

Ion Mobility Mass Spectrometry of Native Protein Complex Anions

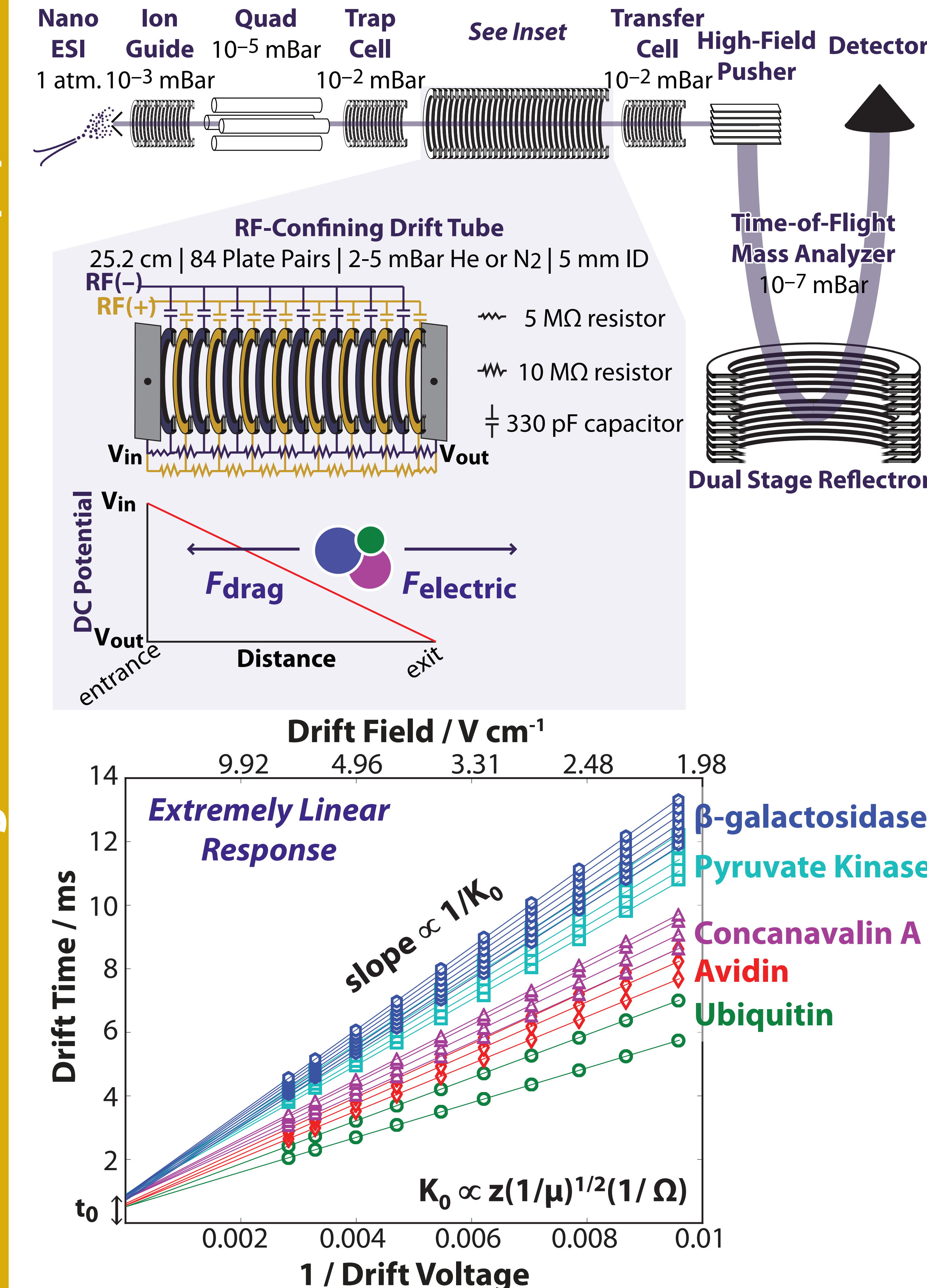
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Key Outcomes

- Ion mobility (IM) mass spectrometry used to characterize **protein complex anions**.
- **Anions adopt smaller average charge states** than the corresponding cations, which may be consistent with lower **field-emission** energies for the loss of charge carriers from anions.
- **Triethylamine** addition results in the appearance of additional, **charge-reduced cations**.
- **Cations, anions, and charge-reduced cations** all have similar collision cross section (Ω) values, suggesting that they all have **similar structures** that depend weakly on charge state.
- These anion Ω values can be used to measure **accurate** calibrated Ω values with **traveling-wave IM**.

