BOOSTING SENSITIVITY IN TOP-DOWN PROTEIN STUDIES USING CYCLIC™ IMS-ENABLED WIDEBAND ENHANCEMENT

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INTRODUCTION

- Top-down protein analysis relies on tandem MS (MSMS) to characterize intact proteins and their post-translational modifications (PTMs)[1]
- Sensitivity in MSMS mode can be limited due to the inherent spreading of ion signals from a precursor to many product ions[2]
- For proteins, ion intensity is also spread over multiple charge states as well as broad isotope distributions
- This work demonstrates a novel implementation of Wideband Enhancement (WBE) on the Cyclic IMS P20 Mass Spectrometer, increasing the MSMS sensitivity of top-down protein analysis for collision- and electron-based fragmentation over the full mass range

WIDEBAND ENHANCEMENT

In normal Time of Flight (TOF) operation, not all of the ions from a continuous ion beam reach the detector from the TOF pusher region.

WBE enables synchronization of ion exiting from the IMS device with the pusher to maximise the duty cycle for a defined *m/z* range, greatly increasing the number of ions reaching the detector.

a) Ions enter IMS region and are separated by size, charge and shape.

b) Ions exit the IMS as distinct packets which arrive in the pusher region in ascending *m/z*.

c) The ion packets are synchronized with the TOF push, resulting in a drastically-increased duty cycle.

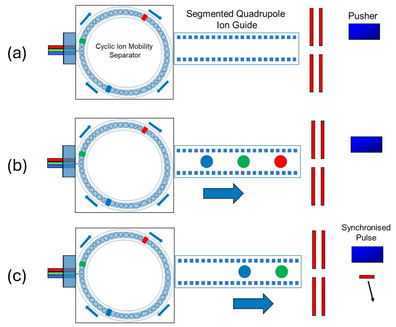

***Figure 1**. Schematic of WBE operation.*

METHODS

Mass Spectrometry

Cyclic IMS P20 Mass Spectrometer equipped with Waters electron capture dissociation cell. Operating in positive ESI mode, samples injected via direct infusion.

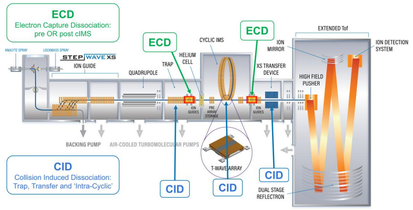

***Figure 2.** Cyclic IMS P20 schematic, indicating the possible fragmentation options. These complementary dissociation techniques combine with scalable ion mobility to deliver unique insights into protein structure.*

Samples

2 proteins were studied by top-down MS under denaturing conditions:

- Bovine ubiquitin (Sigma-Aldrich) – smaller protein ~8.5 kDa
- Bovine carbonic anhydrase II (Sigma-Aldrich) – larger protein ~29 kDa

Single-pass and by-pass ion mobility experiments were conducted, with and without WBE. In single-pass mode, ions are separated within the 0.98 m cyclic travelling wave device, whereas in bypass mode, ions traverse the multi-functional array with minimal separation. CID and ECD fragmentation were utilized in both modes.

Data

- **Acquisition:** MassLynx™ v4.2 Software.
- **Processing and Visualization:** DriftScope™ and waters_connect™ UNIFI™ peptide mapping workflow.

REFERENCES

1.Cui W, Rohrs HW, Gross ML. Top-down mass spectrometry: Recent developments, applications and perspectives. *Analyst.* 2011;136(19):3854-3864.
2.Hoang C, Uritboonthai W, Hoang L, et al. Tandem Mass Spectrometry across Platforms. *Anal Chem.* 2024;96(14):5478-5488.

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Conflict of Interest Statement – Authors are employees of Waters Corporation who manufacture and sell the products highlighted in this work.

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RESULTS – UBIQUITIN

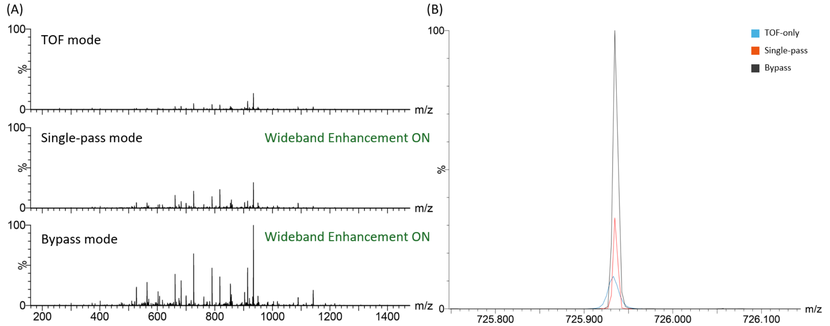

***Figure 3.** MS spectra demonstrating the improvement in sensitivity of ubiquitin CID fragments using Wideband Enhancement. (A) Comparison of CID fragment intensity across different acquisition modes (B) Overlay of the 726 m/z fragment ion peak. This highlights how WBE increases sensitivity beyond that of TOF mode, enabling high sensitivity with IMS separation. WBE with bypass IMS mode achieves the greatest boost in sensitivity.*

