LARGE BIOMOLECULE ANALYSIS USING CHARGE DETECTION MASS SPECTROMETRY

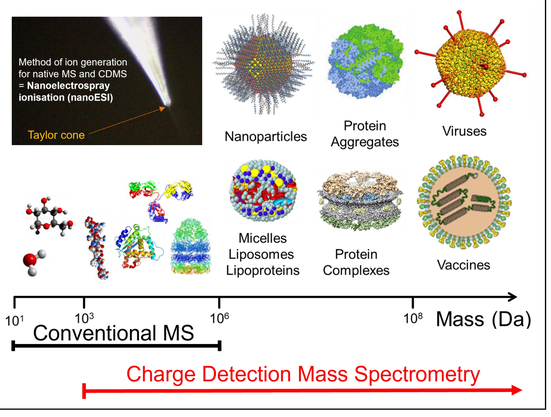
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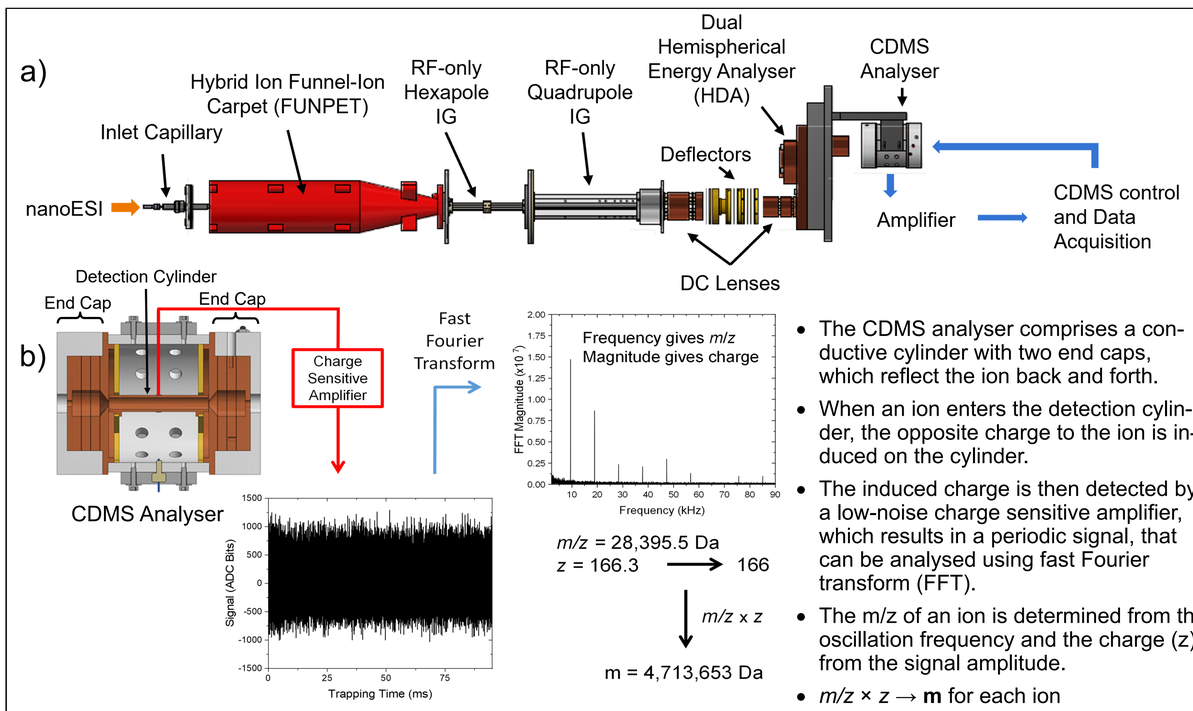
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Introduction

- Charge detection mass spectrometry (CDMS), using an electrostatic linear ion trap (ELIT), is an ultra-high mass analytical technique, which provides direct mass measurement of individual ions through simultaneous determination of their mass-to-charge ratio (*m/z*) and charge (*z*).
- CDMS enables accurate molecular weight information to be determined routinely for analytes well into the megadalton regime, such as viruses and vaccines.
- This work has translational applications to assessing virus assembly and the analysis of gene therapy products, such as characterising the critical quality attributes (e.g. capsid content) of adeno-associated viruses (AAVs).
- Herein, we aim to demonstrate the potential of CDMS for various applications, where it is a challenge for current methodologies and conventional mass spectrometry.



