ENZYME CHEMISTRY

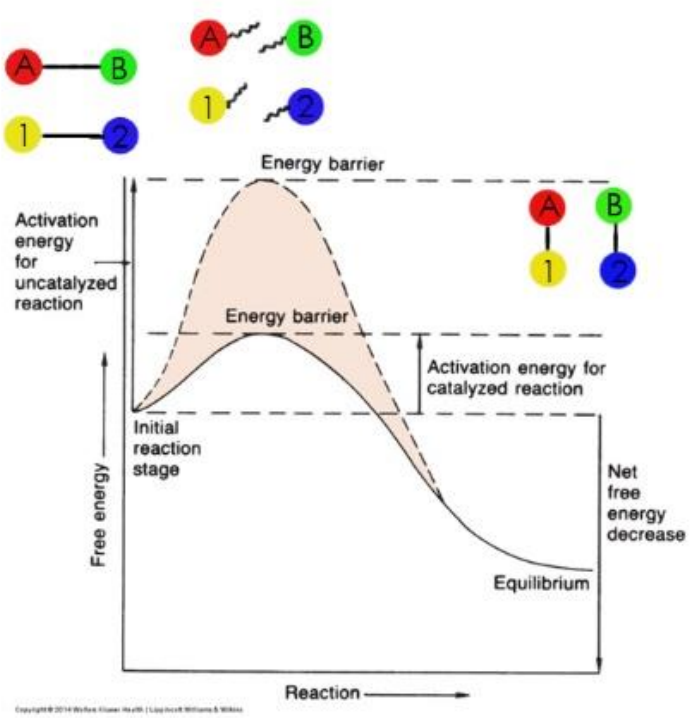
BACKGROUND INTO THE CLASSIFICATION AND KINETICS OF ENZYMES

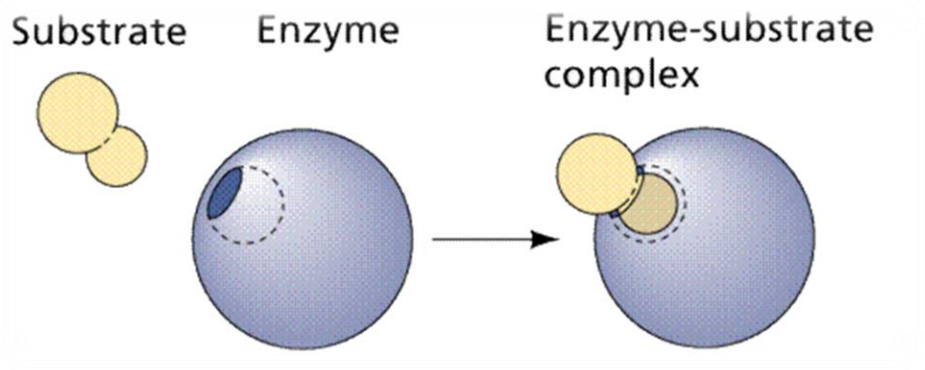
ENZYME CHEMISTRY-FUNCTION

- Proteins (mostly, some RNA)
- Catalyze reaction
  - Lower activation energy
  - Not consumed
  - Maintain reaction equilibrium
- Structure
  - 1°, 2°, 3°, 4°
  - Isoenzymes
    - Different physical properties
      - Electrophoretic, solubility, inactivation

ENZYME-FUNCTION

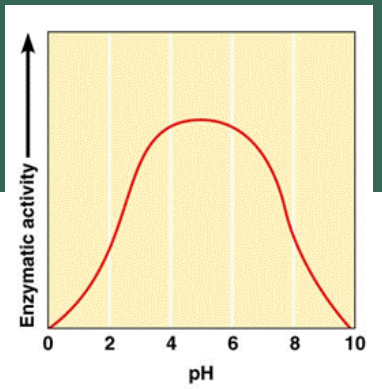
- Activation energy must be reached for a reaction to occur
  - Instead of heating up reactants to provide more energy, use enzymes to decrease energy needed



