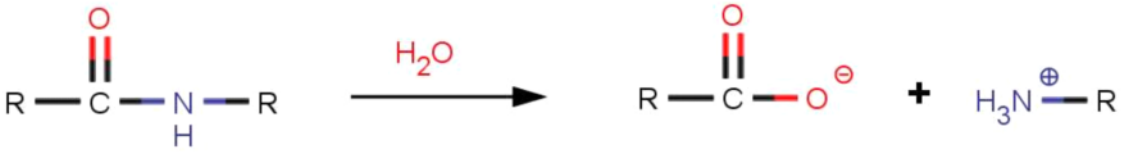
In this activity, you will be using a couple very common biochemical reactions to help you understand the role of enzymes in catalysis.

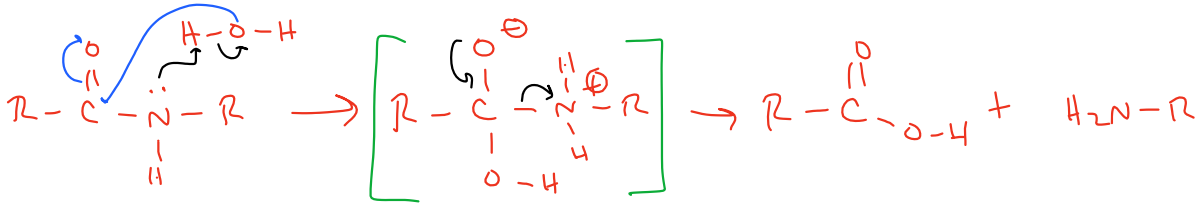
**Serine Proteases**

The common proteases that we discussed earlier in the term are all examples of serine proteases. Let's explore this reaction.




1. The net reaction catalyzed by serine proteases is shown above. What enzyme class are proteases? hydrolase

2. Using the image above as a reference point, predict how this reaction may proceed in a test tube. Make sure to consider activation of a nucleophile and clearly draw out all transition states.



3. Under normal biological conditions, do you think that activation of the nucleophile is likely? Explain your choice.

No. We need $^-OH$ to be a nucleophile + @ pH 7, this is in low [ ].

4. Is the transition state stable? Why or why not?

Nope. The distribution of charge is much less stable than the energy of reactants or products

5. Carefully consider the chemical features that you see in the transition state compared to the reactants and products (consider stereochemistry, geometry, charge, etc). What differences do you see?

Presenting ions to partner with the $O^-$ & $N^+$ would help stabilize them

6. What features do you think the enzyme might present that can stabilize the transition state (i.e. lower the energy)? Can you come up with three possibilities?

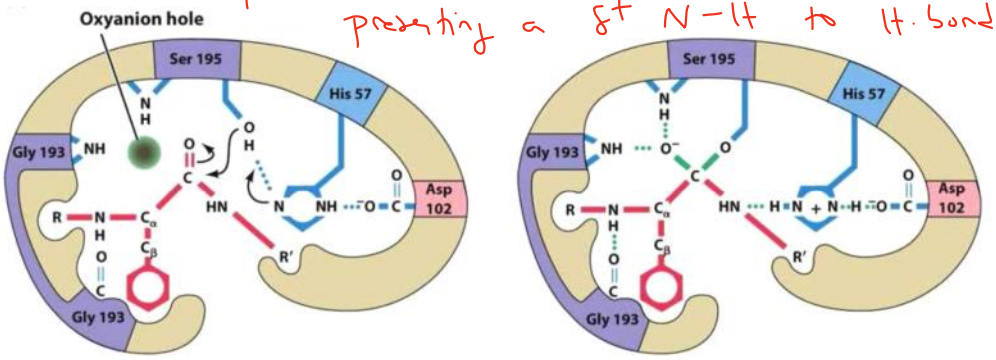
① $Zn^{2+}$ in oxyanion hole

② Lys / Arg in oxyanion hole

③ Glu / Asp in close proximity to $N^⊕$

7. The image below is a cartoon representation of the active site. How do serine proteases actually stabilize the tetrahedral intermediate? Is this consistent with what you predicted in the last question?

The oxyanion hole is stabilized by peptide backbone presenting a $\delta+$ N–H to H-bond with



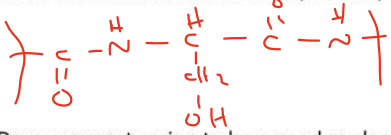

8. Why isn't the oxyanion hole directly above the carbonyl? Is this important in reducing the energy of the transition state?

$sp^2$ vs. $sp^3$ – the position of the oxygen changes, so the oxyanion hole needs to be in the $sp^3$ spot

9. Ok, now we know how the enzyme stabilizes the transition state, but how about activation of the nucleophile? Look back at your answer to number 3. What is the nucleophile and how is it activated? Is this likely at biological pH (~7.3)?

the mechanism suggests that it happens via standard acid/base chemistry. Not very likely b/c very low $[H_3O^+]$ + $[OH^-]$

10. Serine proteases are named such because they make use of a serine residue as the nucleophile. Draw serine as it would look in the middle of a protein (so both termini are part of peptide bonds).

