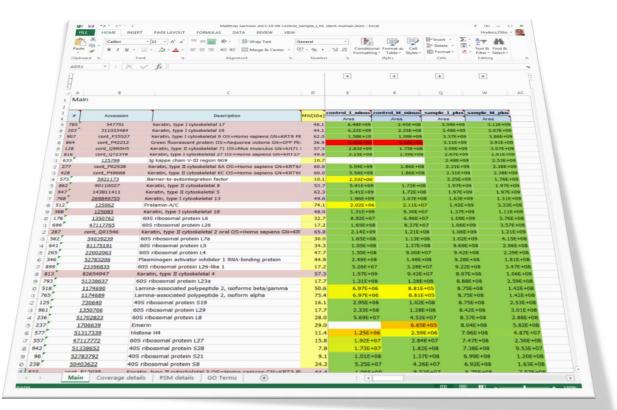
"How to read my .xlsm

mass spec result"





Please also visit our online compilation of FAQs

http://cores.imp.ac.at/protein-chemistry/faqs/

The xlsm file is structured in 6 tabs:

> Main: containing the list of all identified proteins

> Coverage Details showing the detailed sequence coverage of each identified protein

> **PSM Details** containing the list of all identified peptide spectrum matches (PSMs)

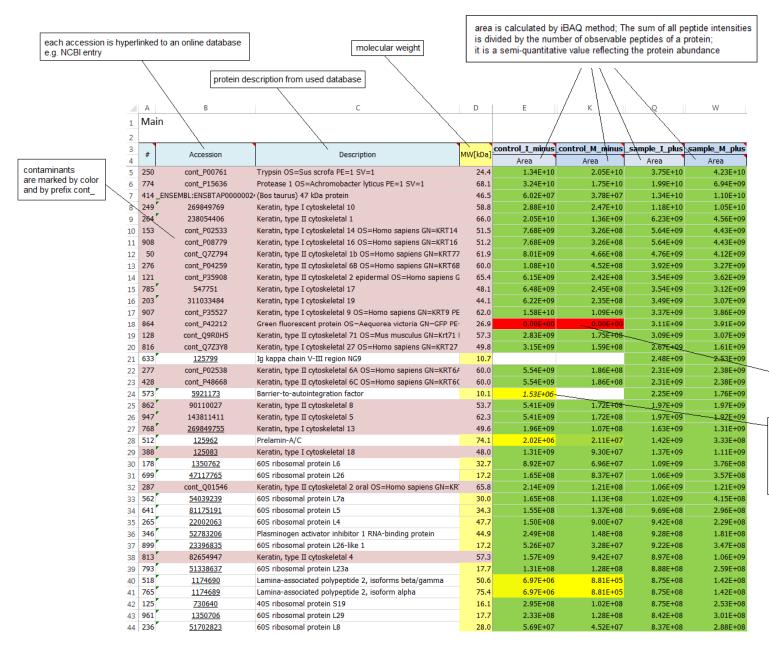
➢ GO Terms Gene Onthology analysis

> Quality Control containing quality control graphs

> Analysis Summary containing parameters used for database search

 Main
 Coverage details
 PSM details
 GO Terms
 Quality control
 Analysis summary

tab1 Main: containing the list of all identified proteins and their according accession number, description, molecular weight and precursor area



maximum
highest value

midpoint
percentile 50

Minimum
lowest value

Some proteins may appear with a red area and a value of 'zero'. This is due to the fact, that the protein in this sample consists only of shared peptides, but no "unique peptides.

