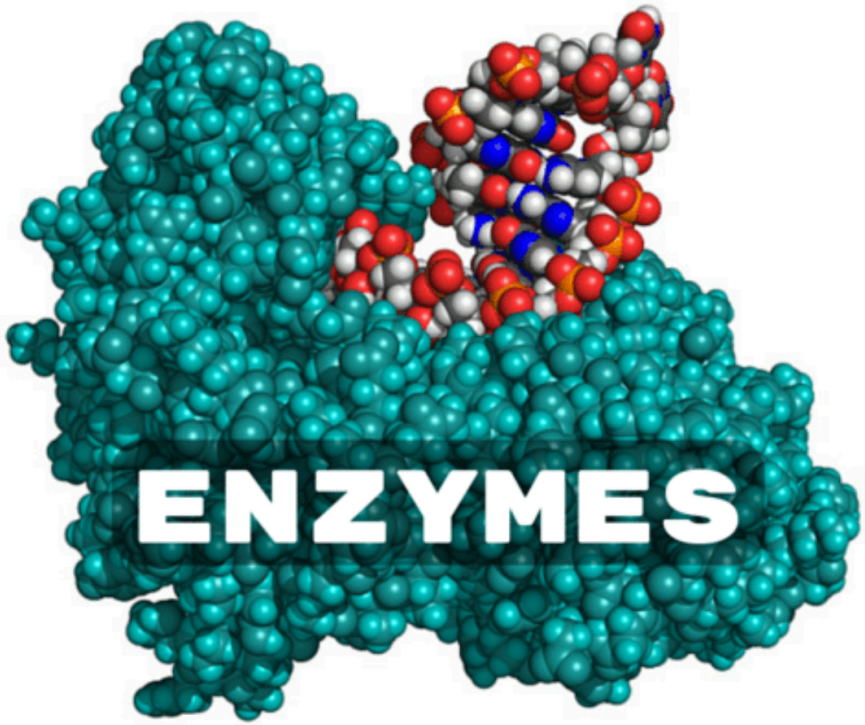
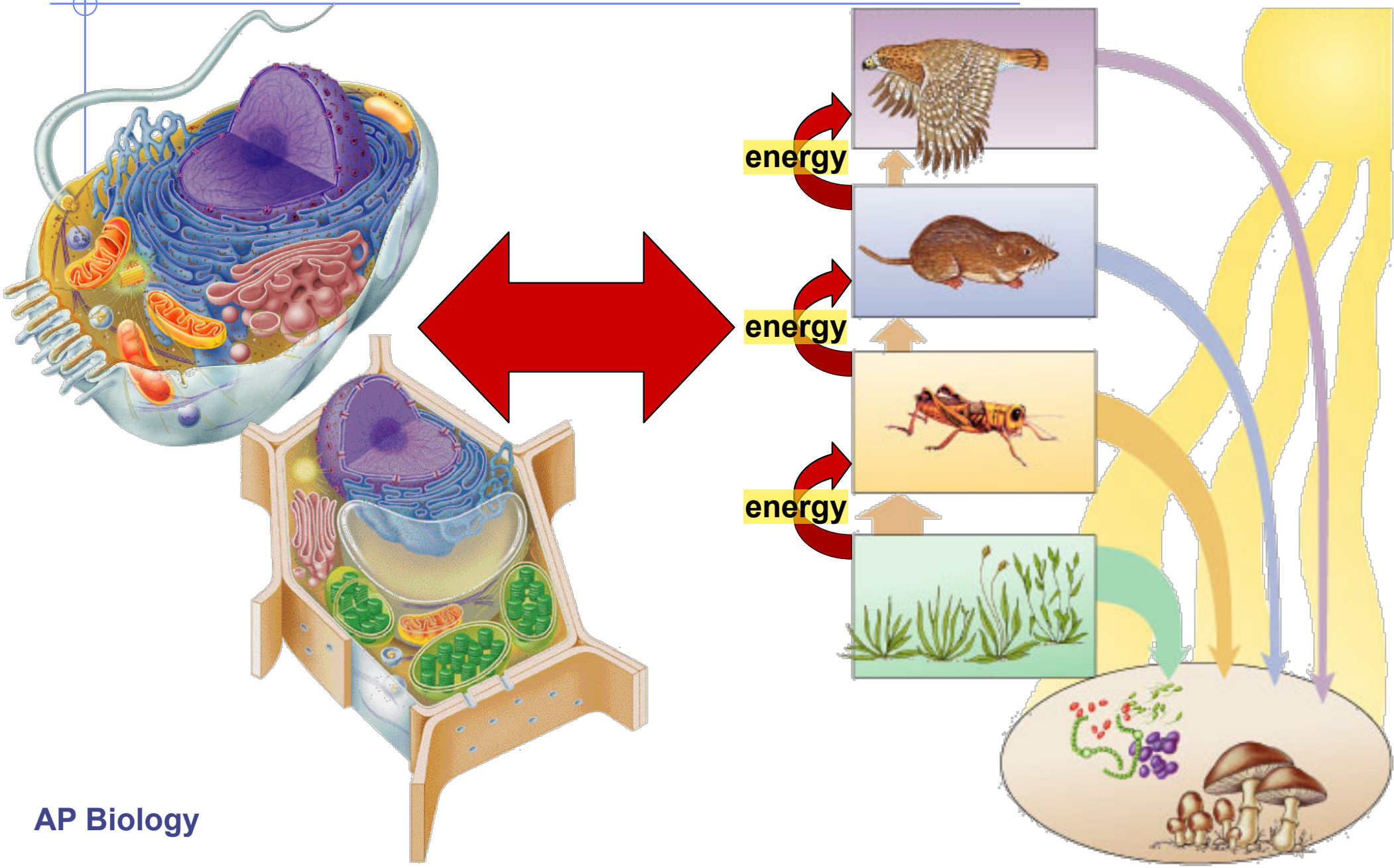
Ch. 8

Metabolism & Enzymes

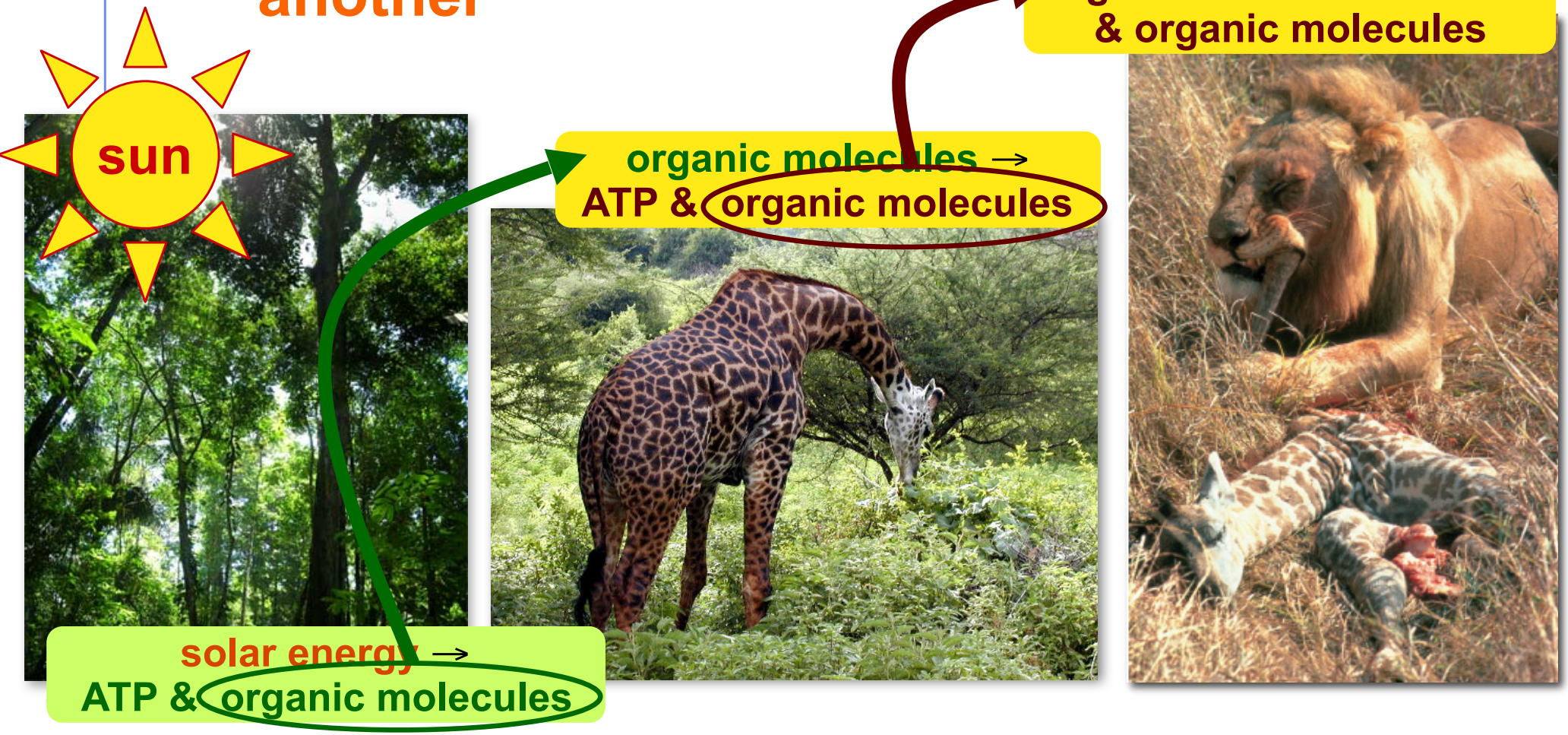


From food webs to the life of a cell



Flow of energy through life

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

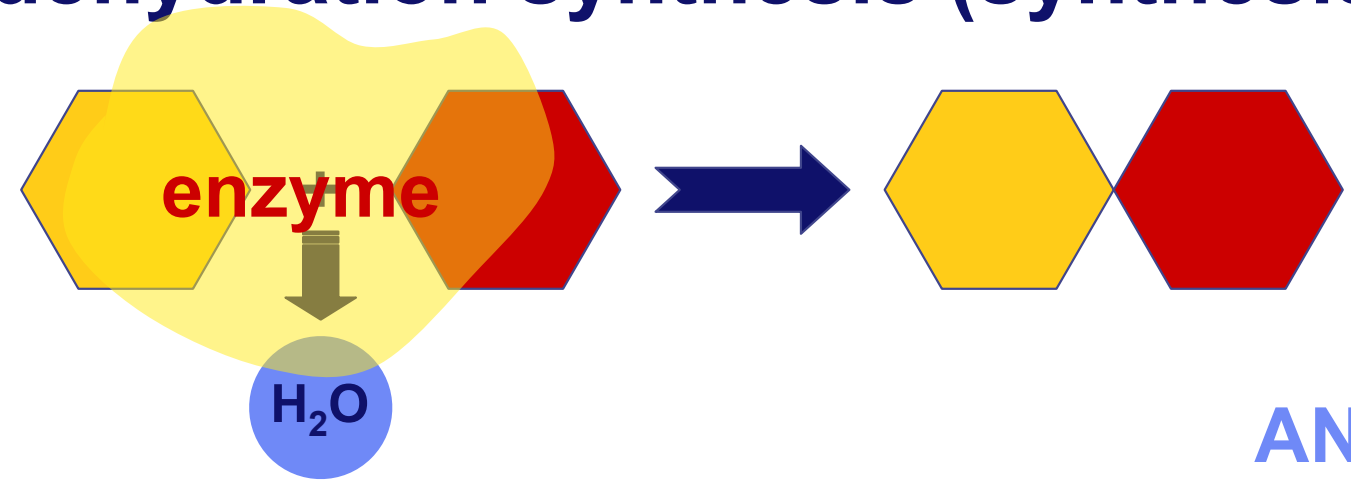


Metabolism (Greek "metabole" = change)

- Chemical reactions of life involve…
  - forming bonds between molecules
    - dehydration synthesis reactions
    - purpose: synthesis
    - anabolic reactions - use up energy to build complicated molecules (biosynthesis pathways)
  - breaking bonds between molecules
    - hydrolysis reactions
    - purpose: digestion
    - catabolic reactions - (breakdown pathways)

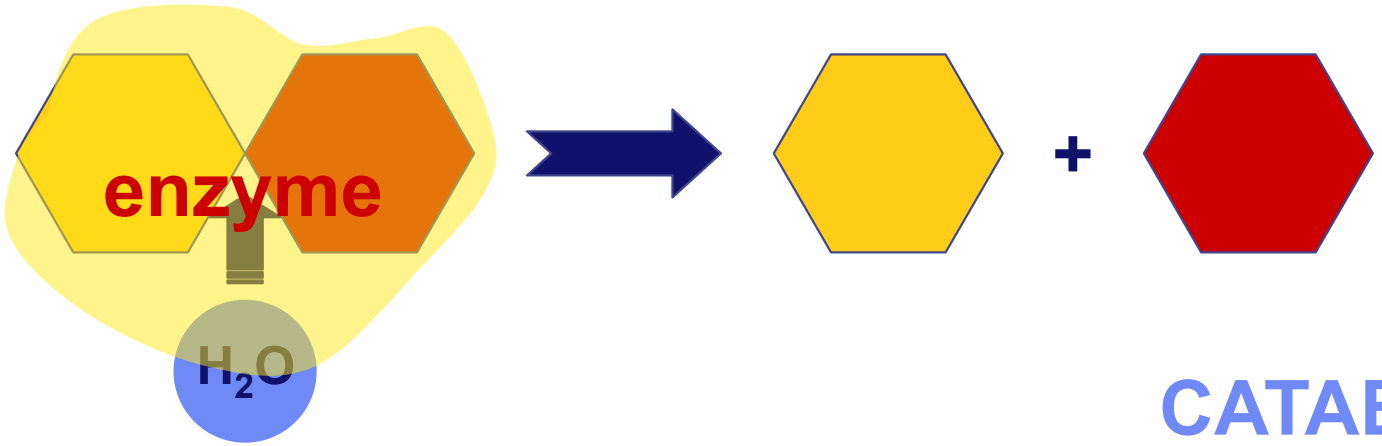
Examples

- dehydration synthesis (synthesis)



ANABOLISM

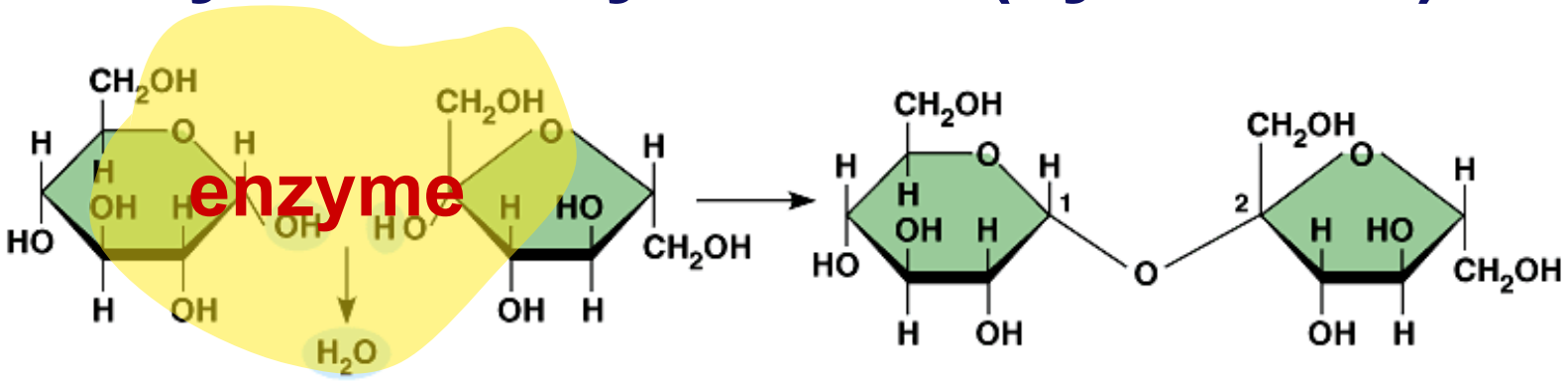
- hydrolysis (digestion *- breaking macromolecules down*)



CATABOLISM

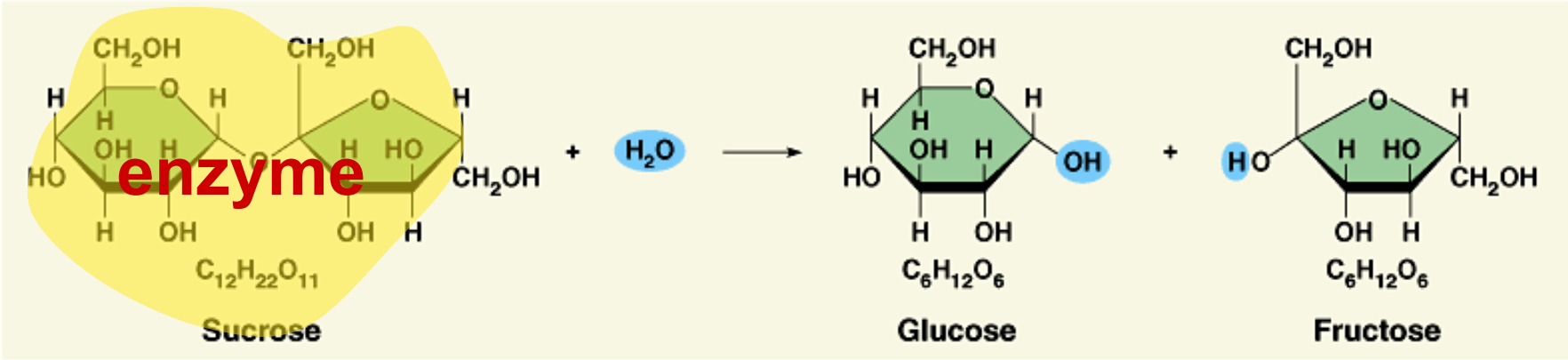
Examples

- dehydration synthesis (synthesis)



ANABOLISM

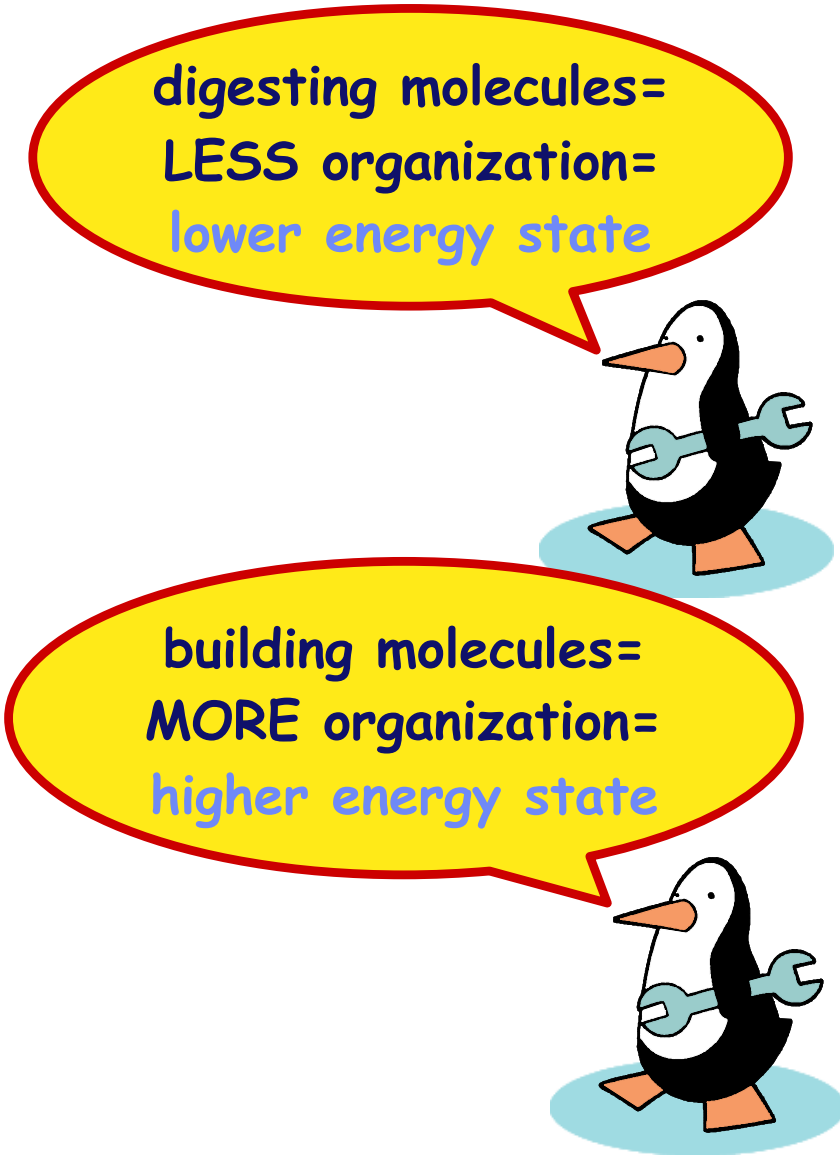
- hydrolysis (digestion)



CATABOLISM

Chemical Energy: The potential energy molecules possess because of the arrangement of their atoms. Potential energy available for release in chemical reactions.

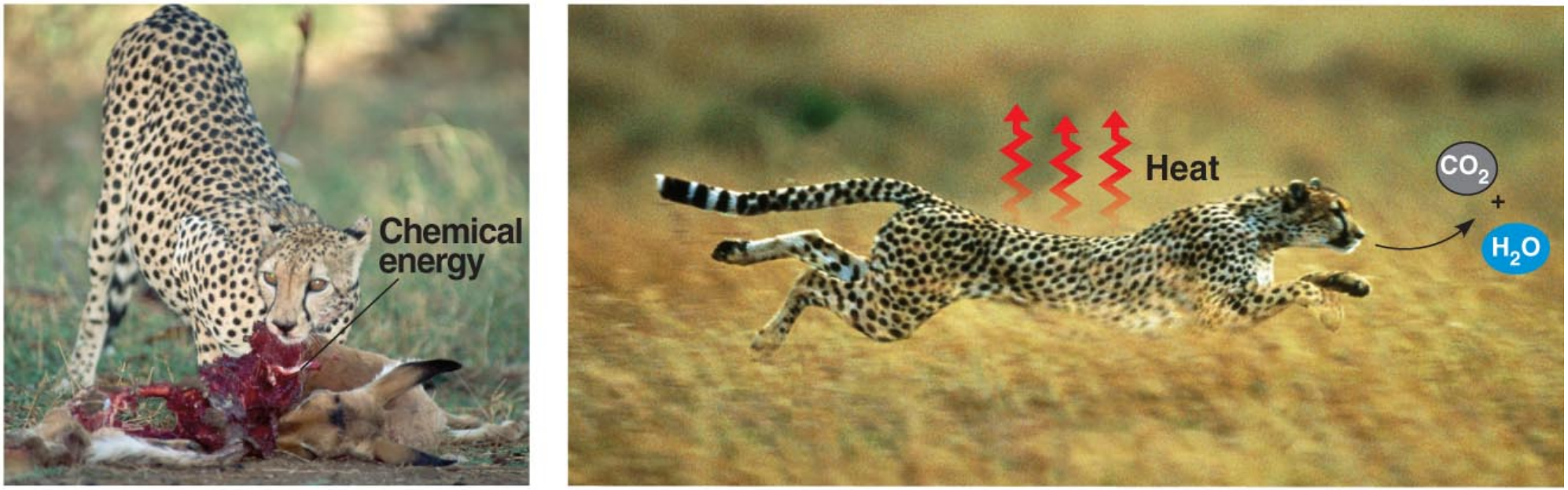
- Some chemical reactions release energy
  - they are exergonic
    - digesting polymers
    - hydrolysis = catabolism
- Some chemical reactions require a net input of energy
  - they are endergonic
    - building polymers
    - synthesis = anabolism



The universe obeys the Laws of Thermodynamics

First law of thermodynamics: Energy an be transferred and transformed but it **cannot** be created nor destroyed.

Second law of thermodynamics: Every energy transfer or transformation increases the disorder (entropy) of the **UNIVERSE**

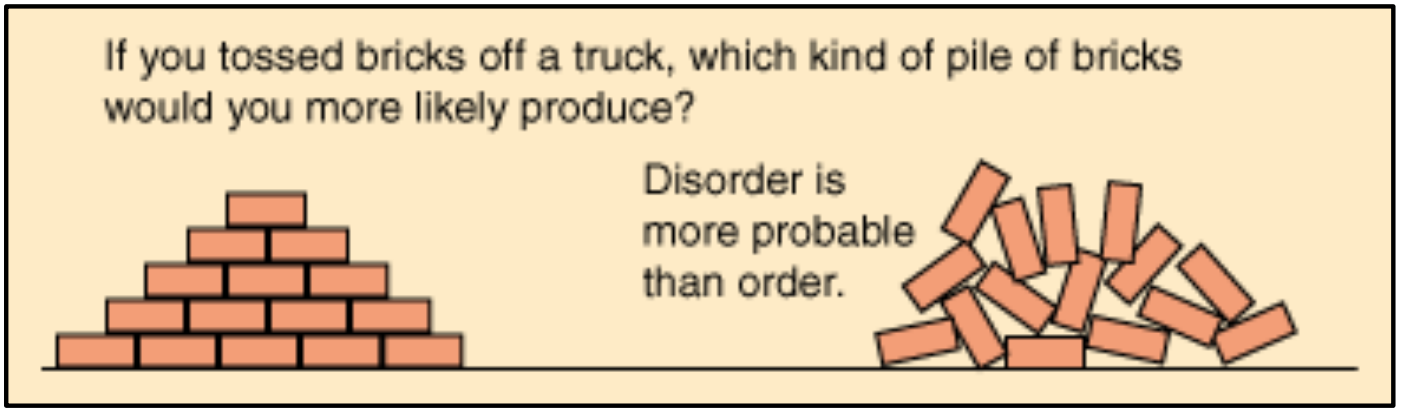


(a) First law of thermodynamics (b) Second law of thermodynamics

The universe obeys the Laws of Thermodynamics

For a process to occur on its own (SPONTANEOUSLY) it must increase the entropy of the universe.

