Chromatographic separation of full and empty AAV8 capsids

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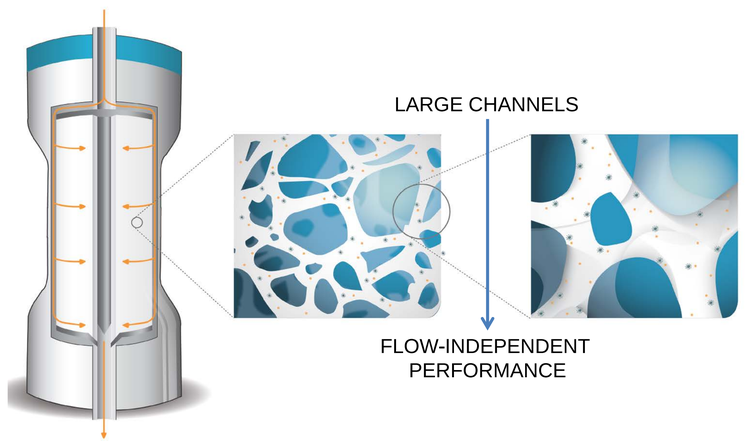
INTRODUCTION

Adeno-associated virus (AAV) vectors of various serotypes are considered to have high potential for gene therapy applications. Currently, manufacturing of AAV vectors faces the challenge of co-production of incompletely formed particles lacking a recombinant viral genome. Empty capsids increase the dose of total AAV administered for efficient transduction and are thought to cause unwanted immunological reactions against the virus.

Removal of empty capsids during manufacturing, as well as analysis of empty/full AAV particle content is therefore a critical requirement for any AAV production process. This poster demonstrates how CIMmultus™ QA monolithic columns can be used to remove empty AAV capsids from the product chromatographically in a single step.

Solution: **Convective Interaction Media Monoliths**

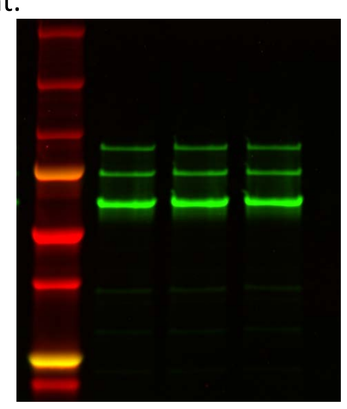
- CIM monoliths are chromatography media cast as a single block, inserted into a housing.
- Highly inter-connected network of channels (1-6 µm) containing functionalised binding sites for **large biomolecules** (viruses, VLPs, pDNA, antibodies).
- Performance unaffected by increasing the flow rate or molecular size.



RESULT

SDS-PAGE

AAV8-containing cell lysate (HEK 293T; 3 freeze-thaw cycles) was benzonase-treated, filtered (0.45µm) and loaded onto AVB Sepharose (1 mL). AAV8 was eluted with 50mM Glycine pH 2.7 and neutralized by addition of Tris pH 8.8 (30 mM final concentration). AAV8 was then buffer exchanged into PBS supplemented with 2.5mM KCl and 1mM MgCl2 until further treatment.

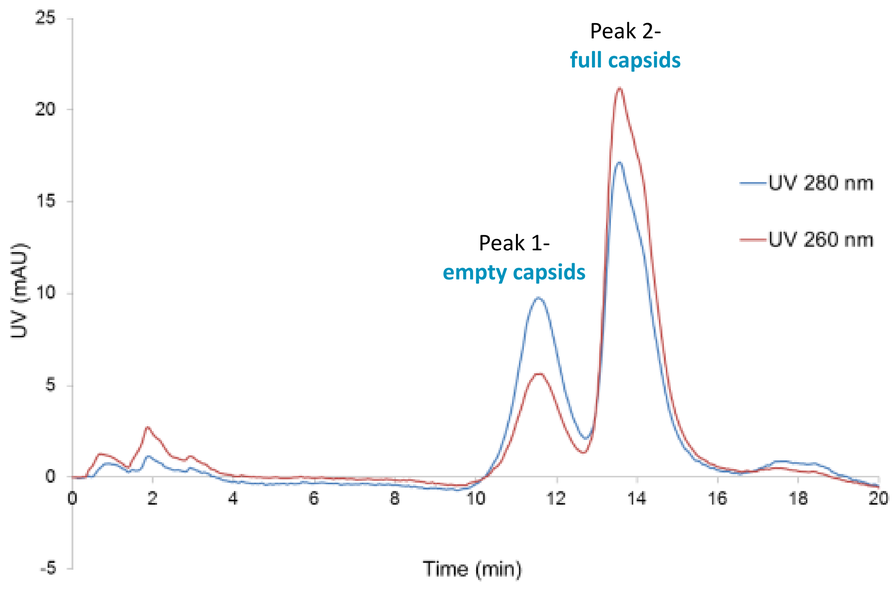


SDS-PAGE analysis of affinity-purified AAV8 shows the presence of VP1-3

CIM® monolith ion exchange chromatographic separation

When 1.29E+12 GC of AAV8 (affinity-purified) were loaded onto **CIMmultus™ QA** (1 mL), 1.05E+12 GC were recovered in the second peak (80% recovery).

