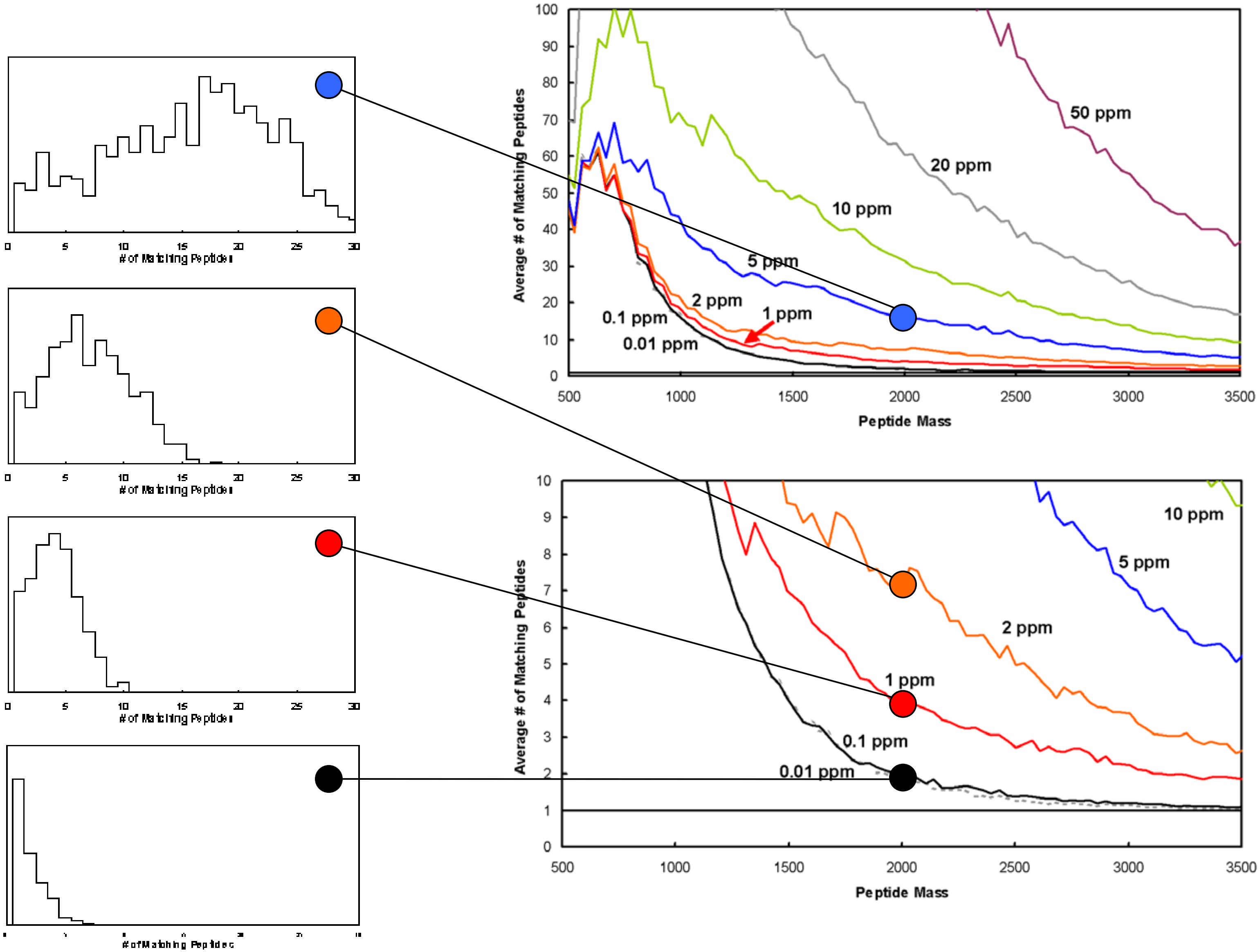
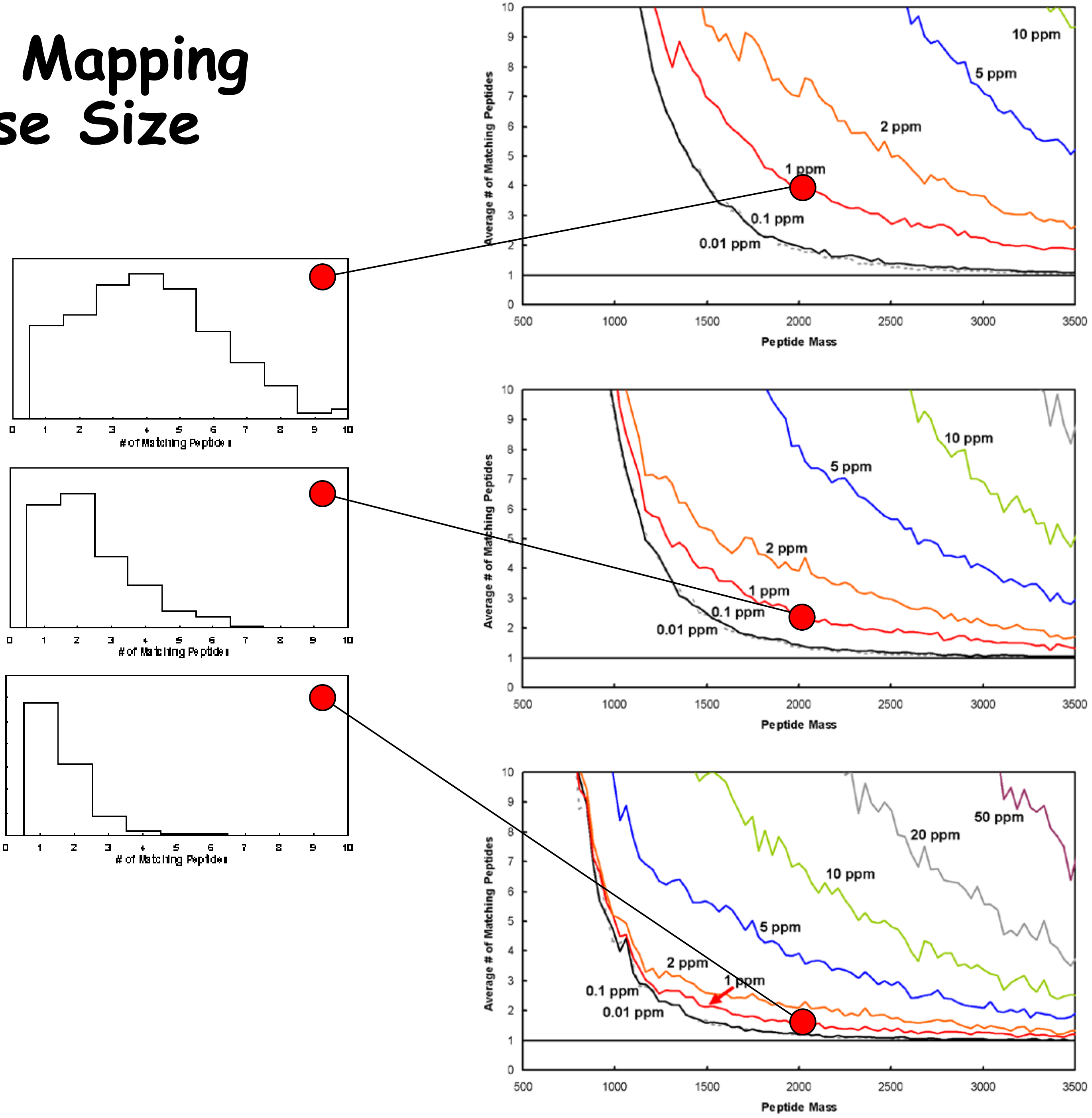
Proteomics Informatics –
Protein identification I: searching protein sequence collections and significance testing (Week 4)

Peptide Mapping - Mass Accuracy



Peptide Mapping Database Size

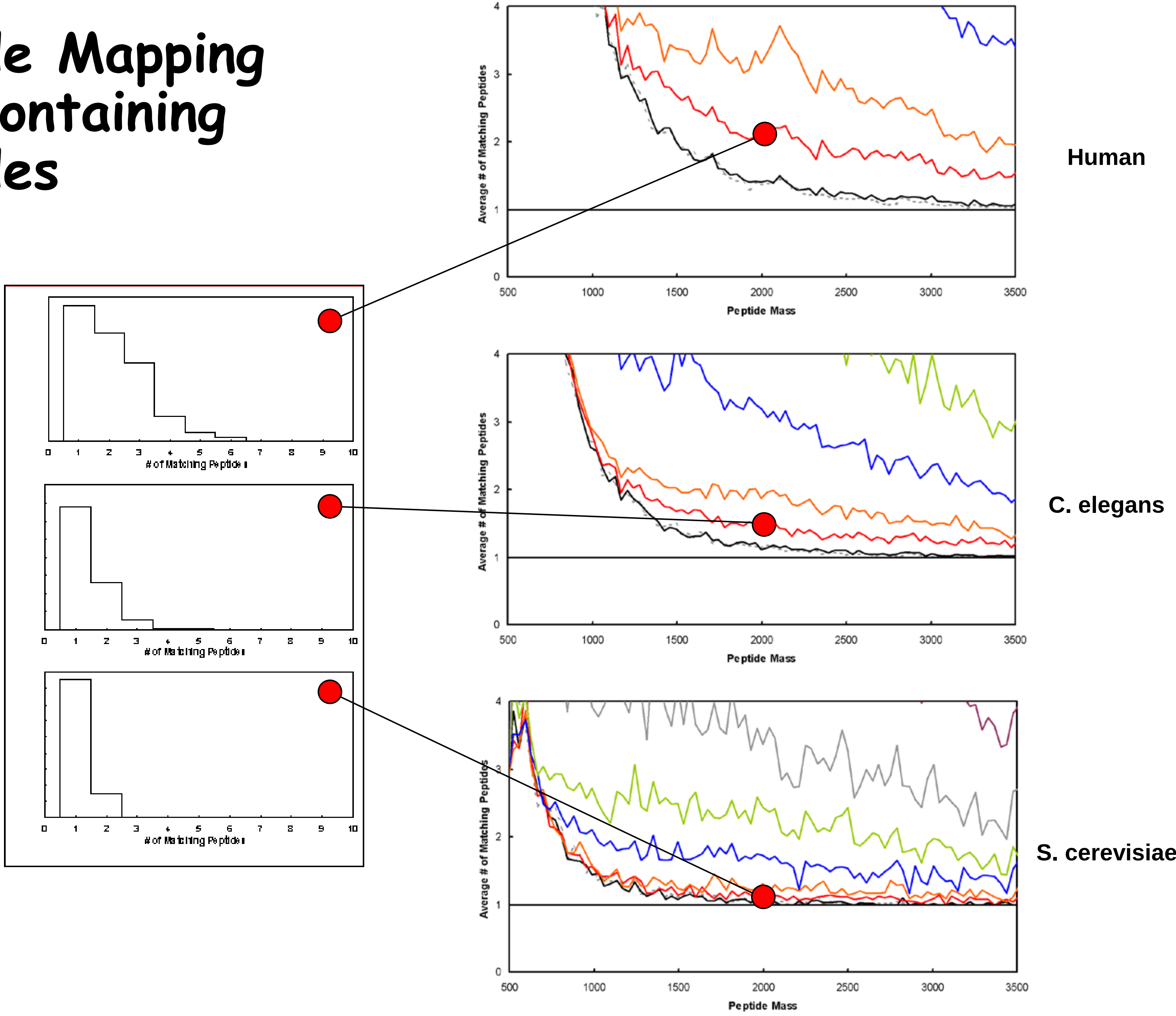


Human

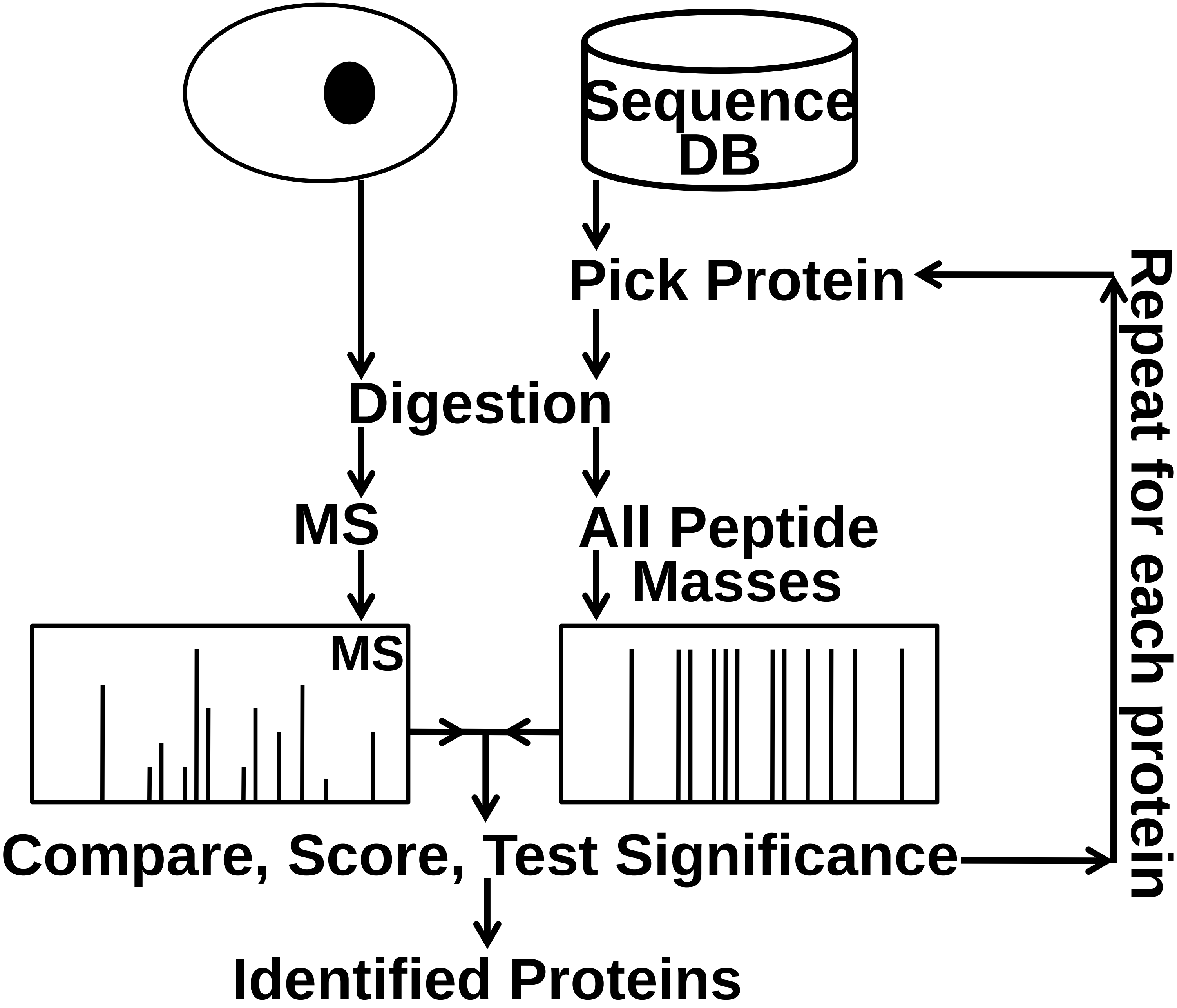
C. elegans

S. cerevisiae

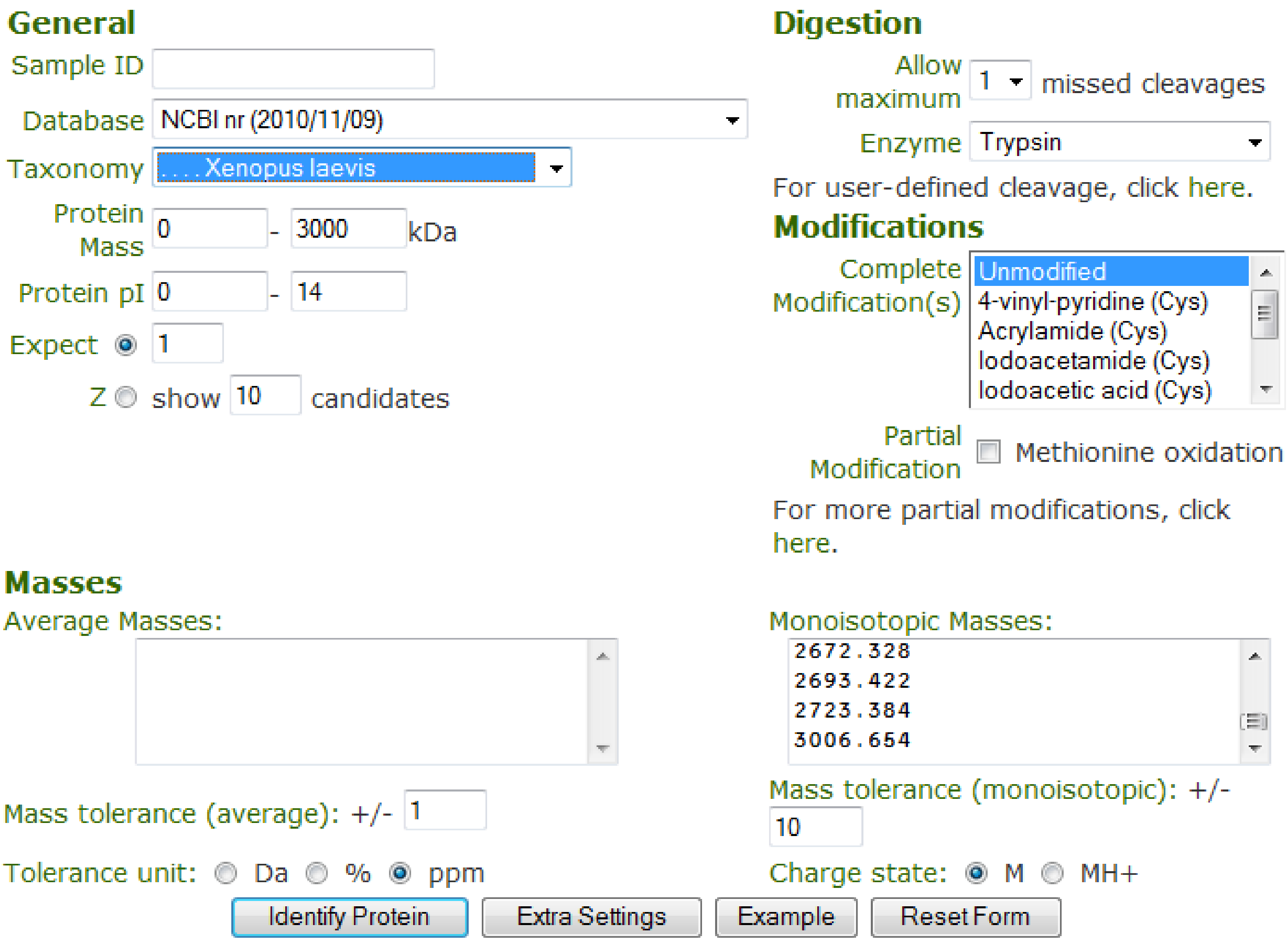
Peptide Mapping Cys-Containing Peptides



Identification – Peptide Mass Fingerprinting



ProFound - Search Parameters



General

Sample ID:

Database: NCBI nr (2010/11/09)

Taxonomy: ....Xenopus laevis

Protein Mass: 0 - 3000 kDa

Protein pI: 0 - 14

Expect ● 1

Z ○ show 10 candidates

Digestion

Allow maximum 1 missed cleavages

Enzyme: Trypsin

For user-defined cleavage, click here.

Modifications

Complete Modification(s): Unmodified; 4-vinyl-pyridine (Cys); Acrylamide (Cys); Iodoacetamide (Cys); Iodoacetic acid (Cys)

Partial Modification: □ Methionine oxidation

For more partial modifications, click here.

Masses

Average Masses:

Monoisotopic Masses:

```
2672.328
2693.422
2723.384
3006.654
```

Mass tolerance (average): +/- 1

Mass tolerance (monoisotopic): +/- 10

Tolerance unit: ○ Da ○ % ● ppm

Charge state: ● M ○ MH+

http://prowl.rockefeller.edu/

ProFound – Protein Identification by Peptide Mapping

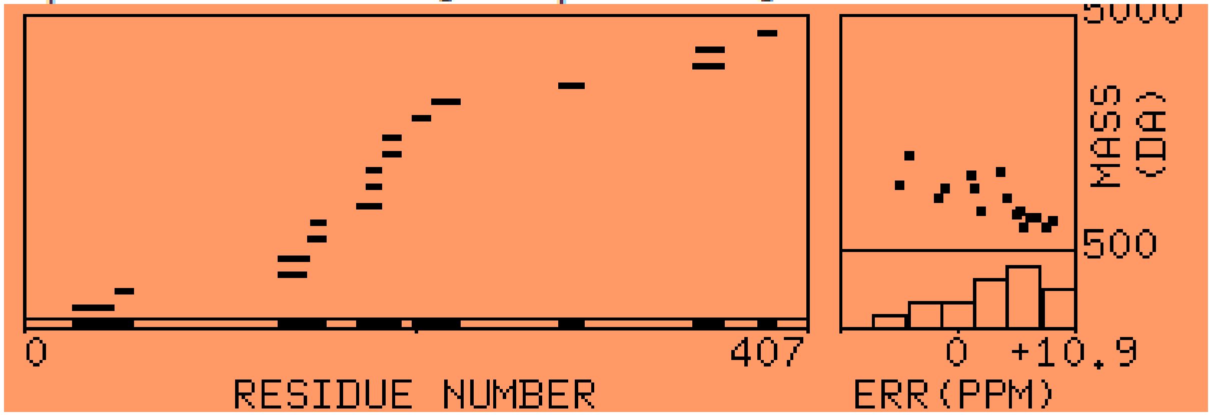
$$P(k \mid DI) \propto P(k \mid I) \frac{(N-r)!}{N!} \prod_{i=1}^{r} g_i \left(\frac{m_{\max} - m_{\min}}{2\sigma} \right)^r \exp\left[\frac{r}{2} - \frac{\sum_{i=1}^{r} (m_i - m_{i0})^2}{2\sigma^2} \right] F_{pattern}$$

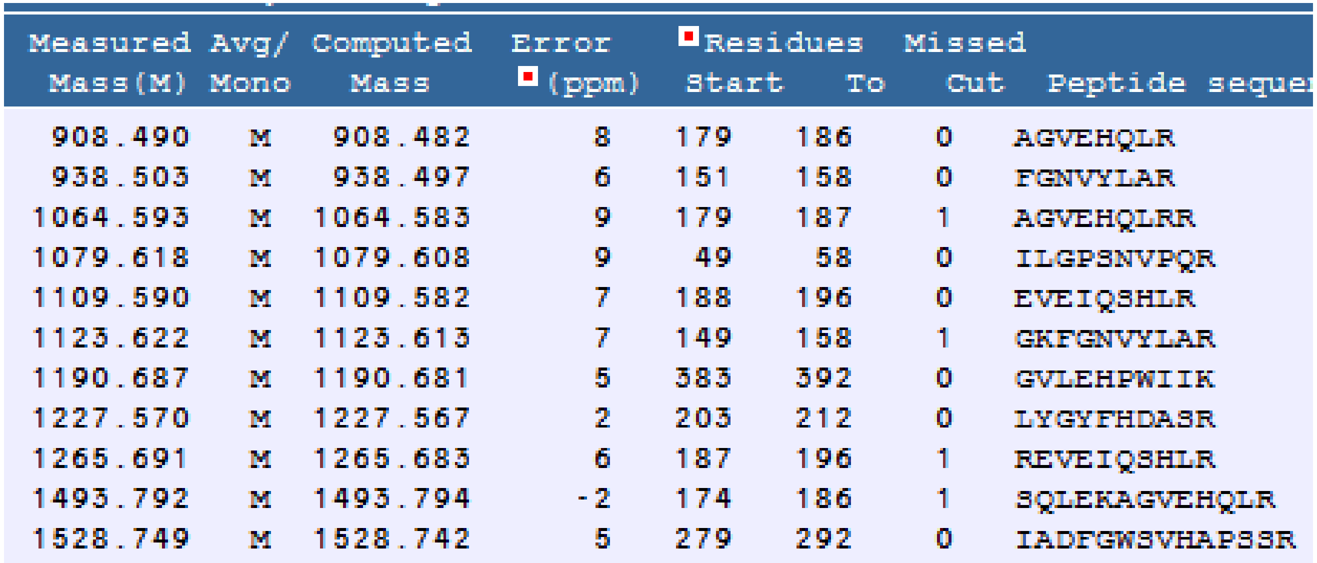
W. Zhang & B.T. Chait,
Analytical Chemistry
72 (2000) 2482-2489