- **Spontaneous processes:**
  - occur without input of energy
- **Nonspontaneous processes:**
  - need energy to occur



Free Energy (G)

G = The portion of a system's energy that can perform work when temperature and pressure are uniform in the system.

$$\Delta G = G_{\text{final state (products)}} - G_{\text{initial state (reactants)}}$$

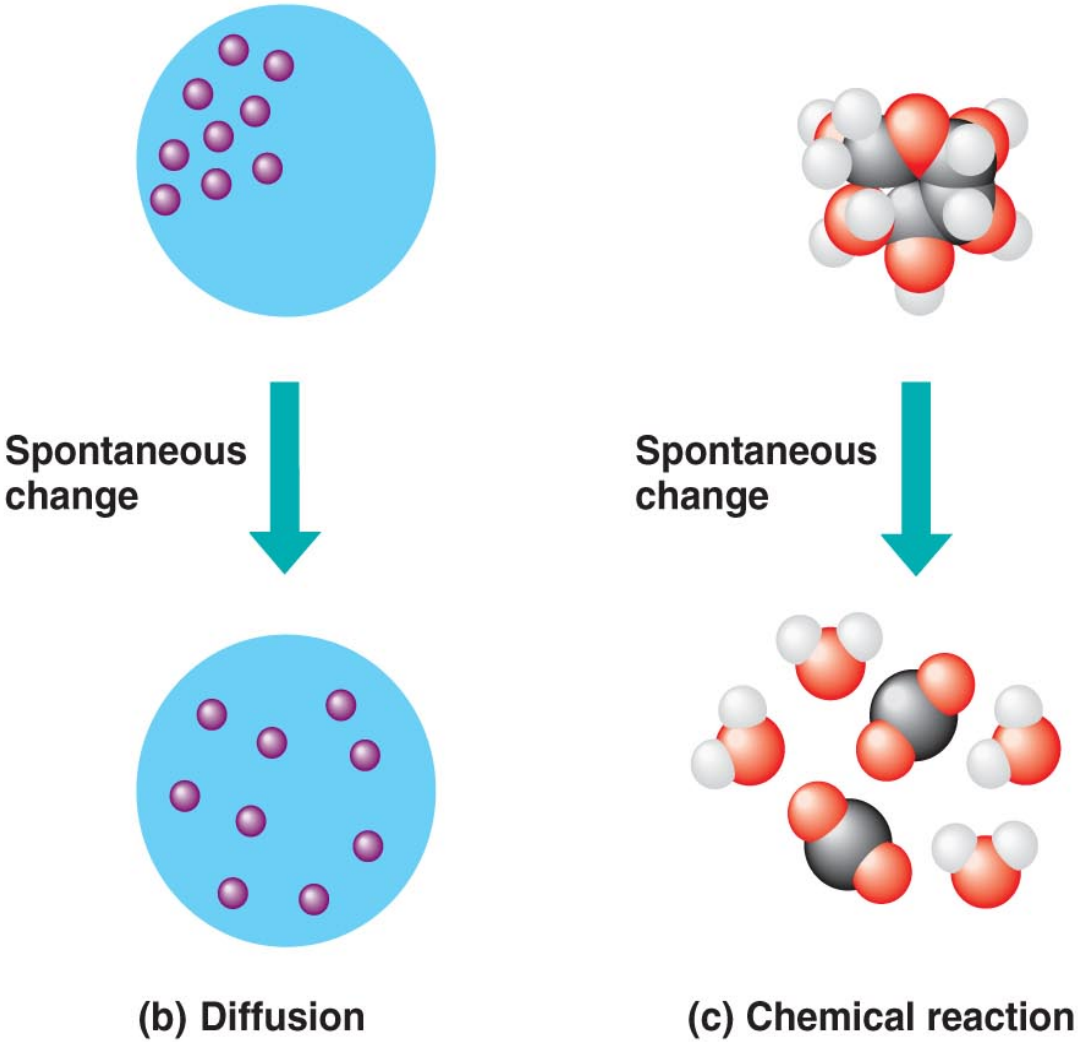
ΔG = Change in free energy.

Only processed with a negative ΔG are spontaneous!

- More free energy (higher *G*)
- Less stable
- Greater work capacity

In a spontaneous change
- The free energy of the system decreases ($\Delta G < 0$)
- The system becomes more stable
- The released free energy can be harnessed to do work

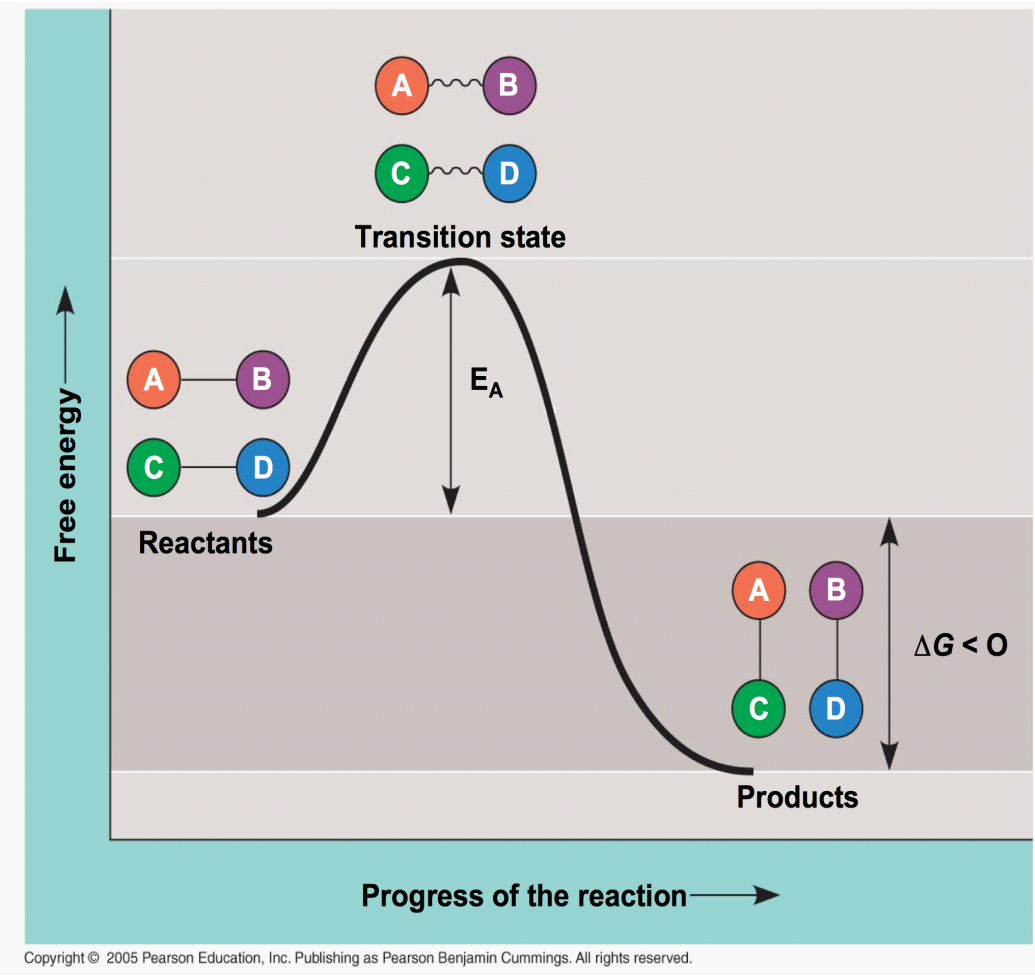
- Less free energy (lower *G*)
- More stable
- Less work capacity



The Activation Energy Barrier

Every chemical reaction between molecules involves bonds breaking and bonds forming

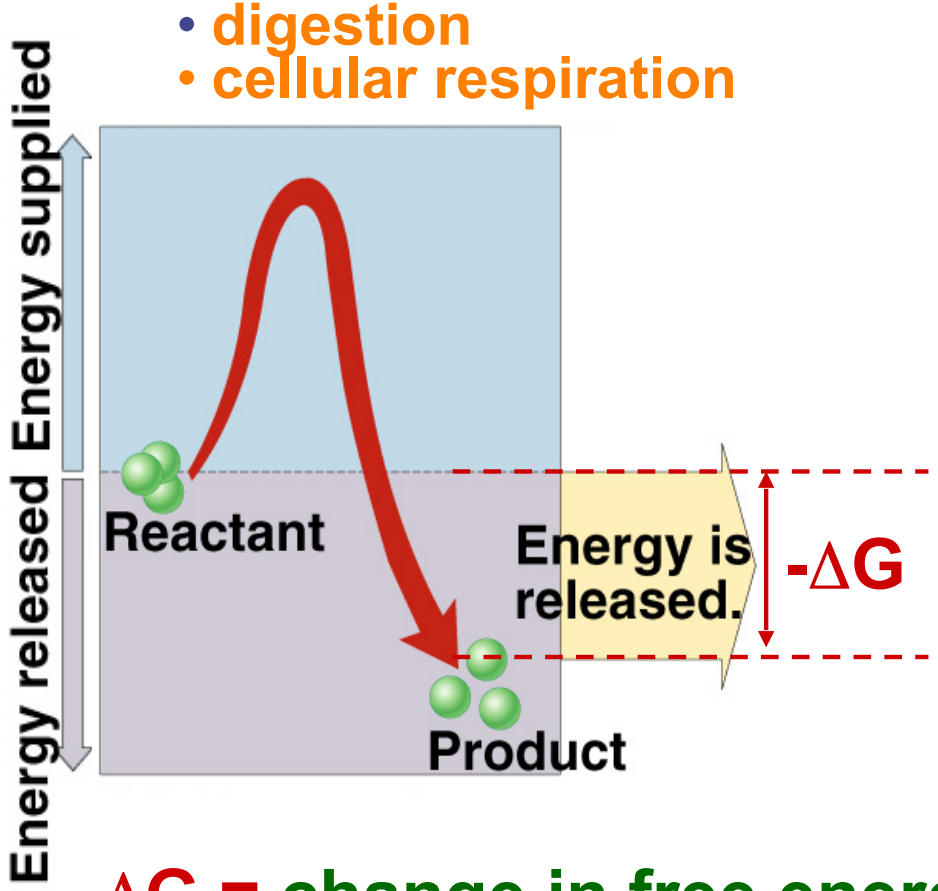
- The initial energy needed to start a reaction is called the free energy of activation or activation energy (E_A)
  - Activation energy is often supplied in the form of heat from the surroundings, though the amount of energy available in the surroundings may not be enough to destabilize the bonds in the reactions in order to cause bonds to break and the reaction to proceed.



Endergonic vs. exergonic reactions

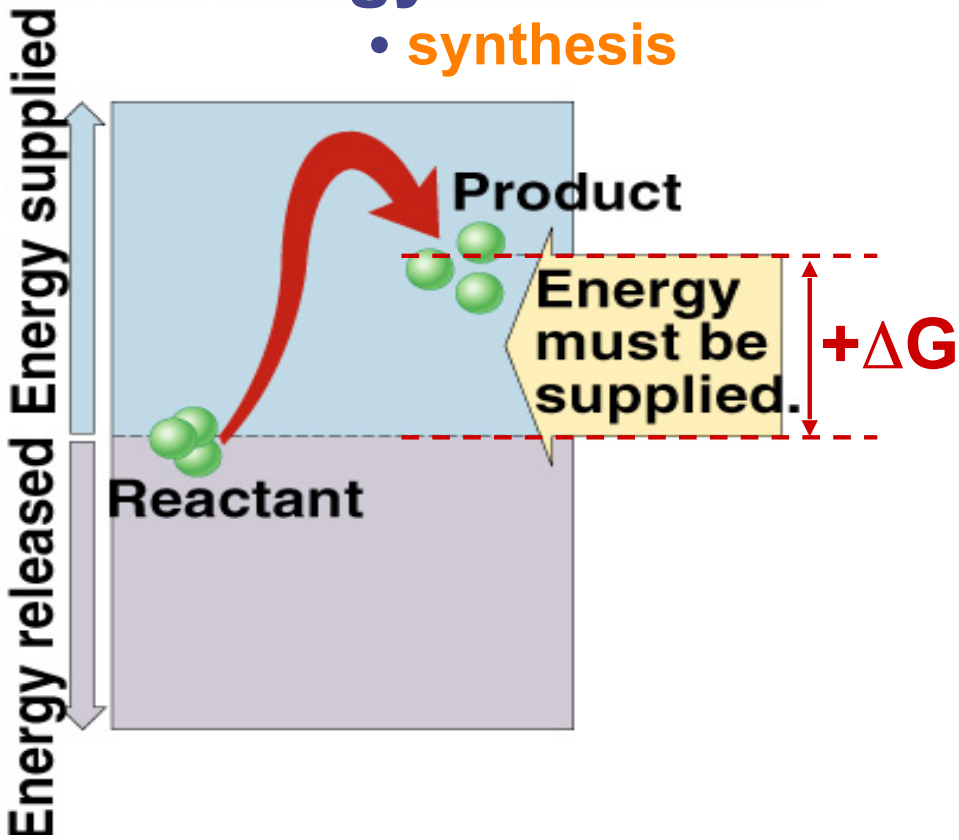
exergonic

- -ΔG
- spontaneous
- energy released
  - digestion
  - cellular respiration



endergonic

- +ΔG
- nonspontaneous
- energy invested
  - synthesis

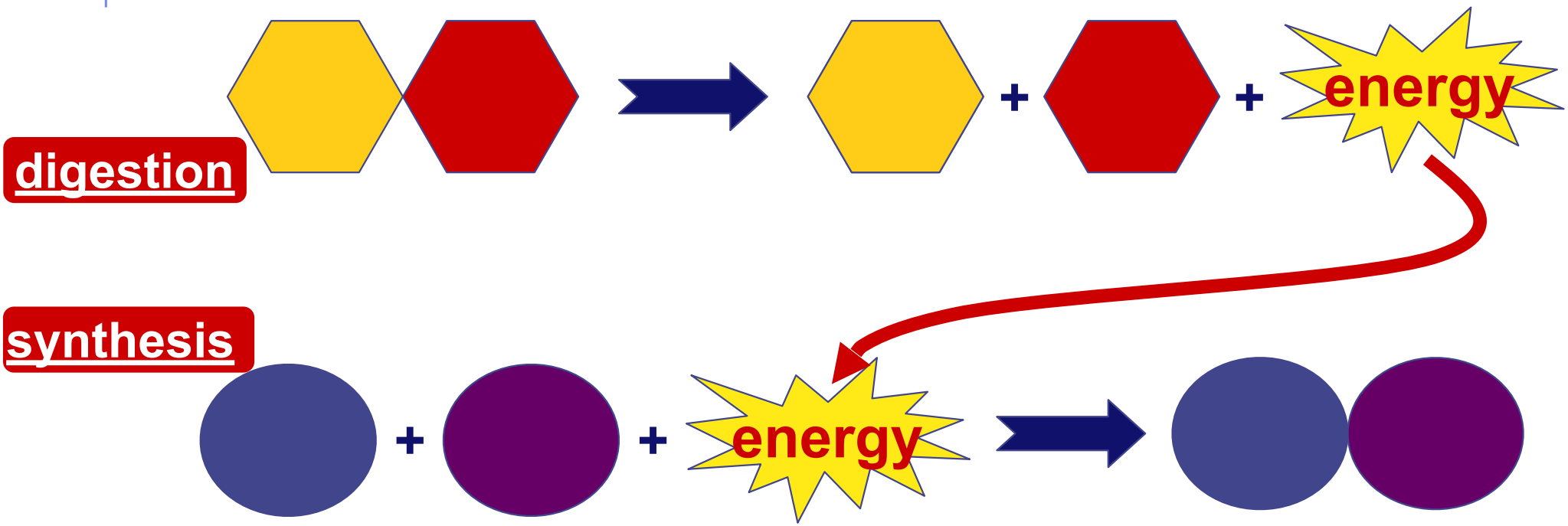


AP

ΔG = change in free energy = ability to do work

“Energy Coupling”

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

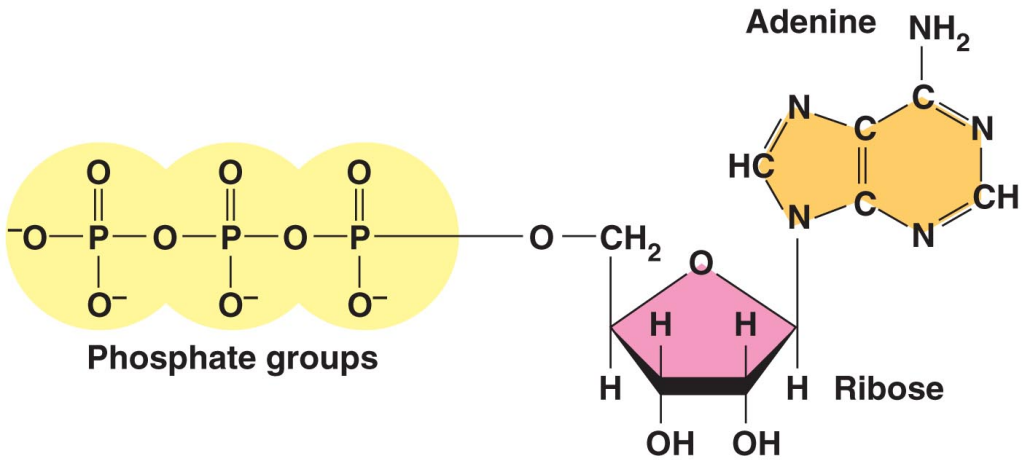


ATP powers work by coupling exergonic reactions to endergonic reactions

- The cell must accomplish 3 TYPES OF WORK
  - **Chemical work:** anabolic processes like building polymers etc..
  - **Transport work:** pumping substances against concentration gradients across membranes
  - **Mechanical work:** Moving substances and particles such as moving chromosomes during cell division, beating cilia, contracting muscle cells etc..

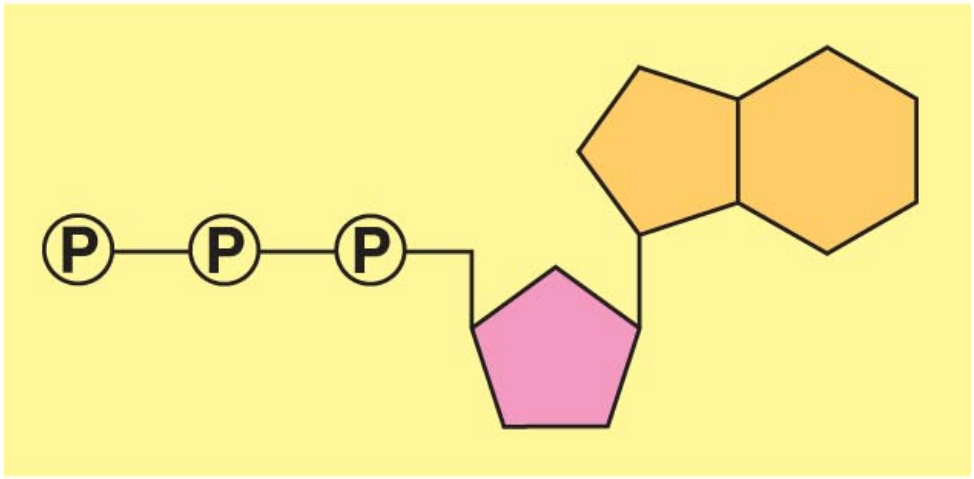
energy

- Work requires **energy**

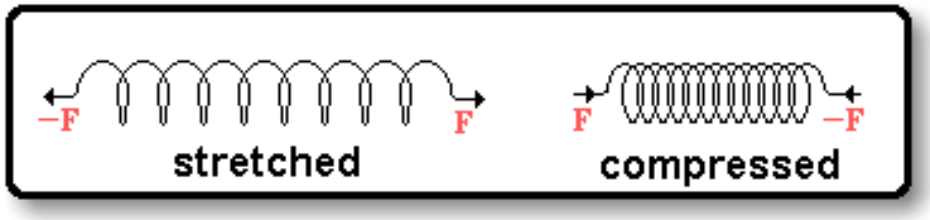


ATP powers work by coupling exergonic reactions to endergonic reactions

- **ATP = adenosine TRI-phosphate**
  - Has a chain of 3 phosphates.
  - Bonds between phosphates are highly unstable and very high in potential energy because of the large amount of negative charge held so closely together



Adenosine triphosphate (ATP)

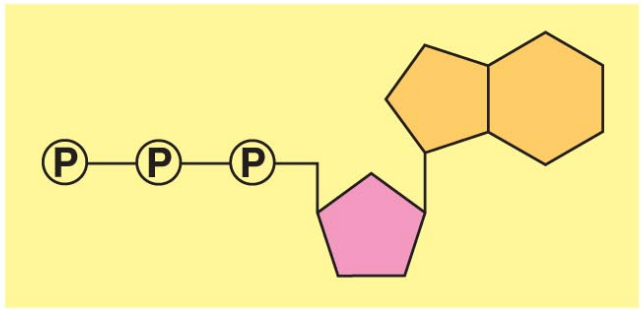


The Phosphate to phosphate bonds contain energy just like a compressed spring!

ATP Hydrolysis

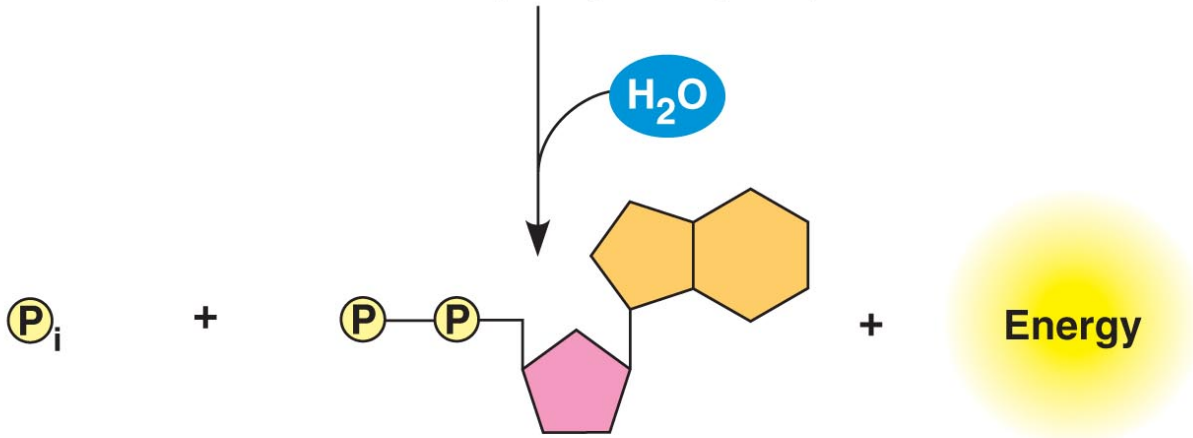
- Breaking the bond between the last two phosphates, through *hydrolysis*, releases an inorganic phosphate & ENERGY!

$\Delta G = -7.3$ kcal/mol



Adenosine triphosphate (ATP)

- ATP now becomes ADP

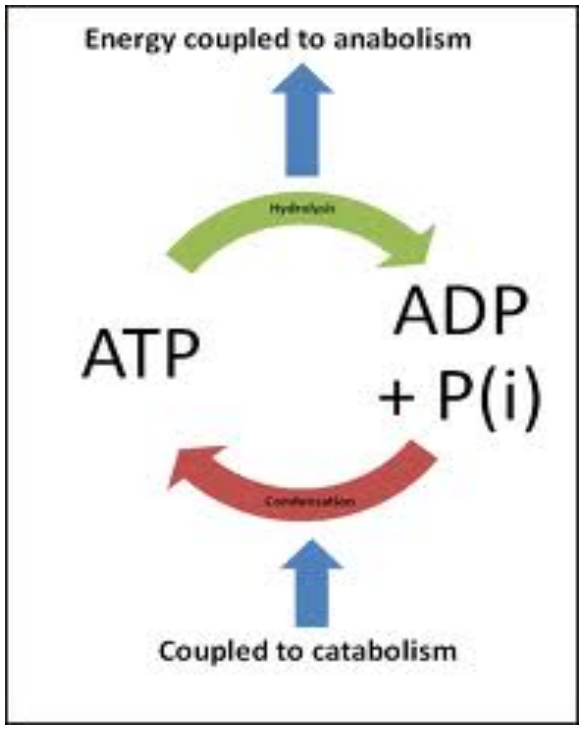


Inorganic phosphate Adenosine diphosphate (ADP)

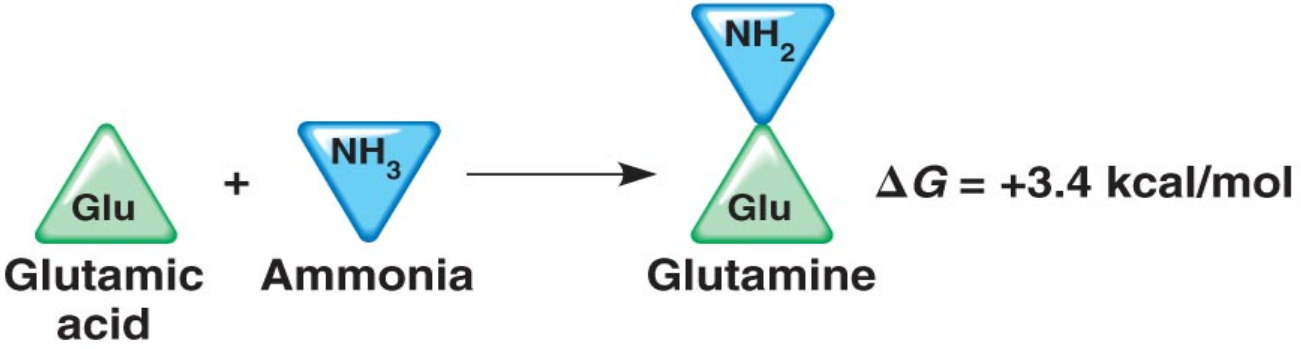
FYI: **Though the ΔG mathematically is negative, the amount of energy released is 7.3kcal/mol.** *[Energy can't be a negative entity. you either have it or you don't.]*

ATP Hydrolysis

- If the energy release from ATP hydrolysis is less than the energy needed for an endergonic reaction, then the two reactions can be coupled so the overall coupled reactions are exergonic

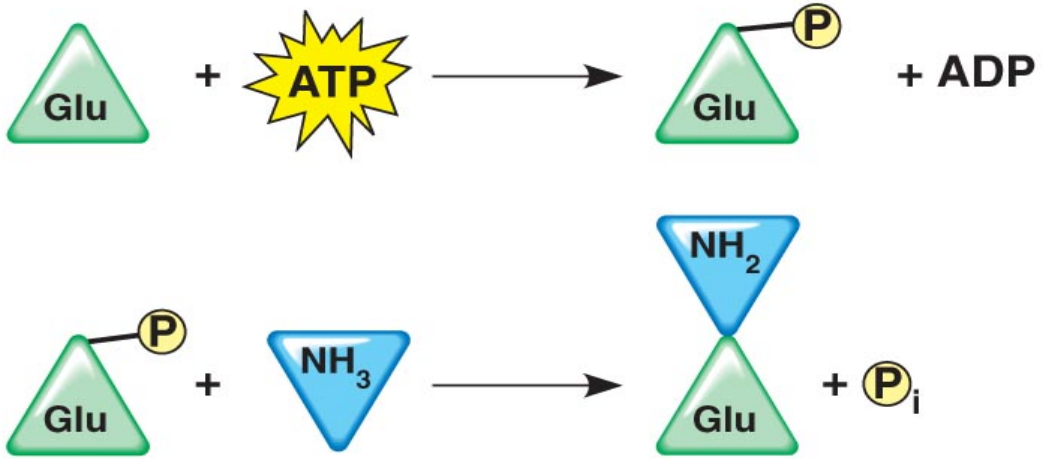


- Coupling often involves phosphorylation
  - Often a phosphate group is transferred from ATP to another molecule, making this molecule more unstable and altering the molecule's shape and ability to interact with other molecules



(a) Endergonic reaction

1. ATP phosphorylates glutamic acid, making the amino acid less stable.
2. Ammonia displaces the phosphate group, forming glutamine.



