Preliminary Evaluation of an Extended Mass Range Ion Mobility Spectrometer Determines CCS of Small Proteins to AAV's

IonDx Inc.

Ben Aguilar* and W. Henry Benner
IonDX Inc., Monterey, California

Motivation

1) Evaluate the performance of IMGenius, an extended range ion mobility spectrometer, by measuring the CCS of electrospray macroions spanning a broad size range.
2) Apply modern computing methods to improve measurement accuracy and optimize instrument operating parameters for a greater upper size limit than previously achieved.

Methods

Instrumentation

IMGenius, a benchtop sized, laminar flow, atmospheric pressure ion mobility spectrometer (Fig. 1) was used for the purpose of measuring the collision cross section (CCS) of lowly-charged electrospray macroions.

Simulation

A computational fluid dynamics (CFD) model using the OpenFOAM engine was developed to simulate the flow of gas inside the spectrometer, illustrated in the upper panel of Fig 2. Dark red areas indicate regions of greater flow velocity, while blue areas indicate lower velocity.

SIMION software was used to simulate the electric field surrounding the center electrode of the spectrometer (Fig 2, lower panel). The lines drawn on the illustration are constant-field lines, analogous to constant-elevation lines on a topographical map.

These two simulations have identical geometry, and the velocity results of the CFD model were overlaid with the electric field calculated by the SIMION model. A custom SIMION user program was created to generate trajectories as the simulated gas flow carried ions through the electric field.

Electrospray Charge Neutralization

Lowly charged gas-phase protein ions were generated using a combination of electrospray and charge neutralization (Fig. 3). Highly-charged electrospray droplets were exposed to bipolar air ions generated by alpha radiation emitted from a radioactive Polonium source (Kaufman) produced singly-charged protein ions that never passed through a high charge state, thus preserving their native conformation.

Protein analysis

Samples are prepared by desalting and buffer exchanging into 25 mM ammonium acetate, then electrospraying at 300 nL/min.

References
Hogan, CJ and JF de la Mora, doi 10.1007/s13361-010-0014-7
Kaufman, SL., et al., doi 10.1021/ac951128f
Kaufman, SL., doi 10.1016/S0021-8502(97)00462-X
Hogan, CJ., et al., doi 10.1021/jp109172k

Figure 1: The IonDx spectrometer with multiple modes of operation rapidly analyzes protein mobility distributions.

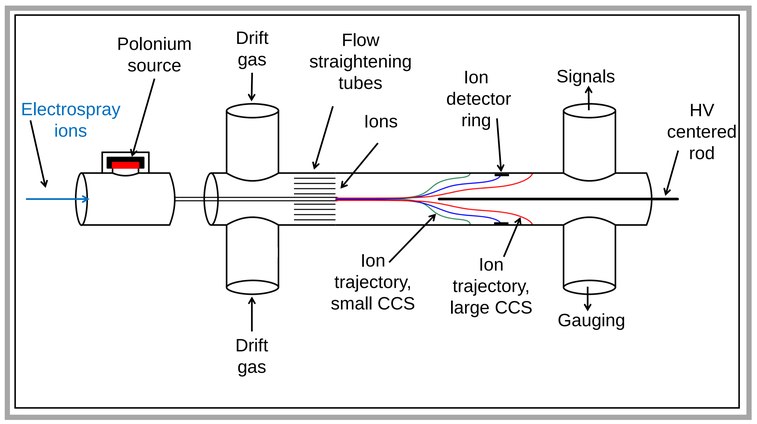
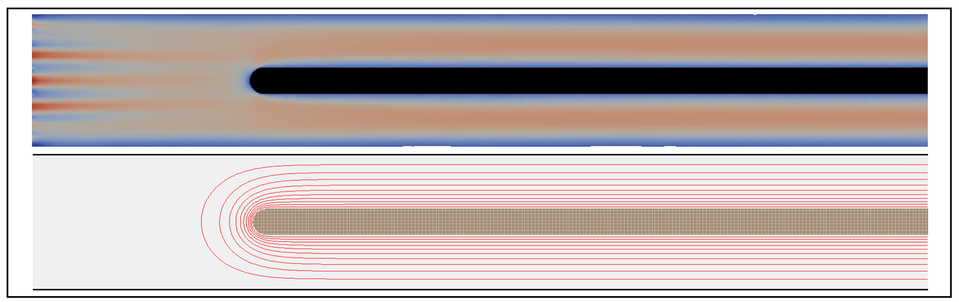
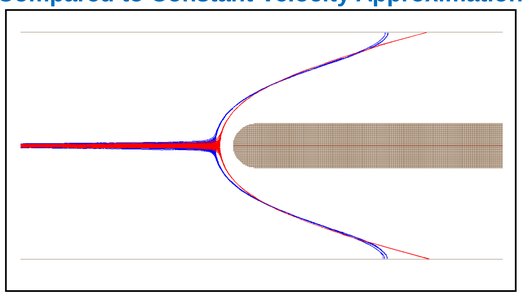