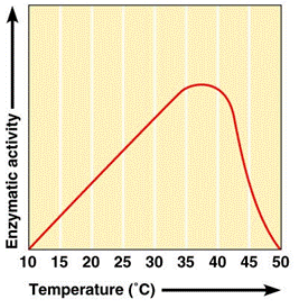


ENZYME CHEMISTRY-KINETICS

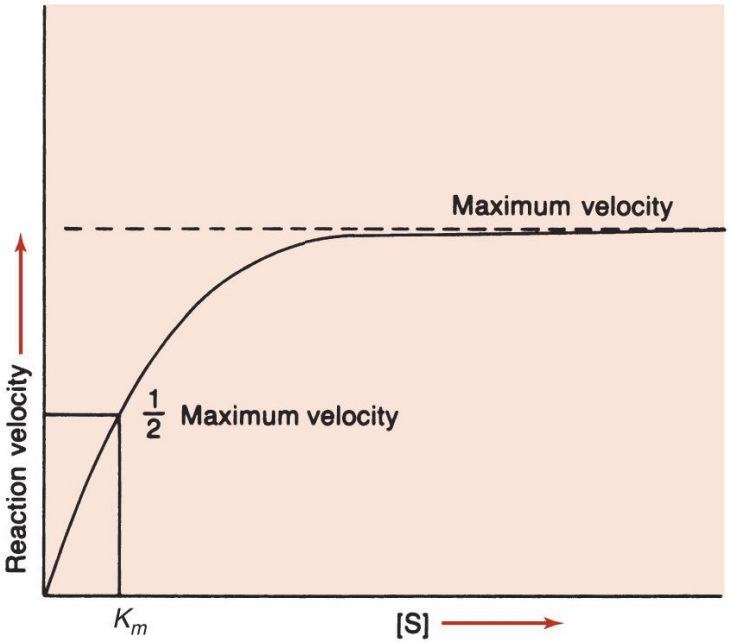
- Factors Affecting Enzymatic Reaction
  - Substrate concentration
    - First order kinetics increase with concentration
    - Zero order kinetics independent of concentration
  - Enzyme concentration
  - pH
  - Temperature
  - Cofactors
  - Inhibitors



(b) pH. The enzyme illustrated is most active at about pH 5.0.

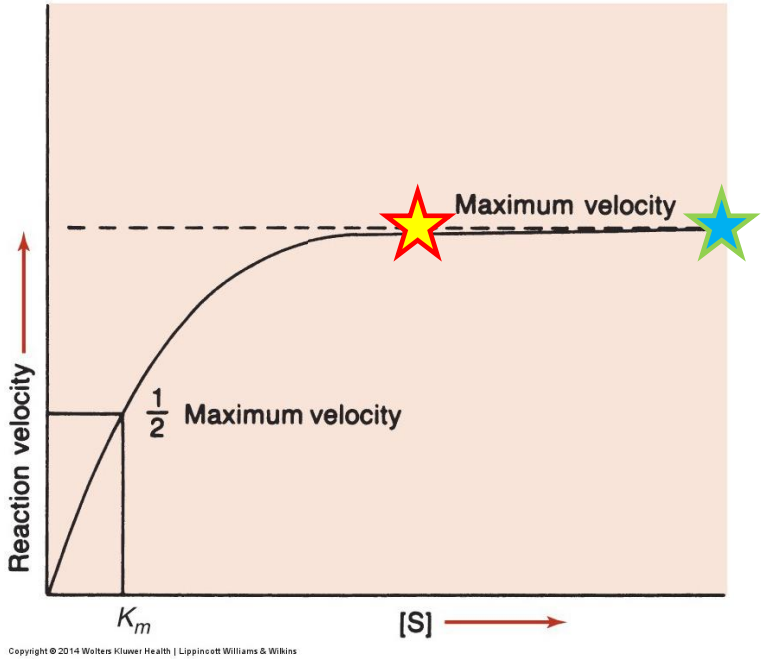


(a) Temperature. The enzymatic activity (rate of reaction catalyzed by the enzyme) increases with increasing temperature until the enzyme, a protein, is denatured by heat and inactivated. At this point, the reaction rate falls steeply.



ENZYME CHEMISTRY-KINETICS

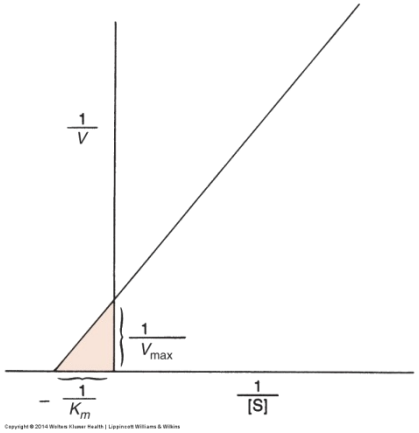
- When measuring Enzyme activity the amount of substrate must *remain* in excess for full reaction
  - If not it will be depleted and reaction will slow

