Characterization and Quantification of Adeno-Associated Virus Capsid-Loading States by Multi-Wavelength Analytical Ultracentrifugation with UltraScan

Henrickson et al.

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BCHM 4000
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Overview

Background

- What are AAVs
- Current characterization methods
- Looking for a better method

Methods

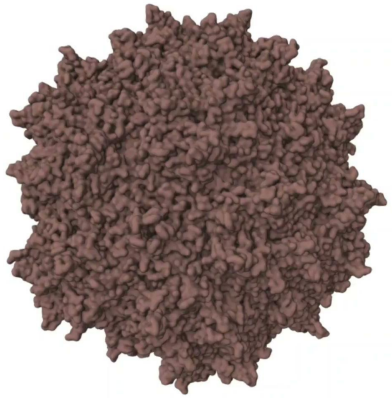
- Analytical Buoyant Density Equilibrium AUC
- Multi-wavelength AUC

Results

- ABDE-MW results
- ABDE, MP, TEM results comparison
- MW results

What are Adeno-associated virus (AAV) capsids???

- AAVs belong to the *Dependovirus* genus that infect and require helper viruses (adenovirus or herpesvirus) to cause productive infection.
- AAV capsids are used in gene therapy, a method of treatment or cure for genetic diseases. AAVs are a novel vector system due to their minimal pathogenicity and broad clinical applicability.
- A capsid is composed of 60 viral protein subunits, packaged with recombinant AAV genome containing a gene of interest as ssDNA.



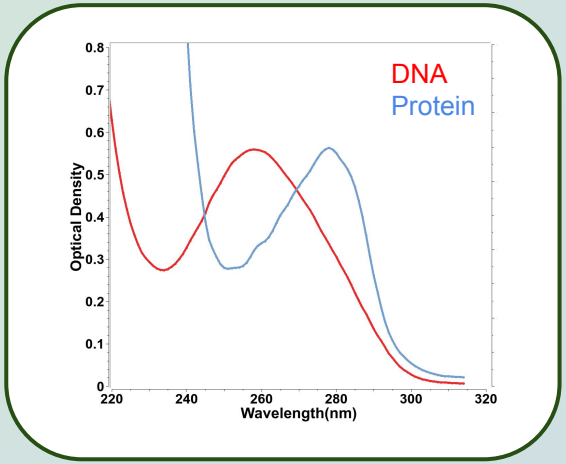
AAV8 and VP1 (PDB 2QA0)
https://doi.org/10.1128/JVI.01304-07

Problem

- The method of assembly and purification of full capsids result in product-related impurities
  - Empty capsids
  - Partially full capsids
  - Contaminants (free nucleic acids or DNA length heterogeneity in partially filled capsids, aggregates, overfilled capsids, or high density capsids)
- Current methods of characterizing AAV formulations limit accuracy and resolutions to quantify components.
  - Transmission electron microscope
  - Size-exclusion chromatography
  - Ion-exchange chromatography
  - Mass photometry

Solution???

- Analytical ultracentrifugation (AUC)!!!
  - Single experiment
  - First-principles
  - UltraScan is able to operate in a GMP environment
  - A traditional sedimentation velocity (SV) experiment?
    - Large amount of sample
    - Overestimates filled capsids due to protein and nucleic acid spectral overlap



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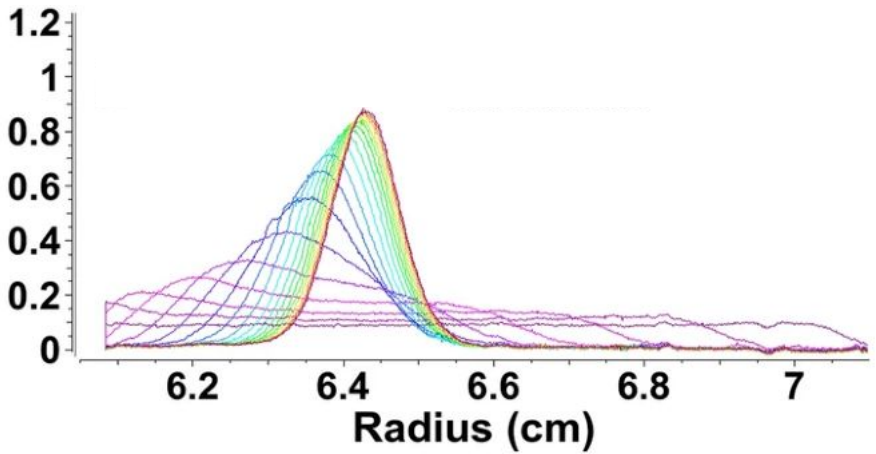
Analytical Buoyant Density Equilibrium (ABDE) AUC



