The Benefit of Peptide CCS Value Prediction and Experimental Determination

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Overview

• Peptide spectra with low MS/MS information content are in need of methods to enable precise and confident identification.
• These comprise singly charged, non-tryptic or low abundant peptides, such as immunopeptides (MHC) or host cell protein (HCP) derived peptides.
• Modern ion mobility mass spectrometers can reproducibly determine collision cross sections (CCS) of peptides with an accuracy of the 0.2 % range, which are specific for a given peptide sequence.
• We developed a CCS value prediction algorithm based on the plain sequence string of the peptides in question, which was evaluated against a very feature-rich proteomic HeLa dataset comprising >80,000 identified peptides.

Methods

Experimental data were generated using trypsin digests of human cancer cell lines (HeLa), which were separated by nano-flow LC fitted with a 25 cm packed emitter C18 column (Proxeon, Australia), applying a linear gradient of 5-35% buffer B (100% ACN and 0.1% FA) at a flow rate of 400 nL/min. MS, MS/MS and CCS data were obtained using the PASEF method on a timsTOF Pro IMS/timsTOF mass spectrometer (Bruker, Fig. 1).

Data analysis was performed using MaxQuant (v1.6.4.0) to obtain a data set consisting of >80,000 unmodified peptides over five charge states. CCS values for twenty near isobaric doubly-charged ions which typically had CCS values in the range 300-550 Å2. At any given m/z value there is a wide distribution of CCS values, exemplified in Fig. 2 which shows the recorded CCS values for peptides within a 0.7 m/z window. The broad distribution of CCS values for similar sized peptides provides the rationale for using prediction and measurement to distinguish isobaric peptides even in the case of failed MS/MS identifications.

Results

The Parallel Acquisition Serial Fragmentation (PASEF) method implemented on the trapped ion mobility spectrometry time of flight mass spectrometer (timsTOF Pro) described previously (Meier et al., JPR, 2015) separates ions based on mobility and CCS to enable the generation of non-chimeric MS/MS spectra for co-eluting peptides. The recorded CCS values are reproducible (average absolute deviation of 0.2%) for the CNN and LSTM models respectively (Fig. 3).

Conclusions

• timsTOF Pro with PASEF provides reproducible CCS values (< 0.2% RMS) and non-chimeric MS/MS identification.
• Near isobaric peptides can in most cases be distinguished by CCS values alone.
• Identification is feasible by high accuracy of mass (< 5 ppm) and CCS value determination alone without MS/MS.
• A sequence test based prediction algorithm for CCS values provided prediction errors < 2%, indicating its future use for peptide identification based on accurate mass and mobility data alone.
• This approach could be applied to the identification of low abundant, non-tryptic or single charged peptides.