

Post-translational Modifications and Proteomics

**Gary Van Domselaar
University of Alberta**

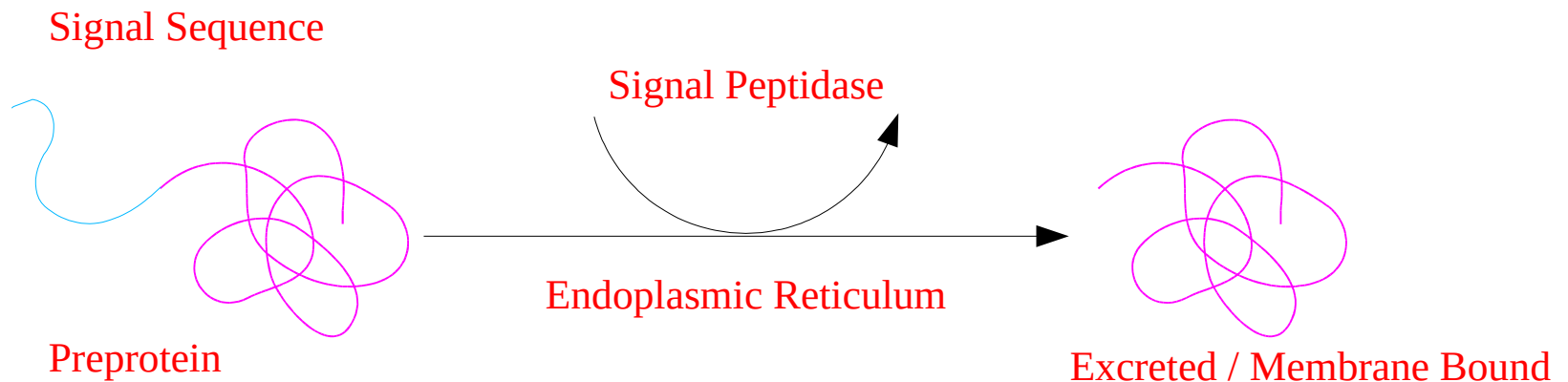
Post-translational Modifications

- **Definition**
 - Covalent modification of side chains in proteins after they are translated and exit from the ribosomes.
- **General Purpose:**
 - To alter the function, destination, or fate of the translated protein
 - To expand the functional group repertoire of proteins beyond the 20 'natural' amino acids.

Post-translational Modifications

- **Proteolytic Cleavage**

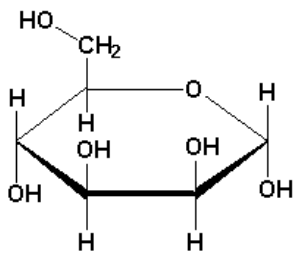
- Conversion of an inactive protein precursor (*proprotein*) to the active form
- Removal of a signal peptide from a *preprotein*.
- Conversion of *zymogens* to active enzymes
- Removal of initiating methionine



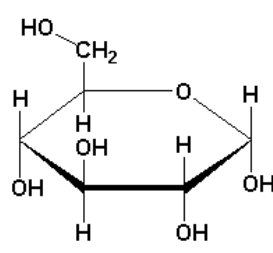
Post-translational Modifications

- **Glycoproteins**

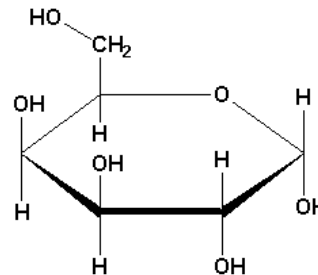
- Proteins covalently linked to carbohydrate (Glucose, galactose, mannose, fucose, GalNAc, GlcNAc, NANA).



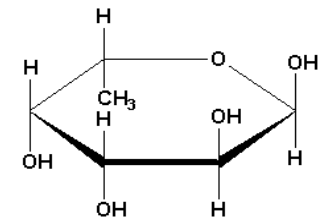
Mannose



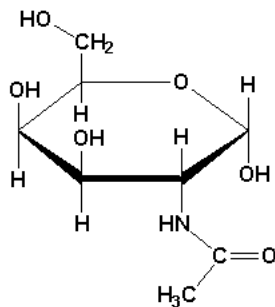
Glucose



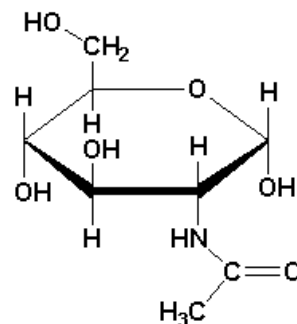
Galactose



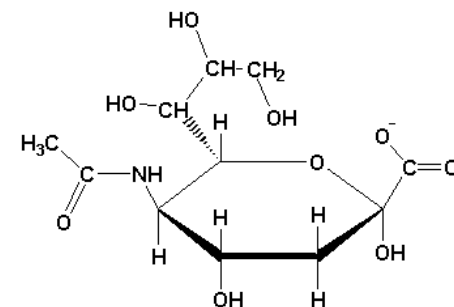
L-Fucose



N-Acetylgalactosamine



N-acetylglucosamine

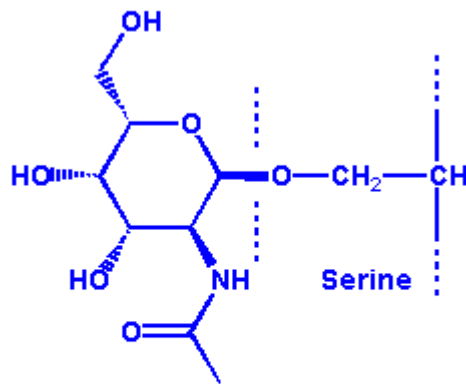


N-Acetyl-Neuraminic Acid

Post-translational Modifications

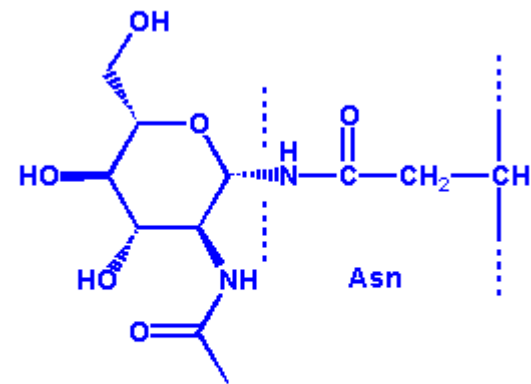
- Glycosidic bonds
 - O-linked (Ser, Thr, Hydroxy-Lys),
 - N-linked (predominant).

GalNAc linkage to Ser, Thr



O-linkage to GalNAc

GlcNAc linkage to Asn

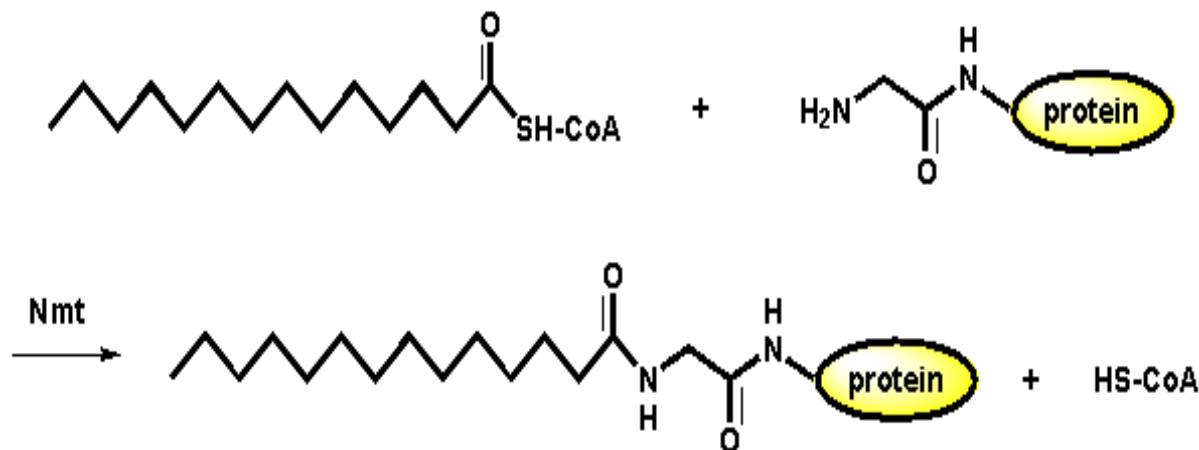
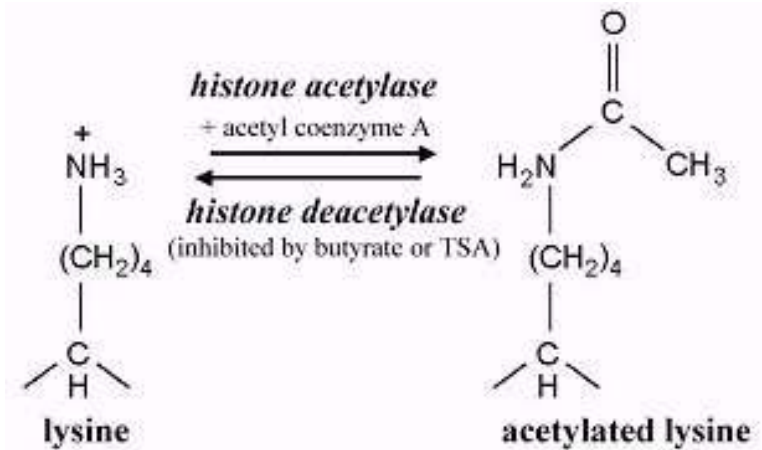


N-linkage to GlcNAc

<http://www.med.unibs.it/~marchesi/protmod.html>

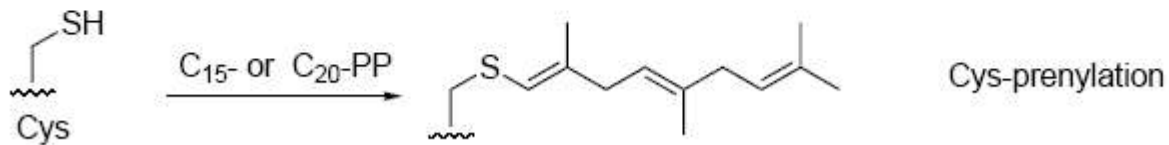
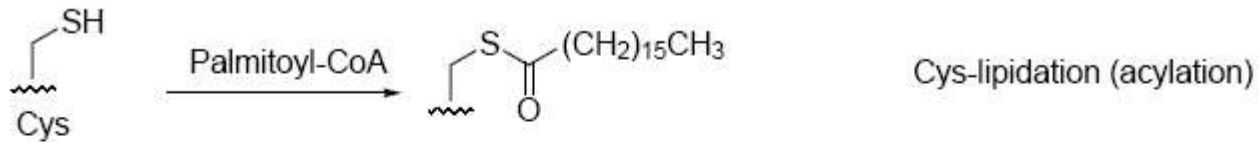
Post-translational Modifications

- N-terminal and Lysine side-Chain Acylation
 - acetylation
 - myristoylation
 - palmitoylation



Post-translational Modifications

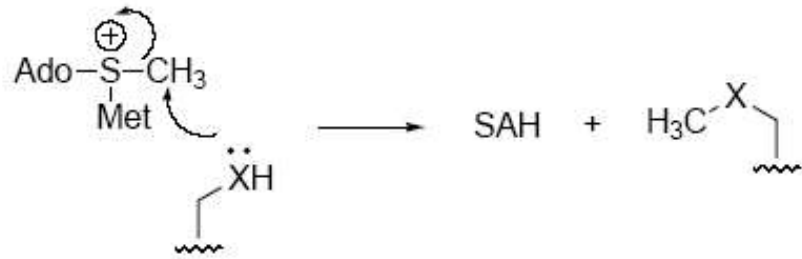
- Cys Lipidation
 - Prenylation
 - Farnesylation
 - Geranylgeranylation
 - Palmitoylation



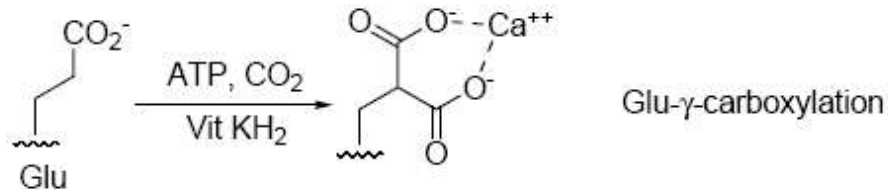
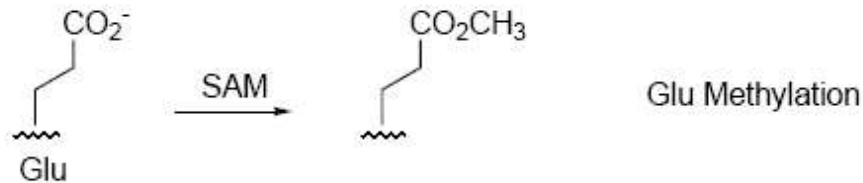
Post-translational Modifications

- **Methylation**

- Lysine
- Arginine
- C- terminal

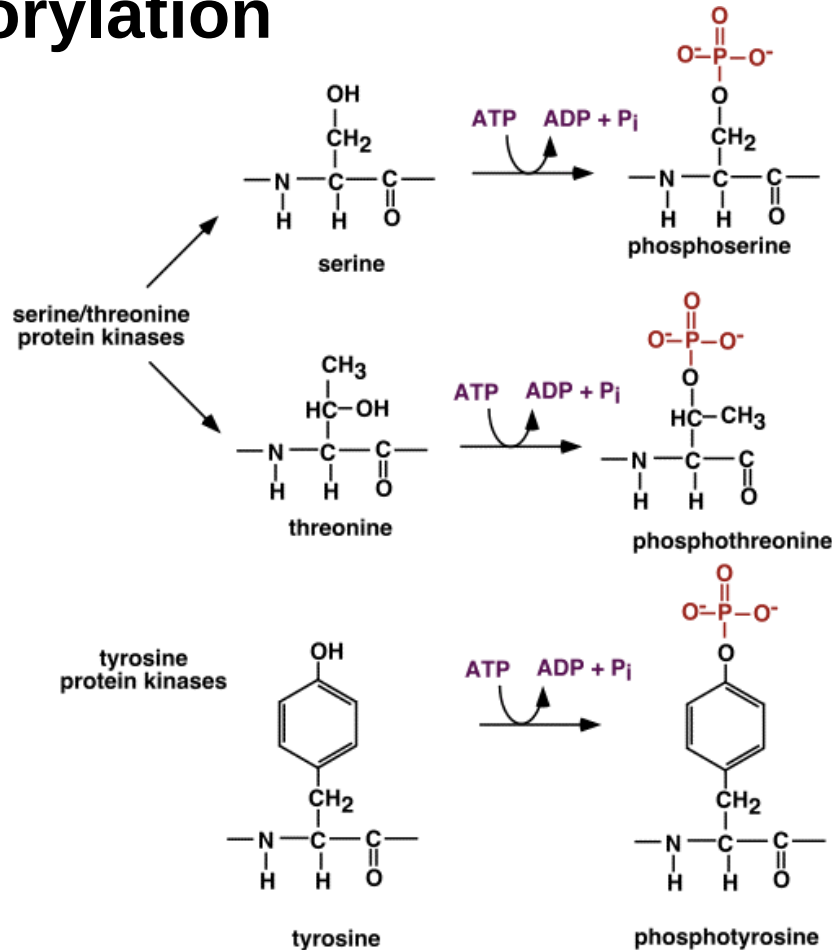


- **Glu Methylation and Carboxylation**



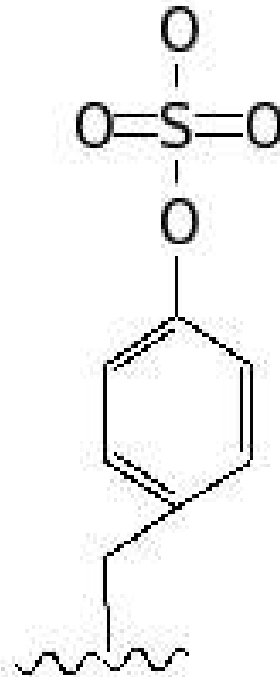
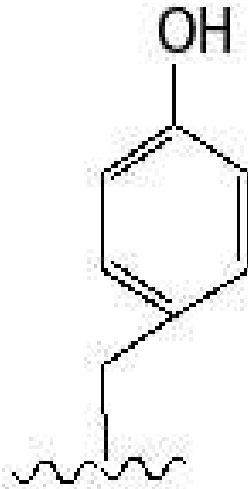
Post-translational Modifications

- Phosphorylation



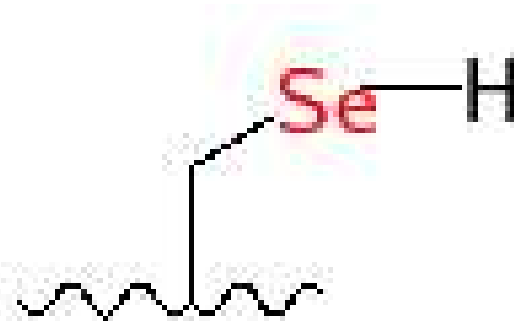
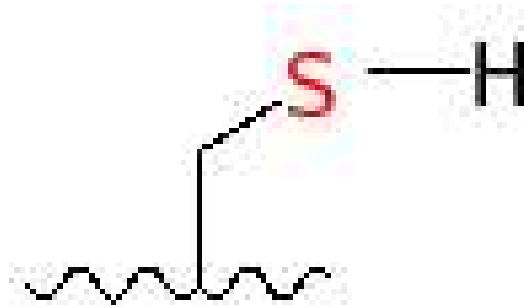
Post-translational Modifications

