Proteomics Core Facility
1st Workshop

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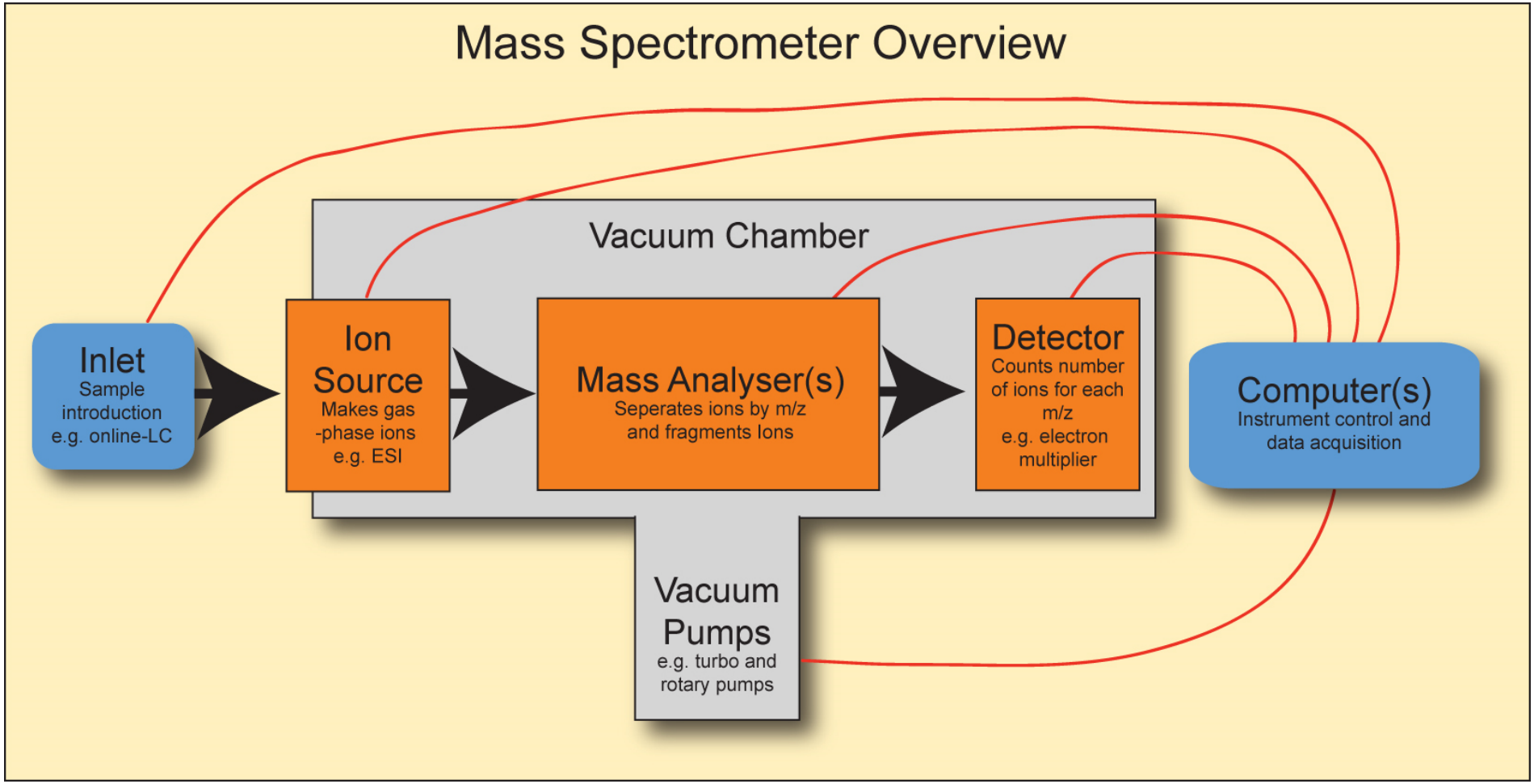
Head of Proteomics Core Facility

OutLook

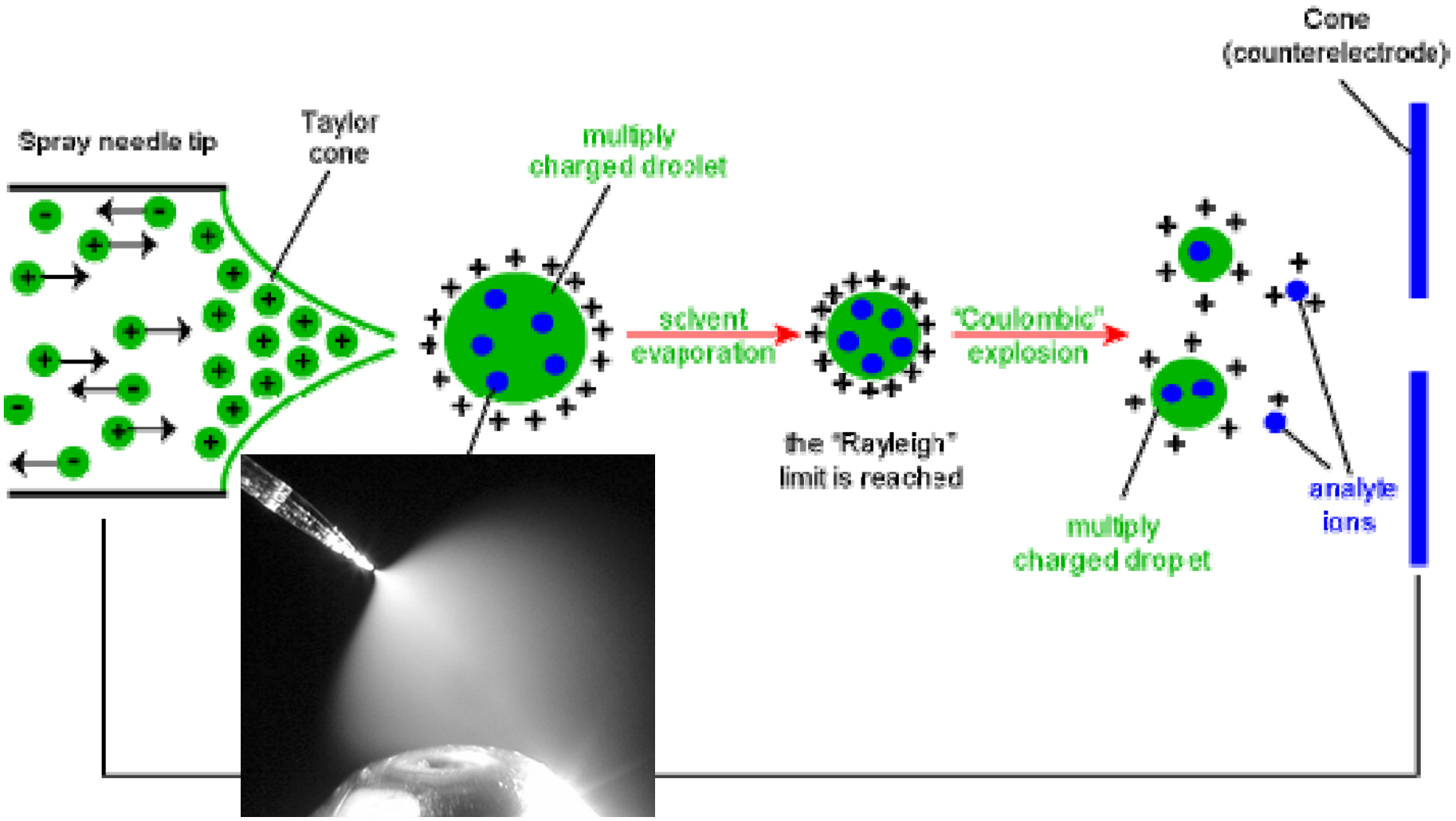
1. Basics of LC and MS
2. How are MS/MS spectra acquired
3. Quantitative proteomics
4. Capability of our facility
5. Detail guideline for LC-MS service

1. Basics of LC and MS

Mass spectrometry- fundamentals

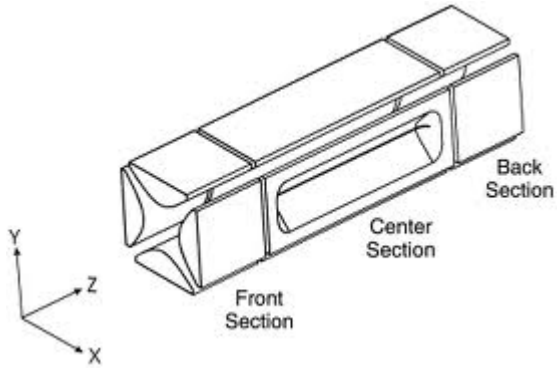


Ion source: electrospray ionization

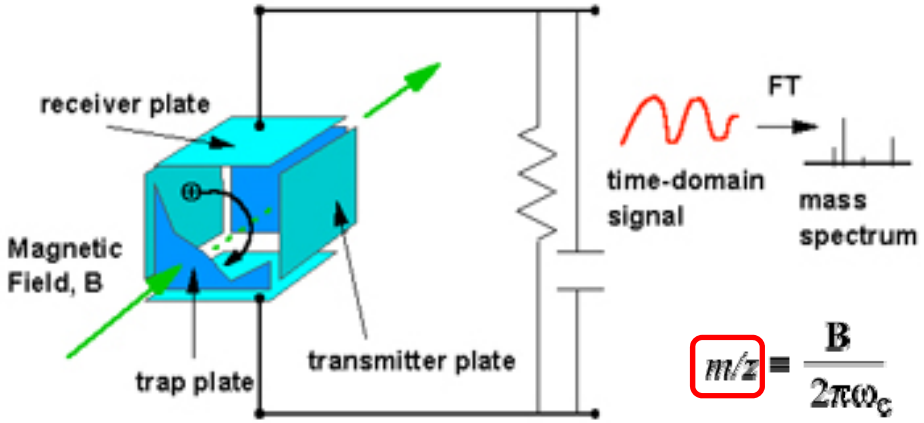


Mass analyzer

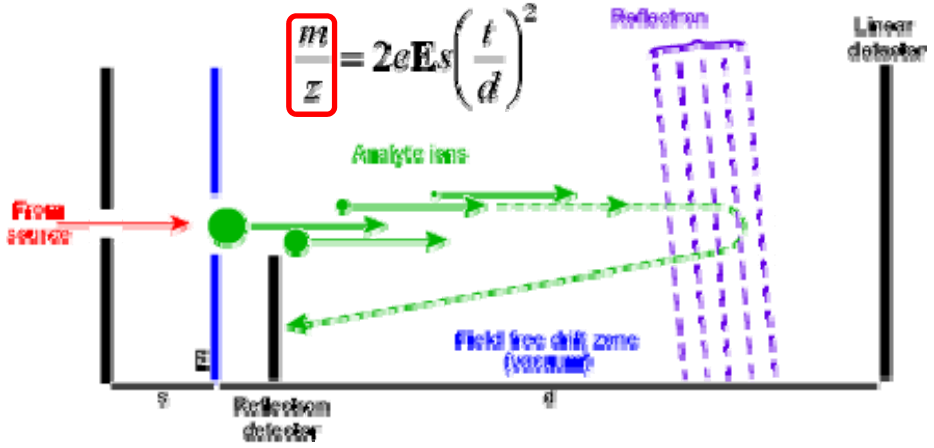
1983, **Ion trap** (1989 Nobel prize)



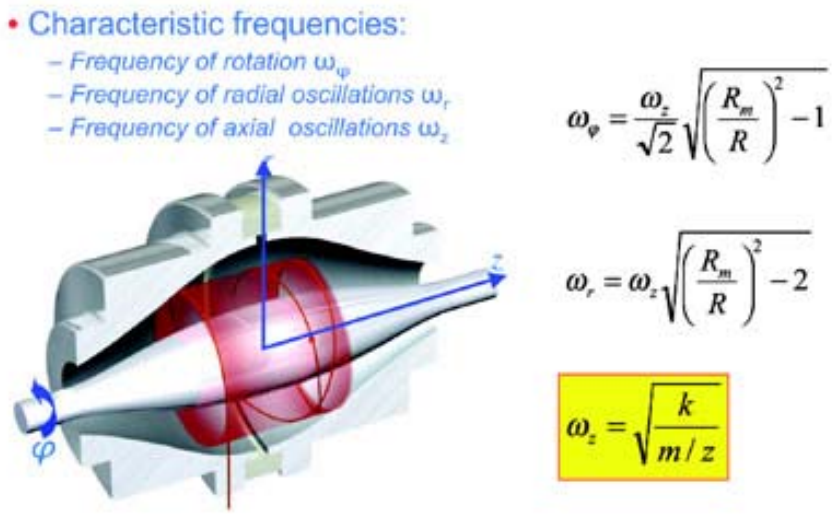
1974, **FT-ICR**
(Fourier-transform ion cyclotron resonance)