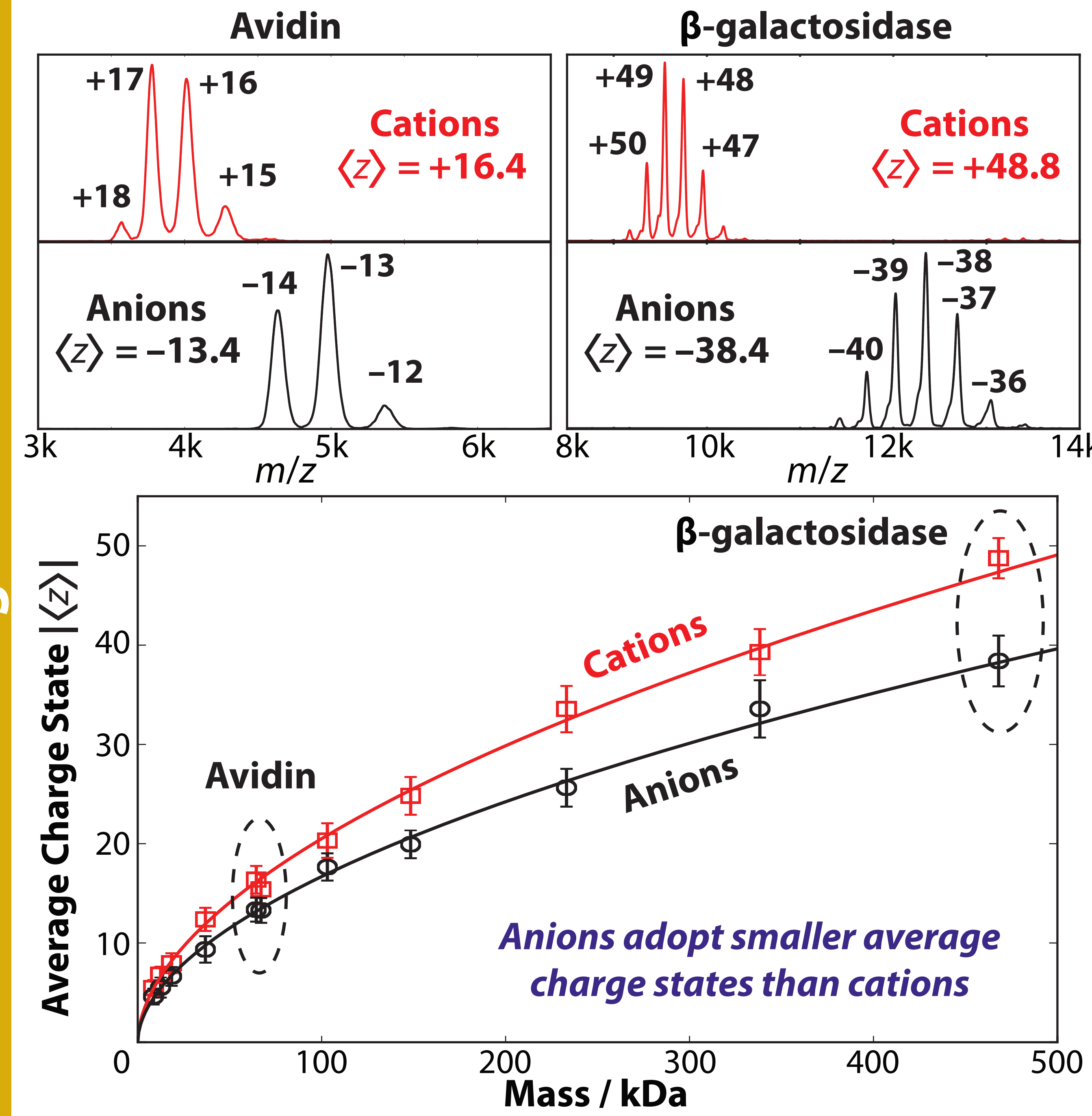
Measuring Collision Cross Sections (Ω)

An RF-confining drift tube has been implemented in place of the traveling-wave ion mobility cell on a Waters Synapt G2 HDMS, which controls all potentials and regulates gas flow.