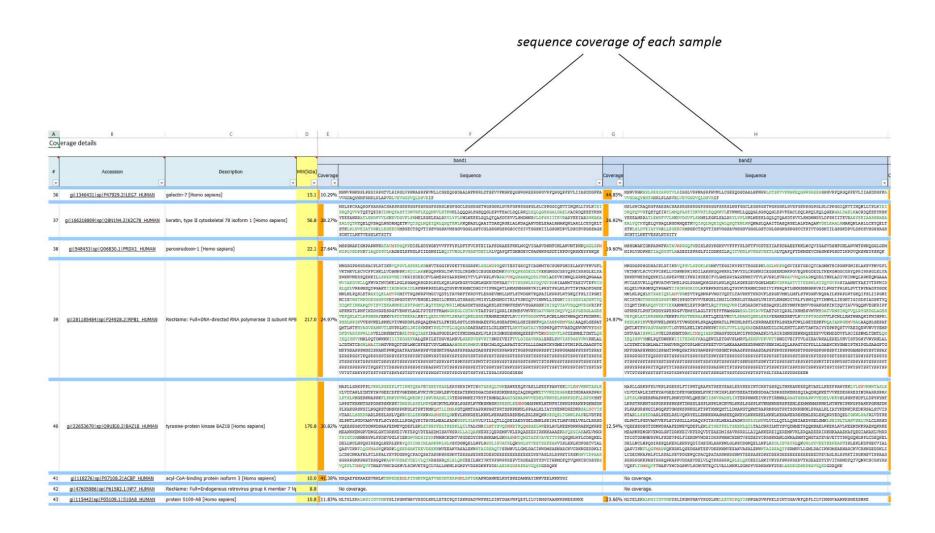
Some proteins may appear in italic. They are usually rather weak and they are not identified, but their mass traces have been found by a method called "match between runs". This approach takes the mass traces of identified peptides and compares them to other samples, where the corresponding protein has not been identified.

> tab1 Main:

containing the list of all identified *proteins* and their according accession number, gene name, description, molecular weight, sequence coverage, number of peptides, number of PSMs and precursor area



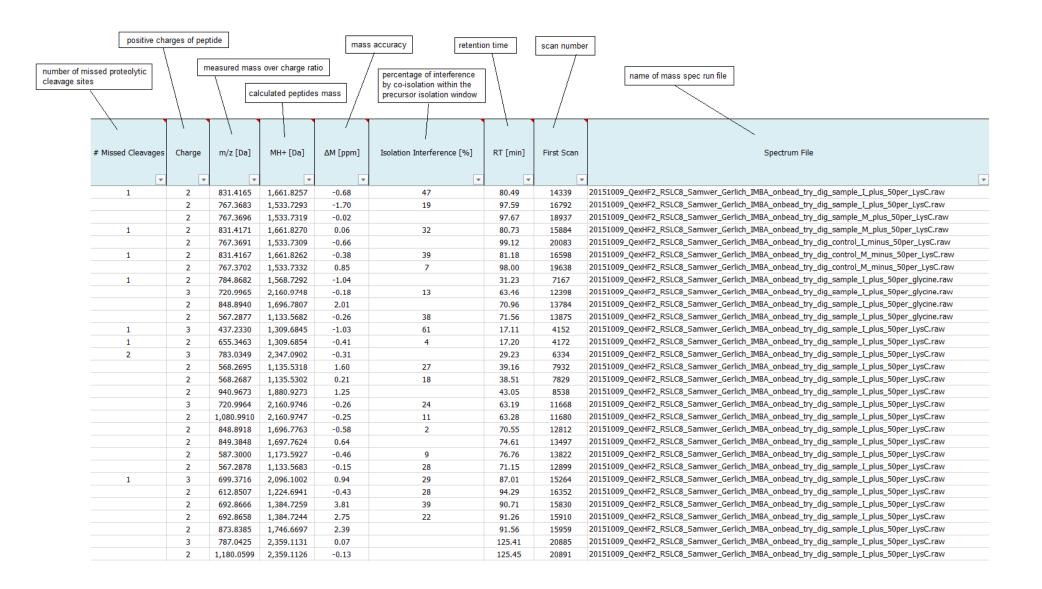
* tab2 Coverage details: showing the detailed sequence coverage of each protein covered regions are highlighted in green modified amino acids are highlighted in red



> tab3 PSM Details containing the list of all identified *PSMs* (peptide spectrum matches) and their related values

