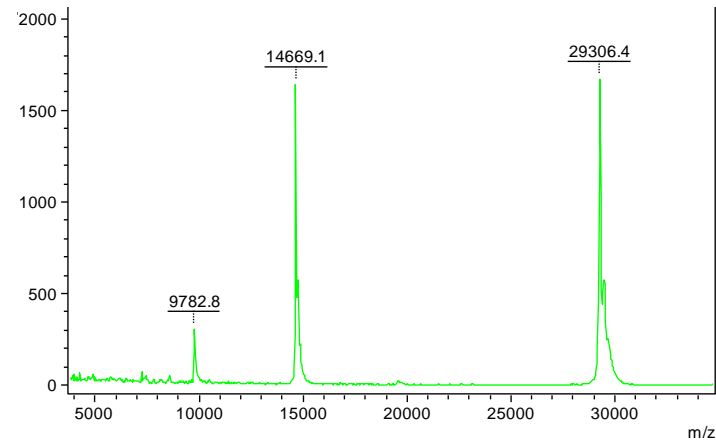
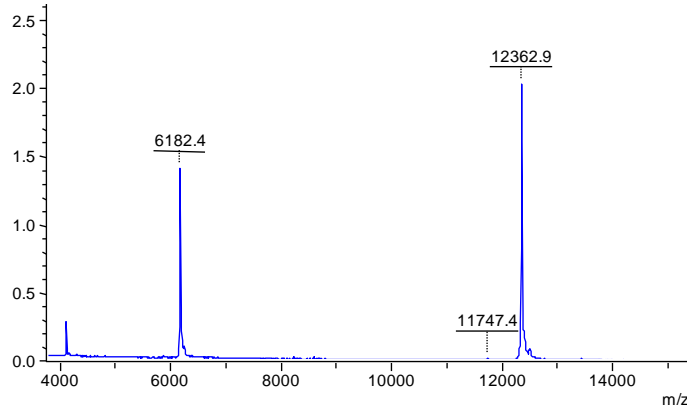


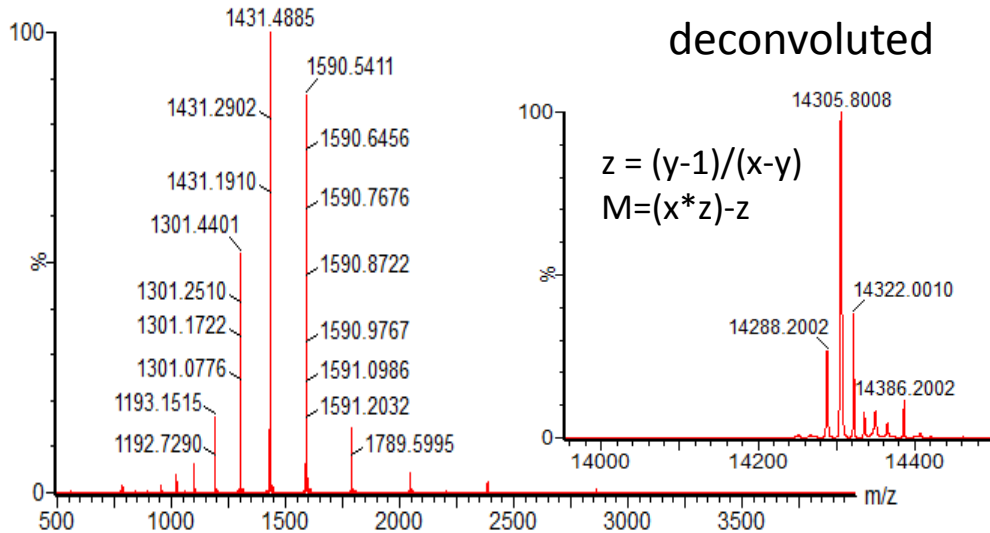
Characterisation of proteins and protein modification

Mass of Intact protein

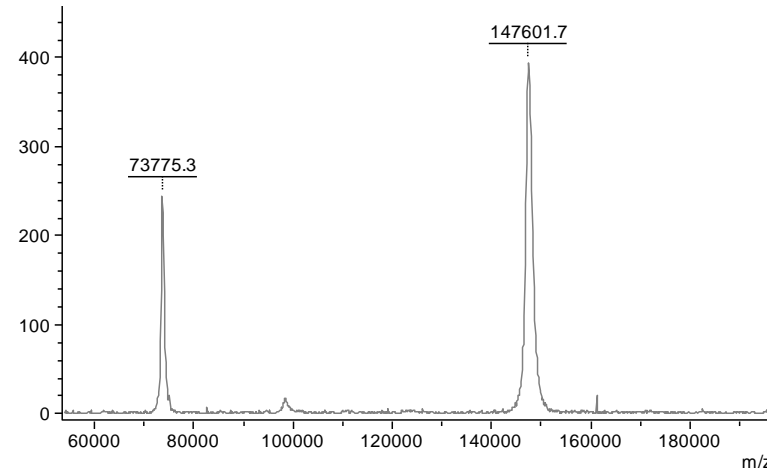
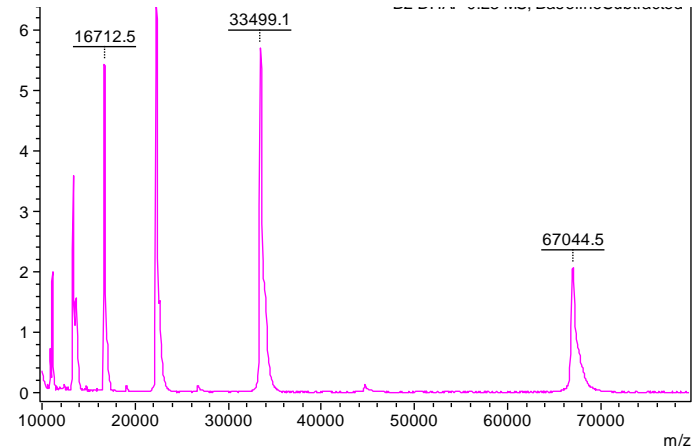
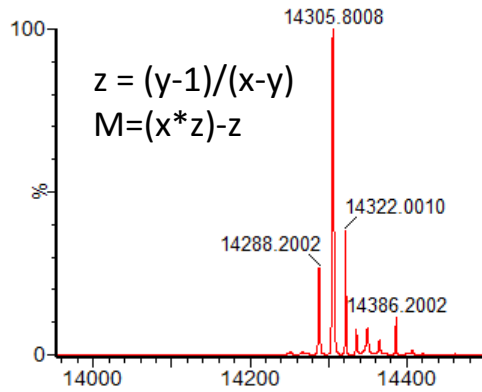
MALDI – TOF



