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Separation of Empty and Full AAV Capsids by Multimodal Metal Affinity Chromatography

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Initial AAV Purification

AAV2/8 or AAV2/9 clarified harvest from Sf9 cells was first processed by tangential flow filtration (TFF) coupled with Kryptonase™ treatment to reduce host cell DNA. Initial AAV capture step was performed on CIMmultus® SO3 cation exchange column. After elution with sodium chloride gradient AAV fraction was cleared of DNA and protein contaminants. Separation of empty and full AAV capsids was performed by multimodal metal affinity chromatography with CIMac™ and CIMmultus® PrimaT.

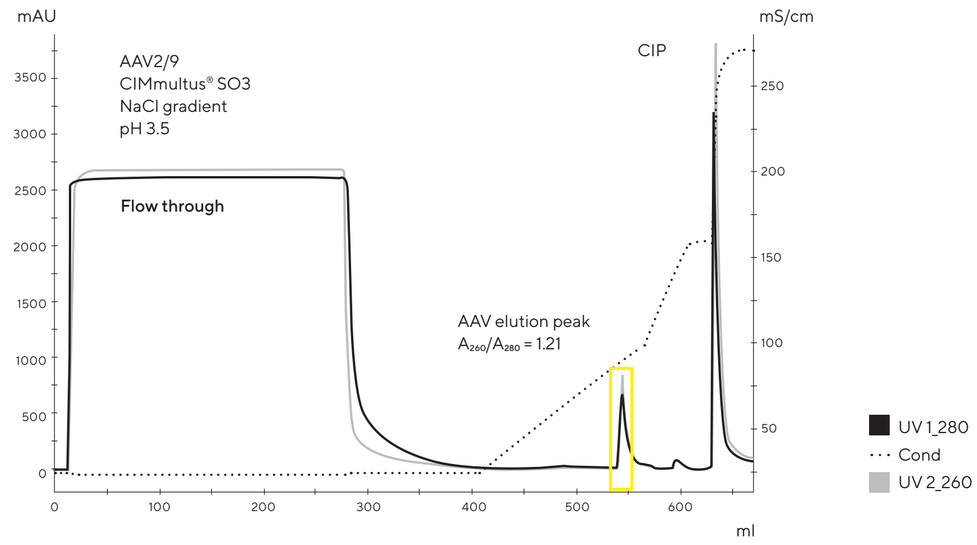


Figure 1: Cation exchange chromatography of AAV2/9 sample on CIMmultus® SO3 8 mL column on Akta Avant FPLC system. Sample: TFF retentate, acidified to pH 3.5, MPA (B): 50 mM formic acid, 1% sucrose, 0.1% poloxamer 188, 200 mM NaCl (2 M NaCl) pH 3.5, MPC: 1 M NaOH + 2 M NaCl. Flow rate: 24 mL/min. Method: direct inject, 15 CV MPA wash, 20 CV gradient 0 to 50%MPB, 5 CV gradient 50 to 100% MPB, 50 CV MPC. Detection: absorbance (260 nm, 280 nm).

Separation of Empty and Full AAV Capsids on PrimaT

Removal of empty capsids is a particular goal of AAV purification. Multimodal PrimaT ligand offers new options for removal of empty and also damaged capsids. Besides, it also contributes to better clearance of contaminating DNA. PrimaT works for different AAV serotypes – for example AAV2/8 and AAV2/9 (see figures below).

