Chem 452 - Lecture 5
Catalytic Strategies
111021

Enzymes have evolved an array of different strategies or enhancing the power and specificity of the reactions they catalyze. For numerous enzymes the details have been worked out at the atomic level. In this lecture we will focus on four examples: chymotrypsin, carbonic anhydrase, the EcoRV restriction endonuclease, and myosin II ATPases.

Introduction

- Enzymes exhibit both catalytic power and specificity
- We will consider closely, four examples.

Introduction

- Chymotrypsin (1gct) 3.4.21.1
  - A Hydrolase, which cleaves peptide bonds in proteins
- Carbonic anhydrase (1ca2) 4.2.1.1
  - A Lyase, which adds water to CO_2.
- EcoRV (1rvb) 3.1.21.4
  - A Hydrolase, which cleave phosphodiester bonds in DNA
- Myosin motor domain ATPase (1fmv & 1fmw) 3.6.4.1
  - An enzyme that couples the hydrolysis of ATP to the mechanical motion.

Introduction

- The Enzyme Commission "names" for enzymes

Table 8.8 Six major classes of enzymes

Class	Type of reaction	Example	Chapter
1. Oxidoreductases	Oxidation–reduction	Lactate dehydrogenase	16
2. Transferases	Group transfer	Nucleoside monophosphate kinase (NMP kinase)	9
3. Hydrolases	Hydrolysis reactions (transfer of functional groups to water)	Chymotrypsin	9
4. Lyases	Addition or removal of groups to form double bonds	Fumarase	17
5. Isomerases	Isomerization (intramolecular group transfer)	Triose phosphate isomerase	16
6. Ligases	Ligation of two substrates at the expense of ATP hydrolysis	Aminoacyl-tRNA synthetase	30

Introduction

- These case studies will provide examples of
  - Generating powerful nucleophiles at neutral pH values.
  - Achieving high absolute reaction rates
  - Specificity for substrate selection
  - Specificity for products produced

Introduction

- Some Basic Catalytic Principles
  - Covalent Catalysis
  - General Acid/Base Catalysis
  - Catalysis by Approximation (Juxtaposition, or the proximity effect)
  - Metal Ion Catalysis
  - Transition state stabilization

Introduction

- Some Basic Catalytic Principles
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Introduction

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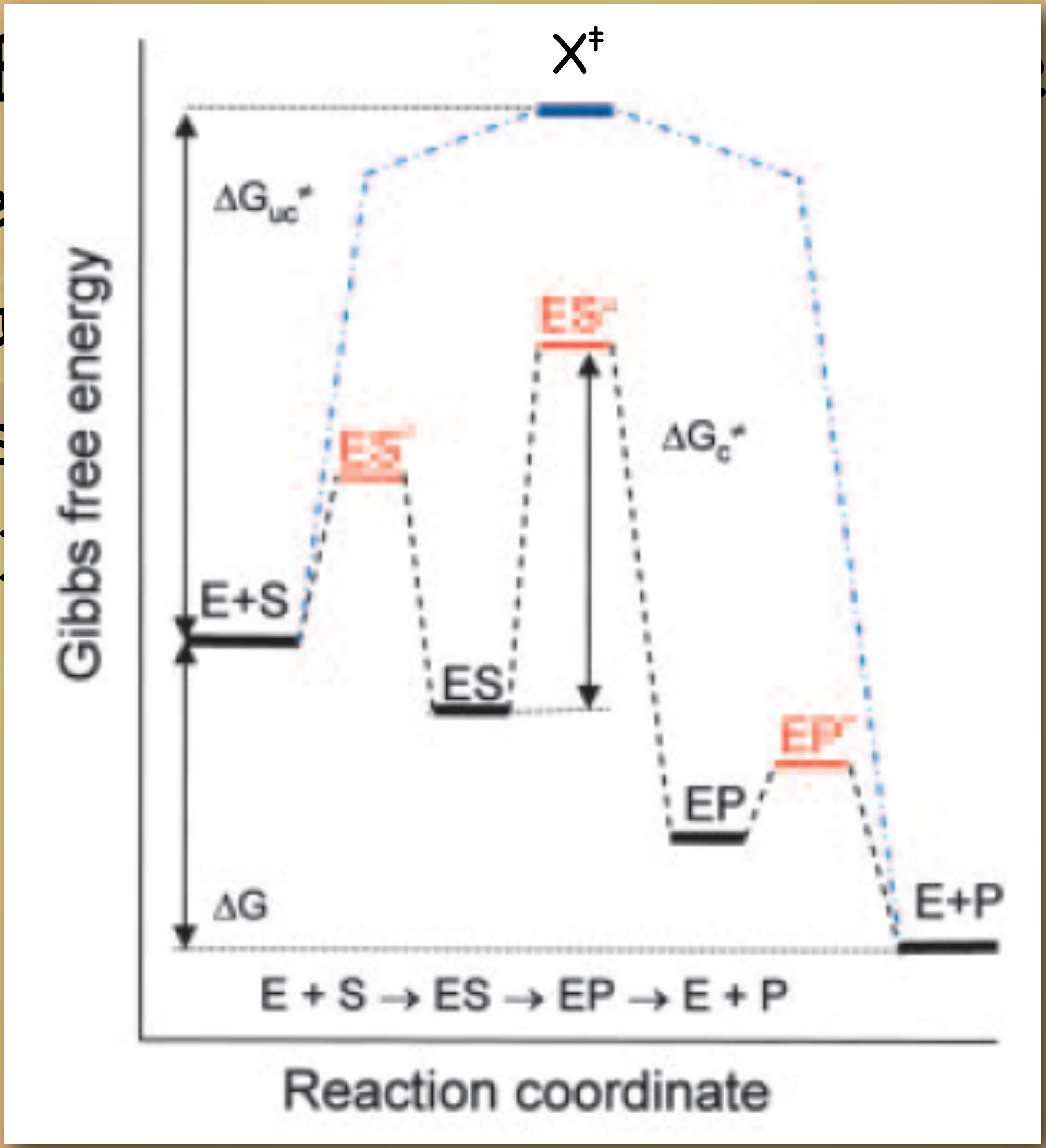
  - Covlale...
  - Genera...
  - Catalys...
  - Metal I...



Introduction

- Some Basic Catalytic Principles
  - Covlalent Catalysis
  - General Acid/Base Catalysis
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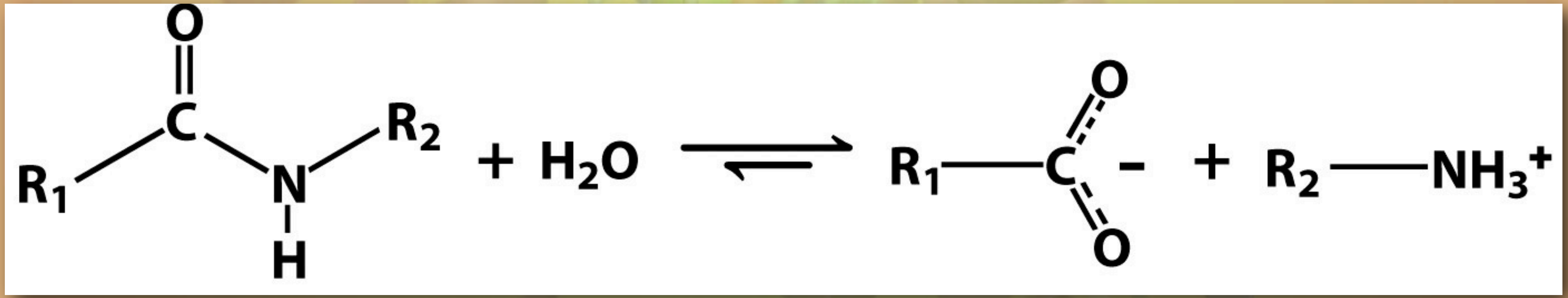
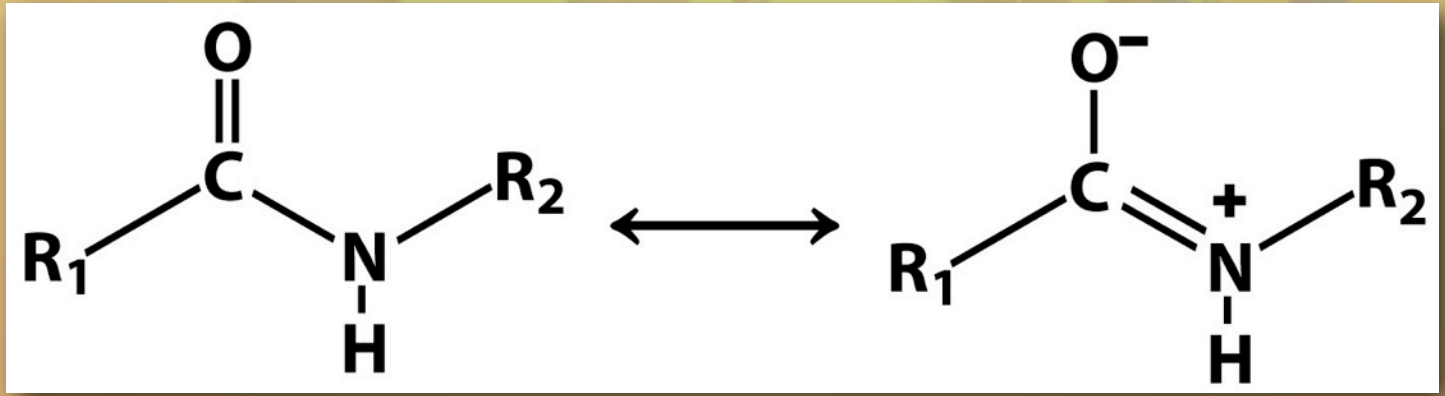
  - Metal Ion Catalysis

Chymotrypsin

- The hydrolysis of the peptide bond is thermodynamically favorable, but kinetically unfavorable.



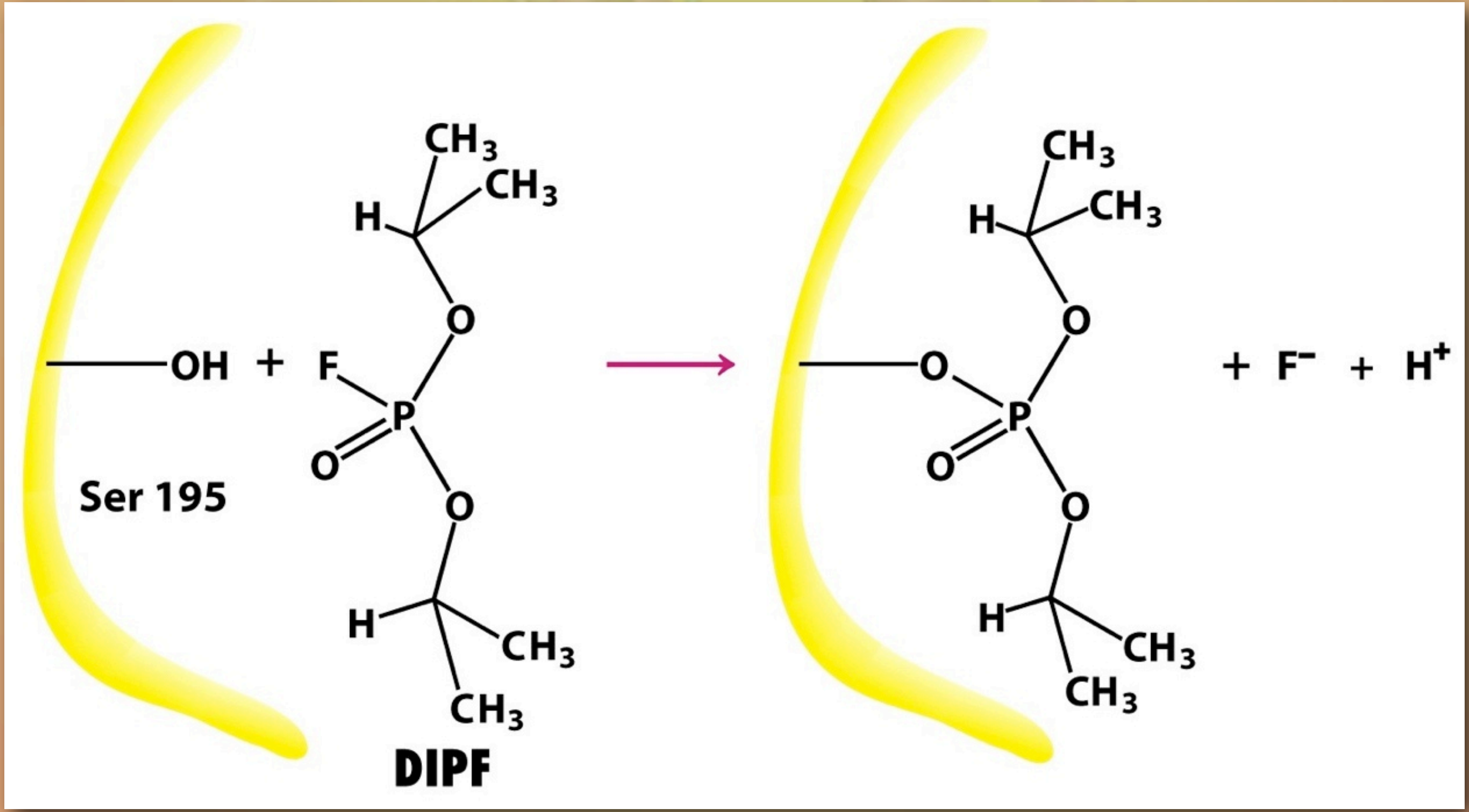


Chymotrypsin

- Chymotrypsin overcomes this by producing a powerful alkoxide nucleophile, in situ.
  - This is an example of **covalent catalysis**

Chymotrypsin

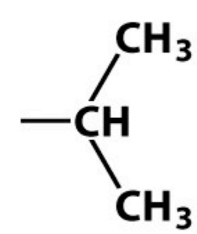
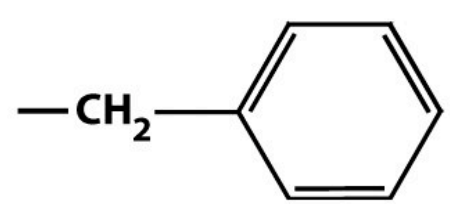
- DIPF selectively reacts with Ser 195 in chymotrypsin.



Chymotrypsin

- Chymotrypsin cleaves peptide bonds to the carboxy side of large non polar amino acid residues.

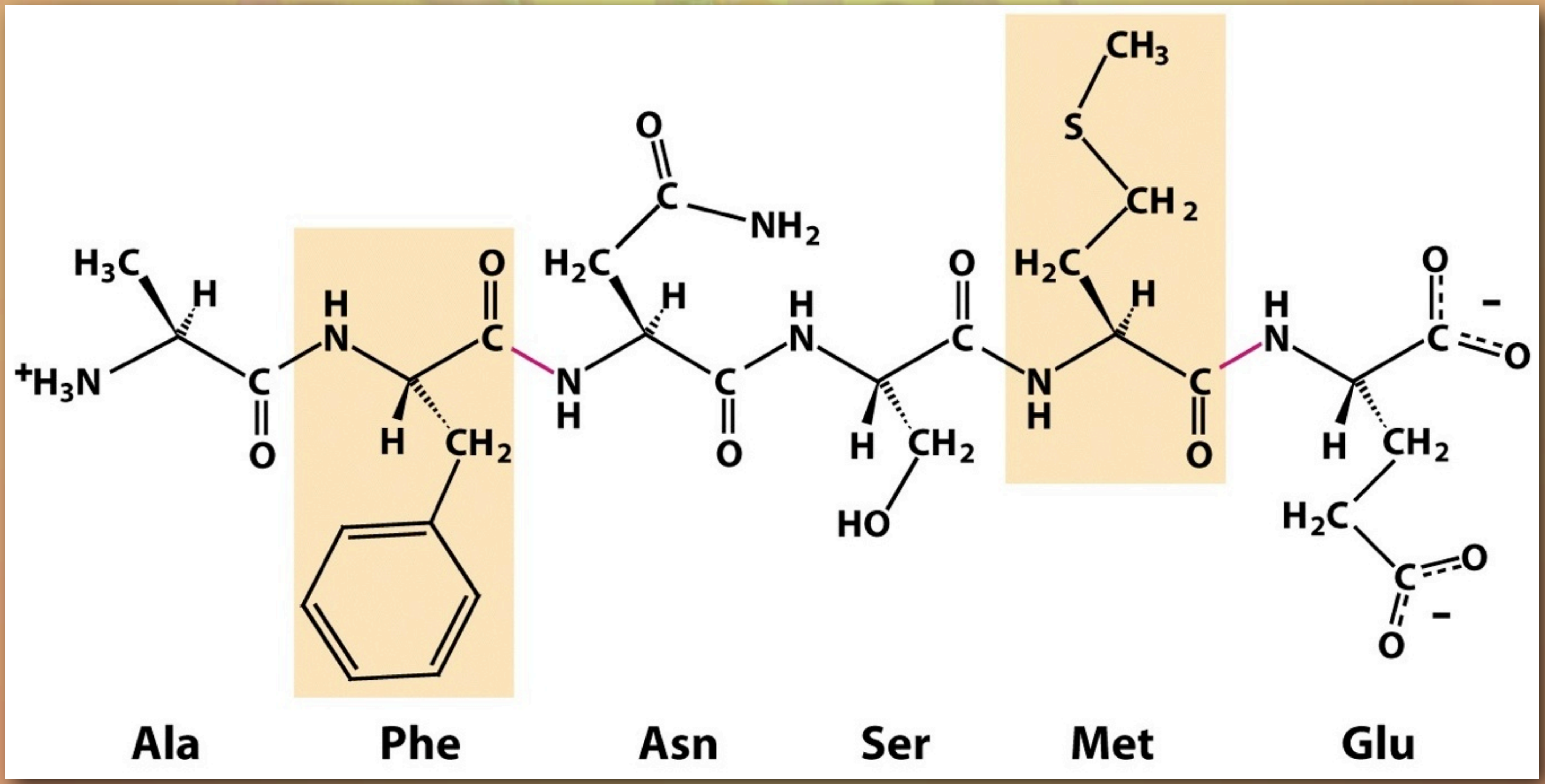
TABLE 8.6 Substrate preferences of chymotrypsin

Amino acid in ester	Amino acid side chain	k_{cat}/K_M (s^{-1} M^{-1})
Glycine	—H	1.3×10^{-1}
Valine		2.0
Norvaline	$—CH_2CH_2CH_3$	3.6×10^{2}
Norleucine	$—CH_2CH_2CH_2CH_3$	3.0×10^{3}
Phenylalanine		1.0×10^{5}

Source: After A. Fersht, *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding* (W. H. Freeman and Company, 1999), Table 7.3.

