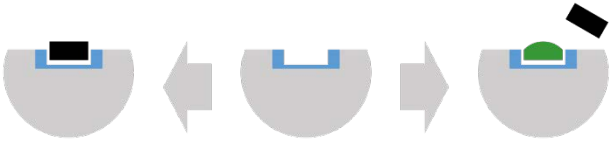
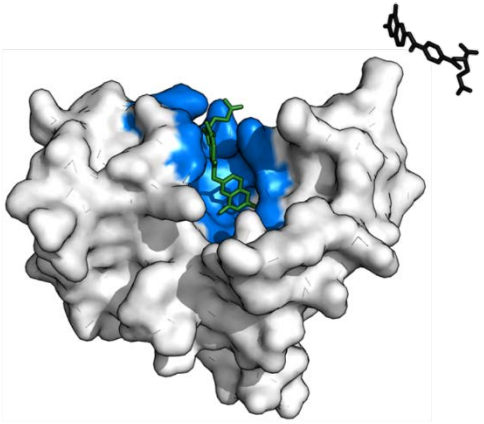
Lecture #6: Enzyme Inhibition and Kinetics

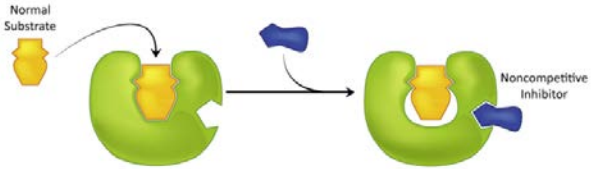


Lecture #6: Enzyme Inhibition and Kinetics

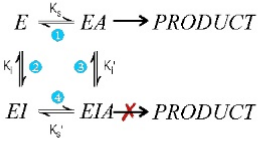
A. Classification of Inhibitors



1. Irreversible
    a. Iodoacetamide
    b. DIFP
    c. Additional examples
2. Classification of Reversible Inhibitors
    a. Competitive
    b. Uncompetitive
    c. Mixed (special case =
    d. Substrate



Mixed Inhibition



1. Always possible
2. Not possible with uncompetitive inhibitors
3. Not possible with competitive inhibitors
4. Not possible with competitive or uncompetitive inhibitors

A noncompetitive inhibitor is capable of all four reactions, but the classical noncompetitive inhibitor, as opposed to a mixed one, is a special case. With these inhibitors *Ks* and *Ks'* are equal to each other, as are *Ki* and *Ki'*

Enzyme Inhibition

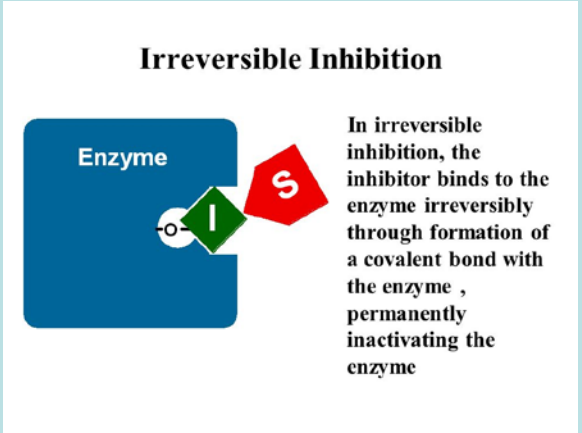
Inhibitors are compounds that decrease an enzyme's activity.

- **Irreversible inhibitors** (inactivators) react with the enzyme.
  - One inhibitor molecule can permanently shut off one enzyme molecule.
  - They are often powerful toxins but also may be used as drugs.

- **Reversible inhibitors** bind to and can dissociate from the enzyme.
  - They are often structural analogs of substrates or products.
  - They are often used as drugs to slow down a specific enzyme.

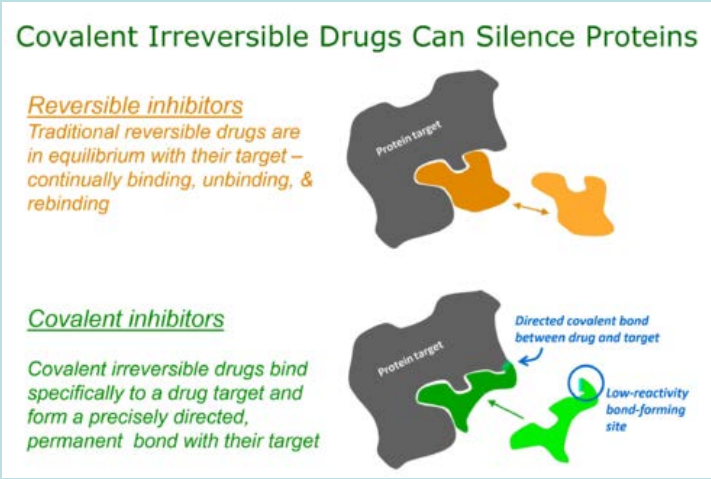
- **Reversible inhibitor** can bind to:
  - the free enzyme and prevent the binding of the substrate.
  - the enzyme-substrate complex and prevent the reaction.

1. Irreversible Inhibitor

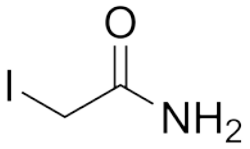


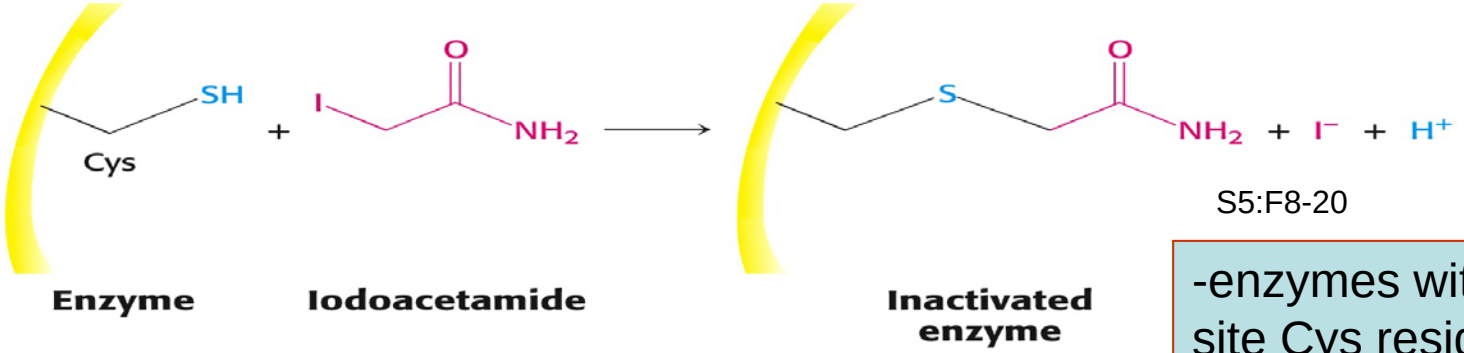
- binds to the enzyme covalently
  - at the active site or elsewhere
- kinetic effect is like that of non-competitive inhibition
  - net effect is loss of active enzyme
  - time-dependent decrease in enzyme activity as $E + I \rightarrow EI$

- cannot be removed by physical means but may be removed by chemical means
  - breaks covalent bond
- depending upon the chemical treatment
  - enzyme may regain its full initial activity
  - have partial activity
  - or be completely inactive



(a) Iodoacetamide

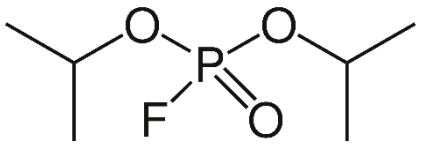


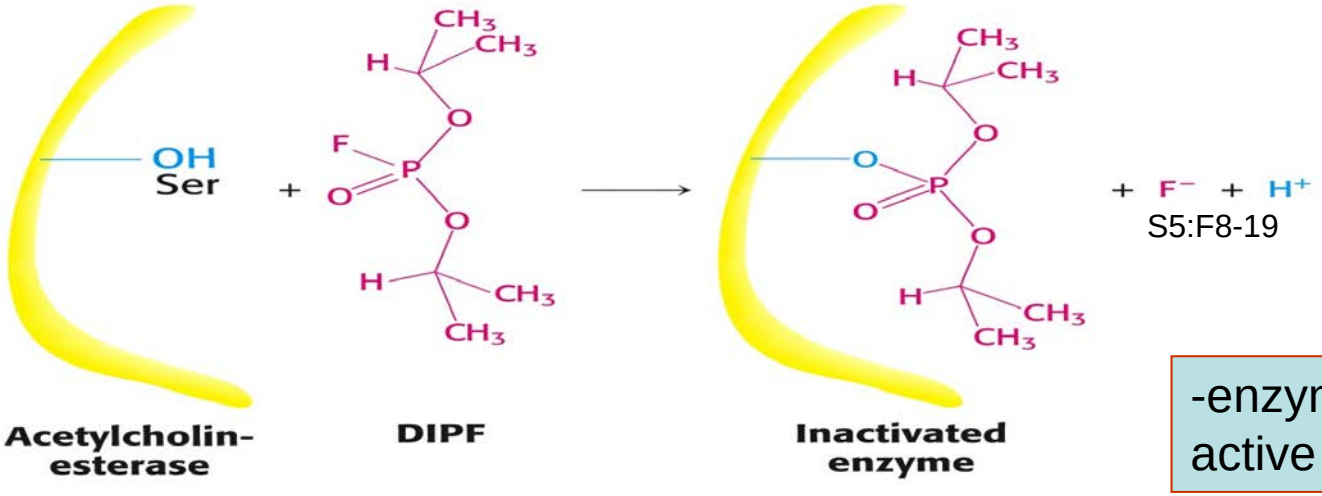


S5:F8-20

-enzymes with active site Cys residues

(b) DIPF (diisopropylfluorophosphate)

