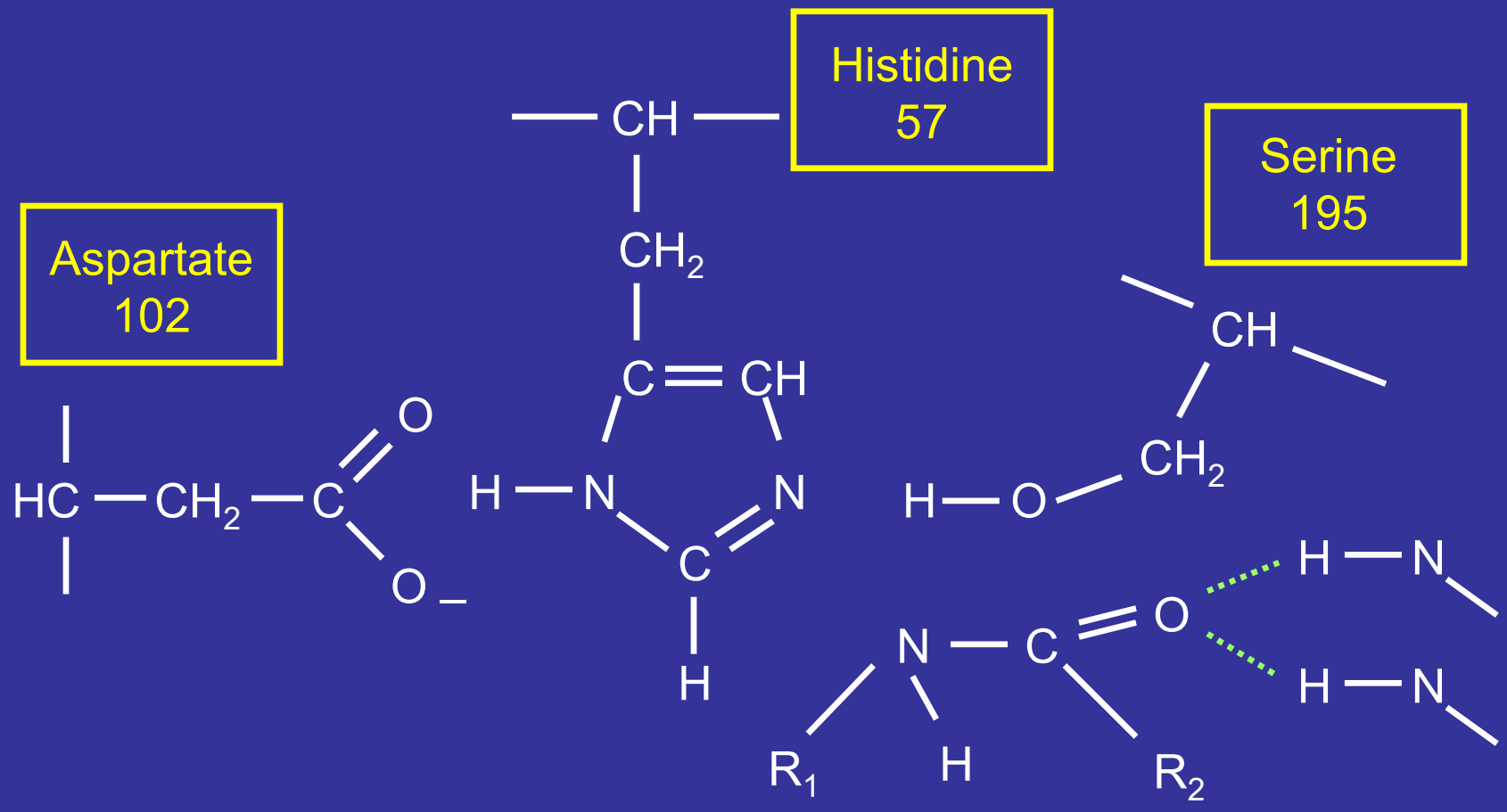
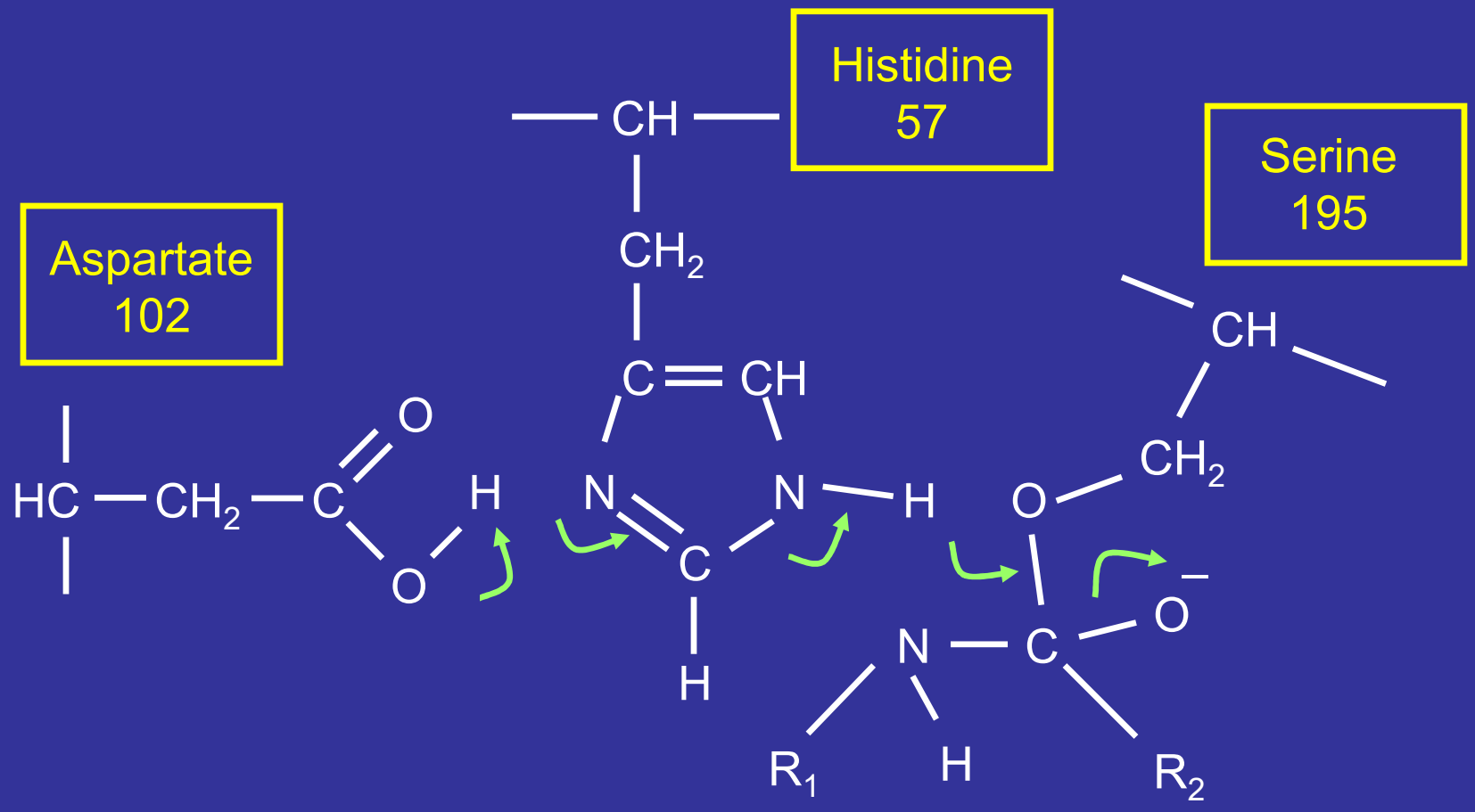
E. Molecular Mechanism of Enzyme Catalysis

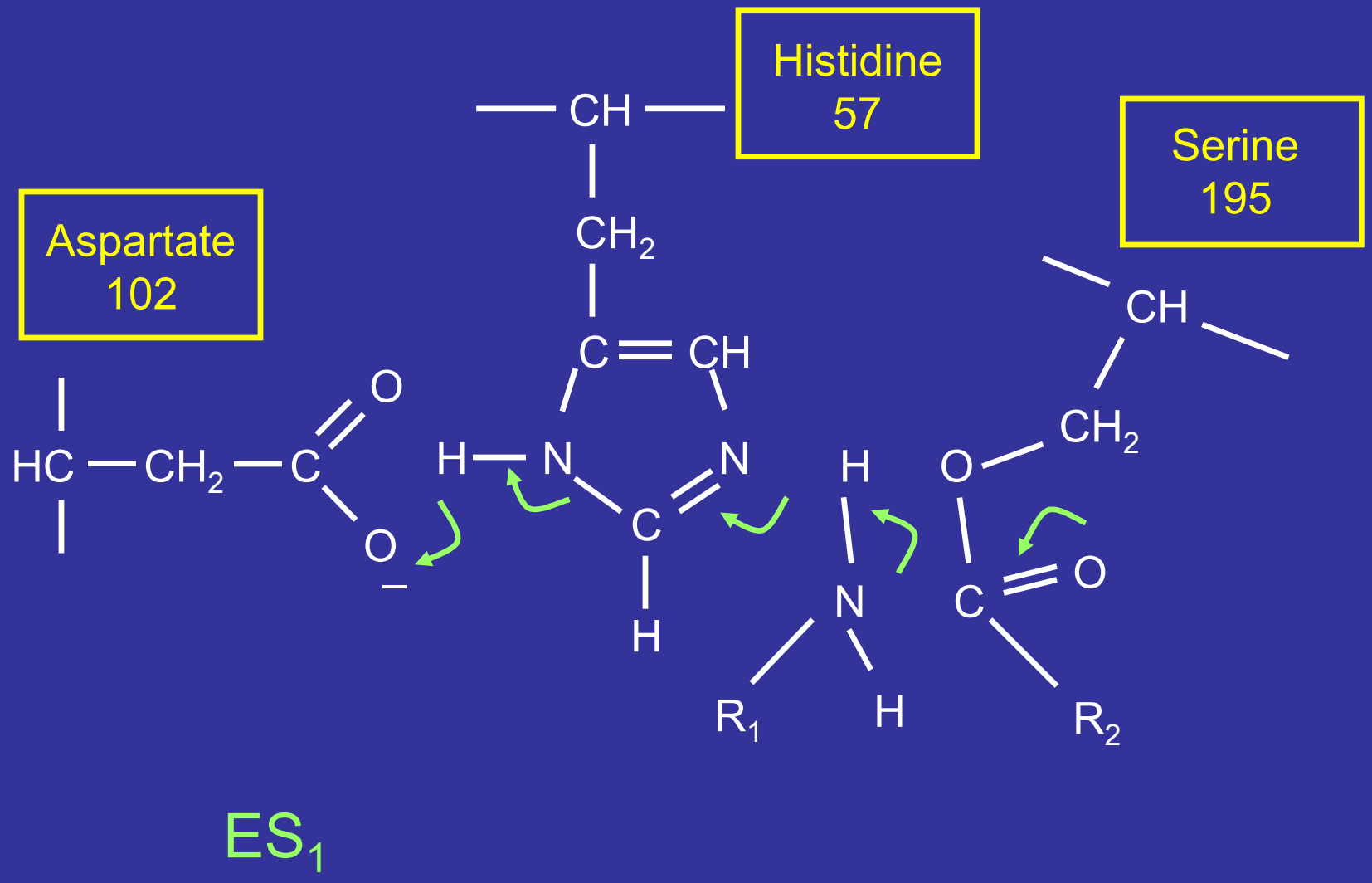
1. Chymotrypsin

An example of base and nucleophilic catalysis via a charge relay system. Serine is often involved as a strong nucleophile in proteases.