Experimental Setup



- The CDMS analyser comprises a conductive cylinder with two end caps, which reflect the ion back and forth.
- When an ion enters the detection cylinder, the opposite charge to the ion is induced on the cylinder.
- The induced charge is then detected by a low-noise charge sensitive amplifier, which results in a periodic signal, that can be analysed using fast Fourier transform (FFT).
- The m/z of an ion is determined from the oscillation frequency and the charge (z) from the signal amplitude.
- $m/z \times z \rightarrow$ **m** for each ion

Figure 1. *a) Schematic representation of the Megadalton Solutions electrostatic linear ion trap charge detection mass spectrometer (courtesy of Megadalton Solutions, Indiana, USA).[1] b) Diagram of the CDMS mass analyser and how m/z and z information is obtained.[2]*

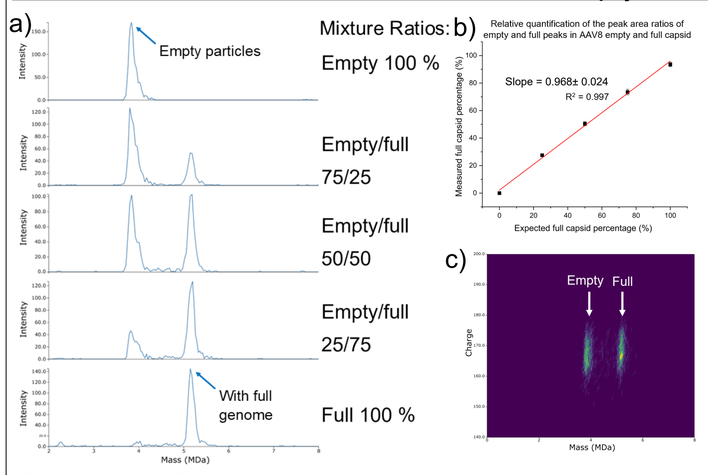
Methods

Samples: AAV empty and full (with CMV promoter-driven expression of GFP) capsids of various serotypes were purchased from different commercial suppliers. The 20S core particle was provided by the Vahidi research group from the University of Guelph, Canada[5] and encapsulins were provided by the Lau research group from the University of Sydney, Australia.[6] All samples were buffer exchanged into either 20 mM or 200 mM ammonium acetate solution using Biospin P-6 micro-size exclusion spin columns (Bio-Rad), Zeba spin desalting columns (Thermofisher), or molecular weight cut-off filters (Amicon).

CDMS: Ions were generated in positive ion mode using nanoelectrospray ionisation and mass analysis was performed using a prototype CDMS with an ELIT, based on the instrument architecture designed by Megadalton Solutions. Signal processing and data visualization was performed using software developed in-house. Ions were trapped for 100 ms and the frequency and amplitude information were converted to *m/z* and *z* values respectively, and ultimately mass (*m/z x z*). These data were subsequently binned to generate the corresponding spectra (histograms). The measured masses and peak areas are reported based on Gaussian fitting of the data.

Adeno-associated Viral Vectors

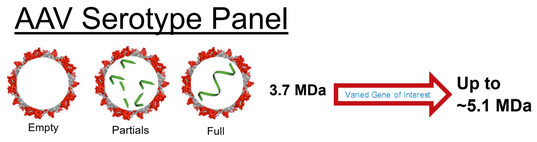
Detection and Relative Quantification of Empty and Full Capsids



- Relative quantification of empty and full AAV8 capsids was investigated by preparing volumetric mixtures ranging from 100 % empty to 100 % full, in increments of 25 % (Figure 2a).
- CDMS data for each mixture were acquired in triplicate (1000-1500 ions in the AAV mass range with a 10-minute acquisition time).
- The data were plotted as mass peak area vs % full capsids and a linear response and correlation was observed (R^2> 0.99) (Figure 2b).
- Both empty and full capsids have approximately the same charge (167e) (Figure 2c) as only the surface of the capsids are charged during the electrospray process.
- Shifts in the charge profile may provide some insights into the structural integrity of the capsids.

