

Protein Mass Spectrometry (Proteomics)

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Mass Spectrometry and Proteomics Facility

The Ohio State University

Summer Workshop

Proteomics:

Proteome=Protein + Genome

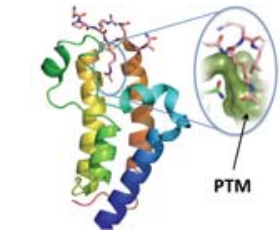
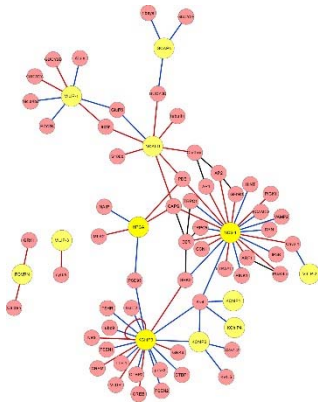
the large-scale study of proteins, particularly their structures and functions.

Why are proteins important:

- The major elements of most cellular structures
- Perform most cellular functions
- Targets of drugs/toxicants

What Can Proteomics Do

Protein Interaction Analysis

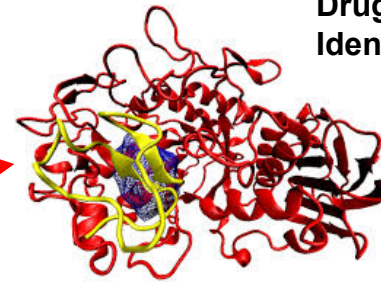


PTM Identification



Protein Crosslink Identification

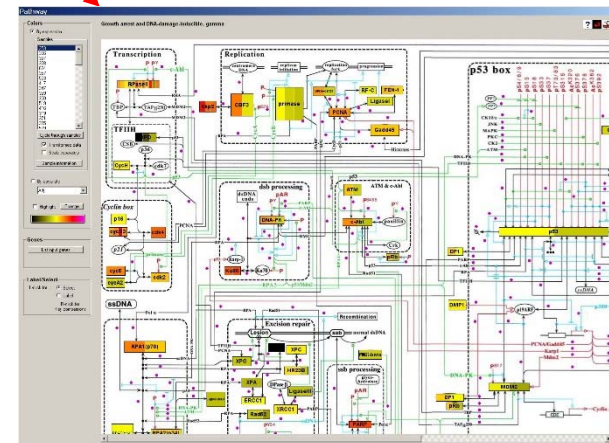
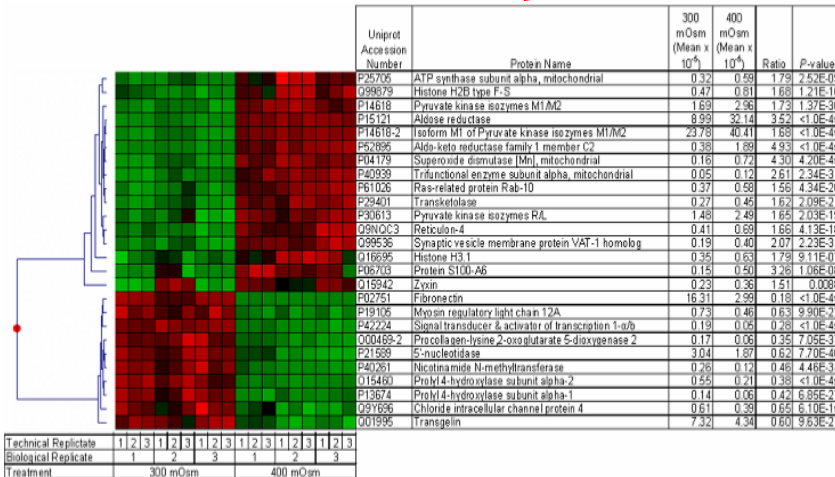
Drug Binding Site Identification



Protein(s) identification

Protein Profile Analysis

Pathway Analysis



Traditional Methods for Proteome Research

.SDS-PAGE

- separates based on molecular weight and/or isoelectric point
- 10 fmol - > 10 pmol sensitivity
- Tracks protein expression patterns

.Protein Sequencing

- Edman degradation or internal sequence analysis

.Immunological Methods

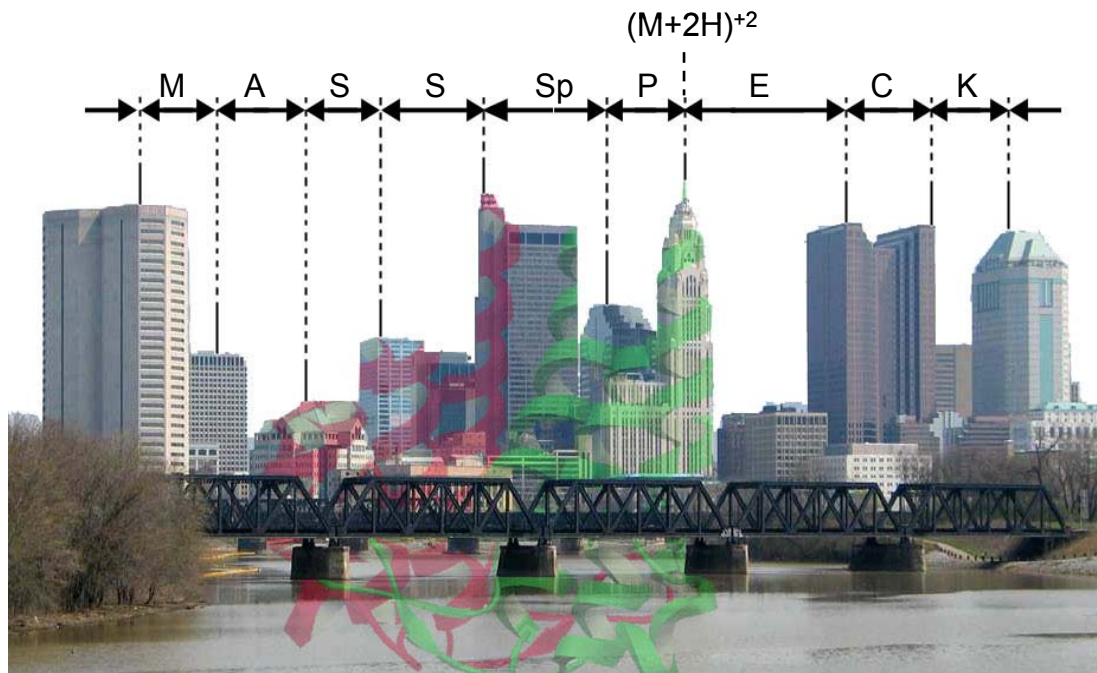
- Western Blots

.SDS-PAGE alone can track the appearance, disappearance or molecular weight shifts of proteins, but can not ID the protein or measure the molecular weight with any accuracy

.Edman degradation requires a large amount of protein and does not work on N-terminal blocked proteins

.Western blotting is presumptive, requires the availability of suitable antibodies and have limited confidence in the ID related to the specificity of the antibody.

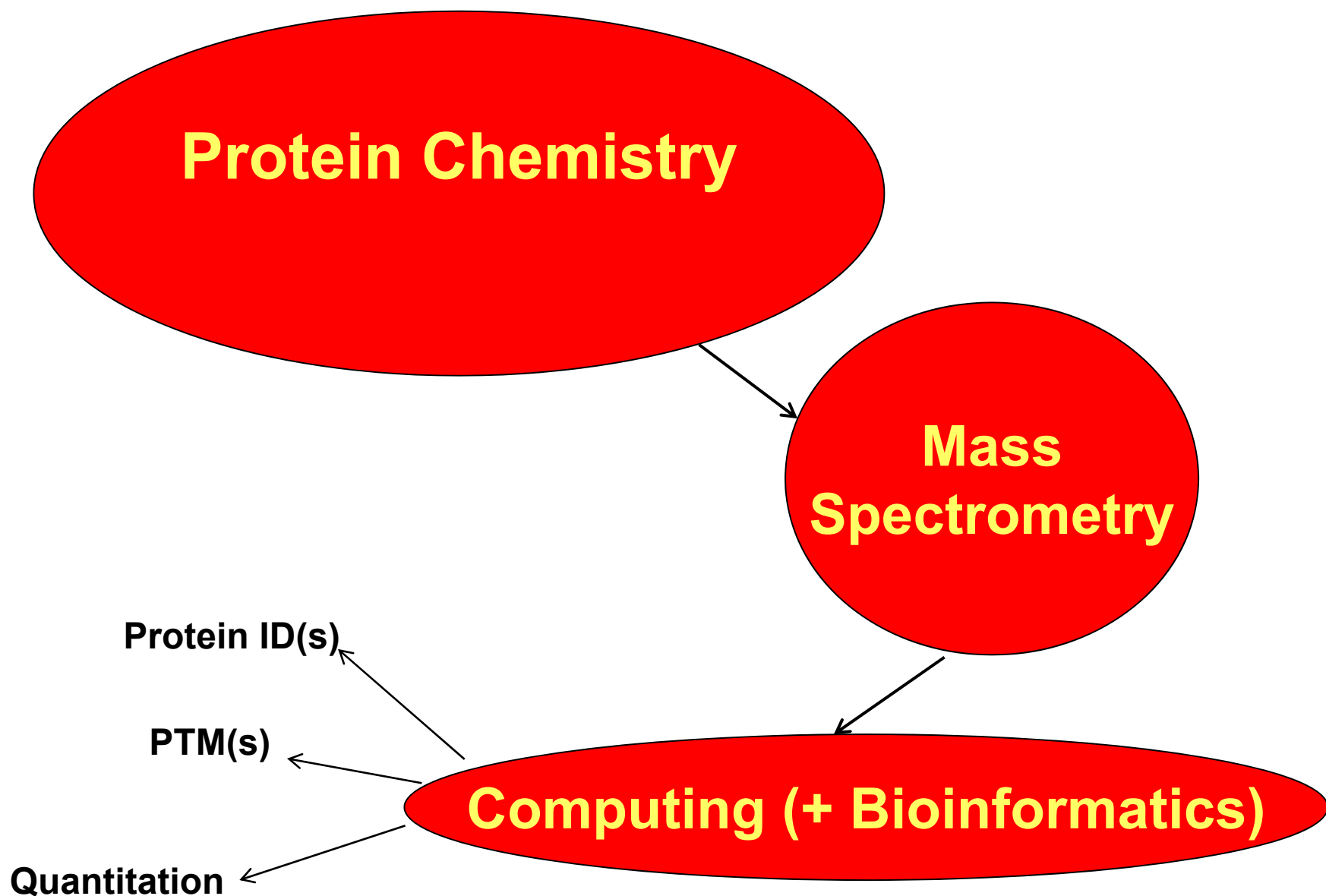
Proteome Research by Mass Spectrometry



High throughput
Sensitivity
Accuracy
Specificity

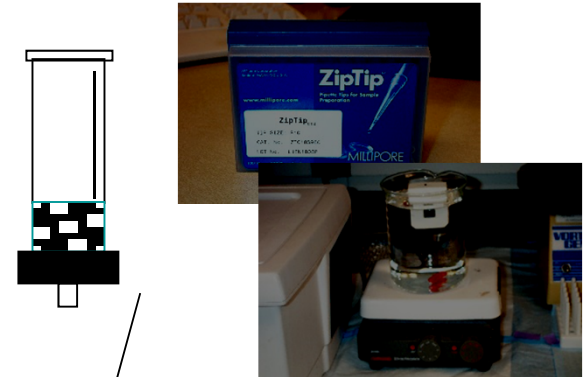
Measure M.W. of the protein/peptide
Sequence amino acid sequence of protein/peptides

Proteomics by Mass Spectrometry

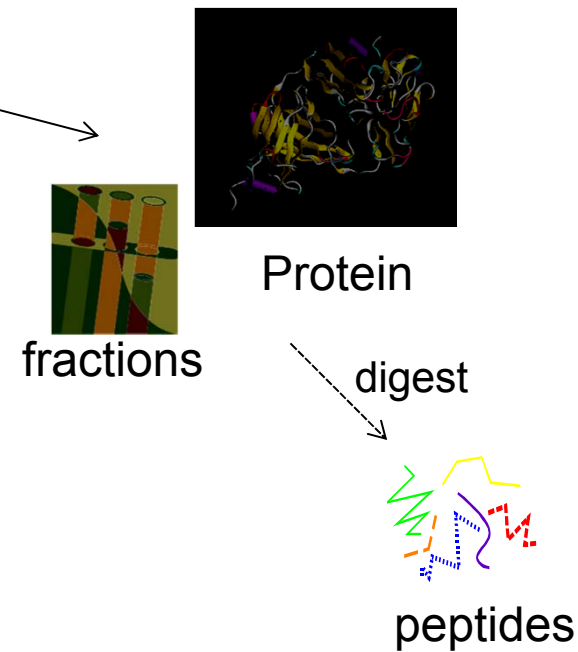
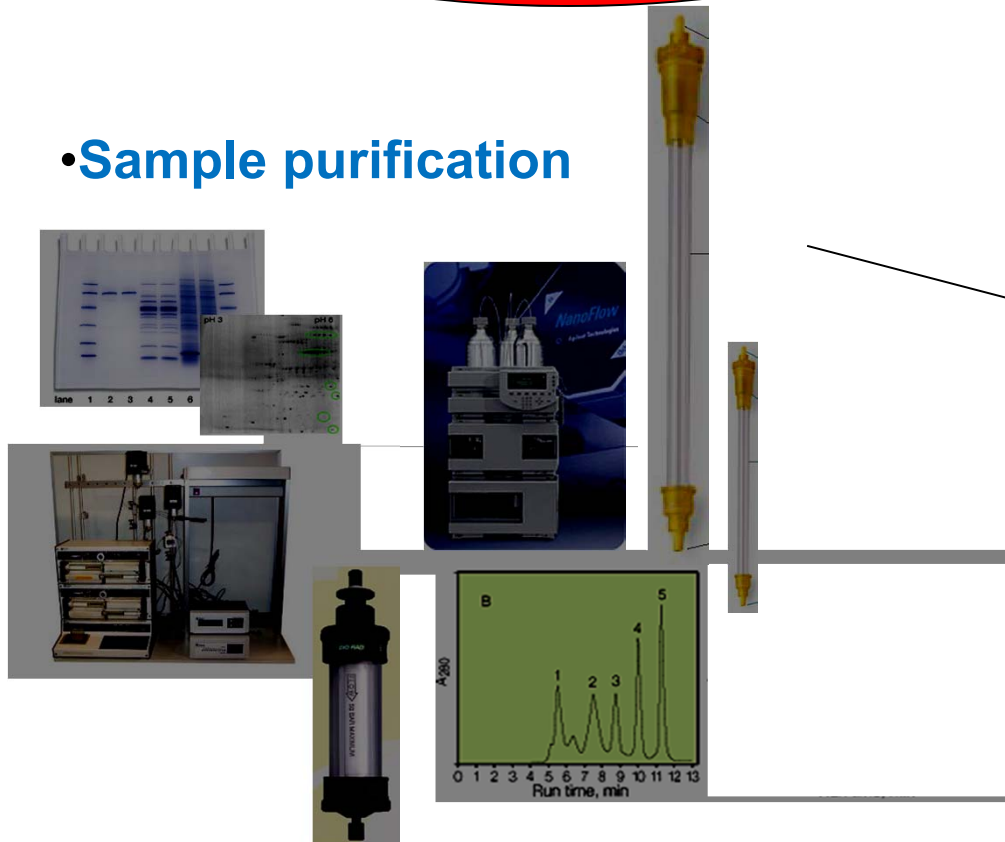


Protein Chemistry

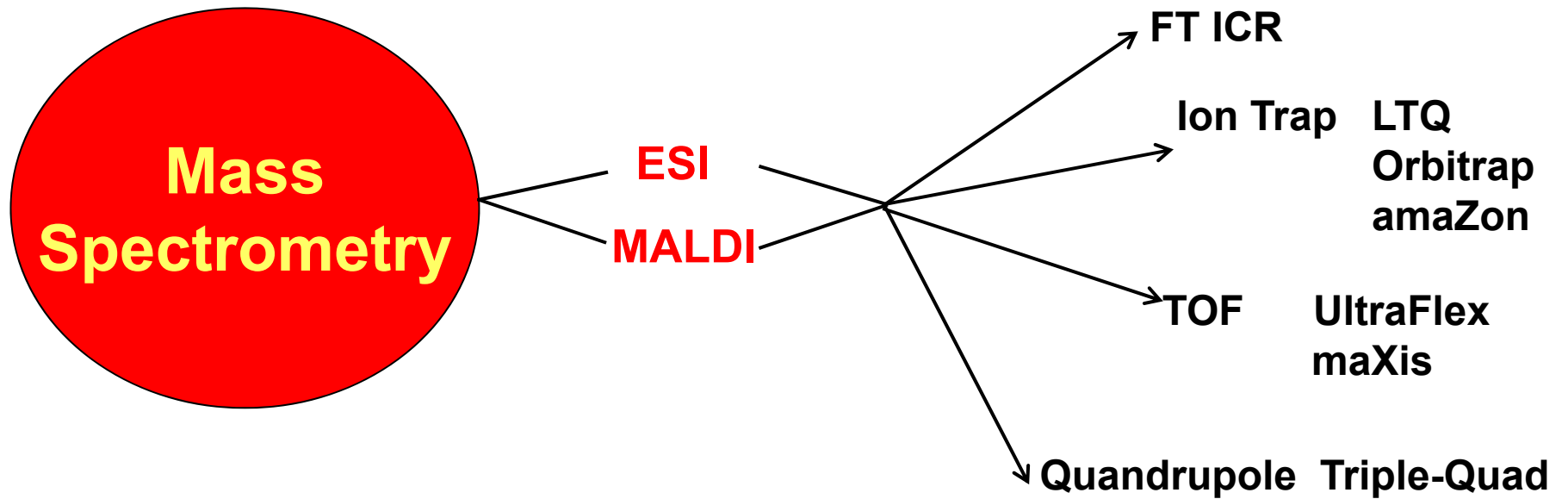
• Sample isolation/clean-up



• Sample purification



All types of hardware used in proteomics



Computing (+ Bioinformatics)

Sequest Summary

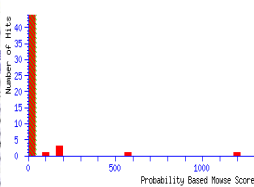
Sample: Kuppamman, K. (YF_204024_BAND5_MR) YF_204024_BAND5_MR.kk
 DataFiles: at_204024_band5 (02/03/04-02/03/04)
 Database: qd (nr) (01/14/2004)
 Directory: kuppammanuf_204024_bands_mr
 Diff Mods: C+H+ST

OutFiles: 14601751
 Enzyme: Trypsin_Sp01
 Mass: 41.5 (Avg)
 Max Hits: 500
 Intensity: 8.7e8 MS2

MASCOT Search Results

| Rank | Accession | Protein | Sequences | TIC | Score | Ident |
|------|---------------------------------|--------------------------------------|-----------|-----|-------|-------|
| 1 | gi11221014 ref NP_072936.4 COLT | histone H4 [Drosophila melanogaster] | 9 | 5% | 214.2 | 17/18 |
| 2 | gi11221014 ref NP_072936.4 COLT | histone H4 [Drosophila melanogaster] | 9 | 5% | 214.2 | 17/18 |
| 3 | gi11221014 ref NP_072936.4 COLT | histone H4 [Drosophila melanogaster] | 9 | 5% | 214.2 | 17/18 |

Probability Based Mowse Score
 Ion score is $-10 \cdot \log(P)$, where P is the probability that the observed match is a random event.
 Individual ion scores > 46 indicate identity or extensive homology (p<0.05).
 Protein scores are derived from ion scores as a non-probabilistic basis for ranking protein hits.



Peptide Summary Report

Format As: Peptide Summary [Help](#)

Significance threshold p< 0.05 Max. number of hits AUTO

Standard scoring MudPIT scoring Ion score cut-off 0 Show sub-sets

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

<http://bioinformatics.org>

<http://www.ncbi.nlm.nih.gov/>

<http://www.expasy.org/>



<http://www.ncbi.nlm.nih.gov/gquery/gquery.fcgi>



The Global Proteome Machine, Simple search page

advanced page
 view saved xml data

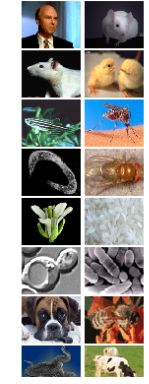
what is the gpm
 powered by tandem
 send us email

Eukaryote proteomes
 1 2 3 4 5 6 7

Boutique proteomes
 human mouse frog
 cow protista plant
 fish rat bacteria

Algorithms
 P3 X! Hunter

Information
 gpmDB messages



- spectra**
 DTA, PKL or Matrix Science format only
- taxon**
 Select one or more.
Eukaryotes:
 H. sapiens (human)
 M. musculus (mouse)
 R. norvegicus (rat)
 S. cerevisiae (budding yeast)
Prokaryotes:
 Bacillus subtilis
 Clostridium perfringens
 Deinococcus radiodurans
 Escherichia coli K12
 reversed sequences with log(e) < -1
 all ¹⁵N
- measurement errors**
 Fragment mass error: 0.4 Da
- residue modifications**
 Complete modifications:

 specify your own

 Potential modifications:

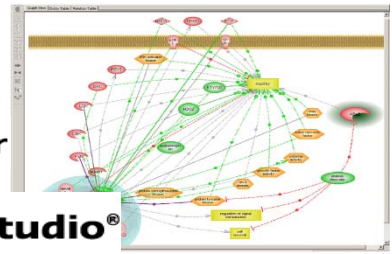
 Oxidation (M)
 Oxidation (W)
 Deamidation (N)
 specify your own

 Oxidation (M)
 Oxidation (W)
 Deamidation (N)
- refinement specification**
 Potential modifications (unimod):

| round 1 | round 2 |
|--|---|
| <input type="text" value="none"/> | <input type="text" value="none"/> |
| <input checked="" type="checkbox"/> Oxidation (M) | <input checked="" type="checkbox"/> Oxidation (M) |
| <input checked="" type="checkbox"/> Oxidation (W) | <input checked="" type="checkbox"/> Oxidation (W) |
| <input checked="" type="checkbox"/> Deamidation (N) | <input checked="" type="checkbox"/> Deamidation (W) |
| <input checked="" type="checkbox"/> Deamidation (O) | <input checked="" type="checkbox"/> Deamidation (W) |
| <input checked="" type="checkbox"/> ICAT-D:2H(8) (C) | <input checked="" type="checkbox"/> Deamidation (N) |

 Point mutations: yes no
 Semi-style cleavage: yes no
- protein cleavage specification**
 Cleavage site:

 tryptsin, [RK](P)
 Semi-style cleavage: yes no
 Spectrum conditioning:
 Remove redundant: yes no, angle: 40 (0-90)
 Spectrum synthesis: yes no
- predefined methods**
 Method: Select device & parent δm .
 FT/ICR (10 ppm)
 Orbitrap (100 ppm)
 Quad-TOF (0.5 Da)



What Can Mass Spectrometry Do for Proteomics

Molecular weight measurement of the protein/peptide

Protein Identification/Confirmation

Protein Identification/PTM Investigation

- Global Digestion/LC-MSMS/Bioinformatics
- Fractionation/Digestion/LC-MSMS/Bioinformatics

Quantitative Proteomics

▫ Gel-Based Technique

-- DIGE

▫ Non Gel-Based Techniques

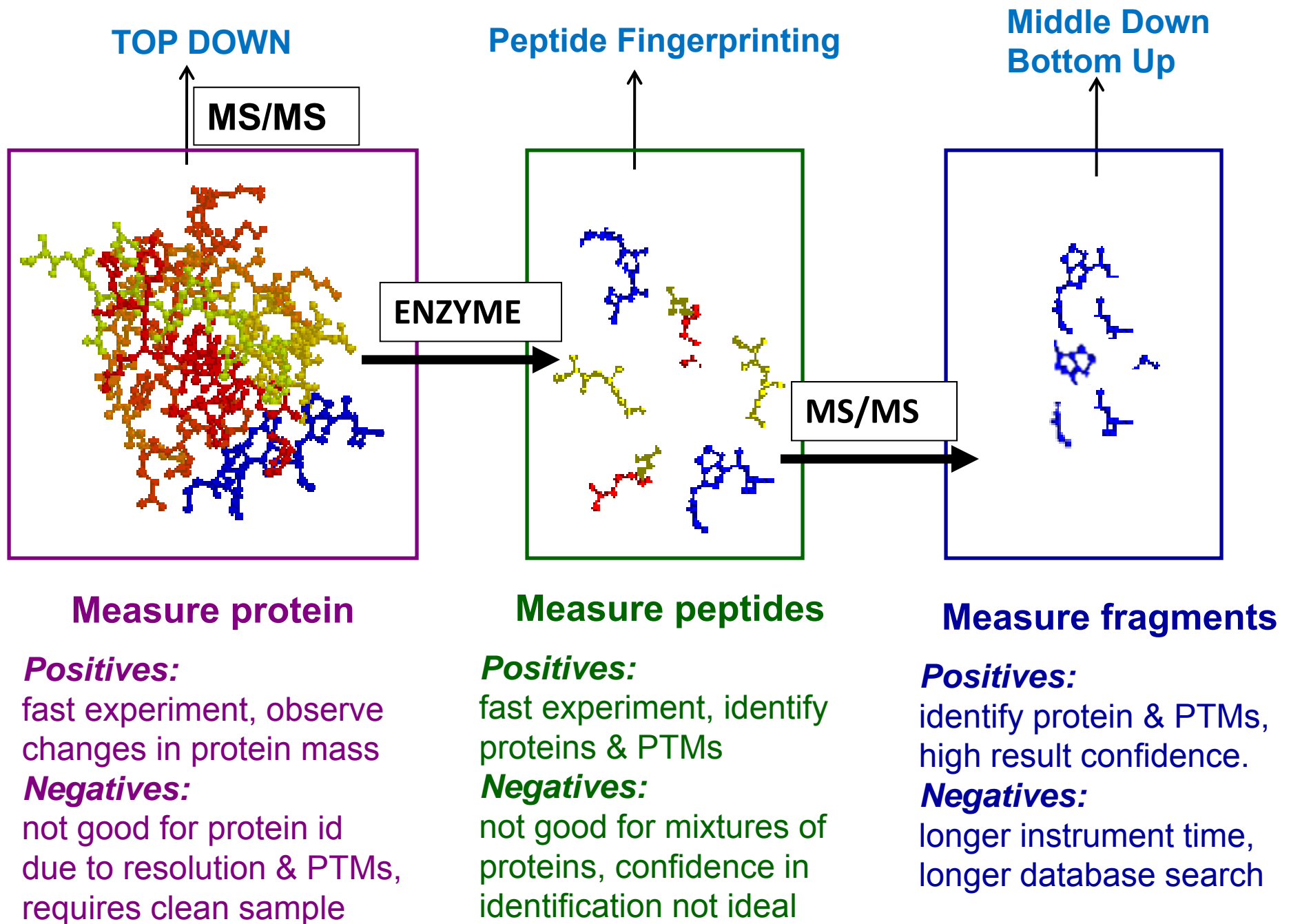
--Isotopic Labeling Techniques:

ITraq/SILAC/ ^{18}O , ^{15}N . ^2H Labeling

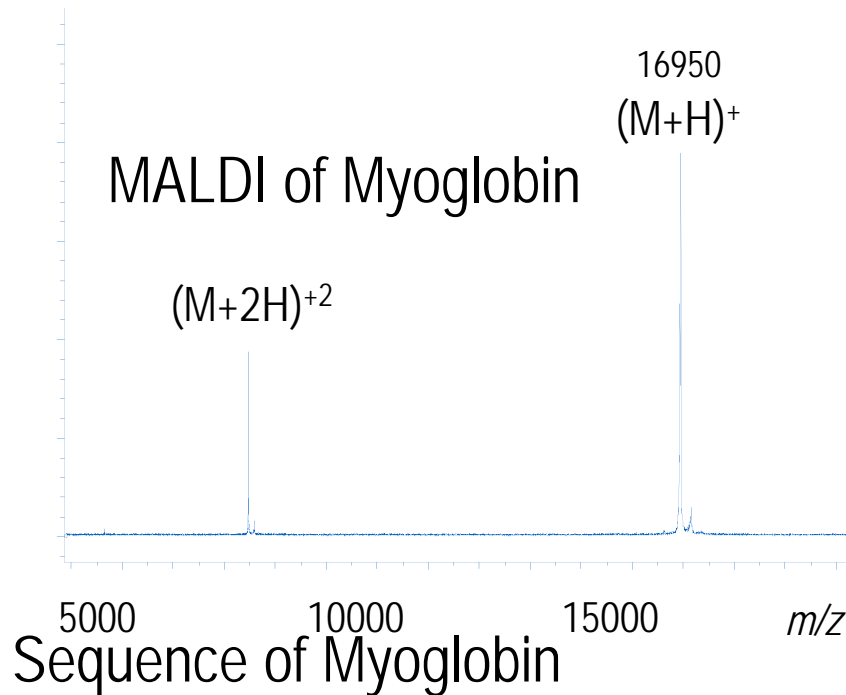
--Label Free Quantitation:

Spectra Counting/Peak Area/EmPAI

MALDI Imaging



Molecular Weight Measurement of the Protein

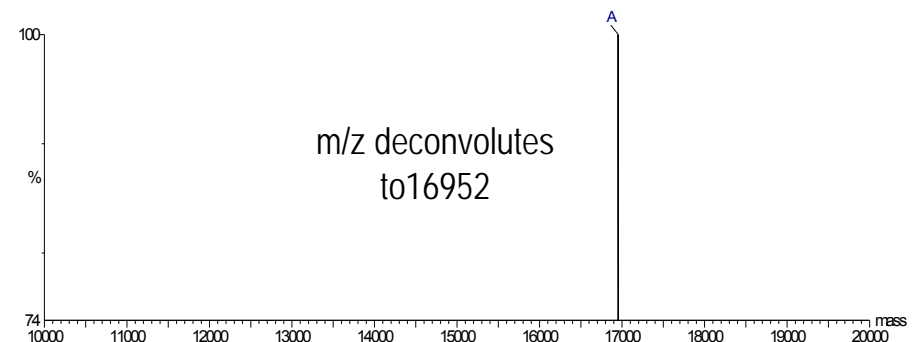
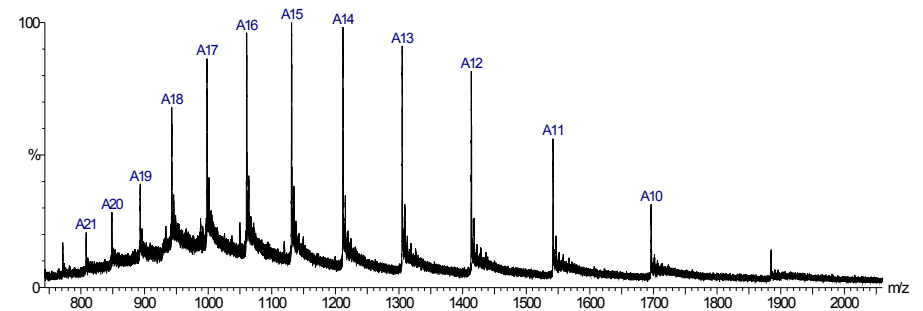


Sequence of Myoglobin

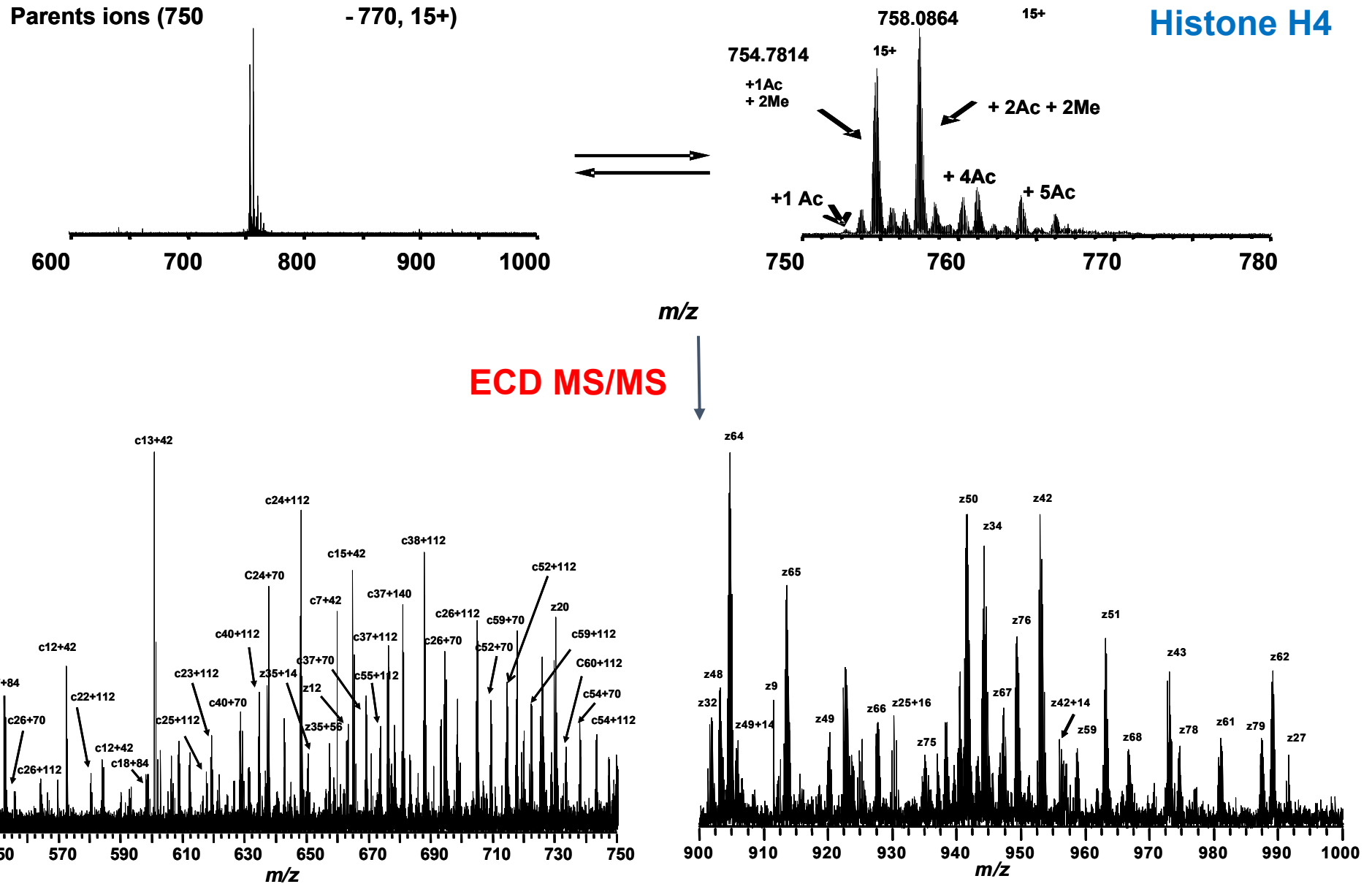
GLSDGEWQQVLNVWGKVEADIAGHGQEVLRIRLFTGHPET
LEKFDKFKHLKTEAEMKASEDLKKHGTVLTALGGILKKG
HHEAELKPLAQSHATKHKIPIKYLEFISDAIHVLHSHKHPGD
FGADAQGAMTKALELFRNDIAAKYKELGFQG

MW = 16951

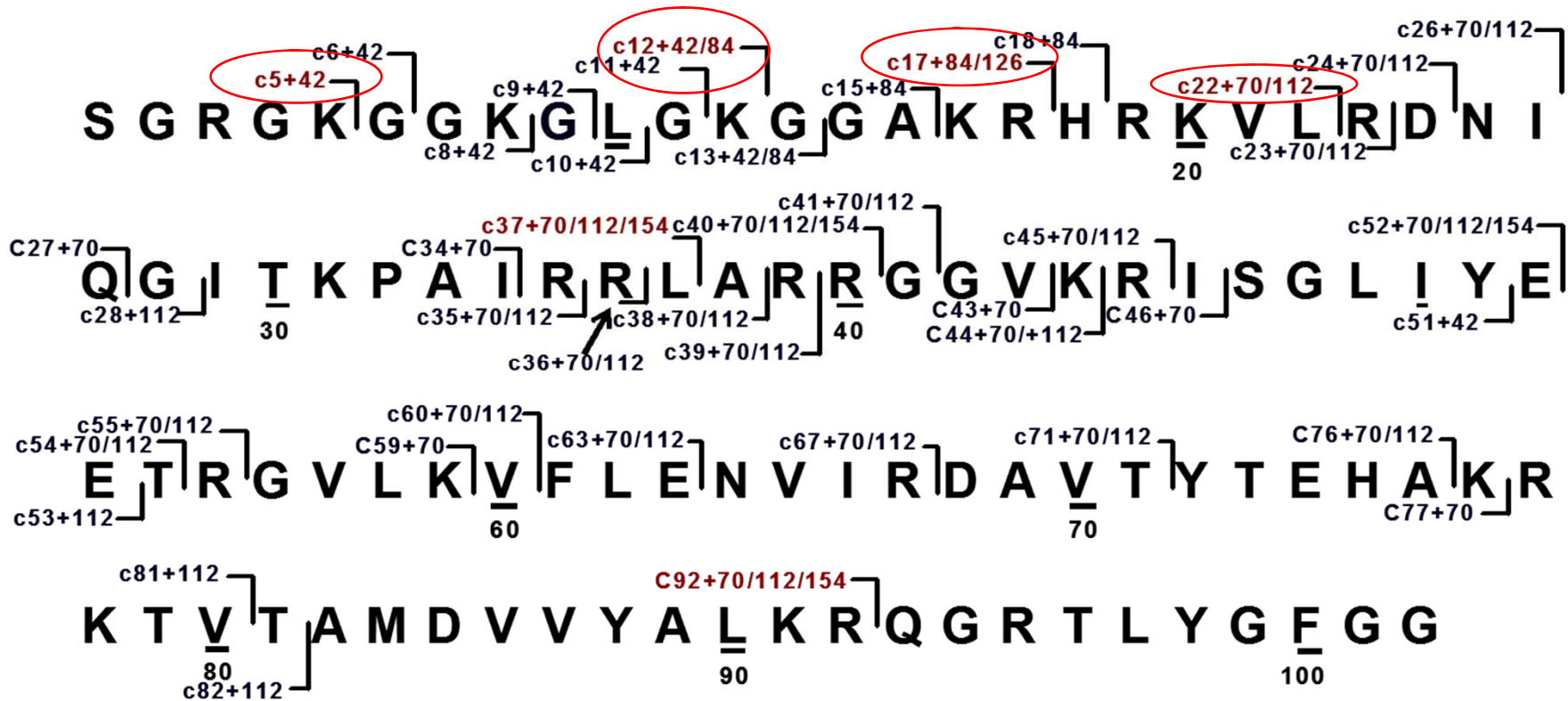
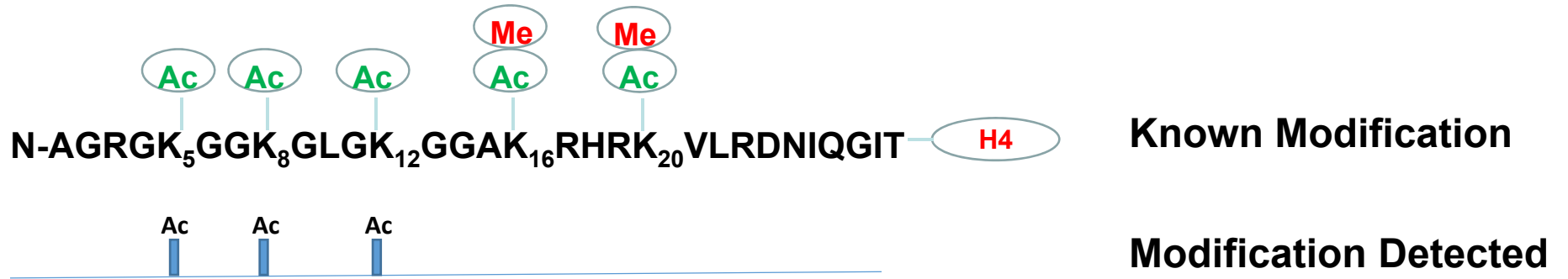
Electrospray of Myoglobin



