Chapter 4: Enzyme Kinetics

Purpose:

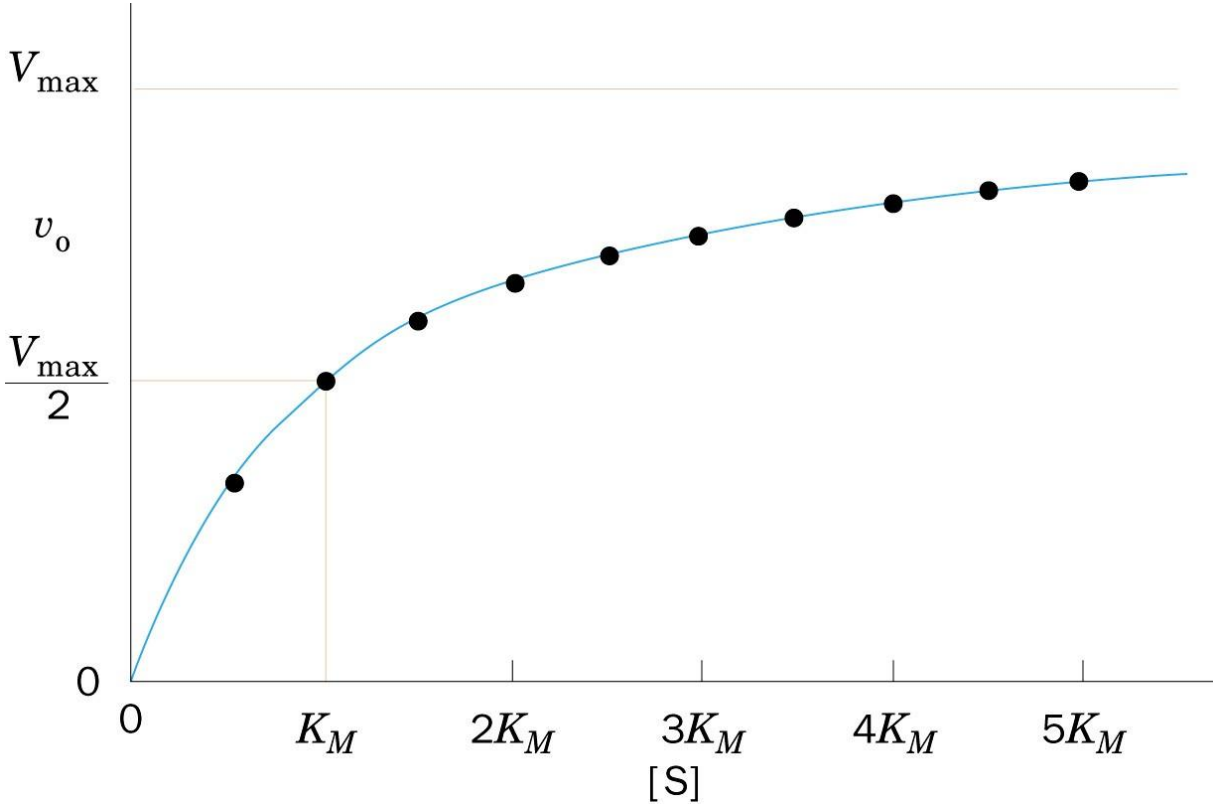
1) Investigate the kinetics of LDH purified from bovine heart and muscle

2) Learn how to determine kinetic information

3) Understand the effects of inhibitors on enzyme activity

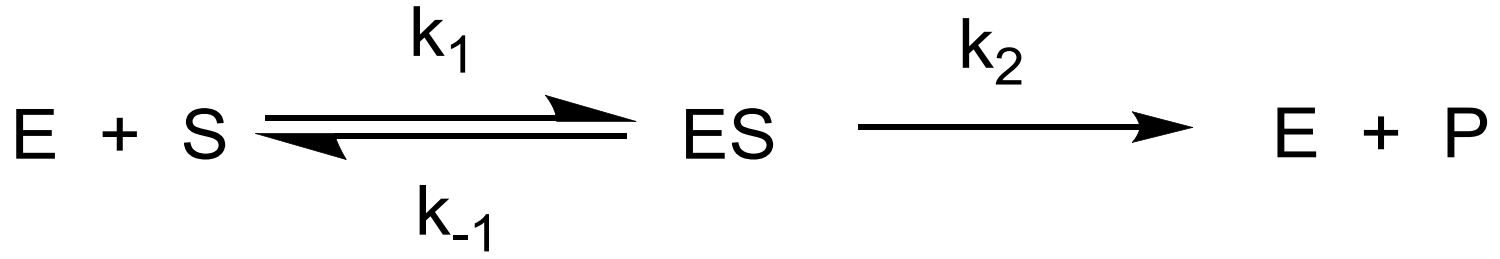
Enzyme Kinetics

- Rate of enzyme catalyzed reaction depends on substrate concentration
- Want to measure initial rate, $\boldsymbol{V_o}$ – [E] low, [S] high
- As [S] increases, V_o increases to certain point and then levels off – $\boldsymbol{V_{max}}$

