G. Other Enzyme Models

Multisubstrate models – many enzymatic reactions have more than one substrate that influences its kinetics

$$A + B \longrightarrow P + Q$$

1. Compulsory Order System

A must react first with the enzyme, and P only is formed after Q is released.

Model:

$$E + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA$$

$$EA + B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EAB$$

$$EAB \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EPQ$$

$$EPQ \xrightarrow{k_4} EP + Q$$

$$EP \xrightarrow{k_5} E + P$$

Note: $E + B \;\cancel{\longrightarrow}$ … etc.

9 Equations can be written (one for each component):

1 $$\frac{dA}{dt} = -k_1[E][A] + k_{-1}[EA]$$

2 $$\frac{dE}{dt} = -k_1[E][A] + k_{-1}[EA] + k_5[EP]$$

3 $$\frac{dEA}{dt} = k_1[E][A] - k_{-1}[EA] - k_2[EA][B] + k_{-2}[EAB]$$

4 $$\frac{dB}{dt} = -k_2[EA][B] + k_{-2}[EAB]$$

5 $$\frac{dEAB}{dt} = k_2[EA][B] - k_{-2}[EAB] - k_3[EAB] + k_{-3}[EPQ]$$

etc.

Assumptions

1 Steady-state

$$\frac{dEA}{dt} = \frac{dEAB}{dt} = \frac{dEPQ}{dt} = \frac{dEP}{dt} = \frac{dE}{dt} = 0$$

2 Equilibrium

Reactions 1, 2, and 3 remain at equilibrium

Result

$$\left.\frac{dP}{dt}\right|_0 = \frac{[E]_0}{\frac{1}{k_{obs}} + \frac{K_3}{K_1[B]} + \frac{K_2K_3}{k_{obs}[A][B]}}$$

where:

K_i = dissociation constant for reaction i

k_{obs} = observed first order rate constant

Note

$$\left.\frac{dP}{dt}\right|_0 = \frac{[E]_0}{\dfrac{1}{k_{obs}} + \dfrac{K_3}{K_1[B]} + \dfrac{K_2K_3}{k_{obs}[A][B]}}$$

If [A]<<[B] then

$$\left.\frac{dP}{dt}\right|_0 \approx \frac{[E]_0}{\dfrac{1}{k_{obs}} + \dfrac{K_3}{K_1B_0} + \dfrac{K_2K_3}{k_{obs}[A]B_0}} = \frac{[E]_0}{\alpha_1 + \beta_1 / [A]}$$

If [B]<<[A] then

$$\left.\frac{dP}{dt}\right|_0 \approx \frac{[E]_0}{\dfrac{1}{k_{obs}} + \dfrac{K_3}{K_1[B]} + \dfrac{K_2K_3}{k_{obs}A_0[B]}} = \frac{[E]_0}{\alpha_2 + \beta_2 / [B]}$$

2. Random Order System

Either A or B can react first with enzyme, and complex formed can further react with other substrate to form enzyme:substrate$_1$:substrate$_2$ complex.

Model:

$$E + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA \qquad E + B \underset{k_{-1}'}{\overset{k_1'}{\rightleftharpoons}} EB$$

$$EA + B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EAB \qquad EB + A \underset{k_{-2}'}{\overset{k_2'}{\rightleftharpoons}} EAB$$

$$EAB \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EPQ$$

$$EPQ \xrightarrow{k_4} EQ + P \qquad EPQ \xcancel{\xrightarrow{k_4'}} EP + Q$$

$$EQ \xrightarrow{k_5} E + Q \qquad EP \xcancel{\xrightarrow{k_5'}} E + P$$

Usually one product is always easiest to release.

3. Ping-Pong Model

Also known as the enzyme-substituted mechanism or double displacement mechanism. Both substrates need not be bound together at the active site. This mechanism is common for phosphate-transferring enzymes.

Model:

$$E + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA$$

$$EA \xrightarrow{k_2} E^* + P$$

$$E^* + B \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E^*B$$

$$E^*B \xrightarrow{k_4} E + Q$$

Using quasi-steady state assumption yields:

$$\frac{dP}{dt} = \frac{[E]_0}{\alpha + \frac{\beta}{[A]} + \frac{\gamma}{[B]}}$$

where:

α, β, γ depend on rate constants

Note that if [A]<<[B] or [B]<<[A], then this equation reduces to Michaelis-Menten Equation

H. pH and temperature effects

1. pH

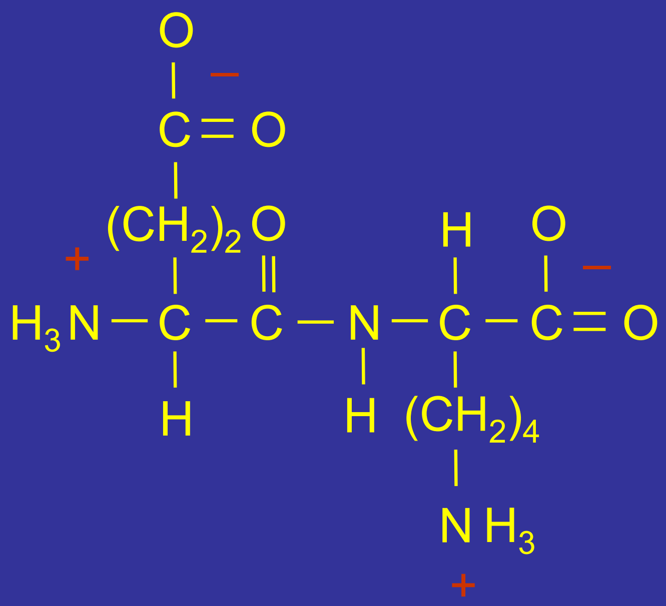
pH has an obvious influence on enzyme structure because:

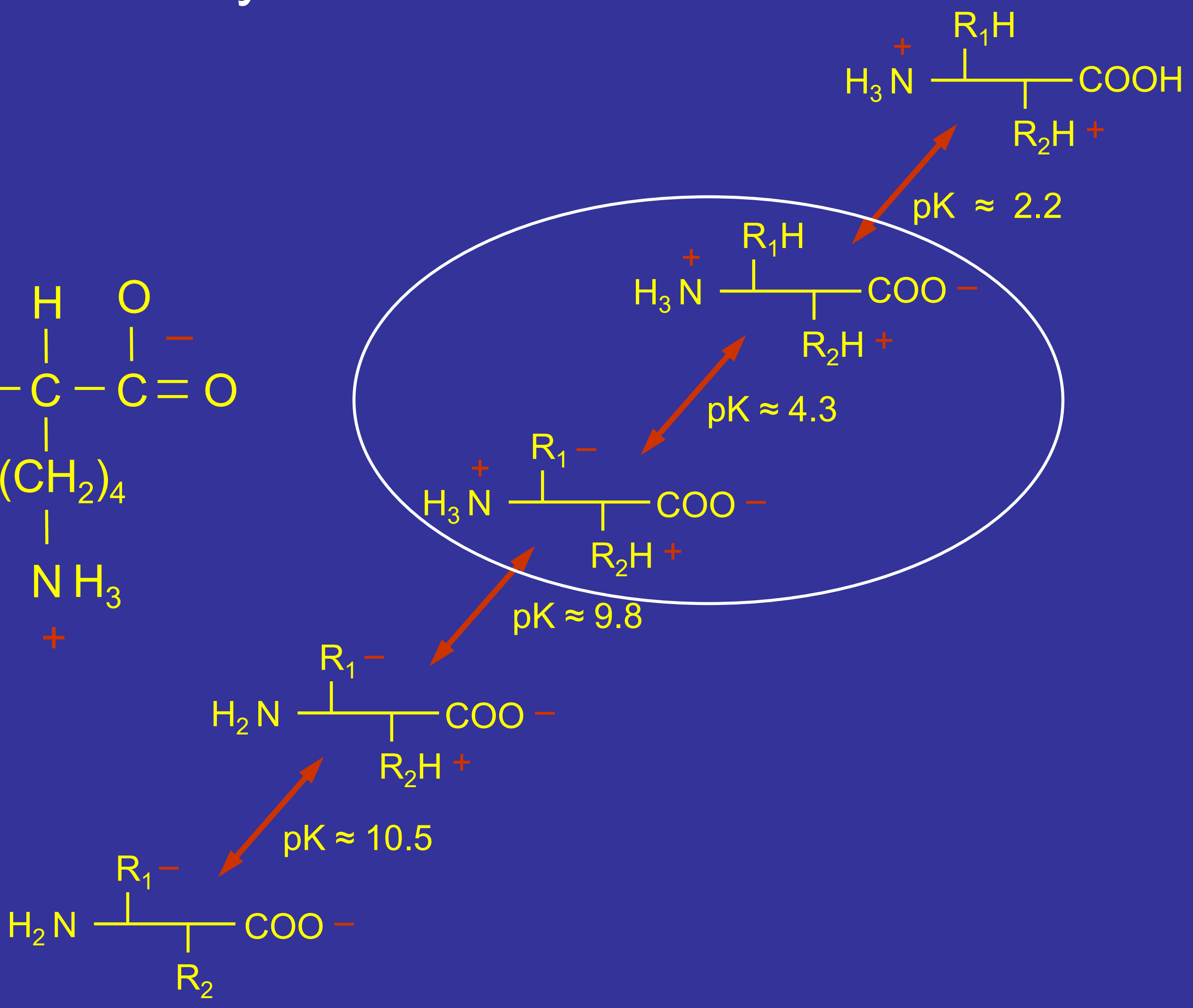
1) the charge of amino acids (as zwitterions) is a function of pH
2) the charge of several residues is a function of pH….

Residue	**pK_R**
Aspartate	3.86
Glutamate	4.25
Histidine	6.0
Cysteine	8.33
Tyrosine	10.07
Lysine	10.53
Arginine	12.48

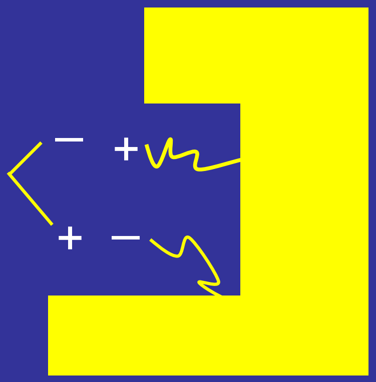
These values for pK_a change slightly when the residue is on a protein.

Consider glutamate-lysine:

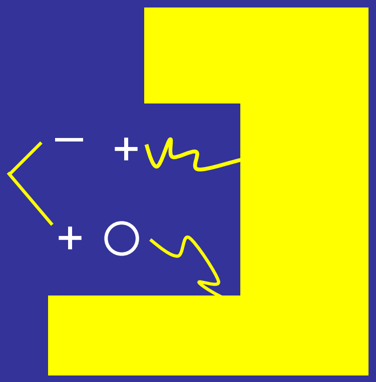




A change in the charge on a residue may alter the charge in the active site

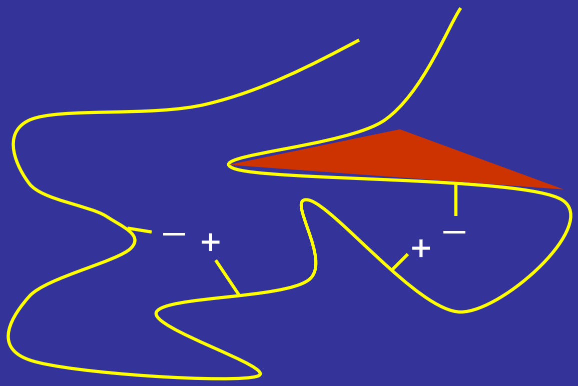


At “medium” pH

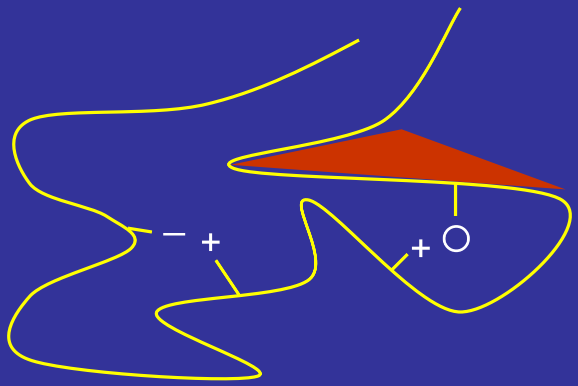


At “low” pH

A change in the charge on a residue may alter the charge internally, reducing the ion pairing which is holding the protein together.