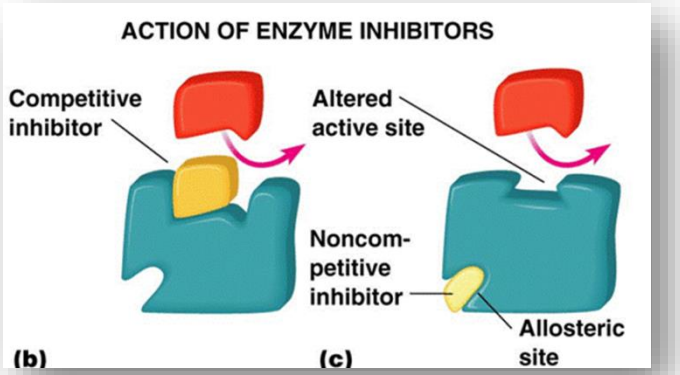


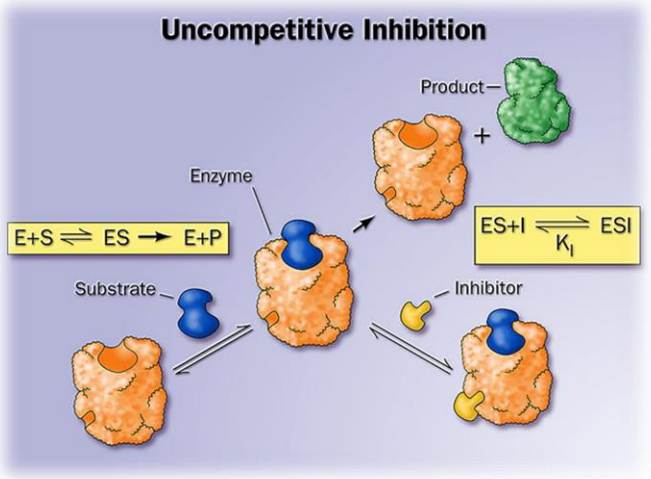
  - When measuring the SUBSTRATE you don't want it to go up past K_m

ENZYME CHEMISTRY-KINETICS

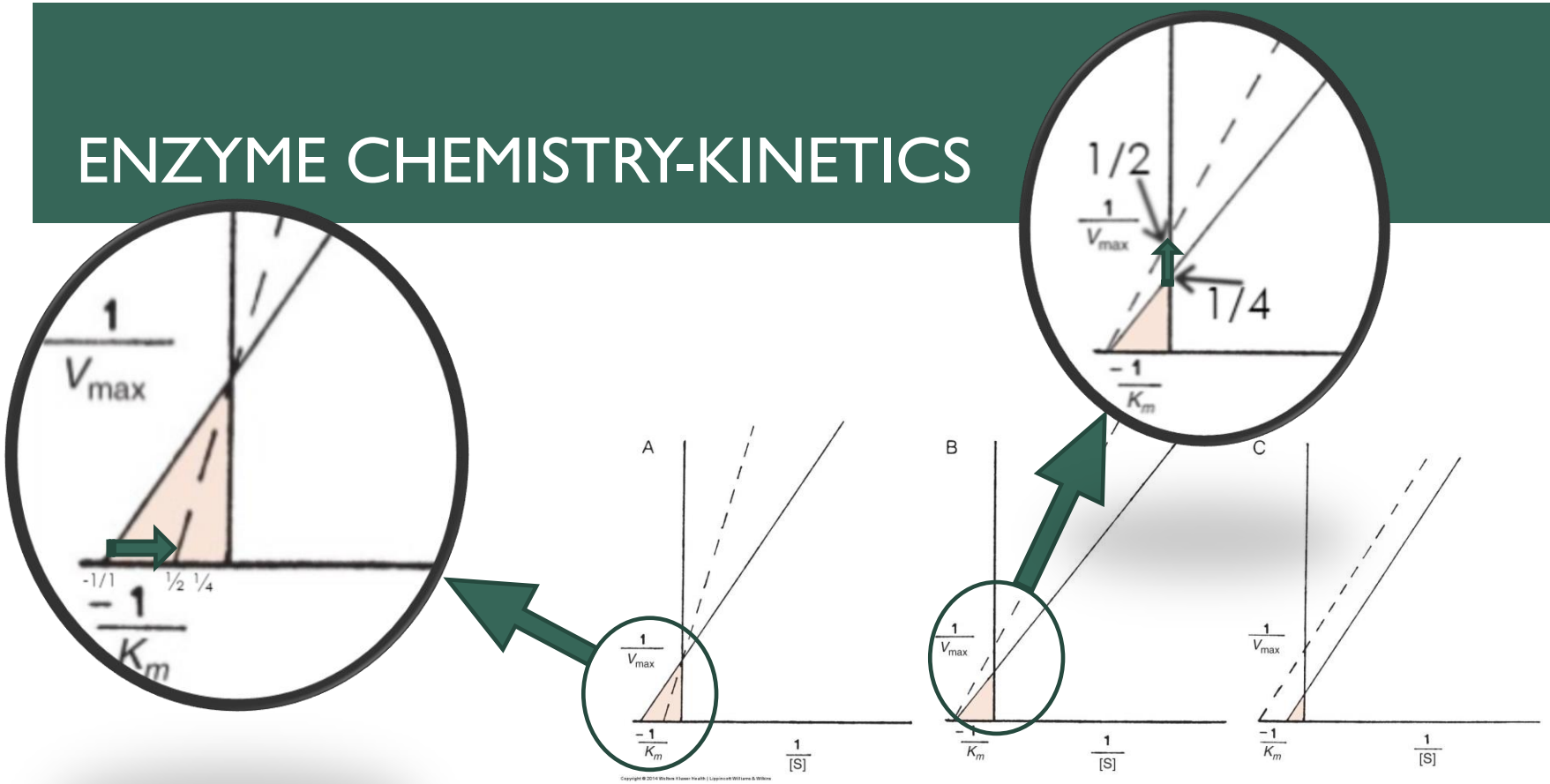
- Michaelis-Menten Constant
  - K_m is substrate concentration at which speed is ½ V_{max}
- Lineweaver-Burk Plot
- Inhibitors
  - Competitive
  - Noncompetitive
  - Uncompetitive







