MDC
mass spectrometry

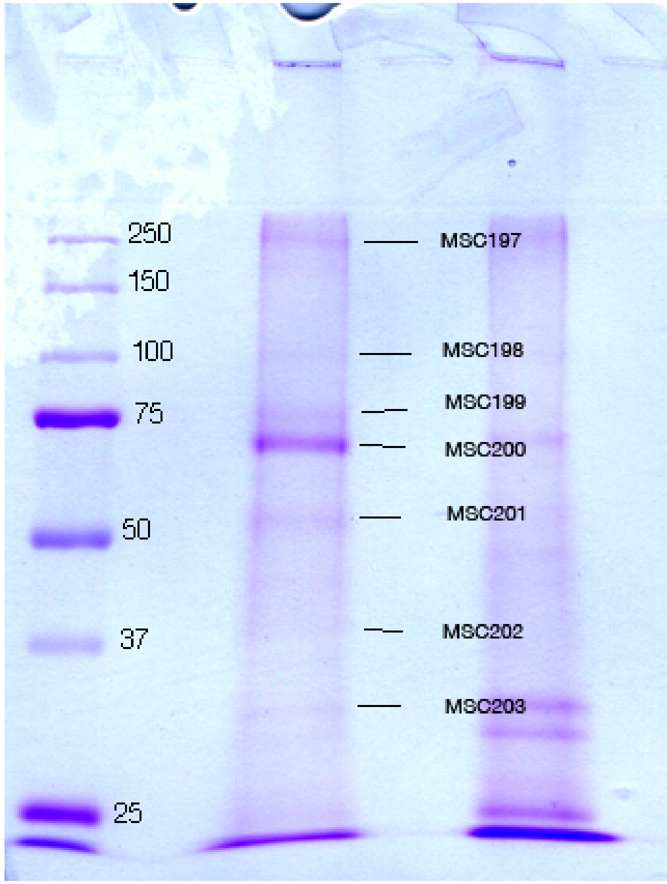
Current techniques in proteomics

Gunnar Dittmar

MDC
mass spectrometry

In-Gel digest

Samples from SDS-PAGE



→ band cut out of the gel → in-gel alkylation → in-gel digest

↓

peptide extraction

↓

MS

In-gel digest from SDS-PAGE

Advantages

- high resolution
- easy to use
- compatible with Immunprecipitations

Disadvantages

- low recovery of peptides
- coomassie stainable band recommended
- not compatible with all staining methods

Amounts

Staining method	Detection limit	Molar amounts
Coomassie	~50 ng	5 pmol (10 kDa Protein) 1 pmol (50 kDa Protein) 0.5 pmol (100 kDa Protein)
colloidal Coomassie	10-20 ng	1 pmol (10 kDa Protein) 0.2 pmol (50 kDa Protein) 0.1 pmol (100 kDa Protein)
Silver Stain	1-5 ng	100 fmol (10 kDa Protein) 20 fmol (50 kDa Protein) 10 fmol (100 kDa Protein)

In-gel digest and alternatives

method	proteins identified
in-gel digest	6
elution with 8M Urea in-solution digest	58
high pH elution in-solution digest	189

Alternatives to the gel digest

Alternatives to the in-gel digest

- **in solution digest**

  \+ fast

  \+ sensitive

  \- no protease inhibitors

  \- no detergents

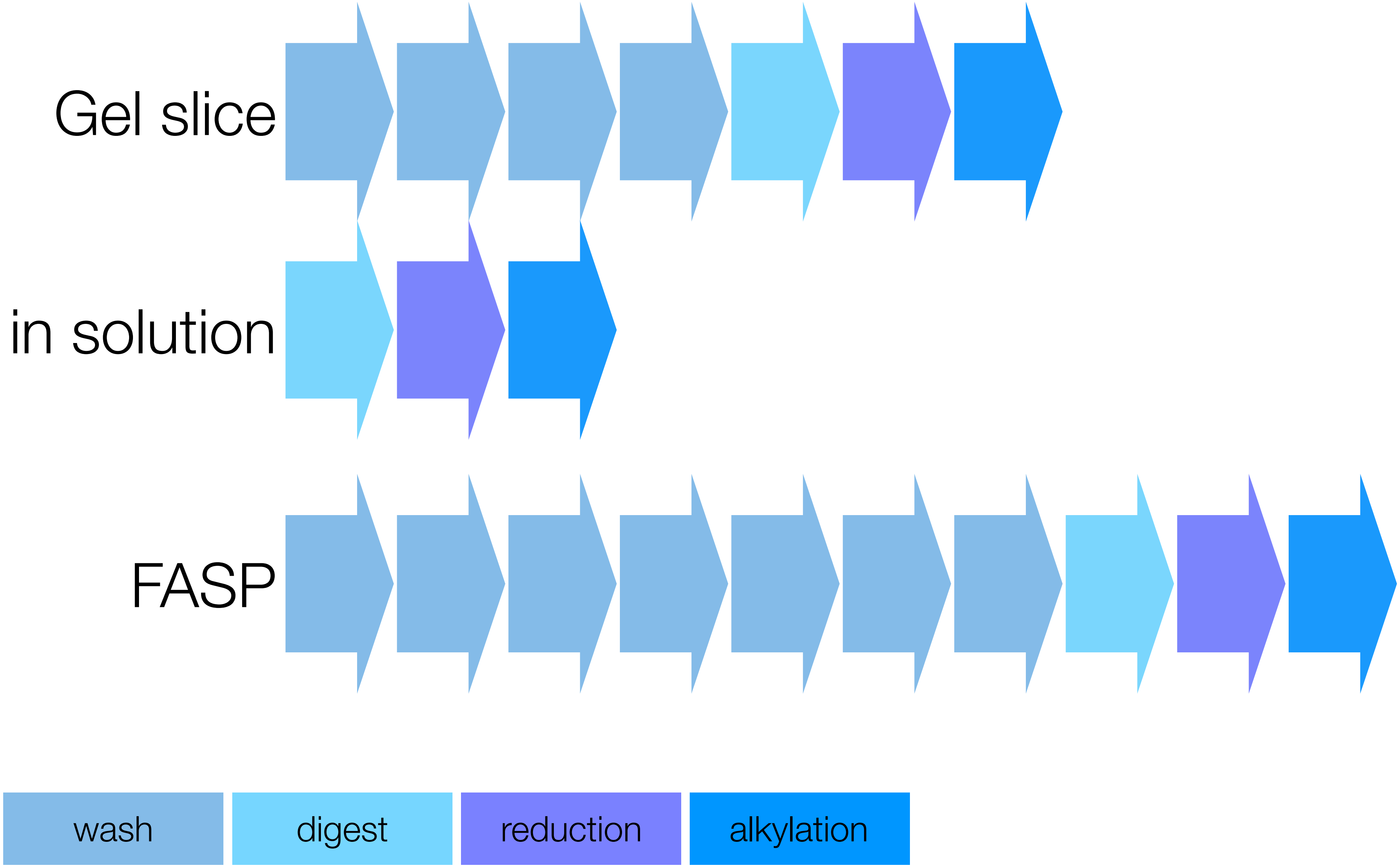
- **filter associated digest (FASP)**

  \+ can deal with detergents and protease inhibitors

  \- many lengthy wash steps

  \- rather significant losses

Work flow



Gel slice

in solution

FASP

wash

digest

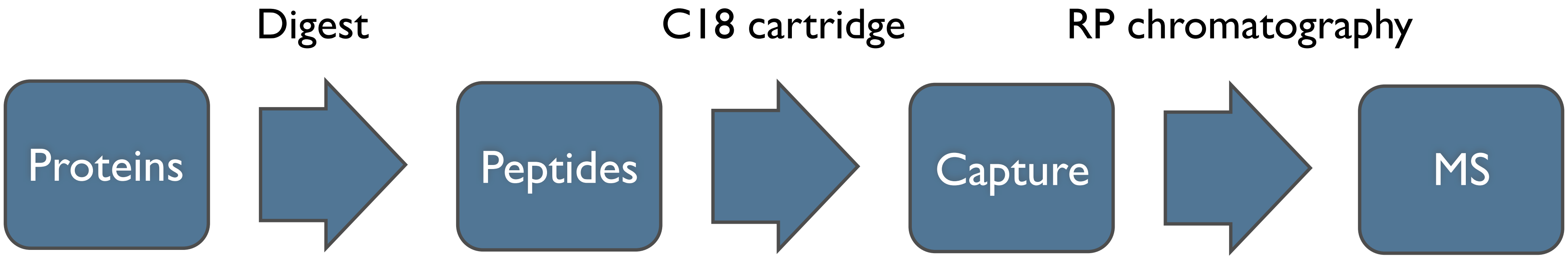
reduction

alkylation

MDC
mass spectrometry

What can go wrong...

MS workflow



Digest

C18 cartridge

RP chromatography

Proteins

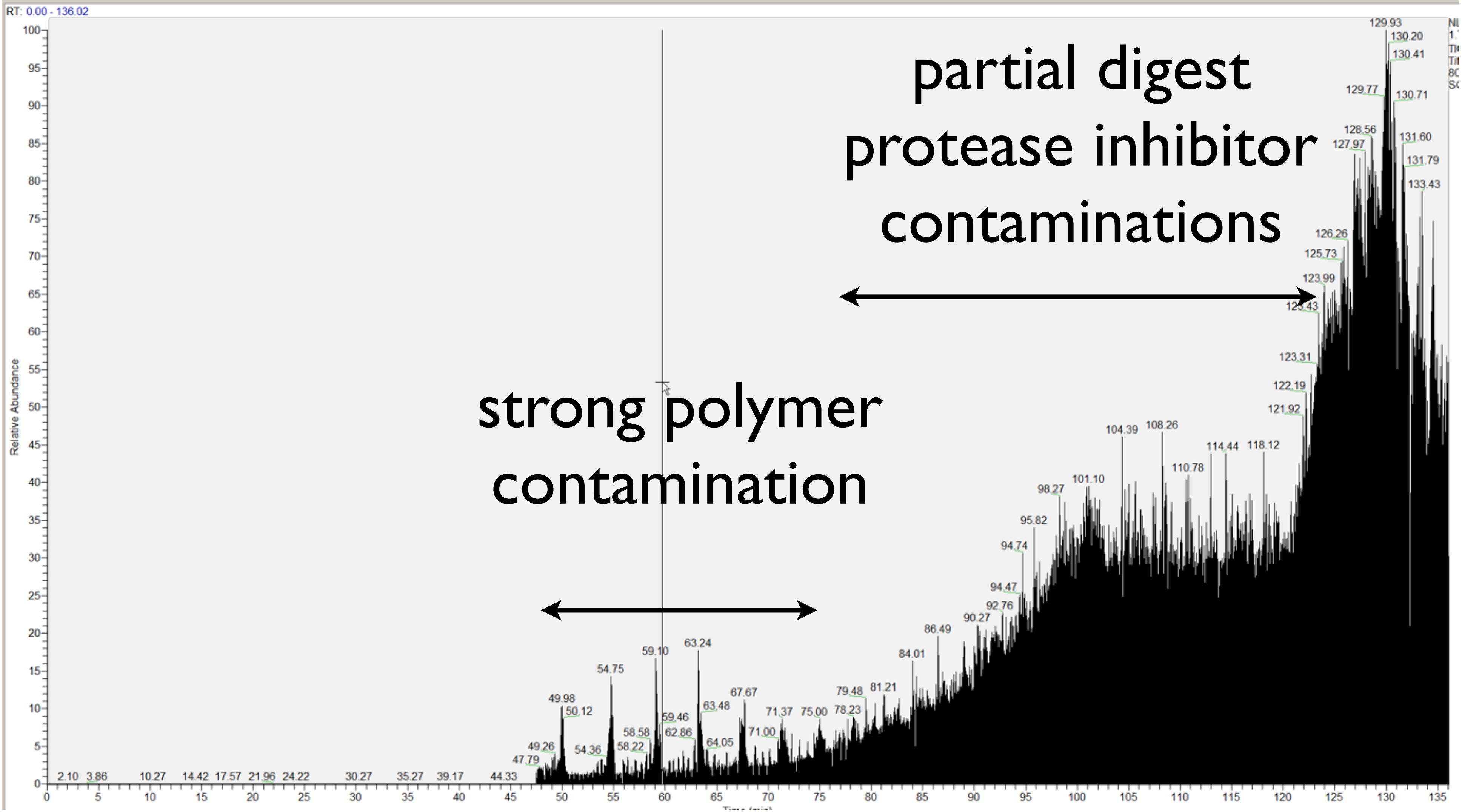
Peptides

Capture

MS

- Protease Inhibitors
- Polymers
- Detergents

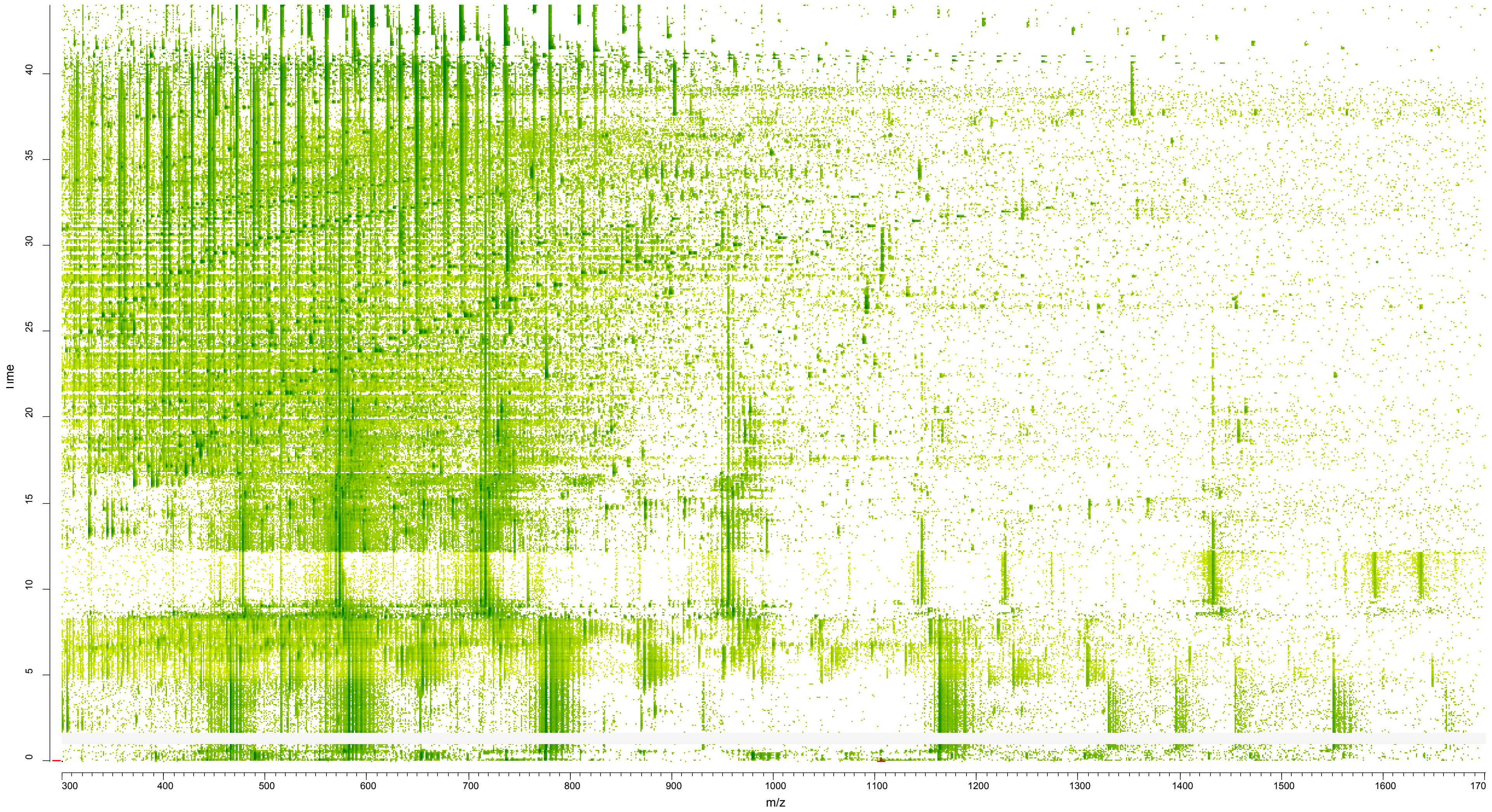
...and if something goes wrong



partial digest
protease inhibitor
contaminations

strong polymer
contamination

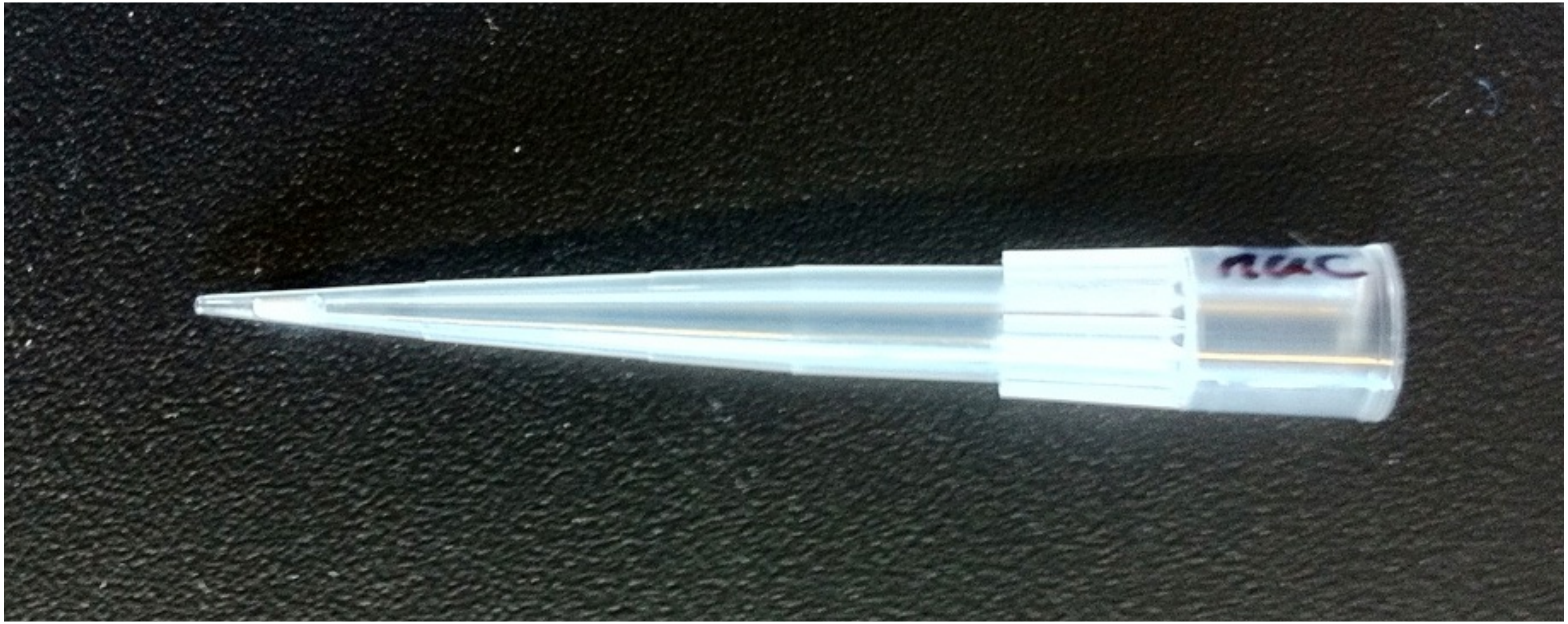
Polymer contamination



Handling tips

- Gloves, no touching!
- NO detergents if possible
- NO protease inhibitors
- small volumes

Peptide capture column



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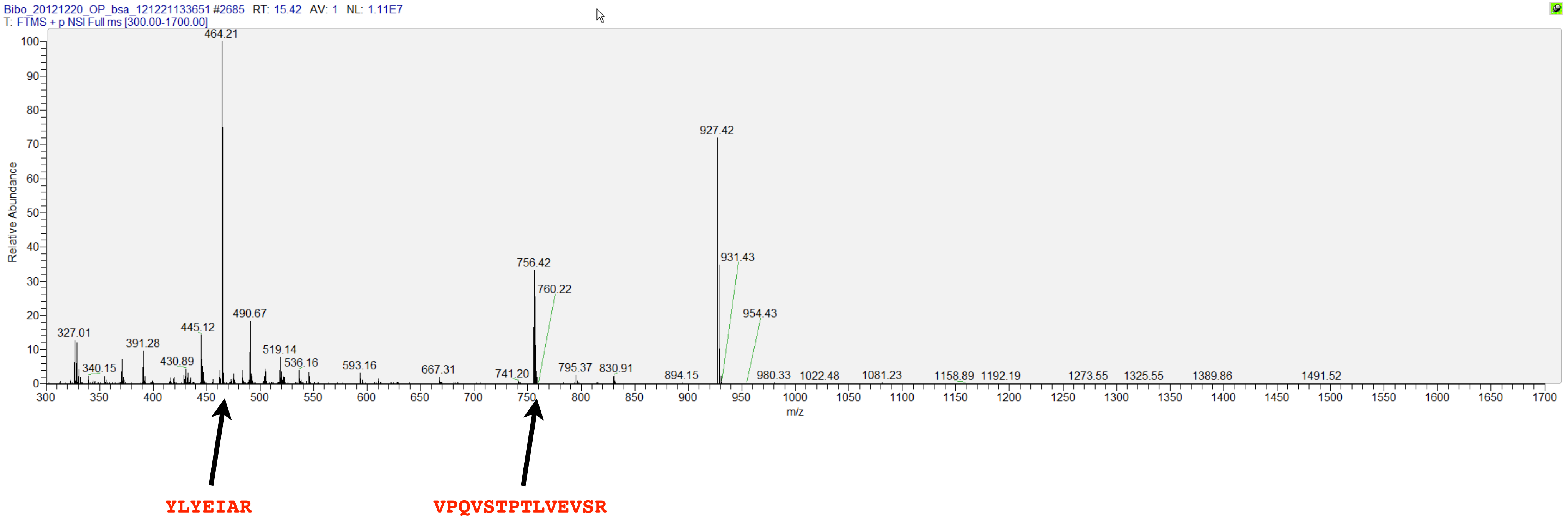
Quantitative Proteomics

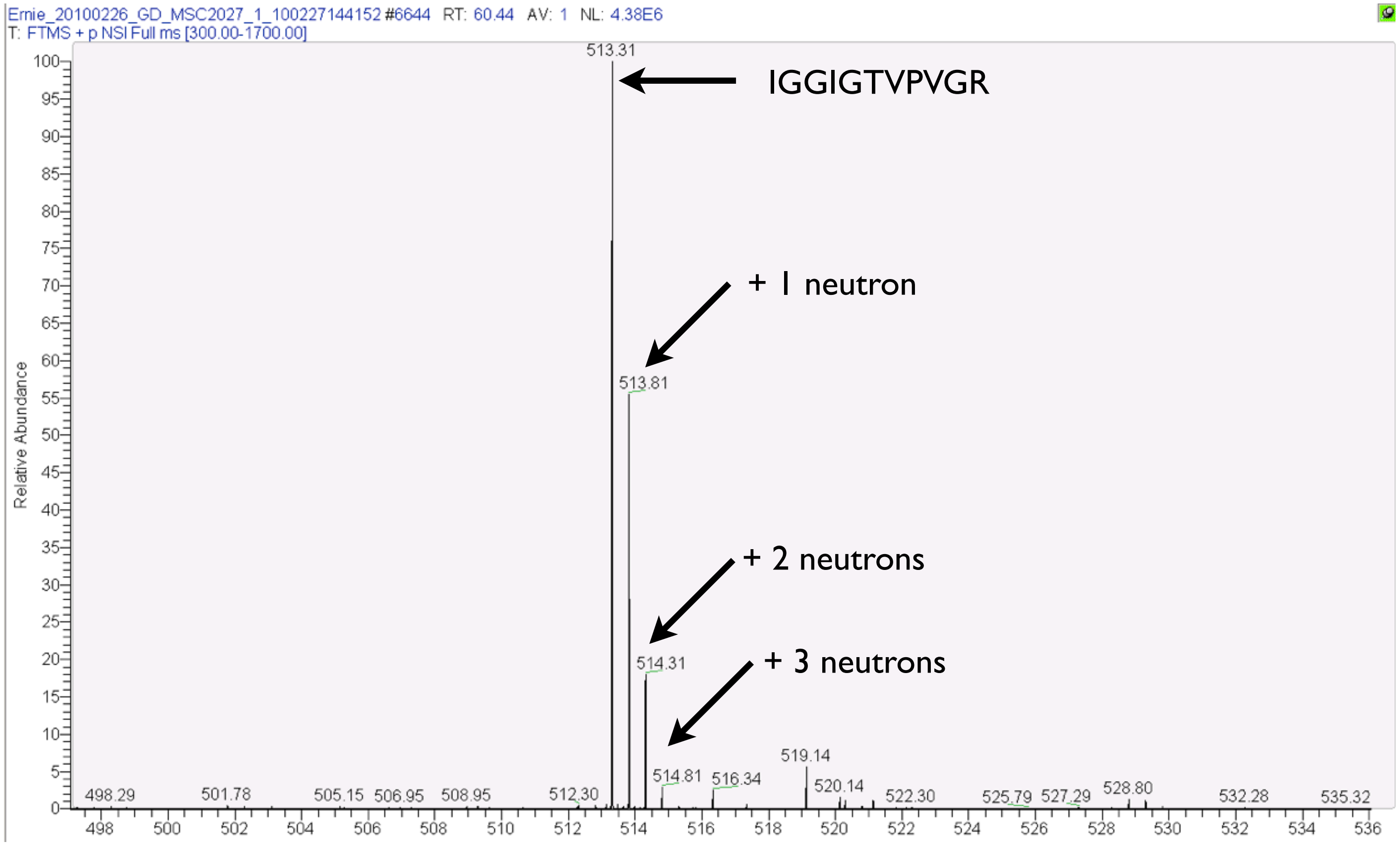
SILAC etc.