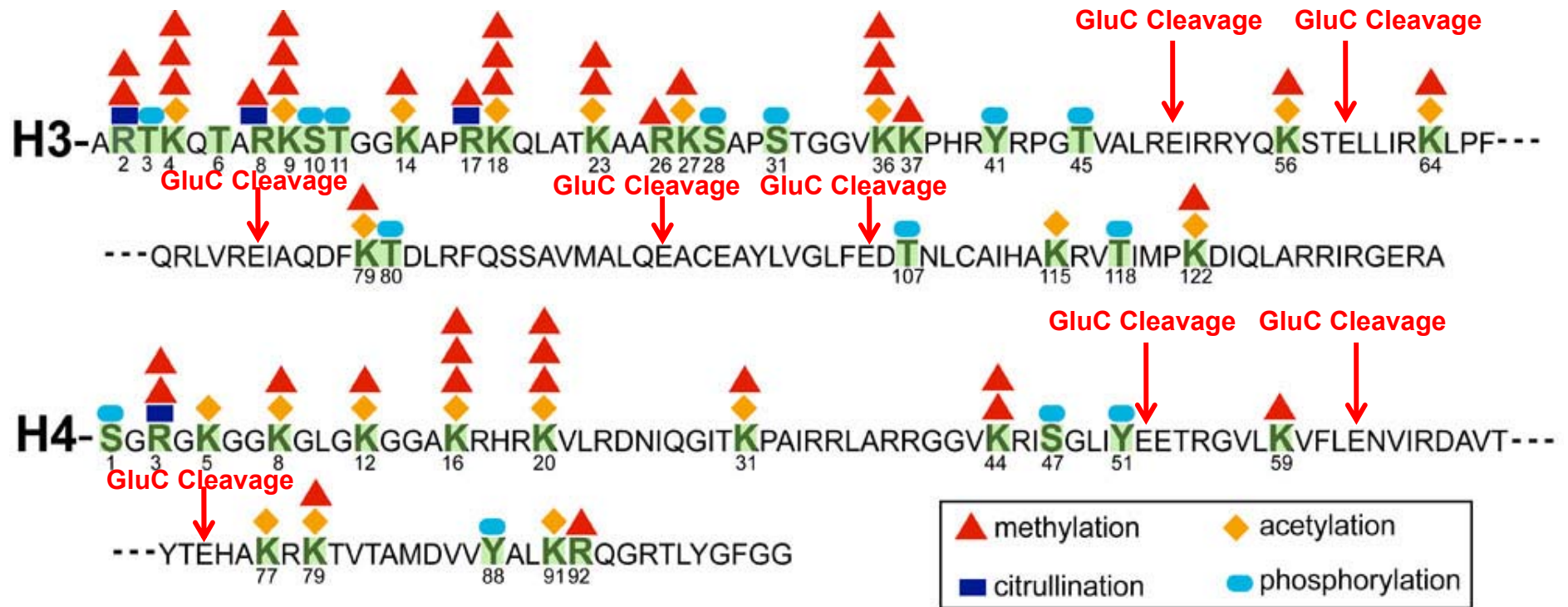
Protein Identification by Top Down MS/MS



Protein Identification by Top Down MS/MS



Middle Down



Why Middle Down:

simplifies the complex mixtures offered by bottom up strategies

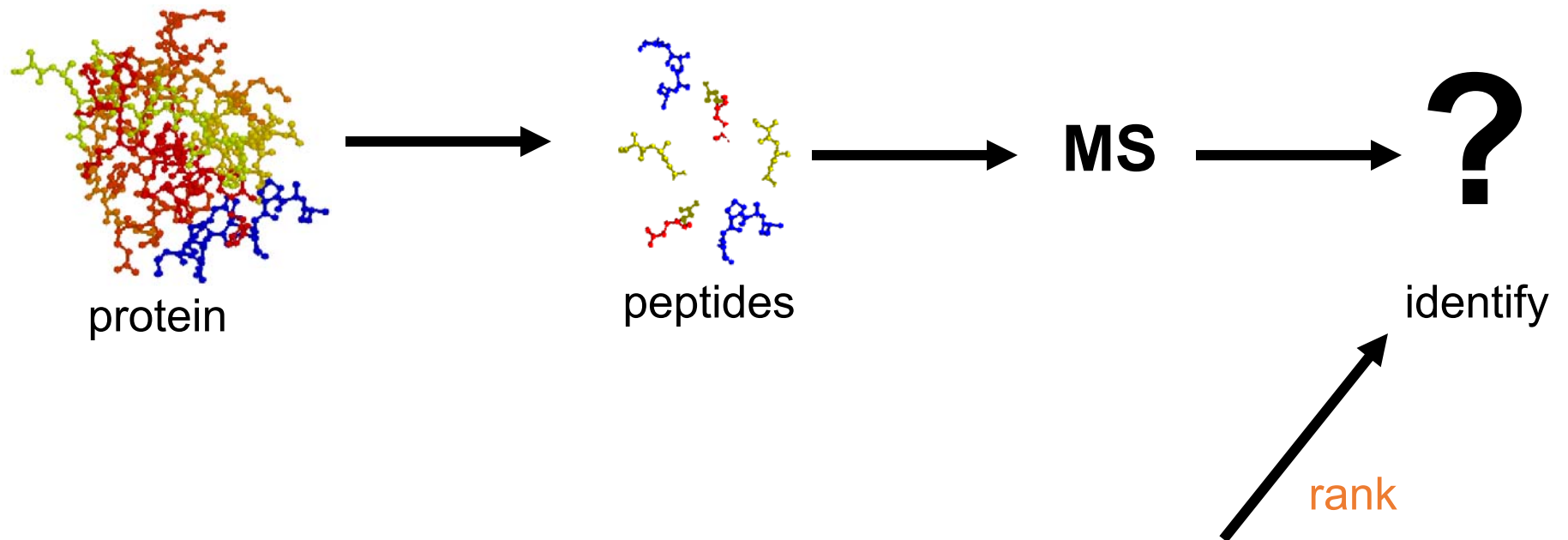
while avoiding the diminished performance of top down experiments

Bottom Up

1 MNSGHSFSQT PSASFHGAGG GWGRPRSFPR APTVHGGAGG ARISLSFTTR
 51 SCPPPGGSWG SGRSSPLLGG NGKATMQNLN DRLASYLEKV RALEEANMKL
 101 ESRILKWHQQ RDPGSKKDYS QYEENITHLQ EQIVDGKMTN AQIILLIDNA
 151 RMAVDDFNLK YENEHSFKKD LEIEVEGLRR TLDNLTIVTT DLEQEVEGMR
 201 KELILMKKHH EQEMEKHHVP SDFNVNVKVD TGPREDLIKV LEDMRQEYEL
 251 IIKKKHRDLT TWYKEQSAAM SQEAASPATV QSRQGDIHEL KRTFQALEID
 301 LQTQYSTKSA LENMLSETQS RYSCKLQDMQ EIISHYEEEL TQLRHELERQ
 351 NNEYQVLLGI KTHLEKEITT YRRLLEGESE GTREESKSSM KVSATPKIKA
 401 ITQETINGRL VLCQVNEIQK HA

| m/z | Sequence |
|-----------|--|
| 924.5149 | 43 <u>ISLSFTTR</u> 50 |
| 1050.5439 | 31 <u>APTVHGGAGGAR</u> 42 |
| 1078.6255 | 83 <u>LASYLEKVR</u> 91 |
| 1090.5375 | 374 <u>LLEGESEGTR</u> 383 |
| 1095.5905 | 284 <u>QGDIELKR</u> 292 |
| 1108.6374 | 104 <u>ILKWHQQR</u> 111 |
| 1244.5477 | 51 <u>SCPPPGGSWGSGR</u> 63 |
| 1360.7141 | 235 <u>EDLIKVLEDMR</u> 245 |
| 1390.6995 | 92 <u>ALEEANMKLESR</u> 103 |
| 1494.8097 | 410 <u>LVLCQVNEIQKHA</u> 422 |
| 1584.9220 | 246 <u>QEYELIKKKHR</u> 257 |
| 1972.9869 | 64 <u>SSPLLGGNGKATMQNLNDR</u> 82 |
| 2277.1279 | 181 <u>TLDNLTIVTTDLEQEVEGMR</u> 200 |
| 2673.2124 | 1 <u>MNSGHSFSQTPSASFHGAGGGWGRPR</u> 26 |
| 2790.4785 | 350 <u>QNEYQVLLGIKTHLEKEITYR</u> 372 |
| 2833.5088 | 384 <u>EESKSSMKVSATPKIKAITQETINGR</u> 409 |
| 2856.3543 | 322 <u>YSCKLQDMQEIISHYEEELTQLR</u> 344 |
| 2870.3261 | 258 <u>DLDTWYKEQSAAMSQEAASPATVQSR</u> 283 |
| 3332.6315 | 293 <u>TFQALEIDLQTQYSTKSALENMLSETQSR</u> 321 |
| 3368.6467 | 152 <u>MAVDDFNLYENEHSFKKDLEIEVEGLR</u> 179 |

Data Analysis for Peptide Mass Mapping



**MS Peptide MW
Found in Selected
Databases**

NDALYFPT...
SWDLTAL...
PTDLVSY...

- **Important data**
 - multiple peaks
 - mass accuracy
 - confirming information (pl, approx. mass, organism, etc.)

.Cut protein into manageable pieces for the mass spectrometer to analyze.

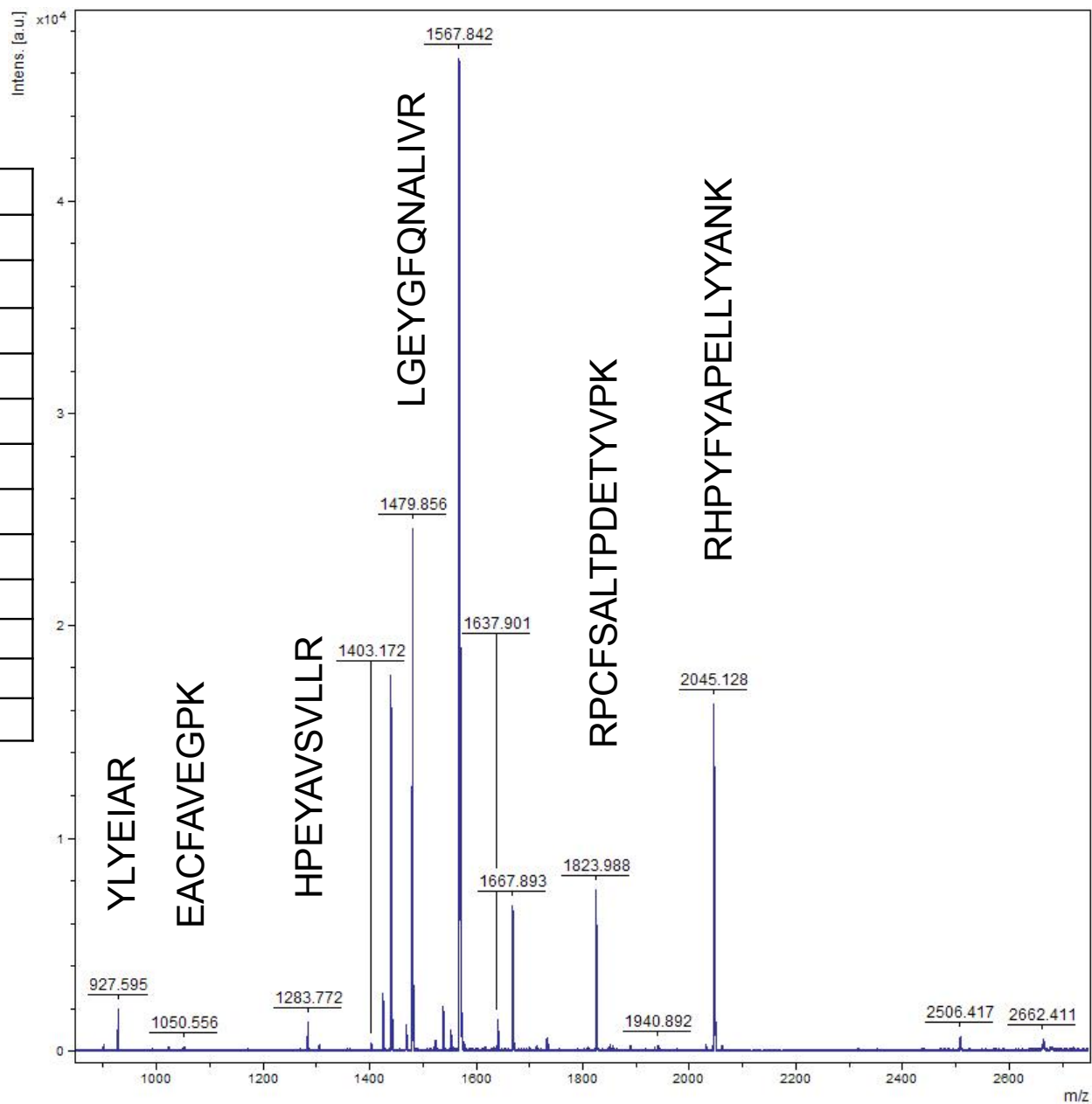
Bovine Albumin

1 MKWVTFISLL LFFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA FSQYLQQCPF DEHVKLVNEL
 71 TEFAKTCVAD ESHAGCEKSL HTLFGDELCK VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLLK
 141 PDPNTLCDEF KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC LLPKIETMRE
 211 KVLTSRARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE FVEVTKLVTD LTKVHKECCH GDLLCADDR
 281 ADLAKYICDN QDTISSKLKE CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
 351 GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAADDPH ACYSTVFDKL KHLVDEPQNL IKQNCDFEK
 421 LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT
 491 PVSEKVTKCC TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT ALVELLKHKP
 561 KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV STQTALA

| | | |
|---|----------|---|
| | | |
| → | 927.486 | 161-167 YLYEIAR |
| → | 1050.485 | 588-597 EACFAVEGPK |
| → | 1282.703 | 361-371 HPEYAVSVLLR |
| | 1305.708 | 402-412 HLVDEPQNLIK |
| | 1439.504 | 360-371 RHPEYAVSVLLR |
| | 1478.788 | 421-433 LGEYGFQNALIVR |
| | 1567.735 | 347-359 DAFLGSFLYEYSR |
| | 1639.931 | 437-451 KVPQVSTPTLVEVSR |
| | 1667.893 | 469-482 MPCTEDYLSLILNR |
| | 1823.892 | 508-523 RPCFSALTPDETYVPK |
| | 2045.021 | 168-183 RHPYFYAPELLYYANK |
| | 2506.243 | 469-489 M _{ox} PCTEDYLSLILNRLCVLHEK |

Peptide Mass Mapping

| | | |
|----------|---------|--------------------------------------|
| 927.486 | 161-167 | YLYEIAR |
| 1050.485 | 588-597 | EACFAVEGPK |
| 1283.703 | 361-371 | HPEYAVSVLLR |
| 1305.708 | 402-412 | HLVDEPQNLIK |
| 1439.504 | 360-371 | RHPEYAVSVLLR |
| 1479.788 | 421-433 | LGEYGFQNALIVR |
| 1567.735 | 347-359 | DAFLGSFLYEYSR |
| 1639.931 | 437-451 | KVPQVSTPTLVEVSR |
| 1667.893 | 469-482 | MPCTEDYLSLILNR |
| 1823.892 | 508-523 | RPCFSALTPDETYVPK |
| 2045.021 | 168-183 | RHPYFYAPELLYYANK |
| 2506.243 | 469-489 | M _{ox} PCTEDYLSLILNRLCVLHEK |



MS-Fit

Database User Protein
 NCBI nr.2013.6.17
 NCBI nr.2013.6.17.random
 NCBI nr.2013.6.17.random.concat

[+] User Protein Sequence
DNA Frame Translation 3 **N Term AA Limit**

Taxonomy
 ARCHAEOGLOBUS FULGIDUS
 BACILLUS SUBTILIS
 BACULOVIRIDAE
 BORRELIJA BURGENDORFERI
BOS TAURUS
 BOTHROPS INSULARIS

Digest Trypsin **Max. Missed Cleavages** 1

Constant Mods
 Acetohydrazide (C-term)
 Acetohydrazide (DE)
 Acetyl (K)
 Acetyl (N-term)
 Acetyl:2H(3) (K)
 Acetyl:2H(3) (N-term)

Output HTML Hits to file Name lastres

[+] Pre-Search Parameters

Start Search

Sample ID (comment)
Display Graph

Maximum Reported Hits 5
Sort By Score Sort
Report Homologous Proteins Interesting
Min. # peptides required to match 4
Report MOWSE Scores **Pfactor** 0.4
Masses are monoisotopic
Tol 0.05 **Da** **Sys Err** 0

Contaminant Masses

Possible Modifications
 Peptide N-terminal Gln to pyroGlu
Oxidation of M
 Protein N-terminus Acetylated
 Acrylamide Modified Cys

User Def Mod 1 Acetyl (K)
User Def Mod 2 Acetyl (K)
User Def Mod 3 Acetyl (K)
User Def Mod 4 Acetyl (K)

OR

Unknown Amino Acid **Single Base Change** **Homology**
Max Mods 1 **Min. # match with NO AA subs** 1

Instrument ESI-Q-TOF **Data Format** PP M/Z Charge

Data Paste Area

927.486
 1050.485
 1283.703
 1305.708
 1439.504
 1479.788

MS-Fit Search Results

[±] Parameters

[±] Pre Search Results (SwissProt.2013.6.27)

Fraction-Spot-Run ID: 1-1-1
 MS-Fit search selects 69 entries (results displayed for top 5 matches).

[−] Results Summary

| Protein Hit Number | MOWSE Score | # mat % | pep % | Mean Err Da | Data Tol Da | # Hom Prot | MS-Digest Index # | Protein MW (Da)/pI | Accession # | Species | Protein Name |
|--------------------|-------------|---------|-----------|-------------|-------------|------------|-------------------|--------------------|-------------|---------|---|
| 1 | 257139 | 9/9/17 | 18.8/17.3 | -0.00579 | 0.0317 | No | 12311 | 69294/5.8 | P02769 | BOVIN | Serum albumin |
| 2 | 5061 | 4/4/8 | 26.5/7.7 | 0.0151 | 0.0263 | No | 70806 | 16337/9.1 | Q05852 | BOVIN | Ubiquinone biosynthesis protein COQ4 homolog, mitochondrial |
| 3 | 894 | 4/4/8 | 10.2/7.7 | 0.0128 | 0.0837 | No | 253729 | 22324/5.1 | Q2YDP3 | BOVIN | Dr1-associated corepressor |
| 4 | 477 | 5/5/10 | 7.0/9.6 | -0.0113 | 0.0735 | No | 41280 | 73414/7.0 | Q58D55 | BOVIN | Beta-galactosidase |
| 5 | 365 | 4/4/8 | 5.6/7.7 | -0.0100 | 0.0688 | No | 192272 | 85894/5.1 | Q8S088 | BOVIN | Integrin beta-6 |

| Protein Hit Number | MOWSE Score | # mat % | pep % | Mean Err Da | Data Tol Da | # Hom Prot | MS-Digest Index # | Protein MW (Da)/pI | Accession # | Species | Protein Name |
|--------------------|-------------|---------|-------|-------------|-------------|------------|-------------------|--------------------|-------------|---------|--------------|
| 12311 | x | . | . | x | . | . | . | . | . | . | . |
| 70806 | . | x | . | . | . | . | . | . | . | . | . |
| 253729 | . | . | x | . | . | . | . | . | . | . | . |
| 41280 | . | . | . | x | . | . | . | . | . | . | . |
| 192272 | . | . | . | . | x | . | . | . | . | . | . |

[−] Detailed Results

1. 9/52 matches (17%).
Acc. #: P02769 **Uniprot ID:** ALBU_BOVIN **Species:** BOVIN **Name:** Serum albumin
Organism: Bos taurus **Gene:** ALB **Existence:** Evidence at protein level **Version:** 4
Index: 12311 **MW:** 69294 Da **pI:** 5.8

| m/z Submitted | MH ⁺ Matched | Intensity | Delta Da | Modifications | Start End | Missed Cleavages | Sequence |
|---------------|-------------------------|-----------|----------|---------------|-----------|------------------|--------------------------------------|
| 927.4860 | 927.4934 | 100.0 | -0.00744 | | 161 167 | 0 | (K)YLVEIAR(R) |
| 1163.5996 | 1163.6307 | 100.0 | -0.0311 | | 66 75 | 0 | (K)LWNETFAK(T) |
| 1283.7030 | 1283.7106 | 100.0 | -0.00765 | | 361 371 | 0 | (R)HPEYAVSVLLR(L) |
| 1305.7080 | 1305.7161 | 100.0 | -0.00813 | | 402 412 | 0 | (K)HLVDEPNLIK(Q) |
| 1479.7880 | 1479.7954 | 100.0 | -0.00744 | | 421 433 | 0 | (K)LGEYGFONALIVR(Y) |
| 1567.7350 | 1567.7427 | 100.0 | -0.00774 | | 347 359 | 0 | (K)DAFLGSLFVEYSR(R) |
| 1576.7986 | 1576.7676 | 100.0 | 0.0310 | | 139 151 | 0 | (K)LKPDNPTLC(Carbamidomethyl)DEFK(A) |
| 1639.9310 | 1639.9377 | 100.0 | -0.00675 | | 437 451 | 1 | (R)KVPQVSTPTLVEYSR(S) |
| 2045.0210 | 2045.0280 | 100.0 | -0.00696 | | 168 183 | 1 | (R)RHPVYAPPELLYYANK(Y) |

Num Unmatched Masses: 43
[Search for disulfide linked peptides.](#)
[Do a non-specific cleavage search.](#)
[Search for another component.](#)

The matched peptides cover 18.0% (109/607AA's) of the protein.
 Summary Mass for This Hit (MS-Digest Index #): 12311

Human Albumin

MKWVTFISLL FLFSSAYSRG VFRRDAHKSE VAHRFKDLGE ENFKALVLIA FAQYLQQCPF EDHVKLVNEV TEFAKTCVAD
 ESAENCCKSL HTLFGDKLCT VATLRETYGE MADCCAKQEP ERNECFLQHK DDNPMLPRLV RPEVDVMCTA FHDNEETFLK
 KYLYEIARRH PYFYAPELLE FAKRYKAAFT ECCQAADKAA CLLPKLDEL R DEGKASSAKQ RLKCASLQKF GERAFAKAWAV
 ARLSQRFPKA EFAEVSKLVT DLTQVHTECC HGDLLCADD RADLAKYICE NQDSISSKLEK ECCEKPLLEK SHCIAEVEND
 EMPADLPSLA ADFVESKDVC KNYAEAKDVF LGMFLYFYAR RHPDYSVLL LRLAKTYETT LEKCCAAADP HECYAKVFDE
 FKPLVEEPQN LIKQNCLEFE QLGEYKFNAL LLVRYTKKVP QVSTPTLVEV SRNLGKVGSK CCKHPEAKRM PCAEDYLSVV
 LNQLCVLHEK TPVSDRVTKC CTESLVNRRP CFSALEVDET YVPKEFNAET FTFHADICTL SEKERQIKKQ TALVELVKHK
 PKATKEQLKA VMDDFAAFVE KCCKADDKET CFAEEGKKLV AASQAALGL

| m/z | Missed Cleavages | Sequence |
|-----------|------------------|---|
| 1013.4244 | 0 | ⁵⁸⁹ <u>ETCFAEEGK</u> ⁵⁹⁷ |
| 1013.5990 | 0 | ⁵⁹⁹ <u>LVAASQAALGL</u> ⁶⁰⁹ |
| 1141.5194 | 1 | ⁵⁸⁹ <u>ETCFAEEGKK</u> ⁵⁹⁸ |
| 1141.6939 | 1 | ⁵⁹⁸ <u>KLVAASQAALGL</u> ⁶⁰⁹ |
| 1149.5759 | 1 | ²⁵ <u>DAHKSEVAHR</u> ³⁴ |
| 1149.6150 | 0 | ⁶⁶ <u>LVNEVTEFAK</u> ⁷⁵ |
| 1352.6661 | 1 | ⁴⁹⁷ <u>VTKCCTESLVNR</u> ⁵⁰⁸ |
| 1352.7685 | 1 | ⁴²⁷ <u>FQNALLVRYTK</u> ⁴³⁷ |
| 1623.7876 | 0 | ³⁴⁸ <u>DVFLGMFLYFYAR</u> ³⁶⁰ |
| 1623.9581 | 1 | ³⁶² <u>HPDYSVLLLRRLAK</u> ³⁷⁵ |
| 1898.9952 | 1 | ¹⁶⁹ <u>RHPYFYAPELLEFFAK</u> ¹⁸³ |
| 1898.9952 | 1 | ¹⁷⁰ <u>HPYFYAPELLEFFAKR</u> ¹⁸⁴ |

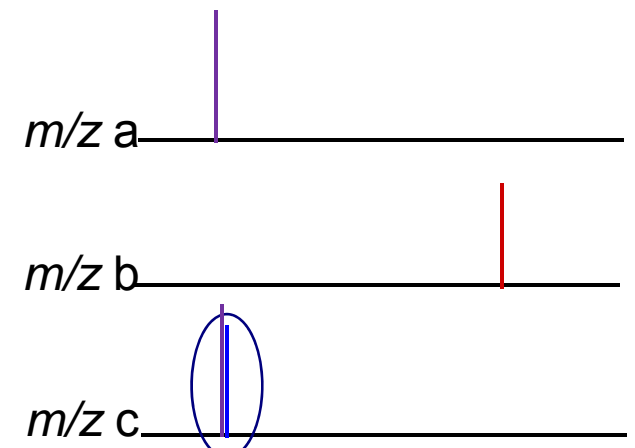
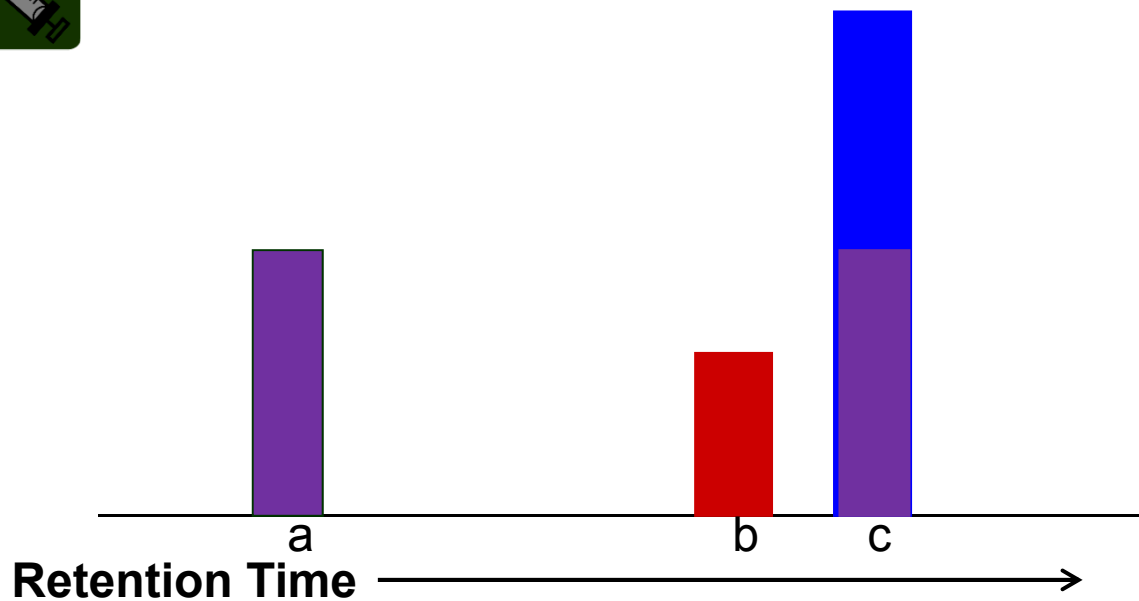
MS alone **CAN NOT** distinguish peptides of similar molecular weight.

- post-translational modification.
- sequence coverage determinations difficult.
- the identification of more than one protein in a sample impossible.
- Mass accuracy requirements

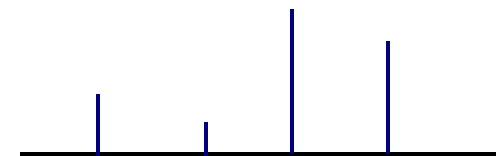
LC/MS or LC/MSMS

 Peptides with different m/z

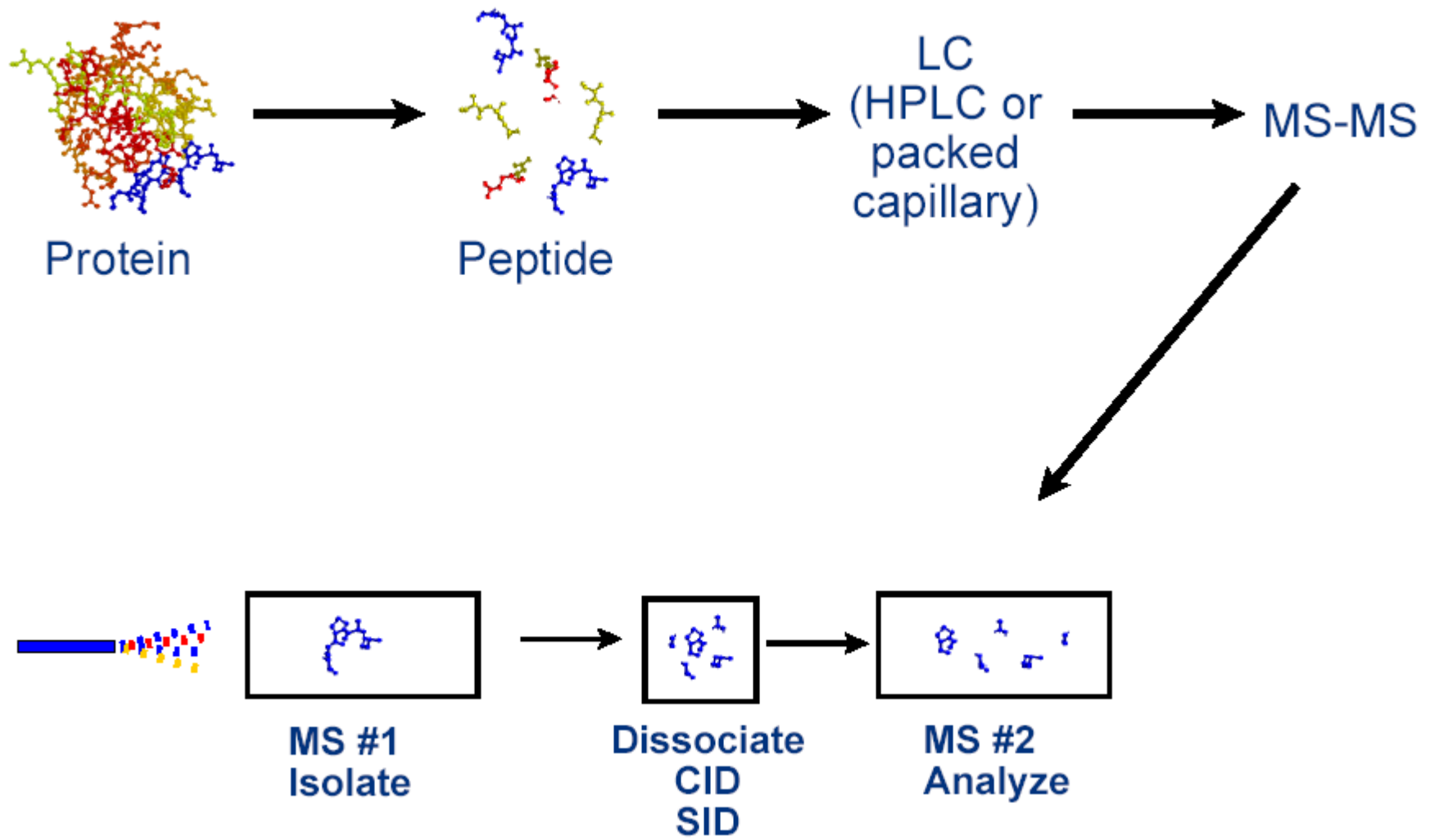
Mixture of Peptides



Collision induced dissociation (CID)



Tandem (MS/MS) – Peptide Fragment Analysis



LC/MSMS

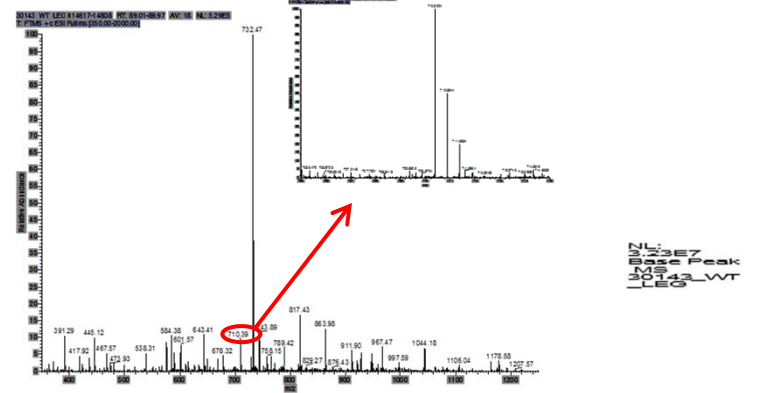
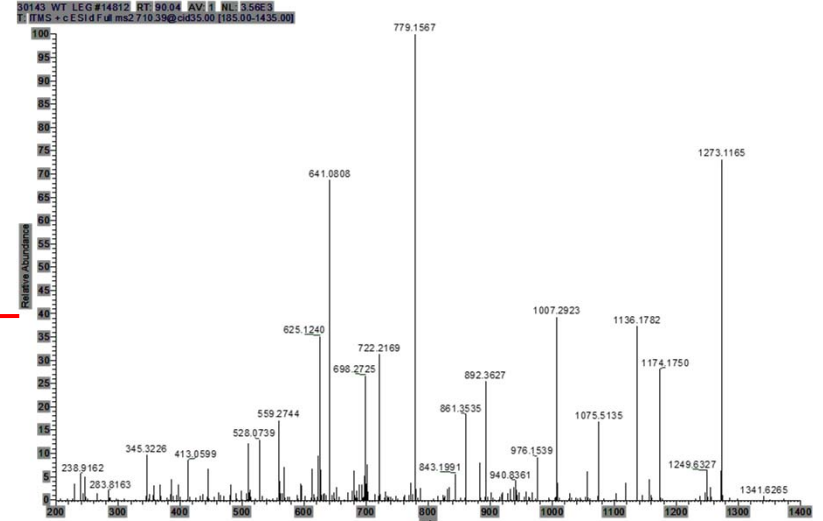
GLLEDLGYDVVVK

CASP4_MOUSE

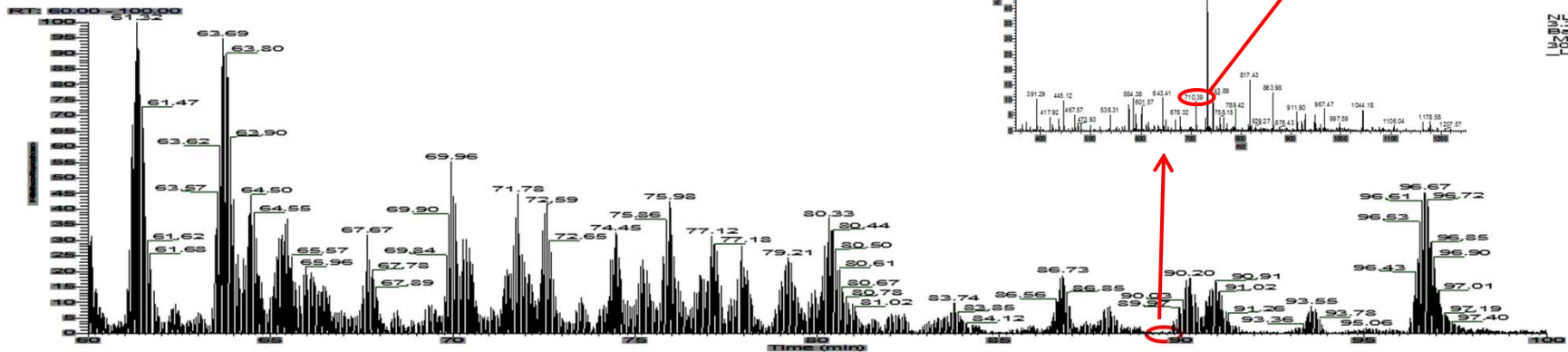
Caspase-4

OS=Mus musculus

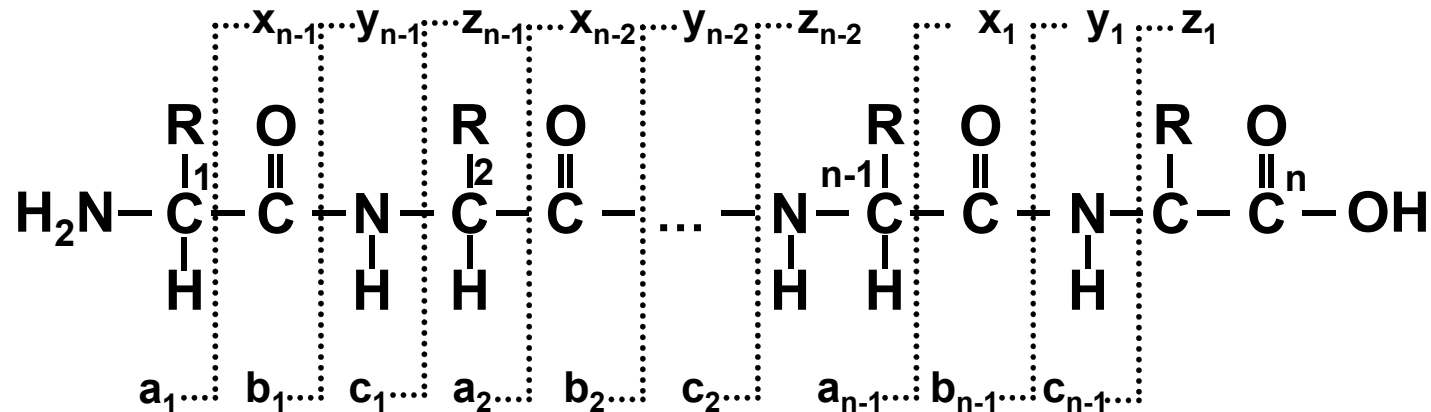
| # | b | Seq. | y | # |
|----|-----------|------|-----------|----|
| 1 | 58.0287 | G | | 13 |
| 2 | 171.1128 | L | 1362.7515 | 12 |
| 3 | 284.1969 | L | 1249.6674 | 11 |
| 4 | 413.2395 | E | 1136.5834 | 10 |
| 5 | 528.2664 | D | 1007.5408 | 9 |
| 6 | 641.3505 | L | 892.5138 | 8 |
| 7 | 698.3719 | G | 779.4298 | 7 |
| 8 | 861.4353 | Y | 722.4083 | 6 |
| 9 | 976.4622 | D | 559.3450 | 5 |
| 10 | 1075.5306 | V | 444.3180 | 4 |
| 11 | 1174.5990 | V | 345.2496 | 3 |
| 12 | 1273.6674 | V | 246.1812 | 2 |
| 13 | | K | 147.1128 | |



NL:
9.23E7
Base Peak
MS
10143.WT
10143

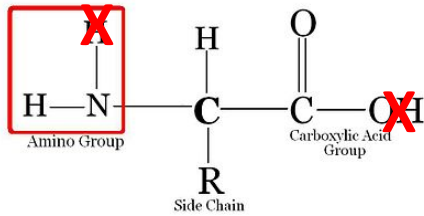


MSMS Fragments and Different Fragmentation Technique



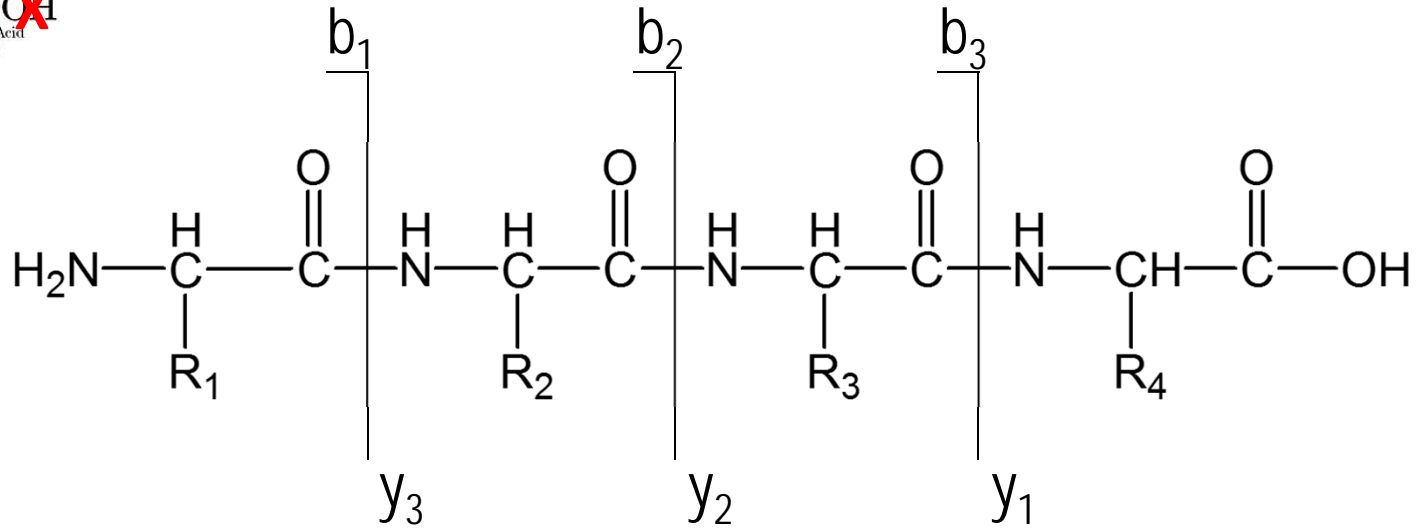
- CID:** a, b and y ion, loss of H₂O, NH₃, side-chain
- PSD:** a, b and y ion
- IRMPD:** b and y ion, loss of side-chain
- ECD:** c, y and z ions
- ETD:** c, y and z ions

