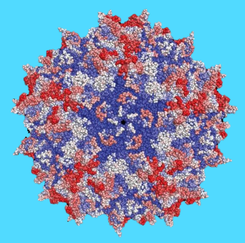

Benchtop high resolution ES-DMA system reveals angstrom-scale size / mass correlation of Adeno-Associated Virus (AAV) vectors for gene therapy capsid analytics

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Particle Perception

Abstract

- Mobility analysis is presented as a tool to characterize DNA content of Adeno Associated Virus (AAV) capsids, the most commonly used vector for gene therapy drug development.
- Electrospray Differential Mobility Analysis (ES-DMA) is compared with charge detection mass spectrometry (CD-MS) for an identical set of AAV samples containing various DNA cargos.
- We identify a condition where a strong correlation between particle mass and diameter is observed enabling robust relative quantification of "empty", "full", and "partial/over-filled" AAVs by ES-DMA.

Introduction

Cargo diameter correlation

- AAV virions are reported to exhibit a fixed diameter irrespective of their DNA cargo by electron microscopy.
- Upon electrospray ionization, desiccation of aerosolized virus capsids may reveal DNA cargo-dependent shifts in particle diameter.
- We hypothesized our high resolution ES-DMA system will detect these differences to provide information on AAV capsid content.

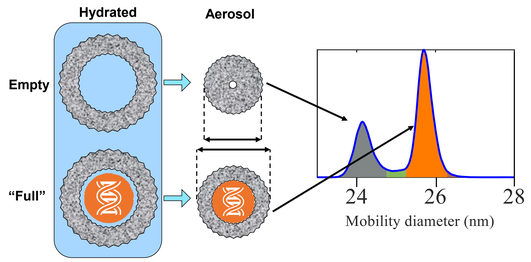


Figure 1: Model for DNA cargo / particle diameter correlation upon desiccation of a core-shell particle producing species of different diameters dependent on DNA content.

Effect of relative humidity on particle diameter

- Empty AAV capsids are monodisperse in mass but ES-DMA spectra show multimodal distributions of peak diameters.
- When aerosolization and analysis is performed at ≥ 40% relative humidity, a single peak is observed for empty AAV9 particles.

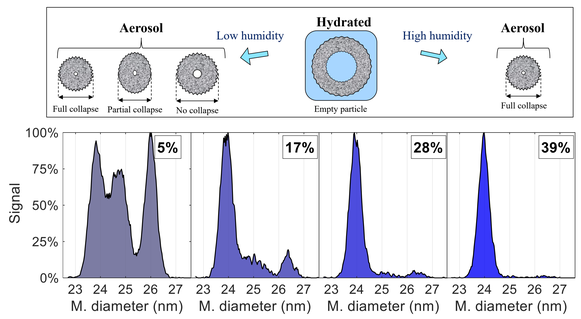
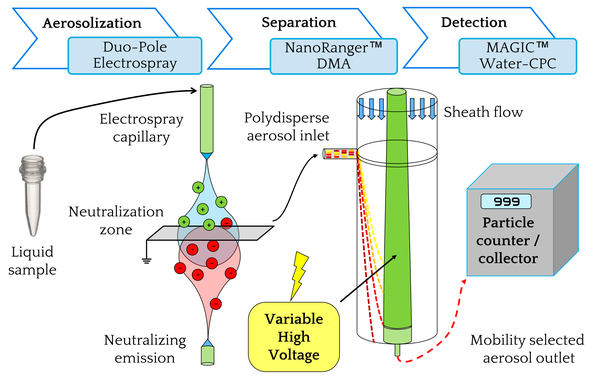


Figure 2: (Top) Model of transition to gas phase for AAV9 empty under high and low humidity. (Bottom) Mobility diameter spectra of AAV9 empty, when aerosolized and analyzed in an atmosphere of various relative humidities as indicated at top right.

Methodology and Results



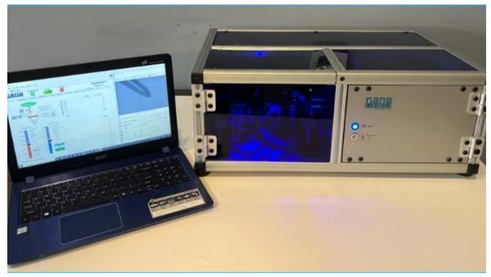


Figure 3: left) diagram showing the transition from liquid sample, electrospray aerosolization, neutralization to single charge, mobility separation and data detection right) image of the setup showing from left to right: detector, NanoRanger mobility analyzer and a live image of the electrospray tip.

