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Characterization and Use of Cross Sections from Features in LC-IMS-MS Experiments

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Laying the foundation for predicting ion mobility cross sections

Overview

- 10-h LC-IMS-MS analyses performed on *Shewanella oneidensis* MR-1 to characterize cross sections of tryptic peptides in a large dataset
- Charge states of deisotoped features plotted as a function of arrival time and monoisotopic mass to characterize their dependence on LC elution time
- Distribution of all post-drift-cell transit time values for observed peptides measured to estimate the achievable accuracy for cross sectional calculation using a single electric field
- Importance of predicting cross sections is explained and a method is proposed using sequence information from *S. oneidensis* accurate mass and time (AMT) tag database and cross sections acquired from 10-h LC-IMS-MS analyses

Introduction

Mass spectrometry (MS)-based technologies are becoming essential tools for molecular and cellular biology research. However, challenges posed by the extreme complexity of many biological samples have demonstrated the need to couple MS with additional separation techniques such as liquid chromatography (LC) and ion mobility spectrometry (IMS) to allow simultaneous characterization of elution time, mass, size, and charge state (Figure 1).

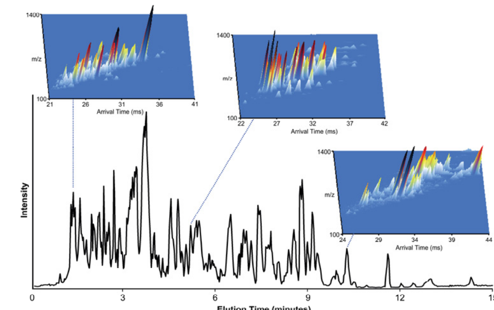


Figure 1: 15-min LC-MS base peak chromatogram with corresponding IMS-MS spectra at 3 different elution times

While MS parameters and LC elution times for complex mixtures have been studied in detail over the last decade, the IMS size and charge state separation for these types of samples has not been studied as extensively. Thus, work to understand how cross sections change as a function of molecular composition, charge state, mass and elution time is essential for using LC-IMS-MS to identify species in complex biological samples.

Methods

Instrumentation: ESI-IMS-TOF MS [1,2]

- High-pressure hourglass ion funnel to focus and trap ions prior to ion injection
- Ion gate
- 1-meter ion mobility drift cell
- Second high-pressure ion funnel to focus the diffuse ion beam post-IMS separation
- Orthogonal TOF MS to measure mass to charge ratios (*m/z*) of the ions after IMS separation

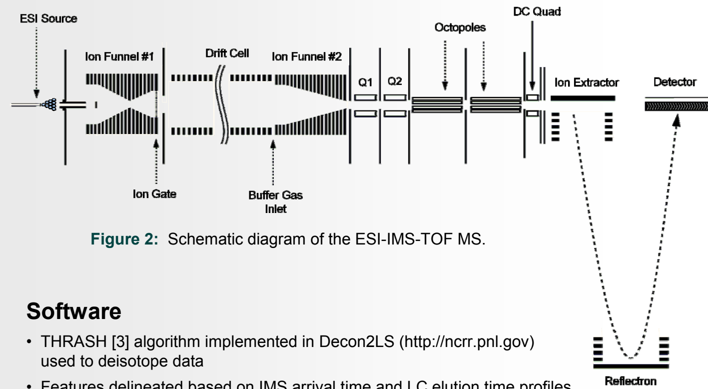


Figure 2: Schematic diagram of the ESI-IMS-TOF MS.

Software

- THRASH [3] algorithm implemented in Decon2LS (http://ncrr.pnl.gov) used to deisotope data
- Features delineated based on IMS arrival time and LC elution time profiles
- Programs under development for calculating [4], characterizing, and adding IMS cross sections to the existing accurate mass and time (AMT) tag database
- The cross section variable is important because aligning each observed peptide feature to 3 variables (monoisotopic mass, elution time, and cross section) increases the confidence of each identification while simultaneously reducing the number of false positives

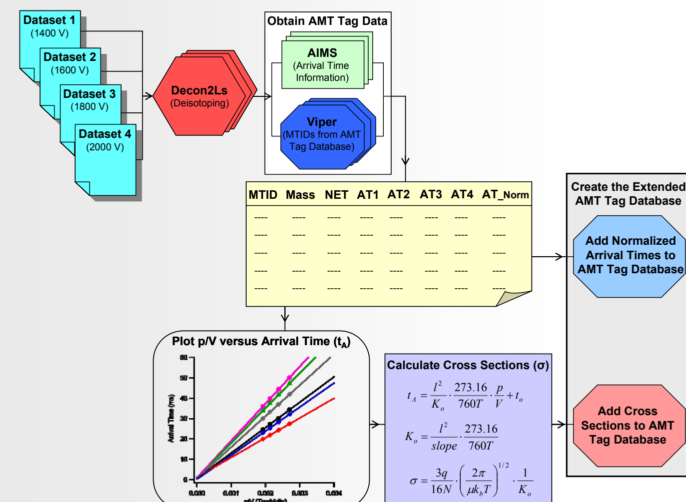


Figure 3: Flow chart of the cross section characterization process.

