Chapter 8 Warm-Up

1. Define the term “metabolism”.

2. List 3 forms of energy.

3. Where does the energy available for nearly all living things on earth come from?

Ch. 8 Warm-Up

1. What are the 1st and 2nd laws of thermodynamics?

2. Give the definition and an example of:
   A. Catabolic reaction
   B. Anabolic reaction

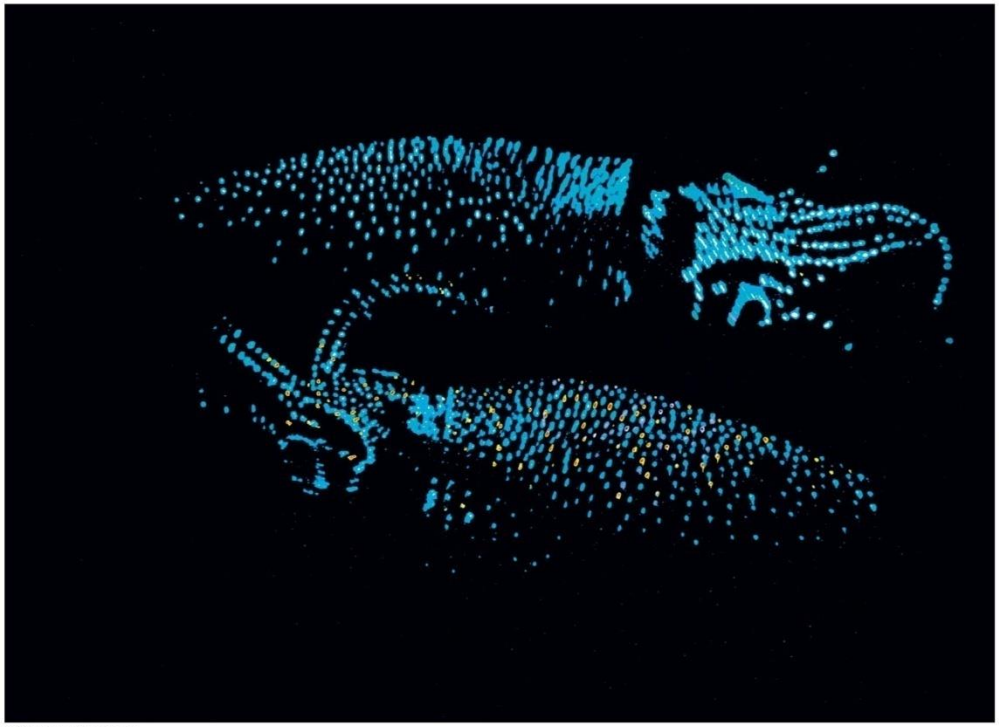
3. Is the breakdown of glucose in cellular respiration exergonic or endergonic?

Ch. 8 Warm-Up

1. Draw and label the following: enzyme, active site, substrate.

2. Describe what is meant by the term ***induced fit***.

3. What types of factors can affect an enzyme's function?



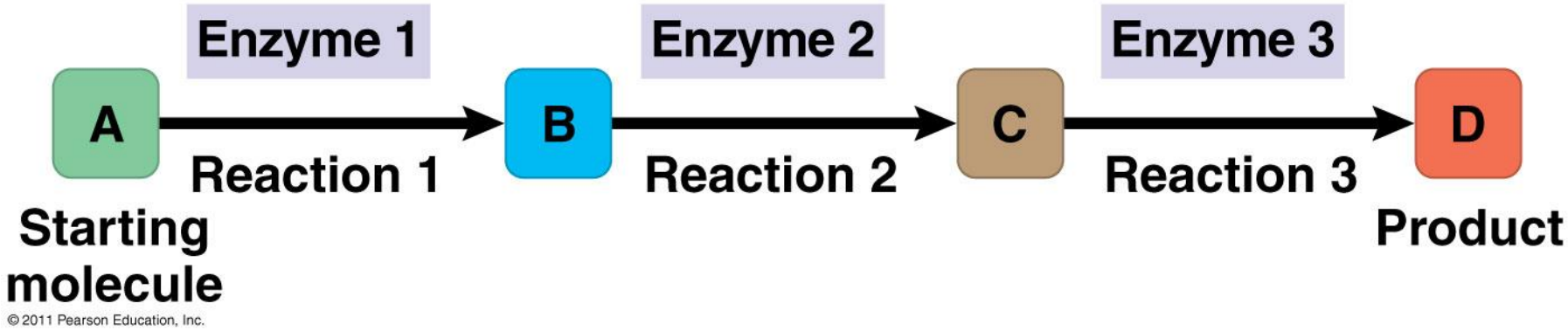
Chapter 8

An Introduction to Metabolism

What You Need To Know:

- Describe the role of **energy** in living systems.
- Describe properties of **enzymes**.
- Explain **how** enzymes affect the rate of biological reactions.
- Explain how **changes** to the structure of an enzyme may affect its function.

What is Metabolism?



Metabolism is the totality of an organism's chemical reactions

- How cells manage the materials and energy resources

Metabolism

- Chemical reactions of life
  - **Forming bonds** between molecules
    - Dehydration synthesis
    - Condensation reactions
    - Anabolic reactions
  - **Breaking bonds** between molecules
    - Hydrolysis
    - Digestion
    - Catabolic reactions

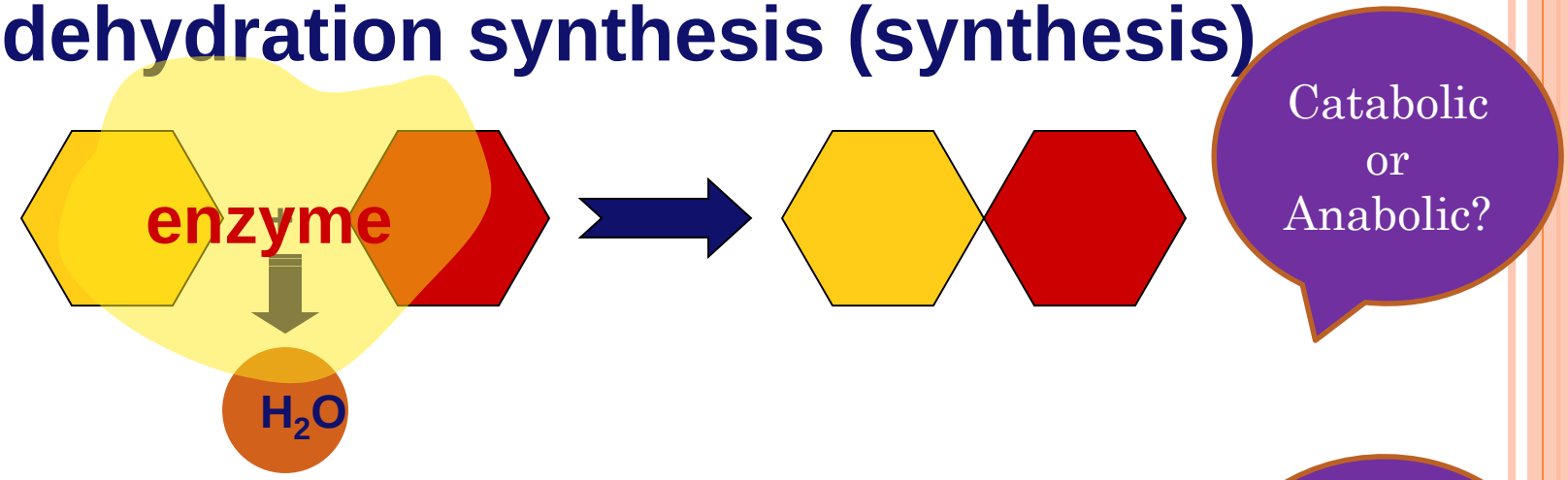
What are 2 metabolic pathways?

- **Catabolic pathways**
  - **release energy** by **breaking down** complex molecules into simpler compounds
    - Eg. digestive enzymes break down food → release energy

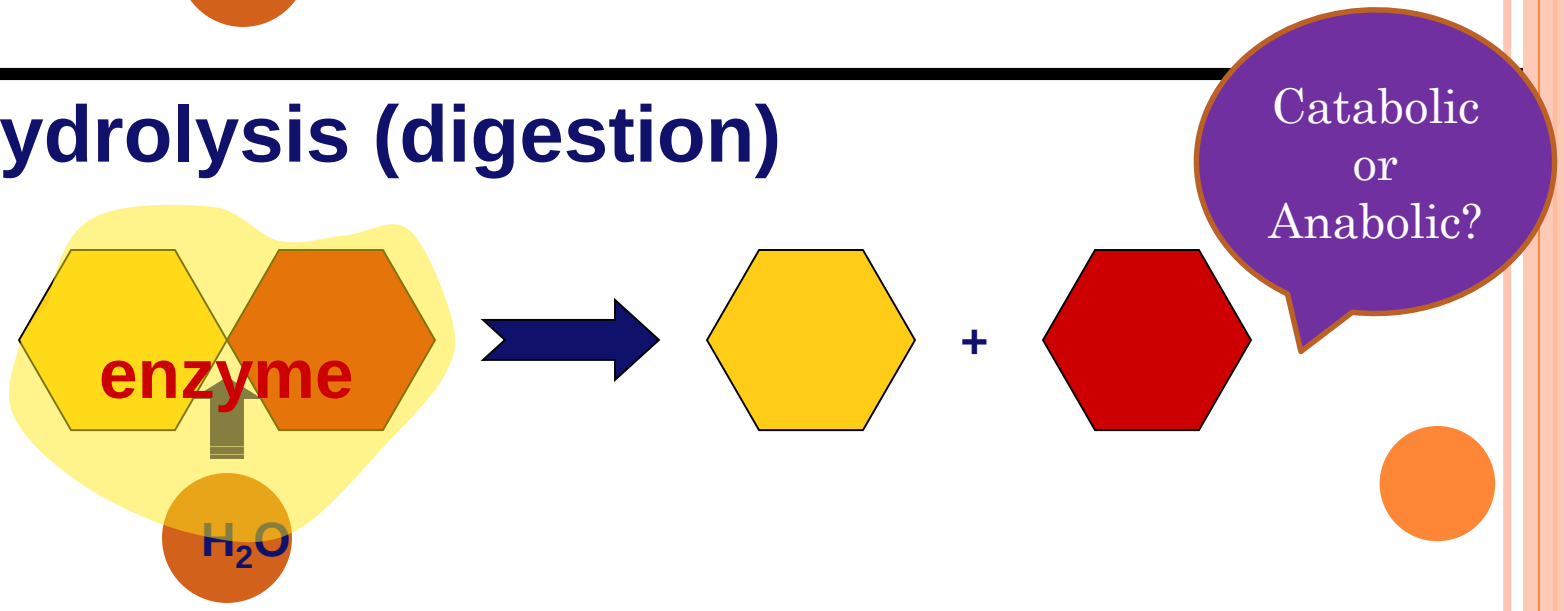
- **Anabolic pathways**
  - **consume energy** to **build** complex molecules from simpler ones
    - Eg. amino acids link to form muscle protein

Examples

- **dehydration synthesis (synthesis)**



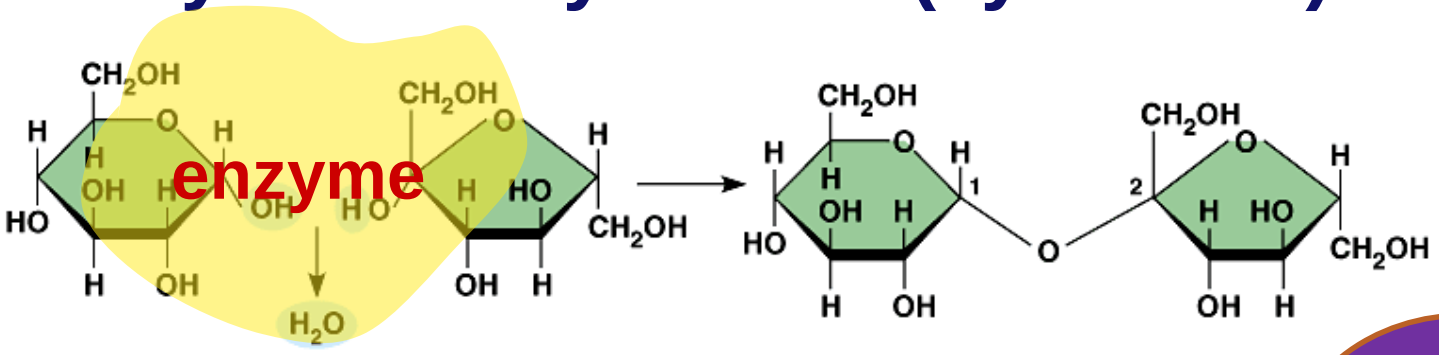
- **hydrolysis (digestion)**



EXAMPLES

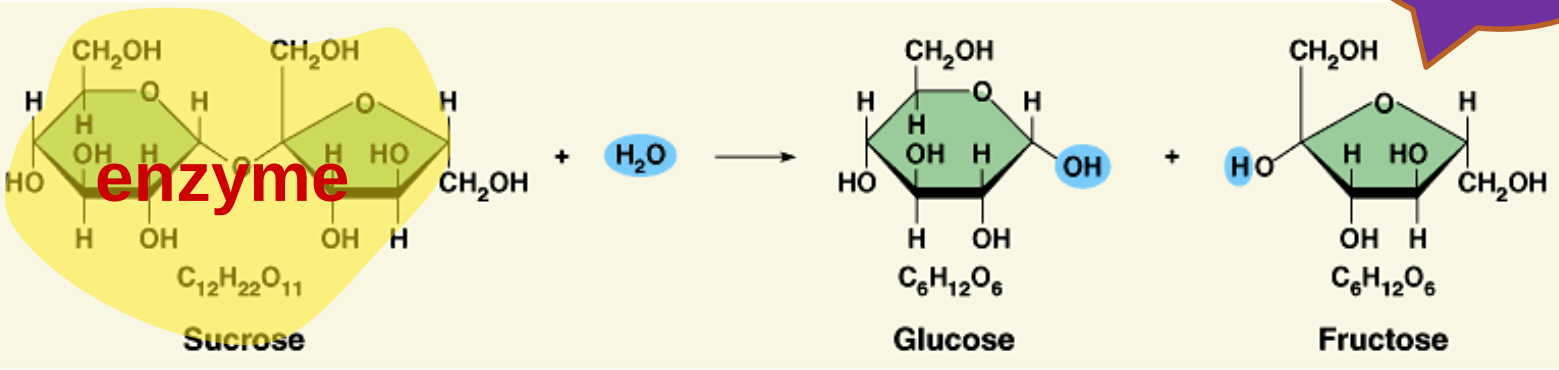
Anabolic

- **dehydration synthesis (synthesis)**



Catabolic

- **hydrolysis (digestion)**

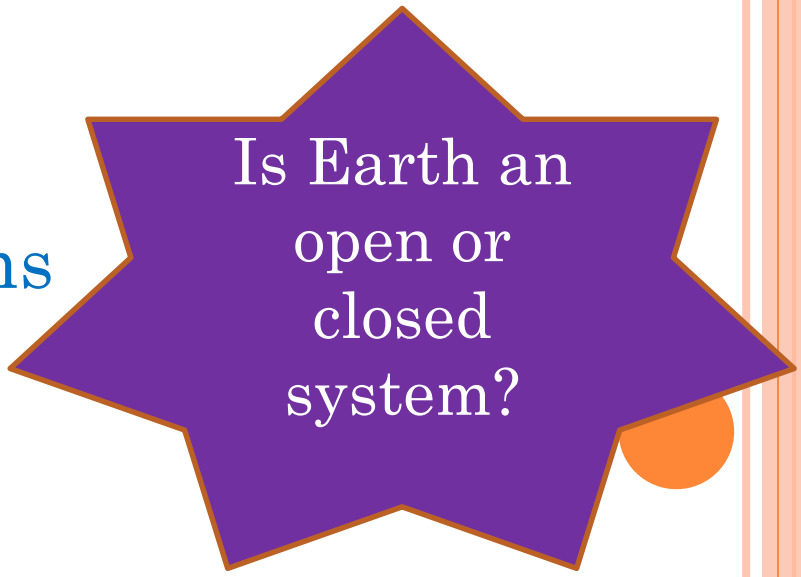


Energy = capacity to do work

- **Kinetic energy (KE)**: energy associated with motion
  - *Heat* (thermal energy) is KE associated with random movement of atoms or molecules
- **Potential energy (PE)**: stored energy as a result of its position or structure
  - *Chemical energy* is PE available for release in a chemical reaction
- Energy can be **converted** from one form to another
  - Eg. chemical → mechanical → electrical

THERMODYNAMICS IS THE STUDY OF ENERGY TRANSFORMATIONS THAT OCCUR IN NATURE

- A closed system, such as liquid in a thermos, is isolated from its surroundings
- In an open system, energy and matter can be transferred between the system and its surroundings
- Organisms are open systems



The First Law of Thermodynamics

- **The energy of the universe is constant**
  - Energy **can** be transferred and transformed
  - Energy **cannot** be created or destroyed

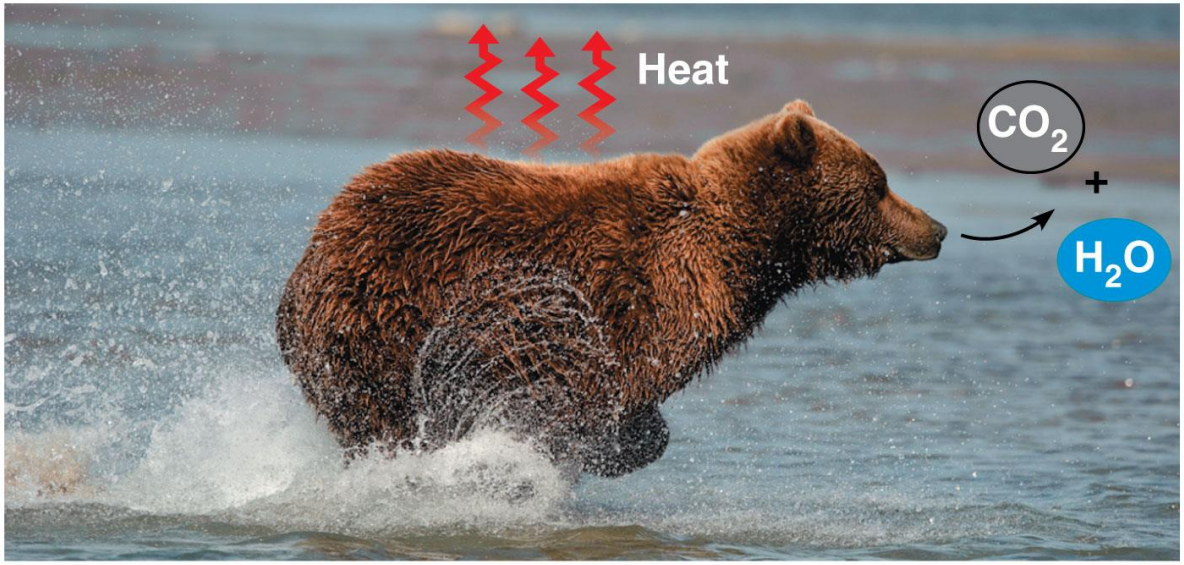
- Also called the principle of **Conservation of Energy**

(a) First law of thermodynamics

The Second Law of Thermodynamics

- Every energy transfer or transformation **increases the entropy** (disorder) of the universe

- During every energy transfer or transformation, some energy is *unusable*, often lost as **heat**



(b) Second law of thermodynamics

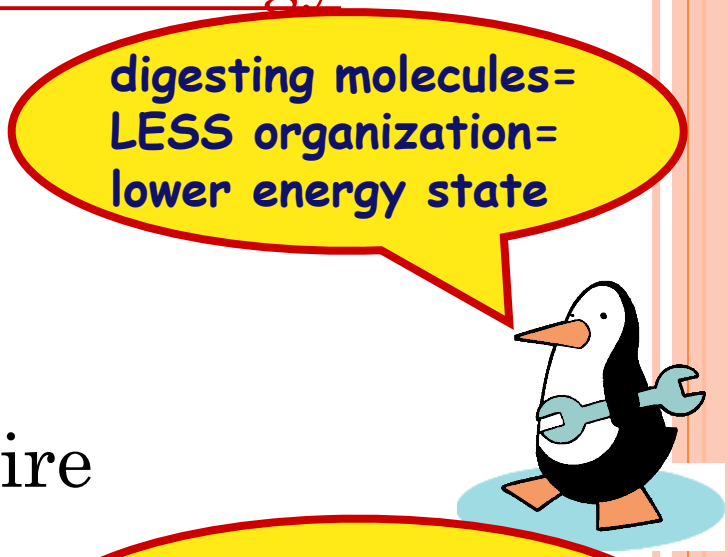
CHEMICAL REACTIONS & ENERGY

- **Free energy**: part of a system's energy available to perform work (not actually "free")
  - ΔG = change in free energy

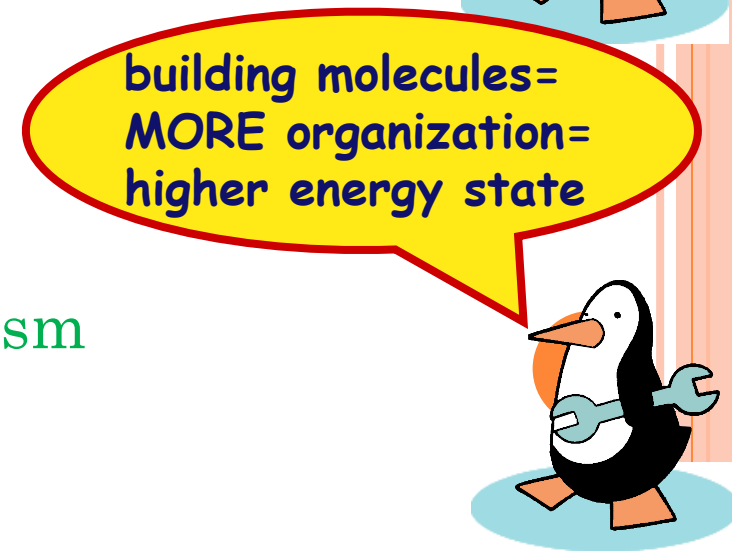
- **Exergonic reaction**: **energy is released**
  - **Spontaneous reaction**
  - $\Delta G < 0$
- **Endergonic reaction**: **energy is required**
  - **Absorb free energy**
  - $\Delta G > 0$

Chemical reactions & energy

- Some chemical reactions release energy
  - exergonic
  - digesting polymers
  - hydrolysis = catabolism



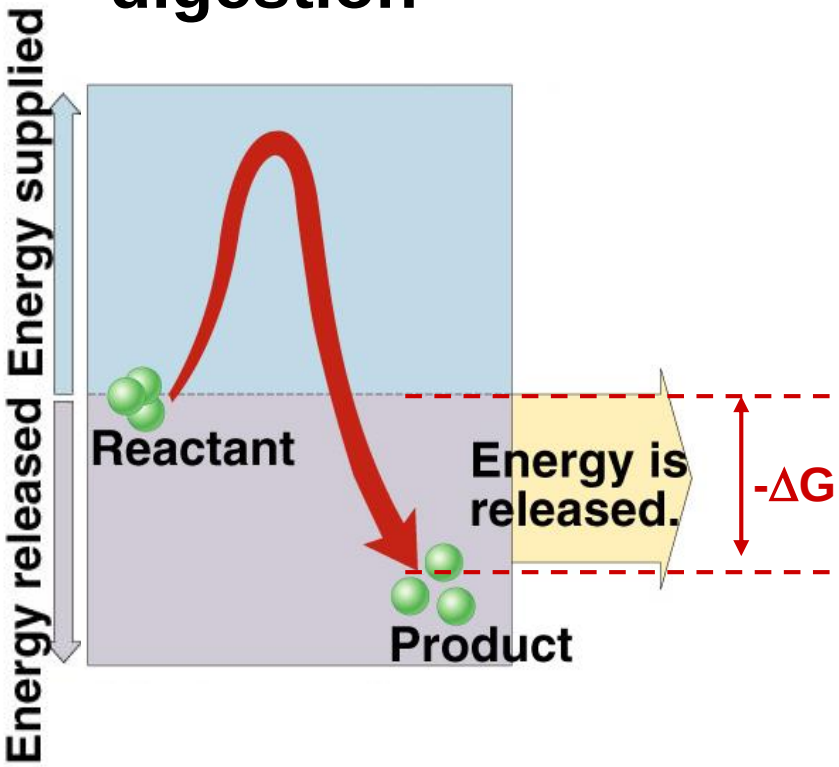
- Some chemical reactions require input of energy
  - endergonic
  - building polymers
  - dehydration synthesis = anabolism



