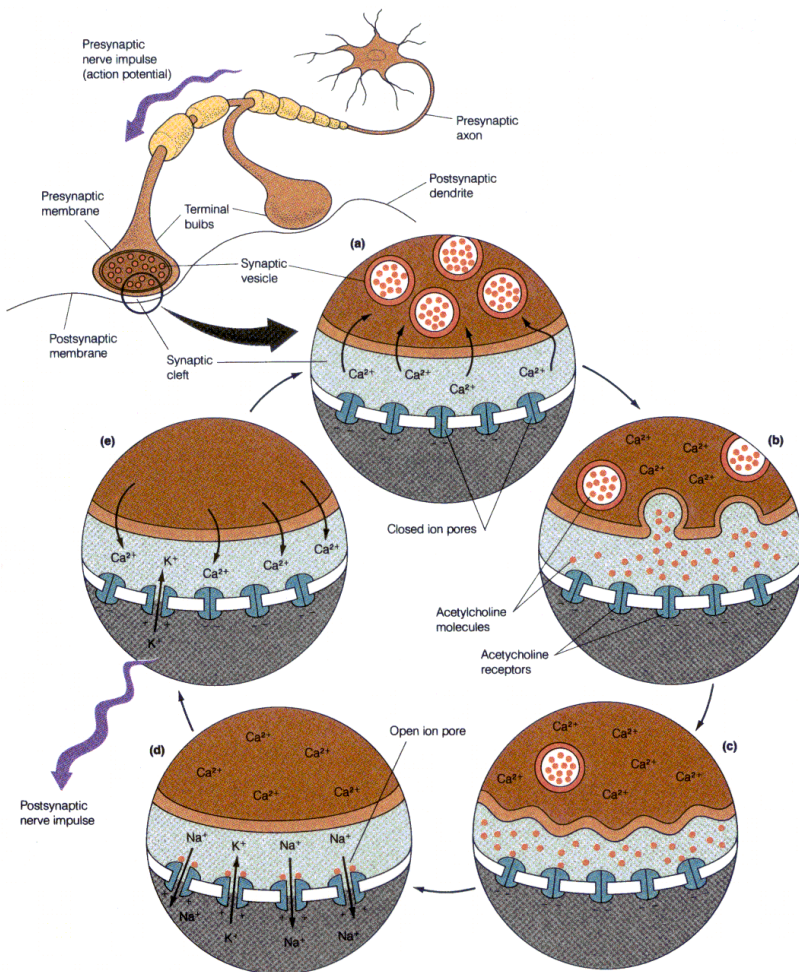


Pre-synaptic nerve  
(action potential)

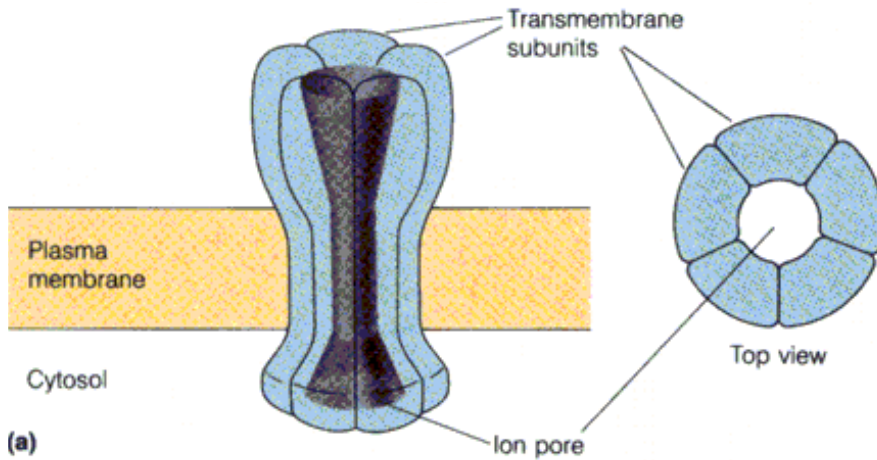
Synapse  
(chemical transmitter)

Post-synaptic nerve  
(action potential)

## ● Acetylcholine



The action potential triggers Ca<sup>2+</sup> release which stimulates acetylcholine release. Acetylcholine diffuses across the synapse and binds to the acetylcholine receptors. This binding triggers the opening of the ion channel. Na<sup>+</sup> rushes in, starting an action potential in the postsynaptic nerve. Acetylcholinesterase hydrolyses the acetylcholine enabling the channel to shut and return to the resting potential.

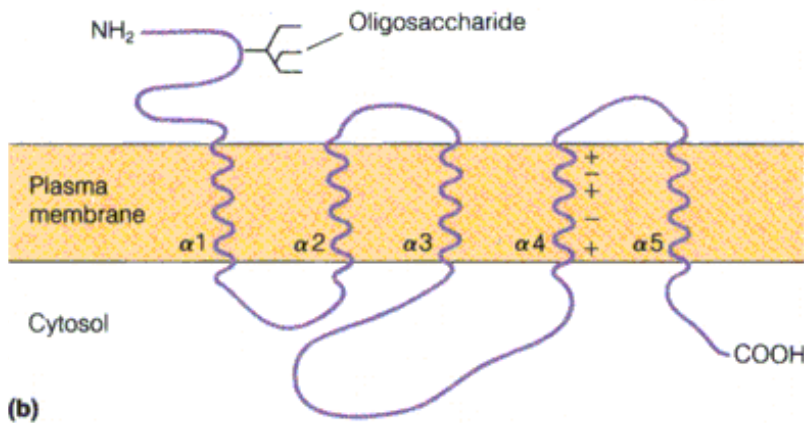


## Nicotinic/Acetylcholine Receptor

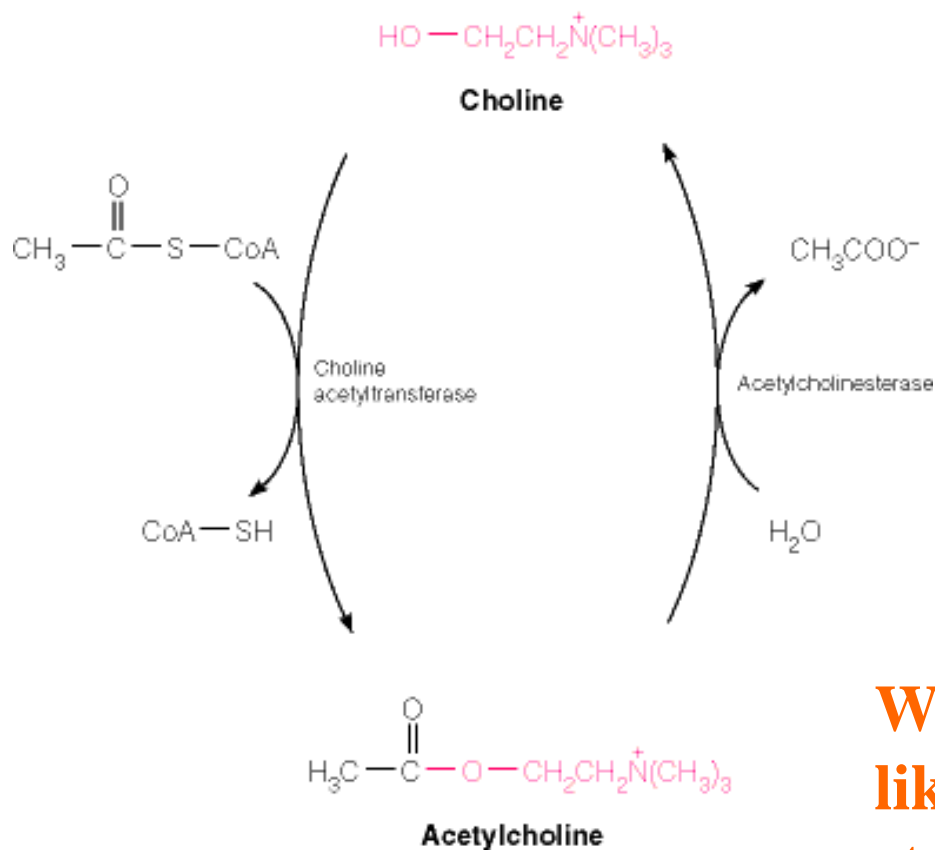
### Integral Membrane Protein

5 subunits

Nicotine blocks open  
Curare blocks closed



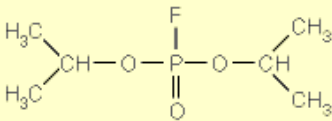
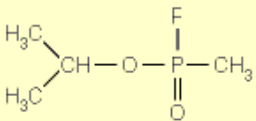
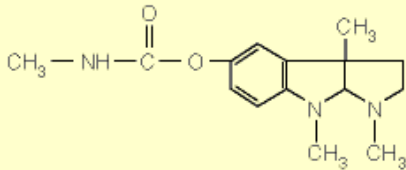
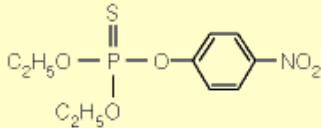
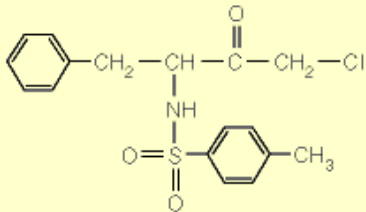
(b)



Rapid hydrolysis of acetylcholinesterase is required for rapid refiring of the nerve.

**What is the likely transition state???**

# Serine Protease Inhibitors

Diisopropyl fluorophosphate (DFP)		Synthetic	Inhibits enzymes with active site serine, including acetylcholinesterase
Sarin		Synthetic (nerve gas)	Like DFP
Physostigmine		Calabar beans	Like DFP
Parathion		Synthetic (insecticide)	Like DFP, but especially inhibitory to insect acetylcholinesterase
N-Tosyl-L-phenylalaninechloromethyl ketone (TPCK)		Synthetic	Reacts with His 57 of chymotrypsin

# Chapter 11--Enzymes

- **Catalysts**
  - **Transition state theory & activation energy**
  - **Kinetics (with oodles of graphs)**
- **Mechanisms**
- **Inhibition**
- **Examples**
  - **Proteases**
  - **Lysozyme**
  - **Prosthetic groups**
  - **RNAs**

