Lecture 3

Tandem MS & Protein Sequencing

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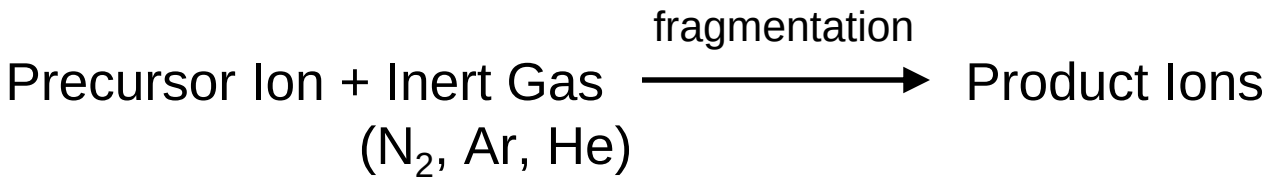
Tandem MS

Steps: 1. Mass Analysis
2. Collision (Fragmentation)
3. Mass Analysis

Collisional Activation-

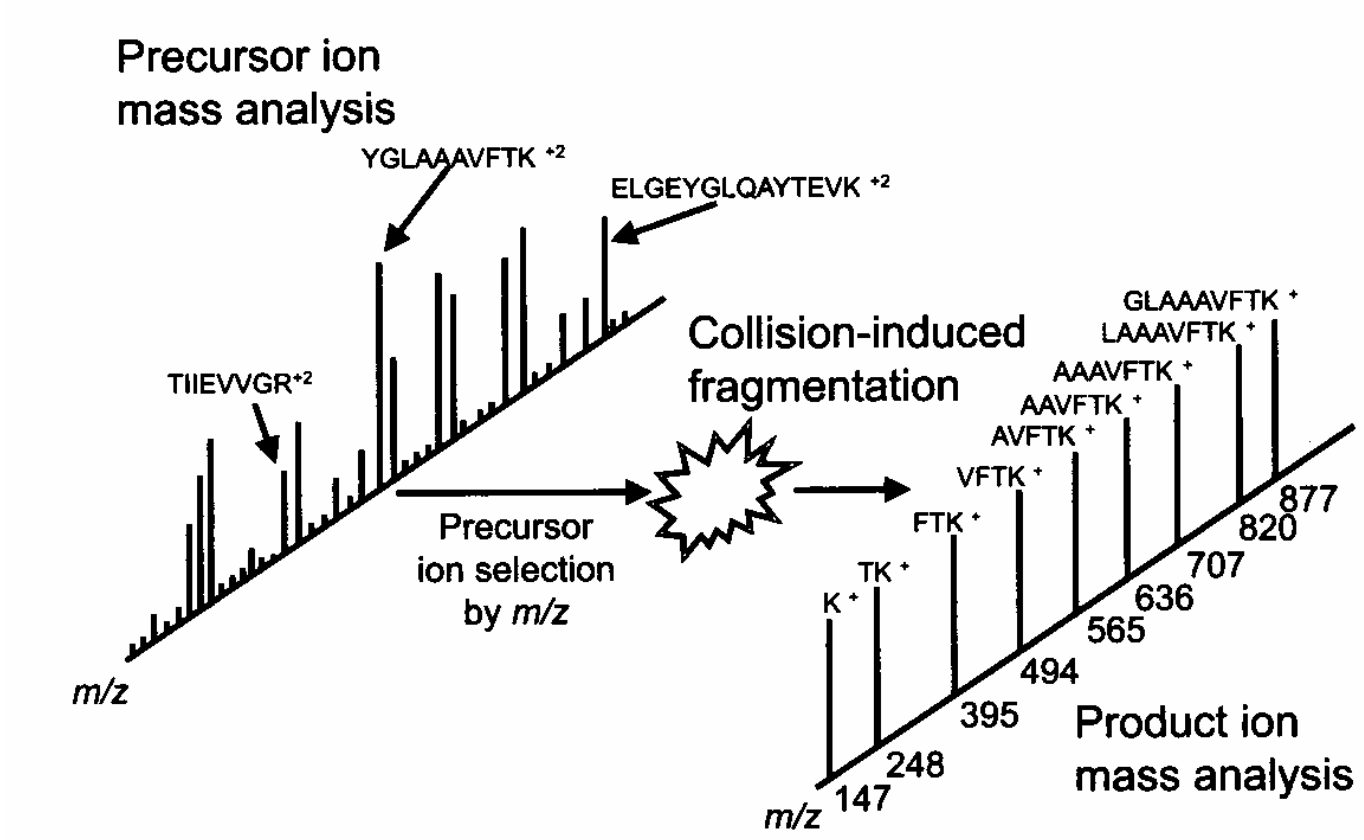
1. Impart kinetic energy to an ion by collision with an inert gas.
2. Kinetic energy is converted to internal energy in the ion.
3. Fragmentation of the unstable ion.



Tandem MS

1. Tandem in Space- >1 mass analyzer

2. Tandem in Time- a. 1 mass analyzer only
b. sequentially trap ions



Tandem in Space

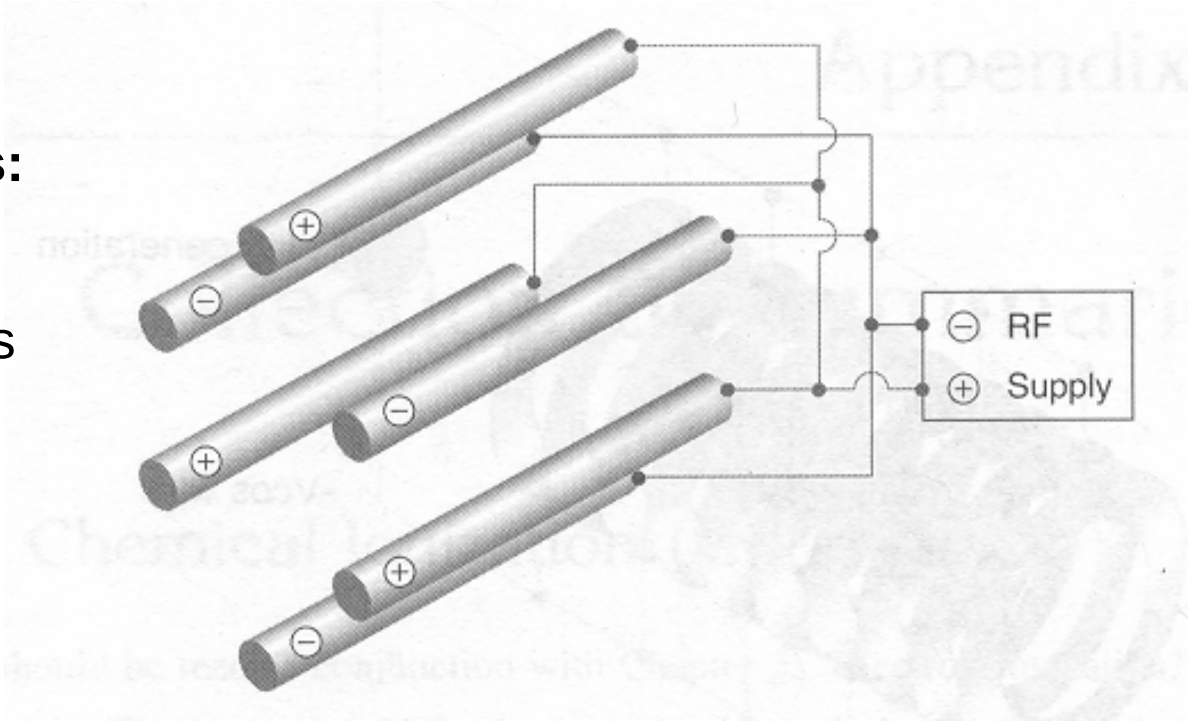
Mass Analyzer 1 - Collision Cell - Mass Analyzer 2

Ex: Quadrupole - Collision Cell - Quadrupole
Quadrupole - Collision Cell - Time of Flight

Collision Cells: RF-only quadrupoles, hexapoles, or octapoles

Collision Cell Functions:

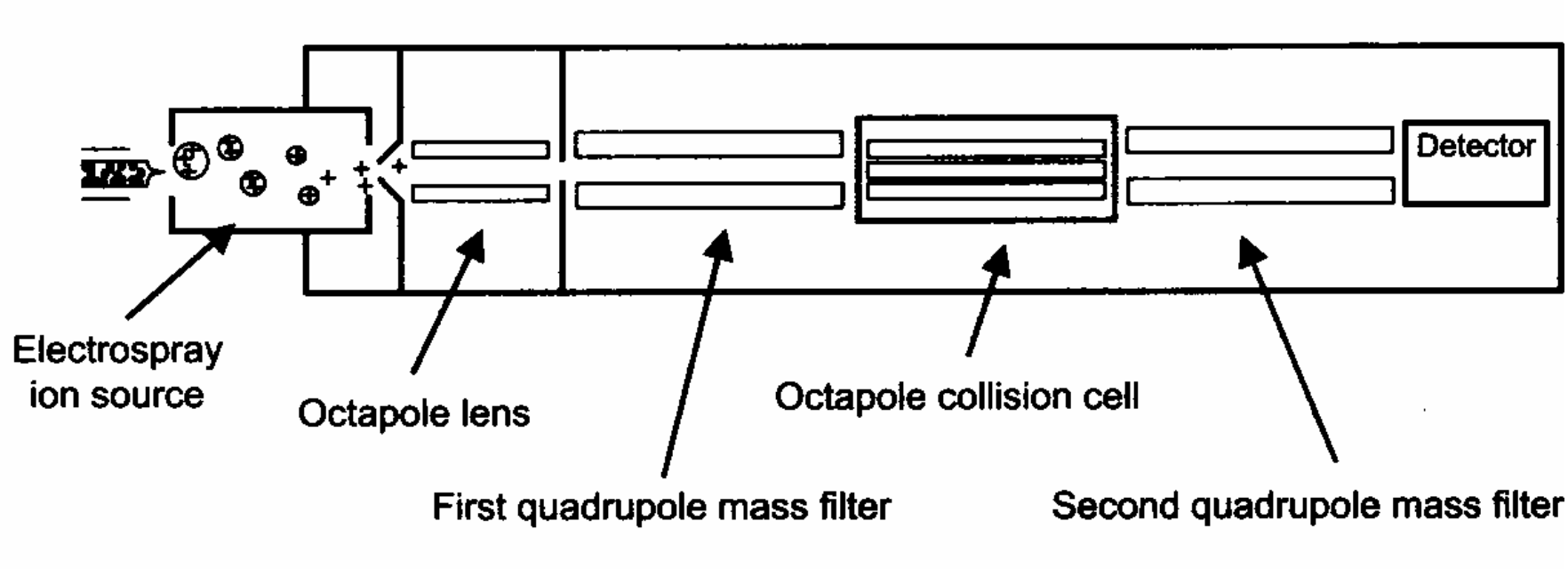
1. Fragment selected ion
2. Contain all product ions
   i.e. all m/z
3. Transmit product ions
   to 2nd mass analyzer



Quadrupole-Quadrupole

RF-Only Octapoles
Ion Focusing
Collision Cell

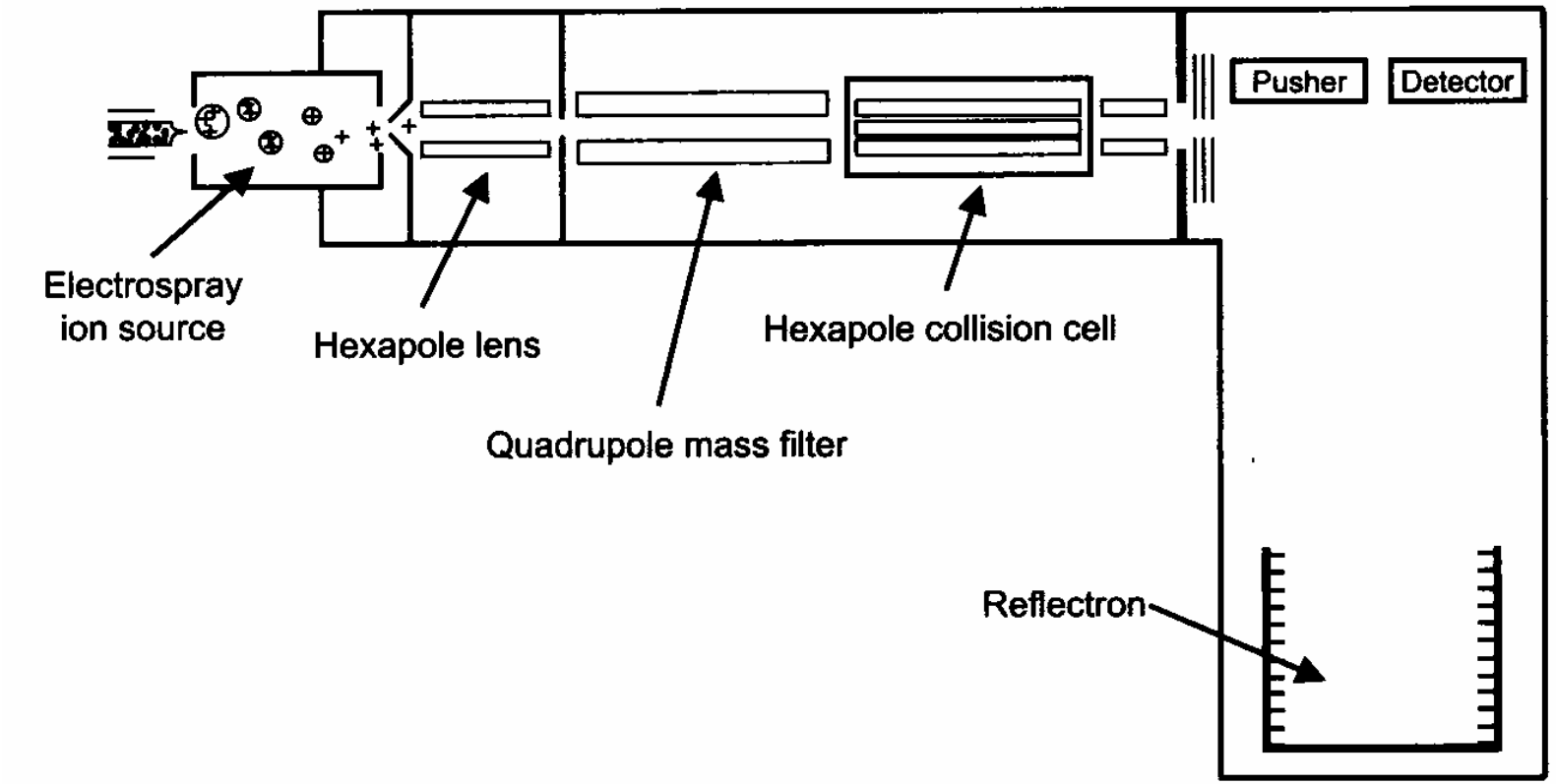
Quadrupoles
Mass Analyzers



Quadrupole-TOF

RF-Only Hexapoles
Ion Focusing
Collision Cell

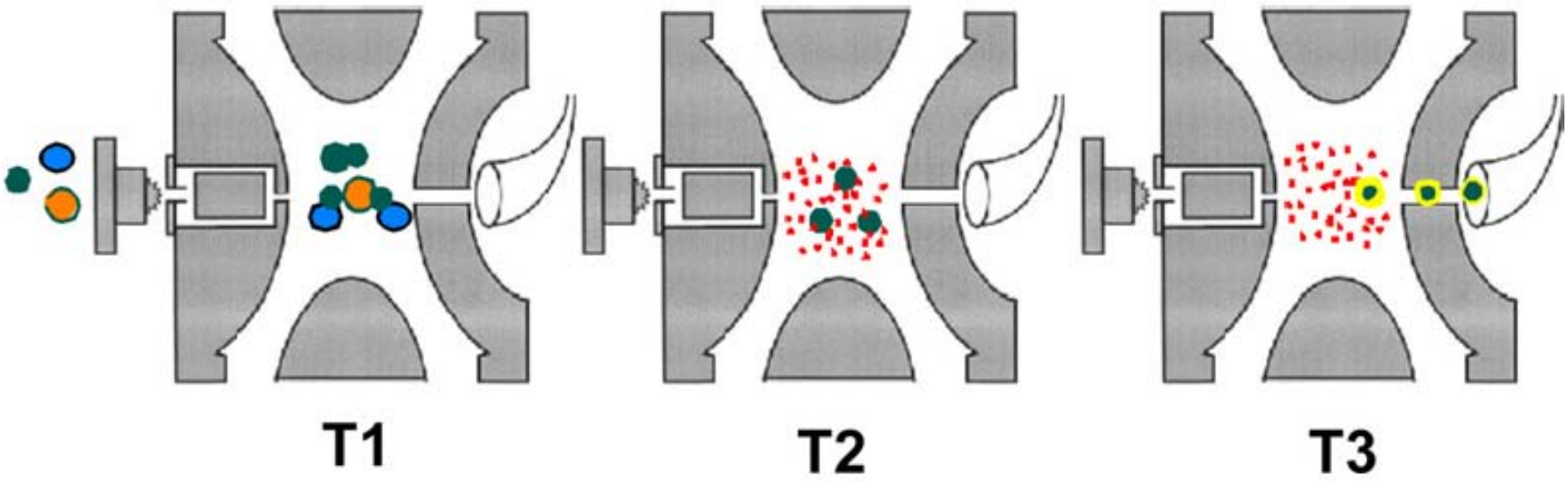
Mass Analyzers
Quadrupole
TOF



Tandem in Time

Single Ion Trap

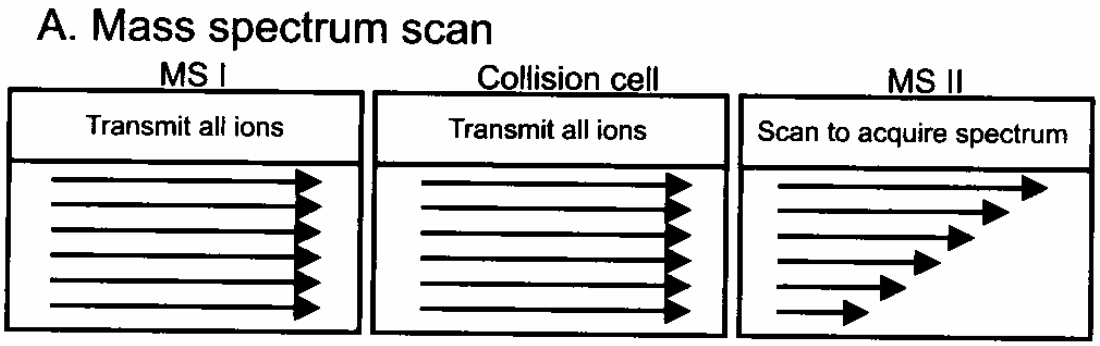
1. Trap all m/z ions.
2. RF scan to eject all m/z except the targeted m/z.
3. Apply RF pulse to accelerate trapped ions
   and fragment ions via gas collisions.
4. Perform m/z scan of product ions.