At “medium” pH



At “low” pH

One (usually not particularly good) way to consider mathematically the effect of pH is to treat hydrogen ion (or lack of ion) as a competitive inhibitor. In other words, the substrate will bind with enzyme having an altered active site, but this binding will yield a dead-end complex:

$$EH + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EHS \xrightarrow{k_2} EH + P$$

$$\left.\begin{array}{l} EH \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E^- + H^+ \\ \\ EH + H^+ \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} EH_2^+ \end{array}\right\}$$

Such reactions are usually at equilibria

$$K_1 = \frac{[EH][H^+]}{[EH_2^+]}$$

$$K_2 = \frac{[E^-][H^+]}{[EH]}$$

Using quasi-steady state assumption yields:

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

Where:

$$K_M^{APP} = K_M \left[1 + \frac{K_2}{[H^+]} + \frac{[H^+]}{K_1} \right]$$

Note similarity with competitive inhibition...

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

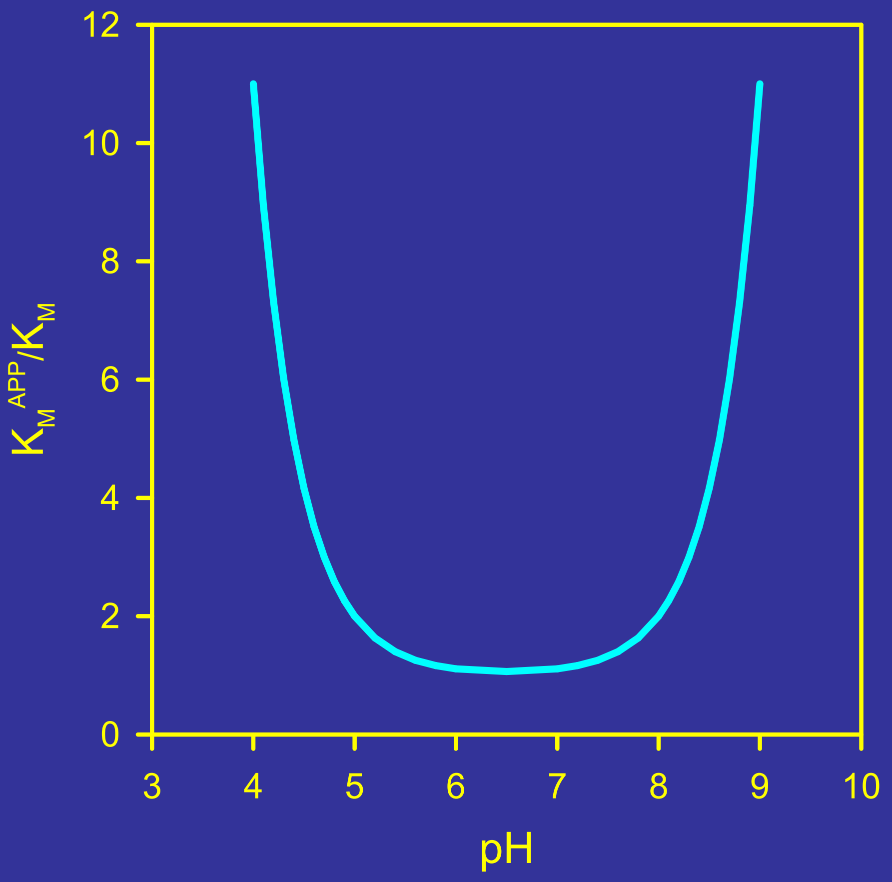
Example of what this model predicts:

Assume $K_1 = 10^{-5}$ or $pK_1 = 5$
$K_2 = 10^{-8}$ or $pK_2 = 8$

Then

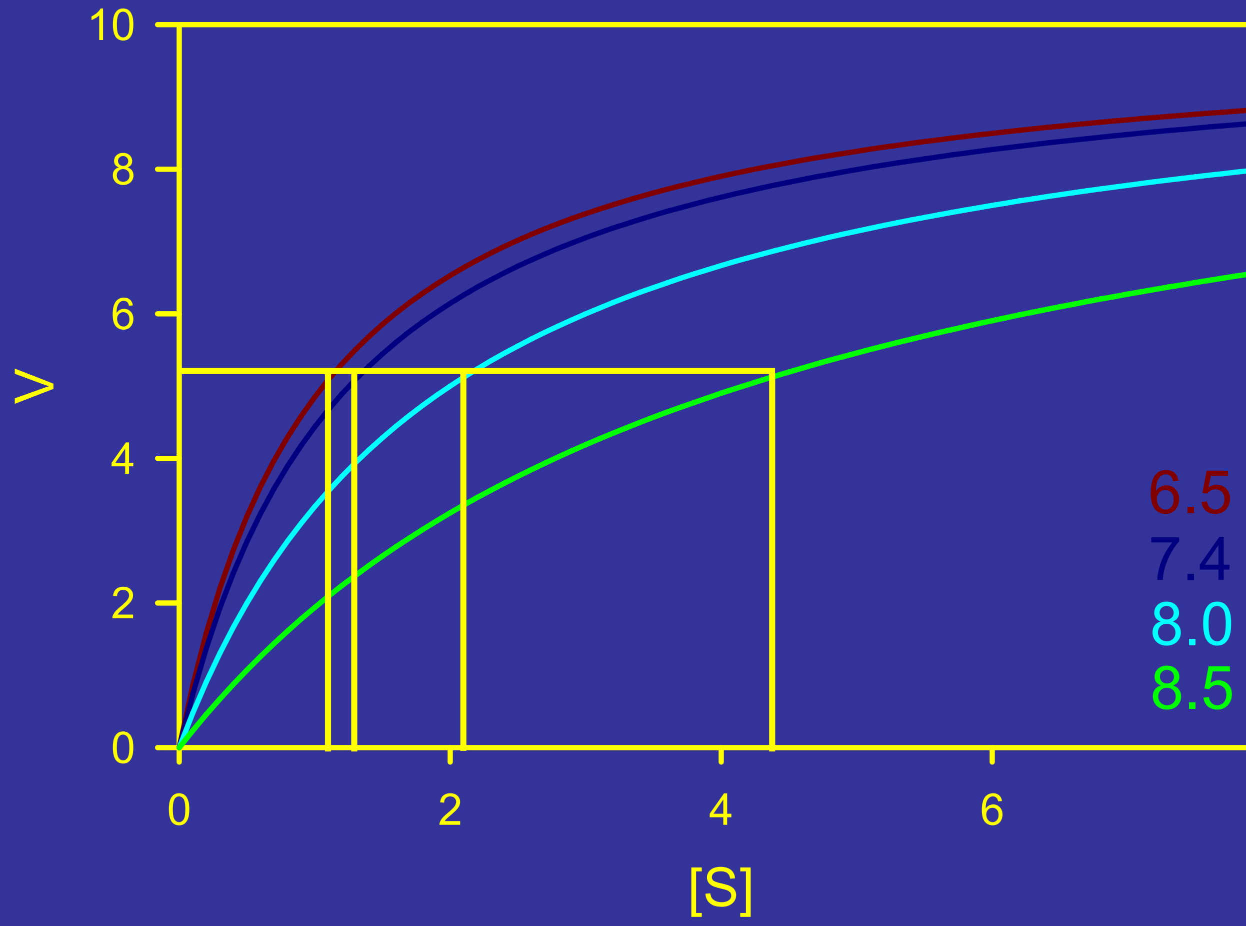
pH	$[H^+]$	K_M^{APP}/K_M
4	10^{-4}	11.0
5	10^{-5}	2.0
6	10^{-6}	1.11
6.5	$10^{-6.5}$	1.06
7	10^{-7}	1.11
8	10^{-8}	2.0
9	10^{-9}	11.0

Enzyme Kinetics at Different pH



...model predicts no change in V_{MAX}!

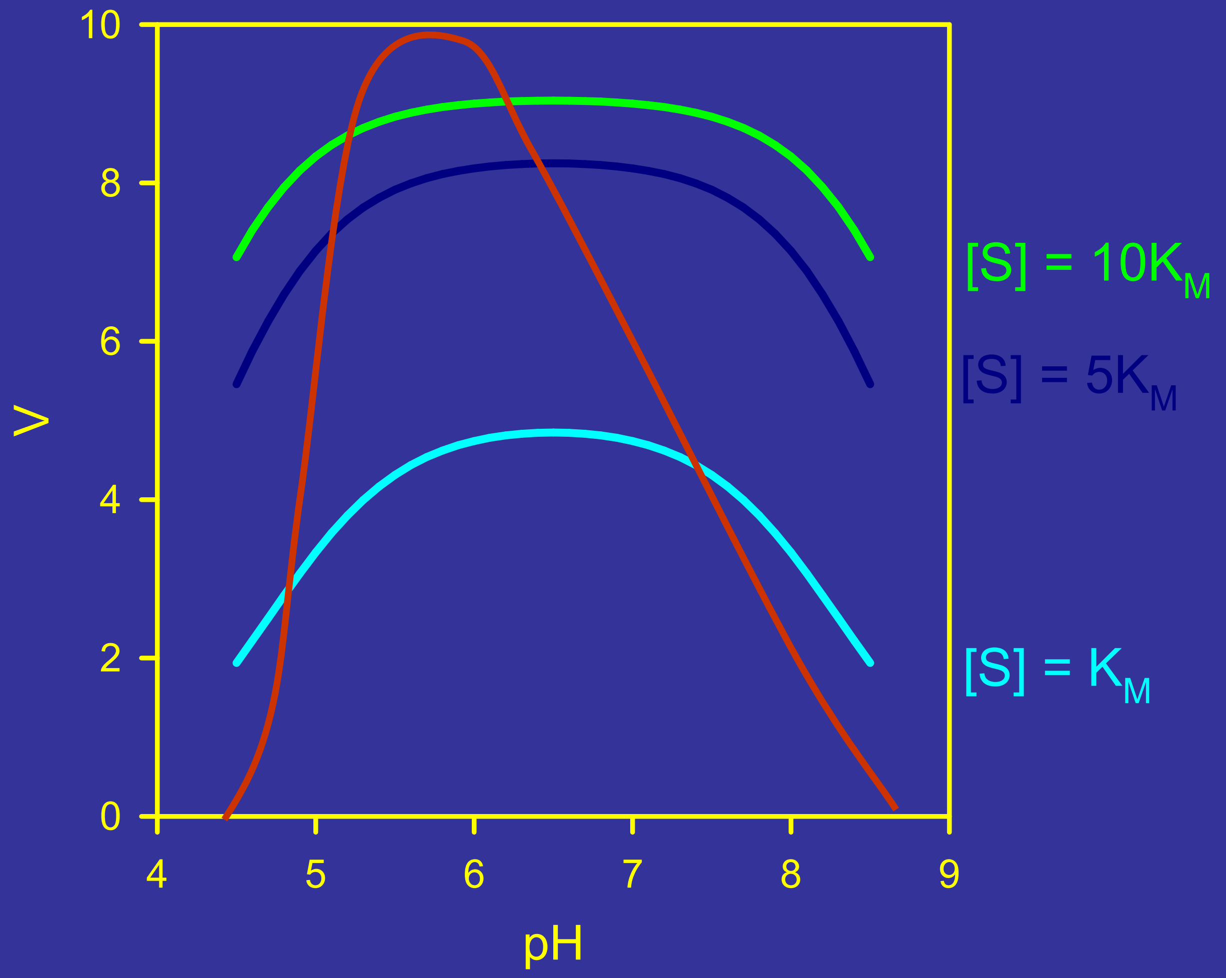
Enzyme Kinetics at Different pH



Problem with model is prediction that at high substrate concentration, maximum reaction rate is restored.

