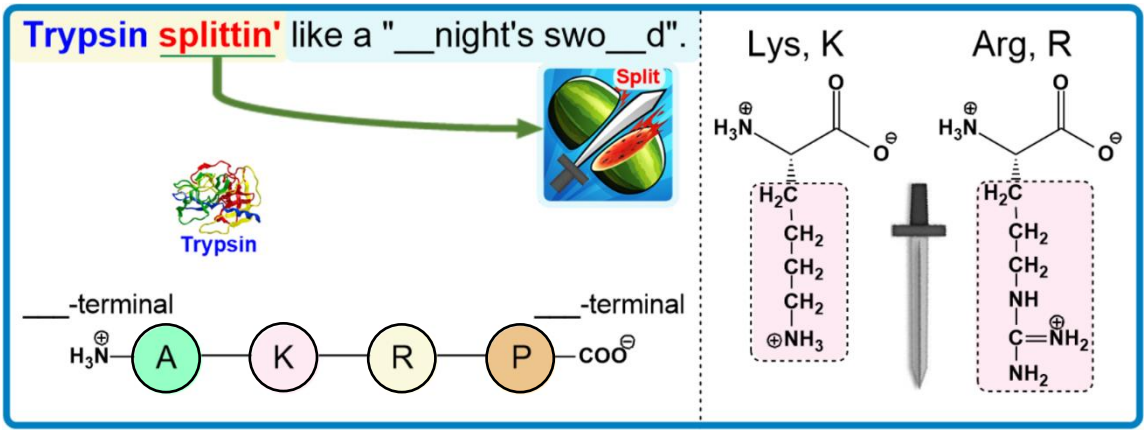
CONCEPT: PEPTIDASES

- _____: enzymes that selectively catalyze the *hydrolysis* of specific peptide bonds.
- *Trypsin* & *Chymotrypsin* are biologically relevant peptidases of our _____ systems.
  - □ _____: *only* cleaves peptide bonds on the *carboxyl* side of both ____ & ____ amino acids.
  - □ Cleavage is typically *blocked/inhibited* for many peptidases if _____ is involved in the peptide bond.

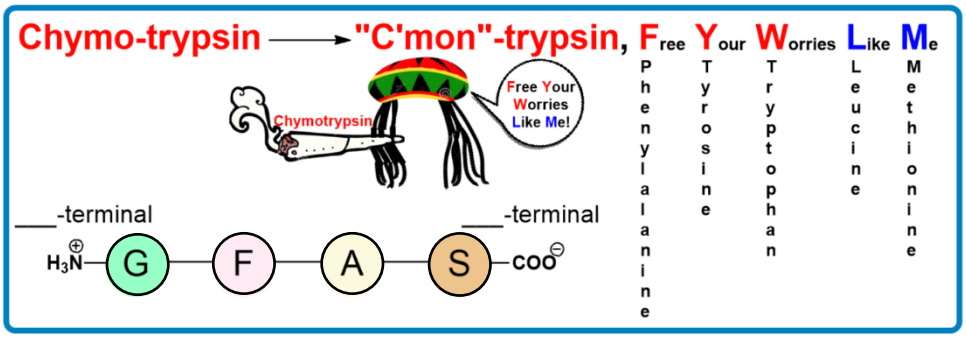
EXAMPLE: Where will trypsin do its peptide bond splittin'?



PRACTICE: Draw out each of the peptide fragments that would be generated if the peptide is treated with trypsin.

Ala-Phe-Lys-Pro-Met-Tyr-Gly-Arg-Ser-Trp-Leu-His $\xrightarrow{\text{Trypsin}}$?

- _____: prefers breaking peptide bonds on the *carboxyl* side of _____ amino acids (F, Y, W).
  - □ Unlike trypsin's specific cleavage, over time chymotrypsin also cleaves *slowly* after L & M residues.



*For practice problems, unless otherwise indicated, first assume Chymotrypsin *only* cleaves its preferred residues (__, __, __). *

PRACTICE: Draw out the resulting peptide fragments that would be generated if the peptide is treated with chymotrypsin.

Ala-Lys-Phe-Pro-Met-Tyr-Gly-Arg-Ser-Trp-Leu-His $\xrightarrow{\text{Chymotrypsin}}$?

CONCEPT: PEPTIDASES

Other Relevant Peptidases

●Below are a list of peptidases and the specific peptide bonds that they hydrolyze.

□ Recall: Peptidase bond cleavage can be inhibited if Proline participates in the peptide bond.

EXAMPLE:

Peptidase	N or C-terminal?	Amino Acid Cleavage Site
Trypsin	___-terminal	____ & ____
Chymotrypsin	___-terminal	Prefers _____ amino acids: F, Y, W. But also *slowly* cleaves L & M.
Elastase	___-terminal	Small, neutral residues: G, A, V, L, I & S
Thrombin	___-terminal	____
Pepsin	___-terminal	F, Y, W & L
Carboxypeptidase A	___-terminal	Cleaves off all C-terminal residues *except* R, K & P

PRACTICE: What would the resulting peptide fragments be if the following peptide were treated with excess Pepsin?

