

Modifications

MASCOT

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Types of Modifications

Post-translational

- Phosphorylation, acetylation

Artefacts

- Oxidation, acetylation

Derivatisation

- Alkylation of cysteine, ICAT, SILAC

Sequence variants

- Errors, SNP's, other variants.

Modifications are a very important topic in database searching.

In some cases, the main focus of a study is to characterise post translational modifications, which may have biological significance. Phosphorylation would be a good example.

In other cases, the modification may not be of interest in itself, but you need to allow for it in order to get a match. Oxidation during sample preparation would be an example.

And, of course, many methods of quantitation involve modifications containing isotopic labels

Some sequence variants, such as the substitution of one residue by another, are equivalent to modifications, and can be handled in a similar way

Unimod protein modifications for mass spectrometry

Logged as **unimod** Log out Change password Advanced search

Search for: Any field Contains [] Search Details found: 633 Records Per Page: 20

Select/Unselect all Delete selected

	Accession #	PSI-MS Name	Interim name	Description	Monoisotopic mass	Average mass	Composition
Edit Copy View	40	Sulfo	Sulfation	O-sulfonation	79.956815	80.0632	O(3) S
Edit Copy View	21	Phospho	Phospho	Phosphorylation	79.966331	79.9799	H O(3) P
Edit Copy View	549		Cys->Trp	Cys->Trp substitution	83.070128	83.0670	H(5) C(8) N S(-1)
Edit Copy View	211	NEIAA	NEIAA-d0	N-ethyl iodacetamide-d0	85.052764	85.1045	H(7) C(4) N O
Edit Copy View	747		Malinyl	Malinylation of C and S residues	86.000294	86.0462	H(2) C(3) O(3)
Edit Copy View	371	HPVK	HPVK86	Michael addition of hydroxymethylvinyl ketone to cysteine	86.036779	86.0892	H(6) C(4) O(2)
Edit Copy View	324	DTBP	DTBP	dimethyl 3,3'-dithiobispropionimidate	87.014270	87.1435	H(5) C(3) N S
Edit Copy View	178	DAET	ser_thr_DAET	phosphorylation to amine thiol	87.050655	87.1866	H(9) C(4) N O(-1) S
Edit Copy View	379	Hypusine	hypusine	hypusine	87.068414	87.1204	H(9) C(4) N O
Edit Copy View	126	Thioacyl	DSP	thioacylation of primary amines (N-term and Lys)	87.990285	88.1283	H(4) C(3) O S
Edit Copy View	185	Label-13C(9) alphaPhospho	13C9_Phospho_Tyr	C13 label (Phosphotyrosine)	88.996524	88.9138	H C(-9) 13C(9) O(3) P
Edit Copy View	212	NEIAA-2H(3)	NEIAA-d5	N-ethyl iodacetamide-d5	90.084148	90.1353	H(2) 2H(3) C(4) N O
Edit Copy View	724		O-Methylphosphate	O-Methylphosphorylation	93.901981	94.0065	H(3) C O(3) P

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Comprehensive and accurate information about post translational and chemical modifications is an essential factor in the success of protein identification. In Mascot, we take our list of modifications from Unimod, which is an on-line modifications database.

UNIMOD protein modifications for mass spectrometry
Unimod, View record [Accession #: 56]

[Back to list](#)

Accession #	56	PSI-MS Name	Acetyl-Lysine	Interim Name	Acetyl_Lysine
Description	Acetate labeling reagent (N-term & K) (heavy form, +3amu)				
Alt. Description	N-Tri-deuteriumacetate				
Composition	C(1) 2H(1) C(2) 0	Monoisotopic	45.029395	Average	45.0552
Specificity Definition 1					
Site	K	Position	Anywhere	Classification	Isotopic label
Hidden	1	Group	1		
Specificity Definition 2					
Site	N-term	Position	Any N-term	Classification	Isotopic label
Hidden	1	Group	2		
Notes and References					
Source	PubMed	Reference	11957757		
Source	PubMed	Reference	11999733		
Source	PubMed	Reference	12175151		
Source	Journal	Reference	Controlling Deuterium isotope effects in comparative proteomics. Zhang, Roujian; Soma, Cathy S.; Thompson, Robert A.; Kiong, Li; Regnier, Fred E. Department of Chemistry, Purdue University, West Lafayette, IN, USA. Analytical Chemistry 72		
Source	Journal	Reference	Global internal standard technology for comparative proteomics. Chakraborty, Anish; Regnier, Fred E. Department of Chemistry, Purdue University, West Lafayette, IN, USA. Journal of Chromatography, A (2002), 949(1-2), 173-184.		
Source	Journal	Reference	Comparative proteomics based on stable isotope labeling and affinity selection. Regnier, Fred E.; Riggs, Larry; Zhang, Roujian; Kiong, Li; Liu, Peiran; Chakraborty, Anish; Sealey, Eric; Soma, Cathy; Thompson, Robert A. Department of Chemistry, Pu		
Curator	jenner	Last Modified	2006-10-16 10:02:50	Verified	Yes

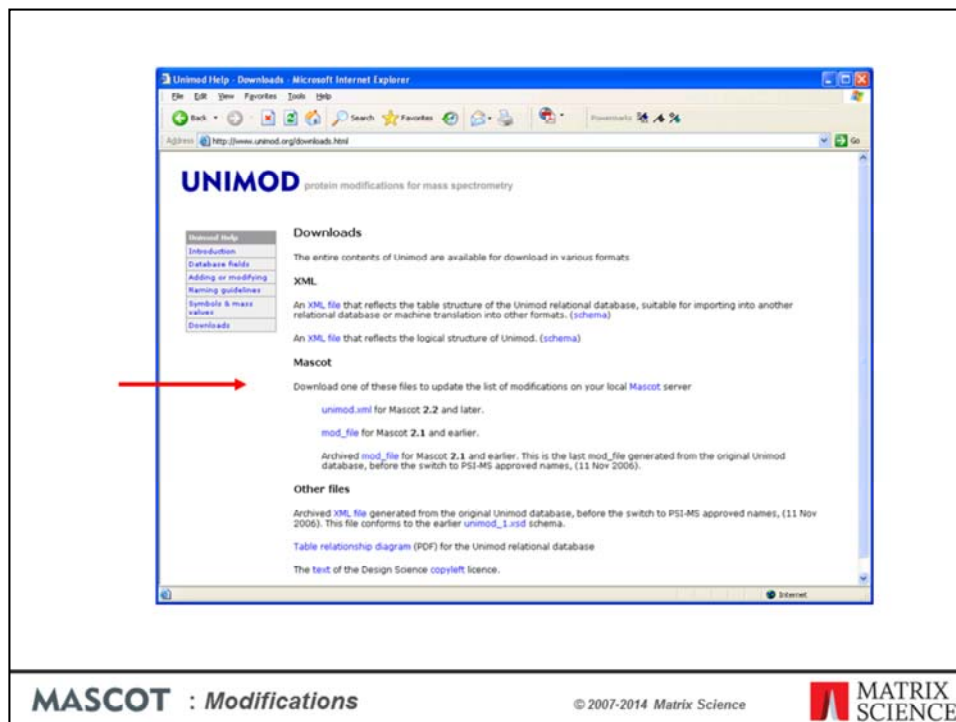
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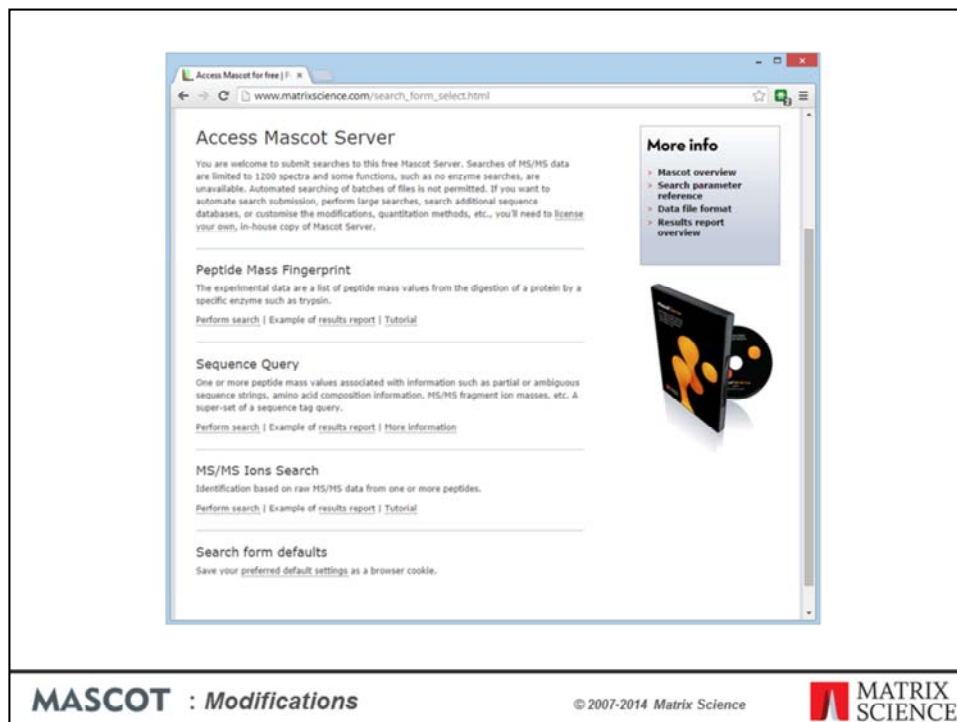


