Characterizing Nucleic Acid Payloads in Lipid Nanoparticles and Viral Vectors via Charge Detection Mass Spectrometry (CDMS)

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Introduction

The characterization of nucleic acid payloads in therapeutic delivery systems such as lipid nanoparticles (LNPs) and adeno-associated viruses (AAVs) is critical for ensuring product quality, structural integrity, and functional performance. These payloads, which include large RNA and DNA molecules, present unique analytical challenges due to their high molecular weight, polydispersity, and susceptibility to degradation. Conventional techniques often rely on indirect quantification or ensemble measurements, limiting resolution and accuracy. Charge Detection Mass Spectrometry (CDMS) overcomes these limitations by enabling direct, single-particle mass and charge measurements of intact nucleic acids and their associated complexes. In this work, we apply CDMS to characterize nucleic acid payloads from LNP and AAV formulations, providing high-resolution insights into size distribution and heterogeneity. Here, we demonstrate a unified CDMS workflow to directly characterize both intact delivery vehicles and their isolated nucleic acid payloads from AAV and LNP formulations.

Methods

To evaluate genome integrity, the USP AAV8 Full reference capsids were digested at 56˚ C for 40 min at 1000rpms in a thermomixer. Then the genomic payloads for the AAV samples were isolated using Monarch® Spin PCR and DNA Cleanup kit (New England Biolabs®). Finally, the isolated AAV genomic payloads were buffer exchanged into 20mM ammonium acetate using Micro Bio-Spin™ P6 Gel Columns (Bio-Rad) before CDMS analysis. Ionizable LNP samples were deformulated by diluting samples in a buffer consisting of 60mM ammonium acetate in 100% IPA then vortexing for 30 seconds. 10 µL of that solution was then analyzed by CDMS. Intact samples were buffer exchanged using the Micro Bio-Spin P6 gel columns (AAV) or by dialysis using Slide-A-Lyzer™ MINI Dialysis Devices (Thermo Scientific™) for at least 30 minutes at 4˚ C Samples were introduced by nano-electrospray ionization into a benchtop commercial Xevo™ CDMS Instrument (Waters) for mass and charge measurement. CDMS data were processed and quantitatively evaluated using the CDMS Toolkit within waters_connect™ Software.

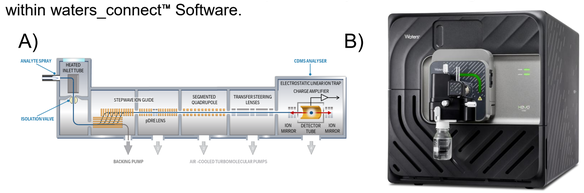
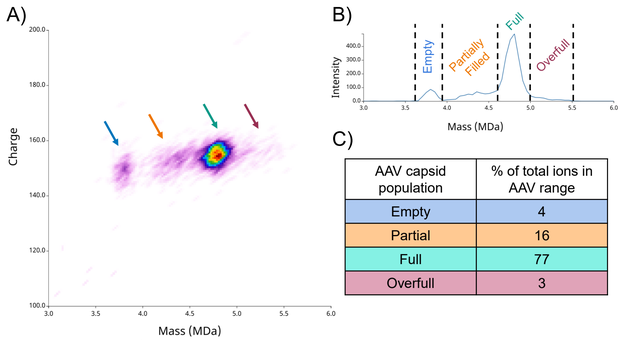


Figure 1: A) Instrument diagram, B) Picture of Xevo CDMS

The authors are employees of Waters Corporation and are presenting this work on behalf of the company. The authors declare no competing interests.

AAV Payload Isolation and Confirmation

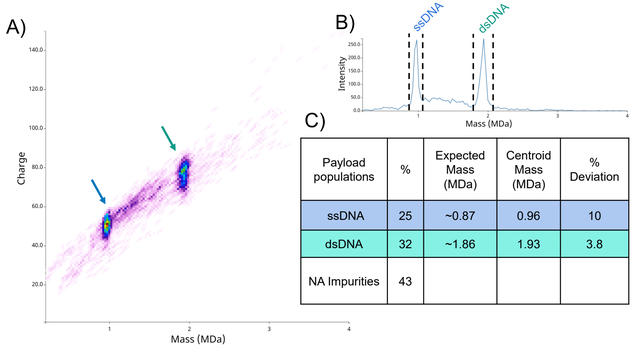
CDMS resolves AAV capsid populations at the single-particle level, enabling quantitative assessment of packaging efficiency.



