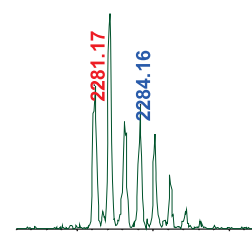
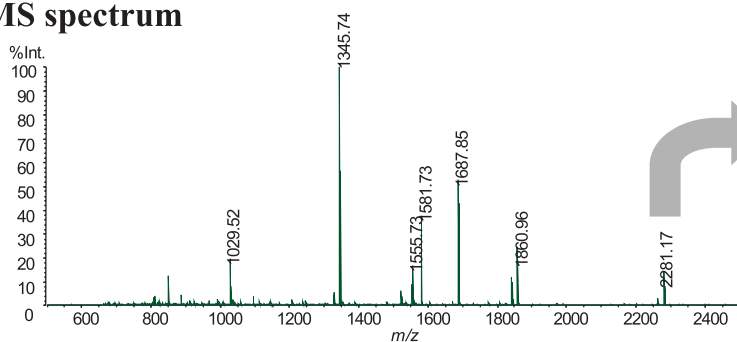


Proteome Analysis Using the AXIMA-QIT

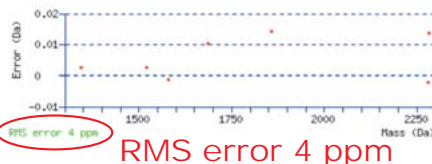
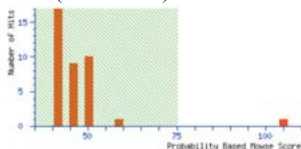
In this application we analyzed a tryptic digest of ovalbumin. A PMF search yielded an identification of the protein with a 4 ppm mass error using an external standard. Two narrowly separated peptide fragments were observed in the ovalbumin digest at m/z 2281 and m/z 2284. The high-resolution selection of the AXIMA-QIT enabled accurate mass selection of the crowded peaks, with only a 3 Da mass difference, even at molecular weights exceeding m/z 2000. MS/MS analysis was conducted on each of the peaks and high-quality MS/MS spectra were obtained as shown in the MS/MS ion search results.

MS spectrum



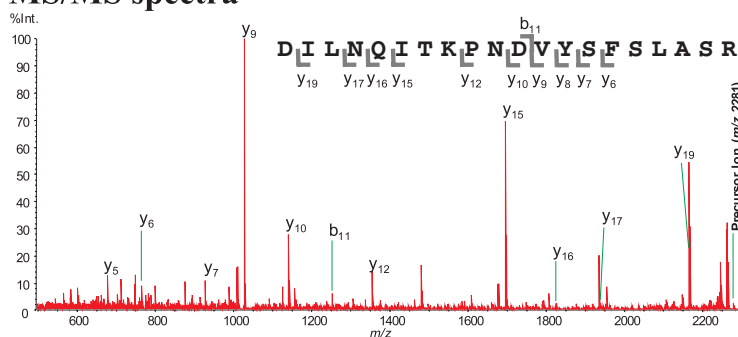
High resolution Selection

PMF (MASCOT™)



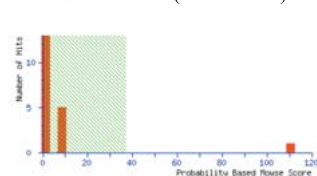
RMS error 4 ppm

MS/MS spectra

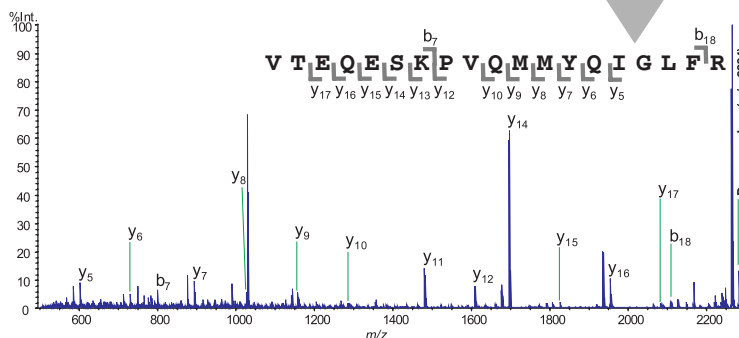


High quality MS/MS

MS/MS Ion Search (MASCOT™)



Score: 110 Queries matched: 2
ovalbumin [validated] - chicken



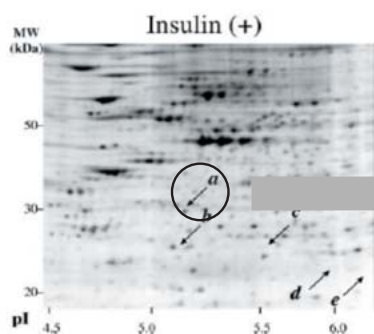
Sample provided by: Kumiko Saeki, Research Institute International Medical Center of Japan

Literature cited: *Am. J. Physiol. Endocrinol. Metab.*, **2005**, Sep; 289(3):E419-28.