(b) Coupled with ATP hydrolysis, an exergonic reaction

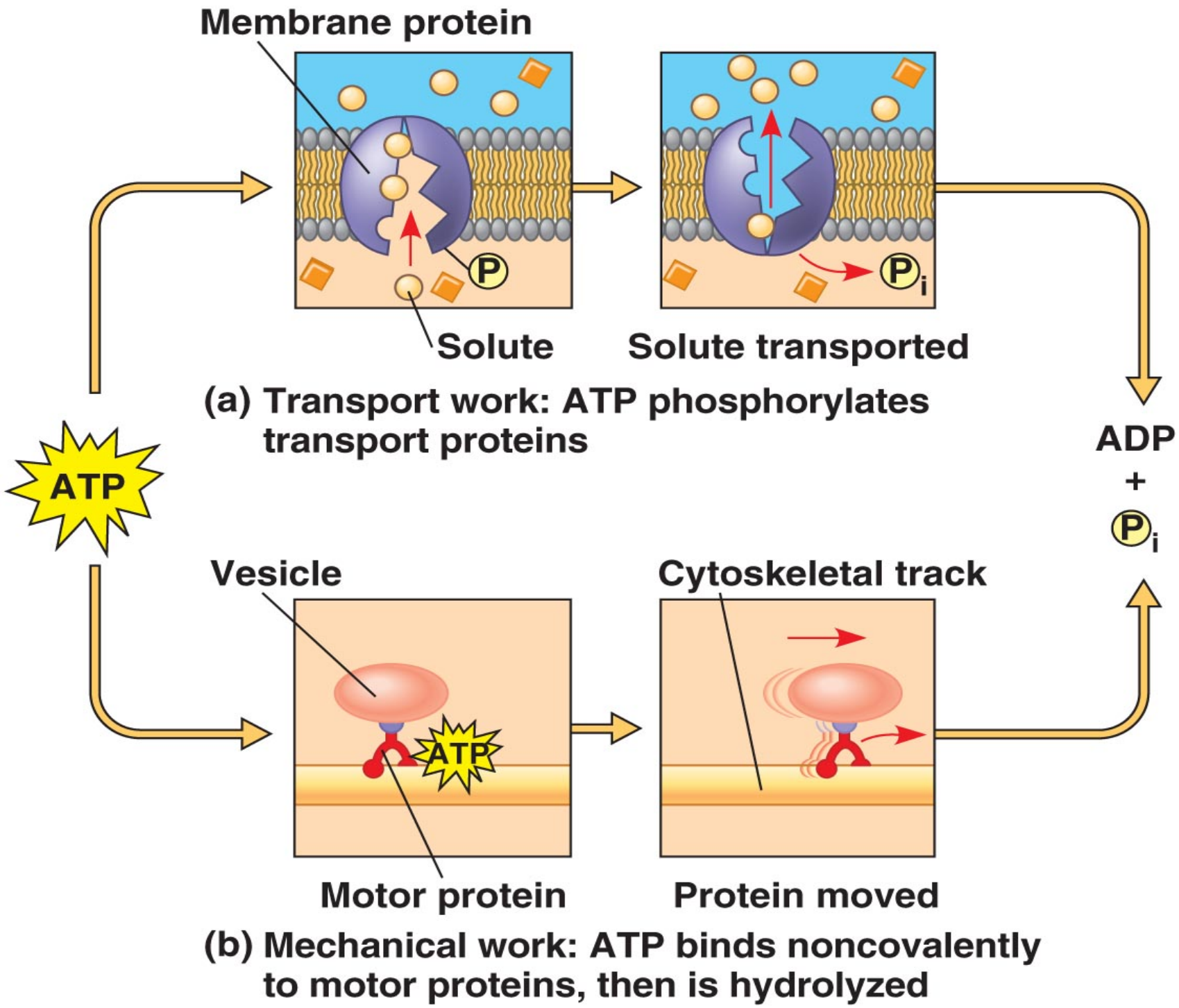
$$\text{Glu} + NH_3 \longrightarrow \text{Glu}{-}NH_2 \qquad \Delta G = +3.4 \text{ kcal/mol}$$

$$\text{ATP} \longrightarrow \text{ADP} + P_i \qquad \Delta G = -7.3 \text{ kcal/mol}$$

$$\text{Net } \Delta G = -3.9 \text{ kcal/mol}$$

(c) Overall free-energy change

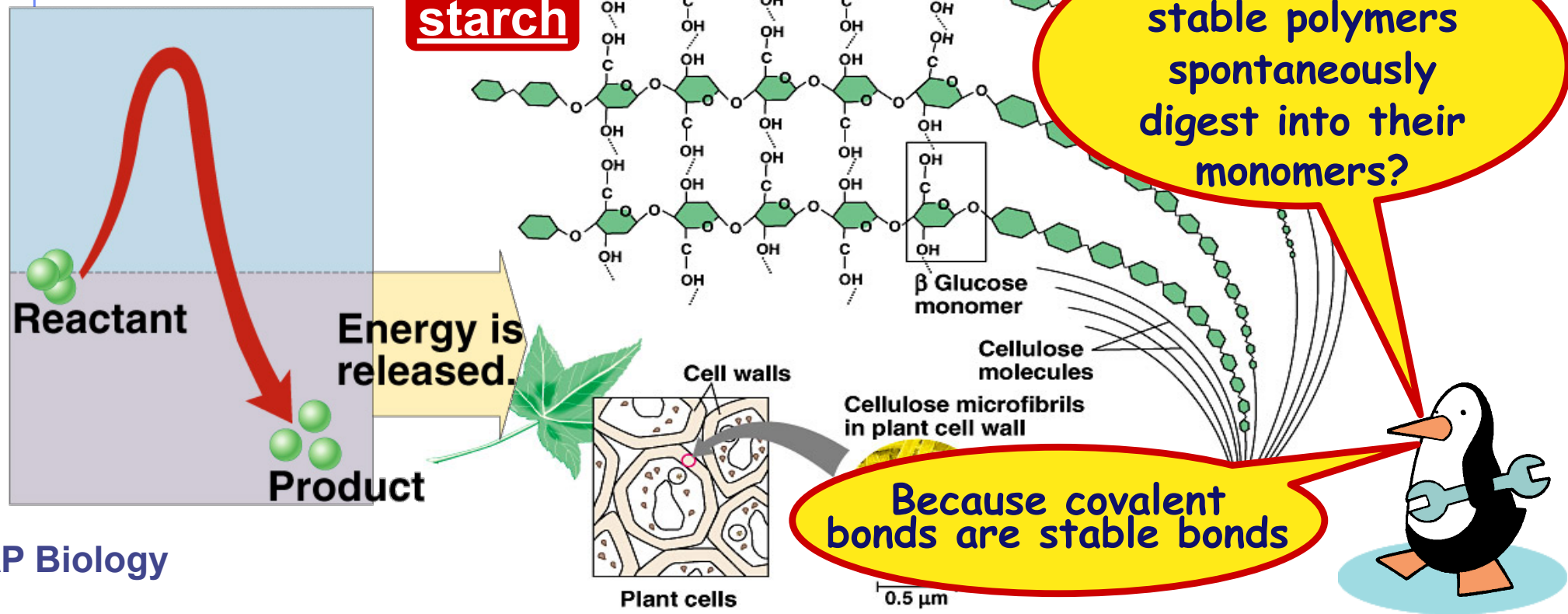
AP Bi



AP Biol

What drives reactions?

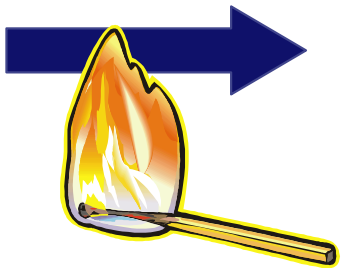
- If reactions proceed "downhill", i.e. they are spontaneous and exergonic, why don't they all just proceed forward and occur on their own & immediately?



Activation energy

- Breaking down large molecules requires an initial input of energy to reach an unstable "TRANSITION STATE" where bonds can be broken.
  - Activation Energy = the energy required to contort the reactant molecule so that bonds can be broken

cellulose



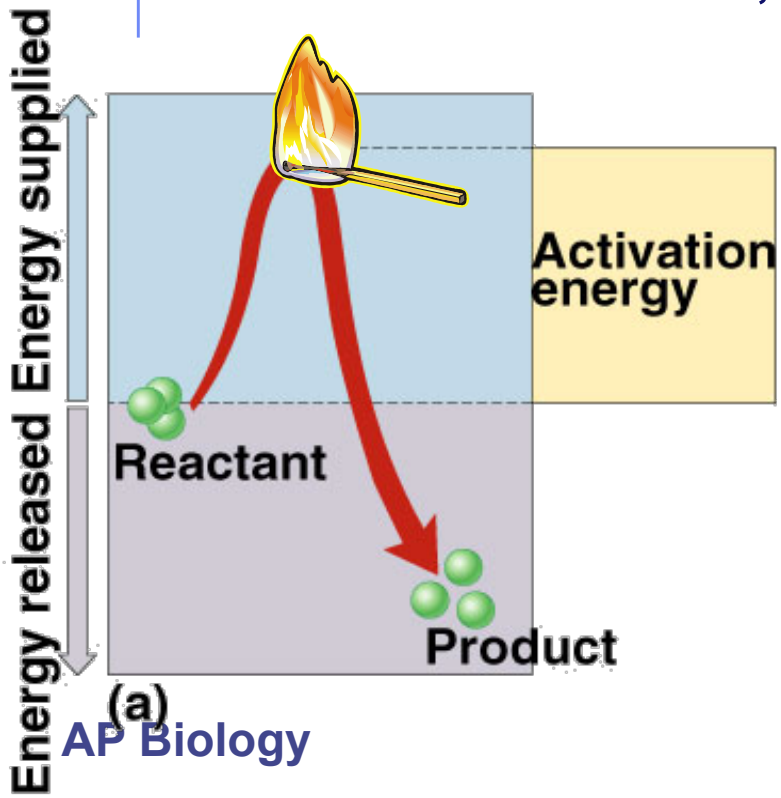
energy

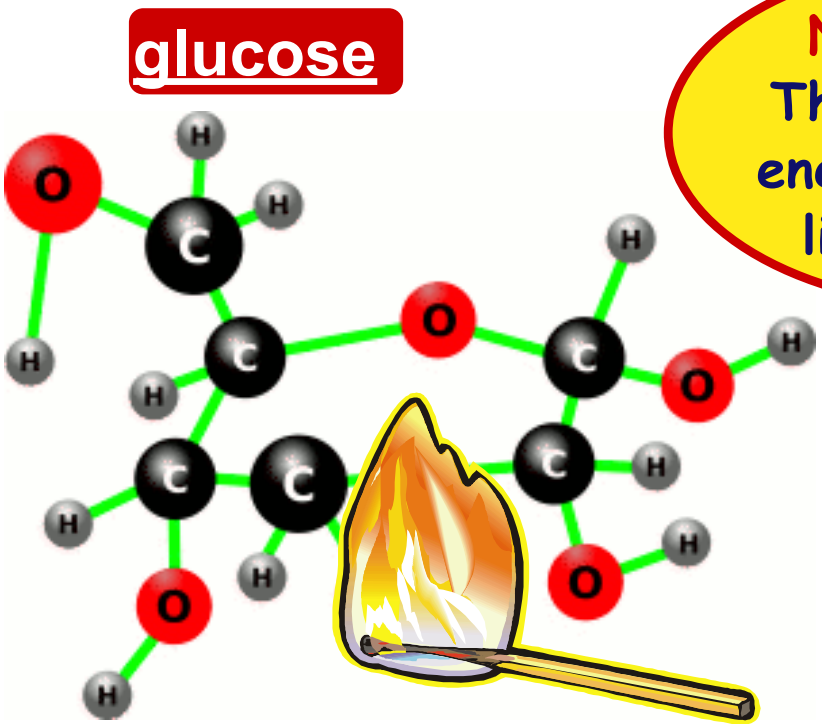
$CO_2 + H_2O +$ heat

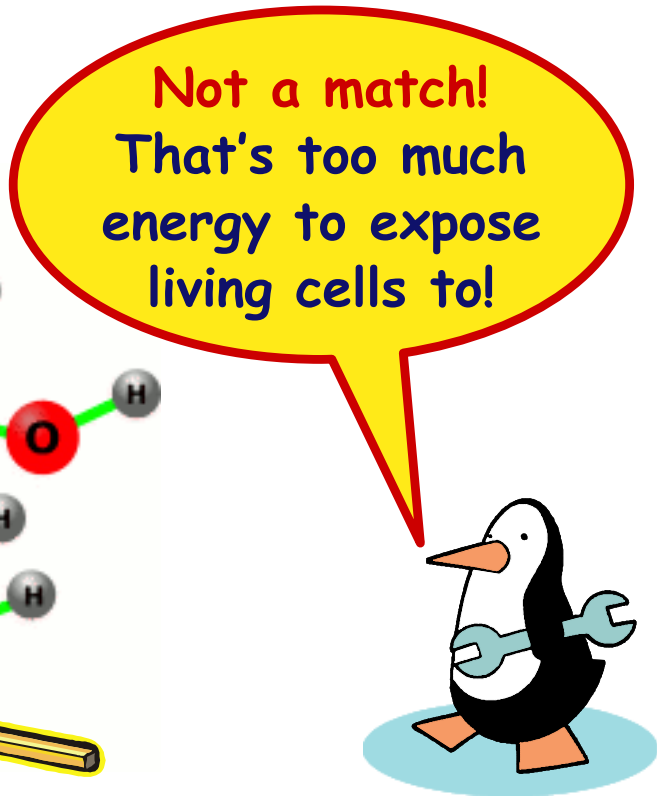
Normally there is too much activation energy for life to occur

- **Activation energy must be overcome to release energy in chemical bonds**
- **But <u>heat cannot be added</u> to biological systems**

  Too much heat would *DENATURE* proteins, cause *membranes to fall apart* (kinetic energy too high), cause unwanted/uncontrolled side chemical reactions, and, therefore, *destroy the cell*

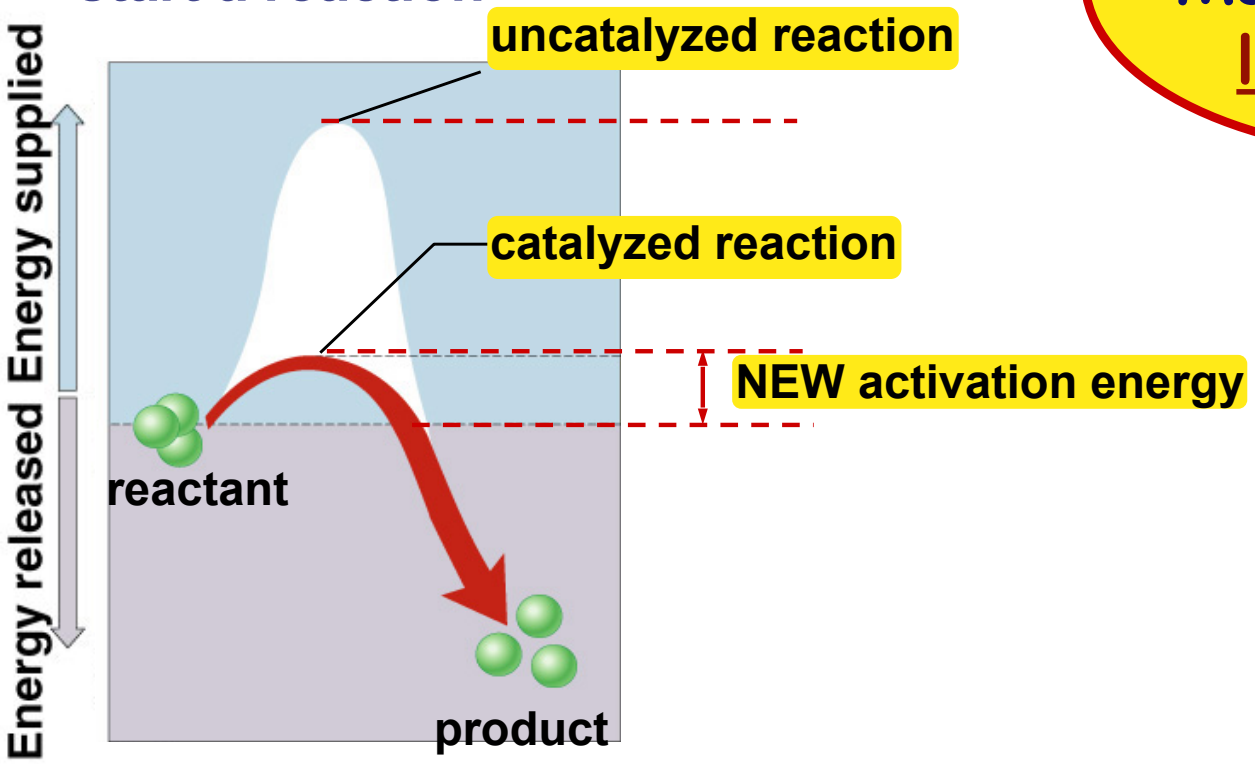


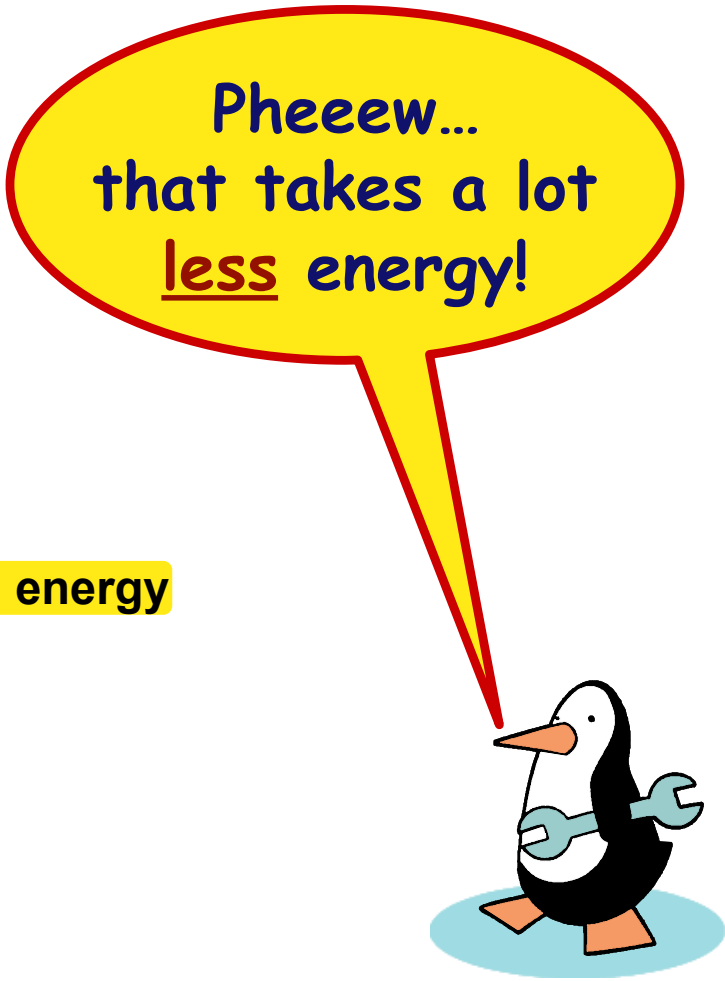




Reducing Activation energy

- E_A is a barrier that determines the rate of reaction.
  - High E_A = reaction occurs more slowly
  - Low E_A = reaction occurs faster
- **Catalysts**
  - reducing the amount of energy to start a reaction

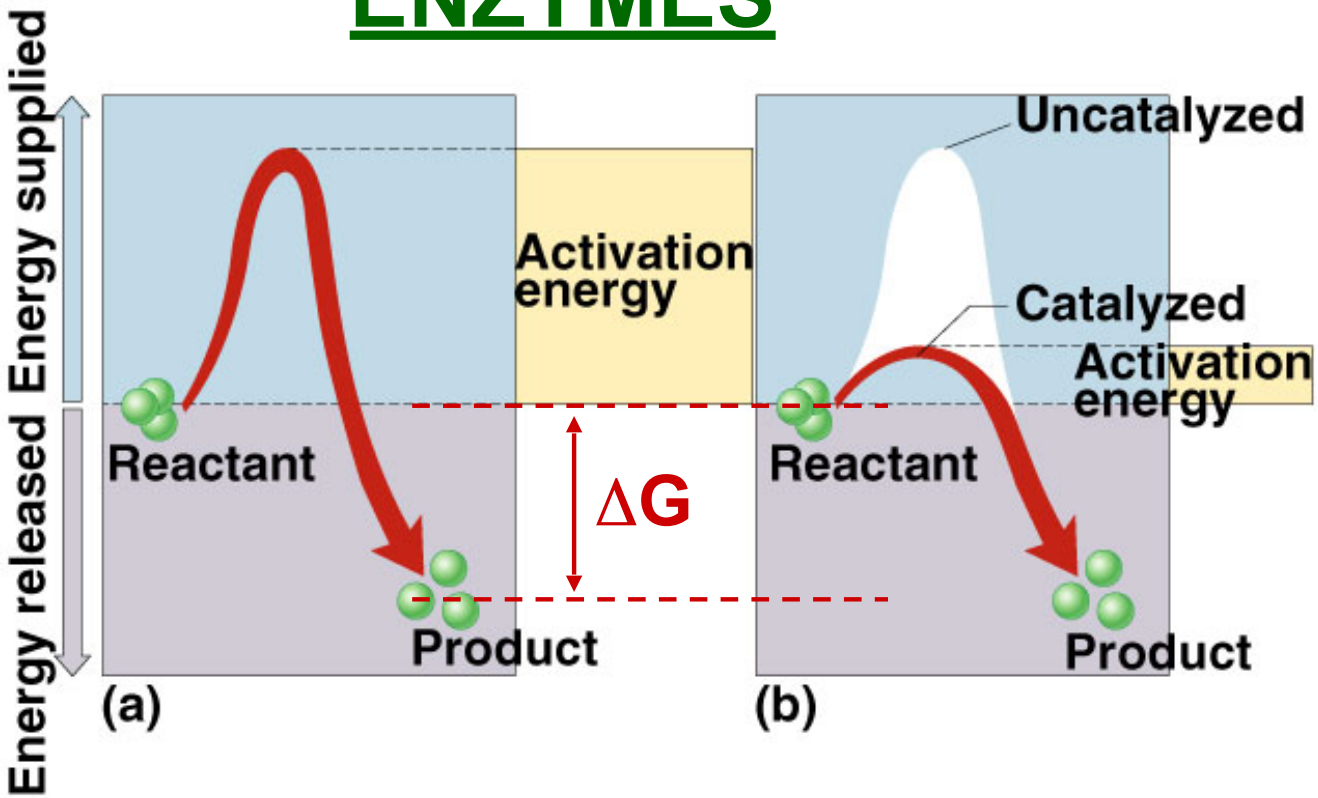




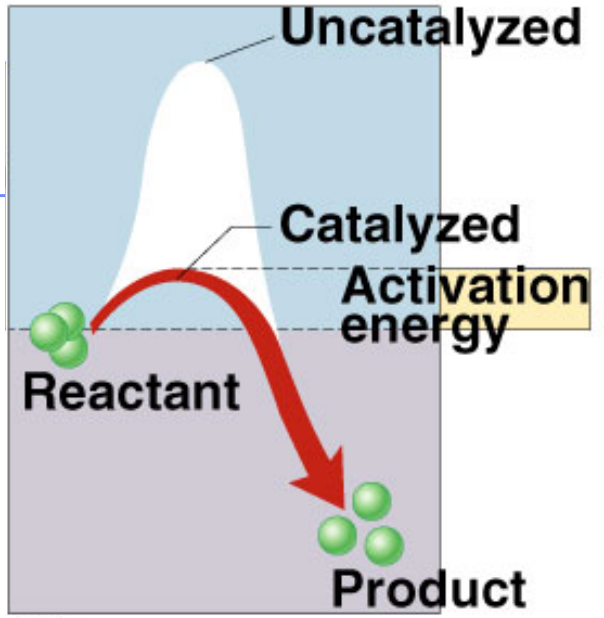
Catalysts speed up chemical reactions

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

ENZYMES



Enzymes



- **Biological catalysts**
  - **Proteins**
    - (but can be **RNA, a.k.a. ribozymes**)
  - **facilitate chemical reactions**
    - **Increase rate of reaction without being consumed**
    - **Reduce activation energy**
      - **DO NOT change free energy (ΔG) released or required!!!**
  - **required for most biological reactions**
  - **they are highly specific!**
    - **there are thousands of different enzymes in cells**
  - **they help control reactions of life**

