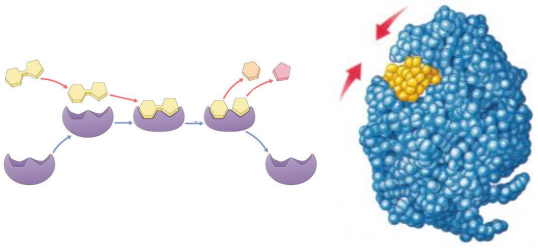
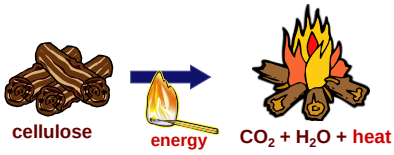
# Enzymes



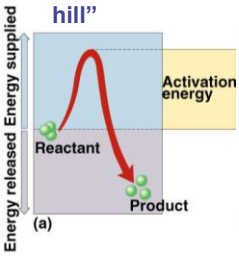
## Activation Energy

- Chemical reactions require an initial input of energy
  - activation energy
  - large biomolecules are stable
  - must absorb energy to break bonds



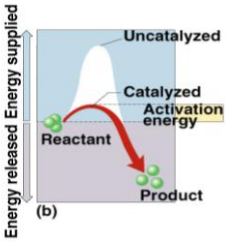

## Activation Energy

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



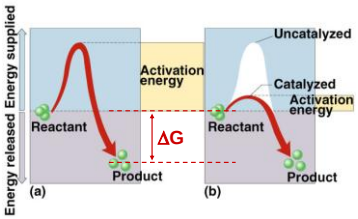

## Reducing Activation Energy

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

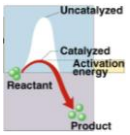



## Catalysts

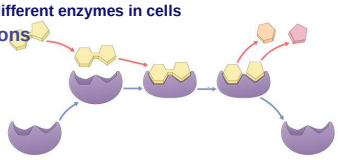
- So what's a cell to do to reduce activation energy?
  - get help! … chemical help… ENZYMES




## Enzymes



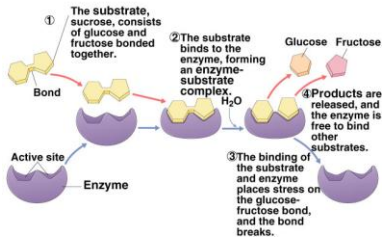

- Biological catalysts
  - proteins (& RNA—ribozymes!)
  - facilitate chemical reactions
    - increase rate of reaction without being consumed
    - reduce activation energy
    - don't change free energy (ΔG) released or required
  - required for most biological reactions
  - highly specific
    -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



## Enzymes & Substrates

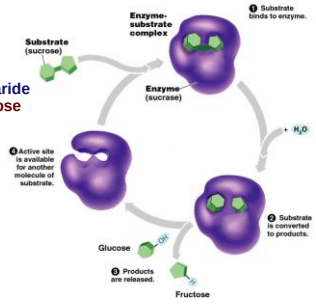
**substrate**
- reactant which binds to enzyme
- enzyme-substrate complex: temporary association

**product**
- end result of reaction



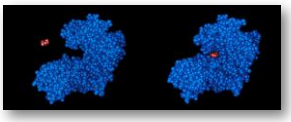

## Enzymes & Substrates

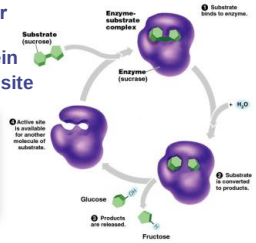
- Enzyme + substrates → products
  - sucrase
    - enzyme breaks down sucrose
    - binds to sucrose and breaks disaccharide into fructose & glucose
  - DNA polymerase
    - enzyme builds DNA
    - adds nucleotides to a growing DNA strand




## Lock and Key Model

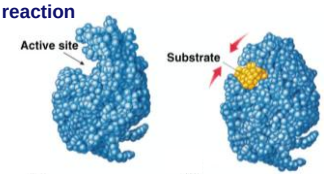
- Simplistic model of enzyme action
  - 3-D structure of enzyme fits substrate
- Active site
  - enzyme's catalytic center
  - pocket or groove on surface of globular protein
  - substrate fits into active site