ProFound Results

Protein Candidates

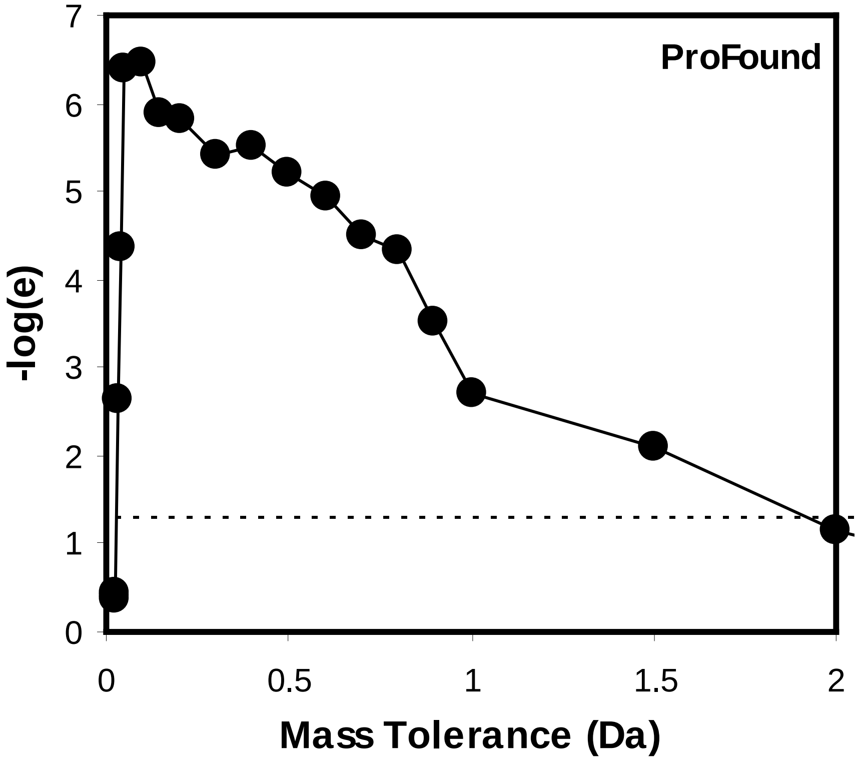
Rank	Expectation	Protein Information and Sequence Analyse Tools (T)	%	pI	kDa
+1	5.110^{-7}.	gi|148236543|ref|NP_001081565.1| serine/threonine-protein kinase 6-A [Xenopus laevis]	36	9.6	46.35
+2	0.057		8	5.3	147.73
3	0.094		9	7.5	126.81

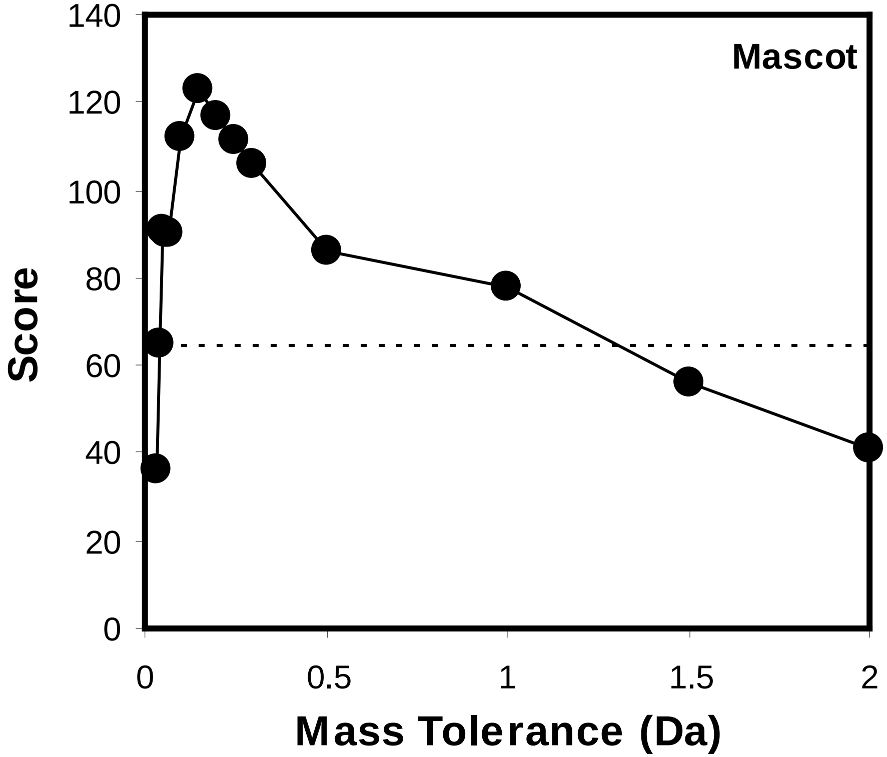




Measured Mass(M)	Avg/ Mono	Computed Mass	Error ▪(ppm)	▪Residues Start	To	Missed Cut	Peptide seque
908.490	M	908.482	8	179	186	0	AGVEHQLR
938.503	M	938.497	6	151	158	0	FGNVYLAR
1064.593	M	1064.583	9	179	187	1	AGVEHQLRR
1079.618	M	1079.608	9	49	58	0	ILGPSNVPQR
1109.590	M	1109.582	7	188	196	0	EVEIQSHLR
1123.622	M	1123.613	7	149	158	1	GKFGNVYLAR
1190.687	M	1190.681	5	383	392	0	GVLEHPWIIK
1227.570	M	1227.567	2	203	212	0	LYGYFHDASR
1265.691	M	1265.683	6	187	196	1	REVEIQSHLR
1493.792	M	1493.794	-2	174	186	1	SQLEKAGVEHQLR
1528.749	M	1528.742	5	279	292	0	IADFGWSVHAPSSR

Peptide Mapping – Mass Accuracy



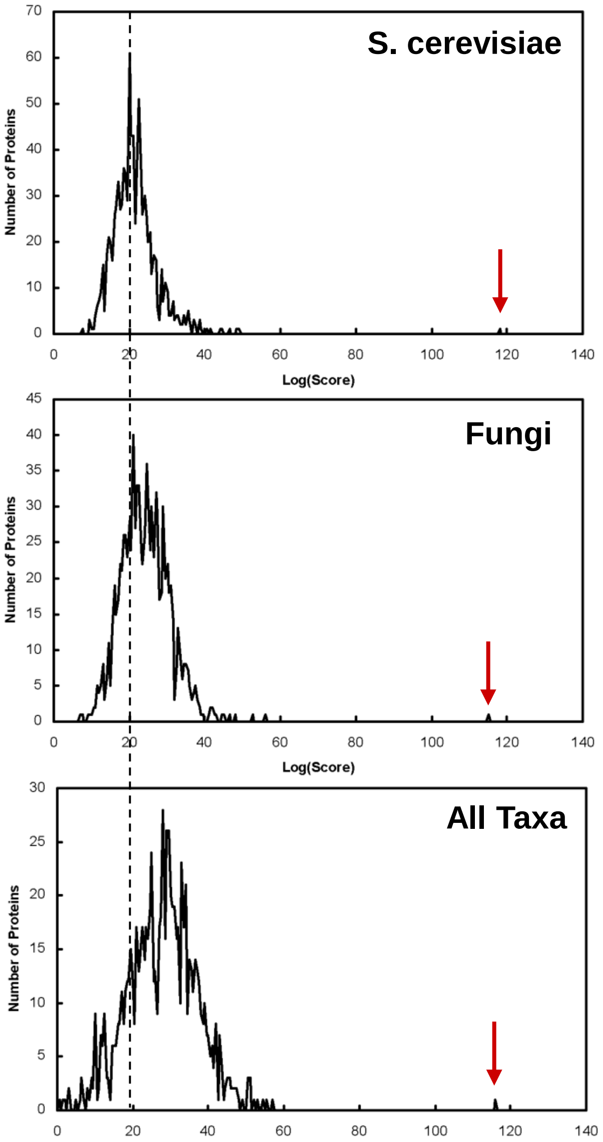


Peptide Mapping - Database Size

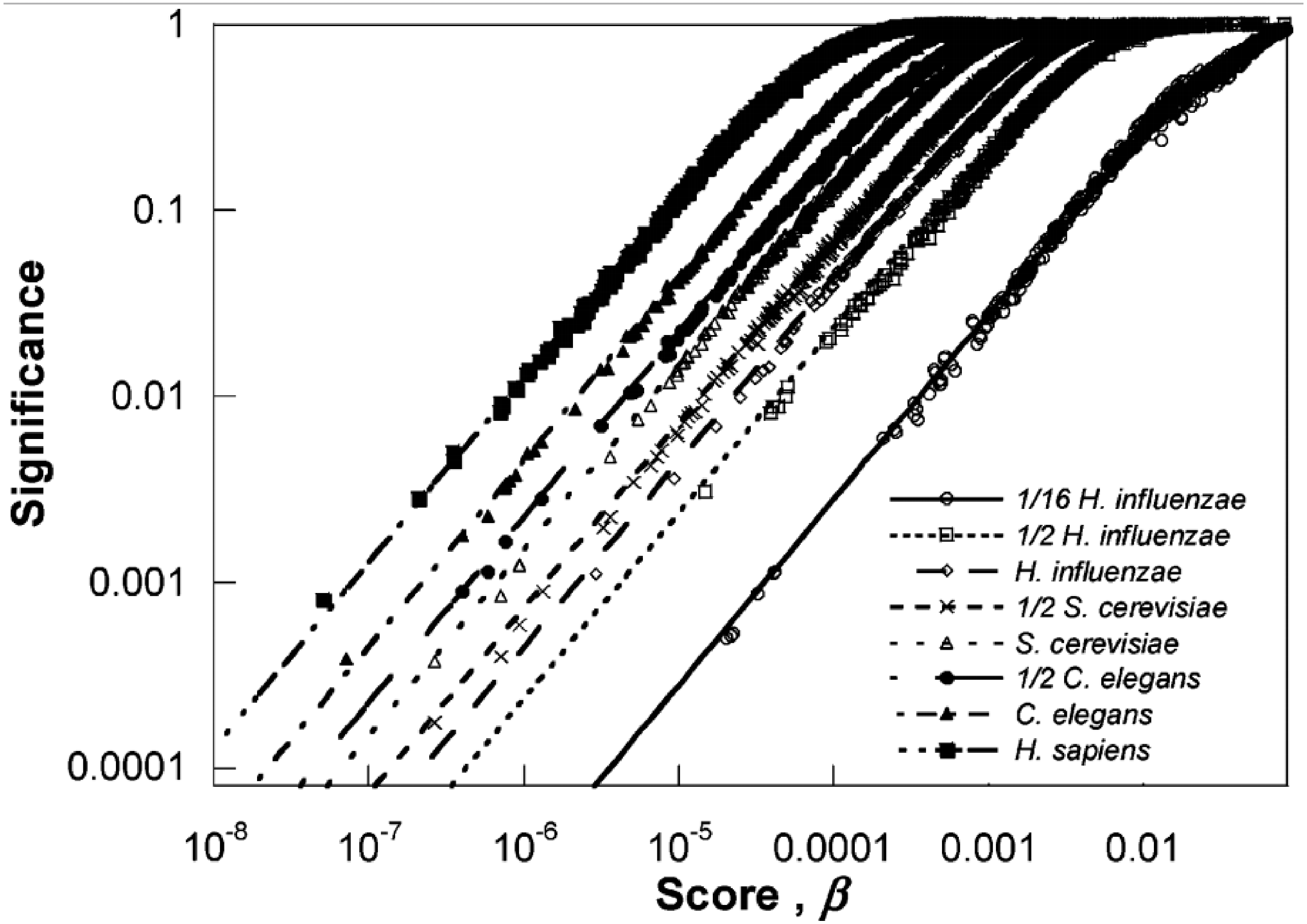
Expectation Values

Peptide mapping example:

S. Cerevisiae	4.8e-7
Fungi	8.4e-6
All Taxa	2.9e-4



Database size

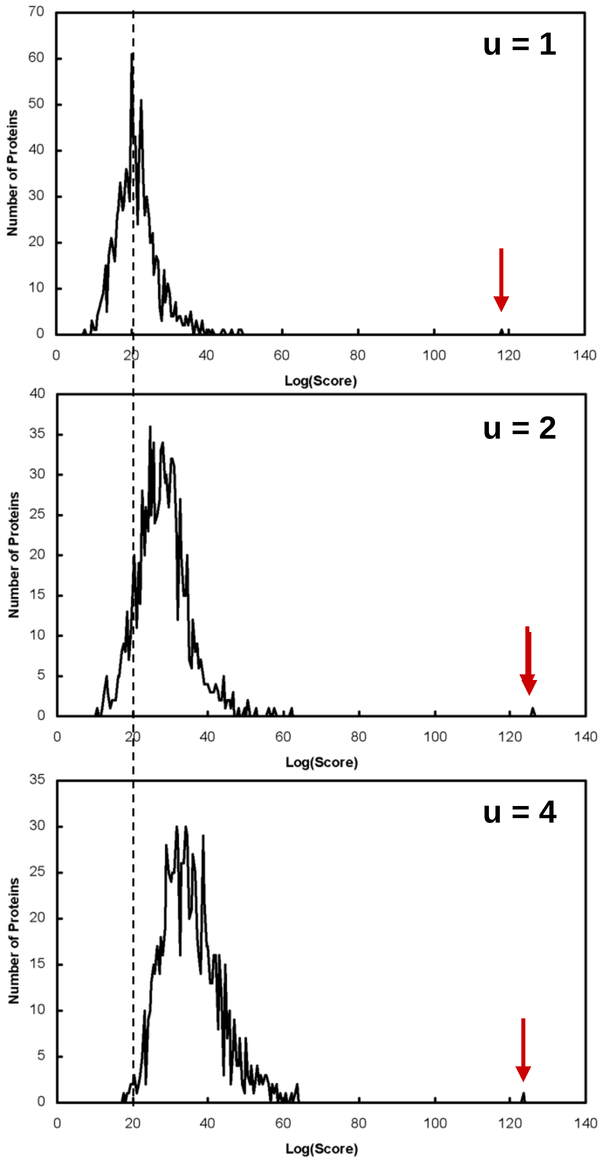


Missed Cleavage Sites

Expectation Values

Peptide mapping example:

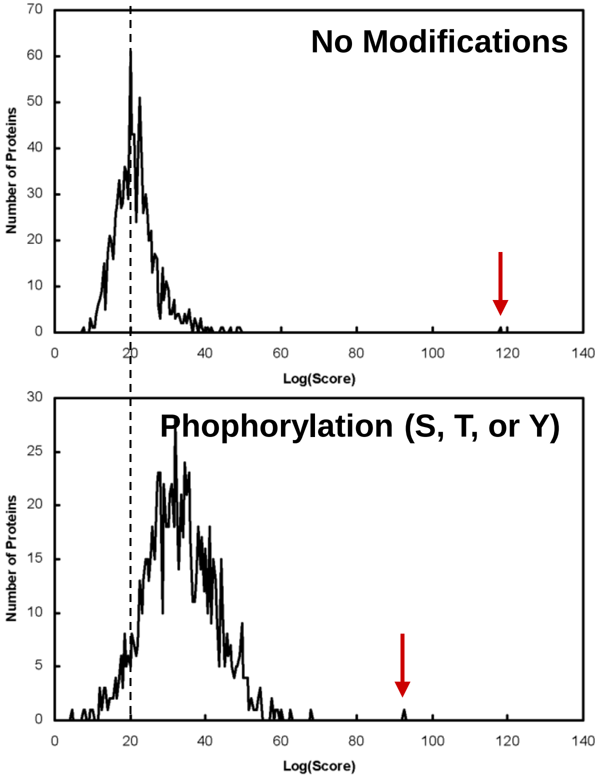
u=1	4.8e-7
u=2	1.1e-5
u=4	6.8e-4



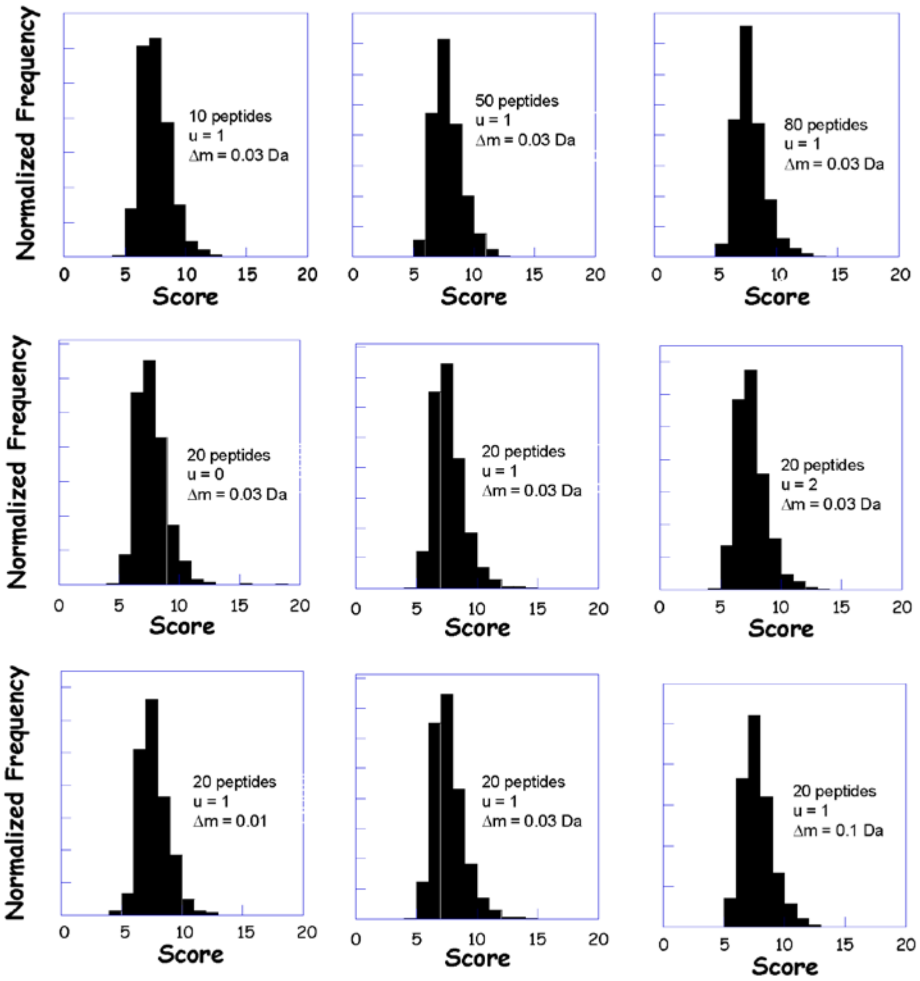
Peptide Mapping - Partial Modifications

	Searched Without Modifications	Searched With Possible Phosphorylation of S/T/Y
DARPP-32	0.00006	0.01
CFTR	0.00002	0.005

Even if the protein is modified it is usually better to search a protein sequence database without specifying possible modifications using peptide mapping data.



Peptide Mapping - Ranking by Direct Calculation of the Significance



General Criteria for a Good Protein Identification Algorithms

The response to random input data should be random.

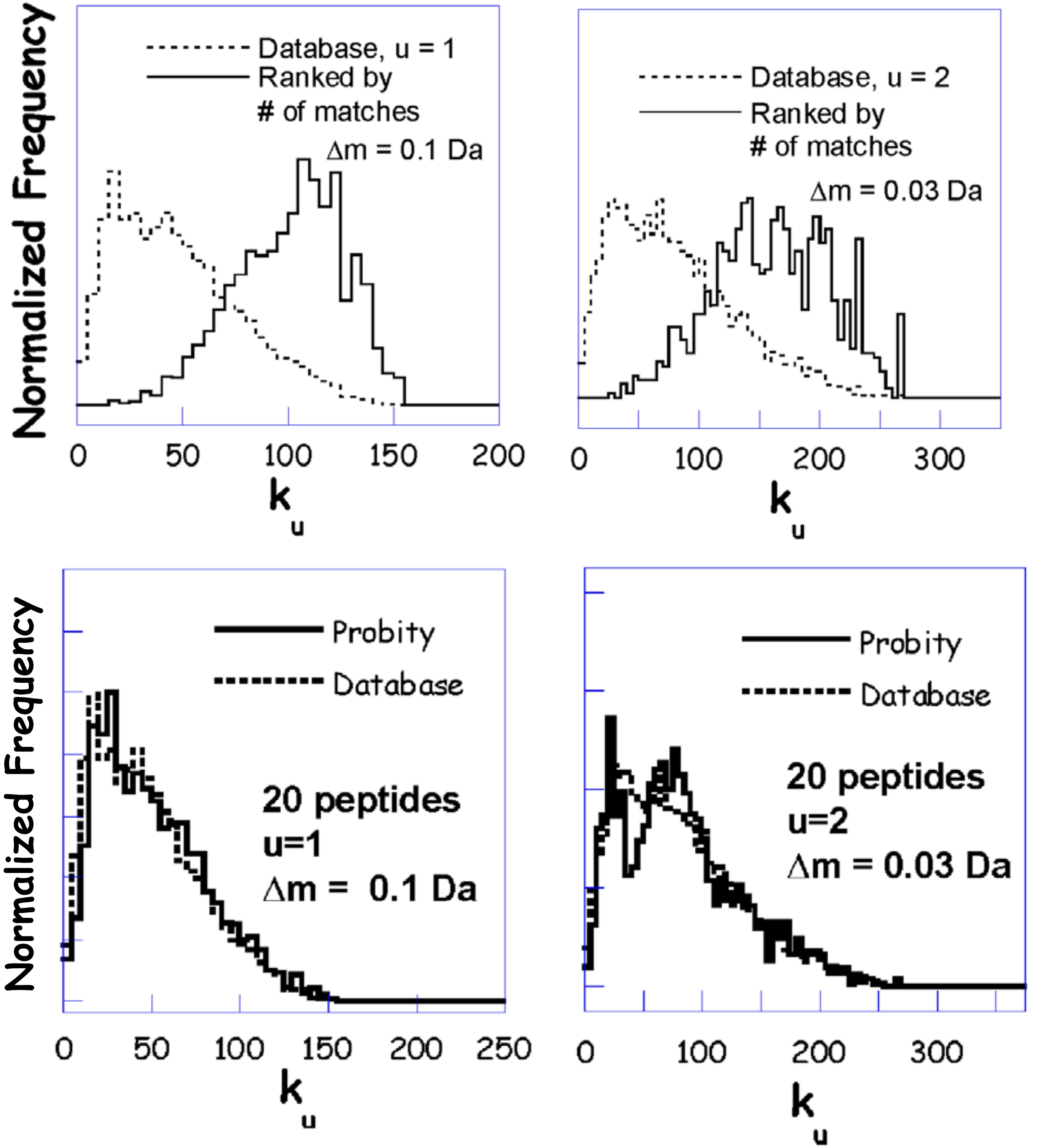
Maximum number of correct identification and minimum number of incorrect identifications for any data set.

Maximal separation between scores for correct identifications and the distribution of scores for random matching proteins for any data set.

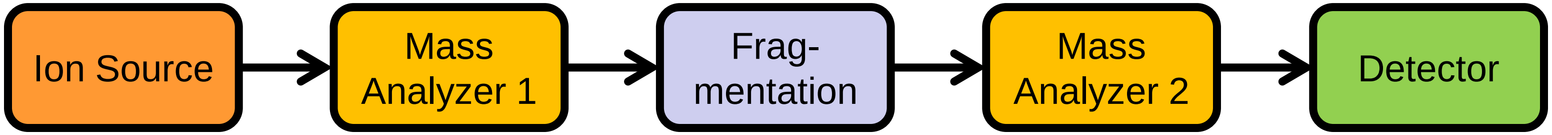
The statistical significance of the results should be calculated.

The searches should be fast.