Peptides have different ionization properties



Quantification by intensity not directly possible

Resolution of a mass spectrometer



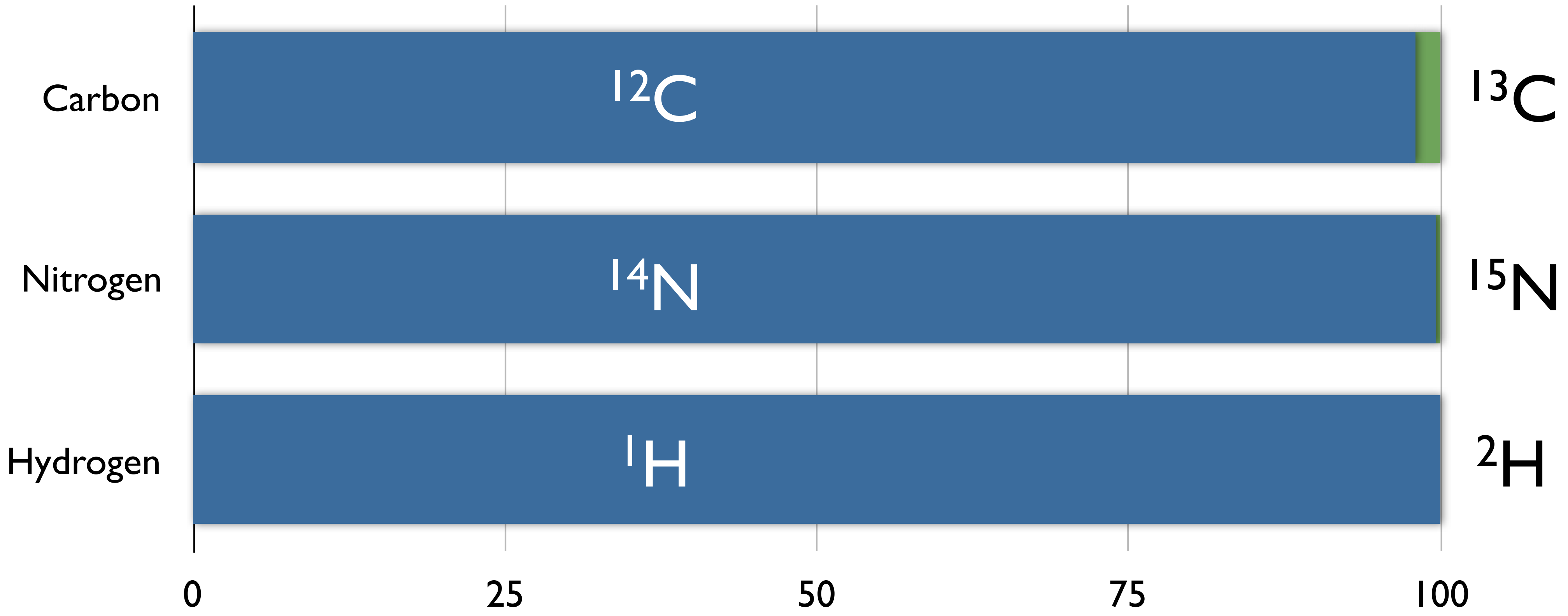
IGGIGTVPVGR

+ 1 neutron

+ 2 neutrons

+ 3 neutrons

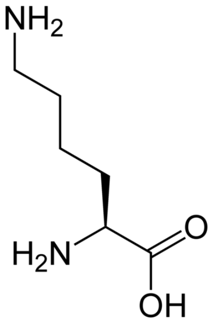
natural occurring isotopes



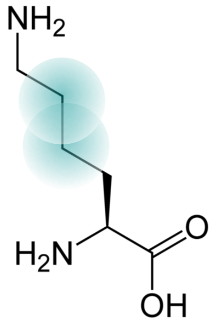
Isotopic labels

● ^{13}C
● ^{15}N
● Deuterium

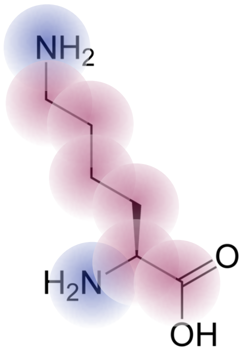
Lysine



light, Lys0

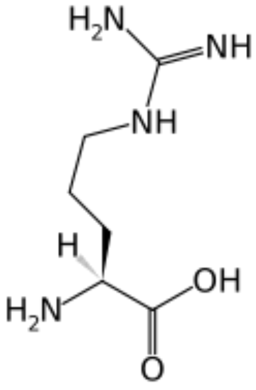


medium, Lys4

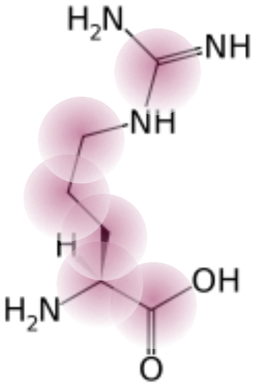


heavy, Lys8

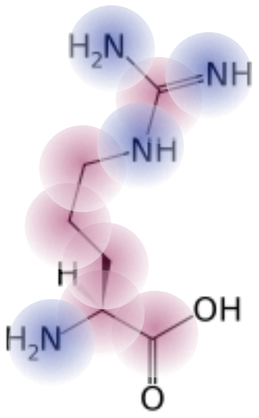
Arginine



light, Arg0

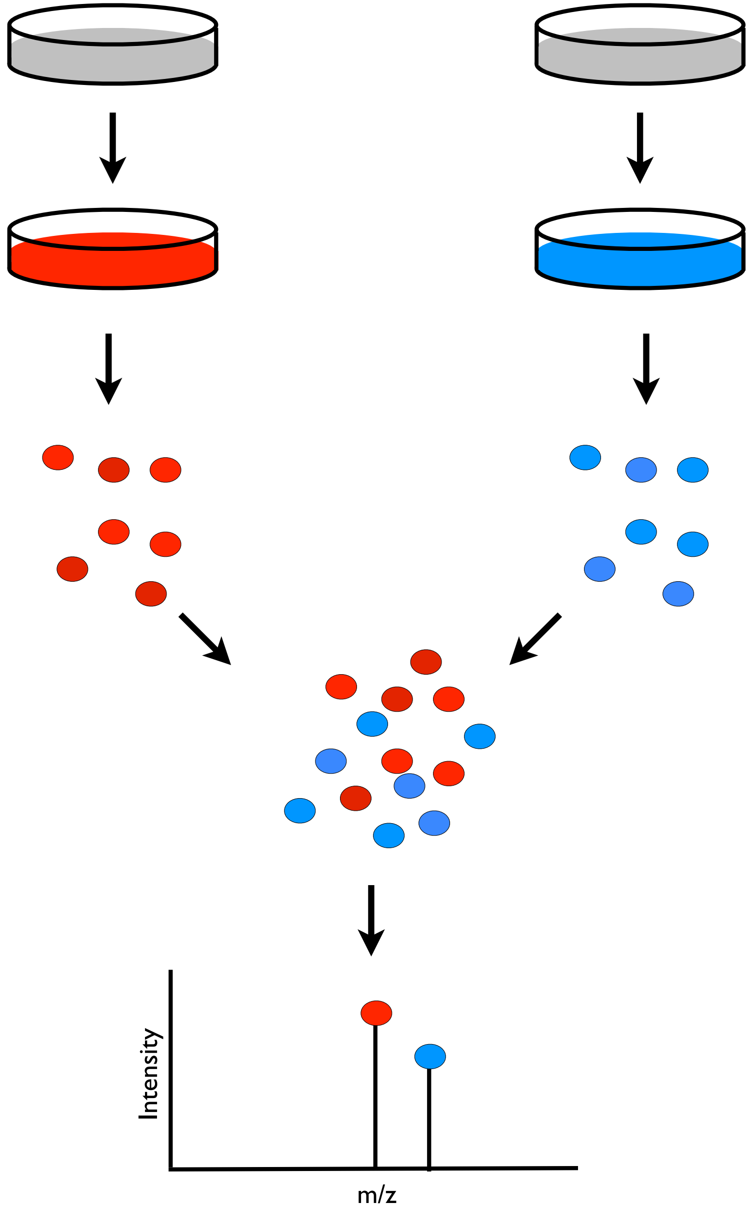


medium, Arg6



heavy, Arg10

SILAC

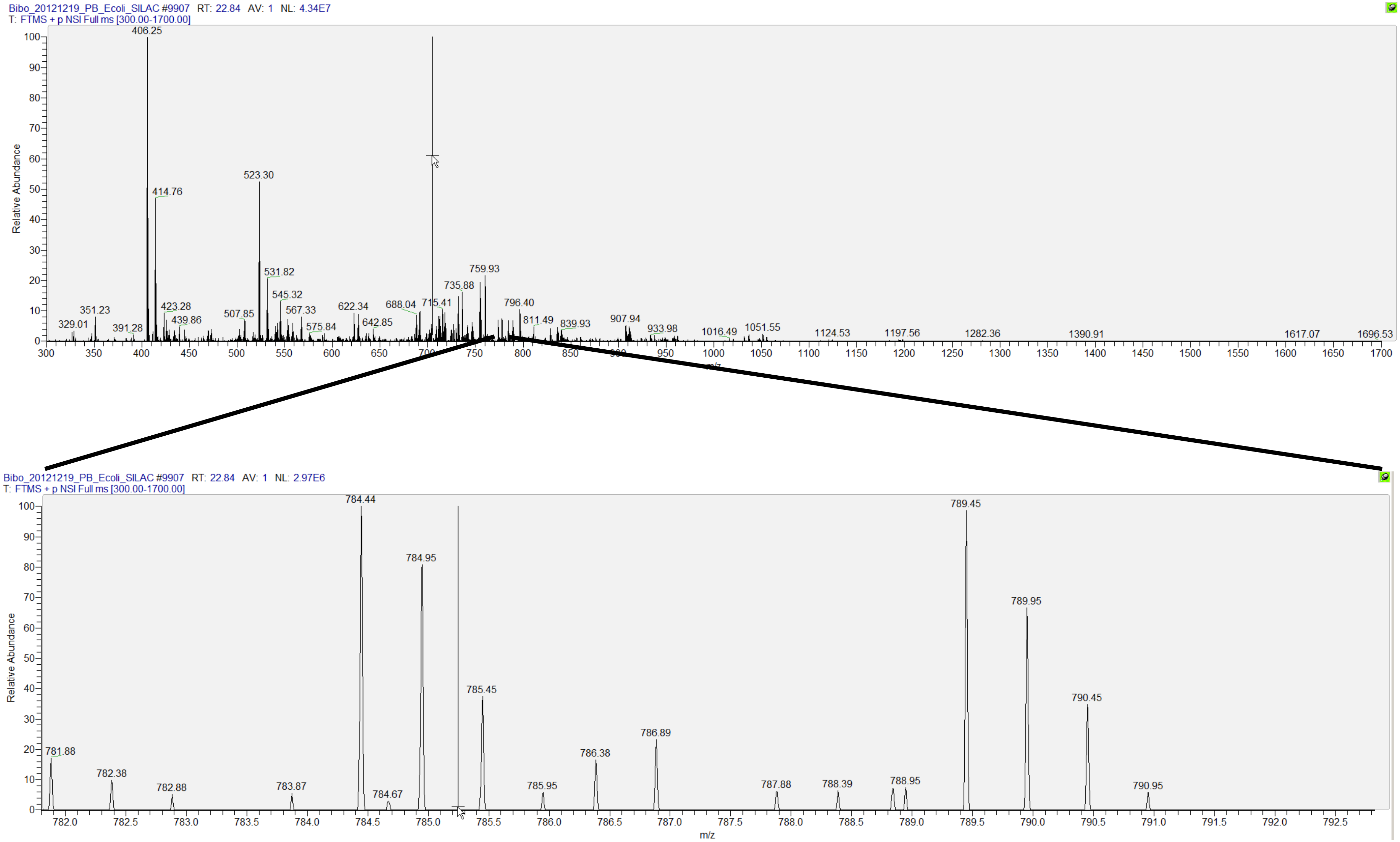


start cultures

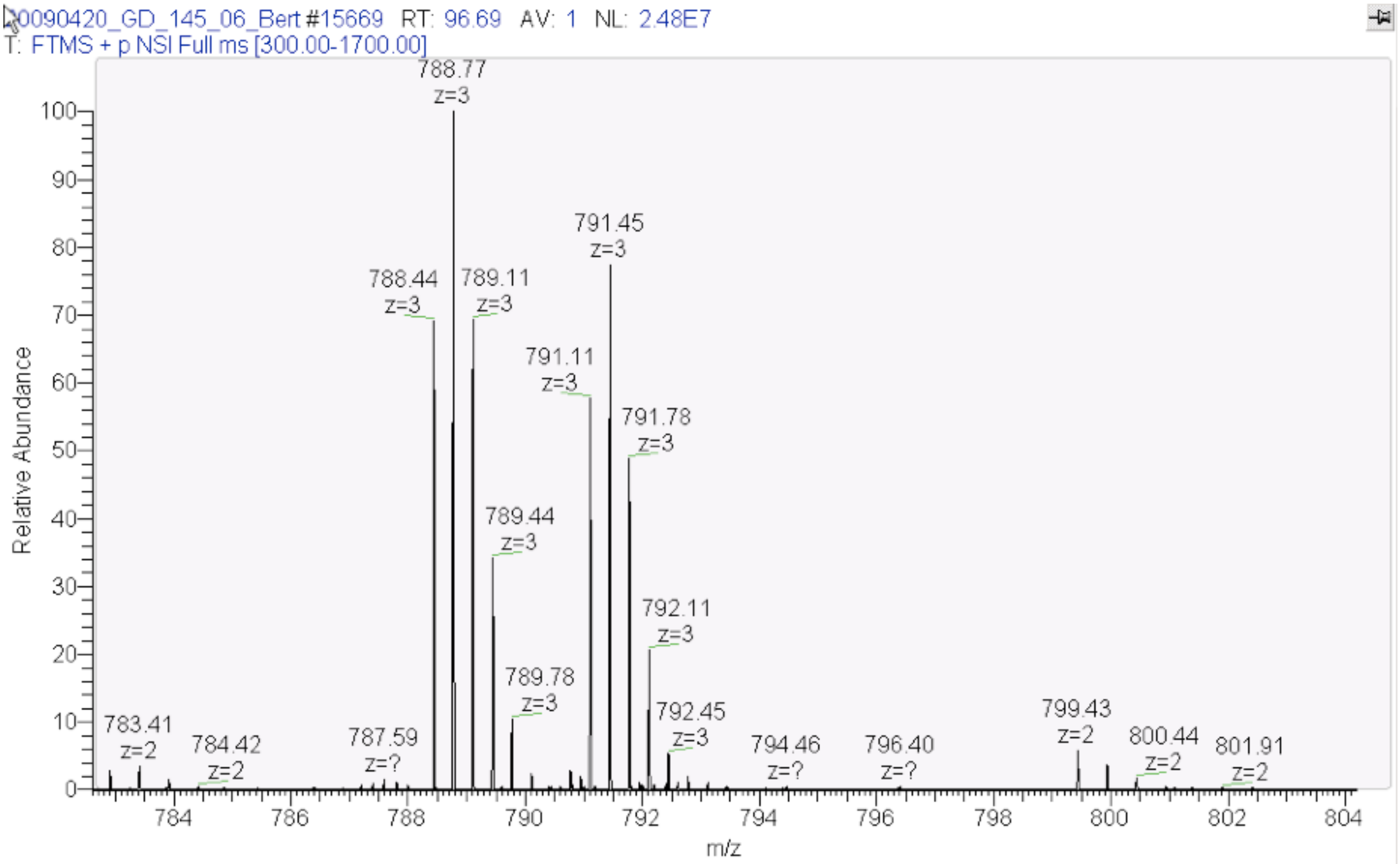
Labeling

Harvest

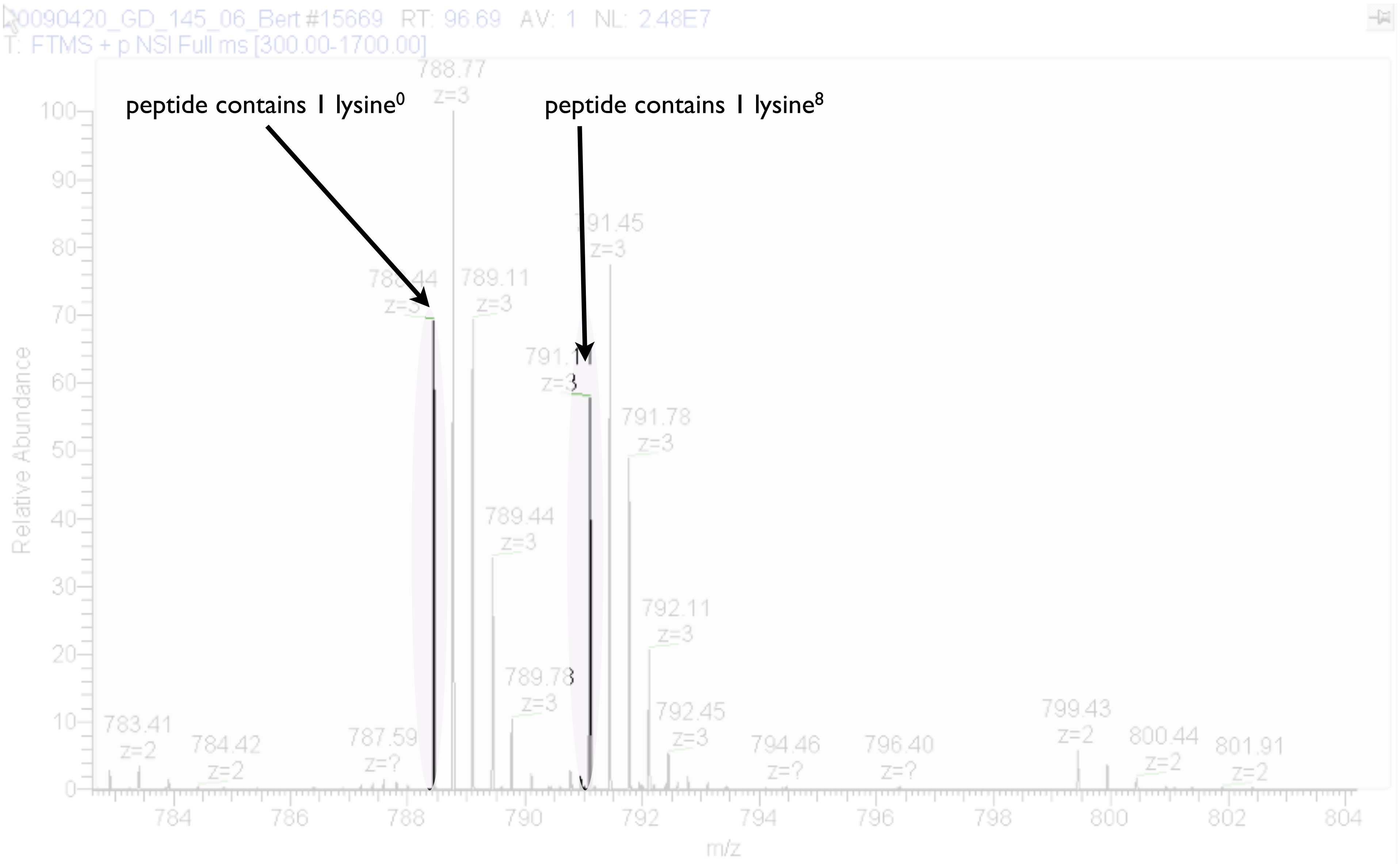
Combining


Bibo_20121219_PB_Ecoli_SILAC #9907 RT: 22.84 AV: 1 NL: 4.34E7
T: FTMS + p NSI Full ms [300.00-1700.00]
Relative Abundance
0
10
20
30
40
50
60
70
80
90
100
406.25
414.76
523.30
531.82
545.32
567.33
575.84
329.01
351.23
391.28
423.28
439.86
507.85
622.34
642.85
688.04
715.41
735.88
759.93
796.40
811.49
839.93
907.94
933.98
1016.49
1051.55
1124.53
1197.56
1282.36
1390.91
1617.07
1696.53
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
1050
1100
1150
1200
1250
1300
1350
1400
1450
1500
1550
1600
1650
1700
m/z
Bibo_20121219_PB_Ecoli_SILAC #9907 RT: 22.84 AV: 1 NL: 2.97E6
T: FTMS + p NSI Full ms [300.00-1700.00]
Relative Abundance
0
10
20
30
40
50
60
70
80
90
100
781.88
782.38
782.88
783.87
784.44
784.67
784.95
785.45
785.95
786.38
786.89
787.88
788.39
788.95
789.45
789.95
790.45
790.95
782.0
782.5
783.0
783.5
784.0
784.5
785.0
785.5
786.0
786.5
787.0
787.5
788.0
788.5
789.0
789.5
790.0
790.5
791.0
791.5
792.0
792.5
m/z

Silac-label Lysine 8



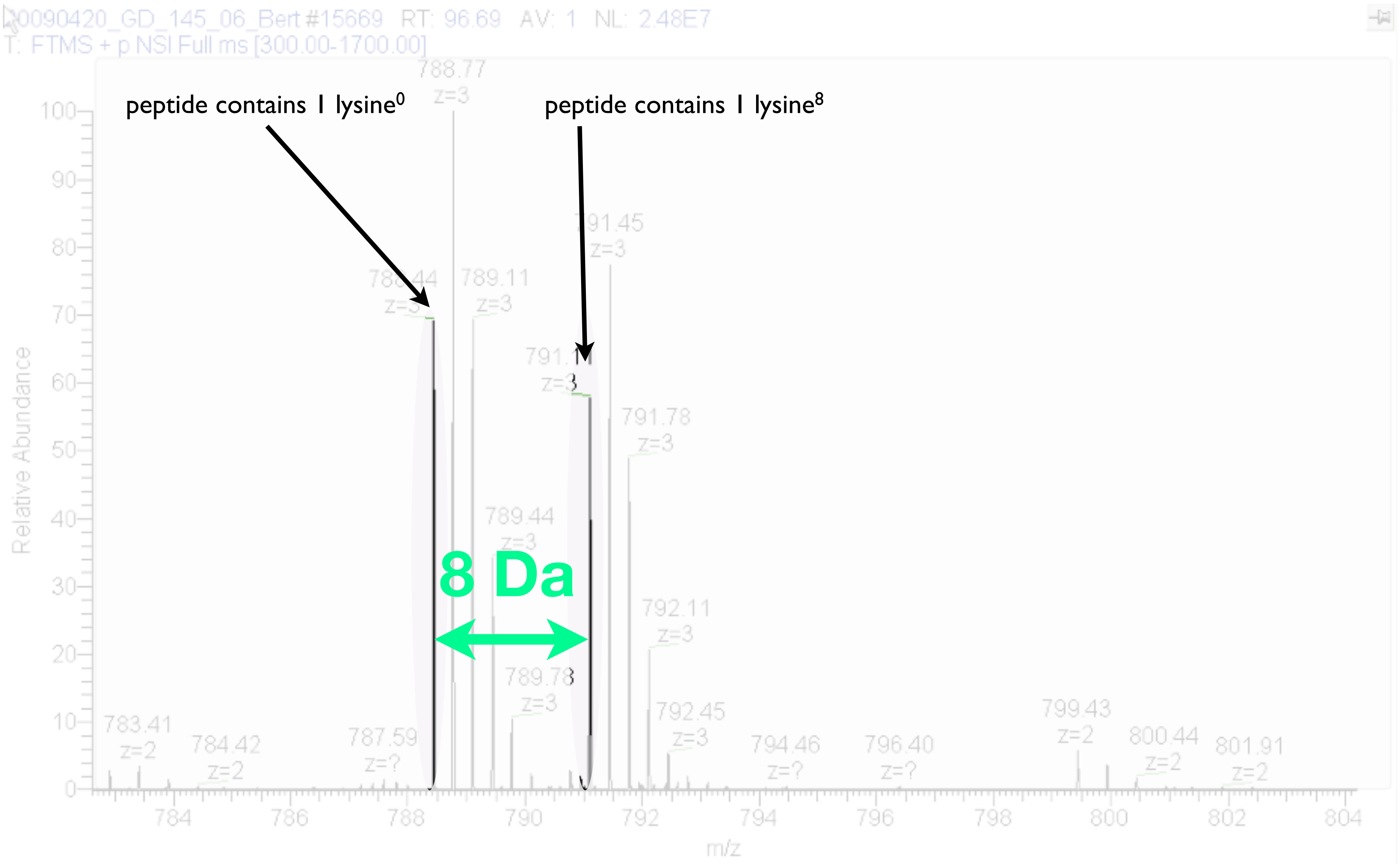
Silac-label Lysine 8



peptide contains 1 lysine^0

peptide contains 1 lysine^8

Silac-label Lysine 8



peptide contains 1 lysine^0

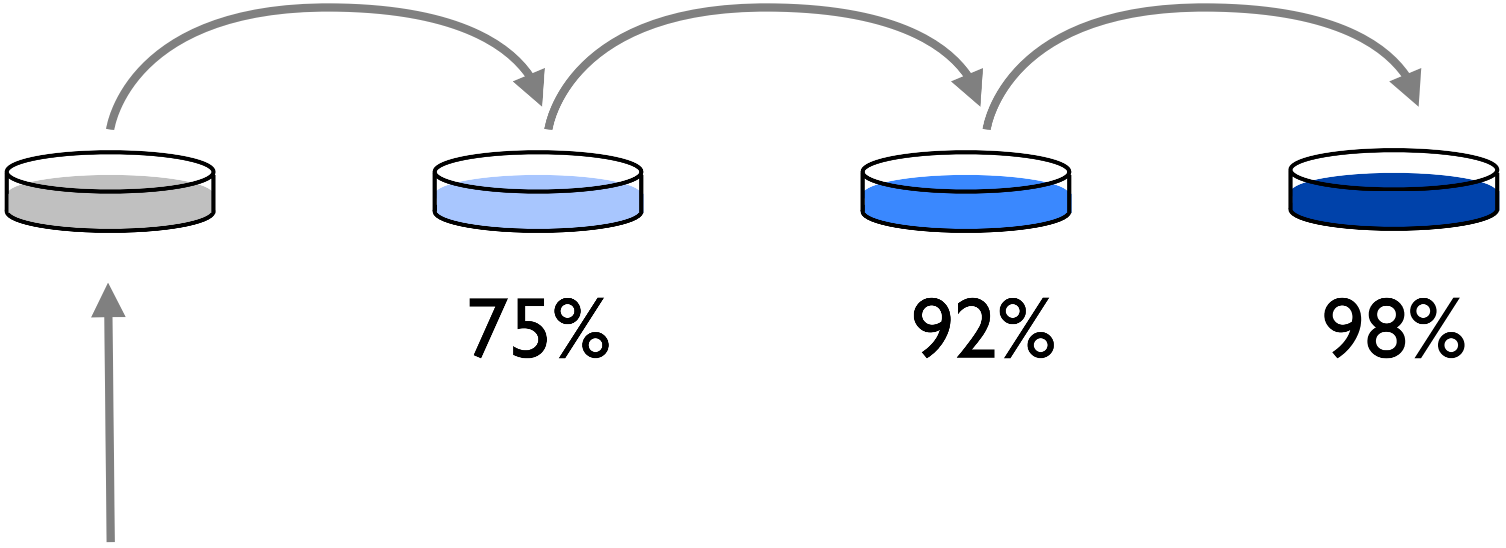
peptide contains 1 lysine^8

8 Da

MDC
mass spectrometry

Comparison of two different cell populations or tissues

Labeling if cells with SILAC amino acids



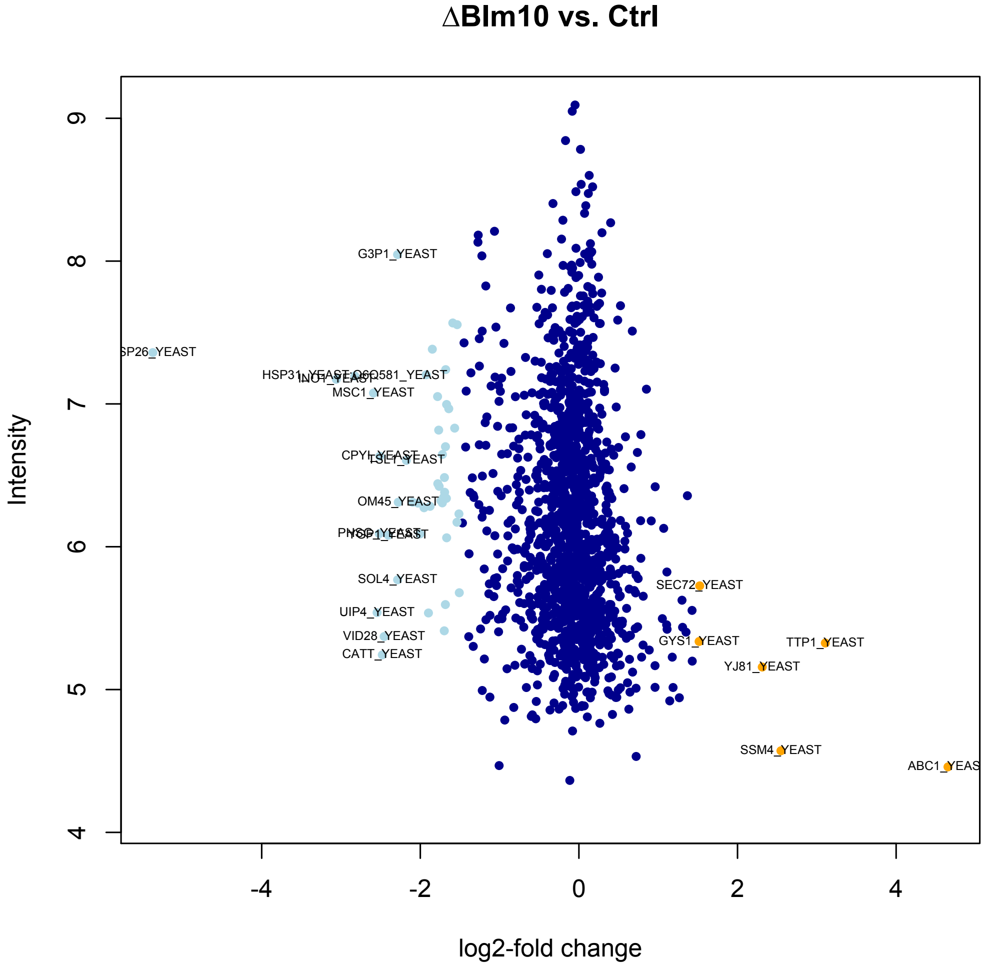
75%

92%

98%

Switch to SILAC
amino acids

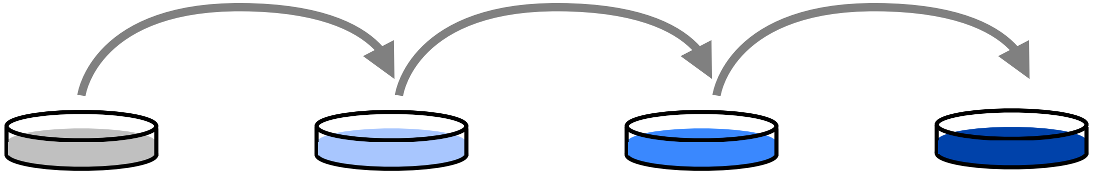
SILAC comparison of wt vs. mutant cells



Usage:

- Quantification of relative protein concentrations
  - large-scale experiments
    - proteome analysis

SILAC Labeling comes with a price



OK

OK, but….

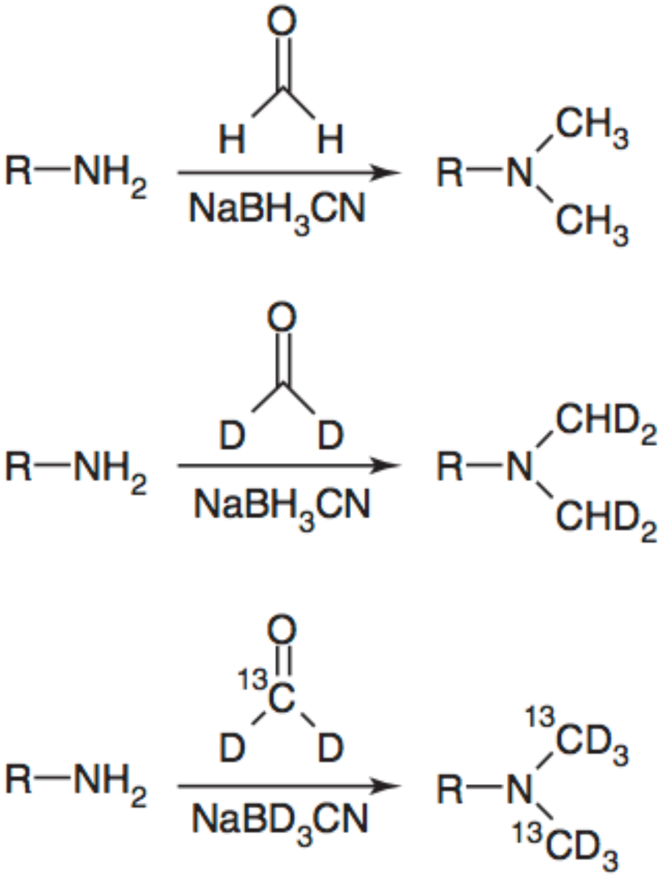
Probably not..