## Induced Fit Model

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




## How does it work?

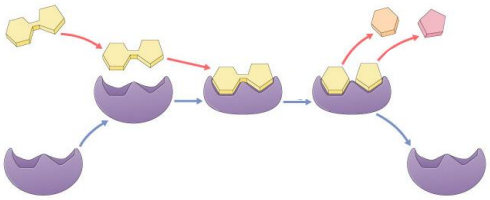
- Variety of mechanisms to lower activation energy & speed up reaction
  - active site orients substrates in correct position for reaction
    - enzyme brings substrate closer together
  - active site binds substrate & puts stress on bonds that must be broken, making it easier to separate molecules
  - groups near the active site can add a chemical charge for re-dox reactions

## Specificity of Enzymes

- Reaction specific
  - each enzyme is substrate-specific
    - due to fit between active site & substrate
      - substrates held in active site by weak interactions
        - H bonds
        - ionic bonds
  - enzymes named for reaction they catalyze
    - sucrase breaks down sucrose
    - proteases break down proteins
    - lipases break down lipids
    - DNA polymerase builds DNA
    - pepsin breaks down proteins (polypeptides)

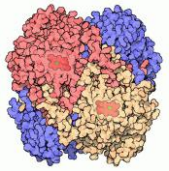
## Reusable

- Not consumed in reaction!
  - single enzyme molecule can catalyze thousands or more reactions per second
  - enzymes unaffected by the reaction



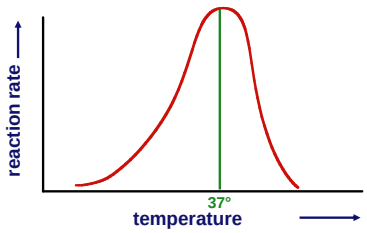
## Factors Affecting Enzymes

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



catalase

## Temperature

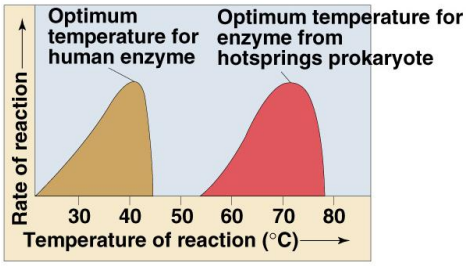



## Temperature

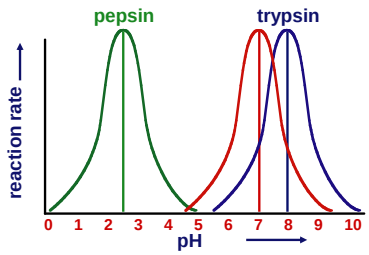
- Effect on rates of enzyme activity
  - Decrease T°
    - molecules move slower
    - decrease collisions
  - Optimum T°
    - greatest number of molecular collisions
    - human enzymes = 35°- 40°C (body temp = 37°C)
  - Increase beyond optimum T°
    - increased agitation of molecules disrupts bonds
      - H, ionic = weak bonds
    - denaturation = lose 3D shape (3° structure)

## Temperature

- Different enzymes functional in different organisms



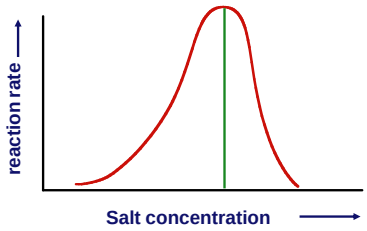

## pH



## pH

- Effect on rates of enzyme activity
  - pH changes
    - changes charges (add or remove $H^+$)
    - disrupt bonds, disrupt 3D shape
      - affect 3° structure
  - most human enzymes = pH 6-8
    - depends on localized conditions
    - pepsin (stomach) = pH 3
    - trypsin (small intestines) = pH 8

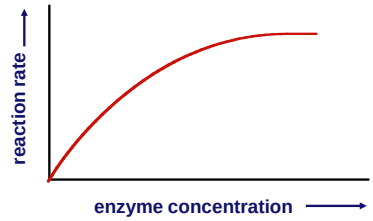
## Salinity (salt concentration)




