Separation of Empty and Full Engineered Adeno-Associated Virus Capsids Using a Weak Anion Exchanger (Abstract 1038)

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Introduction

Gene therapy has shown promising results to treat genetic disorders. In this space, recombinant Adeno-associated virus (rAAV) using engineered capsids has gained a lot of attention due to their ability to target specific tissues and improved transduction efficiencies. Capsida has successfully developed unique capsids that cross brain blood barrier (BBB) while de-targeting other organs[1]. As the need to manufacture such highly specific engineered capsids grows, several unit operation process parameters need to be optimized to suffice the growing demand. One of the key consideration is separating full capsids containing the gene of interest (GOI) from empties. Currently, full and empty capsid separation often relies on ultracentrifugation, however, scaling ultracentrifugation is infeasible. In this work, we report successful enrichment of full capsids using anion exchange chromatography (AEX).

Methods and Materials

Engineered AAV capsids (rCap) were first captured using affinity chromatography. The affinity elution pool was buffer exchanged into a low salt solution at a higher (basic) pH, and AEX screening work began using a two-step approach. Initially, different resins (3 strong AEX and 2 weak AEX) were screened and binding strength assessed using SDS-PAGE and Western blot. Based on this initial binding assessment, resins were selected. Elution conditions (different salts, pH conditions) were then screened on 1 mL columns. Elution fractions were analyzed using absorbance ratios, ddPCR, and ELISA based assays. AEX work was performed with three different rCAPs.

Figure 1. Downstream Process Flow Overview

Results and Discussion

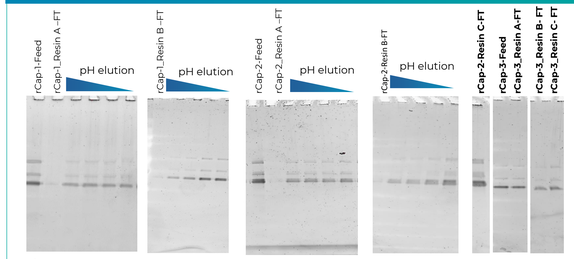
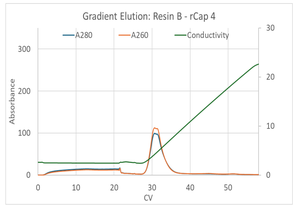
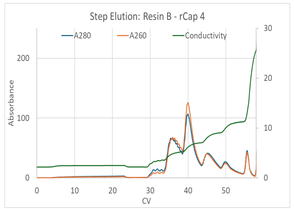


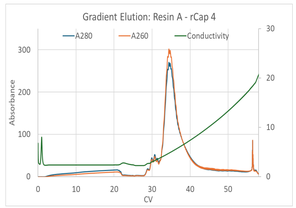
Figure 2. Initial screening of different AEX resins with multiple novel capsids

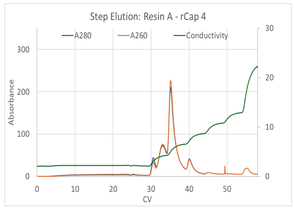
- Strong AEX resins bound 3 engineered capsids at high pH conditions, however a significant portion of the bound capsids were washed off when the pH was lowered by 0.5 or more units
- One of the weak anion exchangers was protonated at the higher loading pH and failed to bind any of the engineered capsids



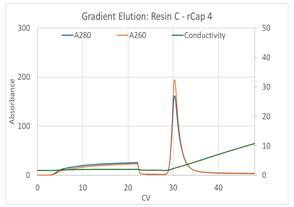


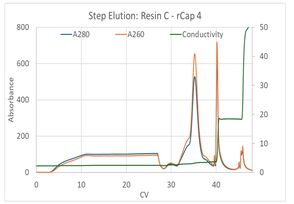
- Full and empty capsids co-eluted at low conductivity during gradient elution
- Shallow wash with increasing salt concentration removed some empty capsids, however co-elution of full capsids was observed
- Based on absorbance, full enrichment of ~ 1.5x was achieved



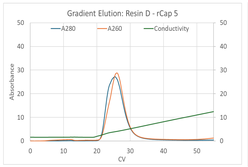


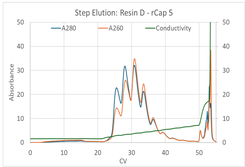
- Breakthrough was observed (rCap-4) with strong anion exchange resin at lower pH. The capsid population in the flow-through was mostly empty capsids. However, gradient elution did not further enhance full/empty separation
- Shifting the pH for the load lowered the breakthrough. Shallow wash with increasing salt concentration removed some empty capsids, however the two capsid populations co-eluted at low conductivities
- Based on absorbance, full enrichment of ~2.3x was achieved

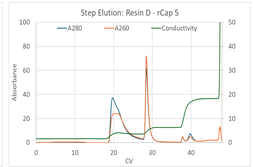




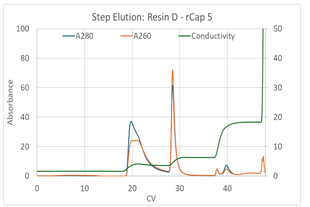
- Breakthrough was significantly higher with weak anion exchange resin at high pH. Gradient elution did not improve separation of the two capsid populations.
- Changing the operating pH helped enrich full capsids
- Based on absorbance, the highest enrichment of ~2.7x was achieved

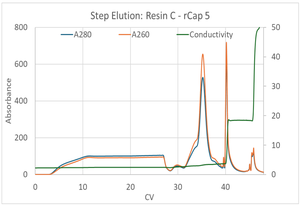






- No breakthrough was observed (rCap-5) with strong anion exchange resin at lower pH
- Under a conductivity gradient, full and empty capsids initially co-eluted then a transition was observed where empty capsids eluted followed by full capsids
- Performing multiple shallow elution steps helped with selectively desorbing empty capsids
- Two step elution was developed which helped in enriching the full capsids significantly
- Based on absorbance, full enrichment was ~2.3x





- With resin D, no capsids were observed in the flowthrough. Using a conductivity shifted wash step, empty capsids were selectively eluted and full capsids were enriched ~2.7x from the starting pool
- For Resin C, binding did not occur at the high pH as the resin was completely protonated. Adjusting the load condition and lowering the pH allowed enriching the full capsids as the majority of the empty capsids did not bind

Conclusions

- Using a two-step AEX development approach, conditions for full enrichment of engineered capsids can be identified. Initially, binding strength for different resins and under different pH conditions is assessed using SDS PAGE and Western blot. Subsequent condition screening is performed with the selected resin(s).
- Engineered capsids may interact differently with the same AEX resin under the same load conditions because of unique modifications
- Understanding the unique modifications on engineered capsids, we are able use capsid specific loading conditions, and empty capsids can be removed from the total population under flow-through mode

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- Volpe et al, Alternative Plasmid Designs Including Two Plasmid Transfection Systems for Improved Production and Packaging of Engineered AAV Capsids (Abs 530)

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- Shi et al, Directed Evolution of AAV2 Libraries Yields Capsids with Improved Performance in the Central Nervous System and Cross-species Translatability (Abs 992)

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Acknowledgements

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