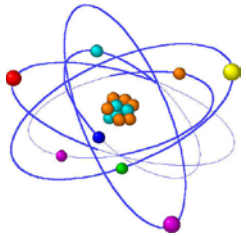
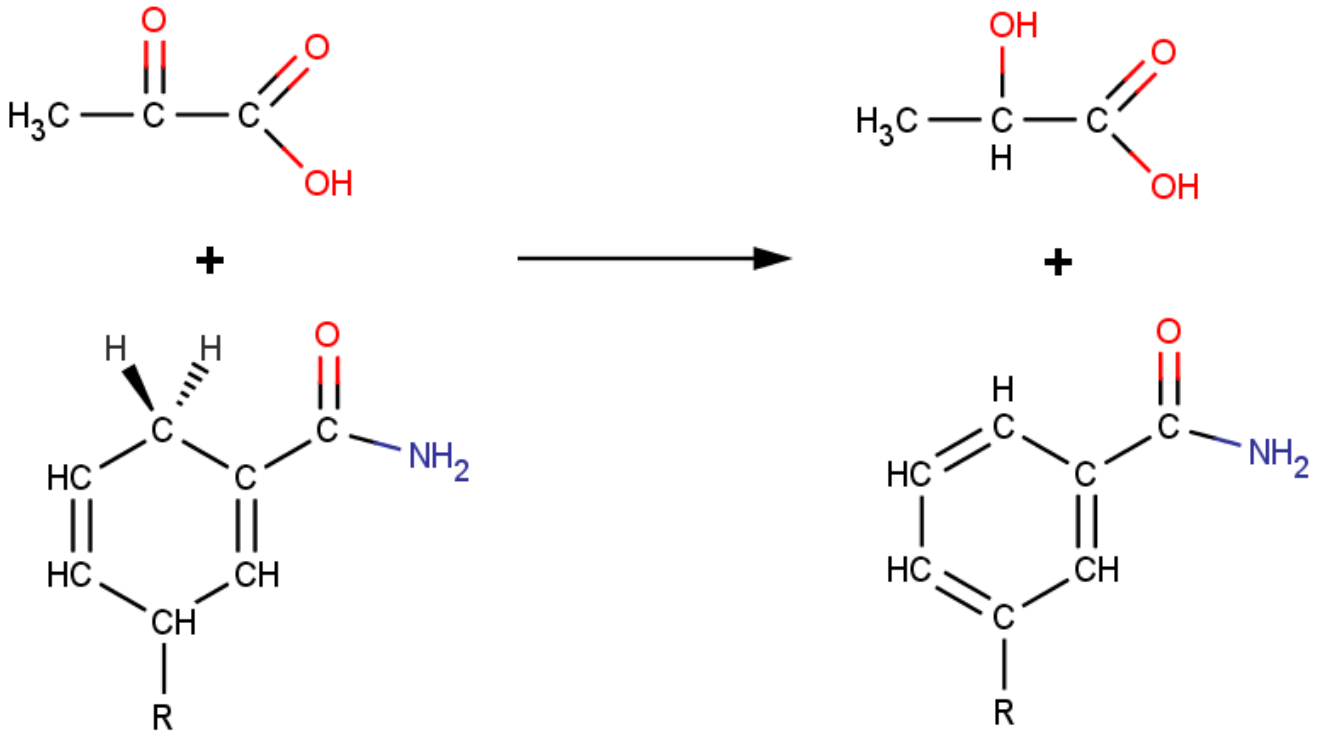
Biochemistry Lab

