Enzyme Kinetics and Inhibition

Pratt & Cornely Ch 7

Enzyme Kinetics

- How fast an enzyme catalyzed reaction goes
- Why study enzyme kinetics?
  - Helps us understand mechanism of enzyme (how it works)
  - Investigation of mutations in metabolic pathways
  - Understanding of regulation of biochemical reactions (up or down regulation of catalyst)

Simple Mechanisms

- Chemical mechanism

$$\text{Substrate} \longrightarrow \text{Product}$$

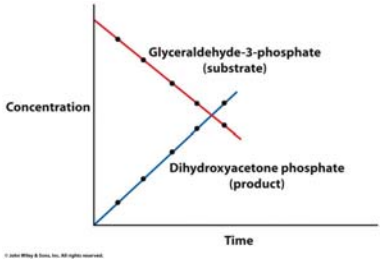
- Enzyme Catalyzed

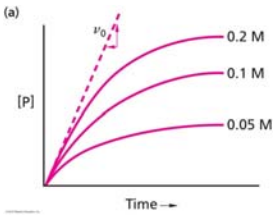
$$\text{Substrate} + \text{Enzyme} \rightleftharpoons \text{Reactive Complex} \longrightarrow \text{Product} + \text{Enzyme}$$

- How do we measure kinetics experimentally?

Chemical Kinetics

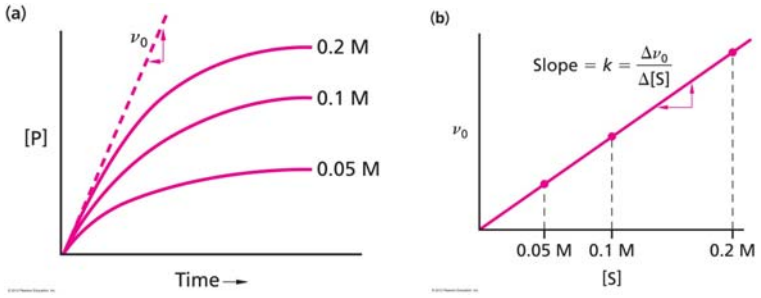
- Rate: measure product formed per second
- Rate slows as reactant disappears
- Measure initial rate
- Do a second experiment with more starting material, and the initial rate is faster





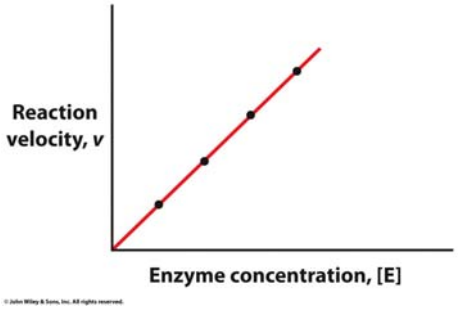
Chemical Kinetics

- Secondary plot: change in rate as a function of how much substrate you started with
- Linear plot—does that make sense?



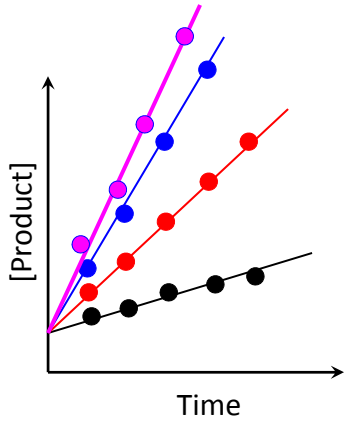
Enzyme Kinetics

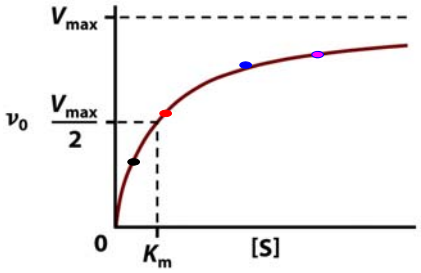
- Complicated—two components, treated separately
- First, how does [enzyme] affect rate (given large [S]?)



