

MS and MS/MS Performance of an AP-MALDI Ion Trap for Proteomics

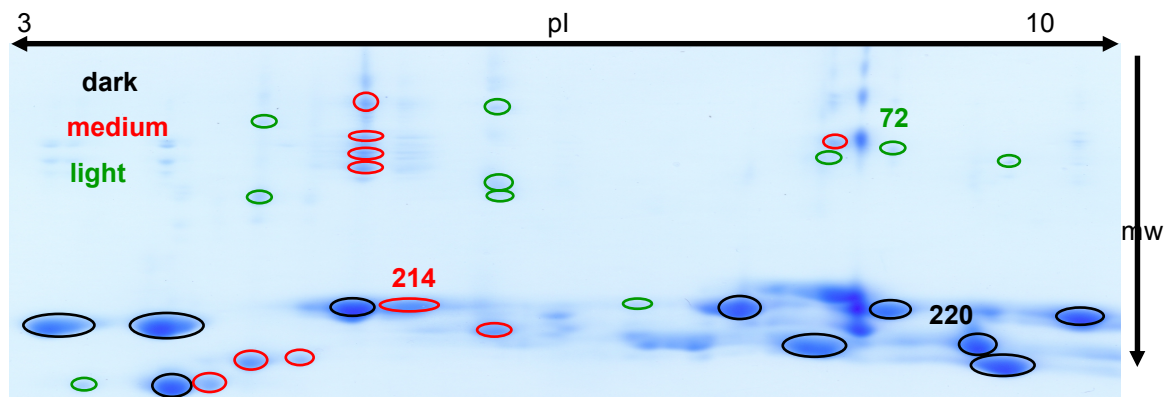
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For proteomics involving 2D gels, a typical workflow has been to rapidly identify medium and dark Coomassie-stained spots via peptide mass fingerprinting on a MALDI-TOF instrument. Faint spots and those yielding few peptides are then identified by MS/MS using capillary LC/MS/MS or nanospray. Now an atmospheric pressure (AP) MALDI source coupled to an ion trap eliminates the need to resort to the slower and more laborious ESI methods. Current AP-MALDI ion trap instruments have achieved sensitivity 1 – 10 fold better than typical MALDI-TOF instruments by adapting the ESI ion optics to better transmit, focus, and trap MALDI generated peptide ions while suppressing low mass matrix ions, thus mitigating space-charging effects. The ability to automatically collect both MS and MS/MS data enables efficient protein identification from 2D gel spots in minutes without resorting to the complexity of plumbing and operating a capillary LC/MS/MS system. Here we describe the performance of an Agilent LC/MSD AP-MALDI ion trap in proteomics applications.

With MALDI ionization peptide ions spanning the entire mass range can not be efficiently trapped using a single trap drive frequency. Typical automated experiments are conducted at 2 or more separate settings and a composite spectrum generated. The flow nature of an ESI-LC/MS/MS experiment requires scans to occur within the time window of a chromatographic peak. In contrast, MALDI ionization is performed from the “stopped-flow condition” of a solid surface affording an unlimited time to acquire data. Consequently, an ESI mode scan on an ion trap is typically done at ~ 13,000 amu/sec and leads to unit resolution. For the AP-MALDI work the resolution can readily be enhanced to >3,000 FWHM using a scan rate of ~5500 amu/sec. This provides better peak shape and more consistent mass accuracy for database searches. On an ion trap, MS/MS fragmentation of a singly-charged AP-MALDI precursor ion tends not to yield as extensive fragmentation as the same peptide would as a doubly-charged ion in ESI-LC/MS/MS mode. The AP-MALDI MS/MS fragmentation is strongly directed by the presence of acidic amino acids (D and E) and prolines (P) with ions representing fragmentation at these residues often dominating a spectrum. Scoring modifications to SpectrumMill were made to facilitate database searches with these tendencies.

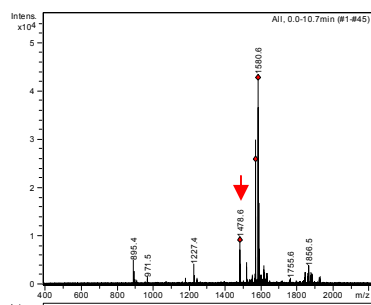
~ 50 mg of proteasome proteins were isoelectrofocused on immobilized pH gradient strips (IPG), pH 3-10 nonlinear, 7 cm (Amersham Biosciences). The IPG strips were treated with reducing and alkylating agents (DTT, iodoacetamide), placed on a 7 cm X 7 cm 16% Tris-Glycine gel (Invitrogen), and separated according to molecular weight. The 2D gel was Coomassie stained with GelCodeBlue (Pierce). Spots were classified by eye as dark, medium, and light; then excised and digested with trypsin. Each recovered digest solution (~30 ml) was analyzed using the AP-MALDI ion trap. Three spots analyzed are show here.



