Chimica Bioorganica

- 2 parti: catalisi e meccanismi in chimica organica

  i meccanismi delle reazioni enzimatiche

- 6 crediti: 48 ore
- esame orale
- orario: lunedì 9-11

  martedì 12-13

  mercoledì 9-11

- testi: Structure and Reactivity in Organic Chemistry
  H. Maskill, Oxford Science Pub., 1999
  Introduction to Enzyme and Coenzyme Chemistry
  T.D.H. Bugg, Wiley, 2012 (3rd ed.)

Introduction

Kinetics and Thermodynamics of Catalysis

Catalysis – Kinetics

Uncatalyzed (n^{th}) order	$R \xrightarrow{k_0} P$		$v_0 = k_0[R]$	1^{st}
Catalyzed ($n+1^{th}$) order	$R + C \xrightarrow{k_{cat}} P + C$		$v_{cat} = k_{cat}[R][C]$	2^{nd}

Rate acceleration: $v_{cat}/v_0 = (k_{cat}/k_0)[C]$

depends on: catalytic efficiency
catalyst concentration

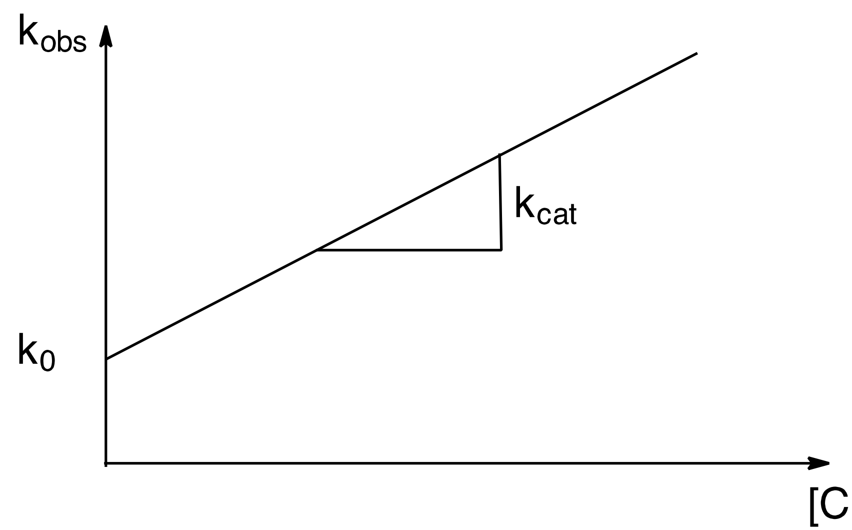
Catalysis – Kinetics

Catalyzed and uncatalyzed reactions run in parallel

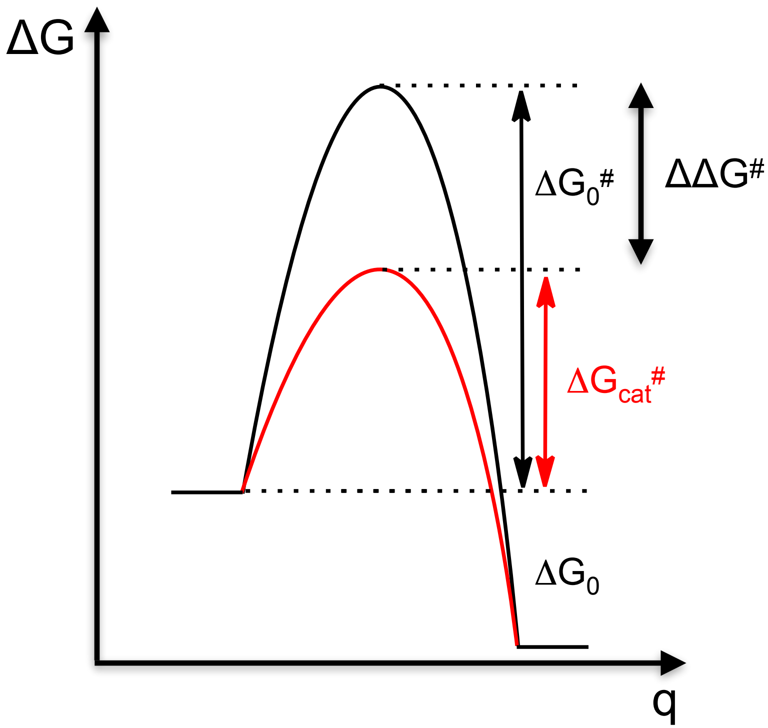
$$
\begin{aligned}
v_{obs} &= v_0 + v_{cat} \\
&= k_0[R] + k_{cat}[R][C] \\
&= [R]\underbrace{(k_0 + k_{cat}[C])}_{k_{obs}}
\end{aligned}
$$

$$v_{obs} = k_{obs}[R]$$

$$k_{obs} = k_0 + k_{cat}[C]$$



Catalysis – Thermodynamics



Eyring eq. $$k = \frac{kT}{h} e^{-\frac{\Delta G^{\#}}{RT}}$$

if	$\Delta G_{cat}^{\#} < \Delta G_{0}^{\#}$
then	$k_{cat} > k_{0}$

A ΔΔG‡ of 5.7 kJ/mol (1/2 of one hydrogen bond) gives a 10-fold rate enhancement.

A ΔΔG‡ of 34 kJ/mol (small fraction of a covalent bond) gives a 10^6-fold rate enhancement

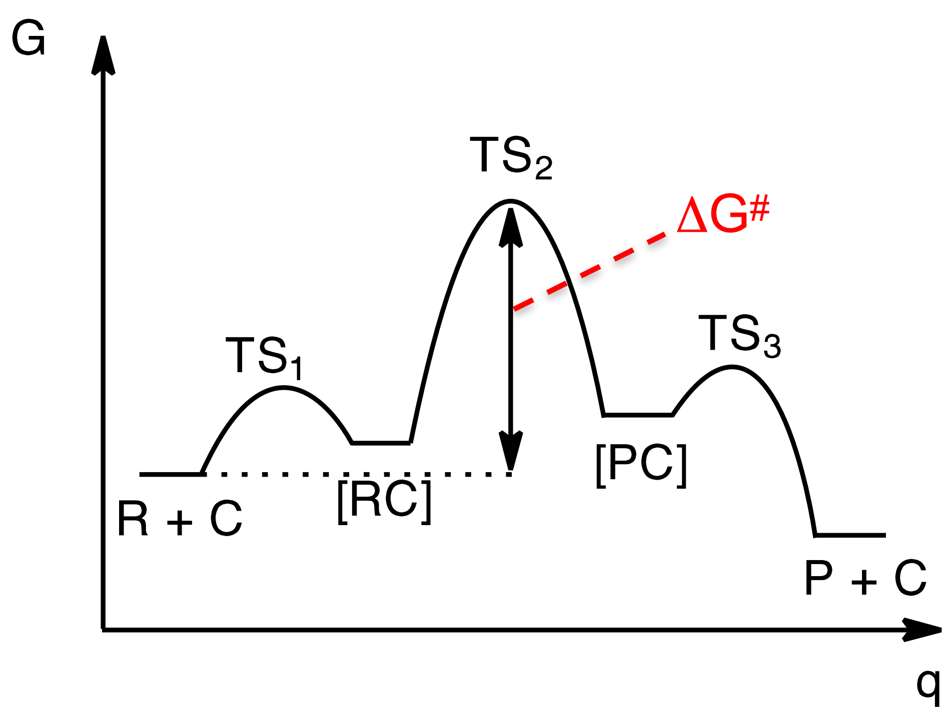
Catalysis – Thermodynamics

R + C ⟶ P + C

A simplified representation

R + C ⇌ [RC] ⟶ [PC] ⇌ P + C

A more realistic representation

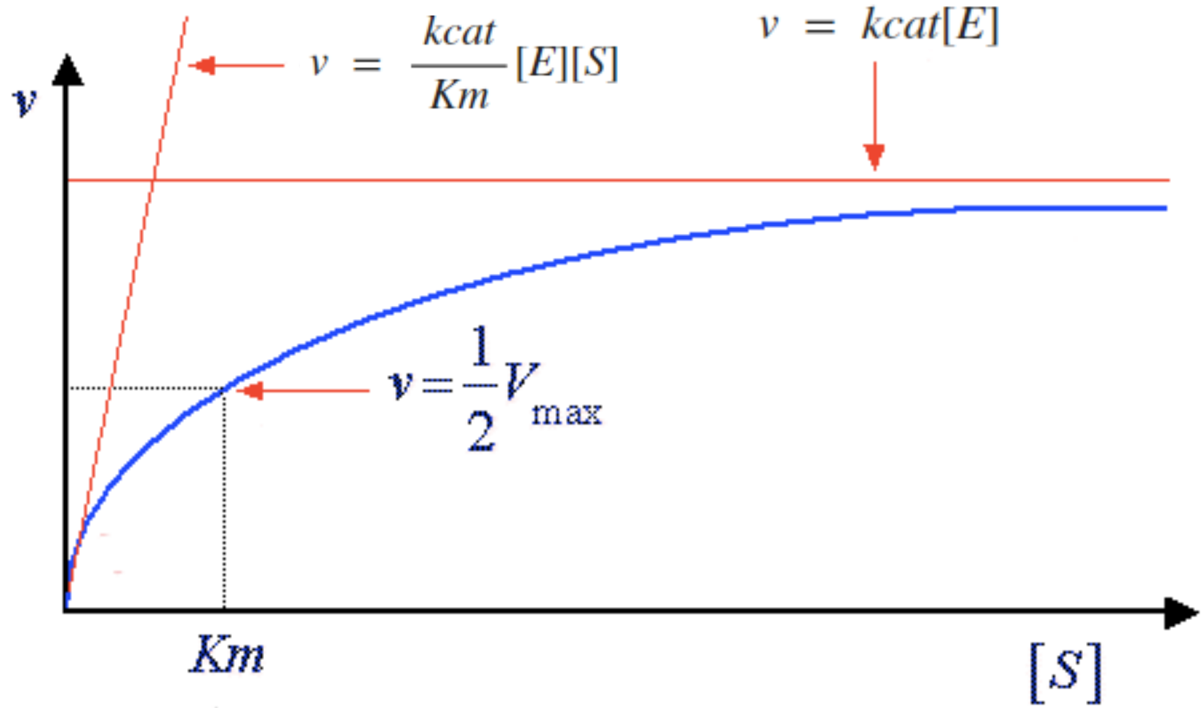


Michaelis-Menten Equation

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} EP \rightleftharpoons E + P$$

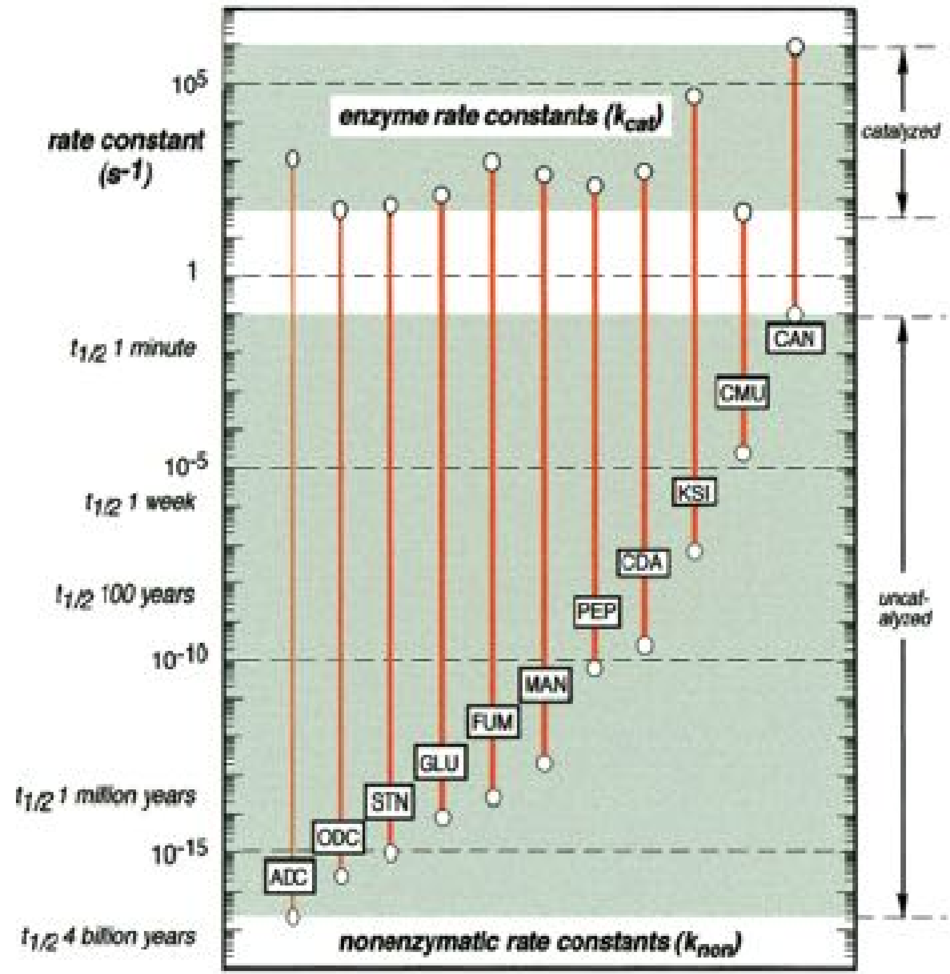
binding catalysis

$$v = \frac{kcat[E][S]}{Km + [S]} \qquad Km = \frac{k{-}1 + kcat}{k1}$$



Catalytic Efficiency: k_{cat}

ADC) arginine decarboxylase;
ODC) orotidine 5'−phosphatedecarboxylase;
STN) staphylococcalnuclease;
GLU) sweetpotato α−amylase;
FUM) fumarase;
MAN) mandelateracemase;
PEP) carboxypeptidaseB;
CDA) E. coli cytidinedeaminase;
KSI) ketosteroidisomerase;
CMU) chorismatemutase;
CAN)carbonicanhydrase.



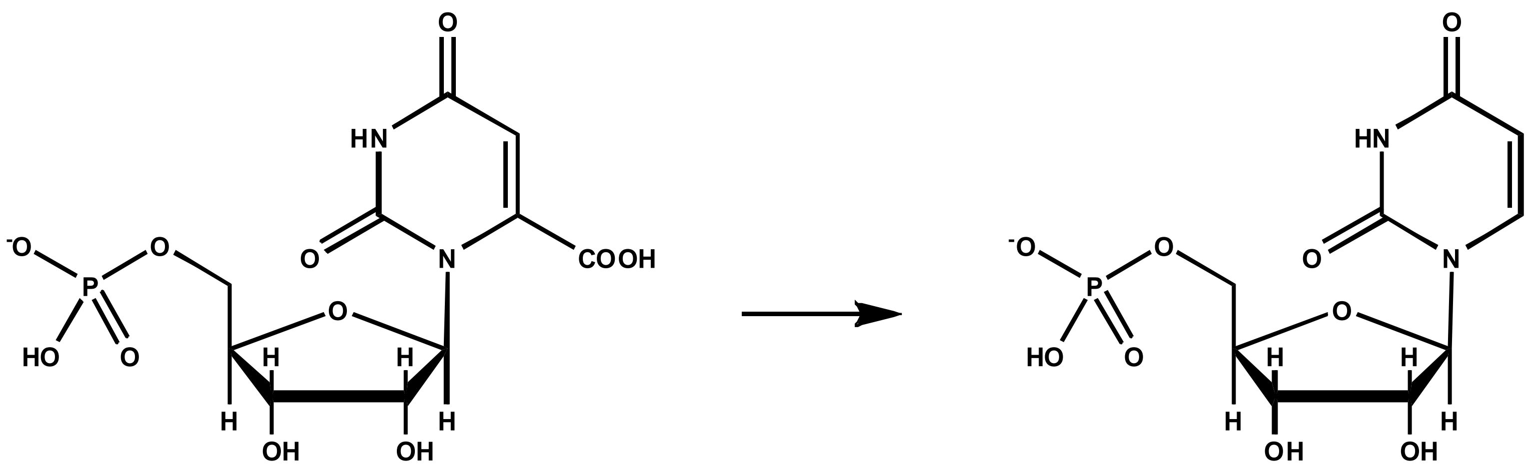
Catalytic Perfection: k_{cat}/K_M

Enzyme	Substrate	Reaction Type	k_{cat}/K_M ($M^{-1}s^{-1}$)	Rate-det. step
superoxide dismutase	***superoxide***	***redox***	***7×10^9***	***diffusion***
fumarase	fumarate	hydration	1×10^9	diffusion
triose phosphate isomerase	***glyceraldehyde 3-phosphate***	***enolization***	***4×10^8***	***diffusion***
b-lactamase	penicillin	lactam hydrolysis	1×10^8	partly diff.
OMP decarboxylas	***orotidine 5'-phosphate***	***decarboxyl ation***	***6×10^7***	***not diff.***
cytochrome c peroxidase	hydrogen peroxide	redox	5×10^7	not diff.
HIV protease	***peptide***	***amide hydrolysis***	***2×10^7***	***not diff.***

Catalytic Efficiency

k_{cat}	up to 10^8 s^{-1}
k_{cat}/k_0	10^6 - 10^{20}
K_m	10^{-3} – 10^{-6} M
k_{cat}/K_m	10^5 – 10^{10}

ODC: Orotidine 5'-phosphate decarboxylase



OMP UMP

$k_{cat}/k_0 = 10^{17}$

$t_{1/2}$ = 78.000.000 years → 0.018 s

$K_{TS} = 10^{23}$

Enzymes are wonderful catalysts

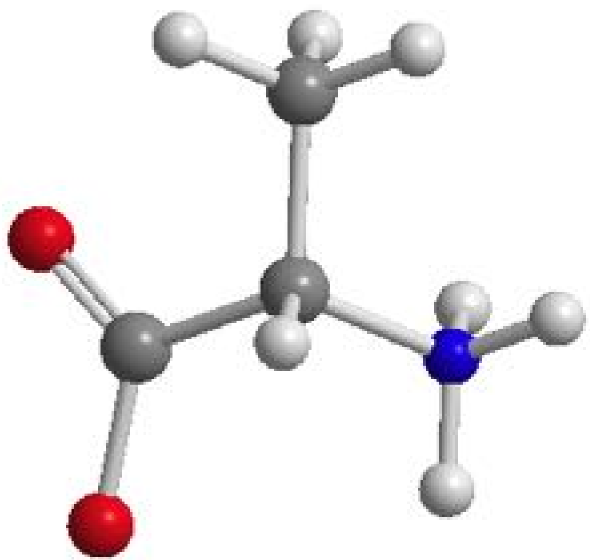
- Catalytic Efficiency $k_{cat}/k_0 = 10^6 - 10^{20}$

- Specificity
- Selectivity

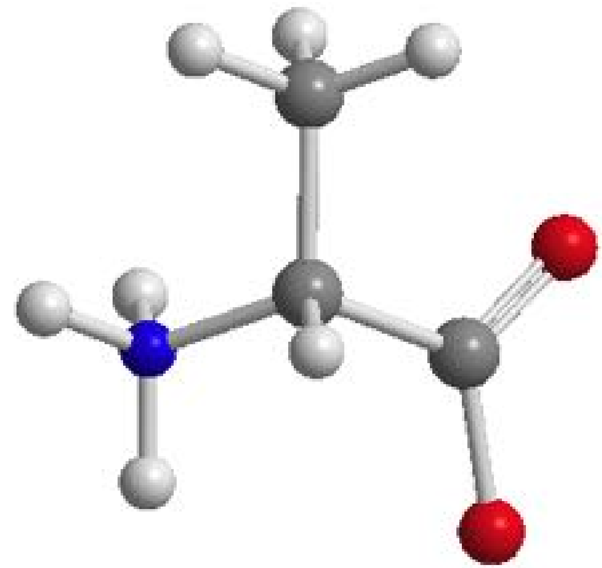
} ⟺ binding

Structure and Properties of Amino Acids, Peptides, Proteins and Enzymes

Aminoacids



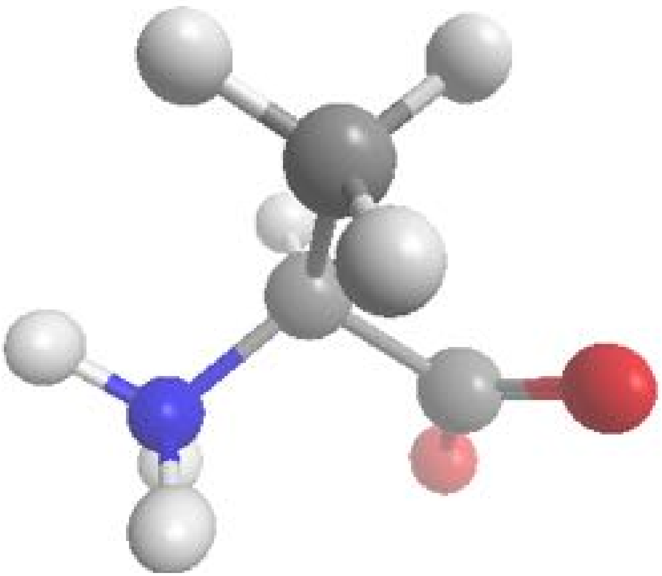
L-alanine



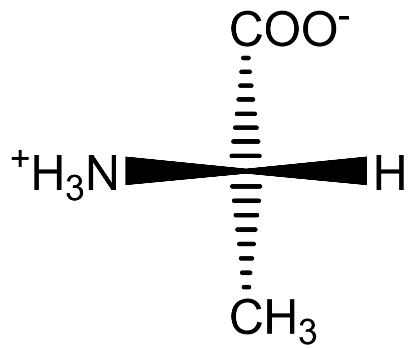
D-alanine

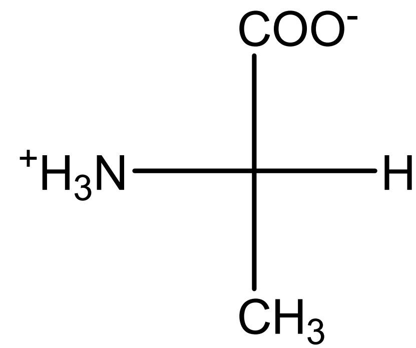
Enantiomers = non superimposable mirror images

Stereochemical Notation

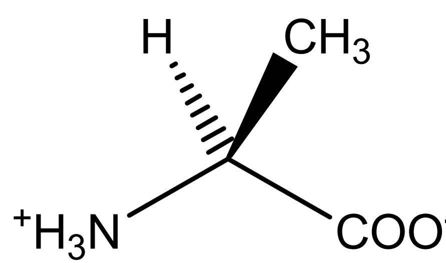


natural alanine



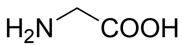


Fischer: L (D)

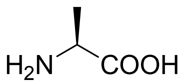


Cahn-Ingold-Prelog (C.I.P.): S (R)

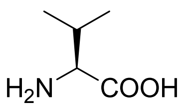
Neutral



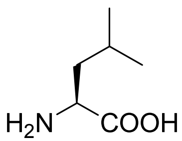
Glycine GLY G



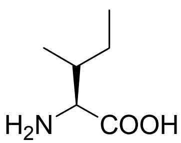
Alanine ALA A



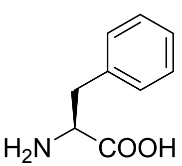
Valine VAL V



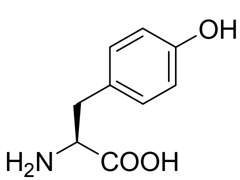
Leucine LEU L



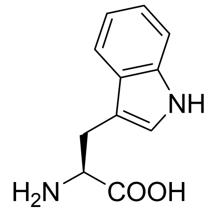
Isoleucine ILE I



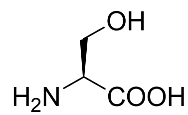
Phenilalanine PHE F



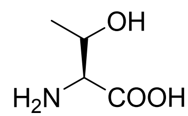
Tyrosine TYR Y



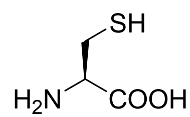
Triptophan TRP W



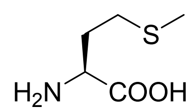
Serine SER S



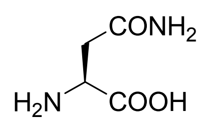
Threonine THR T



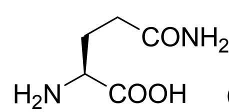
Cysteine CYS C



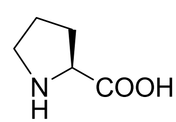
Methionine MET M



Asparagine ASN N

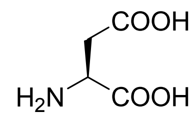


Glutammine GLN Q

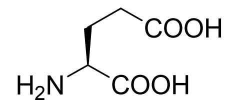


Proline PRO P

Acids

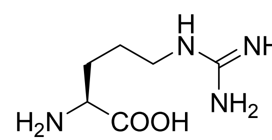


Aspartic Ac. ASP D

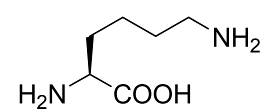


Glutammic Ac. GLU E

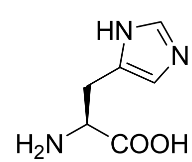
Bases



Arginine ARG R

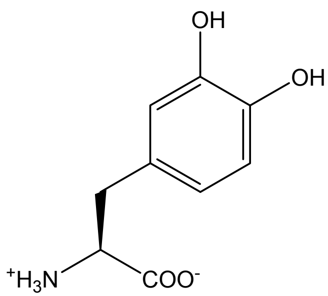


Lysine LYS K

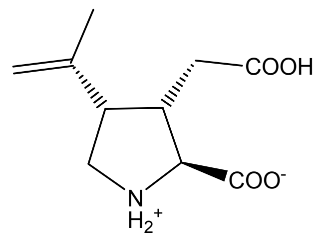


Histidine HIS H

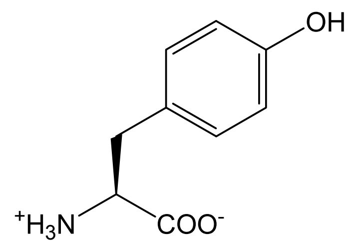
Non Proteinogenic a.a.



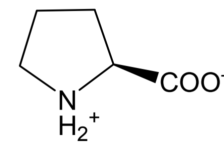
L-Dopa
(anti-Parkinson's)



Kainic Acid
stimulant (seaweed)

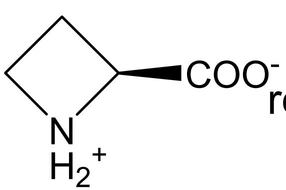


Tyr



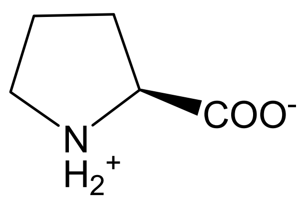
Pro

Non proteinogenic a.a. are occasionally found in proteins

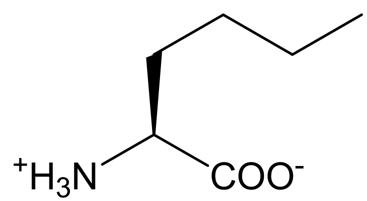


Azetidin-
2-carboxylic acid

replaces

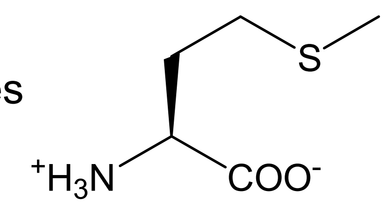


Pro



Norleucine

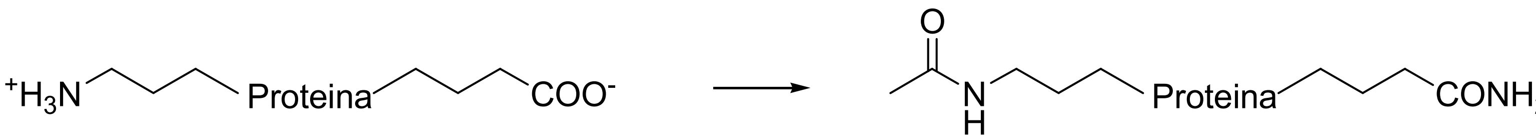
replaces



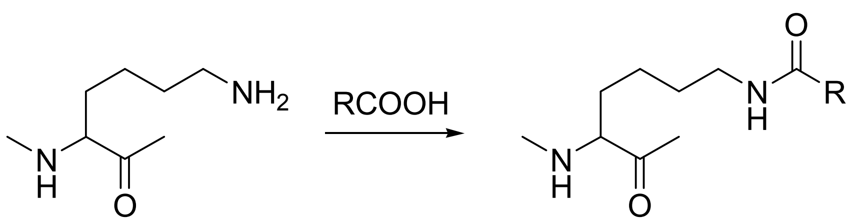
Met

Post-traslational Modifications

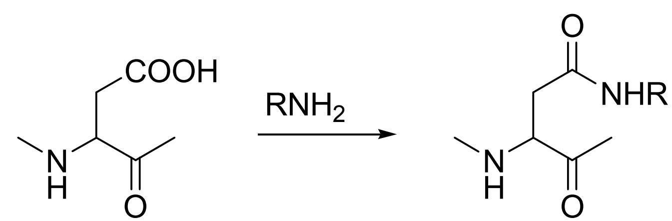
C,N-terminal



Side chains

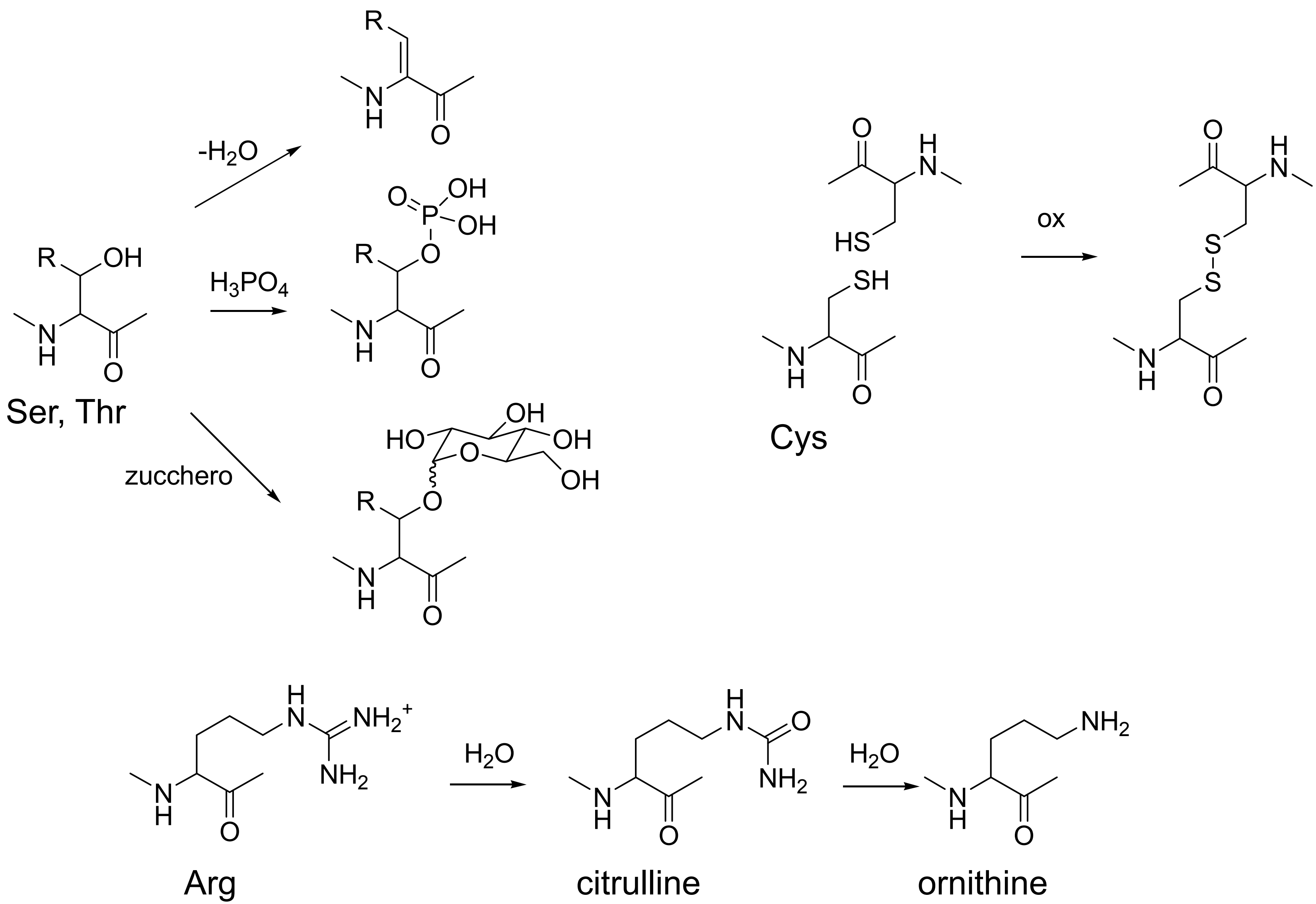


Lys

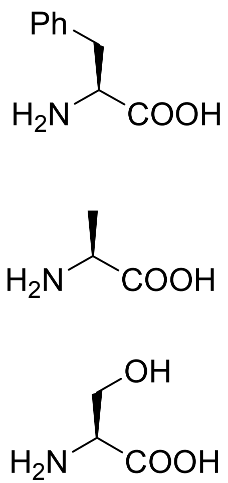


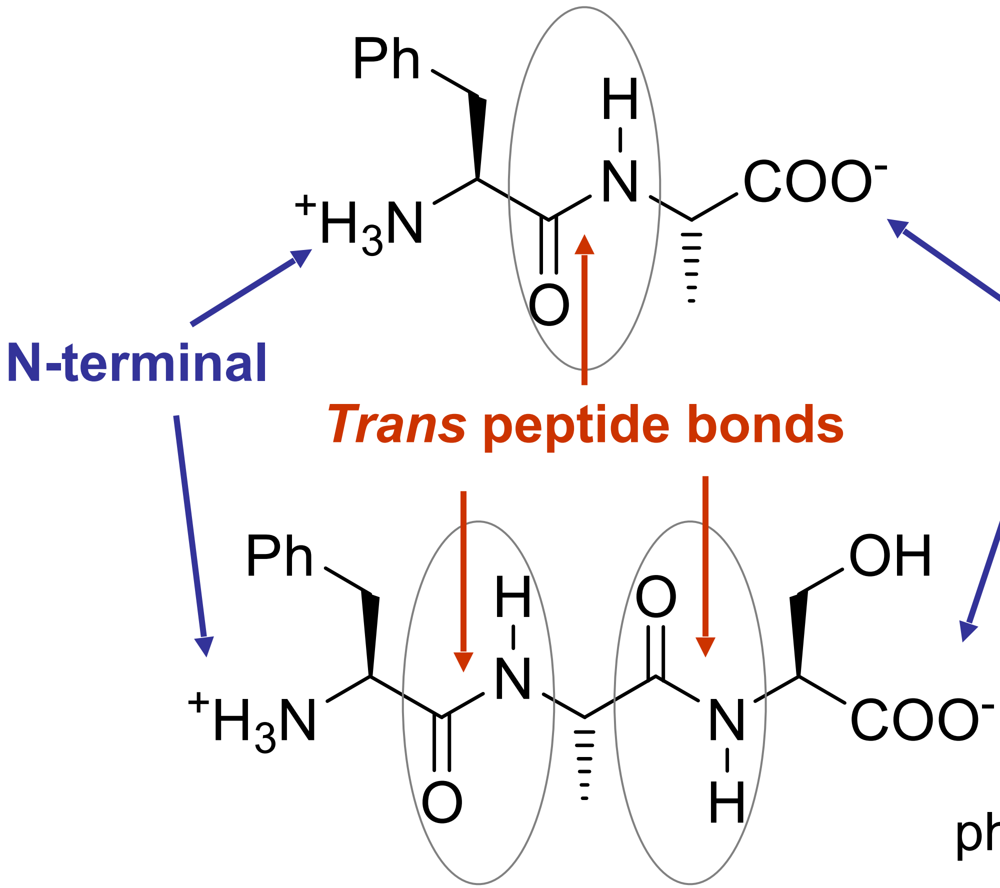
Asp, Glu

Post-traslational Modifications



Peptides



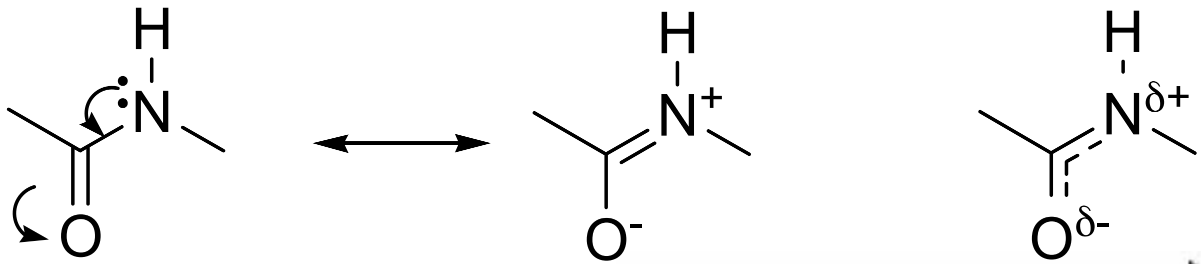


A dipeptide:
phenylalanylalanine
Phe-Ala
FA

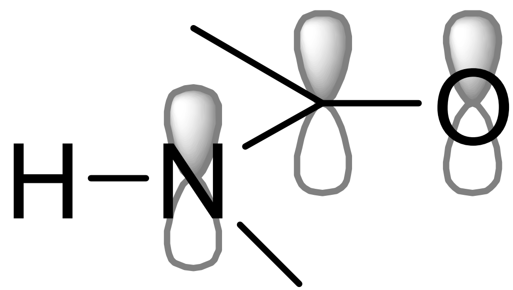
A tripeptide:
phenylalanylalanylserina
Phe-Ala-Ser
FAS

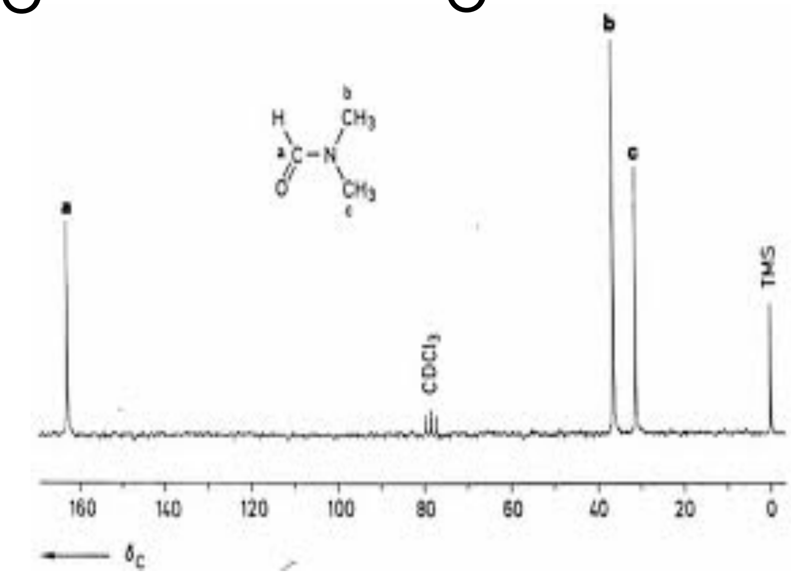
The Peptide Bond

Amide resonance



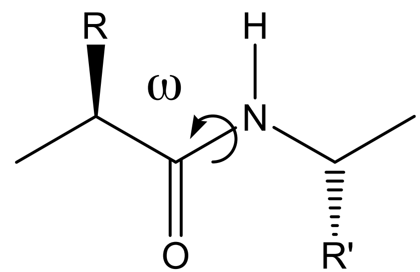
planar (sp2) C, N, O
restricted rotations



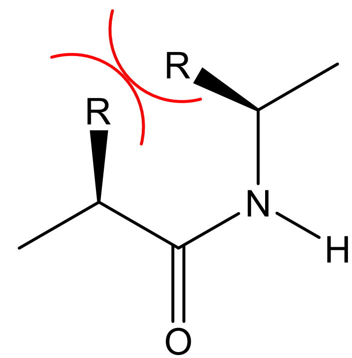


Cis-Trans Peptide Bonds

trans amides are generally more stable (less hindered)

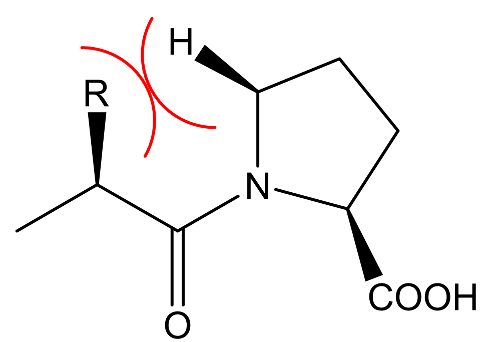


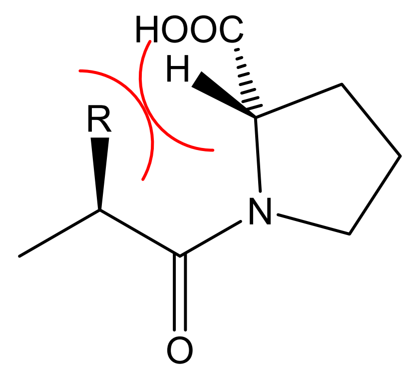
trans ω = 180°



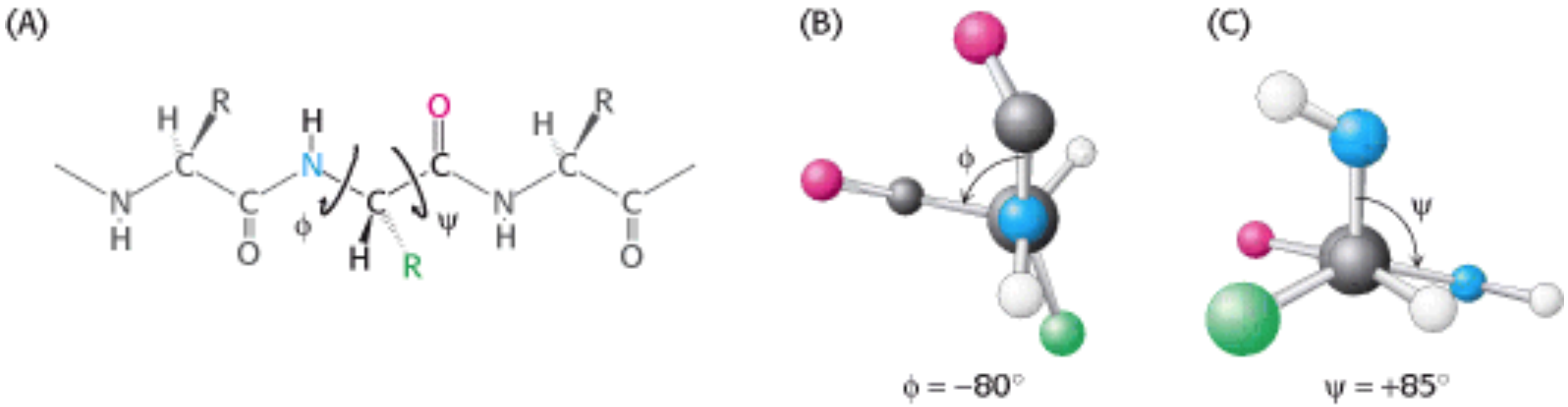
cis ω = 0°

the proline case





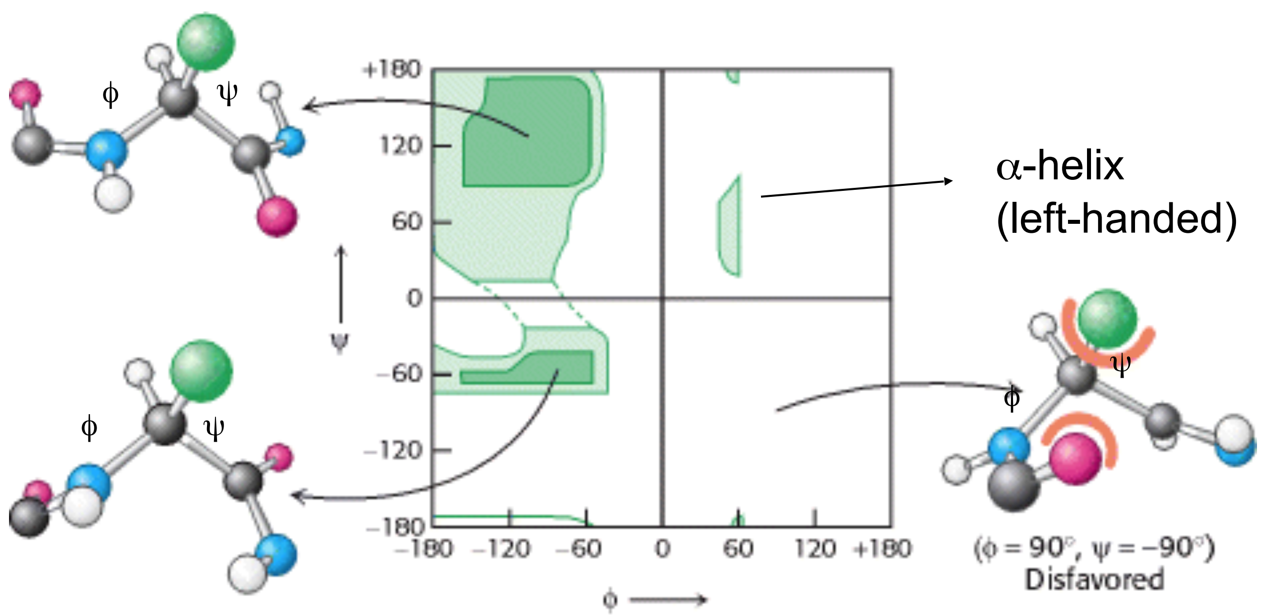
Conformations of Peptides



Ramachandran Plot

β–sheet

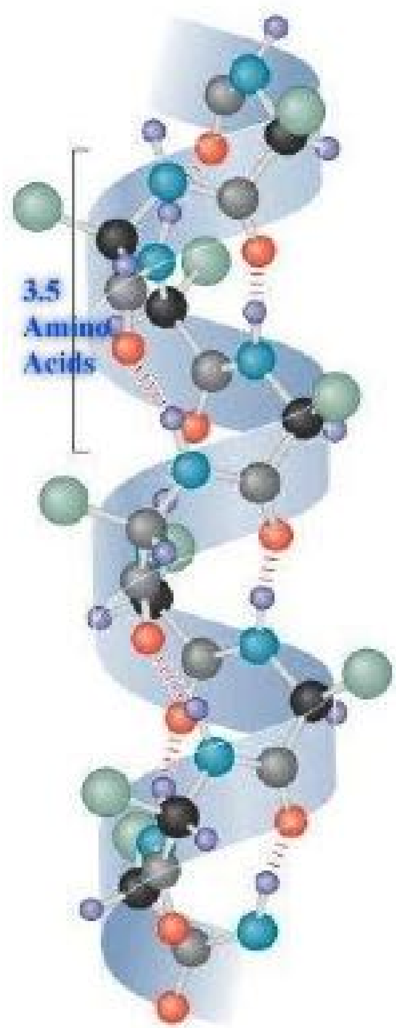
α-helix
(right-handed)



α-helix
(left-handed)

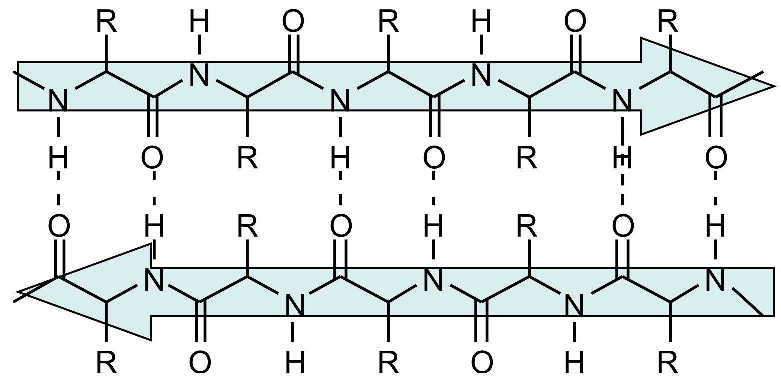
Conformations of Peptides

α-helix

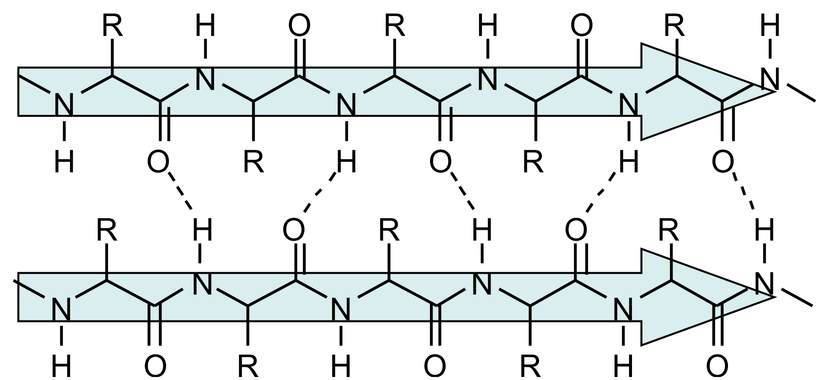


β-sheet

antiparallel

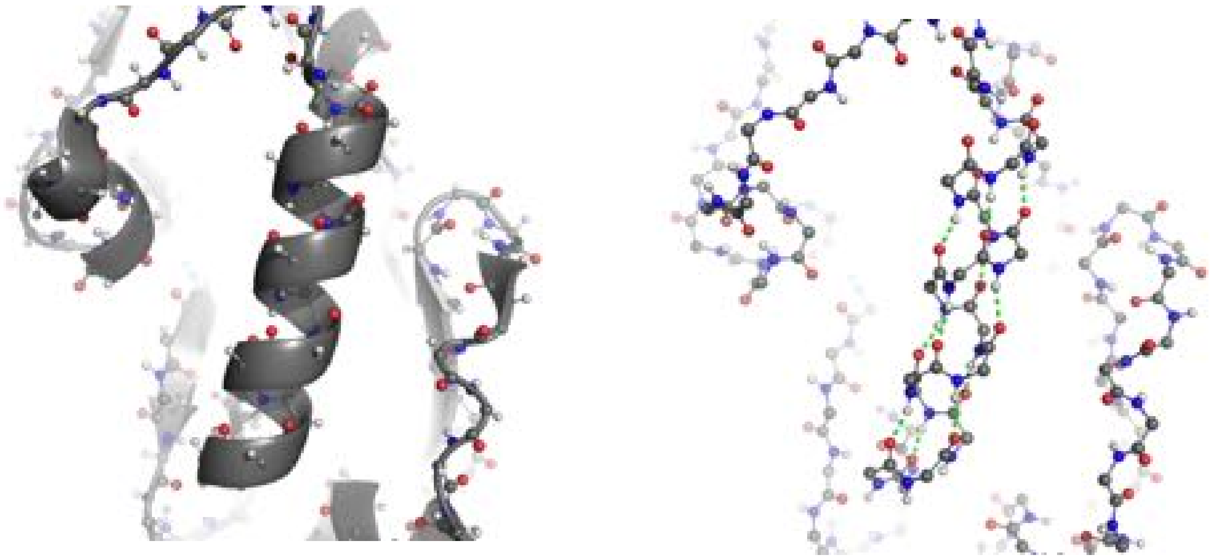


parallel

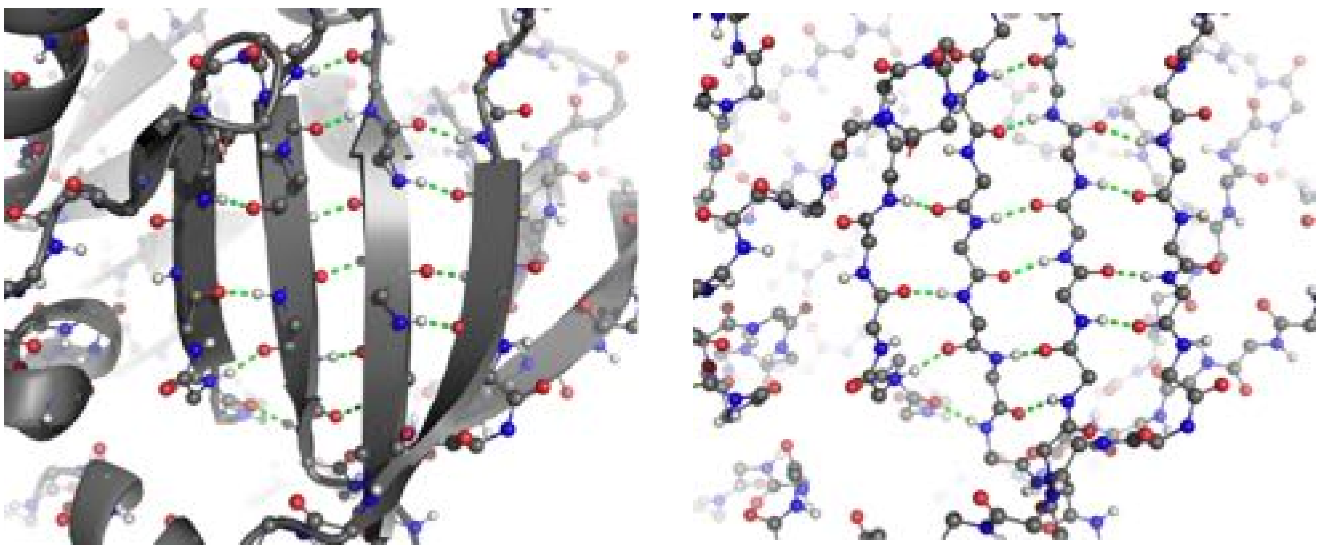


Secondary Structure of Proteins

α-helix

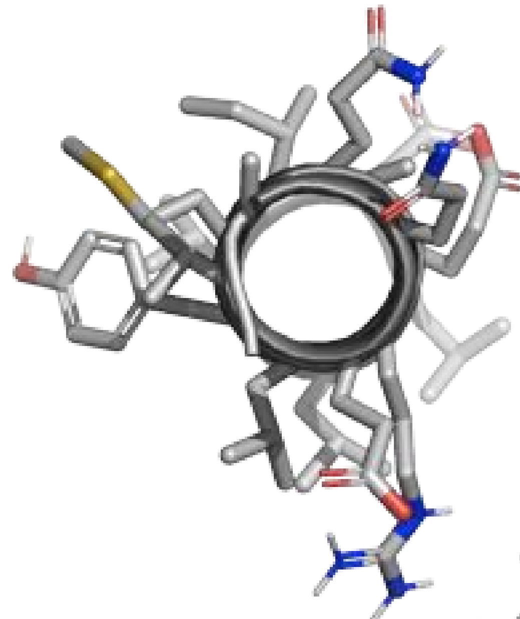


β-sheet

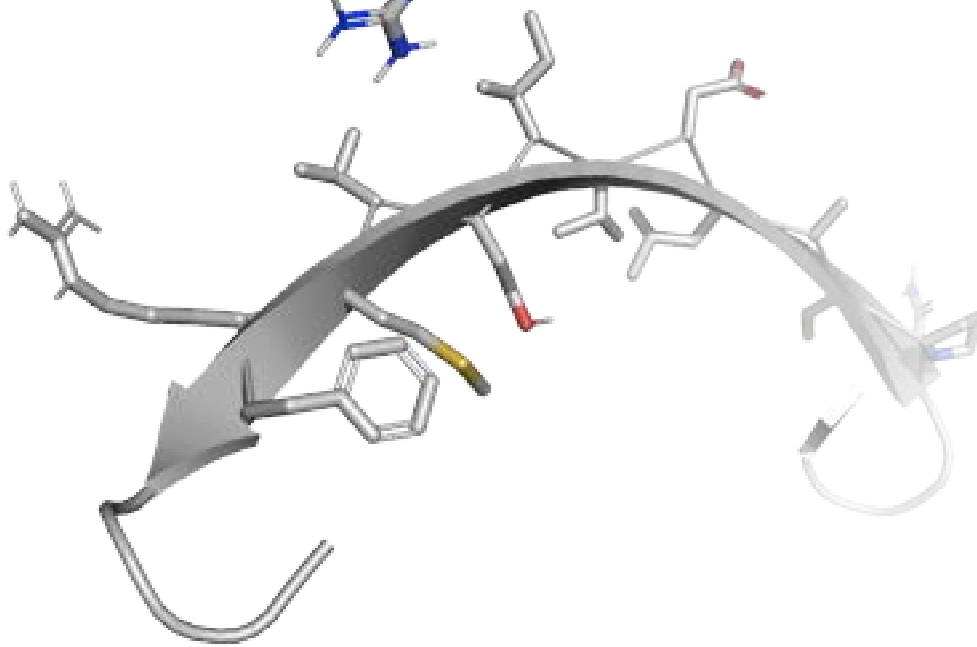


Secondary Structure of Proteins

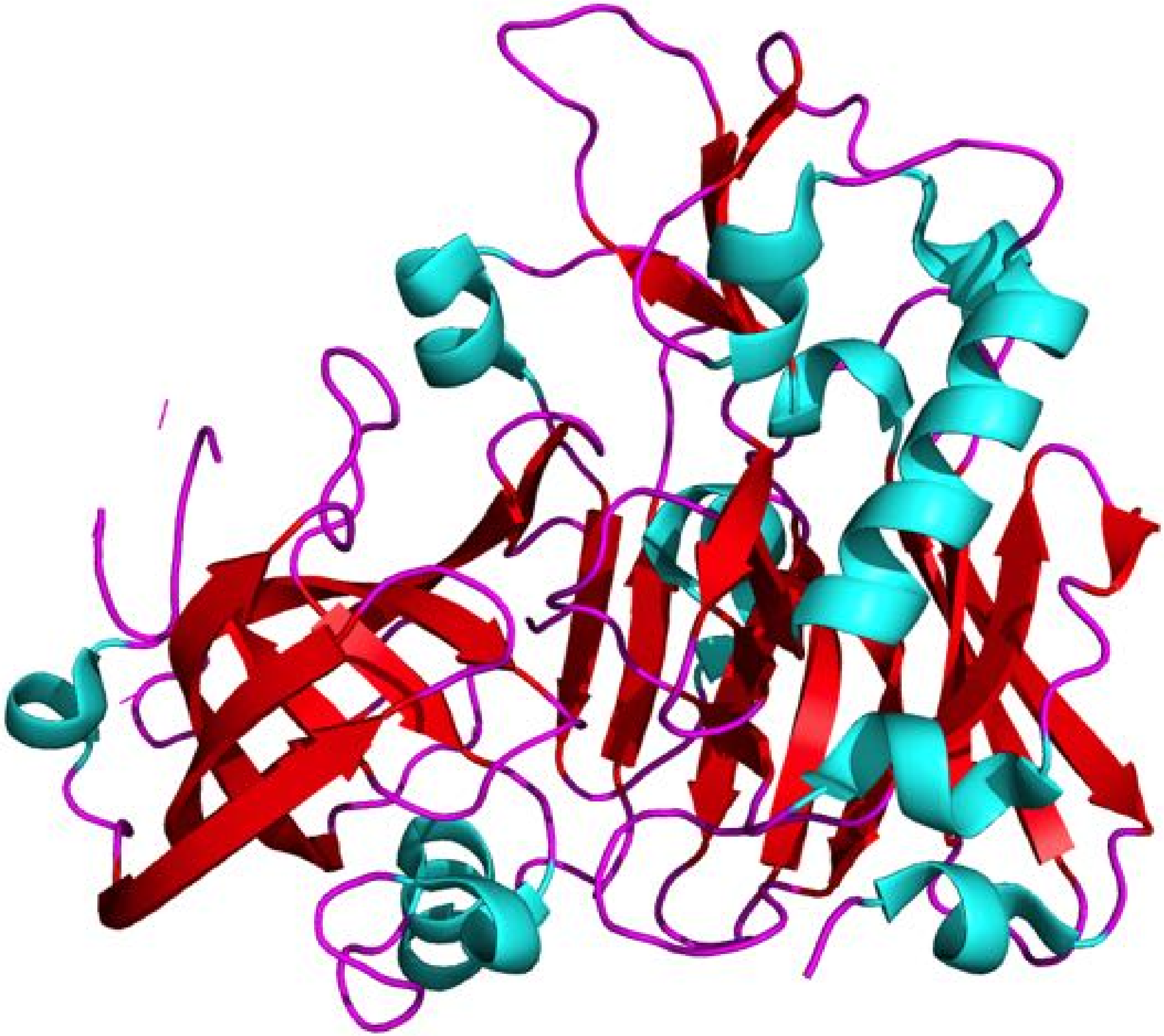
α-helix



β-sheet

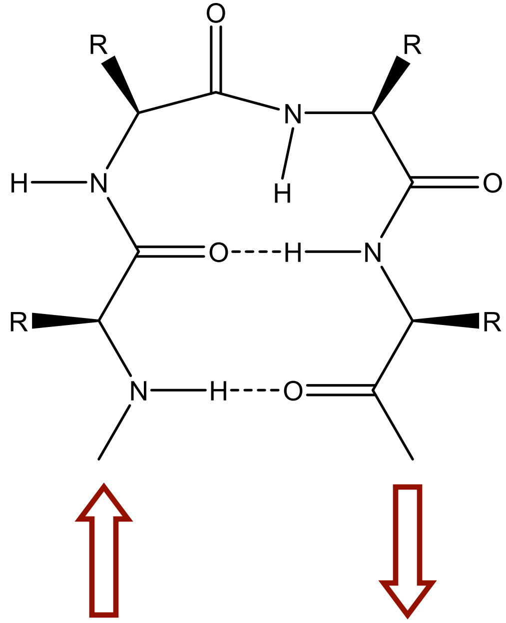


Secondary Structure of Proteins



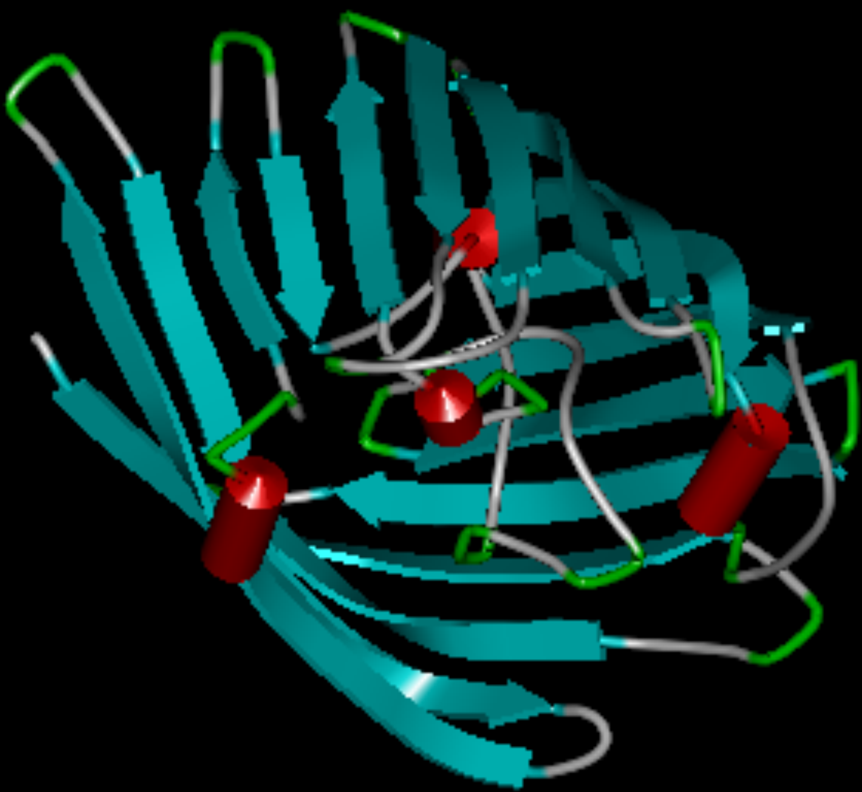
Conformations of Peptides

β-turn

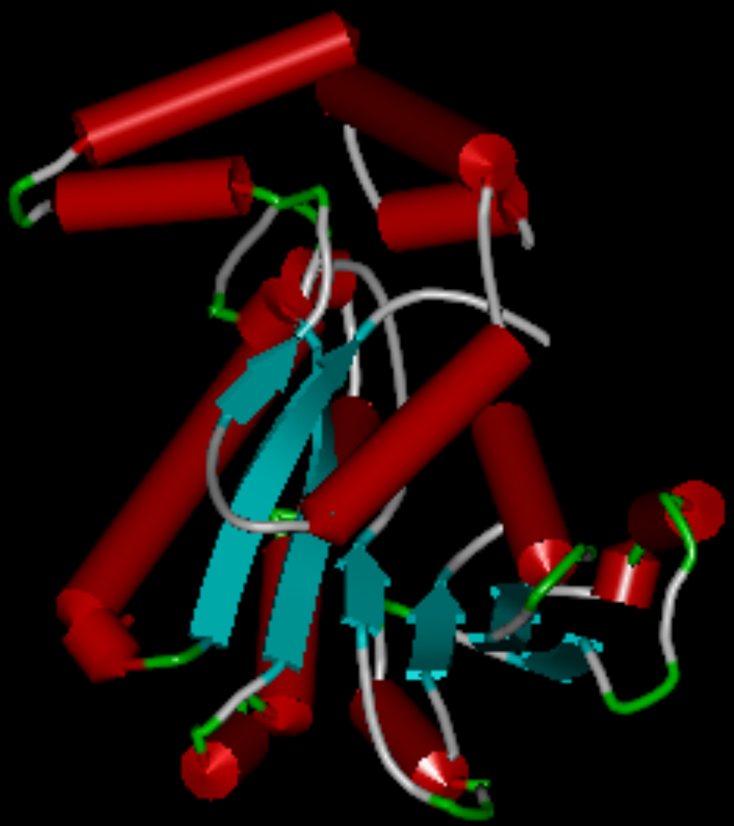


Secondary Structure of Proteins

Antiparallel β Sheets



Helices and Parallel β Sheets



Chou-Fasman Rule

α-helix

Glu Ala Leu His Met Gln Trp Val Phe — promote (helicogenic)

Lys Ile Asp Thr Ser Arg Cys — neutral

Asn Tyr Pro Gly — inhibit

4 helicogenic aa in a sequence of 6 initiate a α-helix

β-sheet

Met Val Ile Cys Tyr Phe Gln Leu Thr Trp — promote

Ala Arg Gly Asp — neutral

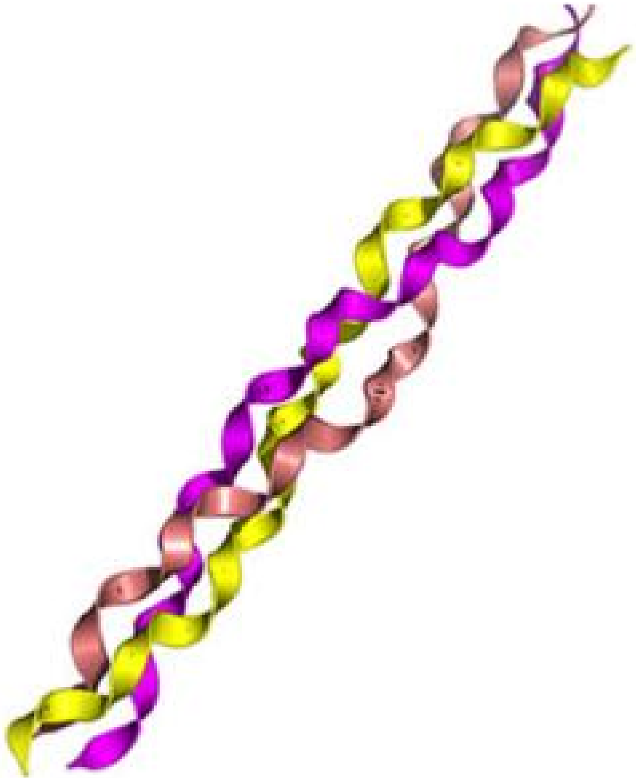
Lys Ser His Asn Pro Glu — inhibit

3 promoters in a sequence of 5 initiate a β sheet.

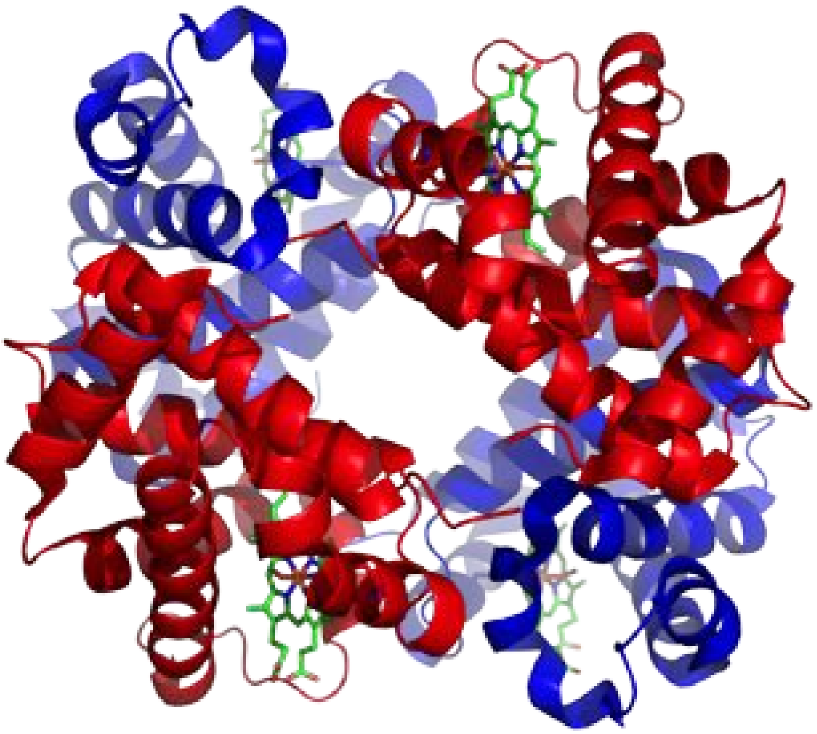
4 inhibitors terminate a β sheet

Tertiary Structure

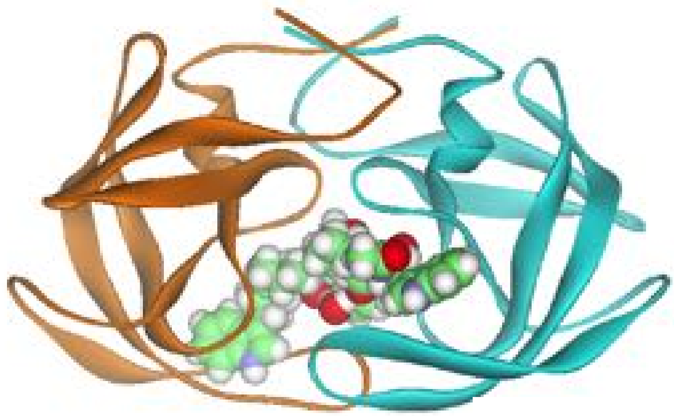
Collagen: LINEAR

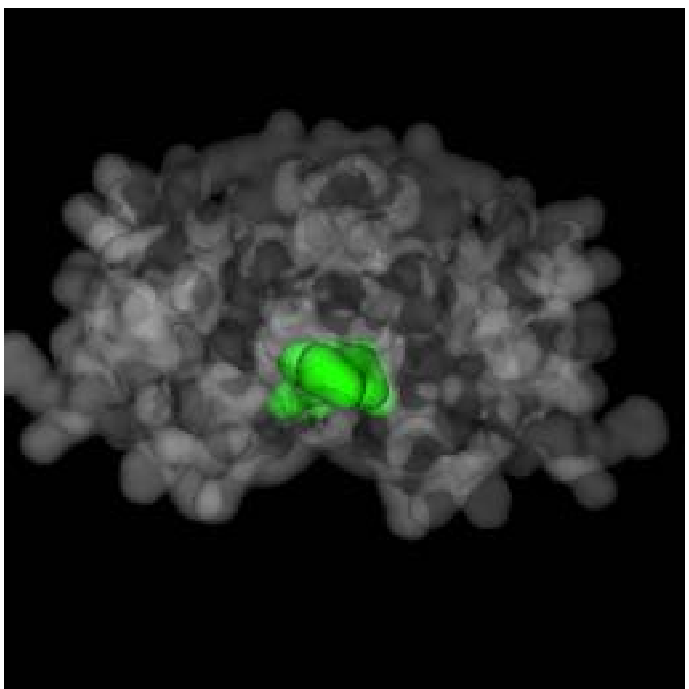


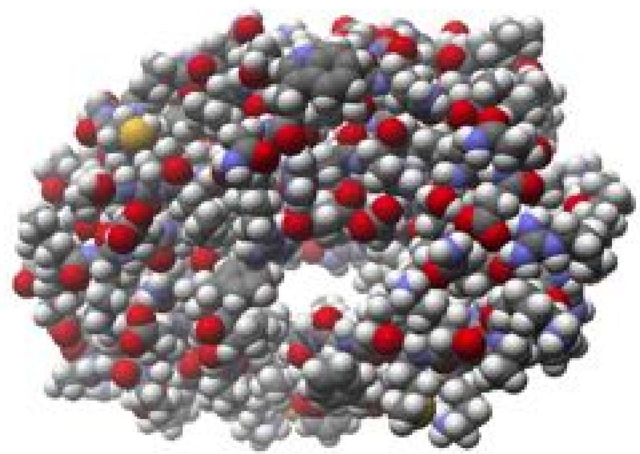
Haemoglobin: GLOBULAR



Hiv-protease complexed with a substrate

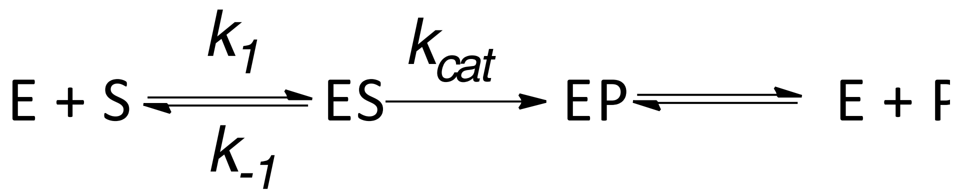






Catalytic Efficiency

Binding and Catalysis



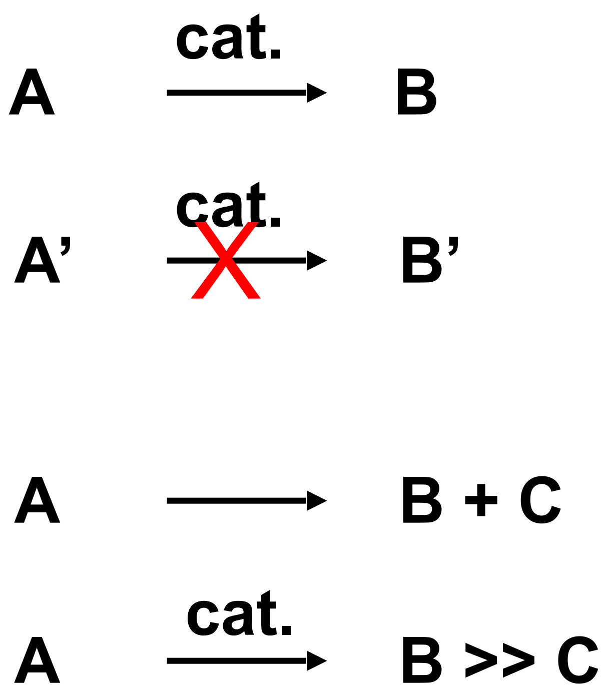
binding catalysis

The activity of enzymes depends on:

their ability to bind a substrate (**binding**)

their ability to promote its transformations (**catalysis**)

Specificity and selectivity



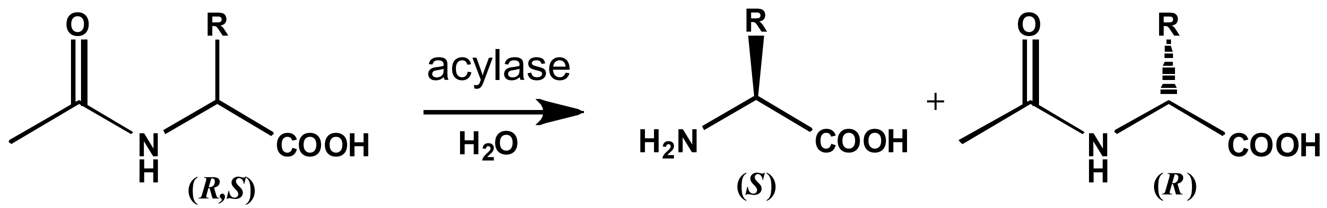
specificity
with respect to reagents

selectivity
with respect to products

Specificity of Serine Proteases

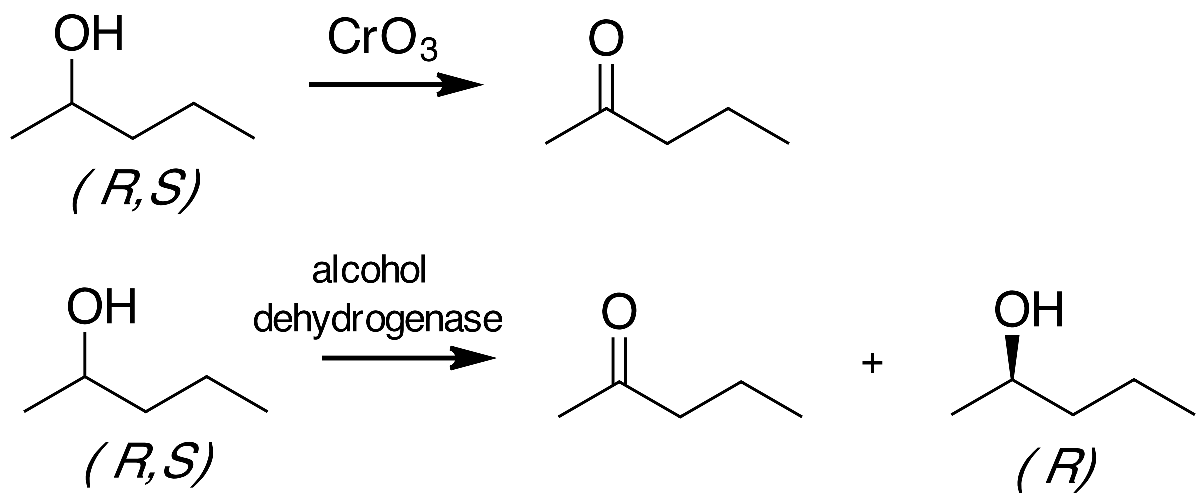
Chymotrypsin:	Phe-Xaa Tyr-Xaa Trp-Xaa
Trypsin:	Lys-Xaa Arg-Xaa
Elastase:	Gly-Xaa Ala-Xaa

Specificity and selectivity



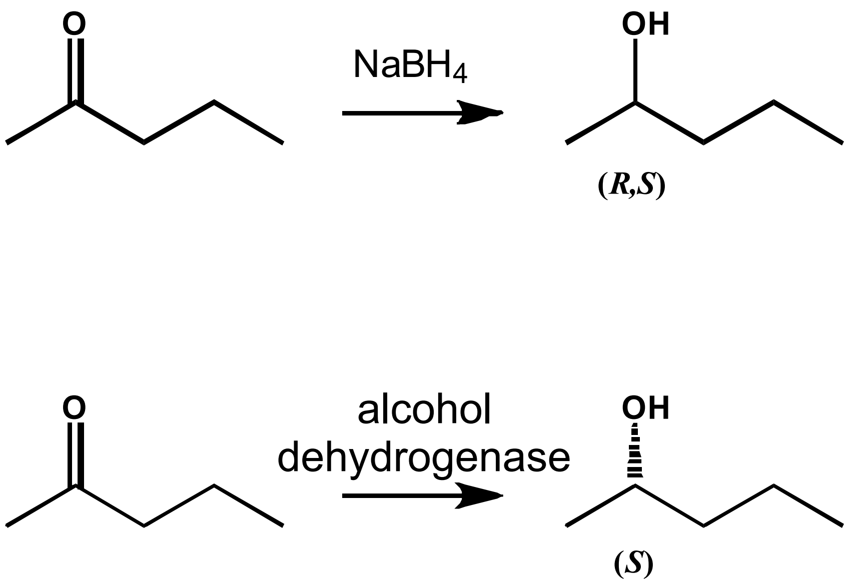
Acylase is stereo**specific**: it recognizes (*S*)-acyl-a.a. but not *R* isomers.

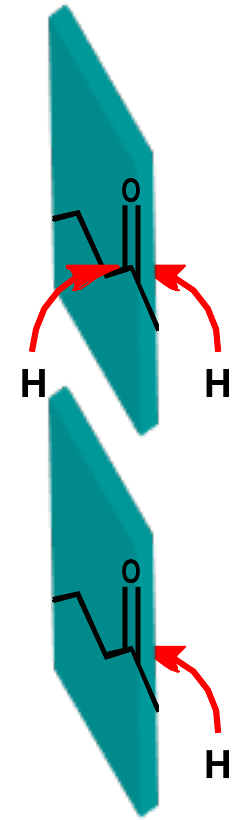
Specificity and selectivity



Alcohol dehydrogenase is stereo**specific**: only the (*S*) alcohol is oxidized

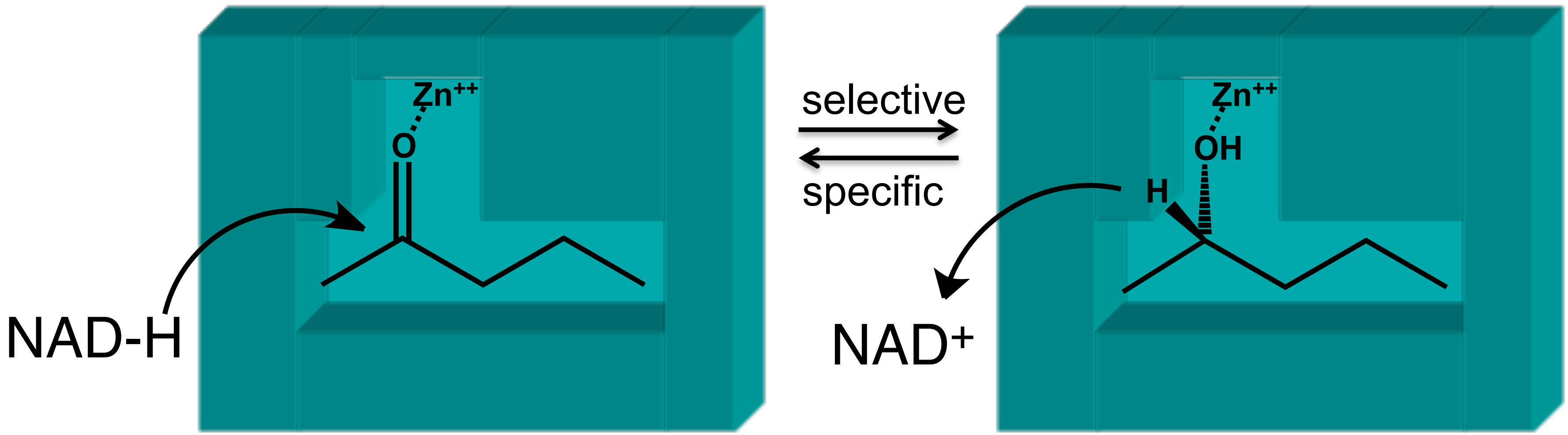
Specificity and selectivity





Alcohol dehydrogenase is stereo**selective**: only the (*S*) alcohol is formed

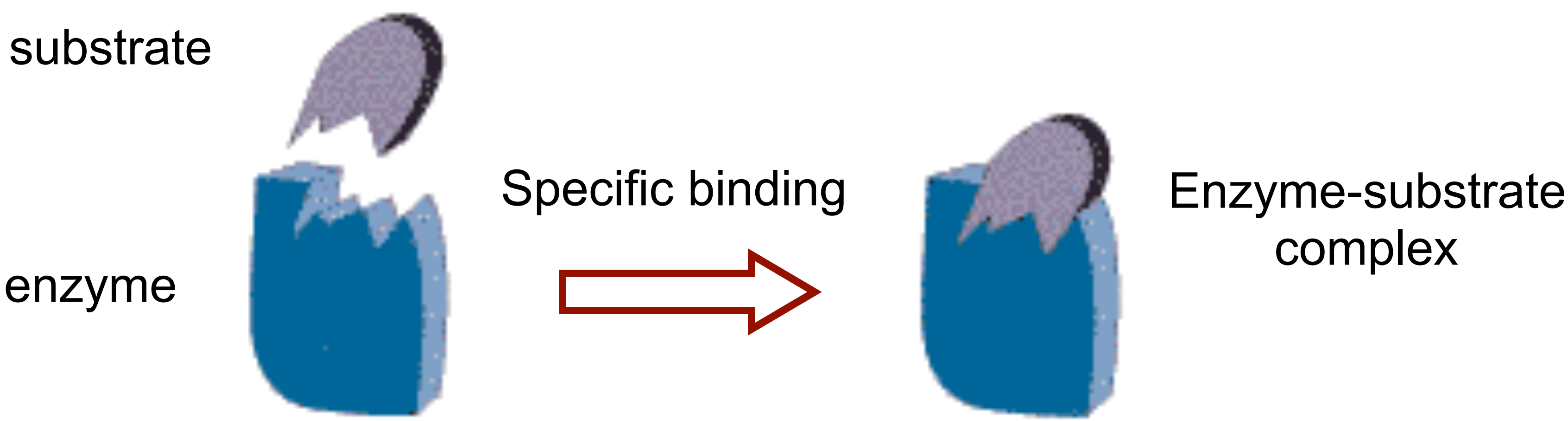
Specificity, Selectivity and Binding



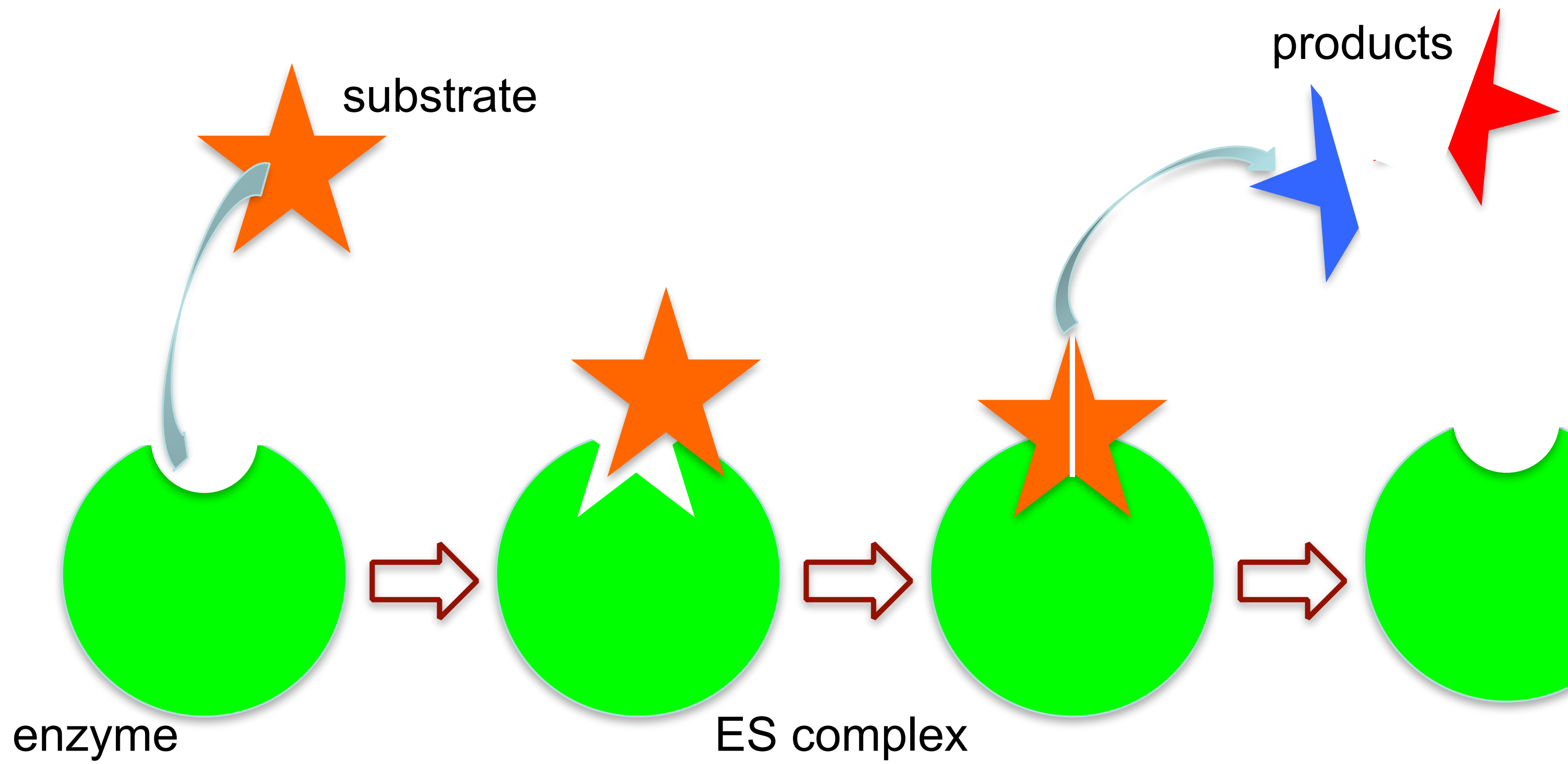
alcohol dehydrogenase

The Lock and Key Principle (Emil Fischer 1894)

Lock and key
are complementary



Flexible binding. Induced Fit



Non-Covalent Binding Interactions

Electrostatic Interactions (< 350 kJ/mol)

- Ion-Ion
- Ion-Dipole
- Dipole-Dipole

Hydrogen Bonding (< 160 kJ/mol)

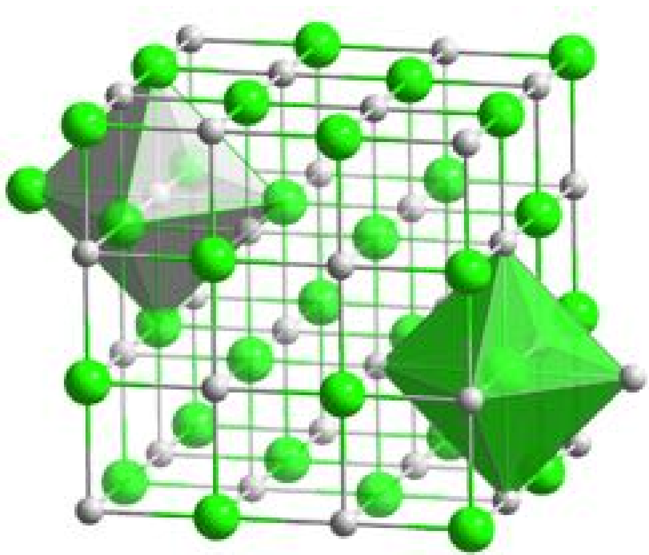
π-Bonds (< 80 kJ/mol)

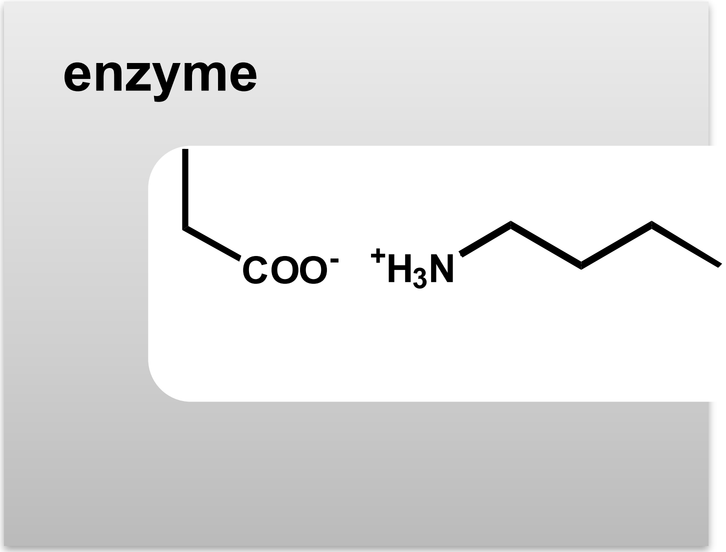
- Cation-π
- π-π Stacking

Van der Waals (< 10 kJ/mol)

- Dipole-Induced Dipole
- London Forces
- The Hydrophobic Effect

Electrostatic Interactions (up to 350 kJ/mol)





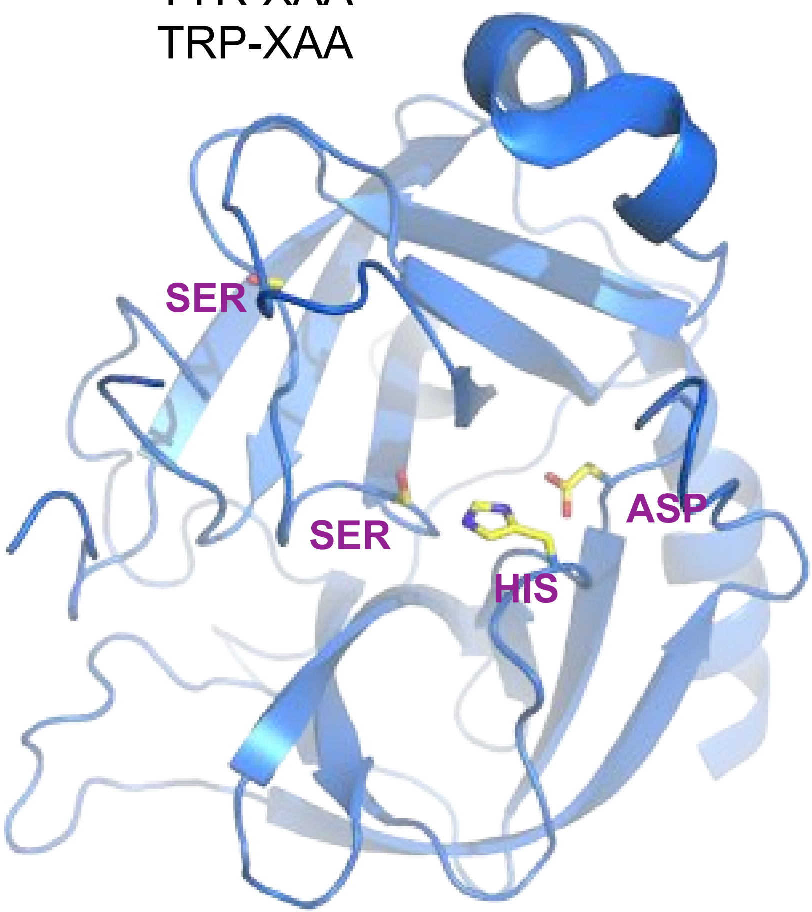
100-350 kJ/mol
$1/r^2$

20-60 Kj/mol
$1/r^2$

Electrostatic Interactions in Proteins

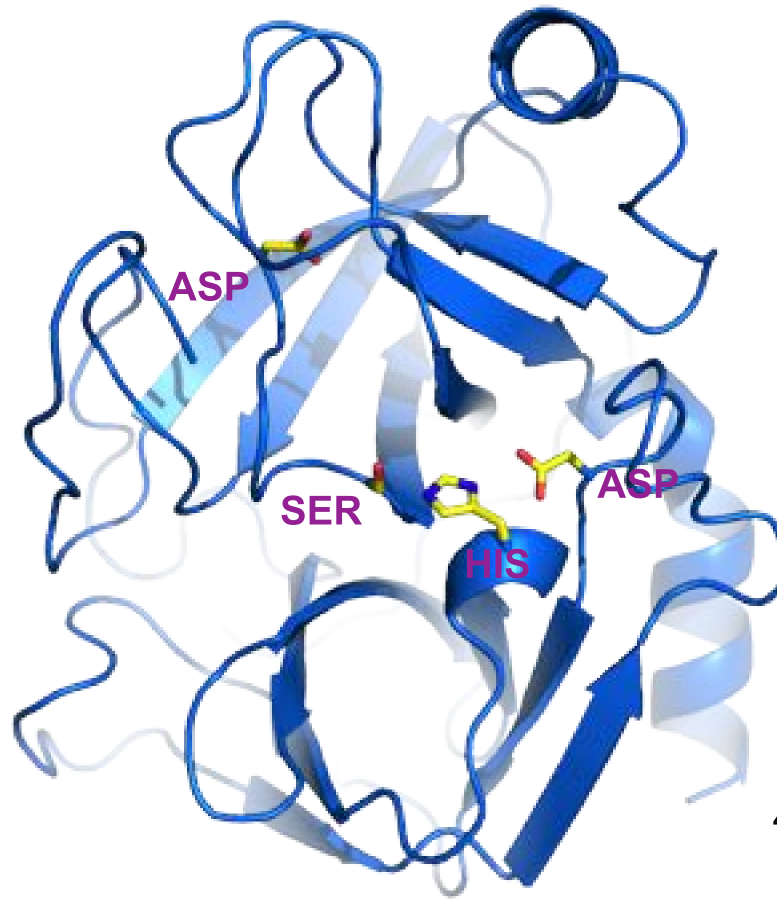
Chymotrypsin

PHE-XAA
TYR-XAA
TRP-XAA

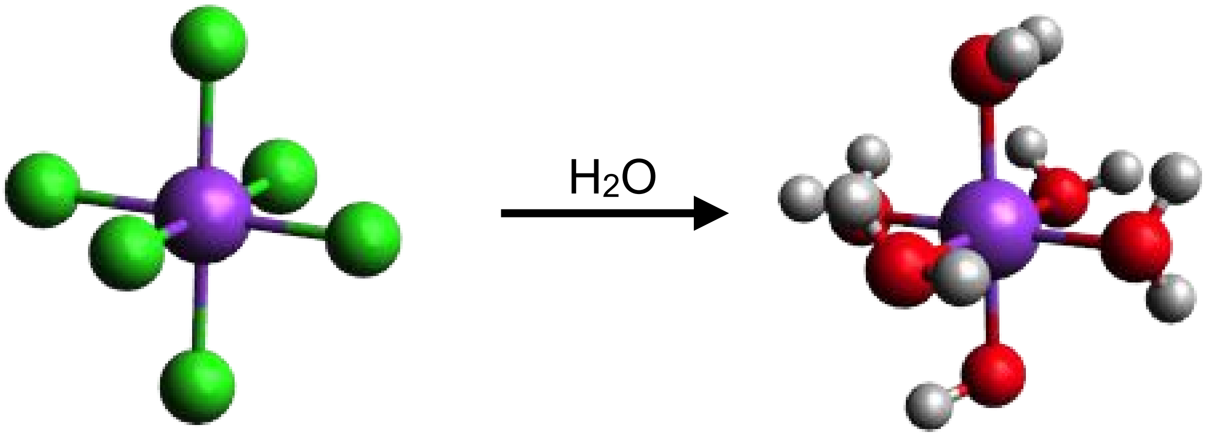


Trypsin

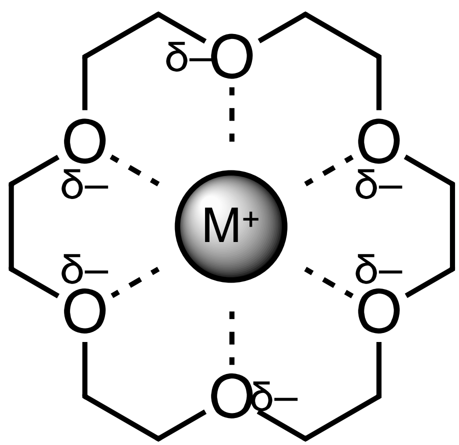
LYS-XAA
ARG-XAA



Ion-Dipole Interactions (50-200 kJ/mol)

