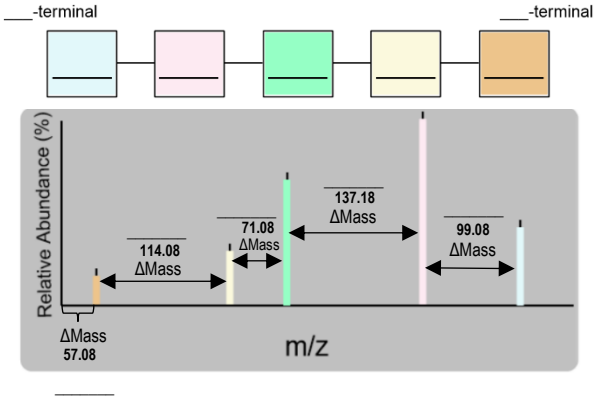
CONCEPT: MASS SPECTRUM

●Mass _____: plots mass spec data (relative _____ on y-axis & _____ ratio on x-axis).

□ Most peaks represent fragments resulting from cleavage of original peptide at only _____ peptide bond.

□ Can reveal _____ protein structure: amino acids identified by m/z _____ between peaks.

EXAMPLE: Use the mass spectrum to reveal the amino acid composition.



Amino Acid	MW of Residue (g/mol)
Alanine	71.08
Asparagine	137.18
Glycine	57.08
Histidine	114.08
Valine	99.08

_____

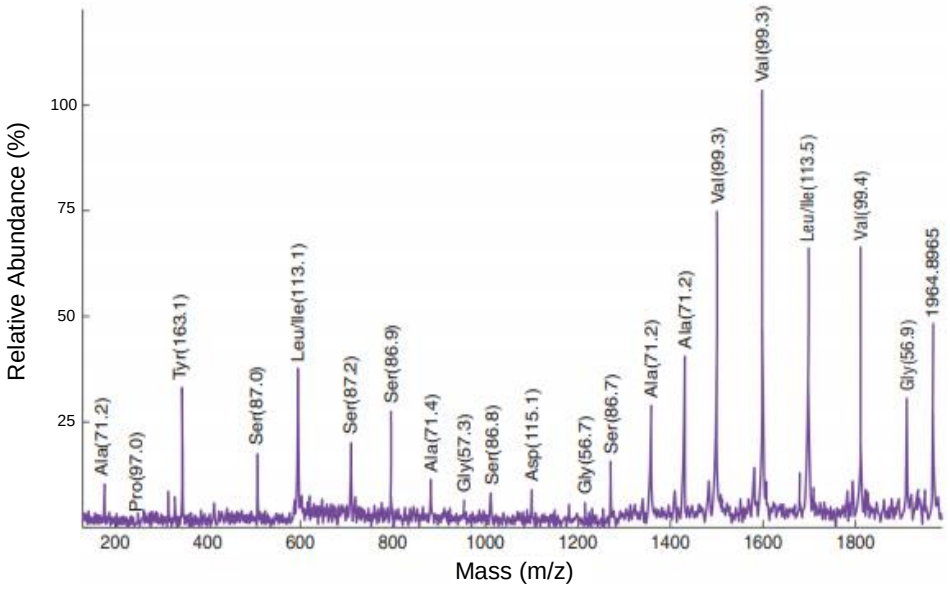
●Mass spectra are typically analyzed from right to _____ to reveal the peptide sequence.

□ MS struggles to differentiate Leu from _____.

PRACTICE: Use the mass spectrum below to determine the sequence of the peptide.

