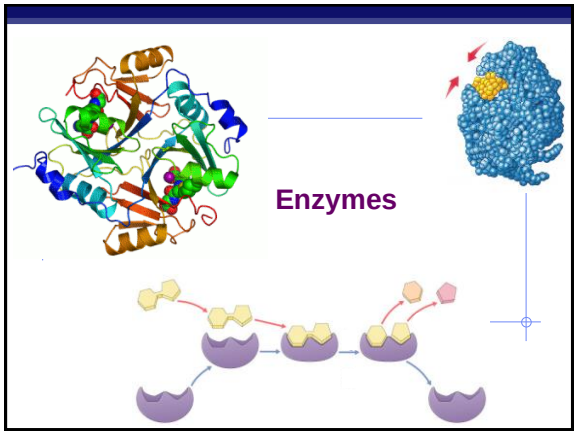
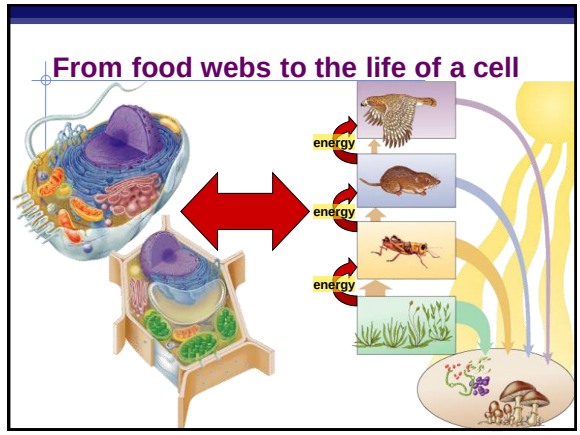
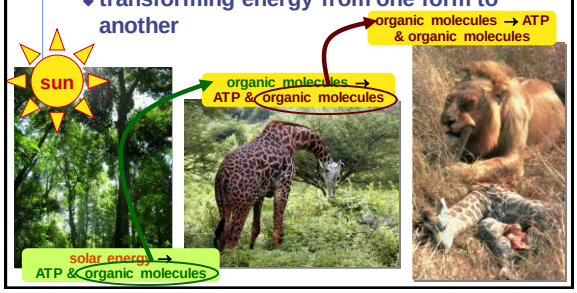
Enzymes





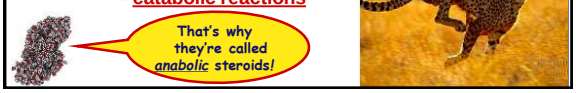
Flow of energy through life

- Life is built on chemical reactions
  - transforming energy from one form to another



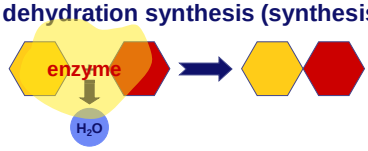
Metabolism

- Chemical reactions of life
  - forming bonds between molecules
    - dehydration synthesis
    - synthesis
    - anabolic reactions
  - breaking bonds between molecules
    - hydrolysis
    - digestion
    - catabolic reactions

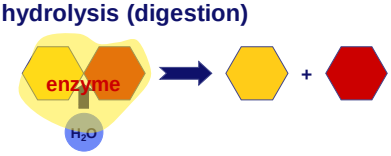


Examples

- dehydration synthesis (synthesis)

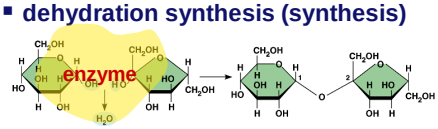


- hydrolysis (digestion)

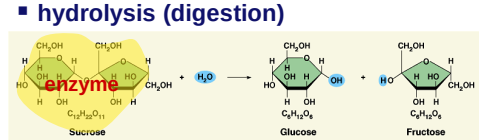


Examples

- dehydration synthesis (synthesis)



- hydrolysis (digestion)



Enzymes

Chemical reactions & energy

- Some chemical reactions release energy
  - exergonic
  - digesting polymers
  - hydrolysis = catabolism
- Some chemical reactions require input of energy
  - endergonic
  - building polymers
  - dehydration synthesis = anabolism