MDC
mass spectrometry

Dimethylation

chemical modification of peptides

Dimethylation reaction with formaldehyde

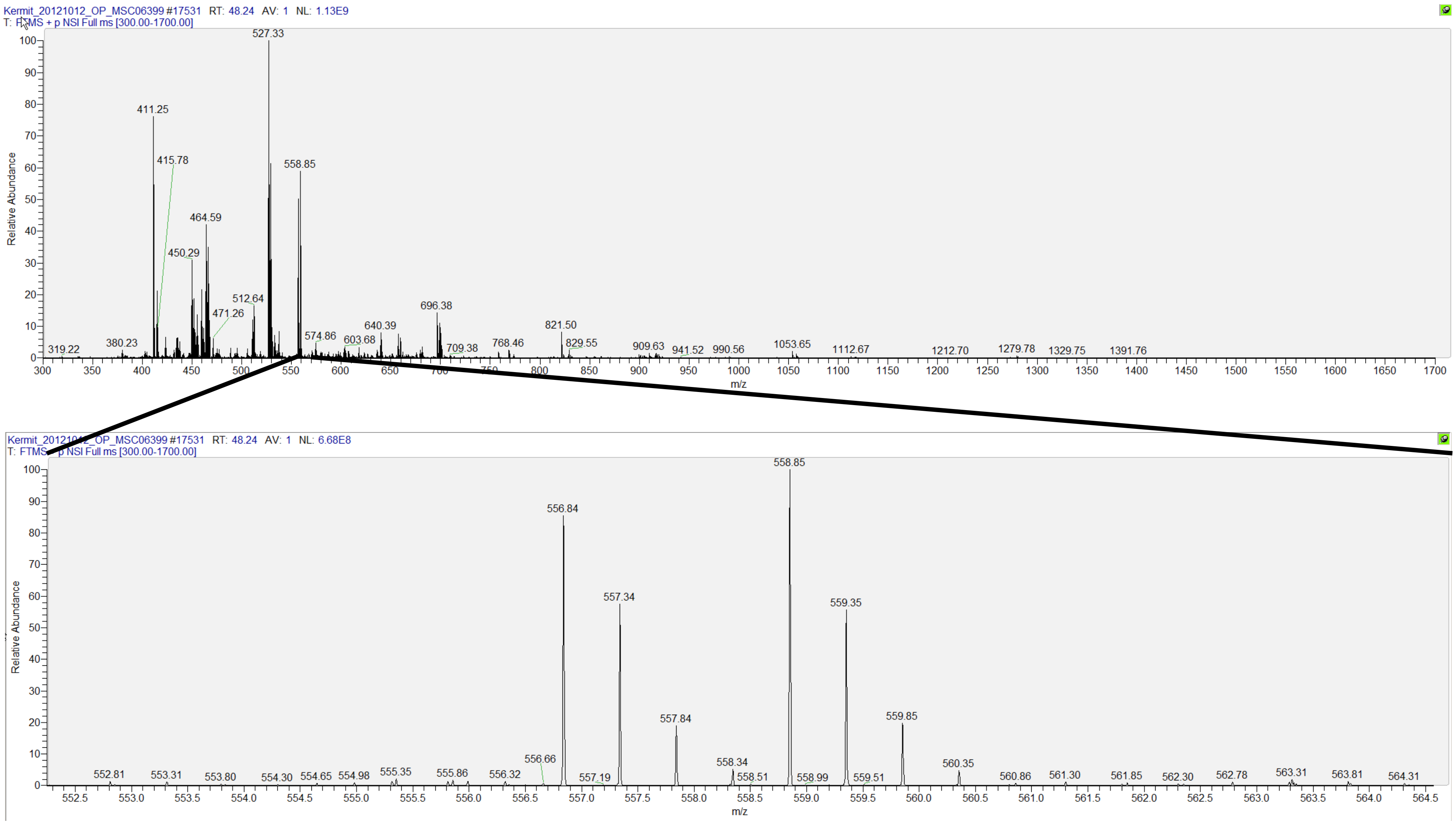


0 Da (28 Da)

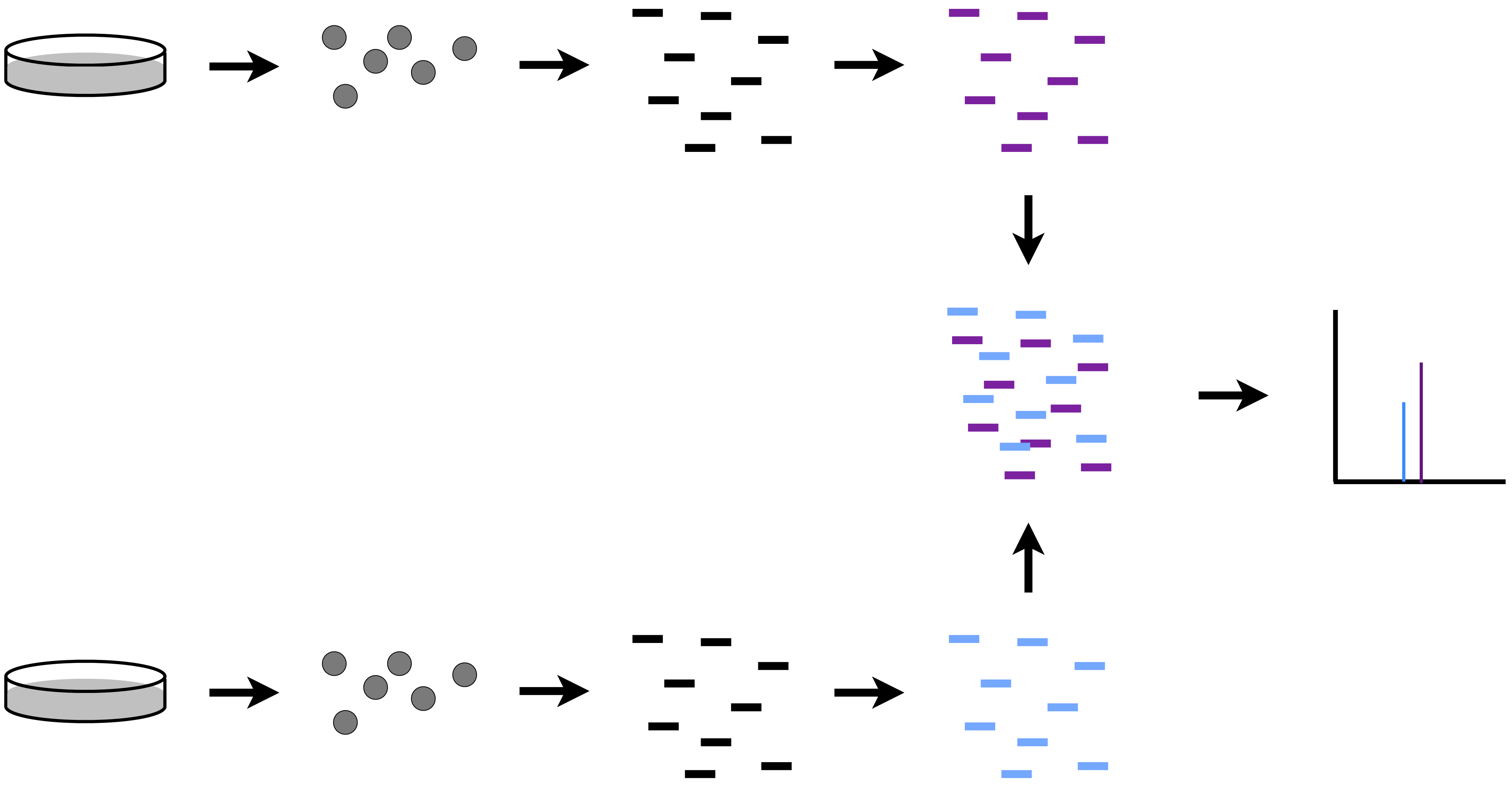
4 Da (32 Da)

8 Da (36 Da)

Isotope douplette in a dimetylation experiment



Dimethylation



Harvest
and
protein extraction

Peptide
digest

dimethylation
reaction

MS analysis

Comparison SILAC and dimethylation analysis

	SILAC	Dimethylation
Introduction of the label	metabolic	chemical reaction
Variations introduced by sample preparation	low	high
material need	low	high

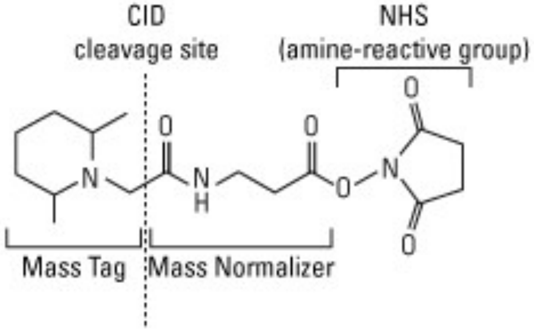
MDC
mass spectrometry

TMT and relatives

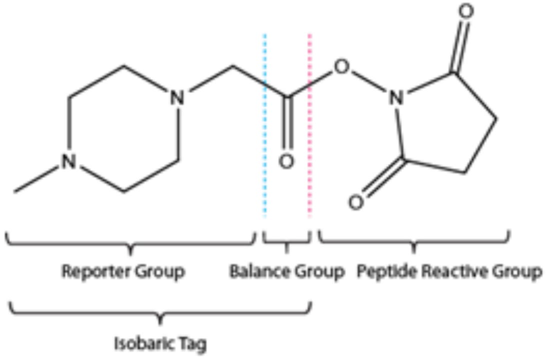
chemical modification of peptides

Isobaric tags

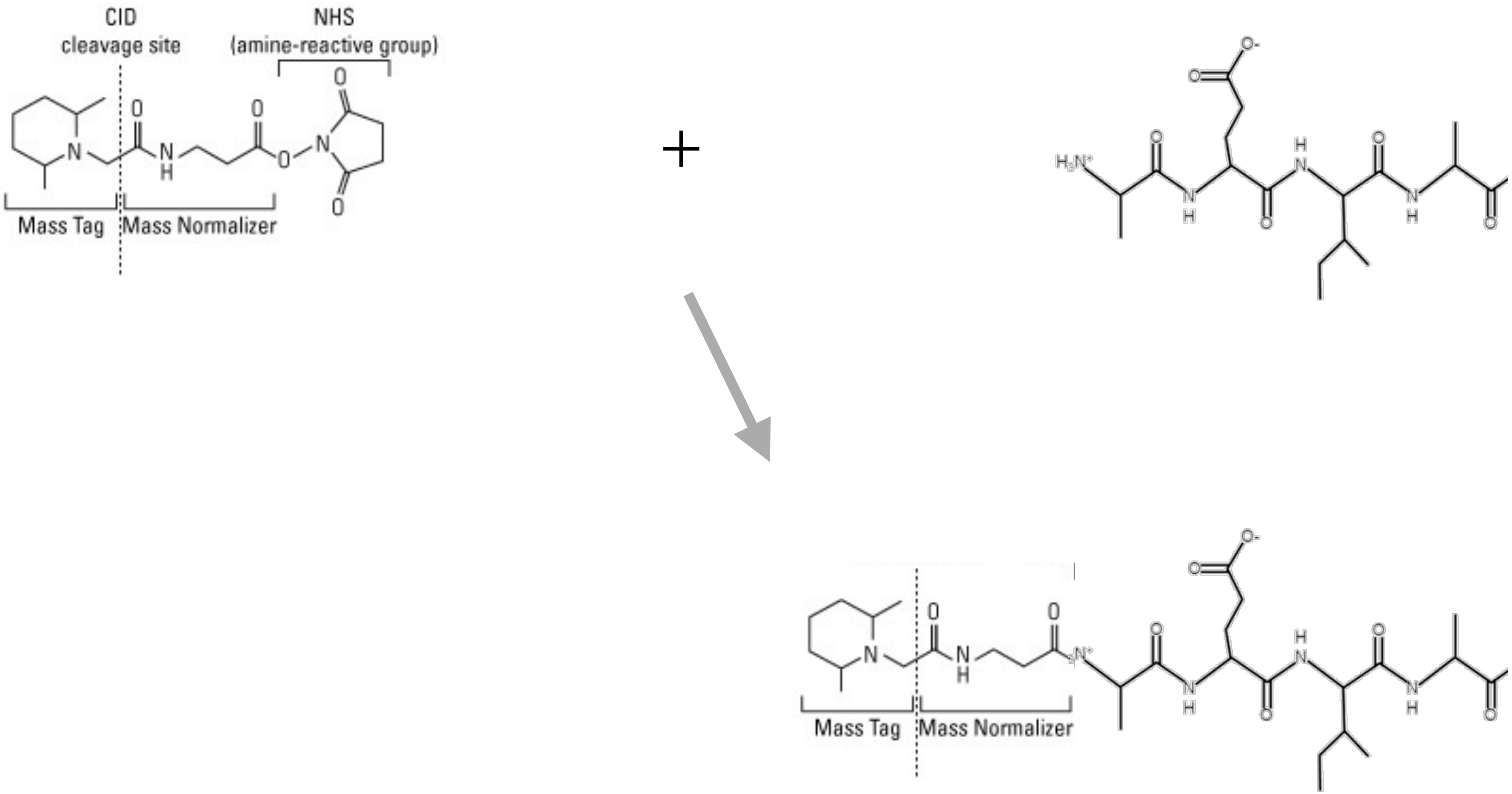
TMT



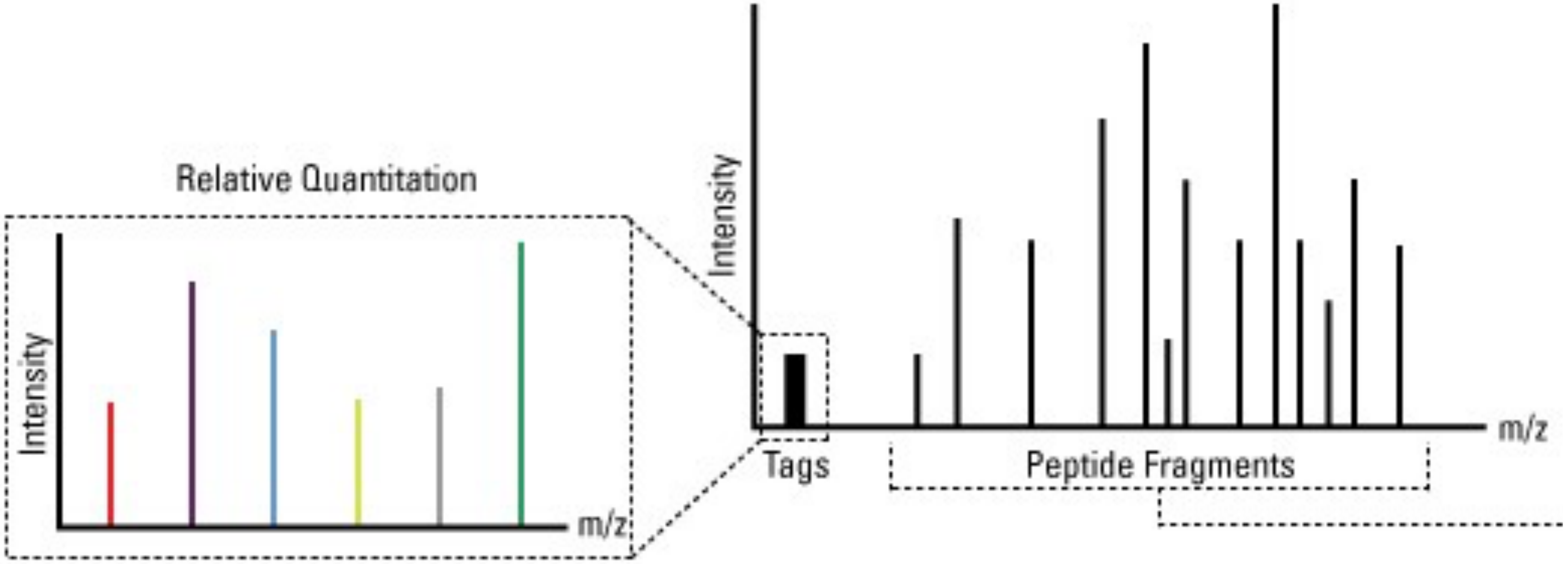
iTraq



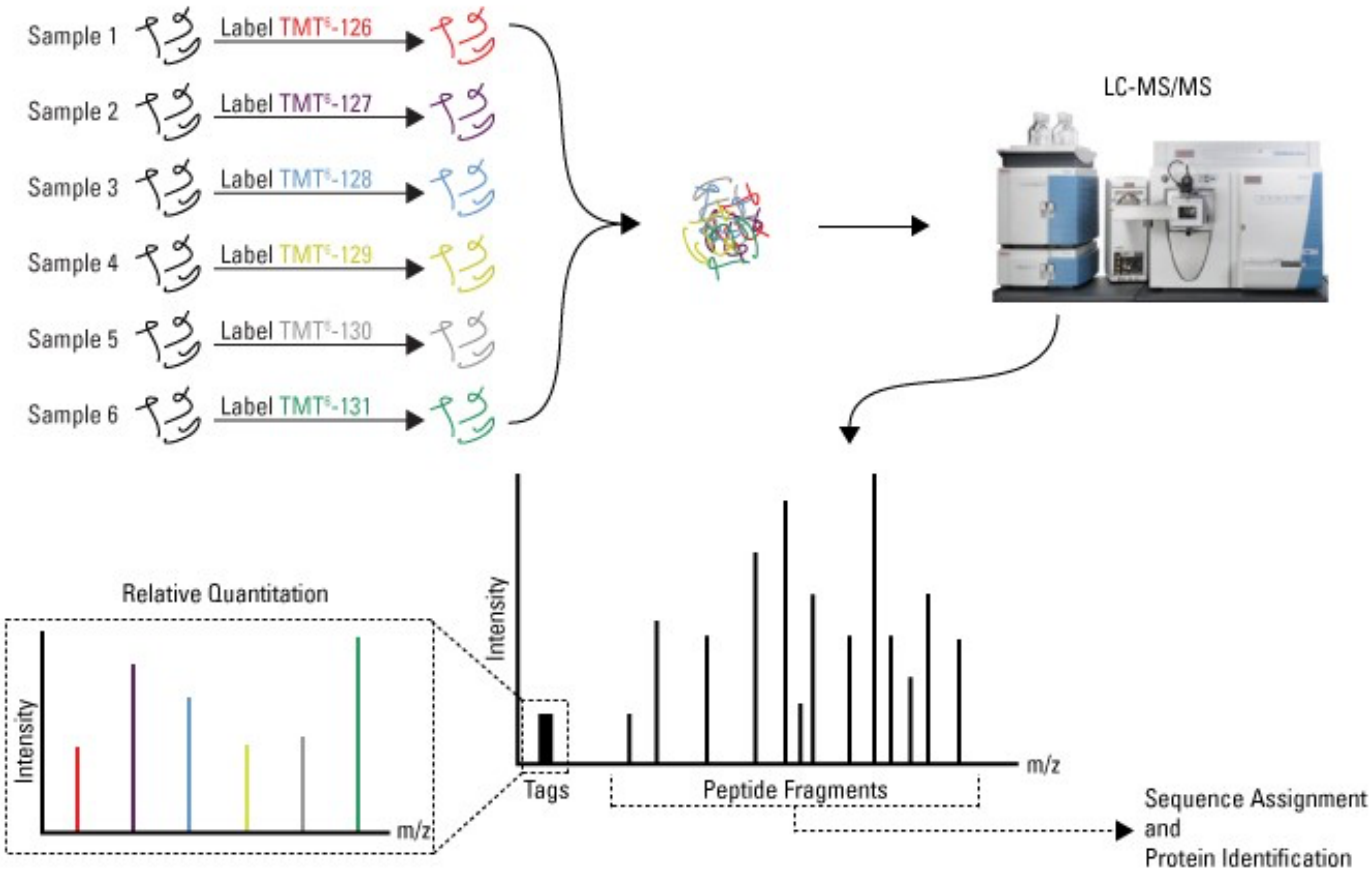
TMT


CID
cleavage site
NHS
(amine-reactive group)
Mass Tag
Mass Normalizer
+
Mass Tag
Mass Normalizer

Qantitation in the MS^2

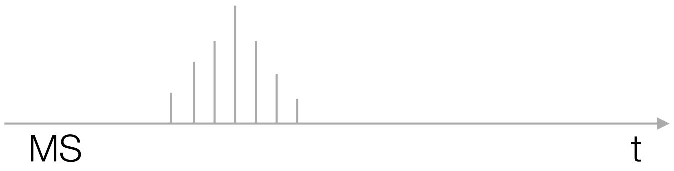


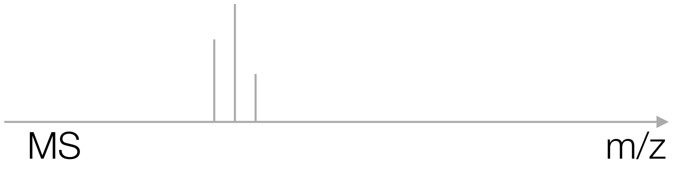
Multiplexing up to 10



Differences and advantages

SILAC/DML





TMT

TMT

Differences and advantages

	SILAC/ Dimethylation	TMT
Quantification	MS	MS^2 or MS^3
Quantification events	many at least 16	one per MS^2
Complexity of the spectra	double or triple	always single
max multiplexing	3	10 or 18

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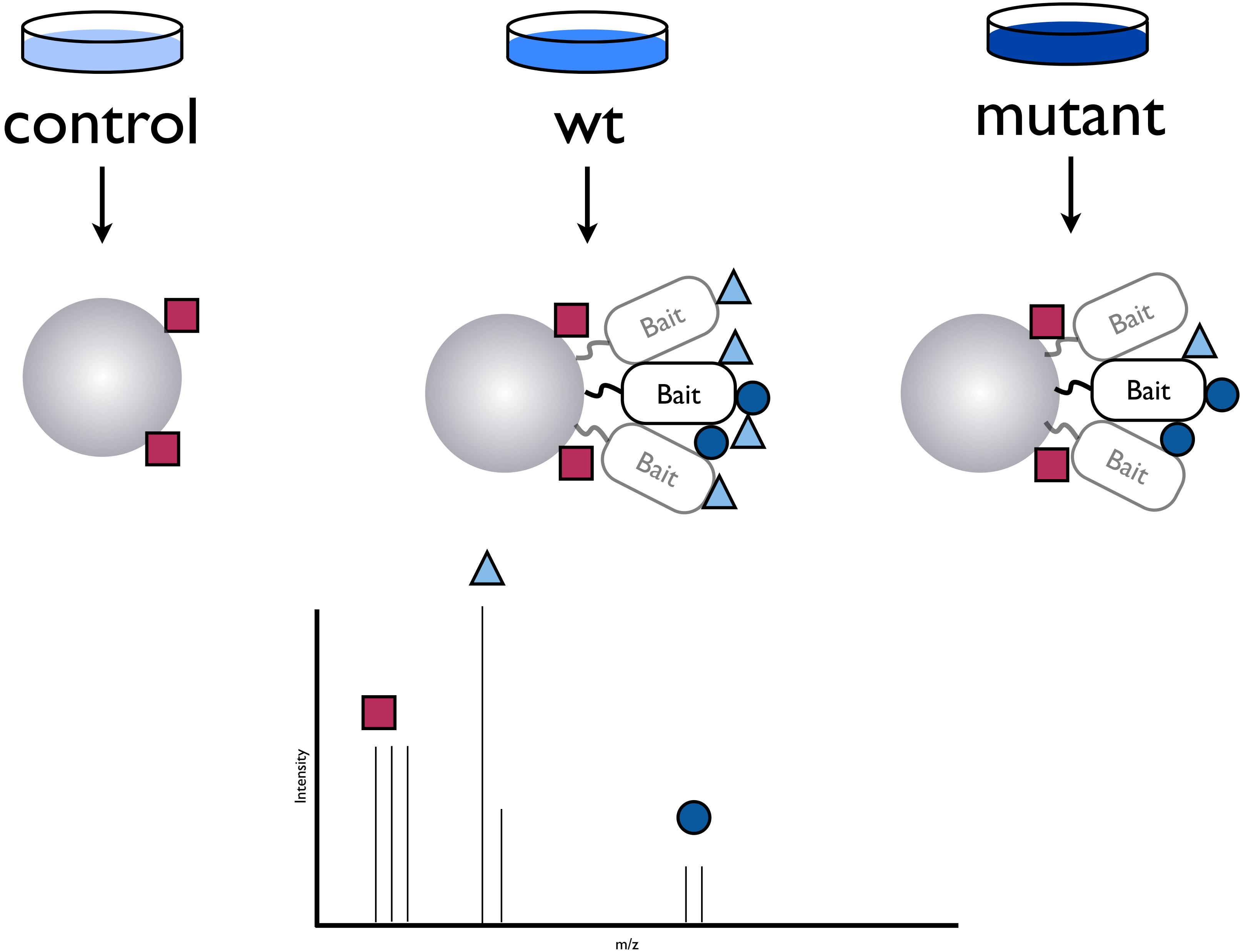
mass spectrometry

SILAC-IP

What if an IP looks like this?

- High background IPs
- Interactions are transient
- many interactions detected

SILAC-IP

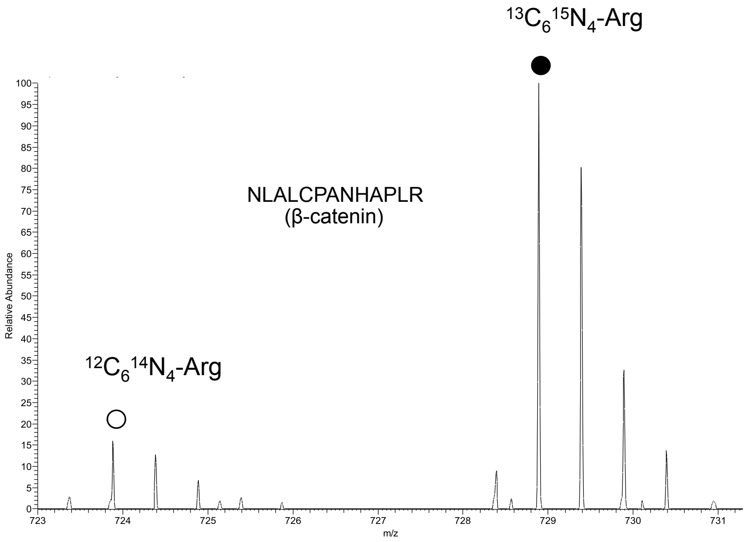


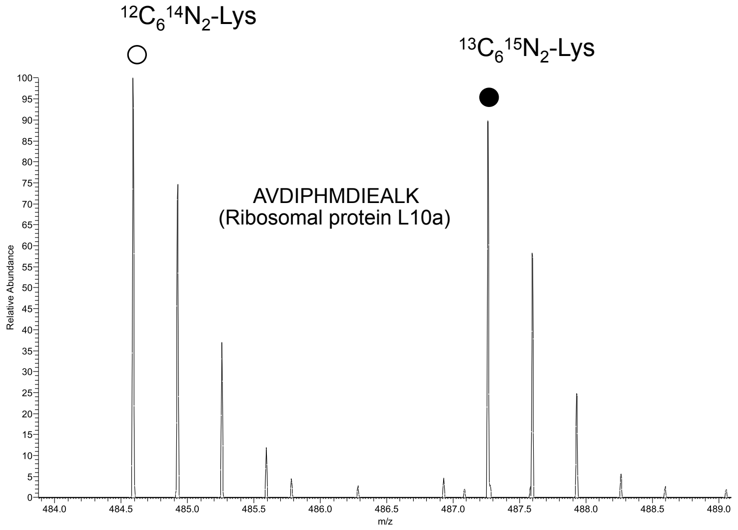
control

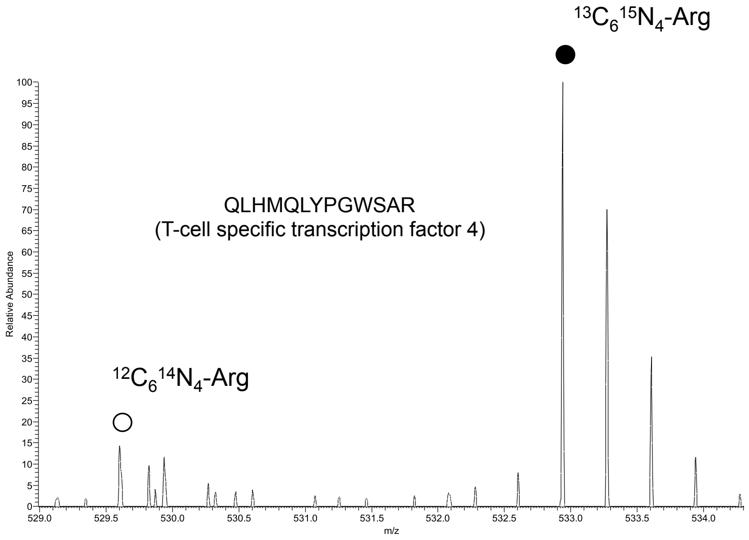
wt

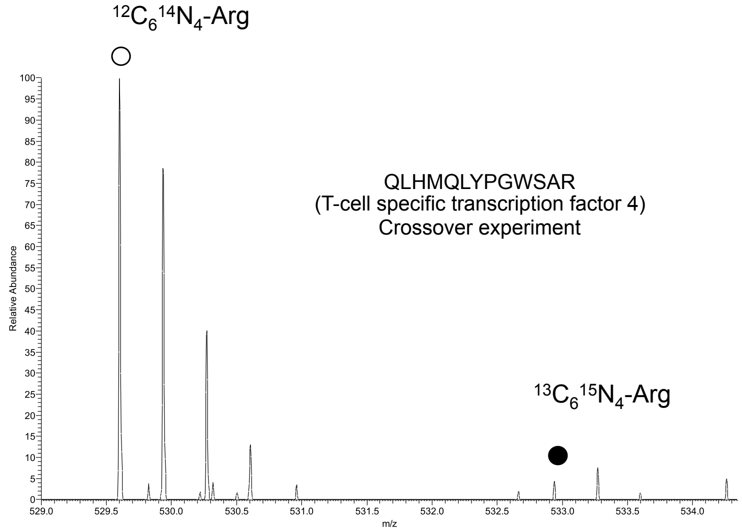
mutant

Spectra of a ß-catenin IP



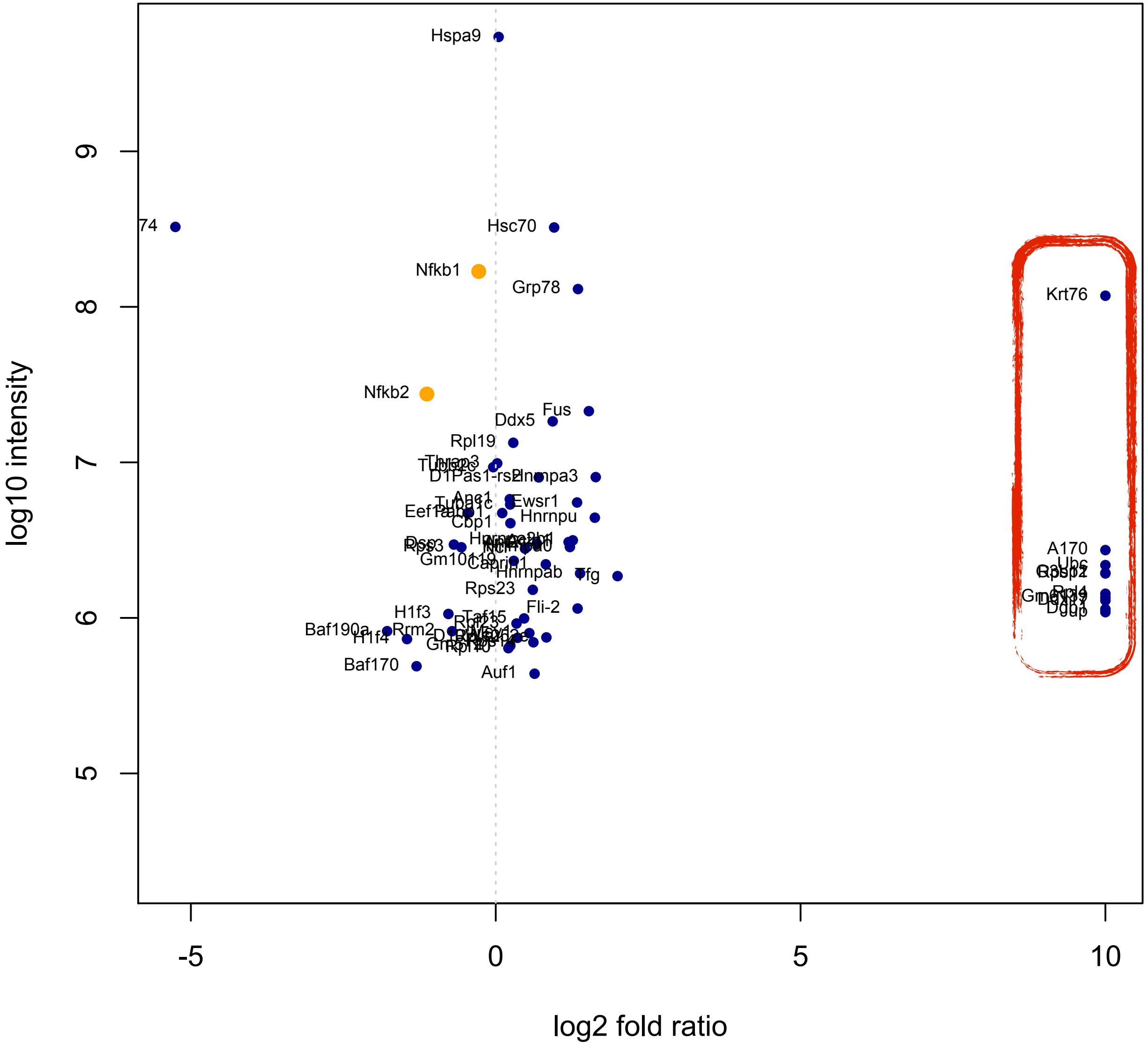






Selbach and Mann, *Nature Methods*, 2006

SILAC IP


Hspa9
74
Hsc70
Nfkb1
Grp78
Krt76
Nfkb2
Fus
Ddx5
Rpl19
Thrap3
Tubb2c
D1Pas1-rs2
Hnrnpa3
Ewsr1
Cbp1
Hnrnpu
Rps3
Gm10119
Caprin1
Hnrnpab
Tfg
Rps23
H1f3
Taf15
Fli-2
Baf190a
Rrm2
H1f4
Baf170
Auf1
A170
Ubc
Rpl4
9
8
7
6
5
-5
0
5
10
log10 intensity
log2 fold ratio

