

# LC-IMS-MS Feature Finder: Detecting Multidimensional Features in LC-IMS-TOF MS Data

Kevin L. Crowell, Anuj R. Shah, Gordon W. Slysz, Brian L. LaMarche, Da Meng, Erin S. Baker, Matthew E. Monroe, Vlad A. Petyuk, John D. Sandoval, Gordon A. Anderson, and Richard D. Smith  
Pacific Northwest National Laboratory, Richland, WA



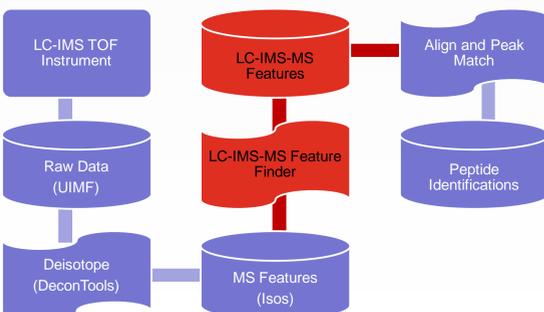
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## Overview

- Software tool for discovery and characterization of possible peptide signatures in LC-IMS-TOF MS
  - Characterize a feature by mass, elution time, drift time, and charge state
  - Detect multiple conformations of a peptide
  - Detect LC co-eluting peptides by leveraging data in IMS dimension
  - Provide confidence scoring for detected conformations
- Software tool developed in C# .NET 4.0 uses Microsoft .NET's Parallel Extensions Library for task parallelizing and multithreading
- Integrated into PNNL's accurate mass and time (AMT) tag pipeline<sup>1</sup>
- Output of software will be used for peptide identifications or AMT tag database creation

## Introduction

- Addition of ion mobility to existing data analysis pipeline presents a number of challenges
- Extended pipeline is required to accurately identify peptides in LC-IMS-TOF MS data
- Extended pipeline should be able to detect co-eluting peptides and multiple conformations from the addition of the ion mobility dimension



## Methods

### Software development

- Microsoft .NET's Parallel Extensions Library
  - Data is partitioned by mass and charge at multiple points by the algorithm
  - Partitioned data are processed simultaneously across multiple processors to speed up runtime
- Accepts DeconTools<sup>2</sup> output (Isos file) as input
- Configurable settings file used as an input to allow any parameter of the algorithm to be altered
- Outputs results in multiple forms that are compatible with downstream AMT tag pipeline
  - SQLite (new generation pipeline)
  - Tab-delimited Text (previous generation pipeline)

### Algorithm development

- Data smoothing implemented to account for features with low signal-to-noise ratios and low abundant features
- Report multiple conformations or co-eluting peptides as separate features
  - Algorithm will not discern between multiple conformations and co-eluting peptides
- Detected conformations should resemble a Gaussian distribution
  - Limited data points in raw data give the need to interpolate points of the detected conformation to build the most accurate profile

### LC-IMS-TOF MS platform<sup>3</sup>

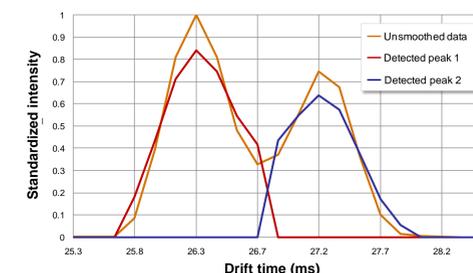
- High pressure converging hourglass ion funnel focuses and traps ions prior to ion injection
- 1-meter IMS drift cell
- Orthogonal Agilent TOF MS provides high mass measurement accuracy after IMS separation
- Data acquired through Multiplexed Ion Mobility Time-of-Flight Mass Spectrometry<sup>4</sup>

For more information, see:  
<http://omics.pnnl.gov/>

## Conformation Detection



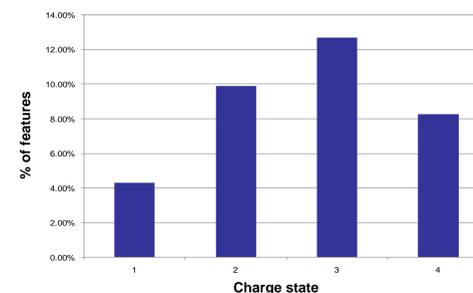
### Detected peaks from smoothed data



- Raw data is first smoothed using a Gaussian Kernel smoother
- Peaks are detected from smoothed data using a simple 3-point peak picking algorithm

