ENHANCING MRNA PRODUCTION QUALITY: COMPREHENSIVE CHARGE DETECTION MASS SPECTROMETRY (CDMS) ANALYSIS OF DNA PLASMID AND MRNA STRUCTURES

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INTRODUCTION

- Messenger ribonucleic acid (mRNA) production is a multistep approach starting with the linearization of the DNA plasmid template.
- The supercoiled DNA plasmid, a precursor to the linearized template, is monitored during manufacturing. A higher percentage of supercoiled plasmid before in-vitro transcription reduces mRNA yield and purity due to its compact and stable nature.
- Common QC techniques involve capillary electrophoresis (CE). The technique lacks in accurately determining the length of circular plasmid, due to its more compact form compared to the linear standard ladders.
- 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*).
- Herein, we use CDMS to examine different plasmid structure populations, including supercoiled, open-circular, and linear forms. By analyzing these populations, valuable insights are gained into the quality of the DNA template and its impact on mRNA production.

CDMS EXPERIMENTAL SETUP

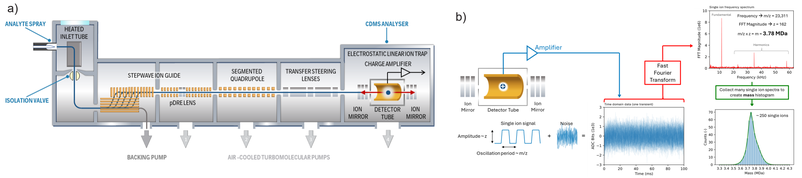


Figure 1. a) A schematic representation of the Waters ELIT-based CDMS instrument b) Diagram of the CDMS mass analyser and how m/z and z information is obtained.

- The CDMS mass analyser houses a conductive cylinder with two end caps, which reflect the ion back and forth.
- When an ion enters the detection cylinder, the charge on the ion is induced on to 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 from the signal amplitude.
- *m/z* × **z** → **m** for each ion

METHODS

Samples: Circular eGFP mRNA, DNA plasmid pUC19—Alu N I linearized and Alu N I + BanI 2x Cut were provided by Biospring. DNA plasmid pBR322 (New England Biolabs). For CDMS analysis, the samples were buffer exchanged into ammonium acetate solution with 0.01 % Pluronic™ F-68 (Gibco) using Bio-Spin® P-6 size-exclusion columns (Bio-Rad Laboratories).

Table 1. Sample information.

Sample	Topology	Length (bases)	Approx. Mw (MDa)
eGFP	Circular mRNA	1637	0.54
pUC19	Circular dsDNA	2686	1.75
pBR322	Circular dsDNA	4361	2.83

Capillary electrophoresis (CE) (Biospring): CE was performed at BioSpring using an Agilent 5200 Fragment analyzer with twelve 33 cm parallel capillaries. For plasmids a DNA specific method with 6.0 kV for 50 min to separate 35 to 5000 bp was used. For circRNA and mRNA a RNA specific method with 8.0 kV for 40 min to separate 15 to 6000 nts was used. Samples were prepared by diluting them into a premixed buffer containing a fluorescent dye for detection. Specific ladders spanning the above mentioned ranges were used for calibration prior to sample evaluation.

CDMS (Waters): Ions were generated in positive ion mode using nanoelectrospray ionisation and mass analysis was performed using a prototype ELIT-based CDMS. Signal processing and data visualization was performed using software developed in-house. Ions were trapped for 100 ms, and total acquisitions times were between 10 and 15 minutes. Detected time-domain signals were Fourier transformed; the measured frequency and the magnitude correspond to an individual ion's m/z and z respectively, enabling direct calculation of mass values. Data for individual ions were histogrammed to generate m/z, charge and mass spectra as well as 2-dimensional heat-maps.

RESULTS AND DISCUSSION

Circular eGFP mRNA

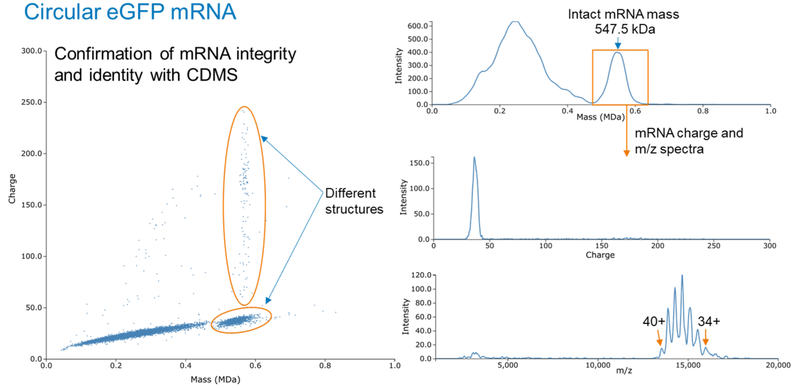


Figure 2. CDMS 2D charge vs. mass scatterplot (where each point represents a single ion), mass histogram, charge spectrum and m/z spectrum for circRNA. (20 kDa mass bin width, 8870 ions in a 10 minute acquisition time).