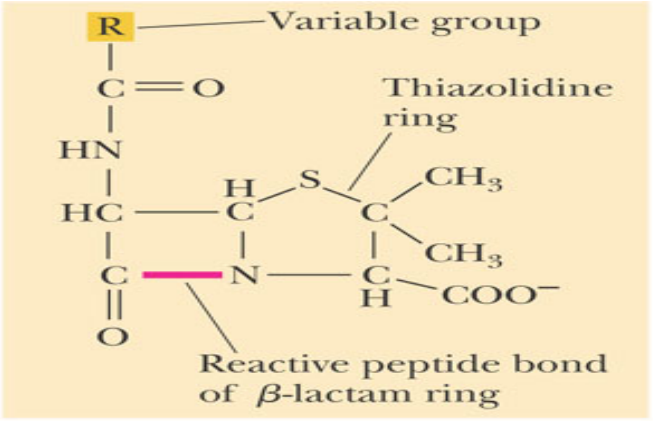




S5:F8-19

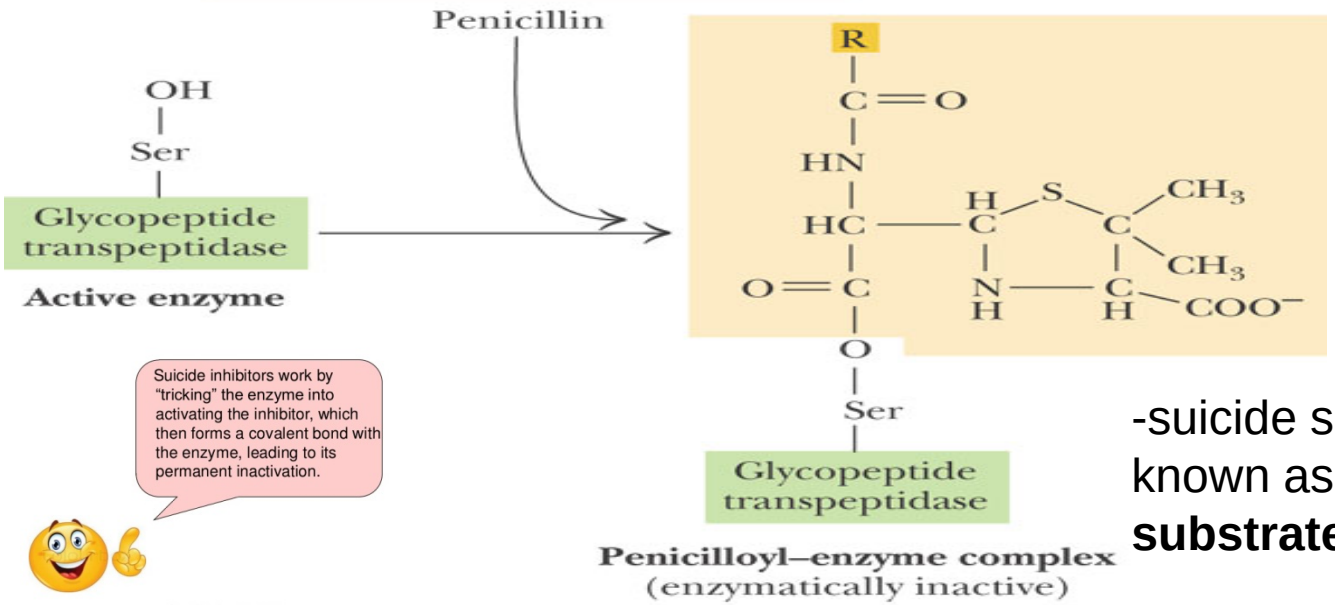
-enzymes with active site Ser

c. Suicide substrates--a substrate that when acted upon by an appropriate enzyme is **converted to product** that essentially **irreversibly inactivates** the enzyme, usually by covalent modification (normal catalytic reaction!)



-penicillin is an irreversible inhibitor of glycoprotein peptidase that is essential for bacterial cell wall synthesis

-conformation of penicillin around its reactive peptide bond resembles the TS of the normal substrate



Trojan horse

-suicide substrates are also known as **"Trojan horse substrates"**

Treating African Sleeping Sickness with a Trojan Horse Inhibitor

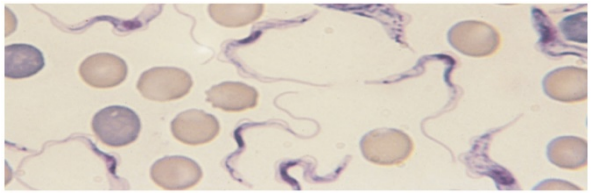
-**African sleeping sickness** is caused by protists called trypanosomes

-vaccines are ineffective because trypanosomes change surface coat proteins continuously

-vulnerable point is polyamine biosynthesis because ornithine decarboxylase (OC) catalyzes the 1st step in polyamine synthesis

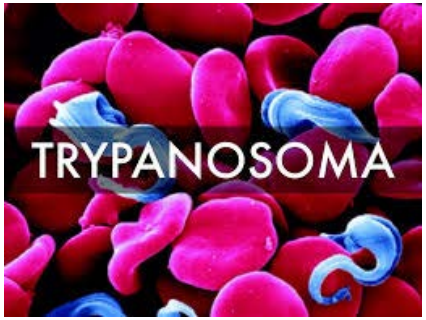
-mammalian OC has short $t_{1/2}$ but trypanosome OC is a much more stable enzyme

