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

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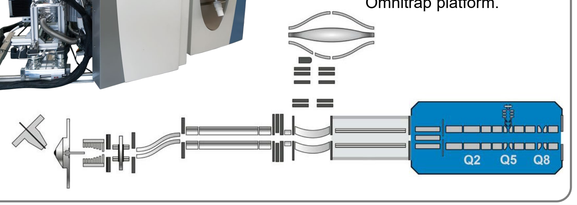
INTRODUCTION

- ✓ 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.

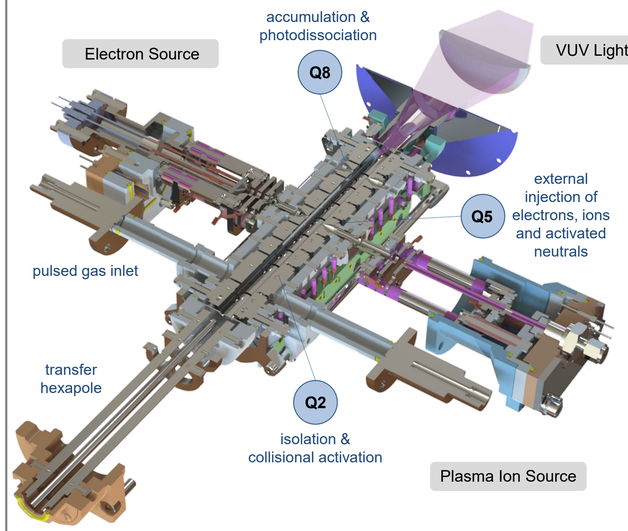
METHODS

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.

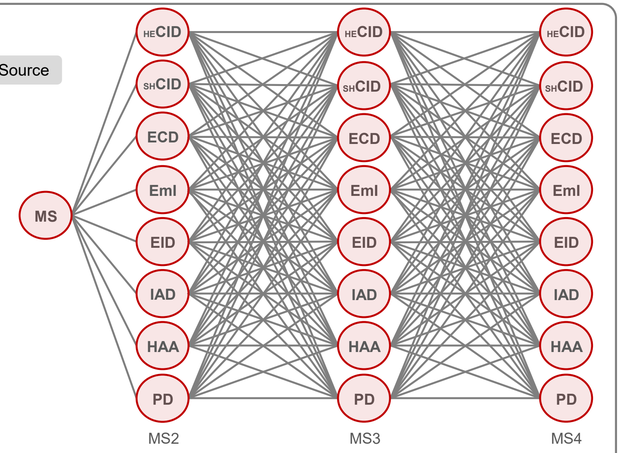
Omnitrap Q-Exactive HF equipped with the FTMS Booster acquisition system



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

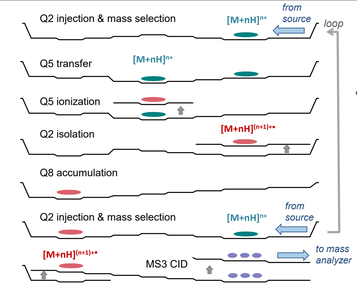


The Omnitrap platform is a segmented RF linear ion trap enabling multidimensional multiple-stage tandem mass spectrometry.

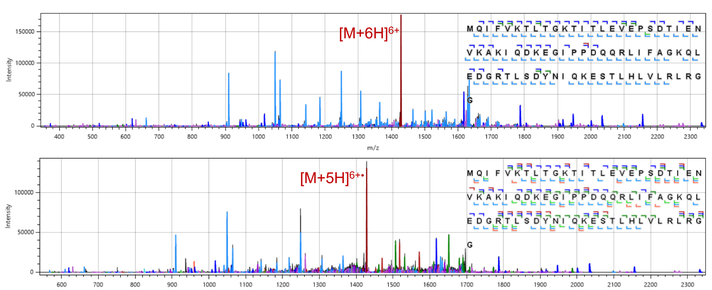


$_{HE}$CID: High-Energy Collision Induced Dissociation
$_{SH}$CID: Slow-Heating Collisional Induced Dissociation
ECD: Electron Capture Dissociation
EmI: Electron meta-Ionization
EID: Electron Induced Dissociation
IAD: Ion Activated Dissociation
HAA: Hydrogen Atom Attachment
PD: Photodissociation