# **What are enzymes??**

**Usually proteins**  
**rarely RNA**  
**often with prosthetic groups**

**Biological catalysts**  
**Speed up reactions**  
**Are not changed**

**No biochemical reaction takes place without an enzyme**

**Many inherited diseases are the result of a defective enzyme**

**Antibiotic and antiviral therapeutics involve inhibiting**  
**Enzymes (theirs but not yours)**

**Biological catalysts must**

**Work at moderate temperatures  
In aqueous solutions**

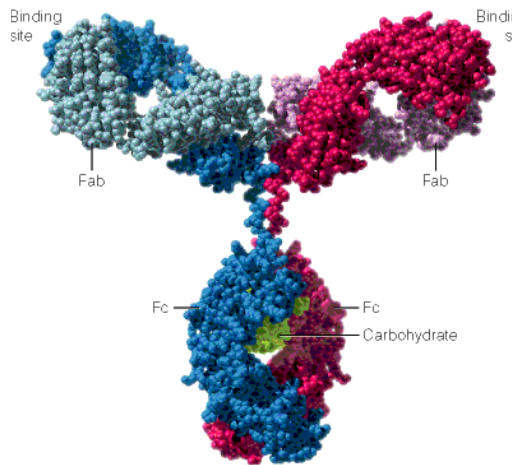
**Be very specific**

**Respond to environmental signals  
(regulation)**

**Antibodies bind ground states**

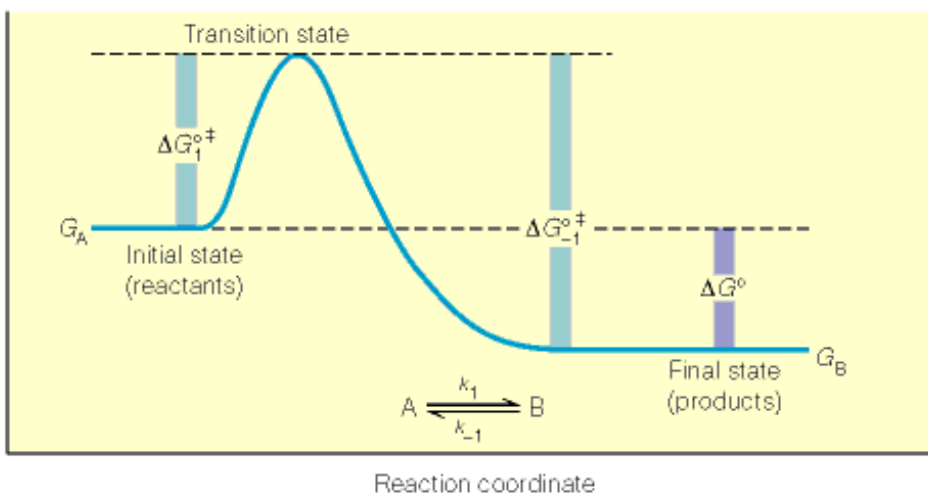
**Enzymes bind transition states**

**Linus Pauling**

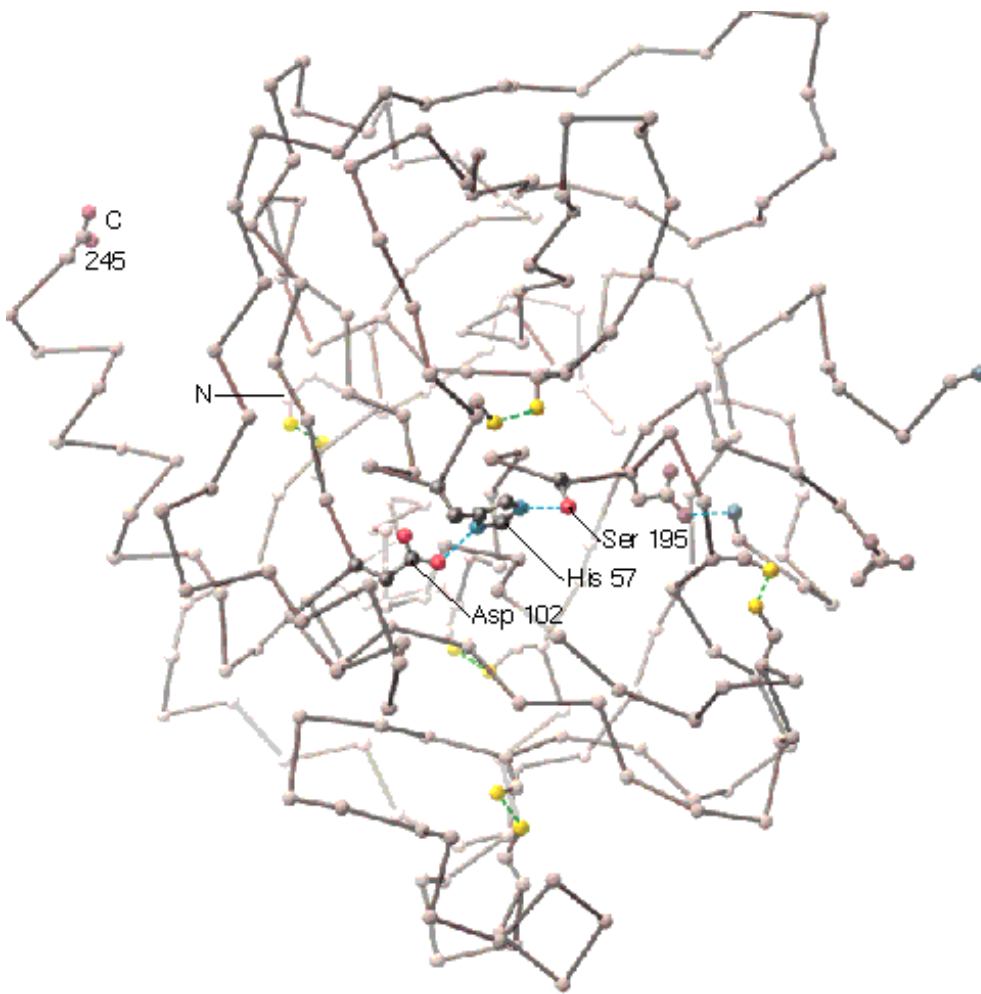


**Antibodies that bind to transition state analogs can act as catalysts.**

**But, are they as good as “natural” enzymes?**



**Catalytic Antibodies**



**Chymotrypsin**

**Serine Protease**

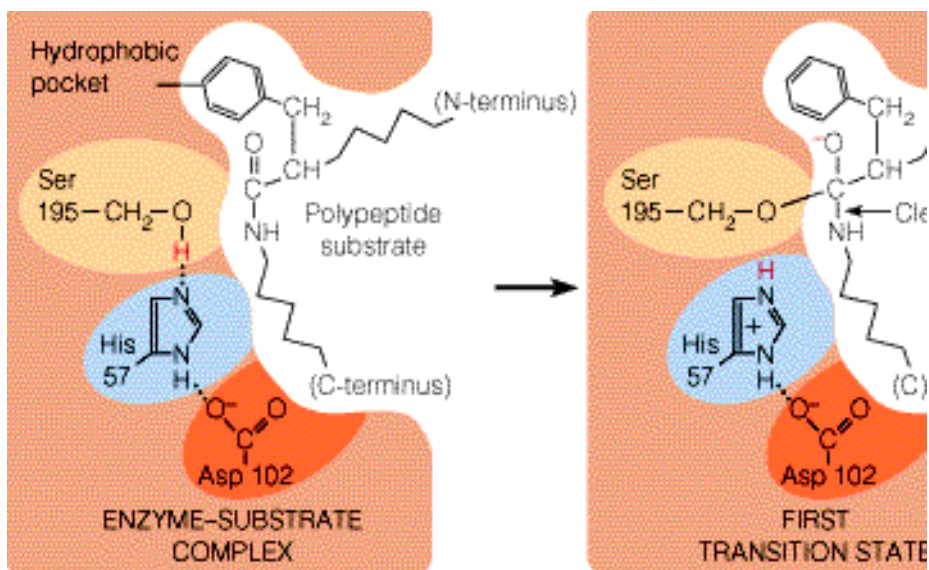
**“Catalytic Triad”**

**Ser 120**

**His 57**

**Asp 102**

## Chymotrypsin Mechanism



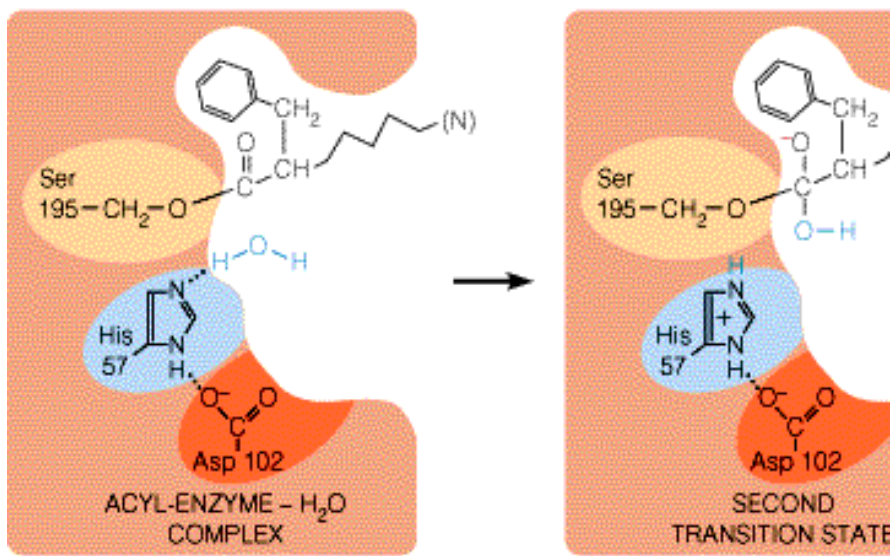
1 Polypeptide substrate binds noncovalently with side chains of hydrophobic pocket.

2 H<sup>+</sup> is transferred from Ser. The substrate forms a tetrahedral transition state with the e

**C-N bond broken**  
**C ter peptide released.**  
**N ter peptide still bound.**

**What kinds of evidence are used to “prove” an enzyme mechanism?**

## Chymotrypsin Mechanism Part II--regeneration



4 A water molecule binds to the enzyme in place of the departed polypeptide.

5 The water molecule trans proton to His 57 and its remaining substrate fragment forms a tetrahedral transition state.

**Release of peptide.  
Migration of  
Serine proton.**

## **PROTEIN “relatedness”**

**BLAST--Compares primary sequences**

**--% identity, %similarity**

**--closely related sequences**

**RCSB, SCOP--Compares 3D structures**

**--Superimpose?**

**--Classify folding patterns**

**--more distantly related proteins**

**> NP\_011485 S. cerevisiae L30**

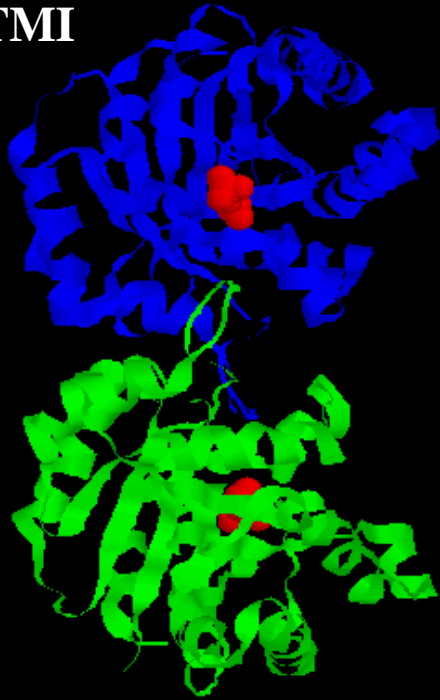
**1 mapvksqesi nqklalviks gkytlgykst**

**vkslrqgksk liiaantpv lrkseleyya**

**mlsktkvyyf qggnnelgta**

**vgklfrvgvv sileagdsdi ltla**

**Yeast TMI**



**TRIOSE  
PHOSPHATE  
ISOMERASE**

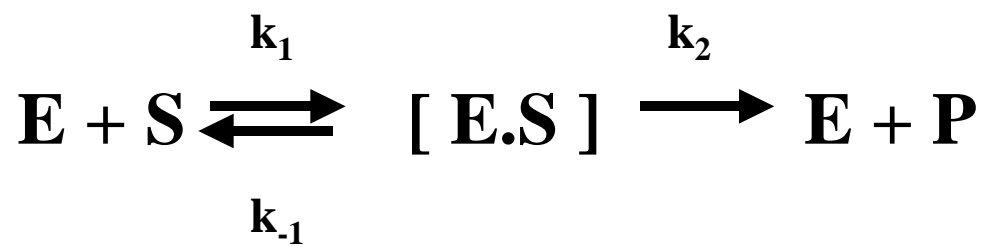
**Has a conserved  
 $\beta$  sheet/  $\alpha$  helix  
fold.**

**Chicken TMI**



**Trypanosome TMI**

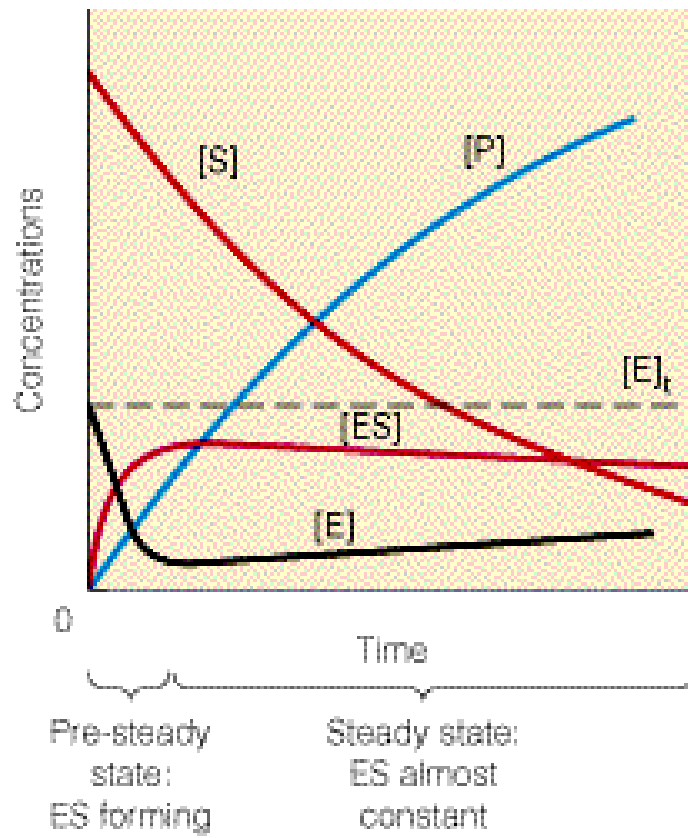




**You should derive this!!!!**

$$\mathbf{V = d [P]/dt = k_2 E S / (K_m + S) = V_{max} S / (K_m + S)}$$

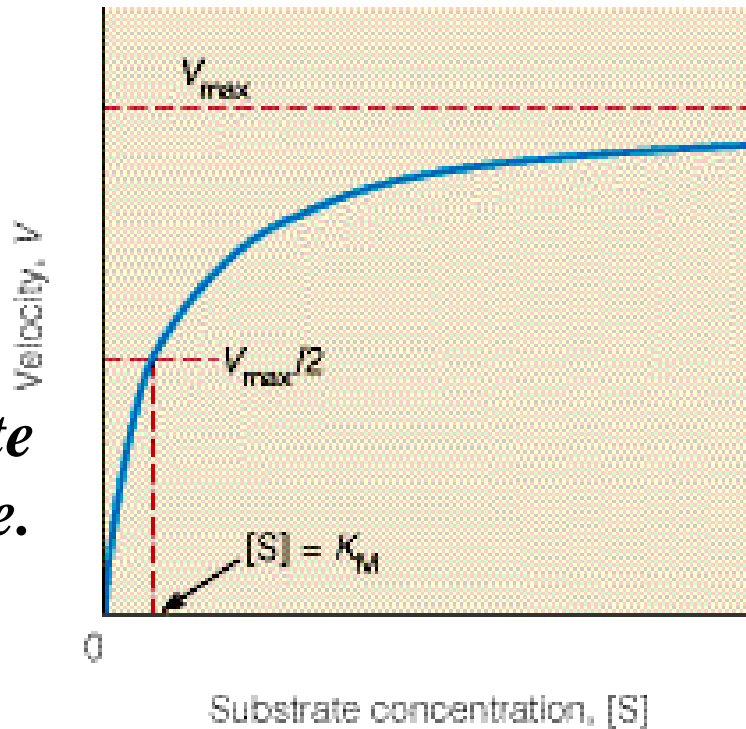
$$\mathbf{Where K_m = (k_2 + k_{-1}) / k_1}$$