-reagent that inactivates OC may be able to kill trypanosomes before host cells

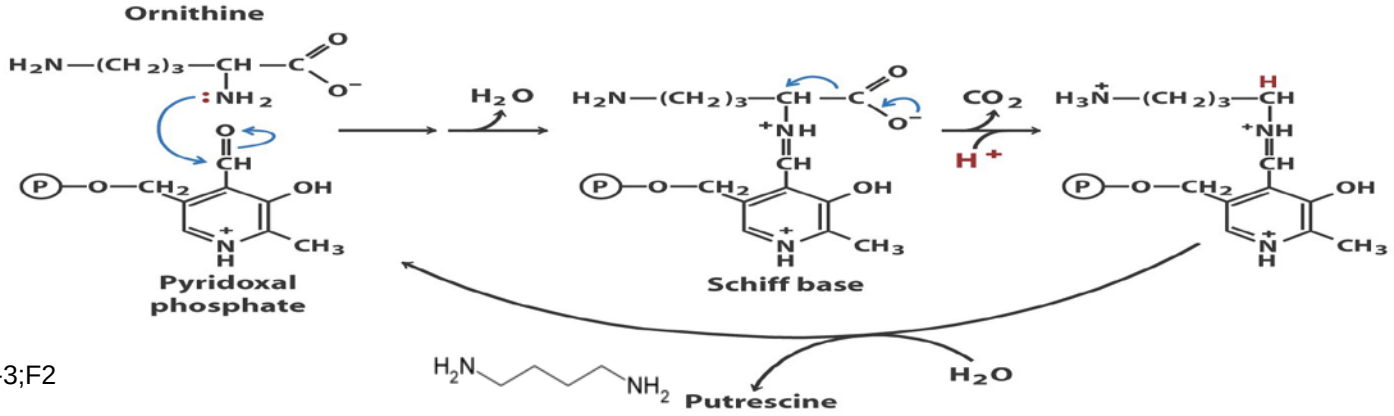


Trypanosoma brucei rhodesiense

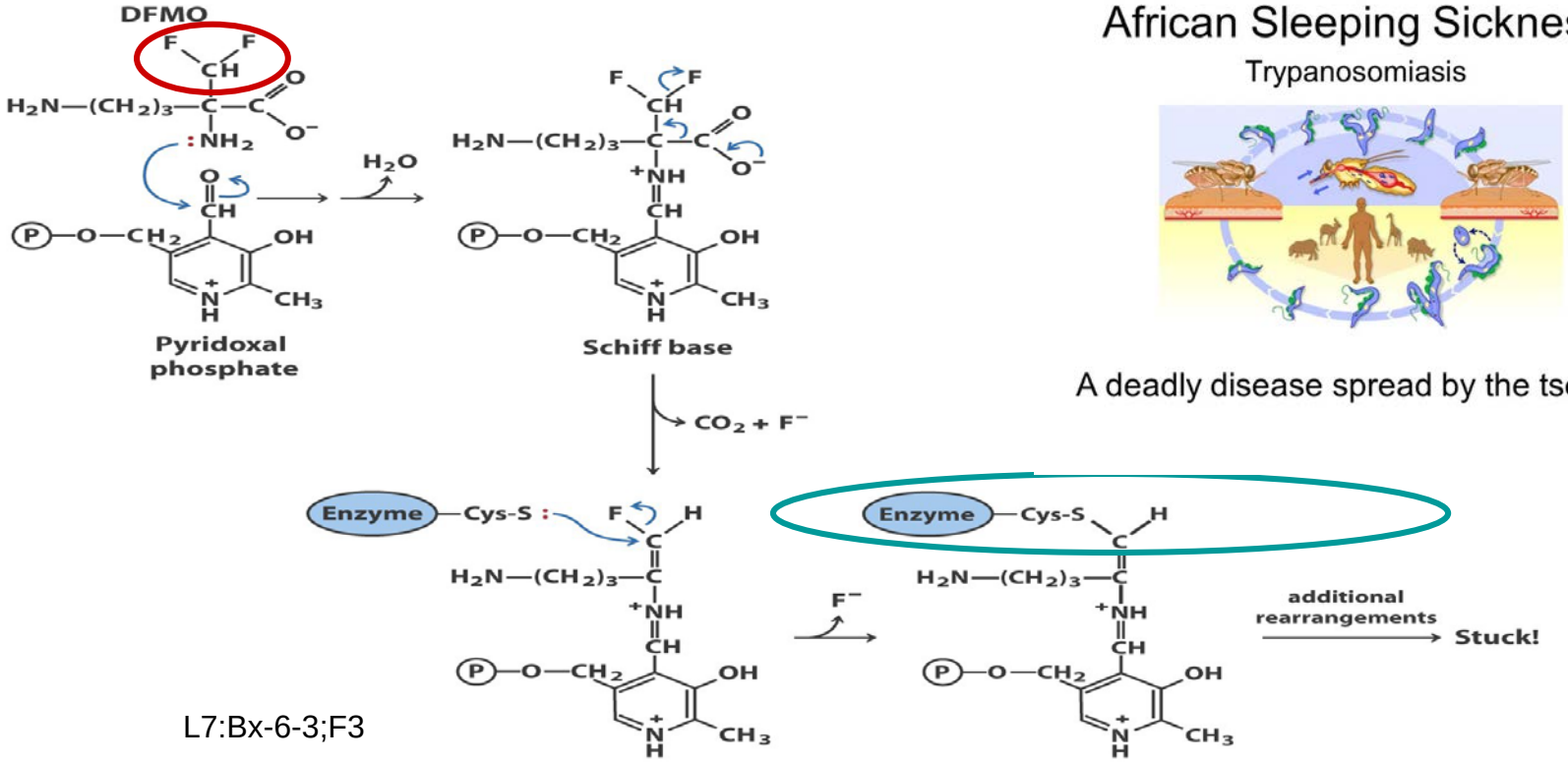
L7:Bx-6-3;F1



Tsetse fly



L7:Bx-6-3;F2



L7:Bx-6-3;F3

African Sleeping Sickness

Trypanosomiasis

A deadly disease spread by the tsetse fly.

-DFMO is a **suicide substrate** that is relatively inert in solution

-when DFMO binds to OC the enzyme is **quickly inactivated**

-inhibitor provides an alternative electron sink in 2 strategically placed **fluorine atoms**, which are excellent leaving groups

-DFMO is now used as a drug to treat African sleeping sickness

Competitive Inhibition

- Inhibitor competes with substrate for binding
  - binds active site
  - does not affect catalysis

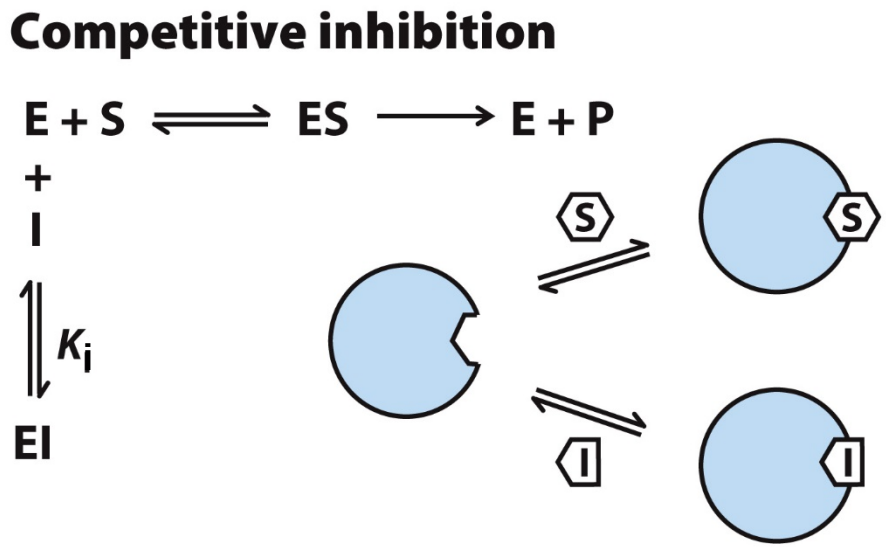


Figure 6-17a
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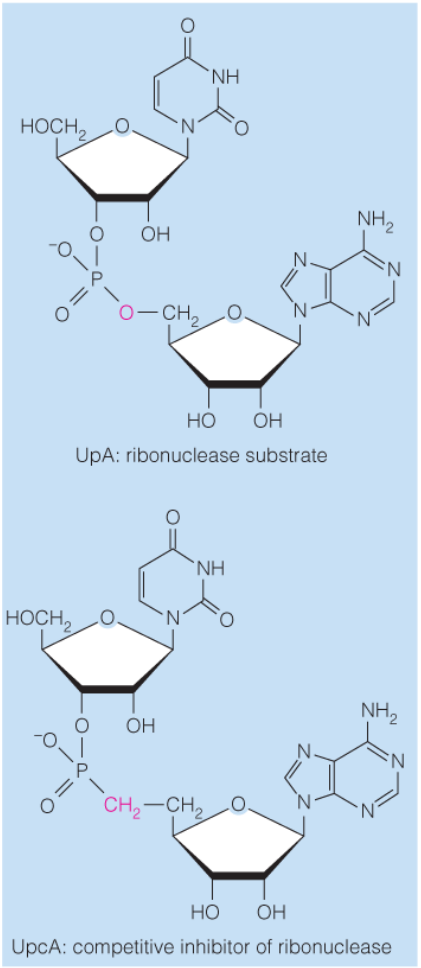


FIGURE 11.31

A substrate and its competitive inhibitor. The substrate UpA and the structurally similar molecule UpcA are competitors for the enzyme ribonuclease. The single difference between the substrate and the inhibitor is shown in magenta.

- No change in V_{max}; apparent increase in K_M
- Lineweaver-Burk: lines intersect at the *y*-axis.

Competitive Inhibition (Fingerprint)

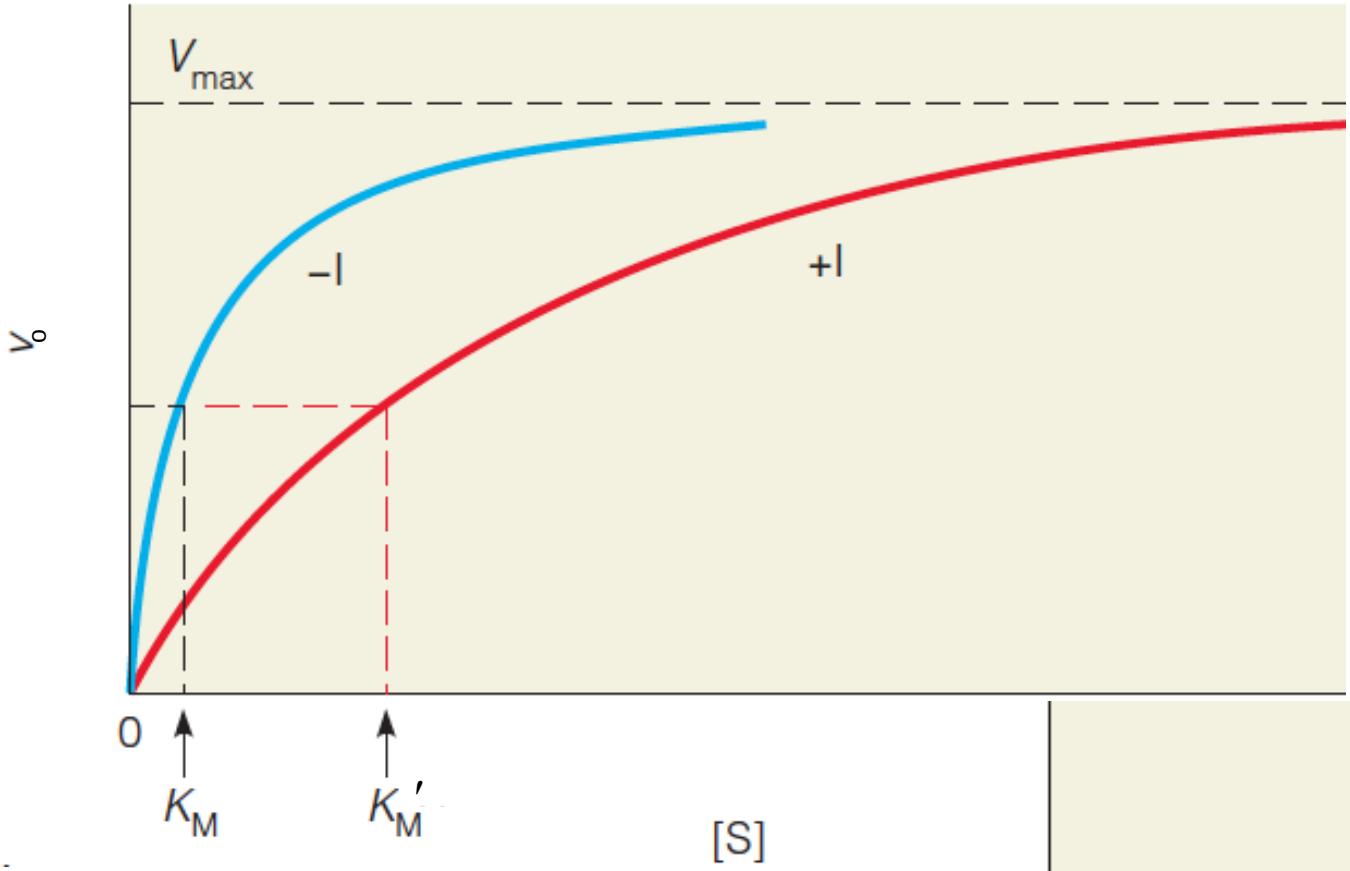
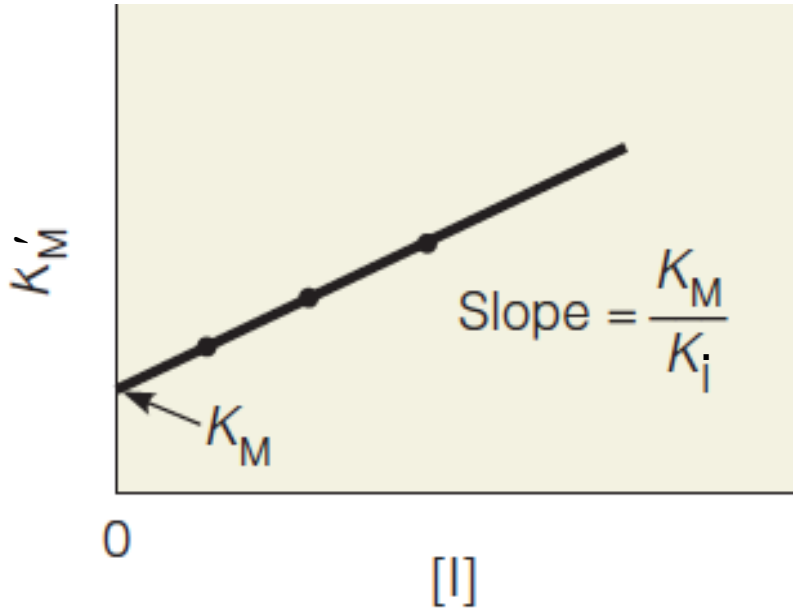


