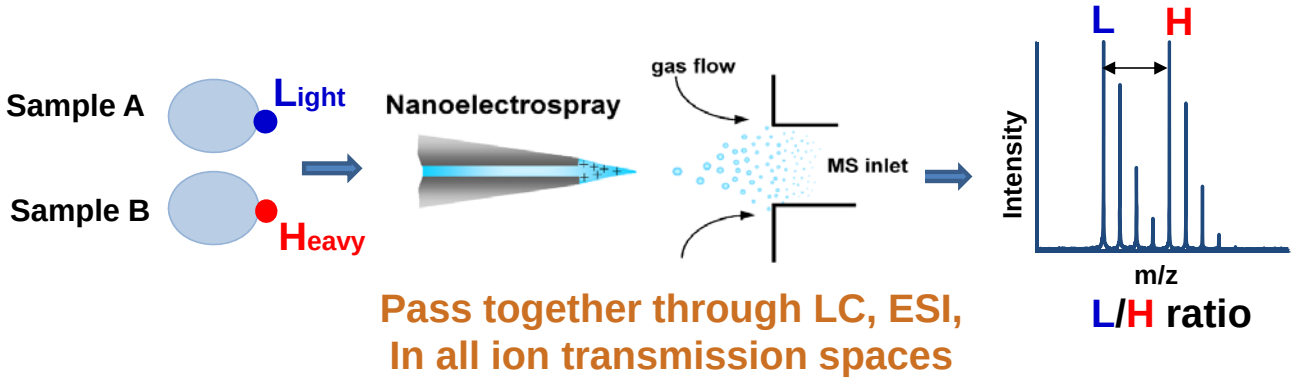
Quantitative proteomics

in-vitro stable isotope labeling

Stable isotope labeling

✓ Light (^{12}C, ^{16}O, ^{14}N) and heavy isotope (^{13}C, ^{18}O, ^{15}N) labeled peptide pairs have <u>exactly identical</u> chemical properties



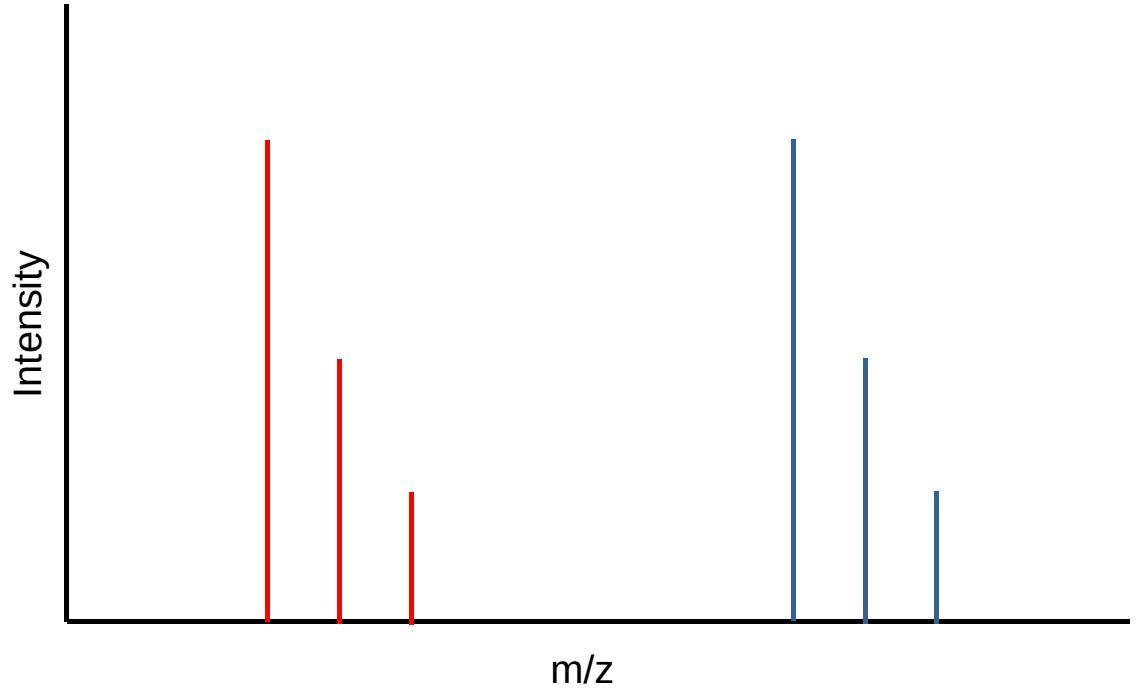
- <u>Chemical labeling</u> (**TMT/iTRAQ, Dimethylation, etc**)
- <u>Enzymatic labeling</u> (**^{18}O-labeling**)
- <u>Metabolic labeling</u> (**SILAC**)

Chemical labeling

- **Isotopic labeling** (quantitation at MS1 level)
  - Cysteine
  - Amine (N-terminal, lysine)

- **Isobaric labeling** (quantitation at MS2 level)
  - iTRAQ (isobaric Tag for Relative and Absolute Quantitation)
  - TMT (Tandem Mass Tag)

Isotopic labeling: quantitation at MS1 level

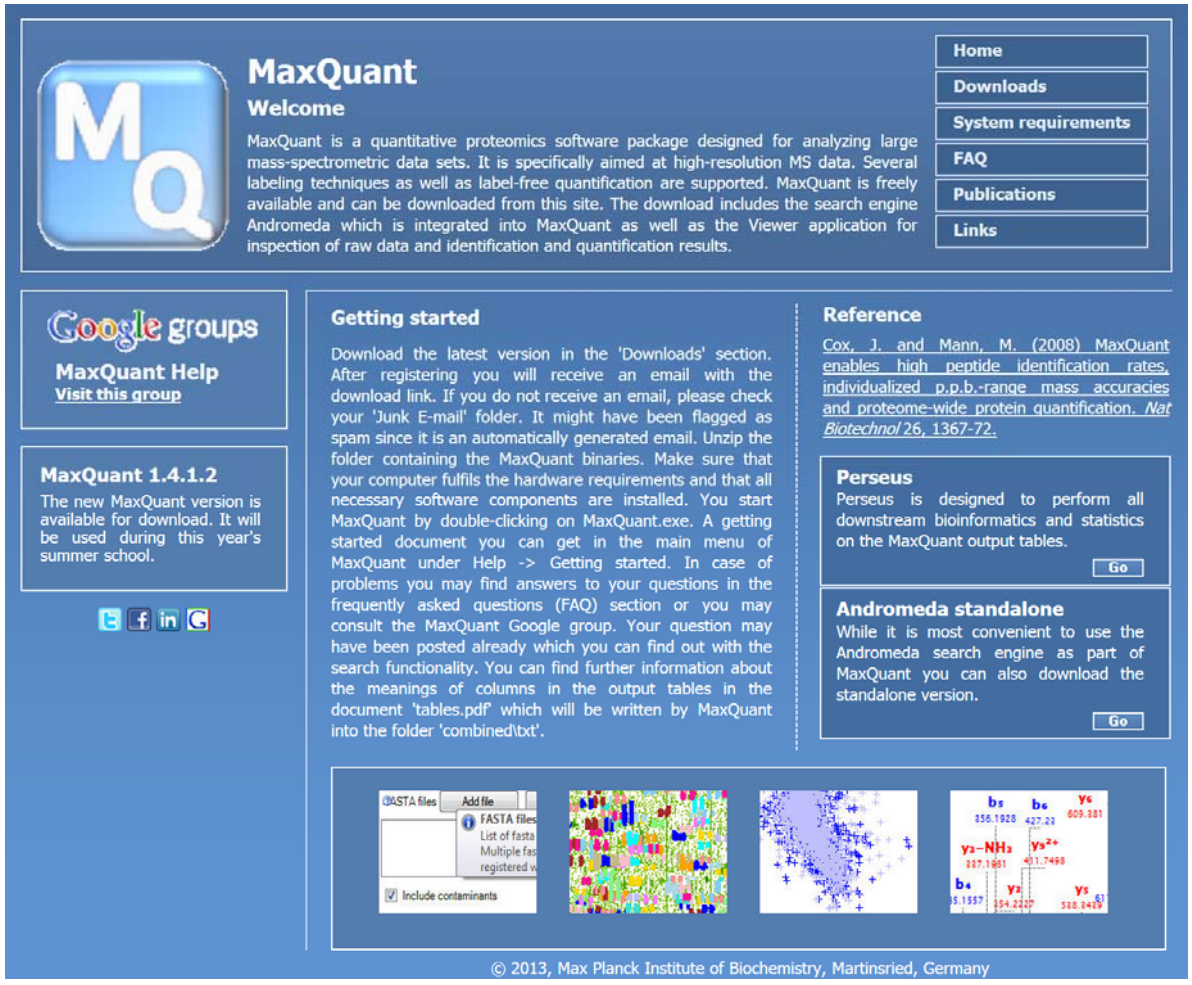


- Double or triple sample complexity, i.e. decrease in identification rate
- Better quantification dynamic range and accuracy
- Low multiplexing power

Isotopic labeling: quantitation at MS1 level

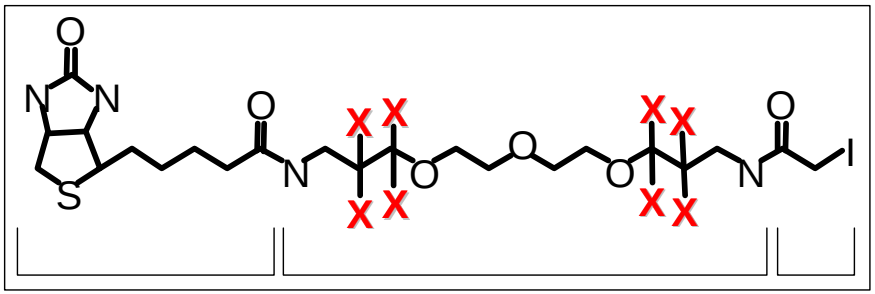
- Cysteine -ICAT
- Reductive dimethylation
- ICPL (isotopic chemical protein labeling)
- mTRAQ labeling
- Others

