

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

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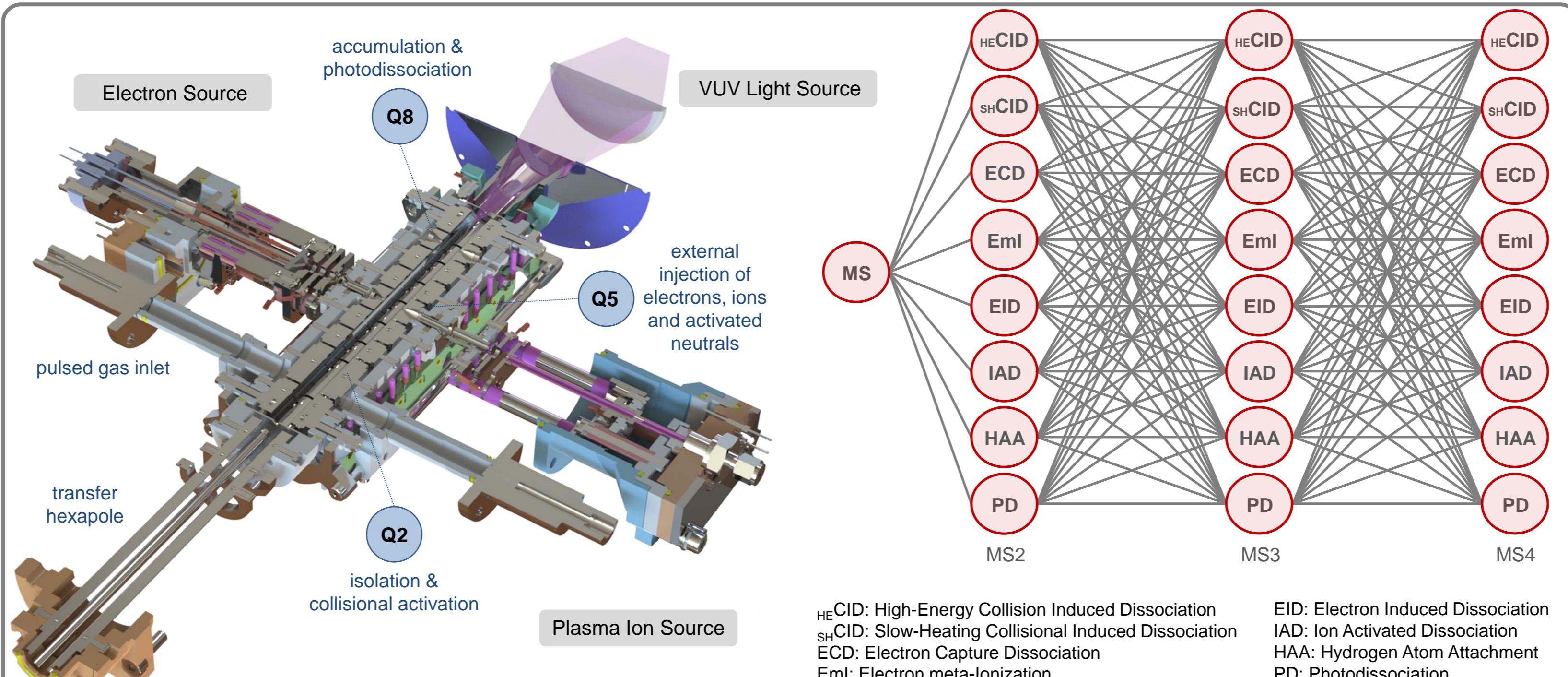
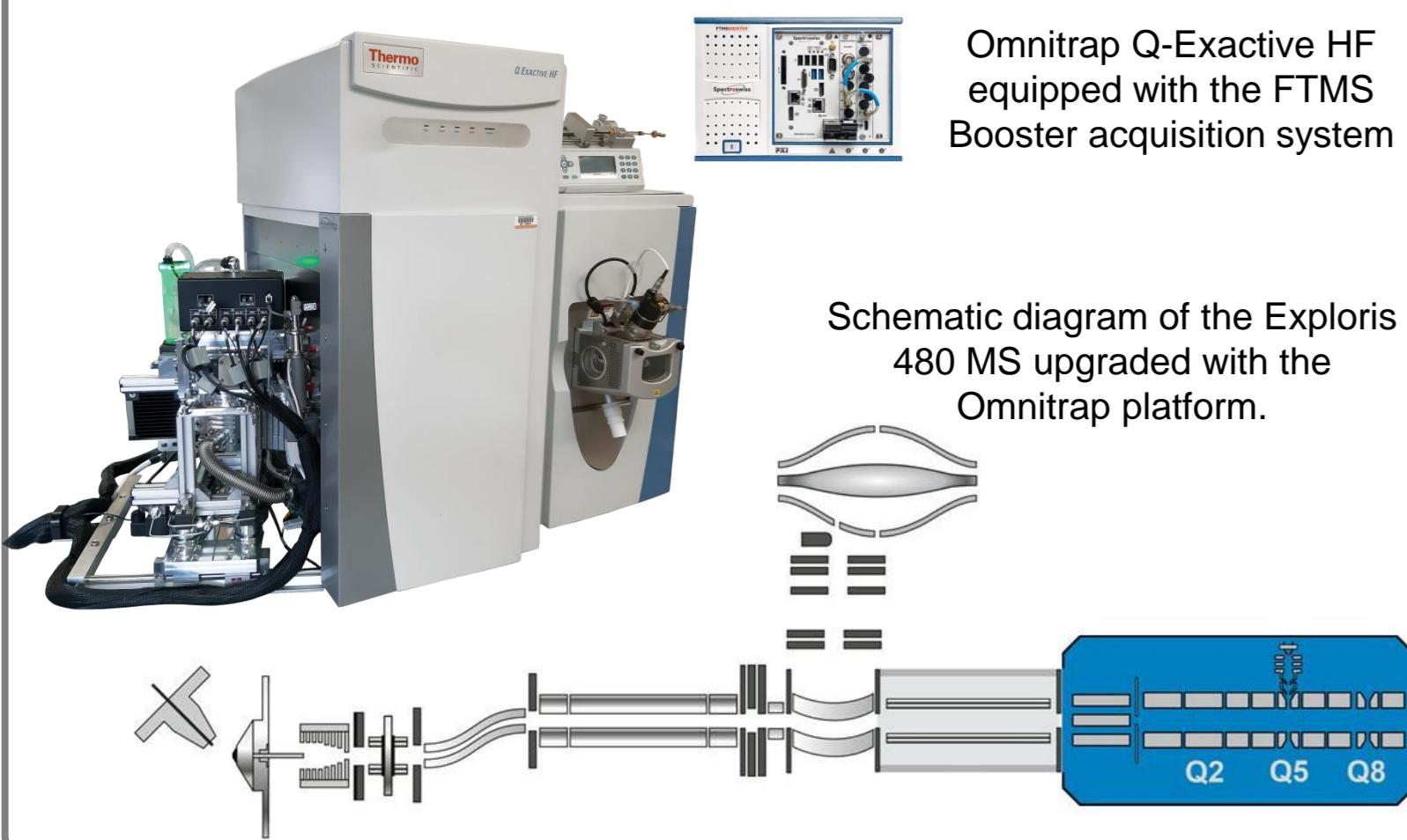
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INTRODUCTION