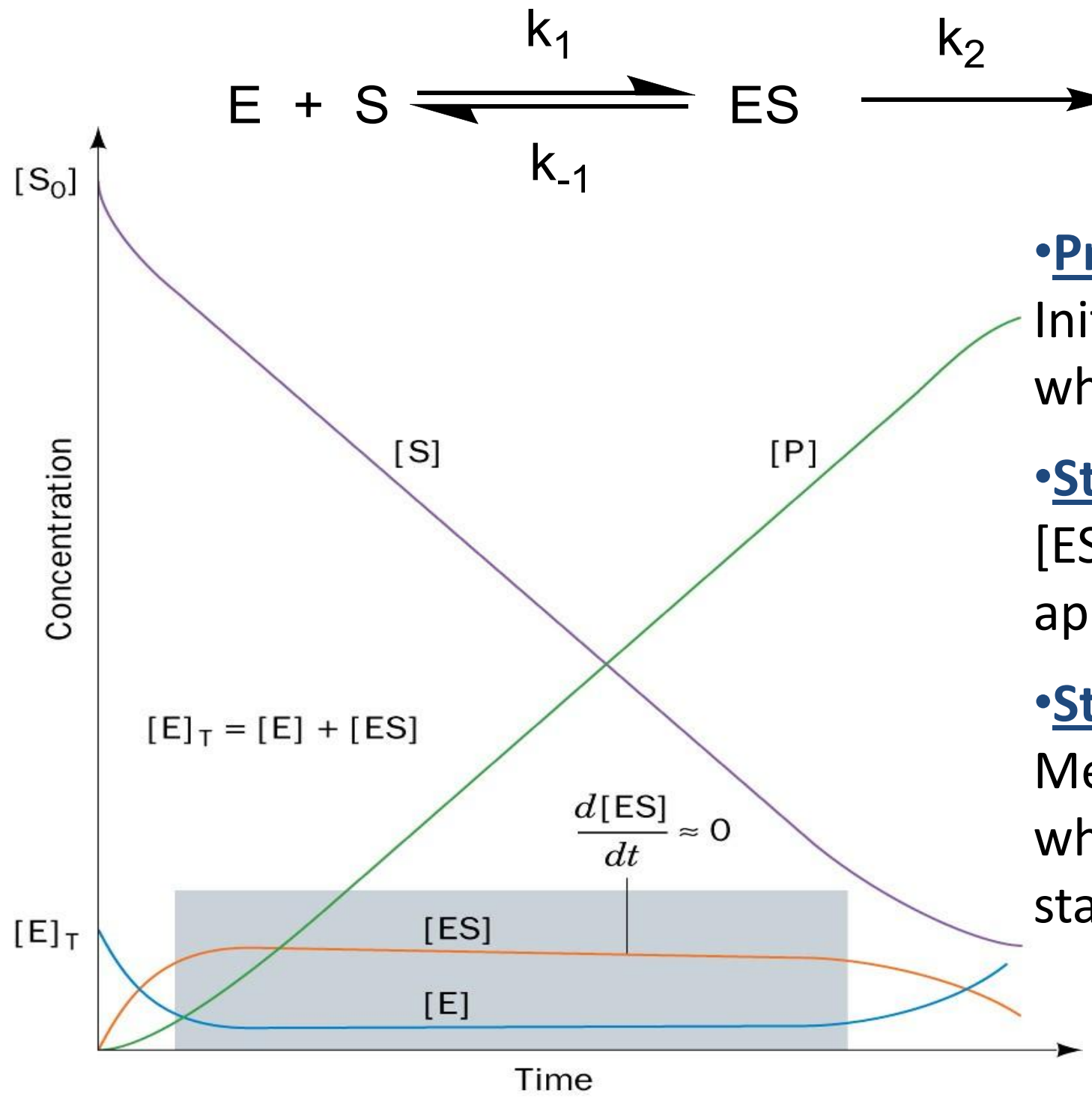


Michaelis-Menton Mechanism for Enzyme Action

- 1st Step: Fast reversible binding of Enzyme to Substrate (**Enzyme-Substrate complex**)
- 2nd Step: Slower breakdown of the ES complex to Enzyme + Product



- At any time during reaction the enzyme is present as both E and ES
- Maximal rate (V_{max}) observed when [ES] is highest, and [E] is lowest
  - **Enzyme is saturated with substrate**



