Real-time monitoring of AAV Empty:Full Capsid Separations using Near infrared High-Precision Tunable Laser Spectroscopy (HPTLS)

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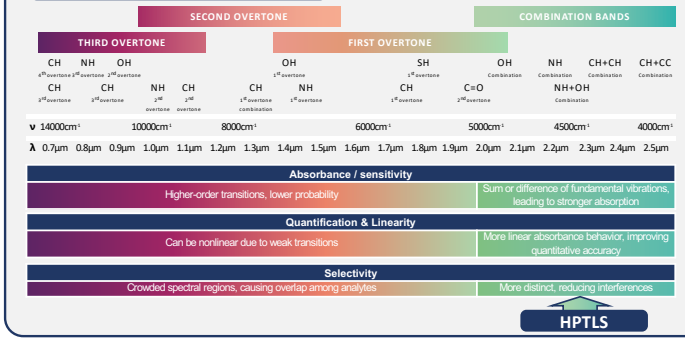
Motivation

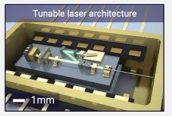
Accurate measurement of AAV empty and full capsids is critical to gene therapy manufacturing, yet current tools—such as UV260/280, mass photometry, AUC, and TEM—are slow, offline, or insensitive to partially filled capsids[1-3]. UV absorbance methods, while fast, often fail to distinguish partial genomes from fully packaged vectors due to overlapping protein and nucleic acid signals[2].

We demonstrate the use of Near-Infrared High-Precision Tunable Laser Spectroscopy (NIR-HPTLS) to monitor AAV empty:full capsid separations in real time. By resolving subtle spectral differences between protein and nucleic acid content, our platform enables rapid, reagent-free fractionation analysis directly during chromatographic purification. This immediate feedback accelerates optimization, boosts yield, and opens the door to real-time release strategies.

Near Infrared Spectroscopy

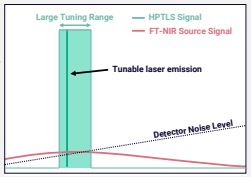


High-Precision Tunable Laser Spectroscopy (HPTLS)



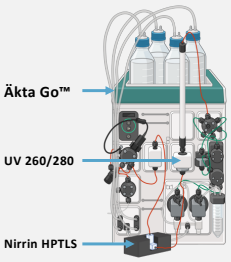
- MEMS-based Fabry-Perot filter capable of tuning 200-300nm
- Proprietary gain chip generates light in the combination band region of NIR
- 0.1-1.0 nm spectral width FWHM across entire tuning range
- Full sweep in 20 msec

➢ **Nirrin makes the only combination band tunable laser capable of 200-300nm tuning range**



- 100X higher power density than broadband light source
  - ➢ **Yields wider dynamic range than other technologies**
- Proprietary wavelength and amplitude referencing provides a calibrated spectrum with each scan
  - ➢ **Allows for accurate library transfer across systems**
  - ➢ **This is the best-in-class signal-to-noise (SNR) spectrometer currently available (±10 µAU repeatability across spectrum)**

Experimental setup



1. Characterize "full" samples from AAV8 and AAV9 by injecting directly into Nirrin flow cell
2. Run AAV8/9 AEX with potassium chloride gradient
3. Monitor and compare in-line UV 260/280 (nucleic acid/protein) to HPTLS characterized signature

Results

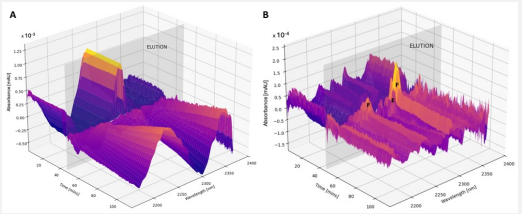


Figure 1. Characterized "full" run: AAV capsids are separated by anion exchange chromatography (AEX) using a salt gradient, where increasing ionic strength displaces capsids based on surface charge. Traditional UV methods detect total absorbance but cannot resolve overlapping signals from salt, protein, and DNA. Using combination band near-infrared (NIR) spectroscopy, we simultaneously monitor the gradient profile (**A, C**) and extract precise spectral signatures for empty and full capsids (**B**) (Empty spectral information – E; Full spectral information - F).

This enables real-time, reagent-free fractionation analysis with spectral correction for buffer effects[1-3].

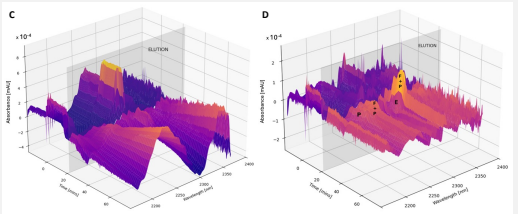


Figure 2. Characterized "partial" run: Traditional analytical methods often misclassify partially filled AAV capsids due to overlapping protein and DNA signals, especially when using UV260/280 or bulk absorbance-based techniques. Partials may co-elute with full capsids during AEX, complicating yield and potency assessments. NIR-HPTLS exploits distinct combination-band absorption features from nucleic acid and protein vibrational overtones (e.g., NH, CH, and OH stretches in the 4,200–5,000 cm^{-1} region) to resolve subtle spectral differences between full and partially filled capsids[1,2,4,5] (**D**) (Empty spectral information – E; Full spectral information – F; partial spectral information- P).

Conclusion

- NIR-HPTLS enables **real-time monitoring** of AAV empty:full separations
- Provides **rapid, label-free** analysis with no reagents or offline prep
- Detects subtle spectral differences between **capsid protein and genome content**.
- Enables **fraction-level feedback** during chromatography for improved control
- Supports **process optimization** and advances toward **real-time release testing**

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2. Sommeregger, W. et al. (2022). *AAV capsid content analysis using UV and orthogonal methods.* Biotechnology and Bioengineering, 119(6), 1402–1413.
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