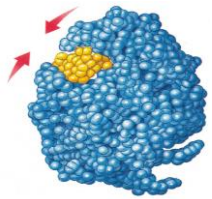
Wednesday, September 23rd

Today we will take a look at your first laboratory practical, **Inquiry into Enzyme Activity**.

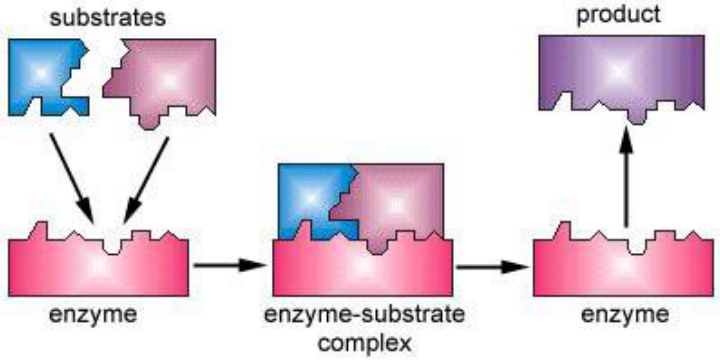
- Please be certain that you have the lab manual for this particular activity. **READ IT**!
- You will need to begin the pre-lab portion of your lab so you are not overwhelmed upon completion of this lab. Refer to your gold LAB EXPECTATIONS packet.
- **Thursday & Friday**: LAB DAYS
- LAB REPORTS are due **WEDNESDAY, September 30th.**
- You will use your report for your lab assessment at that time.



Inquiry into Enzyme Activity Lab

What is an enzyme?

- *Protein that speeds up the rate of a reaction*
  - Decreases the energy required to start rxn
  - Substrate specific
    - Only a molecule with a matching "shape" will bind with the enzyme that acts upon it



1. Catalytic: break down proteins (hydrolysis)
2. Anabolic: build molecules (condensation)

Inquiry into Enzyme Activity Lab

Objectives: Students will…

1. Understand the relationship between enzyme structure and function.
2. Make generalizations about enzymes by studying just one enzyme (peroxidase) in particular.
3. Determine factors that can change the rate of an enzyme reaction.
4. Determine which factors that affect enzyme activity could be biologically important.

Day 1 (Thursday), Procedure 1: Developing a Method for Measuring Peroxidase in Plant Material and Determining a Baseline

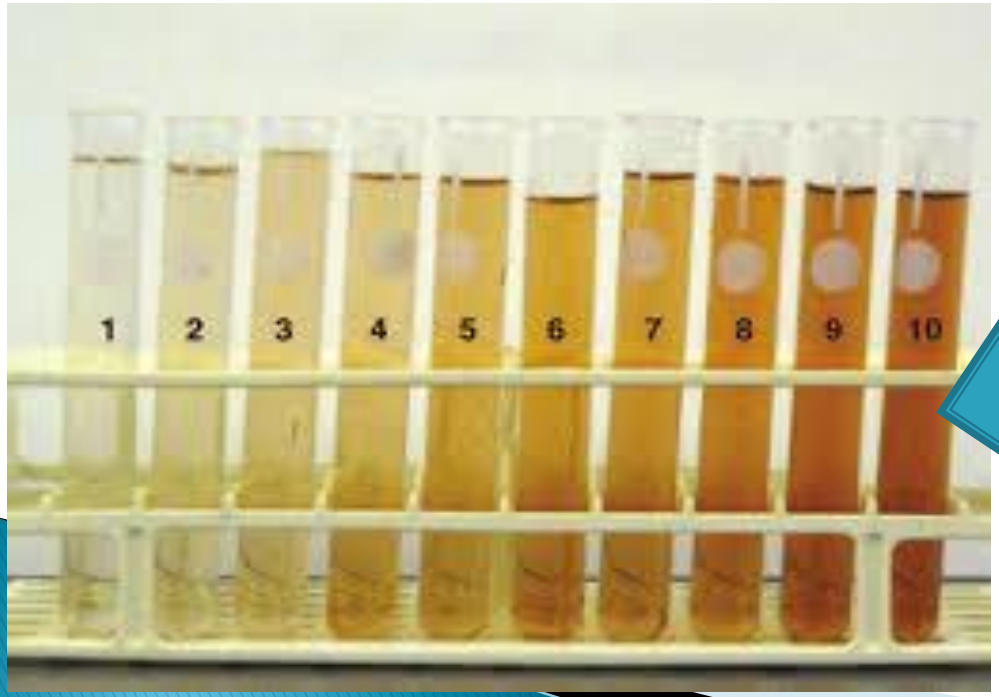
What is peroxide?

- *Toxic byproduct of cellular respiration (aerobic)*
- Peroxidase
  - Enzyme that breaks down H_2O_2 into usable water & oxygen

$$2\ H_2O_2 \xrightarrow{\text{peroxidase}} 2\ H_2O + O_2\ (\text{gas})$$

In this hydrolysis from H_2O_2 into water & oxygen…

- Use the release of oxygen as an **indicator** of peroxidase activity
  - **Guaiacol**
    - Bonds to free O_2
    - Turns a dark brown color as more O_2 "binds"



In which test tube do we see more oxygen released?

Inquiry into Enzyme Activity Lab

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Day 2 (Friday), Procedure 2: Determining the Effect of pH on Enzymatic Activity

What do you predict will occur if the pH in the reaction changes?

- pH in cells is typically **neutral, 7**
- Test your hypothesis by introducing peroxidase to various pH solutions

Remember how pH impacts the tertiary structure of a protein…?!?

Thursday, September 24th

Inquiry into Enzyme Activity: PROCEDURE 1

Objectives: Students will…

1. Understand the relationship between enzyme structure and function.
2. Make generalizations about enzymes by studying just one enzyme (peroxidase) in particular.
3. Determine factors that can change the rate of an enzyme reaction.
4. Determine which factors that affect enzyme activity could be biologically important.