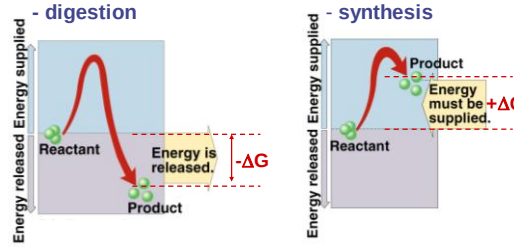
digesting molecules= LESS organization= lower energy state

building molecules= MORE organization= higher energy state

Endergonic vs. exergonic reactions

exergonic
- energy released
- digestion

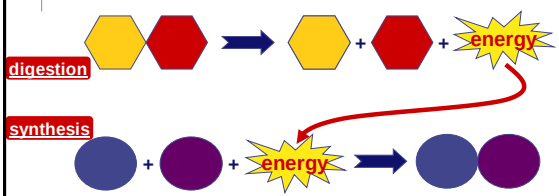
endergonic
- energy invested
- synthesis



ΔG = change in free energy = ability to do work

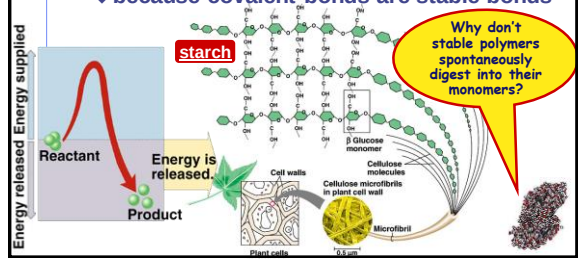
Energy & life

- Organisms require energy to live
  - where does that energy come from?
    - coupling exergonic reactions (releasing energy) with endergonic reactions (needing energy)



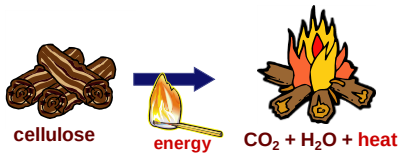
What drives reactions?

- If reactions are "downhill", why don't they just happen spontaneously?
  - because covalent bonds are stable bonds



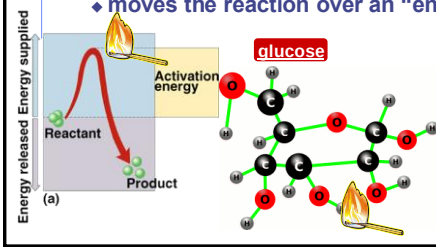
Activation energy

- Breaking down large molecules requires an initial input of energy
  - activation energy
  - large biomolecules are stable
  - must absorb energy to break bonds



Too much activation energy for life

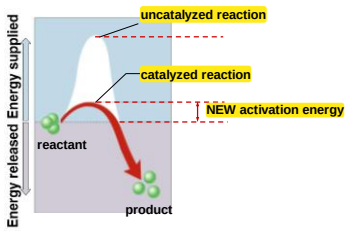
- Activation energy
  - amount of energy needed to destabilize the bonds of a molecule
  - moves the reaction over an "energy hill"



Enzymes

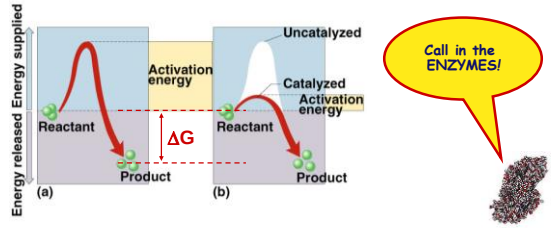
Reducing Activation energy

- Catalysts
  - reducing the amount of energy to start a reaction

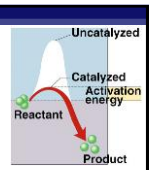


Catalysts

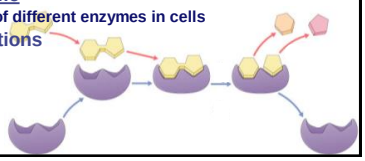
- So what's a cell got to do to reduce activation energy?
  - get help! … chemical help… ENZYMES