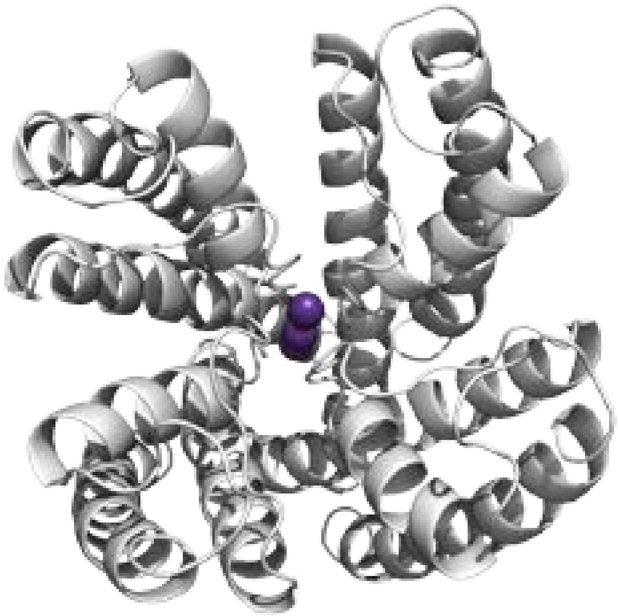


NaCl $\rightarrow$ $Na(H_2O)_6^+$

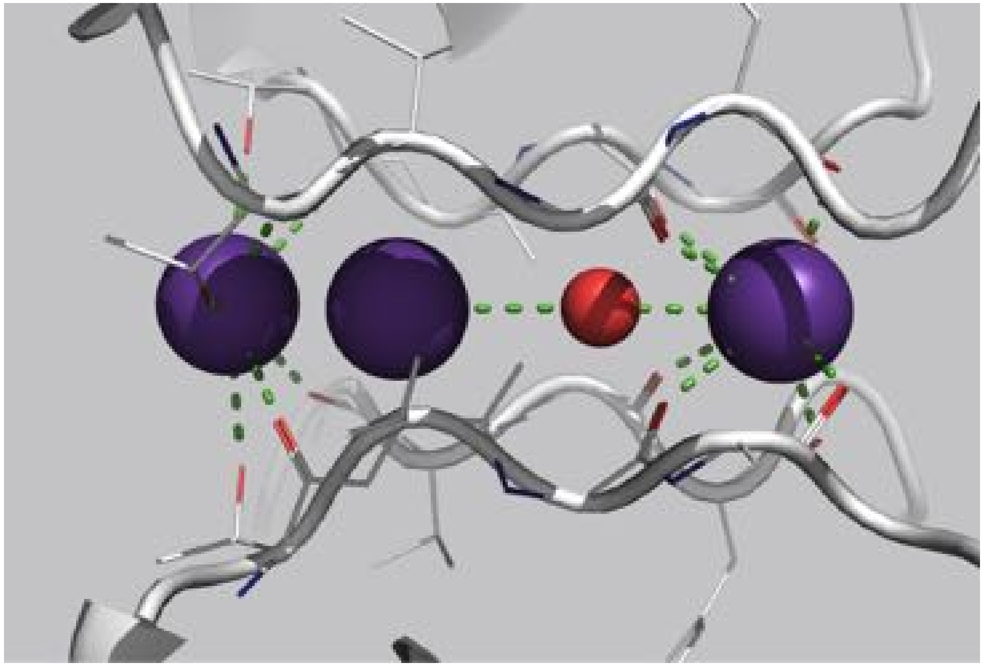


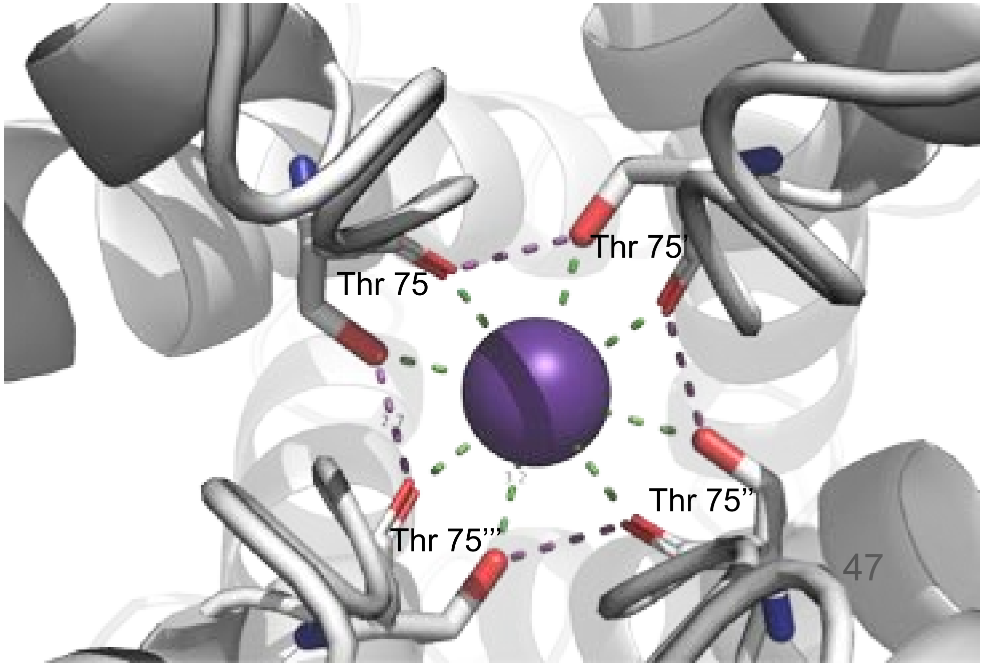
18 Crown 6

Ion-Dipole Interactions in Proteins



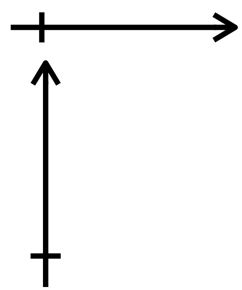
Potassium channel from
Streptomycin Lividans



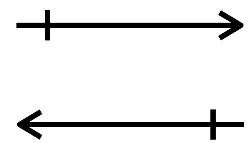


Noskov S.Y. Biophys. Chem. 2006

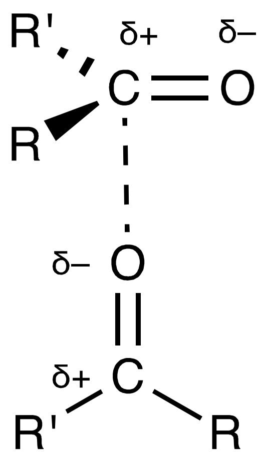
Dipole-Dipole Interactions (5-50 kJ/mol)



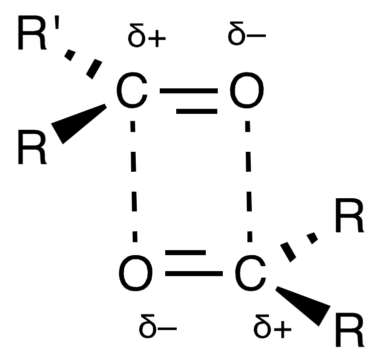
Orthogonal



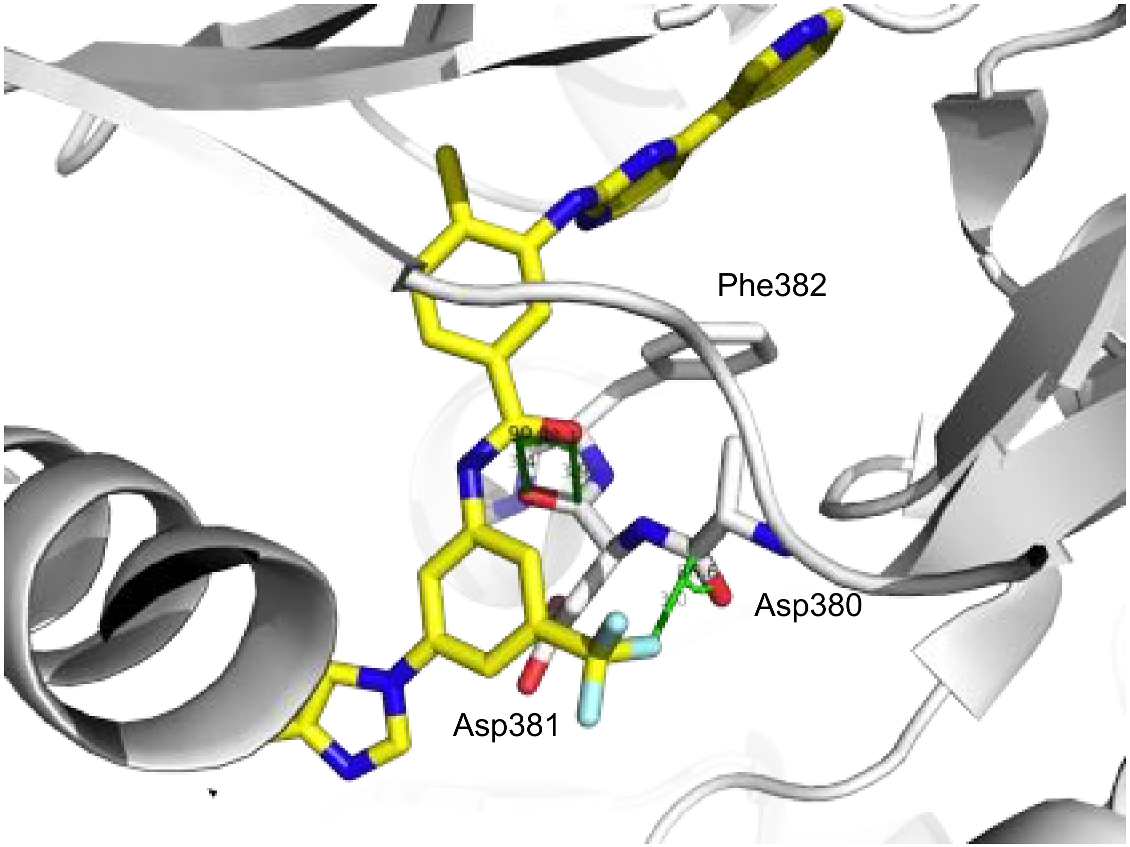
Antiparallel

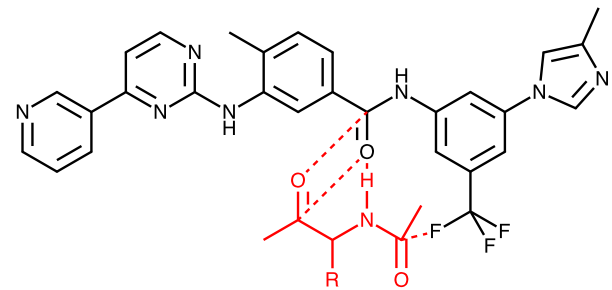


2 - 4 A



Dipole-Dipole Interactions

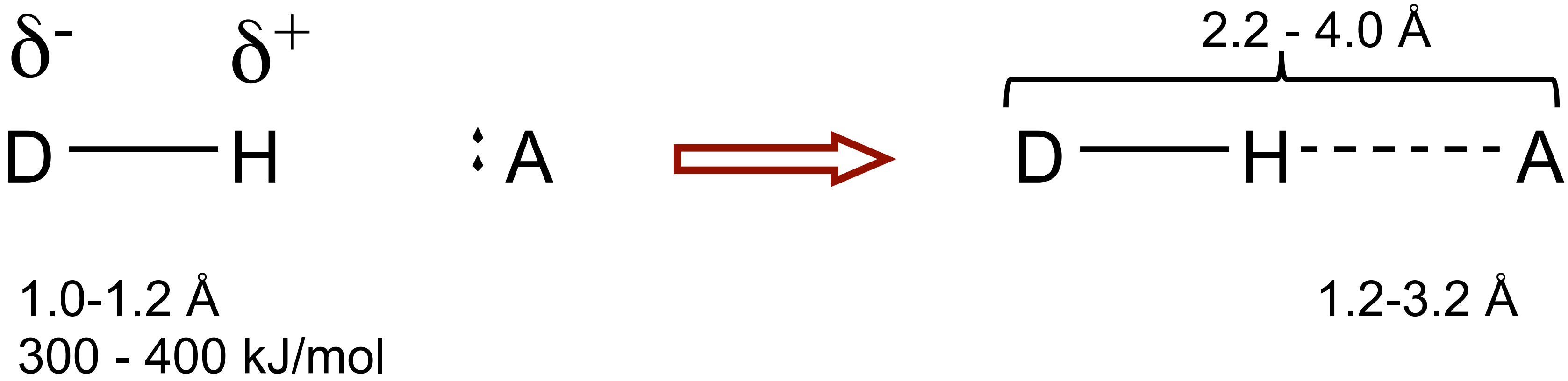




Nilotinib
(a kinase inhibitor)
approved for Leukemia

Diederich, F. Angew. Chem. Int. Ed. 2015

Hydrogen Bond
4-160 kJ/mol

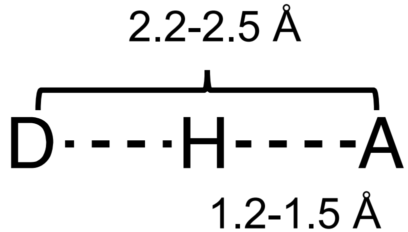


D = donor: an electronegative atom
(in proteins: N, O)

A = acceptor: an atom with non-bonded electron pairs
(in proteins: N, O)

Hydrogen Bonds

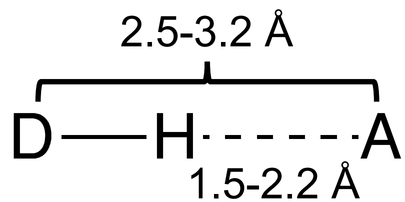
Strong
60-160 kJ/mol

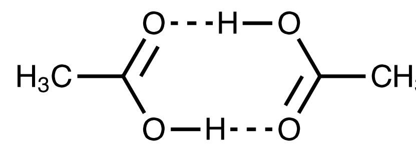


F—H—F 163 kJ/mol

HO—H---OH^- 135 kJ/mol

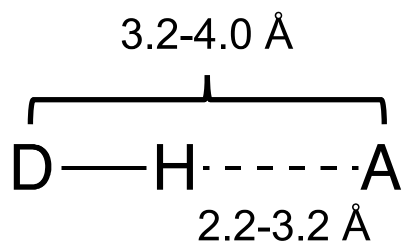
Intermediate
16-60 kJ/mol

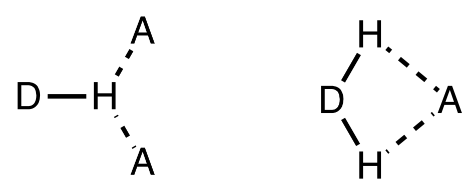




A—D
D—A

Weak
4-16 kJ/mol

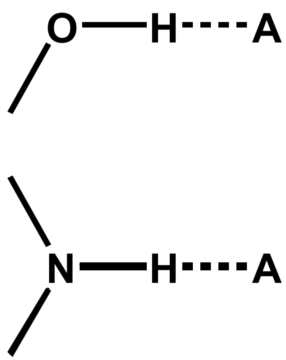




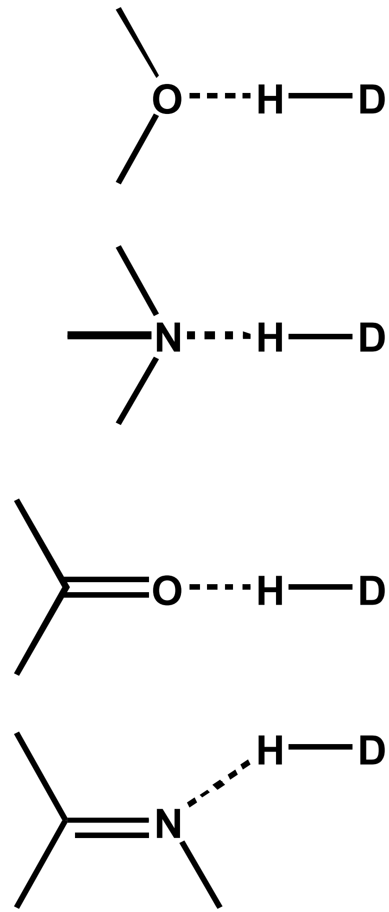
in bifurcated systems

Hydrogen Bonds in Proteins

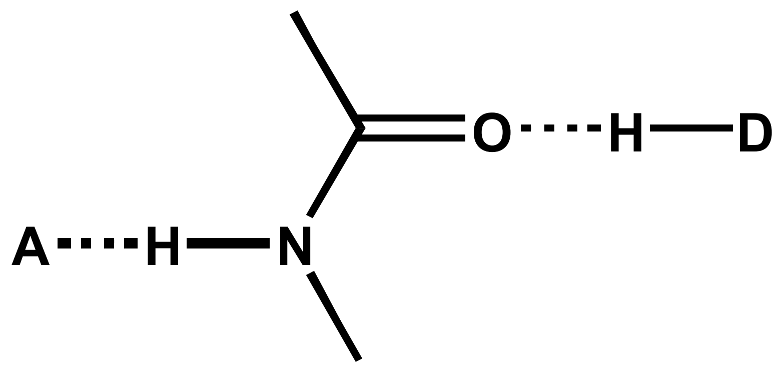
donors



acceptors

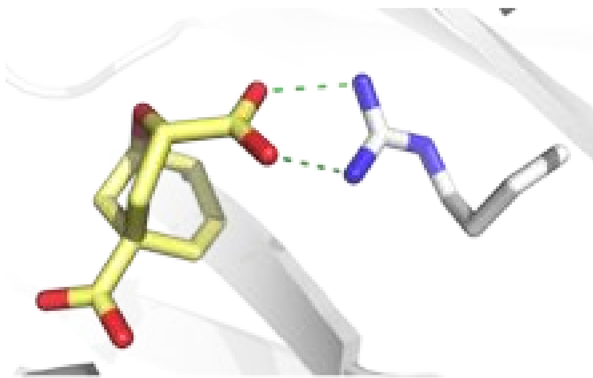


Hydrogen Bonds in Proteins

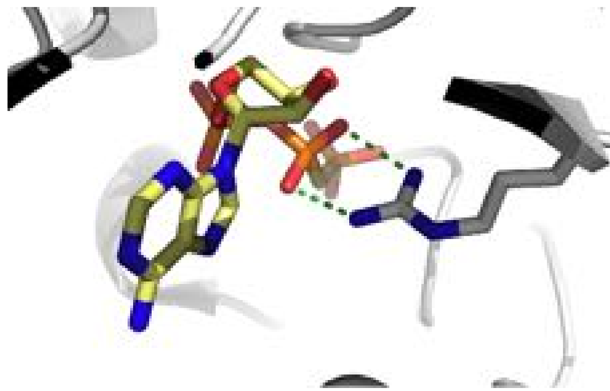


Also amide groups in the *backbone* can form hydrogen bonds with the substrate, if they are not engaged in hydrogen bonds internal to the protein (α-helix, β-sheet).

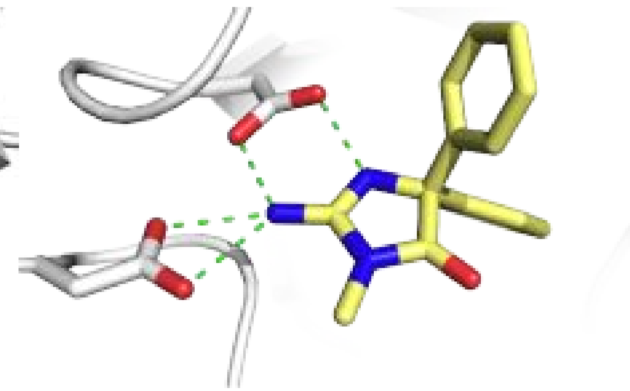
Hydrogen Bond Motifs



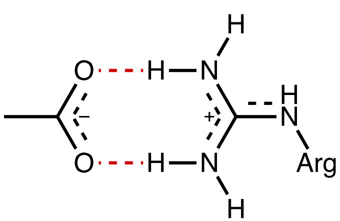
chorismate mutase

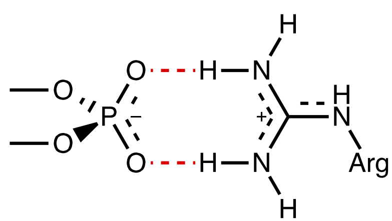


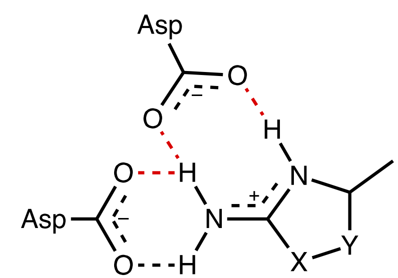
ATPase



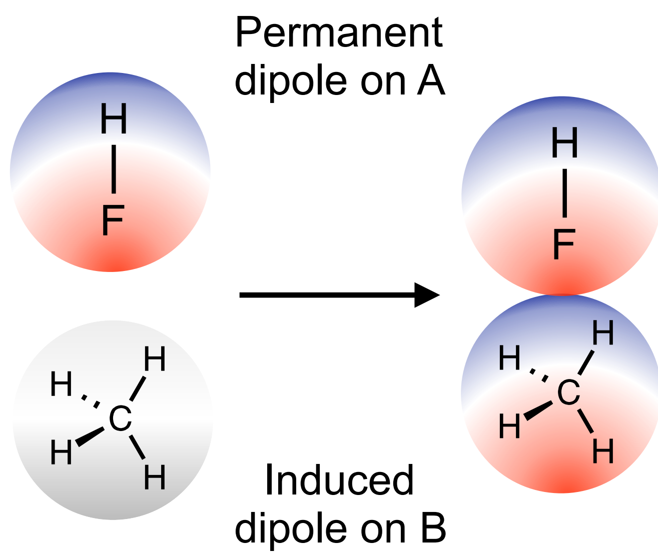
BACE 1



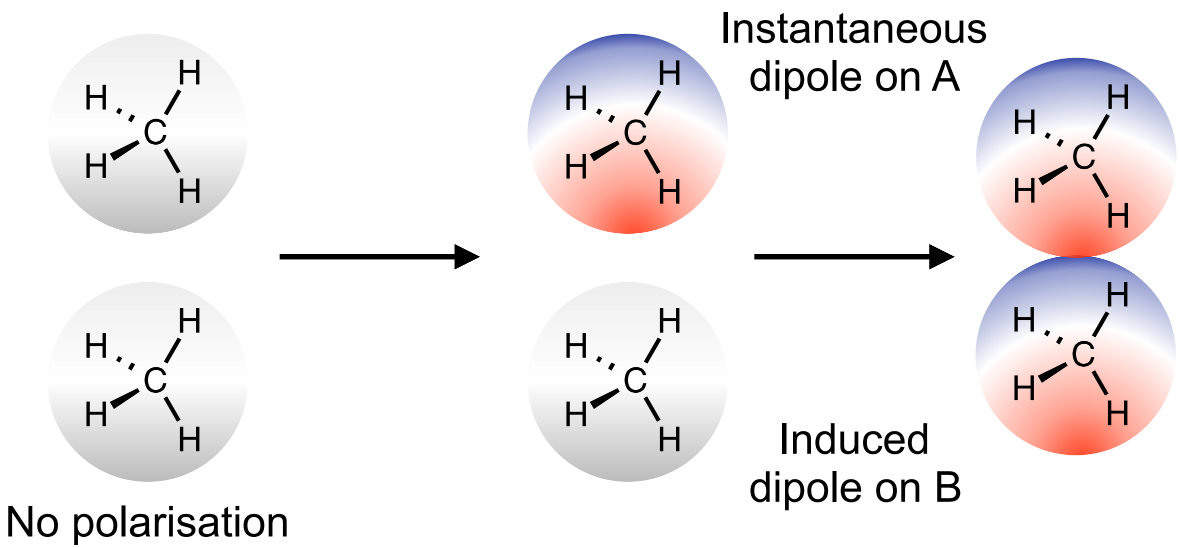


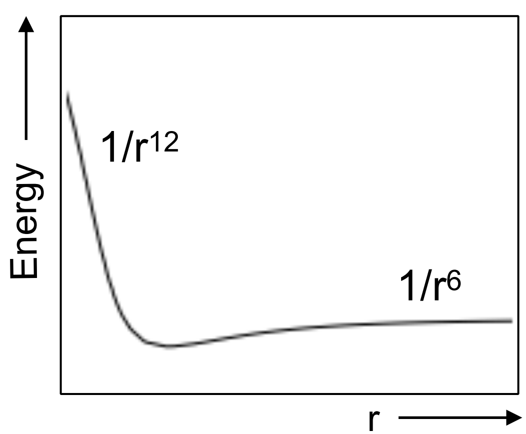


Dipole-Induced Dipole Interactions

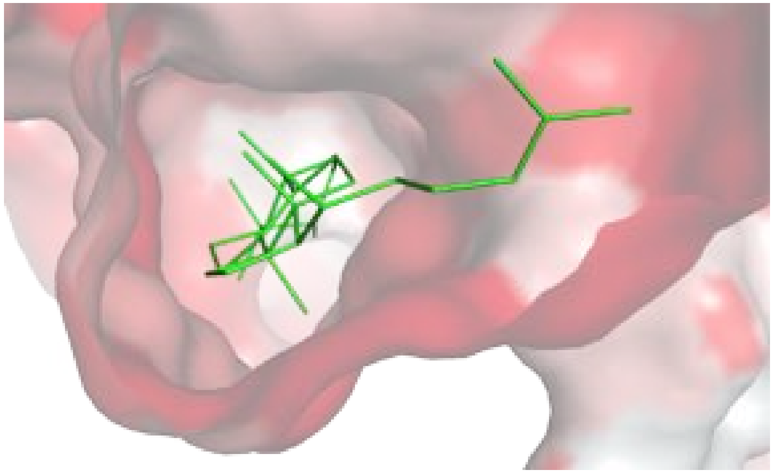


London Forces (< 5 kJ/mol)

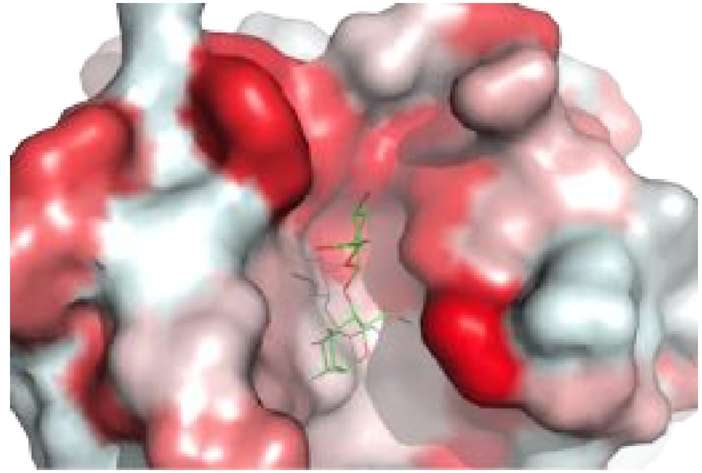




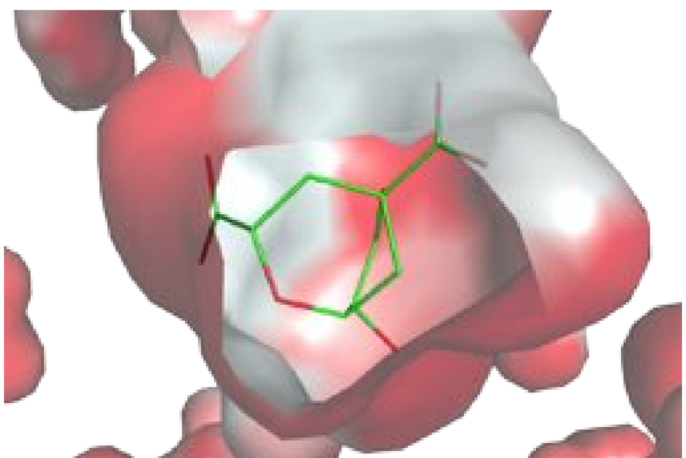
London Forces (Hydrophobic Interactions)



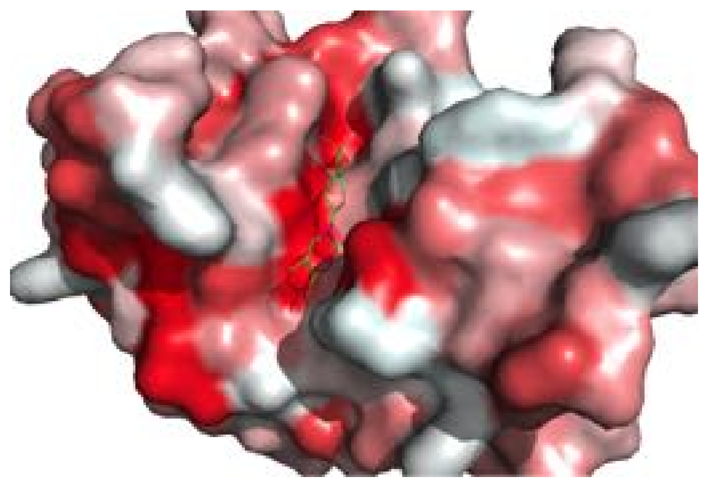
Squalene oxide cyclase
K_M = 250 µM



Lysozyme

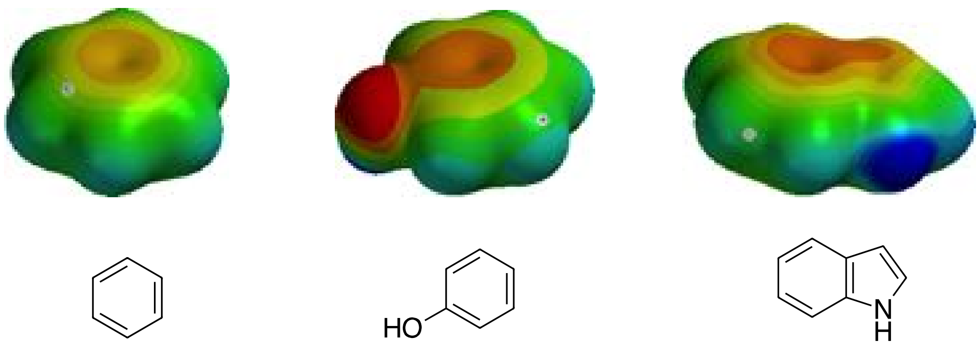


Chorismate mutase
K_M = 2 mM

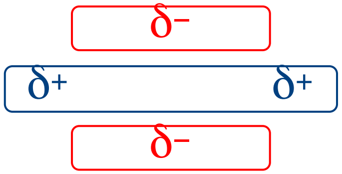


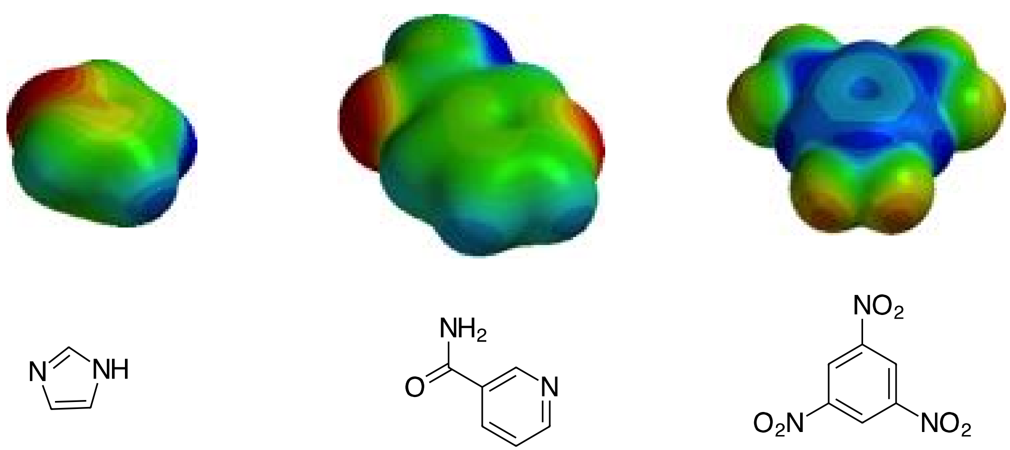
Ribonuclease A

π-π-Stacking
(< 50 kJ/mol)

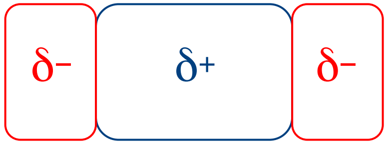


electron
rich

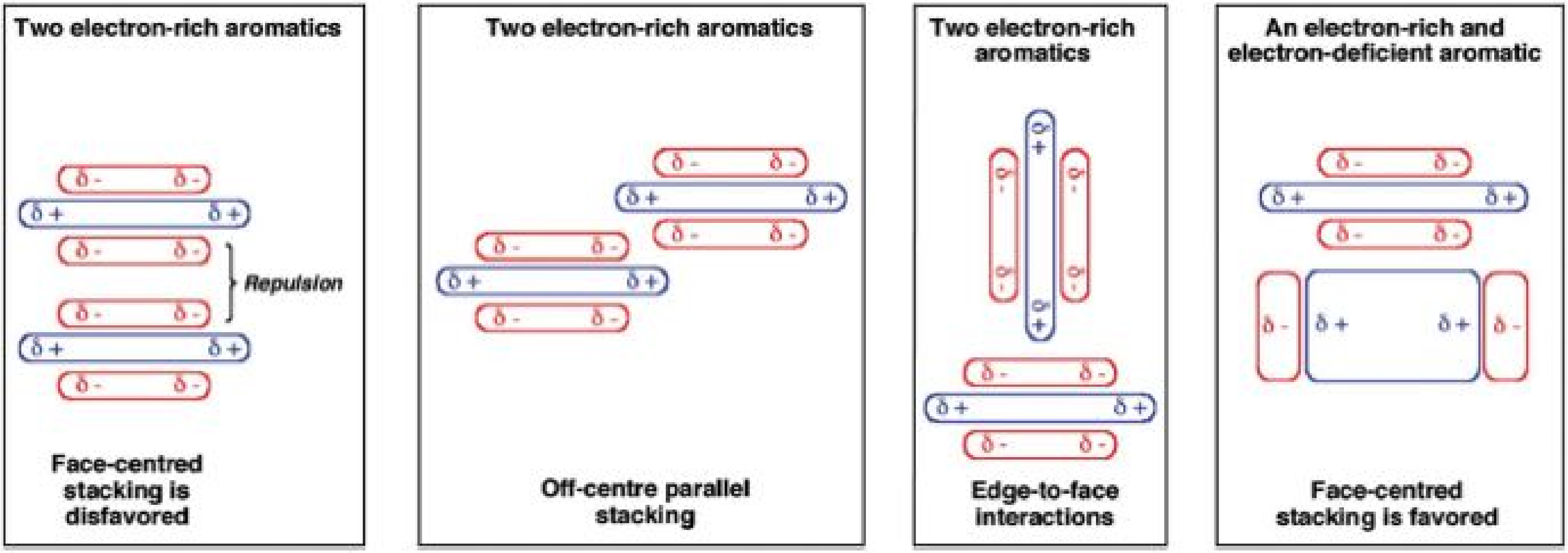


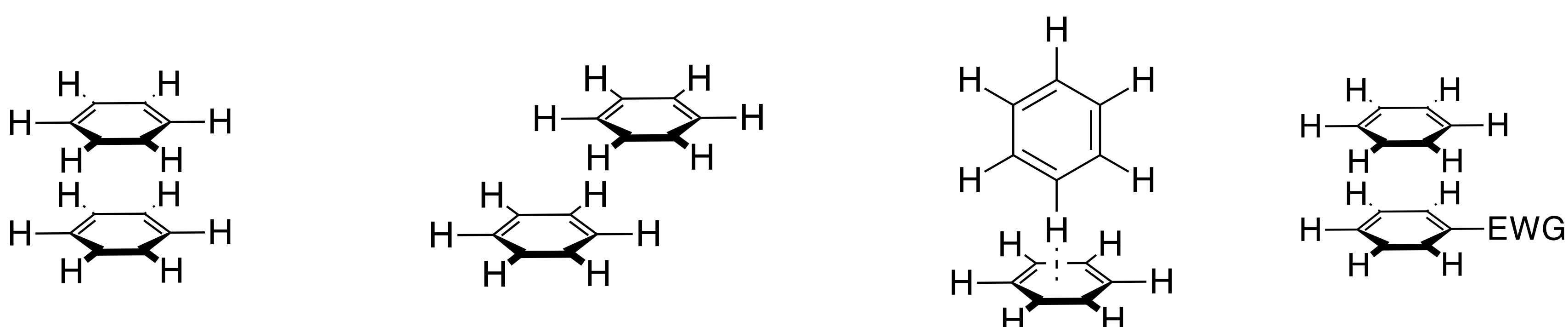


electron
deficient

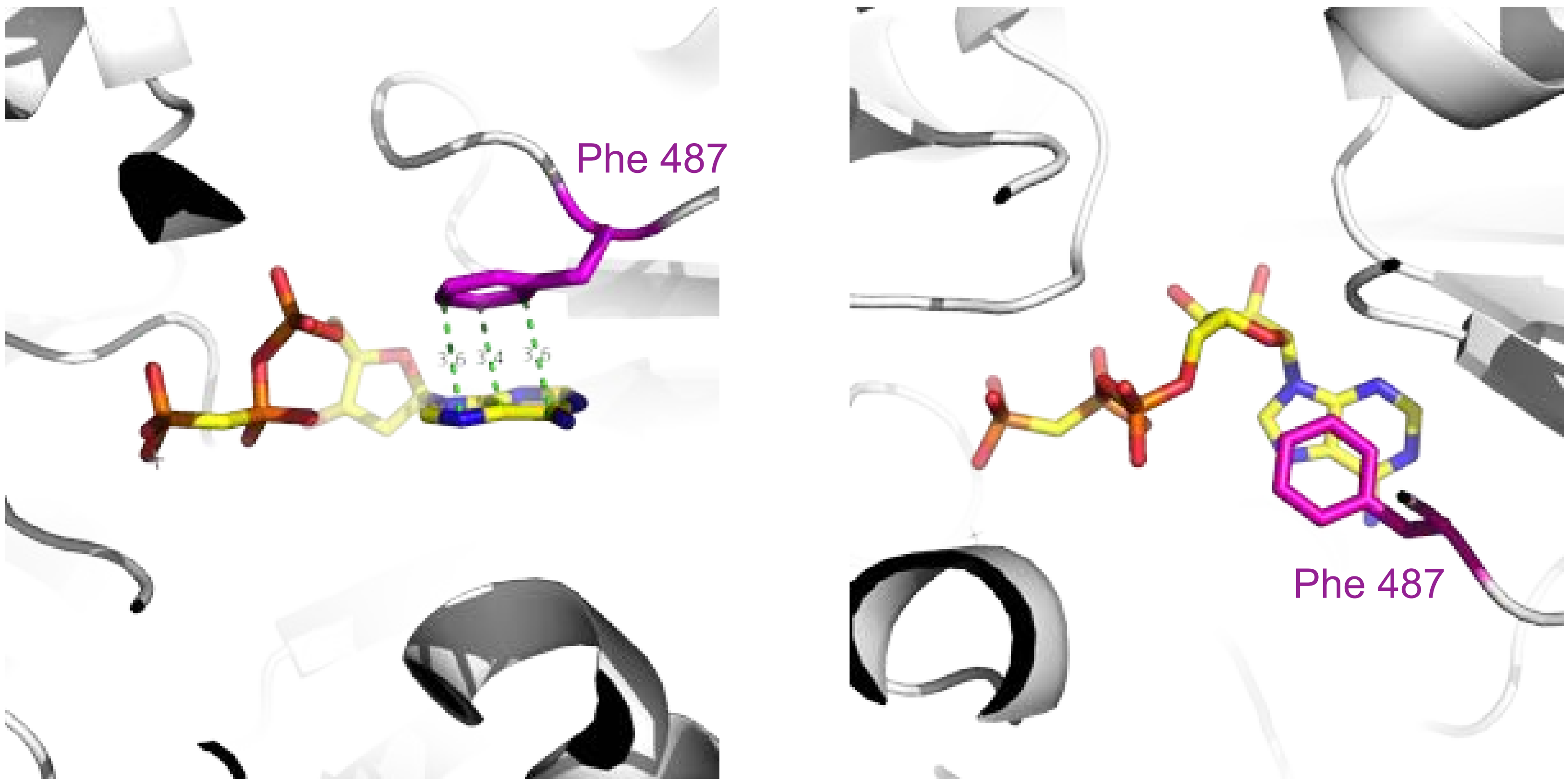


π-π-Stacking


Two electron-rich aromatics
δ - δ -
δ + δ +
Repulsion
Face-centred stacking is disfavored
Two electron-rich aromatics
Off-centre parallel stacking
Two electron-rich aromatics
Edge-to-face interactions
An electron-rich and electron-deficient aromatic
Face-centred stacking is favored


H
EWG

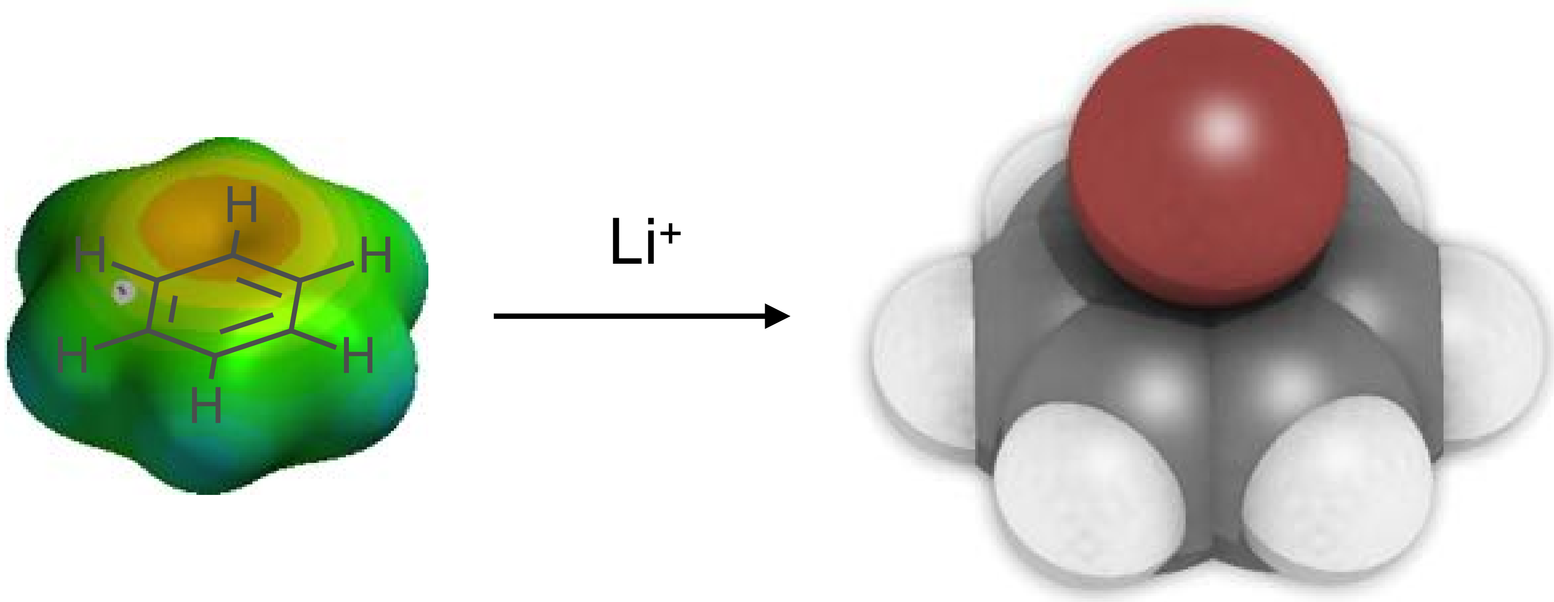
π-π-Stacking in Proteins



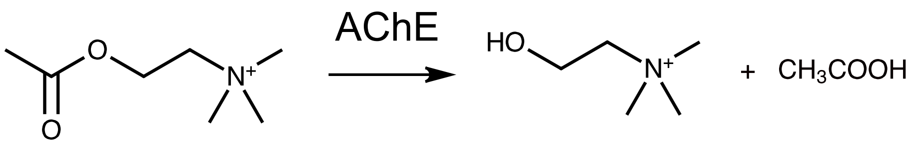
Calcium ATPase (PDB: 1T5S)

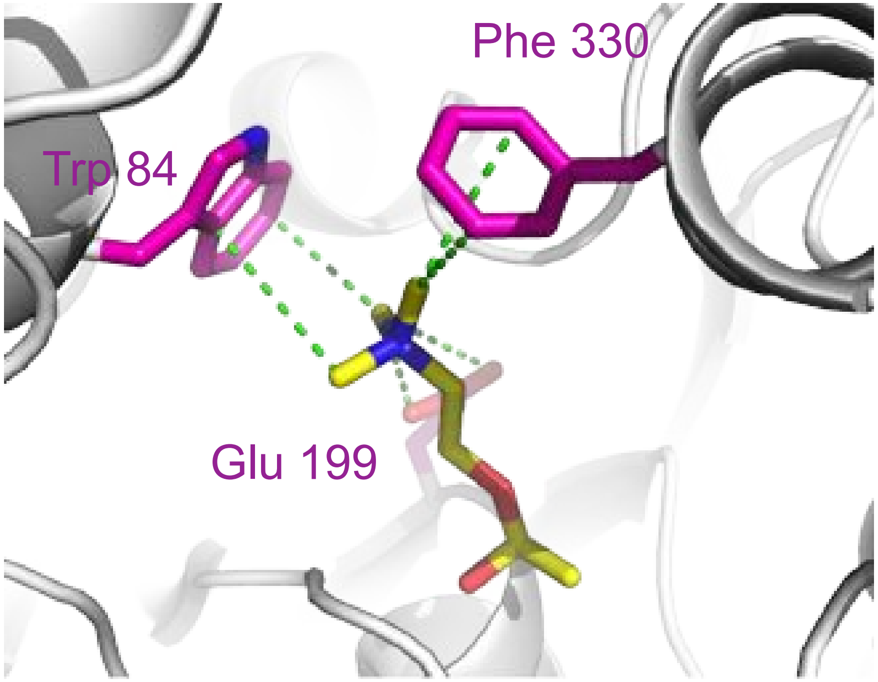
Sorensen, T.L.-M. Science 2004

Cation-π Interactions (5-80 kJ/mol)



Cation-π Interactions in Proteins





Dougherty, D.A. PNAS 1999

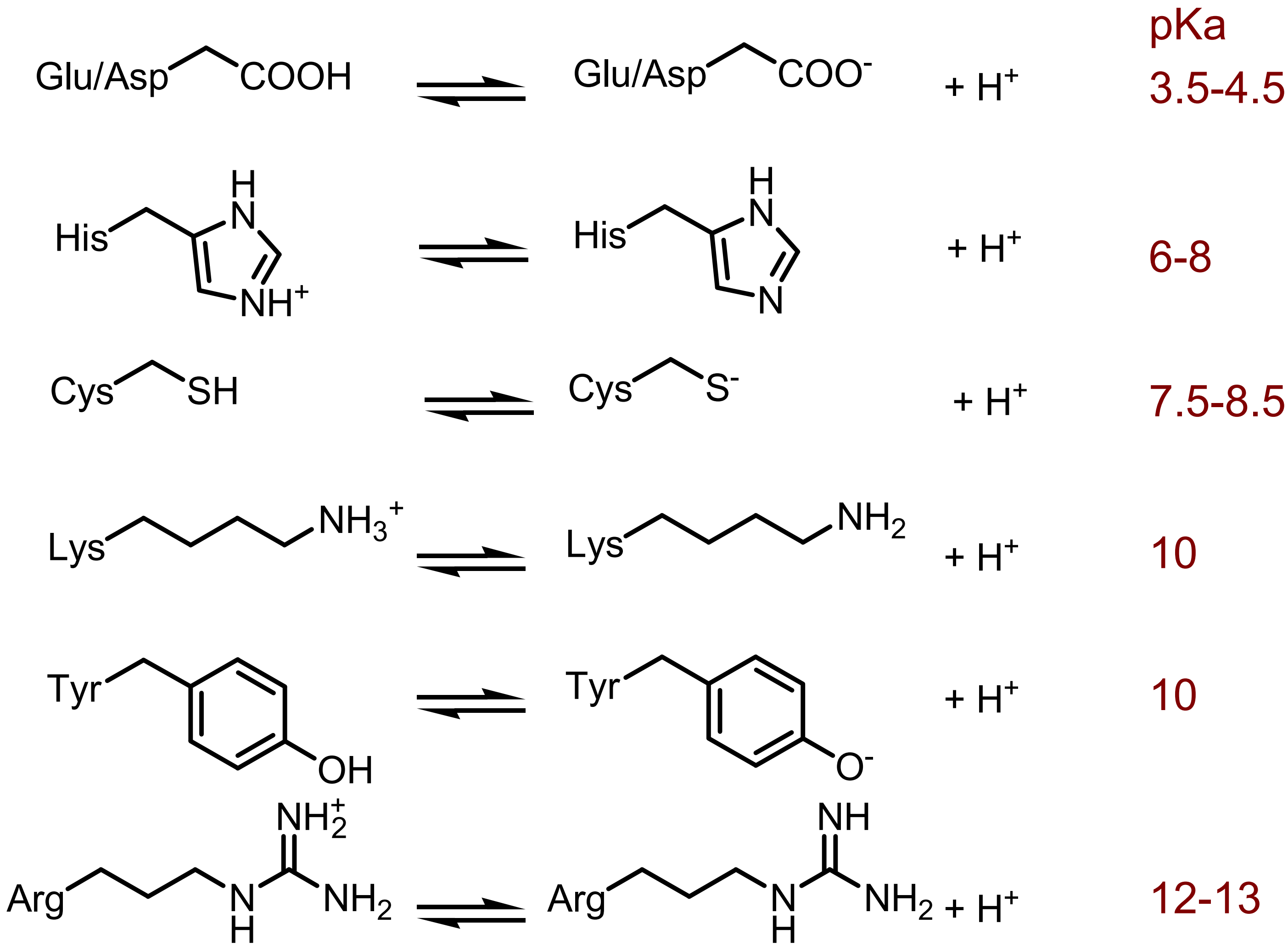
Catalysis

- Acid
- Base

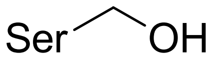
} Proton transfer

- Electrophilic
- Nucleophilic

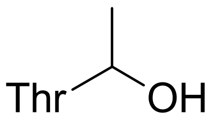
Acid-Base Properties



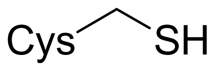
Nucleophiles



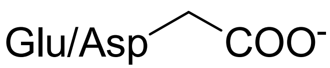
Proteases, lipases, esterases



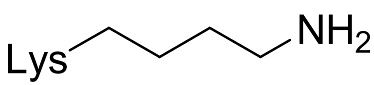
Posphotransferases



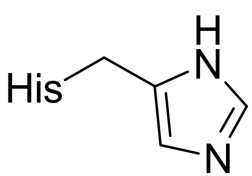
Proteases



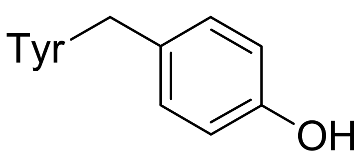
Epoxide hydrolases, haloalkane dehalogenases



Aldolases, acetoacetate decarboxylase



Phosphotransferases, Nucleases

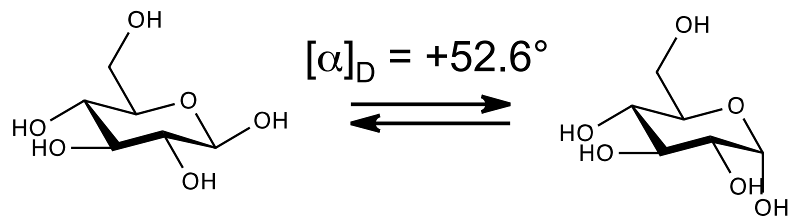


DNA topoisomerase

Catalytic Efficiency

- multifunctional catalysis
- proximity
- transition state complementarity
- substrate distorsion

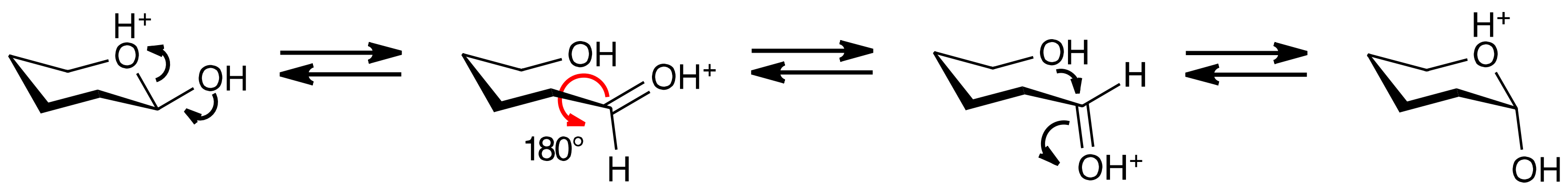
Bifunctional Catalysis: Mutarotation



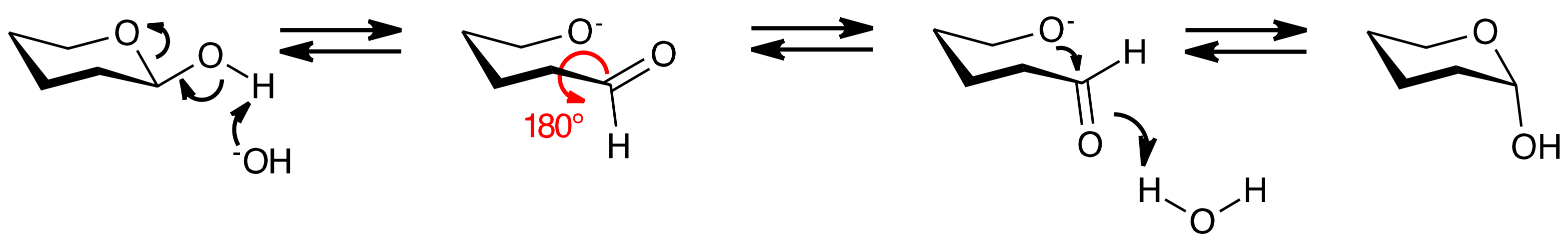
β-D-glucopiranosio (63%)
$[\alpha]_D = +18.7°$

α-D-glucopiranosio (37%)
$[\alpha]_D = +112.2°$

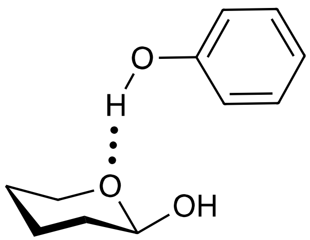
acid-catalyzed:



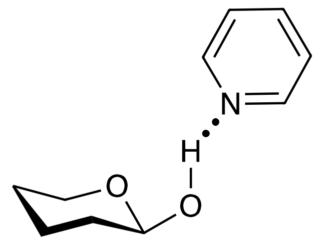
base-catalyzed:



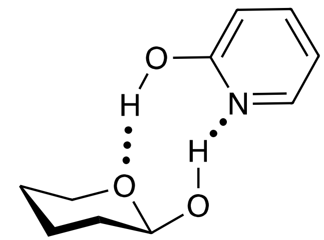
Bifunctional Catalysis: Mutarotation



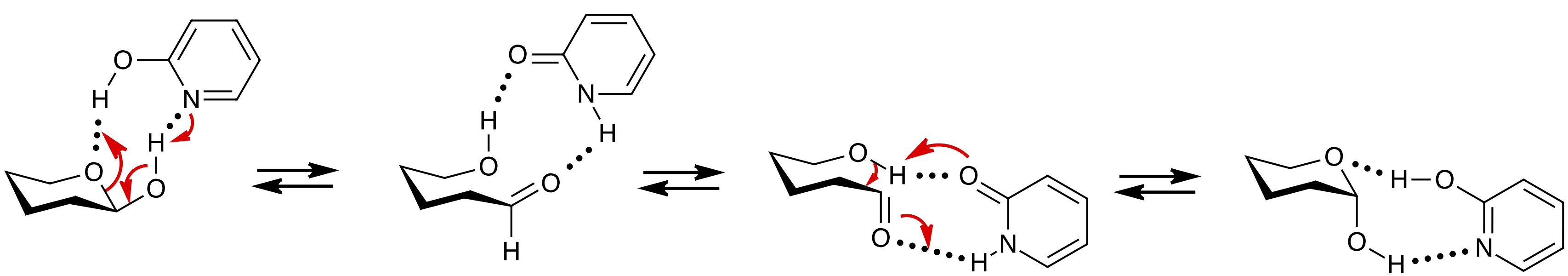
k_{PhOH}



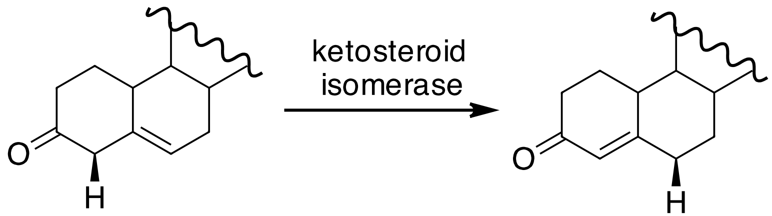
k_{Py}

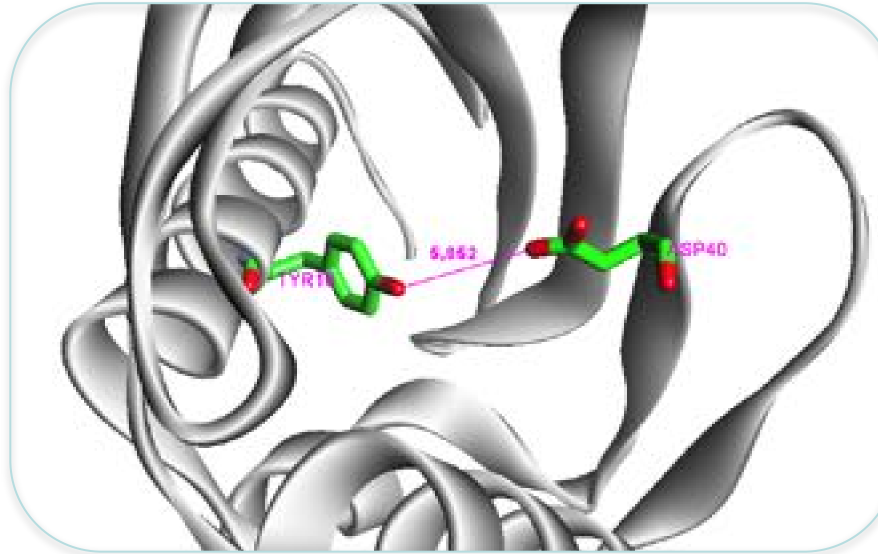


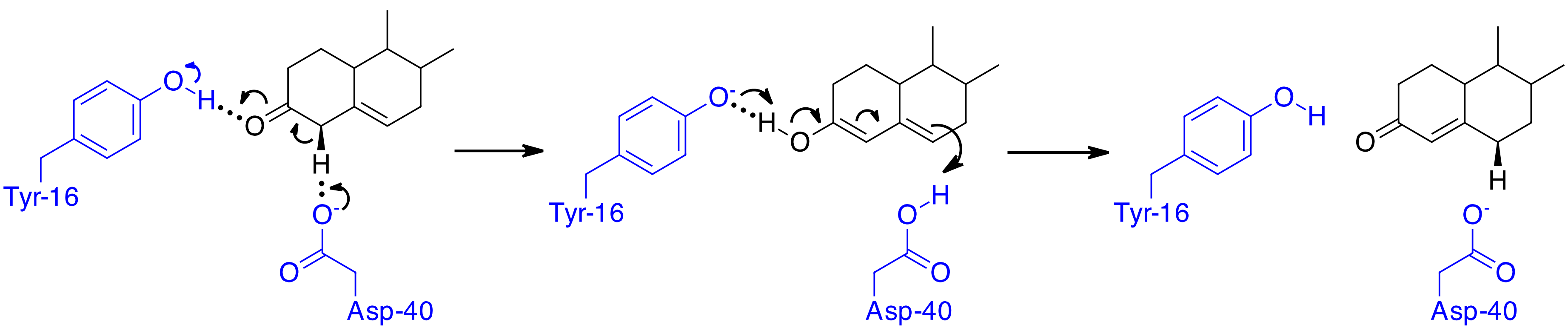
$k_{PyOH} = 7000 \times (k_{Py} + k_{PhOH})$



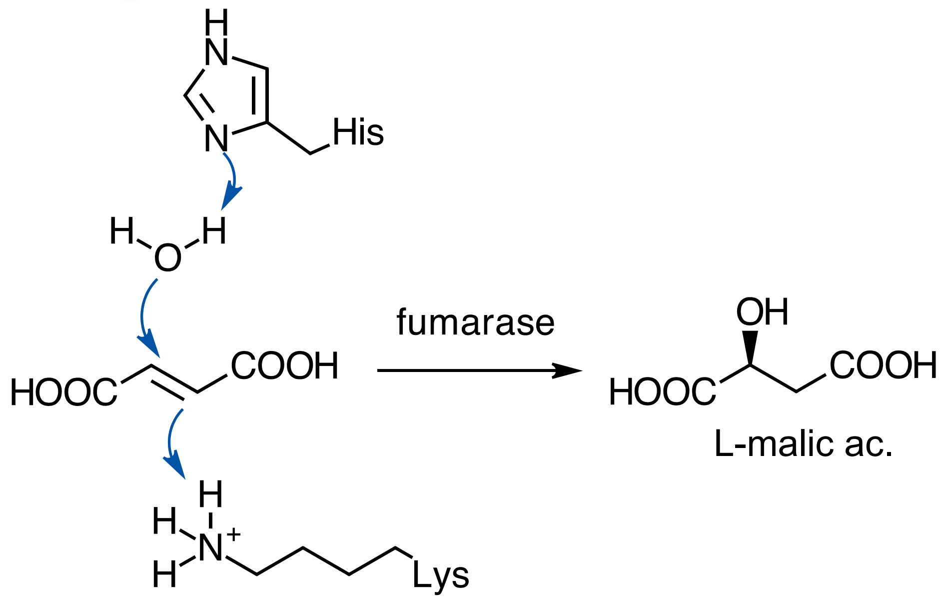
Bifunctional Catalysis: Ketosteroid Isomerase

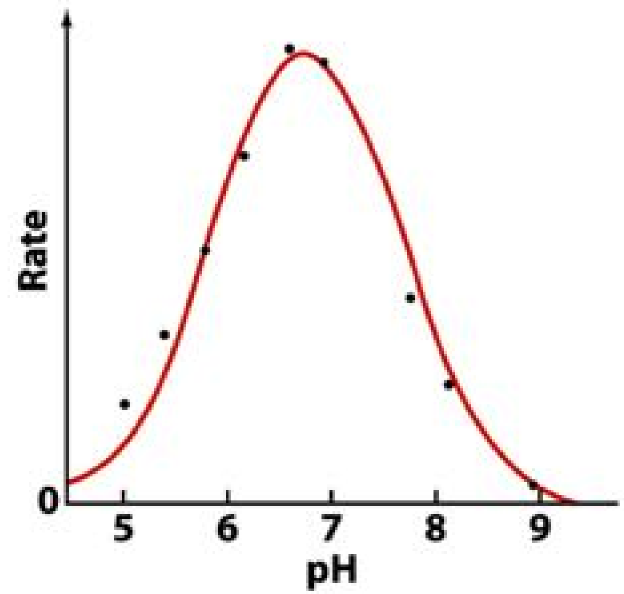






pH Optimum of Fumarase





Catalytic Efficiency

- multifunctional catalysis
- ➢ proximity
- transition state complementarity
- substrate distortion

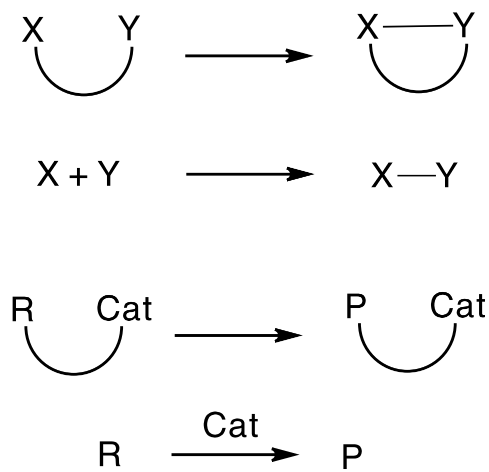
Intramolecular Catalysis

Intramolecular reaction

Intermolecular reaction

Intramolecular catalysis

Intermolecular catalysis



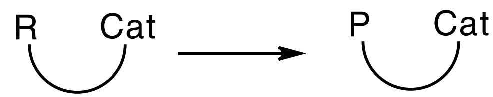
$$\Delta G^{\#}_{inter} = \Delta H^{\#}_{inter} - T\Delta S^{\#}_{inter}$$

$$\Delta G^{\#}_{intra} = \Delta H^{\#}_{intra} - T\Delta S^{\#}_{intra}$$

$$\left.\begin{array}{l} \Delta H^{\#}_{intra} \cong \Delta H^{\#}_{inter} \\ \Delta S^{\#}_{intra} > \Delta S^{\#}_{inter} \end{array}\right\} \quad \Delta G^{\#}_{intra} < \Delta G^{\#}_{inter}$$

Intramolecular Catalysis

Intramolecular catalysis



Intermolecular catalysis

$$v_{intra} = k_{intra}[R—Cat] \qquad [k_{intra}] = s^{-1}$$

$$v_{inter} = k_{inter}[R][Cat] \qquad [k_{inter}] = M^{-1}s^{-1}$$

if $\quad [R—Cat] = [R]$ and $v_{intra} = v_{intra}$

then $\quad k_{intra} = k_{inter}[Cat]$

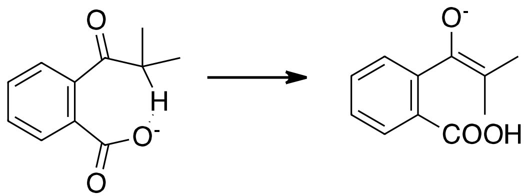
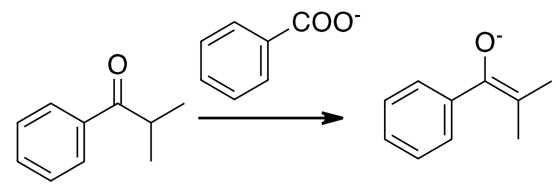
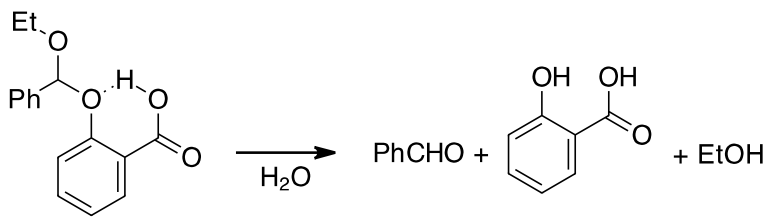
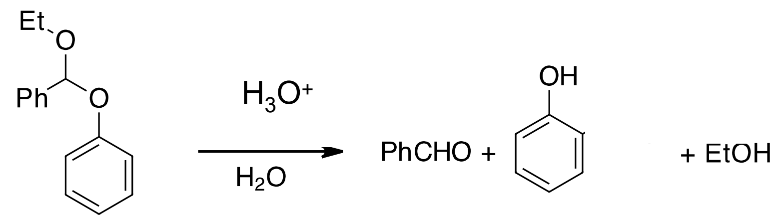
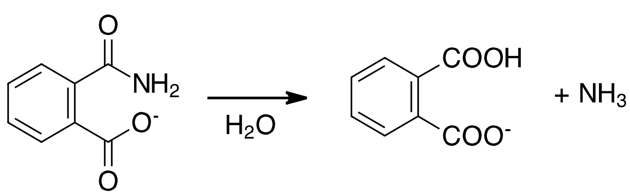
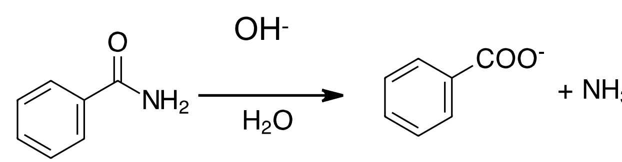
$$k_{intra}/k_{inter} = [Cat]$$

Effective Molarity

$$EM = k_{intra}/k_{inter} > 1$$

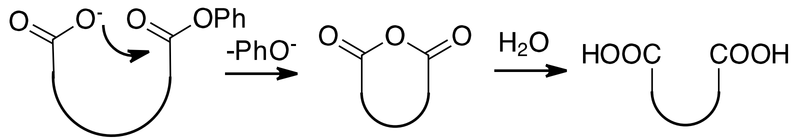
$$[EM] = s^{-1}/M^{-1}s^{-1} = M$$

Intramolecular Catalysis

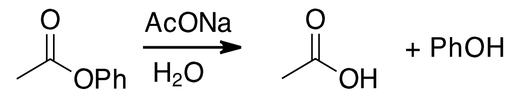
EM	intramolecular catalysis	reference reaction
56		
30.000		
10^9		

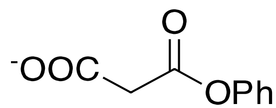
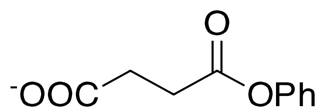
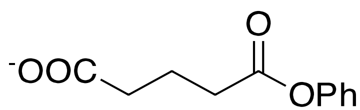
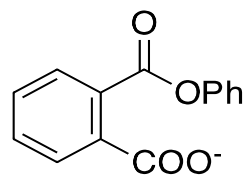
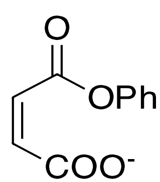
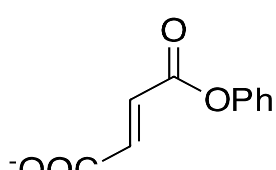
EM for Intramolecular Catalysis

intramolecular catalysis



reference reaction



	9
	4000
	25
	2 x 10^5
	10^{10}
	1

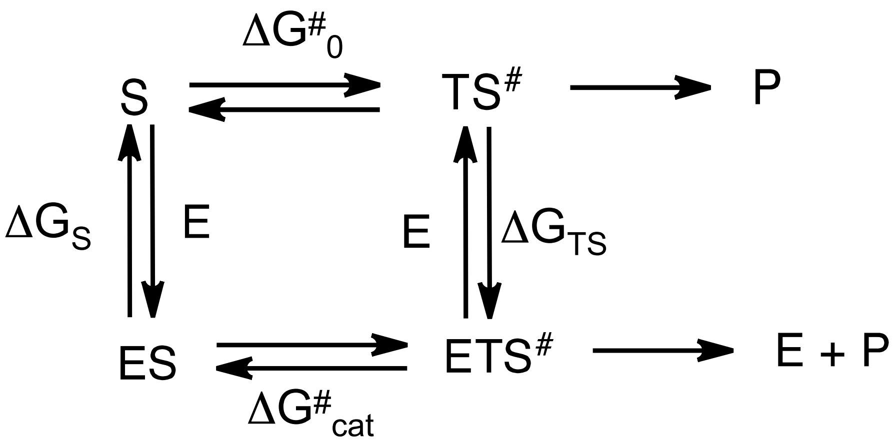
Catalytic Efficiency

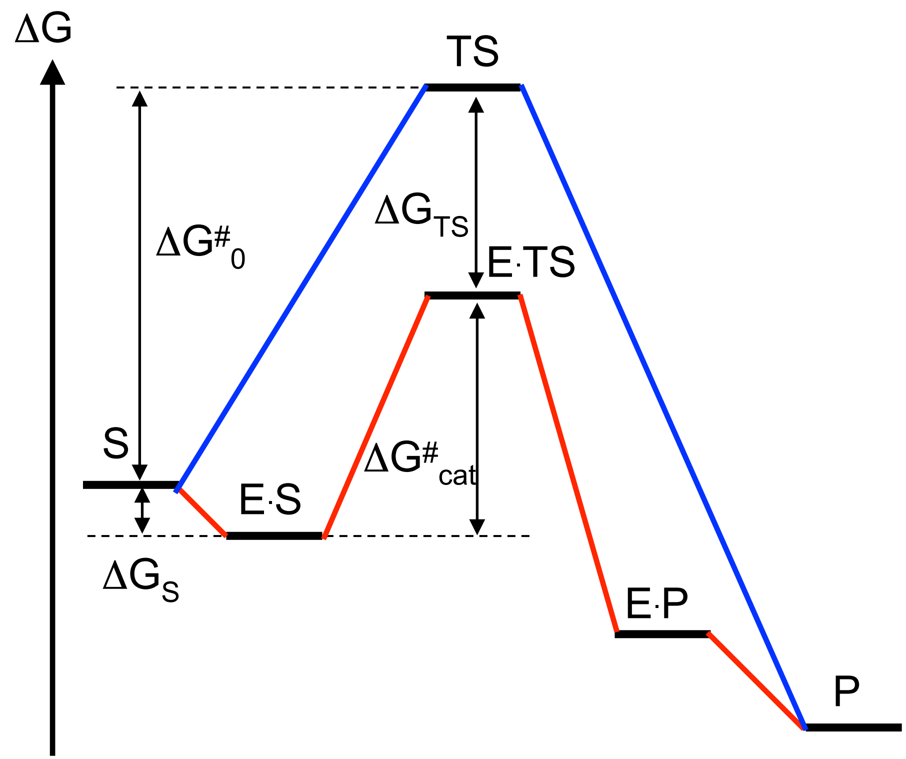
- multifunctional catalysis
- proximity

➢ transition state complementarity

➢ substrate distorsion

Transition State Complementarity





$\Delta G^{\#}_{0} - \Delta G_{TS} - \Delta G^{\#}_{cat} + \Delta G_{S} = 0$

$\Delta G^{\#}_{0} + \Delta G_{S} = \Delta G^{\#}_{cat} + \Delta G_{TS}$

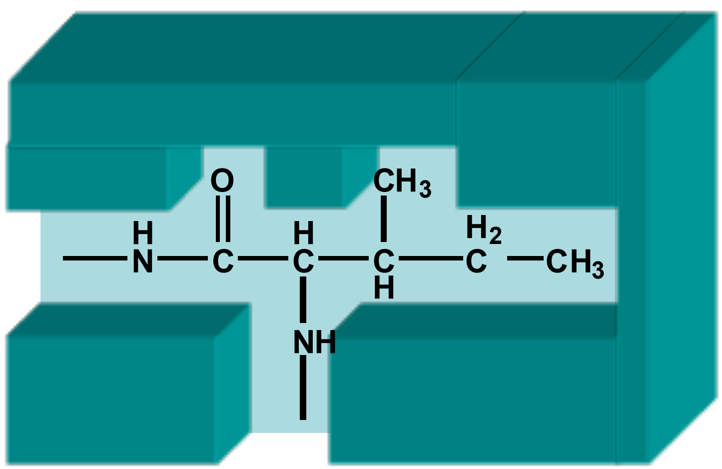
$\Delta G^{\#}_{cat} - \Delta G^{\#}_{0} = \Delta G_{S} - \Delta G_{TS}$

$\Delta G^{\#}_{cat} < \Delta G^{\#}_{0}$ if $\Delta G_{TS} > \Delta G_{S}$

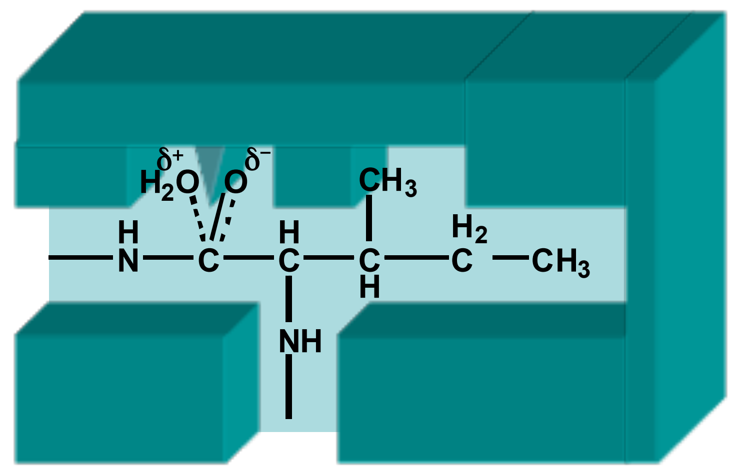
$\Delta G^{\#}_{cat} < \Delta G^{\#}_{0}$ if $\Delta G_{TS} > \Delta G_{S}$

Transition State Complementarity

substrate complementary

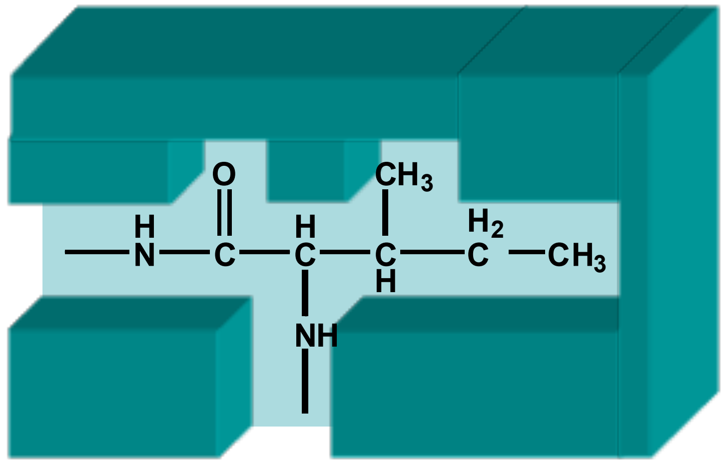


transition state complementary



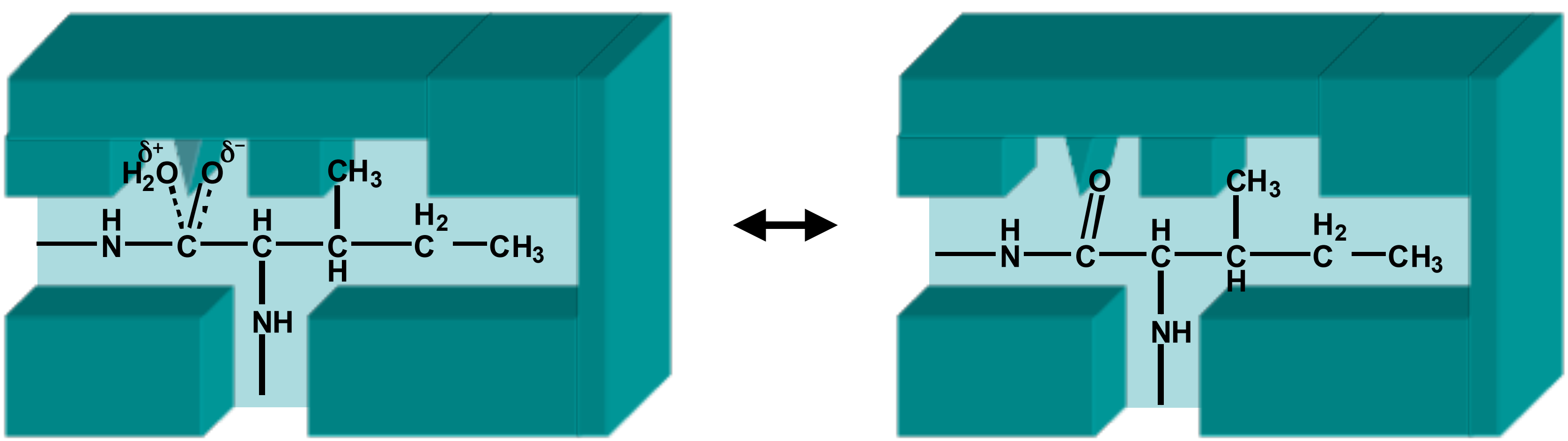
Substrate Destabilization

substrate complementary

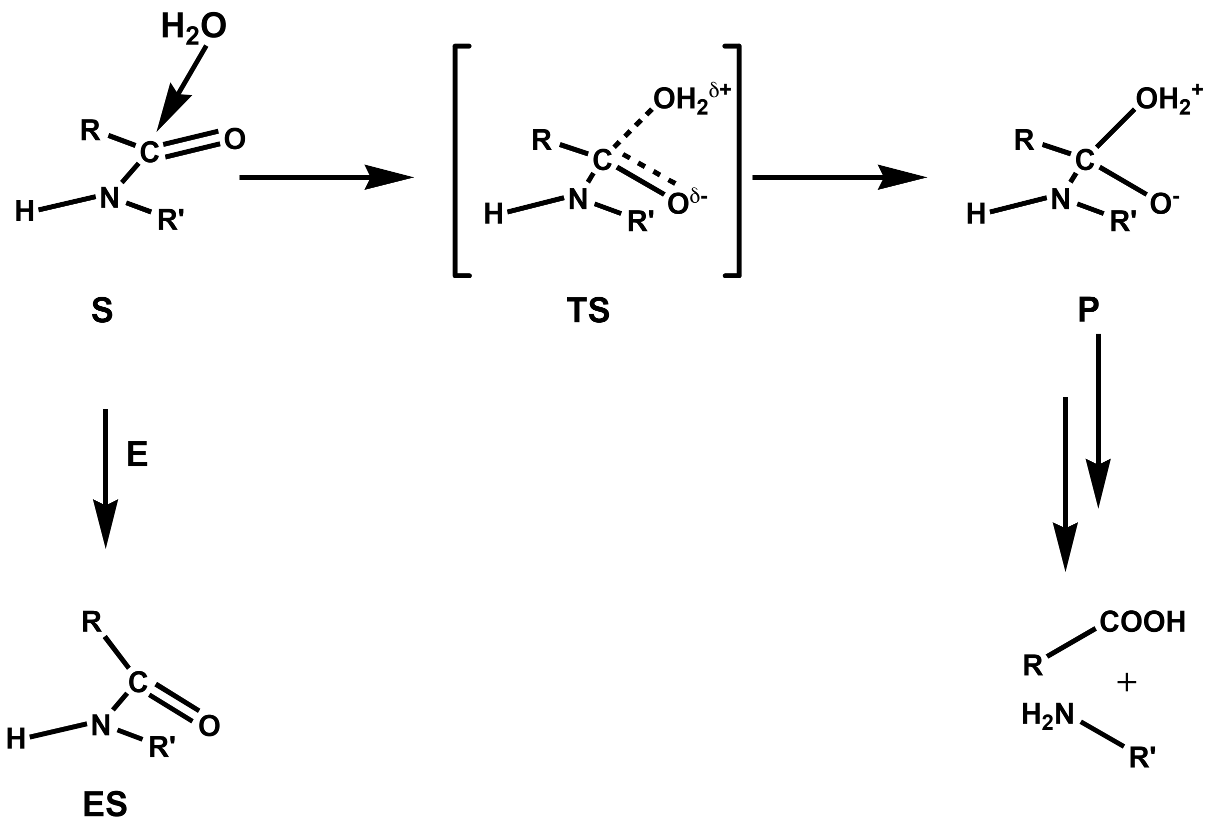


transition state complementary

substrate distortion



Substrate Destabilization



Distorted amide:

- geometrically similar to the TS
- loss of amide resonance ≈ 20 Kcal/mol

Catalysis

- Acid
- Base

} Proton transfer

- Electrophilic
- Nucleophilic

Specific Acid-Base Catalysis

The catalyst is H_3O^+ or OH^-

$R \longrightarrow P \qquad v_0 = k_0[R]$

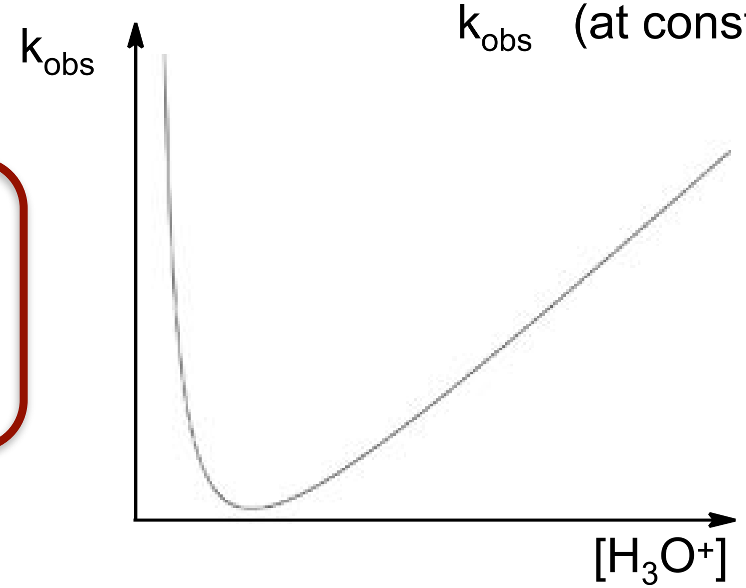
$R \xrightarrow{H_3O^+} P \qquad v_H = k_H[R][H_3O^+]$

$R \xrightarrow{OH^-} P \qquad v_{OH} = k_{OH}[R][OH^-]$

$$v = v_0 + v_H + v_{OH}$$

$$= k_0[R] + k_H[R][H_3O^+] + k_{OH}[R][OH^-]$$

$$= \underbrace{(k_0 + k_H[H_3O^+] + k_{OH}[OH^-])}_{k_{obs}\ \text{(at constant pH)}}[R]$$



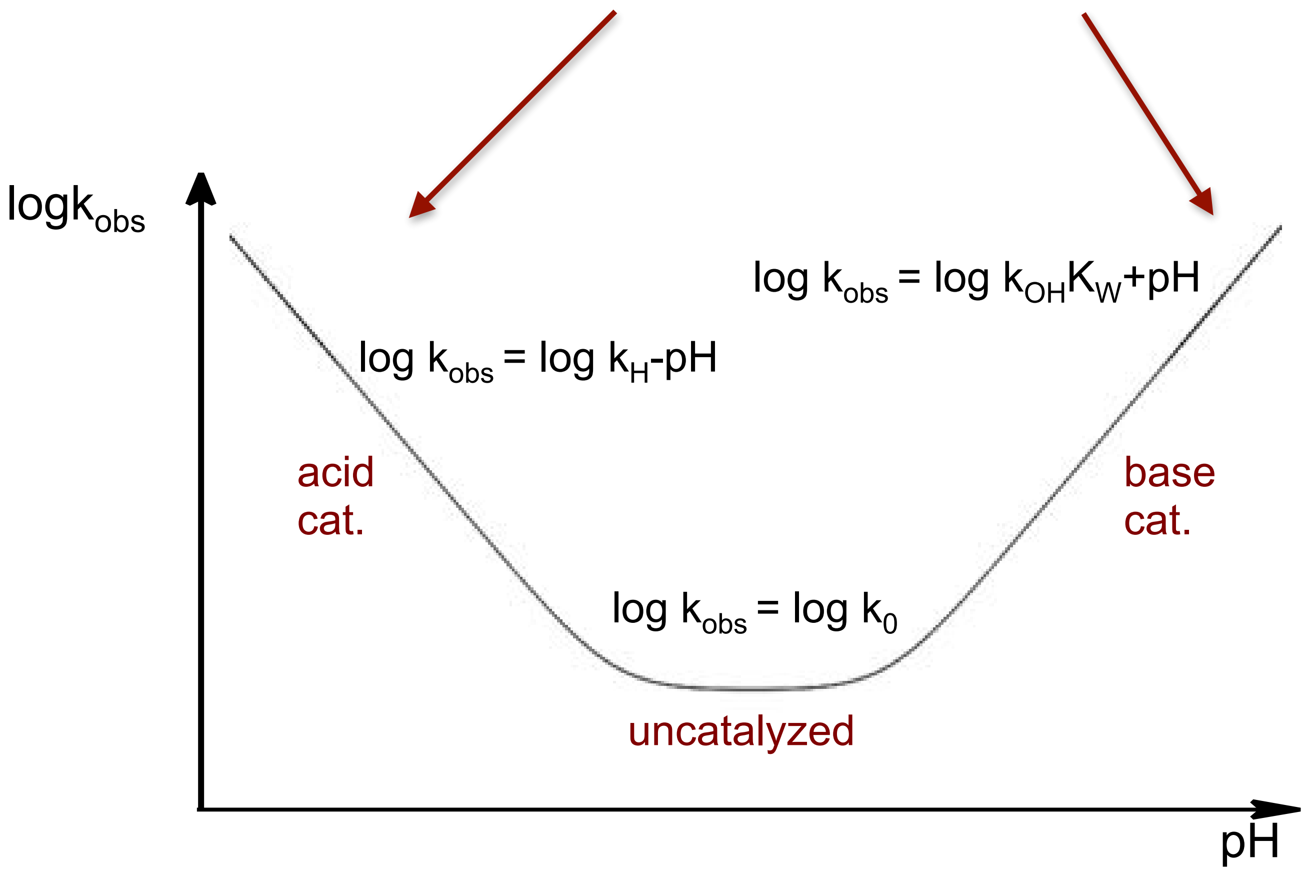
at constant pH:

$v = k_{obs}[R]$

$k_{obs} = k_0 + k_H[H_3O^+] + k_{OH}K_w/[H_3O^+]$

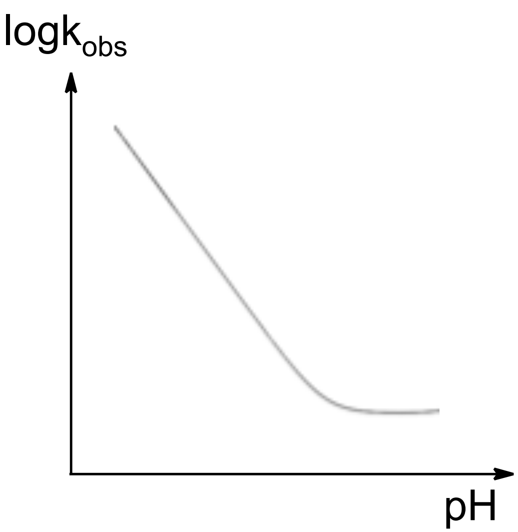
pH Profile

$$k_{obs} = k_0 + k_H[H_3O^+] + k_{OH}K_w/[H_3O^+]$$

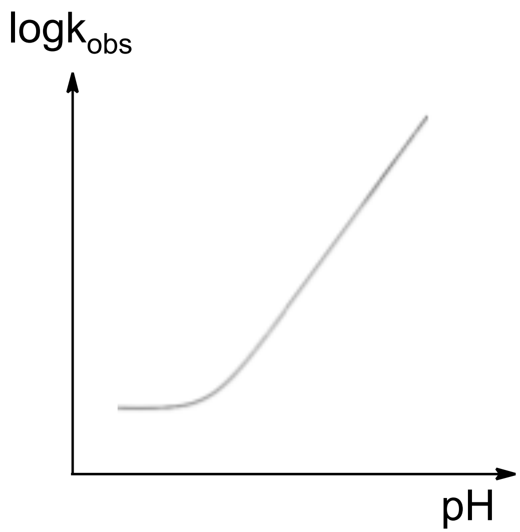


pH Profiles

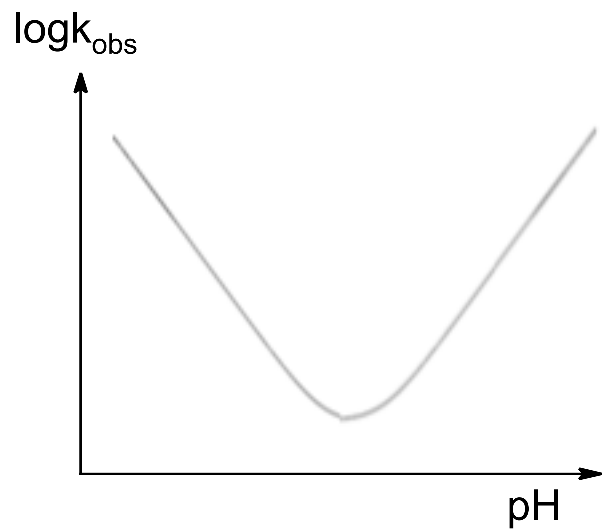
$k_H, k_0 >> k_{OH}$



$k_{OH}, k_0 >> k_H$

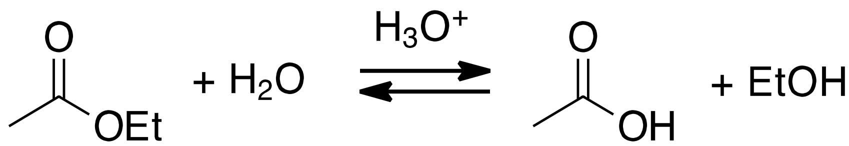


$k_H, k_{OH} >> k_0$



(ester hydrolysis)