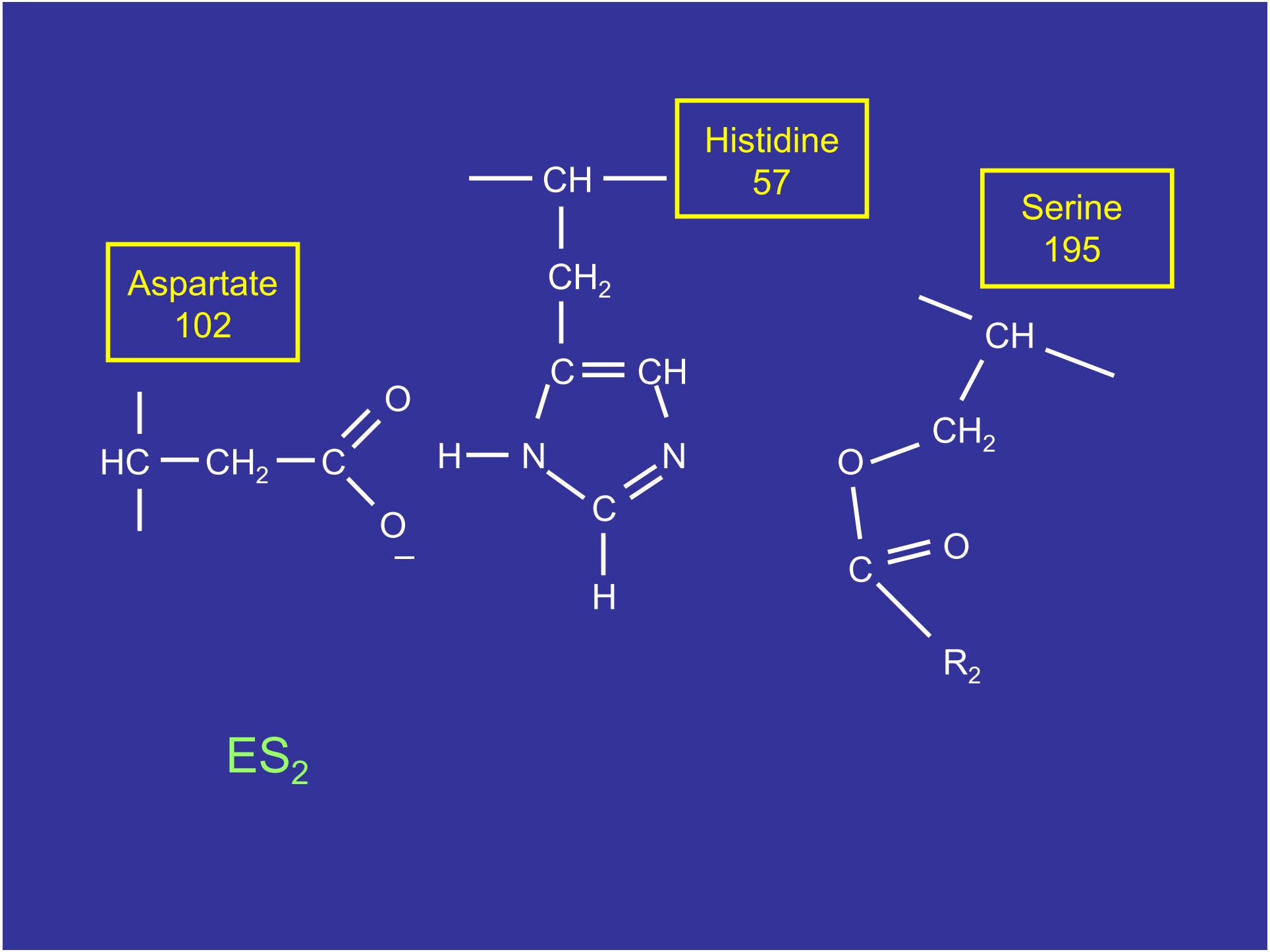

Histidine
57
Aspartate
102
Serine
195
E + S
Protein Stabilized by
Hydrogen Bonding

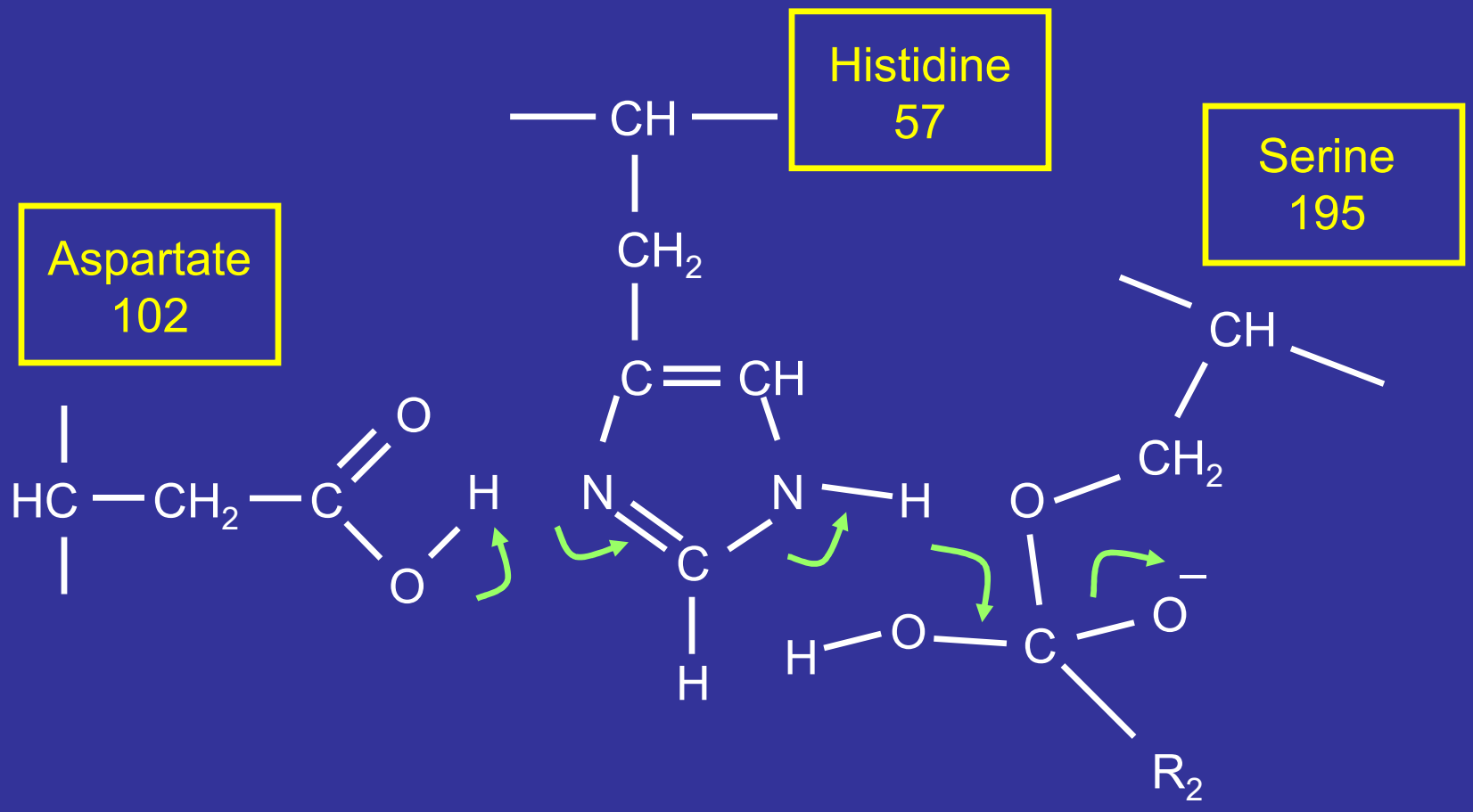

Histidine
57
Serine
195
Aspartate
102
CH
CH2
C
CH
HC
CH2
C
O
O
H
N
N
H
C
H
O
CH2
CH
O
–
N
C
R1
H
R2

E + S

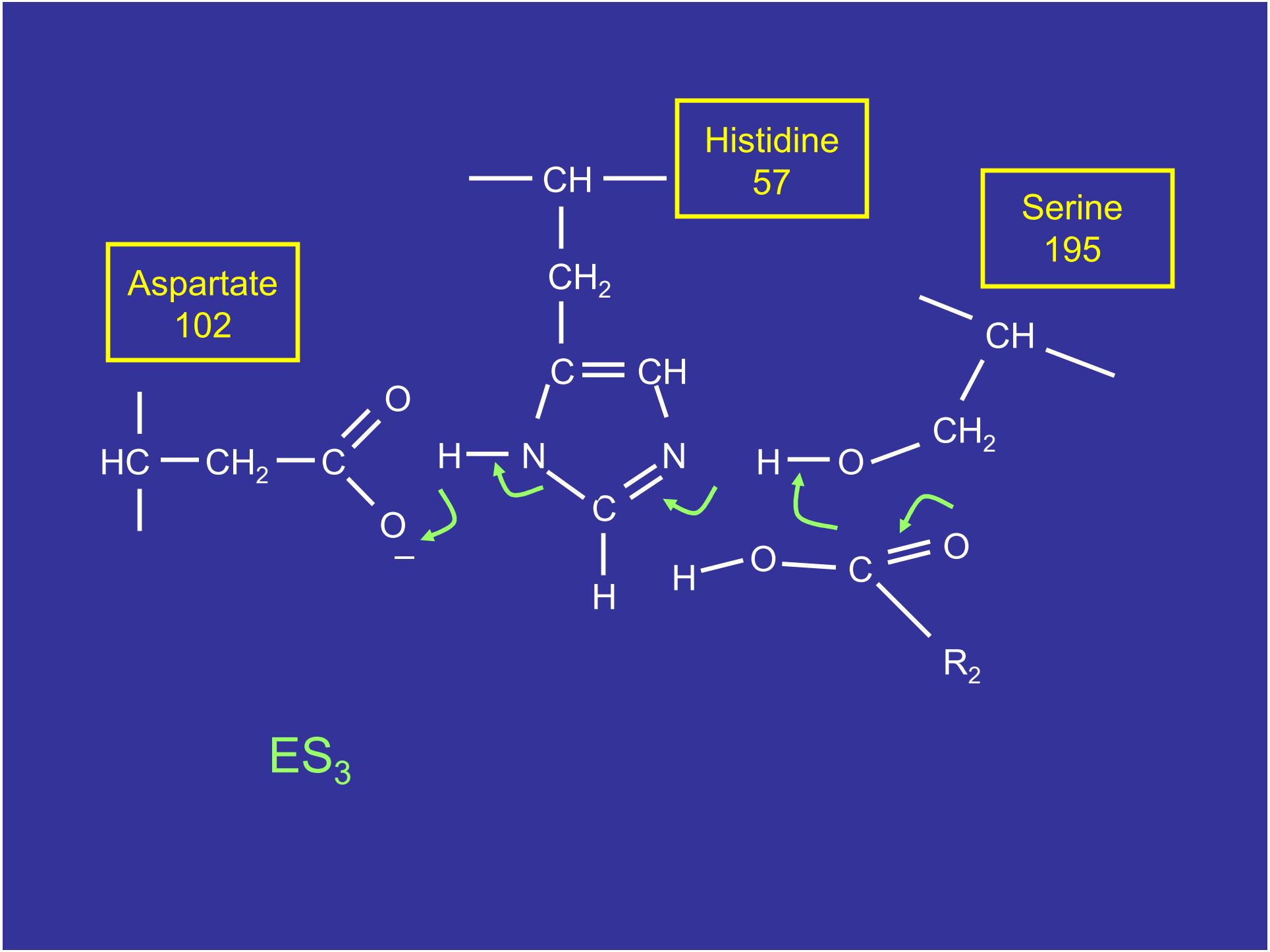

Histidine
57
Serine
195
Aspartate
102
CH
CH2
C
CH
O
HC
CH2
C
H
N
N
H
O
CH
CH2
O
O
C
H
N
H
R1
H
C
R2

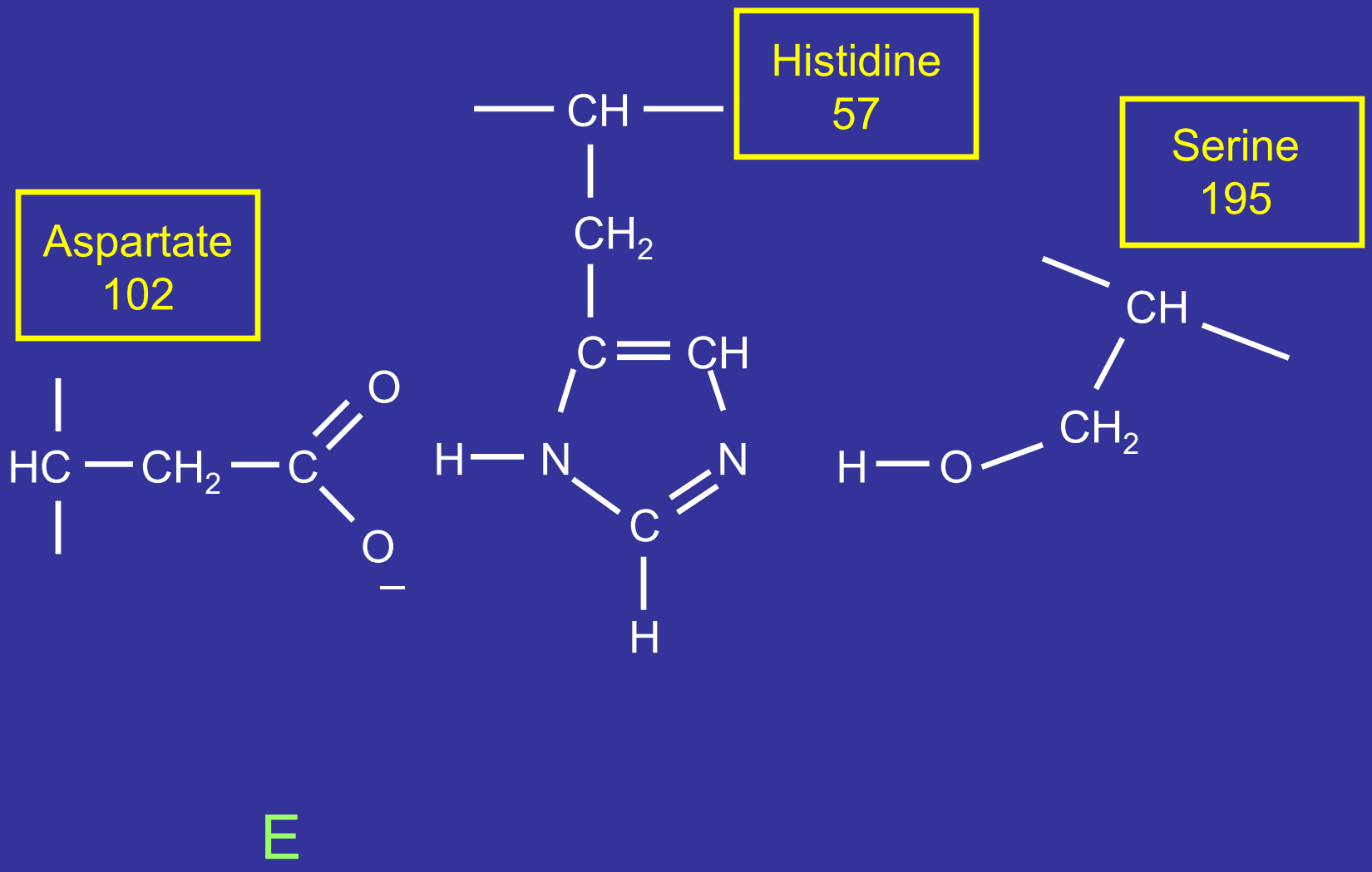
ES_1


Aspartate
102
Histidine
57
Serine
195
HC
CH2
C
O
O
–
CH
CH2
C
CH
H
N
N
C
H
CH
CH2
O
C
O
R2
ES2