MDC
mass spectrometry

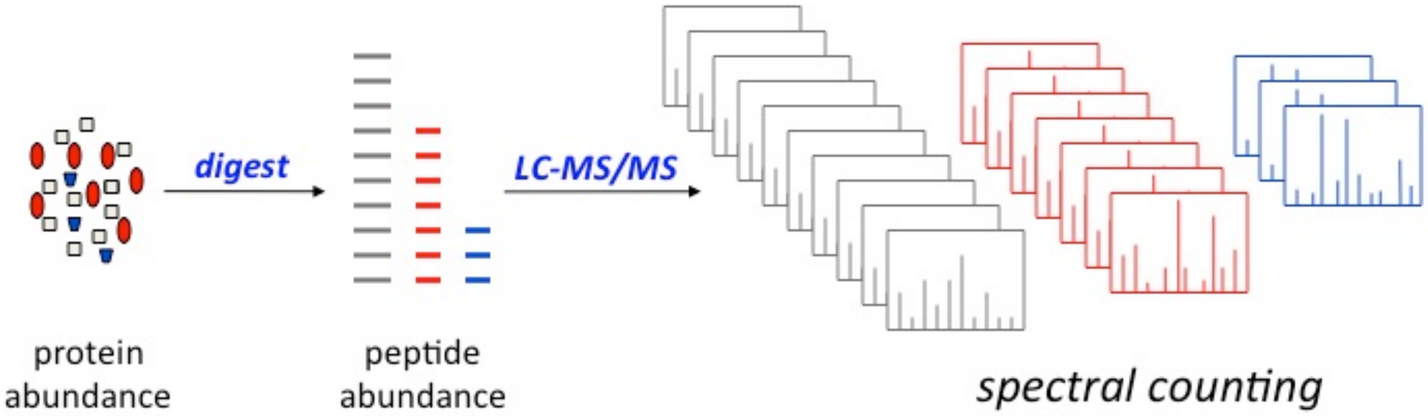
Label free quantification

Label free quantification methods

	needs standard	effort	accuracy
spectral counting	-	low	low
PAI/emPAI	-	low	mid
LFQ MaxQuant	-	mid	mid
iBAQ	+	high	better than mid

spectral counting

Quantitation in shotgun proteomics by spectral counting



MDC
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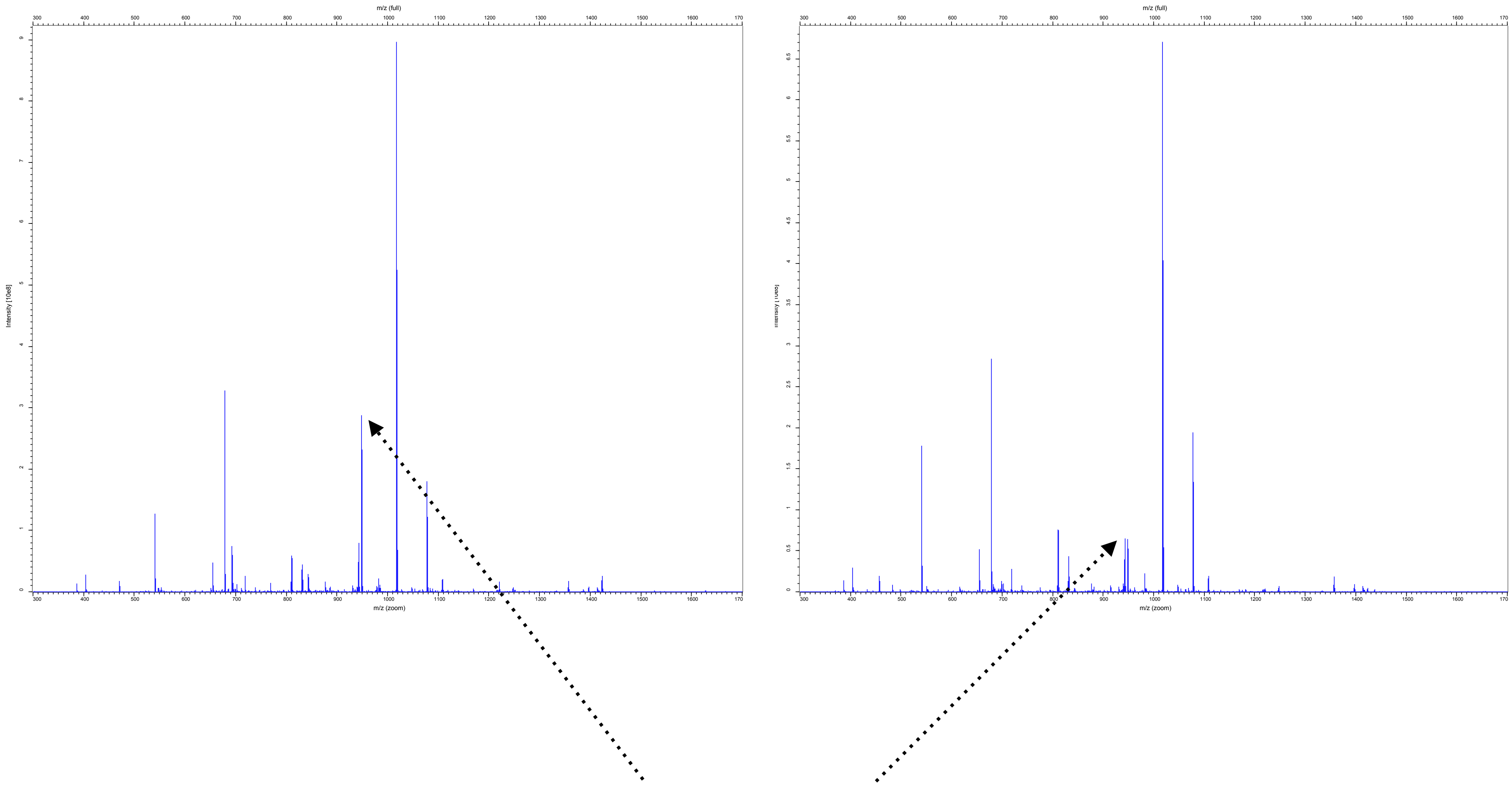
Interactor mapping using label-free proteomics

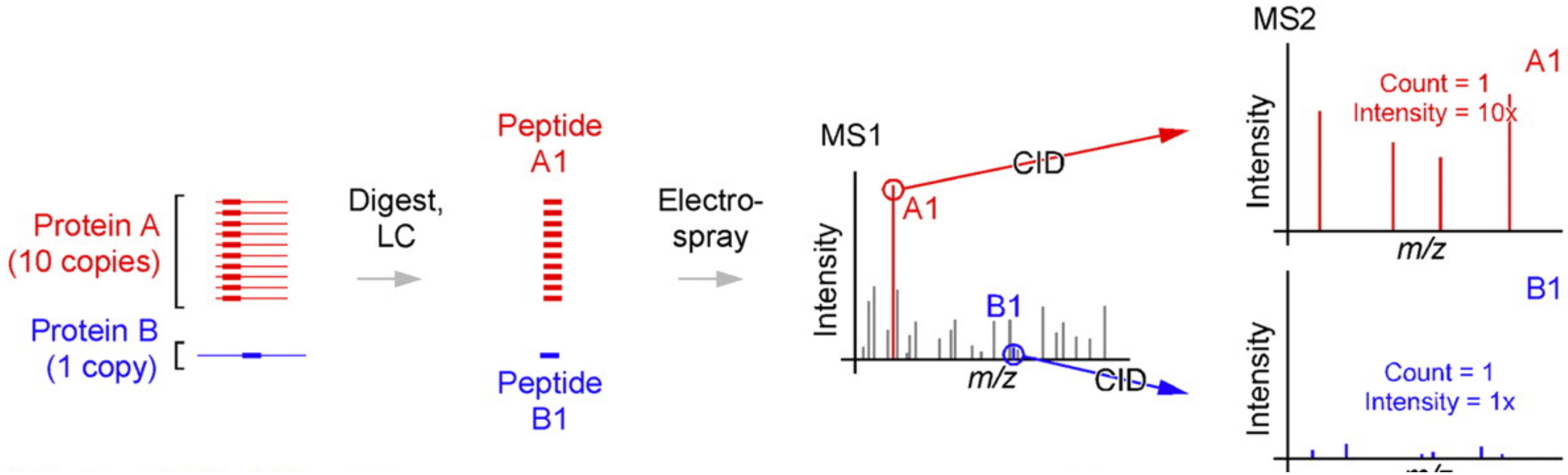
repeats are your friends

Label free quantification

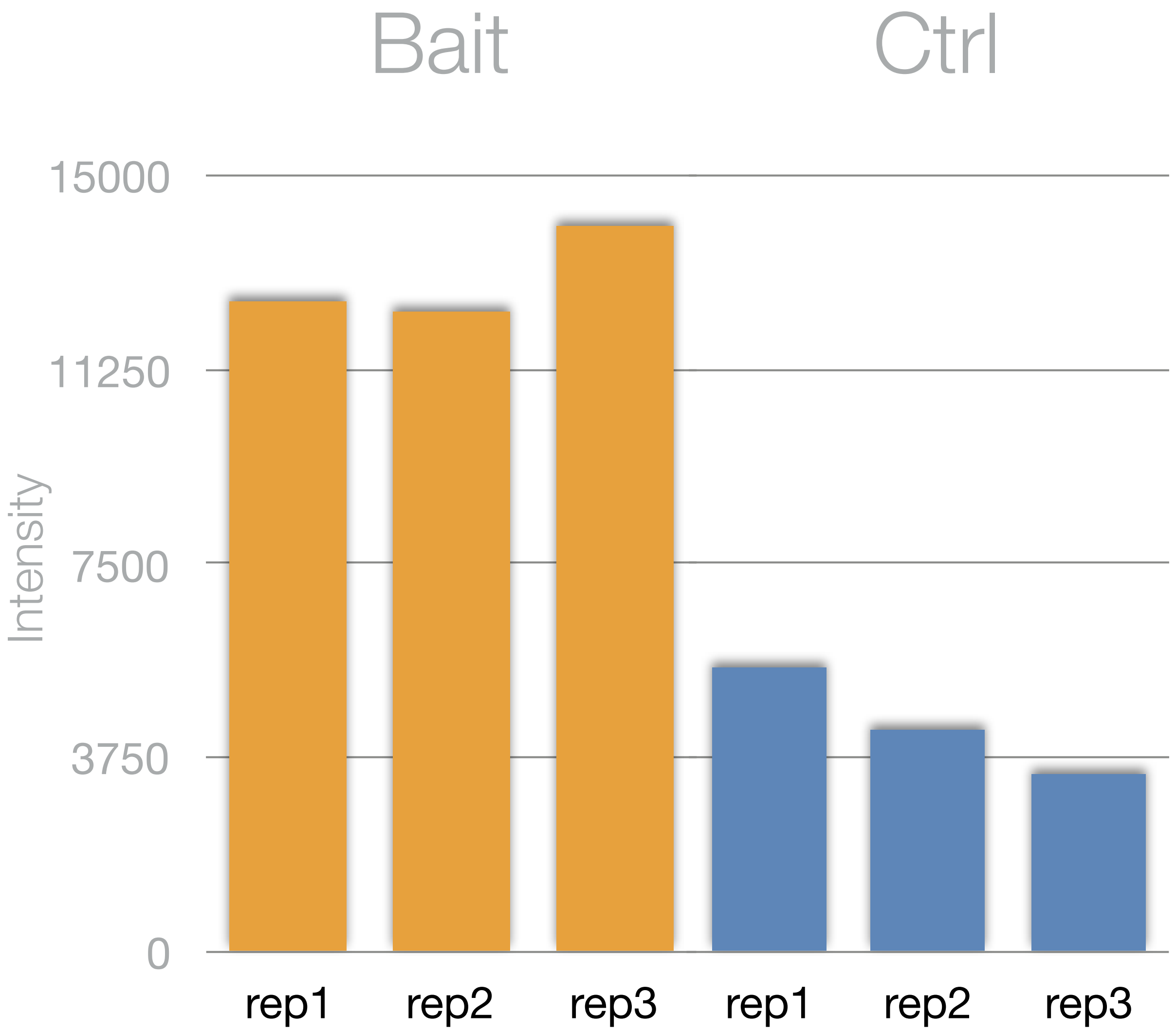


same peptide in two samples

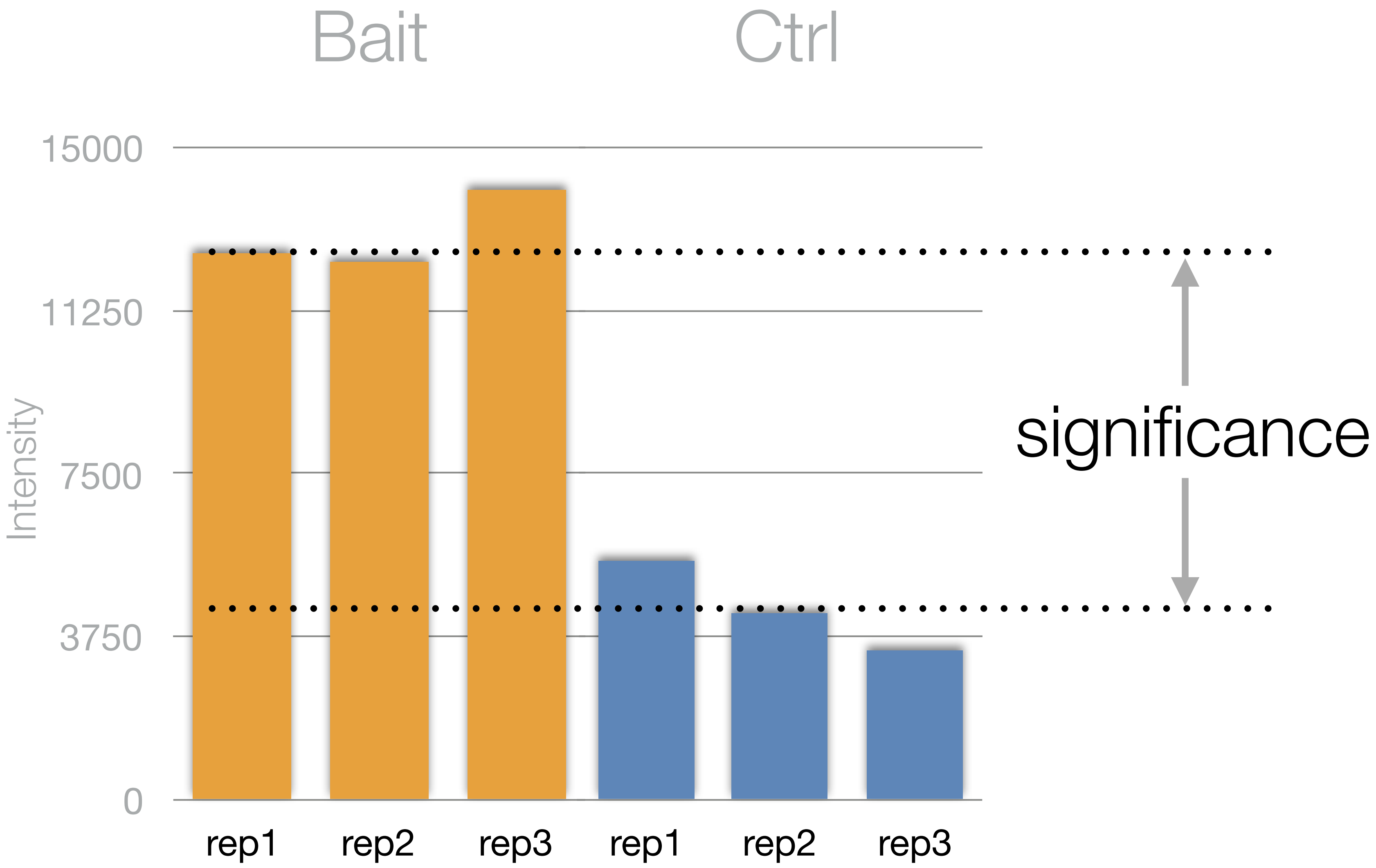
Intensity comparison based on the top 5 peptides



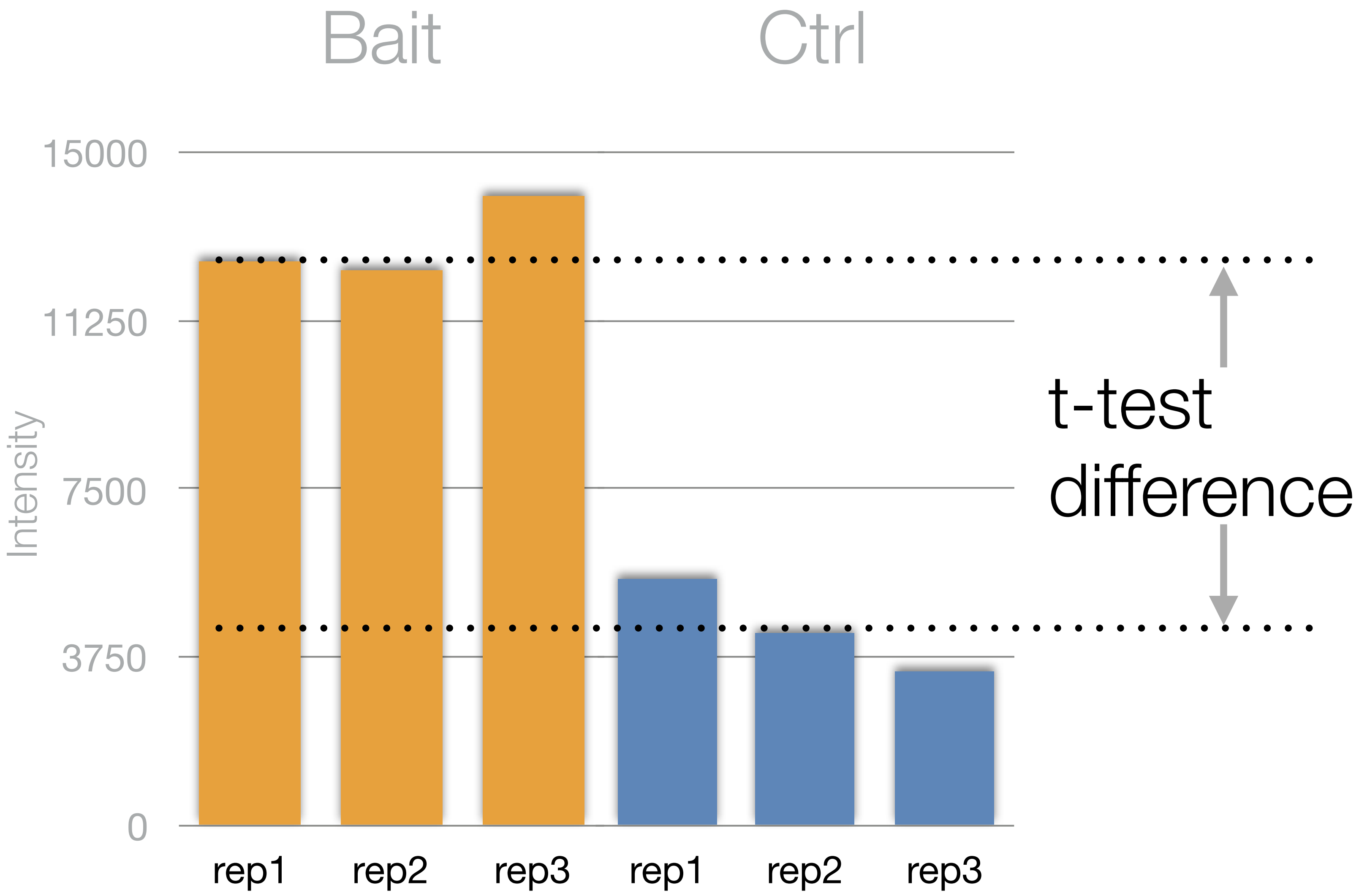
Label-free


Bait
Ctrl
Intensity
15000
11250
7500
3750
0
rep1
rep2
rep3
rep1
rep2
rep3

Label-free

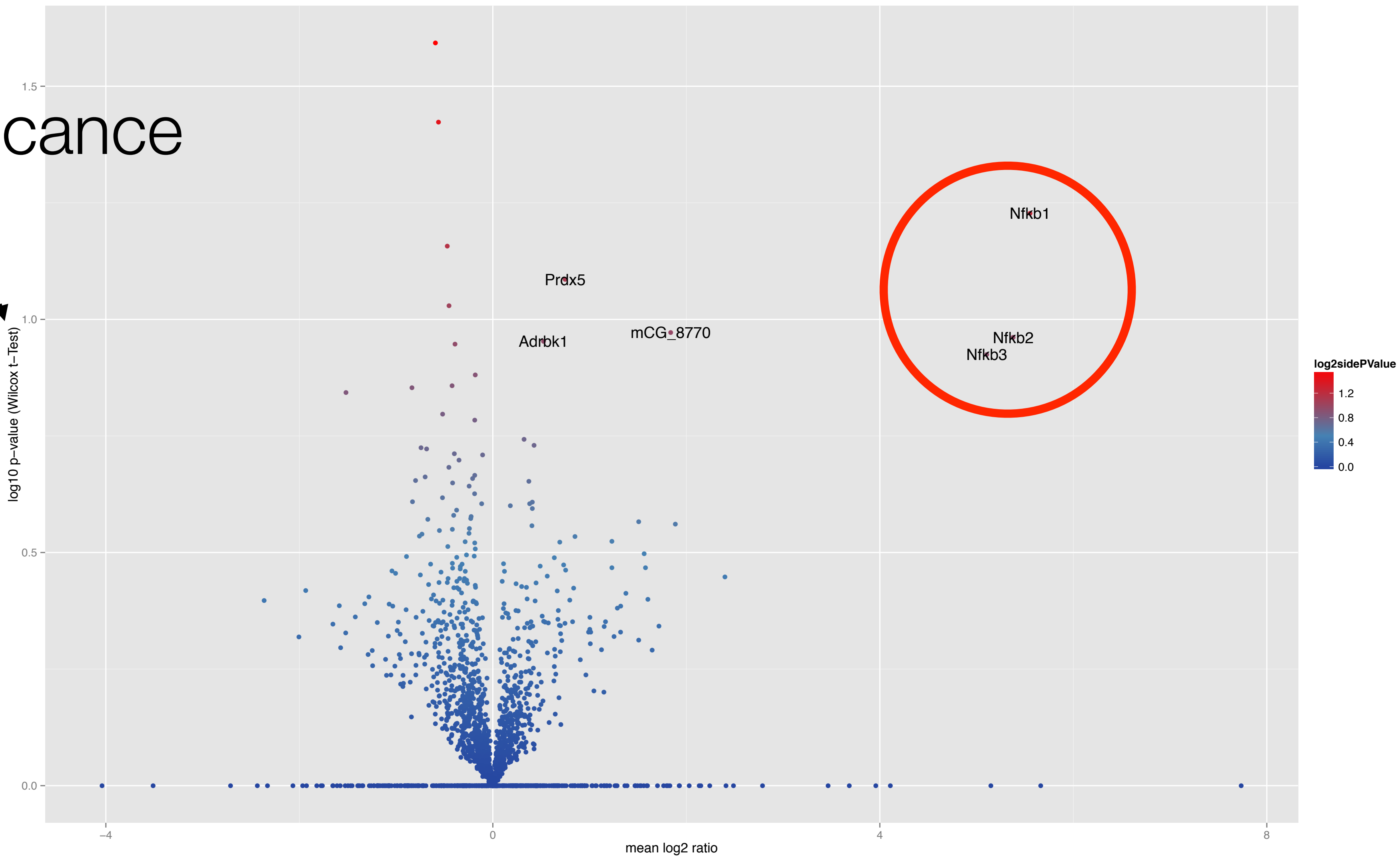

Bait
Ctrl
15000
11250
7500
3750
0
Intensity
rep1
rep2
rep3
rep1
rep2
rep3
significance

Label-free



t-test
difference

SILAC IP



significance

t-test difference

MDC
mass spectrometry

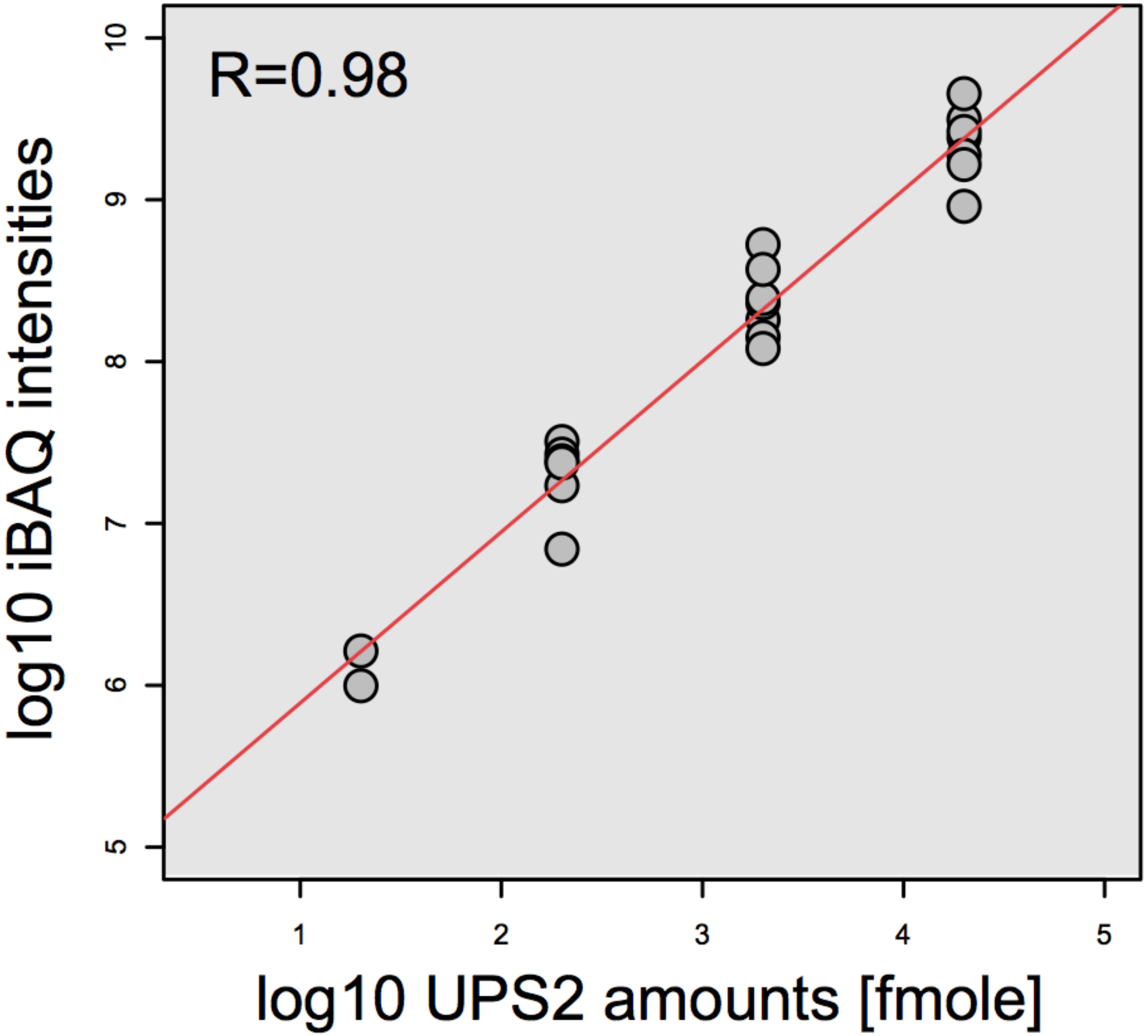
iBAQ

iBAQ - quantification using a standard

48 human proteins has been formulated into a dynamic range of concentrations, ranging from 500 amoles to 50 pmoles