Results

- LC-IMS-MS analyses with a 10-h gradient were performed using *Shewanella oneidensis* MR-1 total protein tryptic digest to characterize the cross sections for a large dataset of tryptic peptides (Figure 4)
- Datasets deisotoped and observed features analyzed based on charge state, monoisotopic mass, arrival time, and elution time
- Linear relationship with unique slope observed for each specific charge state (1+, 2+, and 3+) when graphed as a function of arrival time and monoisotopic mass (bottom of Figure 4)
- Slopes for each charge state plotted against elution time, resulting in virtually flat lines for all charge states except 1+ (influenced by solvent related ions in mobile phases) (Figure 5).
- All deisotoped features aligned to the AMT tag database; only peptide ions that correlated with an MTID were extracted.
- Cross section for each peptide ion calculated by plotting peptide arrival time at 4 different voltages vs. pressure/voltage (p/V) (Figure 6)

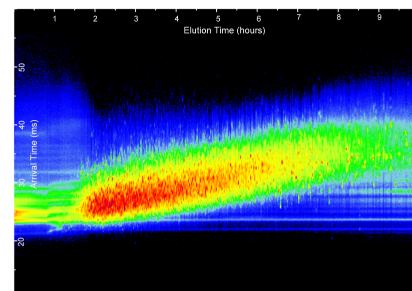
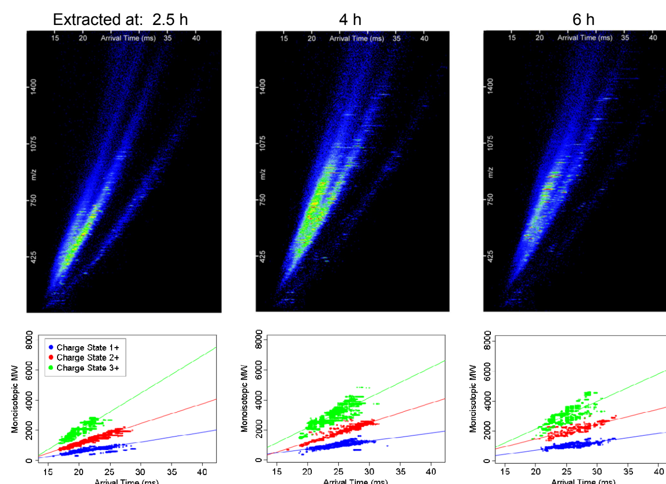


Figure 4: 10-h LC-IMS-MS separation of *S. oneidensis* MR-1 (left) plotted with 3 different nested spectra averaged for 20 s and extracted at 2.5, 4, and 6 h mark to show difference in eluting peptides. Nested spectra were deisotoped and 1+, 2+ and 3+ charge states plotted as a function of arrival time and monoisotopic mass (bottom).



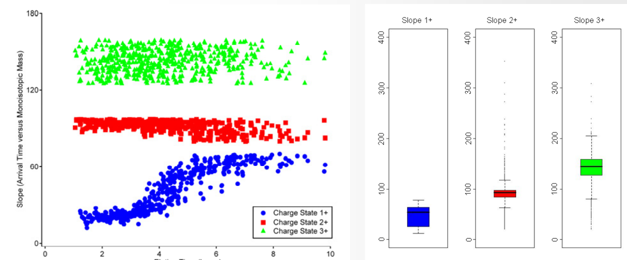


Figure 5: Slope values for 1+, 2+, and 3+ charge state trend lines (after outlier removal) for each deisotoped feature from the *S. oneidensis* analysis plotted as a function of elution time (left). While 2+ and 3+ charge states remained constant, 1+ charge state changed with time due to the contribution of 1+ solvent ions during the gradient. Only the interquartile range of slopes are shown on the left, so boxplots were made to represent all slopes (right).

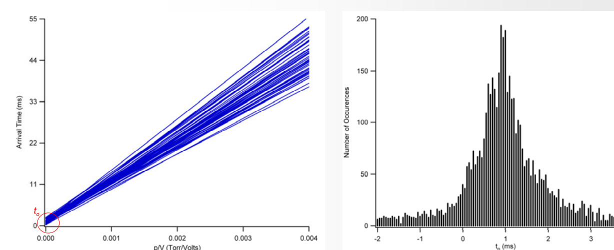


Figure 6: Arrival times of detected *S. oneidensis* MR-1 peptides plotted as a function of *p/V* to determined cross sections and post-drift-cell transit time values (t_o) (left). Lines for only 50 peptides are shown for clarity. The distribution of all t_o values for observed peptides averaged ~1 ms (right), indicating that cross sections can be estimated for datasets at a single electric field.

Future Work

- Due to the extensive process of creating a cross section (normalized arrival time) database with 10-h LC-IMS-MS experiments, the ability to predict cross sections is essential for enabling high throughput IMS analysis of many different types of samples with numerous peptides.
- The numerous tryptic peptides already characterized by LC-IMS-MS are being utilized to setup cross section prediction methods (Figure 7).

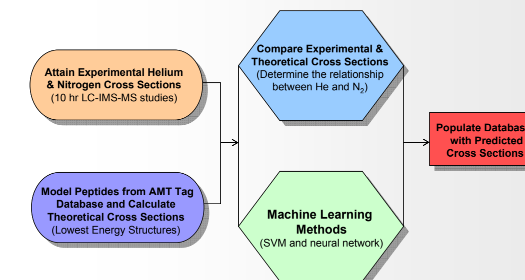


Figure 7: Flow chart of the cross section prediction method.

Conclusions

- Characterization of feature charge states (1+, 2+, and 3+) in the *Shewanella oneidensis* MR-1 sample by graphing their relationship as a function of arrival time and monoisotopic mass resulted in a linear relationship; each charge state had a unique slope.
- Slopes for the 2+ and 3+ charge state trend lines remained constant with elution time, but the 1+ charge state changed due to the contribution of 1+ solvent ions, which varied during the gradient.
- The post-drift-cell transit time values average ~1 ms for all observed peptides, indicating that cross sections can be estimated for single datasets with only one electric field voltage.

Acknowledgements

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