# 20.201

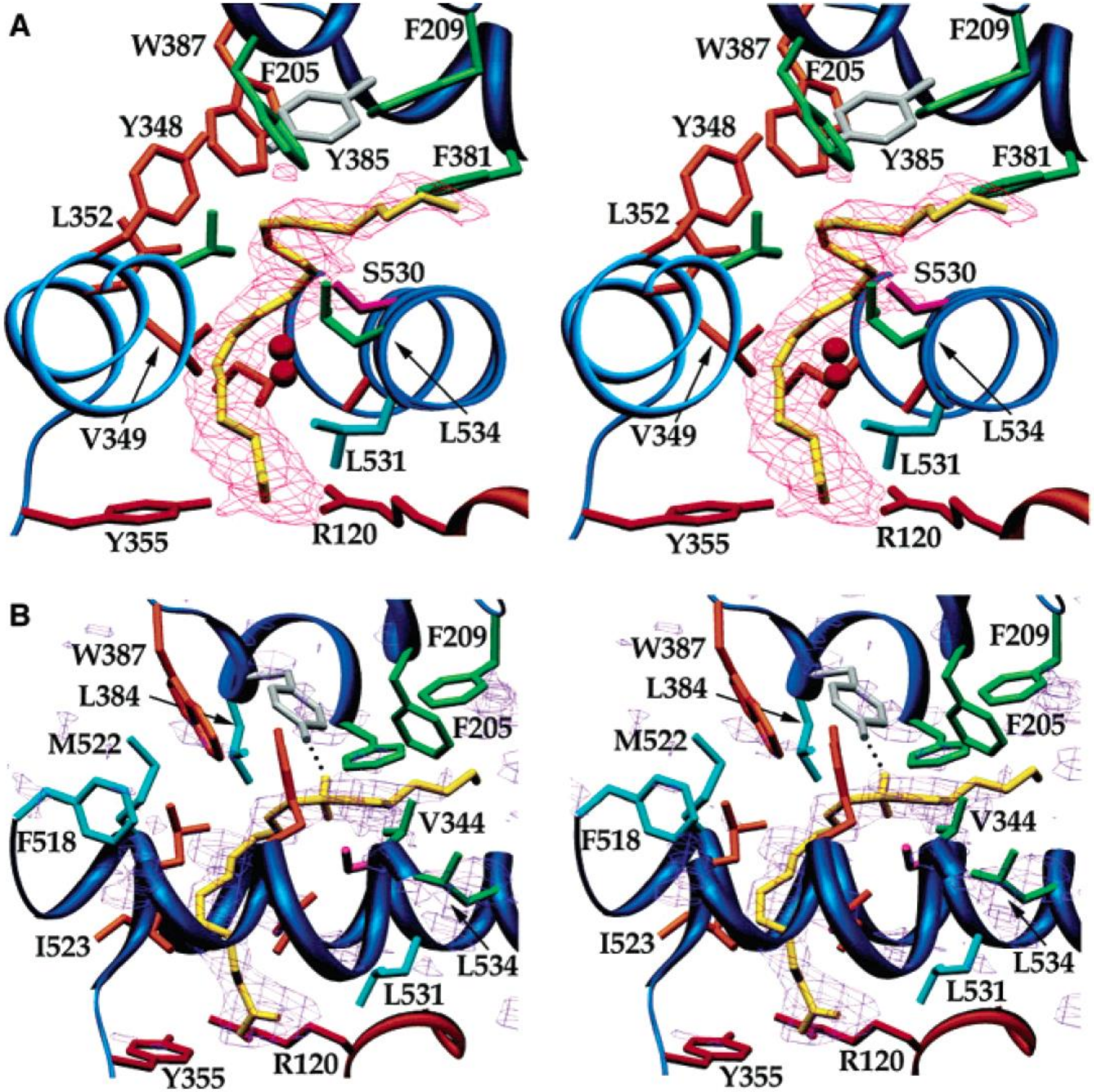
Biochemistry Review

# Michaelis-Menten Kinetics

$$E + S \underset{k-1}{\overset{k1}{\rightleftarrows}} ES \xrightarrow{k2} P + E$$

- E = Enzyme
- S = Substrate
- ES = Enzyme-substrate complex (Michaelis Complex)
- P = Product
- What is the unit of *k*?

