Announcements

- Chapter 3 and 11 are **both** due at the end of Chapter 4 lab

Chapter 4: Enzyme Kinetics

Purpose:

A) Re-assay LDH activity using Chapter 3 cocktail
(**containing potassium phosphate buffer*)

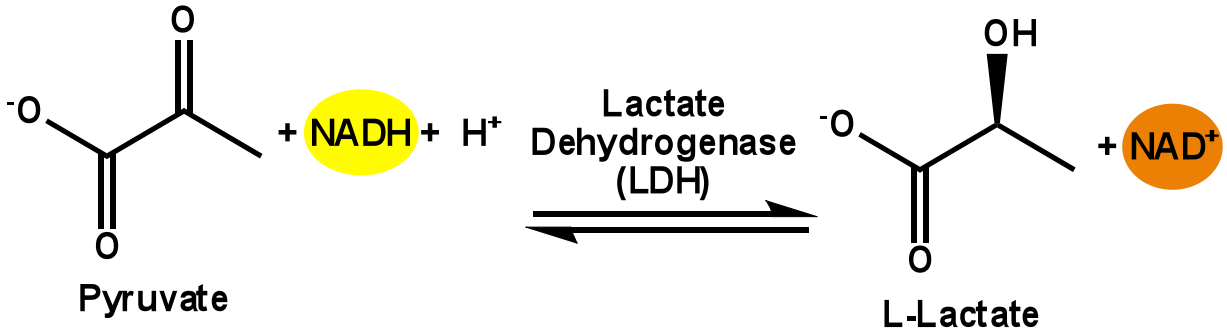
B) Assay LDH activity using varying concentrations of one substrate (pyruvate) → **Tris buffer cocktail*

- To determine K_m & V_{max} of pyruvate

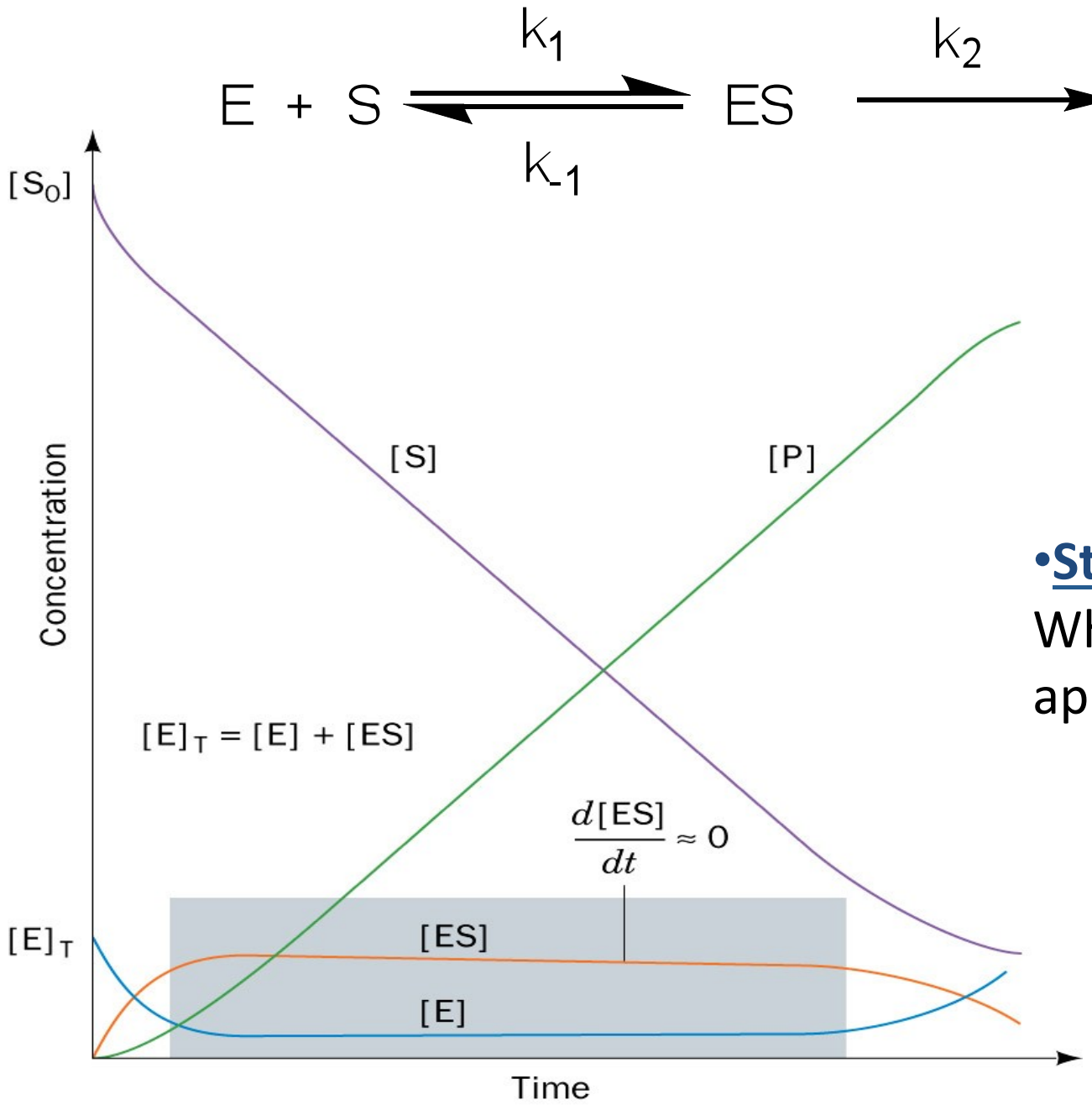
C) Characterize the effect of an unknown inhibitor on enzyme activity → **Tris buffer cocktail*