- **Pre-Steady State**

Initial mixing of E + S, while [ES] builds up

- **Steady-State**

[ES] remains approximately constant

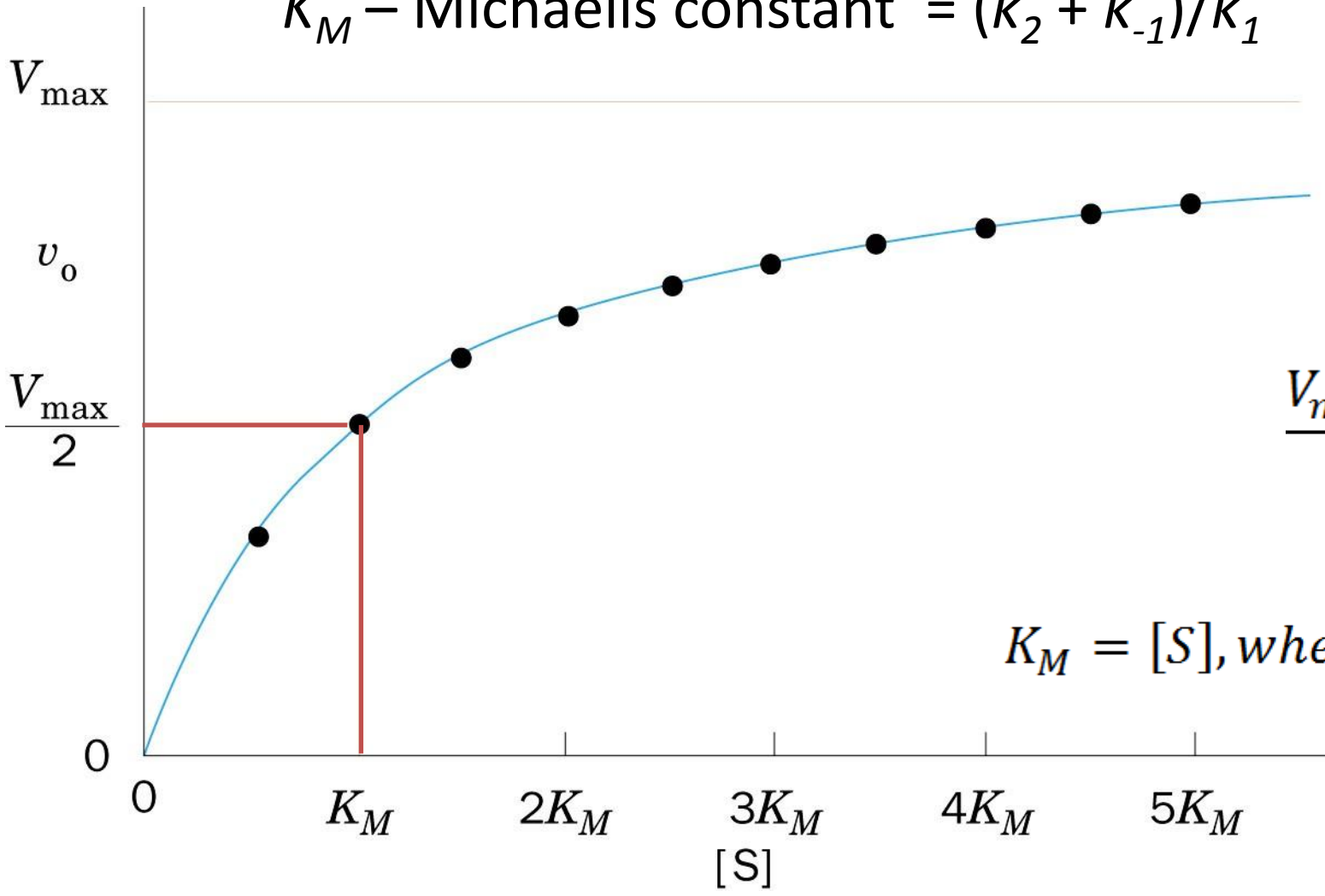
- **Steady-State Kinetics**

Measurements of V_o while [ES] is relatively stable

Michaelis-Menton Kinetics

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

K_M – Michaelis constant $= (k_2 + k_{-1})/k_1$



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

