Rapid Isobaric Peptide Classification by Trapped Ion Mobility Spectrometry

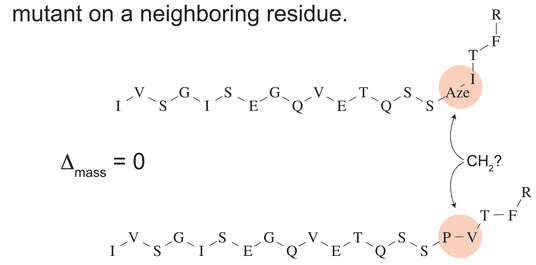
Ryan D. Leib[1], Christopher M. Adams[2], Kratika Singhal, Allis S. Chien[1]

Stanford University[1], Bruker Corporation[2]

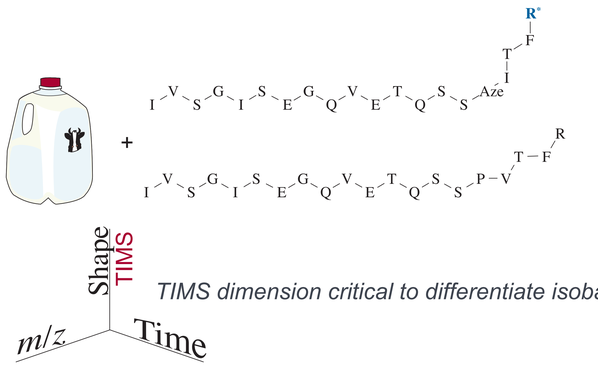
Overview

Assigning isobaric peptides in a complex biological background such as milk or serum presents a significant analytical challenge. In many cases, chromatographic and MS/MS-based assignments are not sufficient to distinguish multiple analogous species.

Here, we use trapped ion mobility to separate a typical example in bovine casein; the non-natural amino acid azetidine has been predicted to replace proline in degenerative disease. Alternatively, there is a known isoleucine-to-valine mutant on a neighboring residue.

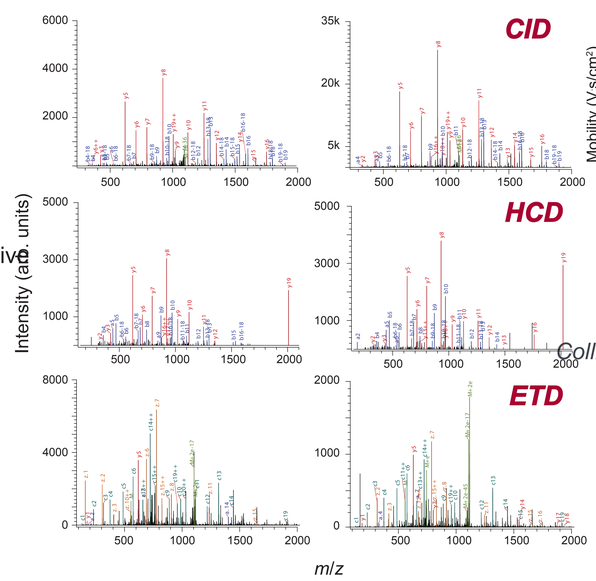


Methods



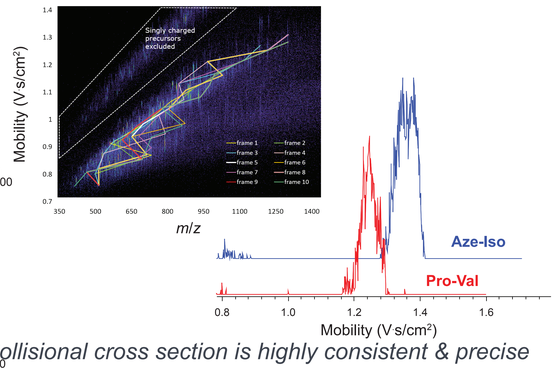
TIMS dimension critical to differentiate isobars!

m/z



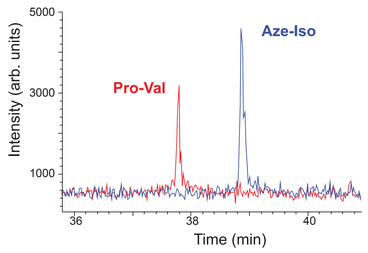
Various fragmentation methods unable to make certain ID, cannot be relied on alone

Shape



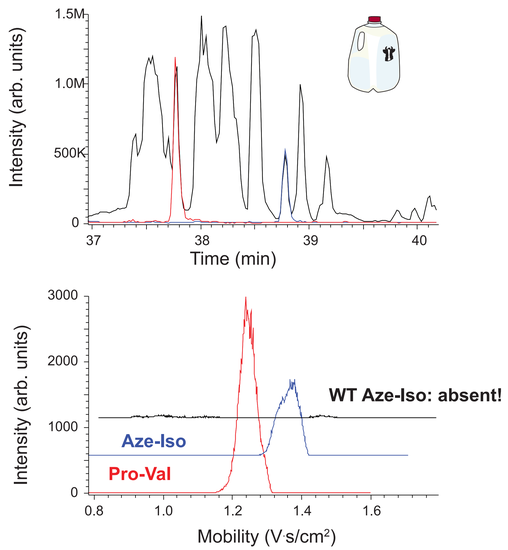
Collisional cross section is highly consistent & precise

Time



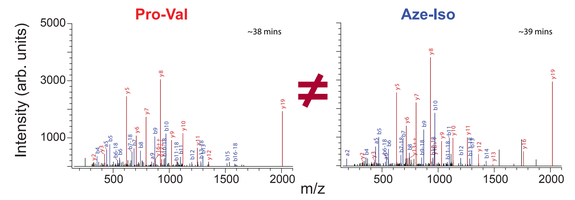
LC elution times may vary based on column & gradient

Milk



Discussion

Shotgun proteomic analyses rely on liquid chromatography to improve peptide observation and depth, but often these details are not considered during peptide ID. In cases involving unknown isobaric peptides, identical MS/MS spectra at different elution times should not be explained away as poor chromatographic separation. Trapped ion mobility offers a method to provide additional confirmation of potential unexpected isobars.



Conclusions

- **timsTOF orthogonal separation greatly increases confidence of assignment**
- **Alternative fragmentation methods do not provide additional details for this modfication**
- **Search engine calls based on MS/MS alone unable to distinguish**

Acknowledgments

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