Figure 2. *a) CDMS mass spectra of AAV8 empty and full capsid mixtures, b) Correlation of AAV8 empty/full mixtures and c) 2D charge vs. mass density plot of the AAV empty/full 50/50 mixture.*

AAV Serotype Panel



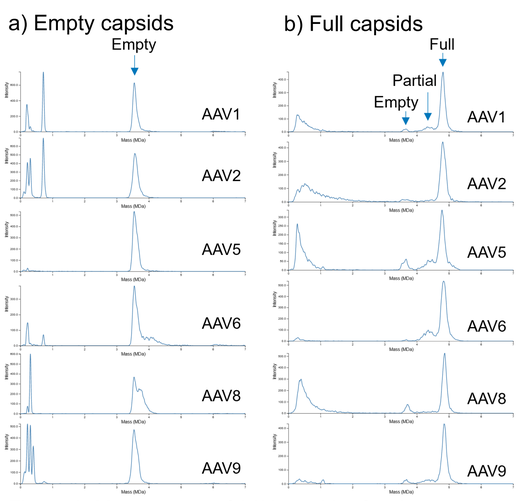


Figure 3. *Mass spectra of AAV capsids of various serotypes a) empty capsids and b) CMV-GFP full capsids.*

- AAV empty, partially filled, and full capsids were readily distinguished using CDMS (Figure 3) with average measured masses in good agreement with expected masses.
- For the 'full' capsids, ~ 15-30 % of the particles observed were actually empty and partially filled (Figure 4a).
- The highest percentage of empty particles in full capsid samples was observed for those with the oldest manufacturing dates (AAV5 and AAV8) - storage conditions of AAVs can have a significant impact on their stability, ultimately affecting the potency of the product.
- Some differences in the charge profile was observed for each AAV serotype, possibly due to the differences in viral protein content within the capsid structure (Figure 4b).

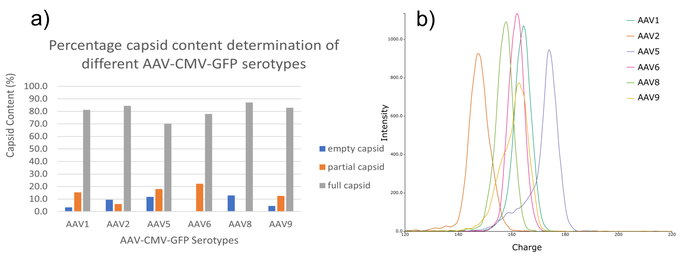


Figure 4. *a) Measured capsid ratios for different AAV serotypes ('full' capsid data - Fig 3b) and b) Overlay plot of the charge distributions.*

