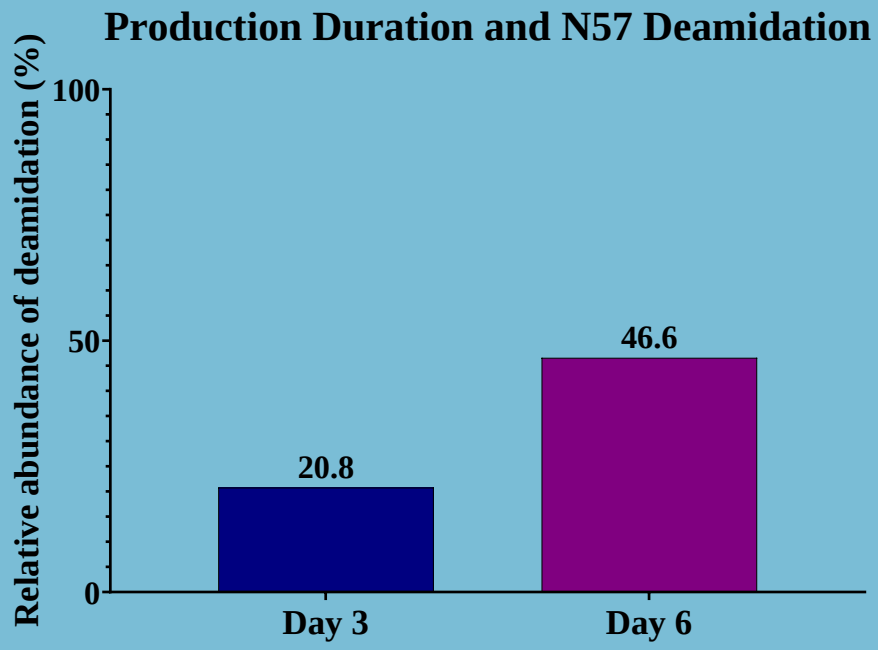
Deamidation is a non-enzymatic post-translational modification in which an asparagine residue undergoes spontaneous hydrolysis to form aspartate or isoaspartate

Common LC-MS monitoring sites: N57 and N94



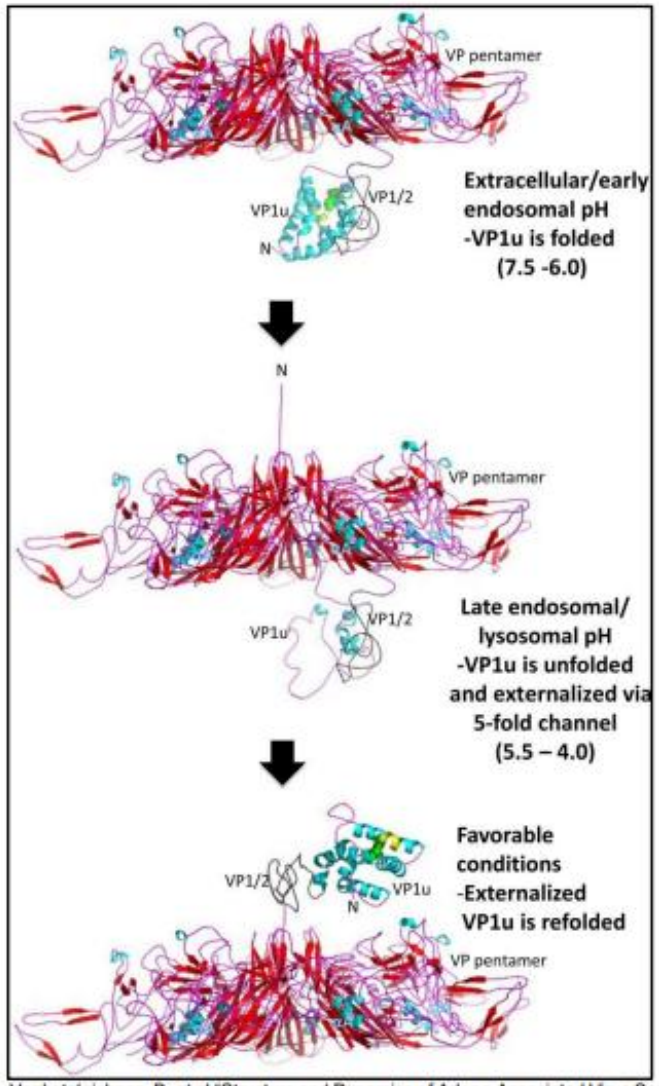
Factors that may cause deamidation:

1. Production duration
2. High temperature
3. High pH
4. Low ionic strength conditions

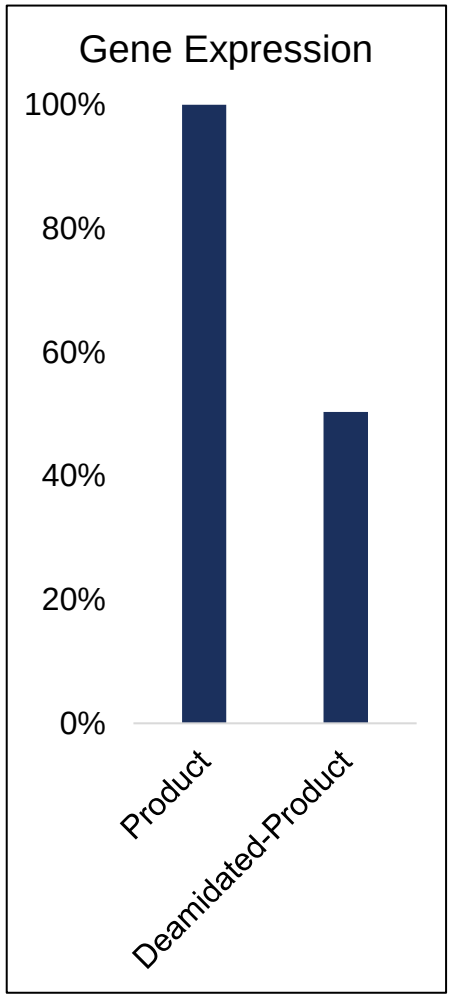


See publication for more details:
Impact of Adeno-Associated Virus Production on Capsid Heterogeneity and Product Quality
https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/bit.70219

Why Should we be Concerned about Deamidation in the Process?



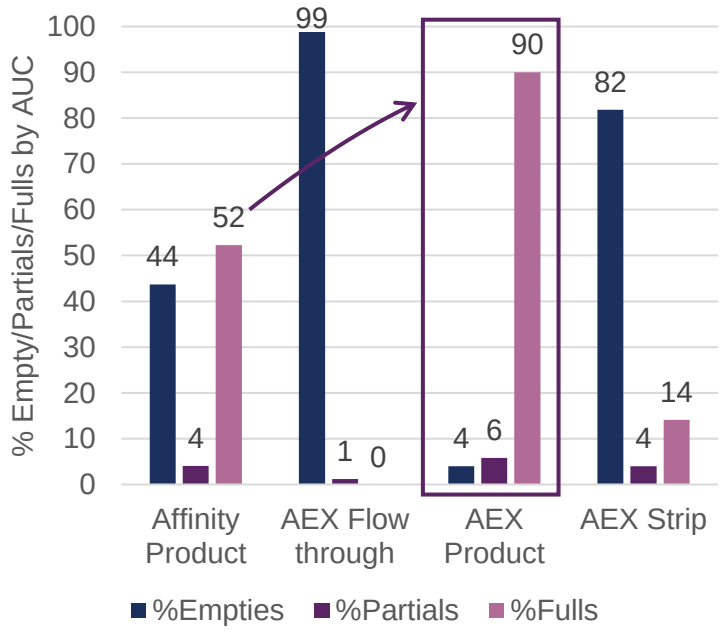
Venkatakrishnan, B. et al "Structure and Dynamics of Adeno-Associated Virus Serotype 1 VP1-Unique N-Terminal Domain and Its Role in Capsid Trafficking"



- Deamidation increases negative charge on the capsid surface and can impact stability, potentially causing conformational changes and the exposure of more hydrophobic areas
- Deamidation can influence VP1u extrusion, which is linked to endosomal escape via the PLA2 domain
- Decreased PLA2 activity can result in reduced infectivity and gene expression