- Sulfation



Post-translational Modifications

- **Selenocysteine**



Post-translational Modifications

Carbohydrates

N-linked oligosaccharides
O-linked oligosaccharides
Glycophosphoinositol-lipid anchors
C-Mannosylation

Lipids

Acylation
Palmitoylation
Myristylation
Farnesylation
Prenylation
Geranylgeranylation

Cross-linking

Disulfide formation
Thiolester formation
Transglutamylation

Amino acid modification

Amidation–deamidation
Cysteinylation
Formylation
Citrullination
Isoaspartylation
Detyrosination
Thioether addition
Gamma-carboxylation
Deimination

Small adducts

Phosphorylation
Sulfation
Nitration
Acetylation
Formylation
Methylation
Oxidation
Hydroxylation
Iodination
Biotinylation

Post-translational Modifications

ABRF Delta Mass Reference - Mozilla

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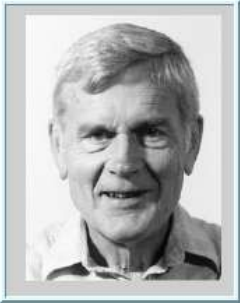
Delta Mass

A Database of Protein Post Translational Modifications

[Contributors](#)

Please send new data or corrections to [Len Packman](#)

The ABRF accepts no responsibility for the accuracy of these data which are freely donated by individuals



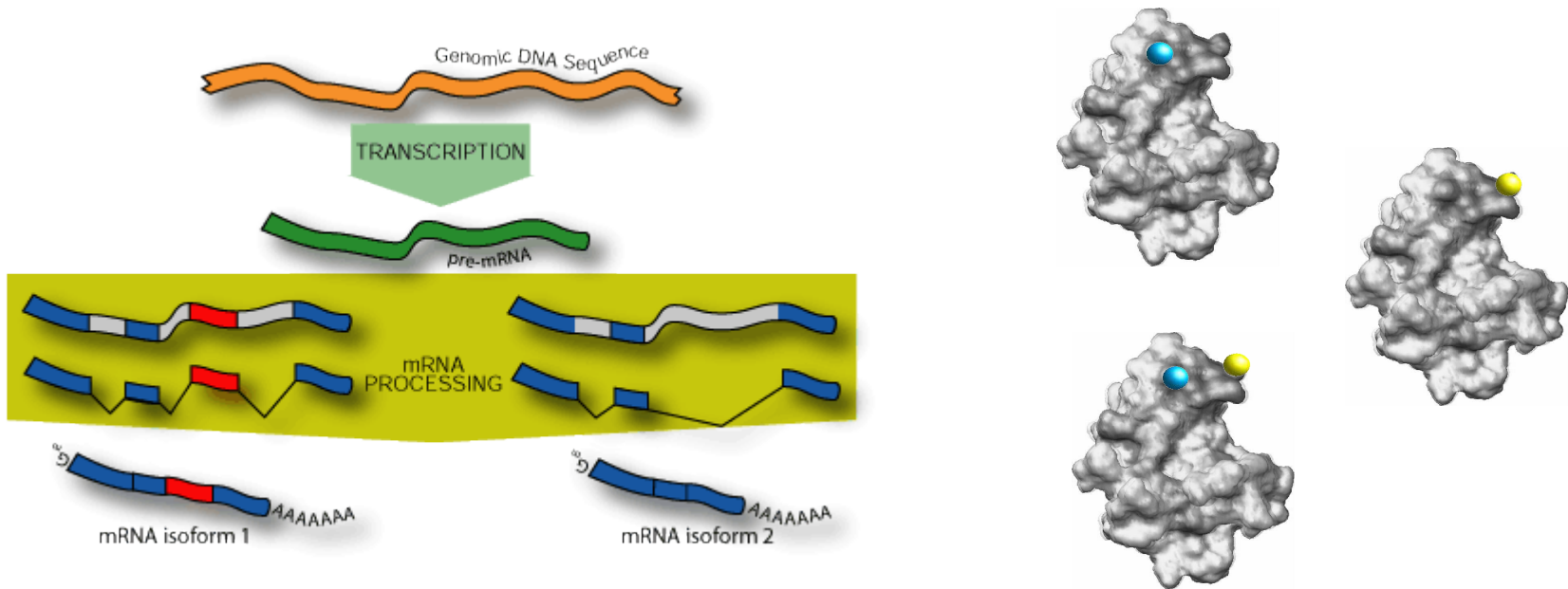
This page is dedicated to the memory of [Finn Wold](#)

Post-translational Modifications

- Many PTMs have been discovered serendipitously during studies of individual proteins and biological processes.
- Direct PTM analysis provides a complete analysis, but is slow and sometimes not even possible for certain proteins because of problems of isolation and obtaining sufficient amounts.
- Proteomics analysis is high throughput, but typically incomplete in its ability to completely characterize the protein primary sequence
- **How can proteomics be applied to the analysis of PTMS?**

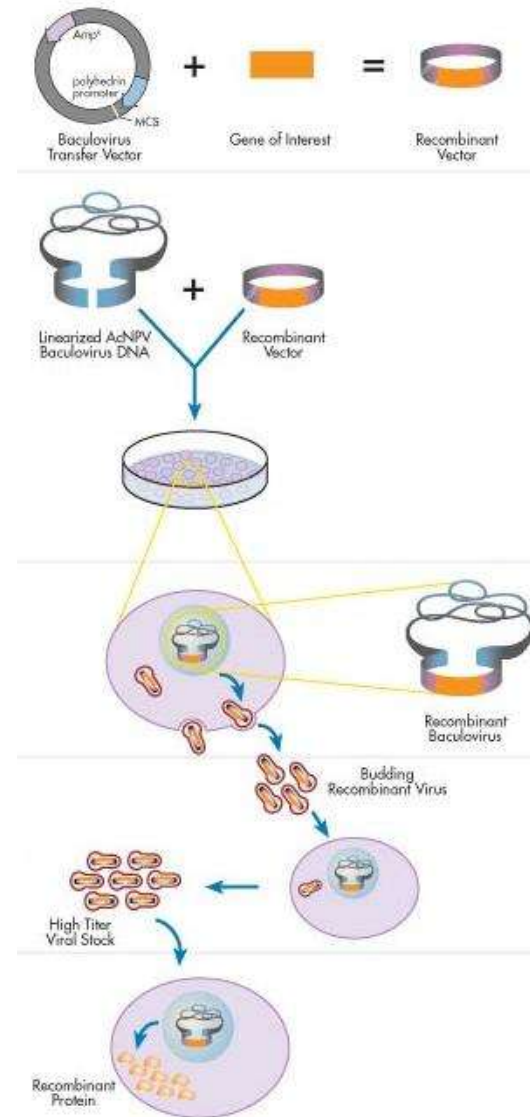
Isolation of Modified Proteins

- **Consideration: A single gene can have many gene products due to alternative splicing and multiple modification combinations.**
- **A lot of protein is necessary (~1ug).**



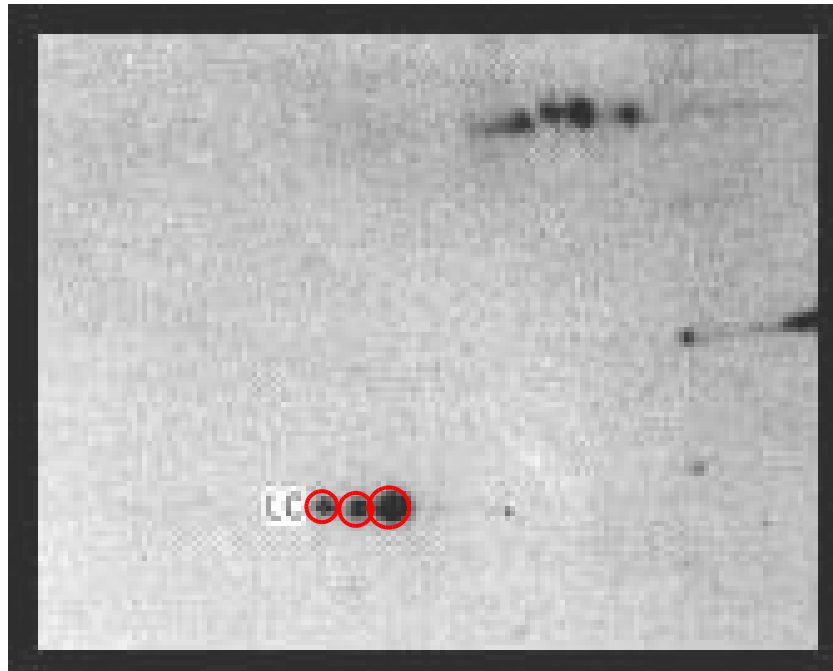
Isolation of Modified Proteins

- A suitable expression (eg baculovirus) can sometimes be employed to recombinantly express the protein(s) to be studied.
- Caveat: recombinant systems do not always produce identical PTMs to the those of mammalian cells.

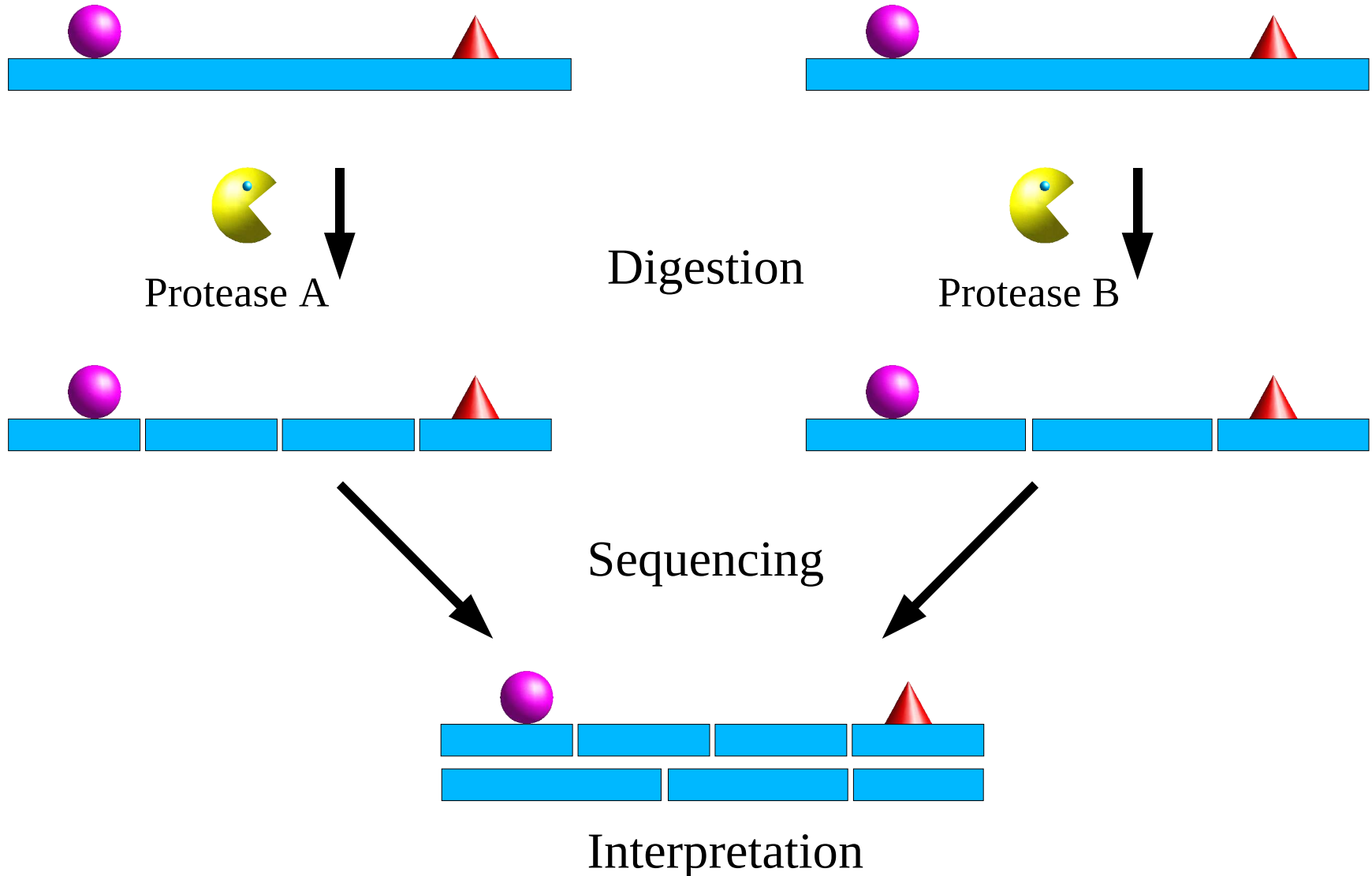


Isolation of Modified Proteins

- **2D Gel electrophoresis typically can be used to separate modified proteins.**