## Salinity (salt concentration)

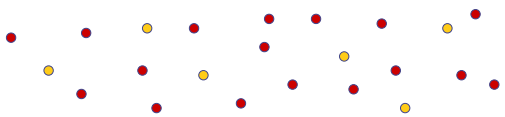
- Effect on rates of enzyme activity
  - protein shape (conformation)
    - depends on attraction of charged amino acids
  - salinity changes
    - change [inorganic ions]
    - changes charges (add + or –)
    - disrupt bonds, disrupt 3D shape
      - affect 3° structure
  - enzymes intolerant of extreme salinity
    - Dead Sea is called dead for a reason!

## [Enzyme]

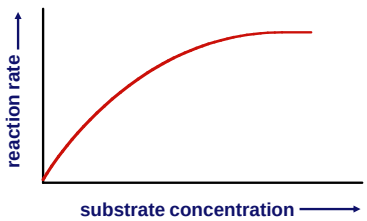



## [Enzyme]

- Effect on rates of enzyme activity
  - 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

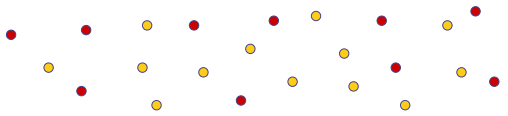


## [Substrate]



## [Substrate]

- Effect on rates of enzyme activity
  - as ↑ substrate = ↑ reaction rate
    - more substrate = more frequently collide with enzymes
  - reaction rate levels off
    - all enzymes have active site engaged
    - enzyme is saturated; is the limiting factor
    - maximum rate of reaction



## [Substrate]

$$E + S \longrightarrow ES \longrightarrow E + P$$

**ES = Enzyme-substrate complex**

formed when substrates fit into the active site of the enzyme

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

## Michaelis – Menten Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

**Assumptions:**

- The binding step of E + S ←→ ES is fast
- ES immediately comes to a steady state, so [ES] is constant
- The catalytic step of ES ←→ E + P is slower, thus rate-limiting

## Michaelis – Menten Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

**Assumptions:**

- At early points of the reaction, where initial velocity, $V_o$ , is measured, [P] = 0
- The enzyme exists in two forms, free E, and substrate-bound E (forming ES); therefore, the total enzyme concentration, ($E_T$), is the sum of both $[E_T] = [E] + [ES]$

## Michaelis – Menten Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

**Assumptions:**

- $[S] >> [E_T]$, so the fraction of S that binds to E (to form ES) is negligible, and [S] is constant at early time points

## Michaelis – Menten Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

- $k_1$ and $k_{-1}$ represent rapid noncovalent association of substrate with enzyme's active site
- $k_2$ = rate constant for the chemical conversion of S to P, the rate-limiting step
- $k_{-2}$ = very small…

## Michaelis – Menten Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

$$K_m = \frac{k_{-1} + k_2}{k_1}$$

**Michaelis Constant $K_m$**

- is the measure of the affinity of E for S
- **inverse relationship:** when $K_m$ is small, affinity is great!
- determined by ½ $V_{max}$!

## Michaelis – Menten Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

- $k_1$ and $k_{-1}$ represent rapid noncovalent association of substrate with enzyme's active site
- $k_2$ = rate constant for the chemical conversion of S to P, the **rate-limiting step**
- $k_{-2}$ = very small…
- $V_o$ = **initial velocity**, ignore reverse reaction, measure rate before P accumulates

$$V_o = k_2 [ES]$$

## Michaelis – Menten Kinetics

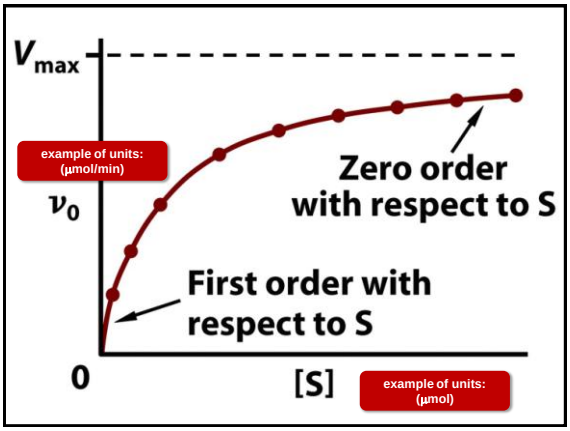
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

