Effect of pH.
Instructor: Nam Sun Wang

**Mechanism #1 -- Acid Enzyme**. Substrate can be protonated, and only the protonated form can combine with an enzyme to form an active complex.

$$\begin{array}{l} \quad S + H^{+} \text{ (inactive)} \\ K_2 \ \downarrow\uparrow \qquad\qquad K_m \qquad\qquad\qquad k_2 \\ SH^{+} + E \longleftrightarrow ESH^{+} \longrightarrow E + PH^{+} \end{array}$$

**Derivation of Reaction Rate Expression with Equilibrium Assumption**.

Given 1. dp/dt=rate=v $\quad v = k_2 \cdot ESH$

2. Conservation of enzyme species $\quad E_0 = E + ESH$

3. Equilibrium Assumption: $\quad K_m = \frac{SH \cdot E}{ESH} \qquad K_2 = \frac{S \cdot H}{SH}$

We have 4 eqns to solve for 4 unknowns -- E, ESH, SH, and v. Find the analytical expression (via |Math|SmartMath|)

$$\text{Find}(E, ESH, SH, v) \rightarrow \begin{bmatrix} E_0 \cdot K_m \cdot \frac{K_2}{(K_m \cdot K_2 + S \cdot H)} \\ S \cdot H \cdot \frac{E_0}{(K_m \cdot K_2 + S \cdot H)} \\ S \cdot \frac{H}{K_2} \\ k_2 \cdot S \cdot H \cdot \frac{E_0}{(K_m \cdot K_2 + S \cdot H)} \end{bmatrix}$$

Thus, the last row is the analytical expression for v.

$$v = \frac{k_2 \cdot E_0 \cdot S \cdot H}{K_m \cdot K_2 + S \cdot H} = \frac{k_2 \cdot E_0 \cdot S}{K_m \cdot \frac{K_2}{H} + S} = \frac{v_m \cdot S}{K_{mapp} + S}$$

where $\quad v_m = k_2 \cdot E_0 \qquad K_{mapp} = K_m \cdot \frac{K_2}{H}$

**Mechanism #2 -- Alkaline Enzyme**. Substrate can give off a proton, and only the ionized form can combine with an enzyme to form an active complex.

$$\begin{array}{l} \qquad\qquad K_m \qquad\qquad\quad k_2 \\ S^- + E \longleftrightarrow ES^- \longrightarrow E + P^- \\ + \\ H^+ \\ K_1 \downarrow \uparrow \\ S \text{ (inactive)} \end{array}$$

**Derivation of Reaction Rate Expression with Equilibrium Assumption**.

Given 1. dp/dt=rate=v $v = k_2 \cdot ES$

2. Conservation of enzyme species $E_0 = E + ES$

3. Equilibrium Assumption: $K_m = \frac{S_i \cdot E}{ES}$ $K_1 = \frac{S_i \cdot H}{S}$

We have 4 eqns to solve for 4 unknowns -- E, ES, Si, and v. Find the analytical expression (via |Math|SmartMath|)

$$\text{Find}(E, ES, S_i, v) \rightarrow \begin{bmatrix} E_0 \cdot K_m \cdot \frac{H}{(K_m \cdot H + K_1 \cdot S)} \\ K_1 \cdot S \cdot \frac{E_0}{(K_m \cdot H + K_1 \cdot S)} \\ K_1 \cdot \frac{S}{H} \\ k_2 \cdot K_1 \cdot S \cdot \frac{E_0}{(K_m \cdot H + K_1 \cdot S)} \end{bmatrix}$$

Thus, the last row is the analytical expression for v.

$$v = \frac{k_2 \cdot E_0 \cdot S \cdot K_1}{K_m \cdot H + K_1 \cdot S} = \frac{k_2 \cdot E_0 \cdot S}{K_m \cdot \frac{H}{K_1} + S} = \frac{v_m \cdot S}{K_{mapp} + S}$$ where $v_m = k_2 \cdot E_0$ $K_{mapp} = K_m \cdot \frac{H}{K_1}$

**Mechanism #3 -- Ionizing Enzyme**. Enzyme can be ionized, and only one form is active.

$E^- + H^+$ (inactive)

$K_2$ $\downarrow\uparrow$

$$EH + S \overset{K_m}{\longleftrightarrow} EHS \overset{k_2}{\longrightarrow} EH + P$$

