

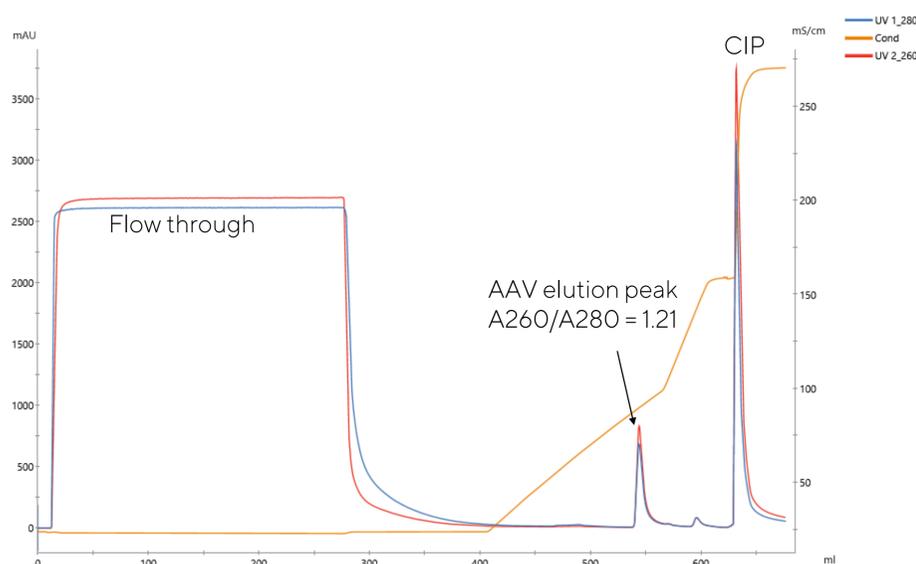
CIM PrimaT - New multimodal ligand for separation of empty and full AAV9 capsids

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Concentration and purification of AAV9

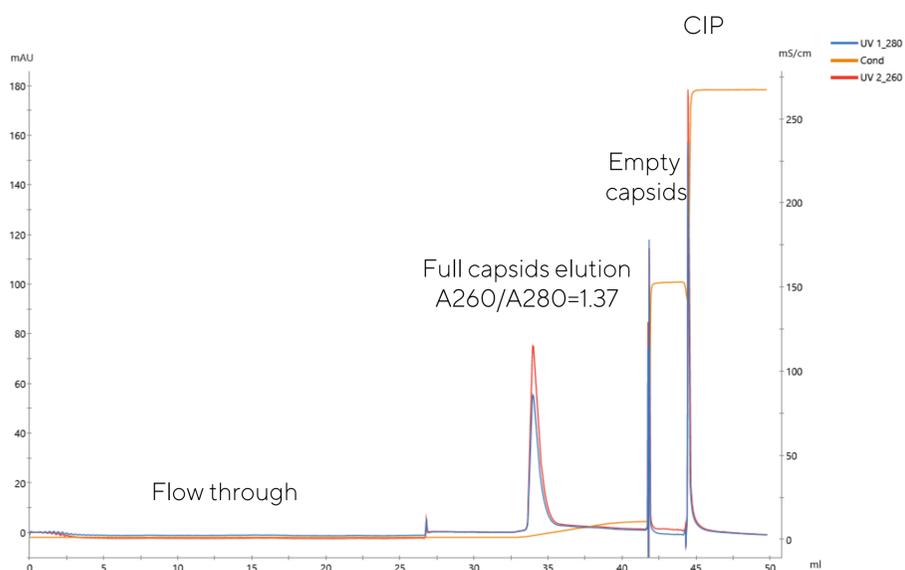
AAV9 clarified harvest from Sf9 cells was concentrated and purified using combination of tangential flow filtration, nuclease treatment and cation exchange capture. First part was TFF coupled with Kryptonase treatment. Capture step was performed on CIMmultus SO3 cation exchange column. AAV elution fraction was cleared of DNA and protein contaminants and prepared for final polishing – empty capsid removal.



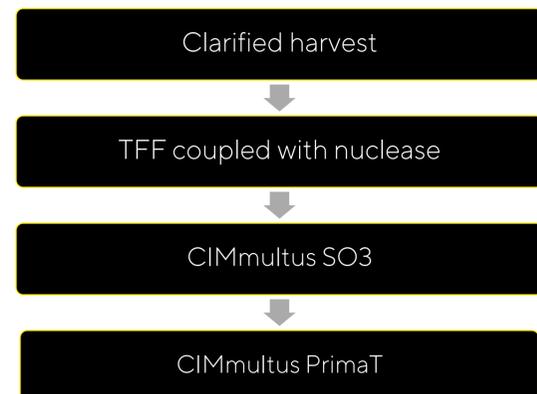
CIMmultus SO3 8 mL on Akta Avant FPLC system, sample: TFF retentate, acidified to pH 3.5, MPA (B): 50 mM formic acid, 1% sucrose, 0.1% poloxamer 188, 200 mM NaCl (2 M NaCl) pH 3.5, MPC: 1 M NaOH + 2 M NaCl. Flow rate: 24 mL/min. Method: Direct inject, 15 CV MPA wash, 20 CV gradient 0 to 50% MPB, 5 CV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

Separation of empty and full AAV9 capsids on PrimaT ligand

PrimaT separation mechanism is based on ligand multimodality, one of them being metal-chelating ability. This was successfully exploited for AAV capsid separation. AAV 9 binds to column at elevated pH conditions and is eluted from column in MgCl₂ gradient. Full AAV9 capsids elute in a single homogenous peak. Elution peak 260/280 ratio is 1.37. Empty capsids are separated in gradient or in salt strip. Step recovery of full AAV9 capsids was 100% (determined by ddPCR).