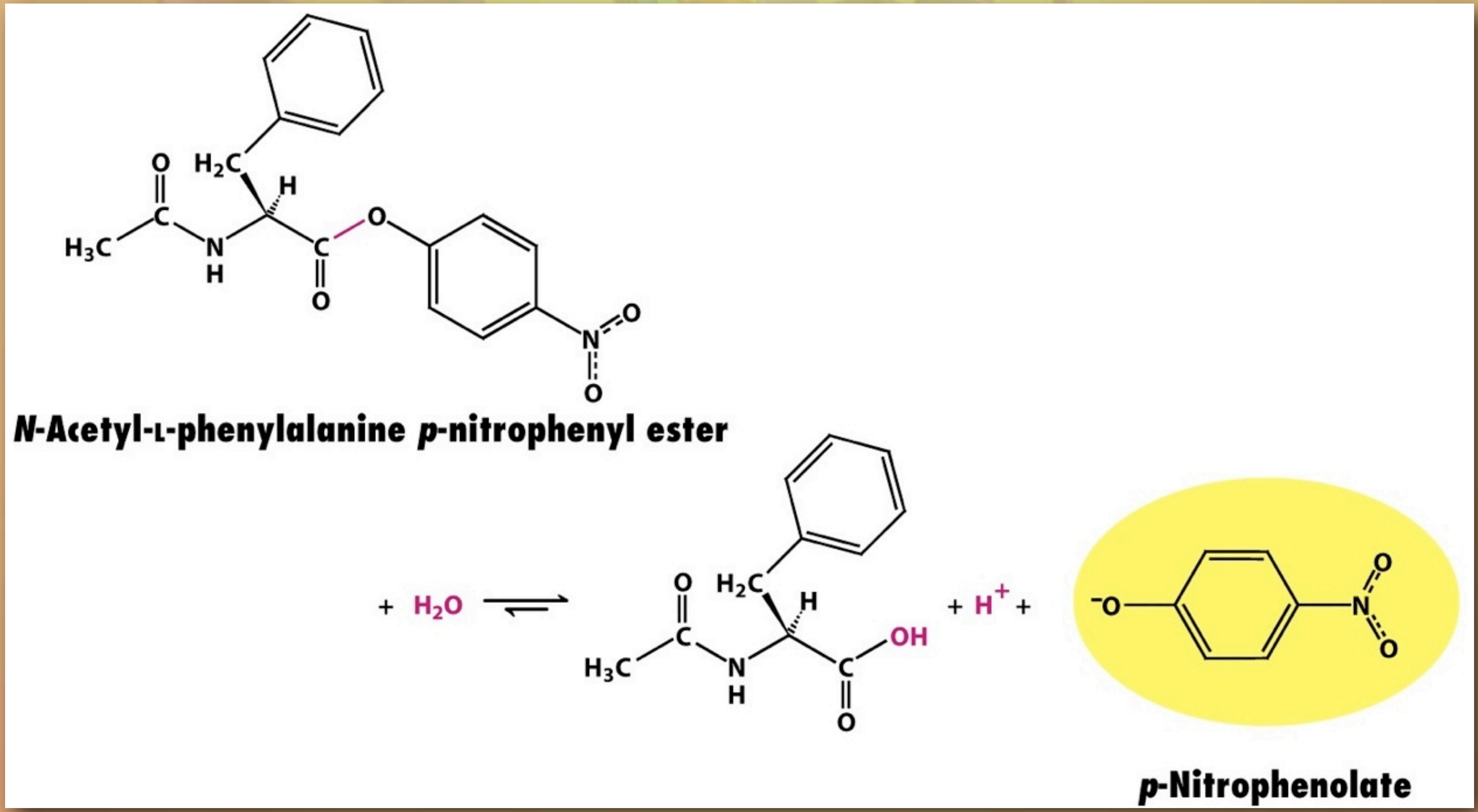
Chymotrypsin

- Chymotrypsin cleaves peptide bonds to the carboxy side of large non polar amino acid residues.



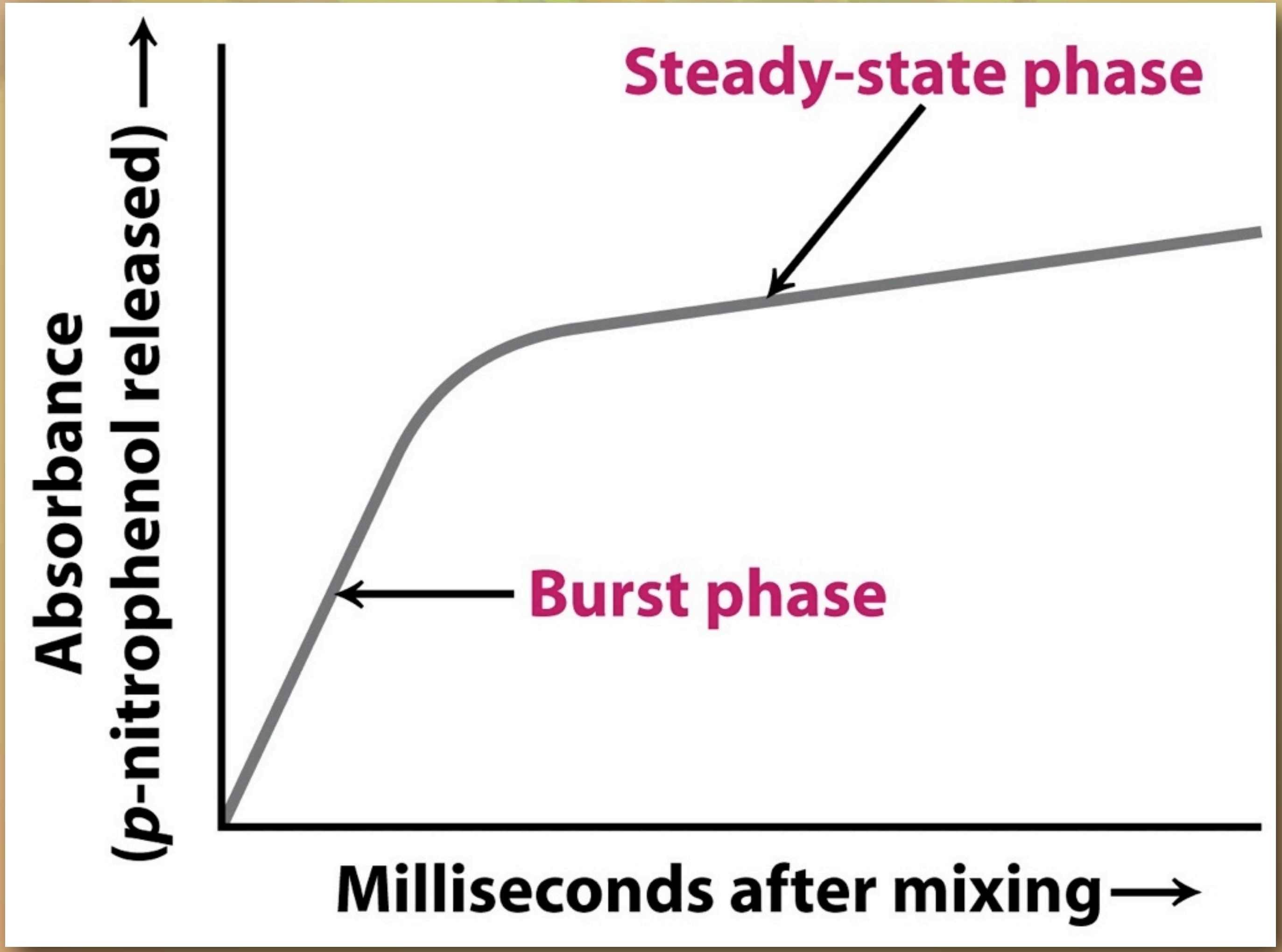
Chymotrypsin

- The chymotrypsin reaction can be followed using a **chromogenic** substrate.



Chymotrypsin

- Stop-flow kinetics experiments suggest a covalently bound intermediate is involved.



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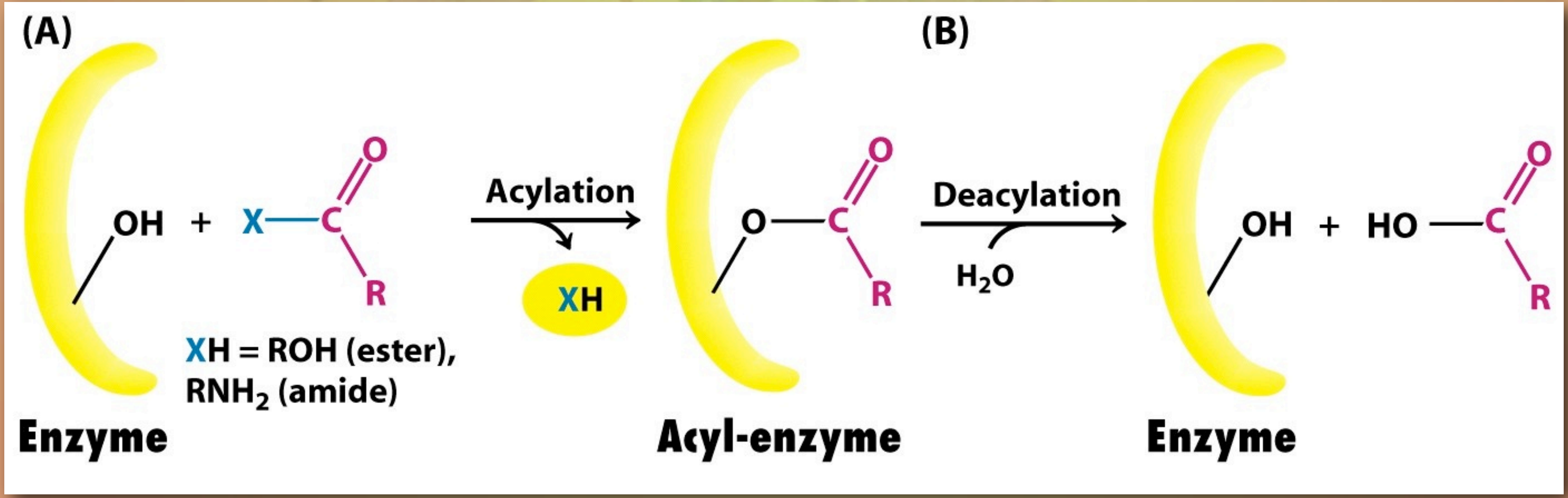
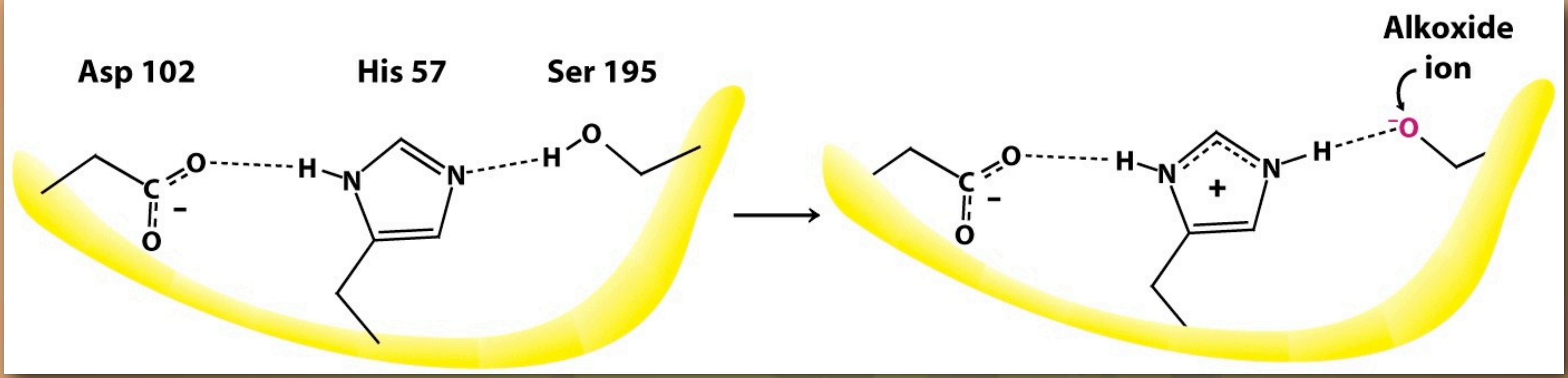


Chymotrypsin

- The reactive Ser 195 is part of a **catalytic triad**.



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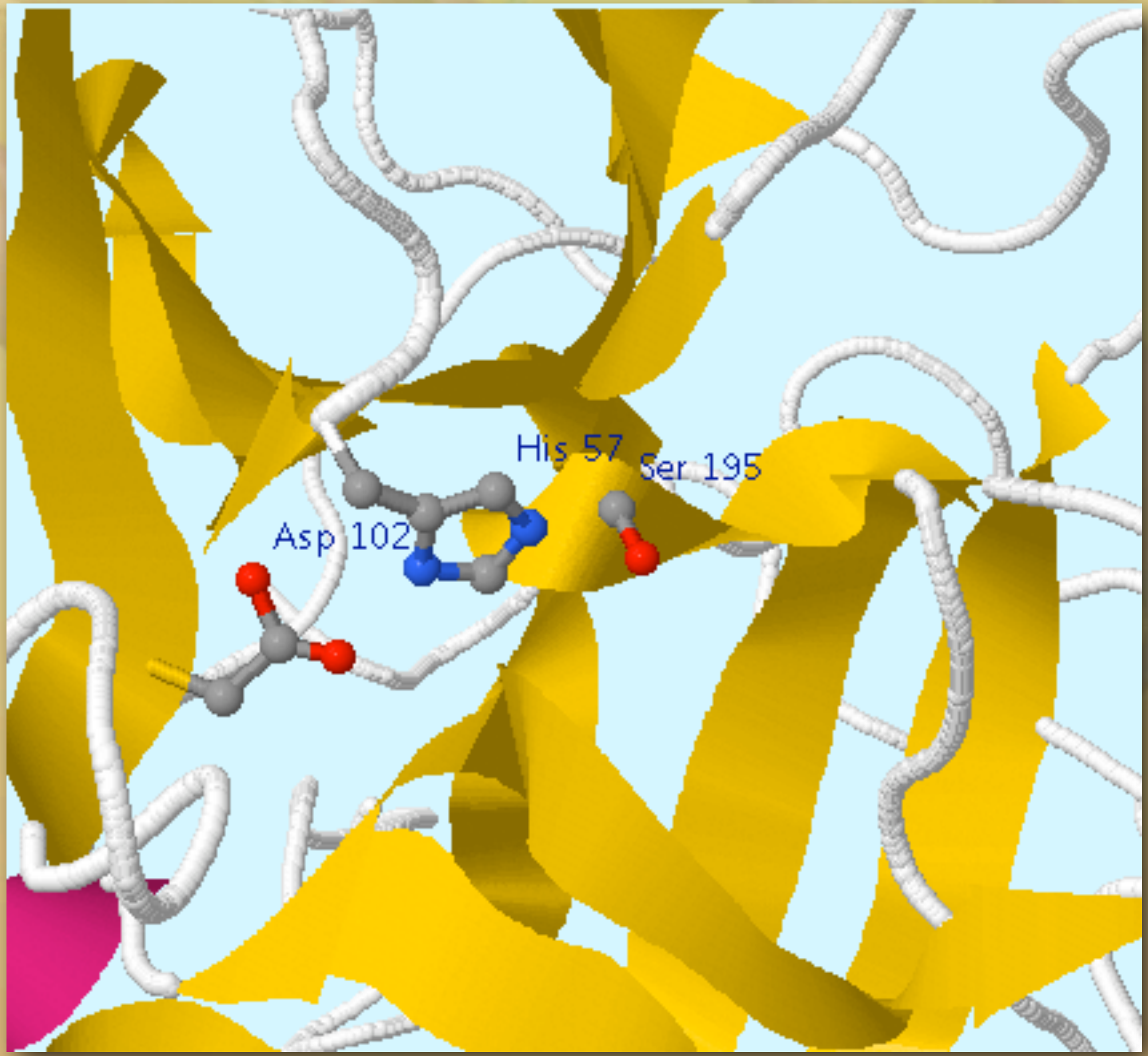

