

Utilizing discrete charge loss events on a minaturized dual sector CDMS analyser to determine charge state by frequency measurement alone

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INTRODUCTION

In CDMS, m/z determination relies on frequency measurements and image charge detection with charge-sensitive pre-amplifiers is used to determine the ion charge. However, frequency measurement is generally easier in comparison to amplitude. It has been observed that prolonged trapping of highly charged ions results in discrete frequency jumps owing to charge loss events (CLE)^{1,2}.

Here we describe a method to utilise CLE for mass determination.

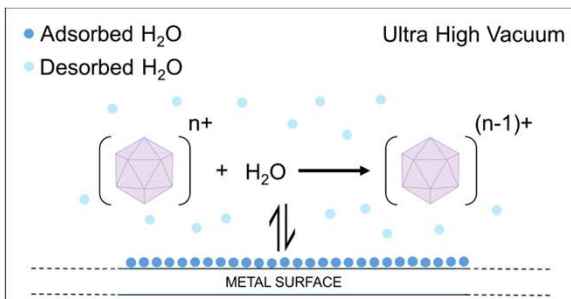
WATER IS RESPONSIBLE FOR CLE IN UHV

Charge loss events manifest as a jump in frequency within a scan. Assuming mass of the charge carrier is significantly smaller than the mass of the molecule, a simple formula can be derived for determination of charge from frequency jump alone.

$$z_1 = \frac{f_1^2}{f_1^2 - f_2^2}$$

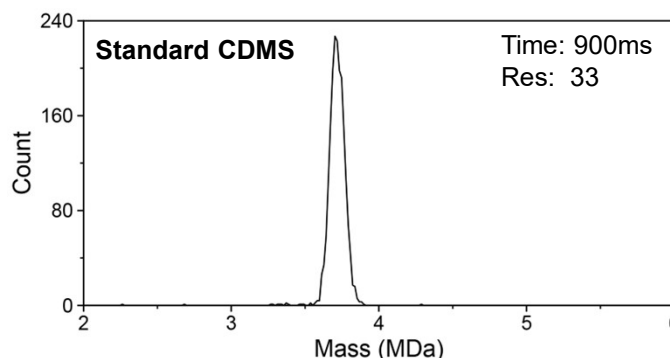
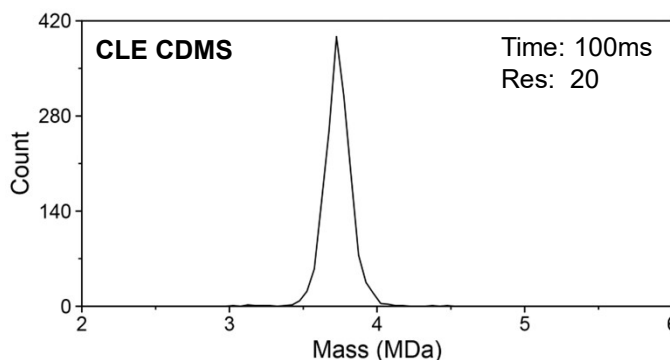
We found that in normal operating conditions at 2E-9 mbar pressure in the analyser, the frequency of CLE is on the order of 1:1000 events/scans. We could induce these events to occur ~1:30 events/scans by temporarily spinning down the analyser turbo until the water layer has built up.

| Analyser pump state | Final Pressure / mbar | CLE rate |
|-----------------------|-----------------------|----------|
| On (normal operation) | 2.00E-09 | 1:1000 |
| Off (minutes) | 2.00E-09 | 1:1000 |
| Off (hour) | 5.00E-08 | 1:30 |



AAV MASSES FROM CLE

By noting the CLE transition time and taking 50ms of FT on either side of the event, charge can be determined from the resultant frequency differences. Mass-to-charge is derived from the original frequency prior to the event. The result is shown below.



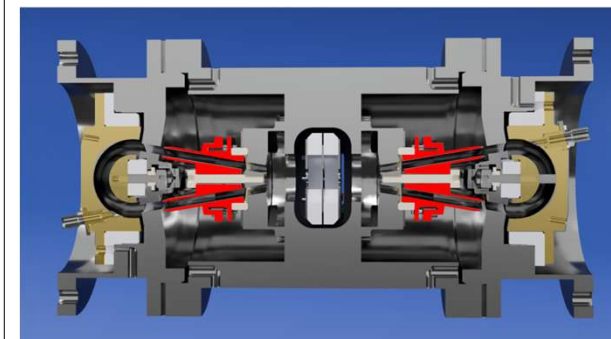
Heck lab previously reported species dependent propensity for CLE³. In their 24s orbitrap experiments, full AAV capsids lost charge less frequently than empty capsids. The difference in reactivity was observed at >5s trapping time, and virtually no CLE were detected at 1s.

At such high charge states, ie $z > 150+$, the reaction is likely collisionally limited, and therefore controlled introduction of a reagent mid-transient should have a negligible bias, at least for AAV samples.

EXPERIMENTAL

Experiments were performed on a second generation TrueMass CDMS analyser. Vertical slice of the Mk2 analyser is shown below. Empty AAV5 capsid purchased from AAVNerGene was diluted to 1E12 VP/mL in 200mM ammonium acetate for analysis via nanoESI.

Time domain data was recorded for 1s at a 2MHz sampling rate using PicoScope 4262. The data was processed in the frequency domain via Fourier transform as well as short-time Fourier transform with a TrueMass CDMS Explorer software package modified for research purposes.



CONCLUSION

Here we have demonstrated a novel way to generate CDMS data that does not rely on charge measurements by the preamplifier.

In future work, we will introduce a gas phase reagent directly into the analyser vacuum chamber for increased throughput and investigate potential species dependent reactivity.

REFERENCES

1. D. Z. Keifer, A. W. Alexander, and M. F. Jarrold, *JASMS* **2017** 28 (3), 498-506.
2. T. P. Wörner, K. Aizikov, J. Snijder, K. L. Fort, A. A. Makarov, and A. J. R. Heck, *Nat. Chem.* **2022** 14, 515-522.
3. E. H. T. M. Eberink, V. C. Yin, E. Deslignière, A. Barendregt, T. P. Wörner, K. L. Fort, A. A. Makarov, and A. J. R. Heck, *JACS* **2025** 147 (13), 10925-10934.

