CONCEPT: STRATEGY FOR ORDERING CLEAVED FRAGMENTS

●There are multiple strategies to order cleaved fragments, but here's a solid strategy involving just ____ steps!

Step #1: Scan problem for helpful _________ that reveal composition/sequence info (ex. use of FDNB, hydrazine, etc.).

Step #2: Recall which specific peptide bonds the reagent ___________.

EXAMPLE: A peptide with 17 amino acids is treated with trypsin to give three fragments and separately treated with chymotrypsin to give four fragments. Identify the sequence of the 17 amino acid residues in the original/starting peptide.

Step #1: Scan the problem for helpful _________.

Step #2: Peptide bonds cleaved?
___-terminal of _____ & _____

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___-terminal of _____, _____ & _____

Trypsin Fragments	Chymotrypsin Fragments
Ala-Ser-Ala-Gly-Phe-Lys	Ile-Trp
Val-Ala-Cys	Lys-Val-Ala-Cys
Ile-Trp-Met-His-Phe-Met-Cys-Arg	Met-His-Phe
	Met-Cys-Arg-Ala-Ser-Ala-Gly-Phe

Step #3: Circle the ___-terminal fragment.

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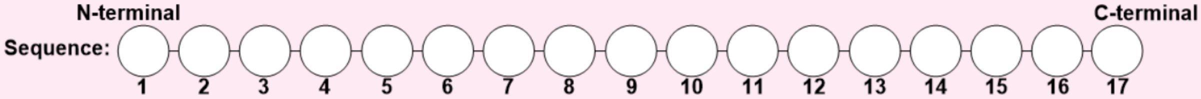
Step #4: _________ terminal fragments like a puzzle to reveal the sequence.

Step #5: If you get stuck, use the ___________ peptide fragment for overlapping.

Trypsin Fragments

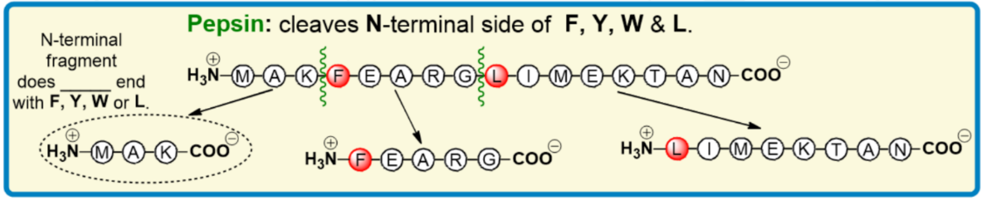
Chymotrypsin Fragments



Step #3: Identify ___-terminal or ___-terminal fragments.

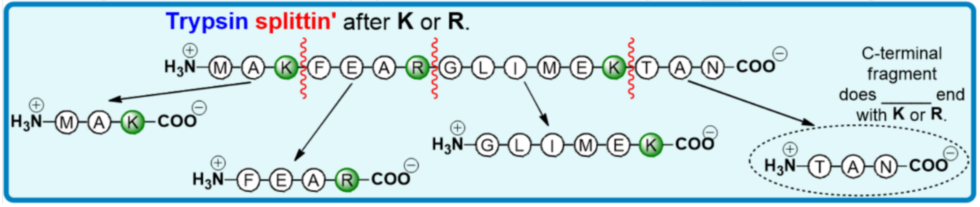
- □ These fragments usually do *NOT* have terminal amino acids that the reagent _______________ for cleavage.
- □ Check if identified terminal fragments from different cleavage reagents _________.

EXAMPLE: If reagents cleave **N-terminal** peptide bonds, look to identify the ___-terminal fragment.


Pepsin: cleaves **N**-terminal side of **F, Y, W** & **L**.

N-terminal fragment does _____ end with **F, Y, W** or **L**.

If reagent cleaves **C-terminal** peptide bonds, look to identify the ___-terminal fragment.


Trypsin splittin' after **K** or **R**.

C-terminal fragment does _____ end with **K** or **R**.

Step #4: Overlap terminal fragments from step #3 with other fragments from ______________ cleavage techniques.

Step #5: If you get stuck, use the longest peptide fragment for overlapping. If there's a tie, pick one & continue to overlap.

- □ If you get stuck again, use the next largest fragment & overlap until the original sequence is revealed.

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PRACTICE: A sample of an unknown peptide was divided into two aliquots. One aliquot was treated with trypsin; the other was treated with cyanogen bromide. Given the following sequences of the resulting peptide fragments, deduce the sequence of the original peptide.

Trypsin treatment:	Cyanogen bromide treatment:
Asn—Thr—Trp—Met—Ile—Lys	Gln—Phe
Gly—Tyr—Met—Gln—Phe	Val—Leu—Gly—Met
Val—Leu—Gly—Met—Ser—Arg	Ile—Lys—Gly—Tyr—Met
	Ser—Arg—Asn—Thr—Trp—Met

Sequence: _____-_____-_____-_____-_____-_____-_____-_____-_____-_____-_____-_____-_____-_____-_____-_____-_____

PRACTICE: A peptide with 31 amino acid residues is independently treated with trypsin to give four fragments and separately treated with chymotrypsin to give six fragments (see chart below). FDNB treatment followed by amino acid hydrolysis resulted in DNP-Met and free amino acids. Identify the sequence of the 31 amino acid residues in the original unfragmented protein using one-letter amino acid codes.

Trypsin Fragments	**Chymotrypsin Fragments**
W-H-I-F-M-A-C-R	K-C-V-A-I-L-V-R-W
M-A-F-V-I-A-V-Y-K	V-I-A-V-Y
C-V-A-I-L-V-R	M-A-C-R-G-I-P-F
G-I-P-F-A-I-V	H-I-F
	A-I-V
	M-A-F

Sequence:

__-__

CONCEPT: STRATEGY FOR ORDERING CLEAVED FRAGMENTS

PRACTICE: The sequence of kassinin, a tachykinin dodecapeptide from the African frog Kassina senegalensis, was determined. A single round of Edman degradation identifies Asp as the N-terminus. A 2nd sample of the peptide is treated with chymotrypsin, releasing two fragments with the following amino acid compositions: fragment 1 (G, T, M, V) and fragment 2 (D_2, Q, K, F, P, S, V). Next, a 3rd sample of peptide is treated with trypsin, which results in two fragments with the following amino acid compositions: fragment 3 (D, P, K, V) and fragment 4 (D, Q, G, T, M, F, S, V). A 4th sample was treated with CNBr, but the dodecapeptide was not cleaved. A 5th sample treated with elastase yields a single Gly residue & three fragments—fragment 5 (T, M), fragment 6 (D, K, P, S, V), and fragment 7, which was sequenced as: D—Q—F—V. What is the sequence of the dodecapeptide?

Hint: Elastase cleaves C-terminal side of small neutral residues: G, A, V, L, I & S.

Sequence: ______-______-______-______-______-______-______-______-______-______-______-______