Enzymes



- Biological catalysts
  - proteins (& RNA)
  - facilitate chemical reactions
    - increase rate of reaction without being consumed
    - reduce activation energy
    - don't change free energy (ΔG) released or required
  - required for most biological reactions
  - highly specific
    - thousands of different enzymes in cells
  - control reactions of life

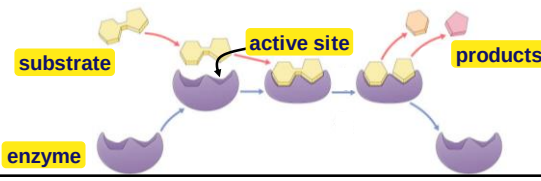


Enzymes vocabulary

substrate
- reactant which binds to enzyme
- enzyme-substrate complex: temporary association

product
- end result of reaction

active site
- enzyme's catalytic site; substrate fits into active site

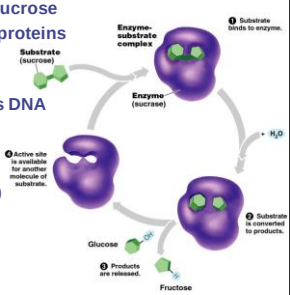


Properties of enzymes

- Reaction specific
  - each enzyme works with a specific substrate
    - chemical fit between active site & substrate
      - H bonds & ionic bonds
- Not consumed in reaction
  - single enzyme molecule can catalyze thousands or more reactions per second
    - enzymes unaffected by the reaction
- Affected by cellular conditions
  - any condition that affects protein structure
    - temperature, pH, salinity

Naming conventions

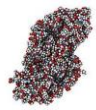
- Enzymes named for reaction they catalyze
  - sucrase breaks down sucrose
  - proteases break down proteins
  - lipases break down lipids
  - DNA polymerase builds DNA
    - adds nucleotides to DNA strand
  - pepsin breaks down proteins (polypeptides)

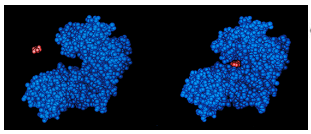


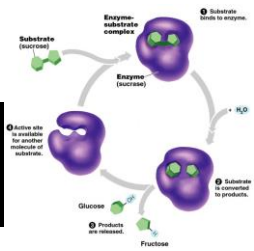
Enzymes

Lock and Key model

- Simplistic model of enzyme action
  - substrate fits into 3-D structure of enzyme' active site
    - H bonds between substrate & enzyme
  - like "key fits into lock"

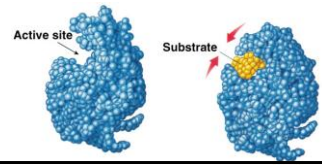






Induced fit model

- More accurate model of enzyme action
  - 3-D structure of enzyme fits substrate
  - substrate binding cause enzyme to change shape leading to a tighter fit
    - "conformational change"
    - bring chemical groups in position to catalyze reaction

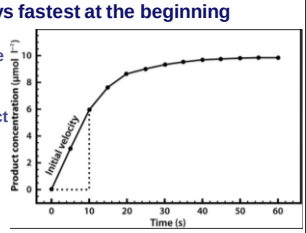


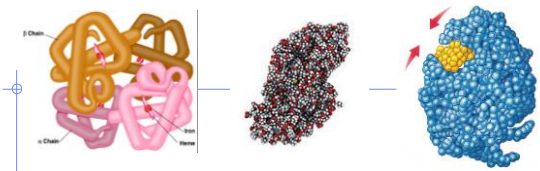
How does it work?