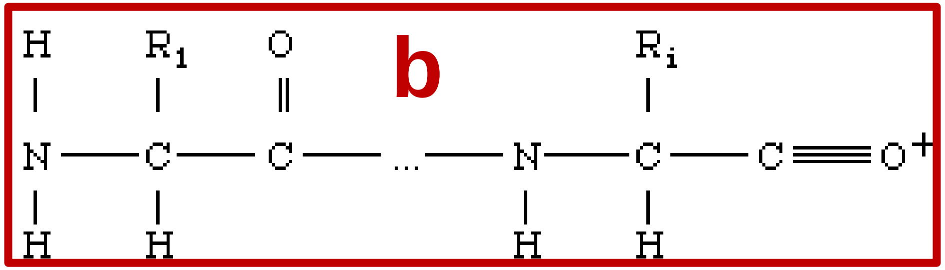
Response to Random Data

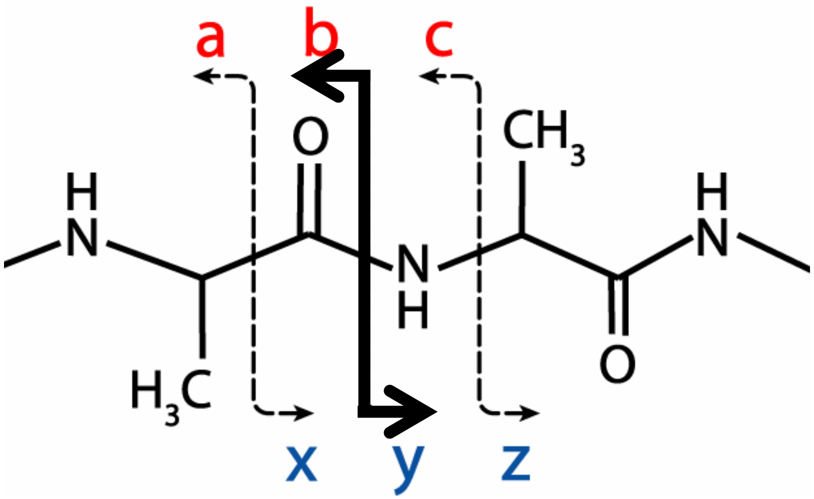


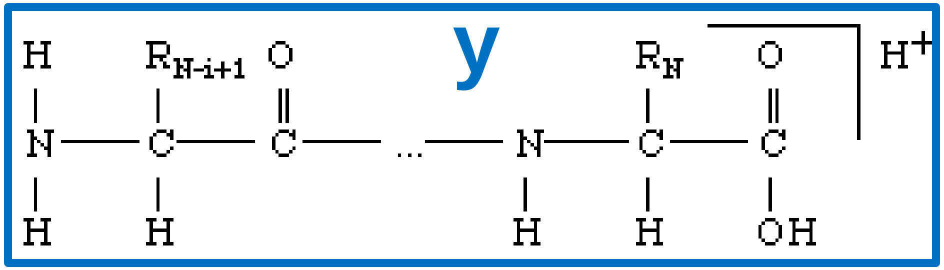
Peptide Fragmentation



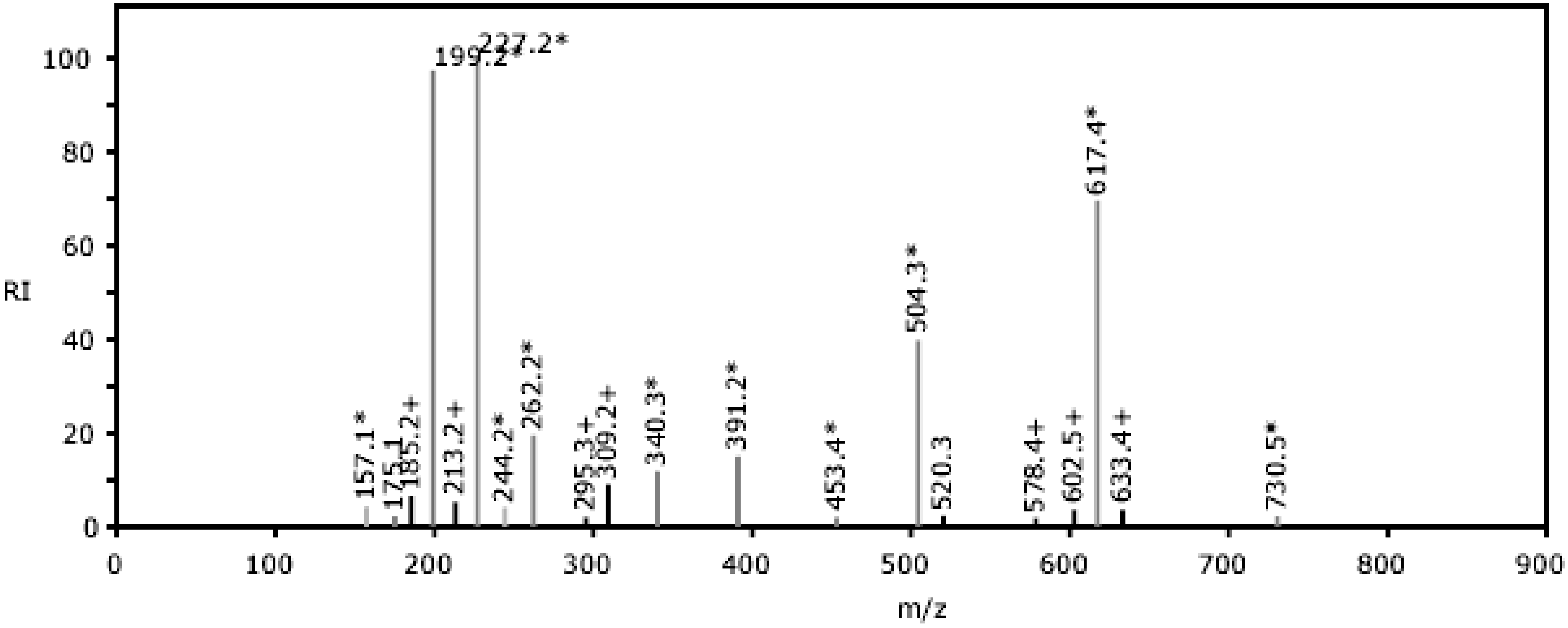
Ion Source → Mass Analyzer 1 → Frag-mentation → Mass Analyzer 2 → Detector





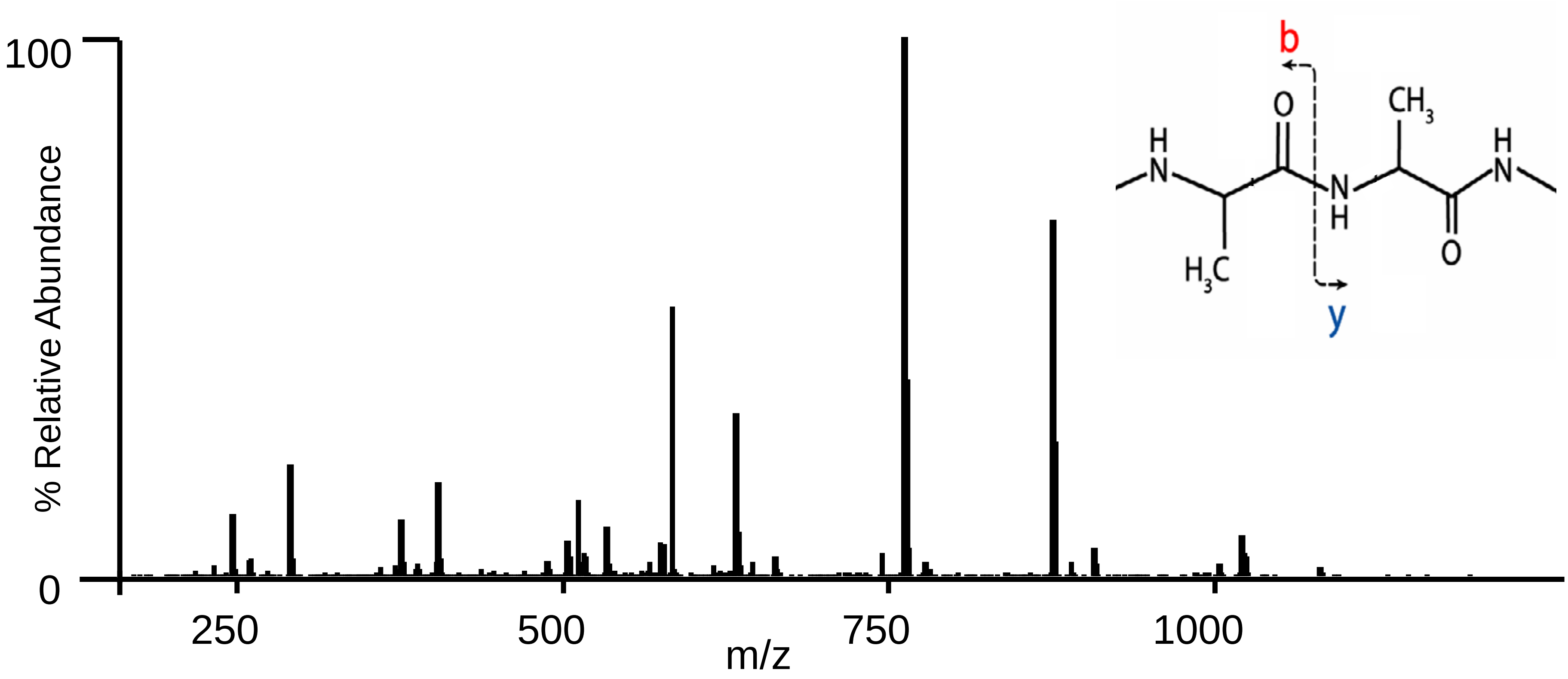


Identification – Tandem MS



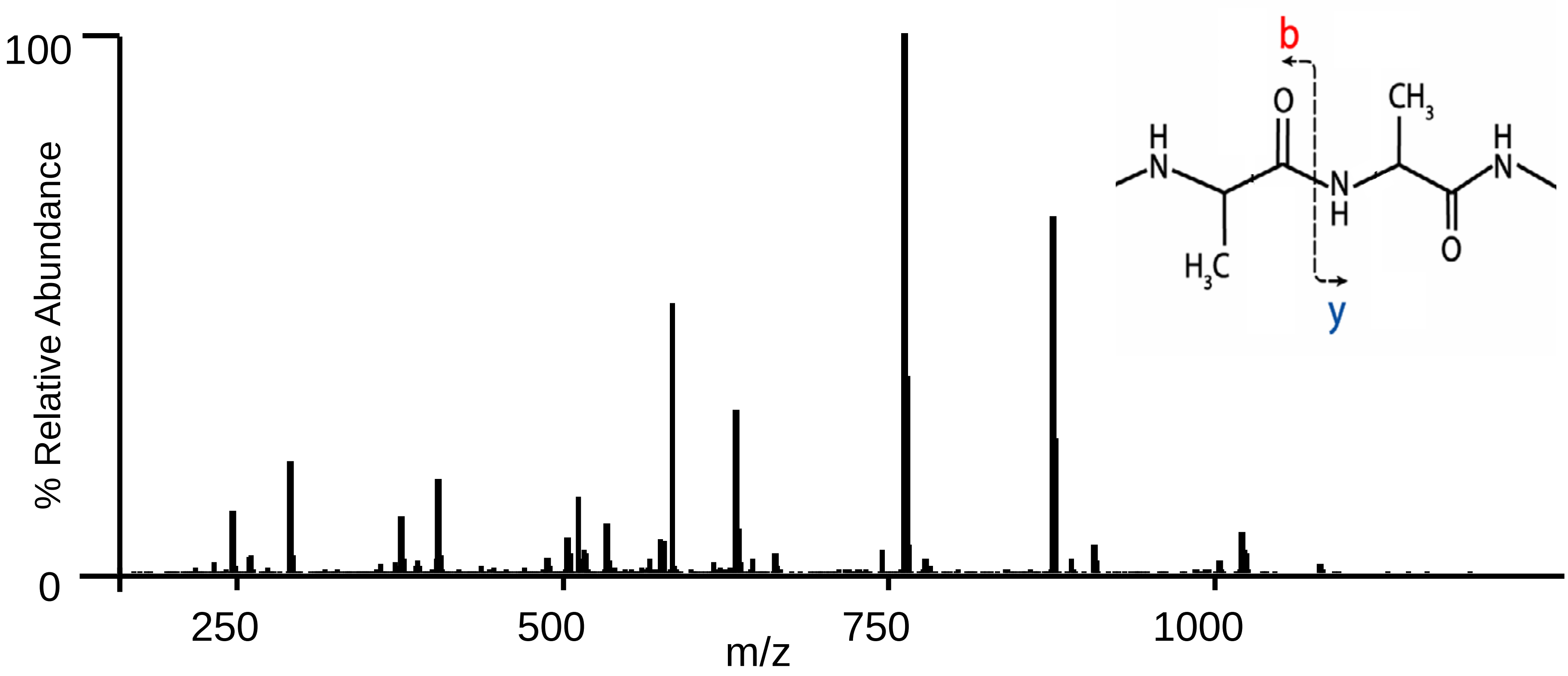
Tandem MS – Sequence Confirmation

S G F L E E D E L K



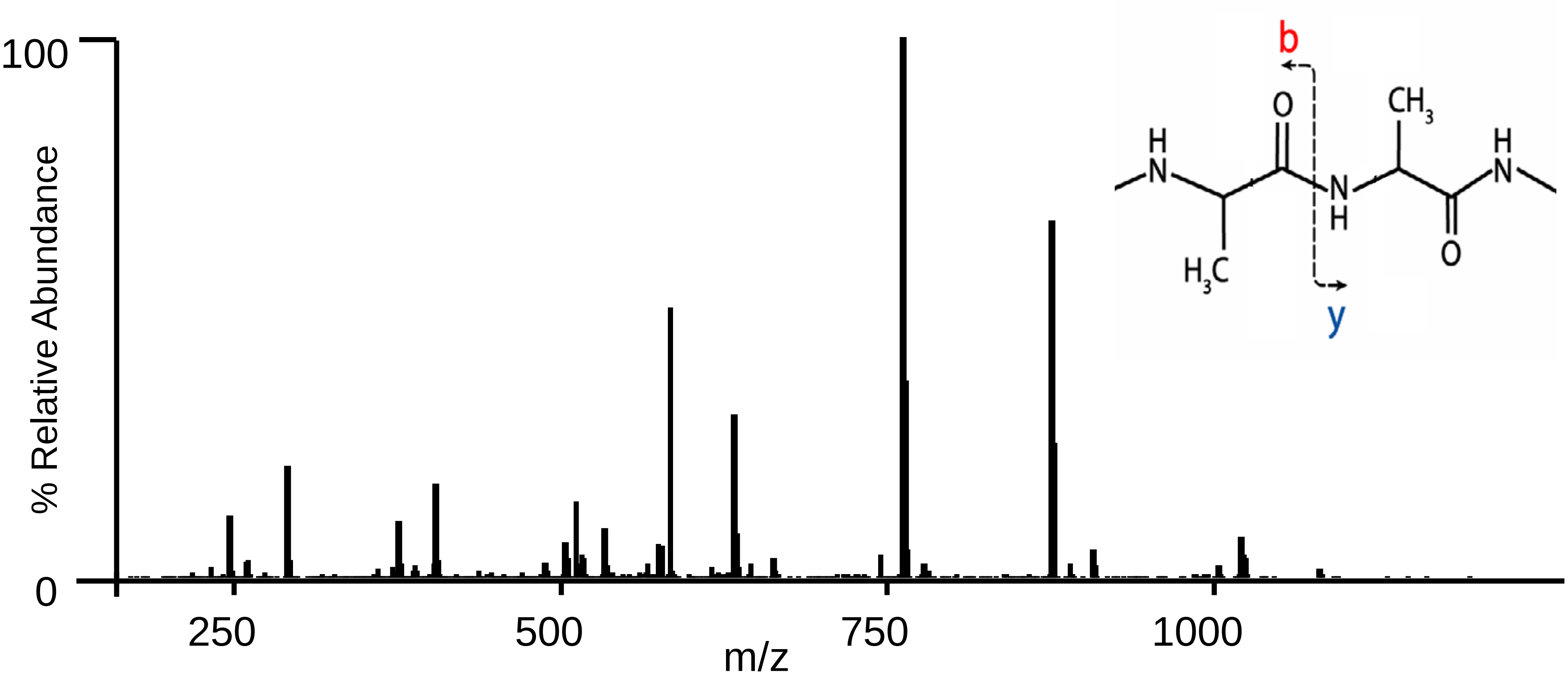
Tandem MS - Sequence Confirmation

S	G	F	L	E	E	D	E	L	K	
88	145	292	405	534	663	778	907	1020	1166	b ions



Tandem MS – Sequence Confirmation

S	G	F	L	E	E	D	E	L	K	
88	145	292	405	534	663	778	907	1020	1166	b ions
1166	1080	1022	875	762	633	504	389	260	147	y ions



Tandem MS – Sequence Confirmation

S	G	F	L	E	E	D	E	L	K	
88	145	292	405	534	663	778	907	1020	1166	b ions
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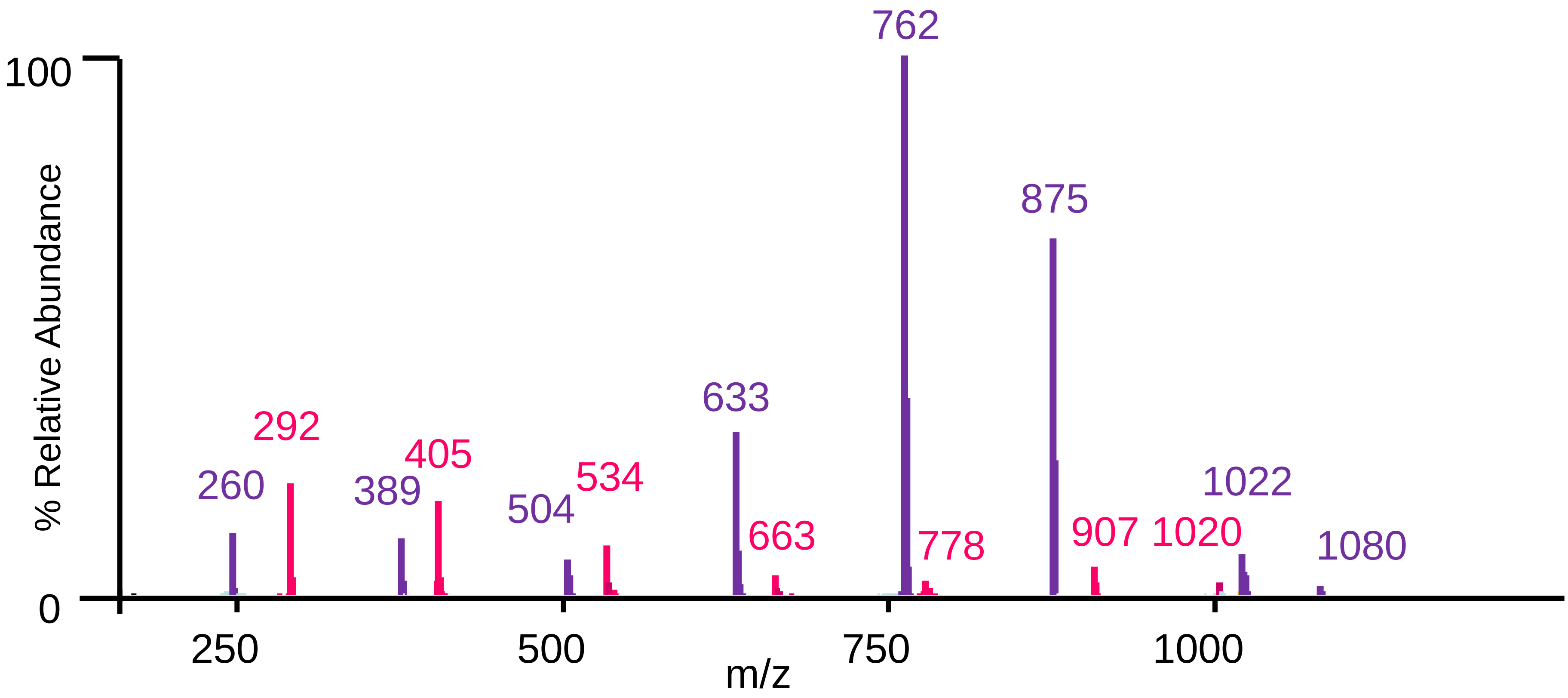

Tandem MS - Sequence Confirmation

S	G	F	L	E	E	D	E	L	K	
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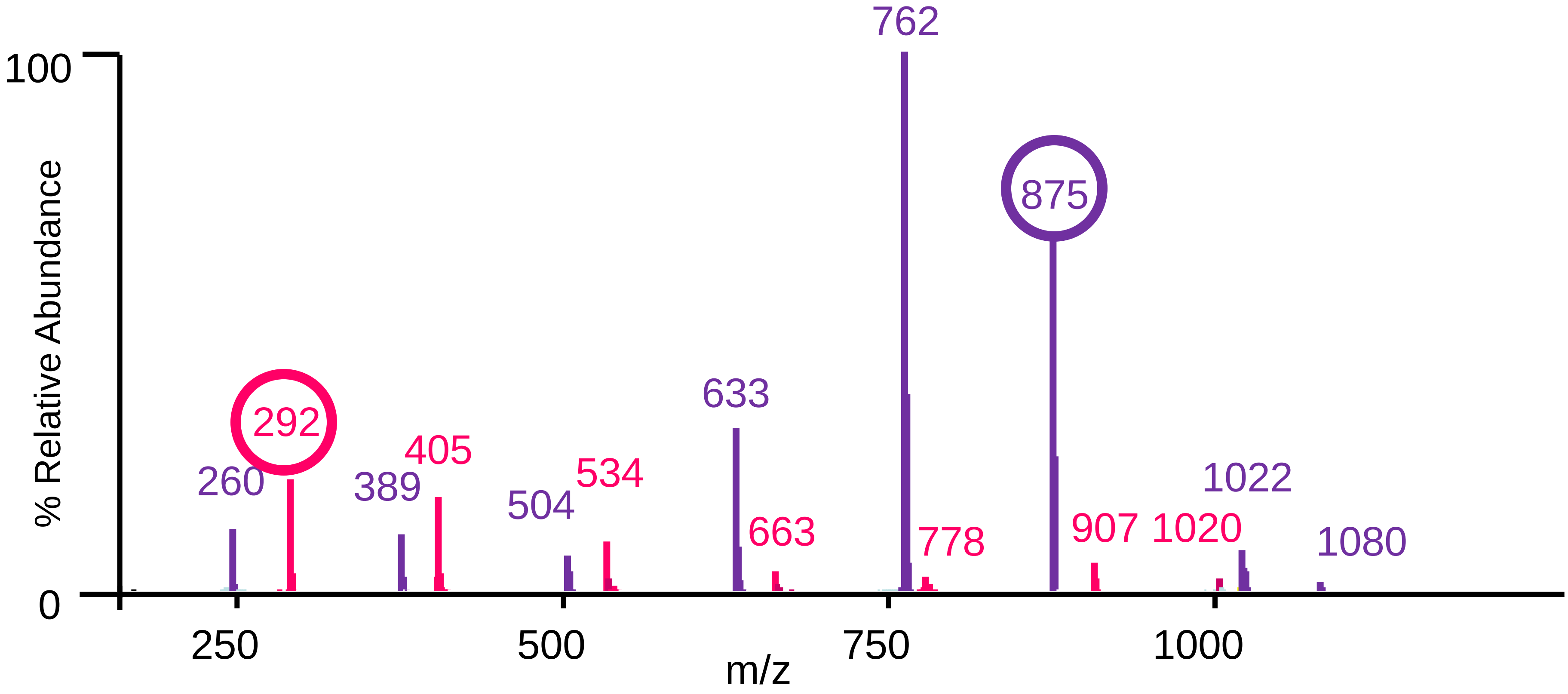

Tandem MS – Sequence Confirmation

S	G	F	L	E	E	D	E	L	K	
88	145	292	405	534	663	778	907	1020	1166	b ions
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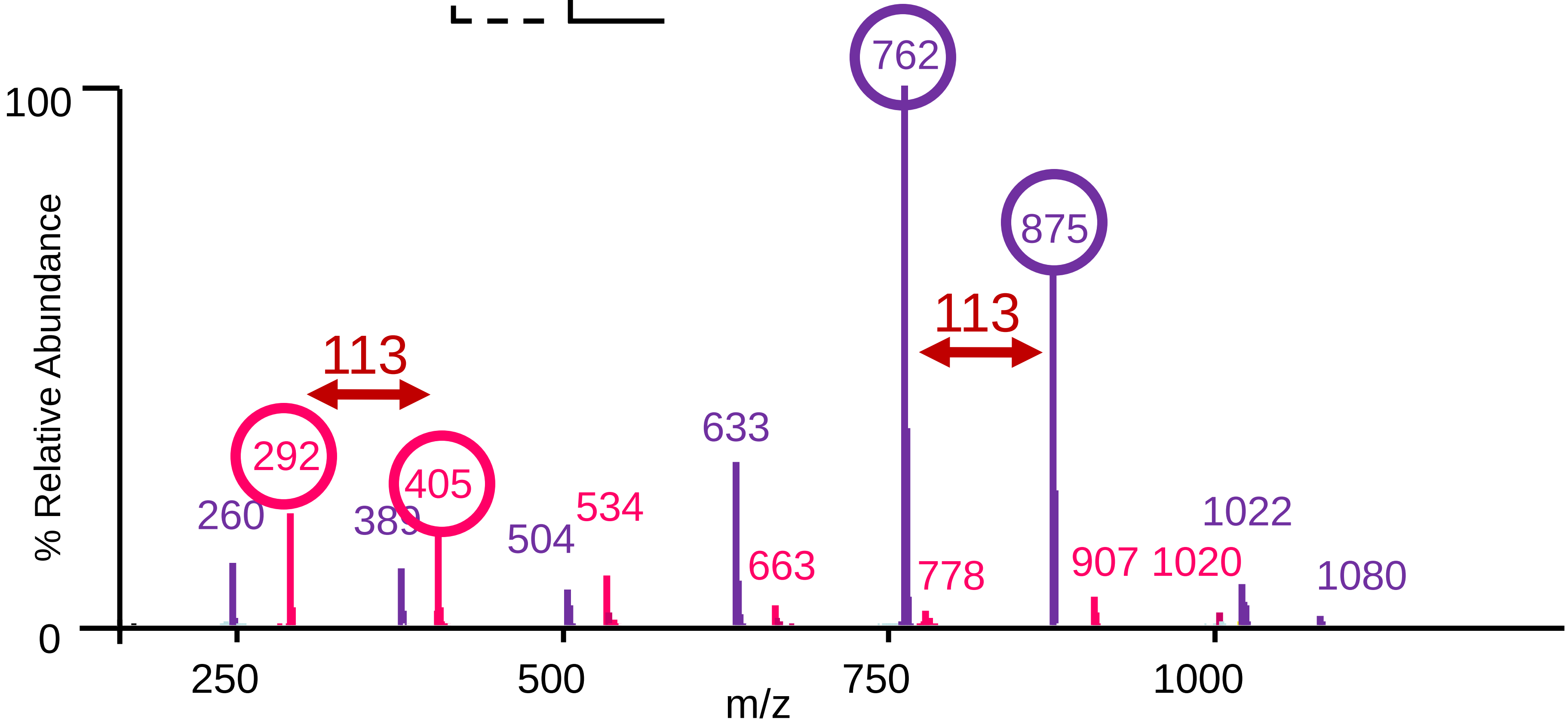