Endergonic vs. exergonic reactions

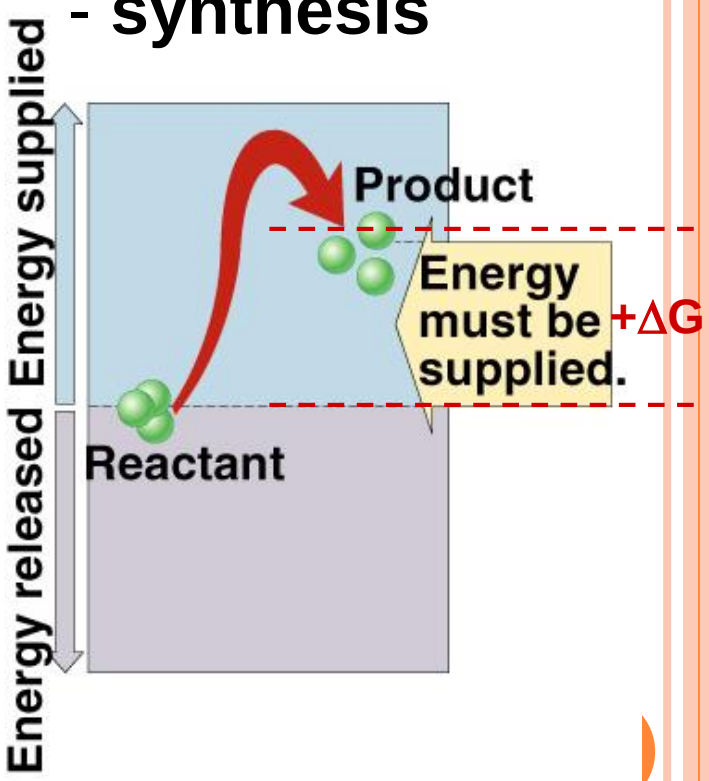
exergonic

- energy released
- digestion



endergonic

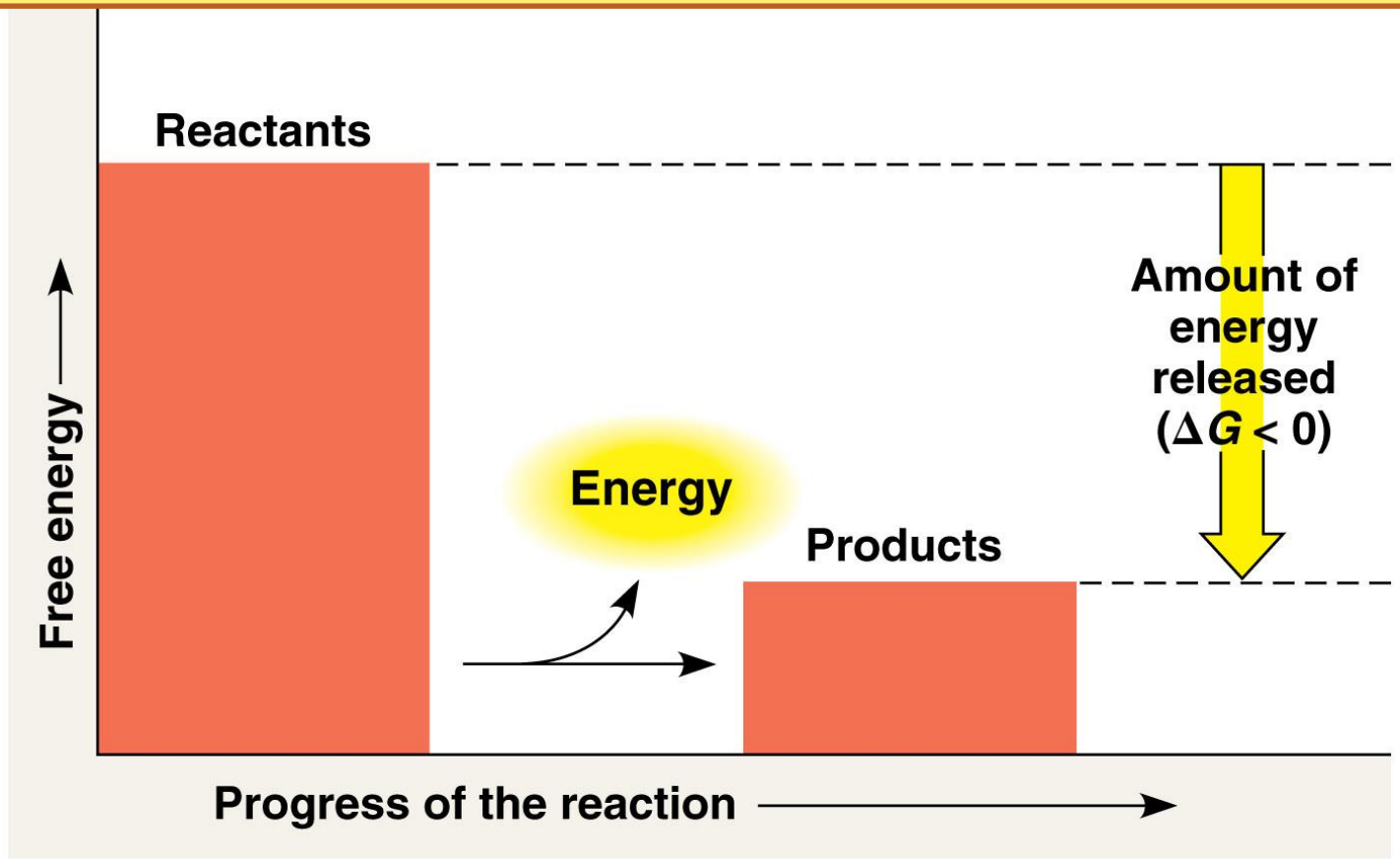
- energy invested
- synthesis



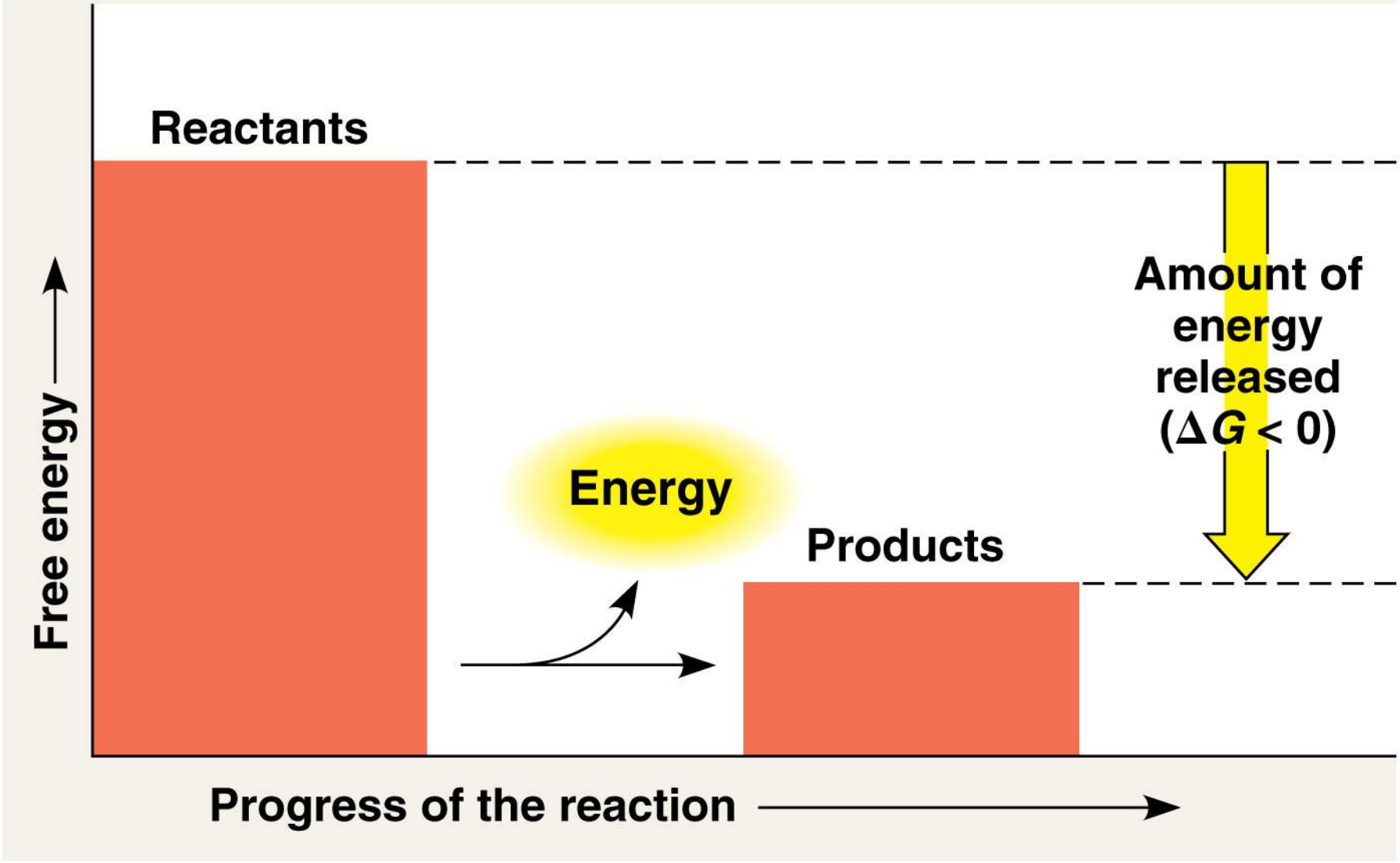
ΔG = change in free energy = ability to do work

TURN AND TALK! (and take notes!!!)

1. What type of reaction is this? (exergonic or endergonic)
2. Is energy being released or required/absorbed?
3. Is this a synthesis or a digestion reaction?
4. Is this reaction spontaneous or not?

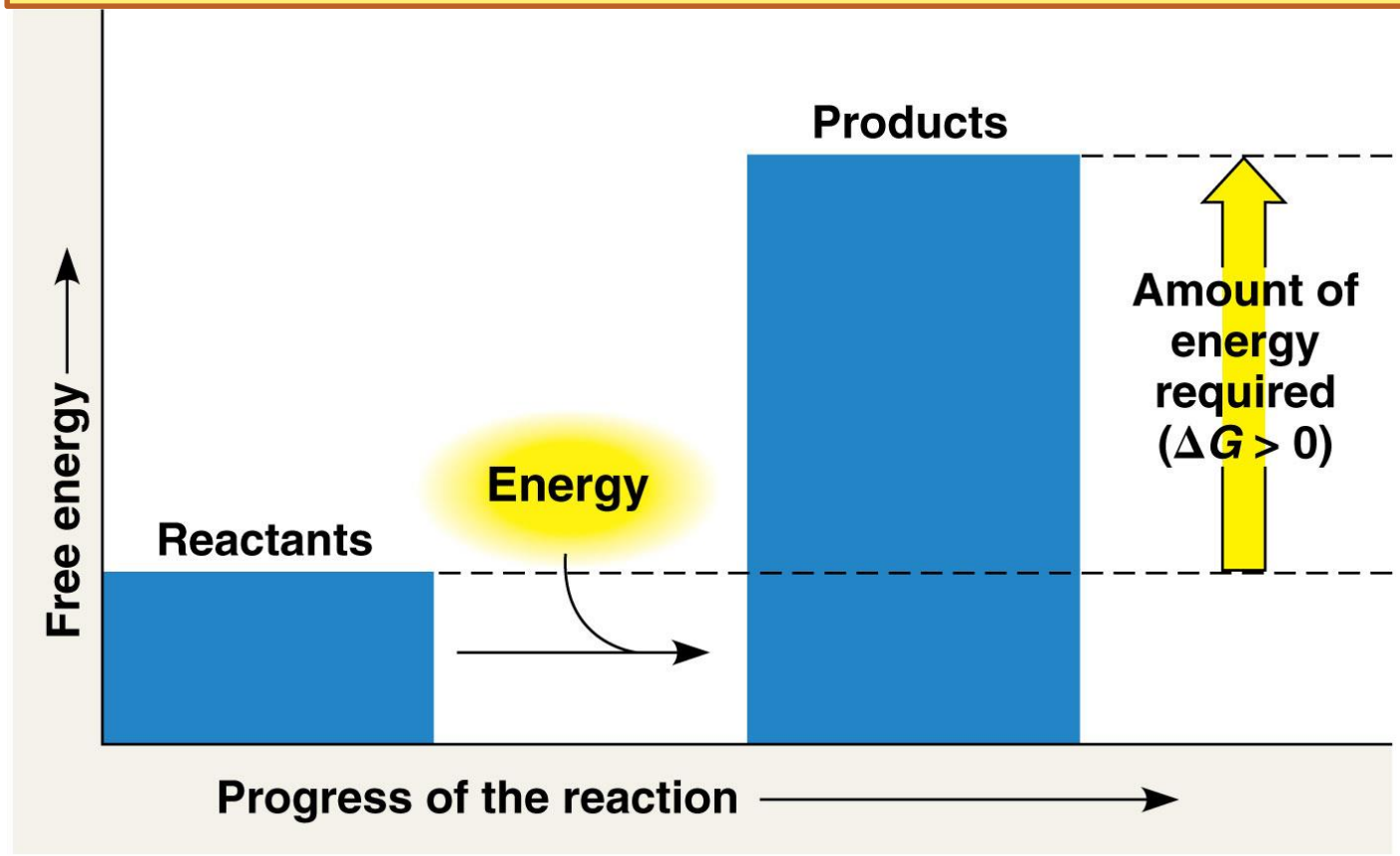


(a) Exergonic reaction: energy released, spontaneous

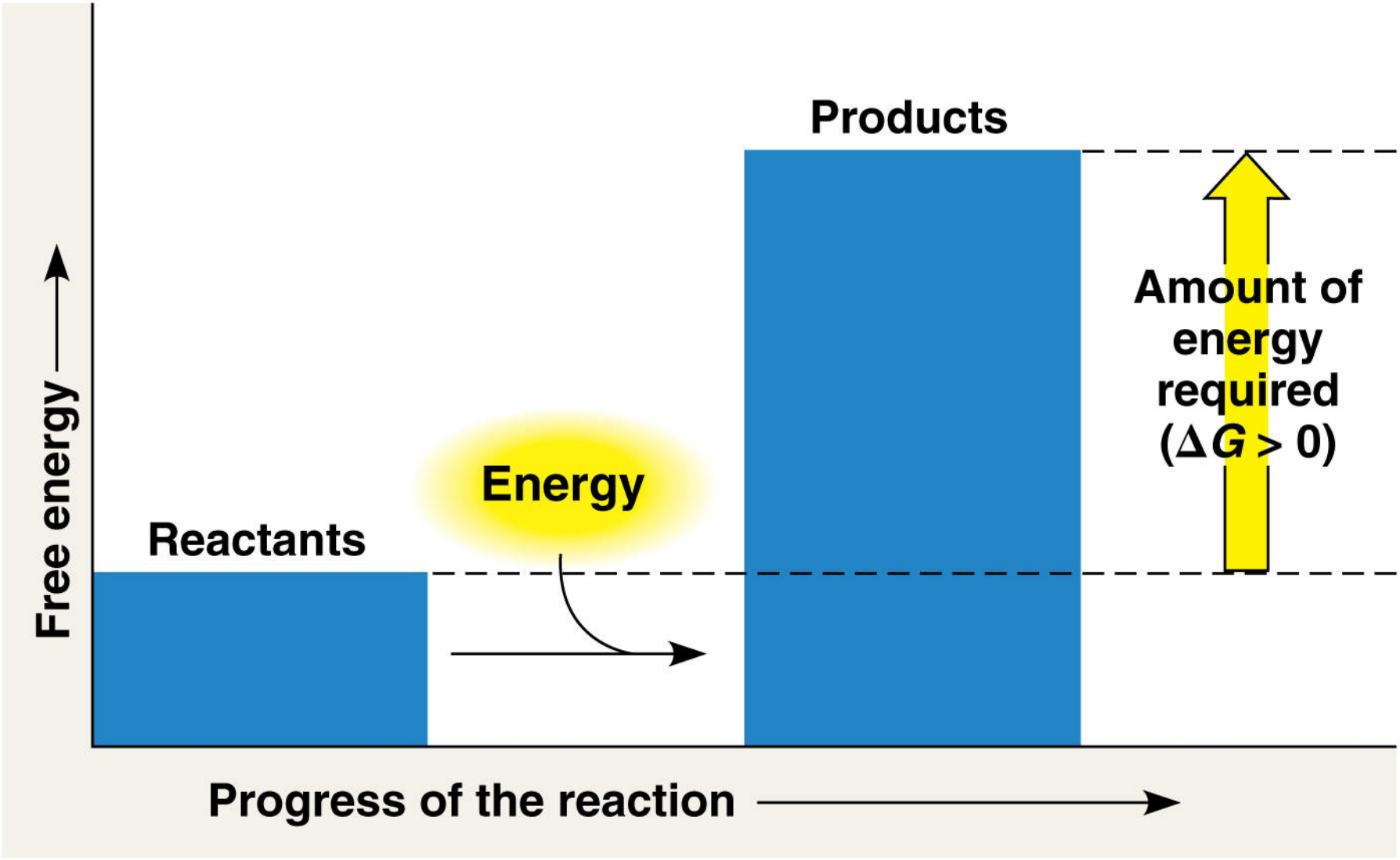


TURN AND TALK! (and take notes!!!)

1. What type of reaction is this? (exergonic or endergonic)
2. Is energy being released or required/absorbed?
3. Is this a hydrolysis or synthesis reaction?
4. Is this reaction anabolic or catabolic?

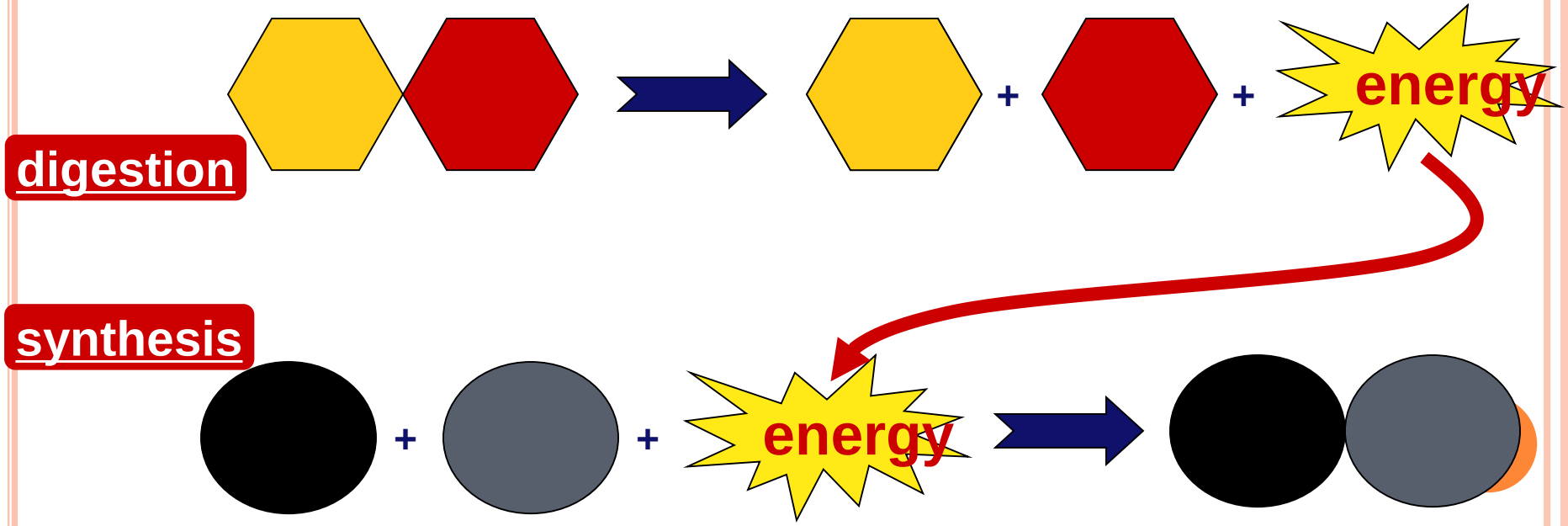


(b) Endergonic reaction: energy required, nonspontaneous



Energy & life

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



Let's Dig Deeper

- Watch Dr. Anderson explain "Free Energy"
  - http://www.bozemanscience.com/012-life-requires-free-energy
  - Complete the Video Review Sheet

- *Optional*: Watch Bozeman's video on "Gibbs Free Energy"
  - http://www.bozemanscience.com/012-life-requires-free-energy

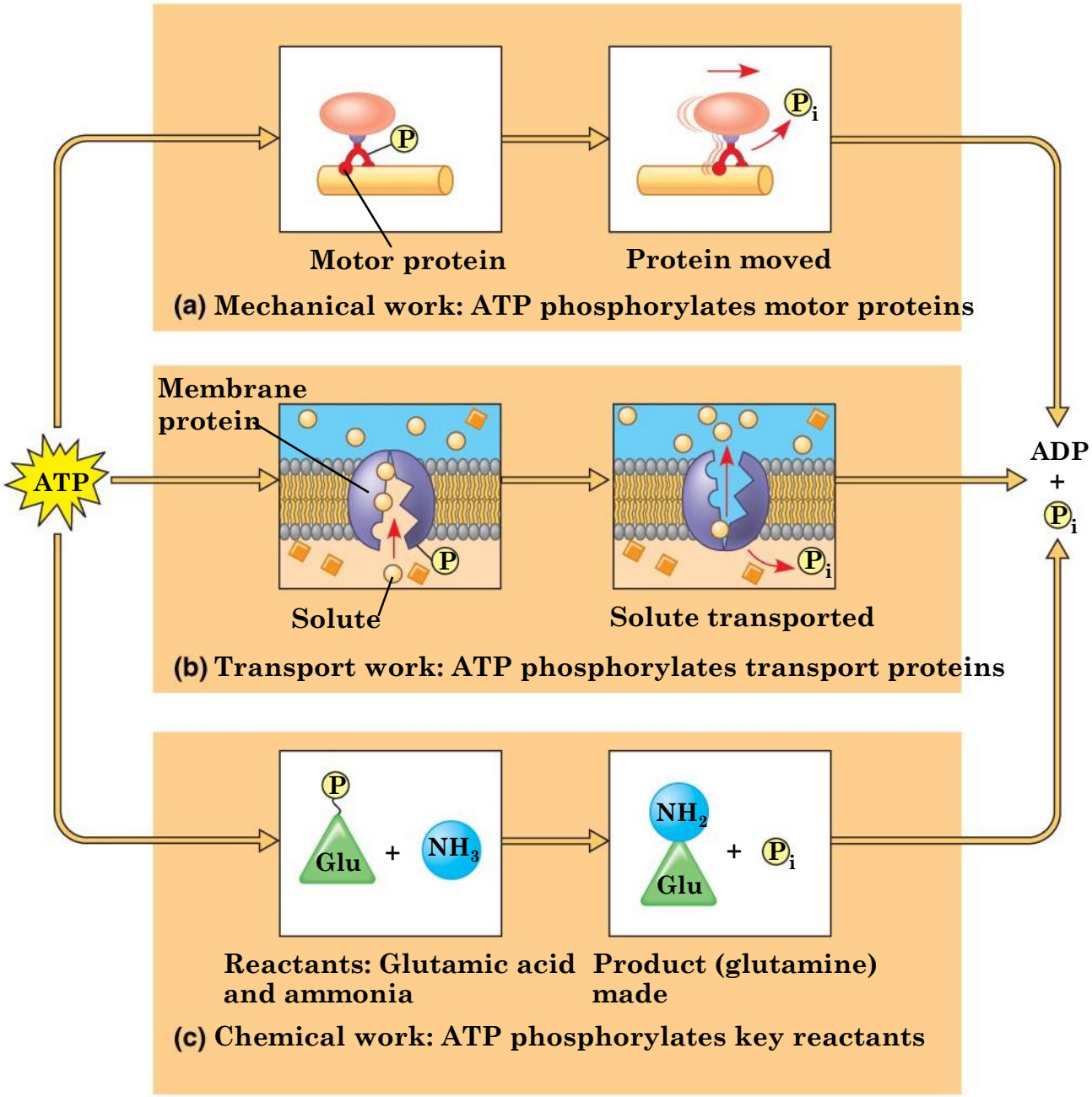
Free Energy POGIL

- Turn the POGIL in for your group if…

You have the longest hair.
(Put a star  by your name!)

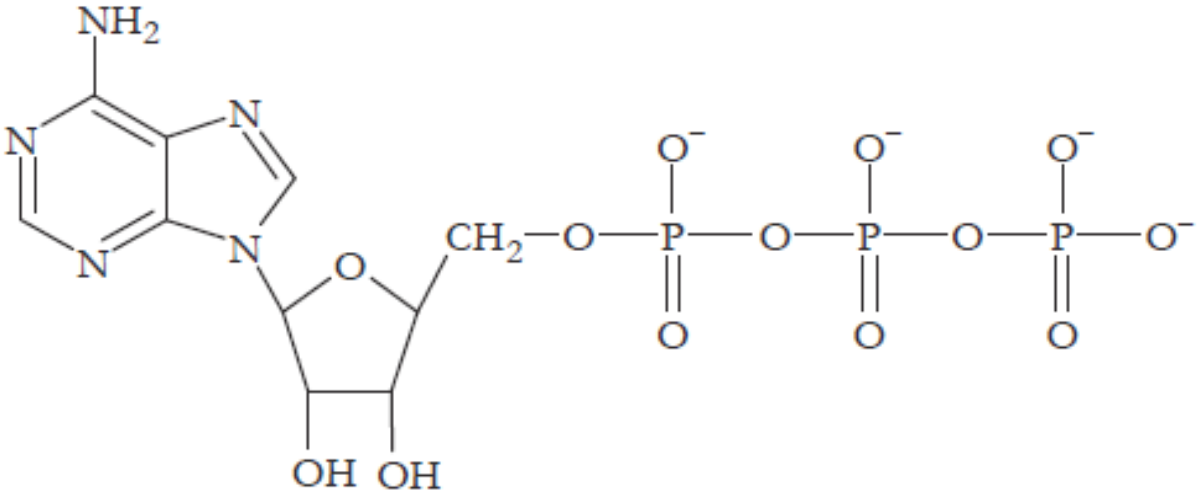
- A cell does three main kinds of work:
  - Mechanical
  - Transport
  - Chemical

- Cells manage energy resources to do work by energy coupling: using an ***exergonic*** process to drive an ***endergonic*** one

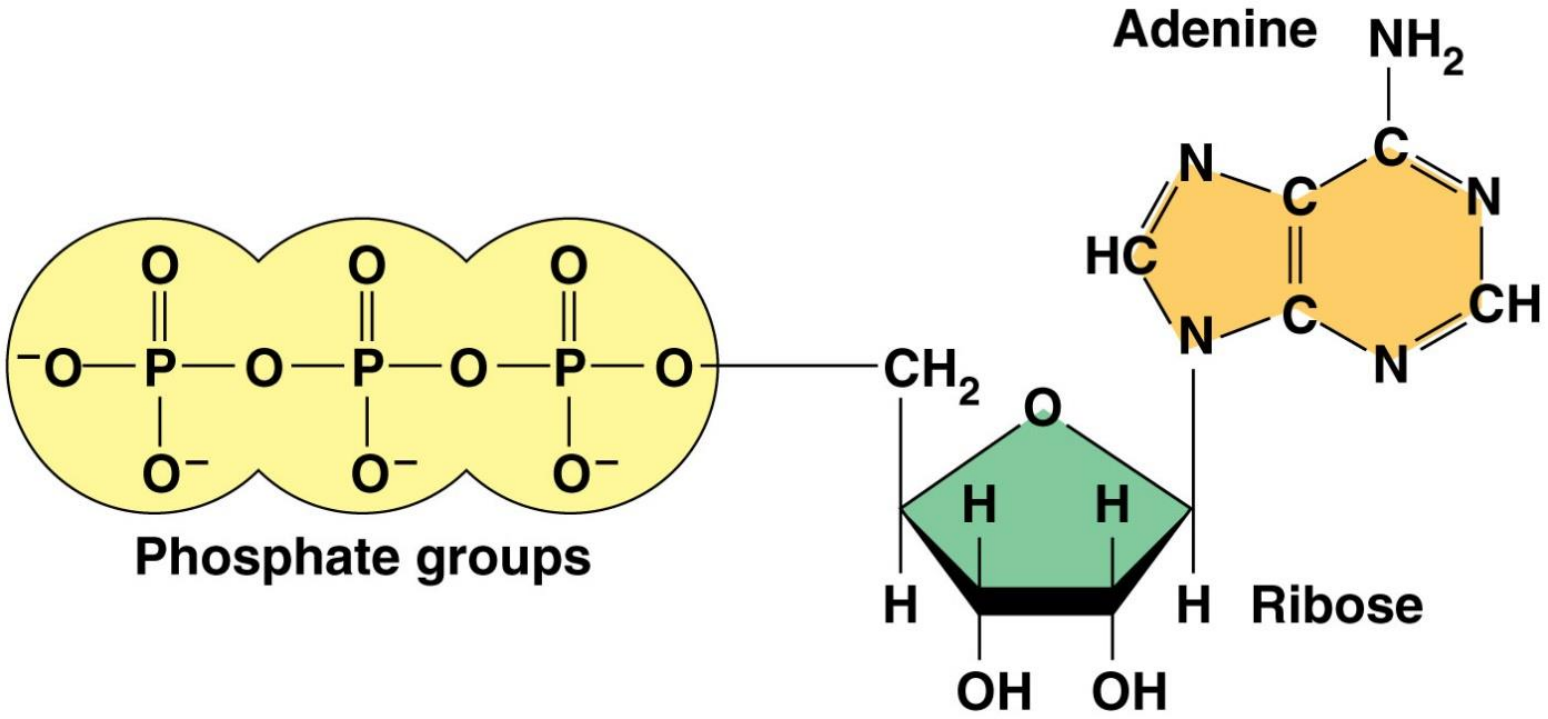


What molecule is this?