Histidine
57
Serine
195
Aspartate
102
CH
CH2
C
CH
HC
CH2
C
O
O
H
N
N
H
C
H
O
CH2
CH
O
H
O
C
O
–
R2

$ES_2 + H_2O$


Histidine
57
Aspartate
102
Serine
195
ES3


Histidine
57
Serine
195
Aspartate
102
CH
CH2
C
CH
O
HC
CH2
C
H
N
N
O
CH
CH2
H
O
O
–
H

E

Chemical Reaction Model

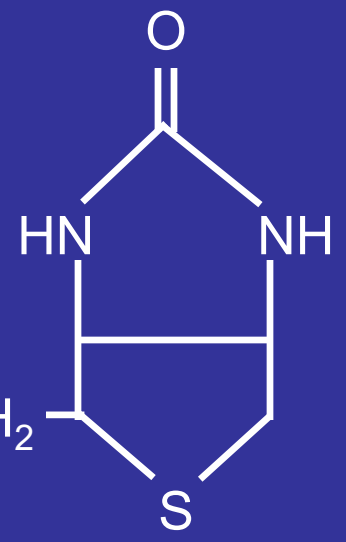
$$E + S \rightleftharpoons ES_1 \longrightarrow ES_2 + P_1$$

$$ES_2 + H_2O \rightleftharpoons ES_3 \longrightarrow E + P_2$$

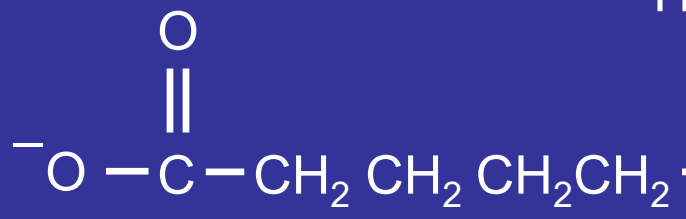
2. Carboxylation with biotin

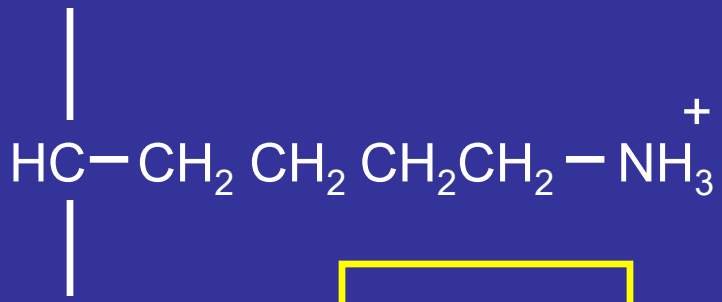
Carboxylation involves the addition of carbon dioxide to an organic biochemical. Several carboxylases use biotin (Vitamin H) and two active sites.