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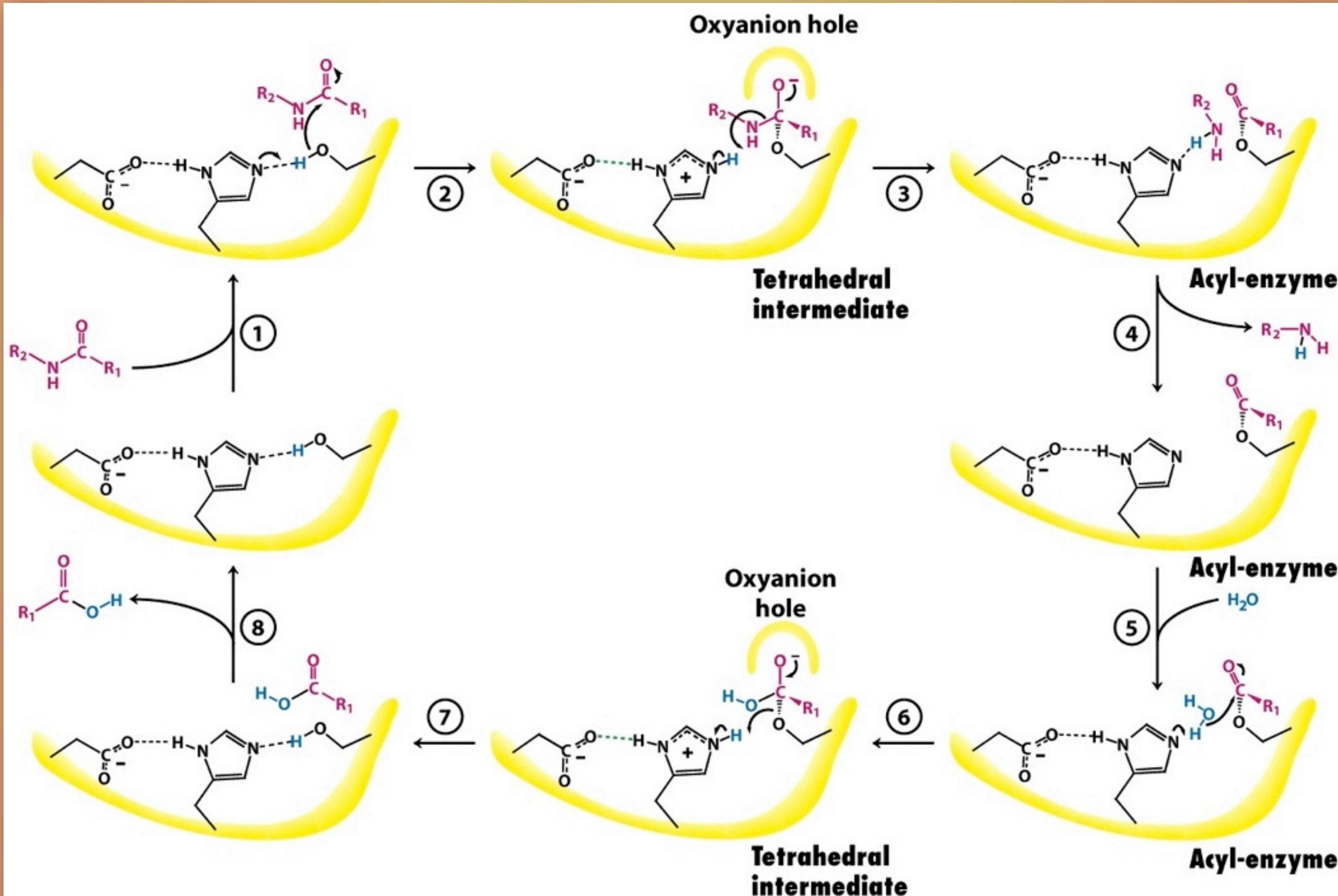
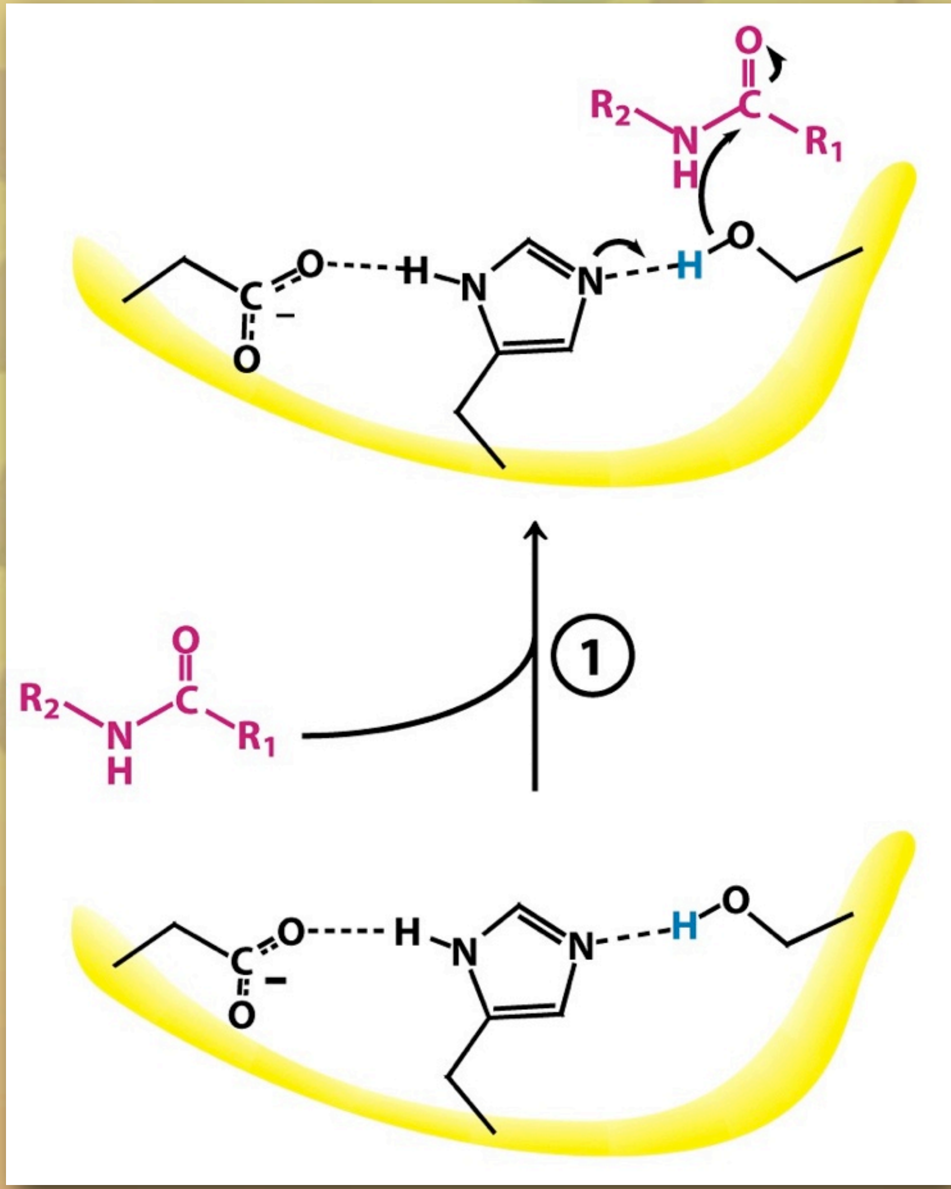
Chymotrypsin Catalytic Cycle



Chymotrypsin Catalytic Cycle

- Step 1: Substrate binding



Chymotrypsin Catalytic Cycle

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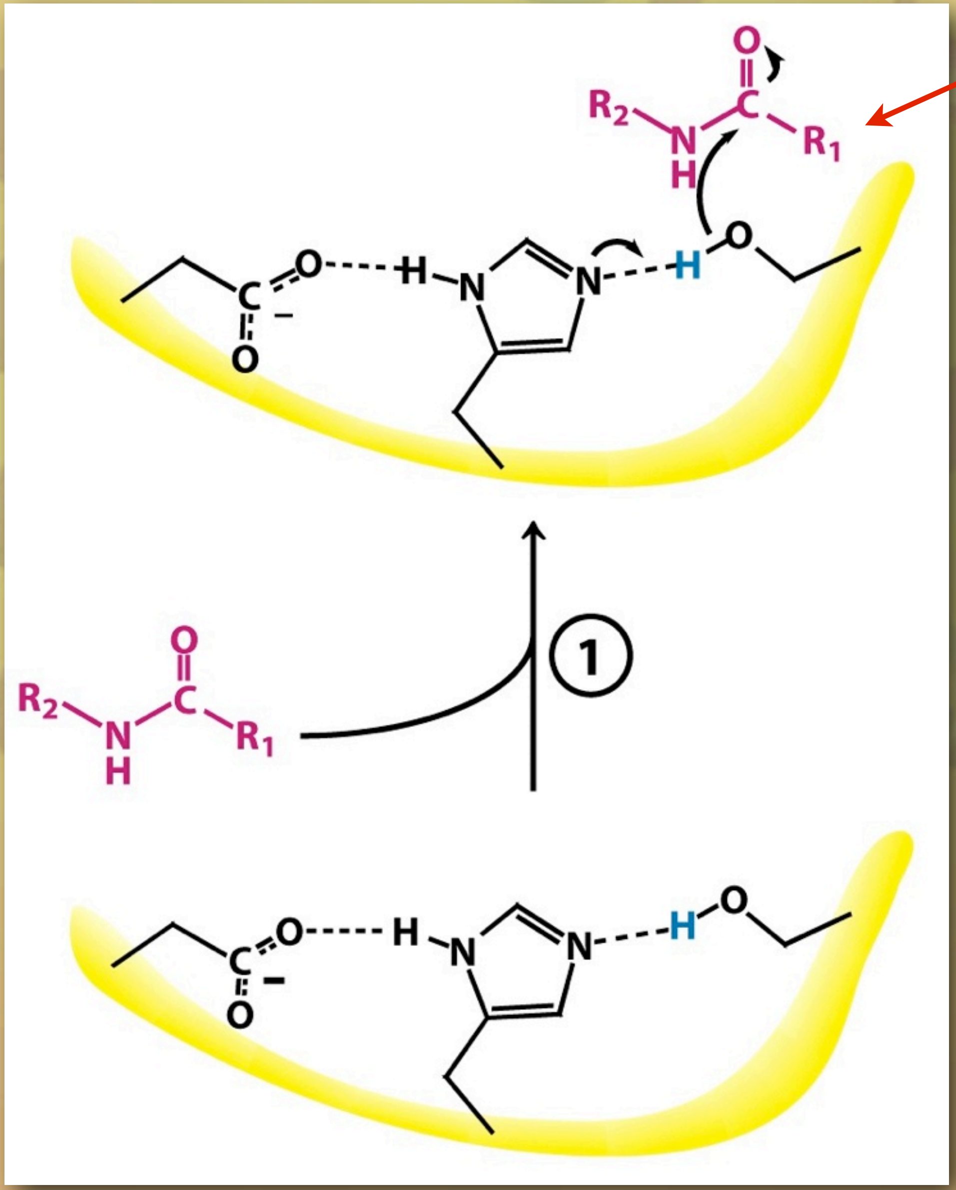
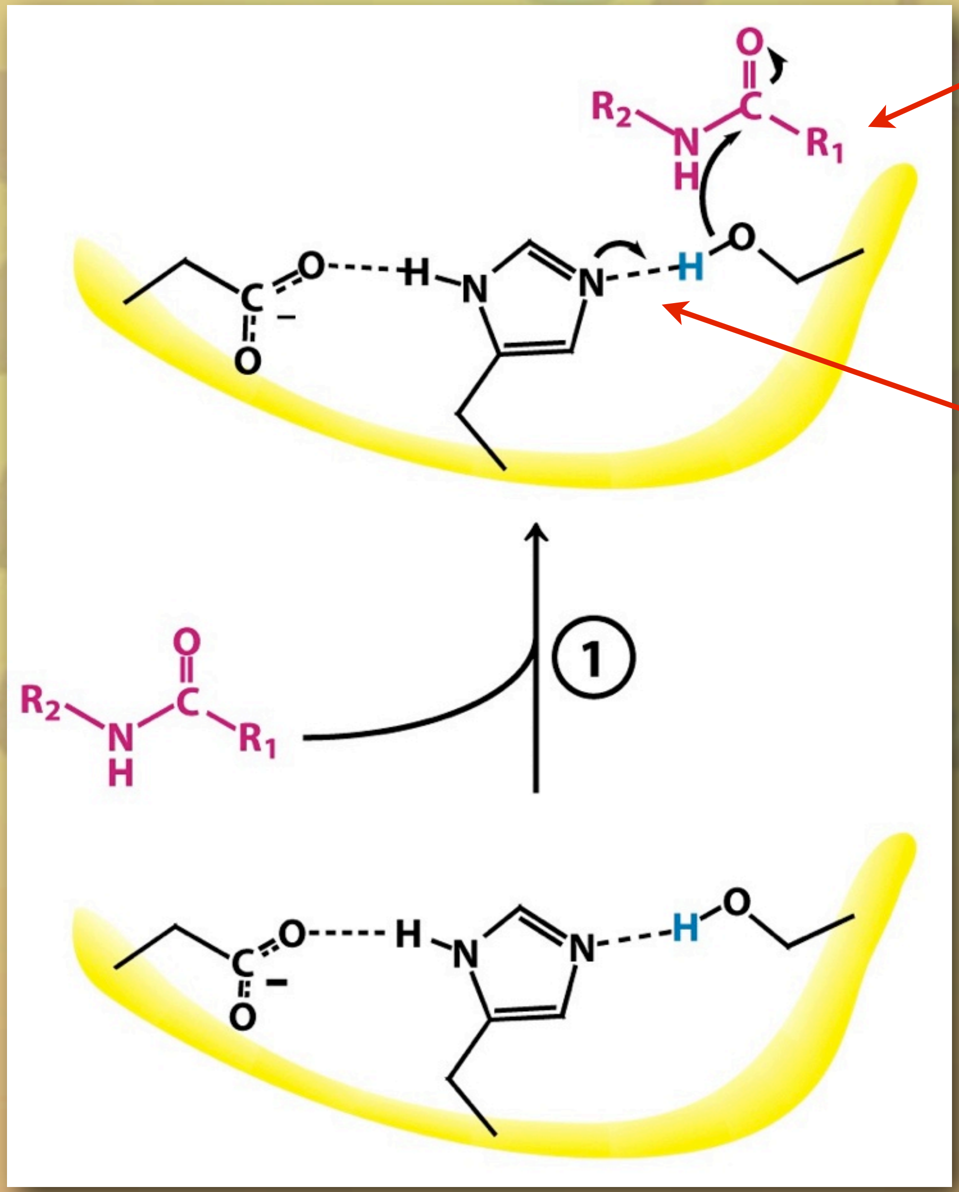