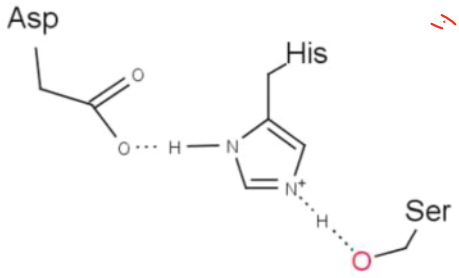


11. Do you expect serine to be a good nucleophile? Why or why not.

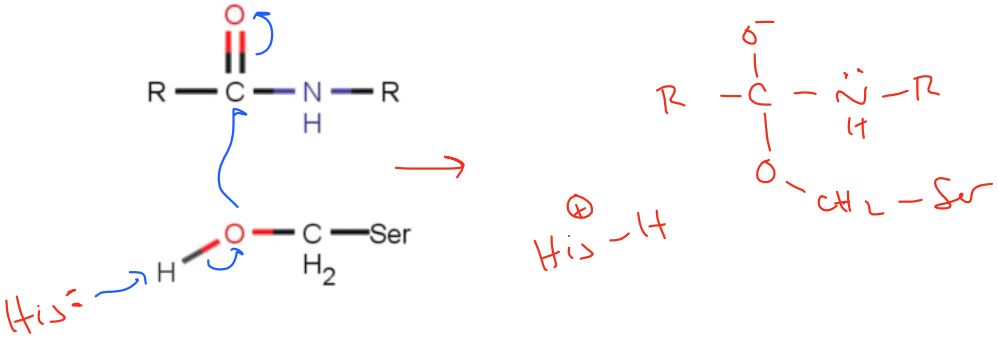
Nope – deprotonation of an alcohol requires really basic conditions.

12. Hopefully you recognized that serine is a terrible nucleophile because it is very hard to deprotonate an alcohol under mild conditions. Enter the amazingness of enzymes. Serine proteases make use of a "catalytic triad". In this case, this triad works together to be a good nucleophile. Carefully inspect the image below. How does the catalytic triad make serine a good nucleophile?

Through a H-bond network that "pushes" at the O–H bond




13. Now that you know serine can be activated to be a nucleophile, show the first step in the catalytic mechanism. Be sure to indicate how the serine is activated and clearly show the transition state.



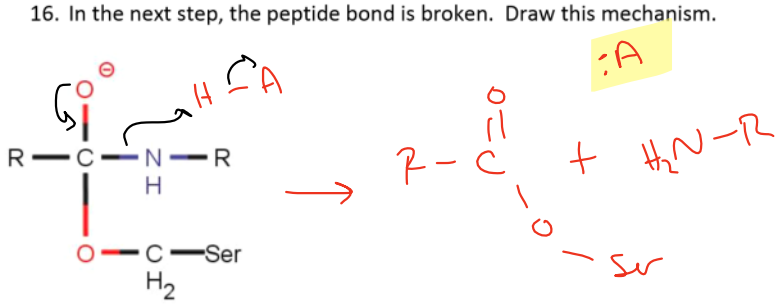

14. Does the transition state look like something you have seen before? How do you think the enzyme stabilizes it?

the oxyanion hole shown in problem 7

15. Your first step should show a covalent bond between serine and the peptide bond. Based on this observation, do serine proteases make use of a covalent catalysis strategy?

Yep

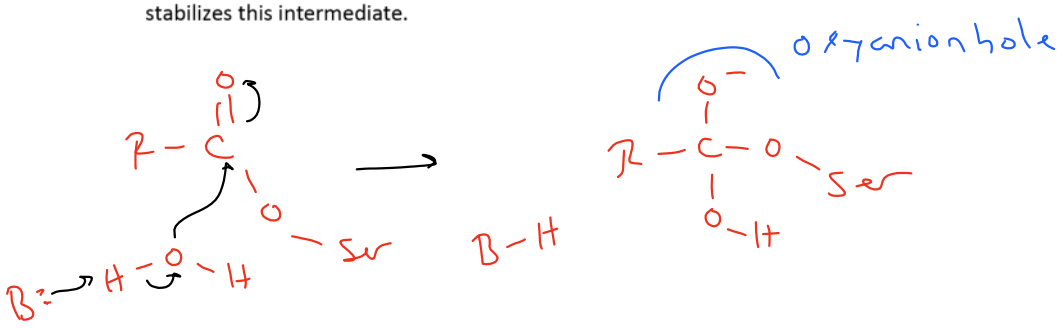
16. In the next step, the peptide bond is broken. Draw this mechanism.




17. Remember these proteins are hydrolases.
    a. Remember that water needs to be activated as a nucleophile. How can the enzyme do this? You now have a base that need to become an acid (highlighted above)
    b. How do you think the enzyme keeps water out prior to this step?

perhaps the R–NH blocks it from entering

18. Now bring in water to form a new tetrahedral intermediate. Show this mechanism and explain how the enzyme stabilizes this intermediate.




19. Almost done! Now collapse the high energy intermediate and draw the resulting products.