Biotin

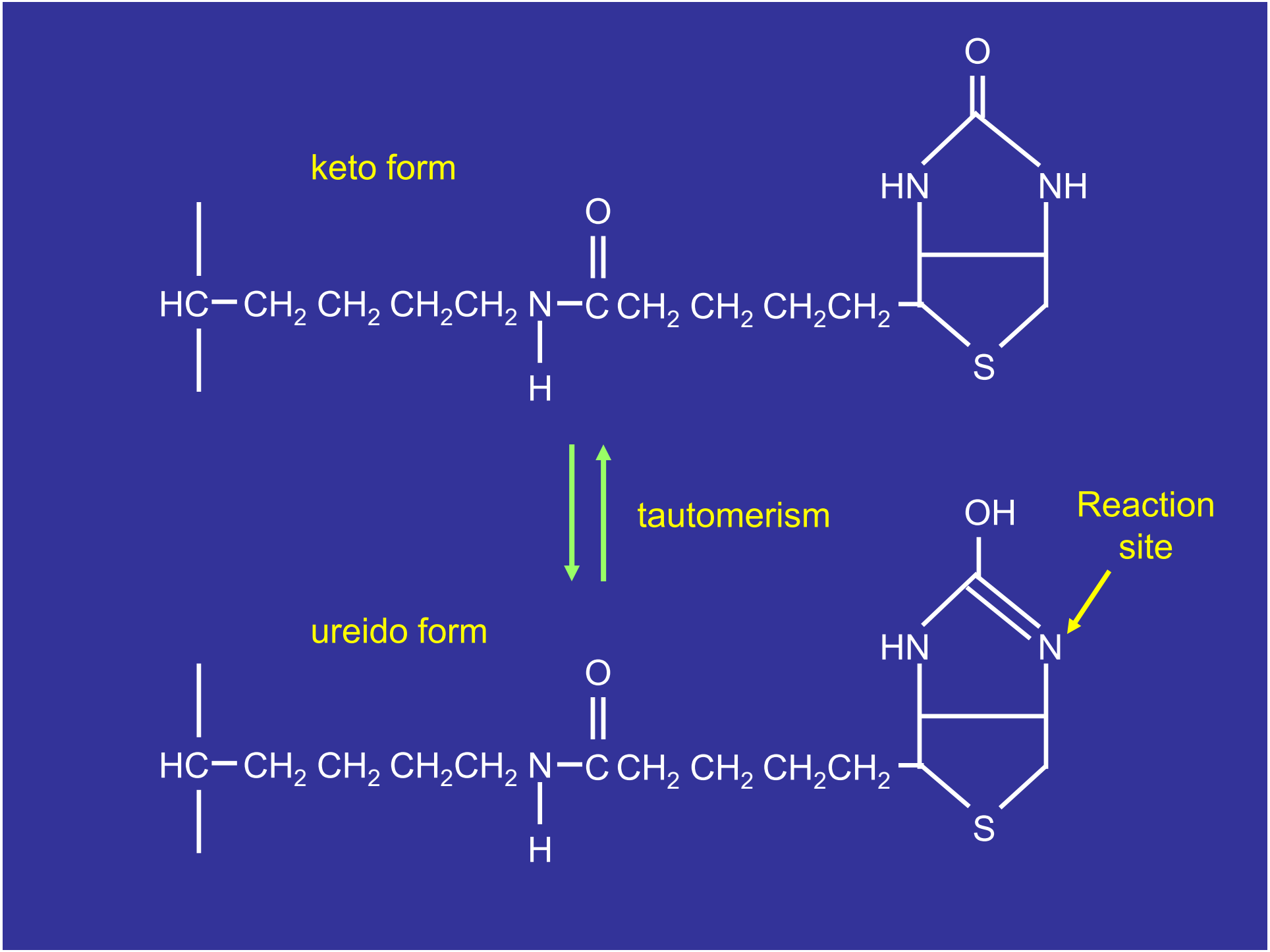


Pyruvate carboxylase

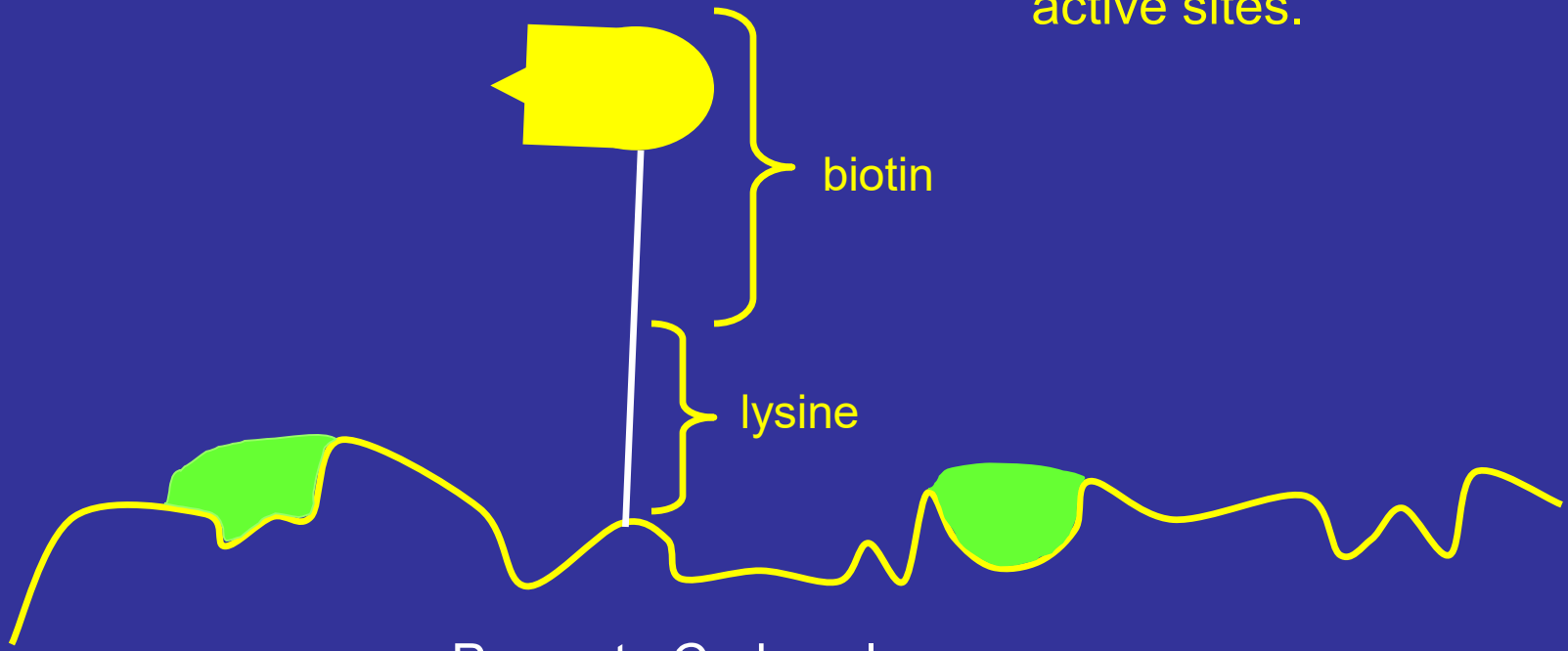




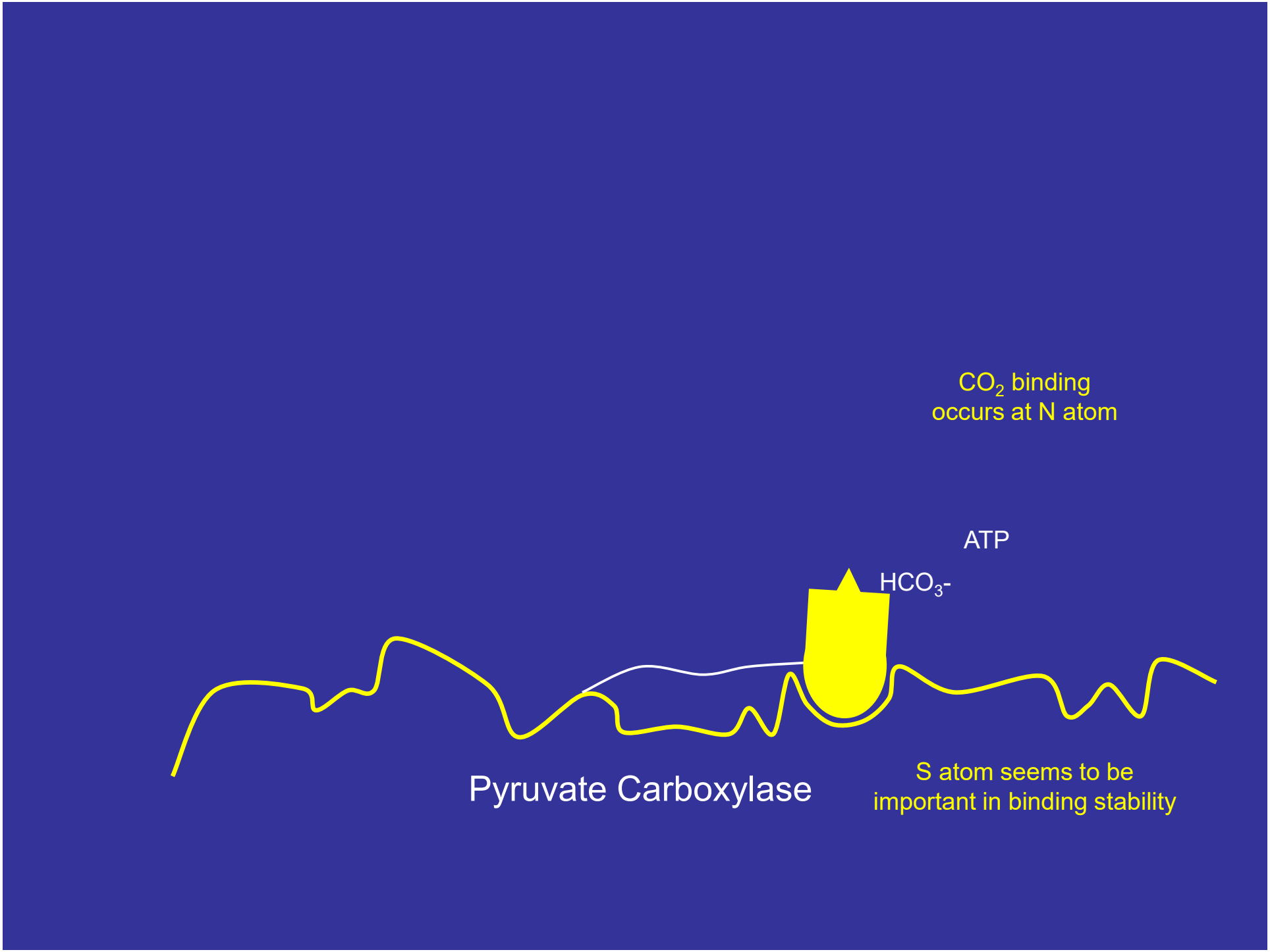
Lysine


keto form
O
HN
NH
S
HC
CH2 CH2 CH2CH2 N
C
CH2 CH2 CH2CH2
H
tautomerism
OH
Reaction site
ureido form
O
HN
N
S
HC
CH2 CH2 CH2CH2 N
C
CH2 CH2 CH2CH2
H

This enzyme has two active sites.

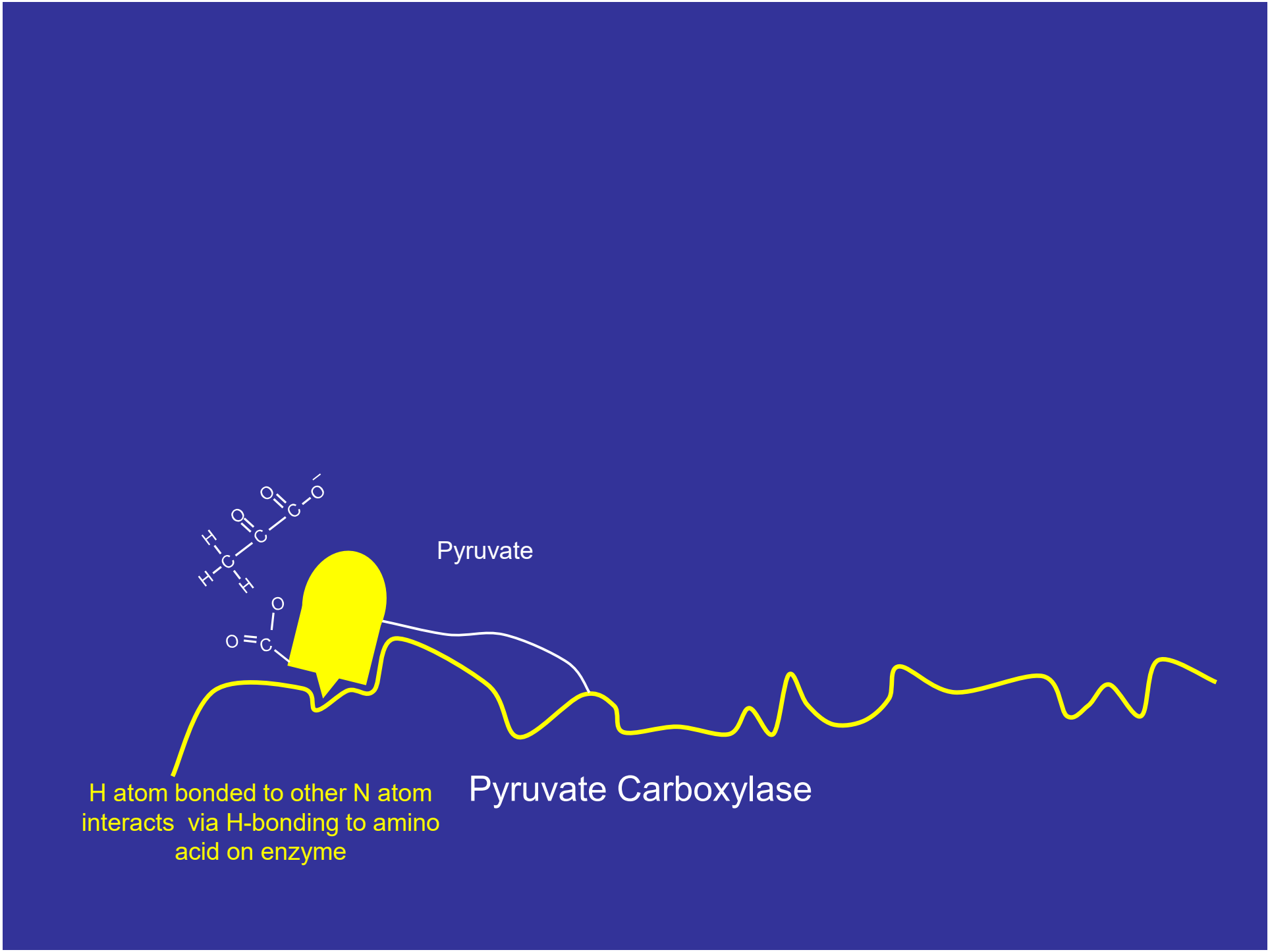


Pyruvate Carboxylase


CO_2 binding
occurs at N atom
ATP
HCO_3-
Pyruvate Carboxylase
S atom seems to be
important in binding stability

Carboxybiotin is not as stable in first active site

Pyruvate Carboxylase


Pyruvate
H atom bonded to other N atom interacts via H-bonding to amino acid on enzyme
Pyruvate Carboxylase

Biotin is not as stable
in second active site

Pyruvate Carboxylase

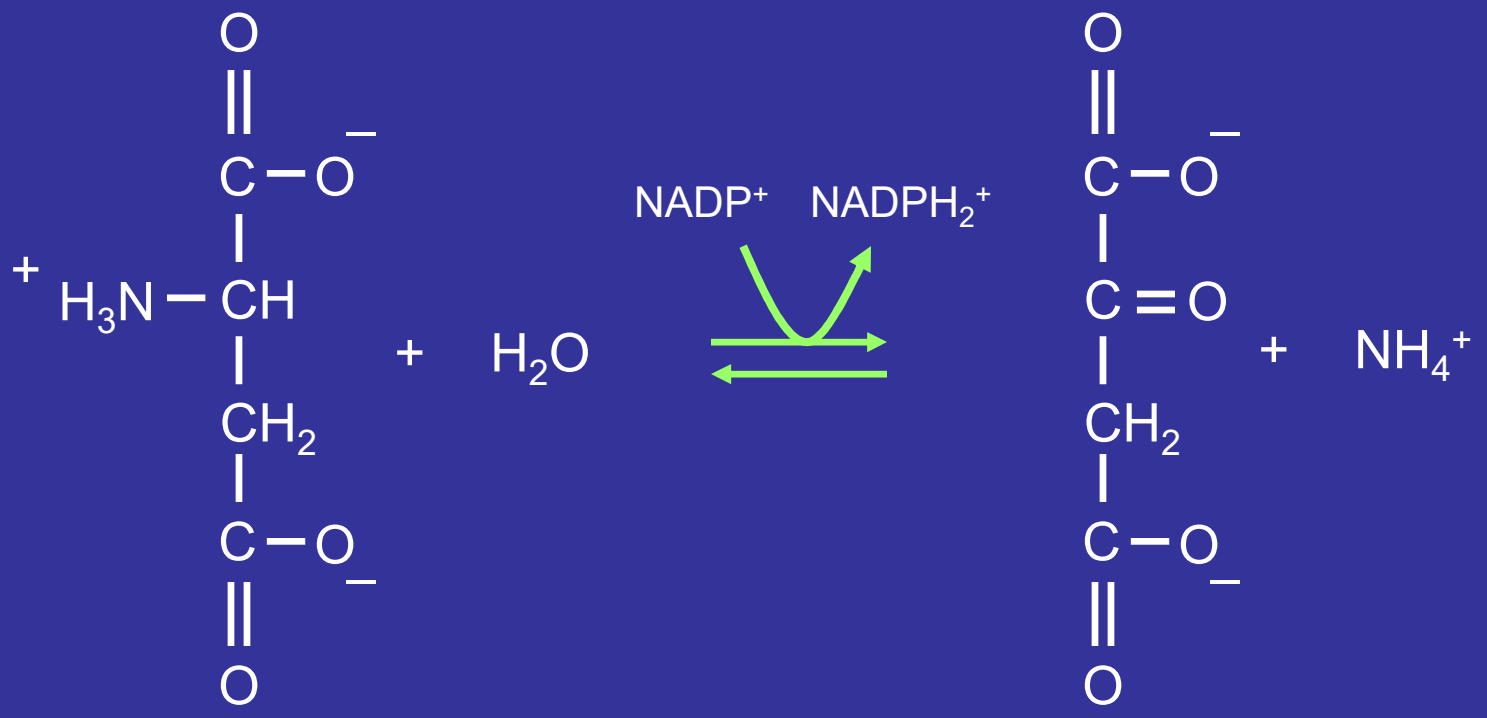
pyruvate carboxylase (protein) + biotin = active enzyme

apoenzyme + cofactor = holoenzyme

Pyruvate Carboxylase

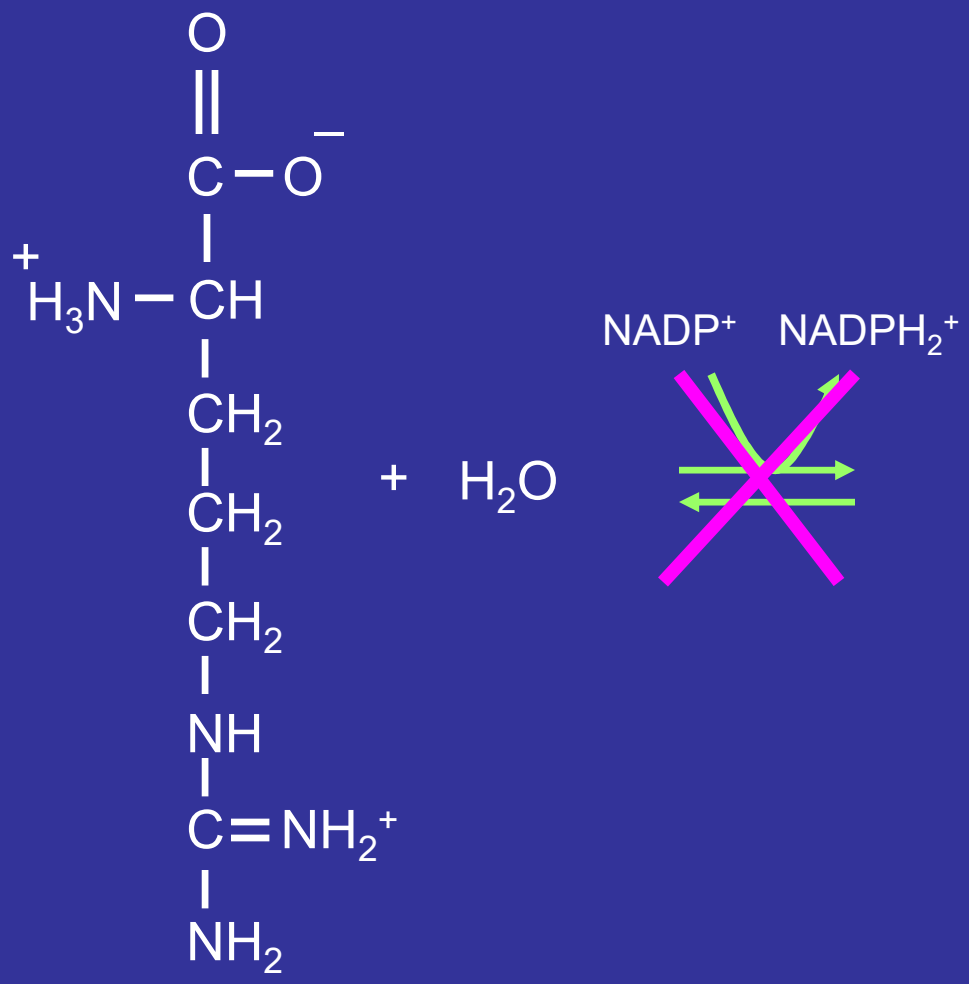
3. Site-directed mutagenesis of aspartate aminotransferase

By altering the gene encoding a protein at one location, the amino acid residue at one location in a protein may be changed to another amino acid residue. If this one change is in the vicinity of the active site, it can have dramatic effects on the reaction.