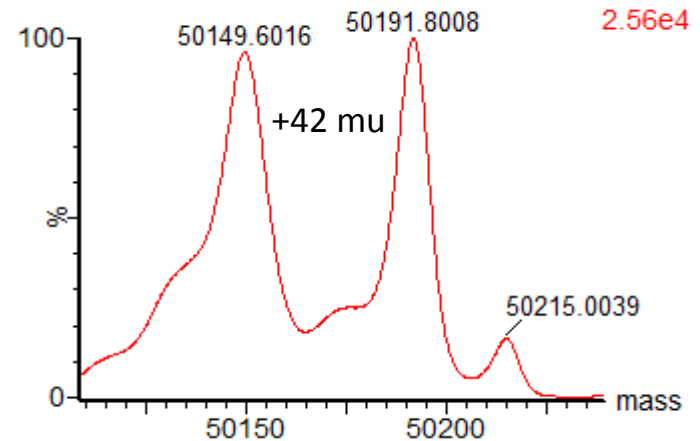
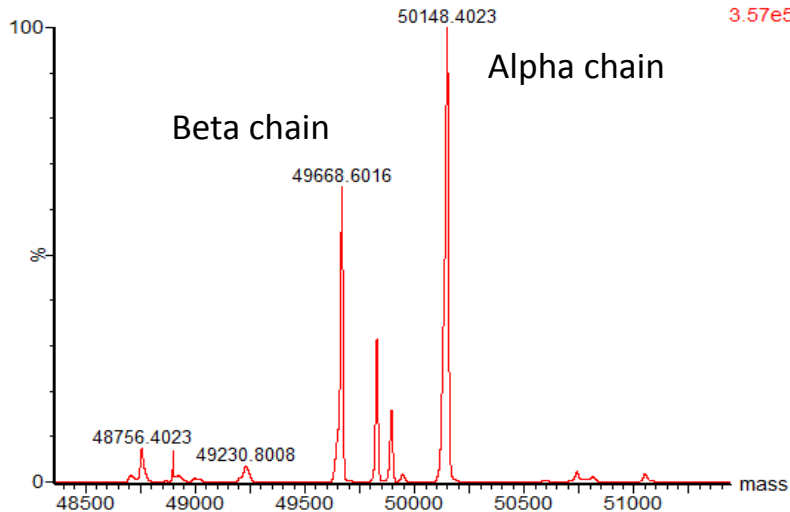
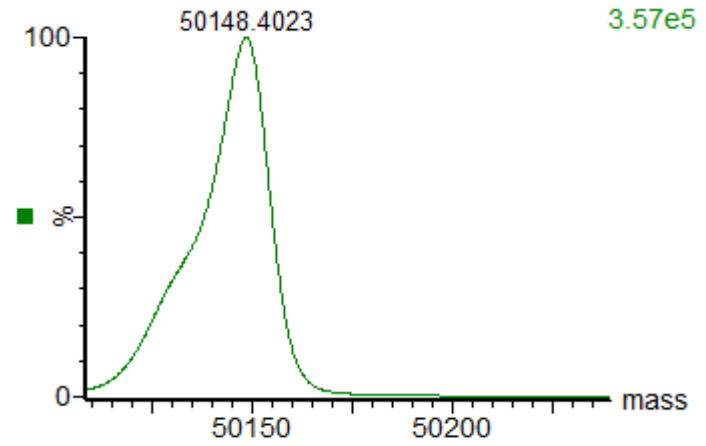
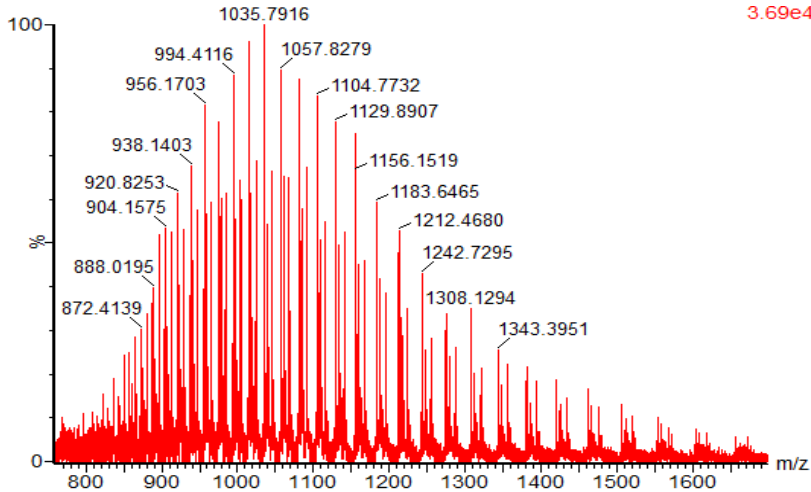
ESI – (TOF, IT, QTOF etc)



deconvoluted



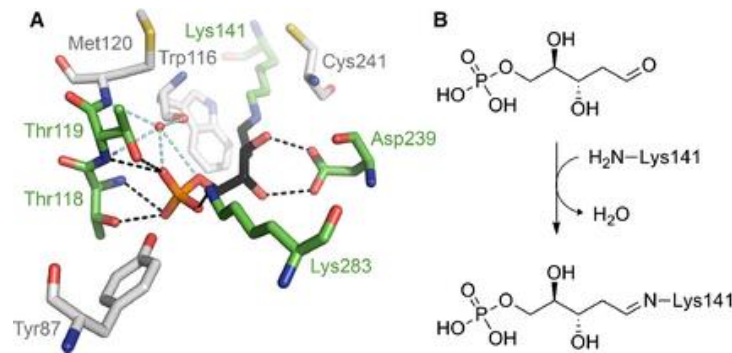
Tubulin- intact protein ESI



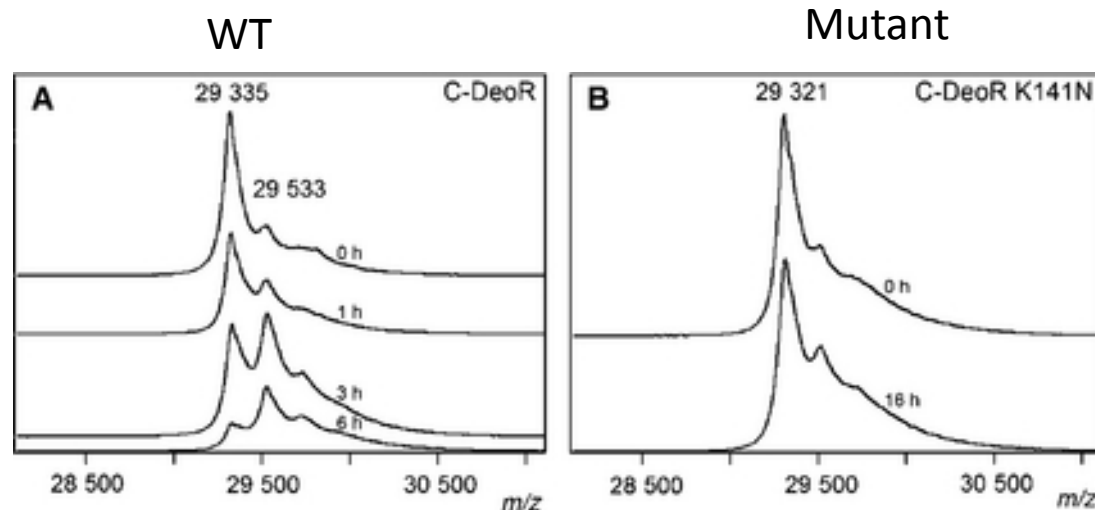
+ Histone deacetylase inhibitor

Deoxyribonucleoside regulator (DeoR)

- Incubation of deoxyribose-5-phosphate with DeoR results in covalent bond formation with Lys141



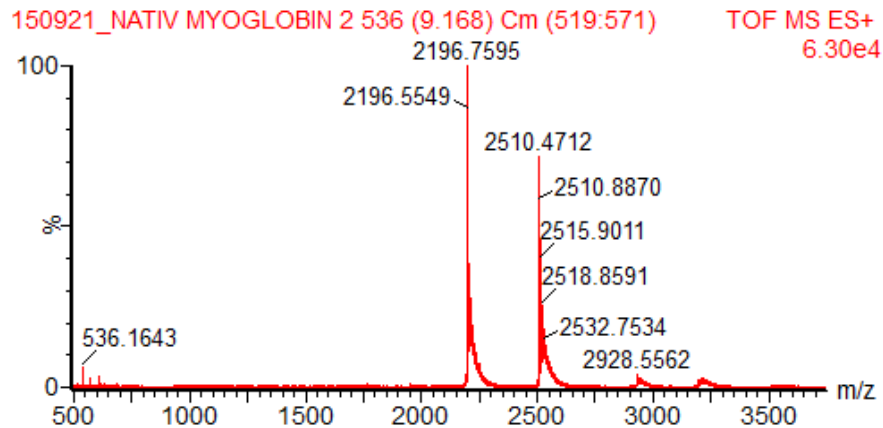
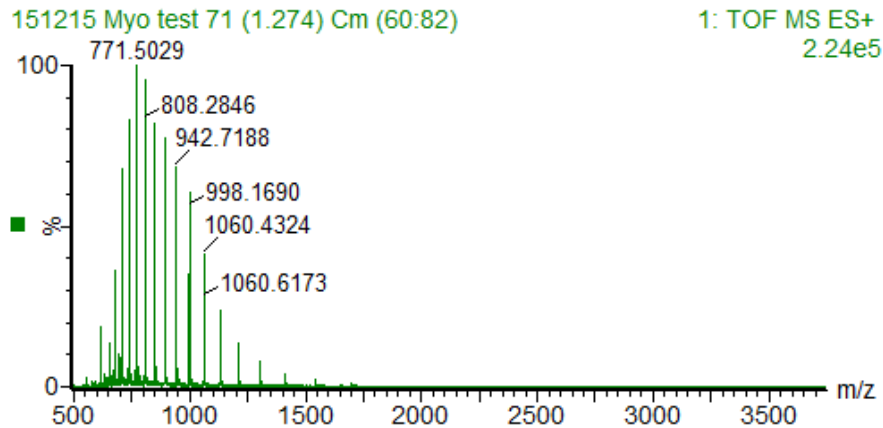
MALDI-TOF analysis



