

III. MECHANISM OF ENZYME CATALYSIS

(Lehninger Ch 6; P&S Ch 5; Fer Ch 2, 9; Palm Ch 10,11; Zub Ch 9; V&V Ch 15)

Lect#9: Enzyme Mechanisms-I

A. Reaction Mechanisms and Catalysis

(1) Enzyme-transition state complementarity

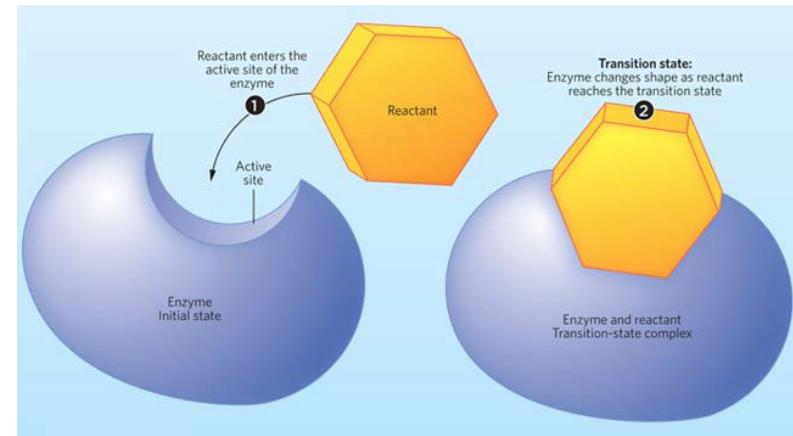
- (a) Structure-activity correlations**
- (b) Transition state analogues**
- (c) Catalytic antibodies**
- (d) Summary**

(2) Preferential transition state binding

(a) Transition state theory

(3) proximity effect

(4) Acid-base catalysts

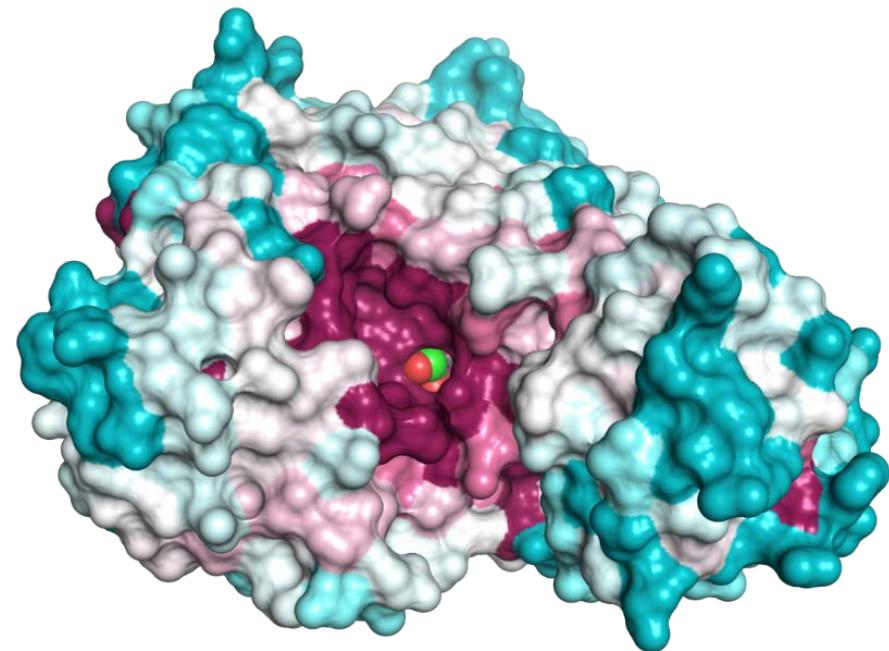


Catalysis: the active site

- **Catalytic residues** tend to be highly evolutionary conserved
- **Binding residues** that determine substrate specificity tend to be less conserved

D	F	-	E	M	D	V	V	A	-	M	V	N	D	T	V	A	T	M	I	S	C	Y	-	-	-	Y	E	D	-
D	F	-	E	M	D	V	V	A	-	M	V	N	D	T	V	A	T	M	I	S	C	Y	-	-	-	Y	E	D	-
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D	I	-	E	M	D	V	V	A	-	M	V	N	D	T	V	A	T	M	I	S	C	Y	-	-	-	Y	E	D	-
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D	F	-	E	M	D	V	V	A	-	M	V	N	D	T	V	A	T	M	I	S	C	Y	-	-	-	Y	E	D	-

ConSurf server (<http://consurf.tau.ac.il>)



The conservation of enzyme residues, mapped on sequence and on structure

Catalysis: mechanisms

- Enzymes lower E_a by various strategies that stabilize the transition state or chemically promote its formation
- For example:
 - Substrate confinement → correct positioning of substrate against catalytic residues
 - Non-covalent stabilization of reaction intermediates
 - Transfer of electrons between enzyme and substrate atoms (**redox catalysis**)
 - Transfer of protons between enzyme and substrate atoms (**acid-base catalysis**)
 - Chemical bonding between enzyme and substrate (**covalent catalysis**)

A. Reaction Mechanism and Catalysis



(1) Enzyme-transition state complementarity

- transition state is short lived
 - Absolute rate theory specifies that it reacts immediately upon formation
- complementarity between an enzyme and its transition state is a **necessity** for catalysis
- evidence for enzyme-transition state complementarity?
 - Transition state analogues bind target enzyme very tightly

Enzymes catalyze reactions by binding more tightly to their transition states than anything else, up to 10^{20} X tighter than their substrates or products.