Comparison of mass and mobility diameter spectra for various nominal cargos

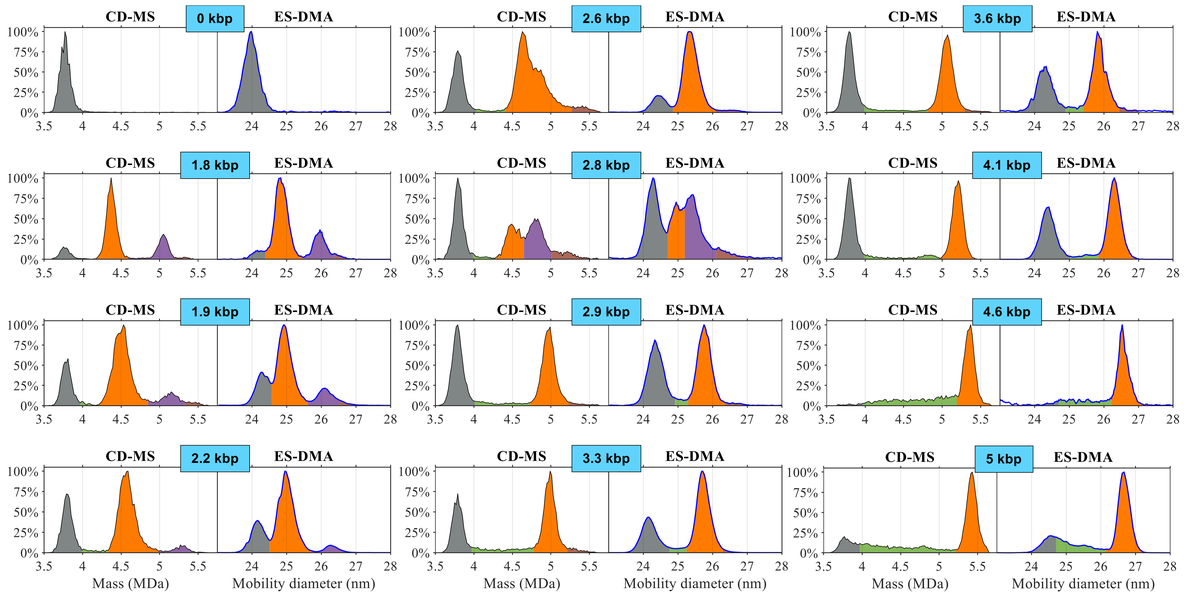


Figure 4: Side by side mass and mobility spectra obtained from identical samples of 12 different cargo-containing preparations of AAV9, where the nominal cargo load is indicated in kilo-basepairs (kbp). The various identifiable features are presented color-coded in both spectra, with resolution precluding peak identification in some cases.

Mass diameter and density correlation

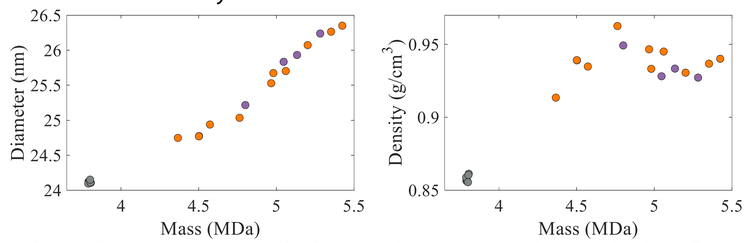


Figure 5: Mass-diameter (left) and mass-density (right) correlation for the most prominent peaks identified in Fig.4

- Observed particle diameter grows monotonously with particle mass, but density seems to reach a maximum.
- For a fully collapsed core shell model, with a fixed amount of lower density protein and an increasing amount of higher density DNA, total density should grow asymptotically.

Conclusion

Quantification of AAV capsid content

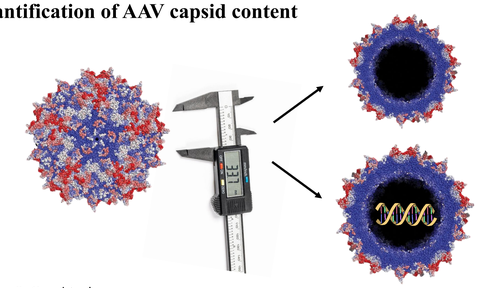


Figure 6: Graphical summary

- Upon aerosolization, AAV particles undergo desiccation and collapse to yield diameters that strongly correlate with levels of encapsidated DNA cargo.
- Exploiting this process, high resolution ES-DMA performs comparably to CD-MS for the identification and relative quantification of various species present in AAV preparations (i.e. empty, full, and partial/over-filled species), shown in Figure 7.
- These results, combined with its relatively low cost and convenience, suggest utility of the ES-DMA technique for AAV capsid analytics

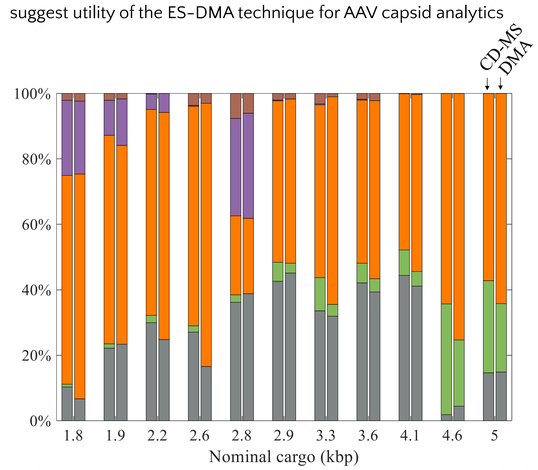


Figure 7 : Relative abundance of various species identified by CD-MS (left) and ES-DMA (right) across 11 different cargo-containing AAV9 samples containing ssDNA genomes of various lengths, indicated at bottom in kbp. Color code matches that of Figure 4.

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