**Introduction**

**Overview**

- Software tool for discovery and characterization of possible peptide signatures in LC-IMS-TOF MS.
- Outputs of the software will be used for peptide identifications or AMT tag database creation.
- Extended pipeline should be able to detect co-eluting peptides in LC-IMS-TOF MS data.
- Addition of ion mobility to existing data analysis instruments.

**Methods**

**Software development**

- Microsoft .NET’s Parallel Extensions Library
-VILLE’s Parallel Extensions Library
- High pressure converging hourglass ion funnel
- 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**

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

**For more info, see:** http://omics.pnl.gov

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


Pacific Northwest National Laboratory, Richland, WA

**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

**Peaks**

- Conformation score = the expected resolution of the IMS-TOF instrument
- Drift time error calculated by matching features together across multiple datasets of technical replicates using MultiAlign

**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

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

**Accuracy**

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

**Conclusion**

- 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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