There are other lists of modifications on the web, like DeltaMass on the ABRF web site and RESID from the EBI, but none is as comprehensive as Unimod

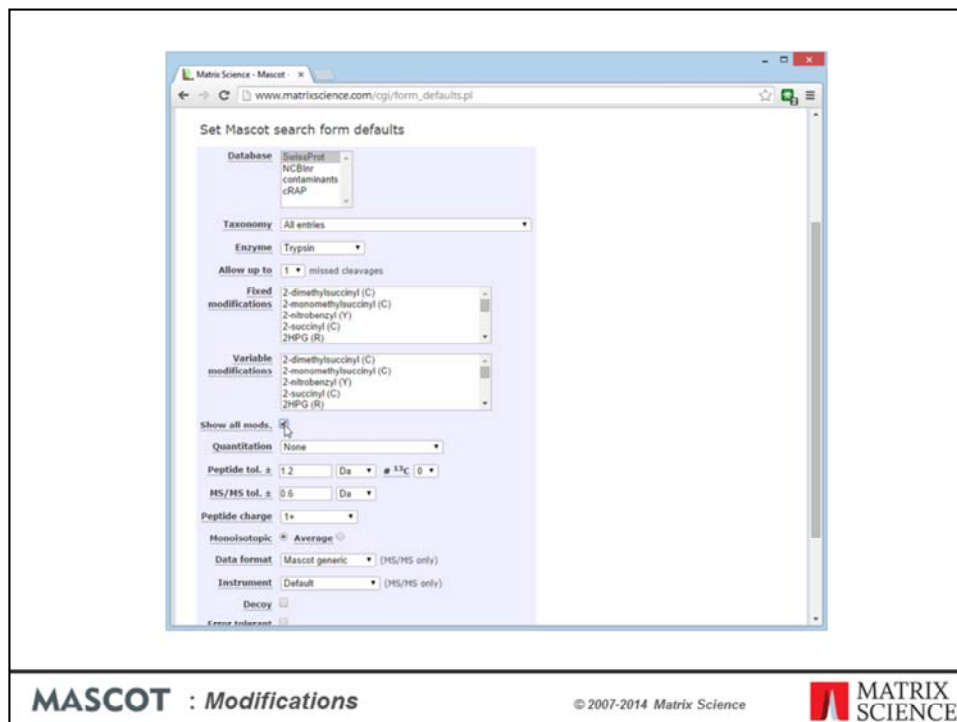
Mass values are calculated from empirical chemical formulae, eliminating the most common source of error. Specificities can be defined in ways that are useful in database searching, and there is the option to enter mass-spec specific data, such as neutral loss information. This screen shot shows one of the better annotated entries, I can't pretend that all of them are this detailed. Nevertheless, it is a very useful, public domain resource that beats having to create your own list in an Excel spreadsheet or on the back of an envelope.



If you go to the help page, there is a link to download the contents of Unimod as a Mascot modifications file. This is the easiest way to keep the modifications list on an in-house Mascot server up-to-date



Here is a tip. The default list of modifications displayed in the Mascot search form is a short list, containing only the most common mods. If you want to see the complete list of mods, and you are using Mascot 2.2 or earlier, you need to follow the link at the bottom of the search form selection page



Check the box for Show all mods, then choose Save. This still sets the default state of the checkbox in Mascot 2.3, but we decided to place the checkbox on the search form, so as to make it easier to swap between the short and long lists.

Be sparing with variable modifications

Some modifications are worse than others

- Mods that affect a terminus are less of a problem, e.g. Pyro-glu
- Mods that apply to residue(s) with a high fractional abundance and at any position are BIG problem, e.g. Phospho (ST) = 13%

Use an error tolerant search to pick up uncommon modifications

- Efficient
- Also catch non-specific peptides

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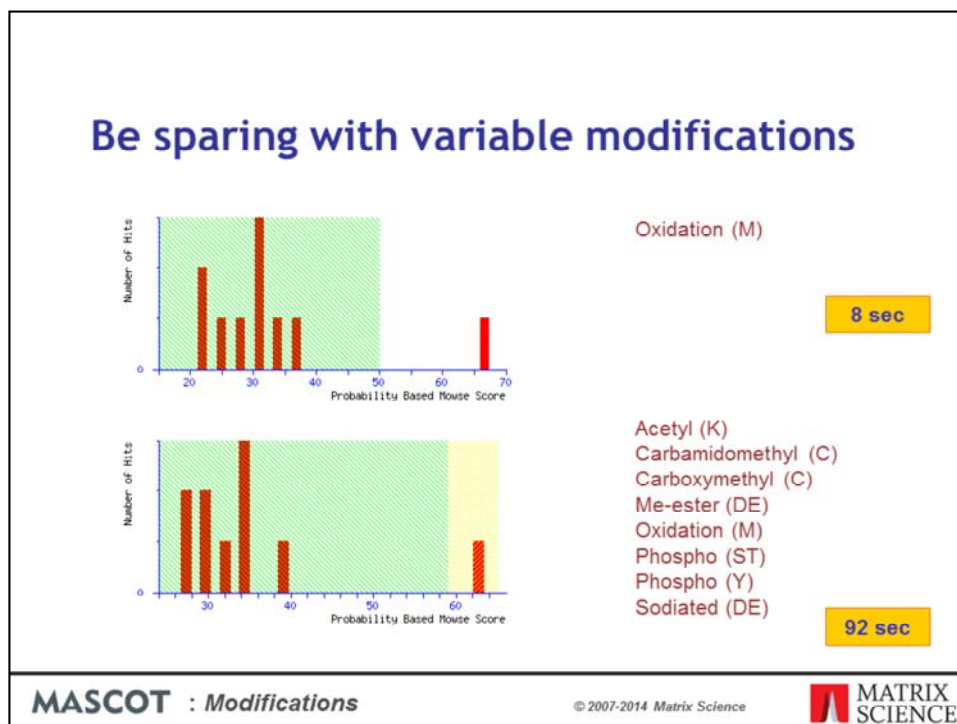


It is extremely important that you do not choose more than the absolute minimum number of variable modification in a search. We talked about this in an earlier presentation, but it is worth repeating.

Variable or differential or non-quantitative modifications are expensive, in the sense that they increase the time taken for a search and reduce its specificity. This is because the software has to permute out all the possible arrangements of modified and unmodified residues that fit to the peptide molecular mass. As more and more modifications are considered, the number of combinations and permutations increases geometrically. The so-called combinatorial explosion.

Some variable modifications are worse than others. Modifications that only apply to a terminus, especially if they only apply when particular residue is at the terminus, like pyro-glu, make little difference to the number of peptides to be tested. The problem modifications are the ones that apply to residues in any position, especially if they apply to multiple residues, like phosphorylation.

Unless you have enriched the sample in a particular PT-mod, e.g IMAC for phosphopeptides, it is usually not a good idea to try and catch PT-mods in a first pass search. Better to use a second pass search, which we call an error tolerant search, to catch the low abundance mods. We will come back to this later.



To illustrate this point. This search of a single MS/MS spectrum, using one variable mod, gives a nice, statistically significant match.

If the search is repeated with 8 mods, the match is the same, with an identical score, but now it is barely significant.

All of these mods have effectively increased the size of the database by a factor of 30

What's worse, the search takes over 10 times as long!

So, use variable mods sparingly. You'll get better results and faster.

By the way, the yellow region in the histogram indicates scores above the homology but below the identity thresholds. You will only see these regions highlighted in an MS/MS search report if it is a search of a single spectrum.

Why is phosphorylation such a challenge?

