Characterization of empty and full AAV capsids in complex samples by anion exchange HPLC with multiple monitors

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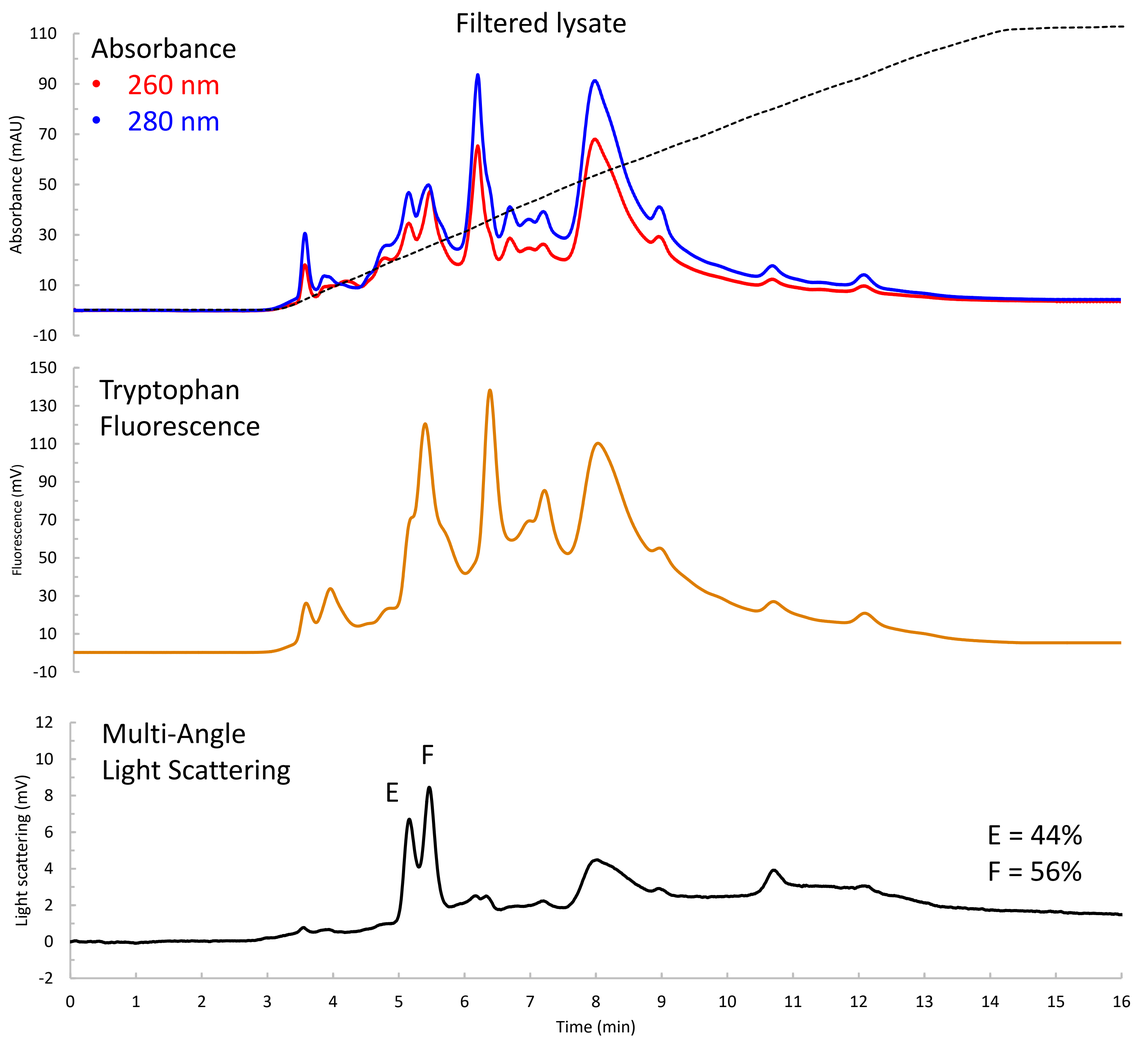
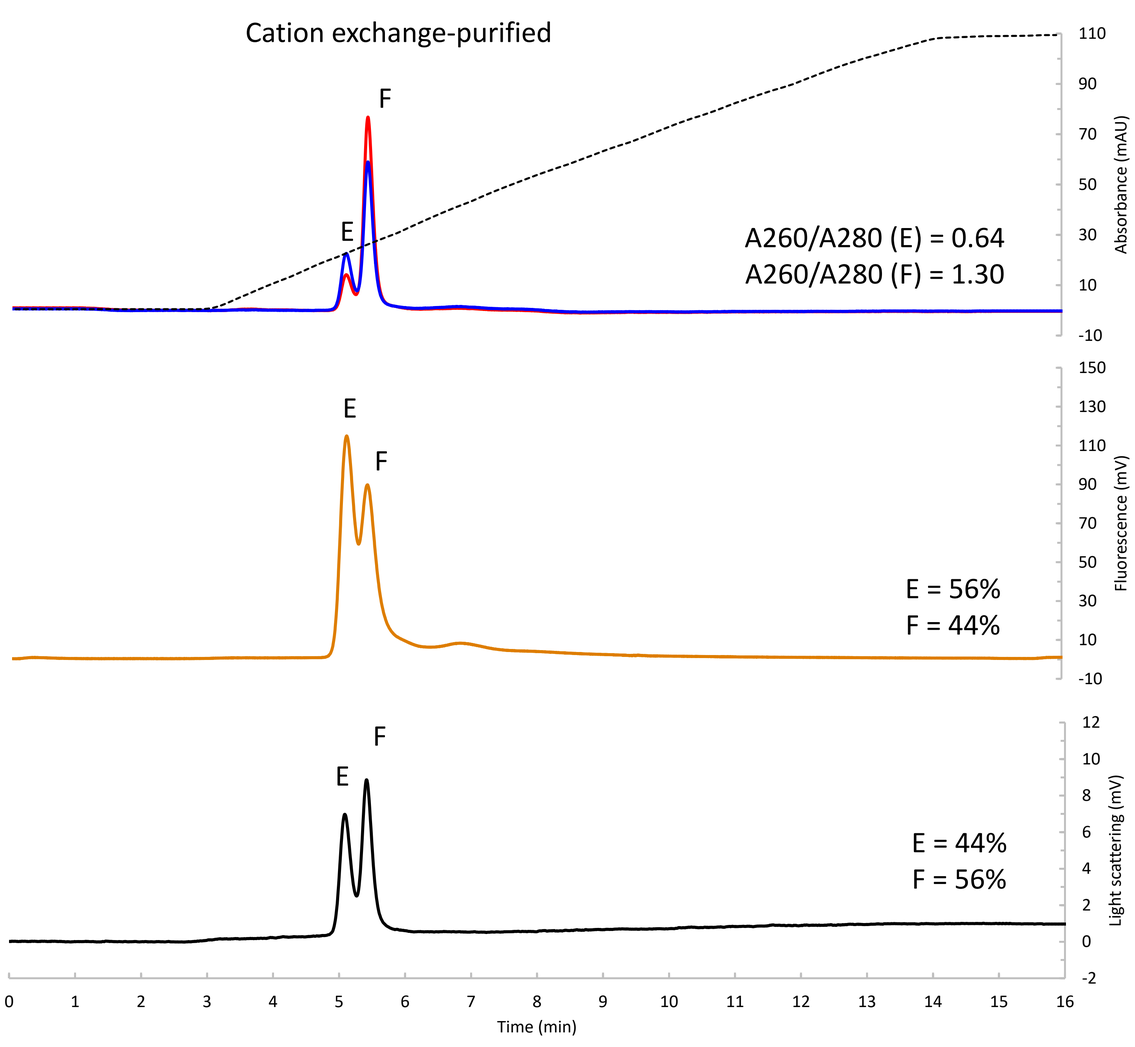
INTRODUCTION

