

# Novel ion activation workflows in the hybrid Orbitrap-Omnitrap platform empower top-down and bottom-up proteomics



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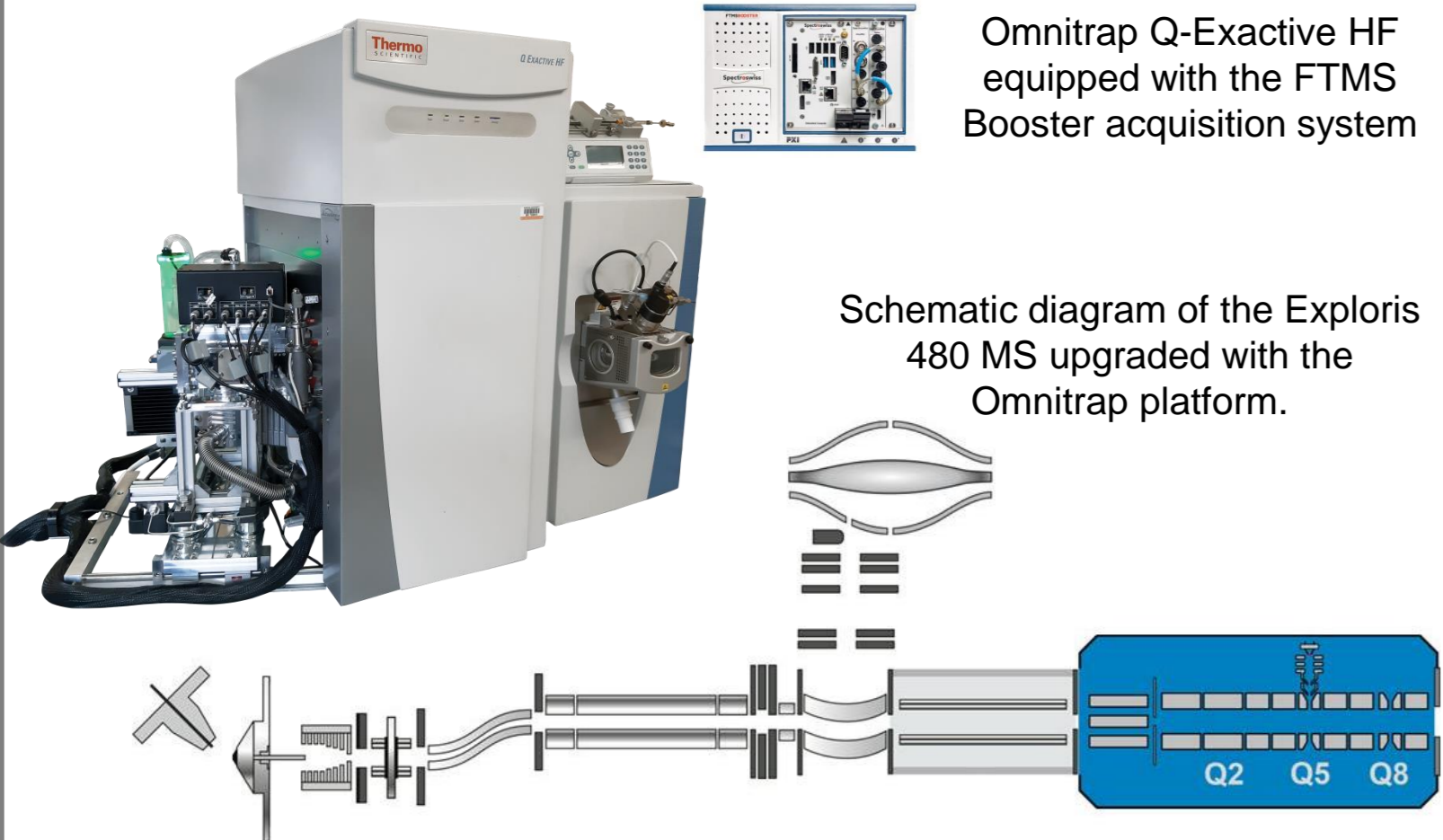
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## INTRODUCTION

- ✓ Advanced processing of ions is demonstrated in the Omnitrap™ platform using a combination of complementarity and synergistic ion activation methods.
- ✓ Enhanced performance is enabled by incorporating variations of the entire range of ion activation methods into a single, highly flexible platform.
- ✓ Unique multidimensional multiple-stage tandem MS workflows are developed for top-down analysis of intact proteins.
- ✓ Highly efficient electron-based reactions are performed on chromatographic time scales enhancing bottom-up MS.
- ✓ The Omnitrap platform is interfaced to the Orbitrap™ mass analyzer offering outstanding capabilities for in-depth characterization of proteins.
- ✓ The high performance FTMS Booster data acquisition system enhances mass spectra quality.