Site heterogeneity

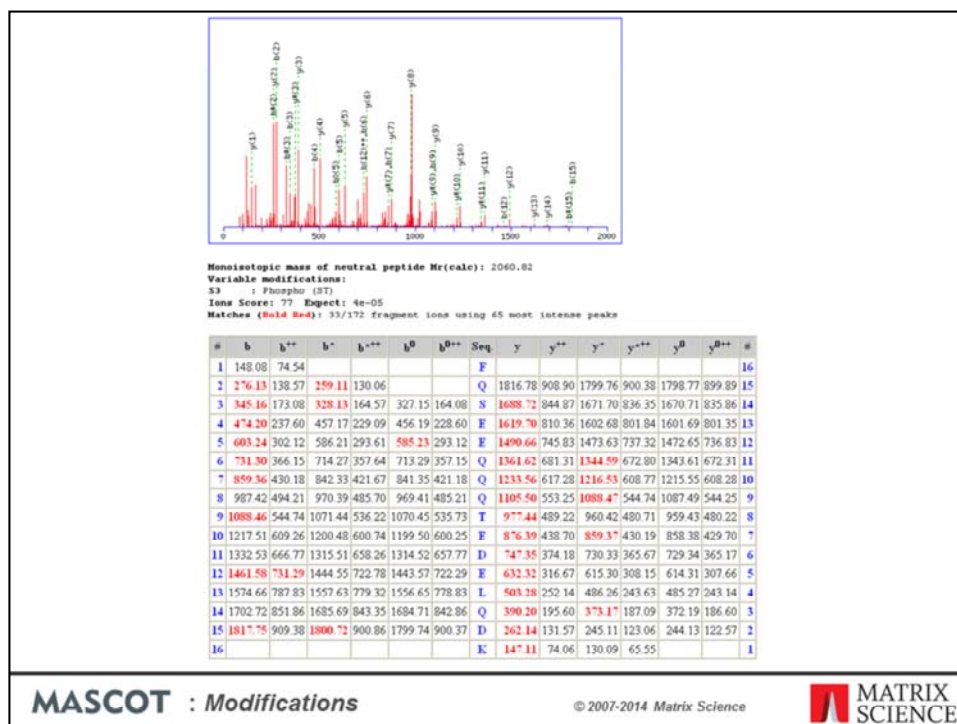
Poor ionisation efficiency

3 fragmentation channels

- intact fragments
- neutral loss of HPO_3 (80 Da)
- neutral loss of H_3PO_4 (98 Da)

Can occur at STY - ~16% of residues.

Of all post-translational modifications, phosphorylation is one of the most interesting and also one of the most difficult. Why is it such a challenge?



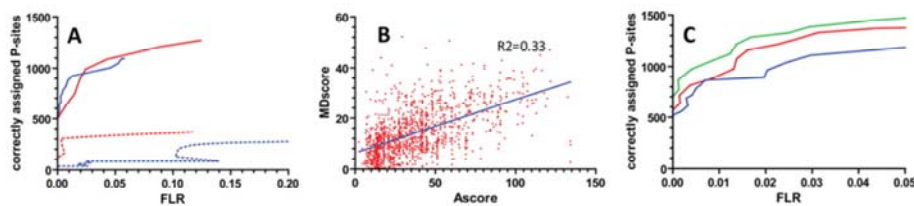
Beautiful spectrum; long run of y ions; move site to T9 and many matches would disappear

Confident Phosphorylation Site Localization Using the Mascot Delta Score[®]

Mikhail M. Savitski[‡], Simone Lemeer[§], Markus Boesche[‡], Manja Lang[‡],
Toby Mathieson[‡], Marcus Bantscheff^{‡||}, and Bernhard Kuster^{§||}

Molecular & Cellular Proteomics 10.2

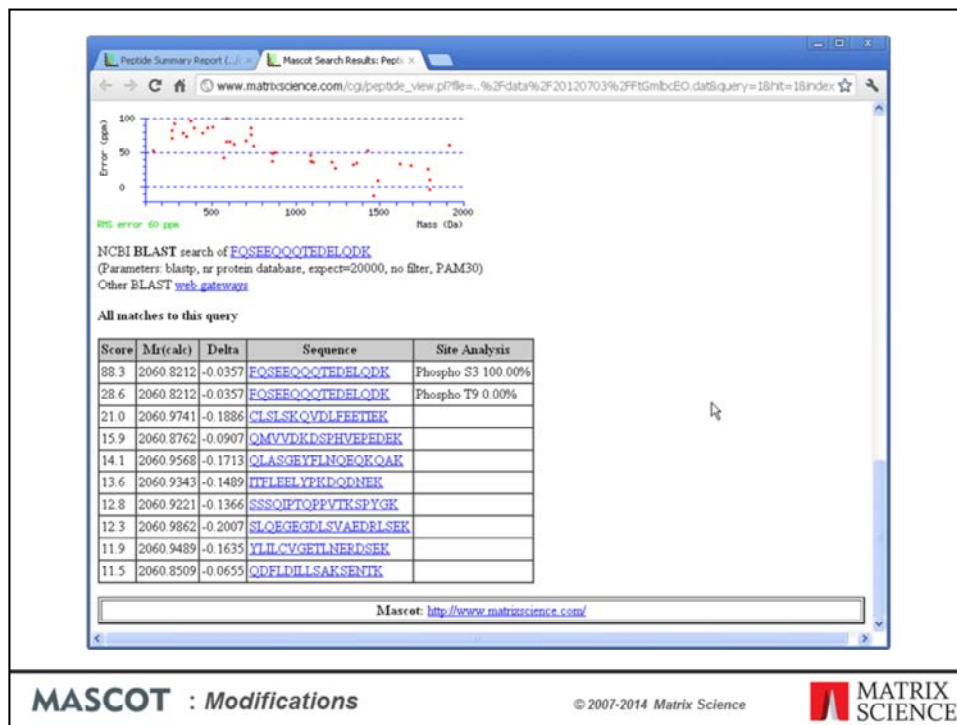
10.1074/mcp.M110.003830-1



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Mascot 2.4 reports site localisation probabilities using the delta score method published in MCP by Bernard Kuster's group. They analysed a collection of synthetic analogs of real phosphopeptides and determined what score difference was required to determine the correct site with an error rate of (say) 5%. Because we don't expect everyone to calibrate their data in this way, we have made the calculation slightly more conservative. A score difference of 10 would give approximately 90% probability that the higher scoring arrangement was correct.



A very large score difference such as the one we were just looking at gives 100% likelihood that the phosphate is on S3.

Peptide Summary Report

Format As: Peptide Summary [Help](#)

Significance threshold $p < 0.05$ Max. number of hits: 20

Standard scoring ☒ MudPIT scoring ☐ Ions score or expect cut-off: 0 Show sub-sets: 0

Show pop-ups: ☒ Suppress pop-ups ☐ Sort unassigned: Decreasing Score ☒ Require bold red ☐

Select All Select None Search Selected ☐ Error tolerant

1. **KAPCA_BOVIN** Mass: 40594 Score: 79 Matches: 1(1) Sequences: 1(1)
 cAMP-dependent protein kinase catalytic subunit alpha OS=Bos taurus GN=PRKACA PE=1 SV=3
☐ Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
<input checked="" type="checkbox"/>	1187.9039	2213.7933	2214.0683	-0.2750	0	80	8.5e-06	1	U	R.IWTLGOTPEYLAPKILSK.G + Ph

Top scoring peptide matches to query 1
 Score greater than 30 indicates homology
 Score greater than 42 indicates identity

Protein	Score	Expect	Delta	Hit	Protein	Peptide	Protein	Peptide
KAPCA	80.4	8.5e-06	-0.2750	1	KAPCA_BOVIN	R.IWTLGOTPEYLAPKILSK.G	PRKACA	PE=2 SV=3
KAPCA	76.9	1.9e-05	-0.2750	1	KAPCA_BOVIN	R.IWTLGOTPEYLAPKILSK.G	PRKACA	PE=2 SV=2
KAPCA	38.7	0.13	-0.2750	1	KAPCA_BOVIN	R.IWTLGOTPEYLAPKILSK.G		
KAPCA	18.0	15	-0.2750	1	KAPCA_BOVIN	R.IWTLGOTPEYLAPKILSK.G		
cAMP	12.6	51	-0.2111	3	GSA_XYLF	K.GGSGHNLGLGIPSPQVPAELSK.L	CA	PE=1 SV=2
KAPCA	12.6	51	-0.2111	3	GSA_XYLF	K.GGSGHNLGLGIPSPQVPAELSK.L		
cAMP	12.6	51	-0.2111	2	GSA_XYLF	K.GGSGHNLGLGIPSPQVPAELSK.L	CA	PE=1 SV=3
KAPCA	11.9	61	-0.2111	3	GSA_XYLF	K.GGSGHNLGLGIPSPQVPAELSK.L		
cAMP	11.9	61	-0.2111	3	GSA_XYLF	K.GGSGHNLGLGIPSPQVPAELSK.L		PE=1 SV=4