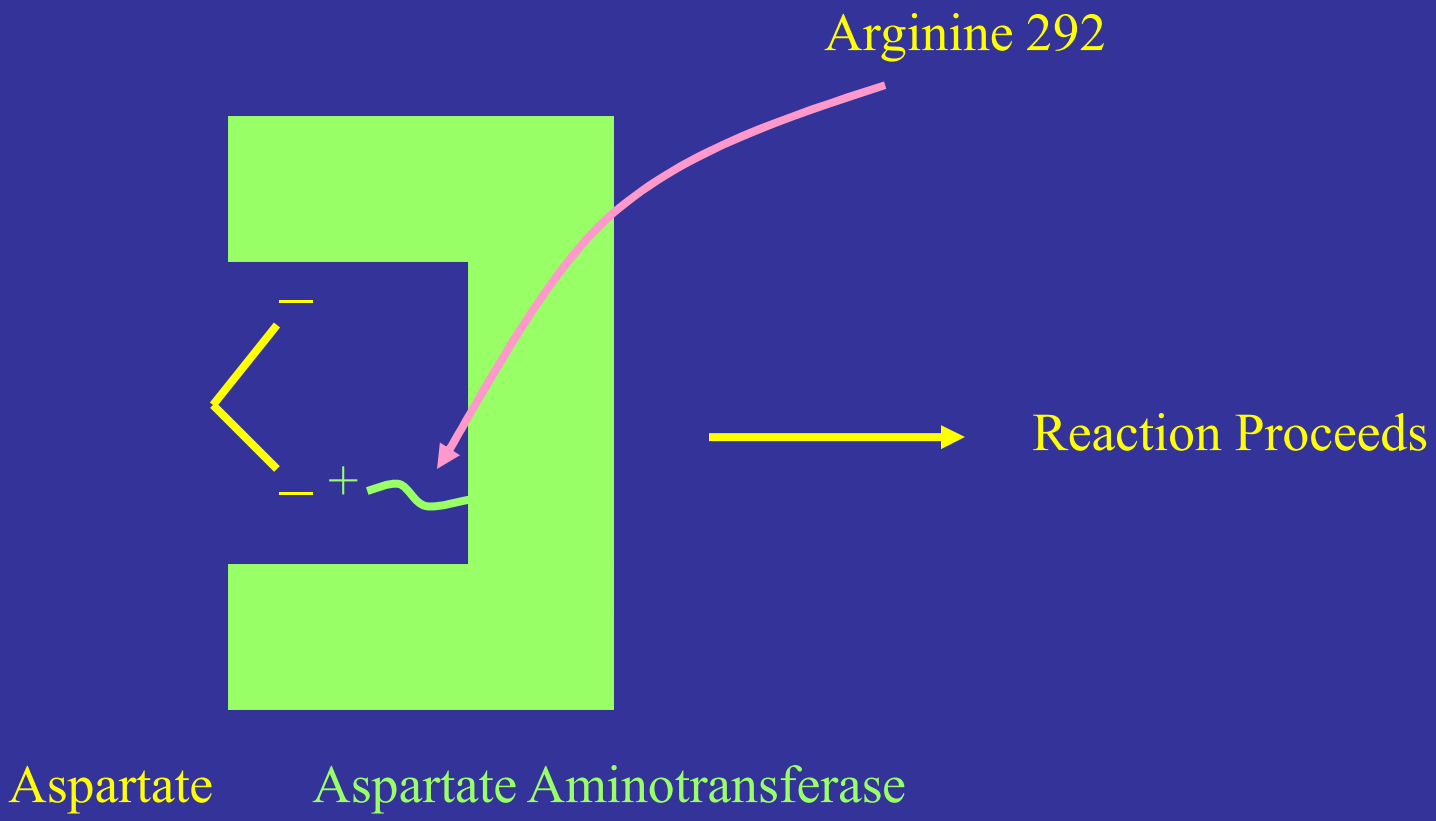
Aspartate

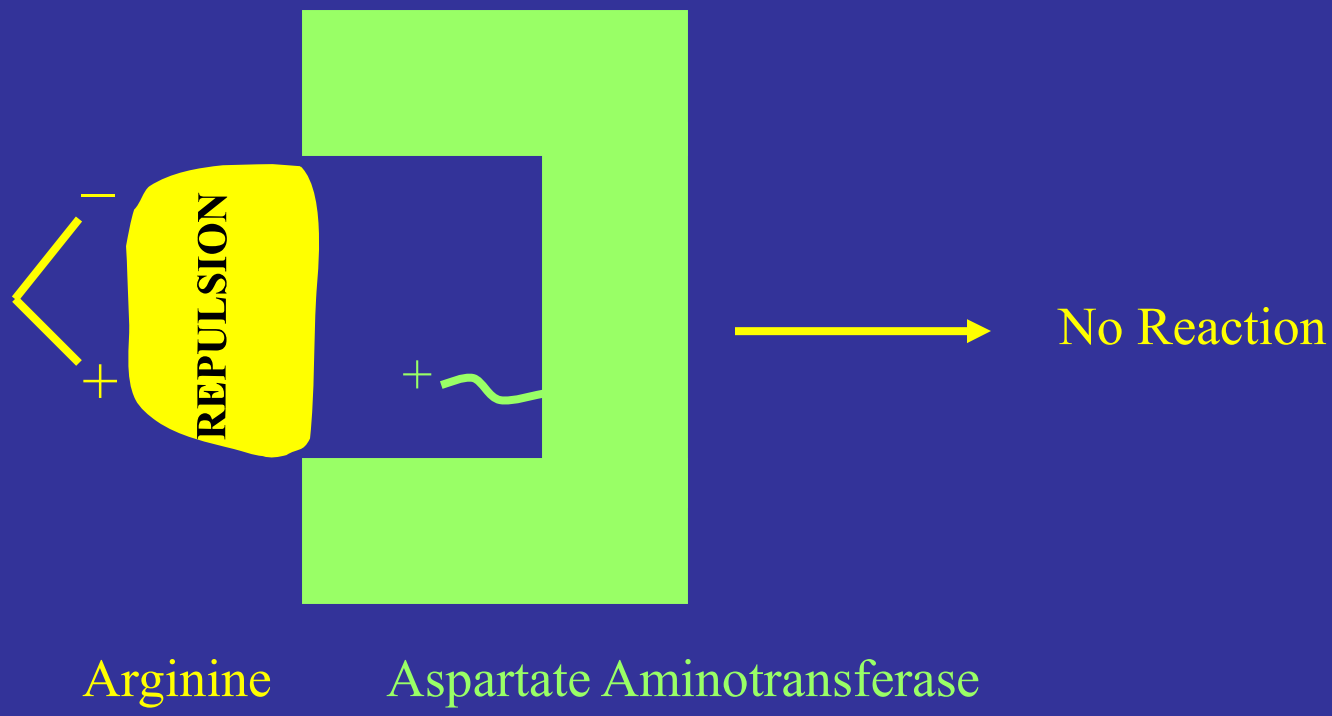
Enzyme also shows activity for glutamate but not for arginine. Why?



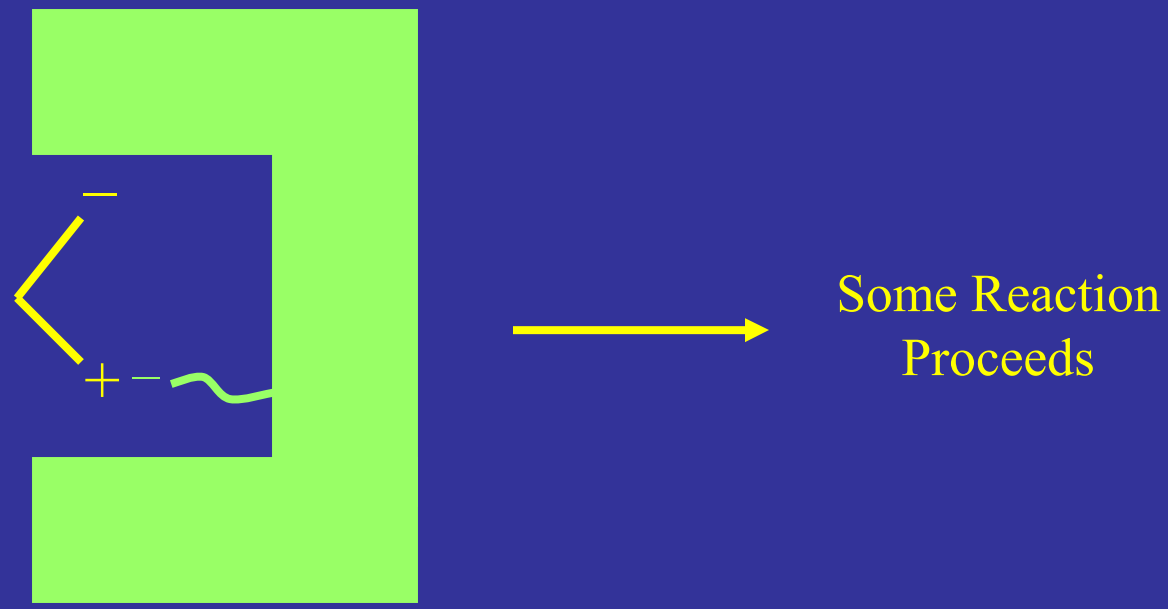
Arginine

Enzyme has an arginine residue at position 292. The positive charge on the side chain (of arginine 292) causes substrate specificity as a result of ion pairing between it and substrate aspartate.


Arginine 292
Reaction Proceeds
Aspartate
Aspartate Aminotransferase


REPULSION
No Reaction
Arginine
Aspartate Aminotransferase

The gene encoding for aspartate aminotransferase was altered so that Arginine 292 was replaced by Aspartate 292. The effect was 1) increase in activity of enzyme towards arginine by 6 times, and 2) decrease in activity of enzyme towards aspartate by 700,000 times.



Arginine Modified Aspartate Aminotransferase

F. Inhibition of Enzyme Catalysis

1. Irreversible Inhibitors:

- Bind irreversibly at active site
- Destroy part of active site
- By degrading a portion (or all!) of the enzyme present, these inhibitors lower the effective concentration of the enzyme

<u>Examples</u>

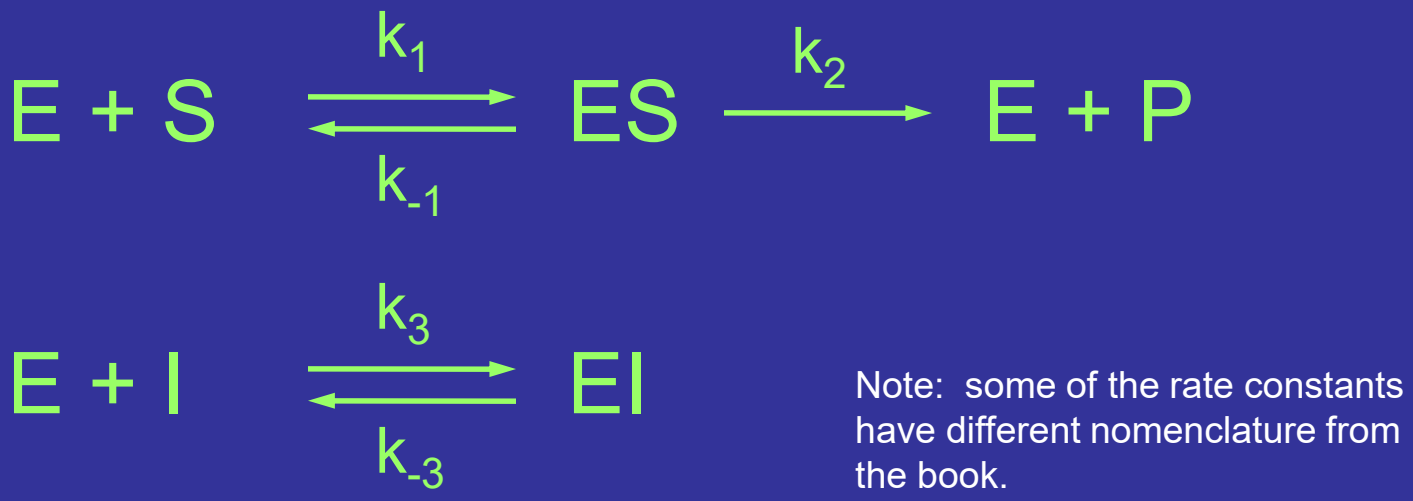
- Iodoacetate attacks sulfhydryl groups
- di-isopropylphosphofluoridate (DPF) is a nerve gas which attacks serine.

Reversible Inhibitors:

- Bind reversibly at active site
- Can be removed from the enzyme, enabling it to recover its activity (somehow).

2. Competitive Inhibition

A chemical which competes with the substrate for the active site. Instead of reacting, the inhibitor forms a “dead-end complex.” Competitive inhibitors are often structurally similar to intended substrate.