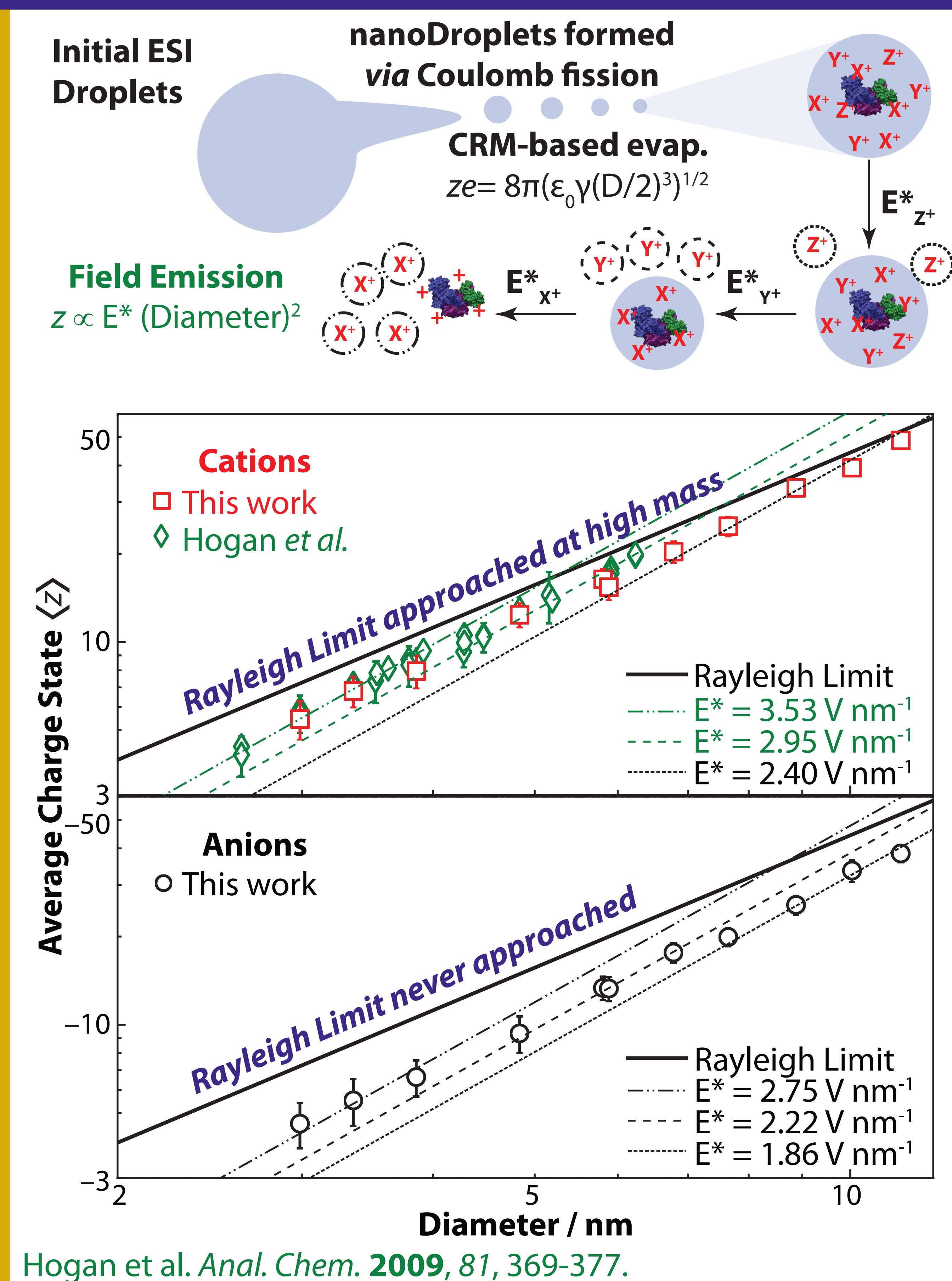


Drift times for anions of native-like proteins were measured at 10 drift voltages ranging from 100 - 350 V in 2 Torr of helium gas.