Mass: 40594 Score: 79 Matches: 1(1) Sequences: 1(1)

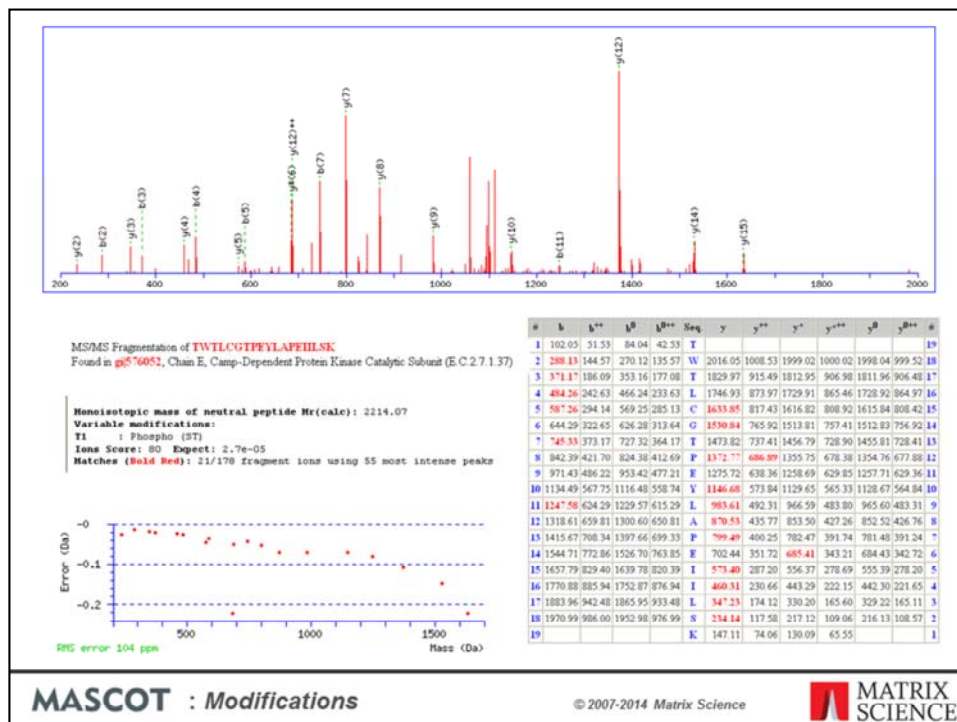
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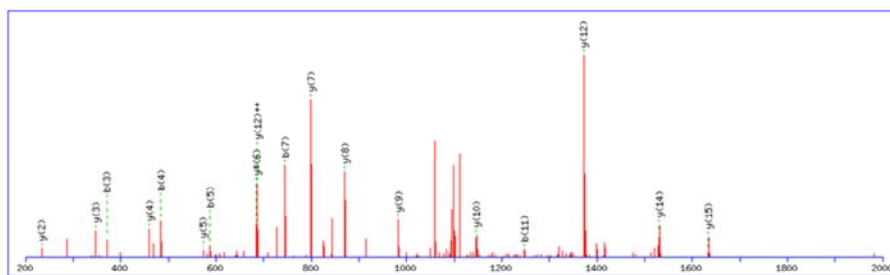
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However, casein peptides are unusually easy to analyse. Here is a more typical example of what you can expect to find - a strong match to a phosphopeptide from a protein kinase.

There is little to choose in terms of score between having the phosphate on T1 or T3.



We can see why there is little difference in score between placing the phosphate on T1 or T3. There is just one extra matched peak, and in probability terms, there isn't a huge difference between 20 matches using 55 experimental peaks and 21. However, if you had to choose one or the other, you'd probably go for T1



MS/MS Fragmentation of **TWTLCGTPEVLAPHLSEK**
 Found in **g576052**, Chain E, Camp-Dependent Protein Kinase Catalytic Subunit (E.C.2.7.1.37)

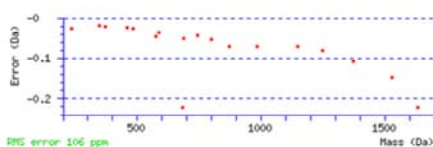
Monoisotopic mass of neutral peptide H₀(calc): 2214.07

Variable modifications:

T3 : Phospho (ST)

Ions Score: 77 Expect: 6.1e-05

Matches (Bold Red): 20/178 fragment ions using 55 most intense peaks

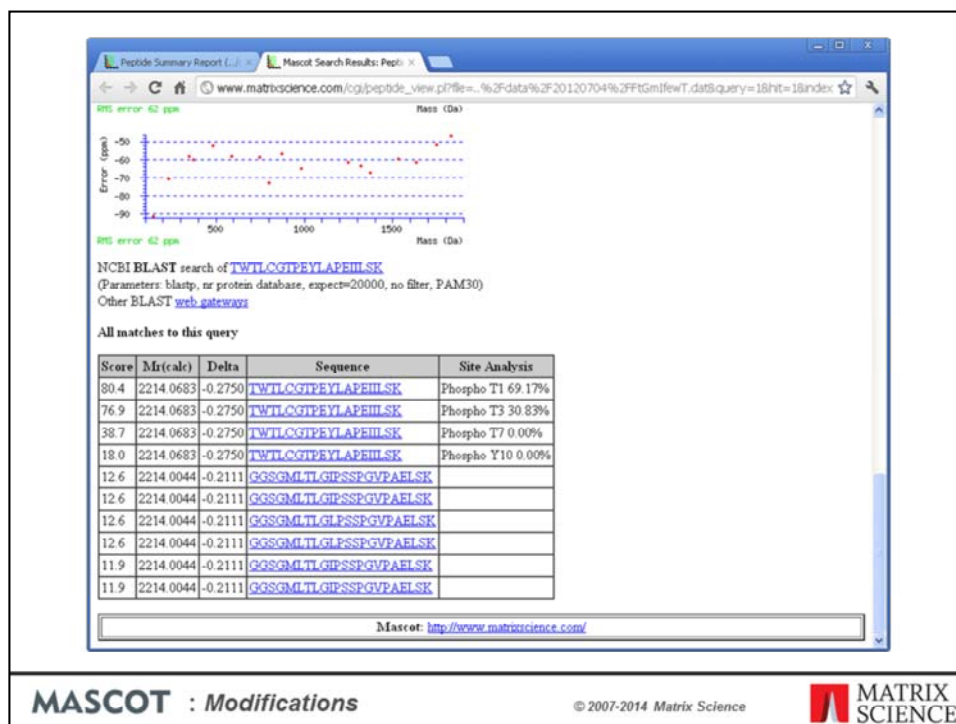


#	b	b ⁺⁺	b ⁺	b ⁰	Seq	y	y ⁺⁺	y ⁺	y ⁰	y ⁻	#
1	84.04	42.53	66.03	33.52	T						19
2	270.12	135.57	252.11	126.56	W	2034.06	1017.53	2017.04	1009.02	2016.05	1008.53
3	371.17	186.09	353.16	177.08	T	1847.98	924.49	1830.96	915.98	1829.97	915.49
4	484.26	242.63	466.24	233.63	L	1746.93	873.97	1729.91	865.46	1728.92	864.97
5	587.26	294.14	569.25	285.13	C	1633.85	817.43	1616.82	808.92	1615.84	808.42
6	644.29	322.65	626.28	313.64	G	1590.84	795.92	1513.81	757.41	1512.83	756.92
7	745.33	373.17	727.32	364.17	T	1473.82	737.41	1456.79	728.90	1455.81	728.41
8	842.39	421.70	824.38	412.69	P	1372.77	686.89	1355.75	678.38	1354.76	677.88
9	971.43	486.22	953.42	477.21	F	1275.72	638.36	1258.69	629.85	1257.71	629.36
10	1134.49	567.75	1116.48	558.74	V	1146.68	573.84	1129.65	565.33	1128.67	564.84
11	1247.58	624.29	1229.57	615.29	L	983.61	492.31	966.59	483.80	965.60	483.31
12	1318.61	659.81	1300.60	650.81	A	870.53	435.77	853.50	427.26	852.52	426.76
13	1415.67	708.34	1397.66	699.33	P	799.49	400.25	782.47	391.74	781.48	391.24
14	1544.71	772.86	1526.70	763.85	F	702.44	351.72	685.41	343.21	684.43	342.72
15	1657.79	829.40	1639.78	820.39	I	573.40	287.20	556.37	278.69	555.39	278.20
16	1770.88	885.94	1752.87	876.94	I	460.31	230.66	443.29	222.15	442.30	221.65
17	1883.94	942.48	1865.95	933.48	L	347.23	174.12	330.20	165.60	329.22	165.11
18	1970.99	986.00	1952.98	976.99	S	234.14	117.58	217.12	109.06	216.13	108.57
19					K	147.11	74.06	130.09	65.55		

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The delta score site analysis suggests 70% probability on T1 and 30% on T3 ... much less clear cut. We can't be confident which site is modified, or whether there is a mixture of both isoforms. But, we can be confident it is not on T7 or Y10 because the score drops dramatically, and these are assigned 0% probability.

