

# Enhanced Detection of Host Cell Proteins enabled by use of Collisional Cross Sections

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## Introduction

Host cell proteins (HCPs) are critical quality attributes for biological therapeutics which can pose a risk to patients or decrease the yield, efficacy or stability of the final drug product. Even very small amounts of HCPs in the low or sub ppm range can be sufficient to cause negative effects, which makes their identification by mass spectrometry challenging. Here we show that collisional cross section (CCS) values can be used as an additional parameter to confirm the presence of low abundant peptides of interest which have low quality MS/MS spectra or for which the precursor intensity was too low to be selected for MS/MS.

## Methods

Purified monoclonal recombinant antibody expressed in Chinese hamster ovary (CHO) cell lines and non-transfected CHO reference samples (InVivo BioTech Services) were reduced using DTT in TFE solution and alkylated with iodoacetamide prior to overnight digestion with trypsin (Promega). Peptides were separated over a 40 min gradient using an ACQUITY UPLC CSH C<sub>18</sub> 1.7µm 2.1 x 150 mm column (Waters) using an Elute UHPLC coupled to a timsTOF Pro ion mobility QTOF mass spectrometer (Bruker). Extracted ion chromatograms (EICs) were generated in DataAnalysis software (Bruker) and the CCS was calculated from observed 1/K0 values. Spectra were searched against the CHO K1 database using Mascot.

## Results

Varying amounts (2, 10 and 30 µg) of monoclonal antibody digest were loaded on column, eluted and analyzed. The timsTOF uses trapped ion mobility, resulting in non-chimeric MS/MS spectra even for low abundant precursors. CCS values were recorded for all ions. A high level of sensitivity is achieved by time and space focussing of the ions in the TIMS cell (Fig 1).

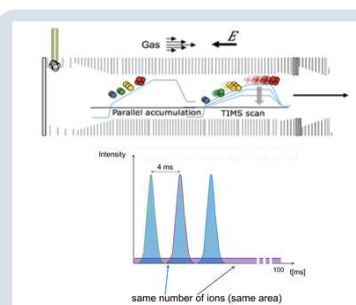


Fig. 1 Space (top) and time (bottom) focusing of ions in the TIMS cell

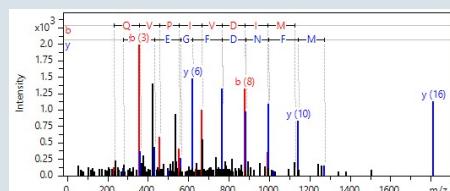


Fig. 2 MS<sup>2</sup> identification of Collagen  $\alpha$ -1(V) peptide K.VEQVPIVDIMFDFGEASQK.F. in mAb sample, 30 µg on column. A 2<sup>nd</sup> peptide was also identified (not shown)

Several CHO HCPs were identified which have been reported to associate with expressed antibodies, including Collagen  $\alpha$ -3(VI) and  $\alpha$ -enolase (1). Here, 2 peptides for Collagen  $\alpha$ -1(V) were identified when 30 and 10 µg was loaded on column but only one when the sample amount was reduced to 2 µg (Fig 2). EICs showed that a trace level of the unidentified precursor was detected in the 2 µg

sample and the peptide ID could be confirmed by CCS in the absence of identification by MS/MS (Fig 3). To further illustrate the use of CCS to extend the limit of detection for HCPs, a CHO reference sample consisting of non-transfected CHO cells in medium was analysed. The sample was digested with trypsin using 3 different protocols (with and without TFE, native digest (2)) and using a 40 minute

gradient, approx. 11,000 peptides were identified for 3,000 CHO HCPs in total. CCS values were recorded for each peptide and this allowed retrospective analysis of the mAb 30 µg dataset, which revealed that a third peptide for Collagen  $\alpha$ -1(V) could also be identified by m/z and CCS (Fig. 4). Identification had previously not been possible because MS/MS was not acquired for this low-abundant precursor and was further hampered due to these experiments being acquired at a different time and there being a retention time shift.

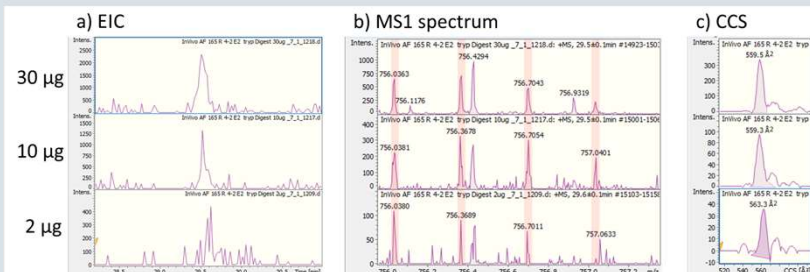


Fig. 3 EIC, MS<sup>1</sup> (isotopes for 3+ precursor highlighted in red) and CCS detection of Collagen  $\alpha$ -1(V) peptide K.VEQVPIVDIMFDFGEASQK.F. in mAb sample, 30 µg, 10 µg and 2 µg on column. MS<sup>2</sup> data was not available for this peptide in the 2-µg sample

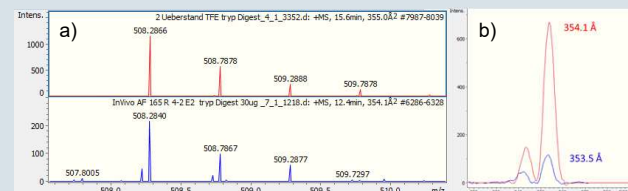


Fig. 4 MS<sup>1</sup> (a) and CCS (b) detection of Collagen  $\alpha$ -1(V) peptide K.TVLEIDTPK.V in CHO reference sample (red) and mAb sample 30 µg on column (blue). MS<sup>2</sup> data was not available for this peptide in the mAb sample but ID was possible after screening for m/z and CCS values of the peptide observed in the CHO reference sample

## References

- (1) Lazar et al. J Pharmaceut. Biomed. 2019, 174:500-508
- (2) Huang et al. Anal. Chem. 2017, 89:5436-5444

## Conclusions

- CCS values can be used together with m/z to increase confidence in the identification of low-abundant HCPs
- The sensitivity of HCP detection can be extended by a factor of ~5-10 by using CCS values to identify ultra-low abundant precursors which did not trigger MS/MS
- Analysis of a CHO reference sample generated 11,000 peptide CCS values for CHO-HCP peptides that can be used as a reference to extend the depth of HCP coverage in future experiments

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