Charge States



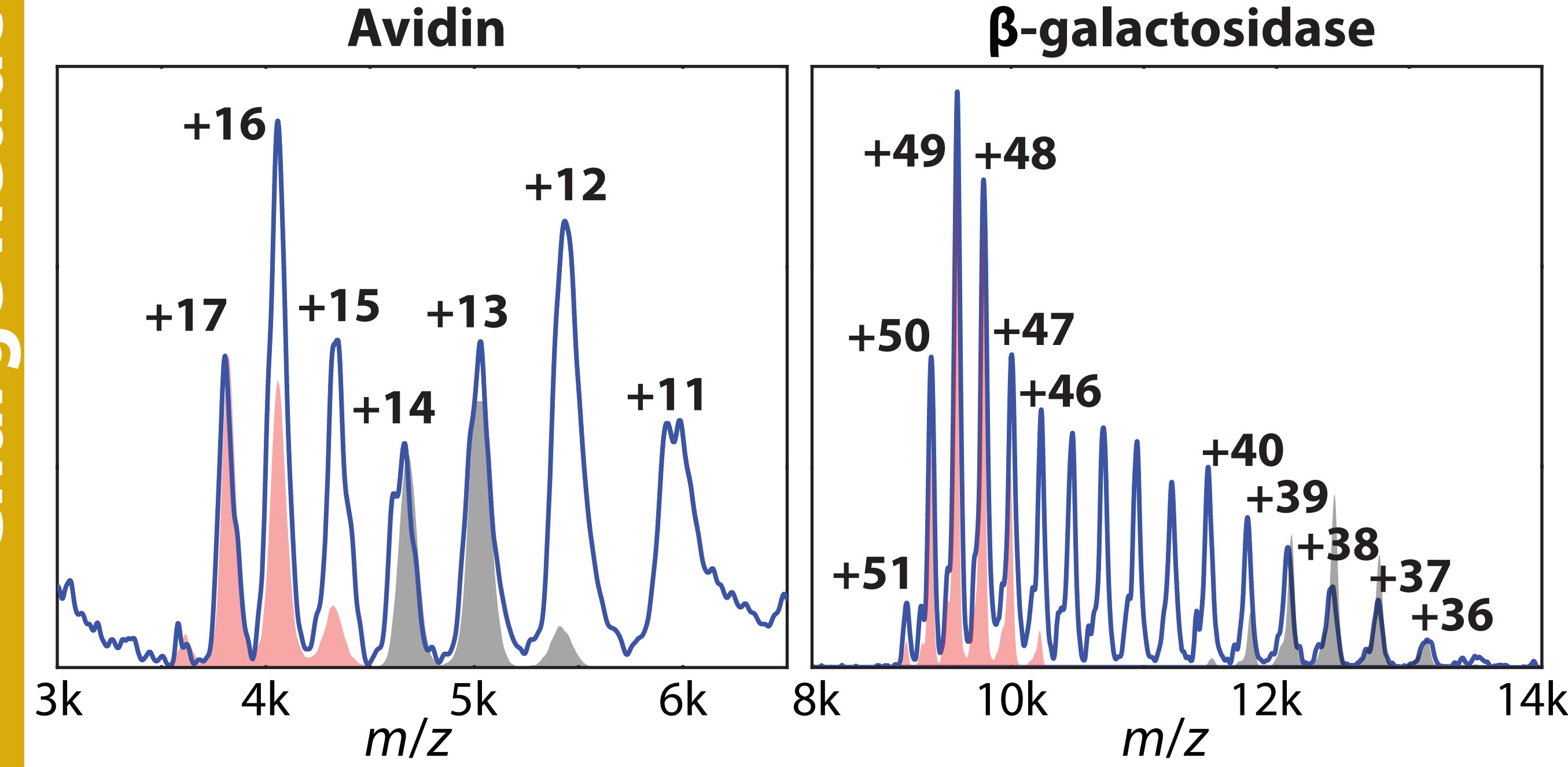
Field-Emission Model



Hogan et al. *Anal. Chem.* **2009**, *81*, 369-377.