(a) No enzyme



(b) Enzyme complementary to substrate



(c) Enzyme complementary to transition state

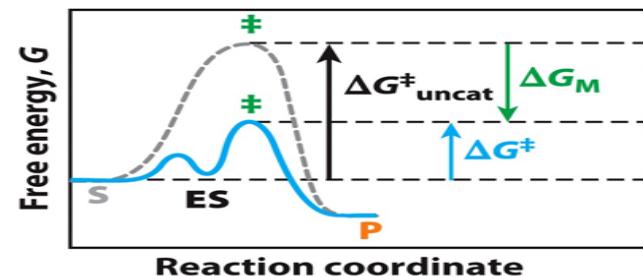
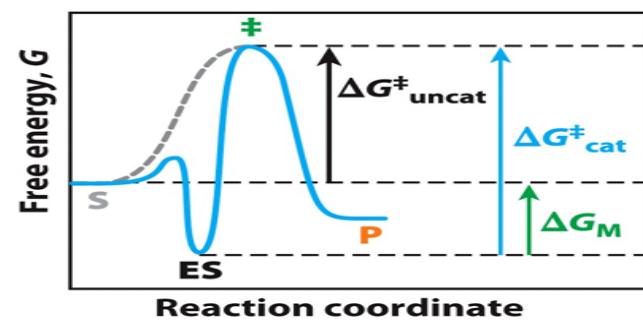
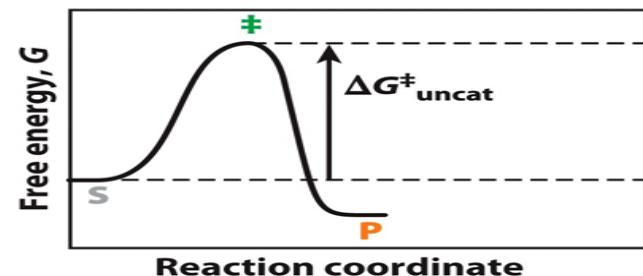
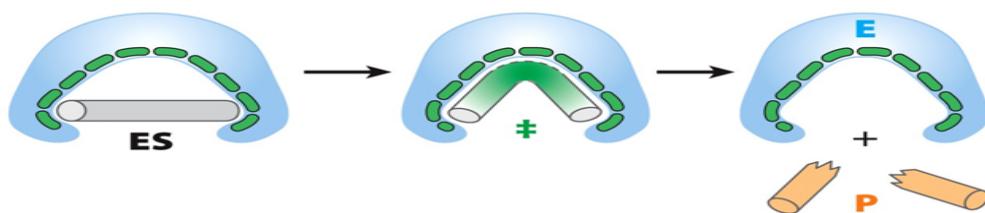
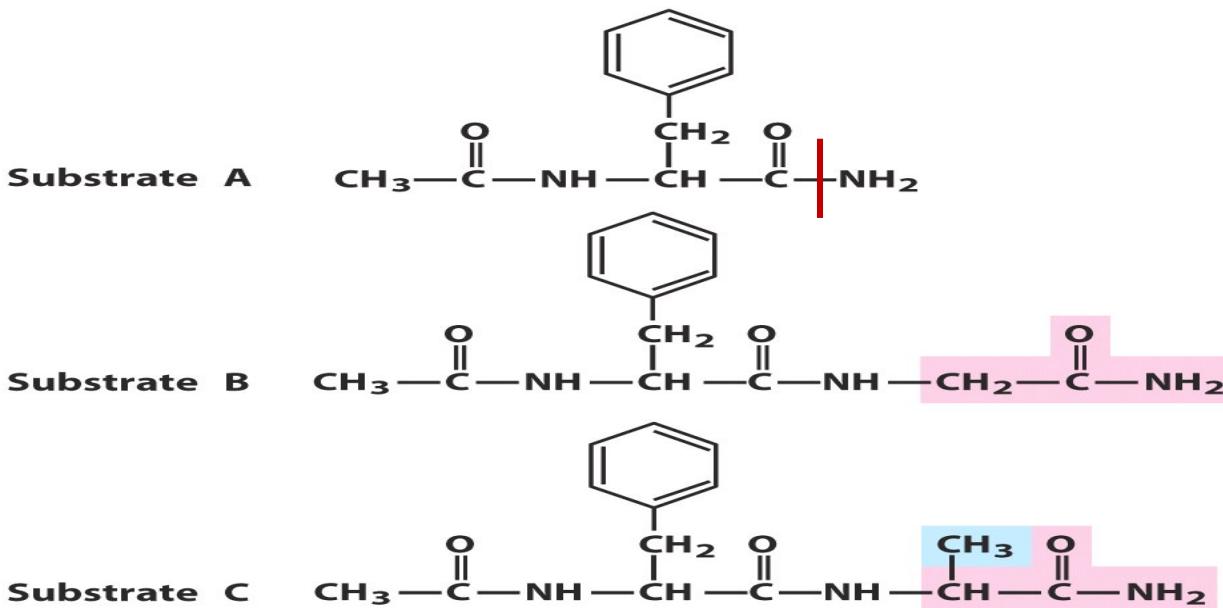


Figure 6-5
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(a) Structure-activity correlations

- if enzymes are complementary to reaction transition states **then** functional groups in both substrate and enzyme must interact preferentially in the transition state
 - altering these groups should not affect K_d for substrate as much as the transition state (TS)
 - the effect on k_{cat} and k_{cat}/K_m should be most obvious