- ✓ 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

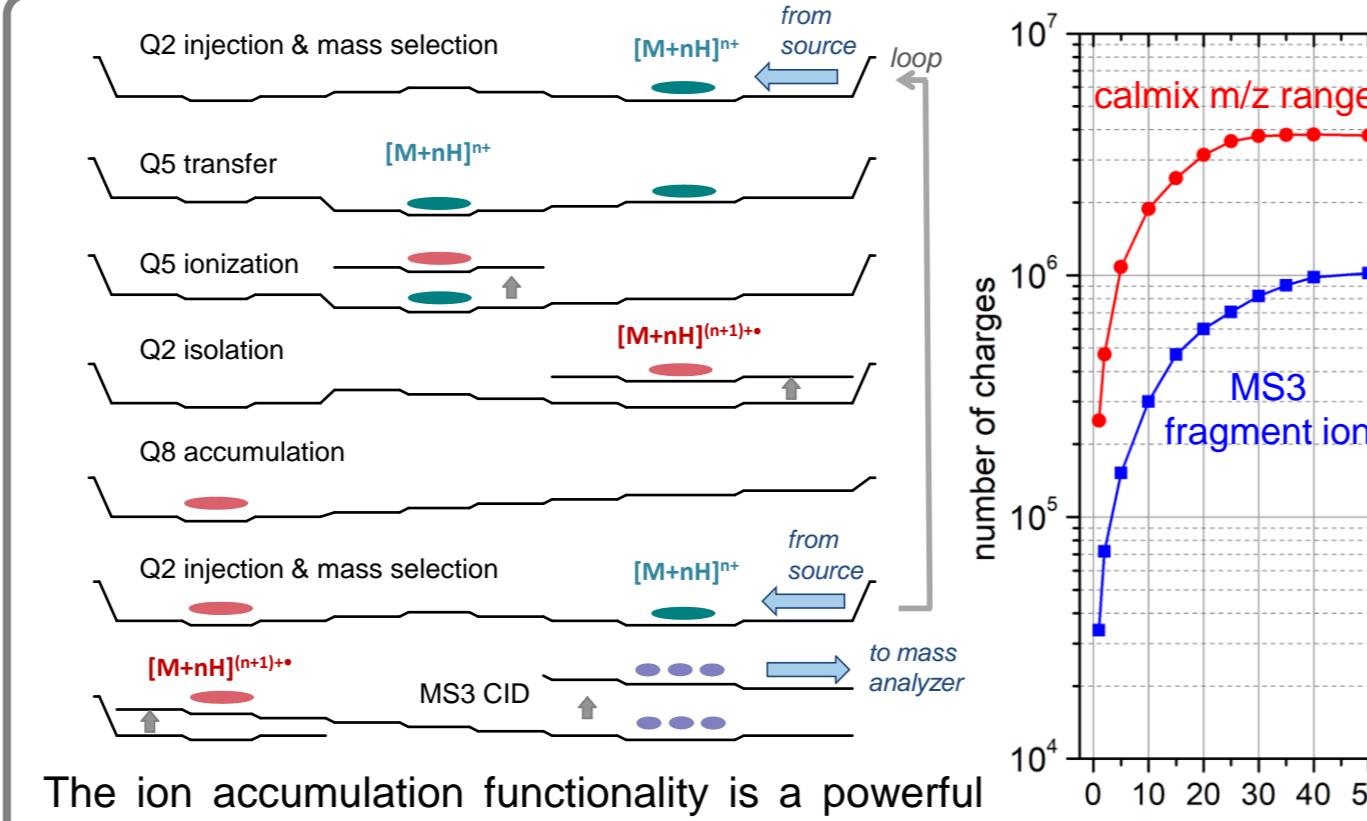
The Omnitrap platform is coupled to the Q Exactive HF™ Orbitrap™ mass spectrometer upgraded with the Biopharma option and more recently to the Exploris™ 480 mass spectrometer. The Orbitrap MS Tune software has been upgraded enabling lossless transfer of ions between the HCD collision cell and the Omnitrap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