$$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_M + [S]}$$

$$K_M = [S], when\ V_o = \frac{1}{2}V_{max}$$

Lineweaver-Burk Manipulation

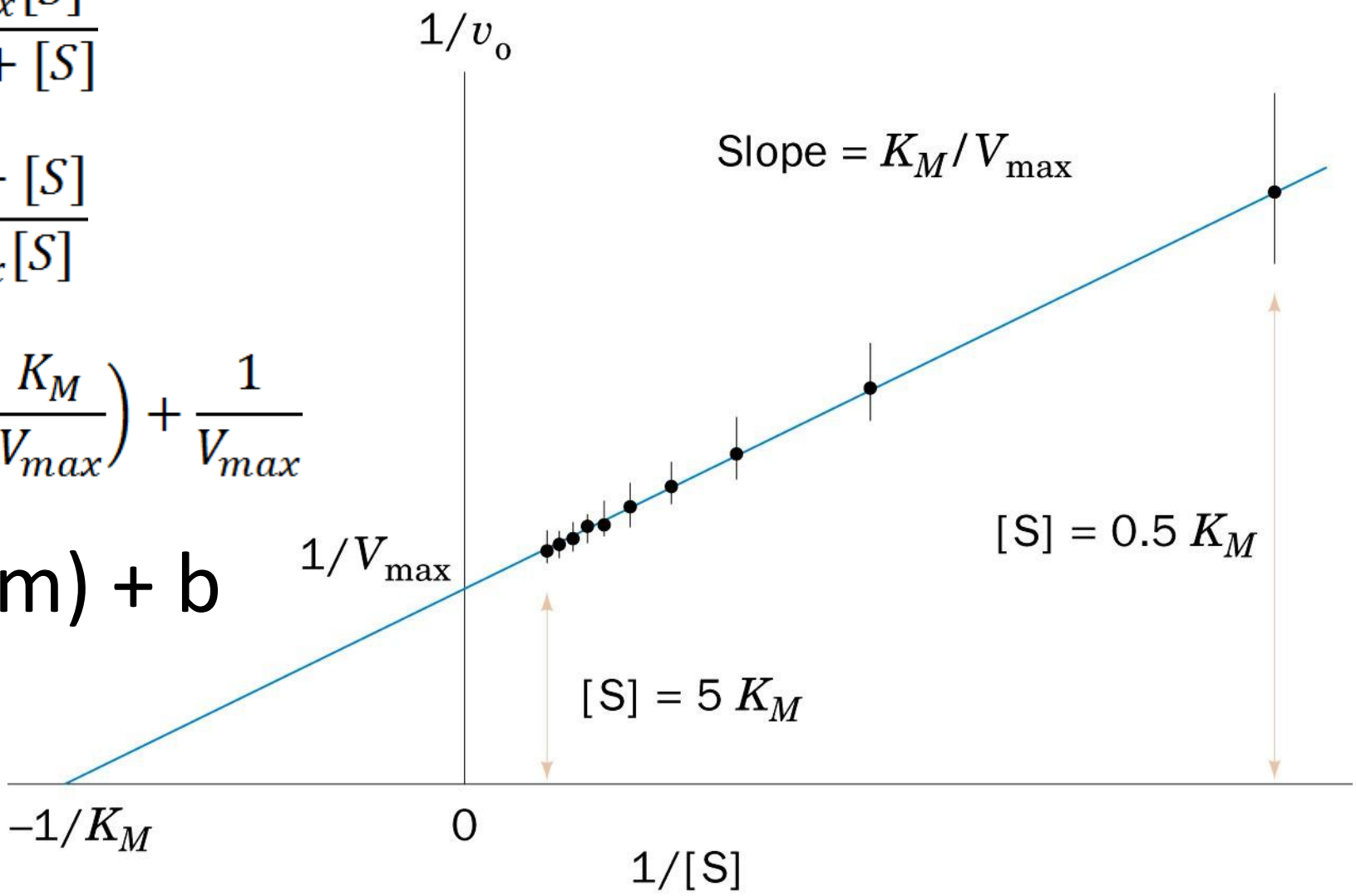
$$V_o = \frac{V_{max}[S]}{K_M + [S]}$$

$$\frac{1}{V_o} = \frac{K_M + [S]}{V_{max}[S]}$$

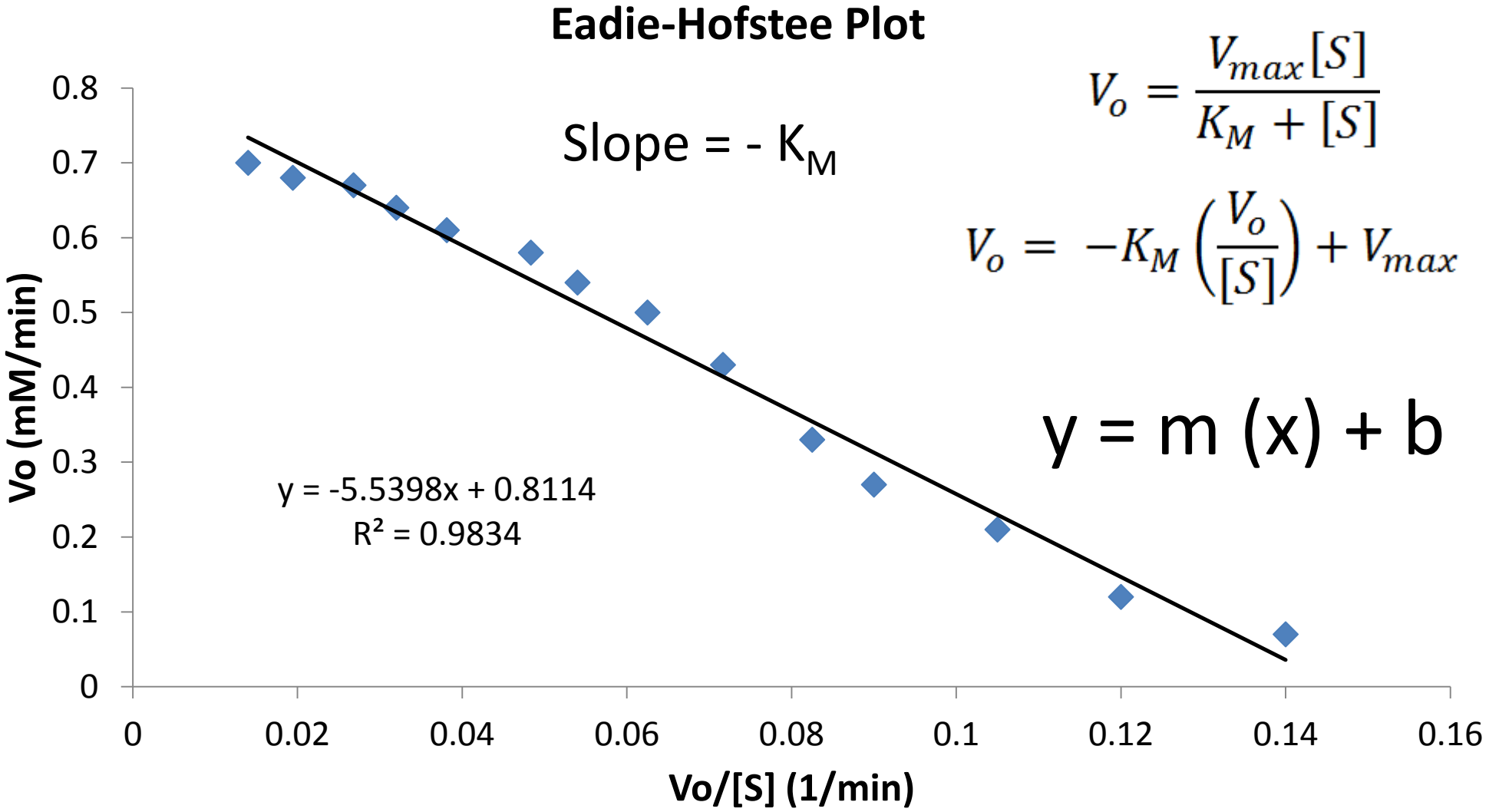
$$\frac{1}{V_o} = \frac{1}{[S]}\left(\frac{K_M}{V_{max}}\right) + \frac{1}{V_{max}}$$