- Solve for K_I

Review: LDH catalyzes the last step of anaerobic glycolysis



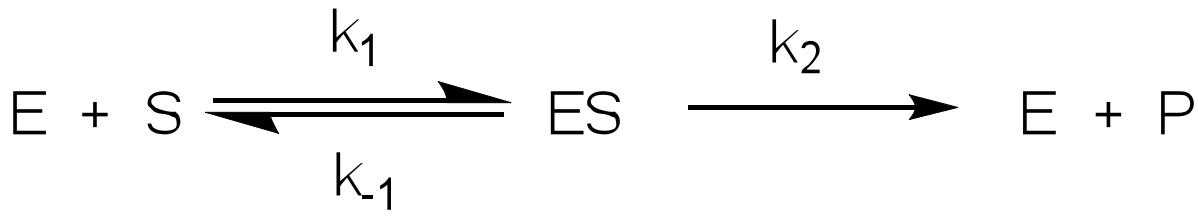
- Multiple forms of LDH found in different tissues → **Isozymes**
  - Each isozyme has slightly different kinetic and structural properties, but same function and overall structure



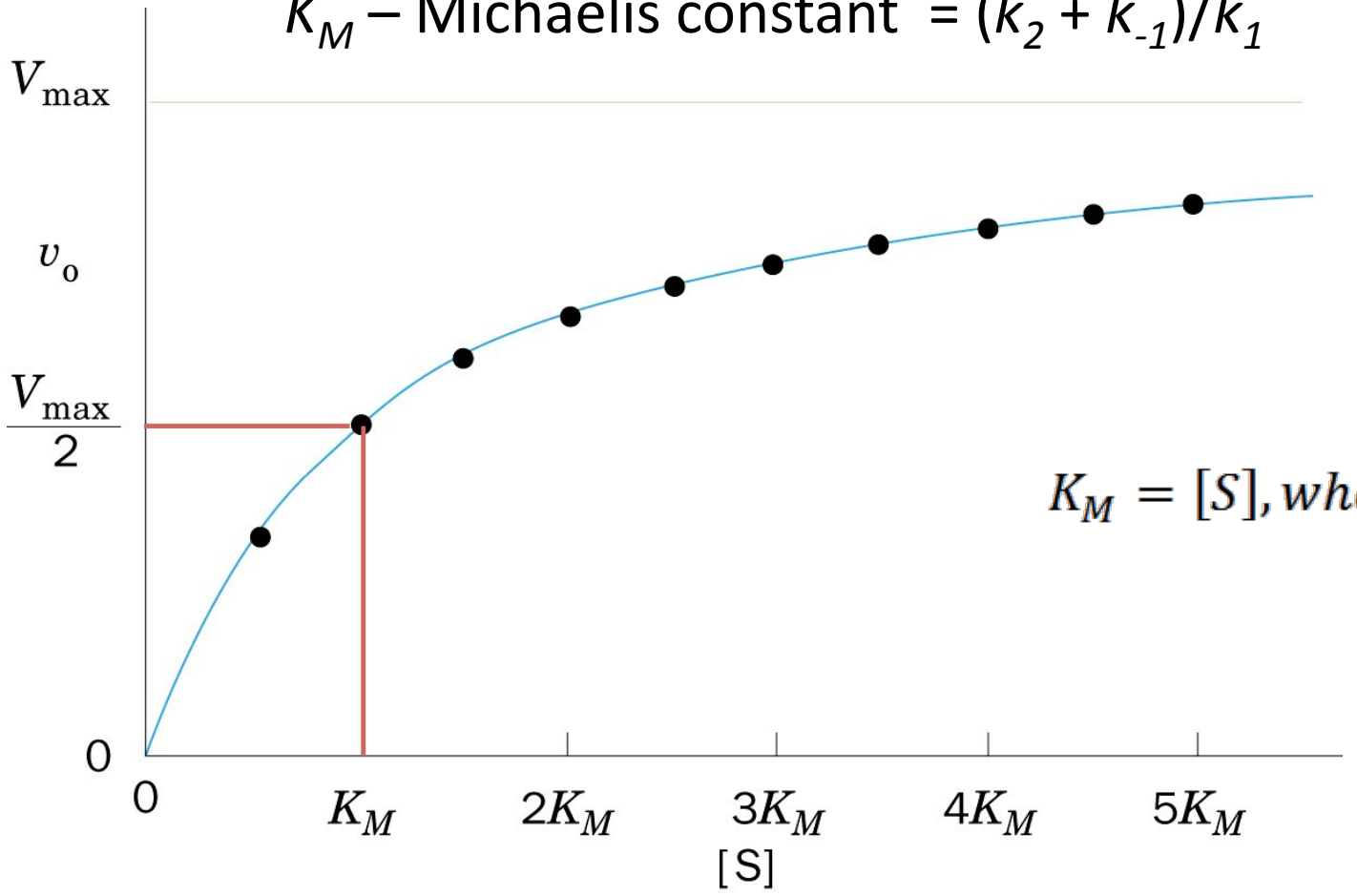
- **Steady-State Kinetics**

Where [ES] remains approximately constant