**Steady  
State  
Approximation:**

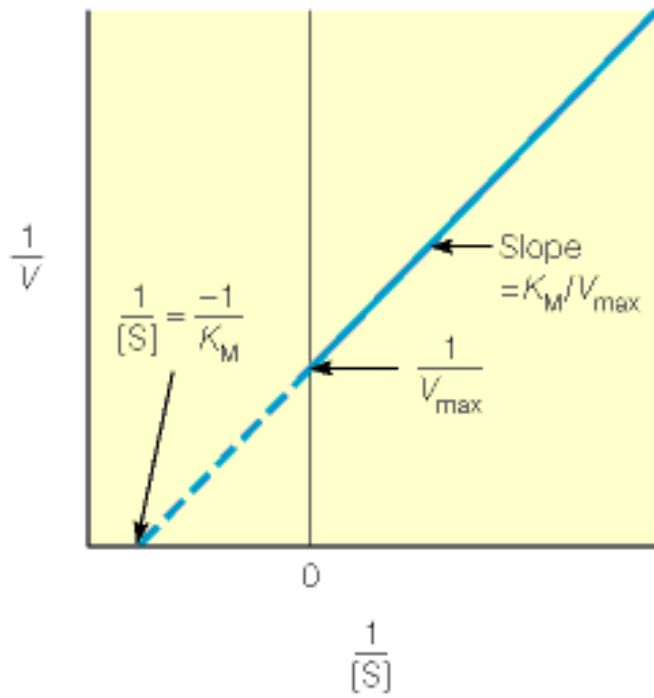
$$-d [ES] / dt = 0$$

$K_m$   
measures  
affinity  
of substrate  
for enzyme.

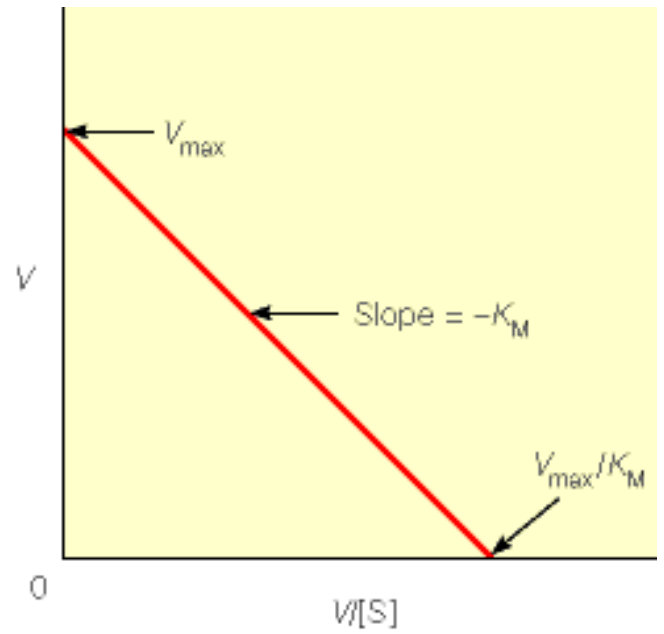


**At  $K_m$  the enzyme is half saturated**

**What [S] is needed to measure  $K_m$ ??  $V_{max}$ ??**



**Lineweaver-Burke Plot**



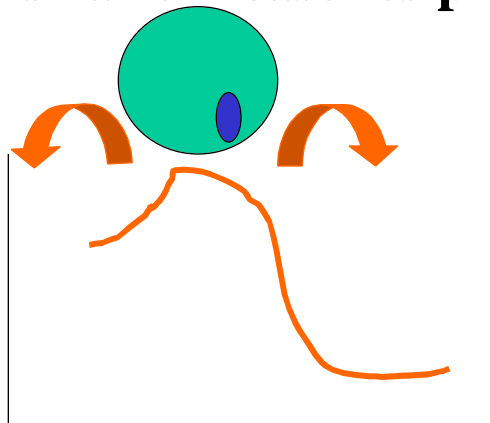
**Eadie-Hofstee Plot**

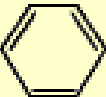
Enzyme “efficiency” is often judged by  $k_{cat} / K_m$

$k_{cat}$  is the turnover number

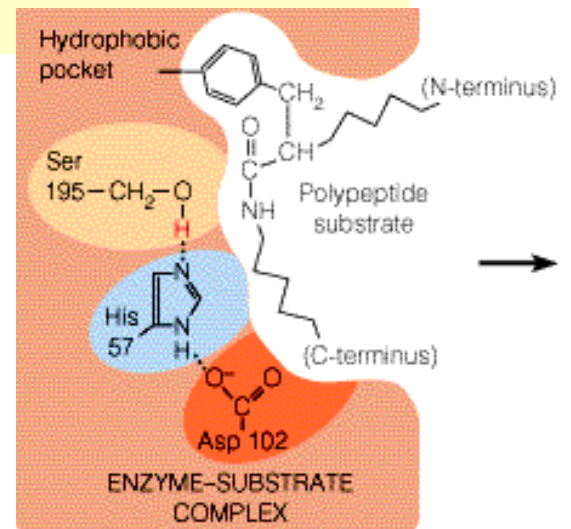
$k_{cat} / K_m$  at low  $[S]$  this ratio measures productivity

of  $E + S$  collision.



Amino Acid in Ester	Amino Acid Side Chain	$k_{\text{cat}}/K_M$ [(mol/L) <sup>-1</sup> s <sup>-1</sup> ]
Glycine	—H	$1.3 \times 10^{-1}$
Norvaline	—CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	$3.6 \times 10^2$
Norleucine	—CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	$3.0 \times 10^3$
Phenylalanine	—CH <sub>2</sub> — 	$1.0 \times 10^5$

**Chymotrypsin's pocket  
For the side chain of  
The R1 amino acid**



- 1 Polypeptide substrate binds noncovalently with side chains of hydrophobic pocket.

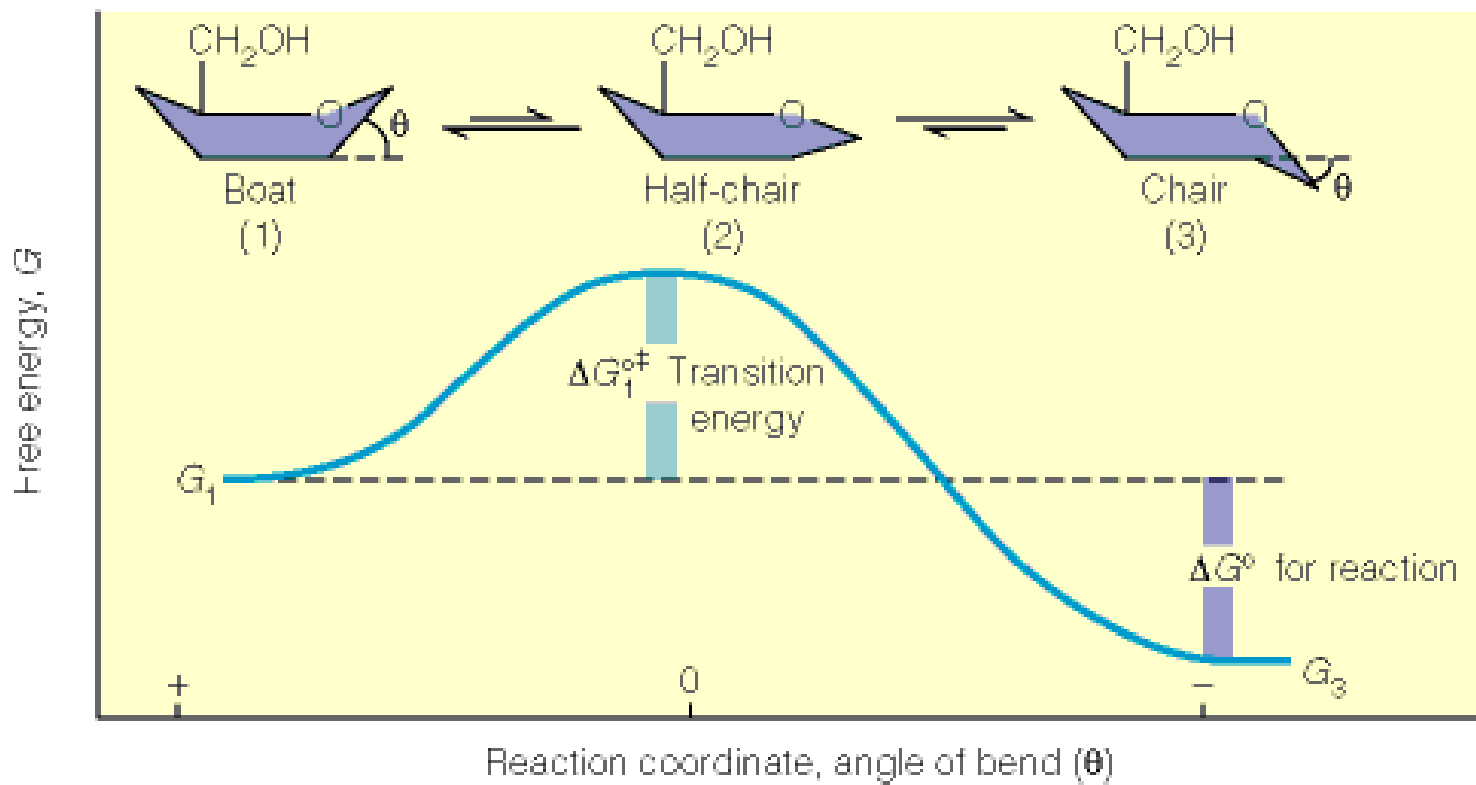
# **What do enzymes actually do?**

**Hexokinase**

**Triose phosphate isomerase**

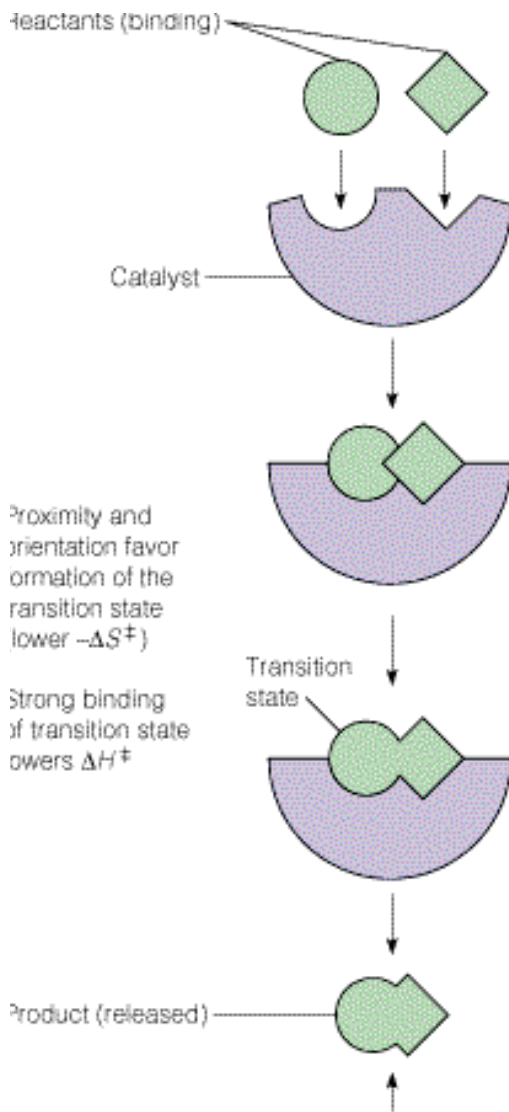
**Lysozyme**

**Ribozymes**



(c)

**Enzyme works on either  $\Delta H^\ddagger$  or  $\Delta S^\ddagger$**



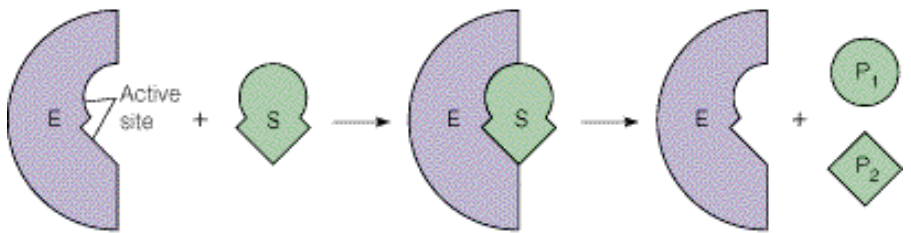
**Enzyme acts  
As a reaction  
surface**