N-terminal C-terminal

_____-_____



CONCEPT: MASS SPECTRUM

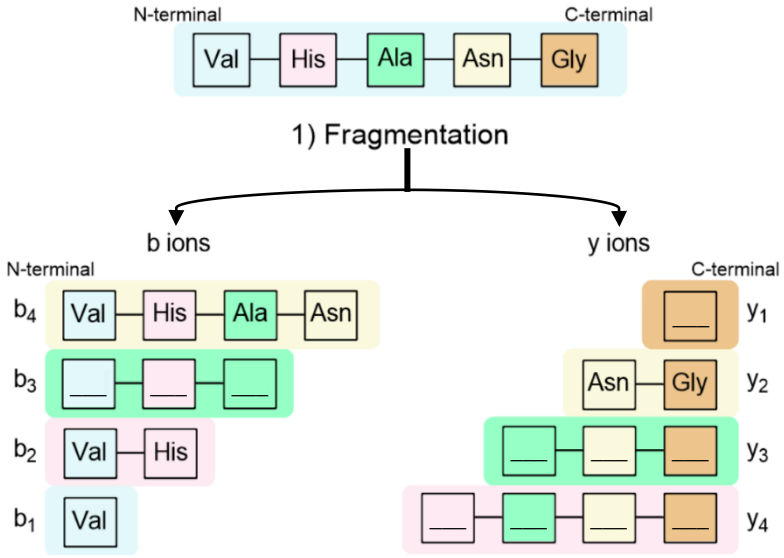
B & Y Ions Contribute to MS Spectra

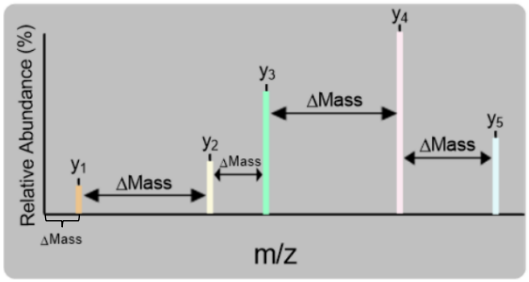
●MS ionization fragments most protein molecules only *once* at a _____ bond; leads to _____ prominent sets of ions.

1) ____ ions (always contain the ____-terminal amino acid residue) & peaks read from left to right.

2) ____ ions (always contain the ____-terminal amino acid residue) & peaks read from right to left.

EXAMPLE:





*In most cases, its safe to assume mass spectrum is analyzed with y ions & read from right to _____.

●Intermixed b & y ions can show up on a mass spectrum, but y ions are more _____ than b ions.

□ y ion intensity/abundance will often be the most prominent peaks in the spectrum.

PRACTICE: Upon fragmentation of a peptide bond during mass spectrometry, what ions can be detected on the spectrum?

a) b ions.

b) y ions.

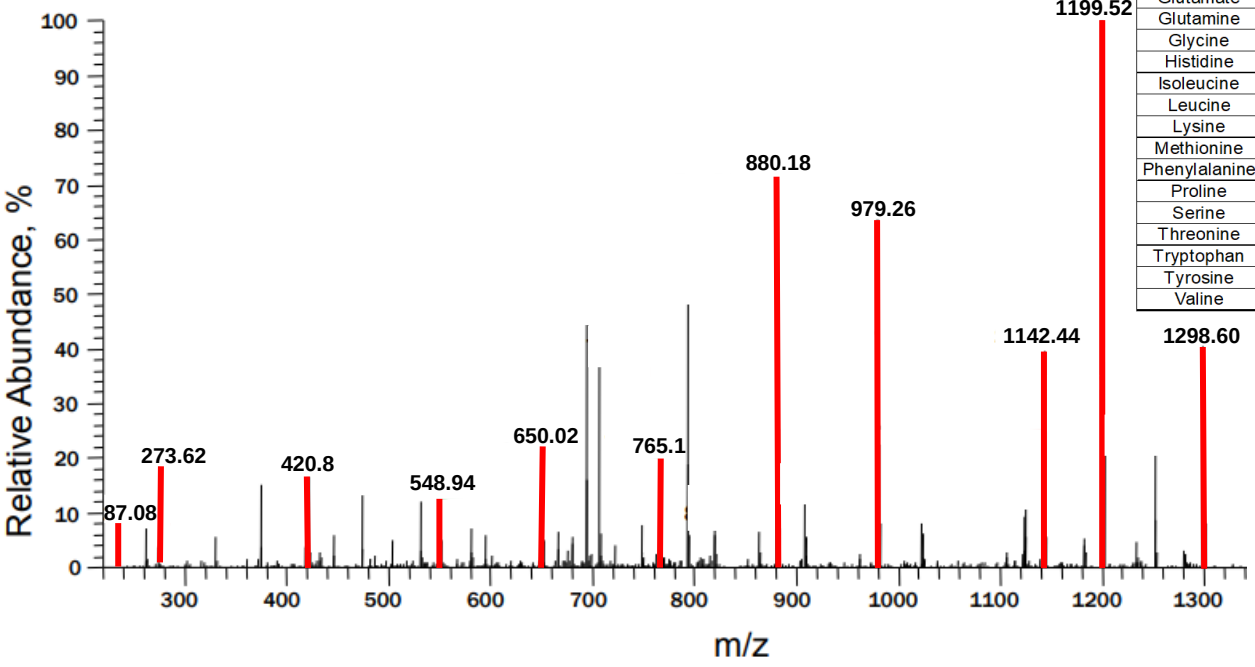
c) b & y ions.