- Variety of mechanisms to lower activation energy & speed up reaction
  - synthesis
    - active site orients substrates in correct position for reaction
      - enzyme brings substrate closer together
  - digestion
    - active site binds substrate & puts stress on bonds that must be broken, making it easier to separate molecules

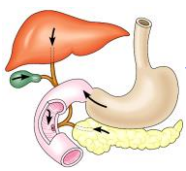
Measuring Reaction Rates

- Generally, enzyme reactions slow over time in their rate
  - Substrate is getting used up
  - Enzymes are less likely to collide with their substrate
- Reaction rate is always fastest at the beginning
  - Initial rate of reaction: calculate by finding the slope of a line tangent to the curve
  - Can also divide product produced in a given quantity of time



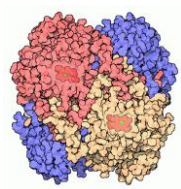


Factors that Affect Enzymes



Factors Affecting Enzyme Function

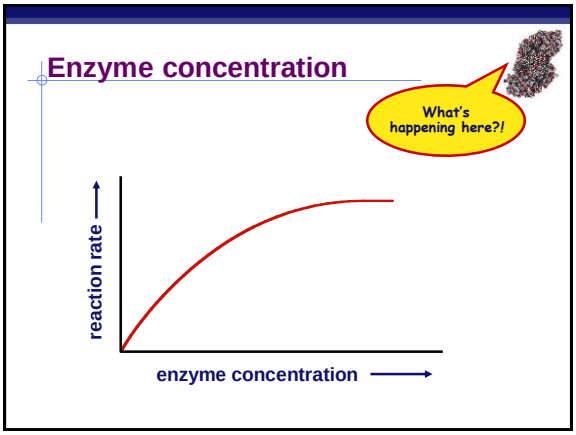
- Enzyme concentration
- Substrate concentration
- Temperature
- pH
- Salinity
- Activators
- Inhibitors



catalase

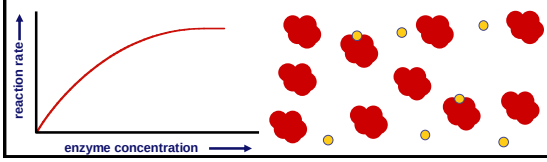
Enzymes

Enzyme concentration

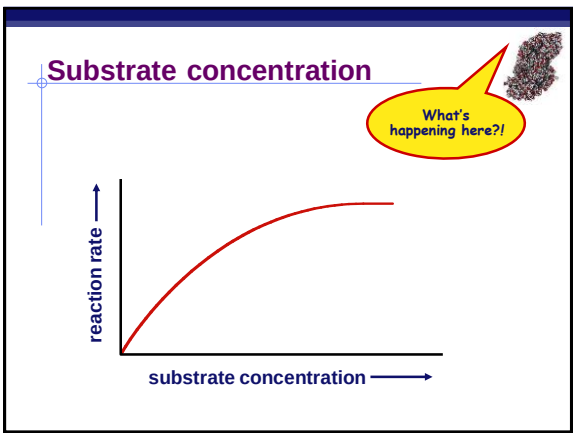


Factors affecting enzyme function

- Enzyme concentration
  - as ↑ enzyme = ↑ reaction rate
    - more enzymes = more frequently collide with substrate
  - reaction rate levels off
    - substrate becomes limiting factor
    - not all enzyme molecules can find substrate

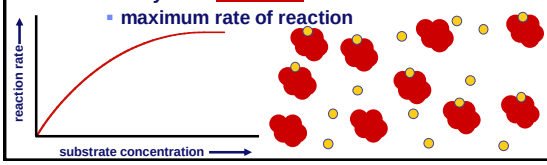


Substrate concentration

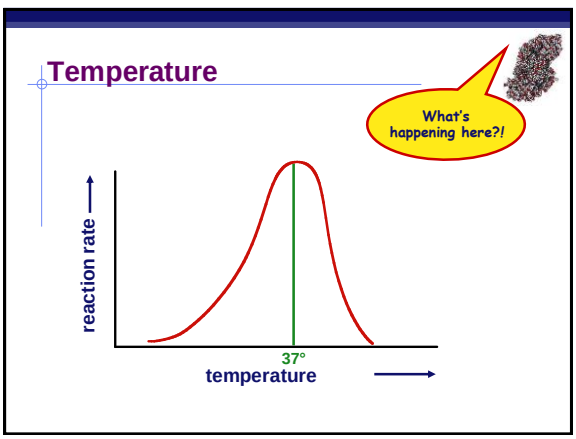


Factors affecting enzyme function

- Substrate concentration
  - as ↑ substrate = ↑ reaction rate
    - more substrate = more frequently collide with enzyme
  - reaction rate levels off
    - all enzymes have active site engaged
    - enzyme is saturated
    - maximum rate of reaction