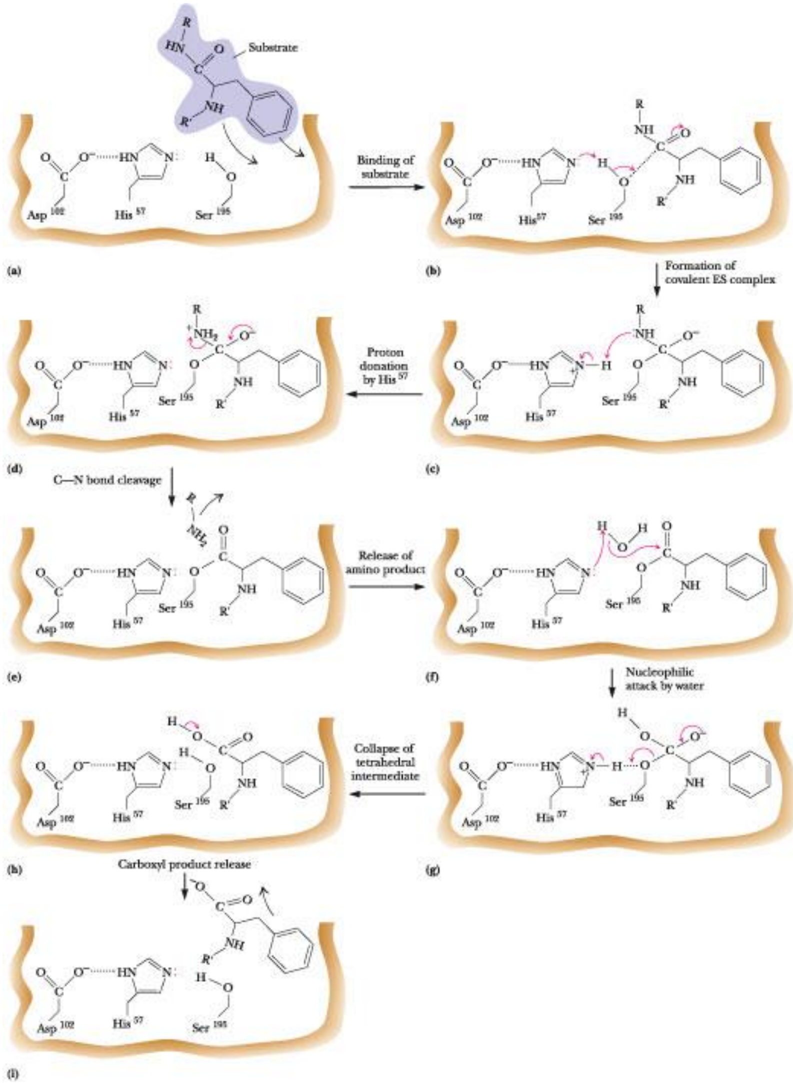
# COX-1 bound to Arachidonic Acid






Source: Rouzer, Carol A., and Lawrence J. Marnett. "Mechanism of Free Radical Oxygenationof Polyunsaturated Fatty Acids by Cyclooxygenases." *Chemical Reviews* 103, no. 6 (2003): 2239-04.