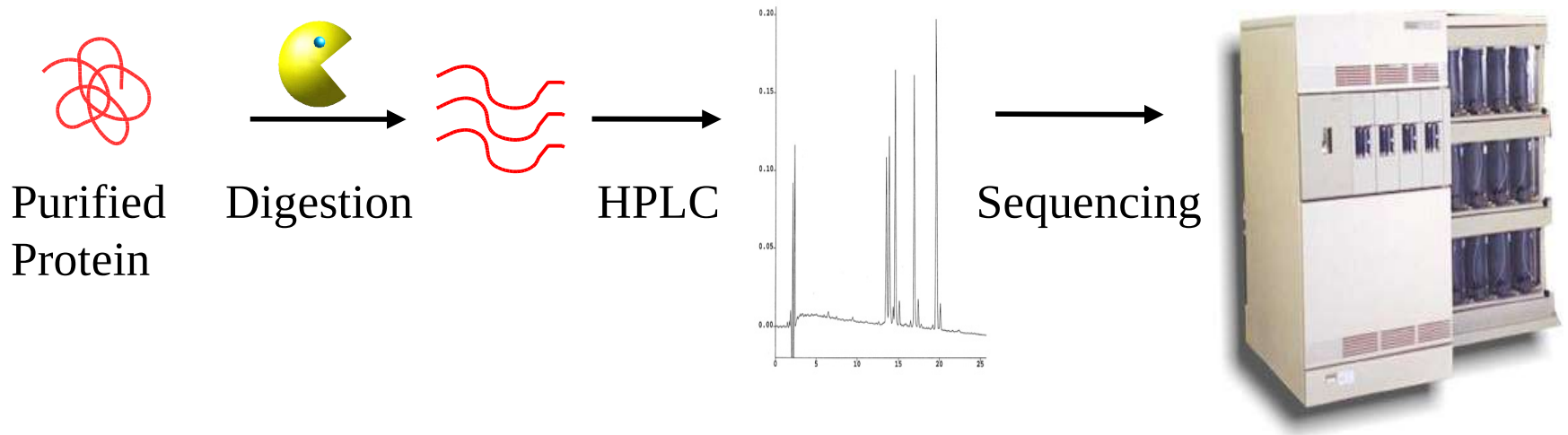


PTM Mapping a Purified Protein



PTM Mapping a Purified Protein

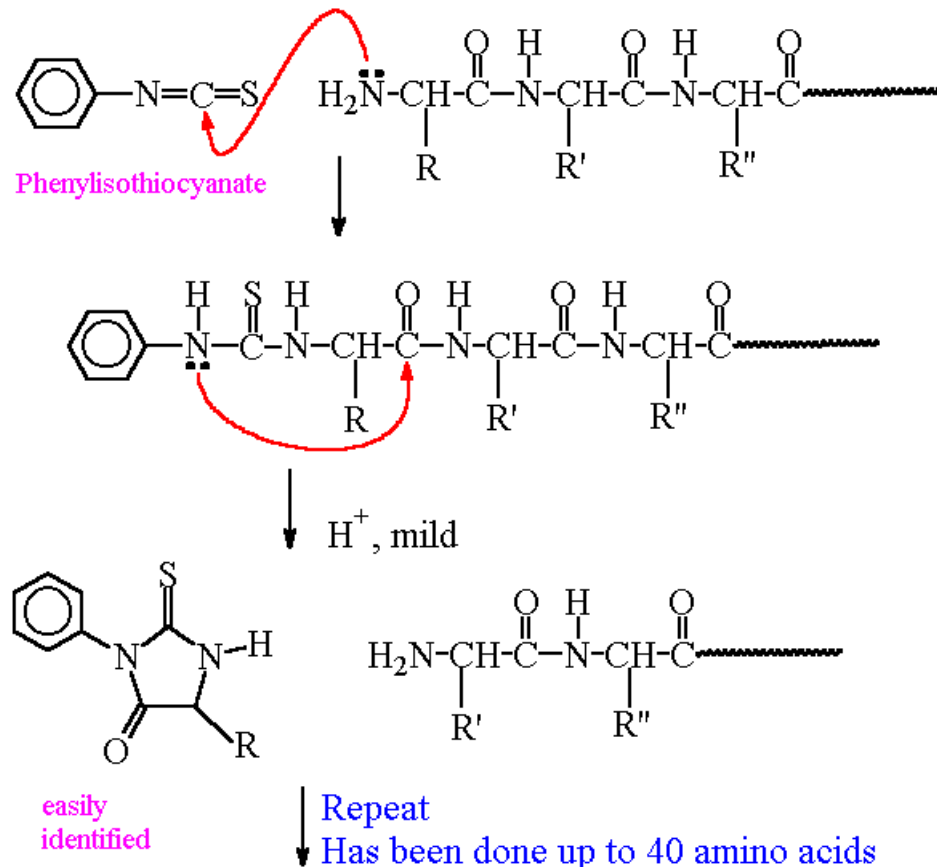
- Sequencing: Edman degradation



PTM Mapping a Purified Protein

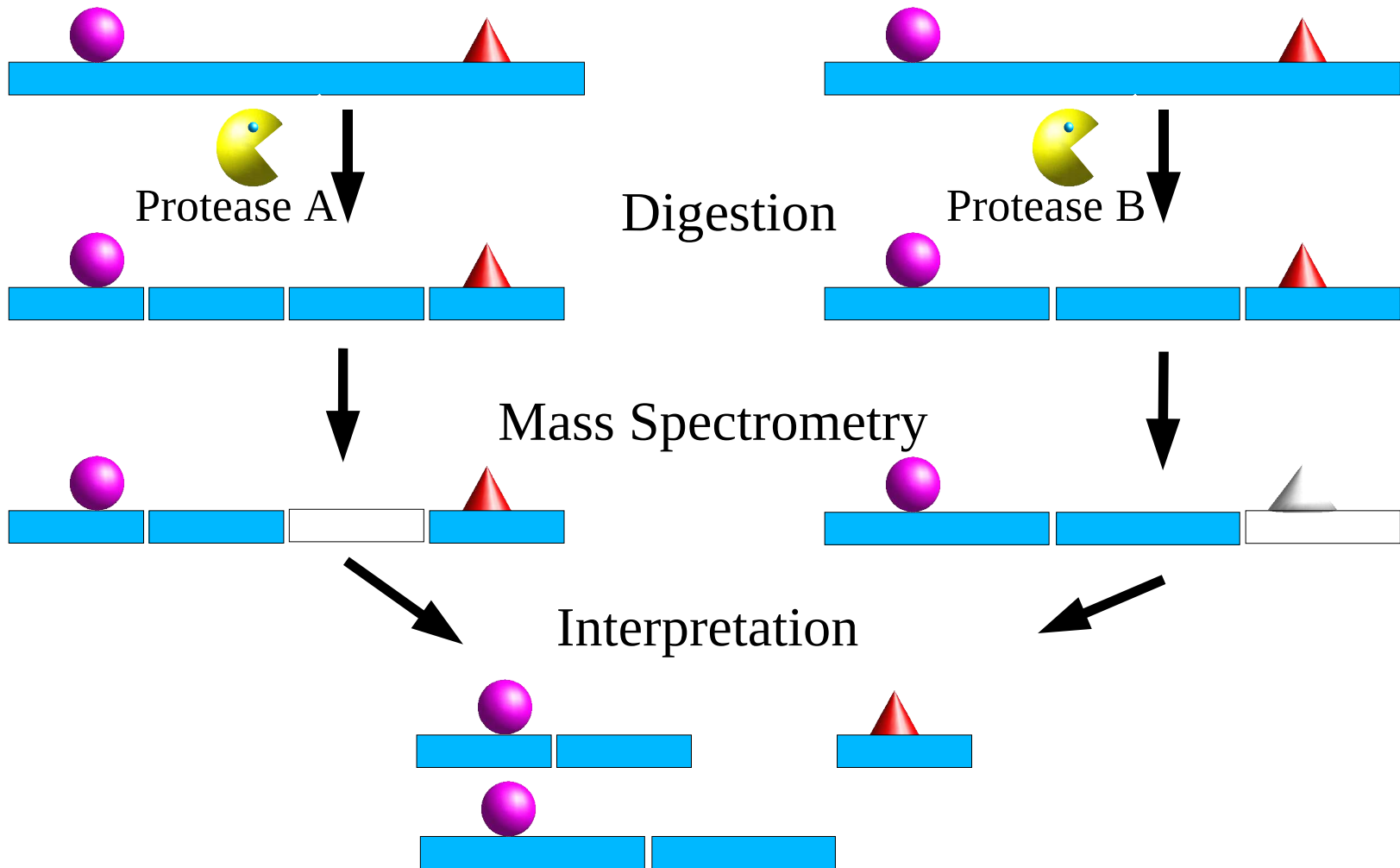
- Sequencing: Edman degradation

Edman Degradation



PTM Mapping a Purified Protein

- **Sequencing: Mass Spectrometry**

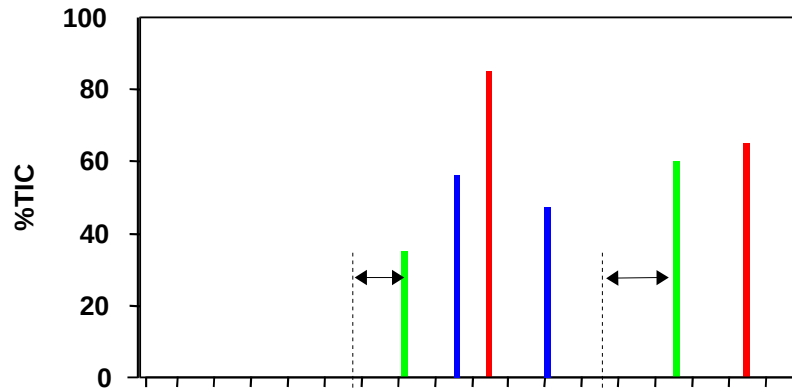


PTM Mapping a Purified Protein



Peptide Mass Fingerprinting
(MALDI, LC/ESI-MS)

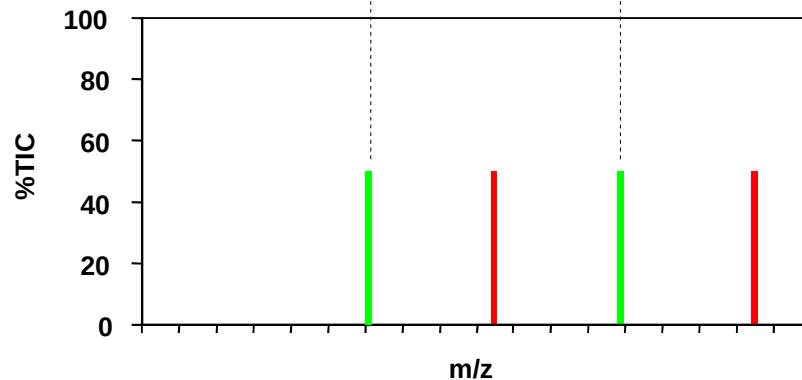
Experimental



Hits

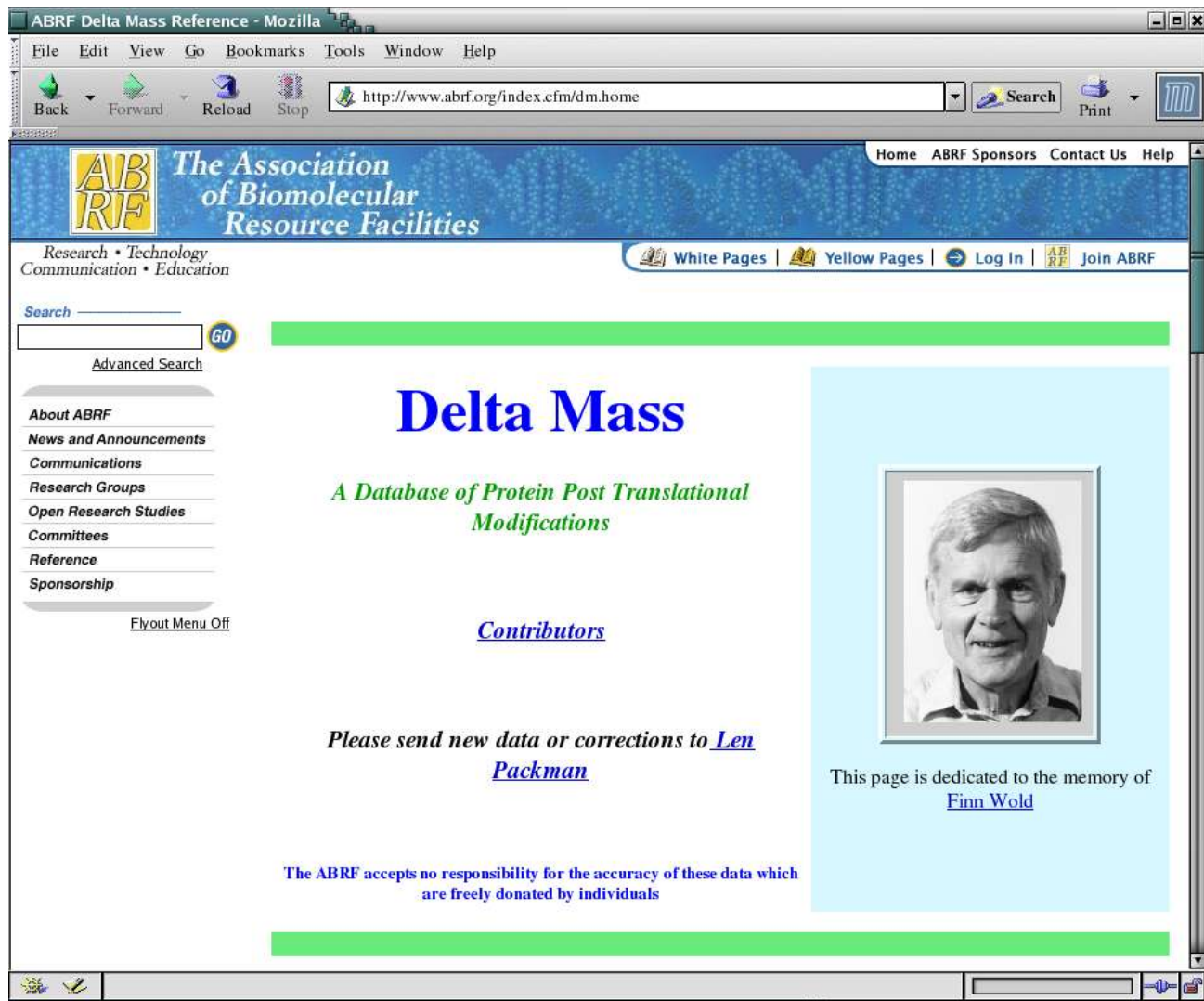
Misses

Theoretical



Other
Unmatched
Masses

Post-translational Modifications



<http://www.abrf.org/index.cfm/dm.home>

PTM Mapping a Purified Protein

Table 1. Some common and important post-translational modifications			
PTM type	Δ Mass ^a (Da)	Stability ^b	Function and notes
Phosphorylation pTyr pSer, pThr	+80 +80	+++ + / ++	Reversible, activation/inactivation of enzyme activity, modulation of molecular interactions, signaling
Acetylation	+42	+++	Protein stability, protection of N terminus. Regulation of protein–DNA interactions (histones)
Methylation	+14	+++	Regulation of gene expression
Acylation, fatty acid modification Farnesyl Myristoyl Palmitoyl etc.	+204 +210 +238	+++ +++ + / ++	Cellular localization and targeting signals, membrane tethering, mediator of protein–protein interactions
Glycosylation N-linked O-linked	>800 203, >800	+ / ++ + / ++	Excreted proteins, cell–cell recognition/signaling O-GlcNAc, reversible, regulatory functions
GPI anchor	>1,000	++	Glycosylphosphatidylinositol (GPI) anchor. Membrane tethering of enzymes and receptors, mainly to outer leaflet of plasma membrane
Hydroxyproline	+16	+++	Protein stability and protein–ligand interactions
Sulfation (sTyr)	+80	+	Modulator of protein–protein and receptor–ligand interactions
Disulfide bond formation	–2	++	Intra- and intermolecular crosslink, protein stability
Deamidation	+1	+++	Possible regulator of protein–ligand and protein–protein interactions, also a common chemical artifact
Pyroglutamic acid	–17	+++	Protein stability, blocked N terminus
Ubiquitination	>1,000	+ / ++	Destruction signal. After tryptic digestion, ubiquitination site is modified with the Gly-Gly dipeptide
Nitration of tyrosine	+45	+ / ++	Oxidative damage during inflammation

^aA more comprehensive list of PTM Δ mass values can be found at: <http://www.abrf.org/index.cfm/dm.home>
^bStability: + labile in tandem mass spectrometry, ++ moderately stable; +++ stable.

Mann M, Jensen ON. (2003) *Nat Biotechnol.* **21**:255-61.

PTM Mapping a Purified Protein

- **Unmatched masses can arise from several sources:**
 - **Contaminating proteins**
 - **Chemical modifications (eg MetO)**
 - **Unexpected cleavage**
- **The remaining unmatched matches can be considered PTMs.**

PTM Mapping a Purified Protein

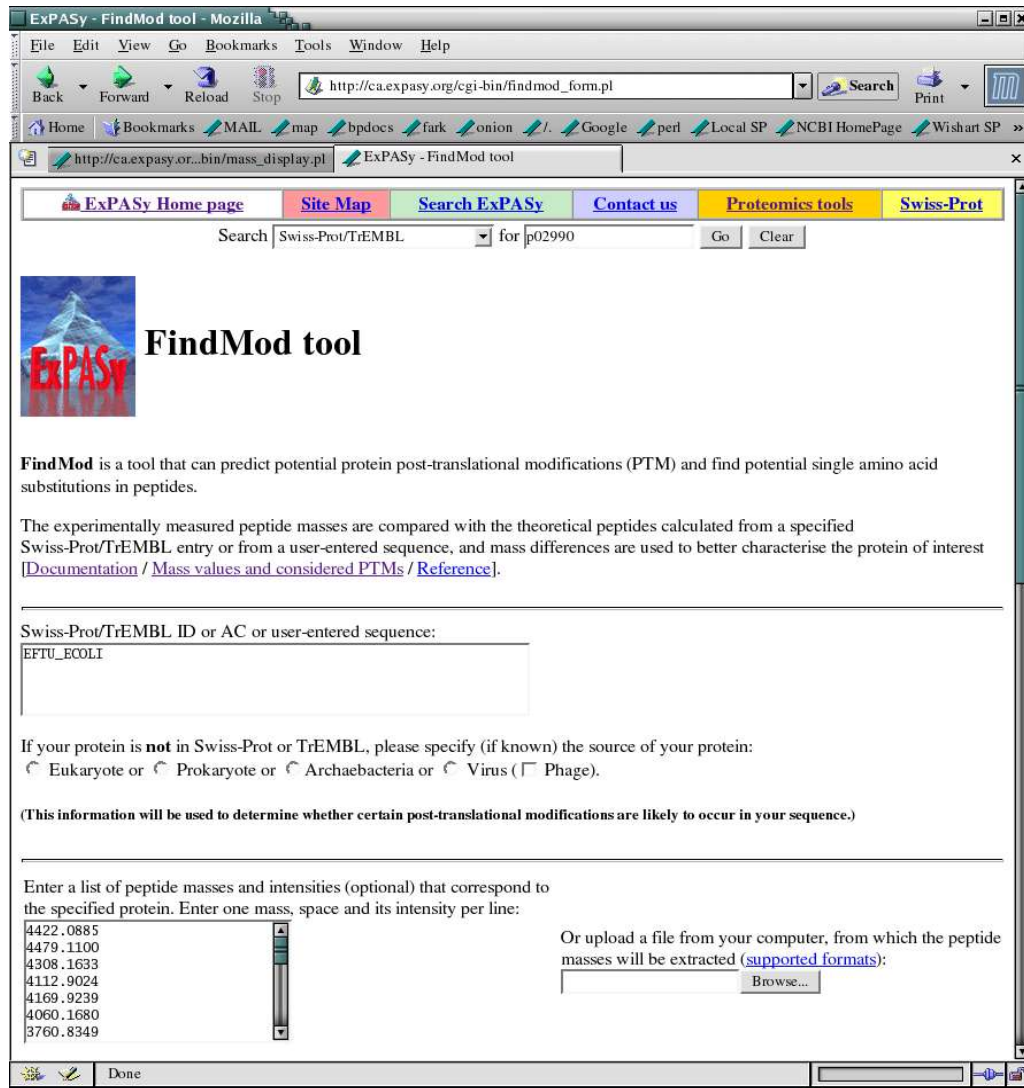
- MS-based protein sequence prediction software can look for a limited set of modifications **if they are known or suspected in advance.**
- The combinatorial explosion involved in matching modified masses between thousands of experimental spectra and millions of database peptides is intractable.

The screenshot shows the Matrix Science Mascot Peptide Mass Fingerprint web interface in a Mozilla browser window. The browser's address bar shows the URL <http://www.matrixscience.com/cgi/>. The page has a navigation bar with links: HOME, WHAT'S NEW, MASCOT, HELP, PRODUCTS, SUPPORT, CONTACT. Below this is a search bar with a 'Go' button. The main heading is 'MASCOT Peptide Mass Fingerprint'. The form includes fields for 'Your name' and 'Email'. The 'Search title' field is empty. The 'Database' is set to 'MSDB'. The 'Taxonomy' is set to 'All entries'. The 'Enzyme' is set to 'Trypsin'. The 'Allow up to' field is set to '1 missed cleavages'. The 'Fixed modifications' list includes: AB_old_ICATd0 (C), AB_old_ICATd8 (C), Acetyl (K), Acetyl (N-term), and Amide (C-term). The 'Variable modifications' list includes: AB_old_ICATd0 (C), AB_old_ICATd8 (C), Acetyl (K), Acetyl (N-term), and Amide (C-term). The 'Protein mass' field is empty, with a unit of 'kDa'. The 'Peptide tol. ±' is set to '1.0 Da'. The 'Mass values' are set to 'MH+' and 'M_r'. The 'Monoisotopic' checkbox is checked, and the 'Average' checkbox is unchecked. The 'Data file' field is empty, with a 'Browse...' button. The 'Query' field is empty, with a note: 'NB Contents of this field are ignored if a data file is specified.' The 'Overview' checkbox is unchecked. The 'Report top' field is set to '20 hits'. There are 'Start Search ...' and 'Reset Form' buttons. The footer contains the copyright notice: 'Copyright © 2003 Matrix Science Ltd. All Rights Reserved.'