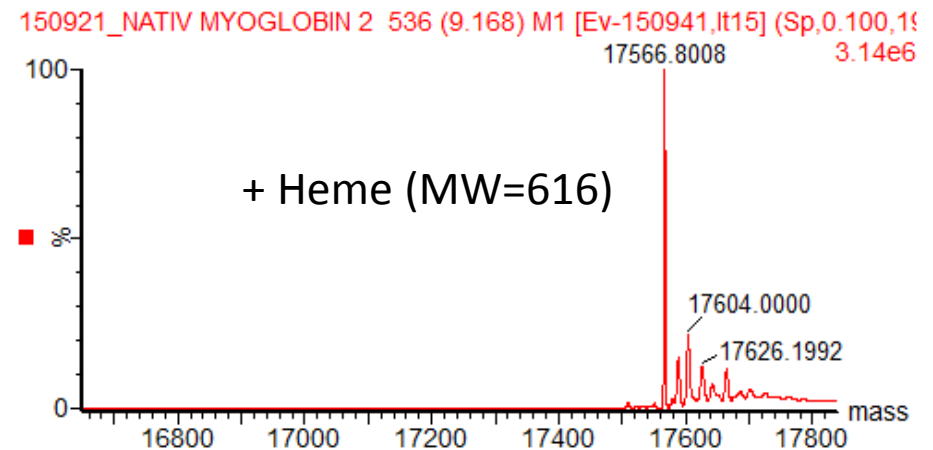
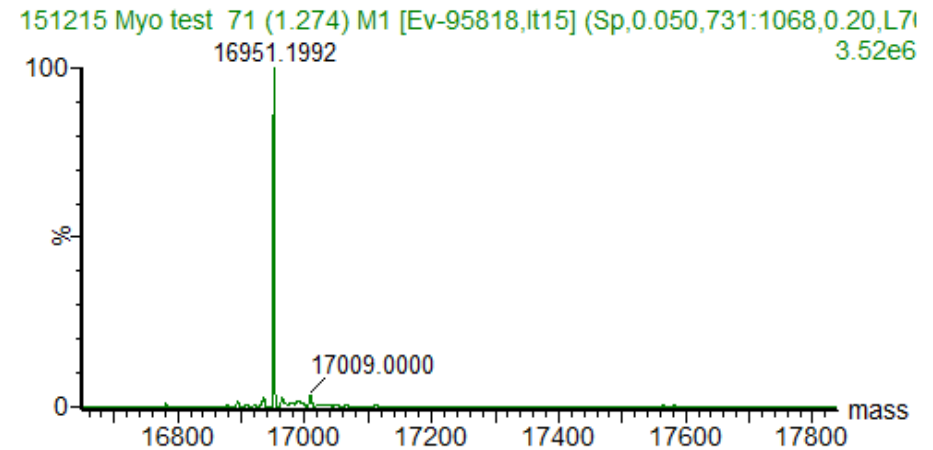
Native Intact ESI

- Keep protein native but at the same time use volatile buffer
 - Ammonium Acetate buffer (up to 200 mM)
 - Needs to be very well desalted
 - glass capillary nanospray needle

unprocessed



deconvoluted



Posttranslational modifications

Disulfide bridge mapping

PDI – aspartic protease inhibitor

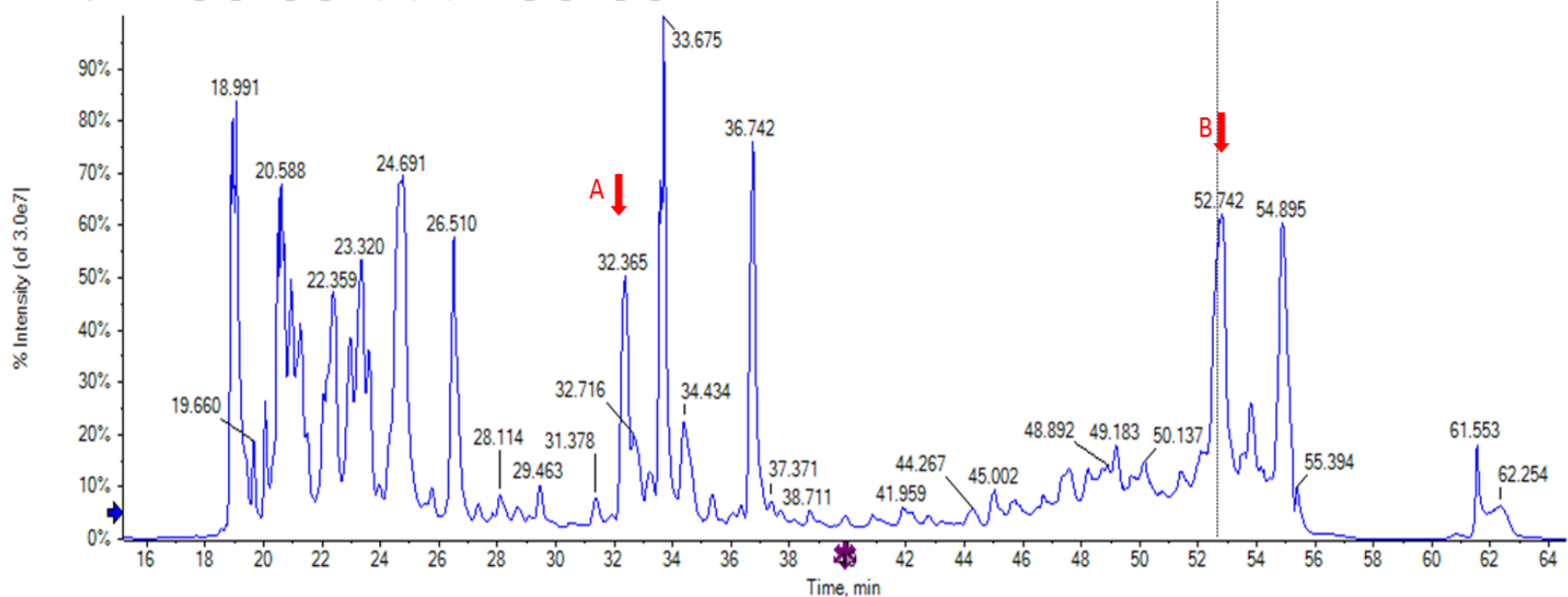
ESPLPKVLDTNGKELNPNSSYRIISIGRGALGGDVYLGKSPNSDAPCPDGVFRYNSDVG
PSGTPVRFIPLSGGIFEDQLLN IQFN IATVKLCVSYTIWKVGNLNAYFRTMLLETGGTIGQ
ADSSYFKIVKLSNFGYNLLYCPITPPFLCPF CRDDNFCAKVG VVIQNGKRRLALVNENPLD
VLFQEV