Isotopic labeling: quantitation at MS1 level

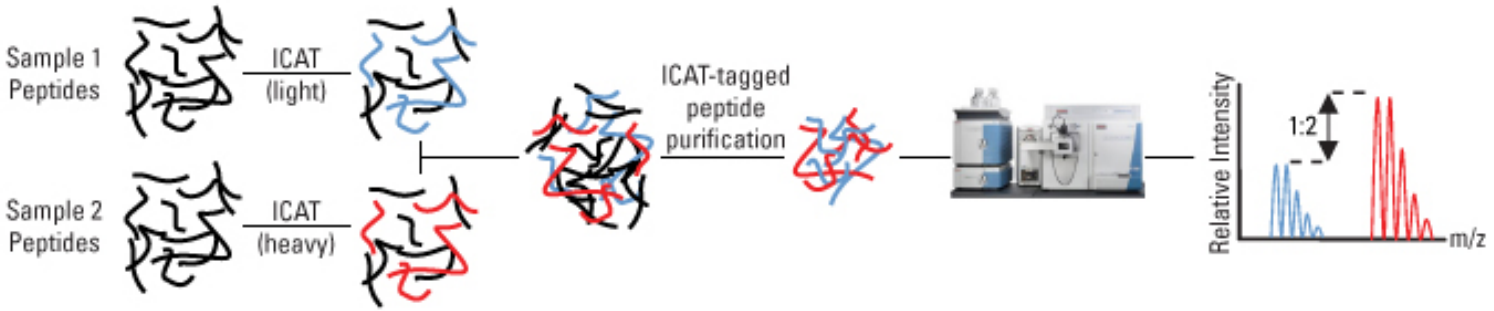


ICAT (**I**sotope-**C**oded **A**ffinity **T**ag)

ICAT Reagents: **Heavy reagent: d8-ICAT (X=deuterium)**
Light reagent: d0-ICAT (X=hydrogen)



Biotin tag | **Linker (heavy or light)** | **Thiol specific reactive group**



Cleavable ICAT® Reagents

Heavy and Light cleavable ICAT reagents consist of four moieties as shown in Figure 1:

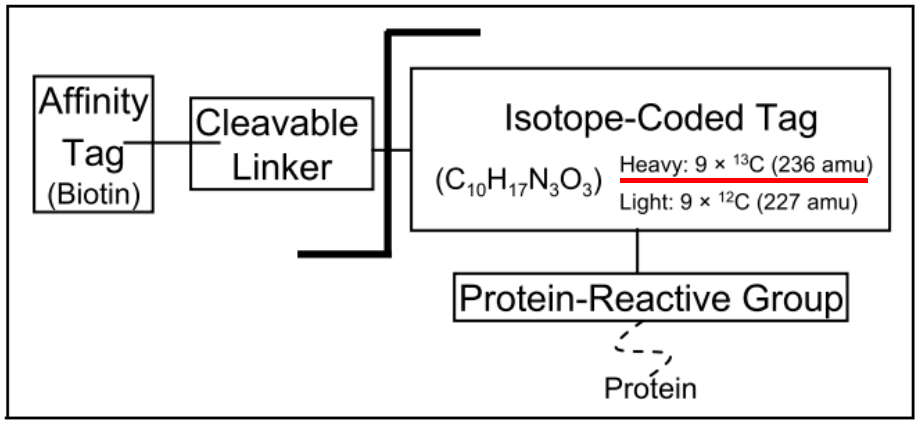
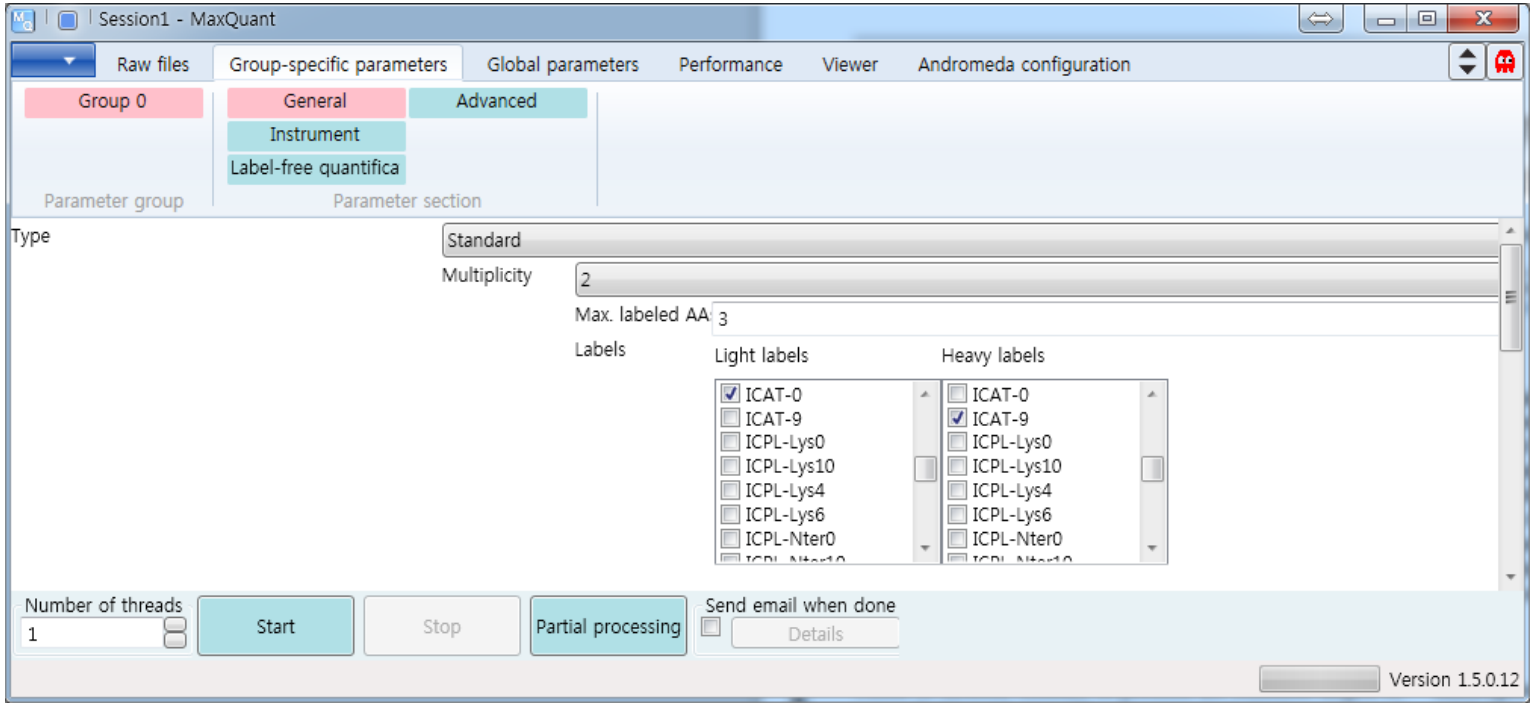


Figure 1 Cleavable ICAT Reagent Structure

ICAT Reagent Specifications

Specification	Light ICAT Reagent	Heavy ICAT Reagent
Composition	$C_{43}H_{70}N_7O_{12}SI$	$^{13}C_9C_{34}H_{70}O_{12}SI$
Average molecular weight	1,036.1	1,045.1
Monoisotopic MH^+	1,036.39	1,045.42
Monoisotopic MH^+ added to peptide before cleaving	907.47	916.50
Composition of tag after cleaving	$C_{10}H_{17}N_3O_3$	$^{13}C_9CH_{17}N_3O_3$
Monoisotopic MH^+ added to peptide after cleaving	227.13	236.16

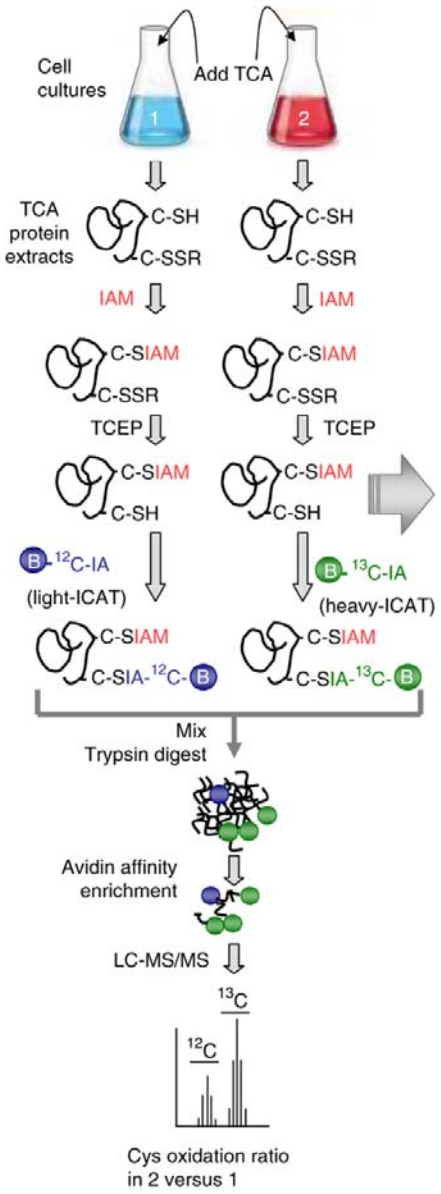
ICAT (**I**sotope-**C**oded **A**ffinity **T**ag)



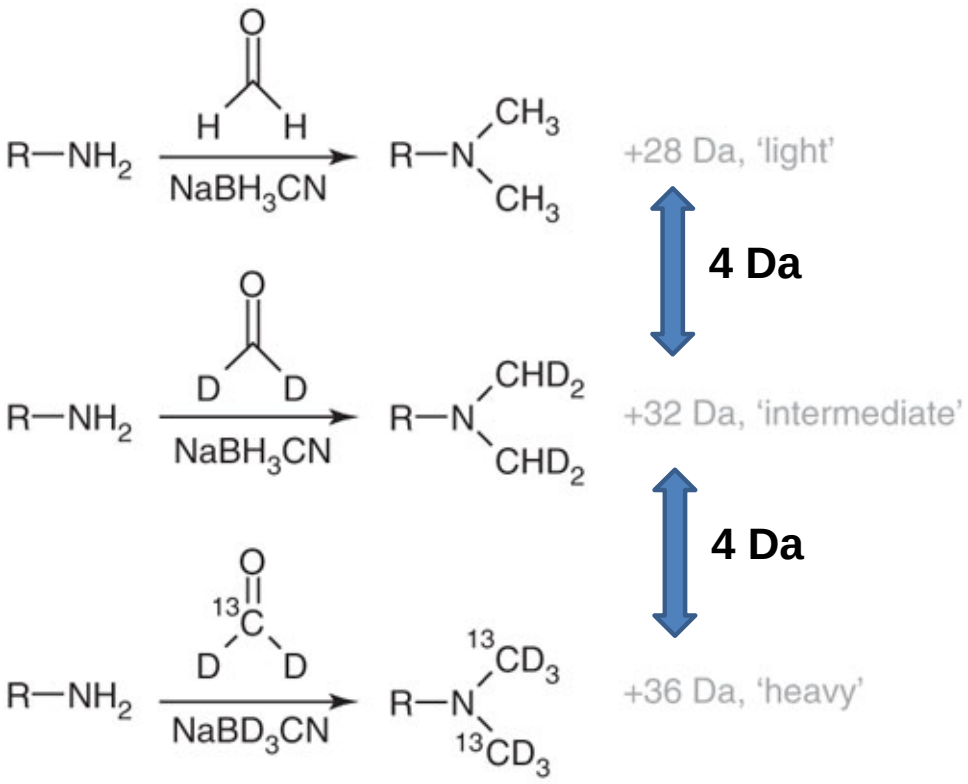
PROTOCOL

Monitoring *in vivo* reversible cysteine oxidation in proteins using ICAT and mass spectrometry