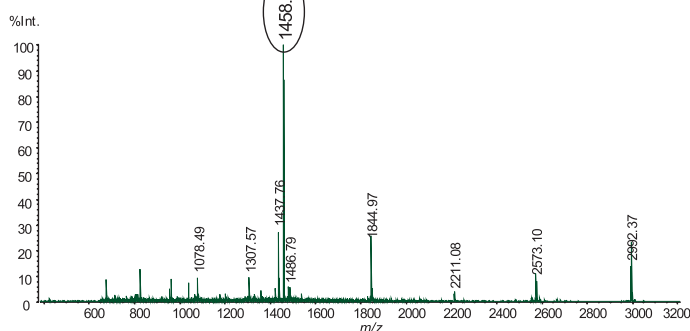
Human hematopoietic cells (HL-60) were processed with insulin and 2-dimensional gel electrophoresis (2DE) was conducted on the protein extract. The gel obtained by 2DE was stained using SYPRO Ruby, and spot "a", which was observed to have intensity as a result of the insulin processing, was subjected to in-gel trypsin digestion. Following digestion, the AXIMA-QIT was employed to collect the MS spectrum. A PMF search yielded an identification of intracellular chloride ion channel (CLIC1) with a 10 ppm mass error using an external standard. We performed an MS/MS measurement of the peak identified as an N-acetylated peptide (m/z 1458) by the PMF search. The b-series ions, shown in blue in the MS/MS spectrum, were detected at masses 42 Da greater (molecular weight difference due to acetylation) than the theoretical values of the unmodified N terminus. In this way, we were able to easily verify acetylation of the N terminus of the peptide.

2DE profile

human hematopoietic HL-60 with insulin treatment

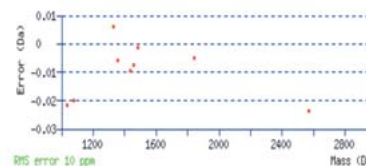


MS spectrum



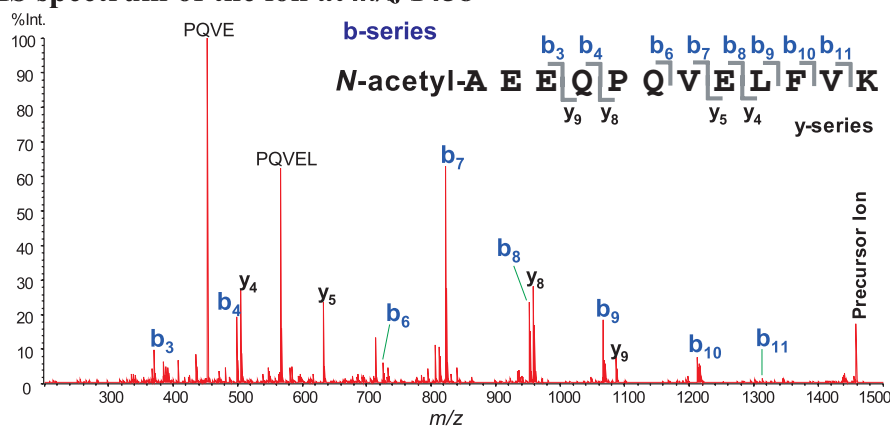
PMF (MASCOT™) result: Intracellular chloride ion channel (CLIC1)

Start - End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
1 - 12	1458.74	1457.73	1457.74	-0.01	0	- .AEEQPQVELVK.A N-Acetyl (Protein)
20 - 28	1078.49	1077.48	1077.50	-0.02	0	K.IGNCPFSQR.L
37 - 49	1437.76	1436.75	1436.76	-0.01	1	K.GVTFNVTIVDKR.R
95 - 112	1844.97	1843.96	1843.97	-0.01	0	K.LAALHPESHTAGLDIPAK.F
119 - 130	1328.65	1327.64	1327.64	0.01	0	K.NSHPALMDHLEK.G
138 - 164	2992.37	2991.36	2991.38	-0.02	0	K.VLDNYLTSPLPEEVDYSAEDEVGVSQR.K
193 - 203	1356.70	1355.69	1355.70	-0.01	1	K.YRGFTIPEAFR.G
193 - 203	1037.52	1036.51	1036.53	-0.02	0	R.GFTIPEAFR.G
193 - 207	1486.79	1485.78	1485.78	-0.00	1	R.GFTIPEAFRGVIR.Y
216 - 237	2573.10	2572.09	2572.12	-0.02	0	R.EEFASTCPDDEEIELAYEQVAK.A



RMS error 10 ppm

MS/MS spectrum of the ion at m/z 1458



JQA-0376