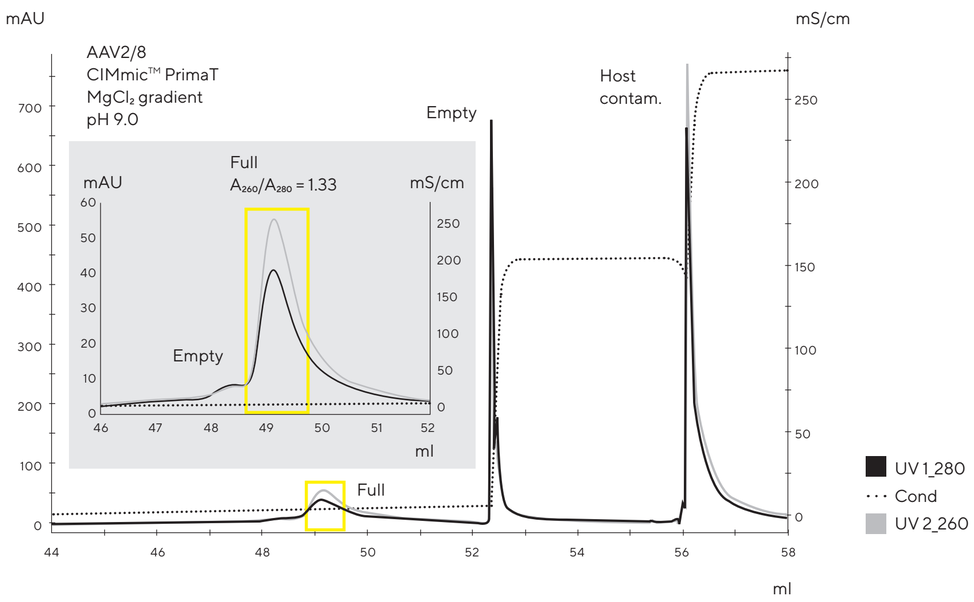


Figure 2: CIMmic™ PrimaT 0.1 mL on Akta Pure FPLC system, sample: AAV2/8 CEX eluate, buffer dilution with MPA, MPA (B1): 50 mM Tris, 12 mM boric acid, 1% sucrose, 0.1% poloxamer 188, (50 mM MgCl2) pH 9.0, MPB2: 50 mM Tris, 12 mM boric acid, 1%sucrose, 0.1% poloxamer 188, 2 M NaCl, pH 9.0, MPC: 1 M NaOH + 2 M NaCl. Flow rate: 1 mL/min. Method: Direct inject, 15 CV MPA wash, 50 CV gradient 0 to 50% MPB1, 30 CV wash with 100% MPB2, 50 CV MPC. Detection: absorbance (260 nm, 280 nm).

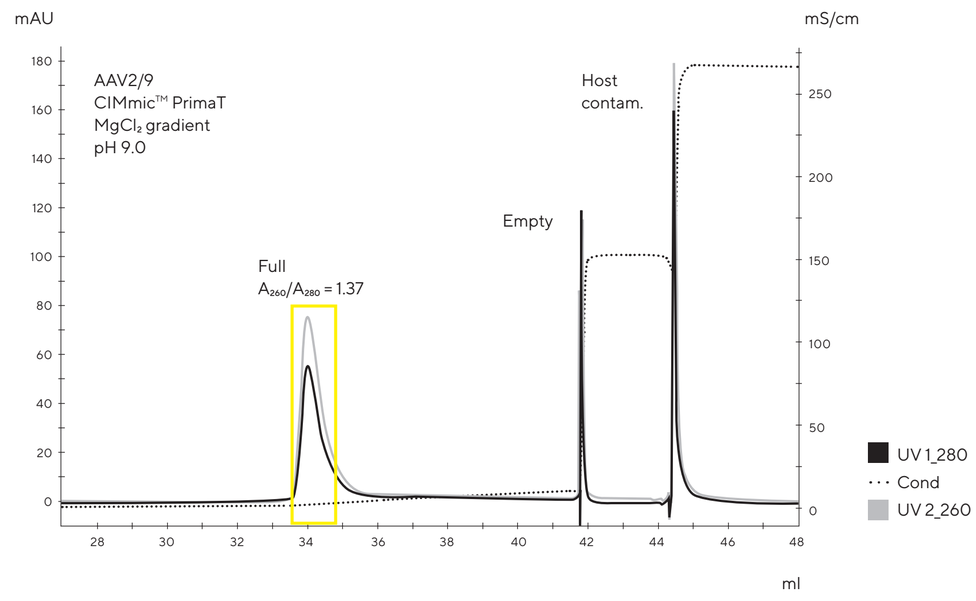
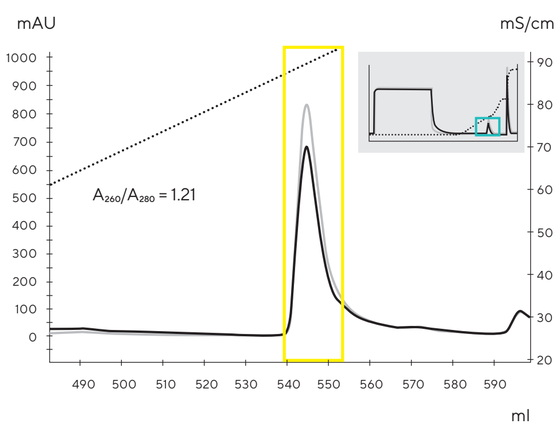