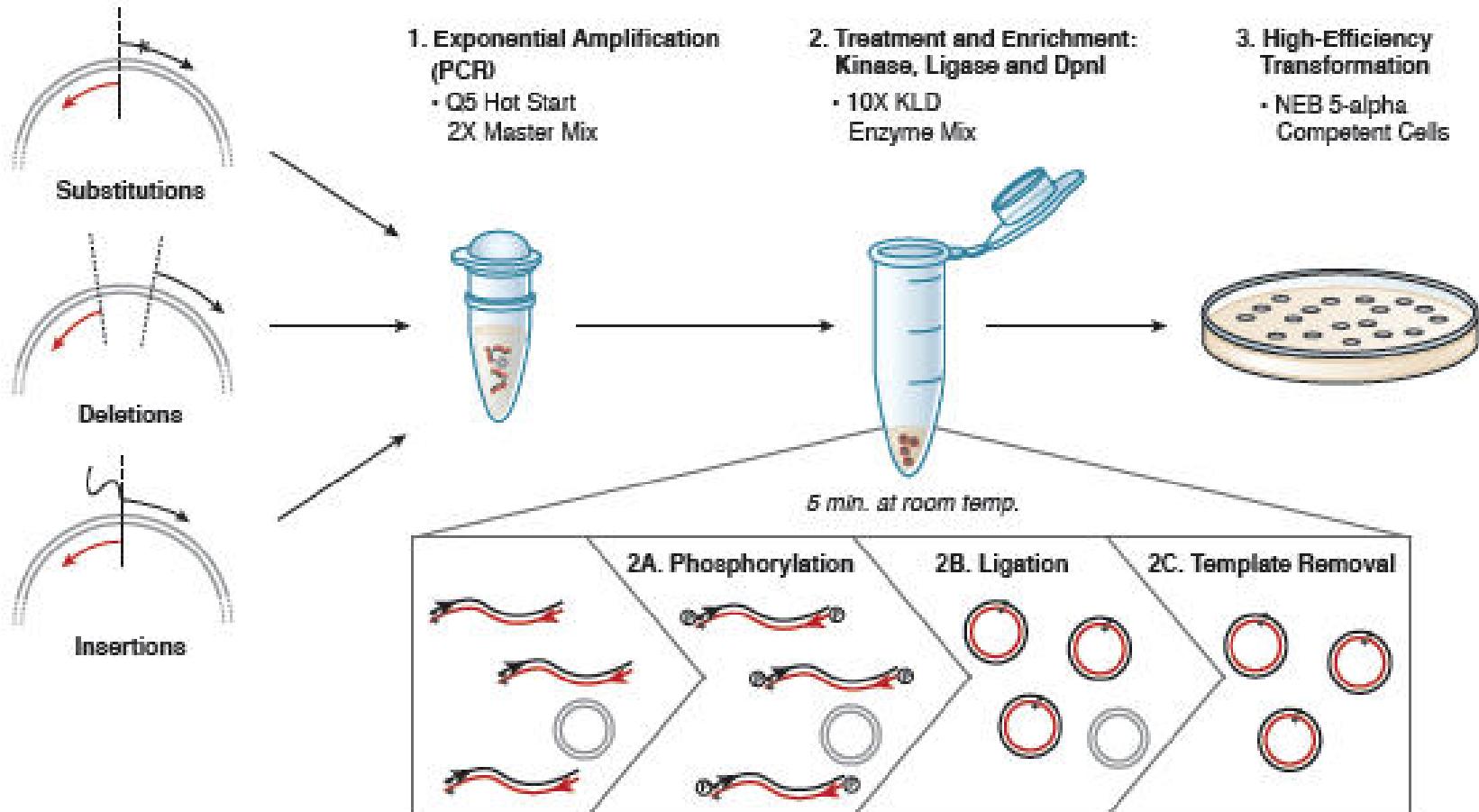
eg., chymotrypsin



k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1} s^{-1}$)
0.06	31	2
0.14	15	10
2.8	25	114

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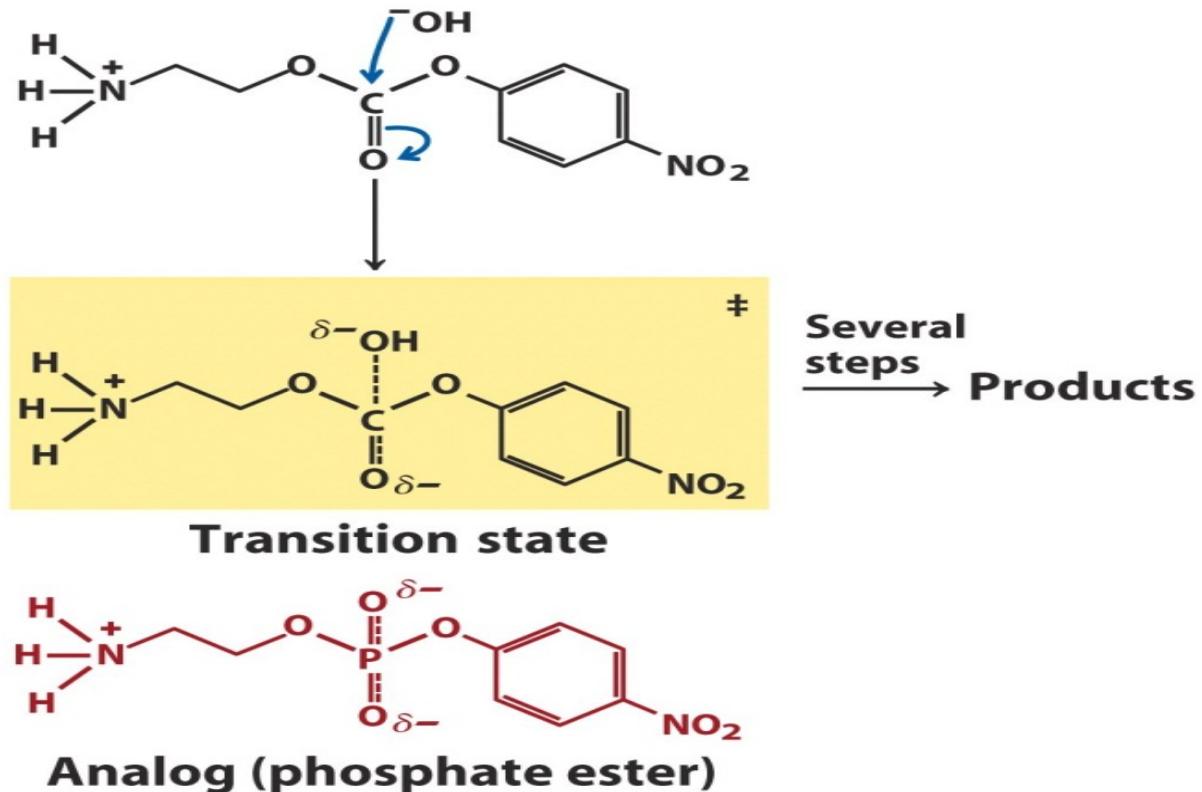
- rate of a reaction can be affected greatly by enzyme-substrate interactions that are **physically remote from the covalent bonds** that are altered
- complementary experimental approach is site-directed mutagenesis
 - change residues in enzyme that interact with the substrate(s)



(b) Transition-state analogues

- transition states can't be observed directly
- can often predict the structure based on knowledge of reaction mechanisms
- transition state** is by definition transient and so unstable that direct measurement of binding interaction between it and the enzyme are not possible