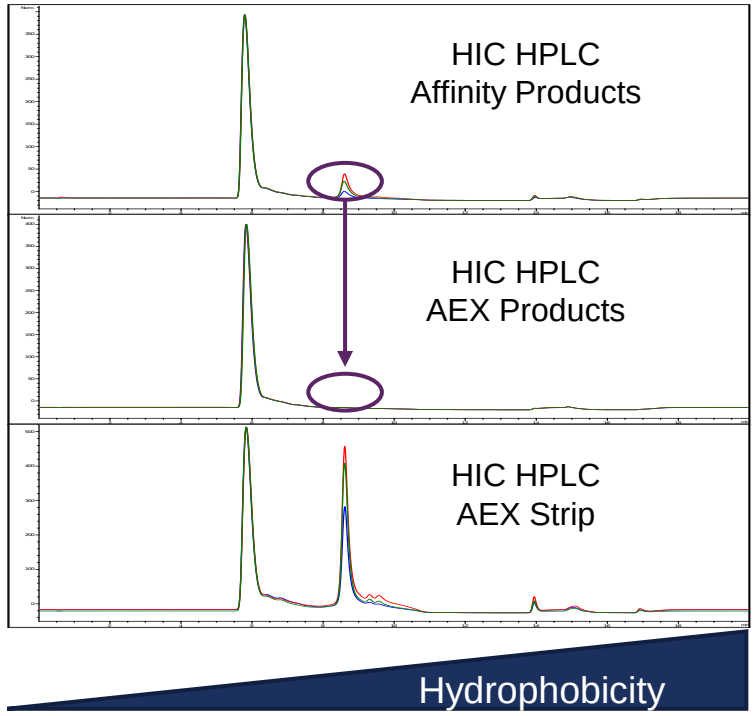
AEX Step Selects Not Only for Full Capsids but Also for Less Hydrophobic and Deamidated Material

Separation by Packaging



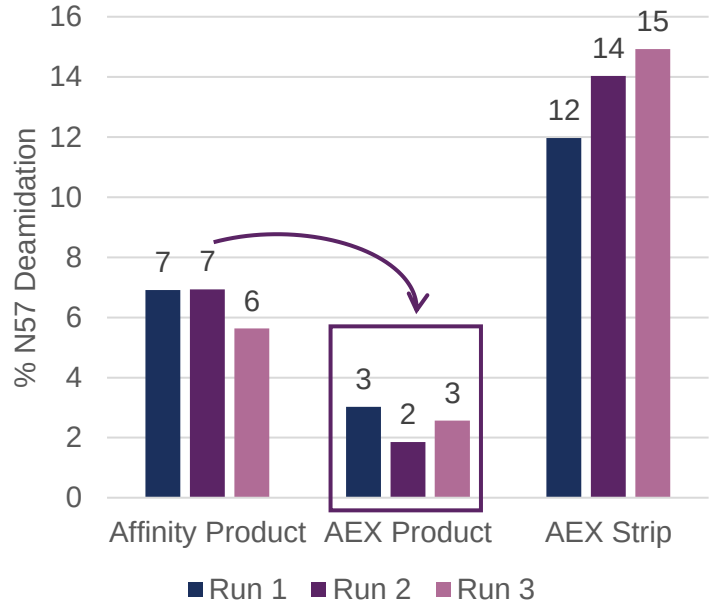
AEX is operated to minimize binding of empty capsids and elute high % fulls

Separation by Hydrophobicity Level



AEX removes hydrophobic species

Separation by Deamidation Level (N57)



AEX selects for less deamidated capsids

Summary

Built on years of experience with AAV, OXB has developed an AEX toolkit capable of delivering high purity across multiple serotypes

- Leveraging platform conditions developed for wild-type vectors enables reduced development timelines, even for tricky novel capsids
- Applying these conditions with our platform anion exchange chromatography resins enables consistent delivery of >90% full

Deamidated AAV tends to be more negatively charged and hydrophobic, allowing its removal using our AAV platform

- Deamidation may reduce potency, making removal of these variants critical to product quality

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