Acid-Catalyzed Hydrolysis of Esters

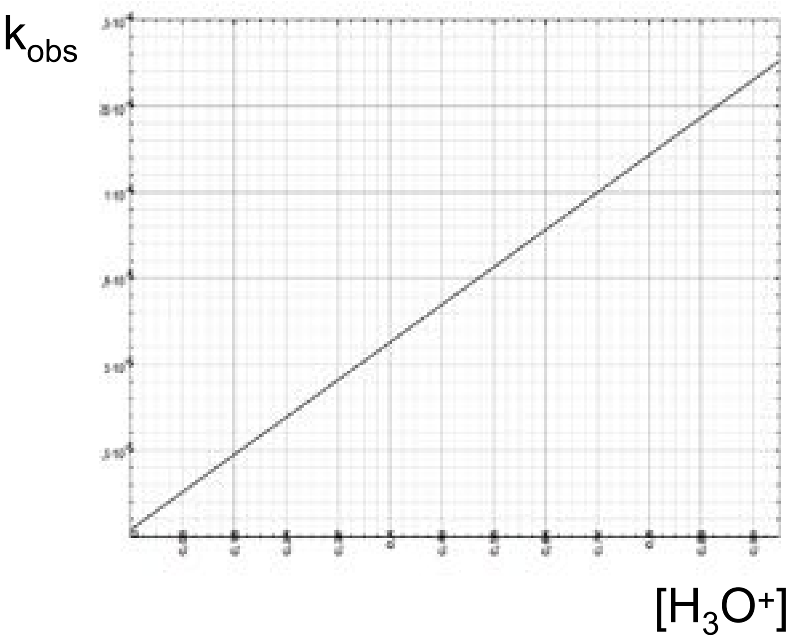


$$v = -d[E]/dt = k_0[E] + k_H[H_3O^+][E]$$

$v = k_{obs}[E]$

$k_{obs} = k_0 + k_H[H_3O^+]$

} At constant pH

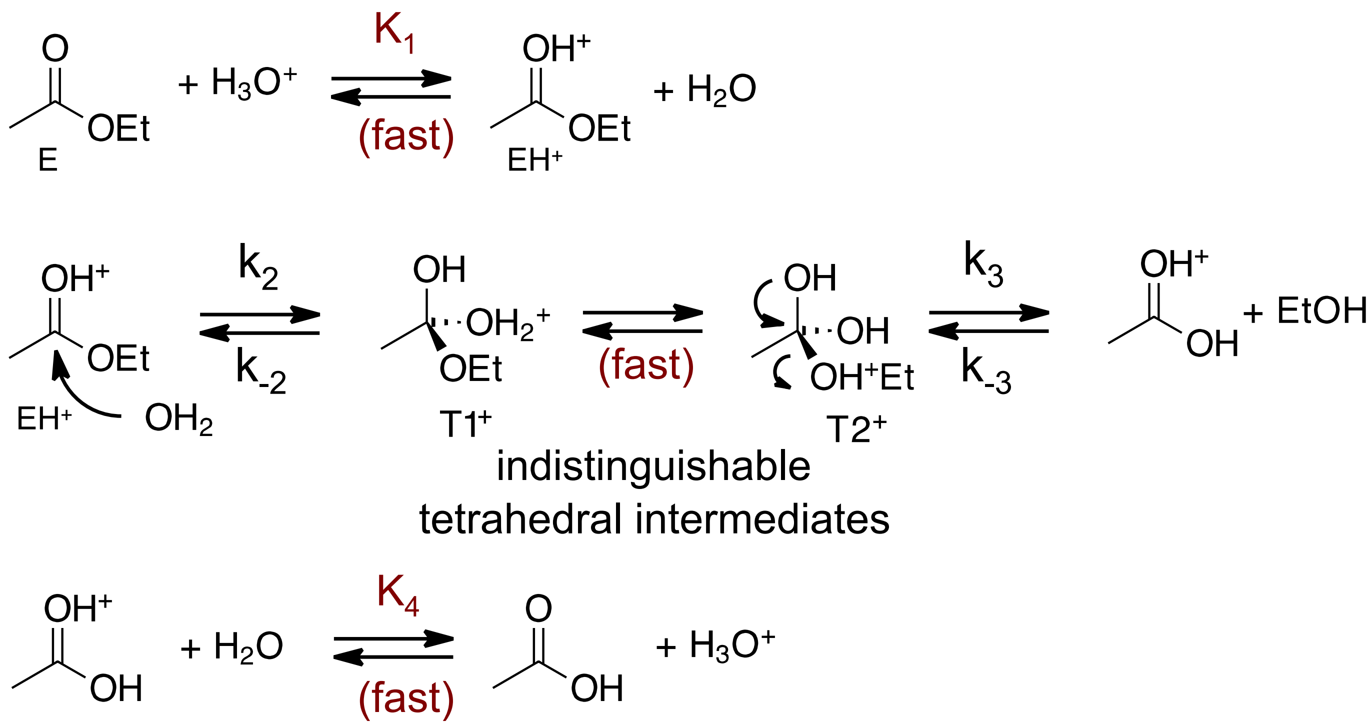


$k_0 = 1 \times 10^{-11}\ s^{-1} \cong 0$

$k_H = 1.36 \times 10^{-4}\ M^{-1}s^{-1}$

$v = k_H[H_3O^+][E]$

$A_{ac}2$ Mechanism

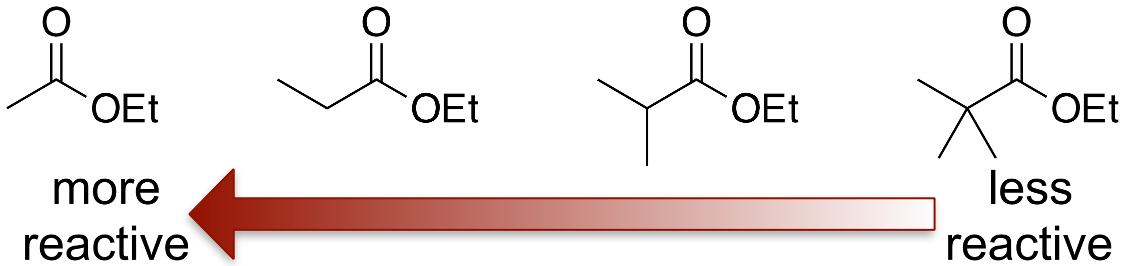


Slow formation of T^+: $v = k_2'[EH^+] = k_2'K_1'[E][H_3O^+] = k_{H,obs}[E][H_3O^+]$

Slow breakdown of T^+: $v = k_3[T^+] = k_3K_2'[EH^+] = k_3K_2'K_1'[E][H_3O^+] = k_{H,obs}[E][H_3O^+]$

Rate Determining Step

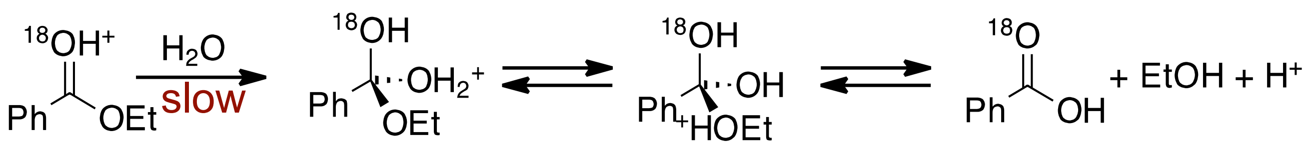
1. Sterically hindered esters



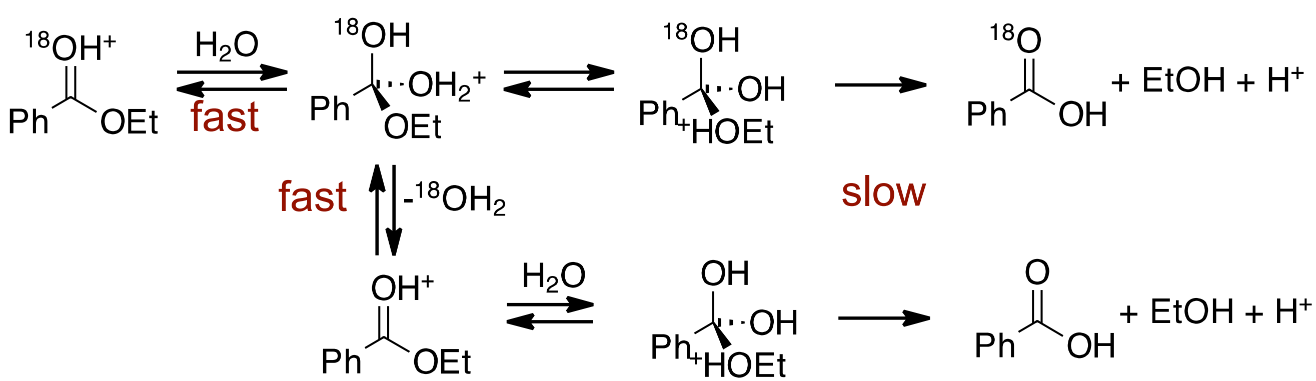
Consistent with slow associative step

Rate Determining Step

2. Isotopic labelling

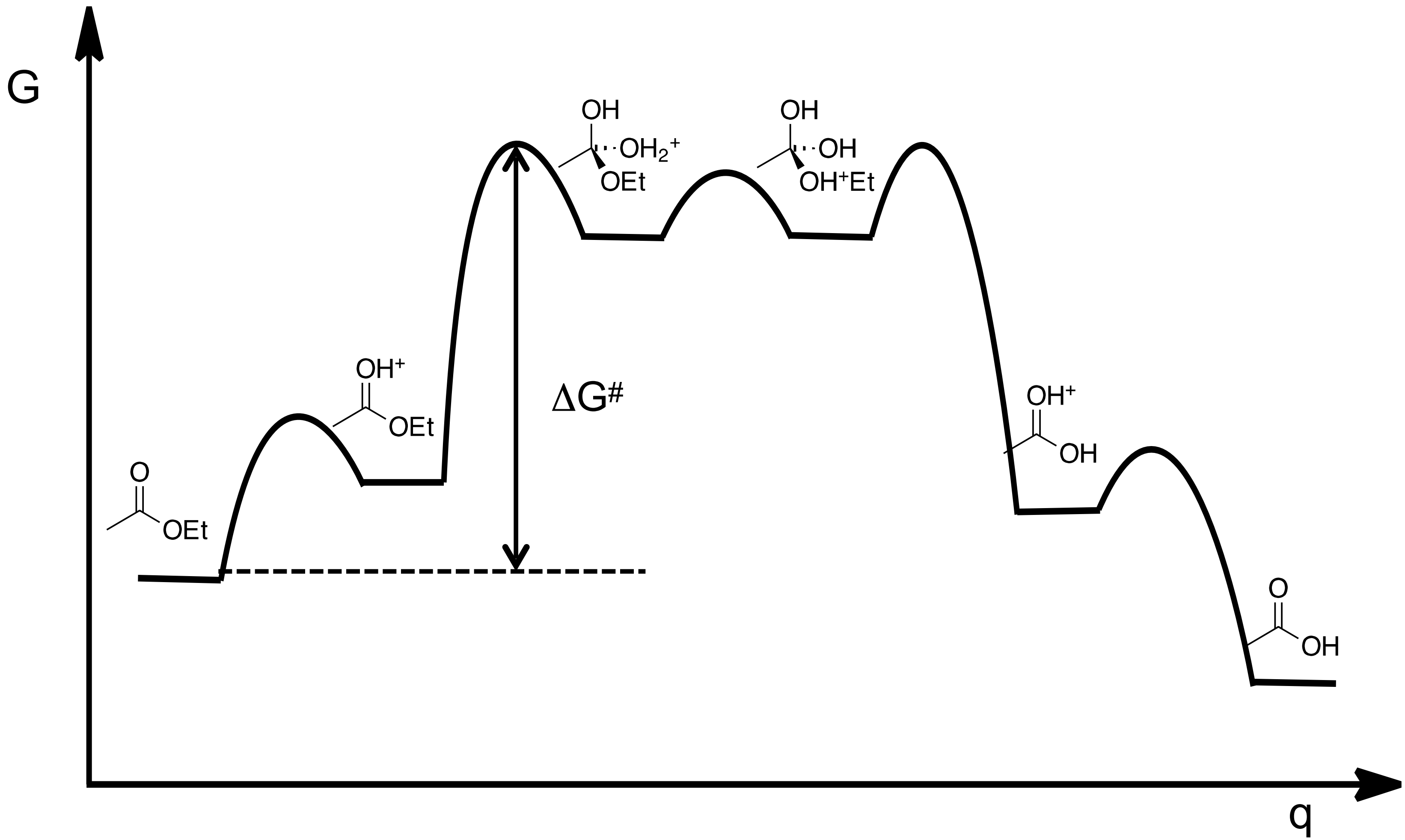


observed retention of ^{18}O

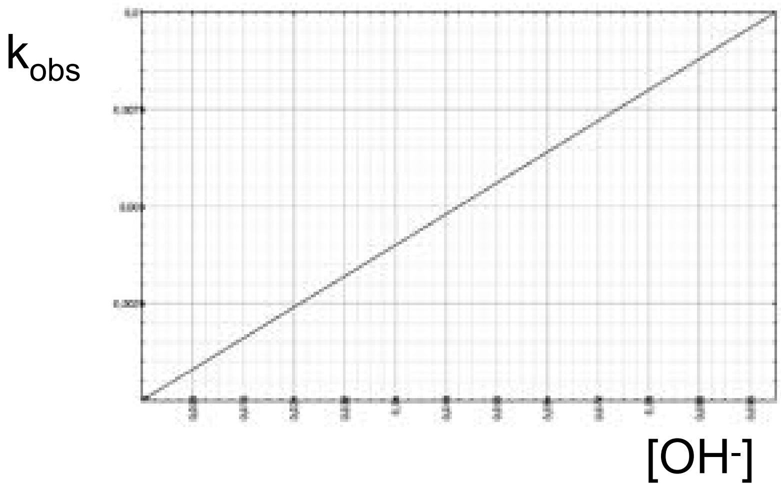


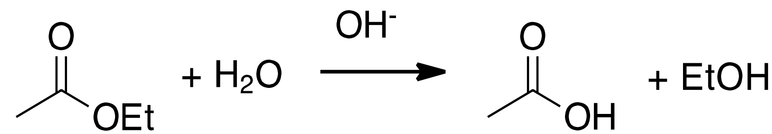
not observed scrambling

Energy Profile



$B_{ac}2$ Mechanism

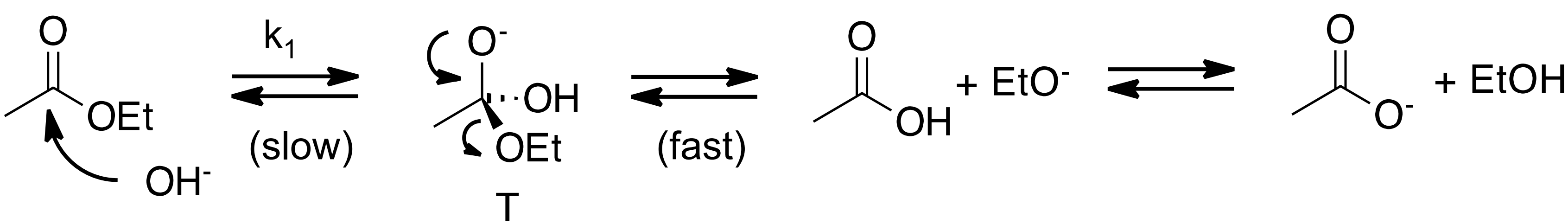




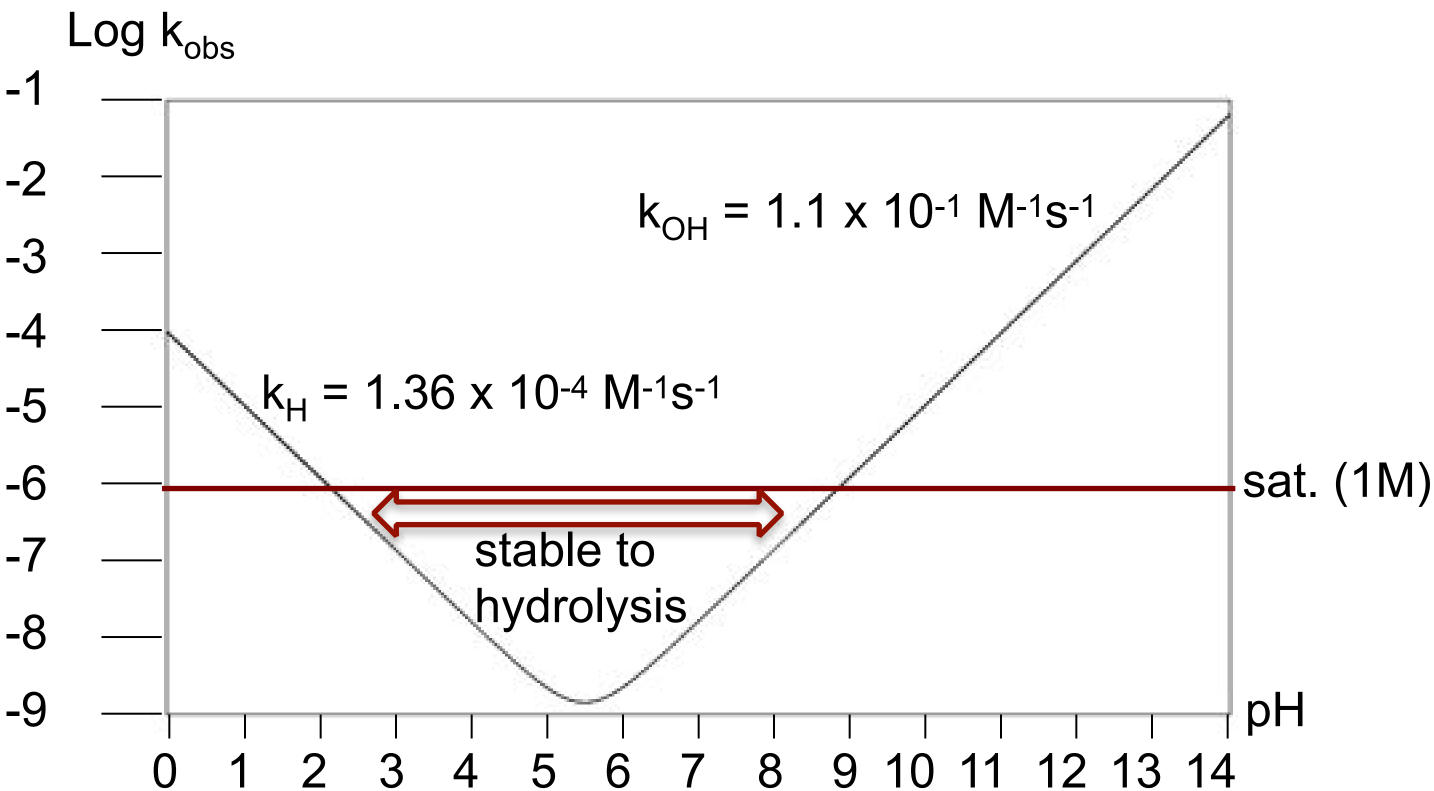
$k_0 = 1 \times 10^{-11}\ s^{-1} \cong 0$

$k_{OH} = 1.1 \times 10^{-1}\ M^{-1}s^{-1}$

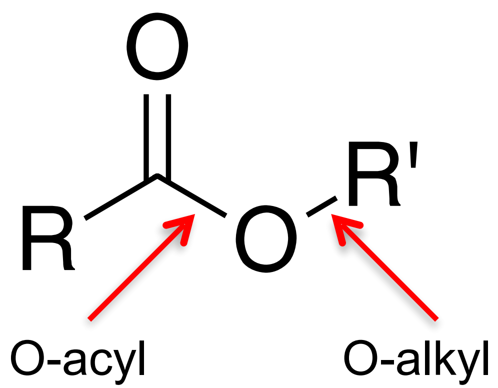
$v = k_{OH}[OH^-][E]$



pH Profile

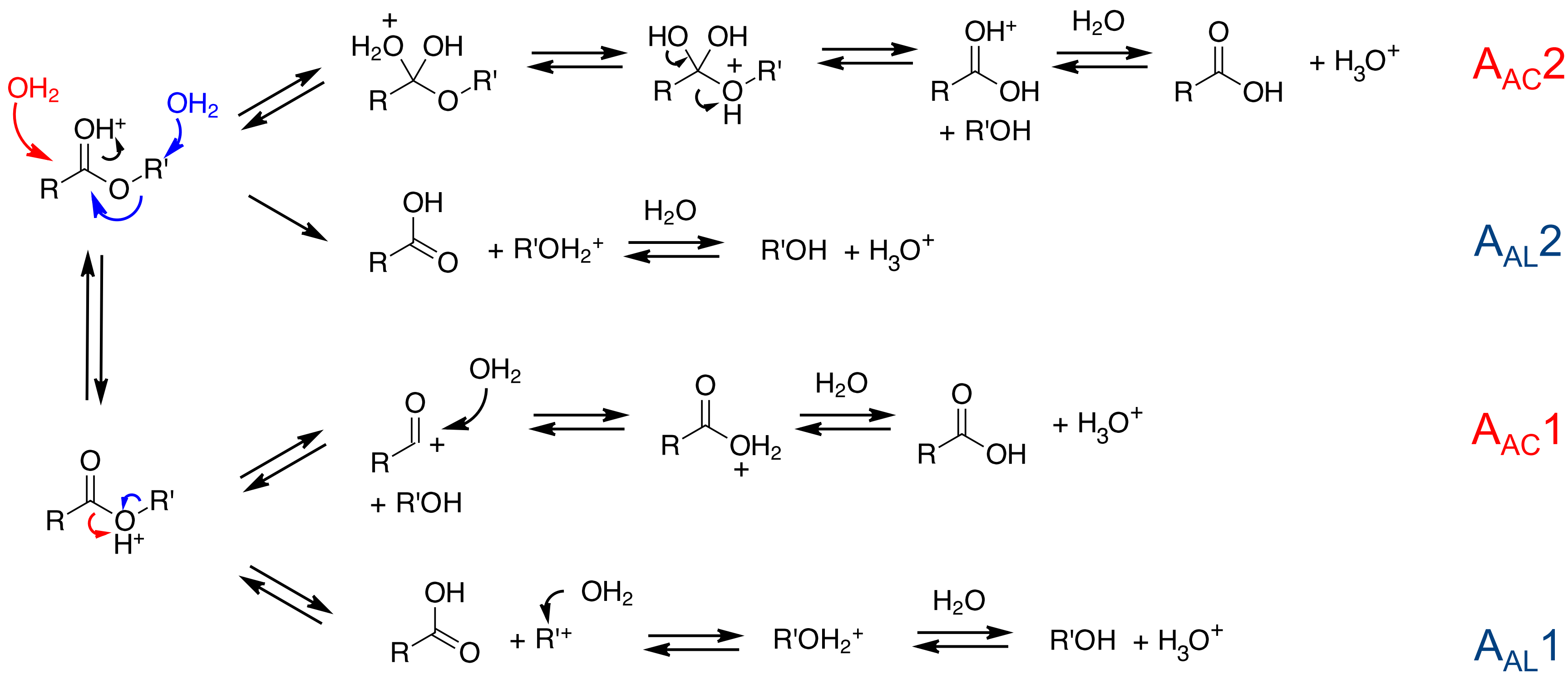

Log k_{obs}
-1
-2
-3
-4
-5
-6
-7
-8
-9
k_{OH} = 1.1 x 10^{-1} $M^{-1}s^{-1}$
k_H = 1.36 x 10^{-4} $M^{-1}s^{-1}$
sat. (1M)
stable to hydrolysis
pH
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Mechanisms for Ester Hydrolysis

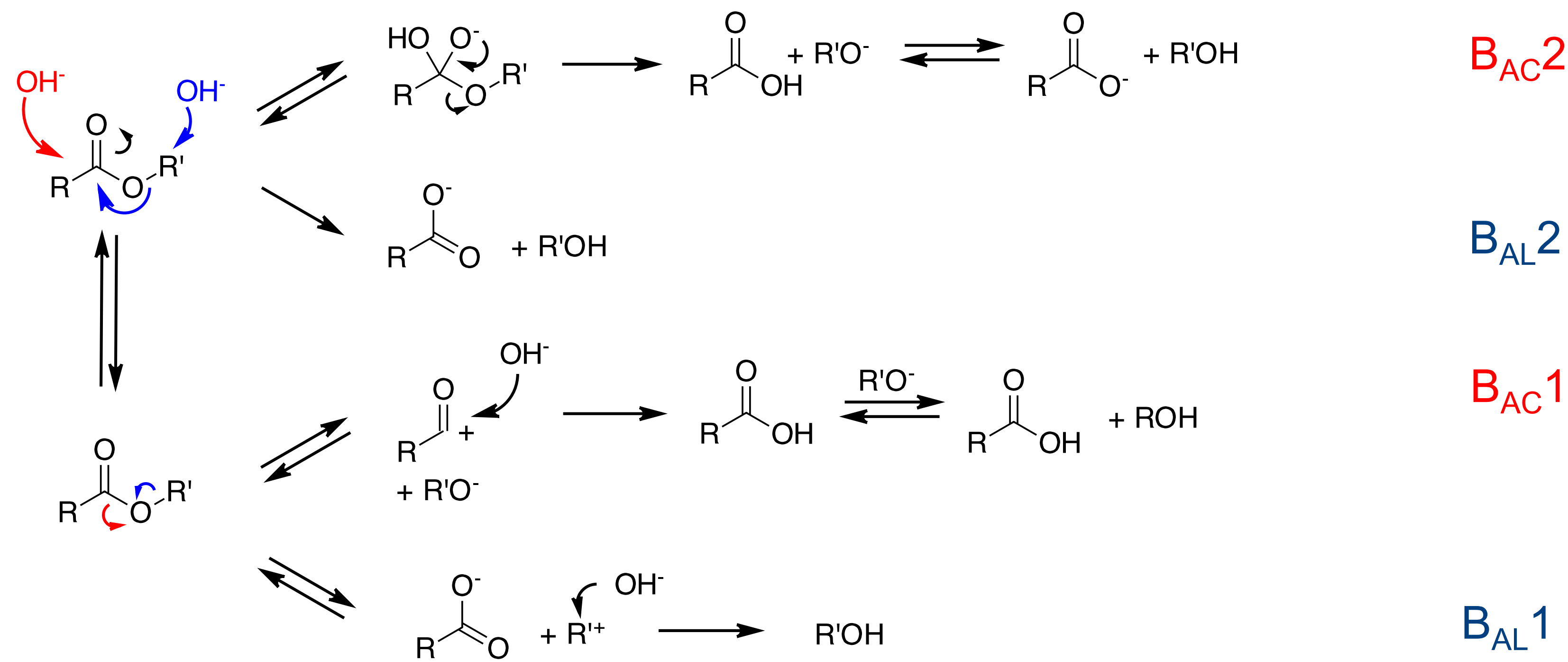


Catalysis	Bond cleavage	Molecularity
A = Acid	***AC = O-acyl***	***1 = monomol.***
B = Base	***AL = O-alkyl***	***2 = bimol.***

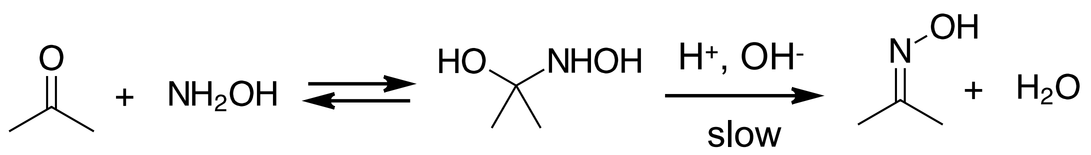
Acid-Catalyzed Ester Hydrolysis

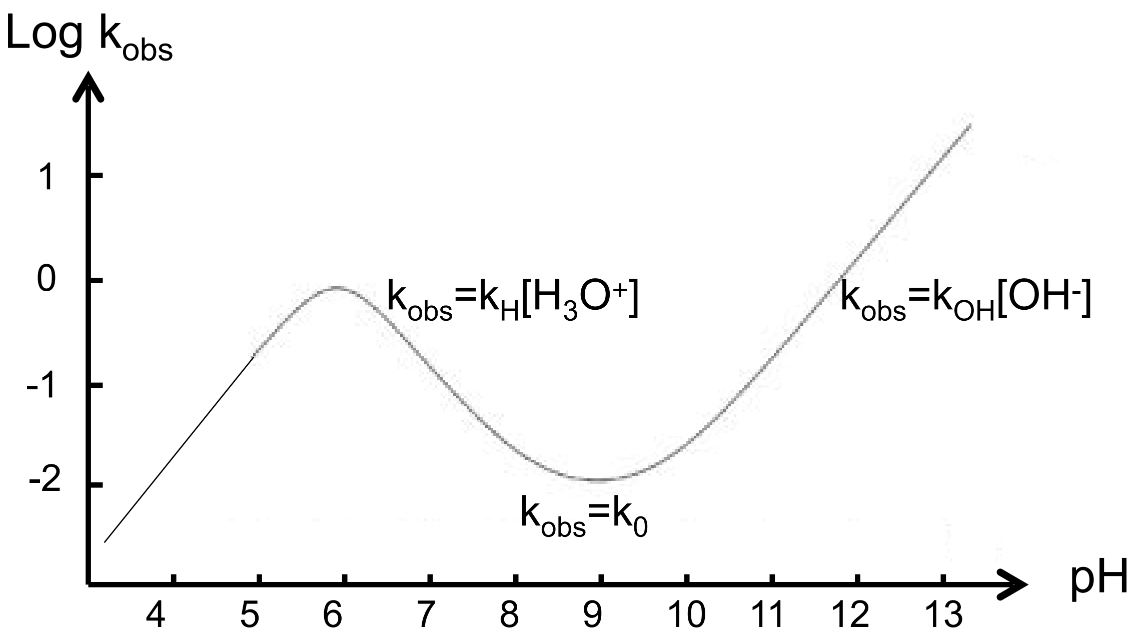


Base-Catalyzed Ester Hydrolysis

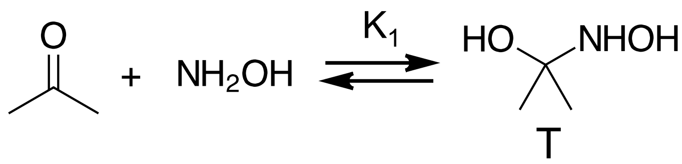


Oxime Formation



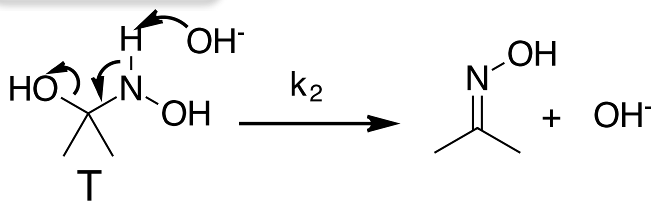


Oxime Formation



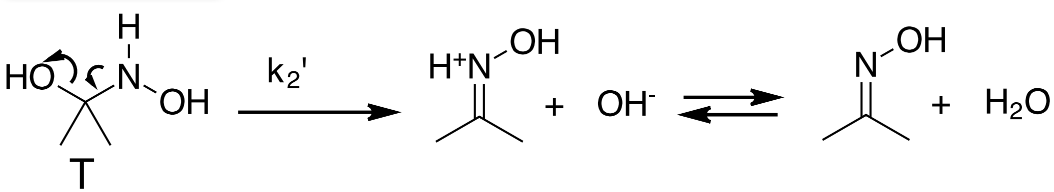
$$[T] = K_1[Ac][NH_2OH]$$

pH >10



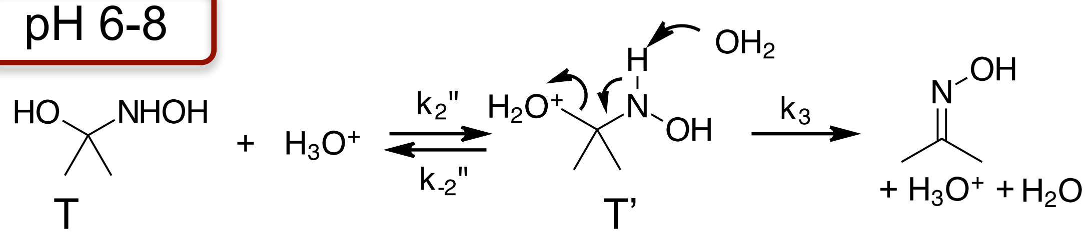
$$v = \underbrace{k_2K_1[OH\text{-}]}_{k_{obs}}[Ac][NH_2OH]$$

pH 8-10



$$v = \underbrace{k_2'K_1}_{k_{obs}}[Ac][NH_2OH]$$

pH 6-8



$$v = k_3[T'] = k_3K_2[T][H_3O^+]$$

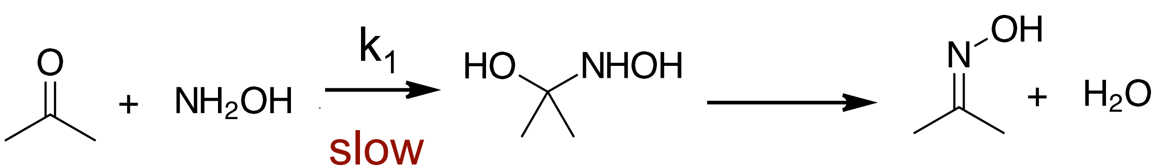
$$= \underbrace{k_3K_1K_2[H_3O^+]}_{k_{obs}}[Ac][NH_2OH]$$

Oxime Formation

pH <6

$$NH_3OH^+ + H_2O \underset{}{\overset{K_a}{\rightleftharpoons}} NH_2OH + H_3O^+ \qquad pK_a=5.96$$

$$[NH_2OH] = \frac{K_a[NH_3OH^+]}{[H_3O^+]} = \frac{K_a([NH_2OH]_{tot}-[NH_2OH])}{[H_3O^+]} \cong \frac{K_a[NH_2OH]_{tot}}{[H_3O^+]}$$



$$v = k_1[Ac][NH_2OH] = \underbrace{\frac{k_1K_a[NH_2OH]_{tot}}{[H_3O^+]}}_{k_{obs}}[acetone]$$

General Acid-Base Catalysis

The catalyst is any species that can transfer a proton:

H_3O^+	strong acid
HA	weaker acid (es. AcOH)
OH^-	strong base
B	weaker base (es. AcO^-)

$$v = v_0 + v_H + v_{AH} + v_{OH} + v_B = k_0[R]+k_H[R][H_3O^+]+k_{HA}[R][HA]+k_{OH}[R][OH^-]+k_B[R][B]$$
$$= [R](k_0+k_H[H_3O^+]+k_{HA}[HA]+k_{OH}[OH^-]+k_B[B])$$

at constant pH:

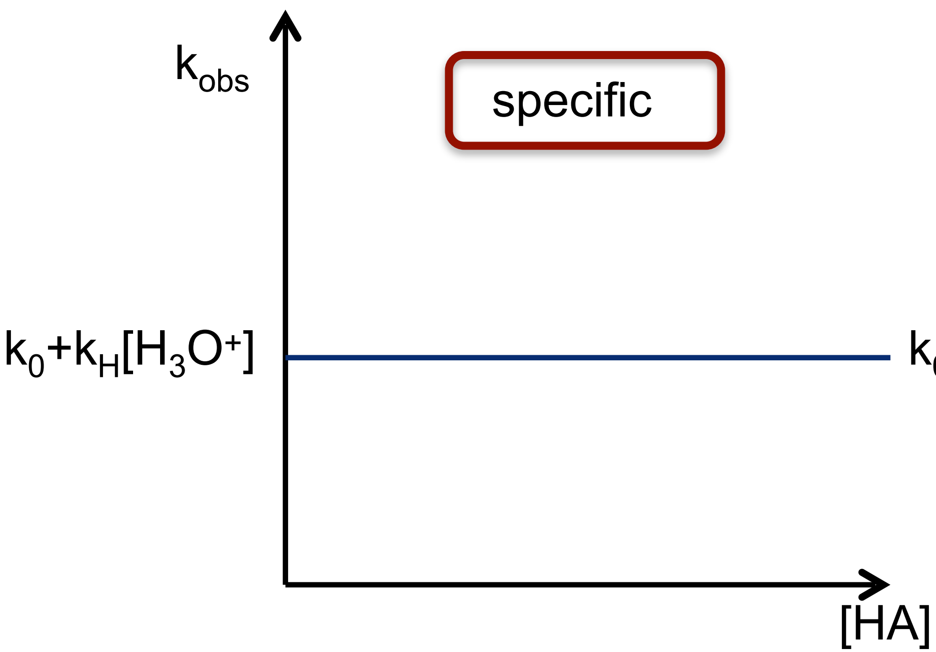
$$v = k_{obs}[R]$$
$$k_{obs} = k_0 + k_H[H_3O^+] + k_{OH}[OH^-] + k_{HA}[HA] + k_B[B]$$

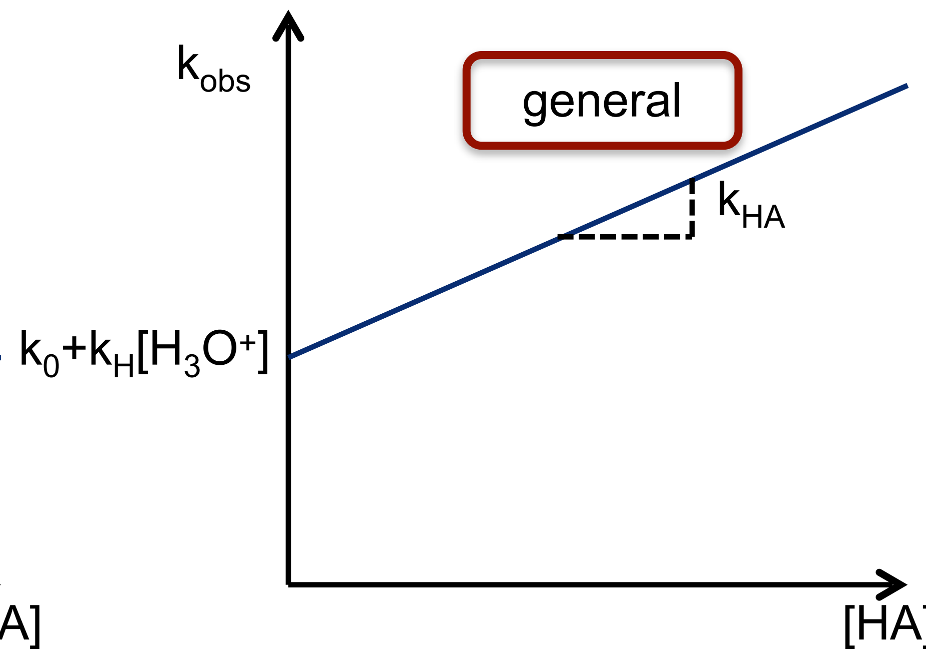
General Acid-Base Catalysis

$$v = k_{obs}[R]$$

$$k_{obs} = k_0 + k_H[H_3O^+] + k_{HA}[HA]$$

At constant pH and varying [HA] (buffer):





Kinetic Origin of General and Specific Catalysis

$$R + HA \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RH^+ + A^-$$

$$RH^+ \xrightarrow{k_2} P + H^+$$

proton transfer is fast (k_2 slow): specific catalysis

proton transfer is slow (k_1 slow): general catalysis

Kinetic Origin of General and Specific Catalysis

$$R + HA \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RH^+ + A^-$$

$$RH^+ \xrightarrow{k_2} P + H^+$$

$$v = d[P]/dt = k_2[RH^+]$$

steady state: $d[RH^+]/dt = 0 = k_1[R][HA]-k_{-1}[RH^+][A^-]-k_2[RH^+]$

$$k_1[R][HA] = [RH^+](k_{-1}[A^-]+k_2)$$

$$[RH^+] = \frac{k_1[R][HA]}{(k_{-1}[A^-]+k_2)}$$

$$v = \frac{k_1k_2[R][HA]}{(k_{-1}[A^-]+k_2)}$$

Kinetic Origin of General and Specific Catalysis

$$R + HA \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RH^+ + A^-$$

$$RH^+ \xrightarrow{k_2} P + H^+$$

$$v = \frac{k_1 k_2 [R][HA]}{(k_{-1}[A^-] + k_2)}$$

1. $k_2 << k_{-1}[A^-]$ (k_2 slow)

$$v = \frac{k_1 k_2 [R][HA]}{k_{-1}[A^-]}$$

$$HA + H_2O = A^- + H_3O^+$$

$$K_{HA} = \frac{[H_3O^+][A^-]}{[HA]}$$

$$\frac{[HA]}{[A^-]} = \frac{[H_3O^+]}{K_{HA}}$$

specific $$v = \frac{k_1 k_2}{k_{-1} K_{HA}} [R][[H_3O^+]$$

Kinetic Origin of General and Specific Catalysis

$$R + HA \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RH^+ + A^-$$

$$RH^+ \xrightarrow{k_2} P + H^+$$

$$v = \frac{k_1 k_2 [R][HA]}{(k_{-1}[A^-] + k_2)}$$

2. $k_2 >> k_{-1}[A^-]$ (k_1 slow)

$$v = \frac{k_1 k_2 [R][HA]}{k_2}$$

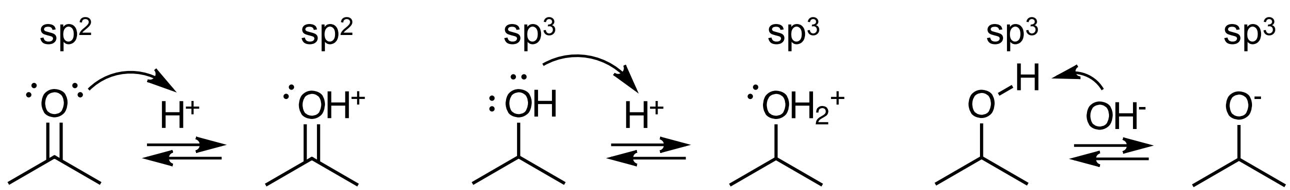
general $v = k_1[R][HA]$

Kinetic Origin of General and Specific Catalysis

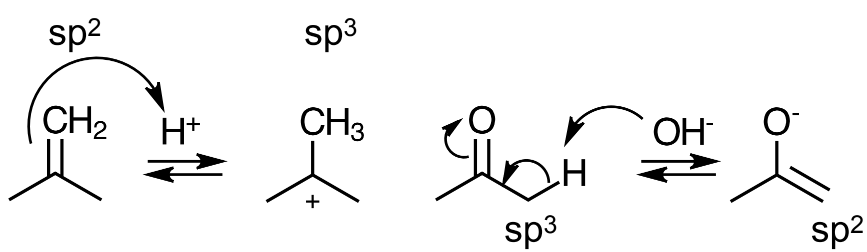
$$R + HA \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RH^+ + A^-$$

$$RH^+ \xrightarrow{k_2} P + H^+$$

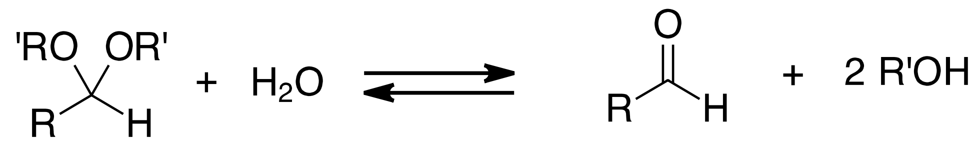
proton transfer is fast (k_2 slow): specific catalysis



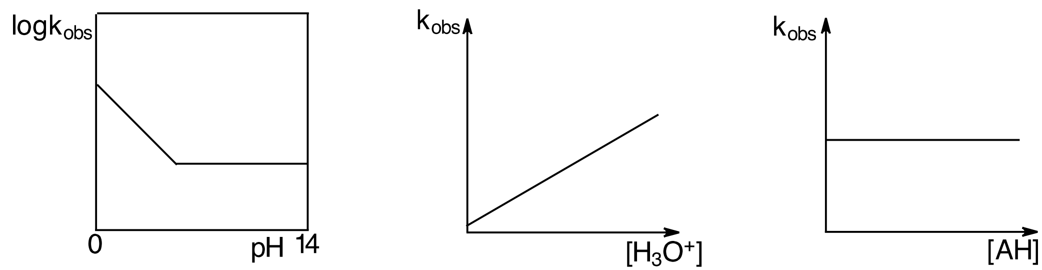
proton transfer is slow (k_1 slow): general catalysis

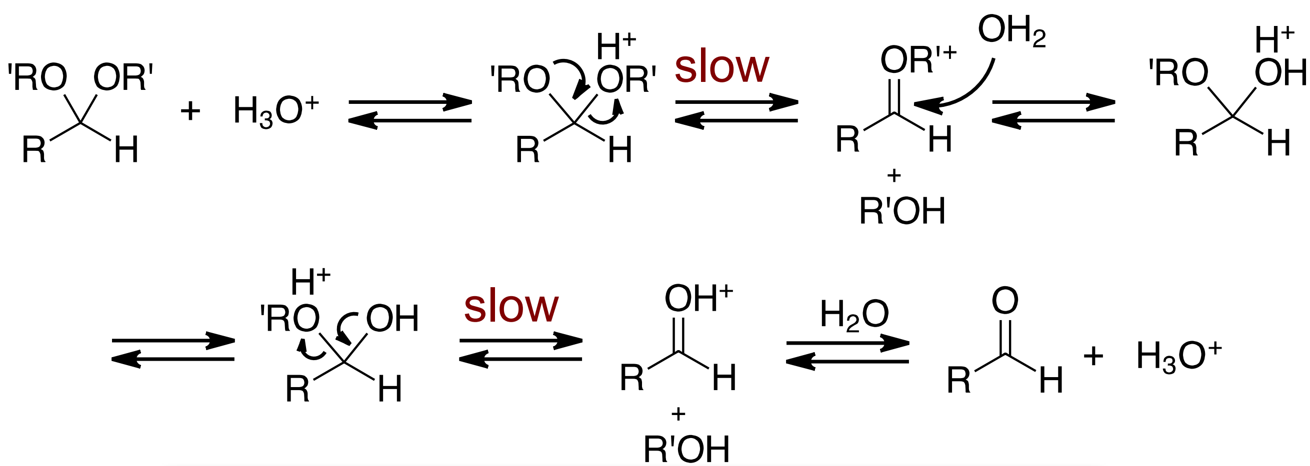


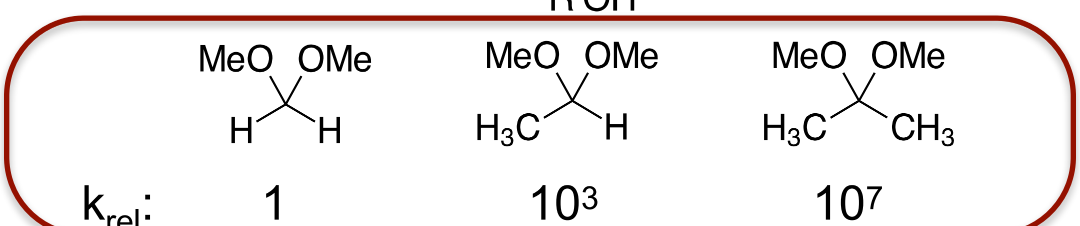
Hydrolysis of Acetals



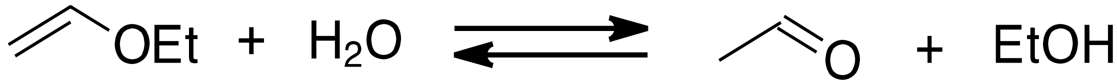
$v = k_{obs}[\text{acetal}]$



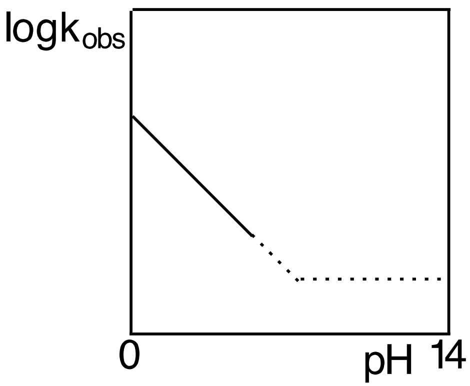


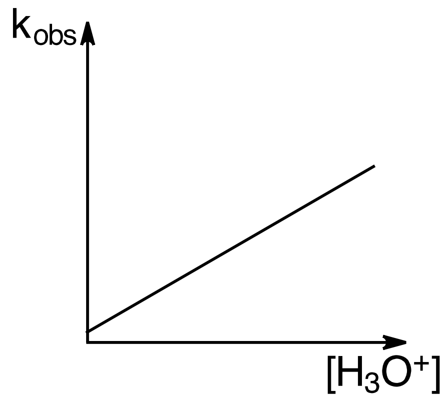


Hydrolysis of Vinyl Ethers



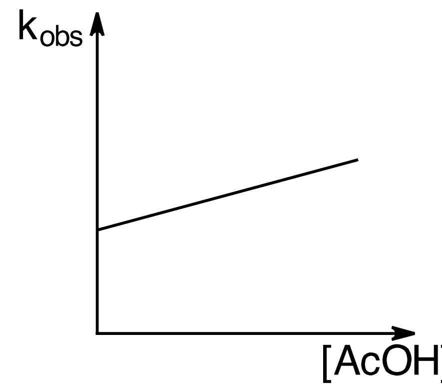
$v = k_{obs}[\text{ether}]$



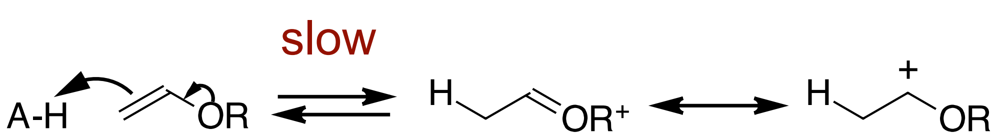


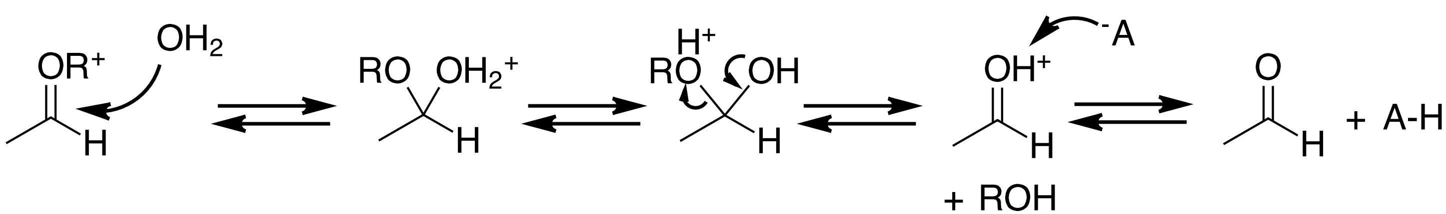
$k_0 \cong 0$

$k_H = 2.1 \times 10^{-1}\ s^{-1}$

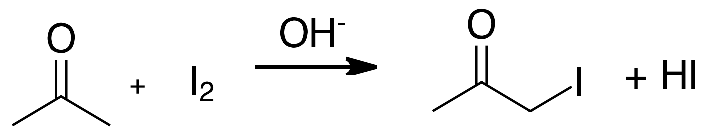


$k_{AcOH} = 1.8 \times 10^{-3}\ M^{-1}s^{-1}$

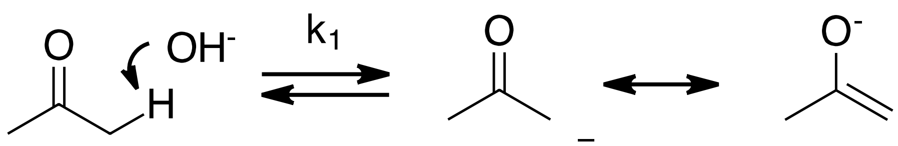




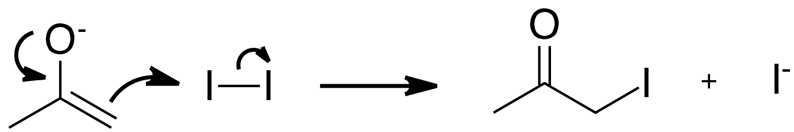
α-Halogenation of Carbonyl Compounds



$$v = k_{OH}[OH^-][acetone]$$



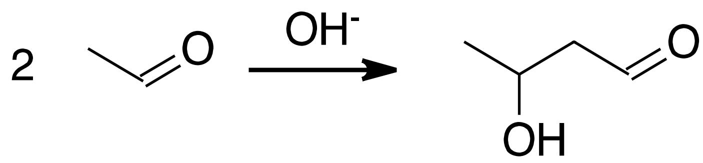
slow $v = k_1[OH^-][acetone]$



fast

General Base Catalysis

The Aldol Reaction



dilute solution

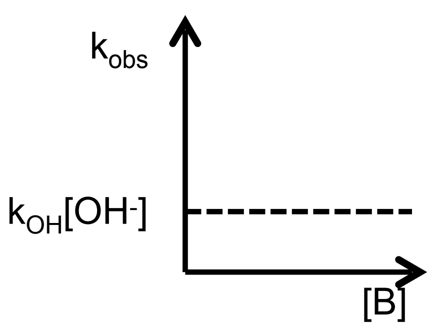
$v = k_{OH}[OH^-][CH_3CHO]^2$

$k_{OH} = 0{,}67\ M^{-2}s^{-1}$

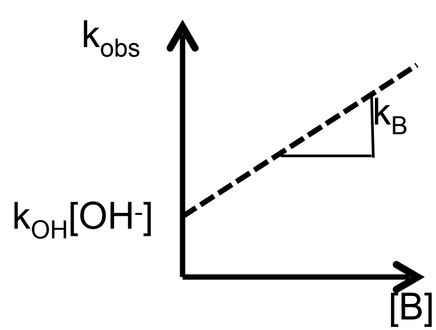
conc. solution (>10M)

$v' = k'_{OH}[OH^-][CH_3CHO]$

$k'_{OH} = 7\ M^{-1}s^-$

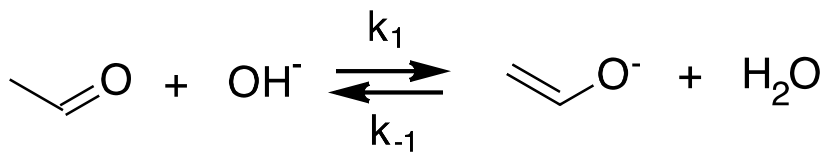


SPECIFIC

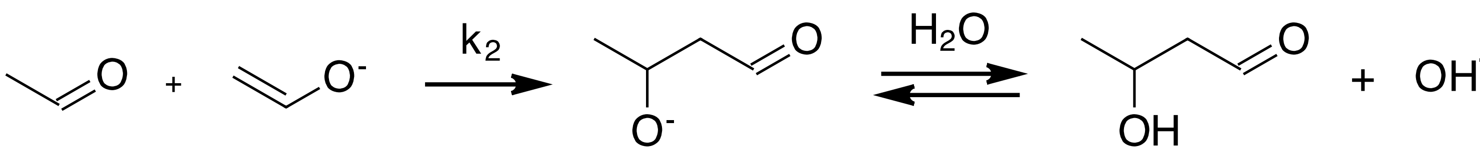


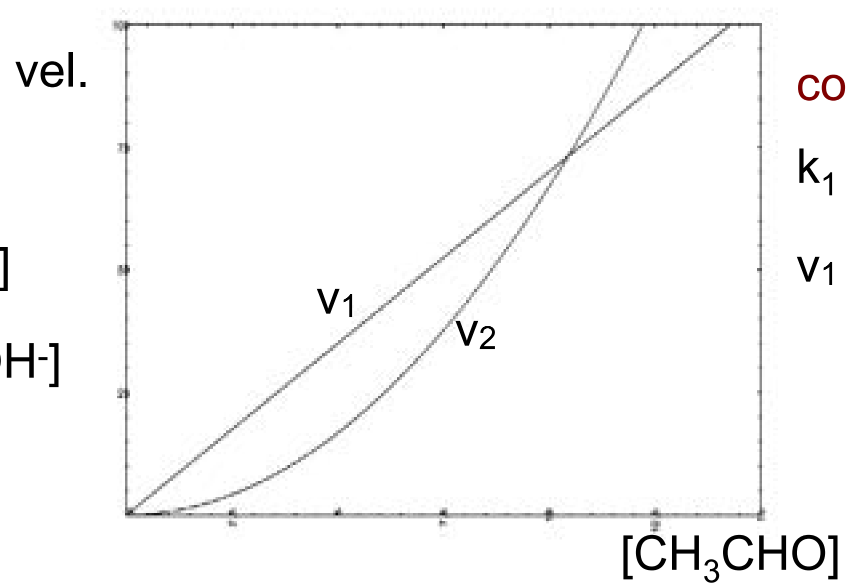
GENERAL

The Aldol Reaction



$$K_1' = \frac{[CH_2CHO^-]}{[CH_3CHO][OH^-]}$$





dil. solution

k_2 is slow

$v_2 = k_2[CH_3CHO][CH_2CHO^-]$

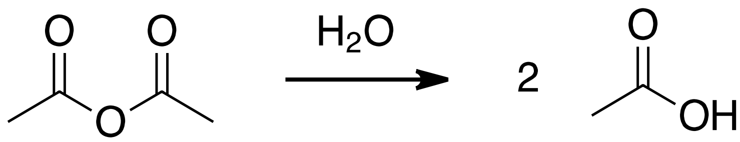
$= k_2K_1' [CH_3CHO]^2[OH^-]$

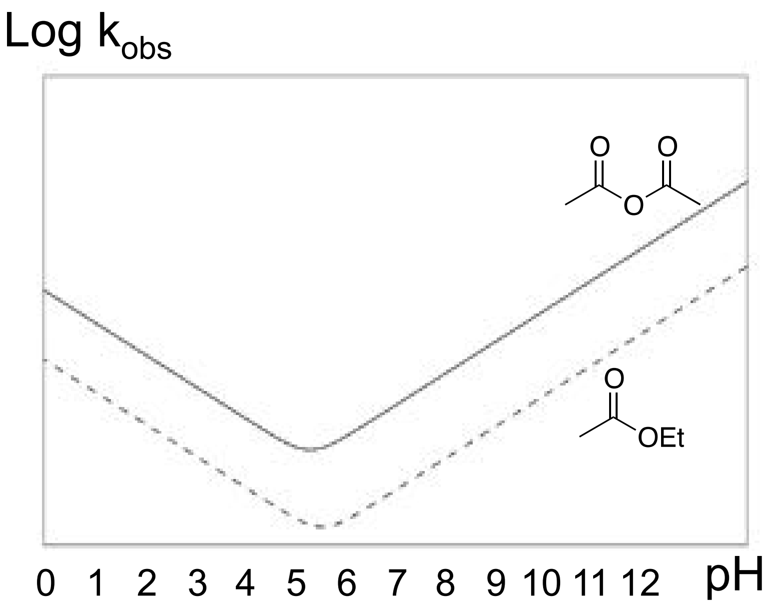
conc. solution

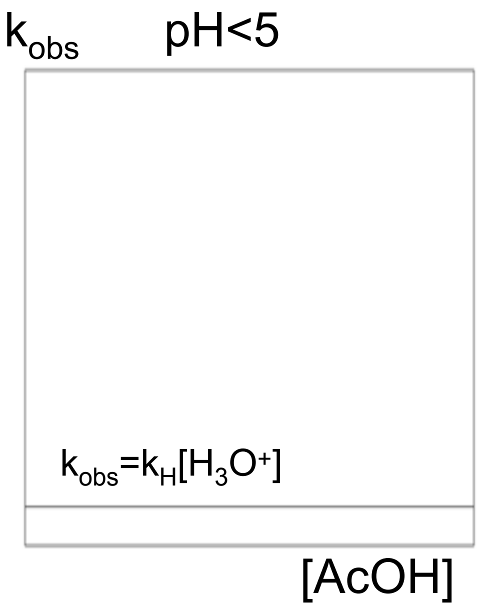
k_1 is slow

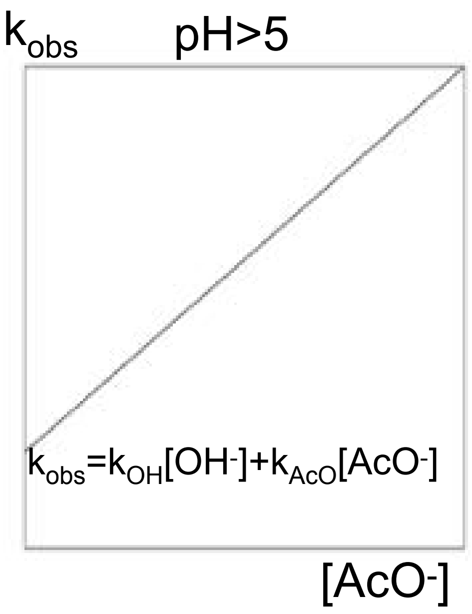
$v_1 = k_1[CH_3CHO][OH^-]$

Hydrolysis of Anhydrides: Mechanistic Catalysis

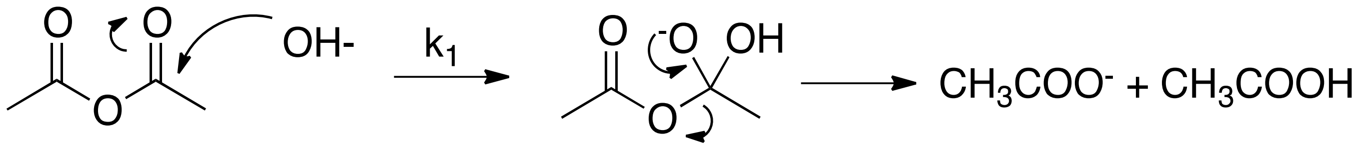




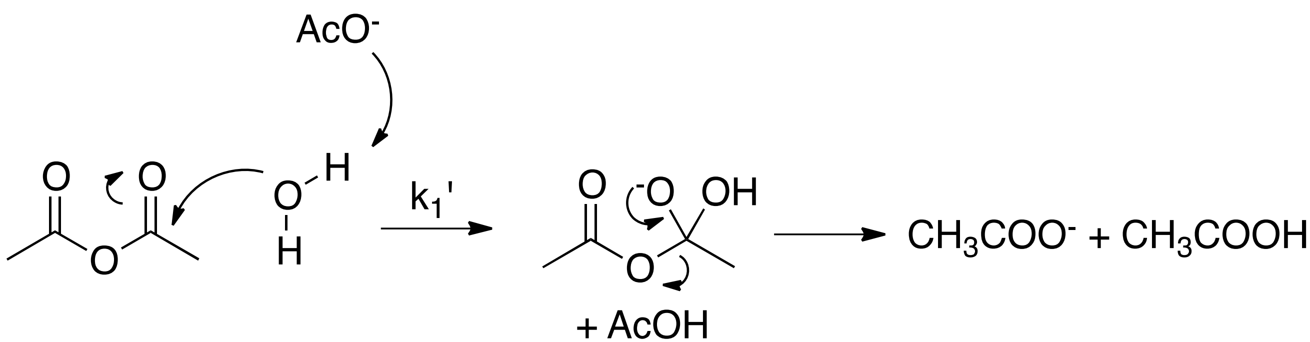




Hydrolysis of Anhydrides: Mechanistic Catalysis



$v_{OH} = k_1[OH^-][Ac_2O]$

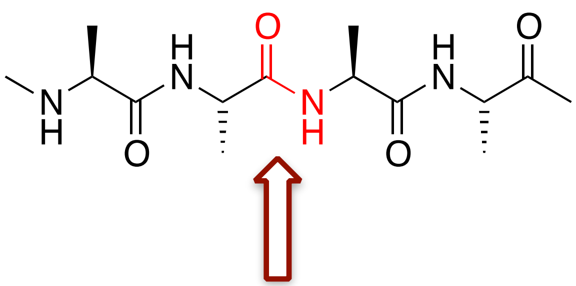


$v_{AcO} = k_1'[AcO^-][Ac_2O]$

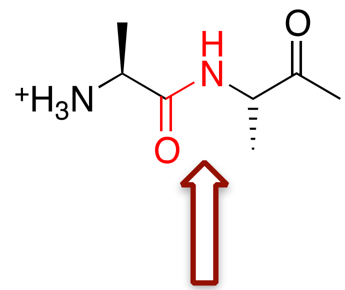
$$v_{obs} = v_{OH} + v_{AcO} = k_1[OH^-][Ac_2O] + k_1'[AcO^-][Ac_2O] = \underbrace{(k_1[OH^-] + k_1'[AcO^-])}_{k_{obs}}[Ac_2O]$$

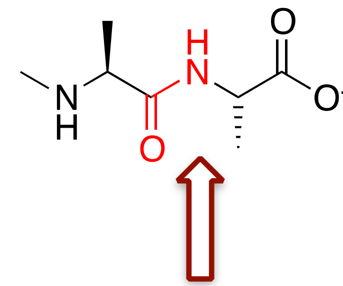
Proteases

Specificity



Endoproteases





Exoproteases

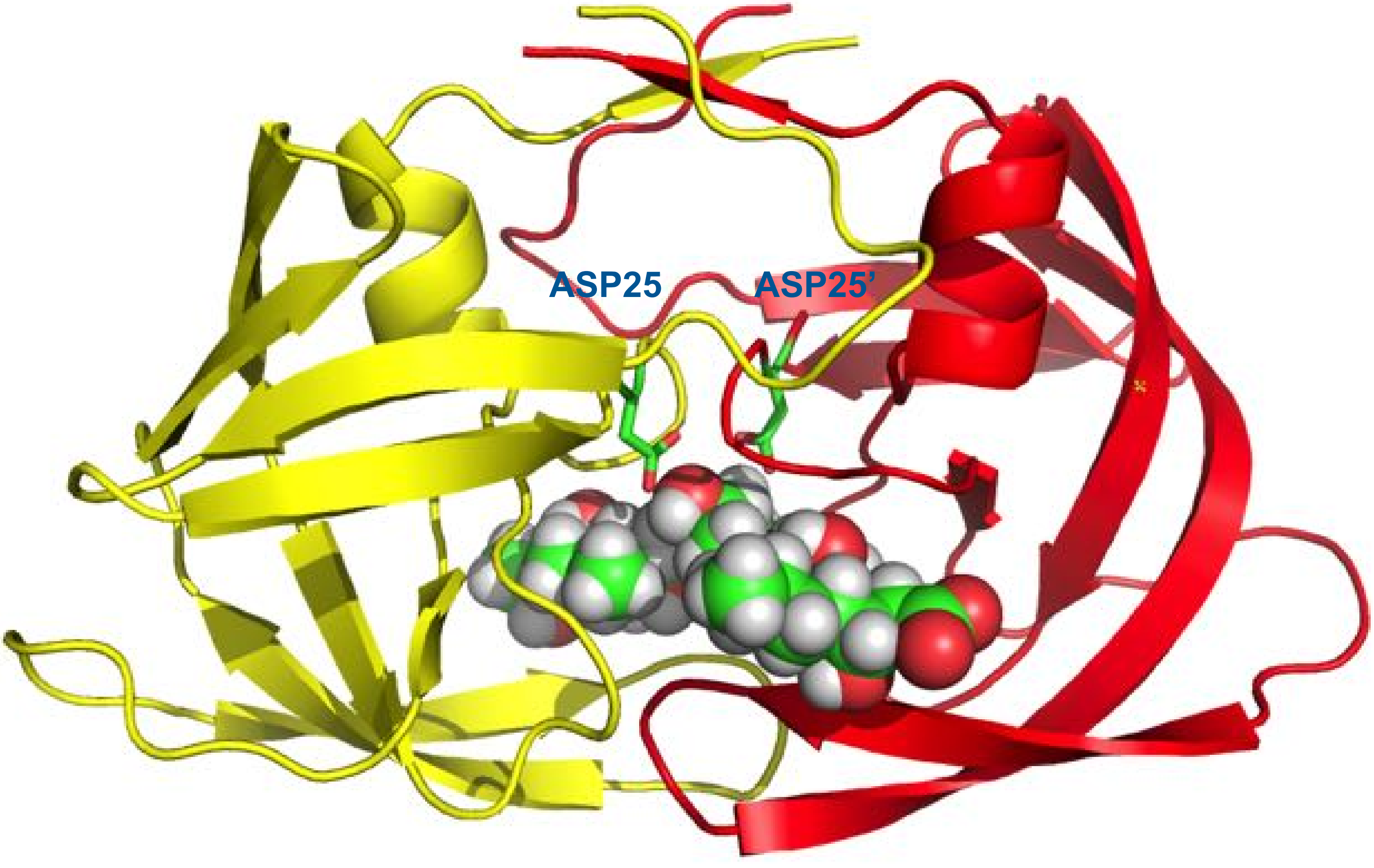
Catalytic mechanism

- Serine protease
- Cysteine proteases

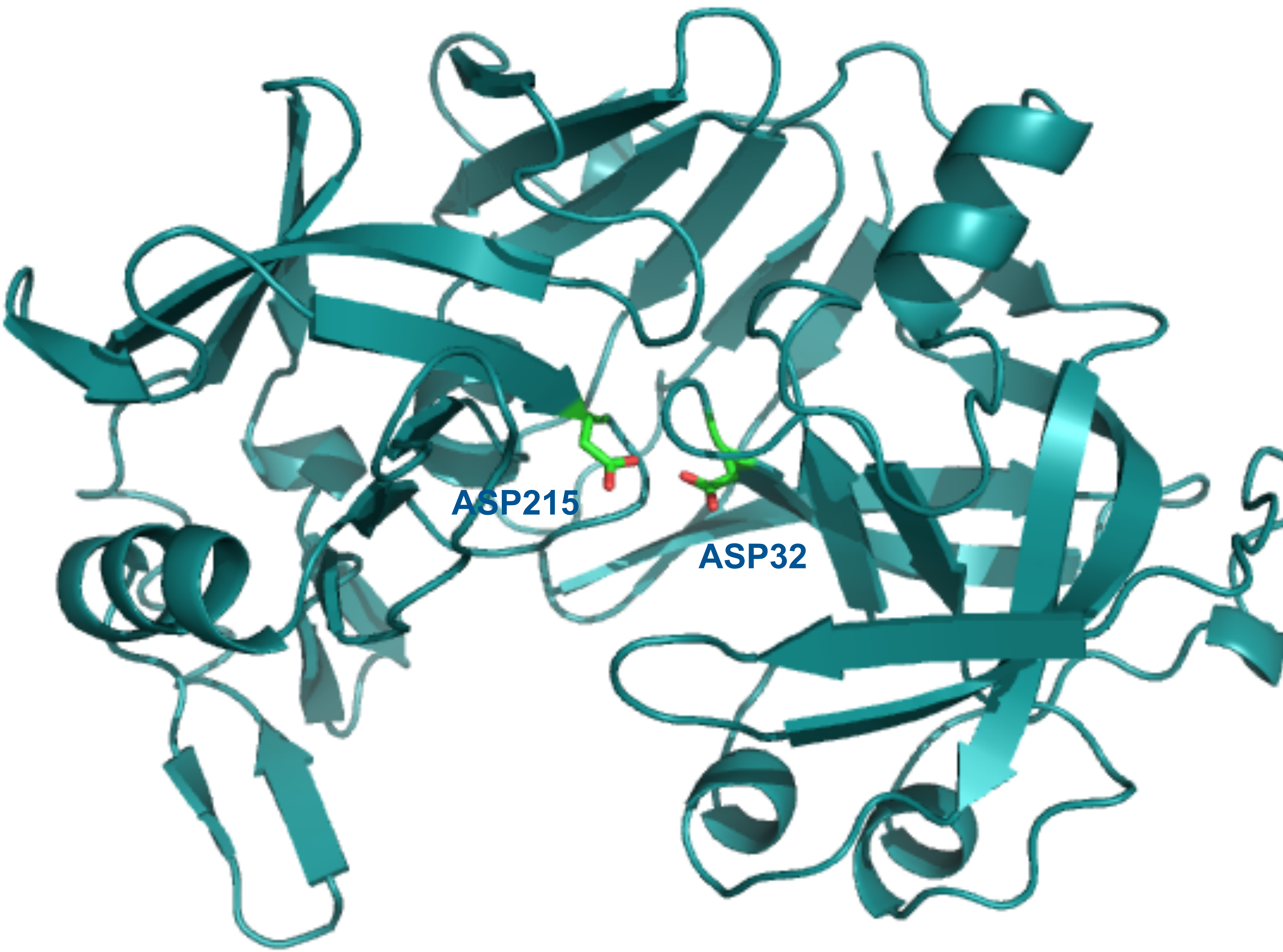
→ nucleophilic catalysis

- Aspartyl proteases → acid-base catalysis
- Metal proteases → electrophilic catalysis

HIV Protease

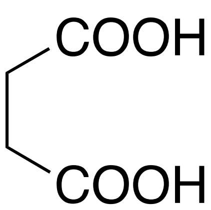


Pepsin

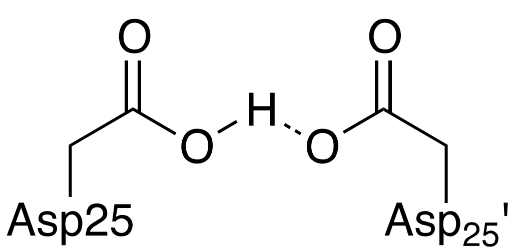


Aspartyl Proteases

- Two Asp residues in the catalytic site
- The two residue can be on the same chain or on different chains
- Optimum pH is acidic: HIV-PR pH 4-5; pepsin pH ≈ 4 (stomach)

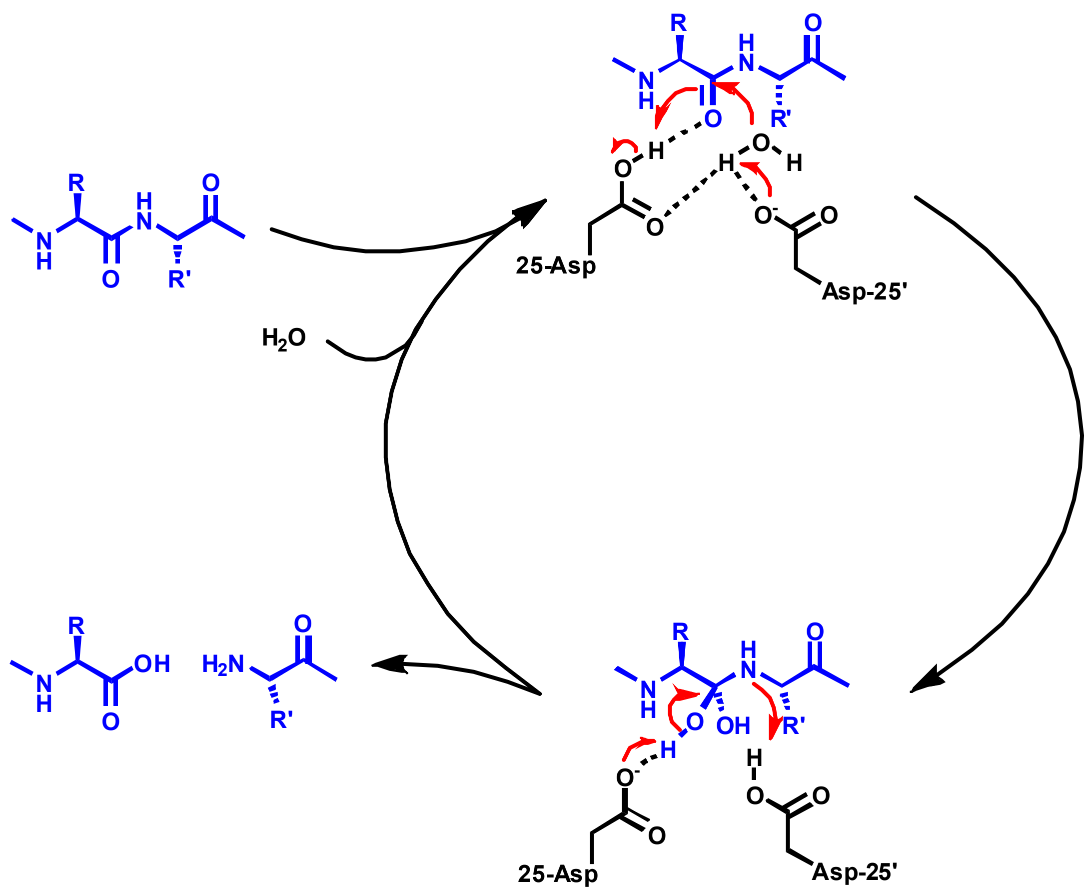


1st pK_a: 3.3 2nd pK_a: 5.3



1st pK_a: 4.21 2nd pK_a: 5.64

HIV-Protease – Catalytic Mechanism



Tetrahedral Intermediate (hydrated amide)

Brønsted Equation

$$HA + H_2O \underset{}{\overset{K_{HA}}{\rightleftharpoons}} A^- + H_3O^+$$

$$HA + R \xrightarrow{k_{HA}} A^- + RH^+$$

$$RH^+ \longrightarrow P$$

$$BH^+ + H_2O \overset{K_{HB}}{\rightleftharpoons} B + H_3O^+$$

$$B + RH \xrightarrow{k_B} BH^+ + R^-$$

$$R^- \longrightarrow P$$

Is there a relation between K_{HA} and k_{HA} (K_{HB} and k_{HB})?

The Brønsted equation (empirical)

$\log k_{HA} = \alpha \log K_{HA}$ + cost.

$\log k_{HA} = -\alpha pK_{HA}$ + cost. $(0 \leq \alpha \leq 1)$

$\log k_B = -\beta \log K_{BH}$ + cost.

$\log k_B = \beta pK_B$ + cost. $(0 \leq \beta \leq 1)$

Brønsted Equation

LFER = Linear Free Energy Relationship

$$\log k_{HA} = \alpha \log K_{HA} + \text{cost}$$

⇩

$$\log \frac{kT}{h} e^{-\frac{\Delta G^{\#}}{RT}} = a \log e^{-\frac{\Delta G}{RT}} + \text{cost}$$

⇩

$$\frac{\Delta G^{\#}}{RT} = \alpha \frac{\Delta G}{RT} + \text{cost}'$$

Brønsted Equation

Meaning of the α, β parameters

$$HA + R \xrightarrow{k_{HA}} A^- + RH^+ \qquad \frac{\Delta G^\#}{RT} = \alpha \frac{\Delta G}{RT} + \text{cost}$$

$$HA' + R \xrightarrow{k_{HA'}} A'^- + RH^+ \qquad \frac{\Delta G'^{\#}}{RT} = \alpha \frac{\Delta G'}{RT} + \text{cost}$$

$$\frac{\Delta G'^\#}{RT} - \frac{\Delta G^\#}{RT} = \alpha\left(\frac{\Delta G'}{RT} - \frac{\Delta G}{RT}\right)$$

⇩

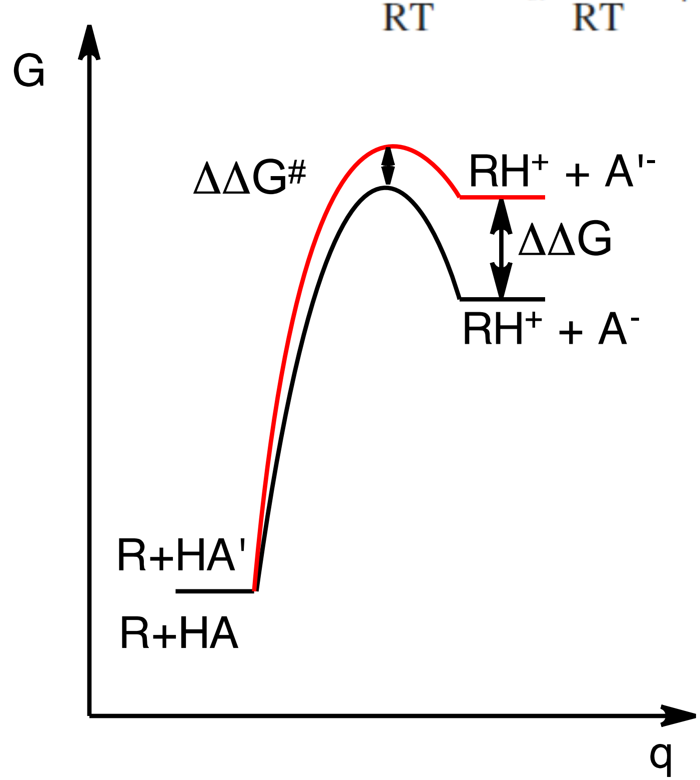
$$\Delta G'^\# - \Delta G^\# = \alpha(\Delta G' - \Delta G)$$

⇩

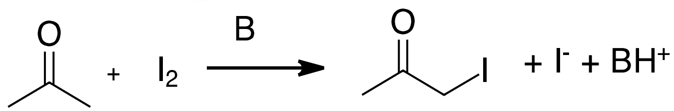
$$\Delta\Delta G^\# = \alpha \Delta\Delta G$$

⇩

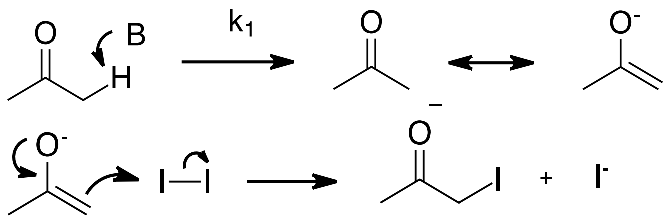
$$\alpha = \frac{\Delta\Delta G^\#}{\Delta\Delta G}$$



Brønsted Equation
Halogenation of Carbonyl Compounds

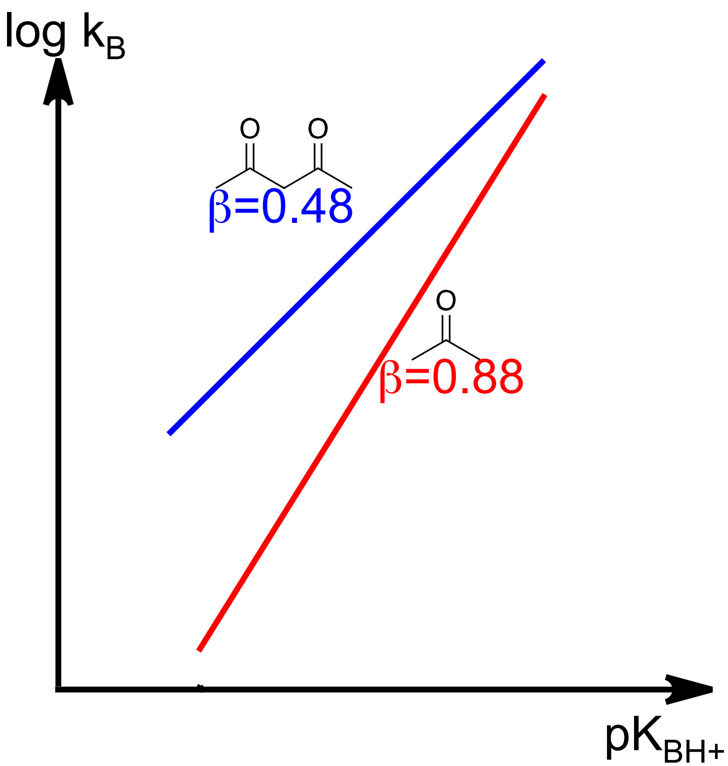


$v = k_{OH}[OH^-][acetone] + k_B[B][acetone]$

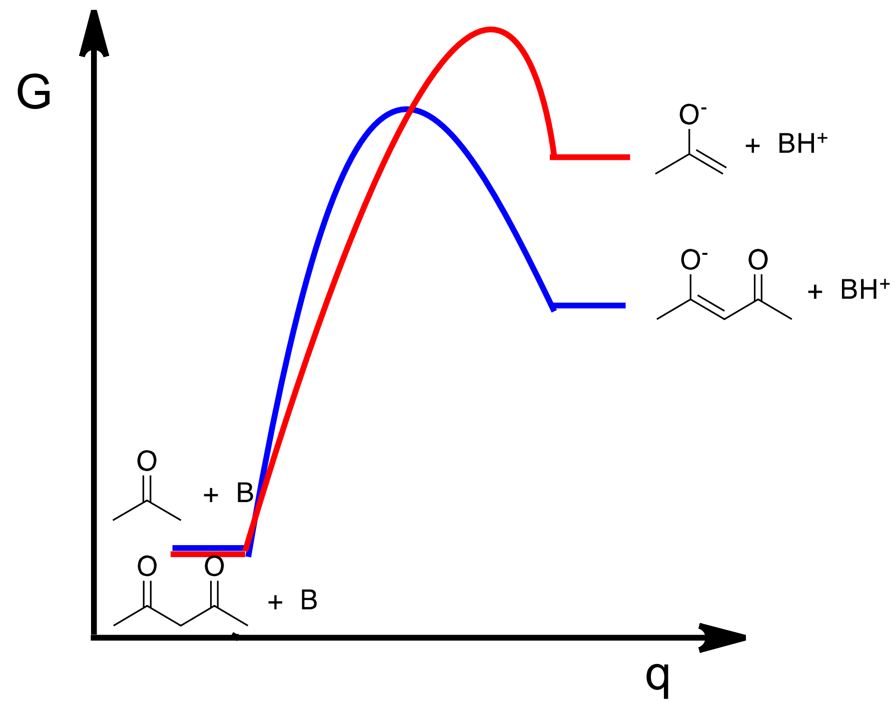


slow $v = k_1[B][acetone]$

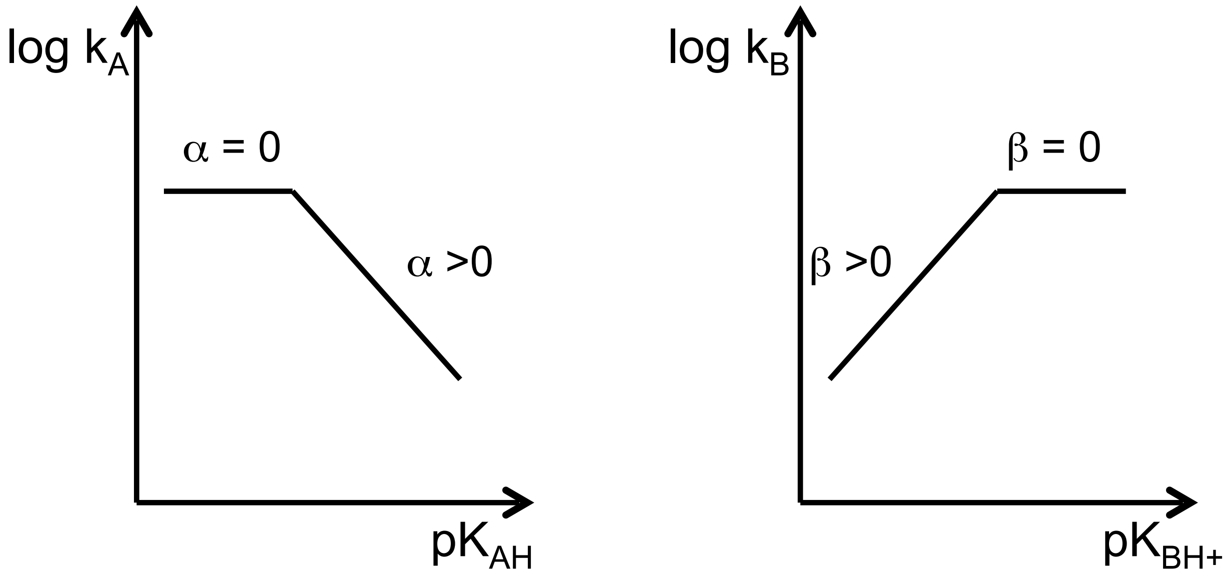
fast



Hammond Postulate



Brønsted Equation: Levelling of α (β)



Kinetic effect

$$HA + R \xrightarrow{k_{HA}} A^- + RH^+$$

$$RH^+ \xrightarrow{k_2} P$$

k_{HA} becomes faster as HA becomes stronger:

general ➔ specific

Thermodynamic effect

$$HA + H_2O \overset{K_{HA}}{\rightleftharpoons} A^- + H_3O^+$$

As HA becomes stronger $[HA] << [H_3O^+]$

(strong acids in water are all the same)

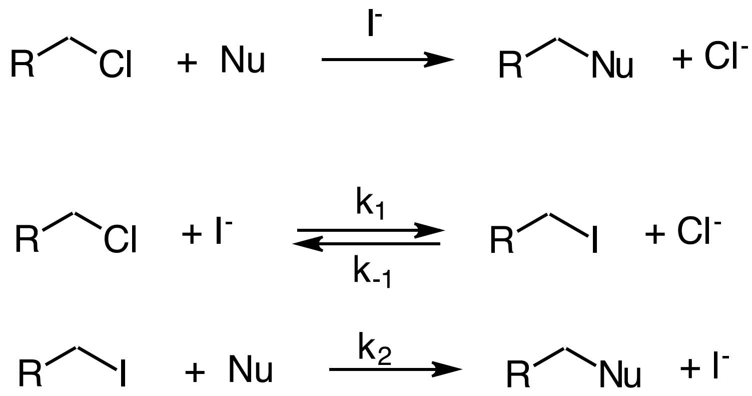
general ➔ specific

Diffusion rate

Reactions in solution can not be faster than diffusion

limit: $k_{diff} \approx 10^{10}\ M^{-1}\ s^{-1}$

Nucleophilic Catalysis

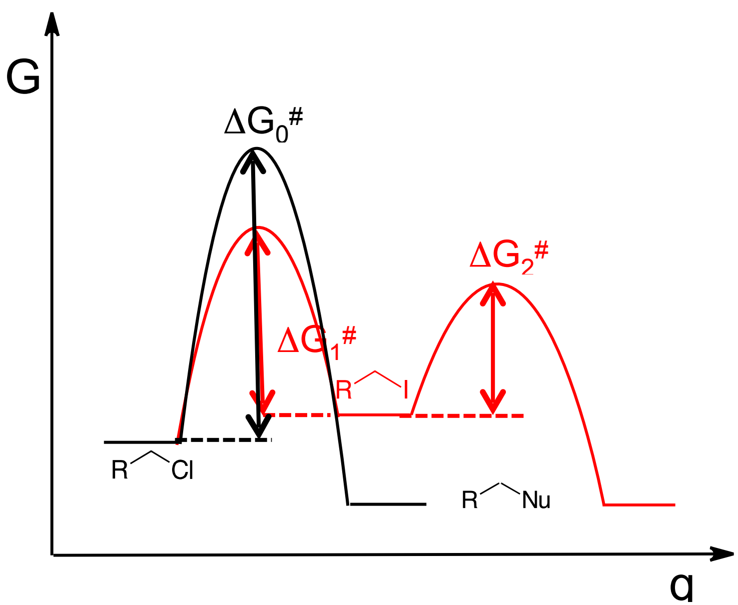


I-: better nucleophile

I-: better leaving group

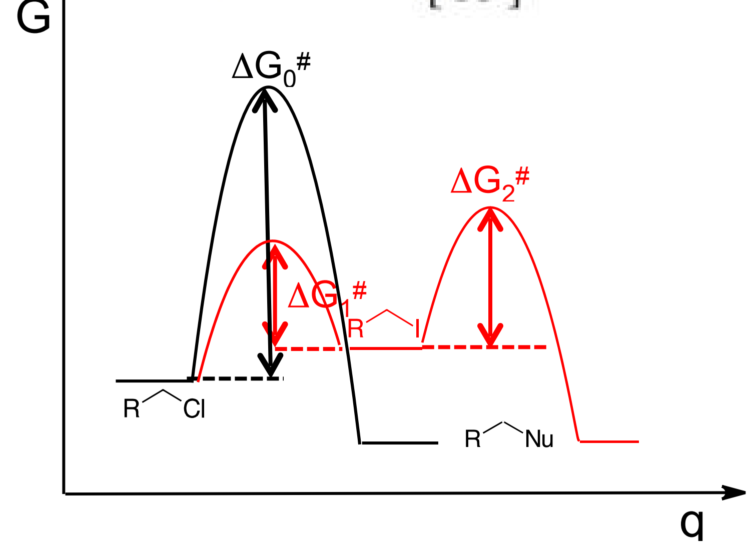
k1 slow

$v = k_1[RCl][I^-]$

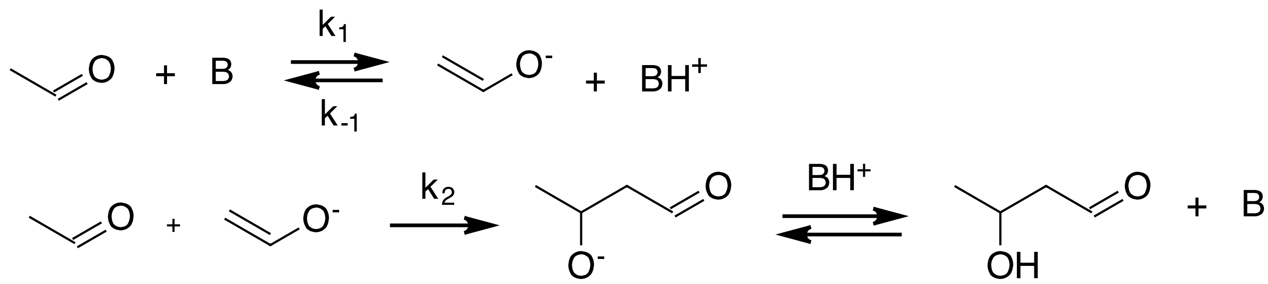


k2 slow

$$v = \frac{k_1 k_2}{k_{-1}} \frac{[RCl][I^-][Nu]}{[Cl^-]}$$



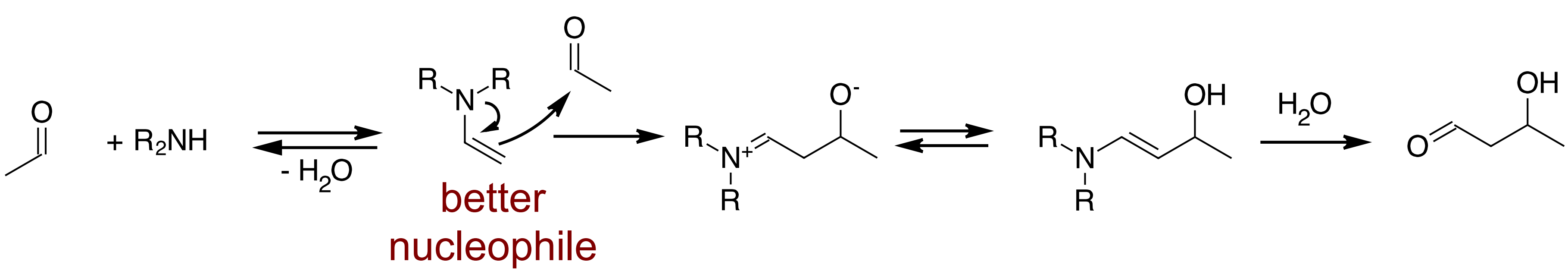
Aldol Reaction



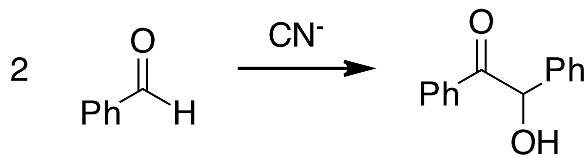
specific base: dil. aqueous sol.
general base: conc. aqueous sol.