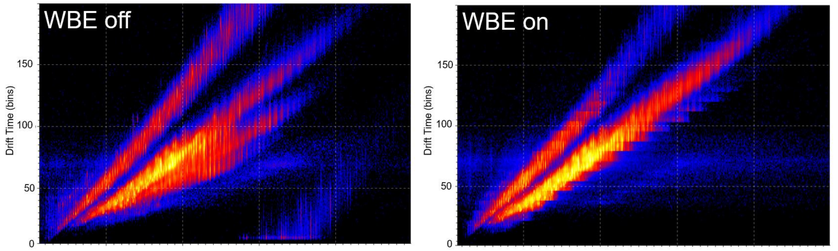

***Figure 4.** DriftScope plot indicating the segmentation of the arrival time vs m/z space during WBE for CID-derived product ions of the 10+ charge state of bovine ubiquitin.*

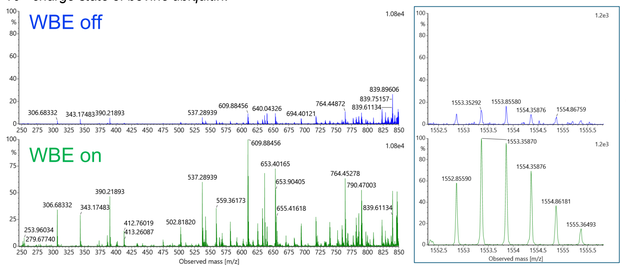

***Figure 5**. Raw MS spectra comparison of low m/z ubiquitin ECD fragments **(LEFT)**, with and without WBE, from a single-pass ion mobility separation in an MSMS experiment. Sensitivity improvements of up to 10-fold are seen across c- and z- ions series. A zoomed spectra **(RIGHT)** of a higher m/z ion 1553.39 highlights the improvement in signal intensity and peak profile with WBE. Data visualised in UNIFI.*

RESULTS – CARBONIC ANHYDRASE

CID fragmentation

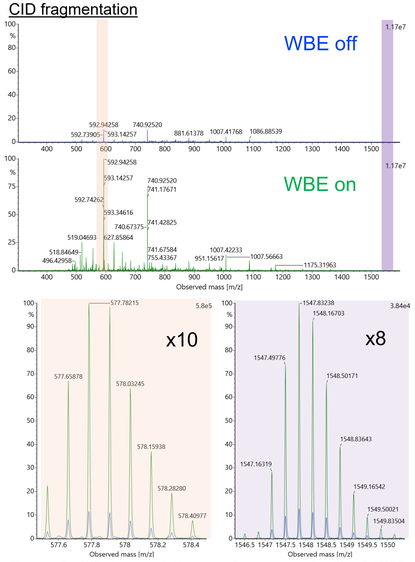

***Figure 6.** CID fragmentation spectra, with and without WBE (TOP). Coloured insets (BOTTOM) display examples of the sensitivity improvements enabled by WBE, highlighting a low m/z fragment ion (x10 signal intensity, orange) and a high m/z fragment ion (x8 signal intensity, purple).*

Whilst CID yields key information about the primary structure of proteins by generating highly reproducible terminal fragments, alternative fragmentation techniques are complementary and provide additional structural information.

ECD fragmentation

ECD fragmentation is a key tool in structural biology, indiscriminately cleaving backbone bonds to increase coverage for top-down sequencing. However, it is typically less efficient than CID, meaning the benefits of using WBE for increased sensitivity are more pronounced.

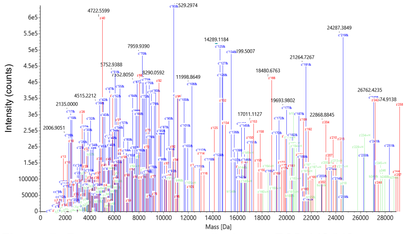

***Figure 7.** Fragmentation viewer displaying ECD MS/MS spectra of carbonic anhydrase II (m/z 807), illustrating dense c- and z-type fragment ions across the mass range.*

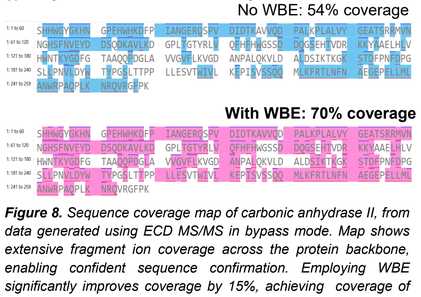

***Figure 8.** Sequence coverage map of carbonic anhydrase II, from data generated using ECD MS/MS in bypass mode. Map shows extensive fragment ion coverage across the protein backbone, enabling confident sequence confirmation. Employing WBE significantly improves coverage by 15%, achieving coverage of 70% in bypass mode.*

CONCLUSIONS

- **Up to 10× higher sensitivity for top-down MS**
  Wideband Enhancement (WBE) enables high-sensitivity MS/MS without compromising resolution
- **Maximize ECD performance**
  By enhancing inherently low-efficiency processes, WBE significantly improves sequence coverage - demonstrated for large proteins such as carbonic anhydrase II
- **Confident, comprehensive structural insight**
  Combining complementary fragmentation (CID, ECD) with WBE on the Cyclic IMS P20 enables more complete top-down protein characterization

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