Note: some of the rate constants have different nomenclature from the book.

Equations:

1 $\frac{dS}{dt} = - k_1[E][S] + k_{-1}[ES]$

2 $\frac{dE}{dt} = - k_1[E][S] + k_{-1}[ES] + k_2[ES] - k_3[E][I] + k_{-3}[EI]$

3 $\frac{dES}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]$

4 $\frac{dI}{dt} = - k_3[E][I] + k_{-3}[EI]$

5 $\frac{dEI}{dt} = k_3[E][I] - k_{-3}[EI]$

6 $\frac{dP}{dt} = k_2[ES]$

Method 1

1) Assume “quasi-steady state” (ala Briggs, Haldane)

$$\frac{dES}{dt} \approx 0$$

$$0 = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

2) Define an inhibition (dissociation) constant:

$$K_I \equiv \frac{k_{-3}}{k_3} = \frac{[E][I]}{[EI]}$$

3) Also, define total enzyme concentration:

$$[E_0] = [E] + [ES] + [EI] \quad \text{or} \quad [E] = [E_0] - [ES] - [EI]$$

From definition of K_I:

$$[EI] = \frac{[E][I]}{K_I}$$

Inserting into Equation 3:

$$[E] = [E_0] - [ES] - \frac{[E][I]}{K_I}$$

Solving for [E]:

$$[E] = \frac{([E_0] - [ES])}{\left(1 + \frac{[I]}{K_I}\right)}$$

Notes:

if I = 0, then $E = E_0 - ES$

if $K_I \rightarrow \infty$, $k_{-3} \rightarrow \infty$ and $[EI] \rightarrow 0$

compare to results without inhibition

Insert equation for [E] into steady-state assumption (1):

$$0 = \frac{k_1[S]([E_0] - [ES])}{\left(1+\frac{[I]}{K_I}\right)} - k_{-1}[ES] - k_2[ES]$$

Solving for [ES]:

$$[ES] = \frac{[E_0][S]}{[S] + \frac{(k_{-1} + k_2)}{k_1}\left(1+\frac{[I]}{K_I}\right)}$$

Insert expression for [ES] into Diff Eqn 6:

$$\frac{dP}{dt} = k_2[ES]$$

$$\frac{dP}{dt} = \frac{k_2[E_0][S]}{[S] + \frac{(k_{-1} + k_2)}{k_1}\left(1+\frac{[I]}{K_I}\right)}$$

$$\boxed{\frac{dP}{dt} = \frac{V_{MAX}\,[S]}{[S] + K_M\left(1+\frac{[I]}{K_I}\right)}}$$

Note:

Strictly, [I] is concentration of inhibitor not associated with enzyme. [I] is not the total concentration of inhibitor!

$$\frac{dP}{dt} = \frac{V_{MAX}\,[S]}{[S] + K_M\left(1+\frac{[I]}{K_I}\right)}$$

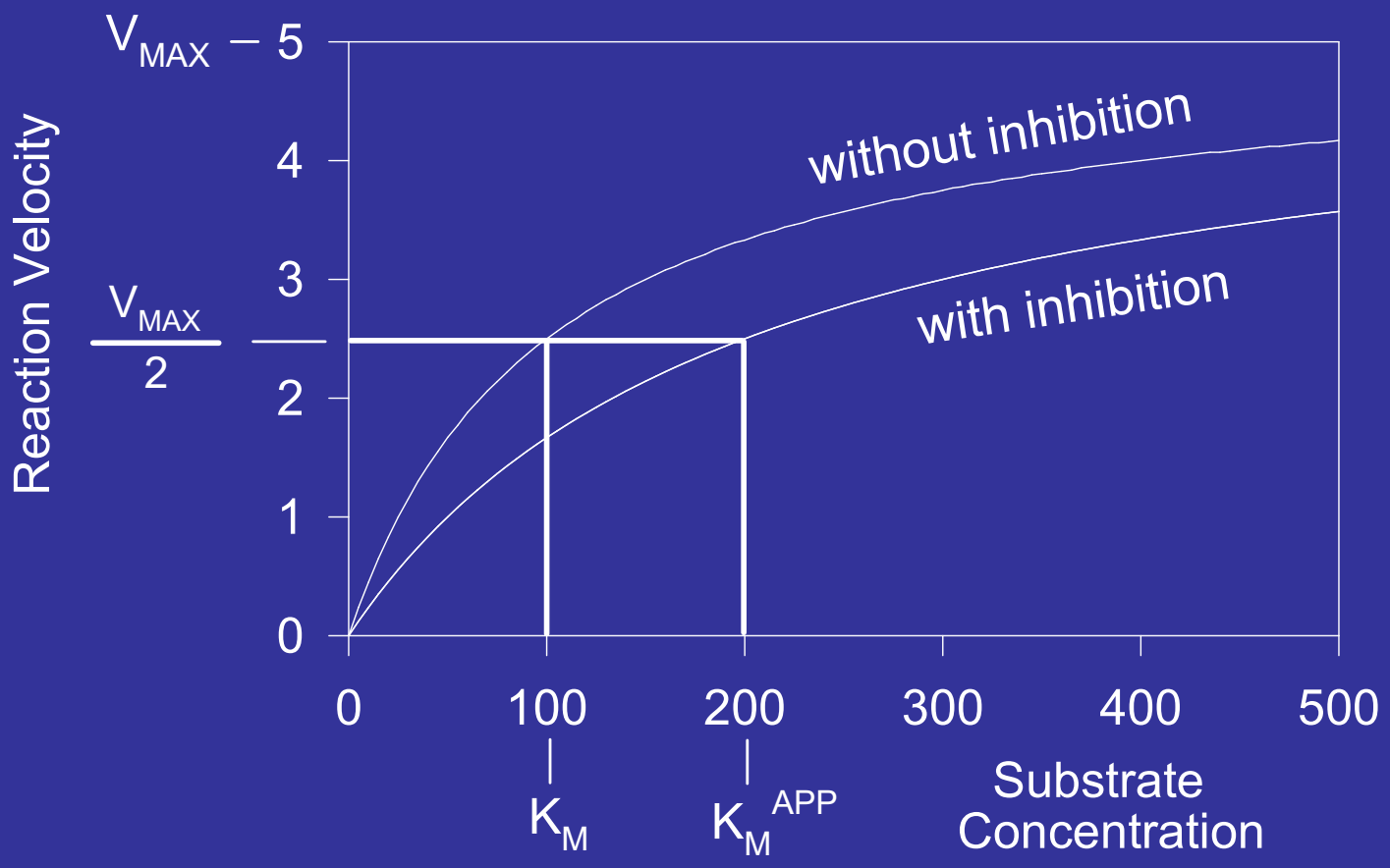
Effects on Michaelis-Menten Parameters:

1) V_{MAX} is unchanged

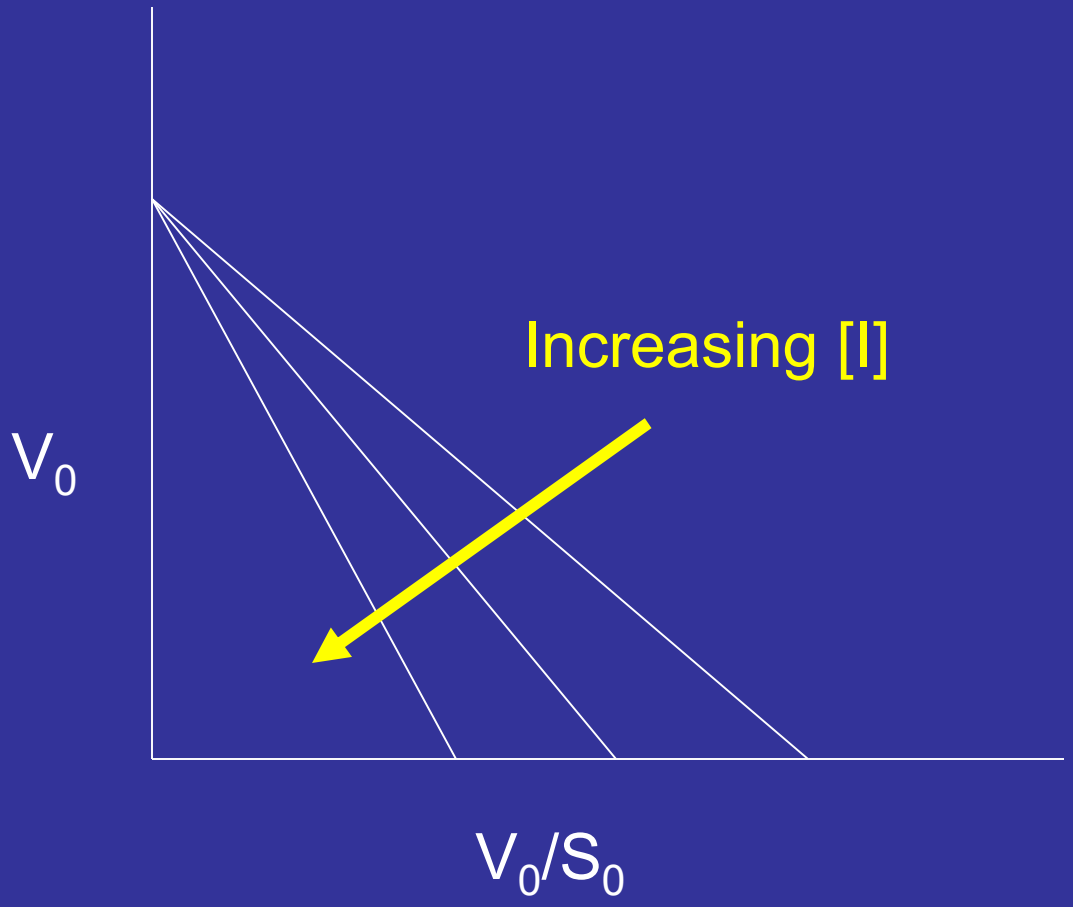
2) K_M is increased by $\left(1+\frac{[I]}{K_I}\right)$

$$K_M^{APP} = K_M\left(1+\frac{[I]}{K_I}\right)$$

Competitive Inhibition:



Eadie-Hofstee Plot for competitive inhibition:



Method 2 Computer Solution

$$\frac{dS}{dt} \approx \frac{\Delta S}{\Delta t} = \frac{S_{i+1} - S_i}{\Delta t}$$

$$\frac{dE}{dt} \approx \frac{\Delta E}{\Delta t} = \frac{E_{i+1} - E_i}{\Delta t}$$

$$\frac{dES}{dt} \approx \frac{\Delta ES}{\Delta t} = \frac{ES_{i+1} - ES_i}{\Delta t}$$

$$\frac{dI}{dt} \approx \frac{\Delta I}{\Delta t} = \frac{I_{i+1} - I_i}{\Delta t}$$

$$\frac{dEI}{dt} \approx \frac{\Delta EI}{\Delta t} = \frac{EI_{i+1} - EI_i}{\Delta t}$$

$$\frac{dP}{dt} \approx \frac{\Delta P}{\Delta t} = \frac{P_{i+1} - P_i}{\Delta t}$$

So, based on 6 derivatives...

$$S_{i+1} = (-k_1 E_i S_i + k_{-1} ES_i)\Delta t + S_i$$

$$E_{i+1} = (-k_1 E_i S_i + k_{-1} ES_i + k_2 ES_i - k_3 E_i I_i + k_{-3} EI_i)\Delta t + E_i$$

$$ES_{i+1} = (k_1 E_i S_i - k_{-1} ES_i - k_2 ES_i)\Delta t + ES_i$$

$$I_{i+1} = (-k_3 E_i I_i + k_{-3} EI_i)\Delta t + I_i$$

$$EI_{i+1} = (k_3 E_i I_i - k_{-3} EI_i)\Delta t + EI_i$$

$$P_{i+1} = k_2 ES_i \Delta t + P_i$$

$k_3 = 10$ L/gmin $\qquad$ $k_{-3} = 40$ /min

```
/*

Program 2

Simple Enzyme Kinetics with Competitive Inhibition

*/


#include<stdio.h>
#include<conio.h>

main()
{
   FILE *fout;
   double km1,km3,k2;              //  units = 1/min
   double kp1,kp3;                 //  units = L/g min
   double Snew,Pnew,Enew,ESnew;    //  units = g/L
   double Sold,Pold,Eold,ESold;    //  units = g/L
   double Iold,Inew,EIold,EInew;   //  units = g/L
   double deltatime;               //  units = minutes
   double currenttime;             //  units = minutes
   double endtime;                 //  units = minutes
   int count=0;

// Define Some Constants

   kp1 = 30.;
   km1 = 160.;
   k2 = 110.;
   kp3 = 10.;
   km3 = 40.;
   endtime = 30;
   deltatime=0.00001;
// Set Initial Conditions

   Sold = 10.;
   Pold = 0.;
   Eold = 0.00875;
   ESold = 0.;
   Iold = 10.;
   EIold = 0.;
```

Again, small value for Δt. And, like Program 1, we have 3 million iterations.

Use an initial value for I and EI.

