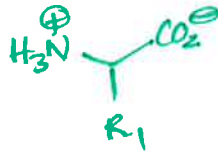
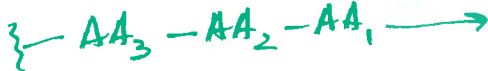
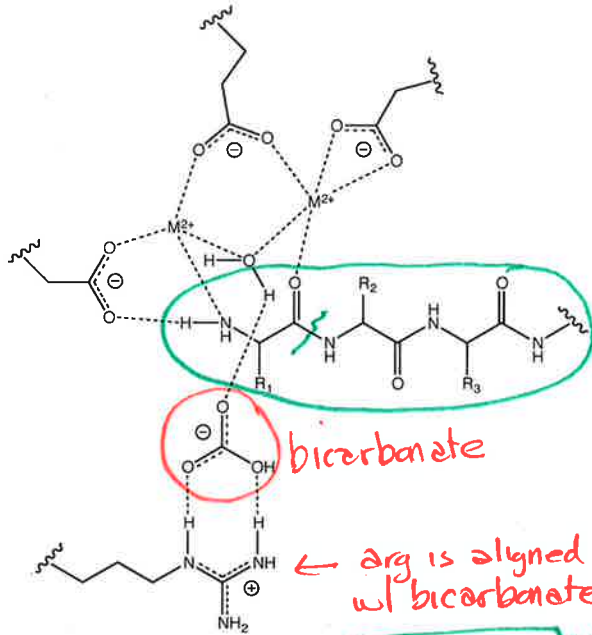


### Active Site SQ

Aminopeptidases are enzymes that cut amino acid residues off of the amino terminus of proteins. They play a number of important roles in cells. One particular example is called Leucine Aminopeptidase or just LAP. LAP is a metallopeptidase because its active site contains metal ions. Shown below is an approximate sketch of the active site of LAP (in 2D, of course, with many details omitted).



Succinctly answer the following questions.

1. Circle the substrate in this diagram.

← see over there

2. Put a squiggly line through the bond that is broken by LAP.

← see over there

3. What kind of reaction does a peptidase catalyze? Be as specific as you can.

Hydrolysis of a peptide (amide) bond

4. Write and balance the reaction catalyzed. You may use abbreviations for the peptide(s).

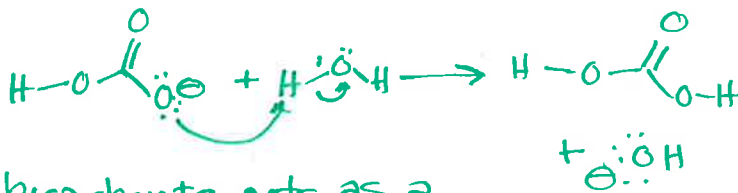
5. If the arg in the active site were mutated to lys, what would you expect to happen to  $k_{cat}$ ?

Since arg + lys are both positive there should be relatively little effect, though

6. If the acidic amino acids in the active site were mutated to val, what would you expect to happen to  $K_m$ ?

One would lose the  $-CO_2^-$  that coordinates w/  $M^{2+}$  & the whole network collapses\*   
 arg aligns with bicarbonate and that would be lost.

7. There is a bicarbonate ion in the active site. Mark it. What role does this ion likely play? Write and balance a reaction relevant to this role.



bicarbonate acts as a base to convert  $H_2O$  to hydroxide which will then attack the  $C=O$  to start the rxn

8. This enzyme works best around pH 8. Give two reasons why a lower pH could negatively affect the action of this enzyme.

If the  $-CO_2^-$  became  $-CO_2H$  due to lower pH the binding network is lost.

If  $HCO_3^-$  is converted to  $H_2CO_3$  the reaction w/  $H_2O$  (question 7) is no longer possible

\*  $K_m$  will go up: worse binding