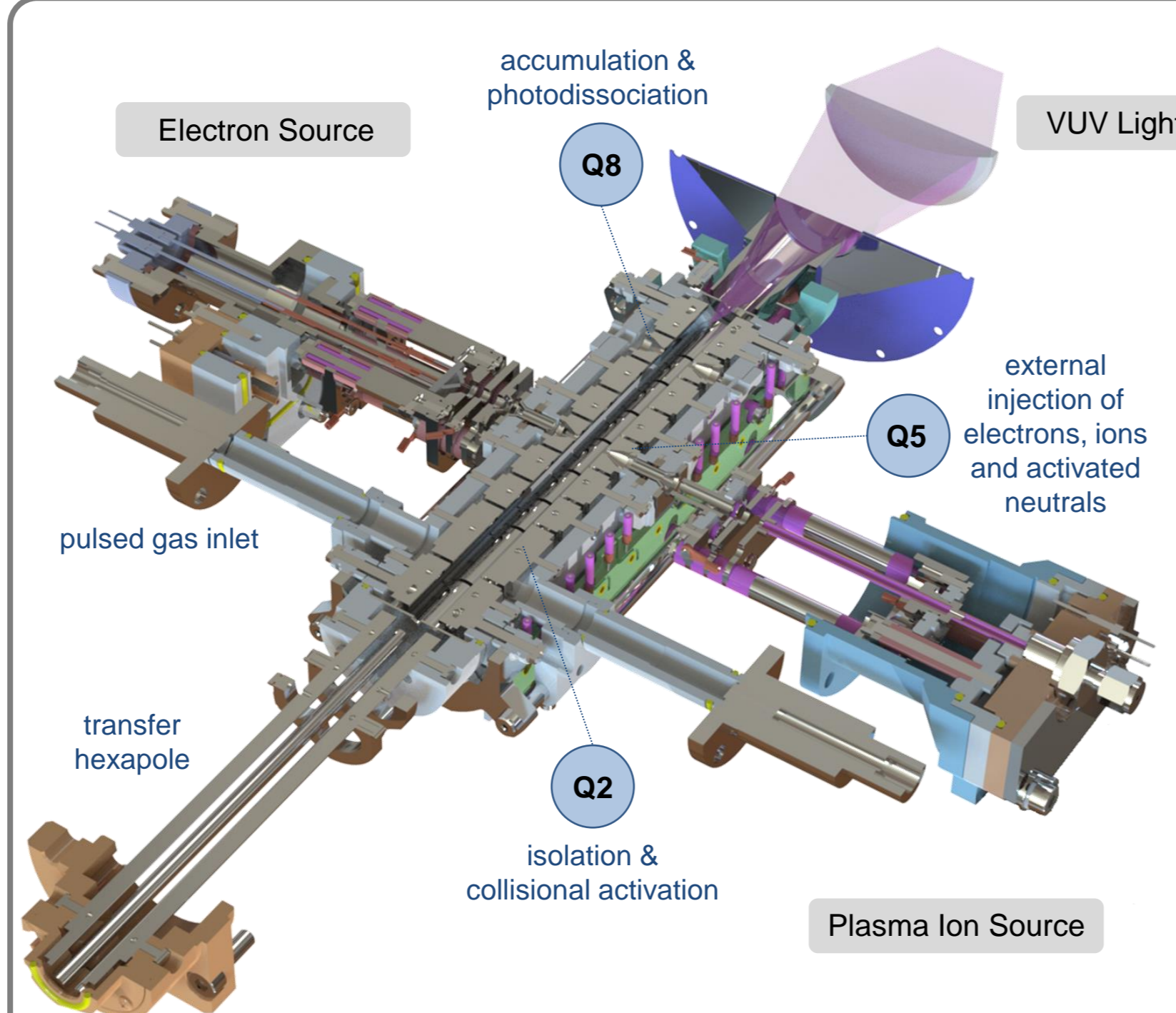
## METHODS

The Omnitrap platform is coupled to the Q Exactive HF™ Orbitrap™ mass spectrometer upgraded with the Biopharma option and more recently to the Exploris™ 480 mass spectrometer. The Orbitrap MS Tune software has been upgraded enabling lossless transfer of ions between the HCD collision cell and the Omnitrap platform.