Please have your lab manual handy. You will begin procedure 1 shortly. Please have out:

- Sheet of notebook paper
- Phone

Friday, September 25th

Inquiry into Enzyme Activity: PROCEDURE 2

Objectives: Students will…

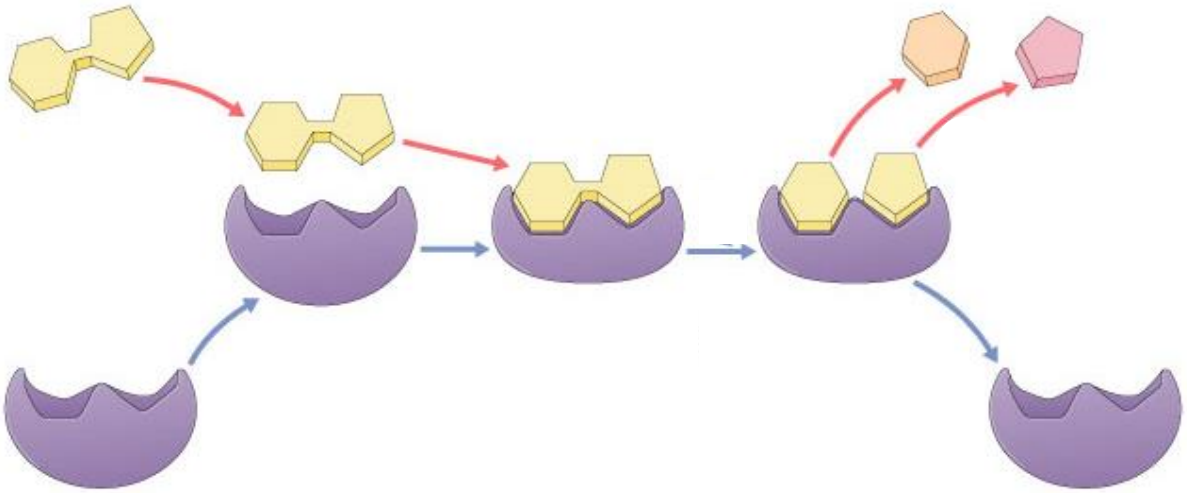
1. Understand the relationship between enzyme structure and function.
2. Make generalizations about enzymes by studying just one enzyme (peroxidase) in particular.
3. Determine factors that can change the rate of an enzyme reaction.
4. Determine which factors that affect enzyme activity could be biologically important.

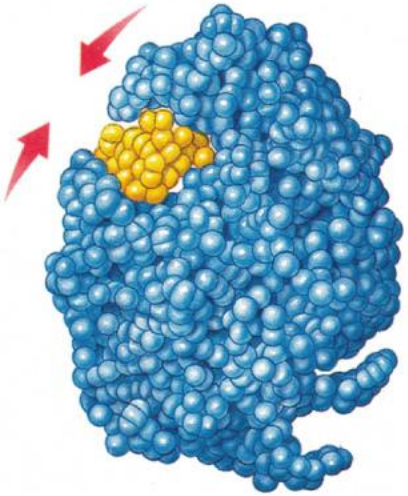
Please have your lab manual handy. You will begin procedure 2 shortly. Please have out:

- Sheet of notebook paper from THURSDAY
- Phone

REMINDER: Finished lab reports are due
WEDNESDAY, September 30th

Metabolism & Enzymes

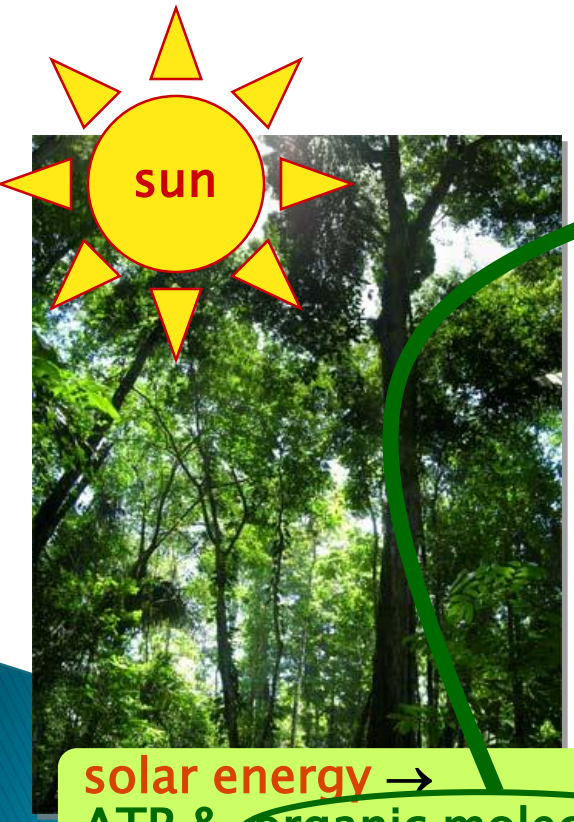




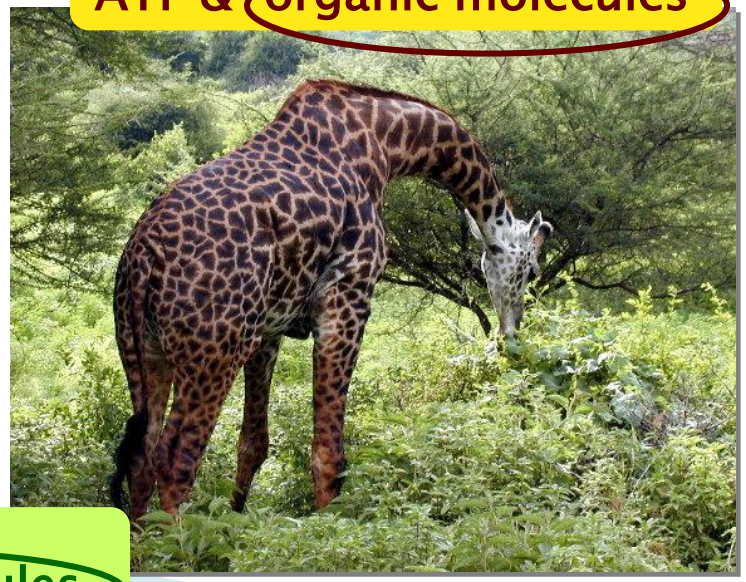
2007-2008

Flow of energy through life

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



solar energy → ATP & organic molecules



organic molecules → ATP & organic molecules

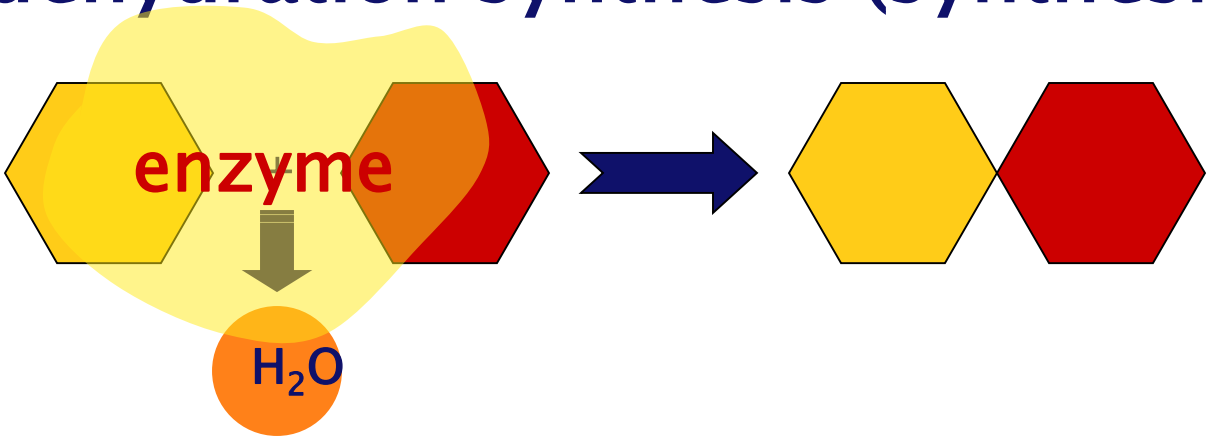
organic molecules → ATP & organic molecules