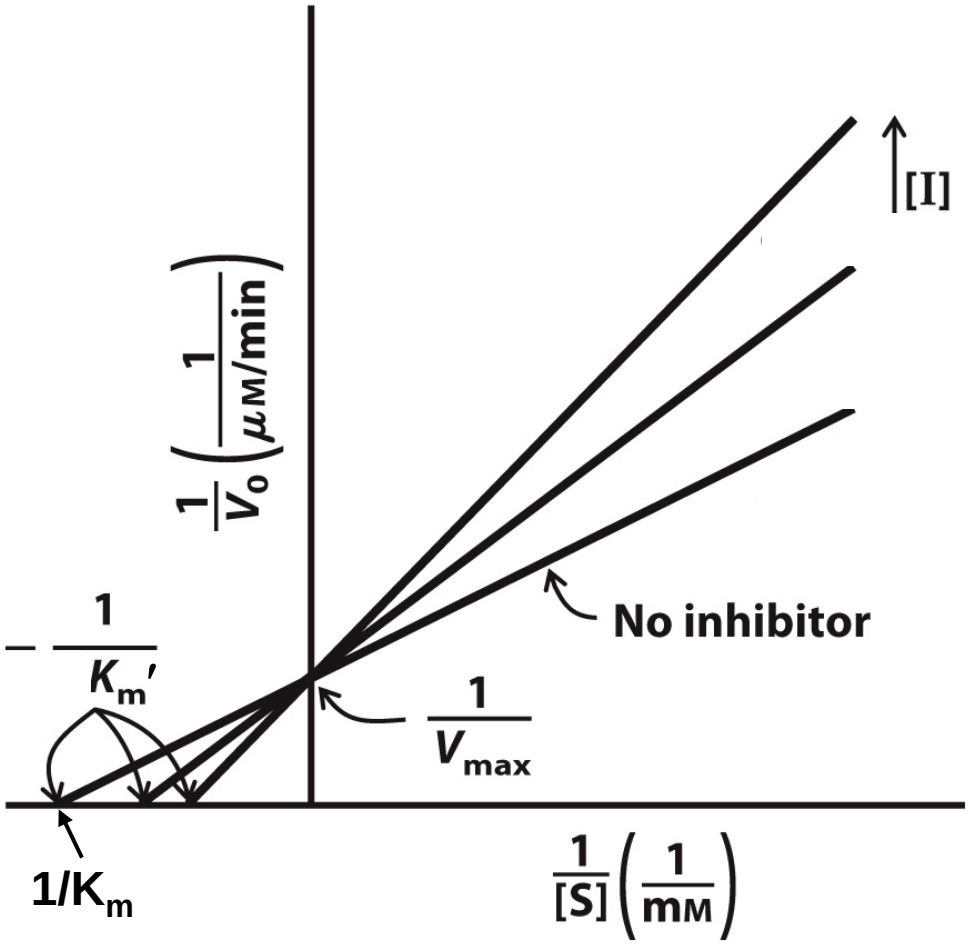
Fig. 11.30
Biochemistry
Matthews 2013



$K_M' = K_M(1 + [I]/K_i) = K_M + (K_M/K_i)[I]$

Competitive Inhibition

LB: $\frac{1}{V_0} = \left(\frac{K_m'}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$



Box 6-2 Figure 1 modified
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Michaelis-Menten Equation:

$$v_0 = \frac{V_{max}[S]}{K_M' + [S]}$$

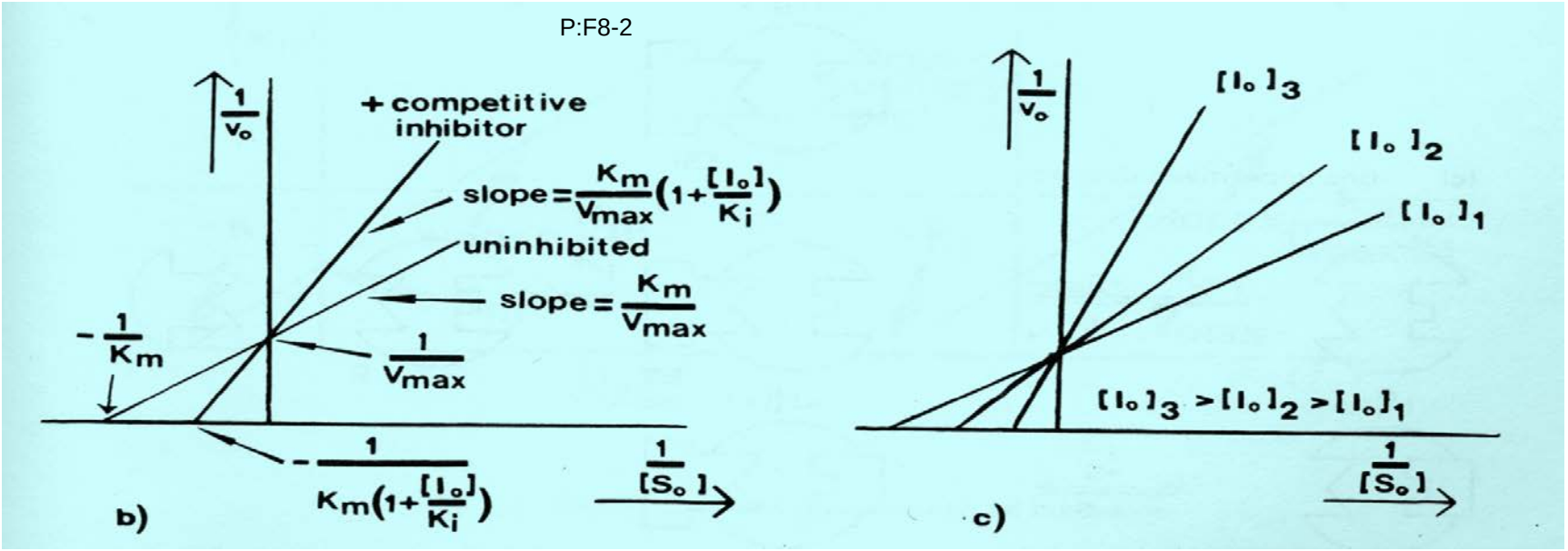
$$K_i = \frac{[E]+[I]}{[EI]}$$

K_M' = apparent K_M

$K_M' = K_M\,(1 + [I]/K_i)$

K_M changes (increases) but V_{max} does not change

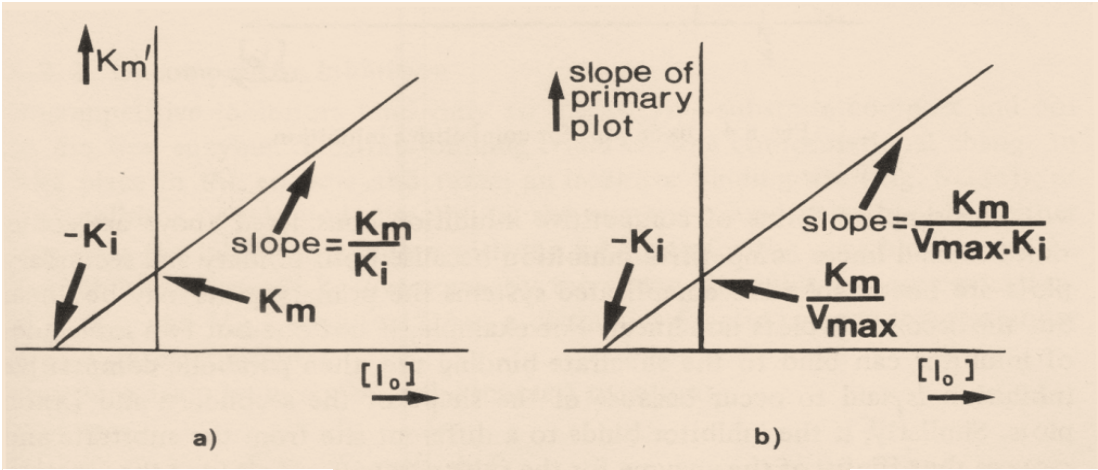
P:F8-2



-to study inhibition and to determine K_i, kinetic studies involve varying $[S_0]$ at several inhibitor concentrations ($[I_0]$)

Secondary Plots

•Graphs of the intercept or slope of the primary plot against $[I_o]$ will also be linear, the intercept on the $[I_o]$ axis giving $-K_i$



Secondary plots for competitive inhibition.

P:F8-3

Dixon Plot

•Alternative graphical means of calculating K_i suggested by Dixon (1953)

$$\frac{1}{v_0} = \frac{K_M}{V_{\max}[S_0]} \cdot \frac{[I_0]}{K_i} + \frac{K_M}{V_{\max}[S_0]} + \frac{1}{V_{\max}}$$

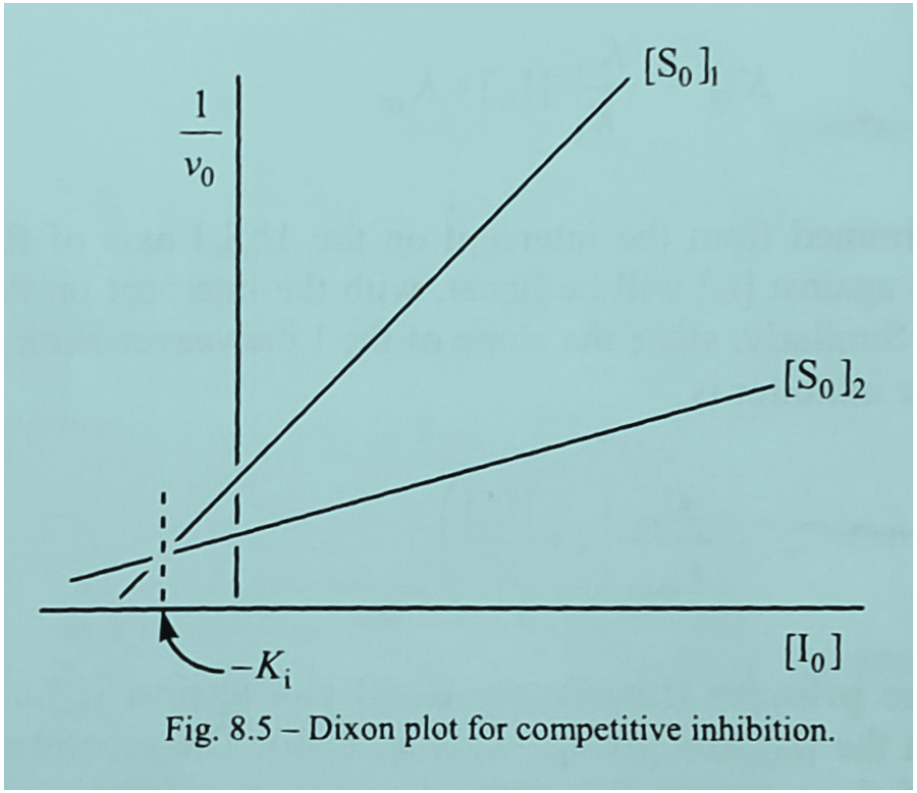
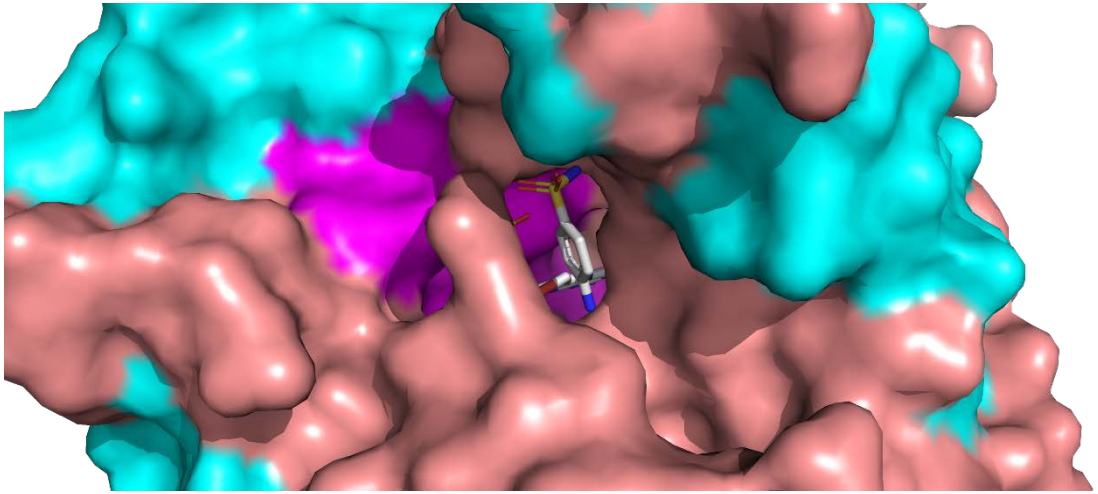
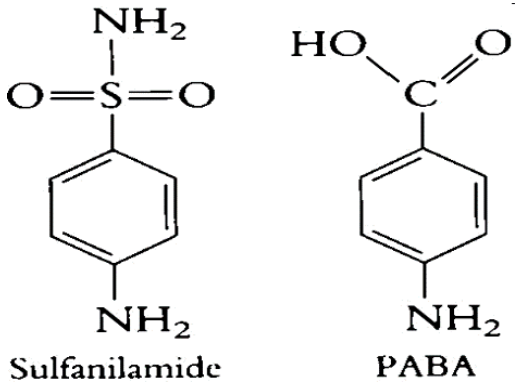


Fig. 8.5 – Dixon plot for competitive inhibition.

P:F8.5

- At fixed $[S_o]$, a plot of $1/v_o$ against $[I_o]$ is linear
- When $[I_o] = -K_i$, then $1/v_0 = 1/V_{max}$ and is independent of $[S_o]$
- Dixon plots for different $[S_o]$ values (fixed $[E_o]$) intersect where $[I_o] = -K_i$





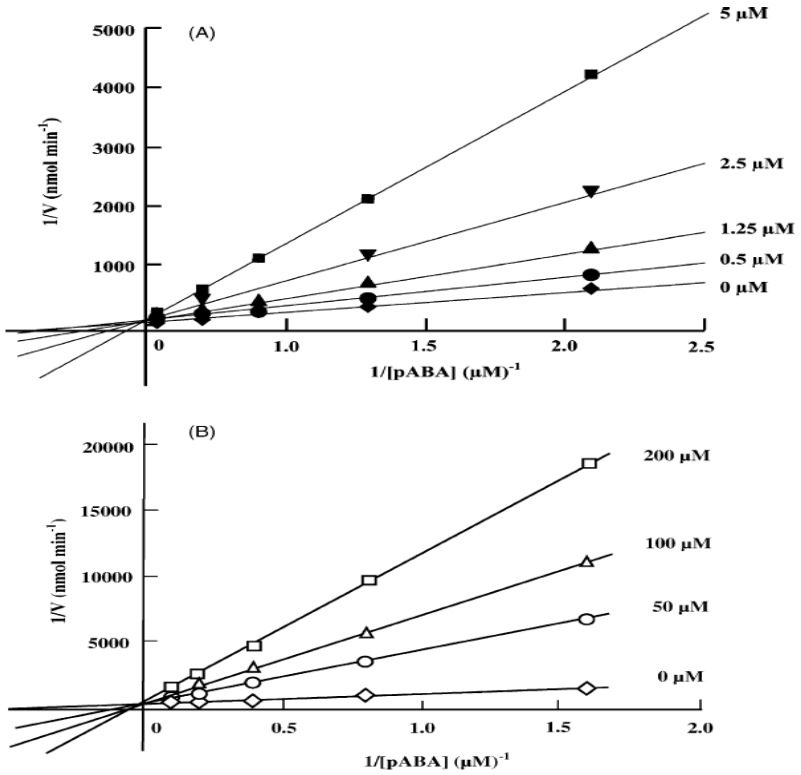
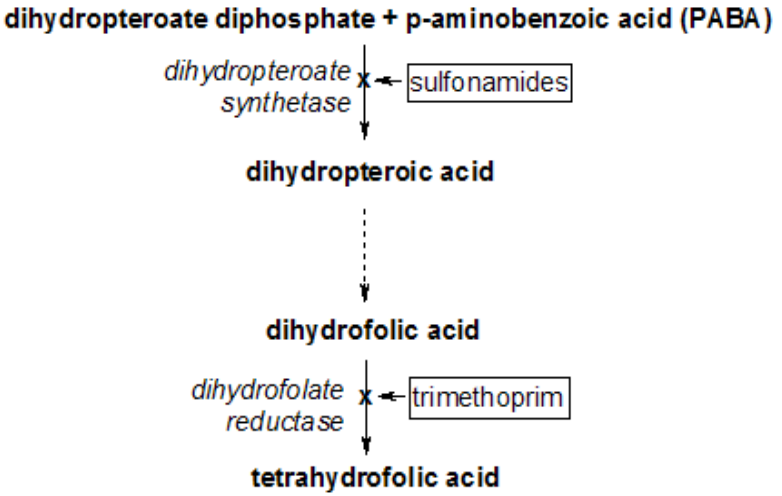