1948, **TOF (time-of-flight)**



2005, **LTQ-Orbitrap**



Mass resolution

Resolution, Isotopic Peaks and Charge-state

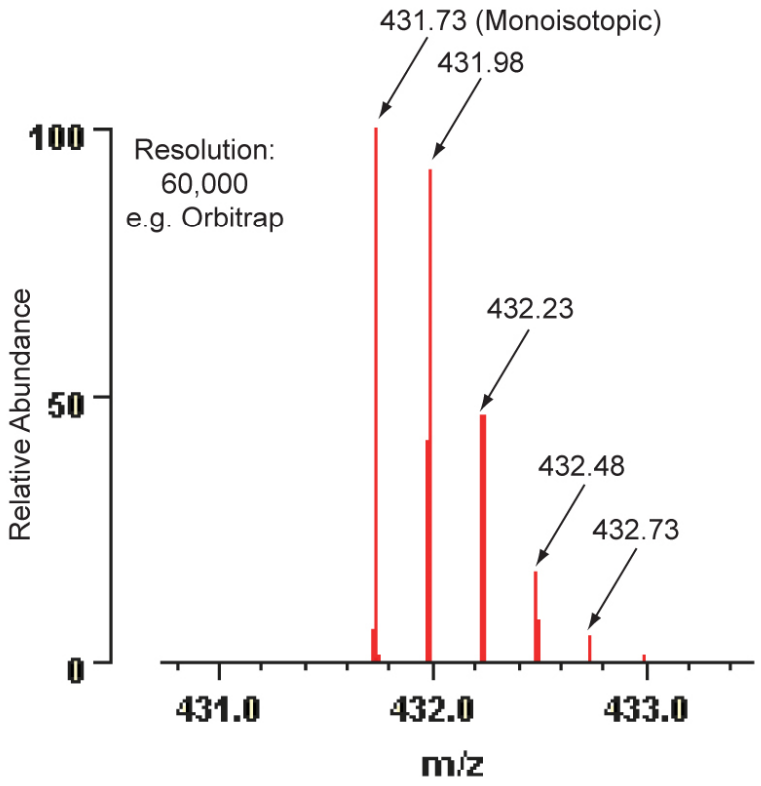
m/z difference between isotopic peaks is 0.25.

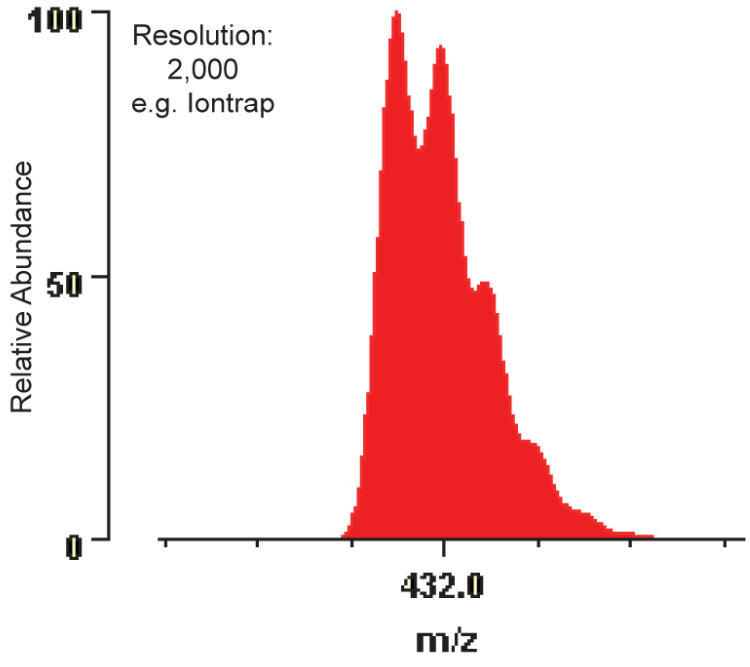
Mass difference between isotopic peaks is 1 amu (1 neutron).

$$z = \frac{1}{0.25} = 4$$

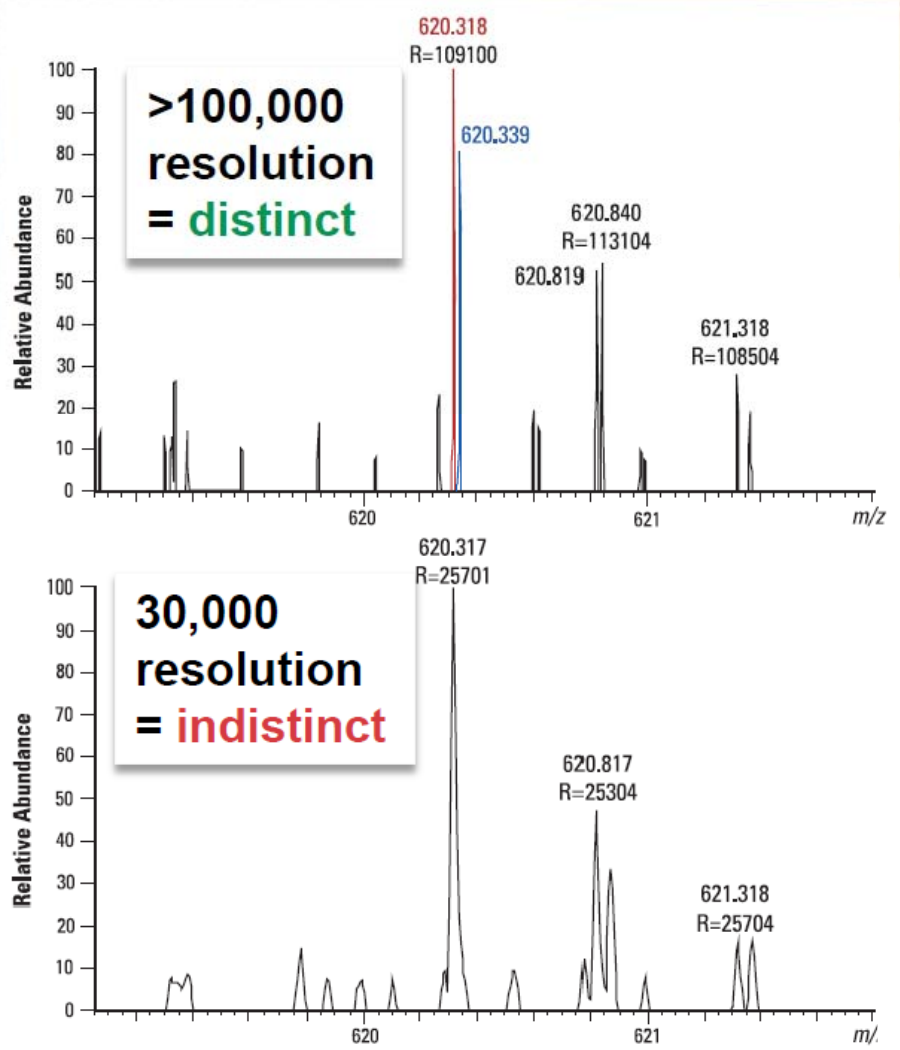
m (monoisotopic) = (431.73 x 4) - 4

$$Resolution = \frac{M}{\Delta M}$$



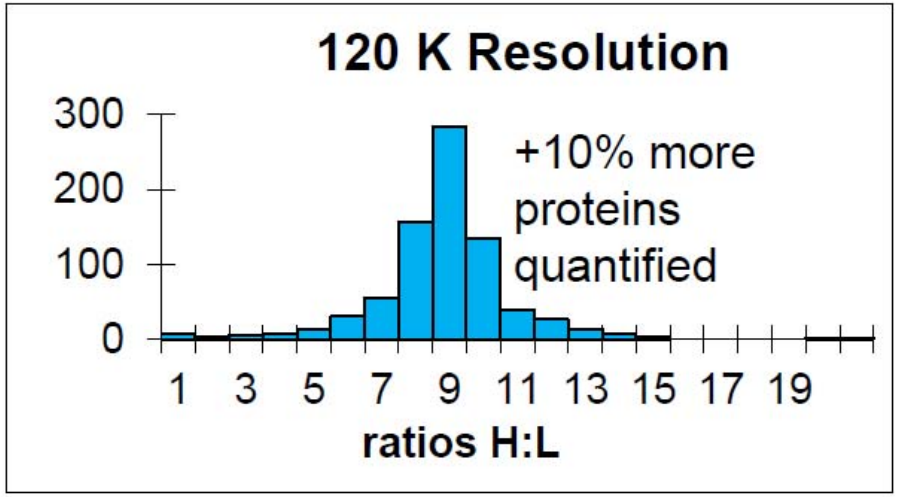


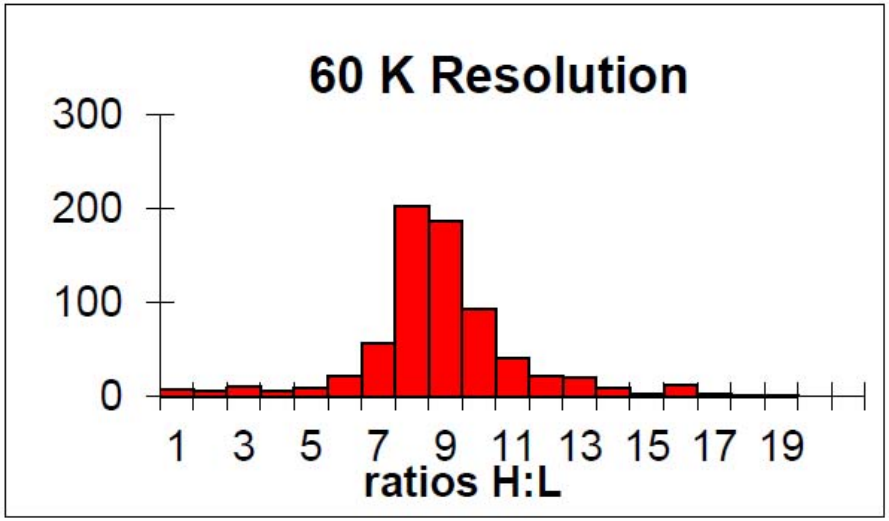
SILAC quantification: Resolution *vs* Accuracy



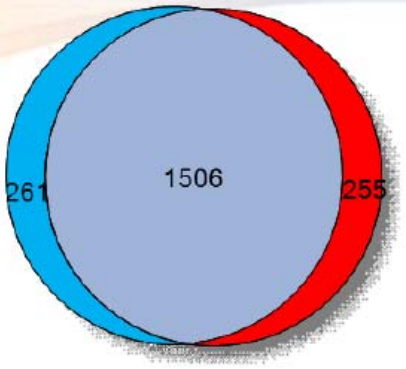
$$Resolution = \frac{M}{\Delta M}$$

SILAC Quantification: Resolution *vs* Accuracy



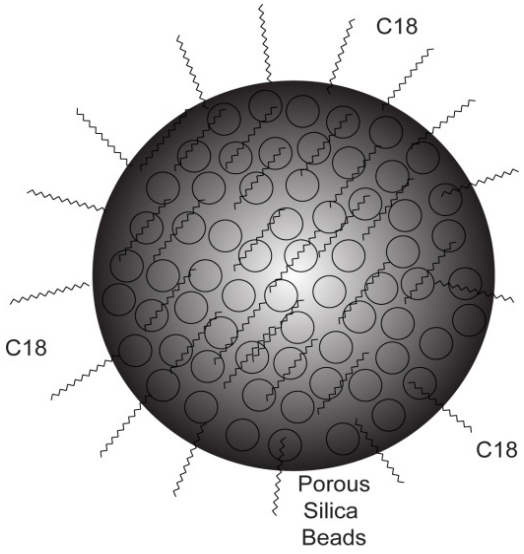


Protein ID results

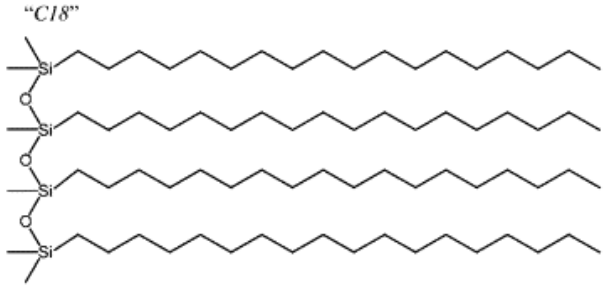


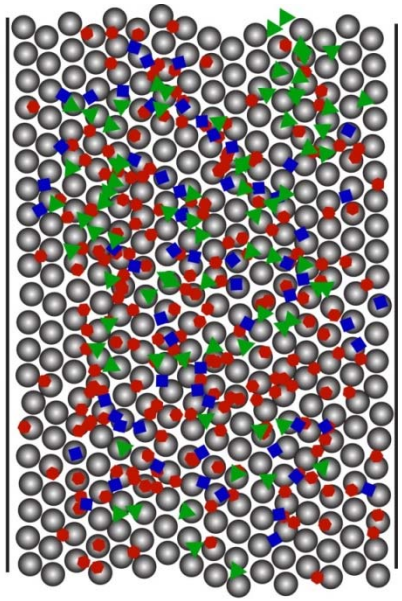
HeLa cell labeled with R^{10}&K^{8}, mixed in H:L=10:1
OT Elite :1x FTMS + 20 x ddrCID,