$+$

$H^+$

$K_1$ $\downarrow\uparrow$

$EH_2^{+}$ (inactive)

**Derivation of Reaction Rate Expression with Equilibrium Assumption**.

Given 1. dp/dt=rate=v $\quad v = k_2 \cdot EHS$

2. Conservation of enzyme species $\quad E_0 = E + EH + EH_2 + EHS$

3. Equilibrium Assumption: $\quad K_{m'} = \dfrac{EH \cdot S}{EHS} \qquad K_1 = \dfrac{EH \cdot H}{EH_2} \qquad K_2 = \dfrac{E \cdot H}{EH}$

We have 5 eqns to solve for 5 unknowns -- E, EH, $EH_2$, EHS, and v. Find the analytical expression (via |Math|SmartMath|)

$$\mathrm{Find}(E, EH, EH_2, EHS, v) \rightarrow \begin{bmatrix} K_2 \cdot K_{m'} \cdot K_1 \cdot \dfrac{E_0}{\left(K_1 \cdot K_2 \cdot K_{m'} + K_1 \cdot K_{m'} \cdot H + K_1 \cdot H \cdot S + K_{m'} \cdot H^2\right)} \\ K_{m'} \cdot K_1 \cdot E_0 \cdot \dfrac{H}{\left(K_1 \cdot K_2 \cdot K_{m'} + K_1 \cdot K_{m'} \cdot H + K_1 \cdot H \cdot S + K_{m'} \cdot H^2\right)} \\ E_0 \cdot K_{m'} \cdot \dfrac{H^2}{\left(K_1 \cdot K_2 \cdot K_{m'} + K_1 \cdot K_{m'} \cdot H + K_1 \cdot H \cdot S + K_{m'} \cdot H^2\right)} \\ K_1 \cdot E_0 \cdot H \cdot \dfrac{S}{\left(K_1 \cdot K_2 \cdot K_{m'} + K_1 \cdot K_{m'} \cdot H + K_1 \cdot H \cdot S + K_{m'} \cdot H^2\right)} \\ k_2 \cdot K_1 \cdot E_0 \cdot H \cdot \dfrac{S}{\left(K_1 \cdot K_2 \cdot K_{m'} + K_1 \cdot K_{m'} \cdot H + K_1 \cdot H \cdot S + K_{m'} \cdot H^2\right)} \end{bmatrix}$$

Thus, the last row is the analytical expression for v. We can further rearrange it by following these steps: copy the denominator, divide by $HK_1$, mark the whole expression and choose |Symbolic|Expand Expression|, mark "$K_m$" and choose |Symbolic|Collect on Subexpression| to collect $K_m$ terms, finally place the resulting denominator back into the expression for v.

$$v = \frac{k_2 \cdot E_0 \cdot S \cdot H \cdot K_1}{K_m \cdot K_2 \cdot K_1 + K_m \cdot H \cdot K_1 + K_m \cdot H^2 + S \cdot H \cdot K_1} = \frac{k_2 \cdot E_0 \cdot S}{K_m \cdot \left(\dfrac{1}{H} \cdot K_2 + 1 + \dfrac{1}{K_1} \cdot H\right) + S}$$

Thus, the above form is transformed into the Michaelis-Menten form by defining:

$$v_m = k_2 \cdot E_0 \qquad K_{mapp} = K_m \cdot \left( \frac{1}{H} \cdot K_2 + 1 + \frac{1}{K_1} \cdot H \right) = K_m \cdot \left( 10^{pH - pK_2} + 1 + 10^{pK_1 - pH} \right)$$

$$v(s) = \frac{v_m \cdot s}{K_{mapp} + s}$$

**Optimum pH**. The rate goes through a maximum when **dv/dpH=0**. Let's find where this maximum lies. Here, we mark "H" in the following equation and choose |Symbolic|Differentiate on Variable|.

$$v = \frac{v_m \cdot s}{K_m \cdot \left( \frac{1}{H} \cdot K_2 + 1 + \frac{1}{K_1} \cdot H \right) + s}$$

$$0 = -s \cdot \frac{v_m}{\left[ K_m \cdot \left( \frac{1}{H} \cdot K_2 + 1 + \frac{1}{K_1} \cdot H \right) + s \right]^2} \cdot K_m \cdot \left( \frac{-1}{H^2} \cdot K_2 + \frac{1}{K_1} \right)$$