Enzyme Kinetics

- Next, keep the [E] constant and low, and test how changing the [S] affects initial rates
- Michaelis-Menton Treatment



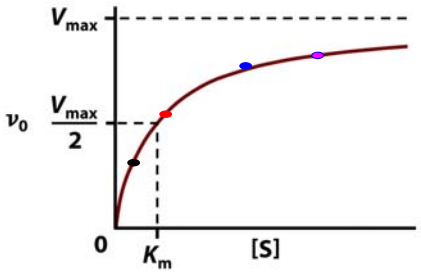


Michaelis-Menton Kinetics

- Rectangular hyperbola
- Parameters

$$y = \frac{ax}{b + x}$$

$$v_o = \frac{V_{max}[S]}{K_m + [S]}$$



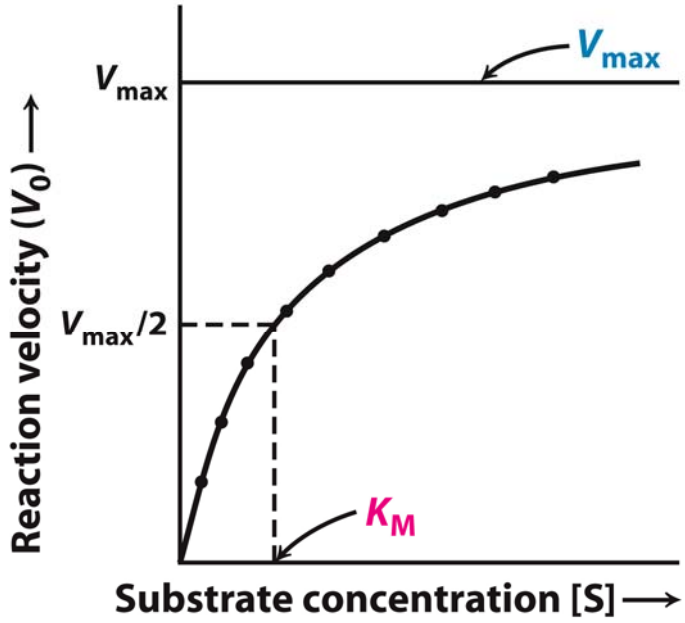
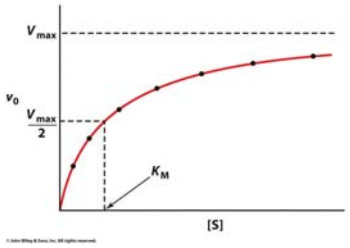


Figure 7.3
Biochemistry: A Short Course, Third Edition

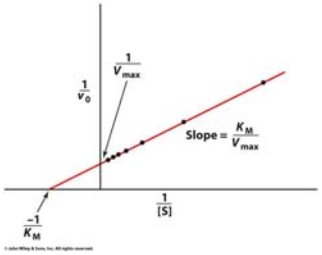
Graphical Determination of Kinetic Parameters

- Analyze hyperbola
- Construct linear plot
- Double reciprical



Lineweaver–Burk equation:

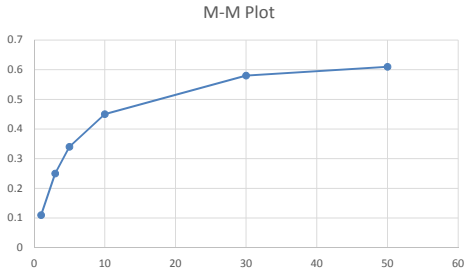
$$\frac{1}{v_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

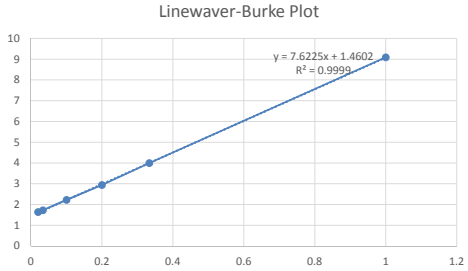


Lineweaver-Burk Analysis

- How can you determine kinetic parameters Vmax and Km?

