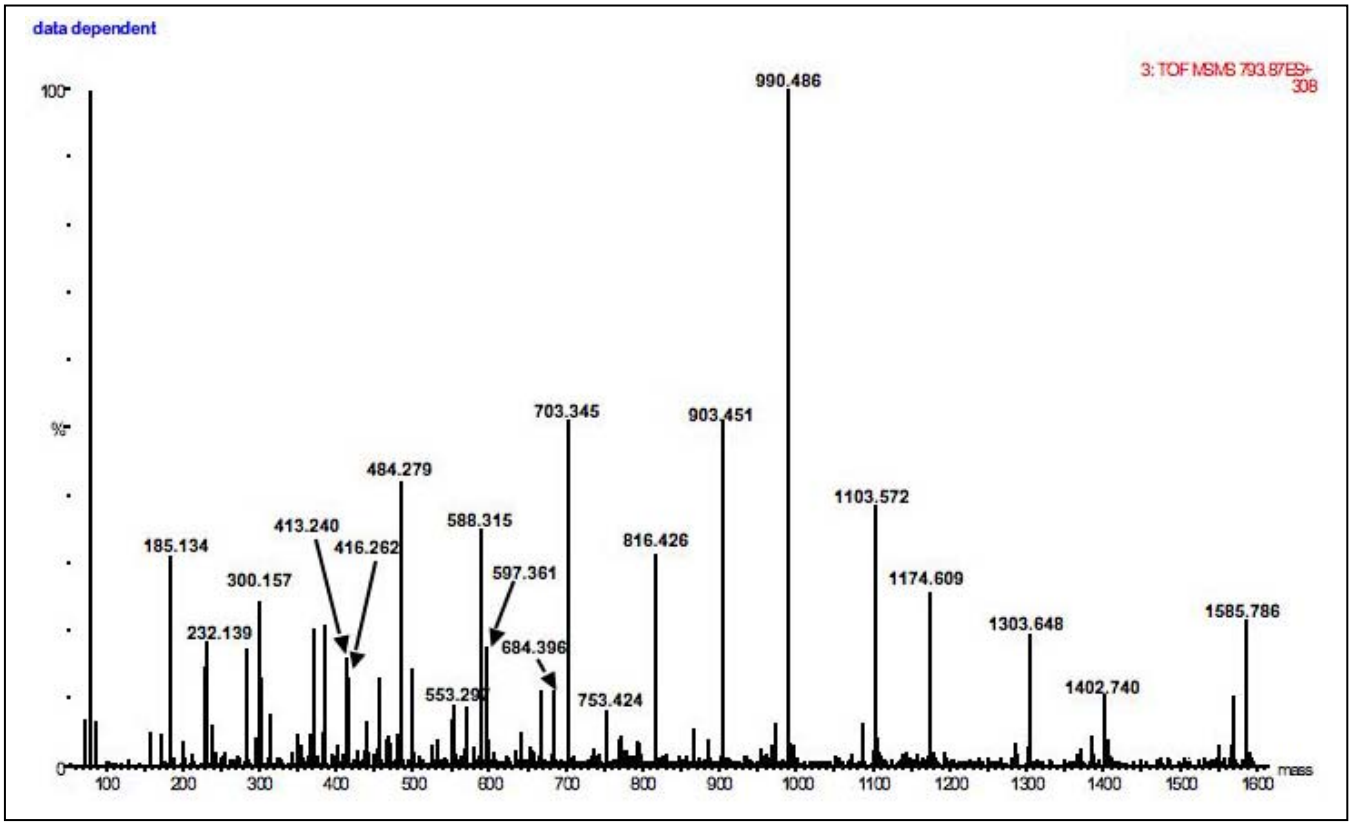
Interpretation of the tandem mass spectrum of peptide (Jan 19, 2007 class)



It was known that the peptide was from a protein in human brain, but it was not clear which protease had been used to generate it. A quick inspection of the data revealed that there were no fragment ions at *m/z* 147 or *m/z* 175. This seemed to rule out trypsin as the protease. The parent ion had an *m/z* of 793.87, implying a molecular weight of 1585.74 and a singly charged molecular ion of 1586.74.

Tandem mass spectra typically contain rich series of *b*-ions and *y*-ions because of collision-induced cleavages at the peptide bonds of the intact peptide. Not all *b*-ions and *y*-ions are observed – this is a function of the susceptibility of each peptide bond and the stability of the resulting ions.