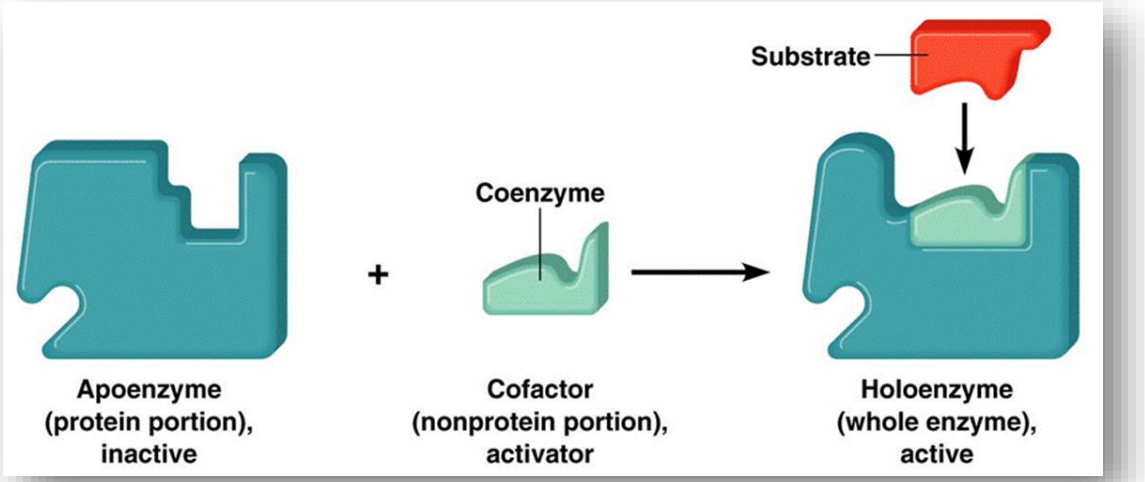
ENZYME CHEMISTRY-KINETICS



(A) Competitive inhibition Vmax unaltered; Km appears increased.
(B) Noncompetitive inhibition Vmax decreased; Km unchanged.
(C) Uncompetitive inhibition Vmax decreased; Km appears decreased.

ENZYME CHEMISTRY-FUNCTION

- Cofactors
  - Non-protein molecules necessary for function
    - Mg^{2+} Cl^{-} are activators (inorganic)
    - Coenzymes are organic like NAD
    - If bound to the enzyme, it is a prosthetic group
      - Coenzyme + apoenzyme = holoenzyme
    - Proenzymes/zymogen secreted inactive forms, altered to become active later



ENZYME CHEMISTRY-CLASSIFICATION

- Classification: Enzyme Commission
  - Oxidoreductases: catalyze redox reactions
  - Transferases: catalyze transfer of a group other than hydrogen
  - Hydrolases: catalyze hydrolysis of bonds
  - Lyases: removal of groups without hydrolysis, product has double bonds
  - Isomerases: catalyze geometric, optical or positional changes among isomers
  - Ligases: joining of two molecules and breaking of an energetic phosphate (or similar) bond

ENZYME CHEMISTRY-CLASSIFICATION

- Relevant Examples
  - Oxidoreuctase:
  - Transferase:
  - Hydrolase:
  - Lysase:
  - Isomerase:
  - Ligase:

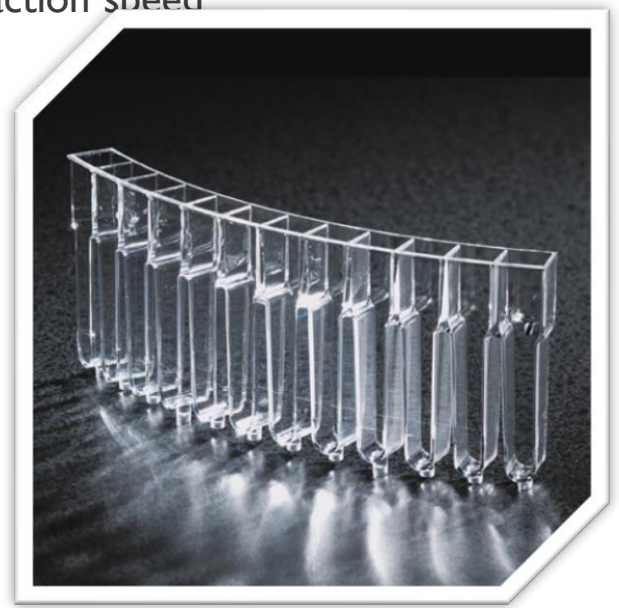
* NOT REALLY RELEVENAT

ENZYME CHEMISTRY-SPECIFICITY

- Group Specificity
  - Reacts will all substrates that have a particular group
- Bond Specificity
  - Catalyze reaction with substrates containing particular bond
- Absolute Specificity
  - Combines with only one substrate and catalyzes only one reaction
- Stereoisometric specificity
  - Enzymes that are specific for a specific isomer of substrate,
    - examples anyone?

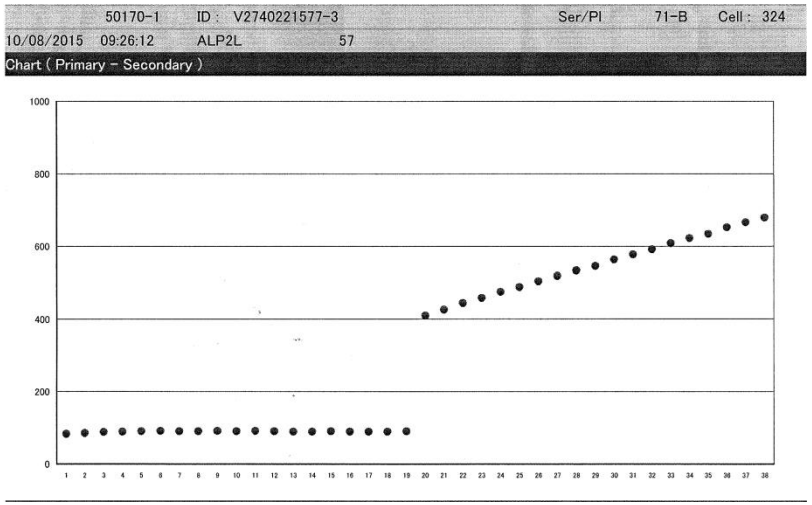
ENZYME CHEMISTRY-MEASUREMENT

- Present in small amounts
  - Measure enzyme activity instead!
  - Activity is related to enzyme concentration
  - Ensure substrate and coenzyme are in excess so amount of enzyme is what drives reaction speed
    - ?? Order Kinetics??
  - Coupled enzyme reactions also need excess
    - NADH absorbs at 340nm NAD doesn't
    - MUST BE IN EXCESS IF MEASURED
- Also must maintain pH and temperature



ENZYME CHEMISTRY-MEASUREMENT

- Fixed Time
  - Reagent added, reaction proceeds, reaction is stopped, and measurement made
- Continuous Monitoring (kinetic)
  - Reagent added, measurements taken every *x* seconds, linearity is seen, slope calculated
- Why wouldn't it be linear?
  - High [enzyme] leads to substrate depletion



ENZYME CHEMISTRY-MEASUREMENT

- Enzyme Activity Quantitation
  - They don't travel in mph so what do we call it?
    - International Unit 1 µmol of substrate catalyzed per minute
      - IU/L final unit
    - Katal (kat) mol/s of substrate concentration
      - kat/L
- Enzyme Mass Quantification
  - Immunoassays quantify by mass
    - May overestimate due to cross reactivity with zymogens etc…

ENZYME CHEMISTRY-MEASUREMENT

- Sometimes the substrate is the analyte
  - i.e. glucose, cholesterol, triglycerides
    - Enzyme must be added in excess
- ELISA assays use enzymes as reagent
  - HRP, ALP, G6PDH