Tandem MS – Sequence Confirmation

S	G	F	L	E	E	D	E	L	K	
88	145	292	405	534	663	778	907	1020	1166	b ions
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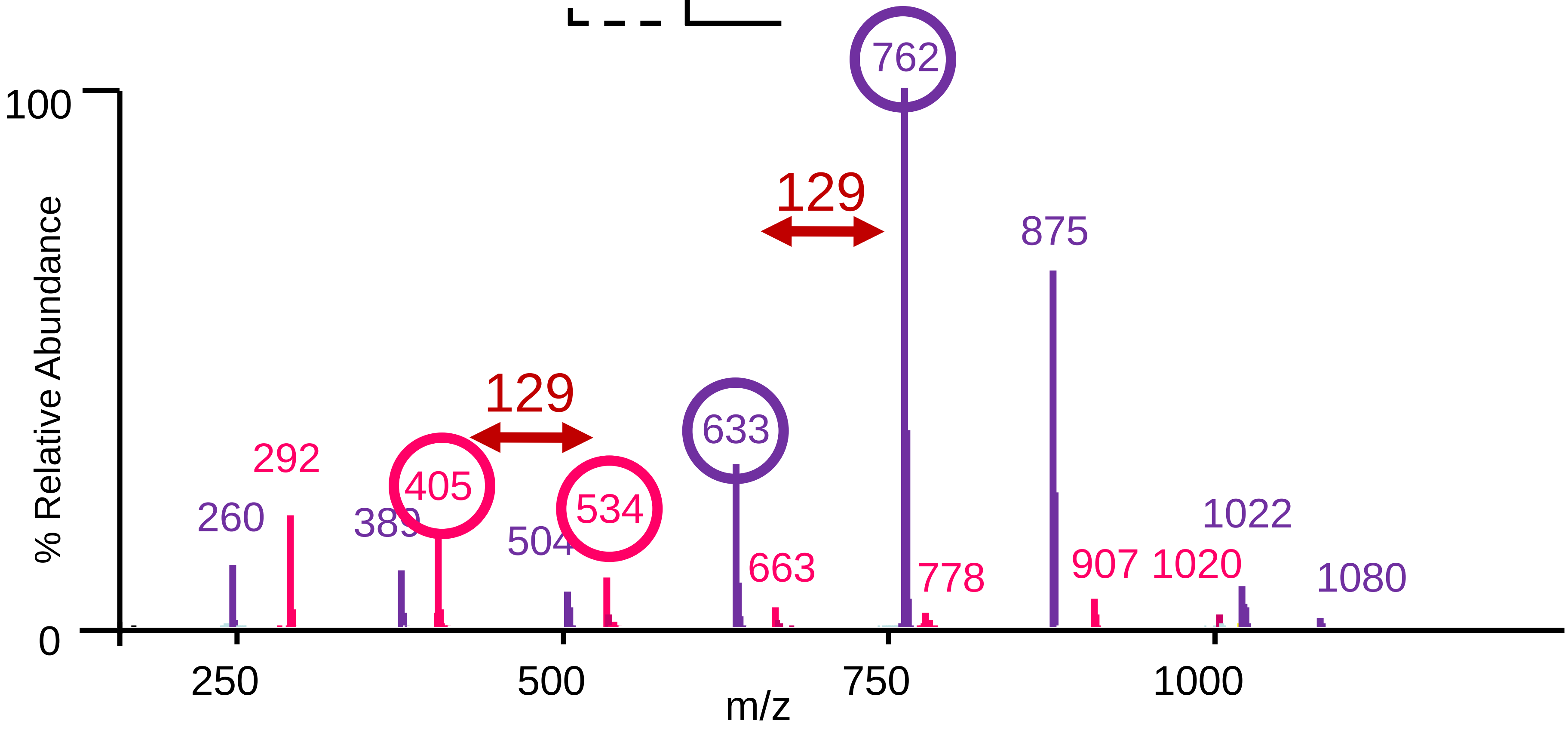
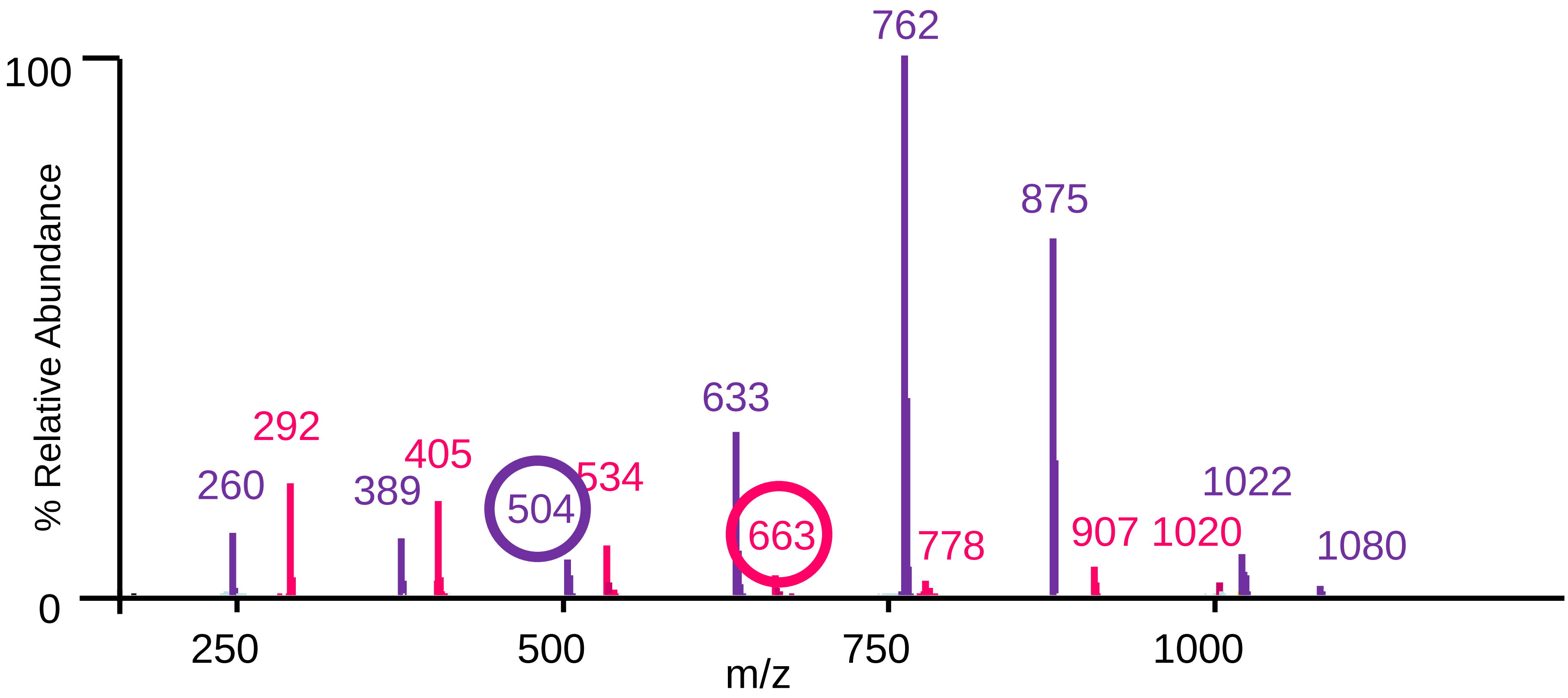


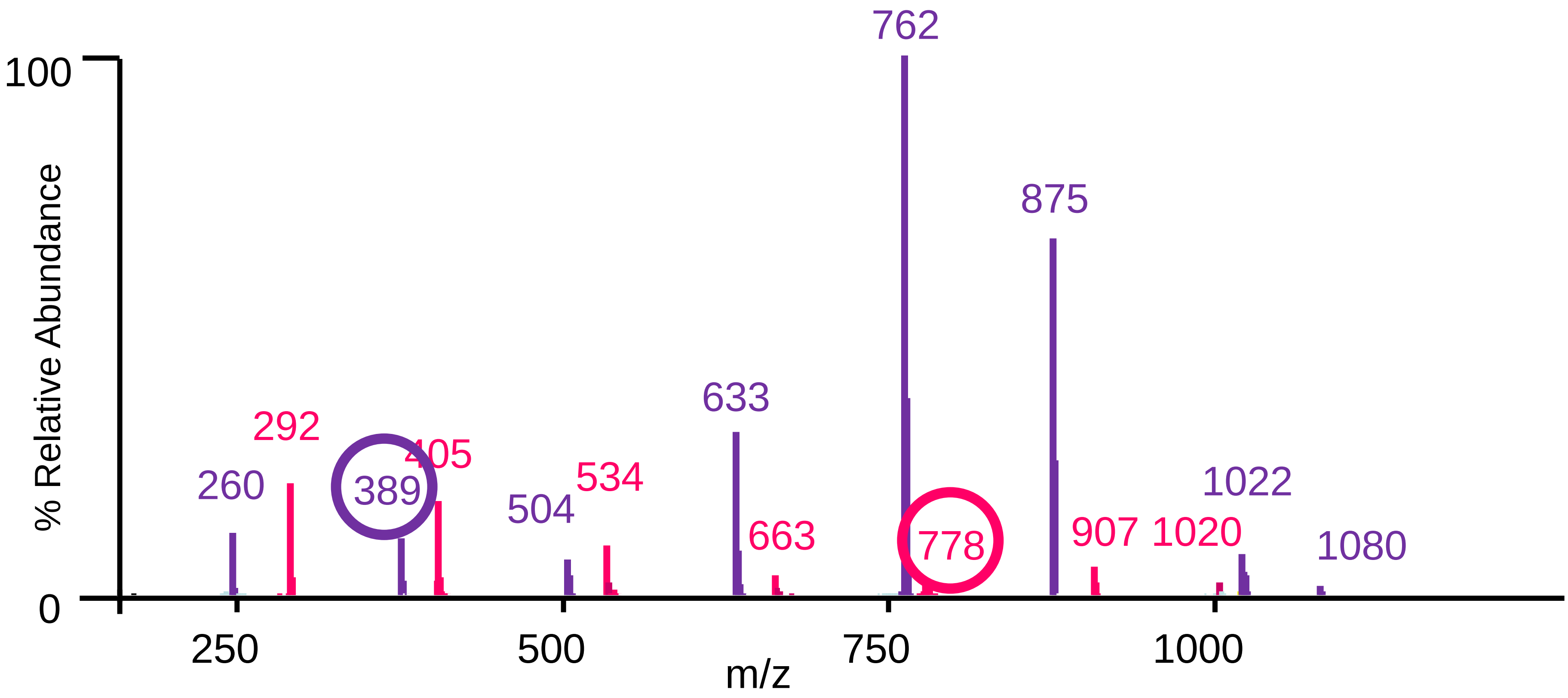
Tandem MS - Sequence Confirmation

S	G	F	L	E	E	D	E	L	K	
88	145	292	405	534	663	778	907	1020	1166	b ions
1166	1080	1022	875	762	633	504	389	260	147	y ions



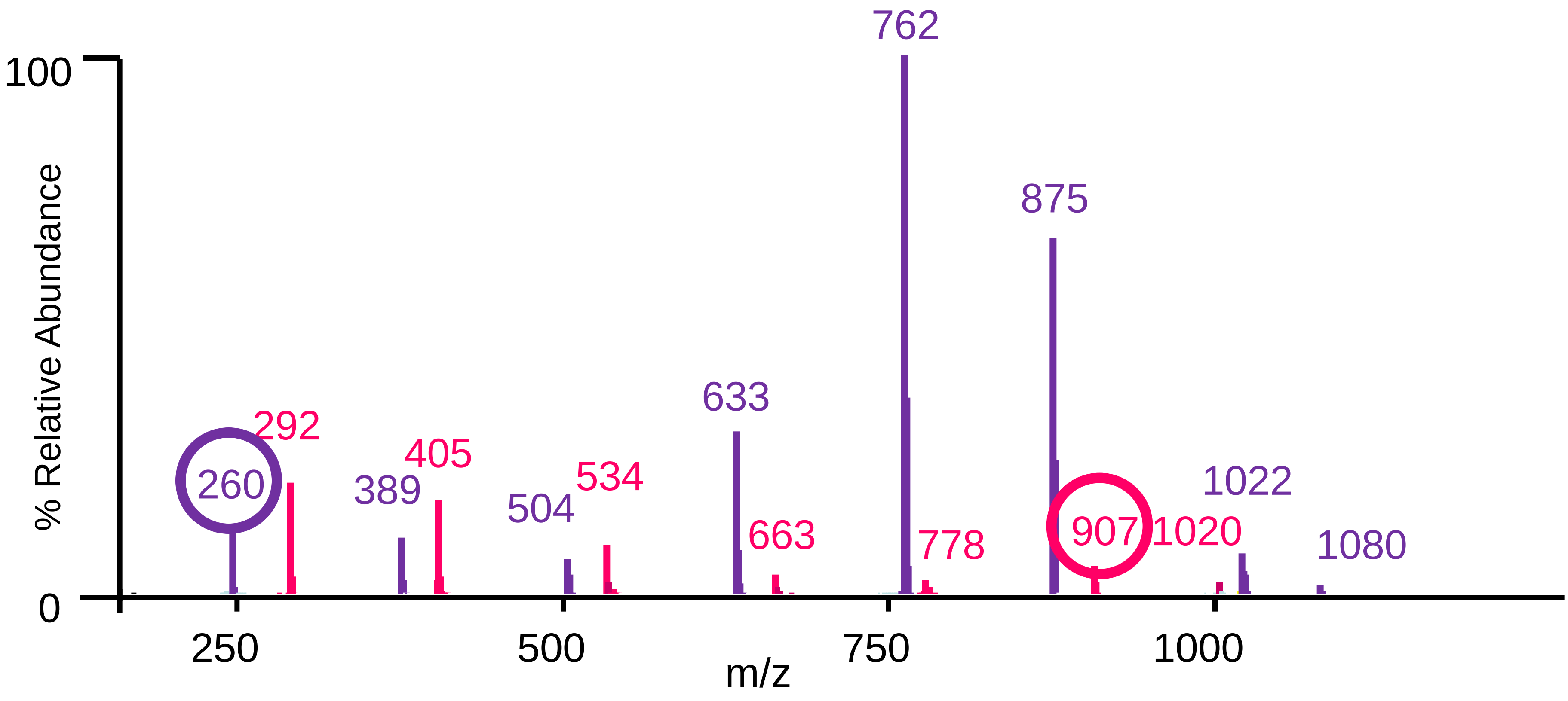
Tandem MS – Sequence Confirmation

S	G	F	L	E	E	D	E	L	K	
88	145	292	405	534	663	778	907	1020	1166	b ions
1166	1080	1022	875	762	633	504	389	260	147	y ions



Tandem MS - Sequence Confirmation

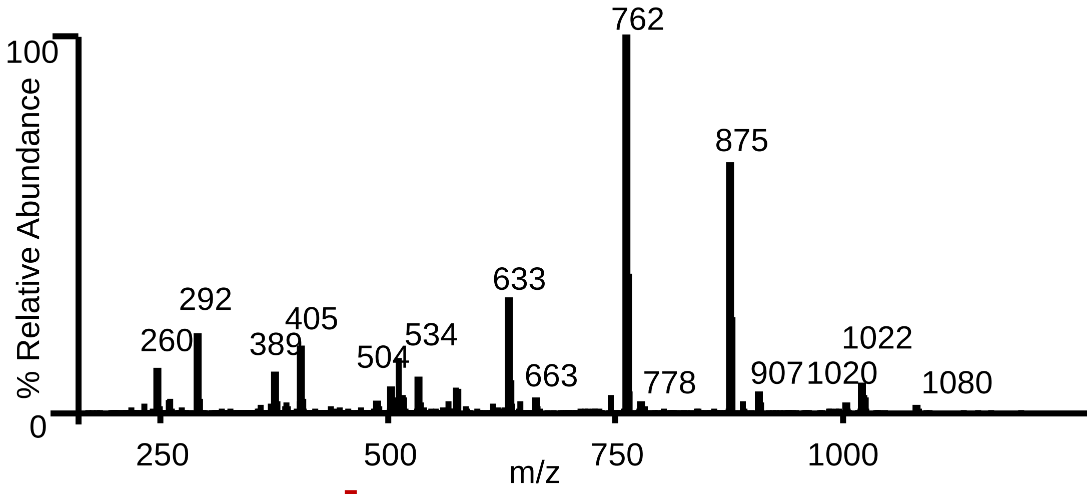
S	G	F	L	E	E	D	E	L	K	
88	145	292	405	534	663	778	907	1020	1166	b ions
1166	1080	1022	875	762	633	504	389	260	147	y ions



Tandem MS – de novo Sequencing

Amino acid masses

1-letter code	3-letter code	Chemical formula	Monoisotopic	Average
A	Ala	C_3H_5ON	71.0371	71.0788
R	Arg	$C_6H_{12}ON_4$	156.101	156.188
N	Asn	$C_4H_6O_2N_2$	114.043	114.104
D	Asp	$C_4H_5O_3N$	115.027	115.089
C	Cys	C_3H_5ONS	103.009	103.139
E	Glu	$C_5H_7O_3N$	129.043	129.116
Q	Gln	$C_5H_8O_2N_2$	128.059	128.131
G	Gly	C_2H_3ON	57.0215	57.0519
H	His	$C_6H_7ON_3$	137.059	137.141
I	Ile	$C_6H_{11}ON$	113.084	113.159
L	Leu	$C_6H_{11}ON$	113.084	113.159
K	Lys	$C_6H_{12}ON_2$	128.095	128.174
M	Met	C_5H_9ONS	131.04	131.193
F	Phe	C_9H_9ON	147.068	147.177
P	Pro	C_5H_7ON	97.0528	97.1167
S	Ser	$C_3H_5O_2N$	87.032	87.0782
T	Thr	$C_4H_7O_2N$	101.048	101.105
W	Trp	$C_{11}H_{10}ON_2$	186.079	186.213
Y	Tyr	$C_9H_9O_2N$	163.063	163.176
V	Val	C_5H_9ON	99.0684	99.1326



Mass Differences

Sequences consistent with spectrum

Tandem MS – de novo Sequencing

	260	292	389	405	504	534	633	663	762	778	875	907	1020	1022	1079
260		32	129	145	244	274	373	403	502	518	615	647	760	762	819
292			97	113	212	242	341	371	470	486	583	615	728	730	787
389				16	115	145	244	274	373	389	486	518	631	633	690
405					99	129	228	258	357	373	470	502	615	617	674
504						30	129	159	258	274	371	403	516	518	575
534							99	129	228	244	341	373	486	488	545
633								30	129	145	242	274	387	389	446
663									99	115	212	244	357	359	416
762										16	113	145	258	260	317
778											97	129	242	244	301
875												32	145	147	204
907													113	115	172
1020														2	59
1022															57

Tandem MS – de novo Sequencing

	260	292	389	405	504	534	633	663	762	778	875	907	1020	1022	1079
260		32	**129**	145	244	274	373	403	502	518	615	647	760	762	819
292			**97**	**113**	212	242	341	371	470	486	583	615	728	730	787
389				16	**115**	145	244	274	373	389	486	518	631	633	690
405					**99**	**129**	228	258	357	373	470	502	615	617	674
504						30	**129**	159	258	274	371	403	516	518	575
534							**99**	**129**	228	244	341	373	486	488	545
633								30	**129**	145	242	274	387	389	446
663									**99**	**115**	212	244	357	359	416
762										16	**113**	145	258	260	317
778											**97**	**129**	242	244	301
875												32	145	**147**	204
907													**113**	**115**	172
1020														2	59
1022															**57**

Tandem MS – de novo Sequencing

	260	292	389	405	504	534	633	663	762	778	875	907	1020	1022	1079
260		32	E	145	244	274	373	403	502	518	615	647	760	762	819
292			~~P~~	I/L	212	242	341	371	470	486	583	615	728	730	787
389				16	D	145	244	274	373	389	486	518	631	633	690
405					~~V~~	E	228	258	357	373	470	502	615	617	674
504						30	E	159	258	274	371	403	516	518	575
534							~~V~~	E	228	244	341	373	486	488	545
633								30	E	145	242	274	387	389	446
663									~~V~~	D	212	244	357	359	416
762										16	I/L	145	258	260	317
778											~~P~~	E	242	244	301
875												32	145	F	204
907													I/L	~~D~~	172
1020														2	59
1022															G

SGF(I/L)EEDE(I/L)…

1166 – 1020 – 18 = 128
⇒K or Q

SGF(I/L)EEDE(I/L)(K/Q)

Tandem MS – de novo Sequencing

Challenges in de novo sequencing

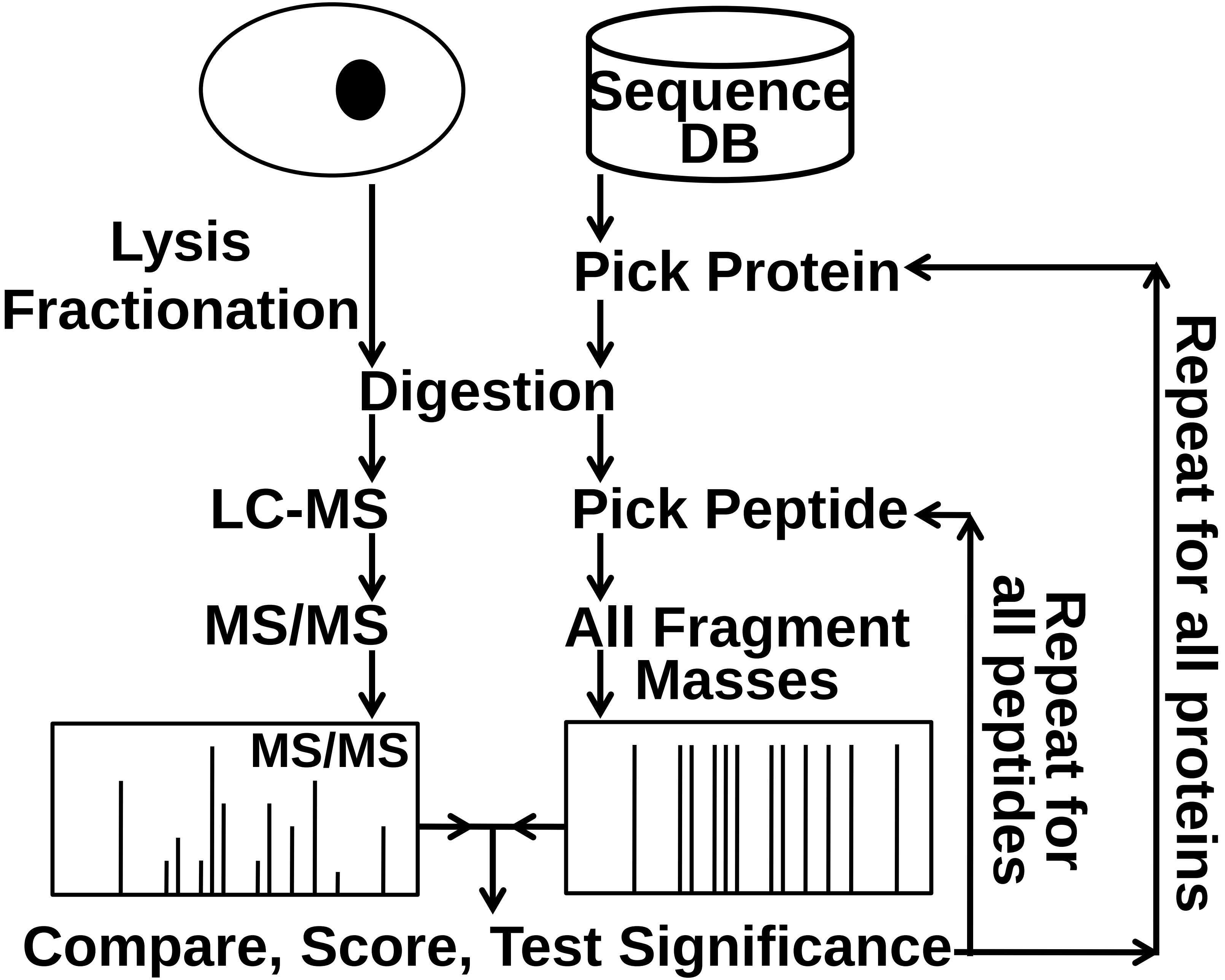
Neutral loss (-H_2O, -NH_3)