Sequence Nomenclature for Mass Ladder

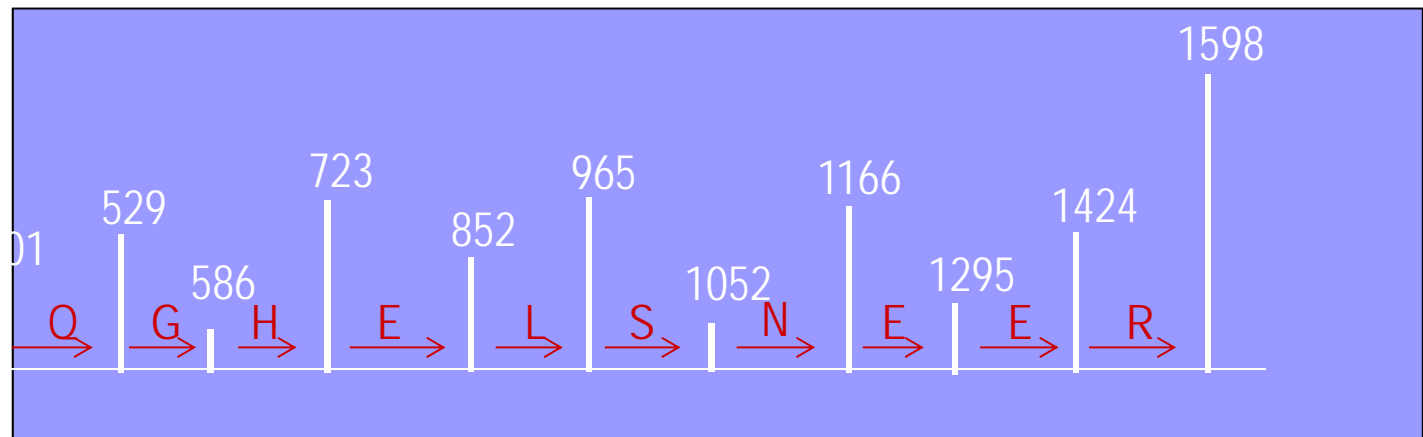


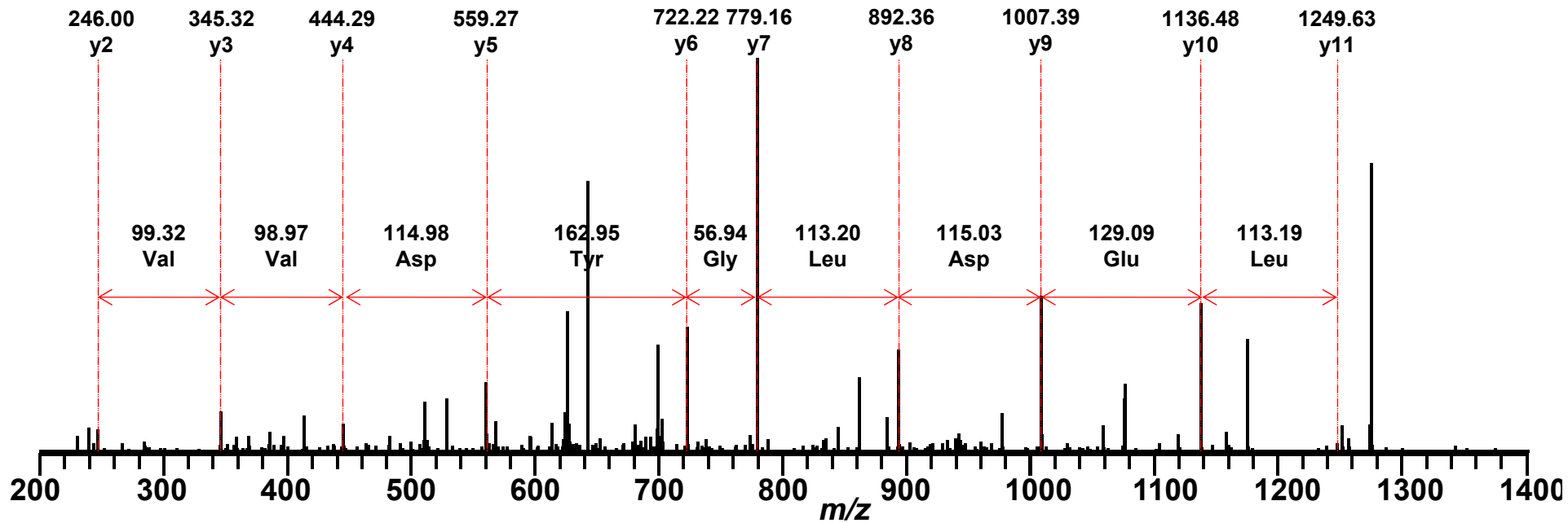
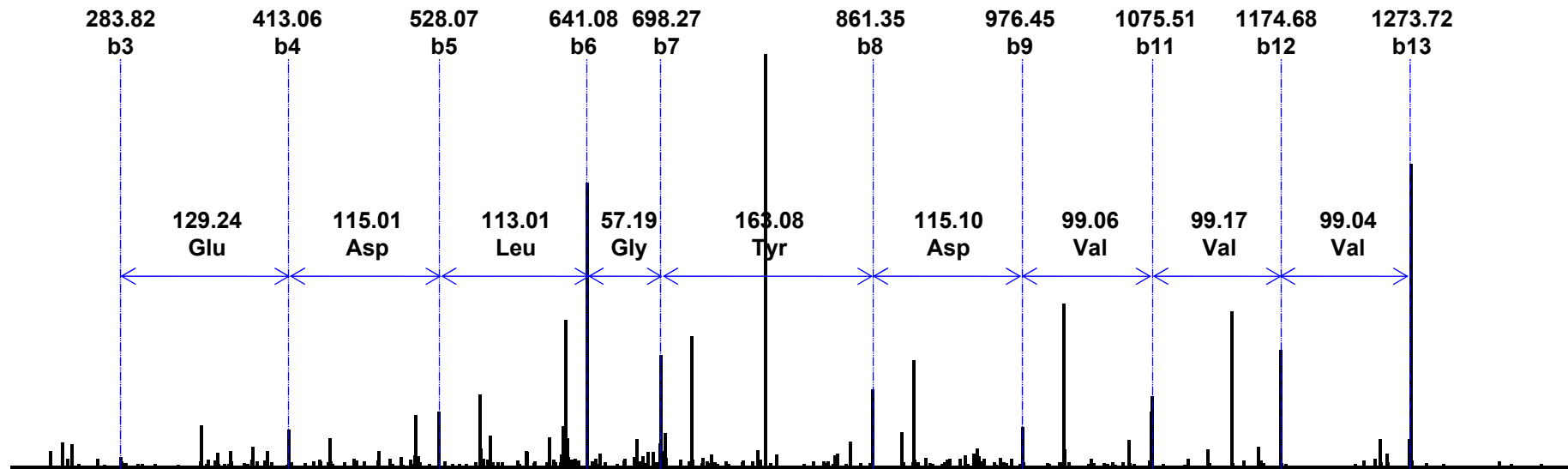
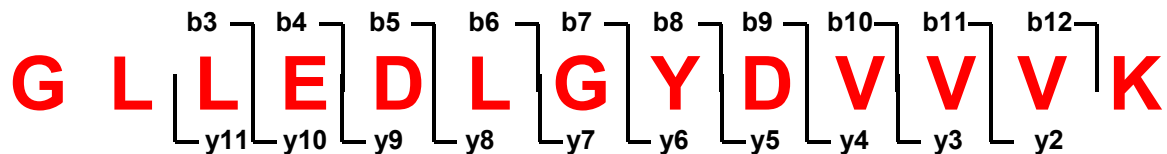
Mass=56Da+R

$b_2 - b_1 = 56\text{Da} + R_2$ $b_3 - b_2 = 56\text{Da} + R_2$



$y_3 - y_2 = 56\text{Da} + R_2$ $y_2 - y_1 = 56\text{Da} + R_2$

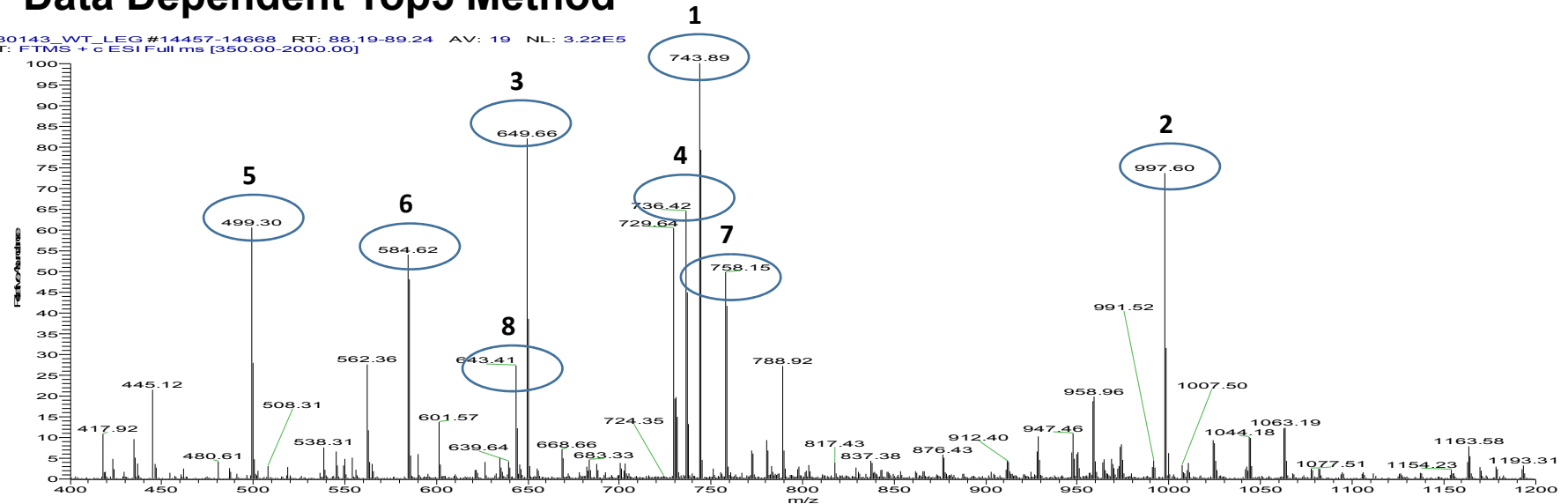




Data Dependent Acquisition and Dynamic Exclusion

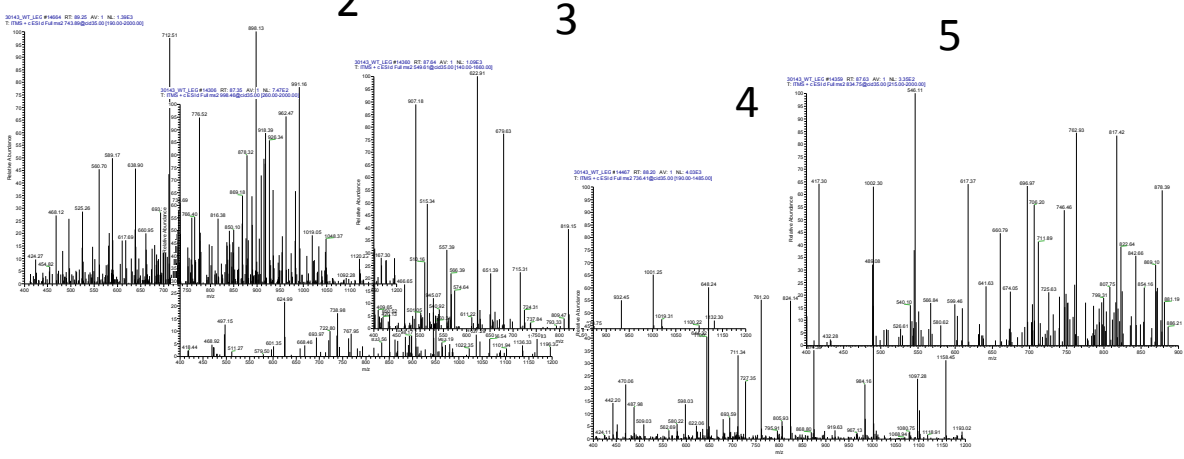
Data Dependent Top5 Method

30143_WT_LEG #14457-14668 RT: 88.19-89.24 AV: 19 NL: 3.22E5
T: FTMS + c ESI Full ms [350.00-2000.00]



MSMS

1 2 3 4 5

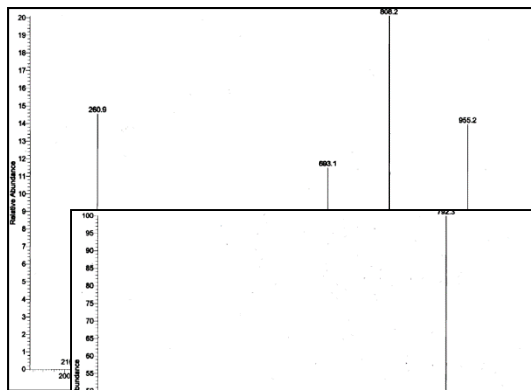


MS Cycle: 0.06S
LC Peak Width >12S

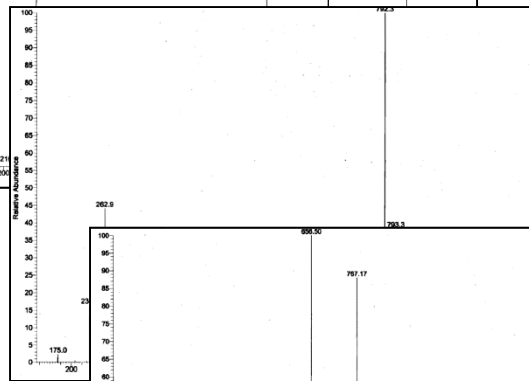
Dynamic Exclusion

Make MSMS for the lower abundant ions possible!!

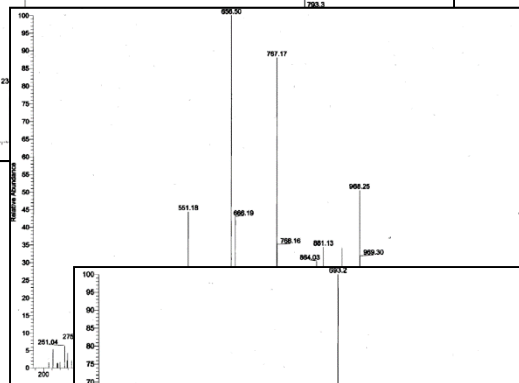
More Peptides Identified Increase Confidence in ID



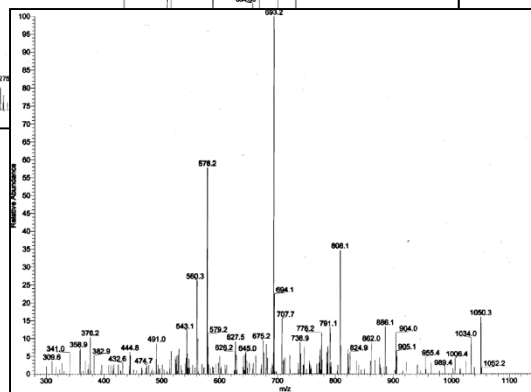
VFGTDMNSR



IFDDSDQTK



LVNLGK

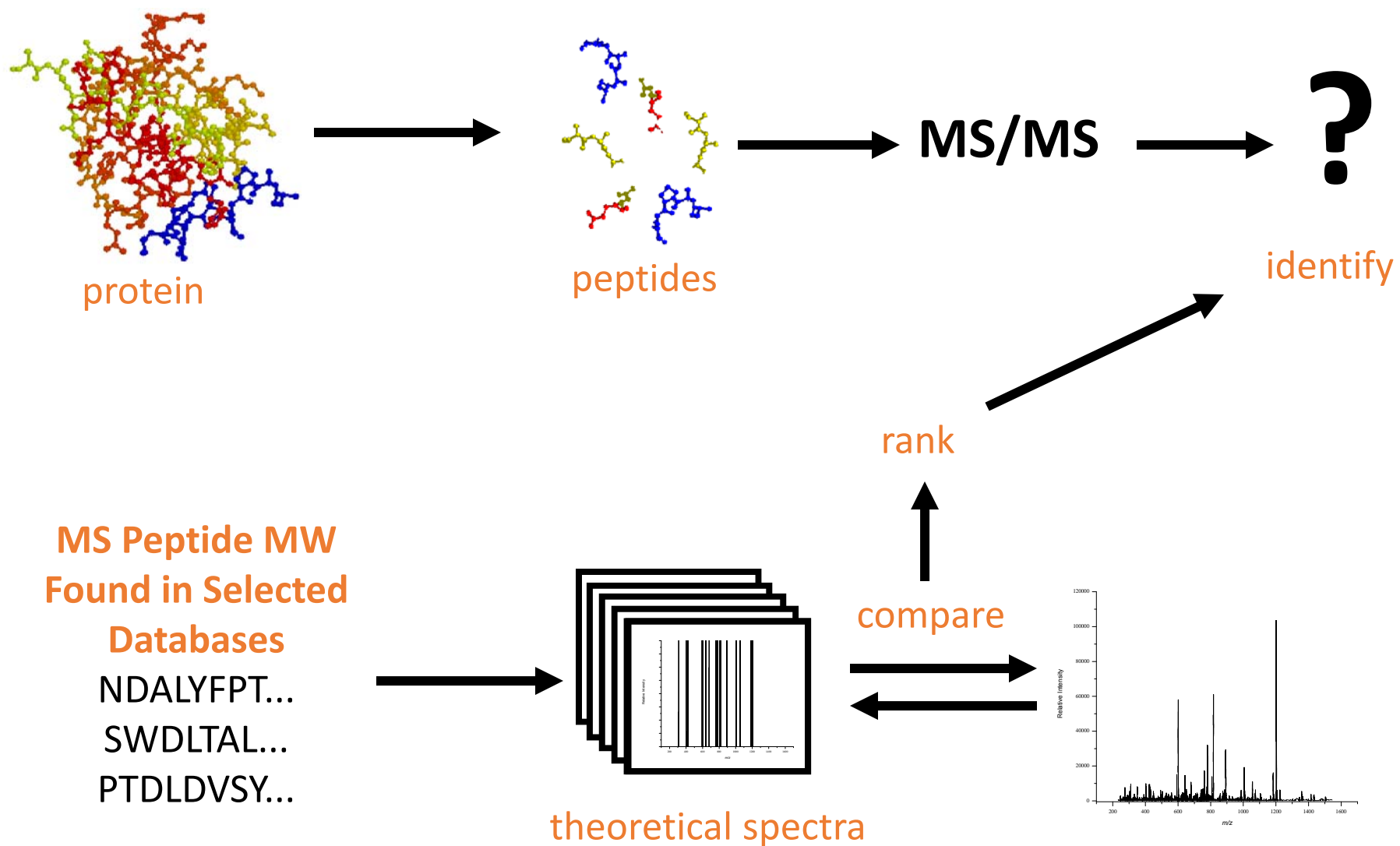


QAEDVNLLDQMSK

If all of these peptides belonged to an unknown protein, MS/MS could potentially reveal protein identity



Data Analysis for MS/MS Method



Database Searching

.Mascot

- 16 node cluster for high-speed data processing

.Protein sequences are digested and fragmented *In Silico* which produces an enormous peak list

.Raw MS/MS data is converted to a peak list and compared against the *In Silico* peak list.

.Critical point of database searching

- The enzyme must work properly (no non-specific cleavage and missed cuts)
- The mass accuracy limits must be set appropriately
- Any modifications must be accounted for (modified cysteine)
- The database must contain the protein

Mascot Database Search Engine

Mascot Daemon

File Edit Help

Status Event Log Task Editor **Parameter Editor**

Parameter set
Filename: M:\Daemon\Parameter\Orbitrap\WITH MOD New Open ... Save

All Searches
User name Kari User email kleinholz.1@osu.edu
Search title trypsin full
Taxonomy Homo sapiens (human) Report top AUTO hits
Database Sabree_All salmonella Smansonisequence Decoy database Protein mass kDa
Fixed modifications Carbamidomethyl (C) Monoisotopic Average Enzyme Trypsin/P Max. missed cleavages 4
Variable modifications Acetyl (K) Deamidated (NQ) GlyGly (K) Oxidation (M) Peptide charge 4+
Select Modifications Peptide tol. ± 20 ppm #13C 1

MS/MS
MS/MS Ions search Data format Mascot generic Quantitation None
Error tolerant search MS/MS tol. ± 0.8 Da Instrument ESI-TRAP

Mascot Daemon: Select Modifications

Acetyl (N-term)
Acetyl (Protein N-term)
Amidated (C-term)
Amidated (Protein C-term)
Ammonia-loss (N-term C)
Biotin (K)
Biotin (N-term)
Carbamyl (K)
Carbamyl (N-term)
Carboxymethyl (C)
Cation:Na (C-term)
Cation:Na (DE)
Cys->Disulfane (C)
cysMT Epilex (C)
Dehydrated (N-term C)
Dehydro (C)
D,Leu4plex (K)
D,Leu4plex (N-term)
D,Leu4plex (Y)
Dimethyl (K)
Dimethyl (R)
Dioxidation (M)

Fixed Modifications
Carbamidomethyl (C)

Variable Modifications
Acetyl (K)
Deamidated (NQ)
GlyGly (K)
Oxidation (M)
Phospho (ST)
Phospho (Y)

Show all modifications OK Cancel

Enzyme Used

20ppm for orbitrap
1.5 Da for LTQ

MASCOT Results

MASCOT Search Results

User : Kari
E-mail : kleinholz.1@osu.edu
Search title : trypsin full
MS data file : \\filesrv\Mass_Spec\Data_New\Orbitrap TEST\071614\STD_071614_B.xml.mgf
Database : SwissProt_ID 2014_06 (545,536 sequences; 194,023,197 residues)
Taxonomy : Other mammalia (13,066 sequences)
Timestamp : 16 Jul 2014 at 19:10:58 GMT

Re-search All Non-significant Unassigned [\[help\]](#) Export As XML

Not what you expected? Try [the select summary](#).

▶ **Search parameters**

▶ **Score distribution**

▶ **Legend**

Protein Family Summary

Filter Significance threshold p< Max. number of families [\[help\]](#)
Ions score or expect cut-off Dendrograms cut at
Show Percolator scores
Preferred taxonomy

▶ **Decoy search summary (reversed protein sequences)**

Proteins (17) [Report Builder](#) [Unassigned \(2249\)](#)

Protein families 1-10 (out of 17)

10 per page 1 [Next](#)

Accession contains

▶ **1**



1 MYG_EQUBU

2474 Myoglobin OS=Equus burchelli GN=MB PE=1 SV=2

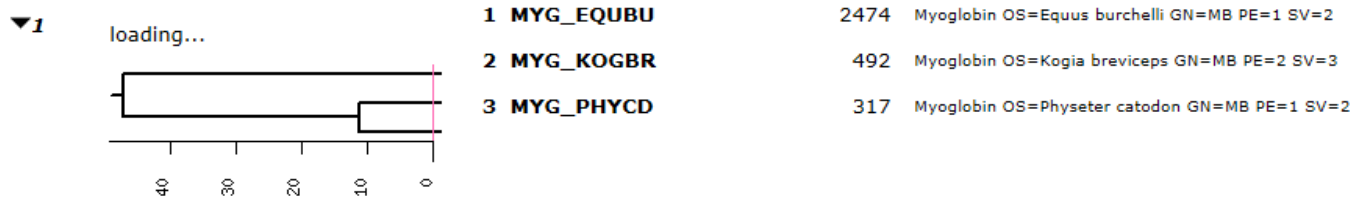
2 MYG_KOGBR

492 Myoglobin OS=Kogia breviceps GN=MB PE=2 SV=3

3 MYG_PHYCD

317 Myoglobin OS=Physeter catodon GN=MB PE=1 SV=2

MASCOT Results



loading...

Threshold (0):

* Protein Name

Protein Mascot Mouse Score

Protein Mol. Weight

of spectra matched

of peptides matched

| Score | Mass | Matches | Sequences | emPAI |
|-------|-------|-----------|-----------|-------|
| 2474 | 17072 | 117 (117) | 13 (13) | 186.7 |
| 492 | 17357 | 41 (41) | 6 (6) | 3.98 |
| 317 | 17320 | 37 (37) | 6 (6) | 3.16 |

Redisplay All None

The Exponentially Modified Protein Abundance Index (emPAI)

$$emPAI = 10^{\frac{N_{observed}}{N_{observable}} - 1}$$

▼138 peptide matches (38 non-duplicate, 100 duplicate)

Auto-fit to window

| Query | Dupes | Observed | Mr (expt) | Mr (calc) | ppm | M | Score | Expect | Rank | U | 1 | 2 | 3 | Peptide |
|-------|-------|----------|-----------|-----------|------|---|-------|----------|------|---|---|---|---|---------------------------------------|
| 55 | | 368.2501 | 734.4856 | 734.4803 | 7.32 | 1 | 24 | 0.012 | 2 | | ■ | ■ | ■ | K.HKIPIK.Y |
| 66 | 22 | 374.7230 | 747.4314 | 747.4279 | 4.74 | 0 | 47 | 0.00044 | 1 | | ■ | ■ | ■ | K.ALELFR |
| 497 | 6 | 471.2427 | 940.4708 | 940.4654 | 5.77 | 1 | 46 | 0.00018 | 1 | U | | | | K.YKELGFQG.- |
| 507 | 3 | 471.7349 | 941.4552 | 941.4494 | 6.18 | 1 | 46 | 0.00048 | 1 | U | | | | K.YKELGFQG.- + Deamidated (NQ) |
| 1406 | 2 | 636.3384 | 1270.6622 | 1270.6557 | 5.12 | 0 | 56 | 8e-005 | 1 | U | | | | R.LFTGHPETLEK.F |
| 1407 | | 424.5614 | 1270.6624 | 1270.6557 | 5.21 | 0 | 30 | 0.032 | 1 | U | | | | R.LFTGHPETLEK.F |
| 1590 | | 454.5883 | 1360.7431 | 1360.7350 | 5.92 | 1 | 31 | 0.034 | 1 | U | | | | K.ALELFRNDIAAK.Y + Deamidated (NQ) |
| 1593 | | 681.3809 | 1360.7472 | 1360.7350 | 8.99 | 1 | 36 | 0.011 | 1 | U | | | | K.ALELFRNDIAAK.Y + Deamidated (NQ) |
| 1622 | 6 | 460.2877 | 1377.8413 | 1377.8344 | 4.99 | 0 | 73 | 2.2e-007 | 1 | U | | | | K.HGTVVLTALGGILK.K |
| *1630 | 4 | 689.9305 | 1377.8464 | 1377.8344 | 8.75 | 0 | 100 | 3.2e-010 | 1 | U | | | | K.HGTVVLTALGGILK.K |
| 1650 | 2 | 464.9611 | 1391.8615 | 1391.8500 | 8.22 | 0 | 27 | 0.0046 | 1 | | ■ | ■ | | K.HGTVVLTALGAILK.K |
| 1785 | 4 | 501.5632 | 1501.6678 | 1501.6620 | 3.85 | 0 | 82 | 1.4e-007 | 1 | U | | | | K.HPGDFGADAQGAMTK.A |
| 1787 | 1 | 751.8440 | 1501.6734 | 1501.6620 | 7.63 | 0 | 94 | 9.6e-009 | 1 | U | | | | K.HPGDFGADAQGAMTK.A |
| 1785 | 1 | 501.8903 | 1502.6491 | 1502.6460 | 2.05 | 0 | 53 | 7.7e-005 | 1 | U | | | | K.HPGDFGADAQGAMTK.A + Deamidated (NQ) |

Peptide Sequence and MS/MS Information

{MATRIX} Mascot Search Results

Peptide View

MS/MS Fragmentation of **HGTVVLTALGGILK**

Found in **MYG_EQUBU** in **SwissProt_ID**, Myoglobin OS=Equus burchelli GN=MB PE=1 SV=2

Match to Query 1630: 1377.846448 from(689.930500,2+) index(2183)

Title: STD_071614_B.3138.3138.2.dta

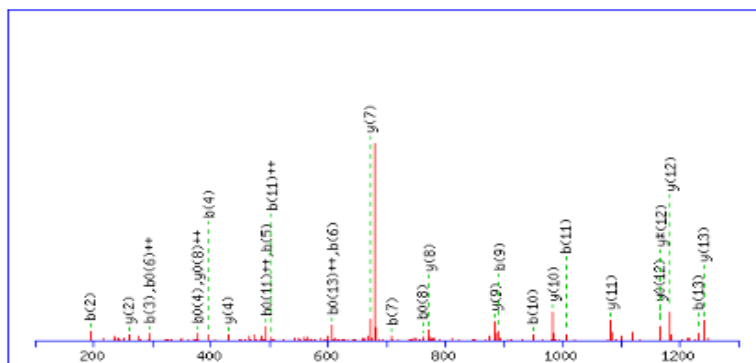
Data file \\filesrv\Mass_Spec\Data_New\Orbitrap TEST\071614\STD_071614_B_xml.mgf

Click mouse within plot area to zoom in by factor of two about that point

Or, 100 to Da

Label all possible matches Label matches used for scoring

Show Y-axis



Monoisotopic mass of neutral peptide $M_r(\text{calc})$: 1377.8344

Ions Score: 100 Expect: $3.2e-010$

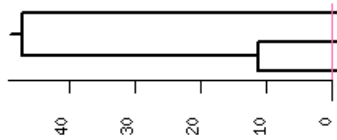
Matches : 28/112 fragment ions using 33 most intense peaks [\(help\)](#)

| # | b | b ⁺⁺ | b ⁰ | b ⁰⁺⁺ | Seq. | y | y ⁺⁺ | y [*] | y ^{++*} | y ⁰ | y ⁰⁺⁺ | # |
|----|-----------|-----------------|----------------|------------------|------|-----------|-----------------|----------------|------------------|----------------|------------------|----|
| 1 | 138.0662 | 69.5367 | | | H | | | | | | | 14 |
| 2 | 195.0877 | 98.0475 | | | G | 1241.7828 | 621.3950 | 1224.7562 | 612.8817 | 1223.7722 | 612.3897 | 13 |
| 3 | 296.1353 | 148.5713 | 278.1248 | 139.5660 | T | 1184.7613 | 592.8843 | 1167.7347 | 584.3710 | 1166.7507 | 583.8790 | 12 |
| 4 | 395.2037 | 198.1055 | 377.1932 | 189.1002 | V | 1083.7136 | 542.3604 | 1066.6871 | 533.8472 | 1065.7030 | 533.3552 | 11 |
| 5 | 494.2722 | 247.6397 | 476.2616 | 238.6344 | V | 984.6452 | 492.8262 | 967.6186 | 484.3130 | 966.6346 | 483.8210 | 10 |
| 6 | 607.3562 | 304.1817 | 589.3457 | 295.1765 | L | 885.5768 | 443.2920 | 868.5502 | 434.7788 | 867.5662 | 434.2867 | 9 |
| 7 | 708.4039 | 354.7056 | 690.3933 | 345.7003 | T | 772.4927 | 386.7500 | 755.4662 | 378.2367 | 754.4822 | 377.7447 | 8 |
| 8 | 779.4410 | 390.2241 | 761.4305 | 381.2189 | A | 671.4450 | 336.2262 | 654.4185 | 327.7129 | | | 7 |
| 9 | 892.5251 | 446.7662 | 874.5145 | 437.7609 | L | 600.4079 | 300.7076 | 583.3814 | 292.1943 | | | 6 |
| 10 | 949.5465 | 475.2769 | 931.5360 | 466.2716 | G | 487.3239 | 244.1656 | 470.2973 | 235.6523 | | | 5 |
| 11 | 1006.5680 | 503.7876 | 988.5574 | 494.7824 | G | 430.3024 | 215.6548 | 413.2758 | 207.1416 | | | 4 |
| 12 | 1119.6521 | 560.3297 | 1101.6415 | 551.3244 | I | 373.2809 | 187.1441 | 356.2544 | 178.6308 | | | 3 |
| 13 | 1232.7361 | 616.8717 | 1214.7256 | 607.8664 | L | 260.1969 | 130.6021 | 243.1703 | 122.0888 | | | 2 |
| 14 | | | | | K | 147.1128 | 74.0600 | 130.0863 | 65.5468 | | | 1 |

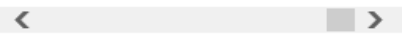
MASCOT Results

▼1

loading...



loading...



Threshold (0):

* Protein Name

| | Score | Mass | Matches | Sequences | emPAI | |
|---------------------------------|-------|-------|-----------|-----------|--------|---|
| ✓ 1.1 MYG_EQUBU | 2474 | 17072 | 117 (117) | 13 (13) | 186.75 | Myoglobin OS=Equus burchelli GN=MB PE=1 SV=2 |
| ▶ 1 same-set of MYG_EQUBU | | | | | | |
| ✓ 1.2 MYG_KOGBR | 492 | 17357 | 41 (41) | 6 (6) | 3.98 | Myoglobin OS=Kogia breviceps GN=MB PE=2 SV=3 |
| ▶ 1 same-set of MYG_KOGBR | | | | | | |
| ✓ 1.3 MYG_PHYCD | 317 | 17320 | 37 (37) | 6 (6) | 3.16 | Myoglobin OS=Physeter catodon GN=MB PE=1 SV=2 |

▼138 peptide matches (38 non-duplicate, 100 duplicate)

Auto-fit to window

| Query | Dupes | Observed | Mr (expt) | Mr (calc) | ppm | M | Score | Expect | Rank | U | 1 | 2 | 3 | Peptide |
|----------------------|-------|----------|-----------|-----------|------|---|-------|----------|------|---|---|---|---|---------------------------------------|
| 55 | | 368.2501 | 734.4856 | 734.4803 | 7.32 | 1 | 24 | 0.012 | ▶2 | | ■ | ■ | ■ | K.HKIPIK.Y |
| 66 | ▶22 | 374.7230 | 747.4314 | 747.4279 | 4.74 | 0 | 47 | 0.00044 | ▶1 | | ■ | ■ | ■ | K.ALELFR |
| 497 | ▶6 | 471.2427 | 940.4708 | 940.4654 | 5.77 | 1 | 46 | 0.00018 | ▶1 | U | | | | K.YKELGFQG.- |
| 507 | ▶3 | 471.7349 | 941.4552 | 941.4494 | 6.18 | 1 | 46 | 0.00048 | ▶1 | U | | | | K.YKELGFQG.- + Deamidated (NQ) |
| 1406 | ▶2 | 636.3384 | 1270.6622 | 1270.6557 | 5.12 | 0 | 56 | 8e-005 | ▶1 | U | | | | R.LFTGHPETLEK.F |
| 1407 | | 424.5614 | 1270.6624 | 1270.6557 | 5.21 | 0 | 30 | 0.032 | ▶1 | U | | | | R.LFTGHPETLEK.F |
| 1590 | | 454.5883 | 1360.7431 | 1360.7350 | 5.92 | 1 | 31 | 0.034 | ▶1 | U | | | | K.ALELFRNDIAAK.Y + Deamidated (NQ) |
| 1593 | | 681.3809 | 1360.7472 | 1360.7350 | 8.99 | 1 | 36 | 0.011 | ▶1 | U | | | | K.ALELFRNDIAAK.Y + Deamidated (NQ) |
| 1622 | ▶6 | 460.2877 | 1377.8413 | 1377.8344 | 4.99 | 0 | 73 | 2.2e-007 | ▶1 | U | | | | K.HGTVVLTALGGILK.K |
| 1630 | ▶4 | 689.9305 | 1377.8464 | 1377.8344 | 8.75 | 0 | 100 | 3.2e-010 | ▶1 | U | | | | K.HGTVVLTALGGILK.K |
| 1650 | ▶2 | 464.9611 | 1391.8615 | 1391.8500 | 8.22 | 0 | 27 | 0.0046 | ▶1 | | ■ | ■ | | K.HGTVTLTALGAILK.K |
| 1785 | ▶4 | 501.5632 | 1501.6678 | 1501.6620 | 3.85 | 0 | 82 | 1.4e-007 | ▶1 | U | | | | K.HPGDFGADAQGAMTK.A |
| 1787 | ▶1 | 751.8440 | 1501.6734 | 1501.6620 | 7.63 | 0 | 94 | 9.6e-009 | ▶1 | U | | | | K.HPGDFGADAQGAMTK.A |
| 1785 | ▶1 | 501.8903 | 1502.6491 | 1502.6460 | 2.05 | 0 | 53 | 7.7e-005 | ▶1 | U | | | | K.HPGDFGADAQGAMTK.A + Deamidated (NQ) |

Peptide Sequence and Sequence Coverage

MATRIX SCIENCE MASCOT Search Results

Protein View: MYG_EQUBU

Myoglobin OS=Equus burchelli GN=MB PE=1 SV=2

Database: SwissProt_ID
Score: 2474
Nominal mass (M_r): 17072
Calculated pI: 7.21
Taxonomy:

Sequence similarity is available as [an NCBI BLAST search of MYG_EQUBU against nr.](#)

Search parameters

MS data file: \\filesrv\Mass_Spec\Data_New\Orbitrap TEST\071614\STD_071614_B_xml.mgf
Enzyme: Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications: [Carbamidomethyl \(C\)](#), [Deamidated \(NQ\)](#), [Oxidation \(M\)](#)

Protein sequence coverage: 88%

Matched peptides shown in **bold red**.

1 MGLSDGEWQQ VLN~~V~~WGKVEA DIAGHGQEV~~L~~ IRLFTGHPET LEKFDKFKHL
51 KTEAEMKASE DLKKHGT~~V~~VL TALGGILK~~R~~K GHHEAELKPL AQSHATKHKI
101 PIKYLEFISD AIIHVLH~~S~~KH PGDFGADAQ~~G~~ AMTKALELFR NDIAARKYKEL
151 GFQG

Unformatted sequence string: [154 residues](#) (for pasting into other applications).

Sort peptides by Residue Number Increasing Mass Decreasing Mass

Show predicted peptides also

| Query | Start - End | Observed | Mr (expt) | Mr (calc) | ppm | M | Score | Expect | Rank | U | Peptide |
|----------------------|-------------|----------|-----------|-----------|------|---|-------|----------|------|---|----------------------------------|
| 2399 | 2 - 17 | 908.4573 | 1814.9000 | 1814.8952 | 2.69 | 0 | 43 | 0.0029 | 1 | U | M.GLSDGEWQQVLNVWGK.V |
| 2401 | 2 - 17 | 908.4642 | 1814.9138 | 1814.8952 | 10.3 | 0 | 65 | 1.9e-005 | 1 | U | M.GLSDGEWQQVLNVWGK.V |
| 1997 | 18 - 32 | 536.2916 | 1605.8530 | 1605.8475 | 3.43 | 0 | 56 | 4.2e-005 | 1 | U | K.VEADIAGHGQEV L IR.L |
| 1998 | 18 - 32 | 536.2916 | 1605.8530 | 1605.8475 | 3.43 | 0 | 72 | 3.2e-006 | 1 | U | K.VEADIAGHGQEV L IR.L |
| 1999 | 18 - 32 | 536.2937 | 1605.8593 | 1605.8475 | 7.36 | 0 | 37 | 0.0096 | 1 | U | K.VEADIAGHGQEV L IR.L |
| 2000 | 18 - 32 | 803.9370 | 1605.8594 | 1605.8475 | 7.47 | 0 | 86 | 1.3e-007 | 1 | U | K.VEADIAGHGQEV L IR.L |
| 2001 | 18 - 32 | 536.2939 | 1605.8599 | 1605.8475 | 7.73 | 0 | 55 | 0.00016 | 1 | U | K.VEADIAGHGQEV L IR.L |
| 2002 | 18 - 32 | 536.2941 | 1605.8605 | 1605.8475 | 8.10 | 0 | 74 | 1.7e-006 | 1 | U | K.VEADIAGHGQEV L IR.L |

MASCOT Results

MASCOT Search Results

User : Kari
E-mail : kleinholz.1@osu.edu
Search title : trypsin full
MS data file : \\filesrv\Mass_Spec\Data_New\Orbitrap TEST\071614\STD_071614_B.xml.mgf
Database : SwissProt_ID 2014_06 (545,536 sequences; 194,023,197 residues)
Taxonomy : Other mammalia (13,066 sequences)
Timestamp : 16 Jul 2014 at 19:10:58 GMT

Re-search All Non-significant Unassigned [\[help\]](#) Export As XML

Not what you expected? Try [the select summary](#).