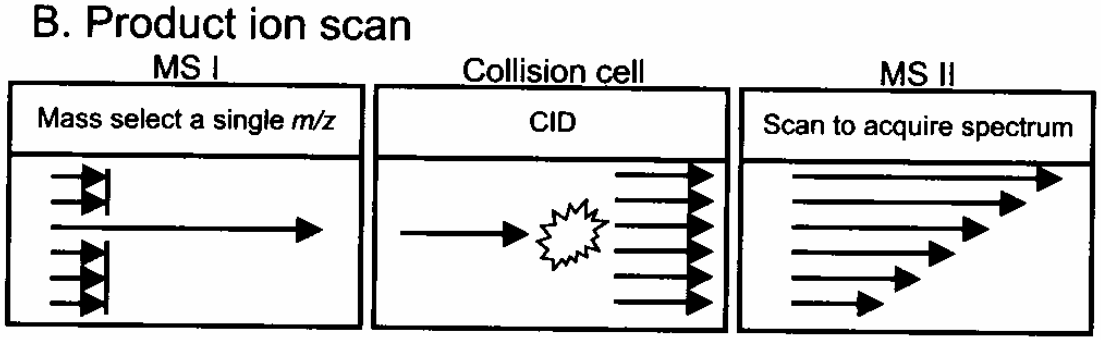
Wysocki et al, Methods, 2005, 35:211.

MS-MS Scan Modes

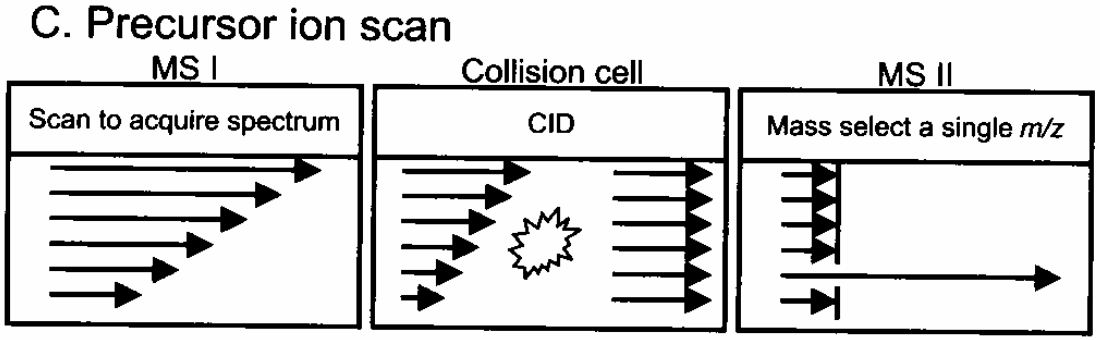
Measure m/z:
No Collisions



Product Ion Scan:
Peptide Sequencing



Precursor Ion Scan:
Phosphorylated Peptides
(PO_3^- m/z = 79)



Protein Sequencing

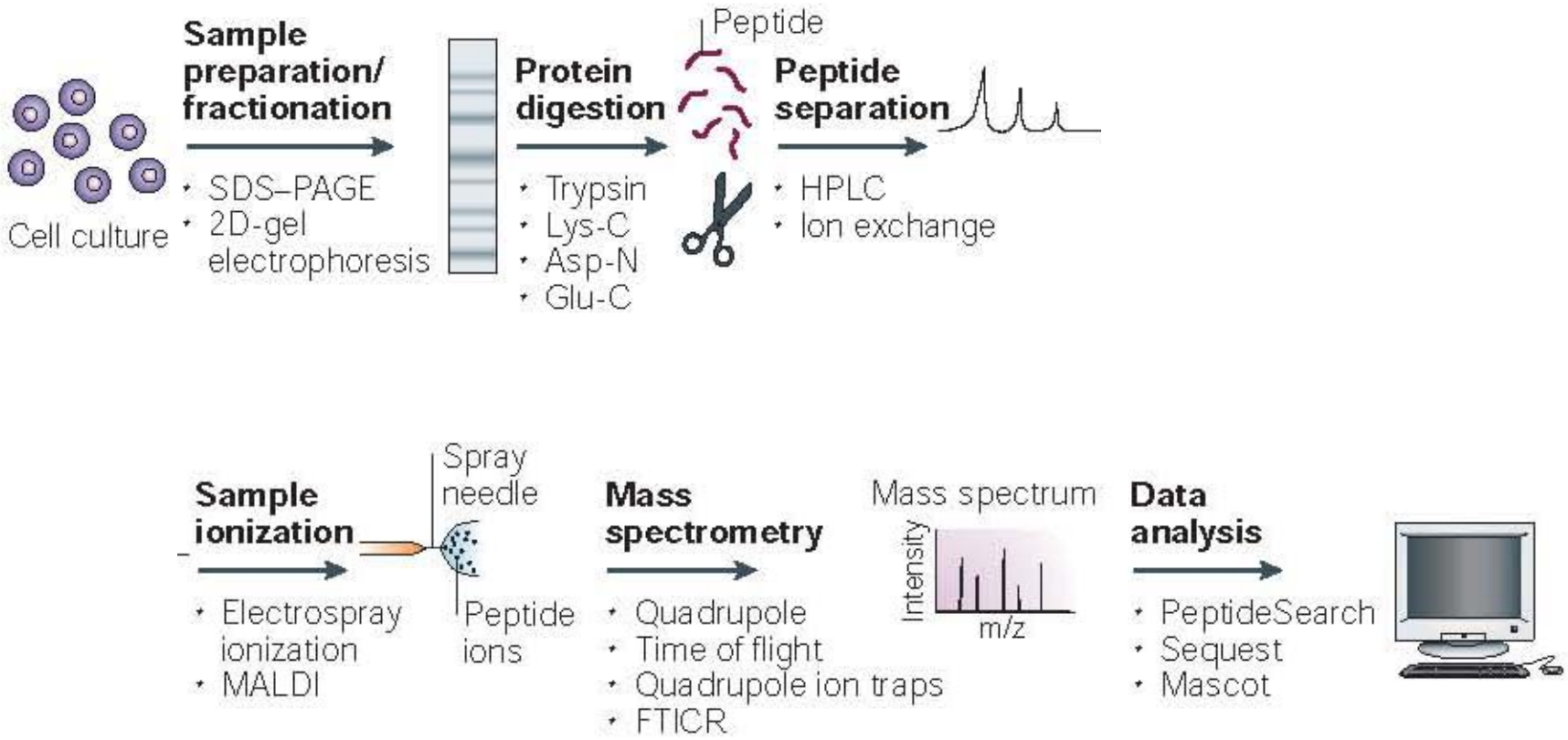
"Bottom-Up" Sequencing- (most common)
a. Cleave protein into peptides.
b. Send peptides into MS for sequencing

"Top-Down" Sequencing- (difficult but fast)
a. Send intact protein into mass spec.
b. Fragment & sequence

Why peptides instead of proteins?

1. Increased stability
2. Better solubility
3. Greater sensitivity
4. Easier to sequence if $\leq$ 20 amino acids
5. Fewer (usually $\leq$1) translational modifications/peptide
5. Cheaper instrumentation
   (proteins require an FTICR for sequencing)

Protein Sequencing By MS



Steen & Mann Nat, Rev, Molec. Cell Bio. 2004, 5:699-711.

Protein Cleavage

Proteases- Must be sequence specific & stable
Ex: Trypsin, Lys-C, Asp-N, Glu-c

Trypsin- Cleaves peptides on the C-terminal side of Arg & Lys

1. Converts proteins to peptides of $\leq$20 amino acids
2. Yields peptides with a C-terminal basic residue
3. With ESI/MS, yields doubly charged peptides
   amino terminus + basic residue

Measured $m/z = (M + 2H^+) / 2^+$

Ex: peptide mass = 1232.55
$m/z = (1232.55 + (2 \times 1.0073)) / 2$
$= 617.28$

Proteolyzed Proteins Need Separation

Cleaved proteins yield a complex peptide mixture
& must be separated prior to MS.