PTM Mapping a Purified Protein

- “Second pass” software has been written to help identify PTMs *after protein identification*.
 - FindMod
 - GlycoMod
 - FindPept
 - Salsa

FindMod



The screenshot shows a Mozilla browser window titled "ExPASy - FindMod tool - Mozilla". The address bar displays "http://ca.expasy.org/cgi-bin/findmod_form.pl". The browser's menu bar includes File, Edit, View, Go, Bookmarks, Tools, Window, and Help. The toolbar contains buttons for Back, Forward, Reload, Stop, Search, and Print. The bookmarks bar shows links to Home, Bookmarks, MAIL, map, bpdocs, fark, onion, /, Google, perl, Local SP, NCBI HomePage, and Wishart SP. The main content area features a navigation bar with links to ExPASy Home page, Site Map, Search ExPASy, Contact us, Proteomics tools, and Swiss-Prot. Below this is a search bar with a dropdown menu set to "Swiss-Prot/TrEMBL" and a text input field containing "p02990". Buttons for "Go" and "Clear" are present. A red ExPASy logo is displayed next to the heading "FindMod tool". The text describes the tool's function: "FindMod is a tool that can predict potential protein post-translational modifications (PTM) and find potential single amino acid substitutions in peptides." It explains that experimentally measured peptide masses are compared with theoretical peptides calculated from a specified Swiss-Prot/TrEMBL entry or a user-entered sequence. Links for [Documentation], [Mass values and considered PTMs], and [Reference] are provided. A form for "Swiss-Prot/TrEMBL ID or AC or user-entered sequence:" contains the text "EFTU_ECOLI". Below this, a section asks for the source of the protein if it is not in the databases, with radio buttons for Eukaryote, Prokaryote, Archaeobacteria, and Virus (with a checkbox for Phage). A note states: "(This information will be used to determine whether certain post-translational modifications are likely to occur in your sequence.)". The next section prompts the user to "Enter a list of peptide masses and intensities (optional) that correspond to the specified protein. Enter one mass, space and its intensity per line:". A text area contains a list of mass and intensity pairs: 4422.0885, 4479.1100, 4308.1633, 4112.9024, 4169.9239, 4060.1680, and 3760.8349. To the right, a section for file upload states: "Or upload a file from your computer, from which the peptide masses will be extracted (supported formats):" with a "Browse..." button. The browser's status bar at the bottom shows "Done".

ExPASy Home page Site Map Search ExPASy Contact us Proteomics tools Swiss-Prot

Search Swiss-Prot/TrEMBL for p02990 Go Clear

 **FindMod tool**

FindMod is a tool that can predict potential protein post-translational modifications (PTM) and find potential single amino acid substitutions in peptides.

The experimentally measured peptide masses are compared with the theoretical peptides calculated from a specified Swiss-Prot/TrEMBL entry or from a user-entered sequence, and mass differences are used to better characterise the protein of interest [Documentation / Mass values and considered PTMs / Reference].

Swiss-Prot/TrEMBL ID or AC or user-entered sequence:
EFTU_ECOLI

If your protein is **not** in Swiss-Prot or TrEMBL, please specify (if known) the source of your protein:
☐ Eukaryote or ☐ Prokaryote or ☐ Archaeobacteria or ☐ Virus (☐ Phage).

(This information will be used to determine whether certain post-translational modifications are likely to occur in your sequence.)

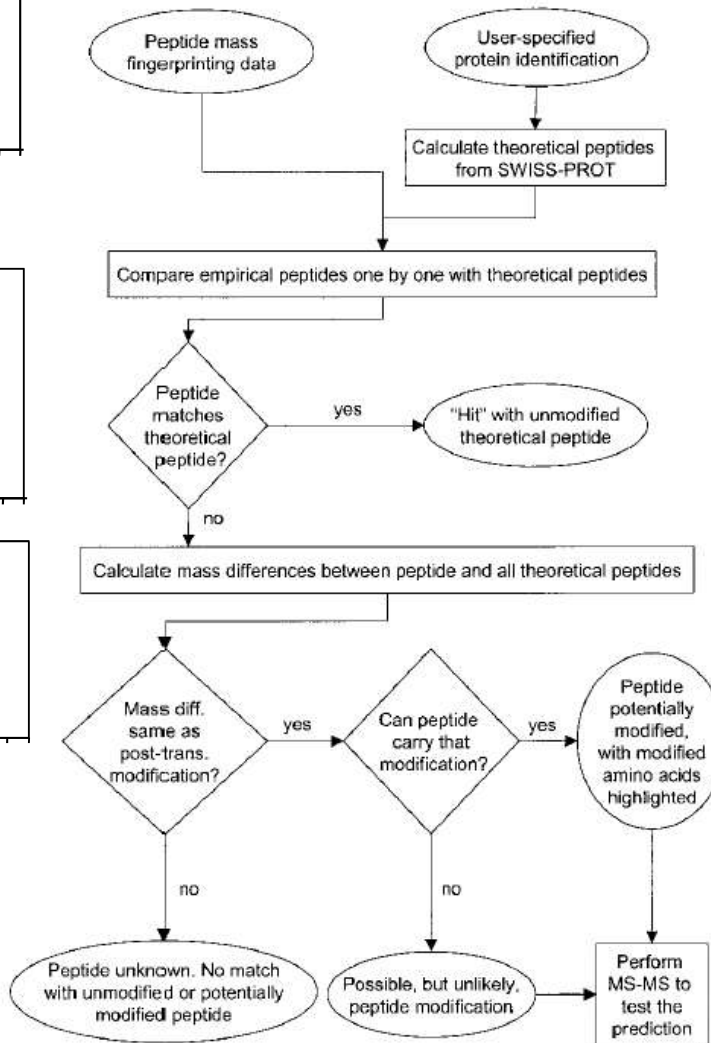
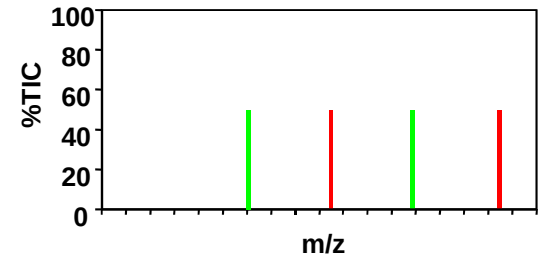
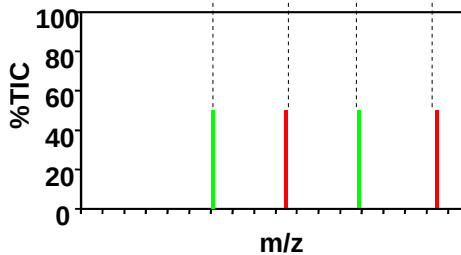
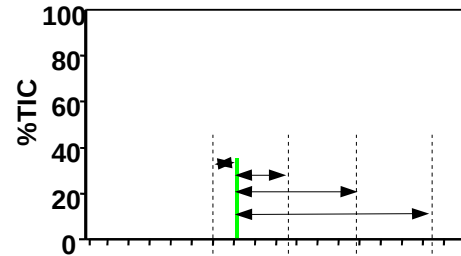
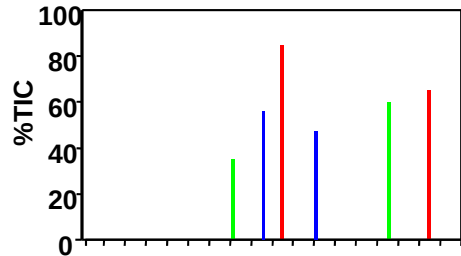
Enter a list of peptide masses and intensities (optional) that correspond to the specified protein. Enter one mass, space and its intensity per line:

4422.0885
4479.1100
4308.1633
4112.9024
4169.9239
4060.1680
3760.8349

Or upload a file from your computer, from which the peptide masses will be extracted (supported formats):
Browse...

<http://ca.expasy.org/tools/findmod/>

FindMod



NiceProt View of Swiss-Prot: P02900 - Mozilla

http://www.expasy.org/cgi-bin/nic

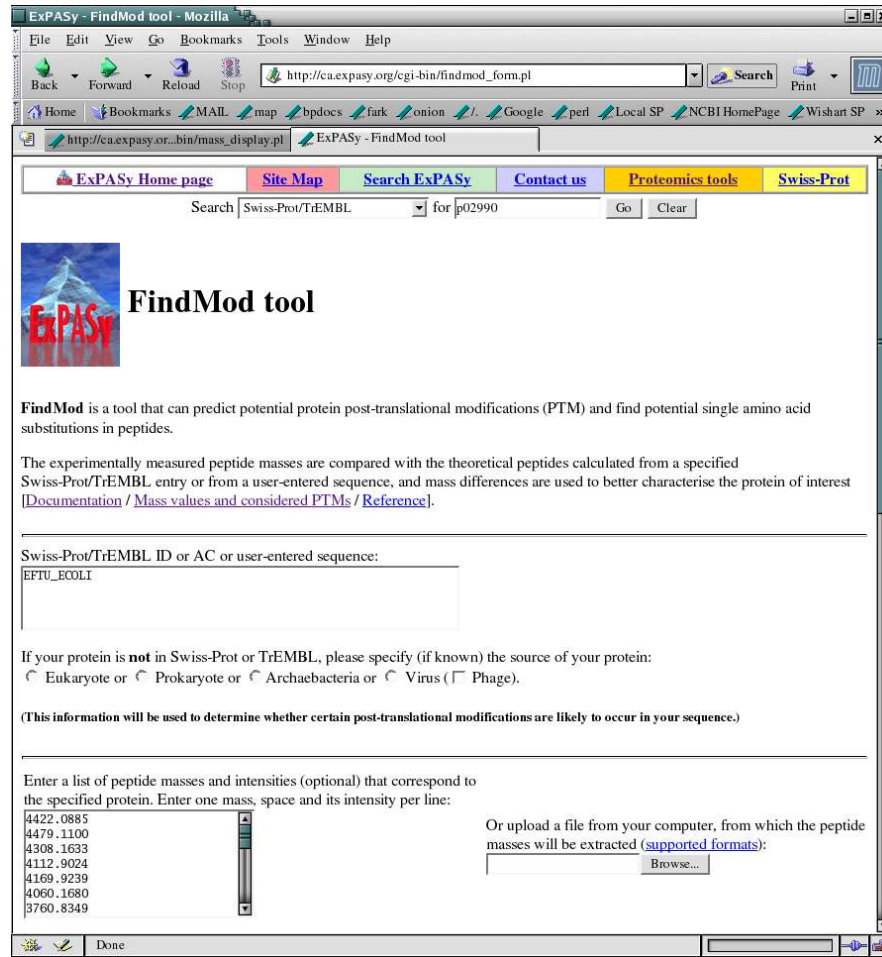
Entrez PubMed | Wiley InterScience | Journal

Key	From	To	Length	Description
INIT_MET	0	0		
NP_BIND	18	23	8	GTP.
NP_BIND	80	84	5	GTP.
NP_BIND	133	138	4	GTP.
BINDING	81	81		ASSOCIATED WITH AMINOACYL-TRNA BINDING.
BINDING	137	137		ASSOCIATED WITH GUANOSINE NUCLEOTIDE BINDING ACTIVITIES.
MOD_RES	1	1		ACETYLATION.
MOD_RES	56	56		N6-methyllysine (partial).
MOD_RES	56	56		N6,N6-dimethyllysine (partial).
MOD_RES	382	382		Phosphothreonine.
VARIANT	393	393	1	S -> G (in tufA).
MUTAGEN	19	19		H->A: No change in binding GDP and 3-fold reduction in binding EF-Ts.
MUTAGEN	20	20		V->G: Lowers GTPase activity 5 to 10-fold.
MUTAGEN	82	82		P->T: Loss of GTPase activity and creation of an autophosphorylation site.
MUTAGEN	114	114		Q->A: Weaker binding for GDP and for EF-Ts.
MUTAGEN	136	136		K->R,Q,E,I: Reduces affinity for GDP.
MUTAGEN	138	138		D->N: Reduces affinity for GDP; increases affinity for XDP.
MUTAGEN	124	124		Q->K: Kirmomycin resistant.
MUTAGEN	222	222		G->D: Inhibits codon-induced conformational changes leading to gtpase activation on the ribosome.
MUTAGEN	230	230		R->C: Pulvomycin resistant.
MUTAGEN	316	316		G->D: Kirmomycin resistant.
MUTAGEN	333	333		R->C: Pulvomycin resistant.
MUTAGEN	334	334		T->A: Pulvomycin resistant.
MUTAGEN	348	348		E->A: No change in binding GDP but higher binding constant for EF-Ts.
MUTAGEN	375	375		A->T,V: Kirmomycin resistant.
STRAND	11	17	7	
TURN	20	21	2	
HELIX	24	39	16	
HELIX	46	50	5	
STRAND	54	57	4	
TURN	58	59	2	

Done

FindMod

- Example: Lysine Dimethylation in *E. coli* elongation factor TU



The screenshot shows the ExPASy FindMod tool interface in a Mozilla browser window. The address bar displays the URL `http://ca.expasy.org/cgi-bin/findmod_form.pl`. The page features a navigation bar with links: ExPASy Home page, Site Map, Search ExPASy, Contact us, Proteomics tools, and Swiss-Prot. Below the navigation bar, there is a search section with a dropdown menu set to "Swiss-Prot/TrEMBL" and a text input field containing "p02990". A "Go" button and a "Clear" button are also present. The main content area is titled "FindMod tool" and includes a description of the tool's function: "FindMod is a tool that can predict potential protein post-translational modifications (PTM) and find potential single amino acid substitutions in peptides." It also provides a link to the "Documentation / Mass values and considered PTMs / Reference". A form for entering the "Swiss-Prot/TrEMBL ID or AC or user-entered sequence:" is shown, with the text "EFTU_ECOLI" entered. Below this, there is a section for specifying the protein source: "If your protein is not in Swiss-Prot or TrEMBL, please specify (if known) the source of your protein:" with radio buttons for "Eukaryote", "Prokaryote", "Archaeobacteria", and "Virus" (with a checkbox for "Phage"). A note states: "(This information will be used to determine whether certain post-translational modifications are likely to occur in your sequence.)". At the bottom, there is a section for entering a list of peptide masses and intensities (optional) that correspond to the specified protein. The text input field contains the following list: 4422.0885, 4479.1100, 4308.1633, 4112.9024, 4169.9239, 4060.1680, 3760.8349. To the right of this list, there is a section for uploading a file: "Or upload a file from your computer, from which the peptide masses will be extracted (supported formats):" with a "Browse..." button.

ExPASy - FindMod tool - Mozilla

File Edit View Go Bookmarks Tools Window Help

http://ca.expasy.org/cgi-bin/findmod_form.pl

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http://ca.expasy.org...bin/mass_display.pl ExPASy - FindMod tool

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 **FindMod tool**

FindMod is a tool that can predict potential protein post-translational modifications (PTM) and find potential single amino acid substitutions in peptides.

The experimentally measured peptide masses are compared with the theoretical peptides calculated from a specified Swiss-Prot/TrEMBL entry or from a user-entered sequence, and mass differences are used to better characterise the protein of interest [Documentation / Mass values and considered PTMs / Reference].

Swiss-Prot/TrEMBL ID or AC or user-entered sequence:
EFTU_ECOLI

If your protein is **not** in Swiss-Prot or TrEMBL, please specify (if known) the source of your protein:
☐ Eukaryote ☐ Prokaryote ☐ Archaeobacteria ☐ Virus ☐ Phage.

(This information will be used to determine whether certain post-translational modifications are likely to occur in your sequence.)

Enter a list of peptide masses and intensities (optional) that correspond to the specified protein. Enter one mass, space and its intensity per line:

4422.0885
4479.1100
4308.1633
4112.9024
4169.9239
4060.1680
3760.8349

Or upload a file from your computer, from which the peptide masses will be extracted (supported formats):
Browse...