▶ Search parameters

▶ Score distribution

▶ Legend

Protein Family Summary

Filter Significance threshold p< Max. number of families [\[help\]](#)
Ions score or expect cut-off Dendrograms cut at
Show Percolator scores
Preferred taxonomy

▶ Decoy search summary (reversed protein sequences)

Proteins (17) [Report Builder](#) [Unassigned \(2249\)](#)

Protein families 1-10 (out of 17)

10 per page 1 [Next](#)

Accession contains

| | | | |
|-----|---|--------------------|---|
| ▶ 1 |  | 1 MYG_EQUBU | 2474 Myoglobin OS=Equus burchelli GN=MB PE=1 SV=2 |
| | | 2 MYG_KOGBR | 492 Myoglobin OS=Kogia breviceps GN=MB PE=2 SV=3 |
| | | 3 MYG_PHYCD | 317 Myoglobin OS=Physeter catodon GN=MB PE=1 SV=2 |

Protein Report

Decoy search summary (reversed protein sequences)

Proteins (17)

Report Builder

Unassigned (2249)

Protein hits (22 proteins)

Columns: Standard (12 out of 12)

Filters: (none)

Export as CSV

| Family | M | DB | Accession | Score | Mass | Matches | Pep(sig) | Sequences | Seq(sig) | emPAI | Description |
|--------------------|---|--------------|-----------------------------|-------|--------|---------|----------|-----------|----------|---------|---|
| 1 | 1 | SwissProt_ID | MYG_EQUBU | 2474 | 17072 | 117 | 117 | 13 | 13 | 186.75 | Myoglobin OS=Equus burchelli GN=MB PE=1 SV=2 |
| 1 | 2 | SwissProt_ID | MYG_KOGBR | 492 | 17357 | 41 | 41 | 6 | 6 | 3.98 | Myoglobin OS=Kogia breviceps GN=MB PE=2 SV=3 |
| 1 | 3 | SwissProt_ID | MYG_PHYCD | 317 | 17320 | 37 | 37 | 6 | 6 | 3.16 | Myoglobin OS=Physeter catodon GN=MB PE=1 SV=2 |
| 2 | 1 | SwissProt_ID | LACB_BOVIN | 1918 | 19870 | 101 | 101 | 8 | 8 | 13.28 | Beta-lactoglobulin OS=Bos taurus GN=LGB PE=1 SV=3 |
| 2 | 2 | SwissProt_ID | LACB_OVIMU | 1651 | 18139 | 93 | 93 | 8 | 8 | 14.43 | Beta-lactoglobulin OS=Ovis orientalis musimon GN=LGB PE=1 SV=1 |
| 3 | 1 | SwissProt_ID | CYC_HORSE | 1111 | 11825 | 68 | 68 | 14 | 14 | 3403.10 | Cytochrome c OS=Equus caballus GN=CYS PE=1 SV=2 |
| 3 | 2 | SwissProt_ID | CYC_EQUAS | 692 | 11811 | 44 | 44 | 12 | 12 | 167.59 | Beta-lactoglobulin OS=Ovis orientalis musimon GN=LGB PE=1 SV=1 |
| 3 | 3 | SwissProt_ID | CYC2_BOVIN | 346 | 11732 | 27 | 27 | 7 | 7 | 35.21 | Cytochrome c OS=Bos taurus GN=CYCT PE=3 SV=3 |
| 4 | 1 | SwissProt_ID | TRY1_BOVIN | 237 | 25769 | 11 | 11 | 4 | 4 | 1.08 | Cationic trypsin OS=Bos taurus PE=1 SV=3 |
| 5 | 1 | SwissProt_ID | CASQ1_CANFA | 47 | 6610 | 1 | 1 | 1 | 1 | 0.54 | Calsequestrin-1 (Fragment) OS=Canis familiaris GN=CASQ1 PE=1 SV=1 |
| 6 | 1 | SwissProt_ID | PROS_RABIT | 43 | 71923 | 1 | 1 | 1 | 1 | 0.05 | Vitamin K-dependent protein S (Fragment) OS=Oryctolagus cuniculus GN=PROS1 PE=2 SV=1 |
| 7 | 1 | SwissProt_ID | H11_BOVIN | 43 | 22087 | 3 | 3 | 2 | 2 | 0.33 | Histone H1.1 OS=Bos taurus GN=HIST1H1A PE=1 SV=1 |
| 8 | 1 | SwissProt_ID | COHA1_BOVIN | 33 | 149062 | 1 | 1 | 1 | 1 | 0.02 | Collagen alpha-1(XVII) chain OS=Bos taurus GN=COL17A1 PE=2 SV=1 |
| 9 | 1 | SwissProt_ID | 4F2_RABIT | 33 | 0 | 2 | 2 | 1 | 1 | | |
| 10 | 1 | SwissProt_ID | CASQ1_RABIT | 29 | 45235 | 1 | 1 | 1 | 1 | 0.07 | Calsequestrin-1 OS=Oryctolagus cuniculus GN=CASQ1 PE=1 SV=1 |
| 11 | 1 | SwissProt_ID | CRSP2_PIG | 28 | 12960 | 1 | 1 | 1 | 1 | 0.26 | Calcitonin receptor-stimulating peptide 2 OS=Sus scrofa GN=CRSP2 PE=2 SV=1 |
| 12 | 1 | SwissProt_ID | RS12_BOVIN | 27 | 14505 | 1 | 1 | 1 | 1 | 0.23 | 40S ribosomal protein S12 OS=Bos taurus GN=RPS12 PE=2 SV=1 |
| 13 | 1 | SwissProt_ID | IF4G1_RABIT | 23 | 153955 | 1 | 1 | 1 | 1 | 0.02 | Eukaryotic translation initiation factor 4 gamma 1 OS=Oryctolagus cuniculus GN=EIF4G1 PE=1 SV=1 |
| 14 | 1 | SwissProt_ID | CP4A4_RABIT | 19 | 58893 | 1 | 1 | 1 | 1 | 0.06 | Cytochrome P450 4A4 OS=Oryctolagus cuniculus GN=CYP4A4 PE=1 SV=3 |
| 15 | 1 | SwissProt_ID | UROM_BOVIN | 17 | 0 | 1 | 1 | 1 | 1 | | |
| 16 | 1 | SwissProt_ID | COCH_BOVIN | 15 | 59556 | 1 | 1 | 1 | 1 | 0.06 | Cochlin OS=Bos taurus GN=COCH PE=2 SV=1 |
| 17 | 1 | SwissProt_ID | OXA1L_BOVIN | 14 | 48956 | 1 | 1 | 1 | 1 | 0.07 | Mitochondrial inner membrane protein OXA1L OS=Bos taurus GN=OXA1L PE=2 SV=1 |

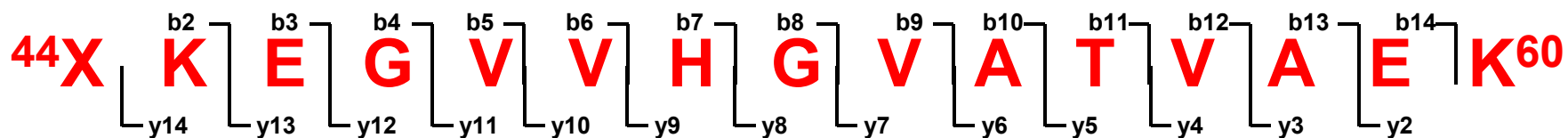
Export as CSV

Post-translational Modifications

| Modification | Modified residue | Mass Shift (Da) |
|---|------------------|-----------------|
| Disulphide bond formation | C | -2.0157 |
| Deamidation of Asn and Gln | Q/N | 0.9840 |
| Methylation | K/R | 14.0157 |
| Hydroxylation | P | 15.9949 |
| Oxidation of Met | M | 15.9949 |
| Acetylation | K | 42.0106 |
| Carboxyamidomethylcysteine (iodoacetamide) | C | 57.0215 |
| Phosphorylation | S/T/Y | 79.9663 |
| Biotinylation (amide bond to lysine) | K | 226.0776 |
| Nitration | Y | 44.9851 |
| Ubiquitination | K | 114.0429 |
| Oxidation of Cys | C | 31.9721/47.9847 |
| nitrosylation | C | 28.9902 |

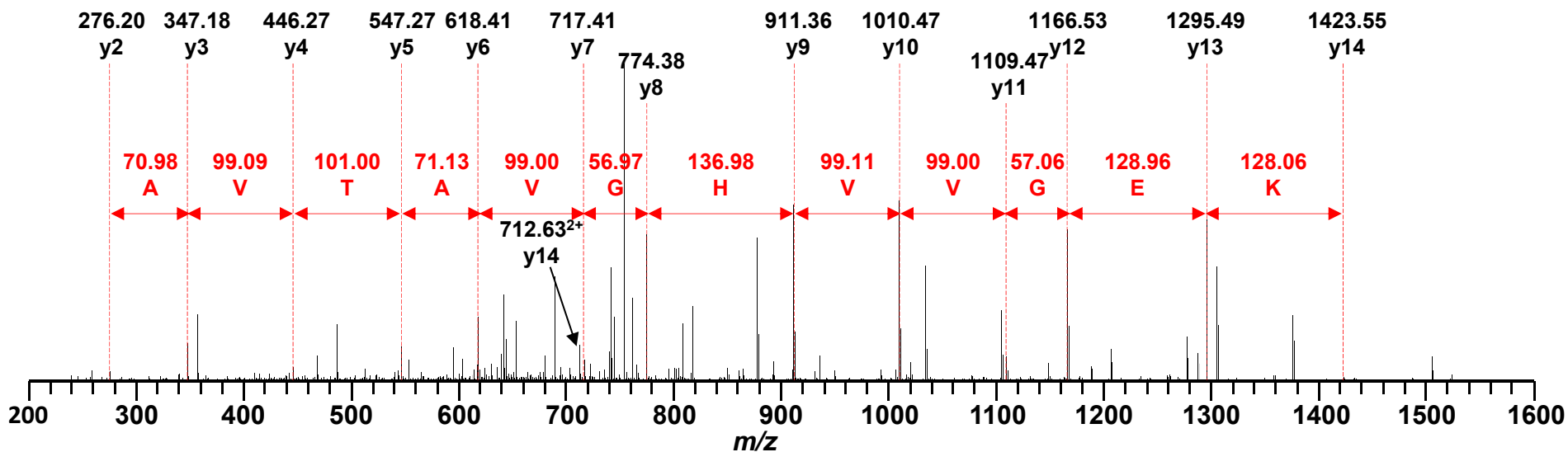
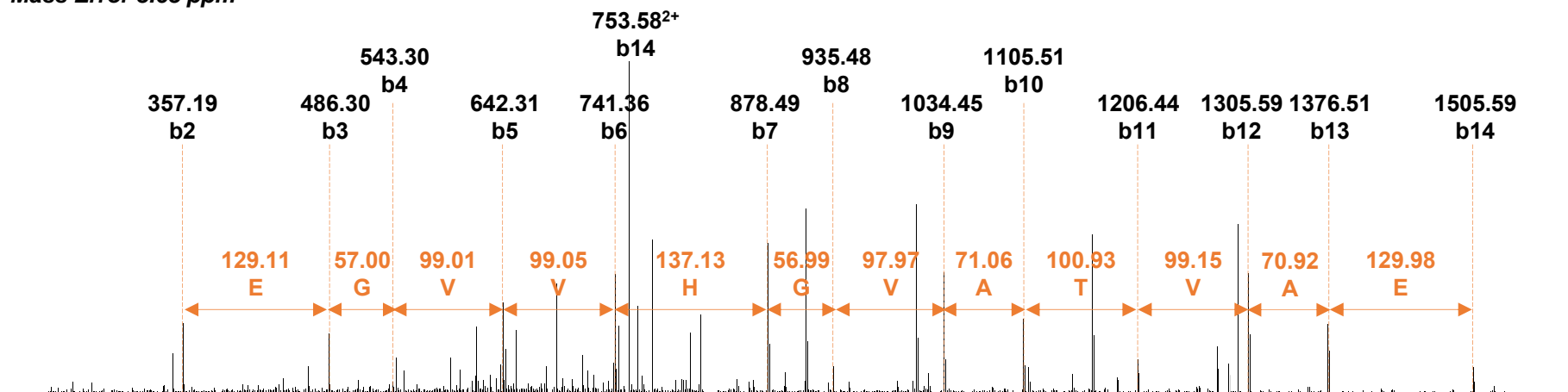
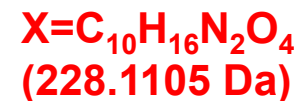
Peptide Modifications

- **Virtually anything that shifts the mass can be determined by MS**
- **Using MS/MS allows for identification of the modification AND location**
- **You must have an idea of what modification you are looking for**
- **First use protein stain to examine various modifications (multiplex analysis)**
- **Western analysis VS MS analysis**

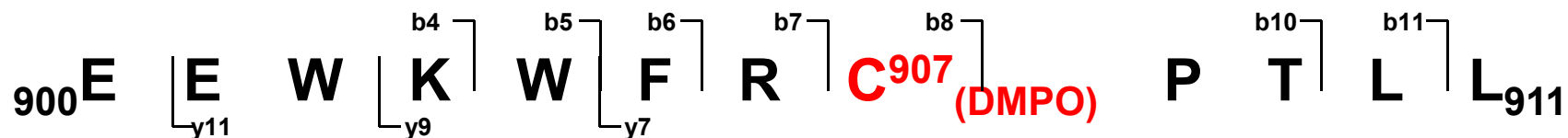


Theoretical m/z 826.4543²⁺
 Observed m/z 826.4573²⁺
 Mass Error 3.63 ppm

Mass of X = (M+H) - y14 = 826.4543 * 2 - 1 - 1423.55 = 228.35

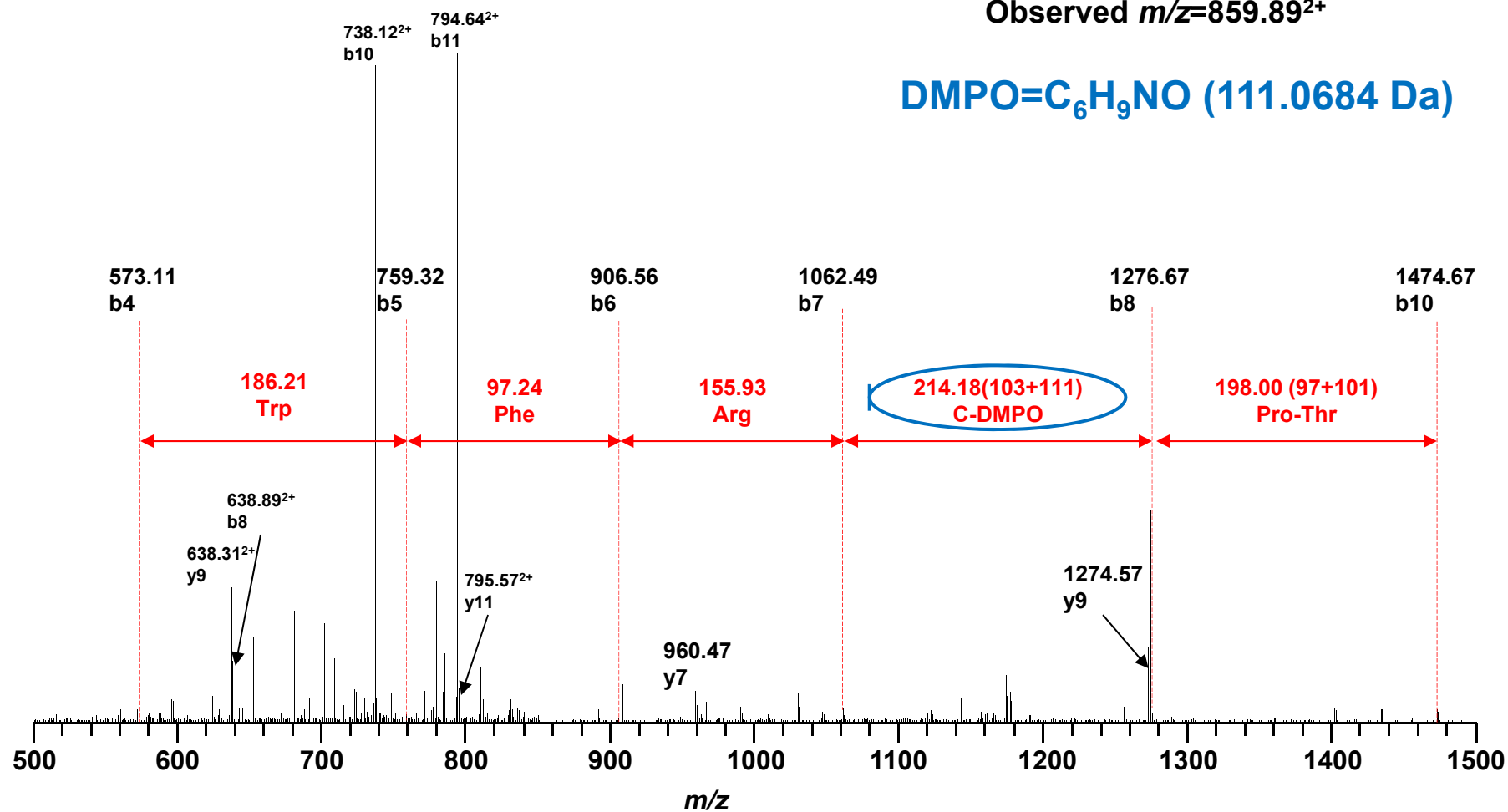


DMPO Adduct

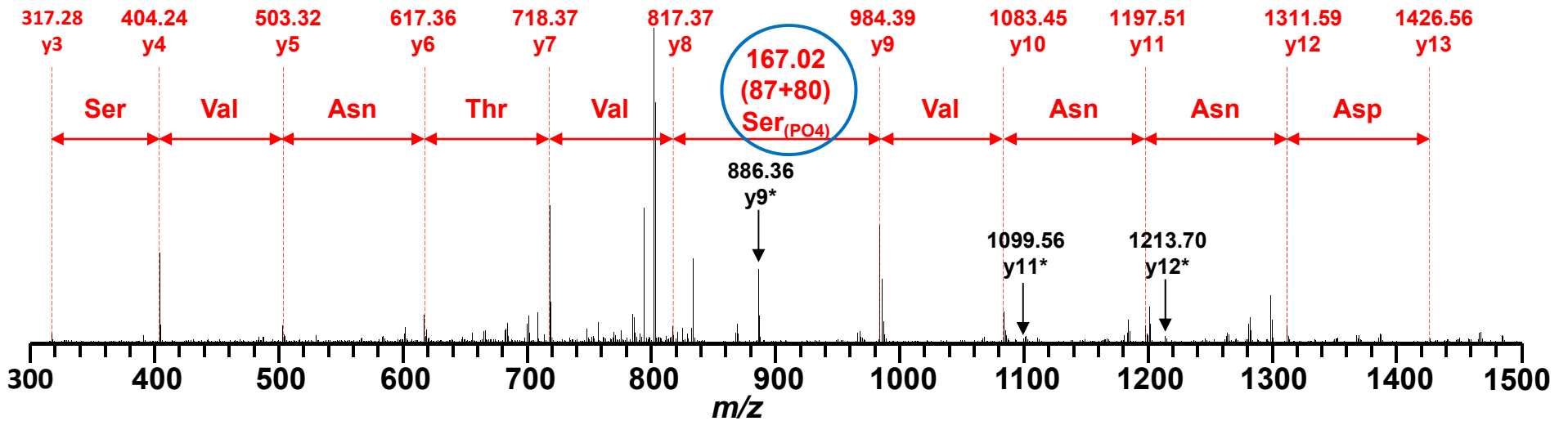
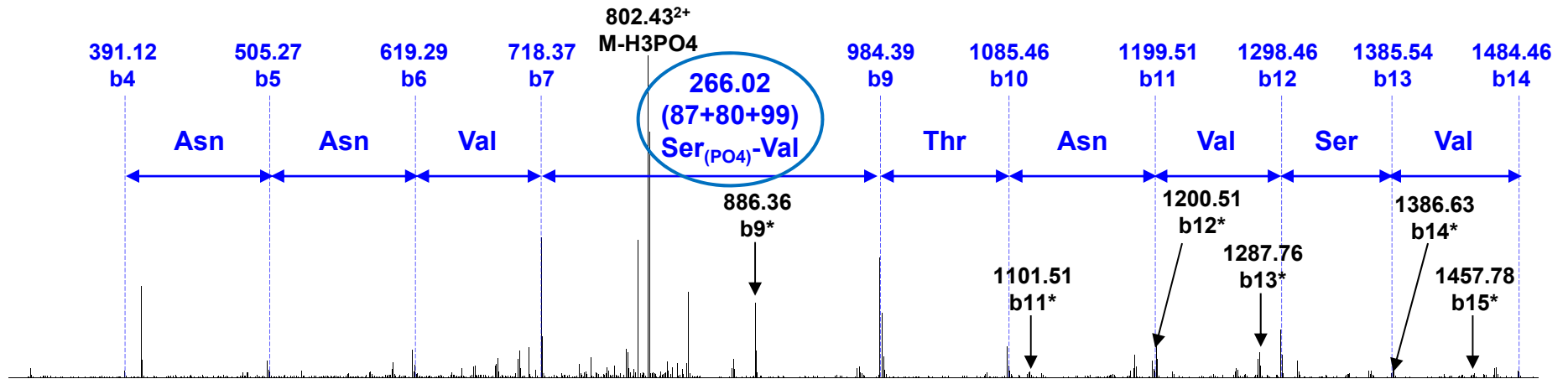
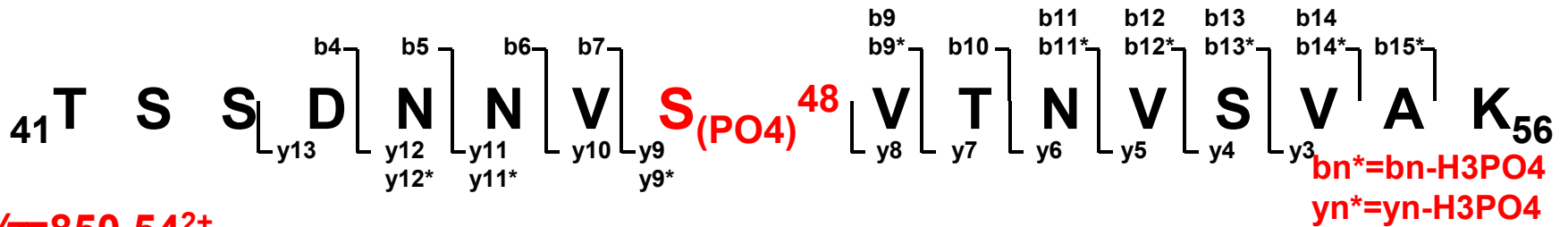


Theoretical $m/z=859.94^{2+}$
Observed $m/z=859.89^{2+}$

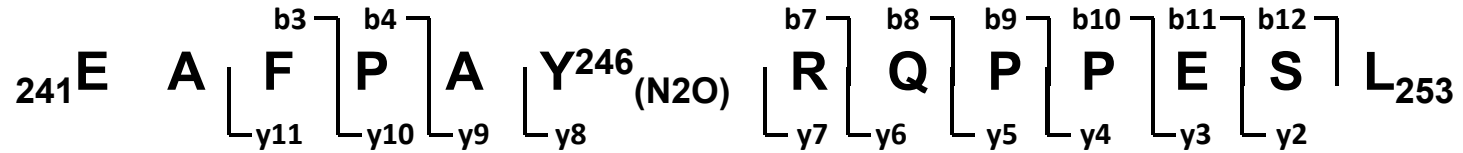
DMPO=C₆H₉NO (111.0684 Da)



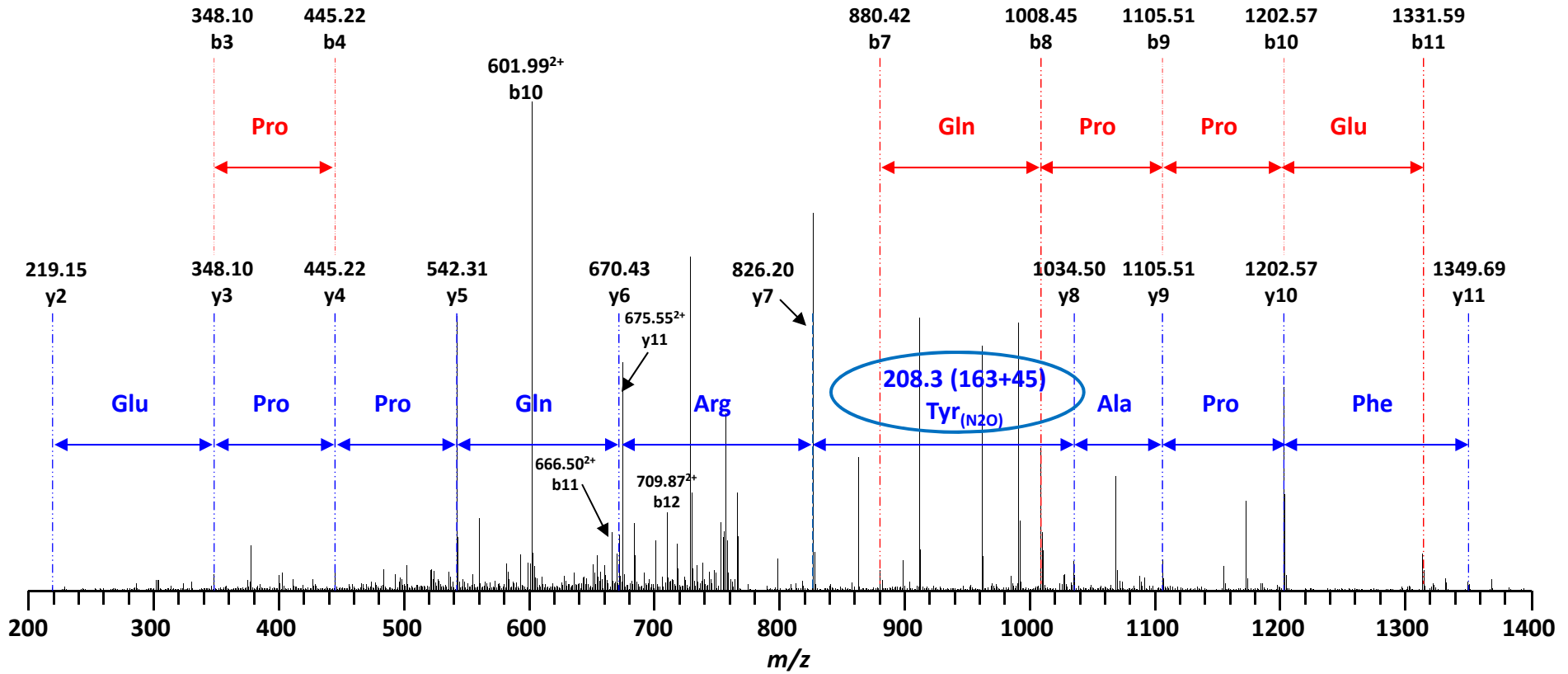
Phosphorylation



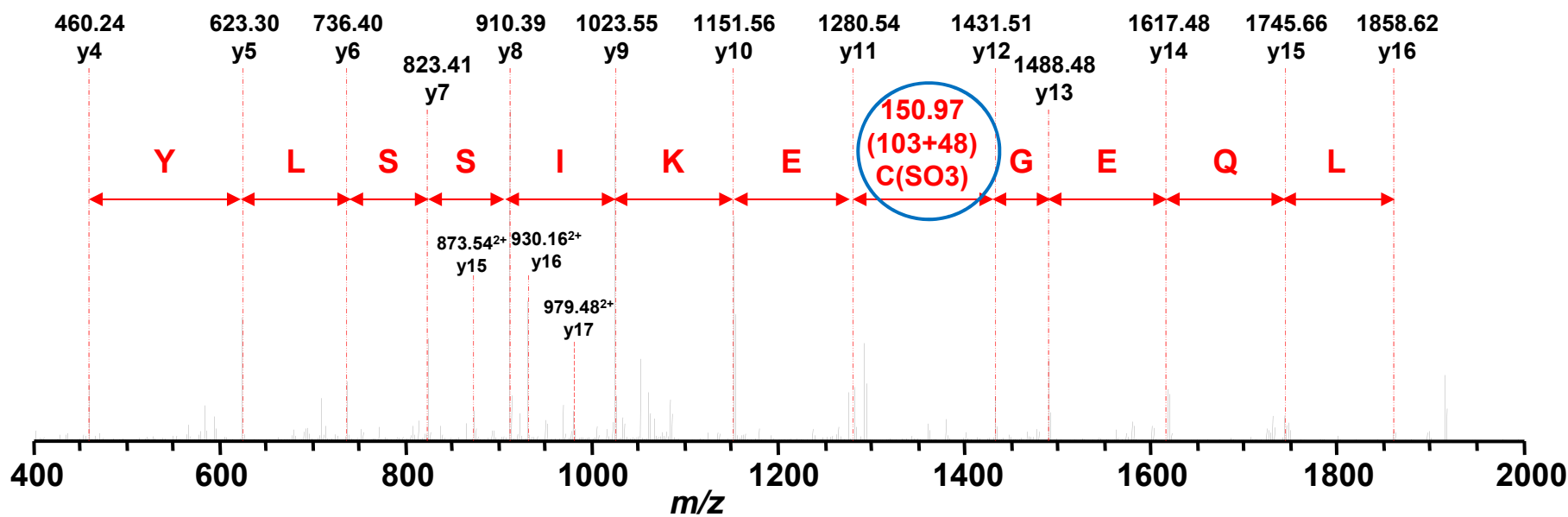
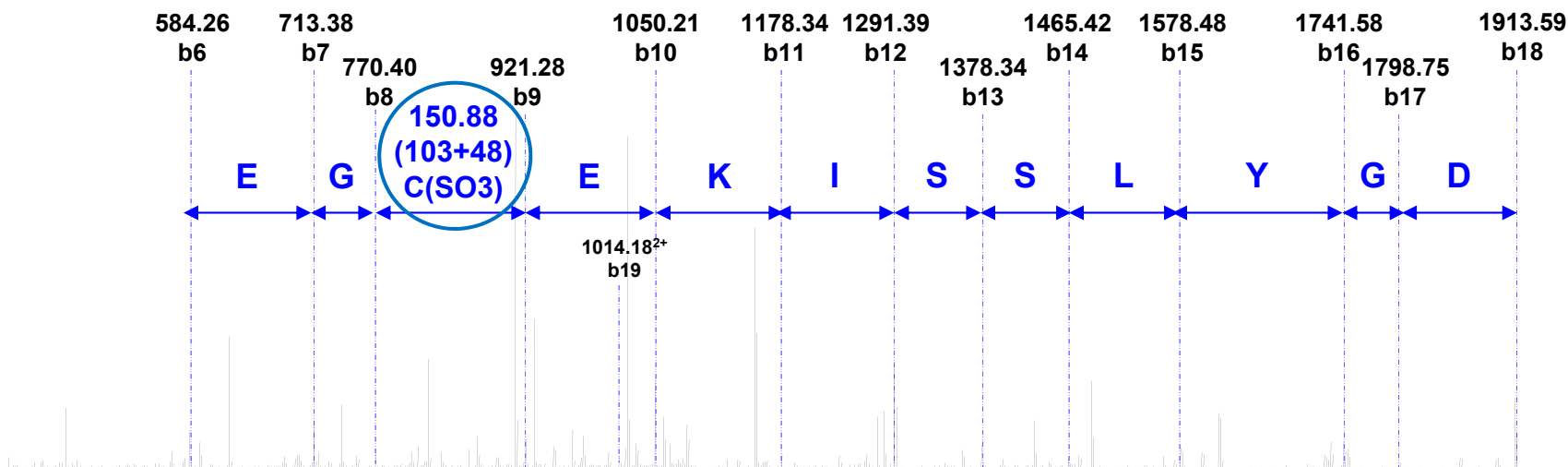
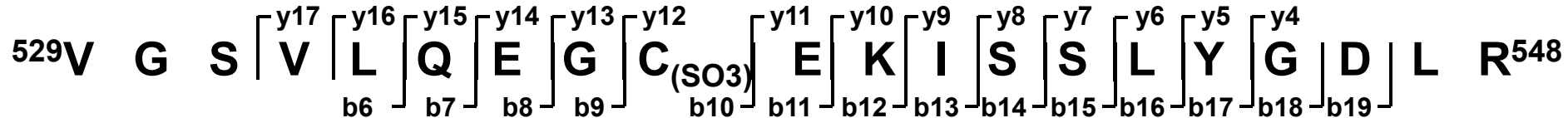
Nitration



Measured $m/z = 775.61^{2+}$
 Theoretical $m/z = 775.37^{2+}$



Cysteine Oxidation



Difficulties for PTMs Identification

- ❖ **Generating Peptides containing modified residue**
 - Different Enzymes
- ❖ **Low population of modifications**
 - Enrichment
 - Targeted Method
- ❖ **Modification not stable**
 - Experiment Condition (reducing reagents, enzymes)
 - Fresh sample
- ❖ **Modification labile in MS analysis**
 - Neutral Loss Method
 - Different Fragmentation Method

Enzymes for Proteome Research

| Trypsin | K-X and R-X |
|--------------------|-----------------------|
| Endoprotease Lys-C | K-X except when X = P |
| Endoprotease Arg-C | R-X except when X = P |
| Endoprotease Asp-N | X-D |
| Endoprotease Glu-C | E-X except when X = P |
| Chymotrypsin | L-X, F-X, Y-X and W-X |
| Cyanogen Bromide | X-M |

Using the CORRECT Enzyme

1 MAALKLLSSG LRLGASARSS RGALHKGVCV YFSVSTRHHT KFYTDPVEAV
51 KDIPNGATLL VGGFGLCGIP ENLIGALLKT GVKDLTAVSN NAGVDNFGLG
101 LLLRSKQIKR MISSYVGENA EFERQFLSGE LEVELTPQGT LAERIRAGGA
151 GVPAFYTSTG YGTLVQEGGS PIKYNKDGSV AIASKPREVR EFNGQHFILE
201 EAITGDFALV KAWKADRAGN VIFRKSARNF NLPMCKAAGT TVVEVEEIVD
251 IGSFAPEDIH IPKIYVHRLI KGEKYEKRIE RLSLRKEGDG KGKSGKPGGD
301 VRERIIKRAA LEFEDGMYAN LGIGIPLLASNFISPNMTVH LQSENGVLGL
351 GPYPLKDEAD ADLINAGKET VTVLPGASFF SSDEFAMIR GGHVNLTMLG
401 AMQVSKYGDL ANWMIPGKMV KGMGGAMD LV SSSKTKVVVT MEHSAKGNAH
451 KIMEKCTLPL TGKQCVNRII TEKGVFDV DK KNGLTLIELW EGLTVDDIKK
501 STGCDFAVSP NLMPMQQIST

Phosphorylation

Trypsin: ₃₀₉AALEFEDGMYANLGIGIPLLASNFISPNMTVHLQSENGVLGLGPYPLK₃₅₆

Chymotrypsin: ₃₂₉ASN₃₃₂

GluC: ₃₁₅DGM₃₁₈YANLGIGIPLLASNFISPNMTVHLQSE₃₄₄

Difficulties for PTMs Identification

- ❖ **Generating Peptides containing modified residue**
 - Different Enzymes
- ❖ **Low population of modifications**
 - Enrichment
 - Targeted Method

PTM Enrichment Techniques (Start with mg of samples)

Acetylation: **Anti-acetyl lysine polyclonal antibody**

Phosphopeptides: **Immobilized Metal Affinity Chromatography (Fe³⁺)
Metal Oxide Affinity Chromatography (TiO₂, ZrO₂)
Reversible Covalent Binding**

[\(Dunn JD¹, Reid GE, Bruening ML](#), Techniques for phosphopeptide enrichment prior to analysis by mass spectrometry. [Mass Spectrom Rev.](#) 2010 Jan-Feb;29(1):29-54.)

Phospho-Threonine Antibody (P-Thr-Polyclonal)

Nitration: **Anti-Nitrotyrosine polyclonal antibody**

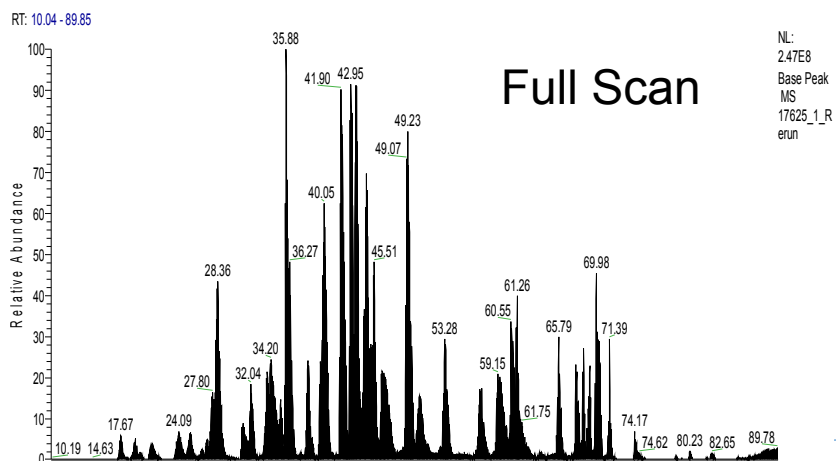
Ubiquitination: **K-ε-GG-specific antibody (enrichment has to be done prior to other treatment on lysine)**

Glycopeptides: **Lectin affinity enrichment
Covalent Interactions
Chromatographic separation**

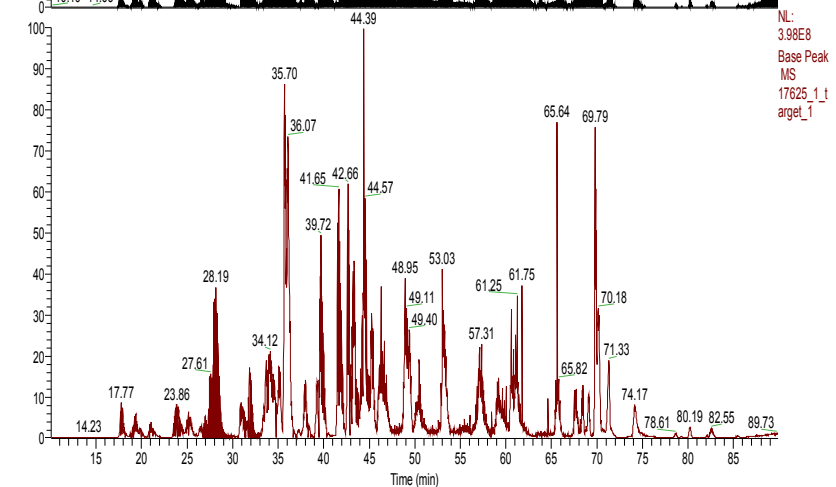
[Ongay S¹, Boichenko A, Govorukhina N, Bischoff R.](#), Glycopeptide enrichment and separation for protein glycosylation analysis., [J Sep Sci.](#) 2012 Sep;35(18):2341-72.