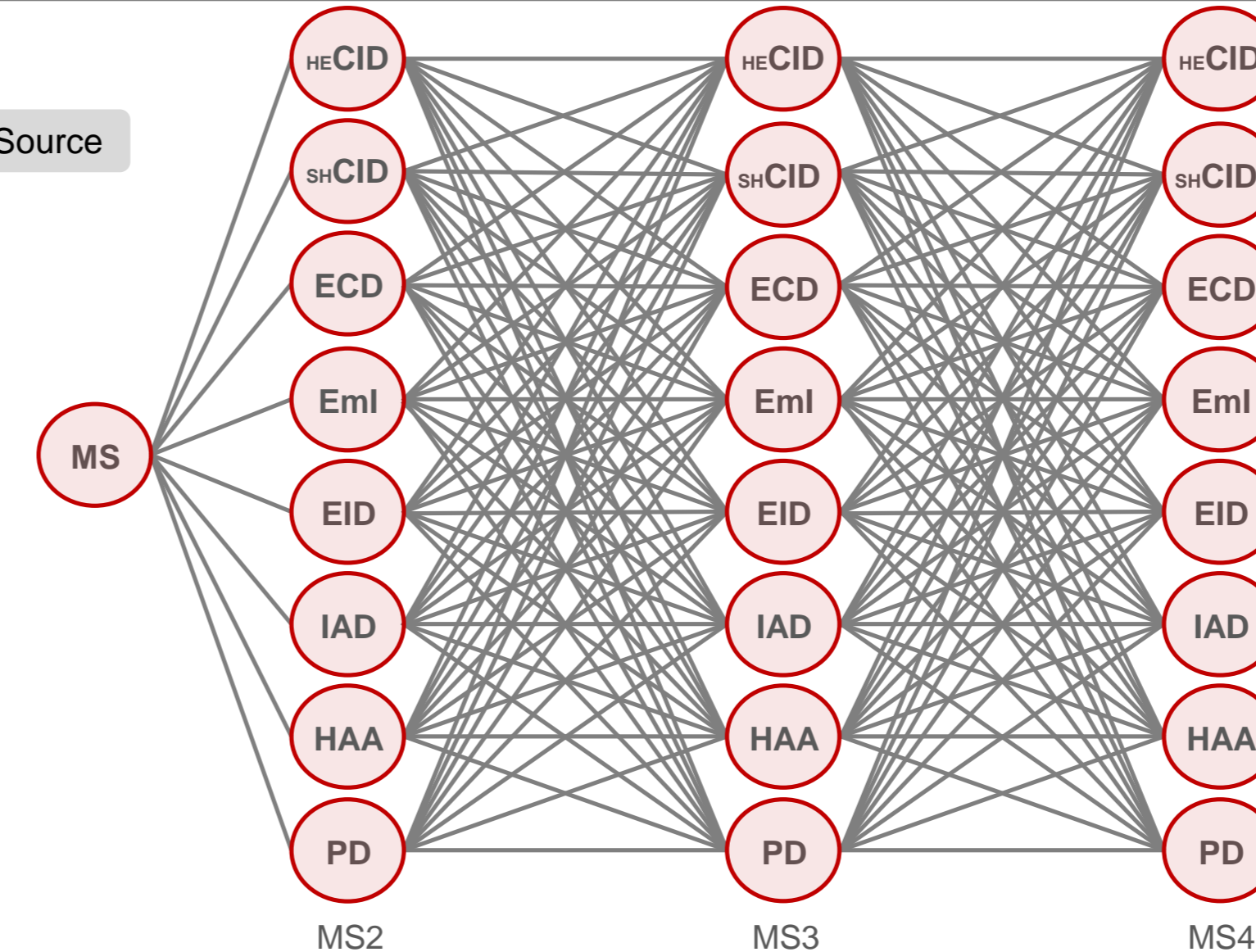


Omnitrap Q-Exactive HF equipped with the FTMS Booster acquisition system

Schematic diagram of the Exploris 480 MS upgraded with the Omnitrap platform.