Proximity Effect

Chymotrypsin Catalytic Cycle

- Step 1: Substrate binding

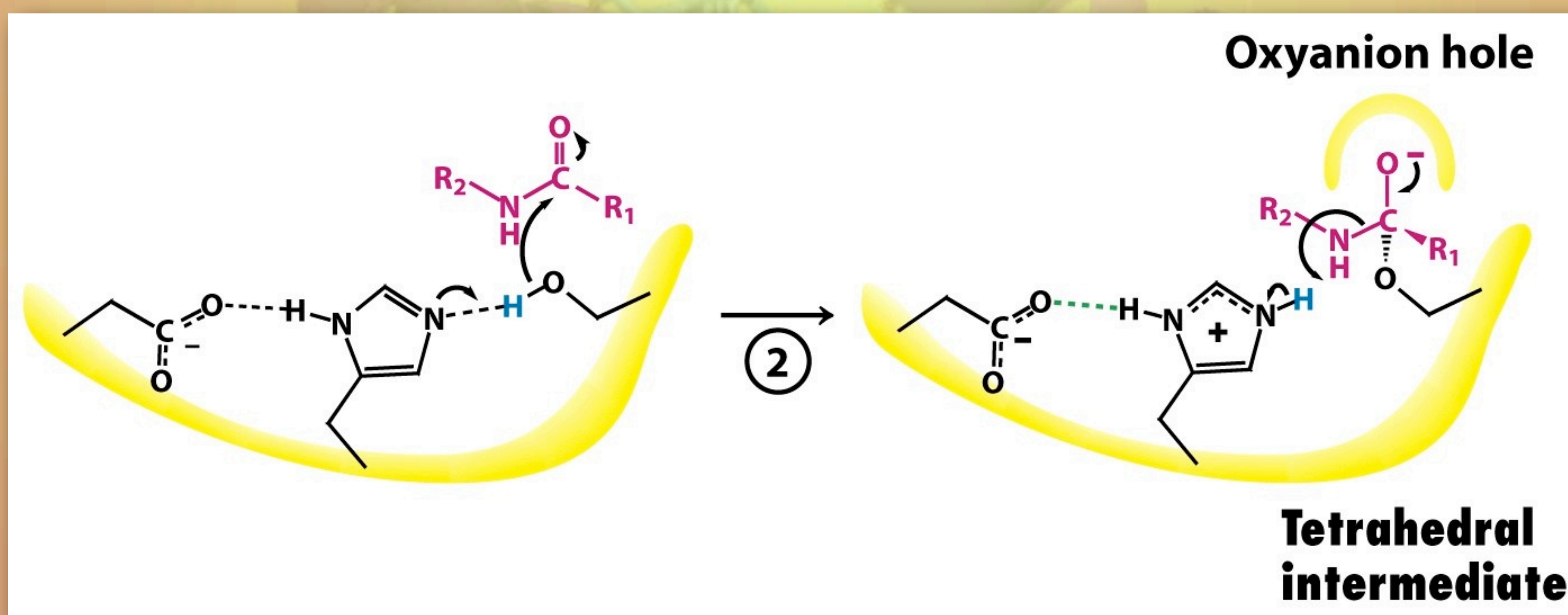


Proximity Effect

Acid/Base Catalysis

Chymotrypsin Catalytic Cycle

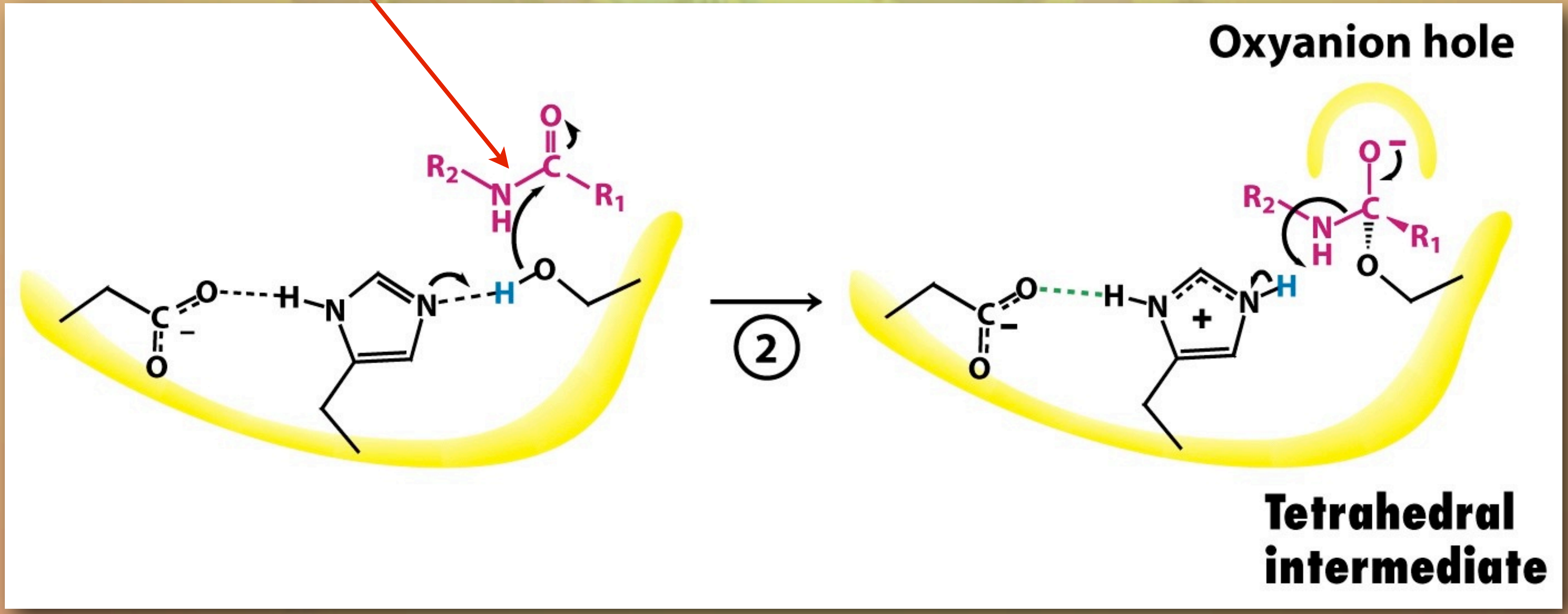
- Step 2: Transition state formation



Chymotrypsin Catalytic Cycle

- Step 2: Transition state formation

Covalent Bond Catalysis

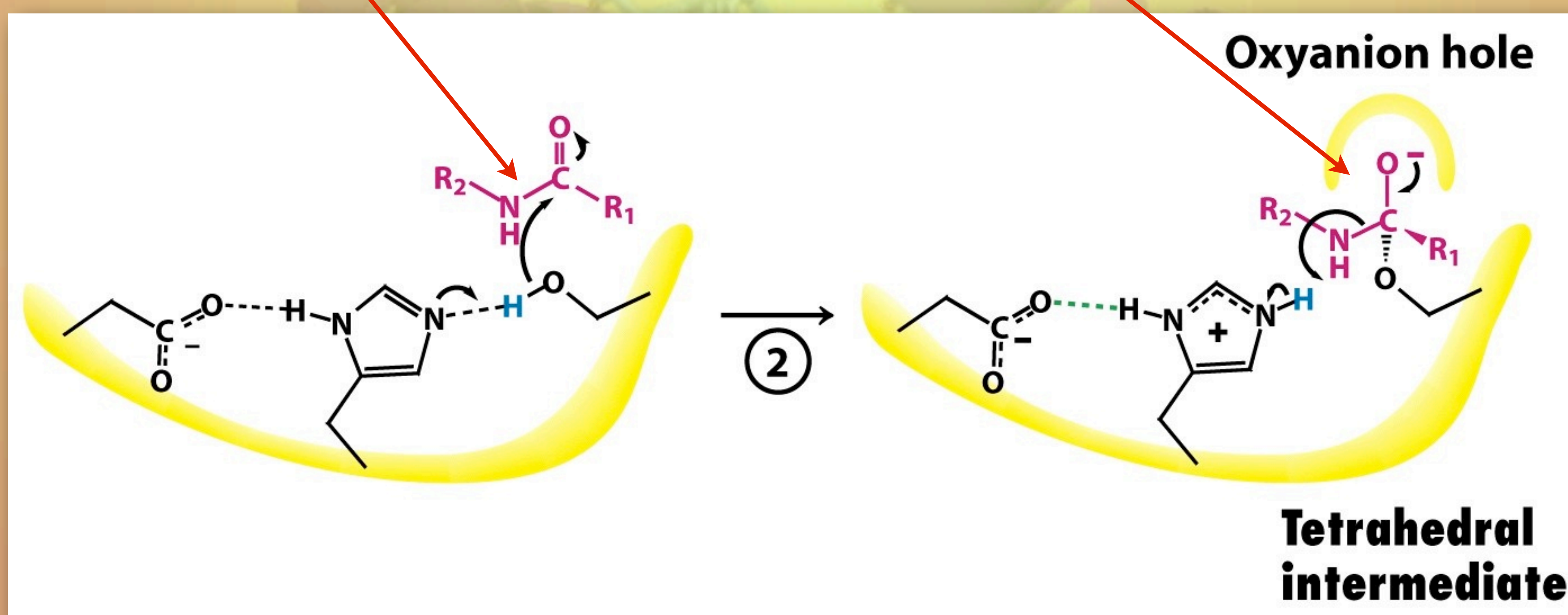


Chymotrypsin Catalytic Cycle

- Step 2: Transition state formation

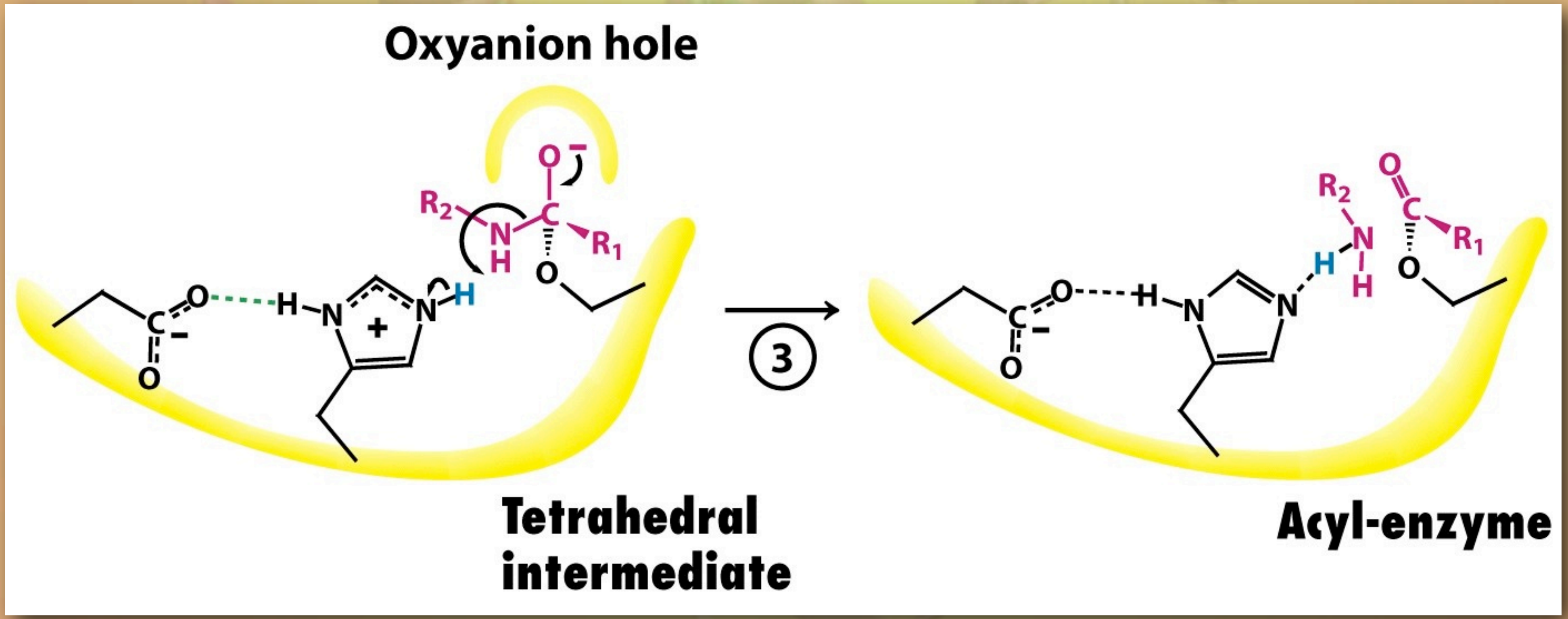
Covalent Bond Catalysis

Transition State Stabilization



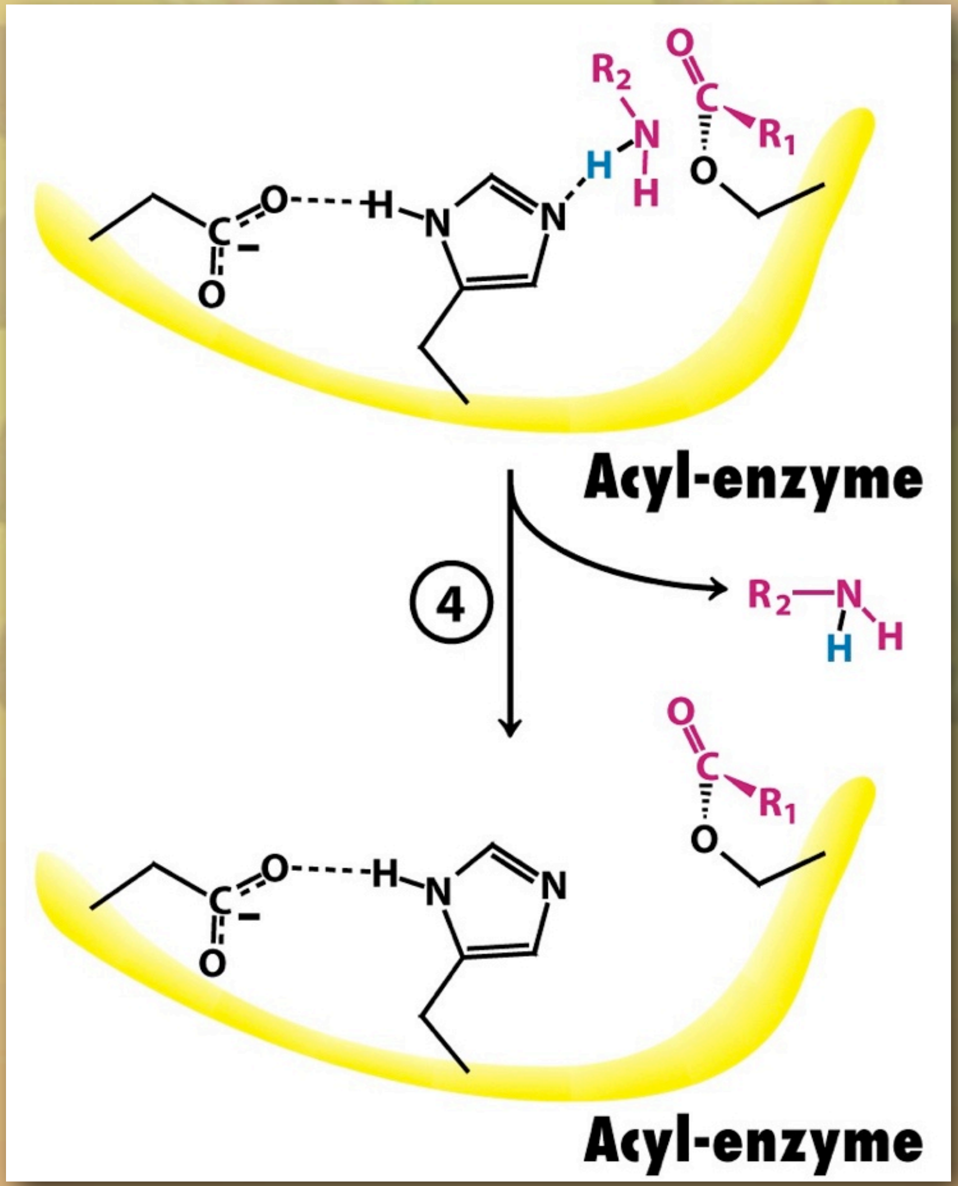
Chymotrypsin Catalytic Cycle

- Step 3: Peptide bond cleavage



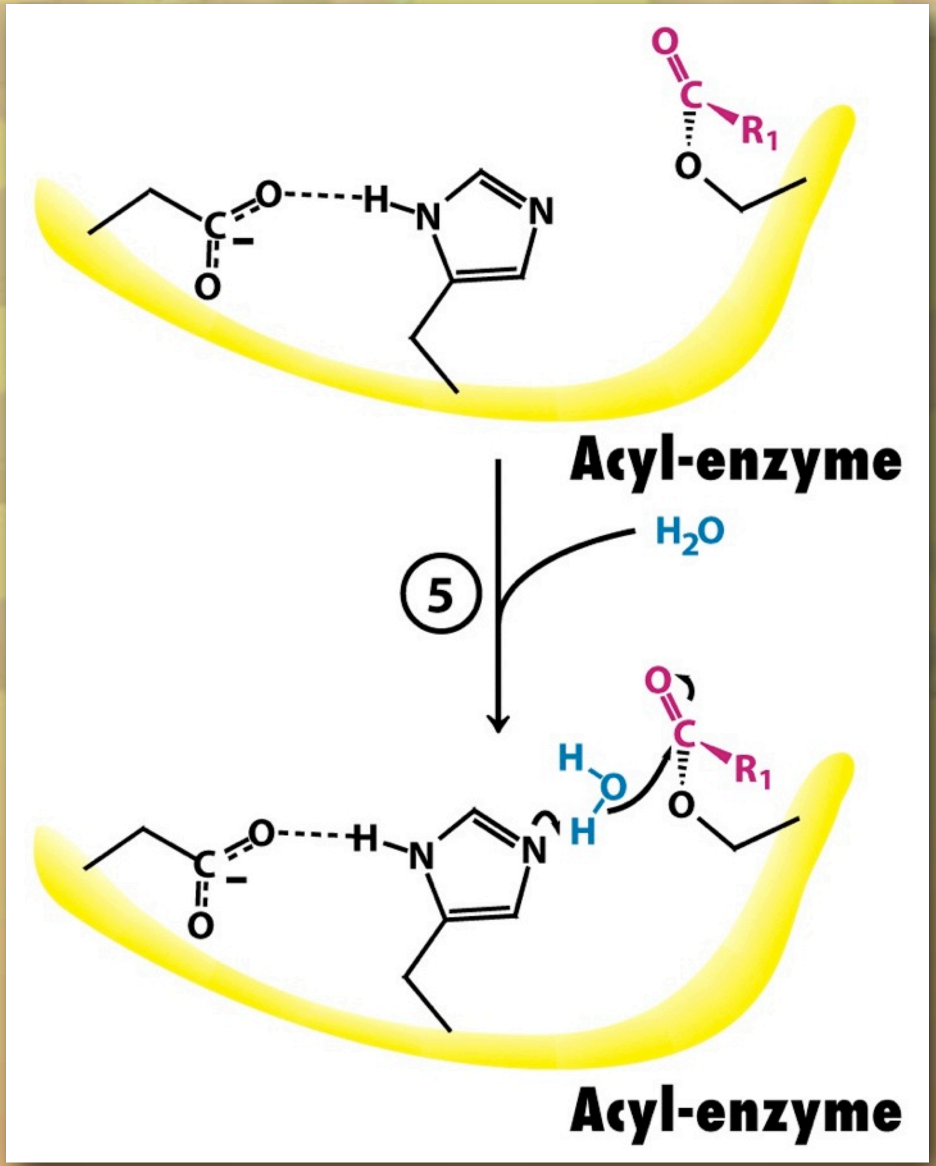
Chymotrypsin Catalytic Cycle

- Step 4: Release of first product (C-terminal half of the peptide)



Chymotrypsin Catalytic Cycle

- Step 5: Binding of the second substrate (H_2O)