y = x (m) + b

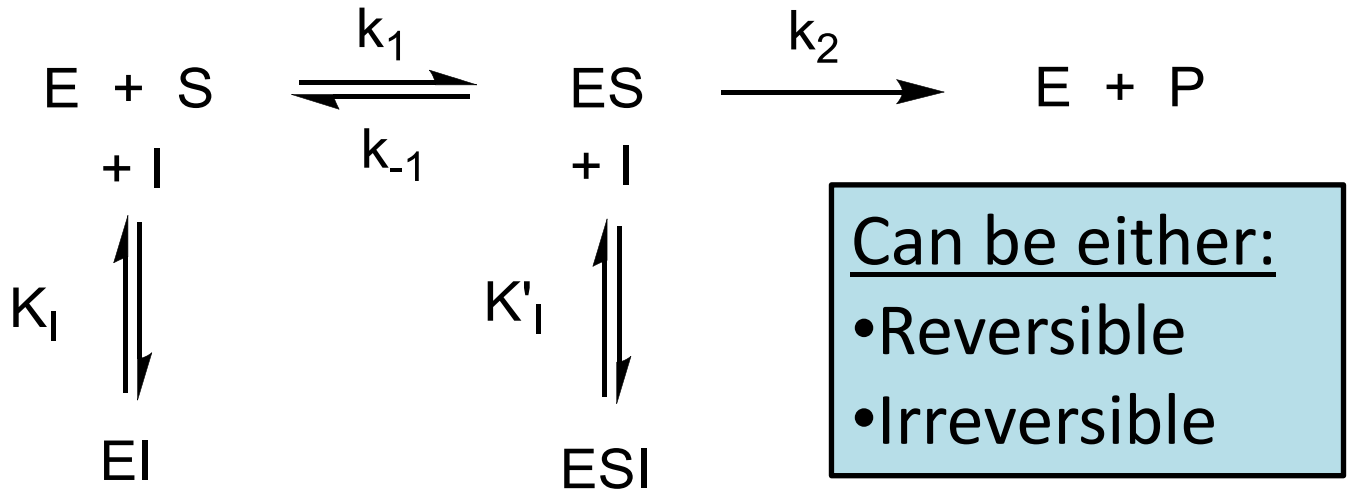
Double-Reciprocal Plot



Eadie-Hofstee Manipulation



Introduction of an Inhibitor



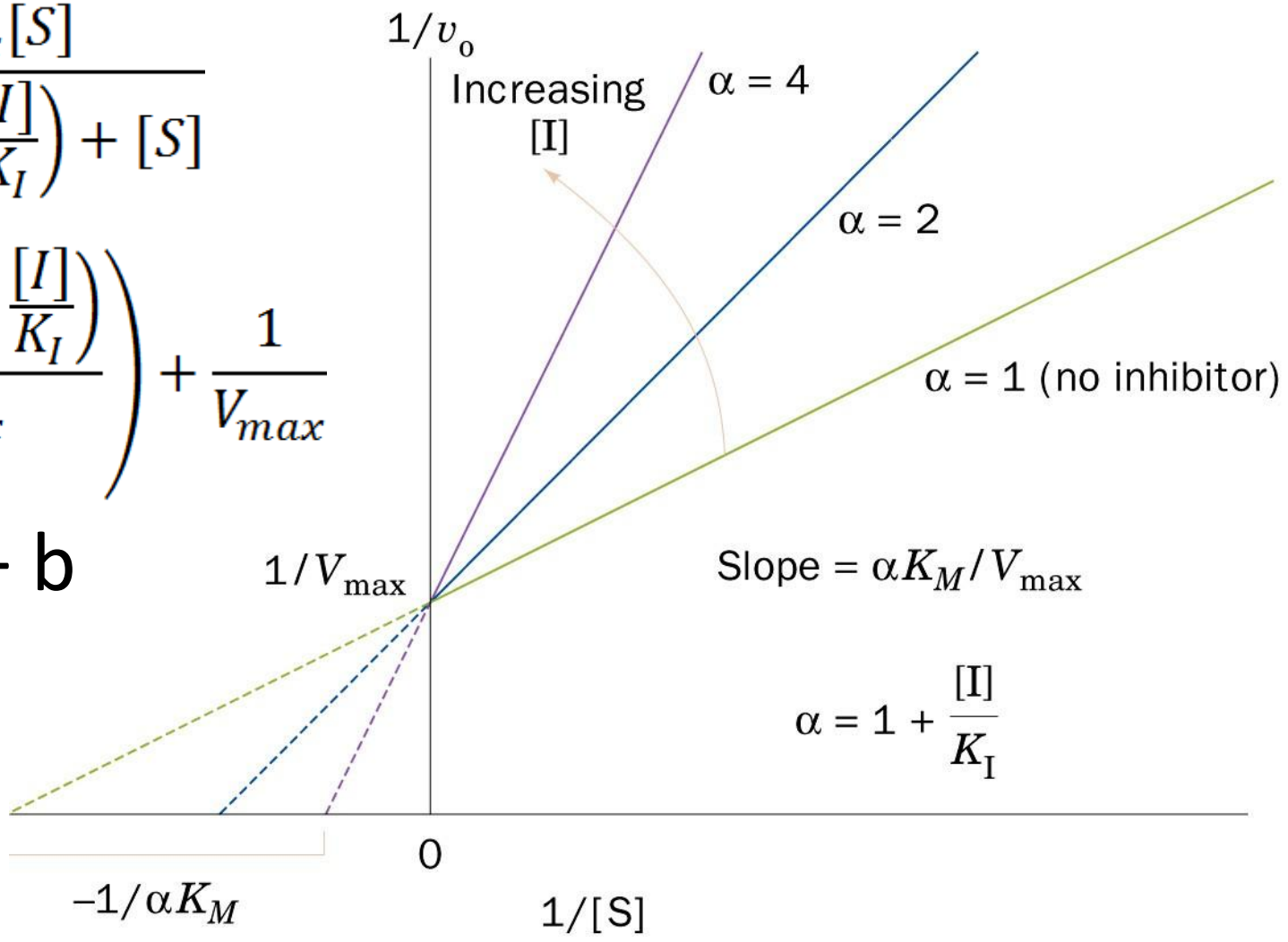
- **Competitive Inhibition** – Competes with substrate for active site
- **Uncompetitive Inhibition** – Binds to distinct site from substrate active site and binds only to ES complex
- **Non-Competitive Inhibition (Mixed)** – Binds to both substrate active site and distinct site
- **Pure Non-Competitive Inhibition** – Binds to a distinct site on the enzyme complex that decreases overall activity

