Ion Mobility Measurement of the GroELTetradecameric Complex by Tandem Differential Mobility Analysis Mass Spectrometry

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Introduction

•Tandem ion mobility-mass spectrometry (IM-MS) has potential for the analysis of biomolecular structures, which are ionized and introduced into the gas-phase by electrospray ionization under non-denaturing conditions.

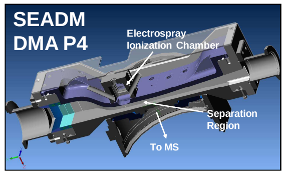
•Several questions remain regarding the interpretation of the mobilities measured, including:

- •is the liquid phase structure preserved in the gas phase ion?
- •What is the influence of lack of perfectly elastic and specular scattering (often assumed for IM-MS data interpretation), on the determination of **collision cross-sections** (CCS) from IM measurements?
  - •In N_2 and Air, nanoparticles (protein sized objects) have mobilities **1.35** times smaller than they would if collisions were elastic and specular.[1,2]
- •What are the effects of polarization and Coulombic stretching on IM measurements of proteins electrosprayed under non-denaturing conditions?

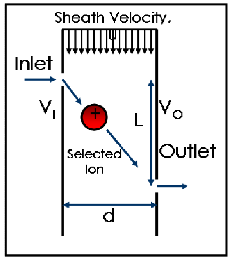
•We use a differential mobility analyzer-mass spectrometer (DMA-MS) to analyze the 800 kDa GroEL tetradecameric chaperone complex from *E. coli* to examine changes in protein structure due to the electrospray process, assess the influence of inelastic scattering on protein complex ion CCSs, and to examine the effects of polarization on IM measurements. (electrosprayed in aqueous ammonium acetate buffer).

DMA-MS Operation

a.



b.



$$Z_p = \frac{U\delta^2}{LV_{DMA}} \qquad V_{DMA} = V_I - V_O$$

Z_p = ion mobility

c.

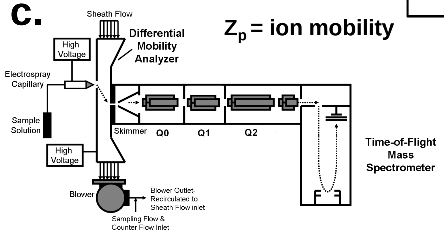


Figure 1. a. CAD image of parallel plate DMA P4 (SEADM). **b.** The DMA operating principle. **c.** Schematic of the DMA P4 mounted on a QSTAR mass spectrometer (Sciex).

Results

Figure 2. Mobility-mass spectra for GroEL 14-mers, with declustering potential V_{dec} = 350 V applied downstream from the DMA. Declustering leads to monomer and occasionally dimer loss from 14-mers, downstream of the DMA; thus, the mobilities at which lower order n-mers appear is dependent upon the mobility of their parent ions. A zoomed in view of the 14-mers is shown below the complete mobility-mass plot. IM-MS measurements reveal that two different conformations persist in the gas-phase.

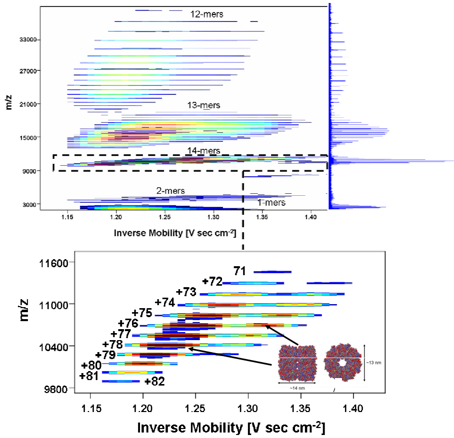
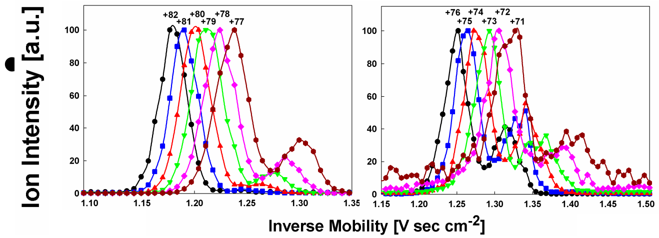


Figure 3. Mass-selected ion abundance versus inverse mobility for GroEL 14-mers, displaying narrow peaks and the existence of two conformers.



Mobility (Stokes-Millikan) Equation

•Stokes-Millikan equation shown valid in air, and N_2 at atmospheric pressure for singly charged aerosol nanoparticles[2,3].

•Semi-empirical, 91% inelastic collisions determine from Millikan apparatus measurements[1], but the influence of polarization is not accounted for (note CCS = $\pi(d_i+d_g)$

$$Z_0\left(1+\frac{m_g}{m_i}\right)^{-1/2} = \frac{zeC}{3\pi\mu\left(d_i+d_g\right)}$$

$$C = 1+\frac{2\lambda}{d_i+d_g}\left(1.257+0.4\exp\left(\frac{-0.55\left[d_i+d_g\right]}{\lambda}\right)\right)$$

Z_0: hard-sphere ion mobility, ***z***: charge state.
d_i: ion mass diameter, ***d_g***: gas diameter,
m_g: gas molecules mass, ***m_i*** ion mass,
μ: gas viscosity, ***e***: electron charge,
λ: mean free path of gas molecules (~66.5 nm)

Analysis & Conclusions

•IM-MS measurements reveal that z/Z_p values depend slightly on z. Hence, both conformers are affected by either Coulombic stretching or by polarization.

- •Dependence is quadratic in z.
- •Linear regression of these plots allows for determination of hard-sphere mobility of both conformers.
- •Both conformers yield approximately the same z/Z_p value at zero charge: $(Z_o/z) = 0.0117\ cm^2\ V^{-1}s^{-1}$
  - •CCS~137 nm^2 (inelastic)
  - •CCS~185 nm^2 (elastic)
  - •CCS=197 nm^2 (crystal)

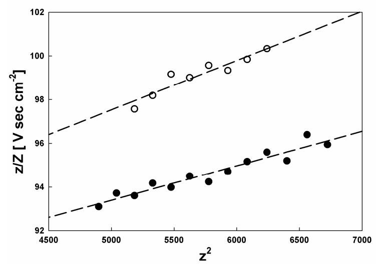


Figure 4. A plot of measured z/Z_p values as a function of z^2 for the more mobile (closed circles) and less mobile (open circles) tetradecamers observed.

- •Prior CCS measurements[3] ~ 244 nm^2 (SYNAPT HD-MS, inelasticity ignored)
  - •Hard Sphere Approximation[4] for Native Structure: 197.5 nm^2.
  - •We conclude that GroEL 14-mers collapse in the gas-phase (partially).
  - •DMA measurements are made immediately following drop evaporation.
    - •Collapse occurs due to the electrospray process itself.
- •Without accounting for inelastic collisions, the collapse of GroEL tetradecamers would go largely unnoticed.
- •Inelastic collisions must be accounted for in IM-MS measurements made in air, N_2, and possibly He!

Acknowledgements & References

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