Bioreactor cell supernatants or post lysis materials entering downstream purification are heterogeneous mixtures of empty, full, and misassembled AAV capsids mixed with host cell proteins, genomic DNA, chromatin complexes, and other contaminants. Monolith-based HPLC columns provide high resolution among these species but overlap of elution position among capsids and contaminants makes it impossible to estimate the relative content of full and empty capsids by UV absorbance. Simultaneous monitoring with multiple detectors however enables quantitative insights that extend far beyond the limitations of UV absorbance.

This poster demonstrates how the combination of fast high-resolution separations with monoliths can be combined with multiple monitors to obtain much deeper characterization than traditional assays. Anion exchange fractionation of filtered lysate and cation exchange-purified AAV8 was monitored by UV absorbance at 260 nm and 280 nm, simultaneously with tryptophan fluorescence to differentially detect proteins without interference by nucleic acids, and with Multi-Angle Light Scattering (MALS) to detect capsids.

Fractionation with CIMac AAV





CIMac AAV (QA) Buffer A: 20 mM BTP + 2 mM $MgCl_2$ pH 9.0 Buffer B: 20 mm BTP + 2 mM $MgCl_2$ + 400 mm NaCl pH 9.0

Estimation of the empty to full capsid ratio has importance at every stage of AAV production and purification. UV absorbance and tryptophan fluorescence can provide accurate estimates of within-peak composition and the relative sizes to different peaks, but both are confounded by co-elution with contaminants in crude samples.

MALS escapes their limitations and makes it possible to estimate the relative sizes of empty and full capsid peaks regardless of its state of purity. This enables anion exchange HPLC-MALS an excellent tool to document transfection efficiency, the effects of different lysis methods, the effects of different sample preparation methods, and the effects of different purification methods.

For MALS to provide truly accurate estimates, it is necessary to apply correction factors for mass and particle size or, more simply, derive a correction factor from tryptophan fluorescent measurement of purified capsids. Estimates from tryptophan fluorescence do not require correction because only capsid proteins are detected, without influence by the DNA plasmid.

Estimation of the DNA to protein ratios in a given peak can be performed only by calculation the ratio of UV absorbance at 260 nm and 280 nm. UV ratios cannot be used to estimate the amounts of empty and full capsids in different peaks is unreliable because the empty capsids lack DNA. The practical result is the UV ratios systematically inflate the apparent proportion of full peaks.

CONCLUSIONS

- Analytical anion exchange fractionation with MALS enables estimation of empty/full capsid ratios even with crude samples. Correction factors for molar mass is required to enable precise estimates but even the raw data is valuable for monitoring E/F content across a series of samples.
- UV absorbance ratios at 260 nm and 280 nm provide an estimate of the amount empty and full capsids in any given peak but only in purified samples.
- Tryptophan fluorescence provides the easiest and most accurate estimate of actual peak size ratio for estimation of empty and full capsids for purified samples but co-elution with protein contaminants limits its utility in crude samples.