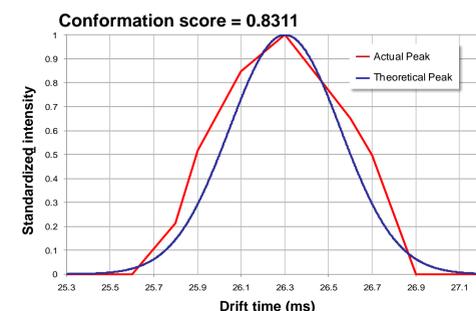
## Results

### Distribution of multiple conformations



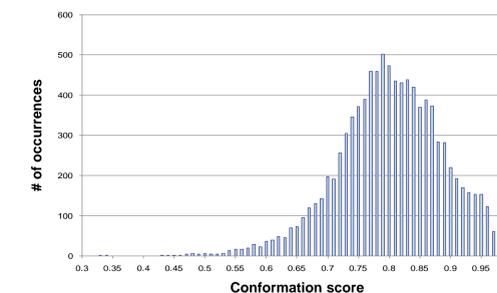
- Multiple conformations are seen the most often in 3+ features
- Multiple conformations are rarely seen in 1+ features
- On average, about 10% of detected features contain multiple conformations

### Actual peak data compared to theoretical peak



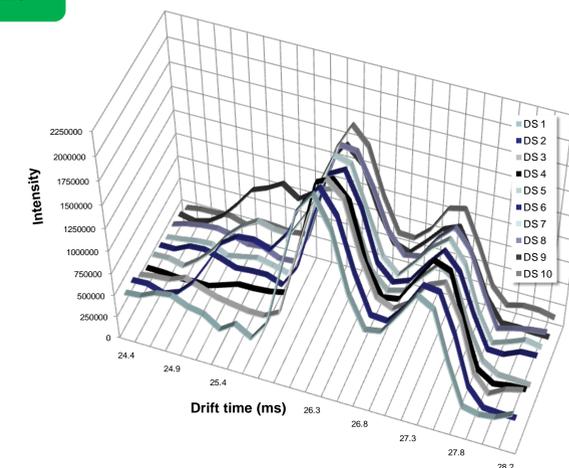
- Theoretical peak is given a standard deviation equal to the expected resolution of the IMS-TOF instrument
- Conformation score =  $1 - \frac{\sum_{i=1}^n |actual_i - theoretical_i|}{n}$  where  $i$  = one of  $n$  (1000) interpolated points

### Conformation score



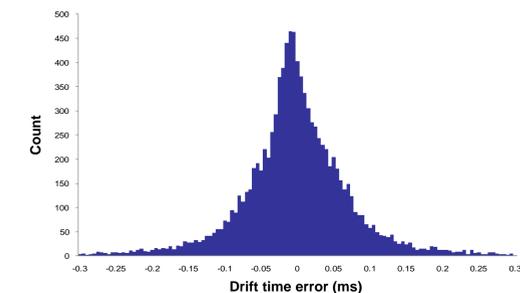
Distribution of conformation scores exhibit a normal distribution skewed towards the end of higher scores

### Reproducibility



- IMS Profile is reproducible across technical replicates
- Multiple conformations are also reproducible

### Drift time accuracy



- Drift time error calculated by matching features together across multiple datasets of technical replicates using MultiAlign
- Errors are only considered for features seen in all (10) datasets
- Since the features are highly reproducible, they should give the best possible error in drift time
- Drift time accuracy of these highly confident features seen to be about 0.1 ms

## Conclusions

- Multiple conformations and co-eluting peptides are often observed in the IMS dimension
- Software is integrated into existing AMT tag pipeline
  - Run time of software is less than 10 min, which allows it to keep up with the high-throughput instrumentation
  - Robustness of software allows for software users to account for future IMS-TOF instrument updates
- Scoring function of a single conformation can be used by downstream analysis tools as a confidence measure
- Ion mobility drift time profile is seen as repeatable across multiple analyses of the same sample type
- Peptides can be identified by using mass, elution time, charge state, and drift time reported by software

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## References

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**CONTACT:** Kevin Crowell  
Biological Sciences Division, K8-98  
Pacific Northwest National Laboratory  
P.O. Box 999, Richland, WA 99352  
E-mail: [Kevin.Crowell@pnnl.gov](mailto:Kevin.Crowell@pnnl.gov)