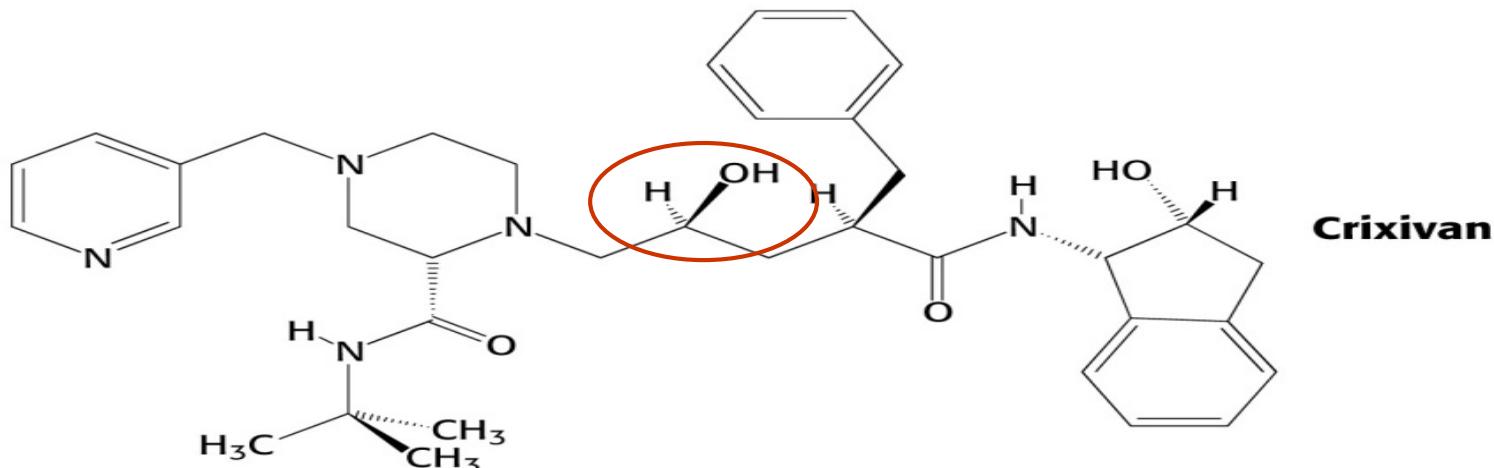
Carbonate hydrolysis



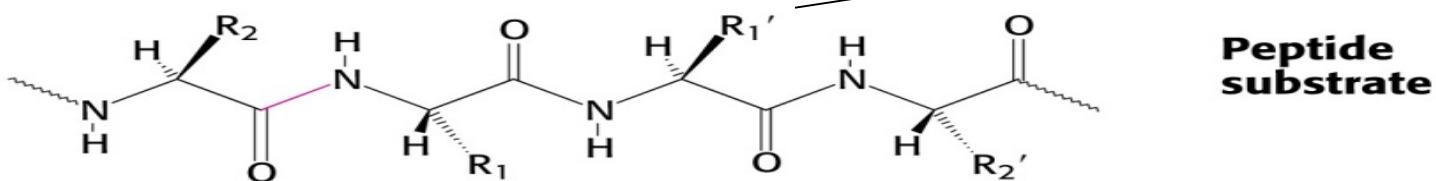
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Transition-state analogues

- molecules that resemble the transition state can be designed and are called **transition-state analogues**
- should bind the enzyme **more tightly** than does the substrate in the E-S complex
- fit active site **better** than substrate, with more noncovalent interactions (higher binding affinity)
- some analogues bind the enzyme 10^6 times more strongly than does the substrate
- pharmaceutical industry **design new drugs** based on the structure of the transition state for the enzyme of interest (eg., anti-HIV drugs against the HIV protease)

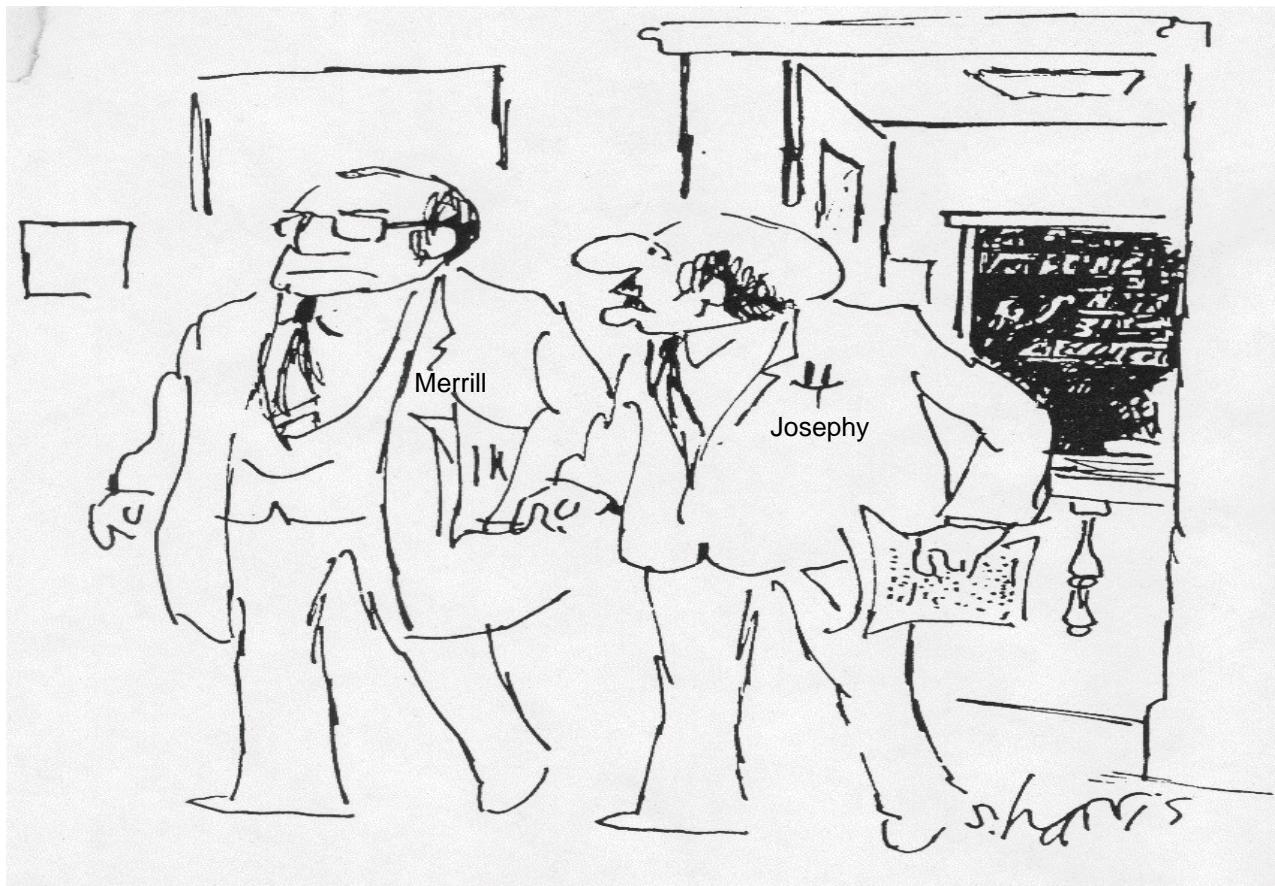


2 Asp from enzyme



S5:F9-20

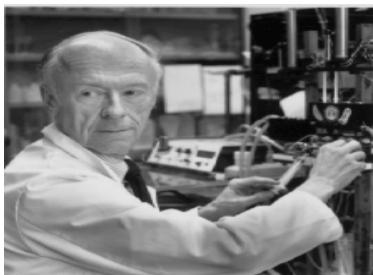
Crixivan is designed around an alcohol that mimics the tetrahedral intermediate formed during peptide bond hydrolysis by the HIV protease



"But don't you see, Dr. Merrill, if the transition-state is too small and too short-lived to detect, we can't just take it on faith that you have discovered it."