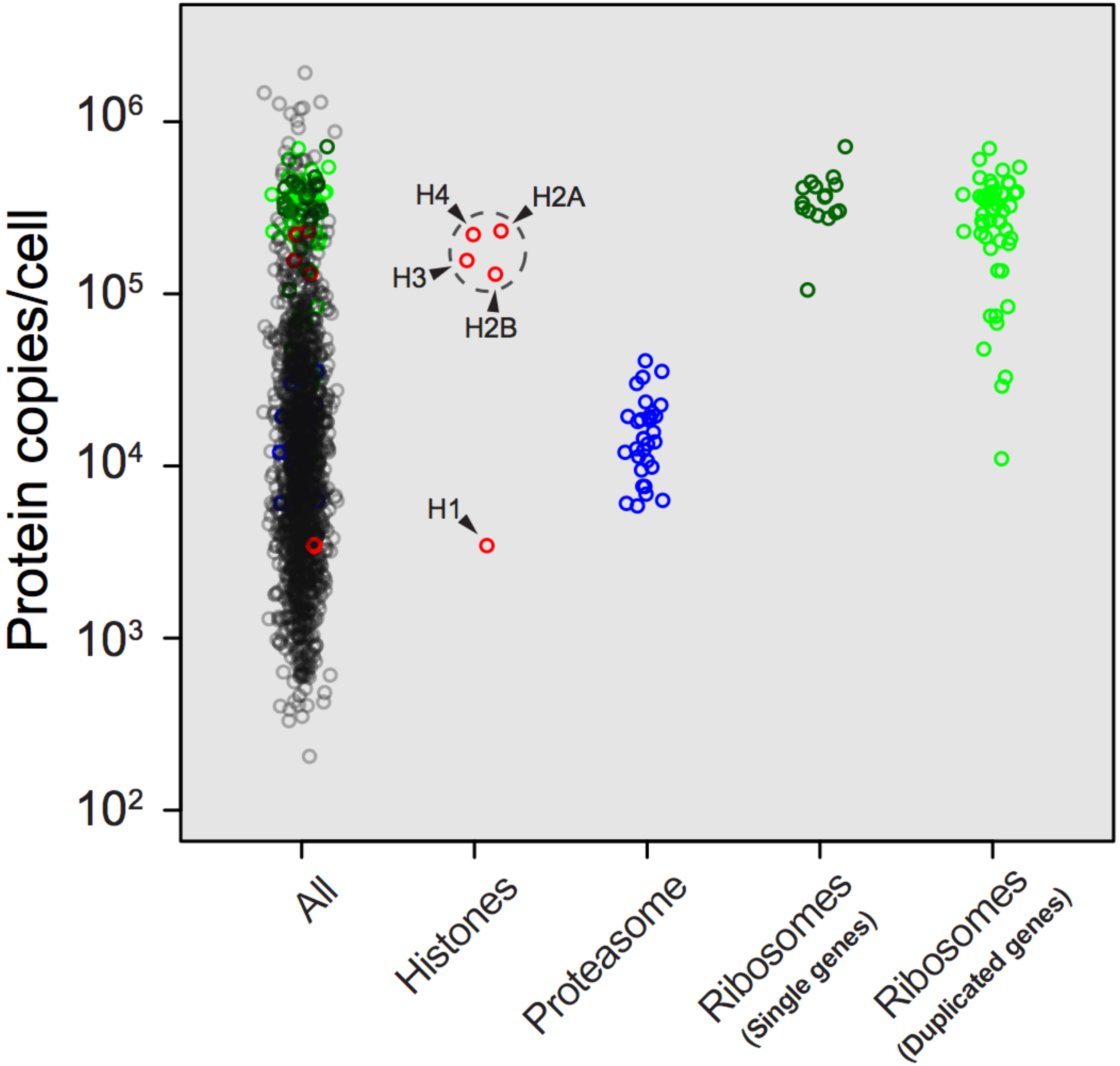
UPS2 Amount (fmol)	UniProt Protein Name [Synonym]	MW (Da) (calculated)	Source or recombinant	Host
50,000	Carbonic anhydrase 1	28,738	Erythrocytes	
50,000	Carbonic anhydrase 2	29,115	Erythrocytes	
50,000	Complement C5 [Complement C5a]	8,536	Recombinant	*E. coli*
50,000	Hemoglobin alpha chain	15,126	Erythrocytes	
50,000	Hemoglobin beta chain	15,867	Erythrocytes	
50,000	Leptin	16,158	Recombinant	*E. coli*
50,000	Serum Albumin	66,357	Recombinant	*Pichia pastoris*
50,000	Ubiquitin	10,597	Recombinant	*E. coli*
5,000	Catalase	59,625	Erythrocytes	
5,000	Cytochrome b_5	16,022	Recombinant	*E. coli*
5,000	Epidermal Growth Factor	6,353	Recombinant	*E. coli*
5,000	Myoglobin C	17,053	Heart	
5,000	NAD(P)H dehydrogenase [quinone] 1 [DT Diaphorase] C	30,736	Recombinant	*E. coli*
5,000	Peptidyl-prolyl cis-trans isomerase A [Cyclophilin A]	20,176	Recombinant	*E. coli*
5,000	Peroxiredoxin 1	21,979	Recombinant	*E. coli*
5,000	Small ubiquitin-related modifier 1 [SUMO-1]	38,815	Recombinant	*E. coli*
500	Alpha-lactalbumin	14,078	Milk	
500	Creatine kinase M-type [CK-MM]	43,101	Heart	
500	Histidyl-tRNA synthetase [Jo-1]	58,233	Recombinant	*E. coli*
500	Lysozyme C	14,701	Milk	
500	Neddylin [Nedd8]	9,072	Recombinant	*E. coli*
500	Retinol-binding protein	21,071	Urine	
500	Ribosyldihydronicotinamide dehydrogenase [quinone] [Quinone oxidoreductase 2] [NQO2]	25,821	Recombinant	*E. coli*
500	Ubiquitin-conjugating enzyme E2 I [UbcH9]	18,007	Recombinant	*E. coli*
50	Antithrombin-III	49,039	Plasma	
50	Beta-2-microglobulin	11,731	Urine	
50	BH3 Interacting domain death	21,995	Recombinant	*E. coli*

iBAQ - quatification using a standard



courtesy of M. Selbach

iBAQ quantification



courtesy of M. Selbach

MDC
mass spectrometry

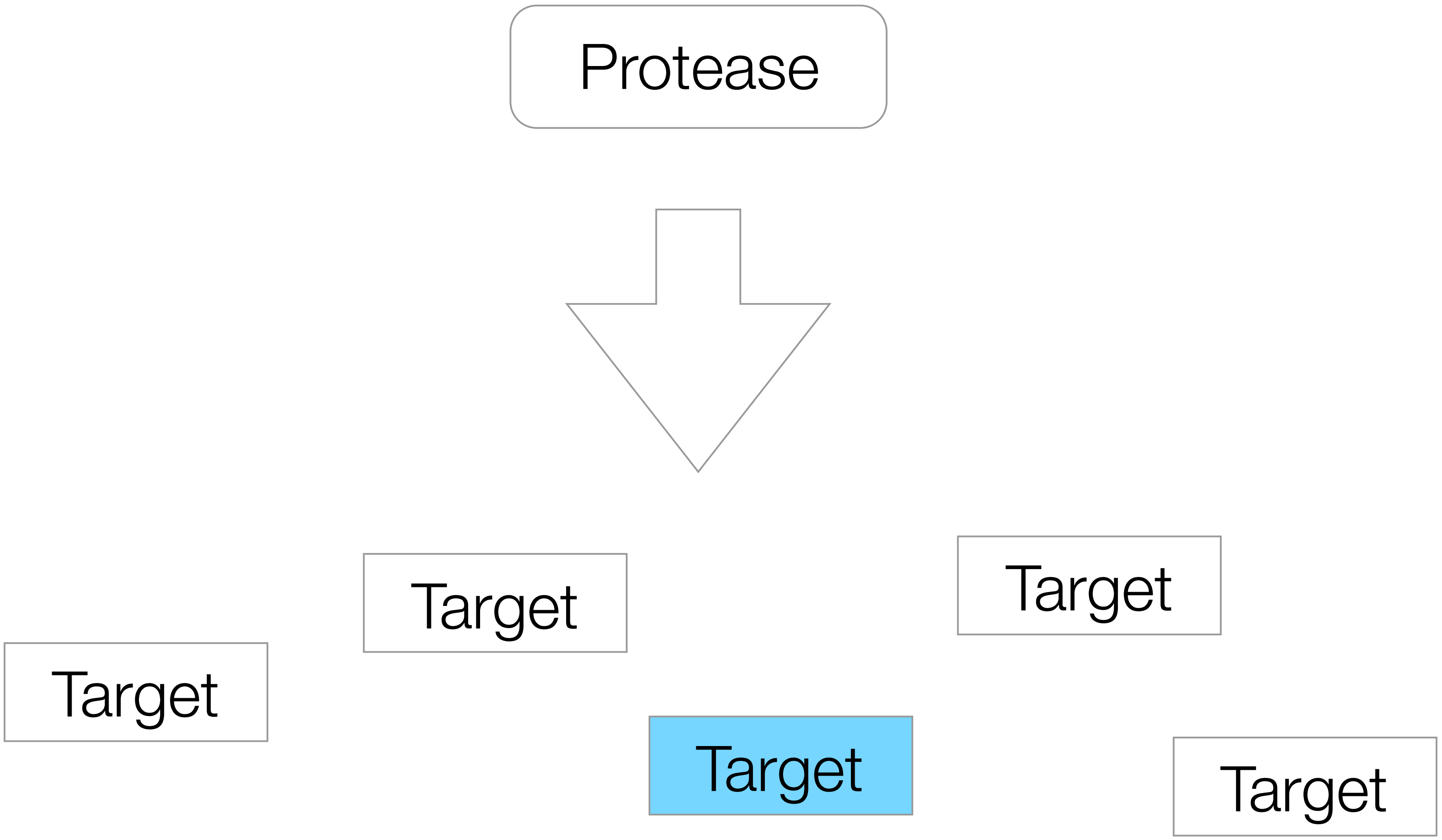
Other applications of mass-spec based proteomics

MDC
mass spectrometry

PICS

proteomic identification of protease cleavage sites

Proteases


Protease

Target

Target

Target

Target

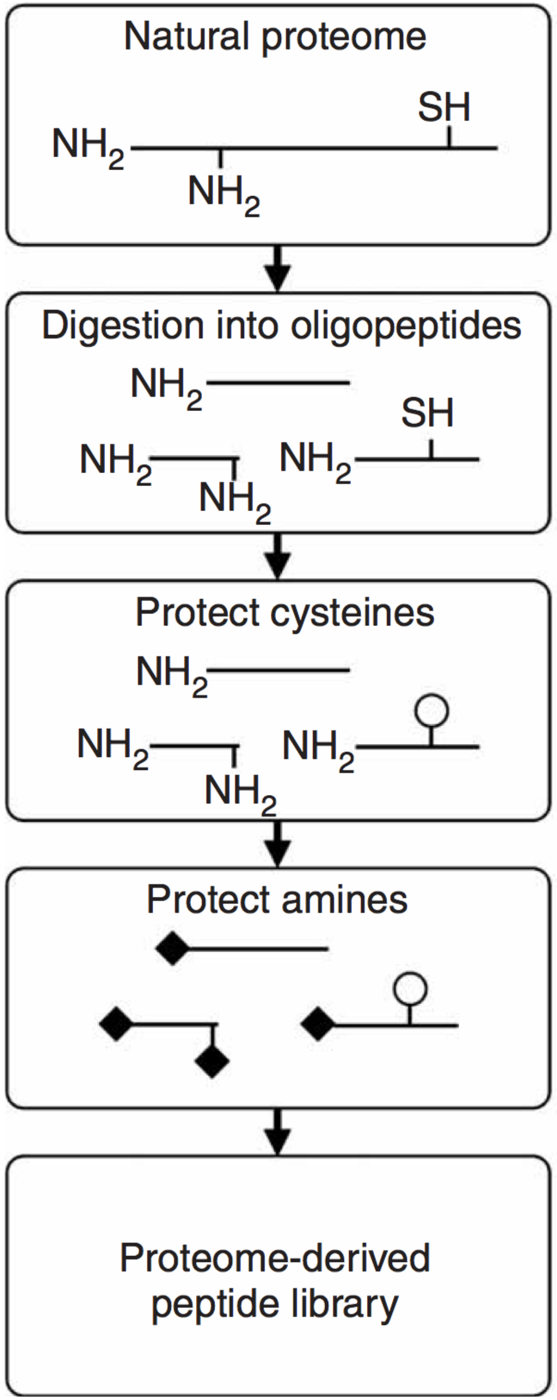
Target

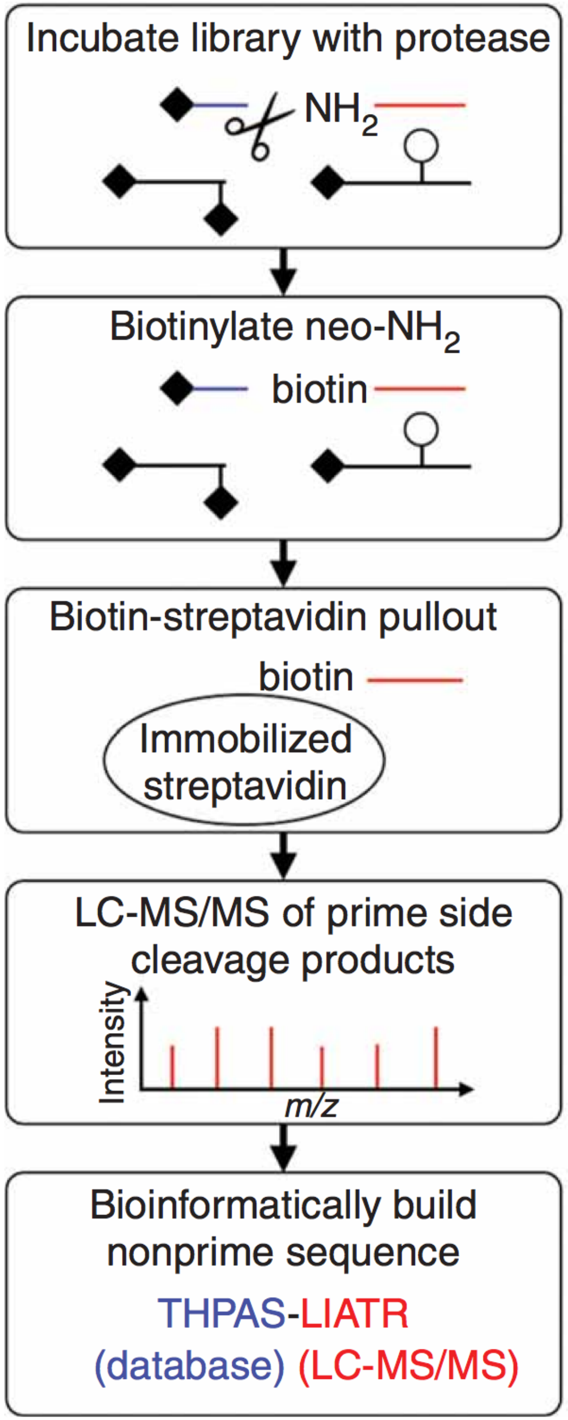
PICS

Two steps

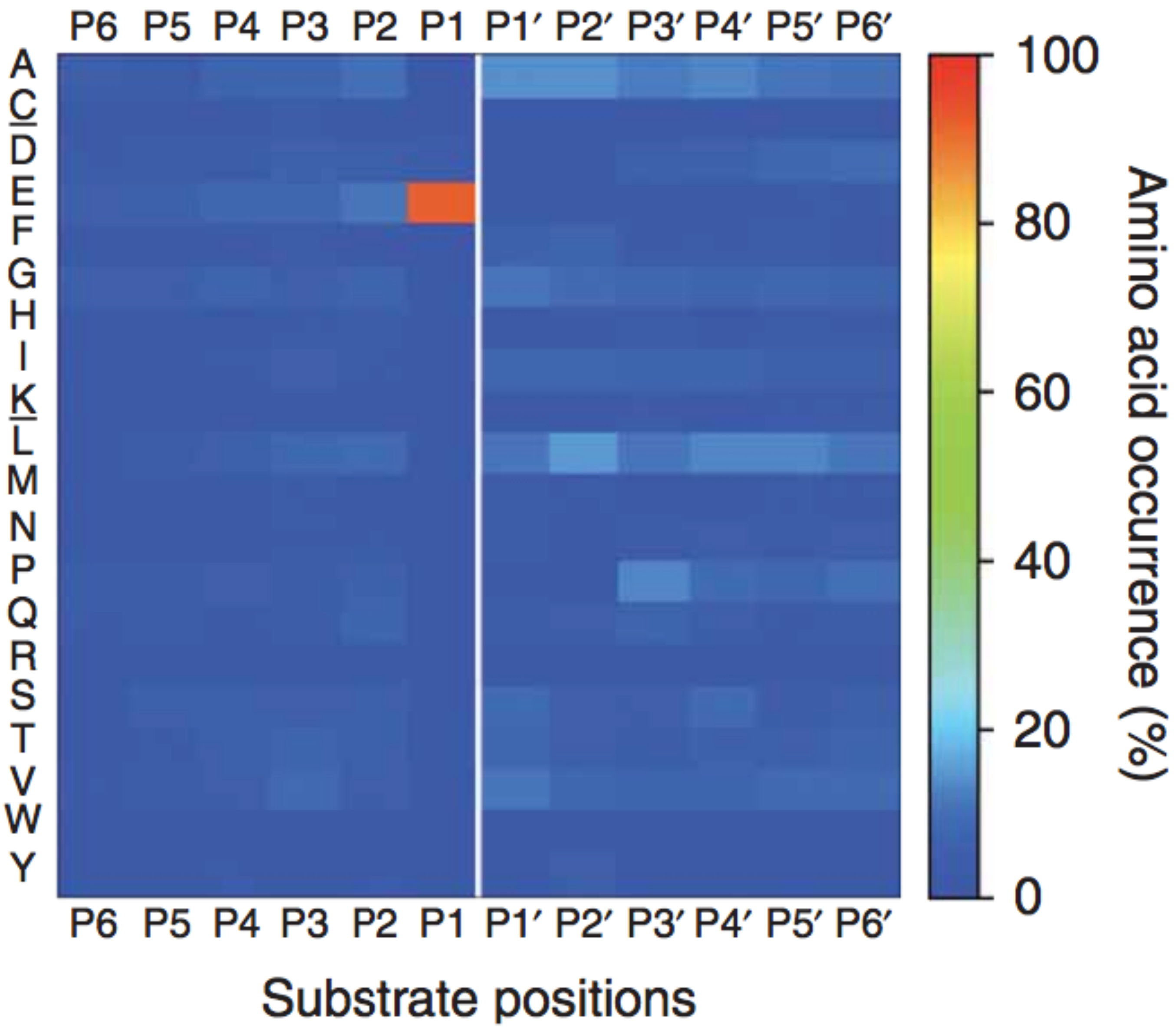
1. library generation
2. identification of specific cleavage products

Schilling, O., and Overall, C.M. (2008).


Natural proteome
SH
NH2
NH2
Digestion into oligopeptides
NH2
SH
NH2
NH2
NH2
Protect cysteines
NH2
NH2
NH2
NH2
Protect amines
Proteome-derived
peptide library


Incubate library with protease
NH2
Biotinylate neo-NH2
biotin
Biotin-streptavidin pullout
biotin
Immobilized streptavidin
LC-MS/MS of prime side cleavage products
Intensity
m/z
Bioinformatically build nonprime sequence
THPAS-LIATR
(database) (LC-MS/MS)

PICS result



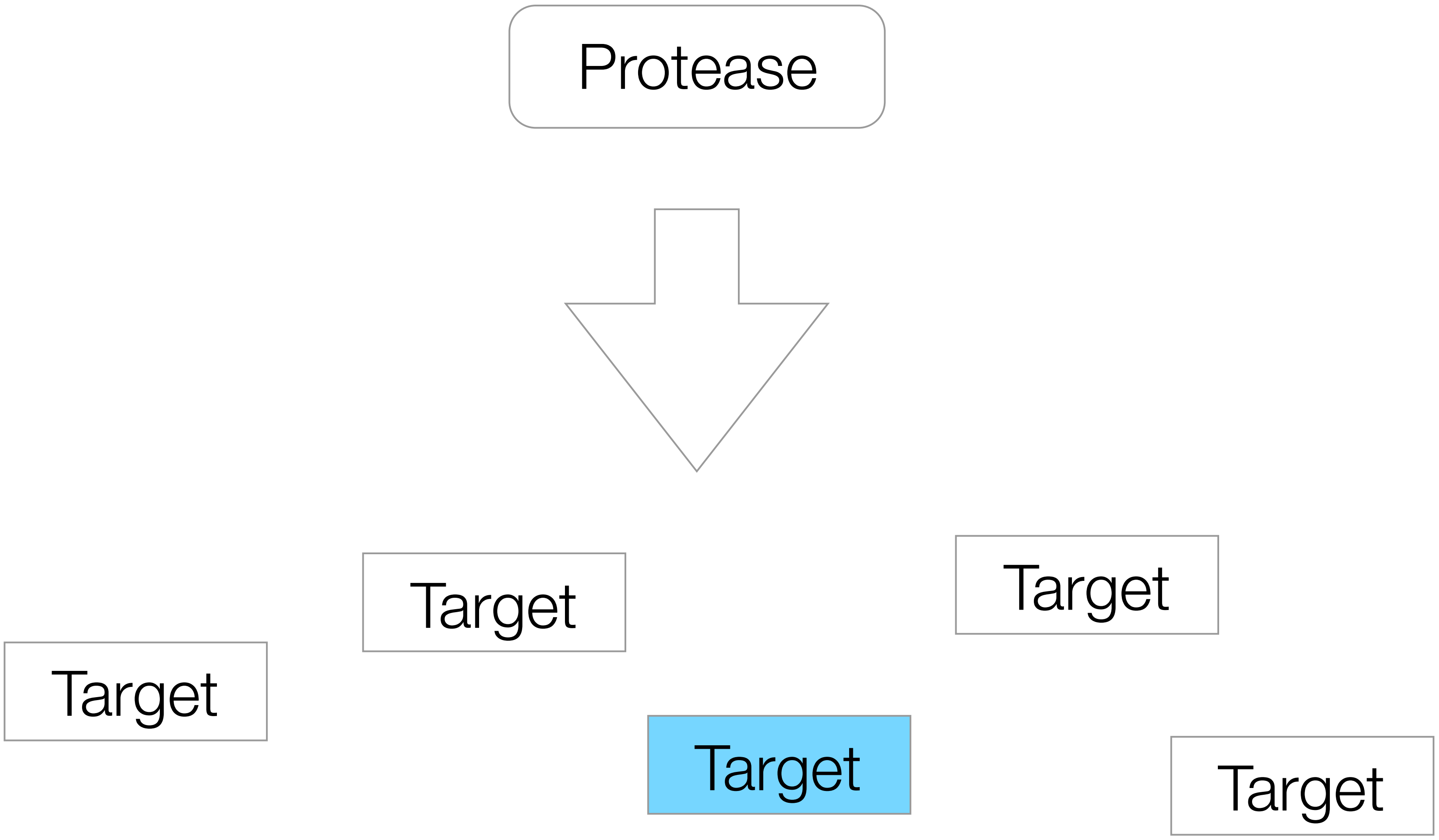
P6 P5 P4 P3 P2 P1 P1′ P2′ P3′ P4′ P5′ P6′
A
C
D
E
F
G
H
I
K
L
M
N
P
Q
R
S
T
V
W
Y
P6 P5 P4 P3 P2 P1 P1′ P2′ P3′ P4′ P5′ P6′
Substrate positions
100
80
60
40
20
0
Amino acid occurrence (%)