2^{ry} and 1^{ry} amines are more efficient than 3^{ry} amines with the same pKa

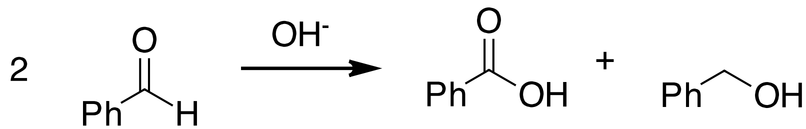
Nucleophilic catalysis via enamine:



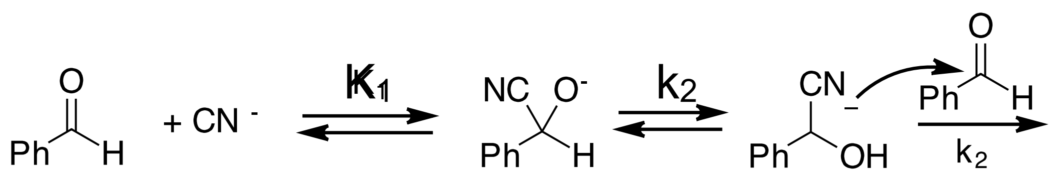
Benzoin Reaction



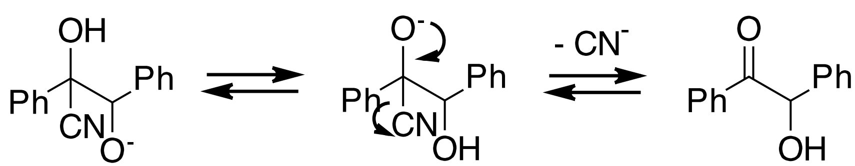
$v = k_{obs}[PhCHO]^2[CN^-]$
nucleophilic catalysis



a different reaction with OH^-



electrophile **nucleophile**

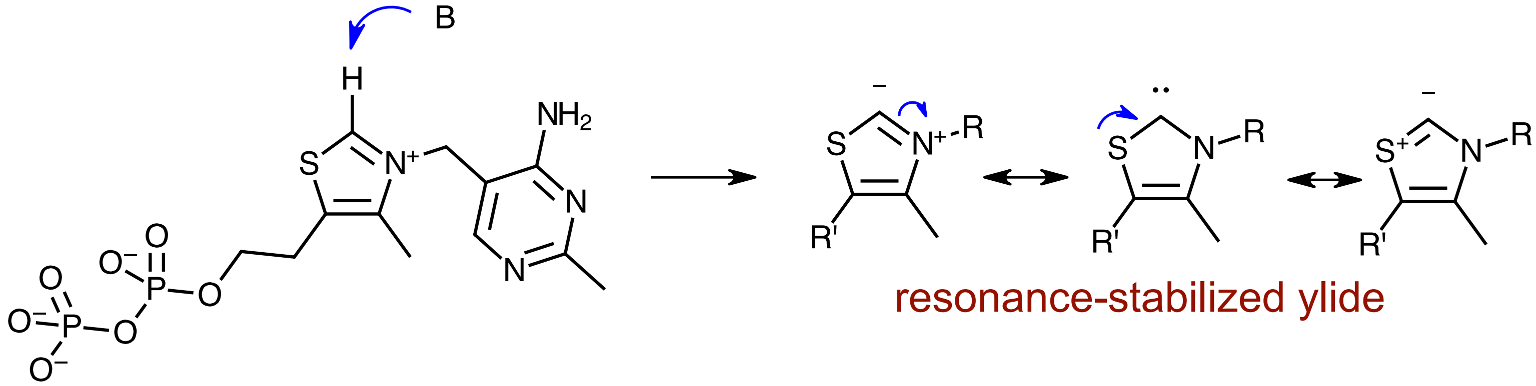


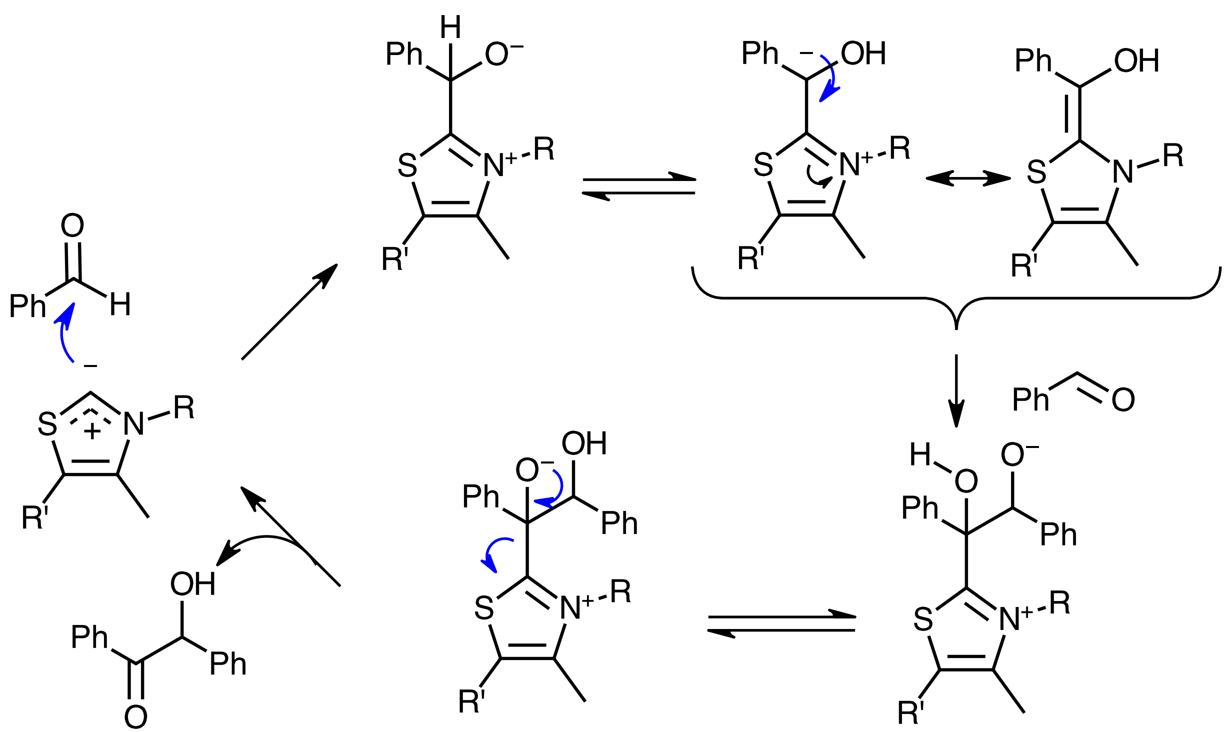
$v = k_2[PhC(OH)CN^-]]PhCHO]$

$[PhC(OH)CN^-] = K_1[PhCHO][CN^-]$

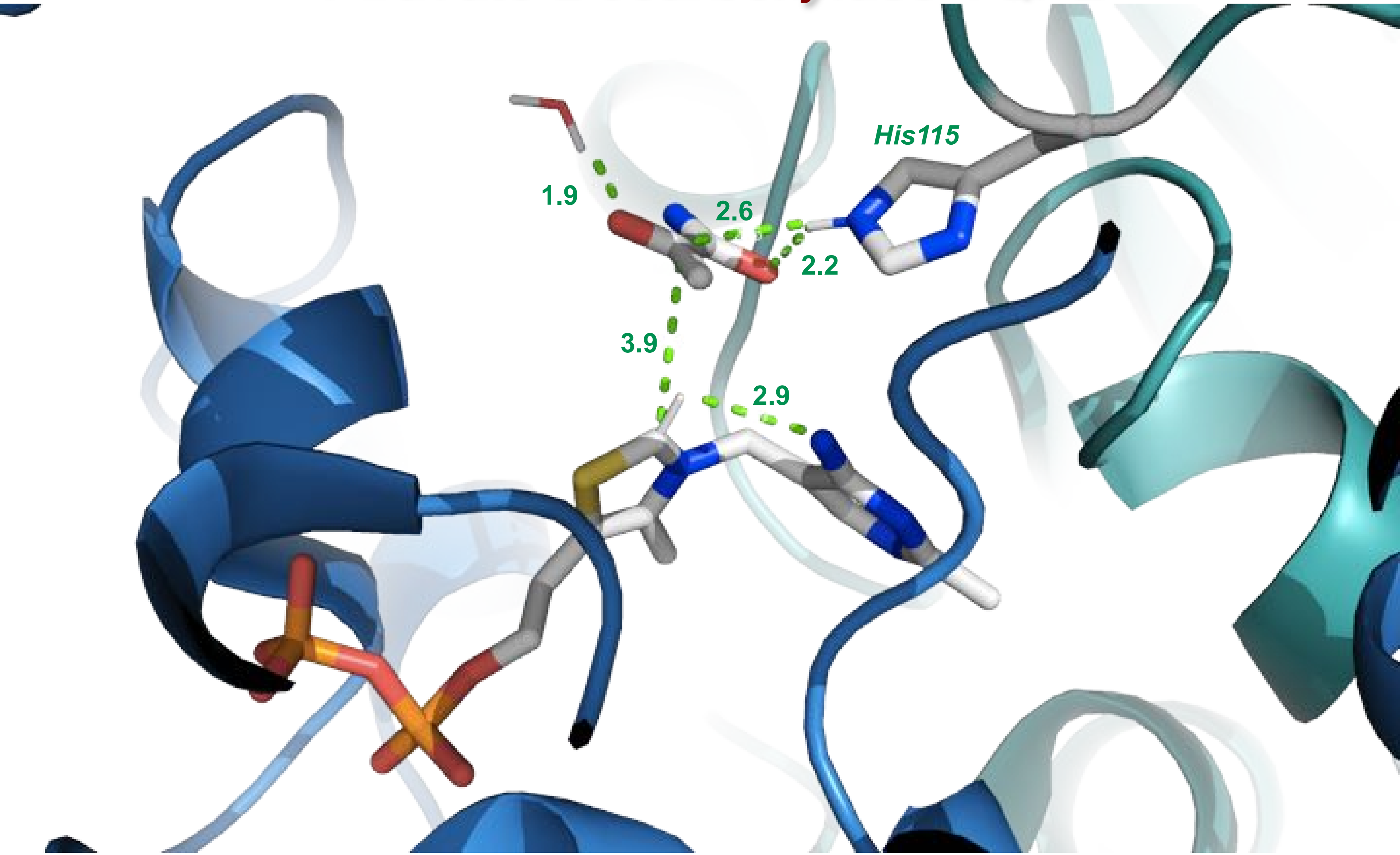
$v = K_1k_2[PhCHO]^2[CN^-]$

Thiamine-Catalyzed Benzoin Reaction

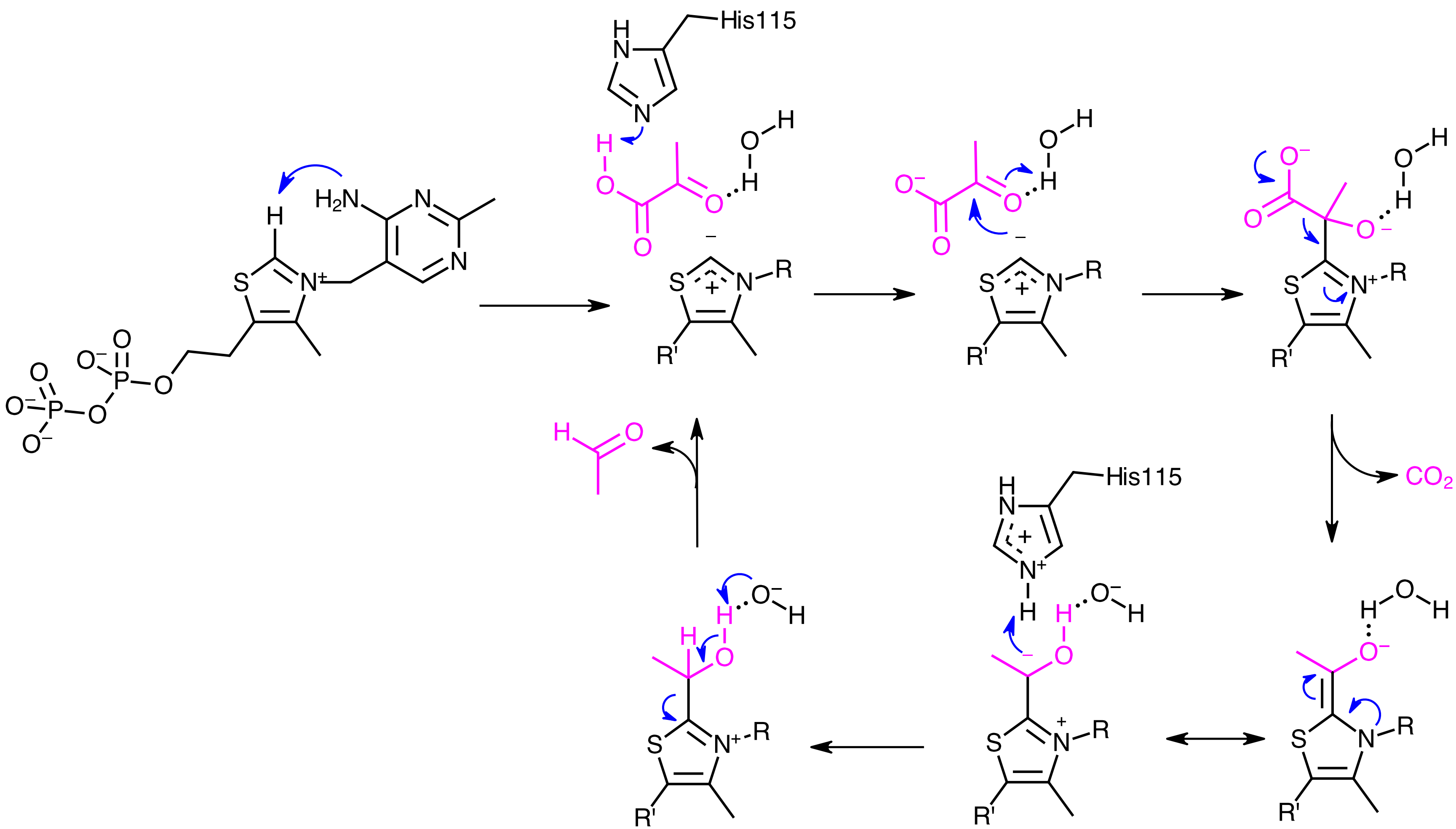




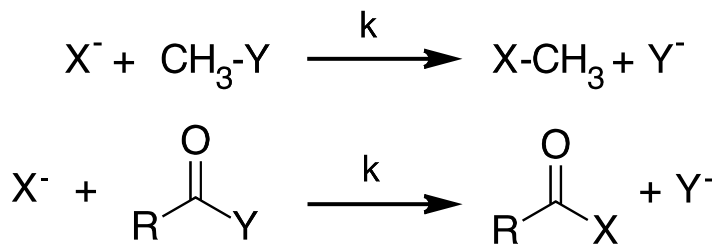
Piruvate Decarboxylase 1QPB



Piruvate Decarboxylase 1QPB



Brønsted Equation for Nucleophiles and Leaving Groups



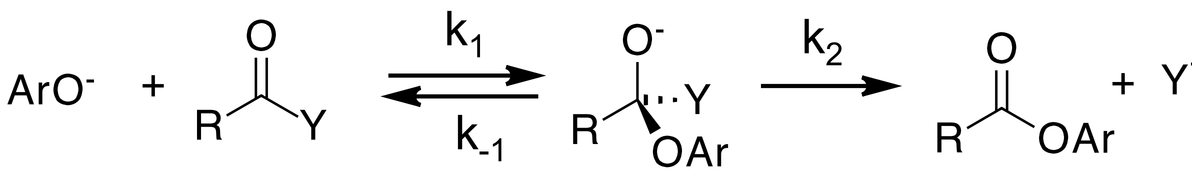
Is there a quantitative relation between nucleophilicity and pKa_{XH} ?

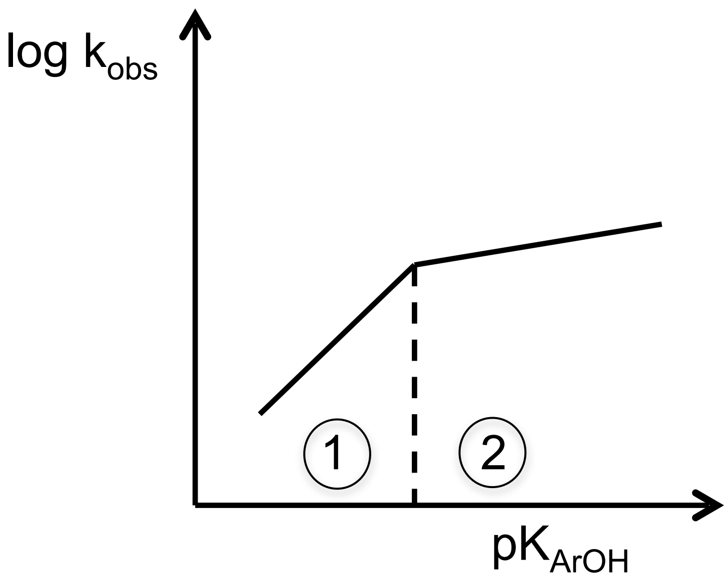
$\log k = \beta_{Nu} pKa_{XH} + \text{cost}$

Is there a quantitative relation between nucleofugality and pKa_{YH} ?

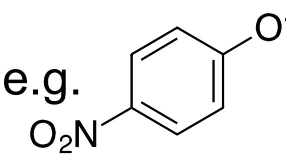
$\log k = -\, \beta_{LG} pKa_{YH} + \text{cost}$

Brønsted Equation for Nucleophiles





1. Poor nucleophiles e.g.

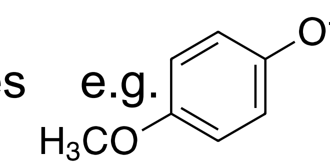


formation of the intermediate is slow:

$v = k_1[ArO^-][RCOY]$

$\log k_1 = \beta_1 pK_{ArOH}$ + cost.

2. Good nucleophiles e.g.



formation of the intermediate is fast:

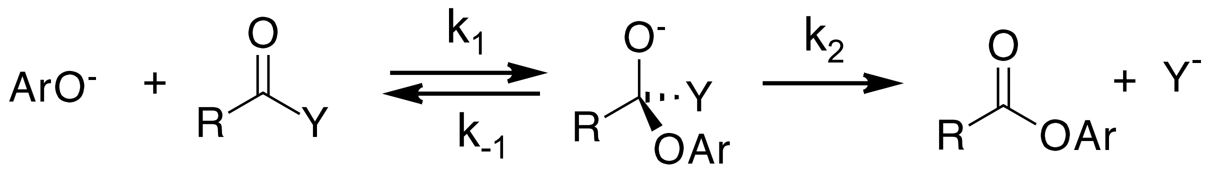
$v = k_{obs}[ArO^-][RCOY]$ $k_{obs} = k_2k_1/k_{-1}$

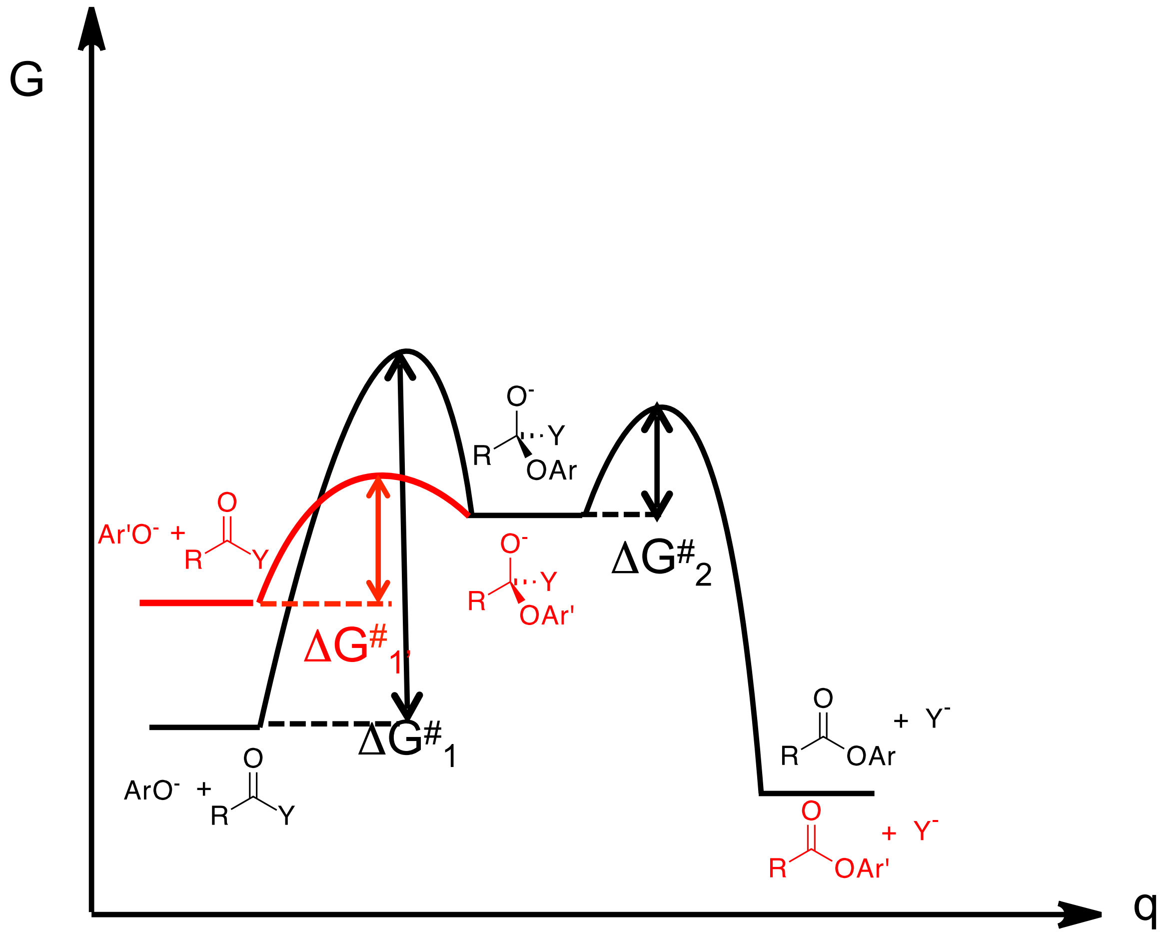
$\log k_{obs} = \beta_{obs} pK_{ArOH}$ + cost.

$\beta_{obs} = \beta_1 - \beta_{-1} + \beta_2$

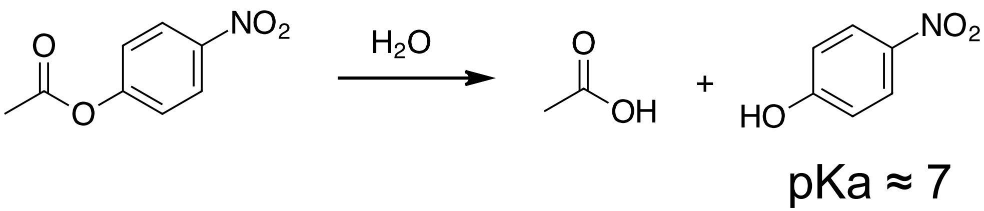
>0 <0 ≈ 0

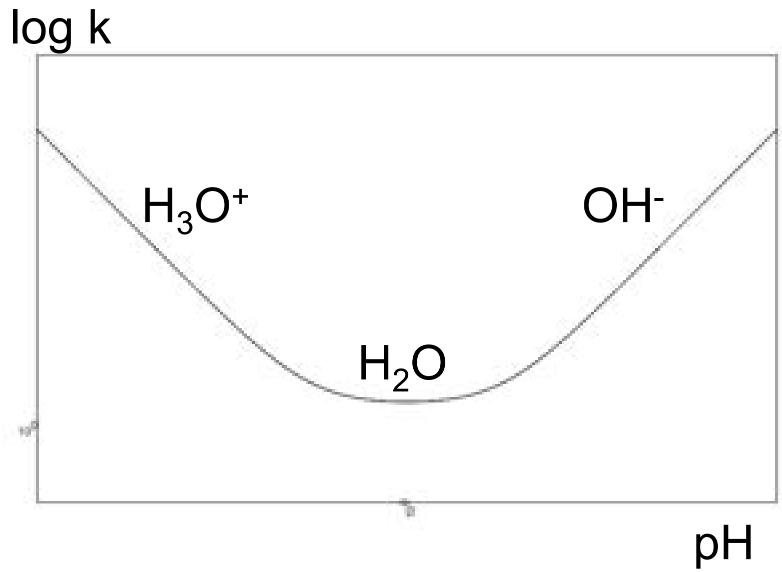
Energy Profile



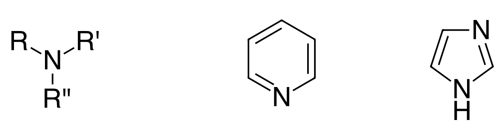


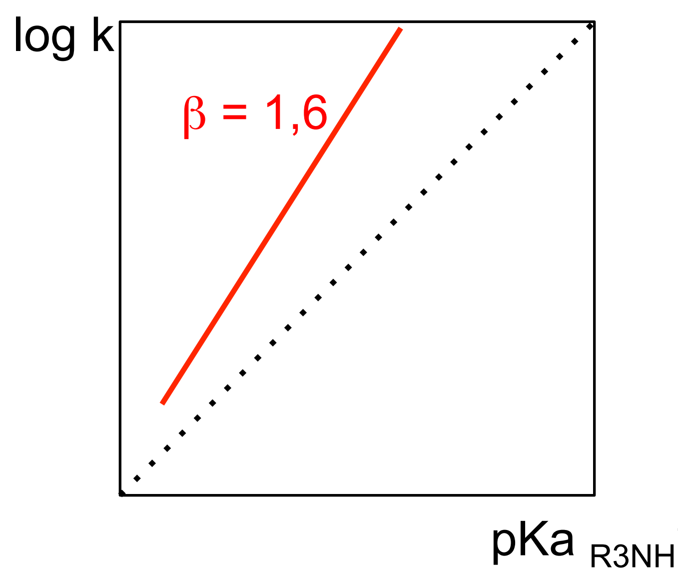
Ester Hydrolysis Catalyzed by Tertiary Amines





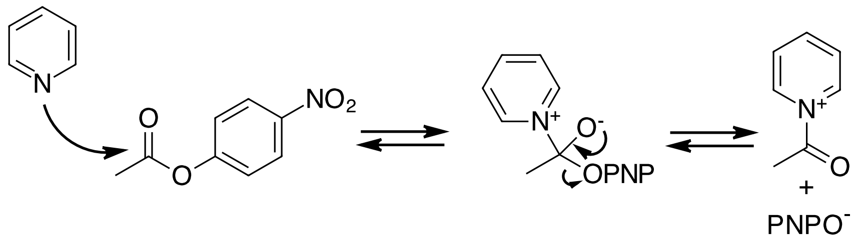
catalyzed by: AcO^- (mechanistic catalysis)
tertiary amines, pyridine, imidazole



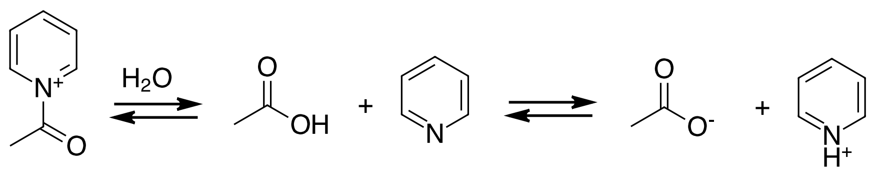


Ester Hydrolysis Catalyzed by Tertiary Amines

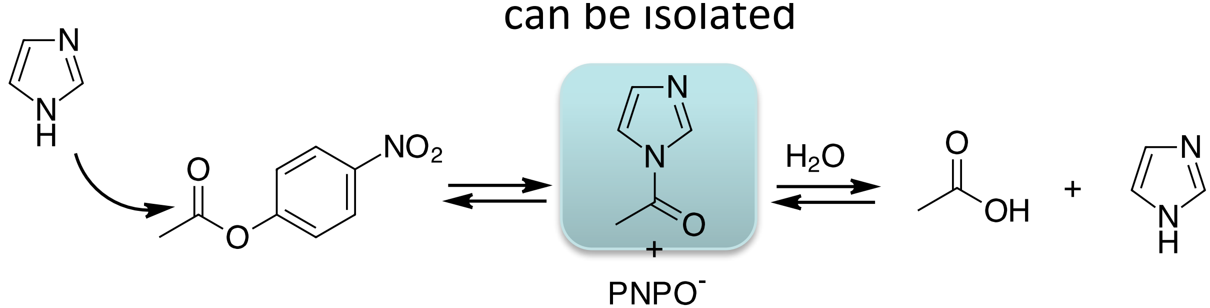
better nucleophile than H_2O



more electrophilic than PNP ester



can be isolated

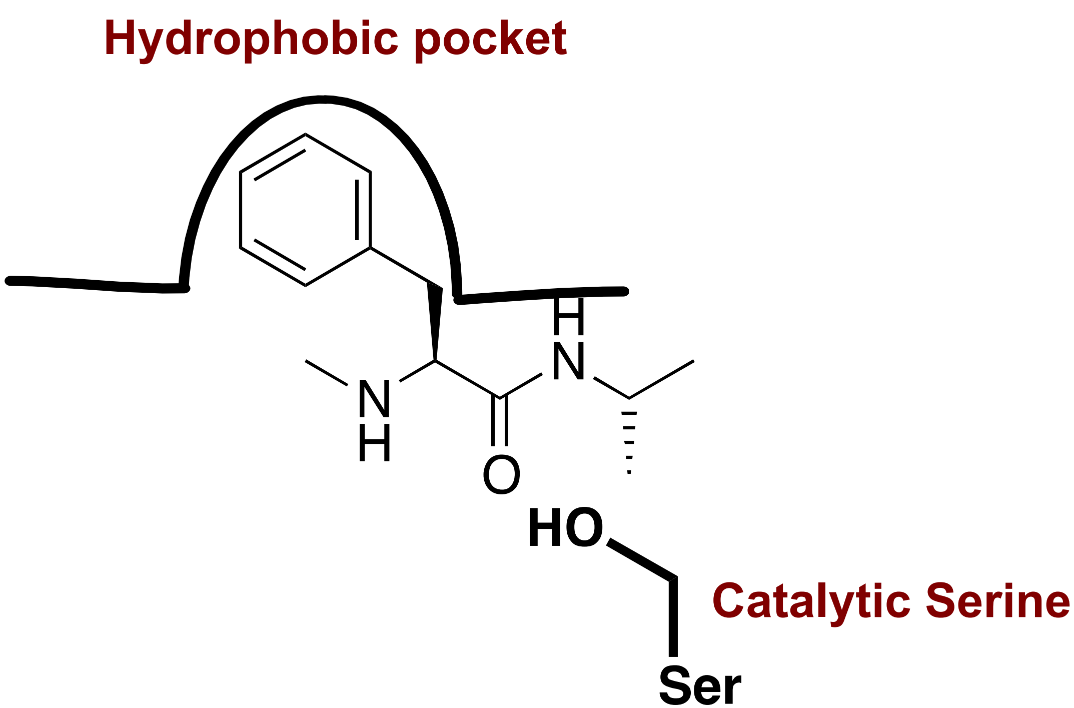


Chymotrypsin

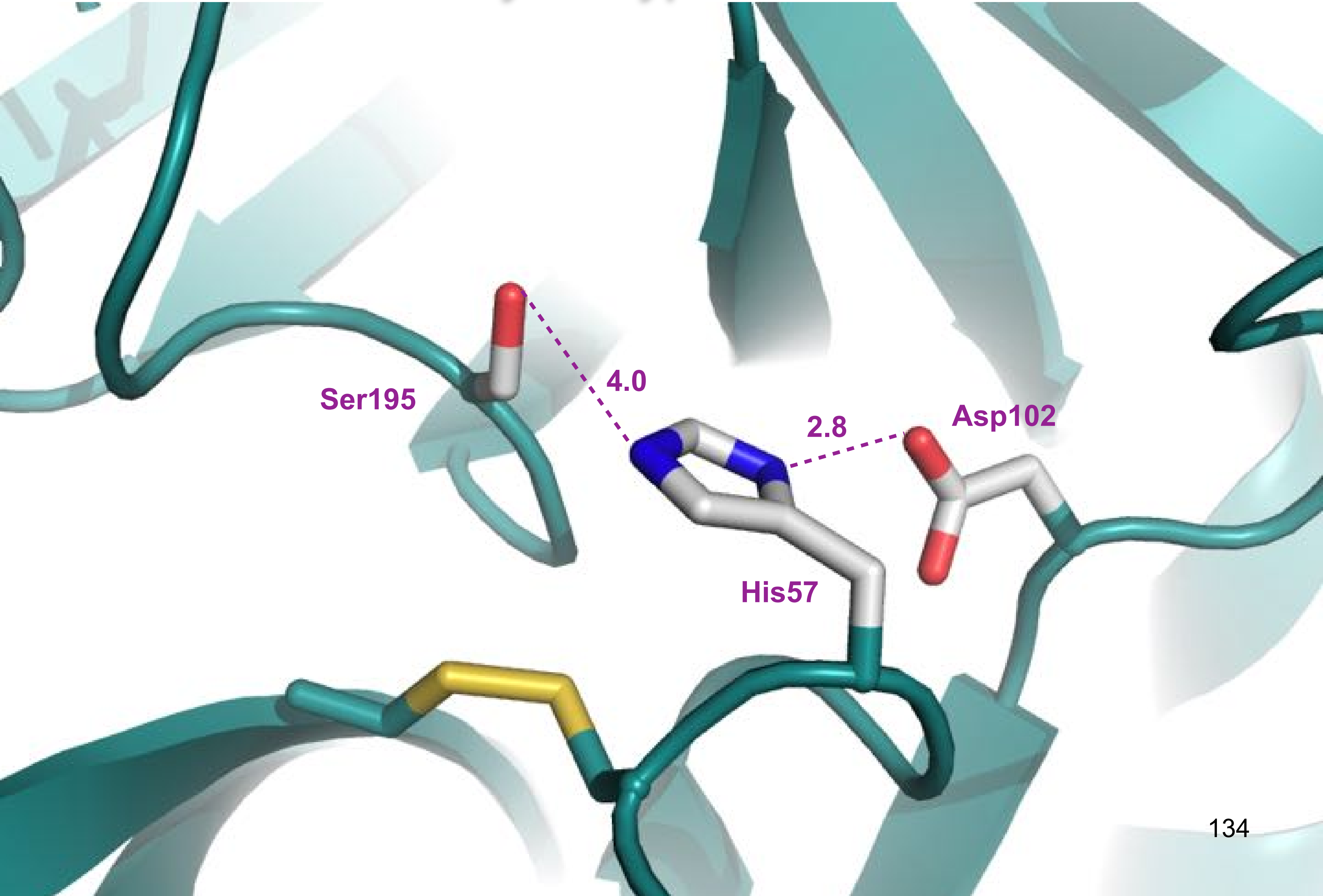
Endoprotease

Specificity: Phe-Xaa, Tyr-Xaa, Trp-Xaa

Mechanism: serine 195 is essential

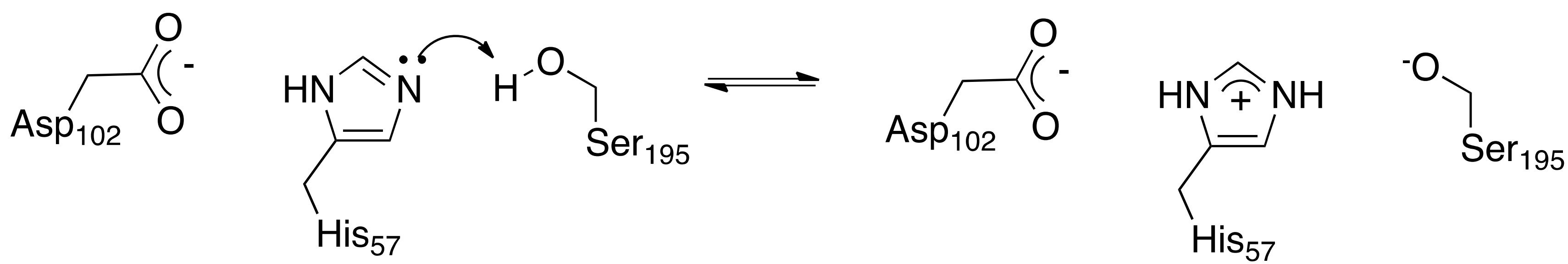


Chymotrypsin



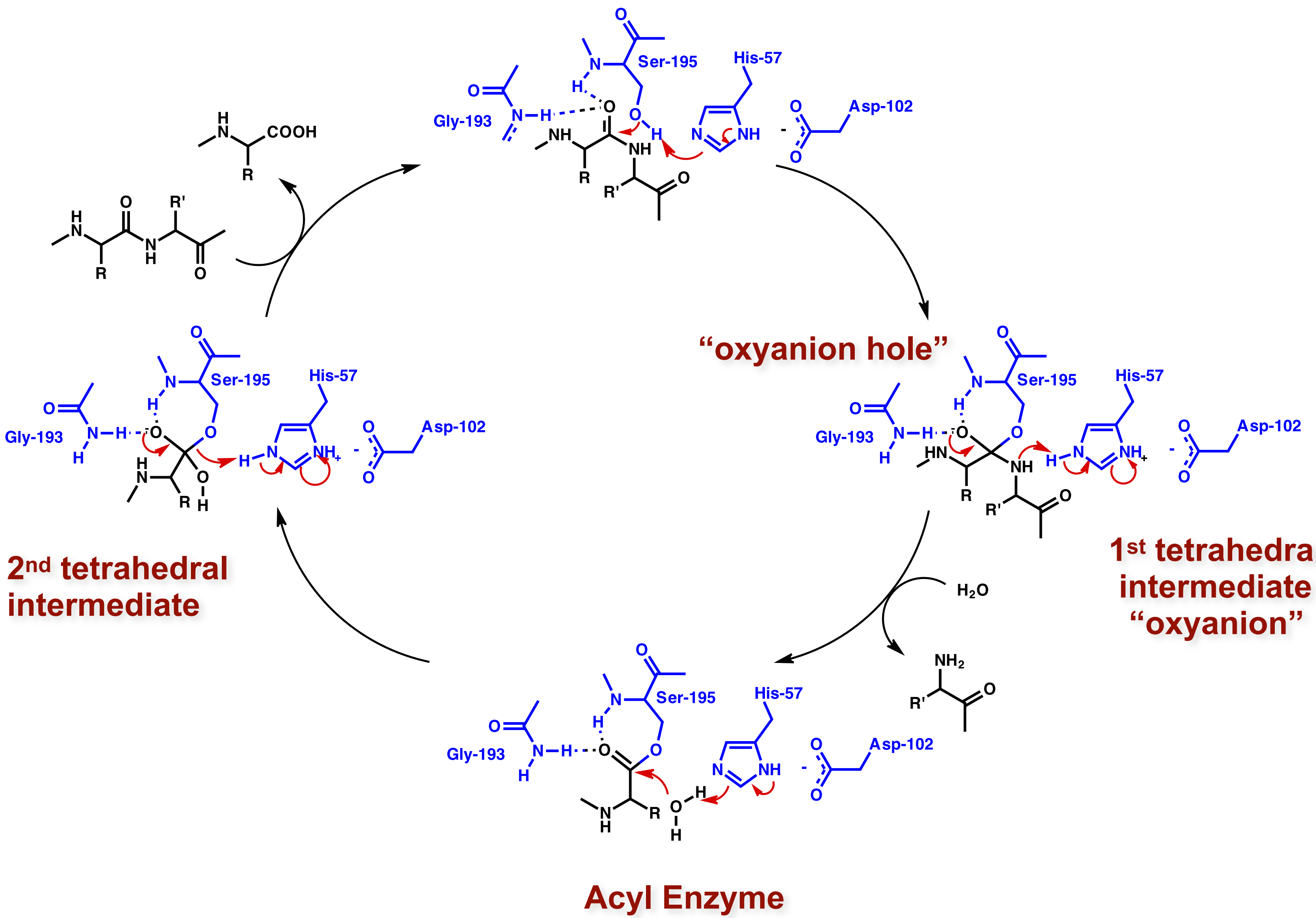
Chymotrypsin: The Catalytic Triad

pKa ≅ 16 pKa ≅ 7



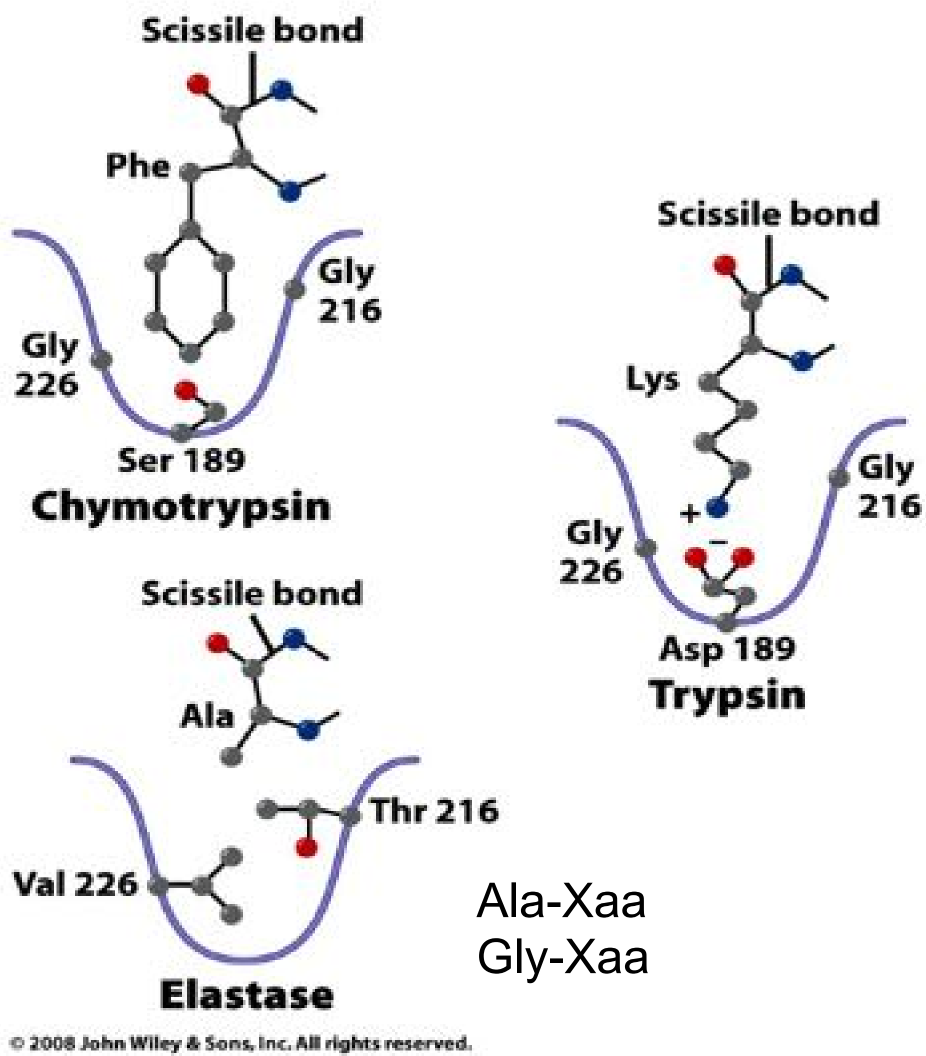
pKa ≅ 16 pKa >> 7

Chymotripsin: Catalytic Mechanism



Serine Proteases: Specificity

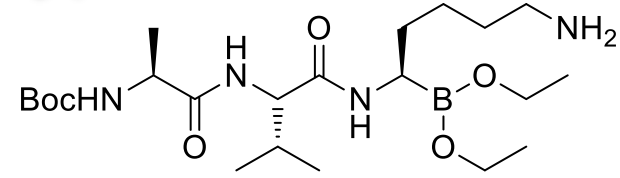
Phe-Xaa
Tyr-Xaa
Trp-Xaa



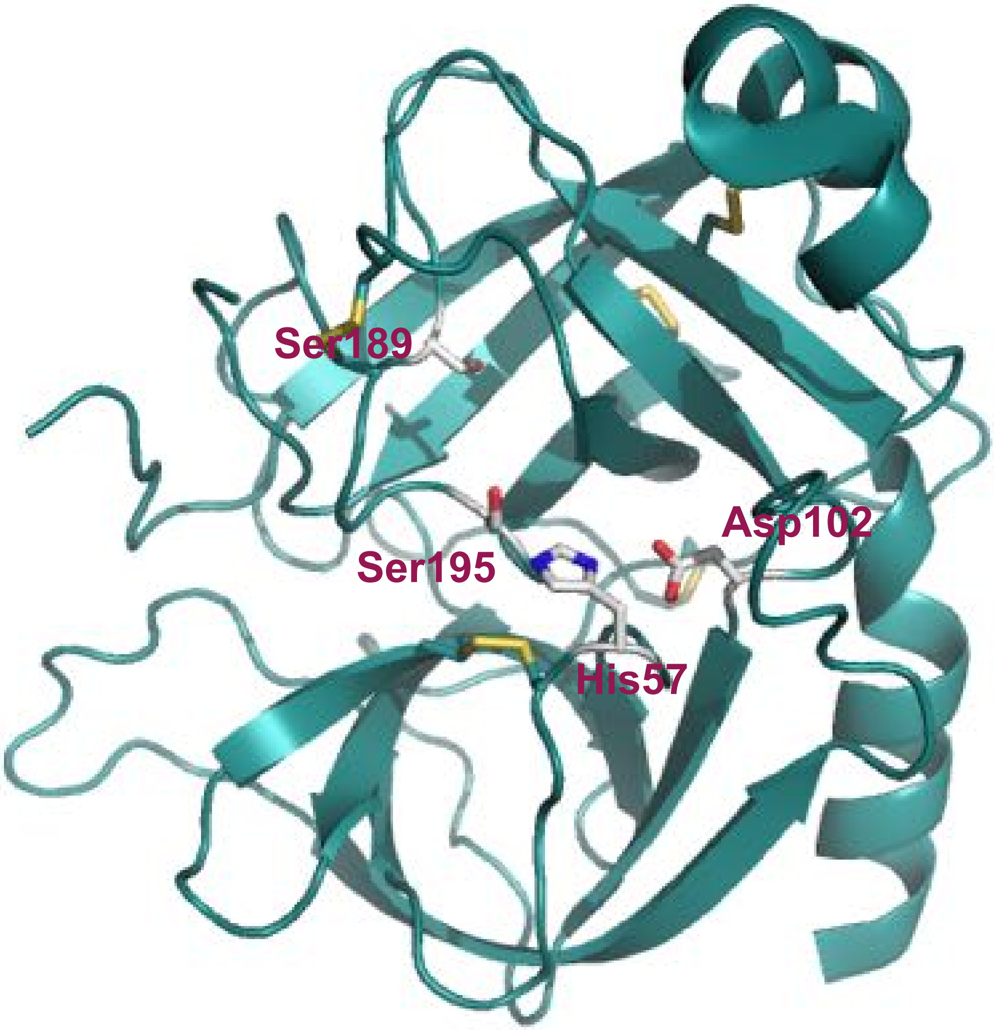
Lys-Xaa
Arg-Xaa

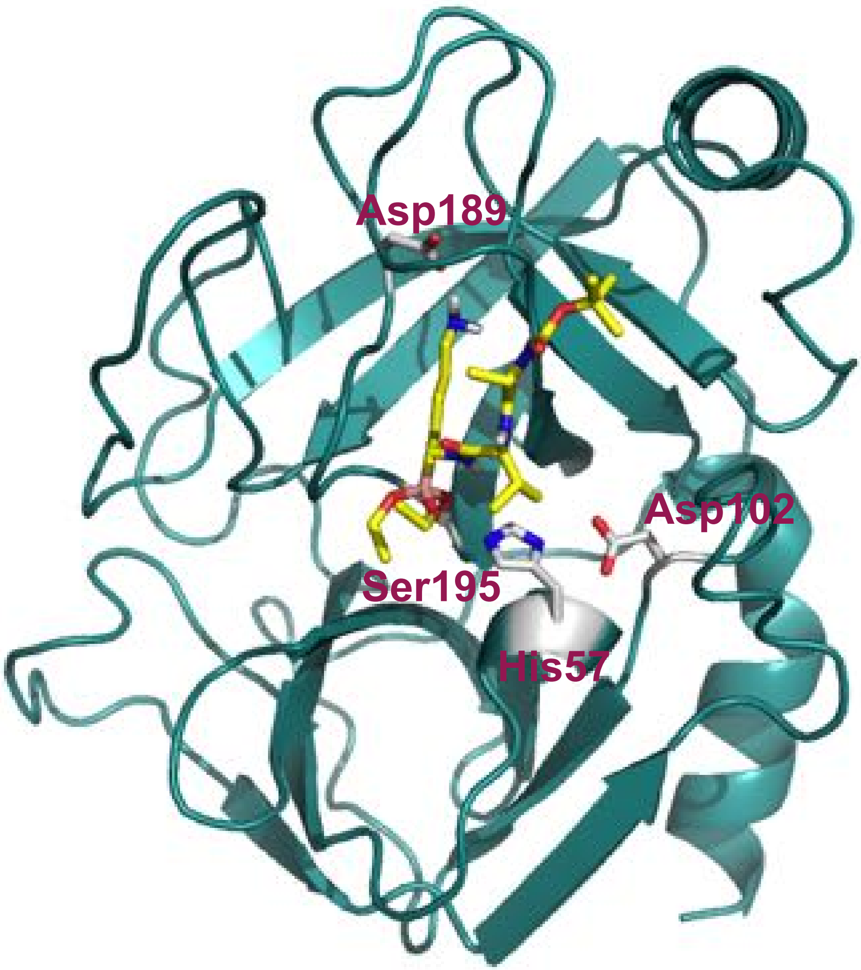
Ala-Xaa
Gly-Xaa

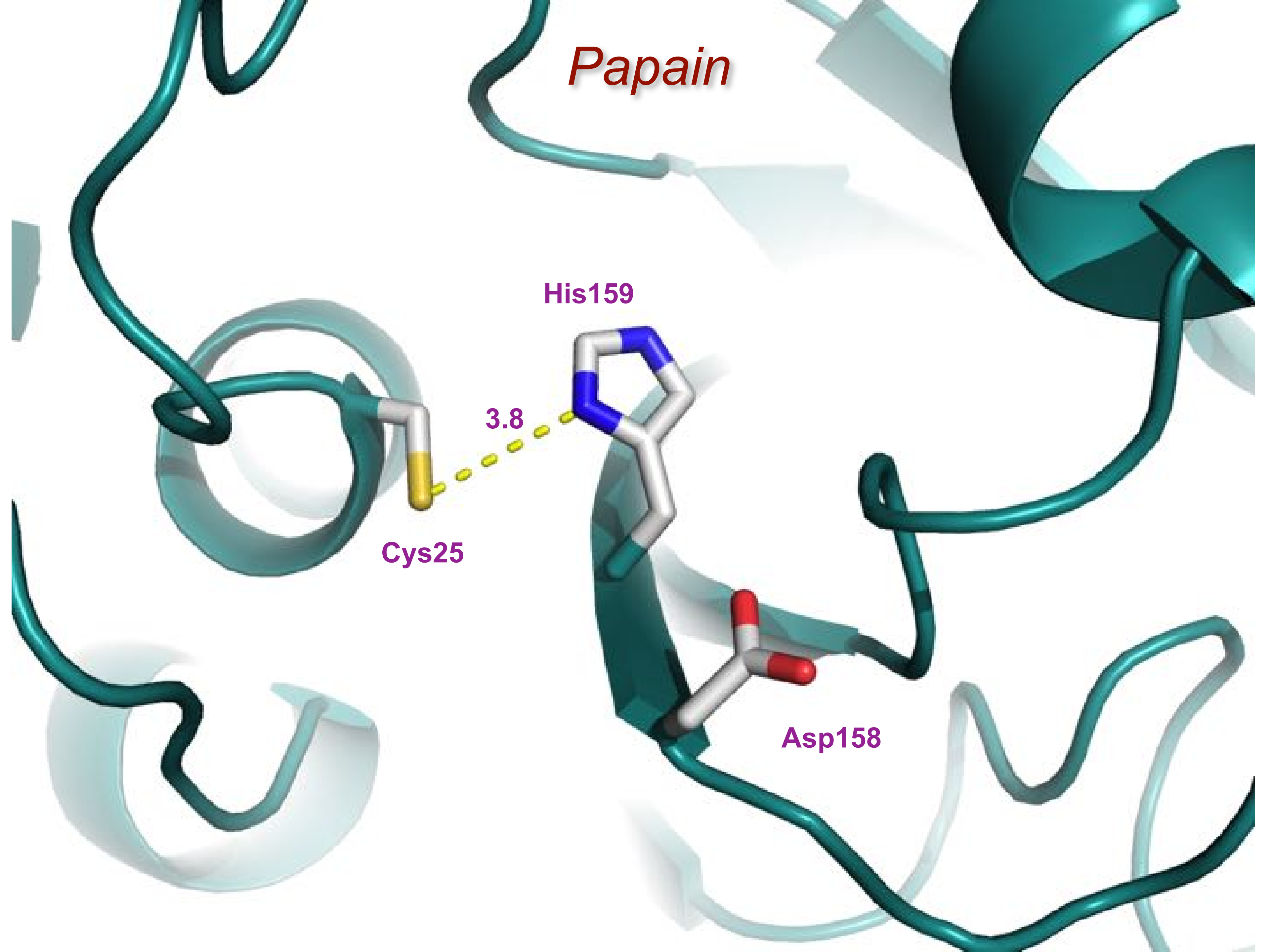
Chymotrypsin and Trypsin



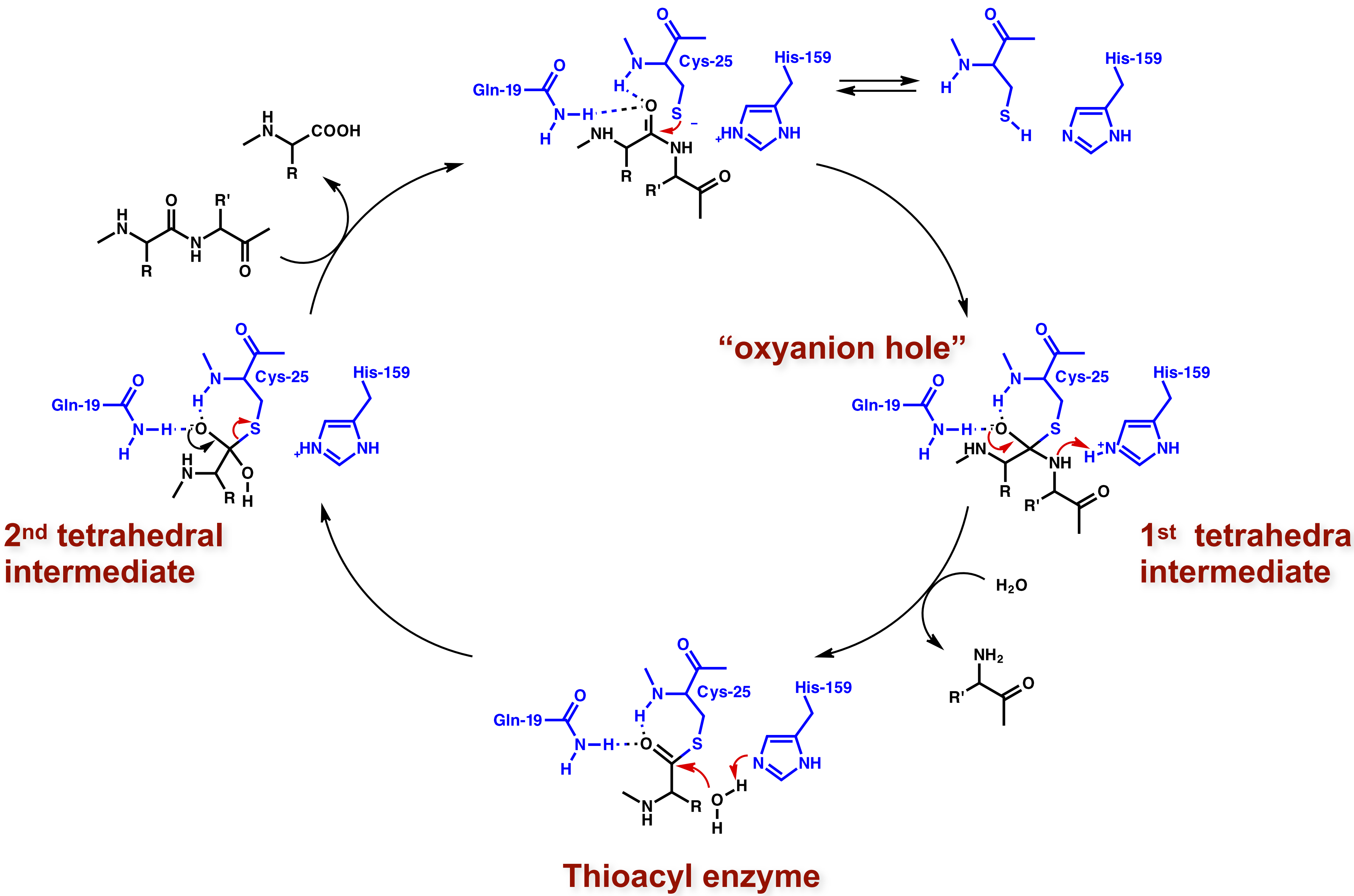
Inhibitor



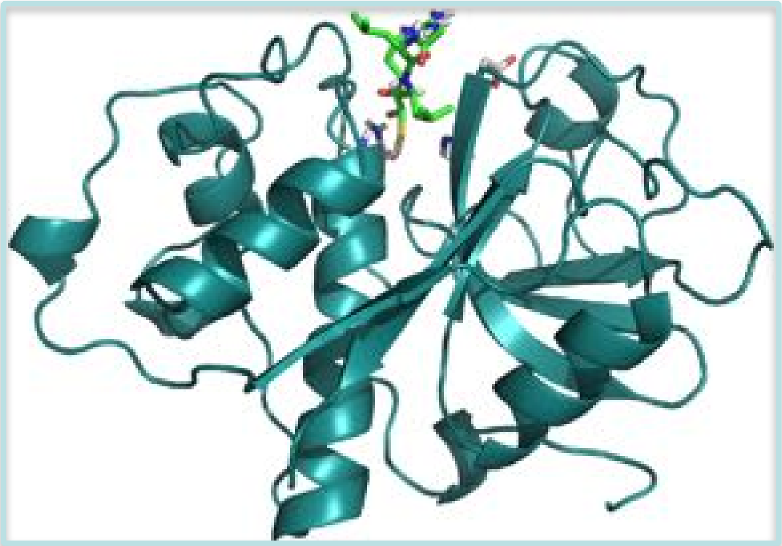


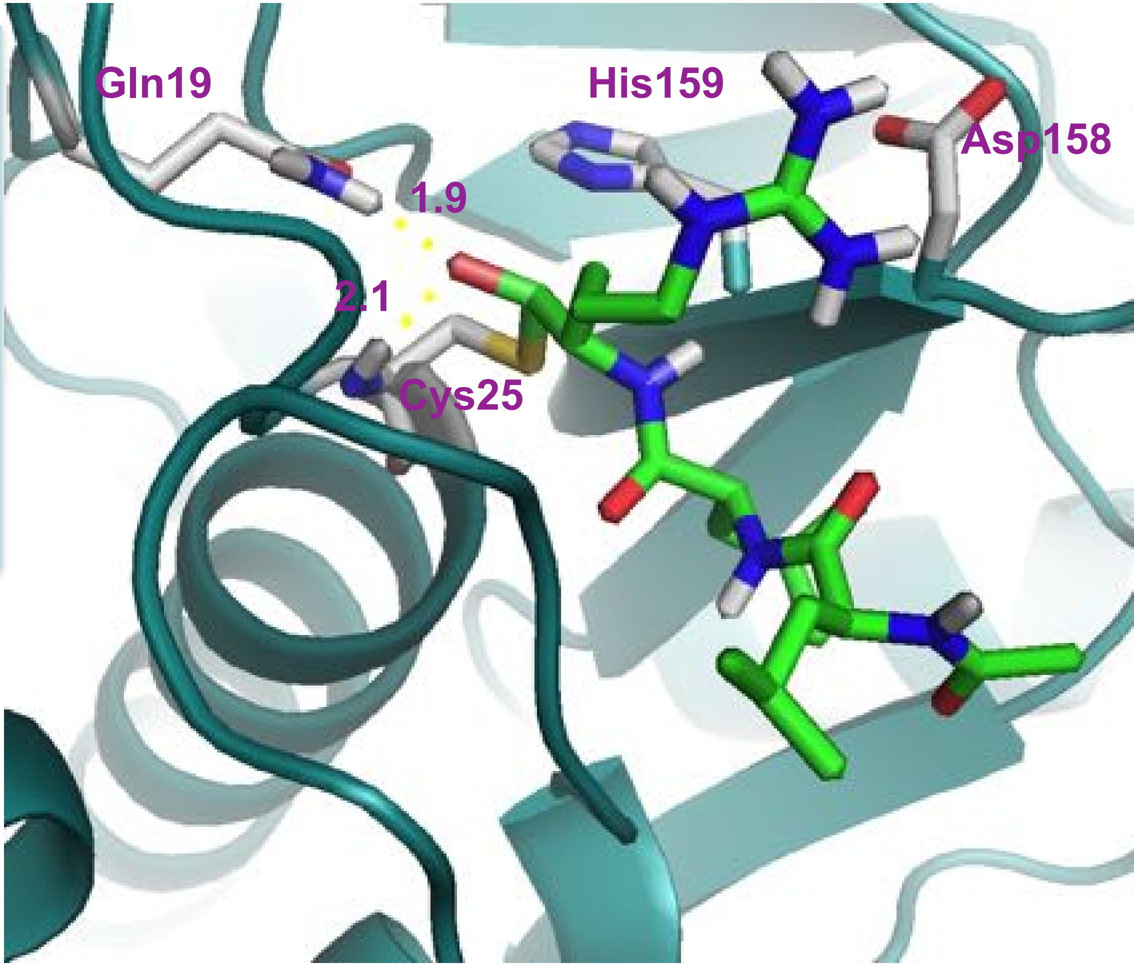

Papain
His159
3.8
Cys25
Asp158

Papain – Catalytic Mechanism

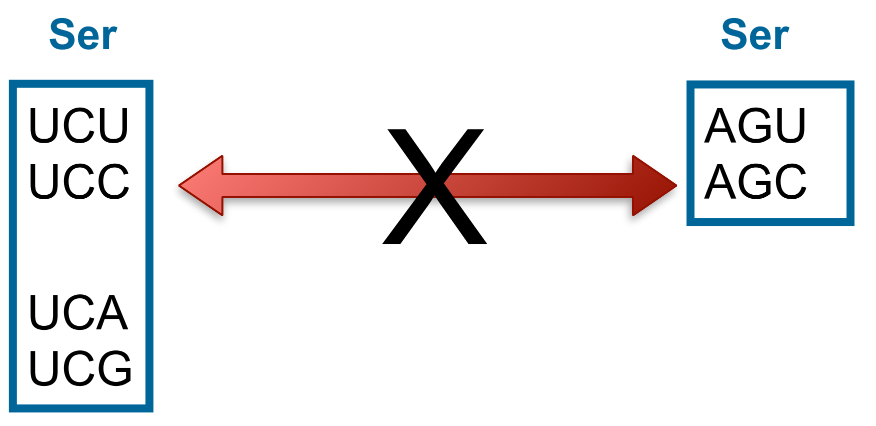


Papain: Acyl Enzyme



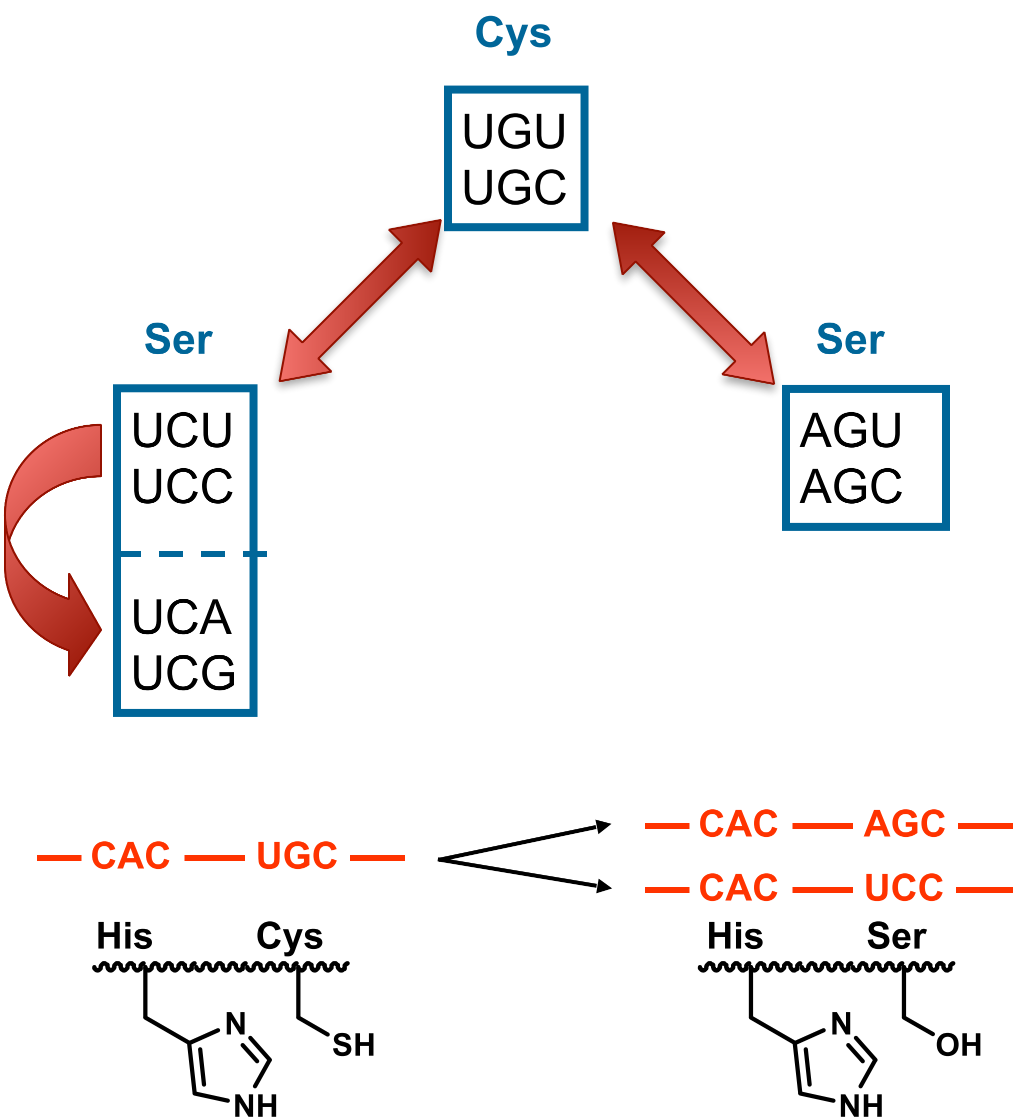


Evolution of Serine and Cystein Proteases



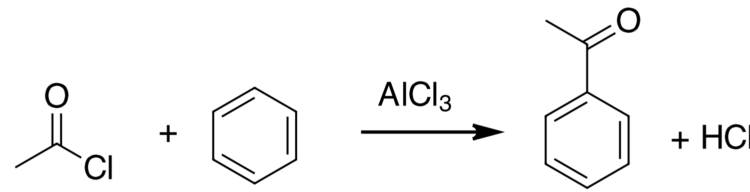
Two families of serine proteases evolutionally distant

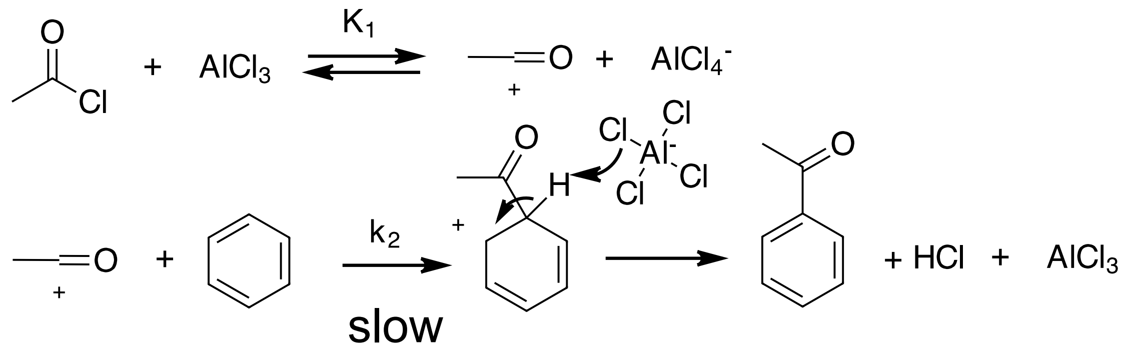
Evolution of Serine and Cystein Proteases



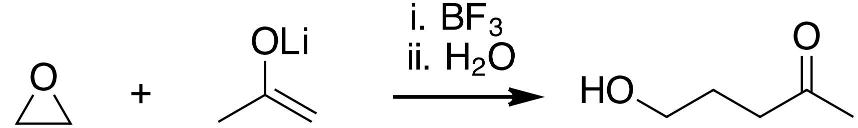
Electrophilic Catalysis

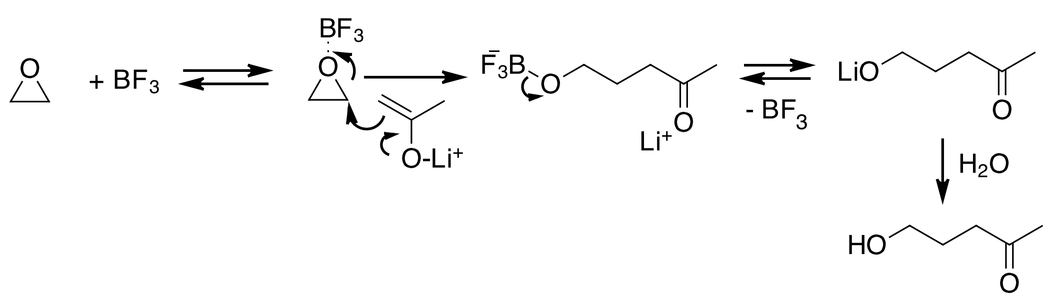
Friedel-Crafts acylation



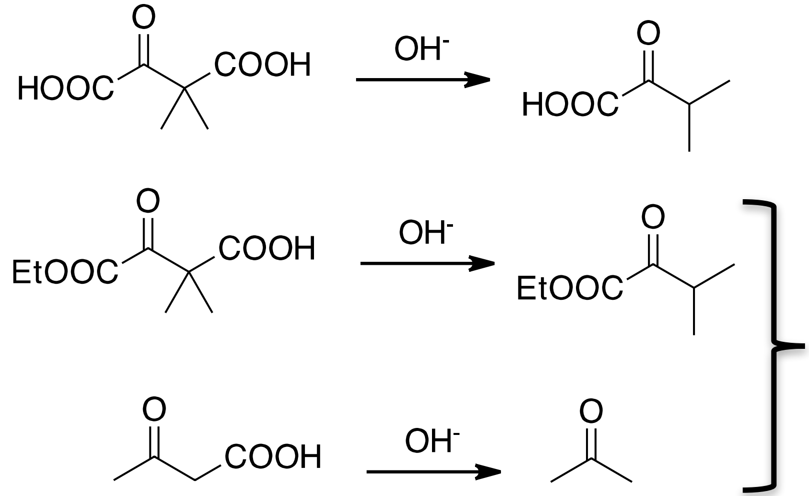


Epoxide ring opening



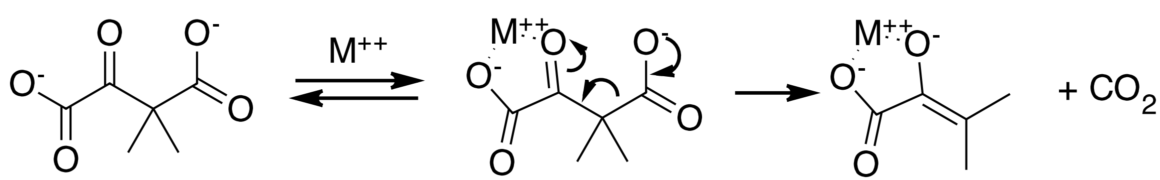


Decarboxylation of Dimethyloxalacetic Acid



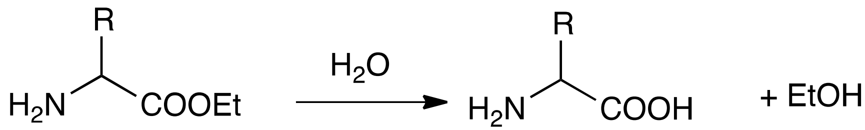
catalyzed by metal ions (Al^{3+}, Cu^{2+})

not catalyzed by metal ions

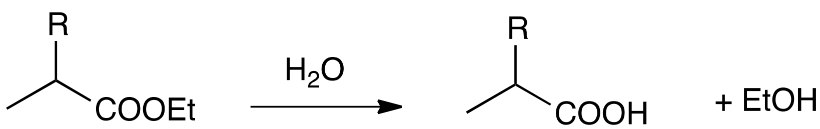


catalysis by metal chelation

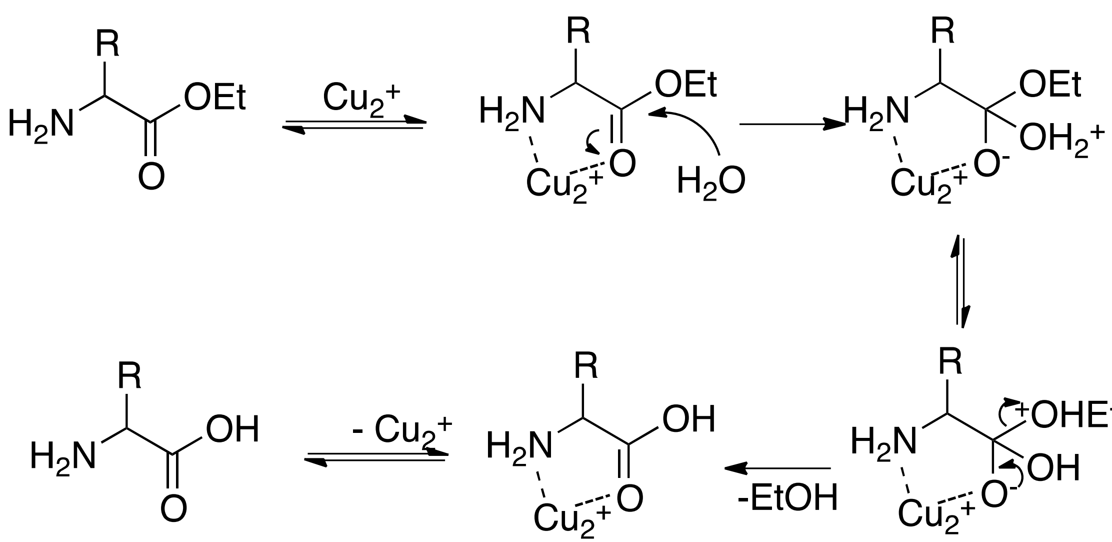
Hydrolysis of Aminoesters



catalyzed by Cu^{2+}

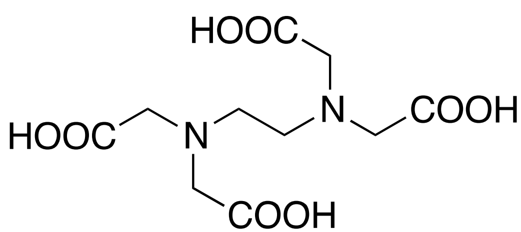


not catalyzed by Cu^{2+}

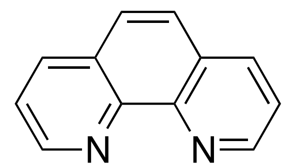


Metal Proteases

- Contain a Zn(II) ion
- Inactivated by chelators sequestering the metal ion



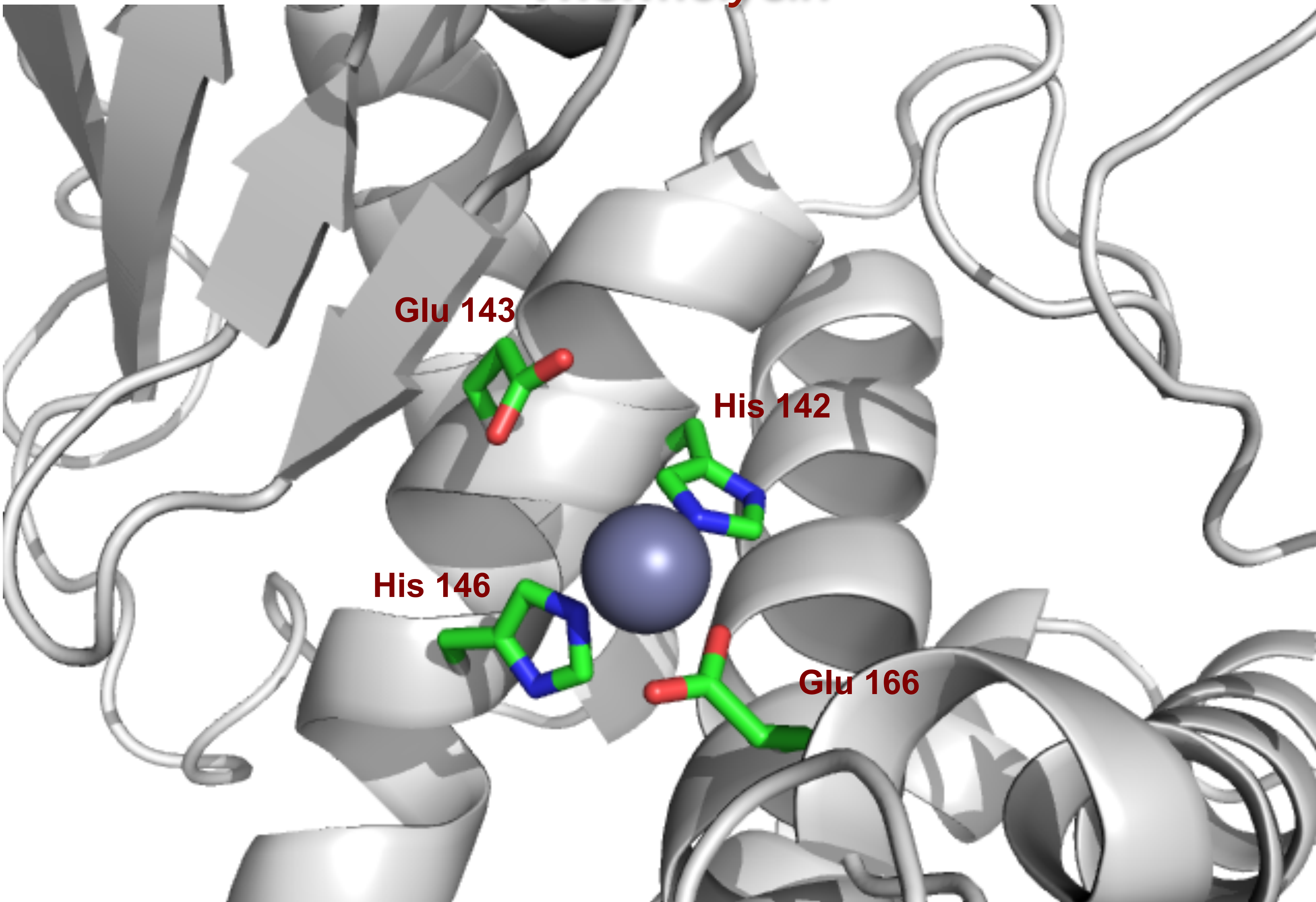
EDTA



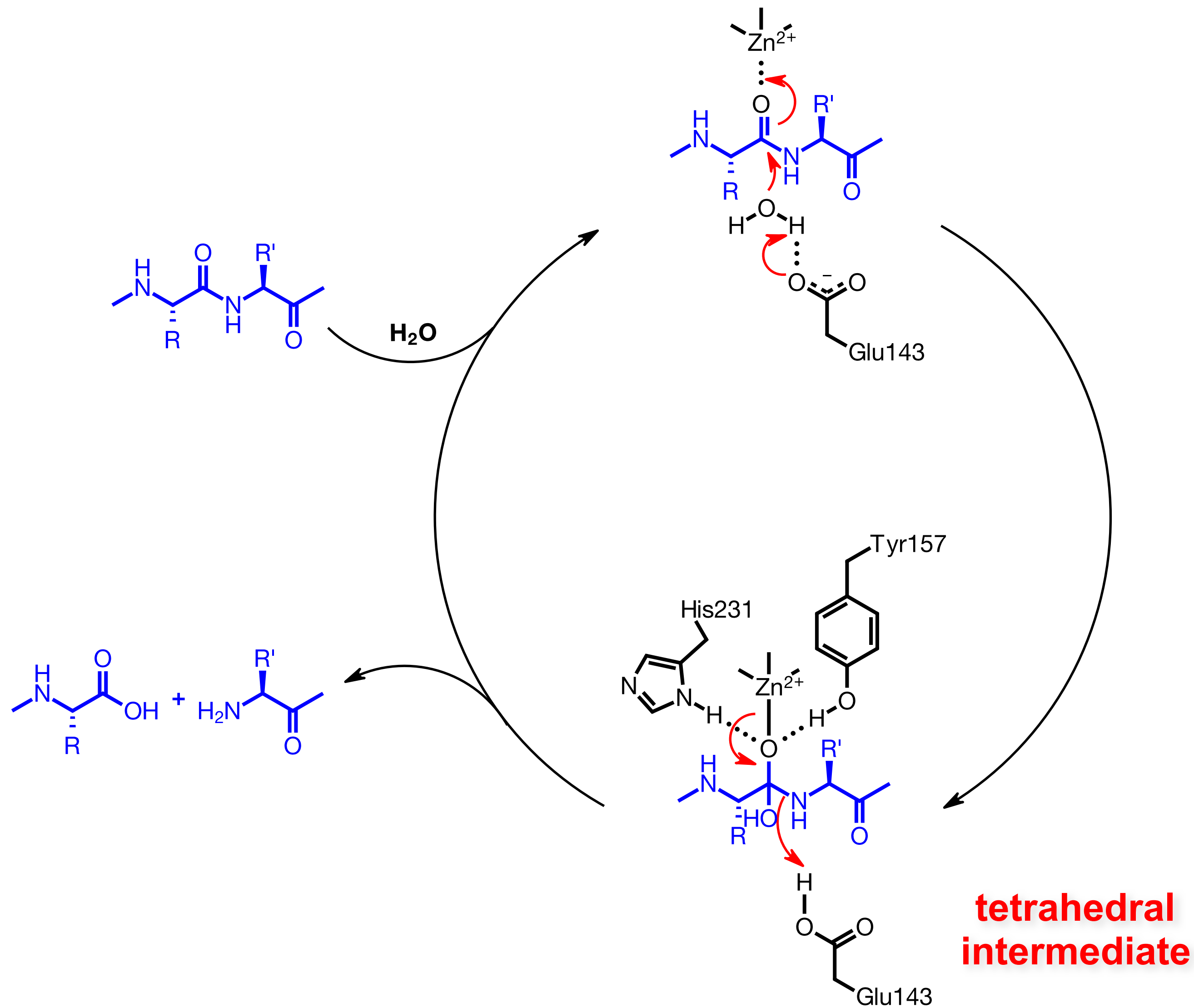
1,10-fenantrolin

- Thermolysin (endopeptidase)
- Carboxypeptidase A (exopeptidase)
- Similar catalytic site architecture
- Different mechanism

Thermolysin

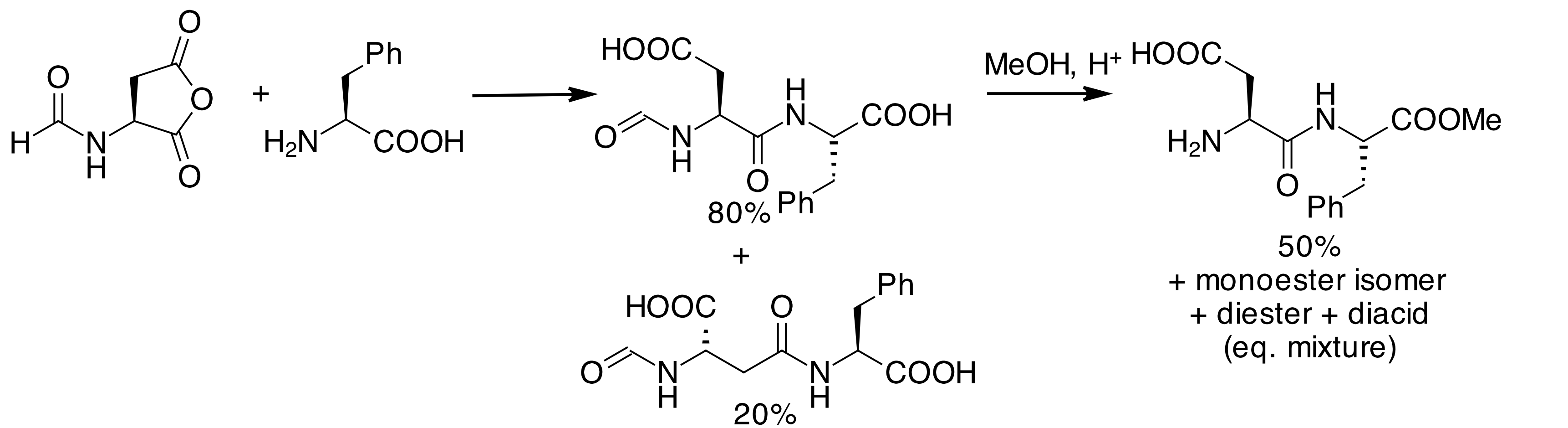


Thermolysin - Catalytic Mechanism

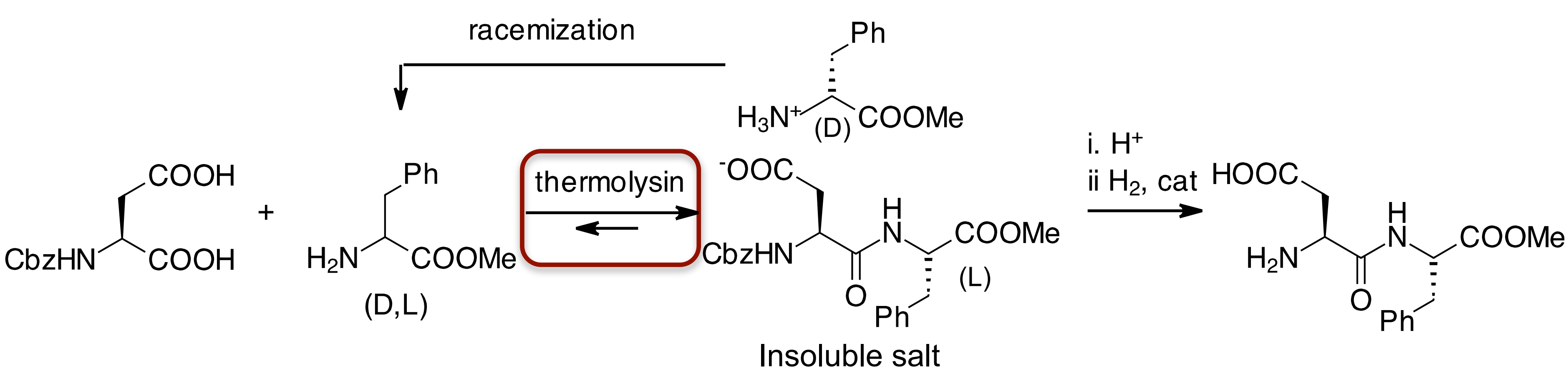


Thermolysin: Industrial Synthesis of Aspartame

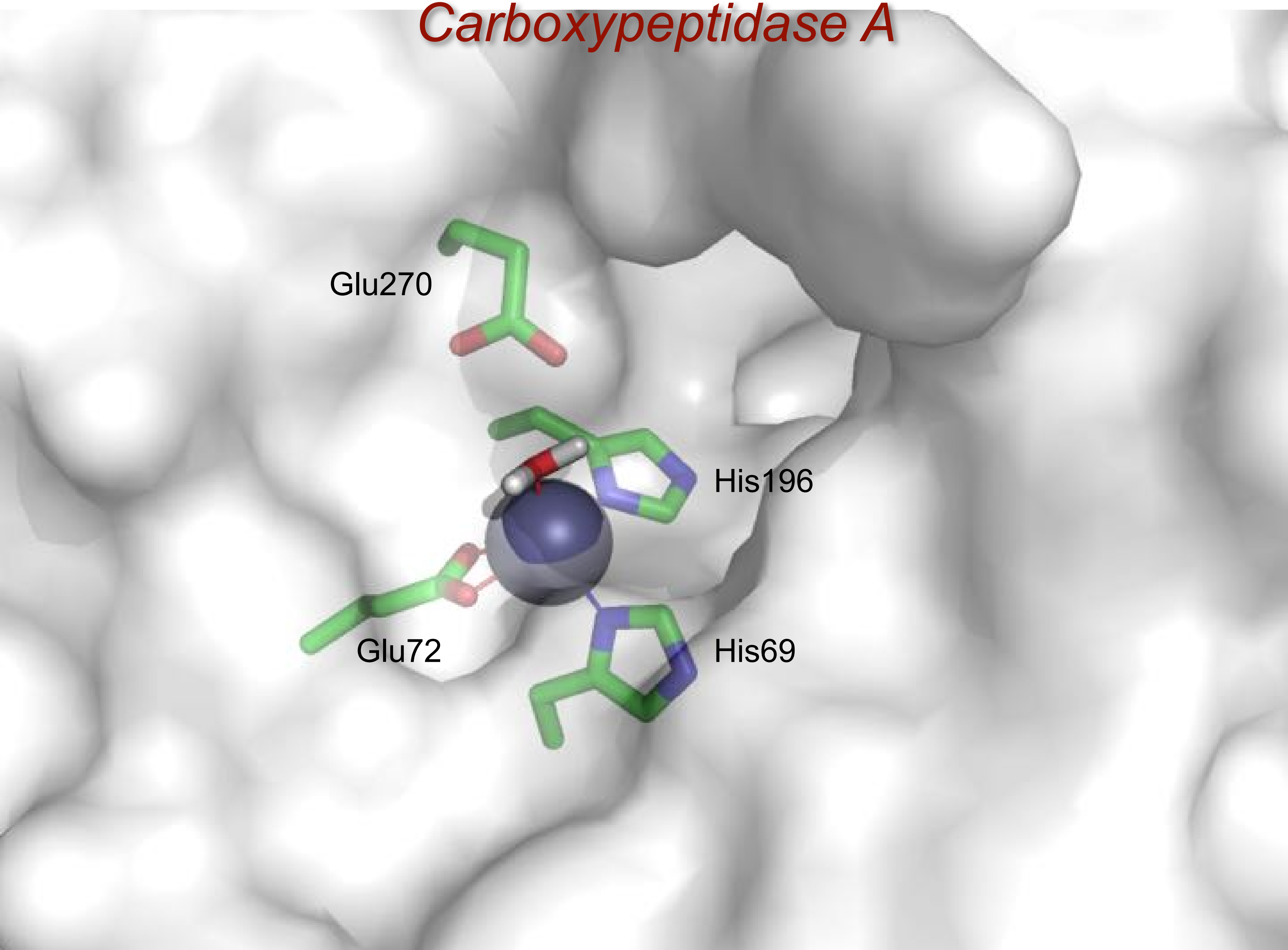
NutraSweet/Ajinomoto "Formyl" process



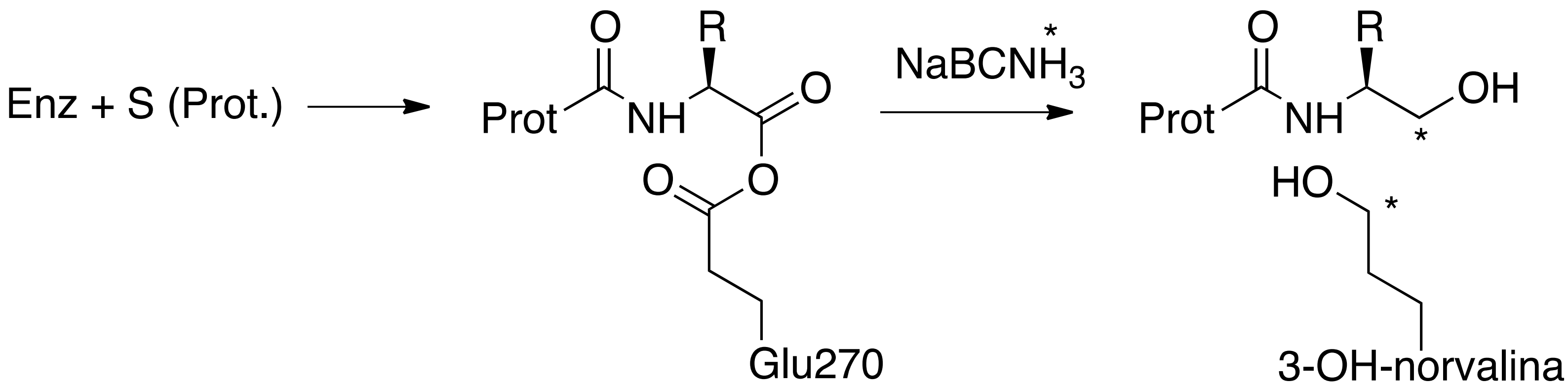
DSM/Tosoh Synthesis chemoenzymatic process



Carboxypeptidase A

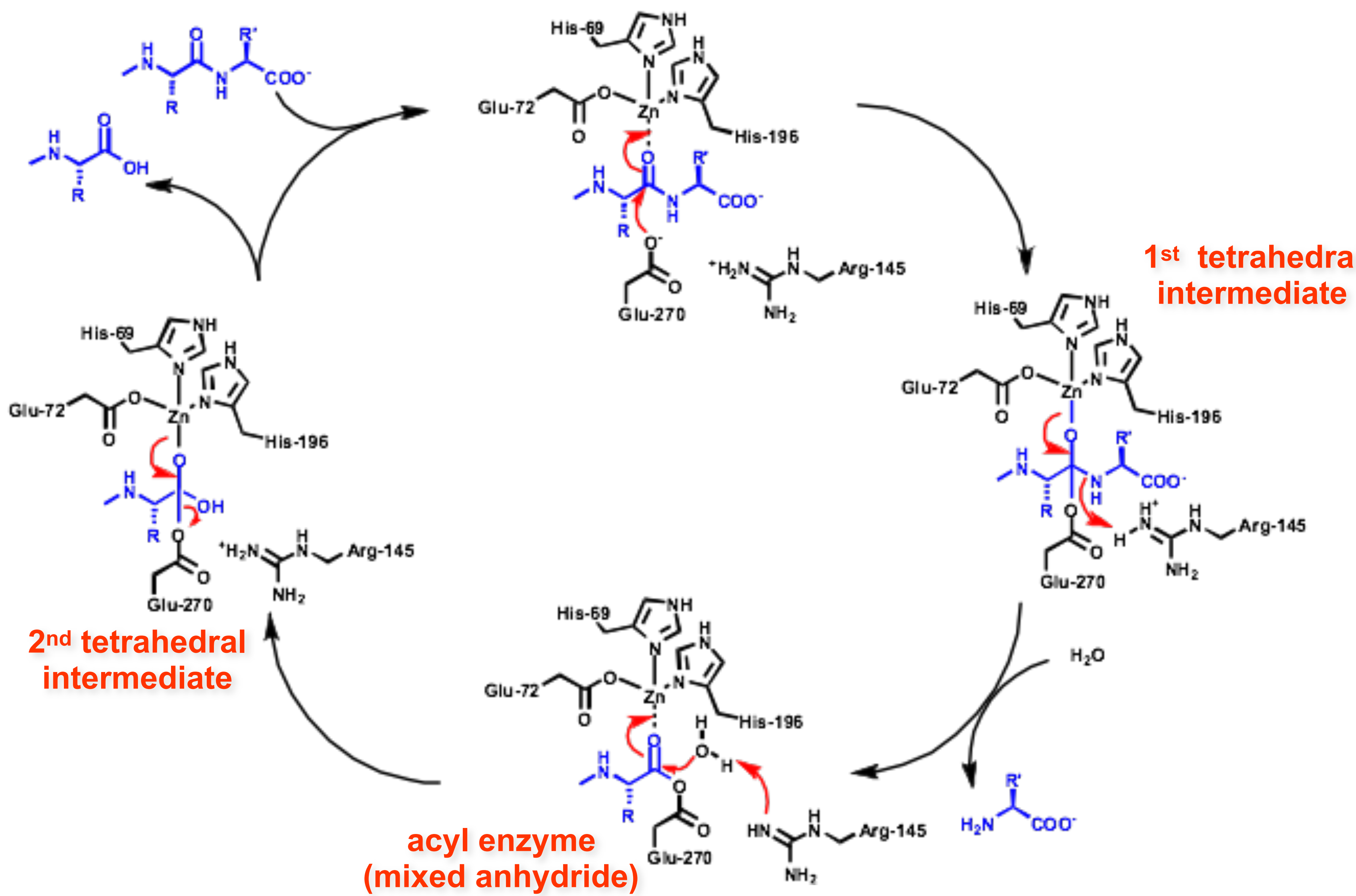


Carboxypeptidase A – Catalytic Mechanism



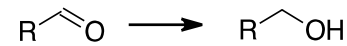
$NaCNBH_3$ does not reduce carboxylic acids, but reduces anhydrides

Carboxypeptidase A – Catalytic Mechanism

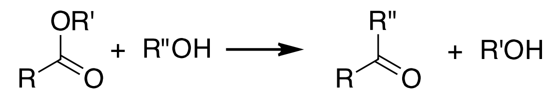


Classification of Enzymes

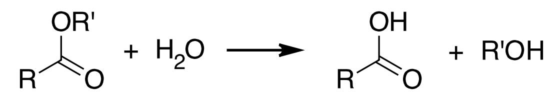
1. Oxidoreductases



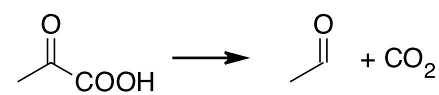
2. Transferases



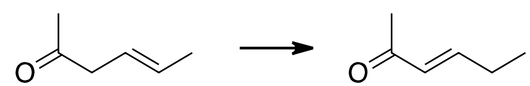
3. Hydrolases



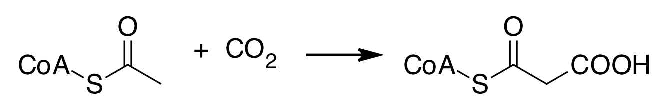
4. Lyases



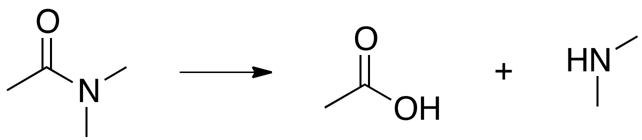
5. Isomerases



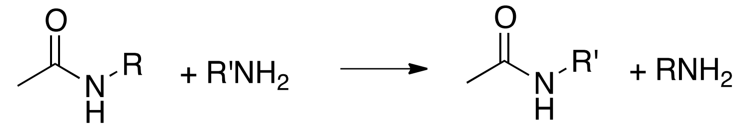
6. Ligases



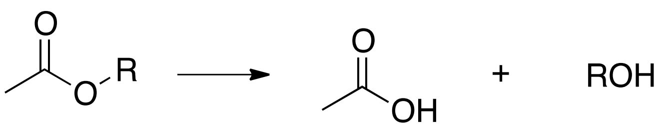
Hydrolysis and Transfer Reactions



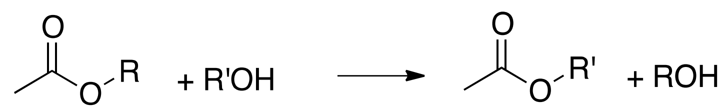
Amidases (Proteases, Peptidases)



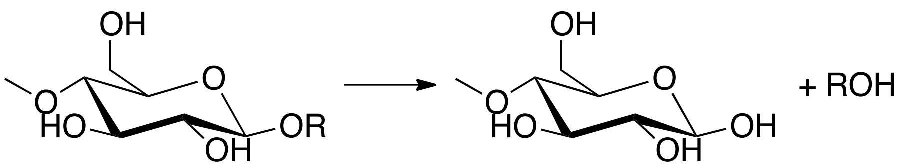
Transpeptidases



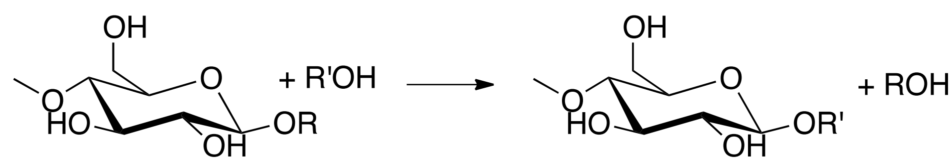
Esterases (Lipases)



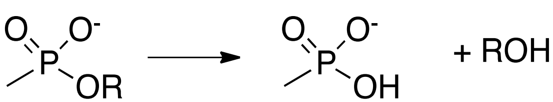
Acyltransferases



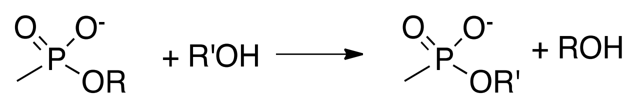
Glycosidases



Glycosyltransferases



Phosphatase (Phosphoesterases, Nucleases)



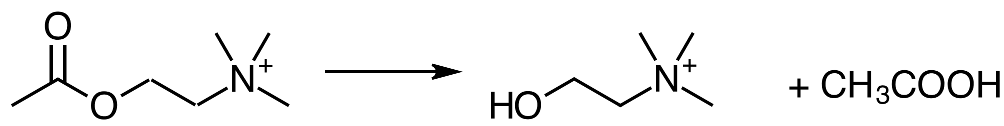
Phosphotransferases (Kinases)

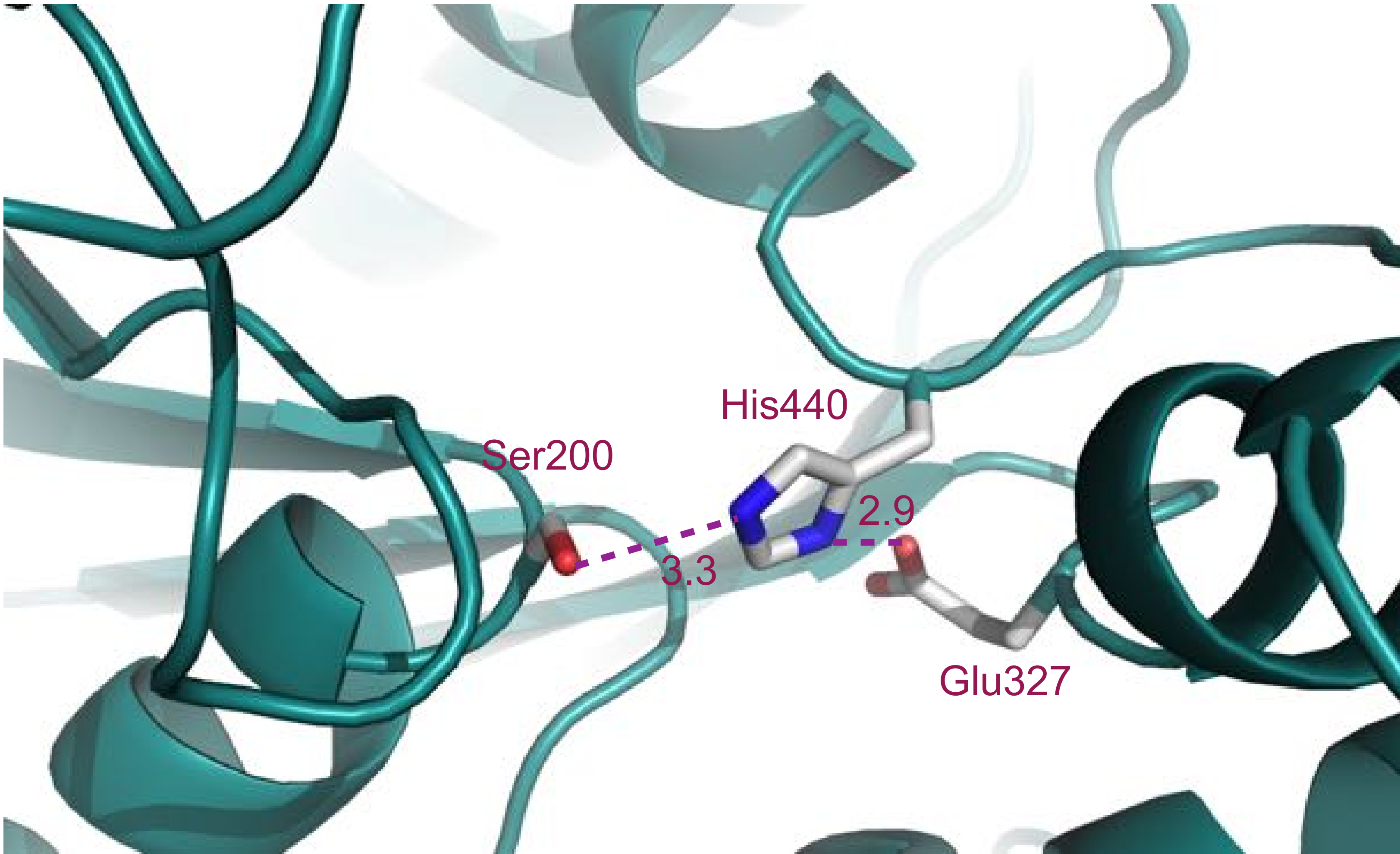
Esterases and Lipases

- Common mechanism.
- Catalytic triad: Asp(Glu)/His/Ser
- Nucleophilic Catalysis (covalent)
- Similar Binding site architecture to serine proteases

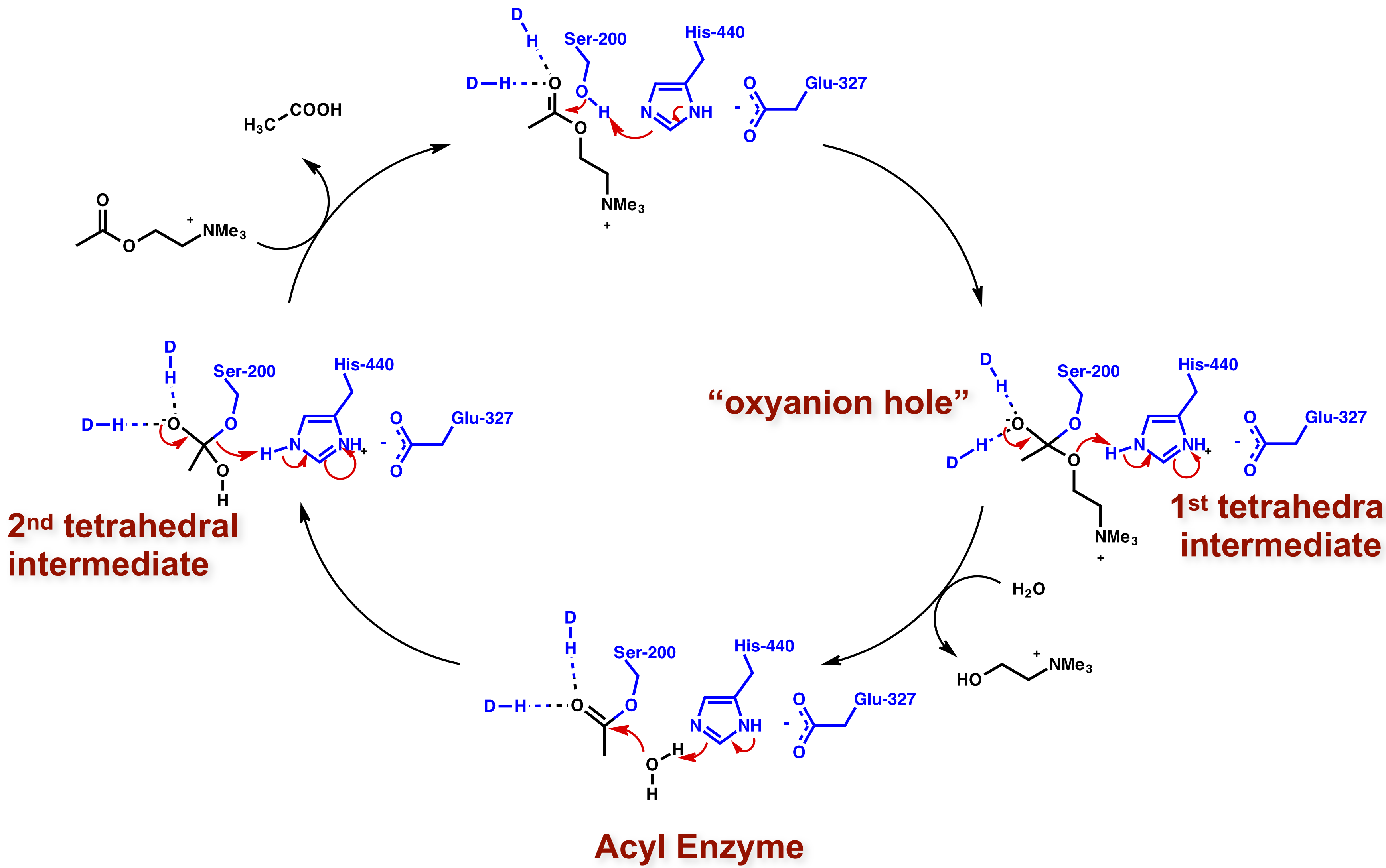
- Esterases: hydrolyze small, water soluble esters
- Lipases: involved in the degradation of fatty acids
  hydrolyze water insoluble triglycerides
  inactive in water
  active at the water-lipid interface

AChE (Acetylcholinesterase)





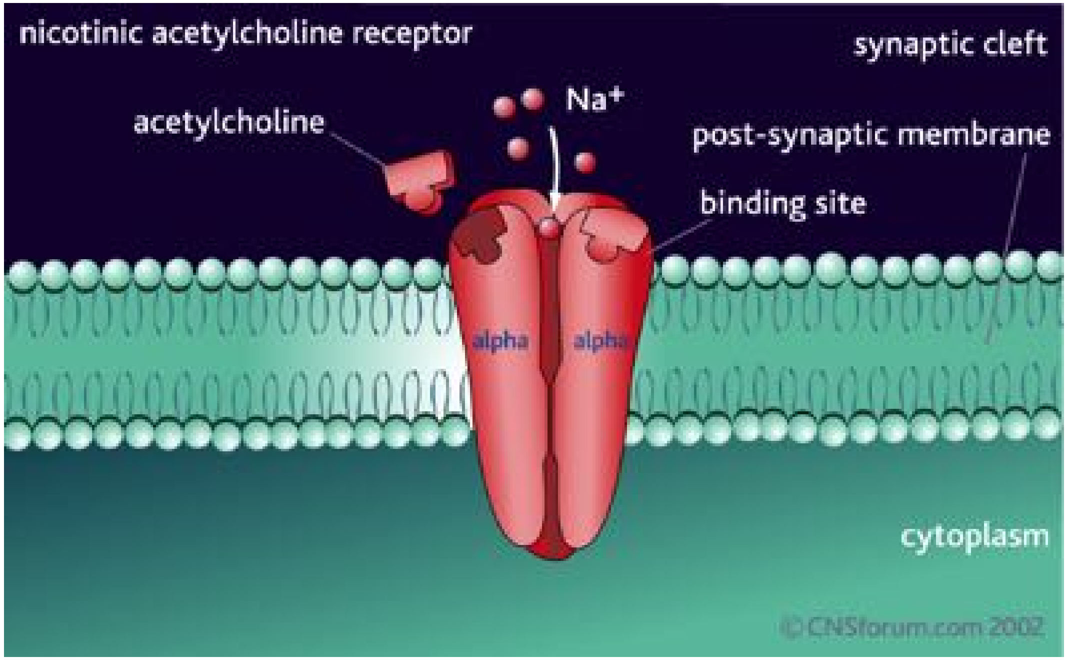
AChE: Catalytic Mechanism



Acetylcholine

Acetylcholine is a neurotransmitter (transmits nerve signals across synapses).