Enzyme vocabulary

substrate

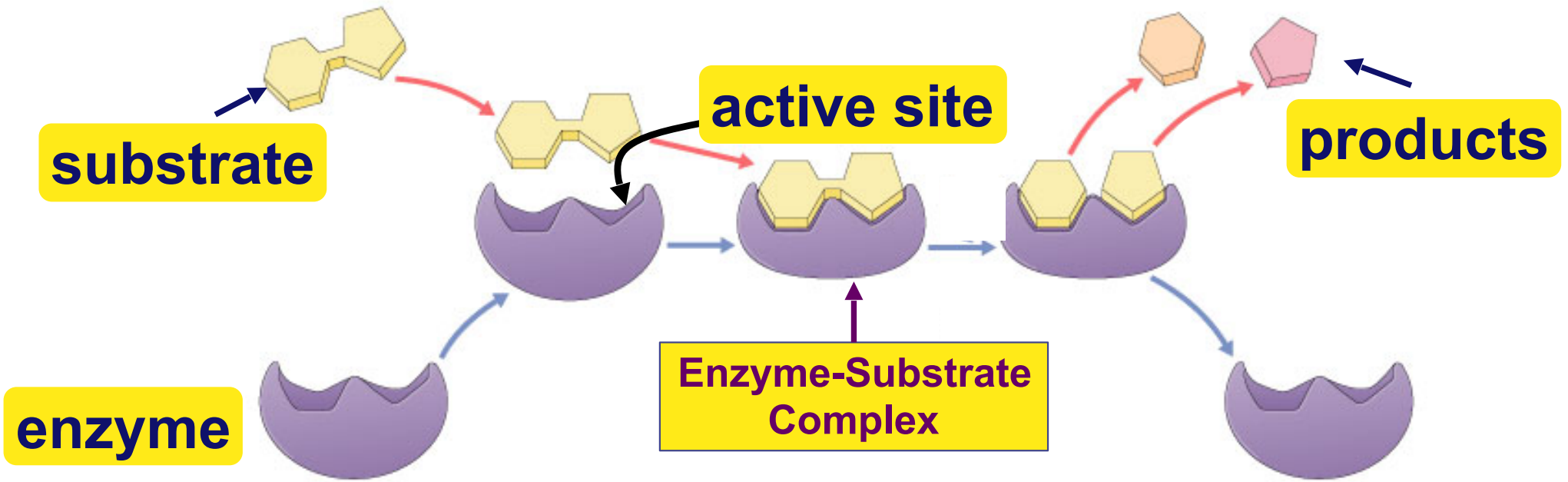
- reactant which binds to enzyme
- enzyme-substrate complex: temporary association

product

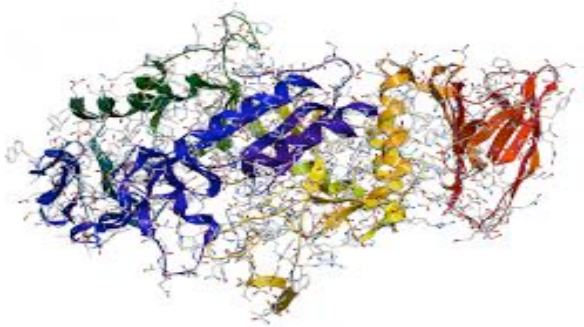
- end result of reaction

active site

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



Properties of enzymes

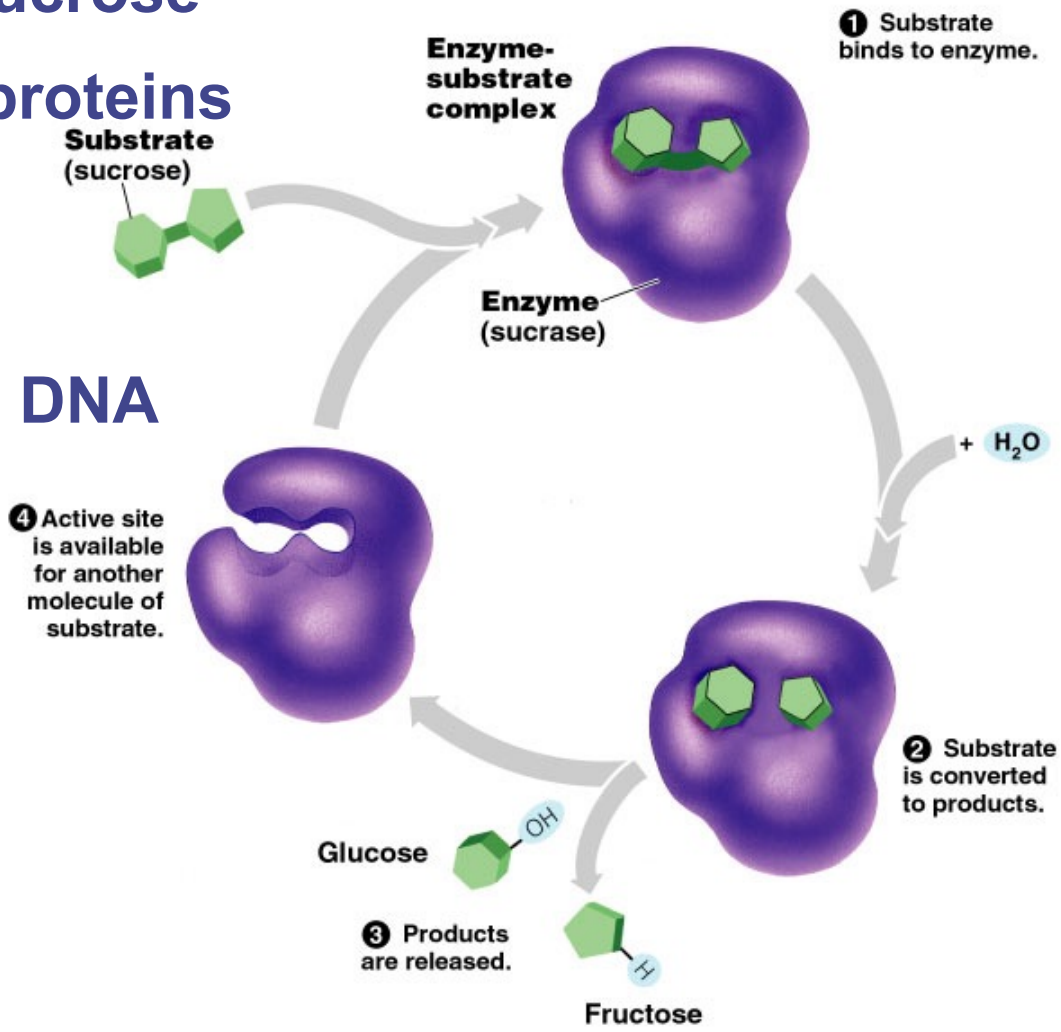


- **Reaction specific**
  - each enzyme works with a specific substrate
    - chemical fit between active site & substrate
      - H bonds, dipole forces, & ionic bonds
- **Not consumed in reaction**
  - single enzyme molecule can catalyze thousands or more reactions per second
    - enzymes unaffected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

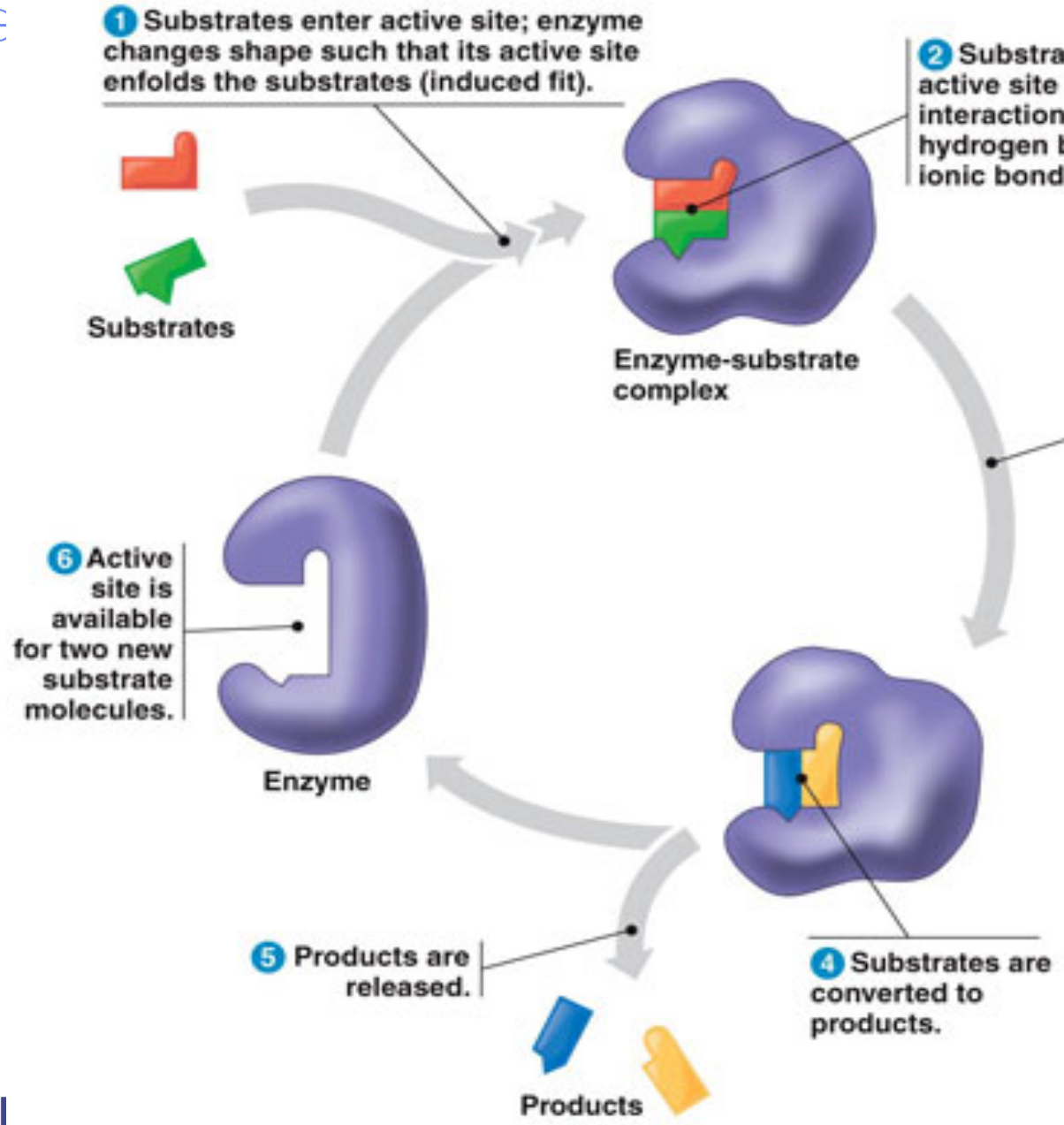
Naming conventions

Enzymes are often 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)



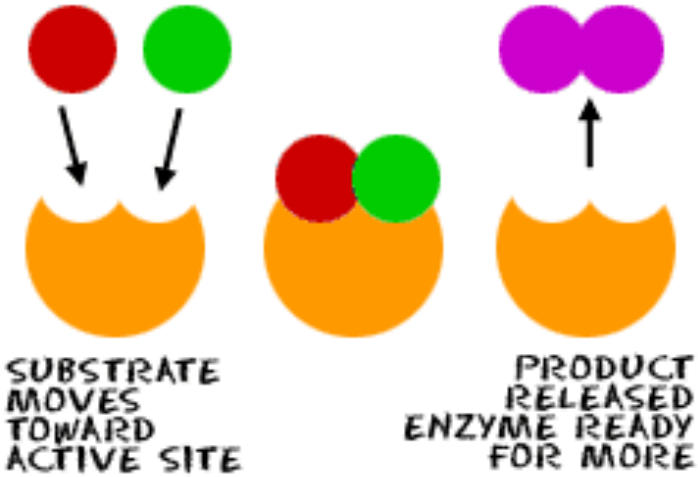
How do enzymes work?



Metabolic reactions are reversible:

- Enzymes catalyze both directions depending on which direction has a negative ΔG.
- Enzymes always helps the reaction proceed in the direction of equilibrium.

How do enzymes work?

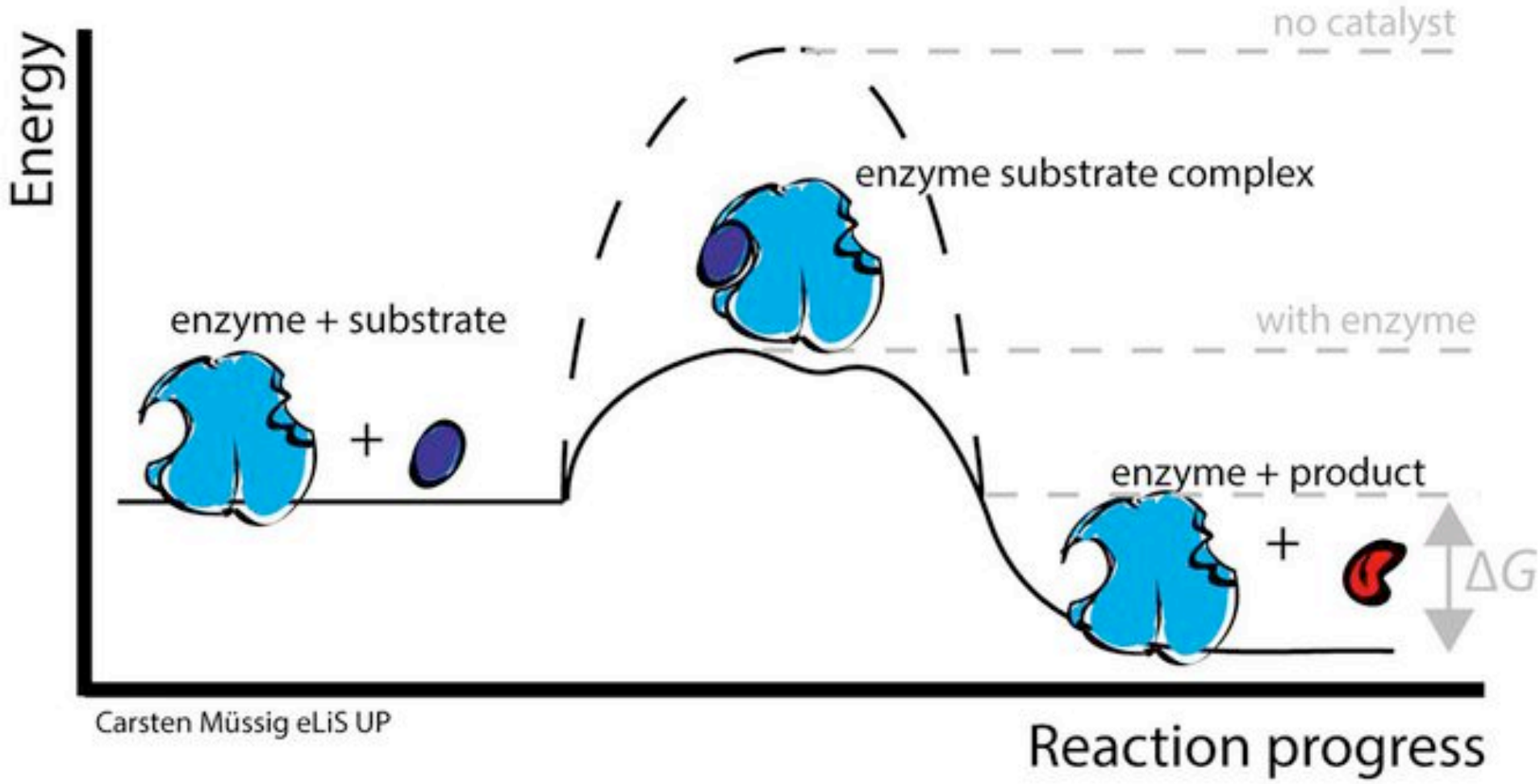


- **Enzymes use a variety of mechanisms to lower activation speed up reaction**

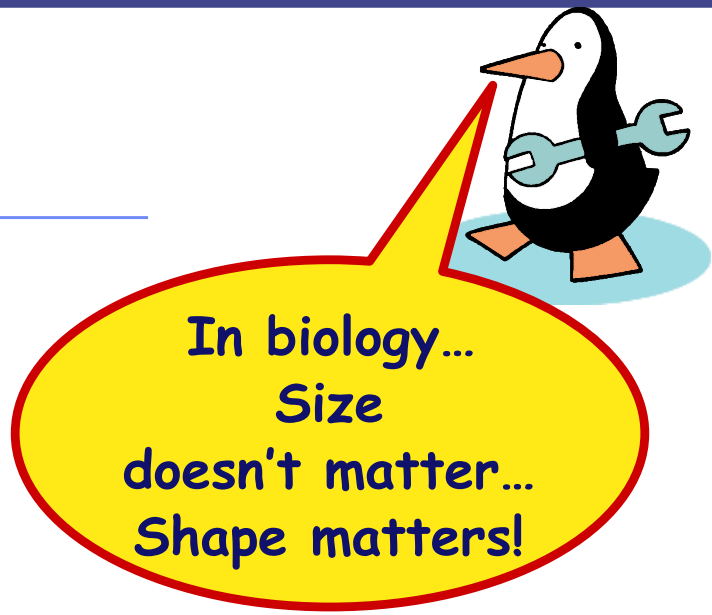
1. active site orients substrates in correct position for reaction
   - enzyme brings substrate closer together
2. active site binds substrate & puts stress on bonds that must be broken, making it easier to separate molecules
3. Provide a favorable microenvironment
4. May participate directly in the chemical reaction

The enzyme effect.

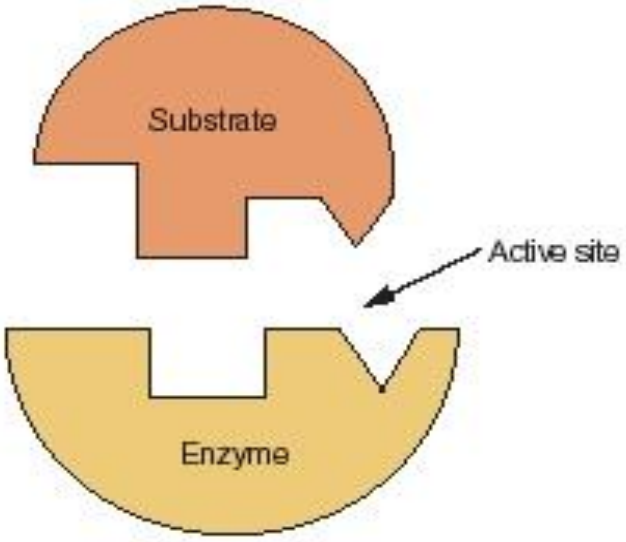
- **Enzymes lower the amount of activation energy required for a chemical reaction to proceed.**



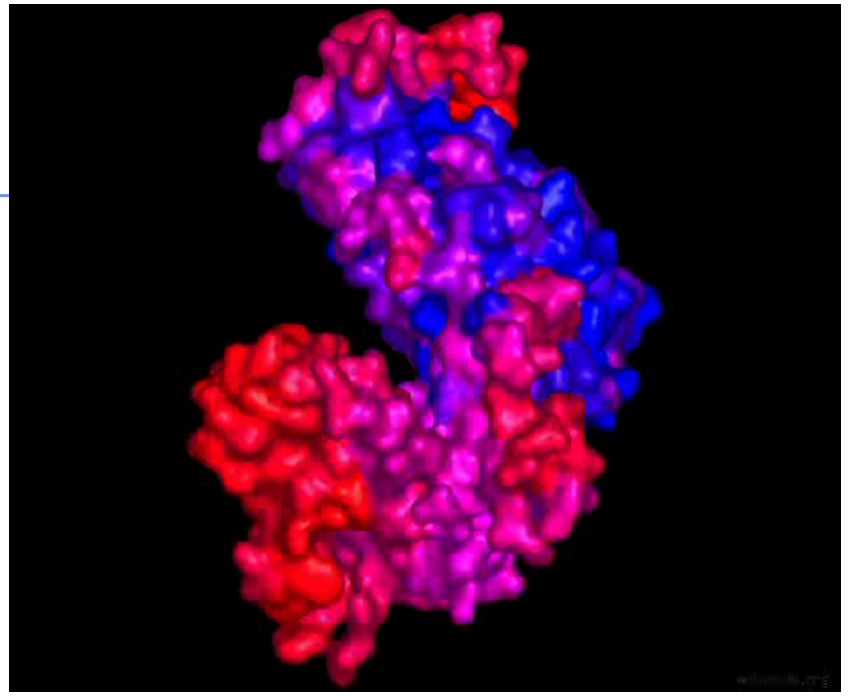
Lock and Key model



- **Simplistic model of enzyme action**
  - **substrate fits into 3-D structure of enzyme' active site**
  - **like "key fits into lock"**

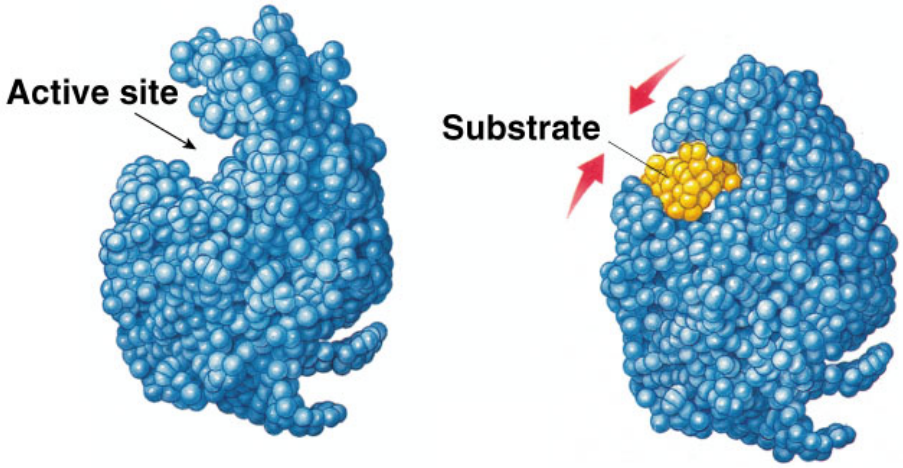


Induced fit model

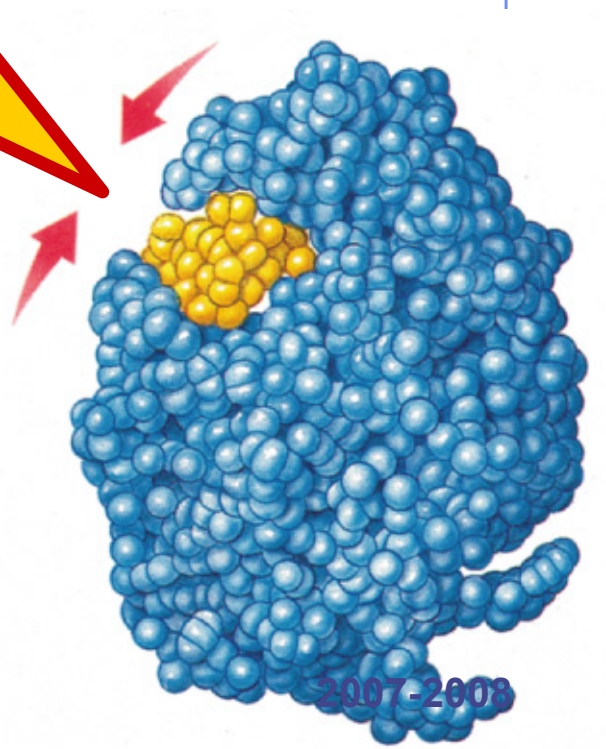


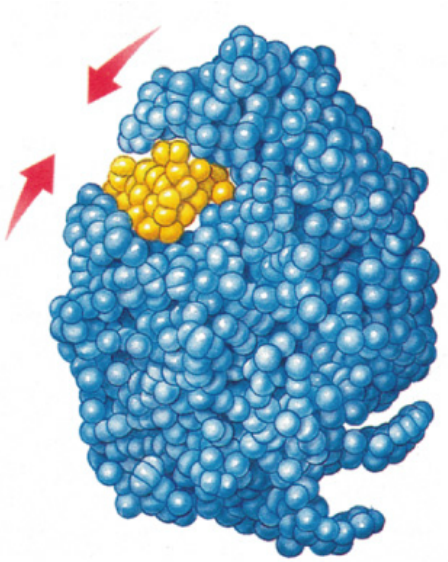
More accurate model of enzyme action

- 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

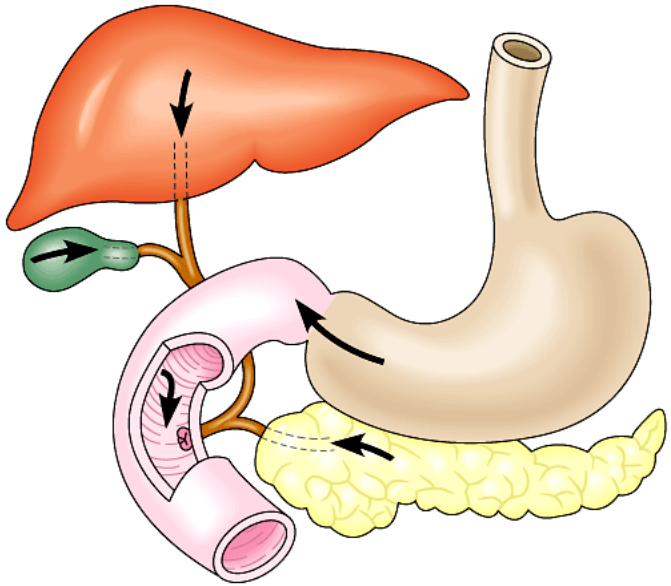


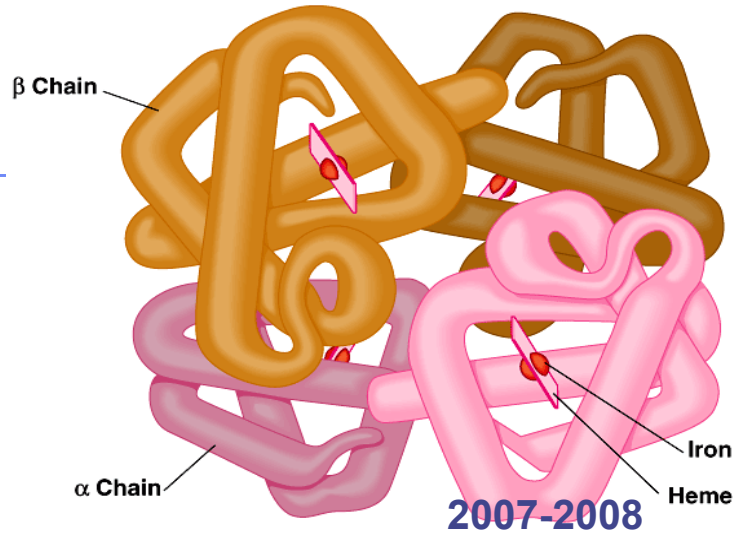
Got any Questions?!