MDC
mass spectrometry

TAILS

The identification of protease cleavage sites

Proteases



Protease

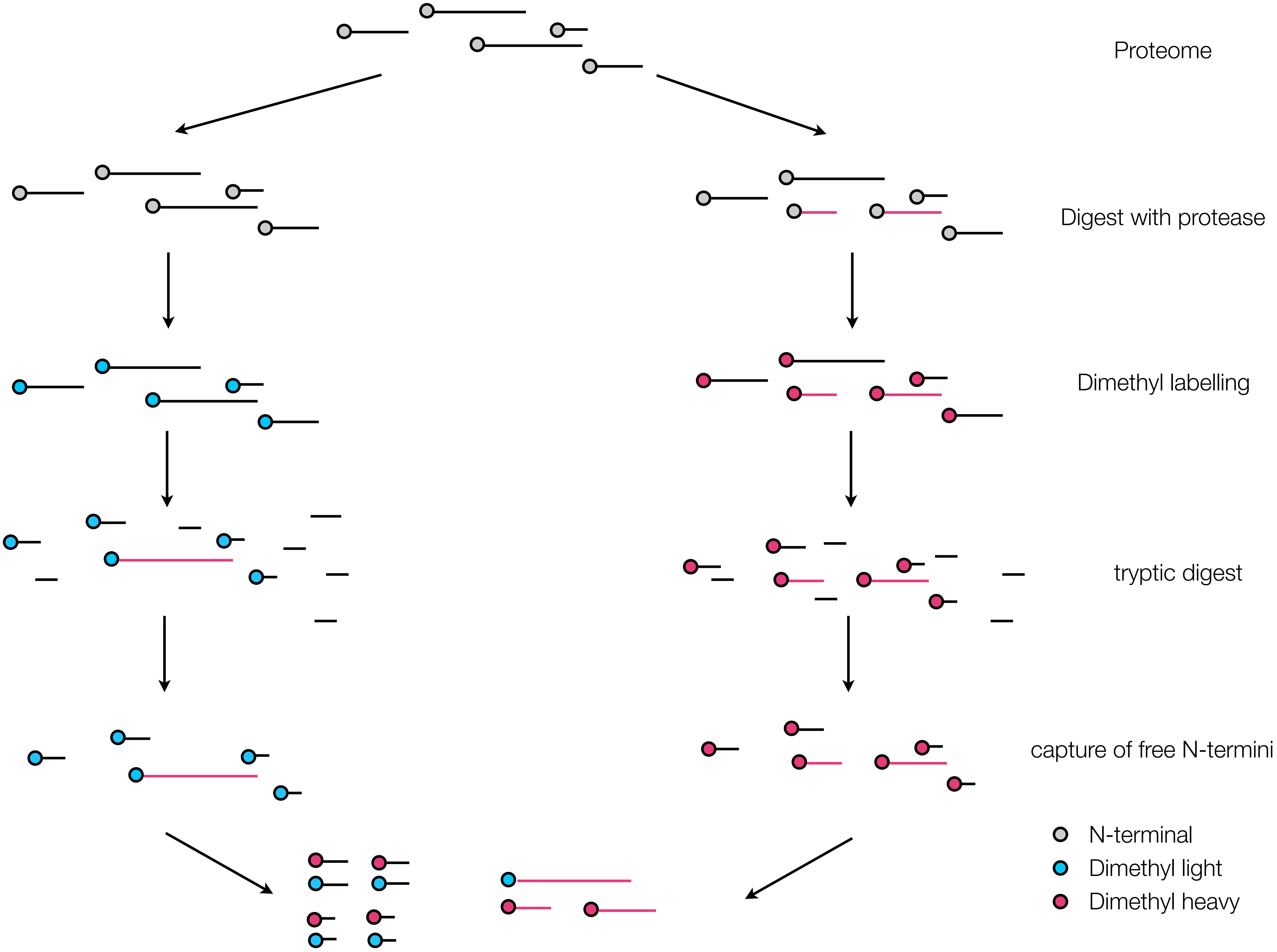
Target

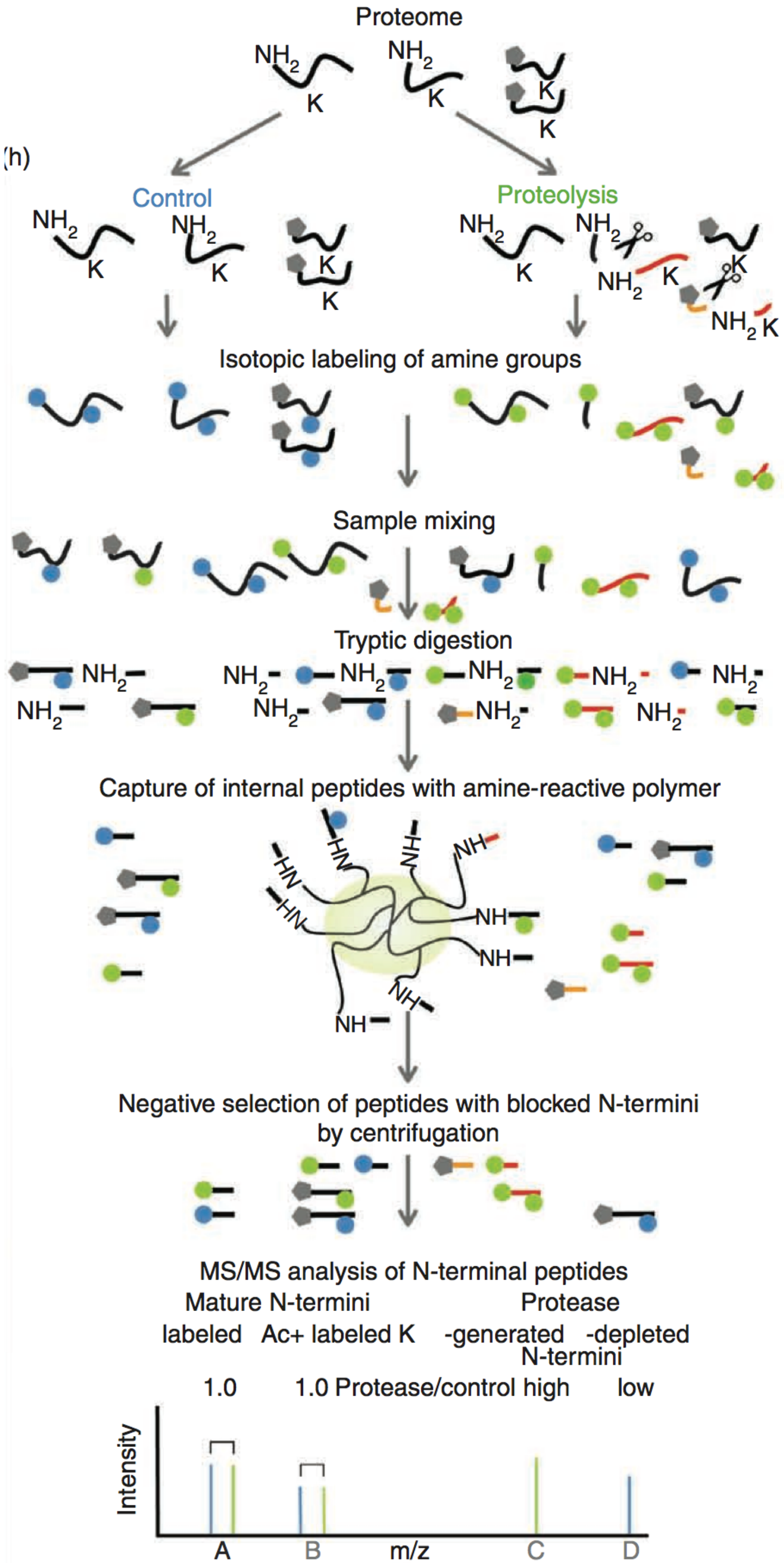
Target

Target

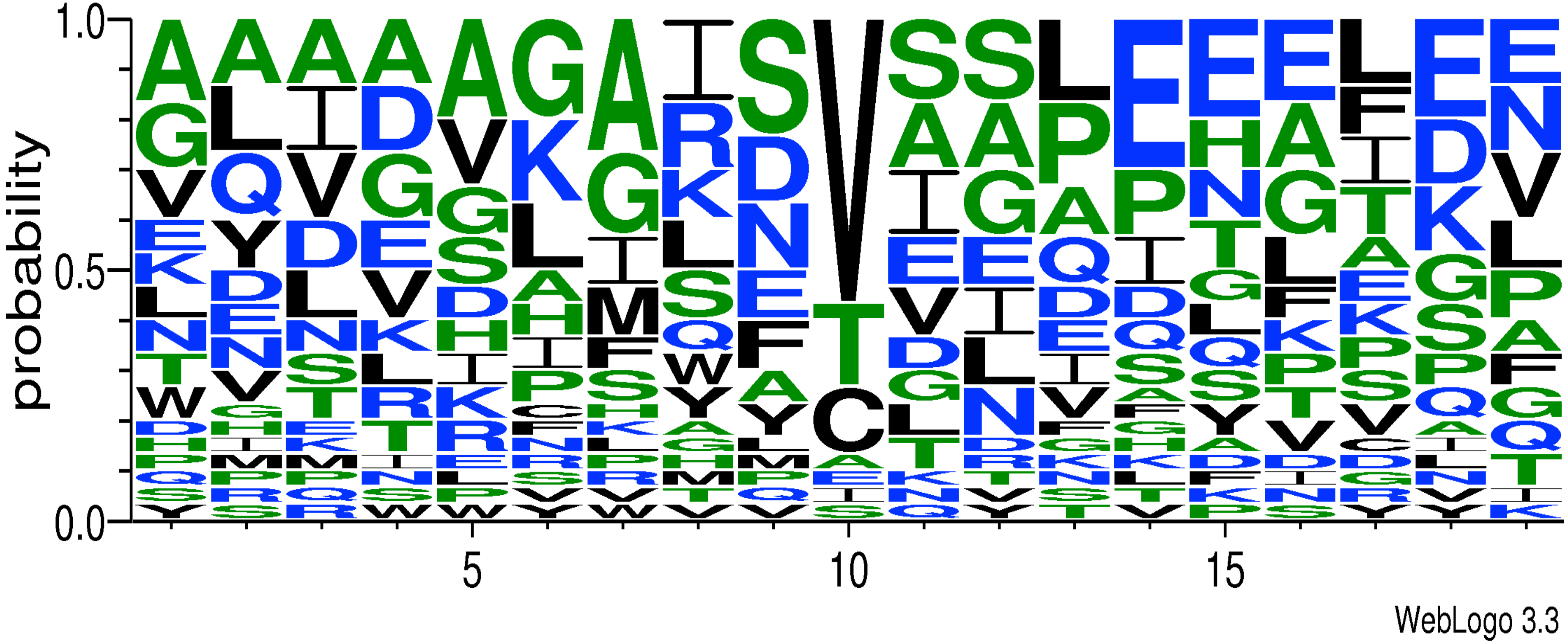
Target

Target


Proteome
Digest with protease
Dimethyl labelling
tryptic digest
capture of free N-termini
N-terminal
Dimethyl light
Dimethyl heavy

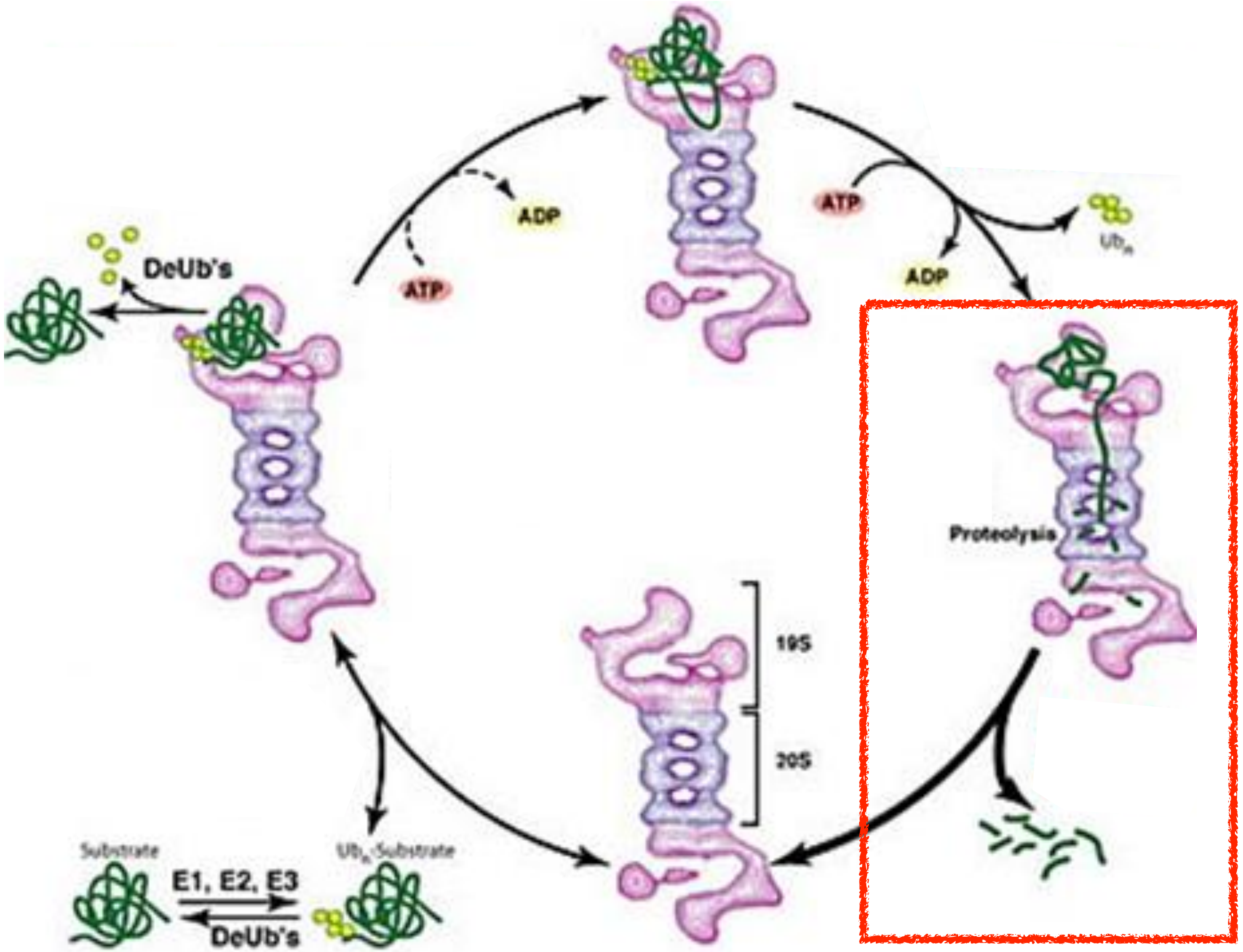

Proteome
NH2
K
(h)
Control
Proteolysis
Isotopic labeling of amine groups
Sample mixing
Tryptic digestion
Capture of internal peptides with amine-reactive polymer
Negative selection of peptides with blocked N-termini by centrifugation
MS/MS analysis of N-terminal peptides
Mature N-termini
labeled
Ac+ labeled K
Protease
-generated
-depleted
N-termini
1.0
1.0 Protease/control high
low
Intensity
m/z
A
B
C
D

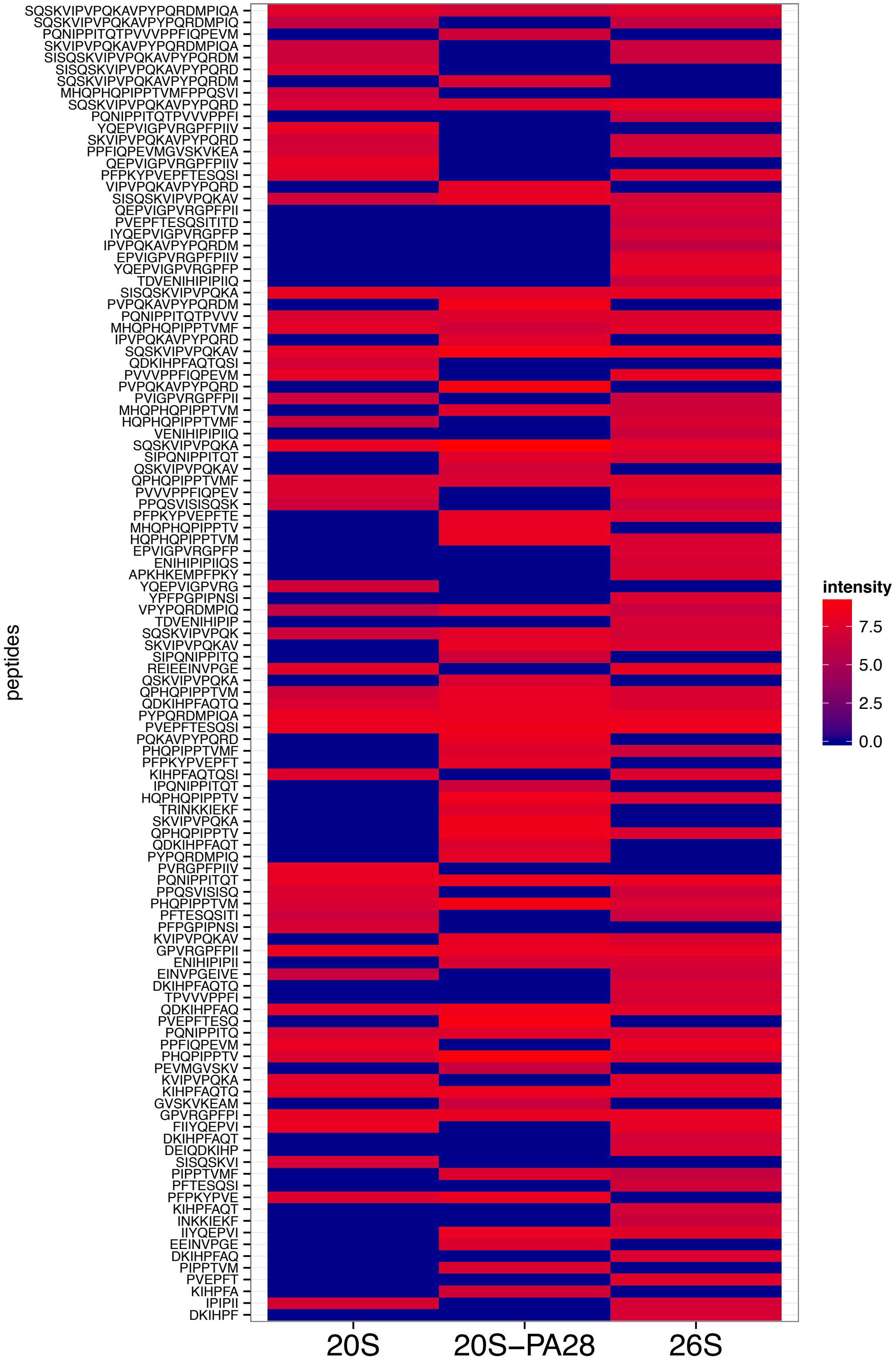
Identified recognition site



Preferred peptide signatures of the proteasome

Protein digestion by the proteasome




SQSKVIPVPQKAVPYPQRDMPIQA
SQSKVIPVPQKAVPYPQRDMPIQ
PQNIPPITQTPVVVPPFIQPEVM
SKVIPVPQKAVPYPQRDMPIQA
SISQSKVIPVPQKAVPYPQRDM
SISQSKVIPVPQKAVPYPQRD
SQSKVIPVPQKAVPYPQRDM
MHQPHQPIPPTVMFPPQSVI
SQSKVIPVPQKAVPYPQRD
PQNIPPITQTPVVVPPFI
YQEPVIGPVRGPFPIIV
SKVIPVPQKAVPYPQRD
PPFIQPEVMGVSKVKEA
QEPVIGPVRGPFPIIV
PFPKYPVEPFTESQSI
VIPVPQKAVPYPQRD
SISQSKVIPVPQKAV
QEPVIGPVRGPFPII
PVEPFTESQSITITD
IYQEPVIGPVRGPFP
IPVPQKAVPYPQRDM
EPVIGPVRGPFPIIV
YQEPVIGPVRGPFP
TDVENIHIPIPIIQ
SISQSKVIPVPQKA
PVPQKAVPYPQRDM
PQNIPPITQTPVVV
MHQPHQPIPPTVMF
IPVPQKAVPYPQRD
SQSKVIPVPQKAV
QDKIHPFAQTQSI
PVVVPPFIQPEVM
PVPQKAVPYPQRD
PVIGPVRGPFPII
MHQPHQPIPPTVM
HQPHQPIPPTVMF
VENIHIPIPIIQ
SQSKVIPVPQKA
SIPQNIPPITQT
QSKVIPVPQKAV
QPHQPIPPTVMF
PVVVPPFIQPEV
PPQSVISISQSK
PFPKYPVEPFTE
MHQPHQPIPPTV
HQPHQPIPPTVM
EPVIGPVRGPFP
ENIHIPIPIIQS
APKHKEMPFPKY
YQEPVIGPVRG
YPFPGPIPNSI
VPYPQRDMPIQ
TDVENIHIPIP
SQSKVIPVPQK
SKVIPVPQKAV
SIPQNIPPITQ
REIEEINVPGE
QSKVIPVPQKA
QPHQPIPPTVM
QDKIHPFAQTQ
PYPQRDMPIQA
PVEPFTESQSI
PQKAVPYPQRD
PHQPIPPTVMF
PFPKYPVEPFT
KIHPFAQTQSI
IPQNIPPITQT
HQPHQPIPPTV
TRINKKIEKF
SKVIPVPQKA
QPHQPIPPTV
QDKIHPFAQT
PYPQRDMPIQ
PVRGPFPIIV
PQNIPPITQT
PPQSVISISQ
PHQPIPPTVM
PFTESQSITI
PFPGPIPNSI
KVIPVPQKAV
GPVRGPFPII
ENIHIPIPII
EINVPGEIVE
DKIHPFAQTQ
TPVVVPPFI
QDKIHPFAQ
PVEPFTESQ
PQNIPPITQ
PPFIQPEVM
PHQPIPPTV
PEVMGVSKV
KVIPVPQKA
KIHPFAQTQ
GVSKVKEAM
GPVRGPFPI
FIIYQEPVI
DKIHPFAQT
DEIQDKIHP
SISQSKVI
PIPPTVMF
PFTESQSI
PFPKYPVE
KIHPFAQT
INKKIEKF
IIYQEPVI
EEINVPGE
DKIHPFAQ
PIPPTVM
PVEPFT
KIHPFA
IPIPII
DKIHPF
peptides
20S
20S−PA28
26S
intensity
7.5
5.0
2.5
0.0

Post-translational modifications

Common post-translational modifications

- Protein N-acetylation
- Phosphorylation (Serine/Threonine/Tyrosine)
- Ubiquitination

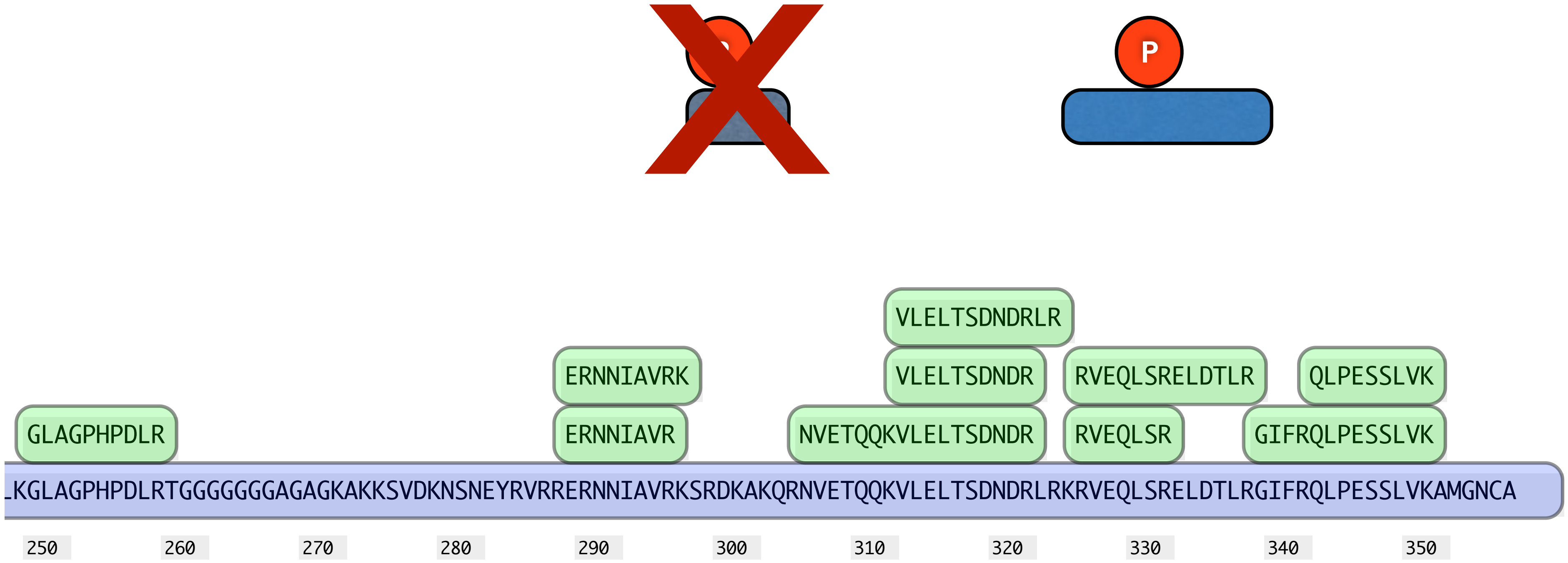
- currently 949 different PTMs listed in data bases[1]

[1] as of:15.08.2012

MDC
mass spectrometry

Peptide coverage is essential….

Peptides mapped to the protein sequence (sequence coverage)

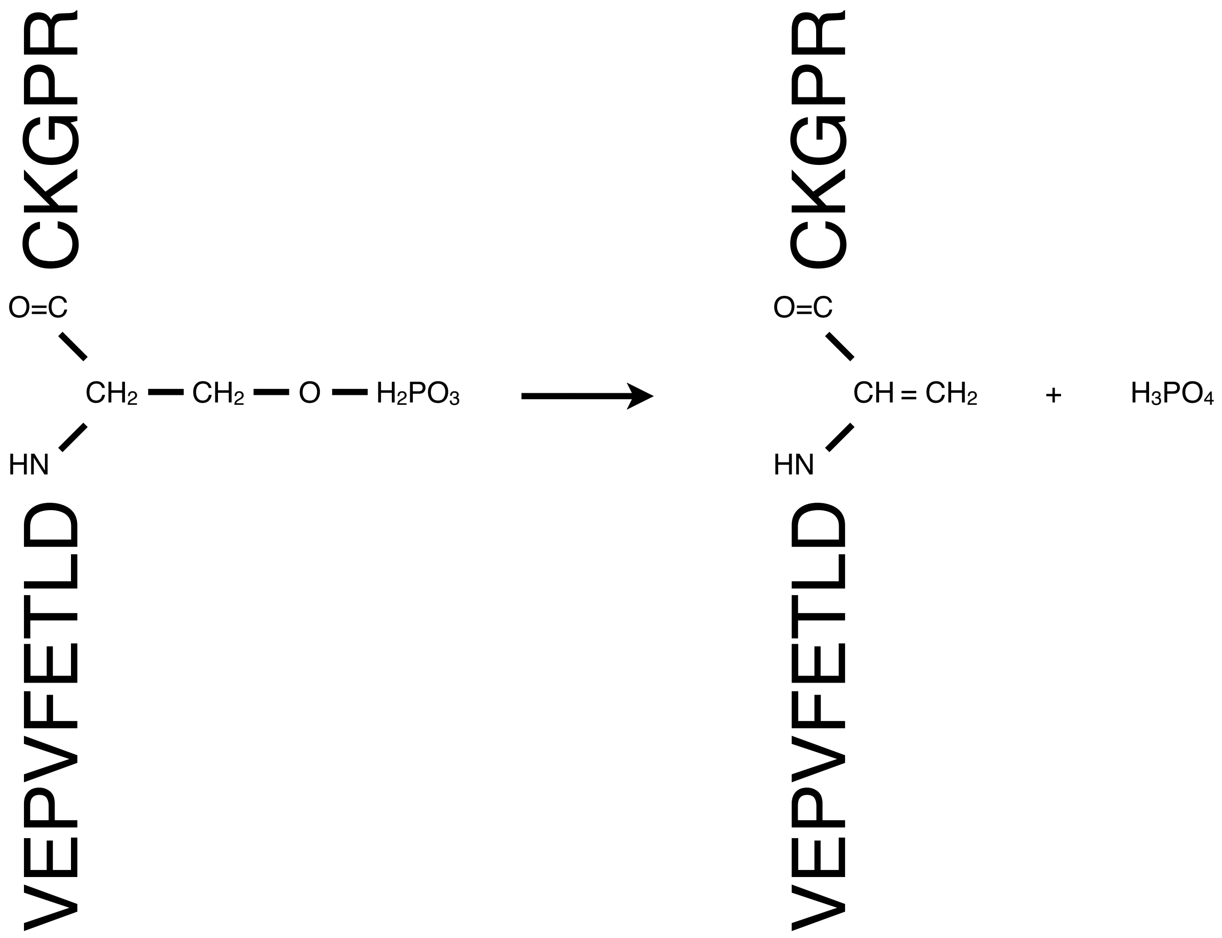


Detection of the PTM in the MS spectra

Post-translational modifications

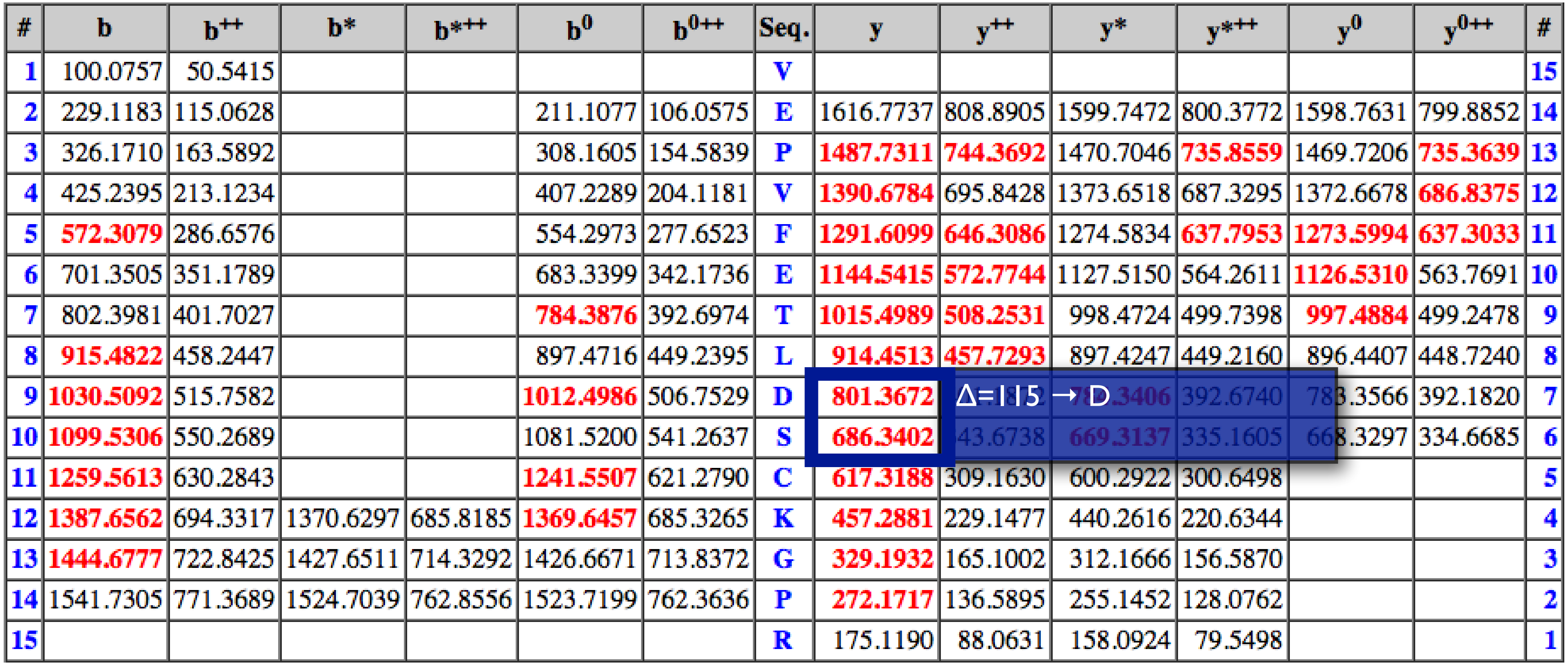
- detection as mass shifts on amino acids
  - +144 Da on lysines for ubiquitin
  - +80 Da for phosphorylations
  - +14 Da for methylations

Neutral loss phospho-serine



Aspertate

VEPVFETL**DSC**KGPR

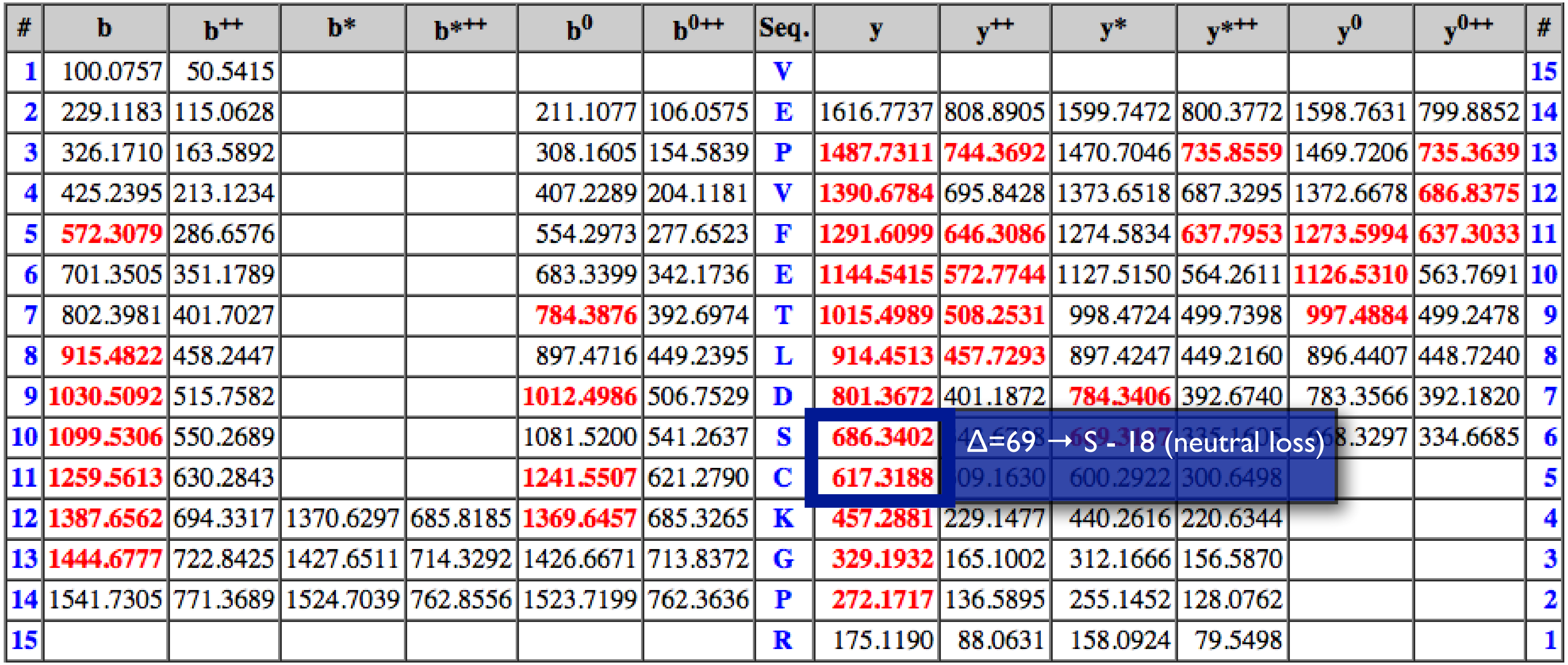


#	b	b++	b*	b*++	b0	b0++	Seq.	y	y++	y*	y*++	y0	y0++	#
1	100.0757	50.5415					V							15
2	229.1183	115.0628			211.1077	106.0575	E	1616.7737	808.8905	1599.7472	800.3772	1598.7631	799.8852	14
3	326.1710	163.5892			308.1605	154.5839	P	1487.7311	744.3692	1470.7046	735.8559	1469.7206	735.3639	13
4	425.2395	213.1234			407.2289	204.1181	V	1390.6784	695.8428	1373.6518	687.3295	1372.6678	686.8375	12
5	572.3079	286.6576			554.2973	277.6523	F	1291.6099	646.3086	1274.5834	637.7953	1273.5994	637.3033	11
6	701.3505	351.1789			683.3399	342.1736	E	1144.5415	572.7744	1127.5150	564.2611	1126.5310	563.7691	10
7	802.3981	401.7027			784.3876	392.6974	T	1015.4989	508.2531	998.4724	499.7398	997.4884	499.2478	9
8	915.4822	458.2447			897.4716	449.2395	L	914.4513	457.7293	897.4247	449.2160	896.4407	448.7240	8
9	1030.5092	515.7582			1012.4986	506.7529	D	801.3672	[illegible]	[illegible]	392.6740	783.3566	392.1820	7
10	1099.5306	550.2689			1081.5200	541.2637	S	686.3402	343.6738	669.3137	335.1605	668.3297	334.6685	6
11	1259.5613	630.2843			1241.5507	621.2790	C	617.3188	309.1630	600.2922	300.6498			5
12	1387.6562	694.3317	1370.6297	685.8185	1369.6457	685.3265	K	457.2881	229.1477	440.2616	220.6344			4
13	1444.6777	722.8425	1427.6511	714.3292	1426.6671	713.8372	G	329.1932	165.1002	312.1666	156.5870			3
14	1541.7305	771.3689	1524.7039	762.8556	1523.7199	762.3636	P	272.1717	136.5895	255.1452	128.0762			2
15							R	175.1190	88.0631	158.0924	79.5498			1