Model prediction (V_{MAX} = 10):

Reality (for example)....



2. Temperature

Temperature has two effects on enzyme kinetics:

1) activation due to the hastening of the release of product from enzyme-substrate complex (just like the temperature effect in chemical reactions).

2) reversible inactivation and irreversible denaturation – loss of enzyme because of destabilization of bonds or more significant loss of structural integrity (such as breaking of cysteine linkages)

Note: textbook only considers irreversible damage.

Model:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$$

$$E \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} E_I \xrightarrow{k_4} E_D$$

Reversible Irreversible

E_I = inactivated enzyme (can return to E)

E_D = denatured enzyme (cannot return to E)

Note: textbook only considers second step and uses k_D instead of k_4.

k_4 and k_2 depend on temperature according to the Arrhenius equation.

$k_2 = A\,e^{-E/RT}$

$k_4 = A''\,e^{-E''/RT}$

E = Energy of activation

E″ = Energy of denaturation

A, A″ (min^{-1})

E, E″ (cal/mol)

Write 6 temperature-dependent differential equations.

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

$$\frac{dE}{dt} = -k_1[E][S] + k_{-1}[ES] - k_3[E] + k_{-3}[E_I] + Ae^{-E/RT}[ES]$$

$$\frac{dES}{dt} = k_1[E][S] - k_{-1}[ES] - Ae^{-E/RT}[ES]$$

$$\frac{dP}{dt} = Ae^{-E/RT}[ES]$$

$$\frac{dE_I}{dt} = k_3[E] - k_{-3}[E_I] - A''e^{-E''/RT}[E_I]$$

$$\frac{dE_D}{dt} = A''e^{-E''/RT}[E_I]$$

Notes:

1) the amount of enzyme lost depends on temperature and duration of exposure. (see Fig. 3-15 p. 77 “The nature of the plot will depend on the length of time the reaction mixture is exposed to the test temperature.” This plot is only valid for “10-min exposure”!)

2) other rate constants (e.g., k_1, k_{-1}) are also affected by temperature. However, the forward and reverse rate constants tend to be influenced similarly. Over a small temperature range, an equilibrium is not altered as much as irreversible rate constants.

A specific example (simulated by the solution to the differential equations):

activation	denaturation
$A = 9.1 \times 10^{8}\ \text{min}^{-1}$	$A'' = 6.6 \times 10^{52}\ \text{min}^{-1}$
$E = 10{,}900$ cal/mol	$E'' = 77{,}900$ cal/mol

T(°C)	k_2 (min^{-1})	k_4 (min^{-1})
30	12.5	0.000429
35	16.8	0.00350
40	22.3	0.0268
45	29.4	0.192
50	38.4	1.29
55	49.7	8.22

A specific example (simulated by the solution to the differential equations):

activation	denaturation
$A = 9.1 \times 10^{8}\ \text{min}^{-1}$	$A'' = 6.6 \times 10^{52}\ \text{min}^{-1}$
$E = 10{,}900\ \text{cal/mol}$	$E'' = 77{,}900\ \text{cal/mol}$