**Enzyme has pockets  
that make H bonds,  
salt bridges, and  
hydrophobic contacts  
with Transition State**

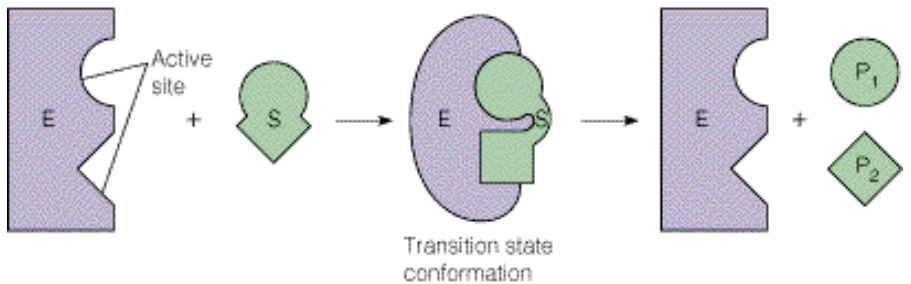
# Enzyme-Substrate Recognition

## Lock and Key--Rigid Enzyme Specificity

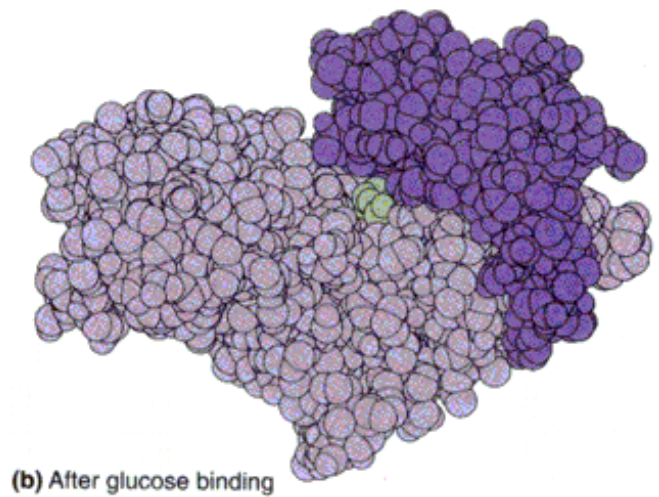
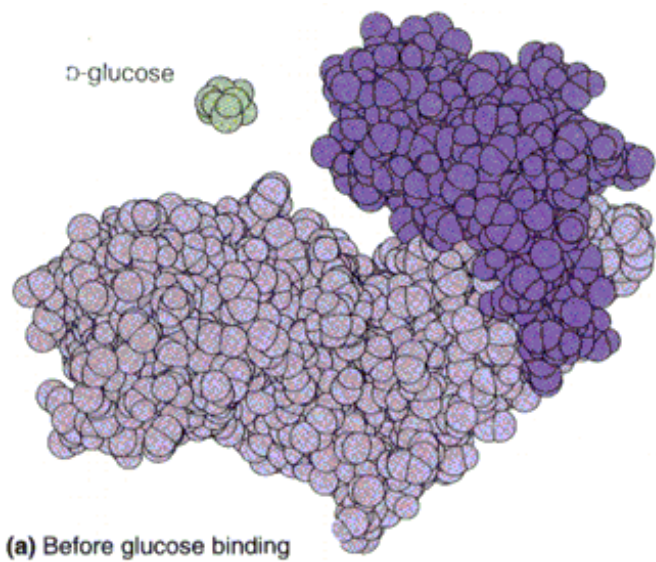
## Induced Fit--Conformational Change Specificity Transition State