d) Ions are deflected but not detected.

CONCEPT: MASS SPECTRUM

PRACTICE: Use the mass spectrum below & the indicated y-ion peaks (red) to reveal the sequence of the peptide.

Peptide sequence: ____-____-____-____-____-____-____-____-____-____-____-____



Amino Acid	Mass of Residue (g/mole)
Alanine	71.08
Arginine	156.18
Asparagine	114.08
Aspartate	115.08
Cysteine	103.18
Glutamate	129.08
Glutamine	128.14
Glycine	57.08
Histidine	137.18
Isoleucine	113.18
Leucine	113.18
Lysine	128.18
Methionine	131.18
Phenylalanine	147.18
Proline	97.08
Serine	87.08
Threonine	101.08
Tryptophan	186.54
Tyrosine	163.18
Valine	99.08

PRACTICE: In your mass-spectrometry of a pure protein with an m/z of 1,582, you found peaks of y ions with the following m/z ratios of 1,582, 1396 and 1283. The mass in Daltons for the possible relevant amino acids are provided: Y (163), N (114), W (186), D (115), G (57), L (113) and M (131). From this data, it is obvious that the C-terminal amino acid residue of the 1,582 fragment is:

a) G.

b) L.

c) N.

d) W.

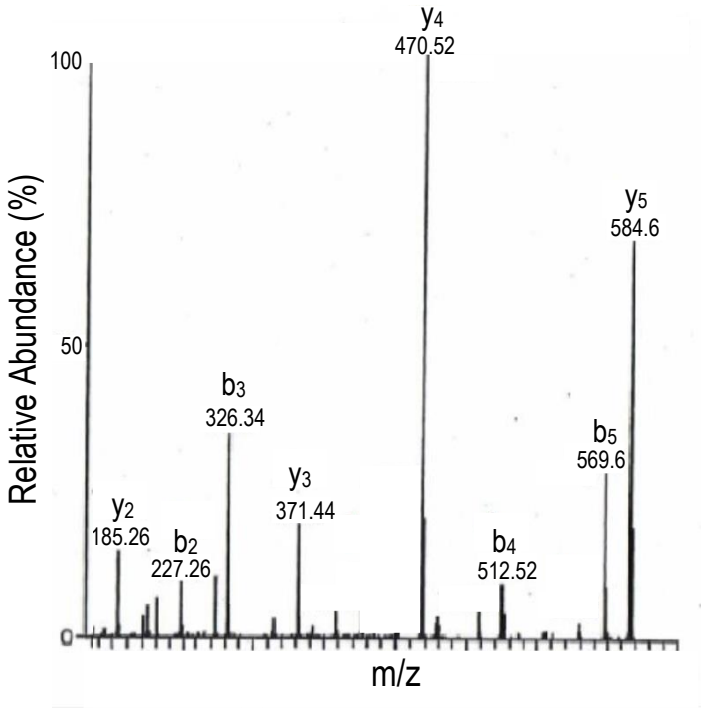
e) Can't be determined.

CONCEPT: MASS SPECTRUM

PRACTICE: Use the mass spectrum below & the provided chart with amino acid masses to determine the sequence of a hexapeptide (6 amino acid residues). In the mass spectrum, y ion peaks are indicated with "y" while b ion peaks are indicated with "b." The N-terminal residue is given as Leu and the C-terminal residue is given as Lys. Determine the remaining amino acid sequence using either the y ions or the b ions.

N-terminus C-terminus

Peptide sequence: L-_____-_____-_____-_____-_____-K



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