Michaelis-Menten Kinetics



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



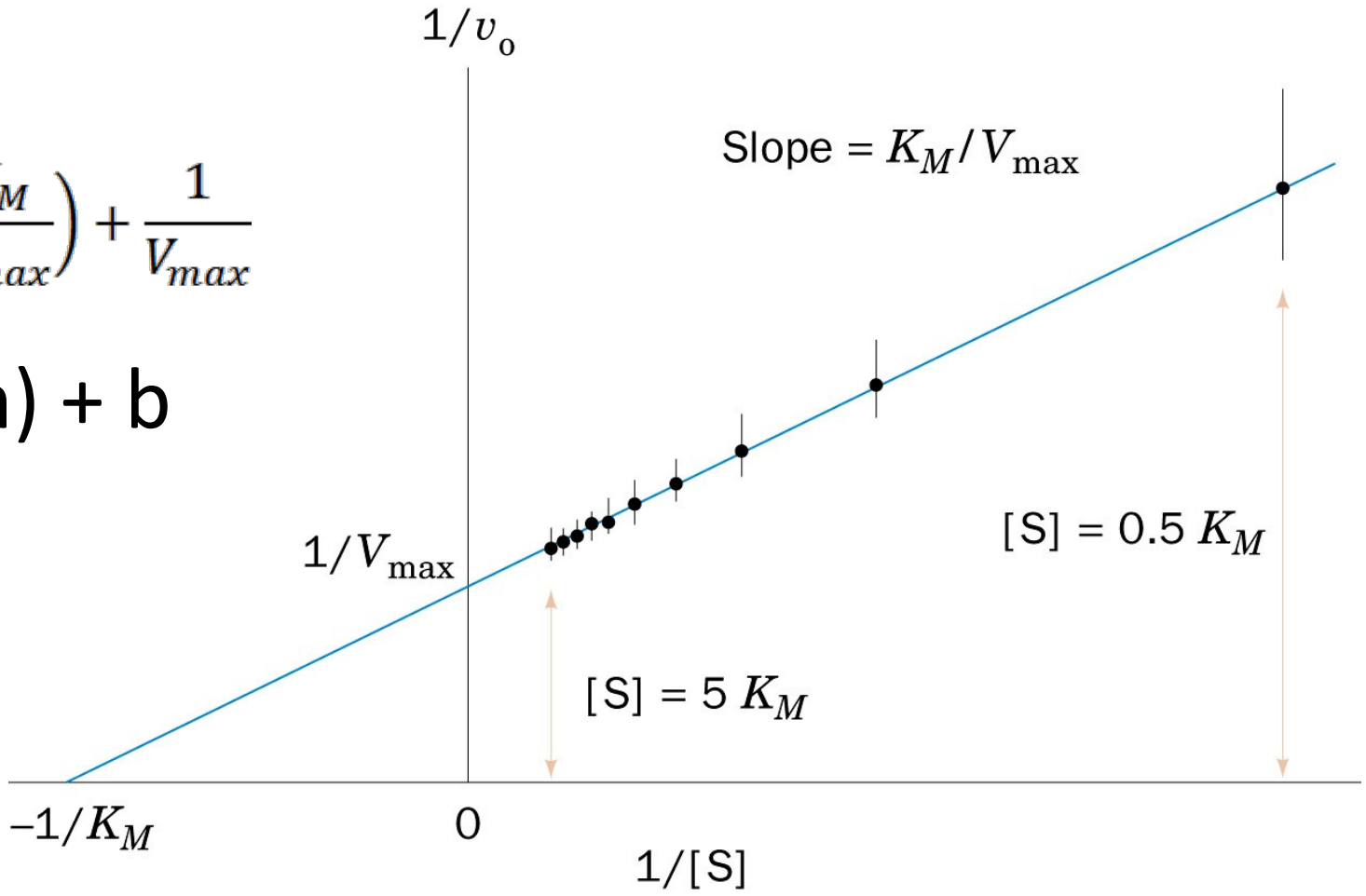
$$K_M = [S], when\ V_o = \frac{1}{2}V_{max}$$

Lineweaver-Burk Manipulation

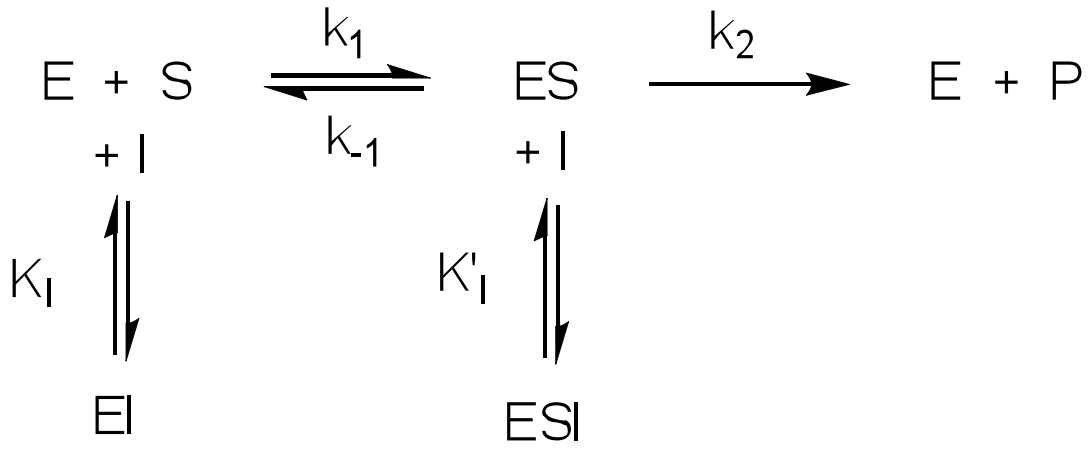
Double-Reciprocal Plot

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

y = x (m) + b



Introduction of an Inhibitor (reversible)