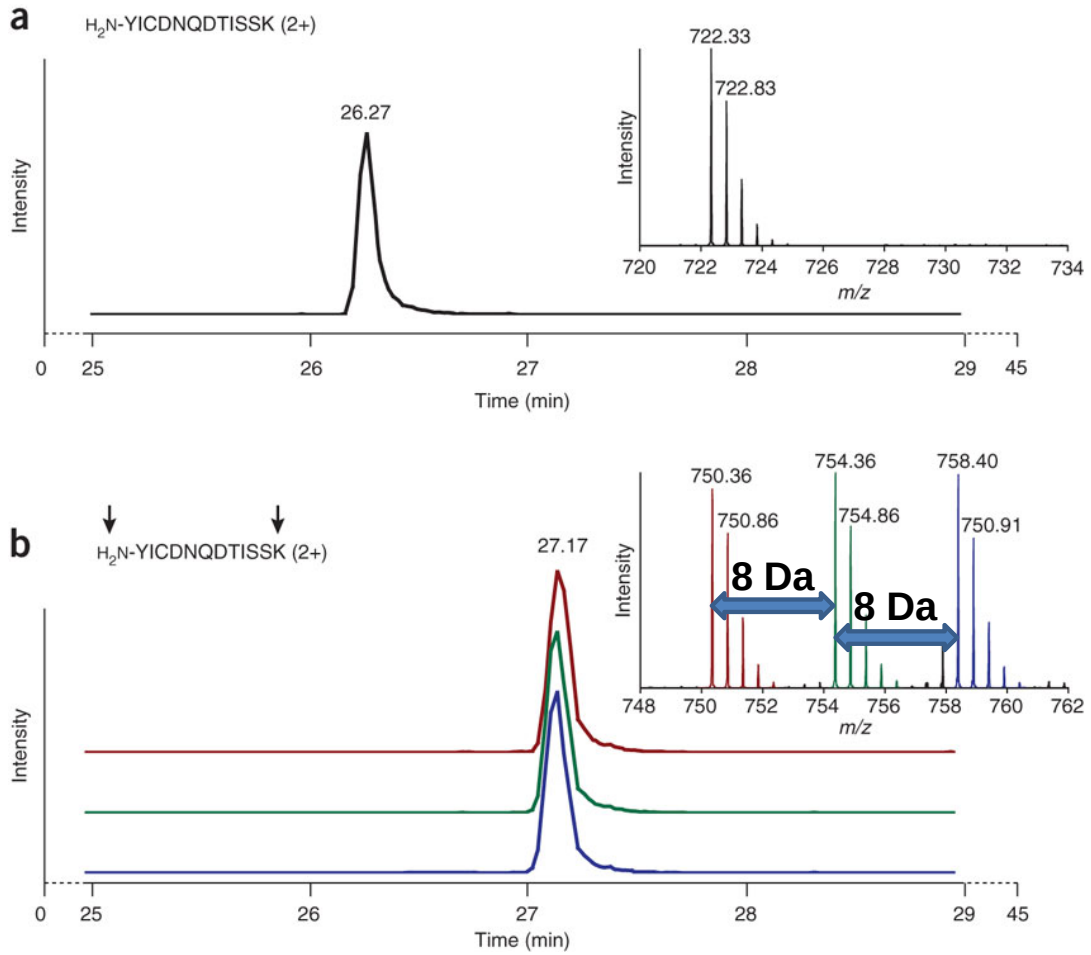
Sarela García-Santamarina[1], Susanna Boronat[1], Alba Domènech[1], José Ayté[1], Henrik Molina[2] & Elena Hidalgo[1]



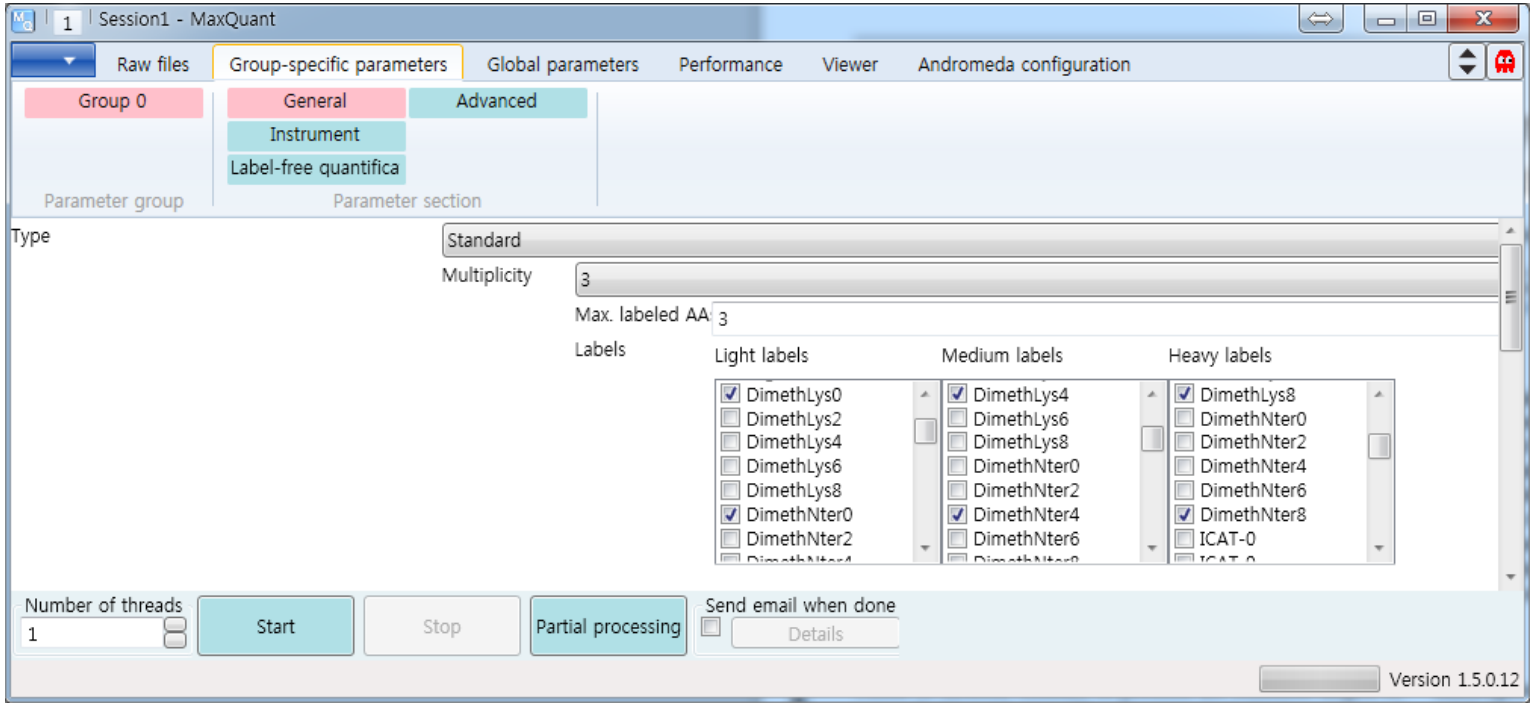
Reductive dimethylation



Reductive dimethylation



Reductive dimethylation

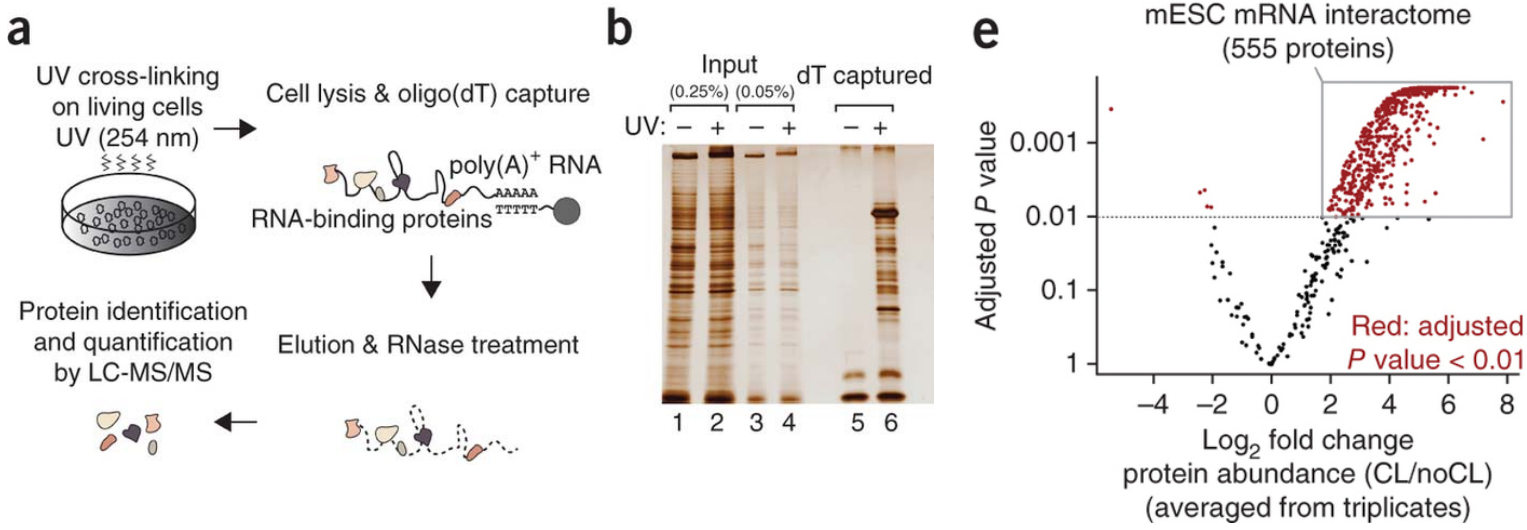


RESOURCE

nature structural & molecular biology

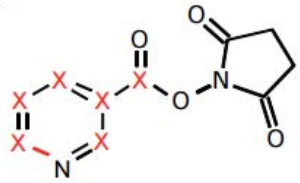
The RNA-binding protein repertoire of embryonic stem cells