AAV capsid population	% of total ions in AAV range
Empty	4
Partial	16
Full	77
Overfull	3

Figure 2: A) 2D plot (mass vs charge) featuring the full USP AAV8 standard, B) the mass histogram for the full USP AAV8 standard, and C) Table of AAV capsid population percentages observed

CDMS reveals full-length genomes alongside truncated and impurity species at reproducible mass intervals.



Payload populations	%	Expected Mass (MDa)	Centroid Mass (MDa)	% Deviation
ssDNA	25	~0.87	0.96	10
dsDNA	32	~1.86	1.93	3.8
NA Impurities	43			

Figure 3: A) 2D plot (mass vs charge) featuring extracted DNA payload, B) the mass histogram for the full USP AAV8 standard, and C) Table of payload populations and associated data

LNP Deformulation and Payload Intact Mass

Intact LNPs display a broad mass distribution consistent with heterogeneous payload loading.

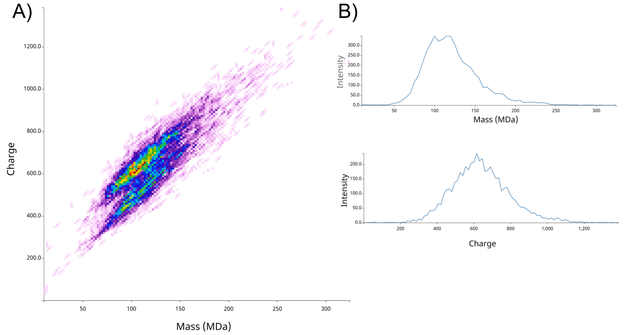
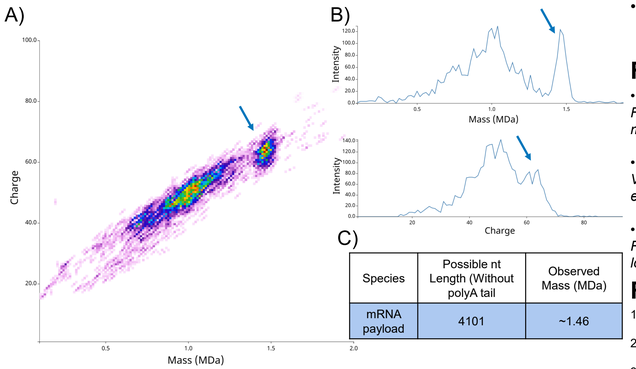


Figure 4: A) 2D plot (mass vs charge) featuring the intact LNP (Spikevax™), B) the mass histogram and C) charge histogram for the intact LNP (Spikevax™)

CDMS detects full-length modified mRNA together with impurities (both nucleic acid and LNP components).



Species	Possible nt Length (Without polyA tail	Observed Mass (MDa)
mRNA payload	4101	~1.46

Figure 5: A) 2D plot (mass vs charge) featuring the mRNA payload and deformulation impurities, B) the mass histogram and C) charge histograms for the mRNA payload and deformulation impurities

Discussion

- Intact mass determination of nucleic acid payloads presents distinct challenges for viral and lipid-based vectors due to differences in encapsulation, heterogeneity, and deformulation requirements.
- Here, we implemented two tailored CDMS workflows optimized for payload release while minimizing fragmentation, enabling quantitative intact mass analysis for both AAV genomes and LNP-encapsulated mRNA.
- The methods presented here extend CDMS beyond intact particle analysis to enable direct intact mass determination of isolated nucleic acid payloads from both viral and lipid nanoparticle systems.
- Despite fundamental differences in vector architecture, both workflows preserve payload integrity sufficiently to resolve full-length nucleic acids, truncated species, and impurities at the single-particle level.
- This highlights CDMS as a versatile analytical tool capable of bridging characterization gaps between viral and non-viral delivery platforms.

Conclusions

- Vector-specific CDMS workflows enable direct intact mass determination of large nucleic acid payloads from both AAV and LNP systems.
- Single-particle resolution allows simultaneous assessment of payload length, heterogeneity, and impurity content.
- These methods establish CDMS as a robust platform for payload integrity assessment across next-generation nucleic acid therapeutics.'

Future Directions

- Identification of nucleic acid impurities

Further interrogate lower-mass and off-target species observed by CDMS to determine their molecular identity and origin (e.g., truncated genomes, degradation products).

- Orthogonal confirmation of payload identity

Validate CDMS-based payload assignments using complementary analytical techniques such as electrophoretic, sequencing-based, or enzymatic methods.

- Correlation of intact particle and payload profiles

Relate intact AAV and LNP mass distributions with extracted payload integrity to better understand loading efficiency and degradation pathways.

References

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