Factors that Affect Enzymes





How is enzyme activity regulated?

- **Crucial to life** is the need to **regulate and control metabolism** and we do so by controlling which enzymes are active or inactive in the body.

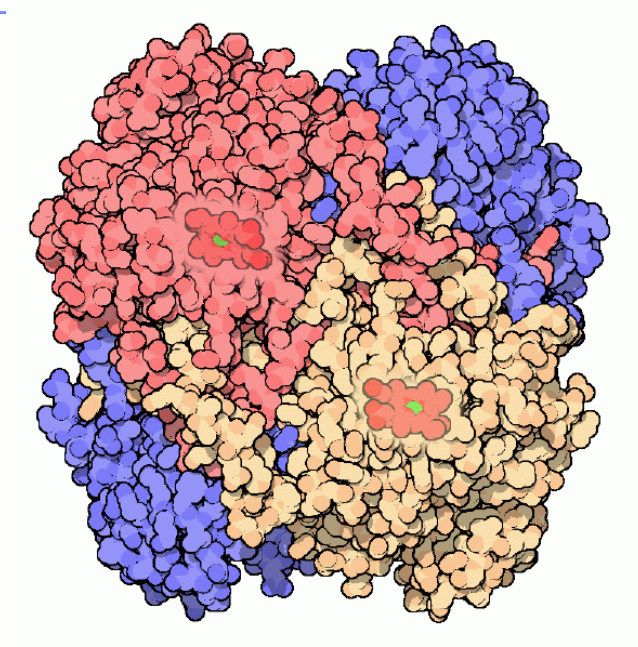
- **Organisms control enzyme activity by**:

1. **Switching on and off certain genes that encode specific enzymes**
2. **Regulating the activity of an already made enzyme.**

Next we will look into how we regulate existing enzymes.

Factors Affecting Enzyme Function

- Enzyme concentration
- Substrate concentration
- Cofactors & Coenzymes
- Activators
- Inhibitors
- Temperature
- pH
- Salinity

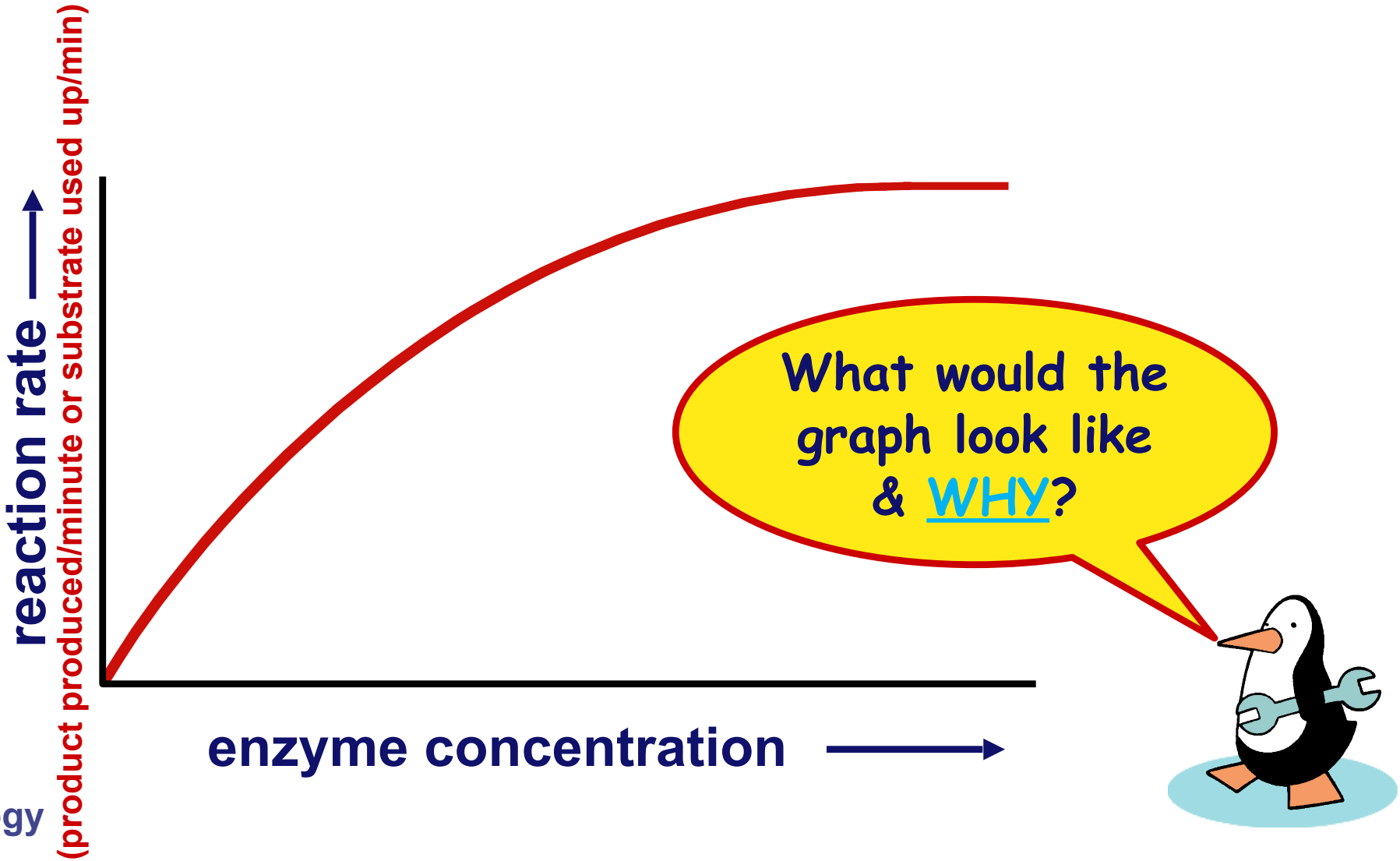


Optimal conditions: the conditions that favor the most active shape for the enzyme

- Enzymes shape is affected by:
  - Temperature
  - pH
  - Salinity

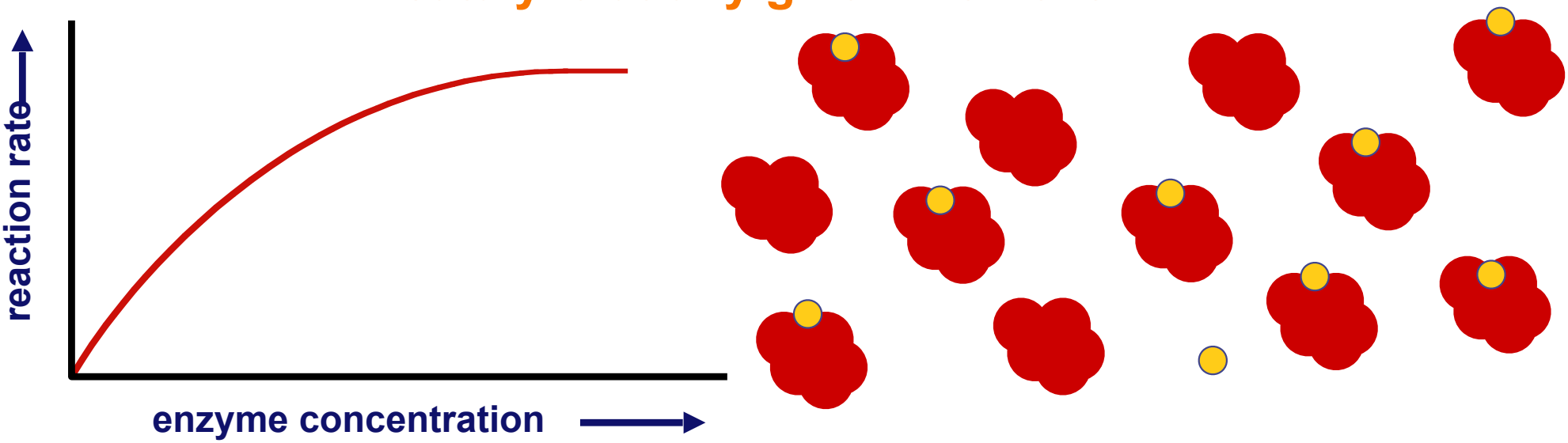
Enzyme concentration affects reaction rate

- Assuming substrate concentration is unchanging.

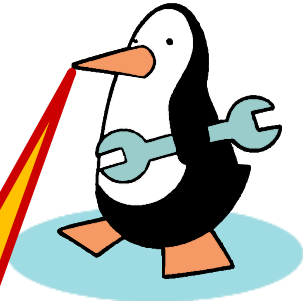


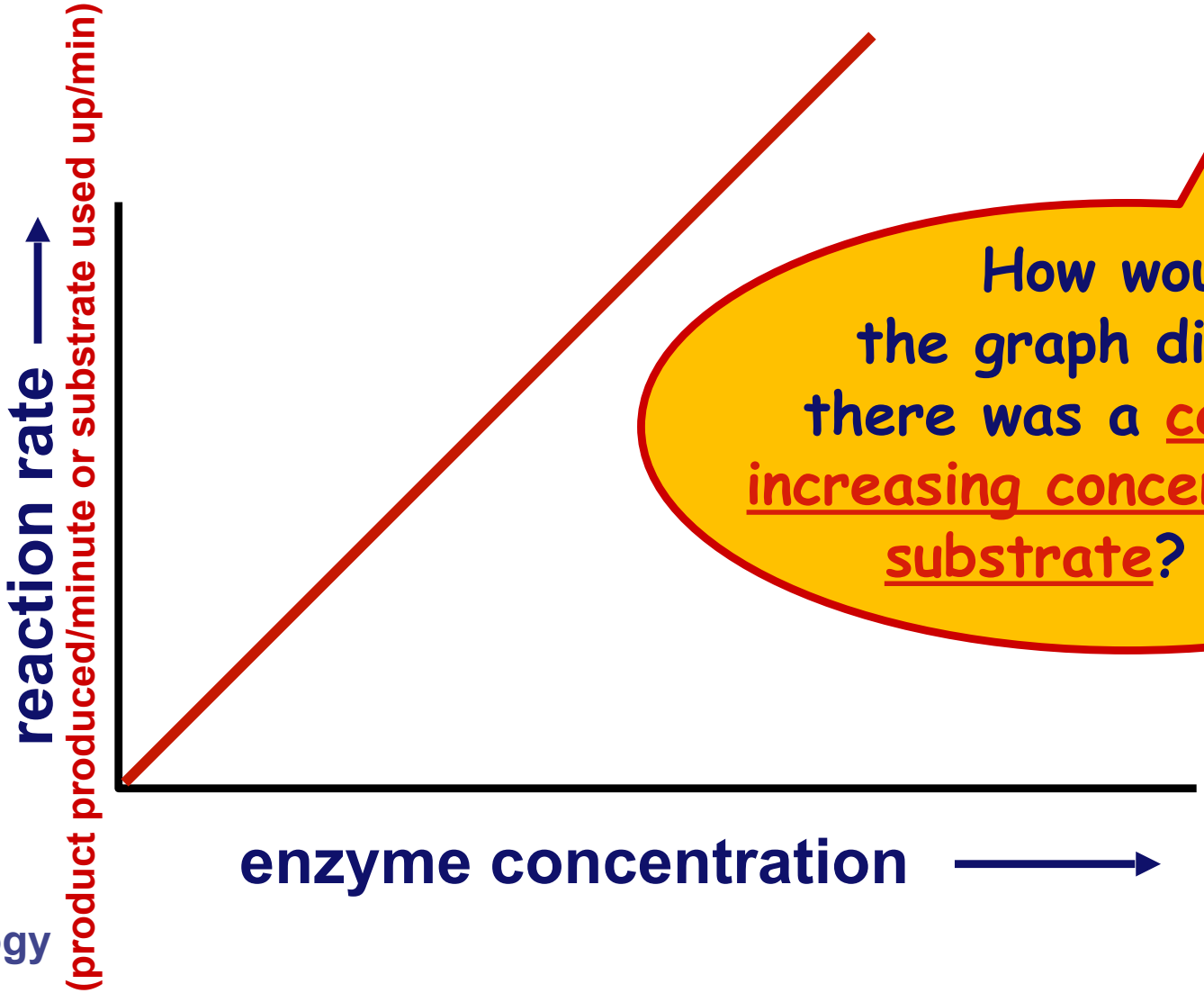
Factors affecting enzyme function

- **Enzyme concentration**
  - **As enzyme conc. ↑ = reaction rate ↑**
    - more enzymes = more frequent collisions with substrates
  - **At a certain point, reaction rate levels off**
    - substrate becomes limiting factor
      - not all enzyme molecules can find substrates to catalyze at any given moment



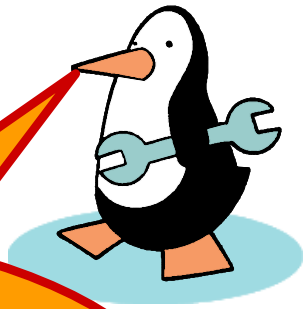
Enzyme concentration affects reaction rate



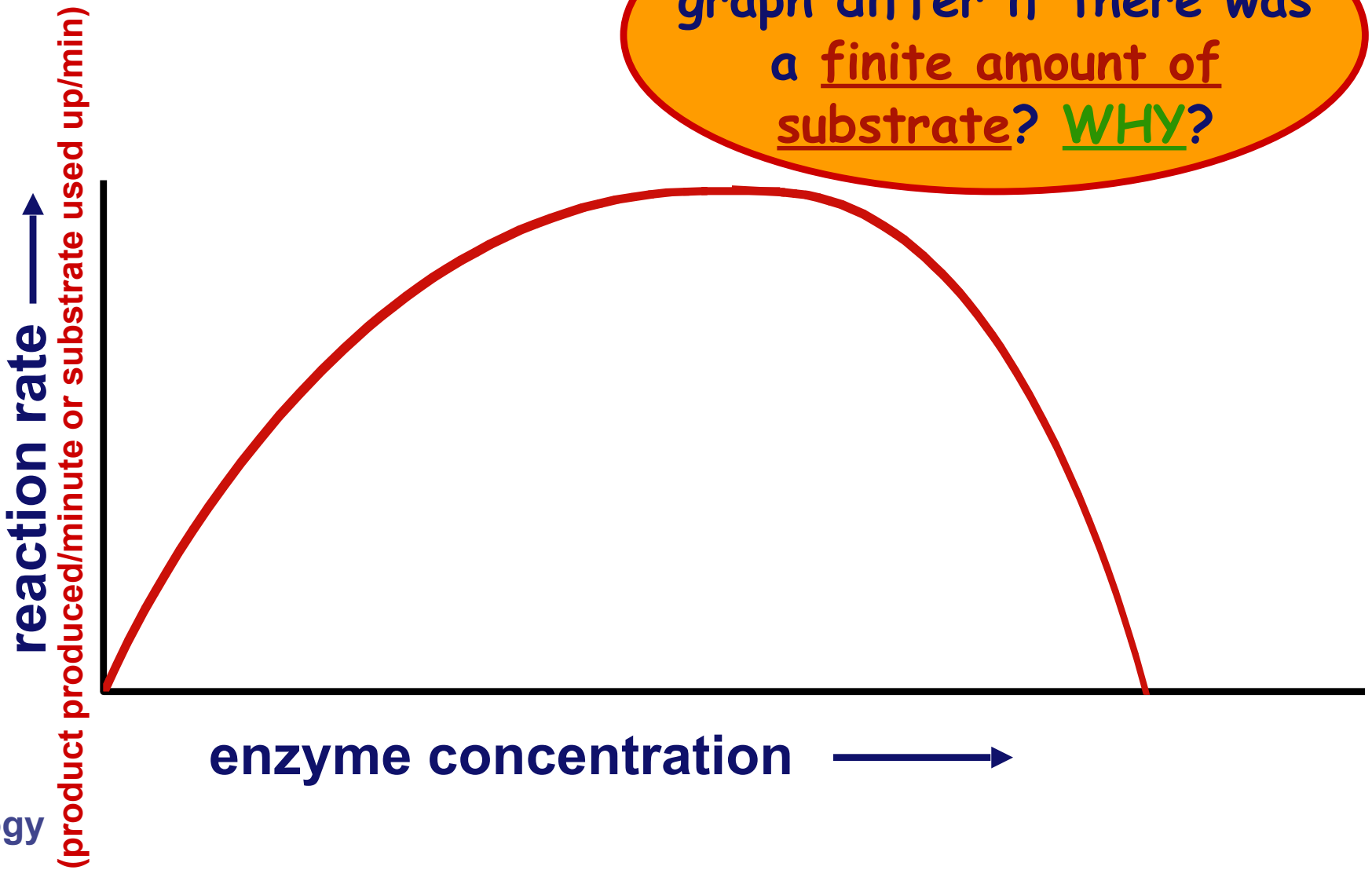


How would the graph differ if there was a constantly increasing concentration of substrate? WHY?

Enzyme concentration affects reaction rate

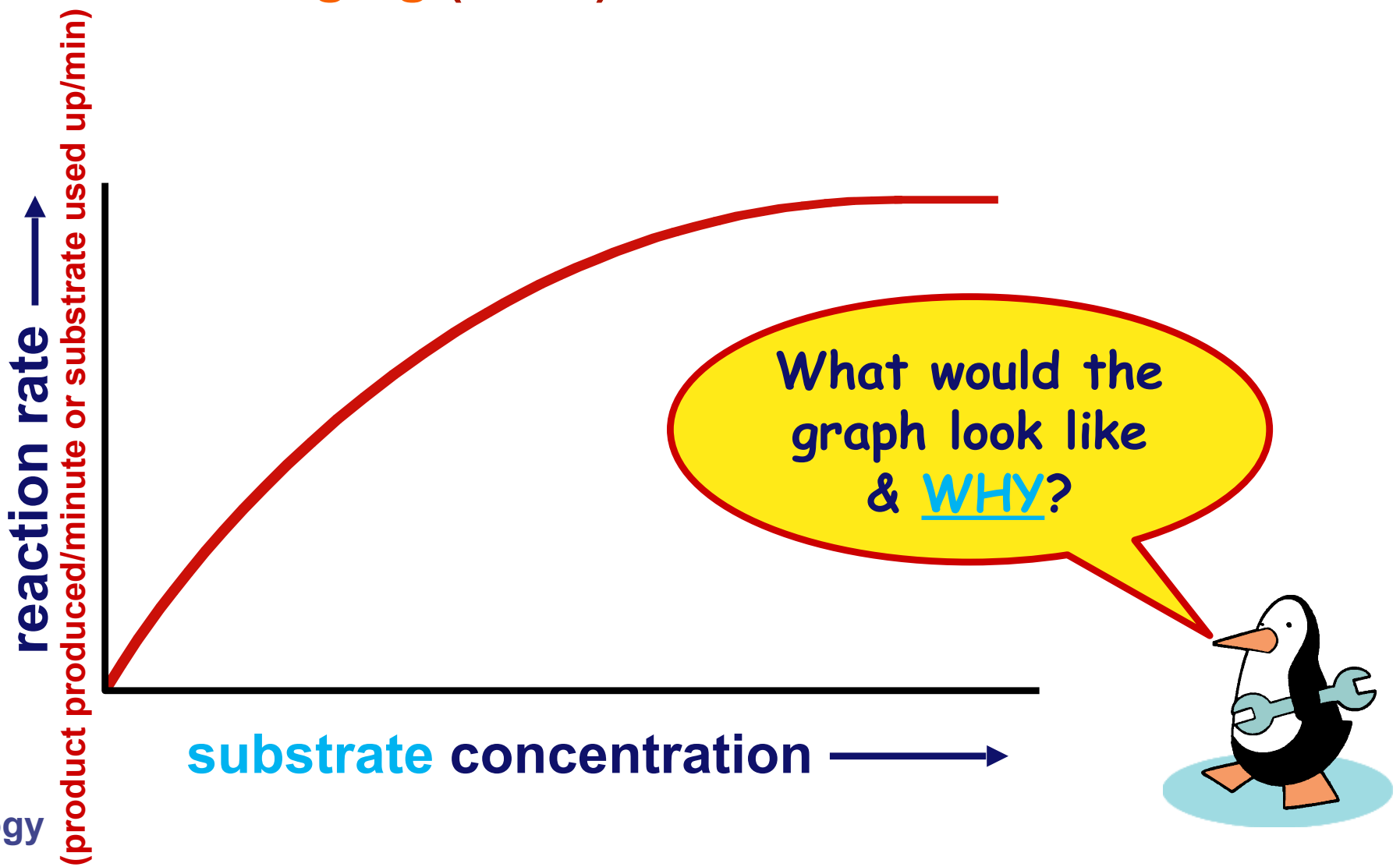


How would the graph differ if there was a finite amount of substrate? WHY?



