

Increasing Throughput in Collision-Induced Unfolding Studies: Enhancing Sensitivity and Streamlining Set-up Waters™

Noelle Elliott¹, Bryan McCullough², Emma Marsden-Edwards², Dale A. Cooper-Shepherd²

1. Waters Corporation, Milford, MA, USA. 2. Waters Corporation, Wilmslow, Cheshire, UK

INTRODUCTION

- Collision-induced unfolding (CIU) is used to study the structure and stability of biomolecules and their complexes
- CIU enables tracking of discrete conformational states along unfolding pathways allowing rapid evaluation of domain stability, ligand binding and proteoform- or mutation-dependent effects
- There is ever-increasing interest in CIU from the biopharmaceutical industry, who wish to assess higher throughput methods for assessing developability of therapeutic products
- Increasing adoption of CIU workflow creates a need for simpler and faster experiment set-up
- Here we present two innovations on the Cyclic™ IMS P20 Mass Spectrometer to increase the speed to results
- A CIU acquisition mode is introduced in the method editor for 15 times faster method creation
- Wideband Enhancement is employed to significantly improve sensitivity to enable reduced run times

METHODS

Mass Spectrometry: All experiments were performed on a Cyclic IMS P20 Mass Spectrometer equipped with a Waters Nanolockspray ionization source and nanoflow capillary adaptor.

Samples: Waters Humanized mAb mass check standard (186009125). Streptavidin (Pierce, 21125).

Software: Data were acquired using MassLynx™ v4.2 Software, and processed using CIUSuite2 (Ruotolo Group, University of Michigan).