S Chul Kwon[1,2], Hyerim Yi[1,2], Katrin Eichelbaum[3], Sophia Föhr[3], Bernd Fischer[3], Kwon Tae You[1,2], Alfredo Castello[3], Jeroen Krijgsveld[3], Matthias W Hentze[3,4] & V Narry Kim[1,2]



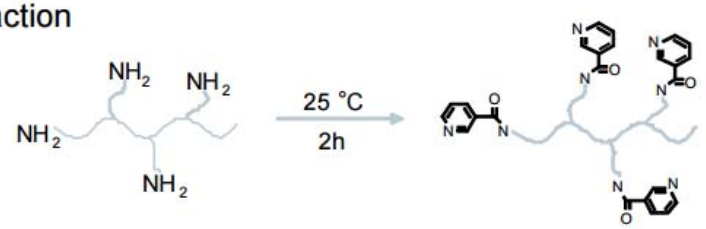
ICPL Scheme (isotope-coded protein label)

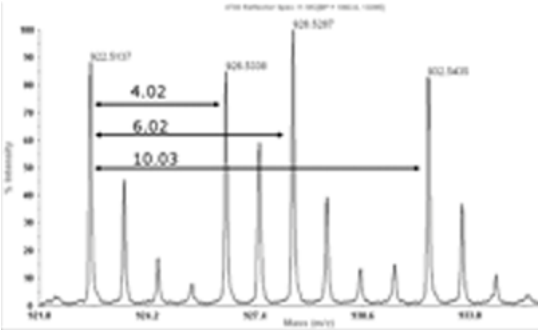
Reagent

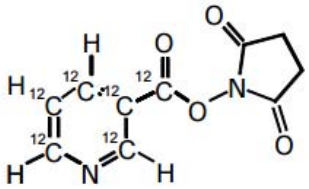


$C_6H_4/C_6{}^2H_4/{}^{13}C_6H_4/{}^{13}C_6{}^2H_4$-
Nicotinoyloxysuccinimide

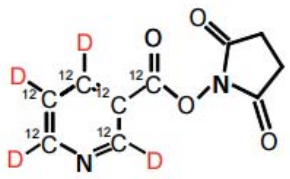
Reaction



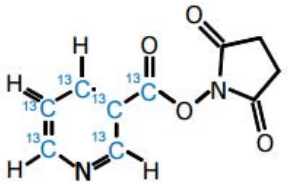




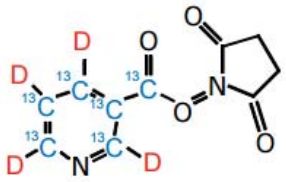
ICPL_0



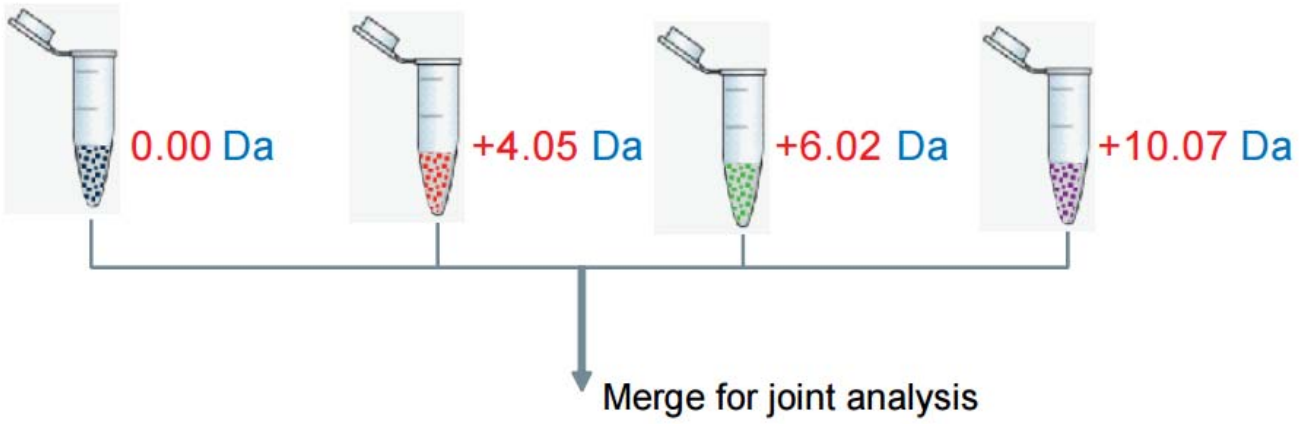
ICPL_4



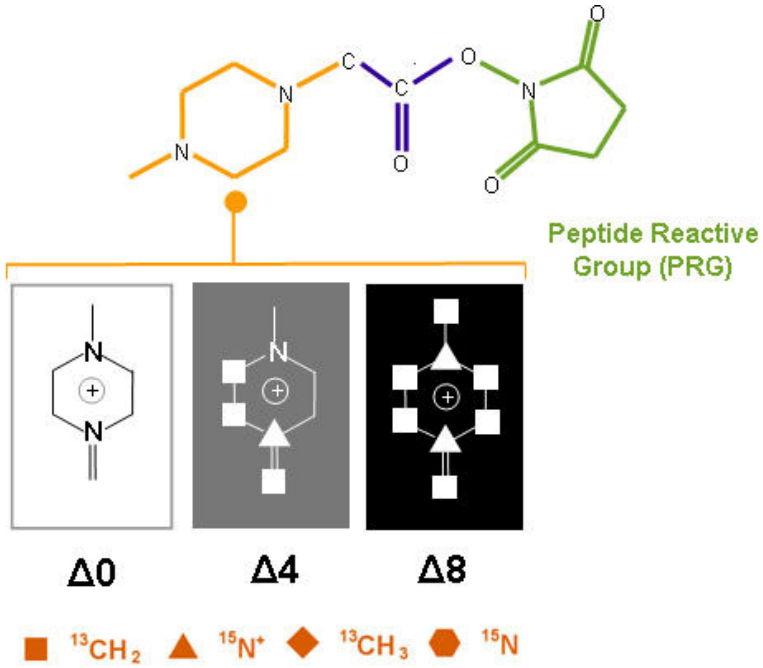
ICPL_6

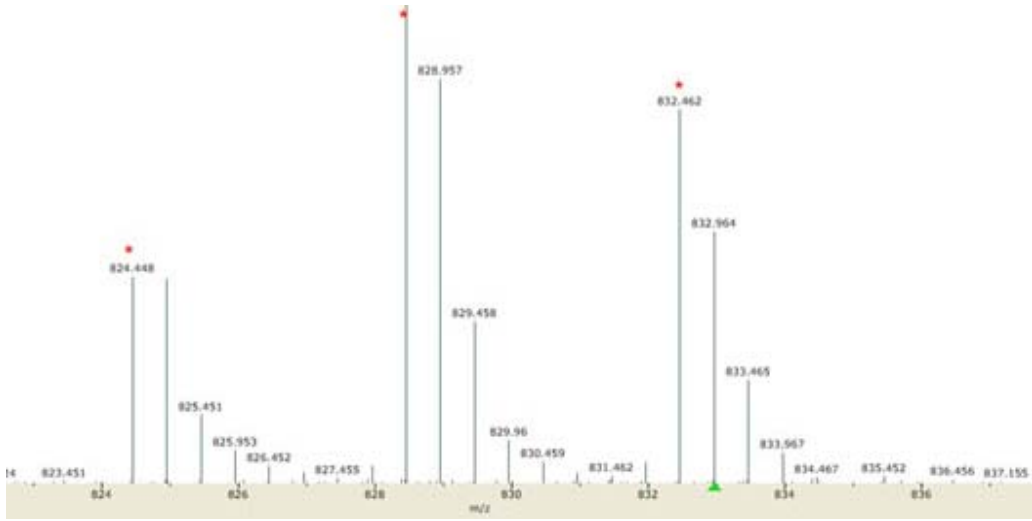


ICPL_10



mTRAQ labeling





ALNEINQFYQK

BMC Bioinformatics. 2011 Feb 15;12

Enzymatic ^{18}O-labeling



Peptides

Dry

Resuspend
in $H_2{}^{18}O$
1:50 Trypsin:Peptide (w/w)