- **Competitive Inhibition** – Binds to free enzyme only & competes with substrate for active site
- **Uncompetitive Inhibition** – Binds to distinct site from substrate active site and binds only to ES complex
- **Pure Non-Competitive Inhibition** – Binds to a distinct site on the free enzyme or ES complex that decreases overall activity ($K_I = K_I'$)
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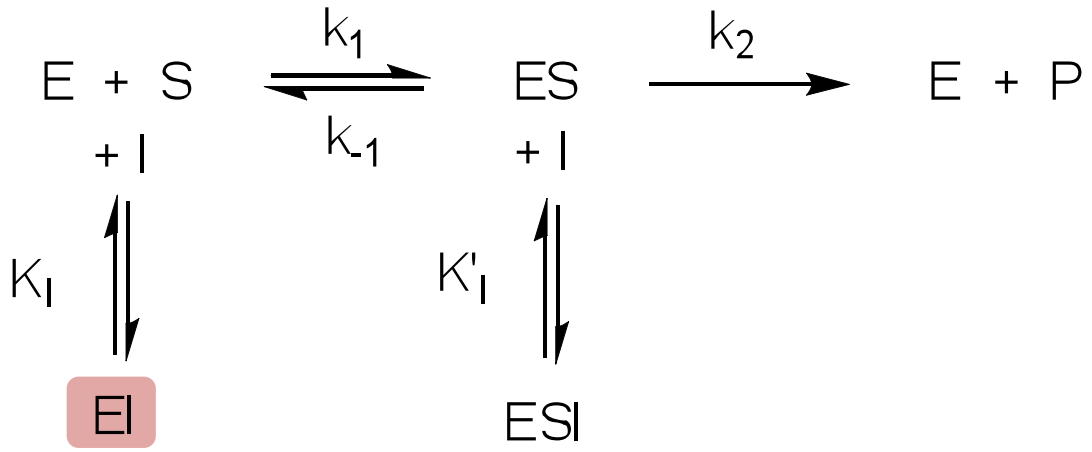

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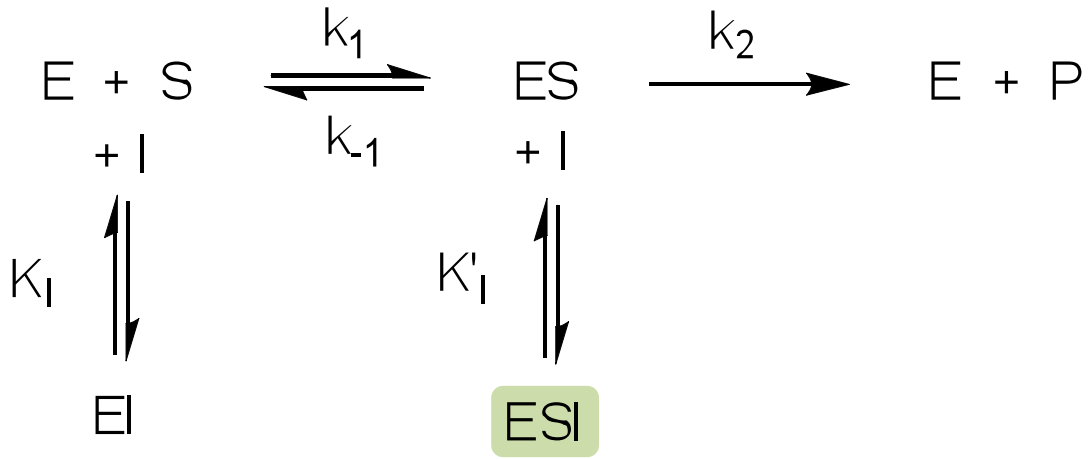

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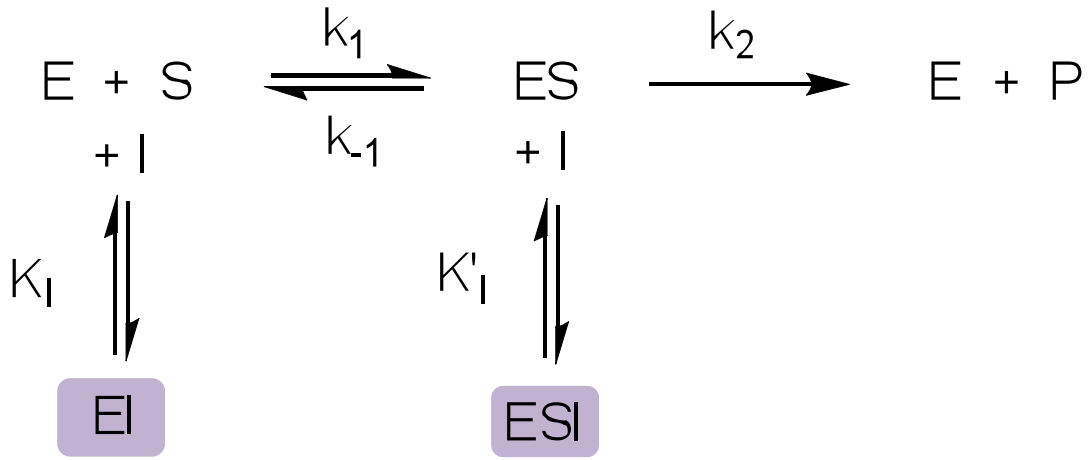

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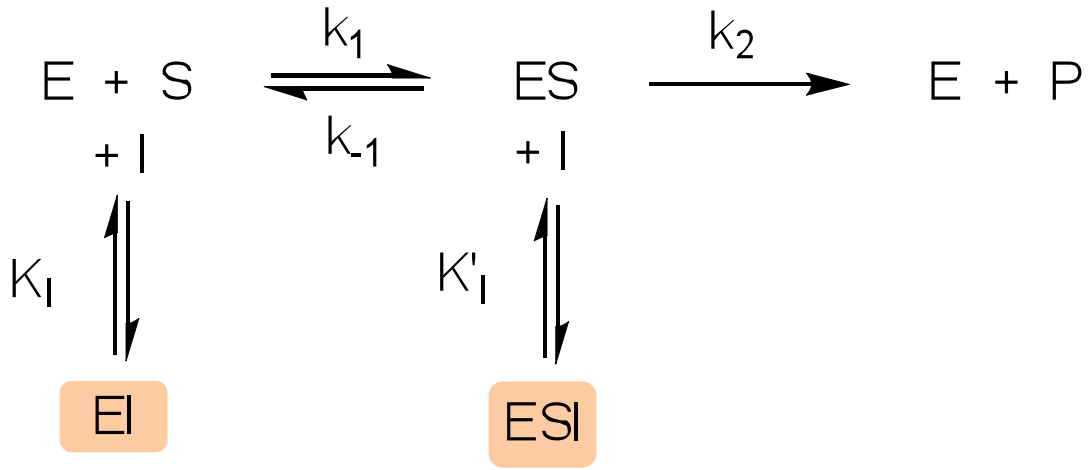