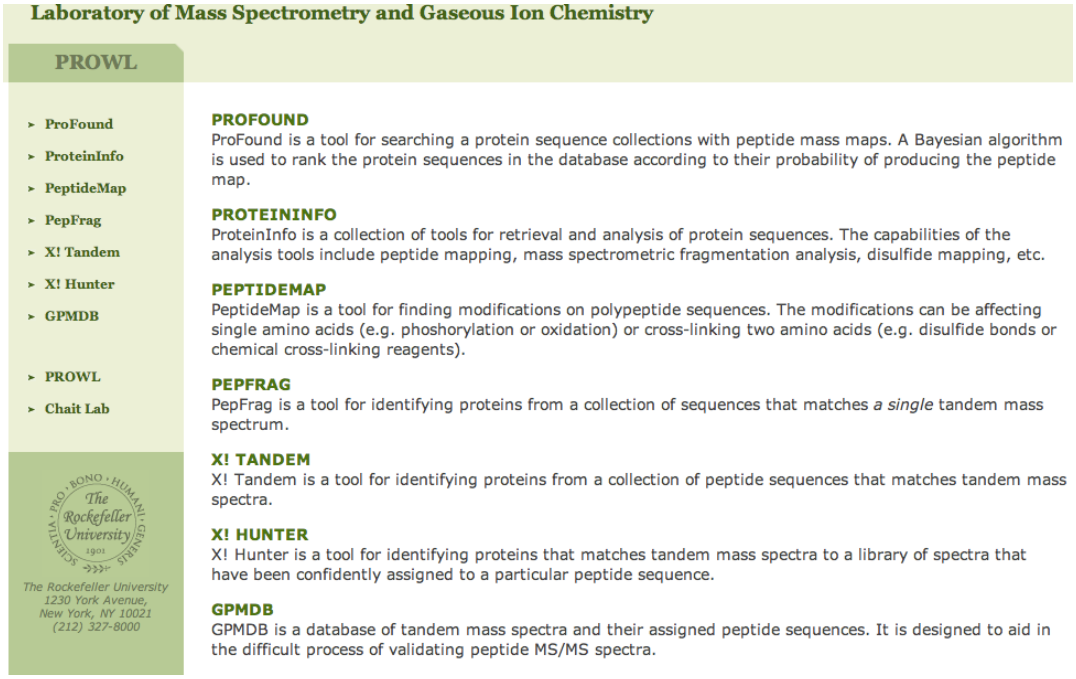
Interpretation of this tandem mass spectrum begins by examining the differences in mass between successive y-ions and matching them to the residue masses of the 20 amino acids (for this exercise we assume that there are no posttranslational modifications). The first pair is 1586.74-1402.74 = 184.00. This difference does not correspond to a single amino acid – see the table to the right - however, it could occur from a combination of proline and serine or isoleucine/leucine and valine. These two residues would be in positions 1 and

Alanine	71.037	Leucine	113.084
Arginine	156.101	Lysine	128.094
Asparagine	114.043	Methionine	131.040
Aspartic acid	115.027	Phenylalanine	147.068
Cysteine	103.009	Proline	97.053
Glutamic acid	129.043	Serine	87.032
Glutamine	128.058	Threonine	101.048
Glycine	57.021	Tryptophan	186.079
Histidine	137.059	Tyrosine	163.063
Isoleucine	113.084	Valine	99.068

2 in the peptide. It should be noticed that there is a corresponding b_2 ion at *m/z* 185. The ions at *m/z* 1402.74, 1303.65, 1174.61, 1103.57, 990.49, 903.45, 816.43, 703.35 and 588.32 are all *y*-ions. They have successive mass differences of 99.09, 129.04, 71.04, 113.08, 87.04, 87.02, 113.08 and 115.03. These correspond to the amino acids VEAI/LSSI/LD. Using *m/z* 588.32, the next *y*-ion was not obvious.

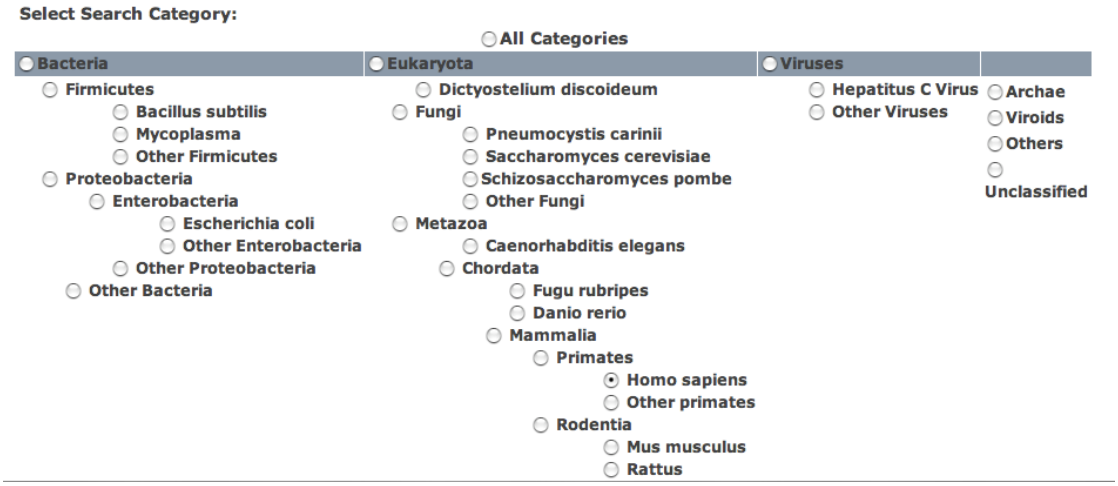
We noted that the *m/z* 185.13 ion was the b_2 ion. From examination of the other marked ions it appeared that the b_3 ion was at *m/z* 300.16. This implied that this was due to an aspartate. However, this didn't match the amino acid identified in the y-ion series, a valine. Thus, b_3 should be at *m/z* 284. In fact, there is an ion at that value – it's just not marked in the spectrum as given. The identification is reinforced by the ions at *m/z* 413.24, 484.28, 597.36 and 684.40. These have mass differences of 129.08, 71.04, 113.08 and 87.04 – thus the *b*-ions give us a sequence of VEAI/LS.