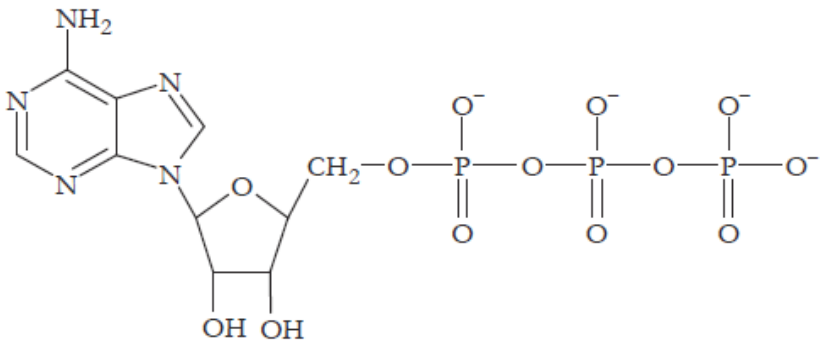
Can you label the 3 parts?

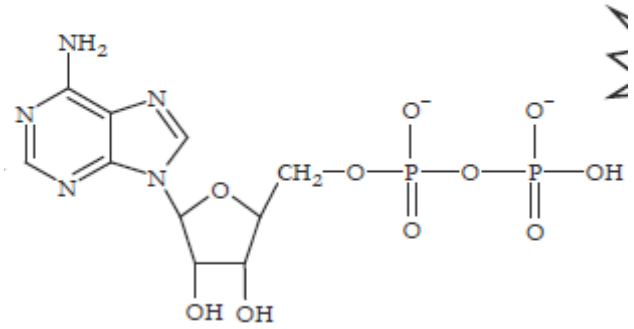


- **ATP** (**adenosine triphosphate**) is the cell's main energy source in energy coupling
- ATP = adenine + ribose + 3 phosphates



(a) The structure of ATP

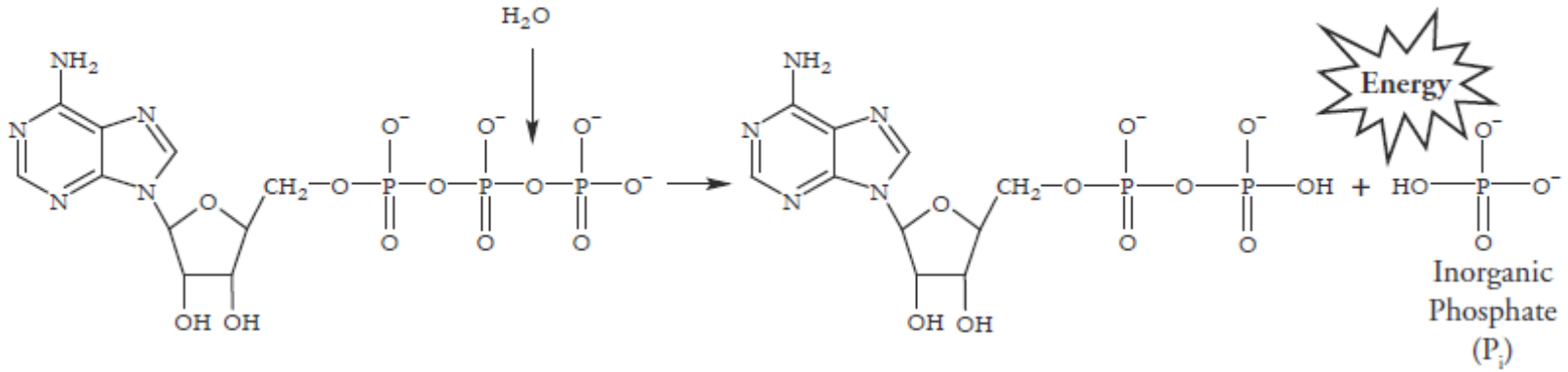




What molecule is this?

How does it compare to ATP?

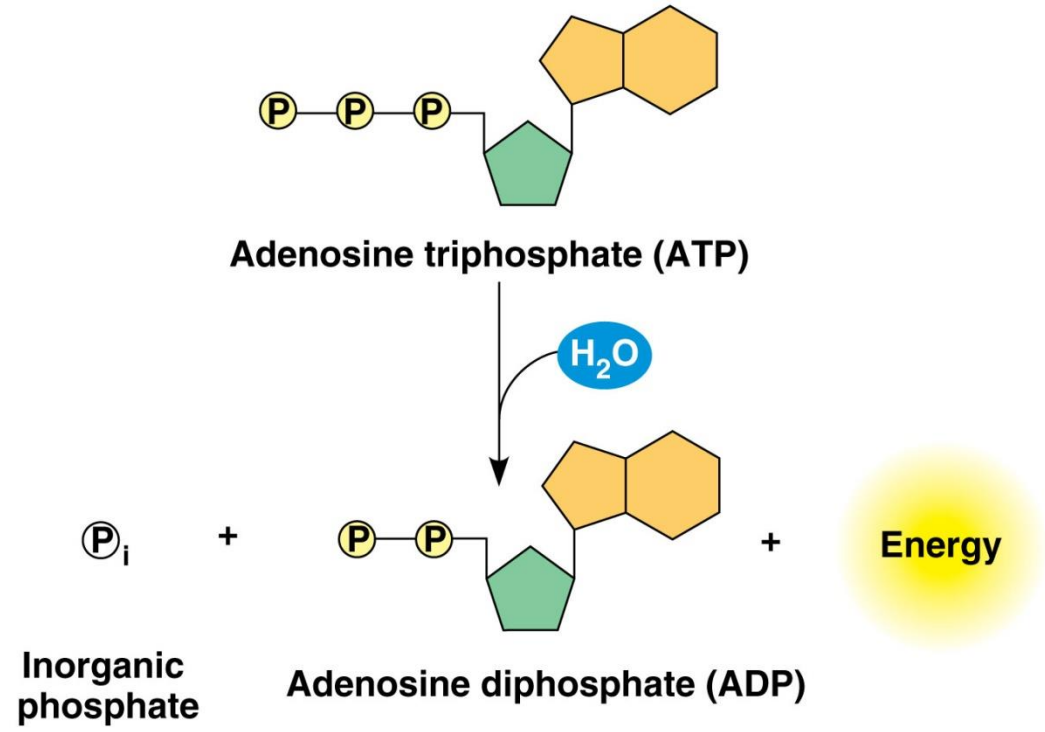
Model 2 – Hydrolysis of ATP



Which molecule, ATP or ADP, has a higher potential energy?

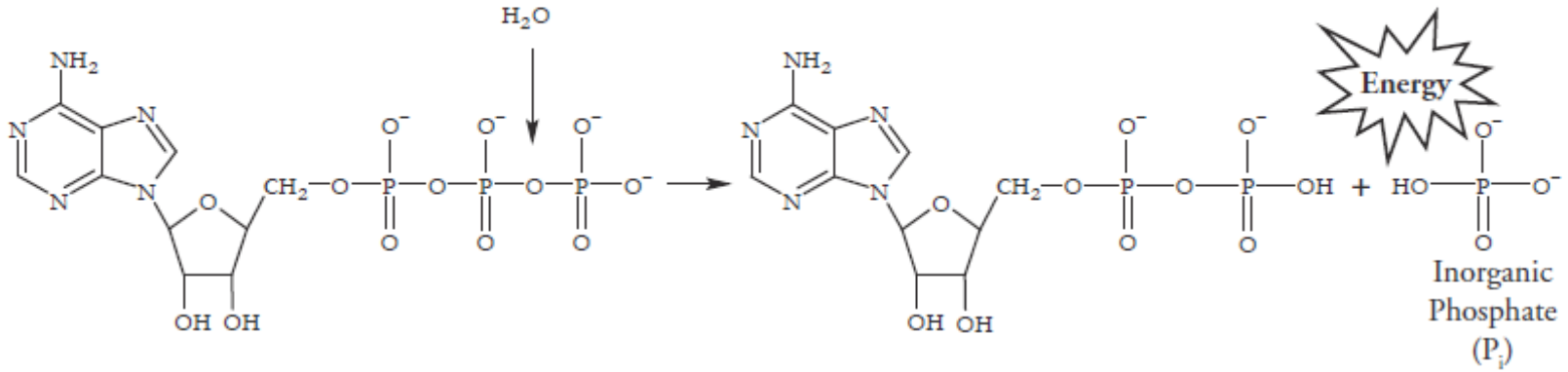
ATP

- When the bonds between the phosphate groups are broken by hydrolysis → ***energy is released***
- This release of energy comes from the chemical change to a state of lower free energy, not in the phosphate bonds themselves



(b) The hydrolysis of ATP

Model 2 – Hydrolysis of ATP



DISCUSS: Is this reaction exergonic (energy releasing) or endergonic (energy absorbing)?

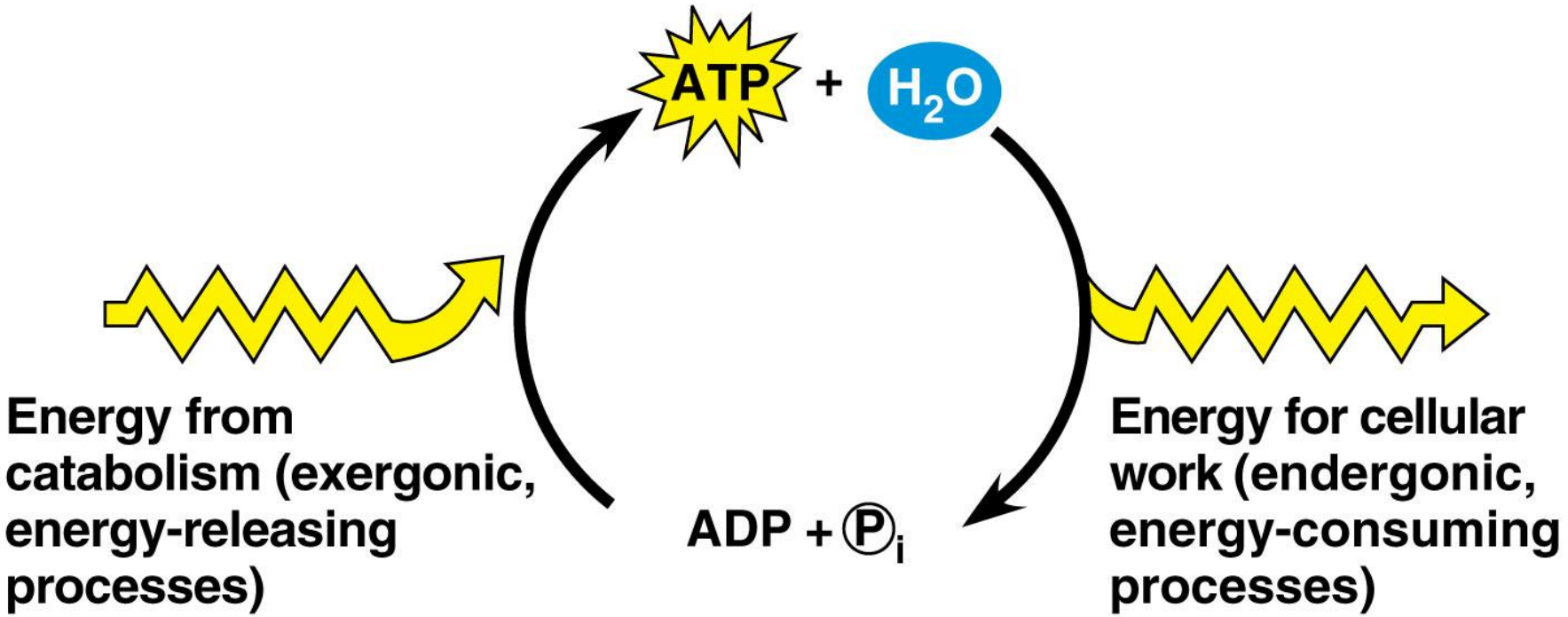
DISCUSS: Is this reaction endothermic (heat absorbing) or exothermic (heat releasing)?

Read This!

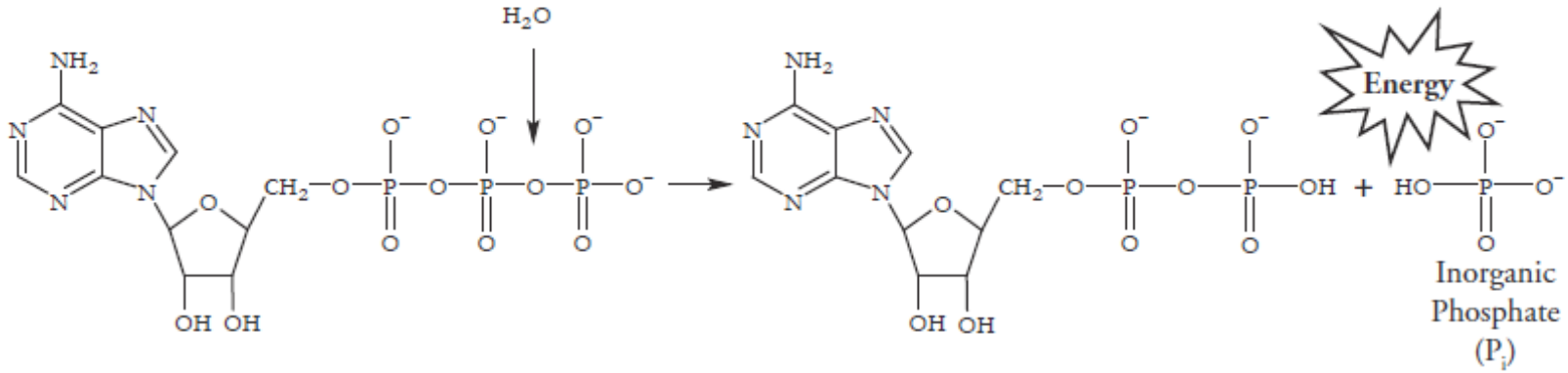
The conversion of ATP to ADP is not only exothermic, but there is also an increase in entropy of the system. Therefore, the hydrolysis of ATP is exergonic, and provides free energy for many processes needed to sustain life.

How ATP Performs Work

- ***Exergonic*** release of P_i (phosphate group) is used to do the ***endergonic (energy requiring)*** work of cell
- When ATP is hydrolyzed, it becomes ADP (adenosine diphosphate)



Model 2 – Hydrolysis of ATP

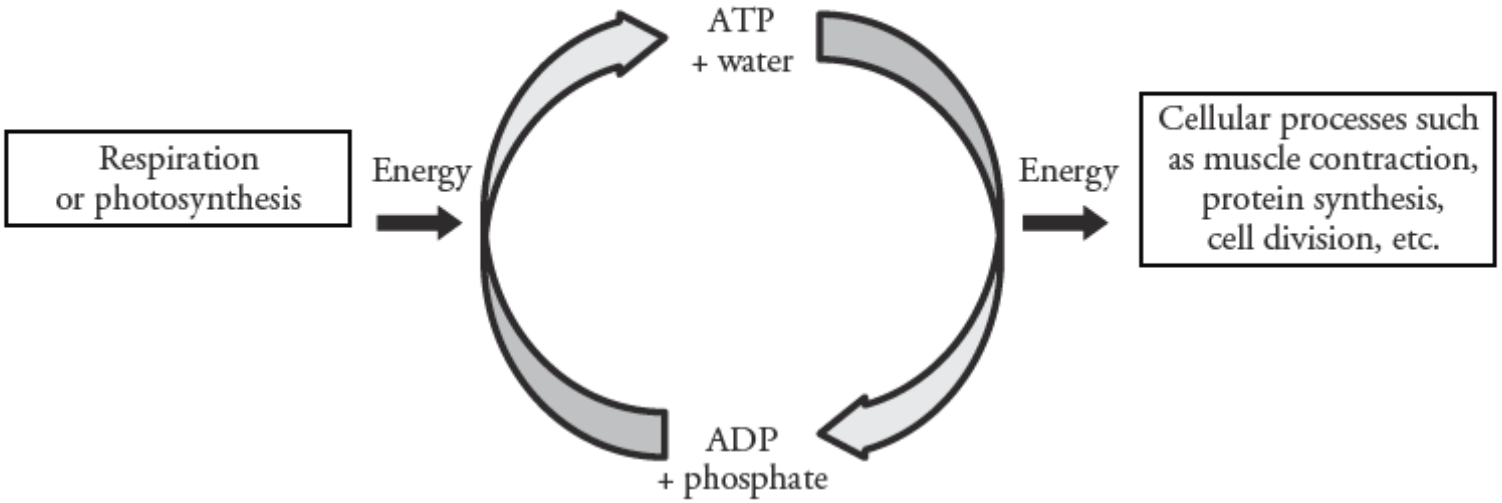


The reaction in Model 2 is reversible.

This reaction is called **phosphorylation**. Explain why this name is appropriate for the reaction above.

Phosphorylate means to add a phosphate group!

Model 3 – The ATP Cycle



10. When ATP is hydrolyzed, free energy is available.

    a. According to Model 3, what does that energy get used for?

    b. Name at least two other cellular processes that could be fueled by the hydrolysis of ATP that are not listed in Model 3.

11. After it is used, an ADP molecule is recycled back into ATP. What cellular, exergonic processes supply the energy needed for the phosphorylation of ADP?

Read This!

It is estimated that more than 2×10^{26} molecules of ATP are hydrolyzed in the human body daily. If each molecule was used only once you would need approximately 160 kg (350 lbs) of ATP daily. The repeated use of ATP molecules through the ATP cycle saves the body a huge amount of resources and energy.

ATP is synthesized in two ways:

- **Substrate-level phosphorylation**—Energy released during a reaction, such as the breakdown of sugar molecules, is used directly to synthesize ATP. A small amount of energy is generated through this process.
- **Electron transfer (oxidative phosphorylation)**—Energy from the movement of electrons from one molecule to another, via electron carriers, is used to synthesize ATP. Most cellular ATP is synthesized by electron transfer in the mitochondria.

Dinitrophenol (DNP) is an "uncoupler," which means it interferes with the flow of electrons during electron transfer. Fifty years ago, DNP was given as a drug to help patients lose weight.

a. Why would taking DNP make someone lose weight?

b. Why would taking DNP be dangerous?

Phosphorylation (adding a phosphate)

SUBSTRATE LEVEL PHOSPHORYLATION

Energy is used to directly add a phosphate to something (like ATP) by an enzyme in the cytoplasm. The phosphate group is the substrate.

- Quicker
- Generates less ATP

OXIDATIVE PHOSPHORYLATION

(electron transfer)

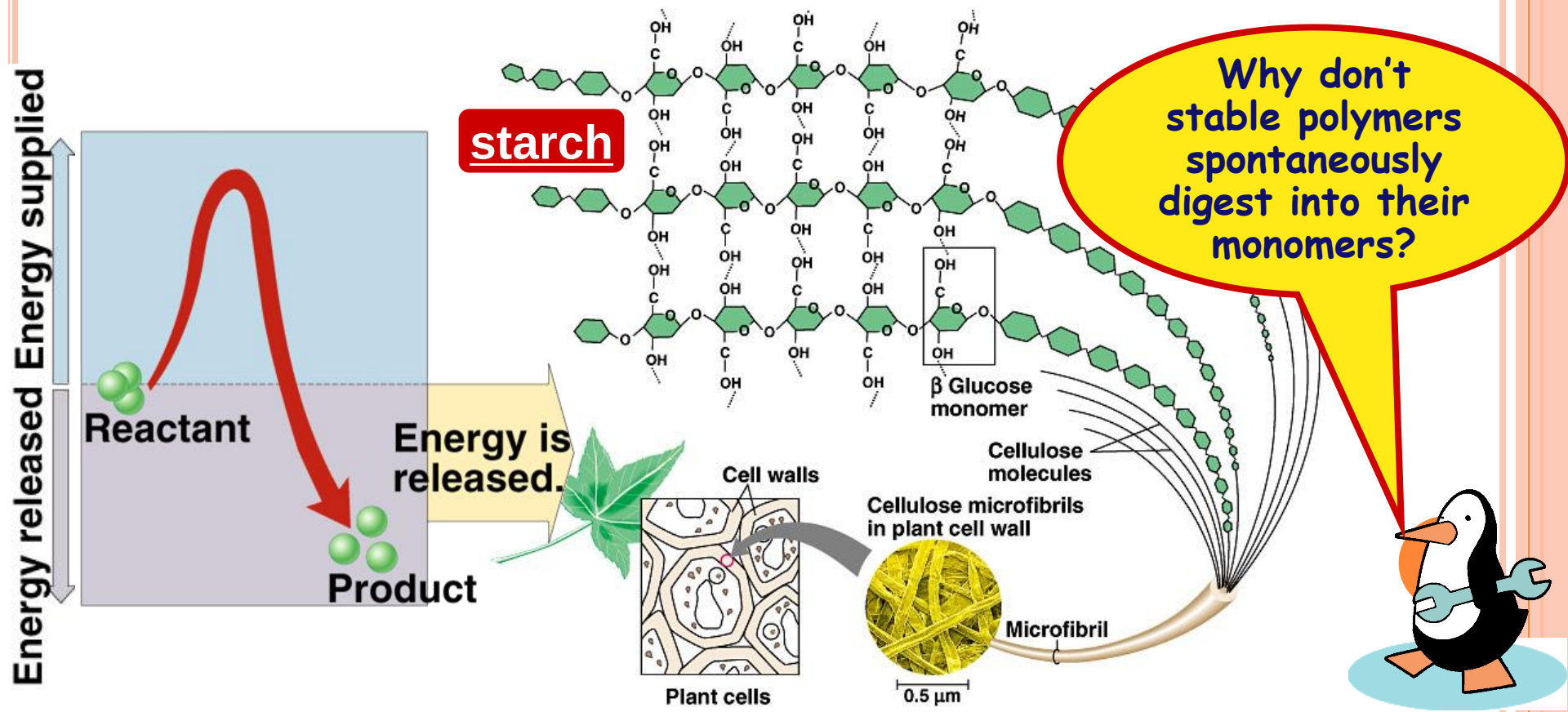
Energy from moving electrons (along electron carriers) phosphorylates ATP.

- Slower
- Generates a lot of ATP

More on these 2 soon!

What drives reactions?

