Do your AAVs contain DNA?

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INTRODUCTION

The use of adeno-associated virus (AAV) vectors for therapeutic gene delivery has been steadily increasing over the last decade. AAVs are complex biotherapeutics, containing both protein and genetic components, in the form of ssDNA packaged inside a protein capsid. We recently published a platform method for the charge heterogeneity analysis of denatured AAV capsid proteins using native fluorescence imaged cIEF, suitable for identity and stability indication. Using the same capillary electrophoresis instrument, Maurice, we sought to answer the titular question. Do your AAVs contain DNA? Here we show an intact AAV method that leverages dual detection modes for intact AAV capsids. This poster demonstrates the concept through the analysis of two commercially available percent genome empty/full capsid series with the Maurice instrument. The quantification from the empty/full series can then be used to estimate the fullness of unknown samples.

METHODS

MATERIALS

AAV5 and AAV8 (2 x 10^{13} VP/mL, Custom Virovek order), Maurice cIEF Method Development Kit (PS-MDK01-C), Compass for iCE Software, Maurice - CE-SDS and icIEF Platform (090-000), Maurice cIEF Cartridge (PS-MC02-C)

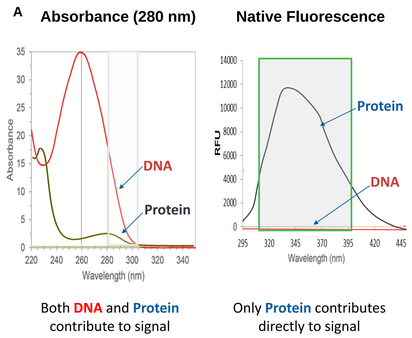
icIEF METHOD

AAVs (2 x 10^{13} GC/mL) were diluted to 3 x 10^{12} GC/mL and added in a 1:4 ratio into a final mixture containing 0.35% MC, 4% Pharmalyte 3-10, 5mM Arginine, 5mM IDA, in a Formamide/SimpleSol blend with pI markers pI 4.05, 5.5, 8.4, 9.5. Samples were focused for 1min at 1.5kV, followed by 9min at 3kV. Absorbance and native fluorescence (NF) images (5, 10, 20, & 40 seconds) were captured using the Compass for iCE Software. The exposure time of 5 seconds for NF was used for this work.

RESULTS

Initial experiments showed that empty and full AAVs releveled that for the same vg/mL the peak heights/area would be dramatically different depending on the detection type used. Using the absorption detection mode, full AAVs had much greater peak height/area, while in NF the empty AAVs peak height/area equaled or surpassed the full AAVs for the same two samples.

Illustration of Maurice Abs/NF profile



Representative Maurice Data from AAV8 E/F Samples

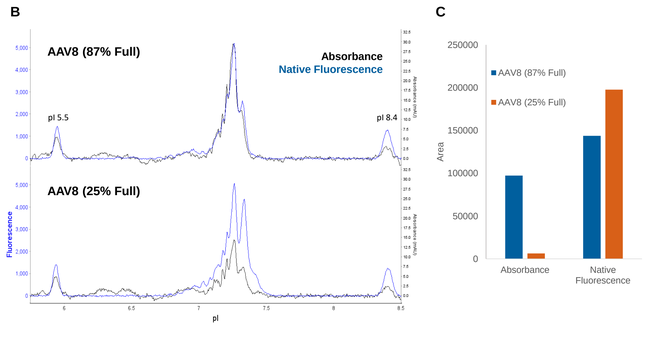
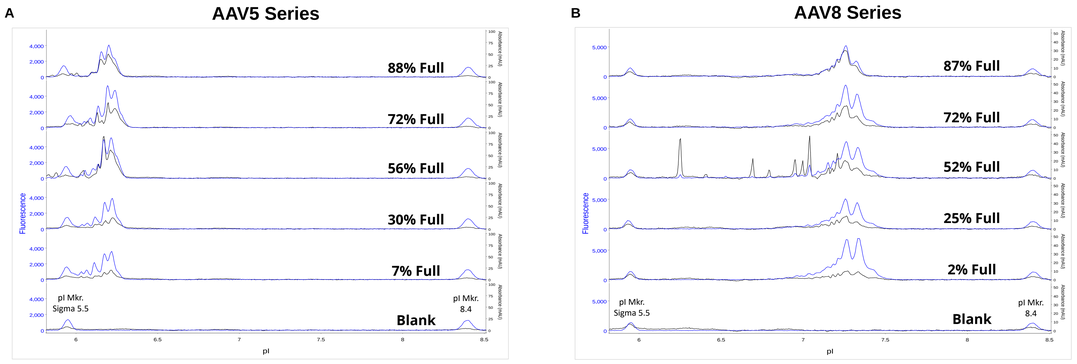