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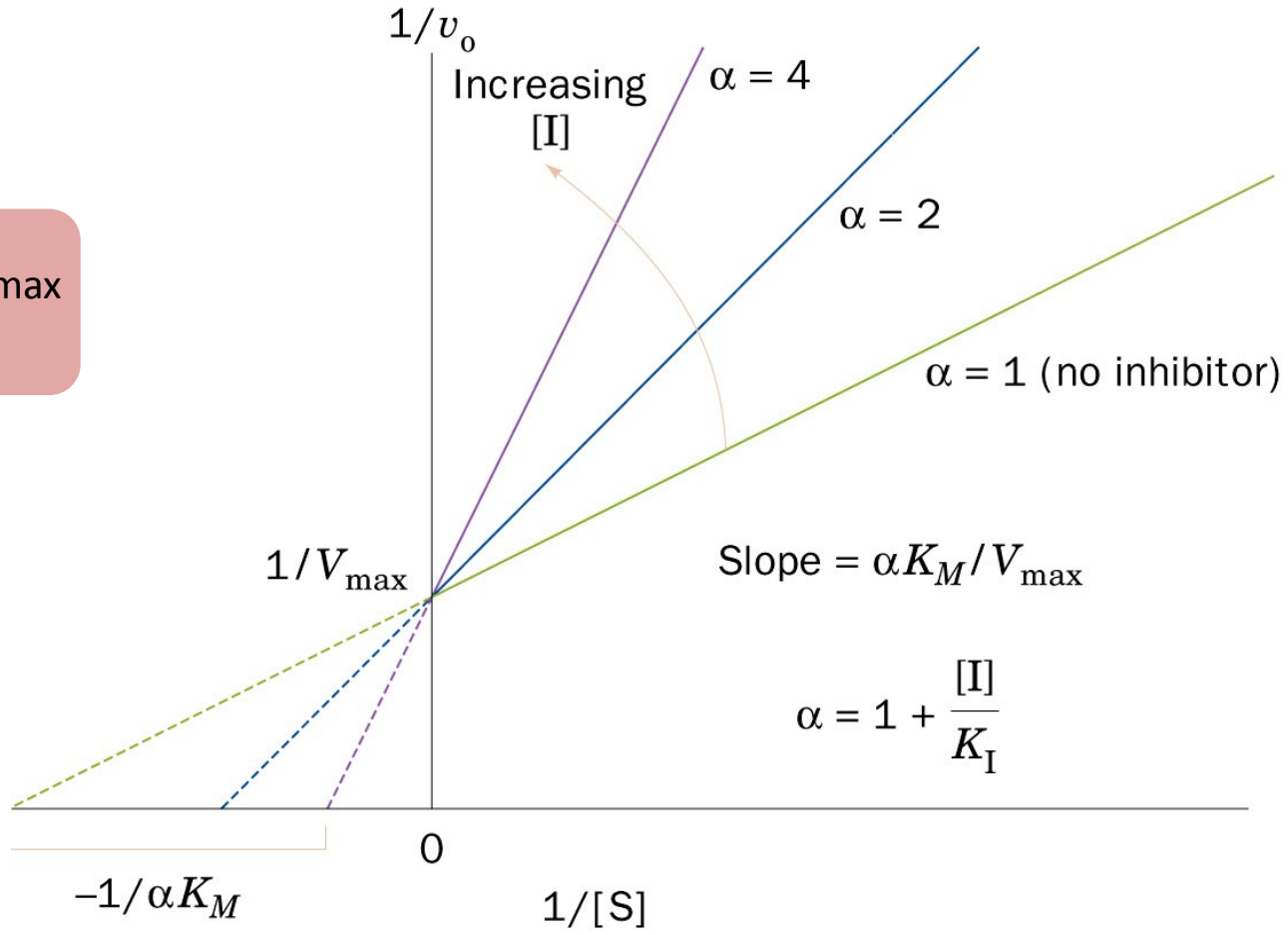
Competitive Inhibition