Fig. 5. Lineweaver–Burk plots of *pf*PPPK-DHPS inhibited by sulfa inhibitors. The assays were performed as described in the text in the presence of varying concentrations of *p*ABA while keeping the concentration of H_2PtCH_2OH constant at 20 µM: (A) sulfathiazole and (B) sulfadoxine. The concentrations of the inhibitors (µM) are as specified.



Uncompetitive Inhibition

- Inhibitor only binds to ES complex
  - does not affect substrate binding
  - inhibits catalytic function

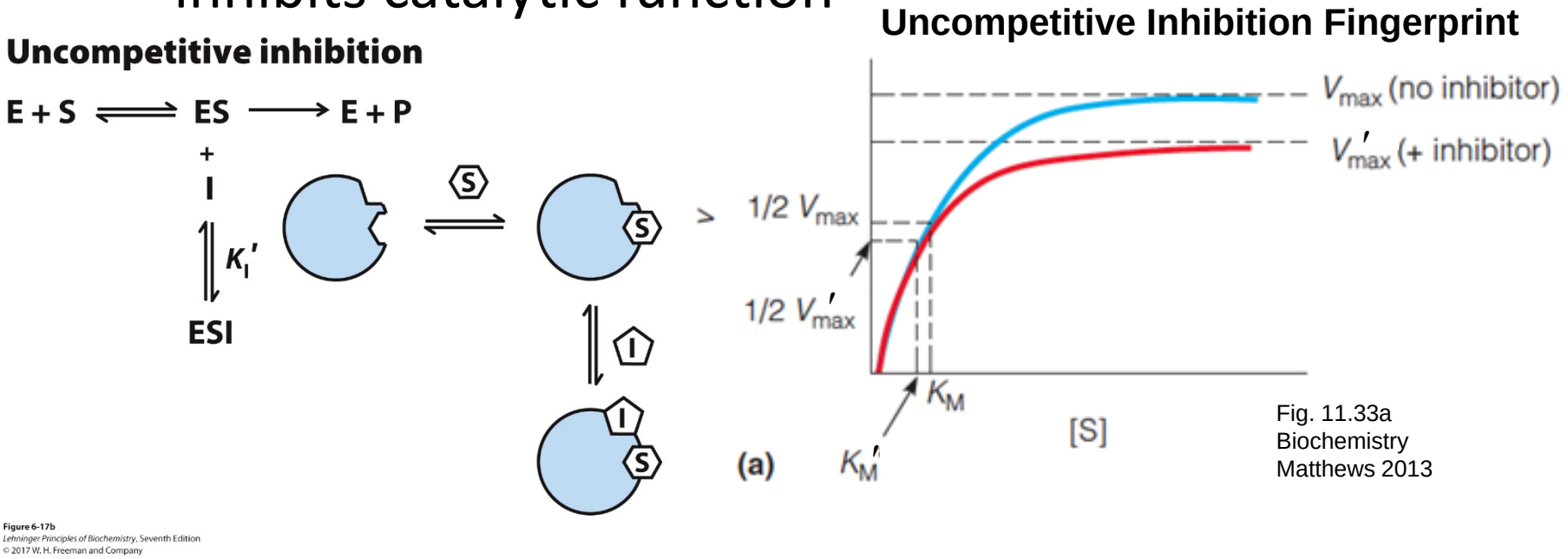


Figure 6-17b
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Fig. 11.33a
Biochemistry
Matthews 2013

- Decrease in V_{max}; apparent decrease in K_M
- No change in K_M/V_{max}
- Lineweaver-Burk: lines are parallel.

Uncompetitive Inhibition

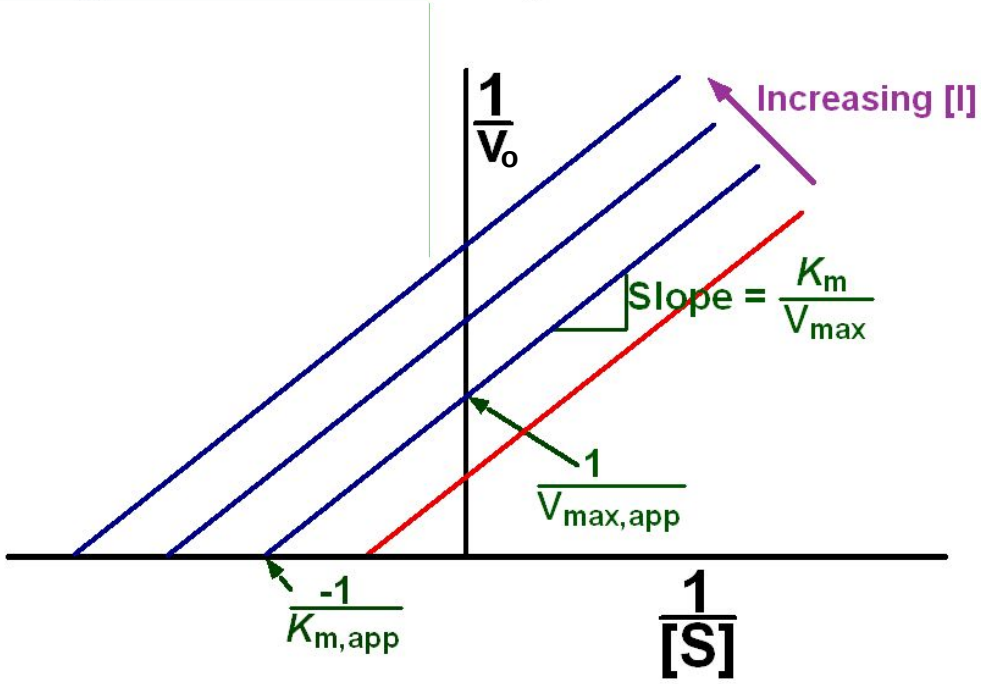
LB: $\frac{1}{v_0} = \frac{K_M'}{V_{max}'}\left(\frac{1}{[S]}\right) + \frac{1}{V_{max}'}$

Michaelis-Menten Equation:

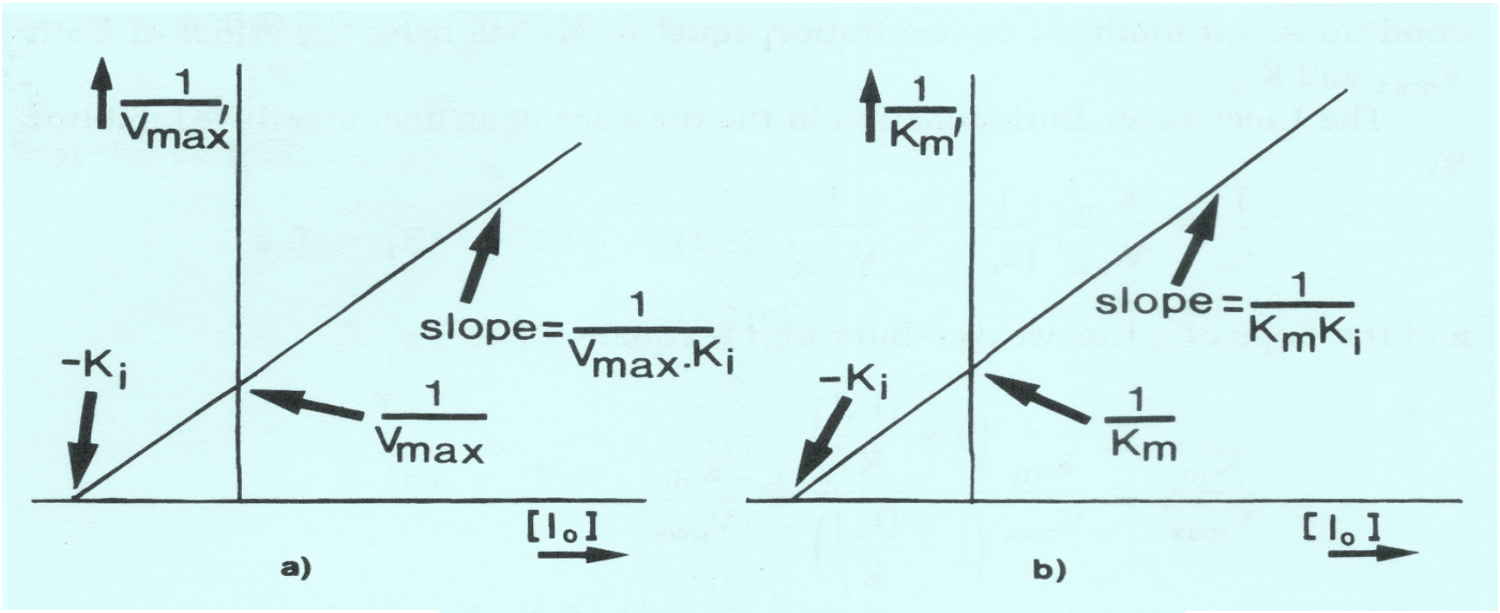
$$v_0 = \frac{V_{max}'[S]}{K_M' + [S]}$$