Metabolism

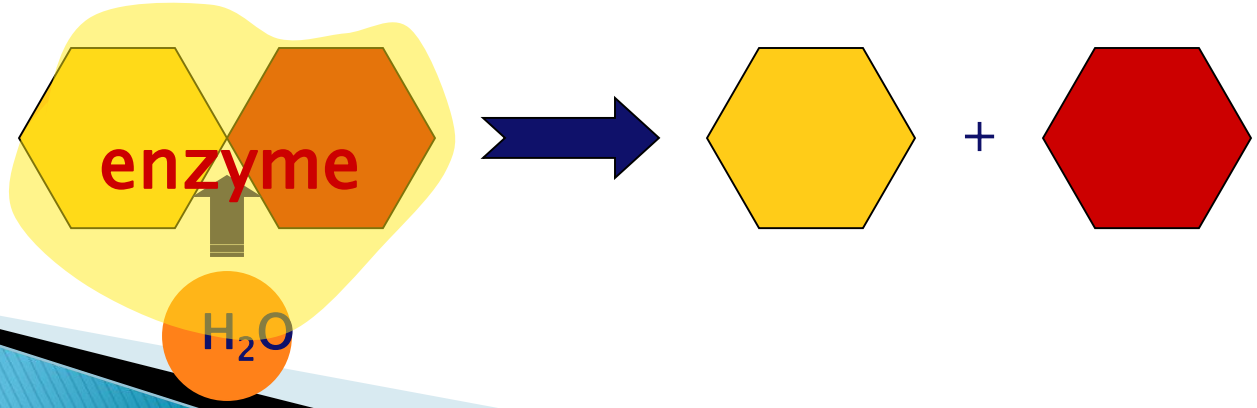
- Chemical reactions of life
  - FORMING bonds between molecules
    - dehydration synthesis
    - synthesis
    - anabolic reactions
  - BREAKING bonds between molecules
    - hydrolysis
    - digestion
    - catabolic reactions

Examples

- dehydration synthesis (synthesis)

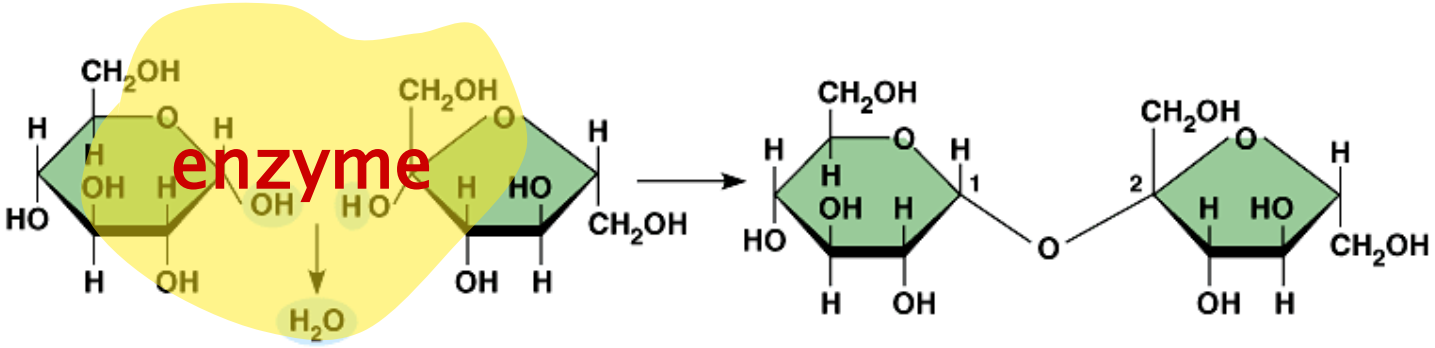


- hydrolysis (digestion)

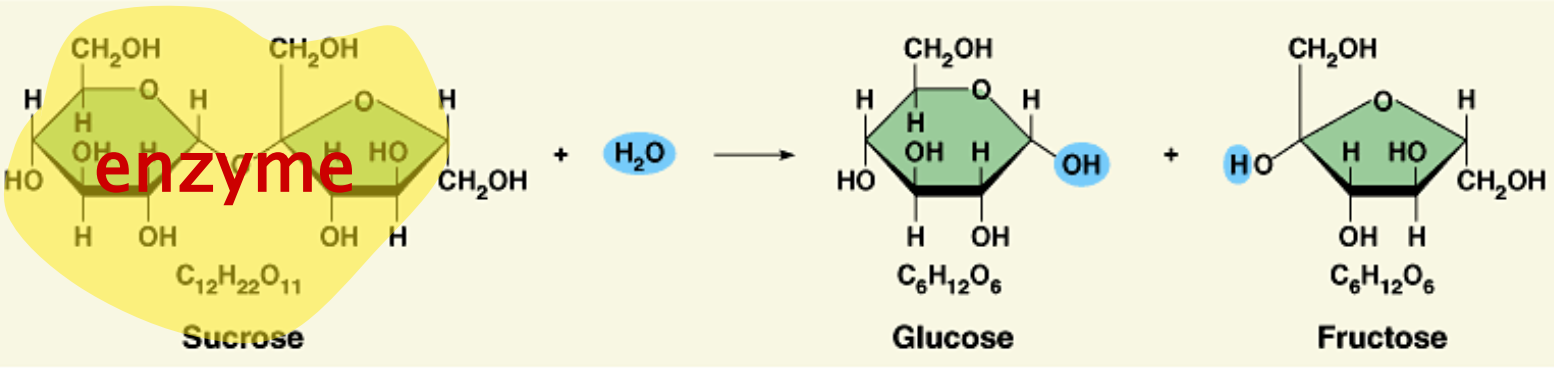


Examples

- dehydration synthesis (synthesis)