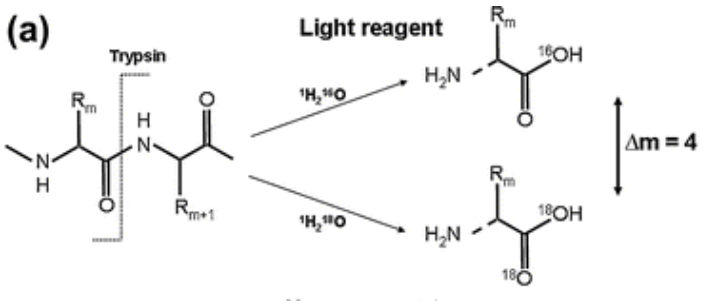


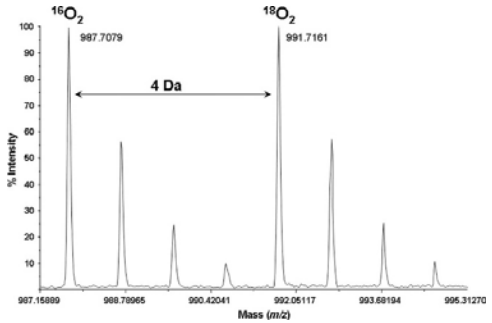
^{18}O-Labeling
37 ^{o}C, 450 rpm

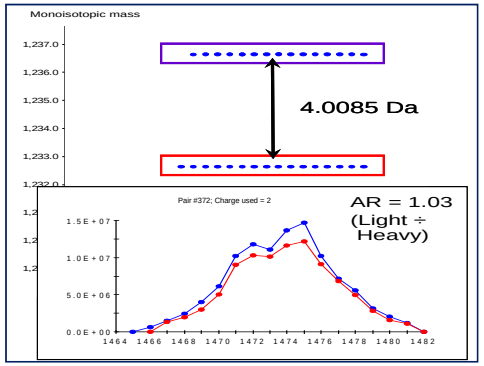


Boil for 10 minutes

Efficient for all tryptic digests

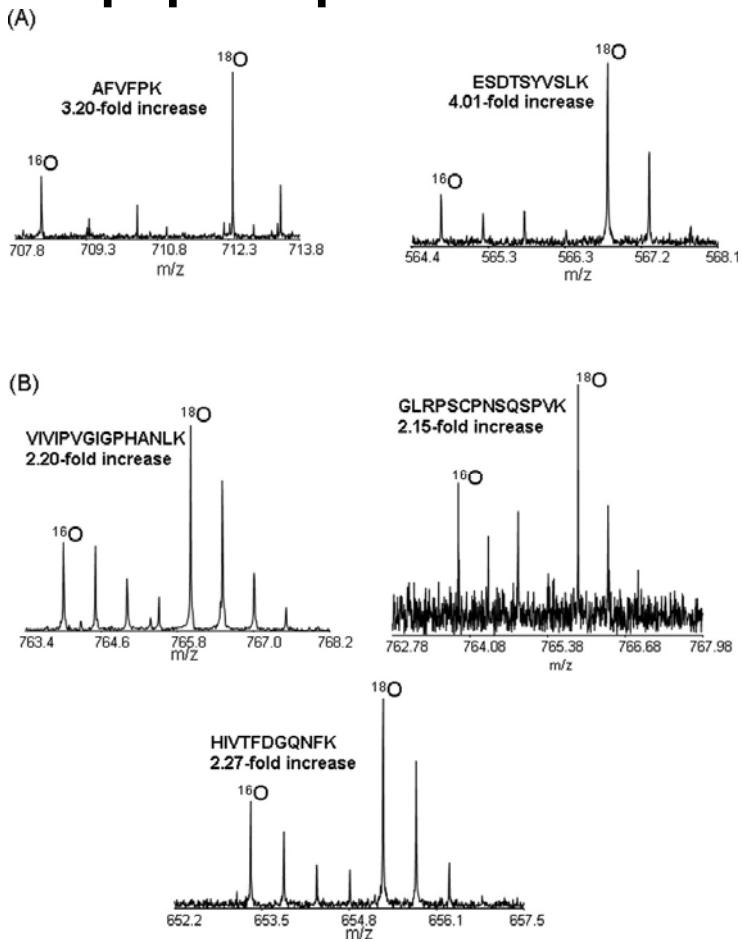






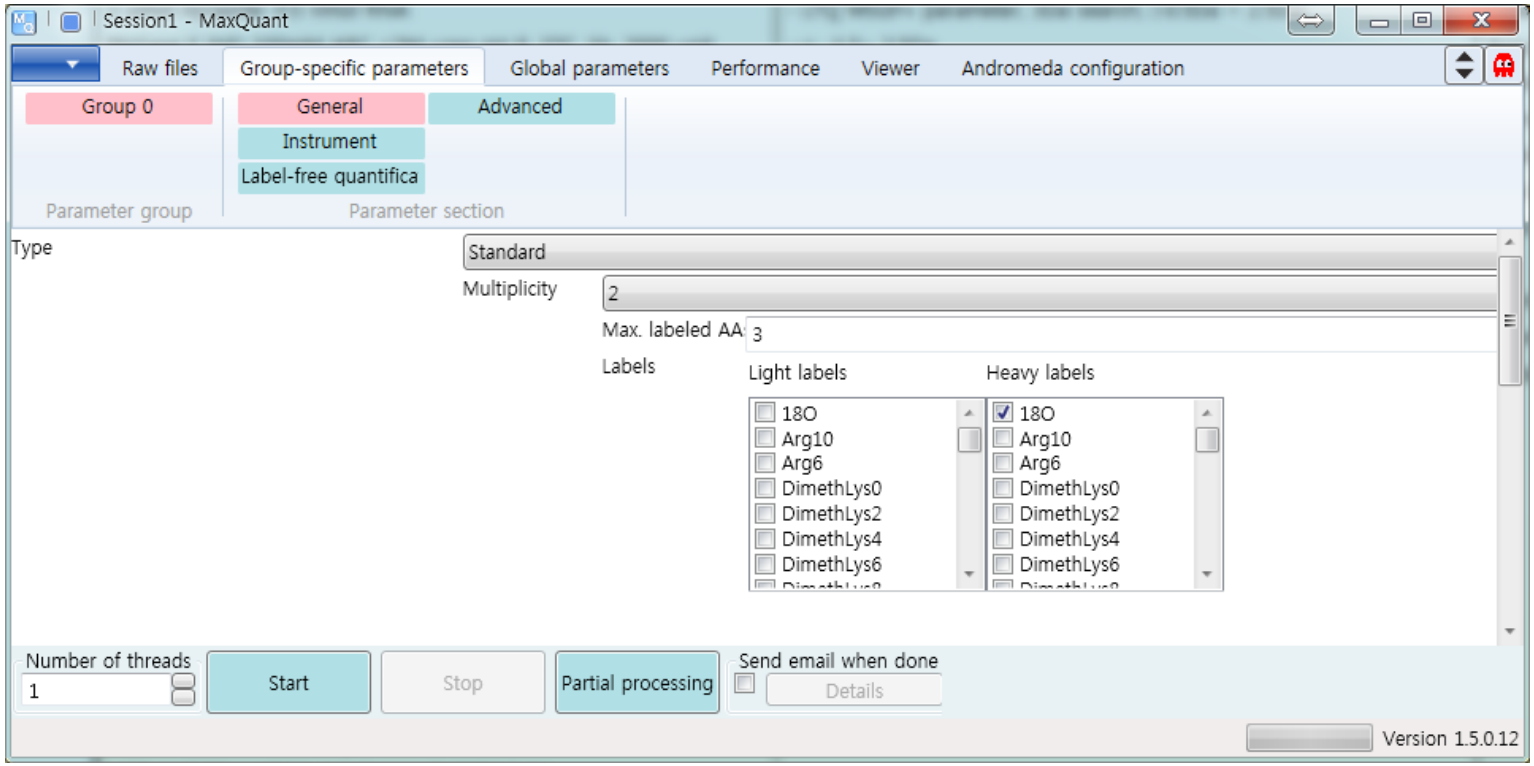
Petritis, *et al.* *J. Proteome Res.* 2009.

A: two different peptide pairs from C-reactive protein.
B: three different peptide pairs from von Willebrand factor.