MW = 20590

Tryptic digest without reduction and alkylation

needed to be boiled to do not inhibit trypsin

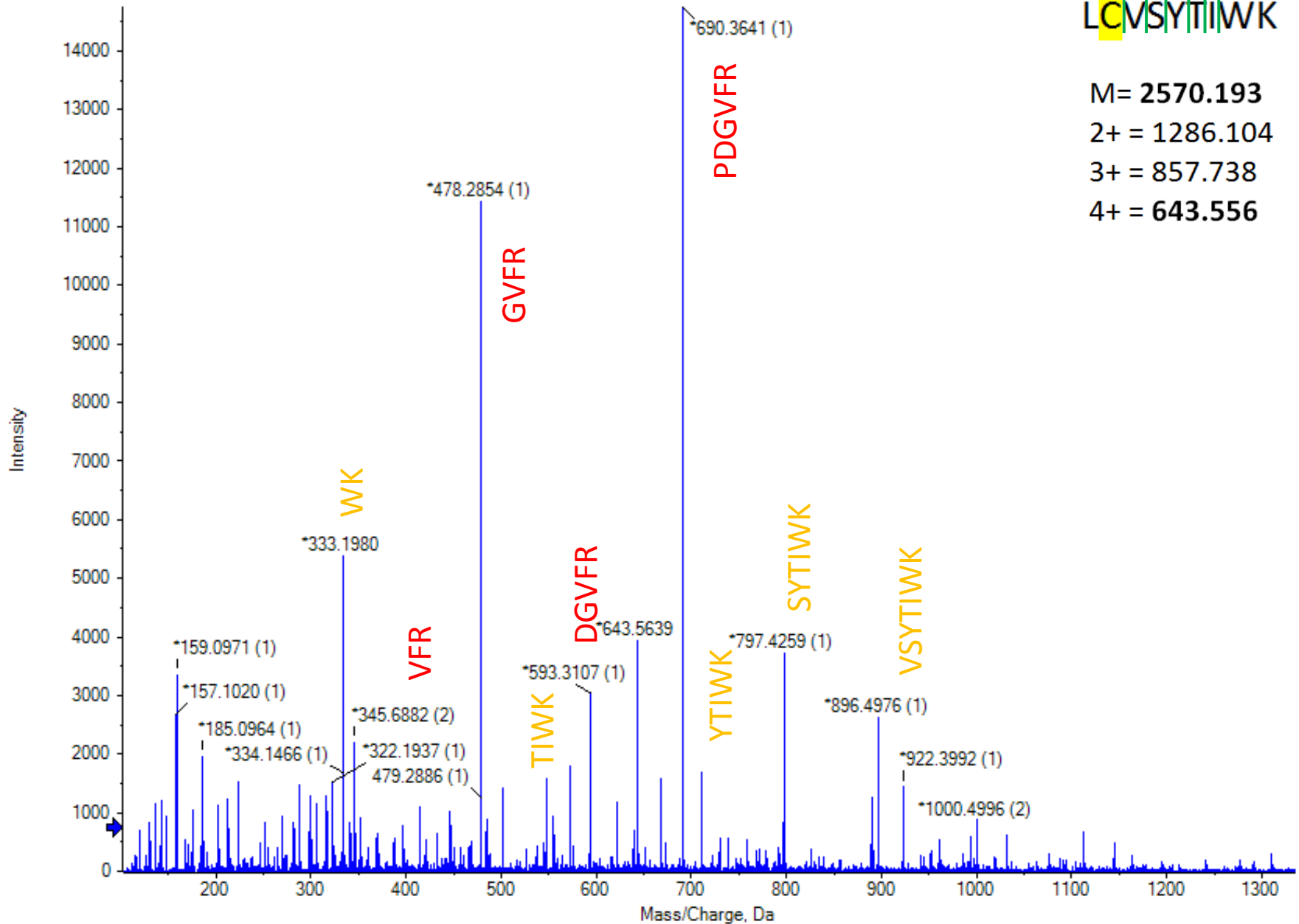
IDA Survey from 140506_PDI_Nered_Var_T.wiff (sample 1) - 140506_PDI_Nered_Var_T



Precursor: 643.6 Da

SPNSDAPCPDGVFR
LCVSYTIWK

M= 2570.193
2+ = 1286.104
3+ = 857.738
4+ = 643.556

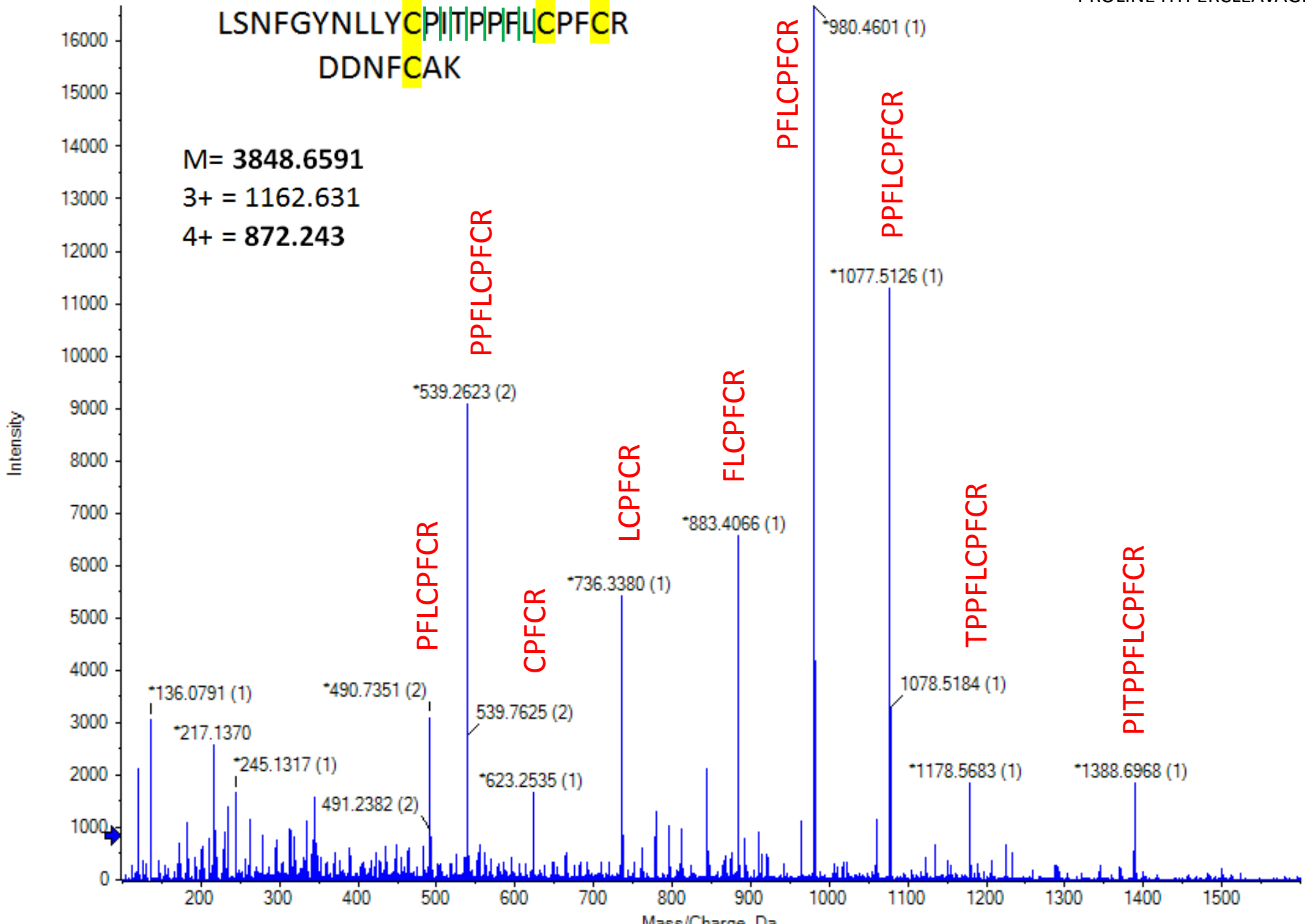


Precursor: 872.2 Da

PROLINE HYPERCLEAVAGE

LSNFGYNLLYCPITPPFLCPFCR
DDNFCAK

M= 3848.6591
3+ = 1162.631
4+ = 872.243



Disulfide formation within protein

ESPLPKPVLDTNGKELNPNSSYRIISIGRGALGGDVYLGKSPNSD
APCPDGVFRYNSDVGPSGTPVRFIPLSGGIFEDQLLNIQFNIAT
VKLCVSYTIWKVGNLNAYFRTMLLETGGTIGQADSSYFKIVKLS
NFGYNLLYCPITPPFLCPCRRDDNFC AKVGVVIQNGKRRRLALVN
ENPLDVLFQEV