(c) Catalytic antibodies (Abzymes)

- if the TS analogue can be designed for the reaction $S \rightarrow P$ then an antibody that tightly binds to this analogue might be expected to catalyze $S \rightarrow P$
- when TS analogue is used as a protein-bound epitope to stimulate the production of antibodies the resulting antibodies are potential catalysts of the reaction



William P. Jencks



Linus Pauling

Linus Pauling conceived the idea and William Jencks developed the method



Chris Farley



River Phoenix



Layne Staley

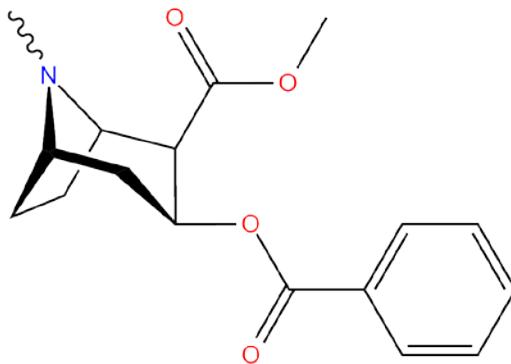


Shannon Hoon



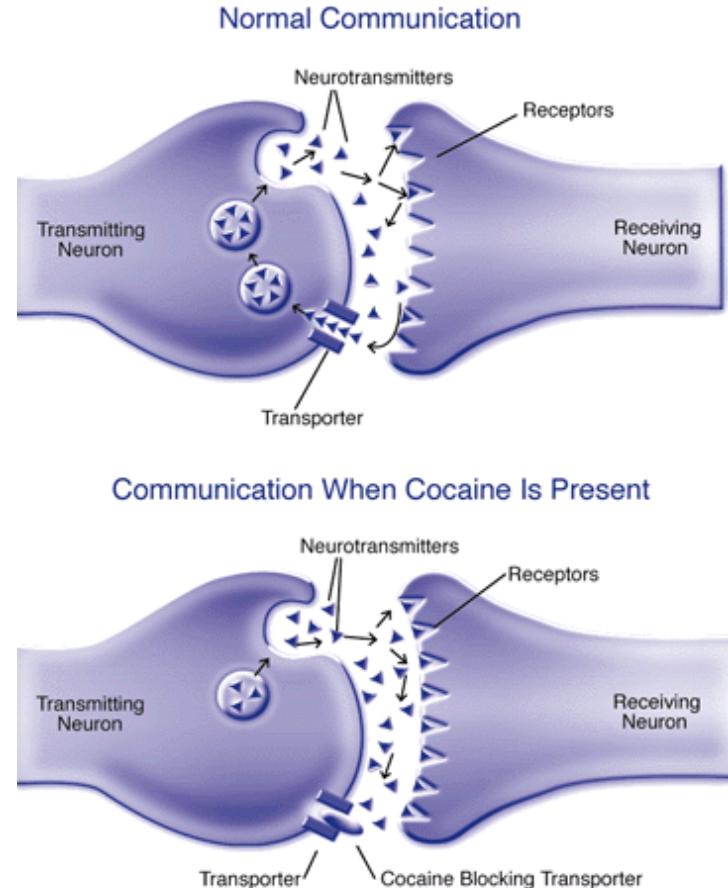
Rob Ford





Cocaine

- Powerful stimulant
- Blocks the neurotransmitter reuptake transporters in the brain
 - Dopamine, serotonin and epinephrine
- Major public health concern worldwide
 - Overdose can result in heart attacks, seizures and death
- Involved in >45% of drug related hospitalizations



A colorful illustration at the top left shows a woman in a straw hat and a red dress, and a man in a hat and vest, working together to assemble or repair a wooden garden fence. They are surrounded by a garden with various flowers and a white house in the background.

**COCAINE
TOOTHACHE DROPS**

Instantaneous Cure!

PRICE 15 CENTS.

Prepared by the

LLOYD MANUFACTURING CO.

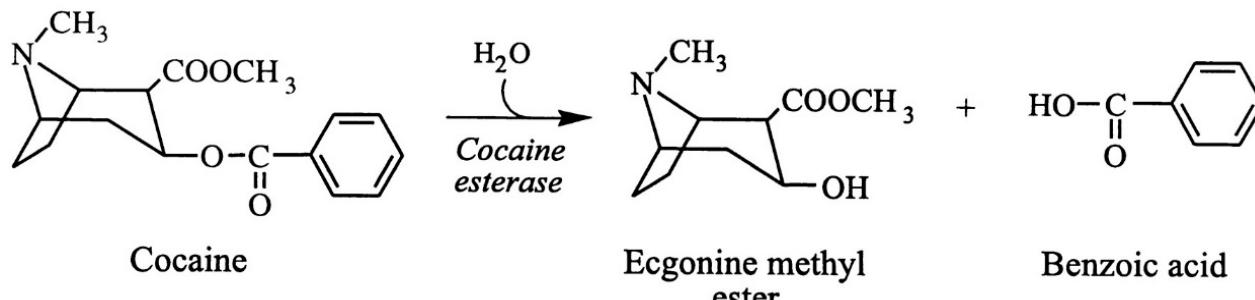
219 HUDSON AVE., ALBANY, N. Y.

For sale by all Druggists.

(Registered March 1885.)

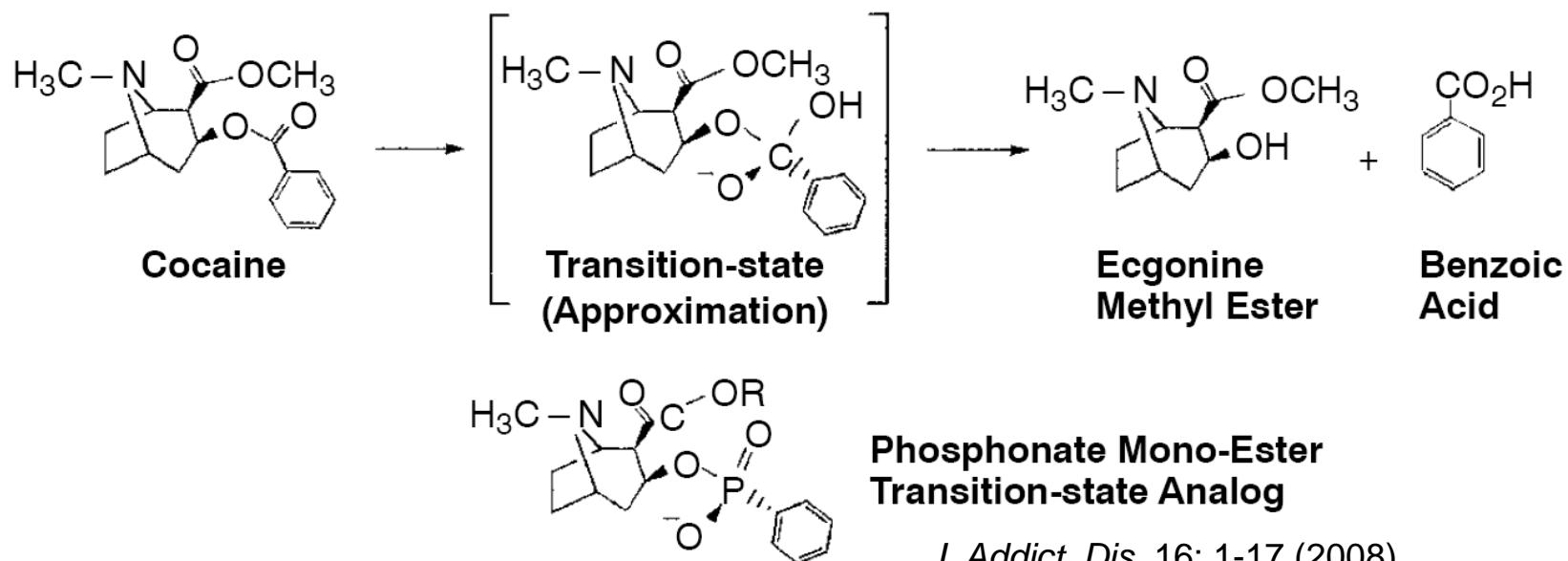
See other side.