Temperature



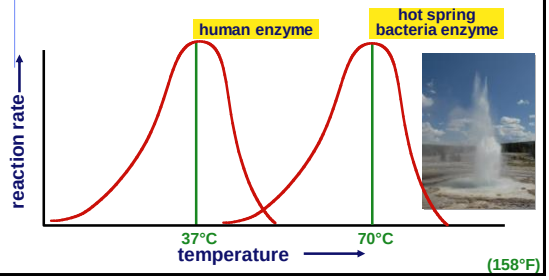
Factors affecting enzyme function

- Temperature
  - Optimum T°
    - greatest number of molecular collisions
    - human enzymes = 35°- 40°C
      - body temp = 37°C
  - Heat: increase beyond optimum T°
    - increased energy level of molecules disrupts bonds in enzyme & between enzyme & substrate
      - H, ionic = weak bonds
    - denaturation = lose 3D shape (3° structure)
  - Cold: decrease T°
    - molecules move slower
    - decrease collisions between enzyme & substrate

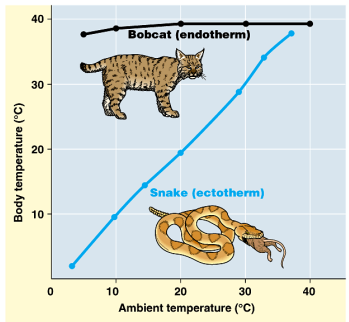
Enzymes

Enzymes and temperature

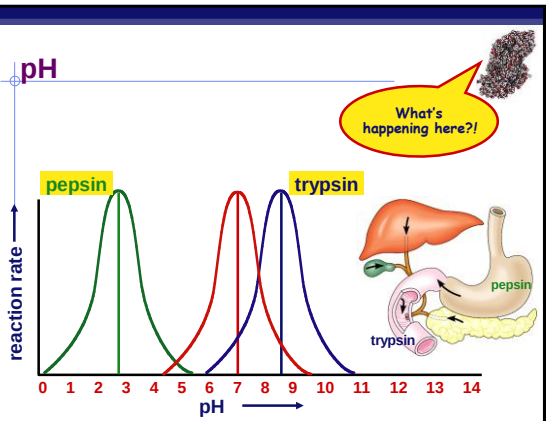
- Different enzymes function in different organisms in different environments



How do ectotherms do it?

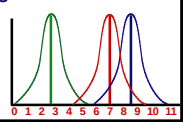


pH

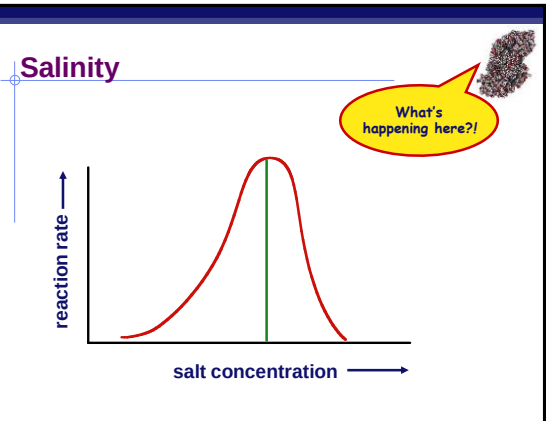


Factors affecting enzyme function

- pH
  - changes in pH
    - adds or remove H^+
    - disrupts bonds, disrupts 3D shape
      - disrupts attractions between charged amino acids
      - affect 2° & 3° structure
      - denatures protein
  - optimal pH?
    - most human enzymes = pH 6-8
      - depends on localized conditions
      - pepsin (stomach) = pH 2-3
      - trypsin (small intestines) = pH 8