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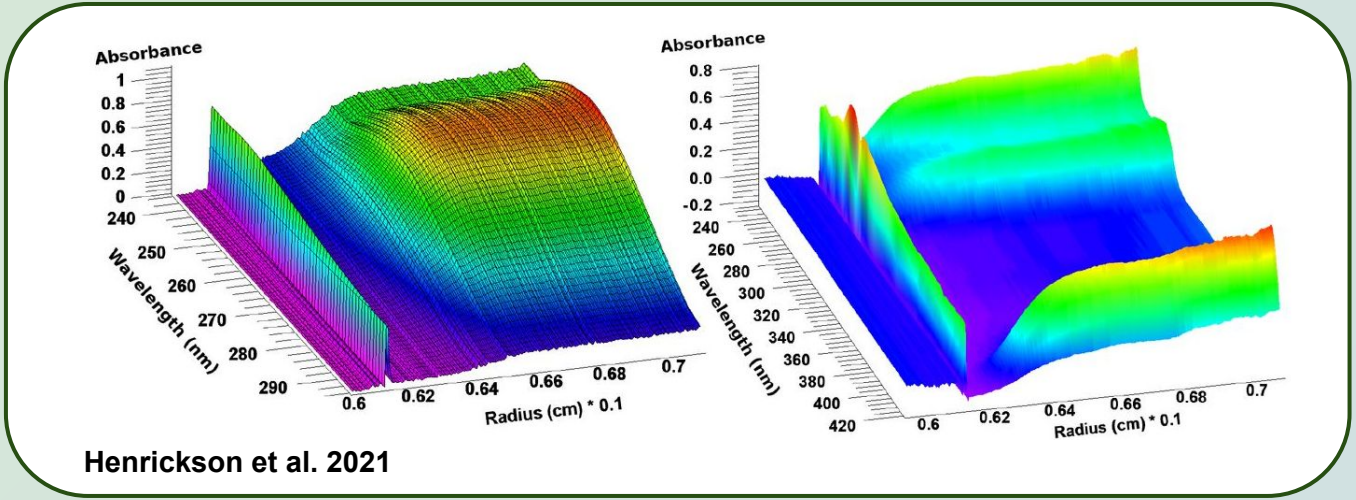
- Utilizes density gradients (Sucrose, Nycodenz, or CsCl) to separate solutes based on their buoyant density, where molecules will either sediment or float to the position on the gradient equal their density.
- This theory can be used to separate empty, partial, or full AAV capsids as the presence of ssDNA will alter the density of the species, resulting a different position on the gradient.

Multi-wavelength (MW) AUC

- Multi-wavelength analysis always the deconvolution of unique chromophores in analyte mixtures that may or may not interact, providing an orthogonal characterization method to resolve analytes.
- Unfortunately, pure AAV protein and DNA extinction spectra is difficult to obtain
    - Instead albumin and DNA plasmid was used



Henrickson et al. 2021

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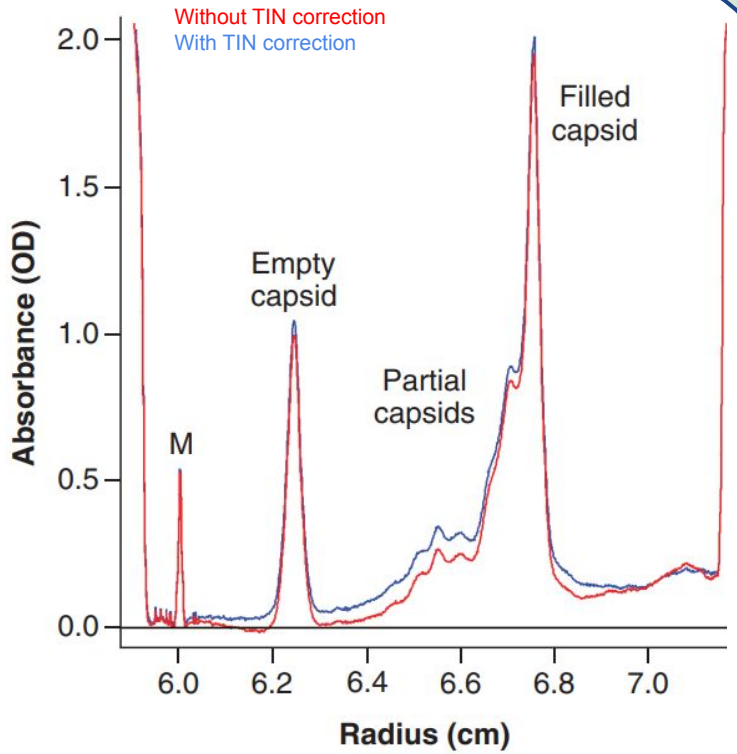
MW-ABDE AUC Results