# Chymotrypsin: Catalytic Triad



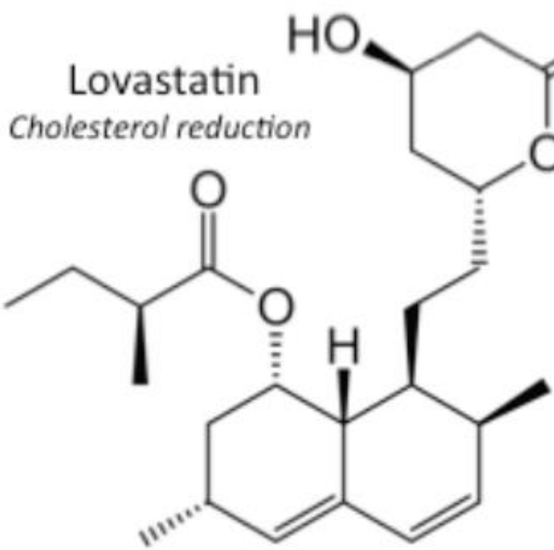


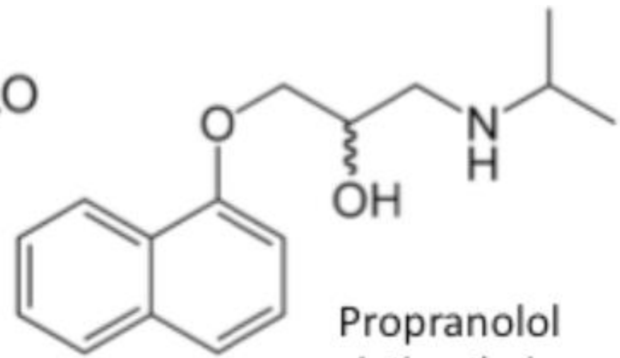



http://en.wikibooks.org/wiki/File:Chymo.jpg

Lovastatin

*Cholesterol reduction*

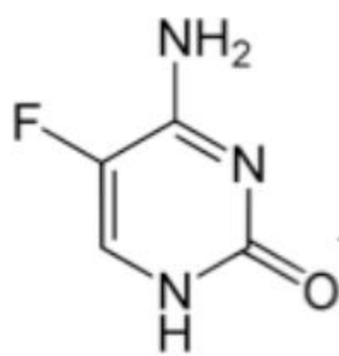




Propranolol

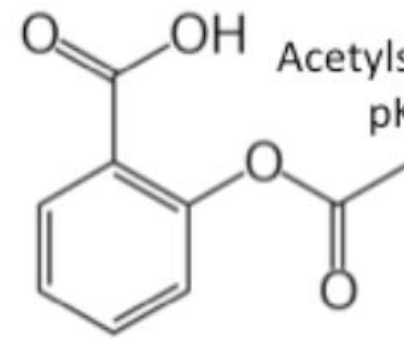
*Antiarrythmic*

pKa ~ 9.5



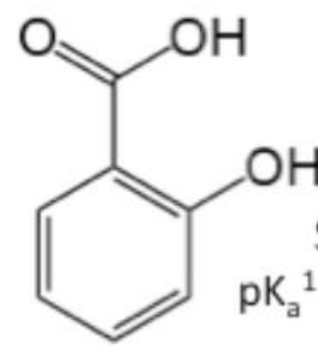
Flucytosine

*Antifungal agent*



Acetylsalicylic acid

$pK_a$ ~ 3.5



Salicylic acid

$pK_a^1$ ~ 3.5, $pK_a^2$ ~ 7-8

# Assumptions

$$E + S \underset{k-1}{\overset{k1}{\rightleftarrows}} ES \xrightarrow{k2} P + E$$

- $k_2 << k_{-1}$
  - Rapid equilibrium
  - Product dissociation is not rate limiting
- Steady state
  - Rate of [ES] formation equals the rate of consumption

# Rate of Reaction (Velocity)

$$E + S \underset{k-1}{\overset{k1}{\rightleftarrows}} ES \xrightarrow{k2} P + E$$

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

How can we determine [ES]?

# Remember 2nd Assumption

$$E + S \underset{k-1}{\overset{k1}{\rightleftarrows}} ES \xrightarrow{k2} P + E$$

- Steady state:

$$\frac{d[ES]}{dt} = 0$$

- Overall rate equation for [ES]:

$$\frac{d[ES]}{dt} = 0 = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

# What do we know about [E]?

$$E + S \underset{k-1}{\overset{k1}{\rightleftarrows}} ES \xrightarrow{k2} P + E$$

$$[E]_T = [E] + [ES]$$

- Rearrange to solve for [E]:

$$[E] = [E]_T - [ES]$$

- Substitute into overall rate equation for [ES]:

# [ES] rate equation

$$E + S \underset{k-1}{\overset{k1}{\rightleftarrows}} ES \xrightarrow{k2} P + E$$

$$\frac{d[ES]}{dt} = 0 = k_1[S]([E]_T - [ES]) - [ES](k_{-1} + k_2)$$

- Combine like terms:

$$k_1[S][E]_T = [ES](k_{-1} + k_2 + k_1[S])$$

- Divide by $k_1$:

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

# Solve for [ES]

$$E + S \underset{k-1}{\overset{k1}{\rightleftarrows}} ES \xrightarrow{k2} P + E$$