							quence of identifed peptide I highlighted amino acid inc		ication	precursor area		site probability of modified residue
protein accession from used database		protein description from used database	'x' marks the identification				1					(localization probability)
								probabilistic identificatio	c search engine on score] \	chemical or post-translational modification	
							1	`	\	1	\	/
#	Accessions	Protein descriptions	20151009_Qext#F.	2015±009_QexHF	20151009_QexHF2	20151009_QexHF2	Sequence		Amanda Score	Peakjuggler Area	Modifications	ptmRS: Best Site Probabilities
~		_	₹ 8 ₹	187	28 ₹	25		▼	_	~	▼	
1	116241284	Chromobox protein hom	nolog 3		X		KVEEAEPEEFVVEK		438.4	2E+06		
	116241284	Chromobox protein hom	nolog 3		X		VEEAEPEEFVVEK		266.6	7E+05		
	116241284	Chromobox protein hom	-				VEEAEPEEFVVEK		299.8	5E+05		
	116241284	Chromobox protein hom	-			X	KVEEAEPEEFVVEK		423.4	1E+06		
	116241284	Chromobox protein hom	-				VEEAEPEEFVVEK		211.9	7E+05		
	116241284	Chromobox protein hom	-	X			KVEEAEPEEFVVEK		493.2	2E+06		
	116241284	Chromobox protein hom	nolog 3	X			VEEAEPEEFVVEK		327.2	1E+06		
	189047131	La-related protein 4			X		TNAAAMNMGRPFQK		292.4	7E+05	M6(Oxidation); M8(Oxidation)	M6(Oxidation): 100; M8(Oxidation): 10
	189047131	La-related protein 4			X		SSGGSEHSTEGSVSLGDGQLNF	R	349.4	1E+06		
	189047131	La-related protein 4			X		ETSTLQVEQNGDYGR		375.9	1E+06		
	189047131	La-related protein 4			X		DLIEDSSVQK		180.1	1E+06		
12	189047131	La-related protein 4			X		ISRPHPSTAESK		161.0	7E+06		
	189047131	La-related protein 4			X		ISRPHPSTAESK		239.0	3E+06		
	189047131	La-related protein 4			X		NEDNGAPENSVEKPHEKPEAR		434.9	2E+06		
	189047131	La-related protein 4			X		SSPMVQVDEK		190.5	6E+06	M4(Oxidation)	M4(Oxidation): 100
16	189047131 189047131	La-related protein 4			X		SSPMVQVDEK		184.8	6E+06	M4(Oxidation)	M4(Oxidation): 100
17	_	La-related protein 4			X		HNPTVTGHQEQTYLQK		402.8	1E+06		
18	189047131 189047131	La-related protein 4			X		SSGGSEHSTEGSVSLGDGQLNF		325.6	9E+06		
	189047131	La-related protein 4			X		SSGGSEHSTEGSVSLGDGQLNF	K	403.3	2E+06		
	189047131	La-related protein 4			X		ETSTLQVEQNGDYGR		348.0	3E+06	N10(Desmidsted)	NI O/Desmidsted), OO FO
22	189047131	La-related protein 4 La-related protein 4			X		ETSTLQVEQNGDYGR MPGELVLENR		384.2 254.3	4E+06 1E+07	N10(Deamidated)	N10(Deamidated): 99.59
23	189047131	La-related protein 4			X		DLIEDSSVQK		203.3	2E+07	M1(Oxidation)	M1(Oxidation): 100
23	189047131	La-related protein 4			X		DGLNQTTIPVSPPSTTKPSR		285.4	2E+07 3E+06		
	189047131	La-related protein 4			X		EPSSVLVQPLR		184.5	2E+07		
26	189047131	La-related protein 4			X		EIPETTPIEEVK		229.8	8E+06		
	189047131	La-related protein 4			X		EIPETTPIEEVK		201.4	8E+06		
28	189047131	La-related protein 4			X		EYEVMYSSSCETTR		369.5	9E+05	M5(Oxidation); C10(Methylthio)	M5(Oxidation): 100
29	189047131	La-related protein 4			X		ASTASPCNNNINAATAVALQEE	PR	314.5	3E+05	C7(Methylthio)	mo(oxidation). 100
	189047131	La-related protein 4			X		ASTASPCNNNINAATAVALQEE		381.7	9E+05	C7(Methylthio)	
	189047131	La-related protein 4			X		LTTDPDLILEVLR		264.9	1E+06	er (meanyiano)	
32	189047131	La-related protein 4			~	Х	SSGGSEHSTEGSVSLGDGQLNF	R	278.5	1E+06		
	189047131	La-related protein 4					MPGELVLENR	-	262.6	2E+06	M1(Oxidation)	M1(Oxidation): 100

tab3 PSM Details containing the list of all identified PSMs (peptide spectrum matches) and their related values