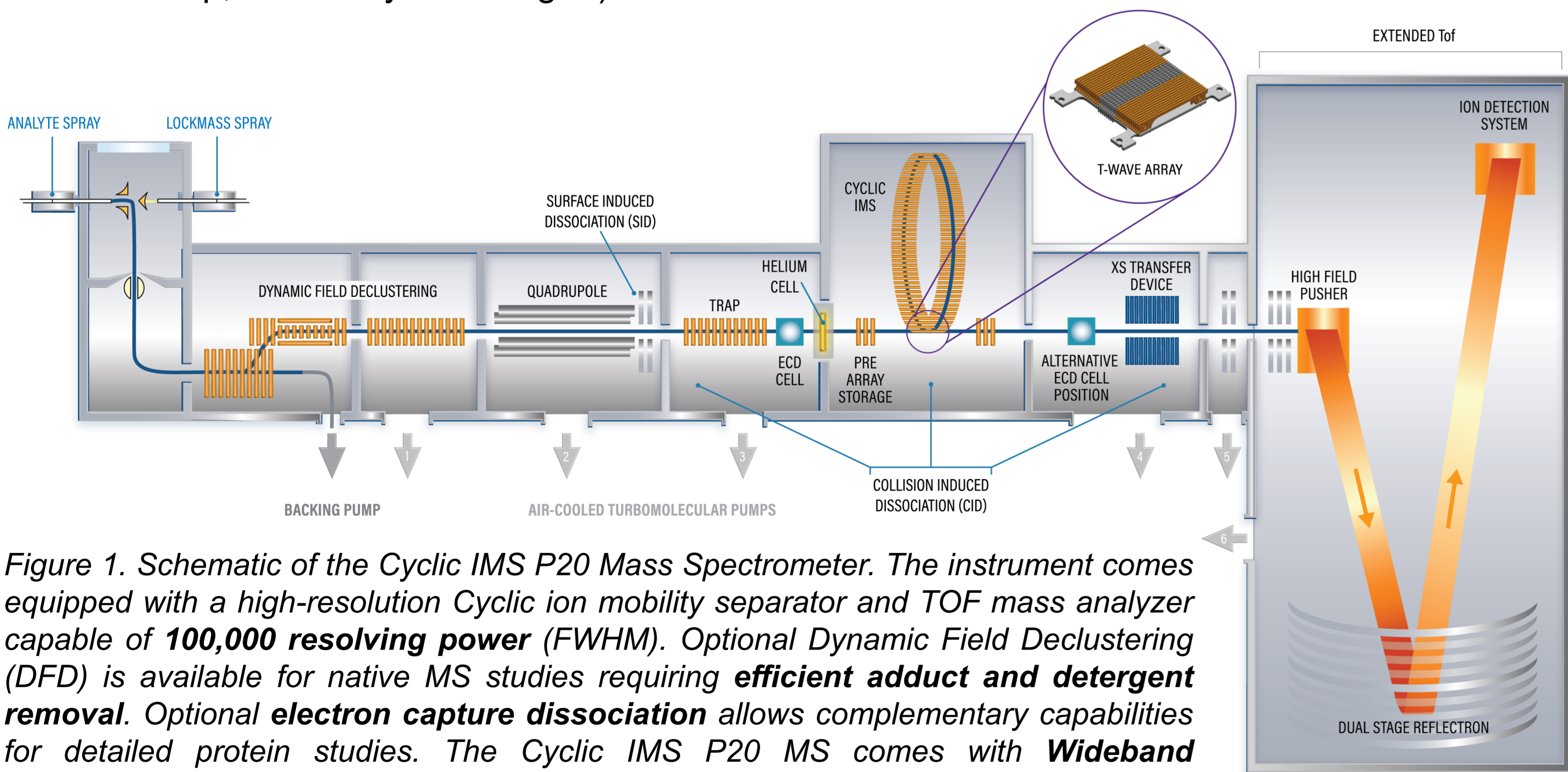


Figure 1. Schematic of the Cyclic IMS P20 Mass Spectrometer. The instrument comes equipped with a high-resolution Cyclic ion mobility separator and TOF mass analyzer capable of 100,000 resolving power (FWHM). Optional Dynamic Field Decustering (DFD) is available for native MS studies requiring efficient adduct and detergent removal. Optional electron capture dissociation allows complementary capabilities for detailed protein studies. The Cyclic IMS P20 MS comes with Wideband Enhancement functionality to improve sensitivity by maximizing TOF duty cycle in ion mobility mode.

RAPID METHOD SET-UP

The Cyclic IMS P20 introduces two new dedicated acquisition types, CIU HDMS and CIU HDMSMS, for multiplexed or single charge state acquisitions, respectively. By specifying the starting voltage, the voltage step, the number of steps and the time per step, the method can be created in less than one minute, decreasing method creation time by at least 15 times.