Figure 1. Maurice dual detection approach for AAV empty/full assay. (A) Illustration to show the adsorption at 280nm and the emission filter for NF detection. The results of the dual detection (B) reveal the relative differences in the two detection modes for a pair of AAV8 (87 and 25% full) analyzed at the same particle number. (C) The increased adsorption (15X between samples) is contributed by the DNA inside the capsid. A small decrease in the AAV8 (85% full) native fluorescence is due to quenching by the DNA.

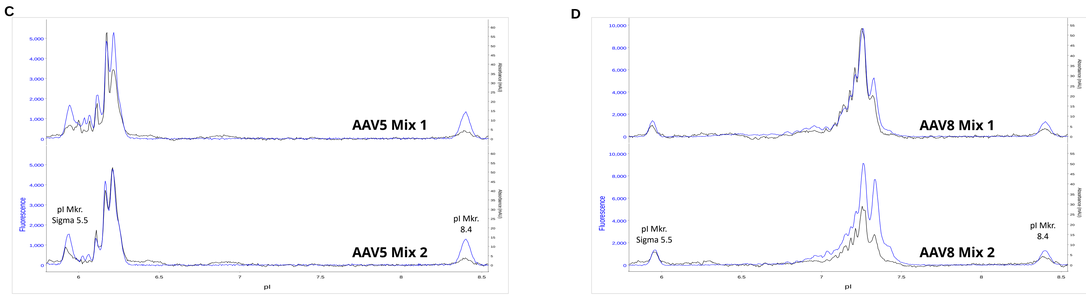
RESULTS

An AAV5 and AAV8 percent full series was purchased and run with an intact AAV method. NF and absorbance electropherograms were integrated and their peak areas used to determine the ratio between them for each percent full sample. The absorbance peak area was divided by the NF peak area, times 1000 for display. The empty and full standards were used to make blinded samples for analysis. The percent full series ratios were used to calculate the values for the unknowns.

MAURICE icIEF native Fluorescence and Absorbance Overlays of AAV Percent Full Series



MAURICE icIEF native Fluorescence and Absorbance Overlays of AAV Sample Mixes



MAURICE icIEF Integration Results

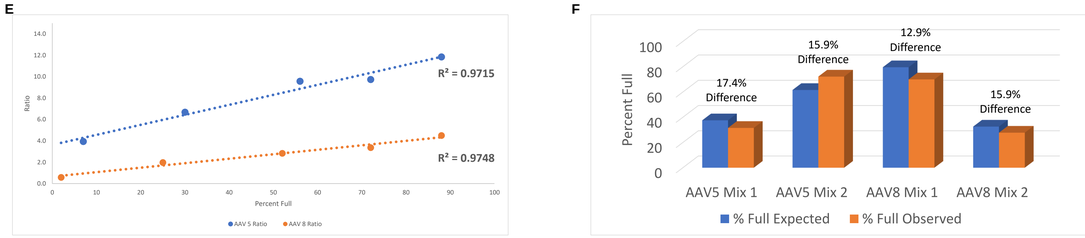


Figure 2. The Virovek AAV percent full titration overlays for AAV5 (A) and AAV8 (B) are shown above. The blinded unknown mix overlays are shown for the AAV5 (C) samples and AAV8 (D) samples. The software normalized dual detection overlays for NF in blue and absorbance in black, illustrate the decreasing NF compared to absorbance for the higher percent full samples. The results for the AAV titration (E) shows a linear relation for the calculated values and percent fullness of the known samples. These values were used to calculate the percent fullness of the unknown mixes (F). The results showed a good correlation between the expected and observed values.

CONCLUSION

- Intact AAV particle charge heterogeneity can be analyzed on the Maurice with absorbance and native fluorescence.
- The capsid's DNA can increase the absorbance at 280nm and cause quenching of the NF signal.
- The ratio between the absorbance and NF signal can be used to derive an estimation of the percent fullness.
- This approach enables AAV charge heterogeneity and percent full calculations to be done in one fast and easy method.

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