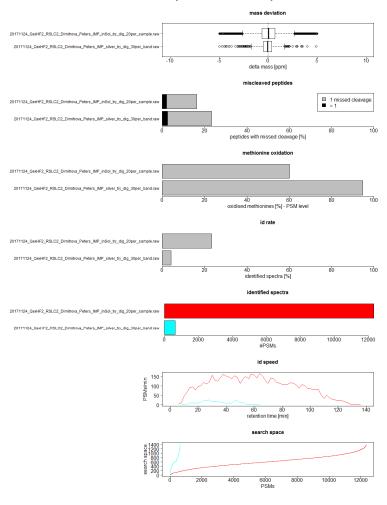


> tab4 GO terms Gene Onthology analysis

	A B one can simp and hit "apply and protein lis		Pfam IDs /	Molecular Function					Cellular Component					Biological Process							
	2 (Apply to main)							/										/	/		_
601	Terms			/			/							/				/			
#	Accession	Description	MW[kDa	Pfam IDs	structural molecule activity catalytic activity	receptor activity receptor activity receptor activity	transporter activity rucleotide binding RNA binding	enzy me regulator activity metal ion binding	signal transducer activity DNA binding antoxidant activity	translation regulator activity rucleus	 cymplasm Golgi membrane 	 cytoskeleton cytosol 	ellular extracellular	d endosome endosome	Corganelle lumen Corganelle lumen	A proteasome	cell organization and biogenesis	4 defanse response 4 metabolic process 6 regulation of biological process 6 possible defaults and the second seco	brocess transport development cell death	cellular homeostasis cell division	cell communication coagulation
1	gi 239938650 sp P35908.2 K22E HUMAN	keratin, type II cytoskeletal 2 epidermal [Homo sapiens]	65.4	Pf00038; Pf08614; Pf12128; Pf1316(>	×					x x	X X	X				x x					
2	gi 269849769 sp P13645.6 K1C10 HUMAN	RecName: Full=Keratin, type I cytoskeletal 10; AltName: Full	58.8	Pf00038; Pf04111 >	K					x x	X					x x					
3	gi 239938886 sp P35527.3 K1C9 HUMAN	keratin, type I cytoskeletal 9 [Homo sapiens]	62.0	Pf00038; Pf13166 >	K					X	X						X				
4	gi 238054406 sp P04264.6 K2C1 HUMAN	keratin, type II cytoskeletal 1 [Homo sapiens]	66.0	Pf00038; Pf01576; Pf05103; Pf0595;>	x x	X X X	(X	X :					X		(X X	X		
5	gi 229463044 sp P02533.4 K1C14 HUMAN	RecName: Full=Keratin, type I cytoskeletal 14; AltName: Full	51.5	Pf00038; Pf13514	K	X				X X		×				X X	X				
6	gi 143811411 sp P13647.3 K2C5 HUMAN	keratin, type II cytoskeletal 5 [Homo sapiens]	62.3	Pf00038; Pf01576; Pf02994; Pf0396:>	x x x	X				X X		X >	K				X				
7	gi 23503075 sp P08779.4 K1C16 HUMAN	keratin, type I cytoskeletal 16 [Homo sapiens]	51.2	Pf00038; Pf07888; Pf13514	K	X				X		X				x x x x	X X				
8	gi 6166599 sp P35579.4 MYH9 HUMAN	RecName: Full=Myosin-9; AltName: Full=Cellular myosin hei	226.4	Pf00063; Pf01442; Pf01496; Pf01576	X X	X X	(X X			X X	X :	X X				x x x	X	X X	X		
9	gi 215274129 sp P35580.3 MYH10 HUMAN	myosin-10 isoform 2 [Homo sapiens]	228.9	Pf00063; Pf00769; Pf01496; Pf01570	XX	X 2	(X			X X	X :	x >	X			x x x	X	X X	X		
10	gi 118177 sp P01040.1 CYTA_HUMAN	cystatin-A [Homo sapiens]	11.0	Pf00031 >		X		X		X X		X				X		X X			
11	gi 20141302 sp P81605.2 DCD_HUMAN	dermcidin isoform 1 preproprotein [Homo sapiens]		Pf15291	X		X						×			X		X			
12	gi 1346344 sp P02538.3 K2C6A_HUMAN	keratin, type II cytoskeletal 6A [Homo sapiens]			X X					X	X					x x		X			
13	gi 34098662 sp Q92576.3 PHF3_HUMAN	PHD finger protein 3 isoform 1 [Homo sapiens]		Pf00628; Pf07500; Pf07744		X		X		X								X	X		
14	gi 391358178 sp P0CG48.3 UBC_HUMAN	polyubiquitin-C [Homo sapiens]		Pf00240; Pf11976; Pf13019; Pf1388:		X	X			X X	X	X				X	X X	(X X	X X	X	