Methylation: **Anti-methyl lysine/arginine antibody**

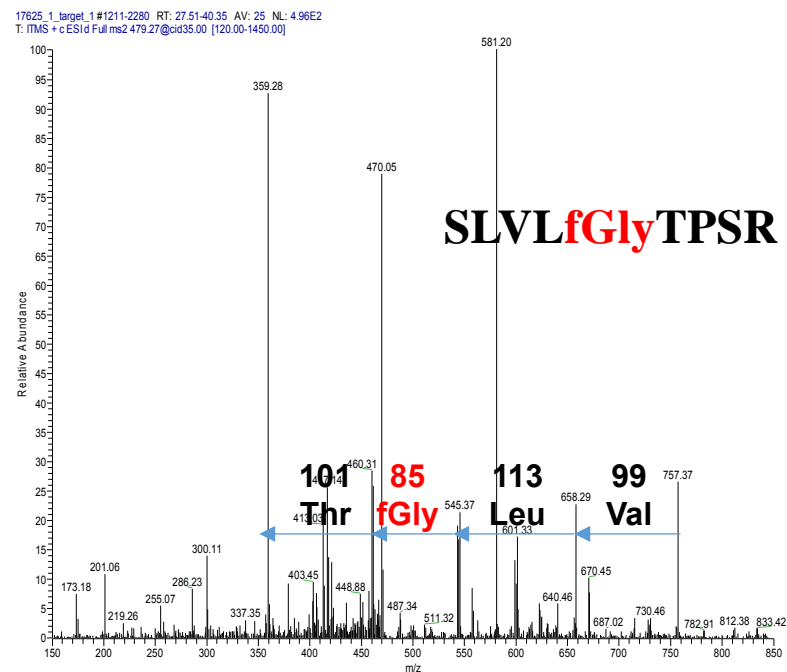
Targeted MS Scan (for Known Modifications)



No MSMS spectrum for 479.2930²⁺



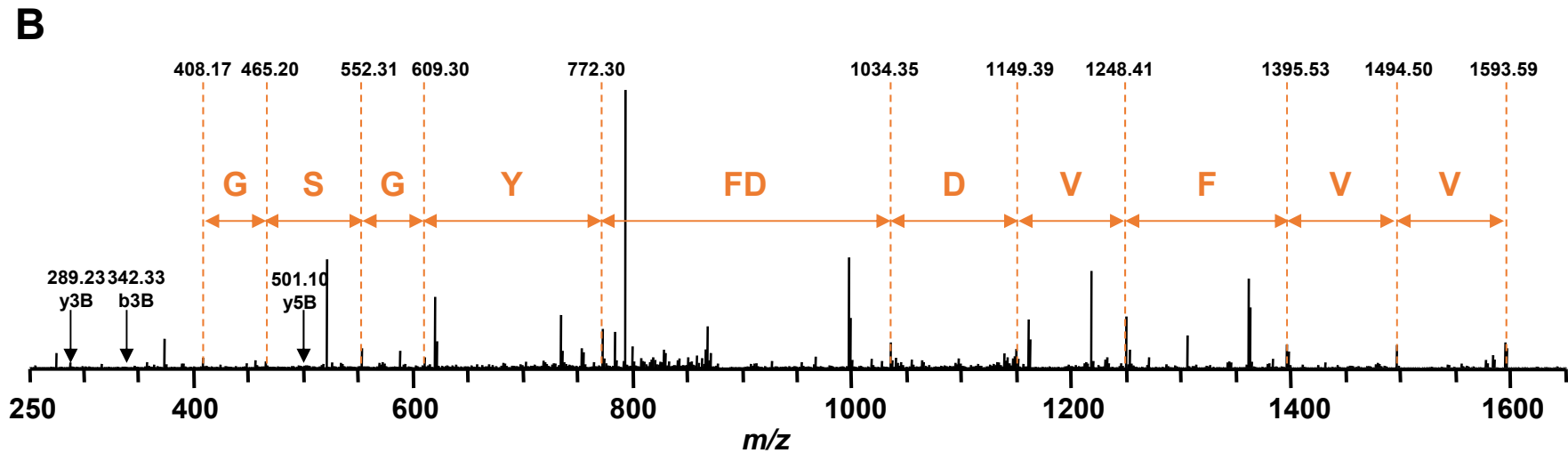
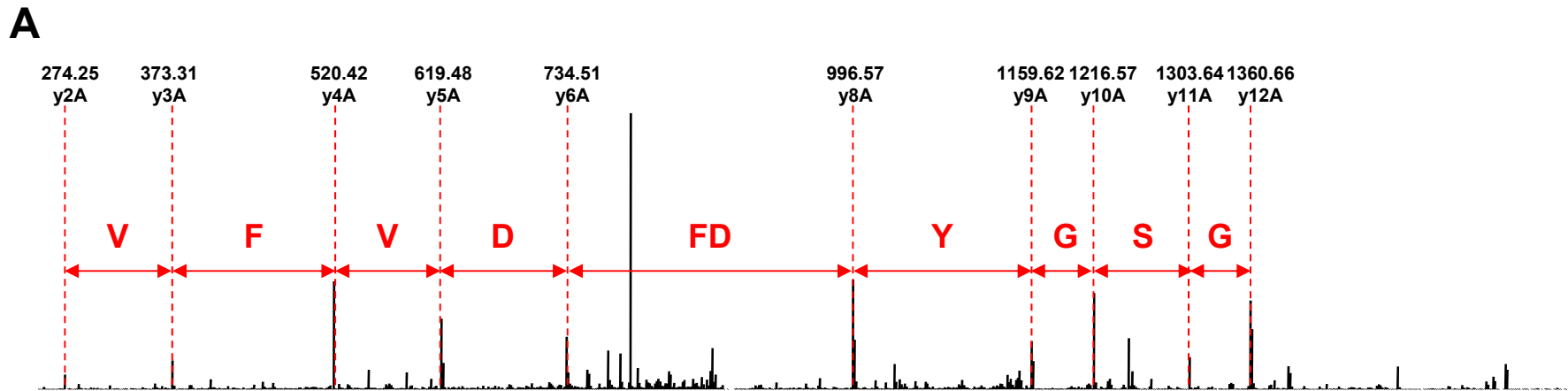
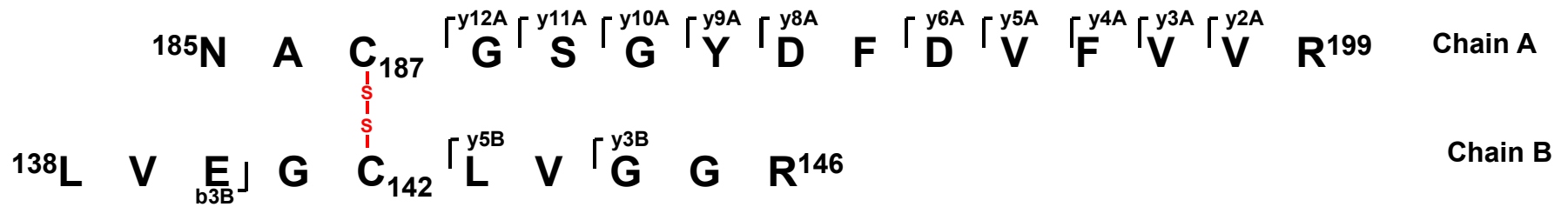
Scan for 479.2730²⁺ (SLVLF^fGlyTPSR)
and 516.7793²⁺ (SLVLC_(CAM)TPSR)



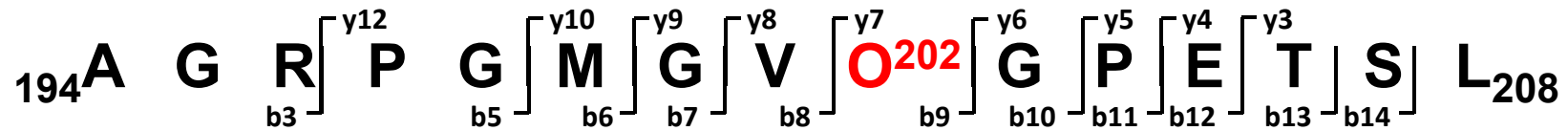
Difficulties for PTMs Identification

- ❖ **Generating Peptides containing modified residue**
 - Different Enzymes
- ❖ **Low population of modifications**
 - Enrichment
 - Targeted Method
- ❖ **Modification not stable**
 - Experiment Condition (reducing reagents, enzymes)
 - Fresh sample

Adjust Experiment Condition to Preserve Modifications

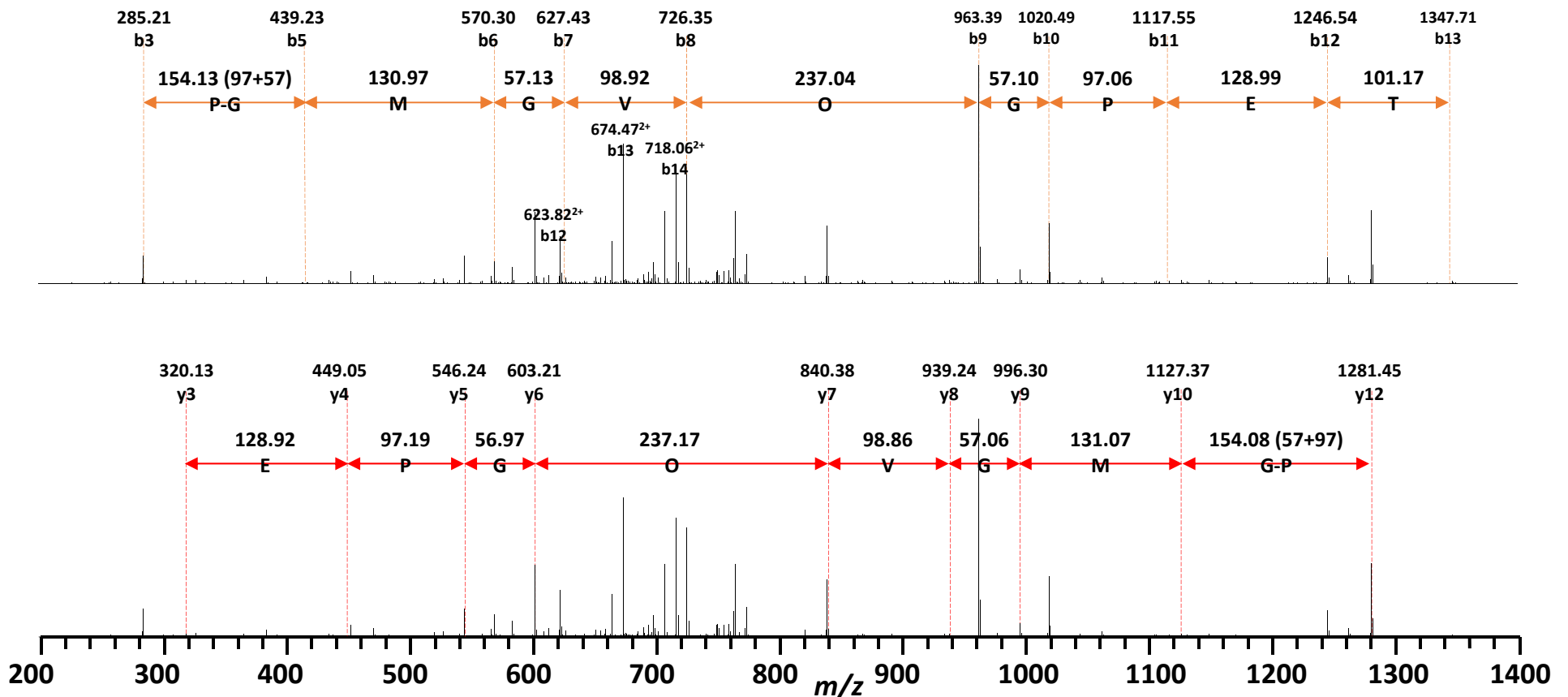
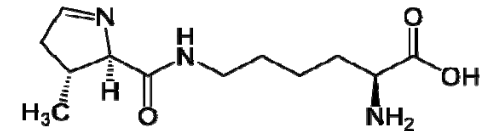


Using Different Enzyme to Protect Modifications



Theoretical $m/z = 783.4089^{2+}$, Observed $m/z = 783.4080^{2+}$, Mass Error = 1.15ppm

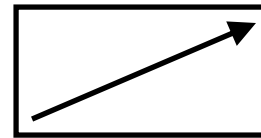
O=Pyrrolysine



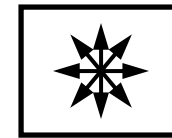
Difficulties for PTMs Identification

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 - Different Enzymes
- ❖ **Low population of modifications**
 - Enrichment
 - Targeted Method
- ❖ **Modification not stable**
 - Experiment Condition (reducing reagents, enzymes)
 - Fresh sample
- ❖ **Modification labile in MS analysis**
 - Neutral Loss Method
 - Different Fragmentation Method

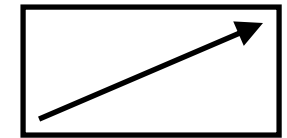
Neutral Loss Scan



Scan

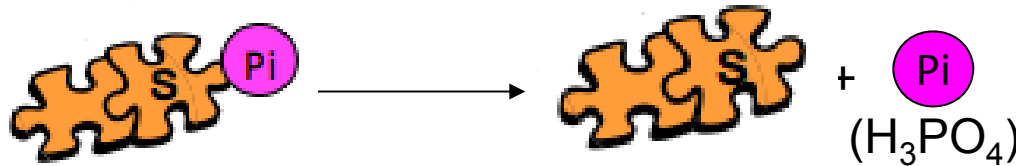


Dissociate

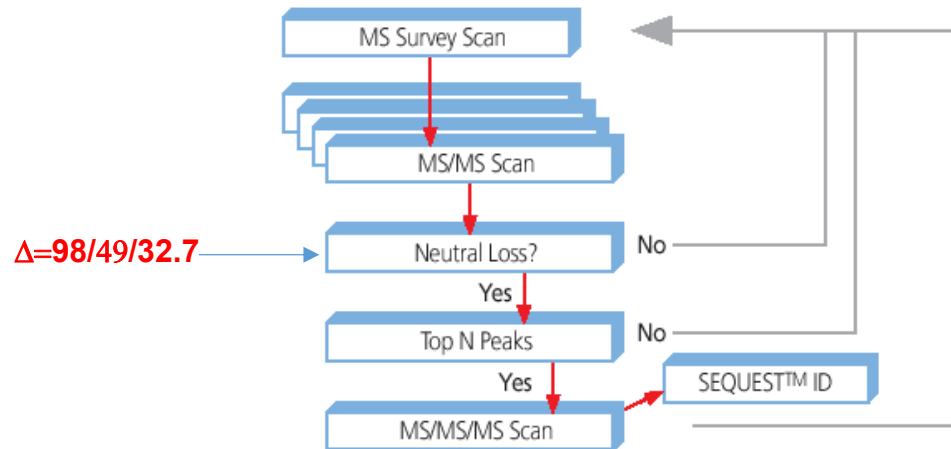


Scan

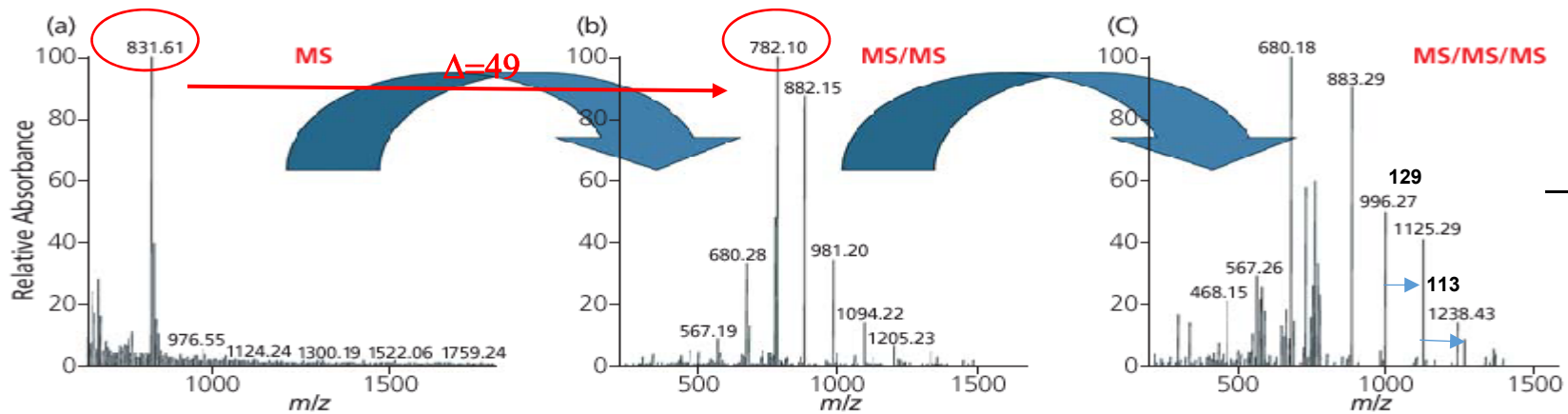
Phosphopeptides can lose phosphoric acid on CID (**Serine, Threonine**)



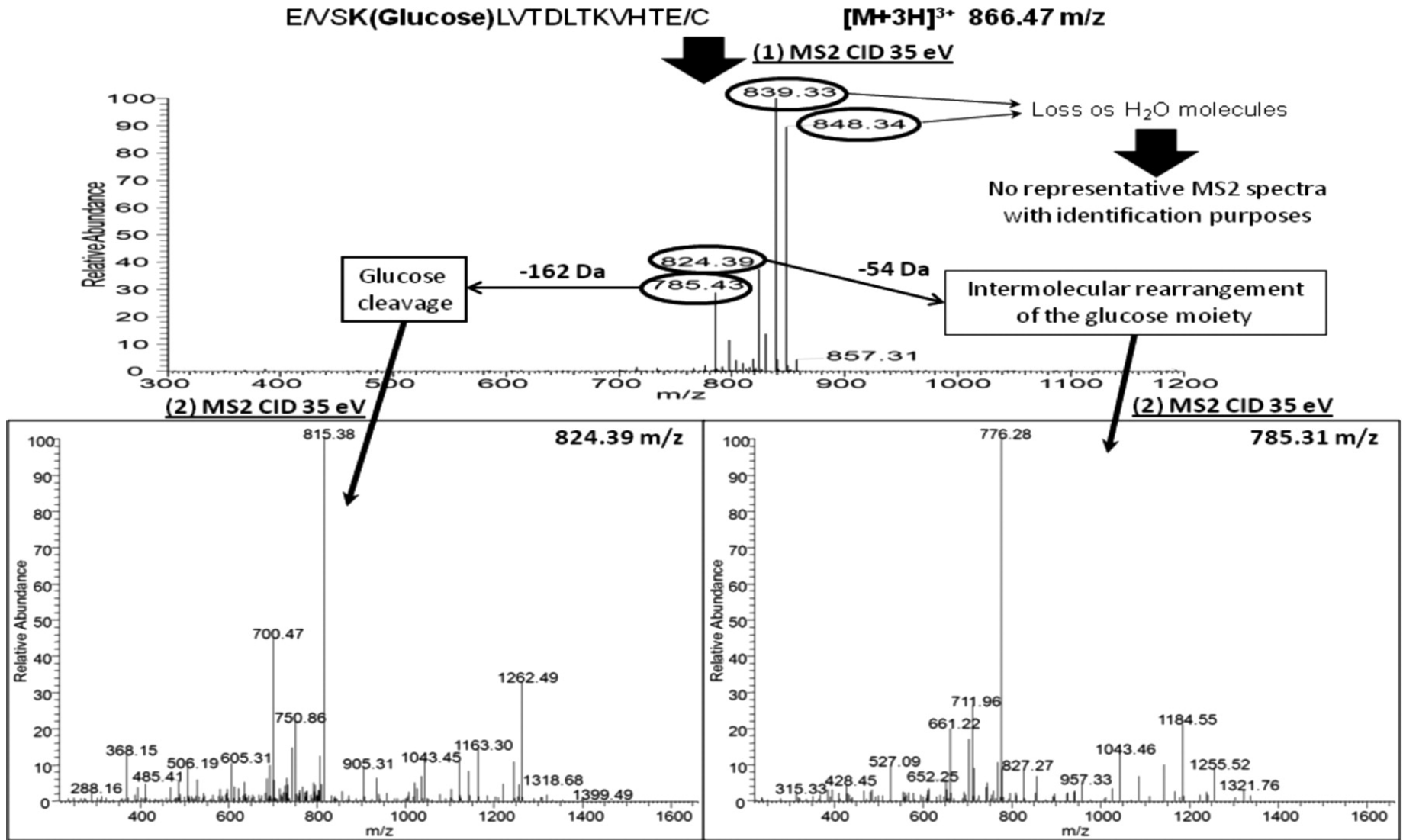
98 amu Neutral (M+H)⁺
49 amu Neutral (M+2H)²⁺



LDIFpSDFGGLK



Neutral Loss Scan



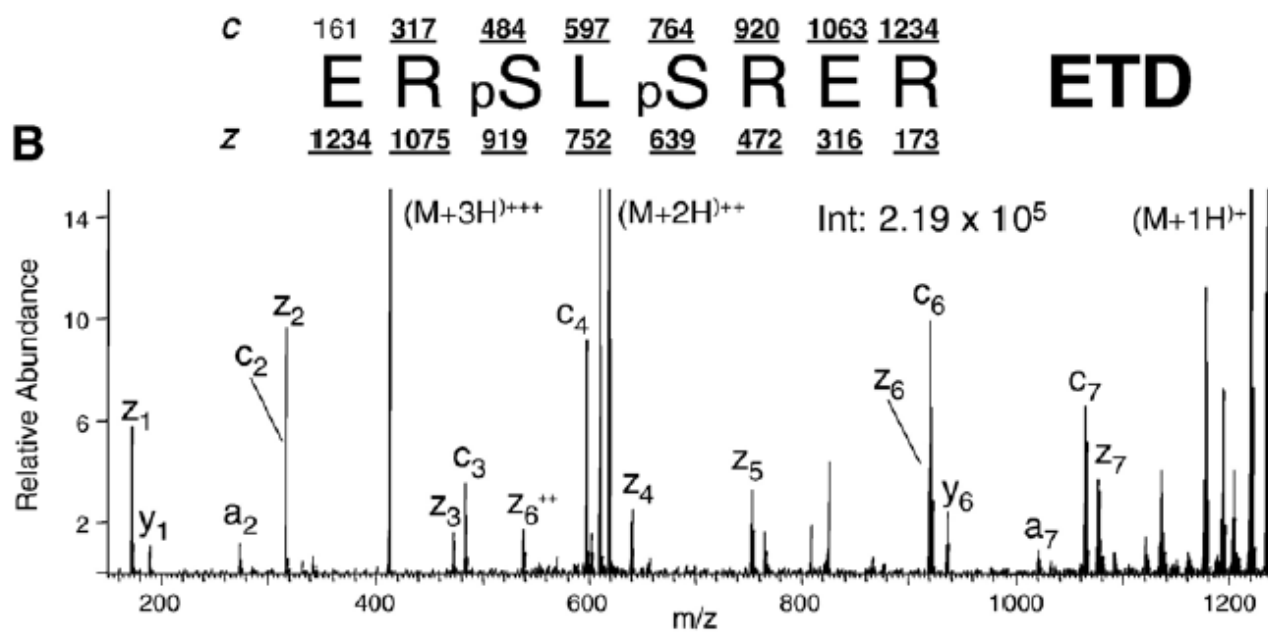
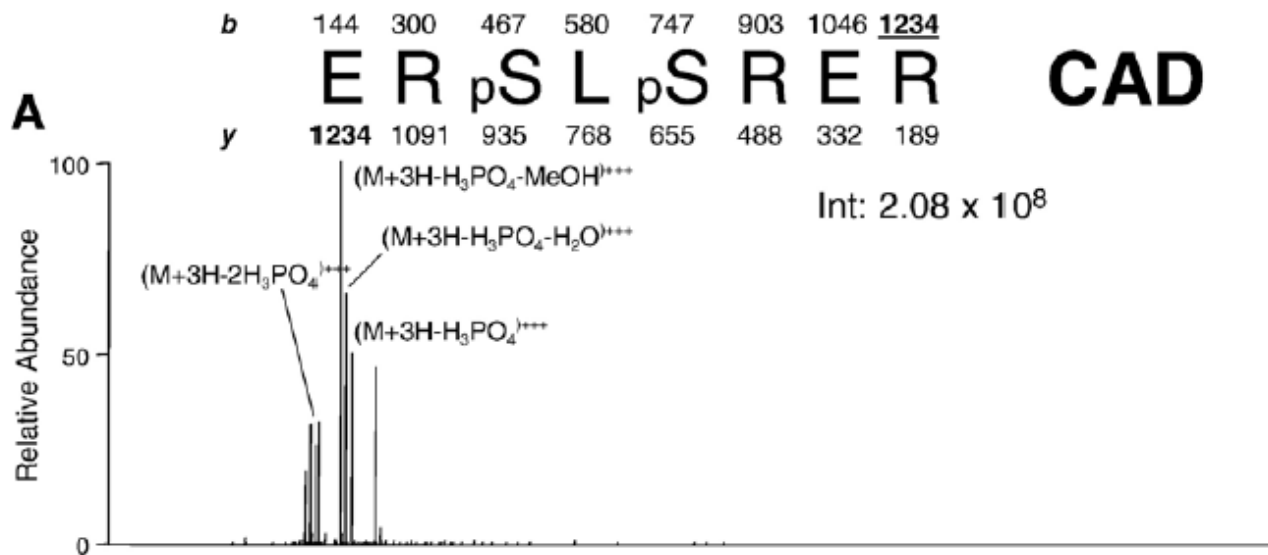
ECD/ETD: Keep the Phosphorylation Intact

PO_3 or H_3PO_4 groups may leave upon CID activation

actual amino acid location of PO_3 group may not be clear

Electron Capture Dissociation (ECD) requires an ICR;
Electron Transfer Dissociation (ETD) in traps

- Peptide fragments without loss of phospho group



Identification of PTMs:

High Mass Accuracy

High Samples Purity

High Concentration

Know the Modification

(Stability, Mass Shift, possible modification site)

Protein Complex Identification

(Whole cell lysate, IP Products)

Clean Sample!!!

(Less Salt, No Detergent
Lipids, DNA Free, Keratin Free)

Better Separation

More sensitive Instruments

On Bead Digestion

Pre-Fractionation

Types of Separation Technologies for Molecules

| Method | Separation based on | Separation done using | Further steps |
|---|--|---|---|
| Gel Electrophoresis (1D) | Molecular mass | Gel (which acts like a molecular sieve) and potential | In-gel digestion of proteins to peptides → LC-MS/MS |
| Gel Electrophoresis (2D) | Isoelectric point (pI; IEF) & Molecular mass | Gel, potential and ampholytes | |
| Reverse Phase (C8 or C4) chromatography | Combination of hydrophobicity and molecular weight | HPLC | Protein(s) → Digest to peptides → LC-MS/MS |
| Gel Filtration | Molecular Weight | HPLC | |
| Ion Exchange | Cation or Anion affinity | FPLC | |
| Affinity Chromatography | DNA, RNA, Anti-body, peptides etc | HPLC | |

Pre-Fractionation Method

LC/MSMS

LC/LC/MSMS

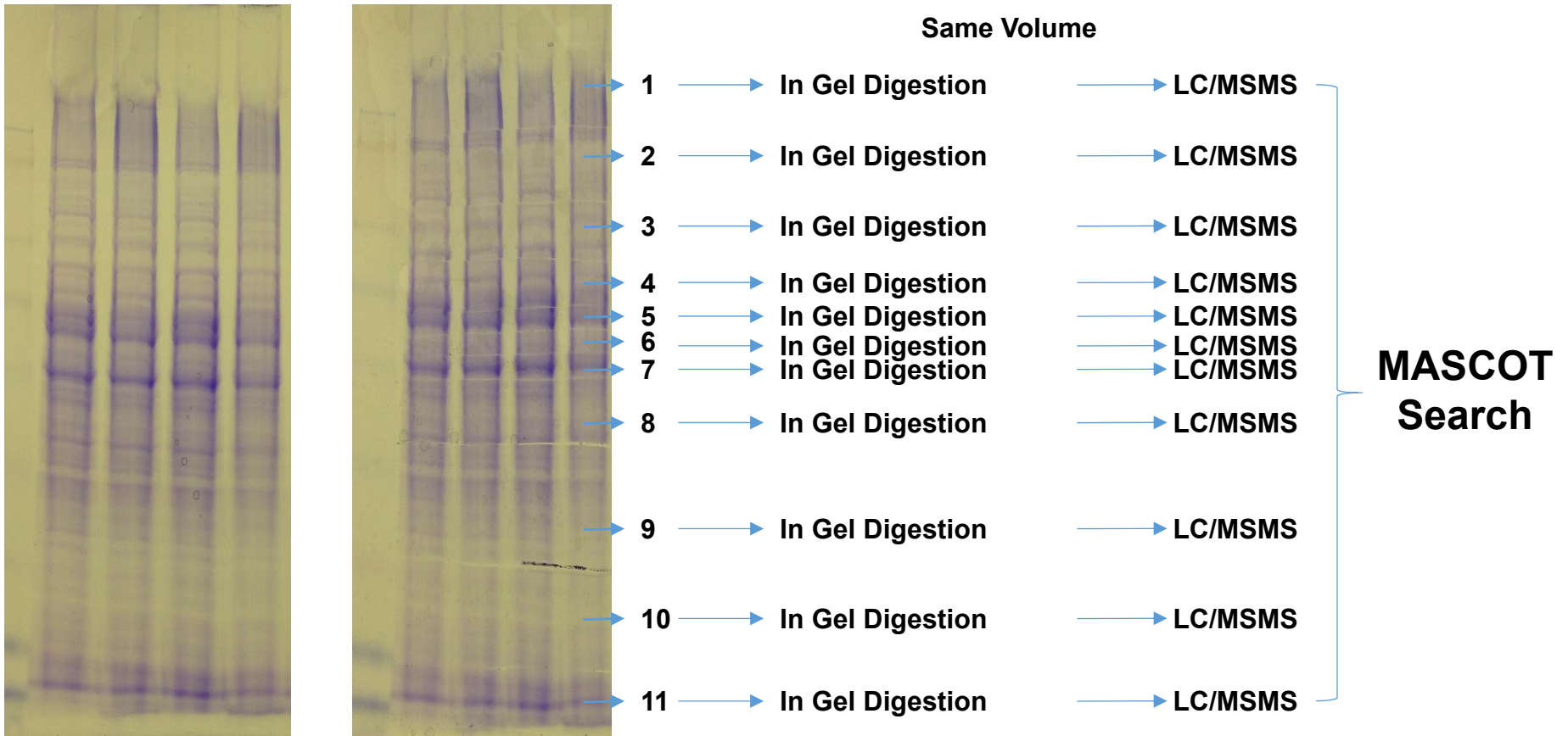
High Abundant Protein Depletion

1D SDS Fractionation

Off-gel Fractionation

SCX Fractionation

1D SDS PAGE Fractionation LC/MSMS Workflow



>1800 Proteins identified

Global Digestion: 920 Proteins
SCX-LC/MSMS: 981 Proteins
1D SDS-LC/MSMS: >1800 Proteins

Quantitative Proteomics

.Quantitation in proteomics has become a popular area in recent proteomics research with the development of quantitation techniques such as DIGE, SILAC, ICAT, iTRAQ and Label Free.

- Difference Gel Electrophoresis**

- Gel based using cy-dye chemistry.

- Isobaric tag for relative and absolute quantitation – iTRAQ**

- is a non-gel based technique used to identify and quantify proteins/peptides from different sources in one single experiment by using isotope coded covalent tags that will label the N-terminus and side chain amines of peptides from protein digestions.

- Stable isotope labeling by amino acids in cell culture – SILAC**

- is a non-gel based approach for in vivo incorporation of a label into proteins for MS quantitative proteomics. It relies on metabolic incorporation of a given 'light' or 'heavy' form of the amino acid into the proteins.

- Label Free Quantitation**

- It has been observed the chromatographic peak areas and number of spectra/peptides observed for a protein in a LC/MS/MS run is correlated with the concentration of that particular protein.

DIGE (Difference Gel Electrophoresis)

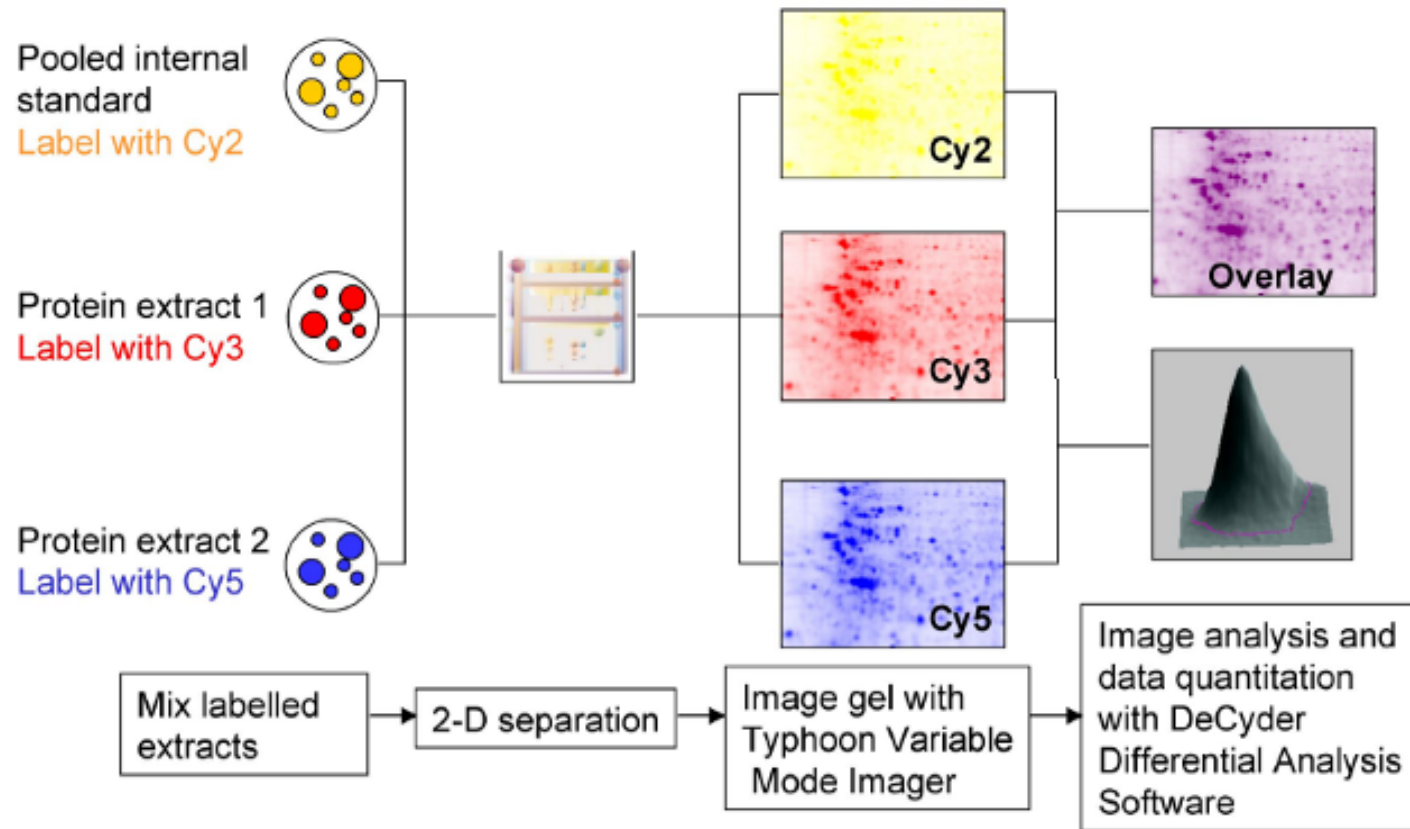
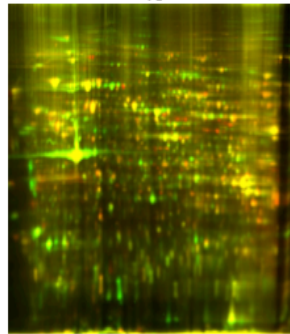
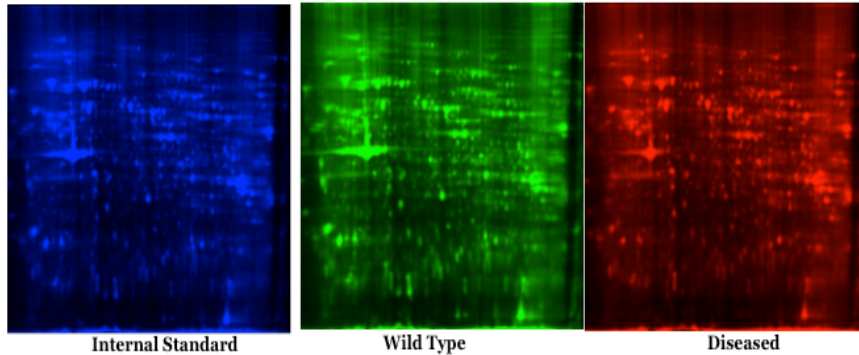


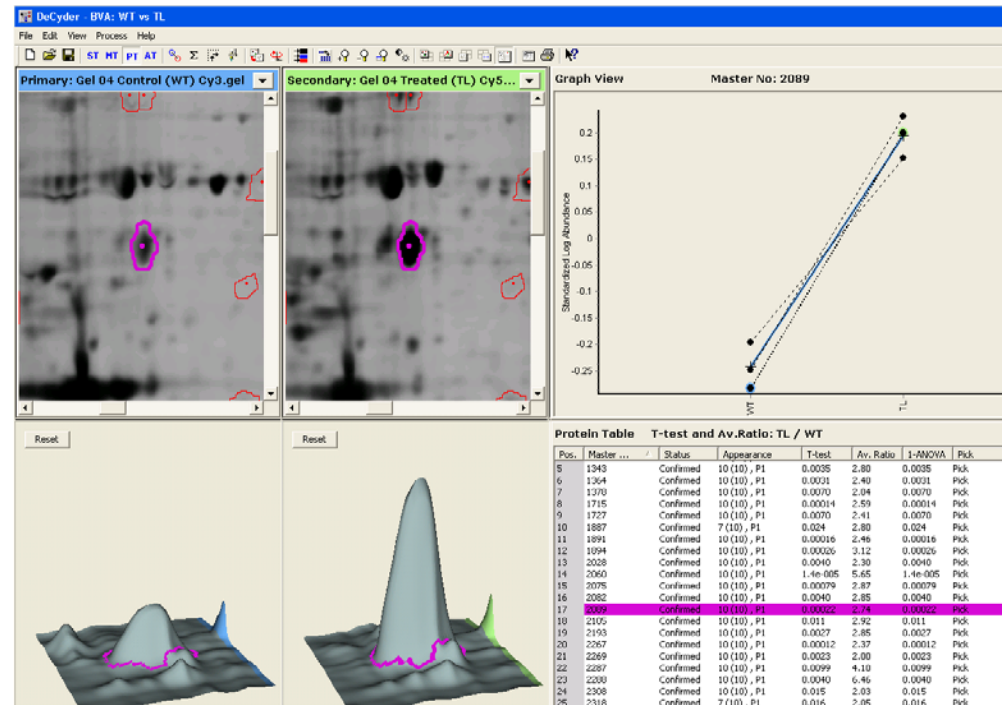
Fig 1-1. Outline of Ettan DIGE system (when used with three CyDye DIGE Fluor minimal dyes separated in a single gel)

DIGE (Difference Gel Electrophoresis)

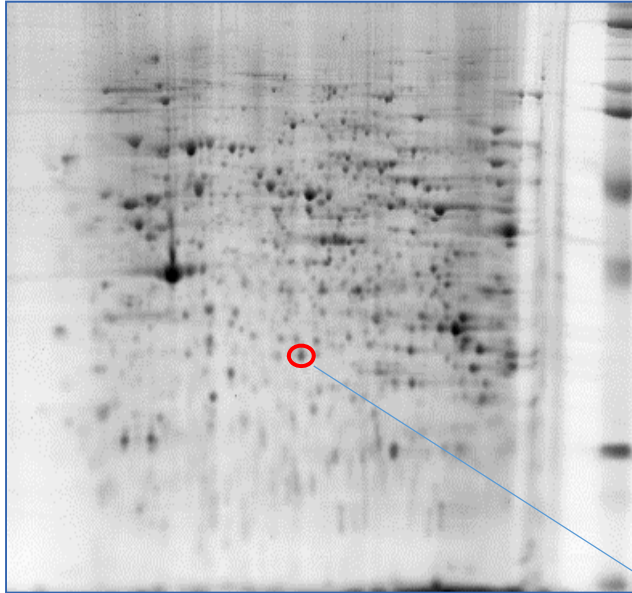


Overlaid

$$\text{Spot Volume} = [\text{spot 1 on treated}]/[\text{spot 1 on standard}]$$



DIGE (Difference Gel Electrophoresis)