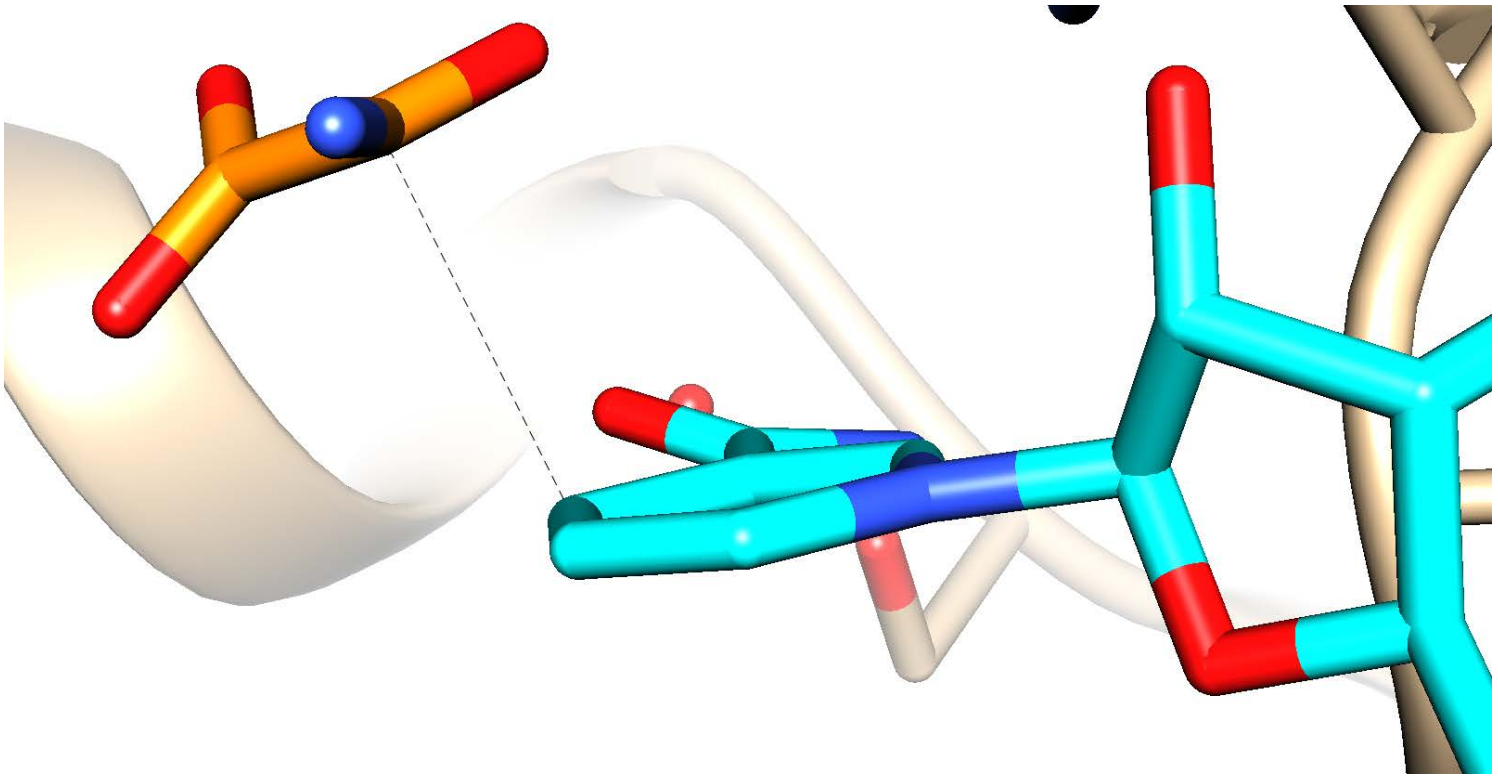


LDH Kinetics

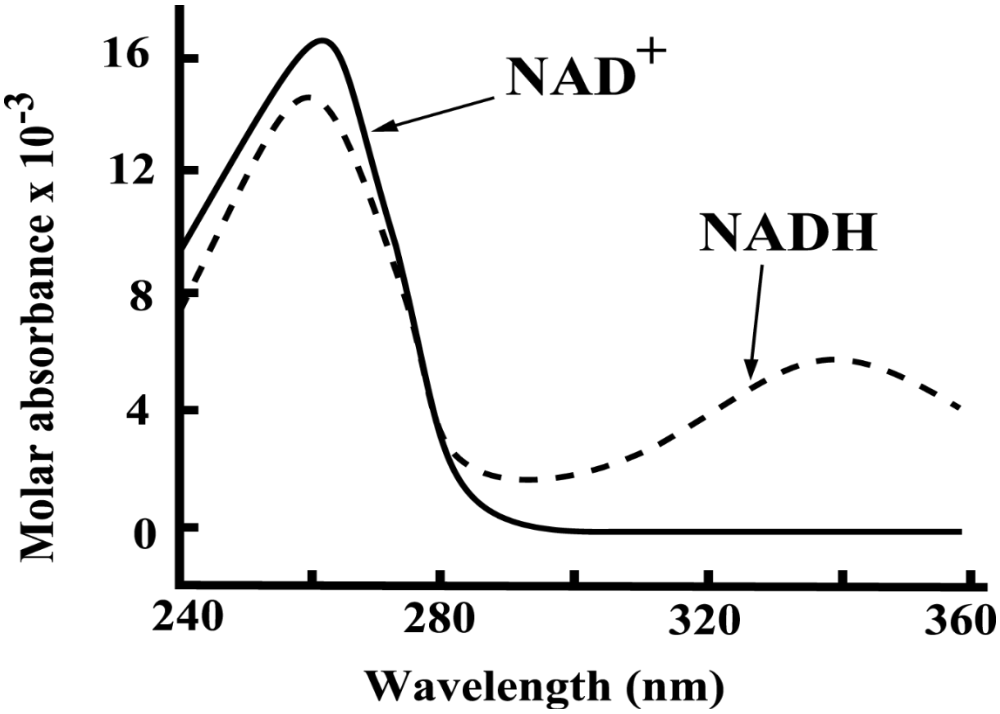
The LDH Reaction



The LDH Reaction

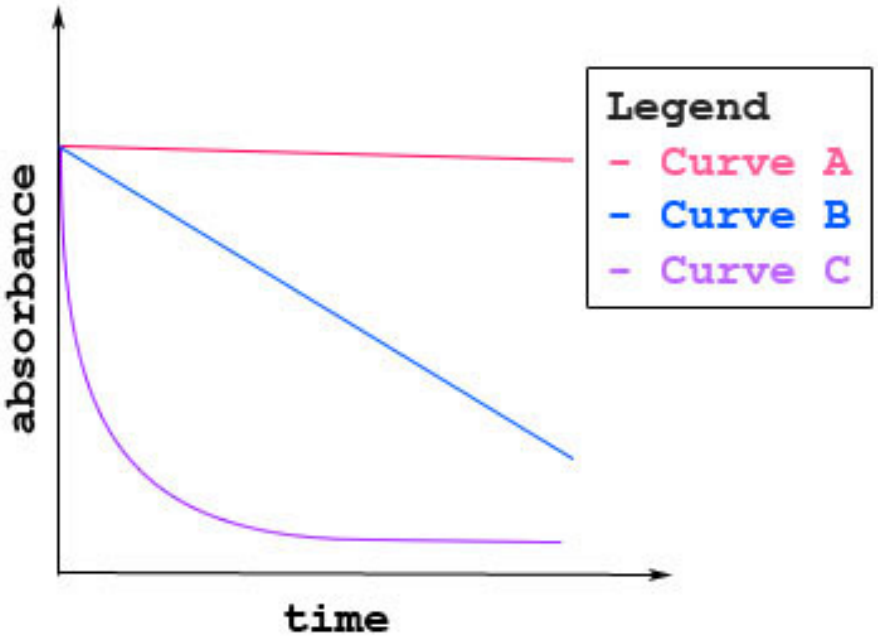


NADH vs. NAD^+



