

Utilizing High Throughput IMS-MS Measurements to Study Noncovalent Protein/Ligand Interaction Kinetics

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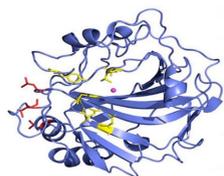


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Overview

- Automation of native protein electrospray
- Protein ligand K_d determination utilizing native electrospray and IMS-MS
- Drift time improves protein-ligand spectra



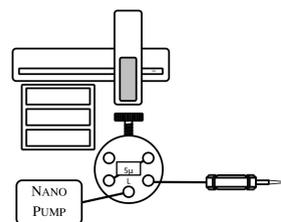
Methods

- Protein samples were brought up in a buffer of 200mM Ammonium Acetate to a concentration sufficient to maintain a 5μM concentration when combined with ligands
- Ligands were also dissolved in 200mM using a small amount of methanol (<1%) as needed
- Proteins and ligands were mixed together and allowed to reach equilibrium resulting in a constant protein concentration of 5μM with varied concentration of ligand
- Samples were introduced into the mass spectrometer utilizing an injection valve (VICI, Houston TX), a nano pump (Agilent, San Jose CA), a PAL auto sampler (Leap Technologies, Carrboro NC) and the instrument control software LCMSnet developed in house to provide unattended operation
- The auto sampler injected 5 microliters of protein solution into the sample loop of the injection valve, the valve was then actuated and the nanopump pushed the solution through the capillary tubing to the electrospray tip at a flow rate of 300nL/min allowing for continuous electrospray for up to 15 minutes

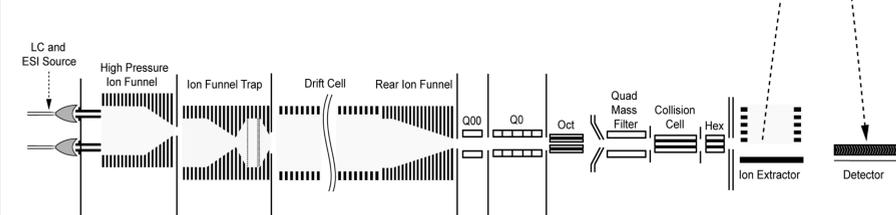


Introduction

- Historically analysis of native proteins by electrospray is a low throughput endeavor, requiring constant operator attention. Due to reduced signal in the absence of potentially denaturing acids spectra are summed for long periods.
- K_d values ranging from low nanomolar to high micromolar can be determined from electrospray. This requires the same concentration of protein to be run against a variety of concentrations of ligands
- This method allows for the unattended operation of the IMS-MS instrument in such a way that the range of K_d values can be determined quickly and with a minimum of sample
- The drift time separation is an excellent tool for isolating the charge states of interest and improving the calculations of K_d



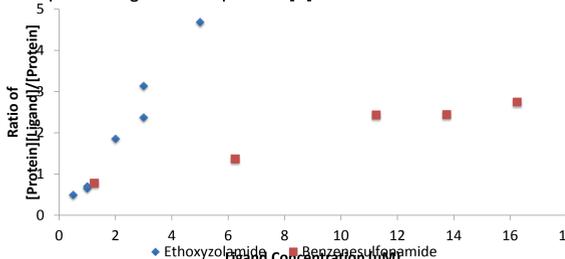
- Samples were acquired for ~12minutes and data summed across 5 minutes
- Tip quality is paramount for spray quality, tips were made using the method of Kelly et al to chemically etch tips
- Spectra were acquired on an in house built IMS-MS platform
- Peak areas were calculated from the raw data and then ratios determined
- Data were fit using the linear regression feature of OriginPro 2015



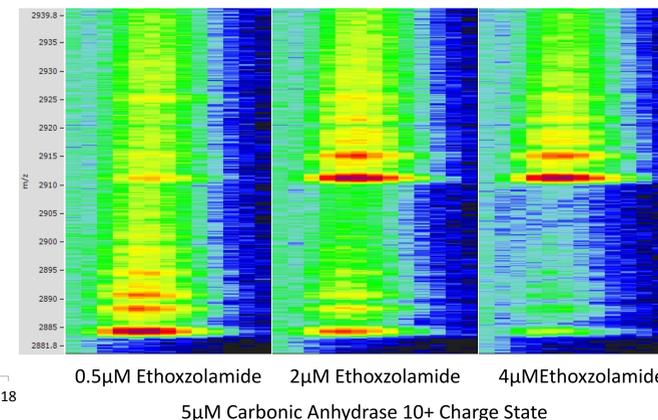
Results

Determination of K_d

The ratio of the signal of the bound protein complex over the unbound protein increases with increasing concentration. This ratio is plotted then used to calculate the K_d values for the various protein ligand complexes [2]

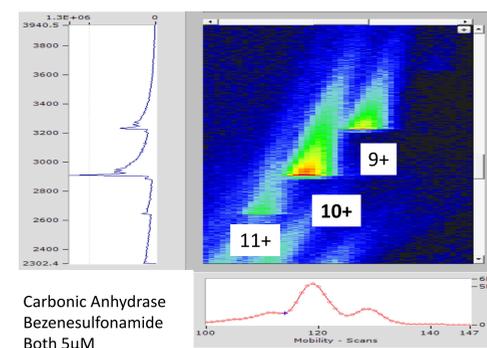


Ligand	K _d (μM)
Benzenesulfonamide	3.94±0.633
Ethoxzolamide	0.000167±0.0066

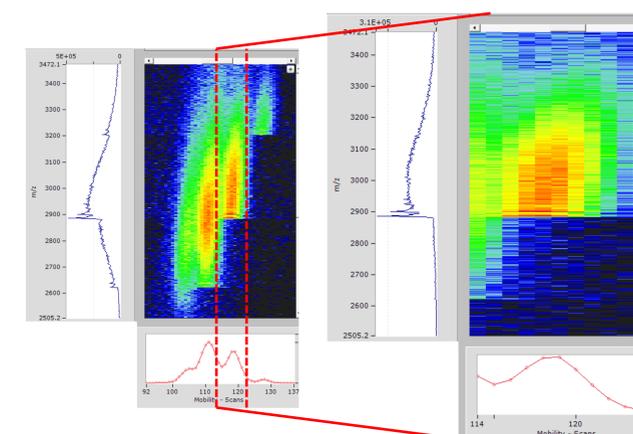


$$\frac{(P.L)}{(P)} = \frac{1}{2} \left(-1 - \frac{[P]_0 - [L]_0}{K_D} + \sqrt{4 \frac{[L]_0}{K_D} + \left(\frac{[L]_0 - [P]_0}{K_D} - 1 \right)^2} \right)$$

Drift Time Improves Signal

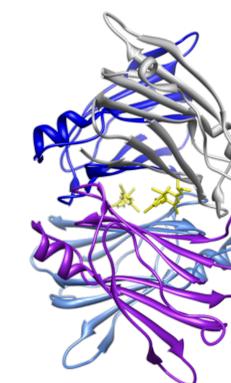


By zooming in on the drift time of interest the mass spectra is improved by the removal of interfering adducts from other charge states of the same protein



Conclusions

- Native protein electrospray can be successfully automated,
- IMS can reduce the noise in MS measurements of proteins by separating the adducts from different charge states
- K_d's ranging from nano molar to micro molar can be determined quickly with nanograms of protein



Acknowledgements

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References

- Kelly, et al., Anal Chem 78:7796-7801 (2006).
- Jecklin, et al., J. Mol. Recognit. 22:319-329 (2009).