Done

FindMod

- Example: Lysine Dimethylation in *E. coli* elongation factor TU

ExPASy - FindMod tool - Mozilla

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http://ca.expasy.org/cgi-bin/mass_display.pl ExPASy - FindMod tool

4479.110	4479.1100	0	1	HYAHVDCPGHADYVKNMITGAAQMDGAILVVAATDGPMPQTR	75-116	(1xCys_CAM)
----------	-----------	---	---	--	--------	-------------

Potentially modified peptides, detected by mass difference and conforming to rules (considering only peptide masses that have not matched above):

User mass	DB mass	mass diff.	mod. diff.	Δ mass (Dalton)	potential mod.	#MC	peptide	position	known modifications
1631.800	1603.7710	28.0290	28.0314	0.002	DIMETH	1	AFDQIDNAPEEKAR	45-58	
1631.800	1617.7867	14.0133	14.0157	0.002	METH	1	AFDQIDNAPEEKAR	45-58	(METH: 56)
1631.800	1616.7407	15.0593	14.9997	-0.059	DEAME	1	ETQKSTCTGVEMFR	249-262	
1631.800	1616.7407	15.0593	14.9996	-0.059	ALKY-DEAM	1	ETQKSTCTGVEMFR	249-262	
1631.800	1616.7407	15.0593	14.9997	-0.059	DEAM-METH	1	ETQKSTCTGVEMFR	249-262	
1631.800	1315.6133	316.1867	316.1402	-0.046	BROM-PALM	1	STCTGVEMFRK	253-263	(1xCys_CAM)
1631.800	1574.9376	56.8624	57.0103	0.148	ACET-DEAME	0	GQVLAKPGTIKPHTK	289-303	
1631.800	1574.9376	56.8624	57.0215	0.159	CAM-METH	0	GQVLAKPGTIKPHTK	289-303	
1631.800	1574.9376	56.8624	57.0705	0.208	METH-TRIMETH	0	GQVLAKPGTIKPHTK	289-303	

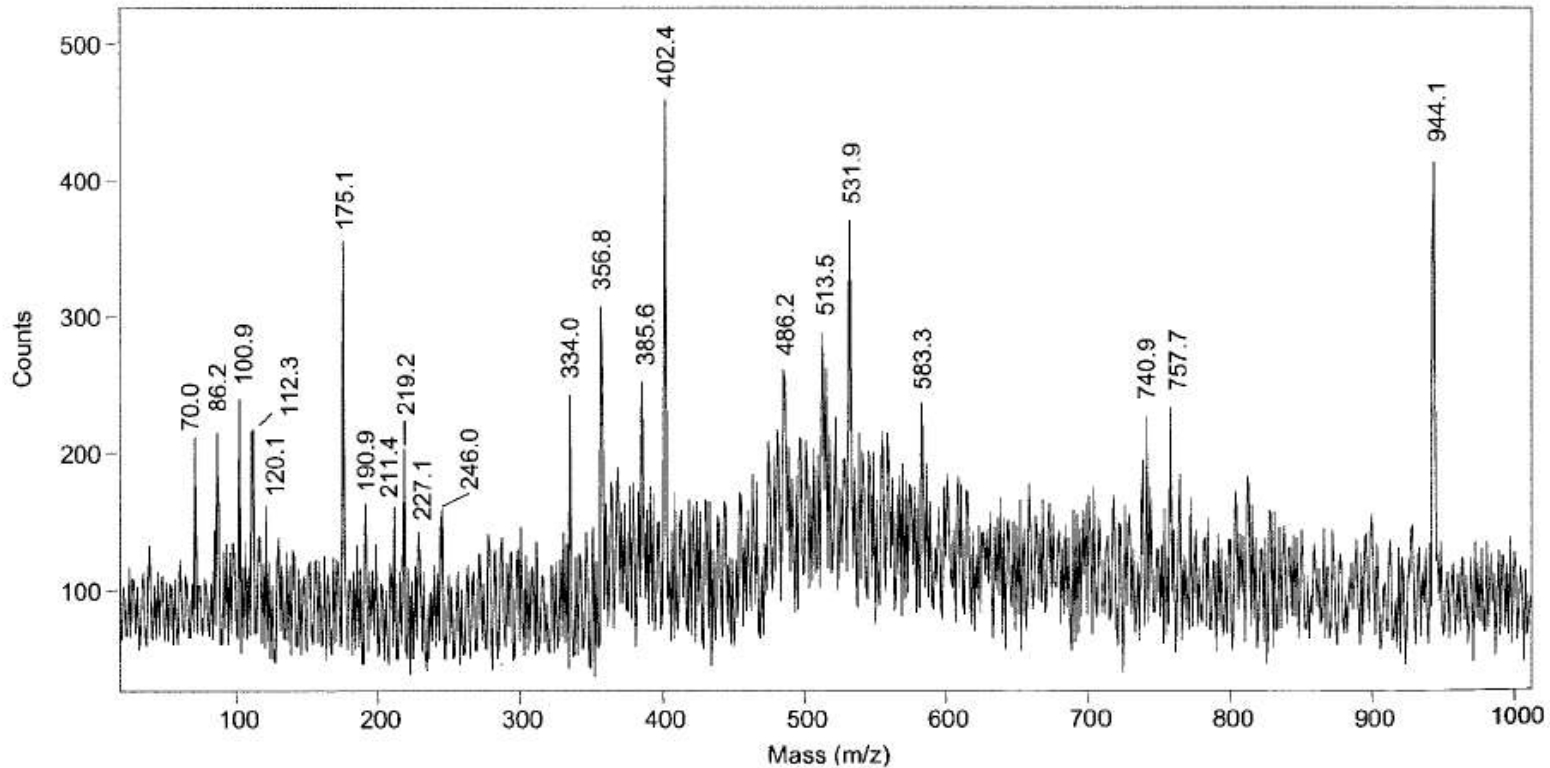
Potential PTMs detected by mass differences, but not confirmed by rules:

User mass	DB mass	mass diff.	mod. diff.	Δ mass (Dalton)	potential mod.	#MC	peptide	position	known modifications
1631.800	1376.6328	255.1672	255.1167	-0.049	BIOT-DIMETP	0	AFDQIDNAPEEK	45-56	
1631.800	1376.6328	255.1672	255.0678	-0.098	BIOT-NTRY	0	AFDQIDNAPEEK	45-56	

Done

FindMod

- Post Source Decay Spectrum of AFDQIDNAPII-K(dimethyl)-AR



FindMod

MS-Product (by [Peter Baker](#) and [Karl Clauser](#)) [Instructions](#)

A tool from the [UCSF Mass Spectrometry Facility](#) that calculates the possible fragment ions resulting from fragmentation of a peptide in a mass spectrometer. Fragmentation possibilities for post-source decay (PSD), high-energy collision-induced dissociation (CID), and low-energy CID processes may be calculated. The fragment ion types seen in **PSD are the default selections**. The program generally uses the standard Biemann-style nomenclature: Biemann, K. *Method Enzymol.* **1990**, *193*, 886-888.

MS-Product can accept a data set as a parameter and show matching peaks.

ProteinProspector Home	MS-Fit	MS-Tag	MS-Seq	MS-Bridge	MS-Pattern
MS-Digest	MS-Comp	DB-Stat	MS-Isotope	MS-Homology	MS-Product at UCSF (San Francisco)

Calculate masses as: Max. Reported Charge: Cysteines Modified by:

----Peptide----

Enter Sequence in **Capital letters** except:
 | m - Met-ox | q - Pyroglutamic acid | h - Homoserine lactone | s, t, y - Phosphorylated S, T, Y | u, v, w, x - user specified amino acids |

N-terminus **Sequence** **C-terminus**

----- **Fragment Ion Types to be Listed** -----
 (some types require presence of specific AA's in ion)

Instrument: ☐ Use instrument specific defaults to override ion types below

AA Composition ions			N-term sequence ions			C-term sequence ions			Internal Fragment-ions	Ladder sequencing ions		
☐ i	☐ m		☐ a	☐ b	☐ c	☐ x	☒ y	☐ Y	☐ z	☐ internal	☐ N-term	☐ C-term
Satellite Sequence Ions (side-chain loss)			Neutral-loss Sequence Ions						Peeling Sequence Ions			
☐ d	☐ v	☐ w	☒ -H ₂ O S, T, E, D	☒ -NH ₃ R, K, Q, N	☐ -H ₃ PO ₄ s, t, y (S, T, Y + PO ₄)	☐ -SOCH ₃ m (M + Ox)	☐ Multiple Losses	☒ b+H ₂ O R, H, K, N				

[User Specified AA elemental composition \(u\):](#)
[User Specified AA elemental composition \(v\):](#)
[User Specified AA elemental composition \(w\):](#)
[User Specified AA elemental composition \(x\):](#)

<http://prospector.ucsf.edu/ucsfhtml4.0/msprod.htm>

FindMod

MS-Product Search Results - Mozilla

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MS-Product Search Results

Parameters

Sequence: **AFDQIDNAPEExAR**
 Amino Acid Composition: **A3 D2 E2 F1 I1 N1 P1 Q1 R1 x1**
 Peptide N terminus: **Hydrogen**
 Peptide C terminus: **Free Acid**
 User AA Formula 4: **C8 H16 N2 O1**
 Cysteine Modification: **carbamidomethylation**
 Instrument Name: **MALDI-TOF**
 Ion Types Considered: **y, h, n, B**
 Elemental Composition: [C70 H111 N20 O25](#)
 All fragment ion masses below are calculated as: **monoisotopic** masses
 Peptide Mass MH^+ (monoisotopic): **1631.8029**
 Peptide Mass MH^+ (average): **1632.7816**

N-terminal ions

1	2	3	4	5	6	7	8	9	10	11	12	13	14
H - A	F	D	Q	I	D	N	A	P	E	E	x	A	R - OH
14	13	12	11	10	9	8	7	6	5	4	3	2	1

C-terminal ions

--- 1560.77 1413.70 1298.67 1170.61 1057.53 942.50 828.46 757.42 660.37 531.33 402.28 246.16 175.12 y ions
 --- 1543.74 1396.67 1281.64 1153.59 1040.50 925.47 811.43 740.39 643.34 514.30 385.26 229.13 158.09 y-NH₃ ions

158.09	y ₁ -NH ₃	514.30	y ₄ -NH ₃	811.43	y ₇ -NH ₃	1153.59	y ₁₀ -NH ₃	1543.74	y ₁₃ -NH ₃
175.12	y ₁	531.33	y ₄	828.46	y ₇	1170.61	y ₁₀	1560.77	y ₁₃
229.13	y ₂ -NH ₃	643.34	y ₅ -NH ₃	925.47	y ₈ -NH ₃	1281.64	y ₁₁ -NH ₃		
246.16	y ₂	660.37	y ₅	942.50	y ₈	1298.67	y ₁₁		
385.26	y ₃ -NH ₃	740.39	y ₆ -NH ₃	1040.50	y ₉ -NH ₃	1396.67	y ₁₂ -NH ₃		
402.28	y ₃	757.42	y ₆	1057.53	y ₉	1413.70	y ₁₂		

Done

FindMod

Ion type	Amino acid or sequence	Measured mass	Theoretical mass
Immonium	P	70.0	70
	I/L	86.2	86
	Q	100.9	101
	R	112.3	112
	F	120.1	120
a 2	AF-	190.0	191.3
b 2	AF-	219.2	219.3
b 3	AFD-	334.0	334.4
y 1	-R	175.1	175.2
y 2	-AR	246.0	246.3
y 3-NH ₃	-K(dimeth)AR	385.6	385.5
y 3	-K(dimeth)AR	402.4	402.5
y 4-NH ₃	-EK(dimeth)AR	513.5	514.6
y 4	-EK(dimeth)AR	531.9	531.6
y 6-NH ₃	-PEEK(dimeth)AR	740.9	740.8
y 6	-PEEK(dimeth)AR	757.7	757.9
Internal Fragments ^a	-PE-	227.1	227.2
	-PEE-	356.8	356.4
	-EEK(dimeth)A-	486.2	486.5
	-PEEK(dimeth)A-	583.3	583.7
	-DQIDNAPEEK(dimeth)A-	1222.2	1222.3

Wilkins et al. (1999) J. Mol. Biol. 289:645-657

FindMod

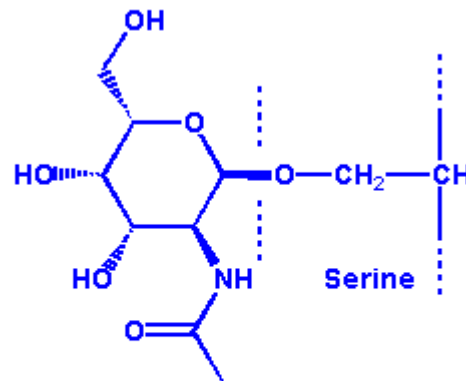
- **Considers about 30 modifications**
- **Cannot handle complex modifications such as glycosylation (except O-GlcNac).**

GlycoMod

- **Glycosylation is one of the most common PTMs (estimated >50% of all proteins are glycosylated).**
- **Glycosylations can be quite complex**
- **GlycoMod finds all possible compositions of a glycan structure from its experimentally determined mass.**

Glycosylation

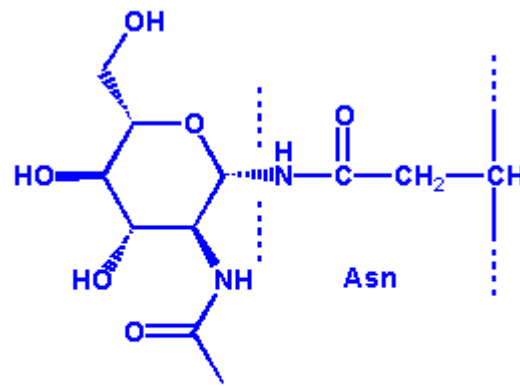
- **O-linked**
 - Ser, Thr, Hydroxy-Lys, or hydroxy-Pro
 - Glycan can be linked to Ser or Thr via a phosphate group (a phosphodiester linkage).



O-linkage to GalNAc

Glycosylation

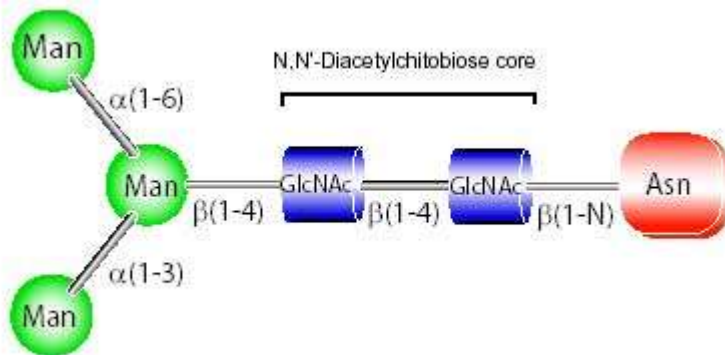
- **N-linked**
 - Linkage is through amide nitrogen of Asparagine
 - Almost always have GlcNAc at the reducing terminus
 - Consensus sequence: -Asn-[!Pro]-[Ser|Thr|(Cys)]



N-linkage to GlcNAc

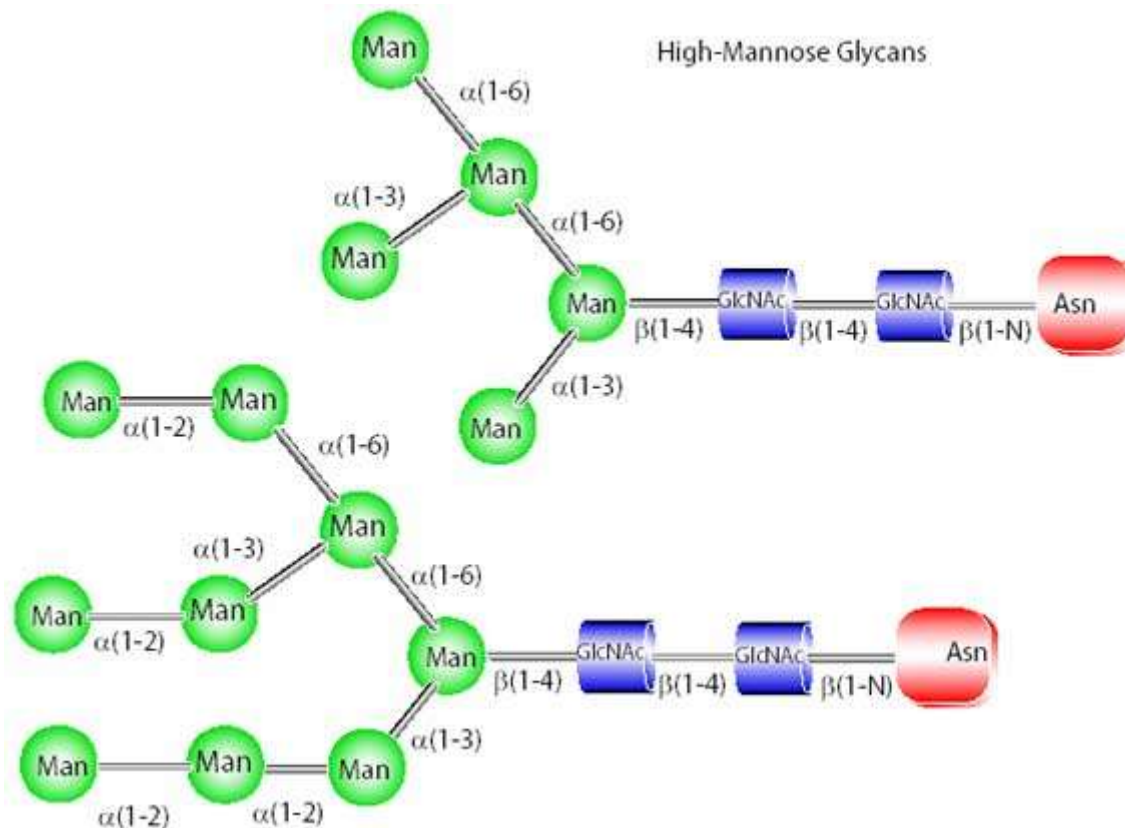
Glycosylation

- **N-linked**
 - Almost always contains the core pentasaccharide
 $\text{Man}(\alpha 1-6)[\text{Man}(\alpha 1-3)]\text{Man}(\beta 1-4)\text{GlcNAc}(\beta 1-4)\text{GlcNAc}(\beta 1-4)$