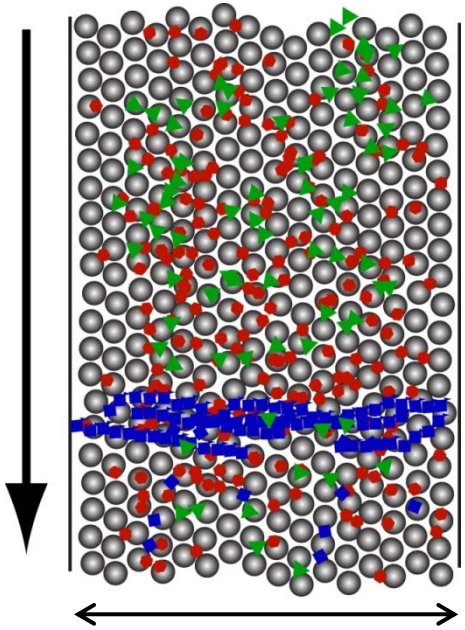
RPLC (Reverse-phase LC)



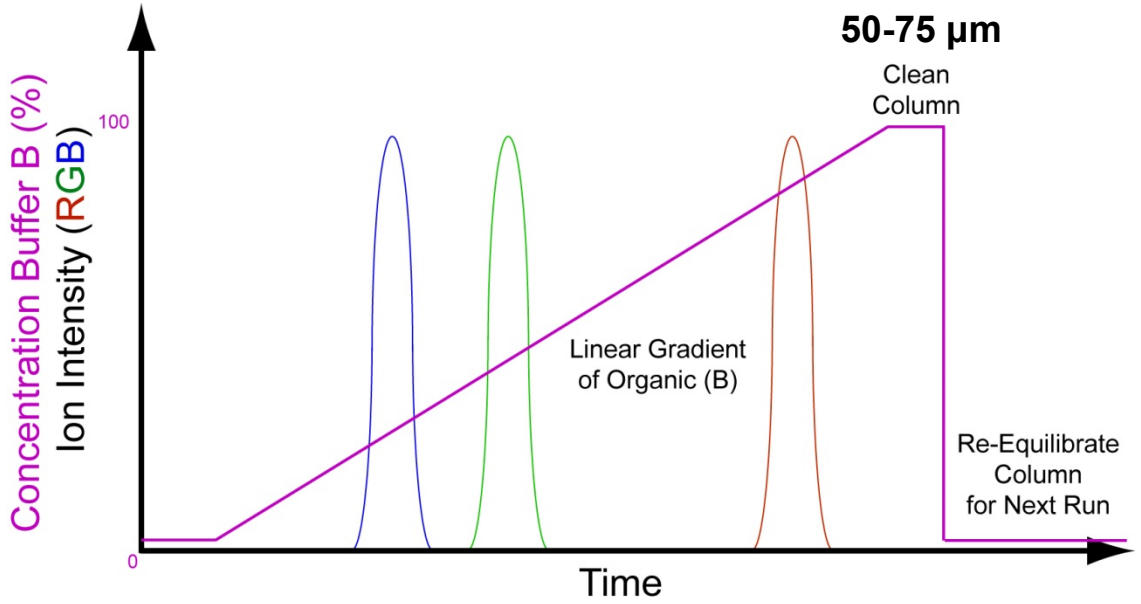
1.7-3.0 µm







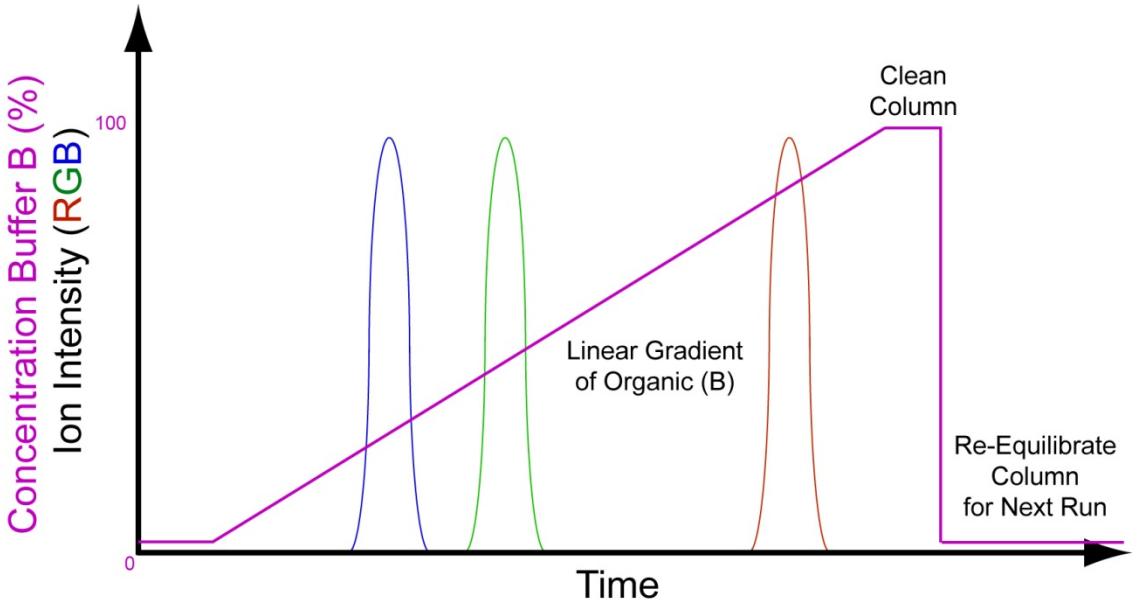
50-75 µm

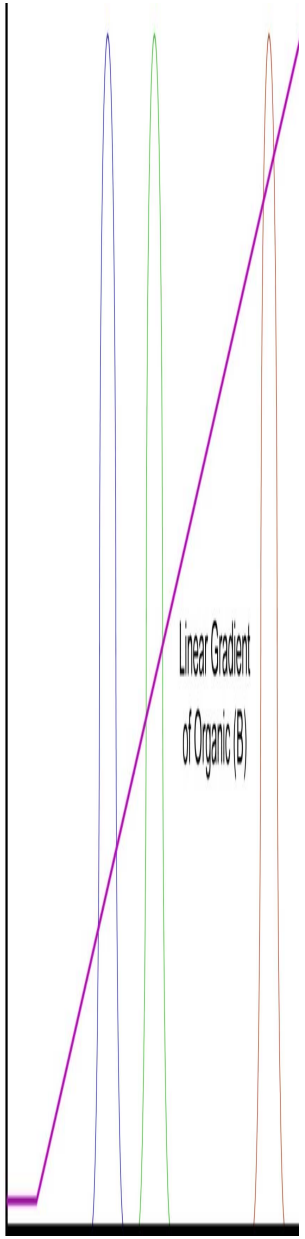


HPLC or UPLC ?

High or ultra performance/sensitivity?

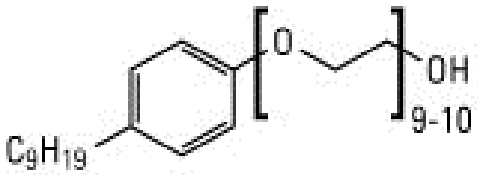
Smaller particle, longer column
→ high or ultra pressure