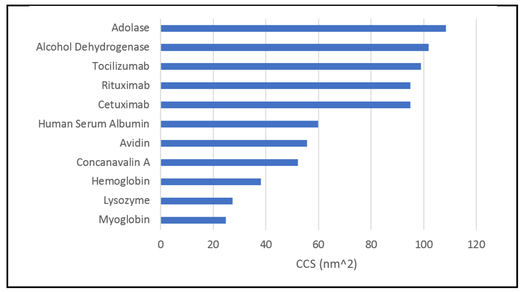
Figure 2: CFD flow profile and SIMION Electric Field Calculation



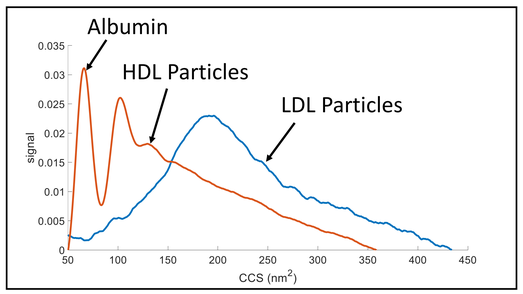
Result 1: Ion Trajectories with CFD results Compared to Constant Velocity Approximation



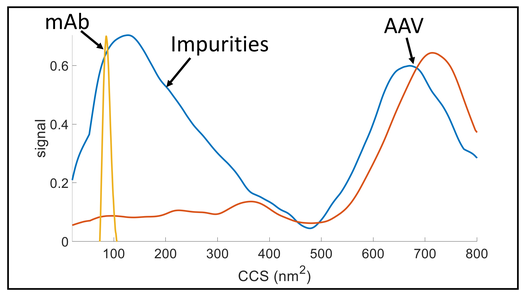
Result 2: CCS of Small Proteins Analyzed in the Singly Charged State



Result 3: Mobility scan data for high-density lipoproteins



Result 4: Analysis of AAV particles reveals large differences in size and purity



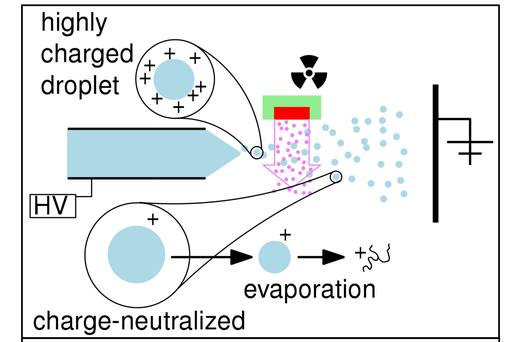


Figure 3: Electrospray detail - A cloud of air ions produced by a radioactive source reduces droplet charge (Kaufman).

Results

1) Integrating high-fidelity CFD results with the SIMION model allows for more accurate calculations (blue trajectories) compared to a constant velocity flow profile (red trajectories), where the gas velocity is approximated as the average velocity across the entire cross-section. The CFD simulation accounts for stagnant boundary layer conditions near the inner wall or near the center rod, as well as the development of a parabolic velocity profile (upper panel in Fig. 2.)
2) The design provides gentle transfer of singly-charged ions into the spectrometer that preserves ion hydration, thus providing a new way to investigate near-native conformations. CCS values for several test proteins are compared to literature values.
3) The upper measurable CCS is 1000 nm^2 , equivalent to mobility of 0.0022 $cm^2/V*sec$ or particle diameters as large as 30 nm.
4) Result 3 shows the analysis of HDL and LDL lipoprotein particles in the CCS range of 100 – 400 nm^2 , equivalent to mobility of 0.02-0.005 $cm^2/V*sec$ or particle diameters as large as 20 nm as used for assessing coronary artery disease.
5) Result 4 shows the size distribution of AAV particles extending to particles having CCS as large as 800 nm^2, equivalent to mobility of 0.003 $cm^2/V*sec$ or particle diameters as large as 29 nm. The variation of AAV particle size is significantly larger than the variation of conformers in a biotherapeutic mAb.

Summary

The performance of a simple benchtop spectrometer was benchmarked for characterizing particles with CCS from 10-1000 nm^2, equivalent to particle diameter from 3 - 30 nm or K between 0.2 and 0.002 potentially extends ion mobility spectrometry to the range of sizes encountered by the new high mass biotherapeutic modalities that includes AAV's, mRNA and lipid nanoparticles, along with large protein complexes such as lipoproteins

* Contact: Ben Aguilar
baguilar@iondx.com

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