Separation Characteristics:

1. Typically reverse phase (hydrophobicity)
   May need multi-dimensional separation.
2. Remove contaminants *i.e.* detergents, salts
3. Reduce complexity but overlapping peaks OK
4. Couple directly to ESI/MS
   a. Elute in smallest possible volume
   b. Peak width of 10-60 s

Ex: μscale- HPLC, capillary electrophoresis, microfluidic chips

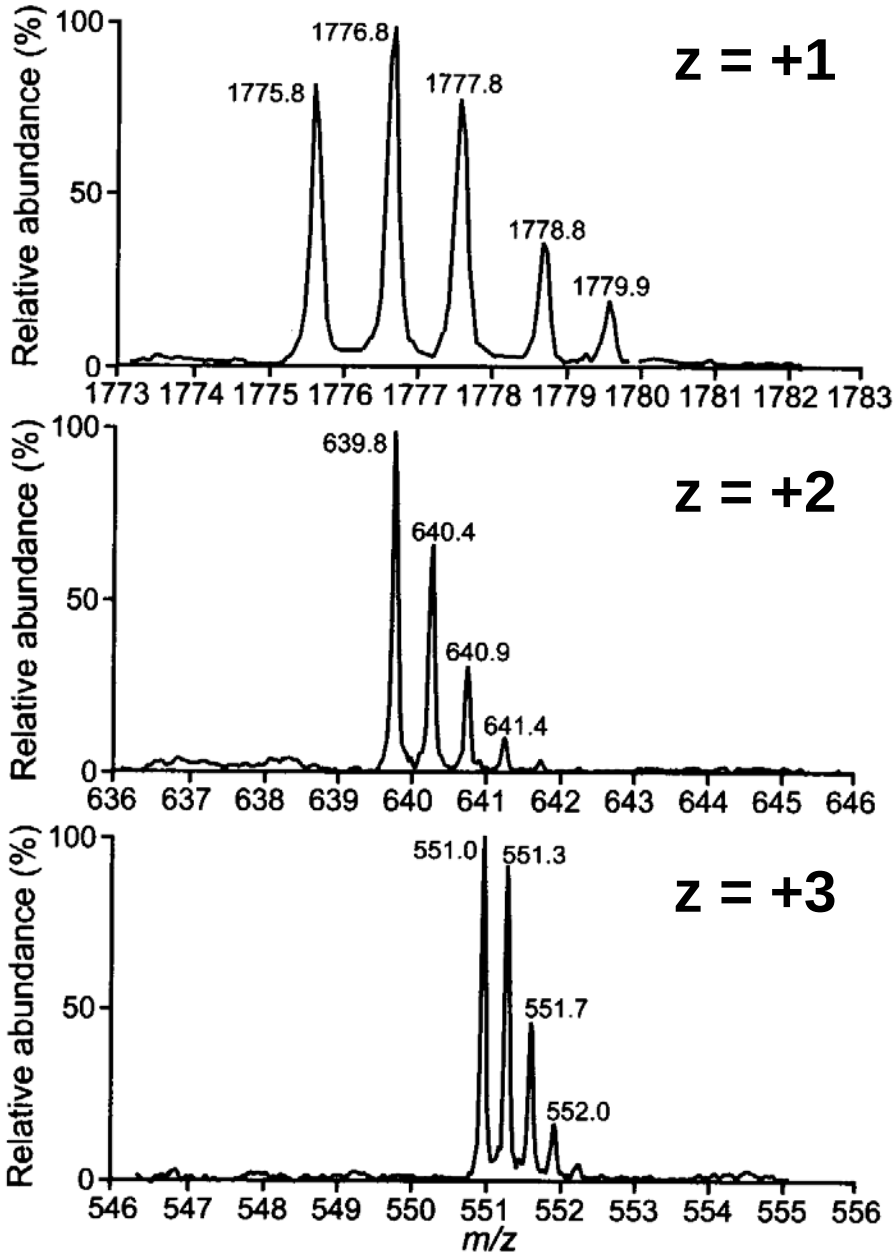
Isotope Clustering of Peptides

1% probability of carbon being ^{13}C instead of ^{12}C.

Peptide peak = Cluster of peaks separated by 1 Da.

For:

- $(M + H^+)$ Δ m/z = 1 Th
- $(M + 2H^+)$ Δ m/z = 0.5 Th
- $(M + 3H^+)$ Δ m/z = 0.33 Th

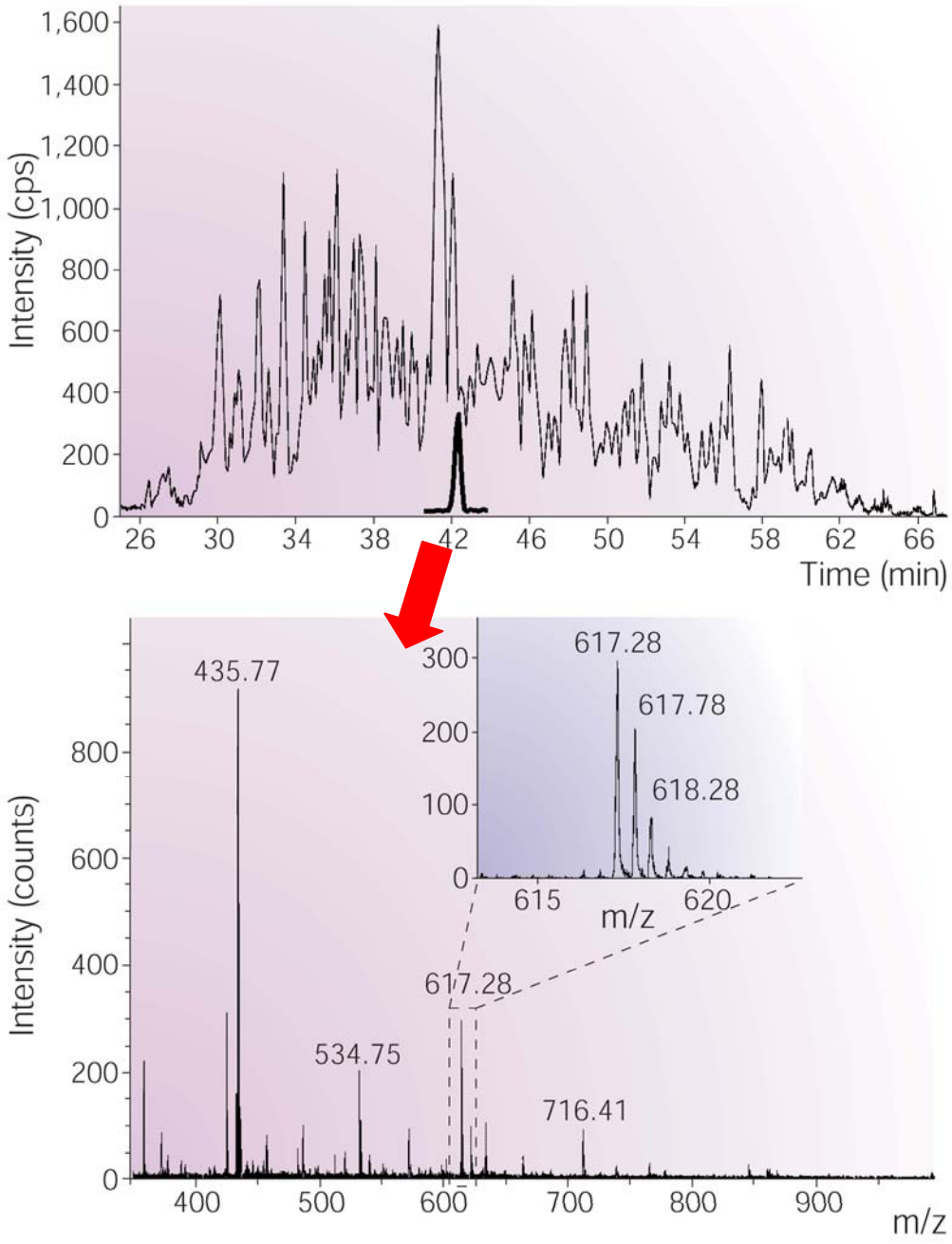


MS Traces for Separated Peptides

1. Total Ion Chromatogram
   ESI Current *vs* Time

2. MS Spectrum of Ions
   at 42.2-42.8 s

3. Isotope Cluster for Peptide
   at m/z = 617.28
   (z = +2)



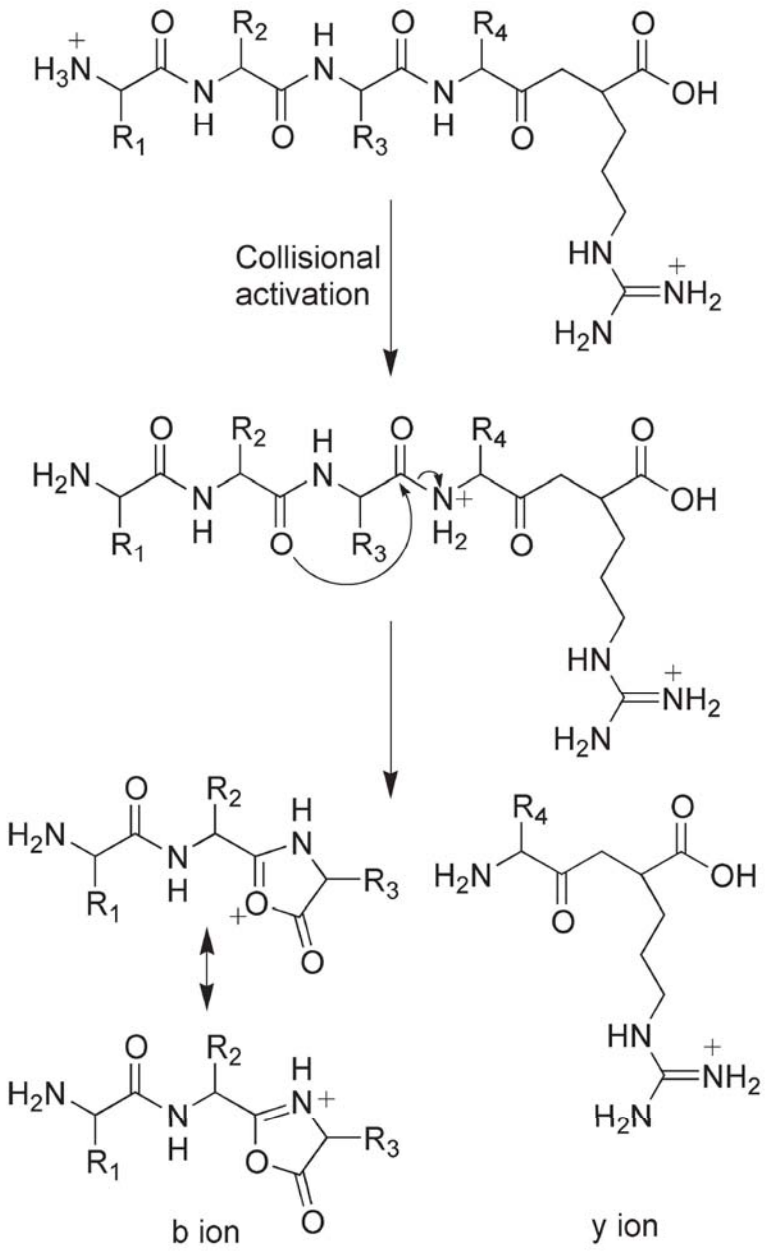
Steen & Mann Nat,
Rev, Molec. Cell Bio.
2004, 5:699-711.