$S_0 = 8.0\ \text{g/L}$

$E_0 = 0.03\ \text{g/L}$

$k_1 = 30\ \text{L/g min}$

$k_{-1} = 160\ \text{min}^{-1}$

$k_3 = 15\ \text{min}^{-1}$

$k_{-3} = 12\ \text{min}^{-1}$

Figure 5a
Enzyme Kinetics at Different Temperatures

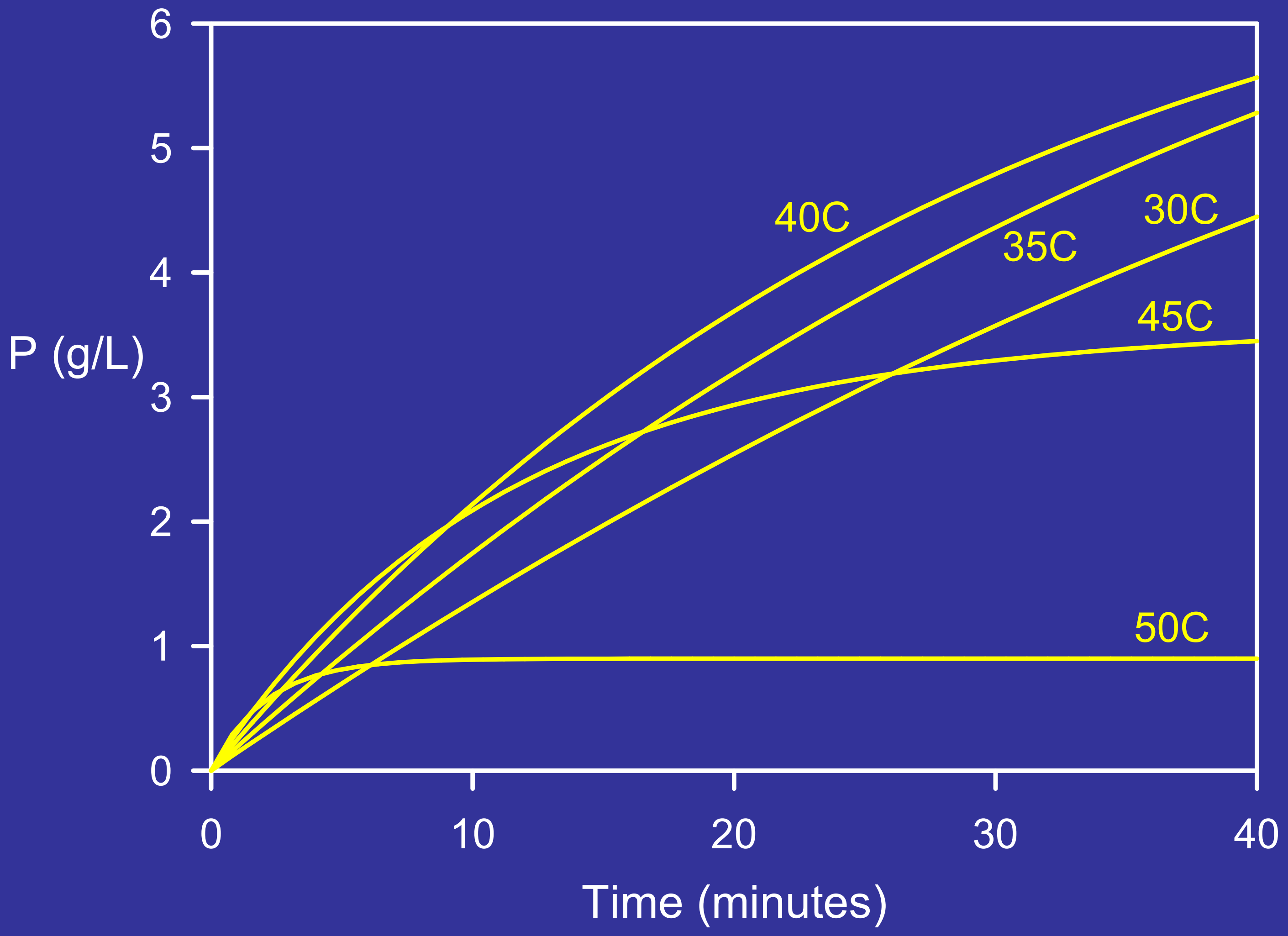


Figure 5b
Enzyme Kinetics at Different Temperatures

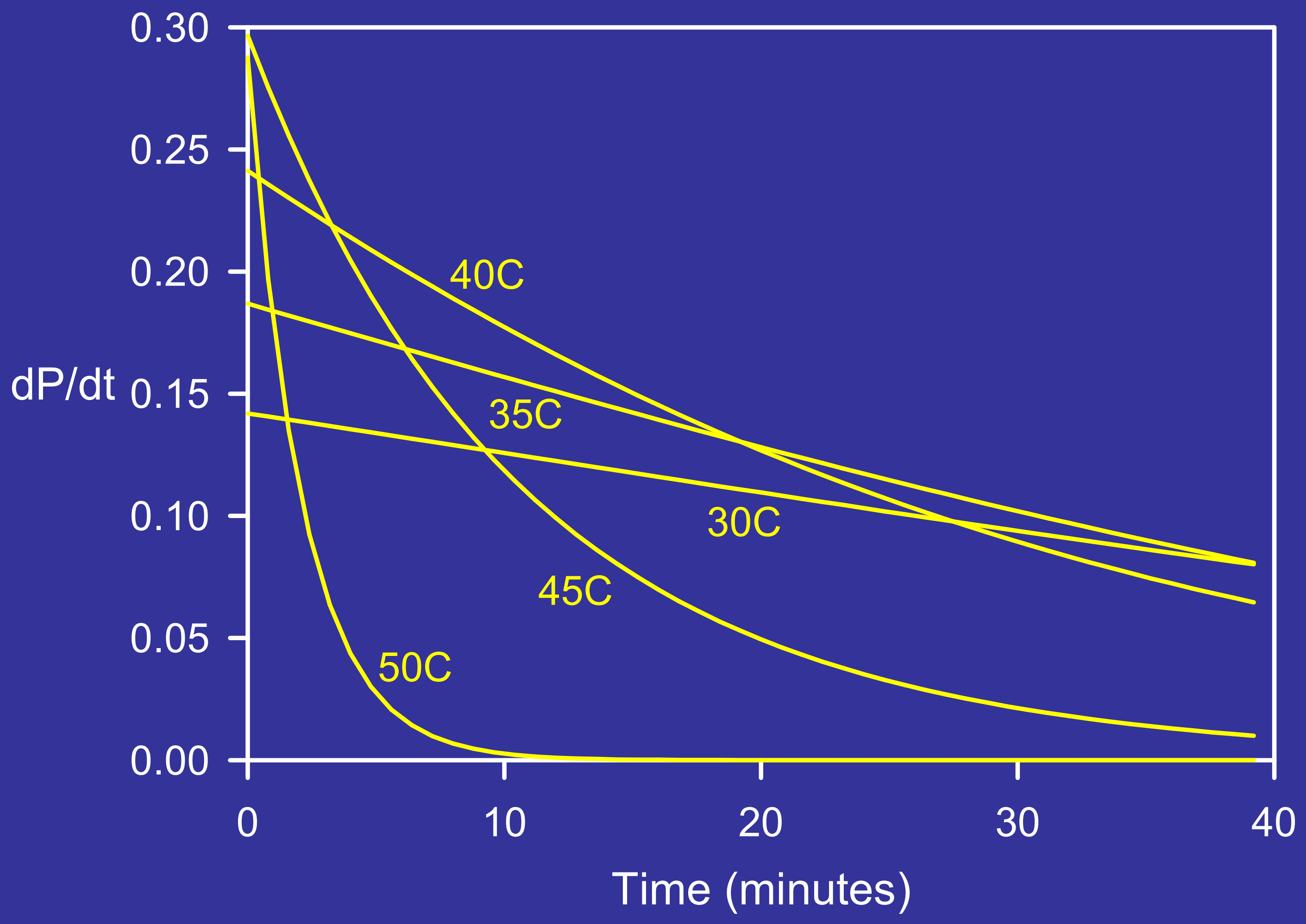


Figure 5c
Enzyme Kinetics at Different Temperatures

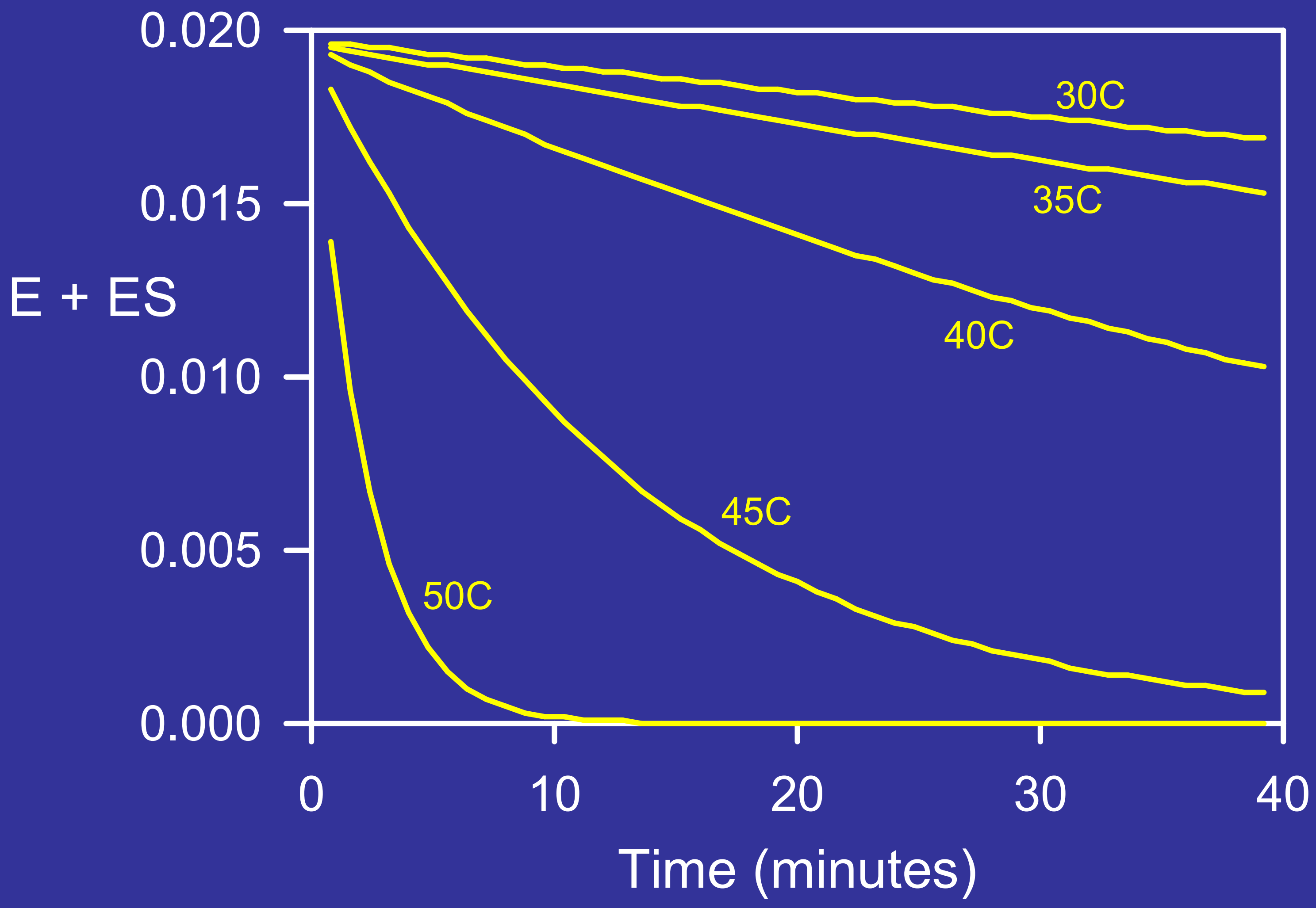


Figure 5d

Enzyme Kinetics at Different Temperatures

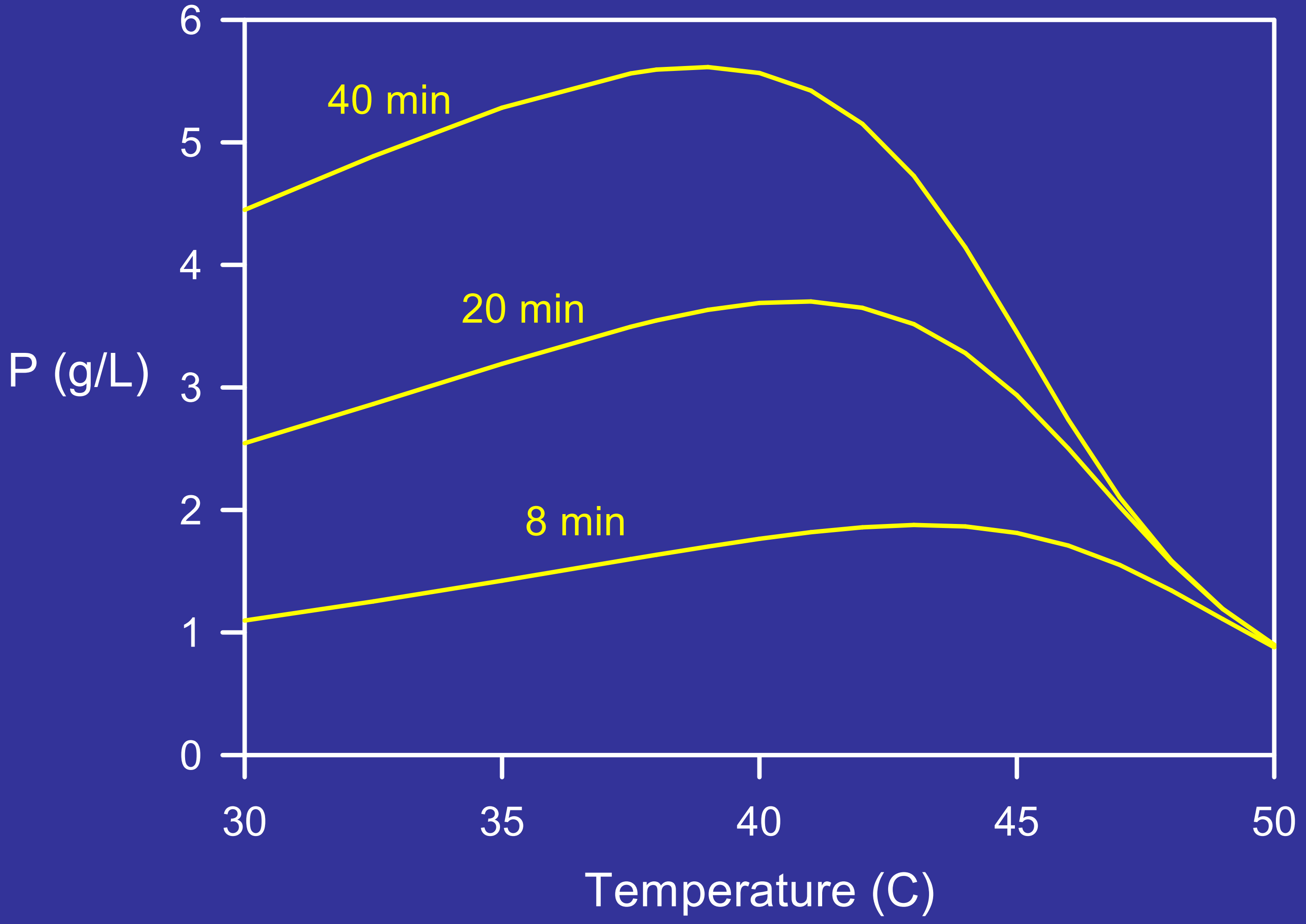
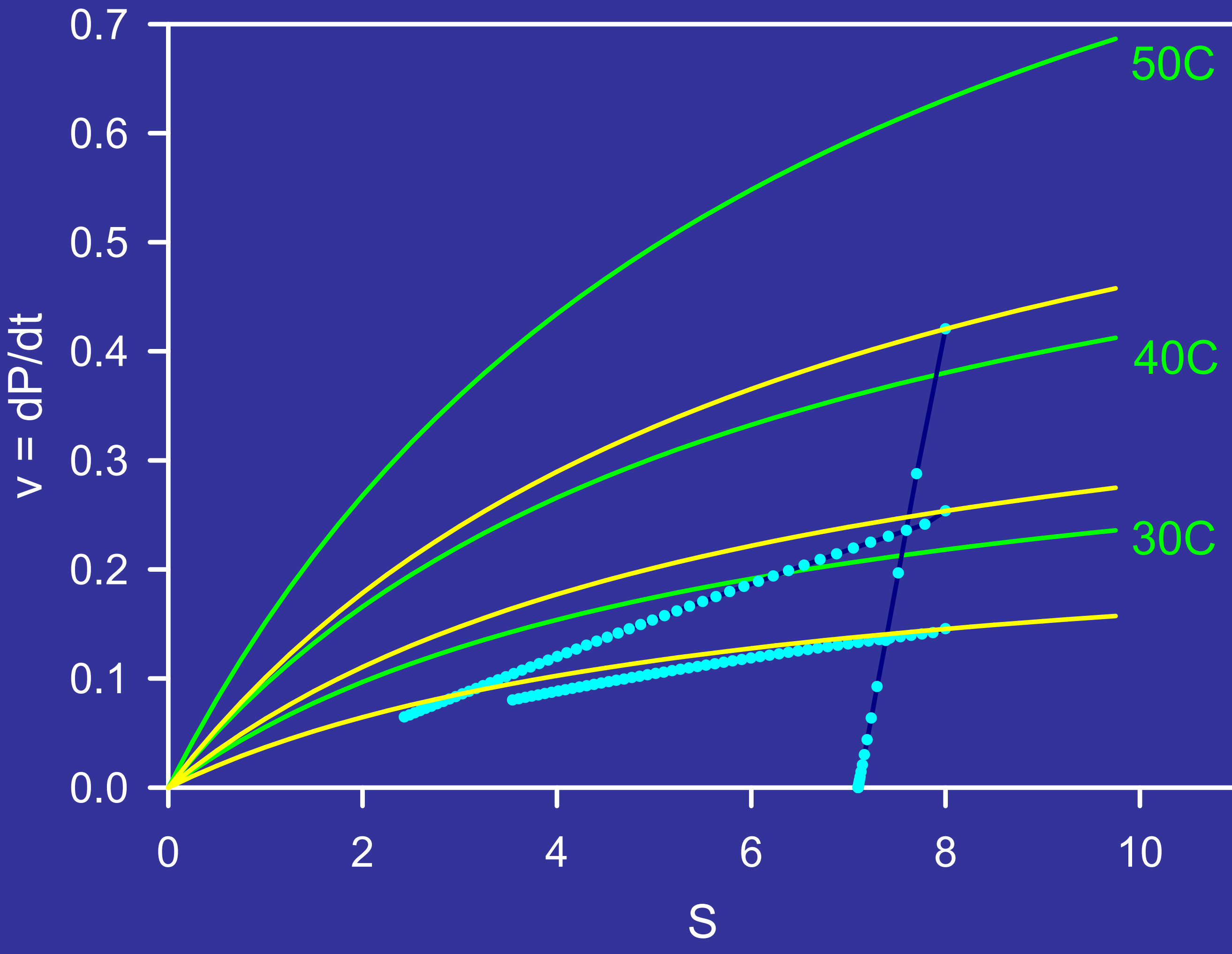


Figure 5e