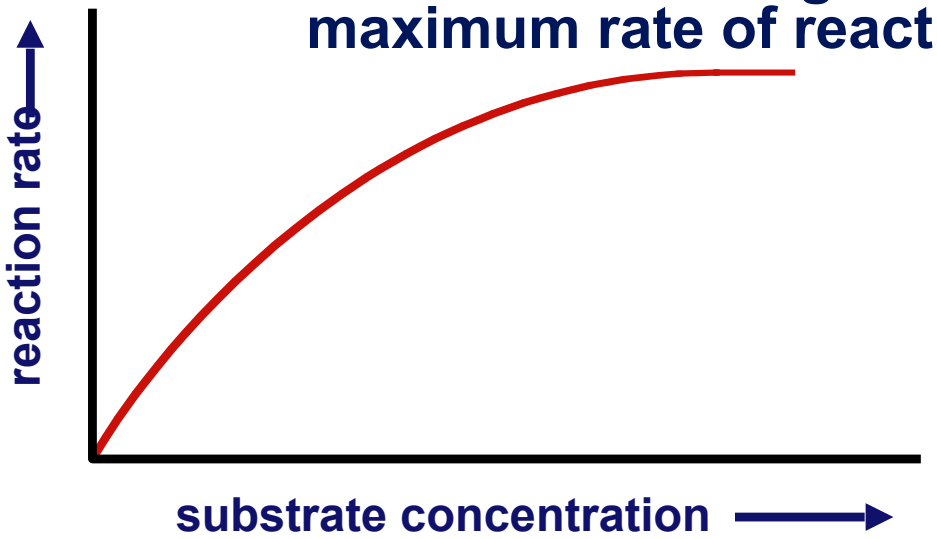
Substrate concentration also affects reaction rate

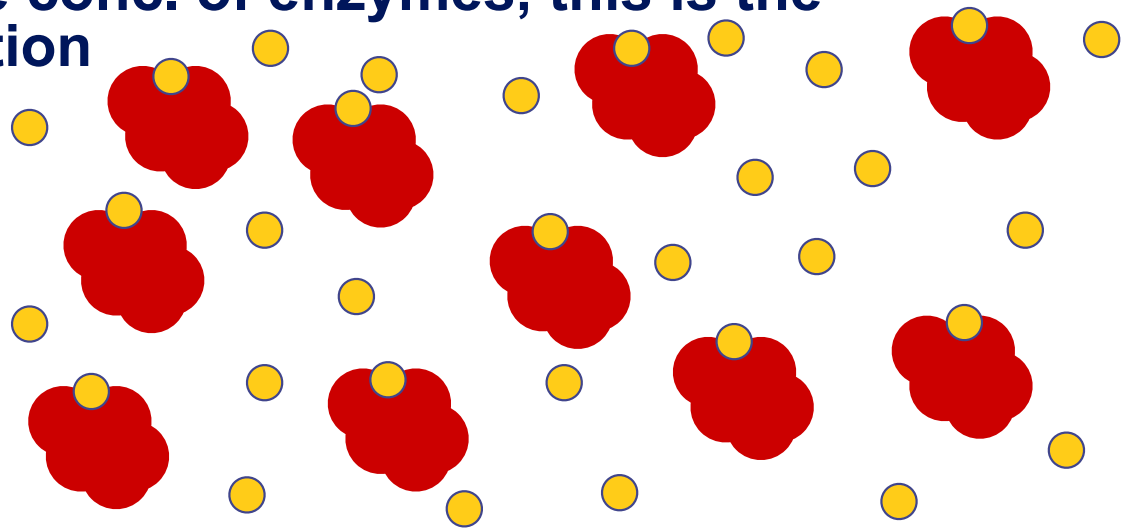
- Assuming enzyme concentration is unchanging (finite)



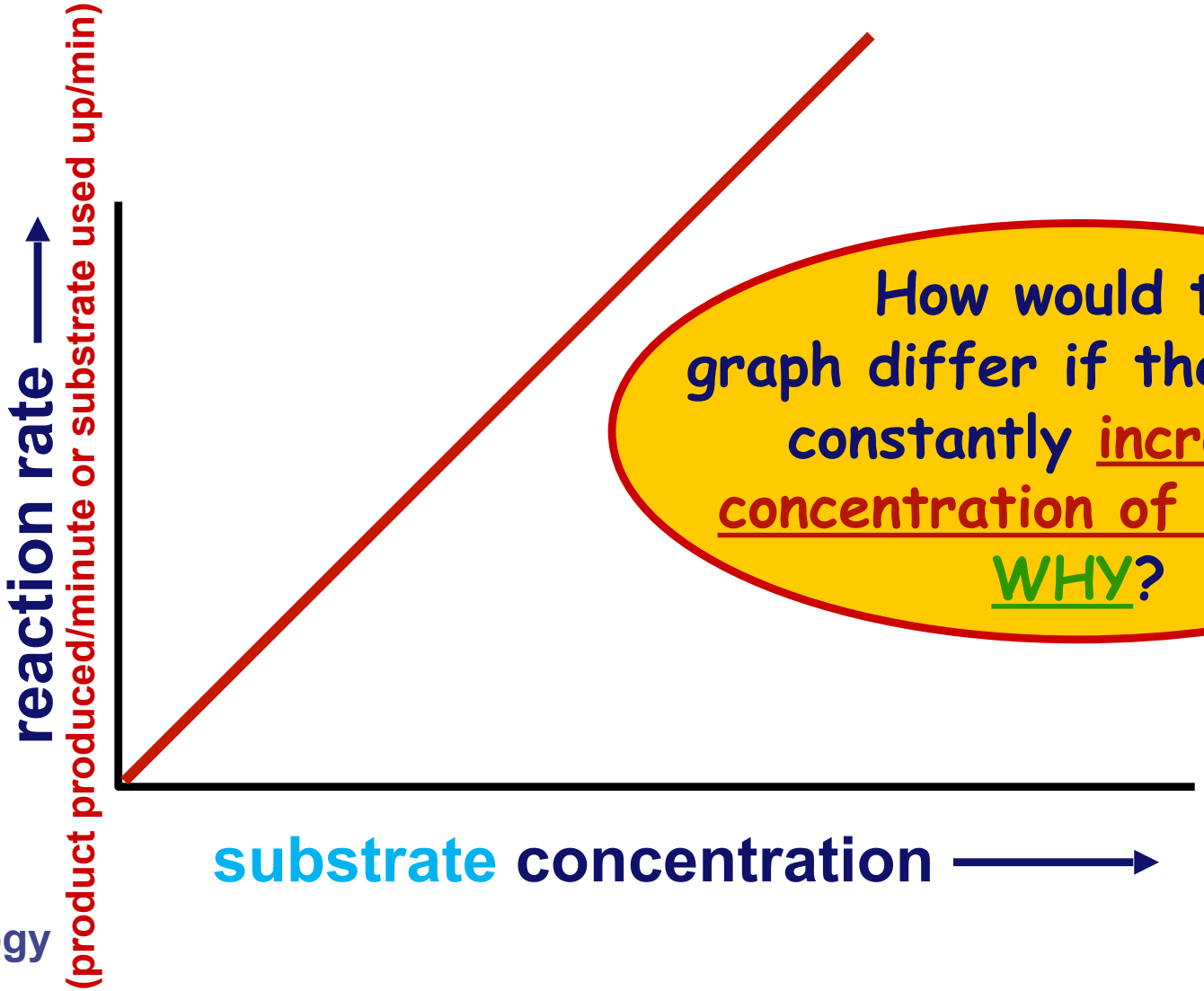
Factors affecting enzyme function

- **Substrate concentration affects reaction rates**
  - **as substrate conc. ↑ = reaction rate ↑**
    - **more substrate = more frequently collide with enzyme**
      - **↑ substrate conc. means ↑ # of substrates accessing the active site**
  - **At a certain point, the reaction rate levels off**
    - **all enzymes have active site engaged, filled, at any moment so additional substrate has to wait for an active site to be available to be catalyzed next**
      - **enzyme is now said to be SATURATED**
    - **Without increasing the conc. of enzymes, this is the maximum rate of reaction**

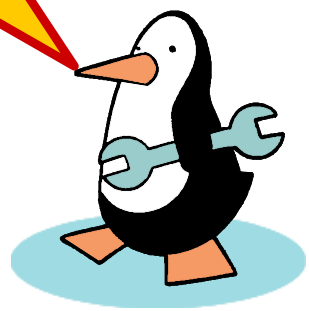




Substrate concentration also affects reaction rate

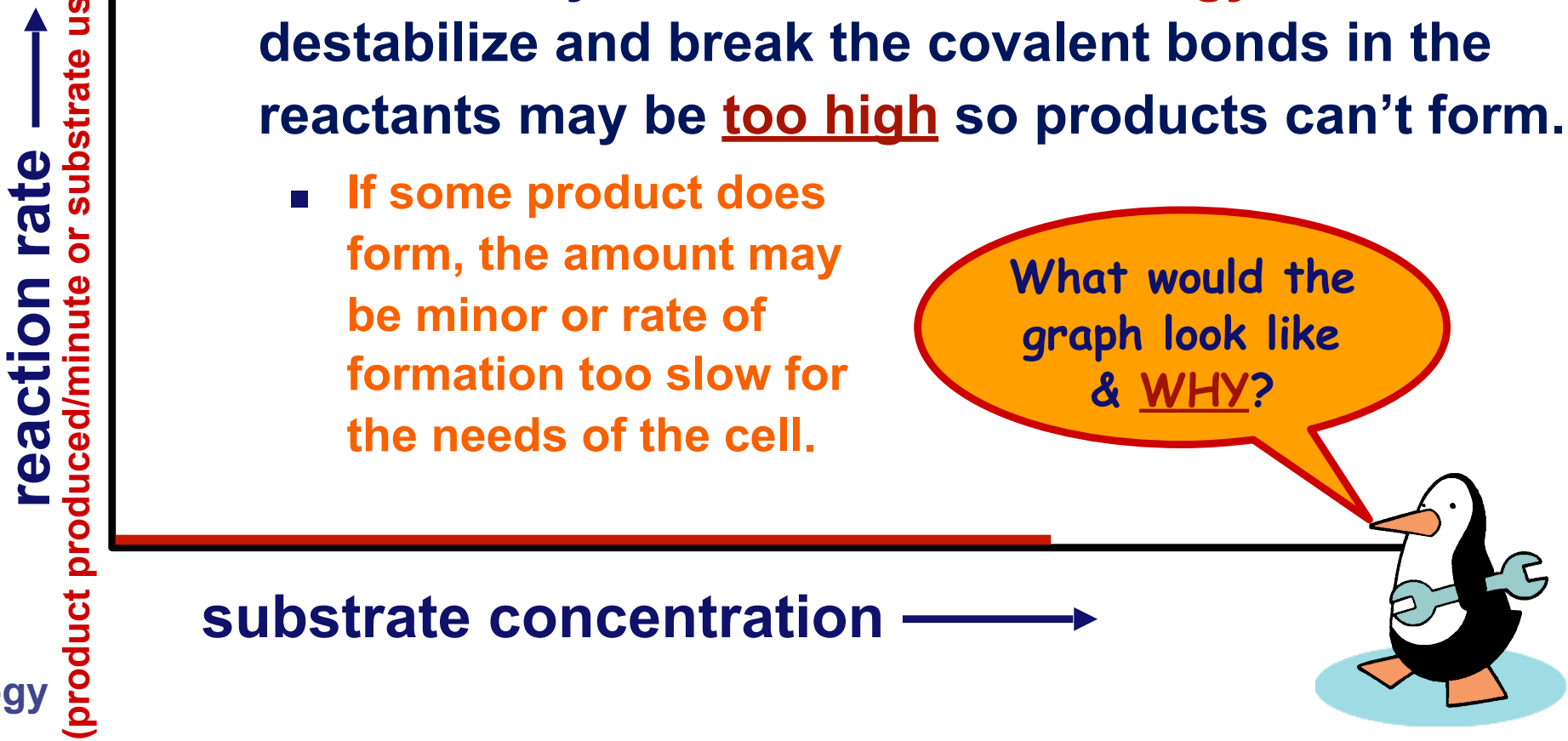


How would the graph differ if there was a constantly increasing concentration of enzyme? WHY?

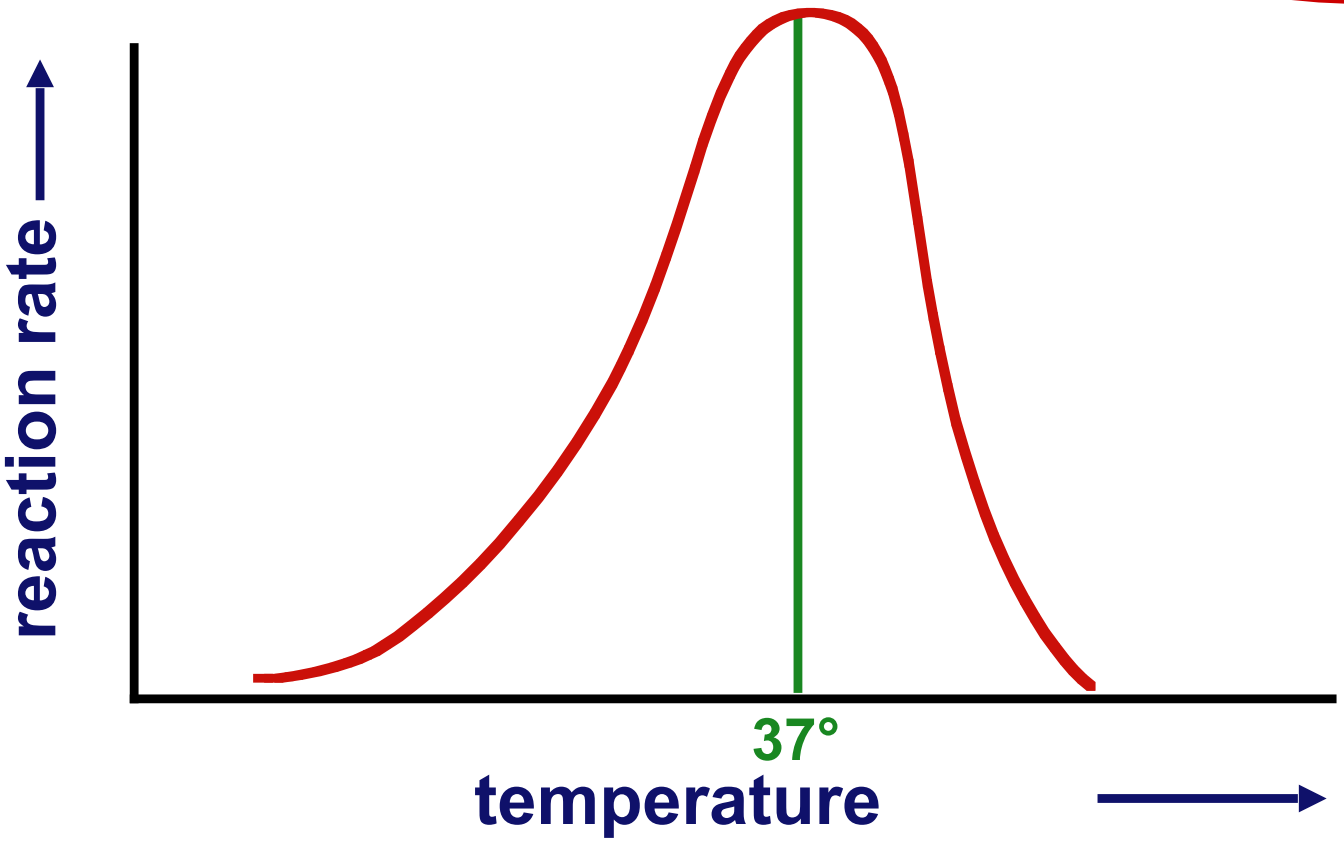


Enzyme concentration affects reaction rate

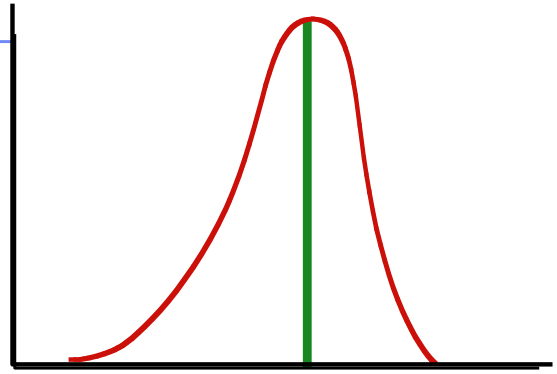
- How would the graph differ in the presence of a finite amount of enzyme which had been rendered non-functional? *(enzymes were denatured or their activity inhibited)*
  - With no enzyme, the activation energy needed to destabilize and break the covalent bonds in the reactants may be too high so products can't form.
    - If some product does form, the amount may be minor or rate of formation too slow for the needs of the cell.



Every enzyme has an *optimal* Temperature



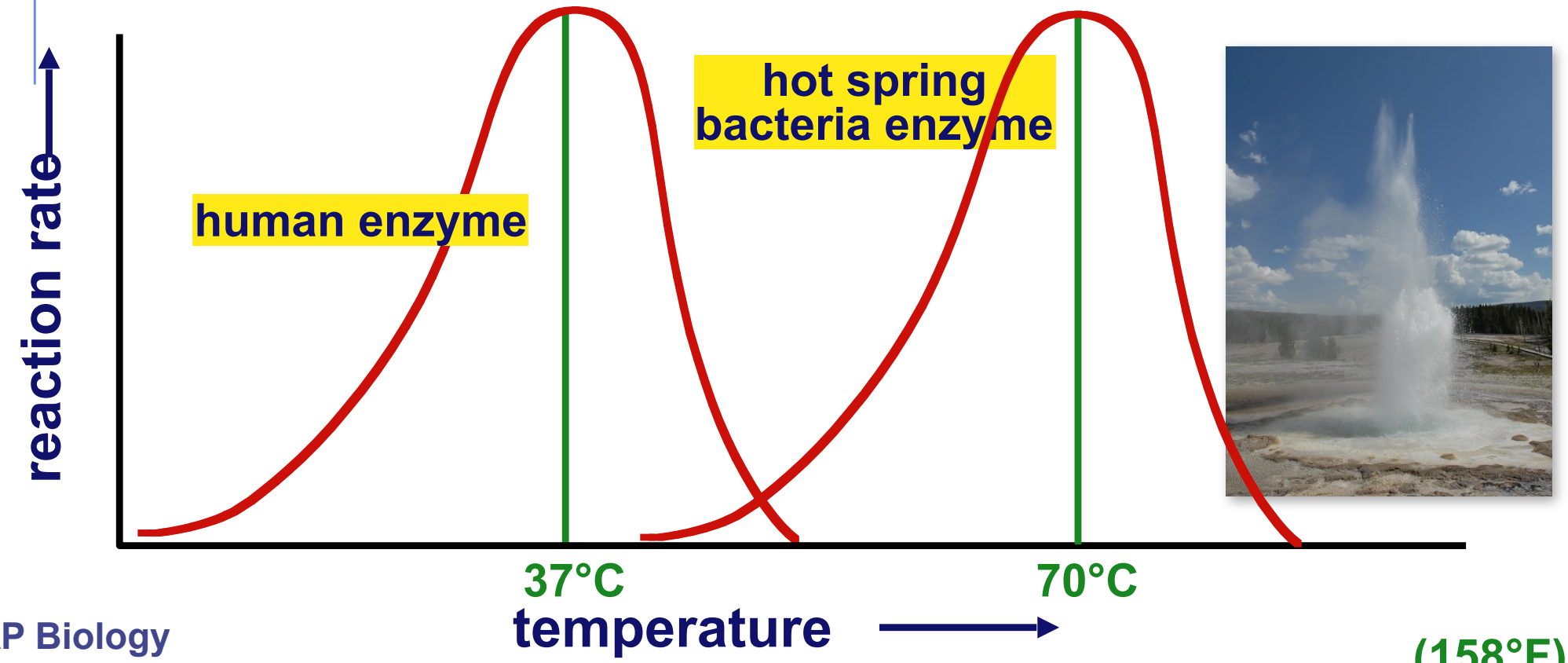
How Temp. Affects Enzyme Functioning



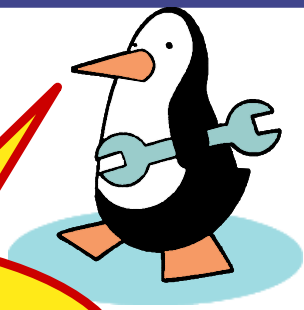
- Optimum T°
  - Enzyme has proper shape while experiencing many molecular collisions due to random motion of substrates and enzymes in solution
    - human enzymes optimal T = 35°- 40°C
      - body temp = 37°C
  - Heat: increase T° beyond optimum T°
    - increased thermal agitation of molecules disrupts weak bonds in enzyme & between enzyme & substrate
      - Hydrogen bonds, ionic attractions (dipole forces), and other weak interactions that stabilize the active shape of the enzyme break
    - Enzyme eventually DENATURES = loses 3D shape (3°structure) and thus its ability to function.
  - Cold: decrease T° under optimum T °
    - molecules move slower and slower the colder it gets causing decreased collisions between enzyme & substrate
      - less product is able to form if there are fewer interactions between substrate and enzymes

Enzymes and temperature

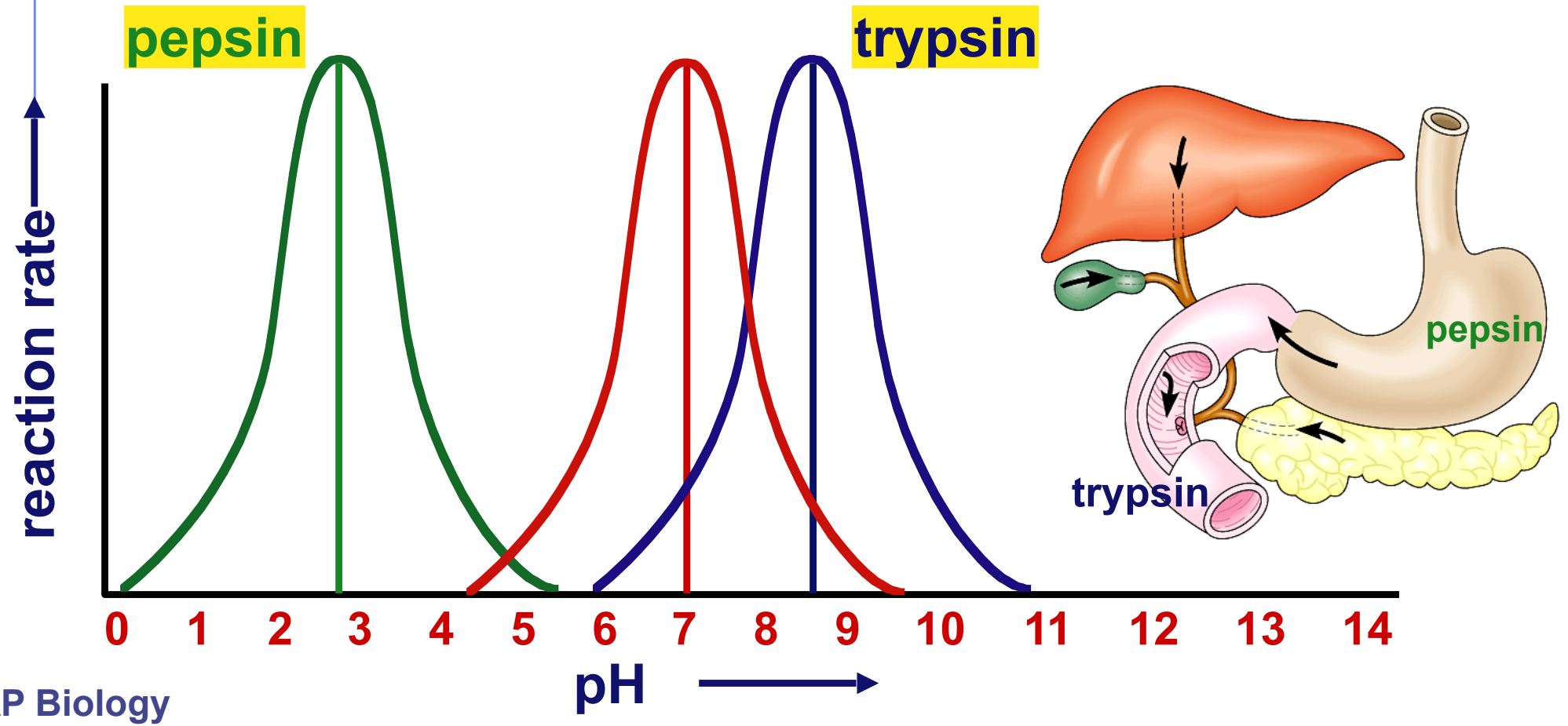
- Different enzymes function in different organisms in different environments
  - Natural selection favors the passing down of DNA from generation to generation that allows the organism to make enzymes that work best at the temperature the organism exists in.



Enzymes function best at their *optimal* pH

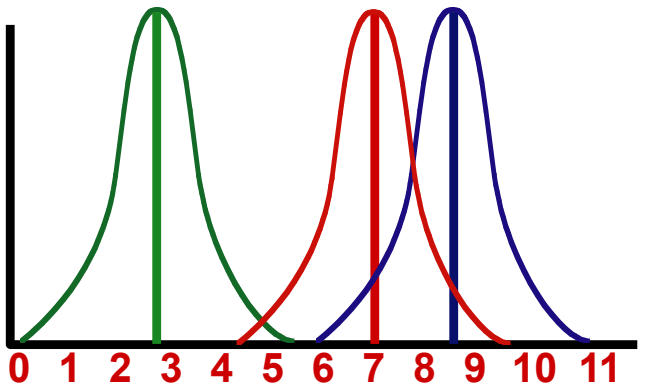


What's happening here?!