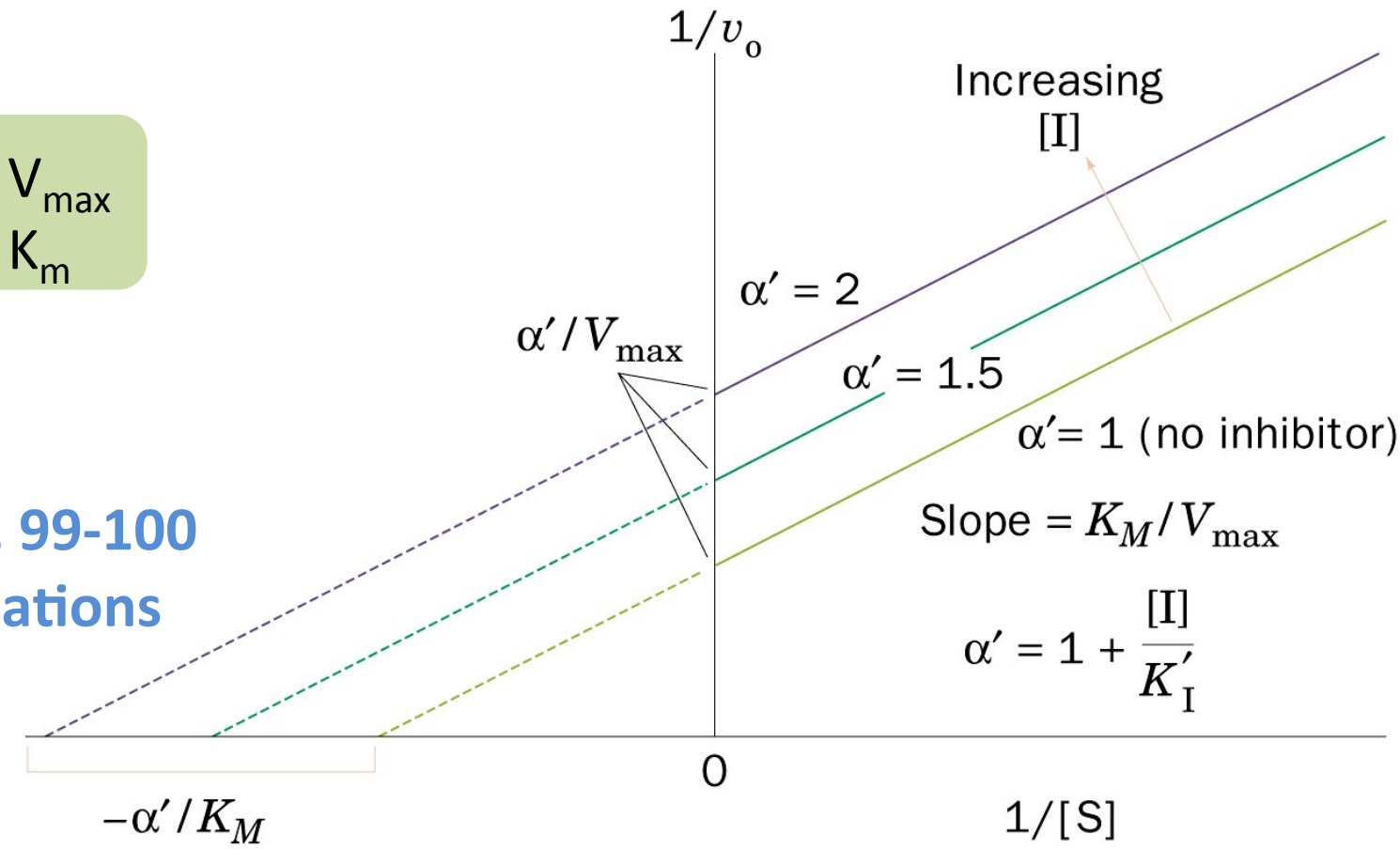
No change in V_{max}
↑ in K_m



See pp. 99-100 for equations

Uncompetitive Inhibition

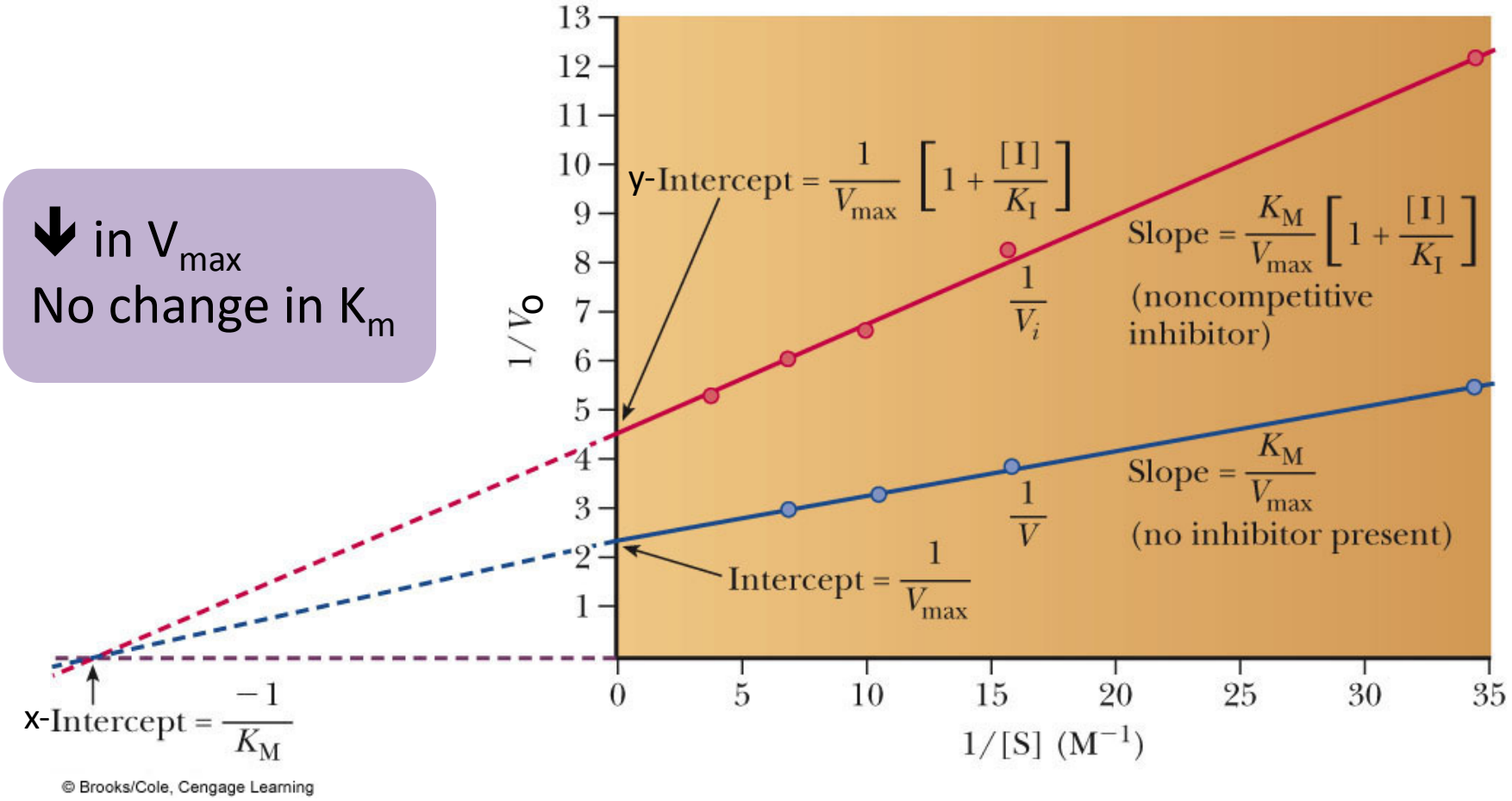
↓ in V_{max}
↓ in K_m



See pp. 99-100 for equations

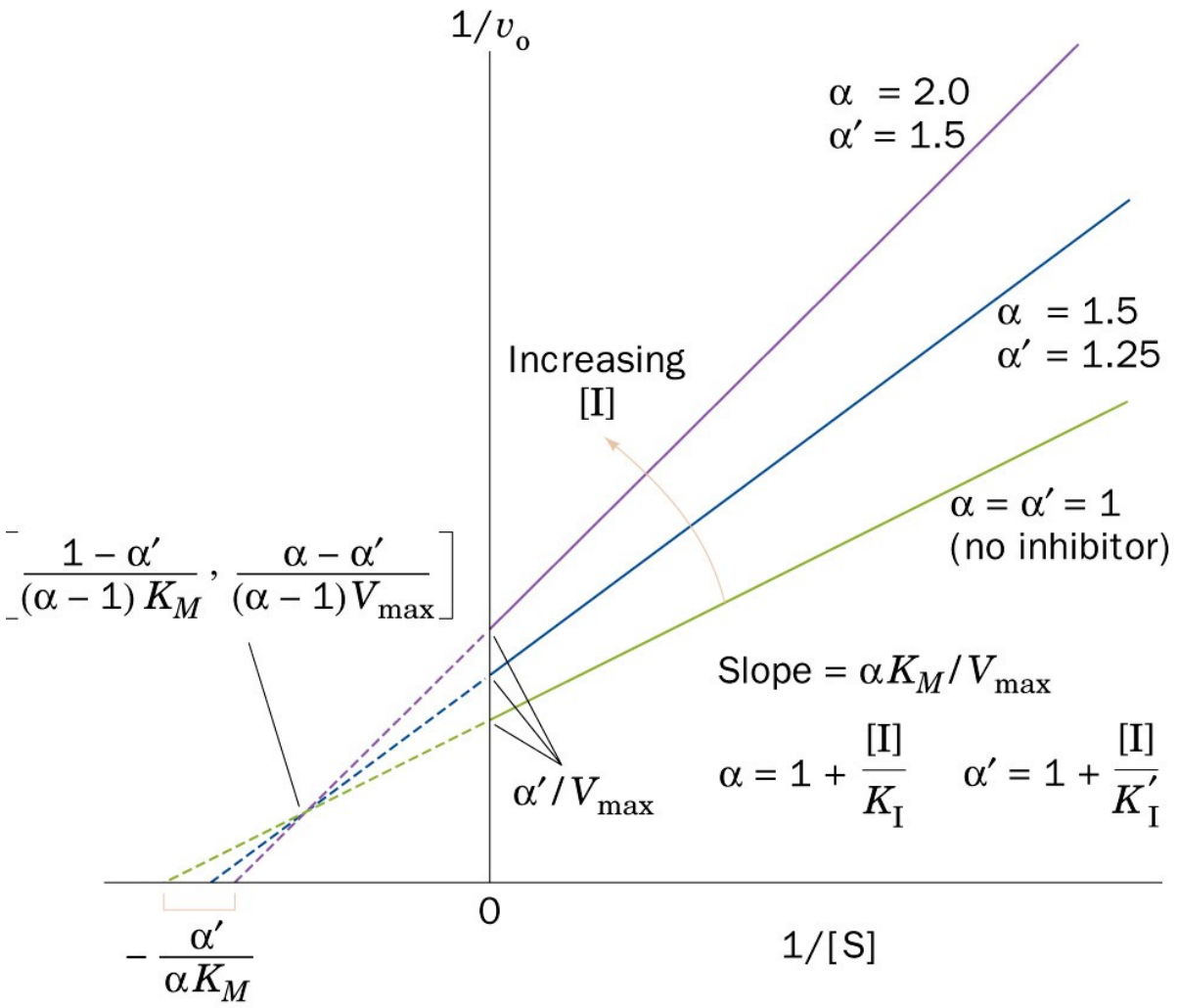
Pure Non-Competitive Inhibition

↓ in V_{max}
No change in K_m



Non-Competitive Inhibition (Mixed)

↓ in V_{max}
↑ in K_m



See pp. 99-100 for equations

Chapter 4A-B: Procedure

Reagent	Chapter 3 recipe	Chapter 4 recipe
Enzyme	LDH	LDH
Cofactor	NADH	NADH
Substrate	Pyruvate	Pyruvate
Buffer	***Potassium phosphate***	***Tris***
Diluent	dH_2O	dH_2O

- Re-assay LDH using Chapter 3 recipe and write down activity concentration
- Make **new** cocktail with Tris-Buffer pH 8.2 – **Cocktail A**
  - Phosphate acts as a mild inhibitor on LDH
- Perform activity assays where you vary [pyruvate] without inhibitor
  - **Starting ΔA_{340}/min = 0.02-0.04** for lowest [pyruvate] (**correction)*
  - Dilute appropriately to get in range

Chapter 4C: Procedure

- Make new cocktail with Tris-Buffer pH 8.2 and inhibitor – **Cocktail B**
  - ***Make sure to write down letter and concentration of your assigned 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!