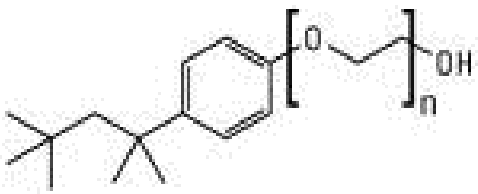


Notorious detergent for LC-MS

Non-ionic detergent

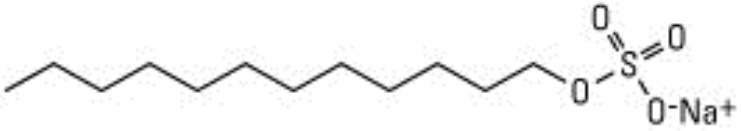


NP-40 Detergent
MW 617

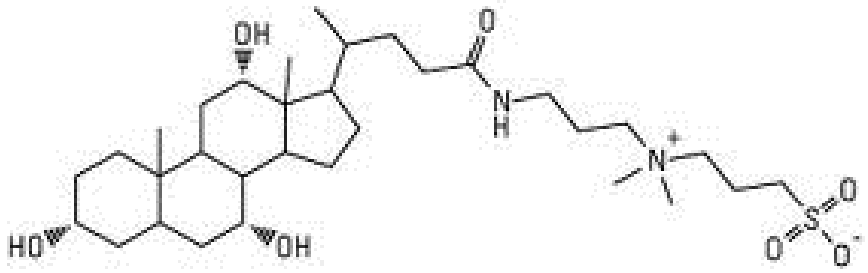


Triton* X-100 Detergent
n = 9-10
MW 647

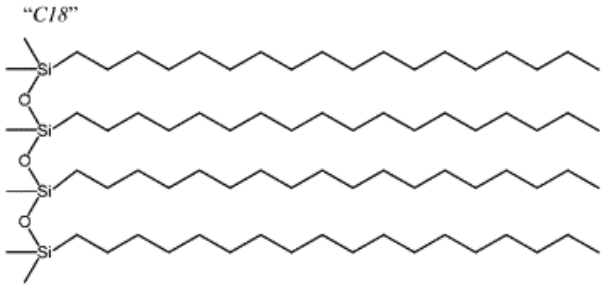
Ionic detergent



Sodium dodecyl sulfate (SDS)
MW 288



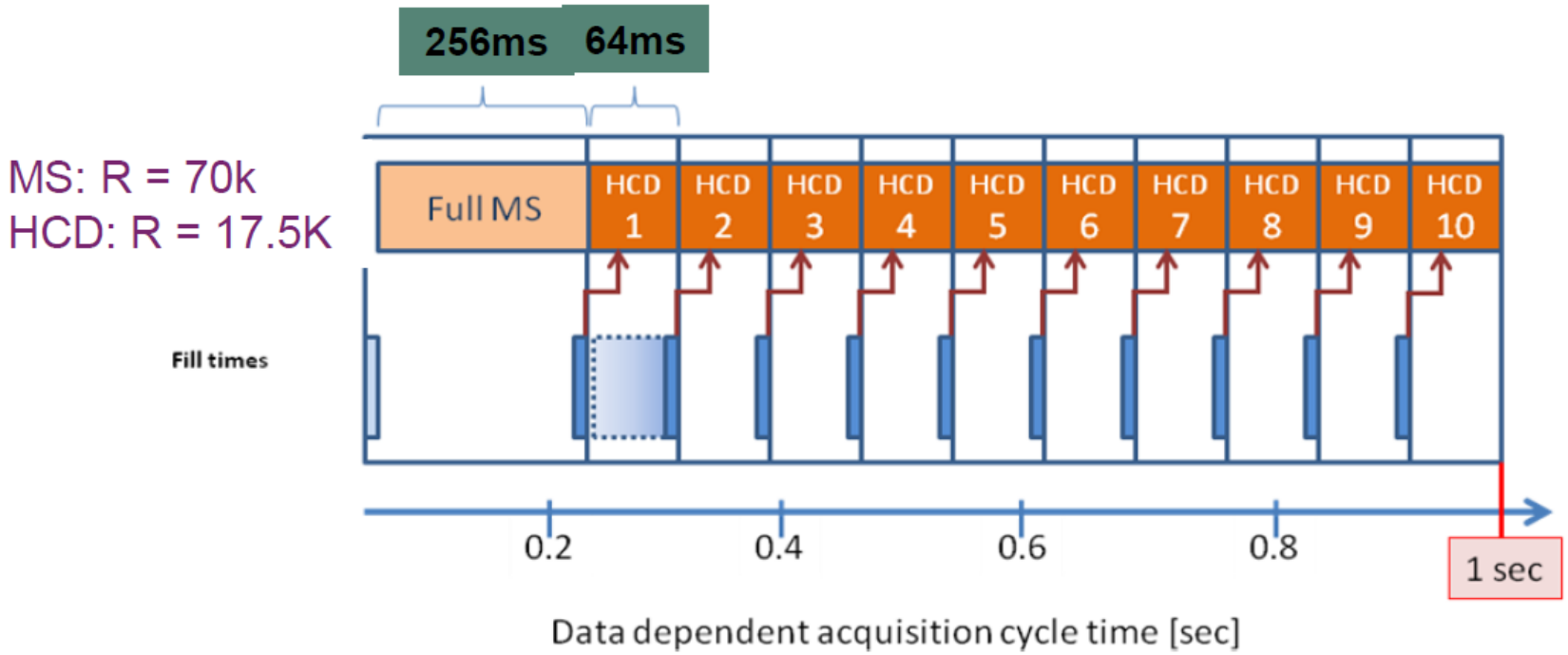
CHAPS
MW 615



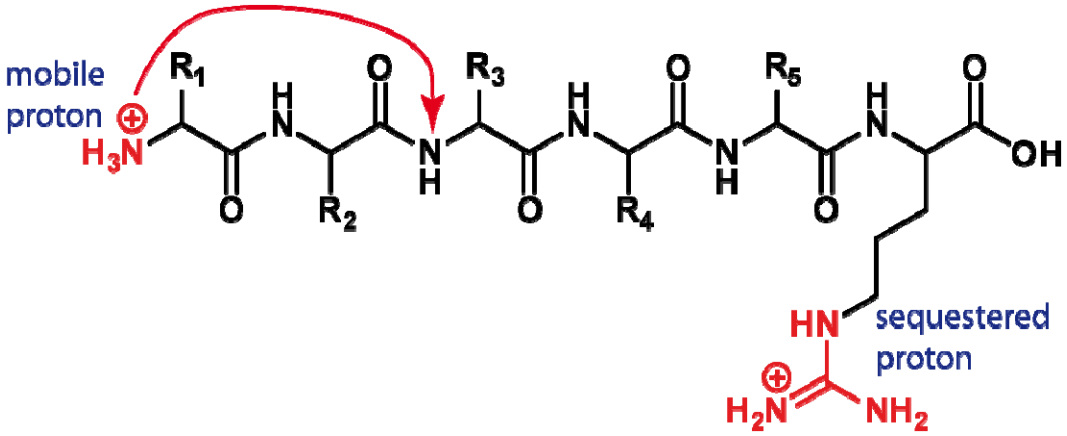
2. How are MS/MS spectra acquired

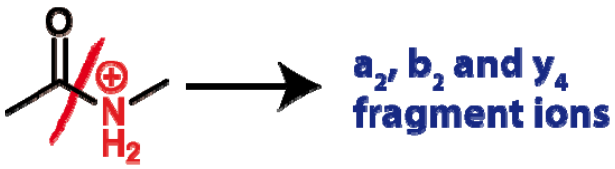
MS/MS: DDA acquisition (Data dependent MS/MS triggering)

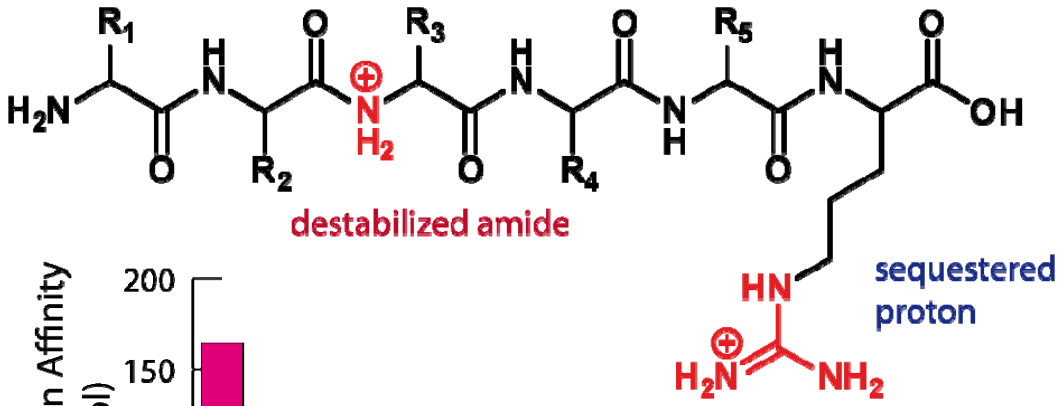
Discovery Proteomics: Top 10 - 10Hz dd-HCD Scan