Δ=115 → D

Neutral loss phospho-serine

VEPVFETL**DSC**KGPR

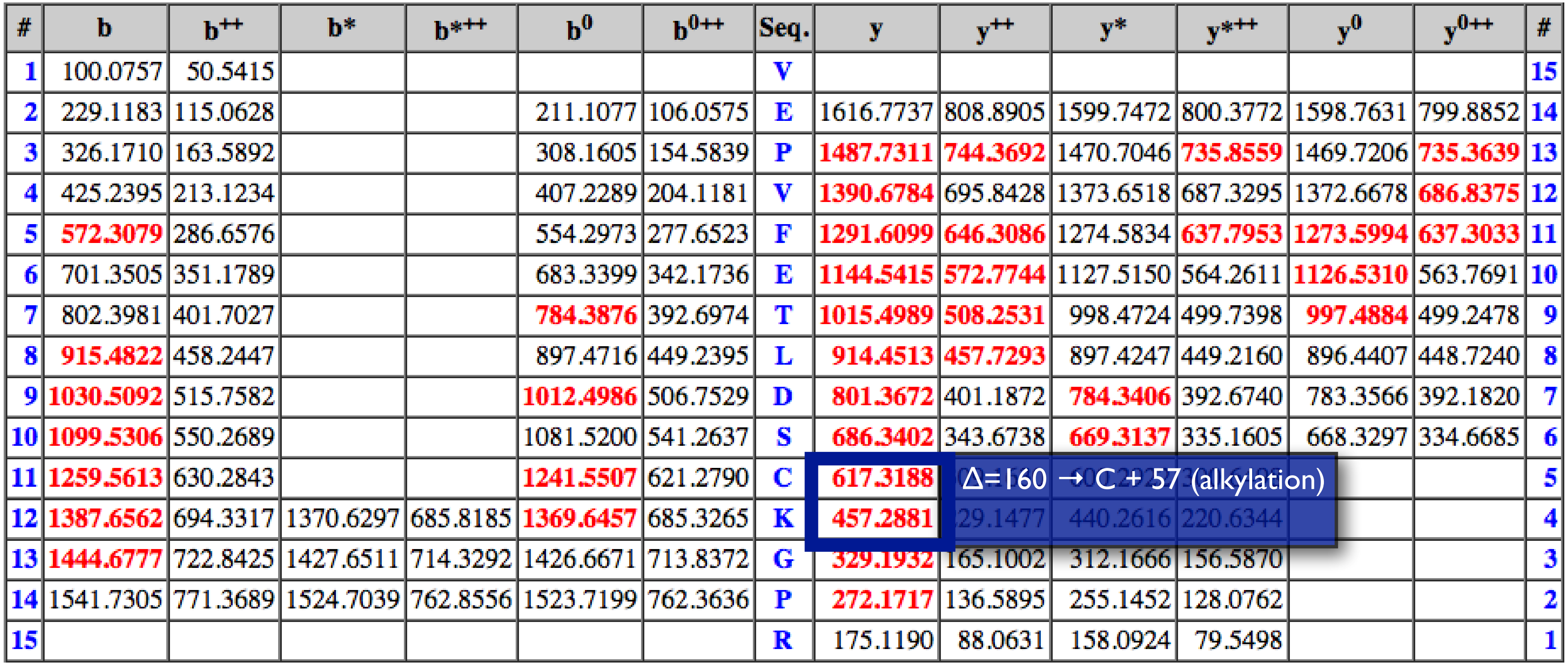


#	b	b^{++}	b^*	b^{*++}	b^0	b^{0++}	Seq.	y	y^{++}	y^*	y^{*++}	y^0	y^{0++}	#
1	100.0757	50.5415					V							15
2	229.1183	115.0628			211.1077	106.0575	E	1616.7737	808.8905	1599.7472	800.3772	1598.7631	799.8852	14
3	326.1710	163.5892			308.1605	154.5839	P	1487.7311	744.3692	1470.7046	735.8559	1469.7206	735.3639	13
4	425.2395	213.1234			407.2289	204.1181	V	1390.6784	695.8428	1373.6518	687.3295	1372.6678	686.8375	12
5	572.3079	286.6576			554.2973	277.6523	F	1291.6099	646.3086	1274.5834	637.7953	1273.5994	637.3033	11
6	701.3505	351.1789			683.3399	342.1736	E	1144.5415	572.7744	1127.5150	564.2611	1126.5310	563.7691	10
7	802.3981	401.7027			784.3876	392.6974	T	1015.4989	508.2531	998.4724	499.7398	997.4884	499.2478	9
8	915.4822	458.2447			897.4716	449.2395	L	914.4513	457.7293	897.4247	449.2160	896.4407	448.7240	8
9	1030.5092	515.7582			1012.4986	506.7529	D	801.3672	401.1872	784.3406	392.6740	783.3566	392.1820	7
10	1099.5306	550.2689			1081.5200	541.2637	S	686.3402	[illegible]	669.3137	335.1605	668.3297	334.6685	6
11	1259.5613	630.2843			1241.5507	621.2790	C	617.3188	309.1630	600.2922	300.6498			5
12	1387.6562	694.3317	1370.6297	685.8185	1369.6457	685.3265	K	457.2881	229.1477	440.2616	220.6344			4
13	1444.6777	722.8425	1427.6511	714.3292	1426.6671	713.8372	G	329.1932	165.1002	312.1666	156.5870			3
14	1541.7305	771.3689	1524.7039	762.8556	1523.7199	762.3636	P	272.1717	136.5895	255.1452	128.0762			2
15							R	175.1190	88.0631	158.0924	79.5498			1

Δ=69 → S - 18 (neutral loss)

Carbamidomethylated cysteine

VEPVFETL**DSC**KGPR



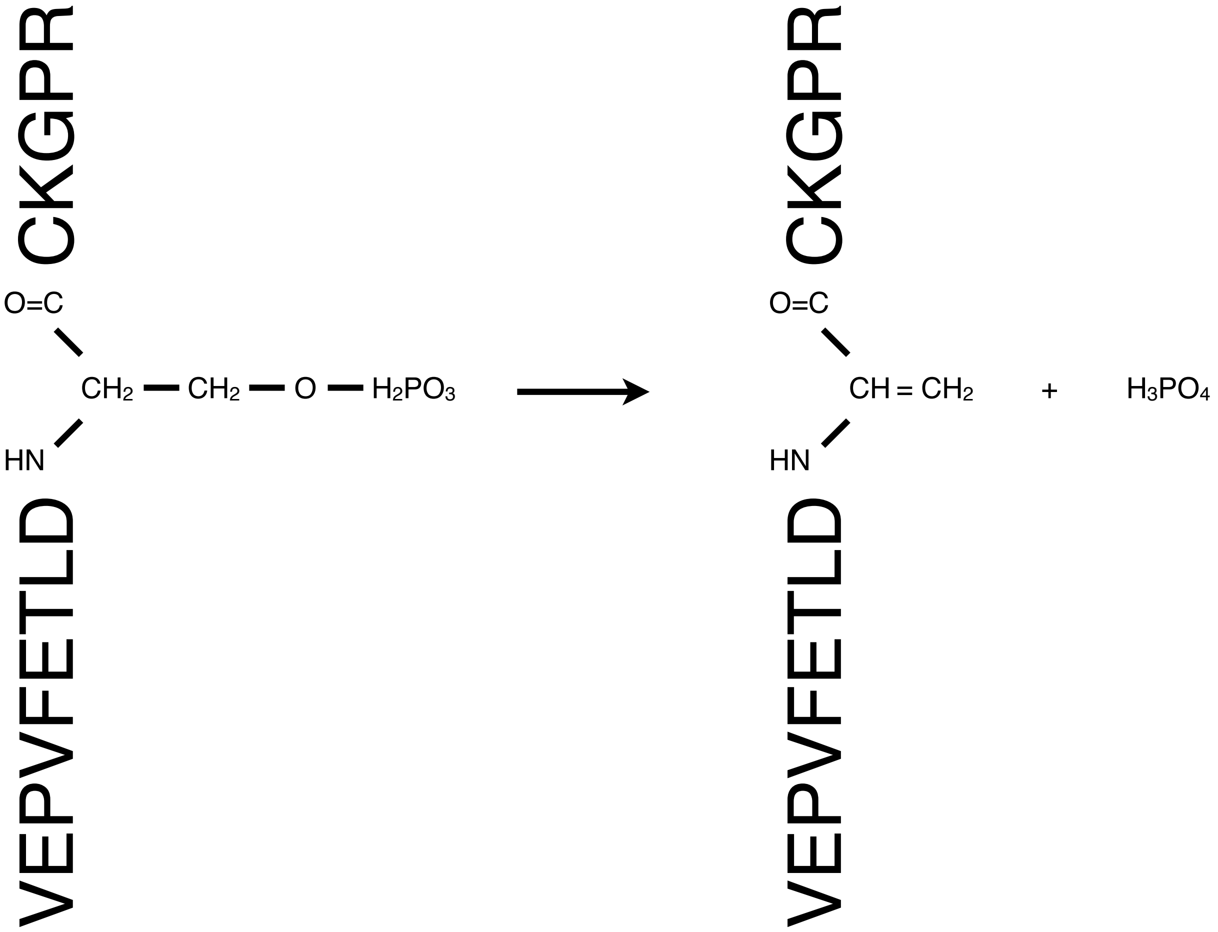
#	b	b^{++}	b^{*}	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y^{*}	y^{*++}	y^{0}	y^{0++}	#
1	100.0757	50.5415					V							15
2	229.1183	115.0628			211.1077	106.0575	E	1616.7737	808.8905	1599.7472	800.3772	1598.7631	799.8852	14
3	326.1710	163.5892			308.1605	154.5839	P	**1487.7311**	**744.3692**	1470.7046	**735.8559**	1469.7206	**735.3639**	13
4	425.2395	213.1234			407.2289	204.1181	V	**1390.6784**	695.8428	1373.6518	687.3295	1372.6678	**686.8375**	12
5	**572.3079**	286.6576			554.2973	277.6523	F	**1291.6099**	**646.3086**	1274.5834	**637.7953**	**1273.5994**	**637.3033**	11
6	701.3505	351.1789			683.3399	342.1736	E	**1144.5415**	**572.7744**	1127.5150	564.2611	**1126.5310**	563.7691	10
7	802.3981	401.7027			**784.3876**	392.6974	T	**1015.4989**	**508.2531**	998.4724	499.7398	**997.4884**	499.2478	9
8	**915.4822**	458.2447			897.4716	449.2395	L	**914.4513**	**457.7293**	897.4247	449.2160	896.4407	448.7240	8
9	**1030.5092**	515.7582			**1012.4986**	506.7529	D	**801.3672**	401.1872	**784.3406**	392.6740	783.3566	392.1820	7
10	**1099.5306**	550.2689			1081.5200	541.2637	S	**686.3402**	343.6738	**669.3137**	335.1605	668.3297	334.6685	6
11	**1259.5613**	630.2843			**1241.5507**	621.2790	C	**617.3188**	[illegible]	[illegible]	[illegible]			5
12	**1387.6562**	694.3317	1370.6297	685.8185	**1369.6457**	685.3265	K	**457.2881**	229.1477	440.2616	220.6344			4
13	**1444.6777**	722.8425	1427.6511	714.3292	1426.6671	713.8372	G	**329.1932**	165.1002	312.1666	156.5870			3
14	1541.7305	771.3689	1524.7039	762.8556	1523.7199	762.3636	P	**272.1717**	136.5895	255.1452	128.0762			2
15							R	175.1190	88.0631	158.0924	79.5498			1

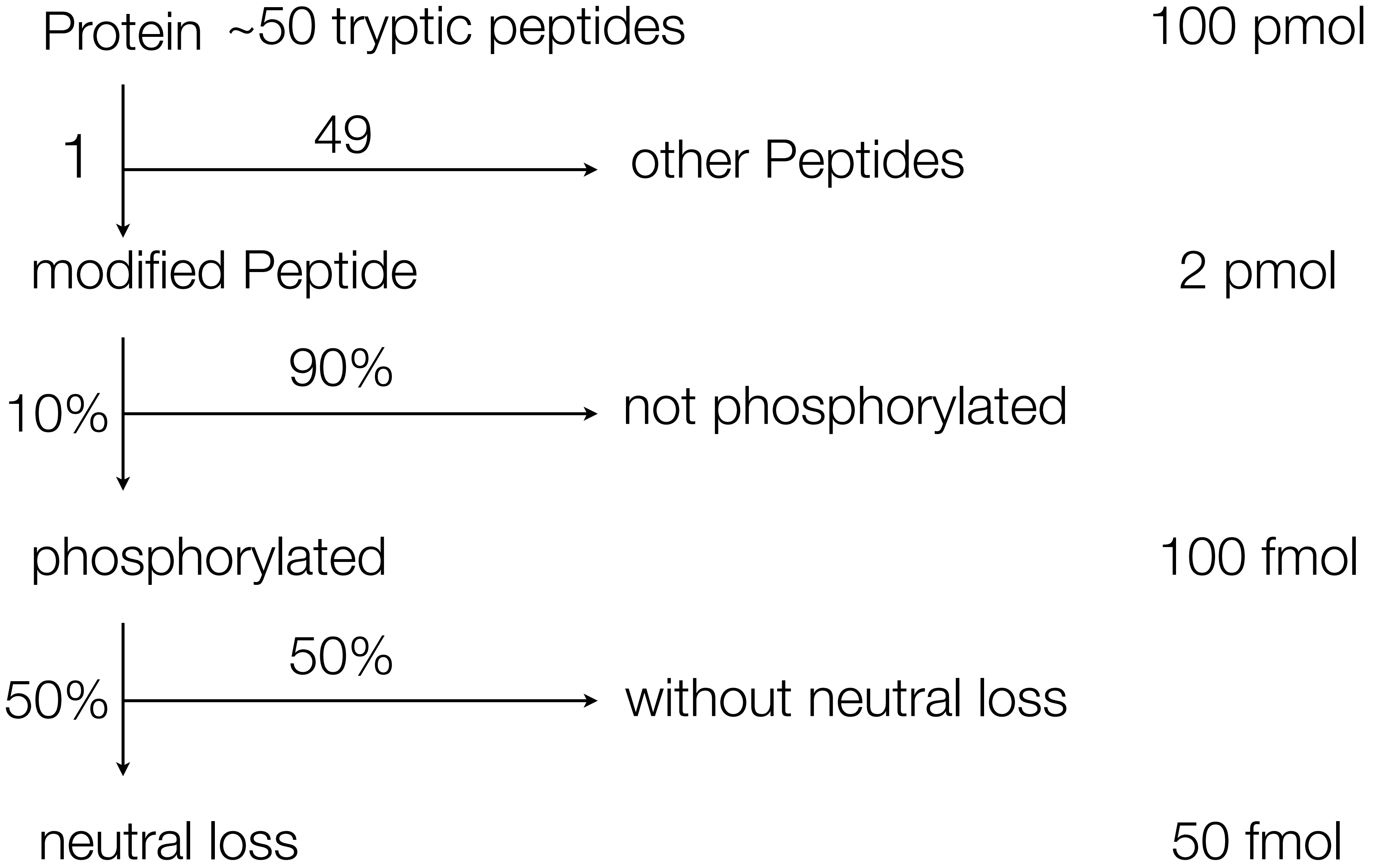
Δ=160 → C + 57 (alkylation)

What is so difficult?

Neutral losses and other MS effects

Neutral loss phospho-serine





Protein ~50 tryptic peptides 100 pmol

1

49

other Peptides

modified Peptide 2 pmol

10%

90%

not phosphorylated

phosphorylated 100 fmol

50%

50%

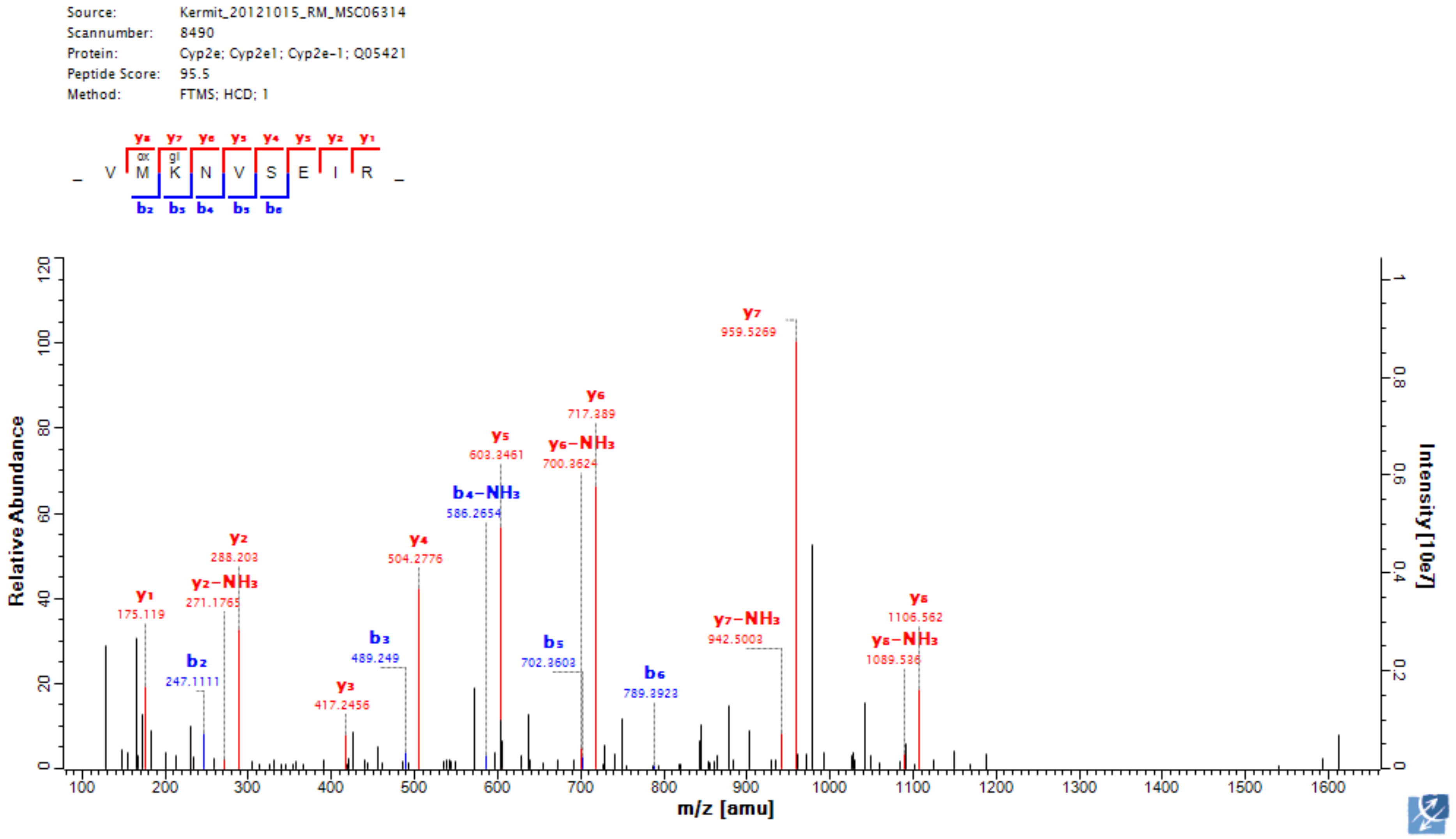
without neutral loss

neutral loss 50 fmol

Modification specific enrichment

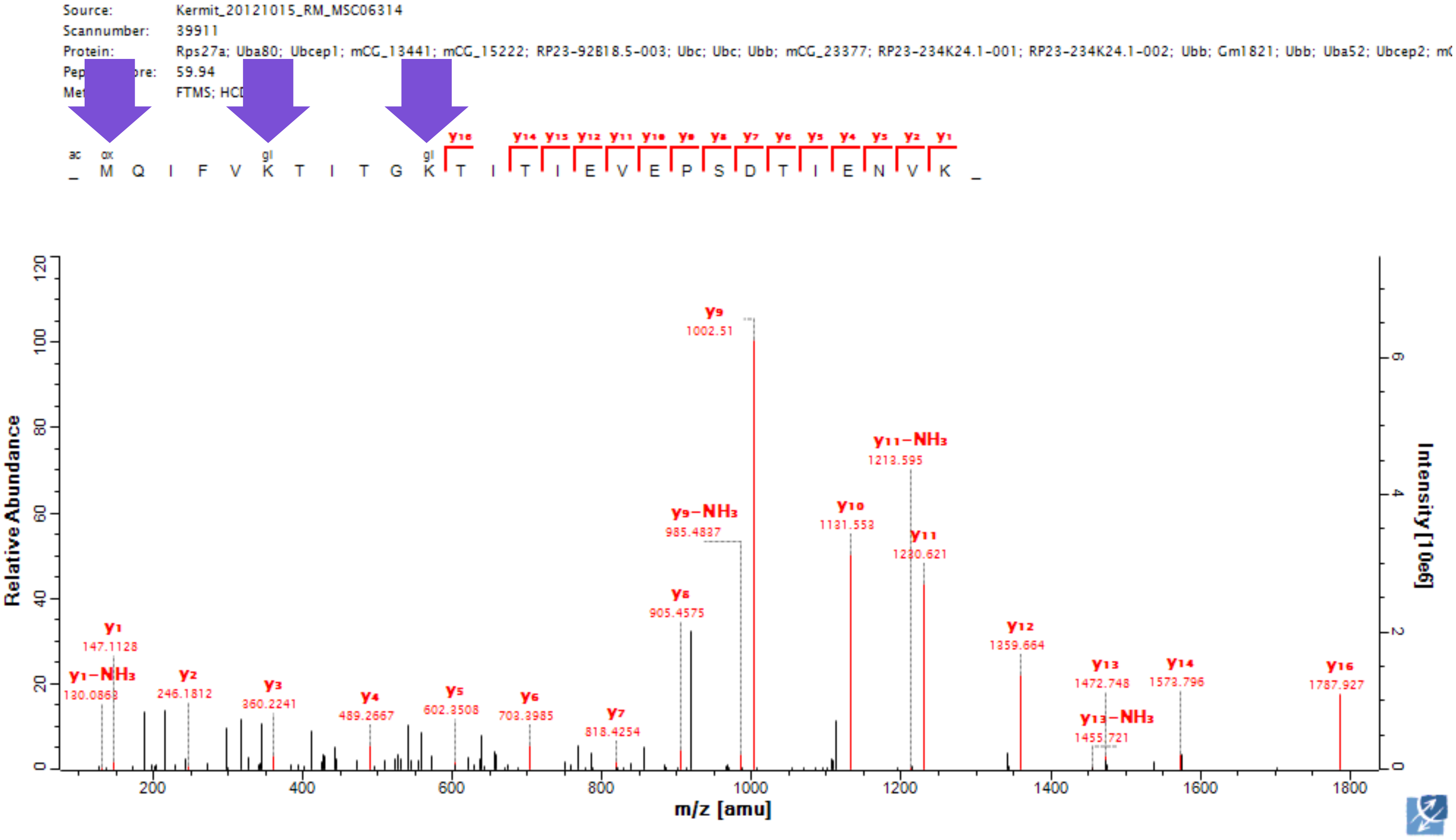
- Phosphotyrosine
  - IP on peptides using an α-pTyr antibody
- Phospho-Serine and Threonine
  - Titanoxide-column
    - works on proteomic level
- Protein-specific peptide antibodies

Good identification



Source: Kermit_20121015_RM_MSC06314
Scannumber: 8490
Protein: Cyp2e; Cyp2e1; Cyp2e-1; Q05421
Peptide Score: 95.5
Method: FTMS; HCD; 1

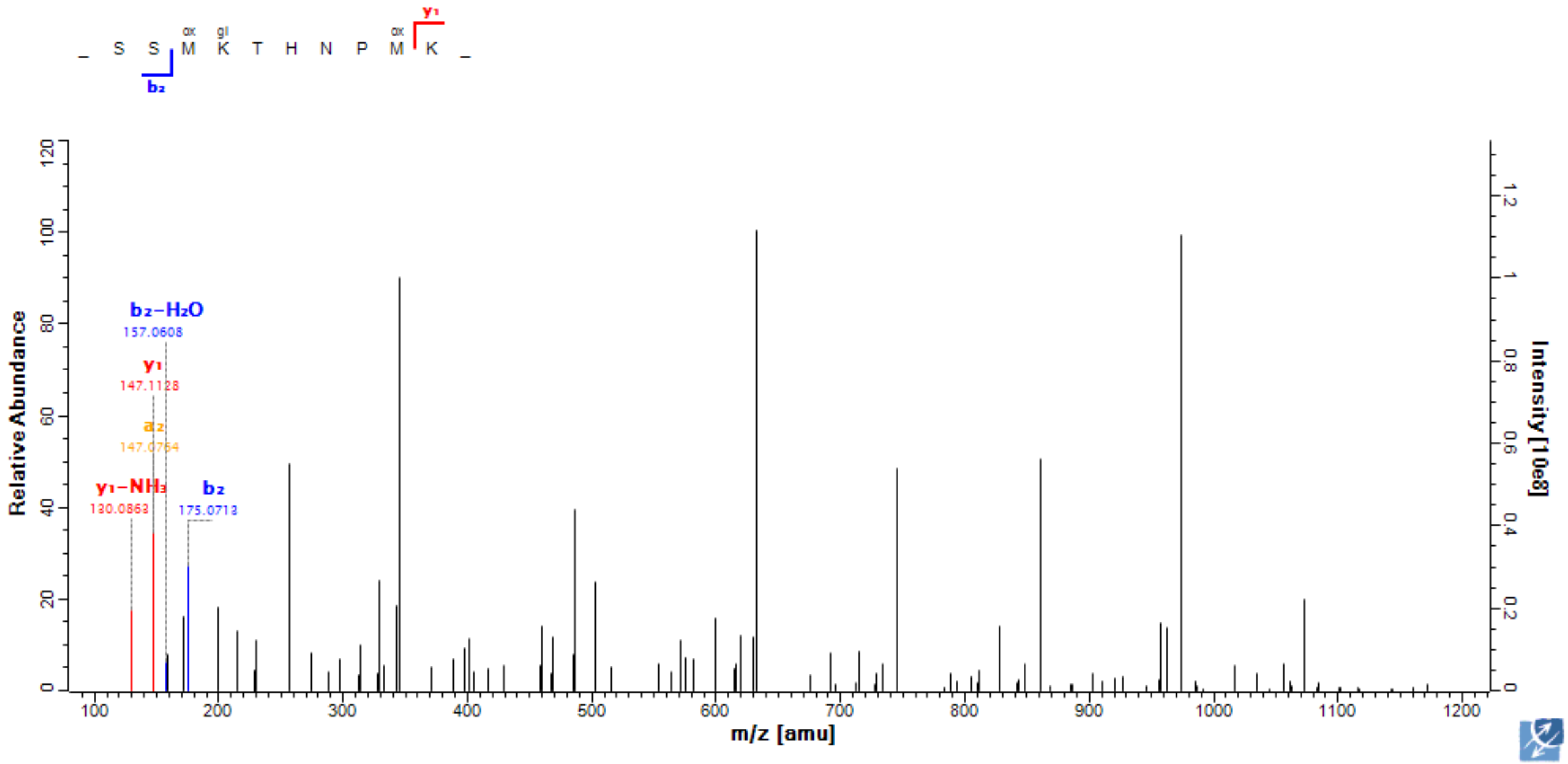
more problematic…

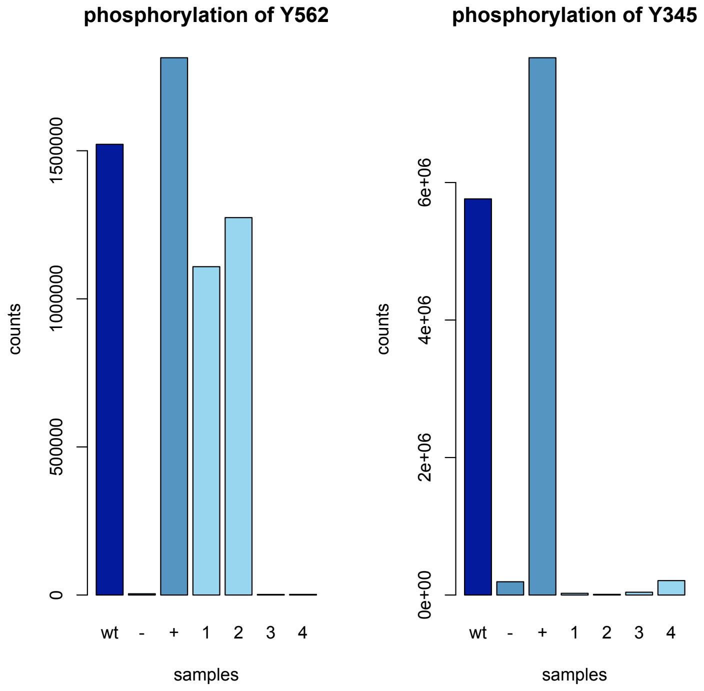


Source: Kermit_20121015_RM_MSC06314
Scannumber: 39911
Protein: Rps27a; Uba80; Ubcep1; mCG_13441; mCG_15222; RP23-92B18.5-003; Ubc; Ubc; Ubb; mCG_23377; RP23-234K24.1-001; RP23-234K24.1-002; Ubb; Gm1821; Ubb; Uba52; Ubcep2; m
Pep ore: 59.94
Met FTMS; HC

Do you believe the interpretation??