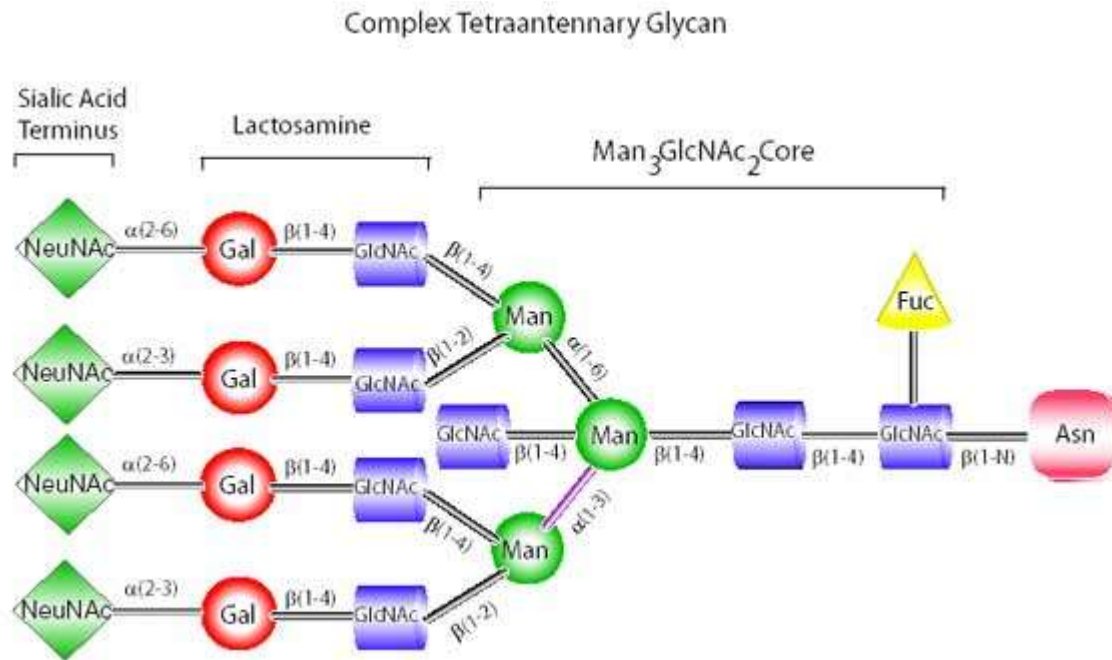
Glycosylation

- N-linked
 - High mannose type



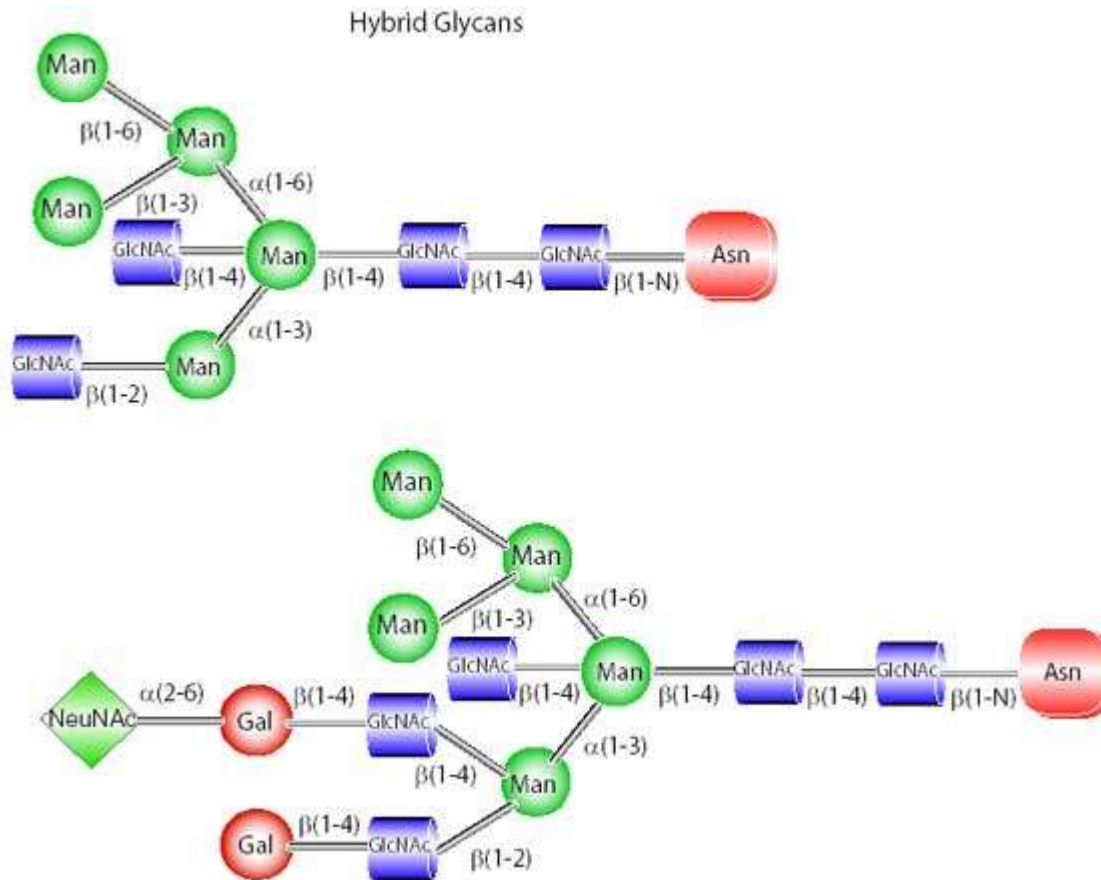
Glycosylation

- N-linked
 - Complex type



Glycosylation

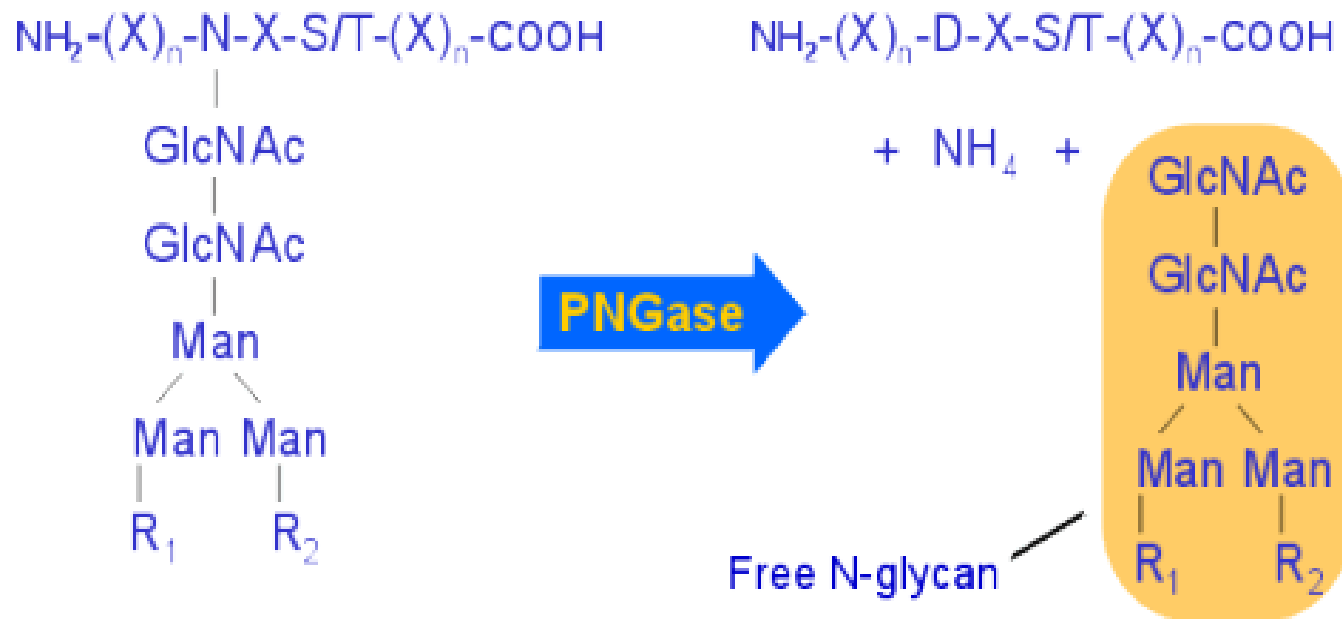
- **N-linked**
 - **Hybrid type**



Glycosylation

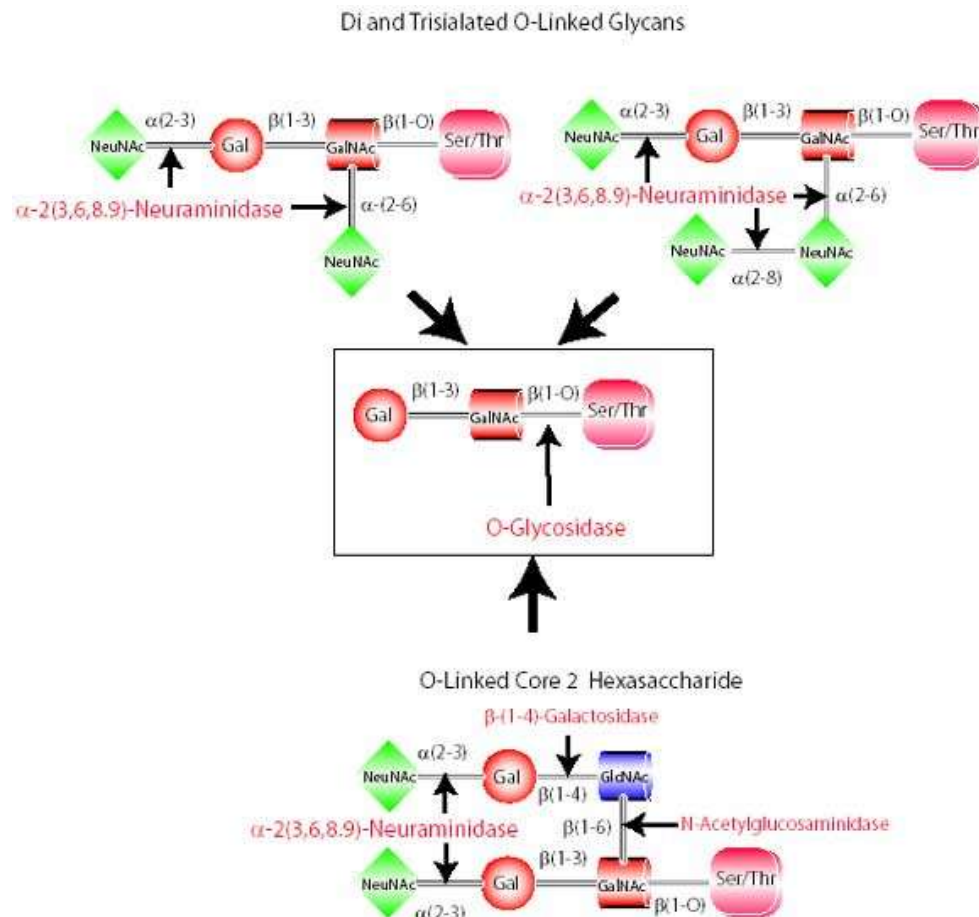
- **PNGase**

Peptide:N-glycanase activity



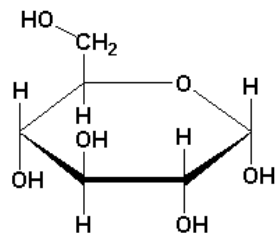
Glycosylation

- O-Glycosidase

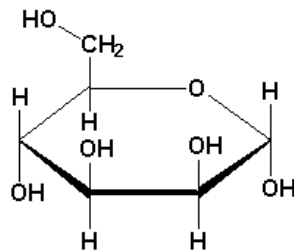


Glycosylation

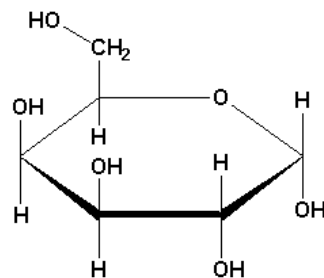
- MS cannot distinguish between monosaccharides with the same mass:



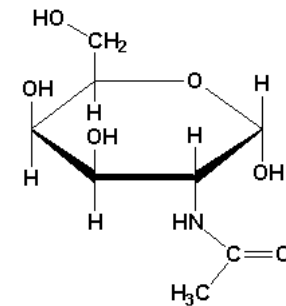
Glucose



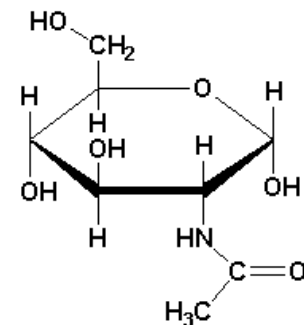
Mannose



Galactose



N-Acetylgalactosamine



N-acetylglucosamine

“Hexoses”

Glycosylation

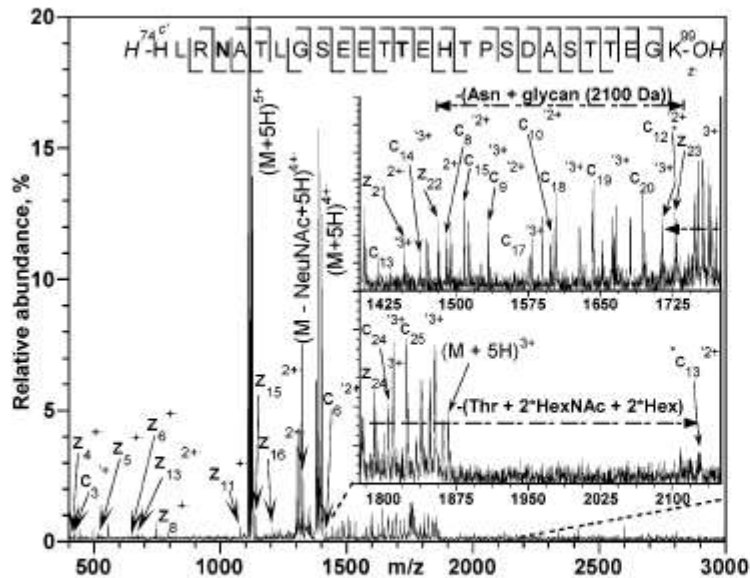


Figure 4. ECD FT mass spectrum of 5+ of peptide 74–99. Two out of 10 potential sites were glycosylated, by N-linkage at Asn77 (add-on mass 2100.7765 Da) and O-linkage at Thr86 (add-on mass 730.2535 Da).

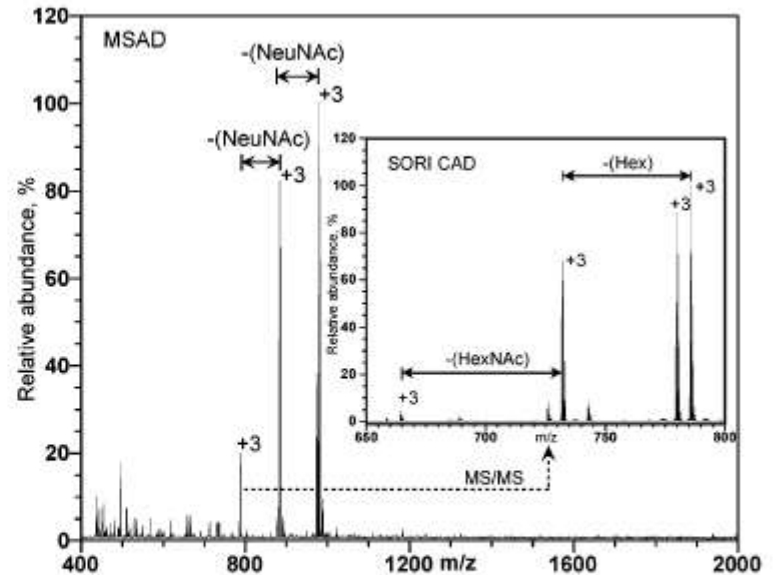


Figure 5. MSAD and SORI CAD (inset) FT mass spectra of 3+ of the peptide from fraction Lys-C 18 showing consecutive losses of two units of NeuNAc (291 Da), one unit of Hex (162 Da), and one unit of HexNAc (203 Da).

Kjeldsen et al. Anal. Chem.2003, 75,2355-2361

GlycoMod

- Finds all possible compositions of a glycan structure from its experimentally determined mass by comparing the the mass against a list of precomputed glycan masses.
- <http://ca.expasy.org/tools/glycomod/>

GlycoMod

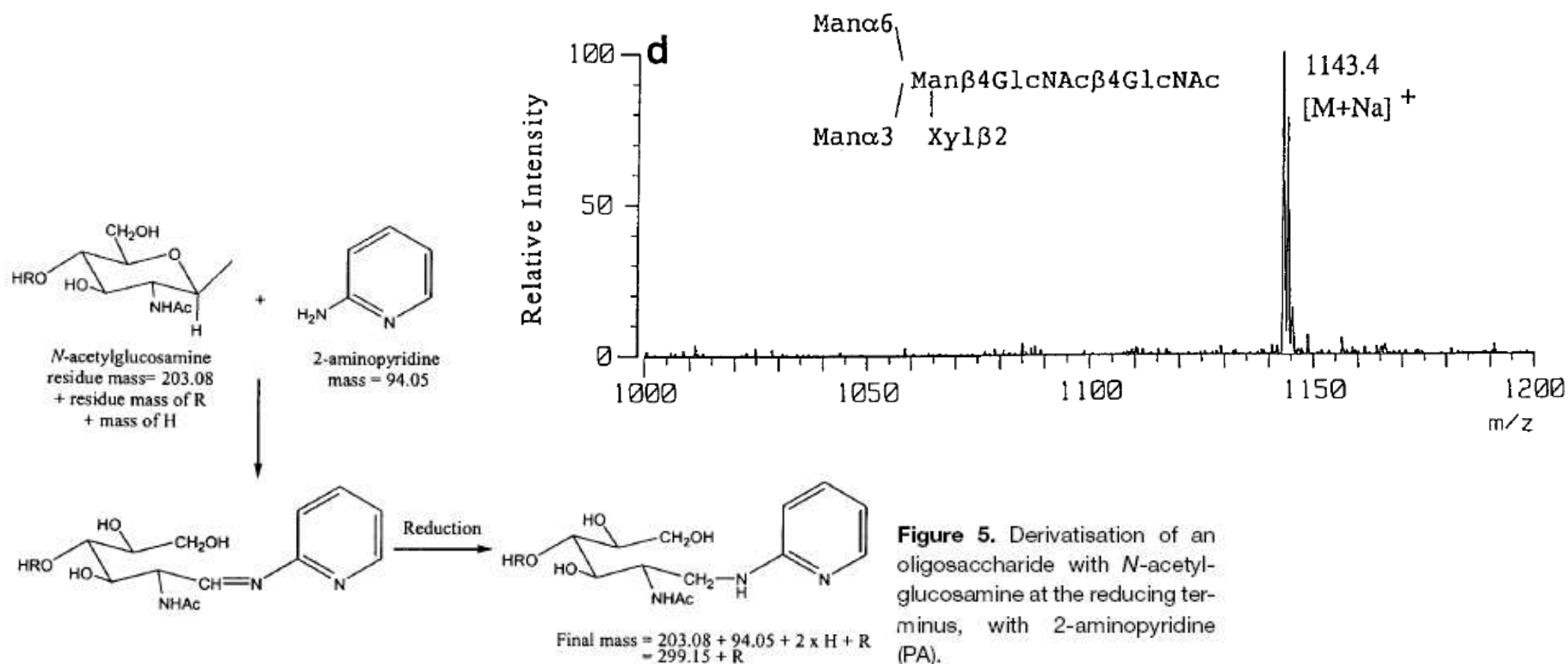
Table 1. Upper limits imposed on the number of residues of a particular monosaccharide (based on a survey of the literature) found in *O*-linked and *N*-linked oligosaccharides using GlycoMod

Monosaccharide residue	<i>O</i> -linked oligosaccharides	<i>N</i> -linked oligosaccharides
Hexose	0–14	0–20
HexNAc	0–14	0–20
Deoxyhexose	0–6	0–6
NeuAc	0–7	0–5
NeuGc	0–7	0–5
Pentose	0–3	0–3
Sulphate	0–6	0–3
Phosphate	0–6	0–2
KDN	0–2	0–0
HexA	0–2	0–0

Cooper et al. (2001) *Proteomics* 1:340-349

GlycoMod

Example: 2-aminopyridine derivatised N-linked oligosaccharide
From horseradish peroxidase



GlycoMod

Example: 2-aminopyridine derivatised N-linked oligosaccharide
From horseradish peroxidase

- Mass Value: 1143.4
- Mass Type: monoisotopic
- IonMode/Adducts: $[M+Na]^+$
- N-linked oligo form: Derivatised oligosaccharide
- Reducing terminal derivative id: 'PA'
- Reducing terminal derivative monoisotopic mass: 94.053

GlycoMod

Example: 2-aminopyridine derivatised N-linked oligosaccharide
From horseradish peroxidase

User mass: 1143.4

Adduct (Na⁺): 22.989768

Derivative mass (PA): 96.06874

glycoform mass	Δ mass (Dalton)	structure	type	Links
1024.323	0.018	(Hex) ₁ (NeuAc) ₁ (NeuGc) ₁ (Pent) ₂	-	
1024.36	-0.018	(Hex) ₃ (HexNAc) ₂ (Pent) ₁	-	GlycoSuiteDB

2 structures found.