$$\varepsilon_{340} = 6220\ M^{-1}cm^{-1}$$

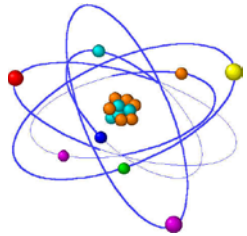
NADH vs. NAD^+

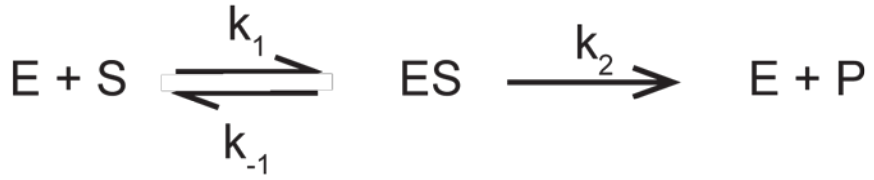


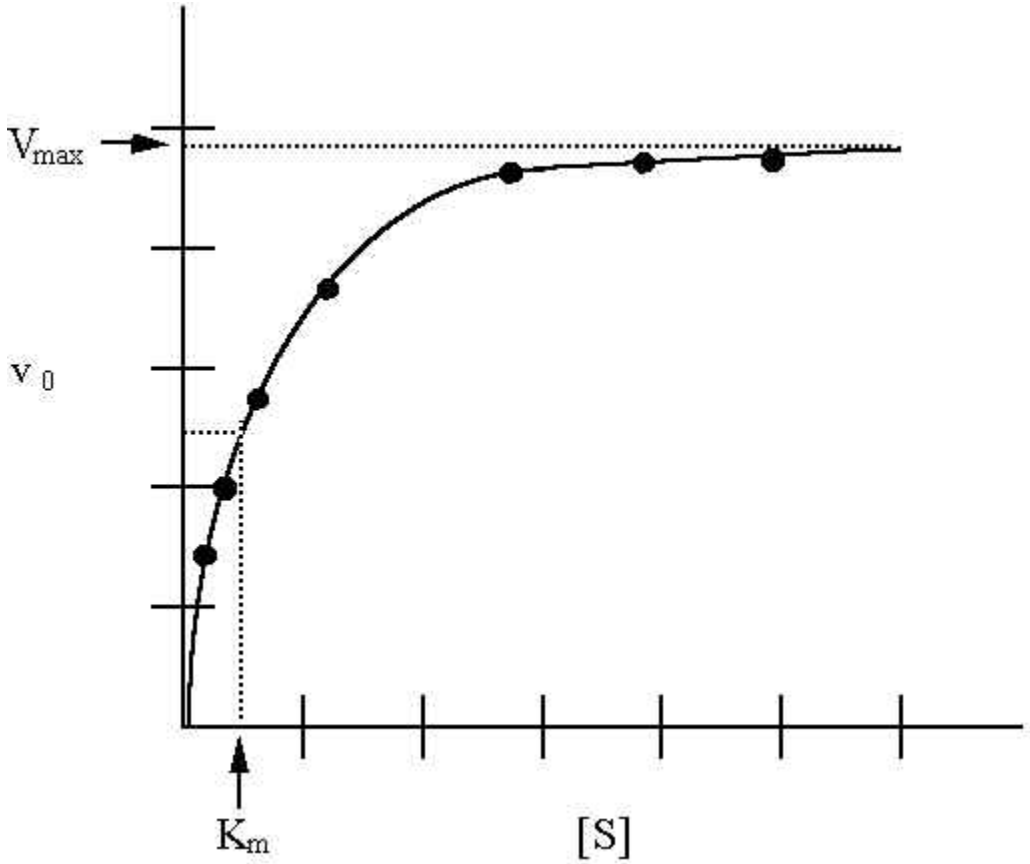
$$slope = \frac{\Delta abs}{\Delta t}$$

$$rate = -\frac{\Delta [NADH]}{\Delta t}$$

Michaelis-Menten Kinetics





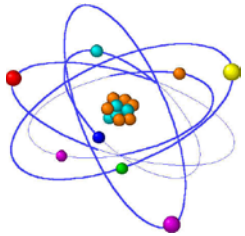


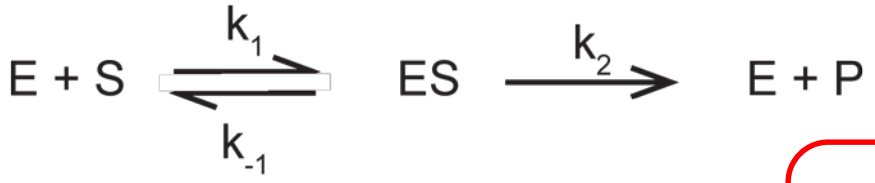
$$v_0 = \frac{V_{\max}[S]}{([S] + K_M)}$$

