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

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Overview

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

Introduction

• Historically analysis of native proteins for 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.
• Kd 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 ligand
• This method allows for the unattended operation of the calculations of Kd values can be determined quickly with a constant operator attention. Due to reduced signal in the absence of potentially denaturing acids spectra are summed for long periods.

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 and injection valve (VICI, Houston TX), a nano pump (Agilent, San Jose CA), a PAL auto sampler (Leap Technologies, Canton 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 auto-sampler of the IMS-MS system for continuous electrospray for up to 15 minutes.
• Samples were acquired for ~12minutes and data summed across 5 scans.
• Data were fit using the linear regression feature of OriginPro 2015

Results

Determination of Kd

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 Kd values for the various protein ligand complexes [2].

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Kd (µM)</th>
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<tbody>
<tr>
<td>Ethoxolamide</td>
<td>0.000167±0.0066</td>
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<tr>
<td>Benzenesulfonamide</td>
<td>3.94±0.633</td>
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</tbody>
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References


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
• Kd’s ranging from nano molar to micro molar can be determined quickly with nanograms of protein

Acknowledgements

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