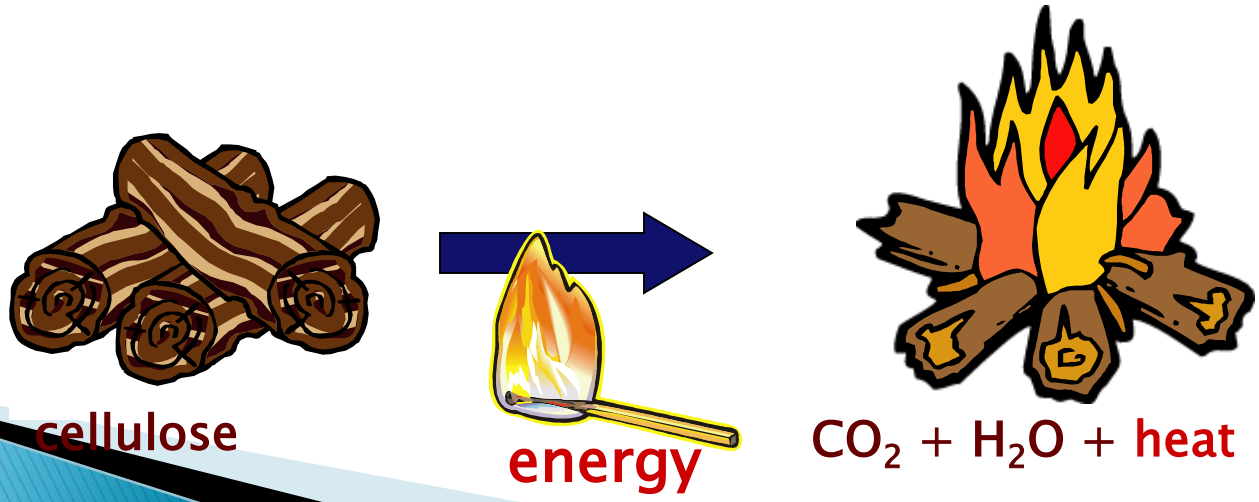


- hydrolysis (digestion)



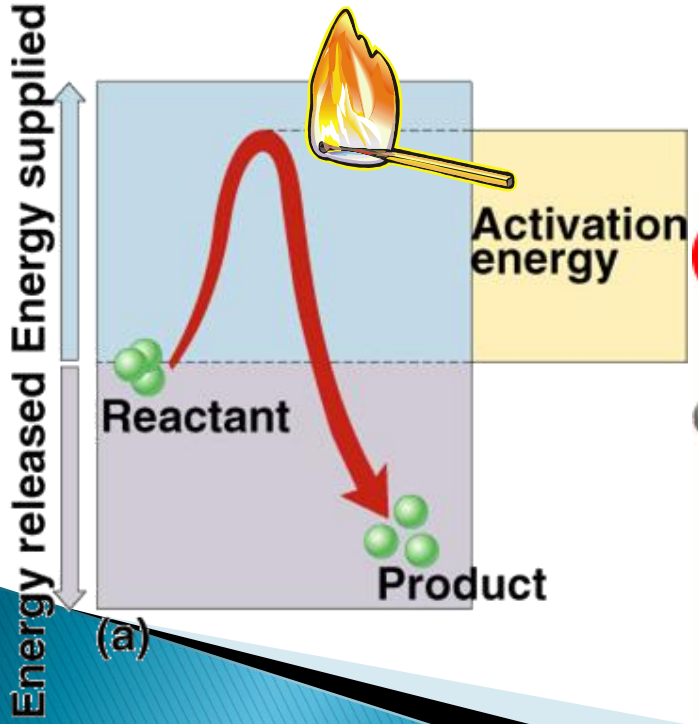
Activation energy

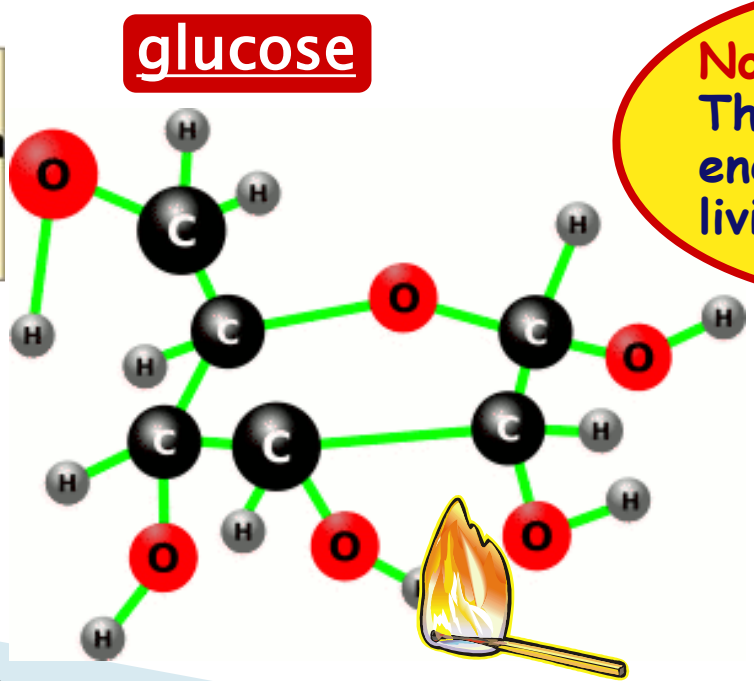
- Breaking down large molecules requires an initial input of energy
  - ACTIVATION ENERGY is required
    - b/c…large biomolecules are stable
    - must absorb energy to break bonds