Chymotrypsin Catalytic Cycle

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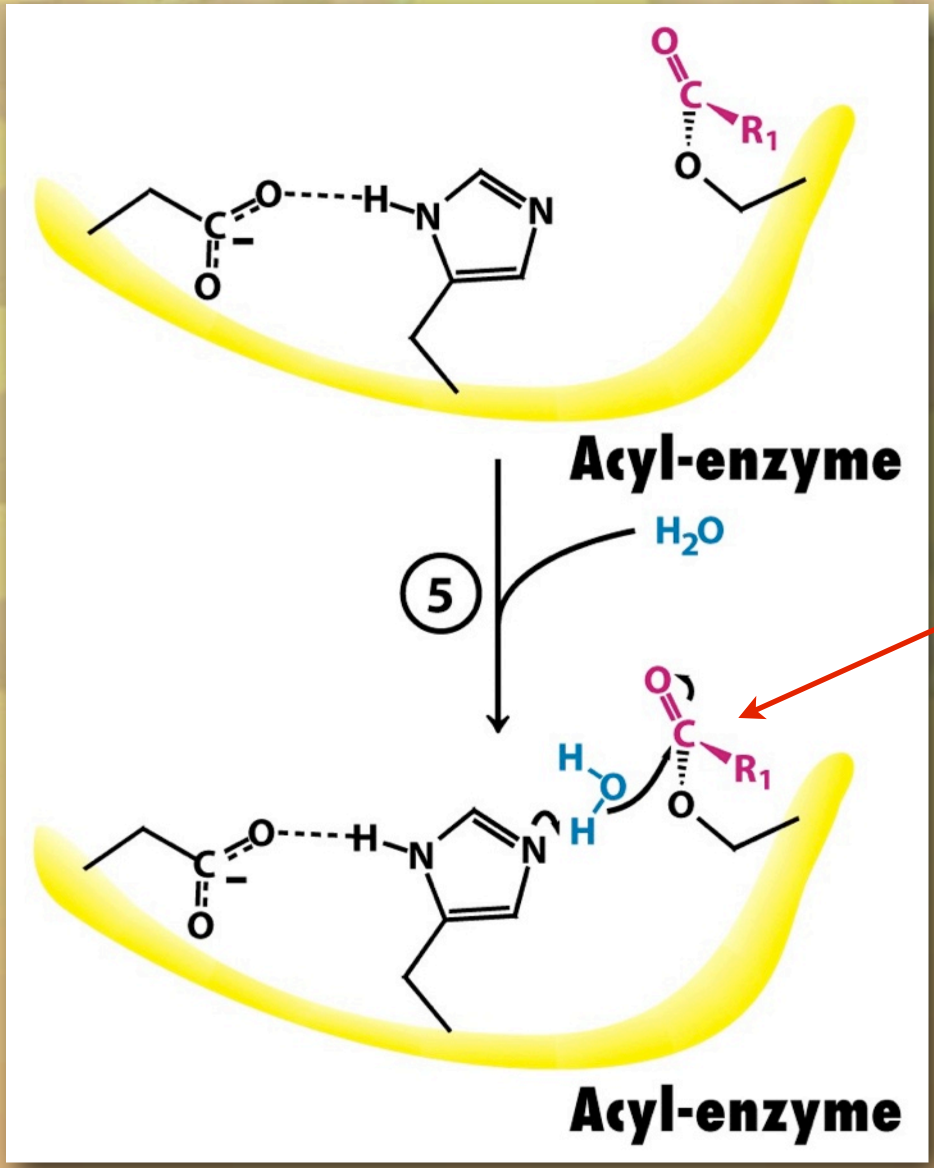
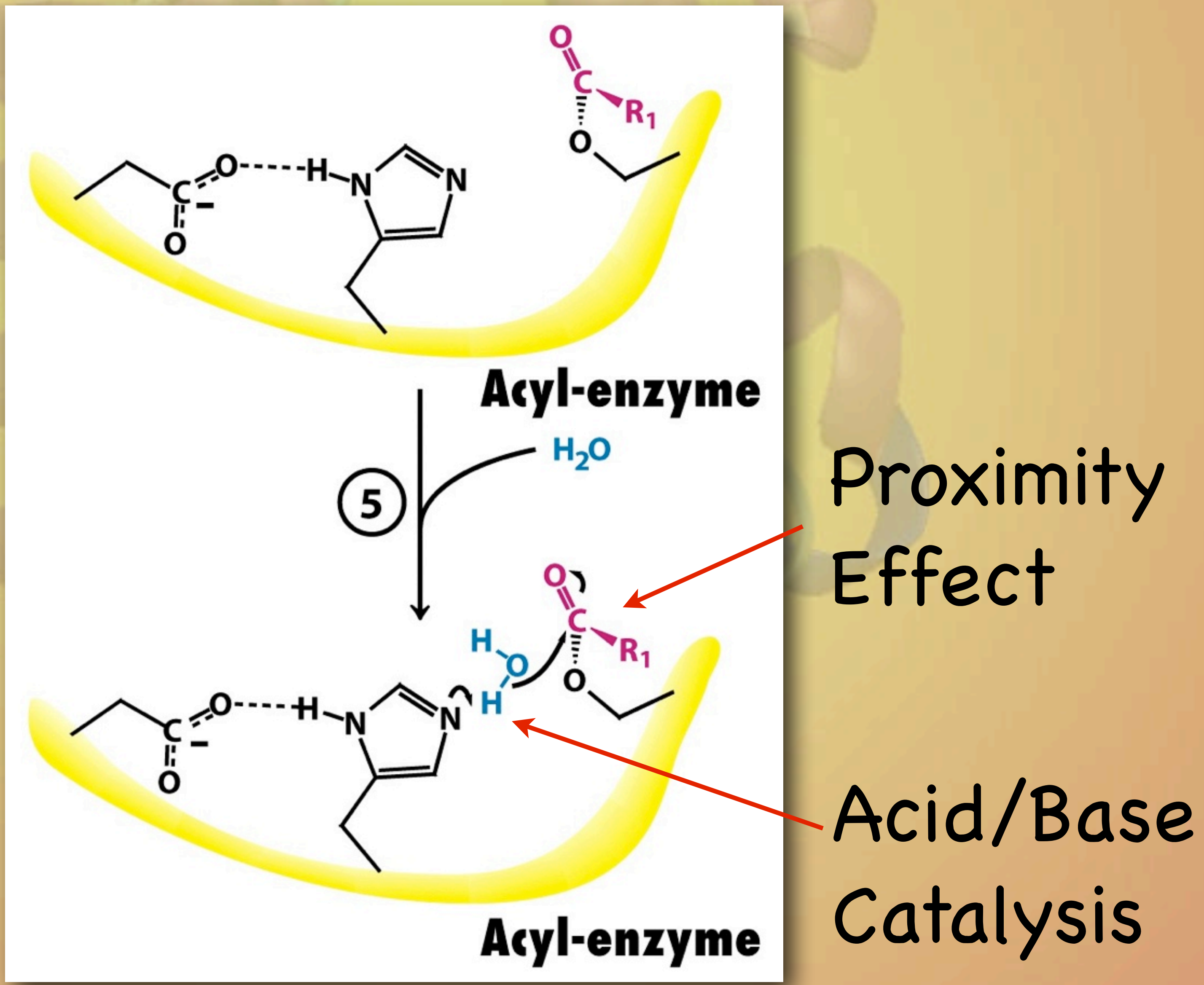


Proximity Effect

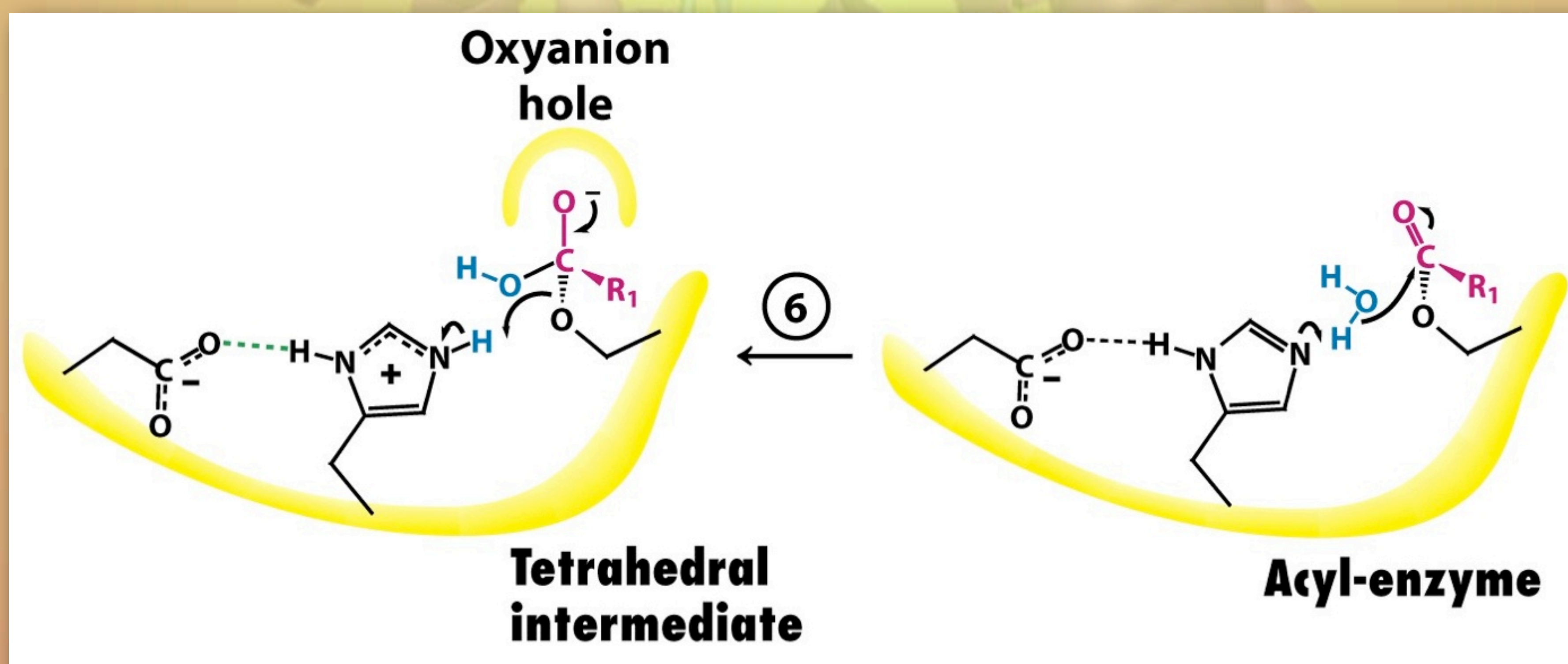
Chymotrypsin Catalytic Cycle

- Step 5: Binding of the second substrate (H_2O)