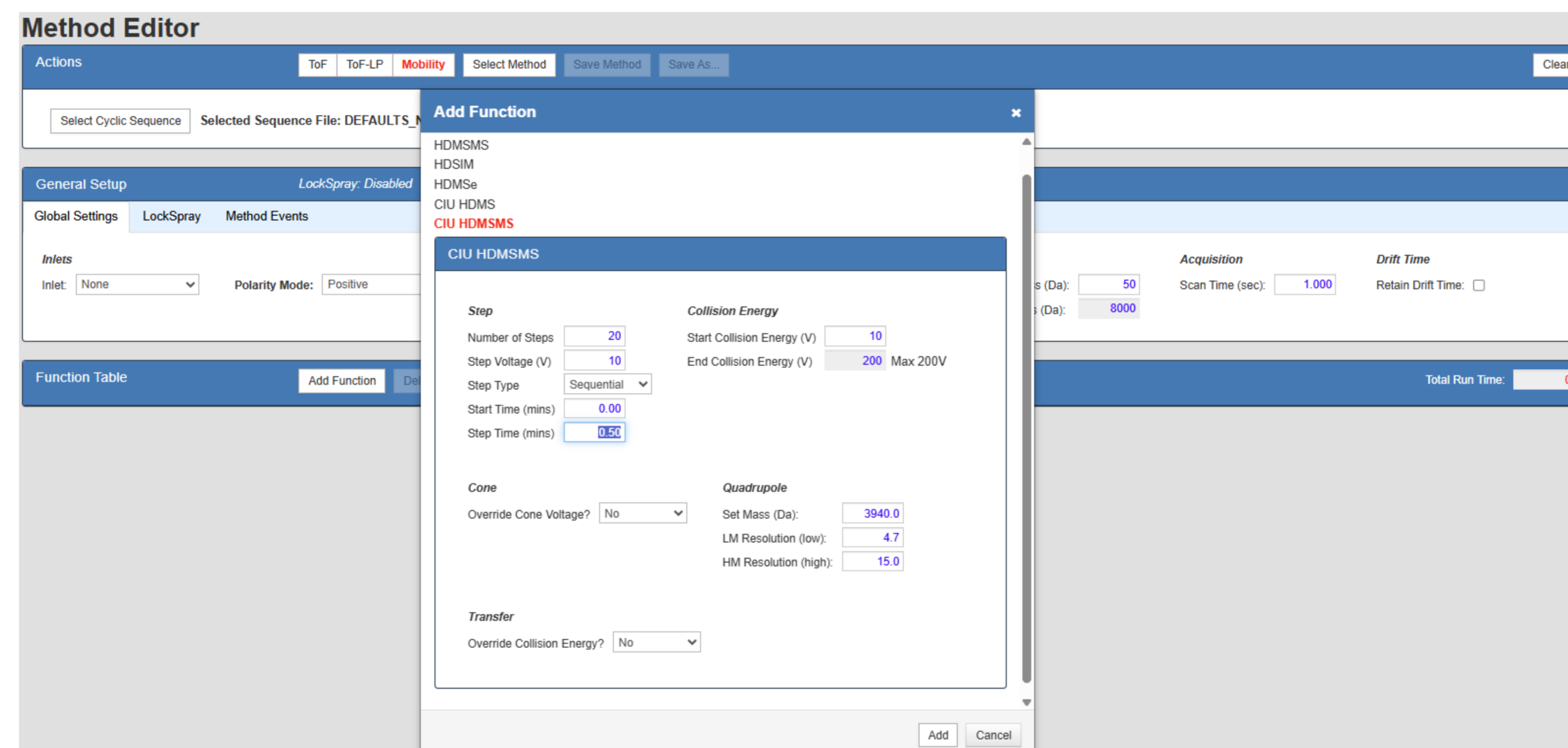


Figure 2. Method editor of the Cyclic IMS P20 MS. The CIU HDMS and CIU HDMSMS acquisition types enable rapid set-up of methods for unsupervised data collection.

The resulting method can be configured as sequential, where one voltage step follows another or parallel, where the voltage ramp is cycled scan-by-scan for the duration of the experiment.

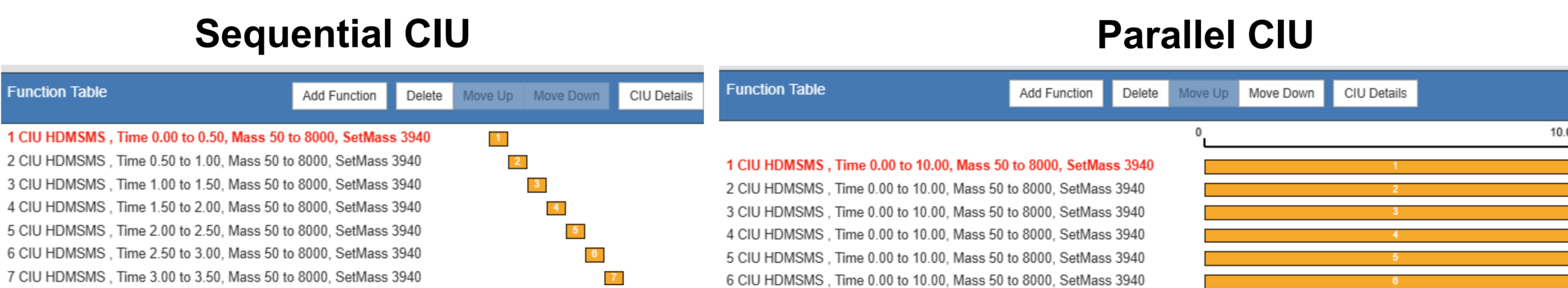


Figure 3. The CIU acquisition types also allow distinction of sequential or parallel CIU. In sequential CIU, voltages are stepped on after the other for a defined duration. In parallel CIU, the collision voltages are cycled scan-by-scan for the duration of the entire run. The parallel type opens up the possibility of online CIU experiments.

CONFLICT OF INTEREST AND TRADEMARK STATEMENTS

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

Waters, Cyclic, and MassLynx are trademarks of Waters Corporation or its affiliates.

INCREASED SENSITIVITY WITH WIDEBAND ENHANCEMENT

CIU experiments typically require a high number of voltage steps in order to produce a high-resolution unfolding fingerprint, meaning full experiments can take 15 minutes or more. Here we show the implementation of a form of Wideband Enhancement (WBE) to increase the TOF sampling duty cycle of over 4 times, revealing the possibility of reducing run times by the same amount. We show a signal increase of 4 times for the 15+ charge state of streptavidin and an increase of >4 times for the 26+ charge state of our standard mAb.