FindPept

- Identifies the origin of “missing” peptide masses resulting from unspecific proteolytic cleavage, missed cleavage, protease autolysis or keratin contaminants.
- Takes into account PTMs and protease autolysis peaks (and keratin peaks).

<http://ca.expasy.org/tools/findpept.html>

FindPept tool

FindPept can identify peptides that result from **unspecific cleavage** of proteins from their experimental masses, taking into account artefactual chemical modifications, post-translational modifications (PTM) and protease autolysis cleavage. If you wish to take into account only specific cleavage, please use [FindMod](#) instead.

The experimentally measured peptide masses are compared with the theoretical peptides calculated from a specified Swiss-Prot/TrEMBL entry or from a user-entered sequence. If autolysis is to be taken into account, an enzyme entry must be specified from the drop-down list of enzymes for which the sequence is known. ([Documentation](#) / [Mass values and considered PTMs](#) / [Reference](#))

Swiss-Prot/TrEMBL ID or AC or user-entered sequence (you may specify post-translational modifications, as specified in [this document](#)):

Enter a list of peptide masses and intensities (optional) that correspond to the specified protein. Enter one mass, space and its intensity per line:

Or upload a file from your computer, from which the peptide masses will be extracted ([supported formats](#)):

If you wish to define other post-translational modifications than [those already in the FindMod/FindPept Database](#), you can specify them here:

	modification name	abbreviation	atom composition	positions
0	example	OXID	H 2, O 1, C 1, N 1, S 1	86 M
1			H 1, O 1, C 1, N 1, S 1	
2			H 1, O 1, C 1, N 1, S 1	

All peptide masses are

with cysteines treated with: ☐ nothing (in reduced form) ☒ with methionines oxidized ☐ with acidic and C-terminal residues esterified ☐ with possible N-Acetylation or N-Formylation ☒ [M+H]⁺ or ☐ [M] or ☐ [M-H]⁻ ☒ average or ☐ monoisotopic.

Mass tolerance: daltons

Display peptides sorted by: ☒ peptide masses or in: ☐ chronological order in the protein.

If you select a protease below, the peptide masses will be checked for specific cleavage as well as autolytic cleavage of the enzyme.

Select an [enzyme](#) (Optional):

Exclude masses that match: ☐ specific cleavage by the enzyme ☐ autolytic cleavage of the enzyme ☐ specific cleavage of human keratins

☐ Send the result by e-mail

Your e-mail address:

Name of the unknown protein:

To run the search:

To clear all fields:

Last modified: 22/06/2010 by EIC

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FindPept

Table 1. List of post-translational and artifactual modifications handled by FindPept. Modifications are applied optionally, except those marked with [*] which are quantitatively applied. For more details on PTMs, see http://www.expasy.org/tools/findmod/findmod_masses.html.

PTMs read in the feature table (FT lines) of a SWISS-PROT entry:	
<i>FT key</i>	<i>description</i>
INIT_MET	Initiator methionine cleavage
MOD_RES and BINDING	Acetylation; amidation; beta-methylthiolation; biotin; carbamylation; citrullination; deamidation; dimethylation; FAD; formylation; gamma-carboxyglutamic acid; hydroxylation; methylation; phosphorylation; pyridoxal phosphate; phosphopantetheine; pyrrolidone carboxylic acid; sulfation; trimethylation
CARBOHYD	N-linked glycation, O-linked N-acetylglucosamination, C-linked mannosylation
LIPID	N-acyl diglyceride, farnesyl, geranylgeranyl, lipoyl, myristyl, palmityl, n-octanoyl
CHAIN and PEPTIDE	Cleavage of chains
Artificial modifications specified by the user:	
– Treatment of cysteine by iodoacetamide, iodoacetic acid or 4-vinylpyridene [*] – Acrylamide adducts on cysteine – Modification of methionine to homoserine lactone by CNBr cleavage – Oxidation of methionine – Methyl esterification of carboxylate side chains and terminus [*]	
PTMs occurring frequently naturally which are considered by the program:	
– Possible N-terminal acetylation – Possible N-terminal formylation	

FindPept

- Example: Atypical cleavage of chicken lysozyme
- Scenario: Chicken lysozyme C (P00698) digestion with lysyl endopeptidase (Lys C) gives a mass at 1262.591 that cannot be explained by the cleavage rule for this enzyme (carboxy side of Lys, including Lys Pro).

You have selected [LYC_CHICK](#) (P00698) from Swiss-Prot:

Lysozyme C precursor (EC 3.2.1.17) (1,4-beta-N-acetylmuramidase C) (Allergen Gal d 4) (Gal d IV).
Signal in positions 1-18 has been removed.

- Chain Lysozyme C at positions [19 - 147](#) [Theoretical pI: 9.32 / Mw (average mass): 14313.14 / Mw (monoisotopic mass): 14303.88]

mass	position	#MC	artif. modification(s)	peptide sequence
6897.1896	52-114	0	Cys_CAM: 82, 94, 98, 112 7125.2755	FESNFNTQATNRNTDGSTDY GILQINSRWWCNDGRTPGSR NLCNIPCSALLSSDITASVN CAK
2280.1090	32-51	0	Cys_CAM: 48 2337.1305	RHGLDNYRGYSLGNWVCAAK
2177.0491	116-134	0	Cys_CAM: 133 2234.0705	IVSDGNGMNAWVAWRNRCK
1474.7583	135-147	0	Cys_CAM: 145 1531.7798	GTDVQAWIRGRL
1295.6598	20-31	0	Cys_CAM: 24 1352.6813	VFGRCELAAAMK

98.4% of sequence covered (you may modify the input parameters to display also peptides < 500 Da):

```

      1      11      21      31      41      51
      |      |      |      |      |      |
1  kV VFGRCELAAAM KHGLDNYRG YSLGNWVCAA KFESNFNTQA      60
61 TNRNTDGSTD YGILQINSRW WCNDGRTPGS RNLNIPCSA LLSSDITASV NCAKkIVSDG      120
121 NGMNAWVAWR NRCKGTDVQA WIRGRL
  
```


FindPept

- Example: Atypical cleavage of chicken lysozyme
- MS-MS analysis confirms (K)GTDVQAWIRGC(R).
- *Myxobacter* Lys C has been shown to cleave C-term of Carboxymethylcysteine.

Matching peptides for specific cleavage:

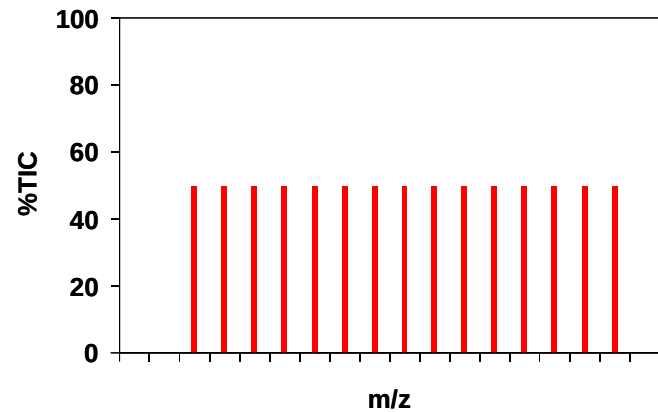
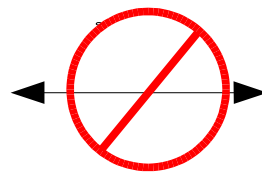
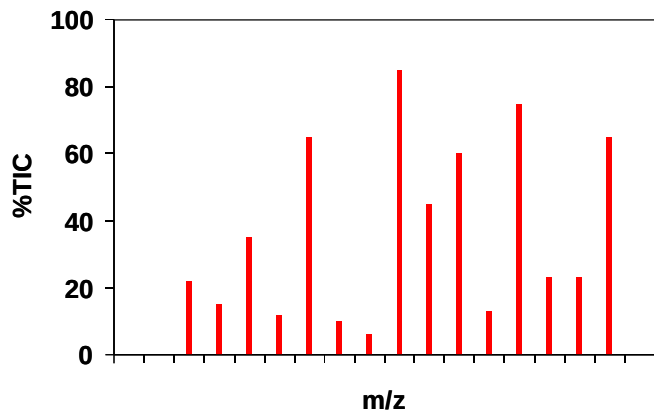
User mass	DB mass	Δ_{mass} (ppm)	peptide	position	modifications	missed cleavages
1352.681	1352.681	0.200	(K)/VFGRCELAAAMK/ (R)	20-31	CYS_CAM	0
1531.780	1531.780	-0.100	(K)/GTDVQAWIRGCRL	135-147	CYS_CAM	0
2234.070	2234.071	0.200	(K)/IVSDGNGMNAWVAWRNRCK/ (G)	116-134	CYS_CAM	0
2337.131	2337.130	-0.200	(K)/RHGLDNYRGYSLGNWVCAAK/ (F)	32-51	CYS_CAM	0
7125.275	7125.275	0.000	(K)/FESNFNTQATNRNTDGDSTDY GILQINSRWWCNDGRTPGSR NLCNIPCSALLSSDITASVN_CAK/ (K)	52-114	CYS_CAM CYS_CAM CYS_CAM CYS_CAM	0

Matching peptides for unspecific cleavage:

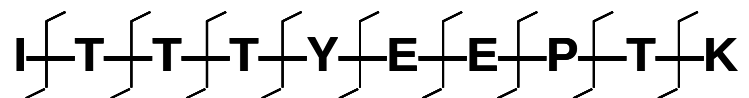
User mass	DB mass	Δ_{mass} (ppm)	peptide	position	modifications	missed cleavages
1262.591	1262.583	-6.000	(K)/IVSDGNGMNAWV (A)	116-127		0
1262.591	1262.595	2.800	(K)/GTDVQAWIRGC (R)	135-145	CYS_CAM	0
1262.591	1262.610	14.900	(I)IQINSRWWC (R)	74-82	CYS_CAM	0
1262.591	1262.616	19.500	(V)NCAKIVSDGNG (M)	111-122	CYS_CAM	1

SALSA

- MS/MS-based sequence prediction packages cannot identify 'unanticipated' modifications.



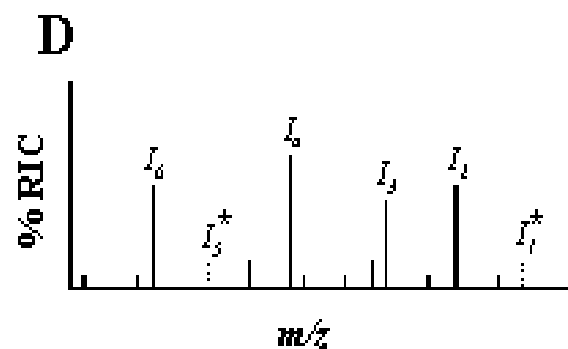
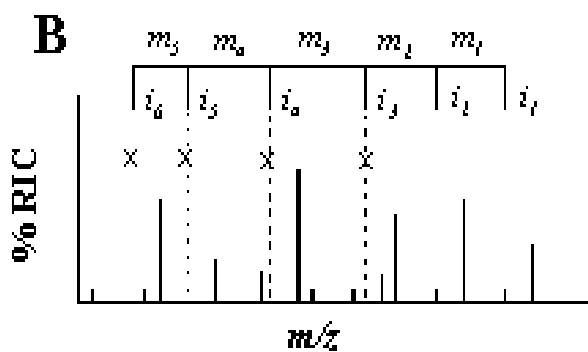
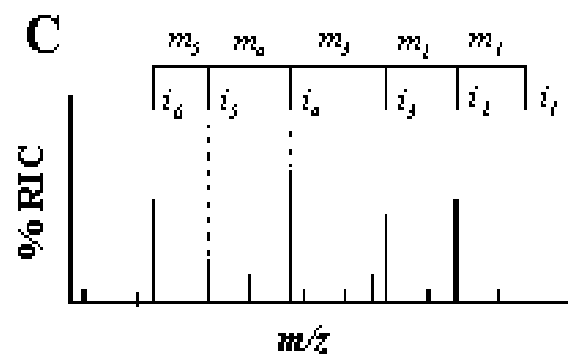
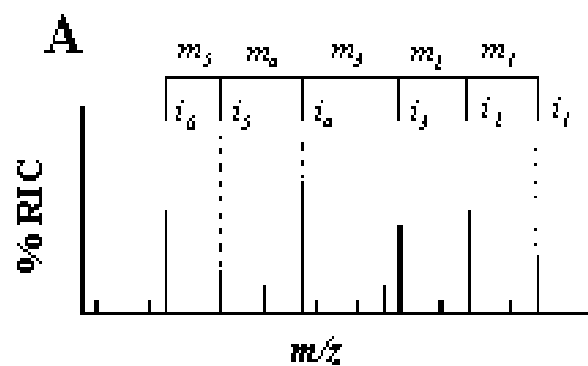
$$(M+H)^+ = X+m$$



$$(M+H)^+ = X$$

- SALSA (Scoring ALgorithm for Spectral Analysis)**
Uses pattern recognition identify motifs and PTMs in MS/MS data.

SALSA



SALSA

- **SALSA uses Sequences to find patterns in MS/MS spectra; this is the opposite of sequence prediction programs like Mascot and SEQUEST.**
- **Can find modified peptides by specifying the peptide sequences manually**
- **SALSA score is not statistically formulated.**

PTMS and The Hypergeometric Distribution

- The hypergeometric distribution models the total number of “successes” in a fixed size sample drawn without replacement from a finite population.
 - Eg. A deck of 52 cards has 16 face cards. Say you were to draw randomly 10 cards from the deck. What is the probability of getting 8 face cards?

The Hypergeometric Distribution

$$y = f(x|M, K, n) = \frac{\binom{K}{x} \binom{M-K}{n-x}}{\binom{M}{n}} \quad \binom{n}{x} = \frac{(n)!}{(n-x)!x!}$$

Where:

M is the size of the population (52 cards).

K is the number of items with the desired characteristic in the population (16 face cards).

n is the number of samples drawn (10).

x is the desired outcome (8 face cards).

$$y = \frac{\binom{16}{8} \binom{36}{2}}{\binom{52}{10}} = \frac{\frac{(16)!}{(8)!(8)!} \frac{(36)!}{(2)!(34)!}}{\frac{(52)!}{(10)!(42)!}} = 5.1 \times 10^{-4}$$

The Hypergeometric Distribution

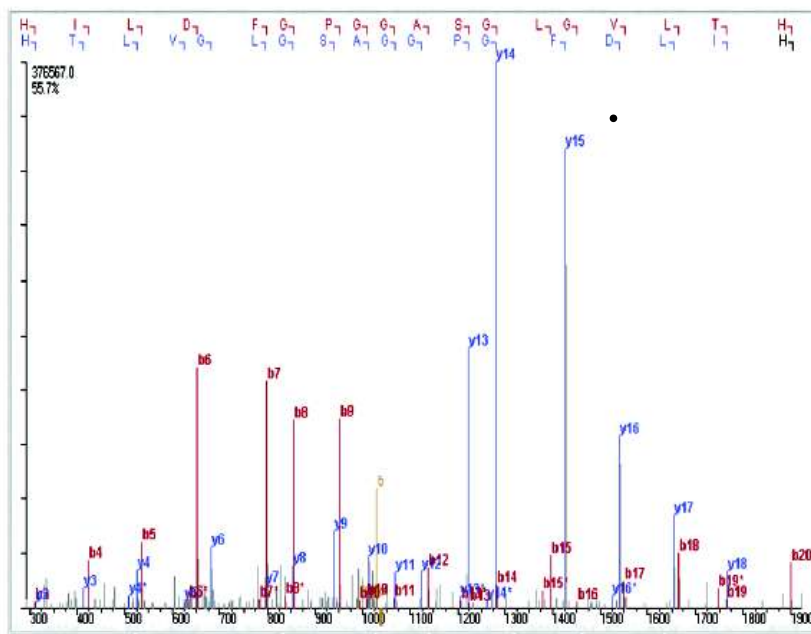
1. Perform an *in silico* digestion of the protein database and identify the $(M+H)^+$ mass for each peptide.
2. Perform an *in silico* fragmentation of each peptide and associate them with the parent ion.
3. Extract all the peptides from the database with a parent $(M+H)^+$ mass matching the experimental parent mass and count the number of fragments for each. The total number all these fragment masses is the population size, M .