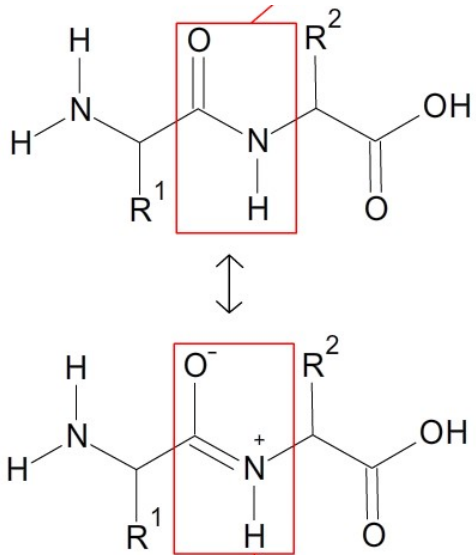


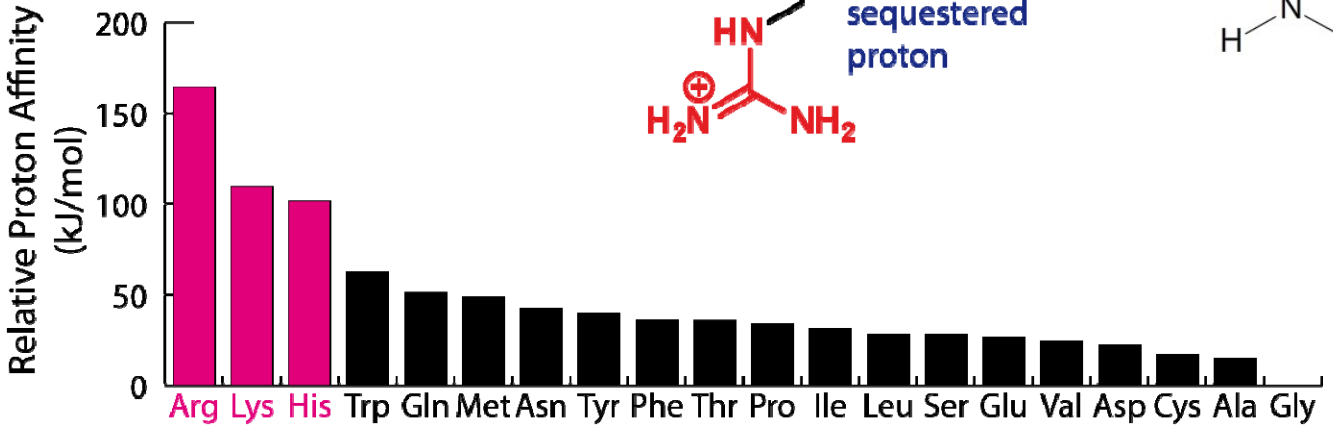
Fragmentation: mobile proton model

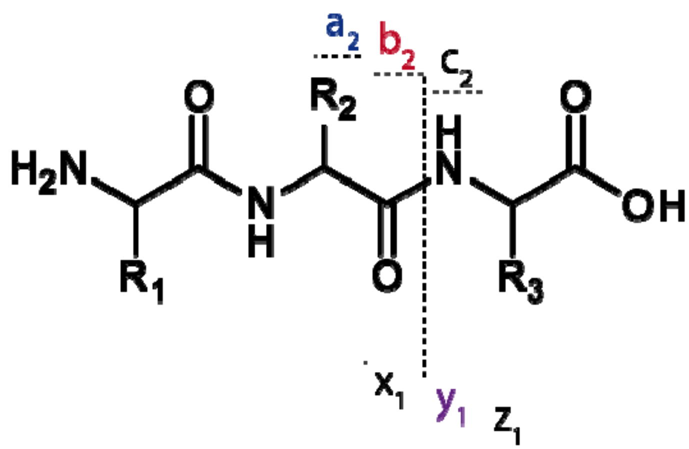


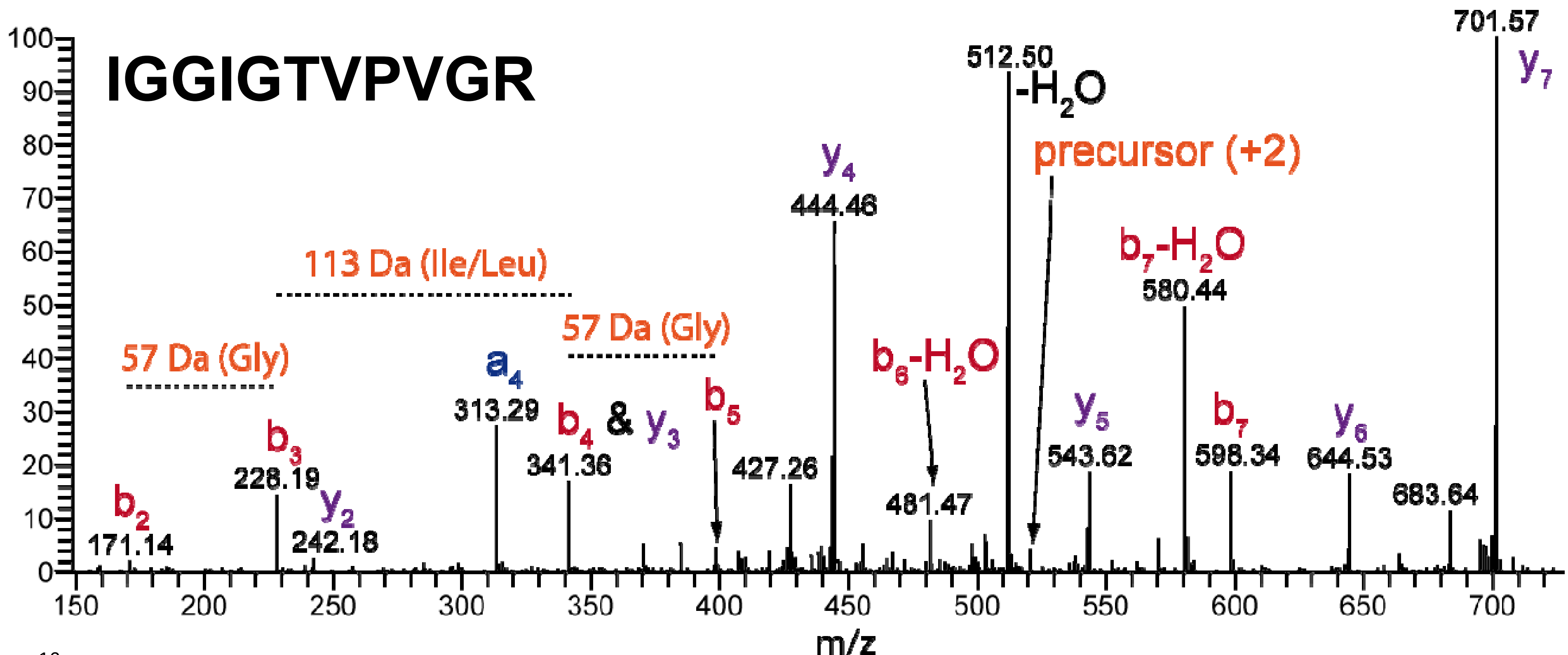




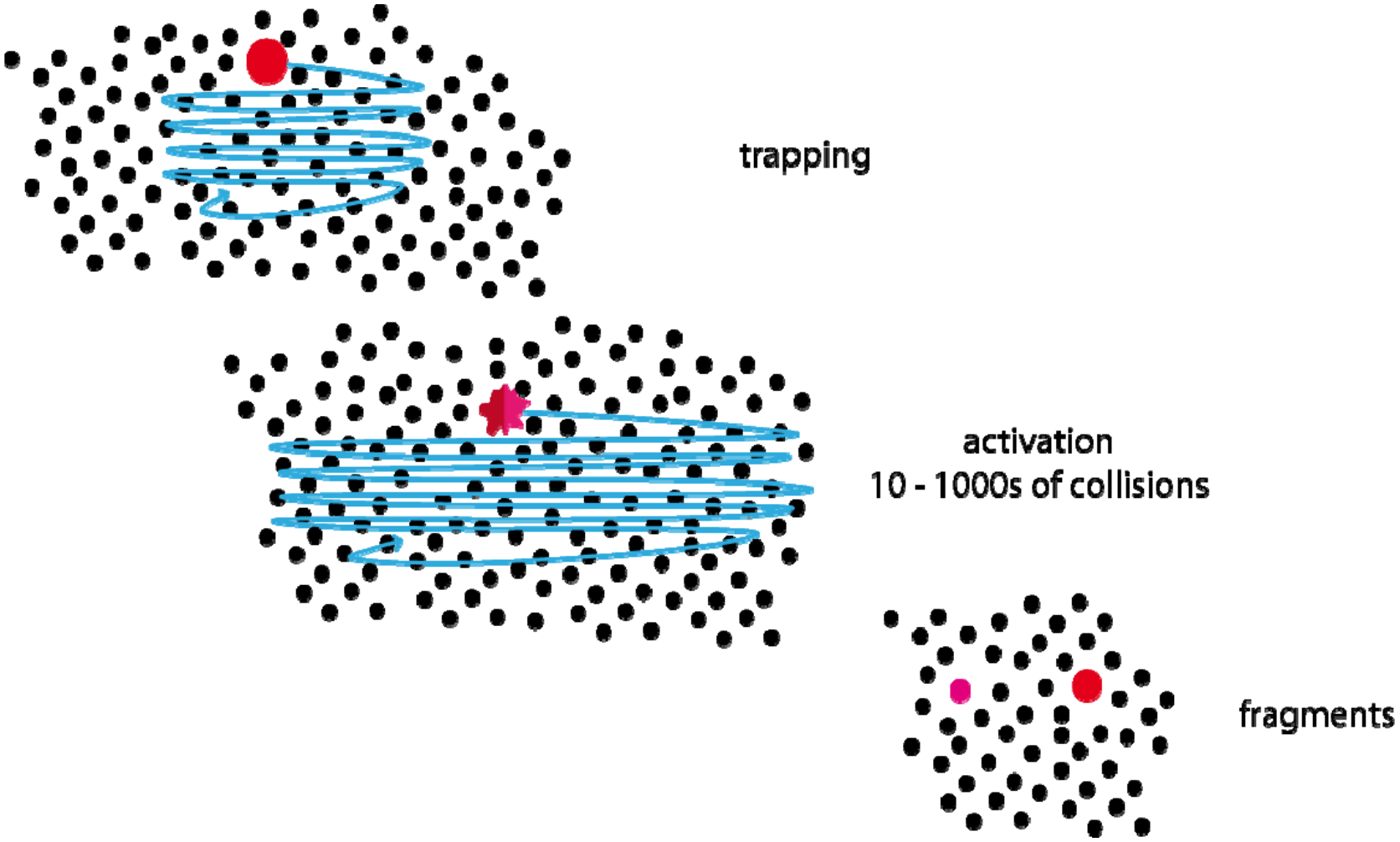




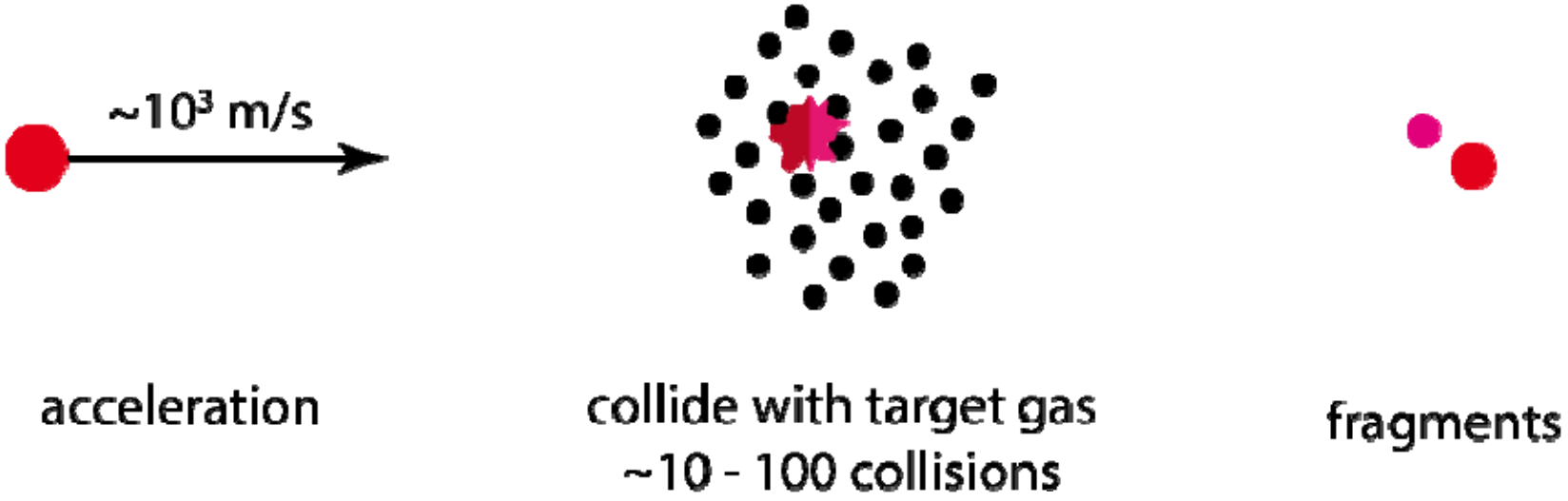

a2
b2
c2
H2N
O
R2
H
N
OH
N
H
R1
O
R3
x1
y1
z1


IGGIGTVPVGR
701.57
y7
512.50
-H2O
precursor (+2)
y4
444.46
b7-H2O
580.44
113 Da (Ile/Leu)
57 Da (Gly)
57 Da (Gly)
a4
313.29
b6-H2O
b5
b4 & y3
341.36
y5
543.62
b7
598.34
y6
644.53
b3
228.19
427.26
481.47
683.64
b2
y2
171.14
242.18
m/z

CID (Collision induced dissociation) for LTQ-MS



HCD (Higher-energy CID) for Q-Exactive MS



3. Quantitative proteomics

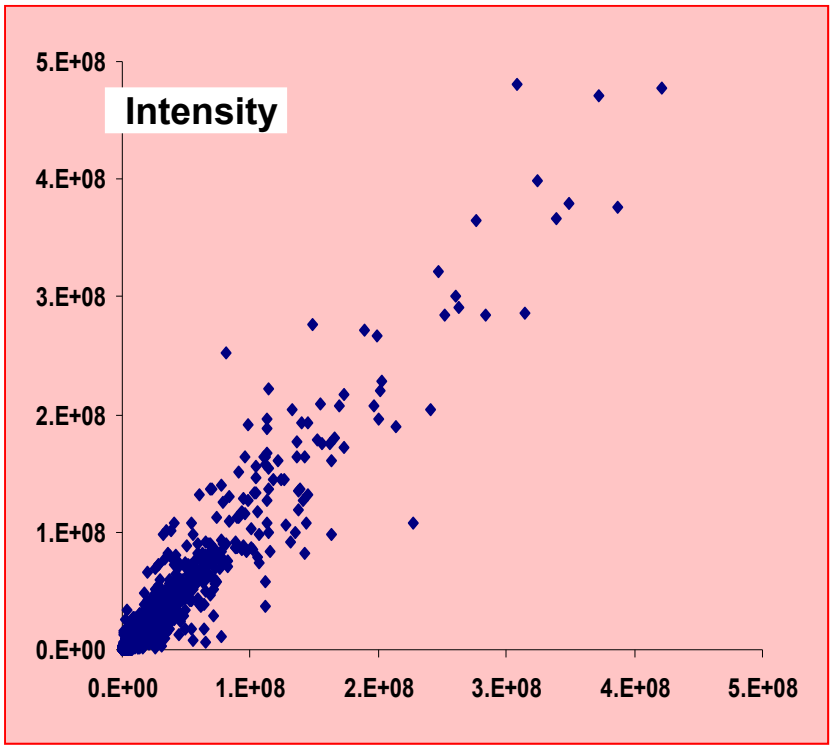
- Label-free
- Spectral counting
- Stable isotope labeling
- Spiking synthetic heavy peptides (AQUA)

  as internal standards (or fully heavy protein)

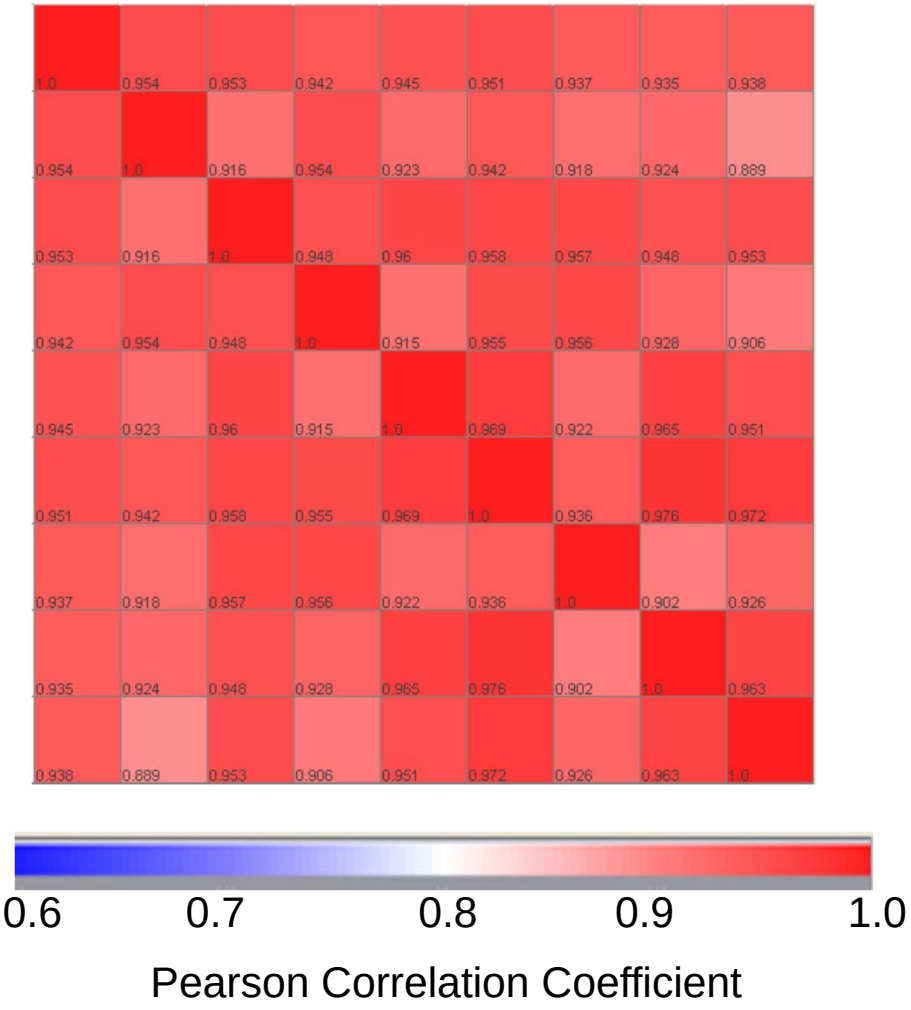
  →enable '*absolute quantification*' of protein