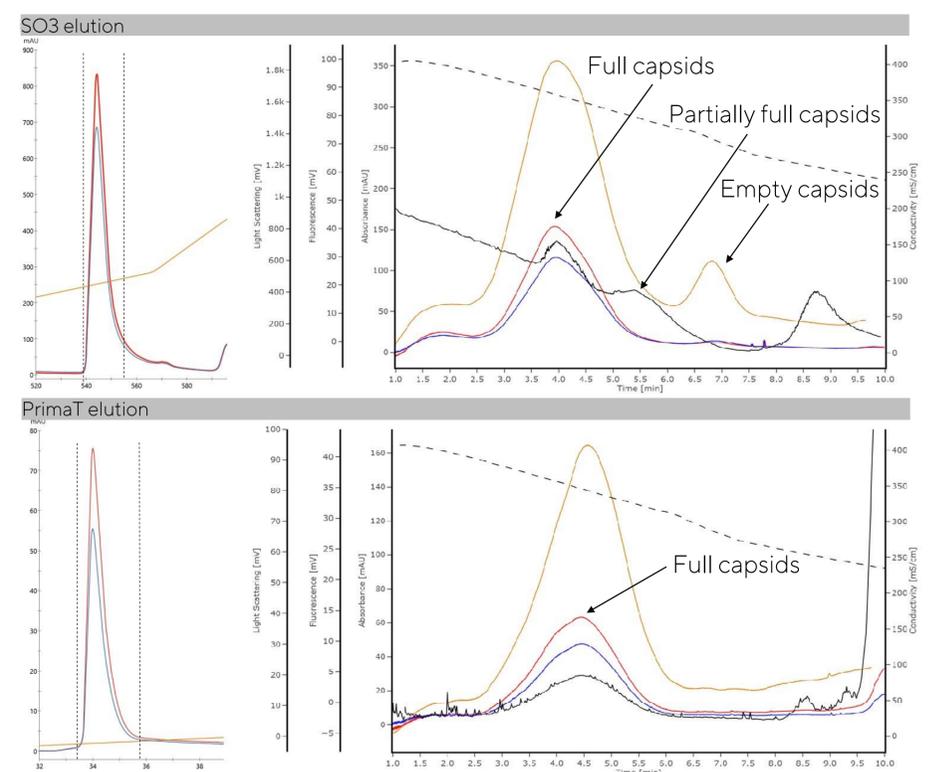


CIMmic PrimaT 0.1 mL on Akta Pure FPLC system, sample: CEX eluate, buffer exchanged to MPA, MPA (B1): 25 mM BTP, 1% sucrose, 0.1% poloxamer 188, (50 mM MgCl₂) pH 9.0, MPB2: 25 mM BTP, 1% sucrose, 0.1% poloxamer 188, 2 M NaCl, pH 9.0, MPC: 1 M NaOH + 2 M NaCl. Flow rate: 1 mL/min. Method: Direct inject, 15 CV MPA wash, 60 CV gradient 0 to 100% MPB1, 30 CV wash with 100% MPB2, 50 CV MPC. Detection: Absorbance (260 nm, 280 nm).



Ultracentrifuge coupled with HPLC detectors for assessment of purity

Efficiency of empty/full capsids separation was checked with ultracentrifuge separation in isopycnic CsCl gradient. Centrifugrams visualised efficiency of empty/full separation. Comparing starting sample (SO3 eluate) and purified AAV9 (PrimaT eluate), the latter exhibited improved AAV9 full capsids purity, with no detection of previously present contaminant particles.



Ultracentrifuge analysis in CsCl gradient. Sample: AAV with concentration 5E+11 vg (determined by ddPCR). Method: spin for 24h at 50.000g. PATfix HPLC (red line – 260 nm absorbance, blue line – 280 nm absorbance) in conjunction with fluorescence (orange line – intrinsic tryptophan fluorescence) and MALS detector (black line – MALS signal) was used for visualisation of separated fractions.

Conclusions

- Excellent separation of full AAV9 from other particles was achieved on PrimaT column.
- Recovery of full AAV9 particles was 100% (determined by ddPCR).
- Ultracentrifuge in conjunction with PATfix HPLC analytical system is a powerful tool for determination of empty/full content.

Background references

- Gagnon P. et al. Streamlining industrial purification of adeno-associated virus. *BioProcess International*, 2020, 18(11-12).
- Gagnon, P. et al. Multiple-monitor HPLC assays for rapid process development, in-process monitoring, and validation of AAV production and purification. *Pharmaceutics*, 2021, 13, 113.