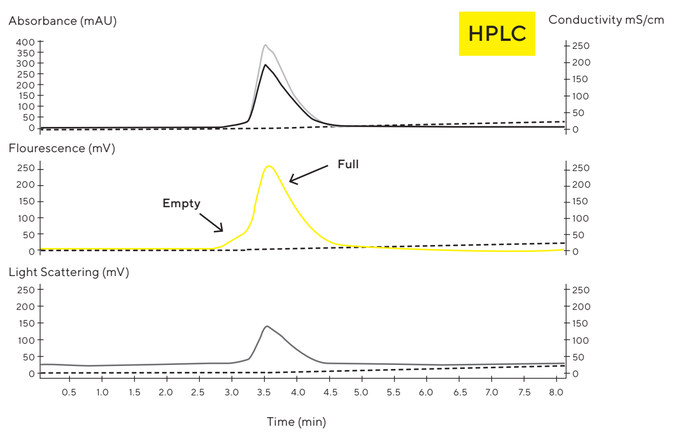
Figure 3: CIMmic™ PrimaT 0.1 mL on Akta Pure FPLC system, sample: AAV2/9 CEX eluate, buffer exchanged to MPA, MPA (B1): 25 mM BTP, 1% sucrose, 0.1% poloxamer 188, (50 mM MgCl2) pH 9.0, MPB2: 25 mM BTP, 1% sucrose, 0.1% poloxamer 188, 2 M NaCl, pH 9.0, MPC: 1 M NaOH + 2 M NaCl. Flow rate: 1 mL/min. Method: Direct inject, 15 CV MPA wash, 50 CV gradient 0 to 50% MPB1, 30 CV wash with 100% MPB2, 50 CV MPC. Detection: absorbance (260 nm, 280 nm).

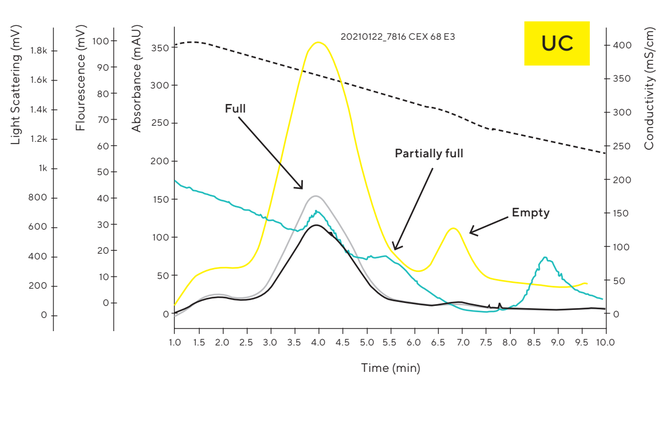
Ortogonal Tools to Study AAV Particles Distribution – HPLC vs Ultracentrifuge