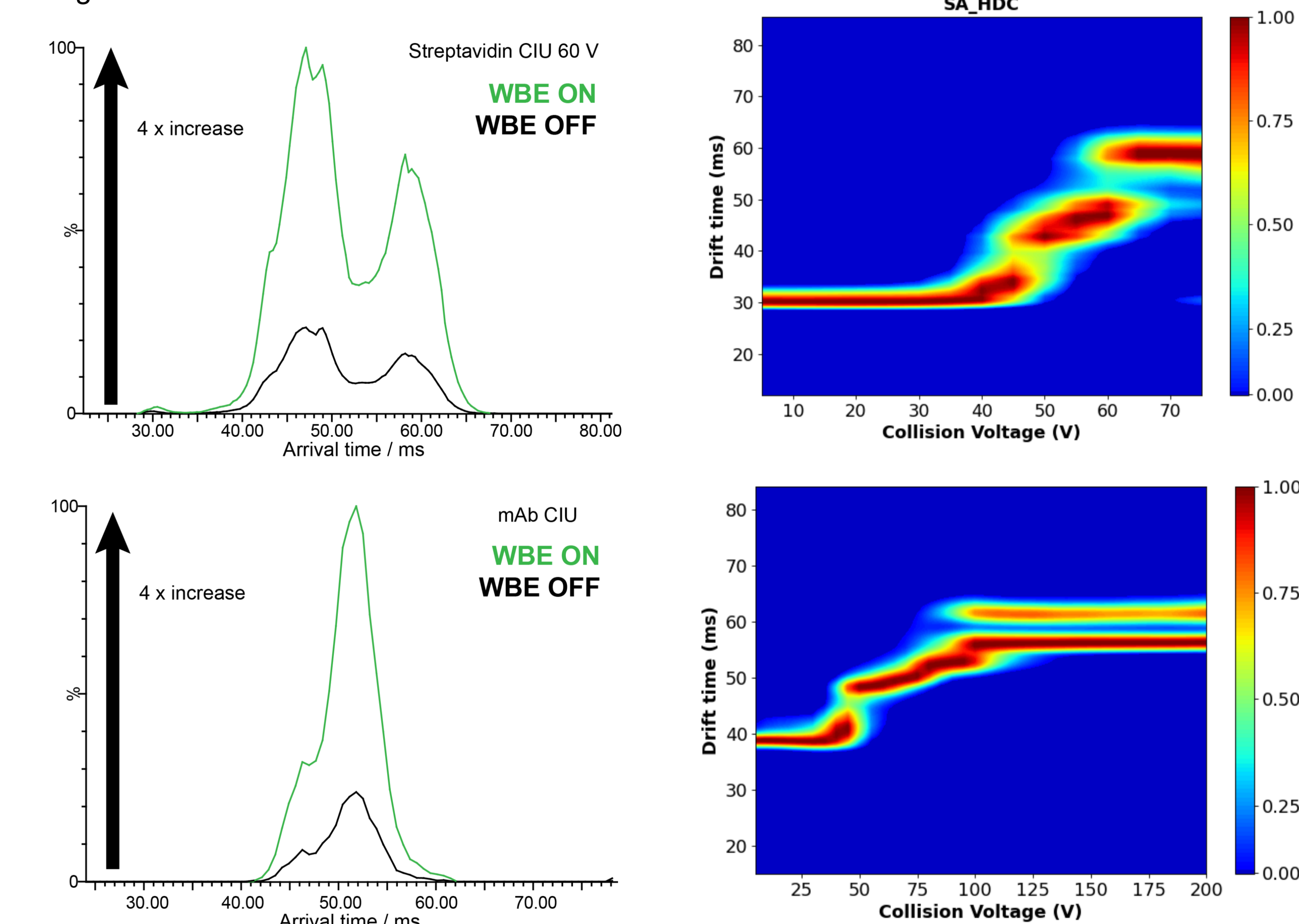


Figure 4. Wideband enhancement maximizes duty cycle for increased sensitivity. Left – Arrival time distributions for the 15+ charge state of streptavidin with and without WBE showing a sensitivity gain of around 4 times (TOP) and the 26+ charge state of the Waters mAb standard with and without WBE showing signal gain of 4 times (BOTTOM). Right – CIU fingerprints obtained in CIUSuite2.

CONCLUSIONS

- The Cyclic IMS P20 Mass Spectrometer enables drastically-increased throughput for CIU studies
- Two new dedicated CIU acquisition types allow for accelerated method set-up by at least 15 times
- Increased sensitivity with Wideband Enhancement functionality decreases run time by up to 4 times