The Hypergeometric Distribution

4. Identify the number of these fragment ion masses from peptide sequences that have a match to a peak in the experimental spectrum, K .
5. For each peptide sequence, the total number of theoretical fragment ion masses is the sample size, n .
6. The number of matches between the theoretical and experimental spectrum is x .

The Hyergeometric Distribution

- Example: ATHILDFGPGGASGLGVLTHR Against the NCBI Non-redundant Protein Database



- M is the total number of theoretical fragment ions from the database that have $(M+H)^+$ masses matching the experimental spectrum (5 284 150).
- K is the subset of these fragment ions that match a fragment ion peak in the experimental spectrum (569 160).
- n is the number of fragment ions for the peptide sequence under consideration (40).
- x is the number of fragment ion matches between the experimental and theoretical fragmentation spectra (34).

The Hypergeometric Distribution

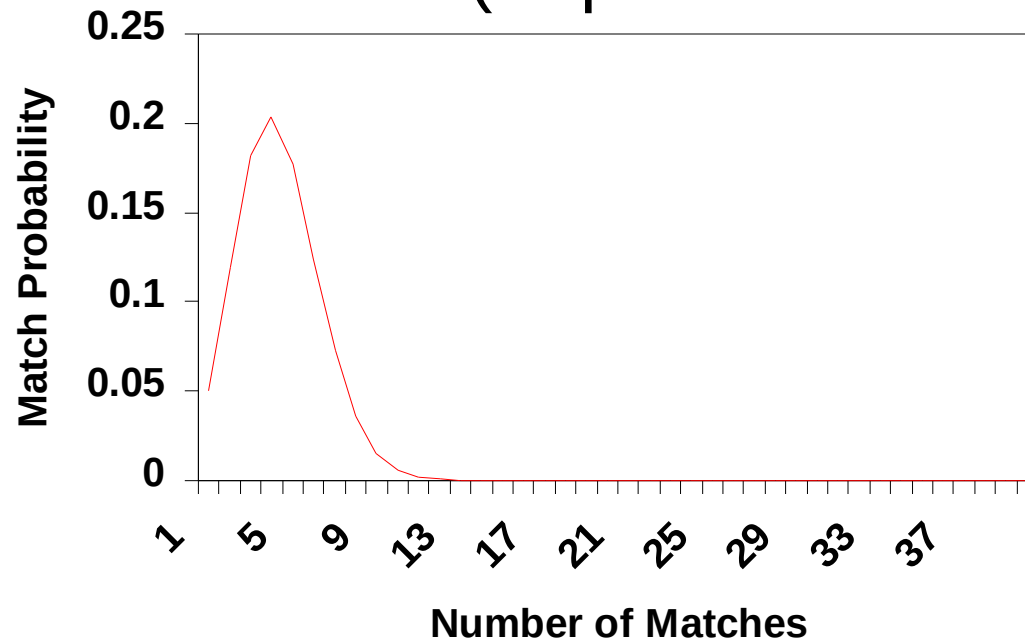
$$y = f(x|M, K, n) = \frac{\binom{K}{x} \binom{M-K}{n-x}}{\binom{M}{n}} \quad \binom{n}{x} = \frac{(n)!}{(x)!(n-x)!}$$

$$y = \frac{\binom{569160}{34} \binom{34714990}{6}}{\binom{5284150}{40}} = \frac{(569160)! (4714990)!}{(34)!(569126)! (6)!(4714984)!} \frac{(5284150)!}{(40)!(5284110)!} = 1.0 \times 10^{-26.62}$$

- M is the total number of theoretical fragment ions from the database that have (M+H)+ masses matching the experimental spectrum (5 284 150).
- K is the subset of these fragment ions that match a fragment ion peak in the experimental spectrum (569 160).
- n is the number of fragment ions for the peptide sequence under consideration (40).
- x is the number of fragment ion matches between the experimental and theoretical fragmentation spectra (34).

The Hypergeometric Distribution

- Significance of a match: How many peptides in a database are expected to have the same or better matches to the experimental spectrum?
- P-Value (Expectation Value)

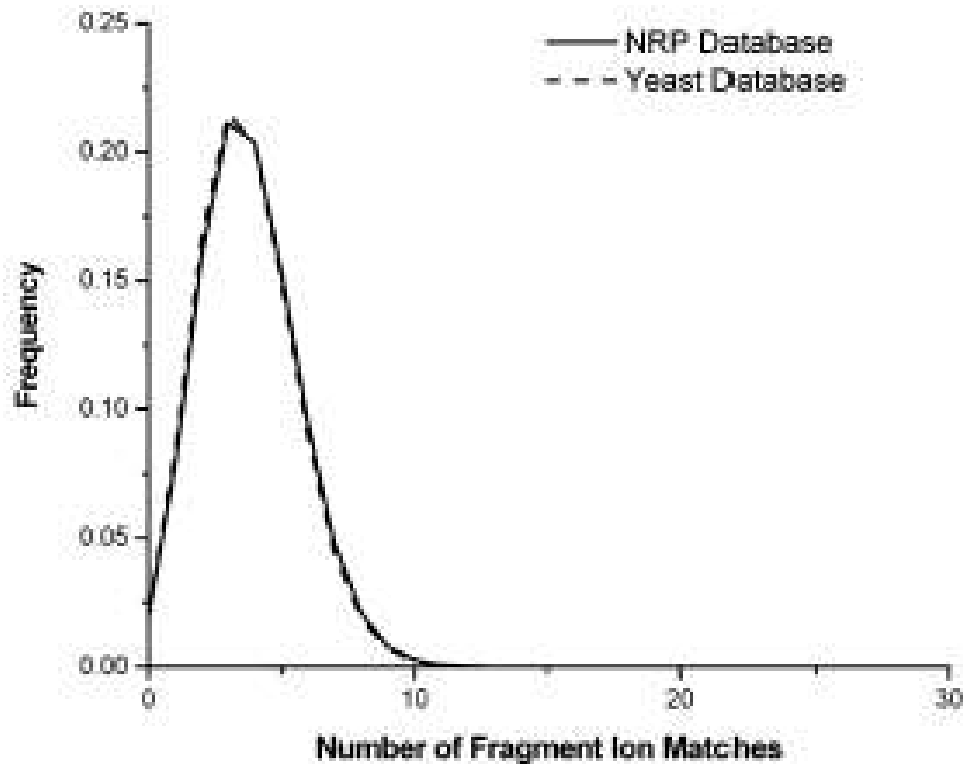


$$\begin{aligned} E &= M \sum_{P < P_0} P \\ &= 5284150 \times 10^{-26.62} \\ &= 1 \times 10^{-19.90} \end{aligned}$$

$E < 0.05$ considered significant

The Hypergeometric Distribution

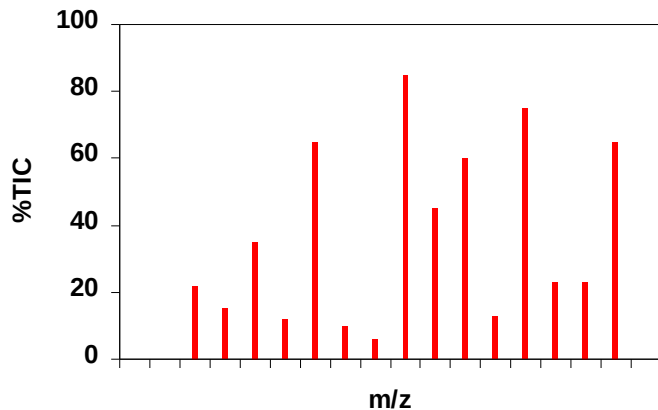
- The hypergeometric model is database independent:



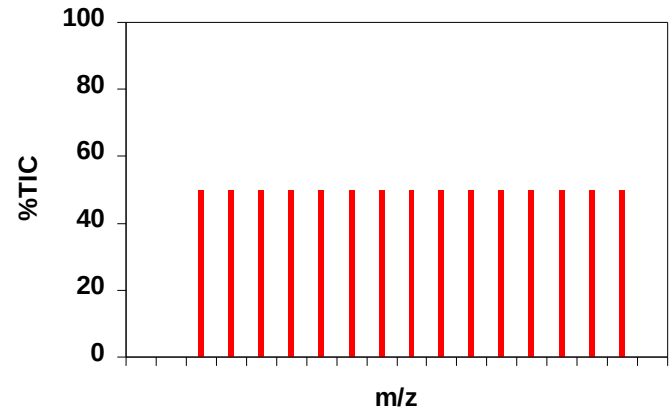
The same experiment conducted against the *S. cerevisiae* databases (6200 sequences) gives a match probability of $10^{-26.51}$ (vs $10^{-26.62}$ for NCBI NR).

Identifying Posttranslational Modifications

- Current protein sequence prediction packages have limited ability to detect posttranslational modifications.

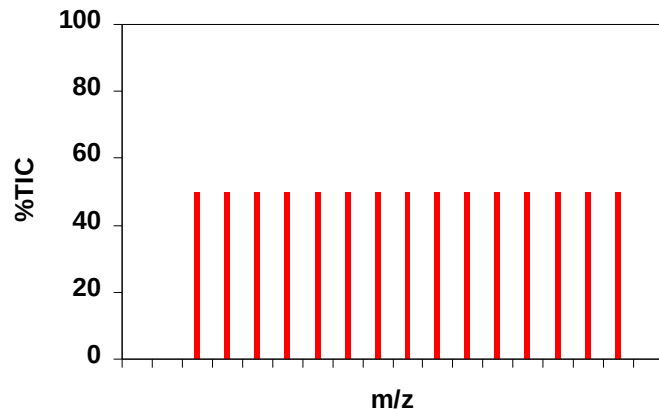
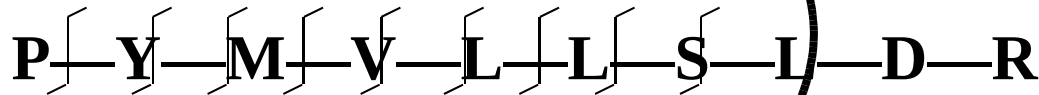
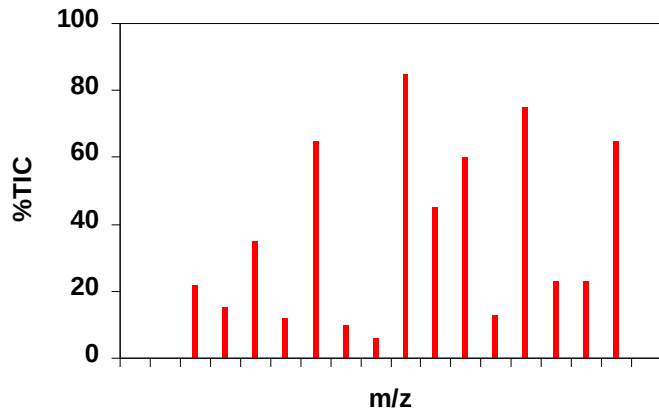


$$(M+H)^+ = X+m$$

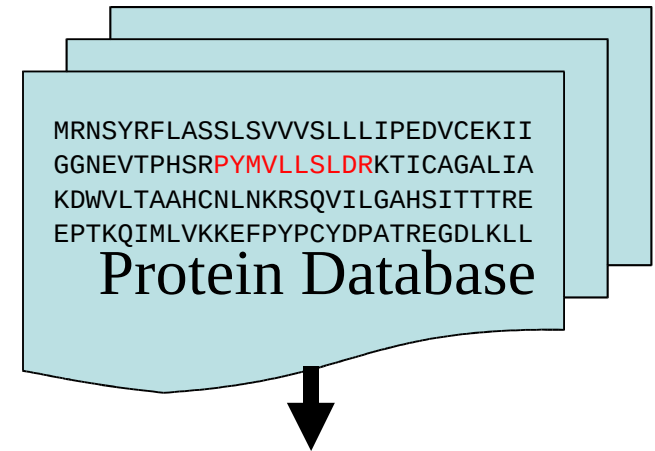


$$(M+H)^+ = X$$

Identifying Posttranslational Modifications



In Silico Fragmentation



In Silico Digestion

LASSLSVVVSLLLIPEDVCEK
IIGGNEVTPHSR
PYMLLSLDR
TICAGALIAK
DWLTAAHCNLNKR
ITTTYEPTK
QIMLVK
FFPYPCYDPATR
EGDLKLL

Identifying Posttranslational Modifications

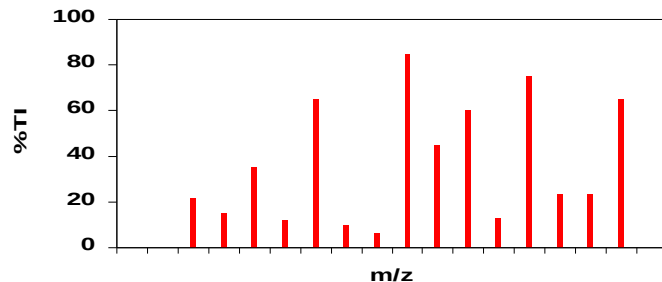
1. Assume each of the spectra corresponds to a candidate peptide.
2. Calculate the $(M+H)^+$ difference between the spectrum and the candidate peptide and determine m .
3. Assign m to each residue in the sequence, in turn.
4. Calculate the hypergeometric probability of the spectrum and this candidate peptide.
5. If the match score improves over the original score, then it is more probable that the spectrum corresponds to a PTM peptide.
6. A look-up table can be used to identify the probable PTM and determine if it is compatible with the modified residue.

Identifying Posttranslational Modifications

LASSLSVVVSLLLIPEDVCEK
 IIGGNEVTPHSR
~~PYMVLLSLDR~~
 TICAGALIAK
 DWVLTAAHCNLNKR
 ITTTYEPTK
 QIMLVK
 EFPYPCYDPATR
 EGDLLKLL

PTM Candidate Peptides

b Ions	Fragment	y Ions
-----	I TTTYEPTK	1062.54
215.14	IT TTYEPTK	961.50
316.19	ITT TYEPTK	860.45
417.23	ITTT YEEPTK	759.40
573.34	ITTTY EEPTK	603.30
702.38	ITTTYE EPTK	474.26
831.42	ITTTYEE PTK	345.21
928.47	ITTTYEEP TK	248.16
1029.52	ITTTYEPT K	147.11
1175.62	ITTTYEPTK	-----



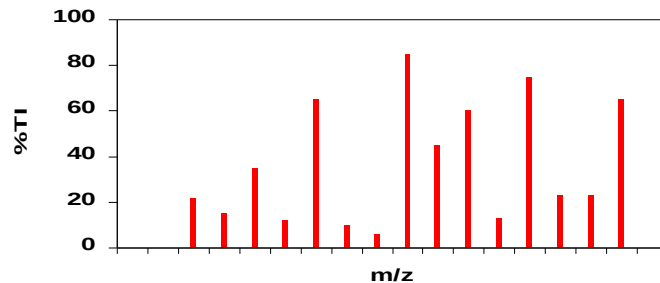
b Ions	Fragment	y Ions
-----	I TTTYEPTK	1062.54+m
215.14	IT TTYEPTK	961.50+m
316.19	ITT TYEPTK	860.45+m
417.23	ITTT YEEPTK	759.40+m
573.34+m	ITTTY EEPTK	603.30
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1175.62+m	ITTTYEPTK	-----

I-T-T-T-Y-E-E-P-T-K

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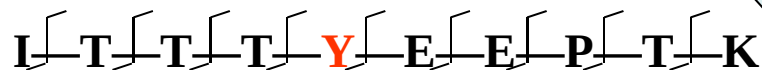
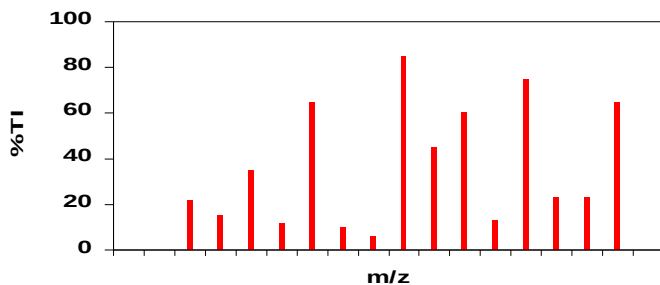
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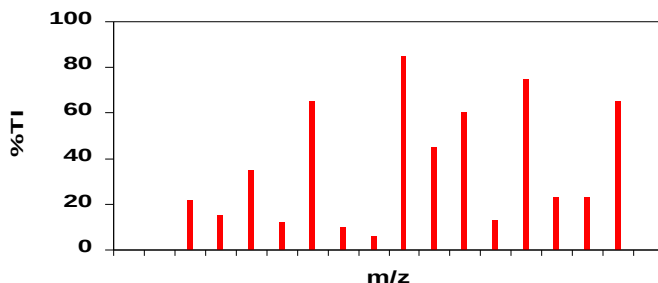
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I-T-T-T-Y-E-E-P-T-K

$$(M+H)^+ = 1175.62+m$$

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