Modifications

Background peaks

Incomplete information

Tandem MS - Database Search



Sequence DB

Lysis

Fractionation

Pick Protein

Digestion

LC-MS

Pick Peptide

MS/MS

All Fragment Masses

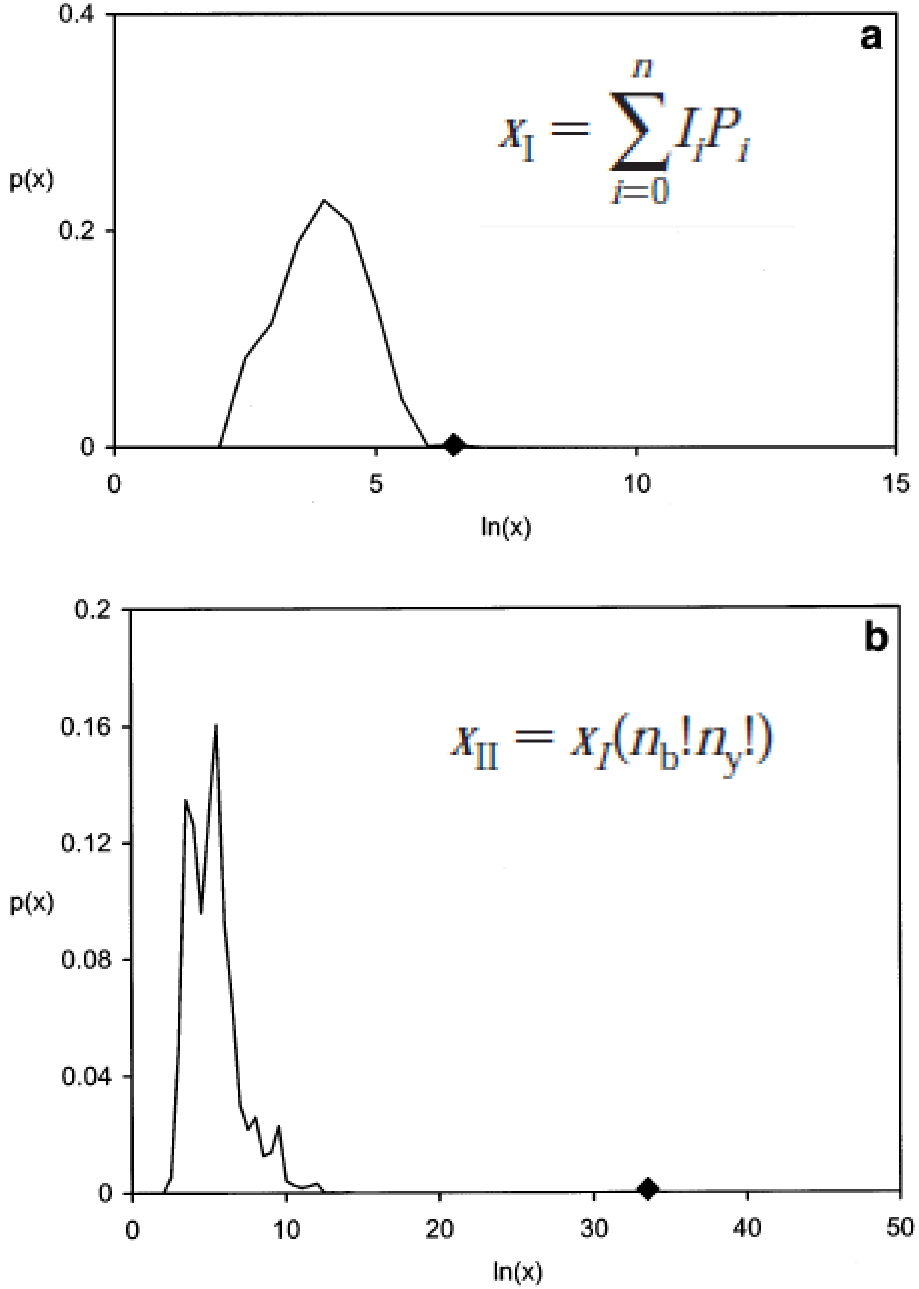
MS/MS

Compare, Score, Test Significance

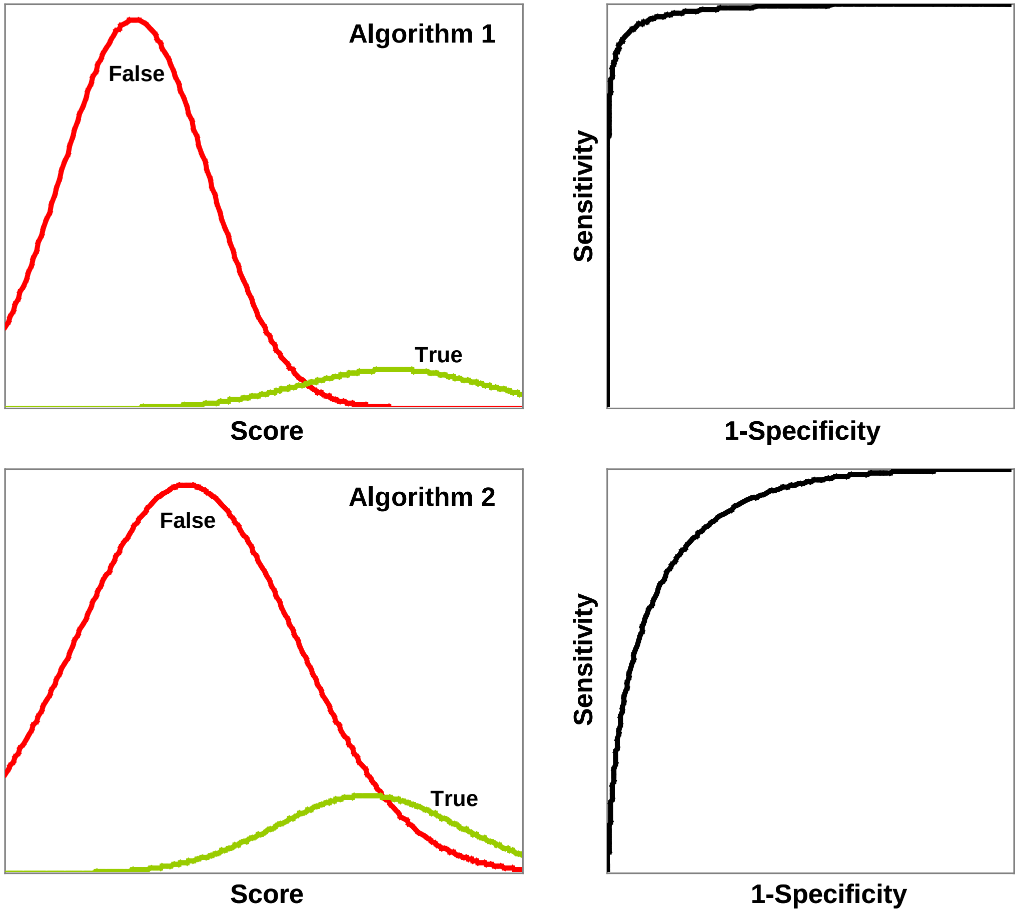
Repeat for all peptides

Repeat for all proteins

Algorithms


a
$x_{\mathrm{I}} = \sum_{i=0}^{n} I_i P_i$
p(x)
0.4
0.2
0
0
5
10
15
ln(x)
b
$x_{\mathrm{II}} = x_I(n_{\mathrm{b}}!n_{\mathrm{y}}!)$
p(x)
0.2
0.16
0.12
0.08
0.04
0
0
10
20
30
40
50
ln(x)

Comparing and Optimizing Algorithms

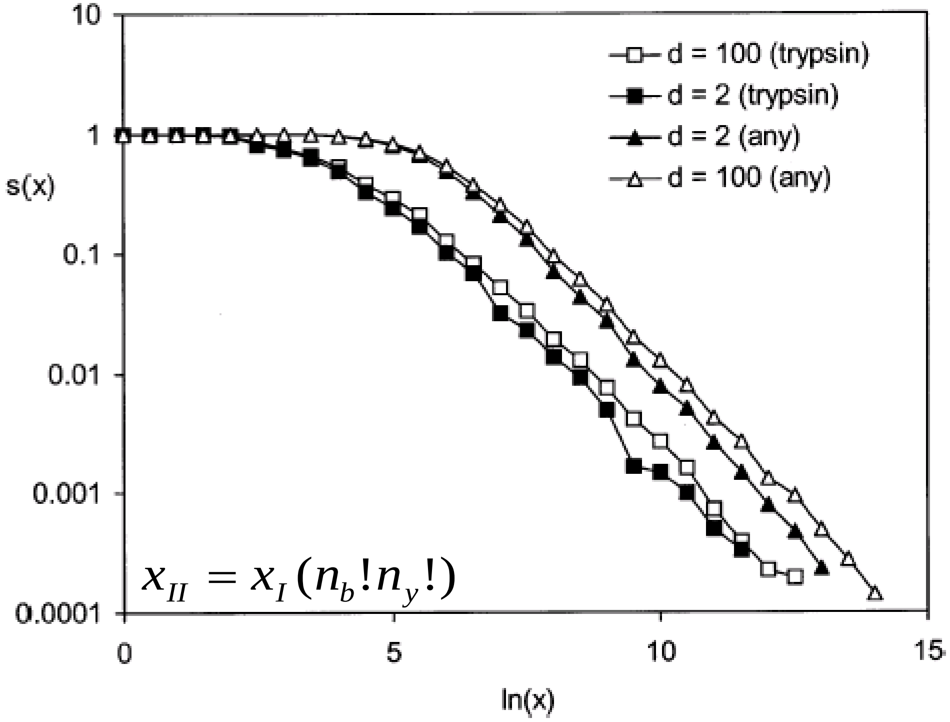


MS/MS - Parent Mass Error and Enzyme Specificity

Expectation Values

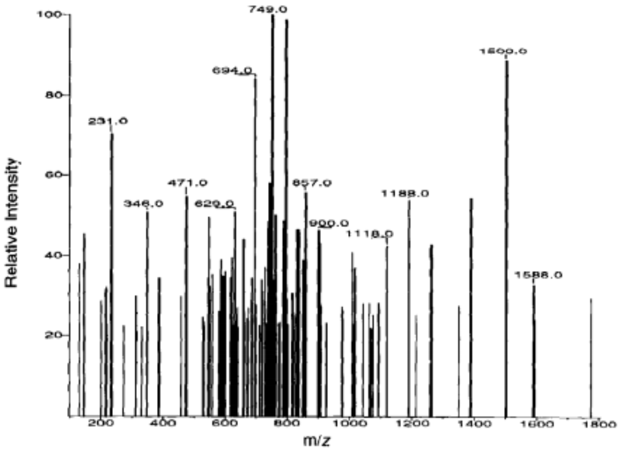
MS/MS example:

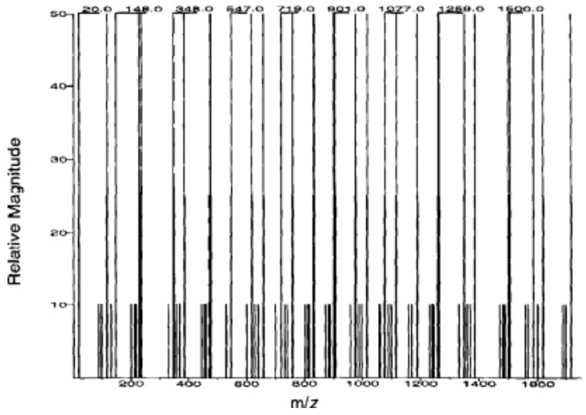
Δm=2, Trypsin	2.5e-5
Δm=100, Trypsin	2.5e-5
Δm=2, non-specific	7.9e-5
Δm=100, non-specific	1.6e-4



$$x_{II} = x_I(n_b!n_y!)$$

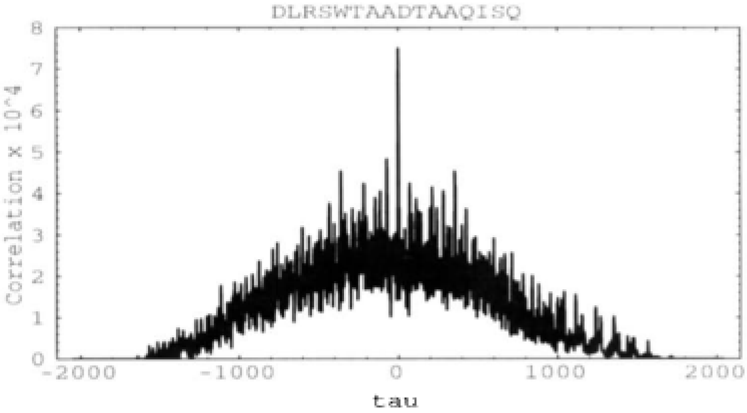
Sequest





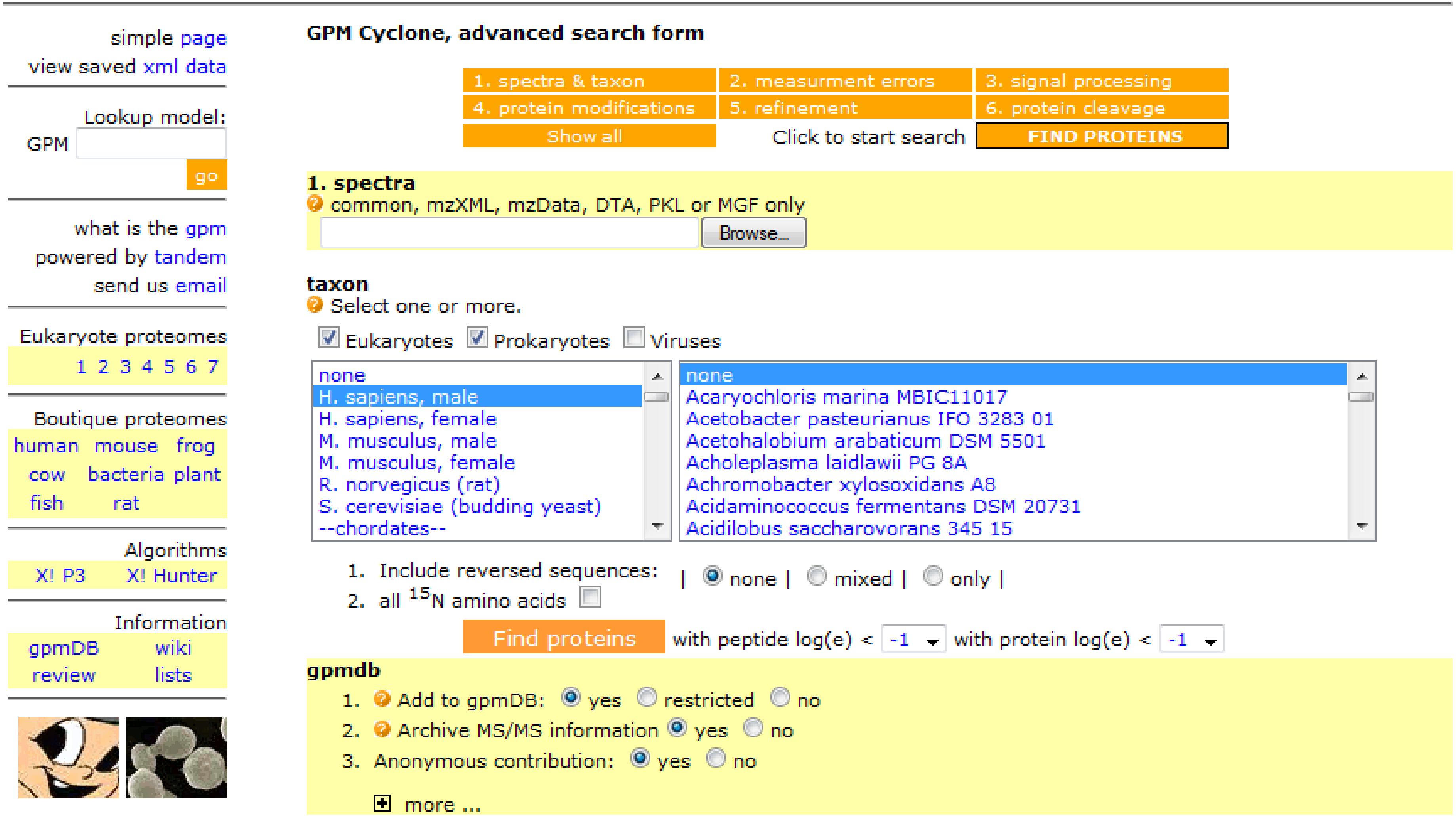
Cross-correlation

$$R_\tau = \sum_{i=0}^{n-1} x[i]\,y[i+\tau]$$



X! Tandem - Search Parameters

http://www.thegpm.org/



simple page
view saved xml data

Lookup model:
GPM
go

what is the gpm
powered by tandem
send us email

Eukaryote proteomes
1 2 3 4 5 6 7

Boutique proteomes
human mouse frog
cow bacteria plant
fish rat

Algorithms
X! P3 X! Hunter

Information
gpmDB wiki
review lists

GPM Cyclone, advanced search form

1. spectra & taxon	2. measurment errors	3. signal processing
4. protein modifications	5. refinement	6. protein cleavage
Show all	Click to start search	FIND PROTEINS

1. spectra
? common, mzXML, mzData, DTA, PKL or MGF only
Browse...

taxon
? Select one or more.

☑ Eukaryotes ☑ Prokaryotes □ Viruses

none
H. sapiens, male
H. sapiens, female
M. musculus, male
M. musculus, female
R. norvegicus (rat)
S. cerevisiae (budding yeast)
--chordates--

none
Acaryochloris marina MBIC11017
Acetobacter pasteurianus IFO 3283 01
Acetohalobium arabaticum DSM 5501
Acholeplasma laidlawii PG 8A
Achromobacter xylosoxidans A8
Acidaminococcus fermentans DSM 20731
Acidilobus saccharovorans 345 15

1. Include reversed sequences: | ● none | ○ mixed | ○ only |
2. all ^{15}N amino acids □

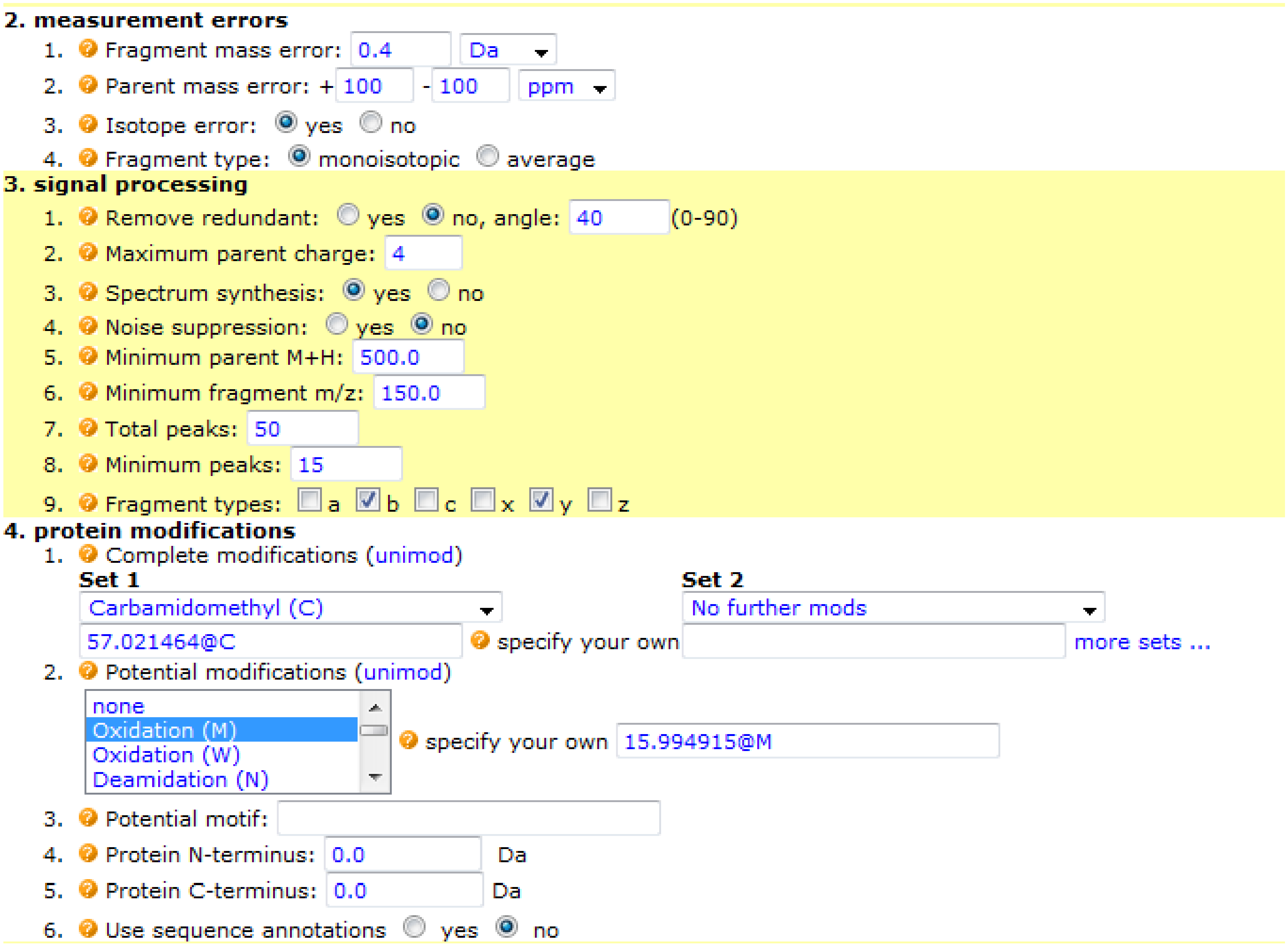
Find proteins with peptide log(e) < -1 with protein log(e) < -1

gpmdb

1. ? Add to gpmDB: ● yes ○ restricted ○ no
2. ? Archive MS/MS information ● yes ○ no
3. Anonymous contribution: ● yes ○ no

⊞ more ...

X! Tandem - Search Parameters



2. measurement errors

1. Fragment mass error: 0.4 Da
2. Parent mass error: + 100 - 100 ppm
3. Isotope error: ● yes ○ no
4. Fragment type: ● monoisotopic ○ average

3. signal processing

1. Remove redundant: ○ yes ● no, angle: 40 (0-90)
2. Maximum parent charge: 4
3. Spectrum synthesis: ● yes ○ no
4. Noise suppression: ○ yes ● no
5. Minimum parent M+H: 500.0
6. Minimum fragment m/z: 150.0
7. Total peaks: 50
8. Minimum peaks: 15
9. Fragment types: □ a ☑ b □ c □ x ☑ y □ z

4. protein modifications

1. Complete modifications (unimod)

   Set 1
   Carbamidomethyl (C)
   57.021464@C

   specify your own

   Set 2
   No further mods

   more sets ...

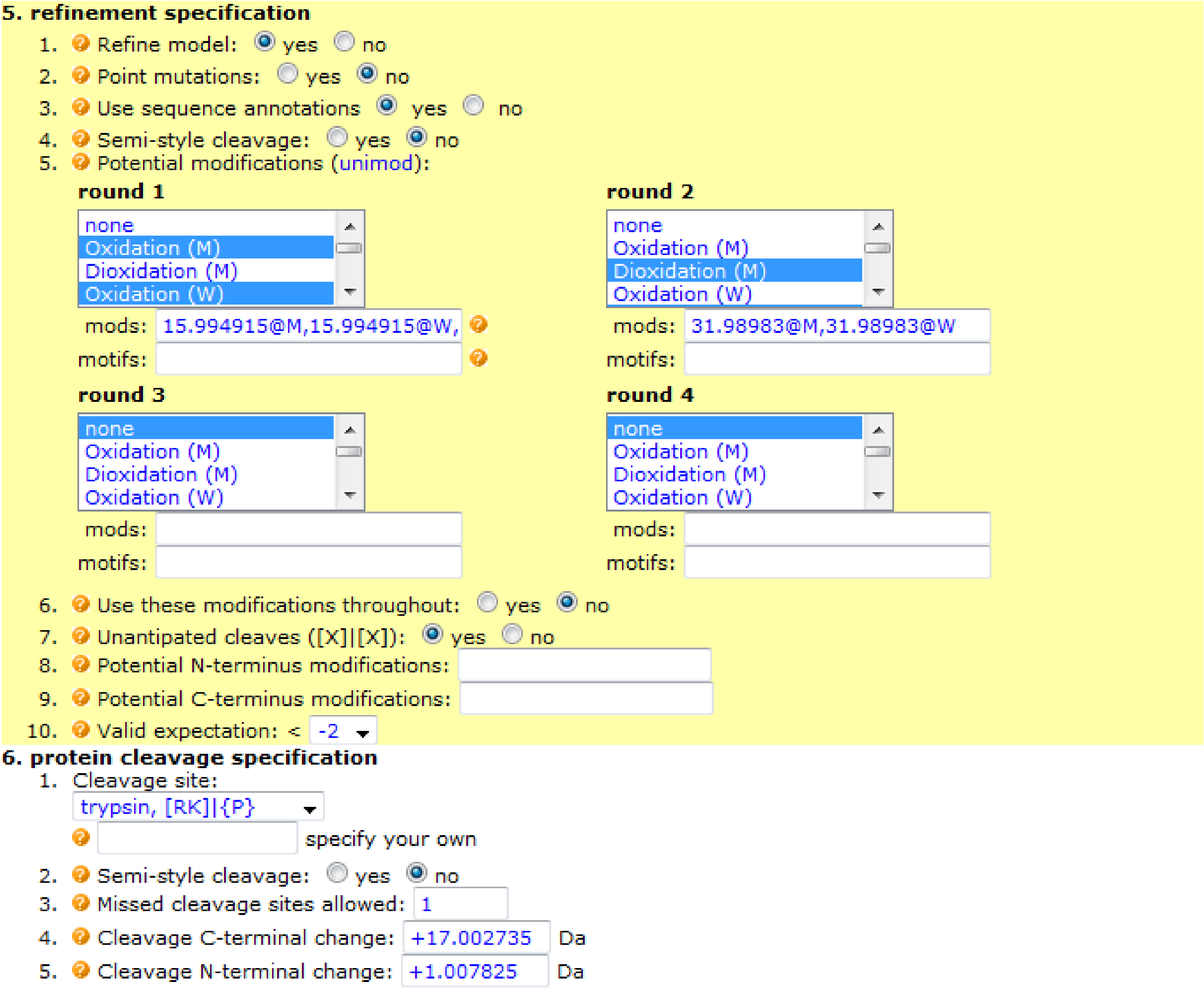
2. Potential modifications (unimod)

   none
   Oxidation (M)
   Oxidation (W)
   Deamidation (N)

   specify your own 15.994915@M

3. Potential motif:
4. Protein N-terminus: 0.0 Da
5. Protein C-terminus: 0.0 Da
6. Use sequence annotations ○ yes ● no

X! Tandem - Search Parameters



5. refinement specification

1. Refine model: ● yes ○ no
2. Point mutations: ○ yes ● no
3. Use sequence annotations ● yes ○ no
4. Semi-style cleavage: ○ yes ● no
5. Potential modifications (unimod):

round 1

none
[✓ Oxidation (M)]
Dioxidation (M)
[✓ Oxidation (W)]

mods: 15.994915@M,15.994915@W,

motifs:

round 2

none
Oxidation (M)
[✓ Dioxidation (M)]
Oxidation (W)

mods: 31.98983@M,31.98983@W

motifs:

round 3

[✓ none]
Oxidation (M)
Dioxidation (M)
Oxidation (W)

mods:

motifs:

round 4

[✓ none]
Oxidation (M)
Dioxidation (M)
Oxidation (W)