Enzyme Kinetics at Different Temperatures



What is effect of activation, inactivation, denaturation?

activation

$V_{MAX} \propto k_2$

As T increases, k_2 increases, V_{MAX} increases

T(°C)	k_2	V_{MAX}
30	12.5	0.375
40	22.3	0.669
50	38.4	1.152

(Green/dashed curves on Figure 5e show increase in reaction rate with temperature due to activation ONLY without inactivation and without denaturation)

inactivation

$$\frac{E_I}{E} = \frac{k_3}{k_{-3}} = \frac{15}{12} = 1.25$$

$$\frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_1} = \frac{160}{30} = 5.33\ \text{g/L}$$

For $S_0 = 8$ g/L, $[E]/[ES] = 0.667$

$$E_0 = E + ES + E_I = 0.667ES + ES + 1.25(0.667)ES$$

$$= \underbrace{0.667ES + ES}_{\substack{\text{useful}\\ \text{enzyme}\\ (67\%)}} + \underbrace{0.833ES}_{\substack{\text{inactive}\\ \text{enzyme}\\ (33\%)}}$$

Equilibrium causes 33% of the enzyme to be in the inactive form
(Yellow/solid curves on Figure 5e show reduction in activity due to inactivation)

denaturation

Effective useful enzyme concentration decreases with time because a portion becomes (irreversibly) denatured.

(Trajectories with “dots” on Figure 5e show reduction in activity with time due to denaturation)