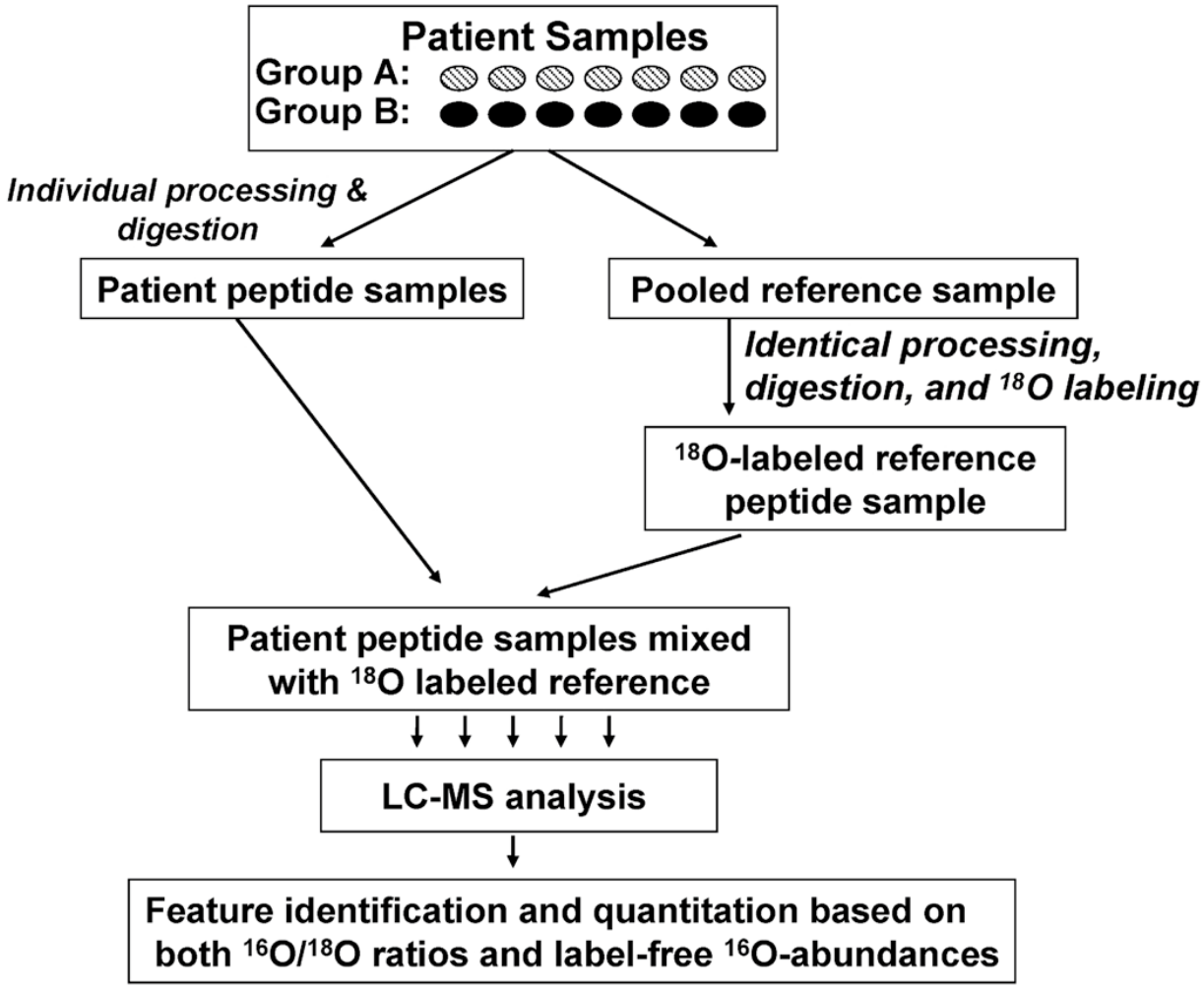


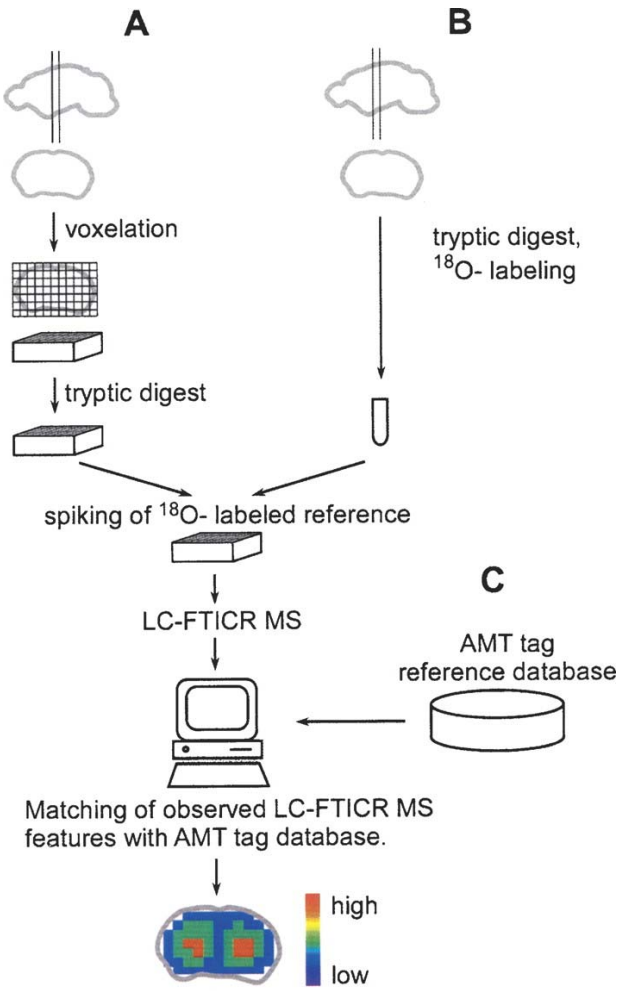
Qian W et al. Mol Cell Proteomics 2005;4:700-709

Ezymatic ^{18}O-labeling

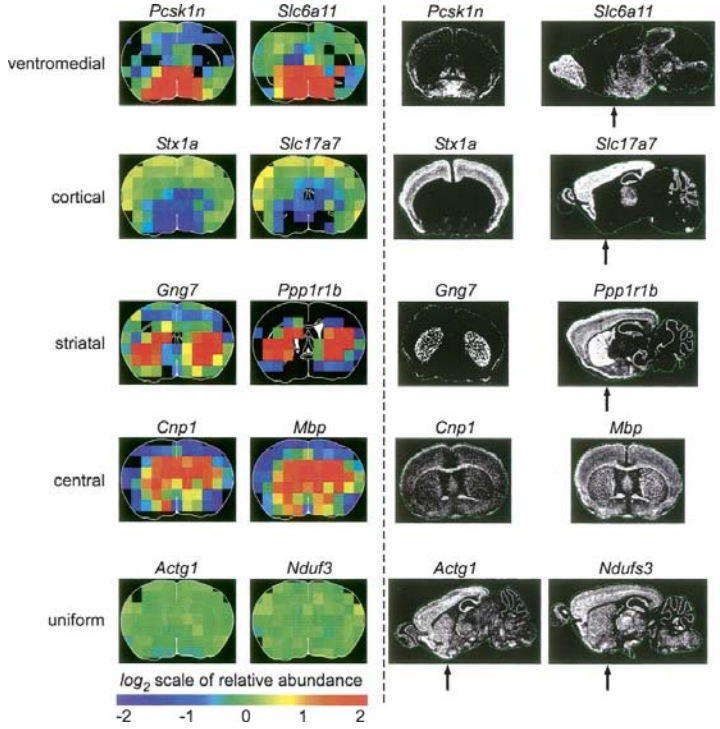


Proteome reference: 18O-labeling



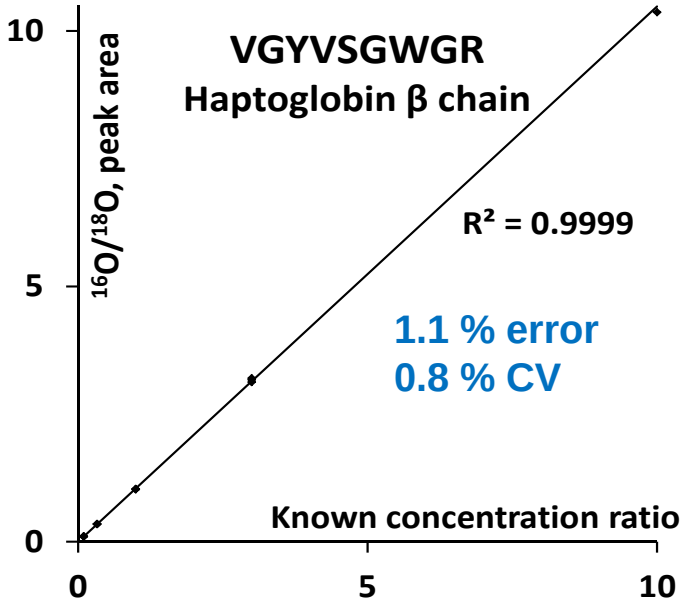


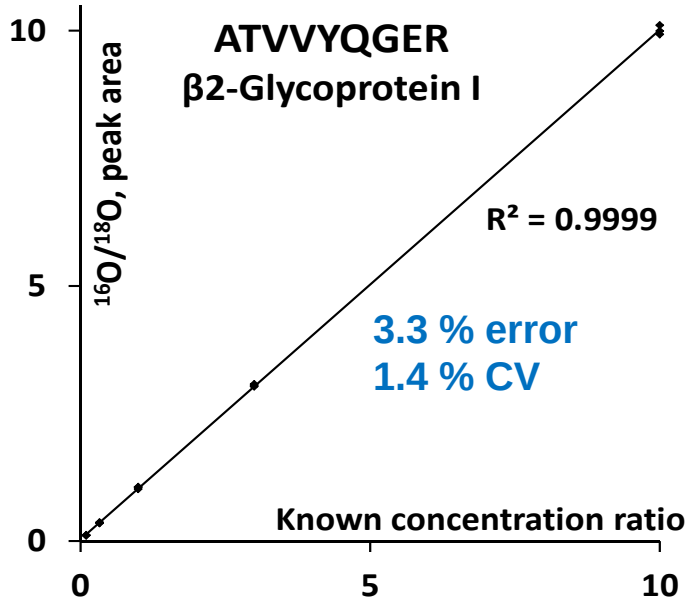
Reconstruction of the relative protein abundance distributions based on isotopic ratios and direct MS intensities of matched LC-FTICR MS features.



Petyuk V A et al. Genome Res. 2007;17:328-336

Accurate relative quantification





Kim JS et al. Mol Cell Proteomics. 2011

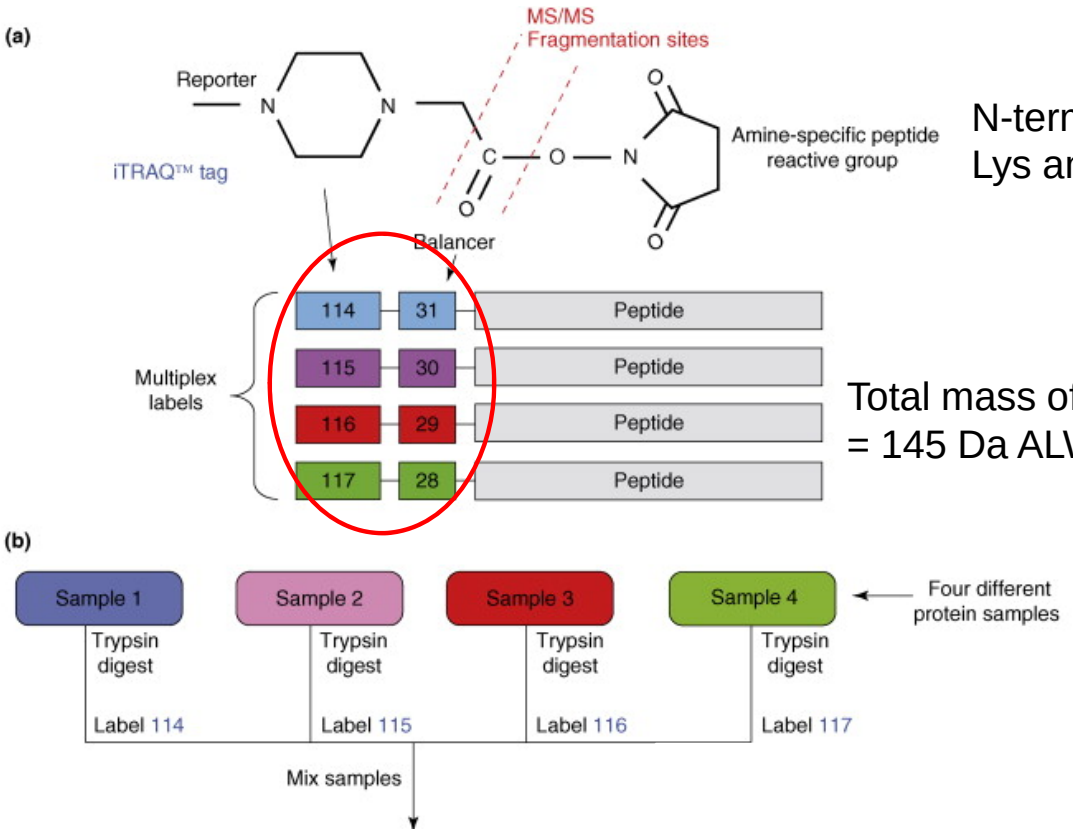
Quantitation based on MS1 level : pros & cons

- Chemical labeling (ICAT, Dimethylation…), Enzymatic labeling (^{18}O-labeling), Metabolic labeling (SILAC)

- **Pros**: better quantification accuracy and dynamic range

- **Cons**: double or triple sample complexity
  → decrease in identification rate, and low multiplexing power (3-plex)