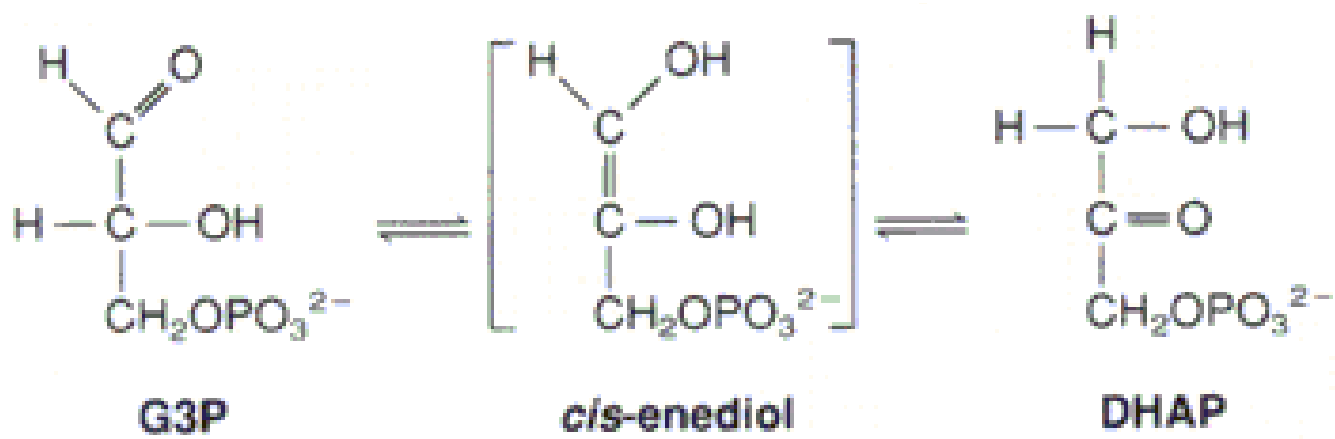
(a) Lock-and-key model



(b) Induced fit model



**Why must the water be excluded from the transition state?**



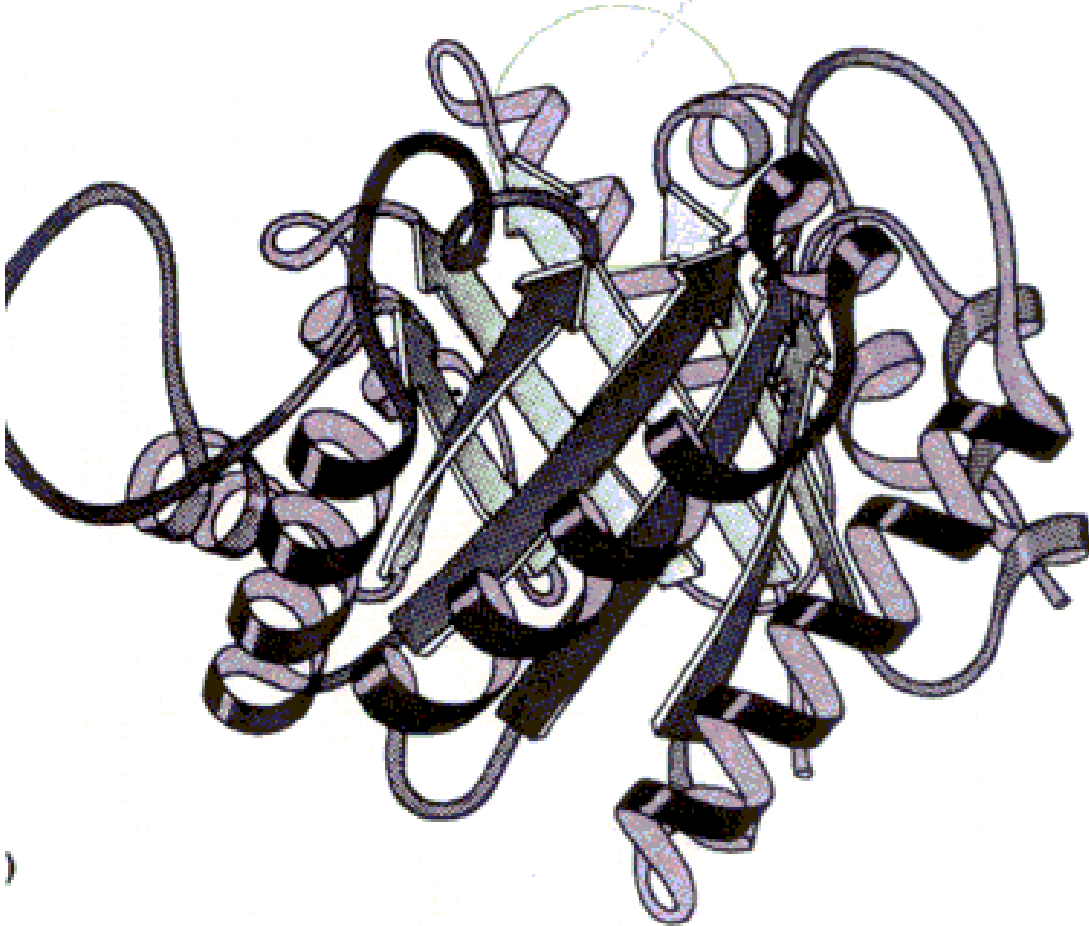
**Glyceraldehyde-3-phosphate**

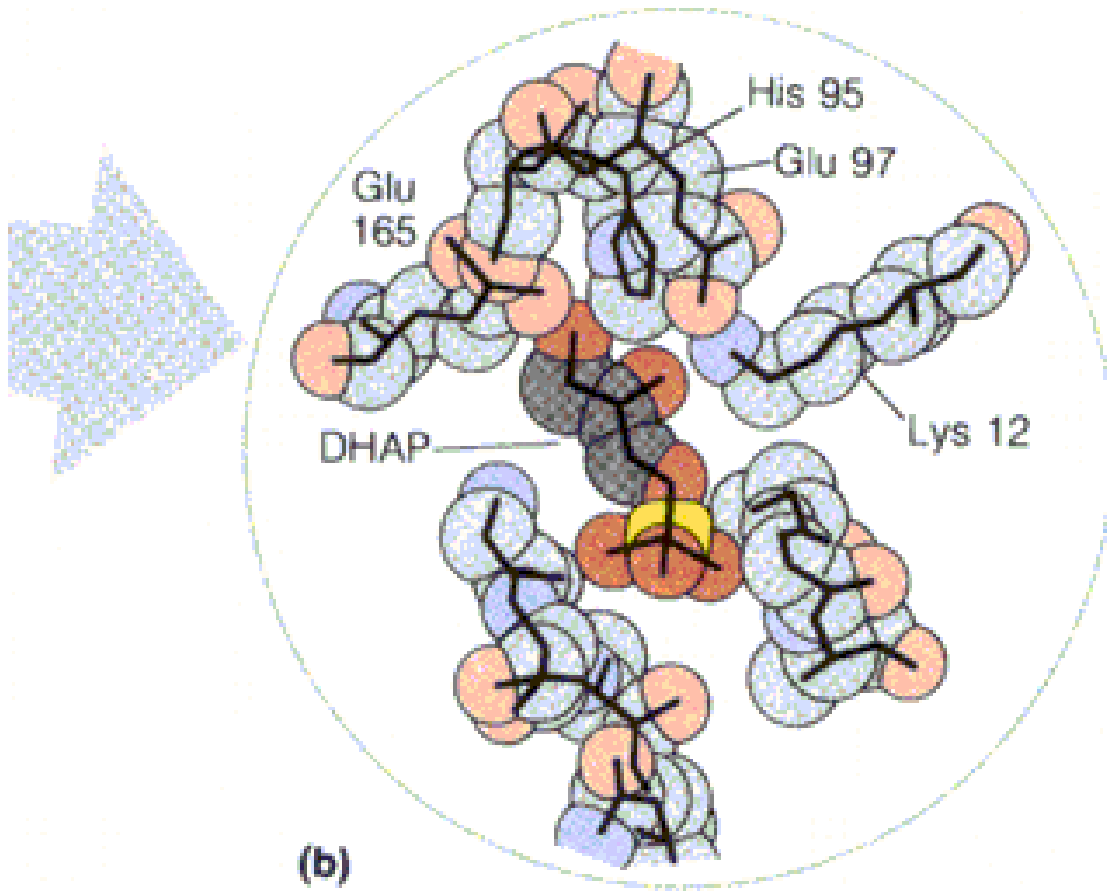
**dihydroxyacetonephosphate**

**Aldose**

**Ketose**

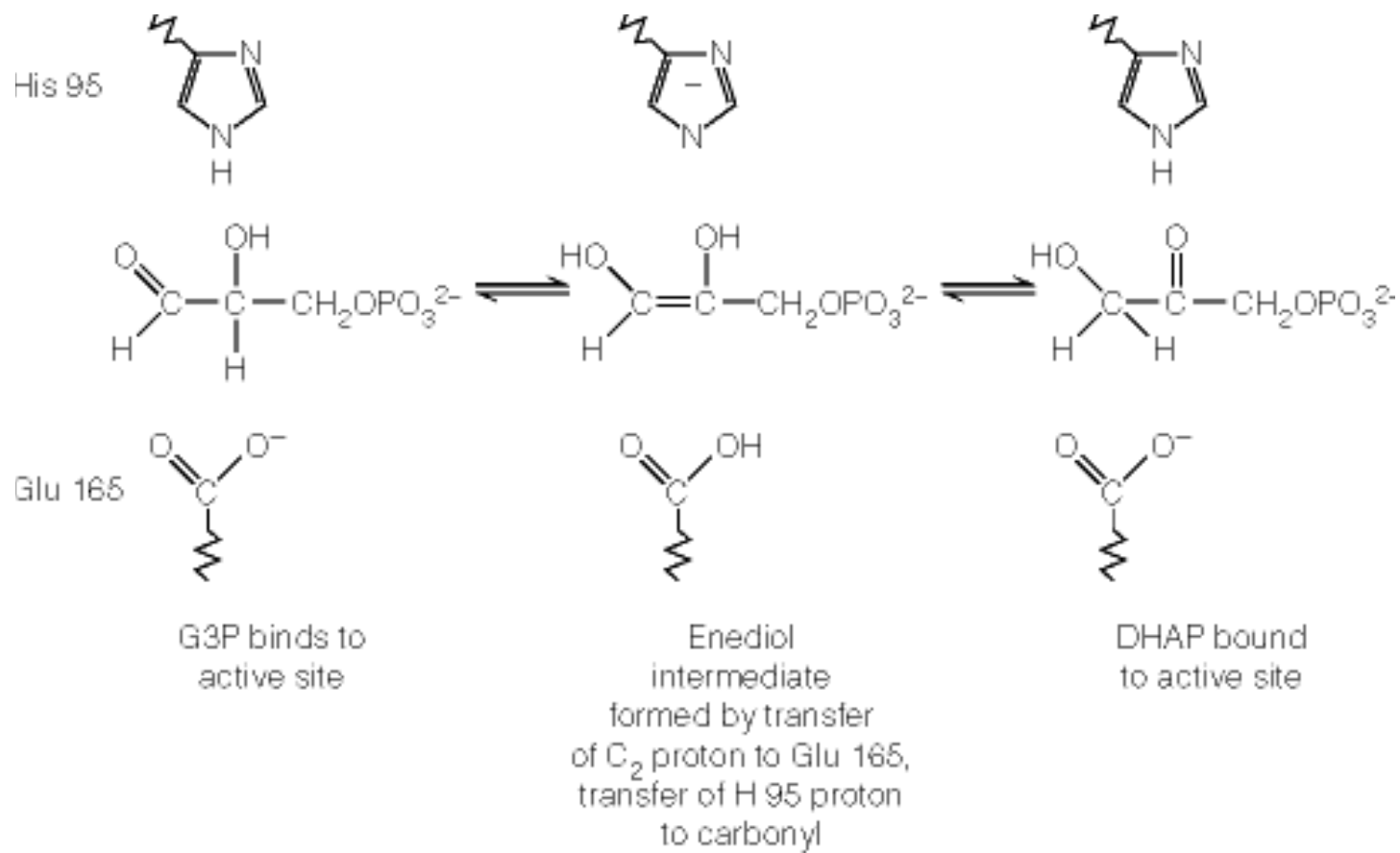
## Triose phosphate isomerase (TIM)



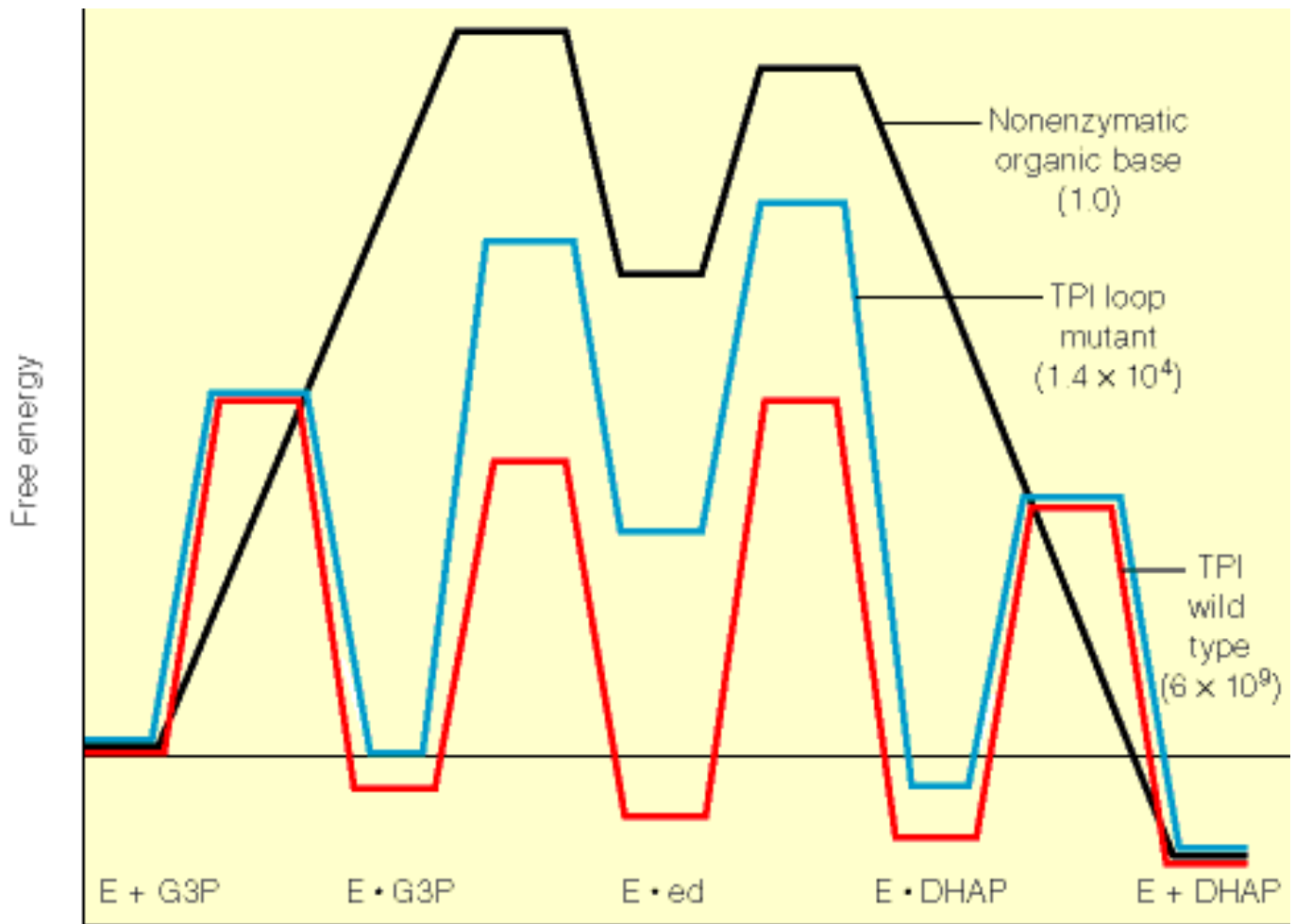


(b)

**TIM active site--His, Glu, Lys**

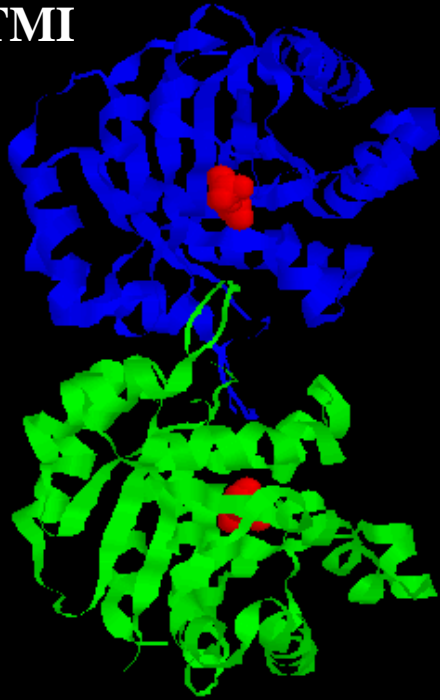


## Role of His and Glu in shuttling protons



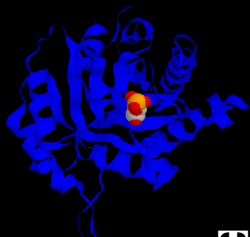
**Enzyme breaks large barrier into series of small ones.**

**Yeast TMI**



**TRIOSE  
PHOSPHATE  
ISOMERASE**

**Has a conserved  
 $\beta$  sheet/  $\alpha$  helix  
fold.**



**Trypanosome TMI**

**Chicken TMI**



Enzyme	Reaction Catalyzed	$K_M$ (mol/L)	$k_{cat}(s^{-1})$	$k_{cat}/K_M$ [(mol/L)
Chymotrypsin	$Ac-Phe-Ala \xrightarrow{H_2O} Ac-Phe + Ala$	$1.5 \times 10^{-2}$	0.14	9.3
Pepsin	$Phe-Gly \xrightarrow{H_2O} Phe + Gly$	$3 \times 10^{-4}$	0.5	$1.7 \times 10^3$
Tyrosyl-tRNA synthetase	$Tyrosine + tRNA \longrightarrow tyrosyl-tRNA$	$9 \times 10^{-4}$	7.6	$8.4 \times 10^3$
Ribonuclease	$Cytidine\ 2',\ 3'\ cyclic\ phosphate \xrightarrow{H_2O} cytidine\ 3'\ phosphate$	$7.9 \times 10^{-3}$	$7.9 \times 10^2$	$1.0 \times 10^5$
Carbonic anhydrase	$HCO_3^- + H^+ \longrightarrow H_2O + CO_2$	$2.6 \times 10^{-2}$	$4 \times 10^5$	$1.5 \times 10^7$
Fumarase	$Fumarate \xrightarrow{H_2O} malate$	$5 \times 10^{-6}$	$8 \times 10^2$	$1.6 \times 10^8$