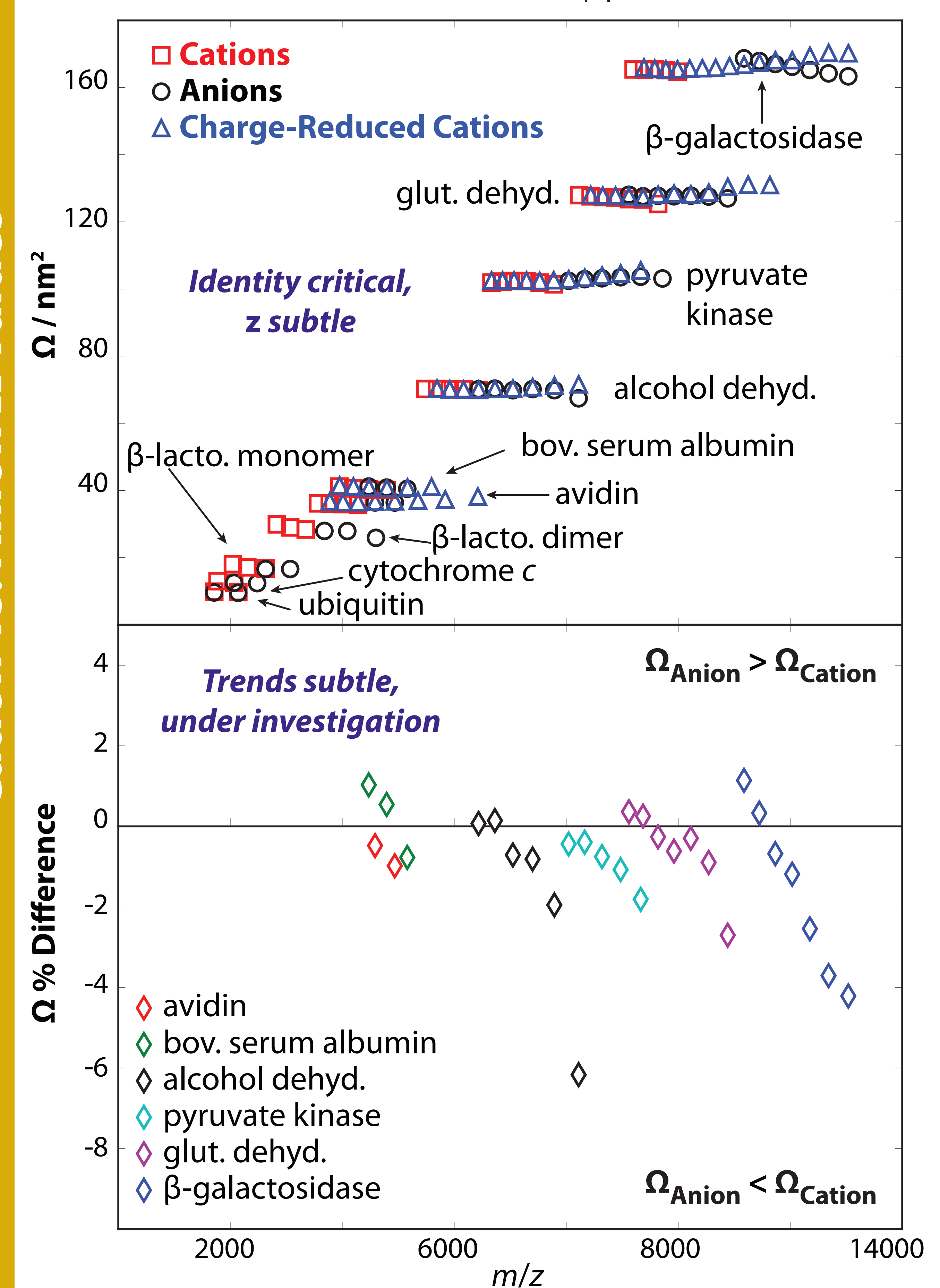
Charge Reduction

The addition of 10 mM triethylamine to the protein solution results in the appearance of a much wider charge-state distribution. Charge-reduced avidin and β -galactosidase (blue) are shown below. The original positive (red) and negative (black) ion mode spectra are shown as a guide to the eye.



