Characterizing Macromolecular Dipole Moments via Differential Ion Mobility Spectrometry with Linked Field/Pressure Scans

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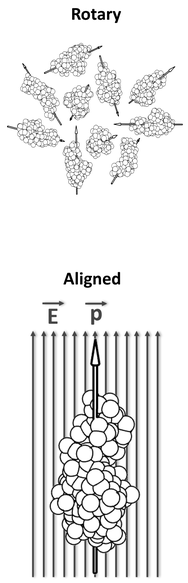
1: Introduction

- Ions separated by IMS methods are usually free to rotate. Then the mobility reflects the properties of orientationally-averaged collision cross section (CCS).
- Nearly all macromolecules have permanent dipole moments. Those for proteins mostly come from the helices and charged residues and strongly depend on the secondary and tertiary structure.
- The electric field in IMS can align sufficiently strong macrodipoles (over ~300 Debye at STP), which are common for proteins and protein complexes above ~30 kDa [1, 2]. Then the field-dependent mobilities can reveal the magnitude of dipole and directional CCS in the plane perpendicular to the field.
- Disentangling this effect from other inevitably present high-field phenomena has been problematic in previous studies at a fixed (ambient) gas pressure.
- By varying the pressure at constant E/N, the dipole alignment governed by absolute E can be isolated from all other FAIMS effects controlled by E/N.

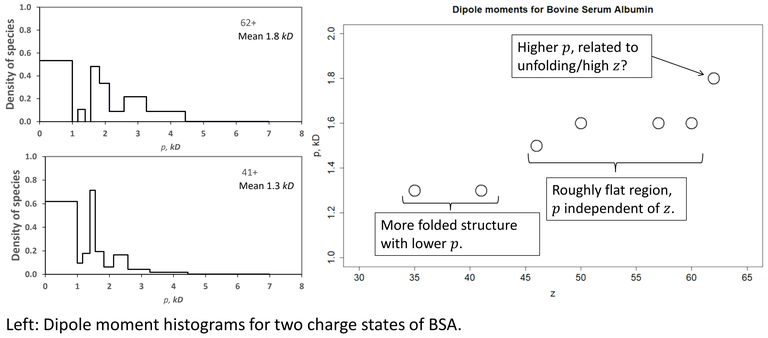


4: Dipole moments

The weakest dipole that can be aligned at any pressure [2] is approximately:

$$p_{crit} = (245\ D)\ \frac{1\ Atm}{P} \qquad (1)$$

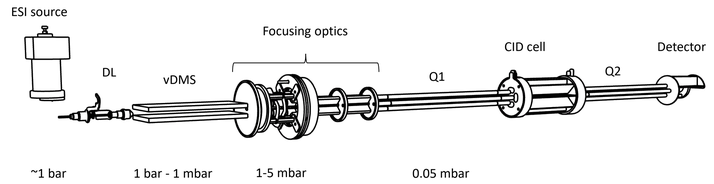
The fraction of conformers with p above a value set by eq. (1) can be assessed as the area of aligned distribution relative to the total. Differentiating that fraction as a function of p_{crit} yields the dipole moment histogram. The average moment for BSA ions increases as outlined above, with an apparent jump over the z ~ 40 - 50 range suggesting the unfolding transition. This would mirror the steps in collision cross sections with increasing z well-known for proteins in linear IMS studies.



Left: Dipole moment histograms for two charge states of BSA.
Right: Mean dipole moment of each charge state studied for BSA.

2: Instrumentation/Methods/Linked scanning

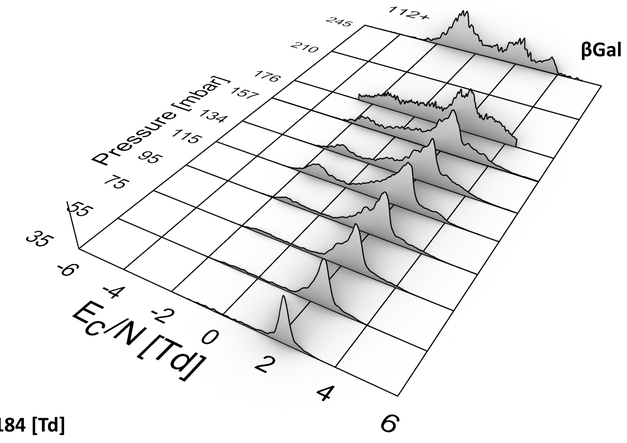
Samples were prepared by dissolving the lyophilised protein (Merck, UK) in 50:49:1 methanol/water/formic acid and infused an ESI source at 9 μL/min.