**How high can this value go??**

**What is the limit?**



**Why are the energetic barriers  
equally high?**

**Why is TIM called “a perfect enzyme”?**

**Superoxide dismutase--superspeedy enzyme**

## **What kinds of evidence are used to “prove” an enzyme mechanism?**

**\*\*mutations**

**\*\*isolate intermediates**

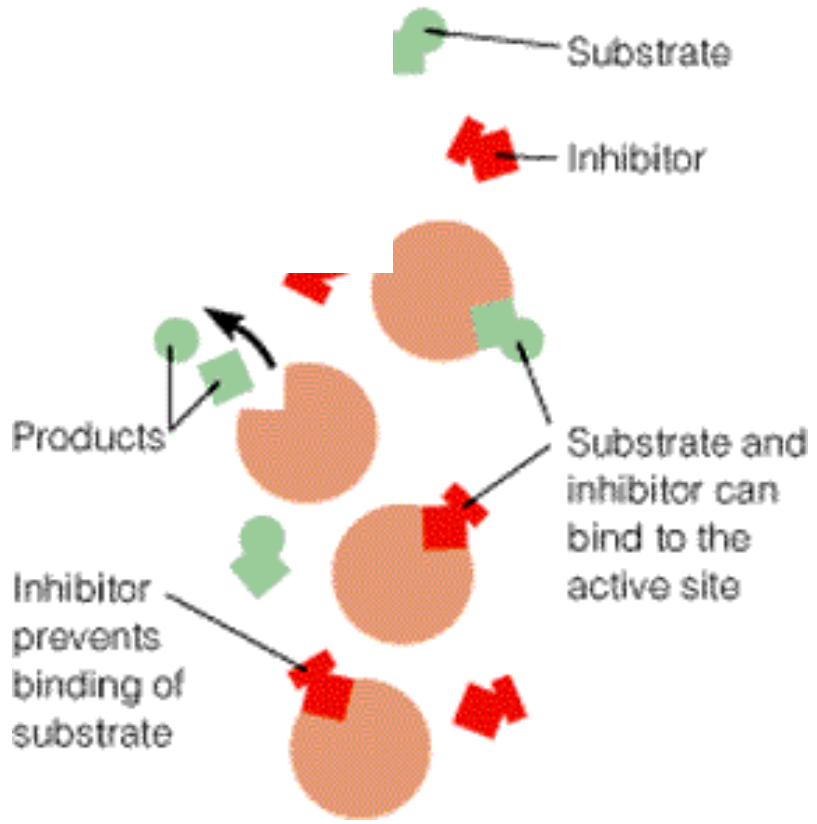
**\*\*isotopic labelling**

**\*\*inhibitors**

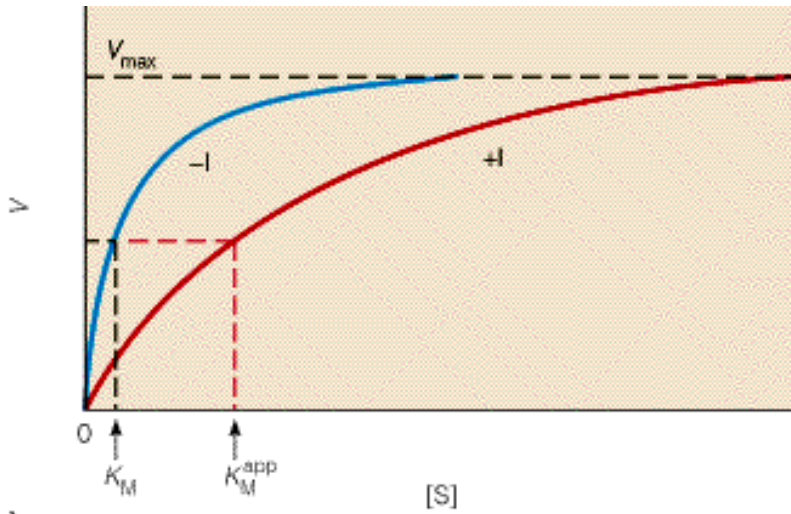
**\*\*pH dependence**

**\*\*kinetics (rate limiting steps)**

**\*\*phylogeny (related proteins)**

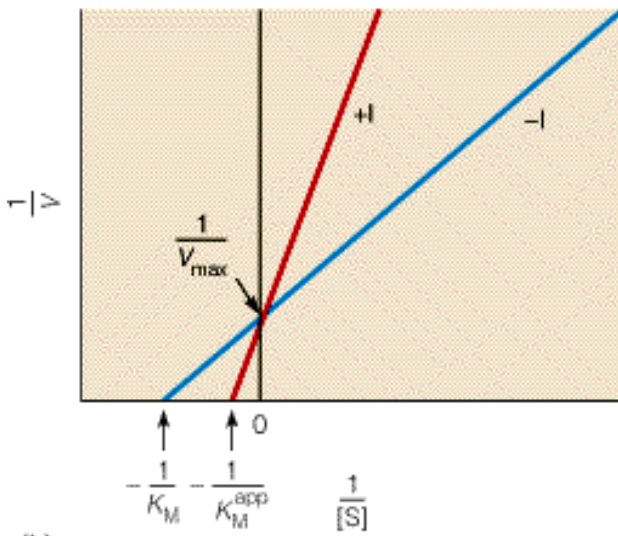


**Derive kinetics?**  
**Graphs?**

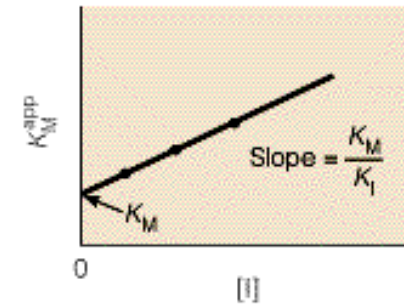


**Competitive inhibitors change  $K_M$  by “reducing” available enzyme.**

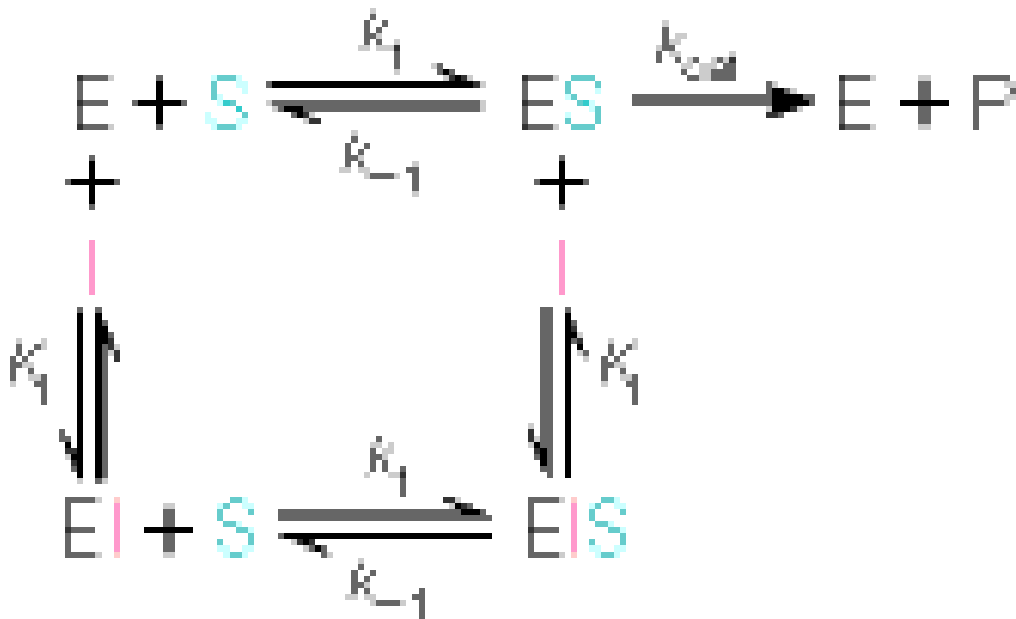
(a)



(b)



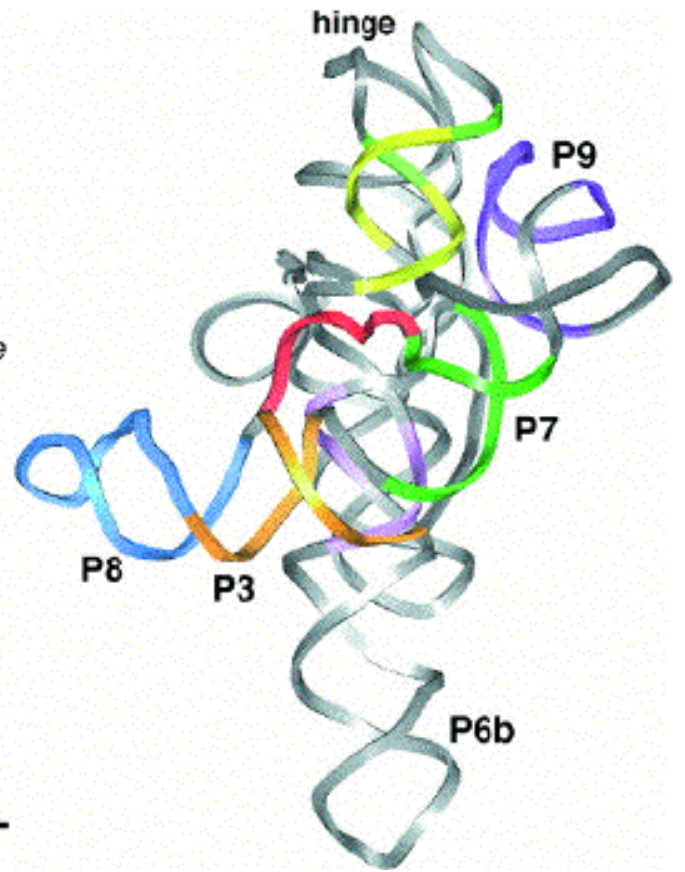
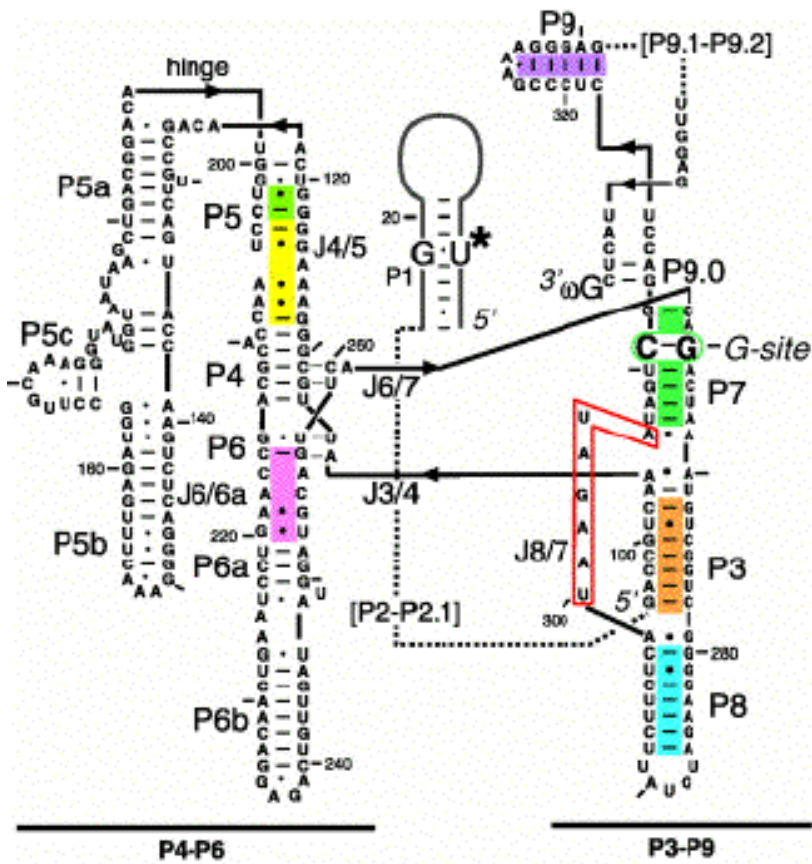
(c)



**Non-competitive inhibition**

**I does not prevent S binding, but does prevent catalysis.**

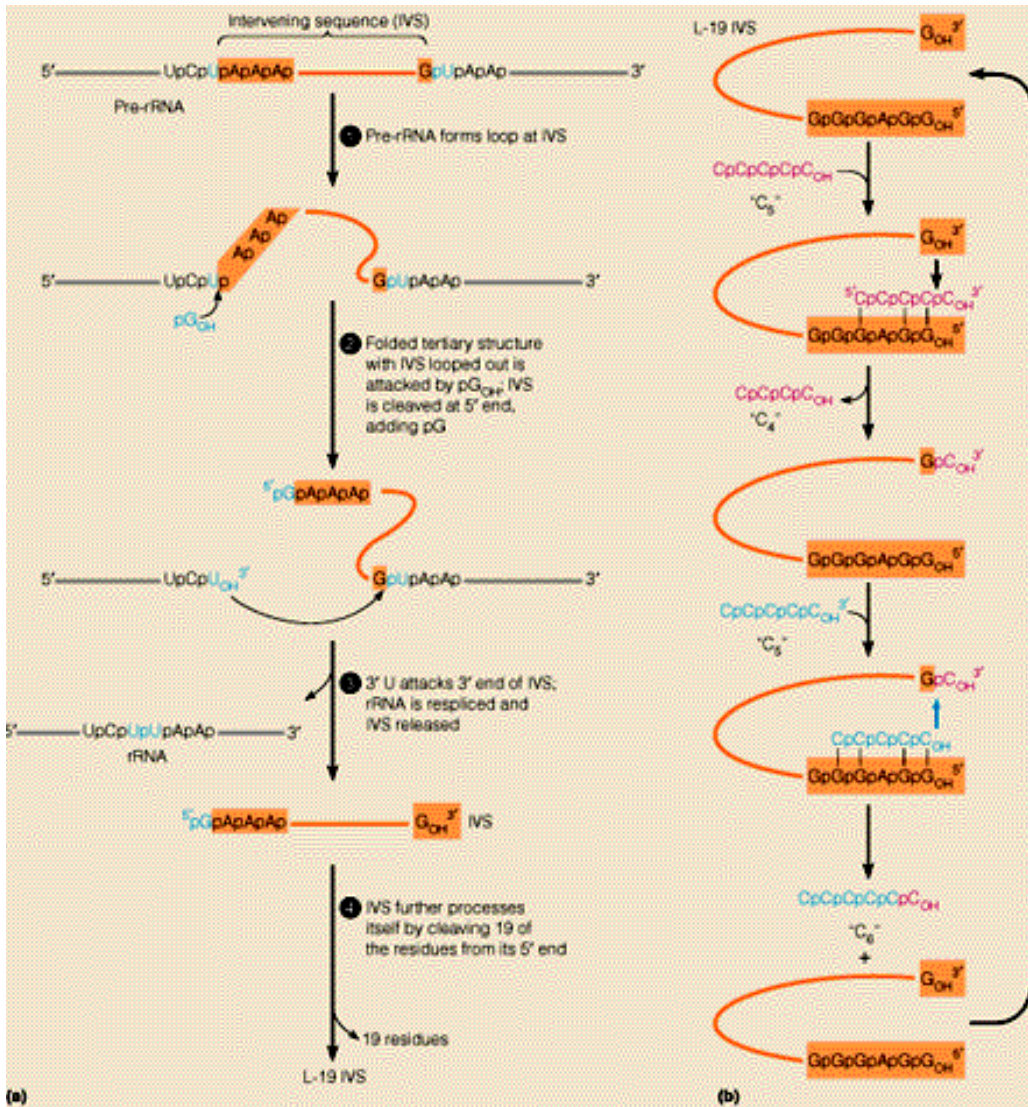
**$k_{cat}$  is reduced, but  $K_m$  is the same.**



## Catalytic RNA

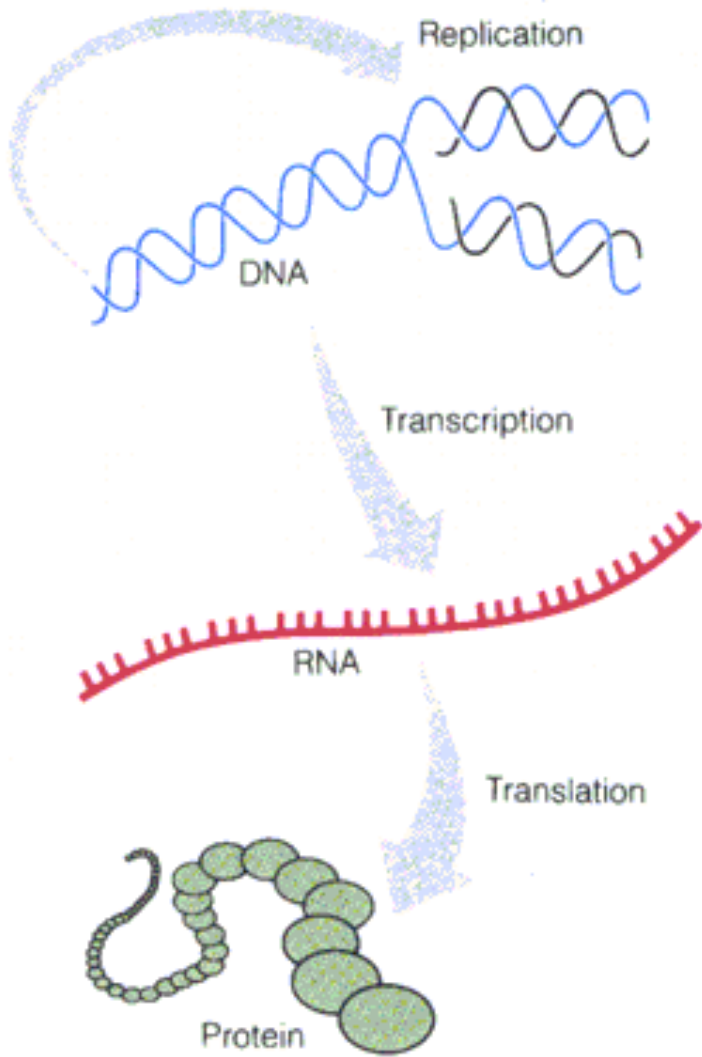
Secondary Structure-- "Roadkill"