1) Label-free quantification

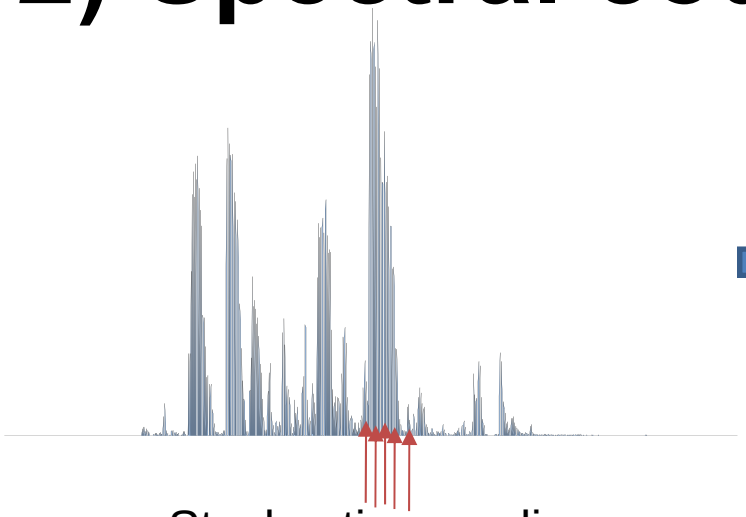
Reproducibility



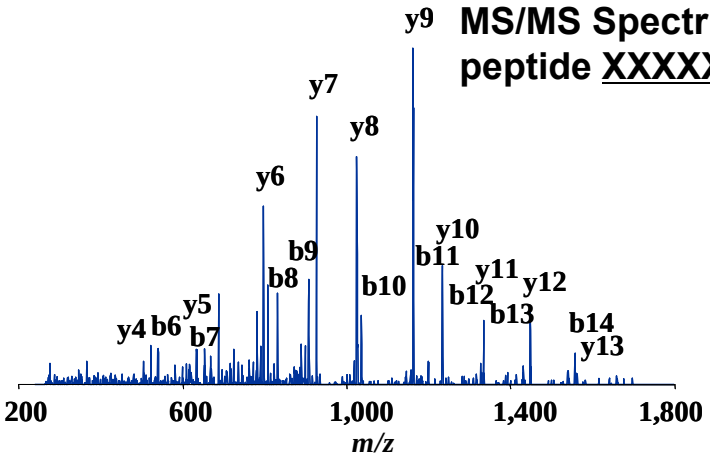
9 Technical replicates



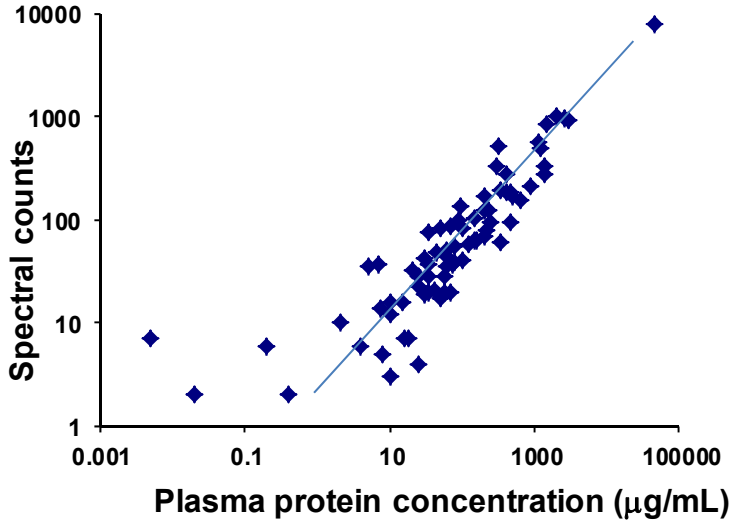
2) Spectral counting

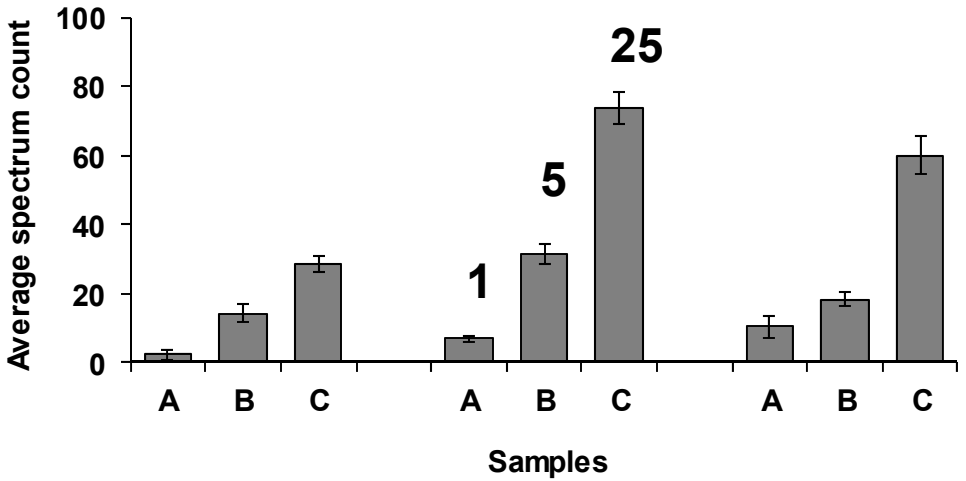


Stochastic sampling



Counting the number of MS/MS spectra

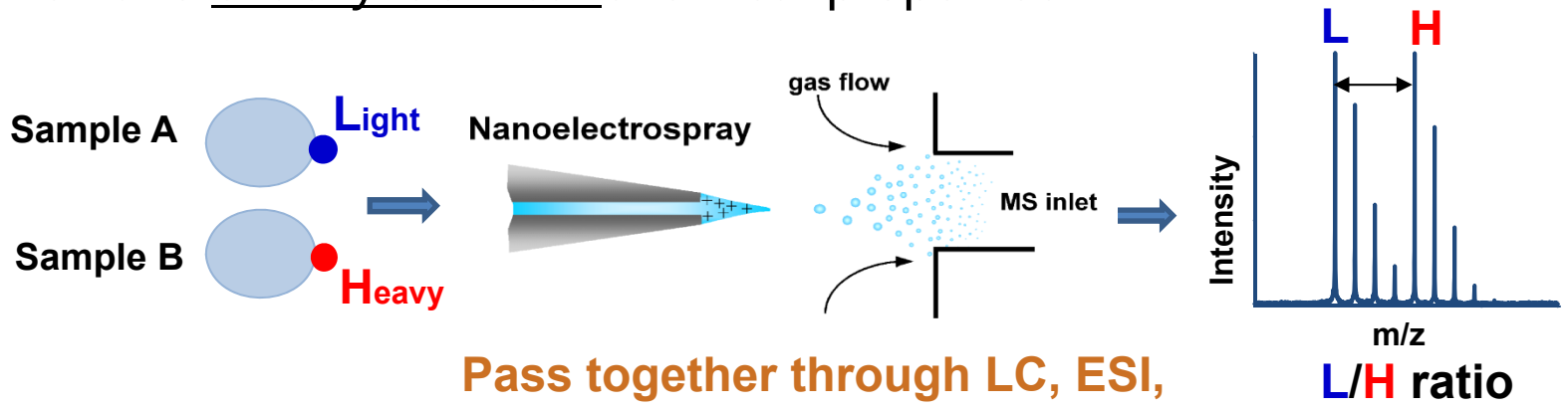




Spectral counting is semi-quantitative!

3) Stable isotope labeling

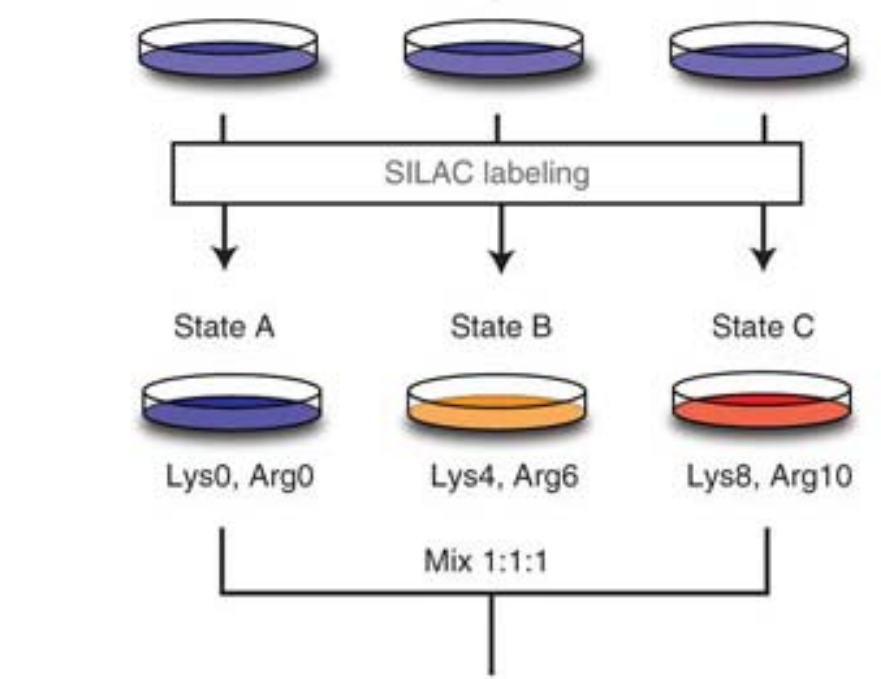
Light (^{12}C, ^{16}O, ^{14}N) and heavy isotope (^{13}C, ^{18}O, ^{15}N) labeled peptide pairs have exactly identical chemical properties

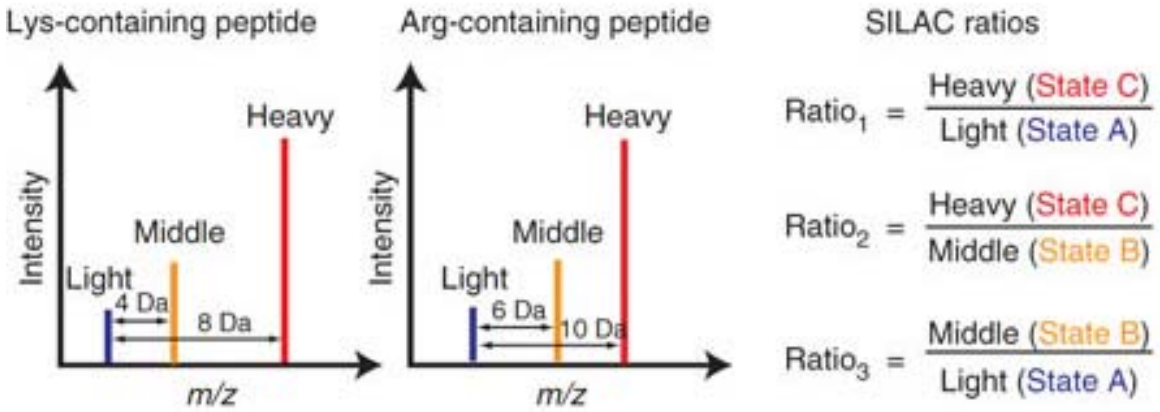


Pass together through LC, ESI, In all ion transmission spaces

- Metabolic labeling (**SILAC**)
- In-vitro labeling (**^{18}O-labeling**)
- Isobaric chemical tagging (**TMT/iTRAQ labeling**)

