Rapid Screening of Megadalton Native mRNA and dsDNA using Charge-Reduced Ion Mobility Spectrometry

Megadalton nucleic acids under various stress conditions were screened rapidly using a novel stability indicating ion mobility method

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Introduction

The recent global pandemic has highlighted the impact of nucleic acid-based modalities. Messenger RNA (mRNA), interfering RNA (RNAi), double stranded DNA plasmids are being used in emerging gene and cell therapies. Historically, large sized nucleic acid moieties are challenging to analyze using traditional LC-MS techniques due to structural heterogeneity, ionization and detection limitations. Enzymatic digestion assays are time consuming and not practical for screening applications. Here, we present a simple yet informative method to rapidly (<15 mins) screen native RNA and DNA that have extensive polydispersity.

Method

Charge-Reduced Electrospray

The charge reducing process starts with electrospraying analyte ions dissolved in volatile buffer that generate primary droplets carrying a single analyte ion. These droplets are then charge reduced by an incoming flow of bipolar air ions (Fig.1). Through gentle droplet evaporation method, singly charged ions that are not coulombically stretched out are generated. Such singly charged ions are not distorted and able to maintain native conformation. Charge reduced analyte ions then get deflected onto a detection ring carefully positioned on the wall of the spectrometer. Once ions are landed, ion mobility spectra are recorded through analog current measurement, thus circumventing the usage of micro channel plates in traditional MS systems. This enables detection of megadalton sized analytes like mRNA without difficulty.

Nucleic Acids

996bp/320kD EGFP mRNA (Trilink Biotechnologies), 3420b/1.1MDa Beta Gal mRNA (Trilink Biotechnologies) and 4361bp/2.83MDa pBR322 DNA plasmid (Thermo Fischer Scientific) samples were analyzed. Samples underwent Ammonium Acetate buffer exchange onto P-6 desalting gel columns (Bio-Rad) prior to infusion. Final sample concentration was 2.5nM-75nM. Various stability studies were done using heat, shear pressure, pH, freeze/thaw cycles and serial dilution

Ion Mobility Spectrometry

IMgenius™, a simple bench-top atmospheric-pressure based ion mobility spectrometer (IonDX, Inc.,)(shown on top right panel) was used to determine the mobility constant K, or 1/ K of singly-charged electrospray ions of nucleic acids. Ion mobility spectrum of intact nucleic acids were processed using the ION Browser™ software.

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Fig. 1

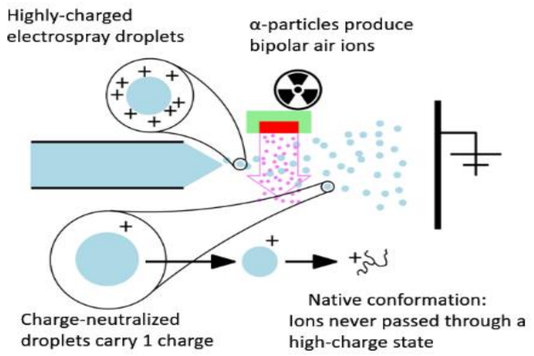
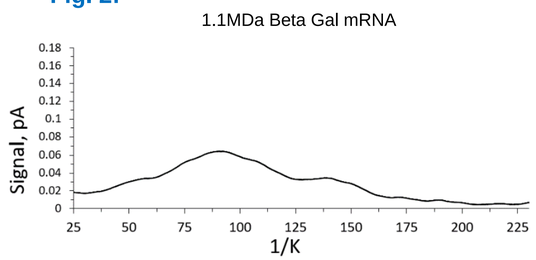
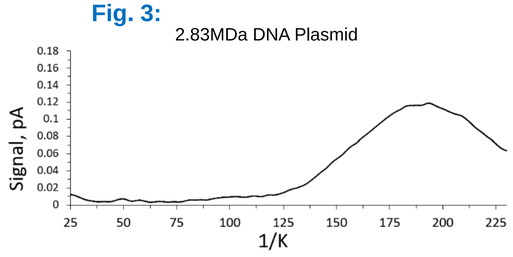


Fig. 2:



smaller ions → larger ions

Fig. 3:



smaller ions → larger ions

Fig. 4:

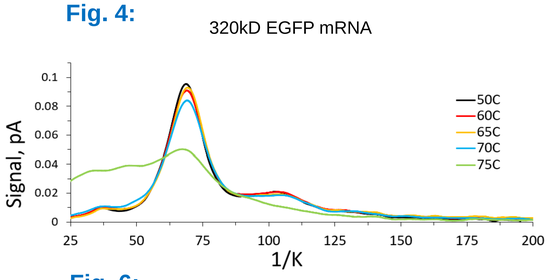
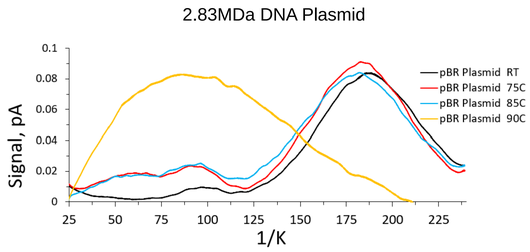