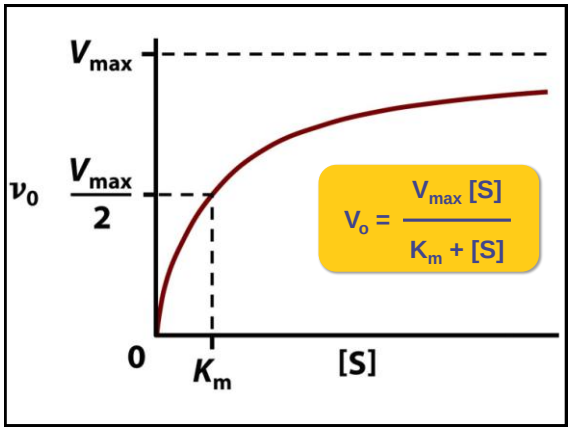
$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

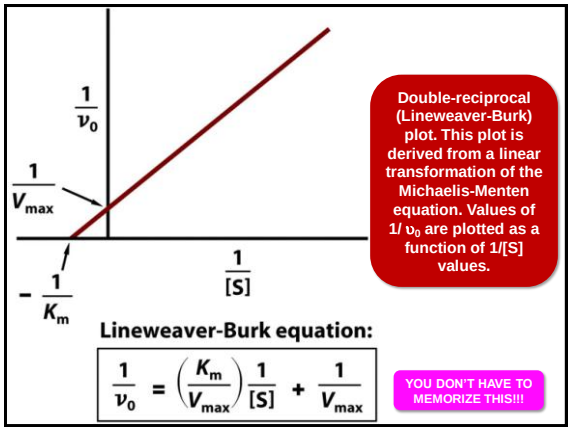
**Michaelis – Menten Equation derived…**

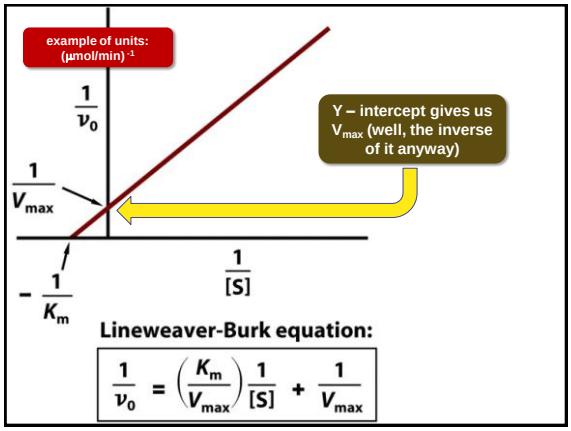
- in terms of initial velocity, maximum velocity, enzyme constant, and substrate concentration

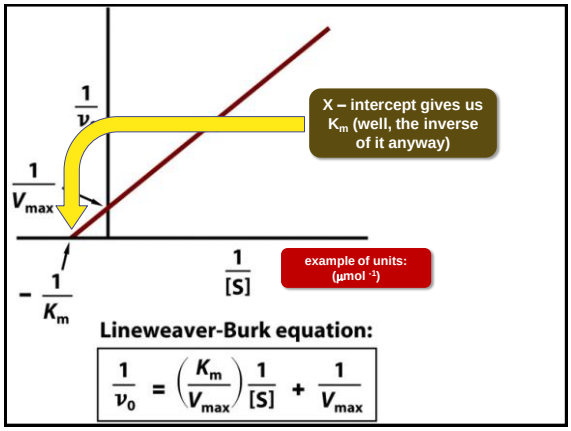
see handout for complete derivation



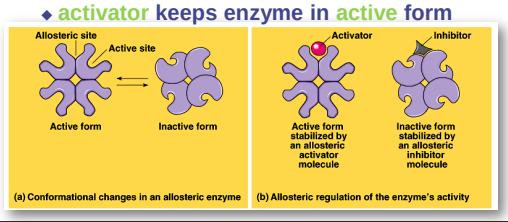





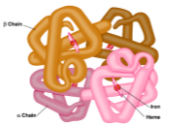

## Action of Allosteric Control

- Inhibitors & Activators
  - regulatory molecules attach to allosteric site (not active site) causing conformational (shape) change
  - inhibitor keeps enzyme in inactive form
  - activator keeps enzyme in active form