Tertiary Structure--3D



**Self-splicing RNA**  
**Transesterifications**  
**O attacks P**  
**Make and break**  
**P - O bonds.**  
**TS = tbp**  
**Mg<sup>2+</sup> stabilizes O-**





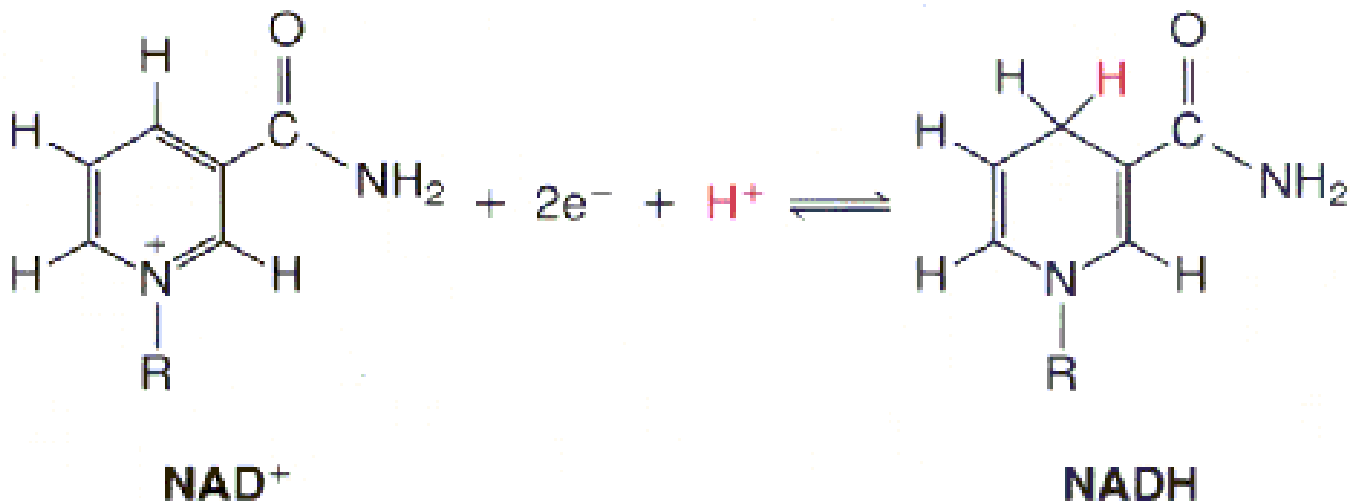
## **RNA World Hypothesis**

**Did RNA come first?**

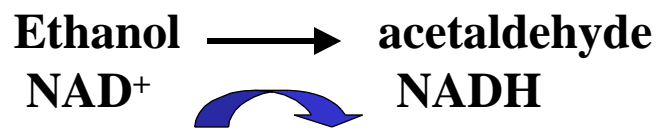
**Genetics  
Catalysis**

**Your enzymes can't do it alone.  
Eat your vegetables and take your vitamins.**

Vitamin	Coenzyme	Reactions Involving These Coenzymes
Thiamine (vitamin B <sub>1</sub> )	Thiamine pyrophosphate	Activation and transfer of aldehydes
Riboflavin (vitamin B <sub>2</sub> )	Flavin mononucleotide; flavin adenine dinucleotide	Oxidation–reduction
Niacin	Nicotinamide adenine dinucleotide; nicotinamide adenine dinucleotide phosphate	Oxidation–reduction
Pantothenic acid	Coenzyme A	Acyl group activation and transfer
Pyridoxine	Pyridoxal phosphate	Various reactions involving amino acid activation
Biotin	Biotin	CO <sub>2</sub> activation and transfer
Lipoic acid	Lipoamide	Acyl group activation; oxidation–reduction
Folic acid	Tetrahydrofolate	Activation and transfer of single-carbon functional groups
Vitamin B <sub>12</sub>	Adenosyl cobalamin; methyl cobalamin	Isomerizations and methyl group transfers

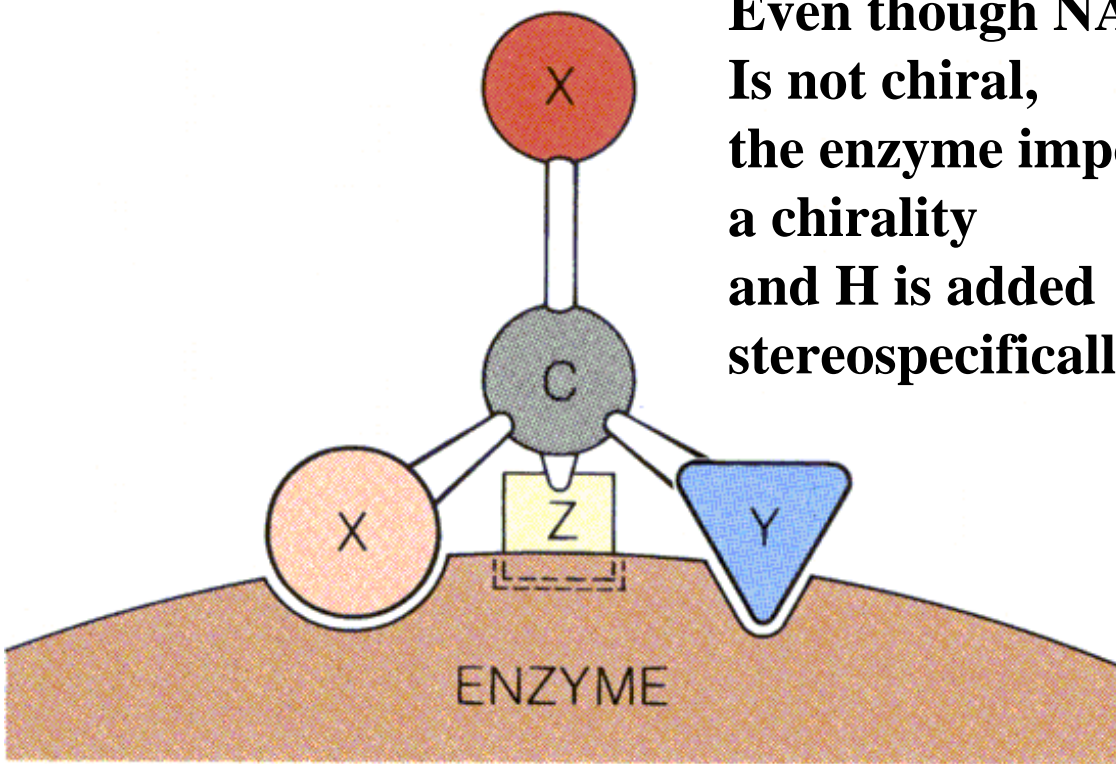


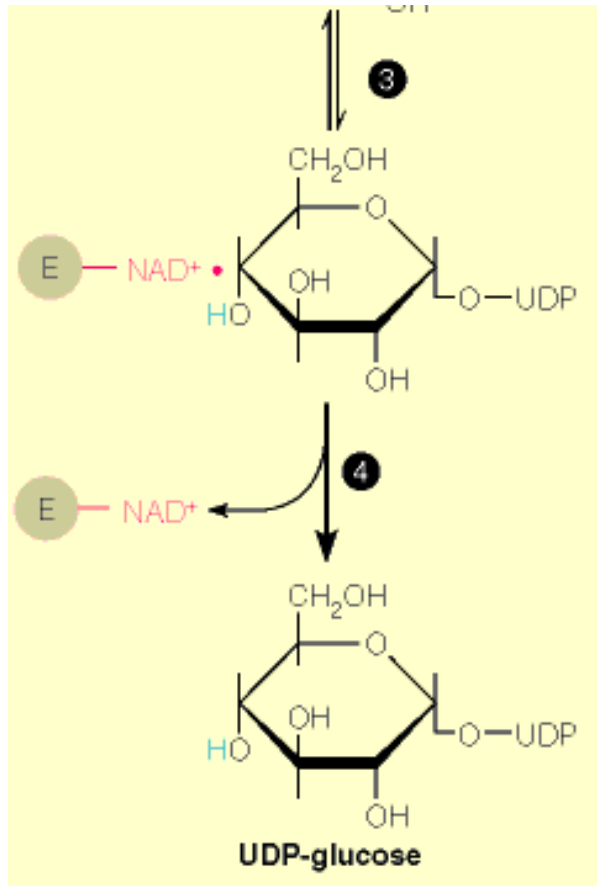
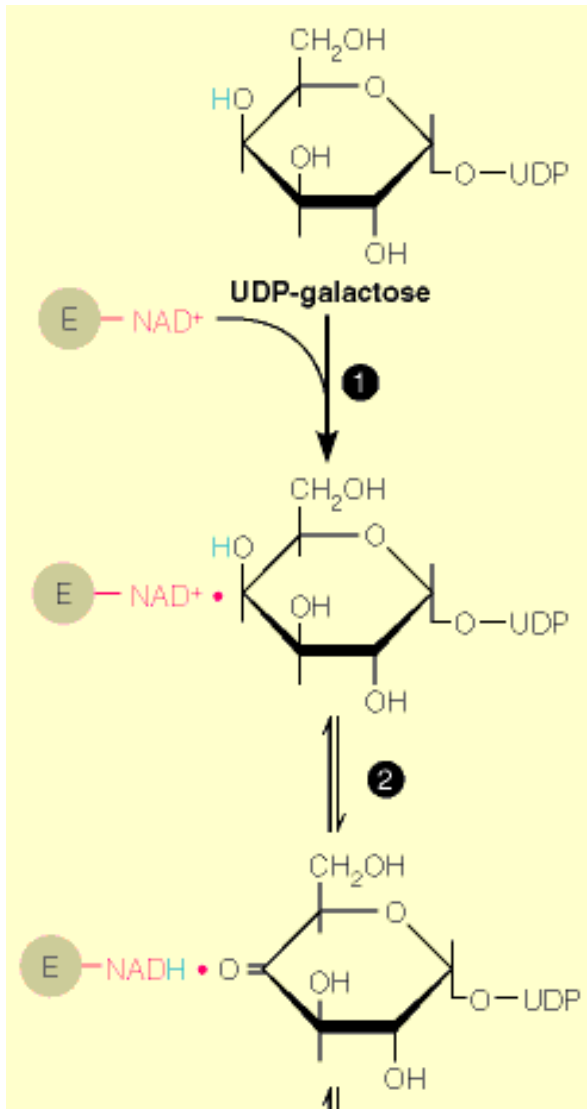
**Nicotinamide adenine dinucleotide--REDOX**  
**R= 2 phosphates, ribose, adenine--Why so big??**