15	gi 238054404 sp P04259.5 K2C6B_HUMAN	keratin, type II cytoskeletal 6B [Homo sapiens]		Pf00038; Pf01576; Pf04111; Pf1316()																	
16	gi 133061 sp P05387.1 RLA2_HUMAN	60S acidic ribosomal protein P2 [Homo sapiens]		Pf00428 >			X				X	X	X				X	X	X		
17	gi 145559510 sp Q14980.2 NUMA1 HUMAN	nuclear mitotic apparatus protein 1 isoform 1 [Homo sapiens		Pf04111; Pf07888; Pf12128 > Pf00038; Pf07888 >		X				X X		XX		X		X	X	×		X	
18 19	gi 547751 sp 004695.2 K1C17 HUMAN	keratin, type I cytoskeletal 17 [Homo sapiens] RecName: Full=Glyceraldehyde-3-phosphate dehydrogenase		Pf00038; Pf07888 > Pf00044; Pf02800	X	x x	X	×		X X		v v					X	X X	X		
20	gi 120649 sp P04406.3 G3P_HUMAN gi 46397316 sp P60711.1 ACTB_RAT	RecName: Full=Actin, cytoplasmic 1; AltName: Full=Beta-act		Pf00022	^	X	X		X	XX							X	^ ^	X		
21	gi 135773 sp P10599.3 THIO HUMAN	thioredoxin isoform 1 [Homo sapiens]		Pf00085; Pf00462; Pf13098; Pf13899	X	×	X		^	X X		X >	v v			x x x		(x x	v	X 2	,
22	gi 12585257 sp 099878.3 H2A1J HUMAN	histone cluster 1, H2aj [Homo sapiens]		Pf00125; Pf00808		×	^		×	X		^ /		X		^ ^ ^	X		^	^ /	A*
23	gi 51315707 sp Q6WV72.3 H4 MYTTR	histone H4 [Homo sapiens]		Pf00125; Pf02969; Pf15630	X	×	X		X	X	×	x	X	X		Y	X	x x			
24	gi 215273944 sp Q9NZT1.2 CALL5 HUMAN	calmodulin-like protein 5 [Homo sapiens]		Pf00036; Pf12763; Pf13202; Pf13405	-		^	×	53.0	0				(**		X		X			
25	gi 115444 sp P06702.1 S10A9 HUMAN	protein S100-A9 [Homo sapiens]		Pf00036; Pf01023; Pf13499; Pf1383:	X	×		XX	X	хх	X	x x	X					(X X	X	1	<
26	gi 113950 sp P07355.2 ANXA2 HUMAN	annexin A2 isoform 2 [Homo sapiens]		Pf00191		×	×	XX		X X			X	x x			X	X			
27	gi 6685537 sp Q14152.1 EIF3A HUMAN	eukaryotic translation initiation factor 3 subunit A [Homo sap		Pf01399 >	K	X	X			X X		х					X	x x			
28	gi 122028 sp P10854.2 H2B1M MOUSE	histone H2B type 1-D [Homo sapiens]		Pf00125; Pf00808		X			×	X				X			X				
29	gi 12231007 sp P31944.2 CASPE_HUMAN	caspase-14 precursor [Homo sapiens]		Pf00656	X					XX						x x		x x	X		
30	gi 125145 sp P01834.1 IGKC HUMAN	RecName: Full=Ig kappa chain C region, partial [Homo sapie		Pf07654		X					X		х			X		(x x			
31	gi 308153590 sp Q7Z794.3 K2C1B HUMAN	keratin, type II cytoskeletal 1b [Homo sapiens]		Pf00038; Pf03962; Pf13166; Pf1481;>	K							X									
32	gi 327478526 sp Q7Z406.2 MYH14 HUMAN	myosin-14 isoform 2 [Homo sapiens]	227.7	Pf00063; Pf01576	x x	X	×			×	X					x x	х	x x			
33	gi 269849755 sp P13646.4 K1C13 HUMAN	RecName: Full=Keratin, type I cytoskeletal 13; AltName: Full		Pf00038 >	<					X		X				X	X				
34	gi 353526311 sp P60174.3 TPIS HUMAN	triosephosphate isomerase isoform 2 [Homo sapiens]		Pf00121	X	×				X		X						×	x		
35	gi 1705916 sp Q00610.5 CLH1 HUMAN	clathrin heavy chain 1 isoform 1 [Homo sapiens]		Pf00637; Pf01394; Pf04053; Pf0926()	K	×	Х			X	×	x >	X			x x x	X	x x	x		
36	gi 1346431 sp P47929.2 LEG7 HUMAN	galectin-7 [Homo sapiens]	15.1	Pf00337						x x			x						X		