Isobaric labeling: quantitation at MS2 level

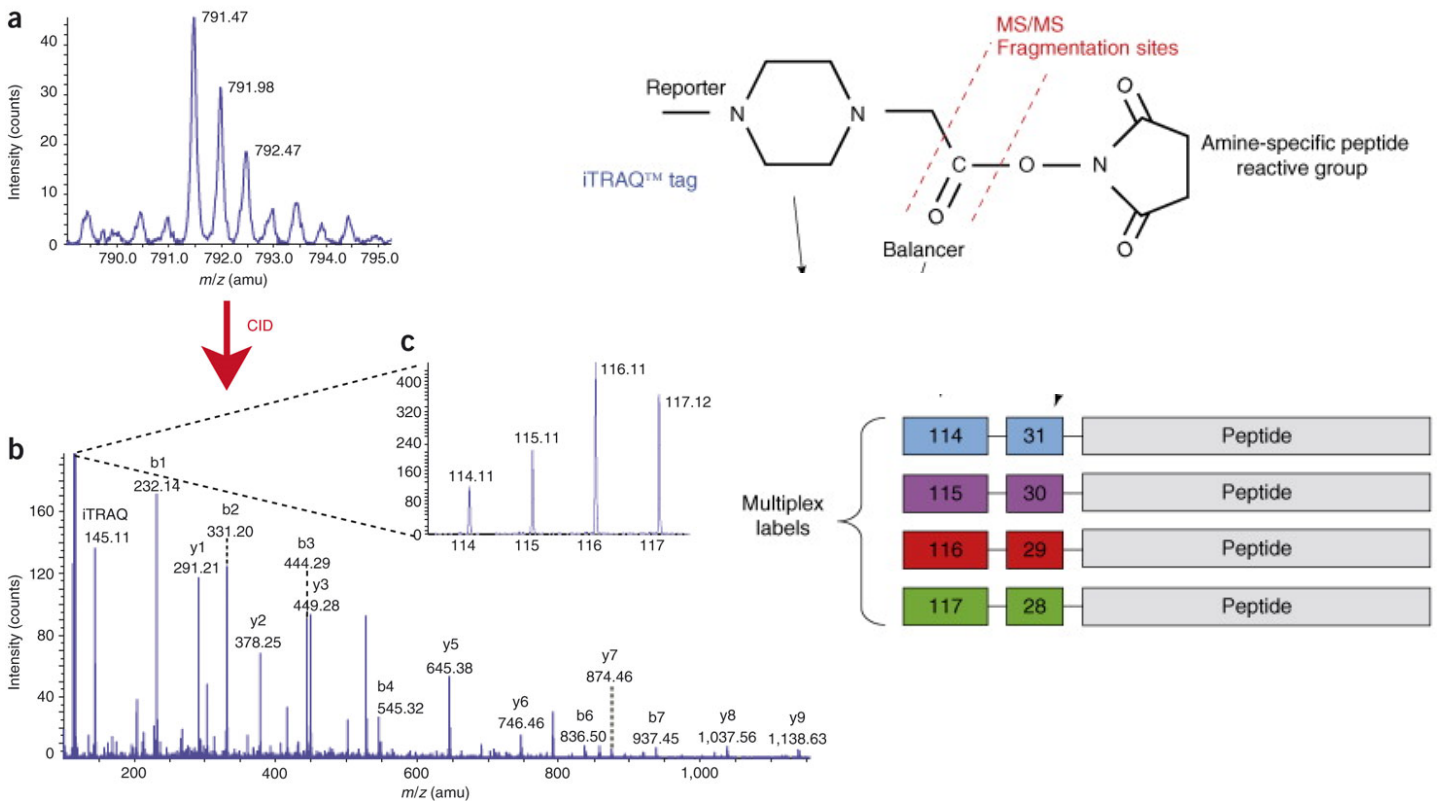


N-terminal amine
Lys amine

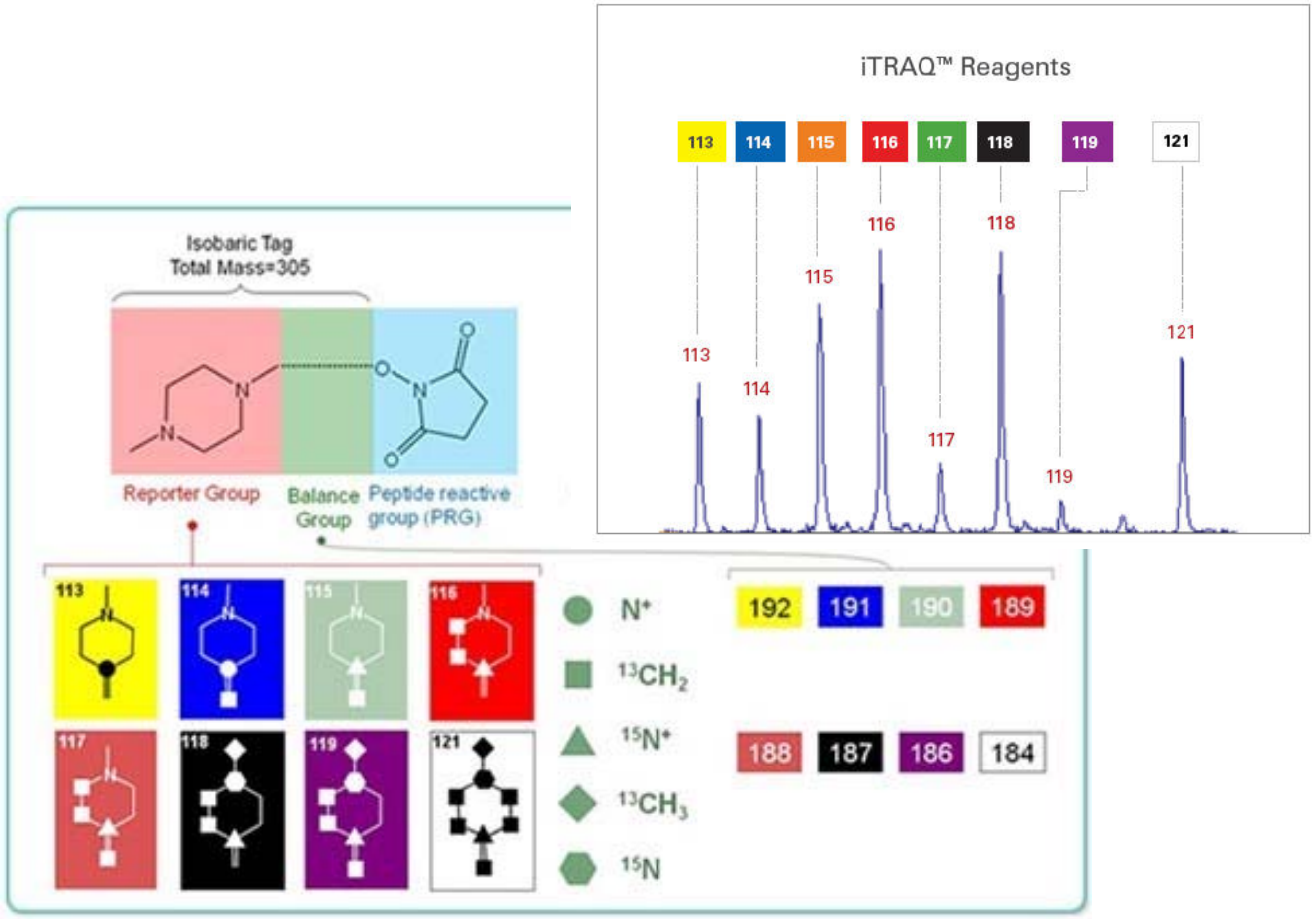
Total mass of label
= 145 Da ALWAYS

Sample prep

iTRAQ 4-plex

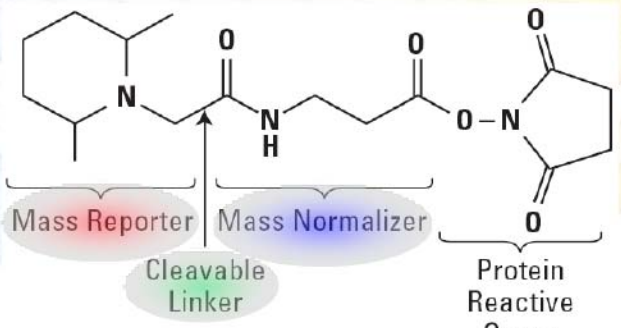

a
791.47
791.98
792.47
Intensity (counts)
m/z (amu)
CID
MS/MS
Fragmentation sites
Reporter
iTRAQ™ tag
Balancer
Amine-specific peptide
reactive group
b
c
iTRAQ
145.11
b1
232.14
b2
331.20
y1
291.21
b3
444.29
y3
449.28
y2
378.25
y5
645.38
b4
545.32
y6
746.46
b6
836.50
y7
874.46
b7
937.45
y8
1,037.56
y9
1,138.63
114.11
115.11
116.11
117.12
Multiplex
labels
114
31
115
30
116
29
117
28
Peptide
Peptide
Peptide
Peptide

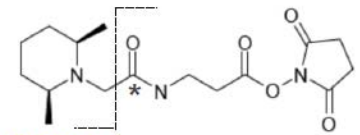
iTRAQ 8-plex

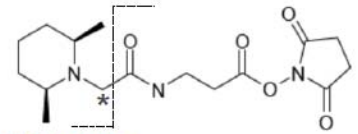

iTRAQ™ Reagents
113
114
115
116
117
118
119
121
Isobaric Tag
Total Mass=305
Reporter Group
Balance Group
Peptide reactive group (PRG)
N+
13CH2
15N+
13CH3
15N
192
191
190
189
188
187
186
184

TMT- Tandem Mass Tags

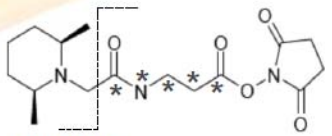
- A family of amine reactive isobaric MS/MS tags based on an identical chemical structure