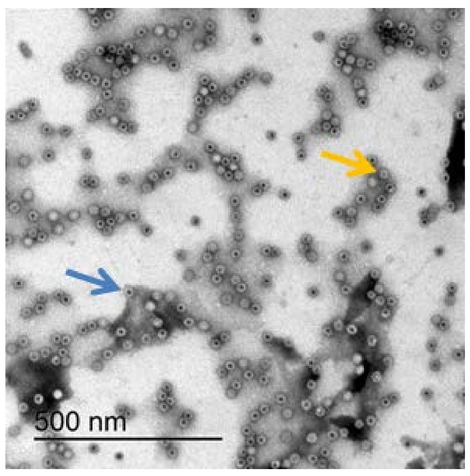
Column:	CIMmultus™ QA Monolithic column, bed volume 1 mL
Mobile phases:	Buffer A: 20 mM Bis-Tris propane (BTP), pH 9.0 Buffer B: 20 mM BTP, pH 9.0, 1 M NaCl
Flow rate:	3 mL/min
Gradient elution method:	Wash after load: 10 columns volumes (CV) buffer A Linear gradient: 0-200 mM NaCl, 60 CV High salt wash: 1M NaCl in 20 mM BTP, pH 9.0 for 10 CV
Sample:	All samples diluted in 1 mL buffer A
Sample loop:	1 mL
Detection:	UV detection, 280 nm and 254 nm



FPLC chromatogram of CIM® monolith ion exchange chromatographic separation of empty and full AAV8 capsids

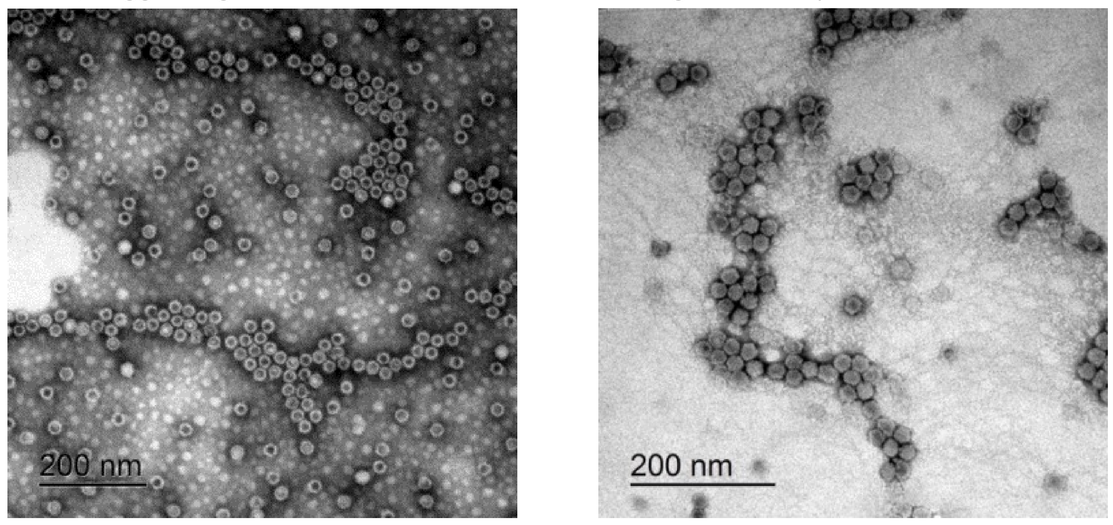
Electron Microscopy

Ion-exchange purified AAV8 capsids were examined under TEM using the negative staining method. Twenty microliters of fractions were applied on formvar-coated and carbon-stabilized copper grids (400 mesh) at room temperature for 5 min and stained with 1% uranyl-acetate (SPI Supplies, West Chester, PA, USA). The samples were observed using a Philips CM 100 transmission electron microscope operating at 80 kV, and images were acquired with an ORIUS SC200 CCD camera using Digital Micrograph Software (Gatan Inc., Pleasanton, CA, USA)



EM analysis reveals the presence of full (yellow arrow) and empty capsids (blue arrow).

EM images demonstrate enrichment of empty capsids in peak 1 (left panel) and of full capsids in peak 2 (right panel). UV absorbance was monitored at 280 and 254 nm. Note the difference in 260/280nm ratios, suggesting a difference in DNA content. GC: genome copies



EM images of CIM® monolith ion exchange chromatographic separation of empty and full AAV8 capsids

CONCLUSIONS

- A rapid method for separation of empty and full AAV8 particles by linear gradient elution on CIMmultus™ QA monoliths with 80% recovery is demonstrated.

ACKNOWLEDGEMENTS

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