Peptide Fragmentation in a Collision Cell

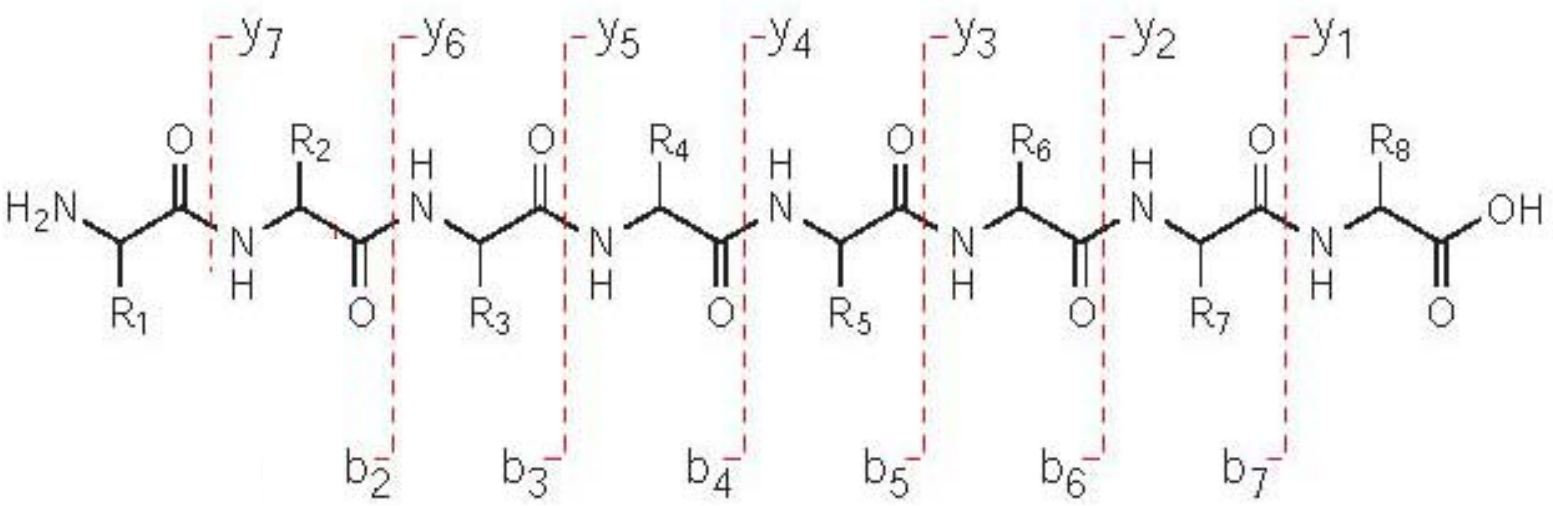
1. Due to collisions with gas.
2. Mobile proton from the amino terminus promotes cleavage.
3. Lowest E bond fragments first (amide bond).
4. At low energies, get mostly b- and y-ions:

   b-ions: amino terminal fragment if it retains H^+ (+1 charge)

   y-ions: carboxy terminal fragment (+1 or + 2 charge)



Peptide Fragmentation



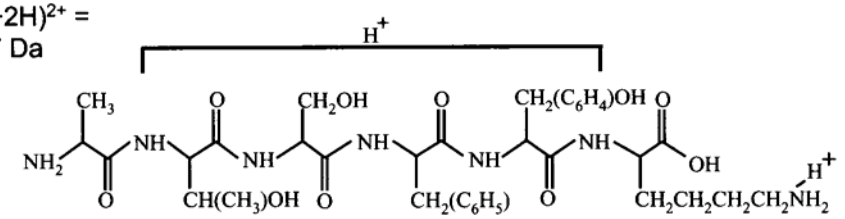
1. A series of b- and y-ions are produced due to the fragmentation of different amide bonds.
2. Subscript refers to the number of R groups on the fragment.
3. y-ions are more common and more stable than b-ions.

Steen & Mann Nat, Rev, Molec. Cell Bio. 2004, 5:699-711.

y-Ion Series

ATSFYK

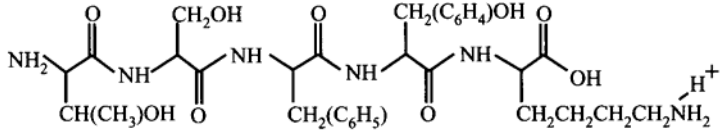
$(M+2H)^{2+}$ = 717 Da



Δ = 72 Da

TSFYK

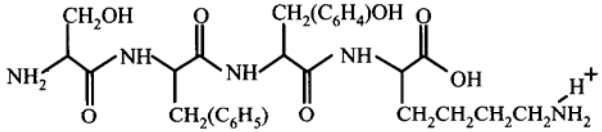
y_5 = 645 Da



Δ = 101 Da

SFYK

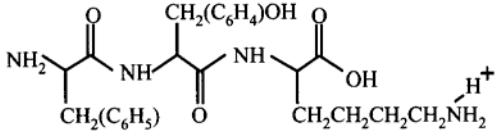
y_4 = 544 Da



Δ = 87 Da

FYK

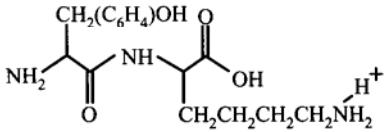
y_3 = 457 Da



Δ = 147 Da

YK

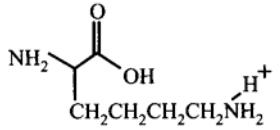
y_2 = 310 Da



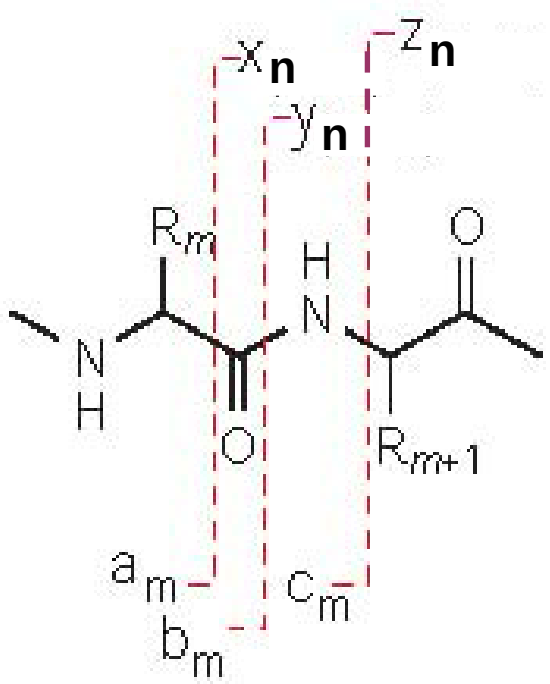
Δ = 163 Da

K

y_1 = 147 Da



Peptides Can Fragment At Other Sites



1. Amino Terminal Fragments:
   a_m, b_m, c_m

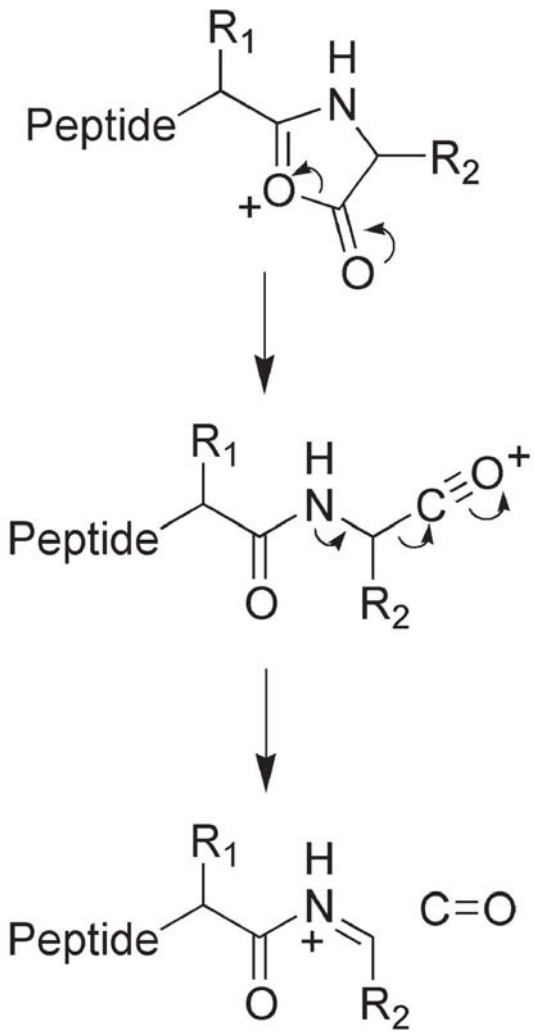
2. Carboxy Terminal Fragments:
   x_n, y_n, z_n