Salinity



Factors affecting enzyme function

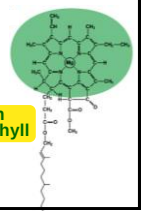
- Salt concentration
  - changes in salinity
    - adds or removes cations (+) & anions (–)
    - disrupts bonds, disrupts 3D shape
      - disrupts attractions between charged amino acids
      - affect 2° & 3° structure
      - denatures protein
  - enzymes intolerant of extreme salinity
    - Dead Sea is called dead for a reason!

Enzymes

Compounds which help enzymes

- Activators
  - cofactors
    - non-protein, small inorganic compounds & ions
      - Mg, K, Ca, Zn, Fe, Cu
      - bound within enzyme molecule
  - coenzymes
    - non-protein, organic molecules
      - bind temporarily or permanently to enzyme near active site
    - many vitamins
      - NAD (niacin; B3)
      - FAD (riboflavin; B2)
      - Coenzyme A

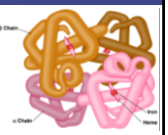
Fe in hemoglobin



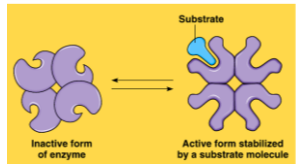
Mg in chlorophyll

Cooperativity

- Substrate acts as an activator
  - substrate causes conformational change in enzyme
    - induced fit
  - favors binding of substrate at 2nd site
  - makes enzyme more active & effective
    - hemoglobin

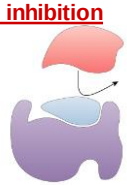


Hemoglobin
- 4 polypeptide chains
- can bind 4 O_2;
- 1st O_2 binds
- now easier for other 3 O_2 to bind



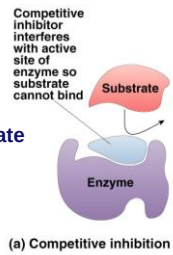
Compounds which regulate enzymes

- Inhibitors
  - molecules that reduce enzyme activity
  - competitive inhibition
  - noncompetitive inhibition
  - irreversible noncompetitive inhibition
  - feedback inhibition



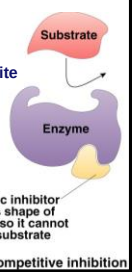
Competitive Inhibitor

- Inhibitor & substrate "compete" for active site
  - penicillin
    blocks enzyme bacteria use to build cell walls
  - disulfiram (Antabuse)
    treats chronic alcoholism
    - blocks enzyme that breaks down alcohol
    - severe hangover & vomiting 5-10 minutes after drinking
- Overcome by increasing substrate concentration
  - saturate solution with substrate so it out-competes inhibitor for active site on enzyme



Non-Competitive Inhibitor

- Inhibitor binds to site other than active site
  - allosteric inhibitor binds temporarily to allosteric site
  - causes enzyme to change shape
    - conformational change
    - active site is no longer functional binding site
      - keeps enzyme inactive
  - some anti-cancer drugs
    inhibit enzymes involved in DNA synthesis
    - stop DNA production
    - stop division of more cancer cells
  - cyanide poisoning
    irreversible inhibitor of Cytochrome C, an enzyme in cellular respiration
    - stops production of ATP



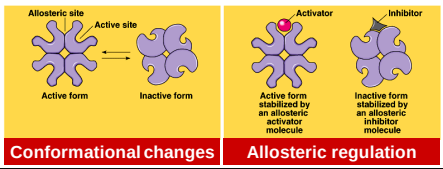
Irreversible inhibition

- Inhibitor permanently binds to enzyme
  - Non-competitive competitor
    - permanently binds to active site
  - allosteric
    - permanently binds to allosteric site
    - permanently changes shape of enzyme
    - nerve gas, sarin, many insecticides (malathion, parathion…)
      - cholinesterase inhibitors
        - doesn't breakdown the neurotransmitter, acetylcholine