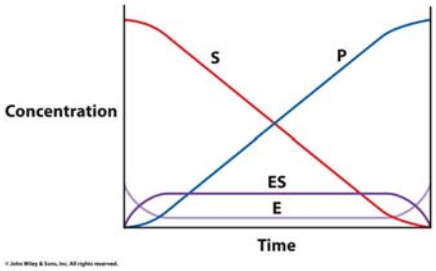
[S] mM	[P] at 1 min (nM)
1	0.11
3	0.25
5	0.34
10	0.45
30	0.58
50	0.61





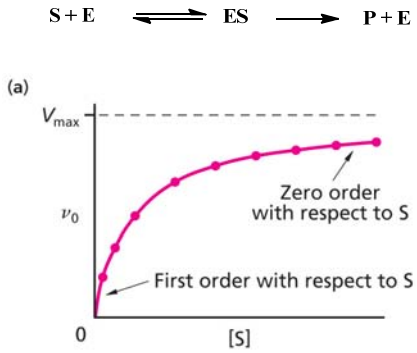
Mechanism and Assumptions

- E + S ←→ ES →E + P
  - Low [E] relative to [S]
    - Steady state
  - Initial rates
    - No back rxn
    - No pdt inhibition
  - **Derive a rate equation**



Interpretation of Shape

- Low [S]
  - Rate very dependent on [S]
  - Binding is rate limiting
- High [S]
  - Rate independent
  - Saturation of E
  - Chemistry is rate limiting



Maximum Velocity and the Catalytic Constant

- What two things contribute to the maximum velocity limit?
  - Amount of enzyme
  - Chemical ability of enzyme (catalytic constant)
- $V_{max} = [E]\ k_{cat}$
- Only k_{cat} tells us about the enzyme
  - Maximum # of substrate molecules per active site per second
  - Turnover number

TABLE 7-1

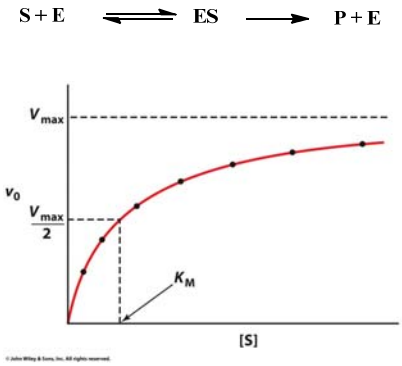
Catalytic Constants of Some Enzymes

Enzyme	k_{cat} (s^{-1})
Staphylococcal nuclease	95
Cytidine deaminase	299
Triose phosphate isomerase	4300
Cyclophilin	13,000
Ketosteroid isomerase	66,000
Carbonic anhydrase	1,000,000

[Data from Radzicka, A., and Wolfenden, R., *Science* **267**, 90–93 (1995).]

Michaelis Constant

- K_m is the [S] at which the reaction reaches half its maximum velocity
- Physical meaning (assuming equilibrium binding): K_m is the dissociation constant for ES
- K_m is [S] at which enzyme is half-bound
- K_m is measure of affinity of enzyme for S
- Low K_m is tight binding

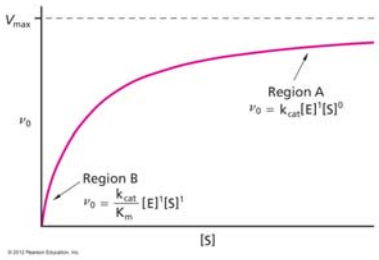


Enzyme Efficiency

- At low [S], the second order rate constant is k_{cat}/K_m
- Efficient enzymes have large k_{cat}/K_m
  - Large k_{cat} and/or
  - Small K_m
- Catalytic perfection at 10^8 or 10^9 M^{-1} S^{-1}
- Diffusion control

$$v = \frac{k_{cat}[E][S]}{K_m + [S]}$$

Assume large [S] and small [S]