Experiments employed a modified LCMS-8060 system with a vacuum differential mobility spectrometry (vDMS) cell inserted between the DL and Q-array elements [3]. The pressure of N_2 in vDMS was P = 35 - 245 mbar, controlled by the pumping/addition of gas. The near-ideal rectangular waveform had the frequency of 200 kHz and aspect ratio of 2.5.

For the linked scans, we fixed the ratio of dispersion field (E_D) to gas number density (N) and varied the dispersion voltage (defining E_D) and pressure (defining N at constant temperature T, 80 °C) in proportion.

5: Aligned ions with positive CV shift

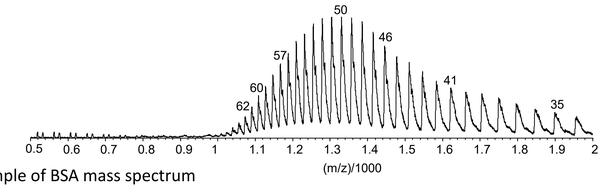


All aligned proteins were previously observed to have more negative E_C values than the rotary conformers [1, 2]. That made sense as the macromolecular dipoles are preferentially oriented roughly along the long molecular axis (principal moment of inertia), wherein the directional CCS in orthogonal plane falls below the orientationally-averaged CCS.

Here, the spectra for βGal at some z exhibit new features at more positive E_C values. These are less common and smaller than those at lower E_C, per the said basic physics of dipoles. Such peaks could not be firmly distinguished from the possible separated rotary conformers in ambient-pressure FAIMS. Now we can characterize their dipole moments and directional CCS too.

3: Dipole alignment switchable by pressure

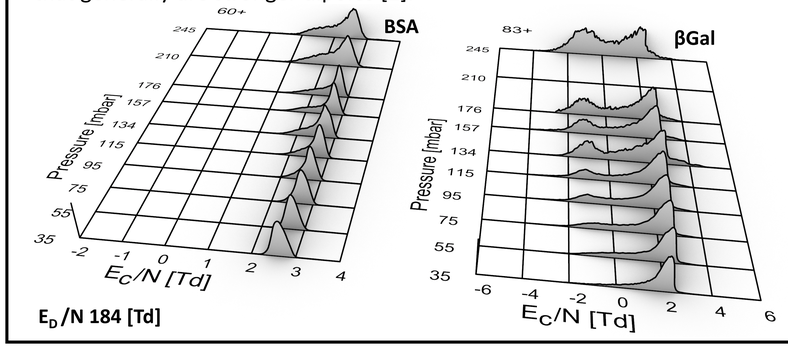
We probed the selected charge states z of albumin (BSA, 66.5 kDa) and β-galactosidase (βGal, 105 kDa) - the largest protein studied by FAIMS to date.



An example of BSA mass spectrum

At low pressures, the FAIMS spectra make single Gaussian distributions. The tails emerge one their low-E_C sides and expand with increasing pressure, eventually forming distinct peaks indicating the dipole alignment. For both proteins, these generally arise at lower pressures and grow larger for higher z, in line with greater P values anticipated for species with higher total charge and more unfolded geometries allowing greater charge separation.

These tails appear at lower pressures and become more prominent for βGal than BSA species at comparable m/z values, as expected for larger proteins that generally are stronger dipoles [2].



6: Conclusions

- Dipole alignment tuneable by varying the gas pressure
- Per the theory, larger proteins align at lower pressures for a given field
- Higher charge states generally exhibit stronger alignment
- Alignment can increase or decrease the ion mobility
- Dipole moments robustly extracted from linked FAIMS scans

Acknowledgments/References

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