Sometimes, it is worth looking at the sequence annotations to see whether these are known phosphorylation sites. If the database sequence doesn't have detailed annotations, you can follow the BLAST link to try and match the peptide to an entry from a better annotated database. In this case, we're searching SwissProt, so we can go straight to the protein view report

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According to Swissprot, both T1 and T3 are possible phosphorylation sites. If you really needed to know which was the case here, or whether it was a mixture, you'd have to acquire more data. Maybe try a different enzyme or target the incomplete cleavage peptide that includes the preceding KG so as to move the sites towards the centre of the peptide, where you might get stronger b and y fragments

Site Analysis

- If alternative sites differ by 20 in score, safe-ish to disregard lower one(s)
- If alternative sites have similar scores, you may be able to choose a preferred site by inspection
- Often, you just can't differentiate between closely spaced sites, even with great data.

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If you are using Mascot 2.3 or earlier, the delta score calculation is not performed in Peptide View. These are our suggested guidelines when using Mascot for site analysis:

If alternative sites differ by 20 in score, safe-ish to disregard lower one(s)

If alternative sites have similar scores, you may be able to choose one by inspection. But, be careful ... one peak is just one peak

Often, you just can't differentiate between adjacent sites, even with great data.

Error Tolerant Search

First pass - simple search of entire database

- Minimal modifications
- Enzyme specificity

Second pass - exhaustive search of selected protein hits

- Wide range of modifications
- Look for SNPs
- Relax enzyme specificity

Reference

➤ Creasy, D. M. and Cottrell, J. S., Error tolerant searching of uninterpreted tandem mass spectrometry data, *Proteomics* 2 1426-1434 (2002)

Now, back to the challenge of finding PT modifications. There are many hundreds of modifications in Unimod, yet I've emphasised the importance of using the minimum number of variable modifications in a search. So, how are we supposed to find unusual modifications?

If you are searching uninterpreted MS/MS data, the efficient way to find unusual modifications, as well as variations in the primary sequence, is a two pass search. The first pass search is a simple search of the entire database with minimal modifications. The protein hits found in the first pass search are then selected for an exhaustive second pass search. During this second pass search, we can look for all possible modifications, sequence variants, and non-specific cleavage products.

Because only a handful of entries are being searched, search time is not an issue. It would be extremely difficult to calculate meaningful statistics for the additional matches in an error tolerant search, and we don't report expect values. The evidence for the presence of any particular protein are the matches from the first pass search. The additional matches from the second pass search serve to increase coverage and may discover interesting modifications or SNPs.

Error Tolerant Search

Unsuspected chemical & P-T modifications

- Iterate serially through comprehensive list
- All fixed and variable mods retained
- Allow for one additional “unsuspected” modification per peptide

For modifications, an error tolerant search looks for one unsuspected modification per peptide in addition to those mods specified as fixed or variable. This is sufficient because it will be rare to get two unsuspected mods on a single peptide

Error Tolerant Search

Primary sequence variants

- Protein database
 - Look for all residue substitutions
 - No attempt to identify single base insertions & deletions because of frame shifts
- Nucleic acid database
 - Look for all single base substitutions, insertions & deletions

The error tolerant search also looks for sequence variants, such as single nucleotide polymorphisms (SNPs) or sequencing errors.

For a protein database, we can't look for the consequences of inserted or deleted bases, because these give rise to frame shifts, and the entire sequence changes from that point on.

Error Tolerant Search

The following constraints apply to the standard, first pass search:

1. Enzyme must be fully specific
2. A reduced ceiling on the number of variable modifications, (default is 2, but this can be changed globally in mascot.dat or for a user group in Mascot security)
3. Cannot be combined with an automatic decoy database search
4. Cannot be combined with quantitation
5. Search cannot include error tolerant sequence tag

There are some constraints on the standard, first pass search

Error Tolerant Search

The screenshot shows the Mascot MS/MS Ions Search web interface. The 'Error tolerant' checkbox is checked and circled in red. The interface includes fields for 'Your name' (John Smith), 'Email' (jhs@res.edu), 'Search title' (Error tolerant example), 'Database(s)' (Fung_EST, Environmental_EST, SwissProt, NCBI, contaminants), 'Enzyme' (Trypsin), 'Allow up to' (2 missed cleavages), 'Taxonomy' (Homo sapiens (human)), 'Fixed modifications' (Carbamidomethyl (C)), 'Variable modifications' (Acetyl (N-term), Oxidation (M)), 'Peptide tol.' (1.2), 'MS/MS tol.' (0.6), 'Peptide charge' (1+), 'Data file' (C:\Dillon\test_data\Mascot\lgtuf20953.mgf), 'Data format' (Mascot generic), 'Instrument' (ESI-QUAD-TOF), 'Decay' (checked), 'Precursor' (m/z), 'Error tolerant' (checked), 'Report type' (AUTO), and 'Hits'.

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Otherwise, submitting the search is just like submitting a standard search except that you check the Error Tolerant Checkbox

Peptide Summary Report

www.matrixscience.com/cgi/master_results.pl?file=%2Fdata%2F20140915%2FFTgcfieOL.dat

Select All

Select None

Search Selected

Error tolerant

1. [PP81_HUMAN](#) Mass: 58259 Score: 519 Matches: 35(10) Sequences: 20(9)