> tab 5 Quality Control plots:

- mass deviation missed cleavages methionine oxidation
- ID rate identified spectra ID speed search space correlation plots



> tab 6 Analysis summary

containing parameters used for database search useful for materials & methods section of a manuscript

For peptide identification, the RAW-files were loaded into Proteome Discoverer (version 2.1.0.81, Thermo Scientific).

All hereby created MS/MS spectra were searched using MSAmanda v2.0.0.9849 (Dorfer V. et al., J. Proteome Res. 2014 Aug 1;13(8):3679-84).

For the 1st step search the RAW-files were searched against the human swissprot database, using following search parameters:

The peptide mass tolerance was set to ±5 ppm and the fragment mass tolerance to 15ppm.

The maximal number of missed cleavages was set to 2.

The result was filtered to 1 % FDR on protein level using Percolator algorithm integrated in Thermo Proteome Discoverer.

A sub-database was generated for further processing.

For the 2nd step the RAW-files were searched against the created sub-database called subdb_20171124_QexHF2_RSLC2_Dimitrova_Peters_IMP_inSol_try_dig_20per_sample_and_band_hsSorrorin_Sf9_Ecoli_conta_step1.fasta 1,057 sequences; 782,301 residues).

The following search parameters were used:

Beta-methylthiolation on cysteine was set as a fixed modification, oxidation on methionine, deamidation on asparagine and glutamine, acetylation on lysine, phosphorylation on serine, threonine and tyrosine, methylation on lysine and arginine, di-methylation on lysine and arginine, tri-methylation on lysine, ubiquitinylation residue on lysine were set as variable modifications.

Monoisotopic masses were searched within unrestricted protein masses for tryptic enzymatic specificity.

The peptide mass tolerance was set to ± 5 ppm and the fragment mass tolerance to ± 15 ppm.

The maximal number of missed cleavages was set to 2.

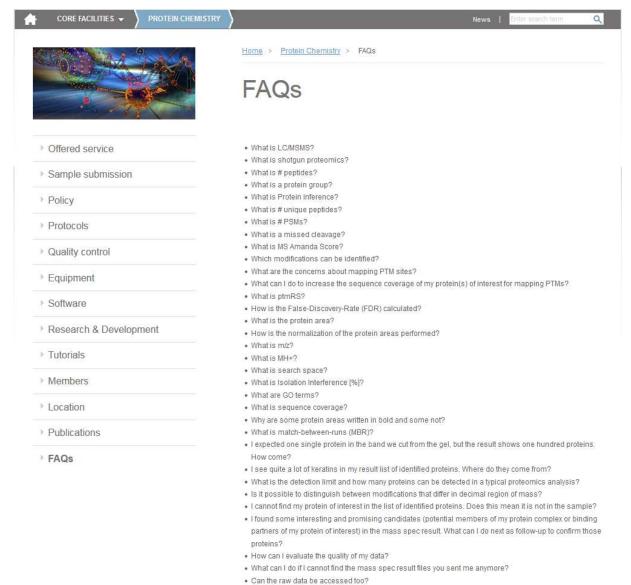
The result was filtered to 1% FDR on peptide level using Percolator algorithm integrated in Thermo Proteome Discoverer.

The localization of the post-translational modification sites within the peptides was performed with the tool ptmRS (Taus T. et al., J. Proteome Res. 2011, 10, 5354-62).

Peptide areas have been quantified using Peakjuggler.

We also would like to draw attention to our online compilation of FAQs, which has proven helpful in better understanding of the mass spec result:

http://cores.imp.ac.at/protein-chemistry/faqs/



Please Notice This