At this point, we have enough information to search the PROWL database at the Rockefeller University (http://prowl.rockefeller.edu)



Click on Proteininfo. This brings up a new page. In the box marked *Sequence entry* insert the sequence that we have derived so far – this is VEAI/LSSI/LD. Since we don't know which of the isoleucine/leucine combinations is the correct sequence, we replace them by X; so the sequence we enter is VEAXSSXD.

We now want to use the correct part of the database for our organism – this is human.



Press the *Select Sequence* button and the system responds by listing the likely candidates in the rat database. The best hit is to the same peptide sequence (VEALSSLD) in human brain-specific creatine kinase. The KIAA0641 protein has proline and glutamate and their masses do not correspond to those observed in the MSMS spectrum

PROTEININFO

Sequence: VEAXSSXD
Database: NCBInr
Category: Homo-sapiens
Max. Matches: 100

gi|7513108|pir||T00378 KIAA0641 protein - human

Matching: SSSPEVEAPSSEDEDTAE (Residues 686 - 693)

gi|21536286|ref|NP_001814.2| brain creatine kinase [Homo sapiens]
gi|125294|sp|P12277|KCRB_HUMAN Creatine kinase B-type (Creatine kinase B chain) (B-CK)
gi|29963|emb|CAA33389.1| creatine kinase B [Homo sapiens] gi|1000862|gb|AAA76852.1|
creatine kinase-B gi|12654701|gb|AAH01190.1| Creatine kinase, brain [Homo sapiens]
gi|13436215|gb|AAH04914.1| Creatine kinase, brain [Homo sapiens]
gi|14249888|gb|AAH08323.1| Creatine kinase, brain [Homo sapiens]
gi|14603055|gb|AAH10002.1| Creatine kinase, brain [Homo sapiens] gi|17939433...

Matching: IEKLAVEALSSLDGDLAG (Residues 159 - 166)

By examining the flanking regions of the matched sequence, we see that the two amino acids at the N-terminus of the peptide are LA, producing the b_2-ion of 185.12 (113.08 + 71.04 + 1). Thus, the sequence we have now is LAVEALSSLD.

The residues at the C-terminus shown in this search are GDLAG – they sum up to give (57.02 + 115.03 + 113.08 + 71.04 + 57.02 = 413.18). This value implies that there is

another amino acid residue(s) causing a mass difference of (588.32-[413.18 + 18 + 1] = 156.14, i.e., it's an arginine. So, now we have the full sequence of the peptide LAVEALSSLDGDLAGR and it is a tryptic peptide, even though the expected *m/z* 175.12 y_1 ion wasn't easily seen. The predicted ions for this peptide can be obtained by inserting the sequence into the box in MS-product, a part of Protein Prospector (at http://prospector.ucsf.edu/prospector/4.0.7/cgi-bin/msprod.cgi).

N-terminal ions																	
a ions	---	157.13	256.20	385.24	456.28	569.37	656.40	743.43	856.51	971.54	1028.56	1143.59	1256.67	1327.71	1384.73	---	
b-H_2O ions	---	---	---	395.23	466.27	579.35	666.38	753.41	866.50	981.53	1038.55	1153.57	1266.66	1337.69	1394.72	---	
b ions	---	185.13	284.20	413.24	484.28	597.36	684.39	771.42	884.51	999.54	1056.56	1171.58	1284.67	1355.71	1412.73	---	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	
H-	**L**	**A**	**V**	**E**	**A**	**L**	**S**	**S**	**L**	**D**	**G**	**D**	**L**	**A**	**G**	**R**	**-OH**
	16	**15**	**14**	**13**	**12**	**11**	**10**	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**	
C-terminal ions																	
y ions	---	1473.75	1402.72	1303.65	1174.61	1103.57	990.49	903.45	816.42	703.34	588.31	531.29	416.26	303.18	232.14	175.12	
y-NH_3 ions	---	1456.73	1385.69	1286.62	1157.58	1086.54	973.46	886.43	799.39	686.31	571.28	514.26	399.24	286.15	215.11	158.09	
y-H_2O ions	---	1455.74	1384.71	1285.64	1156.60	1085.56	972.47	885.44	798.41	685.33	570.30	513.28	---	---	---	---	