Acetylcholine controls Na^+-K^+ channels and is degraded by AChE in the synaptic cleft.



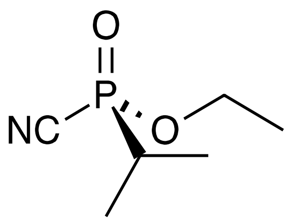
When acetylcholine accumulates, the channel remains open causing muscles to contract.

This leads to spasms, loss of control over body functions, inability to breathe and, eventually, death.

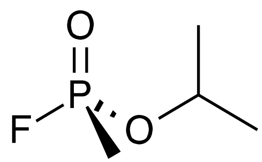
Nerve Agents and Insecticides

Nerve agents (organophosphorus compounds) and certain insecticides are AChE inhibitors

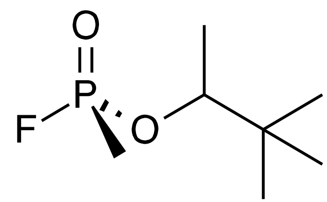
Nerve agents (chemical weapons) were discovered in Germany in 1935-1939



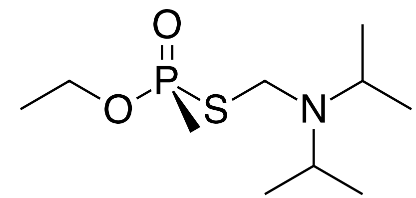
TABUN



SARIN

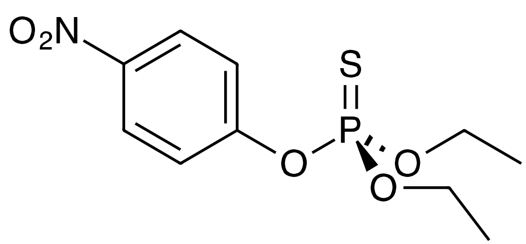


SOMAN

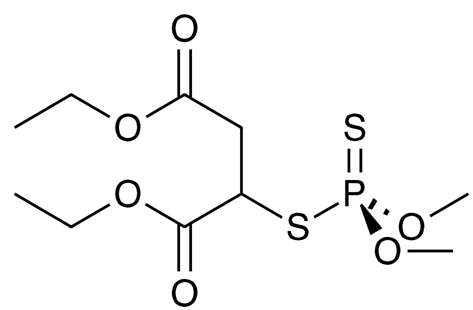


VX (UK)

Insecticides



PARATHION



MALATHION

SARIN

Irreversible AChE inhibitor

26 times more toxic than HCN

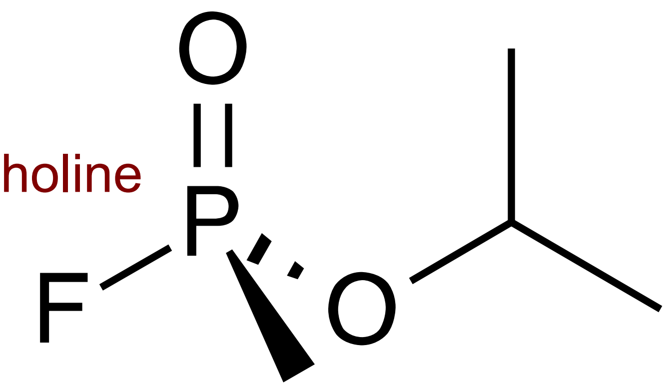
1988. Iraq (Kurdistan and IRAQ-IRAN war)

1995. Japan - Tokyo Metro

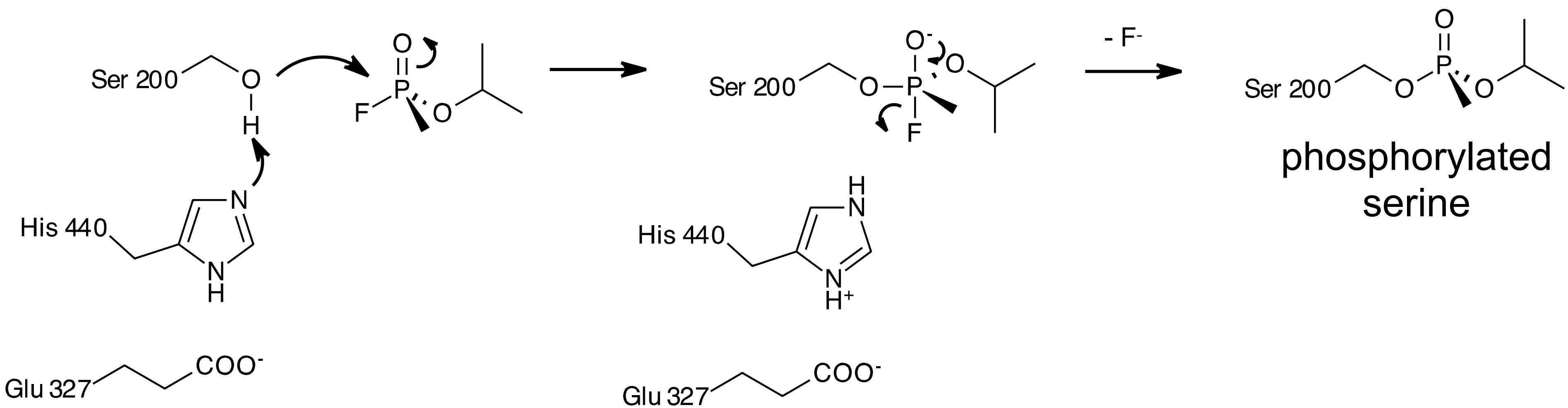
2013 Syria

Tetrahedral P:
Mimicks the TS for
hydrolysis of acetylcholine

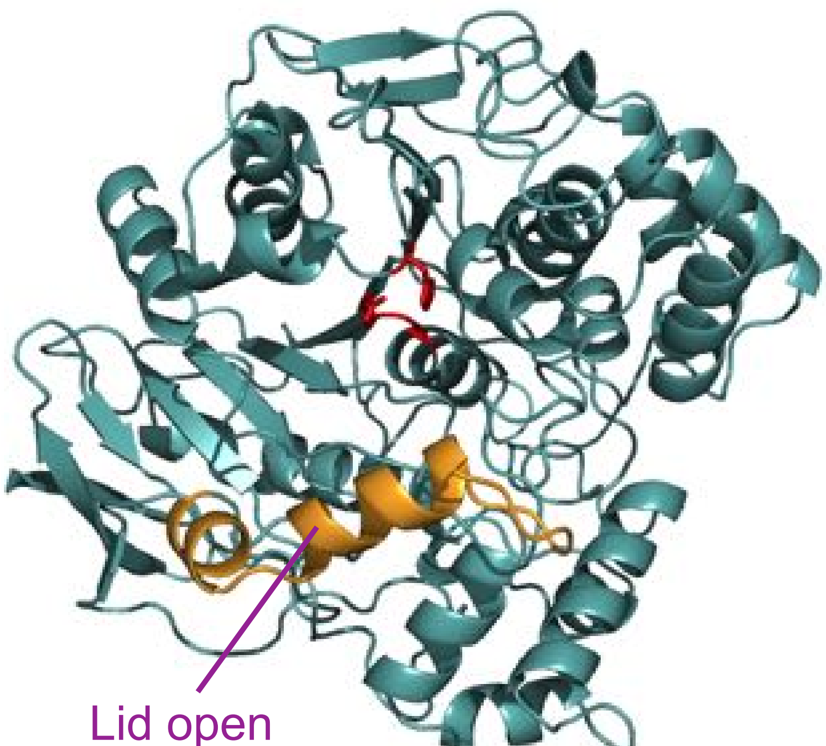
F: good leaving group



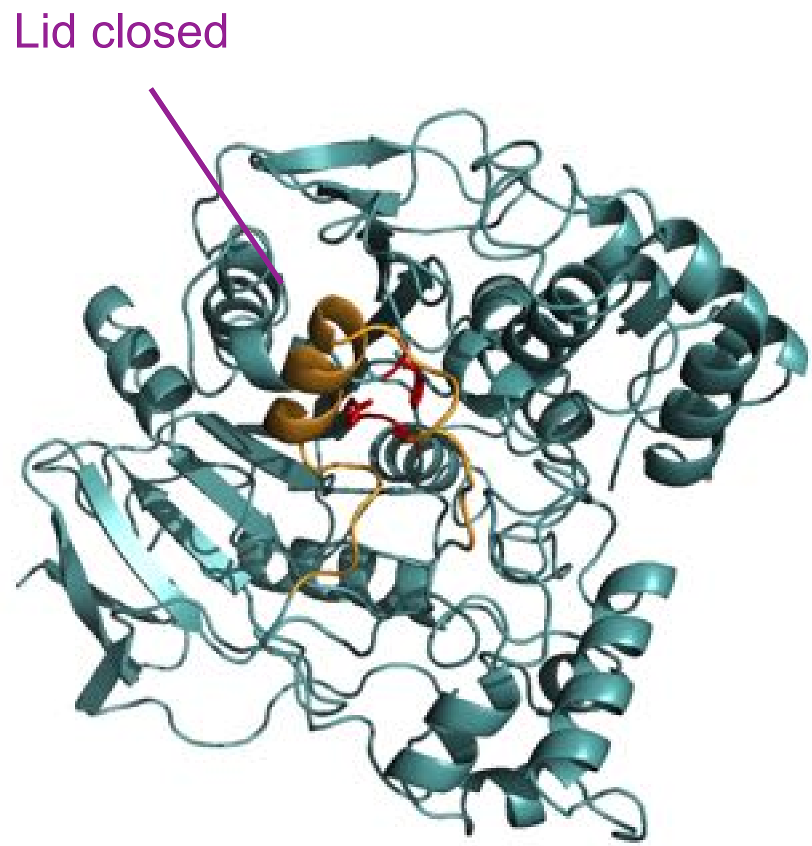
SARIN


Ser 200
O
H
F
P
O
O
His 440
N
N
H
Glu 327
COO-
Ser 200
O
P
O-
O
F
His 440
H
N
N
H+
Glu 327
COO-
- F-
Ser 200
O
P
O
O
phosphorylated serine

Lipases: interfacial activation

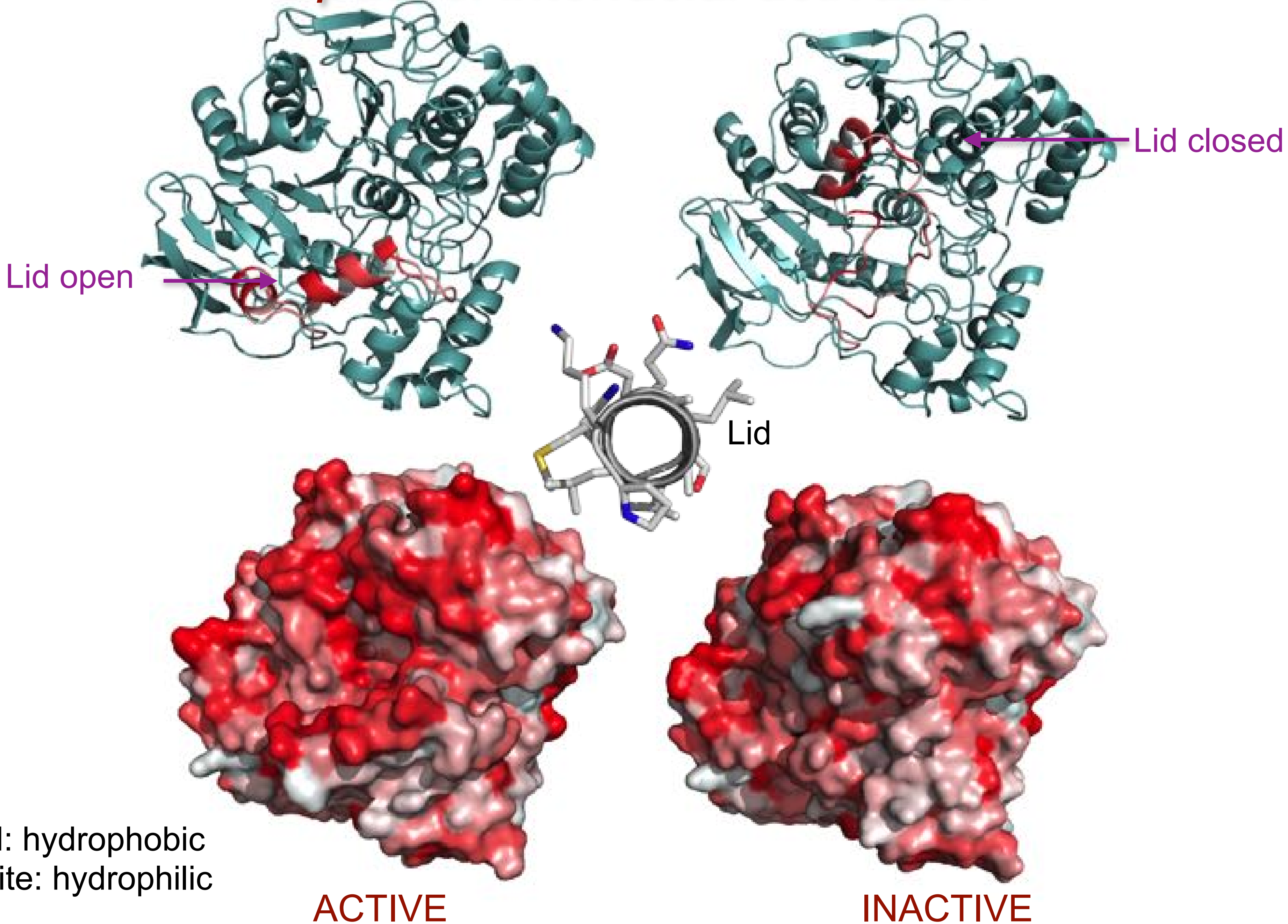


ACTIVE



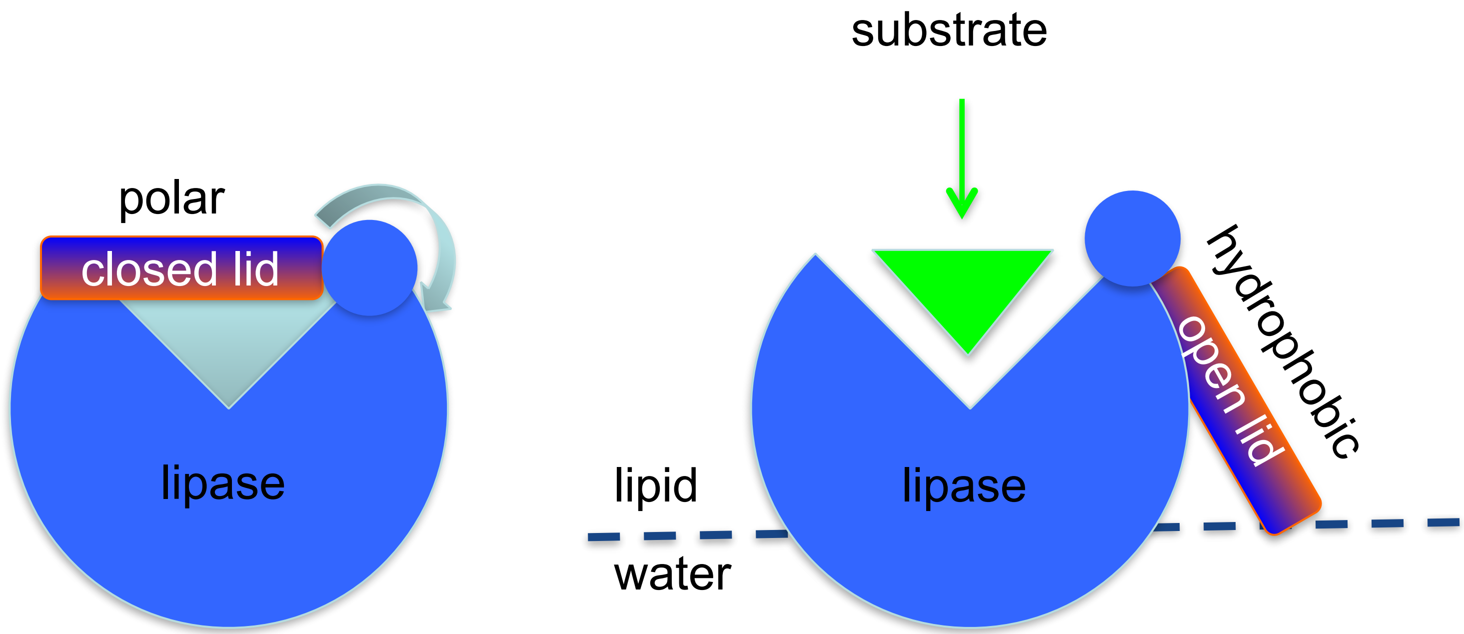
INACTIVE

Lipases: interfacial activation



red: hydrophobic
white: hydrophilic

Lipases: interfacial activation



in water:
closed form

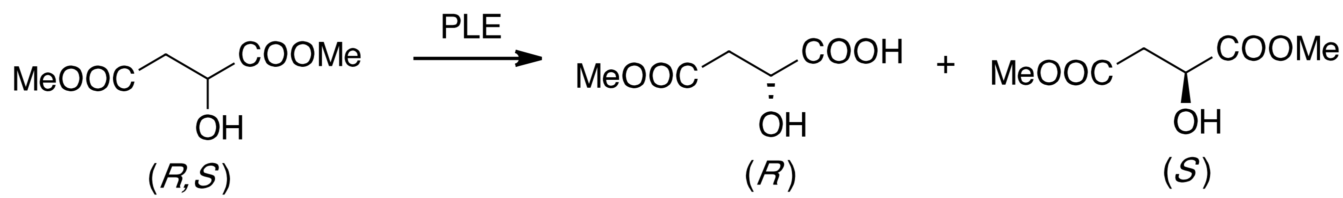
at the water-lipid interface:
open form

Biocatalysis in Organic Synthesis

Lipases and esterases are widely used in organic synthesis for their stereospecificity and stereoselectivity, both at the laboratory and industrial scale

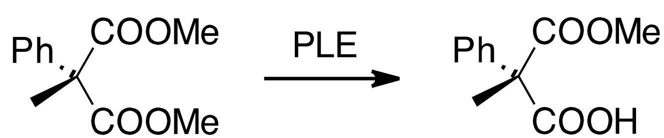
Stereospecificity

Kinetic resolution

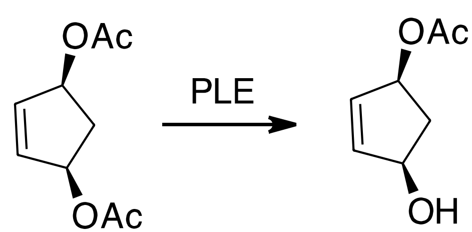


Stereoselectivity (asymmetric synthesis)

Asymmetrization of prochiral compounds

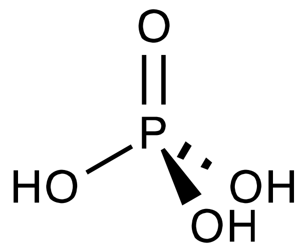


Asymmetrization of meso compounds



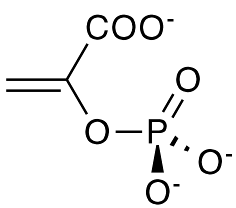
Phosphoesters

phosphoric acid

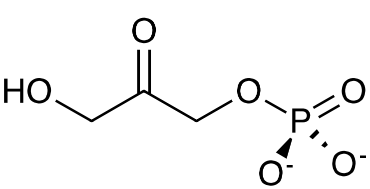


$pKa_1 = 2.15$
$pKa_2 = 7.20$
$pKa_3 = 12.35$

Monesters

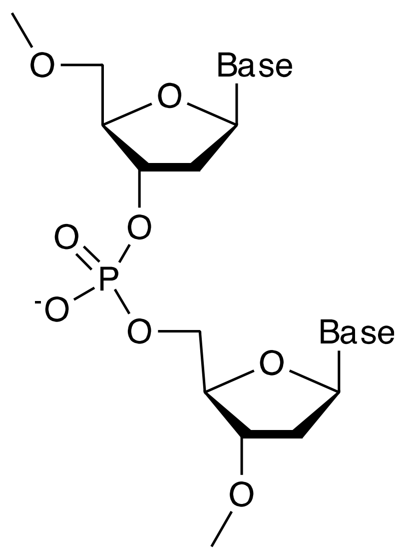


phosphoenolpyruvate



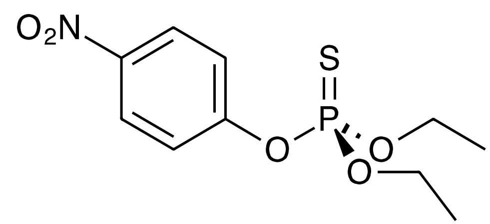
dihydroxyacetone phosphate

Diesters



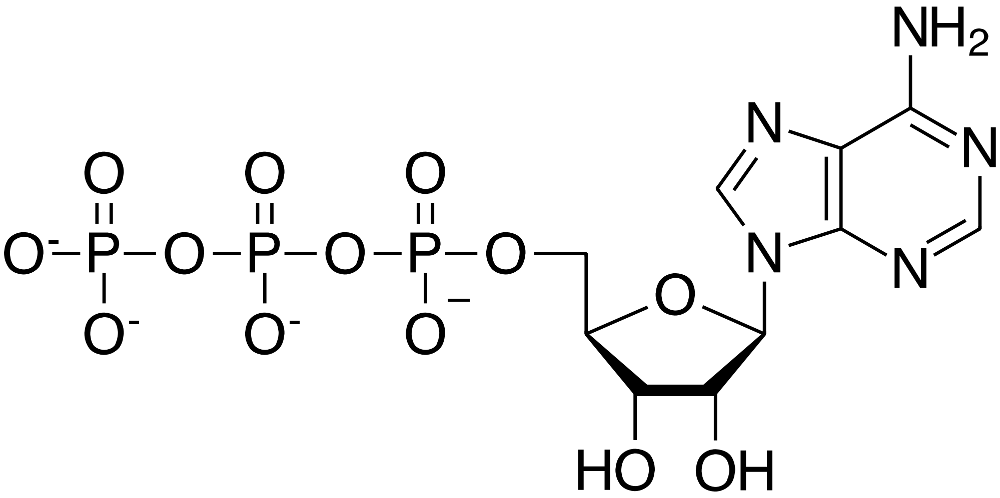
nucleic acids

Triesters



parathion

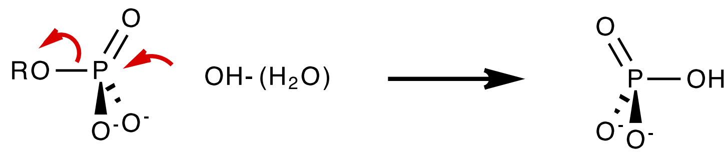
Phosphoesters



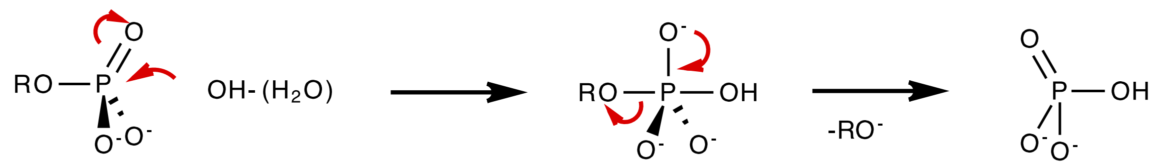
ATP

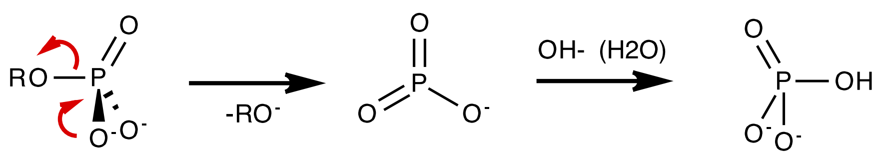
Hydrolysis of Phosphate Esters

Monoesters:

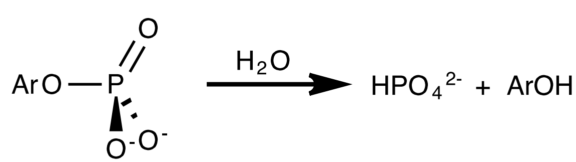


Preferred

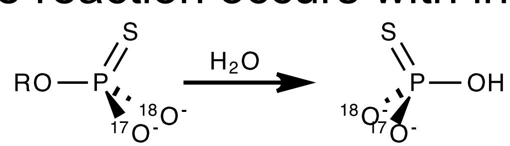




- β_{LG} : - 0.8



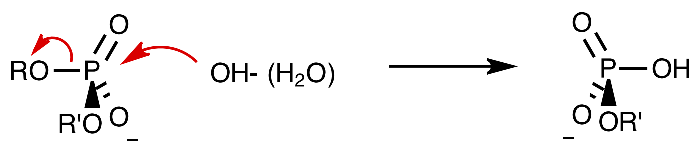
- The reaction occurs with inversion of configuration at P



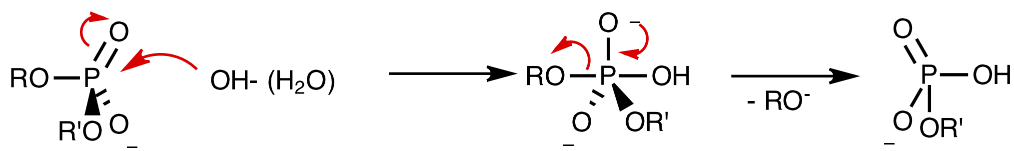
"chiral phosphate"

Hydrolysis of Phosphate Esters

Diesters:

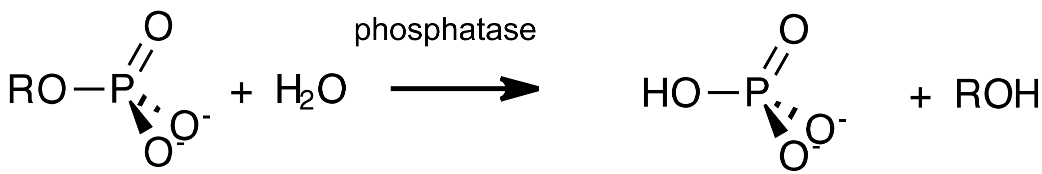


preferred

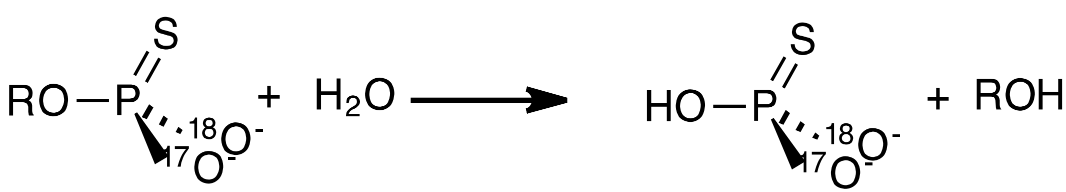


possible

Alkaline Phosphatase

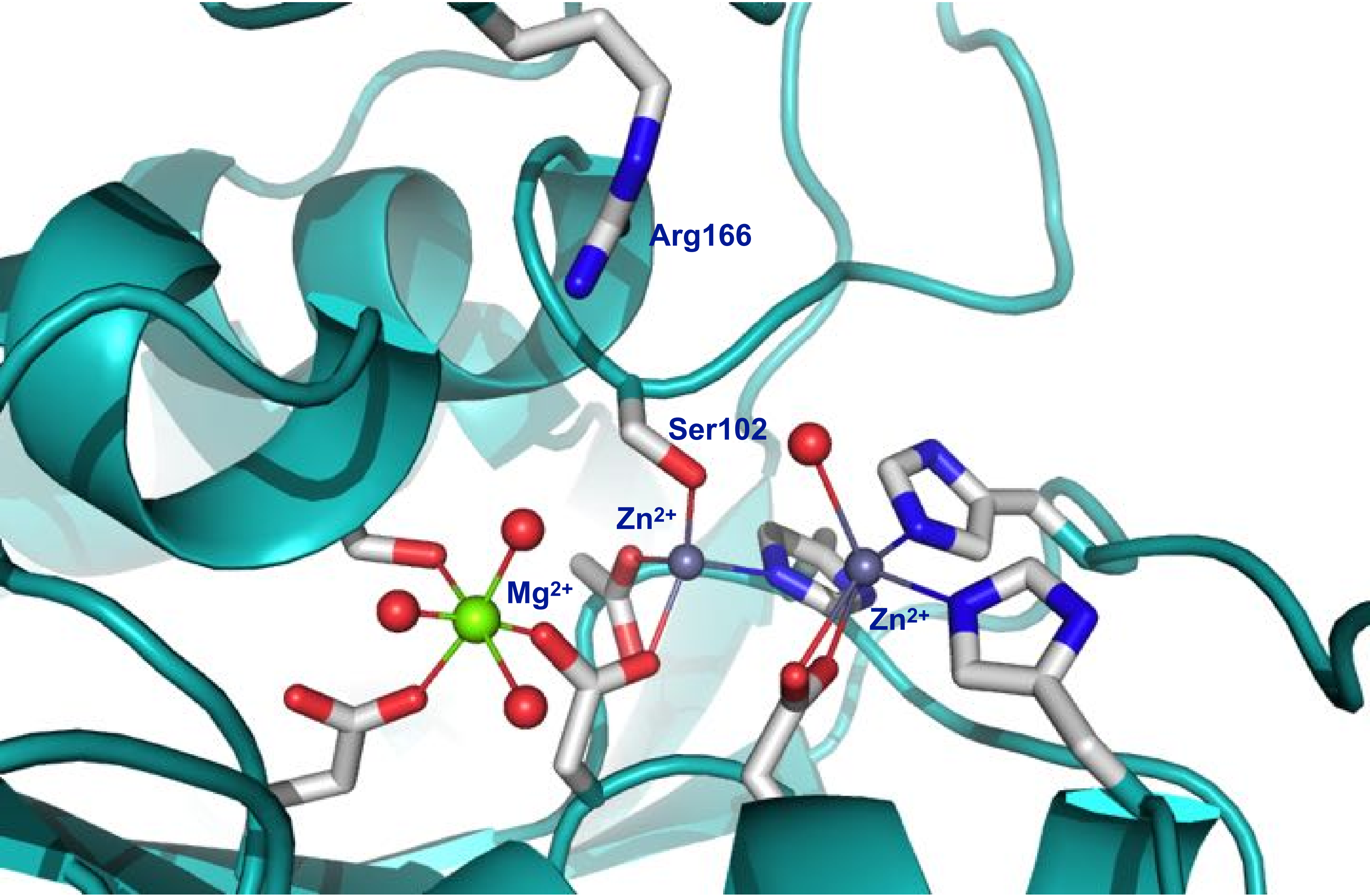


- ROH and HPO_4^{2-} are formed at different rates
- 1 mol of ROH is rapidly released before phosphate is formed
- For the formation of ROH β_{LG} = -1.1
- k_{cat} (for the slow formation of HPO_4^{2-}) is independent from R
- The reaction occurs with retention of configuration at P

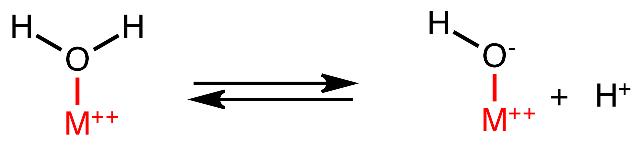


"chiral phosphate"

Alkaline Phosphatase (E. Choli)

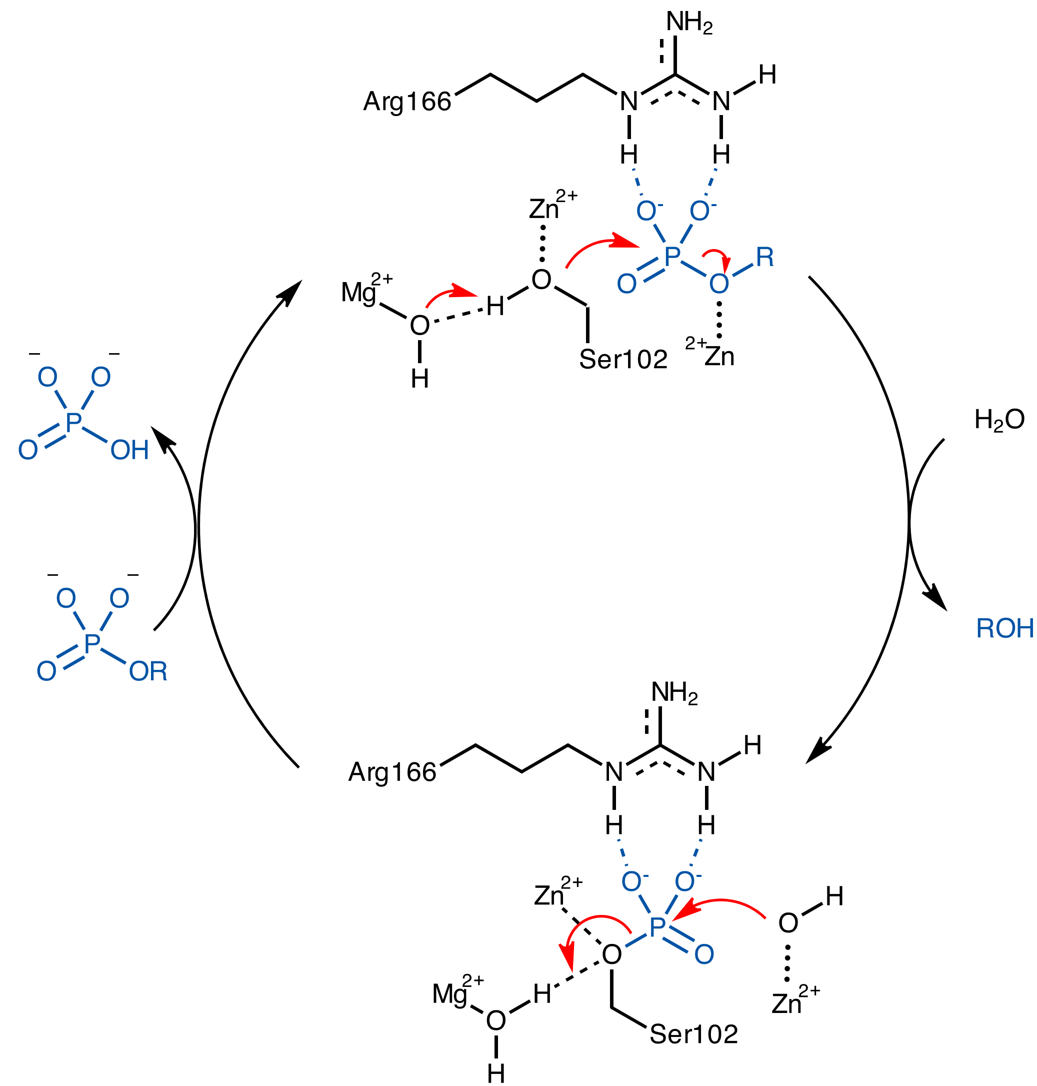


Electrophilic Water Activation by Metal Ions



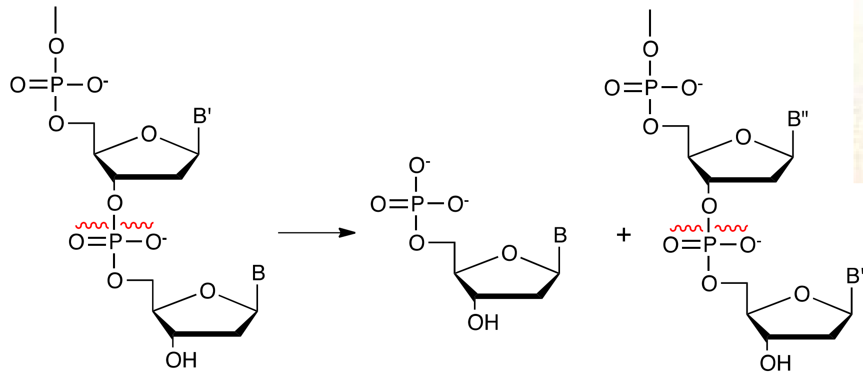
Metal	***pKa***	***Metal***	***pKa***
Ba^{2+}	13.1	Ca^{2+}	12.5
Mg^{2+}	**11.4**	Mn^{2+}	10.1
Cd^{2+}	9.8	**Zn^{2+}**	**9.6**
Co^{2+}	9.4	Ni^{2+}	9.0
Fe^{2+}	8.4	Be^{2+}	4.3

Alkaline Phosphatase

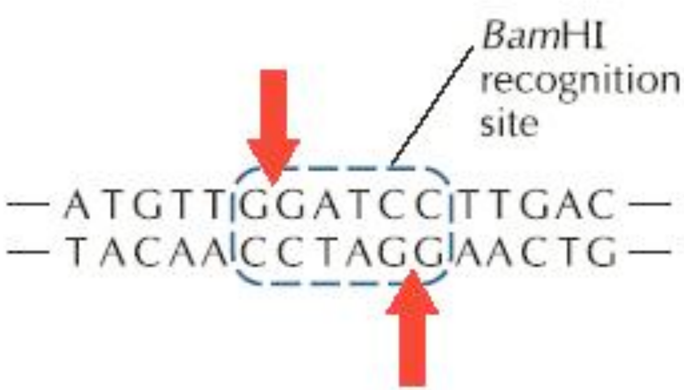


Phosphodioesterases - Nucleases

Exonucleases: hydrolyze phosphate bonds from the 3’ or 5’ terminal. Nucleases from snake’s venom digest single stranded DNA from the 3’ terminal in a completely aspecific way

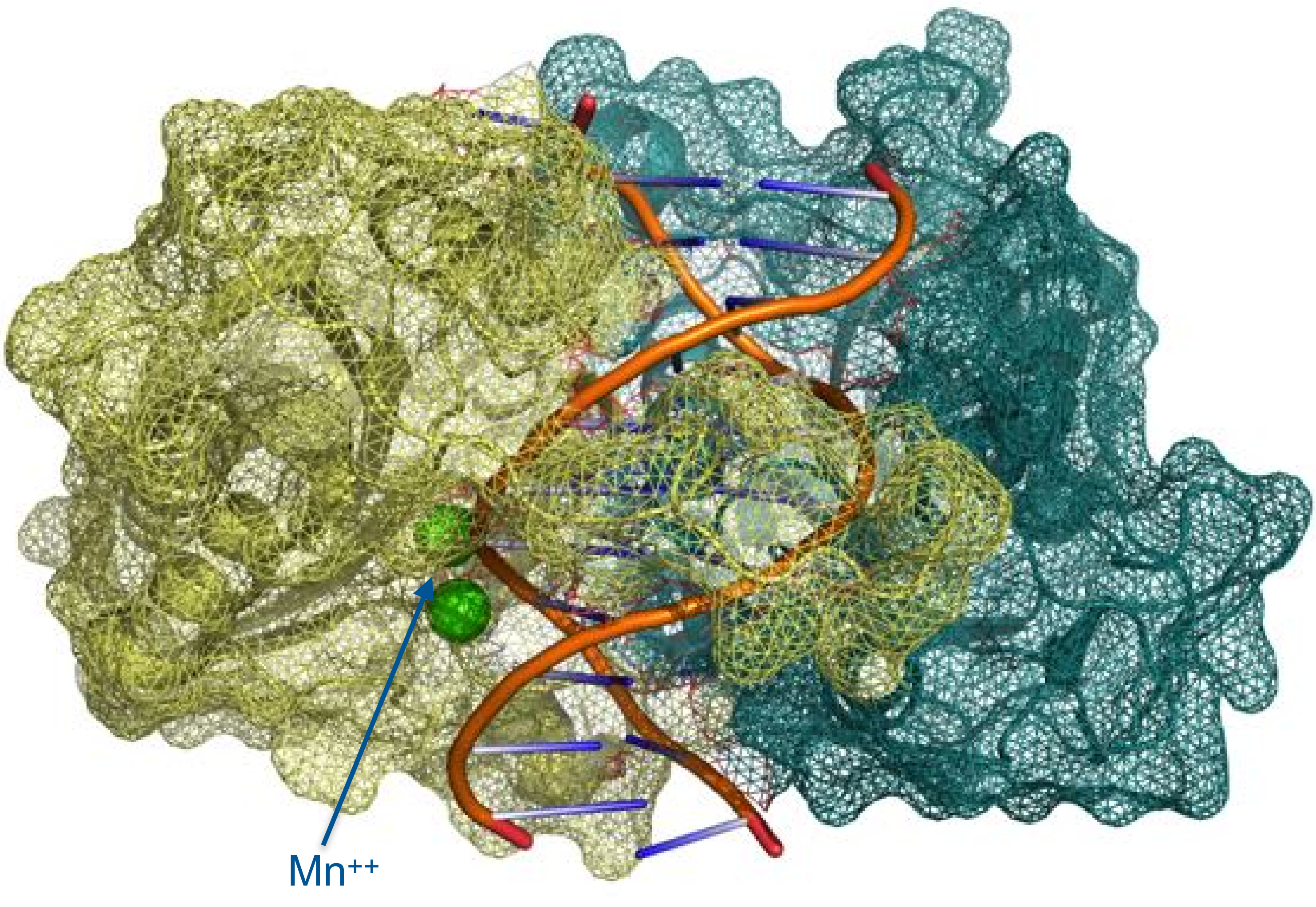


Endonucleases: hydrolyze internal phosphate bonds and are, in general, highly specific. Restriction enzymes cut DNA’s double helix in palindromic positions

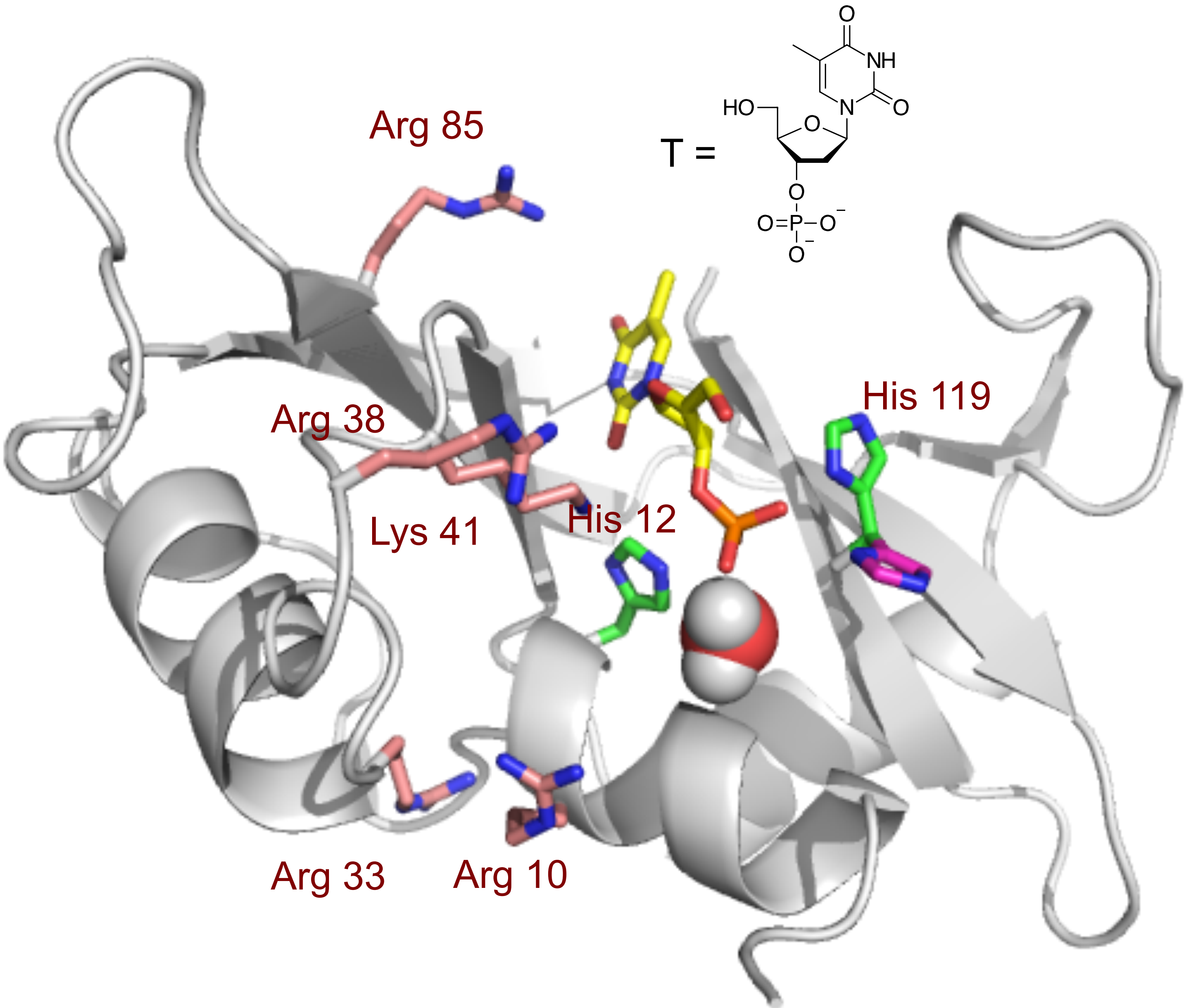


Bacyllus amyloliquefaciens

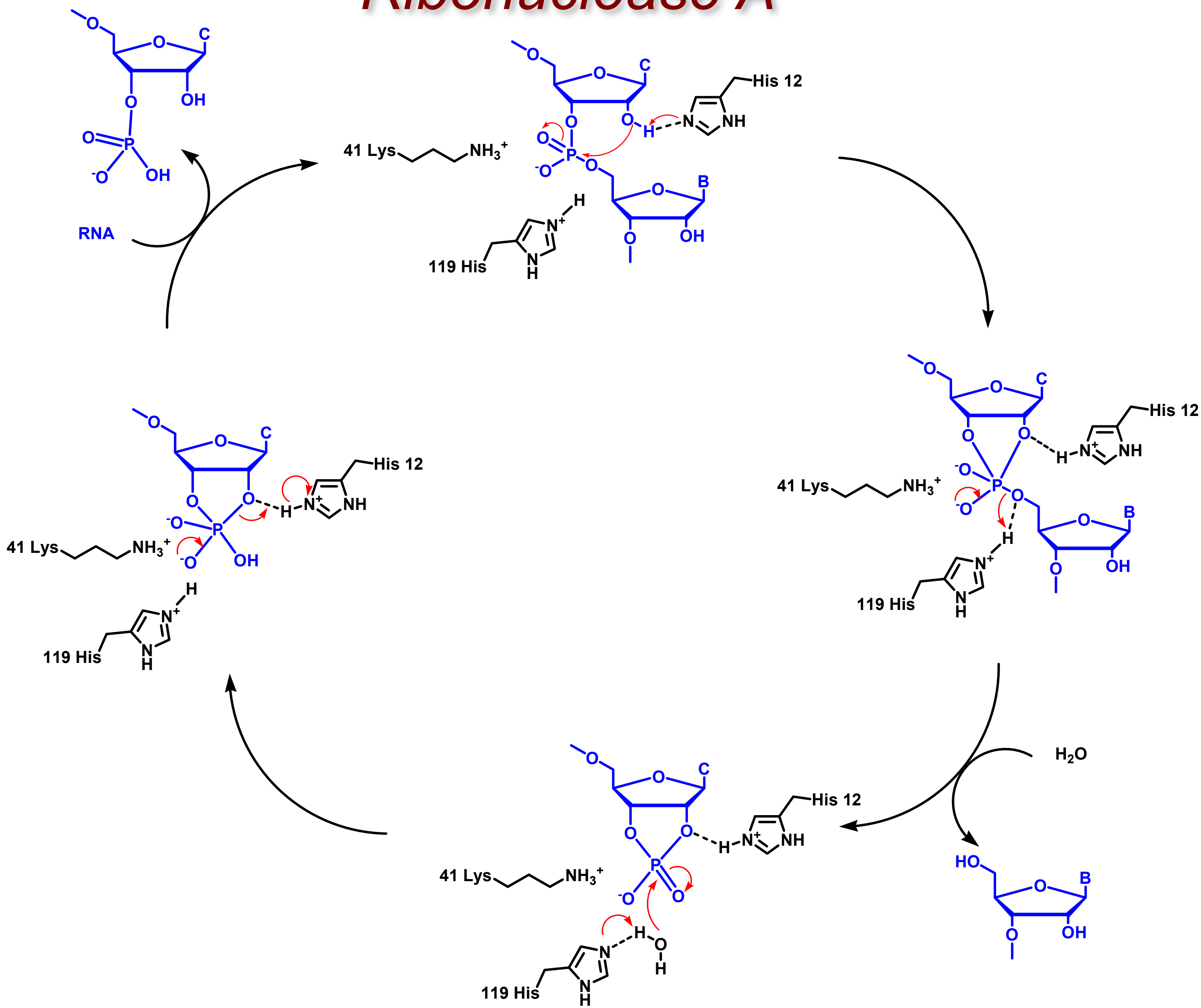
Phosphodiesterases: BamH1



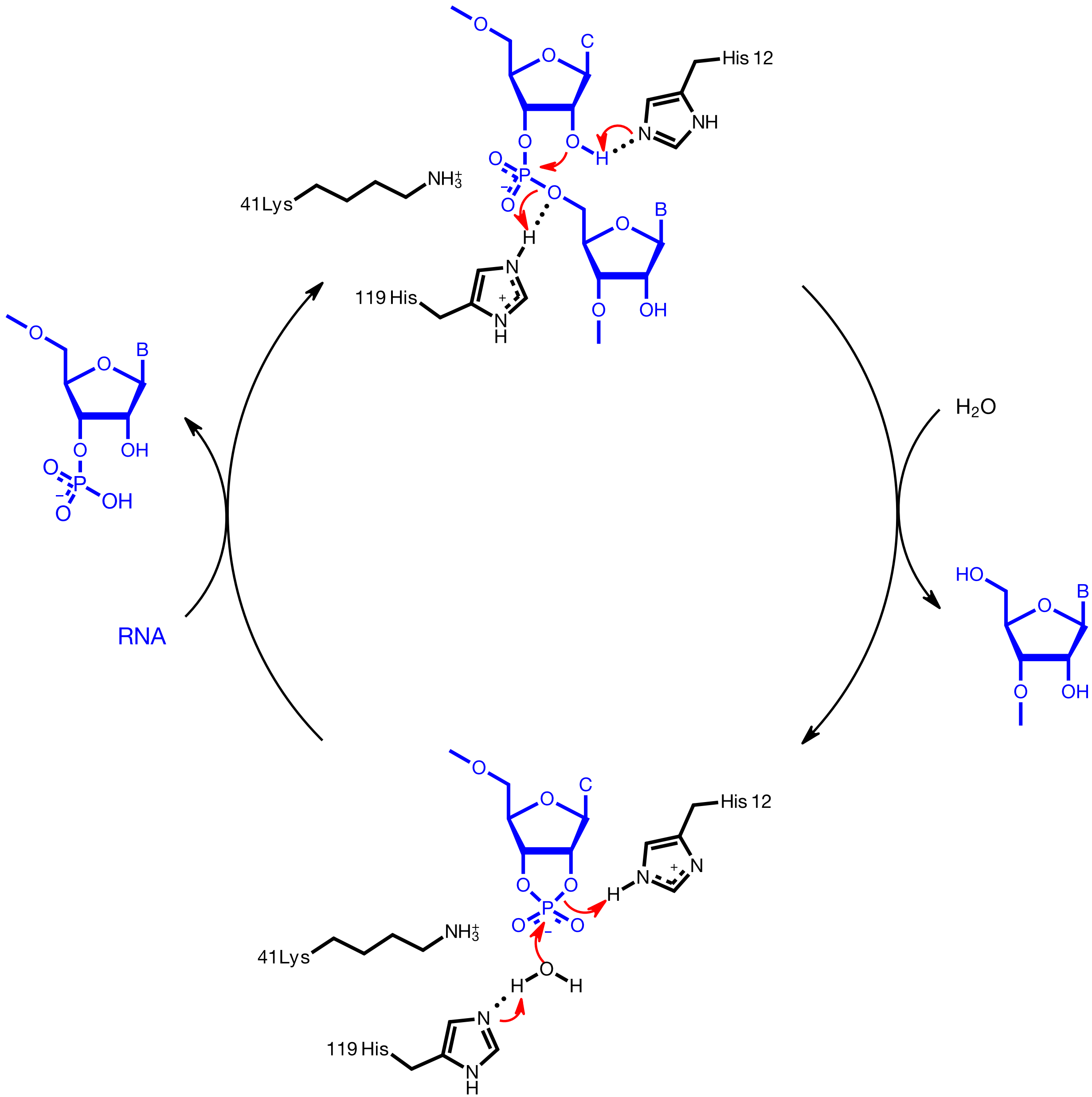
Ribonuclease A



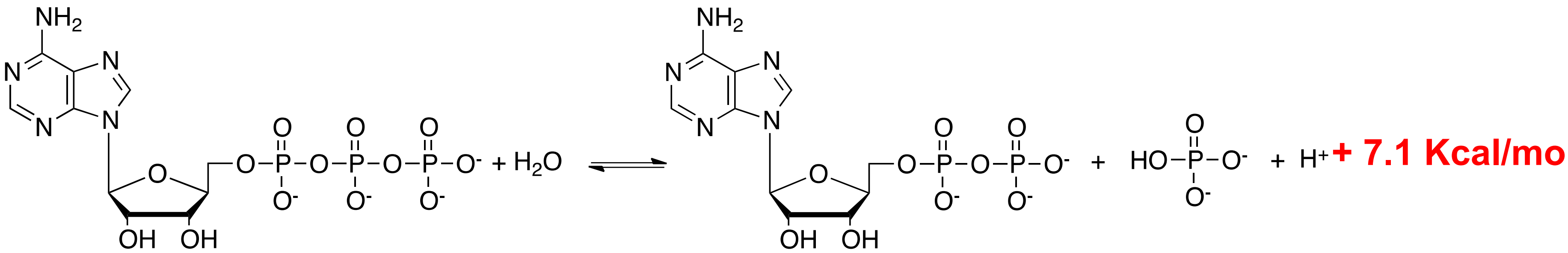
Ribonuclease A



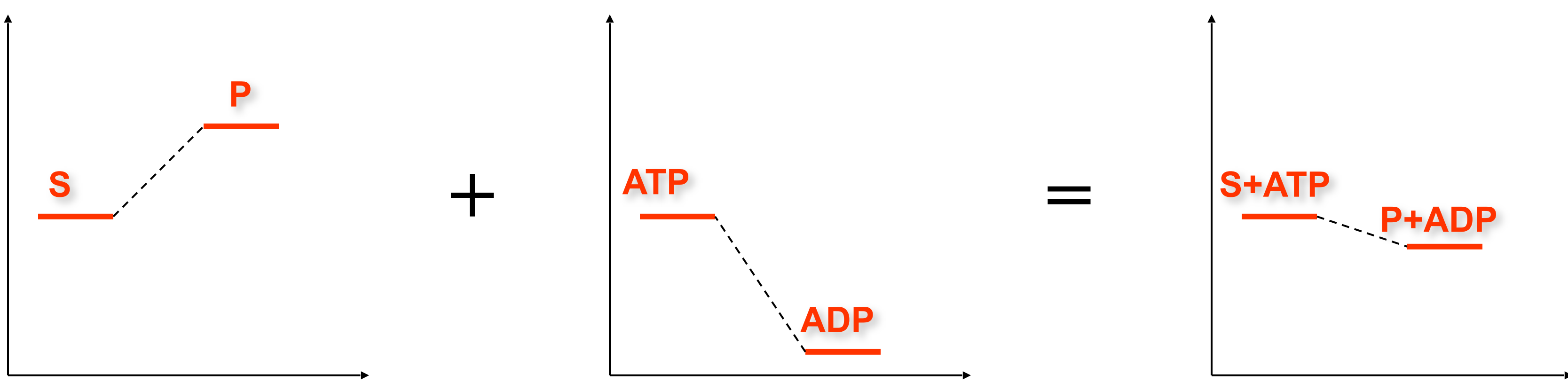
Ribonuclease A



ATP: Energy Storage and Supply

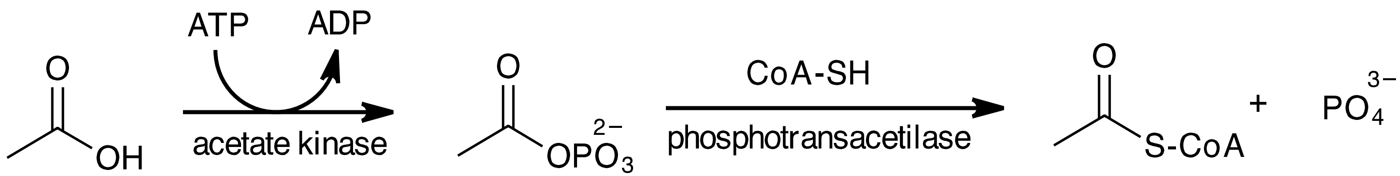


ATP **ADP** **P_i**

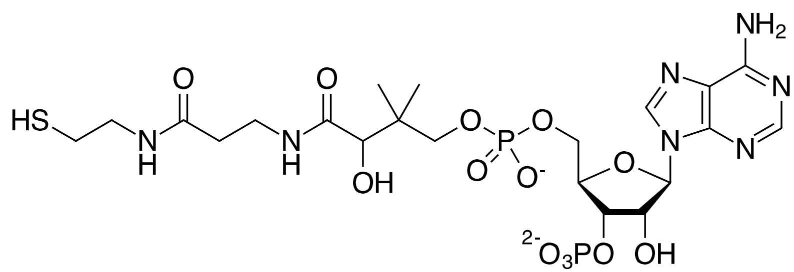


Hydrolysis of ATP allows to overcome thermodynamical barriers

ATP: Energy Storage and Supply



CoA-SH =

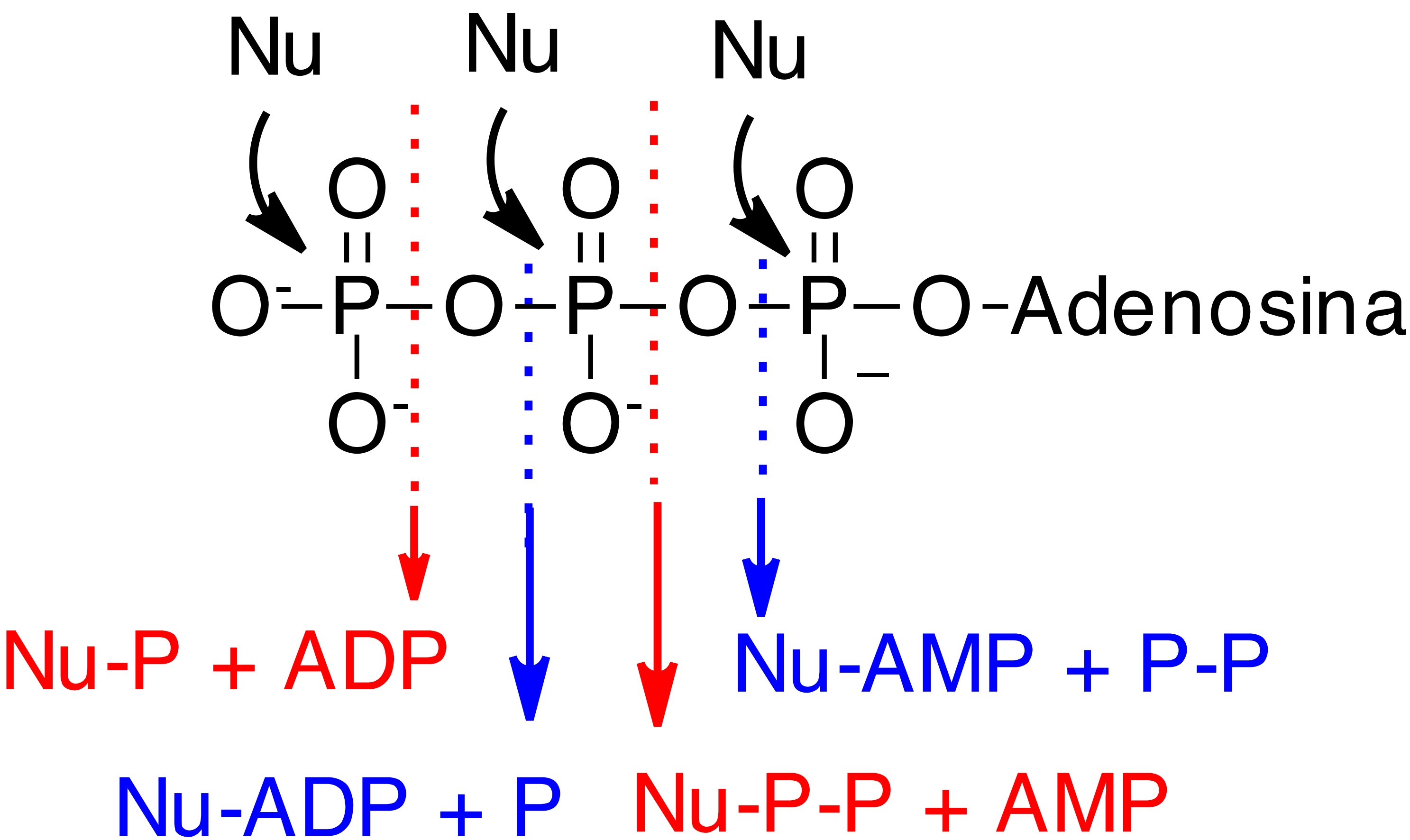


$$R{-}OH + ATP \longrightarrow R{-}OPO_3^{2-} + ADP + H^+$$

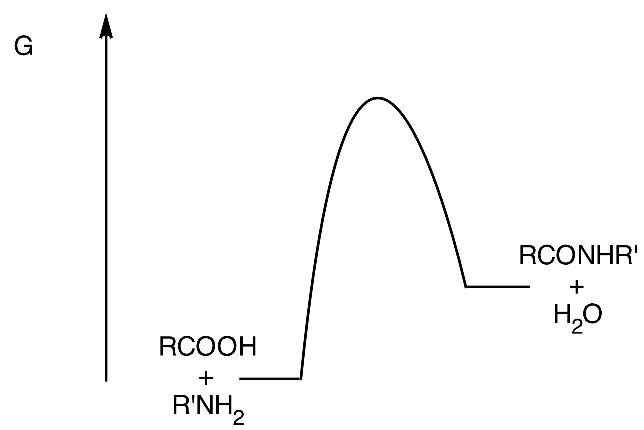
$$R{-}OPO_3^{2-} + Nu \longrightarrow R{-}Nu + PO_4^{3-}$$

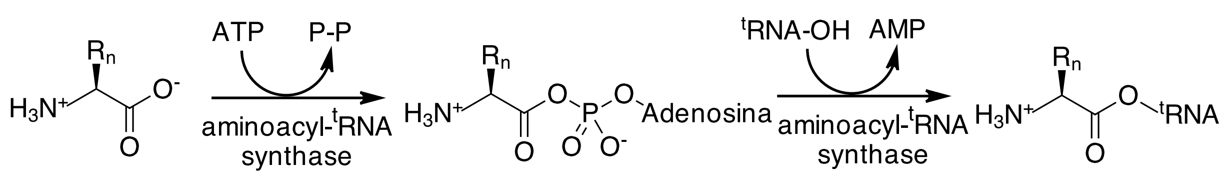
$$R\text{-}OH + Nu + ATP \longrightarrow R\text{-}Nu + ADP + PO_4^{3-} + H^+$$

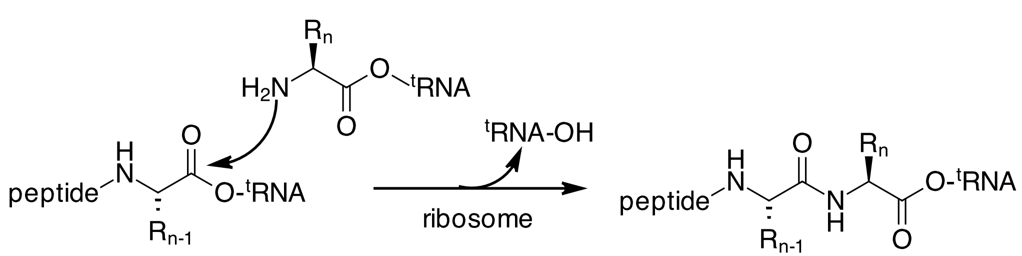
ATP: Energy Storage and Supply



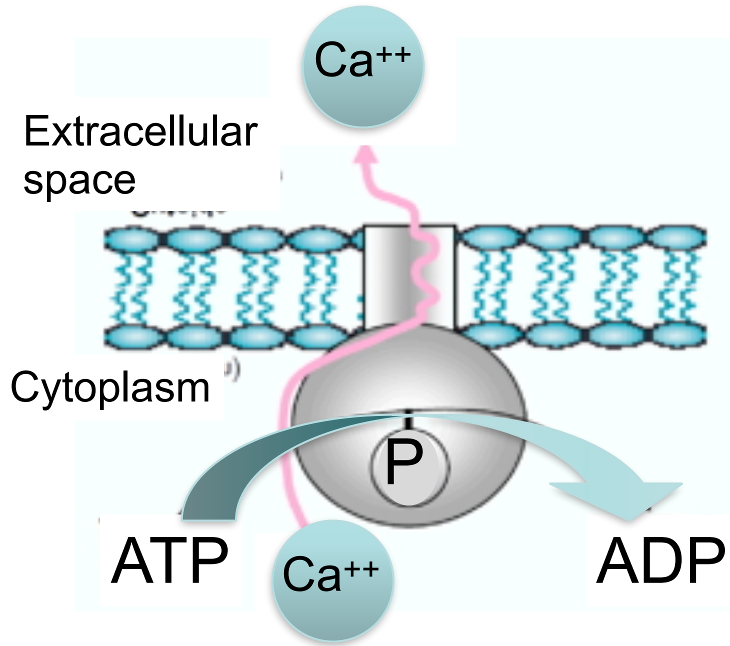
Protein Synthesis

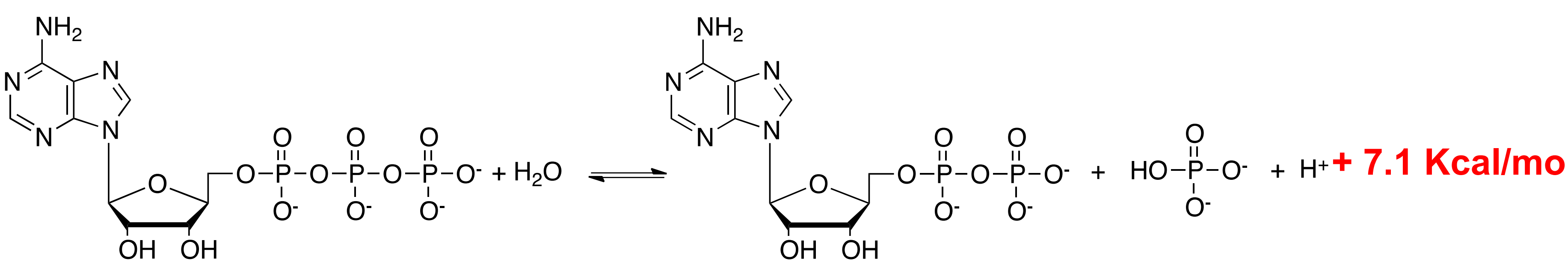






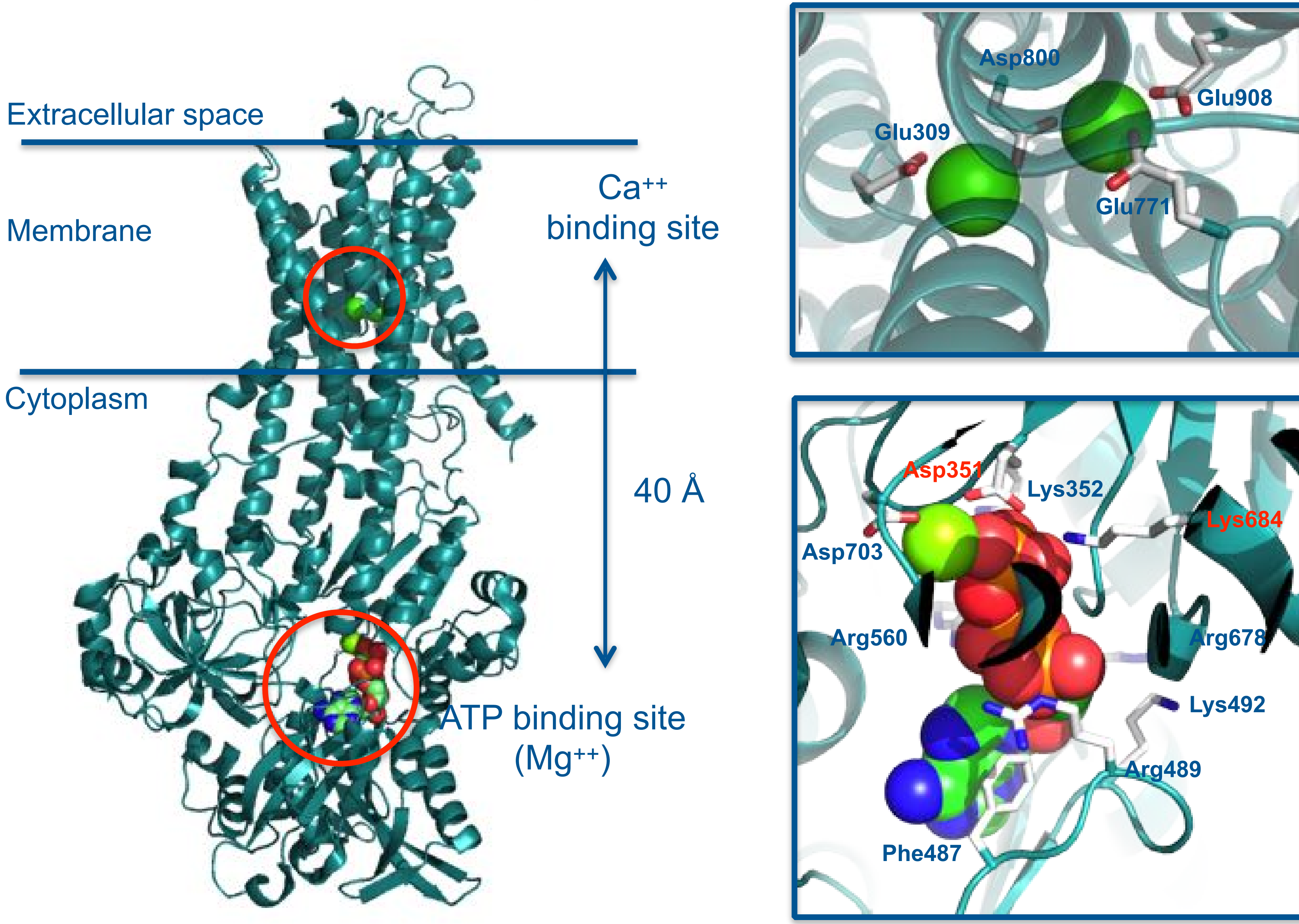
P-Type ATPase



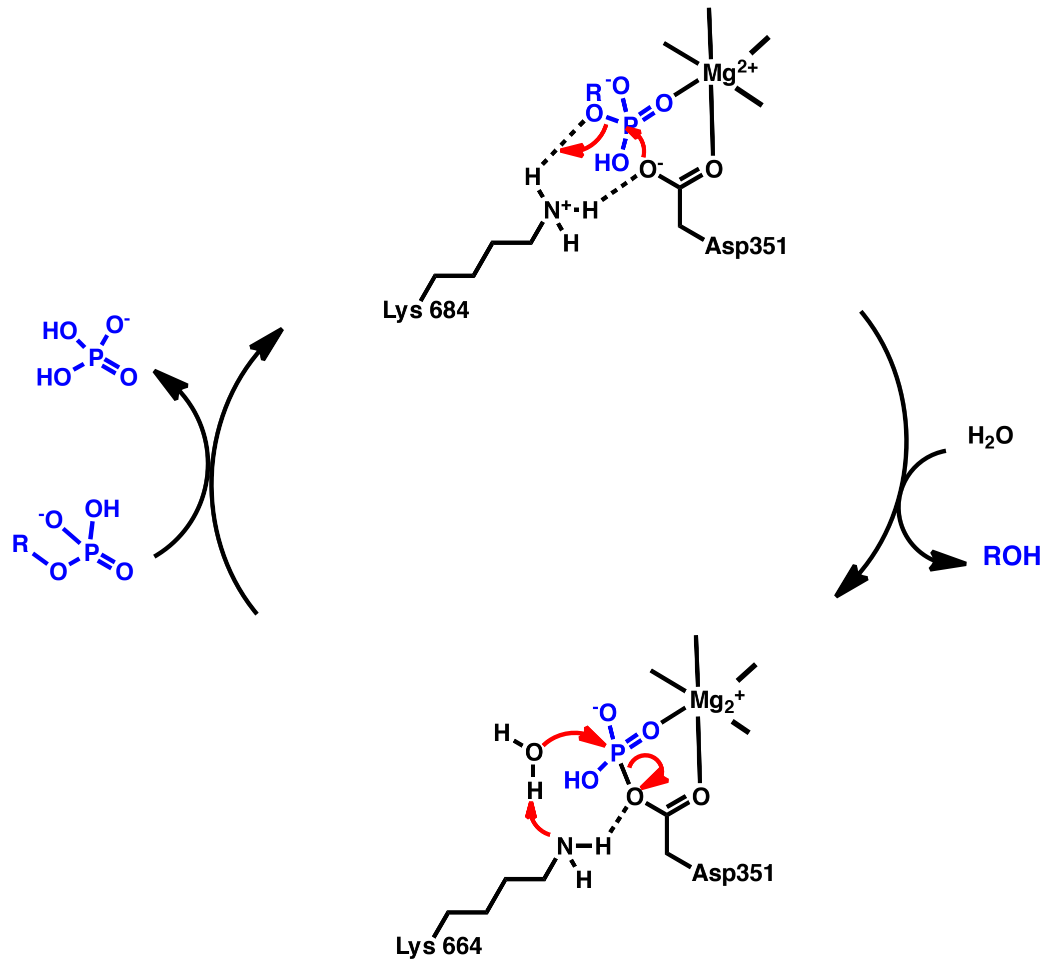


ATP **ADP** **P_i**

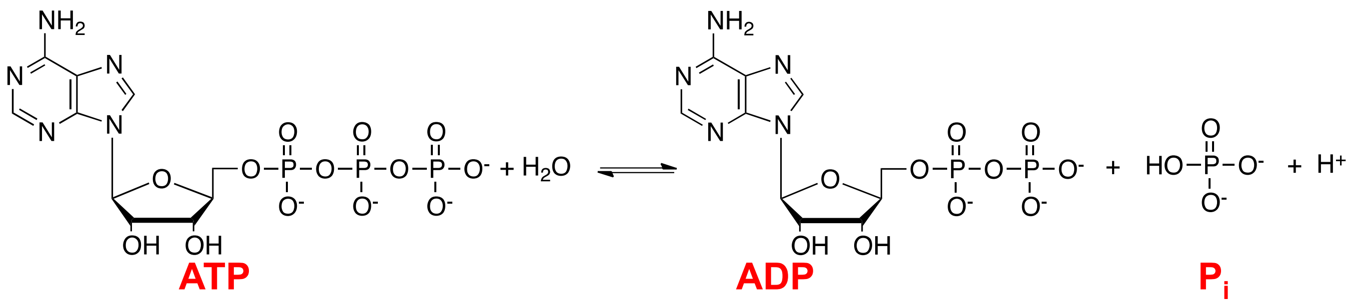
P-Type ATPase



P-Type ATPase

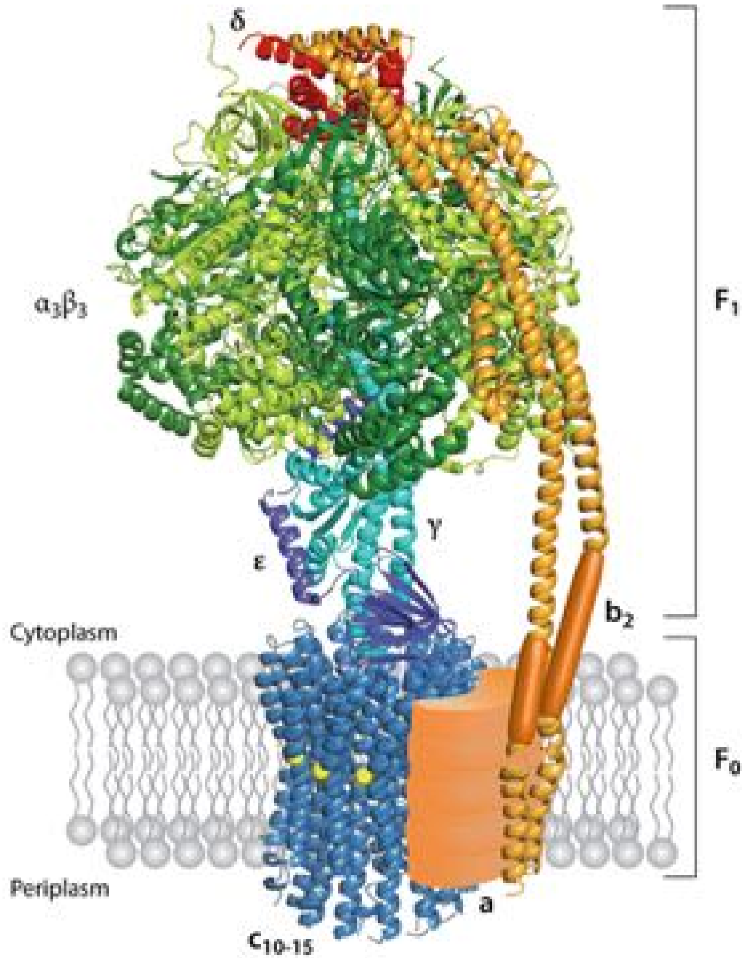


ATP Synthase, a Molecular Machine



+ 7.1 Kcal/mol

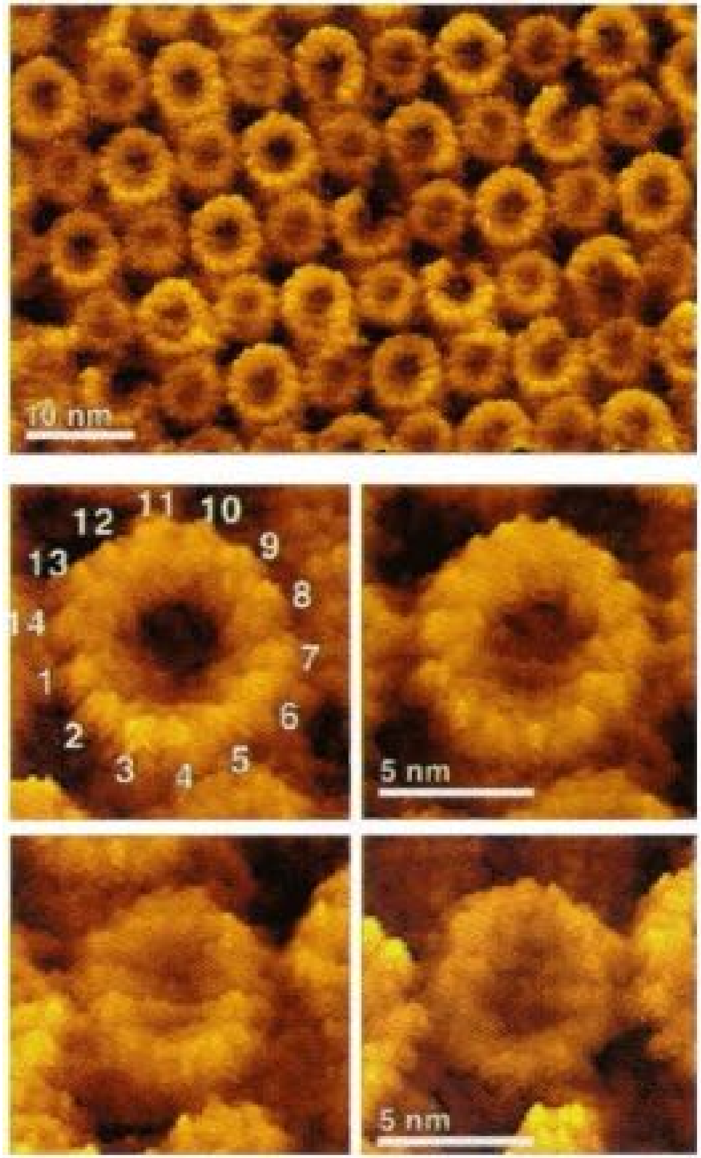
Fig. 1



F_1	
α	***3***
β	3
γ	***1***
δ	1
ε	***1***
F_0	
a	1
b	***2***
c	10-15

ATP Synthase, a Molecular Machine

Subunit-III oligomers of chloroplast ATP synthase

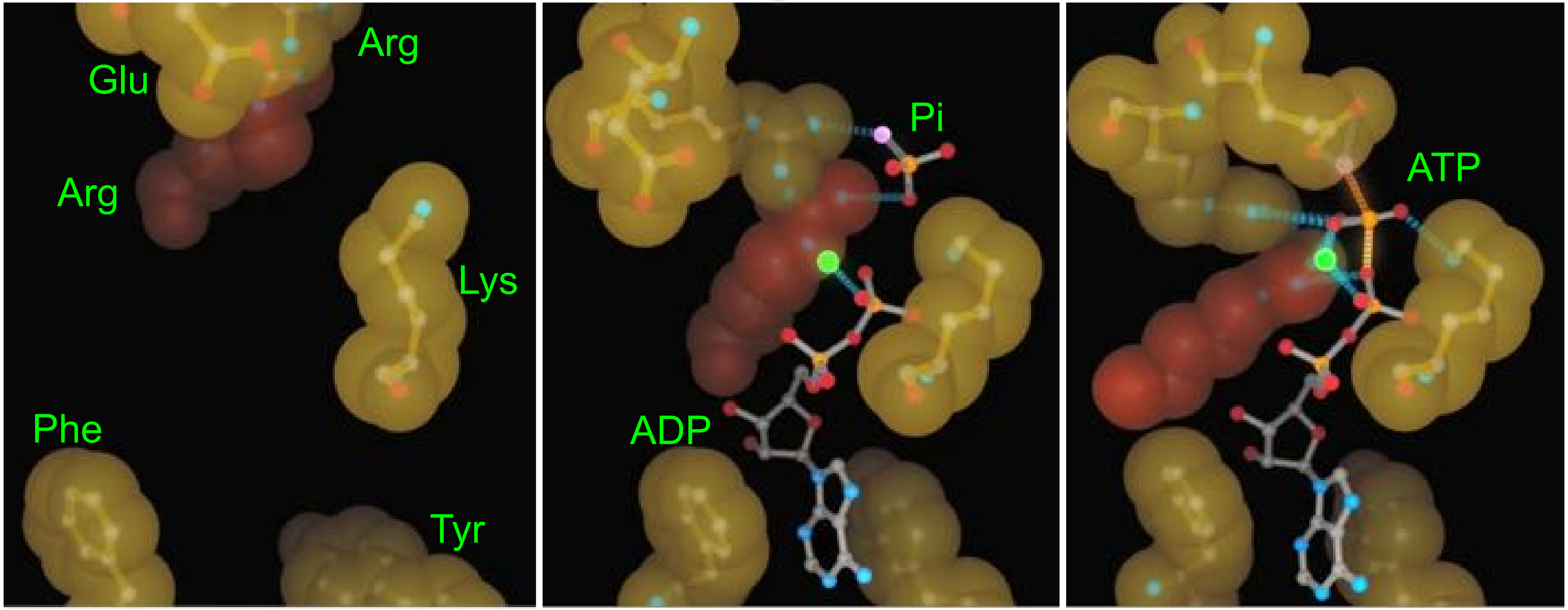


the distinct wide and narrow rings represent the two surfaces of the subunit-IIIx oligomer

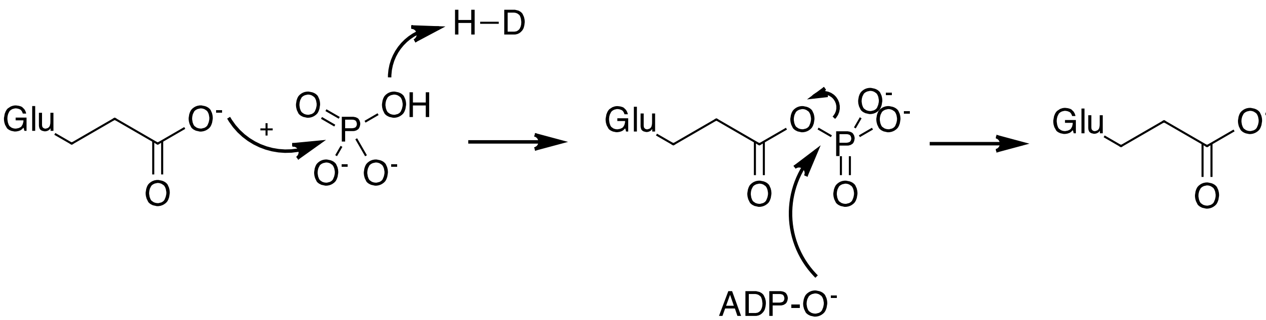
wide oligomer ends

narrow oligomer ends

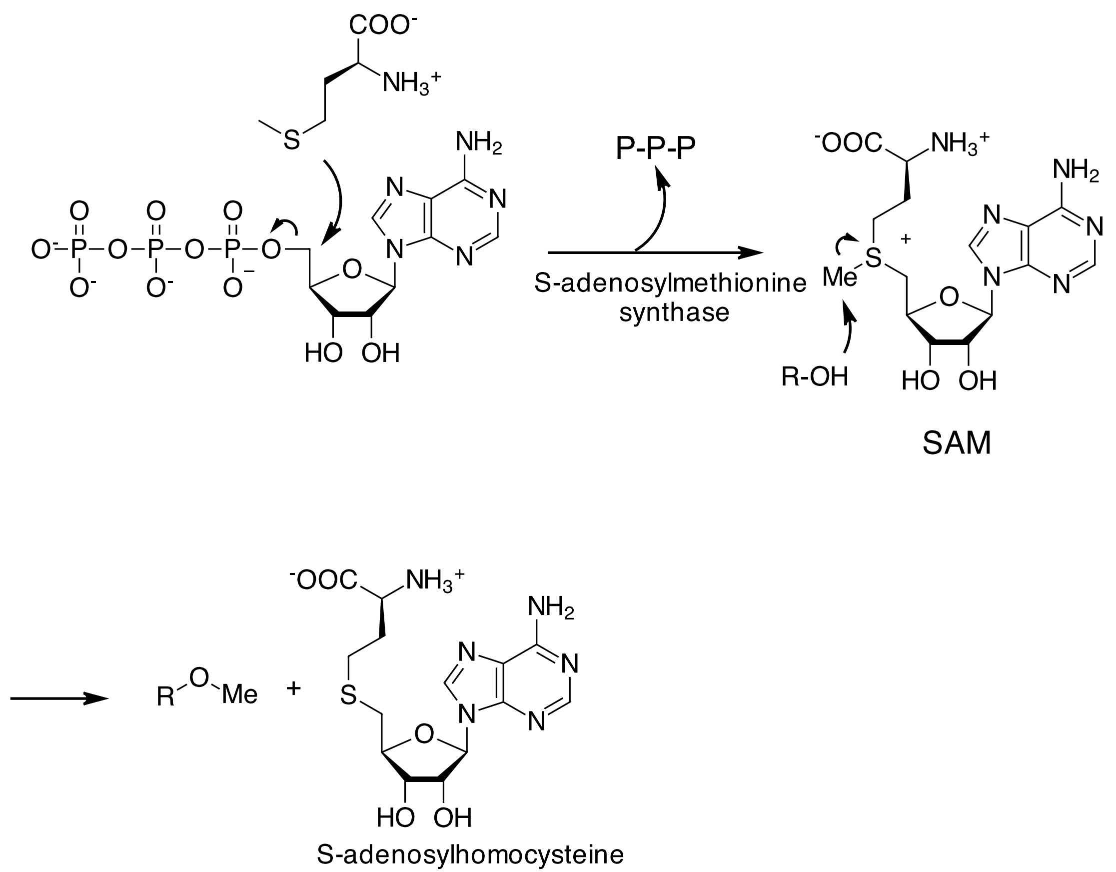
ATP Synthase



open loose binding tight binding

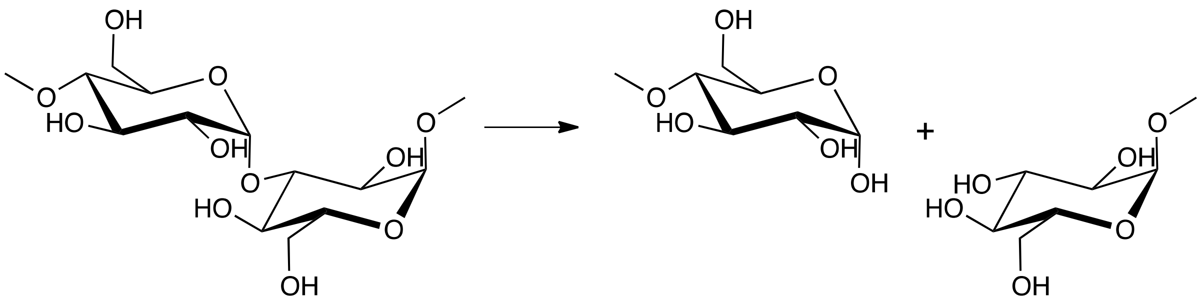


ATP: the Third Cleavage Site. Biological Methylations



Glycosidases

Hydrolyse the glycosidic bond between two sugars: are involved in polysaccharides degradation



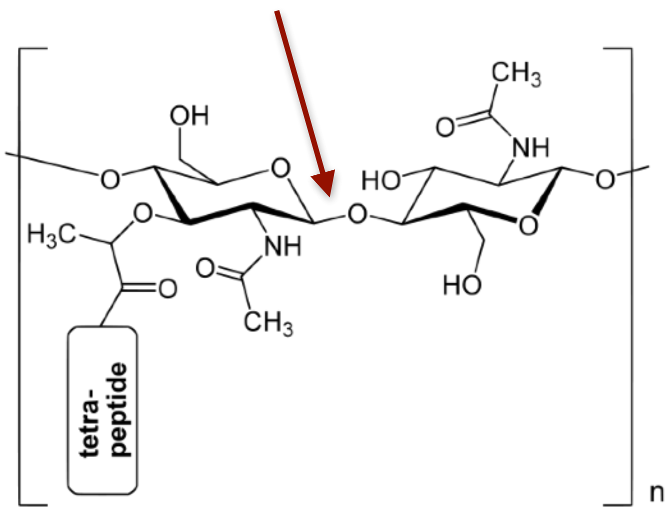
They are, generally, very specific for the disaccharide substrate:

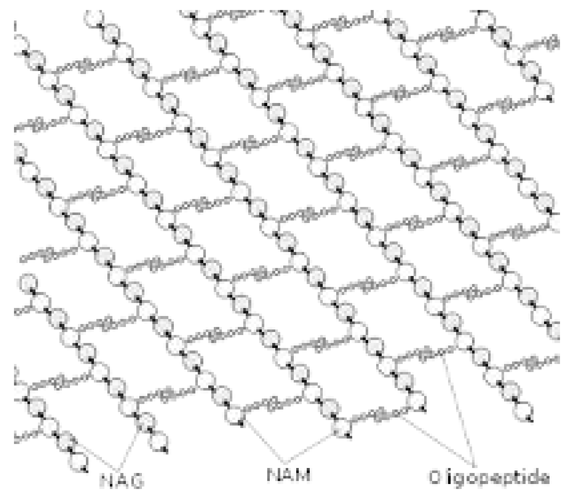
- sugars
- type of bond (1,4-1,6-1,3; α, β)

All glycosidases use acid catalysis (acetal hydrolysis)

Lysozyme

Degrades peptidoglycan of bacterial cell walls by cutting between N-acetylmuramic acid and N-acetylglucosamine residues.