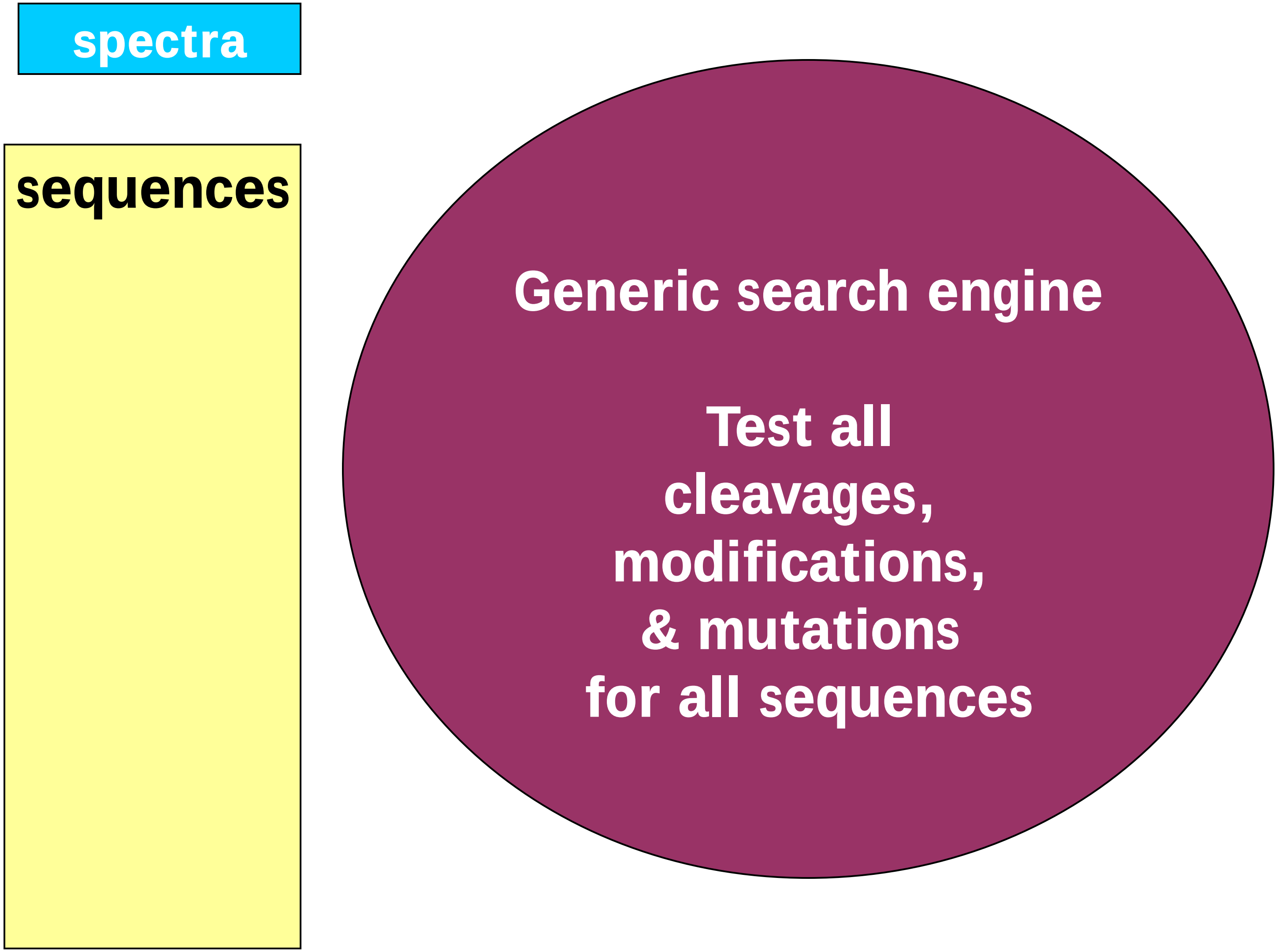
mods:

motifs:

6. Use these modifications throughout: ○ yes ● no
7. Unantipated cleaves ([X]|[X]): ● yes ○ no
8. Potential N-terminus modifications:
9. Potential C-terminus modifications:
10. Valid expectation: < -2

6. protein cleavage specification

1. Cleavage site:
   trypsin, [RK]|{P}
   specify your own
2. Semi-style cleavage: ○ yes ● no
3. Missed cleavage sites allowed: 1
4. Cleavage C-terminal change: +17.002735 Da
5. Cleavage N-terminal change: +1.007825 Da



spectra

sequences

Generic search engine

Test all
cleavages,
modifications,
& mutations
for all sequences

Conventional,
single stage searching

Some hard problems in MS/MS analysis in proteomics

Allowing for unanticipated peptide cleavages

- e.g., chymotryptic contamination in trypsin
- calculation order ~ 200 × tryptic cleavage
- “unfortunate” coefficient

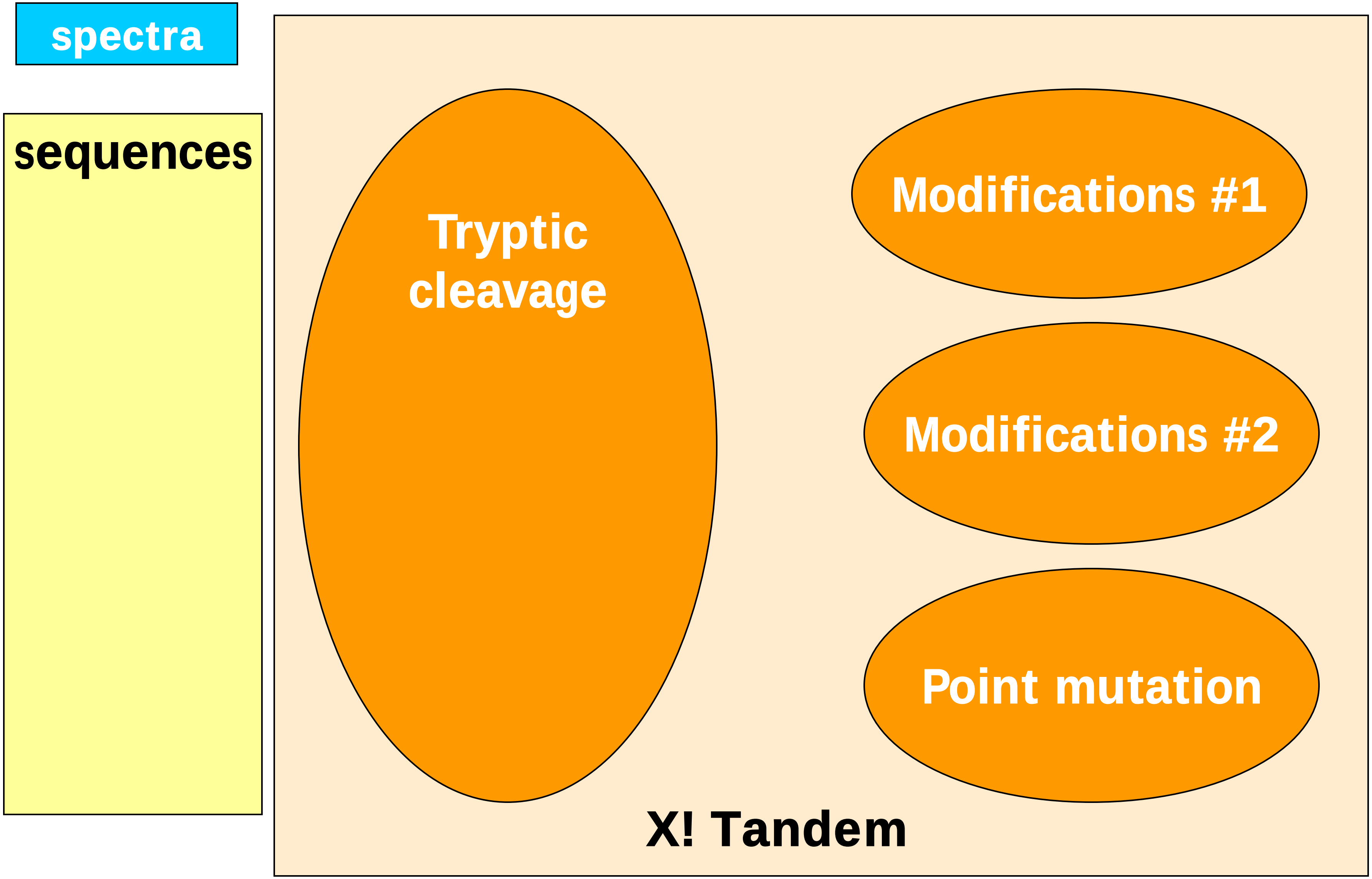
Determining potential modifications

- e.g., oxidation, phosphorylation, deamidation
- calculation order 2^n
- NP complete

Detecting point mutations

- e.g., sequence homology
- calculation order 18^N
- NP complete

Multi-stage searching



spectra

sequences

Tryptic cleavage

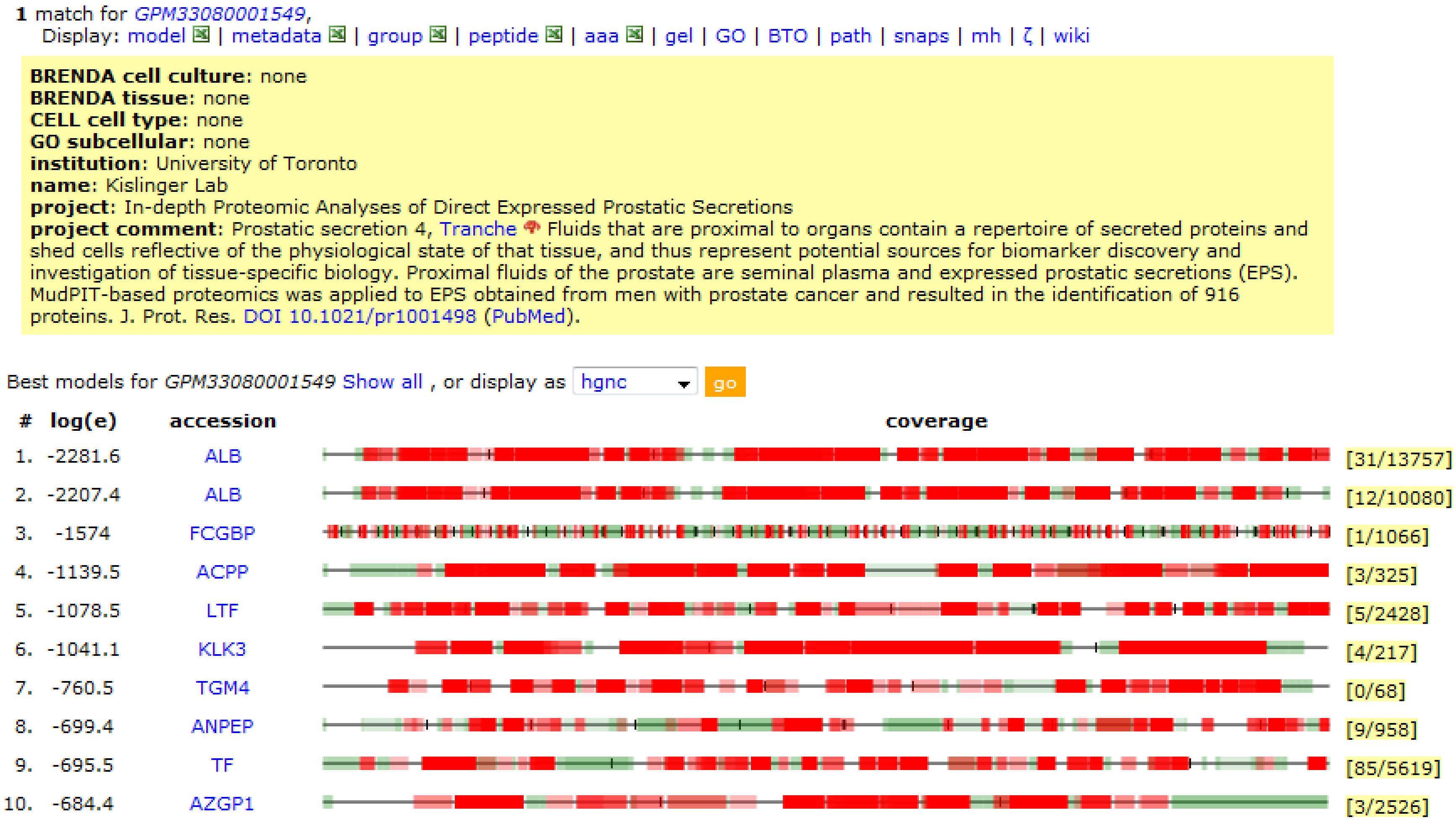
Modifications #1

Modifications #2

Point mutation

X! Tandem

Search Results



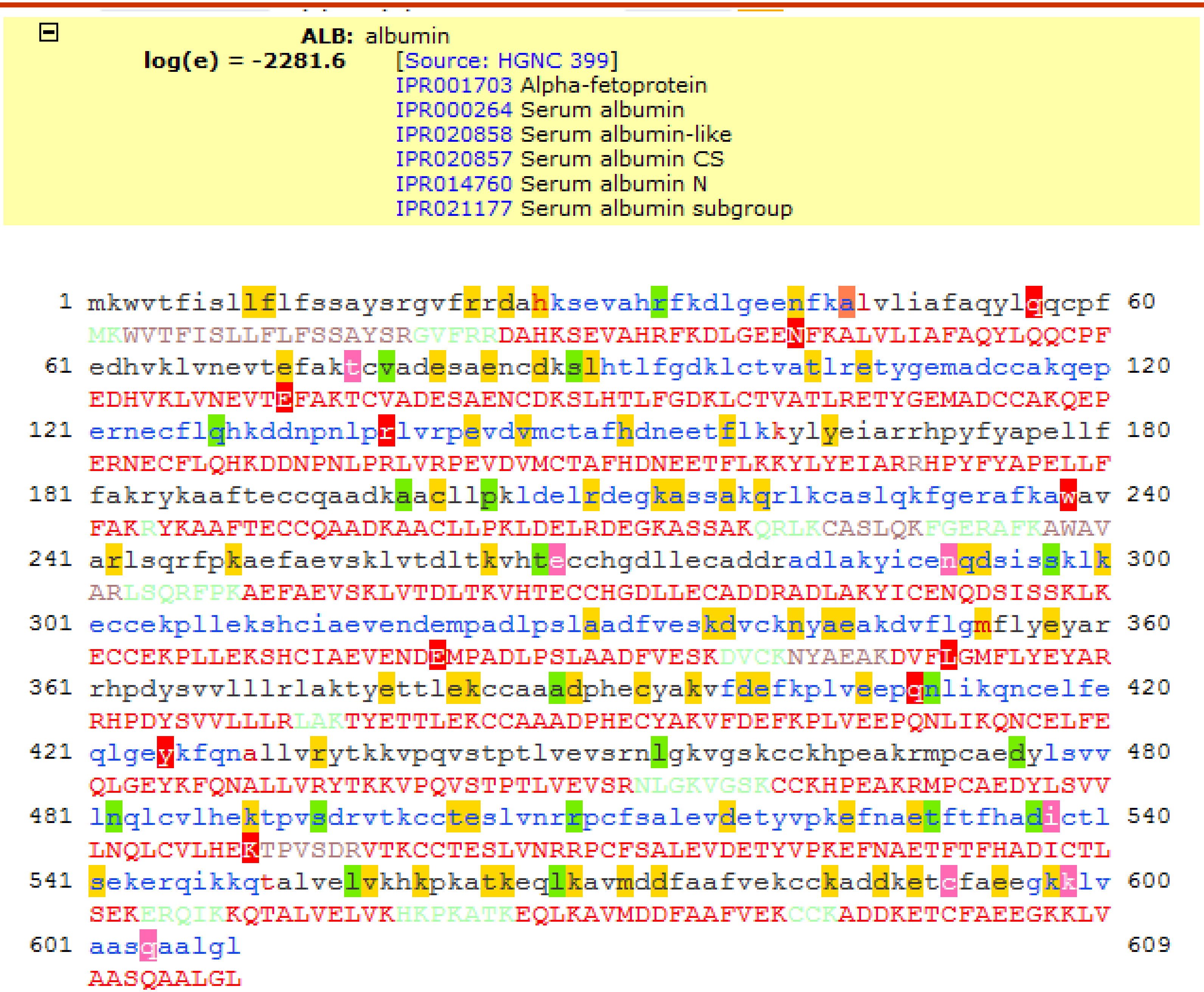
1 match for *GPM33080001549*,
Display: model ☒ | metadata ☒ | group ☒ | peptide ☒ | aaa ☒ | gel | GO | BTO | path | snaps | mh | ζ | wiki

BRENDA cell culture: none
BRENDA tissue: none
CELL cell type: none
GO subcellular: none
institution: University of Toronto
name: Kislinger Lab
project: In-depth Proteomic Analyses of Direct Expressed Prostatic Secretions
project comment: Prostatic secretion 4, Tranche Fluids that are proximal to organs contain a repertoire of secreted proteins and shed cells reflective of the physiological state of that tissue, and thus represent potential sources for biomarker discovery and investigation of tissue-specific biology. Proximal fluids of the prostate are seminal plasma and expressed prostatic secretions (EPS). MudPIT-based proteomics was applied to EPS obtained from men with prostate cancer and resulted in the identification of 916 proteins. J. Prot. Res. DOI 10.1021/pr1001498 (PubMed).

Best models for *GPM33080001549* Show all , or display as hgnc ▾ go

#	log(e)	accession	coverage	
1.	-2281.6	ALB		[31/13757]
2.	-2207.4	ALB		[12/10080]
3.	-1574	FCGBP		[1/1066]
4.	-1139.5	ACPP		[3/325]
5.	-1078.5	LTF		[5/2428]
6.	-1041.1	KLK3		[4/217]
7.	-760.5	TGM4		[0/68]
8.	-699.4	ANPEP		[9/958]
9.	-695.5	TF		[85/5619]
10.	-684.4	AZGP1		[3/2526]

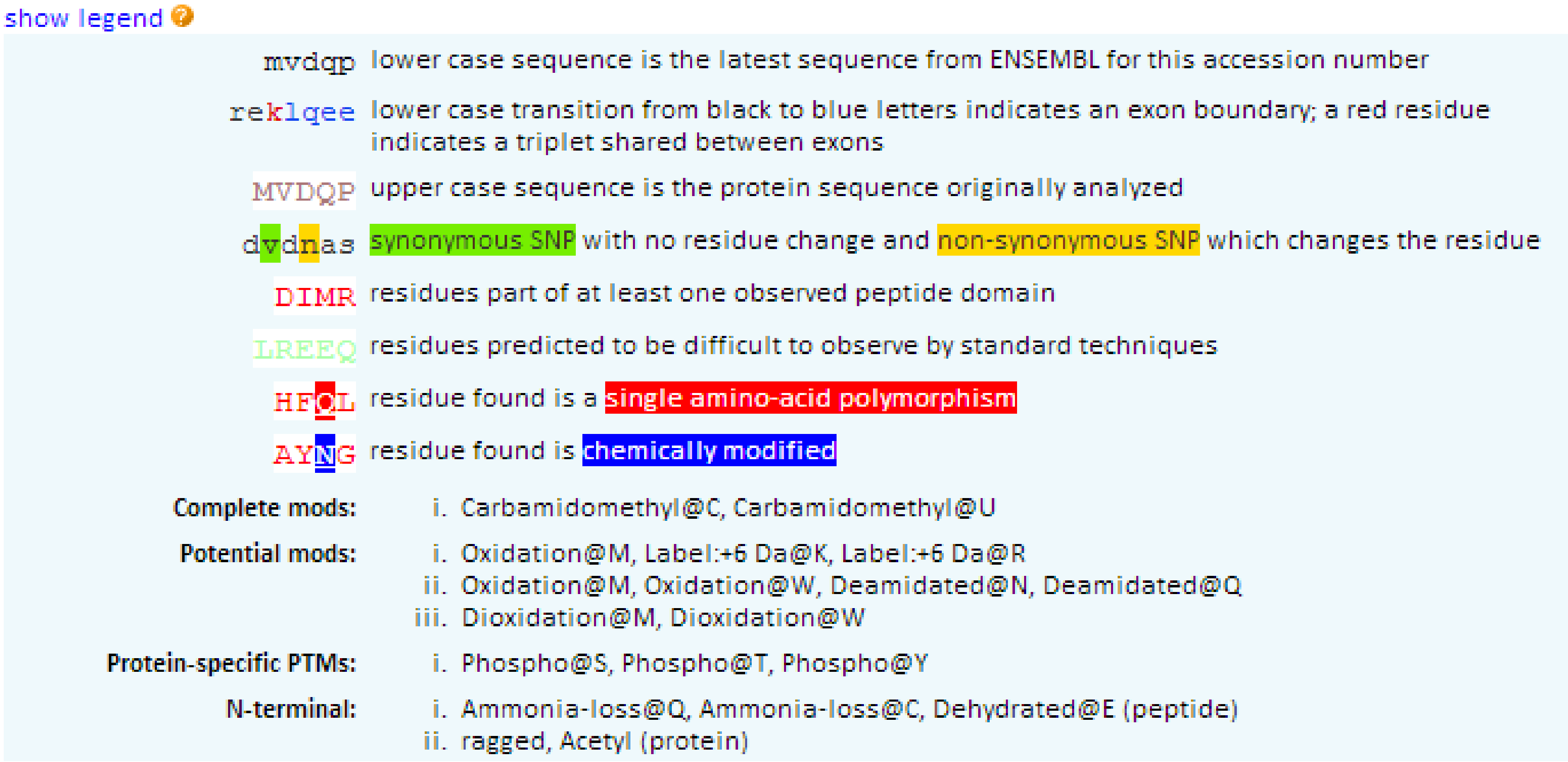
Search Results



⊟ **ALB:** albumin
log(e) = -2281.6 [Source: HGNC 399]
IPR001703 Alpha-fetoprotein
IPR000264 Serum albumin
IPR020858 Serum albumin-like
IPR020857 Serum albumin CS
IPR014760 Serum albumin N
IPR021177 Serum albumin subgroup

```
  1 mkwvtfisllflfssaysrgvfrrdahksevahrfkdlgeenfkalvliafaqylqqcpf  60
    MKWVTFISLLFLFSSAYSRGVFRRDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPF
 61 edhvklvnevtefaktcvadesaencdkslhtlfgdklctvatlretygemadccakqep 120
    EDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEP
121 ernecflqhkddnpnlprlvrpevdvmctafhdneetflkkylyeiarrhpyfyapellf 180
    ERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLF
181 fakrykaaftecc qaadkaacllpkldelrdegkassakqrlkcaslqkfgerafkawav 240
    FAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAV
241 arlsqrfpkaefaevsklvtdltkvhtecchgdllecaddradlakyicenqdsissklk 300
    ARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLK
301 eccekpllekshciaevendempadlpslaadfveskdvcknyaeakdvflgmflyeyar 360
    ECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYAR
361 rhpdysvvlllrlaktyettlekccaaadphecyakvfdefkplveepqnlikqncelfe 420
    RHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFE
421 qlgeykfqnallvrytkkvpqvstptlvevsrnlgkvgskcckhpeakrmpcaedylsvv 480
    QLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVV
481 lnqlcvlhektpvsdrvtkccteslvnrrpcfsalevdetyvpkefnaetftfhadictl 540
    LNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTL
541 sekerqikkqtalvelvkhkpkatkeqlkavmddfaafvekcckaddketcfaeegkklv 600
    SEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLV
601 aasqaalgl                                                    609
    AASQAALGL
```

Sequence Annotations



show legend

mvdqp lower case sequence is the latest sequence from ENSEMBL for this accession number

reklqee lower case transition from black to blue letters indicates an exon boundary; a red residue indicates a triplet shared between exons

MVDQP upper case sequence is the protein sequence originally analyzed

dvdnas synonymous SNP with no residue change and non-synonymous SNP which changes the residue

DIMR residues part of at least one observed peptide domain

LREEQ residues predicted to be difficult to observe by standard techniques

HFQL residue found is a single amino-acid polymorphism

AYNG residue found is chemically modified

Complete mods:

i. Carbamidomethyl@C, Carbamidomethyl@U

Potential mods:

i. Oxidation@M, Label:+6 Da@K, Label:+6 Da@R
ii. Oxidation@M, Oxidation@W, Deamidated@N, Deamidated@Q
iii. Dioxidation@M, Dioxidation@W

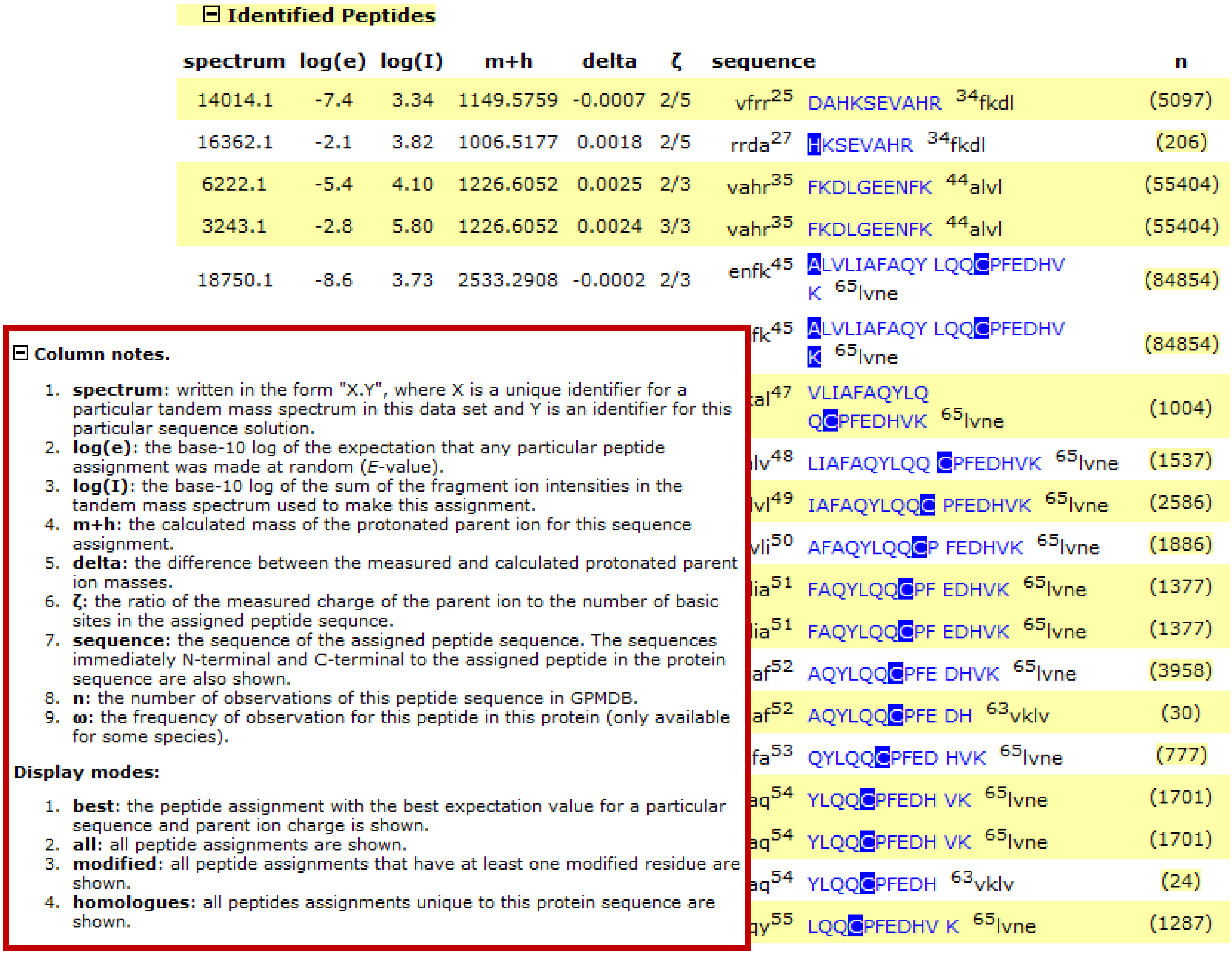
Protein-specific PTMs:

i. Phospho@S, Phospho@T, Phospho@Y

N-terminal:

i. Ammonia-loss@Q, Ammonia-loss@C, Dehydrated@E (peptide)
ii. ragged, Acetyl (protein)