In Gel Digestion, LC/MSMS, MASCOT

MATRIX Mascot Search Results

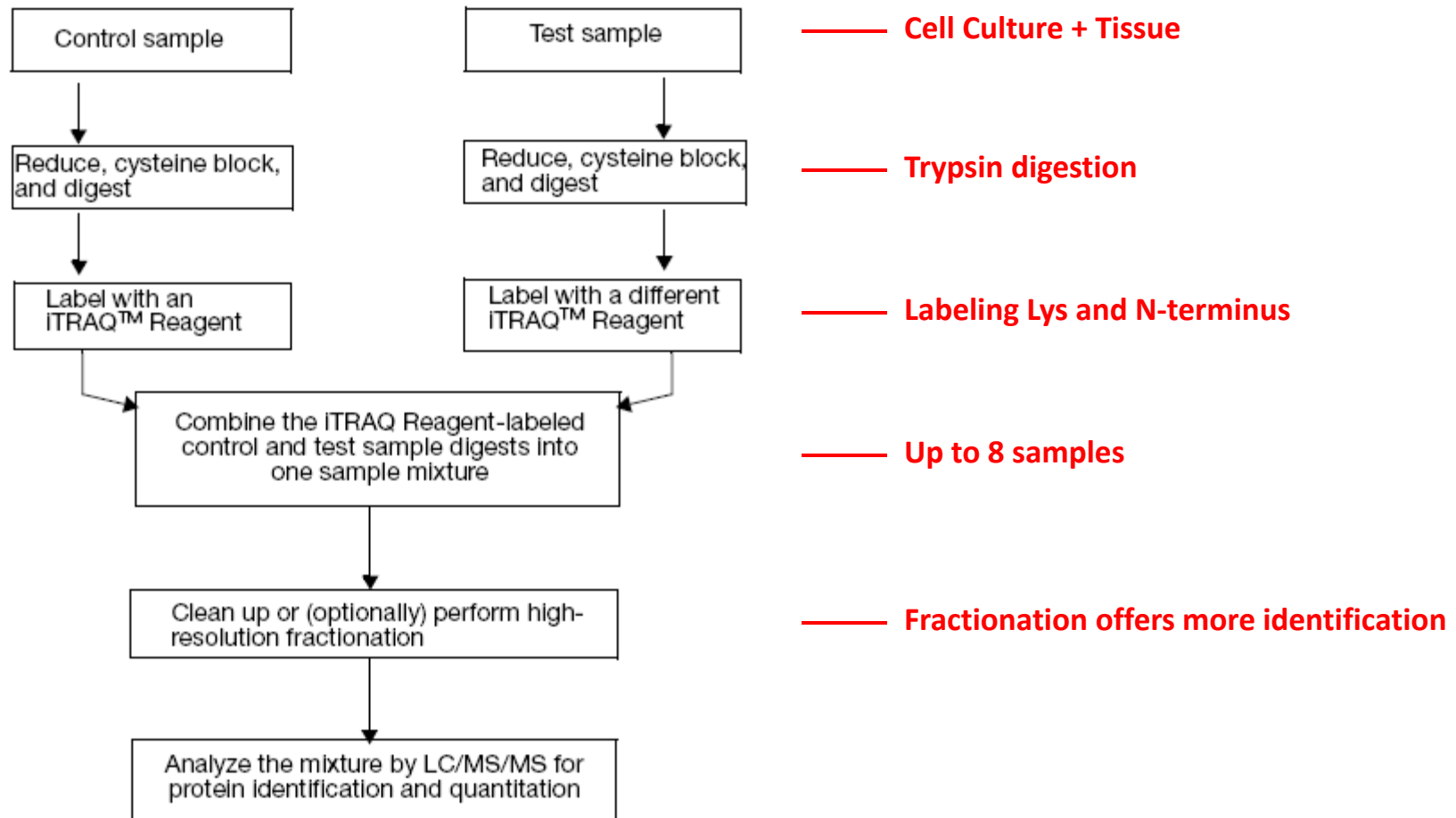
User : Kari
Email : kleinholz.1@osu.edu
Search title : october
MS data file : C:\Documents and Settings\All Users\Desktop\mgfs to process\14510_6_xml.mgf
Database : SwissProt 56.8 (410518 sequences; 148080998 residues)
Timestamp : 16 Mar 2009 at 10:31:12 GMT
Protein hits : [OC17_CHICK](#) Ovocleidin-17 OS=Gallus gallus PE=1 SV=2
[TRYP_PIG](#) Trypsin OS=Sus scrofa PE=1 SV=1
[LYSC_CHICK](#) Lysozyme C OS=Gallus gallus GN=LYZ PE=1 SV=1
[TRY1_CANFA](#) Cationic trypsin OS=Canis familiaris PE=2 SV=1
[TRY3_HUMAN](#) Trypsin-3 OS=Homo sapiens GN=PRSS3 PE=1 SV=2
[GLMM_PELUB](#) Phosphoglucosamine mutase OS=Pelagibacter ubique GN=glmM PE=3 SV=1
[OVAL_CHICK](#) Ovalbumin OS=Gallus gallus GN=SERPINB14 PE=1 SV=2
[YCF1A_ANTFO](#) Putative membrane protein ycf1 N-terminal part OS=Anthoceros formosae GN=ycf1 PE=2 SV=1
[Y9333_DICDI](#) Probable inactive serine/threonine-protein kinase DDB_G0280855 OS=Dictyostelium discoideum GN=DDB_G0280855 PE=3 SV=1

Pros and Cons to DIGE

- **high sensitivity**
- **linearity of the dyes utilized**
- **straightforward significant reduction of experiment error**
- **High reproducibility**
- **300 – 500 μg of total protein is required for each biological replicate**
- **Requires high resolution 2D gels**
- **Not ideal for membrane proteins**
- **Not ideal for serum type samples**
- **Some protein spots identify more than one protein or do not have enough protein to identify the spot**
- **Labor Intensive**

Overview of iTRAQ™ Reagents Methodology

Isobaric tag for relative and absolute quantitation



Chemistry of TMT™ Reagents

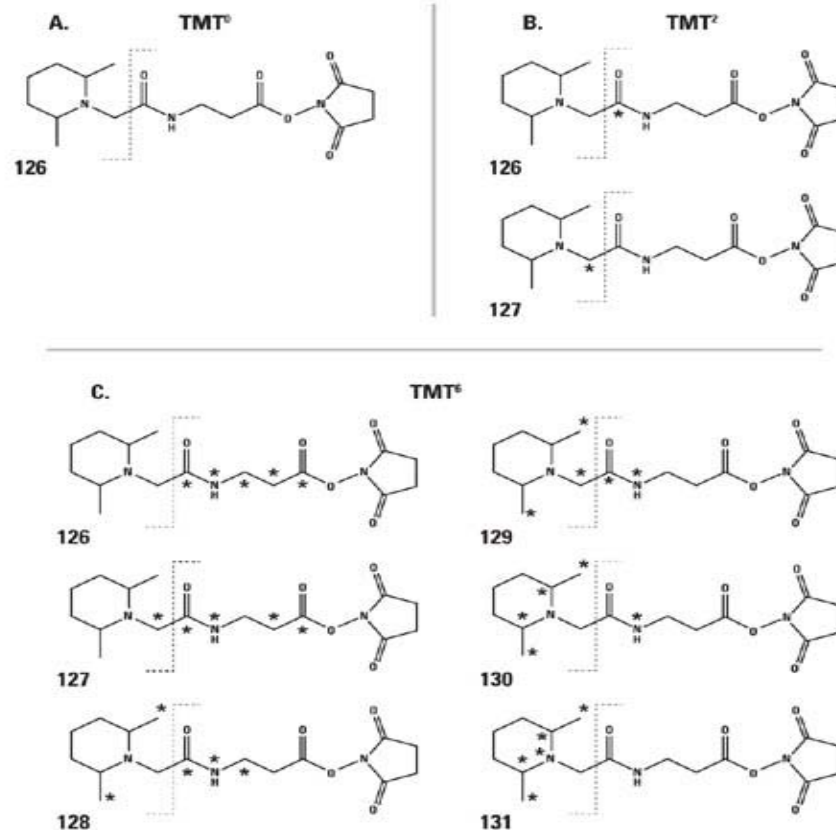
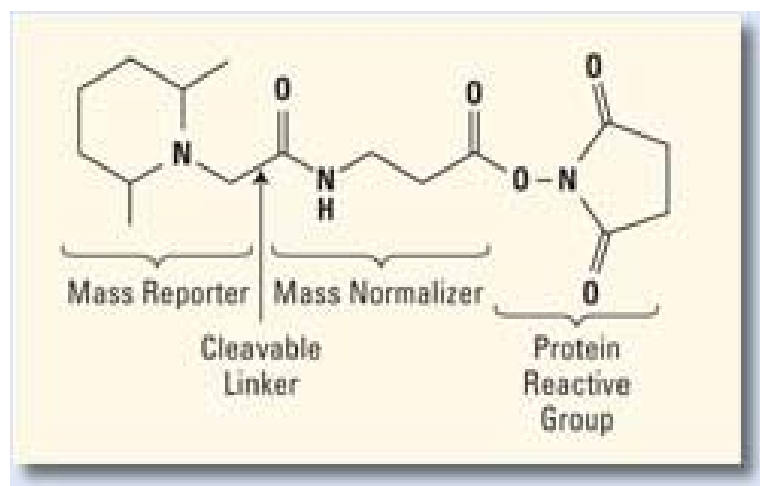


Figure Provided Courtesy of Thermo Fisher Scientifics

Workflow of TMT™ Reagents

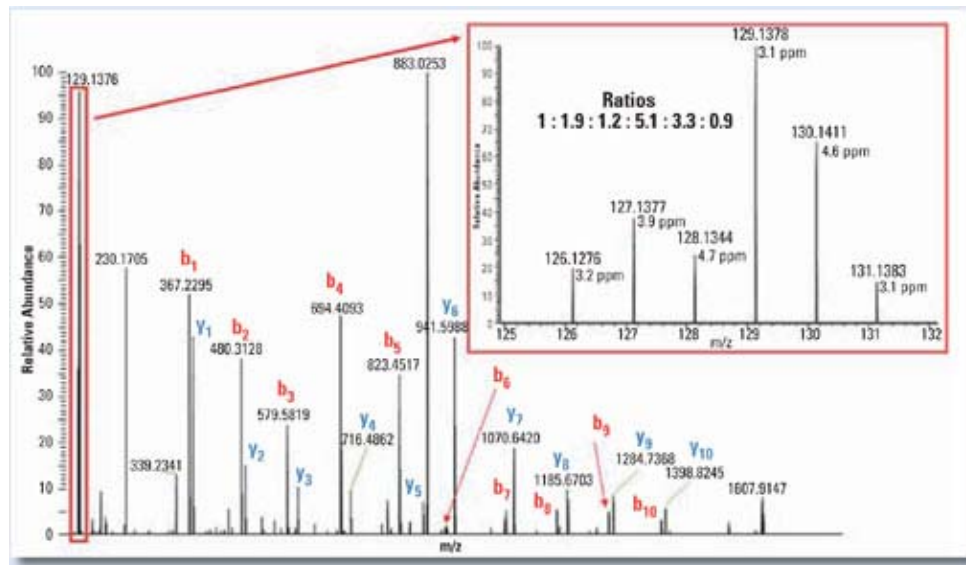
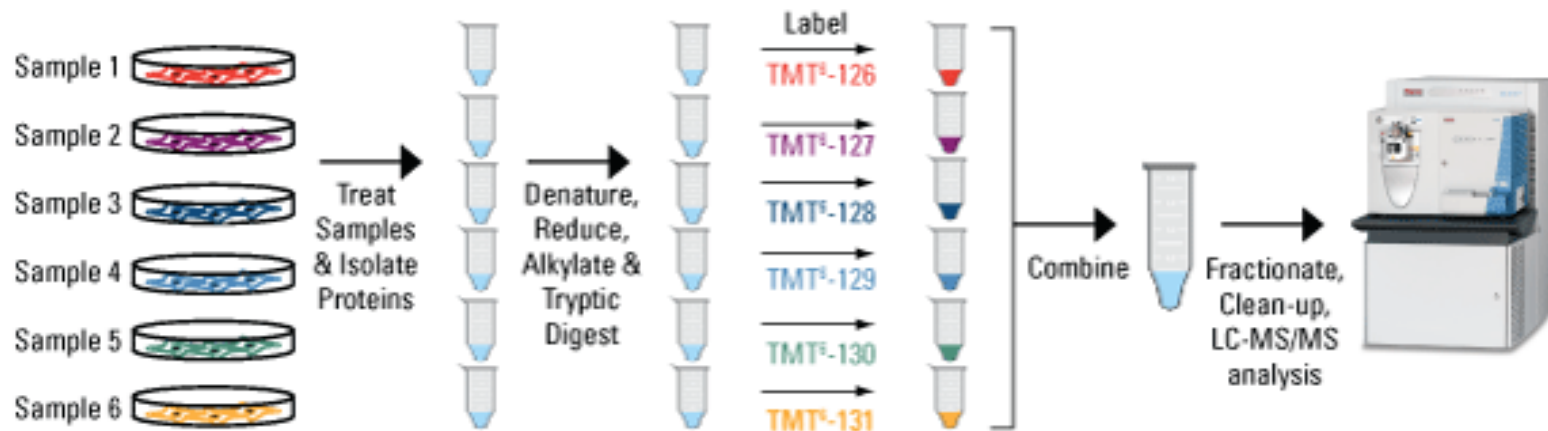
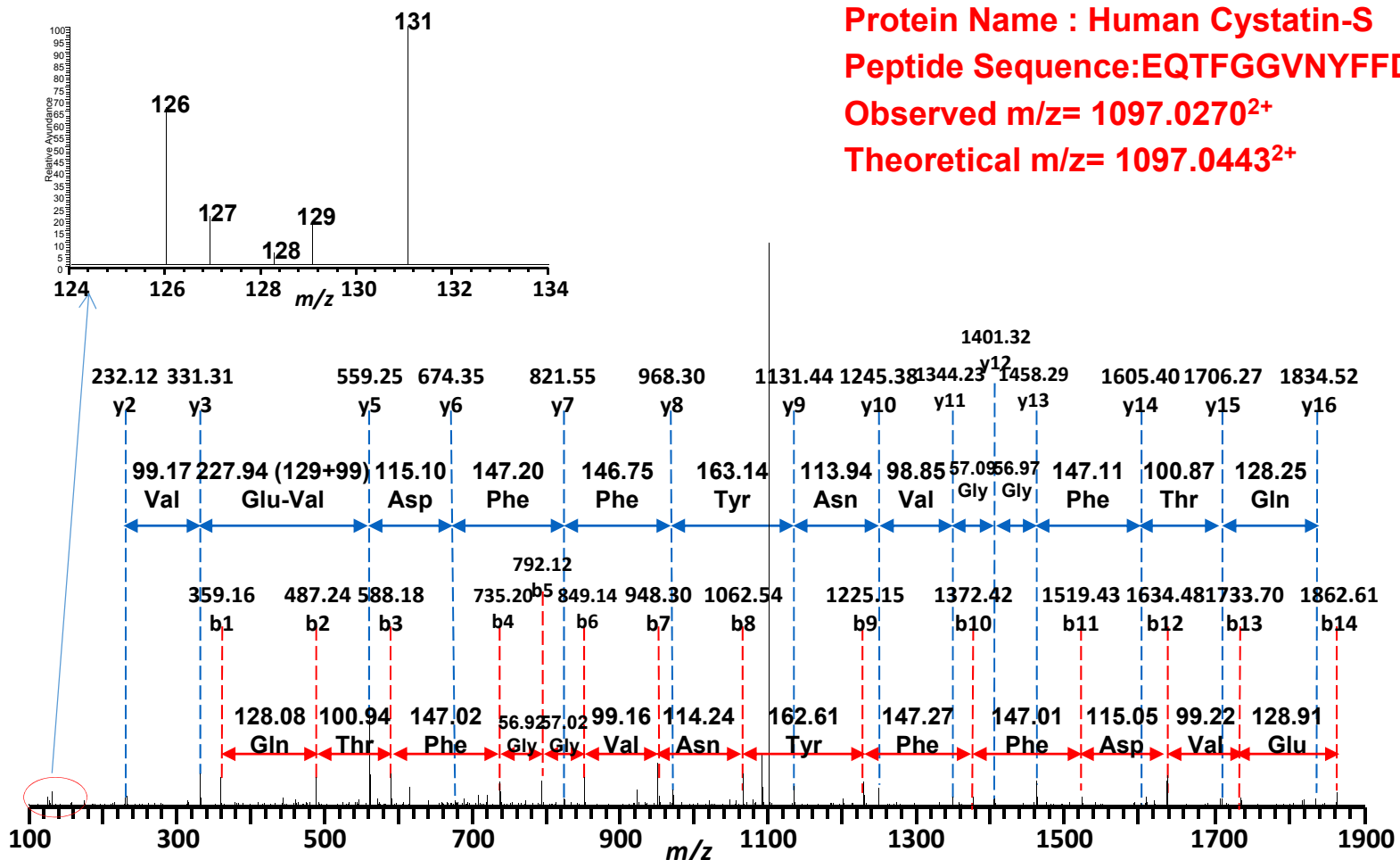


Figure Provided Courtesy of Thermo Fisher Scientifics

Dry Eye Studies: 4 Groups: Normal, Moderate Dry Eye, Mild Dry Eye, Mixed Dry Eyes

| Group | IS | Normal | Mod | Mild | Mix |
|-------|------------|------------|------------|------------|------------|
| 1 | IS 1 (126) | 2004 (127) | 2041 (128) | 2043 (129) | 2069 (130) |
| 2 | IS 2 (126) | 2007 (131) | 2042 (127) | 2040 (128) | 2066 (129) |
| 3 | IS 3 (126) | 2008 (130) | 2065 (131) | 2070 (127) | 2037 (128) |
| 4 | IS 4 (126) | 2063 (129) | 2046 (130) | 2038 (131) | 2014 (127) |
| 5 | IS 5 (126) | 2015 (128) | 2068 (129) | 2071 (130) | 2036 (131) |
| 6 | IS 6 (126) | 2016 (127) | 2067 (128) | 2062 (130) | 2035 (131) |

Ratio 126:127:128:129:130:131=1:0.30:0.09:0.25:0.00:1.49



iTraq MASCOT Results

{MATRIX} Mascot Search Results

User : Kari
 Email : kleinholz.1@osu.edu
 Search title :
 MS data file : C:\Program Files\Matrix Science\Mascot Daemon\mgf\2712 15076 sample 1\mascot_daemon_merge.mgf
 Database : SwissProt 57.5 (471472 sequences; 167326533 residues)
 Taxonomy : Homo sapiens (human) (20399 sequences)
 Quantitation : TMT 6plex [method details](#)
 : Proteome Sciences 6-plex Tandem Mass Tags (R)
 Timestamp : 19 May 2010 at 18:44:12 GMT
 Enzyme : Trypsin
 Fixed modifications : Carbamidomethyl (C),TMT6plex (N-term),TMT6plex (K)
 Variable modifications : Oxidation (M),TMT (K),TMT (N-term)
 Mass values : Monoisotopic
 Protein Mass : Unrestricted
 Peptide Mass Tolerance : ± 1.2 Da
 Fragment Mass Tolerance : ± 0.8 Da
 Max Missed Cleavages : 3
 Instrument type : ESI-TRAP
 Number of queries : 27933
 Protein hits :

| 127/126 | 128/126 | 129/126 | 130/126 | 131/126 | | |
|---------|---------|---------|---------|---------|-------------------------------|---|
| 0.663 | 0.590 | 0.428 | 1.392 | 0.711 | TRFL_HUMAN | Lactotransferrin OS=Homo sapiens GN=LTF PE=1 SV=6 |
| 1.129 | 1.216 | 1.006 | 0.815 | 0.660 | ALBU_HUMAN | Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2 |
| 1.732 | 0.696 | 1.054 | 2.023 | 1.087 | PIGR_HUMAN | Polymeric immunoglobulin receptor OS=Homo sapiens GN=PIGR PE=1 SV=4 |
| 0.957 | 1.203 | 1.134 | 1.060 | 0.791 | C3_HUMAN | Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2 |
| 1.127 | 1.366 | 1.425 | 0.753 | 0.519 | TRFE_HUMAN | Serotransferrin OS=Homo sapiens GN=TF PE=1 SV=2 |
| 1.375 | 0.596 | 1.030 | 1.734 | 0.945 | IGHA1_HUMAN | Ig alpha-1 chain C region OS=Homo sapiens GN=IGHA1 PE=1 SV=2 |
| 0.950 | 1.233 | 1.368 | 0.979 | 1.512 | ENO1_HUMAN | Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2 |
| 1.570 | 0.631 | 1.139 | 1.854 | 1.030 | IGHA2_HUMAN | Ig alpha-2 chain C region OS=Homo sapiens GN=IGHA2 PE=1 SV=3 |
| 1.014 | 1.443 | 1.595 | 0.958 | 0.975 | ACTB_HUMAN | Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1 |
| 1.622 | 1.485 | 1.888 | 0.952 | 0.977 | AIAT_HUMAN | Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3 |
| 0.971 | 1.094 | 1.261 | 0.834 | 0.398 | IGHG1_HUMAN | Ig gamma-1 chain C region OS=Homo sapiens GN=IGHG1 PE=1 SV=1 |
| 1.160 | 1.332 | 1.327 | 0.835 | 0.809 | ANXA1_HUMAN | Annexin A1 OS=Homo sapiens GN=ANXA1 PE=1 SV=2 |
| 1.486 | 0.812 | 0.570 | 1.605 | 1.122 | PIP_HUMAN | Prolactin-inducible protein OS=Homo sapiens GN=PIP PE=1 SV=1 |
| 0.911 | 0.994 | 1.215 | 0.862 | 0.590 | IGHG3_HUMAN | Ig gamma-3 chain C region OS=Homo sapiens GN=IGHG3 PE=1 SV=2 |
| 1.365 | 0.501 | 0.638 | 1.245 | 0.923 | CYTS_HUMAN | Cystatin-S OS=Homo sapiens GN=CST4 PE=1 SV=3 |
| 0.904 | 1.280 | 1.343 | 1.125 | 0.766 | IGHG2_HUMAN | Ig gamma-2 chain C region OS=Homo sapiens GN=IGHG2 PE=1 SV=2 |
| 1.040 | 1.036 | 1.619 | 0.764 | 1.082 | KPYM_HUMAN | Pyruvate kinase isozymes M1/M2 OS=Homo sapiens GN=PFKM2 PE=1 SV=4 |
| 0.672 | 0.874 | 1.435 | 1.272 | 0.799 | HPT_HUMAN | Haptoglobin OS=Homo sapiens GN=HP PE=1 SV=1 |
| 1.023 | 0.748 | 0.598 | 1.095 | 0.722 | BA2G_HUMAN | Zinc-alpha-2-glycoprotein OS=Homo sapiens GN=A2GP1 PE=1 SV=1 |
| 1.209 | 0.749 | 1.372 | 1.605 | 0.674 | IGKC_HUMAN | Ig kappa chain C region OS=Homo sapiens GN=IGKC PE=1 SV=1 |
| 0.790 | 0.433 | 0.401 | 1.104 | 0.638 | LYSC_HUMAN | Lysozyme C OS=Homo sapiens GN=LYZ PE=1 SV=1 |
| 1.042 | 0.510 | 0.437 | 0.814 | 0.507 | LCN1_HUMAN | Lipocalin-1 OS=Homo sapiens GN=LCN1 PE=1 SV=1 |
| 1.307 | 0.650 | 1.004 | 1.262 | 0.911 | LAC_HUMAN | Ig lambda chain C regions OS=Homo sapiens GN=IGLC1 PE=1 SV=1 |
| 0.922 | 1.208 | 1.336 | 0.969 | 1.111 | HEM1_HUMAN | Hemopexin OS=Homo sapiens GN=HPX PE=1 SV=2 |
| 0.824 | 0.926 | 1.101 | 1.337 | --- | CLUS_HUMAN | Clusterin OS=Homo sapiens GN=CLU PE=1 SV=1 |
| 0.940 | 1.411 | 1.580 | 0.928 | 1.028 | ACTA_HUMAN | Actin, aortic smooth muscle OS=Homo sapiens GN=ACTA2 PE=1 SV=1 |
| 0.539 | 0.657 | 0.576 | 2.614 | 2.835 | DMBT1_HUMAN | Deleted in malignant brain tumors 1 protein OS=Homo sapiens GN=DMBT1 PE=1 SV=2 |
| 0.869 | 1.060 | 1.875 | 1.155 | 1.009 | ALDH1A1_HUMAN | Retinal dehydrogenase 1 OS=Homo sapiens GN=ALDH1A1 PE=1 SV=2 |
| 0.715 | 1.086 | 1.694 | 0.856 | 1.471 | ILEU_HUMAN | Leukocyte elastase inhibitor OS=Homo sapiens GN=SERPINF1 PE=1 SV=1 |
| 0.912 | 1.597 | 1.532 | 1.116 | 0.560 | PRDX1_HUMAN | Peroxiredoxin-1 OS=Homo sapiens GN=PRDX1 PE=1 SV=1 |
| 1.625 | 0.796 | 1.666 | 0.807 | 1.010 | IGHM_HUMAN | Ig mu chain C region OS=Homo sapiens GN=IGHM PE=1 SV=3 |
| 1.263 | 1.151 | 1.279 | 1.125 | --- | TGM2_HUMAN | Protein-glutamine gamma-glutamyltransferase 2 OS=Homo sapiens GN=TGM2 PE=1 SV=2 |
| 1.572 | 0.763 | 1.632 | 0.698 | 1.010 | MUCB_HUMAN | Ig mu heavy chain disease protein OS=Homo sapiens PE=1 SV=1 |
| 1.505 | 0.496 | 0.642 | 1.169 | 0.846 | CYTN_HUMAN | Cystatin-SN OS=Homo sapiens GN=CST1 PE=1 SV=2 |

iTraq Results

| Protein Name | DE/NDE (Ratio (# of Detection, p value)) | | | Function |
|---|--|--------------------------|--------------------------|---|
| | Mild/Normal | Mod/Normal | Mix/Normal | |
| Aldehyde dehydrogenase, dimeric NADP-preferring OS=Homo sapiens | (2.41, 6, 0.0030) | | (1.69, 6, 0.0471) | oxidoreductase activity |
| Apolipoprotein A-I OS=Homo sapiens | (1.75, 6, 0.0362) | | | transporter activity; protein binding; enzyme regulator activity; lipid binding |
| Cystatin-S OS=Homo sapiens | (0.51, 6, 0.0014) | (0.43, 6, 0.0005) | (0.60, 6, 0.0283) | enzyme regulator activity |
| Deleted in malignant brain tumors 1 protein OS=Homo sapiens | | (0.60, 6, 0.0472) | | signal transducer activity; protein binding; bacterial cell surface binding |
| Ezrin OS=Homo sapiens | | (1.79, 6, 0.0101) | | protein binding; binding |
| Hemopexin OS=Homo sapiens | (2.15, 6, 0.0384) | (2.11, 6, 0.0486) | | binding; transporter activity; ion binding |
| Haptoglobin OS=Homo sapiens | (1.97, 6, 0.0474) | | | catalytic activity; protein binding; peptidase activity |
| Ig alpha-1 chain C region OS=Homo sapiens | | (0.59, 6, 0.0113) | | antigen binding; protein binding |
| Ig gamma-1 chain C region OS=Homo sapiens | | (1.94, 6, 0.0266) | | antigen binding; protein binding |
| Ig gamma-2 chain C region OS=Homo sapiens | | (1.87, 6, 0.0369) | | antigen binding |
| Ig gamma-3 chain C region OS=Homo sapiens | | (2.08, 6, 0.0408) | | antigen binding |
| Extracellular glycoprotein lacritin OS=Homo sapiens | (0.28, 6, 0.0059) | (0.23, 6, 0.0003) | (0.48, 6, 0.0428) | protein binding; extracellular matrix binding |
| Putative lipocalin 1-like protein 1 OS=Homo sapiens | (0.43, 6, 0.0026) | (0.39, 6, 0.0002) | (0.50, 6, 0.0119) | binding; transporter activity |
| Lipocalin-1 OS=Homo sapiens | (0.33, 6, 0.0030) | (0.32, 6, 0.0003) | (0.43, 6, 0.0188) | binding; transporter activity; protein binding; enzyme regulator activity |
| Lysozyme C OS=Homo sapiens | (0.33, 6, 0.0019) | (0.28, 6, 0.0031) | | catalytic activity; hydrolase activity; protein binding |
| Polymeric immunoglobulin receptor OS=Homo sapiens | (0.51, 6, 0.0290) | (0.43, 6, 0.0016) | | protein binding |
| Prolactin-inducible protein OS=Homo sapiens | (0.36, 6, 0.0004) | (0.38, 6, 0.0008) | (0.58, 6, 0.0409) | protein binding |
| Proline-rich protein 1 OS=Homo sapiens | (0.44, 6, 0.0023) | (0.39, 6, 0.0056) | | enzyme regulator activity |
| Proline-rich protein 4 OS=Homo sapiens | (0.41, 6, 0.0042) | (0.34, 6, 0.0081) | | |
| Secretoglobin family 1D member 1 OS=Homo sapiens | (0.28, 6, 0.0032) | (0.21, 6, 0.0006) | (0.41, 6, 0.0169) | binding |
| Mammaglobin-B OS=Homo sapiens | (0.34, 6, 0.0076) | (0.26, 6, 0.0009) | (0.43, 6, 0.0215) | steroid binding; binding; hormone binding |
| Serotransferrin OS=Homo sapiens | | (2.02, 6, 0.0429) | | protein binding; ion binding |
| Lactotransferrin OS=Homo sapiens | (0.33, 6, 0.0019) | (0.34, 6, 0.0072) | | peptidase activity; ion binding; pattern bindingcarbohydrate binding; hydrolase activity; protein binding |
| Vitamin D-binding protein OS=Homo sapiens | (1.65, 6, 0.0449) | (1.87, 6, 0.0206) | | transporter activity; steroid binding; vitamin binding:: protein binding |
| Zinc-alpha-2-glycoprotein OS=Homo sapiens | (0.40, 6, 0.0047) | (0.36, 6, 0.0029) | (0.59, 6, 0.0502) | lipid binding; carboxylic acid binding; hydrolase activity; transporter activity |
| Zymogen granule protein 16 homolog B OS=Homo sapiens | | (0.44, 6, 0.0383) | | carbohydrate binding |

Pros and Cons to iTRAQ

- **Requires less total protein than DIGE (50 µg or less)**
- **One experiment can determine fold change, protein ID and Post translational modification**
- **Up to 8-10 groups can be compared at the same time**
- **Samples can come from both tissue or cell culture**
- **Mass Spectrometry requirements are rigorous**
 - High resolution
 - Advanced LC chromatography
 - Long instrument time
 - PQD/HCD needed on IT instrument
- **Produces enormous amount of data**
- **Requiring detailed bioinformatic analysis**
- **Expensive**

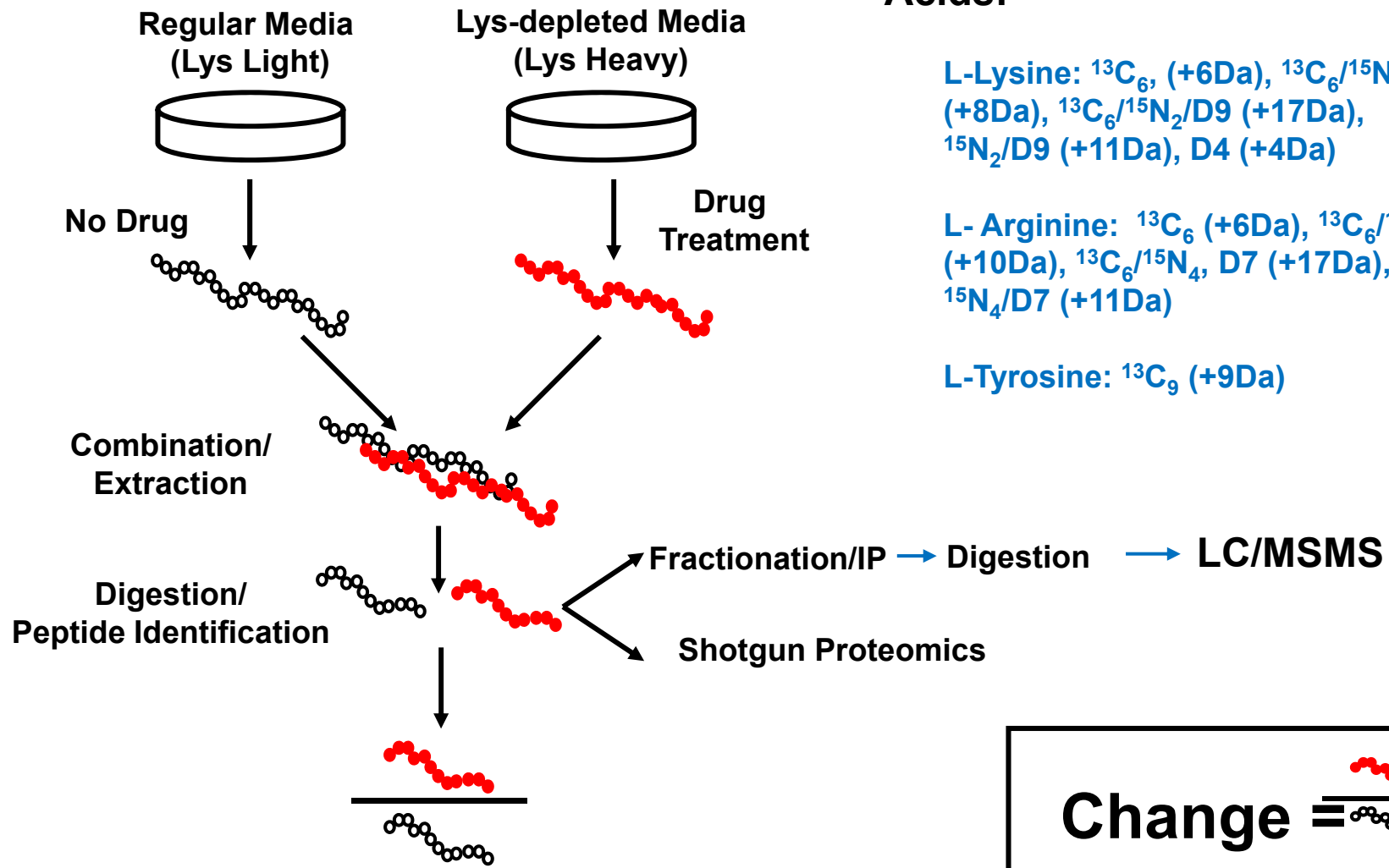
SILAC (Stable isotope labeling by amino acids in cell culture)

Heavy Isotopic Labeled Amino Acids:

L-Lysine: $^{13}\text{C}_6$, (+6Da), $^{13}\text{C}_6/^{15}\text{N}_2$ (+8Da), $^{13}\text{C}_6/^{15}\text{N}_2/\text{D}_9$ (+17Da), $^{15}\text{N}_2/\text{D}_9$ (+11Da), D4 (+4Da)

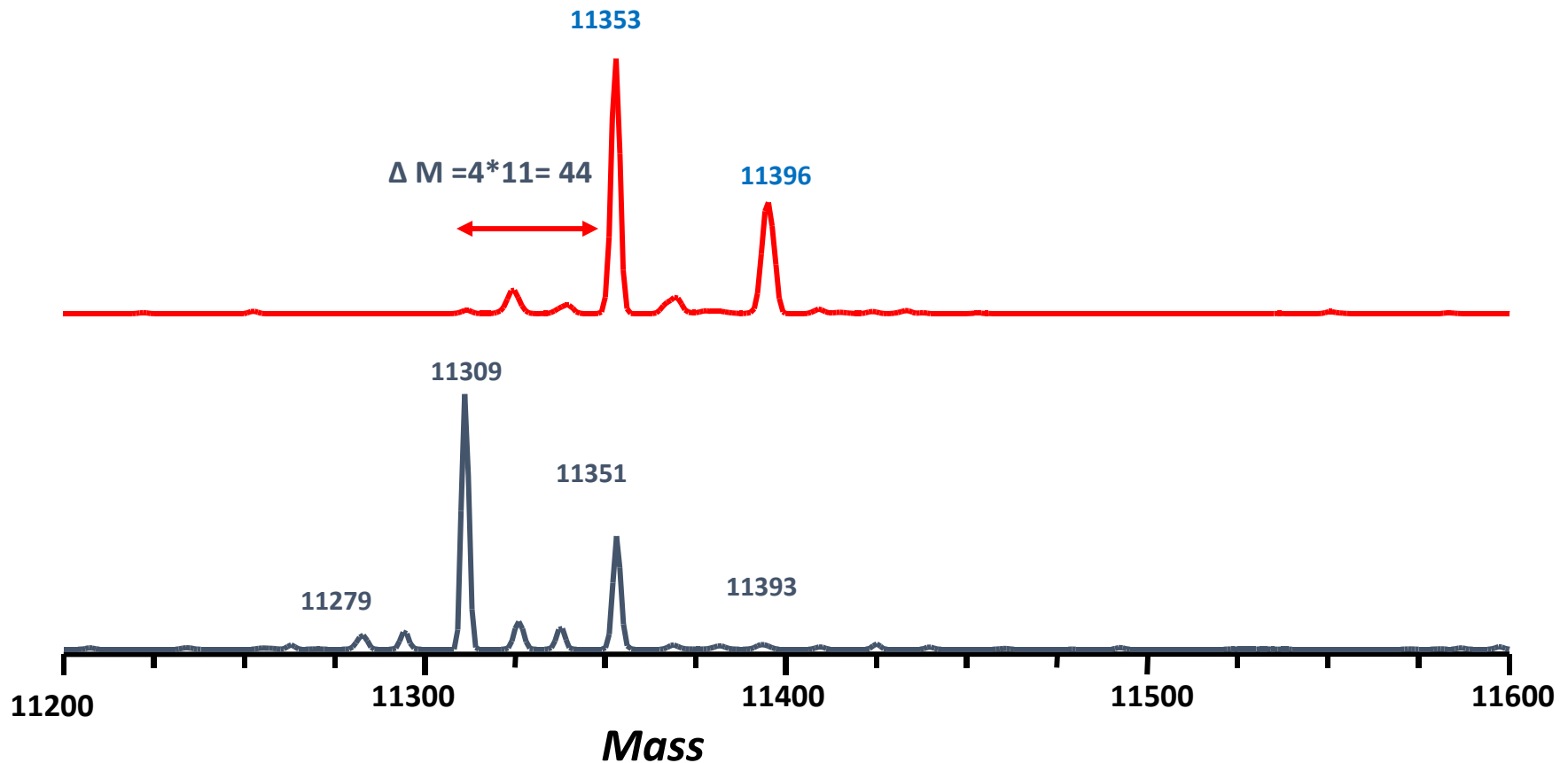
L-Arginine: $^{13}\text{C}_6$ (+6Da), $^{13}\text{C}_6/^{15}\text{N}_4$ (+10Da), $^{13}\text{C}_6/^{15}\text{N}_4/\text{D}_7$ (+17Da), $^{15}\text{N}_4/\text{D}_7$ (+11Da)

L-Tyrosine: $^{13}\text{C}_9$ (+9Da)

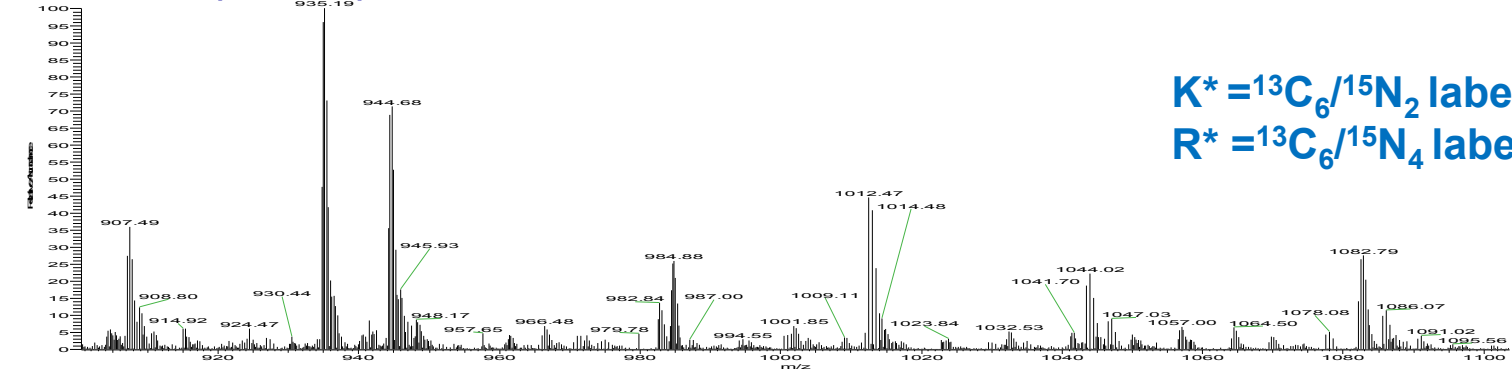


Optimization of Heavy Amino Acids Incorporation

S G R G **K⁵** G G **K⁸** G L G **K¹²** G G A **K¹⁶** R H R **K²⁰** V L R D N I Q G I T **K³¹** P A I R R
L A R R G G V **K⁴⁴** R I S G L I Y E E T R G V L **K⁵⁹** V F L E N V I R D A V T Y
T E H A **K⁷⁷** R **K⁷⁹** T V T A M D V V Y A L **K⁹¹** R Q G R T L Y G F G G