CDMS Mass Range

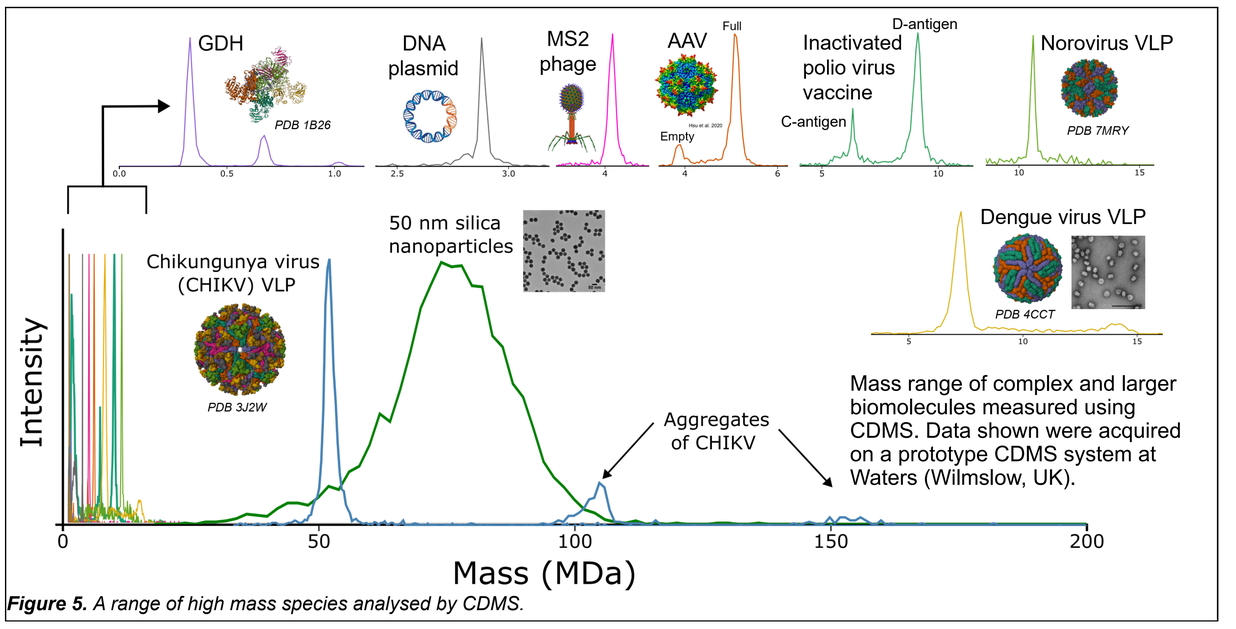


Figure 5. *A range of high mass species analysed by CDMS.*

Proteasomes

- The 20S core particle (CP) is a key component of the proteasome system in *Mycobacterium tuberculosis (Mtb),* which degrades protein substrates that are misfolded or have become damaged.
- It is composed of 28 subunits, arranged as four heptameric rings (α_7-β_7-β_7-α_7).
- CDMS data were obtained for the intact 28-mer of the 20S CP.

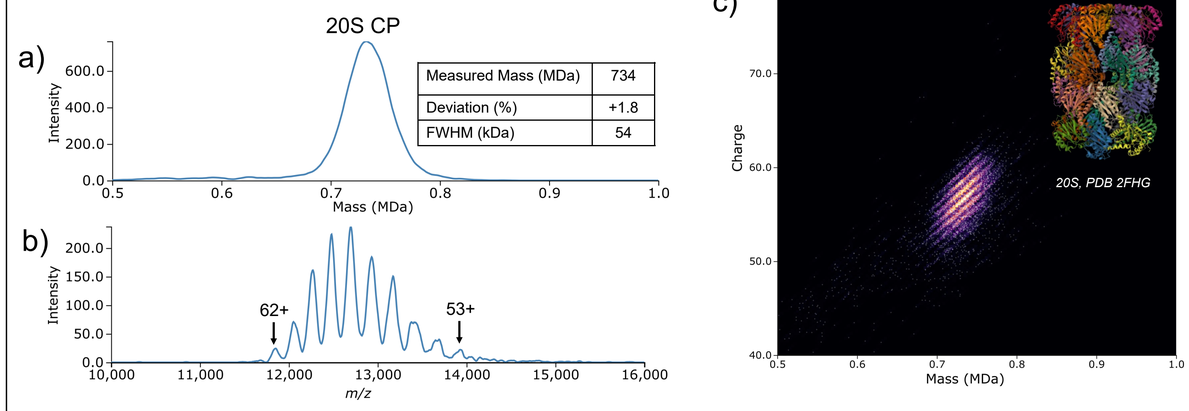


Figure 6. *a) mass spectrum (25 kDa mass bin width), b) m/z spectrum, and c) 2D charge vs mass plot for the 20S core particle.*

DNA

pBR322 DNA Plasmid

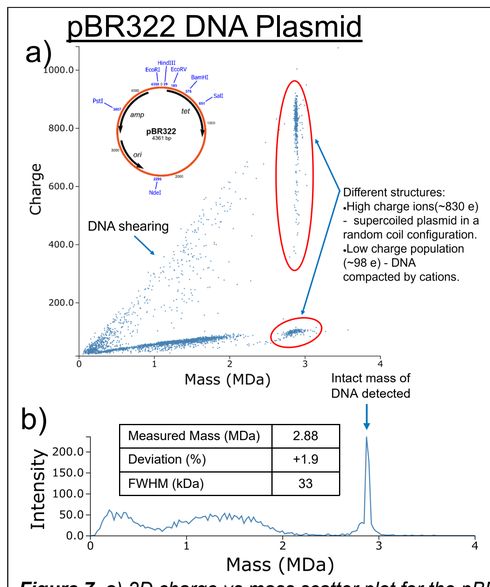


Figure 7. *a) 2D charge vs mass scatter plot for the pBR322 DNA plasmid where each point represents measurement of a single ion and b) mass spectrum (25 kDa bin width).*

DNA Ejection from an AAV

- DNA can be extracted from AAV vectors through in-solution heating - this enables mass determination of the packaged genome.
- More reliability in the measurement of the intact DNA helps with AAV quality control in manufacture.
- MW distribution of the DNA is affected by counterions, which are typically NH^{4+}, as the AAVs are in ammonium acetate solution when they are introduced into the CDMS using nanoESI.