Case Study: Diffusion Controlled Enzymes

Table 6.4 Enzymes with second-order rate constants near the upper limit

Enzyme	Substrate	$k_{cat}/K_m (M^{-1} s^{-1})^*$
Catalase	H_2O_2	4×10^7
Acetylcholinesterase	Acetylcholine	2×10^8
Triose phosphate isomerase	D-Glyceraldehyde 3-phosphate	4×10^8
Fumarase	Fumarate	10^9
Superoxide dismutase	$\cdot O_2^{\ominus}$	2×10^9

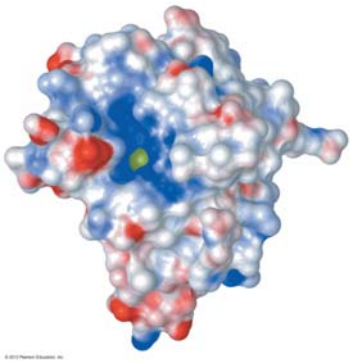
*The ratio k_{cat}/K_m is the apparent second-order rate constant for the enzyme-catalyzed reaction E + S → E + P. For these enzymes, the formation of the ES complex can be the slowest step.

Superoxide Dismutase: Better than Diffusion!

$$4 \cdot O_2^{\ominus} \xrightarrow[\text{Superoxide dismutase}]{4 H^{\oplus}} 2 H_2O_2 \xrightarrow{\text{Catalase}} 2 H_2O + O_2$$

$$E\text{-}Cu^{2+} + \cdot O_2^{\ominus} \longrightarrow E\text{-}Cu^{\oplus} + O_2$$

$$E\text{-}Cu^{\oplus} + \cdot O_2^{\ominus} + 2H^{\oplus} \longrightarrow E\text{-}Cu^{2+} + H_2O_2$$



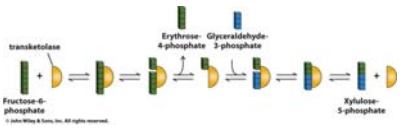
Catalytic Proficiency

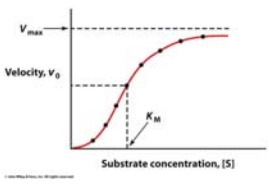
Table 5.2 Catalytic proficiencies of some enzymes

	Nonenzymatic rate constant (k_n in s^{-1})	Enzymatic rate constant (k_{cat}/K_m in $M^{-1}s^{-1}$)	Catalytic proficiency
Carbonic anhydrase	10^{-1}	7×10^6	7×10^7
Chymotrypsin	4×10^{-9}	9×10^7	2×10^{16}
Chorismate mutase	10^{-5}	2×10^6	2×10^{11}
Triose phosphate isomerase	4×10^{-6}	4×10^8	10^{14}
Cytidine deaminase	10^{-10}	3×10^6	3×10^{16}
Adenosine deaminase	2×10^{-10}	10^7	5×10^{16}
Mandelate racemase	3×10^{-13}	10^6	3×10^{18}
β-Amylase	7×10^{-14}	10^7	10^{20}
Fumarase	10^{-13}	10^9	10^{21}
Arginine decarboxylase	9×10^{-16}	10^6	10^{21}
Alkaline phosphatase	10^{-15}	3×10^7	3×10^{22}
Orotidine 5′-phosphate decarboxylase	3×10^{-16}	6×10^7	2×10^{23}
Uroporphyrinogen decarboxylase	10^{-17}	2×10^7	2×10^{24}

Non-MM Kinetics

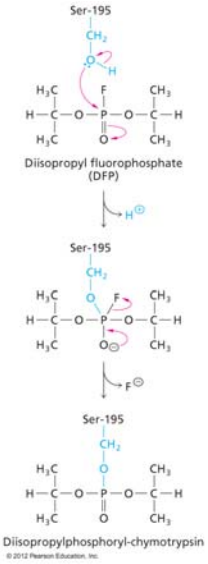
- Multi-substrate
  - Each substrate has its own Km
  - Random, ordered, ping-pong
- Multistep reactions
  - k_{cat} not simplified to k_2
- Allosteric enzymes
  - cooperativity