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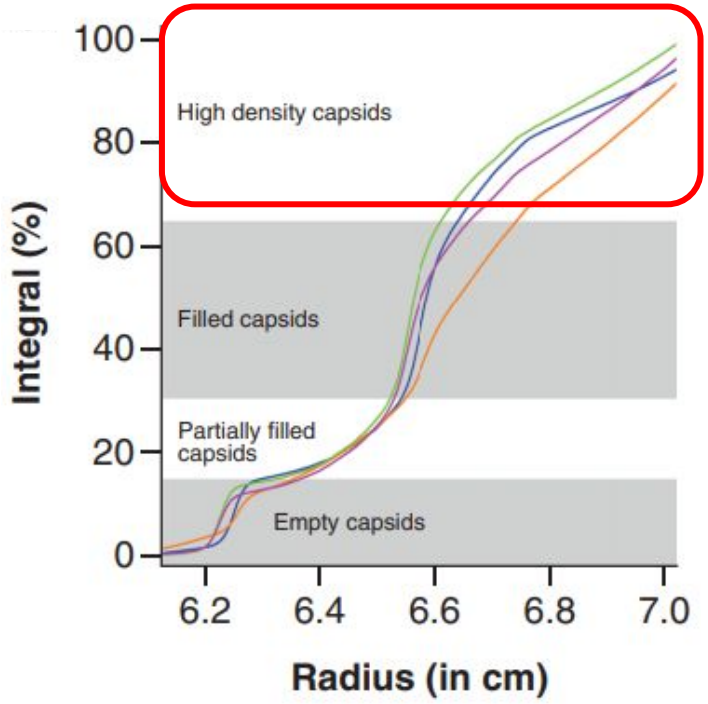
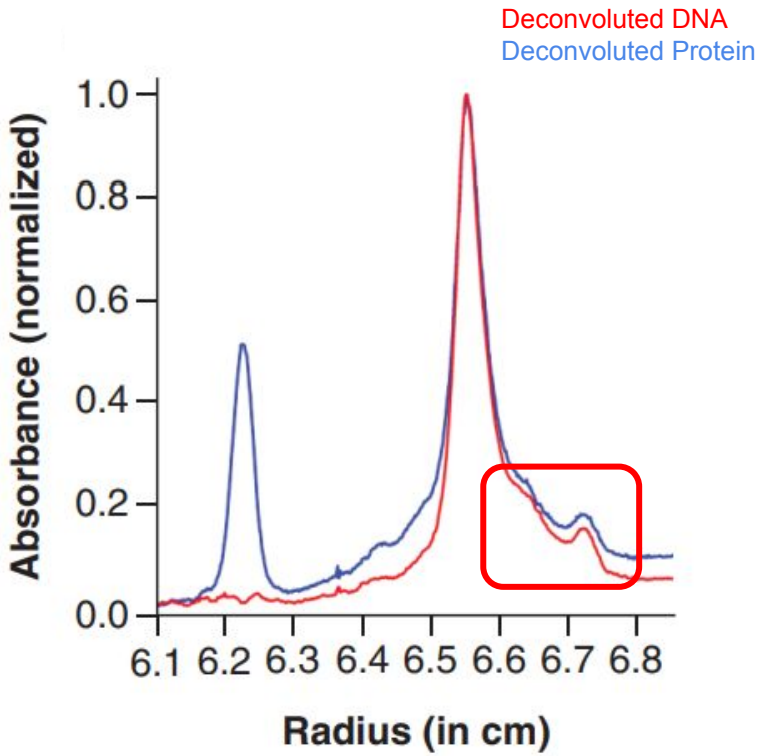
- Empty, partial, and filled capsids were successfully separated in CsCl (1.36 g/mL).
- Baseline corrections are processed before peak picking to ensure accurate quantification.
- UltraScan has a peak fitting module that calculates the integral and buoyant density of each analyte from it's peak size and position based on experimental parameters.