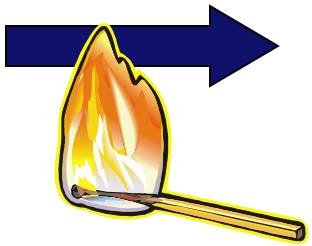
- If reactions are "downhill", why don't they just happen spontaneously?
  - because covalent bonds are stable bonds



Activation energy

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

cellulose

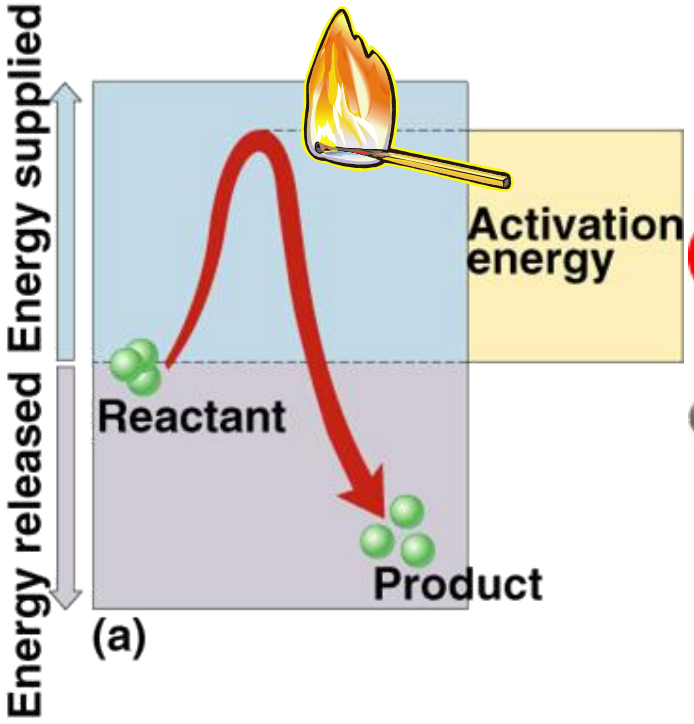


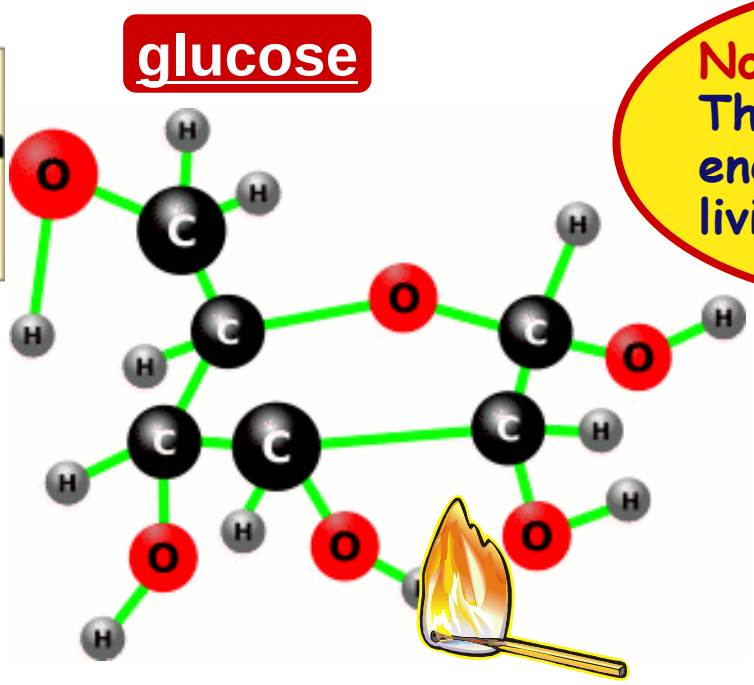
energy

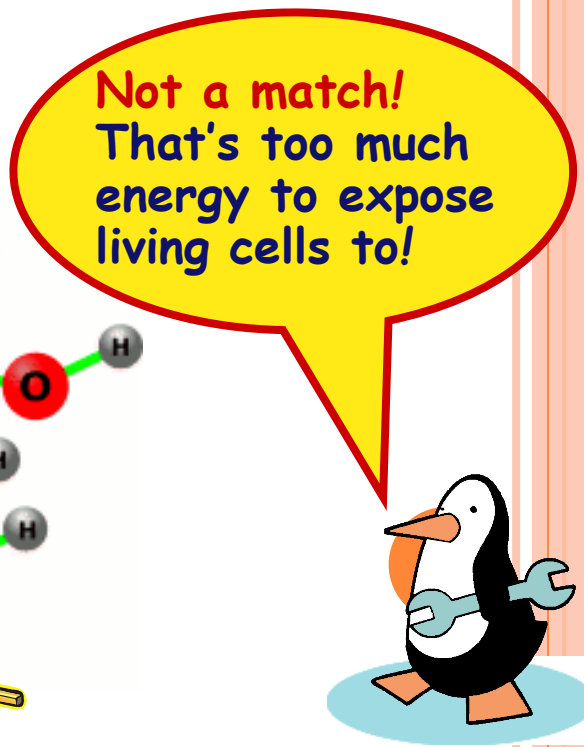
$CO_2 + H_2O$ + heat

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"

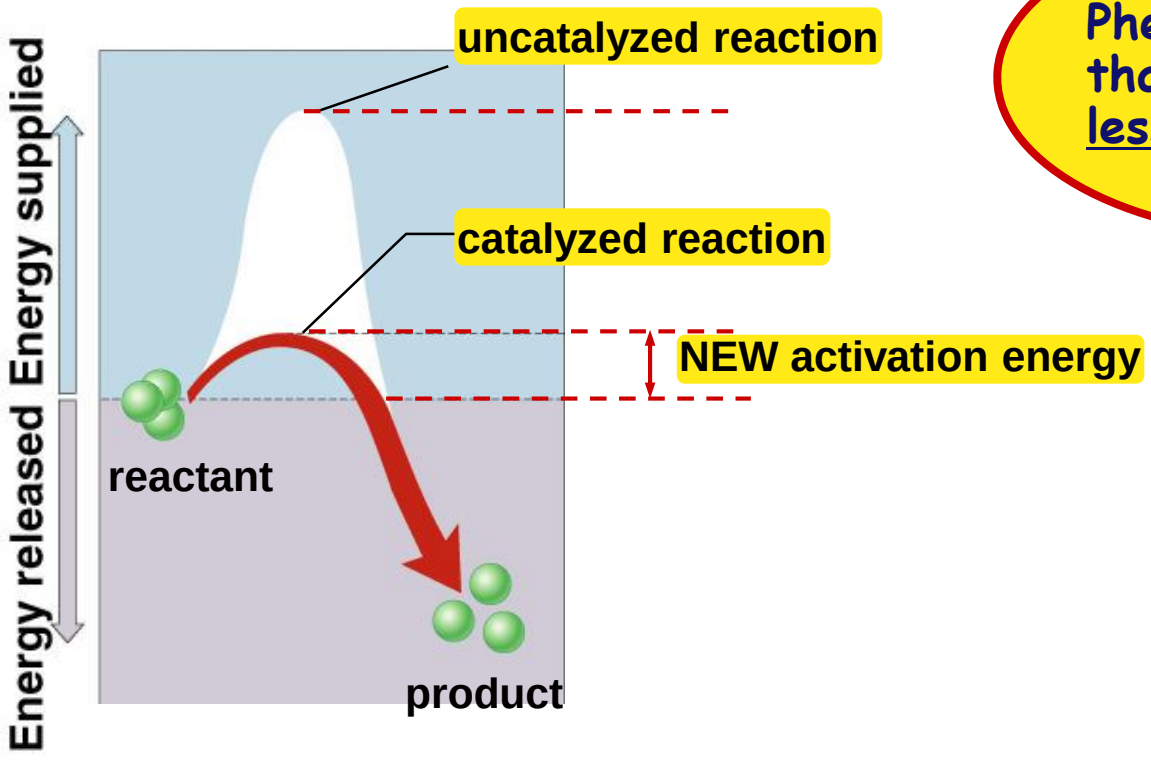


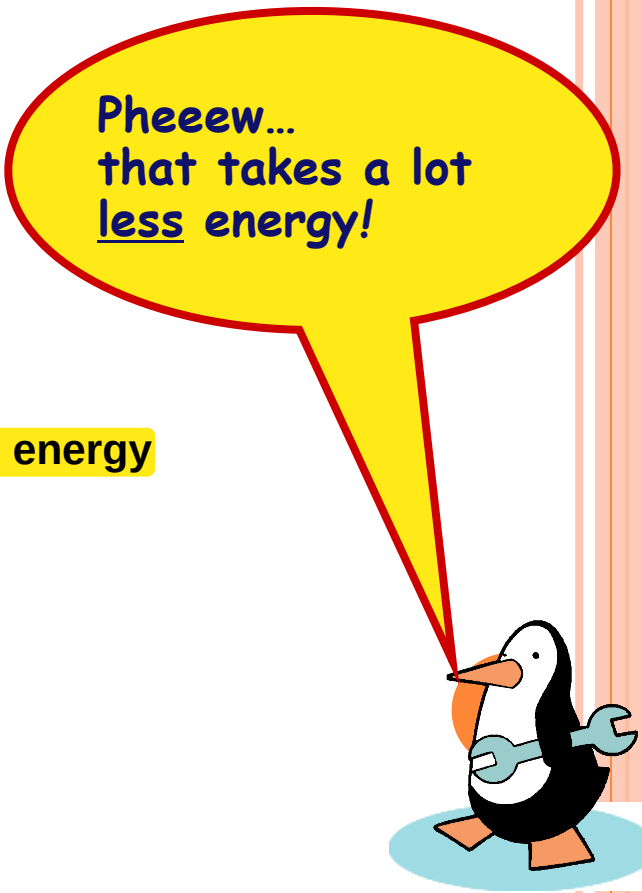




Reducing Activation energy

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

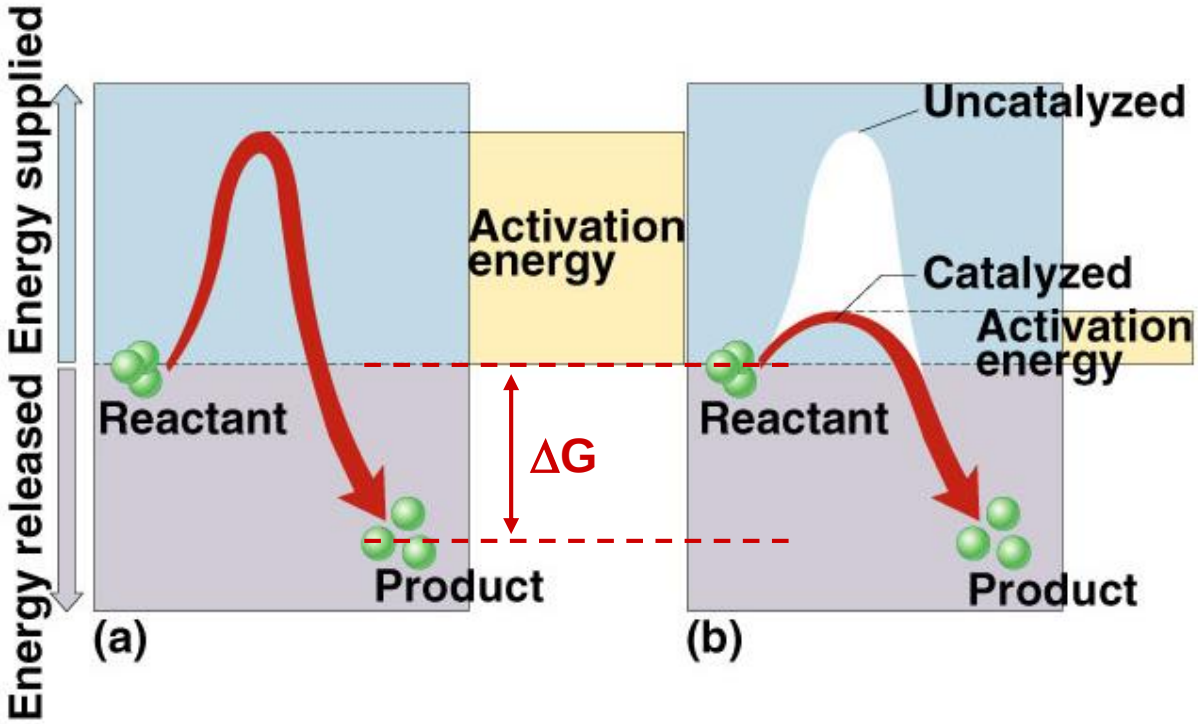


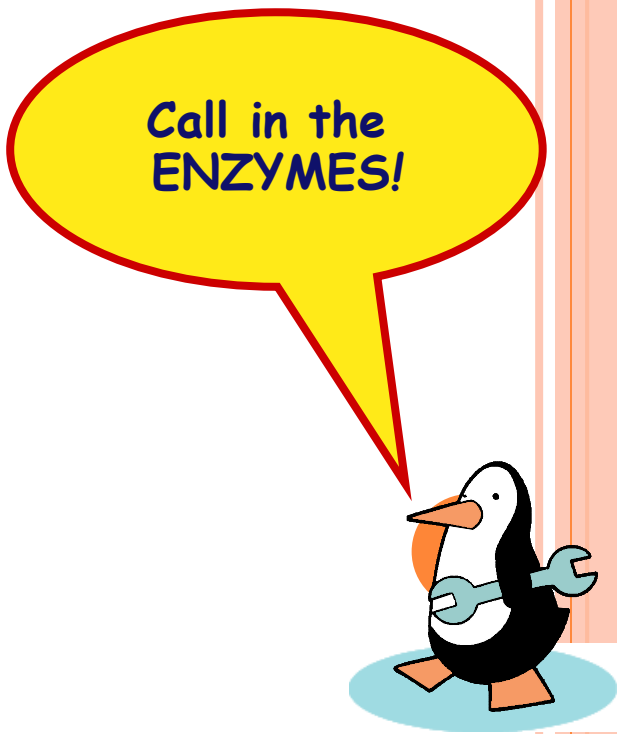


Catalysts

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

ENZYMES





Let's Watch

- Amoeba Sisters: "Enzymes"

- Complete the viewing guide…we will check answers afterwards

POGIL: Enzymes and cellular regulation

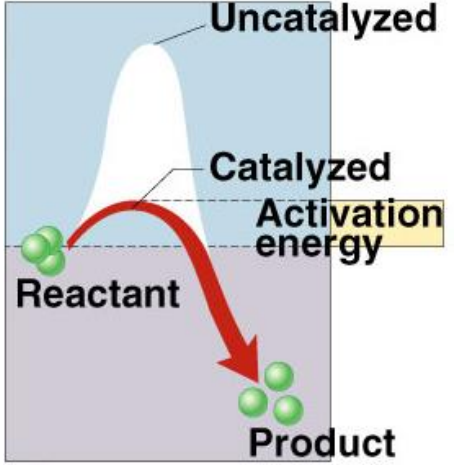
- Stick drawing to determine groups.

- Turn in POGIL if you are

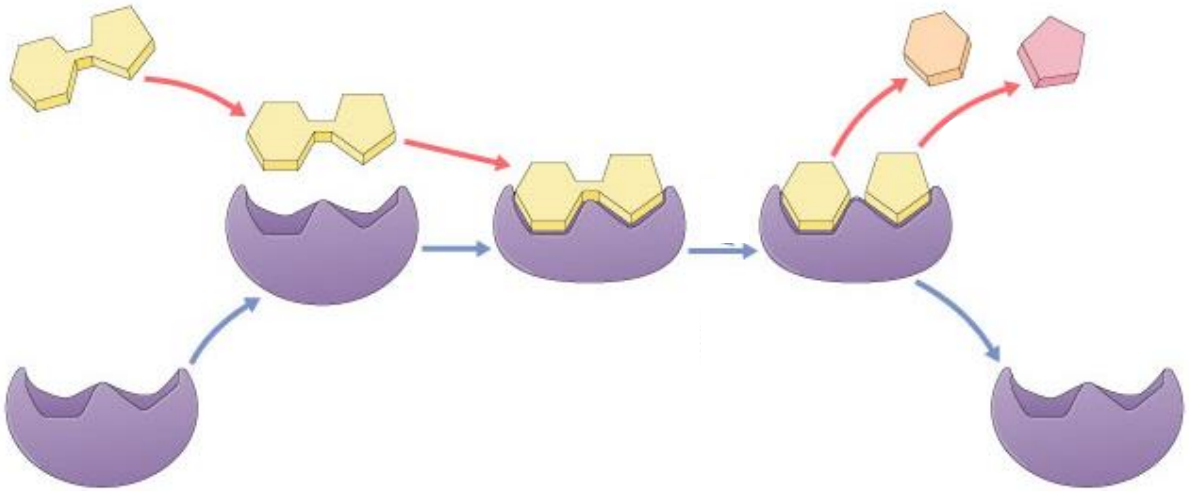
The MANAGER

(Put a star by your name!)

What are 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

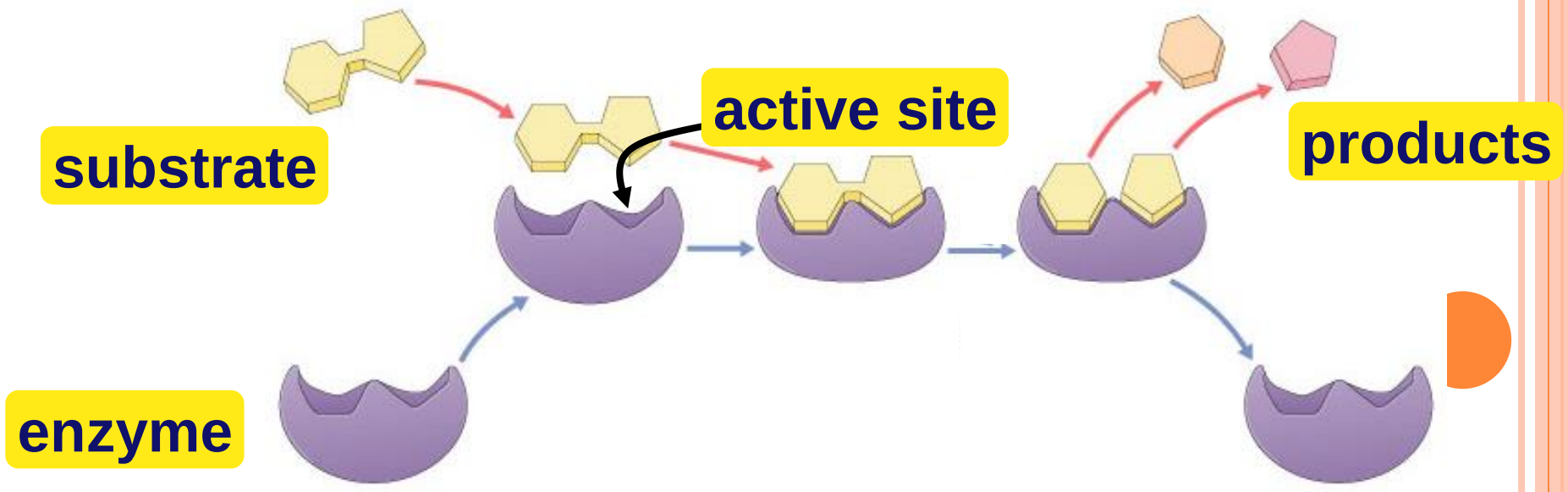


Enzymes vocabulary

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

1. Reaction SPECIFIC
   - each enzyme works with a specific substrate
     - chemical fit between active site & substrate
       - H bonds & ionic bonds

2. Not consumed in reaction (REUSABLE)
   - single enzyme molecule can catalyze thousands or more reactions per second
     - enzymes unaffected by the reaction

3. Affected by cellular conditions
   - any condition that affects protein structure
     - temperature, pH, salinity

Enzyme Specificity

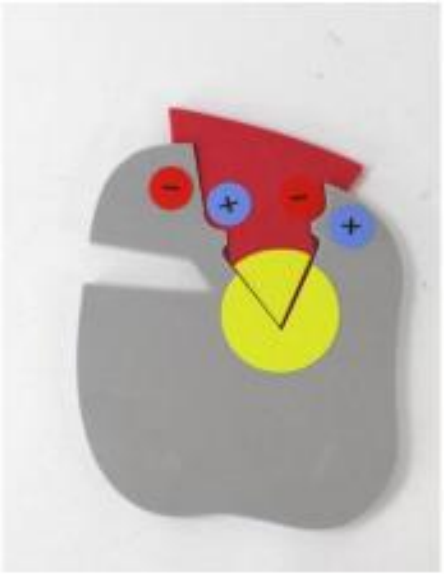


Figure 1: Enzyme-substrate complex

Figure 2: The charges align between the enzyme and the substrate; however, the enzyme's shape will not "fit".

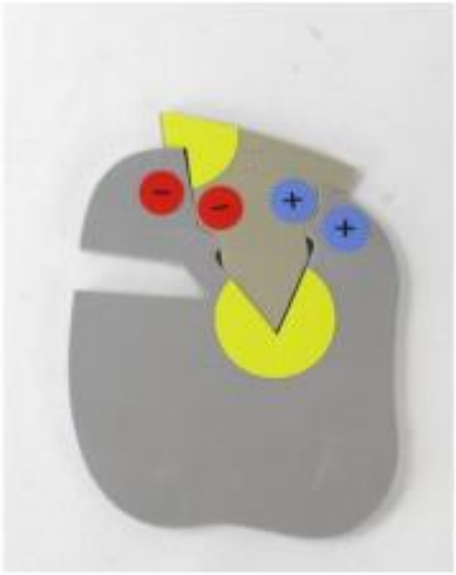
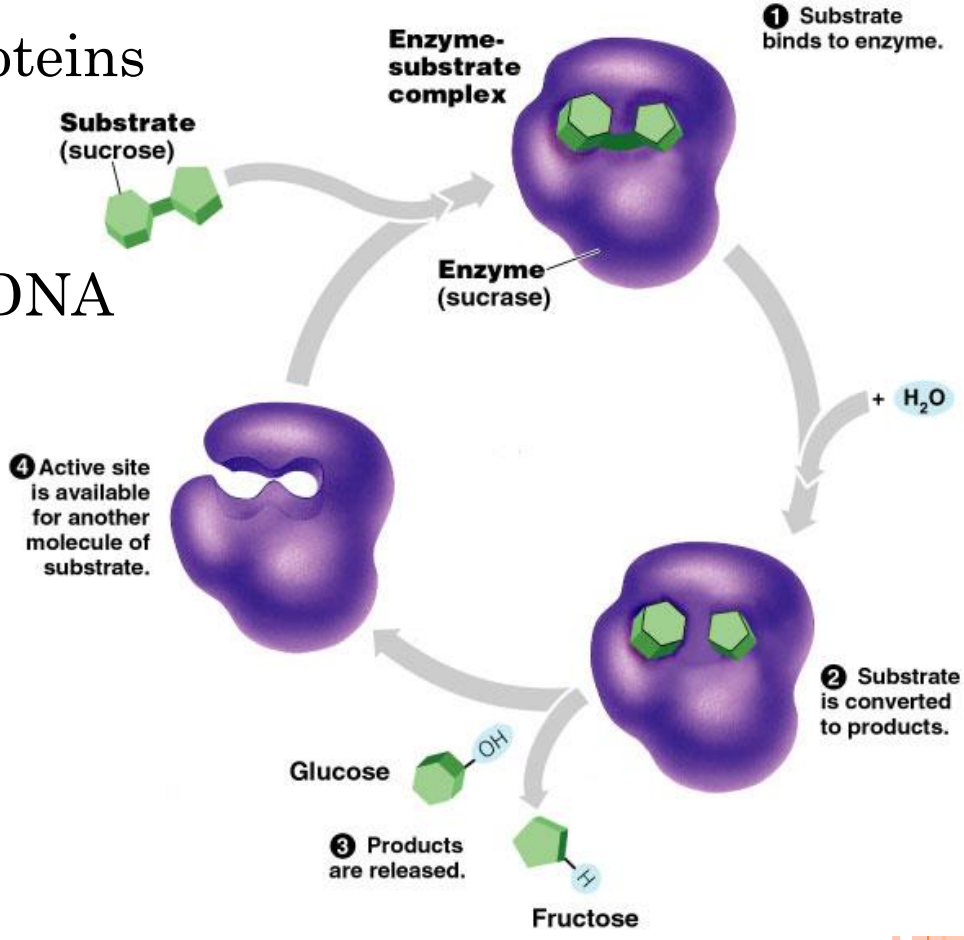


Figure 3: The shape of the substrate appears to fit but the charges do not align in the active site of the enzyme.

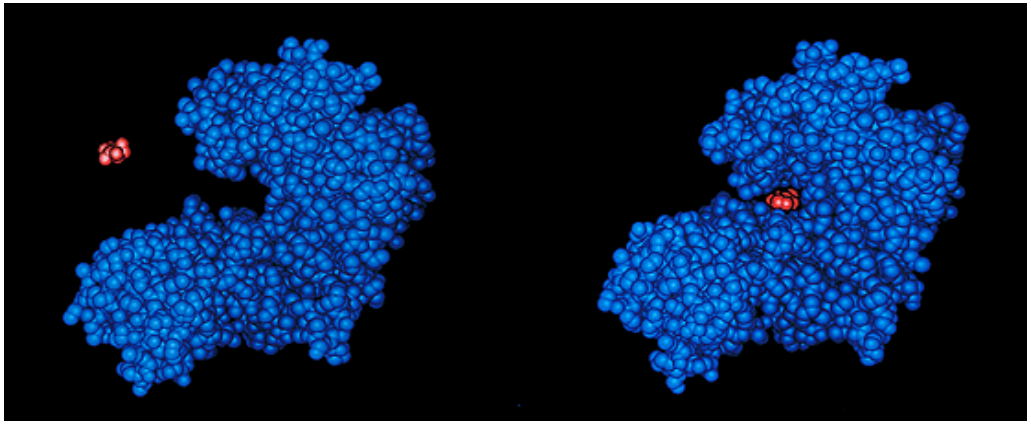
Naming conventions

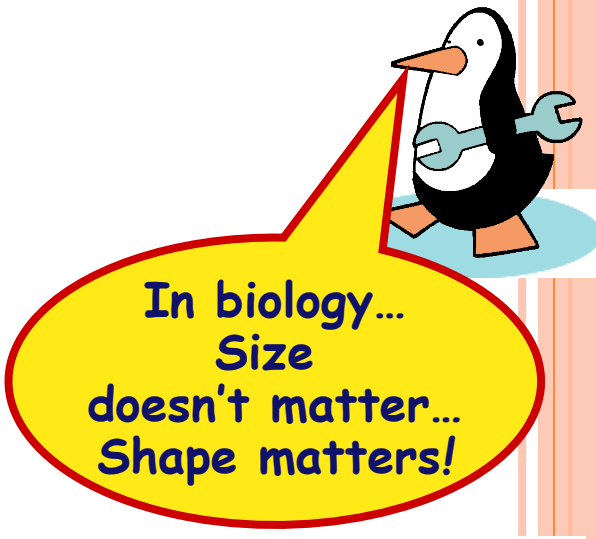
- Enzymes named for reaction they catalyze
  - sucr*ase* breaks down sucrose
  - prote*ases* break down proteins
  - lip*ases* break down lipids
  - DNA polymer*ase* builds DNA
    - adds nucleotides to DNA strand
  - pepsin breaks down proteins (polypeptides)

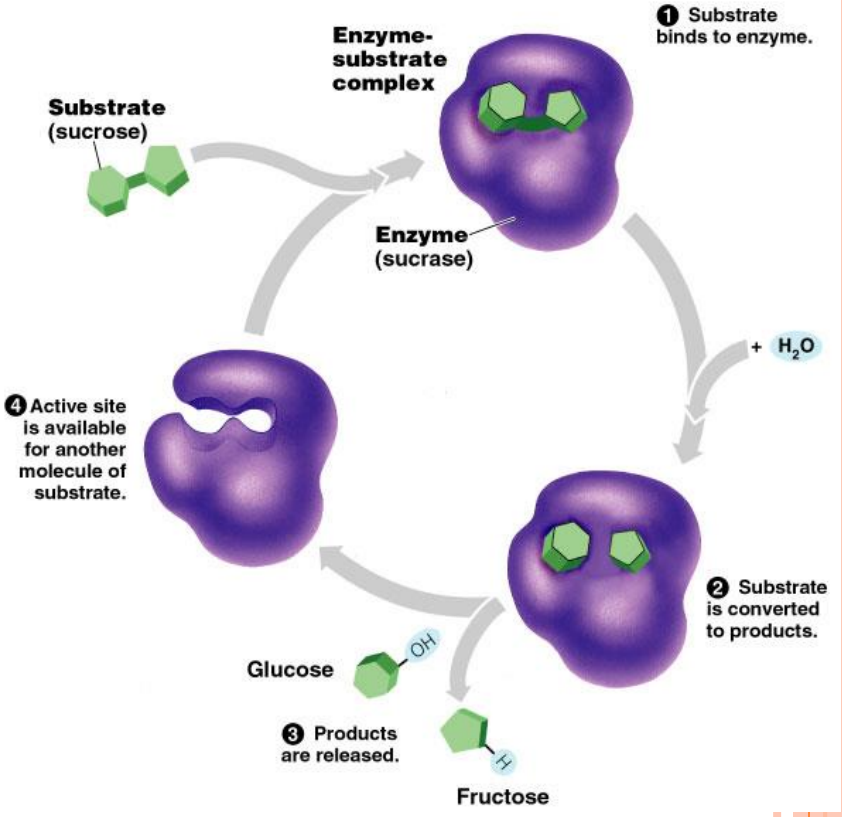


Lock and Key model

- 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"