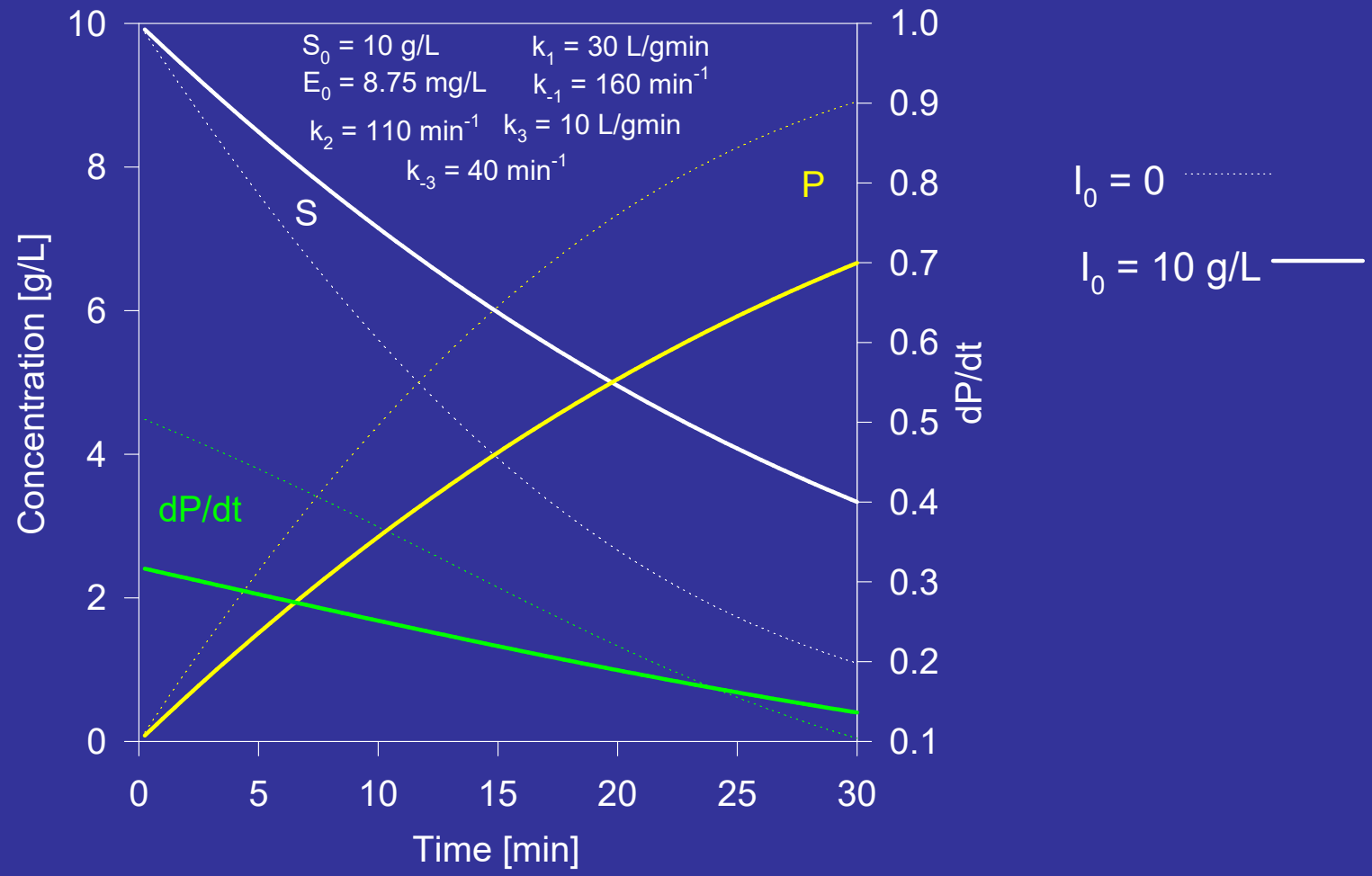
```
// Calculations

   fout=fopen("a:ex3b1.dat","w");
   currenttime = 0.;

   do
      {
      Snew = (-kp1*Eold*Sold+km1*ESold)*deltatime+Sold;
      Enew = (-kp1*Eold*Sold+km1*ESold+k2*ESold-
kp3*Eold*Iold+km3*EIold)*deltatime+Eold;
      ESnew = (kp1*Eold*Sold-km1*ESold-k2*ESold)*deltatime+ESold;
      Inew = (-kp3*Eold*Iold+km3*EIold)*deltatime+Iold;
      EInew = (kp3*Eold*Iold-km3*EIold)*deltatime+EIold;
      Pnew = k2*ESold*deltatime+Pold;
      count++;
      if (count==25000)
         {
       fprintf(fout,"%7.2f %7.4f %7.4f %6.4f\n",currenttime,Snew,Pnew,(Pnew-
Pold)/deltatime);
       count=0;
         }
      Sold=Snew;
      Eold=Enew;
      ESold=ESnew;
      Pold=Pnew;
      EIold=EInew;
      Iold=Inew;
      currenttime+=deltatime;
      }
   while(currenttime<endtime);
   fclose(fout);
}
```

Like Program 1, this is the meat. Calculate the "new" values for each of the six! components (e.g., S_1), given the "old" values for the components (e.g., S_0).

Replace old values with new values.

Figure 3

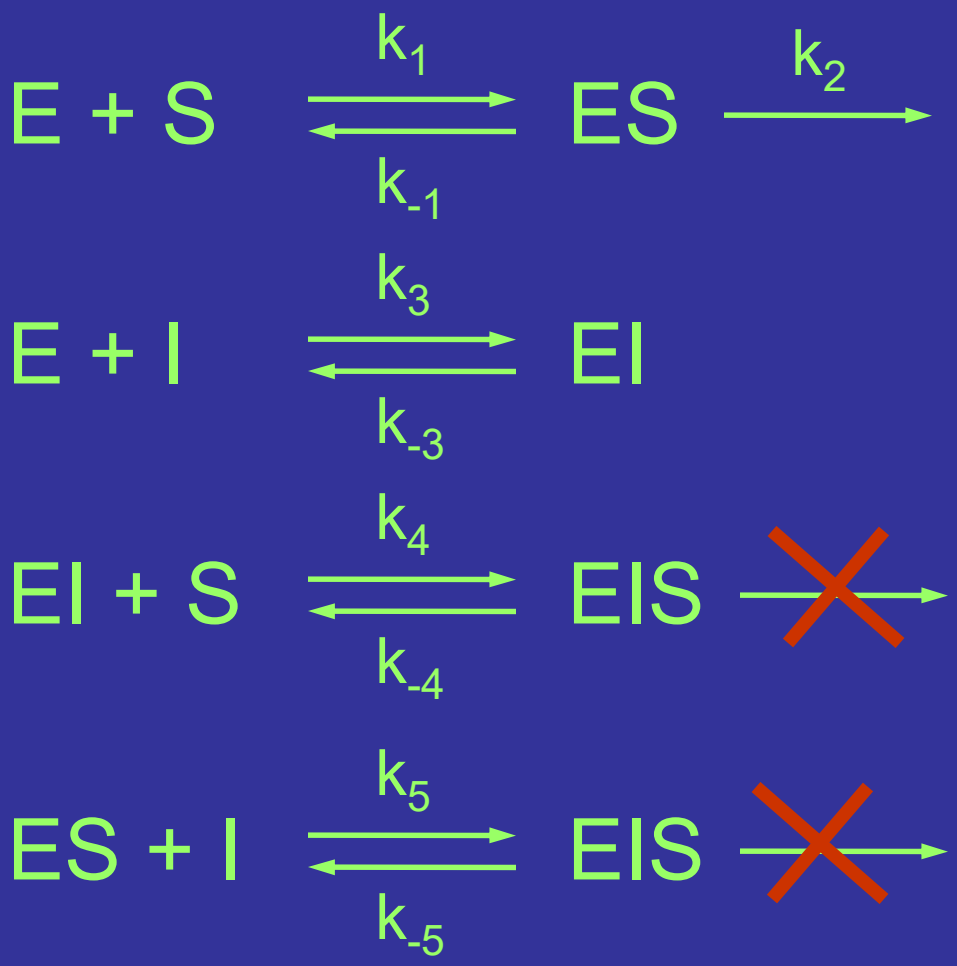
Simple Enzyme Kinetics with Competitive Inhibition



3. Noncompetitive Inhibition

A chemical which binds with enzyme on site(s) other than the active site. The inhibitor can alter enzyme affinity to substrate: the product will not form from enzyme complexed with inhibitor.

3. Noncompetitive Inhibition



$$K_S = \frac{k_{-1}}{k_1}$$

$$K_S' = \frac{k_{-4}}{k_4}$$

a. “Pure” Noncompetitive

Substrate has the same affinity for enzyme as it has for the enzyme-inhibitor complex.

$$K_S = K_S'$$

Presence of inhibitor lowers the effective concentration of ES. Some of the enzyme-substrate is busy in unproductive reactions.

Quasi-steady state assumption...

$$\frac{dP}{dt} = \frac{V_{MAX}[S] \Big/ \left(1+\frac{[I]}{K_I}\right)}{[S] + K_M}$$

$$K_I \approx \frac{[E][I]}{[EI]} = \frac{k_{-3}}{k_3}$$

$$\approx \frac{[ES][I]}{[EIS]} = \frac{k_{-5}}{k_5}$$

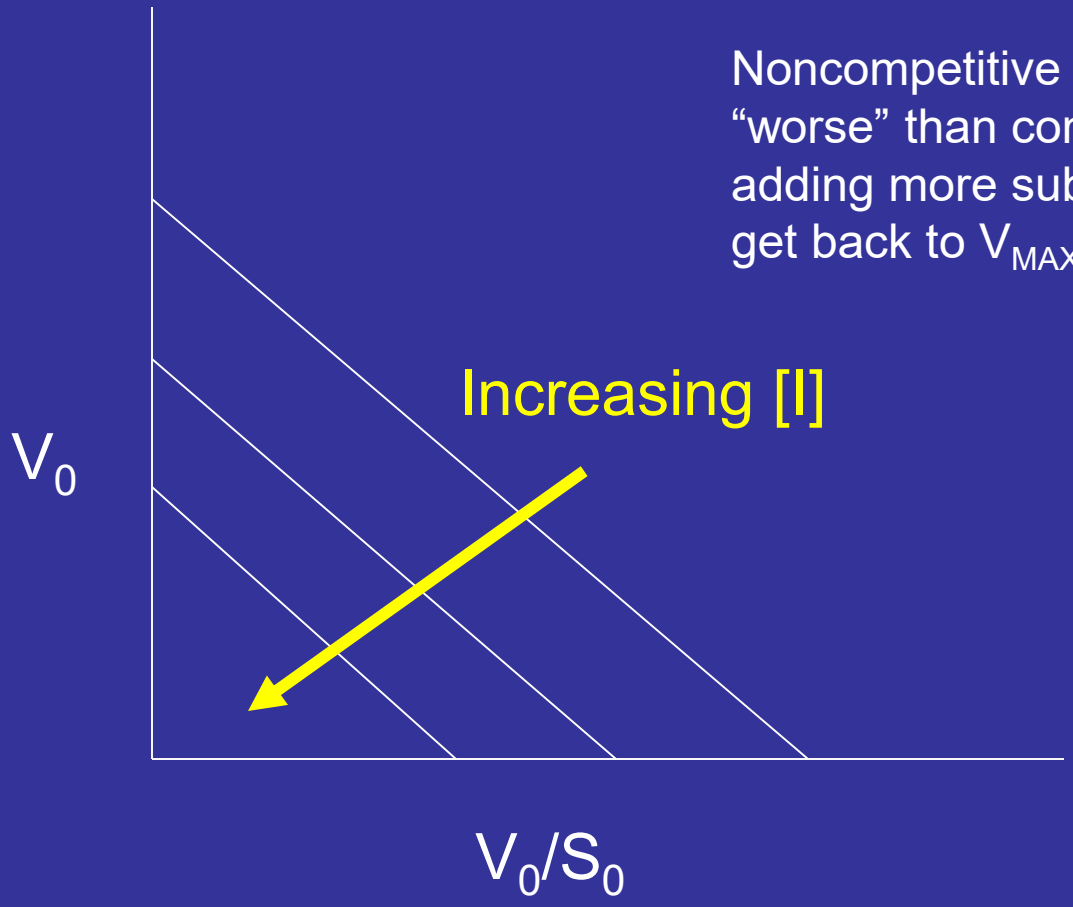
1) K_M is unchanged

2) V_{MAX} is decreased by $\left(1+\frac{[I]}{K_I}\right)$

$$V_{MAX}^{APP} = V_{MAX} \Big/ \left(1+\frac{[I]}{K_I}\right)$$

Effect of noncompetitive inhibitor is to reduce the maximum reaction velocity. From the reaction equations, note that increasing [S] is not very effective at increasing reaction rate because more S will also go towards unproductive reactions.