Chymotrypsin Catalytic Cycle

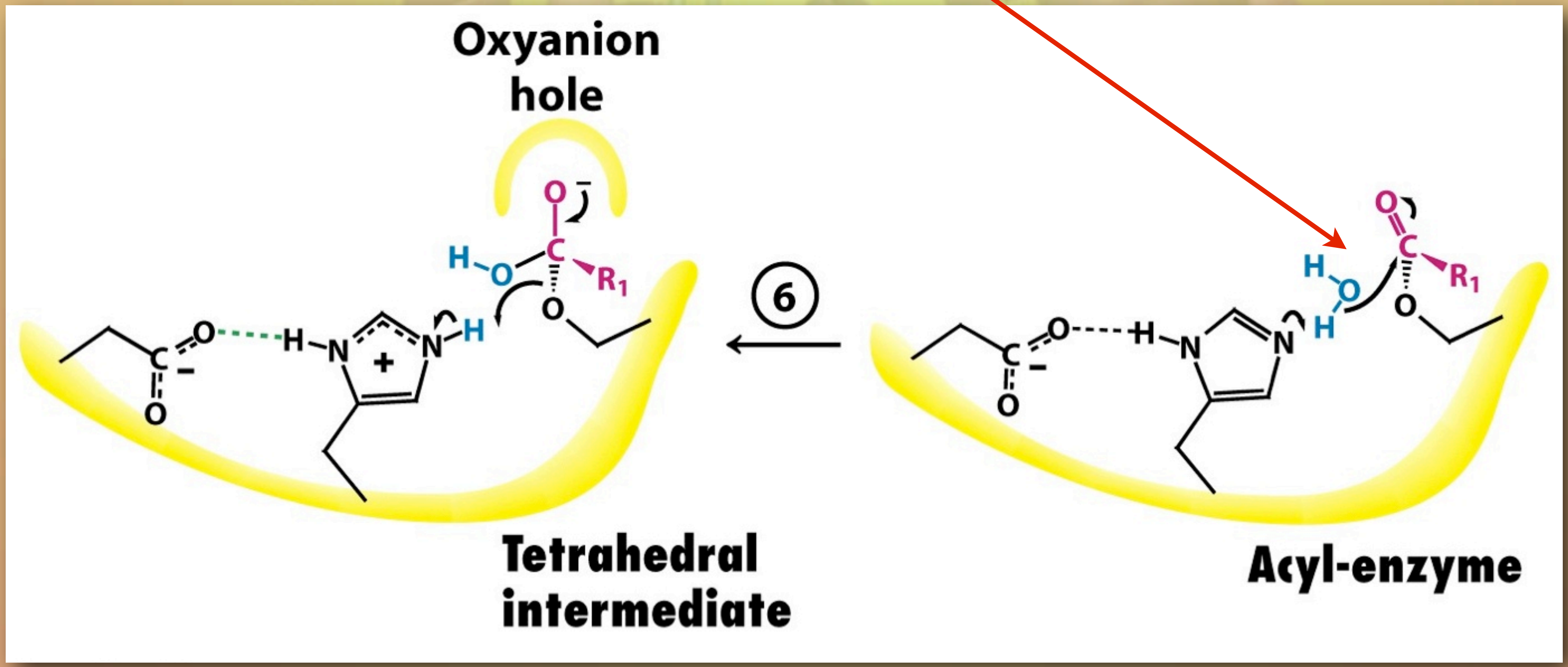
- Step 6: Transition state formation



Chymotrypsin Catalytic Cycle

- Step 6: Transition state formation

Covalent
Bond
Catalysis

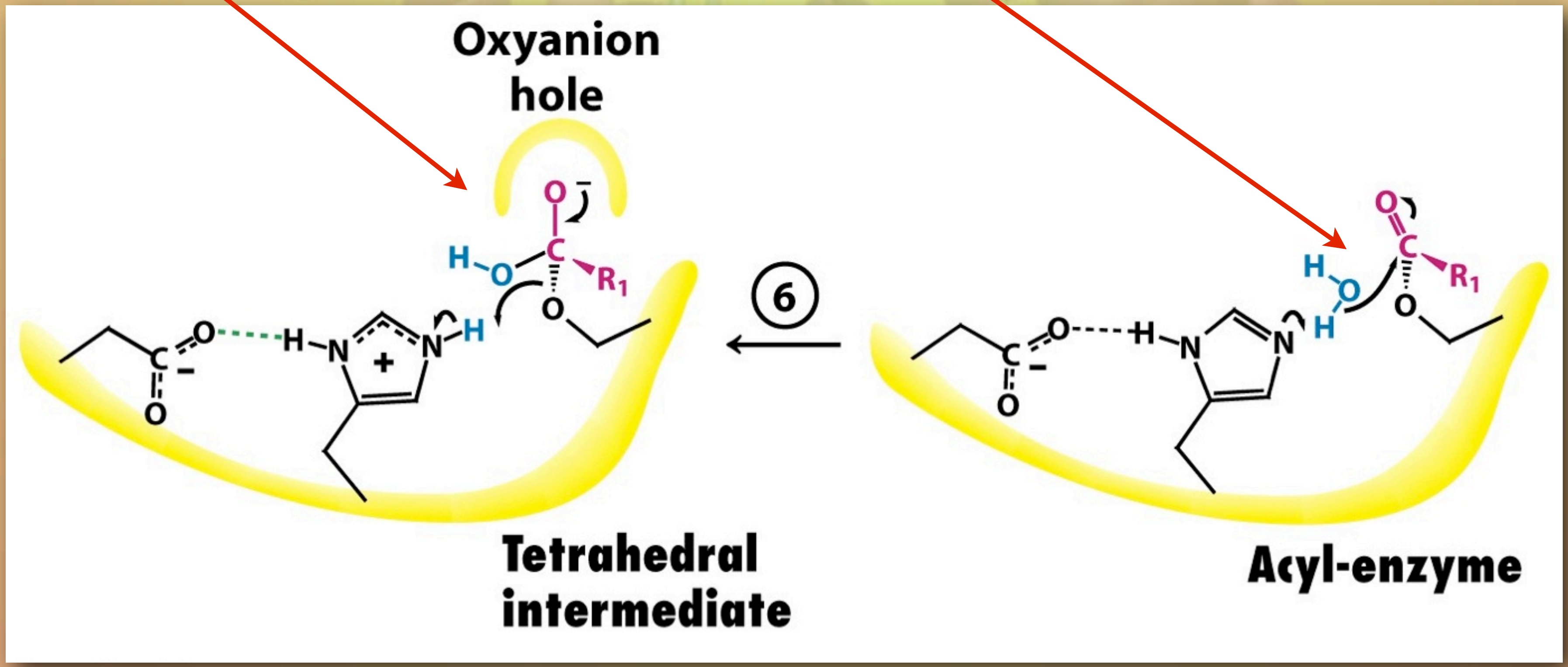


Chymotrypsin Catalytic Cycle

- Step 6: Transition state formation

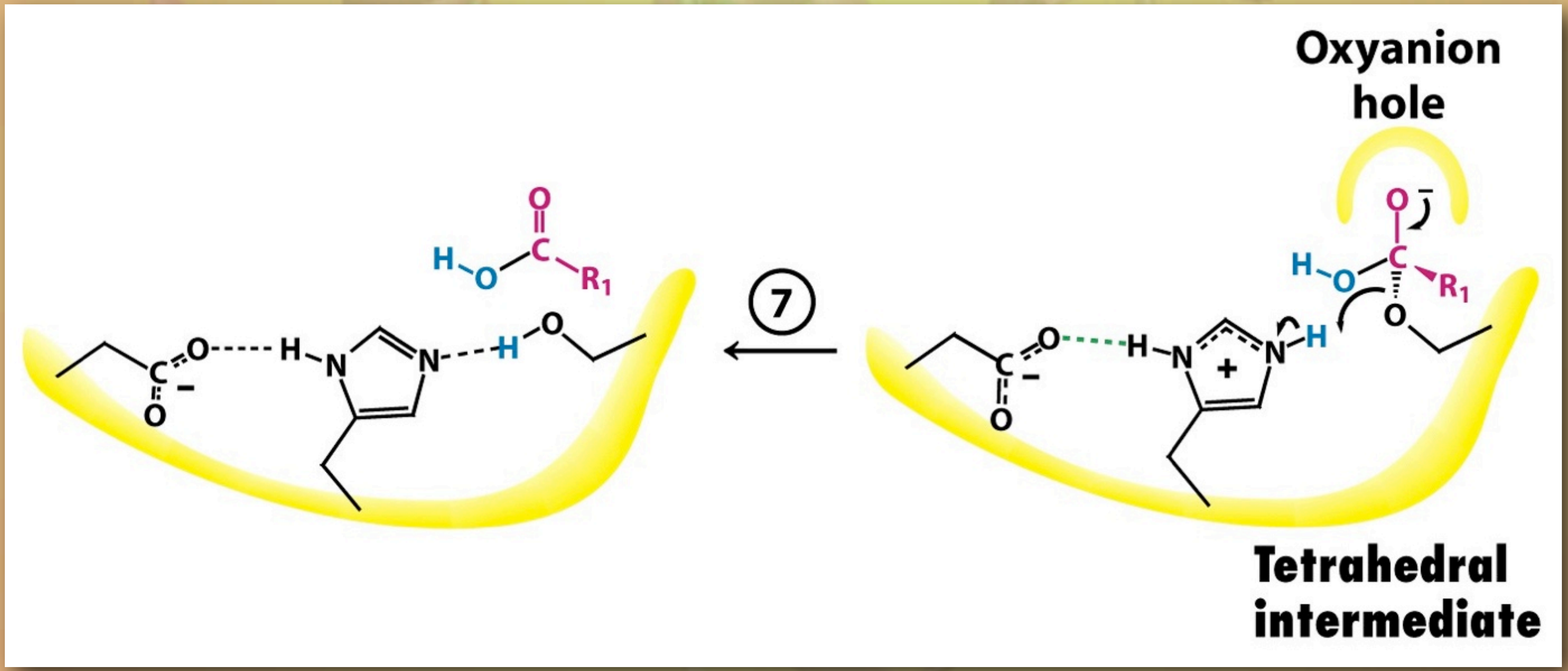
Transition State Stabilization

Covalent Bond Catalysis



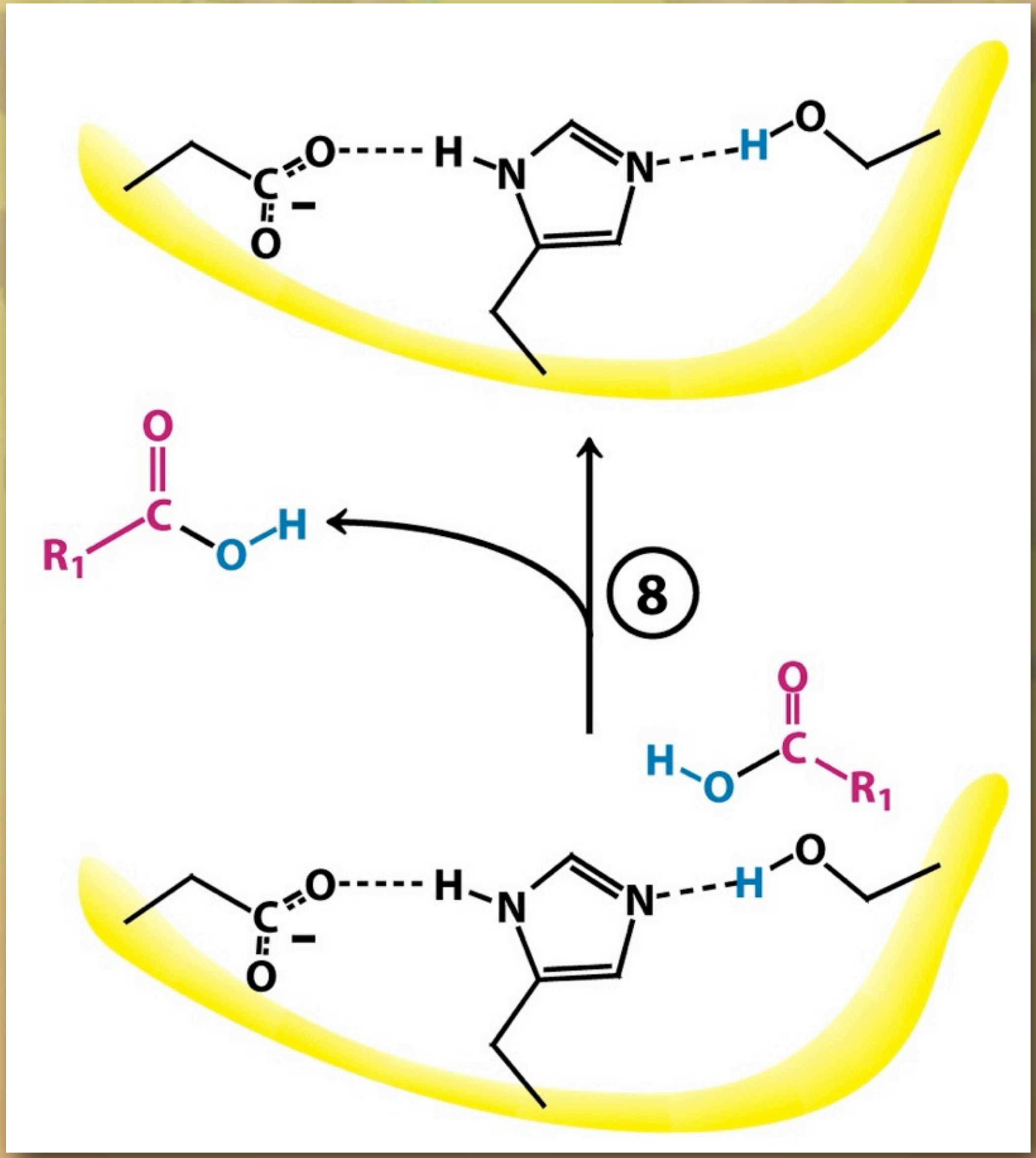
Chymotrypsin Catalytic Cycle

- Step 7: Ester bond cleavage



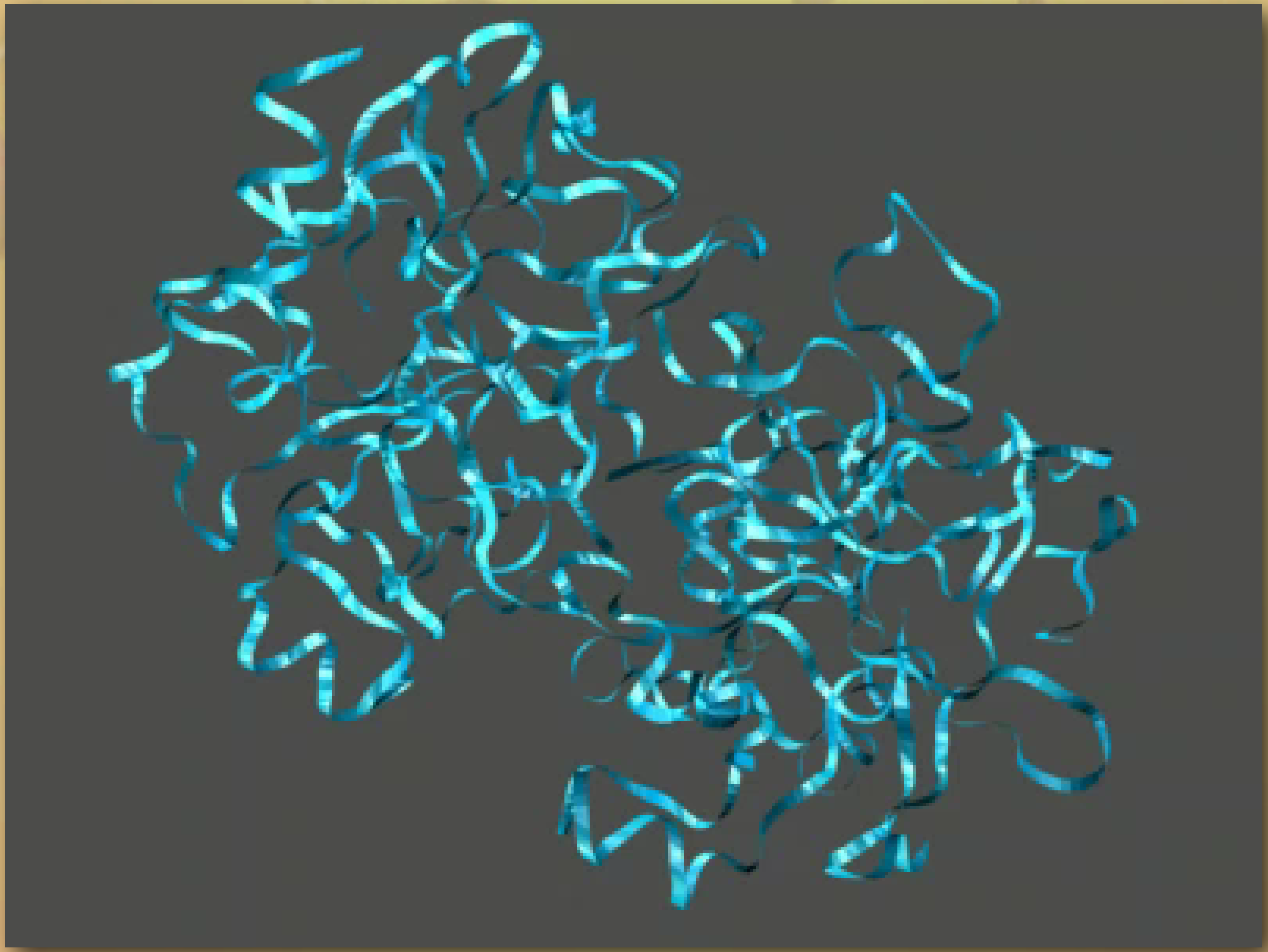
Chymotrypsin Catalytic Cycle

- Step 8: Release of second product (N-terminal half of the peptide)



Chymotrypsin Catalytic Cycle

- Putting it all together:
  - Step-by-Step through the catalytic cycle



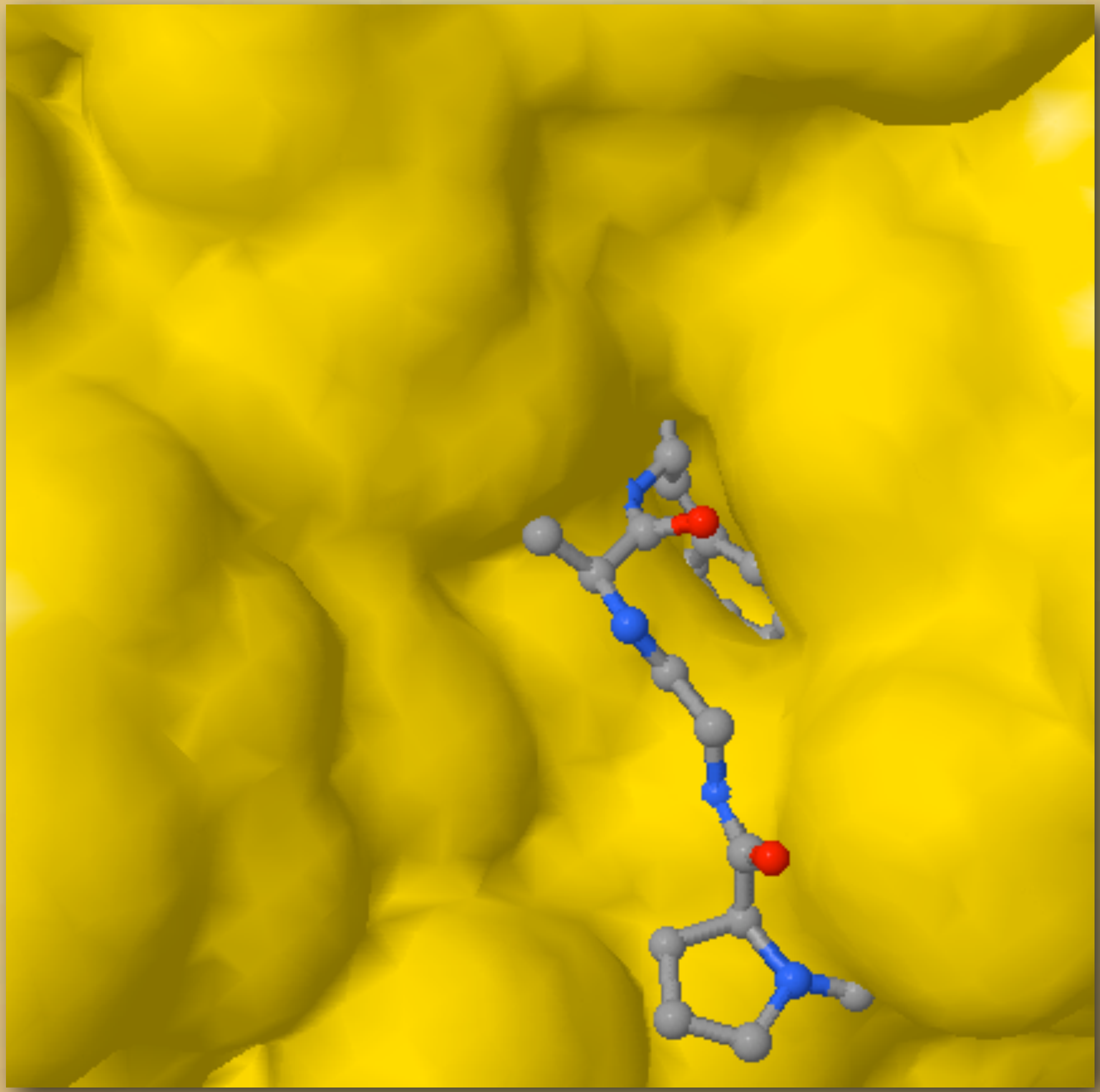
(Click to start animation)

Chymotrypsin

- Covalent Catalysis
  - Ser 195 is converted into powerful nucleophile and leads to a covalent, enzyme-bound intermediate.
- General Acid/Base Catalysis
  - His 57 does both
- Catalysis by Approximation
  - Binds the substrate with specificity and arranges the various players next to one another.
- Metal Ion Catalysis
  - Nothing here
- Transition State Stabilization
  - The oxyanion hole stabilizes the negatively charged, tetrahedral transition state.

Chymotrypsin

- Substrate Specificity



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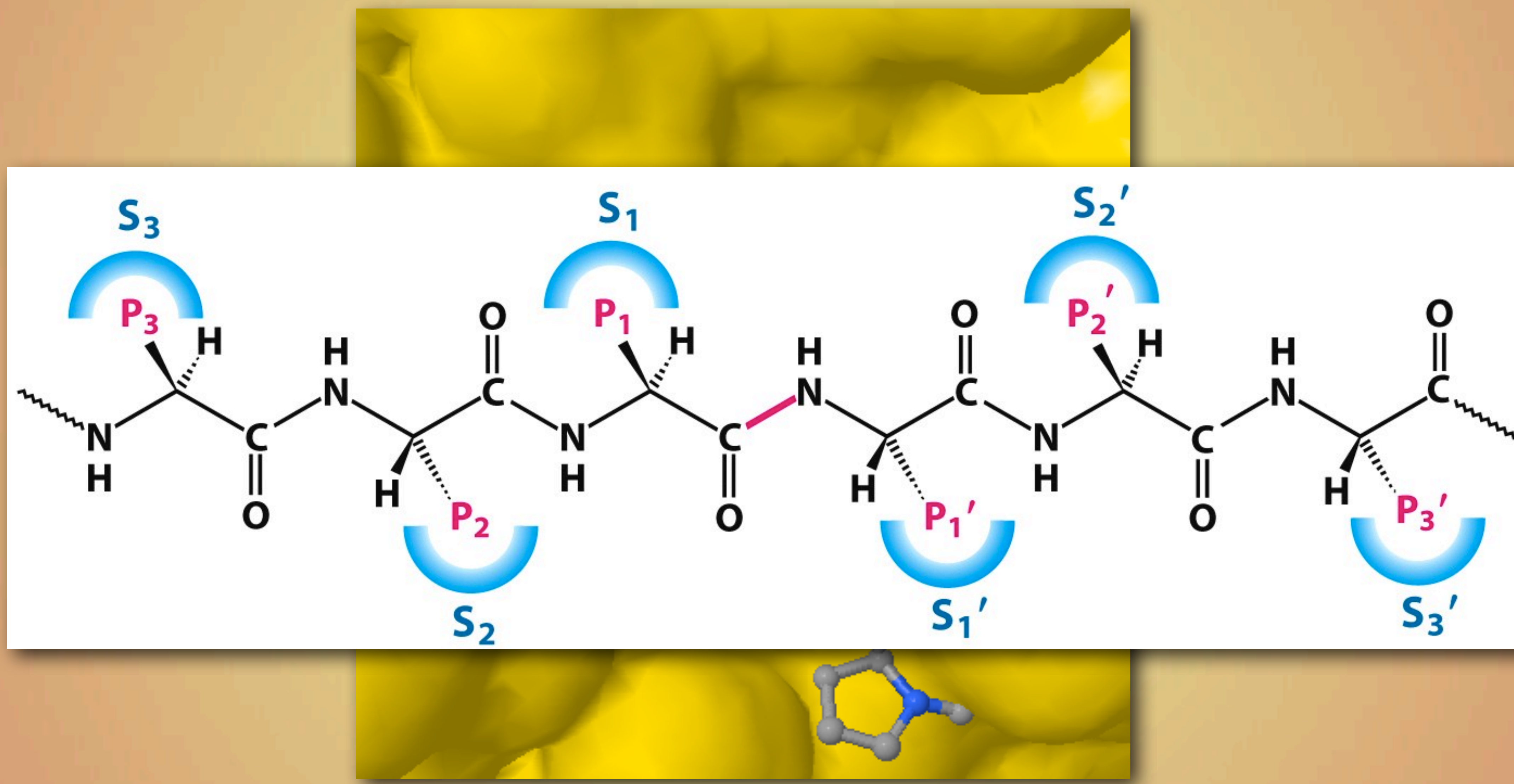
Chymotrypsin

- Substrate Specificity



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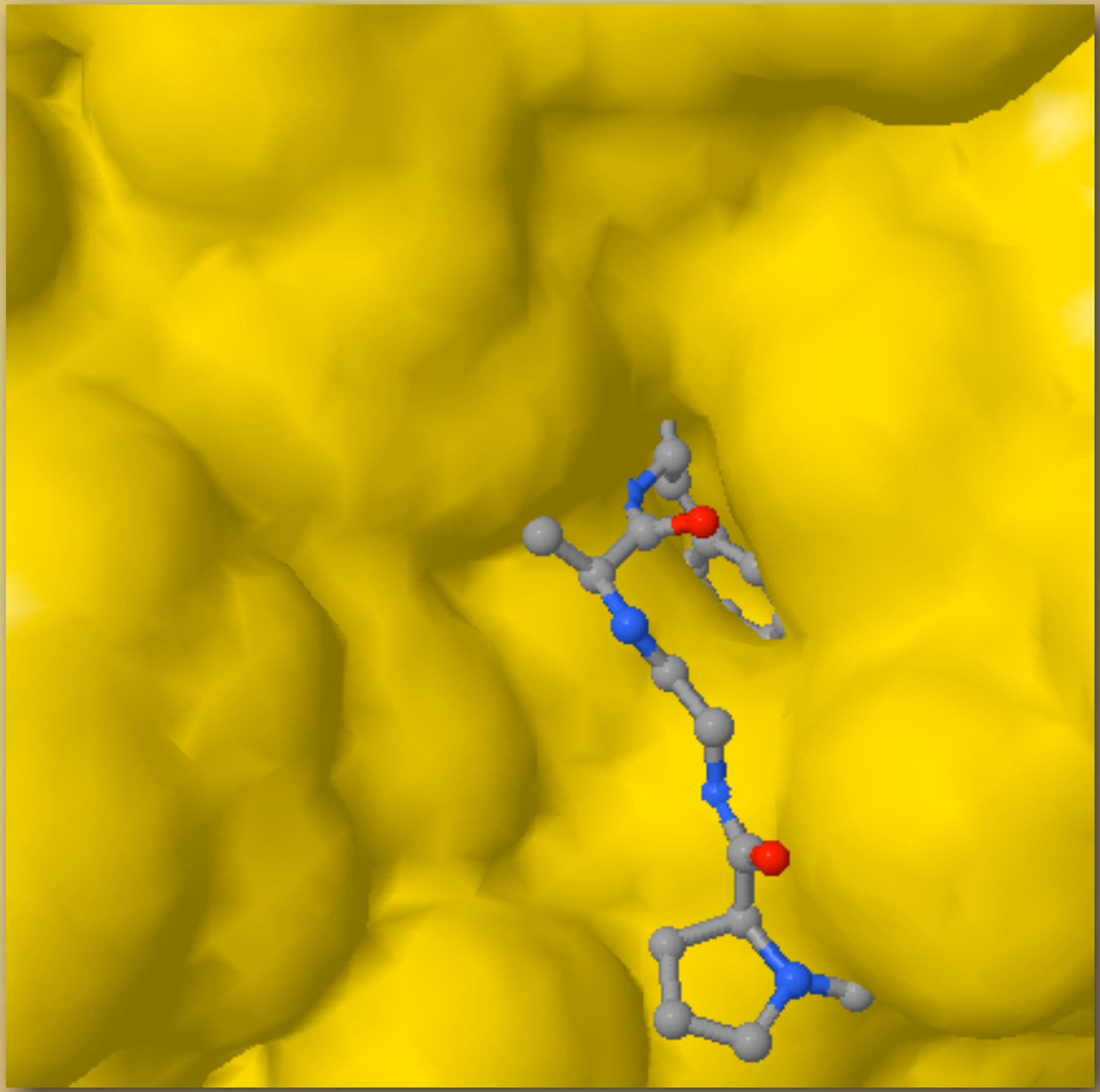
Chymotrypsin