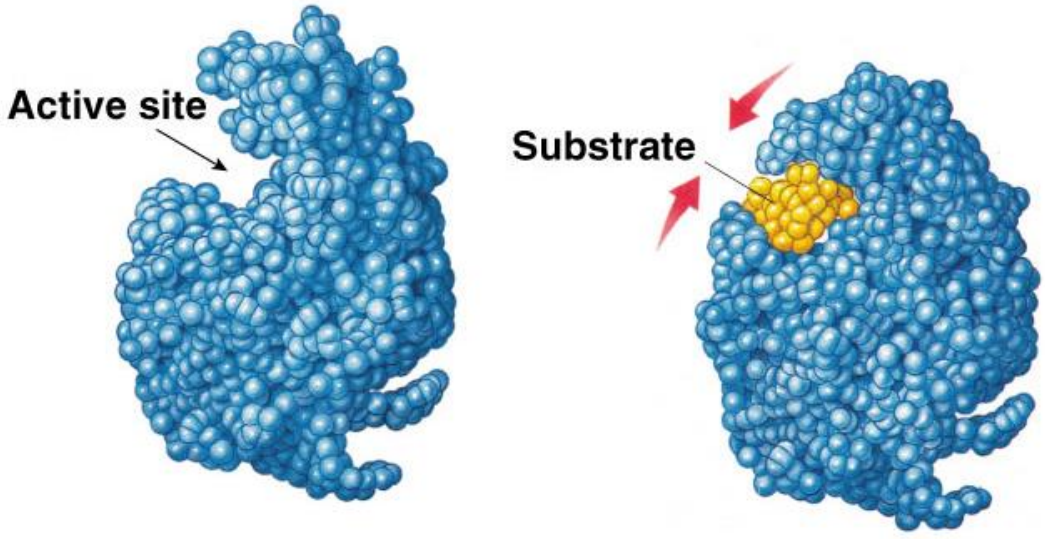




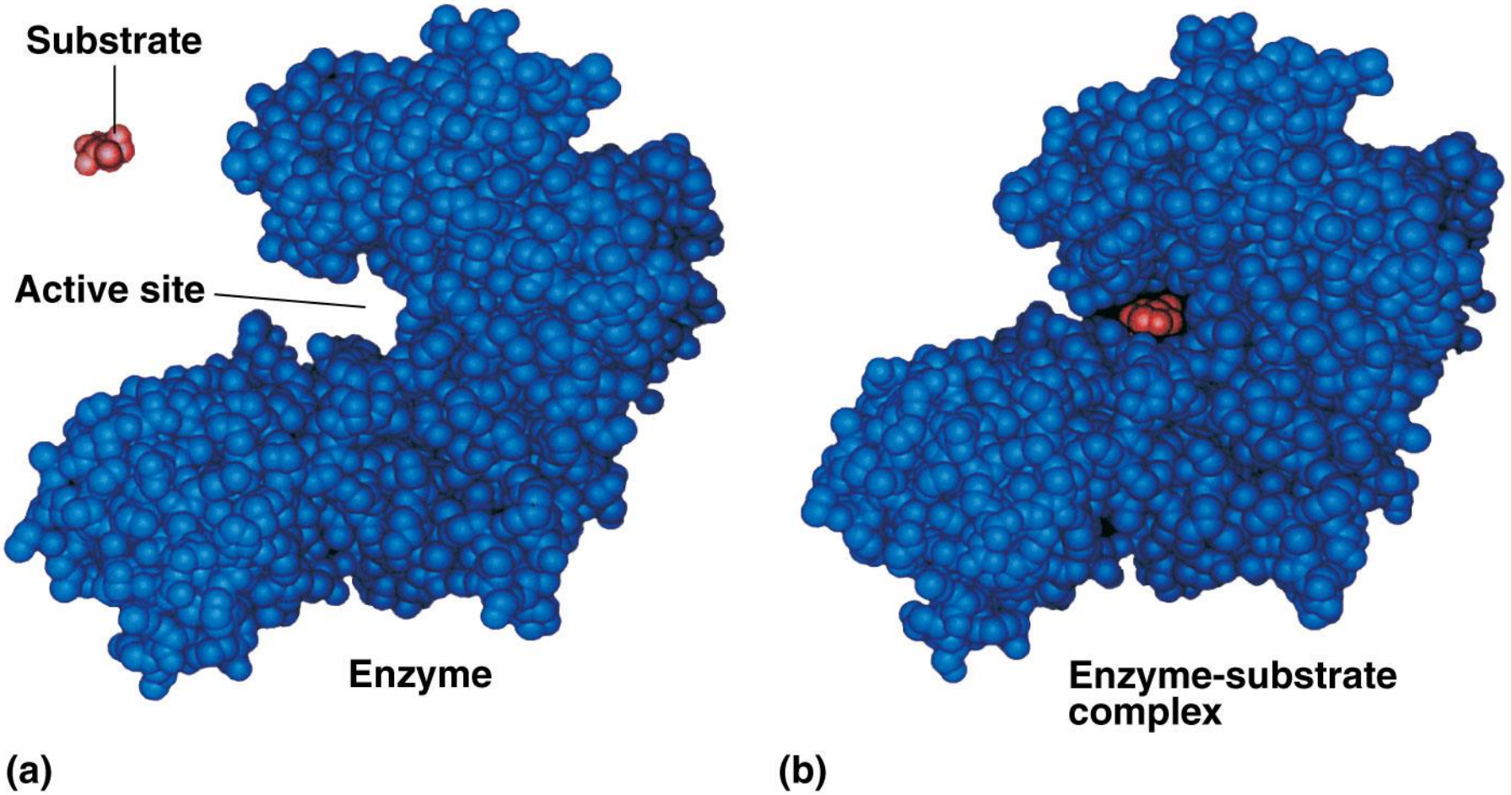


Induced fit model

- 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

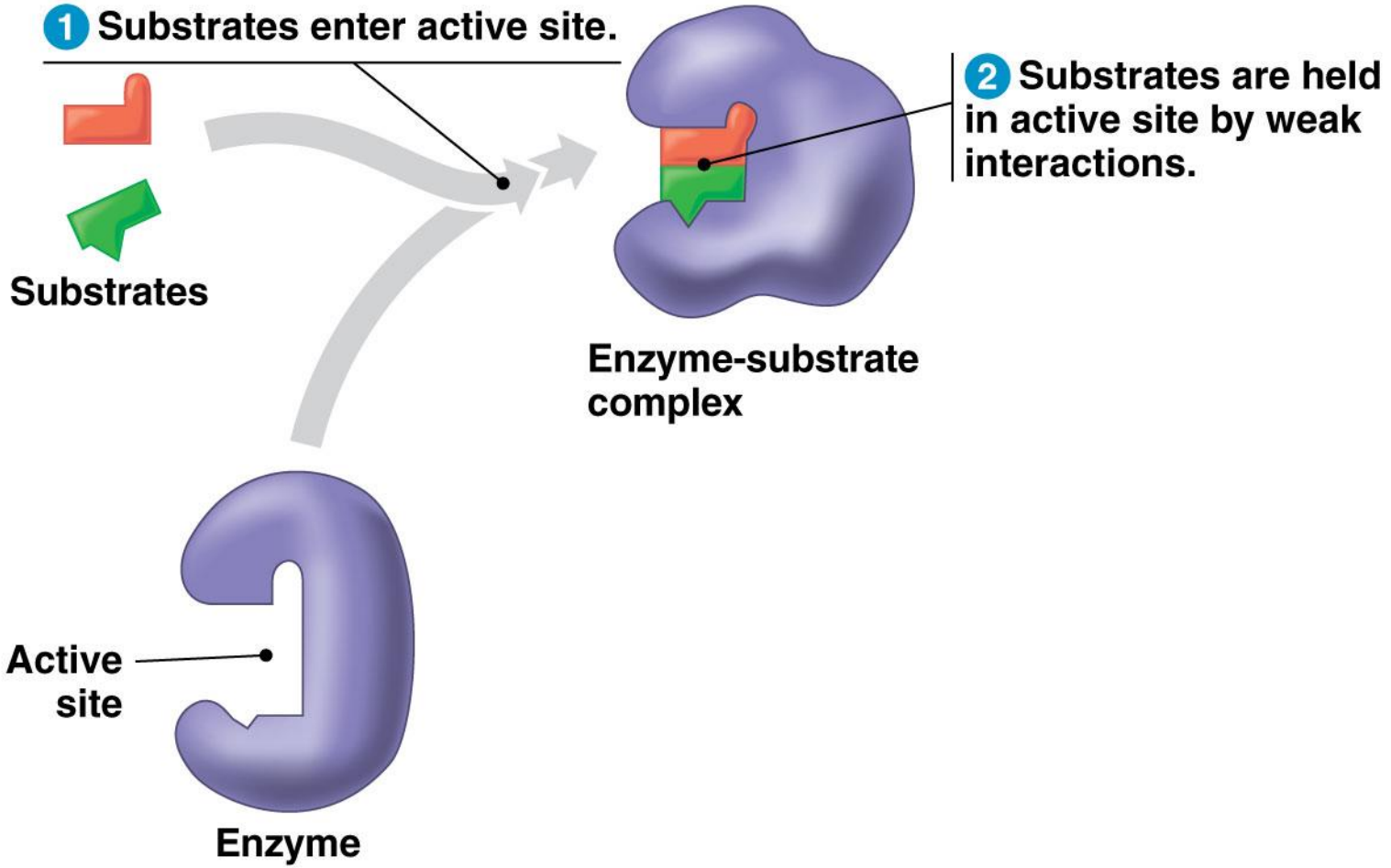


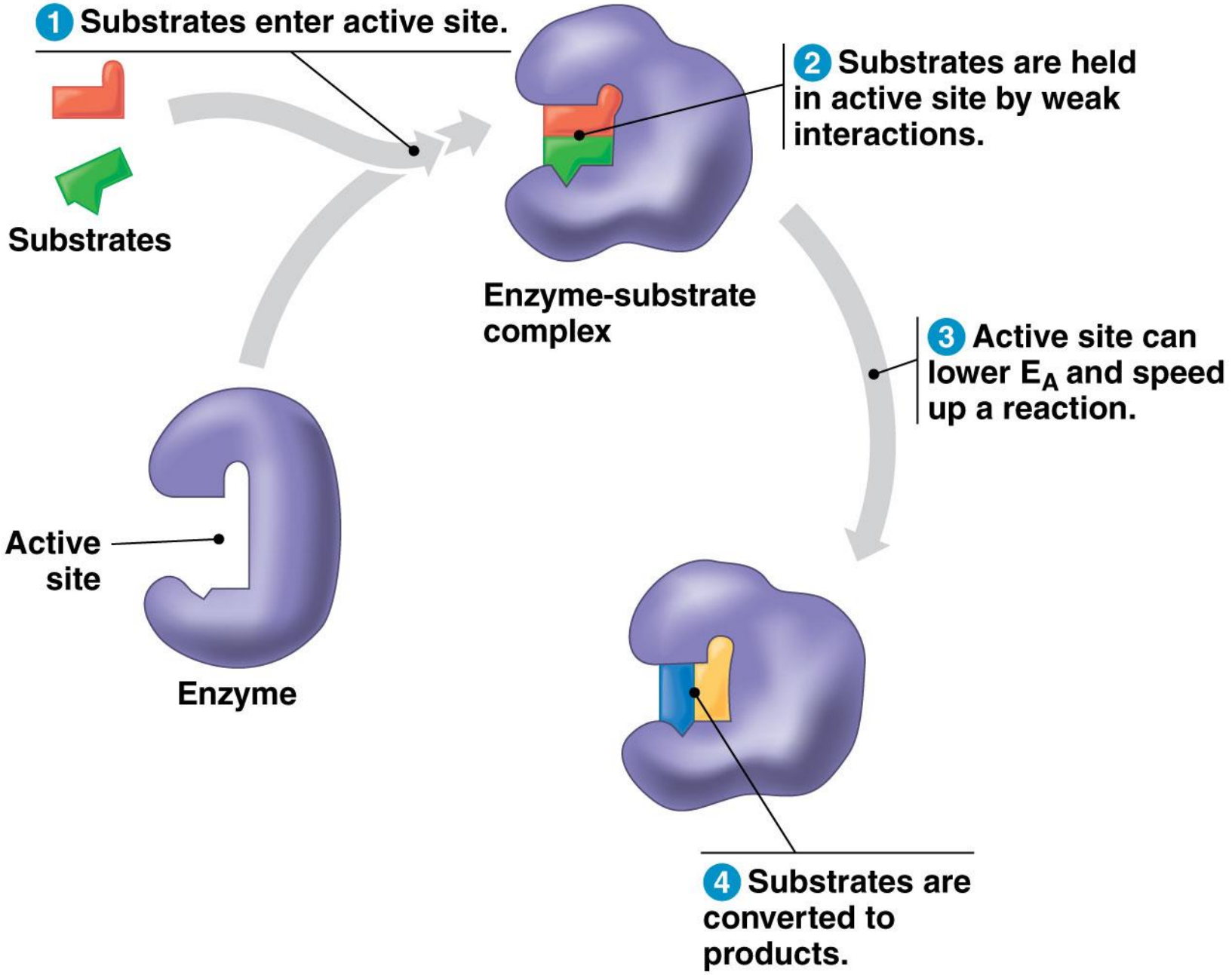
INDUCED FIT: ENZYME FITS SNUGLY AROUND SUBSTRATE -- "CLASPING HANDSHAKE"

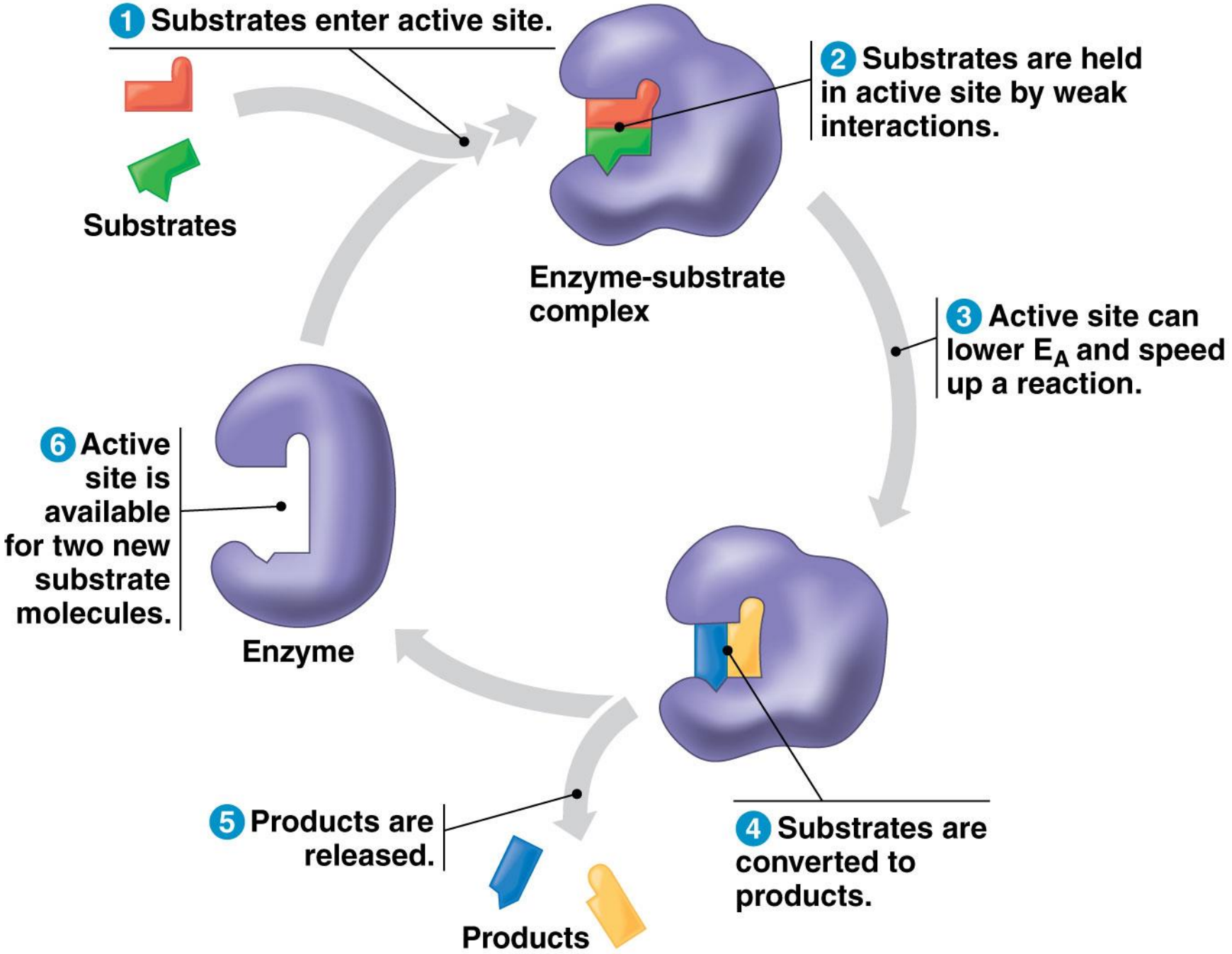


How does it work?

- 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



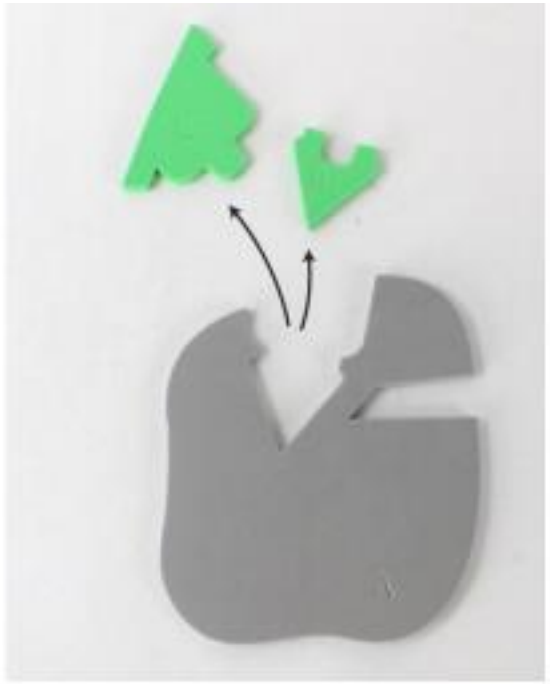




Enzyme Action: Catabolism

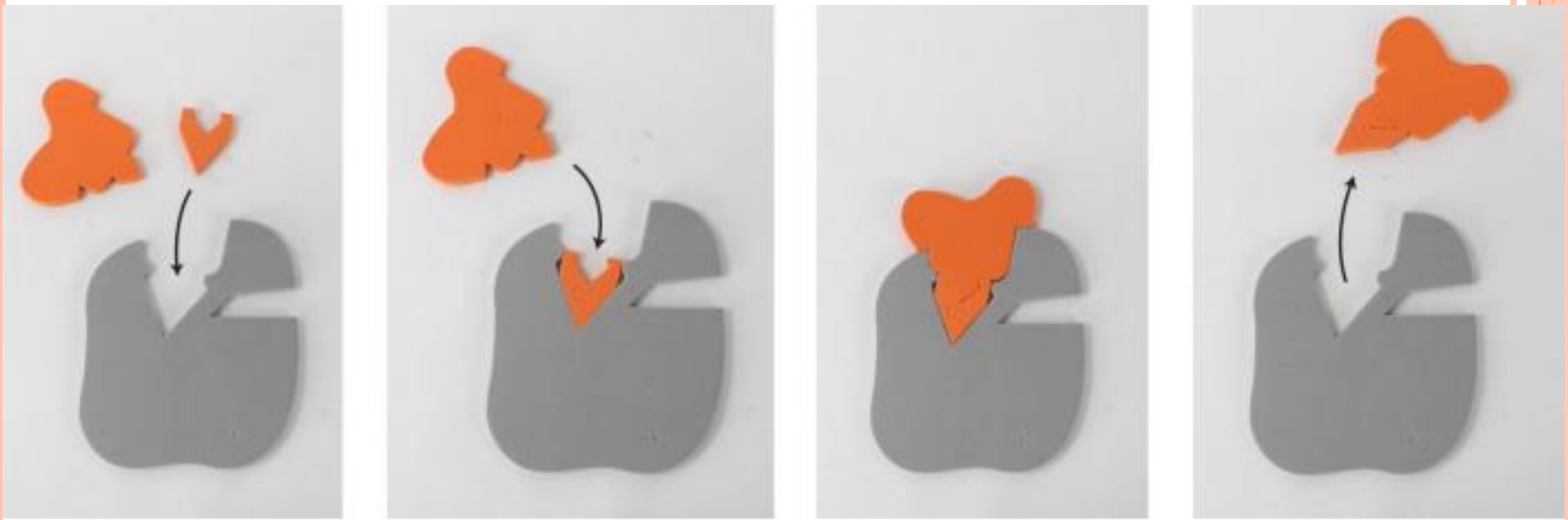
Step 1

Step 2



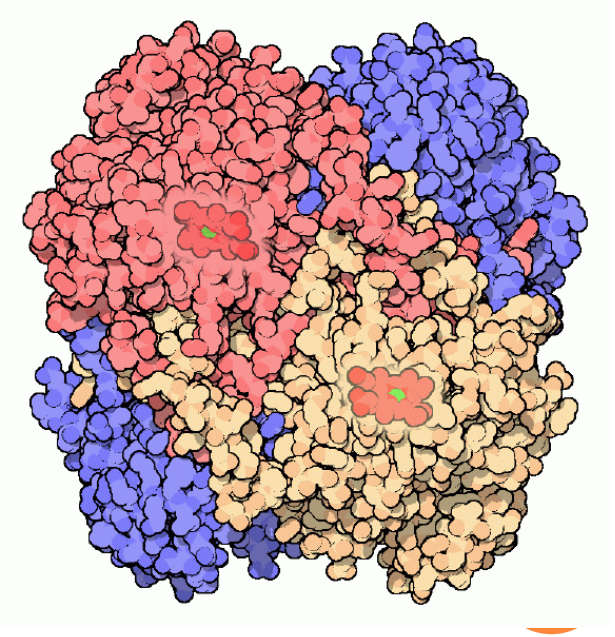
Step 3

Enzyme Action: Anabolism



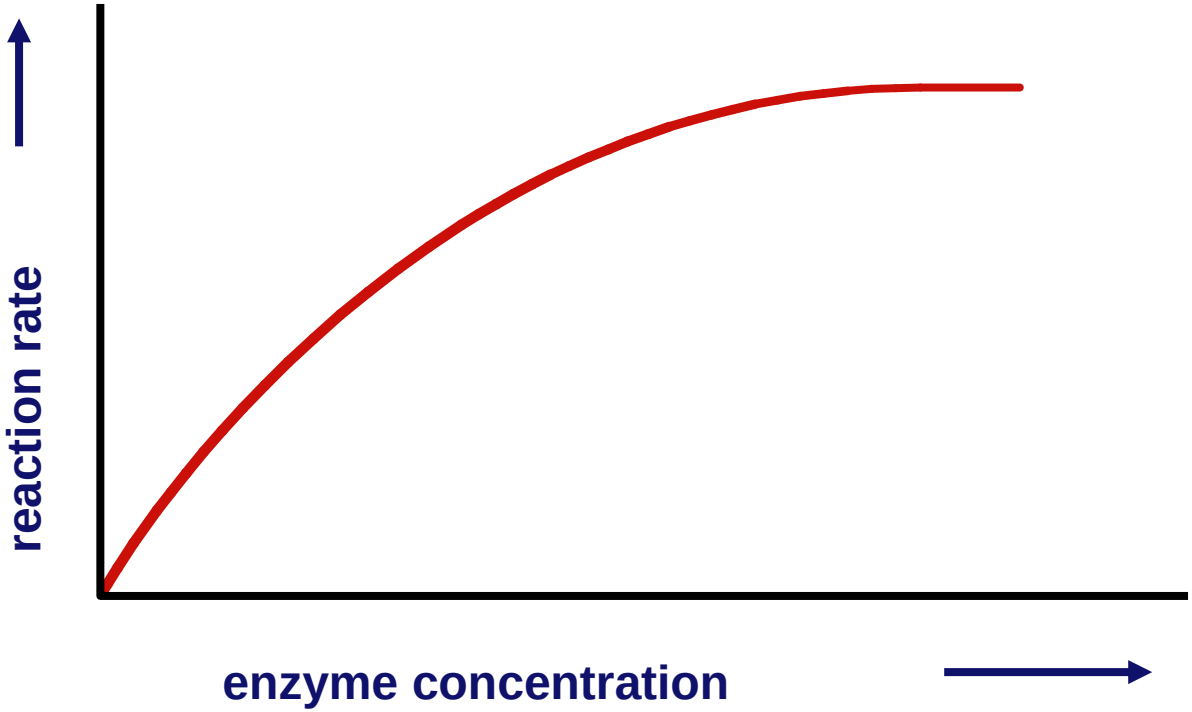
What Factors Affect Enzyme Function?