$$K_I = \frac{[ES]+[I]}{[ESI]}$$

The Lineweaver-Burk plot is diagnostic for uncompetitive inhibition



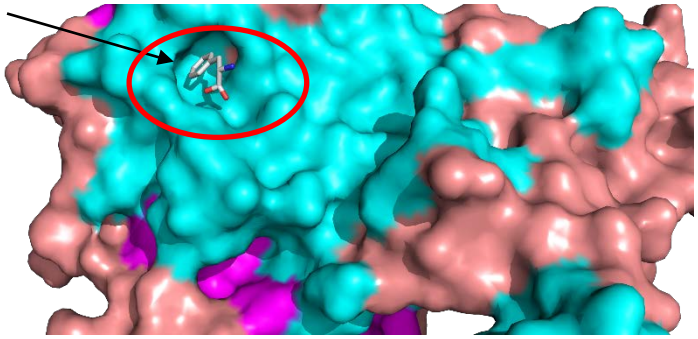
- both K_M and V_{max} are apparent values (changed)
  - both are smaller than uninhibited values
- $V_{max}' = V_{max}/1 + [I]/K_I$
- $K_M' = K_M/ 1 + [I]/K_I$
- K_M/V_{max} is unchanged so lines are parallel



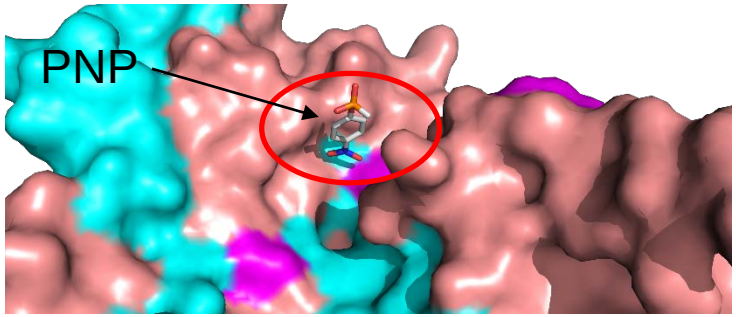
Secondary plots for uncompetitive inhibition.

P:F8-7

L-Phe

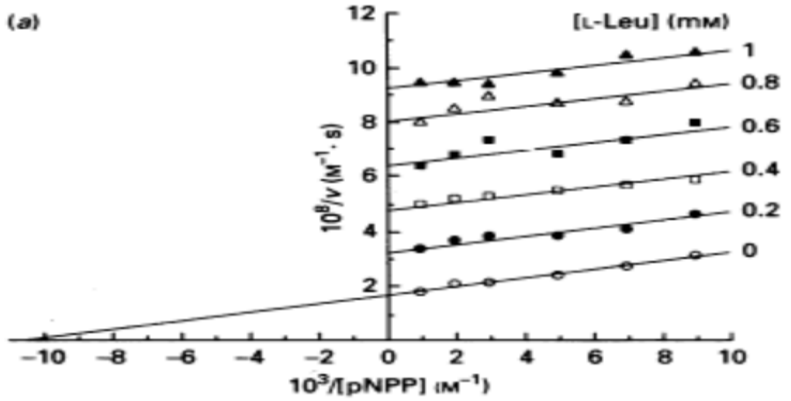


PNP



Biochem. J. (1992) 286:23-30

M. F. Hoylaerts, T. Manes and J. L. Millán



Mixed Inhibition

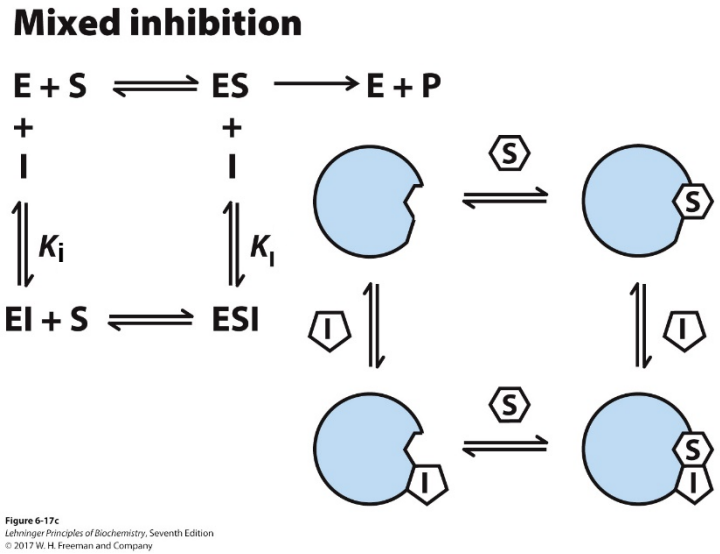


Figure 6-17c
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- Binds enzyme with or without substrate
  - binds to regulatory site
  - inhibits both substrate binding and catalysis
- Decrease in V_{max}; apparent change in K_M
- Lineweaver-Burk: lines intersect left from the *y*-axis.
- **Special case:** Noncompetitive inhibitors are mixed inhibitors such that there is no change in K_M

Non-competitive Inhibition

Non-competitive Inhibition:

- special case of mixed inhibition where $K_i = K_I$
- V_{max} changes but not K_M
- Enzyme, S and E-S are in rapid equilibrium

- inhibitor binds to E and to ES but does **not affect** substrate binding

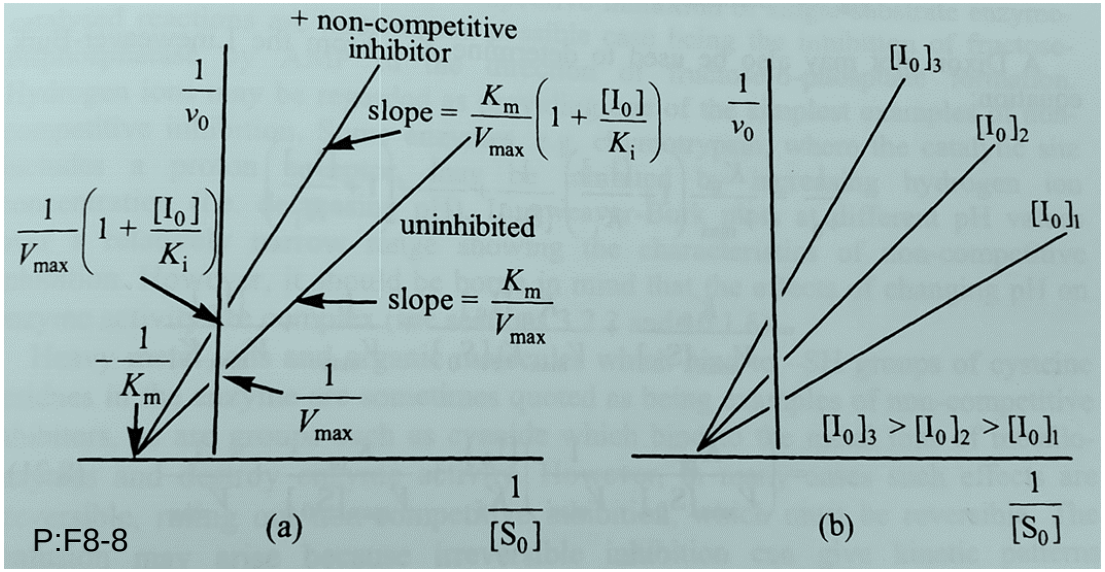
Michaelis-Menten Equation:

$$v_0 = \frac{V_{max}'[S]}{K_M + [S]}$$

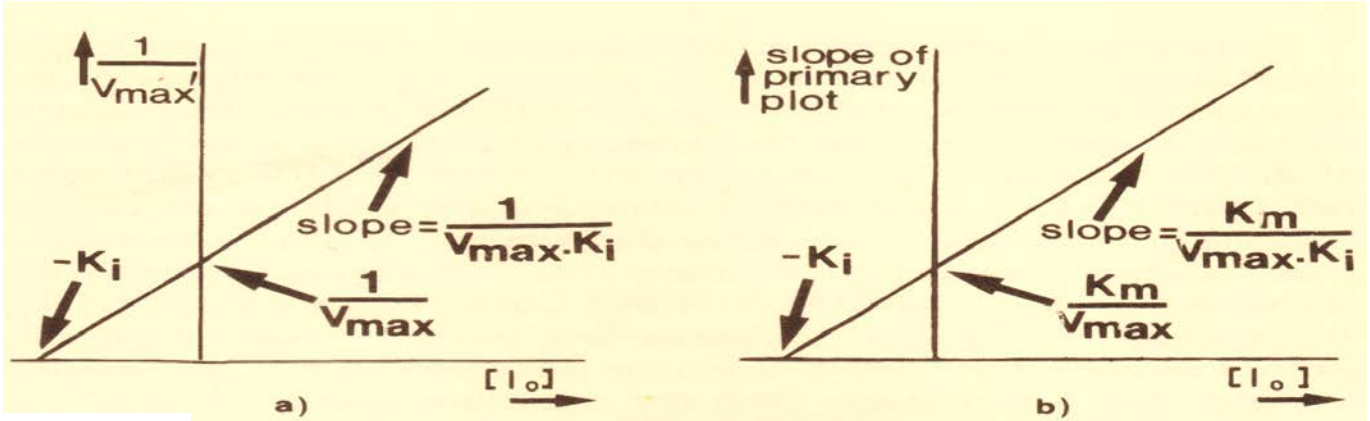
$$Ki = \frac{[E][I]}{[EI]} \quad \text{and} \quad K_I = \frac{[ES]+[I]}{[ESI]}$$

- $V_{max}' = V_{max}/1 + [I]/K_I$

LB: $\frac{1}{v_0} = \frac{K_M}{V_{max}'}\left(\frac{1}{[S]}\right) + \frac{1}{V_{max}'}$



P:F8-8



P:F8-9 Secondary plots (at constant $[E_o]$) for simple linear non-competitive inhibition.