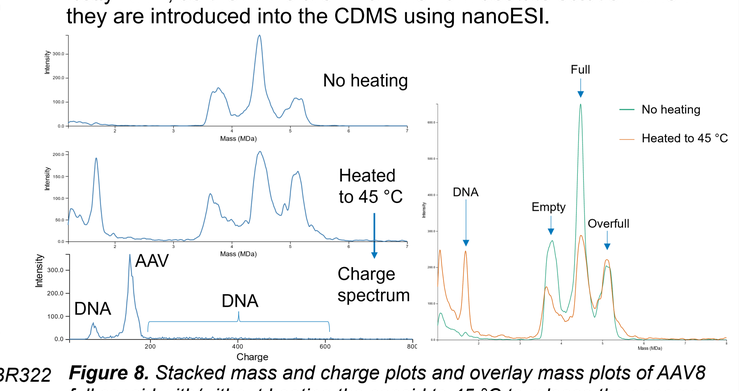


Figure 8. *Stacked mass and charge plots and overlay mass plots of AAV8 full capsid with/without heating the capsid to 45 °C to release the genome.*

Encapsulins

- Encapsulins are bacterial protein nanocages, which house cargo proteins that can have diverse functions.
- *Myxococcus xanthus* is a 180-mer bacterial capsid.
- CDMS data demonstrates the co-existence of two kinetically stable assemblies of the mutant encapsulin (Figure 9a):
  - 36-mer assembly at 1.1 MDa with tetrahedral symmetry
  - 60-mer assembly at 1.9 MDa with icosahedral symmetry
- Characterisation of encapsulins is useful for the development of biotechnological applications that depend on capsid morphologies i.e. for targeted drug delivery and vaccines.

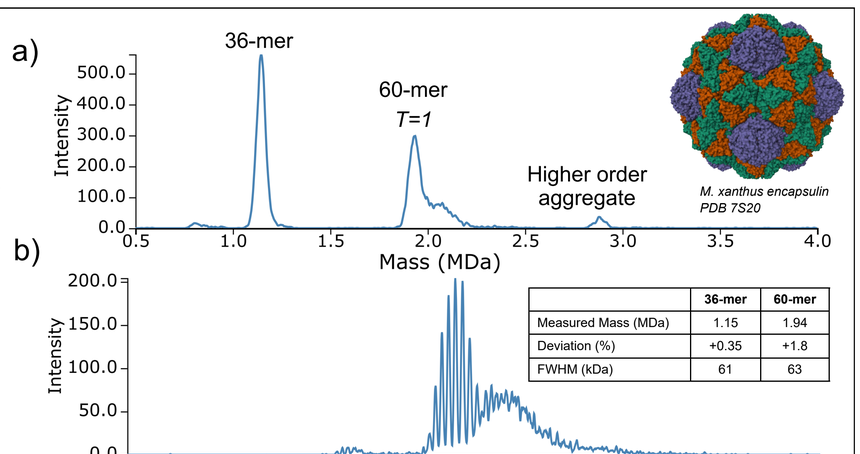


Figure 9. *Mutant protein nanocage a) mass spectrum b) m/z spectrum.*

Conclusions

1. CDMS provides direct mass measurement of very high molecular weight species - ion populations up to 150 MDa shown.
2. Distinguishing between empty, partial and full particles in AAV formulations can be readily achieved, including the relative quantification of empty and full particles based on the peak area ratios.
3. Structural information is discernible based on the distinct charge populations obtained for the pBR322 DNA plasmid.[3]
4. Intact mass measurements obtained for ejected DNA from AAVs, IPOL vaccine antigens, VLPs of various sizes, multimeric protein complexes, and protein nanocages. There is a strong interest in extending and evaluating the range of biomolecule analysis for CDMS.

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