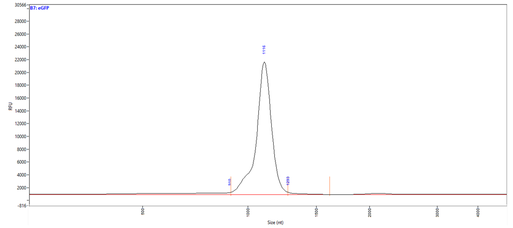


Figure 3. Electropherogram of eGFP circRNA.

The size of the circRNA is lower than excepted due to its circular form. Expected length is 1637 nts and is displayed as 1116 which is a difference of ~ 32 %. Additionally next to the main peak a second peak can be detected, which can arise from truncated species or it could be a second isoform which matches the CDMS data.

Circular pUC19 DNA Plasmid

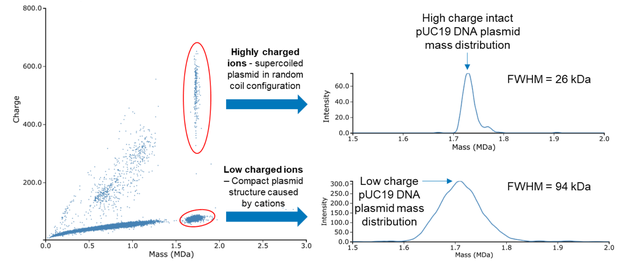


Figure 4. CDMS 2D charge vs. mass scatterplot (where each point represents a single ion), mass histogram, charge spectrum and m/z spectrum for circular pUC19 (20 kDa mass bin width, 2300 ions of intact plasmid in a 10 minute acquisition time). The mass excess of the measured plasmid mass from the expected sequence mass is within 2 %. The mass accuracy is higher for the highly charged form of the DNA plasmid (mass excess within 1 %).

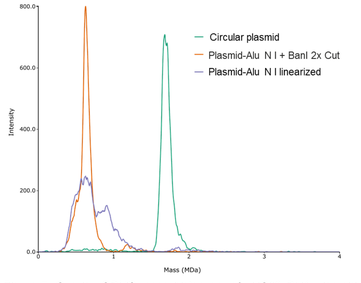


Figure 5. Overlay CDMS mass histogram of pUC19 DNA plasmid, Alu N I linearized and Alu N I + BanI 2x Cut

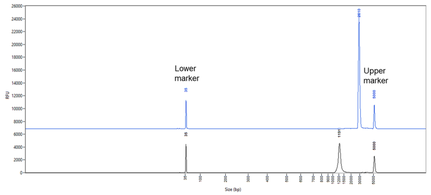
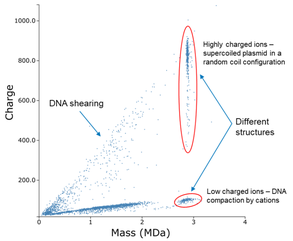


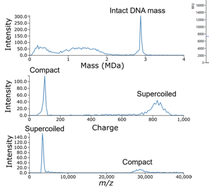
Figure 6. Comparative electropherograms of linearized (upper graph(blue)) and circular (bottom graph(black)) pUC19 plasmid. Linear pUC 19 matches the theoretical length with ~4% deviation. The circular form can not be sufficiently identified using the standard CE approach.

As shown in Figure 5. all variations of the pUC19 DNA plasmid samples were successfully analyzed using CDMS, with intact masses closely matching expected values. Notably, differences in peak widths were observed between the mass spectra of singly and doubly cut plasmids. Additionally, an unidentified peak (~0.6 MDa) detected in the Plasmid-Alu N I linearized sample may result from degradation, as this is a common observation with linearized plasmids.

Circular pBR322 DNA Plasmid



Measured Mass (MDa)	2.88
Deviation (%)	+1.9
FWHM (kDa)	33



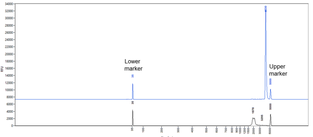


Figure 8. Comparative electropherograms of linearized (upper graph(blue)) and circular (bottom graph(black)) pBR322 plasmid. Linear pBR322 matches the theoretical length with ~7% deviation. The circular form can not be sufficiently identified using the standard CE approach. Due to its size the circular form is present in multiple isoforms (see peak at 1878).

Figure 7. CDMS 2D charge vs. mass scatterplot (where each point represents a single ion), mass histogram, charge spectrum and m/z spectrum for circular pBR322 (20 kDa mass bin width, 8000 ions in a 10 minute acquisition time).

CONCLUSIONS

- Intact circular pUC19 and pBR322 plasmid as well as eGFP mRNA masses were obtained with CDMS. Measured masses correspond well with expected masses (up to ~ 2 % deviation in the measured mass compared to sequence mass).
- CE showed good results for the linearized (deviation of ~4% for pUC19 and ~7% for pBR322) while the circular form could not be sufficiently identified. Here, CDMS shows its superiority by lower mass deviation from the theoretical value and the mass information instead of the length.
- Structural information discernible with CDMS e.g. for the mRNA and DNA plasmids obtained from measurement of distinct charge populations.

Acknowledgements

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