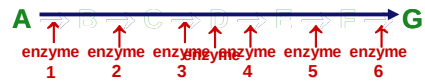
Enzymes

Allosteric regulation

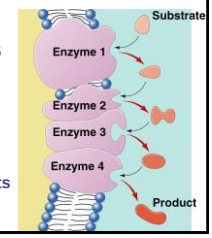
- Conformational changes by regulatory molecules
  - inhibitors
    - keeps enzyme in inactive form
  - activators
    - keeps enzyme in active form



Metabolic pathways

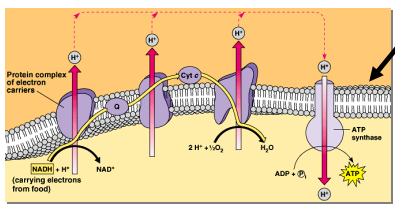


- Chemical reactions of life are organized in pathways
  - divide chemical reaction into many small steps
    - artifact of evolution
    - ↑ efficiency
      - intermediate branching points
    - ↑ control = regulation



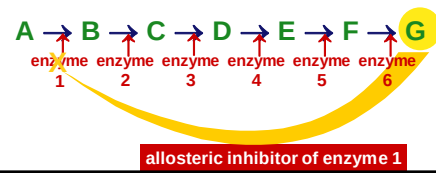
Efficiency

- Organized groups of enzymes
  - enzymes are embedded in membrane and arranged sequentially
- Link endergonic & exergonic reactions



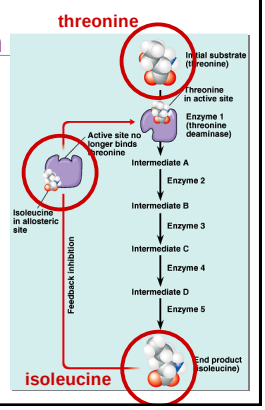
Feedback Inhibition

- Regulation & coordination of production
  - product is used by next step in pathway
  - final product is inhibitor of earlier step
    - allosteric inhibitor of earlier enzyme
    - feedback inhibition
  - no unnecessary accumulation of product

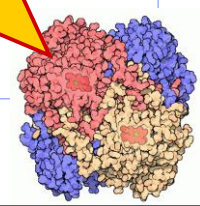


Feedback inhibition

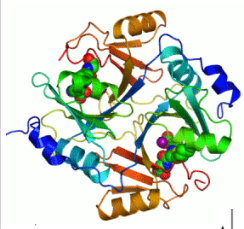
- Example
  - synthesis of amino acid, isoleucine from amino acid, threonine
  - isoleucine becomes the allosteric inhibitor of the first step in the pathway
    - as product accumulates it collides with enzyme more often than substrate does

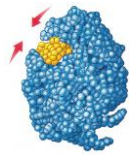


Don't be inhibited! Ask Questions!