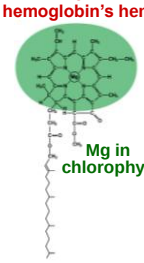



## Activators

- Compounds which help enzymes
- Prosthetic groups
  - non-amino acid groups bound to enzymes
    - heme group in hemoglobin
- Cofactors
  - non-protein, small inorganic compounds & ions
    - Mg, K, Ca, Zn, Fe, Cu
    - bound in 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



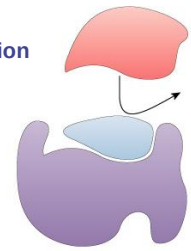
Fe in hemoglobin's heme



Mg in chlorophyll

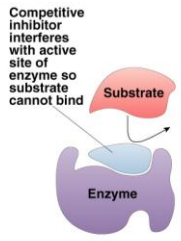
## Inhibitors

- Regulation of enzyme activity
  - other molecules that affect enzyme activity
- Selective inhibition & activation
  - competitive inhibition
  - noncompetitive inhibition
  - irreversible inhibition
  - feedback inhibition



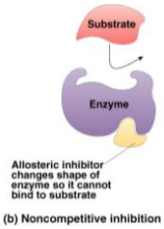
## Competitive Inhibitor

- Effect
  - inhibitor & substrate "compete" for active site
    - ex: penicillin blocks enzyme that bacteria use to build cell walls
    - ex: disulfiram (Antabuse) to overcome alcoholism
  - overcome by increasing substrate concentration
    - saturate solution with substrate so it out-competes inhibitor for active site on enzyme
    - ex: methanol poisoning



## Non-Competitive Inhibitor

- Effect
  - inhibitor binds to site other than active site
    - allosteric site
    - called allosteric inhibitor
      - ex: some anti-cancer drugs inhibit enzymes involved in synthesis of nucleotides & therefore in building of DNA = stop DNA production, stopping abnormal division
      - ex: heavy metal poisoning
      - ex: cyanide poisoning
    - causes enzyme to have a conformational shape change
    - renders active site unreceptive




## Irreversible Inhibition

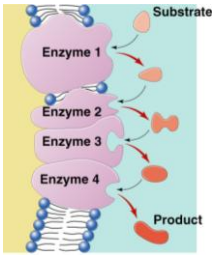
- Inhibitor permanently binds to enzyme
  - competitor
    - permanently binds to active site
  - allosteric
    - permanently changes shape of enzyme
    - ex: nerve gas, sarin, many insecticides (malathion, parathion…)
      - DIPF (diisopropylphosphorofluoridate) is an…
      - acetylecholinesterase inhibitor—doesn't breakdown the neurotransmitter, acetylcholine, which is vital for muscle contraction

## Metabolic Pathways

A → B → C → D → E → F → G

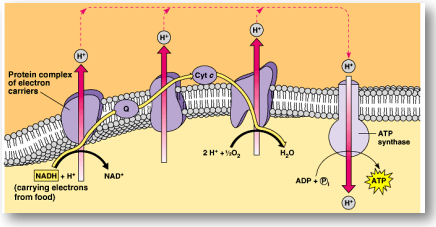
enzyme 1, enzyme 2, enzyme 3, enzyme 4, enzyme 5, enzyme 6

- Chemical reactions of life are organized in pathways
  - divide chemical reaction into many small steps
    - efficiency
    - control = regulation




## Efficiency

- Groups of enzymes organized
  - if enzymes are embedded in membrane they are 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

A → B → C → D → E → F → G

enzyme 1, enzyme 2, enzyme 3, enzyme 4, enzyme 5, enzyme 6

G is an allosteric inhibitor of enzyme 1

## Feedback Inhibition

- Example
  - synthesis of amino acid, isoleucine from amino acid, threonine