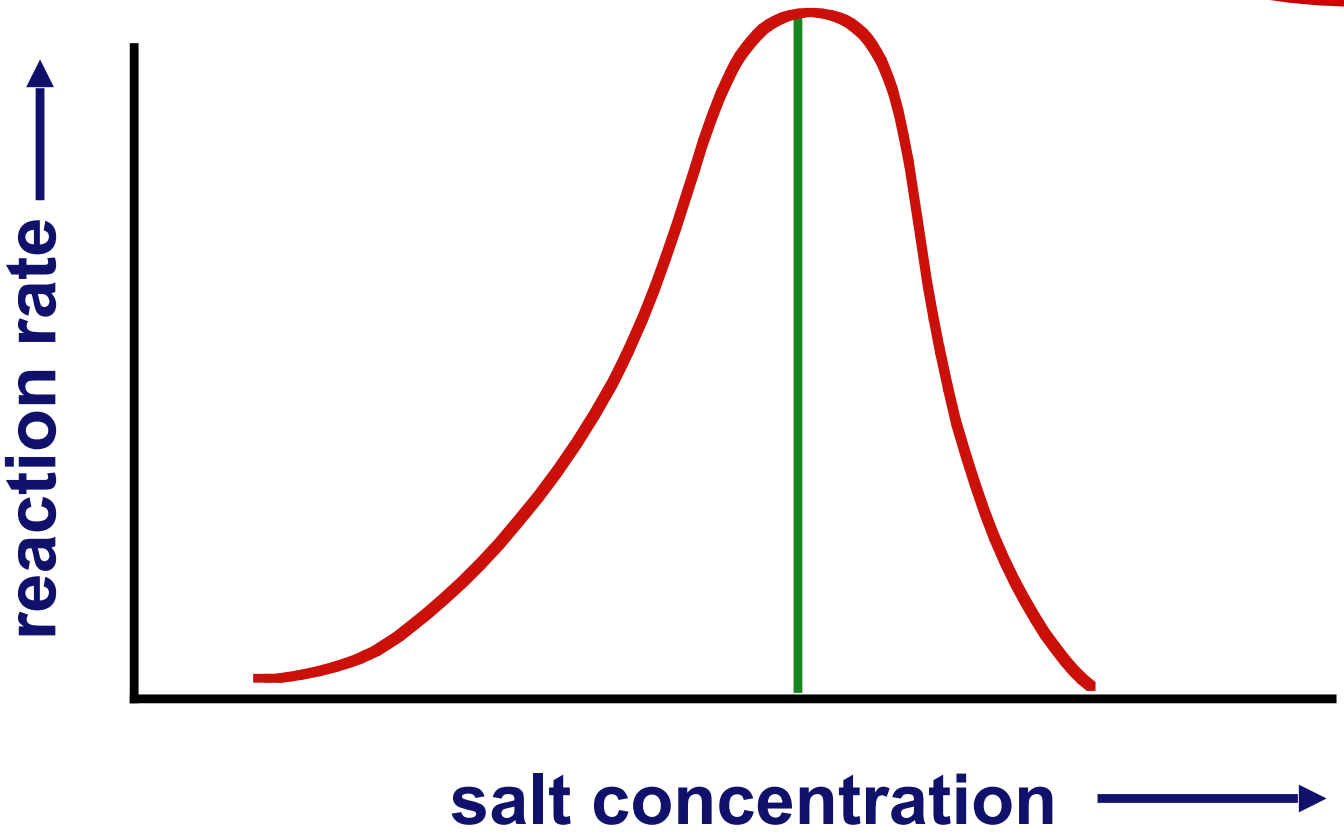


How pH Affects Enzyme Functioning

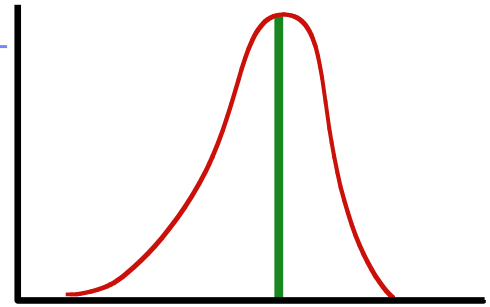
- **Changes in pH**
  - adding or removing H^+ in solution disrupts weak other intermolecular bonds and, therefore, disrupts the 3D shape of enzymes
    - $[H^+]$ disrupts attractions between charged amino acids
    - alters the 2° & 3° & 4° structure of proteins
      - DENATURES proteins causing enzymes to loose their ability to function!!!
- **What is the optimal pH of enzymes?**
  - most human enzymes = pH 6-8
    - BUT, optimal pH depends on localized conditions
      - pepsin (stomach) = pH 2-3
      - trypsin (small intestines) = pH 8



Salinity affects enzyme functioning



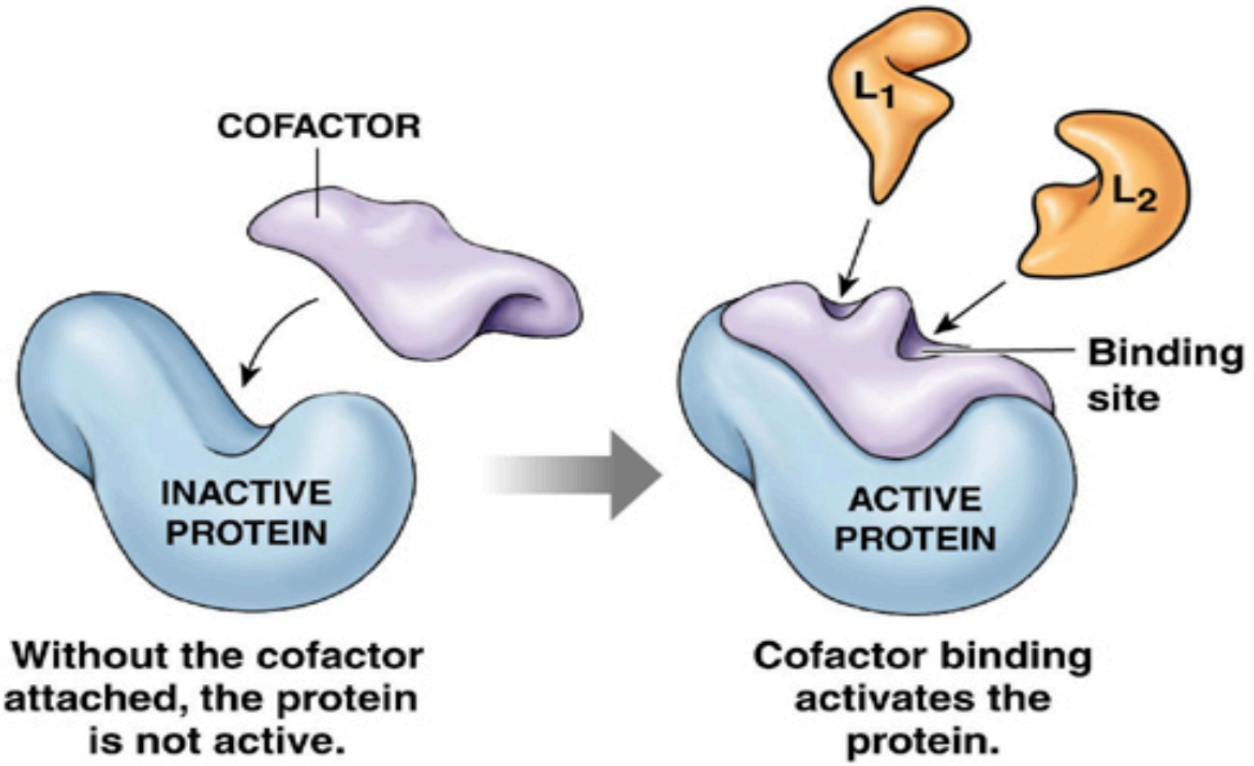
Factors affecting enzyme function



- **Changing salt concentration**
  - adding or removing cations (+) & anions (–) disrupts weaker intermolecular interactions, and, therefore, disrupts the 3D shape of proteins
    - changing ion concentrations <u>disrupts and alters attractions and repulsions</u> experienced between charged amino acids in polypeptides of proteins, including enzymes
    - affect and changes the proteins 2° & 3° structure
      - <u>DENATURES</u> the protein, causing the enzyme to loose its ability to function!!!
- **Enzymes are intolerant of extreme salinity**
  - Dead Sea is called dead for a reason!

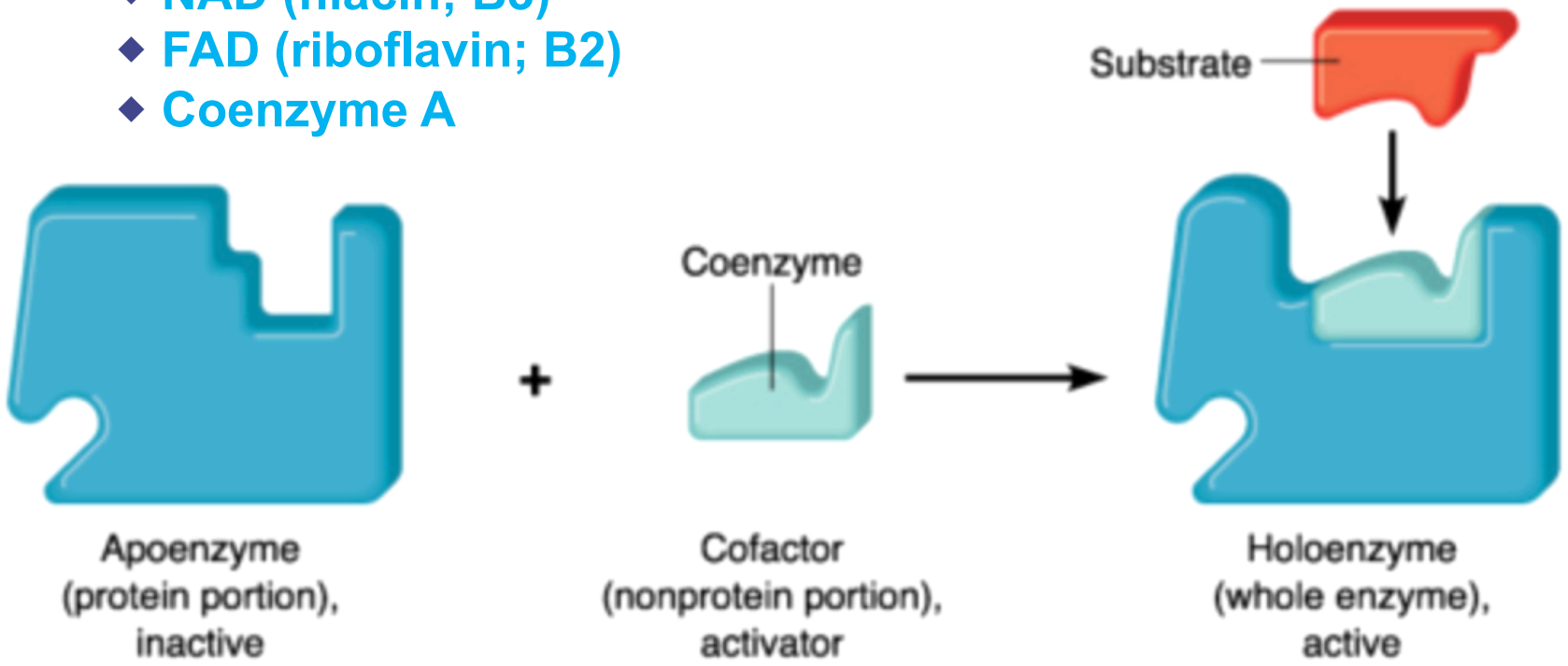
Some Enzymes Need Additional Non-Substrate Substances Near or in Their Active Sites for their Catalytic Activities to Function

- "Enzyme Helpers"
  - cofactors
    - non-protein, small inorganic compounds & ions that help catalytic functions of some enzymes
      - Mg, K, Ca, Zn, Fe, Cu ions
        - Bind within enzyme molecule



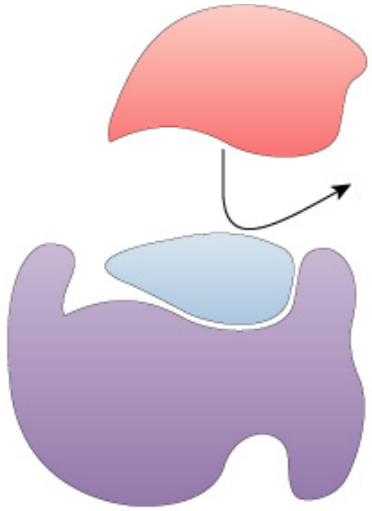
Compounds which help catalytic function of enzymes

- "Enzyme Helpers"
  - coenzymes
    - non-protein, organic molecules that help catalytic functioning of certain enzymes
      - bind temporarily or permanently to enzyme near active site
    - Most vitamins or vitamin derivatives acts as coenzymes
      - NAD (niacin; B3)
      - FAD (riboflavin; B2)
      - Coenzyme A



Compounds which regulate enzymes

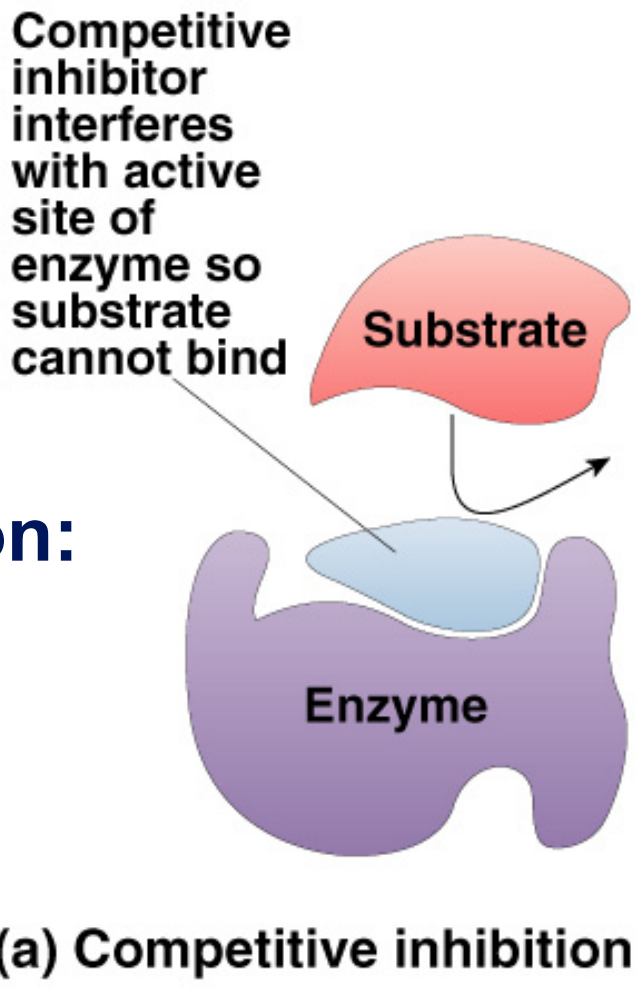
- **Activators:**
  - Molecules that increase enzyme activity
- **Inhibitors**:
  - molecules that reduce enzyme activity
    - competitive inhibition
    - noncompetitive inhibition
    - irreversible inhibition
    - Products of chemical pathways that function in feedback inhibition

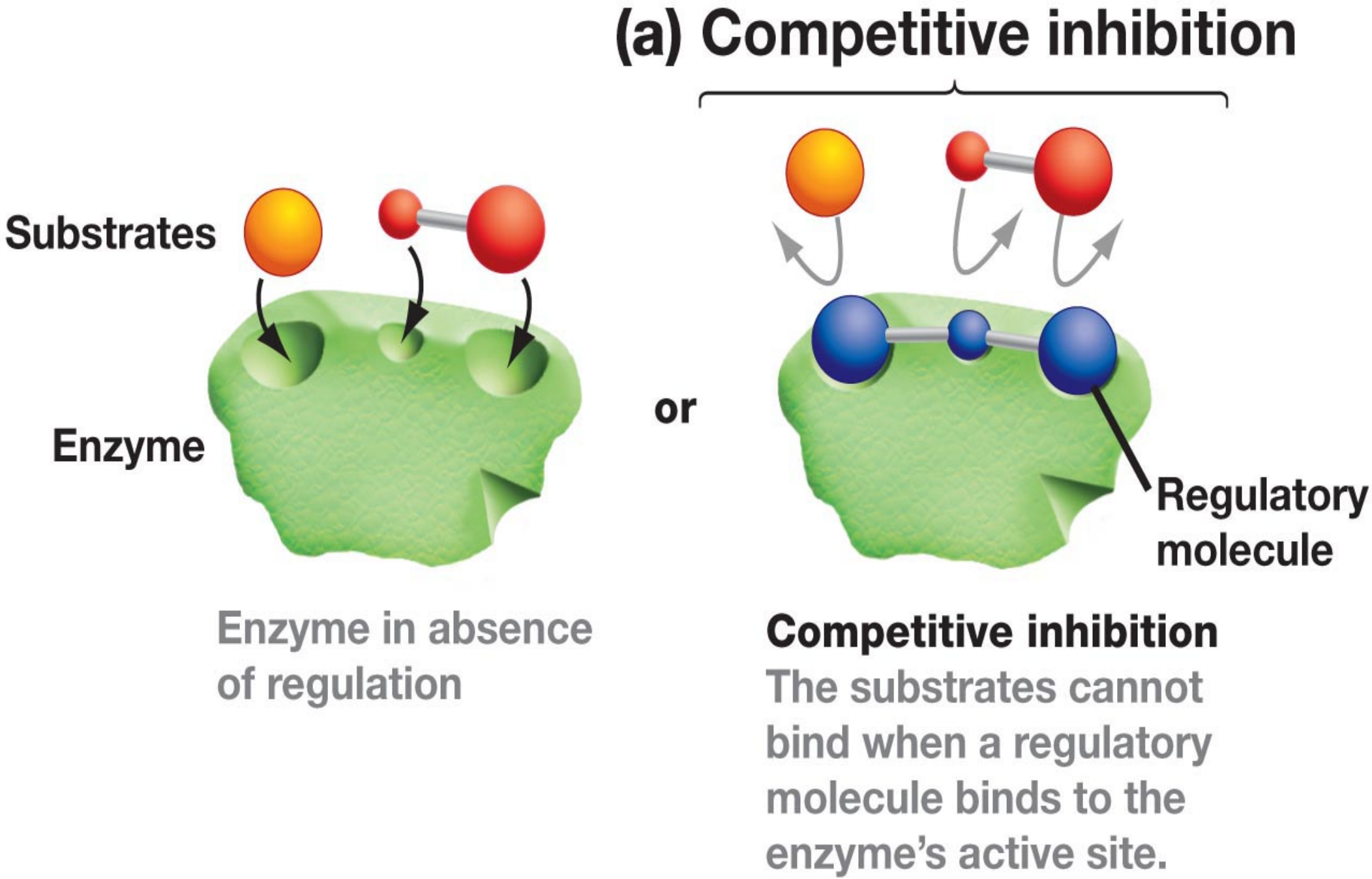


Competitive Inhibitor

- Inhibitor & substrate "compete" for active site
  - Ex: penicillin
    - blocks enzyme bacteria use to build cell walls

- Can be overcome by increasing substrate concentration:
  - saturate solution with substrate so it out-competes inhibitor for active site on enzyme





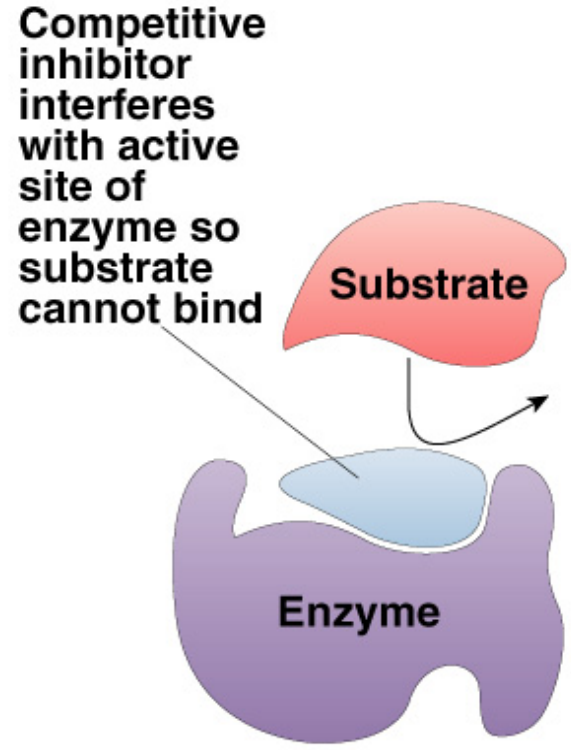
Competitive Inhibitor Example

- Inhibitor & substrate "compete" for active site
  - disulfiram (Antabuse) used to treat chronic alcoholism
    - blocks enzyme that breaks down alcohol
    - severe hangover & vomiting 5 - 10 minutes after drinking

Ethanol is metabolized in the body by oxidation to acetaldehyde, which is in turn further oxidized to acetic acid by aldehyde oxidase enzymes.

Normally, the second reaction is rapid so that acetaldehyde does not accumulate in the body.

A drug, disulfiram (Antabuse) inhibits the aldehyde oxidase which causes the accumulation of acetaldehyde with subsequent unpleasant side-effects of nausea and vomiting.



(a) Competitive inhibition

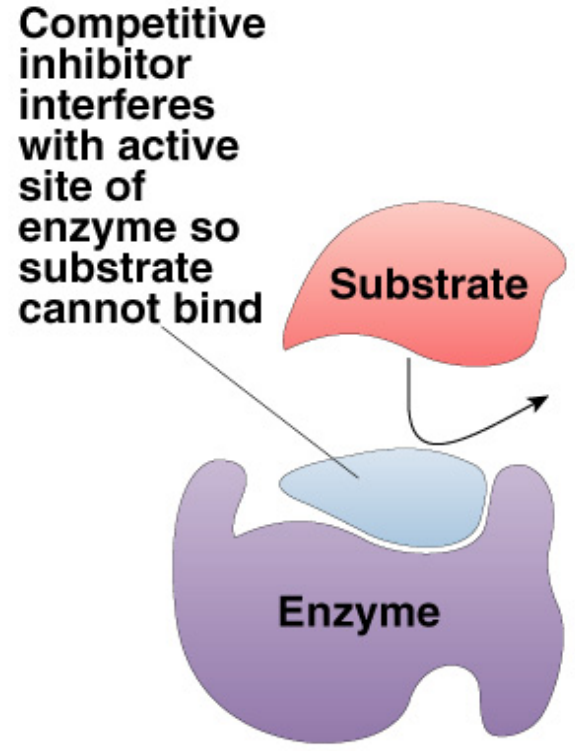
Competitive Inhibitor

- **Enzymes are highly specific!!!**
  Very rarely will an enzyme catalyze more than one reaction *[usually the substrates are extremely similar in size & properties if this happens]*

Methanol (wood alcohol) poisoning occurs because methanol is oxidized to formaldehyde and formic acid which attack the optic nerve causing blindness.

Ethanol is given as an antidote for methanol poisoning because ethanol competitively inhibits the oxidation of methanol.

Ethanol is oxidized in preference to methanol and consequently, the oxidation of methanol is slowed down so that the toxic by-products do not have a chance to accumulate.

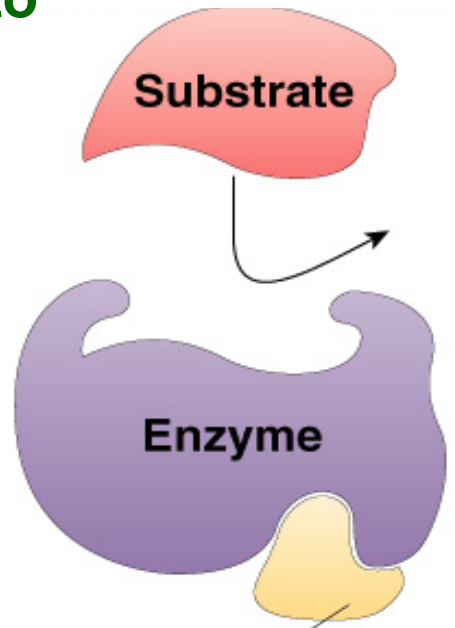


(a) Competitive inhibition

Non-Competitive Inhibitor

Inhibitor binds to site other than active site

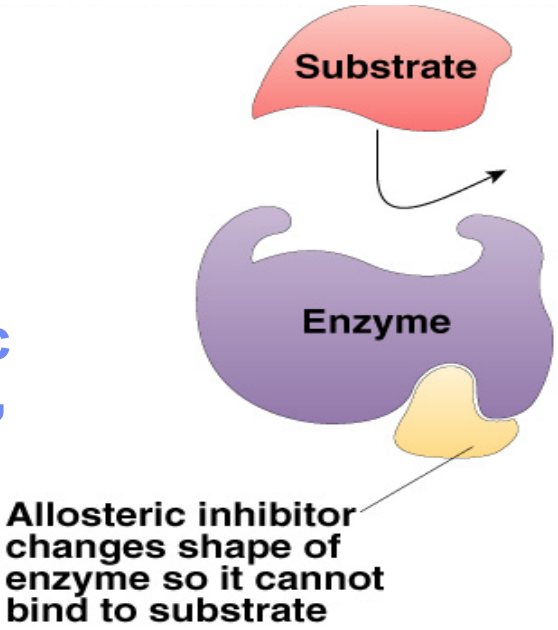
- DO NOT DIRECTLY COMPETE WITH SUBSTRATE
  - Increasing substrate amounts does nothing to overcome the inhibition
- causes enzyme to change shape
  - Enzyme experiences a conformational change
    - active site is no longer functional binding site
      - keeps enzyme inactive
- allosteric inhibitor binds to allosteric site



(b) Noncompetitive inhibition

Non-Competitive Inhibitor

- Ex: some anti-cancer drugs inhibit enzymes involved in synthesis of nucleotides & therefore in building of DNA
  - These drugs stop DNA production & prevent the division of more cancer cell
    - methotrexate and FdUMP inhibit enzymes involved in the synthesis of thymidine and hence DNA.
- ex: heavy metal poisoning also occurs through noncompetitive inhibitors
- ex: cyanide poisoning does too!
  - Cyanide combines with the copper prosthetic groups of the enzyme cytochrome C oxidase, thus inhibiting respiration which causes an organism to run out of ATP



(b) Noncompetitive inhibition

CN- causes you to run out of ATP

QUICK FACT

Today I found out...

Cyanide poisoning works by not allowing the body to use oxygen. So the blood remains oxygenated after it passes through your body and back to the lungs. Thus, it causes the body to suffocate, even though the lungs are working properly.