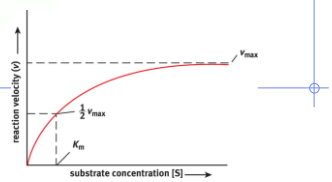


Enzymes



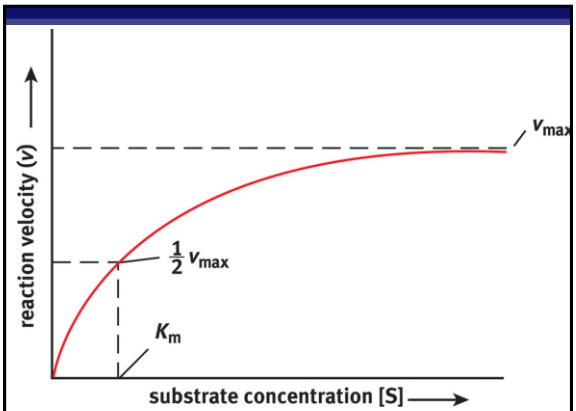


Enzyme Kinetics



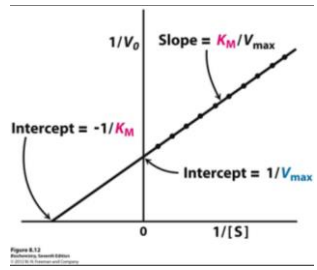
Enzyme Affinities

- Previous factors and the biochemistry of the enzyme and substrate affect affinity
  - Turnover rate: how fast an enzyme converts substrate into product
  - V_{max}: theoretical maximum rate of an enzyme catalyzed reaction
    - Enzyme is saturated with Substrate
- The relationship between rate of reaction and concentration of substrate depends on the affinity of the enzyme for its substrate.
  - Expressed as the K_m, or the Michaelis-Menten constant
  - Inverse measure of affinity
  - K_m is the concentration of substrate which lets the enzyme reach ½ V_{max}

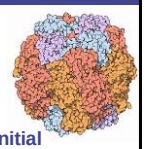


The Lineweaver-Burke Plot

- Double reciprocal plot used to extrapolate Km and Vmax



Uses of Km and Vmax



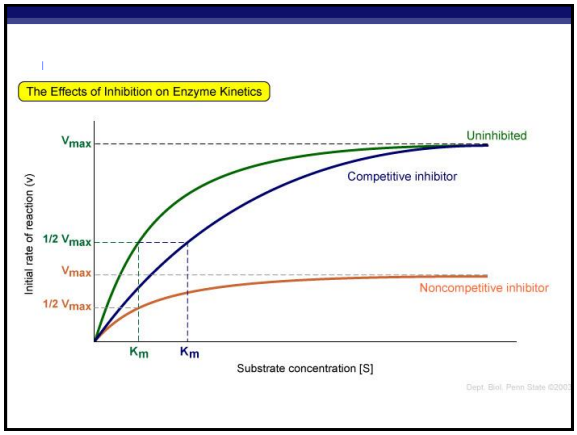
- Higher affinities mean lower K_m
  - Lower [S] will reach ½ V_{max}
  - On a graph this will create a steeper initial reaction rate
- Can also be used to compare substrate affinities

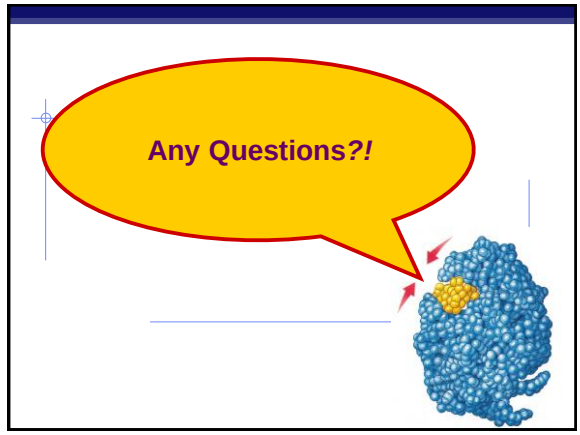
  - RuBisCO: Ribulose Bisphosphate Carboxylase Oxidase
    - Enzyme binds to both CO_2 and O_2
    - RuBisCO fixes CO_2 to make glucose, but in high [O_2] environments, it bonds to O_2 instead
    - Km CO_2 for RUBISCO = 12 μM
    - Km O_2 for RUBISCO = 250 μM
    - This makes PROBLEMS in our current atmosphere!

How do inhibitors affect kinetics?

- Competitive inhibitor
  - K_m increases in the presence of competitive inhibitor
    - Need HIGHER substrate concentration to OUTCOMPETE inhibitor
  - V_{max} stays the same
    - Saturation point
    - At infinite [S], combination with inhibitor is statistically impossible
- Noncompetitive inhibitor
  - K_m stays the same
    - Higher [S] doesn't affect reaction rate because it is noncompetitive
  - V_{max} decreases
    - No way of outcompeting inhibitor

Enzymes


The Effects of Inhibition on Enzyme Kinetics
Uninhibited
Competitive inhibitor
Noncompetitive inhibitor
Vmax
1/2 Vmax
Vmax
1/2 Vmax
Initial rate of reaction (v)
Km
Km
Substrate concentration [S]


Any Questions?!