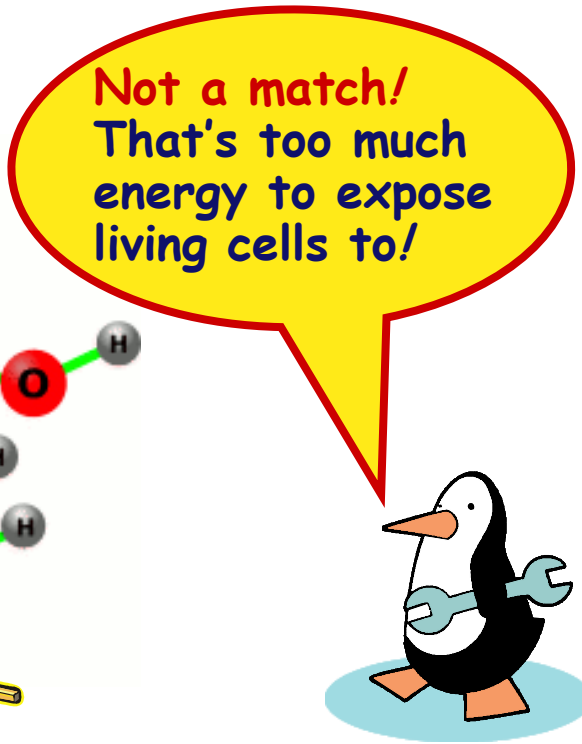


Activation energy

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

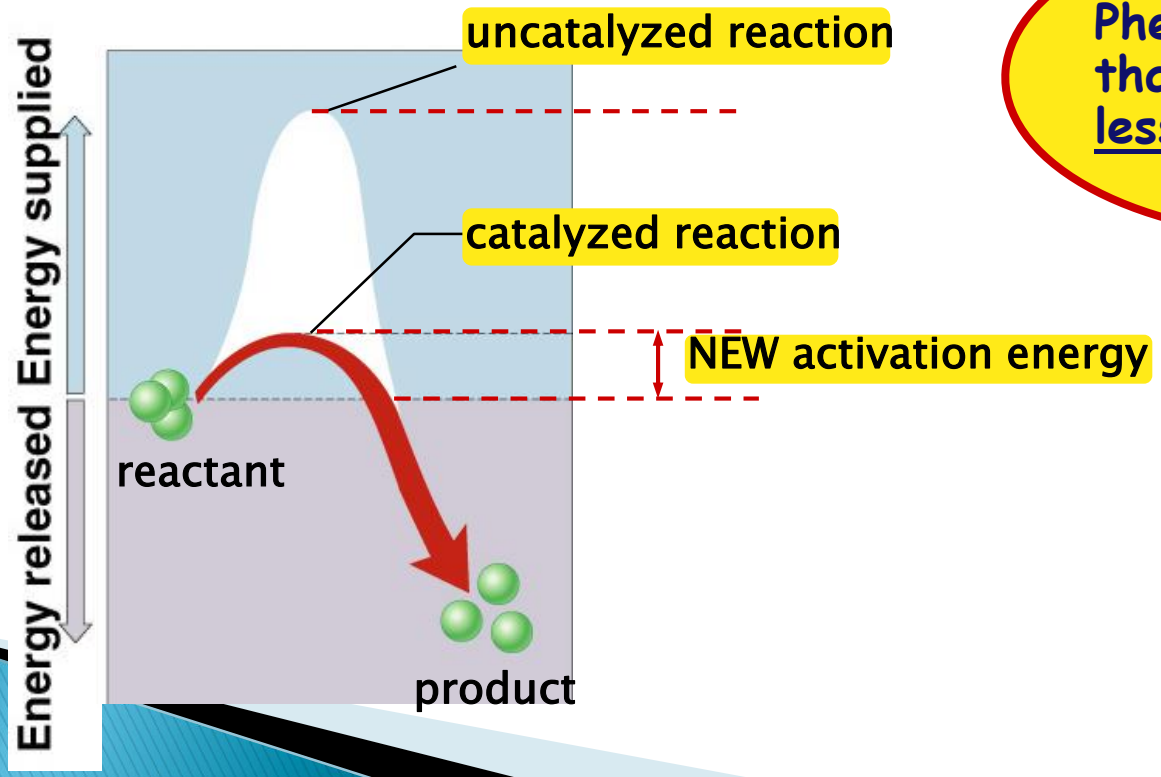


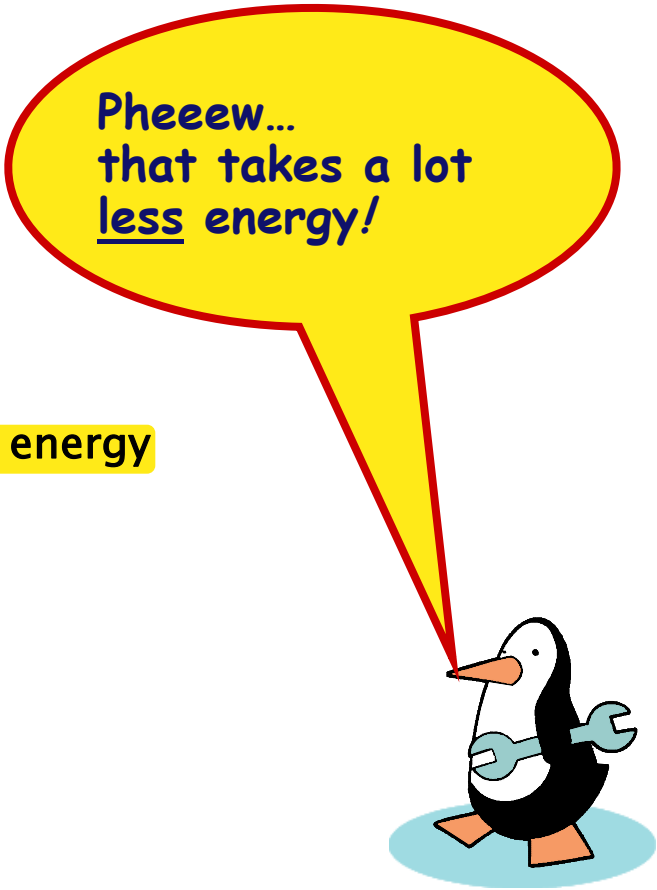




Reducing Activation energy

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

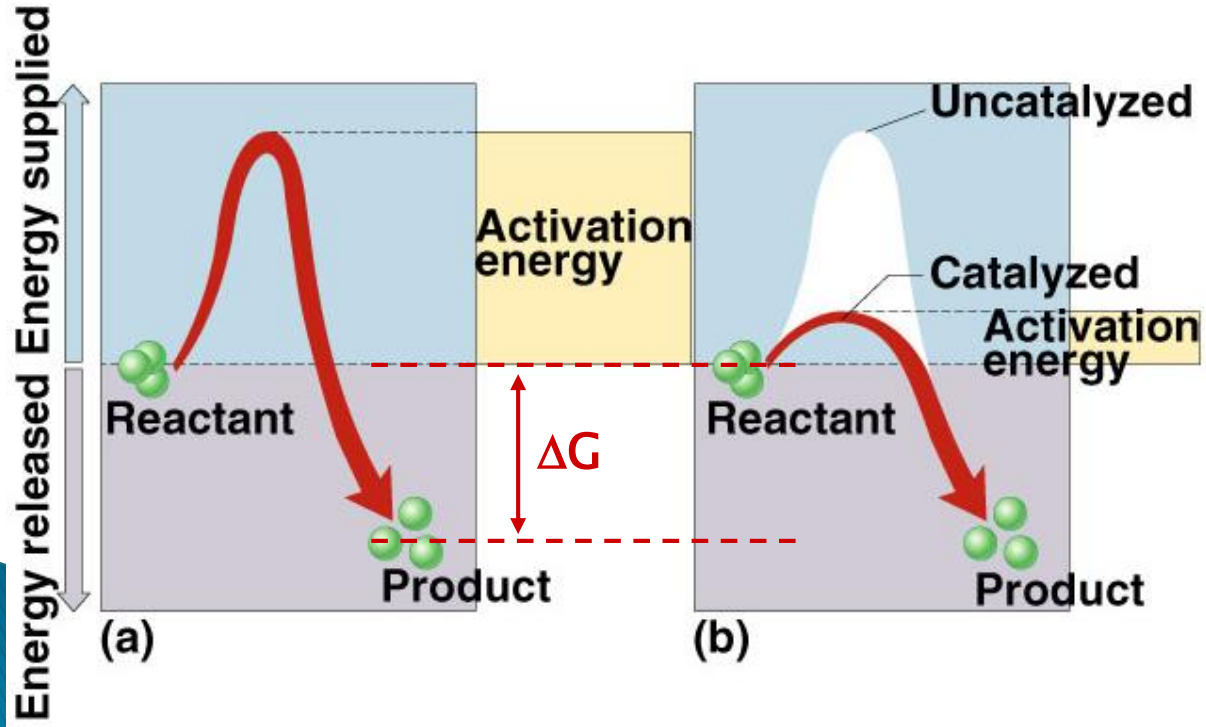


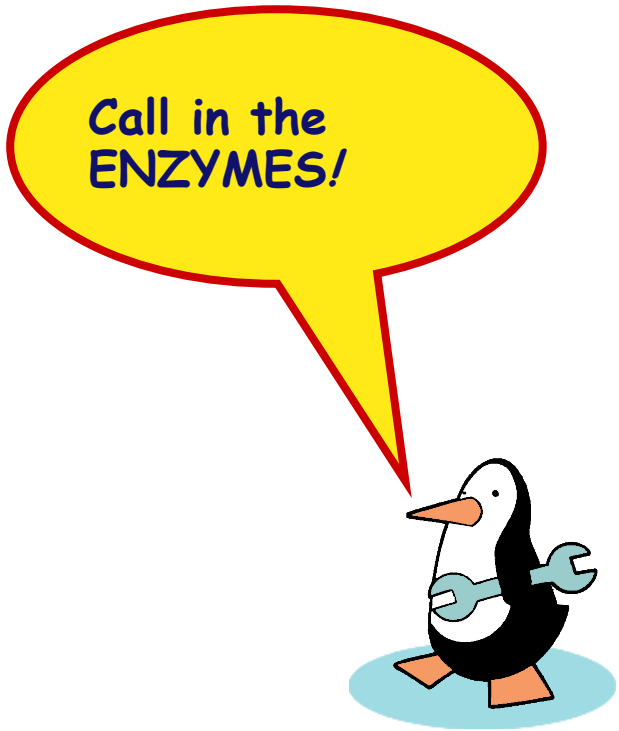


Catalysts

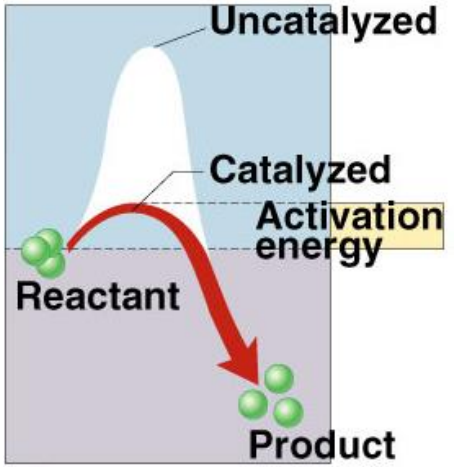
- So what's a cell got to do to reduce activation energy?
  - get help! … chemical help…