1. Enzyme concentration
2. Substrate concentration
3. Temperature
4. pH
5. Salinity
6. Activators
7. Inhibitors



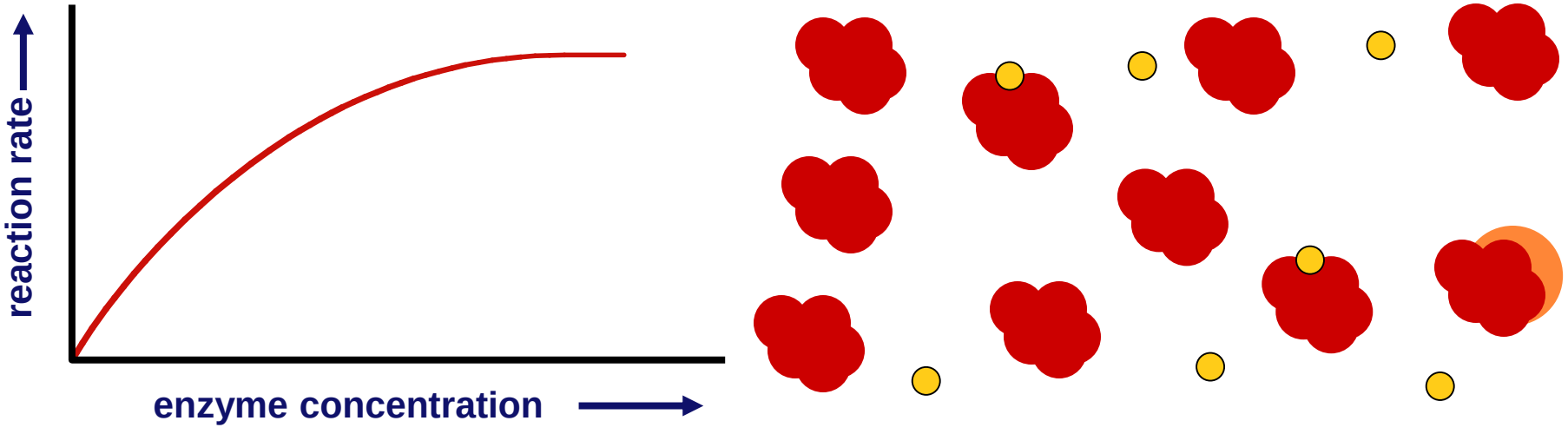
catalase

1) Enzyme concentration

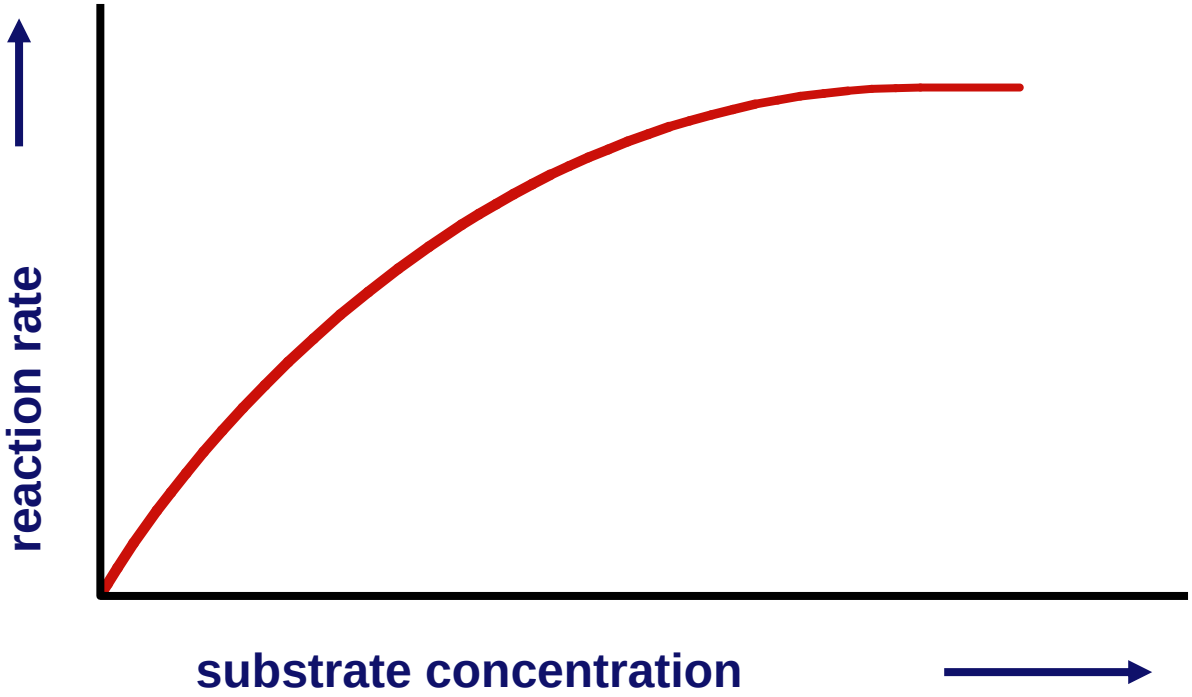


Enzyme concentration

- 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

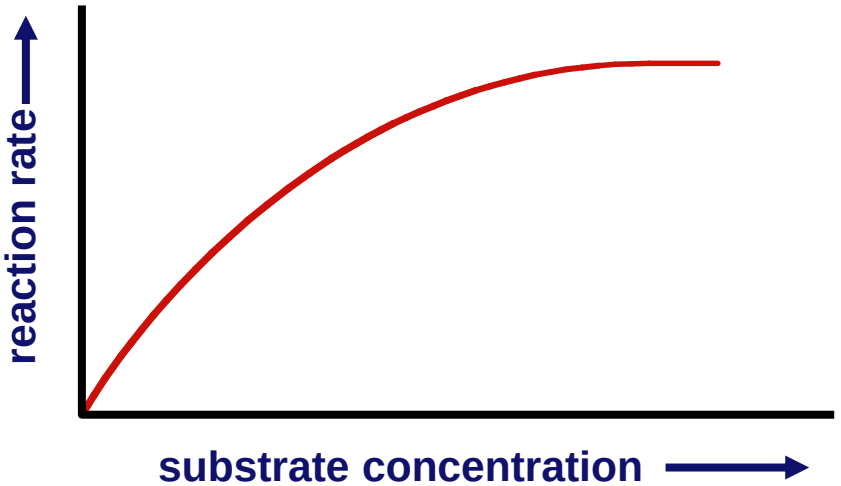


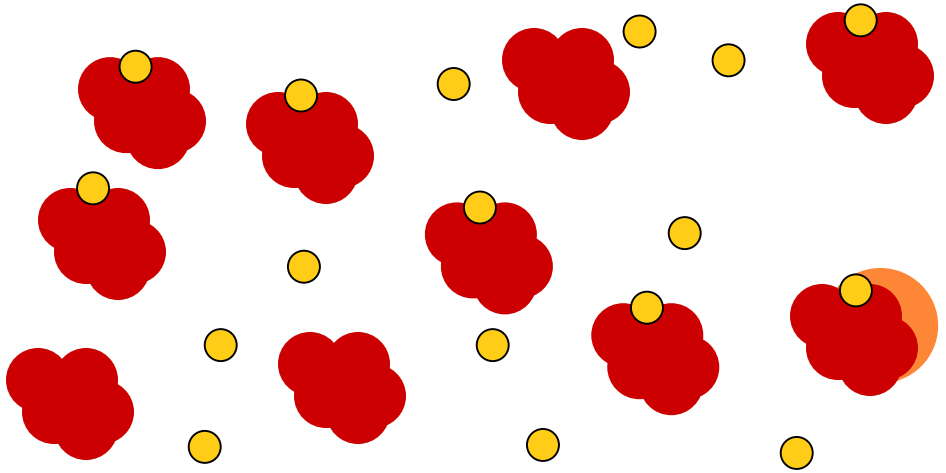
2) Substrate concentration



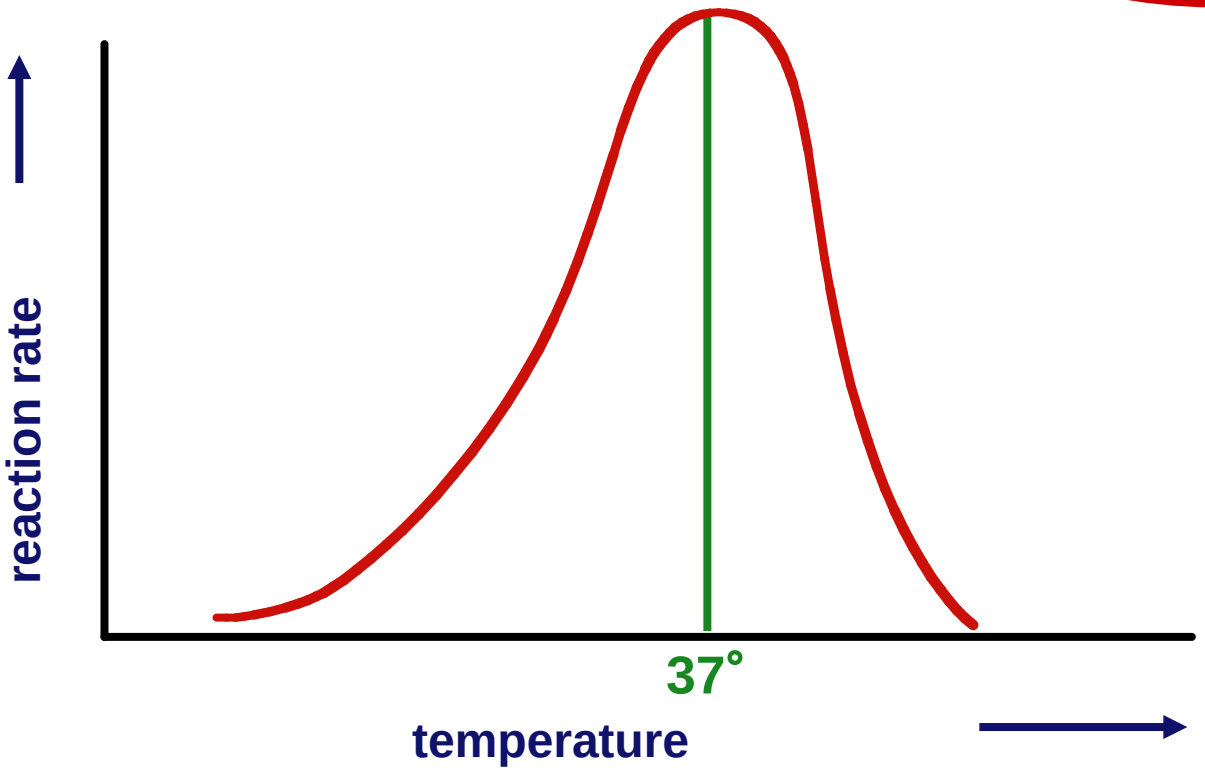
Substrate concentration

- 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



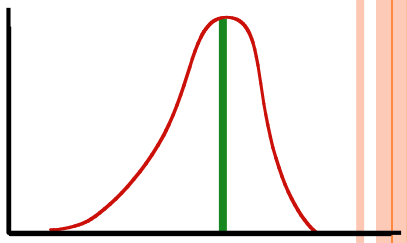


3) Temperature



Temperature

- **Optimum T°**
  - greatest number of molecular collisions
  - human enzymes = 35° - 40° C
    - body temp = 37° C

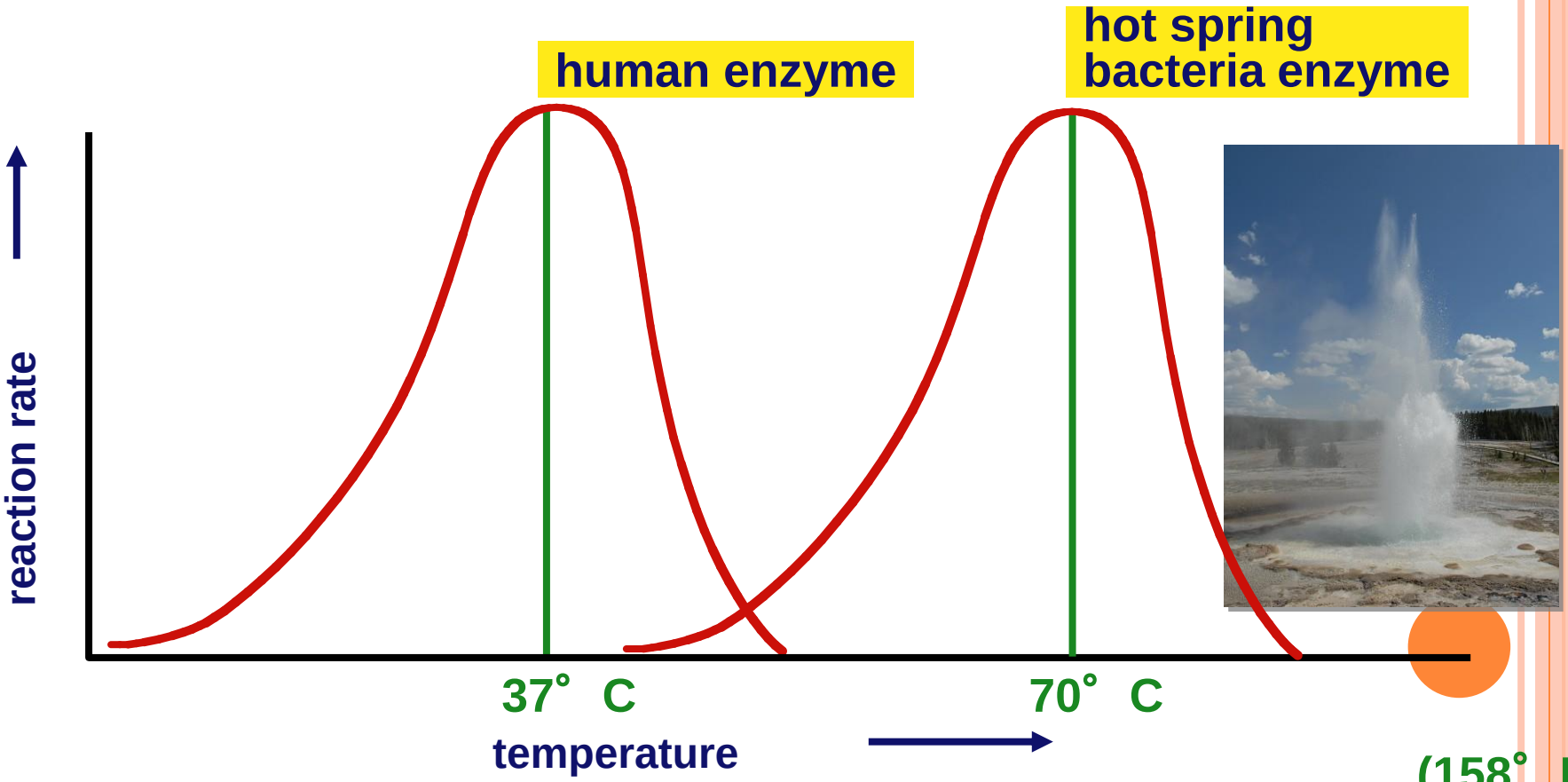


- Heat: increase beyond optimum T°
  - increased energy level of molecules disrupts bonds in enzyme & between enzyme & substrate
    - H, ionic = weak bonds
  - **denaturation** = lose 3D shape (2^o, 3^o, 4^o structure) and stop functioning

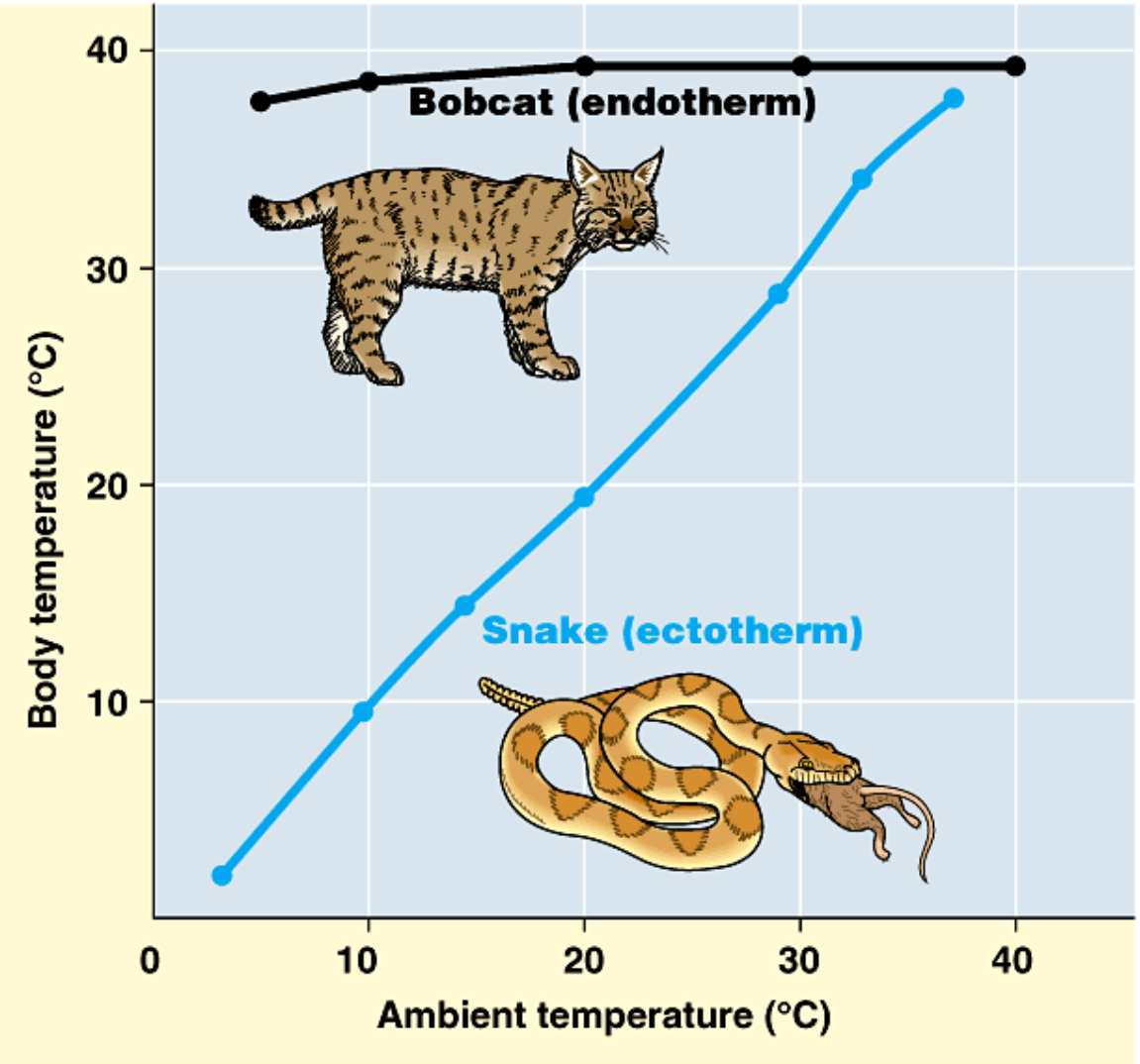
- Cold: decrease T°
  - molecules move slower
  - decrease collisions between enzyme & substrate

Enzymes and temperature

- Different enzymes function in different organisms in different environments

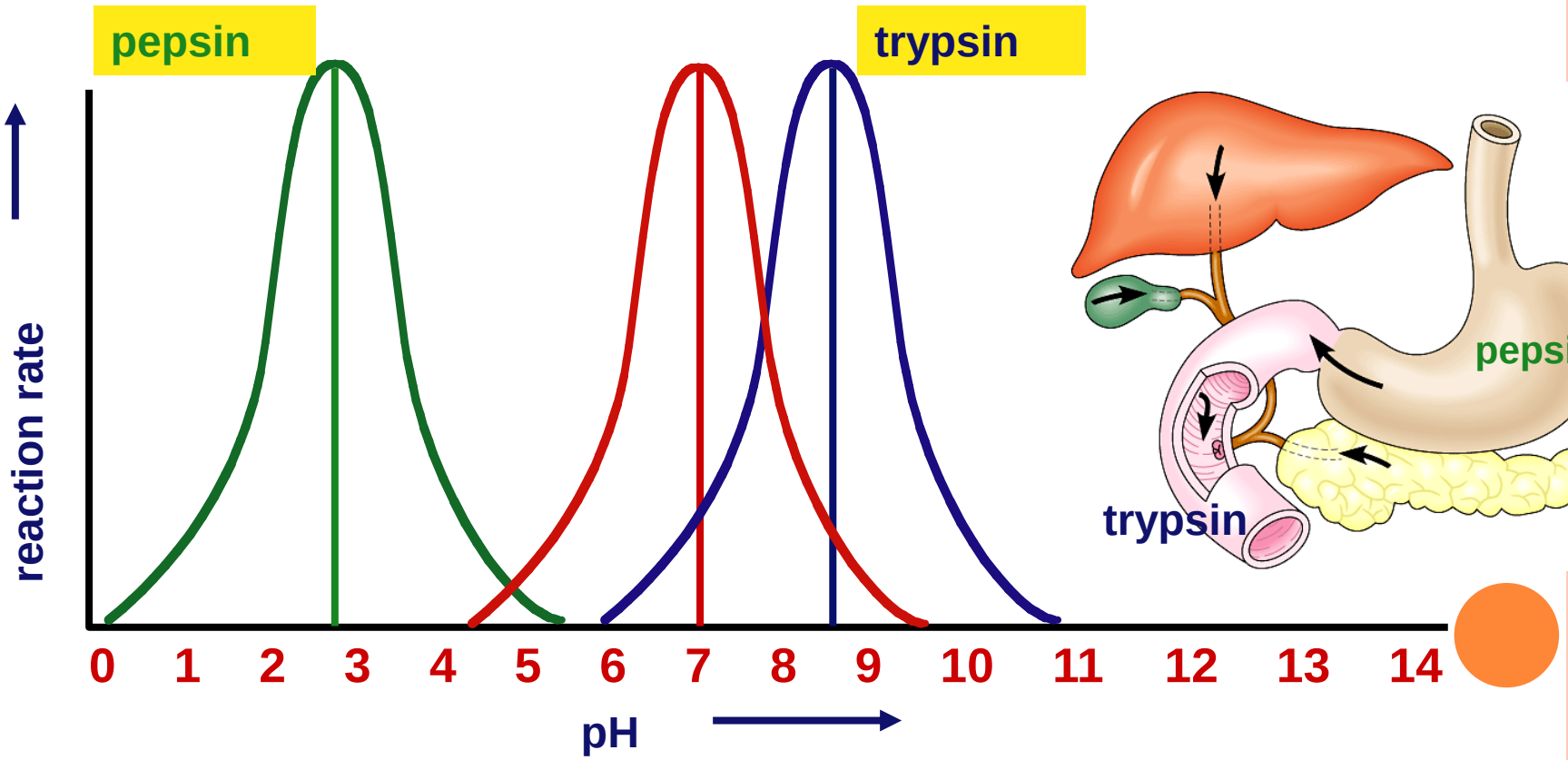


How do ectotherms do it?



4) pH


What's happening here?!

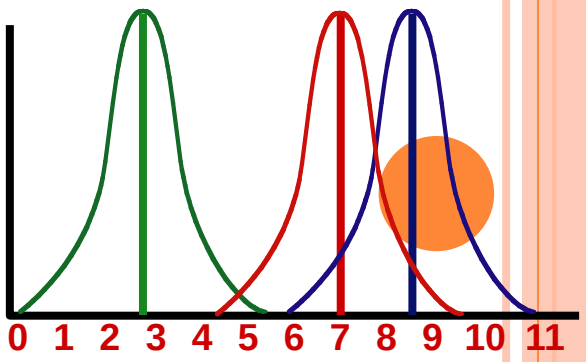

pepsin
trypsin
reaction rate
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
pH

pepsin
trypsin

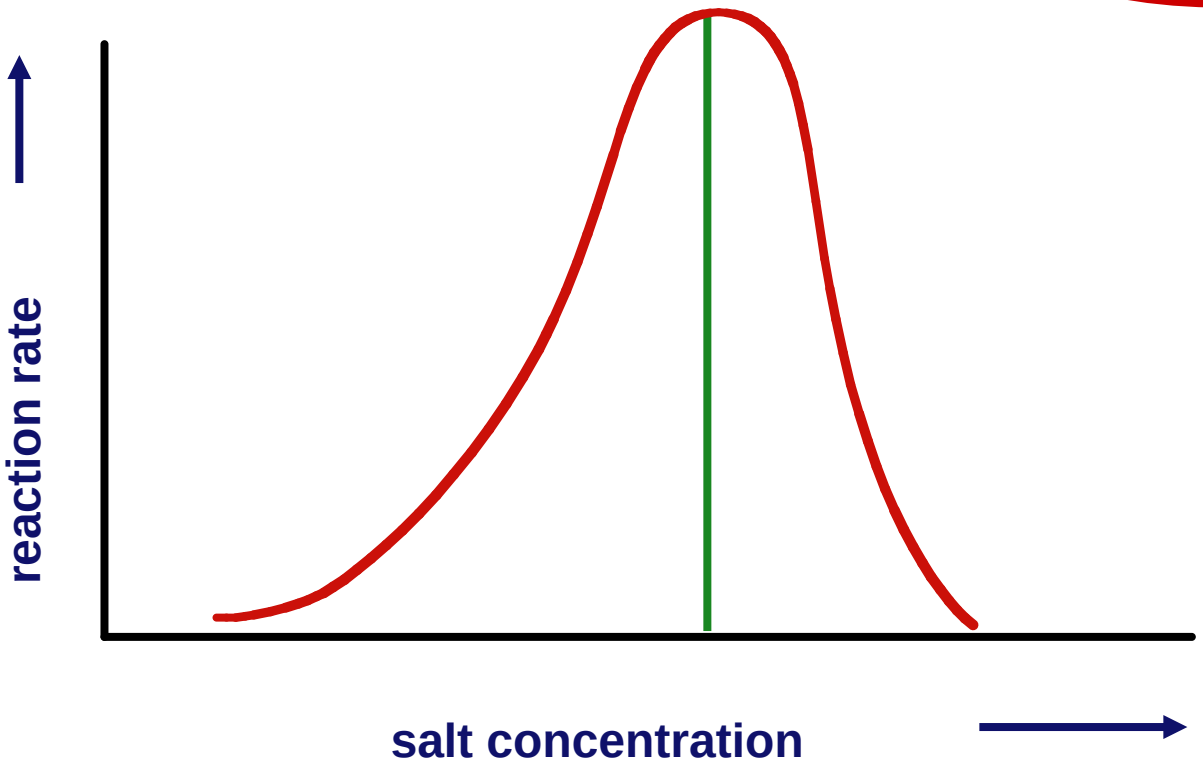
pH

- 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



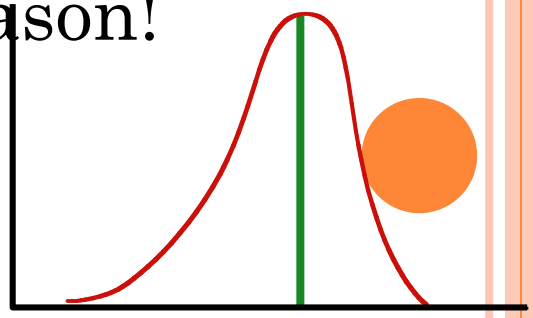
5) Salinity



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!