AAV2/9 – SO3 elution

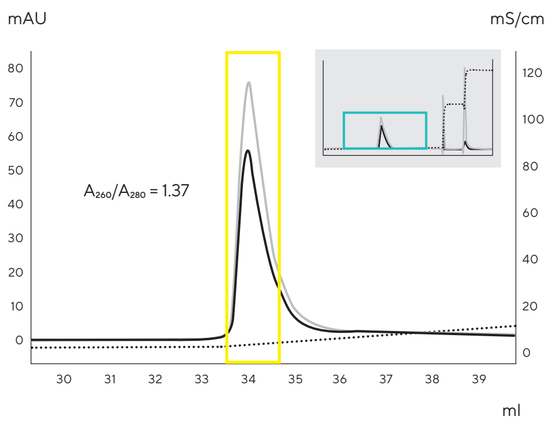


to HPLC and UC

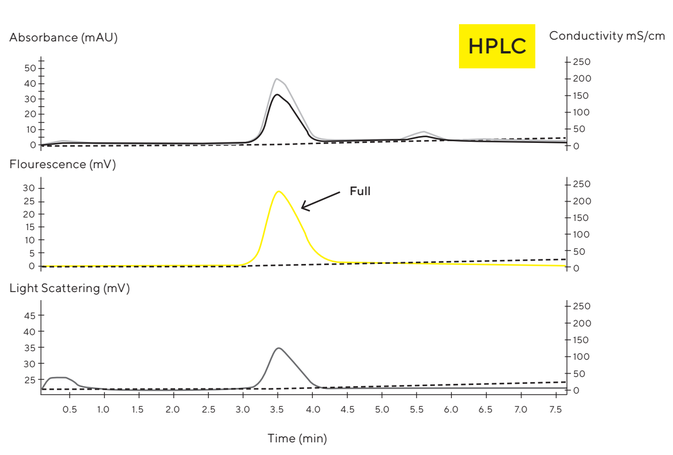




AAV2/9 – PrimaT elution



to HPLC and UC



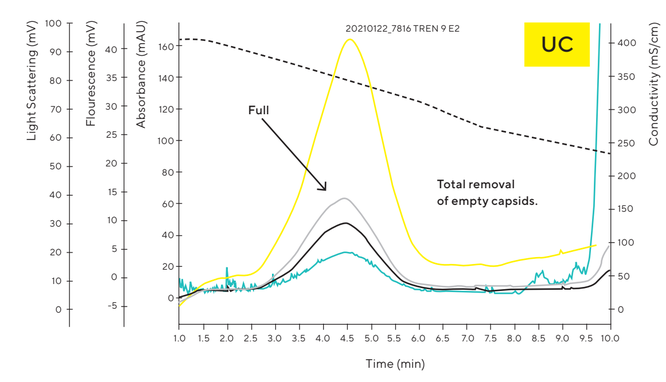


Figure 4: HPLC and ultracentrifuge analysis in CsCl gradient. Sample: AAV2/9 full fraction after separation on CIMmic® PrimaT. HPLC analysis: CIMac™ AAV (QA) column, NaCl gradient, MPA (B): 20 mM BTP, 2 mM MgCl2, (400 mM NaCl) pH 9.5. UC analysis: spin for 24 h at 50000 g. Legend: PATfix® HPLC (grey line –260 nm absorbance, black line – 280 nm absorbance) in conjunction with fluorescence (yellow line – intrinsic tryptophan fluorescence) and MALS detector (teal line –MALS signal) was used for visualization of separated fractions.

Conclusion

- Excellent separation of full AAV2/8 or AAV2/9 capsids from other particles was achieved by PrimaT.
- Density gradient centrifugrams provide orthogonal confirmation of empty and full capsid content.

References

Gagnon P et al. Streamlining industrial purification of adeno-associated virus. BioProcess International 2020; 18: S14-S20
Gagnon P et al. Multiple-monitor HPLC assays for rapid process development, in-process monitoring, and validation of AAV production and purification. Pharmaceutics 2021; 17: 113
Peljhan S et al. Multiple-parameter profiling of density gradient fractionation for characterization of empty and full capsid distribution in AAV Preparations, Cell Gene Ther. Insights 2021; 3: 161–168.