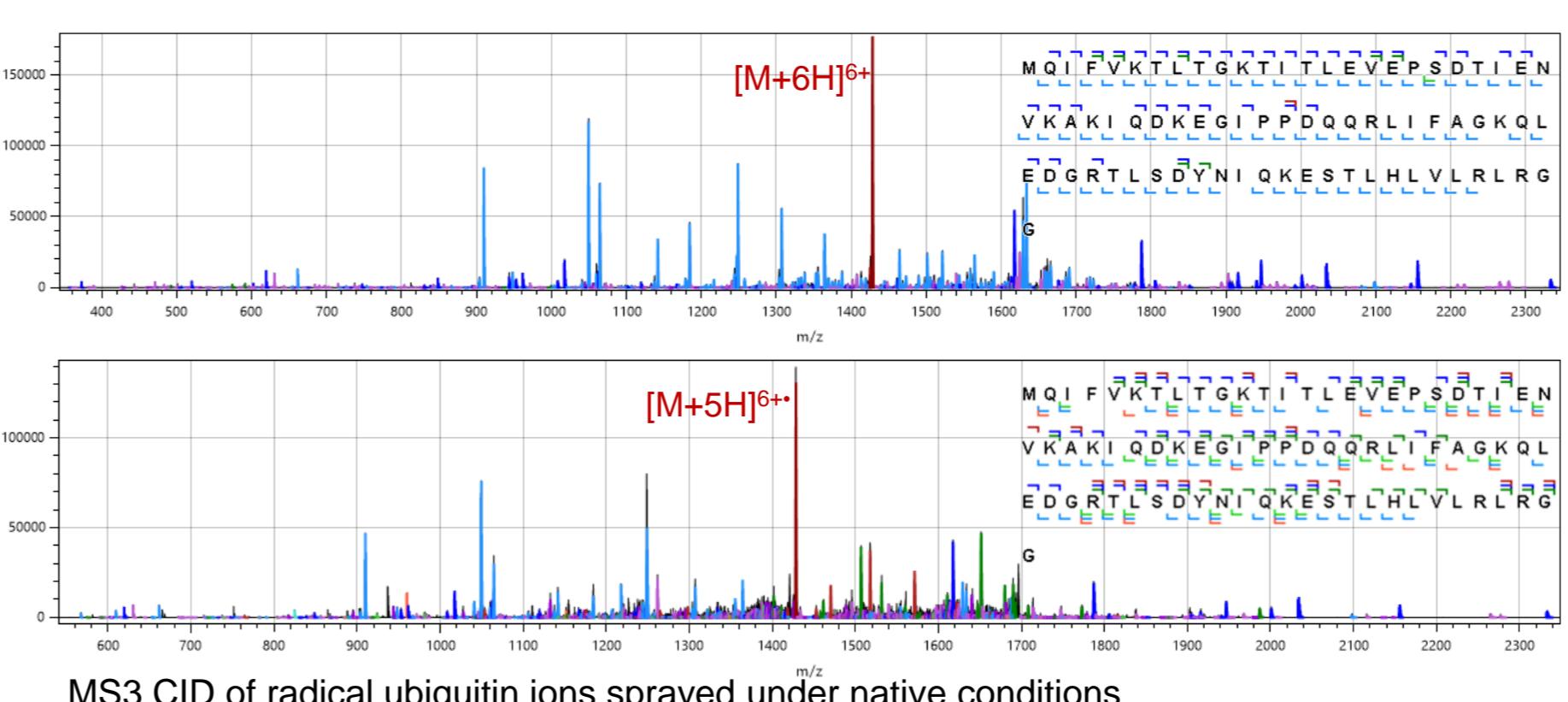
The Omnitrap platform is a segmented RF linear ion trap enabling multidimensional multiple-stage tandem mass spectrometry.

HECID: High-Energy Collision Induced Dissociation
SHCID: Slow-Heating Collisional Induced Dissociation
ECD: Electron Capture Dissociation
IAD: Ion Activated Dissociation
HAA: Hydrogen Atom Attachment
PD: Photodissociation