SILAC Method





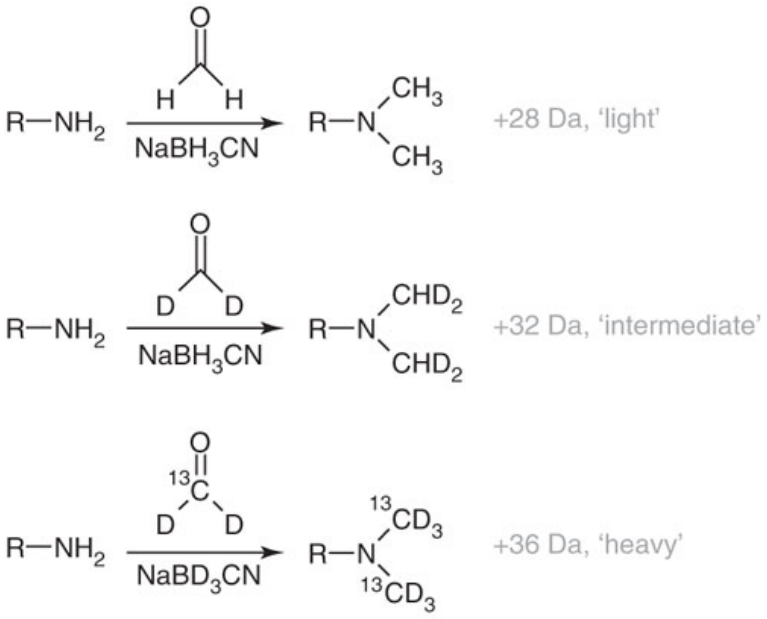
Limitation

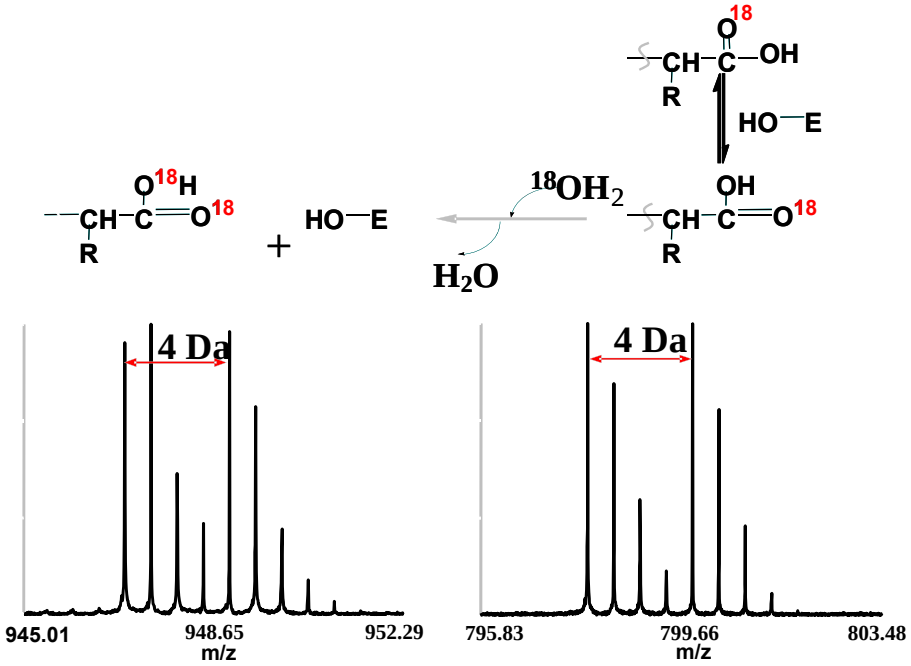
Not applicable

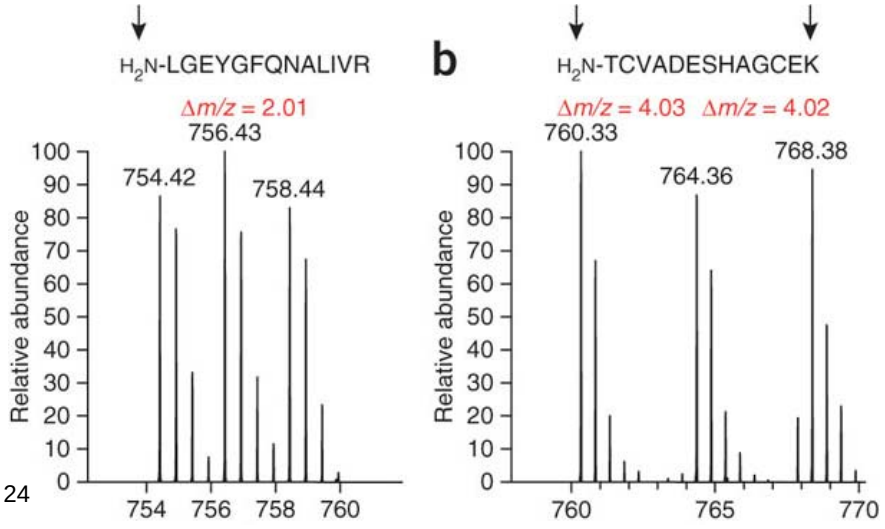
to non-cultural samples

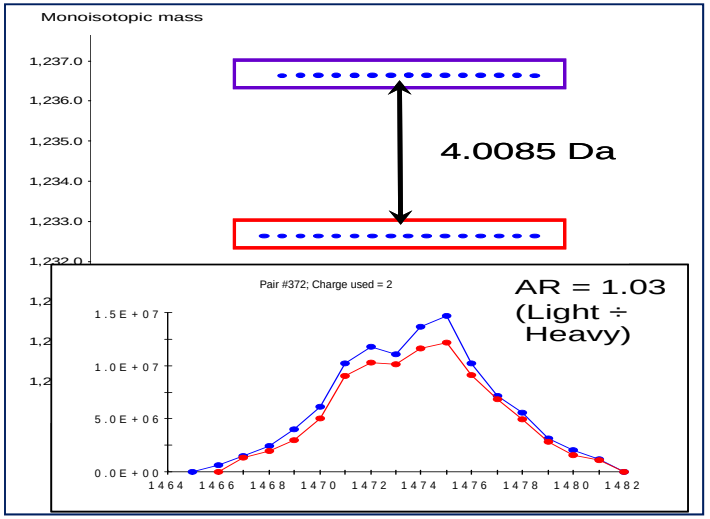
e.g., serum

In-vitro isotopic labeling

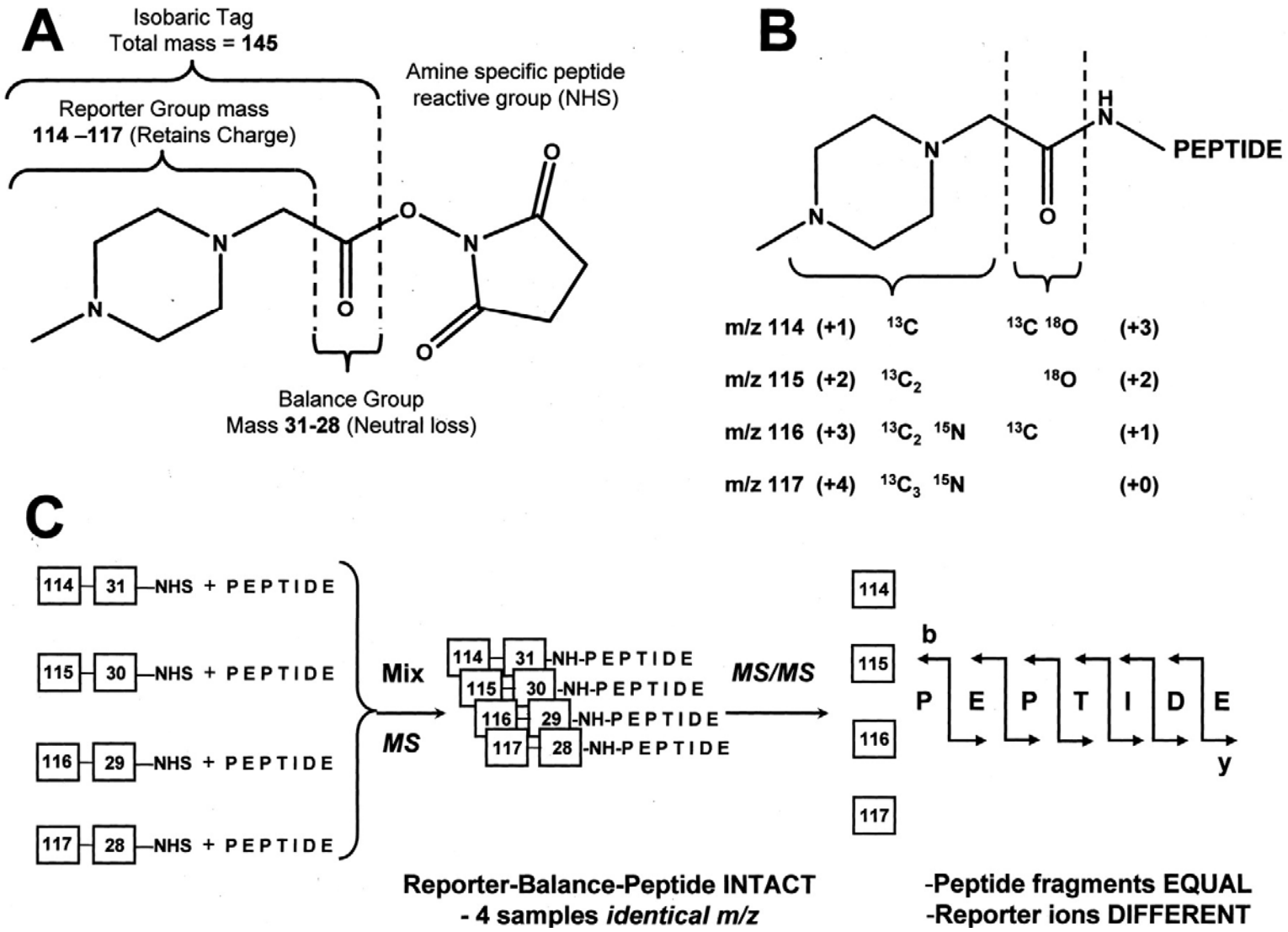








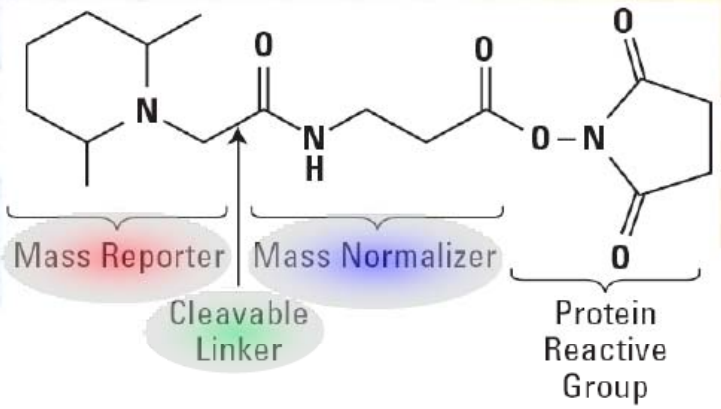
Isobaric chemical labeling-iTRAQ

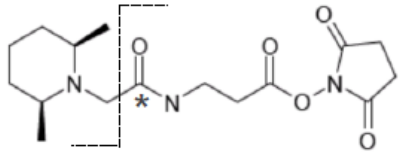


Ross P L et al. Mol Cell Proteomics 2004;3:1154-1169

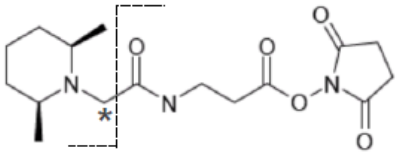
TMT- Tandem Mass Tags

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

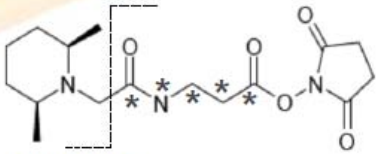




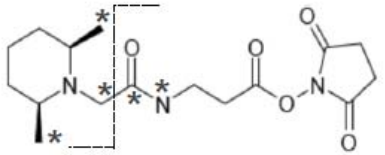
126 Da



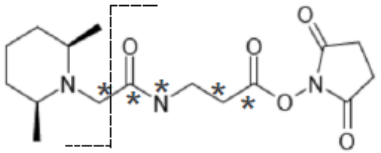
127 Da



126 Da



129 Da



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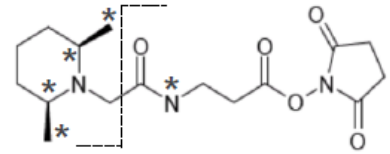

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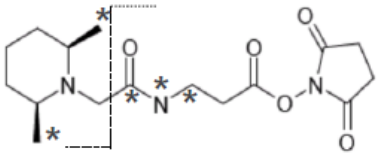

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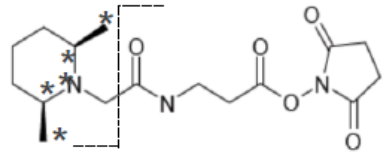
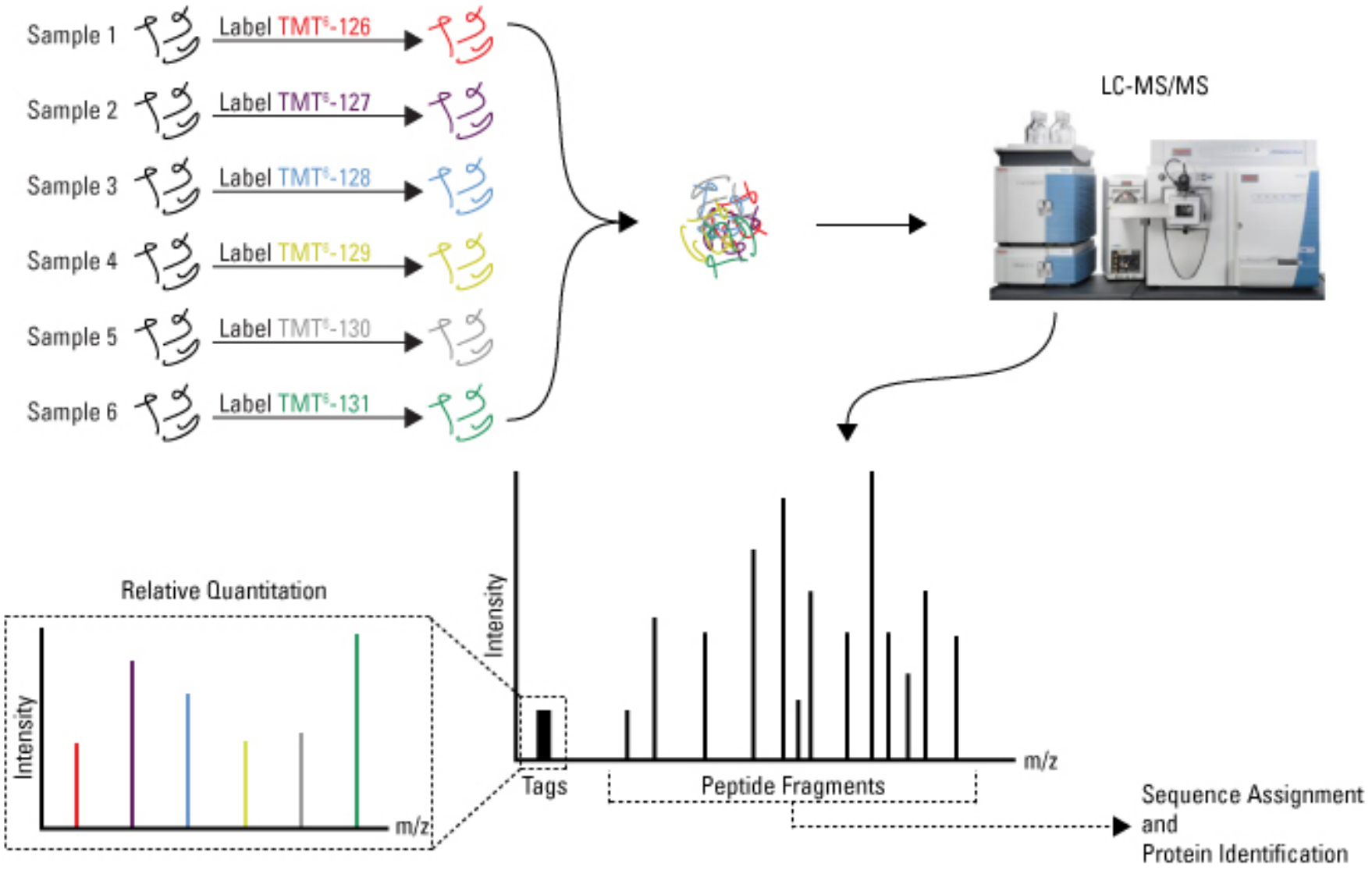