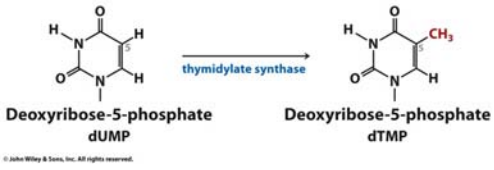
Irreversible Enzyme Inhibition

- Affinity labels
  - Test enzyme mechanisms
  - Serine protease
- Mechanism-based Inhibitors
- Transition State Analogs



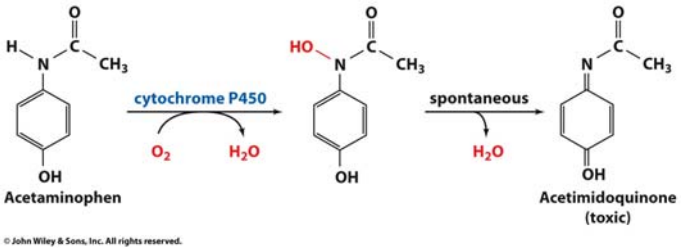
Mechanism Based Inhibitors

- Suicide inhibitors
- Selectivity
- Targeting fast-growing cells

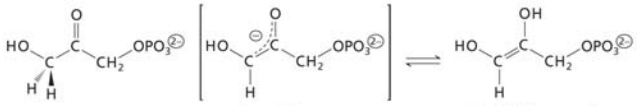


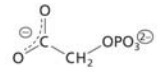
Drug Byproducts

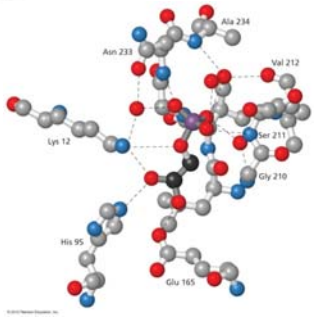
- Oxidation of xenobiotics by P450 enzymes
- Pharmacology
- Liver damage—covalent binding to cysteine



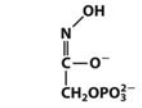
Transition State Analog



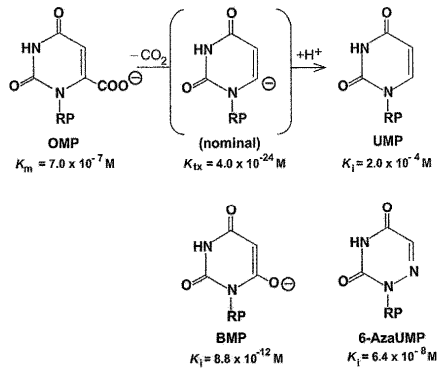




- Your book presents high energy intermediate analog



Case Study: Orotidine Decarboxylase



Mechanism of Catalysis

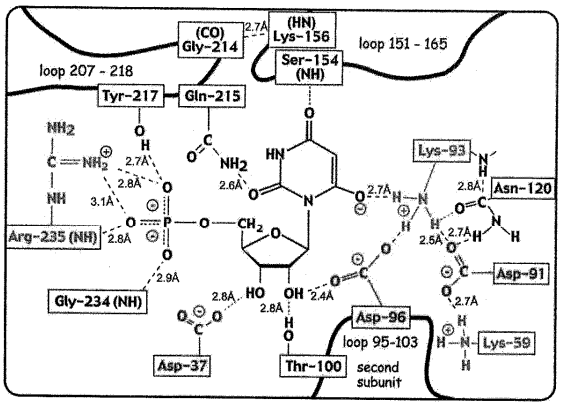
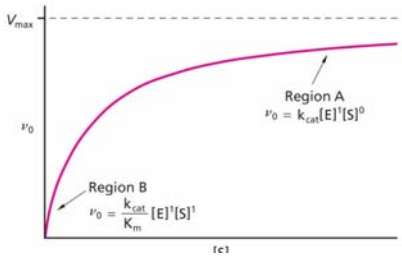


Figure 9 Schematic view of the active site of yeast ODCase (71), showing the active site residues that contact the inhibitor 6-hydroxyUMP (BMP). Positively charged residues are shown in red, negatively charged residues in green.