ENZYMES

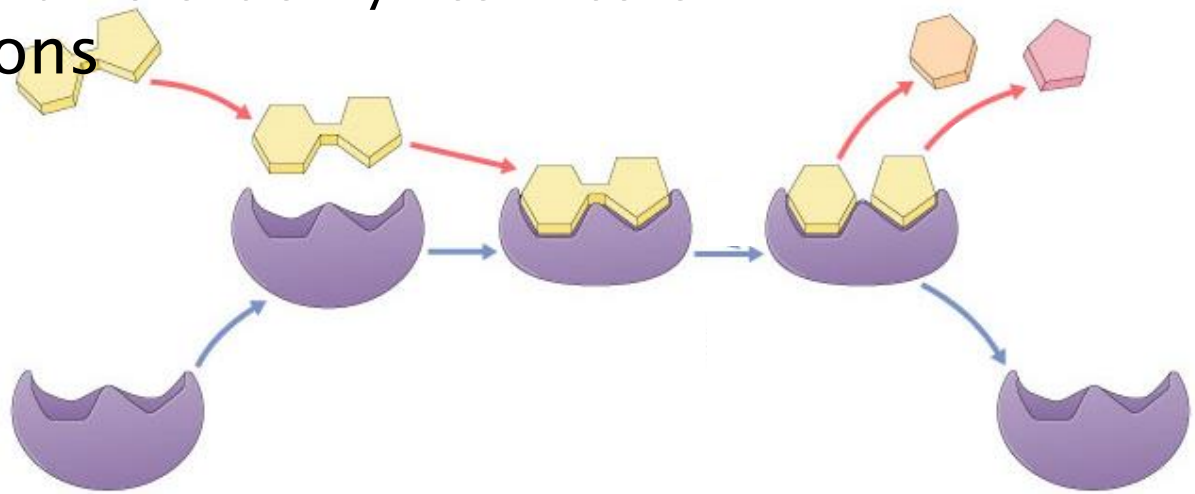




Enzymes



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



Enzymes vocabulary

substrate

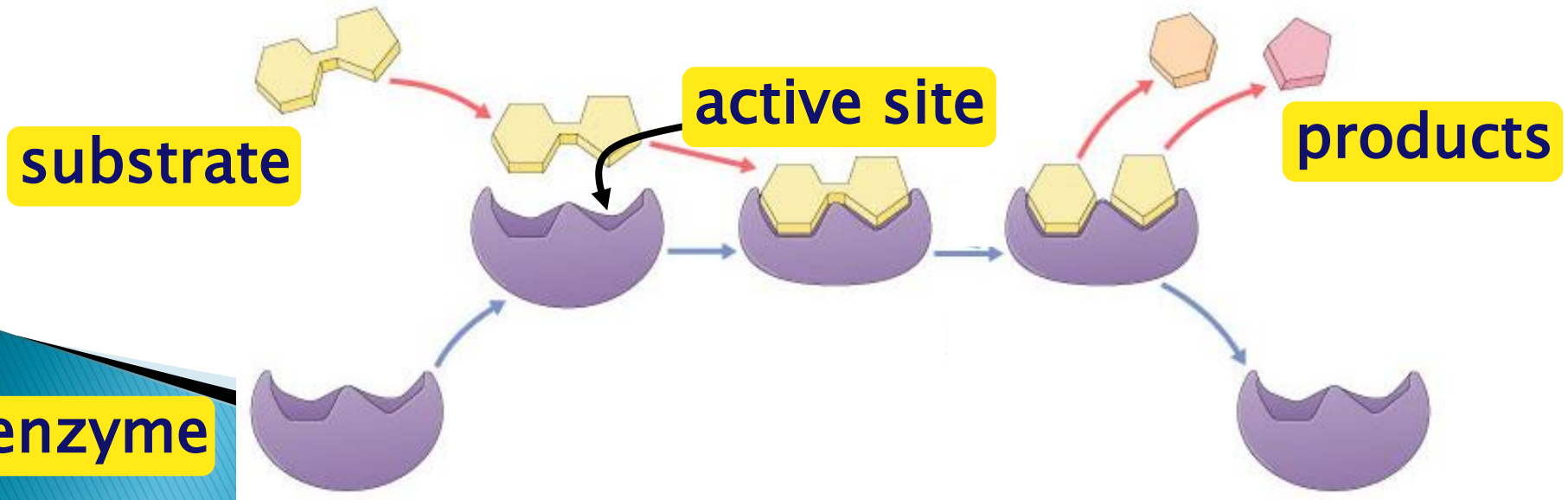
- reactant which binds to enzyme
- enzyme-substrate complex: temporary association

product

- end result of reaction

active site

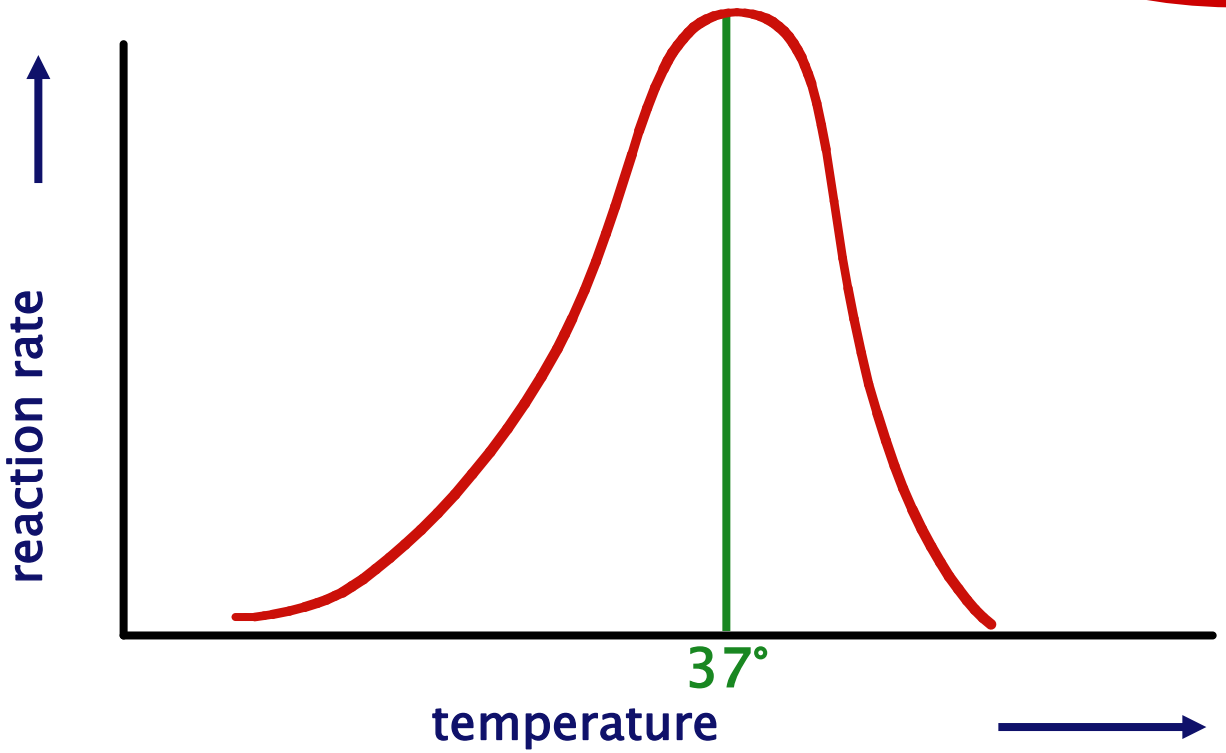
- enzyme's catalytic site; substrate fits into active site