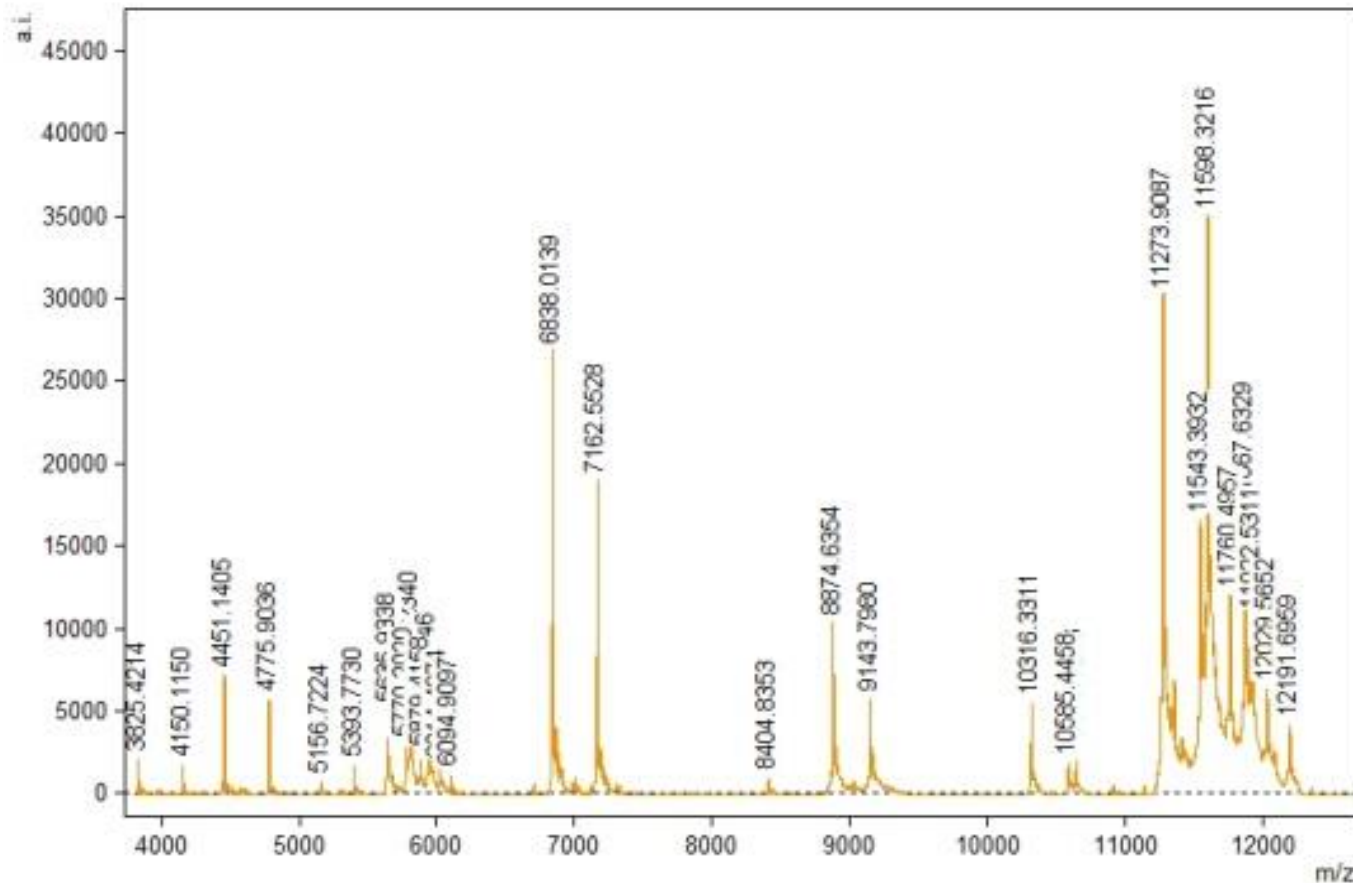
The diagram illustrates the formation of two disulfide bonds within a protein sequence. The sequence is shown in five lines. Cysteine residues are highlighted in red. Green lines connect the cysteines to show disulfide bonds: one between the first and second cysteines, and another between the third and fourth cysteines.

Cystatin (*Fasciola hepatica*)

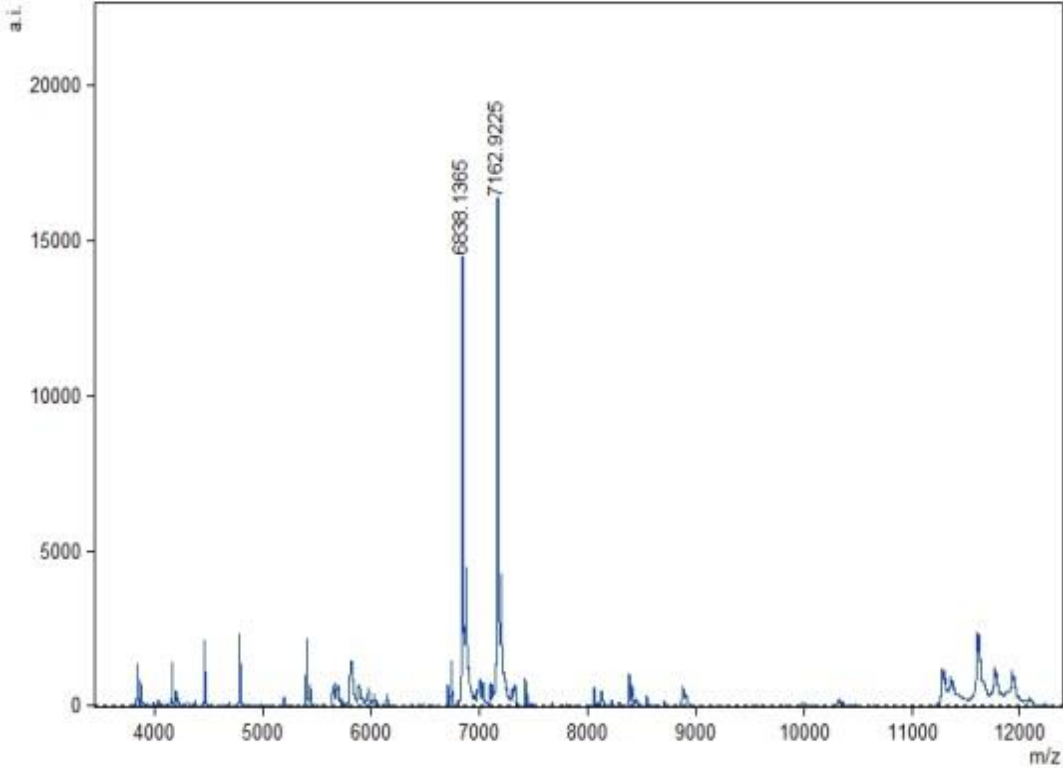
IRGEMLVGGYTEPRSVTPEERSVVFQPMILSKLLTAGSVVSSCELELLQVSTQVVAGTNY
KFKVSGGATCPGCWEVVVVFVPLYSSKSATSVGTPTRVSC TAAAHHHHHH

MW = 11528

MALDI-TOF spectrum of tryptic digest of non-reduced protein



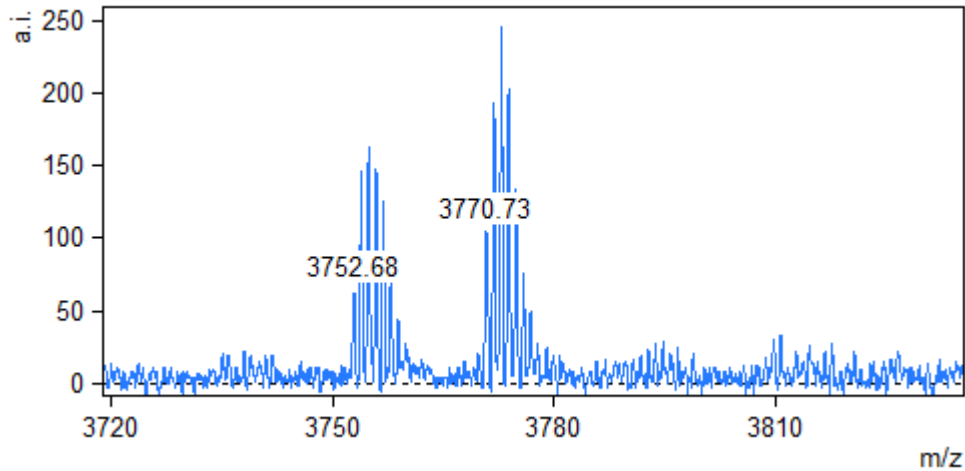
MALDI-TOF spectrum of tryptic digest followed by prolyl endopeptidase