Fig. 5:



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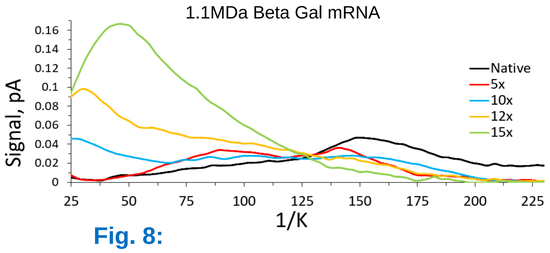


Fig. 7:

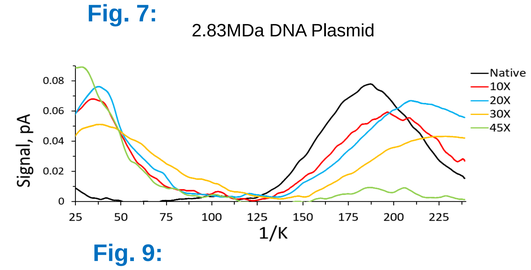


Fig. 8:

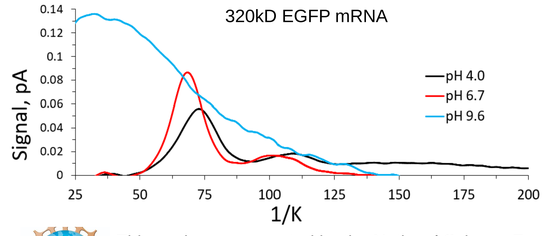
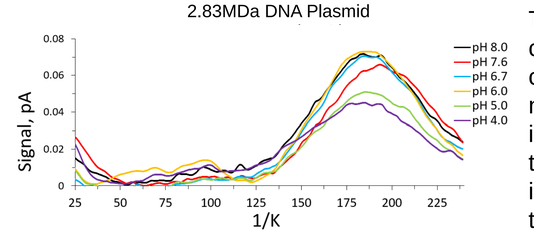


Fig. 9:



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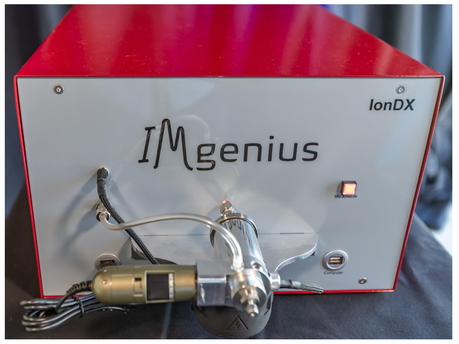

Results

Fig. 2. Ion mobility spectra of native mRNA shows presence of polydispersity and subpopulation of higher order structures.

Fig. 3. Ion mobility spectra of native plasmid DNA shows a symmetrical distribution of conformations confirming the presence of a primary supercoiled structure (larger ions in the higher 1/K range).

Fig. 4. Ion mobility spectra of thermally stressed mRNA sample shows melting temperature in the range of 70-75 degrees C. Appearance of new material at 1/K < 50 indicates formation of small fragments.

Fig. 5. Ion mobility spectra of thermally stressed plasmid DNA sample shows a relatively stable sample with degradation occurring around 90 degrees C.

Fig. 6. Ion mobility spectra of shear stressed mRNA shows effective degradation after 5-10 cycles. Appearance of new material at 1/K < 125, suggests formation of fragments.

Fig. 7. Ion mobility spectra of shear stressed DNA . As shear cycles increase, 1/K values shift toward larger size, indicating unfolding. After 10 shear cycles nearly all the dsDNA was fragmented, leading to a new peak at 1/K = 40.

Fig. 8. Ion mobility spectra of pH stressed mRNA shows mobility shifts to the left of the spectrum with increased pH thereby indicating presence of lower molecular weight degradant species. Lowering the pH from 6.7 to 4.0 shifted the mobility peak from 68.4 to 72.8, indicating unfolding. Raising the pH 6.7 to 9.6 caused a portion of the original mRNA to unfold (new peak appears between 1/K of 75 and 110) and a portion to degrade (new peak at 1/K< 70).

Fig. 9. Ion mobility spectra of pH stressed DNA shows a comparatively resistant moiety with minimal mobility shifts or changes. Starting at pH = 6, raising the pH caused unfolding while lowering the pH led to molecular compaction.

Summary

This ion mobility-based analytical technique extends macromolecular characterization to megadalton nucleic acids. Analyzing singly-charged ions minimizes coulombic stretching possibilities and allows near native conformations to be investigated. Using a single instrument, it was possible to evaluate the effect of shearing, temperature, and pH on the stability of several types of nucleic acids, including mRNA, dsDNA and plasmid DNA. In the future, this technique will be applied to study native mRNA and other RNA/DNA-based binding reactions as well as forced degradation of such complex structures.