H—E—L—P—M—E—P—L—E—A—S—E

a) H—E—L—P—M—E—P—L & E—A—S—E

b) H—E—L—P, M—E—P, & L—E—A—S—E

c) H—E, L—P—M—E—P—L—E—A—S—E

d) H—E, L—P—M—E, P—L—E, A—S—E

PRACTICE: Which is the expected result of chymotrypsin cleavage of the following peptide?

Lys—Gly—Phe—Thr—Tyr—Pro—Asn—Trp—Ser—Tyr—Phe

a) Lys—Gly—Phe, Thr—Tyr—Pro—Asn—Trp, Ser—Tyr, & Phe

b) Lys—Gly—Phe, & Thr—Tyr—Pro—Asn—Trp—Ser—Tyr—Phe

c) Lys—Gly—Phe, Thr—Tyr—Pro—Asn—Trp, Ser, & Tyr—Phe

d) Lys—Gly—Phe—Thr—Tyr—Pro—Asn—Trp, Ser, & Tyr—Phe

CONCEPT: PEPTIDASES

PRACTICE: You perform multiple tests to derive the amino acid sequence of a purified peptide (see results below). Which of the following peptides listed best represents the sequence of the unknown peptide?

a) Trp—Tyr—Ala—Ala—His.
b) Ala—His—Trp—Tyr—Ala.
c) His—Ala—Trp—Tyr—Ala.
d) Ala—His—Tyr—Trp—His.
e) His—Ala—Tyr—Trp—Ala.

Test Performed	Result
Carboxypeptidase A digestion	A tetrapeptide & free Ala
FDNB, 6M HCl & HPLC	DNP-His + free amino acids
Elastase digestion	A tripeptide and a dipeptide
Chymotrypsin digestion	Free Trp & Ala and a tripeptide comprised of Ala, Tyr & His

*Elastase cleaves C-terminal peptide bond of G, A, V, L, I & S.

PRACTICE: A) The octapeptide AVGWRVKS was digested with the enzyme trypsin. Would ion exchange or size-exclusion chromatography be most appropriate for separating the fragments? Explain.

a) Ion-Exchange chromatography.
b) Size-exclusion chromatography.

B) Suppose the same peptide was digested with chymotrypsin. Which would be the optimal separation technique? Explain.

a) Ion-Exchange chromatography.
b) Size-exclusion chromatography.

CONCEPT: PEPTIDASES

PRACTICE: A nonapeptide was determined to have the following amino acid composition: $(Lys)_2$, $(Gly)_2$, $(Phe)_2$, His, Thr, Met. The native peptide was incubated with 1-fluoro-2,4-dinitrobenzene (FDNB) and then hydrolyzed; 2,4-dinitrophenylhistidine was identified by HPLC. When the native peptide was exposed to cyanogen bromide (CNBr), an octapeptide and free glycine were recovered. Incubation of the native peptide with trypsin gave a pentapeptide, a tripeptide, and free Lys. 2,4-Dinitrophenyl-histidine was recovered from the pentapeptide, and 2,4-dinitrophenylphenylalanine was recovered from the tripeptide. Digestion with the enzyme pepsin produced a dipeptide, a tripeptide, and a tetrapeptide. The tetrapeptide was composed of $(Lys)_2$, Phe, and Gly. The native sequence was determined to be:

a) Gly—Phe—Lys—Lys—Gly—Thr—Met—Phe—His.
b) His—Thr—Gly—Lys—Lys—Phe—Phe—Gly—Met.
c) His—Thr—Phe—Gly—Lys—Lys—Phe—Met—Gly.
d) His—Phe—Thr—Gly—Lys—Lys—Phe—Met—Gly.
e) Met—Thr—Phe—Lys—Phe—Gly—Gly—Lys—His.

Recall: Pepsin cleaves N-terminal peptide bond of F, Y, W & L residues.

PRACTICE: The following reagents are often used in protein chemistry. Match the reagent with the purpose for which it is best suited. Some answers may be used more than once or not at all and more than one reagent may be suitable for a given purpose.

a) CNBr (Cyanogen bromide).
b) Carboxypeptidase A.
c) FDNB.
d) Performic acid.
e) Chymotrypsin.
f) Trypsin.
g) Iodoactetate.
h) β-mercaptoethanol.
i) Hydrazine.
j) Phenylisothiocyanate (PITC).

_____ Hydrolysis of peptide bonds on C-terminal side of Lys & Arg.
_____ Cleavage of peptide bonds on C-terminal side of Met.
_____ Breakage of disulfide bonds (-S-S-).
_____ Carboxymethylation of cysteines to prevent disulfide reformation.
_____ Determining the N-terminal amino acid in a polypeptide.
_____ Determining the C-terminal amino acid in a polypeptide.
_____ Determination of the amino acid sequence of a peptide.