Eadie-Hofstee Plot for noncompetitive inhibition:



Noncompetitive inhibition can be "worse" than competitive since adding more substrate will not get back to V_{MAX}

b. “Mixed” Noncompetitive

$$K_S \neq K_S'$$

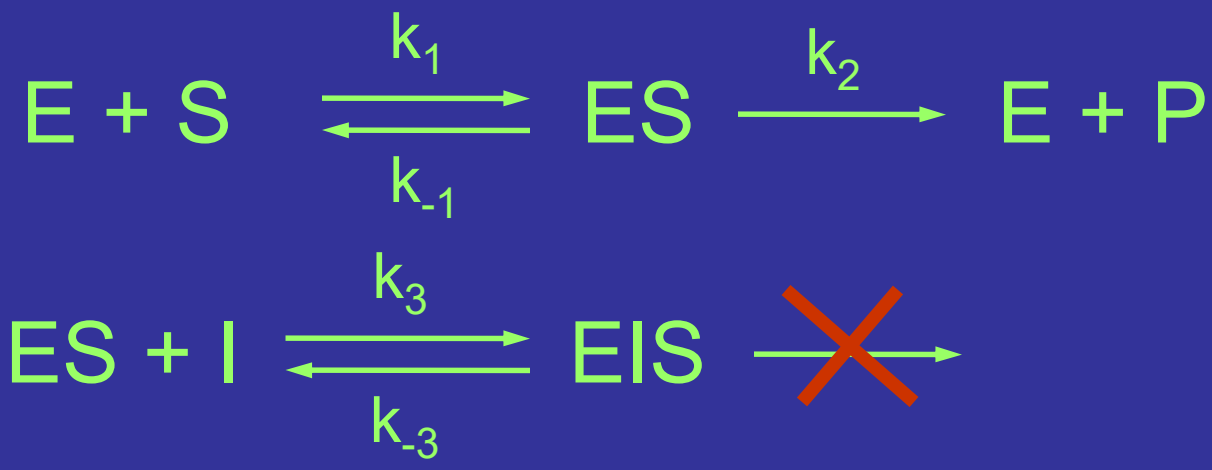
Substrate has a different affinity for enzyme than for the enzyme-inhibitor complex.

Both V_{MAX} and K_M are altered by inhibitor concentration. Inhibition lies between noncompetitive and uncompetitive.

4. Uncompetitive Inhibition

A chemical which binds with enzyme-substrate complex but not with the enzyme itself. The presence of the inhibitor lowers the effective ES concentration.

Uncompetitive Inhibition



Quasi-steady state assumption...

$$\frac{dP}{dt} = \frac{V_{MAX}^{APP}[S]}{K_M^{APP} + [S]}$$

1) K_M is *decreased* by $\left(1+\frac{[I]}{K_I}\right)$

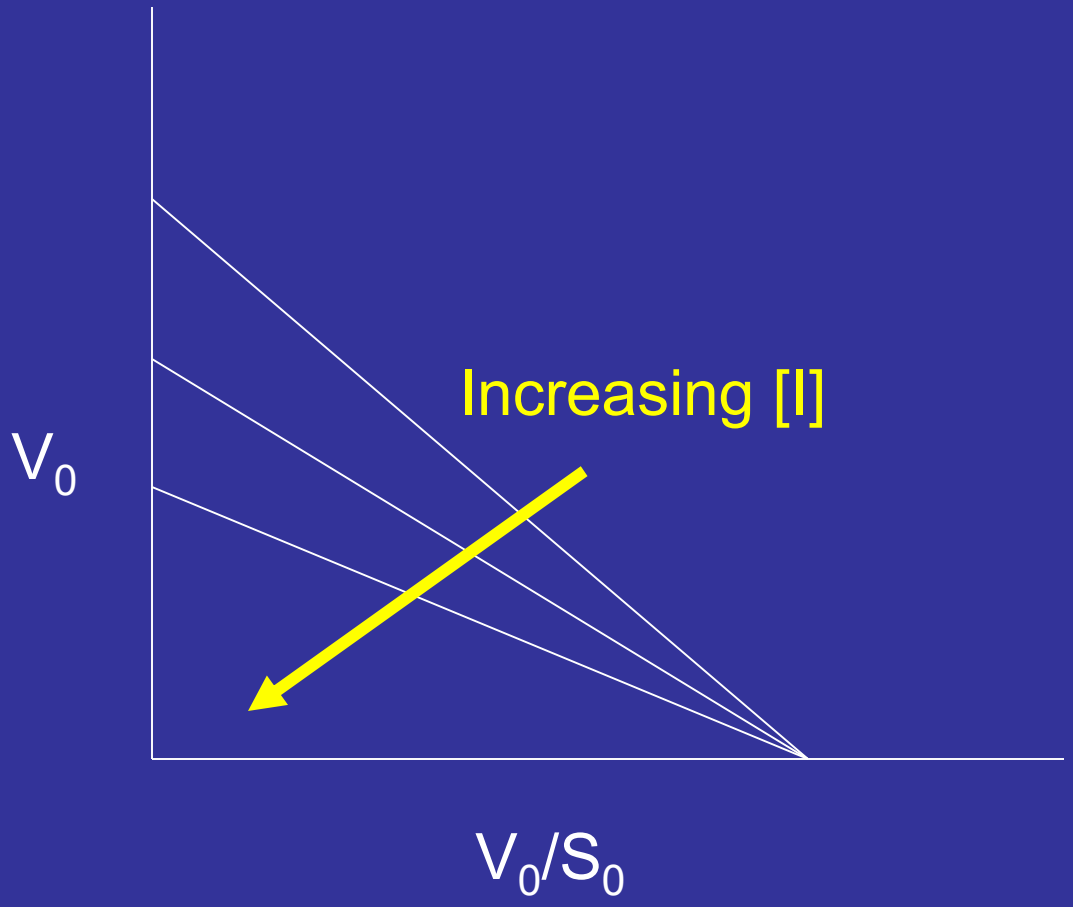
2) V_{MAX} is decreased by $\left(1+\frac{[I]}{K_I}\right)$

$$K_I \approx \frac{[ES][I]}{[EIS]} = \frac{k_{-3}}{k_3}$$

$$V_{MAX}^{APP} = V_{MAX} \Big/ \left(1+\frac{[I]}{K_I}\right)$$

$$K_M^{APP} = K_M \Big/ \left(1+\frac{[I]}{K_I}\right)$$

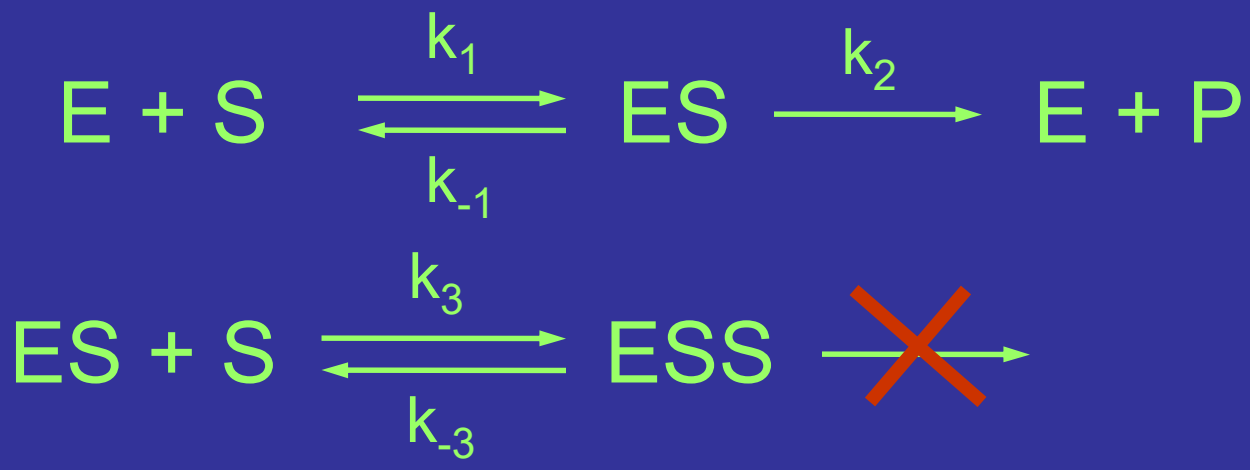
Eadie-Hofstee Plot for uncompetitive inhibition:



5. Substrate Inhibition

Identical to uncompetitive inhibition except that the substrate is the actual inhibitor. A special case of uncompetitive inhibition.

Substrate Inhibition



Quasi-steady state assumption...

$$\frac{dP}{dt} = \frac{V_{MAX}^{APP}[S]}{K_M^{APP} + [S]}$$

Note: since chemical reaction is the same as uncompetitive inhibition, the solution is the "same" except [S] = [I]

$$K_I \approx \frac{[ES][S]}{[ESS]} = \frac{k_{-3}}{k_3}$$

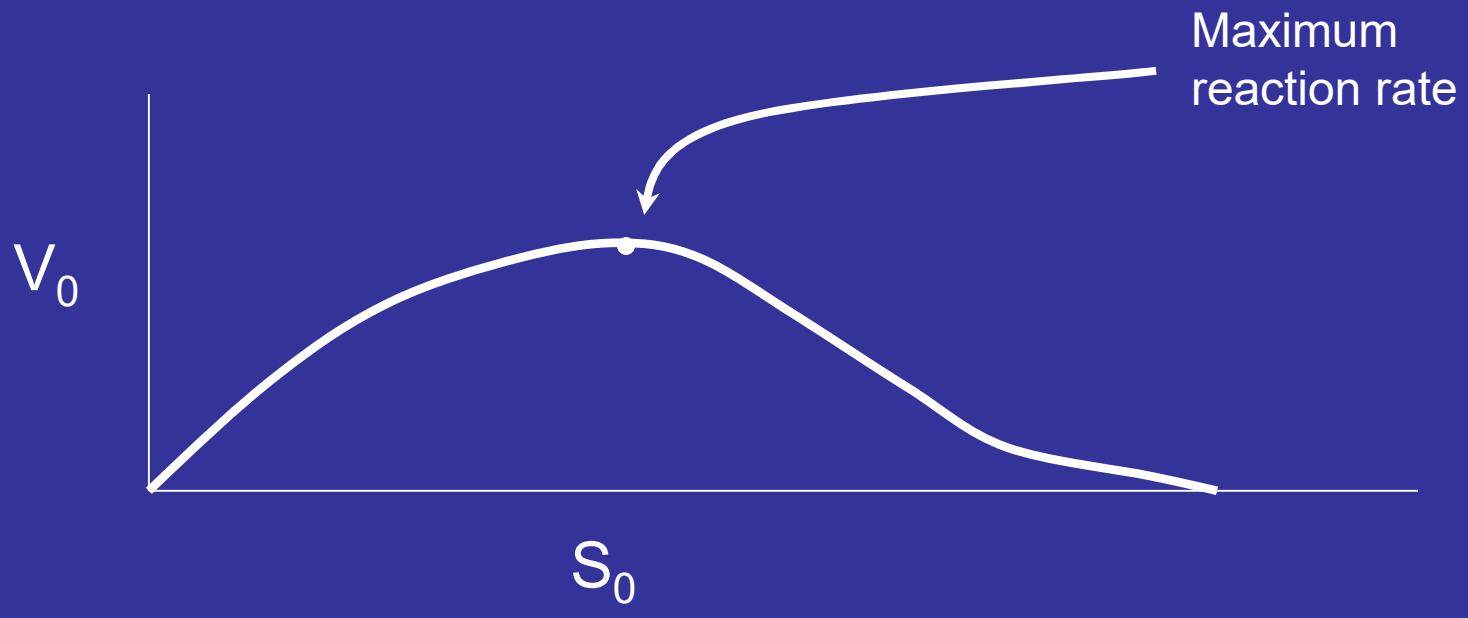
$$V_{MAX}^{APP} = V_{MAX} \Big/ \left(1 + \frac{[S]}{K_I}\right)$$

$$K_M^{APP} = K_M \Big/ \left(1 + \frac{[S]}{K_I}\right)$$

Simplification...

$$V = \frac{dP}{dt} = \frac{V_{MAX}\,[S]}{K_M + [S] + [S]^2/K_I}$$

Initially, increasing substrate concentration increases rate.
Eventually, increasing substrate concentration decreases rate.
Thus, a maximum reaction rate exists.



A maximum v is found for S when dv/dS = 0

$$\frac{dv}{dS} = \frac{V_{MAX}(K_M + [S] + [S]^2/K_I) - V_{MAX}[S](1 + 2[S]/K_I)}{(K_M + [S] + [S]^2/K_I)^2}$$

$$= \frac{V_{MAX}}{(K_M + [S] + [S]^2/K_I)^2} (K_M + [S] + [S]^2/K_I - [S] - 2[S]^2/K_I)$$

$$= \frac{V_{MAX}}{(K_M + [S] + [S]^2/K_I)^2} (K_M - [S]^2/K_I)$$

$$\frac{dv}{dS} = 0 \text{ when } (K_M - [S]^2/K_I) = 0$$

$$(K_M - [S]^2/K_I) = 0$$

$$S_{MAX} = \sqrt{K_I K_M}$$

Comparison of common types of inhibition:

	V_{MAX}	K_M
Competitive	–	↑
Noncompetitive	↓	–
Uncompetitive	↓	↓

For given values of V_{MAX}, K_I, K_M and [S]:

V (noncompetitive) < V (uncompetitive)

Figure 4

Comparison of Enzyme Inhibition