Competitive Inhibition

$$V_o = \frac{V_{max}[S]}{K_M\left(1 + \frac{[I]}{K_I}\right) + [S]}$$

$$\frac{1}{V_o} = \frac{1}{[S]}\left(\frac{K_M\left(1 + \frac{[I]}{K_I}\right)}{V_{max}}\right) + \frac{1}{V_{max}}$$

y = x (m) + b



See pp. 99-100 for equations

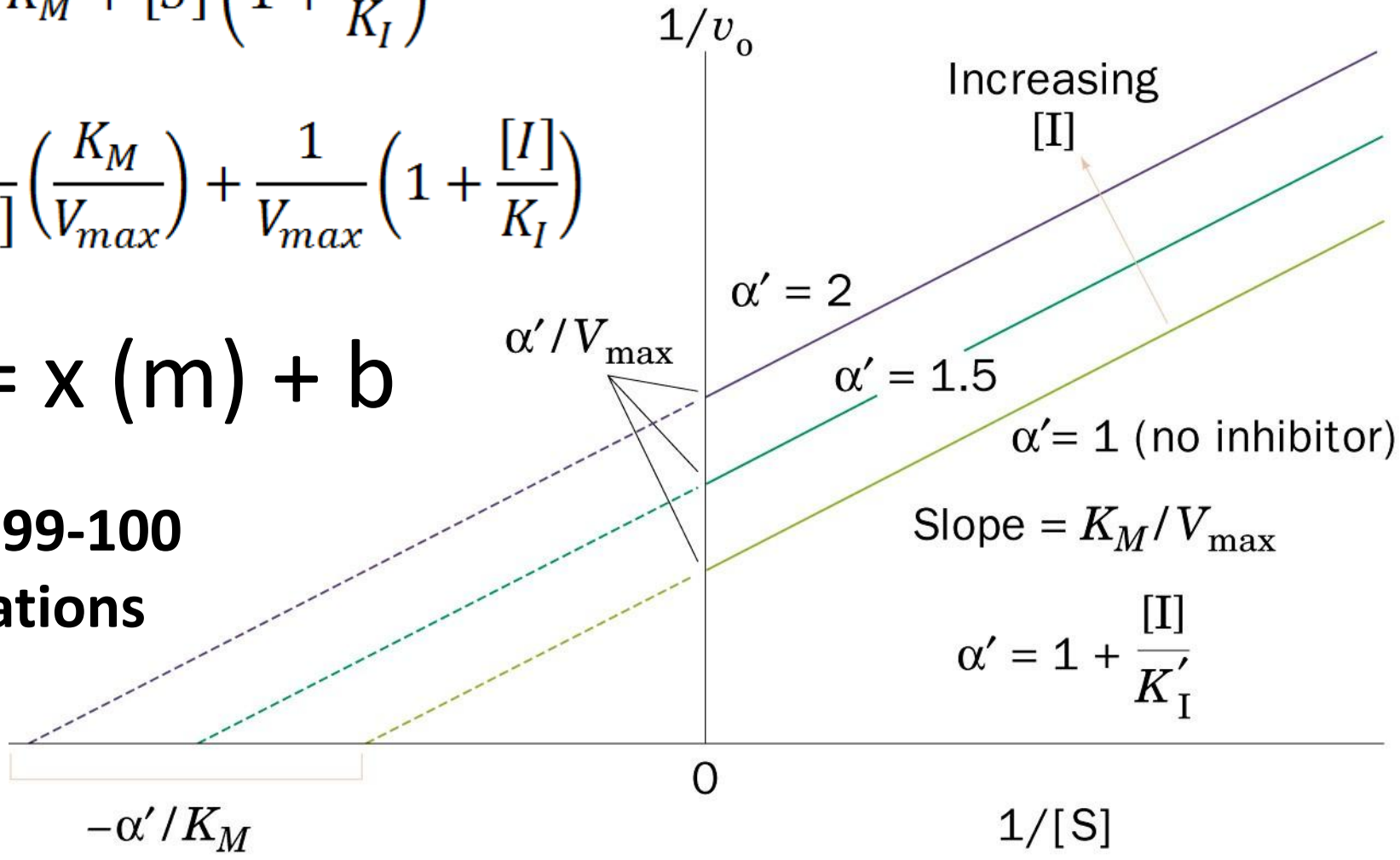
Uncompetitive Inhibition

$$V_o = \frac{V_{max}[S]}{K_M + [S]\left(1 + \frac{[I]}{K_I}\right)}$$

$$\frac{1}{V_o} = \frac{1}{[S]}\left(\frac{K_M}{V_{max}}\right) + \frac{1}{V_{max}}\left(1 + \frac{[I]}{K_I}\right)$$