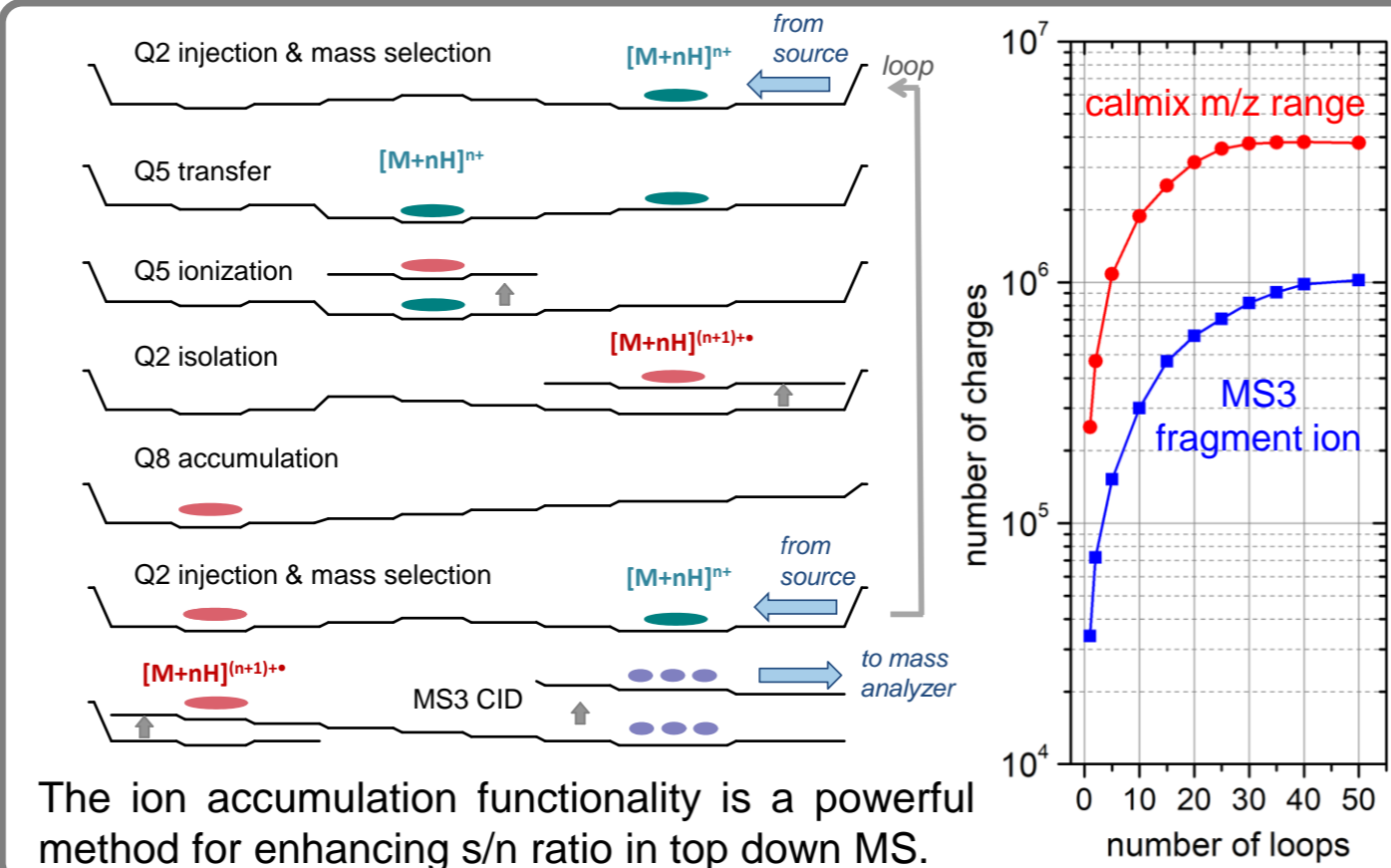
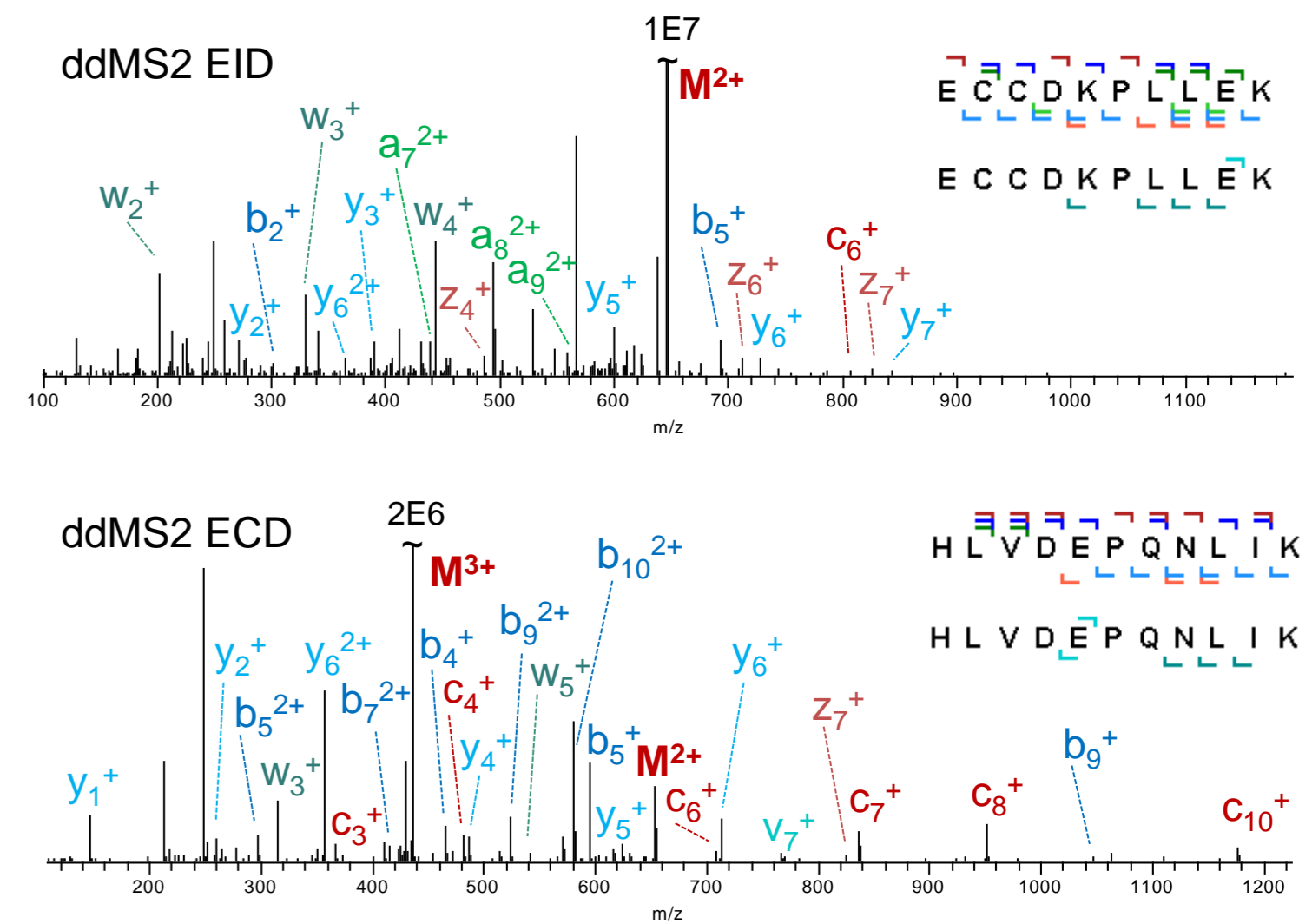
The Omnitrap platform is a segmented RF linear ion trap enabling multidimensional multiple-stage tandem mass spectrometry.