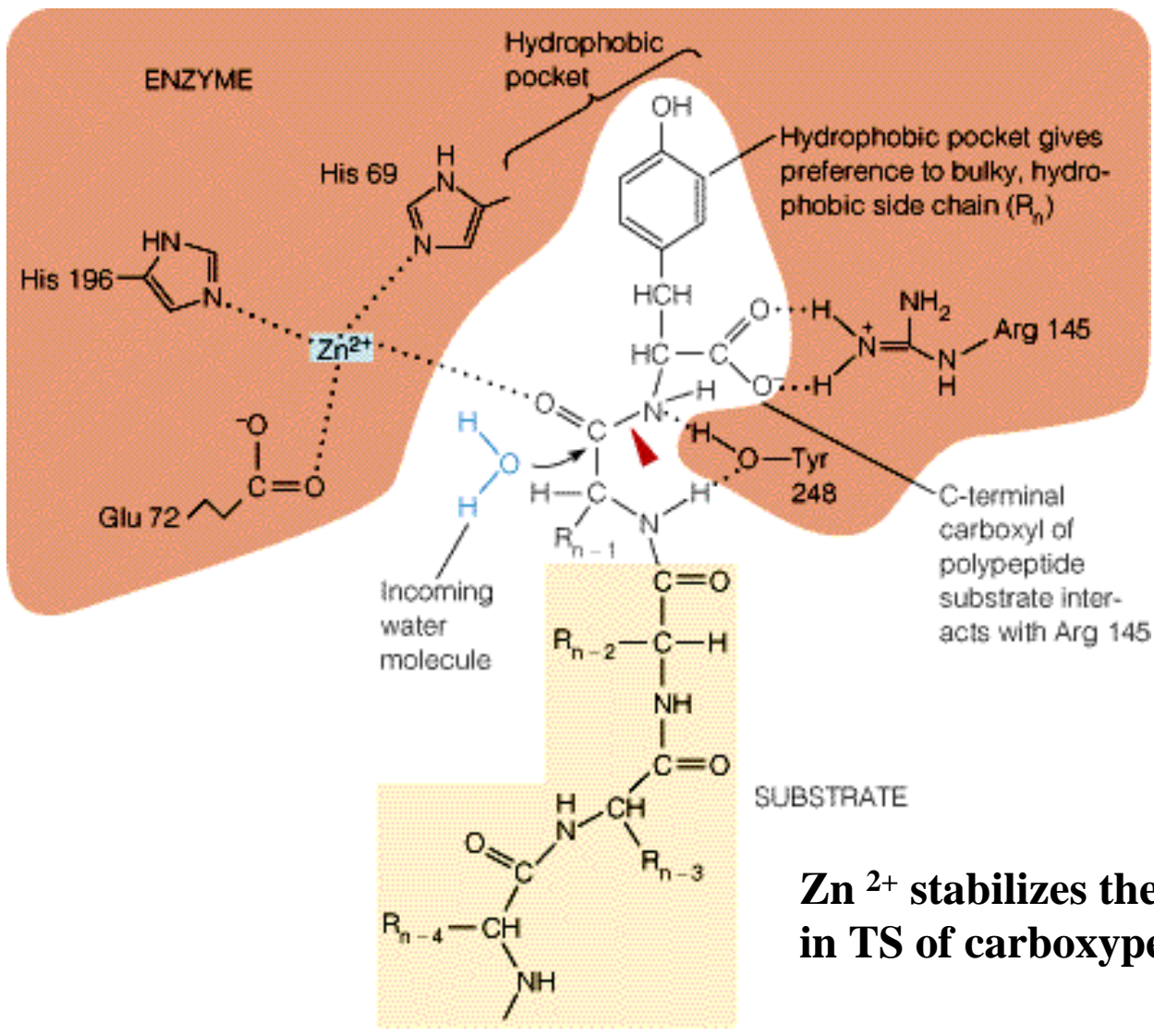
**Alcohol dehydrogenase--If you don't have it?**

**Even though NAD<sup>+</sup>  
Is not chiral,  
the enzyme imposes  
a chirality  
and H is added  
stereospecifically.**



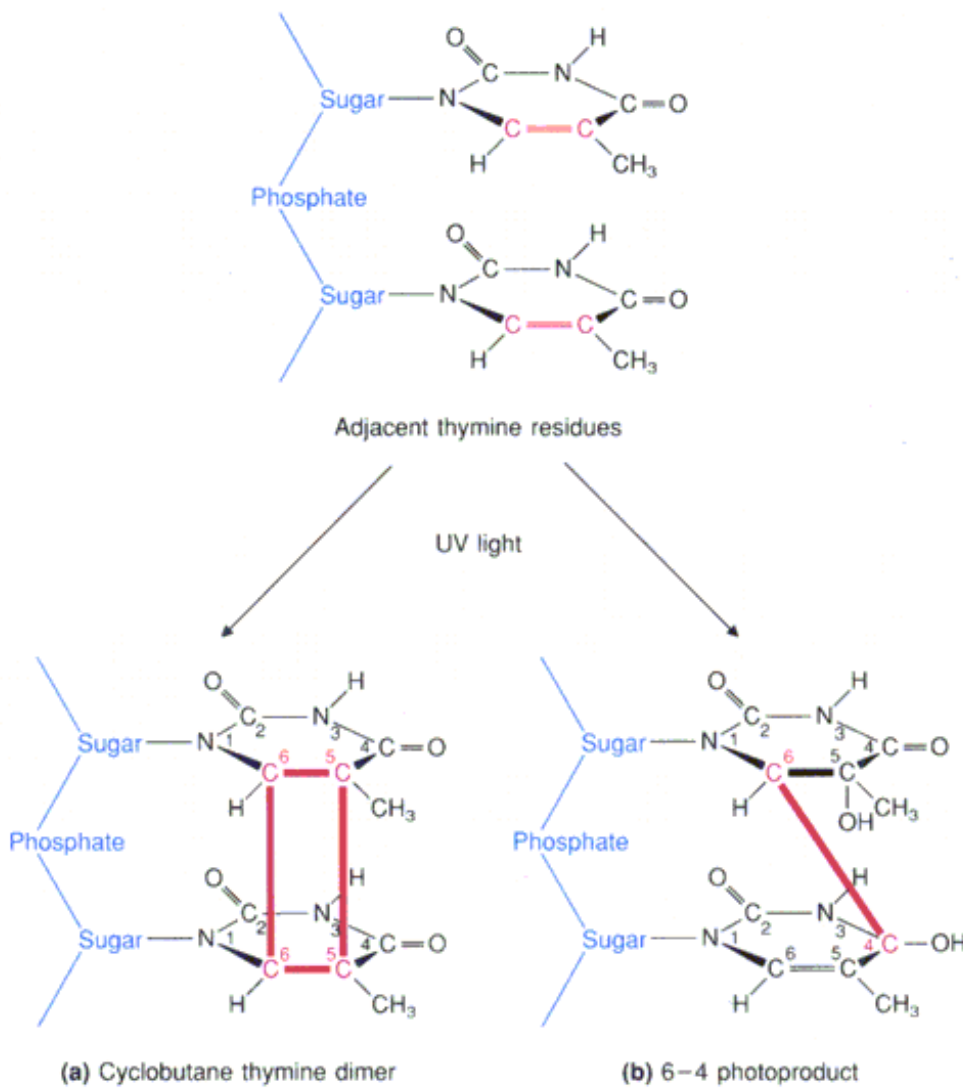


**Epimerase--NAD<sup>+</sup> needed to Form C=O**



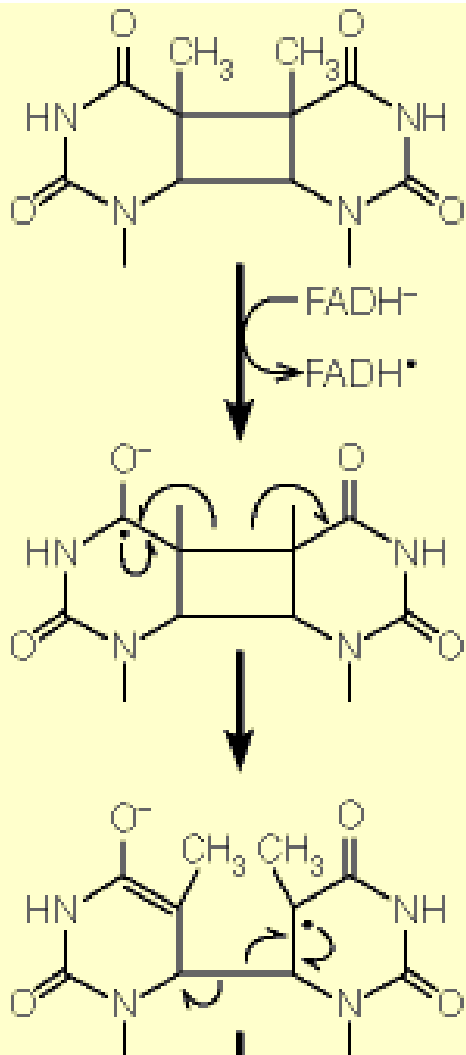
**$Zn^{2+}$  stabilizes the  $O^-$  in TS of carboxypeptidase**

Spring 2024

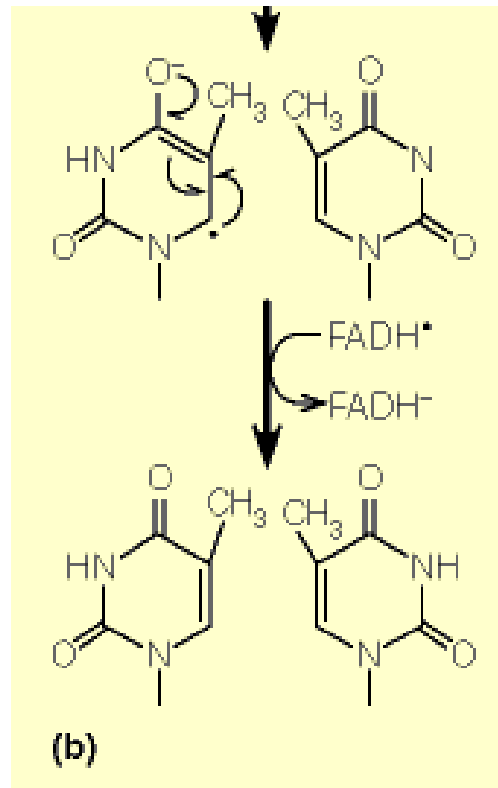


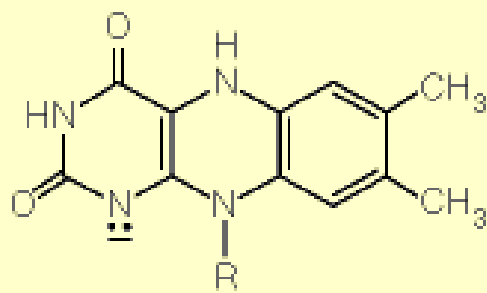
**Unprotected fun in the sun may lead to crosslinking of DNA pyrimidines.**

**This can lead to mutations, cell death, or cancer.**

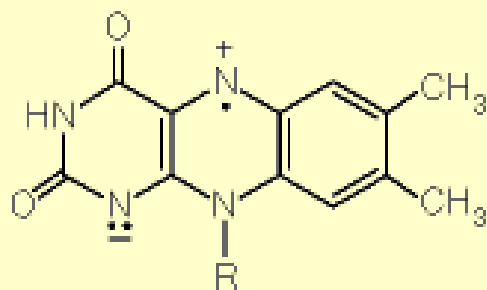


## Reversal of the Photocrosslink Use photolyase/FAD





FADH<sup>-</sup>



FADH<sup>+</sup>

(a)

## Photolyase Co-factor

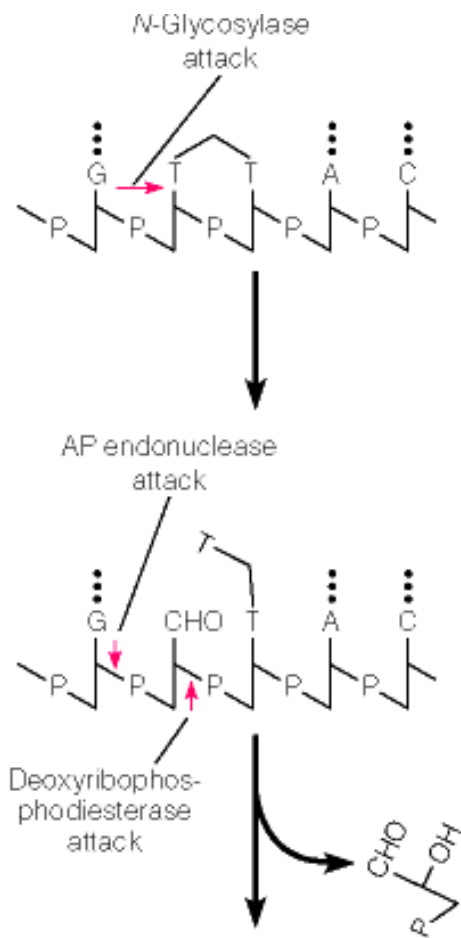
### Flavin adenine dinucleotide

(R= a linear ribose followed by two phosphates and an adenine)

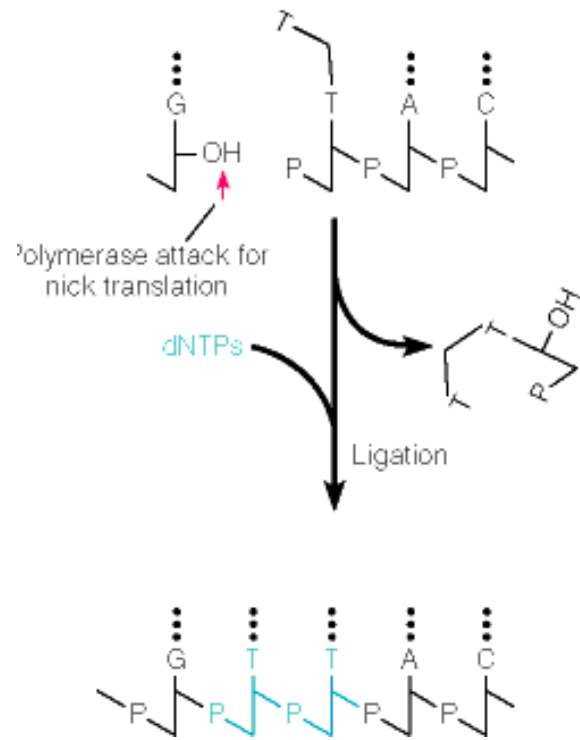
Absorbs light at 370 nm

Shuttles electrons to convert 2 C--C to 2 C=C.

**BUT We don't have it.  
Bacteria do.**



**We have  
base and nucleotide  
excision-repair**



**Defects in the proteins of the UV repair system may result  
In diseases such as Xeroderma pigmentosum (XP)  
Or breast cancer (BRCA1).**