y = x (m) + b

See pp. 99-100 for equations

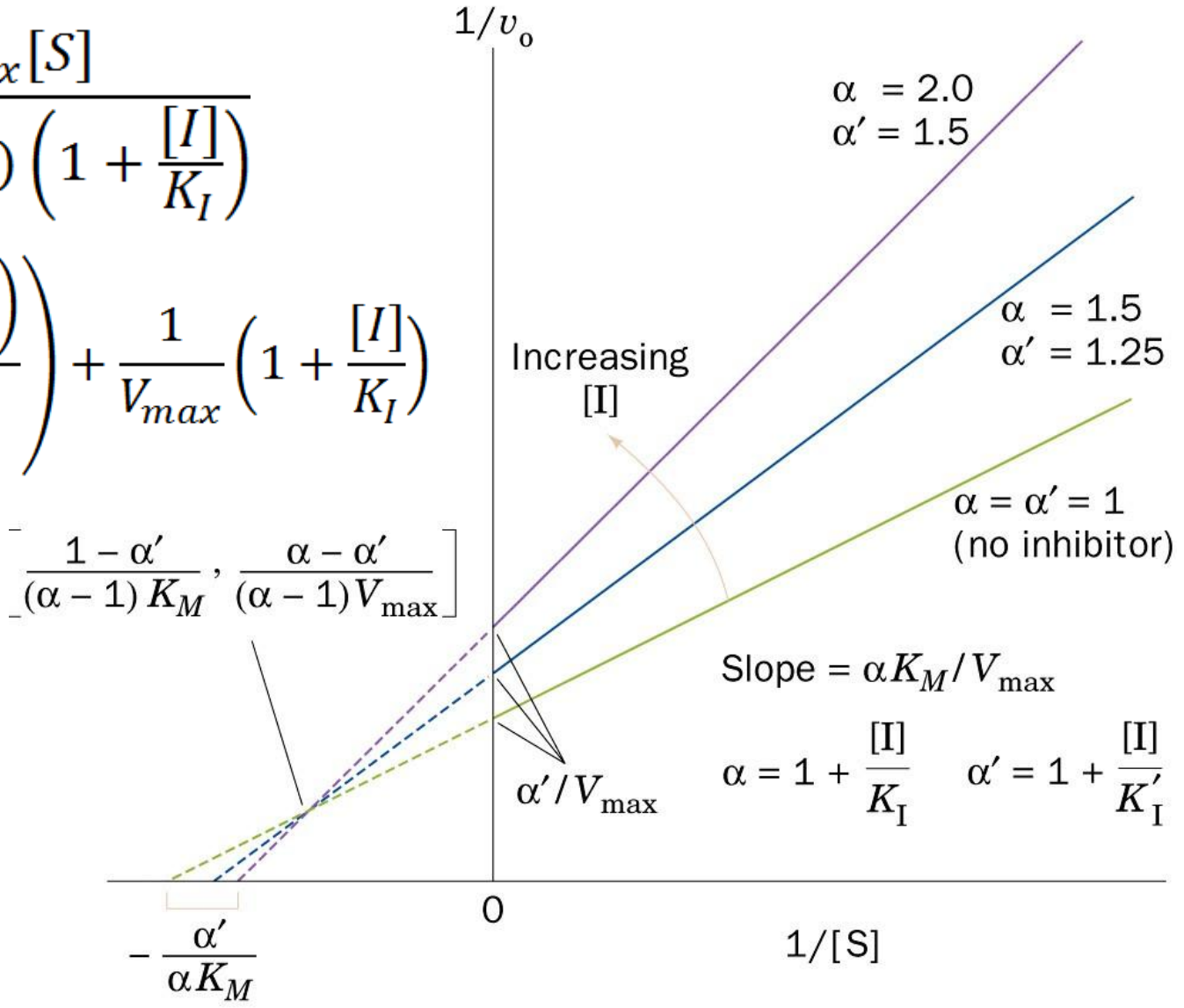


Non-Competitive Inhibition (Mixed)