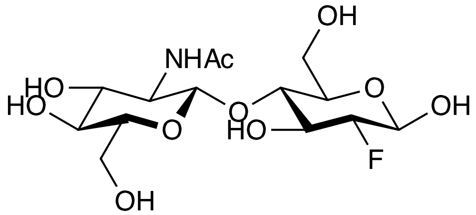


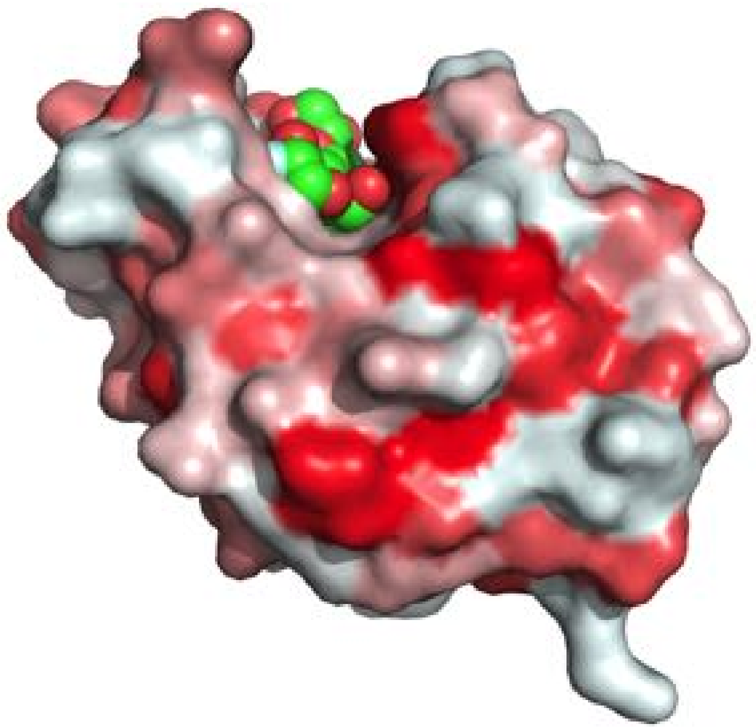
Lysozyme is a natural antibiotic present in tears, nasal mucus, egg white; it is used as a preservative in the food industry.

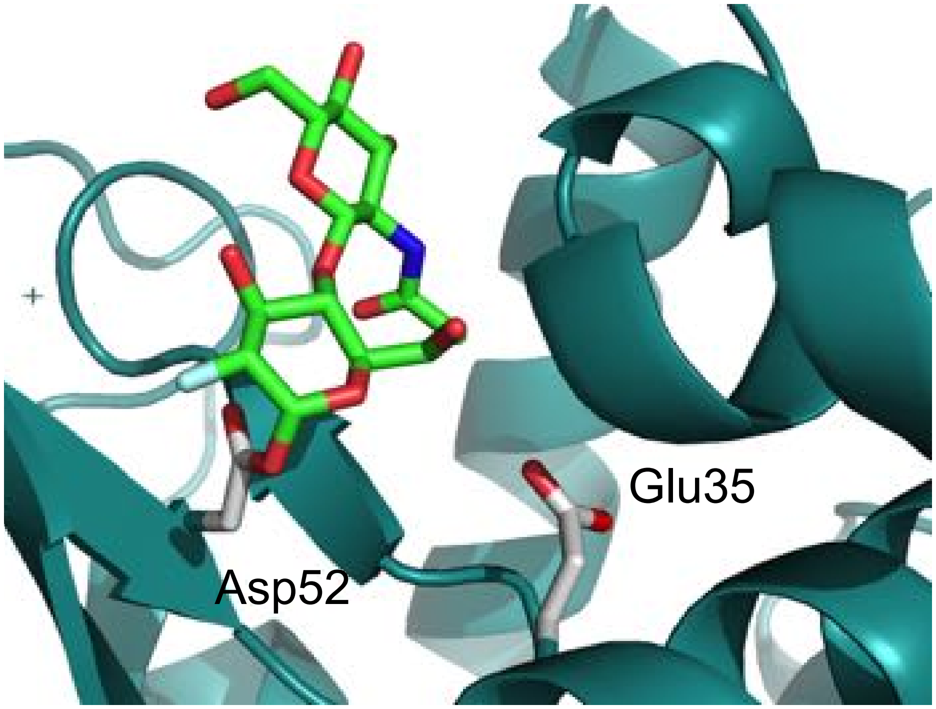
Discovered in 1922 by Alexander Fleming

Lysozyme

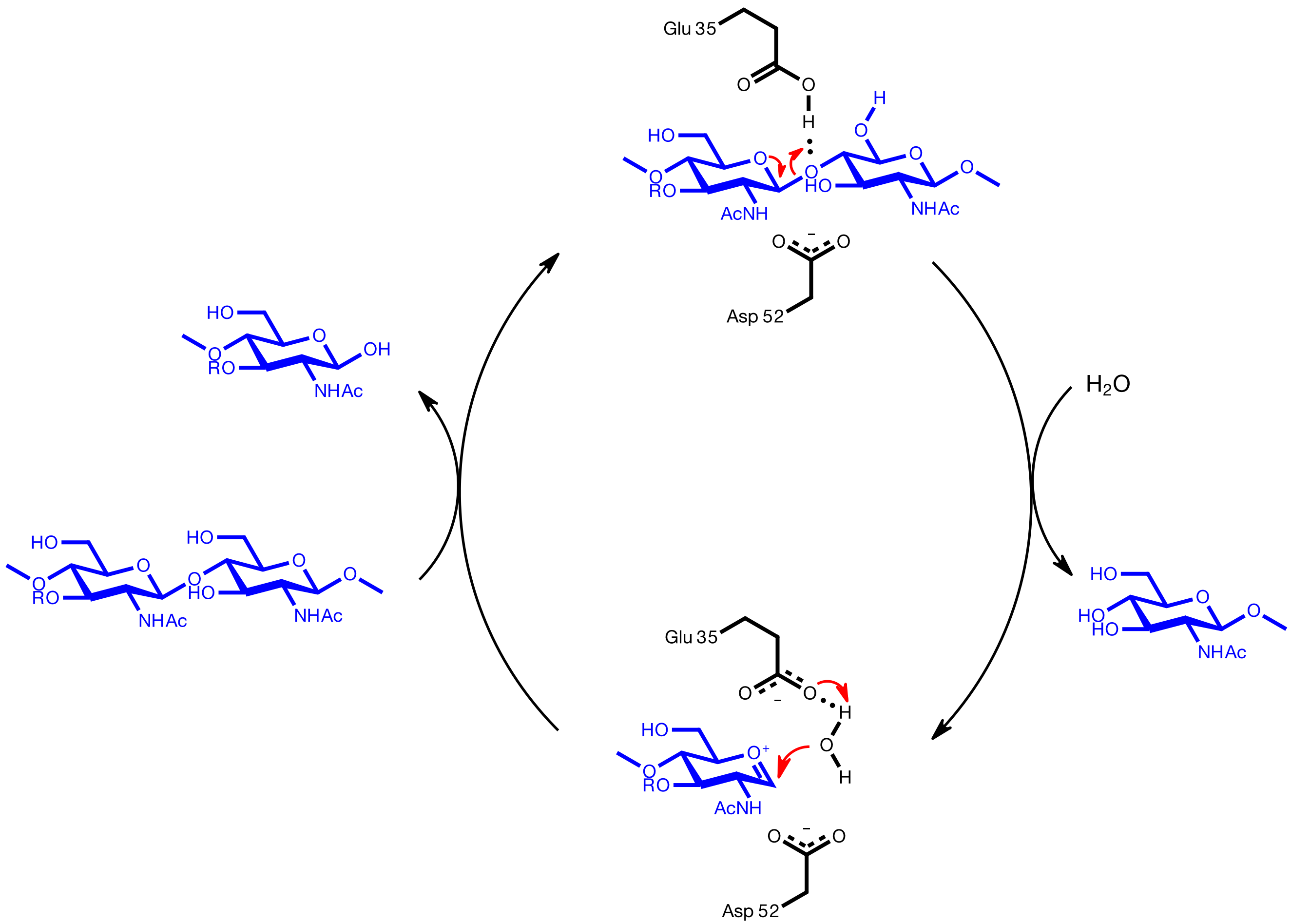
Inhibitor:



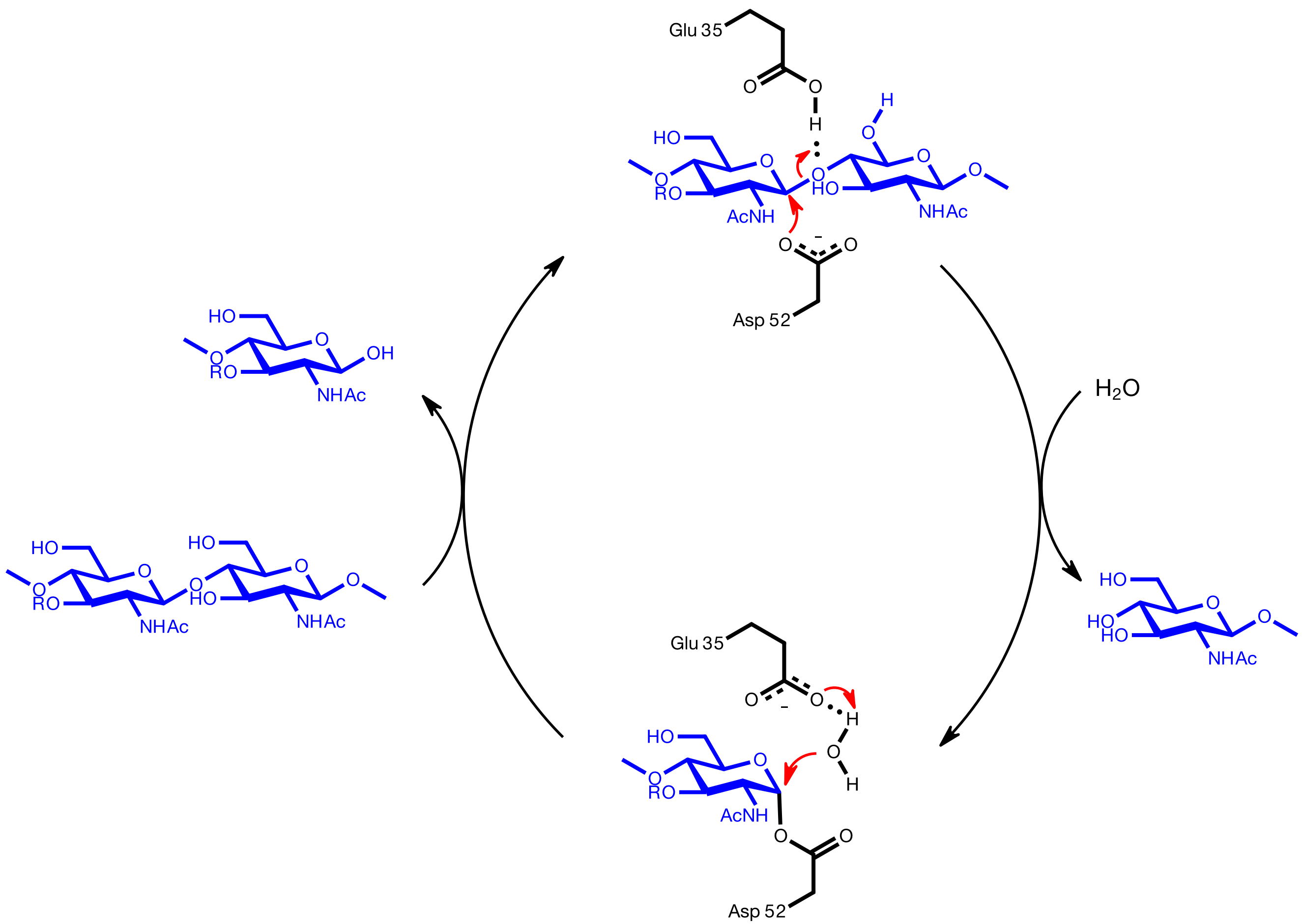




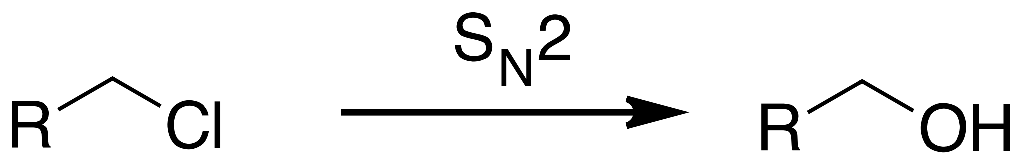
Lysozyme - Carbocation Mechanism



Lysozyme - Nucleophilic Mechanism

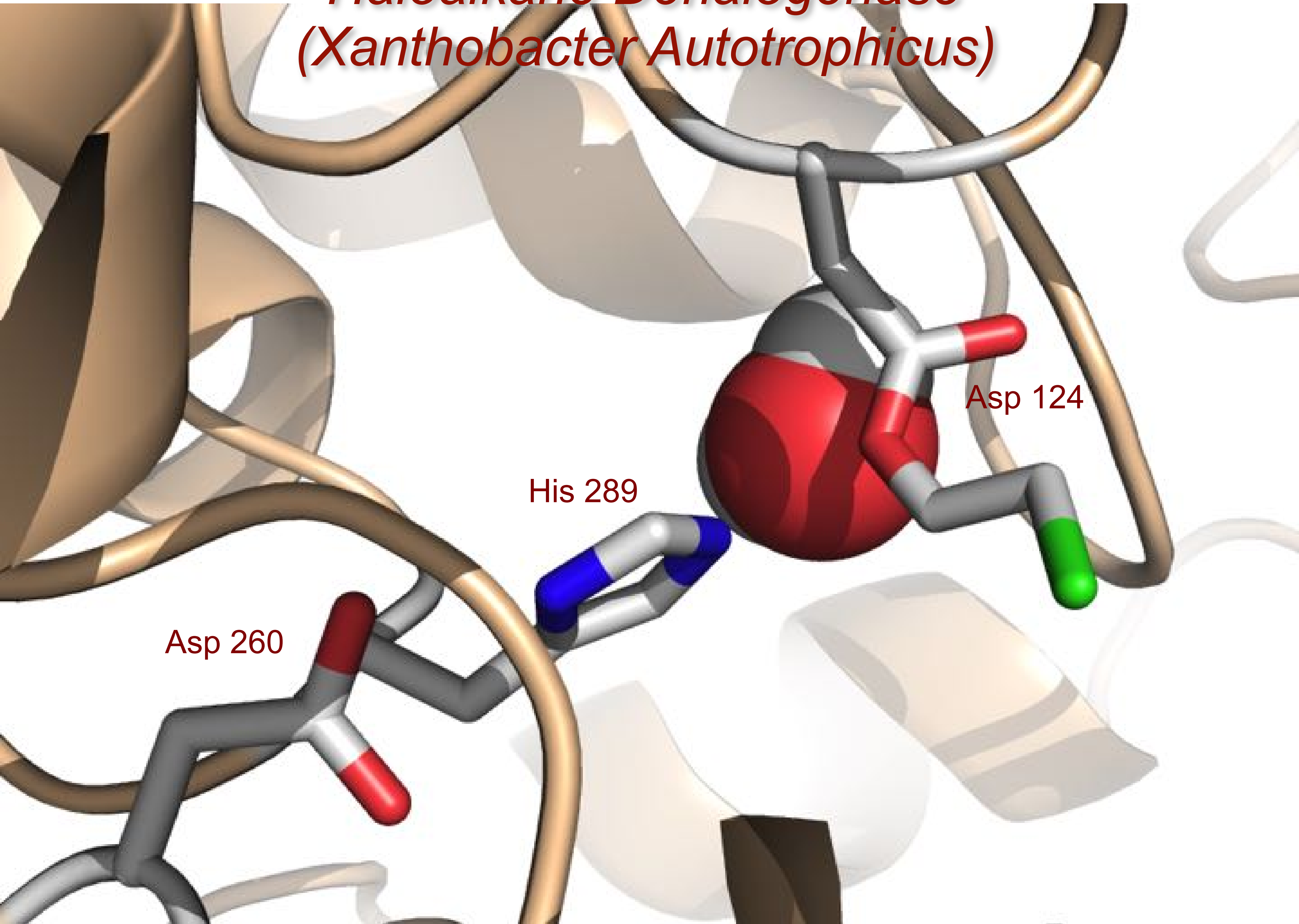


Nucleophilic Substitutions: Haloalkane Dehalogenase

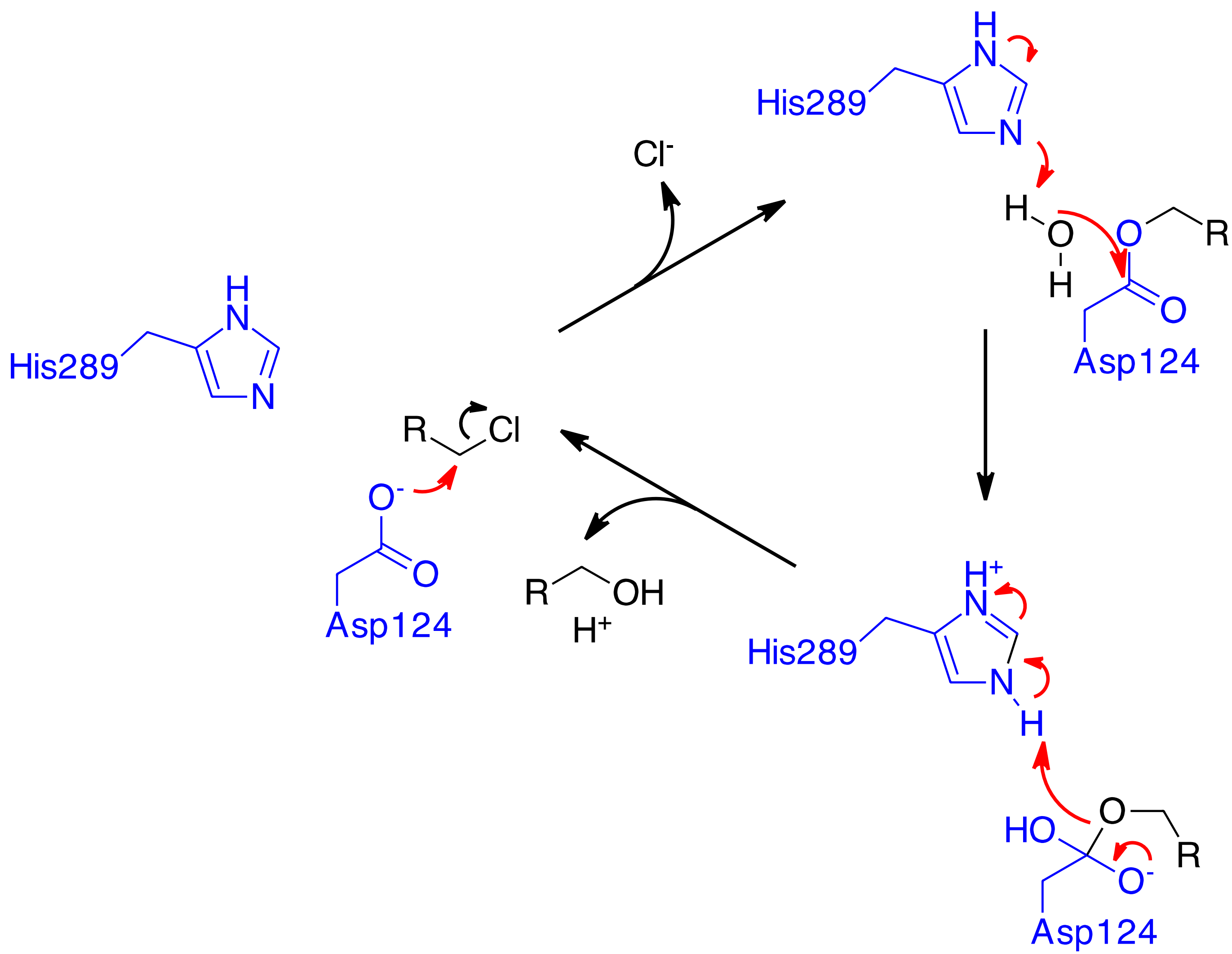


Haloalkane Dehalogenase is found in bacteria that grow in industrial wastes

Haloalkane Dehalogenase (Xanthobacter Autotrophicus)



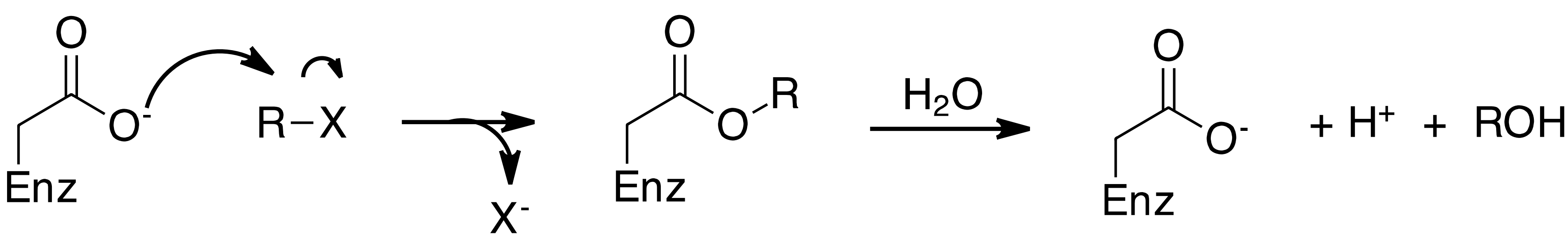
Haloalkane Dehalogenase



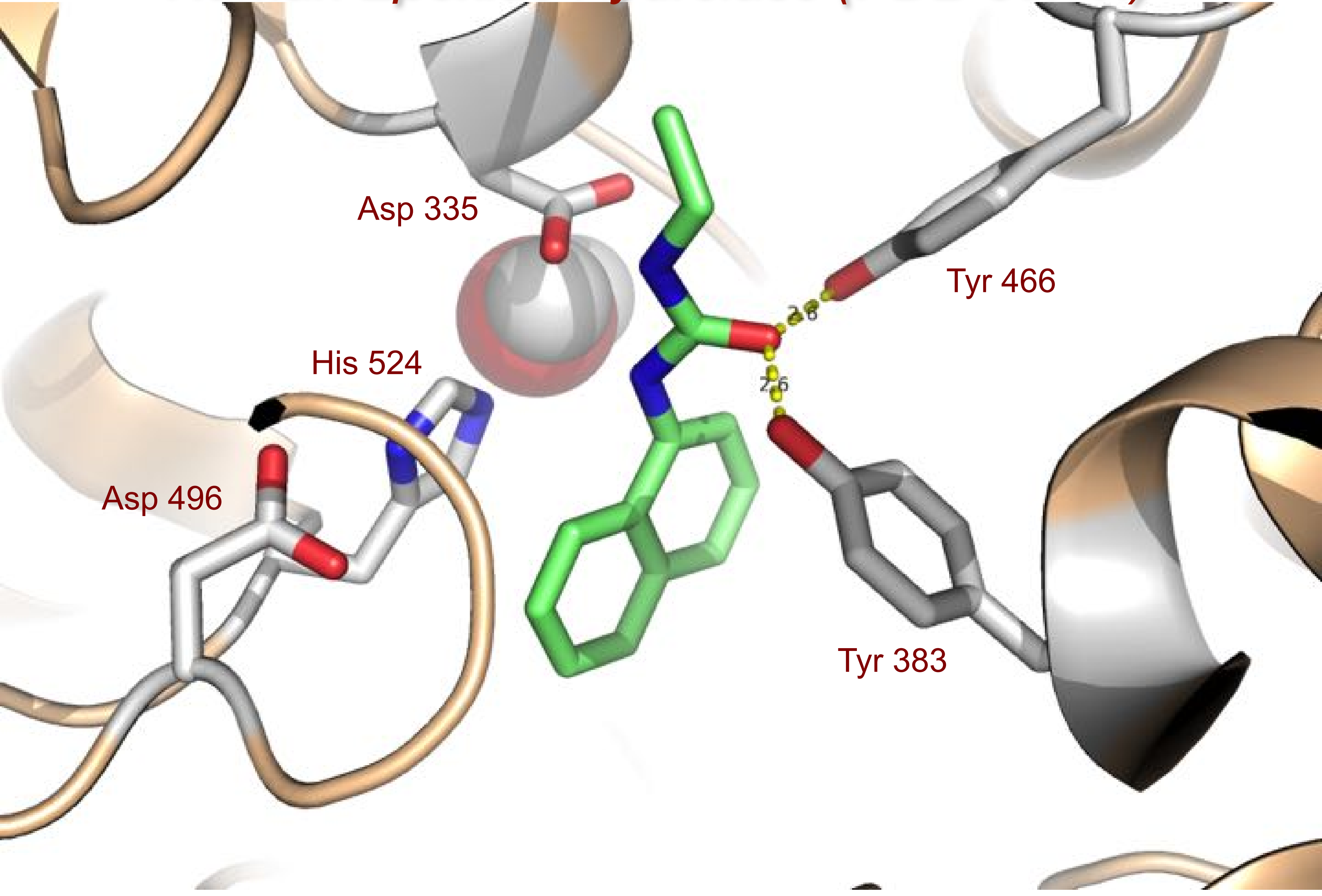
S_N2 Reactions

Detoxification of hydrophobic compounds containing a suitable leaving group

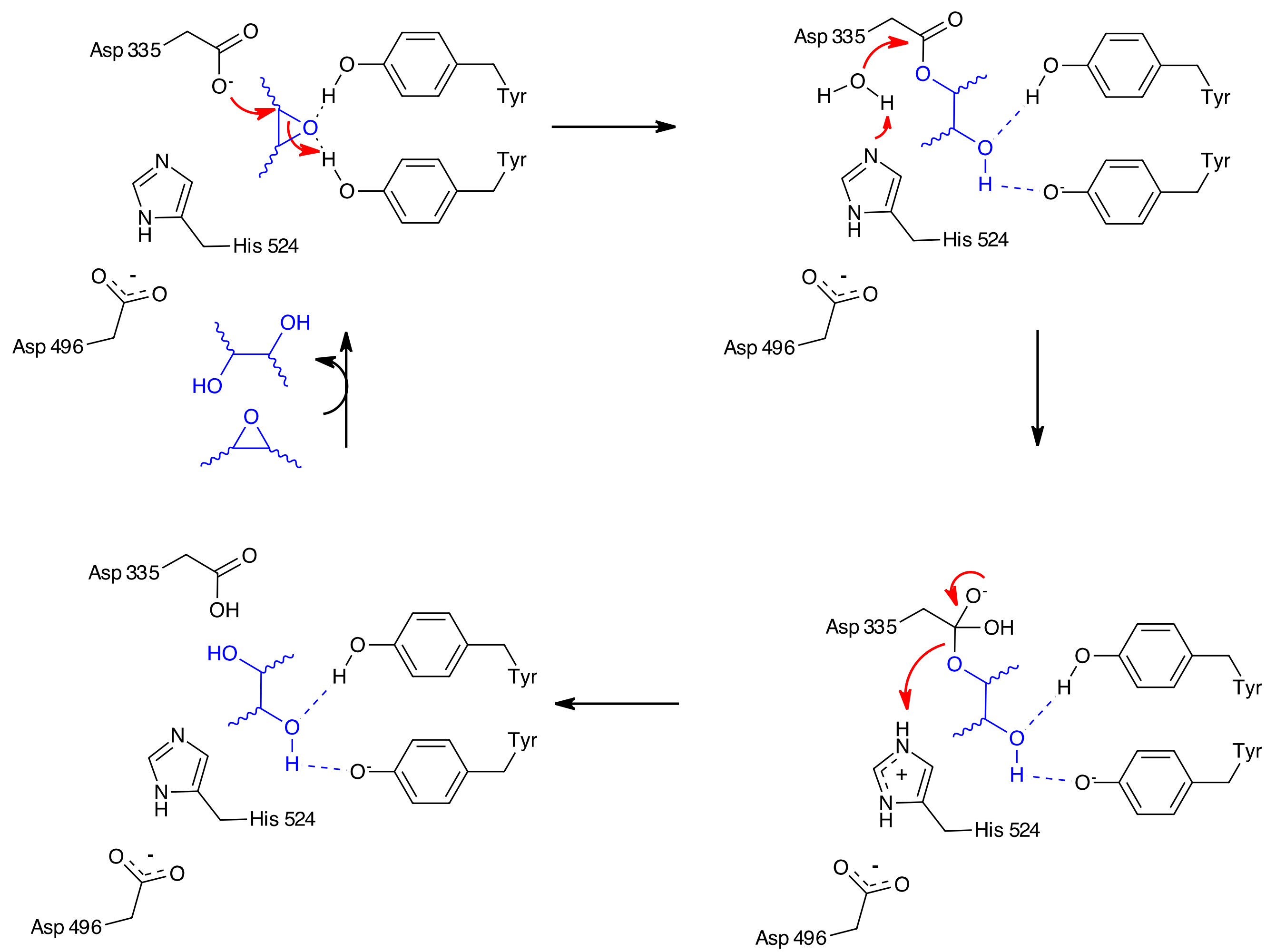
$$R{-}X \xrightarrow{H_2O} R{-}OH + HX$$



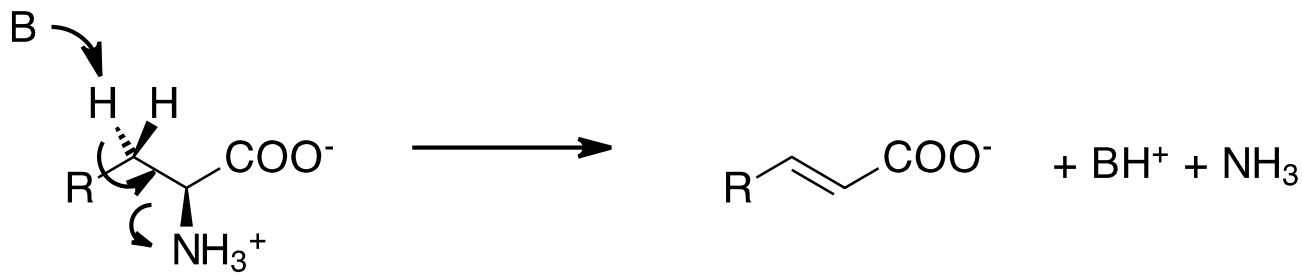
Human Epoxide Hydrolase (PDB 3WK4)



Human Epoxide Hydrolase (PDB 3WK4)

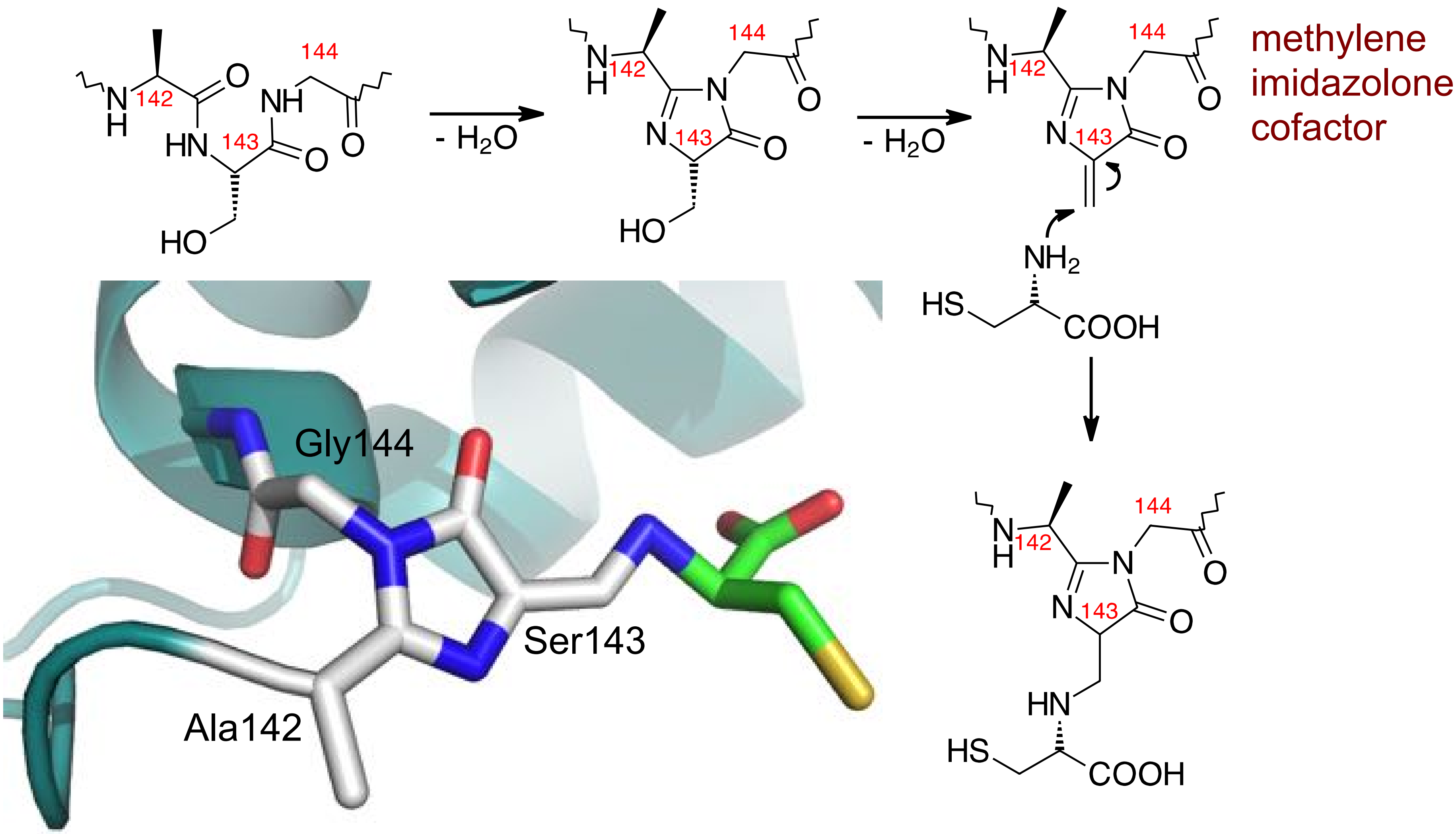


1,2-Eliminations: Histidine Ammonia Lyase

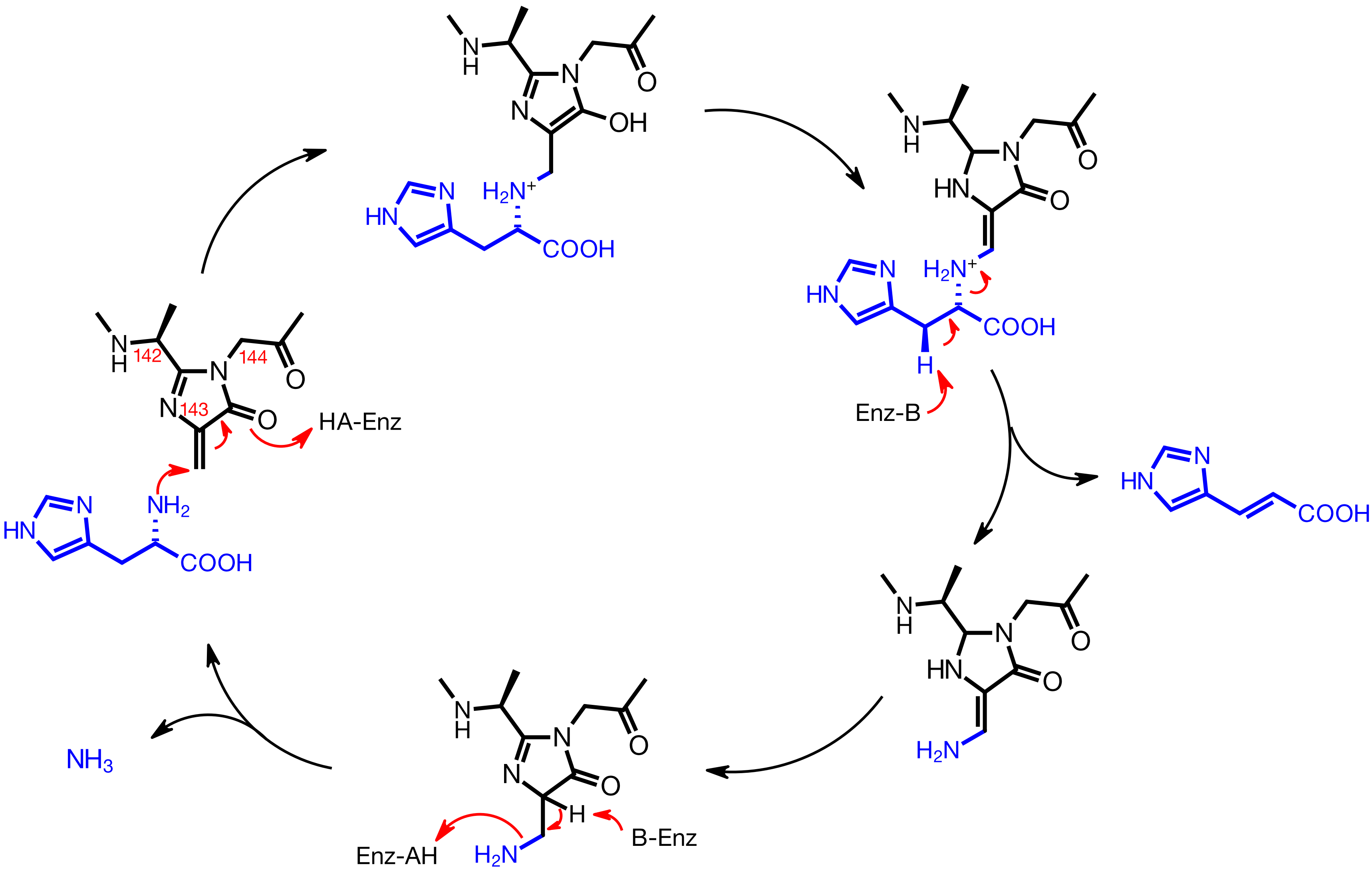


R = Ph: phenylalanine ammonia lyase
R = Im: histidine a. l.
R = COO^- aspartase

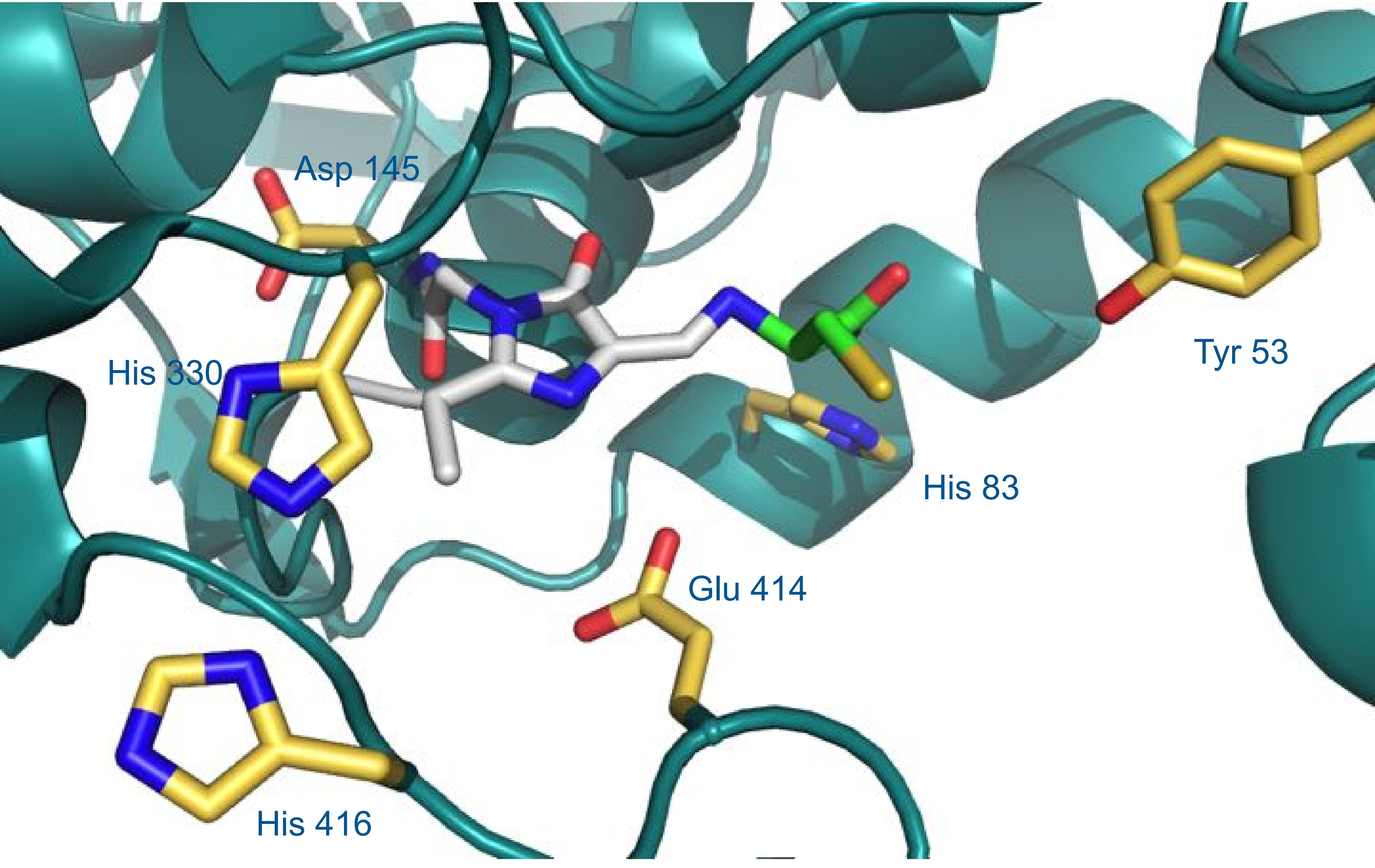
1,2-Eliminations: Histidine Ammonia Lyase



1,2-Eliminations: Histidine Ammonia Lyase

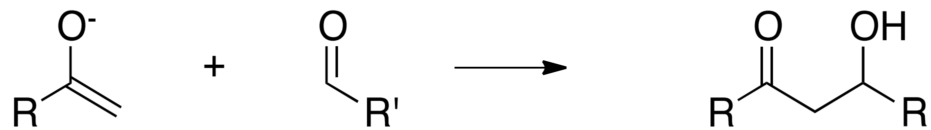


Pseudomonas Putida Histidine Ammonia Lyase

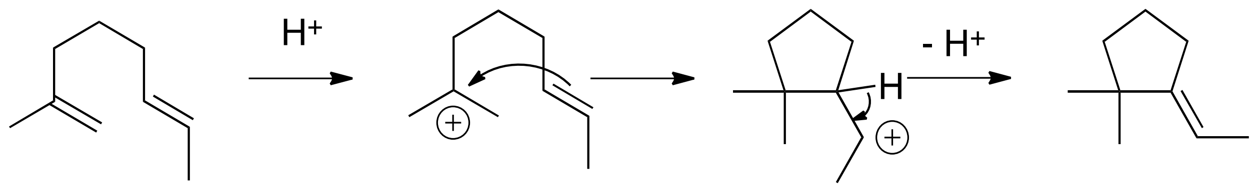


C-C Bond Formation

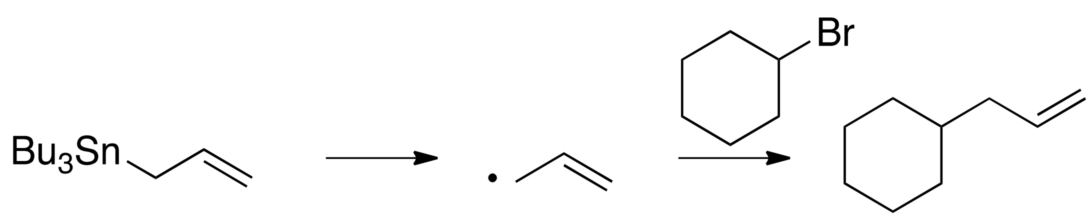
anionic



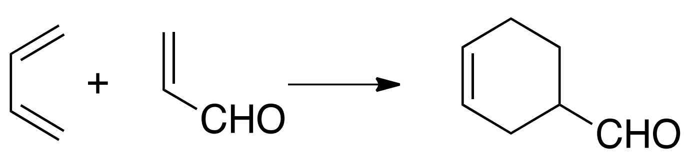
cationic



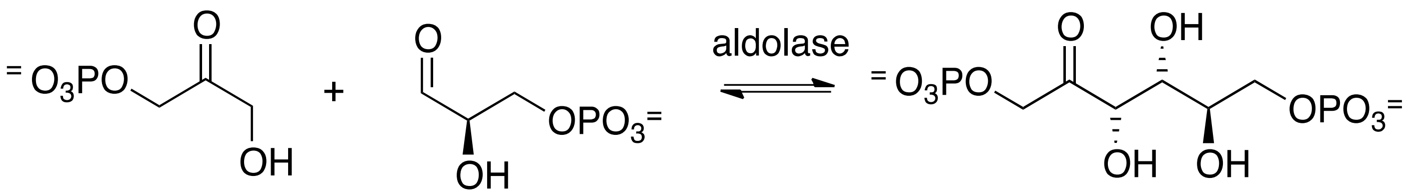
radicalic



pericyclic



Aldolases (Fructose 1,6-Diphosphate Aldolases)

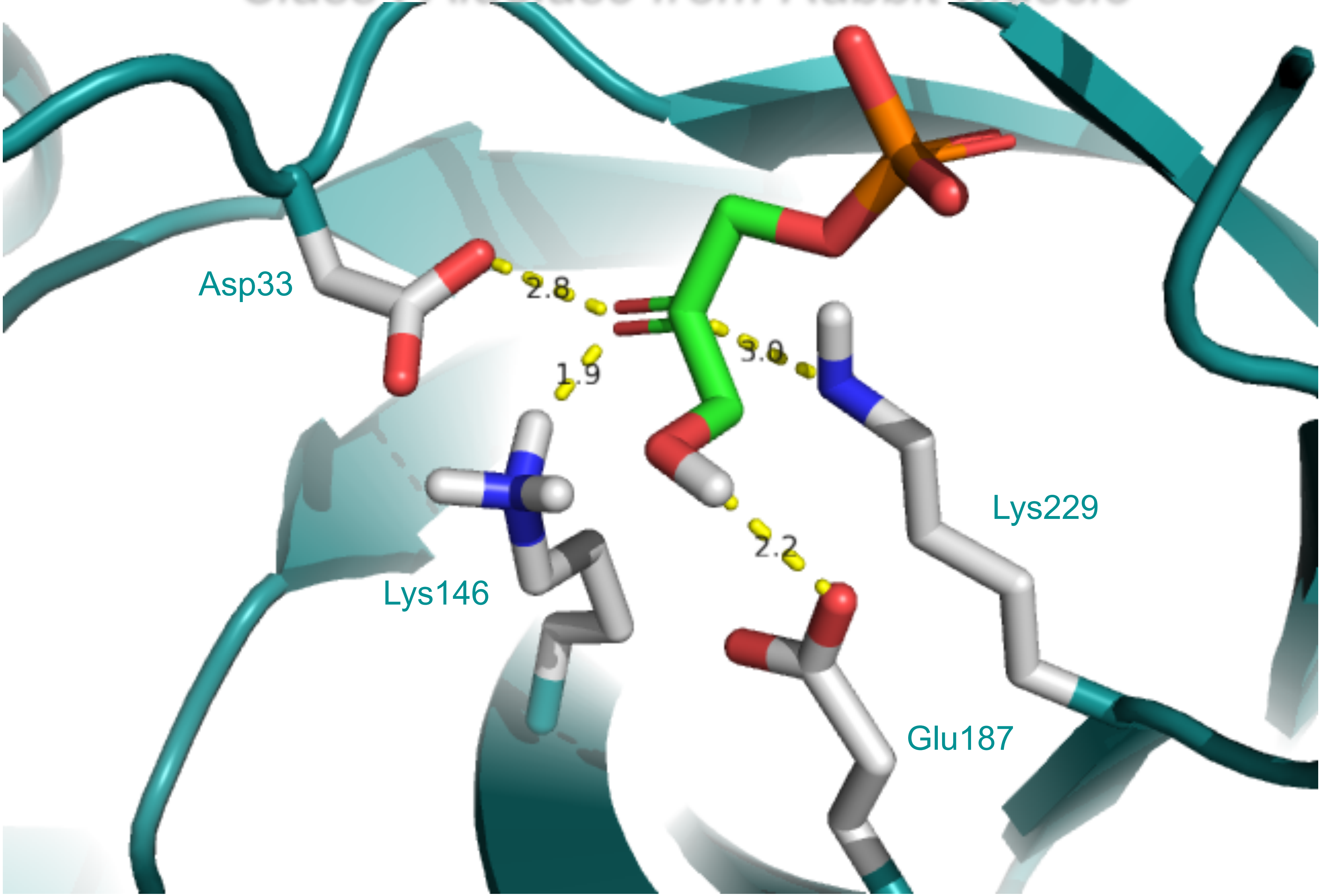


Aldolases: Class I – via enamine Lys-NH_2

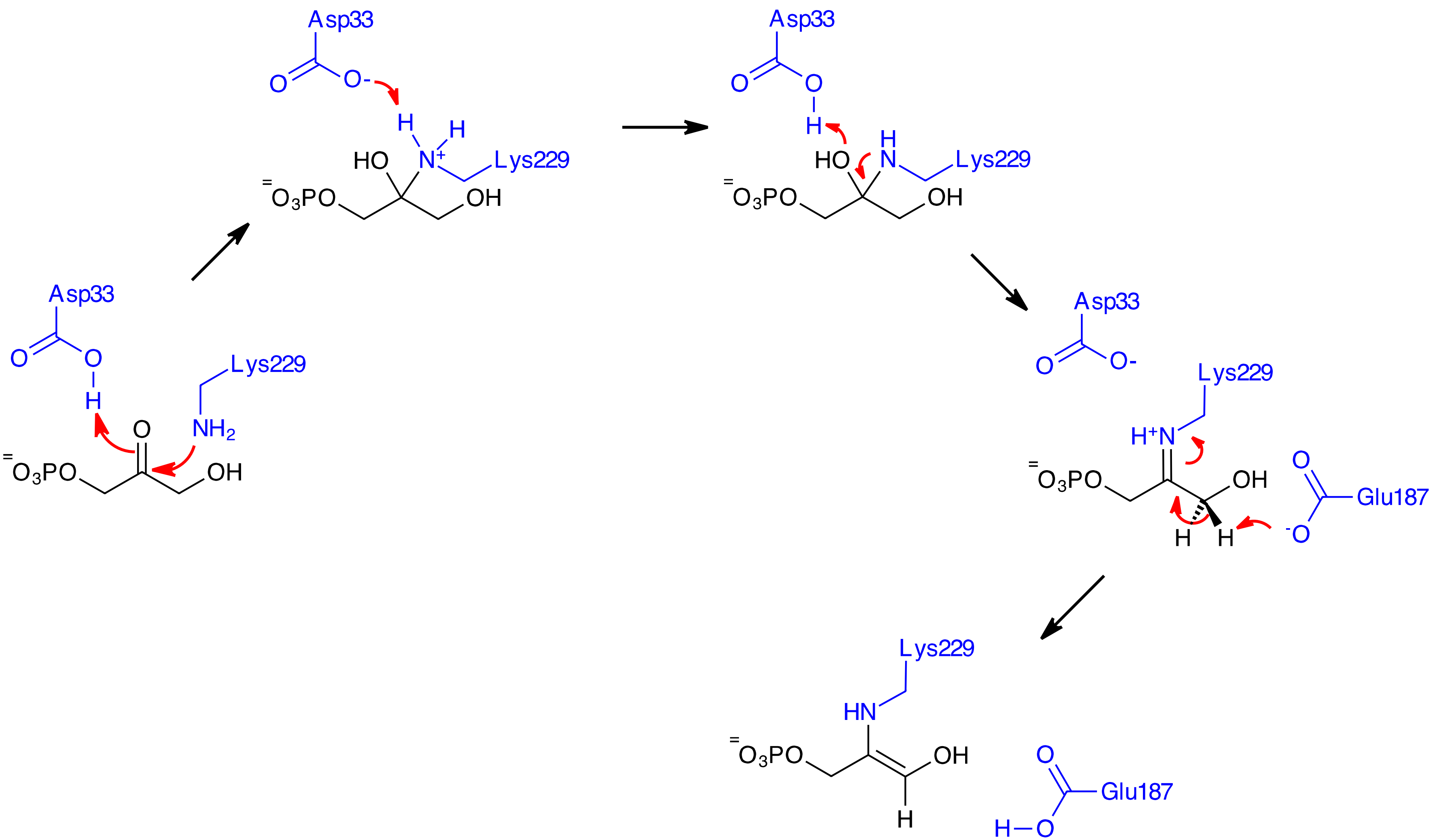
Classe II – metal enzymes (Mg^{2+}, Zn^{2+}, Mn^{2+})

Fructose 1,6-diphosphate aldolase: class I in mammals
class II in bacteria

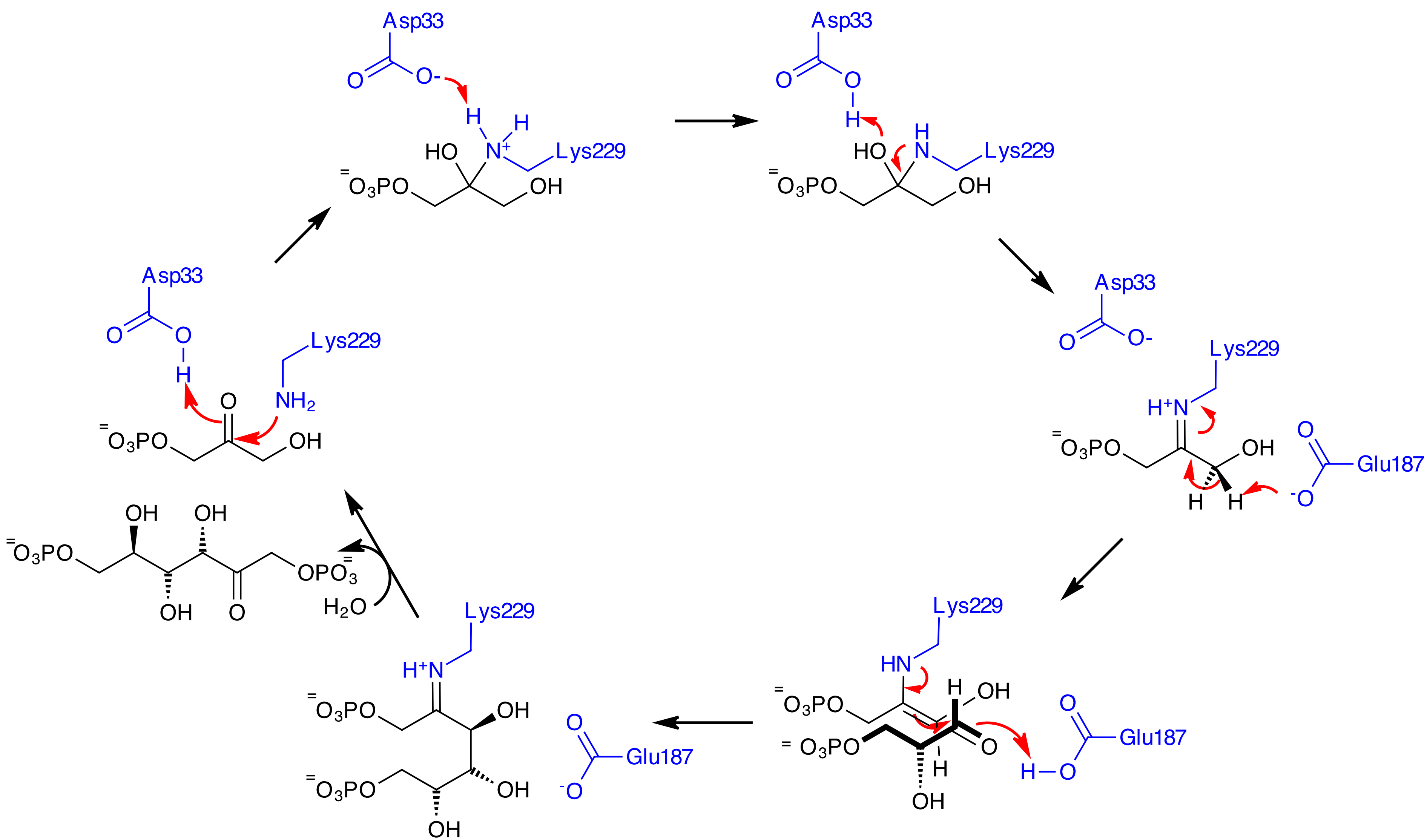
Class I Aldolase from Rabbit Muscle



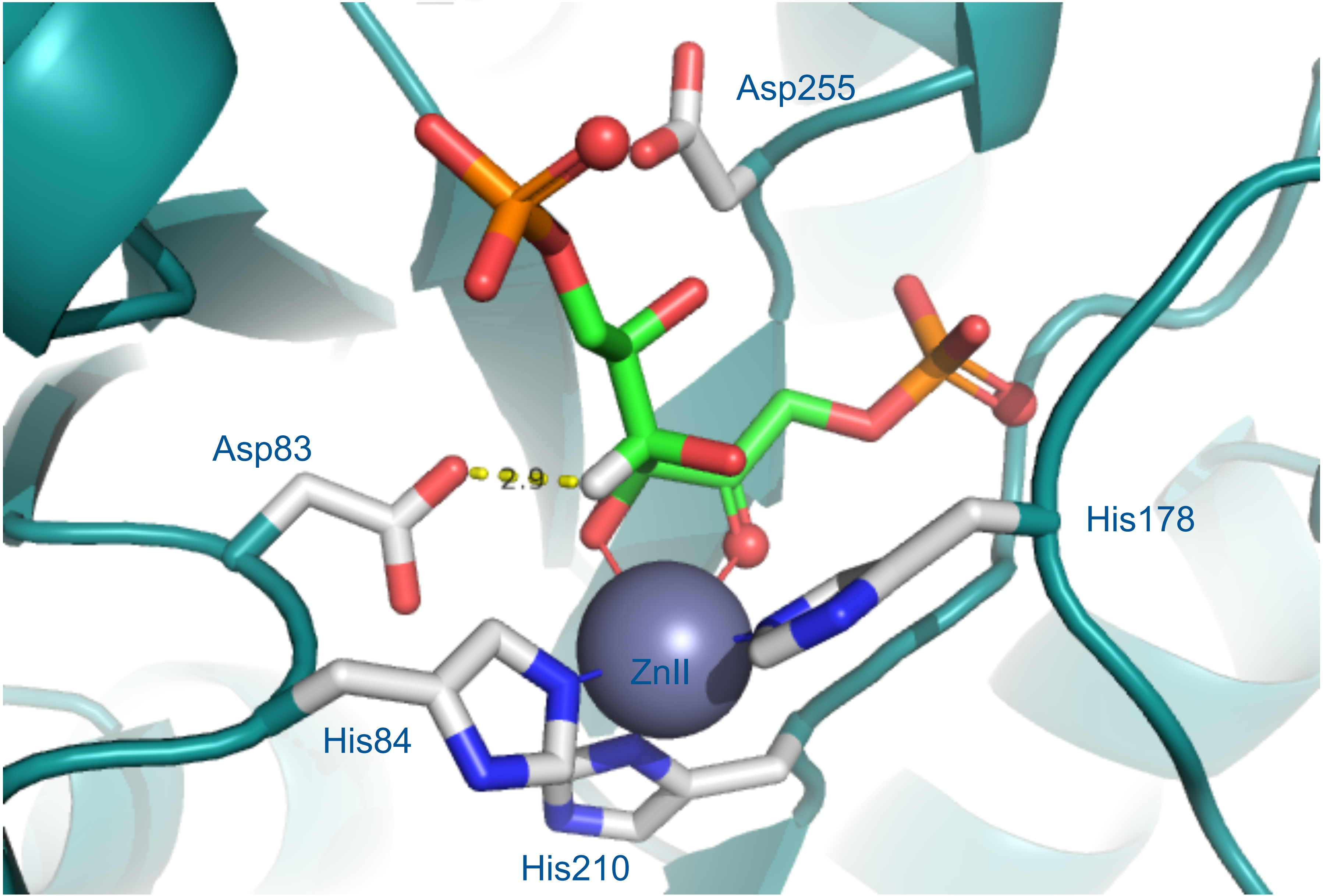
Class I Aldolase: Mechanism



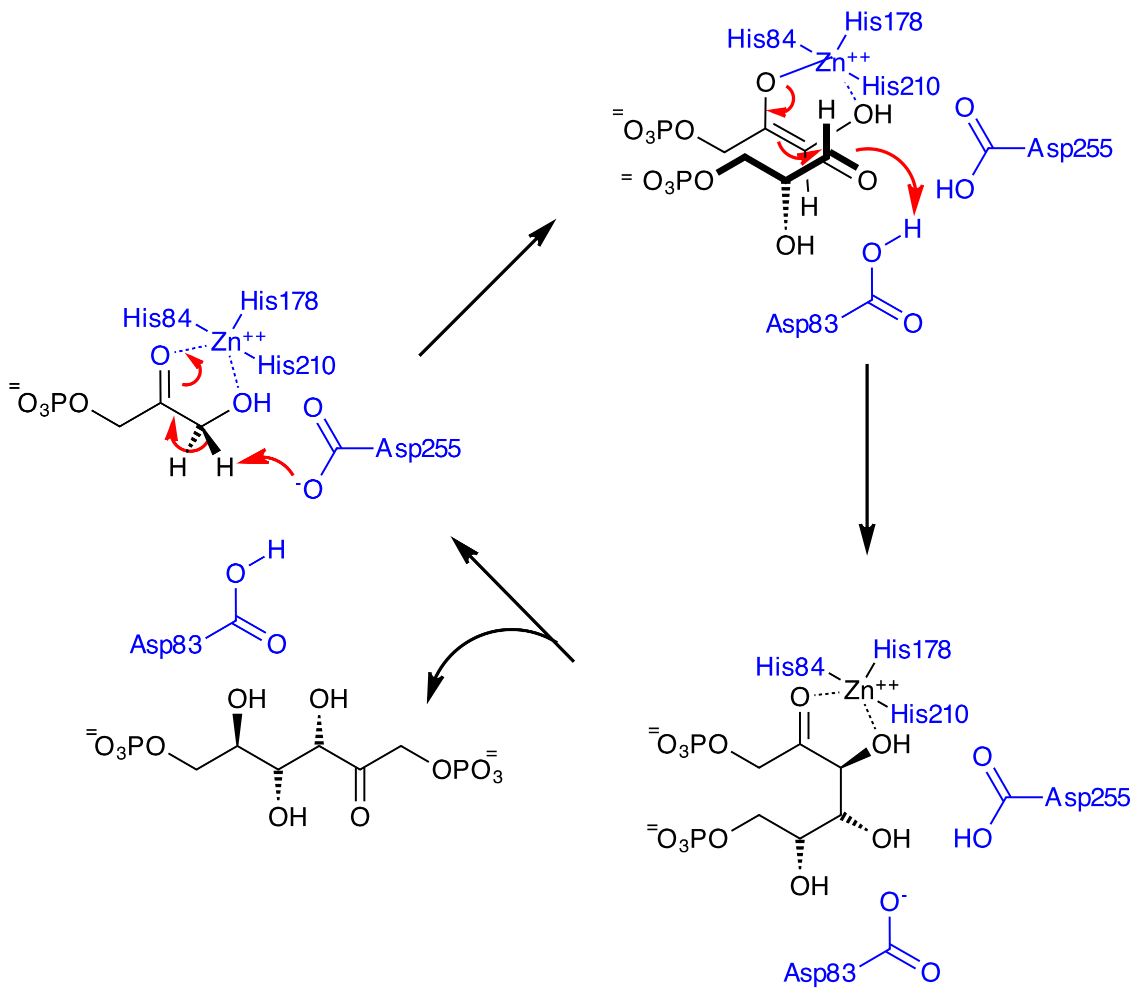
Class I Aldolase: Mechanism



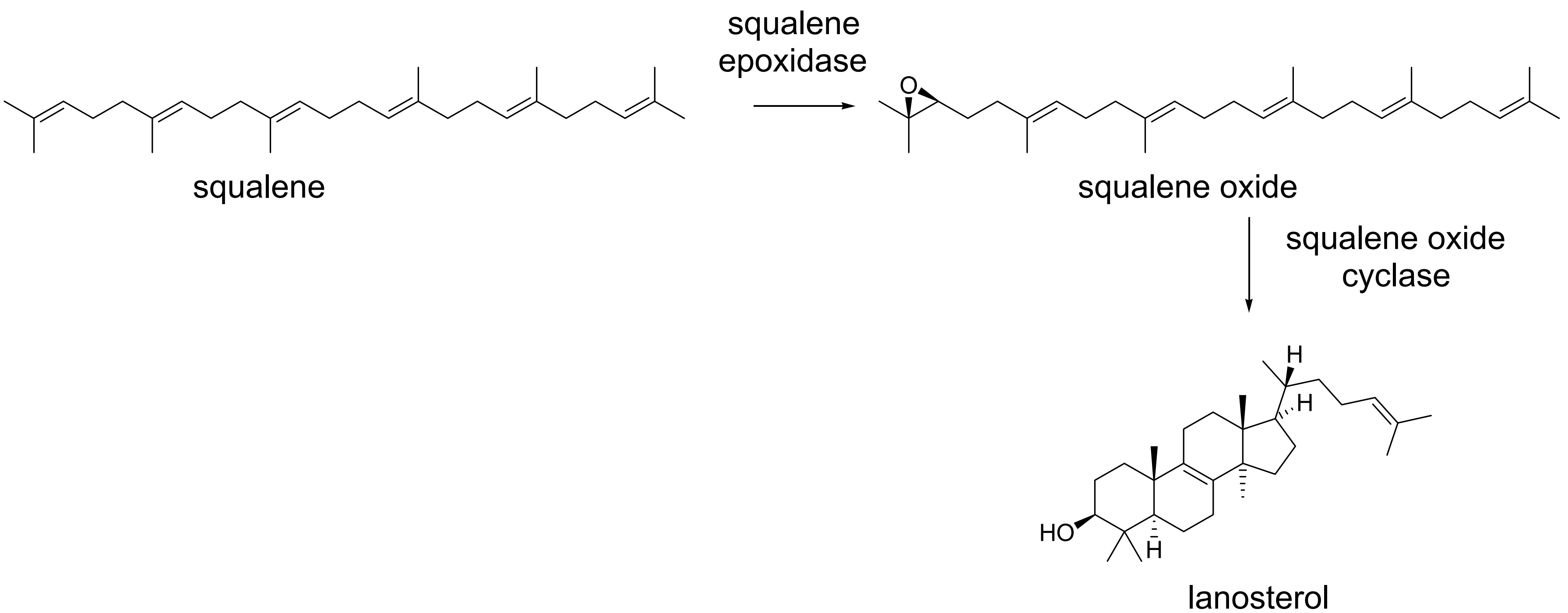
Class II Aldolase from Giardia lamblia Complexed with Tagatose-1,6-diphosphate



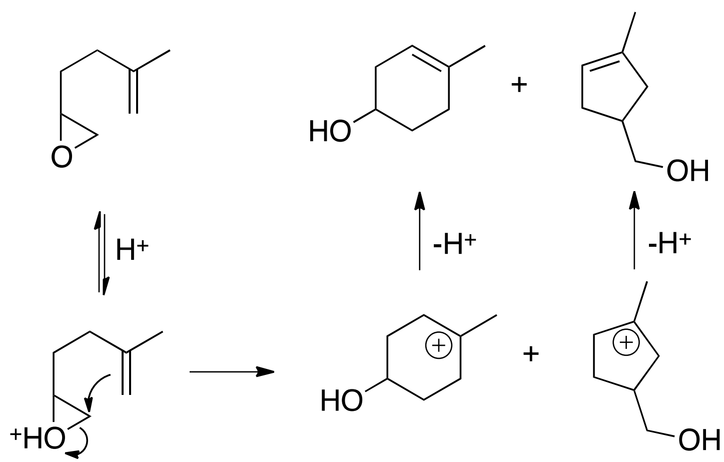
Class II Aldolase



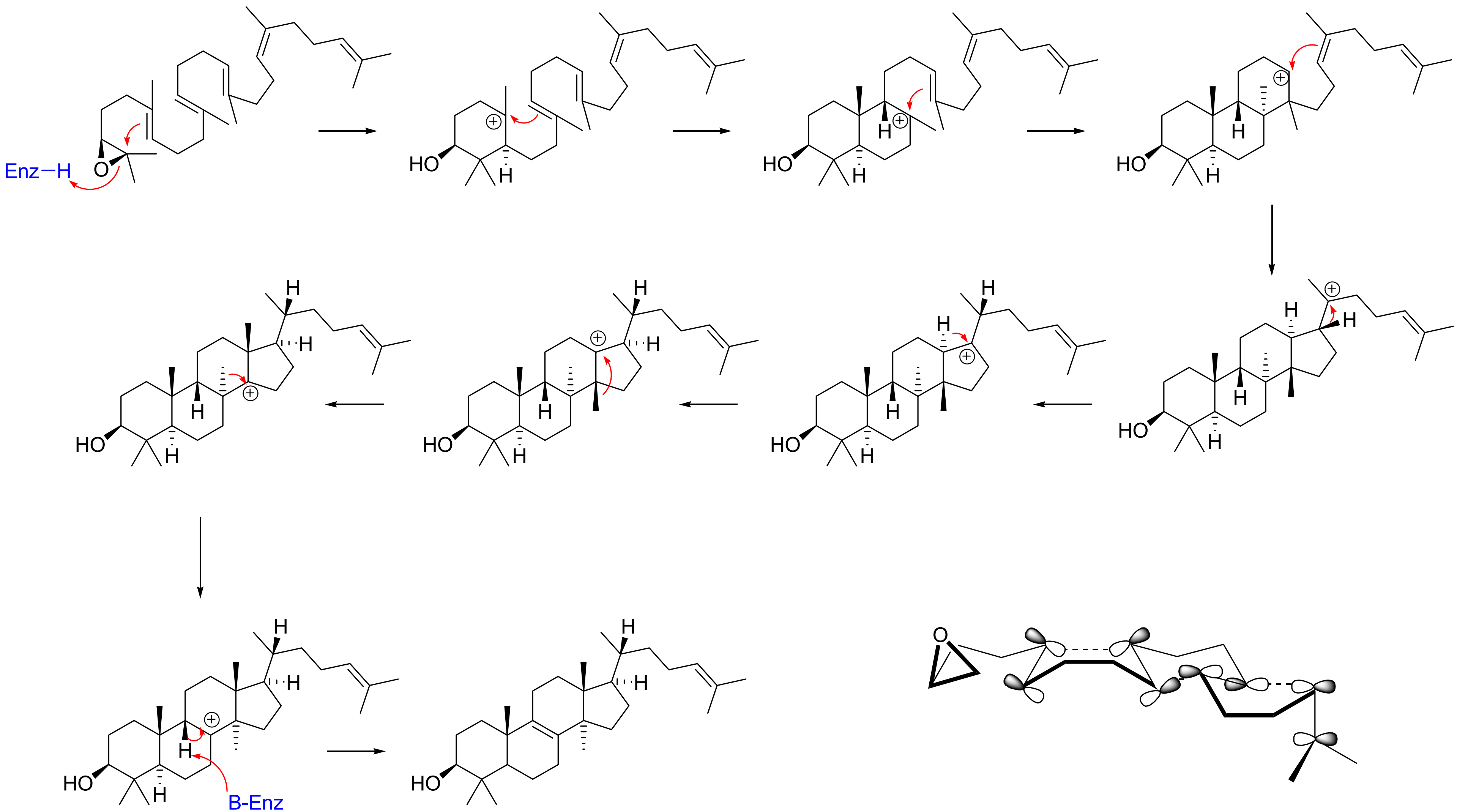
Squalene Oxide Cyclase



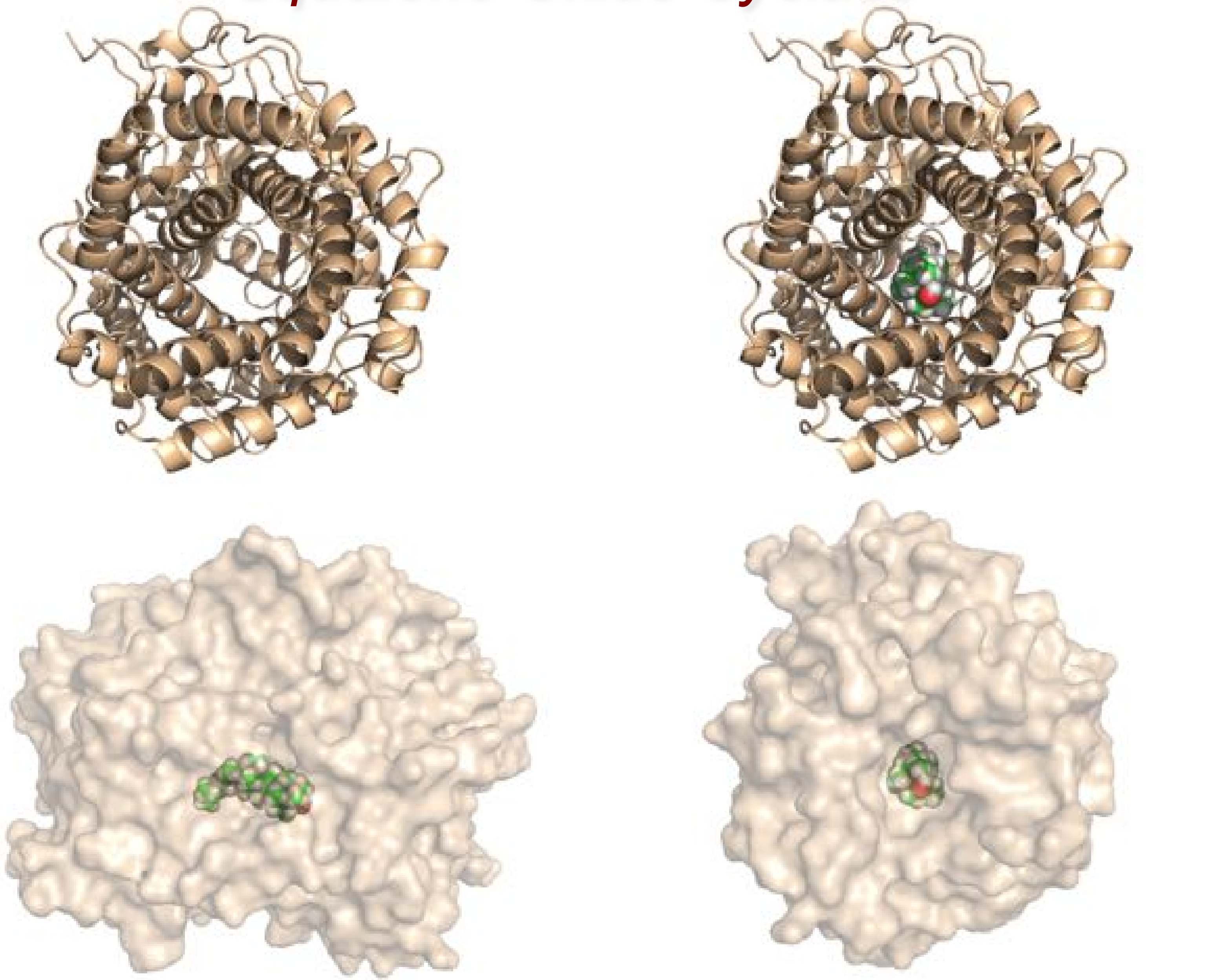
model reaction:



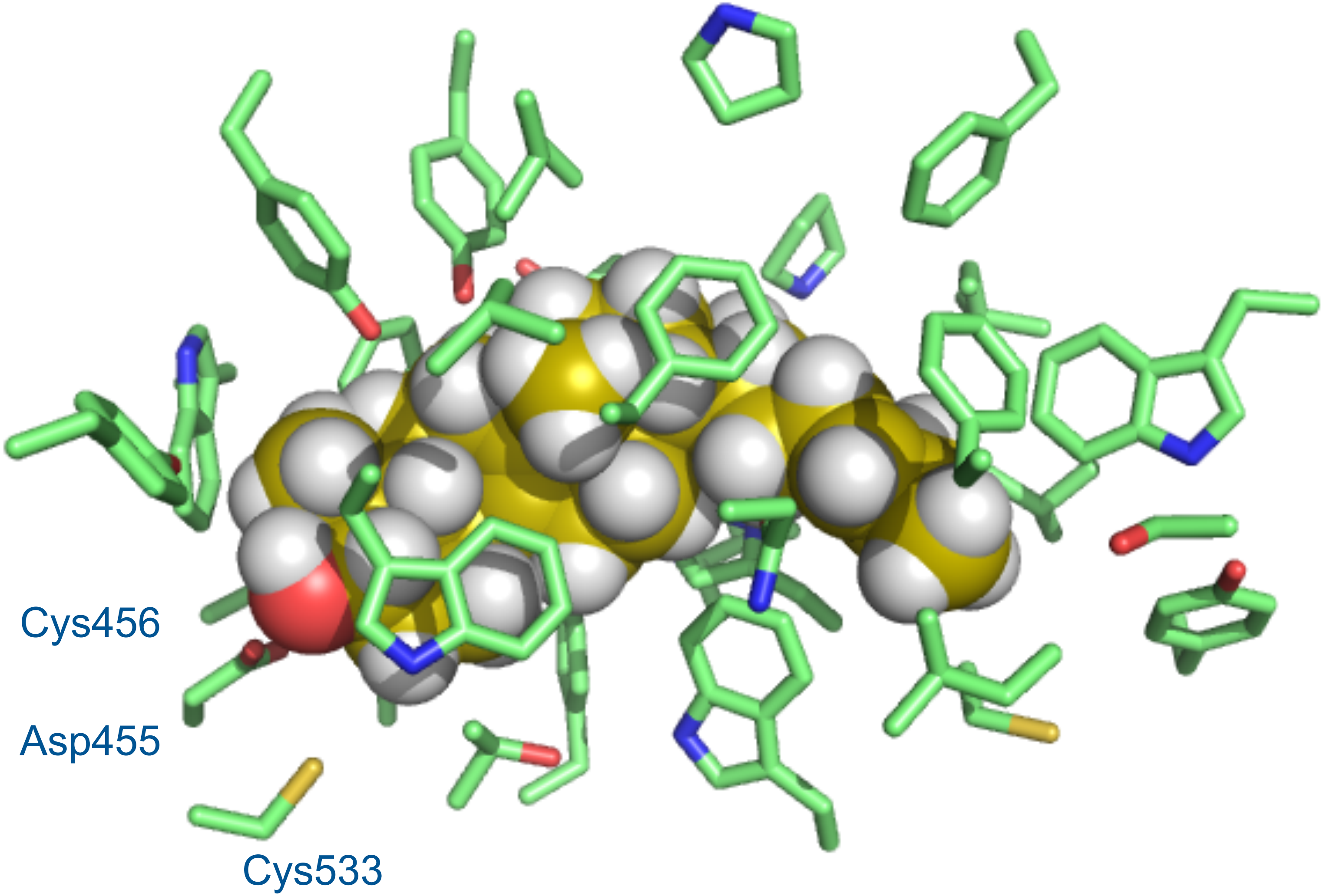
Squalene Oxide Cyclase Mechanism



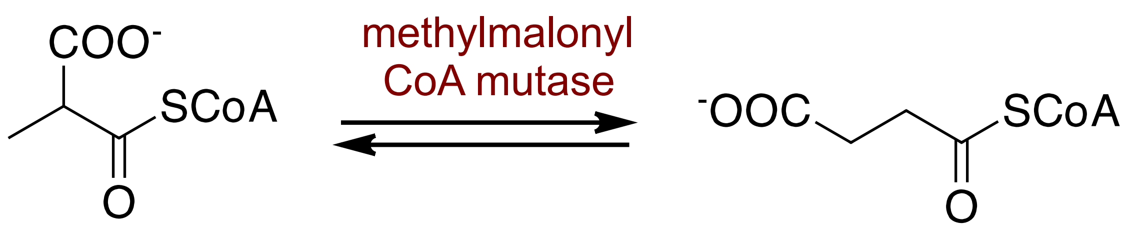
Squalene Oxide Cyclase



Squalene Oxide Cyclase



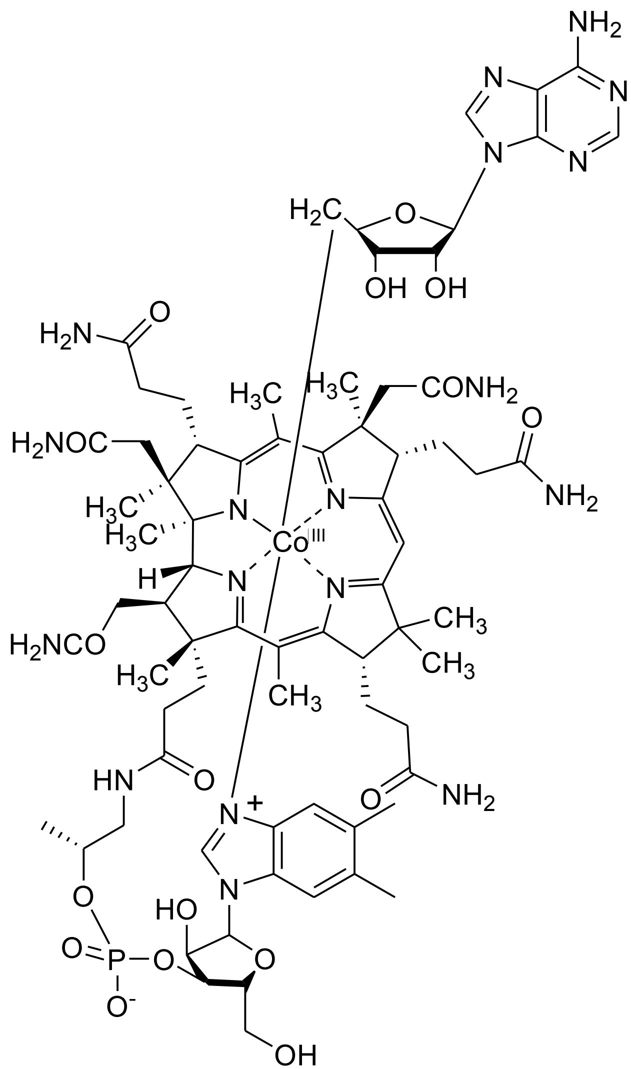
Radical Reactions: Methylmalonyl CoA Mutase

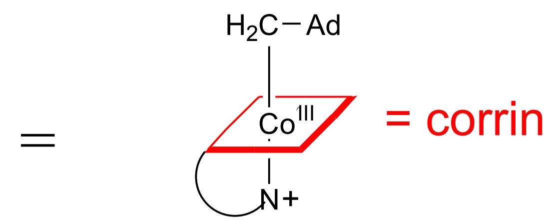


methylmalonylCoA

succinylCoA

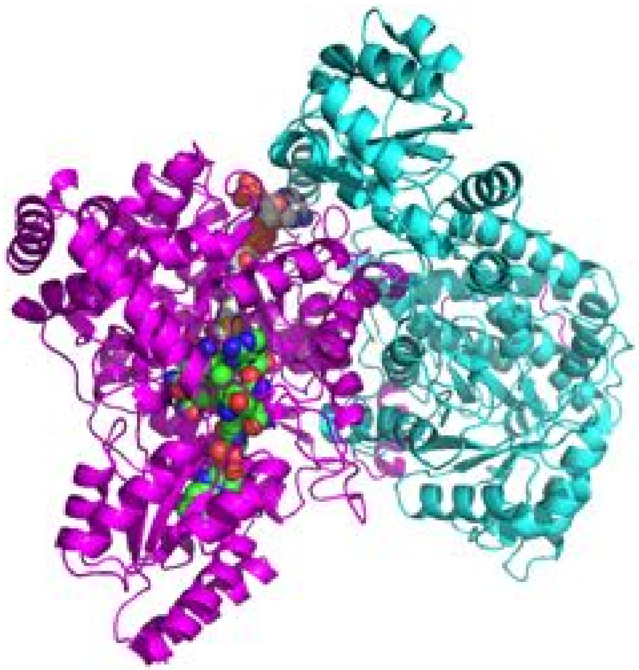
B12 Vitamin

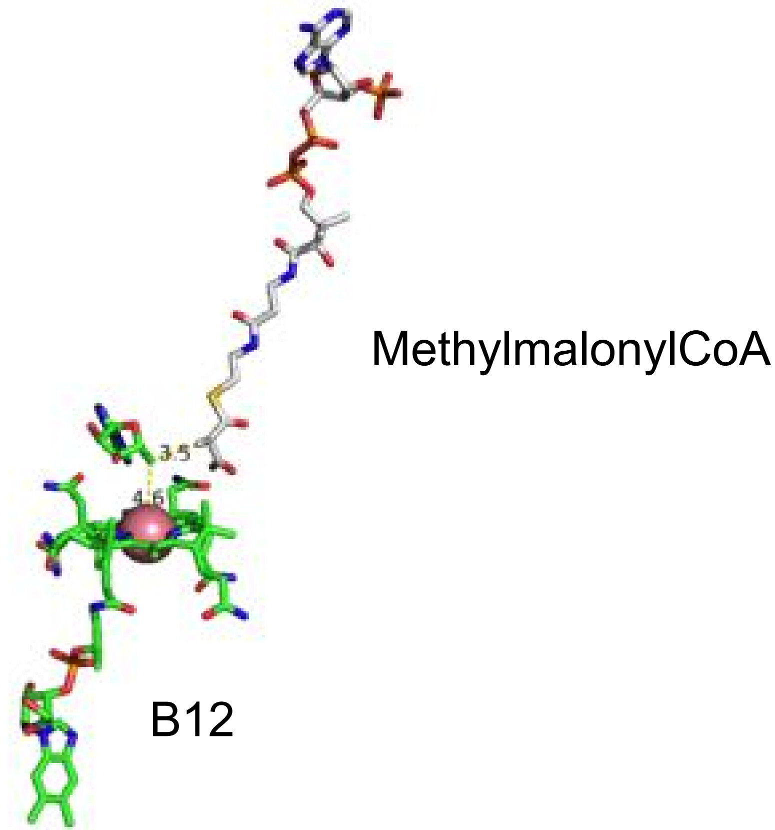




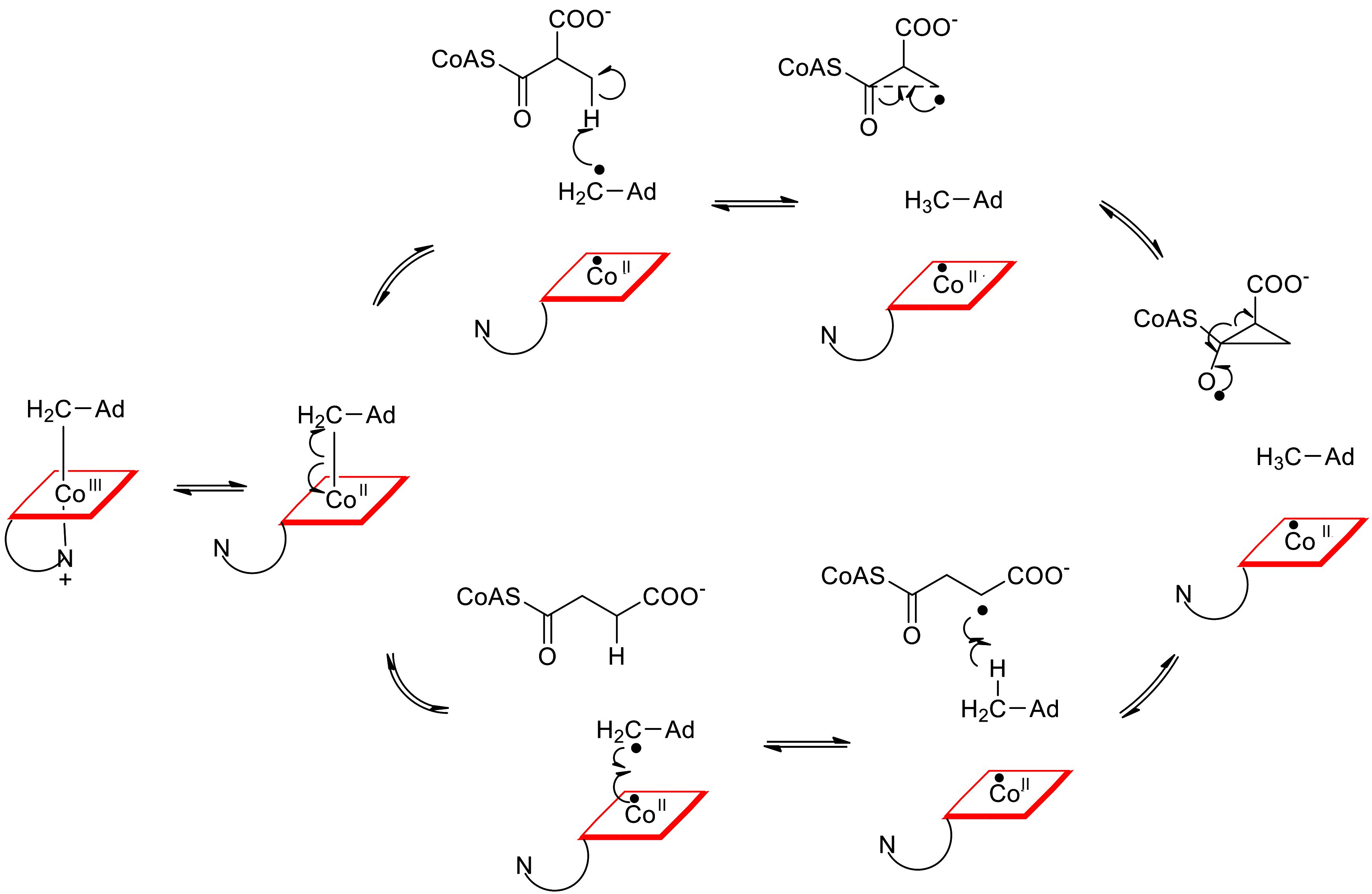
5' deoxyadenosylcobalamine

Methylmalonyl CoA Mutase



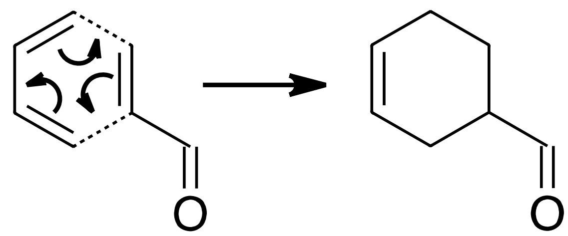


Methylmalonyl CoA Mutase

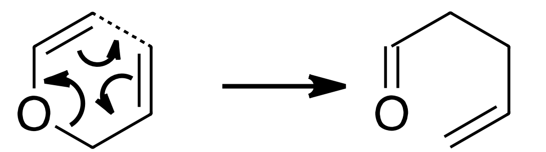


Pericyclic Reactions

Diels-Alder cycloaddition

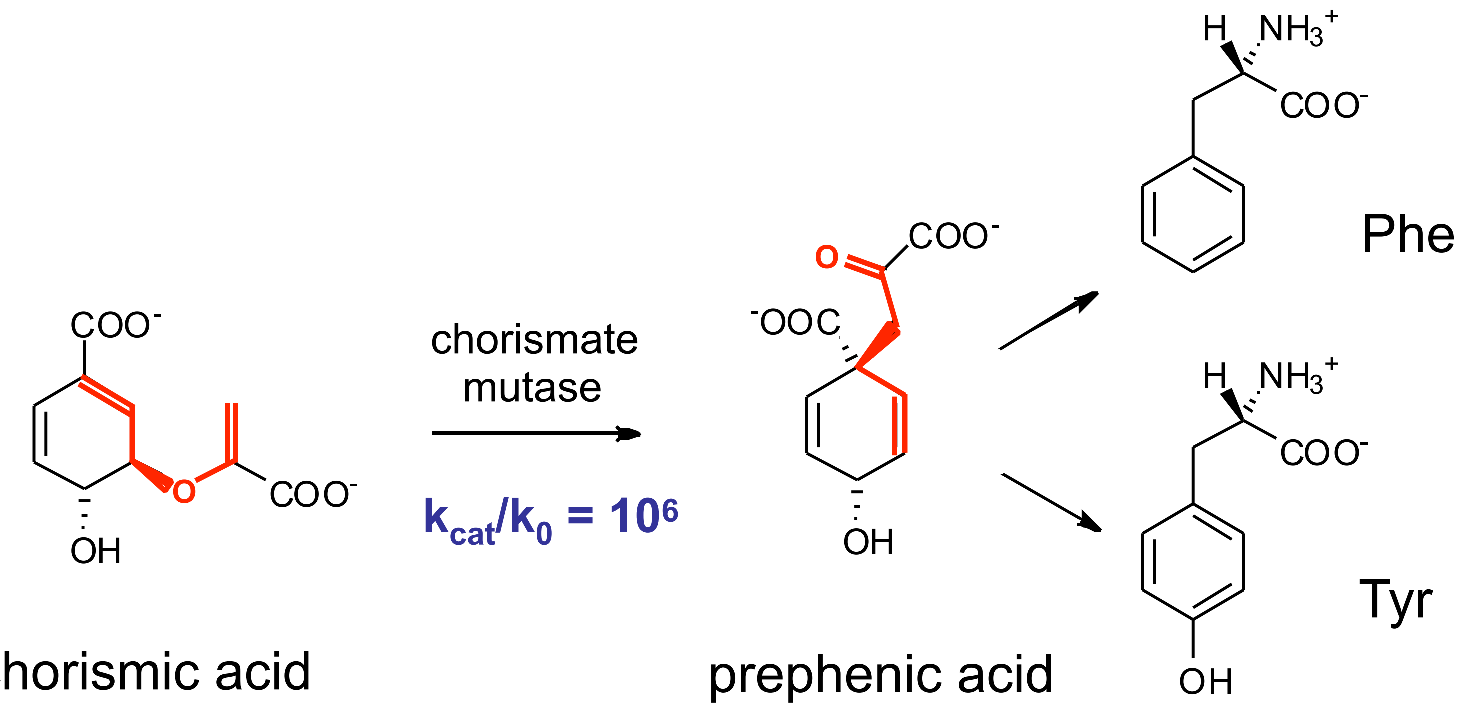


Claisen rearrangement



$\Delta H^{\#}$ is generally small, $\Delta S^{\#}$ is generally large and negative