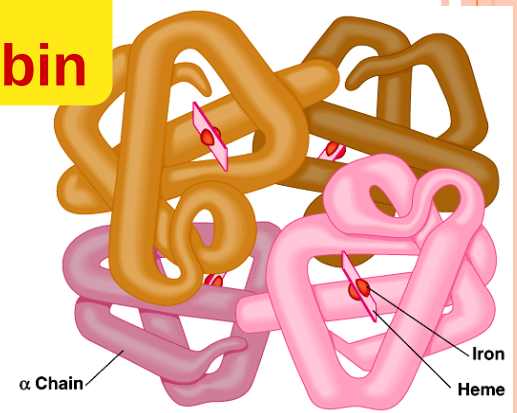
6) Activators

Compounds which help enzymes

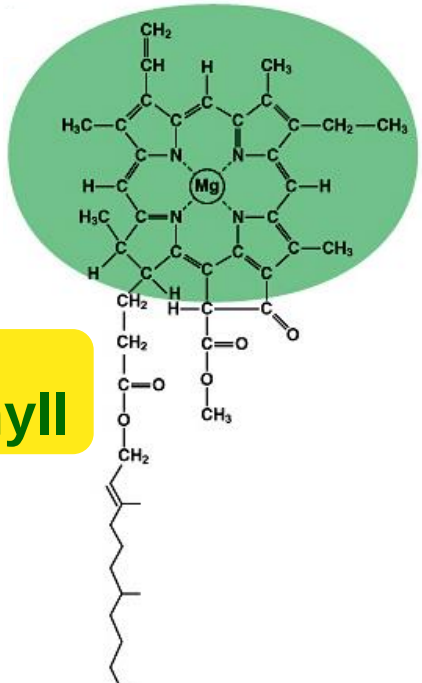
- **cofactors**
  - non-protein, small inorganic compounds & ions
    - Mg, K, Ca, Zn, Fe, Cu
    - bound within enzyme molecule

- **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



7) Inhibitors

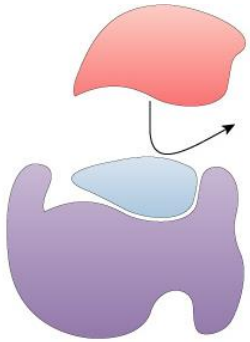
Compounds which regulate enzymes

- molecules that reduce enzyme activity

A. Competitive inhibition
   I. Reversible
   II. Irreversible

B. Noncompetitive inhibition (=Allosteric)
   I. Reversible
   II. Irreversible

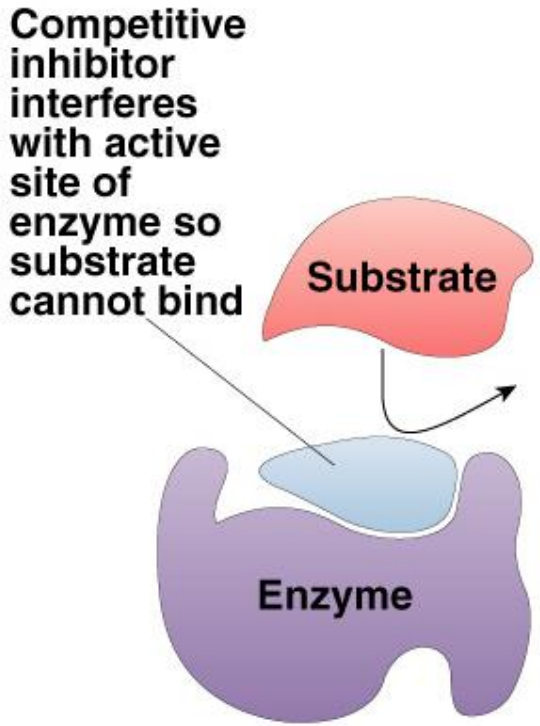
C. Feedback inhibition



A) 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 (if reversible)
  - saturate solution with substrate so it out-competes inhibitor for active site on enzyme

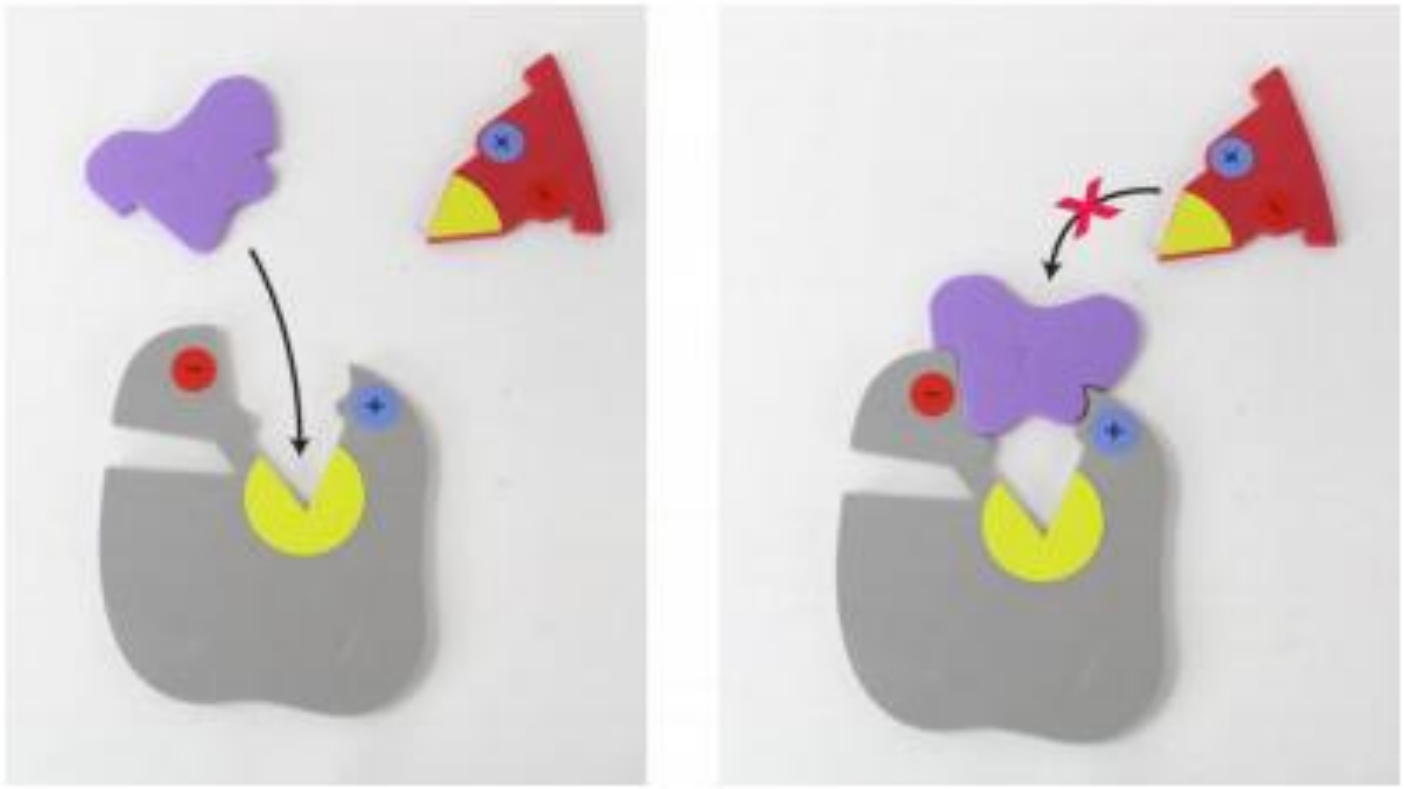


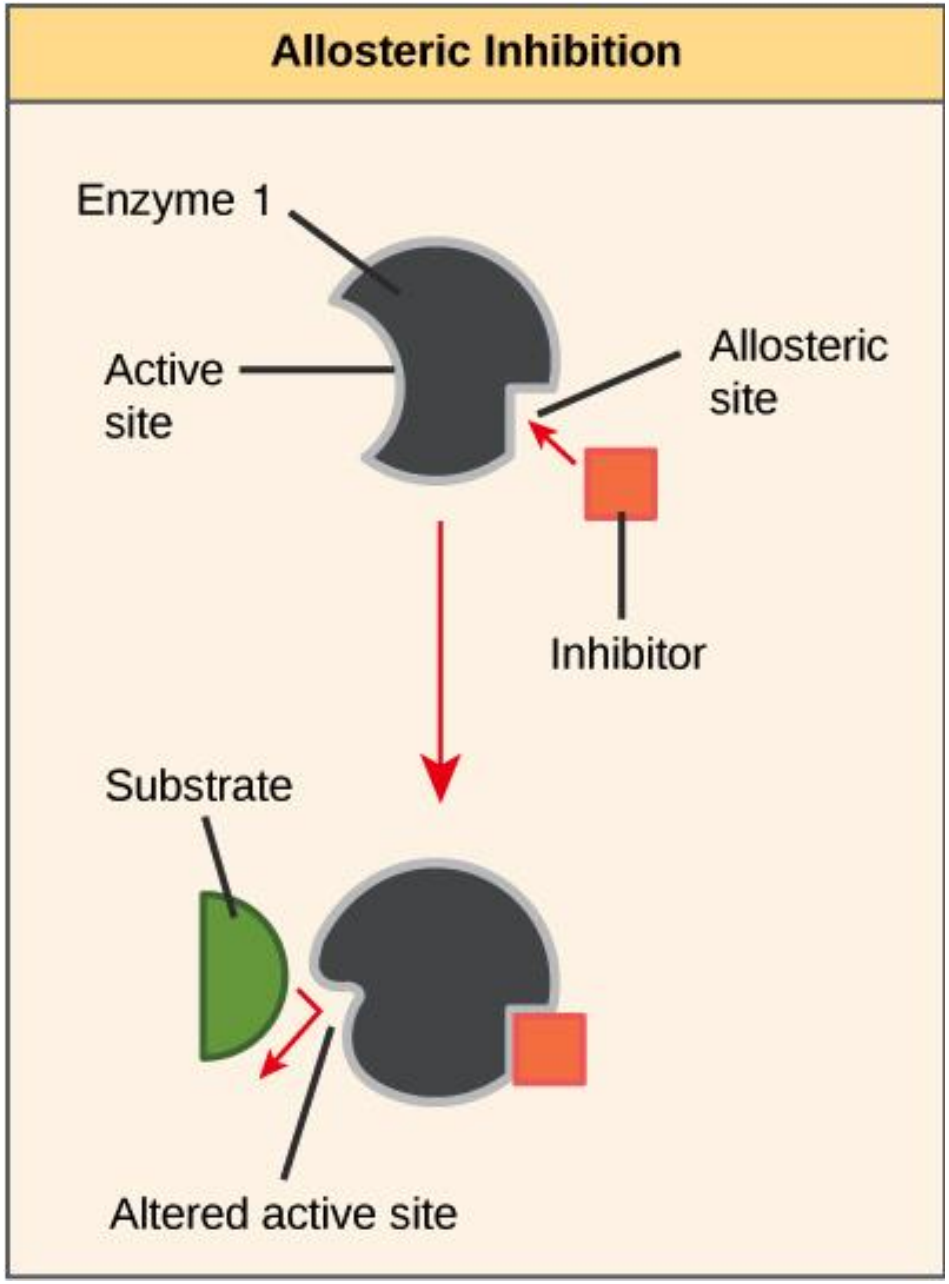
(a) Competitive inhibition

A) Irreversible Competitive inhibition

- Inhibitor permanently binds to enzyme
  - competitor
    - permanently binds to active site
    - Examples:
      - Nerve Gas – permanently binds to an enzyme needed to deactivate a the neurotransmitter acetylcholine; without it, nerve impulses can't be transmitted and muscles stay in prolonged contraction leading to paralysis & death
      - Some insecticides work in a similar way

Competitive Inhibition





TURN & TALK

1) What is an active site?

2) What do you think an allosteric site is?

3) What happens when something binds to an allosteric site?

B) Irreversible Non-Competitive inhibition (Allosteric Inhibition)

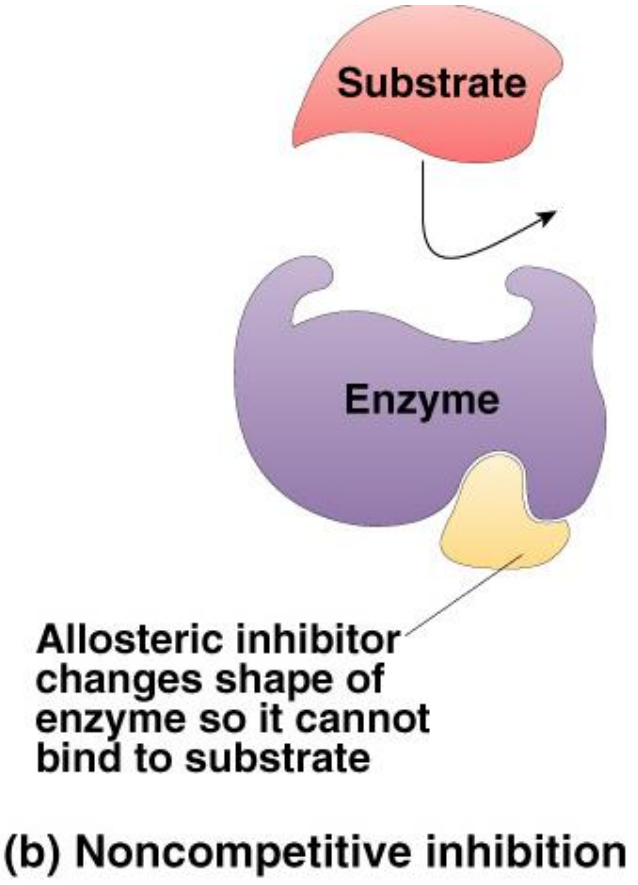
- Allosteric Inhibition
  - permanently binds to allosteric site
    - Allosteric site is a place on the enzyme OTHER than the active site.
    - Binding at these places alters the shape/structure & function of the enzyme and its active site so that the substrate can no longer bind.
  - permanently changes shape of enzyme and keeps it inactive

Irreversible Non-Competitive inhibition (Allosteric Inhibition)

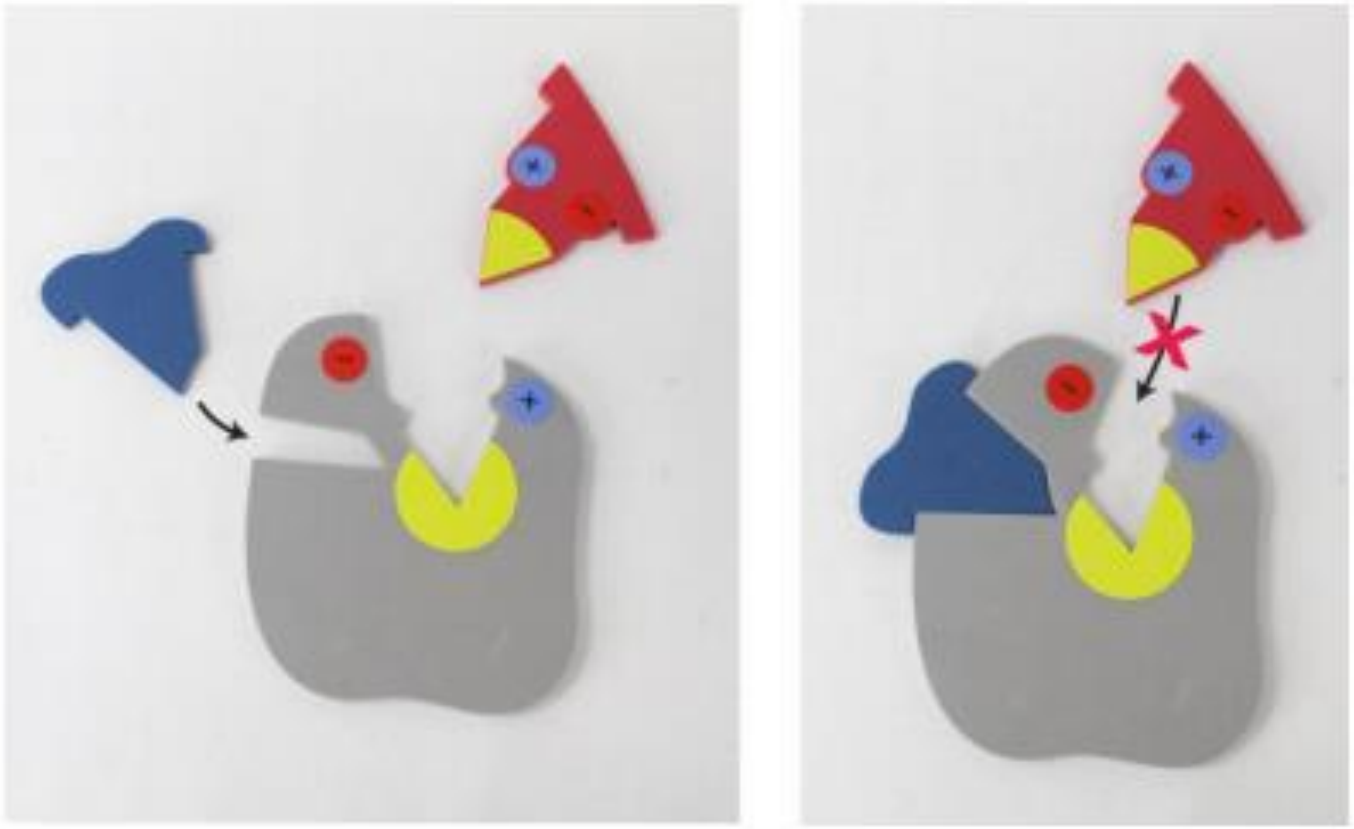
Examples:

- 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

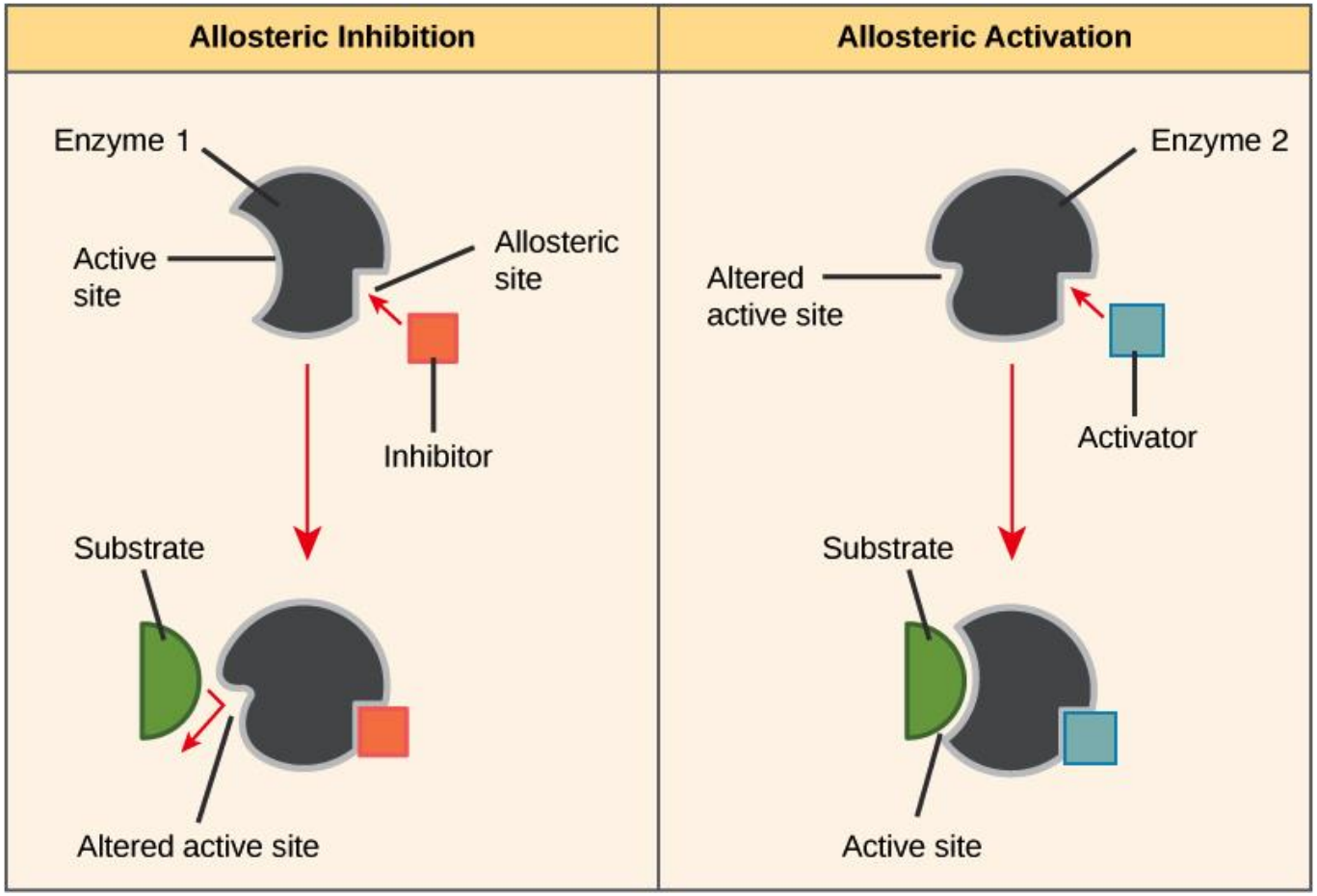


Noncompetitive Inhibition



Allosteric regulation

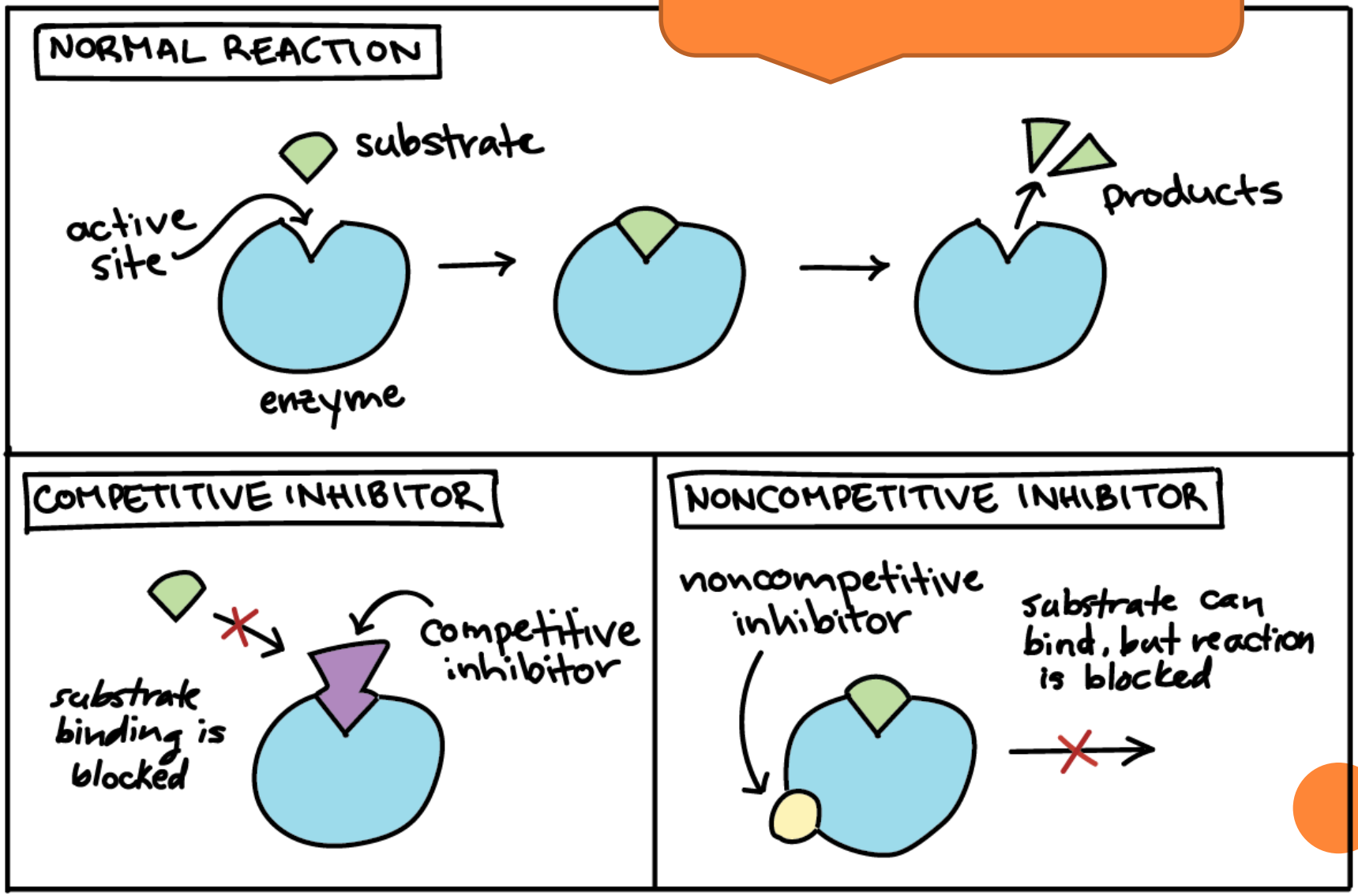
Conformational changes



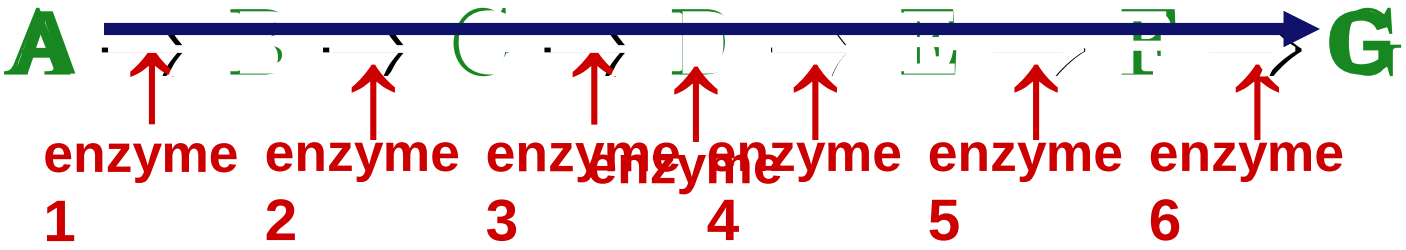
Activators = regulatory molecules that keep enzyme in active form

Inhibitors = regulatory molecules that keep enzyme in inactive form

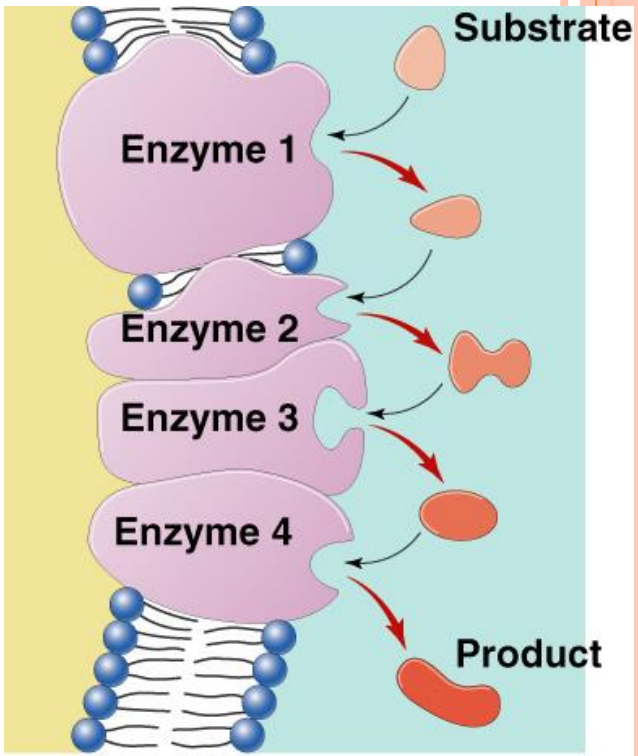
TURN & TALK


NORMAL REACTION
substrate
active site
enzyme
products
COMPETITIVE INHIBITOR
substrate binding is blocked
competitive inhibitor
NONCOMPETITIVE INHIBITOR
noncompetitive inhibitor
substrate can bind, but reaction is blocked