Search Results



⊟ **Identified Peptides**

spectrum	log(e)	log(I)	m+h	delta	ζ	sequence	n
14014.1	-7.4	3.34	1149.5759	-0.0007	2/5	vfrr^{25} DAHKSEVAHR ^{34}fkdl	(5097)
16362.1	-2.1	3.82	1006.5177	0.0018	2/5	rrda^{27} HKSEVAHR ^{34}fkdl	(206)
6222.1	-5.4	4.10	1226.6052	0.0025	2/3	vahr^{35} FKDLGEENFK ^{44}alvl	(55404)
3243.1	-2.8	5.80	1226.6052	0.0024	3/3	vahr^{35} FKDLGEENFK ^{44}alvl	(55404)
18750.1	-8.6	3.73	2533.2908	-0.0002	2/3	enfk^{45} ALVLIAFAQY LQQCPFEDHV K ^{65}lvne	(84854)
						fk^{45} ALVLIAFAQY LQQCPFEDHV K ^{65}lvne	(84854)
						al^{47} VLIAFAQYLQ QCPFEDHVK ^{65}lvne	(1004)
						lv^{48} LIAFAQYLQQ CPFEDHVK ^{65}lvne	(1537)
						vl^{49} IAFAQYLQQC PFEDHVK ^{65}lvne	(2586)
						vli^{50} AFAQYLQQCP FEDHVK ^{65}lvne	(1886)
						lia^{51} FAQYLQQCPF EDHVK ^{65}lvne	(1377)
						lia^{51} FAQYLQQCPF EDHVK ^{65}lvne	(1377)
						af^{52} AQYLQQCPFE DHVK ^{65}lvne	(3958)
						af^{52} AQYLQQCPFE DH ^{63}vklv	(30)
						fa^{53} QYLQQCPFED HVK ^{65}lvne	(777)
						aq^{54} YLQQCPFEDH VK ^{65}lvne	(1701)
						aq^{54} YLQQCPFEDH VK ^{65}lvne	(1701)
						aq^{54} YLQQCPFEDH ^{63}vklv	(24)
						qy^{55} LQQCPFEDHV K ^{65}lvne	(1287)

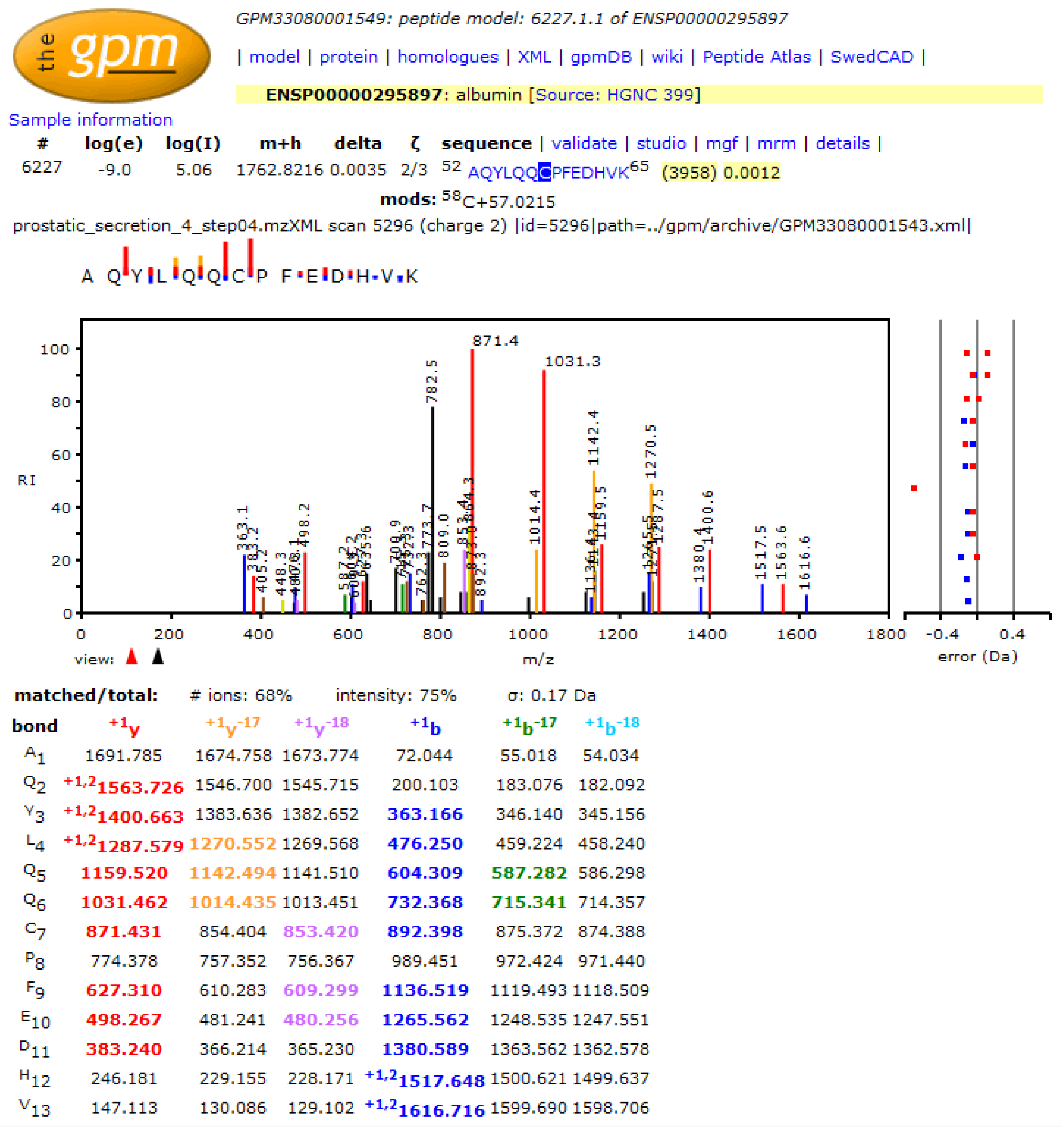
⊟ **Column notes.**

1. **spectrum**: written in the form "X.Y", where X is a unique identifier for a particular tandem mass spectrum in this data set and Y is an identifier for this particular sequence solution.
2. **log(e)**: the base-10 log of the expectation that any particular peptide assignment was made at random (*E*-value).
3. **log(I)**: the base-10 log of the sum of the fragment ion intensities in the tandem mass spectrum used to make this assignment.
4. **m+h**: the calculated mass of the protonated parent ion for this sequence assignment.
5. **delta**: the difference between the measured and calculated protonated parent ion masses.
6. **ζ**: the ratio of the measured charge of the parent ion to the number of basic sites in the assigned peptide sequnce.
7. **sequence**: the sequence of the assigned peptide sequence. The sequences immediately N-terminal and C-terminal to the assigned peptide in the protein sequence are also shown.
8. **n**: the number of observations of this peptide sequence in GPMDB.
9. **ω**: the frequency of observation for this peptide in this protein (only available for some species).

Display modes:

1. **best**: the peptide assignment with the best expectation value for a particular sequence and parent ion charge is shown.
2. **all**: all peptide assignments are shown.
3. **modified**: all peptide assignments that have at least one modified residue are shown.
4. **homologues**: all peptides assignments unique to this protein sequence are shown.

Search Results



GPM33080001549: peptide model: 6227.1.1 of ENSP00000295897

| model | protein | homologues | XML | gpmDB | wiki | Peptide Atlas | SwedCAD |

ENSP00000295897: albumin [Source: HGNC 399]

Sample information

#	log(e)	log(I)	m+h	delta	ζ	sequence | validate | studio | mgf | mrm | details |
6227	-9.0	5.06	1762.8216	0.0035	2/3	^{52} AQYLQQCPFEDHVK^{65} (3958) 0.0012

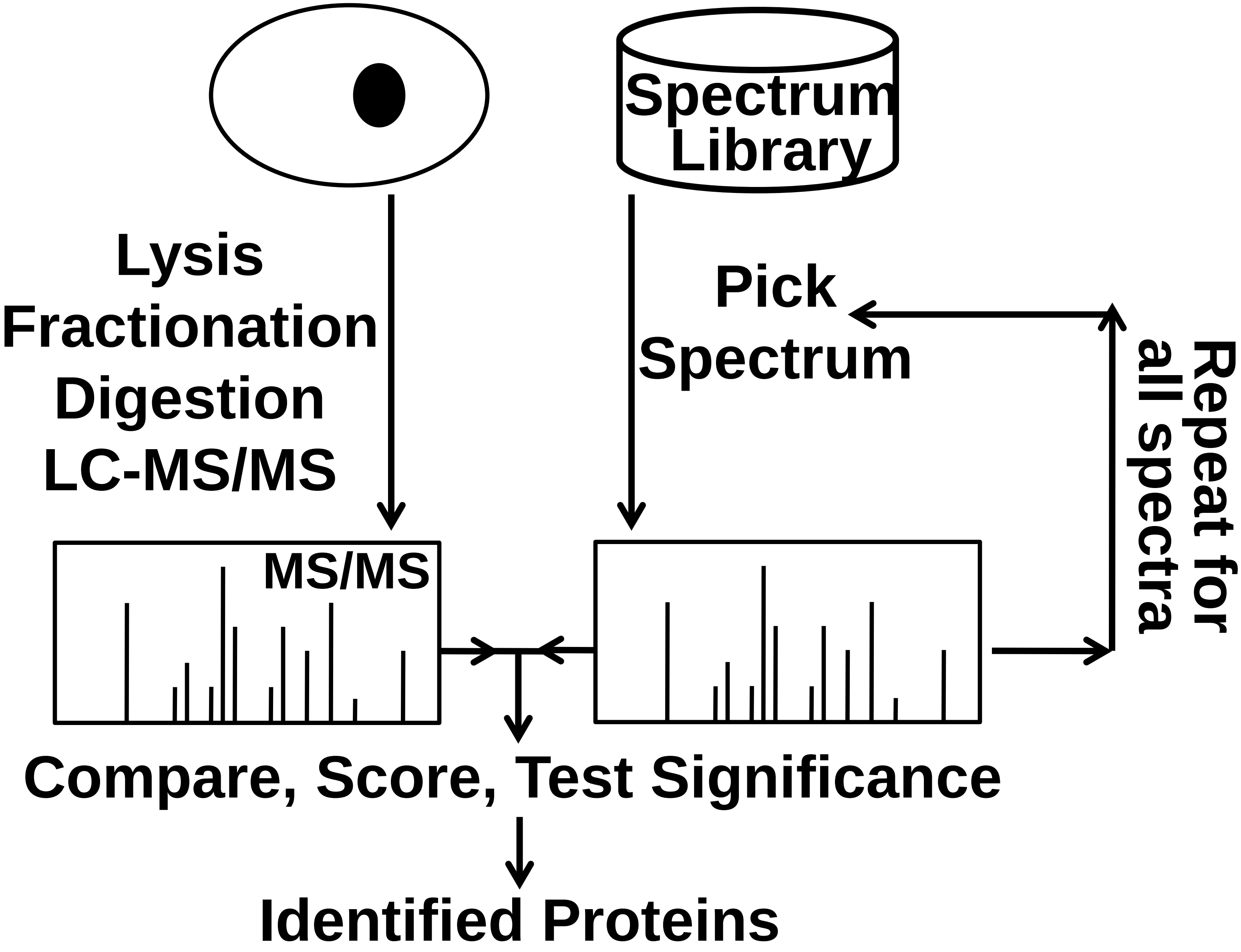
mods: ^{58}C+57.0215

prostatic_secretion_4_step04.mzXML scan 5296 (charge 2) |id=5296|path=../gpm/archive/GPM33080001543.xml|

matched/total: # ions: 68% intensity: 75% σ: 0.17 Da

bond	$^{+1}$y	$^{+1}$y-17	$^{+1}$y-18	$^{+1}$b	$^{+1}$b-17	$^{+1}$b-18
A_1	1691.785	1674.758	1673.774	72.044	55.018	54.034
Q_2	$^{+1,2}$**1563.726**	1546.700	1545.715	200.103	183.076	182.092
Y_3	$^{+1,2}$**1400.663**	1383.636	1382.652	**363.166**	346.140	345.156
L_4	$^{+1,2}$**1287.579**	**1270.552**	1269.568	**476.250**	459.224	458.240
Q_5	**1159.520**	**1142.494**	1141.510	**604.309**	**587.282**	586.298
Q_6	**1031.462**	**1014.435**	1013.451	**732.368**	**715.341**	714.357
C_7	**871.431**	854.404	**853.420**	**892.398**	875.372	874.388
P_8	774.378	757.352	756.367	989.451	972.424	971.440
F_9	**627.310**	610.283	**609.299**	**1136.519**	1119.493	1118.509
E_{10}	**498.267**	481.241	**480.256**	**1265.562**	1248.535	1247.551
D_{11}	**383.240**	366.214	365.230	**1380.589**	1363.562	1362.578
H_{12}	246.181	229.155	228.171	$^{+1,2}$**1517.648**	1500.621	1499.637
V_{13}	147.113	130.086	129.102	$^{+1,2}$**1616.716**	1599.690	1598.706

Identification – Spectrum Library Search



Steps in making an
Annotated Spectrum Library (ASL):

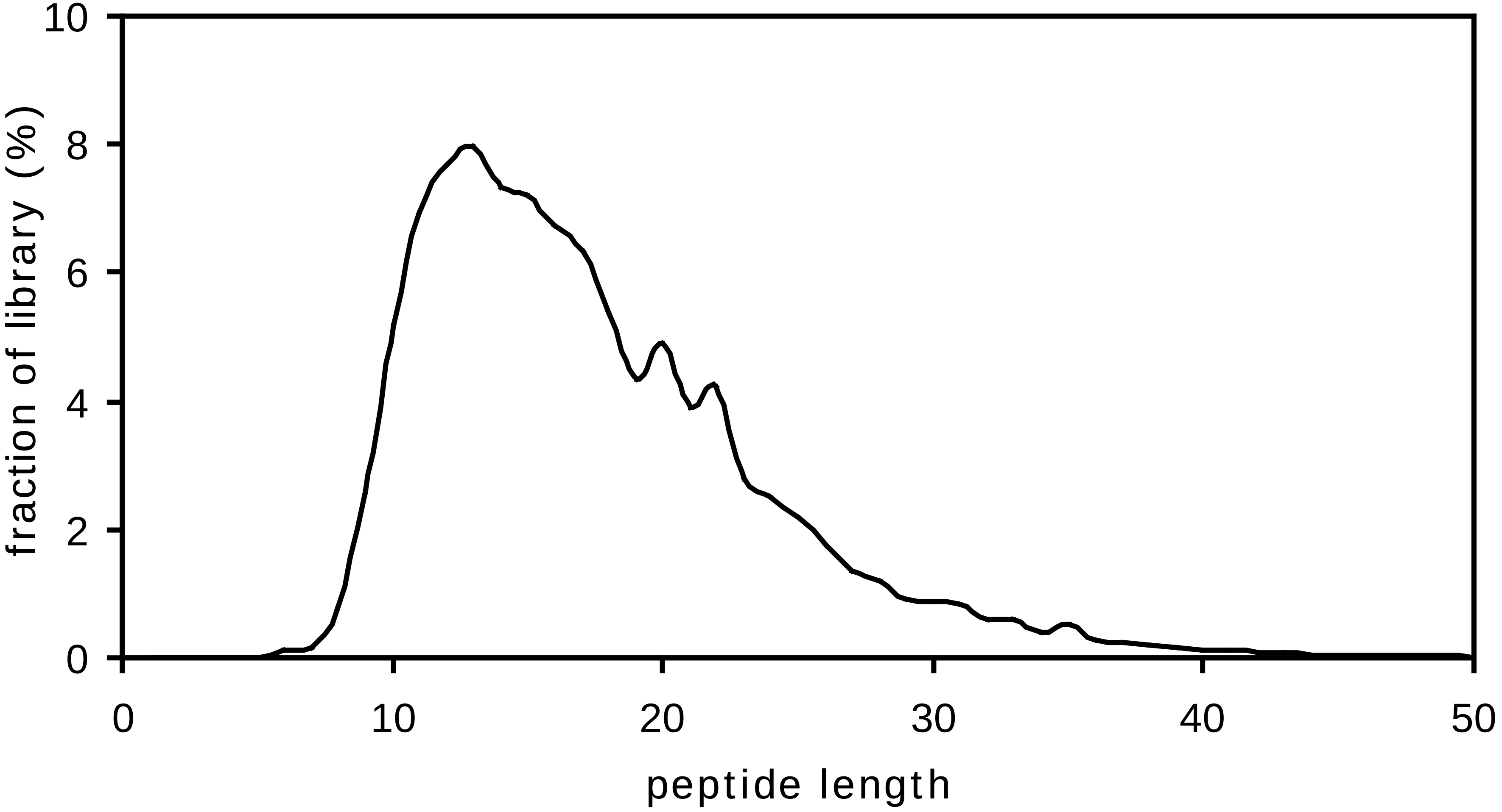
1. Find the best 10 spectra for a particular sequence, with the same PTMs and charge.

2. Add the spectra together and normalize the intensity values.

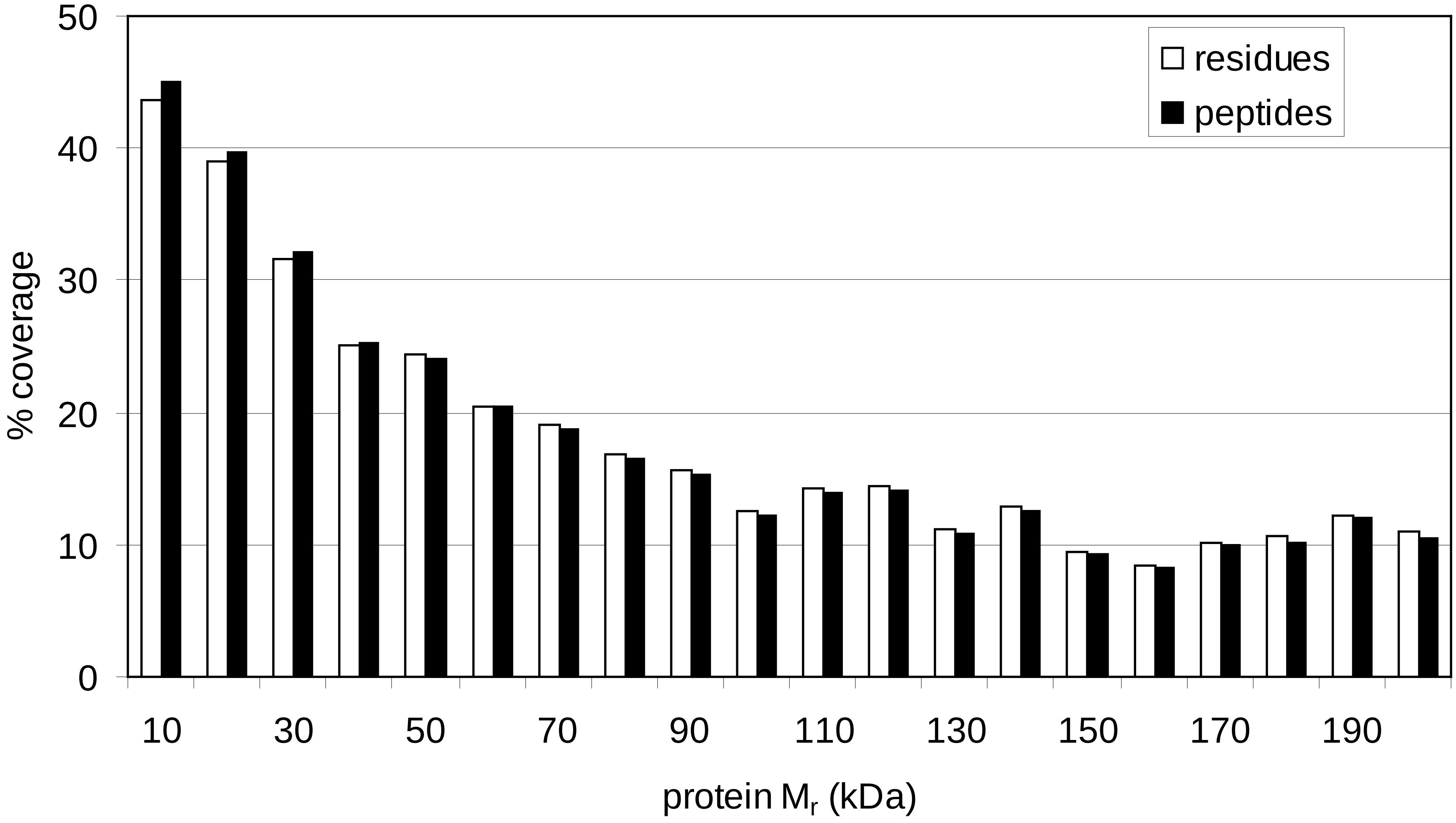
3. Assign a “quality” value: the median expectation value of the 10 spectra used.

4. Record the 20 most intense peaks in the averaged spectrum, it’s parent ion *z*, *m/z*, sequence, protein accessions & quality.

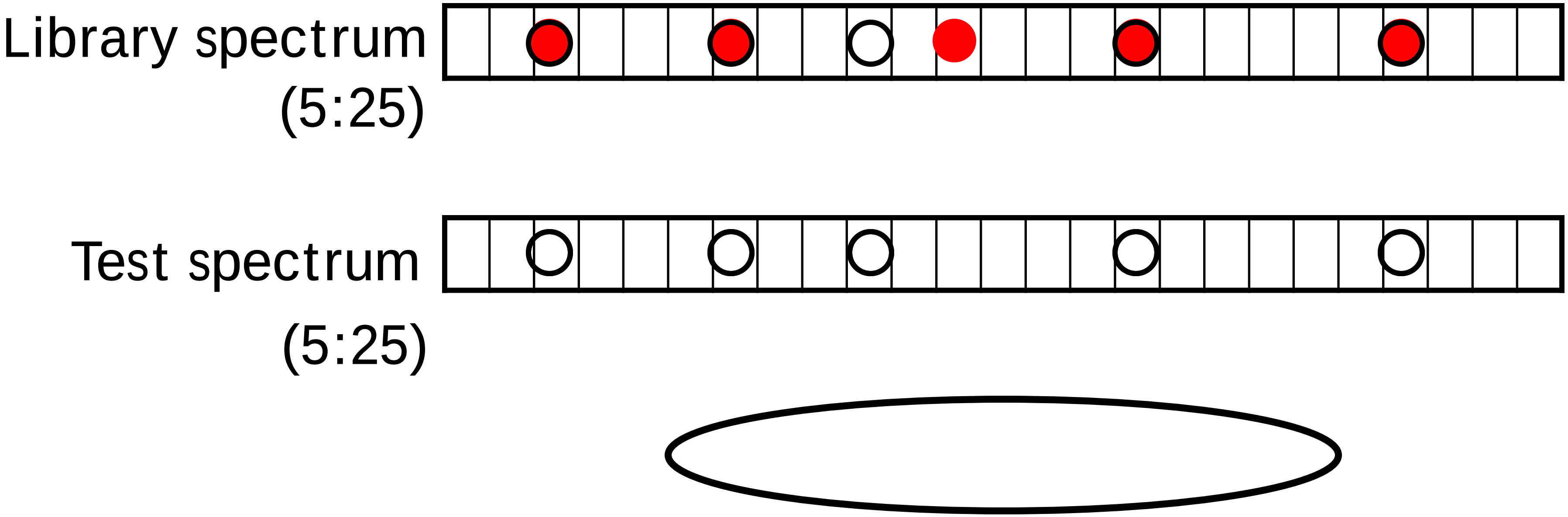
Spectrum Library Characteristics – Peptide Length



Spectrum Library Characteristics - Protein Coverage



Identification – Spectrum Library Search

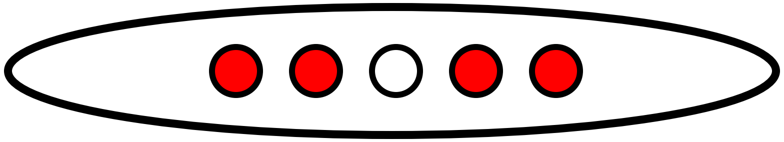


Library spectrum
(5:25)

Test spectrum
(5:25)

Results: 4 peaks selected, 1 peak missed

Identification – Spectrum Library Search



How likely is this?