Properties of enzymes

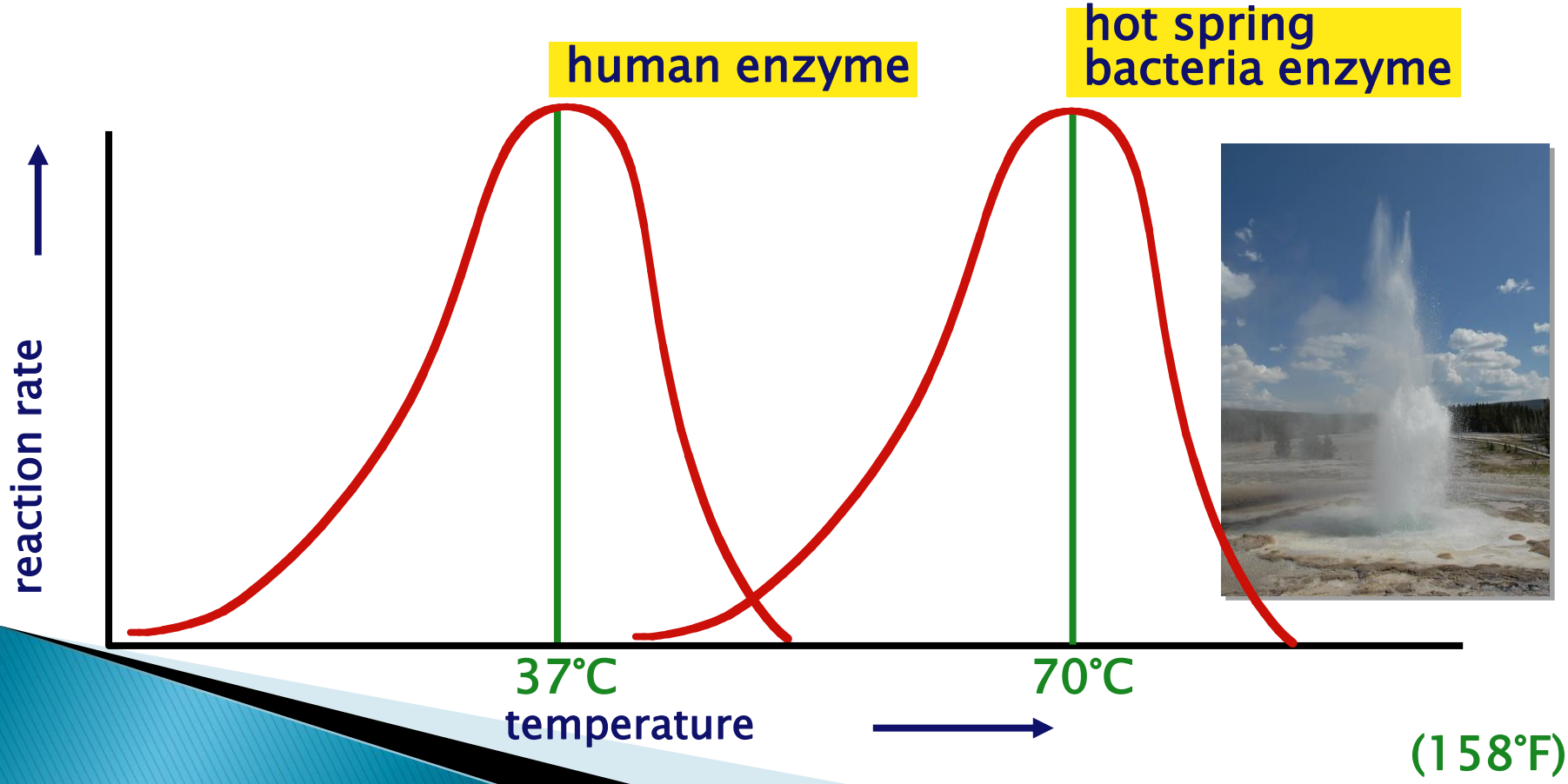
- Reaction specific
  - each enzyme works with a specific substrate
    - chemical fit between active site & substrate
      - H bonds & ionic bonds
- Not consumed in reaction
  - single enzyme molecule can catalyze thousands or more reactions per second
    - enzymes unaffected by the reaction
- Affected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