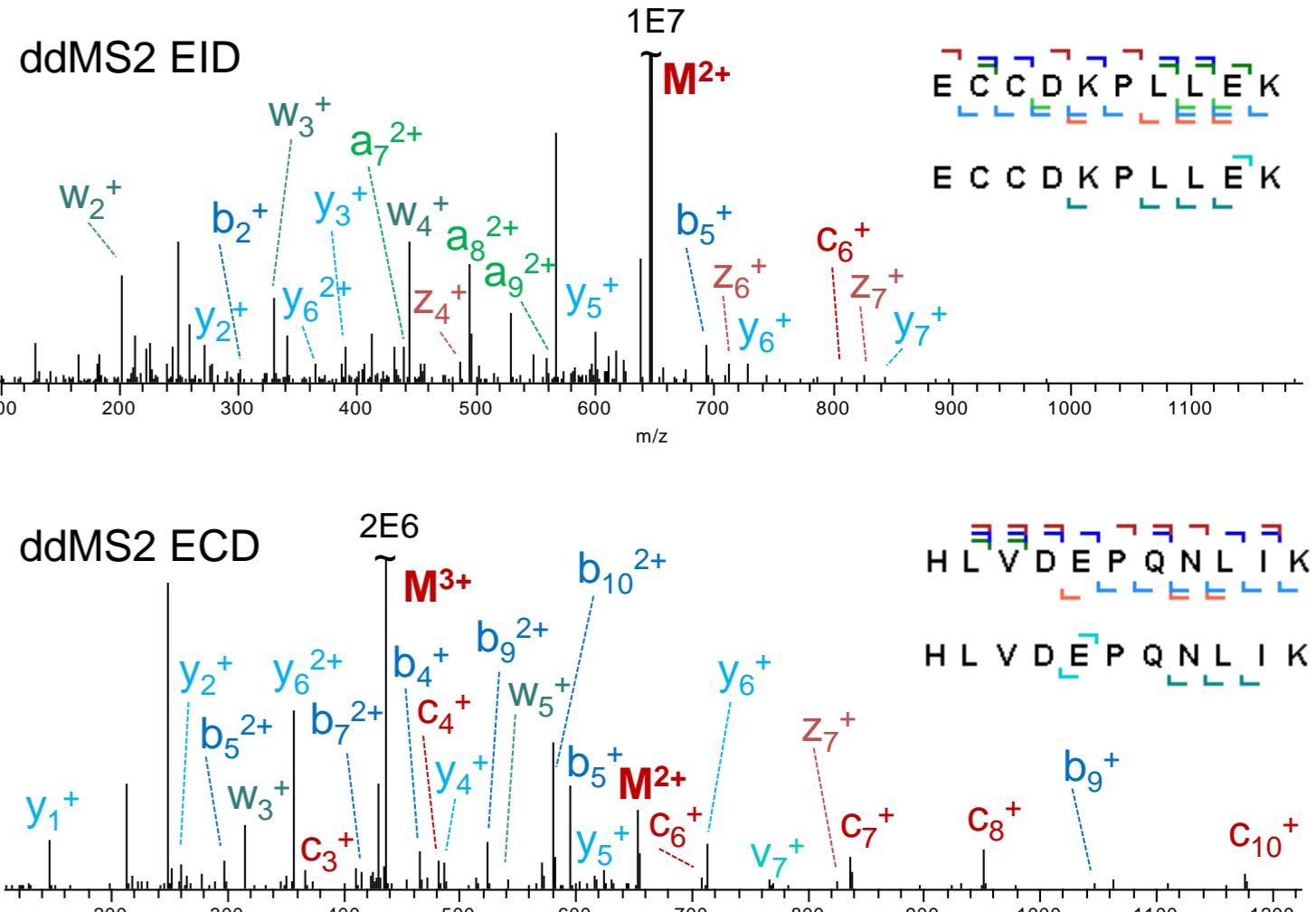
The ion activation network of the Omnitrap platform incorporates variations of the entire range of ion activation methods into a single platform.



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



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.



Dissociation Method	z	% Sequence coverage						
		Total	a	b	c	x	y	z
HCD	2+	91	25	47	8	85		
EID	2+	97	48	69	25	52	86	64
ECD	2+	87	19	45	31	12	67	41
HCD	3+	73	21	37	10	67		
EID	3+	95	46	69	25	42	78	56
ECD	3+	93	26	61	72	19	72	59
HCD	4+	62	17	34	6	50		
EID	4+	82	41	60	29	35	58	45
ECD	4+	93	21	54	74	15	62	46
Sequence Coverage (%)		89	88	88	82			