17881_13Days #21693-22317 RT: 127.45-130.89 AV: 100 NL: 6.03E5



K* = ¹³C₆/¹⁵N₂ labeled Lys
R* = ¹³C₆/¹⁵N₄ labeled Arg

14.1 FAS_HUMAN Score 2140 Mass 275877 Matches 114 (114) Sequences 48 (48) emPAI 1.22 Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=3

▼114 peptide matches (68 non-duplicate, 46 duplicate)

Auto-fit to window

| Query Dupes | Observed | Mr (expt) | Mr (calc) | ppm | M | Score | Expect | Rank | U | Peptide |
|-------------|----------|-----------|-----------|--------|---|-------|----------|------|---|--|
| 1982 | 466.2486 | 930.4826 | 930.4798 | 3.01 | 0 | 53 | 0.001 | 1 | U | R.GYAVLGGER.G + Label:13C(6)15N(4) (R) |
| 2348 | 479.2917 | 956.5688 | 956.5655 | 3.53 | 0 | 35 | 0.043 | 1 | U | R.GLVQALQTK.V |
| 2416 1 | 483.2988 | 964.5830 | 964.5797 | 3.49 | 0 | 54 | 0.00058 | 1 | U | R.GLVQALQTK.V + Label:13C(6)15N(2) (K) |
| 3147 | 510.8319 | 1019.6492 | 1019.6470 | 2.19 | 0 | 34 | 0.015 | 1 | U | R.VAAAVDLLIK.S + Label:13C(6)15N(2) (K) |
| 3445 | 522.2977 | 1042.5808 | 1042.5771 | 3.58 | 0 | 37 | 0.012 | 1 | U | R.AQVADVVS.R |
| 3611 | 527.3015 | 1052.5884 | 1052.5854 | 2.91 | 0 | 66 | 6.3e-005 | 1 | U | R.AQVADVVS.R + Label:13C(6)15N(4) (R) |
| 4263 1 | 544.7761 | 1087.5376 | 1087.5367 | 0.92 | 0 | 42 | 0.0029 | 1 | U | R.LSFFDFP.R + Label:13C(6)15N(4) (R) |
| 4951 | 562.3458 | 1122.6770 | 1122.6740 | 2.73 | 0 | 24 | 0.04 | 1 | U | R.SEGVAVLLTK.K + Label:13C(6)15N(2) (K) |
| 6507 | 600.7976 | 1199.5806 | 1199.5770 | 3.06 | 0 | 46 | 0.0046 | 1 | U | R.LGMLSPEGTCK.A + Label:13C(6)15N(2) (K) |
| 7107 | 616.2621 | 1230.5096 | 1230.5088 | 0.71 | 0 | 35 | 0.0081 | 1 | U | R.AFDTAGNGYCR.S |
| 7281 | 621.2665 | 1240.5184 | 1240.5170 | 1.13 | 0 | 52 | 0.00021 | 1 | U | R.AFDTAGNGYCR.S + Label:13C(6)15N(4) (R) |
| 7702 1 | 420.5490 | 1258.6252 | 1258.6226 | 2.02 | 0 | 37 | 0.011 | 1 | U | R.FDASFFGVHPK.Q + Label:13C(6)15N(2) (K) |
| 7710 | 630.3246 | 1258.6346 | 1258.6226 | 9.54 | 0 | 40 | 0.0051 | 1 | U | R.FDASFFGVHPK.Q + Label:13C(6)15N(2) (K) |
| 7969 1 | 637.3804 | 1272.7462 | 1272.7430 | 2.59 | 0 | 52 | 0.0014 | 1 | U | R.LQVVDQPLPVR.G + Label:13C(6)15N(4) (R) |
| 8640 | 650.7929 | 1299.5712 | 1299.5759 | -3.61 | 0 | 31 | 0.0057 | 1 | U | R.VYQWDDPDR.L + Label:13C(6)15N(4) (R) |
| 8676 | 651.8397 | 1301.6648 | 1301.6649 | -0.041 | 0 | 40 | 0.029 | 1 | U | R.AALQELQLCK.G |

Cell Culture Labeled for 7 days

3.1 FAS_HUMAN Score 5532 Mass 275877 Matches 222 (222) Sequences 70 (70) emPAI 1.68 Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=3

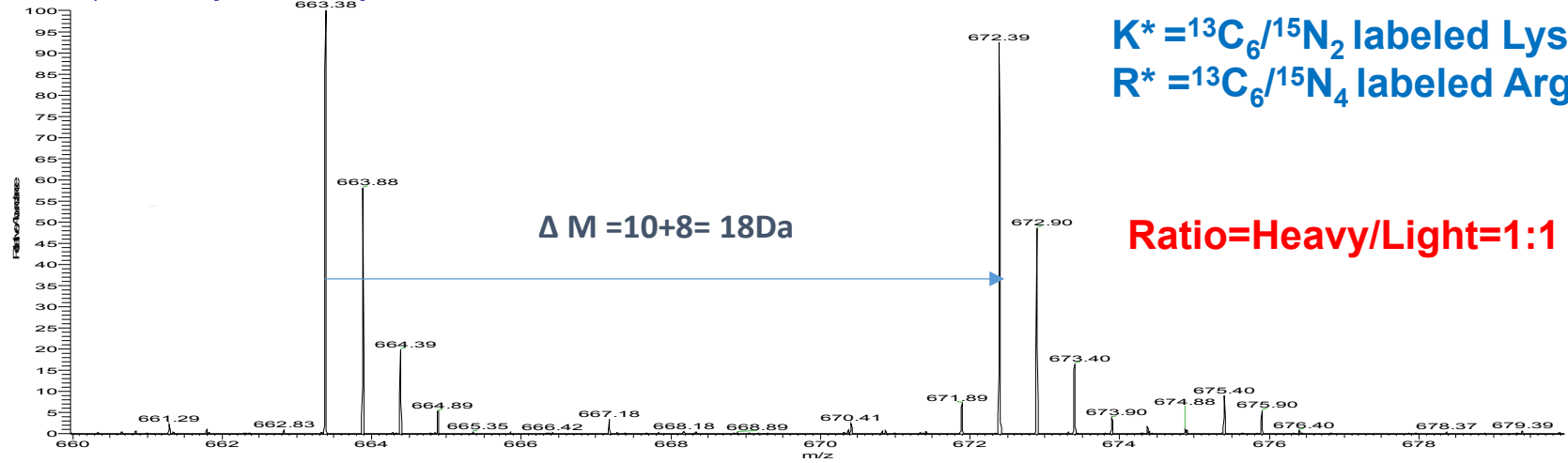
▼222 peptide matches (84 non-duplicate, 138 duplicate)

Auto-fit to window

| Query Dupes | Observed | Mr (expt) | Mr (calc) | ppm | M | Score | Expect | Rank | U | Peptide |
|-------------|----------|-----------|-----------|-------|---|-------|----------|------|---|--|
| 2888 | 523.8415 | 1045.6684 | 1045.6627 | 5.52 | 0 | 27 | 0.032 | 1 | U | R.GTPLISPLIK.W + Label:13C(6)15N(2) (K) |
| 2903 1 | 524.7698 | 1047.5250 | 1047.5224 | 2.50 | 0 | 57 | 0.00035 | 1 | U | R.KYSGTLNLDL.R + Label:13C(6)15N(4) (R) |
| 3005 1 | 527.3020 | 1052.5894 | 1052.5854 | 3.86 | 0 | 62 | 0.00013 | 1 | U | R.AQVADVVS.R + Label:13C(6)15N(4) (R) |
| 3569 | 544.7788 | 1087.5430 | 1087.5367 | 5.88 | 0 | 53 | 0.0011 | 1 | U | R.LSFFDFP.R + Label:13C(6)15N(4) (R) |
| 3677 | 547.3189 | 1092.6232 | 1092.6270 | -3.45 | 0 | 36 | 0.016 | 1 | U | R.QPELLIGSTK.S + Label:13C(6)15N(2) (K) |
| 4209 2 | 562.3469 | 1122.6792 | 1122.6740 | 4.69 | 0 | 65 | 3.2e-005 | 1 | U | R.SEGVAVLLTK.K + Label:13C(6)15N(2) (K) |
| 5574 | 600.7982 | 1199.5818 | 1199.5770 | 4.06 | 0 | 46 | 0.0045 | 1 | U | R.LGMLSPEGTCK.A + Label:13C(6)15N(2) (K) |
| 5674 | 402.8709 | 1205.5909 | 1205.5851 | 4.83 | 0 | 41 | 0.0039 | 1 | U | R.QARTMDPQLR.L + Label:13C(6)15N(4) (R) |
| 5682 | 402.8964 | 1205.6674 | 1205.6627 | 3.88 | 0 | 28 | 0.049 | 1 | U | R.SDEAVKPFGLK.V + 2 Label:13C(6)15N(2) (K) |
| 6321 1 | 621.2684 | 1240.5222 | 1240.5170 | 4.20 | 0 | 56 | 6.9e-005 | 1 | U | R.AFDTAGNGYCR.S + Label:13C(6)15N(4) (R) |
| 6688 4 | 420.5490 | 1258.6252 | 1258.6226 | 2.02 | 0 | 46 | 0.00068 | 1 | U | R.FDASFFGVHPK.Q + Label:13C(6)15N(2) (K) |
| 6963 2 | 637.3810 | 1272.7474 | 1272.7430 | 3.53 | 0 | 56 | 0.00057 | 1 | U | R.LQVVDQPLPVR.G + Label:13C(6)15N(4) (R) |
| 7753 | 650.7973 | 1299.5800 | 1299.5759 | 3.16 | 0 | 45 | 0.00029 | 1 | U | R.VYQWDDPDR.L + Label:13C(6)15N(4) (R) |

Cell Culture Labeled for 7 days

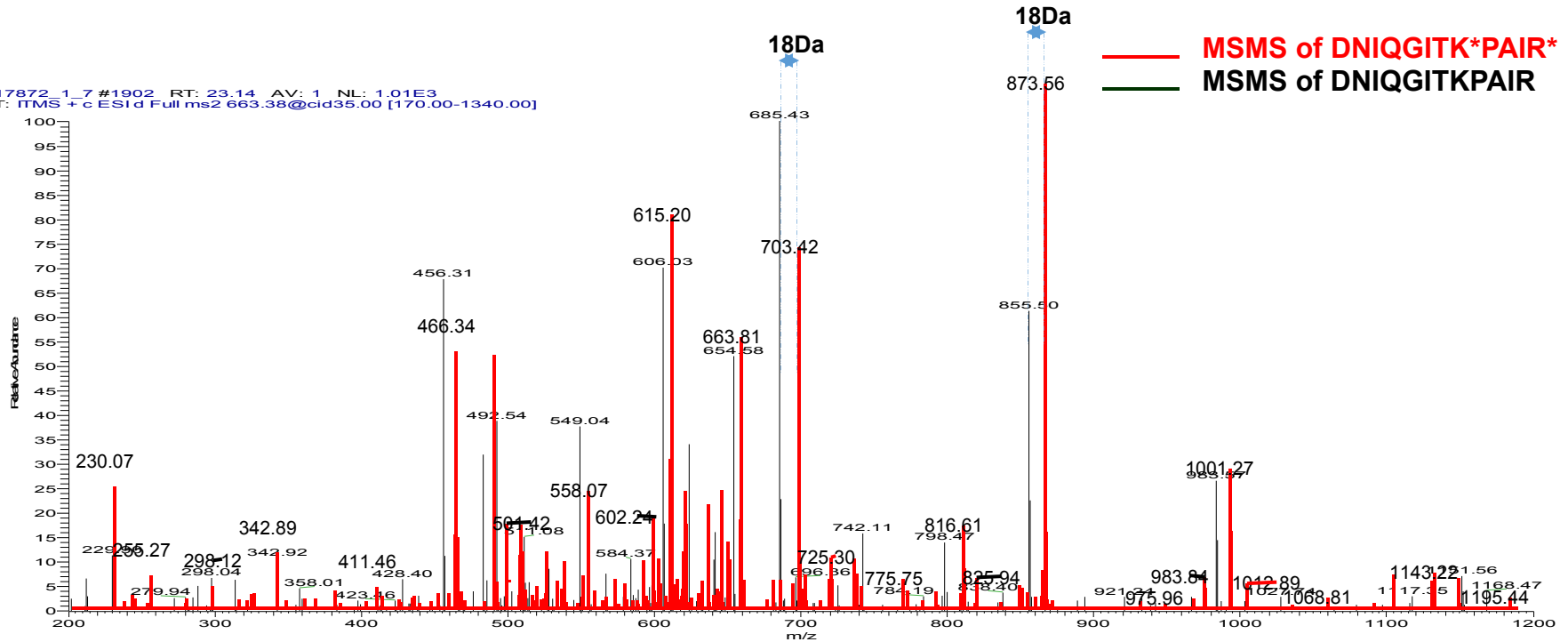
17872_1_7 #1858-1959 RT: 22.95-23.41 AV: 13 NL: 5.19E4
T: FTMS + p ESI Full ms [350.00-2000.00]

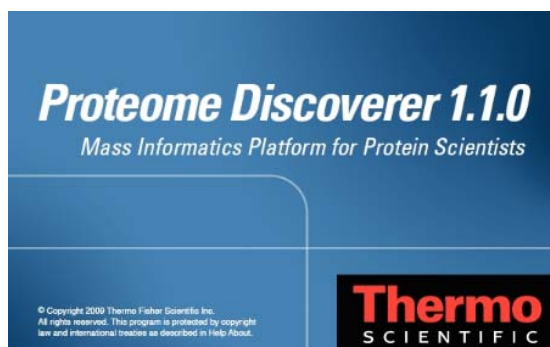


DNIQGITKPAIR

DNIQGITK*PAIR*

17872_1_7 #1902 RT: 23.14 AV: 1 NL: 1.01E3
T: ITMS + c ESI d Full ms2 663.38@cid35.00 [170.00-1340.00]





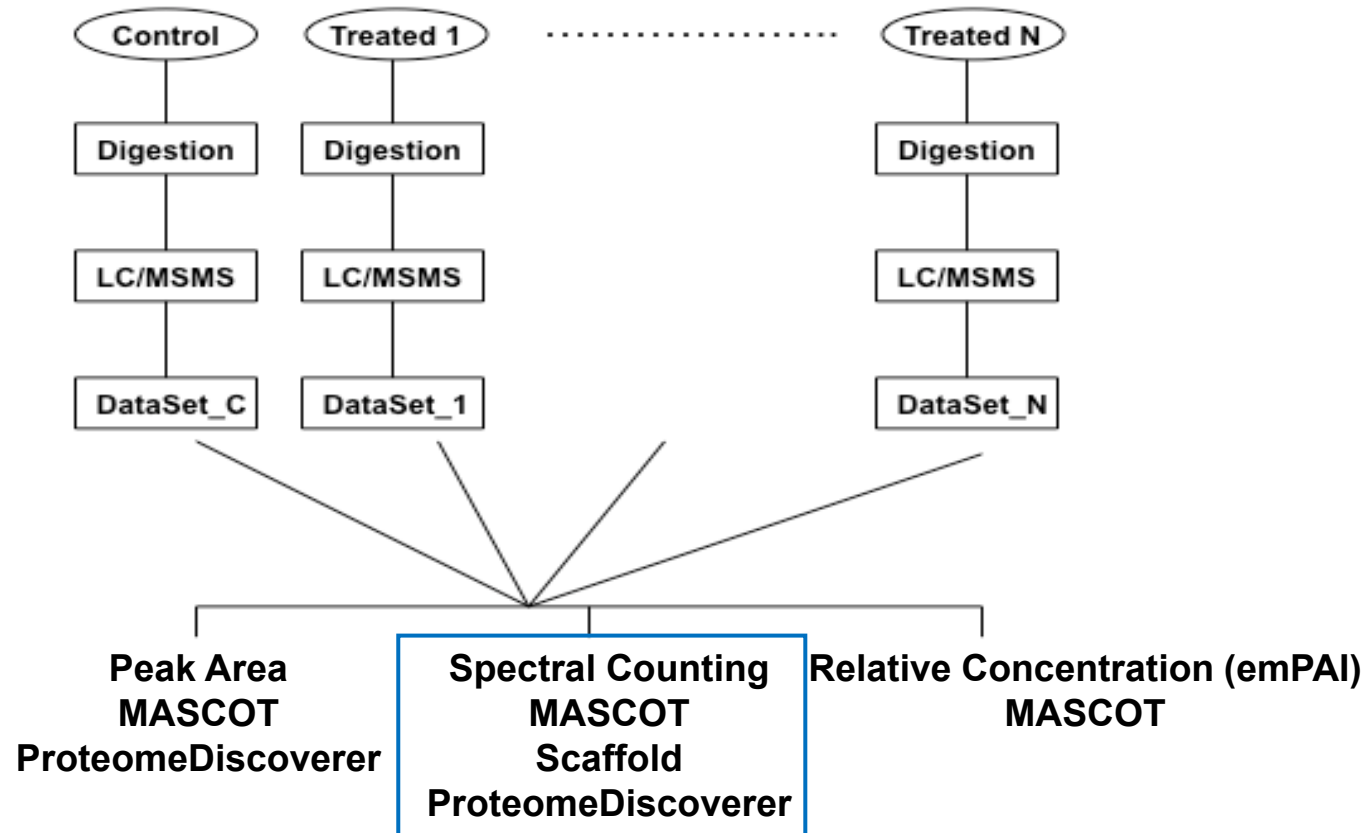
| Accession | Description | Ratio: Heavy/Light | |
|-----------|--|--------------------|----------|
| | | Sample 1 | Sample 2 |
| P35527 | Keratin, type I cytoskeletal 9 OS=Homo sapiens | 0.096 | 0.049 |
| P22314 | Ubiquitin-like modifier-activating enzyme 1 OS=Homo sapiens | 0.106 | 0.182 |
| P23526 | Adenosylhomocysteinase OS=Homo sapiens | 0.250 | 0.151 |
| P13645 | Keratin, type I cytoskeletal 10 OS=Homo sapiens | 0.257 | 0.265 |
| P52597 | Heterogeneous nuclear ribonucleoprotein F OS=Homo sapiens | 0.260 | 0.254 |
| O43175 | D-3-phosphoglycerate dehydrogenase OS=Homo sapiens | 0.262 | 0.276 |
| P31943 | Heterogeneous nuclear ribonucleoprotein H OS=Homo sapiens | 0.284 | 0.337 |
| P23771 | Trans-acting T-cell-specific transcription factor GATA-3 OS=Homo sapiens | 0.295 | 0.379 |
| O00299 | Chloride intracellular channel protein 1 OS=Homo sapiens | 0.306 | 0.316 |
| P07741 | Adenine phosphoribosyltransferase OS=Homo sapiens | 0.319 | 0.343 |
| Q14103 | Heterogeneous nuclear ribonucleoprotein D0 OS=Homo sapiens | 0.359 | 0.390 |
| Q9BQE3 | Tubulin alpha-1C chain OS=Homo sapiens | 0.380 | 0.404 |
| P13639 | Elongation factor 2 OS=Homo sapiens | 0.391 | 0.609 |
| P31949 | Protein S100-A11 OS=Homo sapiens | 0.394 | 0.626 |
| P08107 | Heat shock 70 kDa protein 1A/1B OS=Homo sapiens | 0.412 | 0.435 |
| P11586 | C-1-tetrahydrofolate synthase, cytoplasmic OS=Homo sapiens | 0.415 | 0.835 |
| P23246 | Splicing factor, proline- and glutamine-rich OS=Homo sapiens | 0.418 | 0.387 |
| P31942 | Heterogeneous nuclear ribonucleoprotein H3 OS=Homo sapiens | 0.424 | 0.382 |
| P58546 | Myotrophin OS=Homo sapiens | 0.429 | 0.420 |
| P11021 | 78 kDa glucose-regulated protein OS=Homo sapiens | 0.442 | 0.514 |
| P00558 | Phosphoglycerate kinase 1 OS=Homo sapiens | 0.450 | 0.574 |
| P61978 | Heterogeneous nuclear ribonucleoprotein K OS=Homo sapiens | 0.472 | 0.445 |
| P62826 | GTP-binding nuclear protein Ran OS=Homo sapiens | 0.475 | 0.496 |
| P35080 | Profilin-2 OS=Homo sapiens | 0.484 | 0.409 |
| O60506 | Heterogeneous nuclear ribonucleoprotein Q | 1.198 | 1.051 |
| Q99623 | Prohibitin-2 OS=Homo sapiens | 1.222 | 0.706 |
| Q06830 | Peroxiredoxin-1 OS=Homo sapiens | 1.251 | 1.183 |
| P62244 | 40S ribosomal protein S15a OS=Homo sapiens | 1.265 | 1.124 |
| P07355 | Annexin A2 OS=Homo sapiens | 1.299 | 1.194 |
| P28072 | Proteasome subunit beta type-6 OS=Homo sapiens | 1.302 | 1.133 |
| P21333 | Filamin-A OS=Homo sapiens | 1.310 | 1.243 |

Pros and Cons to iTRAQ

- **One experiment can determine fold change, protein ID and Post translational modification**
- **Up to several groups can be compared at the same time**
- **Mixing at the beginning, allow more sample preparation steps**
- **significant reduction of experiment error**
- **High reproducibility**
- **Expensive**
- **Special data analysis platform**
- **Samples come from cell cultures**
- **Time consuming**

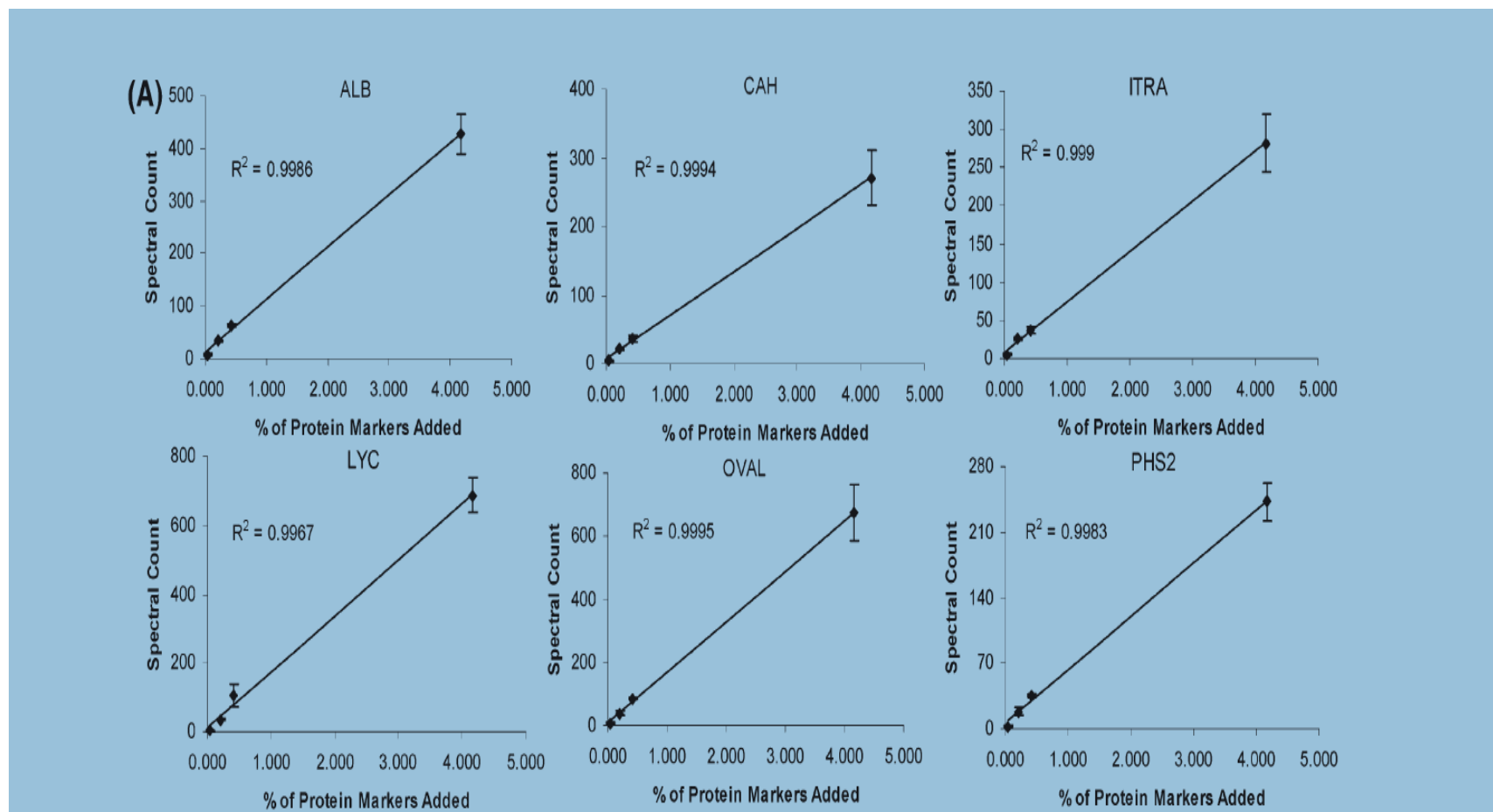
Label-free quantification

doesn't use a stable isotope containing compound to chemically label the protein. It can determine the relative amount of proteins in two or more biological samples by comparing peptide peak areas or spectral counting.



Label Free Quantitation by Spectral Counting

Relative protein quantification is achieved by comparing the number of identified MS/MS spectra from the same protein in each of multiple LC/MS/MS



Hongbin Liu, Rovshan G. Sadygov and John R. Yates, III, A Model for Random Sampling and Estimation of Relative Protein Abundance in Shotgun Proteomics *Anal. Chem.* **2004**, 76, 4193-4201

Label Free Quantitation by Spectral Counting

▶ Decoy search summary (reversed protein sequences)

[Proteins \(17\)](#)

Report Builder

[Unassigned \(2249\)](#)

Protein hits (22 proteins)

▶ Columns: Standard (12 out of 12)

▶ Filters: (none)

Export as CSV

| Family | M | DB | Accession | Score | Mass | Matches | Pep(sig) | Sequences | Seq(sig) | emPAI | Description |
|--------------------|---|--------------|-----------------------------|-------|--------|---------|----------|-----------|----------|---------|---|
| 1 | 1 | SwissProt_ID | MYPG_EQUBU | 2474 | 17072 | 117 | 117 | 13 | 13 | 186.75 | Myoglobin OS=Equus burchelli GN=MB PE=1 SV=2 |
| 1 | 2 | SwissProt_ID | MYPG_KOGBR | 492 | 17357 | 41 | 41 | 6 | 6 | 3.98 | Myoglobin OS=Kogia breviceps GN=MB PE=2 SV=3 |
| 1 | 3 | SwissProt_ID | MYPG_PHYCD | 317 | 17320 | 37 | 37 | 6 | 6 | 3.16 | Myoglobin OS=Physeter catodon GN=MB PE=1 SV=2 |
| 2 | 1 | SwissProt_ID | LACB_BOVIN | 1918 | 19870 | 101 | 101 | 8 | 8 | 13.28 | Beta-lactoglobulin OS=Bos taurus GN=LGB PE=1 SV=3 |
| 2 | 2 | SwissProt_ID | LACB_OVIMU | 1651 | 18139 | 93 | 93 | 8 | 8 | 14.43 | Beta-lactoglobulin OS=Ovis orientalis musimon GN=LGB PE=1 SV=1 |
| 3 | 1 | SwissProt_ID | CYC_HORSE | 1111 | 11825 | 68 | 68 | 14 | 14 | 3403.10 | Cytochrome c OS=Beta-lactoglobulin OS=Ovis orientalis musimon GN=LGB PE=1 SV=1 |
| 3 | 2 | SwissProt_ID | CYC_EQUAS | 692 | 11811 | 44 | 44 | 12 | 12 | 167.59 | Cytochrome c OS=Equus asinus GN=CYCS PE=1 SV=2 |
| 3 | 3 | SwissProt_ID | CYC2_BOVIN | 346 | 11732 | 27 | 27 | 7 | 7 | 35.21 | Cytochrome c 2 OS=Bos taurus GN=CYCT PE=3 SV=3 |
| 4 | 1 | SwissProt_ID | TRY1_BOVIN | 237 | 25769 | 11 | 11 | 4 | 4 | 1.08 | Cationic trypsin OS=Bos taurus PE=1 SV=3 |
| 5 | 1 | SwissProt_ID | CASQ1_CANFA | 47 | 6610 | 1 | 1 | 1 | 1 | 0.54 | Calsequestrin-1 (Fragment) OS=Canis familiaris GN=CASQ1 PE=1 SV=1 |
| 6 | 1 | SwissProt_ID | PROS_RABIT | 43 | 71923 | 1 | 1 | 1 | 1 | 0.05 | Vitamin K-dependent protein S (Fragment) OS=Oryctolagus cuniculus GN=PROS1 PE=2 SV=1 |
| 7 | 1 | SwissProt_ID | H11_BOVIN | 43 | 22087 | 3 | 3 | 2 | 2 | 0.33 | Histone H1.1 OS=Bos taurus GN=HIST1H1A PE=1 SV=1 |
| 8 | 1 | SwissProt_ID | COHA1_BOVIN | 33 | 149062 | 1 | 1 | 1 | 1 | 0.02 | Collagen alpha-1(XVII) chain OS=Bos taurus GN=COL17A1 PE=2 SV=1 |
| 9 | 1 | SwissProt_ID | 4F2_RABIT | 33 | 0 | 2 | 2 | 1 | 1 | | |
| 10 | 1 | SwissProt_ID | CASQ1_RABIT | 29 | 45235 | 1 | 1 | 1 | 1 | 0.07 | Calsequestrin-1 OS=Oryctolagus cuniculus GN=CASQ1 PE=1 SV=1 |
| 11 | 1 | SwissProt_ID | CRSP2_PIG | 28 | 12960 | 1 | 1 | 1 | 1 | 0.26 | Calcitonin receptor-stimulating peptide 2 OS=Sus scrofa GN=CRSP2 PE=2 SV=1 |
| 12 | 1 | SwissProt_ID | RS12_BOVIN | 27 | 14505 | 1 | 1 | 1 | 1 | 0.23 | 40S ribosomal protein S12 OS=Bos taurus GN=RPS12 PE=2 SV=1 |
| 13 | 1 | SwissProt_ID | 1F4G1_RABIT | 23 | 153955 | 1 | 1 | 1 | 1 | 0.02 | Eukaryotic translation initiation factor 4 gamma 1 OS=Oryctolagus cuniculus GN=EIF4G1 PE=1 SV=1 |
| 14 | 1 | SwissProt_ID | CP4A4_RABIT | 19 | 58893 | 1 | 1 | 1 | 1 | 0.06 | Cytochrome P450 4A4 OS=Oryctolagus cuniculus GN=CYP4A4 PE=1 SV=3 |
| 15 | 1 | SwissProt_ID | UROM_BOVIN | 17 | 0 | 1 | 1 | 1 | 1 | | |
| 16 | 1 | SwissProt_ID | COCH_BOVIN | 15 | 59556 | 1 | 1 | 1 | 1 | 0.06 | Cochlin OS=Bos taurus GN=COCH PE=2 SV=1 |
| 17 | 1 | SwissProt_ID | OXA1L_BOVIN | 14 | 48956 | 1 | 1 | 1 | 1 | 0.07 | Mitochondrial inner membrane protein OXA1L OS=Bos taurus GN=OXA1L PE=2 SV=1 |

Export as CSV

of reports = # of Treatment Conditions X # of Bio-replicates



Scaffold

Spectral Counting Results-Scaffold

Scaffold Viewer - Samples - 17169 1D fractionation

File Edit View Experiment Export Quant Window Help

Protein Threshold: 99.0% Min # Peptides: 2 Peptide Threshold: 95%

Display Options: Quantitative Value (Normalized Total Spectra) Req Mods: No Filter Search:

Load Data

Samples

Proteins

Similarity

Quantify

Publish

Statistics

1458 Proteins at 99.0% Minimum 2 Min # Peptides 1.0% Decoy FDR 379681 Spectra at 95.0% Minimum 0.02% Decoy FDR

Protein Identification Probability

Percent Coverage

Percentage of Total Spectra

Exclusive Unique Peptide Count

Exclusive Unique Spectrum Count

Exclusive Spectrum Count

Total Spectrum Count

Quantitative Value (Normalized Total Spectra)

Bio View: Identified Proteins (1458) Including 15 Decoys

Accession

Molecular Weight

Protein Grouping Ambiguity

T-Test (P-Value)

Aerobic

Phototrophic

Conditions

Bio-Replicates

of Spectra

P-Value

Sample Information:

Biological Sample:

Sample Category:

Sample Description:

MS/MS Sample:

MS/MS Sample Notes:

| # | Visible? | Stained? | Accession | Molecular Weight | Protein Grouping Ambiguity | T-Test (P-Value) | 17169_A1 | 17169_A2 | 17169_A3 | 17169_A4 | 17169_A5 | 17169_A6 | 17169_P1 | 17169_P2 | 17169_P3 | 17169_P4 | 17169_P5 | 17169_P6 |
|----|----------|----------|---|------------------|----------------------------|------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| 1 | ✓ | ✓ | [gene=gro1] [protein=molecula... NC_007493... | 53 kDa | | < 0.00010 | 1486 | 1278 | 1342 | 1215 | 1210 | 1359 | 2554 | 2400 | 3219 | 2383 | 3303 | 2948 |
| 2 | ✓ | ✓ | [gene=tufA] [protein=elongation... NC_007493... | 43 kDa | | 0.00045 | 1016 | 933 | 942 | 985 | 1128 | 1061 | 1185 | 1219 | 1462 | 1441 | 1628 | 1402 |
| 3 | ✓ | ✓ | [gene=atpD] [protein=ATP synth... NC_007493... | 50 kDa | | 0.28 | 686 | 758 | 713 | 689 | 621 | 625 | 784 | 734 | 811 | 742 | 713 | 577 |
| 4 | ✓ | ✓ | [gene=atpA] [protein=ATP synth... NC_007493... | 55 kDa | | 0.053 | 430 | 413 | 455 | 417 | 501 | 455 | 374 | 436 | 395 | 391 | 421 | 433 |
| 5 | ✓ | ✓ | [gene=Pnp] [protein=polynucleo... NC_007493... | 77 kDa | | 0.0019 | 386 | 399 | 387 | 395 | 378 | 396 | 330 | 338 | 312 | 293 | 263 | 172 |
| 6 | ✓ | ✓ | [gene=rpoC] [protein=DNA-direc... NC_007493... | 157 kDa | | < 0.00010 | 397 | 436 | 366 | 335 | 407 | 417 | 302 | 266 | 263 | 261 | 251 | 178 |
| 7 | ✓ | ✓ | [gene=rpoB] [protein=DNA-direc... NC_007493... | 154 kDa | | 0.00038 | 391 | 399 | 301 | 325 | 388 | 420 | 269 | 268 | 252 | 255 | 219 | 163 |
| 8 | ✓ | ✓ | [gene=dnaK] [protein=molecula... NC_007493... | 69 kDa | | 0.0072 | 233 | 226 | 258 | 244 | 271 | 236 | 347 | 272 | 393 | 242 | 361 | 393 |
| 9 | ✓ | ✓ | [gene=RSP_0745] [protein=acet... NC_007493... | 40 kDa | | 0.092 | 276 | 211 | 247 | 309 | 287 | 302 | 267 | 320 | 282 | 308 | 353 | 293 |
| 10 | ✓ | ✓ | [gene=tig] [protein=trigger facto... NC_007493... | 49 kDa | | 0.00054 | 298 | 246 | 273 | 254 | 295 | 269 | 166 | 185 | 170 | 223 | 208 | 239 |
| 11 | ✓ | ✓ | [gene=RSP_1613] [protein=TRA... NC_007493... | 34 kDa | | 0.016 | 195 | 152 | 183 | 190 | 166 | 170 | 180 | 275 | 175 | 249 | 239 | 243 |
| 12 | ✓ | ✓ | [gene=dctP] [protein=TRAP dicar... NC_007493... | 36 kDa | | 0.0057 | 264 | 247 | 328 | 321 | 217 | 259 | 146 | 159 | 133 | 175 | 175 | 275 |
| 13 | ✓ | ✓ | [gene=RSP_0842] [protein=porin... NC_007493... | 34 kDa | | 0.0042 | 374 | 370 | 425 | 267 | 259 | 218 | 31 | 162 | 171 | 270 | 129 | 136 |
| 14 | ✓ | ✓ | [gene=fusA1] [protein=elongatio... NC_007493... | 78 kDa | | 0.0051 | 250 | 245 | 262 | 250 | 214 | 234 | 217 | 215 | 197 | 209 | 172 | 138 |
| 15 | ✓ | ✓ | [gene=bztA] [protein=glutamate... NC_007493... | 36 kDa | | 0.033 | 151 | 149 | 185 | 177 | 173 | 144 | 211 | 231 | 160 | 277 | 308 | 181 |
| 16 | ✓ | ✓ | [gene=RSP_2923] [protein=bran... NC_007493... | 40 kDa | | 0.0036 | 186 | 151 | 103 | 182 | 180 | 149 | 220 | 192 | 227 | 331 | 301 | 312 |
| 17 | ✓ | ✓ | [gene=RSP_2663] [protein=suga... NC_007493... | 63 kDa | | < 0.00010 | 395 | 412 | 435 | 380 | 386 | 376 | 13 | 24 | 13 | 15 | 17 | 5 |
| 18 | ✓ | ✓ | [gene=glnA] [protein=L-glutamin... NC_007493... | 52 kDa | | 0.29 | 184 | 192 | 205 | 174 | 202 | 181 | 199 | 179 | 180 | 166 | 150 | 201 |
| 19 | ✓ | ✓ | [gene=RSP_1806] [protein=acon... NC_007493... | 99 kDa | | 0.38 | 214 | 218 | 168 | 188 | 193 | 186 | 206 | 194 | 224 | 175 | 148 | 129 |
| 20 | ✓ | ✓ | [gene=gabD4] [protein=succinat... NC_007493... | 55 kDa | | < 0.00010 | 247 | 240 | 281 | 249 | 256 | 255 | 115 | 104 | 115 | 91 | 100 | 131 |
| 21 | ✓ | ✓ | [gene=gapB] [protein=glycerald... NC_007493... | 36 kDa | | 0.038 | 151 | 129 | 146 | 149 | 173 | 154 | 157 | 191 | 172 | 191 | 196 | 297 |
| 22 | ✓ | ✓ | [gene=alaS] [protein=alanyl-tri... NC_007493... | 95 kDa | | 0.56 | 151 | 162 | 158 | 151 | 169 | 159 | 191 | 173 | 162 | 137 | 129 | 114 |
| 23 | ✓ | ✓ | [gene=sucC] [protein=succinyl-C... NC_007493... | 42 kDa | | 0.034 | 174 | 144 | 170 | 166 | 190 | 166 | 159 | 165 | 141 | 149 | 147 | 121 |
| 24 | ✓ | ✓ | [gene=puhA] [protein=reactio c... NC_007493... | 28 kDa | | < 0.00010 | 0 | 1 | 0 | 1 | 0 | 2 | 189 | 283 | 230 | 329 | 307 | 306 |
| 25 | ✓ | ✓ | [gene=gltB] [protein=glutamate ... NC_007493... | 165 kDa | | < 0.00010 | 197 | 216 | 189 | 188 | 218 | 205 | 113 | 87 | 103 | 78 | 69 | 114 |
| 26 | ✓ | ✓ | [gene=OppA] [protein=oligopepti... NC_007493... | 58 kDa | | < 0.00010 | 113 | 109 | 87 | 98 | 88 | 92 | 179 | 220 | 205 | 256 | 203 | 209 |
| 27 | ✓ | ✓ | [gene=RSP_1352] [protein=D-3-... NC_007493... | 57 kDa | | < 0.00010 | 106 | 104 | 83 | 94 | 116 | 109 | 196 | 137 | 186 | 216 | 218 | 171 |
| 28 | ✓ | ✓ | [gene=RSP_0161] [protein=sper... NC_007493... | 39 kDa | | 0.015 | 131 | 120 | 128 | 125 | 126 | 119 | 142 | 140 | 140 | 150 | 152 | 204 |
| 29 | ✓ | ✓ | [gene=mdh] [protein=malate de... NC_007493... | 36 kDa | | 0.013 | 128 | 119 | 145 | 142 | 110 | 127 | 138 | 150 | 164 | 149 | 172 | 140 |
| 30 | ✓ | ✓ | [gene=RSP_2948] [protein=indol... NC_007493... | 123 kDa | | 0.076 | 154 | 154 | 127 | 135 | 149 | 153 | 150 | 131 | 140 | 125 | 109 | 74 |
| 31 | ✓ | ✓ | [gene=gltA] [protein=citrate syn... NC_007493... | 48 kDa | | 0.25 | 142 | 141 | 136 | 131 | 141 | 137 | 142 | 133 | 130 | 134 | 140 | 88 |
| 32 | ✓ | ✓ | [gene=RSP_0381] [protein=hypo... NC_007493... | 16 kDa | | 0.80 | 106 | 96 | 84 | 94 | 203 | 174 | 145 | 64 | 117 | 63 | 138 | 290 |
| 33 | ✓ | ✓ | [gene=ahcY] [protein=S-adenos... NC_007493... | 51 kDa | | 0.15 | 111 | 113 | 118 | 120 | 123 | 114 | 142 | 139 | 123 | 124 | 96 | 146 |
| 34 | ✓ | ✓ | [gene=cysK] [protein=cysteine s... NC_007493... | 38 kDa | | 0.020 | 153 | 130 | 148 | 148 | 145 | 159 | 103 | 97 | 130 | 107 | 162 | 112 |