131 Da

TMT^2 - Two Plex Quantitation

TMT^6 - Six Plex Quantitation

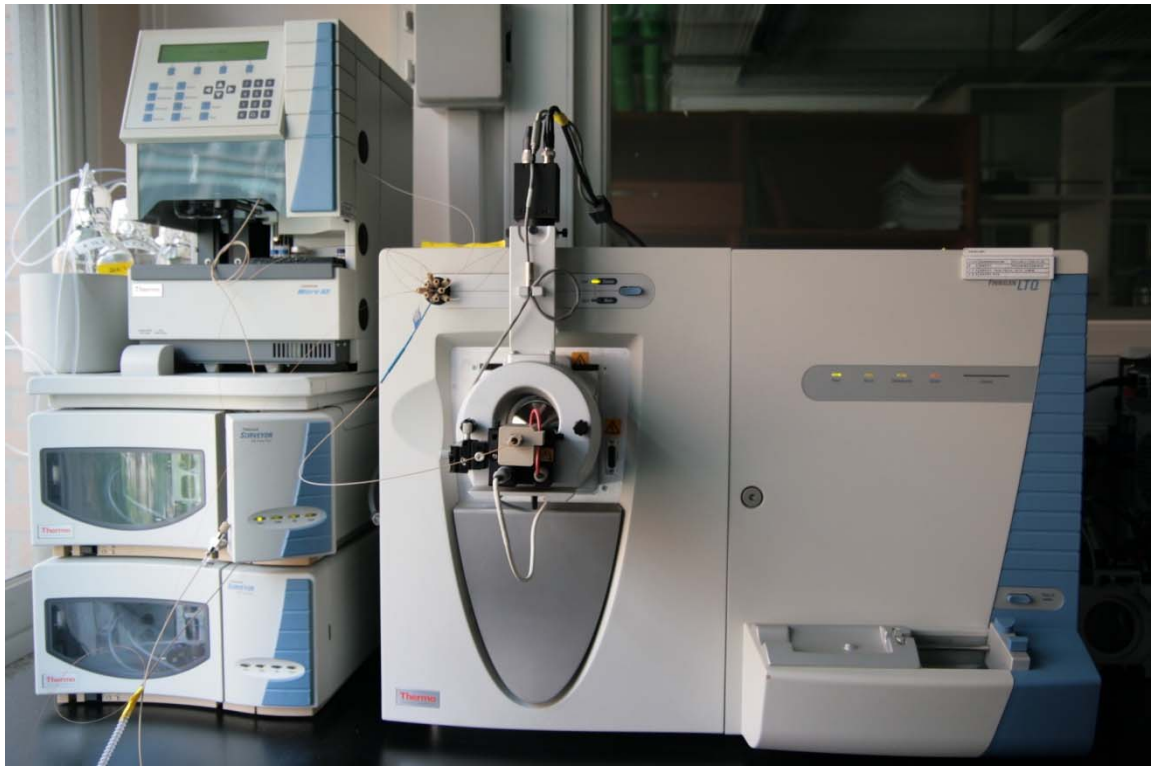
Mutiplexing capability



4. Capability of our facility

http://biosci.snu.ac.kr/proteomics

LTQ XL/HPLC (2006)



Q-Exactive/UPLC(2013)

LTQ XL/HPLC system: main equipment, gel-spot/band (3 Hz in MS/MS)

Q-Exactive/UPLC system: optional for gel section or IP pull-down samples (12 Hz in MS/MS)

The most trustworthy DB searching

1. **MS-GF+**, which is a standard DB searching tool for *Clinical Proteomic Tumor Analysis Consortium* (*CPTAC*), NCI, US

2. Easily beats Sequest, Mascot and any other DB search algorisms.

3. Invented by Korean computational proteomics scientist, **Dr. Sangtae Kim** in PNNL.

Services and prices

1. Protein Identification using **LTQ XL/HPLC system**
   1) **Single gel band** or **spot**
      10만원 for 1st sample, **5만원** from 2nd samples
   2) Gel section **10만원** for all cases

2. Protein Identification using **Q-Exacitve/UPLC system**
   (optional, can be delayed)
   1) Gel section **15만원** for all cases
   2) **Immuno precipitated samples** **15만원** for all cases

3. Protein phosphorylation or other PTM without enrichment
   15만원 for all cases

4. Quantitative proteomics study: Please consult

Price example & comparison

1. Protein Identification using **LTQ XL/HPLC system**
   Single gel band or spot: **10만원** for 1st sample, **5만원** from 2nd samples

   1 sample : 10만원, 2 samples: 15만원 (7.5만원/sample)

   3 samples: 20만원 (6.7만원/ sample)

   5 samples: 30만원 (6.0만원/ sample)

   10 samples: 55만원 (5.5만원/ sample)

   20 samples: 105만원 (5.2만원/ sample)

   Before 2011: 6만원/gel band, 20만원/IP sample

   공동기기원: 20만원/sample, NICEM: 10만원/sample

5. Detail guideline

1. Strongly recommend to use coomassie stain rather than silver.

2. For coomassie, destain gel to a clear background so that bands can be easily seen.

3. For silver, Must **not** use any solutions containing formaldehyde or glutaraldehyde to fix the gel. Only stain the gel long enough (usually only a few minutes) to detect the bands of interest.

4. For PTM analysis, sample must be coomassie-stainable.
   The more protein you can load in the gel and the more pure the protein is the better chance we will have of finding modification sites

5. For immuno precipitated samples, ionic and non-ionic detergents like SDS, NP-40, Triton X-100, CHAPS, and cholate should be removed before trypsin digestion using TCA/acetone precipitation or size cut-off membrane filters.

Required pre-processing for gel band/spot sample

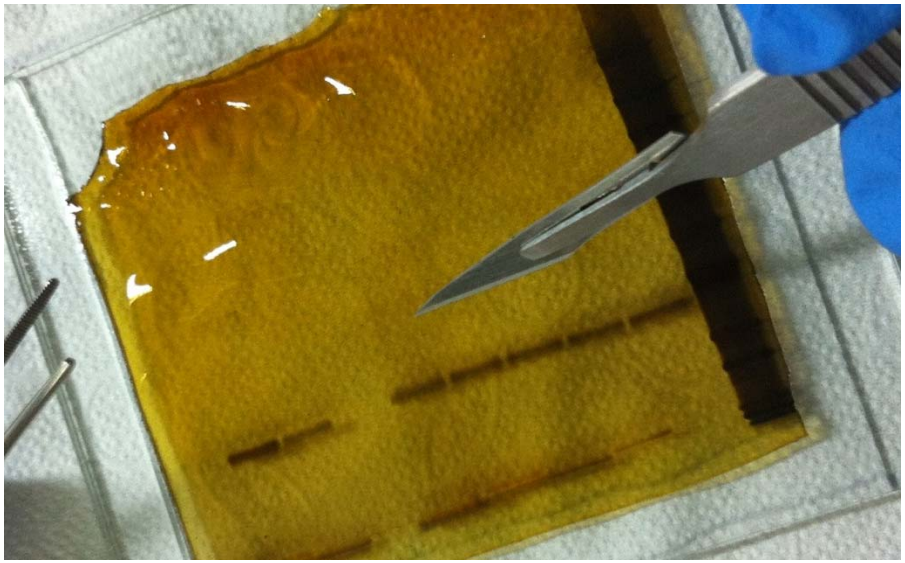
1. Take a picture of the gel prior to excision of gel bands and submit photo along with the sample.

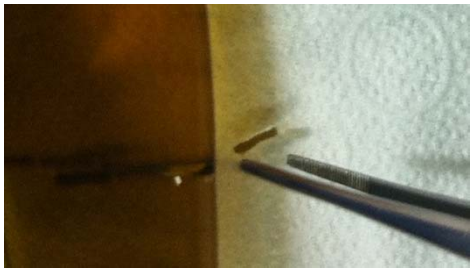
2. Excise gel band(s)/spot(s) with as little excess empty gel as possible and make it pieces as smaller as possible (less than 1 mm^3)

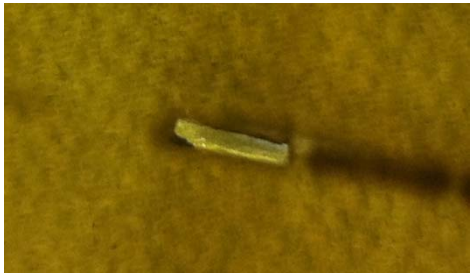
3. Place the gel band(s)/spot(s) into a micro centrifuge tube with some DW (<10 μL).

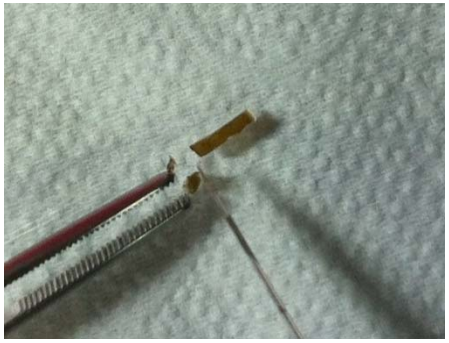
4. Contact to **김선아 연구원** (Tel. 880-**4434**)

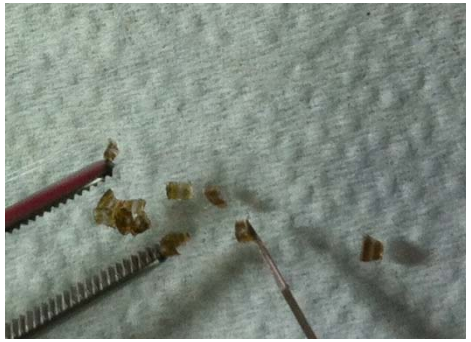
5. Drop off samples to **504-206A, 단백질체지원실** (No need to send samples on dry ice).

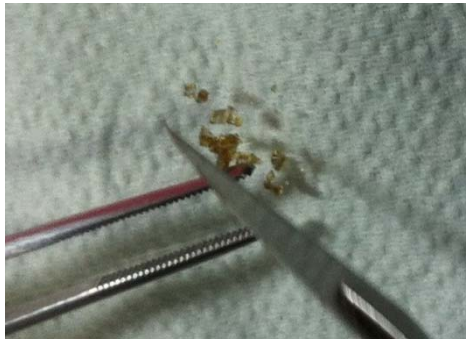


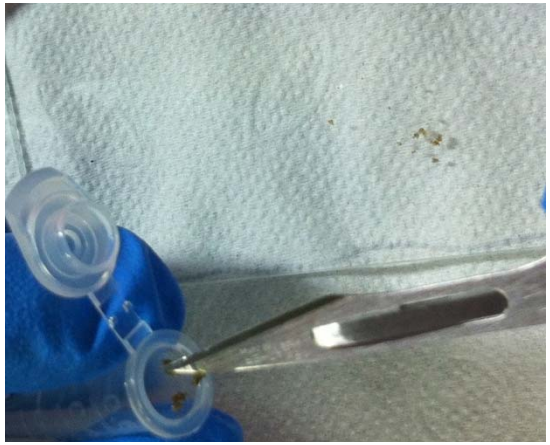


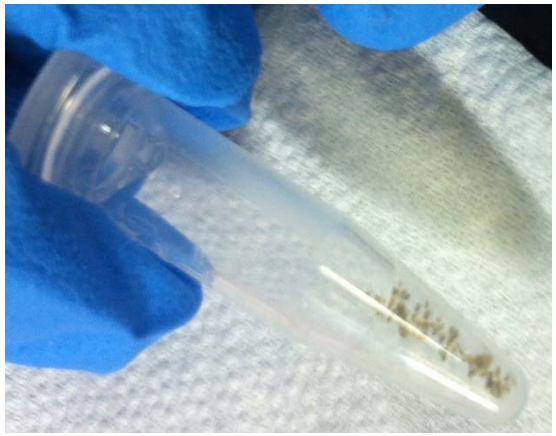












What we do

1. In-gel reduction and alkylation of disulfide bonds (2 hr)
2. In-gel trypsin digestion (4hr or overnight)
3. C18 zip tip clean-up (~20 min for each sample)
4. LC-MS/MS analysis (>2 hr for each sample)
5. MSGF+ database searching (30 min)
6. Filtering out for user-friendly reporting (1 hr)

Acknowledgements

- Prof. V. Narry Kim

- Prof. Kun-Soo Rhee, chair of department
- Prof. Young-Yun Kong, vice chair of department

- RNA-Proteomics team
  - Yong-Woo Na
  - Sun Ah Kim

- IBS supporting team