- Substrate Specificity



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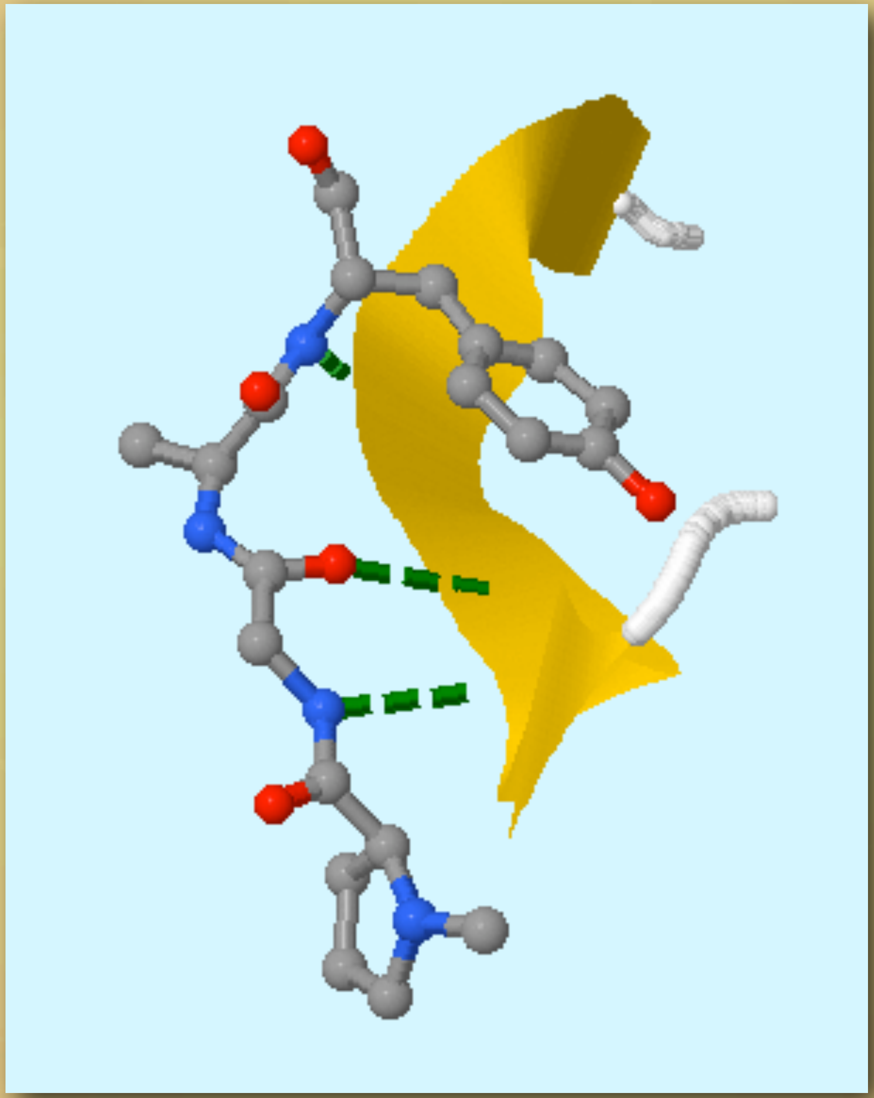
Chymotrypsin

Also illustrates substrate specificity



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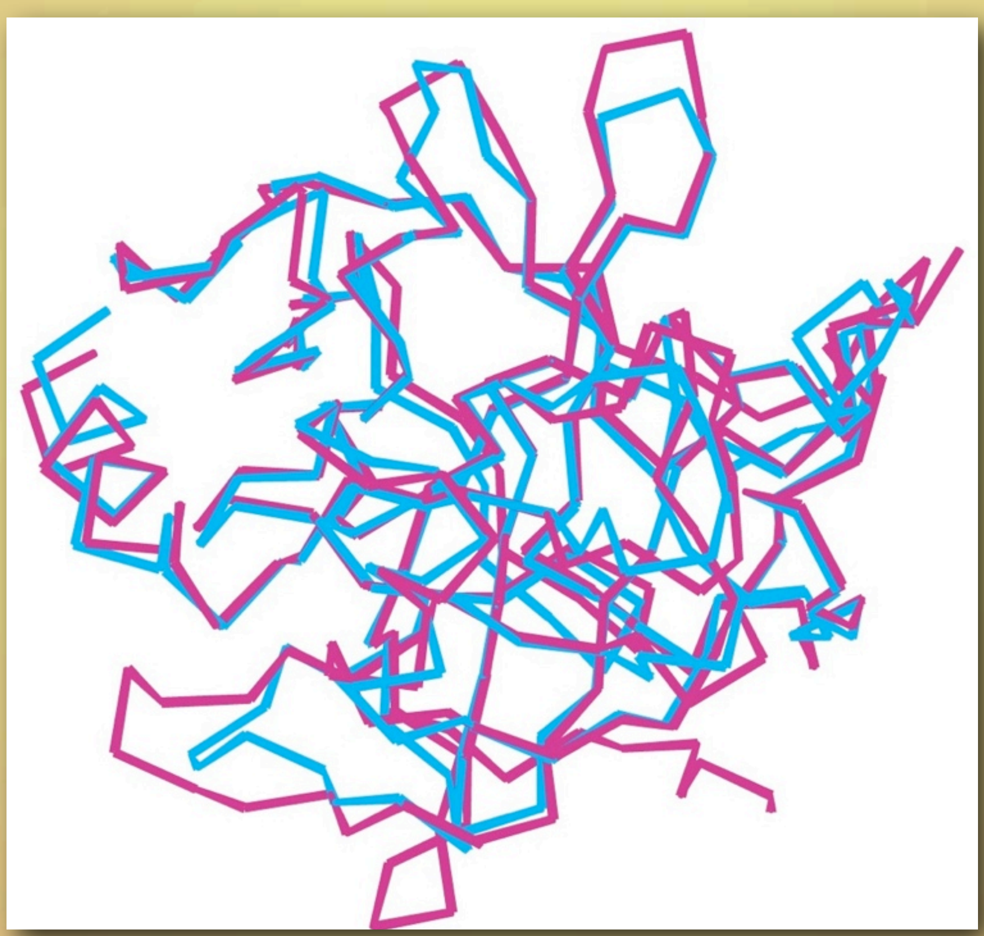
Other Serine Proteases

- Other Homologous Serine Proteases include **trypsin** and **elastase**



Trypsin's structure overlaid on Chymotrypsin's

Other Serine Proteases

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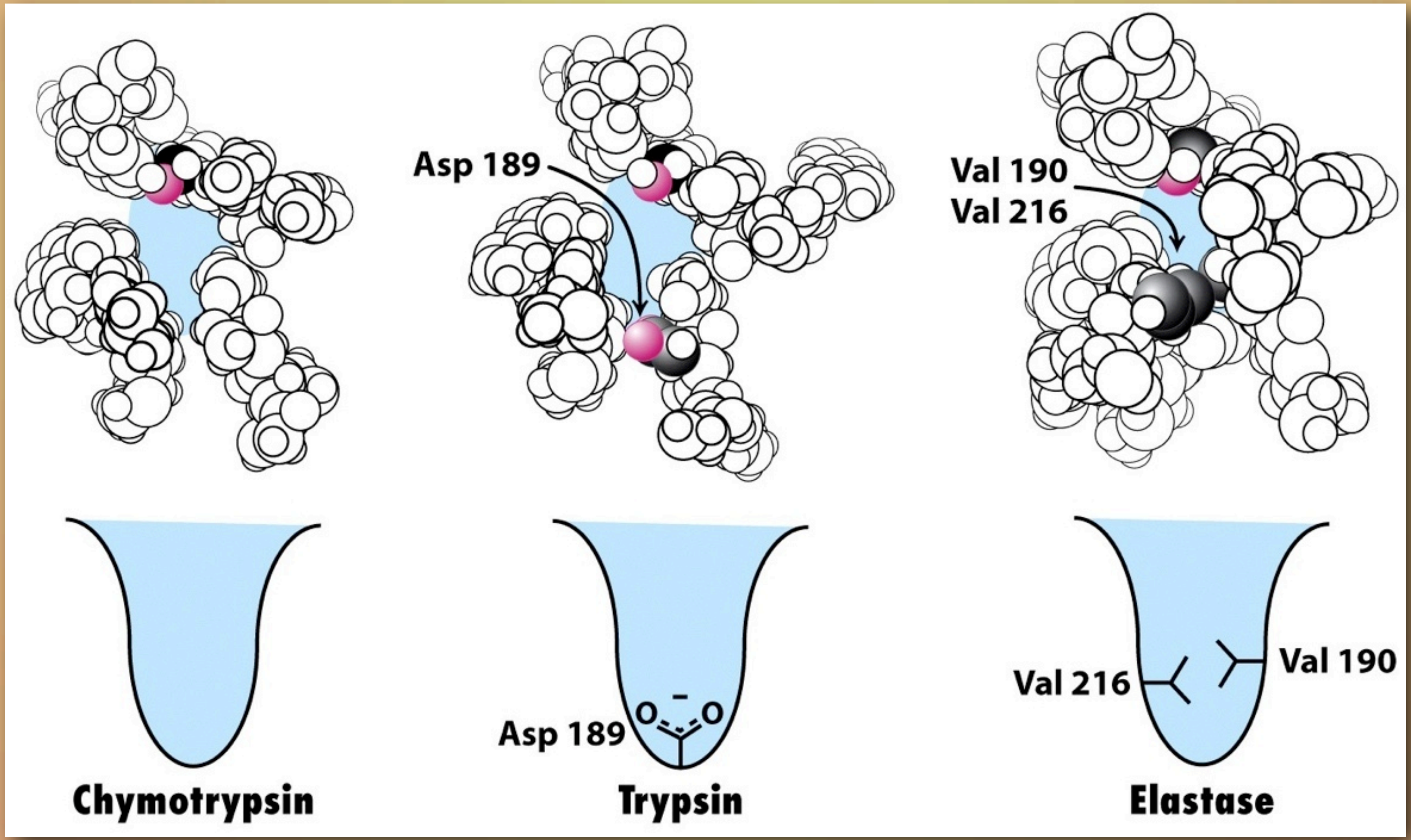
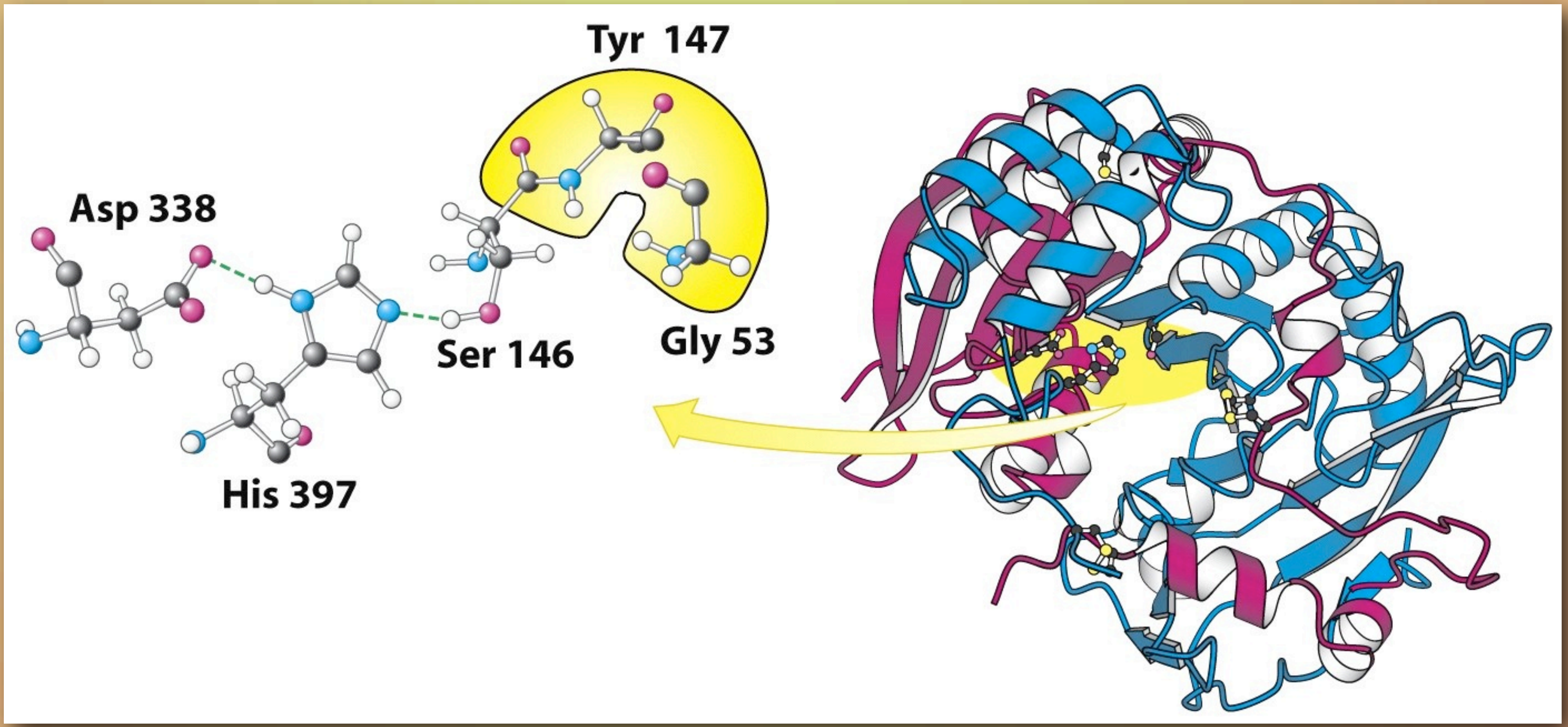


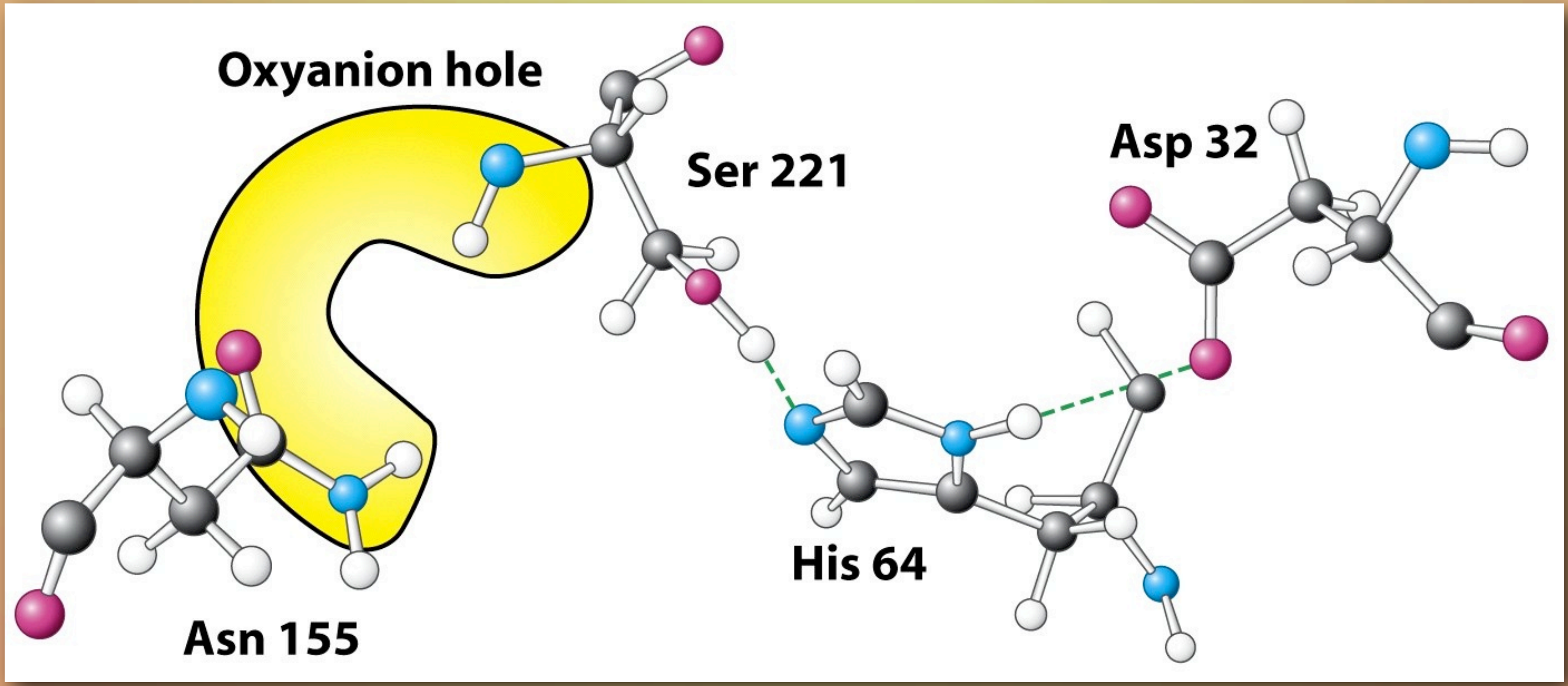
Other Serine Proteases

- Some serine proteases are not homologues of chymotrypsin
  - **carboxypeptidase II**