Alkaline phosphatase, placental type OS=Homo sapiens GN=ALPP PF=1 SV=2

☐ Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta Miss Score	Expect	Rank	Unique	Peptide		
27	462.6807	923.3468	923.5156	-0.1649	0	33	0.17	1	R.FPVVALSK.T	
51	517.1760	1032.3375	1032.5604	-0.2229	0	70	4e-05	2	R.GSSIFGLAPGK.A	
52	545.6819	1089.3491	1089.5819	-0.2327	0	(53)	1	R.GSSIFGLAPGK.A + [+57.0215 at 52]		
62	564.6804	1127.3463	1127.5764	-0.2301	0	30	32	1	R.GFLFVEGGR.I	
65	567.6567	1133.2987	1133.5499	-0.2511	0	44	0.011	1	R.GNEVSVQWR.A + Oxidation (M)	
88	614.2001	1226.3856	1226.6329	-0.2473	0	27	0.56	1	U	K.LGPEIFLAGGR.F + Oxidation (M)
102	657.2191	1304.4057	1304.6837	-0.2780	0	(87)	5.8e-07	1	K.QNQQTSLGAAR.F	
124	710.2235	1418.4324	1418.7266	-0.2942	0	95			K.QNQQTSLGAAR.F + [+114.0429 at N-term G]	
125	726.1806	1450.3465	1450.6477	-0.3011	0	73	1.2e-05	1	R.NAYSDGNVPSAR.Q	
133	499.1349	1494.3828	1494.6694	-0.2866	0	88	1		L.DPSLITTEALR.L + 2 Oxidation (M)	
136	754.6864	1507.3582	1507.6691	-0.3109	0	(44)	1		R.NAYSDGNVPSAR.Q + [+57.0215 at N-term N]	
145	526.1538	1575.4396	1575.7814	-0.3418	0	(61)	1		R.ALTTETTFDGAIR.A + [-48.0000 at F8]	
154	820.7283	1639.4420	1639.7763	-0.3343	0	306	6.2e-09	1	R.ALTTETTFDGAIR.A + Oxidation (M)	
165	841.2310	1680.4474	1680.8029	-0.3554	0	(75)	1		R.ALTTETTFDGAIR.A + Oxidation (M); [+41.0266 at N-term A]	
176	864.2888	1726.5629	1726.9294	-0.3664	0	44	0.0092	1	K.AVTLVGGPVPVLR.D	
178	879.2425	1756.4635	1756.8420	-0.3760	0	(48)	1		G.IIPVEENQDVAR.E	
179	593.4834	1777.4285	1777.7764	-0.3478	0	45	1		K.HVPSGATATAYLGGV.G + [+31.9357 at C-term K]	
204	956.2437	1910.4729	1910.8601	-0.3872	0	30	0.23	1	U	R.DSLDPSLITTEALR.L + 2 Oxidation (M)
208	975.8100	1949.6055	1950.0245	-0.4190	0	85	6.5e-07	1		K.NLIFLGGQSVSTTAAR.I + Oxidation (M)
209	976.2340	1950.4534	1950.8555	-0.4021	0	(27)	0.41	1		K.DGAPFVTESSGSPVLR.Q
211	656.1752	1965.5039	1964.8712	-0.6327	0	(72)	1		K.DGAPFVTESSGSPVLR.Q + [+14.0357 at TR]	
212	664.5518	1996.6336	1991.0510	-0.4274	0	(56)	1		K.NLIFLGGQSVSTTAAR.I + Oxidation (M); [+41.0266 at N-term N]	
215	1003.2027	2000.3908	2000.8058	-0.4150	0	(67)	4.1e-05	1	U	R.IGSTPPEVVDYSGGTR.L + Oxidation (M)
217	167.8046	2000.3919	2000.8058	-0.4139	0	76	4.9e-06	1	U	R.IGSTPPEVVDYSGGTR.L + Oxidation (M)
218	670.1561	2007.6466	2007.8770	-0.4304	0	75	1		K.DGAPFVTESSGSPVLR.Q + Acetyl (N-term); [+15.0109 at N-term O]	
222	681.8205	2042.4397	2041.8324	-0.6073	0	(61)	1	U	R.IGSTPPEVVDYSGGTR.L + Acetyl (N-term); Oxidation (M); [-0.3860 at E7]	
224	1029.7081	2057.4016	2057.8273	-0.4256	0	(45)	1		R.IGSTPPEVVDYSGGTR.L + Oxidation (M); [+57.0215 at N-term N]	
227	711.5744	2131.7013	2132.1340	-0.4327	1	36	4.9	1	U	K.LGPEIFLAGGRFPVVALSK.T + Oxidation (M)
252	784.5440	2350.6103	2351.1030	-0.4927	0	(69)	1	U	R.QQAVPLDETHAGDVAVAR.G + [-17.0265 at N-term Q]	
253	790.2187	2367.6341	2368.1295	-0.4954	0	94	7.4e-08	1	U	R.QQAVPLDETHAGDVAVAR.G
260	809.2208	2424.6406	2425.1510	-0.5104	0	(66)	1	U	R.QQAVPLDETHAGDVAVAR.G + [+57.0215 at N-term Q]	
274	914.9140	2741.7263	2741.2306	-0.4956	0	(41)	1		R.QVQGDATQLSMDITNVLSGGH.R + Oxidation (M); [+29.5568 at C4]	
375	670.5878	2794.7415	2795.5583	-0.6167	0	80	1		R.DFCCDNTSTQVAMDTNVLGGGR.R + Acetyl (N-term); Oxidation (M); [-0.4876 at F37]	

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And here is the first hit of the results report. The additional matches, found in the error tolerant search, are the ones without expect values. One of these, query 133, is a simple, non-specific peptide with a very good score. There's another example for query 176. The error tolerant search is a much better way of picking up non-specific peptides than searching the entire database with semi-trypsin or no enzyme. We only fail to get such matches in an error tolerant search if there are no matches to the protein in the first pass search. However, you have to ask yourself whether you would believe a protein hit in which the only peptide match was non-specific. I think the answer is no.

Error Tolerant Search

To reduce 'junk' matches

- An individual peptide can be semi-specific OR have one unsuspected modification OR have one primary sequence mutation.
- If the mass delta of the modification is less than the smaller of the precursor mass tolerance and the fragment mass tolerance, the modification is rejected. This eliminates modifications that are meaningless given the estimated mass error, like Q->K, in most cases.
- Match must have a score of at least the identity threshold for the same query in the first pass search
- Match must have a score in excess of the highest scoring match to the same query in the first pass search

The matches from an error tolerant search are aggressively filtered to remove junk matches

Peptide Summary Report

www.matrixscience.com/cgi/master_results.pl?file=.%2Fdata%2F20140915%2FFTgofieOL.dat

Select All Select None Search Selected Error tolerant

1. **PP81_H5050** Mass: 58259 Score: 519 Matches: 35(10) Sequences: 20(9)
Alkaline phosphatase, placental type OS=Homo sapiens GI=ALPP PE=1 SV=2
☐ Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
22	402.6807	923.3468	923.5116	-0.1649	0	33	0.17	1		R.FPVVALSK.T
51	517.1760	1032.3375	1032.5604	-0.2229	0	70	4e-05	2		R.GSSIFGLAPGK.A
53	545.6819	1089.3401	1089.5819	-0.2327	0	(53)				R.GSSIFGLAPGK.A + [+57.0215 at 52]
52	564.6804	1127.3463	1127.5764	-0.2301	0	10	32	1		R.GFLFVEGGK.I
55	567.6567	1133.2987	1133.5499	-0.2511	0	44	0.011	1		R.GNEVISVNR.A + Oxidation (N)
88	614.2001	1226.3856	1226.6329	-0.2473	0	27	0.56	1	U	R.LGPEIPLAGK.F + Oxidation (N)
100	653.2101	1304.4057	1304.6837	-0.2780	0	(87)	5.6e-07	1		R.GNPQTGLSAAK.F
124	710.2235	1418.4324	1418.7266	-0.2942	0	95				R.GNPQTGLSAAK.F + [+118.0429 at N-term G]
125	726.1806	1450.3465	1450.6477	-0.3011	0	73	1.2e-05	1		R.NWSDNPFASAR.Q
133	499.1349	1494.3828	1494.6694	-0.2866	0	88				L.DPSLSHETEAR.L + 2 Oxidation (N)
136	754.6864	1507.3582	1507.6691	-0.3109	0	(44)				R.NWSDNPFASAR.Q + [+57.0215 at N-term N]
145	526.1538	1575.4396	1575.7854	-0.3418	0	(61)				R.ALTTETEDDAER.A + [+48.0215 at F81]
154	820.7283	1639.4420	1639.7763	-0.3343	0	306	6.2e-09	1		R.ALTTETEDDAER.A + Oxidation (N)
165	841.2318	1680.4474	1680.8029	-0.3554	0	(75)				R.ALTTETEDDAER.A + Oxidation (N)
172	864.2888	1736.5629	1736.9294	-0.3664	0	44	0.0002	1		R.AYFLVYGGPVLK.D
175	586.4951	1756.4635	1756.8420	-0.3786	0	(48)				G.IIPVEENPFQNR.E
176	879.2425	1756.4705	1756.8420	-0.3715	0	83				G.IIPVEENPFQNR.E
179	593.4834	1777.4285	1777.7764	-0.3478	0	45				R.NVDSGATATVLCGV.G + [+31.0215 at N-term G]
204	956.2437	1910.4729	1910.8601	-0.3872	0	30	0.23	1	U	R.DSTLPSLSETEAR.L + 2 Oxidation (N)
208	975.8100	1949.6055	1950.0245	-0.4190	0	85	6.5e-07	1		R.NLIFLGGQGVSTAAK.L + Oxidation (N)
209	976.2340	1950.4534	1950.8555	-0.4021	0	(27)	0.41	1		R.DGAPQVTESESGPEYR.Q
211	956.1752	1965.5039	1964.8712	-0.6327	0	(72)				R.DGAPQVTESESGPEYR.Q + [+14.0152 at T8]
212	664.5518	1996.6336	1991.0510	-0.4214	0	(58)				R.NLIFLGGQGVSTAAK.L + Oxidation (N); [+81.0266 at N-term N]
215	1001.2027	2000.3908	2000.8058	-0.4150	0	(67)	4.1e-05	1	U	R.NSTPPEYVDDVSGQTR.L + Oxidation (N)
217	667.8046	2000.3919	2000.8058	-0.4139	0	76	4.9e-06	1		R.NSTPPEYVDDVSGQTR.L + Oxidation (N)
218	670.1561	2007.4466	2007.8770	-0.4304	0	75				R.DGAPQVTESESGPEYR.Q + Acetyl (N-term); [+15.0109 at N-term D]
221	681.8205	2042.4397	2041.8324	-0.6073	0	(61)			U	R.NSTPPEYVDDVSGQTR.L + Acetyl (N-term); Oxidation (N); [+0.5860 at E7]
224	1029.7081	2057.4016	2057.8273	-0.4256	0	(85)				R.NSTPPEYVDDVSGQTR.L + Oxidation (N); [+57.0215 at N-term N]
227	711.5744	2131.7013	2132.1340	-0.4327	1	16	4.9	1	U	R.LGPEIPLAGRFPVVALSK.T + Oxidation (N)
251	784.5440	2350.6193	2351.1030	-0.4927	0	(69)				R.QQAVPLDETHAGDVFAR.G + [+17.0205 at N-term Q]
251	790.2187	2367.6341	2368.1295	-0.4954	0	94	7.4e-08	1	U	R.QQAVPLDETHAGDVFAR.G
260	809.2208	2424.6406	2425.1510	-0.5104	0	(66)				R.QQAVPLDETHAGDVFAR.G + [+57.0215 at N-term Q]
274	934.9160	2741.7263	2741.2306	-0.4956	0	(41)				R.QEQQDEATQLSMQDVIKGGK.R + Oxidation (N); [+29.9568 at C4]
274	934.9160	2741.7263	2741.2306	-0.4956	0	96				R.QEQQDEATQLSMQDVIKGGK.R + Acetyl (N-term); Oxidation (N); [+0.5876 at E3]