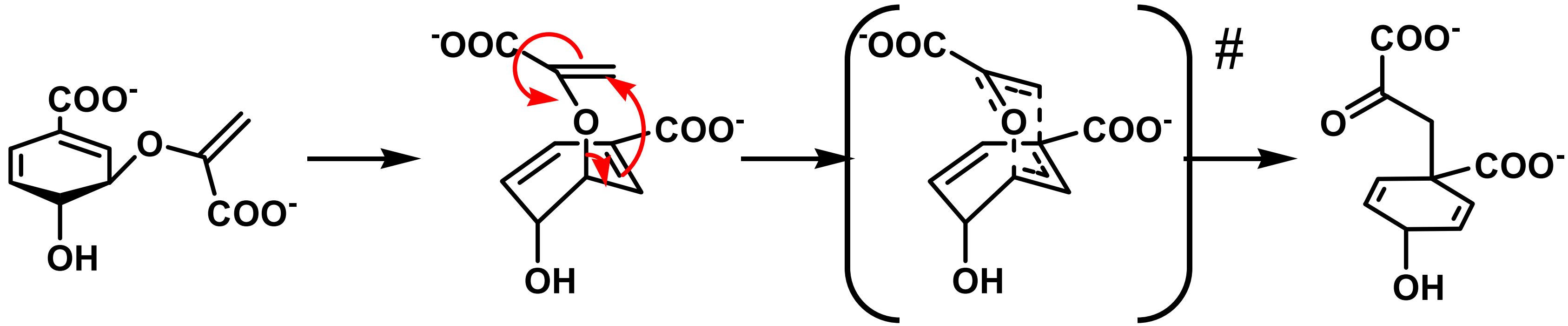
Chorismate mutase



chorismic acid

prephenic acid

Claisen Rearrangement

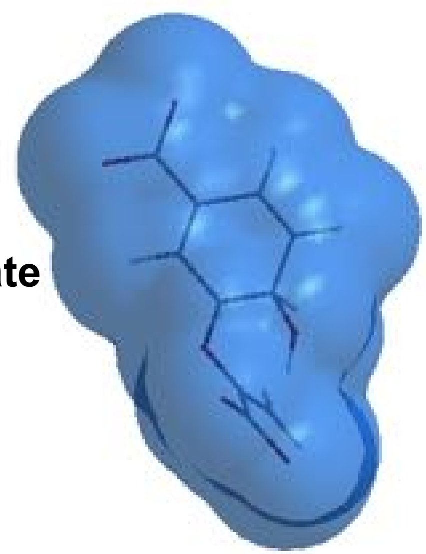


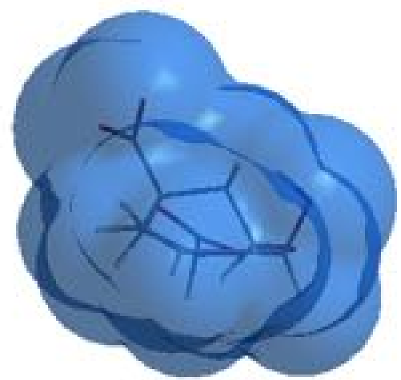
chorismic acid

TS

prephenic acid

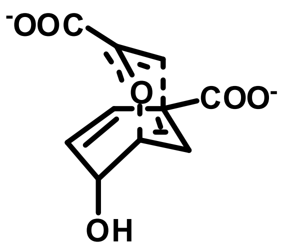
substrate



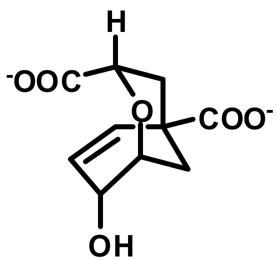


transition state

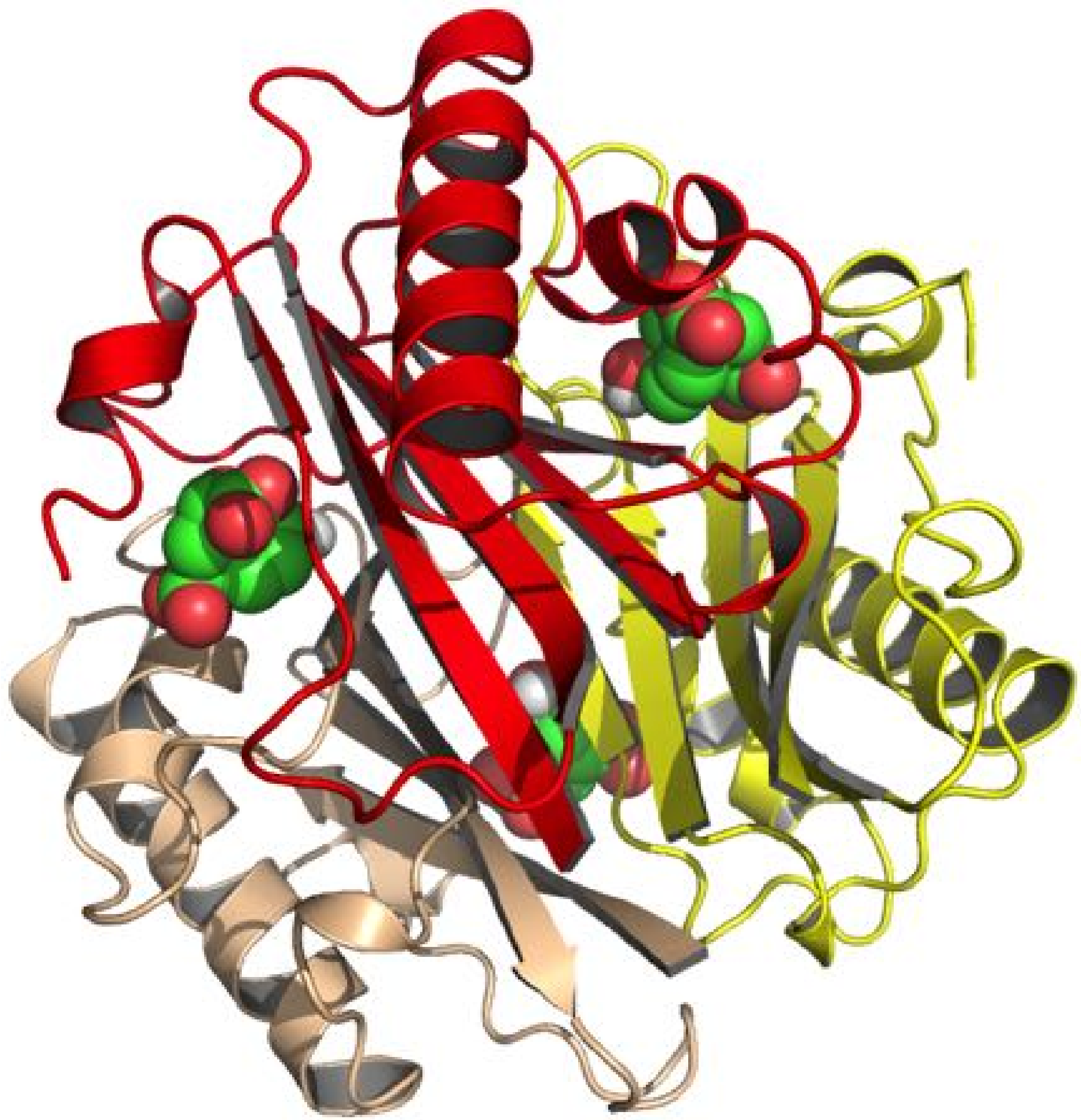
Chorismate mutase



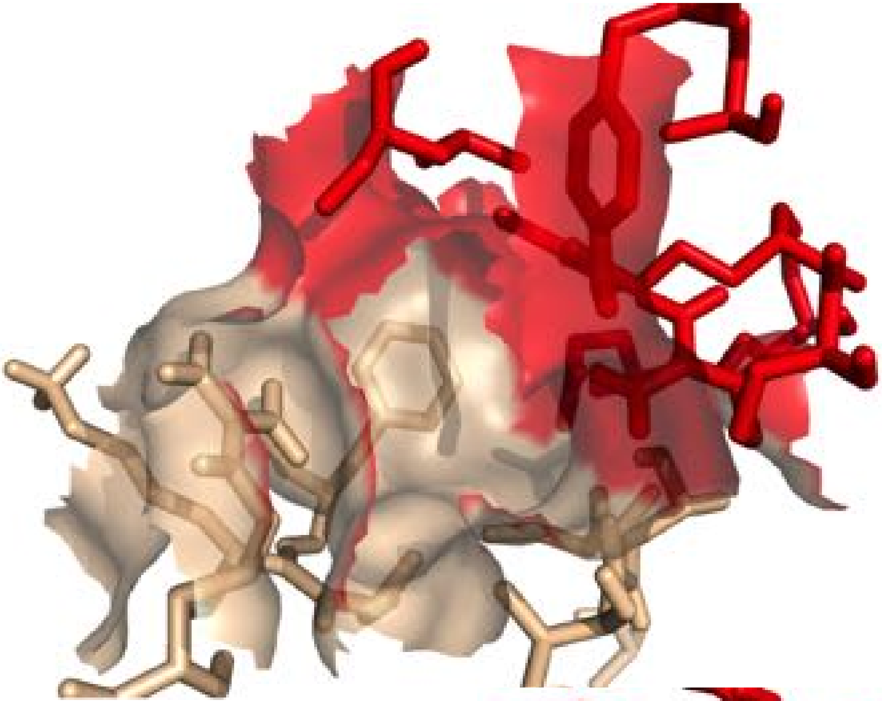
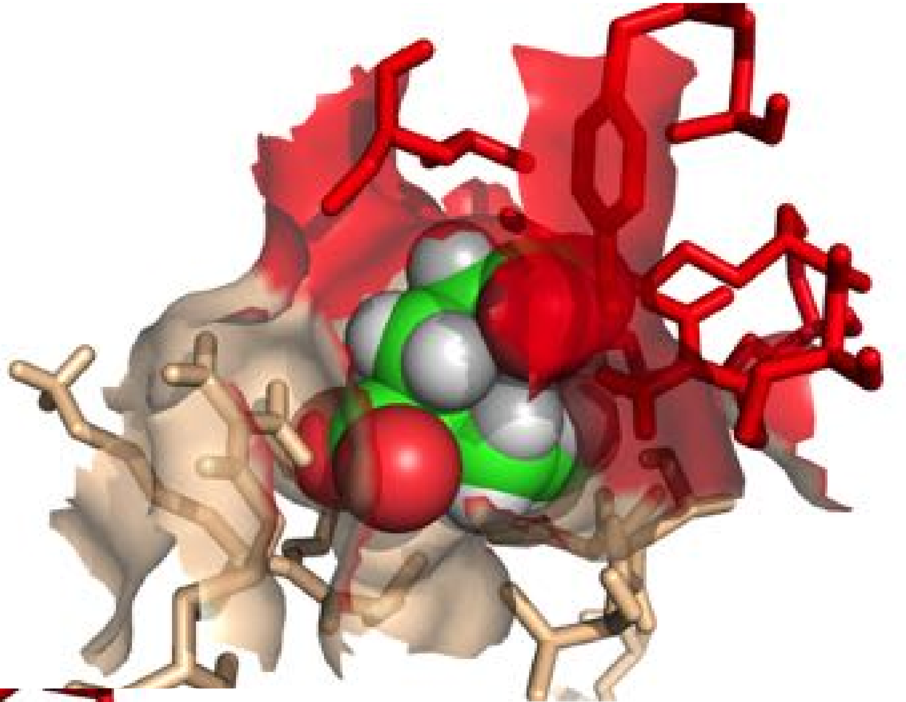
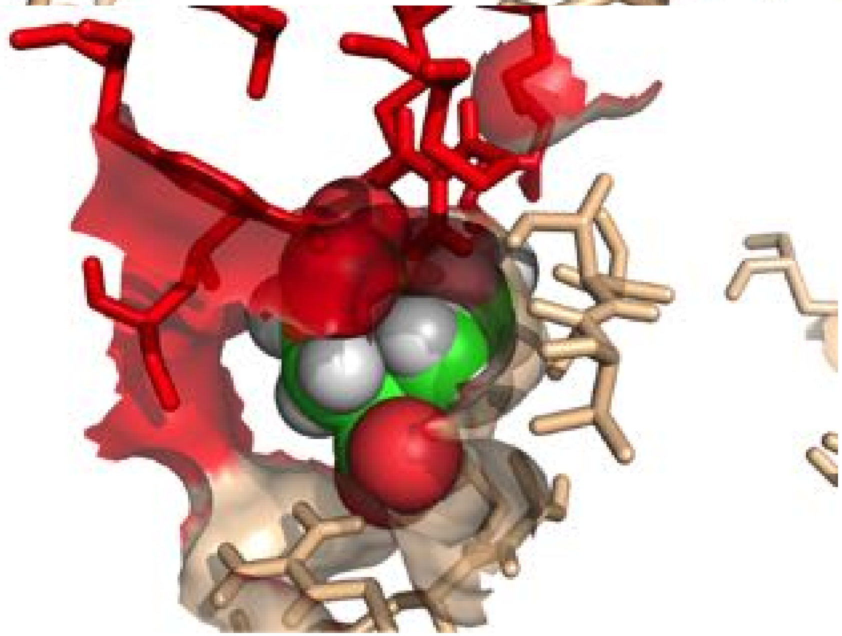
TS



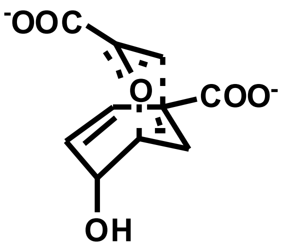
inhibitor



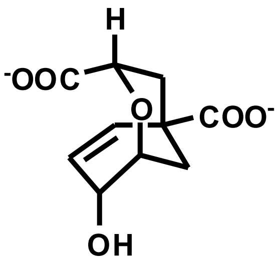
Chorismate mutase

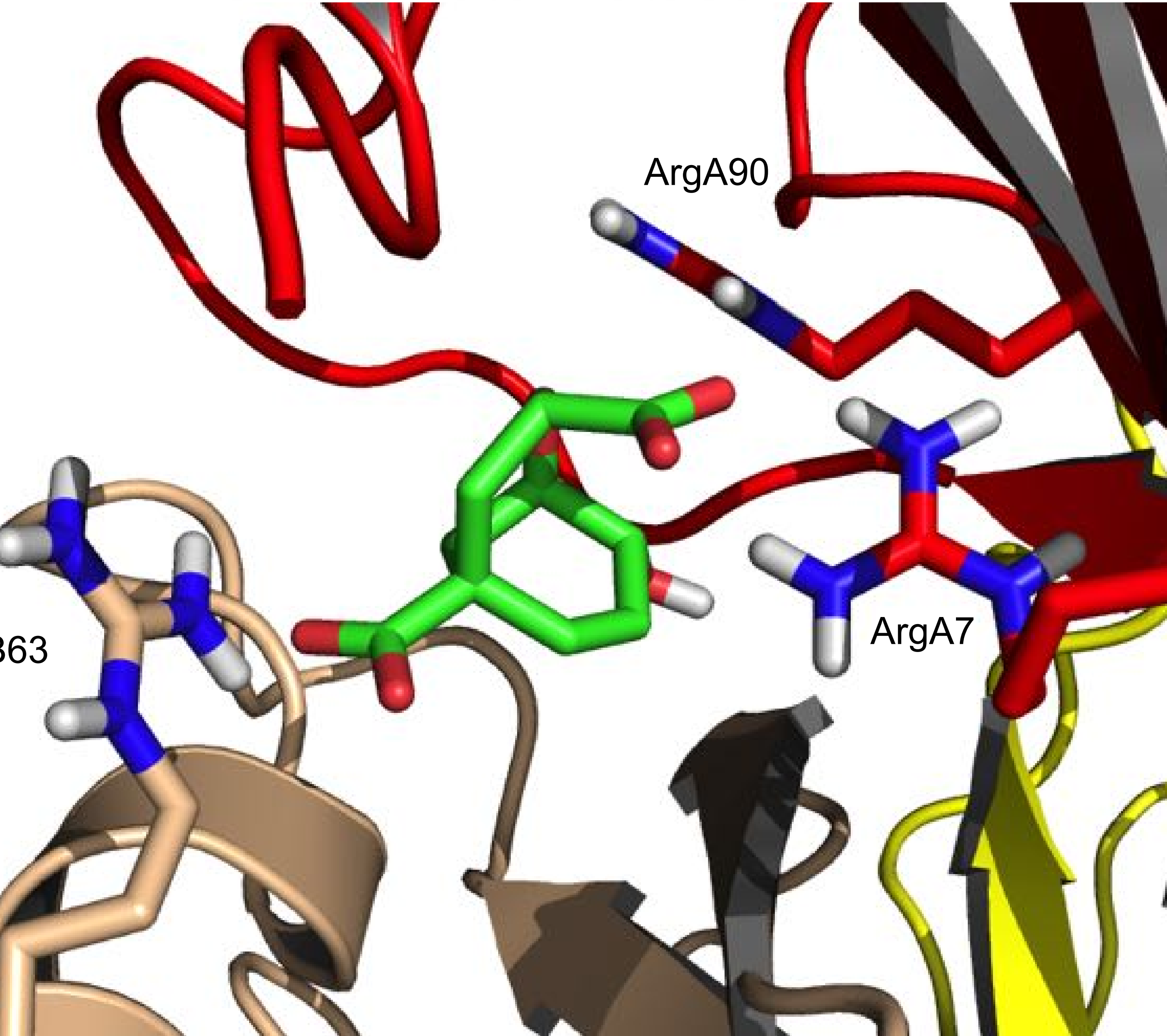
Chorismate mutase



TS



inhibitor



Redox Reactions

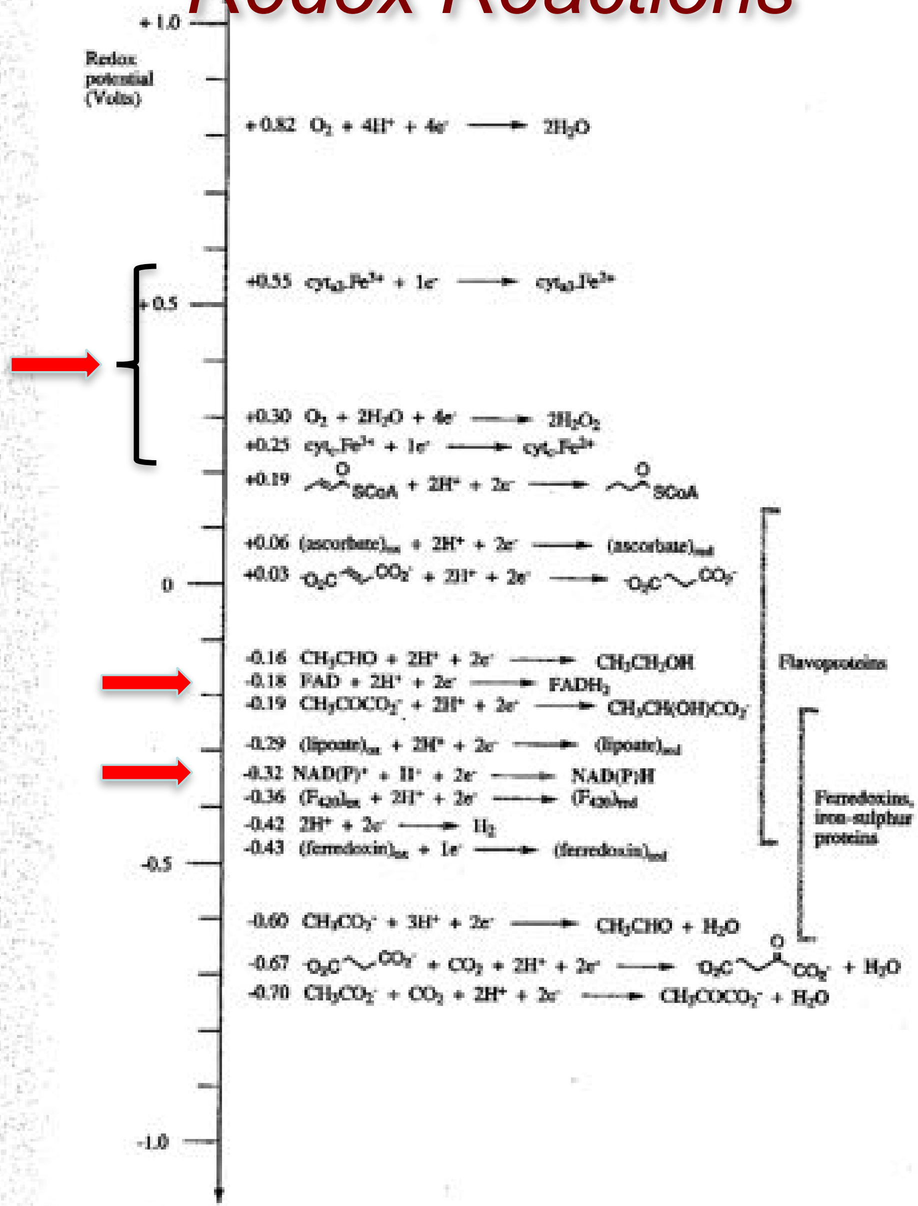
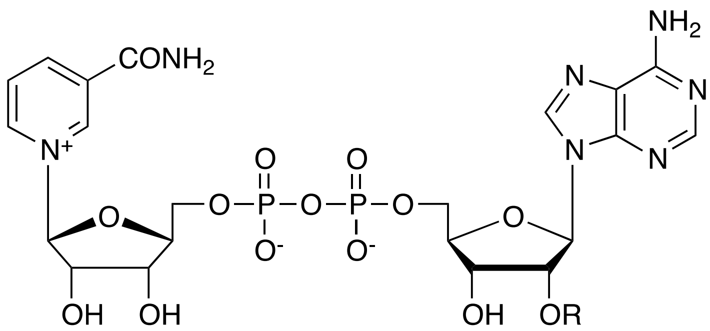


Fig. 6.1 Some biologically important redox potentials. CoA, coenzyme A; FAD, flavin adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate.

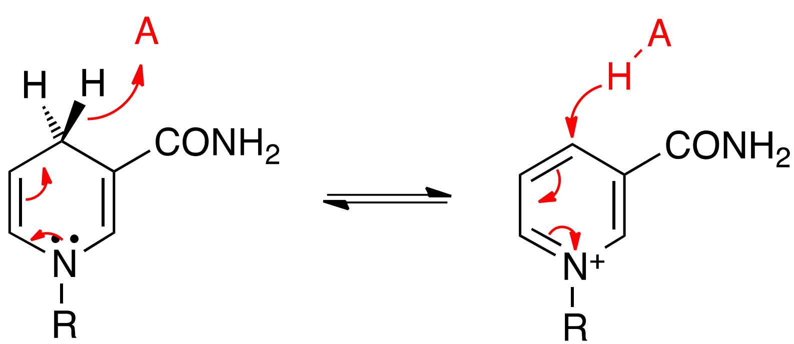
Redox Reactions: NADH/NAD$^+$



NAD$^+$ (R = H): catabolism

NADP$^+$ (R = $PO_3^=$) : anabolism

NAD(P)H is the strongest biological reducing agent



E_0 = + 0.32V

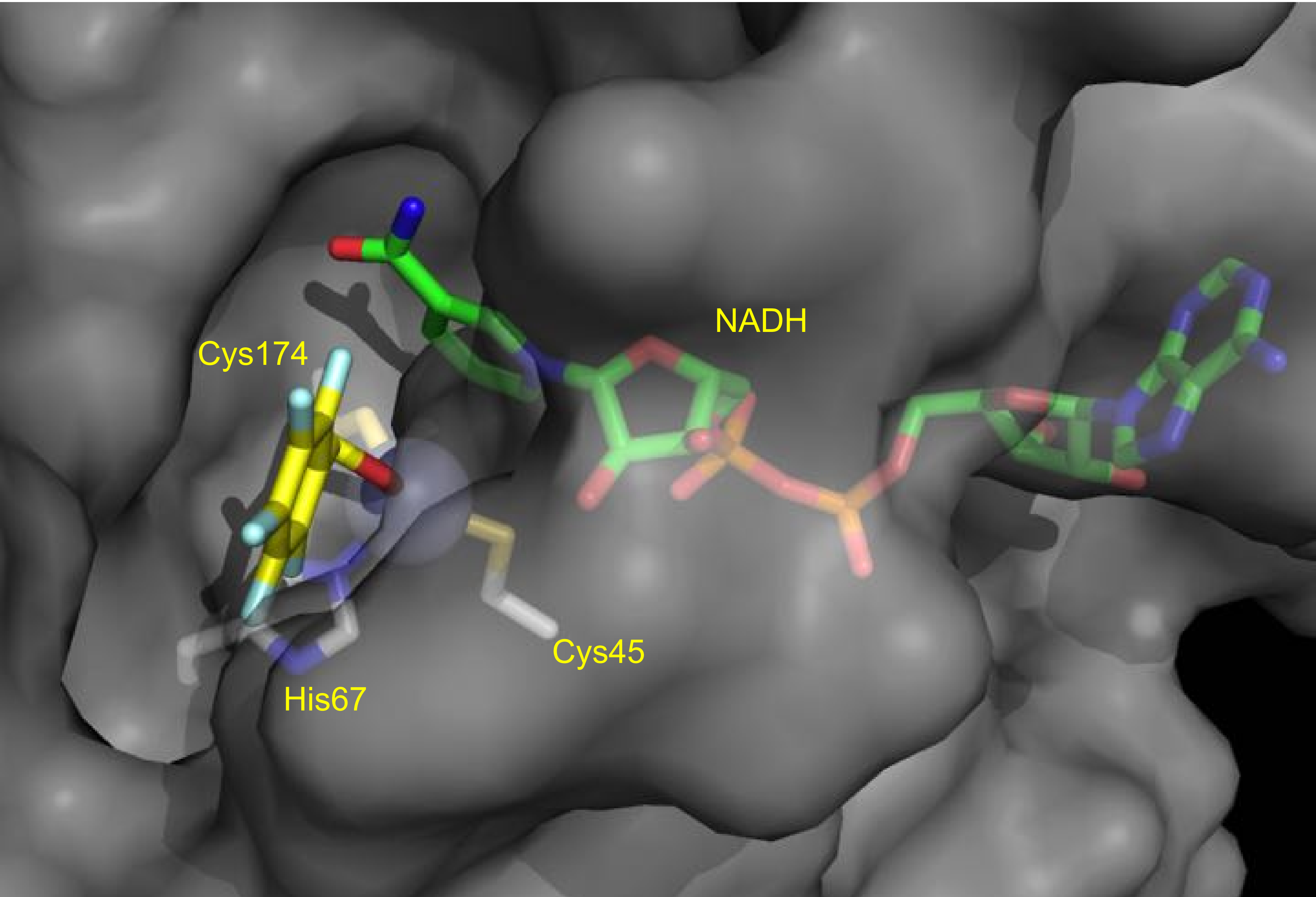
Alcohol dehydrogenase

$CH_3CHO + 2\ H^+ + 2\ e^- \longrightarrow CH_3CH_2OH$ $E_0 = -0.16$ V

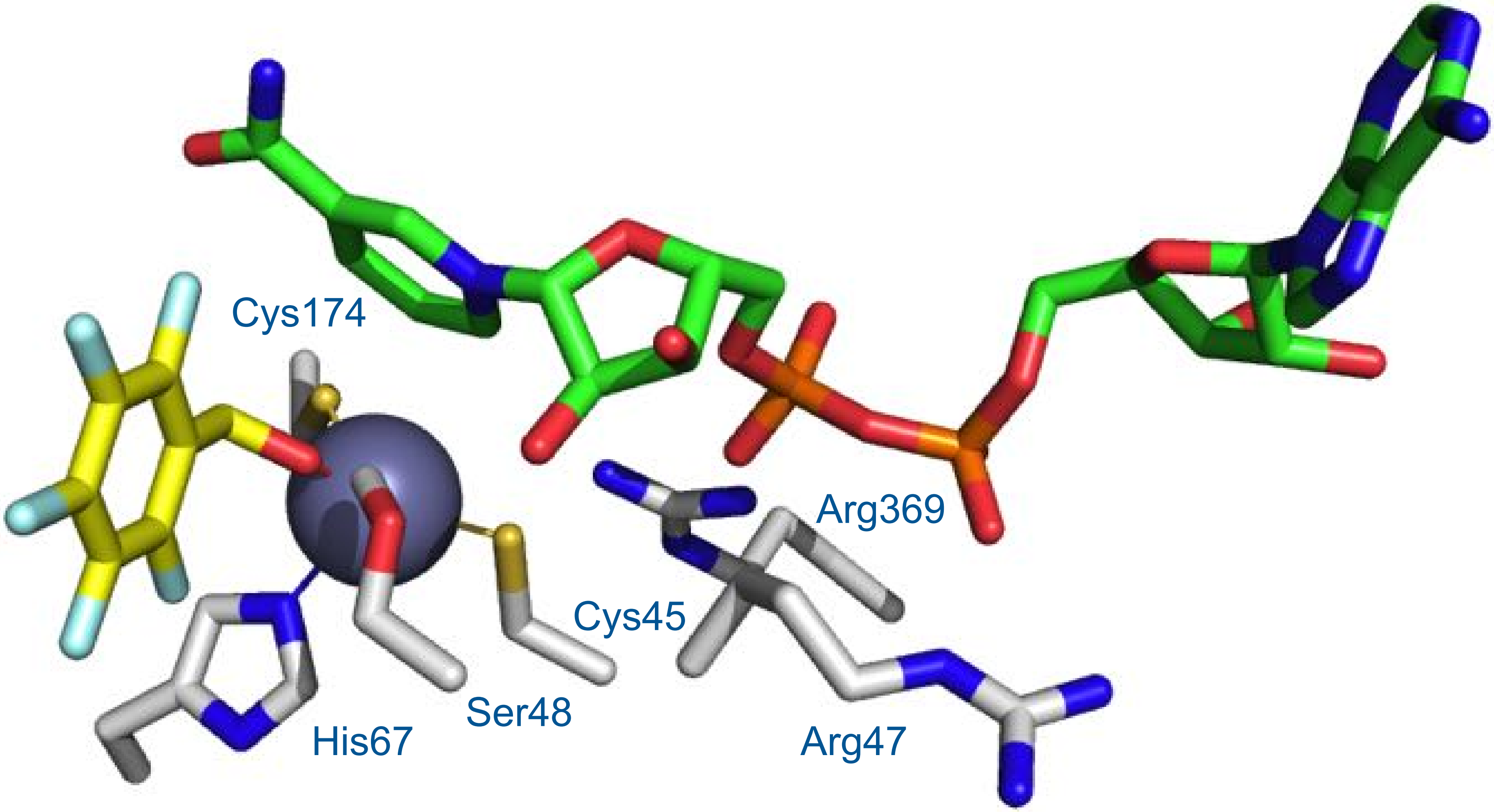
$NADH \longrightarrow NAD^+ + H^+ + 2e^-$ $E_0 = +0.32$ V

$CH_3CHO + NADH + H^+ \longrightarrow CH_3CH_2OH + NAD^+$ $E_0 = +0.16$V

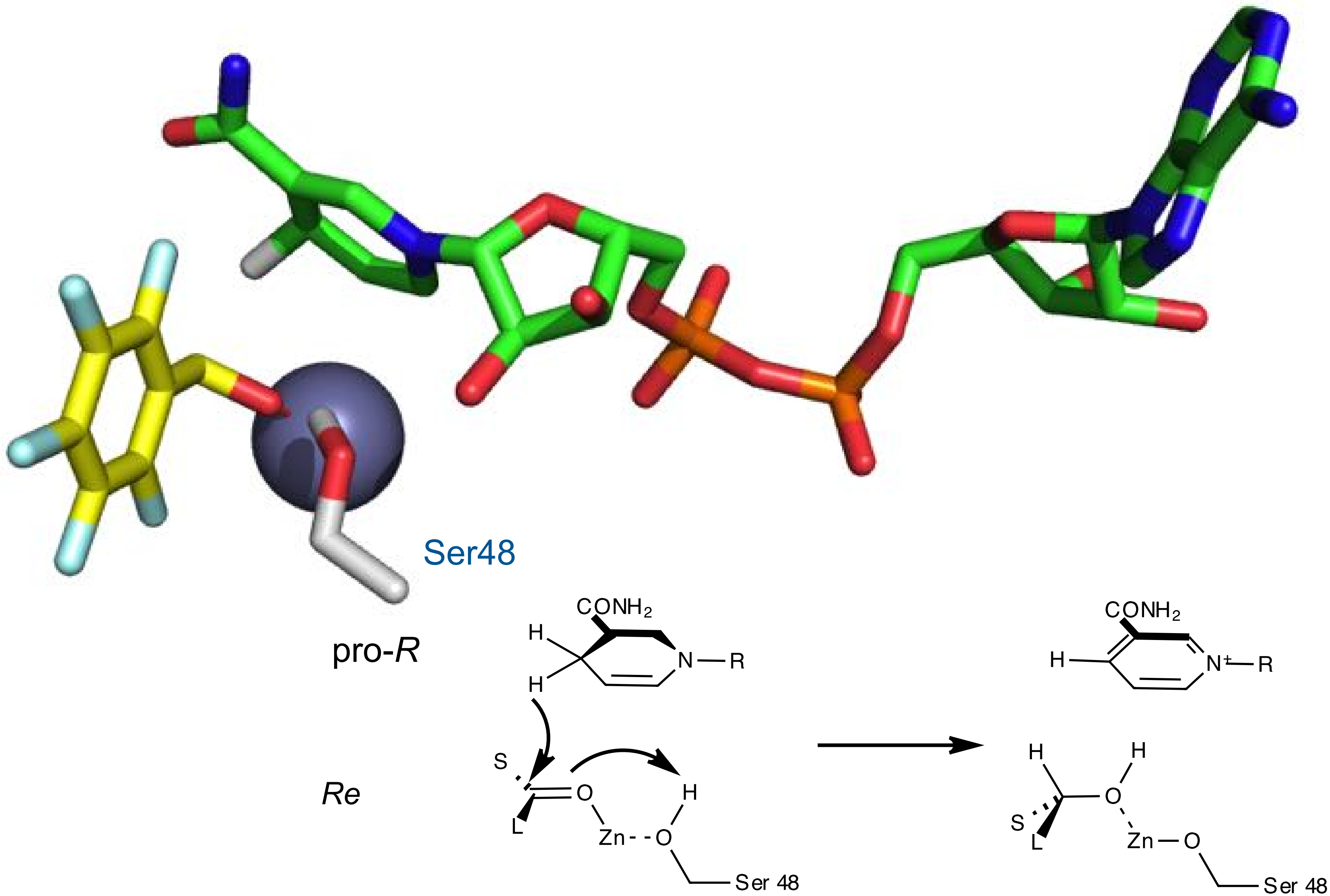
HLADH



HLADH



HLADH

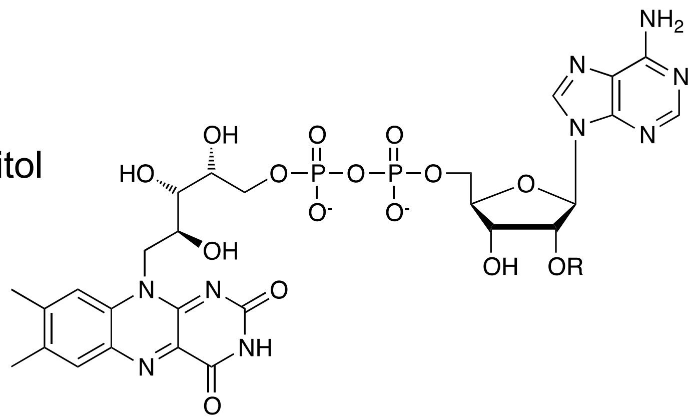


Ser48
pro-R
Re
CONH2
H
H
N
R
S
L
O
Zn
O
H
Ser 48
CONH2
H
N+
R
H
H
O
S
L
Zn
O
Ser 48

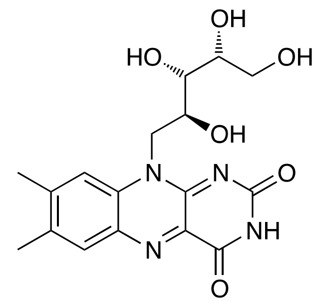
Flavin-Adenin Dinucleotide FAD

ribitol

isoalloxazine

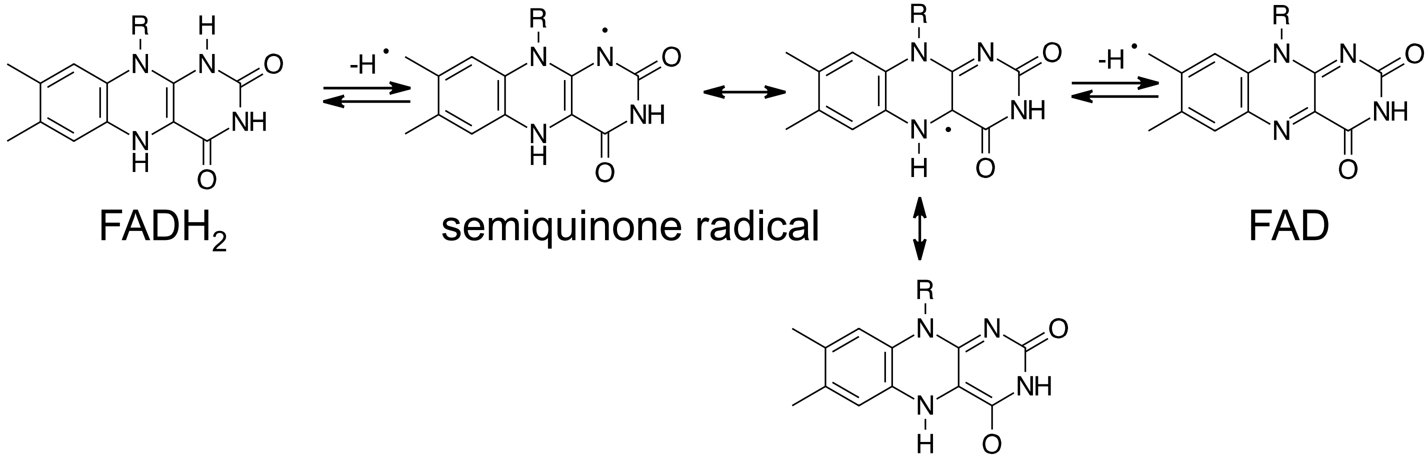


FAD



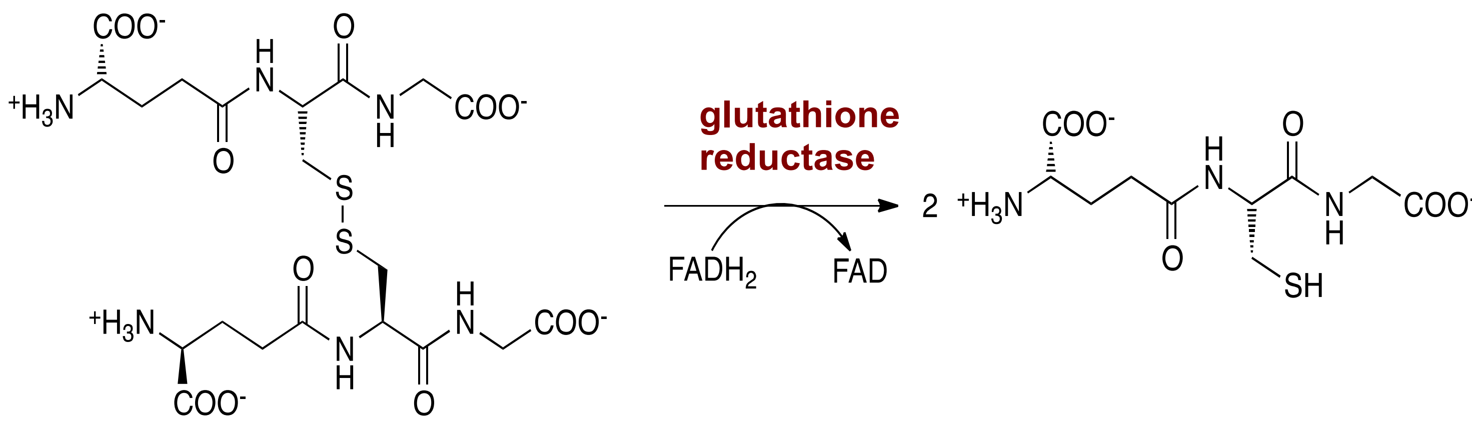
Vit. B2

$FADH_2 \rightarrow FAD + 2H^+ + 2e^-$ $\quad E_0 = +0.18$



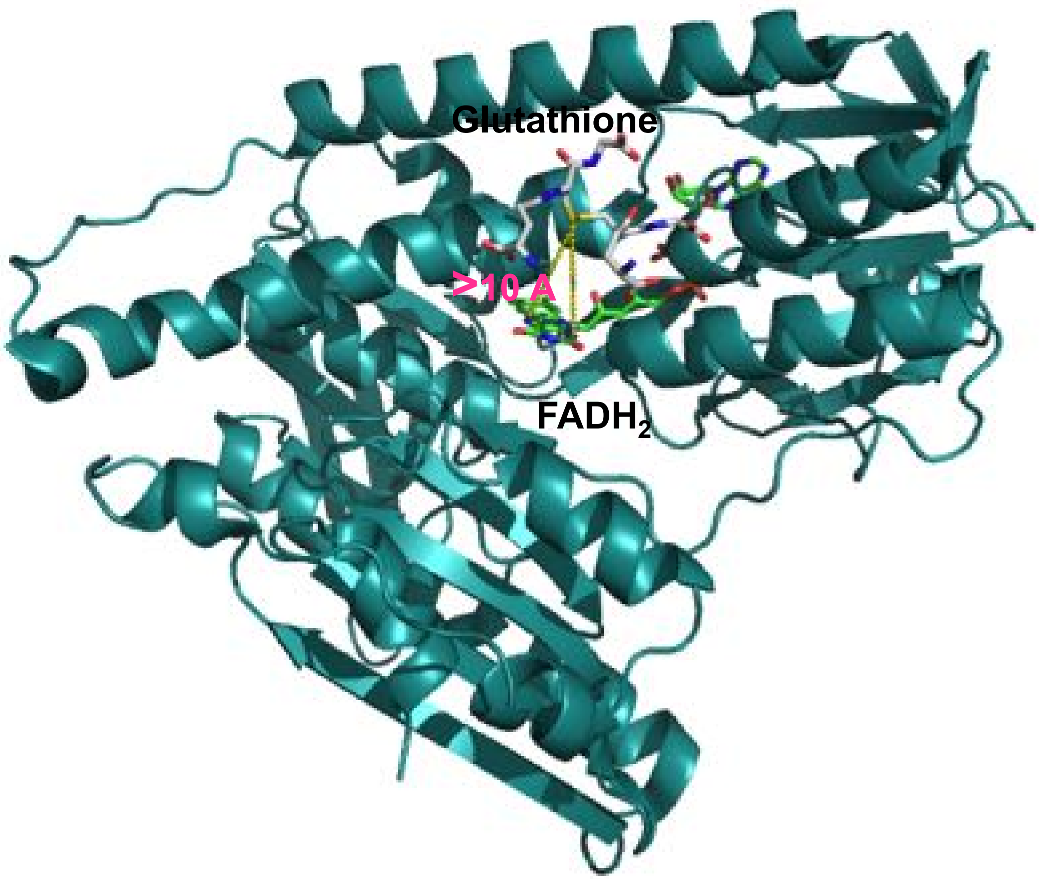
$FADH_2$ semiquinone radical FAD

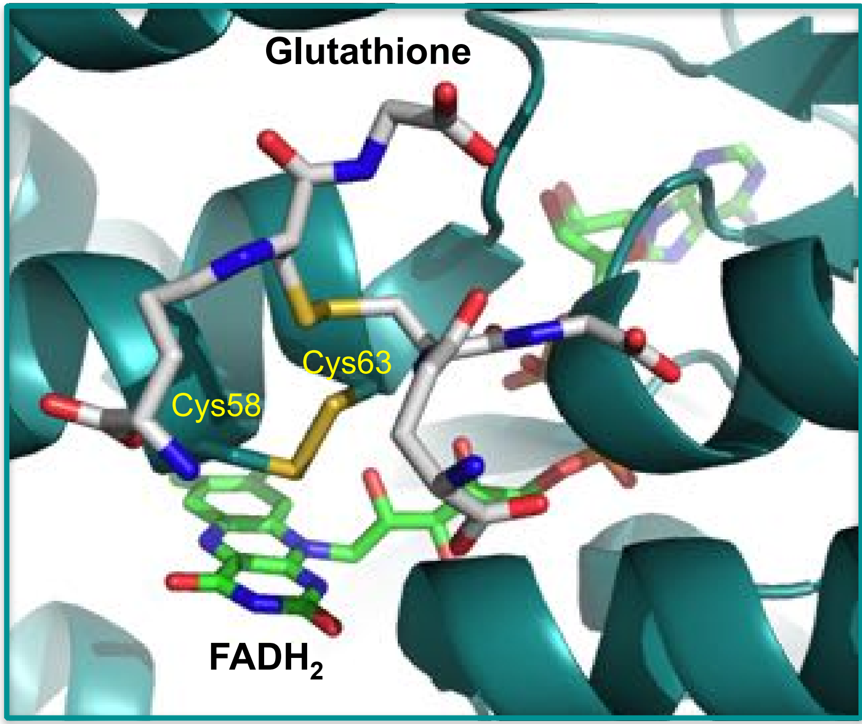
Glutathione Reductase



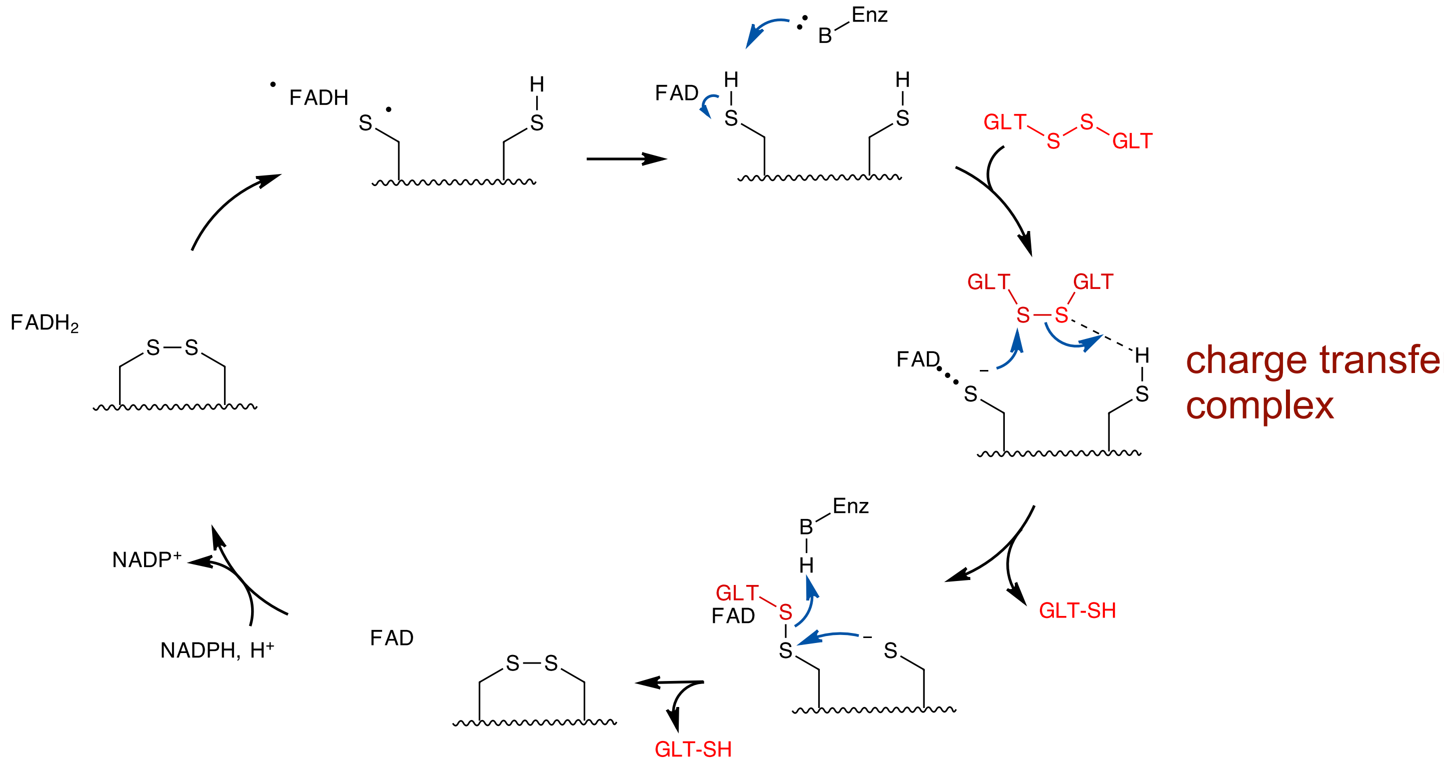
Glutathione: protects cells from oxidative stress and from molecular oxygen

Glutathione Reductase

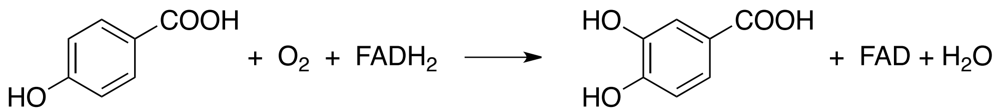




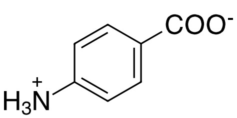
Glutathione Reductase

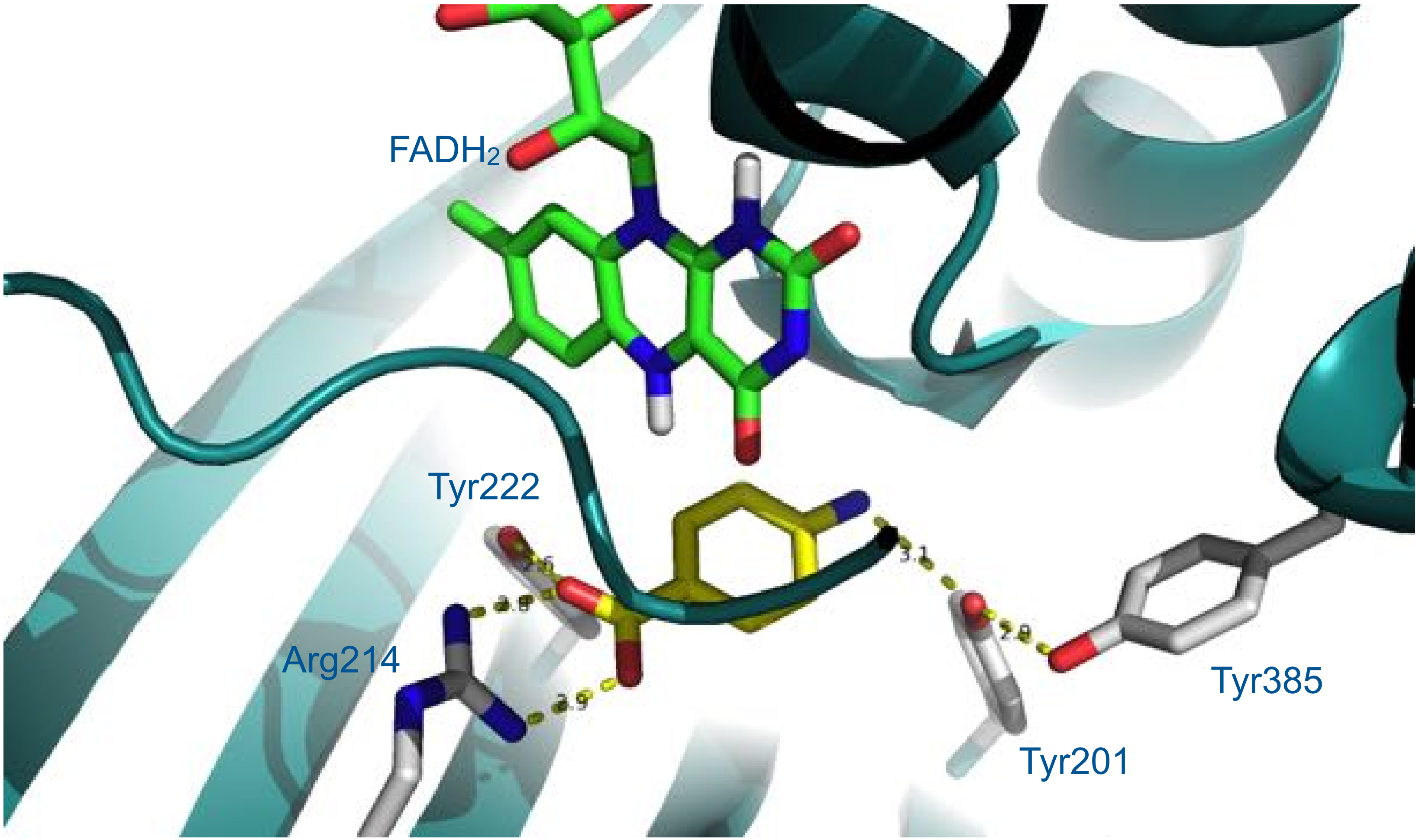


p-Hydroxybenzoate Hydroxylase (Monooxygenase)

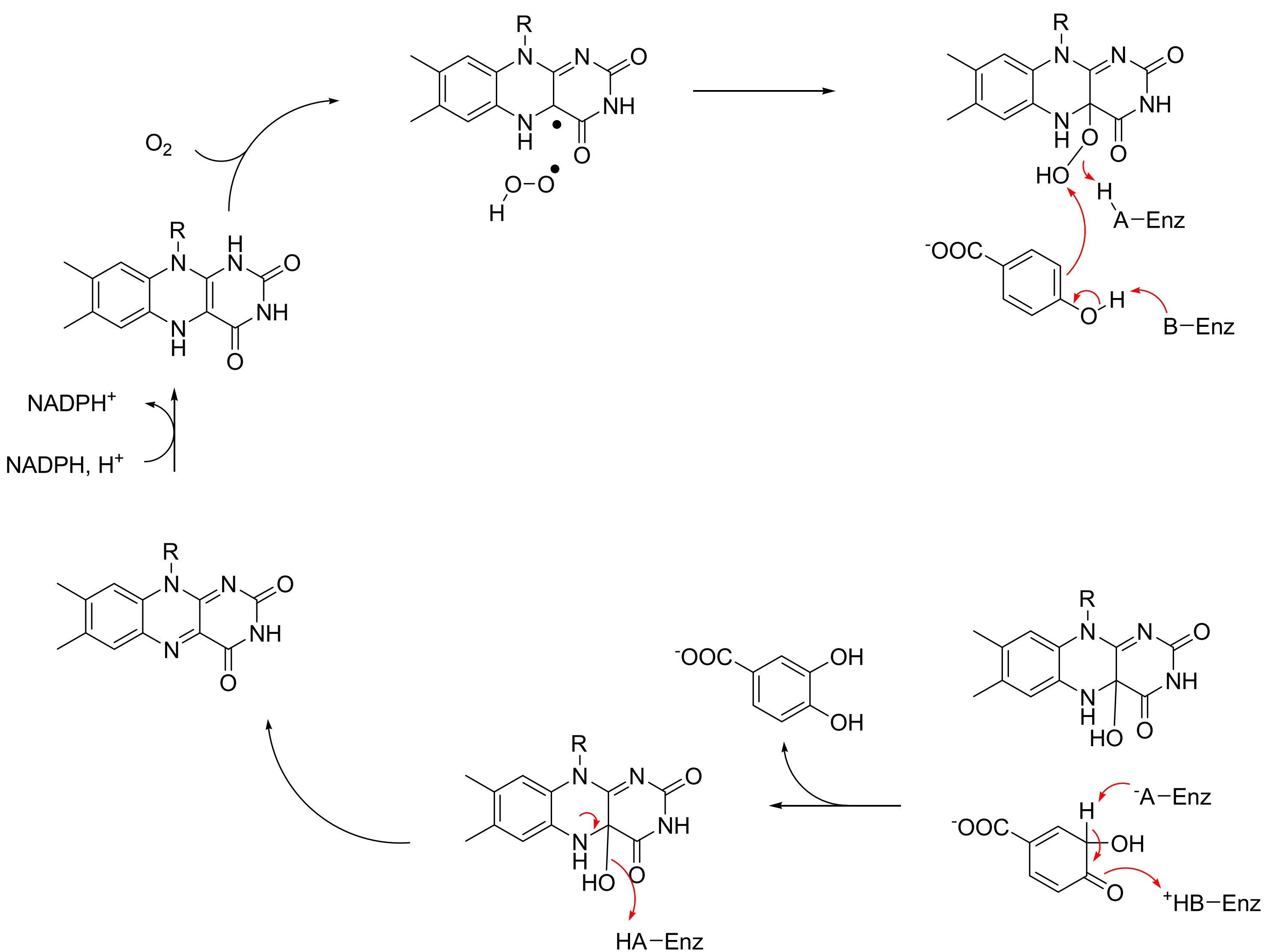


Inhibitor:

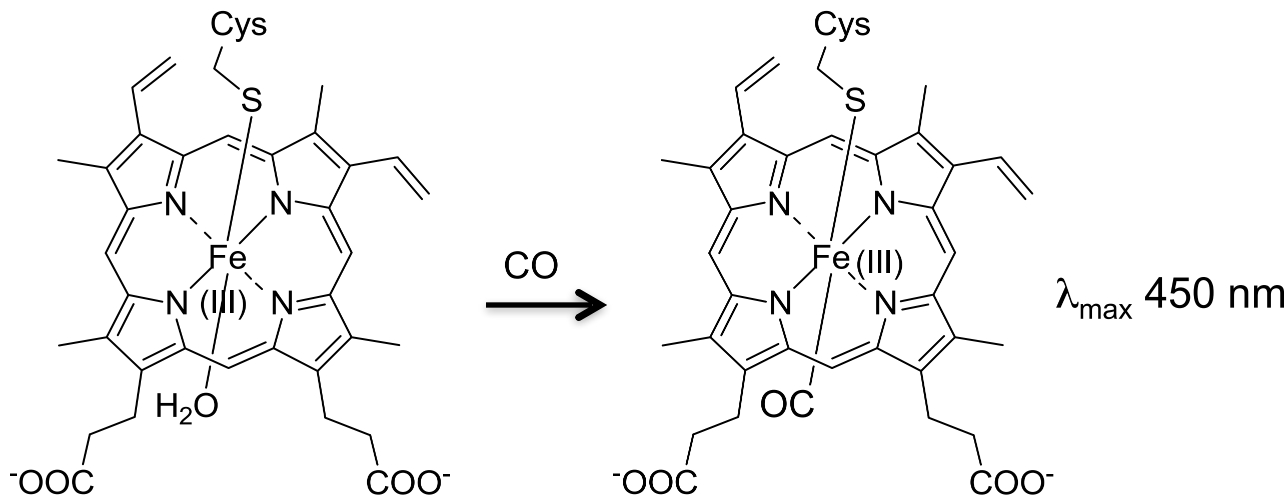




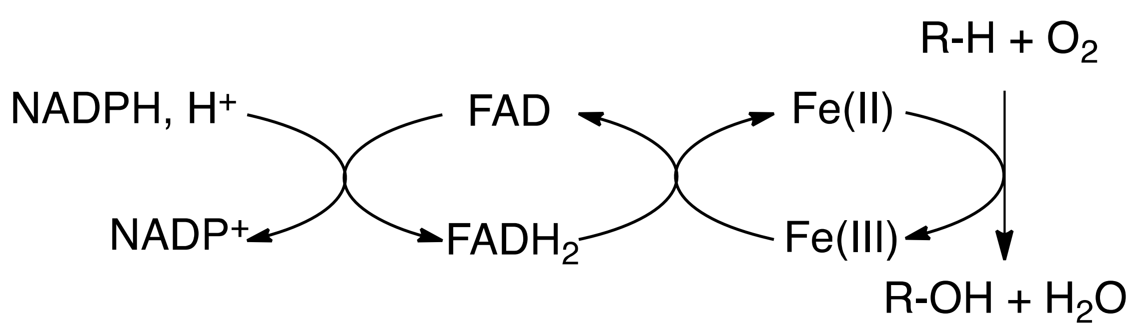
p-Hydroxybenzoate Hydroxylase (Monooxygenase)



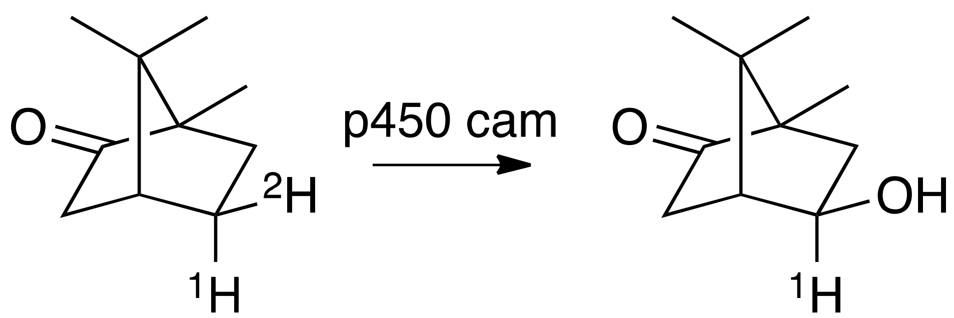
Metal Dependent Monooxygenases Cytochrome p450



Cytochrome p450 catalyzes the hydroxylation of unactivated alkanes (detoxification)

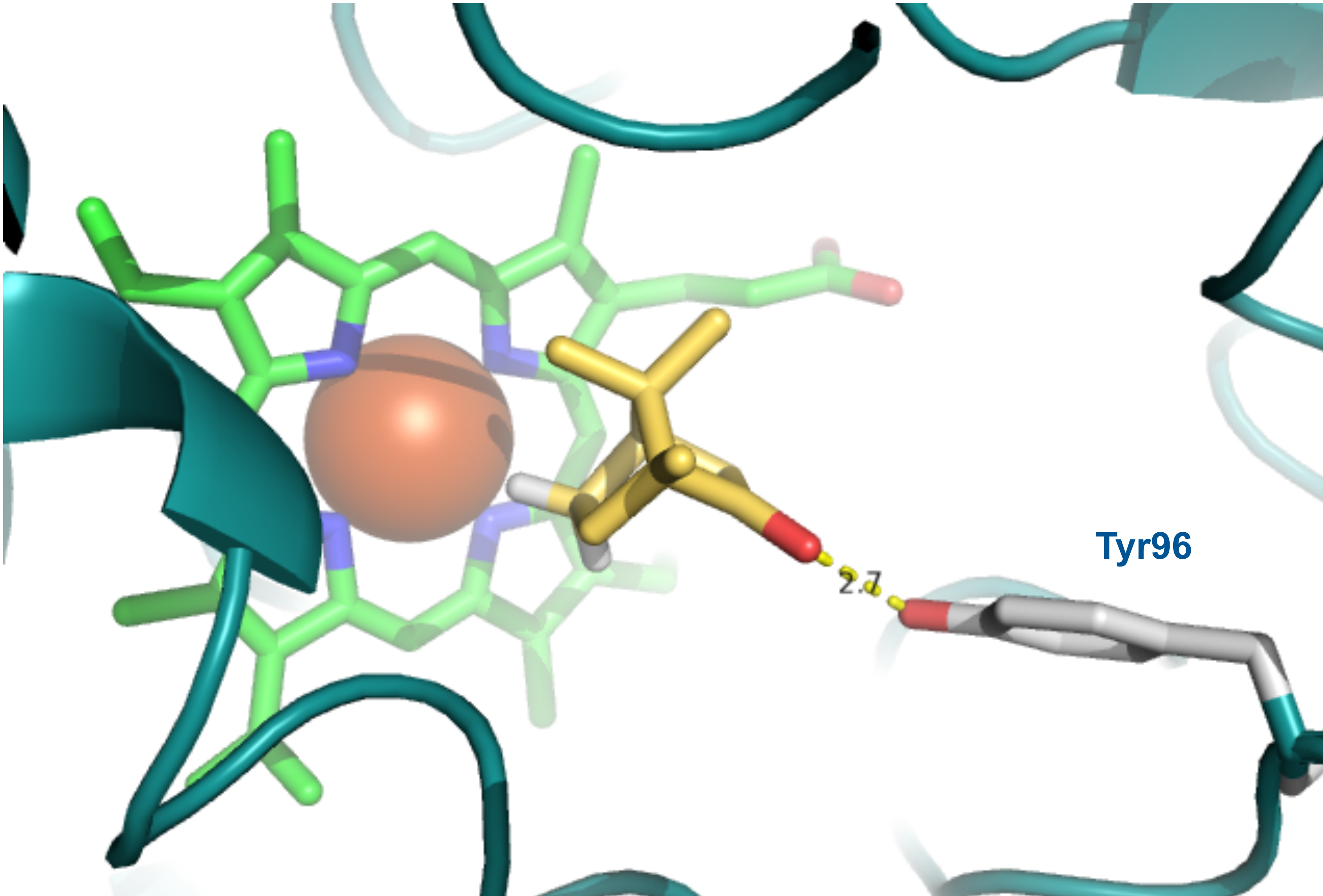


Camphor Hydroxylase (P450cam)

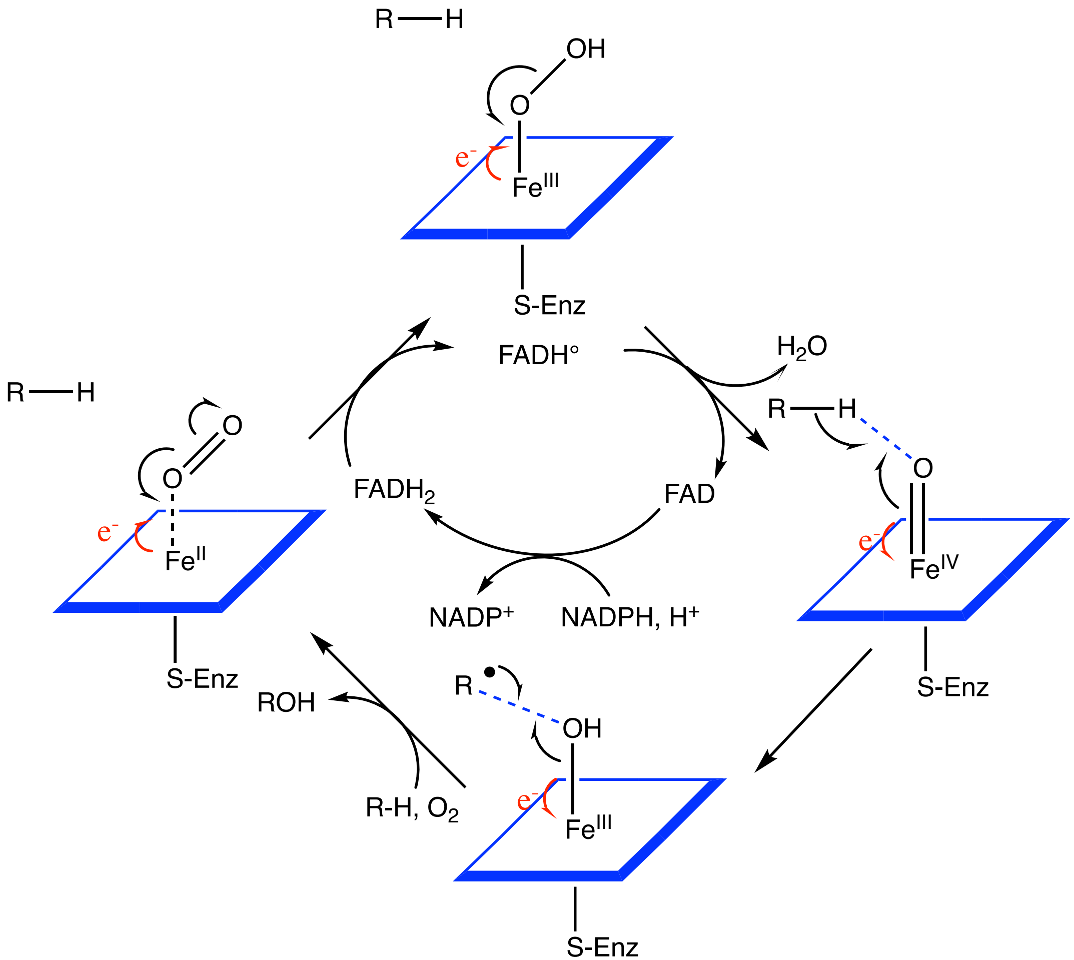


Stereospecific! Retention of configuration

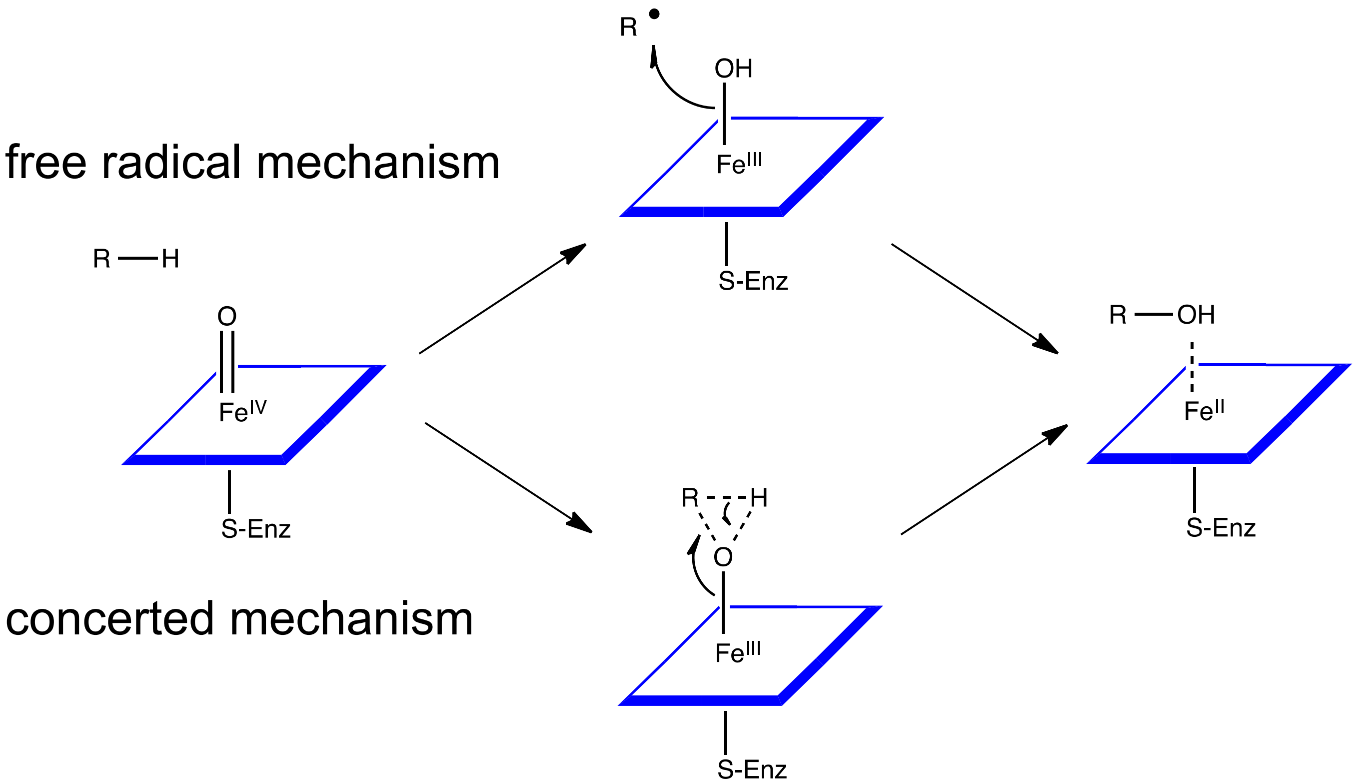
Camphor Hydroxylase (P450cam)



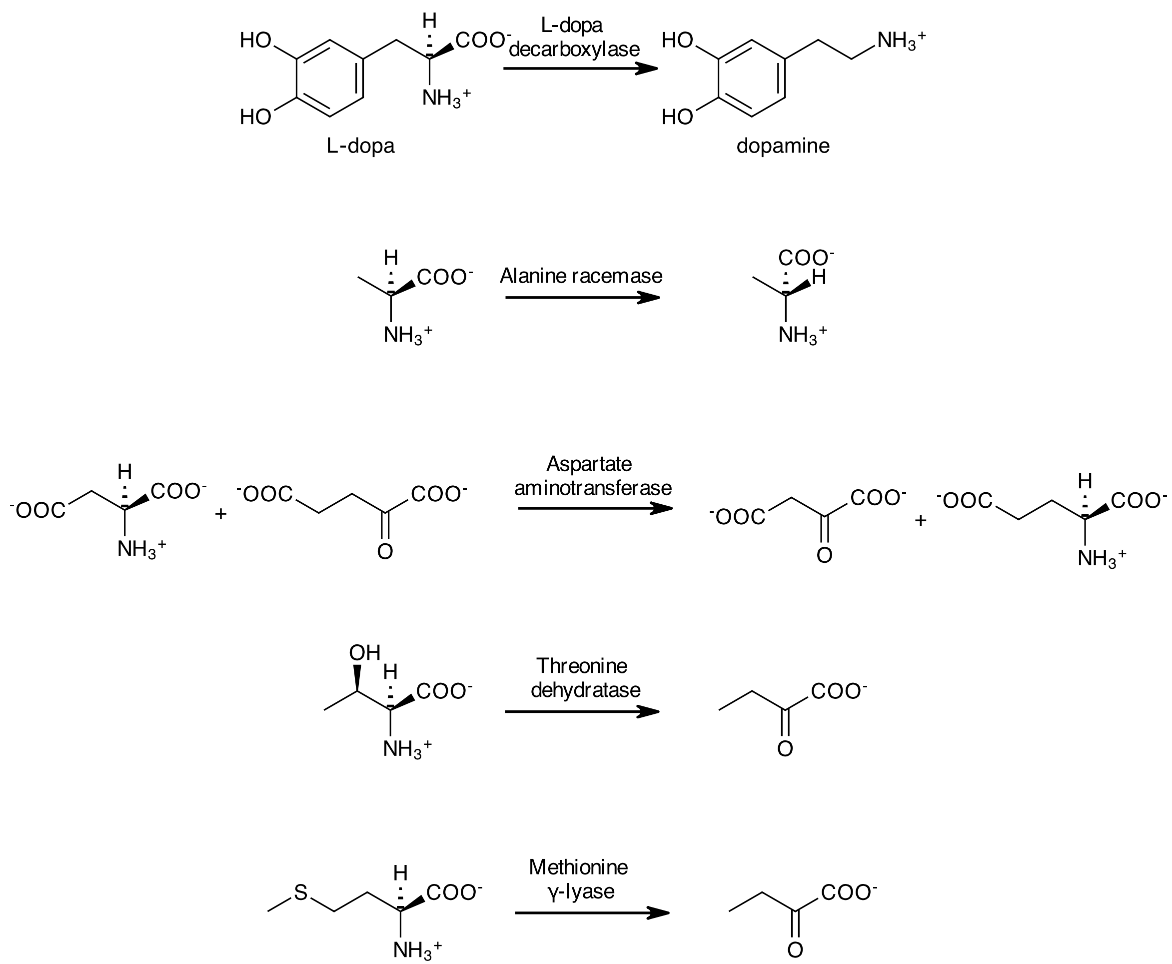
Camphor Hydroxylase (P450cam)

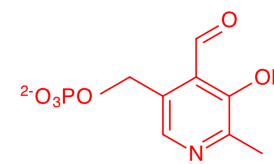


Camphor Hydroxylase (P450cam)



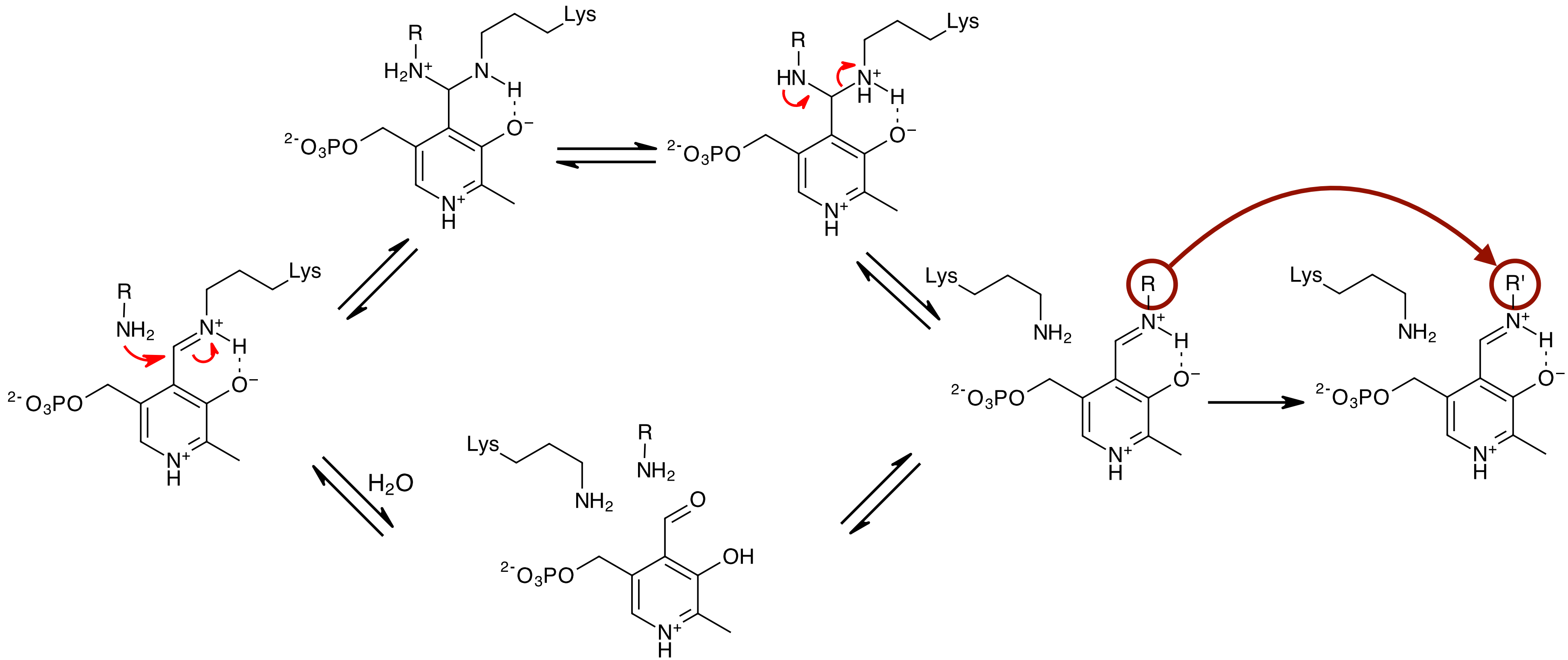
Pyridoxal-Catalyzed Reactions



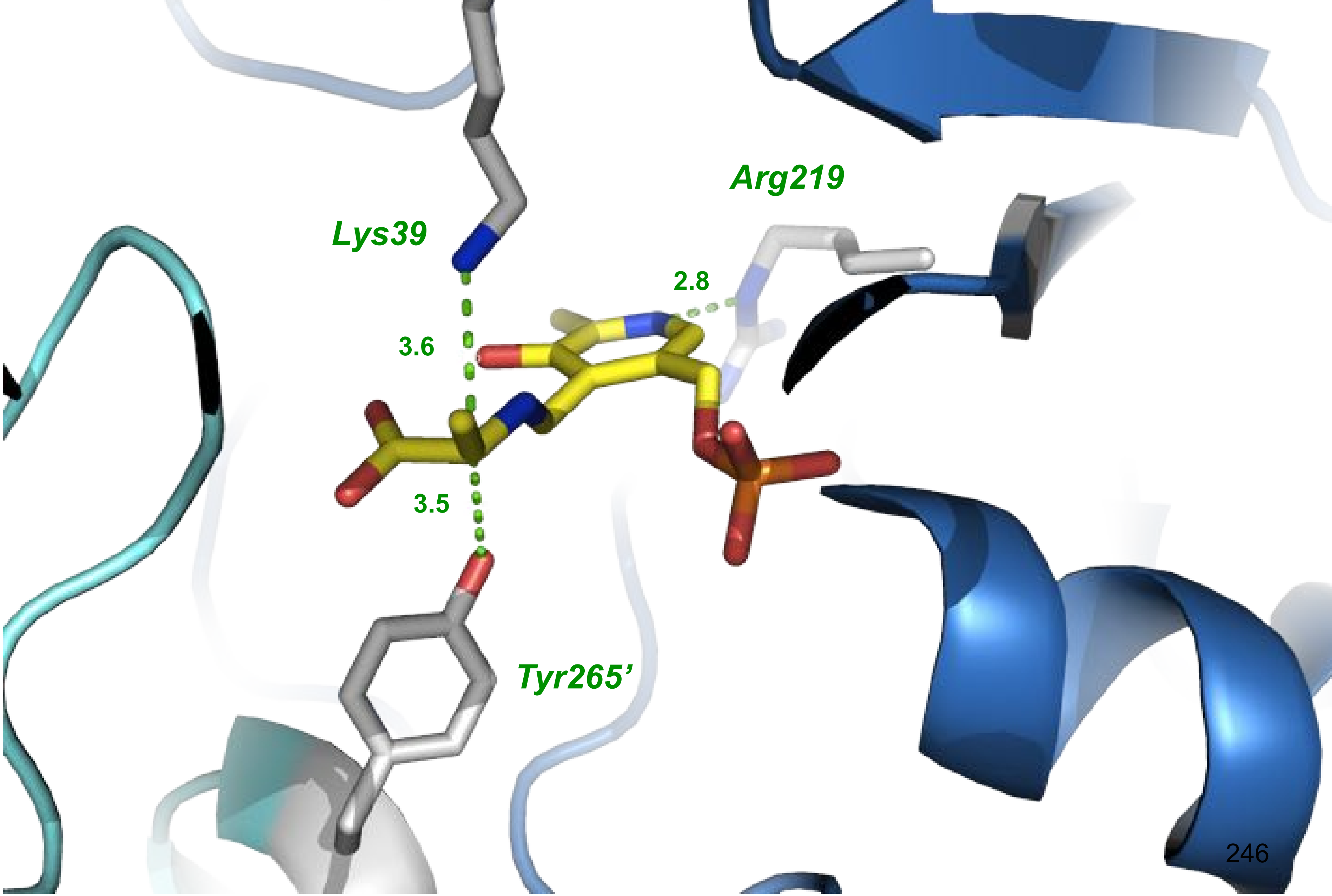


Pyridoxal 5'-phosphate

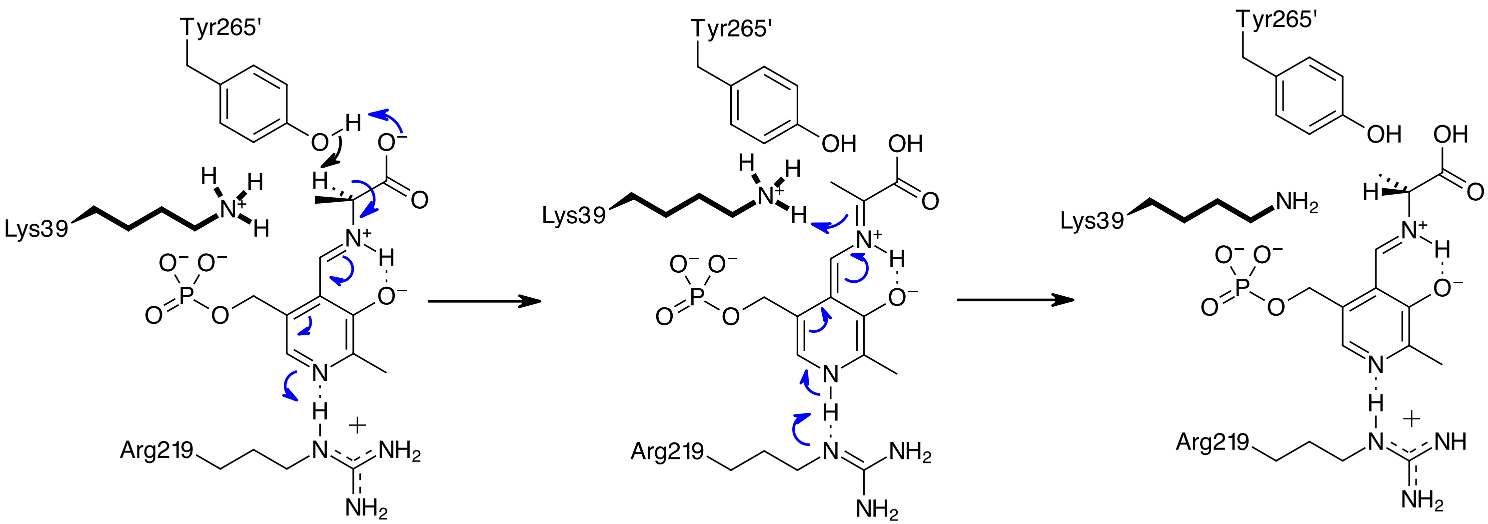
Pyridoxal-Catalyzed Reactions



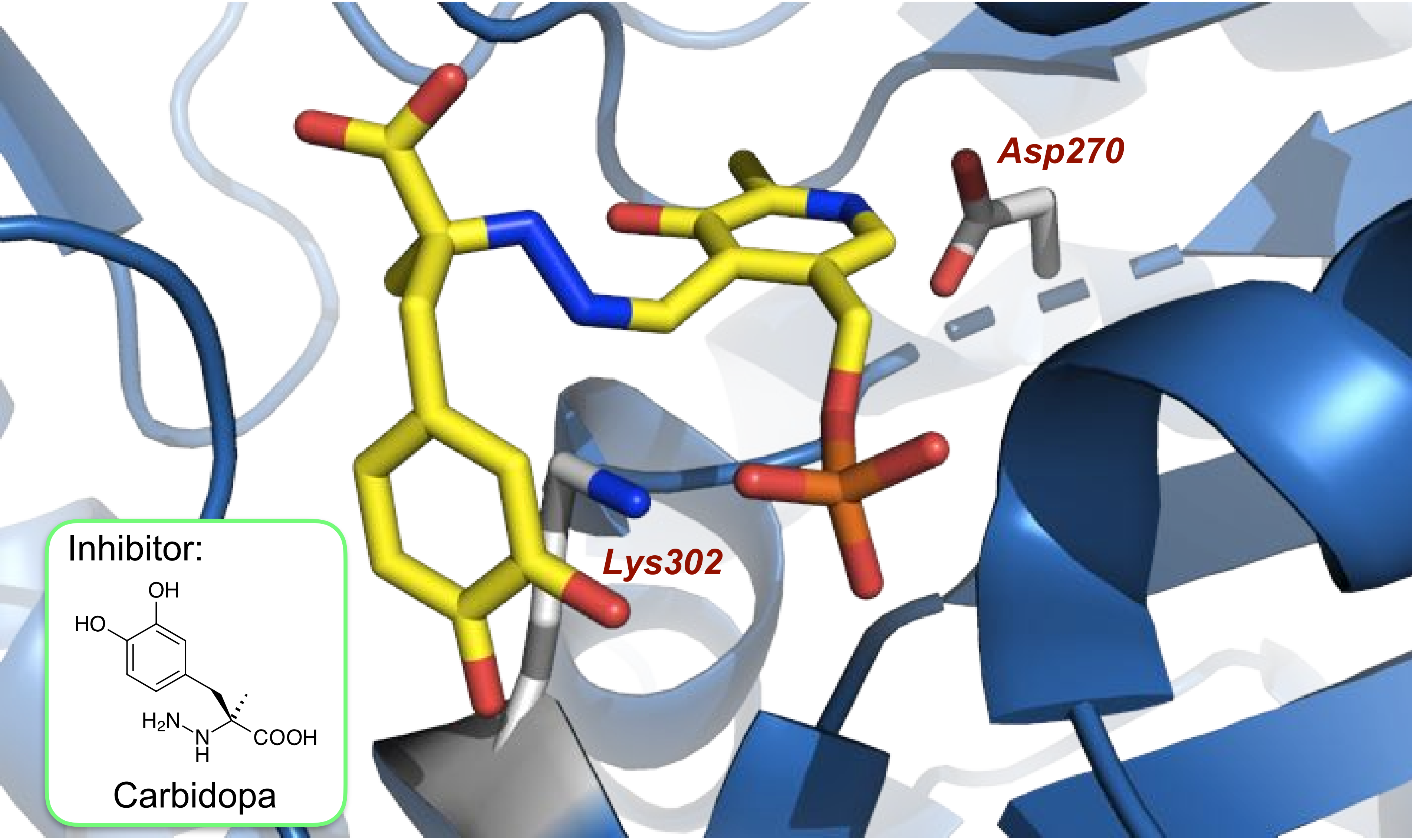
Alanine Racemase 1L6F



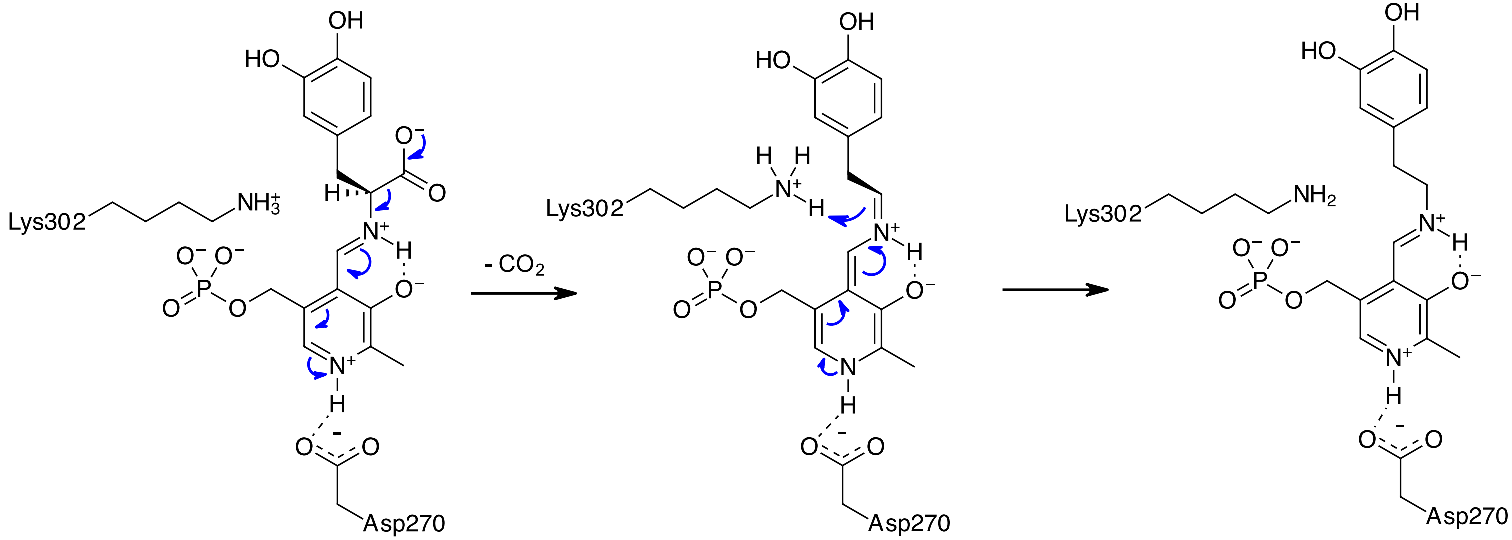
Alanine Racemase
(Two-Base Mechanism)



L-DOPA Decarboxylase 1JS3

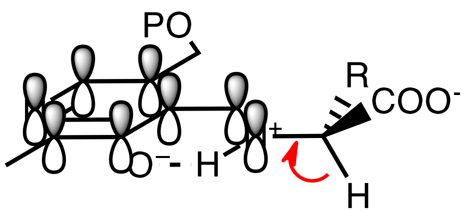


L-DOPA Decarboxylase 1JS3

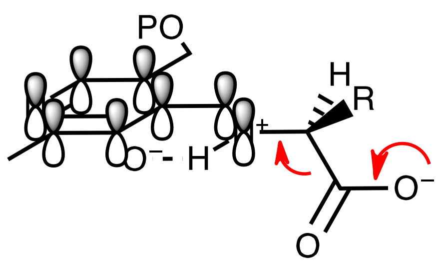


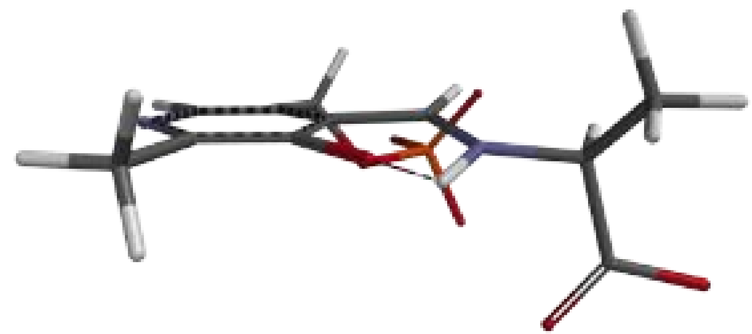
Stereoelectronic Control of Reactivity

racemases
transaminases
etc.