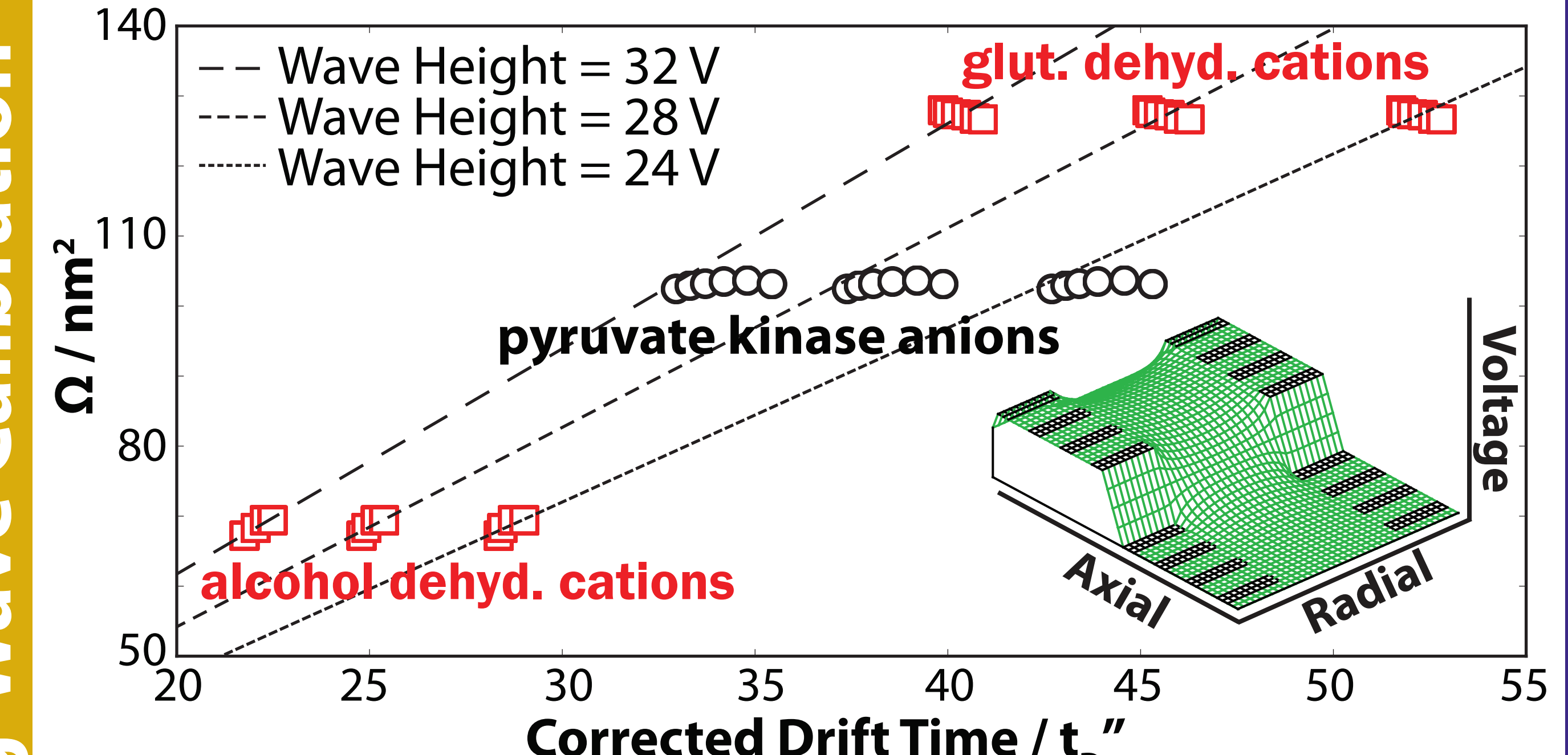
Cation vs. Anion Ω Values

(Top) Ω values of charge-reduced cations (blue) are compared to those of the original cations (red) and anions (black). Cation Ω values measured with and without triethylamine are indistinguishable. (Bottom) The differences between the Ω values of charge-reduce cations and anions of the same $|z|$ are small.