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

$$K_M = \left(\frac{k_{-1} + k_2}{k_1}\right) = \left(\frac{k_{-1}}{k_1}\right) = K_d$$ (when $k_{-1} >> k_2$)

# Calculating Reaction Velocity (*v*)

$$E + S \underset{k-1}{\overset{k1}{\rightleftarrows}} ES \xrightarrow{k2} P + E$$

$$[ES] = \frac{[S][E]_T}{(K_M + [S])}$$

$$v = \frac{dP}{dt} = k_2[ES] = \frac{k_2[S][E]_T}{(K_M + [S])}$$

# Calculating Reaction Velocity (*v*)

$$E + S \underset{k-1}{\overset{k1}{\rightleftarrows}} ES \xrightarrow{k2} P + E$$

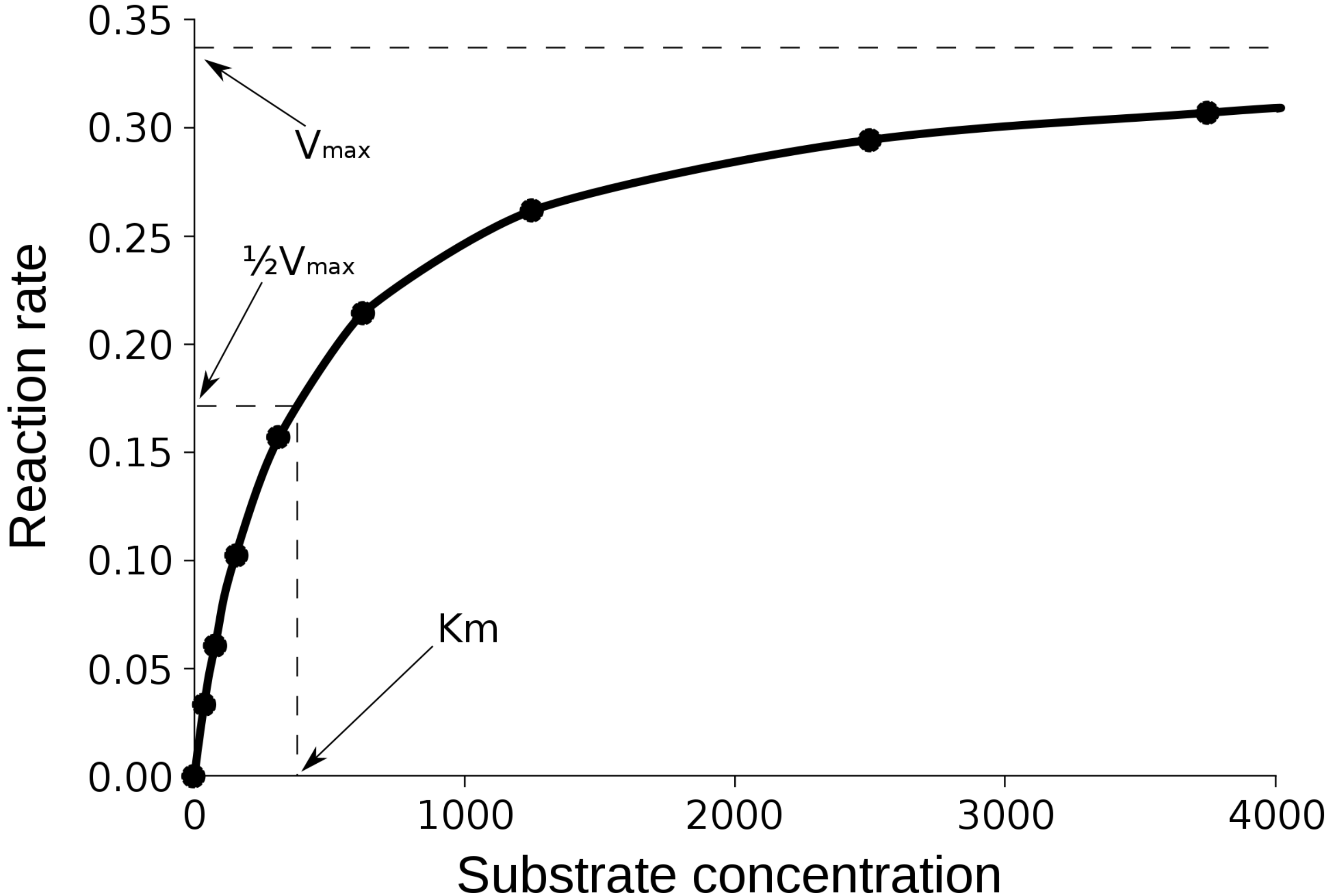
When $[S] >> [E]$ there is no free $[E]$ so $[E]_T = [ES]$