$$k_{cat} = \frac{V_{\max}}{[E]_{total}}$$

$$\frac{k_{cat}}{K_M}$$

Steady State Approximation





$$v_0 = \frac{k_2[E]_{total}[S]}{([S]+K_M)} \qquad V_{\max} = k_2[E]_{total}$$

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

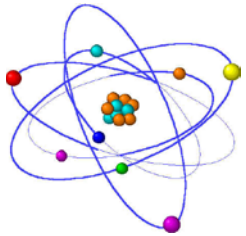
Applications of M-M Kinetics

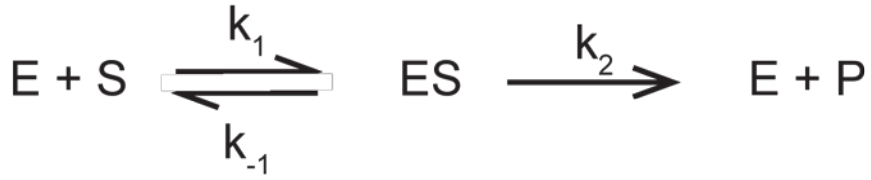
Turnover Number (k_{cat}) → reports on number of processes per enzyme

$$k_{cat} = \frac{V_{\max}}{[E]_{total}} = k_2$$

Catalytic Efficiency ($\frac{k_{cat}}{K_M}$) → Apparent 2nd order rate constant

Determining K_M and V_{max}

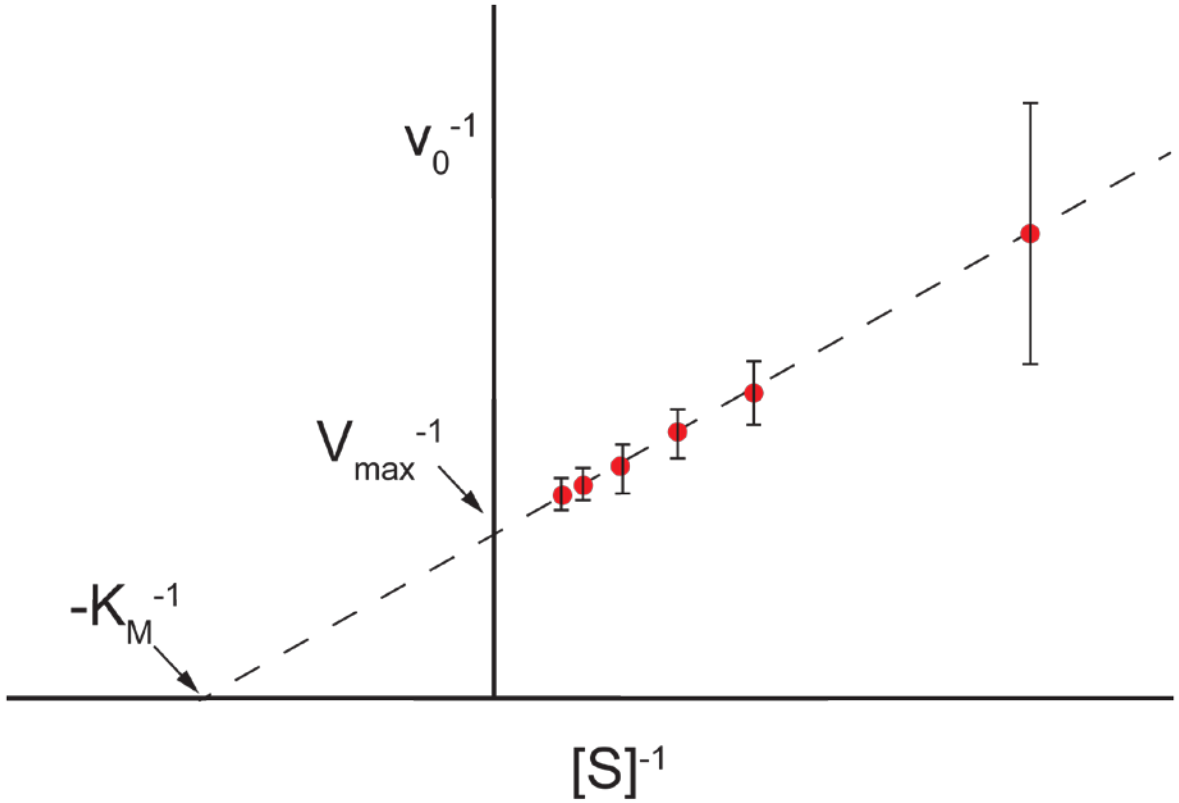




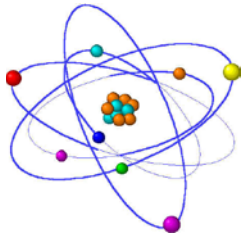
Lineweaver-Burk Relationship

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

$$\frac{1}{v_0} = \frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$$



Experimental Details



Goals for lab:

- Determine the concentration of LDH (measure Abs_{280})