LLTAGSVESS**C**ELELLQVSTQVVAGINYK
VSGGAT**C**PG**C**WEVVVFVPLYSSK
V**S**CTAAAHHHHHH

LLTAGSVESS**C**ELELLQVSTQVVAGINYK
VSGGAT**C**PG**C**WEVVVFVPLYSSK
V**S**CTAAAHHHHHH

MALDI-TOF spectrum of tryptic digest followed by GluC digestion

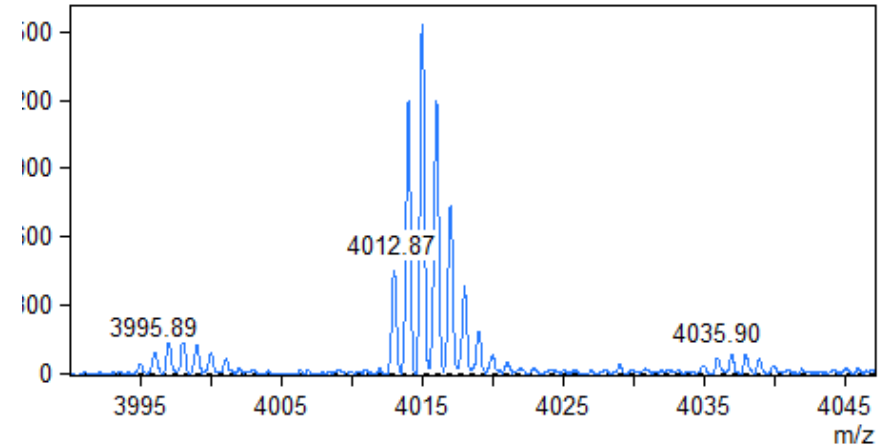


$[M+H]^+ = 3770,73$

LLTAGSVVSS**CE**

VSGGAT**CPGCWE**

VS**CT**AAAHHHHHH



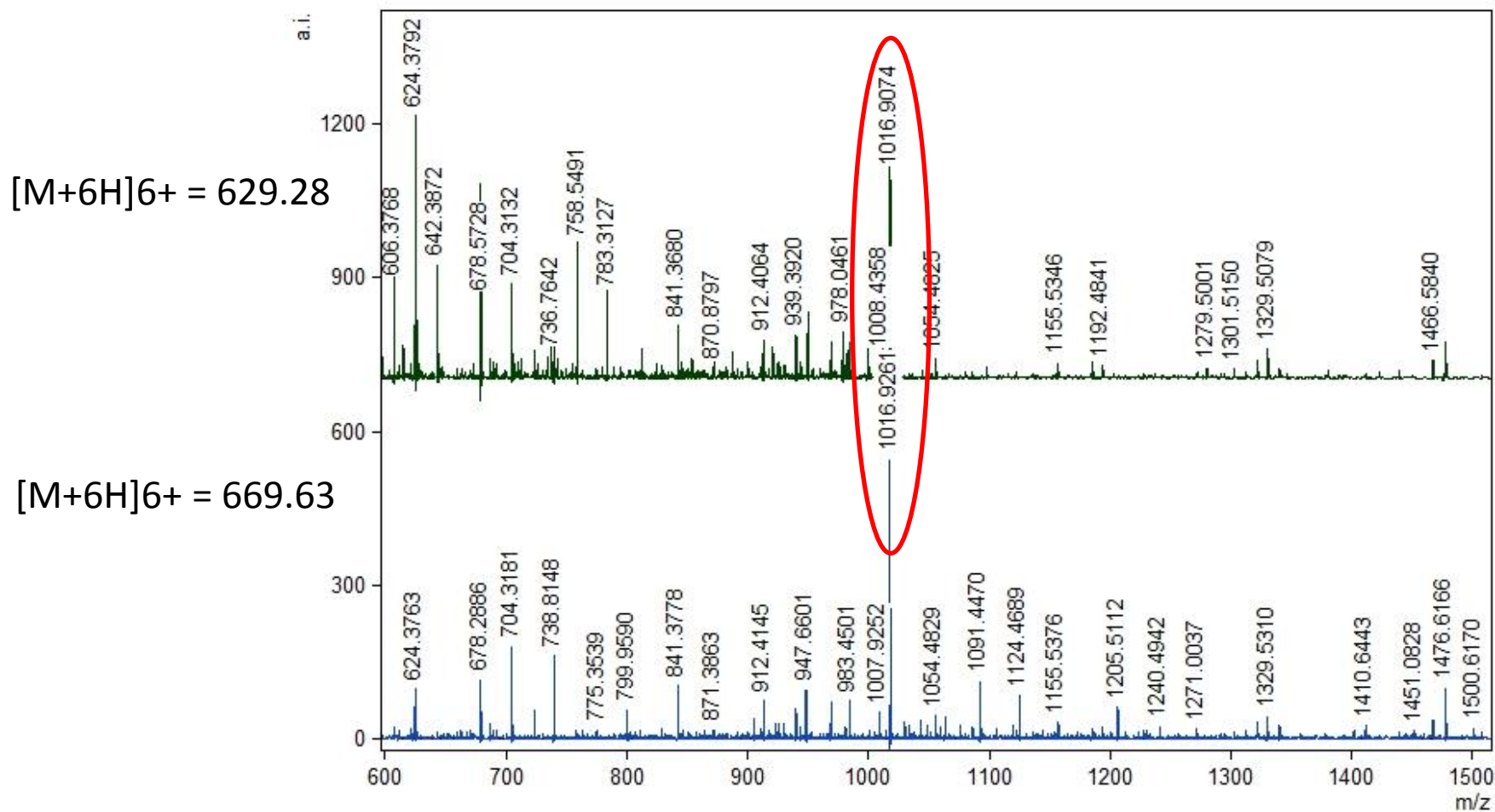
$[M+H]^+ = 4012,87$

LLTAGSVVSS**CELE**

VSGGAT**CPGCWE**

VS**CT**AAAHHHHHH

ESI-MS/MS spectrum of selected ions in T-GluC digest



$[M+2H]2+ = 1016,9$

PGCWE

VSC TAAAHHHHHH

Disulfide formation within protein

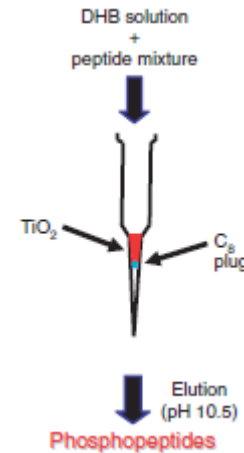
LLTAGSVVSS**CE**
VSGGAT**CPGCWE**
VS**CTAA**HHHHHH