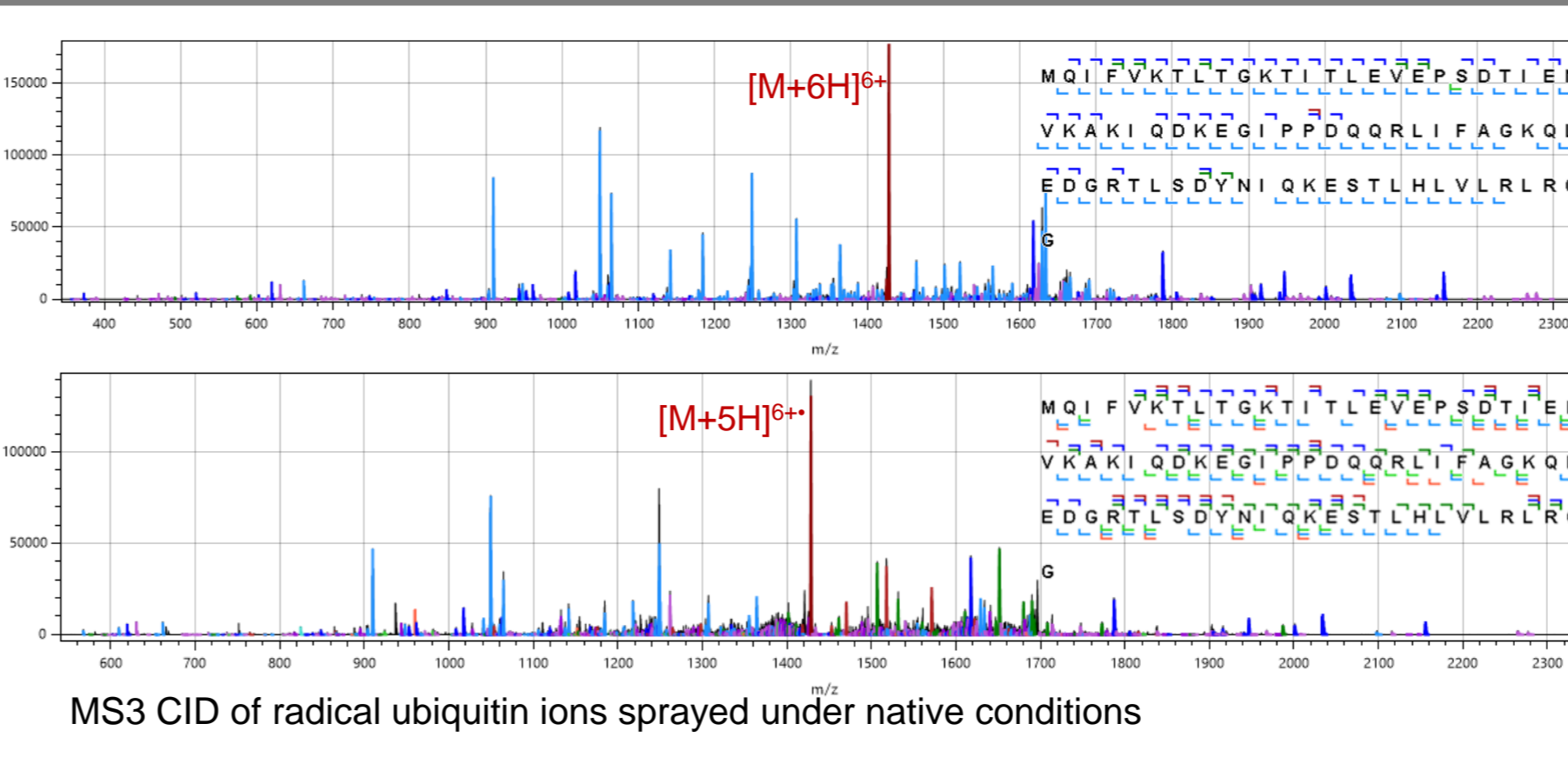
HE-CID: High-Energy Collision Induced Dissociation  
SH-CID: Slow-Heating Collisional Induced Dissociation  
ECD: Electron Capture Dissociation  
Eml: Electron meta-ionization  
EID: Electron Induced Dissociation  
IAD: Ion Activated Dissociation  
HAA: Hydrogen Atom Attachment  
PD: Photodissociation