MW-ABDE AUC Results





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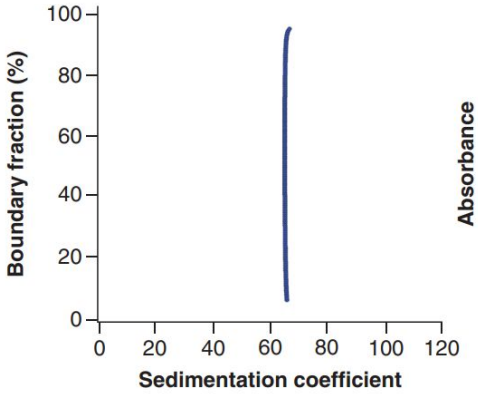
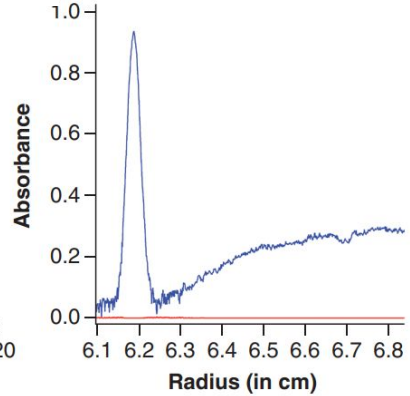
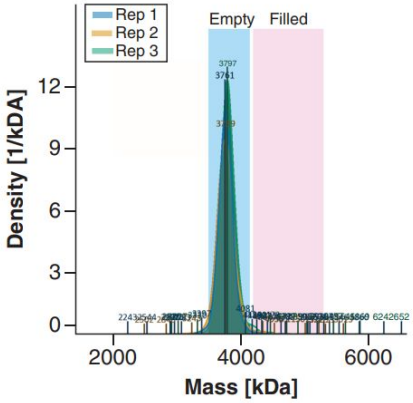
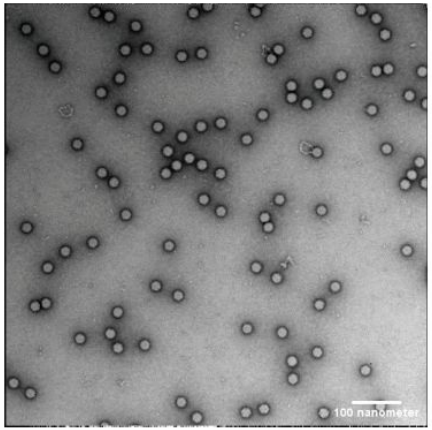
MW-ABDE AUC, MP, TEM Results









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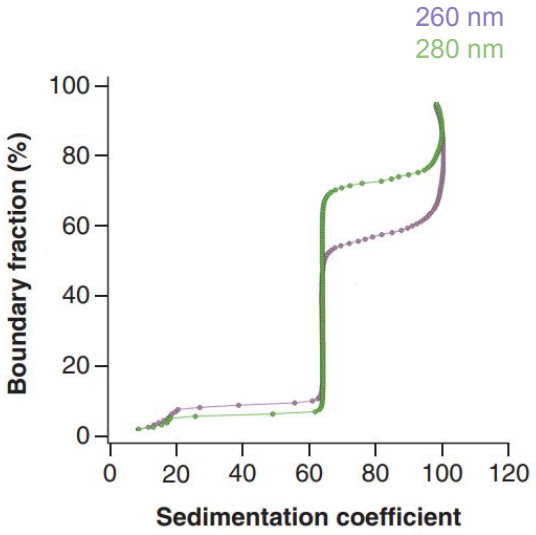
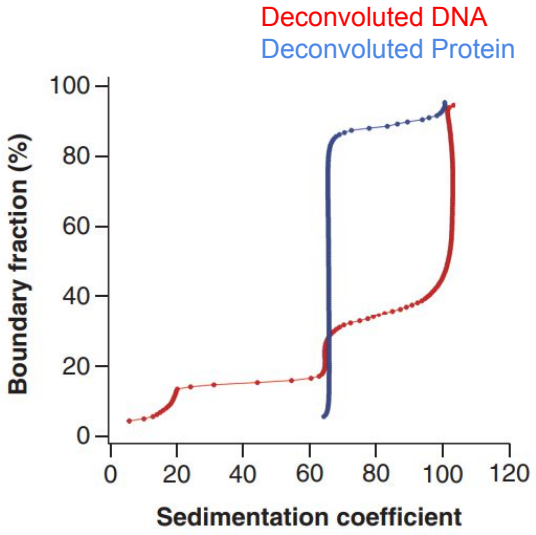
MW-AUC Results

- vHW of MW data shows a more accurate representation of composition compared to two wavelength analysis.

		Filled Capsids (100 S)	Partially Filled Capsids (65 S)
260/280 nm	260 nm	45%	45%
	280 nm	30%	65%
MW	Protein Signal	10%	90%
	DNA Signal	65%	20%





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Key Points

- MW-ABDE methods separate protein and DNA signal, require 20- to 40-fold less sample, and have a higher throughput than SV experiment.

- MW can detect and quantify partially filled capsids, in contrast to TEM.

- MW AUC methods quantify AAV capsid loading ratios and identify product related impurities, correcting for over-emphasis of filled capsids in a dual-wavelength AUC.

- MW-ABDE have identified high-density capsids, which require further investigation to identify.