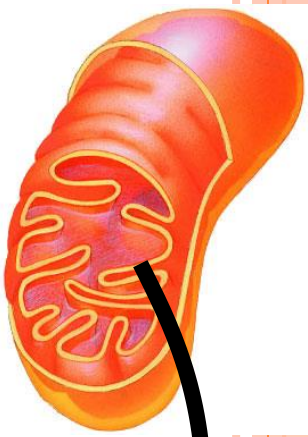
Metabolic pathways



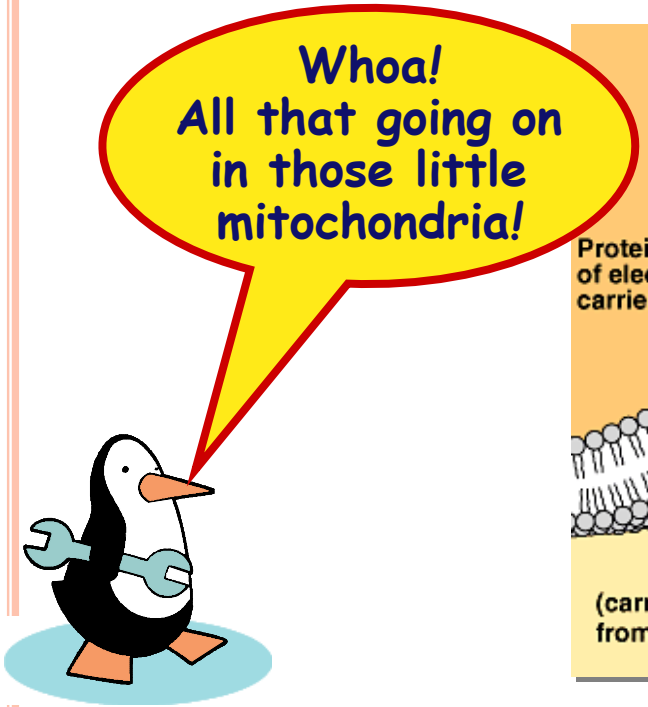
- **Chemical reactions of life are organized in pathways**
  - **divide chemical reaction into many small steps**
    - **artifact of evolution**
    - **↑ efficiency**
      - **intermediate branching points**
    - **↑ control = regulation**

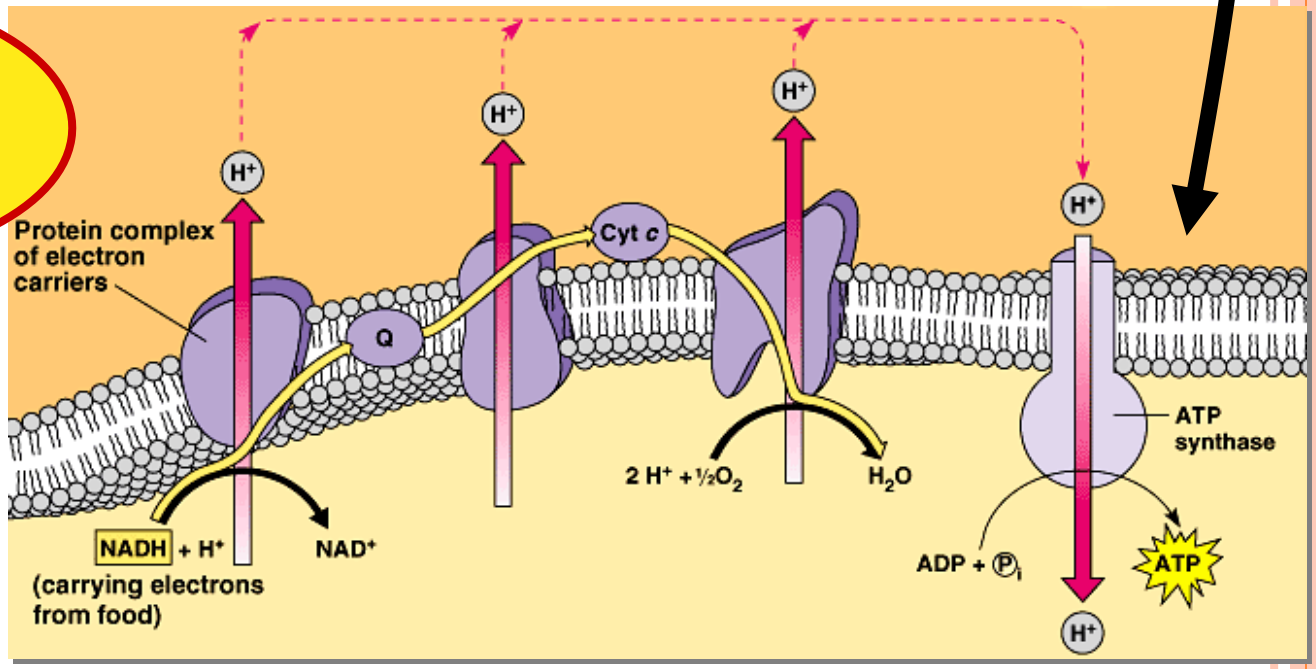


Efficiency



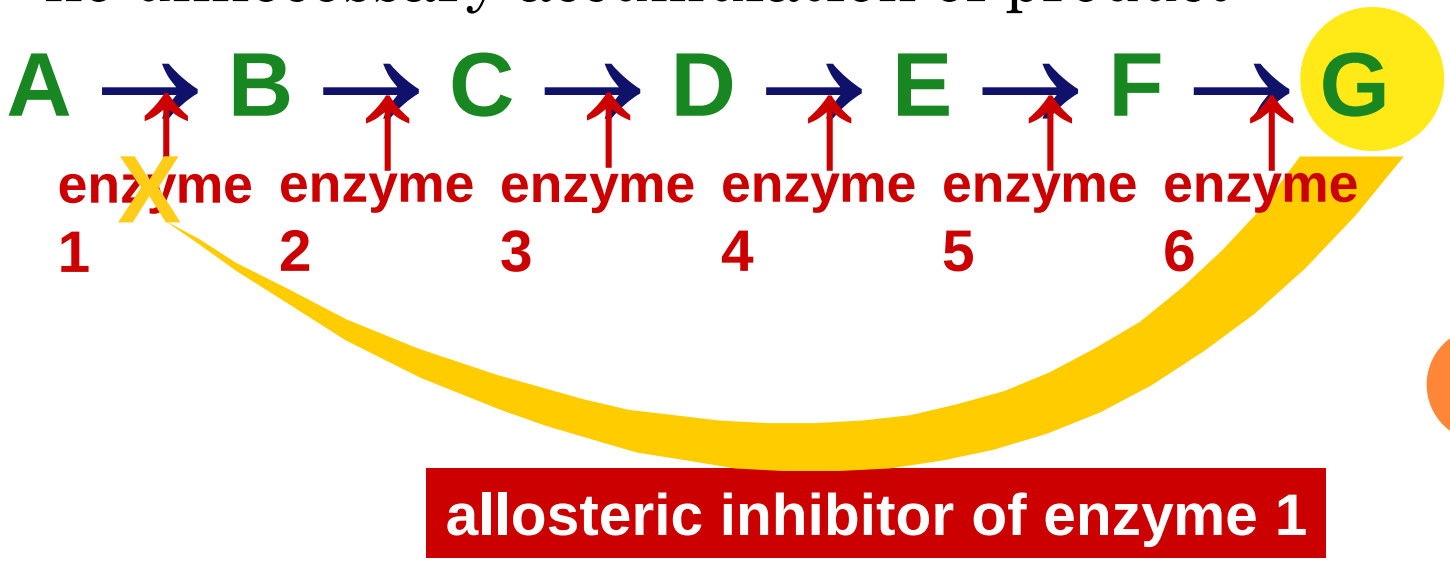
- Organized groups of enzymes
  - enzymes are embedded in membrane and arranged sequentially
- Link endergonic & exergonic reactions





C) Feedback Inhibition

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



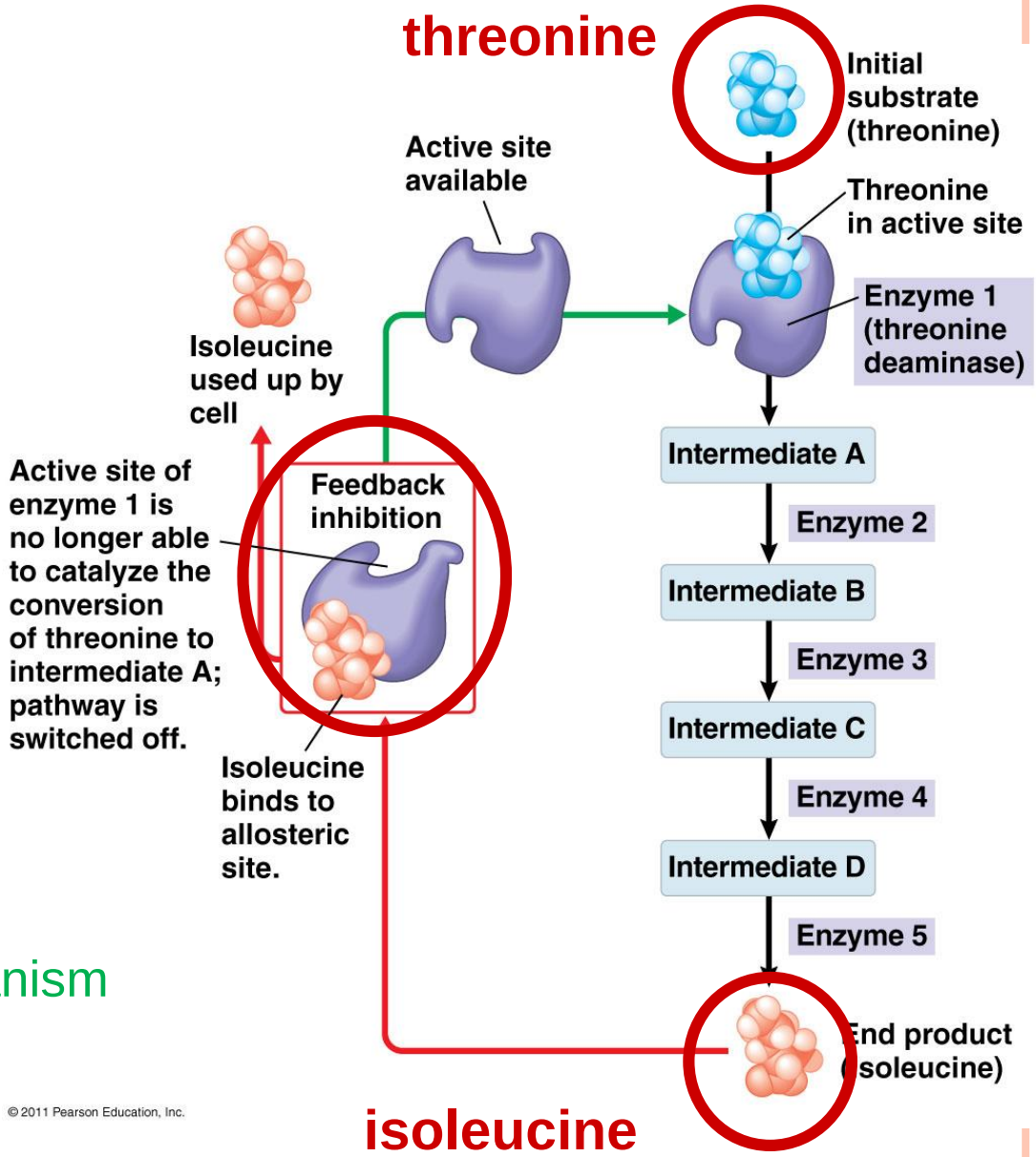
Feedback Inhibition

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

= Negative Feedback mechanism



FeedBack loops

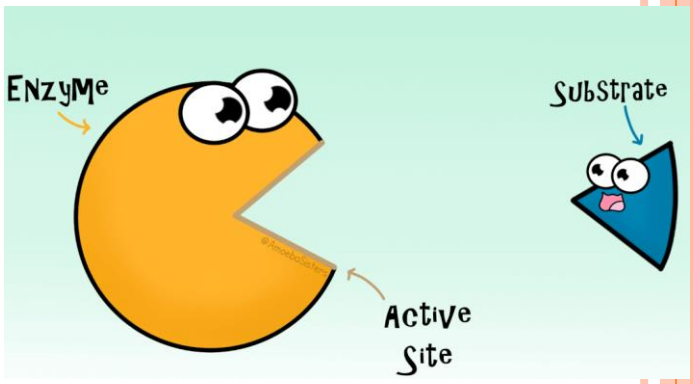
- http://www.bozemanscience.com/positive-and-negative-feedback-loops
- Use the Cornell Notes taking template to take notes during the video.

TOPIC/OBJECTIVE: Differentiate between positive and negative feedback loops with examples.

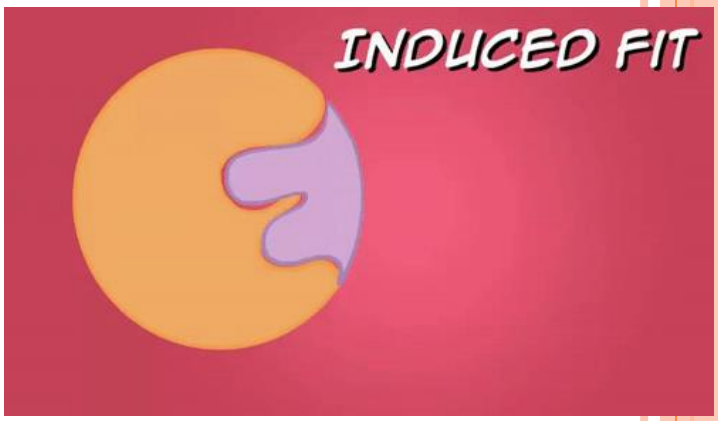
ESSENTIAL QUESTION: How do living things use feedback loops to maintain homeostasis?

Good Morning/Afternoon!

- Please complete the Enzyme Warm-up.



- You may use your notes…but please no talking *yet*.



Enzyme Lab

- READ THOROUGHLY
- Set up

PROCEDURE: ENZYME CONCENTRATION

1. Obtain 7 clean beakers.
2. Label the beakers with tape and pen: 100% catalase, 80% catalase, 60% catalase, 40% catalase, 20% catalase, and 0% catalase. Label the last beaker "reaction beaker."
3. Create the appropriate enzyme concentrations following the protocol in the table here below. Use a graduated cylinder to measure each amount. Be sure to rinse the cylinder well when switching between enzyme and water. Stir all solutions well.

Test	Catalase Enzyme Concentration	Volume of Enzyme	Volume of Water
# 1	100%	40 ml	0 ml
# 2	80%	32 ml	8 ml
# 3	60%	24 ml	16 ml
# 4	40%	16 ml	24 ml
# 5	20%	8 ml	32 ml
# 6	0%	0 ml	40 ml

4. Obtain the 3% hydrogen peroxide substrate.
5. Pour 30 ml of the 3% H_2O solution into a clean beaker, labeled "reaction beaker.
6. Pick up a paper disk with a clean forceps (or tweezers) and dunk the disk into the enzyme extract for 5 seconds, until the disk is uniformly moistened but not beaded with drops of liquid. Gently tap it on a paper towel for about 5 seconds to remove excess enzyme.
7. Use the forceps to place the filter disk (containing enzymes) onto the **BOTTOM** of the "reaction beaker" containing the H_2O_2 liquid substrate.

Measure the time it takes for the disk to **LIFT OFF**; go completely horizontal. **NOT** rise all the way to the top.

9. Watch the filter disk to see tiny bubbles of oxygen being released. Record the time in seconds in data table 2.
10. Repeat steps 5-8 for all of the Test #s. Remember to use a clean disk each time.
11. You will record data from 3 other groups (under the other trials columns) to obtain results for multiple trials and then calculate the average enzymatic rate for each enzyme concentration.

PROCEDURE: H_2O_2 CONCENTRATION

1. Obtain 6 clean beakers.
2. Label the beakers with tape and pen: 3.0% H_2O_2, 2.0% H_2O_2, 1.5% H_2O_2, 1.0% H_2O_2, 0.5% H_2O_2, and 0.3% H_2O_2.
3. Create the appropriate substrate concentrations following the protocol in the table here below. Use a graduated cylinder to measure each amount. Be sure to rinse the cylinder well when switching between H_2O_2 substrate and water. Stir all solutions well.

Test	H_2O_2 Substrate Concentration	Volume of H_2O_2	Volume of Water
# 1	3.0%	30 ml	0 ml
# 2	2.0%	20 ml	10 ml
# 3	1.5%	15 ml	15 ml
# 4	1.0%	10 ml	20 ml
# 5	0.5%	5 ml	25 ml
# 6	0.3%	3 ml	27 ml

4. For this part of the lab, use a 60% catalase solution (from Part 2 if available).
5. Pick up a paper disk with a clean forceps (or tweezers) and dunk the disk into the 60% enzyme extract for 5 seconds, Gently tap it on a paper towel for about 5 seconds to remove excess enzyme.
6. Use the forceps to place the filter disk (containing enzymes) onto the **BOTTOM** of the "reaction beaker" containing the H_2O_2 liquid substrate. (Start with the 3.0% H_2O_2.)

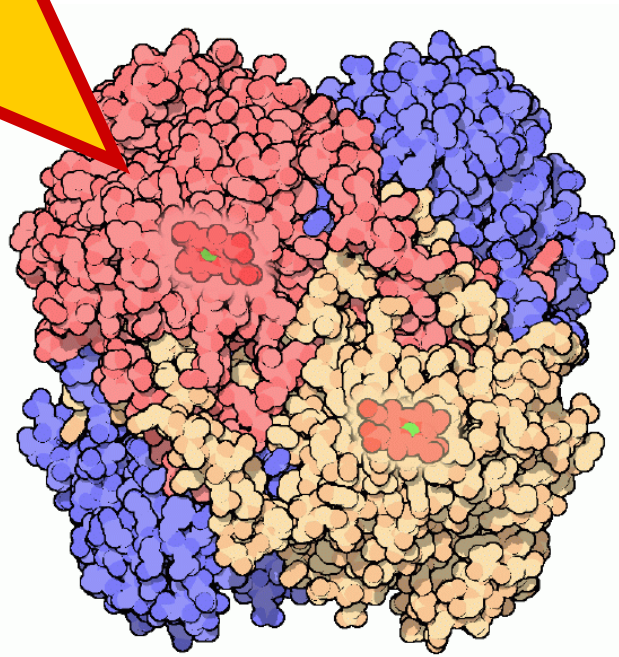
Measure the time it takes for the disk to **LIFT OFF**; go completely horizontal. **NOT** rise all the way to the top.

8. Record the time in seconds in data table 3.
9. Repeat steps 5-8 for all of the Test #s. Remember to use a clean disk each time.
10. You will record data from 3 other groups (under the other trials columns) to obtain results for multiple trials and then calculate the average enzymatic rate for each enzyme concentration.

Practice

- Review your notes.
- Use your NOTES to answer the questions in the packet.
- This is NOT an appropriate time to be on your phone or computer.

Don’t be inhibited!
Ask Questions!



Feedback Inhibition

- End product of a metabolic pathway shuts down pathway by binding to the allosteric site of an enzyme
- Prevent wasting chemical resources, increase efficiency of cell

Enzyme Lab

1. Close Read of Background
    1. Highlight vocab (define)
    2. Comments, Questions, Connections, drawings, etc. in margins
2. Answer Pre-Lab Questions
3. Demos
    1. Egg whites
    2. Controls
4. Lab Groups
5. Lab Materials
6. Procedures

NEED MORE?

- Bozeman Science to the Rescue!
  - Watch "Enzymes here:

http://www.bozemanscience.com/048-enyzmes

- Bozeman: Enzyme Catalysis Lab

Demonstrations

- Egg white contains a protein called ovalbumin.
- It also has the enzyme catalase.
- Both of these are susceptible to environmental conditions like temperature and pH.

Catalase + Hydrogen Peroxide → water + Oxygen

(enzyme) (substrate) ("bubbles")

- Record your observations (or drawings) of each demonstration. They will only occur once.

What happened?

1. Why did the egg white NOT "bubble" in buffer 7?
2. Why did the egg white NOT "bubble" in acid?
3. Explain the reaction happening beaker 2 with the hydrogen peroxide.

1. What happened when hydrogen peroxide was added to egg white + buffer 7? Why?
2. Why did it still not "bubble" when hydrogen peroxide was added to egg white + vinegar?
3. What does this tell us about enzyme activity?

Controls

- Watch how the catalase enzyme extract is prepared from potatoes.
- Pay attention to the general procedures because this is what you will do in your groups tomorrow.
- **We will measure the time it takes for the disk to "lift off".** NOT the time it takes to get all the way to the top of the solution/beaker.

Lab Groups – 1st block –

Lab Station 1	Lab Station 2	Lab Station 3	Lab Station 4	Lab Station 5
Group 1 Perform Test #1	**Group 2 Perform Test #2**	**Group 3 Perform Test #3**	**Group 4 Perform Test #4**	**Group 5 Perform Test #5**
Maya Mariana Daniel	Janie Jampel Rose	Carly Maggie Olwyn	Yelena Katie Darby	Valeria Joelle Joshua
Group 6 Perform Test #1	**Group 7 Perform Test #2**	**Group 8 Perform Test #3**	**Group 9 Perform Test #4**	**Group 10 Perform Test #5**
Kate Morgan Grayson	Sallie Sam Jackson Addi	Ellie Manpreet Emma Maddison	Liza Matthew Rachel Alzahraa	Molly Dalton Wayne

5 Lab Groups –4TH block

Station 1 Perform Test #1	Station 2 Perform Test #2	Station 3 Perform Test #3	Station 4 Perform Test #4	Station 5 Perform Test #5
Halle Akshra Bella Collin	Ian Ashlyn Stuart Charlotte	Beth Kate Caroline Cameron Nick	Aiden Brooke David Darien Jackson	Joel Kent Sophia Matthew

Part 1 – Enzyme concentration

- Your potato extract (catalase enzyme) is "100%
- Each group will only test 1 of the listed enzyme concentrations
  - Group 1 → test #1 (20%)
  - Group 2 → test #2 (40%)
  - Etc.

You will follow the "recipe" on your procedure for making each solution.

- For Part 1 you will need 1% H_2O_2 _ be sure to obtain the correct substrate and use the appropriately labeled beaker. (* last bullet #2)

- EVERYONE in the group should read ALL directions carefully *before* beginning the procedure.

Reminders:

- Use a stopwatch to time how long it takes for the disk to fully "lift off" – not float all the way to the top of the solution.
- Step 3: bullet 1 → do not need to use the graduated cylinder to measure 30 ml. Use the markings on the side of the beaker.
- Be sure to QUICKLY remove the disk once it lifts off. This is so it won't continue to use up the substrate.
- We will do all graphs AFTER the lab is done.

Part 1 – 1ST Block
Data Table / Enzyme Concentration

Test Number	Group Average	CLASS Average	1/t
1 (20%)	2.3 13.6	7.95	
2 (40%)	7.21 9.923	8.57	
3 (60%)	7.33 7.53	7.43	
4 (80%)	4.33 5.02	4.68	
5 (100%)	.58 2.7	1.64	

Part 1 – 4th Block
Data Table / Enzyme Concentration

Test Number	Average	1/t
1 (20%)	17.9	
2 (40%)	4.59	
3 (60%)	10.11	
4 (80%)	6.39	
5 (100%)	4.08	

Part 2 - substrate concentration

- #1 - We will be testing substrate concentrations of 2%, 1.5%, 1%, 0.8%, and 0.5%
- Each group will test only 1 concentration & share data with the class.
- You will need to MAKE a 60% catalase (potato) solution. Follow the "recipe" in the table from Part 1 on how to do this.

Part 2 – 1st Block
Data Table / Substrate Concentration

Test Number	Group Average	CLASS AVERAGE	1/t
1 (2%)	2 .74	1.37	
2 (1.5%)	7.70 6.31	7.01	
3 (1.0%)	5.3 8.1	6.70	
4 (0.8%)	5.72 7.66	6.69	
5 (0.5%)	16.1 .41	8.26	

Part 2 – 4th Block
Data Table / Substrate Concentration

Test Number	Group Average	1/t
1 (2%)	3.5	
2 (1.5%)	4.86	
3 (1.0%)	5.51	
4 (0.8%)	6.56	
5 (0.5%)	8.56	

Part 3 – pH influence on enzyme

1. Use the labeled pipettors in the solutions up front. DO NOT MIX THEM UP!
2. Use the "recipe" from part 1 to prepare your 60% catalase solution
   - 24mL potato enzyme + 16mL d.H_2O
   - Use the "catalase" plastic pipette at your station to measure 2mL
   - While you let the solution sit for 5 min…work on graphs from Part 1 & 2.
3. Use the plastic "H_2O_2" pipette at your stations. The top line is 1mL. Work on graphs while you let it sit for 5 min.

Clean up directions

1. Pour all liquids down the drain, but thoroughly rinse out the sink
2. Remove labels from all glassware at your station
3. Use SOAP & BRUSHES to wash out ALL glassware & graduated cylinders – even if you didn't use it (supplies at Lab Station 4)
4. Throw away plastic pipettes & paper plates
5. Stack everything neatly on your tray

Warnings Continued:

- Be sure to answer all the questions thoroughly in complete sentences that clearly indicate what the question is asking.
- Example:
  - Question: "Would the same enzymes that began the digestion of proteins in the stomach work as efficiently in the small intestine?"
  - Bad Answer: "No, they would work differently."
  - Good Answer: "Enzymes that digest proteins in the stomach would not work as efficiently in the small intestine because…."

CLEAN UP

- When you finish Part 3 **WASH** all glassware…use soap.
- Lay glassware against the back wall of your lab table on the towel to dry.
- Dispose of all plastic and paper products.
- Wipe down your lab table with a sanitizing wipe
- Don't leave any paper, pencils, miscellaneous objects behind.

Good Morning!

- Enzyme Lab
- 3 Line Graphs
  - Part 1: Enzyme Concentration vs. Reaction Time (1/t) – Class Average
  - Part 2: Substrate Concentration vs Reaction Time (1/t) – Class Average
  - Part 3: pH vs. Height of Solution/Foam