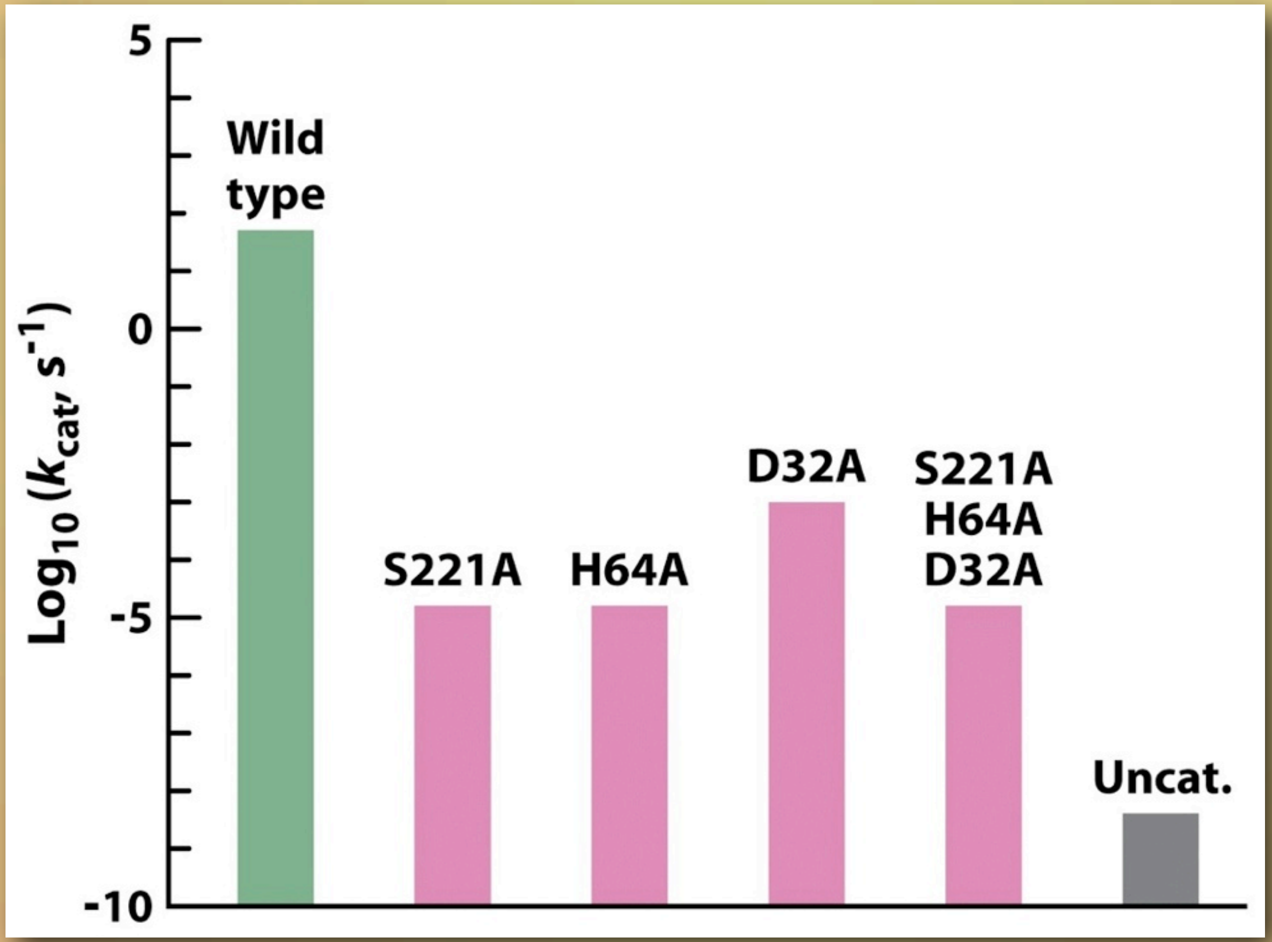
Other Serine Proteases

- Some serine proteases are not homologues of chymotrypsin
  - **subtilisin**



Other Serine Proteases

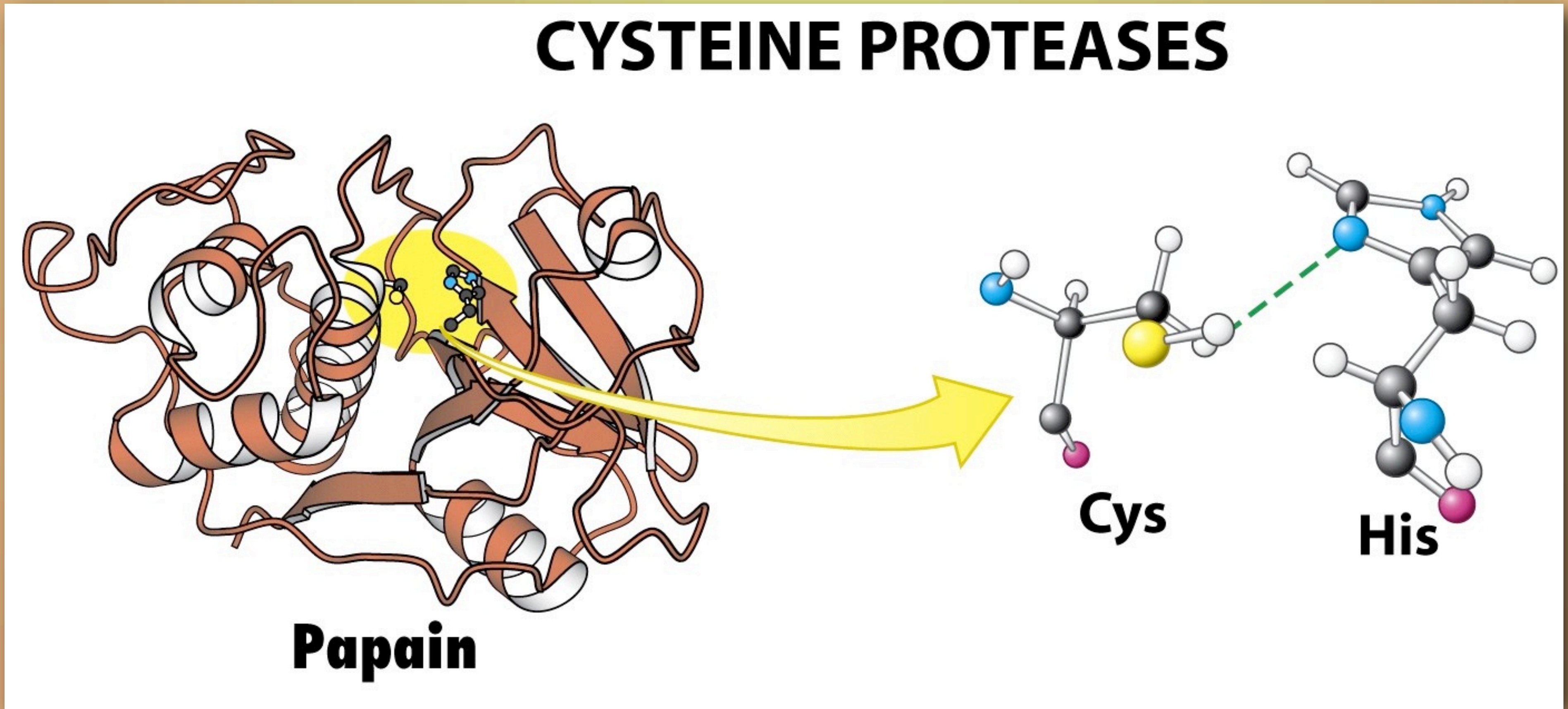
- Investigating the catalytic triad by site-directed mutagenesis



Subtilisin

Other Proteases

- Other strategies are used to hydrolyze peptide bonds:



Other Proteases

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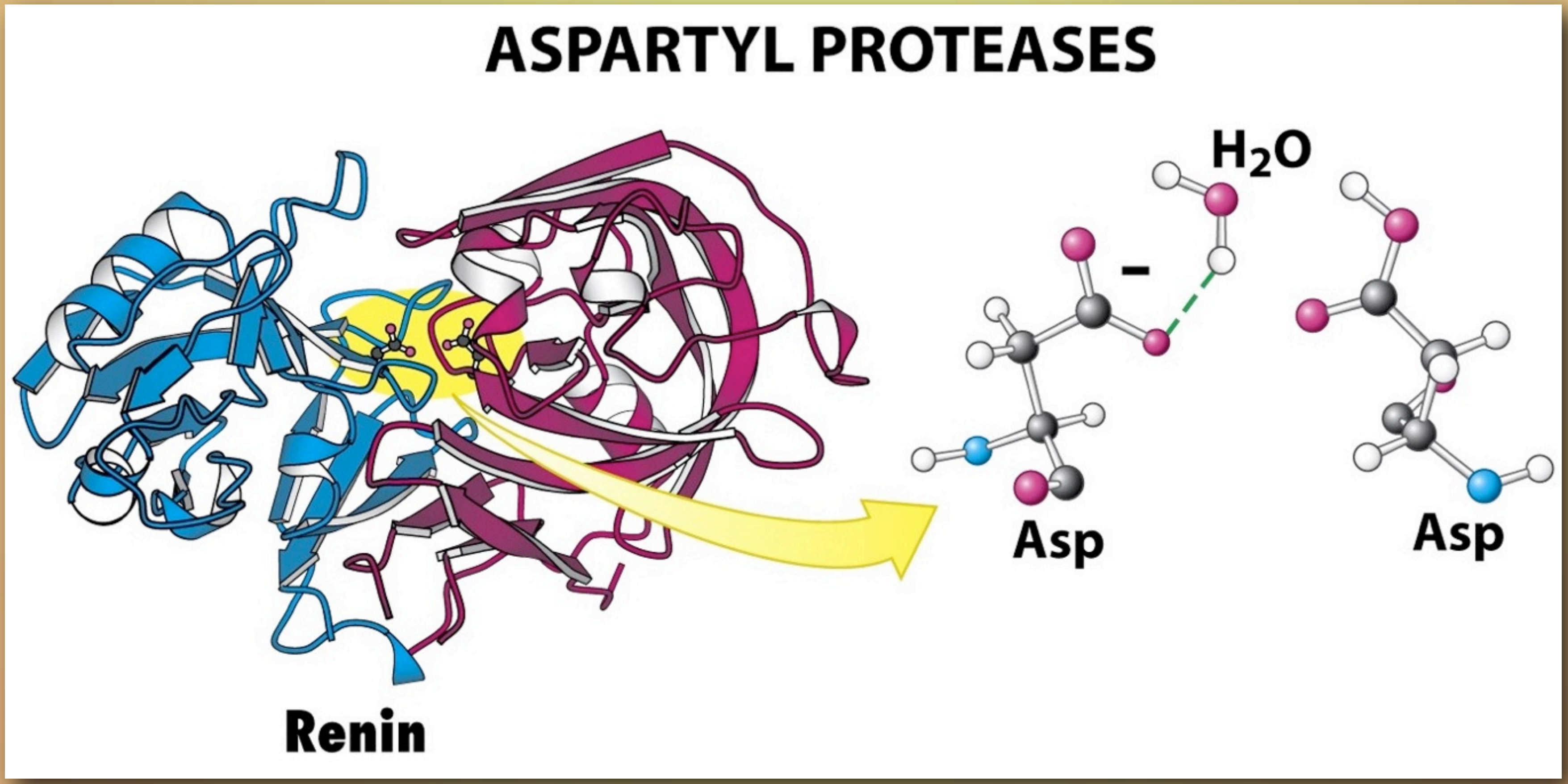

Other Proteases

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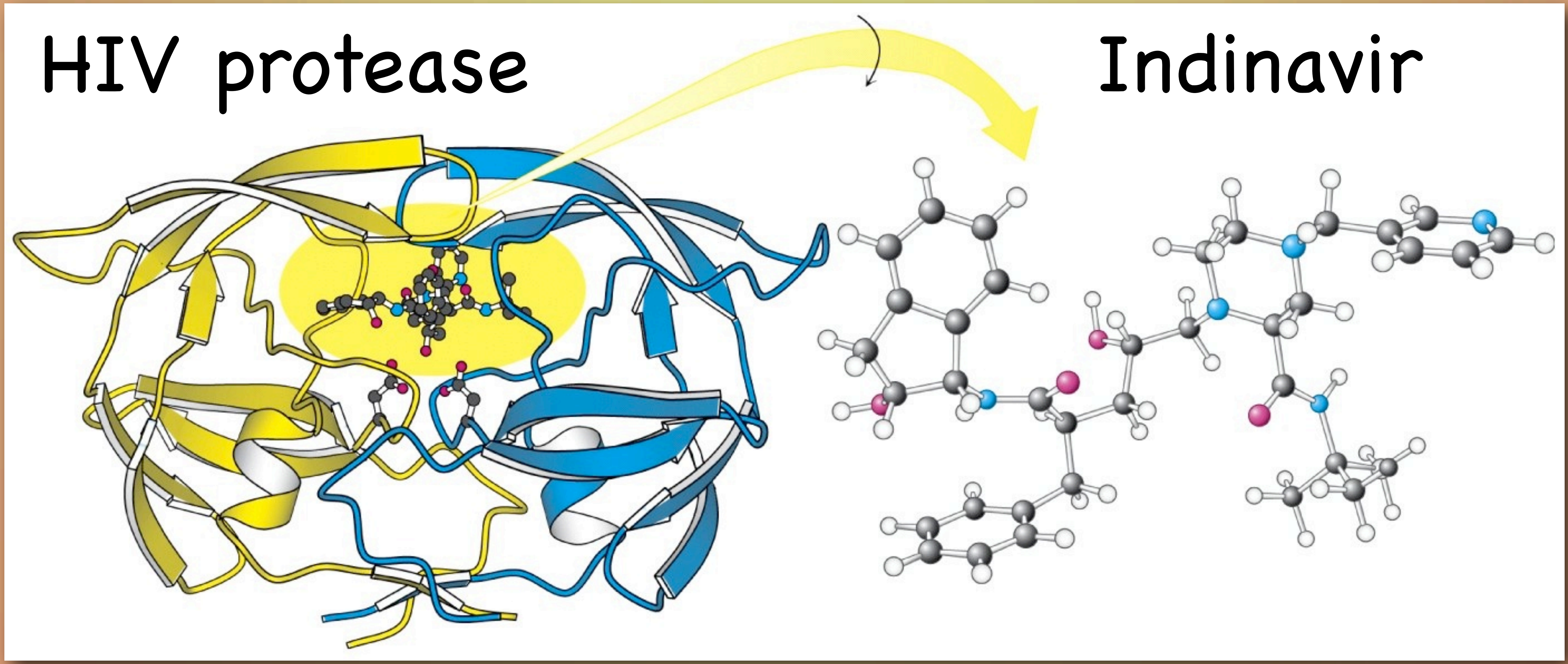

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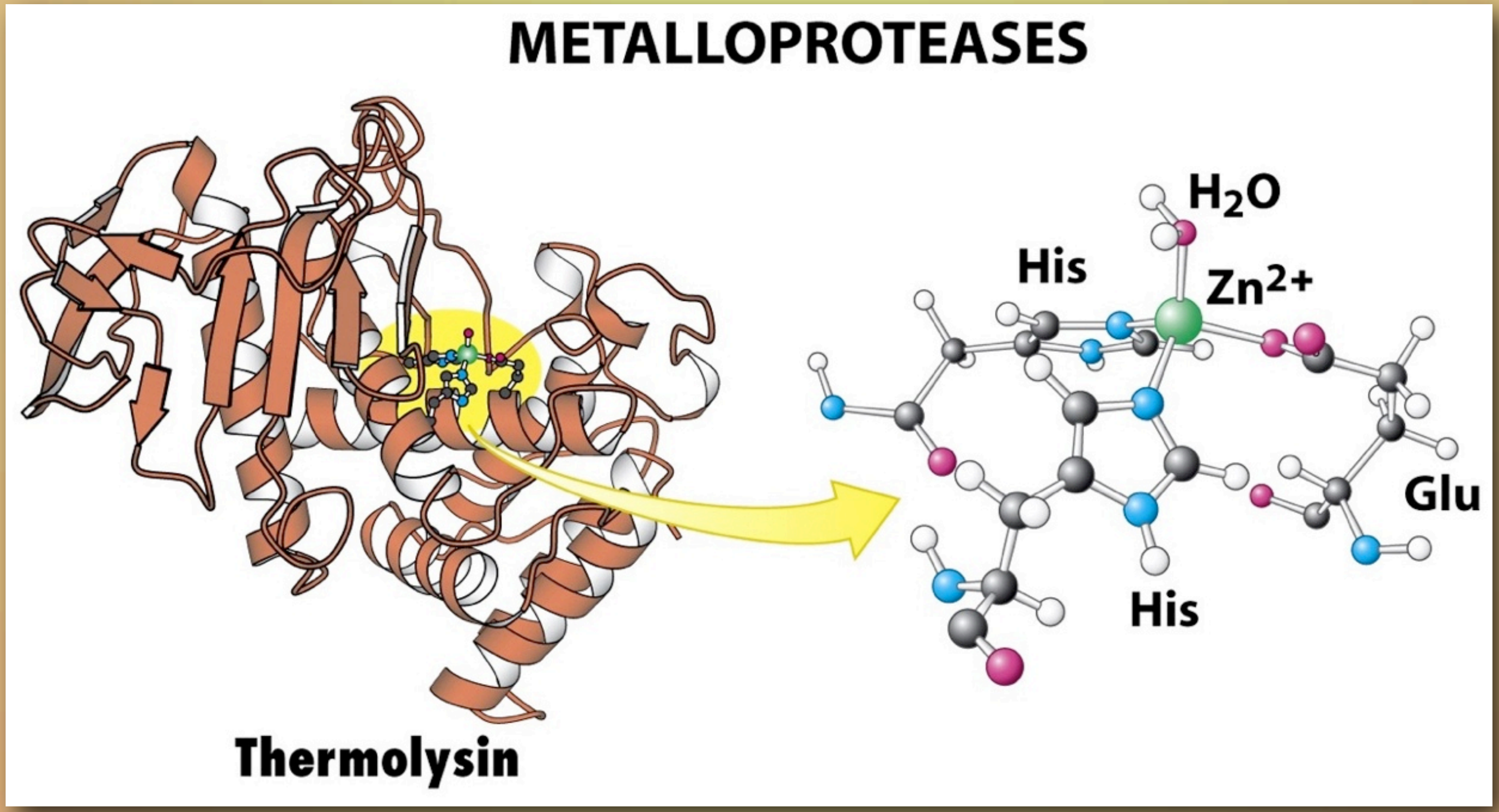

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Next up

- Catalytic Strategies, con'd (Chapter 9)
  - Carbonic Anhydrase
  - EcoRV
  - Myosin II ATPase