# Trapped Ion Mobility Spectrometry and PASEF for Enhanced Observation of Chemically Crosslinked Peptides

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Chris M. Adams[1], Stijn van Dorp[2], Richard Lewis[2], Kratika Singhal[3], Allis S Chien[3], Ryan D Leib[3]
[1]Bruker Daltonics, San Jose, CA
[2]Molecular and Cellular Physiology, Stanford University, CA
[3]Stanford University Mass Spectrometry, Stanford University, CA

## Introduction

Chemical crosslinking and mass spectrometry complemented with electron microscopy and molecular modeling have advanced the field of structural biology immensely in recent years. The identification of crosslinked peptides post digestion has improved because of advances in technology, namely alternative fragmentation types, mass accuracy and increased sensitivity of mass spectrometers. This combined with molecular modeling can suggest a structure of highest probability and lowest energetics. Trapped ion mobility spectrometry (TIMS) and parallel accumulation serial fragmentation (PASEF)[1] are new principles to mass spectrometry and in particular as coupled to a Q-TOF. In summary, ions pushed by a gas, entering the TIMS device are met with an electric field. This focuses and traps packets of ions in time and space by their collisional cross section (CCS). Here we show that chemical crosslnked peptides have a unique CCS that differentiates them from proteotypic peptides. This physcial phenomenom, different than charge state screening, can be exploited to increase the number of observered crosslinked forms.

## Methods

Protein complexes were crosslinked with zero-length or BS3 crosslinks, run on a PAGE gel excised and digested. The mass spectrometer was a timsTOF Pro run in “PASEF” mode where the TIMS domain was tuned to capture peptides of higher collisional cross sections (CCS). Data was searched with Byonic and filtered to a 1% FDR.

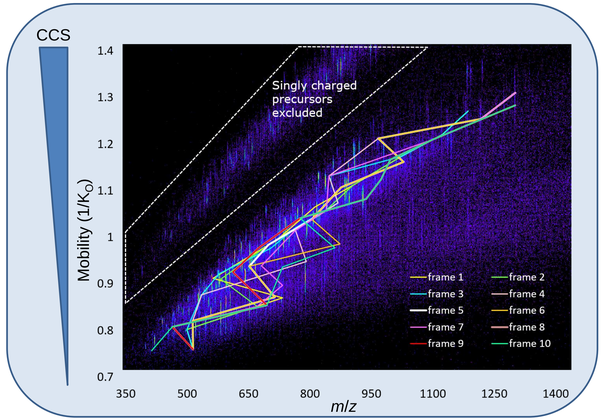


**Fig. 1 tims-PASEF and ion separation by CCS.** Within the TIMS device ions are separated by size, charge and weight, where they are focused in time and space. Chemically crosslinked polypeptide ions most commonly exist in charge state greater or equal to 3+, this in combination with the differential mobility term allowed for exploitation of the TIMS device to specifically tune for chemically cross linked forms. As Fig. 1 describes packets of ions are separated by their mobility term, stored and transmitted for MS and MSMS. This unique trapping allows for near 100% duty cycle, which is particularly relevant for chemically crosslinked peptides as they common exist as sub-stoichiometric species.

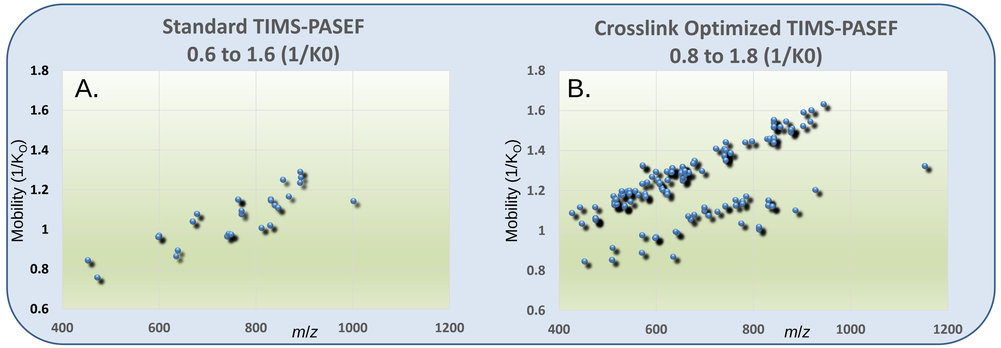


**Fig. 2 Optimizing the TIMS device for identification of chemically crosslinked peptides.** The same chemically crosslinked digested sample was run in technical duplicates using different mobility term selections (targeted mobility spectrometry- TMS). Panel A represents the default tims-PASEF approach optimized for identification of polypetides from a tryptic digest in a complex mixture, as such identification of 42 (25 unique) high confidence crosslinked forms. The TIMS device was tuned to account for the differential CCS of chemically crosslinked forms and presented in panel B. The selectivity of the tims trap was apparent as the number of crosslinked forms identified jumped to 312 (132 unique), a 7.4 times increase in observed crosslinked peptides.

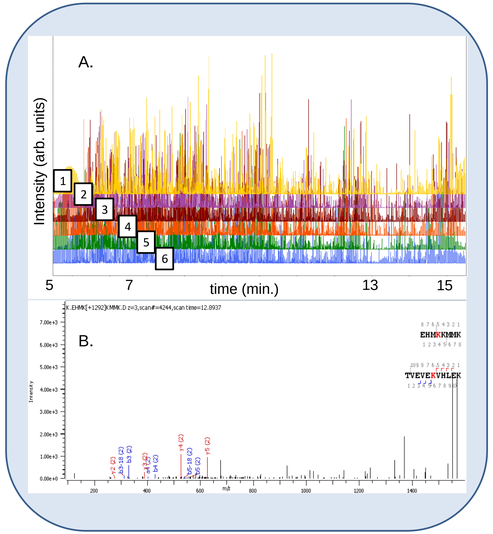


**Fig. 3 Speed and Sensitivity A)** Base chromatogram of six chemically crosslinked in gel digests run at 10 min. gradients with no sacrifice to number of crosslink identifications as of the result of the fast sequencing speed using PASEF (>100 Hz). This allows for 5-6 times the throughput over current workflow. **B)** MSMS fragment ion spectra of chemically crosslinked peptides.

## Results

The timsTOF Pro mass spectrometer was well suited for the detection of chemically crosslinked peptides as it could preferentially distinguish modified from non-modified forms (Fig. 1 and 2) as a function of their CCS, or gas phase confirmation. Using multiple crosslinker types (zero-length and BS3) in technical replicates and various trapping modalities (Fig. 2) we were able to increase the number of crosslinked observed peptides >7 times (42 v. 312) over baseline proteotypic tims methodologies. This suggests an application toward glycopeptides and other species where a unique gas phase confirmation is a result of modification. Speed and sensitivity are also realized advantages to timsTOF with PASEF as our data suggests that the LOD is substantially higher than competing technologies in the lab, we attest this to the concentration effect of tims and the near 100% duty cycle and sequencing speed with PASEF. Generally speaking crosslinked proteolytic samples of protein complexes are not rich in the number of peptides and as such fast gradients (10 min., Fig. 3) are applicable given the MSMS sensitivity and sequencing speed.

### References

(1) Meier et al.; Journal of Proteomics Research 2015

## Conclusions

- Chemically crosslinked peptides have a unique mobility term
- The tims cell can be preferentially set to selectively capture chemically crosslinked peptides
- tims and PASEF increase both the sensitivity and speed of chemical crosslinked runs (5-6x’s)
- Targeted mobility spectrometry (TMS) is applicable to other modified peptide species (glycopeptides)

timsTOF Pro