3. The fragments can also fragment espec if have a mobile H^+

4. Various side chain reactions

Steen & Mann Nat, Rev, Molec. Cell Bio. 2004, 5:699-711.

b-Ions Can Fragment to a-Ions



Sequencing From A y-Ion Series

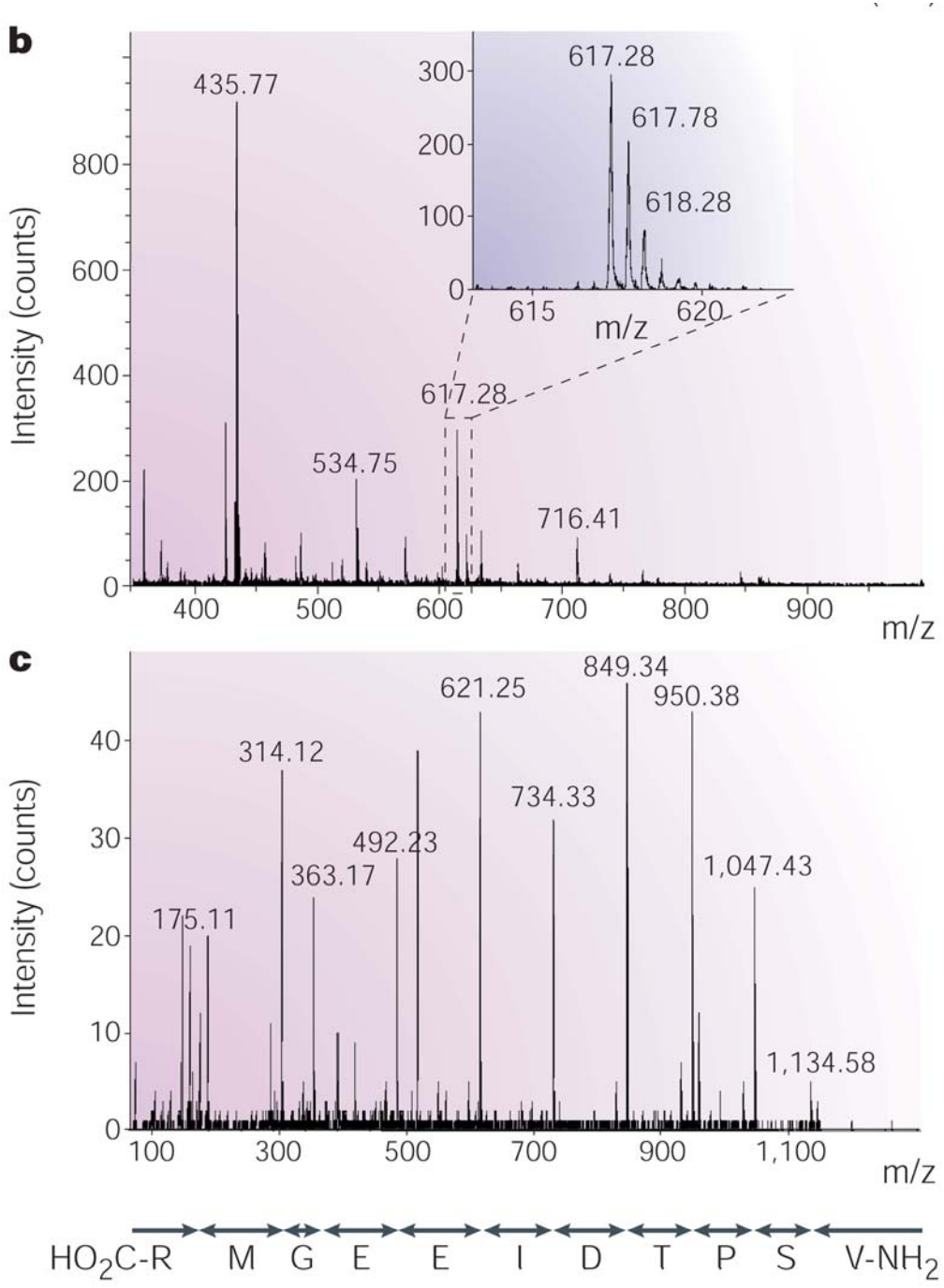
1st MS Analysis

↓

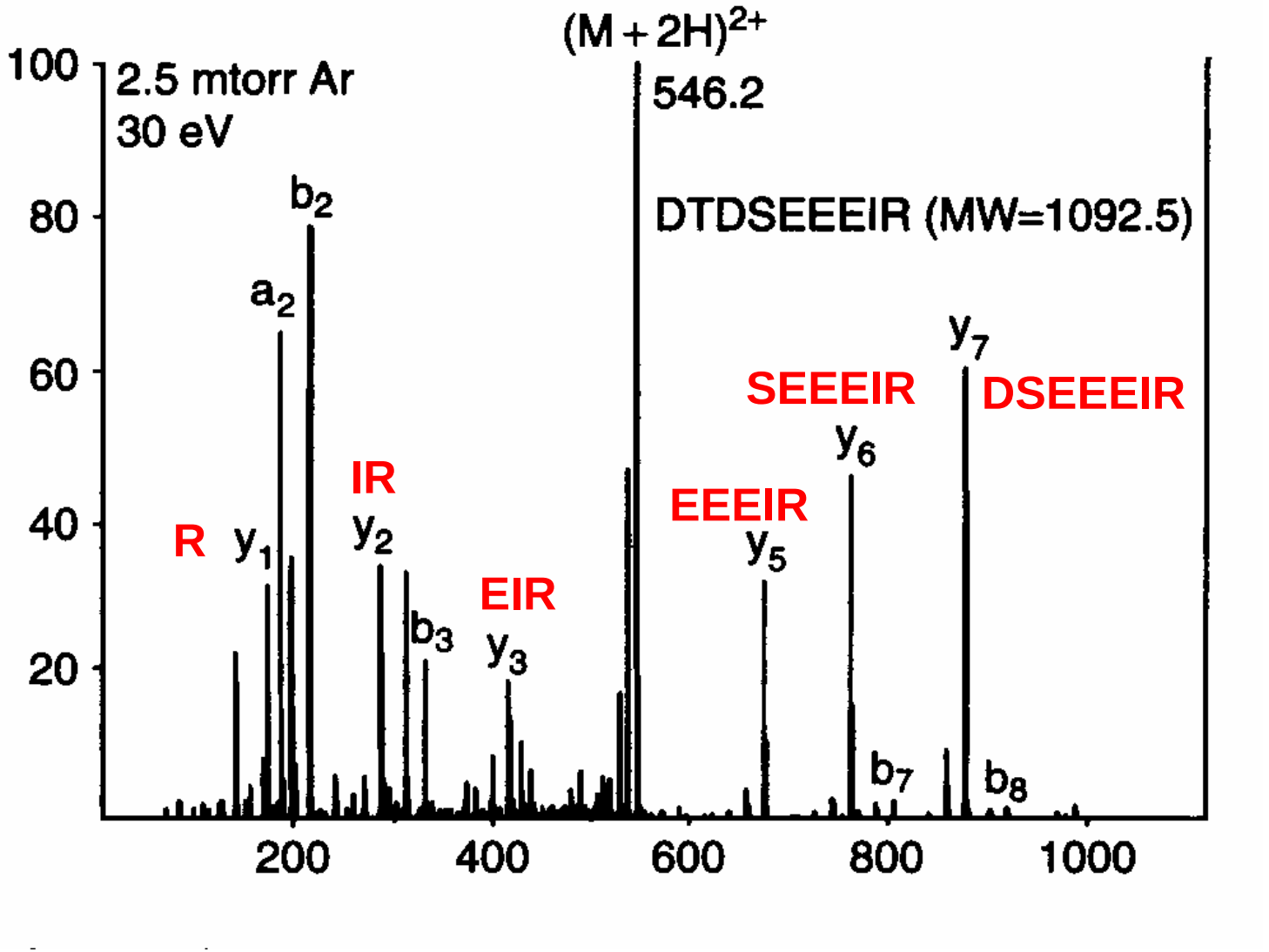
Select Ions at 617.28 & Send to Collision Cell

↓

2nd MS Analysis of the Fragments (mostly y-ions)



Sequencing From A y-Ion Series



MS/MS Spectra Can Be Complex

1. Many types of fragments.
   (Some expected ones will be absent.)

2. Amino acid isomers- Leucine & Isoleucine, m = 113.08

3. Amino acid isobars- Glutamine (m = 128.06)
   Lysine (m = 128.09)

4. **Table 4.3. Amino acids combinations that are equal to a single amino acid residue mass.***

Amino acid combination	Residue mass (Da)	Equivalent amino acid
GG	114	N
GA	128	Q, K
GV	156	R
GE	186	W
AD	186	W
SV	186	W
SS	174	C[a]

Convert Peptide Sequencing Problem To A Database Searching Problem

Only a very small fraction of the possible amino acid sequences actually occur in nature!

1. Peptide fragment spectrum may be insufficient to sequence de novo.
2. But it might be enough to match it to a database of fragments of known proteins.
3. Expected proteins/fragments are derived from the sequenced genomes.