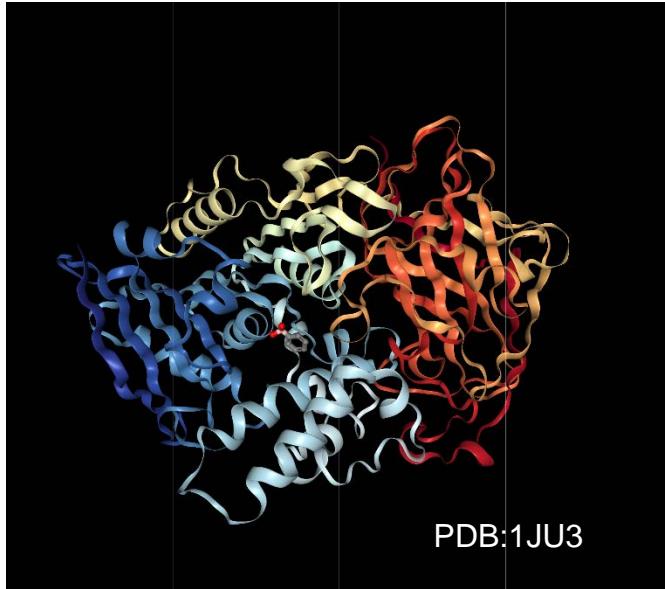
Cocaine esterase (CocE) reaction



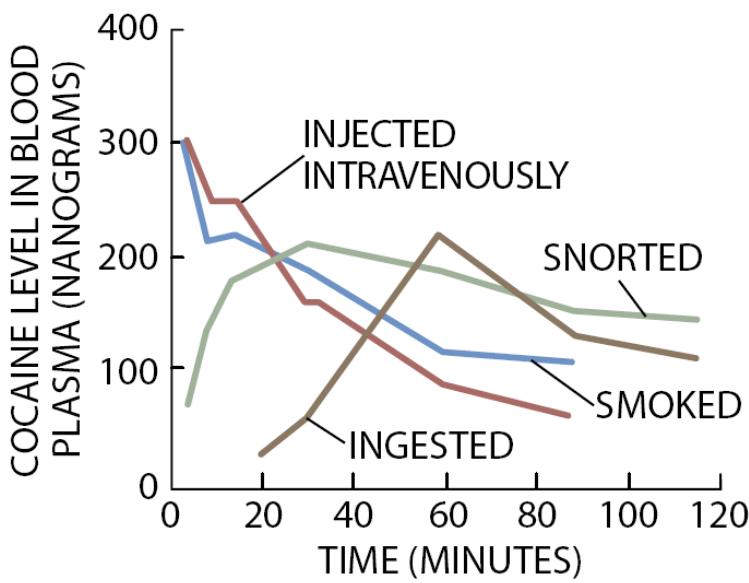
- *Rhodococcus MB1* thrives in the rhizosphere soil of the cocaine-producing plant *Erythroxylum coca* and it produces CocE enzyme (“Bacterial Junkie”)

FIGURE 2. Cocaine hydrolysis and transition state analog.





Bacterial cocaine esterase with transition state analogue



Transition-state analogue of cocaine hydrolysis is used to make abzyme that has CocE activity

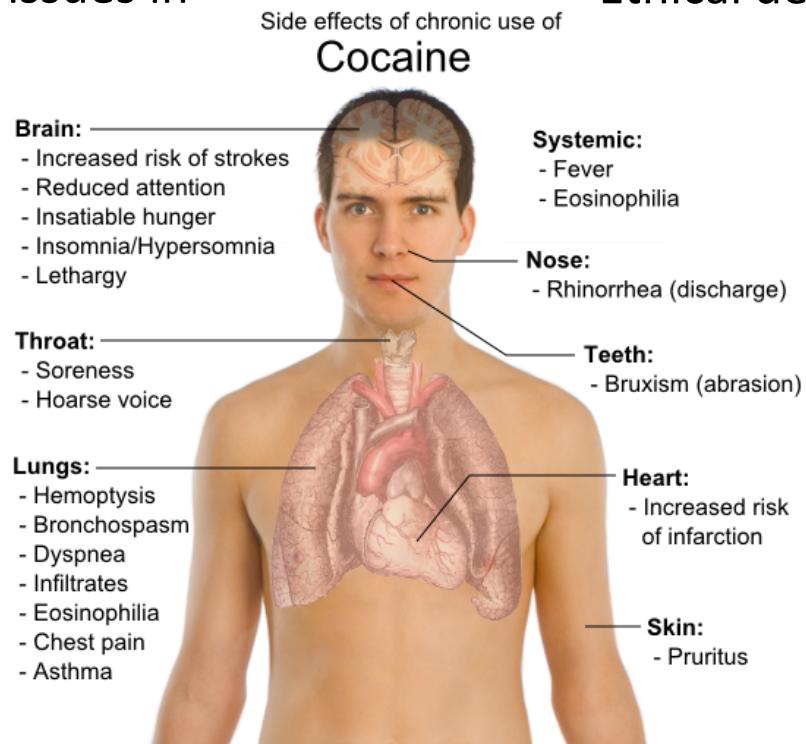
Short Term/Emergency

Inject patient with CocE

- Suitable treatment candidate due to high specificity and activity
- Shown to decrease toxic effects by rapid metabolism
- Possible issues with induction of immune response
- Short $t_{1/2}$ may pose issues in treatment timing