Choose |Symbolic|Simplify| yields:

$$0 = s \cdot v_m \cdot K_1 \cdot K_m \cdot \frac{\left( K_1 \cdot K_2 - H^2 \right)}{\left( K_m \cdot K_2 \cdot K_1 + K_m \cdot H \cdot K_1 + K_m \cdot H^2 + s \cdot K_1 \cdot H \right)^2}$$

Since there is only one equation, mark "H" in the above equation and choose |Symbolic|Solve for Variable| yields:

$$\begin{bmatrix} \sqrt{K_1} \cdot \sqrt{K_2} \\ -\sqrt{K_1} \cdot \sqrt{K_2} \end{bmatrix}$$

Since the H concentration must be non-negative, we choose the first solution.

$$H_{max} = \sqrt{K_1 \cdot K_2}$$

To find the maximum v at optimum pH, copy "$\sqrt{K_1 \cdot K_2}$" into the clipboard, mark "$H_{max}$" in the following equation for $v_{opt}$, and choose |Symbolic|Substitute for Variable|.

$$v_{opt} = \frac{v_m \cdot s}{K_m \cdot \left( \frac{1}{H_{max}} \cdot K_2 + 1 + \frac{1}{K_1} \cdot H_{max} \right) + s}$$

$$v_{opt} = \frac{v_m \cdot s}{K_m \cdot \left( 2 \cdot \frac{\sqrt{K_2}}{\sqrt{K_1}} + 1 \right) + s} = \frac{v_m \cdot s}{K_m \cdot \left( 2 \cdot 10^{\frac{pK_1 - pK_2}{2}} + 1 \right) + s}$$

← the maximum rate at optimum pH

Plot of pH dependence $v_m := 1$ $K_m := 1$ $pK_1 := 6$ $pK_2 := 8$

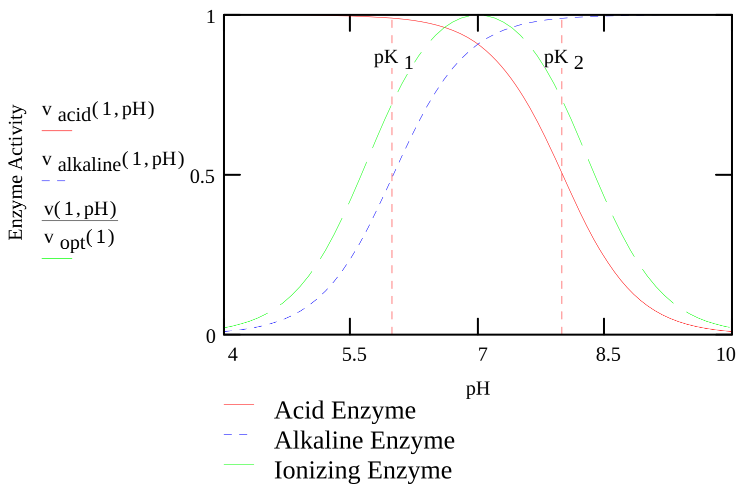
Acid enzyme: $$v_{acid}(s, pH) := \frac{v_m \cdot s}{K_m \cdot 10^{-pK_2 + pH} + s}$$

Alkaline enzyme: $$v_{alkaline}(s, pH) := \frac{v_m \cdot s}{K_m \cdot 10^{pK_1 - pH} + s}$$

Ionizing enzyme: $$v(s, pH) := \frac{v_m \cdot s}{K_m \cdot \left(10^{pH - pK_2} + 1 + 10^{pK_1 - pH}\right) + s}$$

ionizing enzyme at optimum pH: $$v_{opt}(s) := \frac{v_m \cdot s}{K_m \cdot \left(2 \cdot 10^{\frac{pK_1 - pK_2}{2}} + 1\right) + s}$$

$pH := 4, 4.1 .. 10$




Acid enzymes have high activities at acidic conditions. Whereas, alkaline enzymes have high activities at alkaline conditions. For these enzymes, the enzyme activity drops to half of the maximum level at pH=pK,. Ionizing enzymes exhibit a maximum at a pH halfway between $pK_1$ and $pK_2$. The smaller the difference between $pK_1$ and $pK_2$, the narrower the active range. Finally, all of these mechanisms give rise to a change in the apparent $K_m$ value; thus, **competitive inhibition**.