MALDI Fingerprinting

1. Purify protein.

2. Digest with trypsin.

3. Perform MALDI-MS (NOT tandem MS).

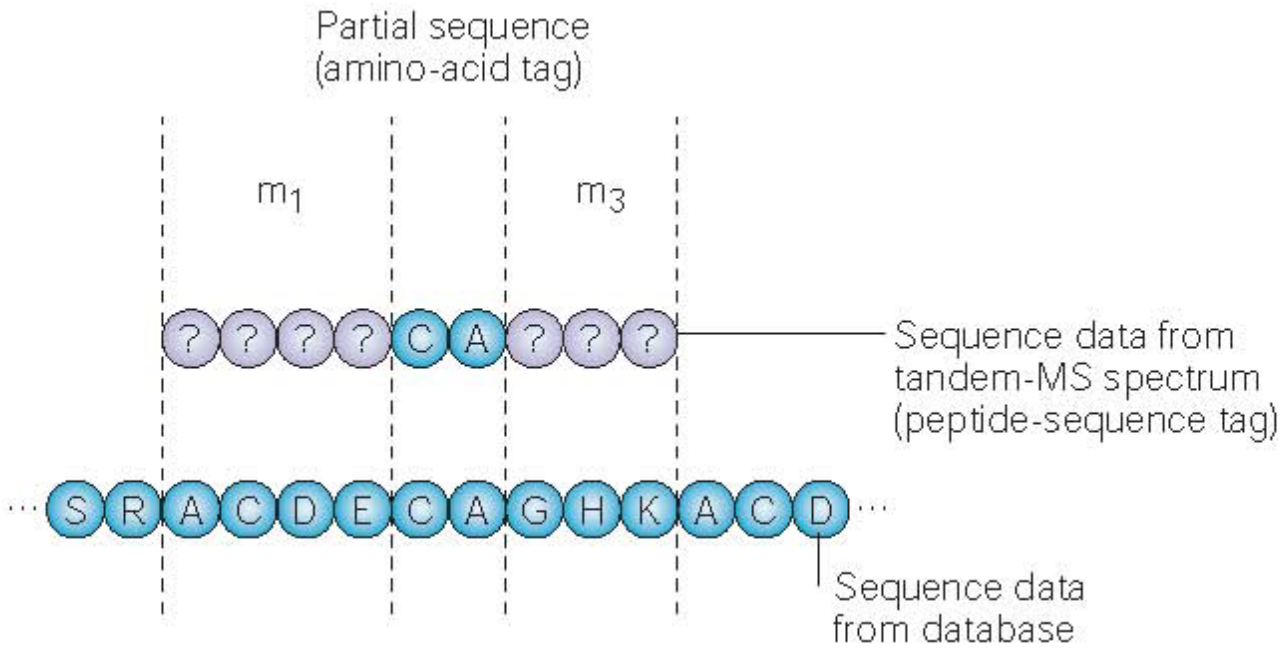
4. Obtain a signature for that protein composed of the peptide masses.

5. Compare peptide masses to a database of expected peptide masses from each known protein for that species.

6. Frequently this identifies the protein and its amino acid sequence unambiguously.

Database Searching- Peptide Sequence Tags

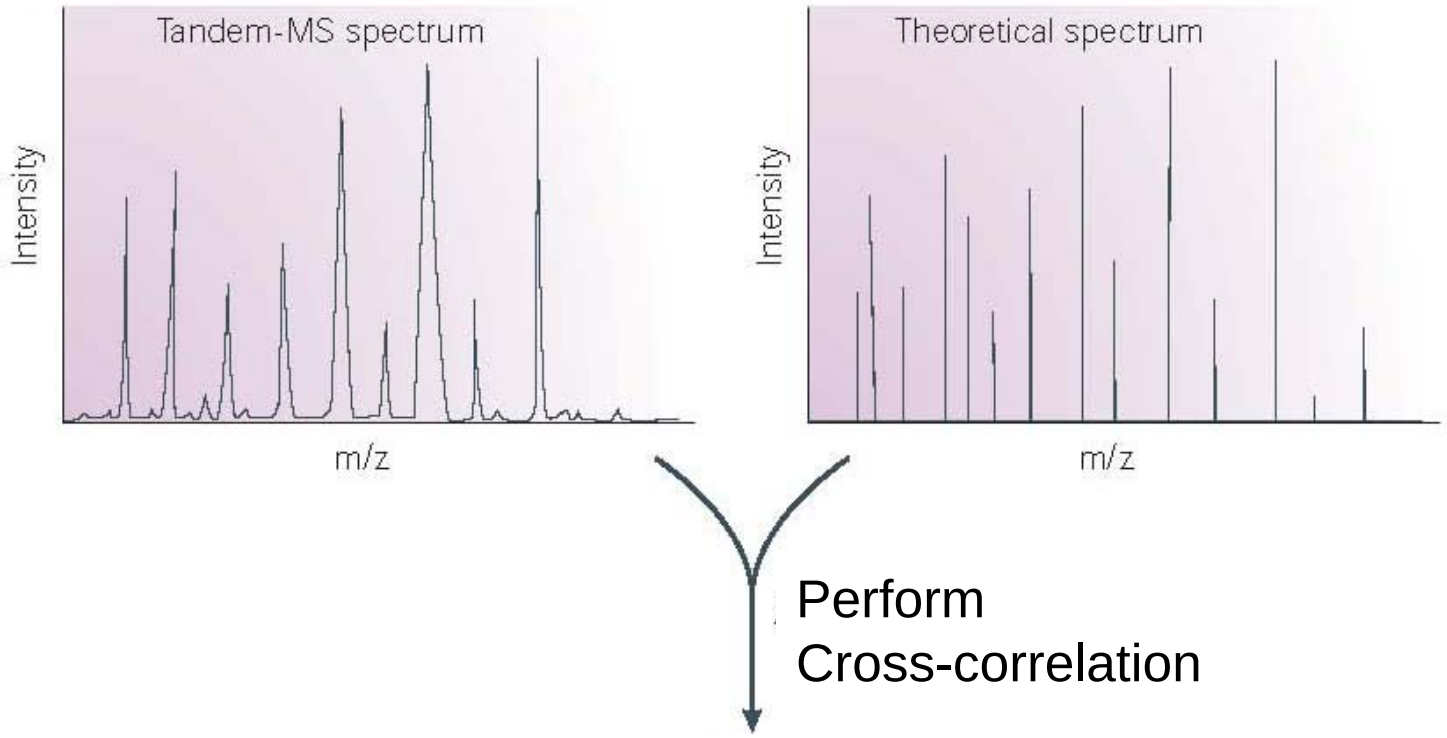
1. Identifies small portions of easily interpreted sequences
   i.e. "amino acid tags"
2. Also identify distance in mass to each peptide terminus.
3. Compare to database.



Mann & Wilm Anal. Chem. 1994, 66:4390-9.

Database Searching- Sequest Algorithm

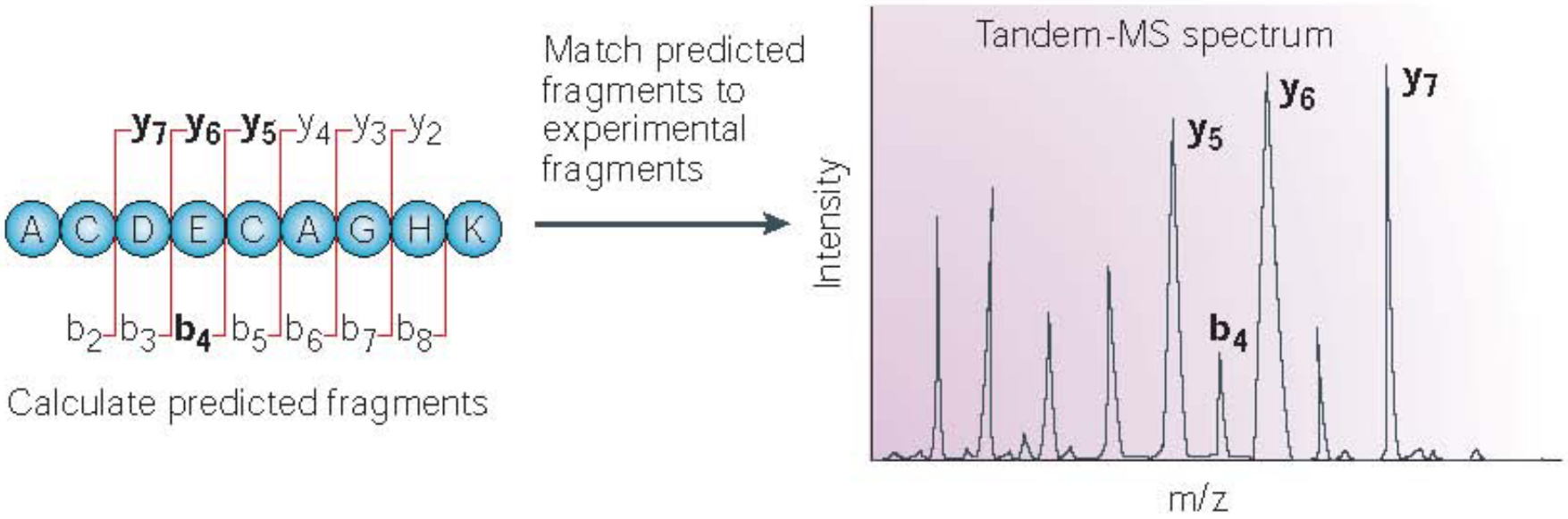
Compare experimental spectra to theoretical spectra
of each protein in a database.