Long Term:

- Immunization with TS analogue
- Produce catalytic antibodies
- Currently, catalytic efficiency has not been reached
- Continuing research to increase abzyme activity
- Ethical debate



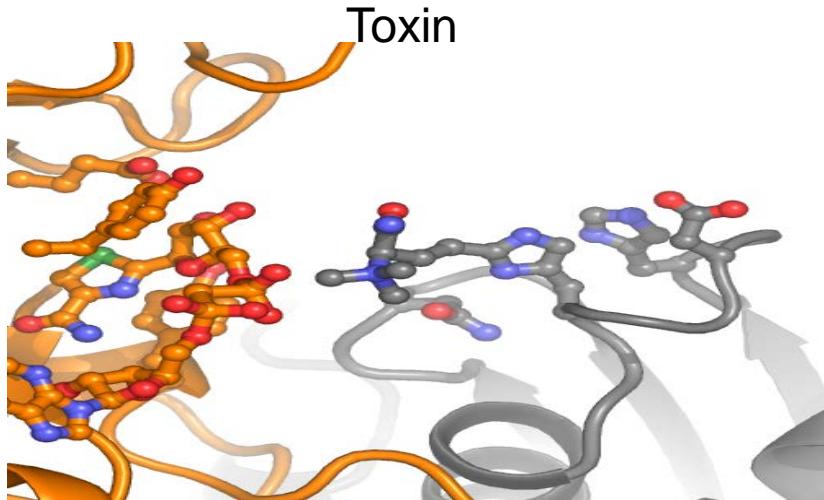
(d) Summary

- Understanding of the complete mechanism of a enzyme-catalyzed reaction requires:

- identification of all substrates, cofactors, products, and regulators
- knowledge of temporal sequence of reaction intermediates
- structure of each intermediate and transition state
- rates of interconversion between intermediates
- structural relationship of enzyme with each intermediate
- energy that all reacting and interacting groups contribute to intermediate complexes and transition states

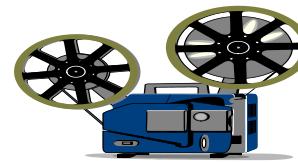
No enzyme for which our current understanding meets all of these requirements!!

Reaction Mechanism for *P. aeruginosa*

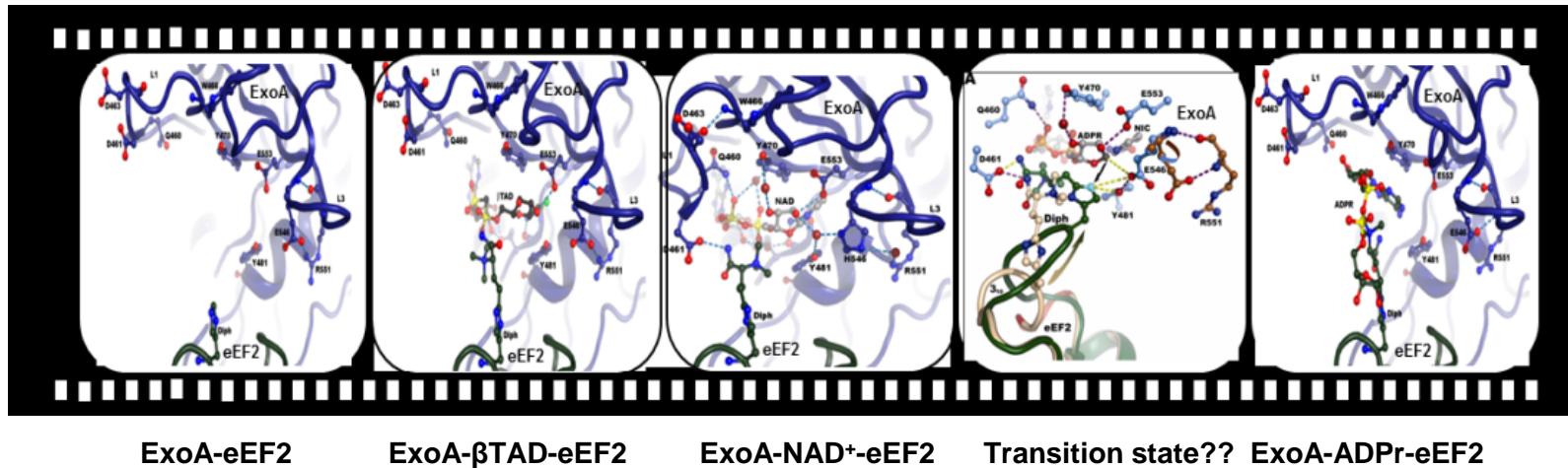


Jorgensen & Merrill (2005) *Nature* 436, 979-984

Making Movies of Enzyme Reaction Mechanisms



A.R. Merrill
Dept. of Mol. & Cell. Biol.
Univ. of Guelph



<https://www.youtube.com/watch?v=Z5fVZegUhzU>



Movie of exotoxin A reaction (*P. aeruginosa*)

(2) Preferential transition state binding

(a) Transition State Theory (TS Theory)

- goal of kinetic theory is to describe the reaction rates in terms of physical properties of reacting molecule
- theory developed by Henry Eyring in 1930 known as "Transition State Theory" or "Absolute Rate Theory"

Consider a bimolecular reaction:



$$[2] \quad v_o = d[P]/dt = k[A][B] = k'[X^\ddagger]$$

- where k is the ordinary rate constant and k' is the rate constant for the decomposition of X^\ddagger to products
- activated complex occurs at an energy maximum and is only metastable \equiv **transition-state species**
- TS theory assumes that X^\ddagger is in rapid equilibrium with the reactants

- enzymes bind the transition state with greater affinity than either substrates or products
- enzymes that preferentially bind the transition state structure **increase its concentration** and therefore proportionally increase the reaction rate ($v_o = k'[X^\ddagger]$)

$\Delta\Delta G^*_{cat} = \Delta G^*_{U} - \Delta G^*_{C} = 34.2 \text{ kJ/mol}$ at 25°C for a 10^6 -fold enhancement in rate by an enzyme

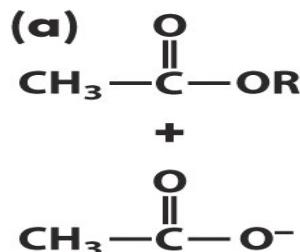
- corresponds to a **net energy** of two H-bonds!