$$v_{max} = k_2[E]_T$$

$$v = \frac{v_{max}[S]}{(K_M + [S])}$$

$$When\ [S] = K_M \therefore v = \frac{v_{max}[S]}{([S] + [S])} = v = \frac{v_{max}}{2}$$

# Michaelis-Menten Plot



# Acid Base Equilibrium

$$HA + H_2O \leftrightarrows H_3O^+ + A^-$$

$$K = \frac{[H_3O^+][A^-]}{[HA][H_2O]}$$

$$K[H_2O] = K_a = \frac{[H_3O^+][A^-]}{[HA]}$$

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

$$[H_2O] = 1000\ g{\bullet}L^{-1}$$

$$18.015\ g{\bullet}mol^{-1}$$

$$= 55\ M$$

# Water Dissociation Constant

$$H_2O \leftrightarrows H^+ + A^-$$

$$K = \frac{[H^+][A^-]}{[H_2O]}$$

$$K[H_2O] = K_w = [H^+][A^-]$$

at 25° C $K_w = 10^{-14}$ $M^2$

Neutrality is defined when $[H^+] = [A^-]$

Therefore, $[H^+] = [A^-] = 10^{-7}$

$$pH = -\log[H^+]$$

# Henderson-Hasselbalch Equation

pH of a solution is dependent on the concentration of the acid and its conjugate base

$$HA \leftrightarrows H_3O^+ + A^-$$

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

$$pH = -\log[H^+]$$

$$[H^+] = K_a \bullet ([HA]/[A^-])$$

$$-\log[H^+] = -\log (K_a \bullet ([HA]/[A^-]))$$

$$pH = -\log K_a – \log([HA]/[A^-])$$

$$= pKa + \log ([A^-]/[HA])$$

# Zero-Order Reactions

$r = k[A]^0 = k\ (1) = k$

Constant $k$ has units of $M \bullet sec^{-1}$

# First-Order Reactions

$$A \rightarrow P$$

$$r = -\frac{d[A]}{dt} = \frac{dP}{dt} = k[A]$$

$$\frac{d[A]}{[A]} = -kdt$$

$$\int_{[A]_o}^{[A]} \frac{d[A]}{[A]} = -k \int_0^t dt$$

$$\ln[A] = \ln[A]_o - kt$$

$$[\mathrm{A}] = [A]_o e^{-kt}$$

(*k* has units $sec^{-1}$)

# Half-Life of First-Order Reaction

At the reaction half-life, $t_{1/2}$, $[\mathrm{A}] = \frac{[A]_o}{2}$

$$\frac{[\mathrm{A}]_o}{2} = [A]_o e^{-kt_{1/2}}$$

$$\frac{[\mathrm{A}]_o}{2[A]_o} = e^{-kt_{1/2}}$$

$$\ln 2 = kt_{1/2} \quad \therefore \quad t_{1/2} = \frac{\ln 2}{k} = \frac{0.693}{k}$$

# Second-Order Reactions

$$2A \rightarrow P$$

$$r = -\frac{d[A]}{dt} = \frac{dP}{dt} = k[A]^2$$

$$\frac{d[A]}{[A]^2} = -kdt$$

$$\int_{[A]_o}^{[A]} \frac{d[A]}{[A]^2} = -k \int_0^t dt$$

$$\frac{1}{[A]} = \frac{1}{[A]_o} + kt$$

(*k* has units $M^{-1} \bullet sec^{-1}$)

# Association vs. Dissociation Constants

$$A + B \underset{\leftarrow}{\overset{\rightarrow}{}} AB$$

$$K_a = \frac{[AB]}{[A][B]}$$

($K_a$ has units of $M^{-1}$)

$$K_d = \frac{[A][B]}{[AB]}$$

($K_d$ has units of M)

$$K_a = \frac{1}{K_d}$$