Possible Assignments:
Carbamidomethyl (N-term) [+57.0215]
Carboxymethyl (N-term) [+58.0055]
Delta(H6)(C13)(O1) (Protein N-term) [+58.0419]

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Take a look at the match to query 136. The mass tolerance for this search was fairly wide, so the observed mass difference could correspond to either carbamidomethylation or carboxymethylation at the N-terminus. Since this sample was alkylated with iodoacetamide, we would choose carbamidomethylation as the more likely suspect, especially as this brings the error on the precursor mass into line with the general trend, whereas carboxymethylation would give an error of +0.6 Da. The assignment to carbamidomethylation is also very believable, because this is a known artefact of over-alkylation. The same modification can be seen in this screen shot for three other queries

Peptide Summary Report

www.matrixscience.com/cgi/master_results.pl?file=-%2Fdata%2F20140915%2FFTgcfieOL.dat

Rank	Score	Mass	Charge	Modifications	Peptide
1	567.6567	1133.2987	1133.5499	-0.2511 0 44 0.011 1	R.GNEVTSVQNR.A + Oxidation (M)
2	514.2901	1226.2856	1226.4329	-0.2473 0 27 0.56 1	K.LGPEIPLAGNR.F + Oxidation (M)
3	653.2391	1304.4057	1304.6837	-0.2780 0 (87) 5.8e-07 1	K.QNFQTQLSAAAR.A
4	739.2235	1418.4324	1418.7266	-0.2942 0 95 1	K.QNFQTQLSAAAR.F + [-114.0429 at N-term G]
5	726.1806	1450.3465	1450.6477	-0.3011 0 73 1.2e-05 1	R.NAYSDQNPASAR.Q
6	499.1349	1494.2828	1494.6694	-0.2866 0 88 1	L.DPILSDIETAAAR.L + 2 Oxidation (M)
7	754.6864	1507.3582	1507.6691	-0.3109 0 (44) 1	R.NAYSDQNPASAR.Q + [-57.0215 at N-term N]
8	526.1538	1575.4396	1575.7814	-0.3418 0 (81) 1	R.ALTEIIPEDDAIER.A + [-48.0000 at F8]
9	820.7283	1639.4420	1639.7763	-0.3343 0 106 6.2e-09 1	R.ALTEIIPEDDAIER.A + Oxidation (M)
10	841.2310	1680.4474	1680.8029	-0.3554 0 (75) 1	R.ALTEIIPEDDAIER.A + Oxidation (M); [-41.0266 at N-term A]
11	864.2888	1726.5629	1726.9294	-0.3664 0 44 0.0092 1	K.AYFLLVGGQVYVLR.D
12	586.4951	1756.4635	1756.8410	-0.3786 0 (48) 1	G.IIPVEIENPDAAR.E
13	879.2425	1756.4795	1756.8420	-0.3715 0 83 1	G.IIPVEIENPDAAR.E
14	593.4834	1777.4285	1777.7764	-0.3479 0 45 1	K.HNPDSGATATVLCQV.G + [-31.3232 at C-term K]
15	956.2437	1910.4729	1910.8601	-0.3872 0 30 0.23 1	R.DSTLDPSLQETAAAR.L + 2 Oxidation (M)
16	975.8100	1949.6055	1950.0245	-0.4190 0 85 6.3e-07 1	K.NLIFLGGQGVYVTAAR.I + Oxidation (M)
17	976.2340	1950.4534	1950.8555	-0.4021 0 (27) 0.41 1	K.DGAPFVTESESGPEYR.Q
18	696.1752	1965.5039	1964.8732	-0.6327 0 (72) 1	K.DGAPFVTESESGPEYR.Q + [-14.0137 at TR]
19	664.5518	1990.6336	1991.0510	-0.4174 0 (58) 1	K.NLIFLGGQGVYVTAAR.I + Oxidation (M); [-41.0266 at N-term N]
20	1001.2027	2000.3908	2000.8058	-0.4150 0 (67) 4.1e-05 1	R.GSIFPQVPEVDYSGGTR.L + Oxidation (M)
21	667.8046	2000.3919	2000.8058	-0.4139 0 76 4.9e-06 1	R.GSIFPQVPEVDYSGGTR.L + Oxidation (M)
22	670.1561	2007.4466	2007.8770	-0.4304 0 75 1	K.DGAPFVTESESGPEYR.Q + Acetyl (N-term); [-15.0309 at N-term D]
23	681.8205	2042.4397	2041.8324	-0.6073 0 (61) 1	R.GSIFPQVPEVDYSGGTR.L + Acetyl (N-term); Oxidation (M); [-0.2840 at E7]
24	1029.7081	2057.4016	2057.8273	-0.4256 0 (45) 1	R.GSIFPQVPEVDYSGGTR.L + Oxidation (M); [-57.0215 at N-term M]
25	711.5744	2131.7013	2132.1340	-0.4327 1 36 4.9 1	K.LGPEIPLAGNR.FPVVLSK.T + Oxidation (M)
26	784.5440	2150.6103	2151.1030	-0.4927 0 (69) 1	R.QQNAVPLDETHAGDVAVFAR.G + [-17.0205 at N-term Q]
27	790.2187	2167.6341	2168.1295	-0.4954 0 94 7.4e-08 1	R.QQNAVPLDETHAGDVAVFAR.G
28	809.2208	2424.6406	2425.1510	-0.5104 0 (65) 1	R.QQNAVPLDETHAGDVAVFAR.G + [-57.04 at E2]
29	914.9160	2742.7263	2741.2306	-0.4956 0 (41) 1	R.QGQDQIATQLISMDIVLGGGR.K + Oxidation (M)
30	920.5878	2758.7415	2759.3582	-0.6167 0 90 1	R.QGQDQIATQLISMDIVLGGGR.K + Acetyl (N-term); Oxidation (M)
31	1078.6327	3232.8763	3233.5629	-0.6867 0 10 16 1	R.AGQLTSEEDTLVLTADHSHVPSGGVPLR.G

2. **FPRLM250** Mass: 57626 Score: 362 Matches: 27(8) Sequences: 15(8)
Alkaline phosphatase, placental-like OS=Homo sapiens GO=ALPPL2 PE=1 SV=4
[] Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
22	482.6807	923.3468	923.5116	-0.1649	0	33	0.17	1	1	R.FPVVLSK.T
61	517.1700	1032.3375	1032.5604	-0.2229	0	70	4e-05	2	1	R.GSSIFPLAPQK.A
51	545.6819	1089.3491	1089.5819	-0.2327	0	53	1	1	1	R.GSSIFPLAPQK.A + [-57.0215 at E2]

possible Assignments:
Glu->pyro-Glu (N-term Q) [-17.0205] at E2

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Another easily believable assignment is pyro-Glu for the match to query 252.