  - $\frac{\Delta abs}{\Delta t} \approx 0.5 \ {}^{Abs}/_{min}$
- Determine the dilution factor of LDH that you need to use.
  - Dilute 2x, 5x, 10x, 20x, 50x

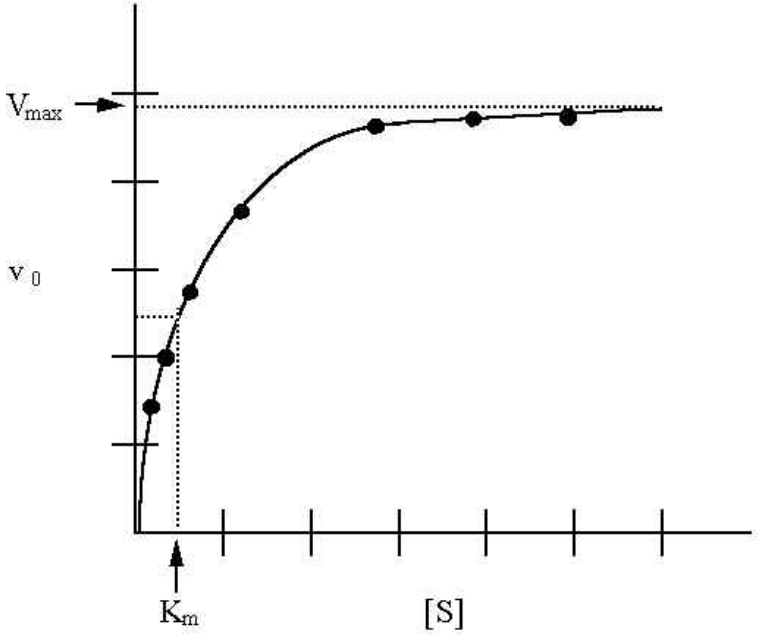
In your reactions:

- 33 µL of 6.6 mM NADH
- 33 µL of 300 mM Pyruvate
- 33 µL of LDH (still need to determine the concentration and dilution)
- 900 mL of experimental buffer (made a few weeks ago – you should know what is in it!)

- Program: monitor the kinetics of the reaction at 340 nm for 2 minutes
  - Blank should be without NADH – it's your chromaphore
  - Why is 340 nm an ideal choice?

Analysis

- Convert slope to rate
  - $A = \varepsilon c l$
- Plot rate vs. [Pyruvate] (be careful with units!!!!!)
- Use the Solver tool in Excel to determine K_M and V_{max}. Use this tutorial if you're not familiar with how to do this.
- Calculate k_{cat} and k_{cat}/K_M (be careful with units!)



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