Spectral Counting Results-Scaffold

Scaffold Viewer - Samples - 17169 1D fractionation

File Edit View Experiment Export Quant Window Help

Protein Threshold: 99.0% Min # Peptides: 2 Peptide Threshold: 95%

Display Options: Quantitative Value (Normalized Total Spectra) Req Mods: No Filter Search:

Probability Legend:
 over 95%
 80% to 94%
 50% to 79%
 20% to 49%
 0% to 19%

Bio View:
 Identified Proteins (1458)
 Including 15 Decoys

| # | Visible? | Starred? | Protein Name | Accession Number | Molecular Weight | Protein Grouping Ambiguity | T-Test (p-Value) | 17169_A1 | 17169_A2 | 17169_A3 | Aerobic | | | | | | Phototrophic | | | | | | | | |
|----|----------|----------|---|------------------|------------------|----------------------------|------------------|----------|----------|----------|---------|-----|-----|-----|-----|-----|--------------|-----|-----|--|--|--|--|--|--|
| 1 | ✓ | ✓ | [gene=groEL] [protein=molecula... NC_007493... | 58 kDa | | | < 0.00010 | 1486 | 1278 | 1342 | | | | | | | | | | | | | | | |
| 2 | ✓ | ✓ | [gene=tufA] [protein=elongation... NC_007493... | 43 kDa | | | 0.00045 | 1016 | 933 | 942 | | | | | | | | | | | | | | | |
| 3 | ✓ | ✓ | [gene=atpD] [protein=ATP synth... NC_007493... | 50 kDa | | | 0.28 | 686 | 758 | 713 | | | | | | | | | | | | | | | |
| 4 | ✓ | ✓ | [gene=atpA] [protein=ATP synth... NC_007493... | 55 kDa | | | 0.053 | 430 | 413 | 455 | | | | | | | | | | | | | | | |
| 5 | ✓ | ✓ | [gene=Pnp] [protein=polynucleo... NC_007493... | 77 kDa | | | 0.0019 | 386 | 399 | 387 | | | | | | | | | | | | | | | |
| 6 | ✓ | ✓ | [gene=rpoC] [protein=DNA-direc... NC_007493... | 157 kDa | | | < 0.00010 | 397 | 436 | 366 | | | | | | | | | | | | | | | |
| 7 | ✓ | ✓ | [gene=rpoB] [protein=DNA-direc... NC_007493... | 154 kDa | | | 0.00038 | 391 | 399 | 301 | | | | | | | | | | | | | | | |
| 8 | ✓ | ✓ | [gene=dnaK] [protein=molecula... NC_007493... | 69 kDa | | | 0.0072 | 233 | 226 | 258 | | | | | | | | | | | | | | | |
| 9 | ✓ | ✓ | [gene=RSP_0745] [protein=acet... NC_007493... | 40 kDa | | | 0.092 | 276 | 211 | 247 | | | | | | | | | | | | | | | |
| 10 | ✓ | ✓ | [gene=tig] [protein=trigger facto... NC_007493... | 49 kDa | | | 0.00054 | 298 | 246 | 273 | | | | | | | | | | | | | | | |
| 11 | ✓ | ✓ | [gene=RSP_1613] [protein=TRA... NC_007493... | 34 kDa | | | 0.016 | 195 | 152 | 183 | | | | | | | | | | | | | | | |
| 12 | ✓ | ✓ | [gene=dctP] [protein=TRAP dicar... NC_007493... | 36 kDa | | | 0.0057 | 264 | 247 | 328 | | | | | | | | | | | | | | | |
| 13 | ✓ | ✓ | [gene=RSP_0842] [protein=porin... NC_007493... | 34 kDa | | | 0.0042 | 374 | 370 | 425 | | | | | | | | | | | | | | | |
| 14 | ✓ | ✓ | [gene=fusA1] [protein=ekongatio... NC_007493... | 78 kDa | | | 0.0051 | 250 | 245 | 262 | | | | | | | | | | | | | | | |
| 15 | ✓ | ✓ | [gene=bztA] [protein=glutamate... NC_007493... | 36 kDa | | | 0.033 | 151 | 149 | 185 | | | | | | | | | | | | | | | |
| 16 | ✓ | ✓ | [gene=RSP_2923] [protein=bran... NC_007493... | 40 kDa | | | 0.0036 | 186 | 151 | 163 | | | | | | | | | | | | | | | |
| 17 | ✓ | ✓ | [gene=RSP_2663] [protein=suga... NC_007493... | 63 kDa | | | < 0.00010 | 395 | 412 | 435 | | | | | | | | | | | | | | | |
| 18 | ✓ | ✓ | [gene=glnA] [protein=L-glutamin... NC_007493... | 52 kDa | | | 0.29 | 184 | 192 | 205 | | | | | | | | | | | | | | | |
| 19 | ✓ | ✓ | [gene=RSP_1806] [protein=acon... NC_007493... | 99 kDa | | | 0.38 | 214 | 218 | 168 | | | | | | | | | | | | | | | |
| 20 | ✓ | ✓ | [gene=gabD4] [protein=succinat... NC_007493... | 55 kDa | | | < 0.00010 | 247 | 240 | 281 | | | | | | | | | | | | | | | |
| 21 | ✓ | ✓ | [gene=gapB] [protein=glycerald... NC_007493... | 36 kDa | | | 0.038 | 151 | 129 | 146 | 149 | 173 | 154 | 157 | 191 | 172 | 191 | 196 | 297 | | | | | | |
| 22 | ✓ | ✓ | [gene=ala5] [protein=alanyl-tRN... NC_007493... | 95 kDa | | | 0.56 | 151 | 162 | 158 | 151 | 169 | 159 | 191 | 173 | 162 | 137 | 129 | 114 | | | | | | |
| 23 | ✓ | ✓ | [gene=sucC] [protein=succinyl-C... NC_007493... | 42 kDa | | | 0.034 | 174 | 144 | 170 | 166 | 190 | 166 | 159 | 165 | 141 | 149 | 147 | 121 | | | | | | |
| 24 | ✓ | ✓ | [gene=puhA] [protein=reaction c... NC_007493... | 28 kDa | | | < 0.00010 | 0 | 1 | 0 | 1 | 0 | 2 | 189 | 283 | 230 | 329 | 307 | 306 | | | | | | |
| 25 | ✓ | ✓ | [gene=gltB] [protein=glutamate ... NC_007493... | 165 kDa | | | < 0.00010 | 197 | 216 | 189 | 188 | 218 | 205 | 113 | 87 | 103 | 78 | 69 | 114 | | | | | | |
| 26 | ✓ | ✓ | [gene=OppA] [protein=oligopepti... NC_007493... | 58 kDa | | | < 0.00010 | 113 | 109 | 87 | 98 | 88 | 92 | 179 | 220 | 205 | 256 | 203 | 209 | | | | | | |
| 27 | ✓ | ✓ | [gene=RSP_1352] [protein=D-3-... NC_007493... | 57 kDa | | | < 0.00010 | 106 | 104 | 83 | 94 | 116 | 109 | 196 | 137 | 186 | 216 | 218 | 171 | | | | | | |
| 28 | ✓ | ✓ | [gene=RSP_0161] [protein=sper... NC_007493... | 39 kDa | | | 0.015 | 131 | 120 | 128 | 125 | 126 | 119 | 142 | 140 | 150 | 152 | 204 | | | | | | | |
| 29 | ✓ | ✓ | [gene=mdh] [protein=malate de... NC_007493... | 36 kDa | | | 0.013 | 128 | 119 | 145 | 142 | 110 | 127 | 138 | 150 | 164 | 149 | 172 | 140 | | | | | | |
| 30 | ✓ | ✓ | [gene=RSP_2948] [protein=indol... NC_007493... | 123 kDa | | | 0.076 | 154 | 154 | 127 | 135 | 149 | 153 | 150 | 131 | 140 | 125 | 109 | 74 | | | | | | |
| 31 | ✓ | ✓ | [gene=gltA] [protein=citrate syn... NC_007493... | 48 kDa | | | 0.25 | 142 | 141 | 136 | 131 | 141 | 137 | 142 | 133 | 130 | 134 | 140 | 88 | | | | | | |
| 32 | ✓ | ✓ | [gene=RSP_0381] [protein=hypo... NC_007493... | 16 kDa | | | 0.80 | 106 | 96 | 84 | 94 | 203 | 174 | 145 | 64 | 117 | 63 | 138 | 290 | | | | | | |
| 33 | ✓ | ✓ | [gene=ahcY] [protein=S-adenos... NC_007493... | 51 kDa | | | 0.15 | 111 | 113 | 118 | 120 | 123 | 114 | 142 | 139 | 123 | 124 | 96 | 146 | | | | | | |
| 34 | ✓ | ✓ | [gene=cysK] [protein=cysteine s... NC_007493... | 38 kDa | | | 0.020 | 158 | 130 | 148 | 148 | 145 | 159 | 103 | 97 | 130 | 107 | 162 | 112 | | | | | | |

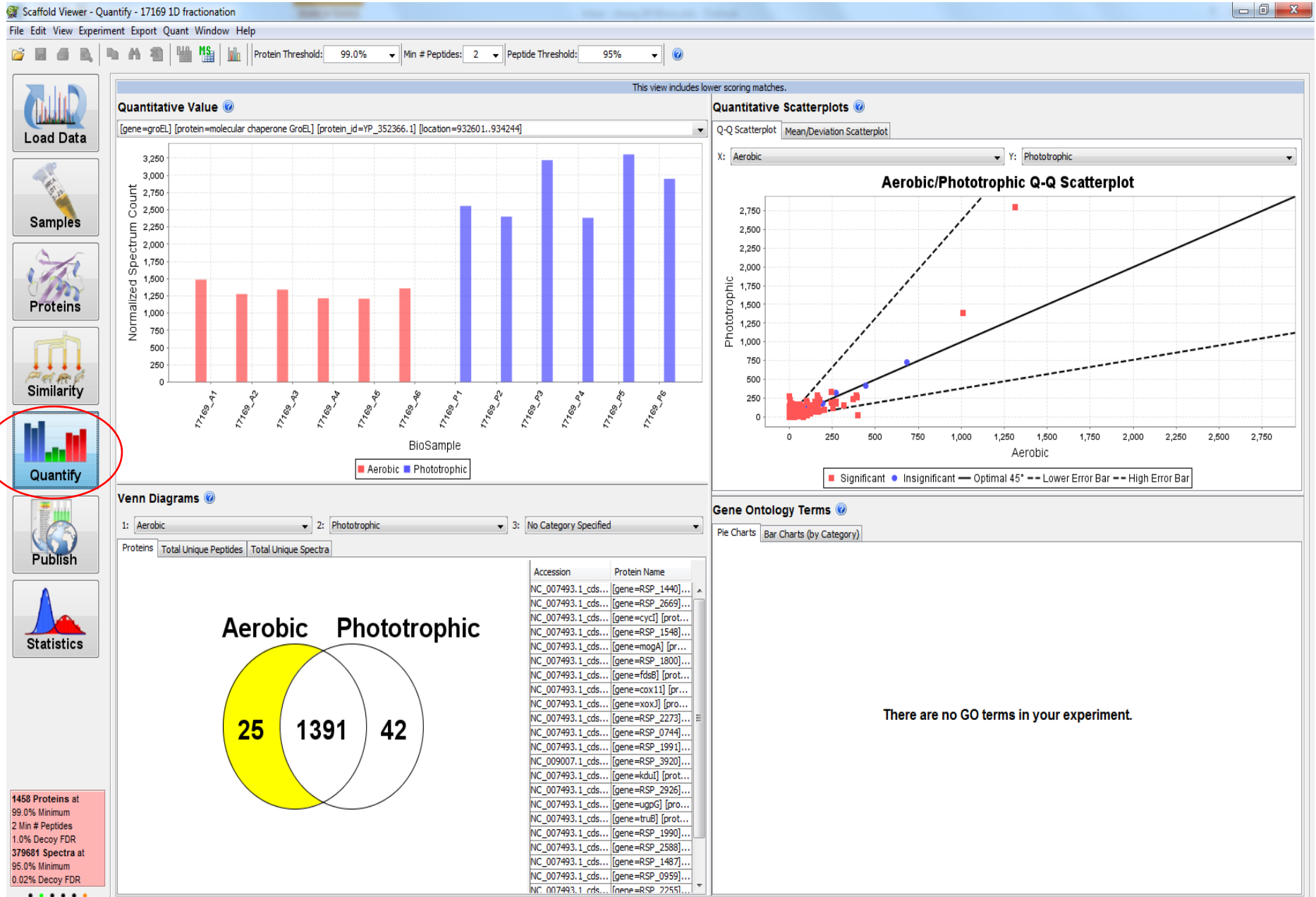
Protein Information:
 Lookup Accession Number In: NCBI (e.g. |1351907;ALBU_BOVIN,P02769)

Sample Information:
 Biological Sample:
 Sample Category:
 Sample Description:
 MS/MS Sample:
 MS/MS Sample Notes:

Quantitative Analysis Setup
 No Test Applied
 Fold Change by Sample
 Fold Change by Category
 Coefficient of Variance
 T-Test
 Analysis of Variance (ANOVA)
 Fisher's Exact Test
 Use Normalization
 Minimum Value: 0.0
 Quantitative Method: Total Spectra
 Add Remove
 Apply Cancel

1458 Proteins at 99.0% Minimum
2 Min # Peptides
1.0% Decoy FDR
379681 Spectra at 95.0% Minimum
0.02% Decoy FDR

Spectral Counting Results-Scaffold



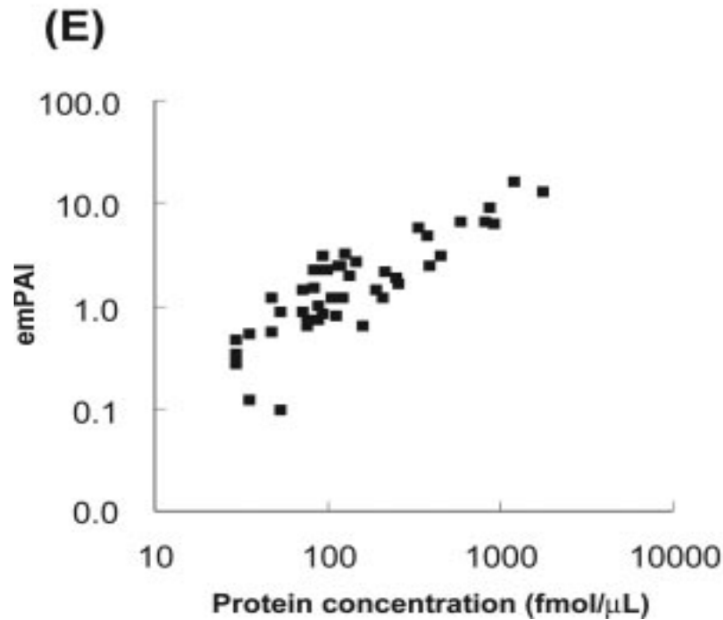
Spectral Counting Results

| Protein ID | Accession Number | P Value | | # of spectra count (Normalized value) | | | | | | | | Fold Change (RspL/Sa pA) | # of Total Spectra | |
|--|------------------|----------|---------------------|---------------------------------------|--------|--------|--------|--------|--------|--------|--------|--------------------------|--------------------|------|
| | | T-Test | Fisher's Exact Test | Rsp L1 | Rsp L2 | Rsp L3 | Rsp L4 | Sap A1 | Sap A2 | Sap A3 | Sap A4 | | RspL | SapA |
| DNA translocase FtsK | gi 68249821 | 2.70E-03 | 5.09E-02 | 0 | 0.74 | 0 | 0.95 | 1.97 | 3.66 | 2.24 | 2.54 | 0.2 | 2 | 10 |
| Tryptophanase | gi 68249281 | 5.90E-03 | 8.29E-05 | 9 | 9.59 | 7.2 | 4.73 | 0 | 0 | 4.48 | 0 | 6.8 | 31 | 4 |
| HemY | gi 68249301 | 7.60E-03 | 9.97E-03 | 0 | 2.21 | 3.09 | 4.73 | 6.89 | 5.86 | 8.95 | 10.18 | 0.3 | 10 | 32 |
| Multidrug resistance protein A | gi 68249485 | 1.10E-02 | 7.76E-03 | 0 | 0.74 | 1.03 | 0 | 1.97 | 4.4 | 5.6 | 2.54 | 0.1 | 2 | 15 |
| Cytochrome c552 | gi 68249627 | 2.40E-02 | 1.05E-02 | 7.2 | 3.69 | 5.15 | 7.57 | 0 | 2.2 | 4.48 | 0 | 3.5 | 24 | 7 |
| ATP-dependent protease La | gi 68249064 | 3.10E-02 | 3.02E-05 | 0 | 0 | 0 | 0 | 6.89 | 4.4 | 6.71 | 0 | 0 | 0 | 18 |
| HflK | gi 68248759 | 3.80E-02 | 2.76E-02 | 0 | 6.64 | 3.09 | 3.79 | 5.91 | 6.59 | 12.31 | 12.72 | 0.4 | 14 | 38 |
| F0F1 ATP synthase subunit delta. | gi 68249084 | 4.00E-02 | 2.83E-02 | 0 | 1.48 | 3.09 | 2.84 | 0 | 0 | 0 | 0 | --- | 7 | 0 |
| Phosphoglucomutase | gi 68249340 | 4.50E-02 | 5.77E-03 | 0 | 0 | 1.03 | 0 | 4.92 | 3.66 | 3.36 | 0 | 0.1 | 1 | 12 |
| | | | | | | | | | | | | | | |
| Thiamine biosynthesis lipoprotein ApbE | gi 68248777 | 1.50E-02 | 7.08E-02 | 9 | 13.28 | 14.41 | 14.19 | 7.88 | 8.79 | 7.83 | 8.9 | 1.5 | 51 | 33 |
| Hemoglobin-haptoglobin binding protein B | gi 68249240 | 1.50E-02 | 1.48E-01 | 5.4 | 2.95 | 9.26 | 5.68 | 10.83 | 8.79 | 13.43 | 12.72 | 0.5 | 23 | 46 |
| Hypothetical protein NTHI1930 | gi 68250232 | 1.70E-02 | 1.70E-01 | 1.8 | 5.16 | 5.15 | 3.79 | 5.91 | 8.79 | 6.71 | 7.63 | 0.5 | 16 | 29 |
| Penicillin-binding protein 5 | gi 68248580 | 1.90E-02 | 9.78E-02 | 0 | 2.21 | 1.03 | 3.79 | 4.92 | 3.66 | 4.48 | 5.09 | 0.4 | 7 | 18 |
| F0F1 ATP synthase subunit beta | gi 68249081 | 3.50E-02 | 6.18E-02 | 16.2 | 8.85 | 17.49 | 18.93 | 8.86 | 6.59 | 7.83 | 11.45 | 1.8 | 61 | 35 |
| Hypothetical protein NTHI1140 | gi 68249554 | 3.60E-02 | 1.78E-01 | 3.6 | 3.69 | 3.09 | 1.89 | 1.97 | 2.2 | 1.12 | 0 | 2.3 | 12 | 5 |
| IgA-specific serine endopeptidase | gi 68249575 | 4.00E-02 | 9.62E-02 | 23.41 | 26.55 | 24.7 | 19.87 | 10.83 | 21.98 | 8.95 | 17.81 | 1.6 | 95 | 60 |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| Fumarate reductase iron-sulfur subunit | gi 68249429 | 5.20E-02 | 2.79E-02 | 0 | 4.43 | 1.03 | 0.95 | 2.95 | 4.4 | 5.6 | 8.9 | 0.3 | 6 | 22 |
| Heme-hemopexin utilization protein C | gi 68248869 | 6.40E-02 | 3.84E-02 | 0 | 5.9 | 7.2 | 5.68 | 0 | 2.9 | 0 | 0 | 6.5 | 19 | 3 |
| Transketolase. | gi 68249596 | 6.80E-02 | 1.03E-02 | 0 | 0 | 0 | 0 | 2.95 | 0 | 4.48 | 1.27 | 0 | 0 | 9 |
| 5,10-methylenetetrahydrofolate reductase | gi 68250024 | 7.00E-02 | 9.13E-04 | 0 | 2.21 | 6.17 | 2.84 | 0 | 0 | 0 | 0 | --- | 11 | 0 |

Relative Quantitation Using emPAI

$$\text{emPAI} = 10^{\text{PAI} - 1}$$

$$\text{Protein content (mol \%)} = \frac{\text{emPAI}}{\sum (\text{emPAI})} \times 100$$

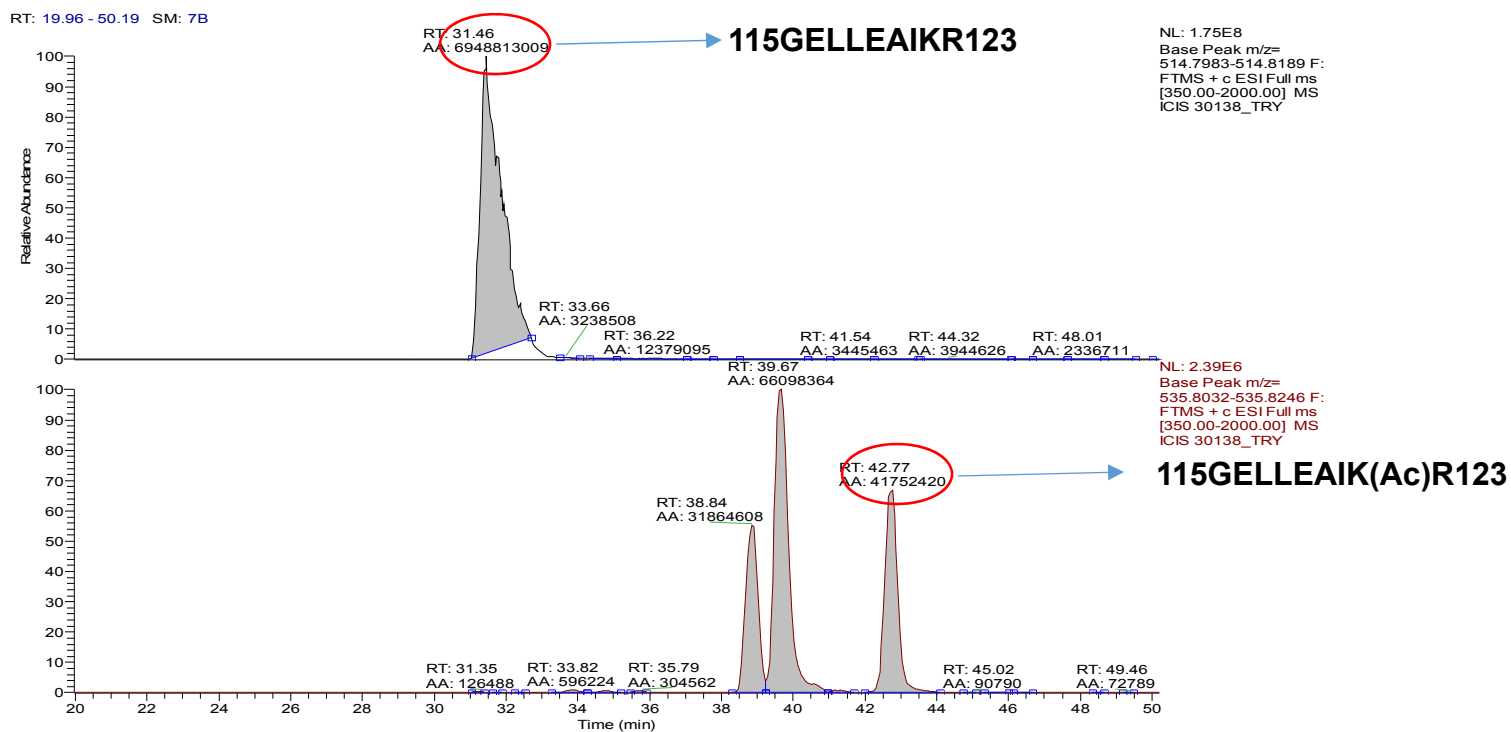


Ishihama Y¹, Oda Y, Tabata T, Sato T, Nagasu T, Rappsilber J, Mann M. Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein. *Mol Cell Proteomics*. 2005 Sep;4(9):1265-72

| Protein name | Conc Fmol | emPAI | Protein Content | | Rank | |
|---|--------------|-------|-----------------|-------|-------|-------|
| | | | Conc. | emPAI | Conc. | emPAI |
| Elongation factor 1-a 1 | 870 | 9.00 | 15.04 | 16.18 | 2 | 1 |
| a enolase | 596 | 6.50 | 10.30 | 11.69 | 3 | 2 |
| Heat shock protein HSP 90-a | 940 | 6.26 | 16.25 | 11.26 | 1 | 3 |
| Vimentin | 336 | 5.58 | 5.81 | 10.03 | 6 | 4 |
| 14-3-3 protein | 381 | 4.62 | 6.58 | 8.31 | 5 | 5 |
| 40 S ribosomal protein S16 | 456 | 2.98 | 7.88 | 5.36 | 4 | 6 |
| Pyruvate kinase, M2 isozyme | 216 | 2.06 | 3.73 | 3.70 | 9 | 7 |
| 40 S ribosomal protein S9 | 135 | 1.89 | 2.33 | 3.40 | 12 | 8 |
| GTP-binding nuclear protein RAN | 255 | 1.85 | 4.41 | 3.33 | 8 | 9 |
| ADP, ATP carrier protein, fibroblast isoform | 264 | 1.64 | 4.56 | 2.95 | 7 | 10 |
| Peripherin | 84 | 1.48 | 1.45 | 2.66 | 18 | 11 |
| Stress-70 protein, mitochondrial precursor | 195 | 1.42 | 3.37 | 2.55 | 11 | 12 |
| Fructose-bisphosphate aldolase A | 210 | 1.15 | 3.63 | 2.07 | 10 | 13 |
| IgE-binding protein | 122 | 1.15 | 2.11 | 2.07 | 13 | 14 |
| Calreticulin precursor | 114 | 1.15 | 1.97 | 2.07 | 14 | 15 |
| 60 S ribosomal protein L11 | 108 | 1.15 | 1.87 | 2.07 | 15 | 16 |
| 60 S ribosomal protein L17 | 90 | 1.00 | 1.56 | 1.80 | 17 | 17 |
| Peroxiredoxin 4 | 72 | 0.85 | 1.24 | 1.53 | 20 | 18 |
| Voltage-dependent anion-selective channel protein | 54 | 0.85 | 0.93 | 1.53 | 21 | 19 |
| T-complex protein 1, e subunit | 96 | 0.81 | 1.66 | 1.46 | 16 | 20 |
| ATP synthase oligomycin sensitivity conferral protein | 78 | 0.70 | 1.35 | 1.26 | 19 | 21 |
| Phosphate carrier protein, mitochondrial precursor | 48 | 0.55 | 0.83 | 0.99 | 22 | 22 |
| T-complex protein 1, a subunit B | 36 | 0.52 | 0.62 | 0.94 | 23 | 23 |
| Nucleolar RNA helicase II | 30 | 0.45 | 0.52 | 0.81 | 24 | 24 |

Relative Quantitation of PTM Using Peak Area

(Based on Retention Time and SIC)



| Sequence | m/z | | Mass Error | Peak Area | RT (min) | Total Peak Area | Percentage (%) |
|----------------------|------------------------|------------------------|------------|------------|----------|-----------------|----------------|
| | Theoretical | Observed | | | | | |
| 115GELLEAIKR123 | 514.8086 ²⁺ | 514.8063 ²⁺ | -4.47 | 6948813009 | 31.46 | | 99.40 |
| 115GELLEAIK(Ace)R123 | 535.8139 ²⁺ | 535.8136 ²⁺ | -0.56 | 41739255 | 42.77 | 6.99E+09 | 0.60 |

MALDI Imaging

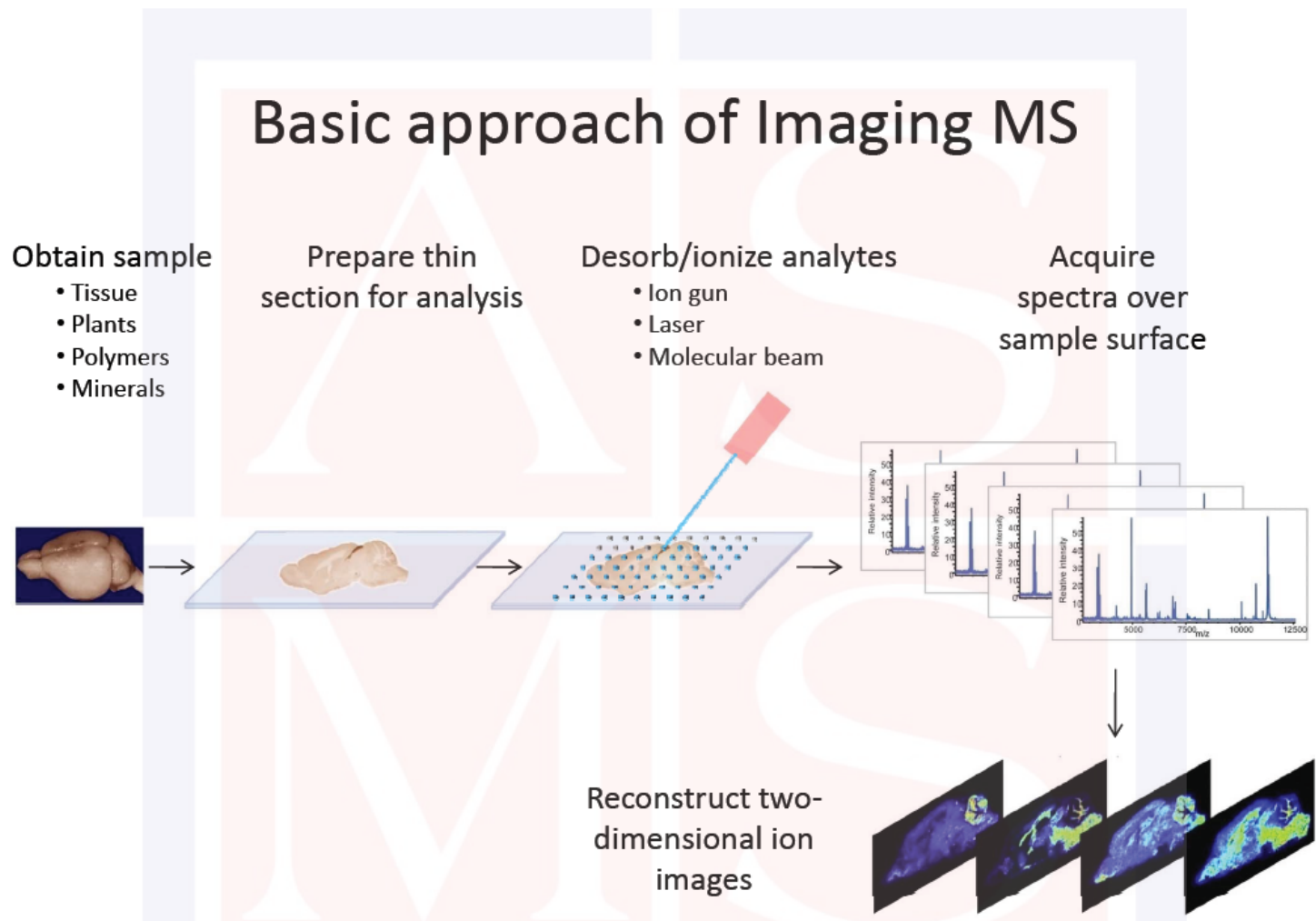
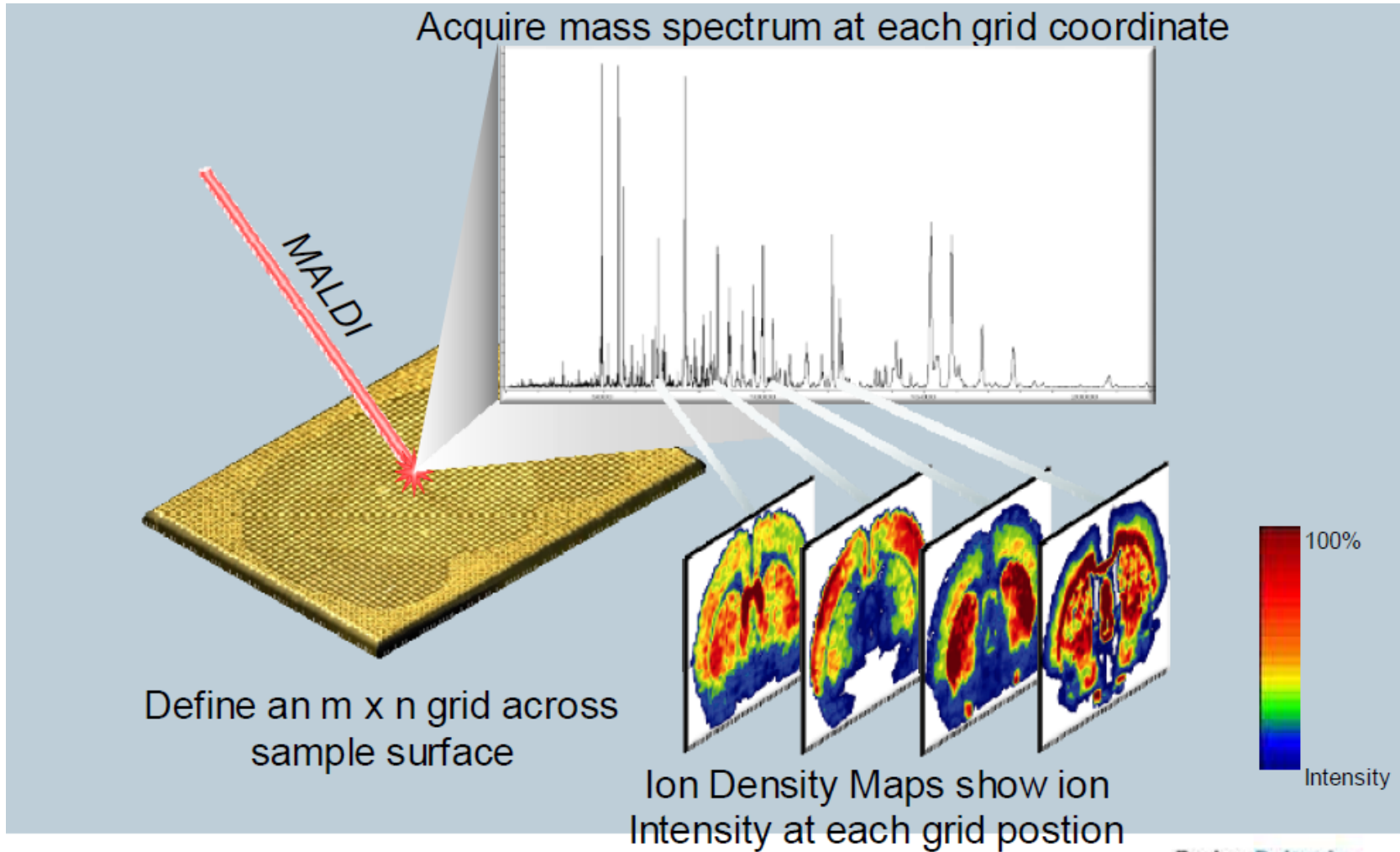
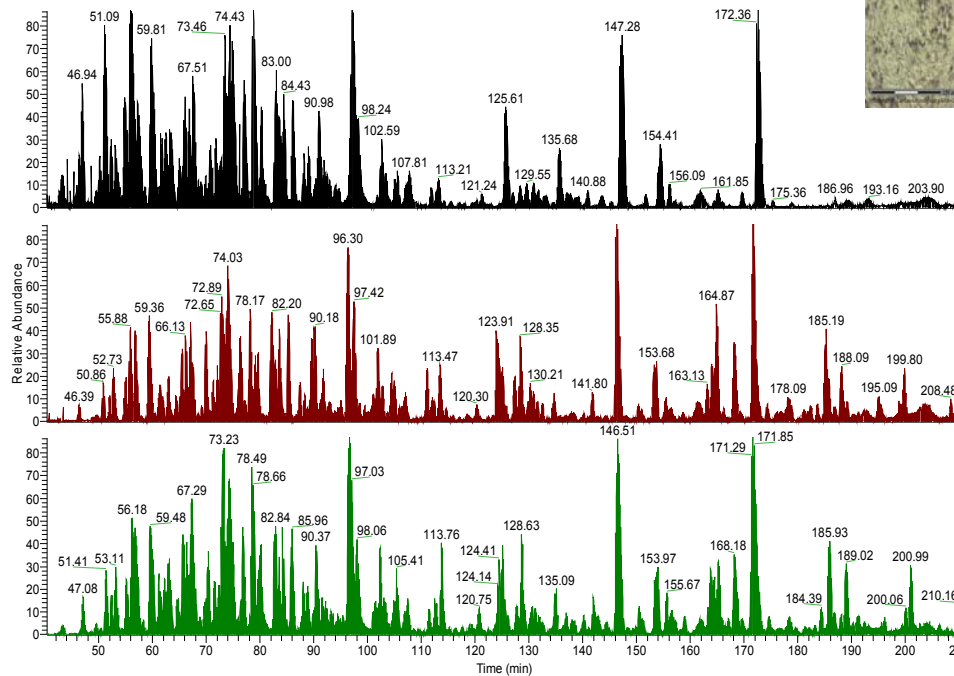
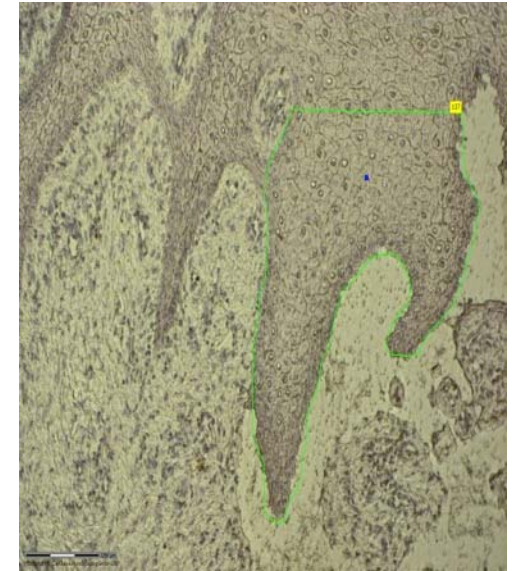
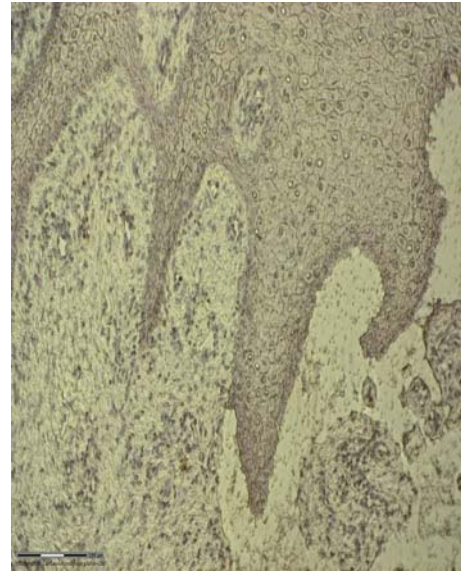
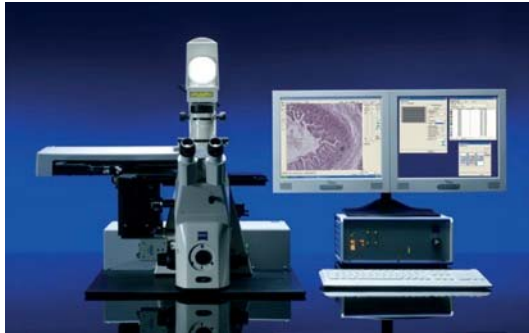


Image from 2014 ASMS Workshop

MALDI Imaging



Laser Capture Microdissection Proteomics



Facility Instruments

- Thermo LTQ Orbitrap
- Bruker ultrafleXtreme MALDI-TOF TOF
- Thermo Scientific LTQ
- Bruker maXis UHR QTOF
- Bruker amaZon ion trap with ETD
- Ettan Spot Handling Workstation
- Thermo Trace GC-MS
- Thermo DSQ II GC-MS
- Micromass Q-TOF II
- FPLC System