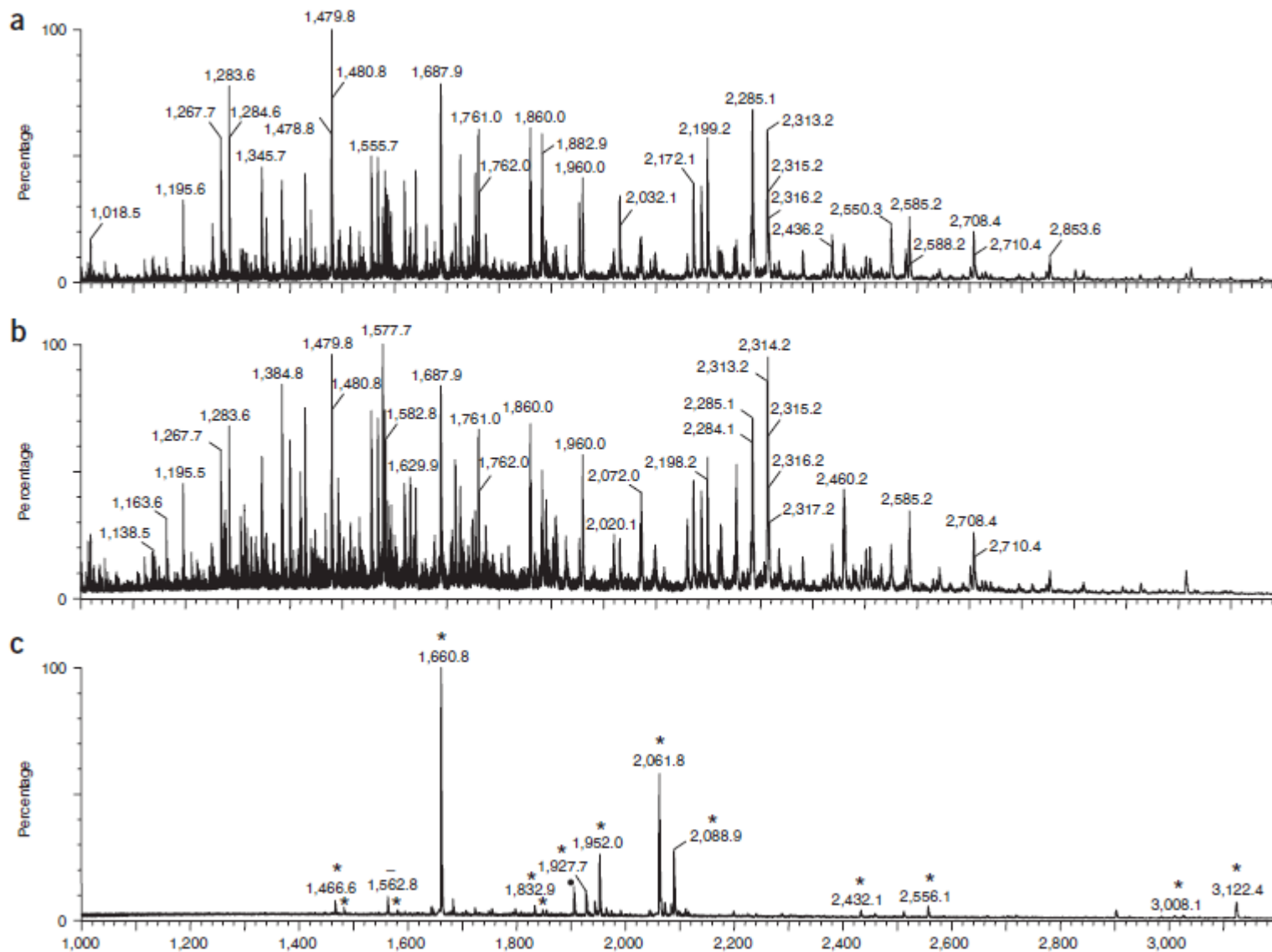
IRGEMLVGGYTEPRSVTPEERSVFQPM
ILSKLLTAGSVVSS**CE**LELLQVSTQVVAG
TNYKFKVSGGAT**CPGCWE**VVVVFVPLY
SSKSATSVGTPTRVS**CTAA**HHHHHH

Posttranslational modification

phosphorylation

- identification
- Enrichment for Phosphopeptides
 - TiO₂ column
 - Measurement on MALDI-TOF/TOF to get the peptide MS and confirm by MS/MS





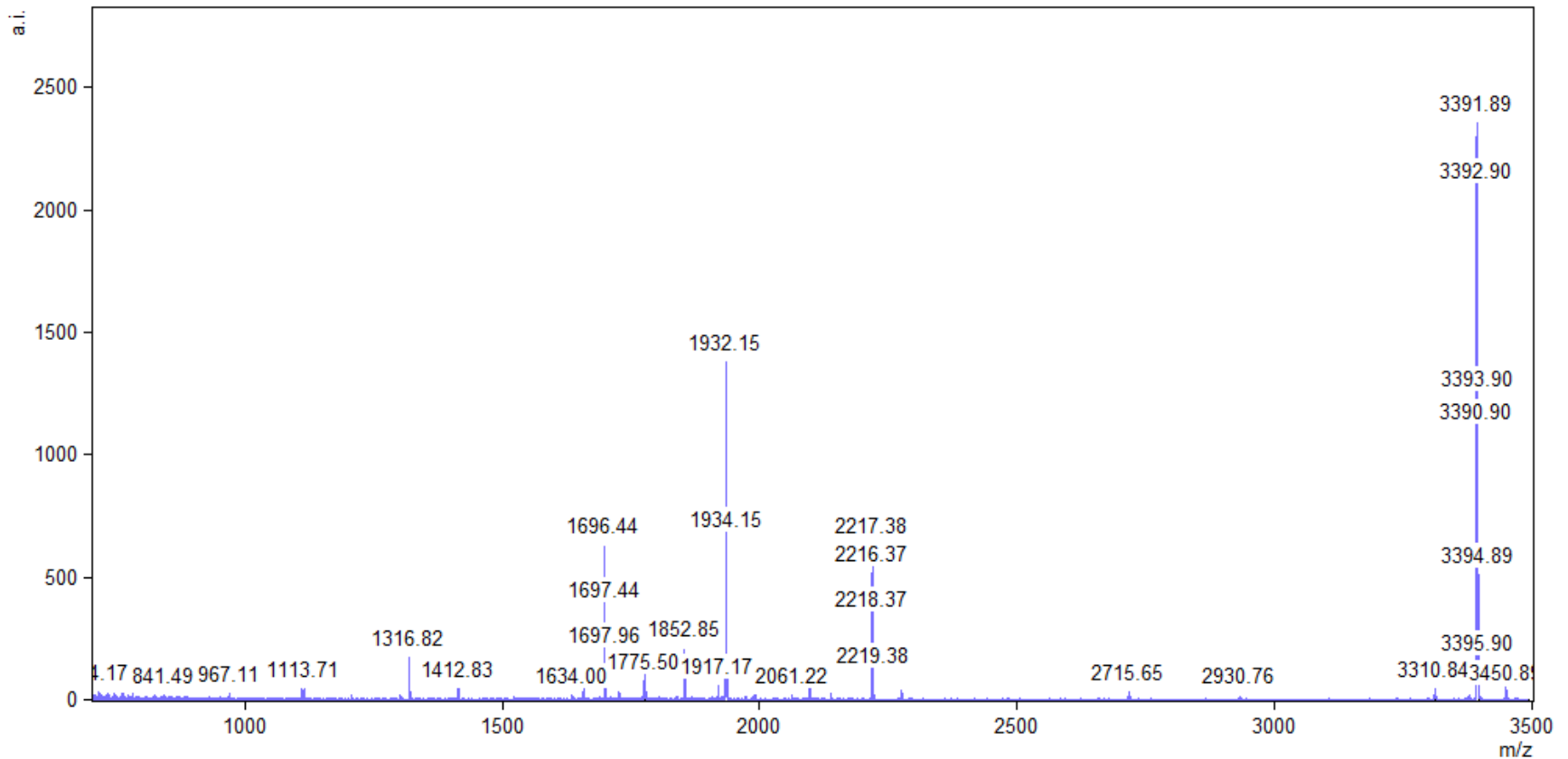
Highly selective enrichment of phosphorylated peptides using titanium dioxide

Tine E Thingholm, Thomas J D Jørgensen, Ole N Jensen & Martin R Larsen

NATURE PROTOCOLS | VOL1 NO.4 | 2006 | 1929

Example: Phosphatidylinositol 4-kinase Beta

- Phosphoenrichment



- Measurement of phosphopeptides in the peptide mixture with other peptides during LC-MSMS
- phospho modification set during searching
- quantification possible, e.g. SWATH