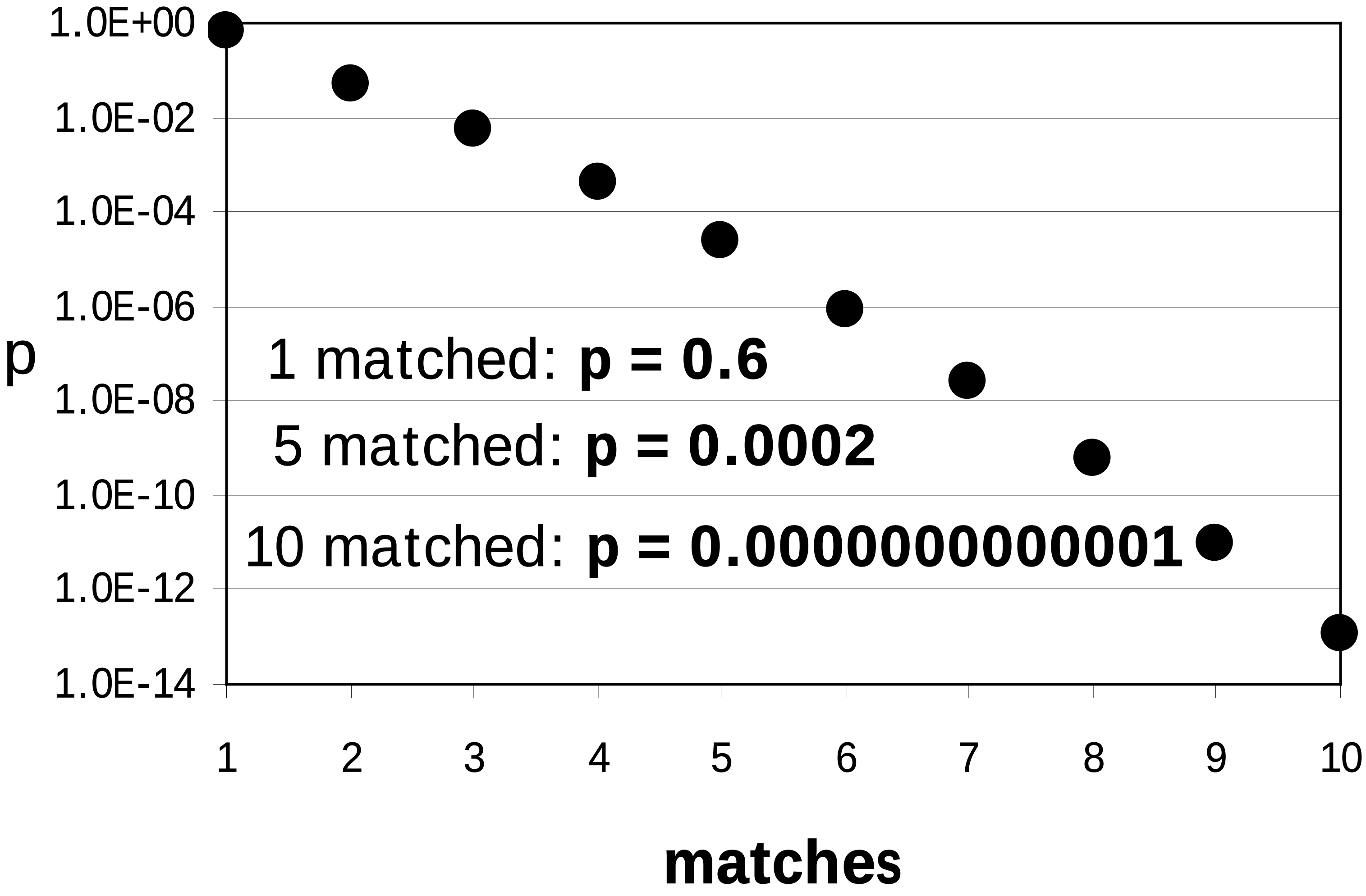
Apply a hypergeometric probability model:

- 25 possible m/z values;
- 5 peaks in the library spectrum; and
- 4 selected by the test spectrum.

Matches	Probability
1	0.45
2	0.15
3	0.016
4	0.00039
5	0.0000037

Identification – Spectrum Library Search

If you have 1000 possible m/z values and 20 peaks in test and library spectrum?



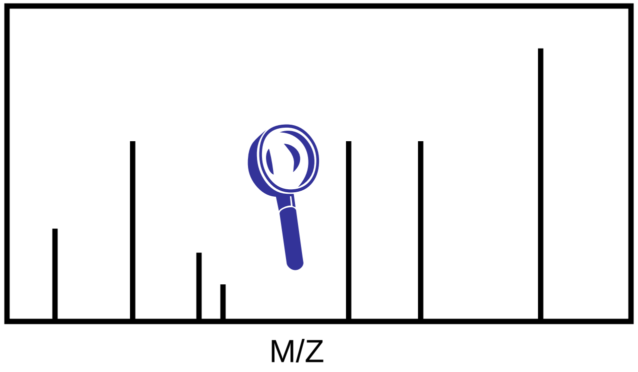
1 matched: **p = 0.6**

5 matched: **p = 0.0002**

10 matched: **p = 0.000000000001**

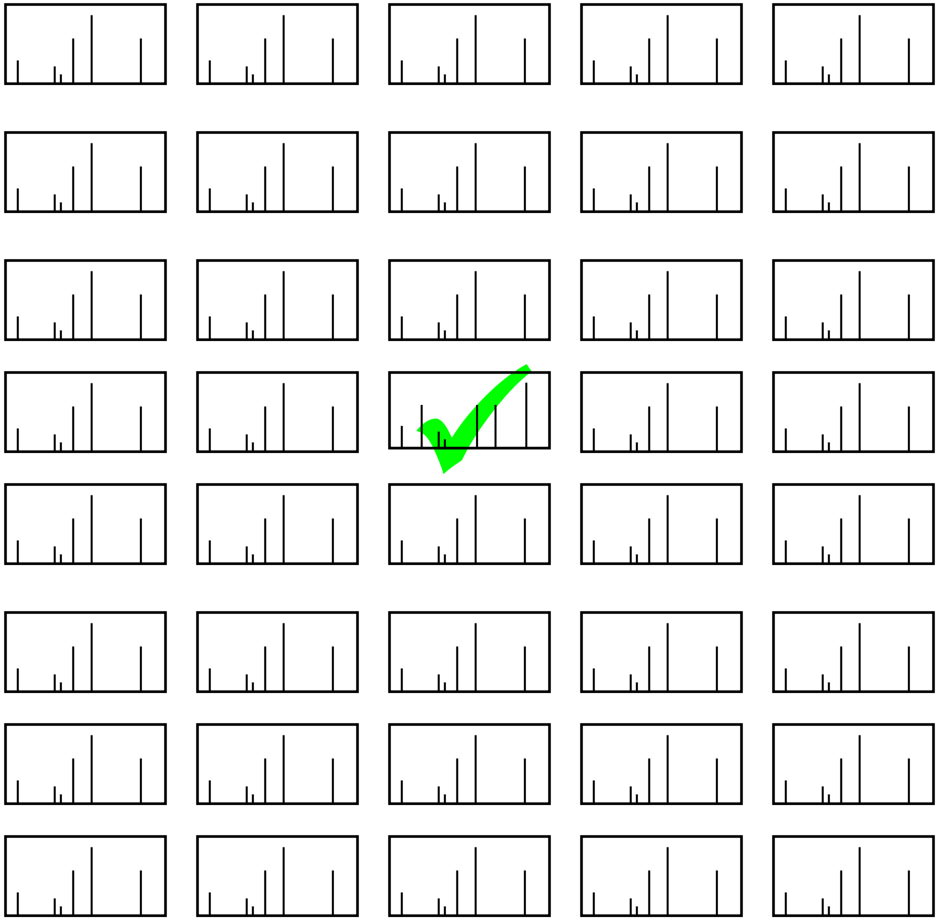
Identification - Spectrum Library Search

Experimental
Mass Spectrum

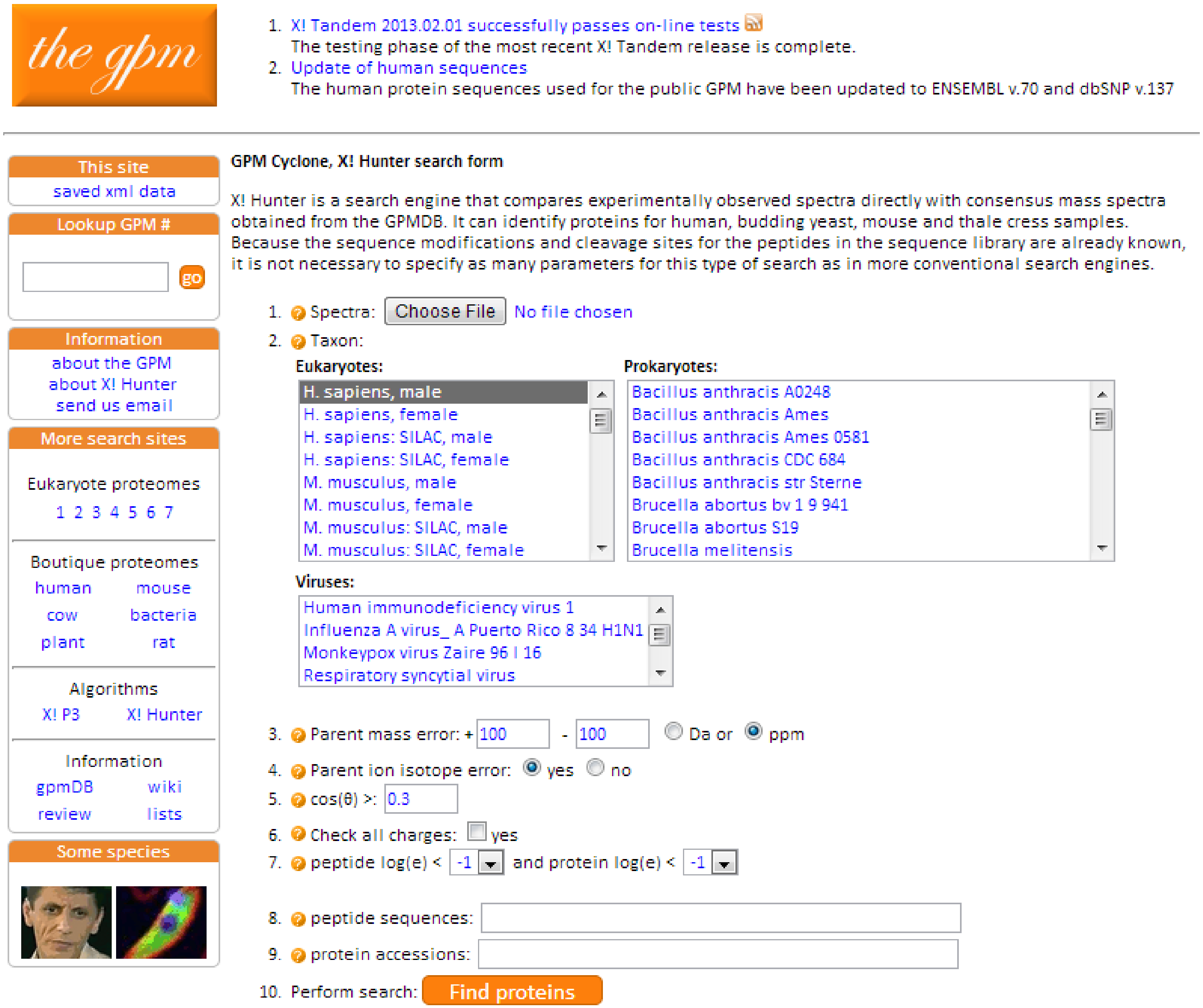


Best search result

Library of Assigned
Mass Spectra



X! Hunter



1. X! Tandem 2013.02.01 successfully passes on-line tests
   The testing phase of the most recent X! Tandem release is complete.
2. Update of human sequences
   The human protein sequences used for the public GPM have been updated to ENSEMBL v.70 and dbSNP v.137

GPM Cyclone, X! Hunter search form

X! Hunter is a search engine that compares experimentally observed spectra directly with consensus mass spectra obtained from the GPMDB. It can identify proteins for human, budding yeast, mouse and thale cress samples. Because the sequence modifications and cleavage sites for the peptides in the sequence library are already known, it is not necessary to specify as many parameters for this type of search as in more conventional search engines.

1. Spectra: Choose File No file chosen
2. Taxon:

Eukaryotes:

H. sapiens, male
H. sapiens, female
H. sapiens: SILAC, male
H. sapiens: SILAC, female
M. musculus, male
M. musculus, female
M. musculus: SILAC, male
M. musculus: SILAC, female

Prokaryotes:

Bacillus anthracis A0248
Bacillus anthracis Ames
Bacillus anthracis Ames 0581
Bacillus anthracis CDC 684
Bacillus anthracis str Sterne
Brucella abortus bv 1 9 941
Brucella abortus S19
Brucella melitensis

Viruses:

Human immunodeficiency virus 1
Influenza A virus_ A Puerto Rico 8 34 H1N1
Monkeypox virus Zaire 96 I 16
Respiratory syncytial virus

3. Parent mass error: + 100 - 100 ○ Da or ● ppm
4. Parent ion isotope error: ● yes ○ no
5. cos(θ) >: 0.3
6. Check all charges: □ yes
7. peptide log(e) < -1 and protein log(e) < -1
8. peptide sequences:
9. protein accessions:
10. Perform search: Find proteins

X! Hunter algorithm:

1. Use dot product to find a library spectrum that best matches a test spectrum.

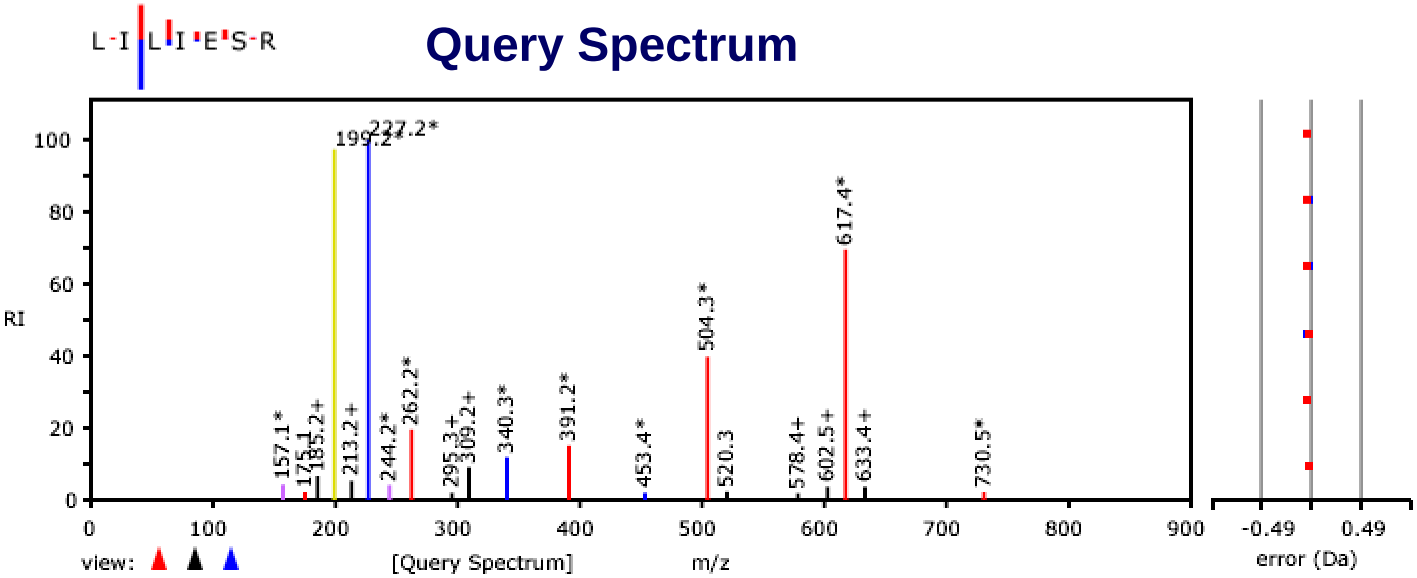
2. Calculate p-value with hypergeometric distribution.

3. Use p-value to calculate expectation value, given the identification parameters.

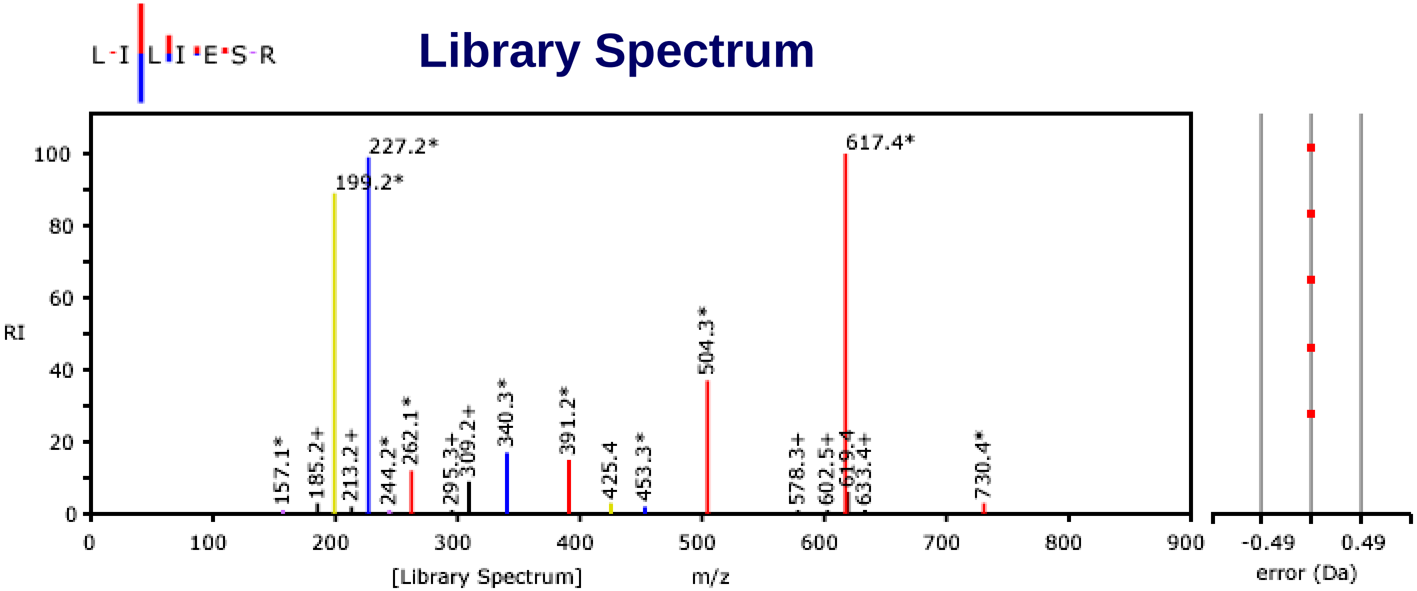
4. If expectation value is less than the median expectation value of the library spectrum, report the median value.

X! Hunter Result

Query Spectrum



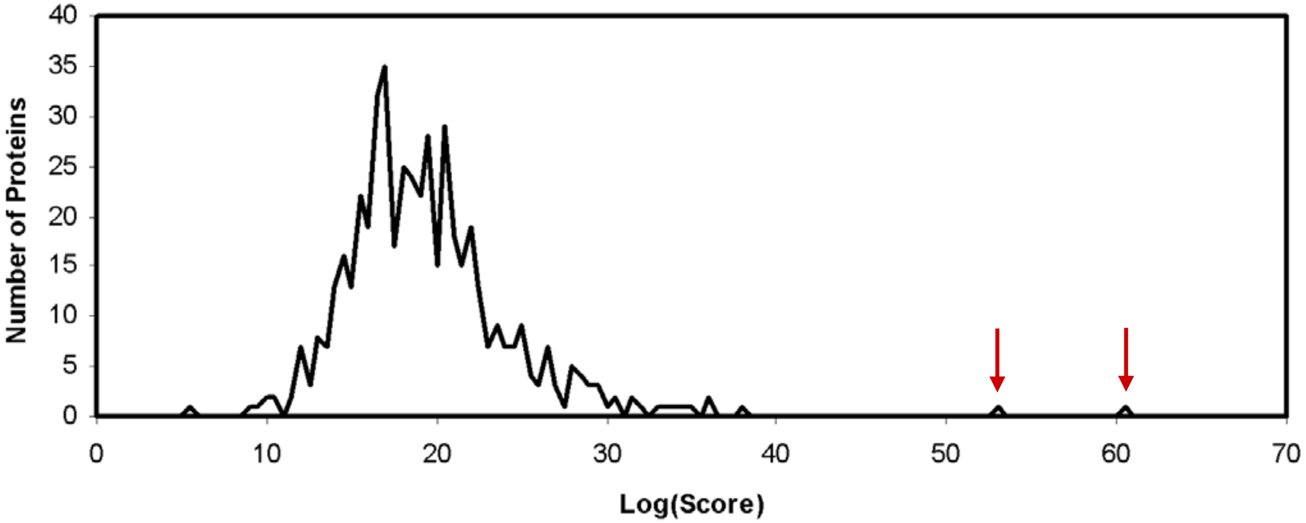
Library Spectrum



Significance Testing

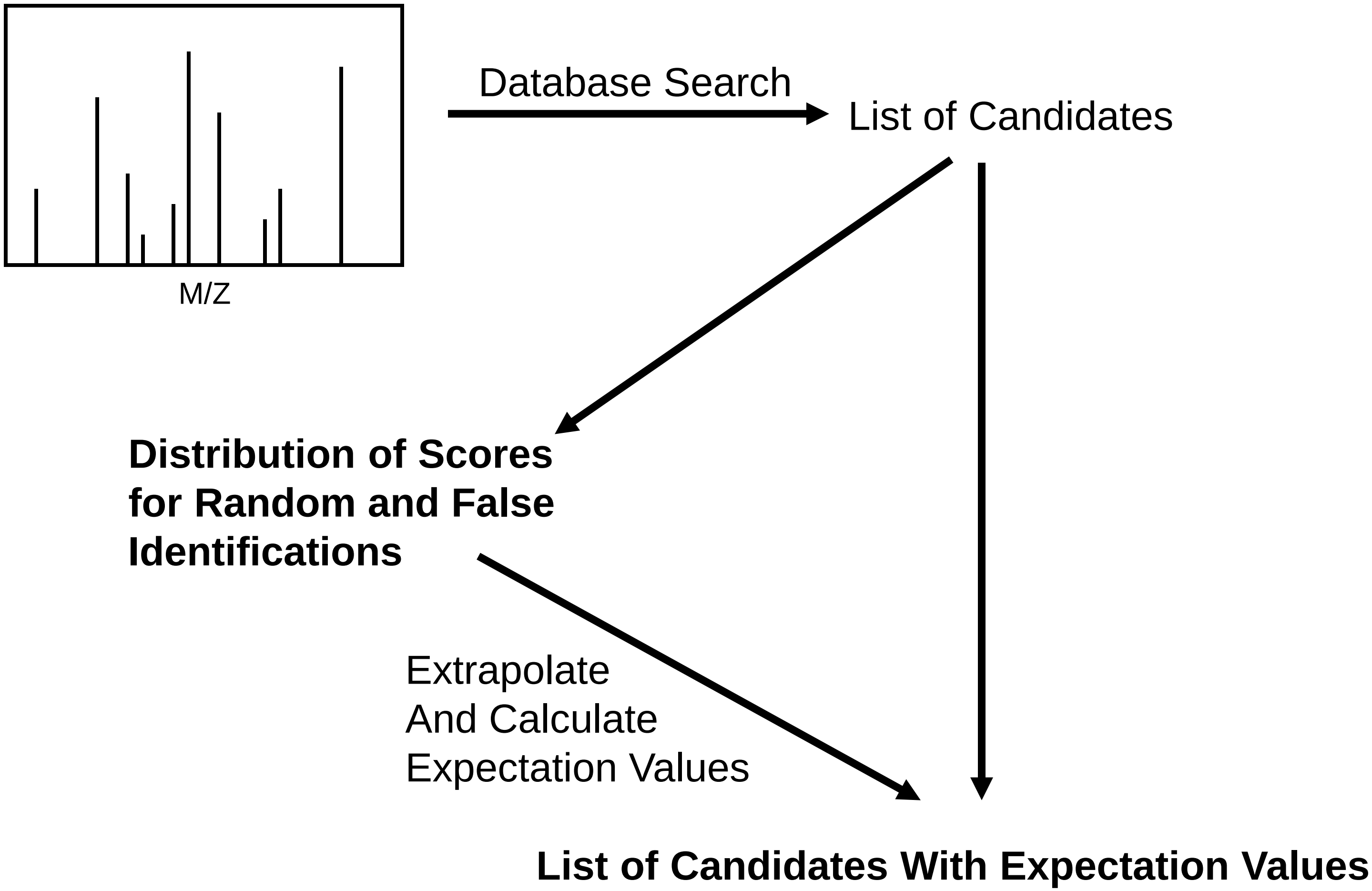
False protein identification is caused by random matching

Significance Testing - Expectation Values



The majority of sequences in a collection will give a score due to random matching.

Significance Testing - Expectation Values



Database Search

List of Candidates

Distribution of Scores for Random and False Identifications

Extrapolate
And Calculate
Expectation Values

List of Candidates With Expectation Values

Proteomics Informatics – Protein identification I: searching protein sequence collections and significance testing (Week 4)