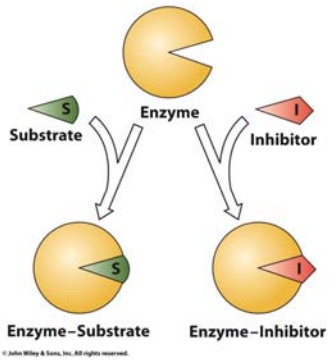
Reversible Inhibition Kinetics

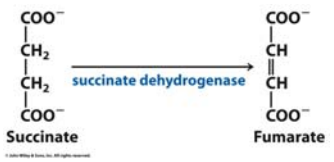
- Know types of Reversible Inhibition
- Know effect on kinetic parameters
- Understand why
- Interpret MM plots

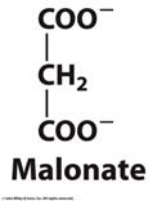


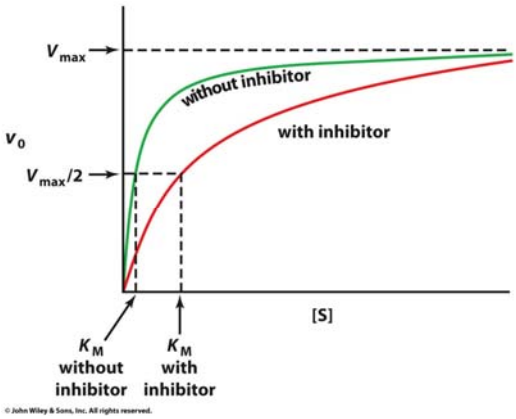
Competitive Inhibition

- Added substrate can outcompete inhibitor
- Draw mechanism with equilibrium arrows
  - Km_{app}: How does added I affect ES dissociation?
  - Vmax: How does adding infinite S affect ES formation?
- Draw altered MM and LB plots



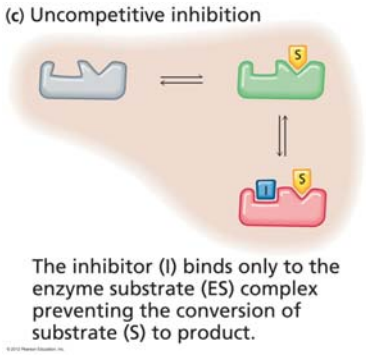






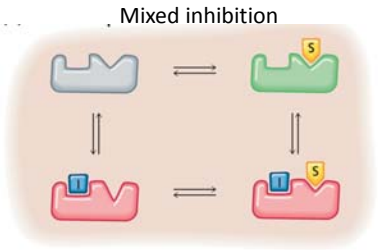
Uncompetitive Inhibition

- S and I help each other bind
- Draw mechanism with equilibrium arrows
  - Km_{app}: How does added I affect ES dissociation?
  - Vmax: How does adding infinite S affect ES formation?
- Draw altered MM and LB plots



Mixed Inhibition

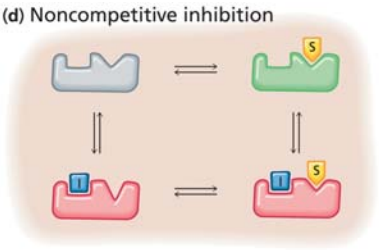
- The continuum between competitive and uncompetitive
  - Inhibitor may bind either E or ES
  - Either more competitive or more uncompetitive
- Noncompetitive is middle of continuum



The inhibitor (I) can bind to either E or ES. The enzyme becomes inactive when I binds. Substrate (S) can still bind to the EI complex but conversion to product is inhibited.