Peptide Summary Report

www.matrixscience.com/cgi/master_results.pl?file=-%2Fdata%2F20140915%2FFTgcfileOL.dat

62	567.6567	1133.2987	1133.5499	-0.2511	0	44	0.011	1	R.GNEVTSVQNR.A + Oxidation (N)
81	614.2901	1226.2856	1226.6329	-0.2473	0	27	0.56	1	U K.LGPEIPLAGNR.F + Oxidation (N)
100	653.2391	1304.4057	1304.6837	-0.2780	0	(87)	5.8e-07	1	K.QNFQITGLSAAAR.A
124	710.2235	1418.4324	1418.7266	-0.2042	0	95		1	K.QNFQITGLSAAAR.F + [-114.0429 at N-term G]
126	726.1806	1450.3465	1450.6477	-0.3011	0	73	1.2e-05	1	R.NAYSDQNPASAR.Q
131	499.1349	1494.2828	1494.6694	-0.2866	0	88		1	L.DPILSISITTEALRL.L + 2 Oxidation (N)
136	754.6864	1507.3582	1507.6691	-0.3109	0	(44)		1	R.NAYSDQNPASAR.Q + [-57.0215 at N-term N]
145	526.1538	1575.4396	1575.7814	-0.3418	0	(81)		1	R.ALTEITIFSDAIAER.A + [-48.0000 at F8]
156	820.7283	1639.4420	1639.7763	-0.3343	0	106	6.2e-09	1	R.ALTEITIFSDAIAER.A + Oxidation (N)
165	841.2310	1680.4474	1680.8029	-0.3554	0	(75)		1	R.ALTEITIFSDAIAER.A + Oxidation (N); [-41.0266 at N-term A]
170	864.2888	1726.5629	1726.9294	-0.3664	0	44	0.0092	1	K.AYFLLVGGPQVPLER.D
172	586.4951	1756.4635	1756.8410	-0.3786	0	(48)		1	G.IIPVEIENPDIAR.E
176	879.2425	1756.4795	1756.8420	-0.3715	0	83		1	G.IIPVEIENPDIAR.E
178	593.4834	1777.4285	1777.7764	-0.3479	0	45		1	K.HNPDGATATATLCQV.G + [-31.3237 at C-term K]
208	956.2437	1910.4729	1910.8601	-0.3872	0	30	0.23	1	U R.DSTLDPSLQITTEALRL.L + 2 Oxidation (N)
208	975.8100	1949.6055	1950.0245	-0.4190	0	85	6.3e-07	1	K.NLIFLGGQGVSTVTAAAR.L + Oxidation (N)
209	976.2340	1950.4534	1950.8555	-0.4021	0	(27)	0.41	1	K.DGAPFVTSVSSGPEYR.Q
211	696.1752	1965.5039	1964.8712	-0.6327	0	(72)		1	K.DGAPFVTSVSSGPEYR.Q + [-14.0437 at T8]
211	664.5518	1990.6336	1991.0510	-0.4174	0	(58)		1	K.NLIFLGGQGVSTVTAAAR.L + Oxidation (N); [-41.0266 at N-term N]
212	1001.2027	2000.3908	2000.8058	-0.4150	0	(67)	4.1e-05	1	U R.GSTPDPVPEVDYSGGTR.L + Oxidation (N)
217	667.8046	2000.3919	2000.8058	-0.4139	0	76	4.9e-06	1	U R.GSTPDPVPEVDYSGGTR.L + Oxidation (N)
218	670.1561	2007.4466	2007.8770	-0.4304	0	75		1	K.DGAPFVTSVSSGPEYR.Q + Acetyl
222	681.8205	2042.4397	2041.8324	-0.6073	0	(61)		1	U R.GSTPDPVPEVDYSGGTR.L + Acetyl
228	1029.7081	2057.4016	2057.8273	-0.4256	0	(45)		1	U R.GSTPDPVPEVDYSGGTR.L + Oxidation (N)
227	711.5744	2131.7013	2132.1340	-0.4327	1	16	4.9	1	U K.LGPEIPLAGNRFPVVALSK.T + Oxidation (N)
252	784.5440	2150.6103	2151.1030	-0.4927	0	(69)		1	R.QQSAVPLDETHAGEVAVFAR.G + [-114.0429 at N-term G]
252	790.2187	2167.6341	2168.1295	-0.4954	0	94	7.4e-08	1	U R.QQSAVPLDETHAGEVAVFAR.G
260	809.2208	2424.6406	2425.1510	-0.5104	0	(65)		1	U R.QQSAVPLDETHAGEVAVFAR.G + [-57.0215 at N-term Q]
274	914.9160	2742.7263	2741.2306	-0.4956	0	(41)		1	R.QGQDQATQLISAGDIDVILGGGR.K + Oxidation (N); [-29.0568 at C4]
275	920.5878	2758.7415	2759.3582	-0.6167	0	90		1	R.QGQDQATQLISAGDIDVILGGGR.K + Acetyl (N-term); Oxidation (N); [-8.9476 at E2]
281	1078.6327	3232.8763	3233.5629	-0.6867	0	10	16	1	R.AGQLTSEDTSLVTADHSHNFSGGVPLR.G

2. [PEPTIDE MATCHES](#) Mass: 57626 Score: 362 Matches: 27(8) Sequences: 15(8)


Alkaline phosphatase, placental-like OS=Homo sapiens GN=ALPPL2 PE=1 SV=4

☐ Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
27	482.6807	923.3468	923.5116	-0.1649	0	33	0.17	1	1	R.FPVVALSK.T
61	517.1700	1032.3375	1032.5604	-0.2229	0	70	4e-05	2	1	R.GSSIFPLAPQK.A
51	545.6819	1089.3491	1089.5819	-0.2327	0	(53)		1	1	R.GSSIFPLAPQK.A + [-57.0215 at S2]

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As is methylation ay T8 for query 211

Peptide Summary Report

www.matrixscience.com/cgi/master_results.pl?file=%2Fdata%2F20140915%2FTgcfieOL.dat

258 795.1595 2382.4566 2382.9447 -0.4880 0 77 3.9e-06 1 U R.GGGGGGYSGGSSYSGGGSSYSGGGGGGG.R.G

252 1192.2367 2382.4588 2382.9447 -0.4858 0 (22) 1.2 1 U R.GGGGGGYSGGSSYSGGGSSYSGGGGGGG.R.G

5. **BAIP2_HUMAN** Mass: 61115 Score: 221 Matches: 11(5) Sequences: 10(5)
Brain-specific angiogenesis inhibitor 1-associated protein 2 OS=Homo sapiens GN=BAIP2 PE=1 SV=1

☐ Check to include this hit in error tolerant search

Query	Observed	Mr(calc)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
28	456.6505	911.2864	911.4753	-0.1888	0	26	0.75	1	U	K.GYDYLVE.H
32	482.6753	963.3361	963.5389	-0.2028	0	51	0.0032	1	U	K.ALAGVTYAK.G
60	562.6743	1123.3340	1123.5662	-0.2322	0	50	0.0026	1	U	R.AFPQATAGFK.Q
118	676.2223	1350.4300	1350.6779	-0.2479	0	74	1.2e-05	1	U	K.LSDQYSNTLPVR.E
125	713.2435	1424.4724	1424.7875	-0.3150	0	94	1.1e-07	1	U	K.EGDLTLVLPVAR.D
148	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V + Acetyl (N-term); [-42.0106 at E5]
149	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
150	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
151	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
152	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
153	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
154	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
155	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
156	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
157	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
158	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
159	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
160	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
161	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
162	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
163	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
164	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
165	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
166	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
167	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
168	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
169	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
170	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
171	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
172	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
173	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
174	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
175	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
176	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
177	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
178	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
179	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
180	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
181	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
182	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
183	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
184	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
185	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
186	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
187	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
188	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
189	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
190	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
191	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
192	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
193	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
194	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
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198	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
199	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
200	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
201	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
202	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
203	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
204	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
205	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
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220	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
221	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
222	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
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224	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
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233	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
234	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
235	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
236	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
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238	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
239	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
240	929.4994	1545.4764	1545.8100	-0.3336	0	38		1	U	K.SFMHLLTQLEK.V
241	929.4994	1545.4764	1545.8100							

Error Tolerant Search

- Can successfully locate mass differences corresponding to a single unsuspected modification or a single SNP per peptide
- User must decide on best explanation for the observed differences
- Limited to proteins which have at least one good peptide match ... not very useful for (say) MHC peptides.

MASCOT : *Modifications*

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In summary, an error tolerant search

- Can successfully locate mass differences corresponding to a single unsuspected modification or a single SNP per peptide
- User must decide on best explanation for the observed differences
- Limited to proteins which have at least one good peptide match ... not very useful for (say) MHC peptides