Chapter 4: Lab manual typos

- Cocktail tables (pg 104 & 105) --
  - For **Na^+ pyruvate**, volume range should be **0.025 – 0.25 mL**
  - For **H_2O**, volume range should be **0.475 – 0.25 mL**

- Part B.1 & Table 1 in notebook section –
  - For your initial trials, the lowest pyruvate concentration should be **0.05 mM**, not 0.1 mM

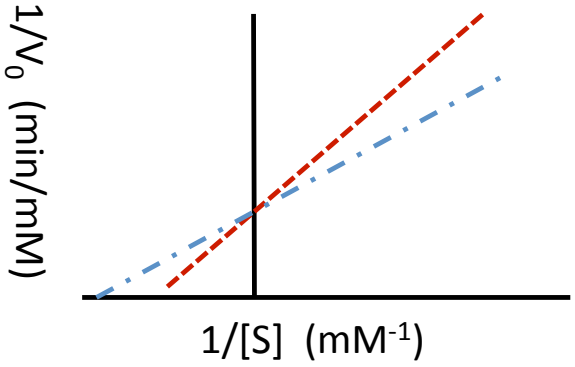
- ΔA/min range for initial trials –
  - For your **initial trials**, use a ΔA/min range between **0.02 and 0.04** at the lowest pyruvate concentration

Lab Notebook: Chapter 4

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

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

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



Uninhibited LDH

Inhibited LDH

What type of inhibition is pictured here?

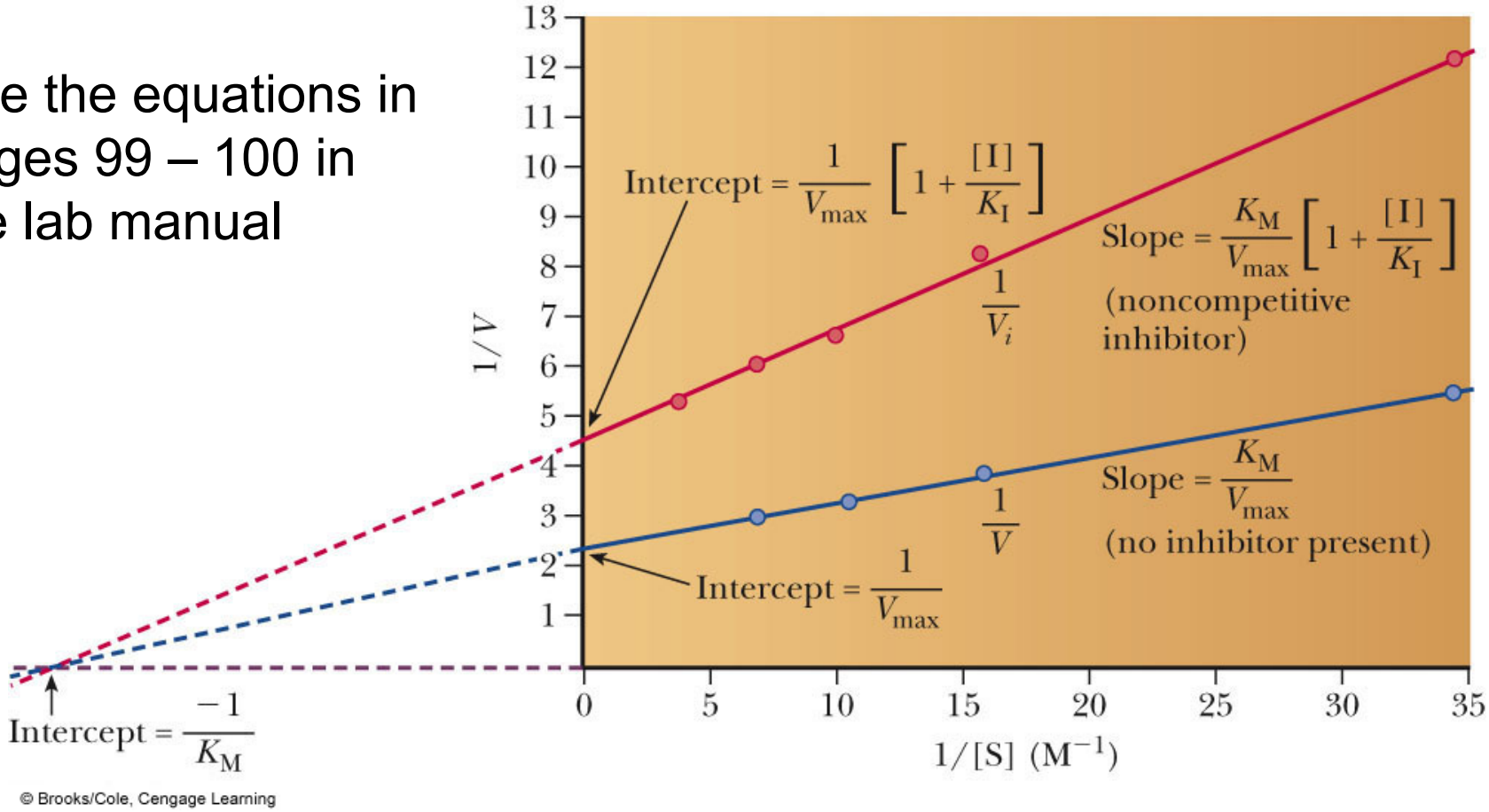
- Calculation of K_M and V_{max} – **Show calculations!**
- Calculation of K_I for **<u>your</u> type of inhibition**

Lab Notebook: Chapter 4

- To calculate K_I you will need your V_{max}, K_m and inhibitor concentration values, depending on your type of inhibitor

- Plot your data first to figure out which type of inhibitor you have

- Use the equations in pages 99 – 100 in the lab manual



Chapter 4:

Before the lab period, you should have:

✓Completed your prelab
- ✓Title, date, introduction, procedures
- ✓Be sure to account for all corrections and changes in lab manual

At the end of lab, you should have:

✓Re-assayed your LDH using Chapter 3 cocktail

✓Collected LDH data for varying pyruvate concentrations

✓Recorded your inhibitor letter and concentration

✓Collected LDH data for varying [pyruvate] with inhibitor present

Questions?