The ion activation network of the Omnitrap platform incorporates variations of the entire range of ion activation methods into a single platform.

The efficiency of ExD reactions available in the Omnitrap platform are evaluated using a BSA tryptic digest separated in a 25 cm long PepSep C18 column and by operating the Orbitrap MS in DDA mode. EID reactions are performed within a 50 ms time window and ECD reactions within 80 ms. Examples of MS2 EID and MS2 ECD spectra demonstrate enhanced sequence coverage and complementarity for all peptides independent of charge state. Results are compared with HCD fragmentation.



The ion accumulation functionality is a powerful method for enhancing s/n ratio in top down MS.



MS3 CID of radical ubiquitin ions sprayed under native conditions

Dissociation Method	z	% Sequence coverage							HCD	EID	ECD
		Total	a	b	c	x	y	z			
BSA PSMs	2+	HCD	91	25	47	8	85		2044	1600	1214
		EID	97	48	69	25	52	86	3241	3094	2598
		ECD	87	19	45	31	12	67	41		
MS/MS	3+	HCD	73	21	37	10	67		18907	14608	13073
		EID	95	46	69	25	42	78	56		
		ECD	93	26	61	72	19	72	59		
Peptides	4+	HCD	62	17	34	6	50		76	72	63
		EID	82	41	60	29	35	58	45		
		ECD	93	21	54	74	15	62	46	89	88
Sequence Coverage (%)											