Eng, McCormack & Yates. J. Am. Soc. Mass Spec. 1994, 5:976-989.

Database Searching- Mascot Search

1. Also compares experimental spectra to theoretical spectra of each protein in a database.
2. Most intense fragments of b- & y-ions are matched first.
3. Probability that the fragment matches could all be random is calculated & reported.



Perkins et al, Electrophoresis. 1999, 20:3551-67.

Making MS Quantitative

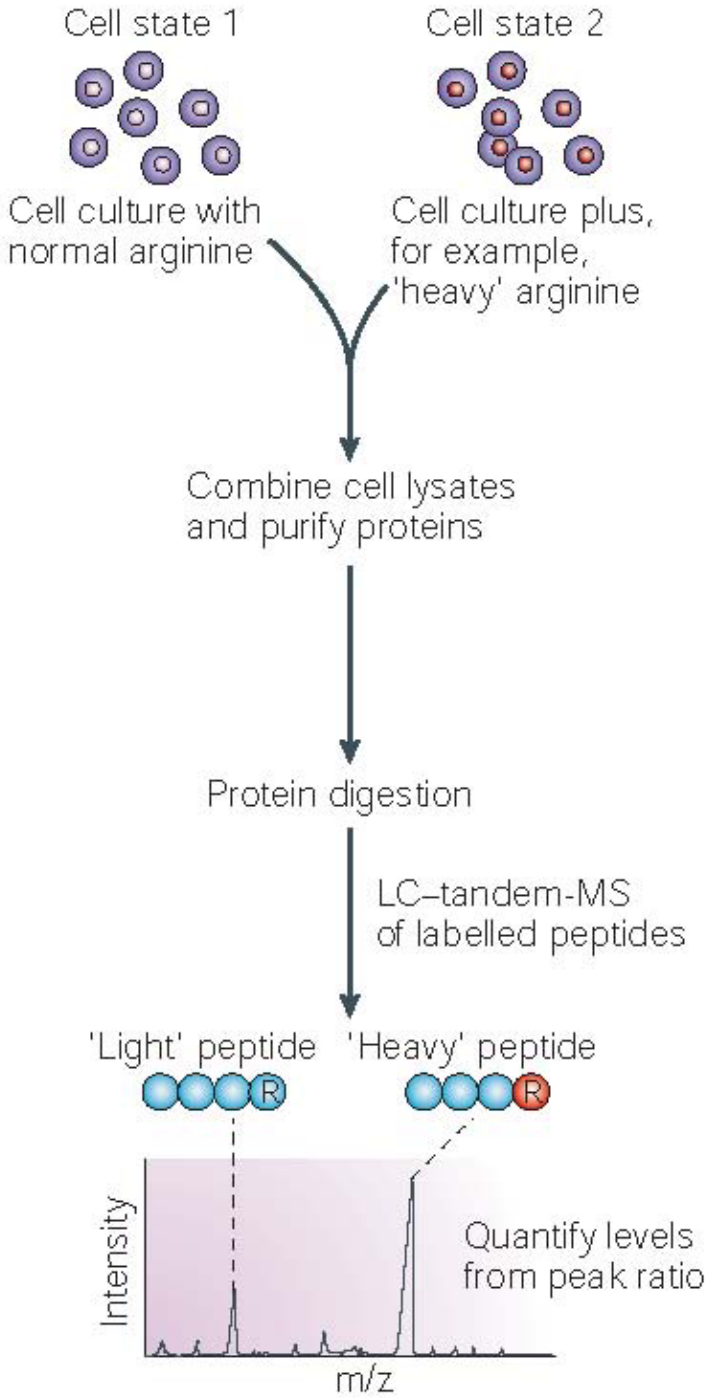
Signal Intensity Does Not Correlate With Amount!

1. Absolute Quantitation-
   Isotopically labeled internal standards

2. Relative Quantitation-
   Use stable isotopes
   Replace ^{1}H with ^{2}H
   ^{12}C with ^{13}C
   ^{14}N with ^{15}N
   ^{16}O with ^{18}O

Relative Quantitation-SILAC

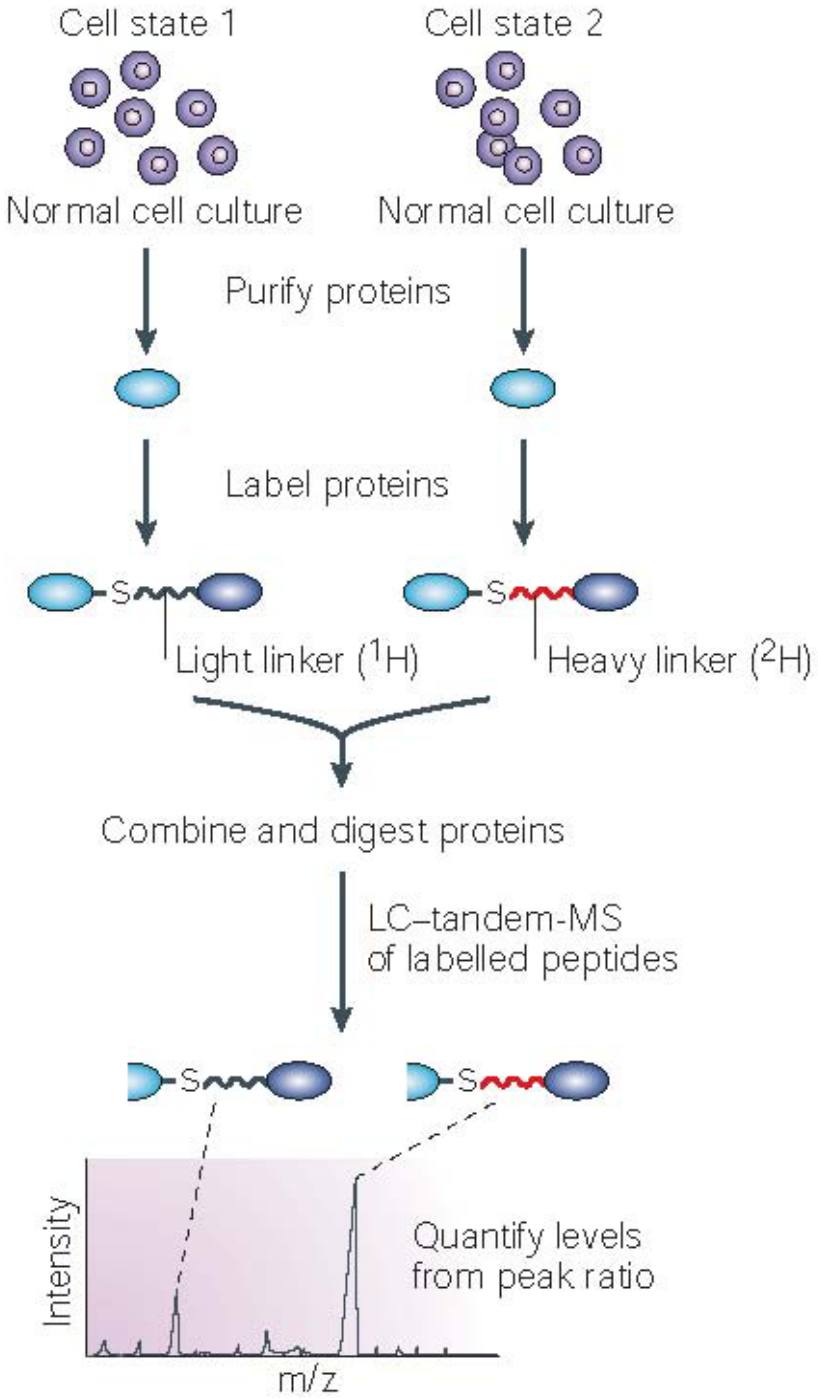
SILAC = Stable Isotope Labeling in Cell Culture



Ong et al. Mol. Cell Proteomics 2002, 1:376-386.
Ong et al. J. Proteome Res. 2003, 2:173-181.

Relative Quantitation-ICAT

ICAT = Isotope-Coded Affinity Tag



Gygi et al. Nat. Biotech. 1999, 17:994-999.

References

1. Standing KG. 2003. Peptide and protein de novo sequencing by mass spectrometry. Current Opinion in Structural Biology. 13:595-601.
3. Lane CS. 2005. Mass spectrometry-based proteomics in the life sciences. Cellular and Molecular Life Sciences. 62:848-869.
4. 7. Steen H, Mann M. 2004. The ABC's (and XYZ's) of peptide sequencing. Nature Reviews: Molecular Cell Biology. 5:699-711.
5. Herbert CG, Johnstone RAW. Mass Spectrometry Basics. 2003. CRC Press, Boca Raton.
6. de Hoffman, E, Stroobant, V. Mass Spectrometry Principles and Application. 2001. 2nd Ed., John Wiley & Sons, New York.
7. Kinter, M., Sherman, N.E. Protein Sequencing and Identification Using Tandem Mass Spectrometry. Wiley, New York, 2000.
8. Yates, JR. 2004. Mass Spectral Analysis in Proteomics. Annu. Rev. Biophys. Biomol. Struct. 33:297-316.
9. Patterson, SD, Aebersold, RH 2003. Proteomics: the first decade and beyond. Nat. Genetics. 33:311-323.
10. Ong, SE, Mann M. 2005. Mass spectrometry-based proteomics turns quantitative. Nat. Chem. Bio. 5:252-262.
11. Kelleher, NL, 2004 Top-down proteomics. Anal. Chem. June 1, 197A-203A.