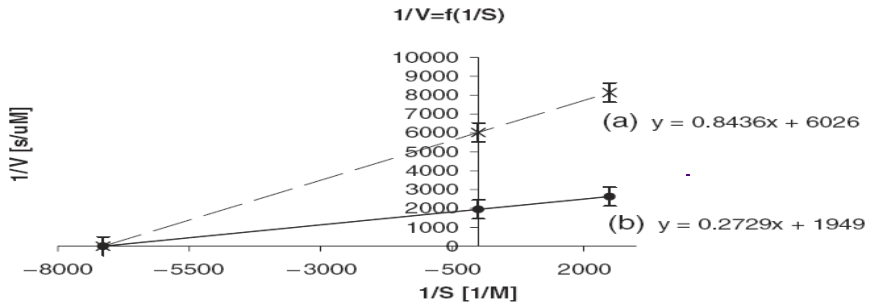
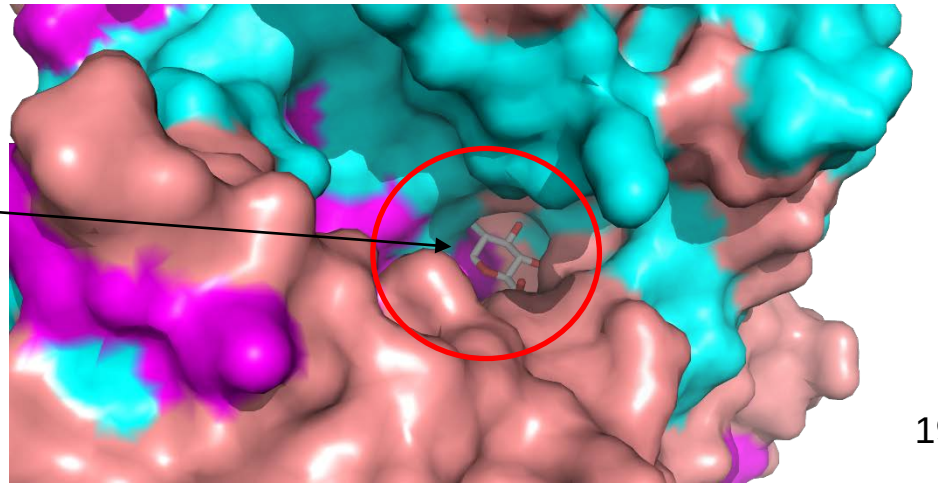


Fig. 6. Kinetics of ONPG hydrolysis by soluble Antarctic β-galactosidase in the presence (a) and absence (b) of glucose.

Makowski et al. (2006)

FEMS Microbiol. **59**, 535-542.

2-deoxy-2-fluoro-D-glucose



Substrate inhibition

- binding of second substrate molecule to the E results in formation of an inactive complex.

$$E + S \Leftrightarrow ES \rightarrow E + P$$
$$+S \downarrow\uparrow -S$$
$$SES$$

-an $[S]^2$ term in the rate equation means that it isn't Michaelis-Menten

-can be detected by non-linearity on standard plots

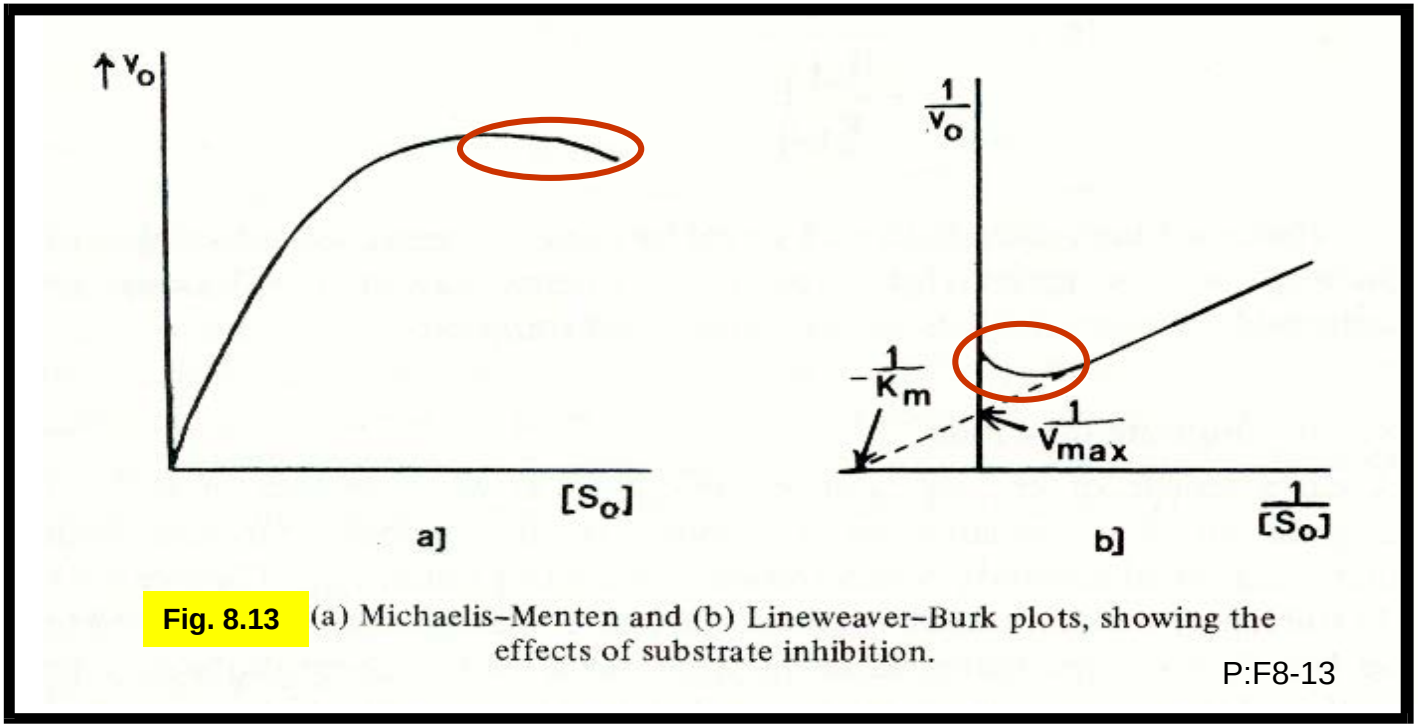
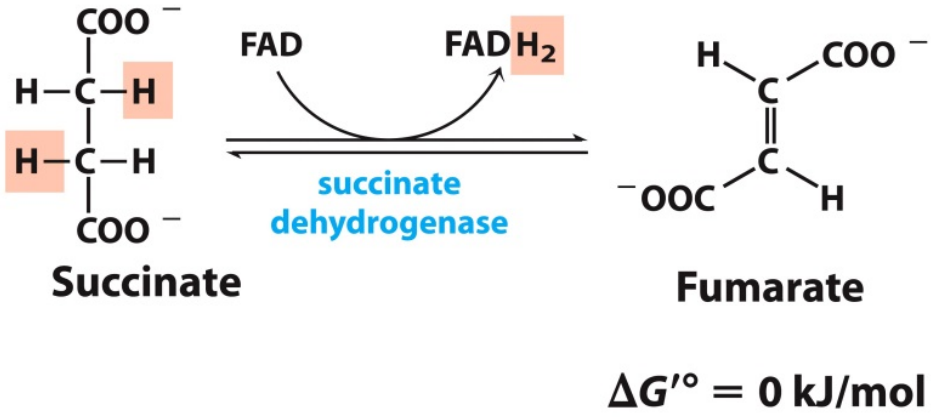


Fig. 8.13 (a) Michaelis–Menten and (b) Lineweaver–Burk plots, showing the effects of substrate inhibition.

P:F8-13

- succinate dehydrogenase shows substrate inhibition with its **succinate** substrate
- at high succinate concentrations above the K_M , the v_o starts to decrease with time
- often caused by active-site crowding



-common occurrence so maximum **amount of substrate** that can be added to assay must be determined