Specificity in transition-state binding: the Pauling model revisited. *Biochemistry* **52**, 2021-2035 (2013)

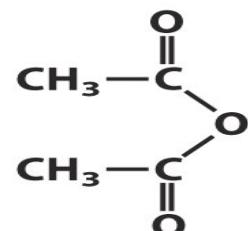
(3) Proximity effect

- enzymes bring reacting species close together and hold in appropriate orientation
 - intramolecular reactions are faster than intermolecular reactions

Reaction

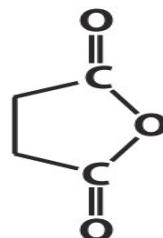
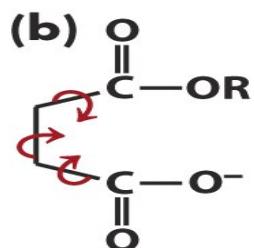


Rate enhancement



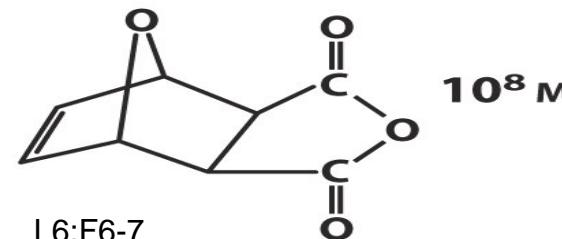
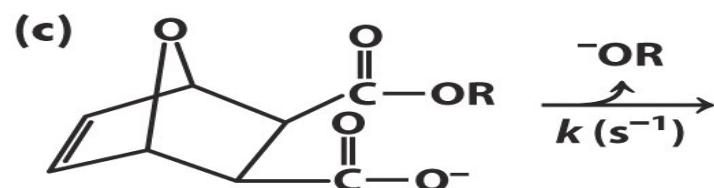
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intermolecular



10^5 M

intramolecular



10^8 M

eg., reaction of an ester with a COO^- to form an anhydride

- when reaction is in a single molecule the rate is much faster
 - the loss of ΔS (entropy) for (b) is much less during product formation than for (a)
 - rate for reaction (c) is further enhanced due to yet smaller ΔS effects
-
- enzymes bind substrates in the **proper orientation** for reaction and align dipoles, etc
-
- **Specific** binding as compared with **nonspecific** binding
 - may enhance rates by 100 fold
 - enzymes freeze out the relative translational and rotational motions of their substrates and catalytic groups (the transition state has little relative motion)
 - may enhance rates by 10^7 fold

(4) Acid-Base Catalysis

- General acid catalysis: process in which partial proton transfer from an acid lowers the free energy of a reaction's transition state
 - General base catalysis: process in which partial proton abstraction by a base enhances the reaction rate
- side chains of Asp, Glu, His, Cys, Tyr, and Lys have pK's in or near the physiological range and can act as acid and/or base catalysts
- enzyme can arrange several catalytic groups around a substrate to produce a *concerted acid-base catalysis* mechanism
 - reaction mechanism will be highly pH-sensitive

General Acid-Base Catalysis

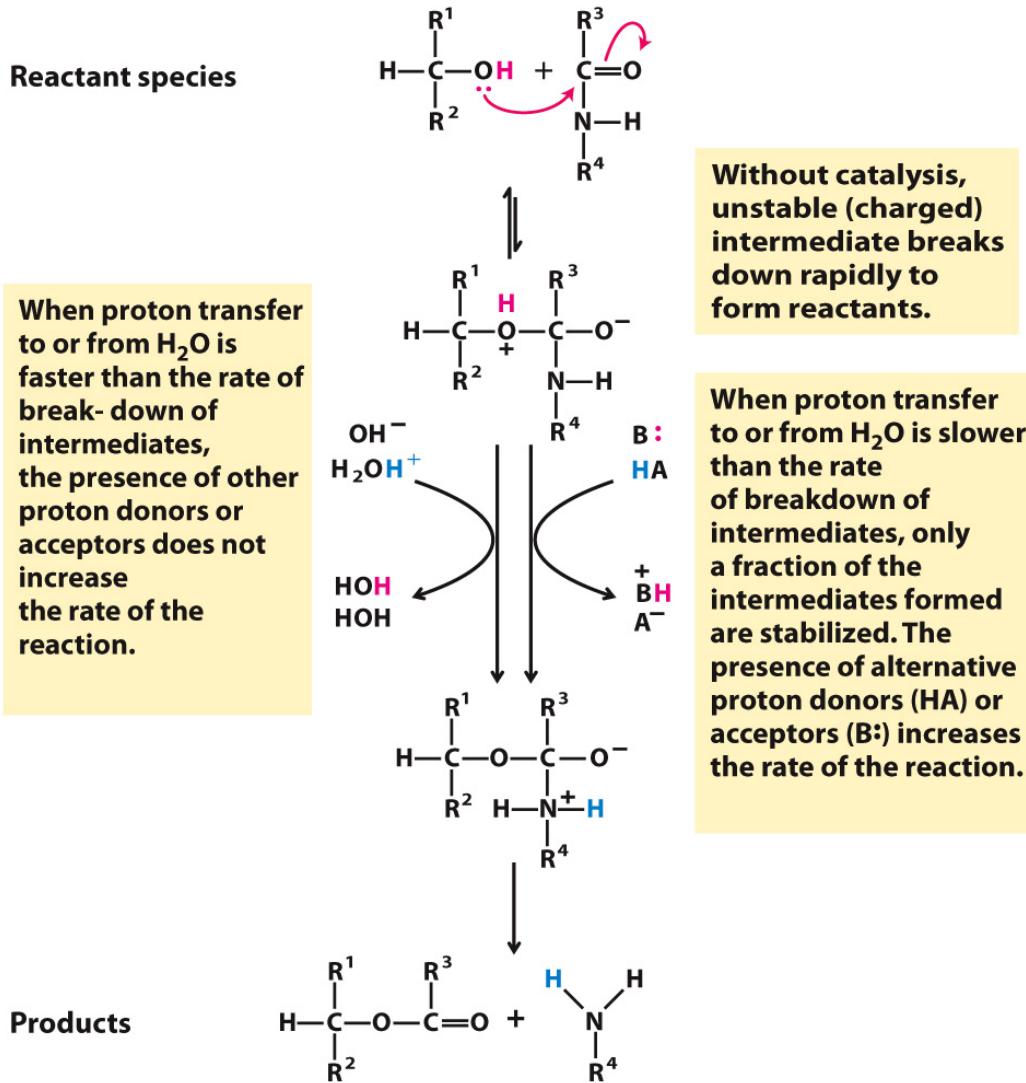


Figure 6-8

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Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp	$R-COOH$	$R-COO^-$
Lys, Arg	$R-\overset{+}{N}(\text{H})_2$	$R-\ddot{\text{N}}\text{H}_2$
Cys	$R-SH$	$R-S^-$
His		
Ser	$R-OH$	$R-O^-$
Tyr		

Figure 6-9

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