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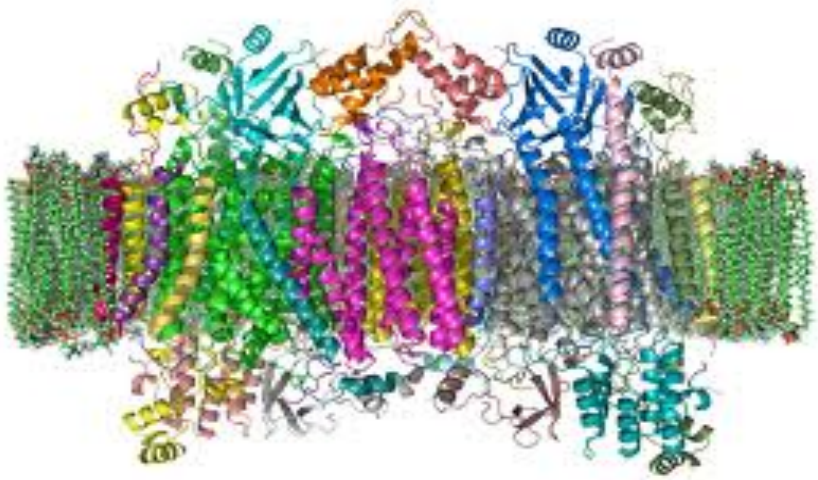
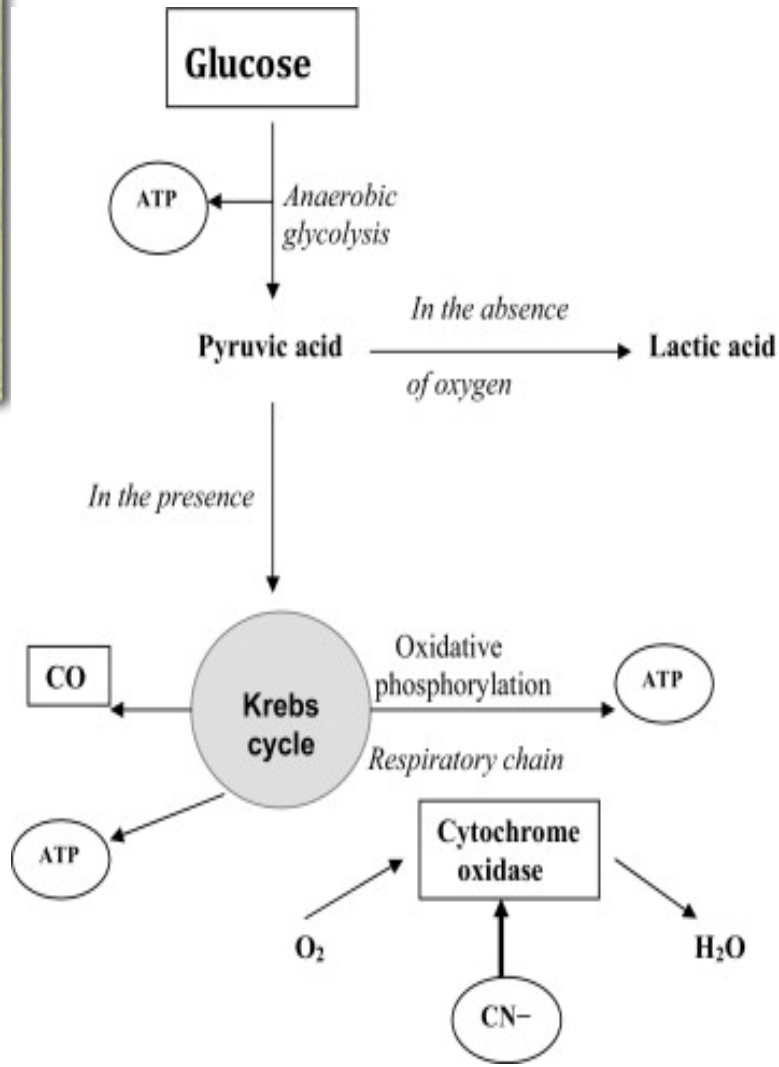
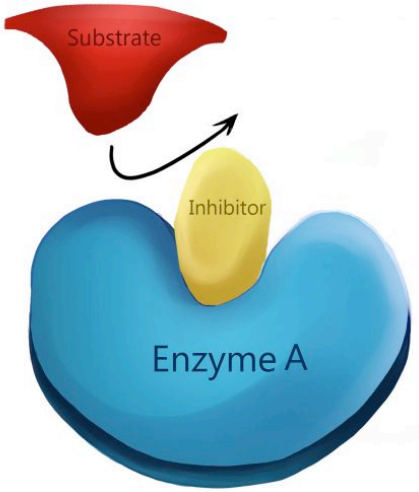
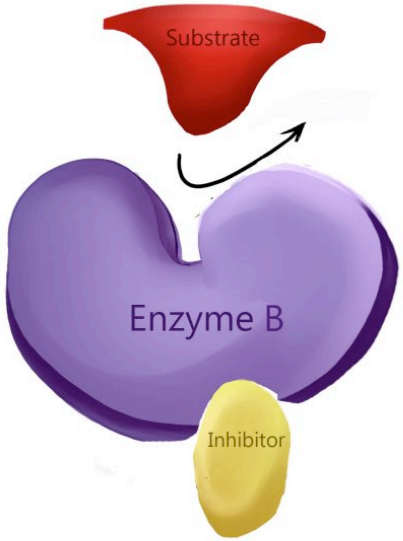




Irreversible inhibition



- These inhibitor permanently bind to enzymes
  - competitor
    - permanently binds to active site
    - Permanently blocks active site
  - Noncompetitive or allosteric
    - permanently binds to allosteric site
    - permanently changes shape of enzyme
      - nerve gas, sarin, many insecticides (malathion, parathion…)

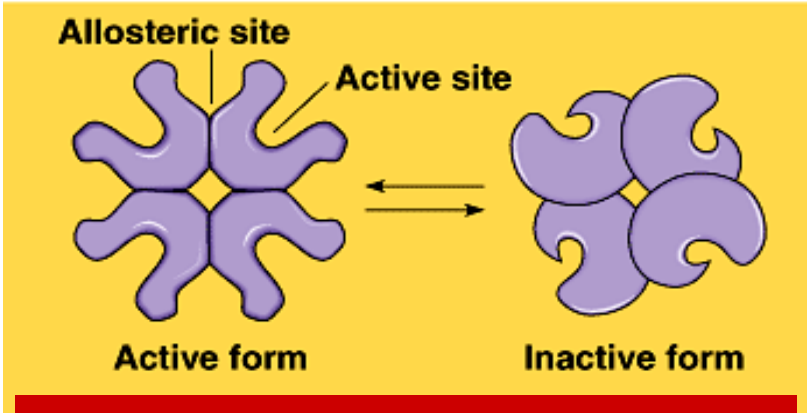


Irreversible inhibition

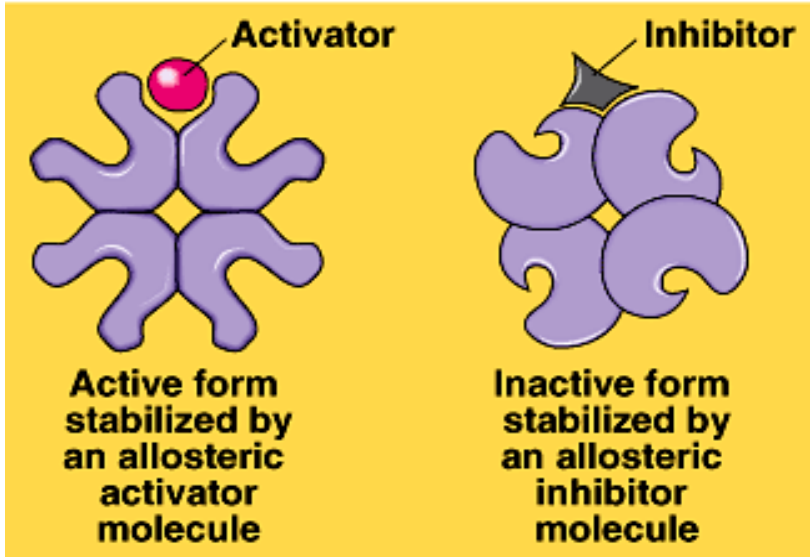
- EX: cholinesterase inhibitors:
  - Another example of irreversible inhibition is provided by the nerve gas diisopropylfluorophosphate (DFP)
    - For use in warfare.
    - Combines with the amino acid serine (contains the –SH group) at the active site of the enzyme acetylcholinesterase.
      - The enzyme deactivates the neurotransmitter acetylcholine.
- Neurotransmitters are needed to continue the passage of nerve impulses from one neuron to another across the synapse.
- Once the impulse has been transmitted, acetylcholinesterase functions to deactivate the acetycholine almost immediately by breaking it down.
  - If the enzyme is inhibited, acetylcholine accumulates and nerve impulses cannot be stopped, causing prolonged muscle contraction [the chemical message to the muscle isn't destroyed].
    - Paralysis occurs because skeletal muscle remain contracted and death may result since the respiratory muscles are affected [can't exhale].

Allosteric regulation: Common with multi-subunit enzymes

- Affecting the function of a protein by the binding of a regulatory molecule to a site other than the active site.
  - Can stimulate or inhibit enzyme
- Often a single activator or inhibitor that binds to one regulatory site will affect the active site of all the other subunits
- Conformational changes by regulatory molecules
  - inhibitors
    - keeps enzyme in inactive form
  - activators
    - keeps enzyme in active form

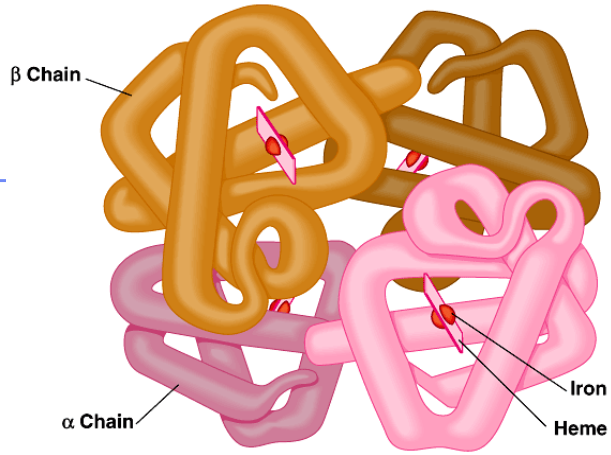


Conformational changes



Allosteric regulation

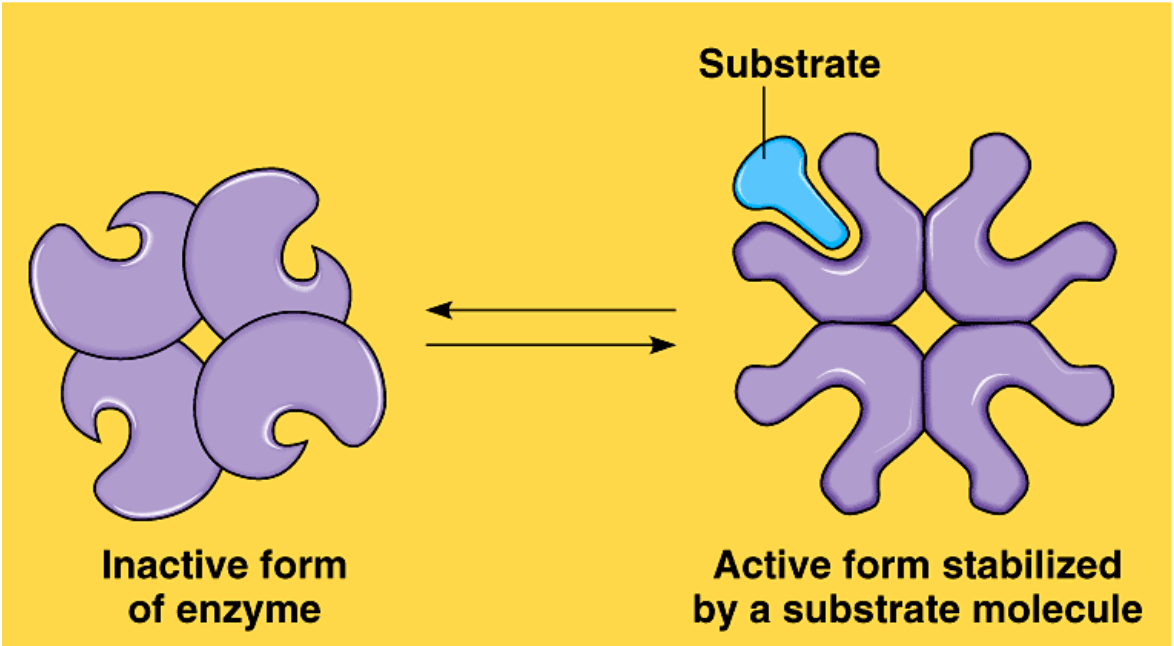
Cooperativity is seen in many protein complexes



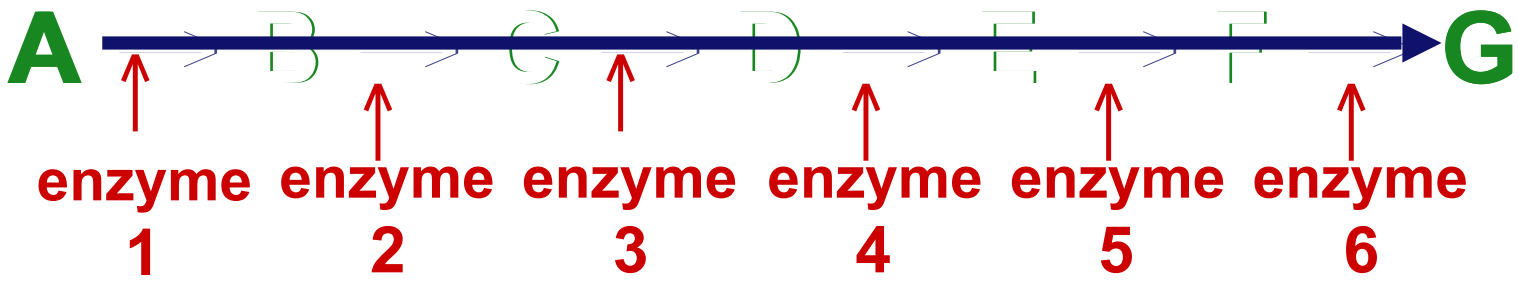
- **Substrate or ligand acts as an activator**
  - substrate causes conformational change in enzyme (or other protein)
    - induced fit
  - a conformational change in on enzyme [or other protein] the causes a conformational change in a neighboring protein in the complex etc.
    - this favors binding of substrate at 2nd enzyme site or favors enhanced activity of the second protein in the complex
  - makes all enzymes (or proteins) in the complex more active & effective
    - we see cooperatively in the transport protein hemoglobin too

Hemoglobin

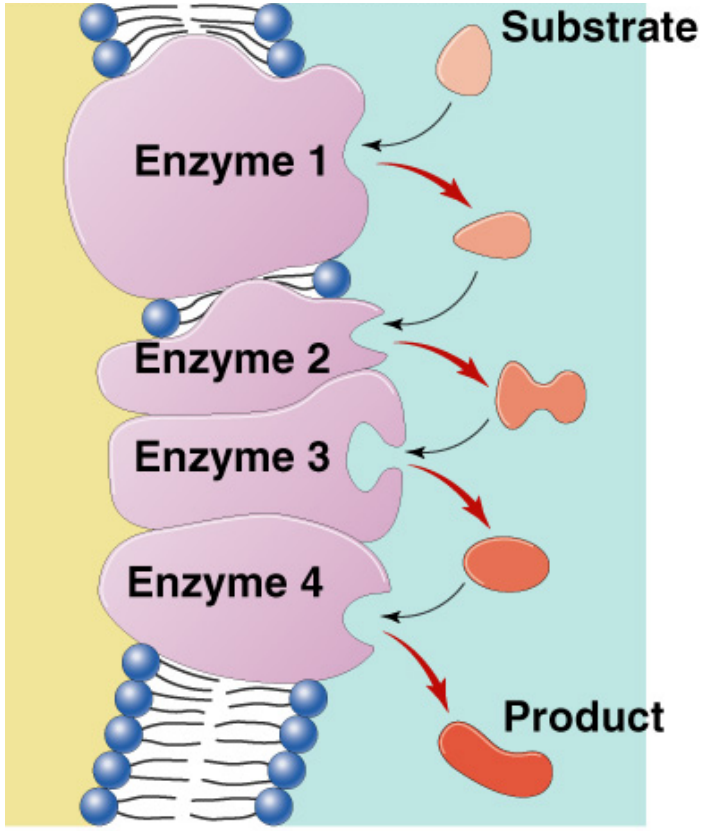
- 4 polypeptide chains
- can bind 4 O_2;
- 1st O_2 binds
- now easier for other 3 O_2 to bind



Metabolic pathways & feedback inhibition

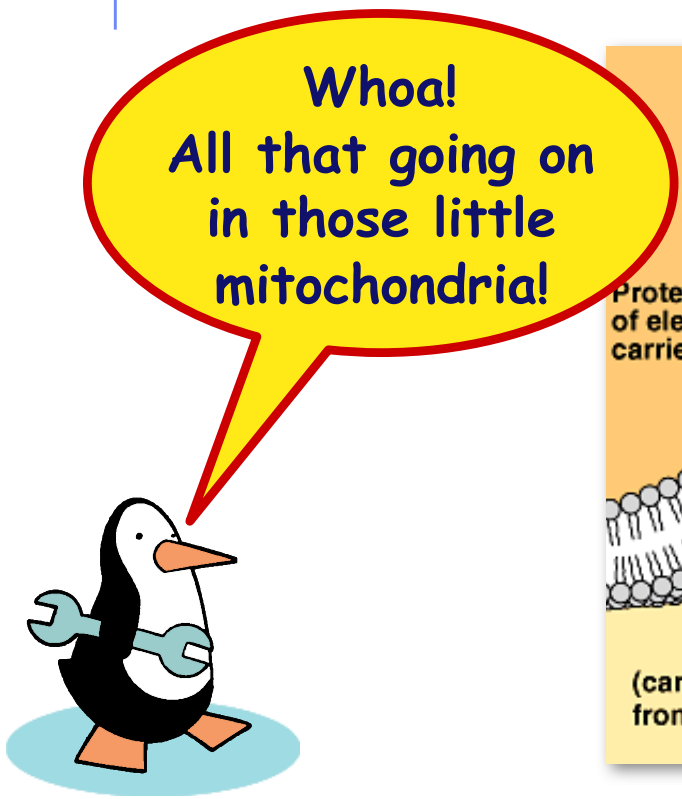


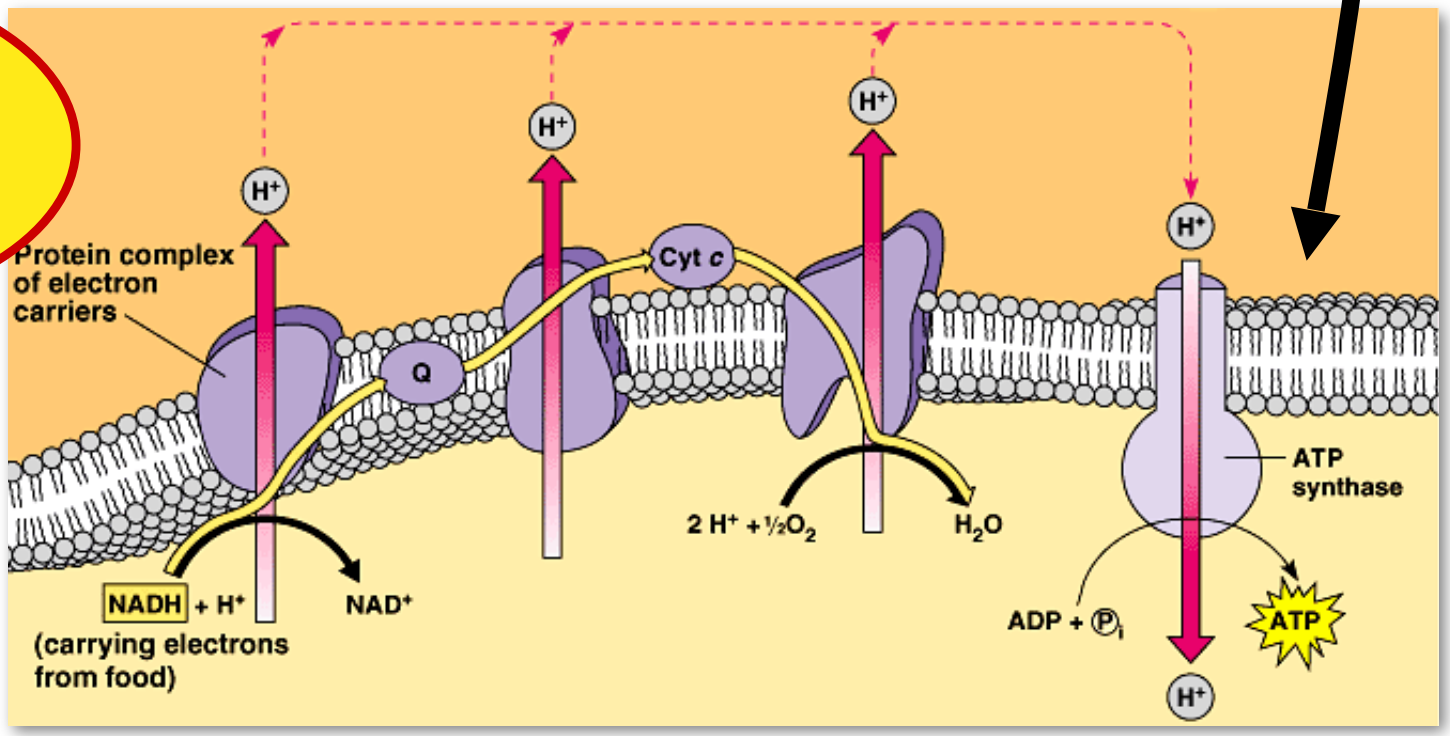
- **Chemical reactions of life are organized in pathways**
  - **divide chemical reaction into many small steps**
    - **artifact of evolution**
    - **↑ efficiency**
      - **intermediate branching points**
    - **↑ control = regulation**



Efficiency

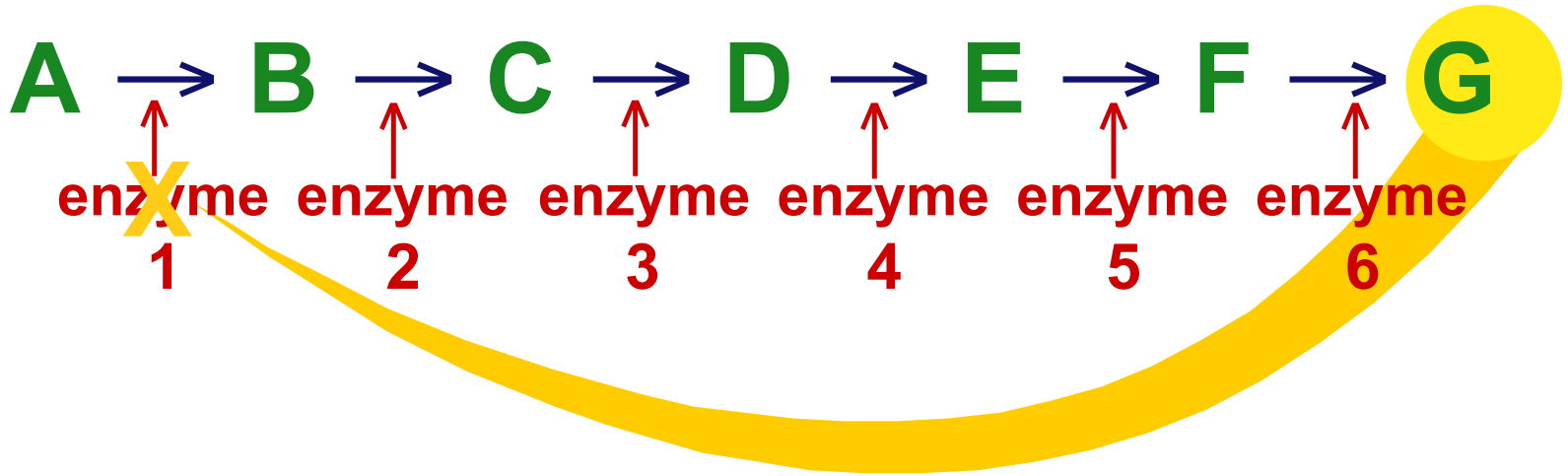
- **Some enzymes are organized in groups**
  - **Enzymes are embedded in membrane or grouped in complexes [held together by scaffold proteins] and arranged sequentially**





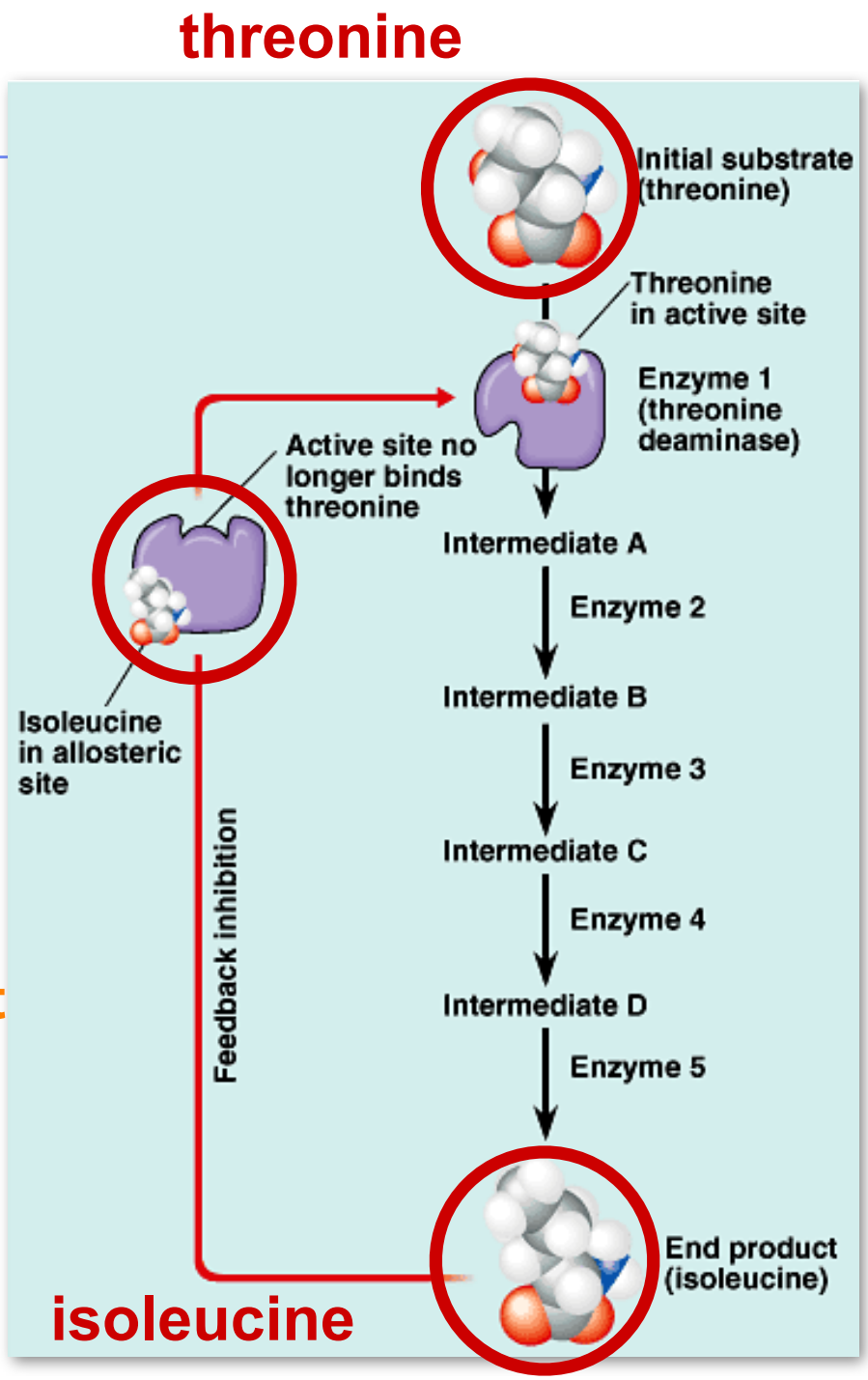
Feedback Inhibition

- Chemical reactions often occur in sequences
  - One product becomes the reactant in the next step in a pathway
- Feedback Inhibition allows for the regulation & coordination of production
  - Here, the final product is an inhibitor of earlier step
    - Usually product is an allosteric inhibitor of earlier enzyme
  - no unnecessary accumulation of product
  - Saves energy and resources



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!