Noncompetitive Inhibition

- Assumes simple case of mixed inhibition in which inhibitor binding equally to E and ES
- Physical explanation: inhibitor binding causes change that affects reaction, but not S binding
- Very rare (nonexistent)
- Draw mechanism with equilibrium arrows
  - Km_{app}: How does added I affect ES dissociation?
  - Vmax: How does adding infinite S affect ES formation?
- Draw altered MM and LB plots



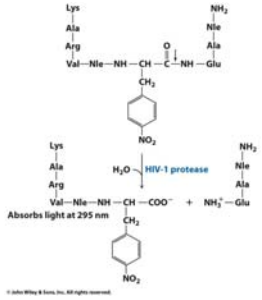
The inhibitor (I) can bind to either E or ES. The enzyme becomes inactive when I binds. Substrate (S) can still bind to the EI complex but conversion to product is inhibited.

Fill in the Chart

Inhibition	Effect on K_M	Effect on V_{max}	Effect on V_{max}/K_M
Competitive			Down
Uncompetitive			Down
Noncompetitive			Down
Mixed			Down

Problem 56

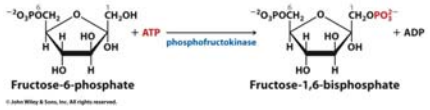
[S] μ M	V (no I)	V (with I)
10	4.63 nmol/min	2.70
15	5.88	3.46
20	6.94	4.74
25	9.26	6.06
30	10.78	6.49
40	12.14	8.06
50	14.93	9.71

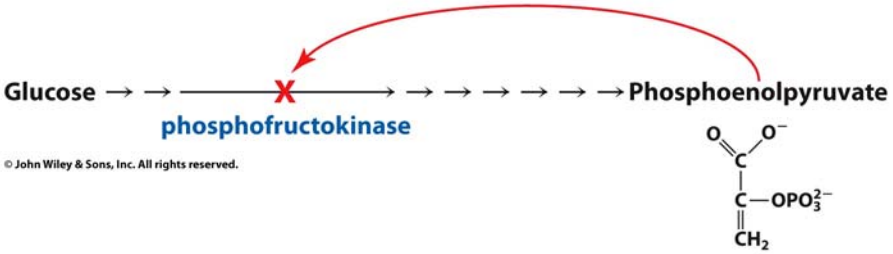


- Use LB plot to determine parameters
- What type of inhibition?
- Calculate Ki.

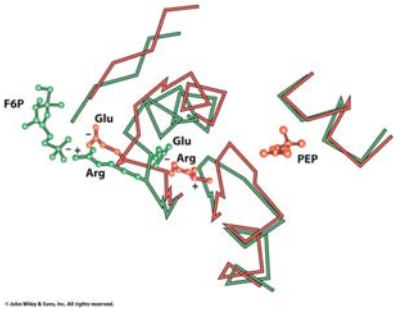
Allosteric Regulation

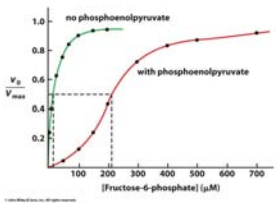
- Can be inhibition
  - Negative effector
  - Feedback inhibition
  - PFK regulation





Mechanism



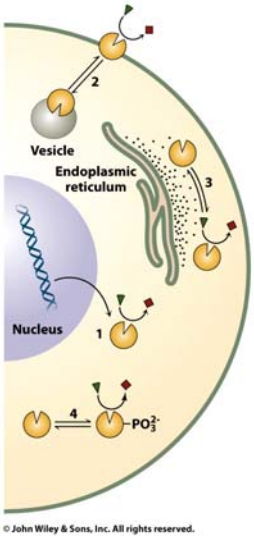


- PEP binding in allosteric site causes conformational shift in neighbor
- An Arg essential for F6P binding is replaced with Glu
- T vs. R state
- Cooperative, no effect on V_{max}, but only apparent K_M

Positive Effector

- ADP acts with positive cooperativity
- Favors R state by binding in the same allosteric site, but holding it open to lock Arg into place
- Does ADP effector make sense physiologically?

Other Modes of Regulation



- Transcriptional level
- Compartmentalization
- Intracellular signal
- Covalent modification