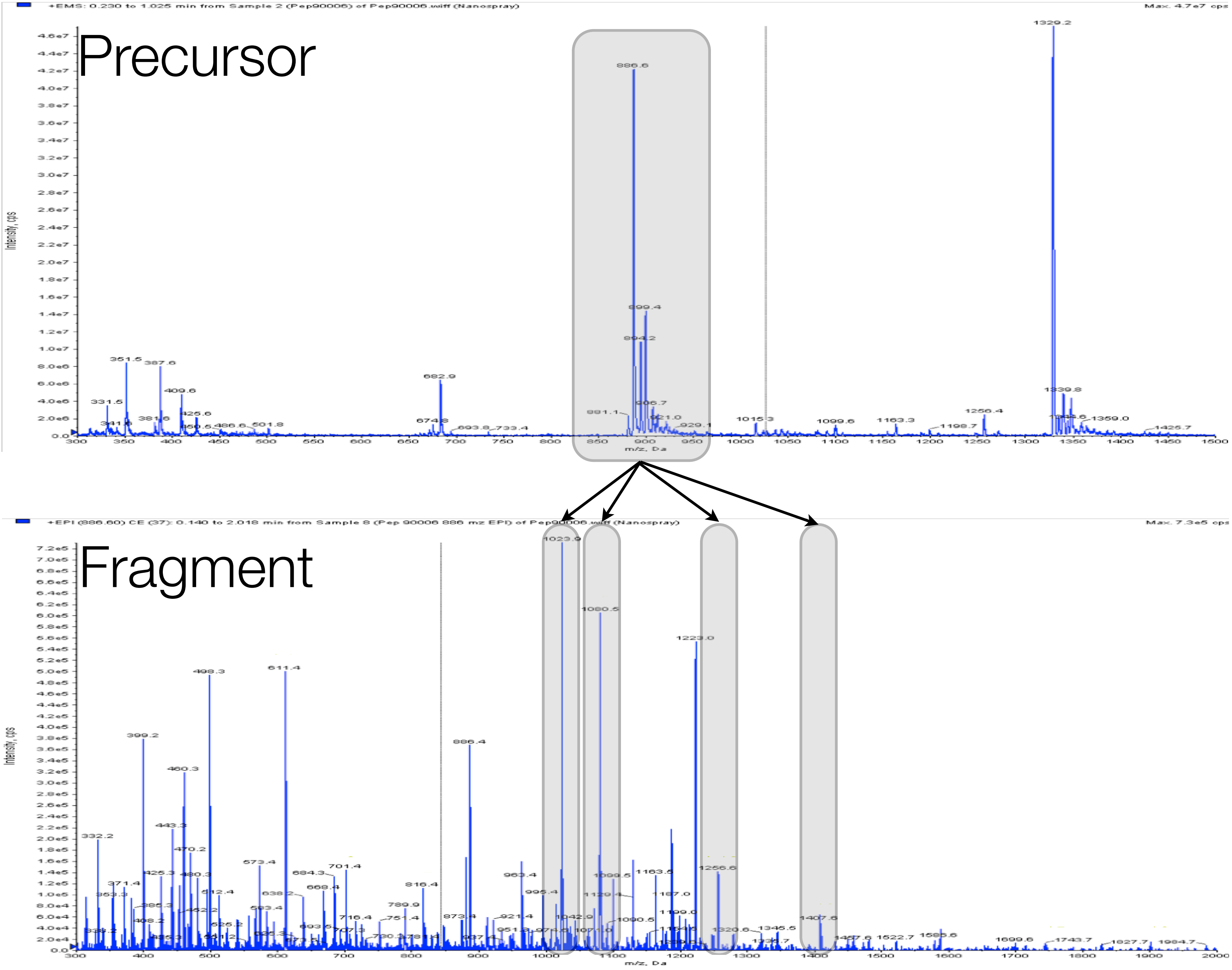
Source: Kermit_20121015_RM_MSC06312
Scannumber: 21581
Protein: Kcnb1; mCG_14614; RP23-19L12.6-001; Kcnb1; B0LAE2; Q0N2S5; Q80X33; Q8K0D1; Q03717
Peptide Score: 13.09
Method: FTMS; HCD; 1

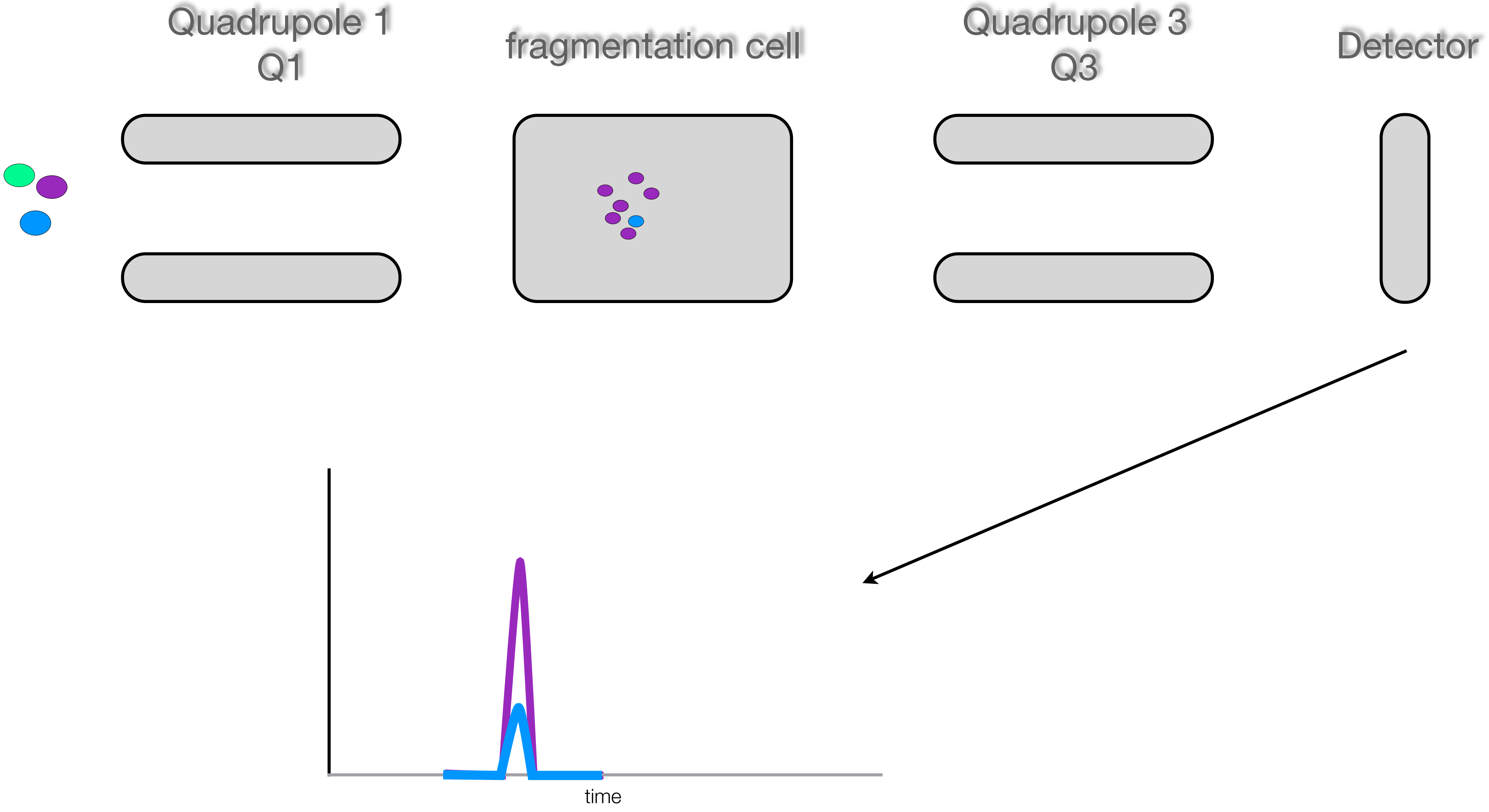




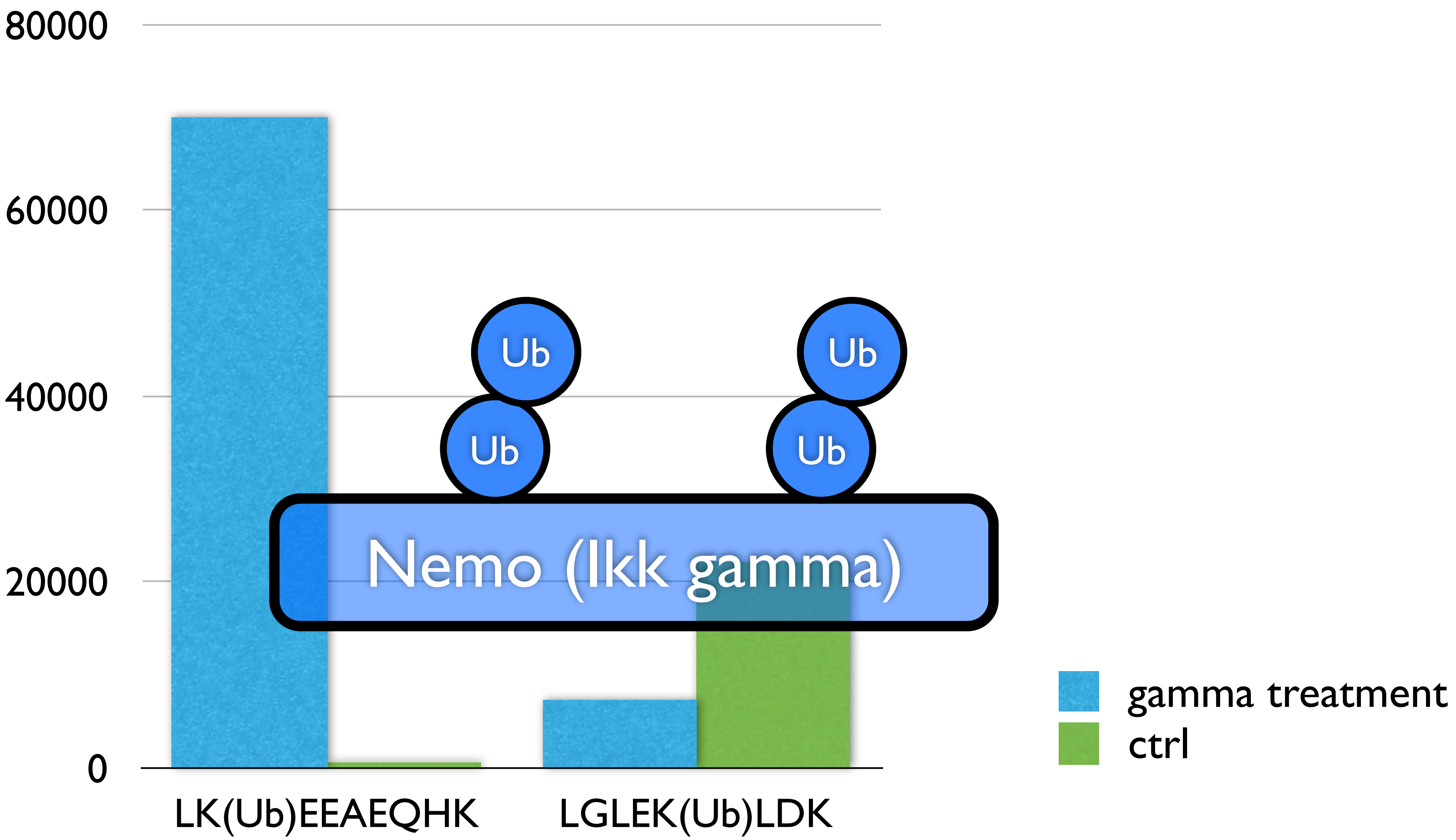
SRM

Selected Reaction Monitoring


+EMS: 0.230 to 1.025 min from Sample 2 (Pep90006) of Pep90006.wiff (Nanospray)
Max. 4.7e7 cps
Precursor
Intensity, cps
m/z, Da
886.6
899.4
894.2
906.7
881.1
921.0
929.1
1329.2
1339.8
1359.0
1425.7
1256.4
1198.7
1163.3
1099.6
1015.3
682.9
674.8
693.8
733.4
351.5
387.6
409.6
331.5
381.6
425.6
486.6
501.8
+EPI (886.60) CE (37): 0.140 to 2.018 min from Sample 8 (Pep 90006 886 mz EPI) of Pep90006.wiff (Nanospray)
Max. 7.3e5 cps
Fragment
Intensity, cps
m/z, Da
1023.9
1080.5
1223.0
1256.6
1407.6
498.3
611.4
399.2
460.3
443.3
470.2
332.2
371.4
425.3
480.3
512.4
573.4
684.3
701.4
668.4
638.2
816.4
789.9
716.4
751.4
886.4
873.4
921.4
963.4
995.4
1042.9
1099.5
1129.4
1090.5
1163.5
1187.0
1199.0
1320.6
1346.5
1457.6
1522.7
1585.6
1699.6
1743.7
1827.7
1984.7

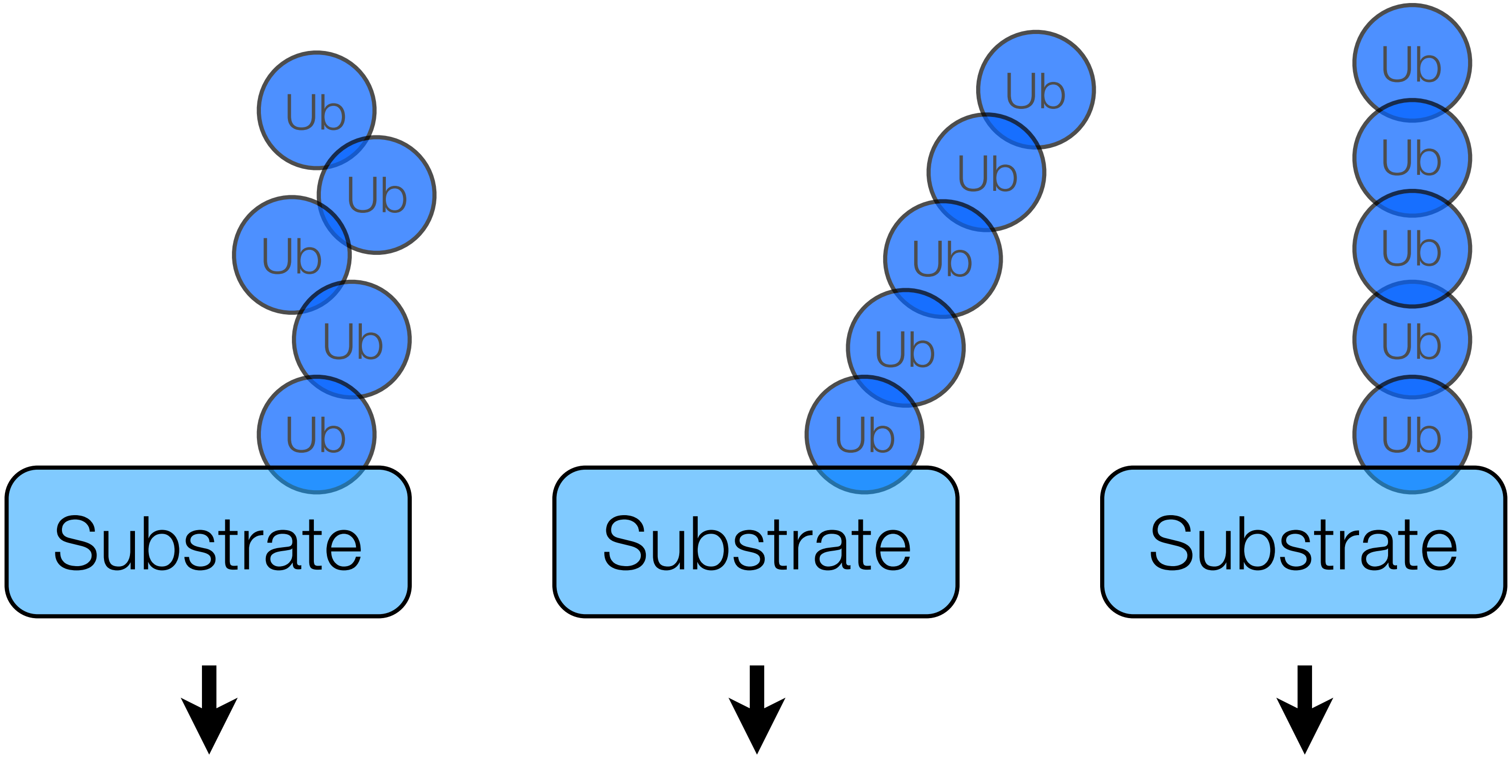

Quadrupole 1
Q1
fragmentation cell
Quadrupole 3
Q3
Detector
time

Regulation of Nemo by Ub-chains



Quantifying branched Ubiquitin

Ubiquitin chains

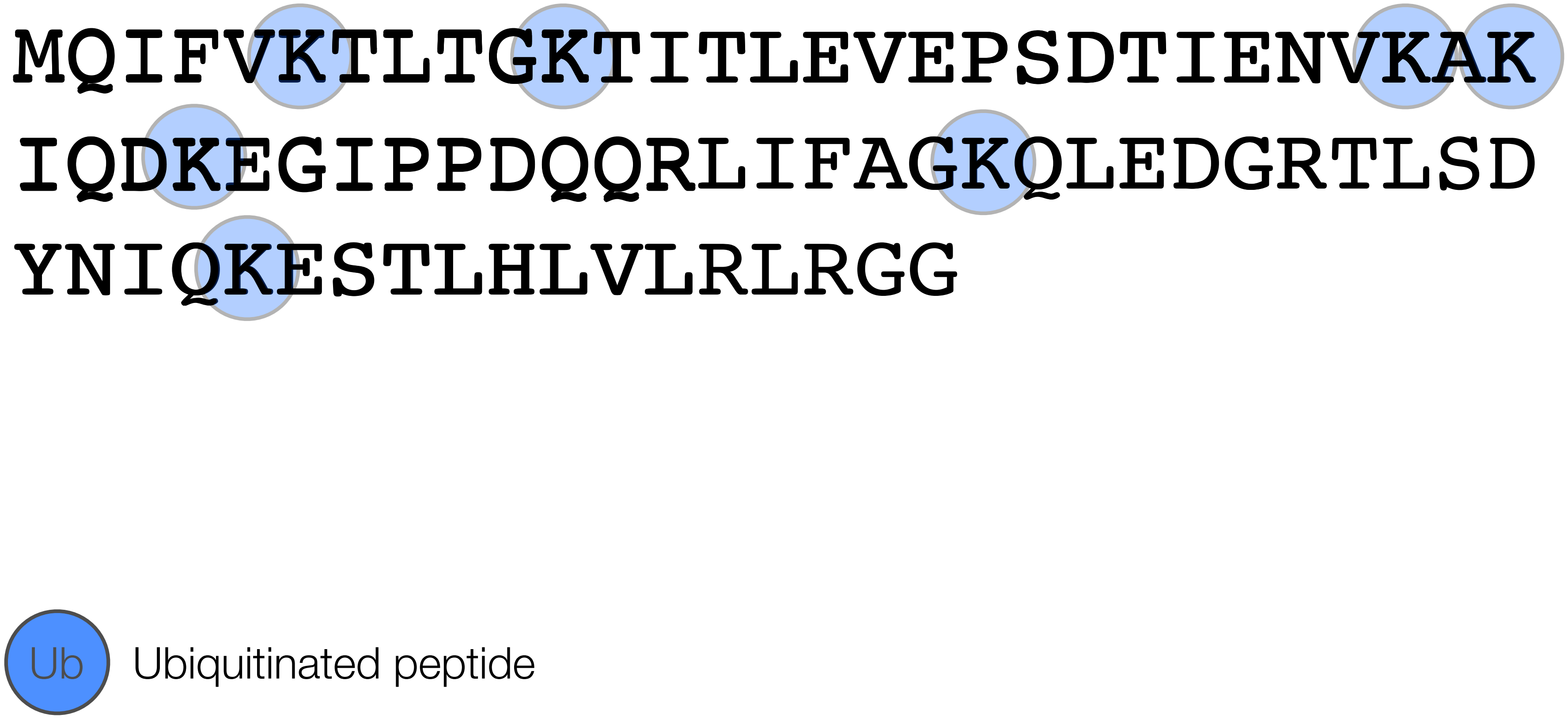


degradation

stabilization

localization

Ubiquitination sites in ubiquitin



MQIFVKTLTGKTITLEVEPSDTIENVKAK
IQDKEGIPPDQQRLIFAGKQLEDGRTLSD
YNIQKESTLHLVLRLRGG

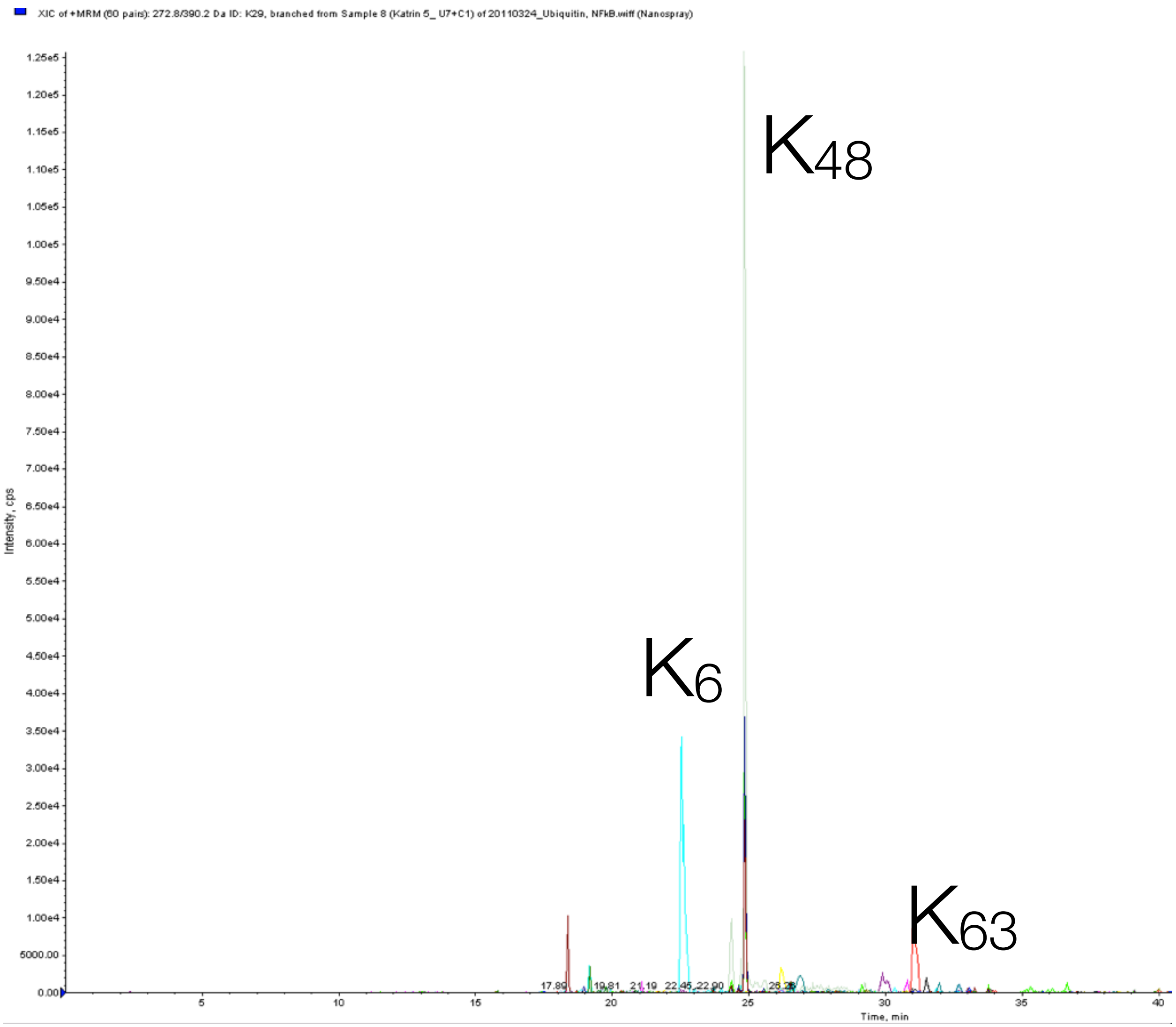
Ubiquitinated peptide

Tryptic peptides of ubiquitin

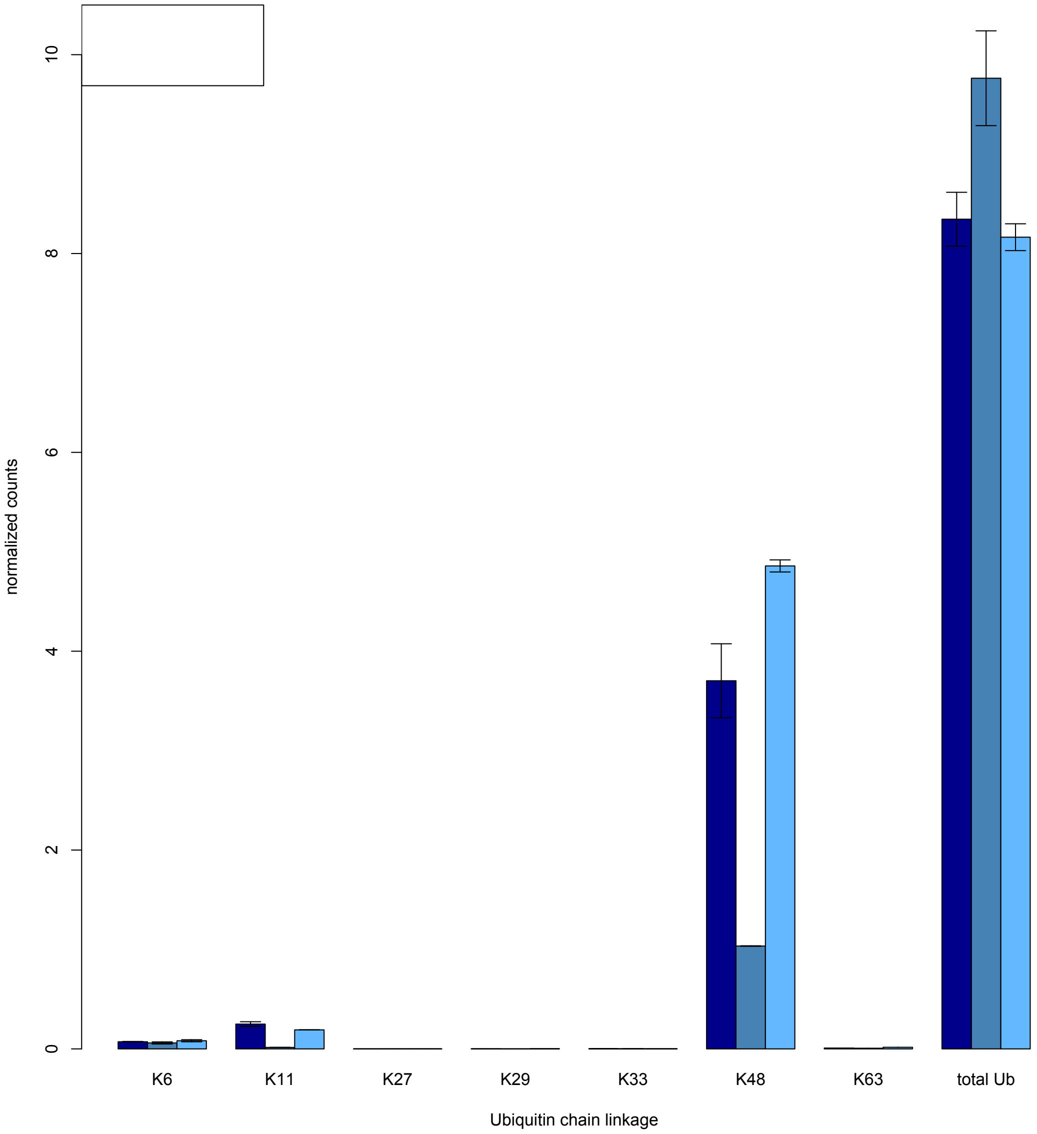
MQIFVKTLTGK	K6
TLTGKTITLEVEPSDTIENVK	K11
TITLEVEPSDTIENVKAK	K27
AKIQDK	K29
IQDKEGIPPDQQR	K33
LIFAGKQLEDGR	K48
TLSDYNIQKESTLHLVL	K63

Ub Ubiquitinated peptide

SRM measurement for Ub quantification



Quantification of Ub-chains



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