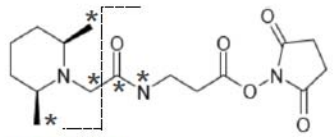


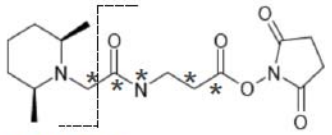


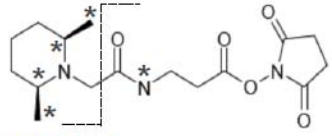


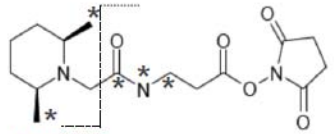
TMT² - Two Plex Quantitation

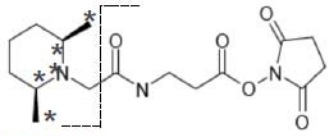






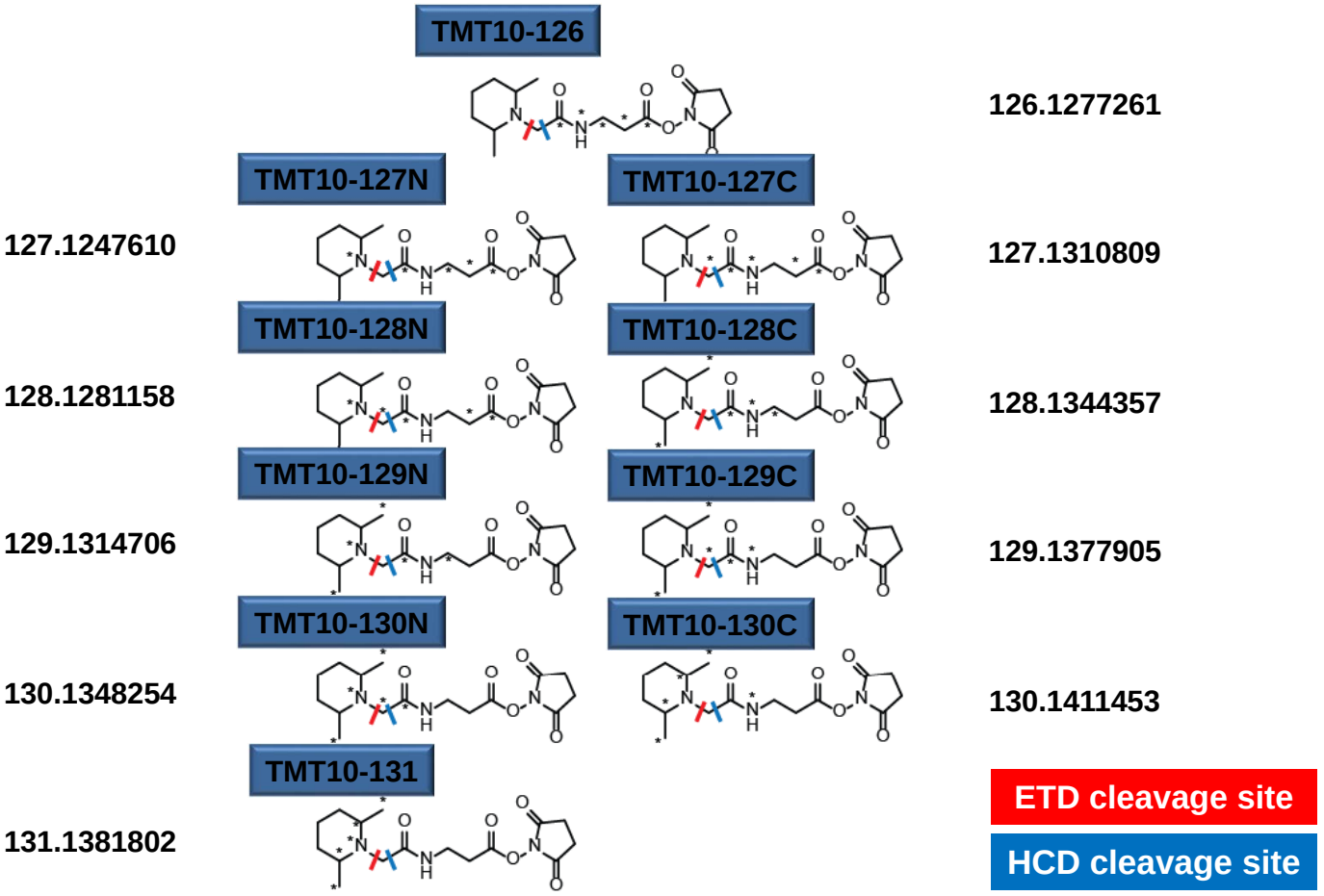




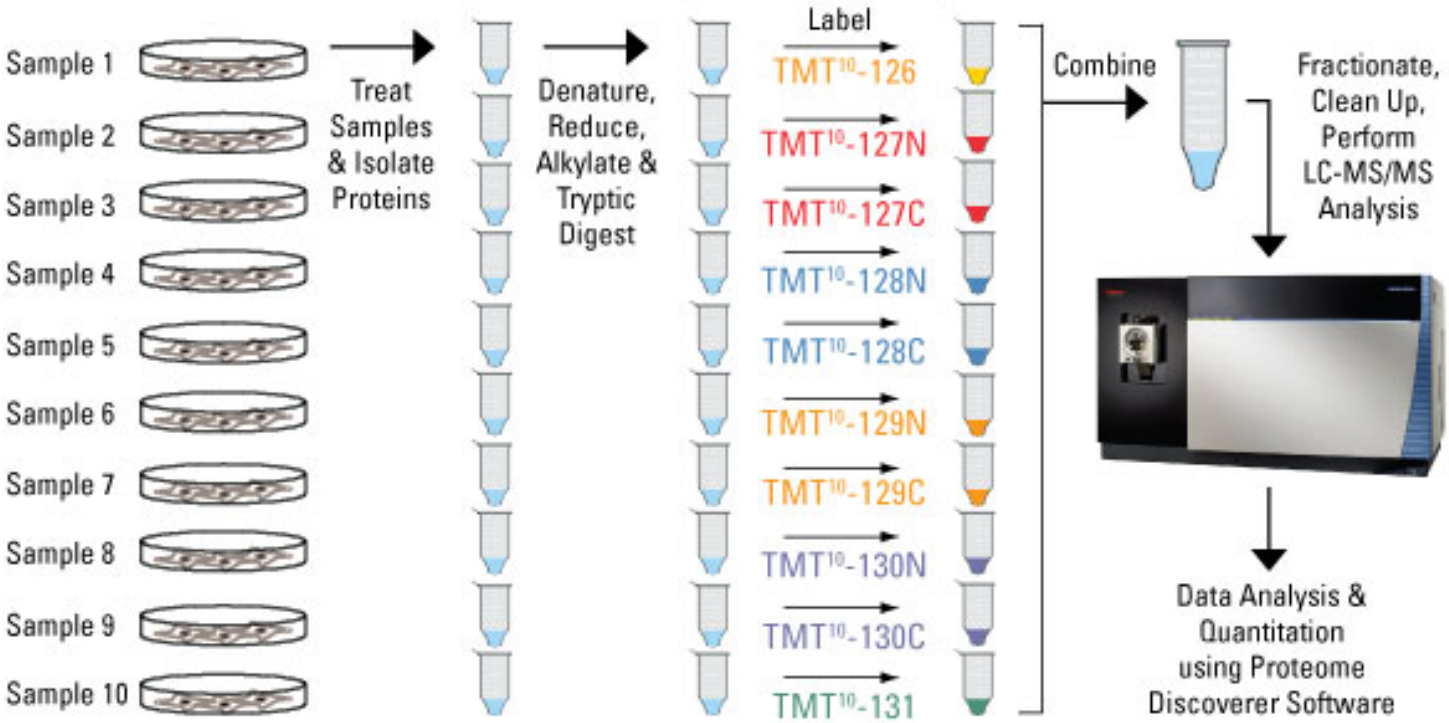


TMT⁶ - Six Plex Quantitation

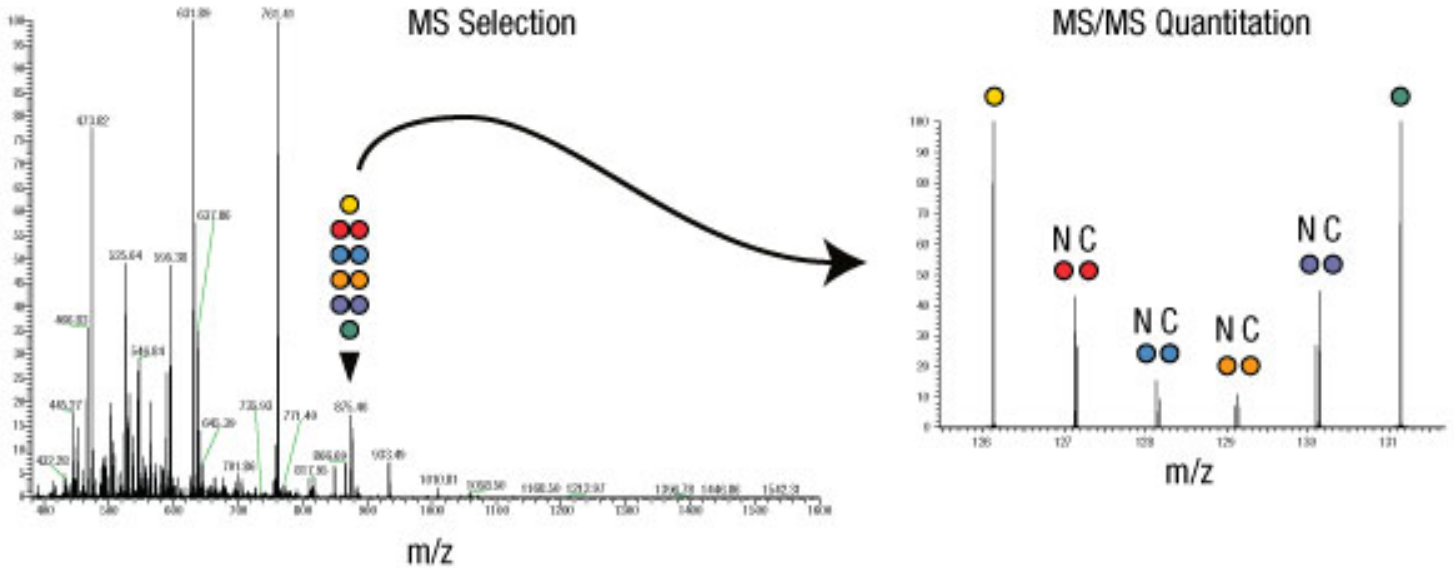
TMT 10-plex Reagents



TMT 10-plex Reagents



TMT 10-plex Reagents



TMT 10-plex labeled peptides were mixed 16:8:4:2:1:1:2:4:8:16