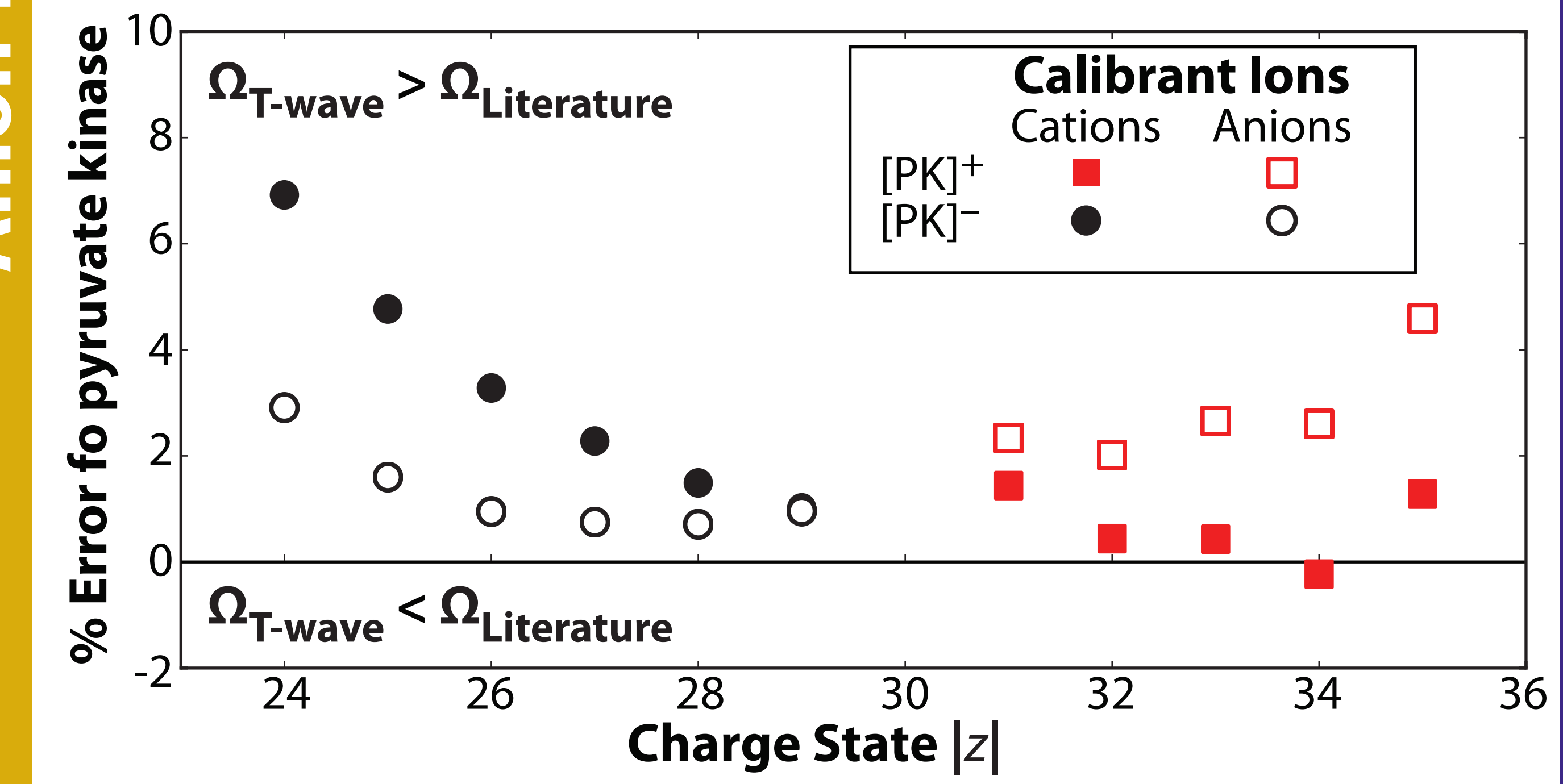


Anion Traveling-Wave Calibration

Traveling-wave IM was used to determine Ω values (Ruotolo et al. *Nat. Prot.* **2008**, *3*, 1139) of selected protein complexes. For example, pyruvate kinase anion drift times (black) were calibrated using literature cation Ω values (red). Calibration errors are evident from the observed deviations from the calibration lines.



Anion drift times calibrated using anion Ω values (O) are more accurate than those calibrated using cation Ω values (●). Similarly, cation drift times calibrated using cation Ω values (■) are more accurate than those calibrated with anion Ω values (□).



Conclusions

Here, we observed that anions of protein complexes formed by nanoelectrospray ionization have significantly smaller average charge states than the corresponding cations. This result is consistent with anions having lower field-emission energies for the loss of charge carriers. Interestingly, cations, anions, and charge-reduced cations all have similar Ω values, suggesting that they all have similar structures that depend weakly on charge state. We also demonstrated that these Ω values can be used to measure accurate calibrated Ω values with traveling-wave IM.

Thanks