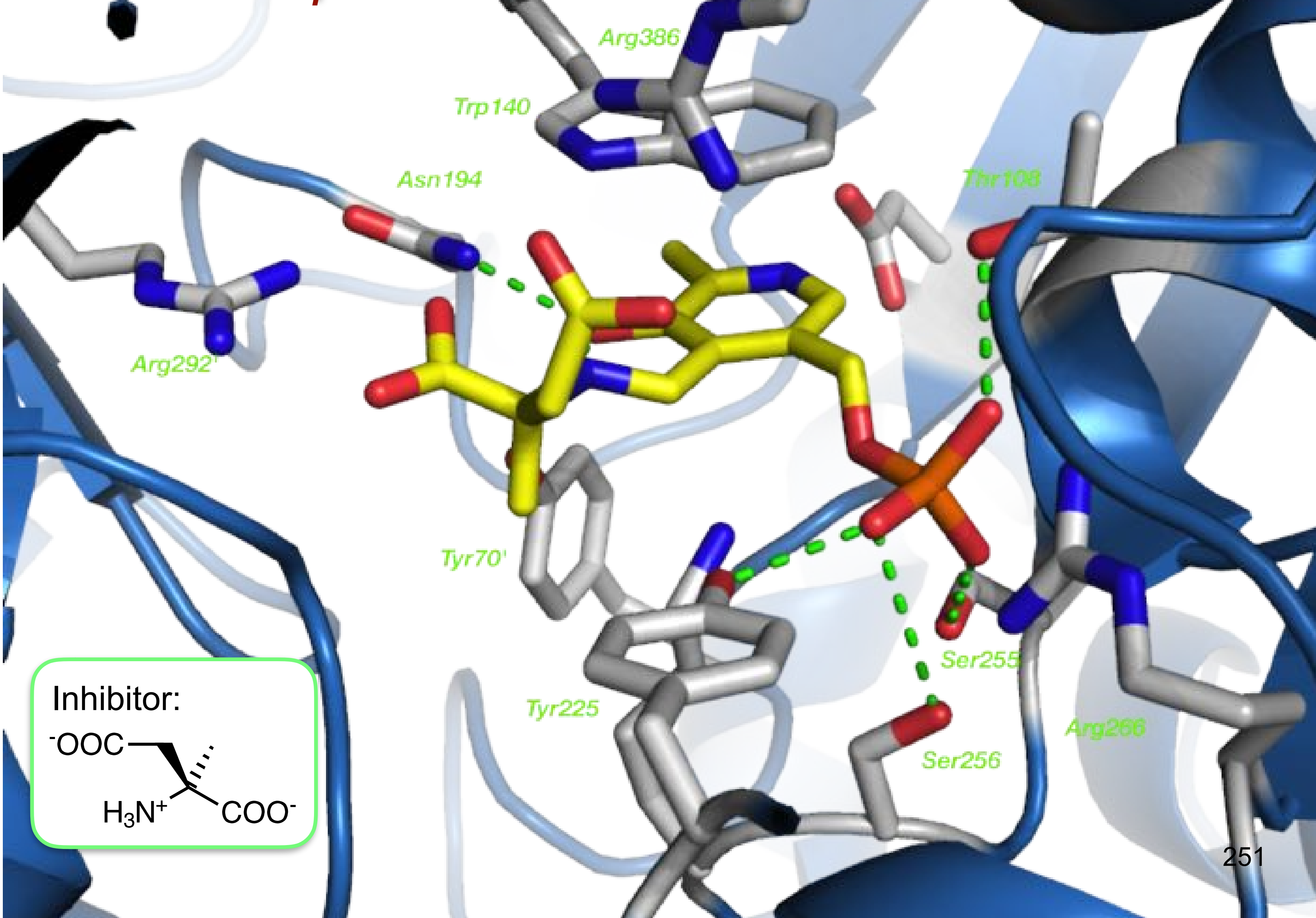


decarboxylases

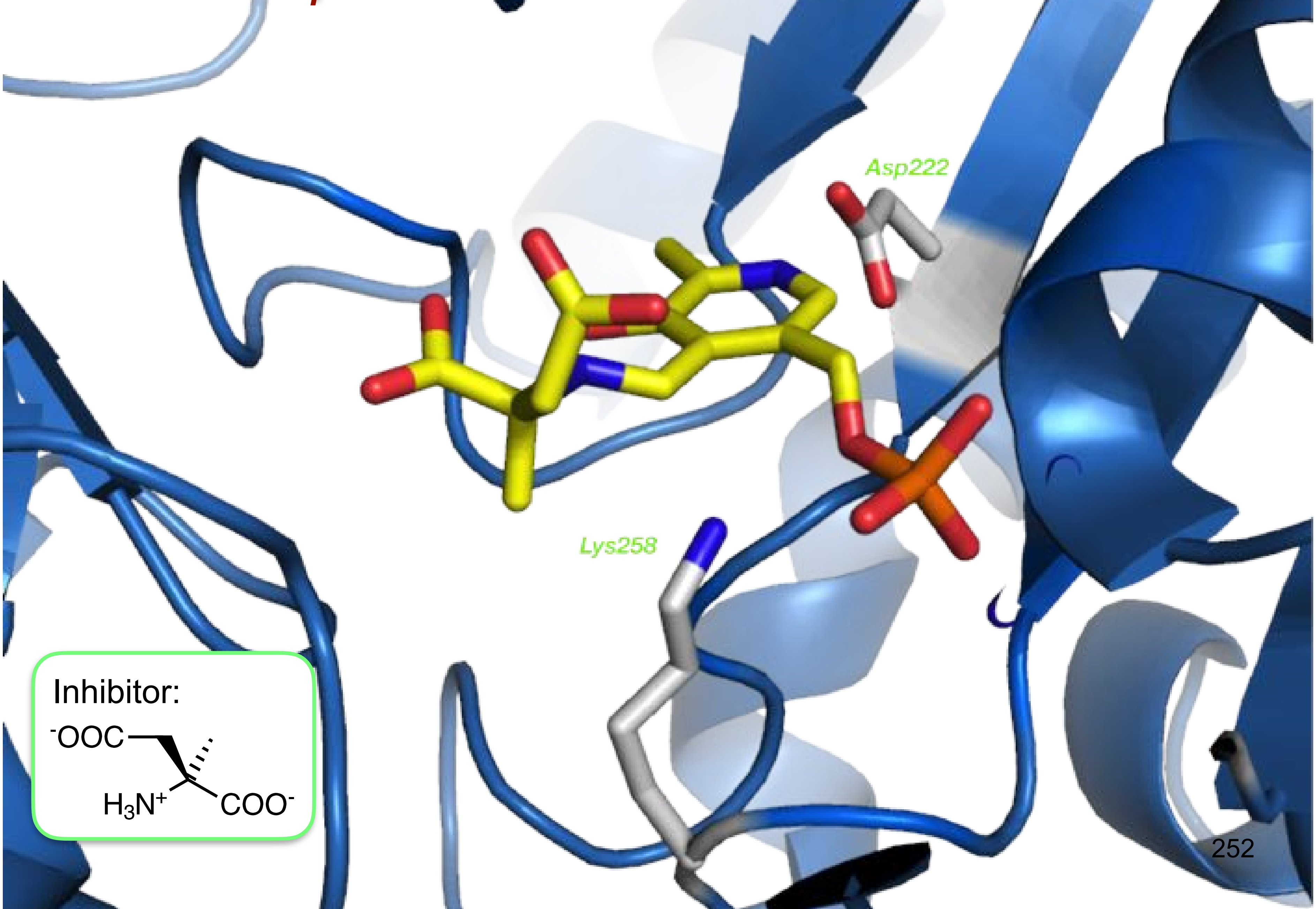




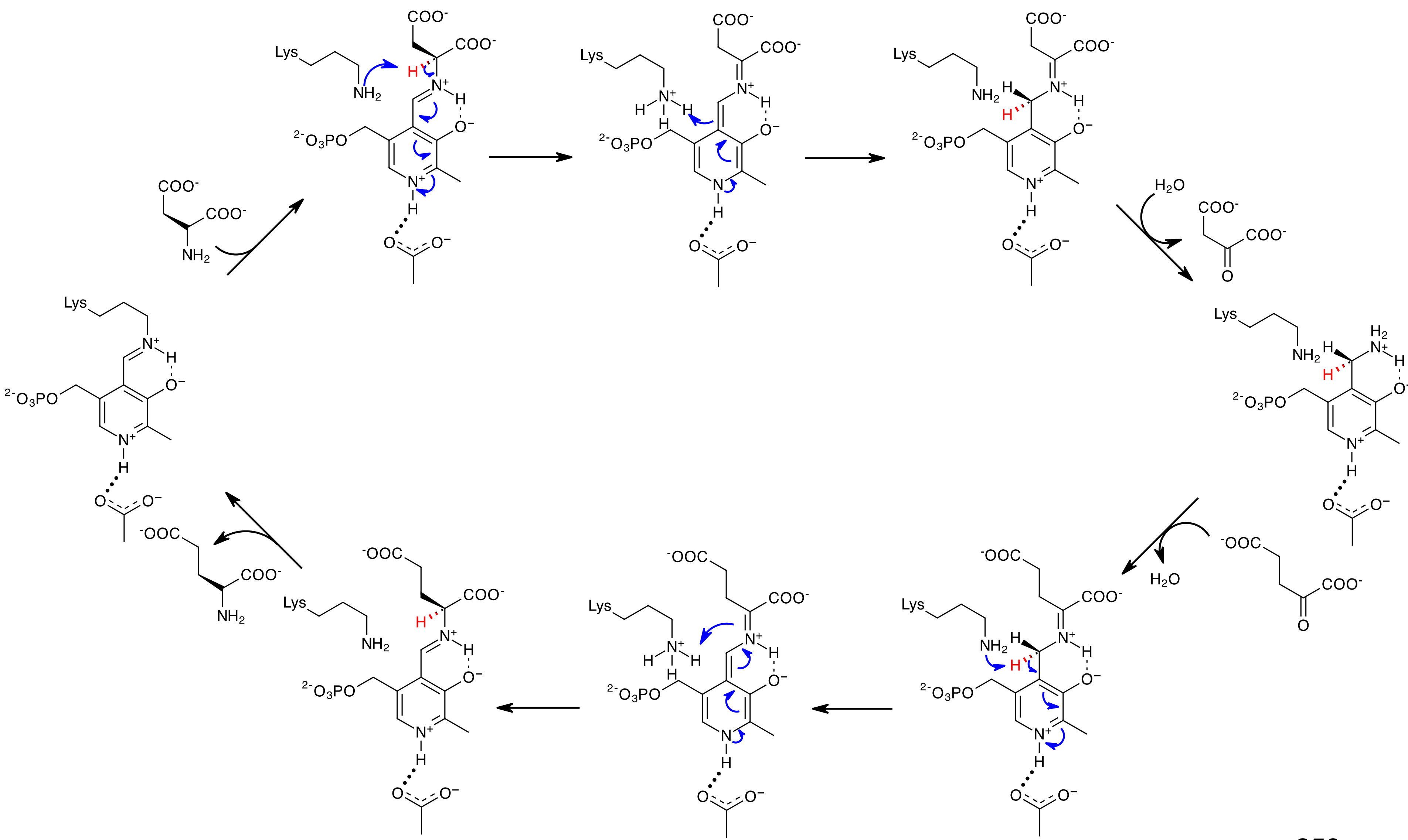
Aspartate Aminotransferase 1AJS



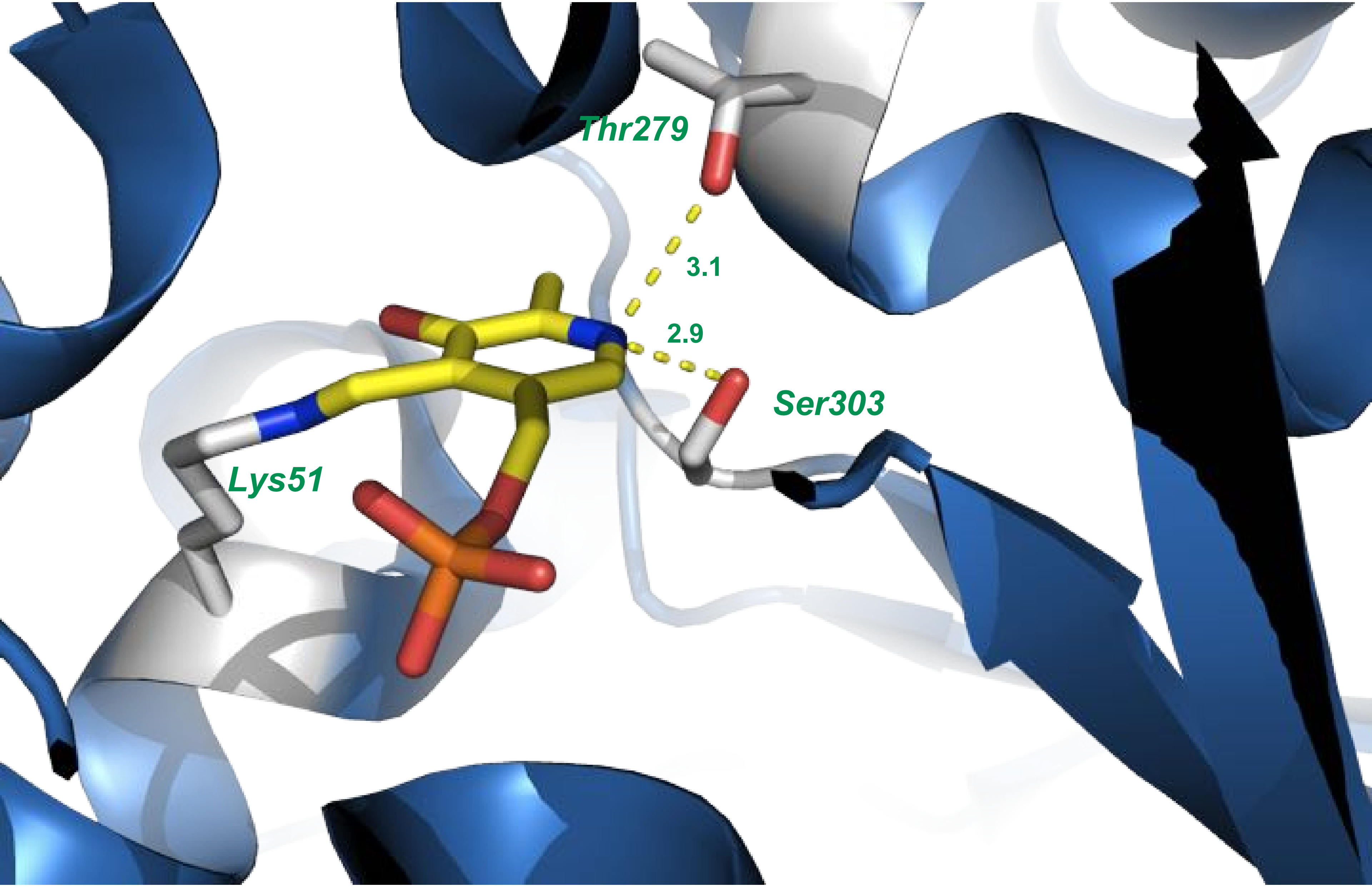
Aspartate Aminotransferase 1AJS



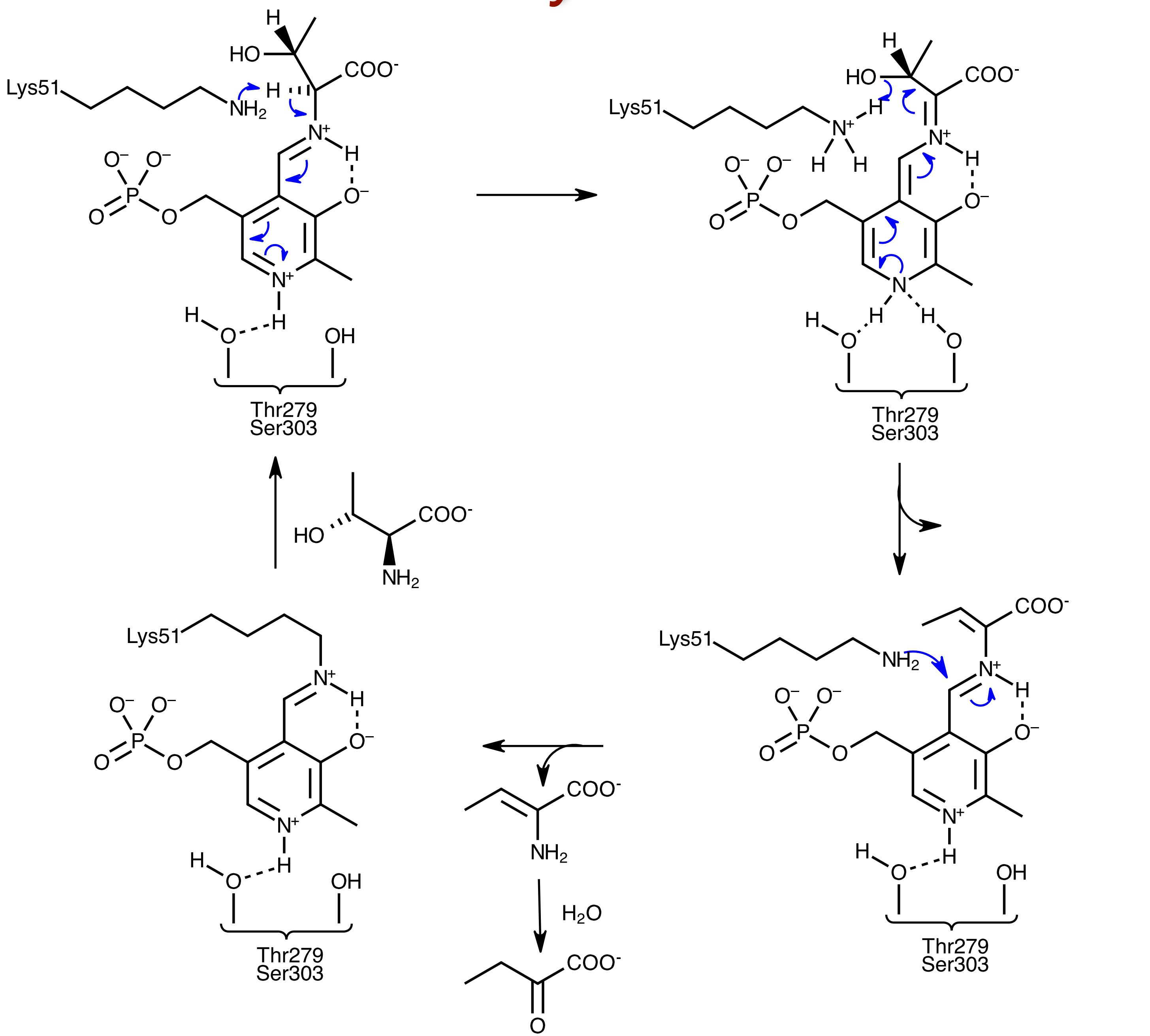
Aspartate Aminotransferase



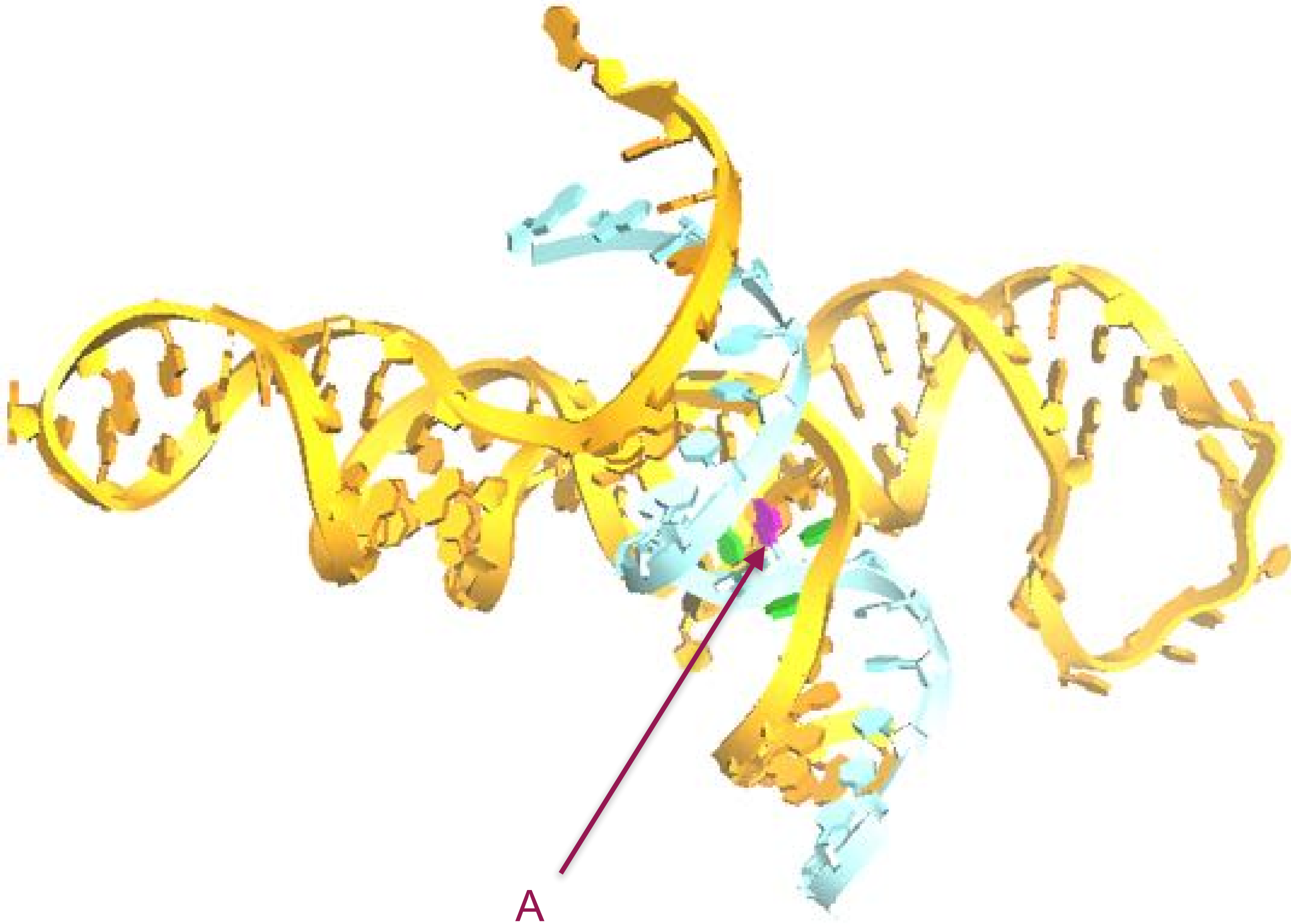
Threonine Dehydratase 1ve5



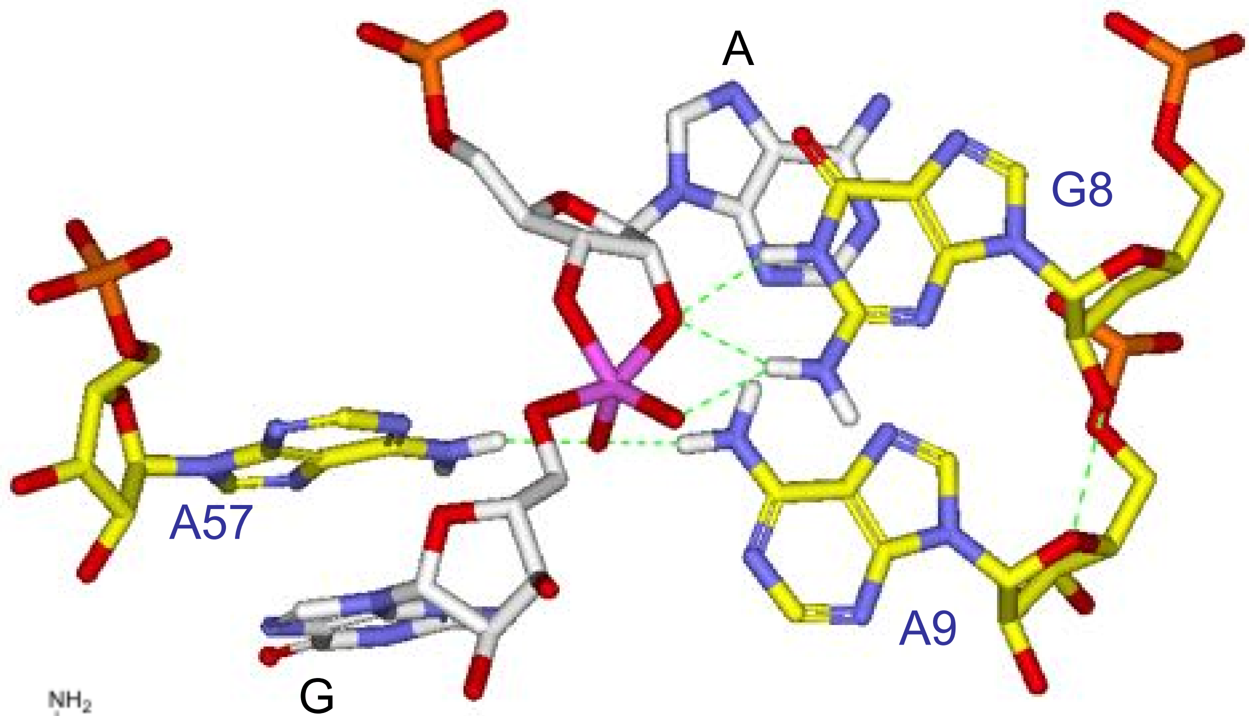
Threonine Dehydratase 1ve5

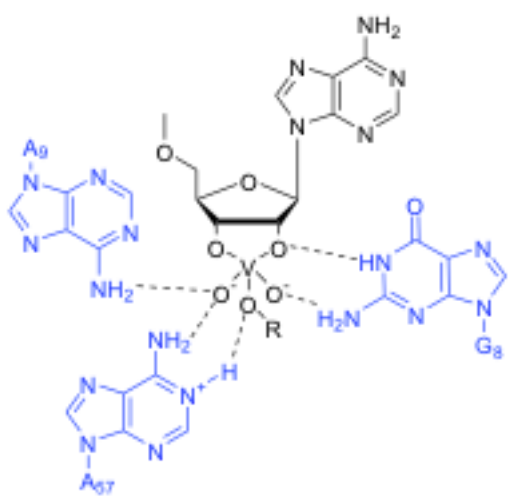


Hairpin Ribozyme

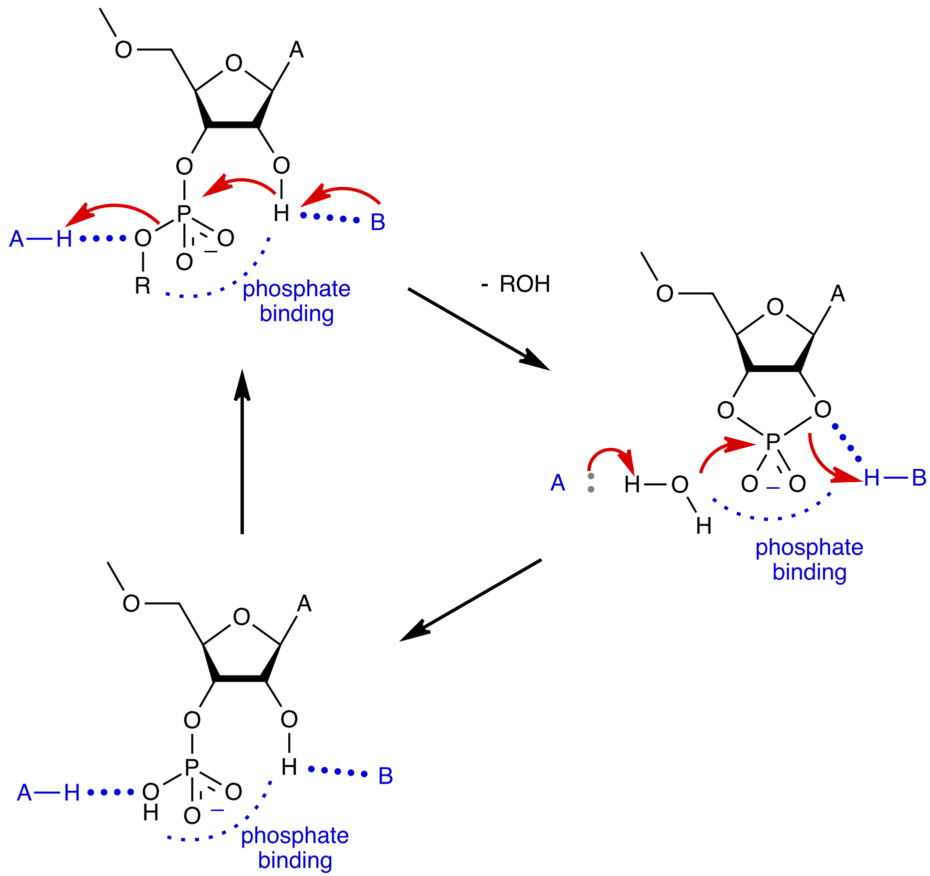


Hairpin Ribozyme

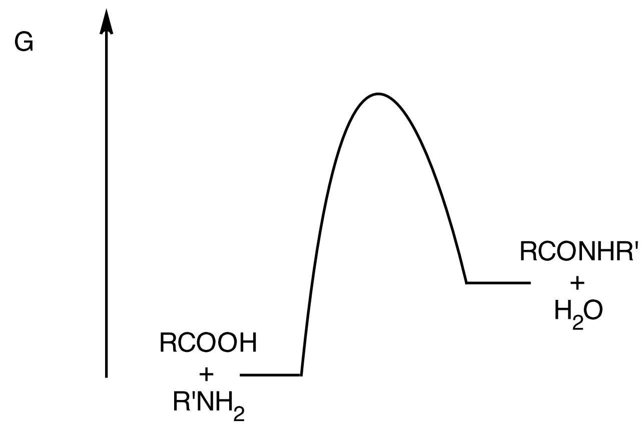


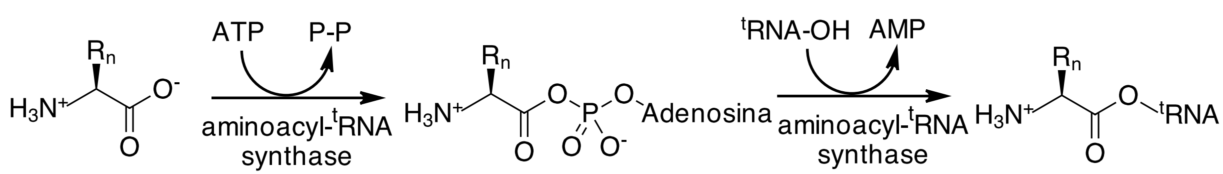


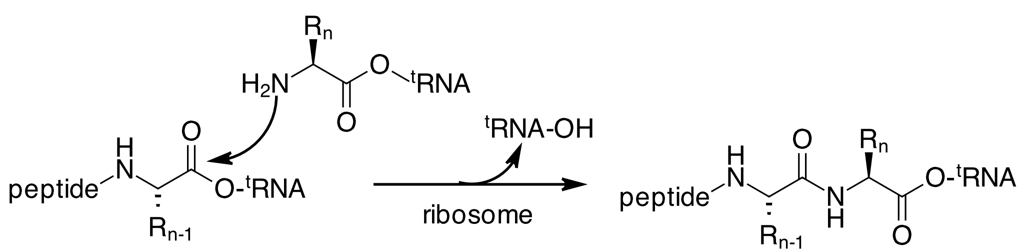
Hairpin Ribozyme: Simplified Mechanism



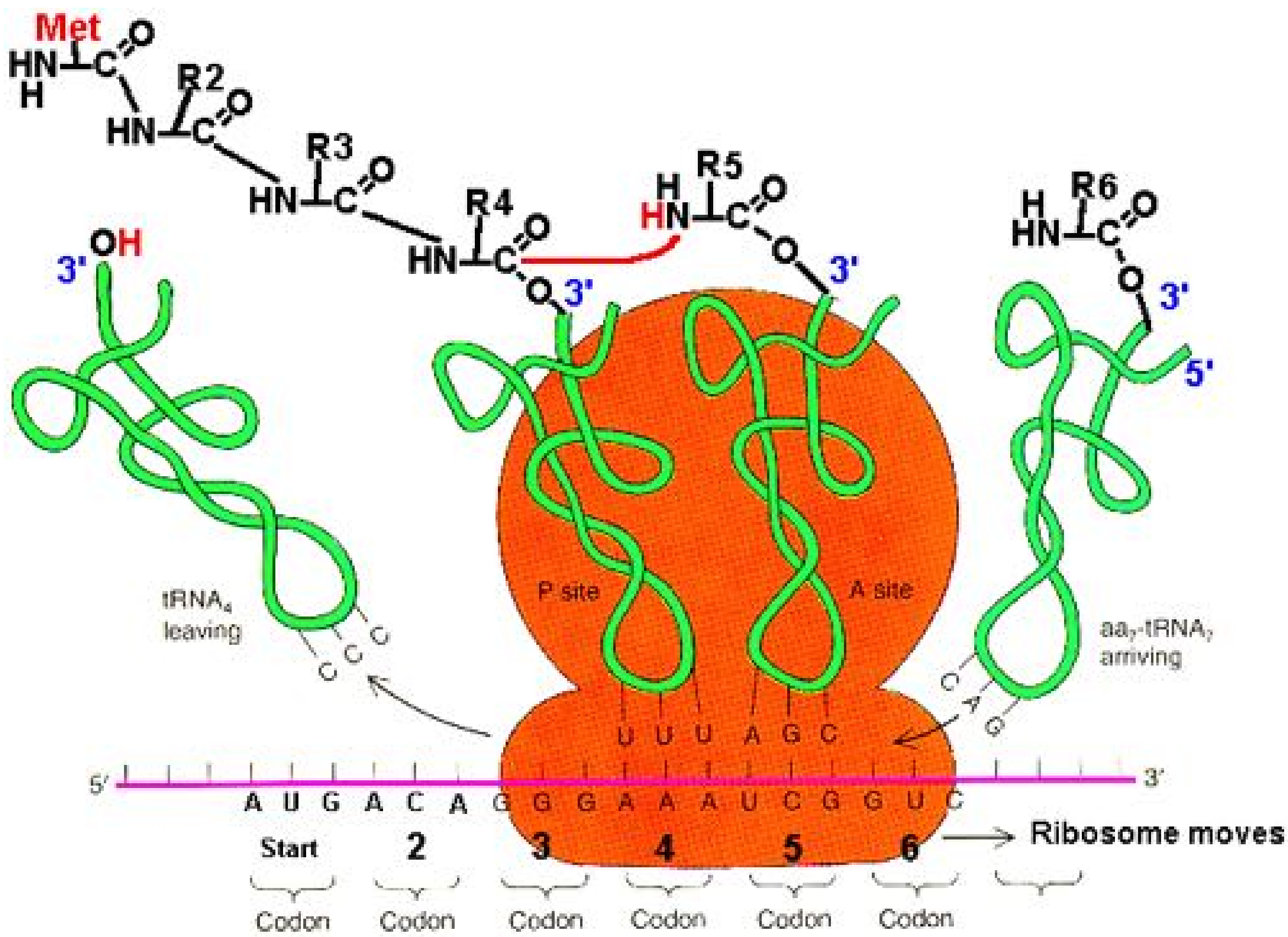
Protein Synthesis





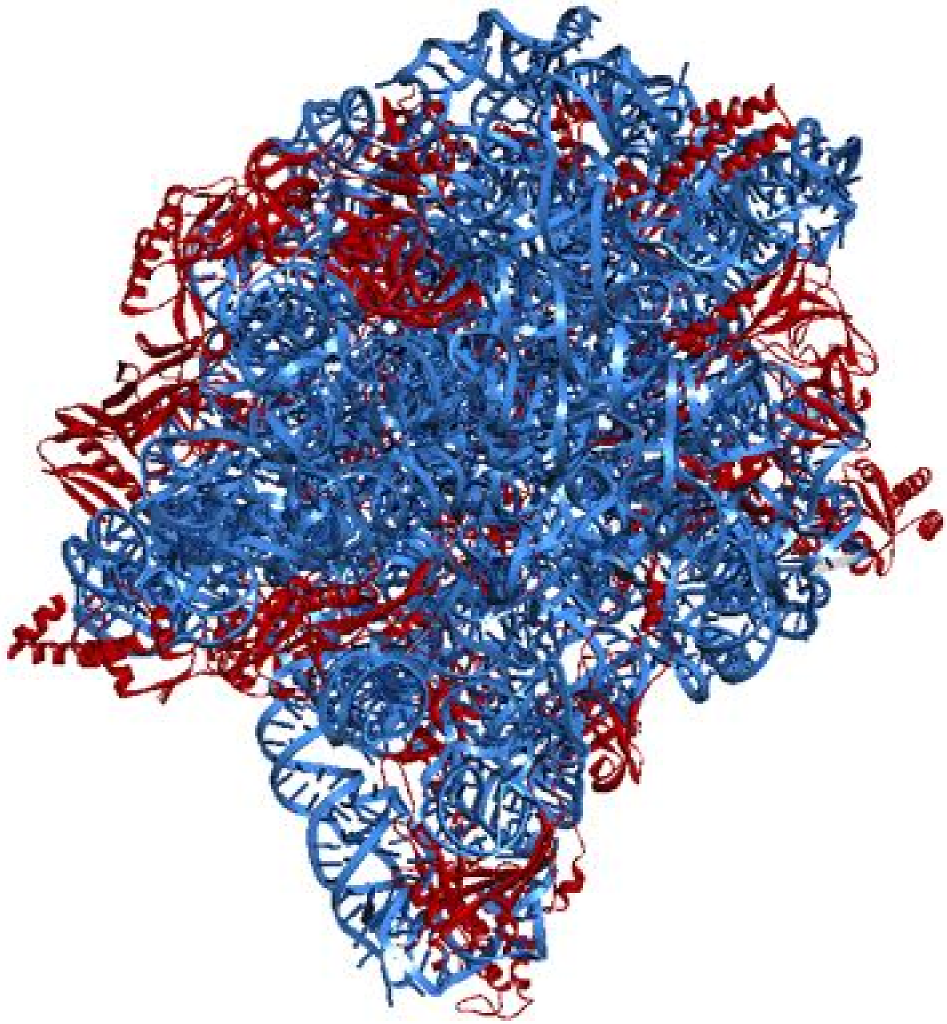


Ribosome and Protein Synthesis



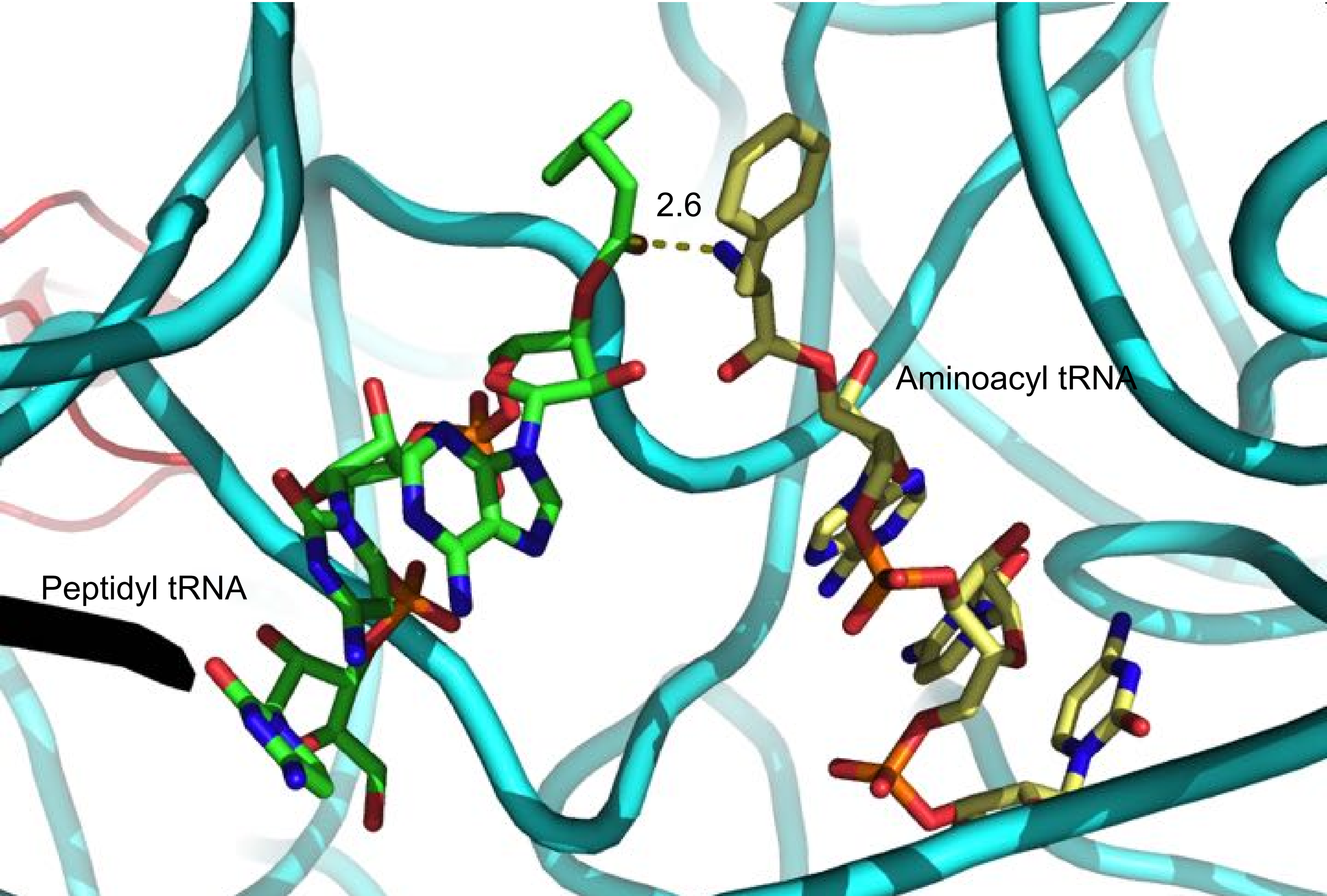
Modified from Griffiths et al., AN INTRODUCTION TO GENETIC ANALYSIS, 6th Ed., W.H. Freeman & Co., 1996.

The Ribosome (Holoarcula Marismortui)

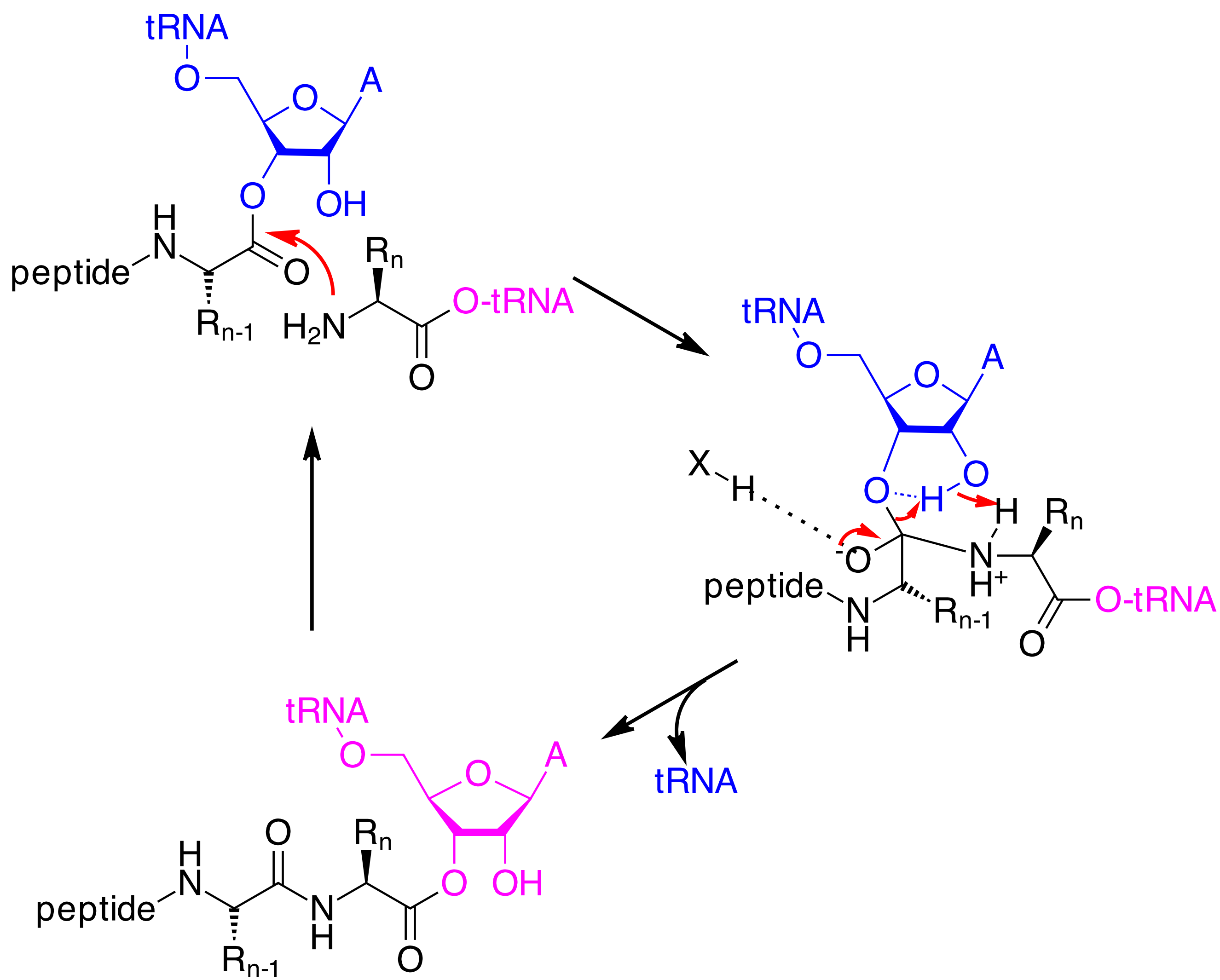


Red: proteins
Cyan: RNA

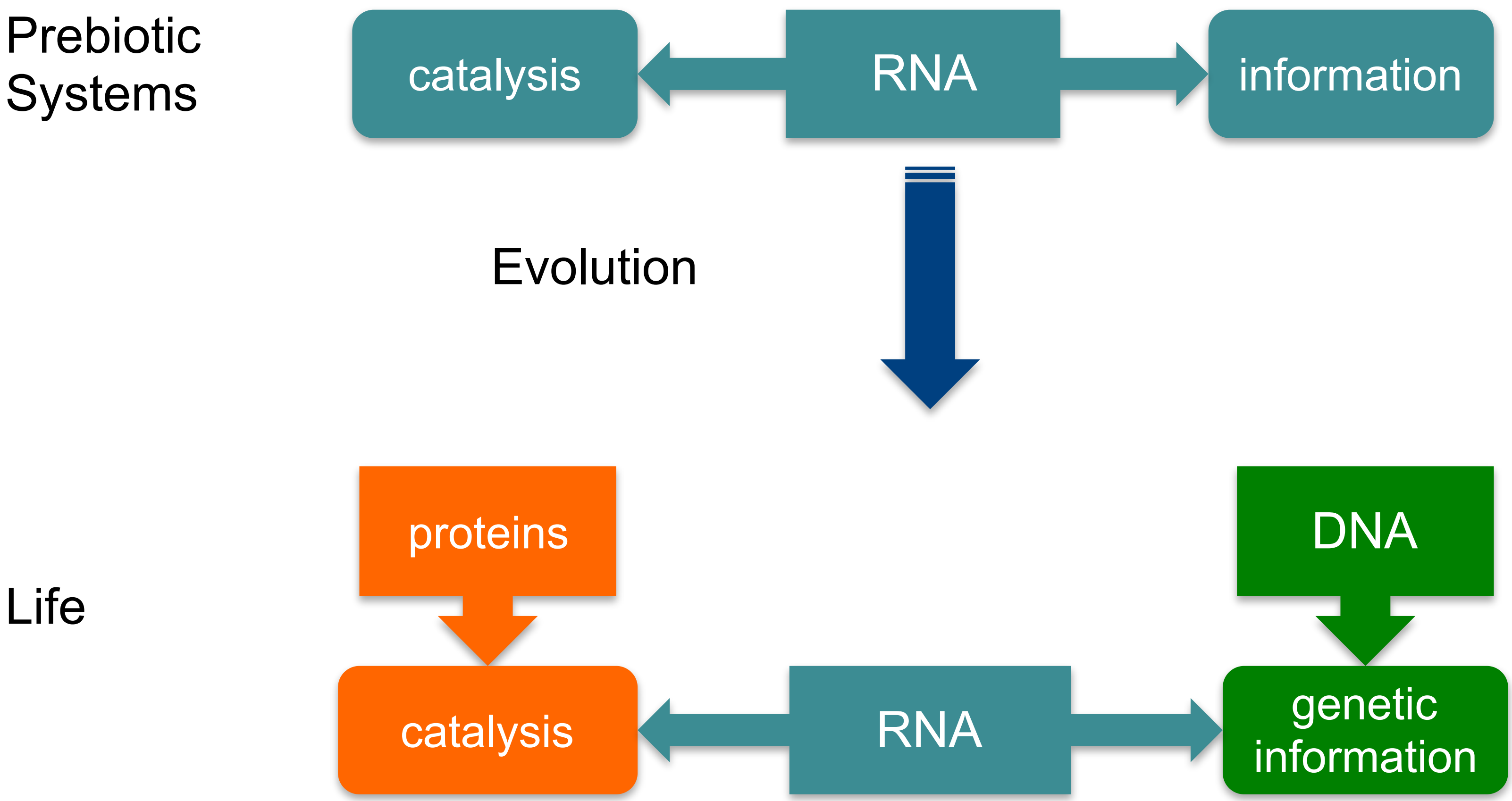
Ribosome and Protein Synthesis



Ribosome and Protein Synthesis

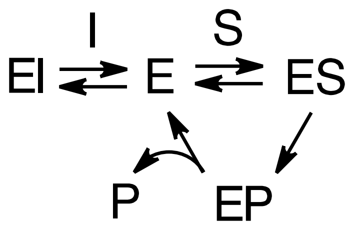


Catalysis and Evolution



Enzyme Inhibitors

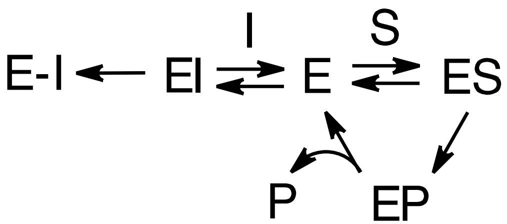
Reversible



Competitive

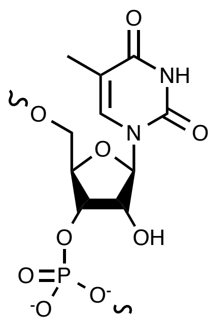
Non Competitive

Irreversible

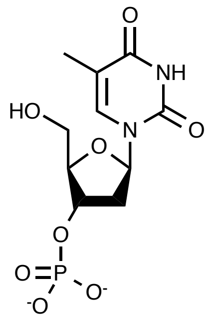


Reversible Inhibitors

substrate analogs

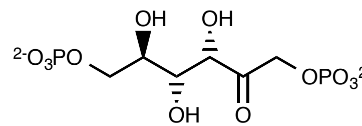


S

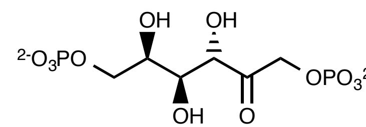


I

Ribonuclease A



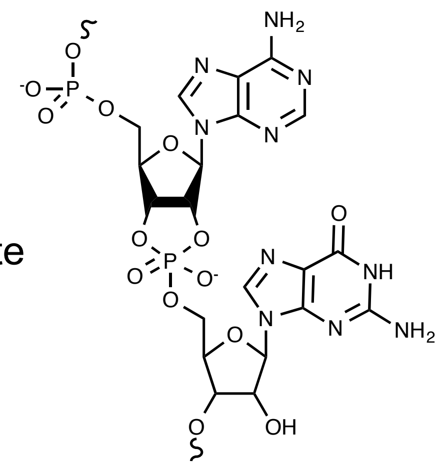
S



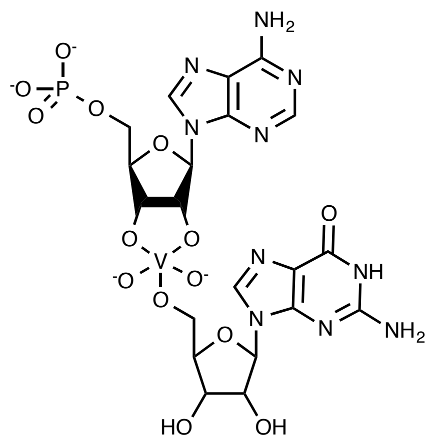
I

F6P-Aldolase

transition state analogs

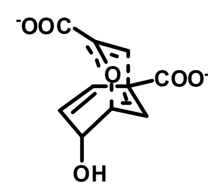


TS

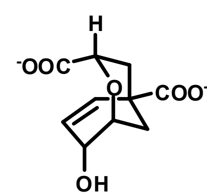


I

Hairpin Ribozyme



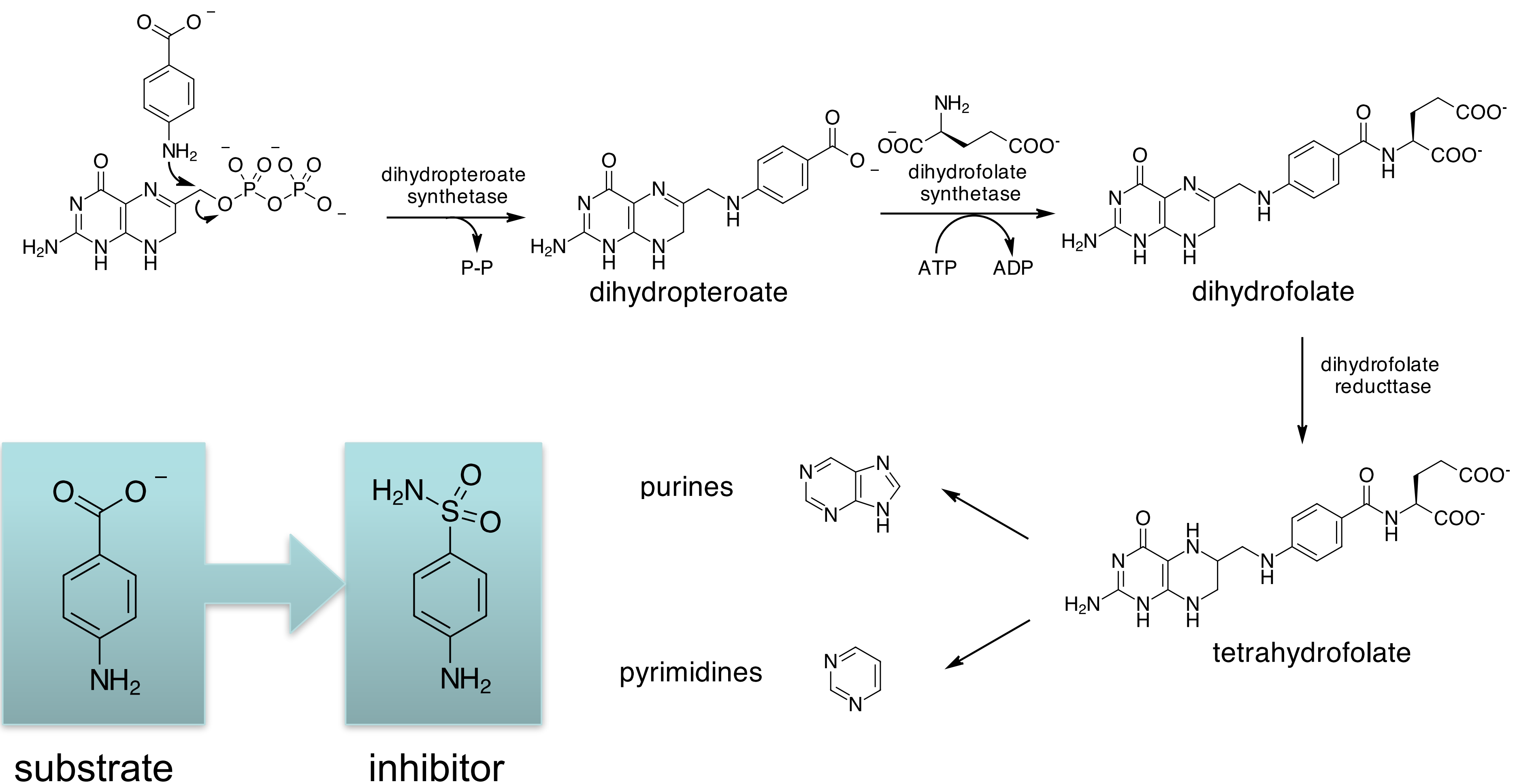
TS



I

Chorismate Mutase

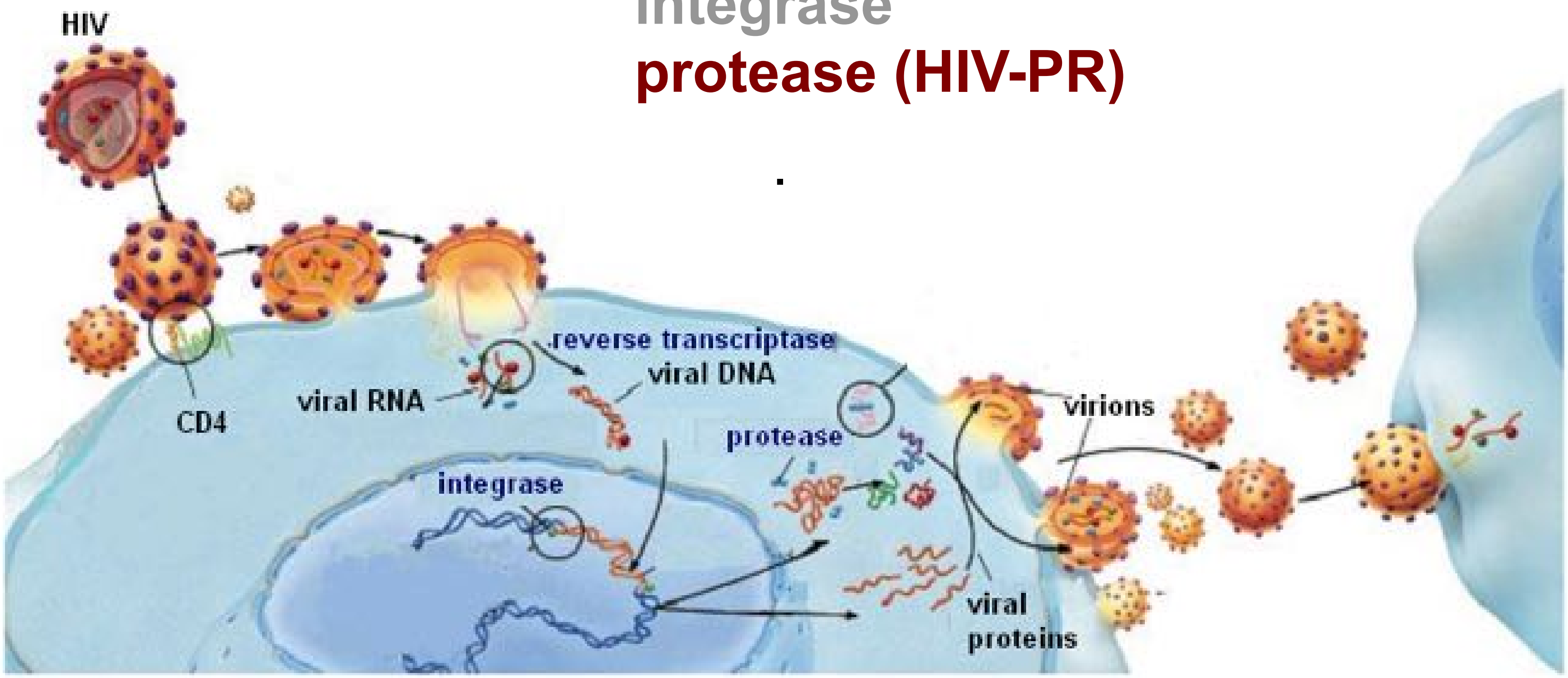
Substrate Analogs: Sulfa Drugs



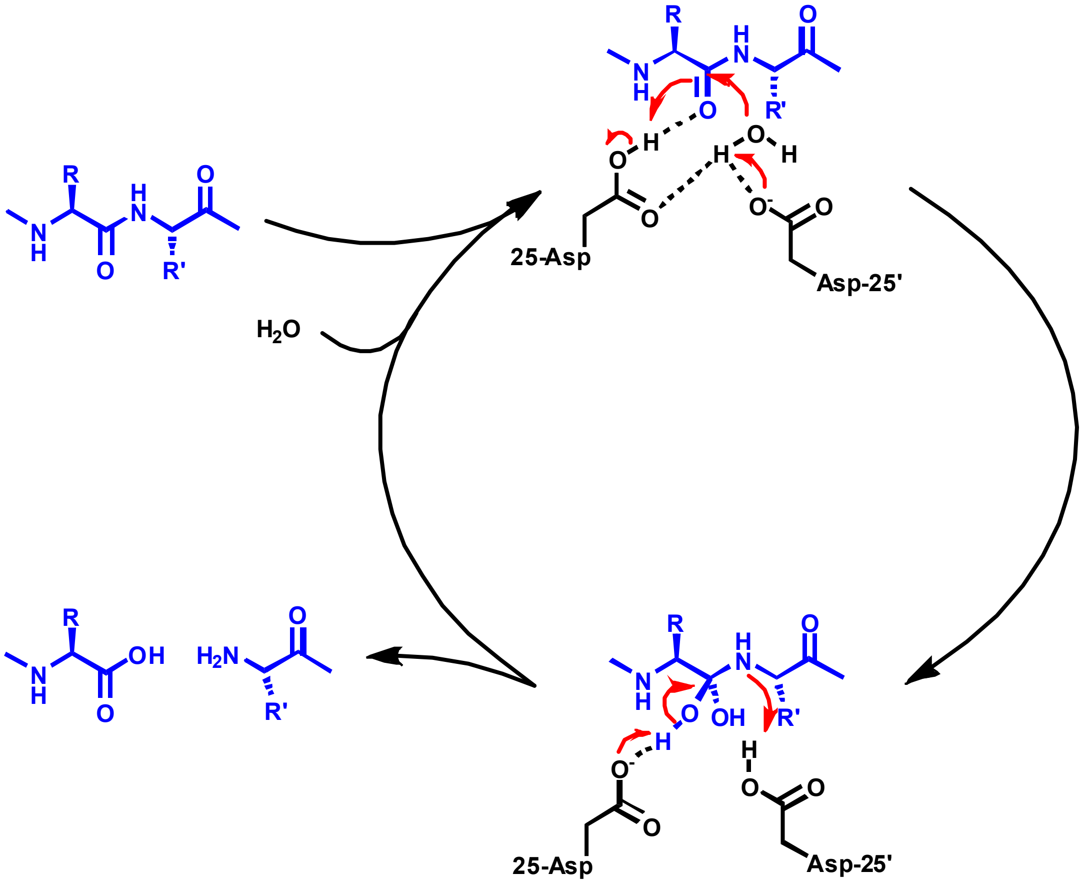
TS Analogs: HIV Protease Inhibitors

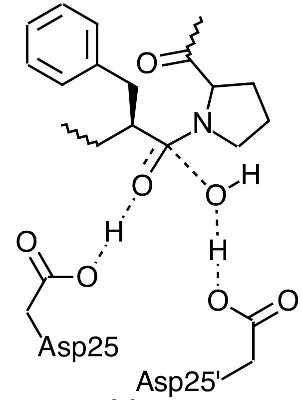
3 viral enzymes:
inverse transcriptase
integrase
protease (HIV-PR)

.

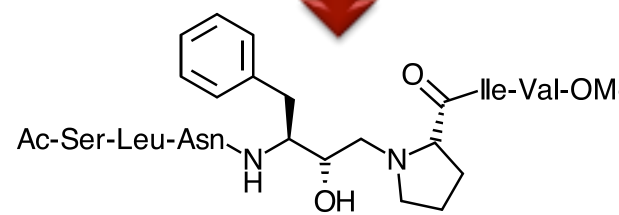


TS Analogs: HIV Protease Inhibitors

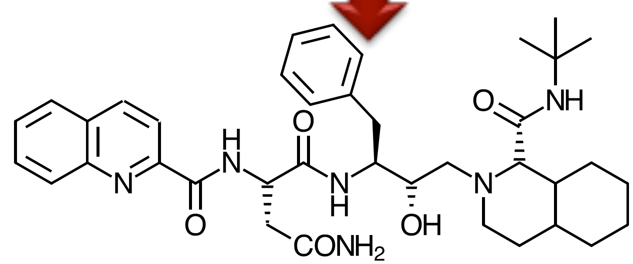




transition state

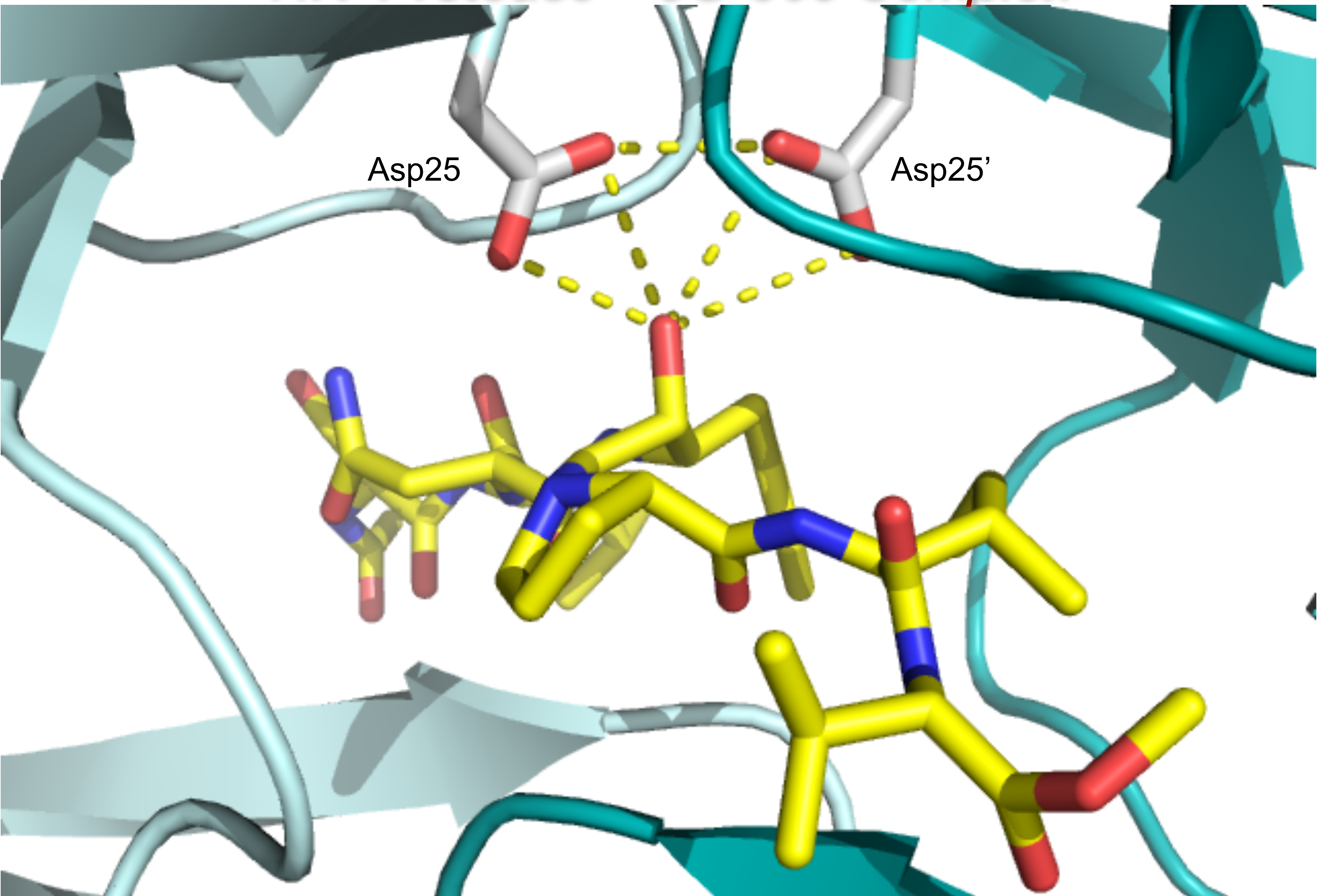


transition state analog (JG-365) K_i = 0.66nM

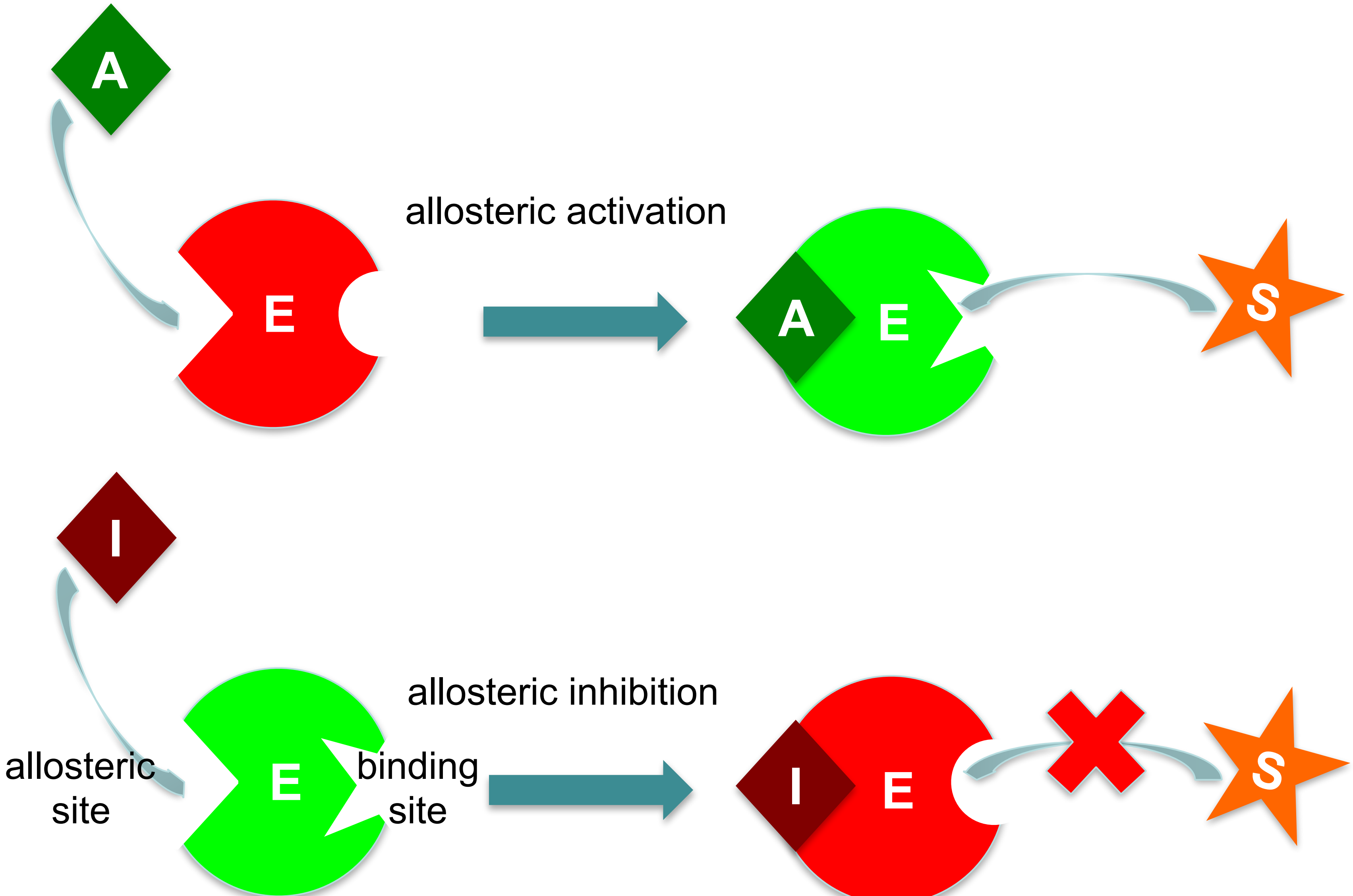


Saquinavir®

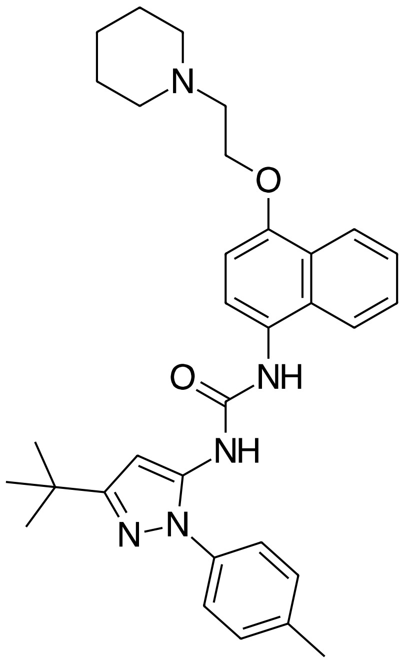
HIV-Protease – JG-365 Complex



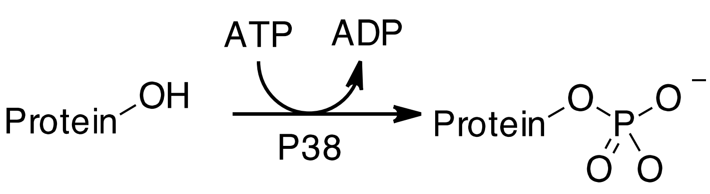
Non Competitive (Allosteric) Inhibition

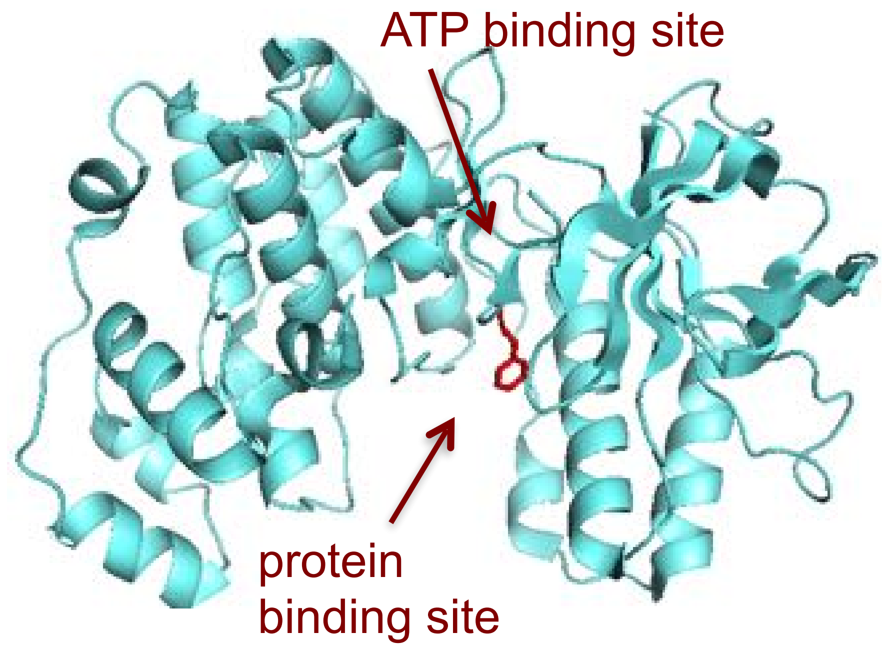


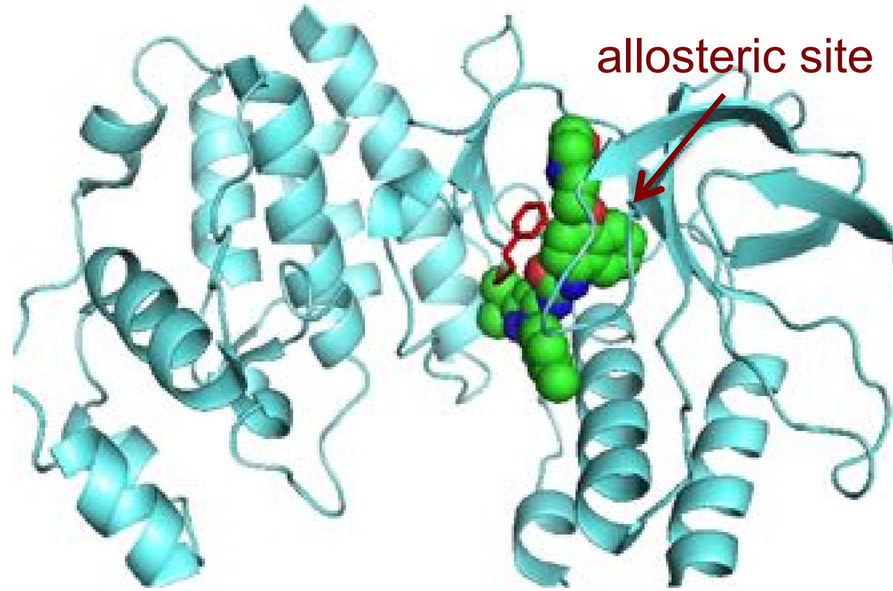
A P38 Kinase Non Competitive Inhibitor



BIRB 796 (Doramapimod®)
antiinflammatory

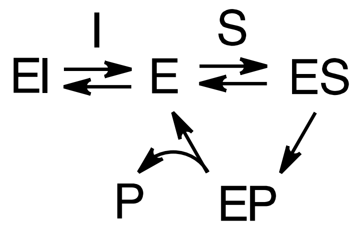






Enzyme Inhibitors

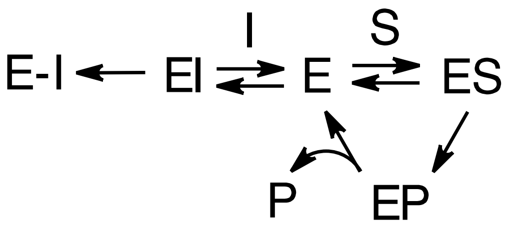
Reversible



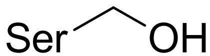
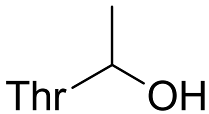
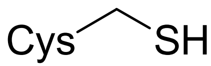
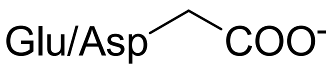
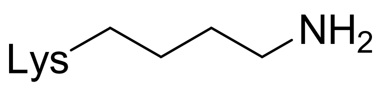
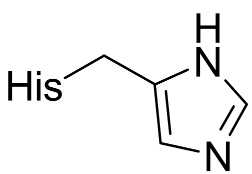
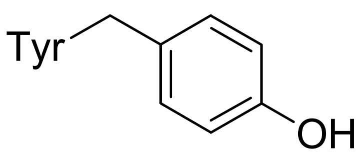
Competitive

Non Competitive

Irreversible

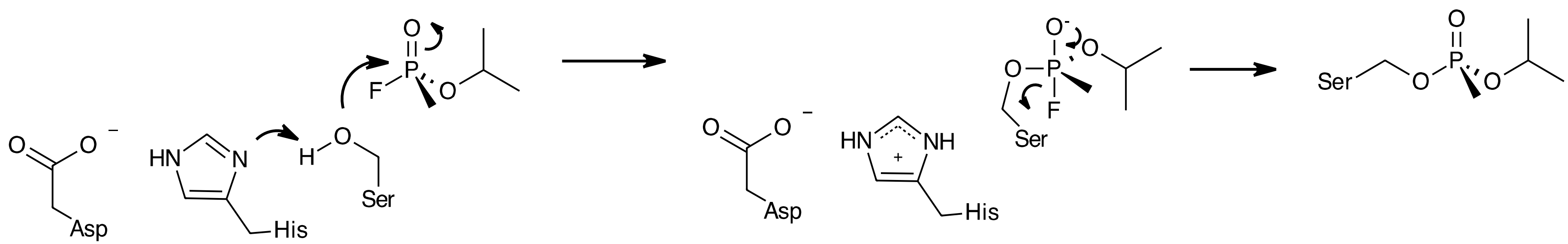


Nucleophiles

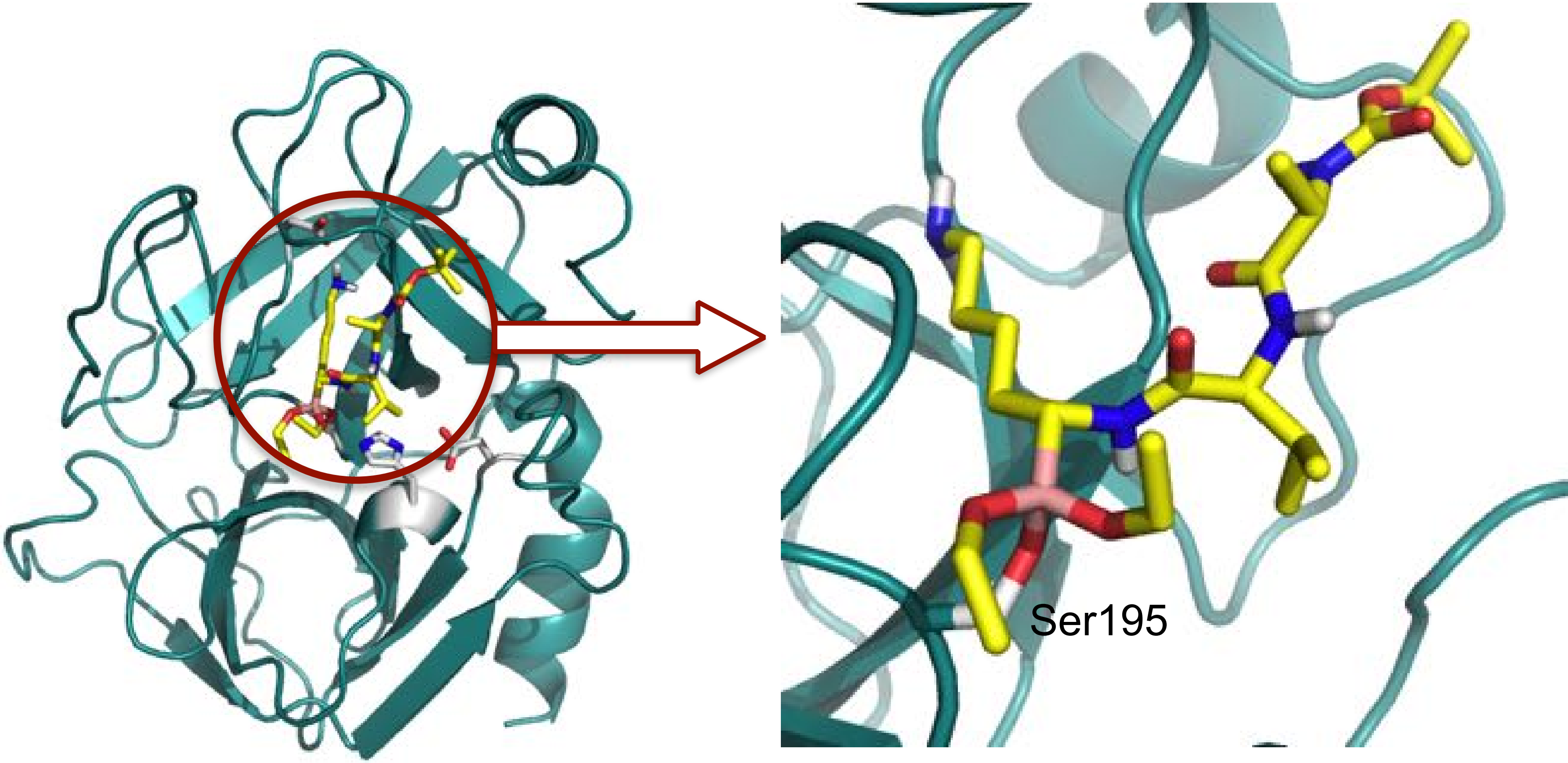
	Proteases, lipases, esterases
	Posphotransferases
	Proteases
	Epoxide hydrolases, haloalkane dehalogenases
	Aldolases, acetoacetate decarboxylase
	Phosphotransferases, Nucleases
	DNA topoisomerase

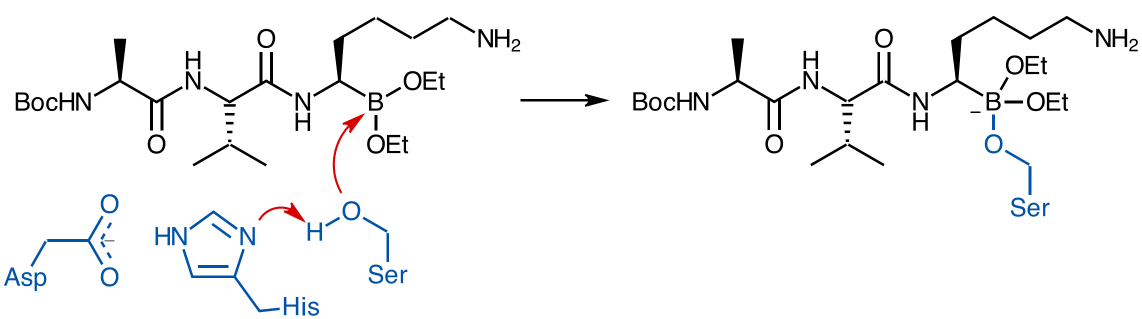
Irreversible Inhibitors

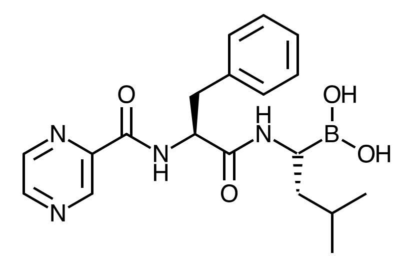
AChE:



Irreversible Inhibitors: Trypsin



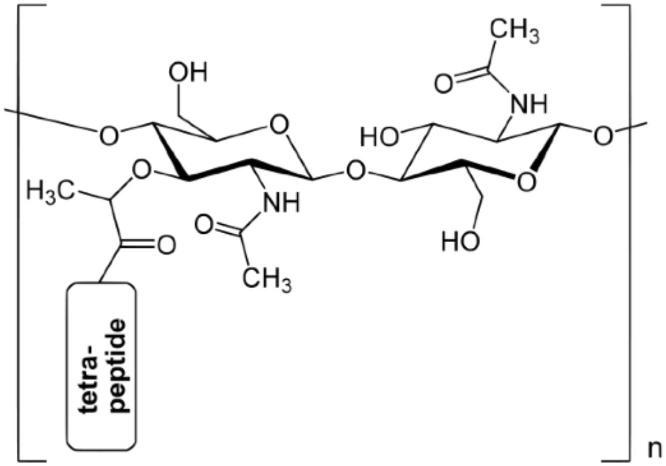


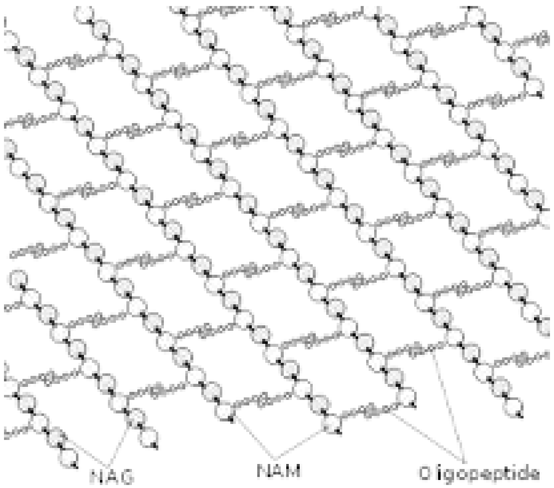


Bortezomib

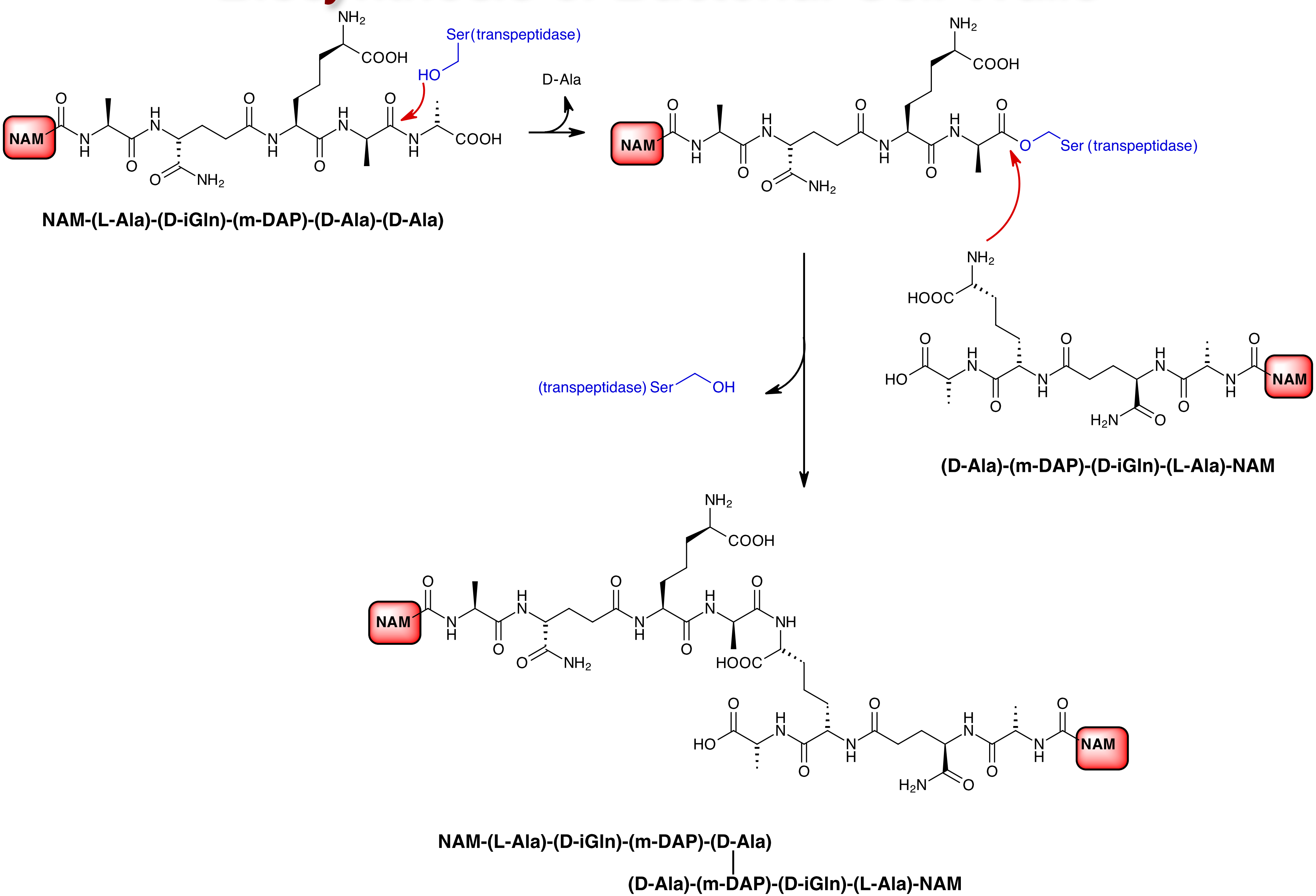
Penicillin (Transpeptidase Inhibitor)

Bacterial Cell Wall:

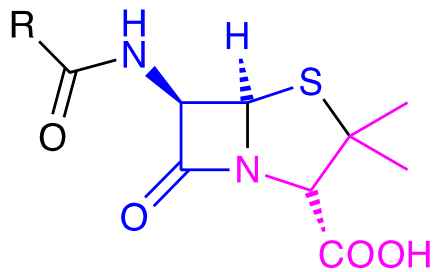




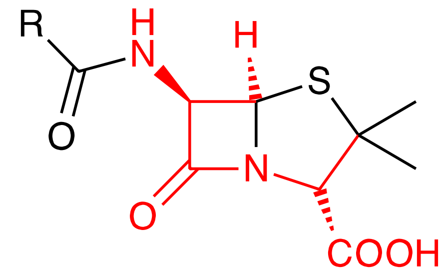
Biosynthesis of Bacterial Cell Walls



Penicillin (Transpeptidase Inhibitor)

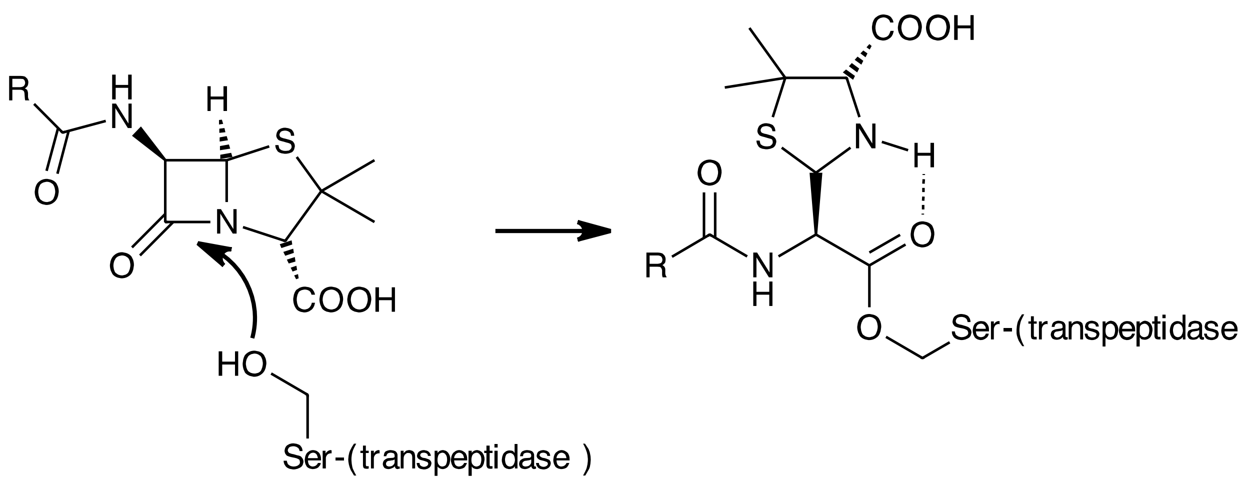


from cys and val



mimics the Ala-Ala substrate

Inhibition:



Curcumin

- antibatterico-antivirale
- antitumorale
- antiossidante
- antiinfiammatoria