$$V_o = \frac{V_{max}[S]}{(K_M + [S])\left(1 + \frac{[I]}{K_I}\right)}$$

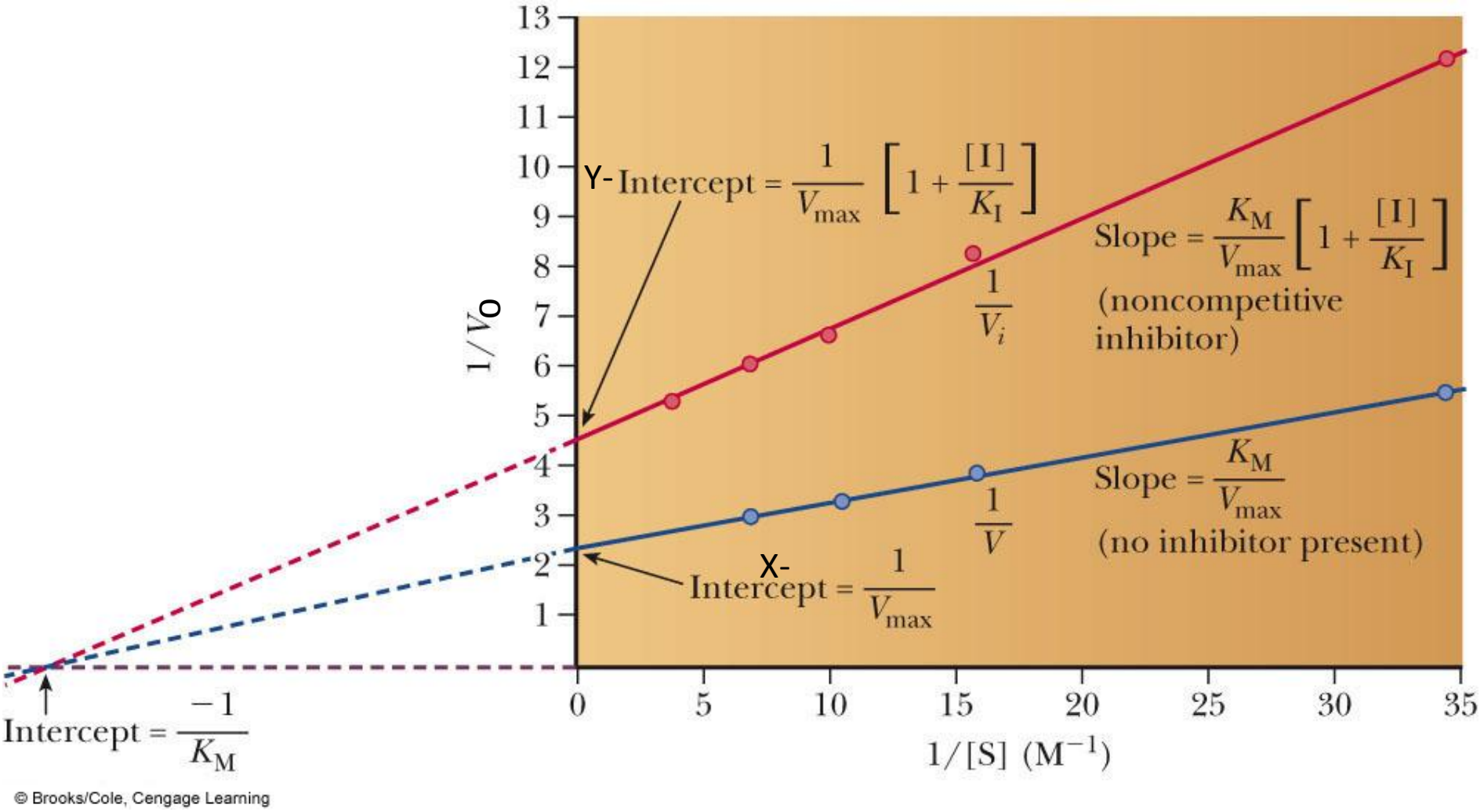
$$\frac{1}{V_o} = \frac{1}{[S]}\left(\frac{K_M\left(1 + \frac{[I]}{K_I}\right)}{V_{max}}\right) + \frac{1}{V_{max}}\left(1 + \frac{[I]}{K_I}\right)$$

y = x (m) + b



See pp. 99-100 for equations

Pure Non-Competitive Inhibition



Chapter 4: Procedure

- Make **new** cocktail with Tris-Buffer pH 8.2 – **Cocktail A**
  - This cocktail gives a higher K_M value for LDH
- Perform activity assays where you vary [pyruvate] without inhibitor
  - Starting ΔA_{340}/min = 0.02-0.04
  - Dilute appropriately to get in range

Chapter 4: Procedure

- Make new cocktail with Tris-Buffer pH 8.2 and inhibitor (your choice) – **Cocktail B**
  - Make sure to write down letter and concentration of inhibitor
- Perform activity assays where you vary [pyruvate] in presence of the inhibitor
  - Rates with inhibitor < Rates of uninhibited reactions

Make sure to prepare data tables p. 106-7 BEFORE LAB!

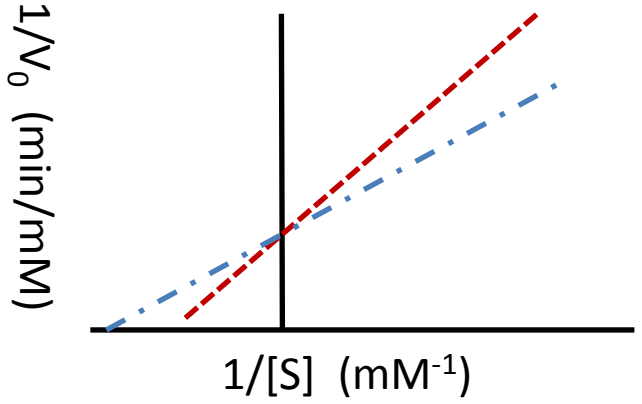
Include all cocktail recipes in your notebook!

Lab Notebook: Chapter 4

- Raw Data for uninhibited and inhibited LDH
- Calculation of rates in mM:

$$\left(\frac{\left(\frac{\Delta A_{340}}{min}\right)}{\left(\varepsilon_{app\ in\ mM^{-1}}\right)}\right)\left(\frac{(3\,mL\ total\ volume)(Dilution\ Factor)}{(0.1\,mL\ enzyme\ used)}\right) = Rates\ in\ mM/min$$

- Michaelis-Menton and Lineweaver-Burk Plots for uninhibited and inhibited LDH



Uninhibited LDH

Inhibited LDH

What type of inhibition is pictured here?

- Calculation of K_M and V_{max} – **Show unit calculations!**
- Calculation of K_I for **your type of inhibition**