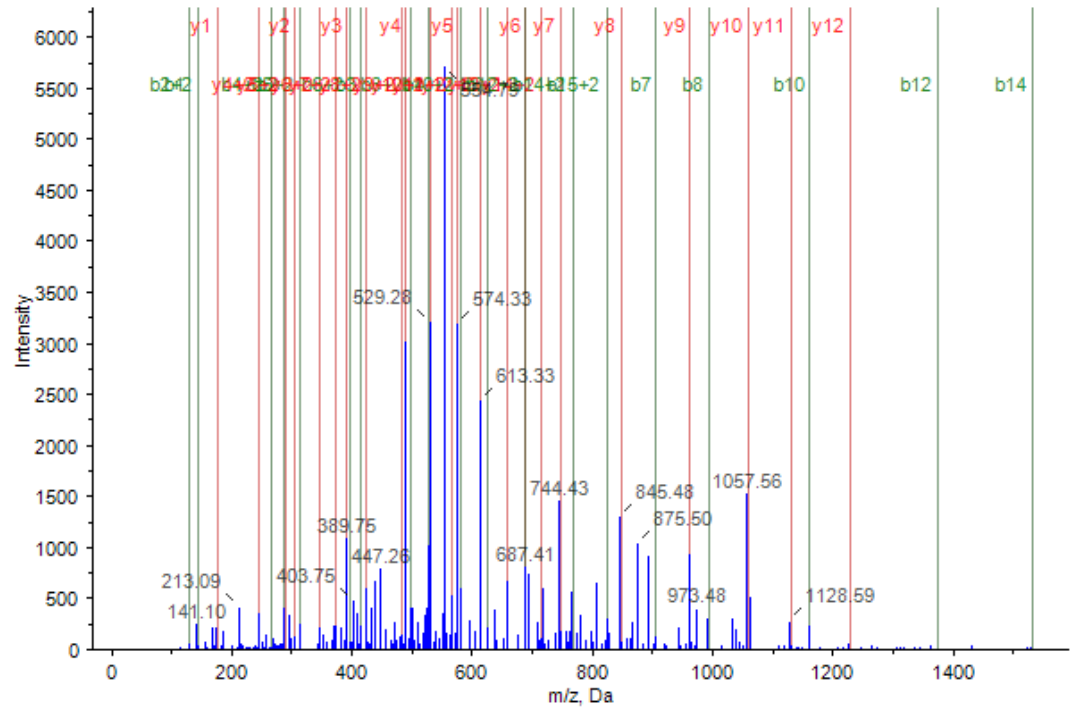
LC-MSMS experiments without enrichment

KRELPSLSPAPDTGLSPSKR

2215.123

KRELPS[Pho]LSPAPDTGLSPSKR

Residue	b	b+2	y	y+2
K	129.1022	65.0548	2216.1435	1108.5754
R	285.2034	143.1053	2088.0485	1044.5279
E	414.2459	207.6266	1931.9474	966.4773
L	527.3300	264.1686	1802.9048	901.9560
P	624.3828	312.6950	1689.8207	845.4140
S[Pho]	791.3811	396.1942	1592.7680	796.8876
L	904.4652	452.7362	1425.7696	713.3884
S	991.4972	496.2522	1312.6856	656.8464
P	1088.5500	544.7786	1225.6535	613.3304
A	1159.5871	580.2972	1128.6008	564.8040
P	1256.6399	628.8236	1057.5636	529.2855
D	1371.6668	686.3370	960.5109	480.7591
T	1472.7145	736.8609	845.4839	423.2456
G	1529.7359	765.3716	744.4363	372.7218
L	1642.8200	821.9136	687.4148	344.2110
S	1729.8520	865.4297	574.3307	287.6690
P	1826.9048	913.9560	487.2987	244.1530
S	1913.9368	957.4721	390.2459	195.6266
K	2042.0318	1021.5195	303.2139	152.1106
R	2198.1329	1099.5701	175.1190	88.0631

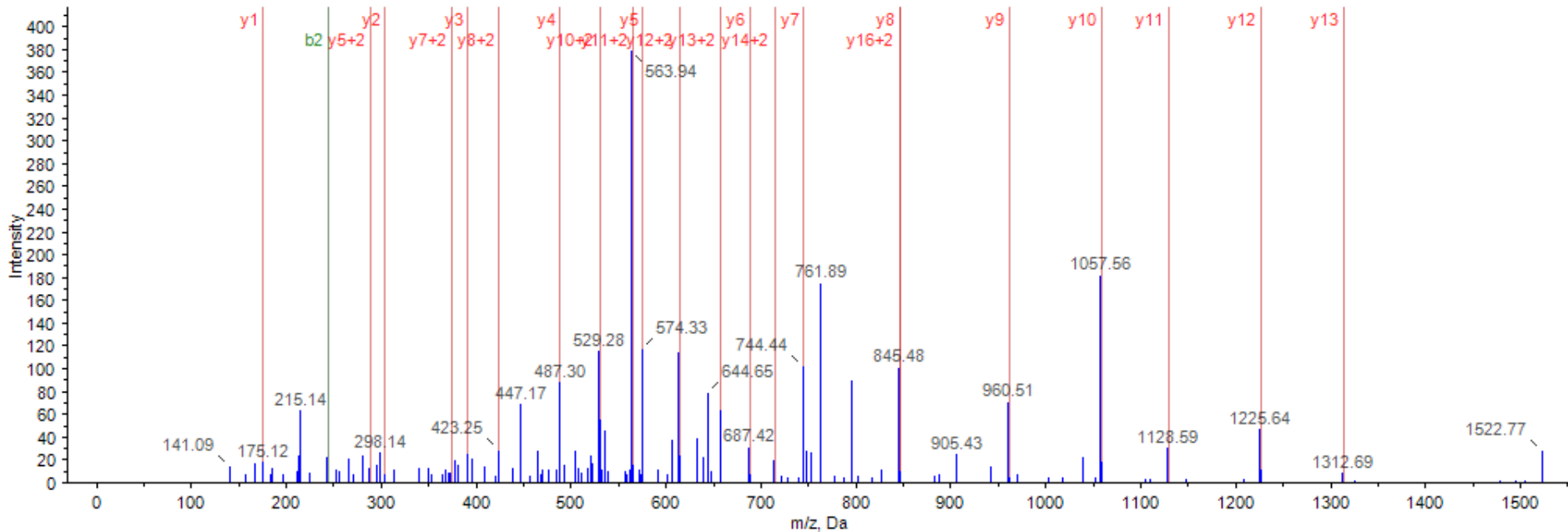


ELPSLSPAPDTGLSPSKR

1930.932

ELPS[Pho]LSPAPDTGLSPSKR

Residue	b	b+2	y	y+2
E	130.0499	65.5286	1931.9474	966.4773
L	243.1339	122.0706	1802.9048	901.9560
P	340.1867	170.5970	1689.8207	845.4140
S[Pho]	507.1851	254.0962	1592.7680	796.8876
L	620.2691	310.6382	1425.7696	713.3884
S	707.3011	354.1542	1312.6856	656.8464
P	804.3539	402.6806	1225.6535	613.3304
A	875.3910	438.1992	1128.6008	564.8040
P	972.4438	486.7255	1057.5636	529.2855
D	1087.4707	544.2390	960.5109	480.7591
T	1188.5184	594.7628	845.4839	423.2456
G	1245.5399	623.2736	744.4363	372.7218
L	1358.6239	679.8156	687.4148	344.2110
S	1445.6560	723.3316	574.3307	287.6690
P	1542.7087	771.8580	487.2987	244.1530
S	1629.7408	815.3740	390.2459	195.6266
K	1757.8357	879.4215	303.2139	152.1106
R	1913.9368	957.4721	175.1190	88.0631



Fragmentation Evidence

RAS[Pho]NPKVENEDEELSSSTESIDNSFSSPVR

Residue	b	b+2	y	y+2
R	157.1084	79.0578	3391.4809	1696.2441
A	228.1455	114.5764	3235.3798	1618.1935
S[Pho]	395.1439	198.0756	3164.3427	1582.6750
N	509.1868	255.0970	2997.3443	1499.1758
P	606.2396	303.6234	2883.3014	1442.1543
K	734.3345	367.6709	2786.2486	1393.6280
V	833.4029	417.2051	2658.1537	1329.5805
E	962.4455	481.7264	2559.0853	1280.0463
N	1076.4884	538.7479	2430.0427	1215.6250
E	1205.5310	603.2692	2315.9998	1158.5035
D	1320.5580	660.7826	2186.9572	1093.9822
E	1449.6006	725.3039	2071.9302	1036.4687
E	1578.6432	789.8252	1942.8876	971.9474
L	1691.7272	846.3673	1813.8450	907.4262
S	1778.7593	889.8833	1700.7610	850.8841
S	1865.7913	933.3993	1613.7289	807.3681
S	1952.8233	976.9153	1526.6969	763.8521
T	2053.8710	1027.4391	1439.6649	720.3361
E	2182.9136	1091.9604	1338.6172	669.8122
S	2269.9456	1135.4764	1209.5746	605.2909
I	2383.0297	1192.0185	1122.5426	561.7749
D	2498.0566	1249.5320	1009.4585	505.2329
N[Dea]	2613.0836	1307.0454	894.4316	447.7194
S	2700.1156	1350.5614	779.4046	390.2060
F	2847.1840	1424.0956	692.3726	346.6899
S	2934.2160	1467.6117	545.3042	273.1557
S	3021.2481	1511.1277	458.2722	229.6397
P	3118.3008	1559.6541	371.2401	186.1237
V	3217.3692	1609.1883	274.1874	137.5973
R	3373.4704	1687.2388	175.1190	88.0631

RASPKVENEDEELSSSTESIDNSFSSPVR

3390.469