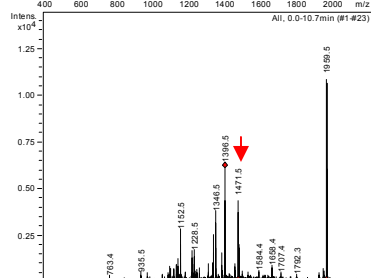
Spectrum Mill interpretation results are shown below along with representative data. Search Parameters: Database: NCBIInr.mammals; Search mode: homolgyMulti_mq; MALDI ion trap +/- 1.5, 0.7 Da (parent, fragment); Enzyme: Trypsin 1 missed cleavage.

spot ID	Protein Name	Protein MW (Da)	Protein pI	# peptides identified	Summed MS-Tag Score
220	Proteasome subunit alpha type 2 (a2)	26,012	7.72	2	20.0
214	Proteasome subunit alpha type 3 (a7)	28,491	5.40	4	30.3
72	Proteasome subunit alpha type 1 (a6)	29,598	6.15	2	13.9

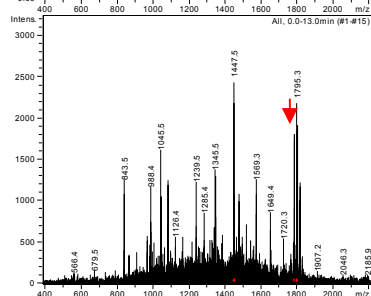
MS composite of trap drives



Dark #220

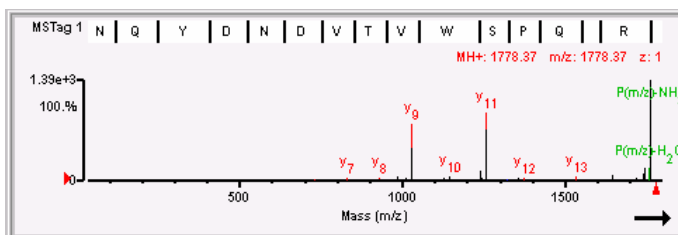
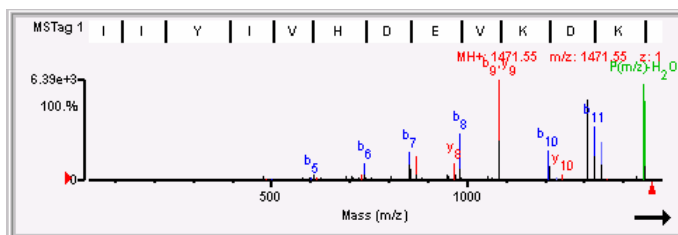
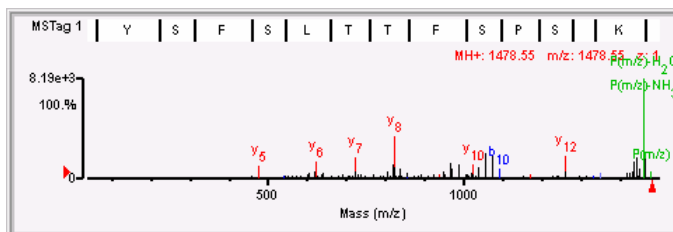


Medium #214



Light #072

MS/MS



Data Dependent MS/MS Acquisition Conditions

Sample: 1/3 of digest (10 ul) was loaded onto a C18 mZipTip to concentrate and desalt.
 Matrix: Eluted onto target in 1:8 α-cyano-4-hydroxy-cinnamic acid (Agilent) in 60% ACN/0.1% formic acid.
 Laser firing rate: 10Hz Mass range: 400-2200 Target ions (ICC): 300,000 Cycle: 1 MS, 3 MS/MS
 MS/MS, frag amplitude: 1.55V SmartFrag: on (30%-200%) Precursor threshold rel = 2%
 Active exclusion on, 1 spectra, 20 minute duration, Trypsin exclusion list

	dark		medium		light	
gel stain intensity:						
segment:	1	2	1	2	1	2
time (min):	0-8	8-11	0-8	8-11	0-8	8-14
trap drive frequency:	65	80	65	80	65	80
Precursor intensity threshold (counts):	500	200	500	200	200	200
Max accumulation time – MS (msec):	535		1605		1605	
Max accumulation time – MS/MS (msec):	856		2568		2568	
MS scans averaged:	10		10		10	
MS/MS scans averaged:	16		16		32	
Max laser shots – MS:	60		180		180	
Max laser shots – MS/MS:	96		288		576	