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

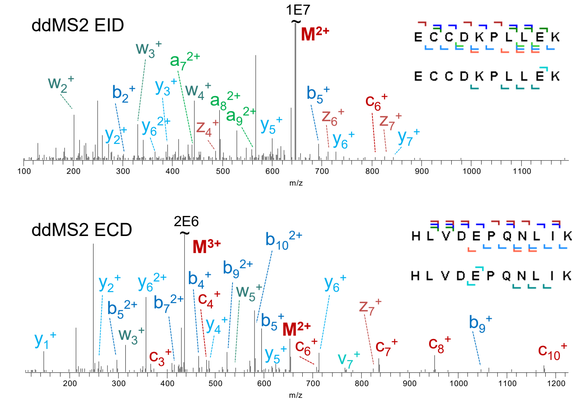


The ion accumulation functionality is a powerful method for enhancing s/n ratio in top down MS.



MS3 CID of radical ubiquitin ions sprayed under native conditions

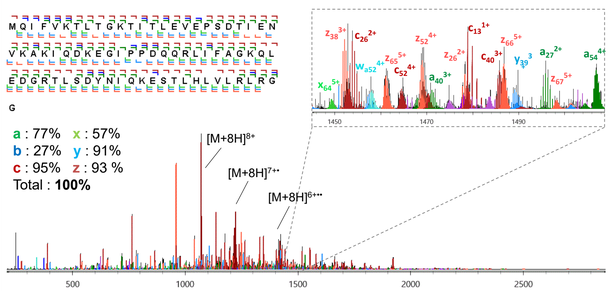
The efficiency of ExD reactions available in the Omnitrap platform are evaluated using a BSA tryptic digest separated in a 25 cm long PepSep C18 column and by operating the Orbitrap MS in DDA mode. EID reactions are performed within a 50 ms time window and ECD reactions within 80 ms. Examples of MS2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



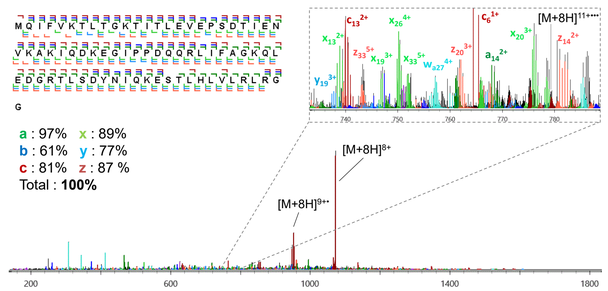
Dissociation Method	z	% Sequence coverage						
		Total	a	b	c	x	y	z
HCD	2+	91	25	47	8		85	
EID		**97**	48	69	25	52	86	64
ECD		87	19	45	31	12	67	41
HCD	3+	73	21	37	10		67	
EID		**95**	46	69	25	42	78	56
ECD		93	26	61	72	19	72	59
HCD	4+	62	17	34	6		50	
EID		82	41	60	29	35	58	45
ECD		**93**	21	54	74	15	62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

	HCD	EID	ECD
BSA PSMs	2044	1600	1214
Total PSMs	3241	3094	2598
MS/MS	18907	14608	13073
Peptides	76	72	63
Sequence Coverage (%)	89	88	82

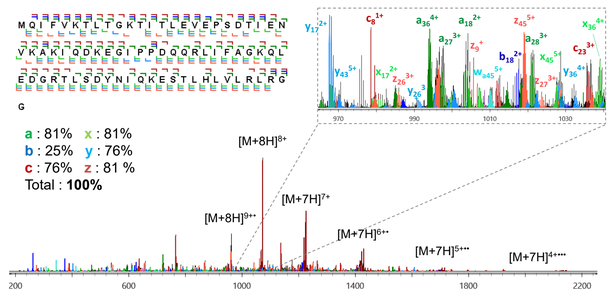
Electron Capture Dissociation



Electron Induced Dissociation



Hydrogen Ion Activated Dissociation



Vacuum Ultraviolet Photodissociation