Temperature



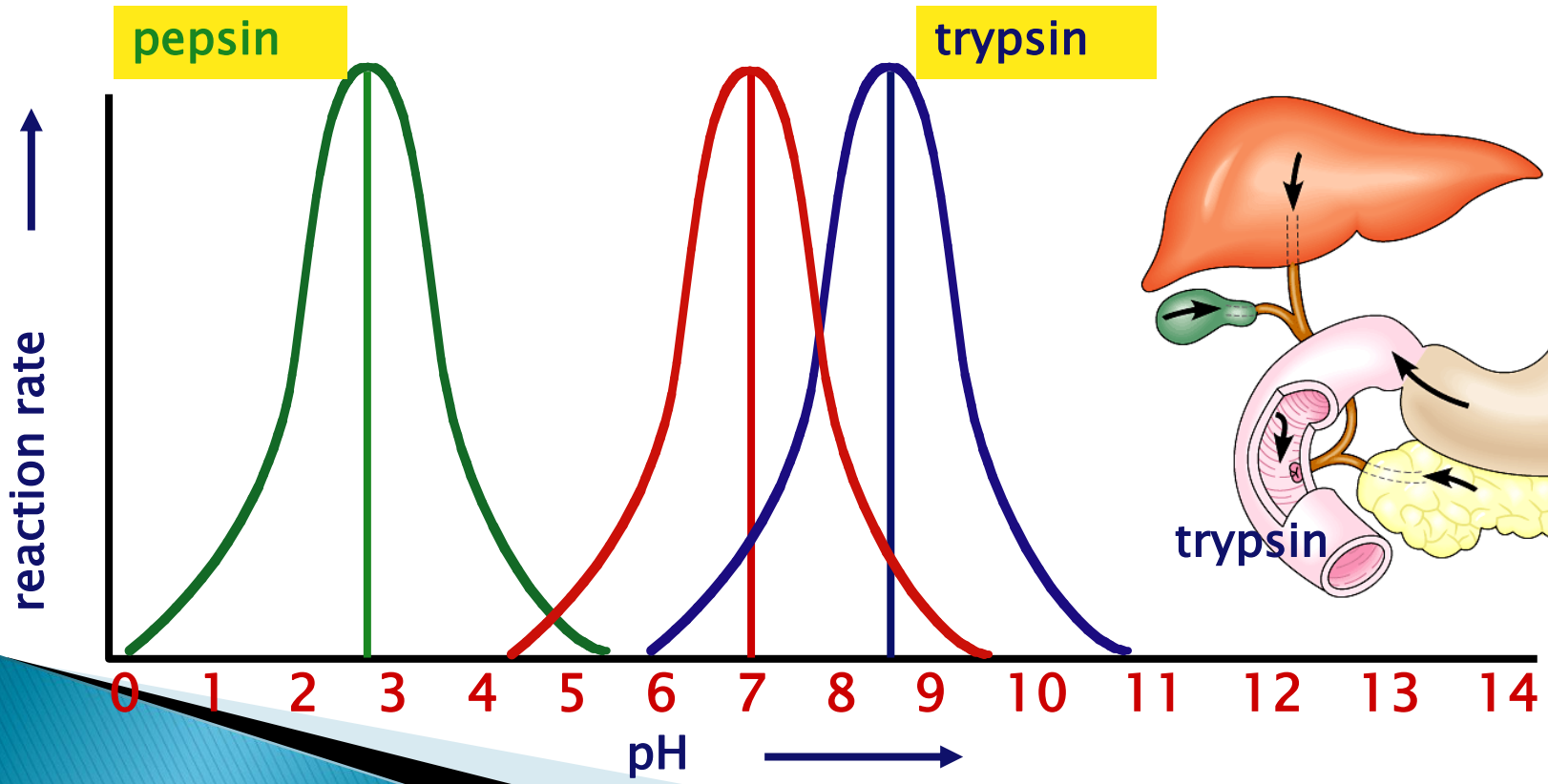
Enzymes and temperature

- Different enzymes function in different organisms in different environments



pH

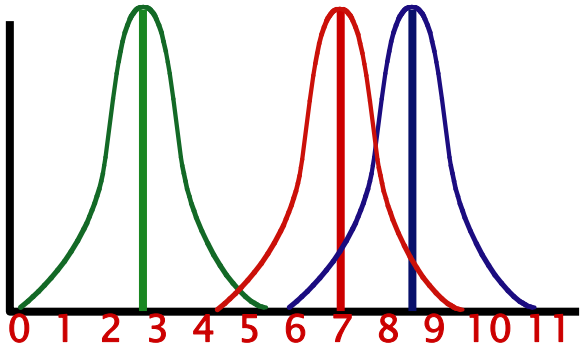

What's happening here?!


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

pepsin
trypsin

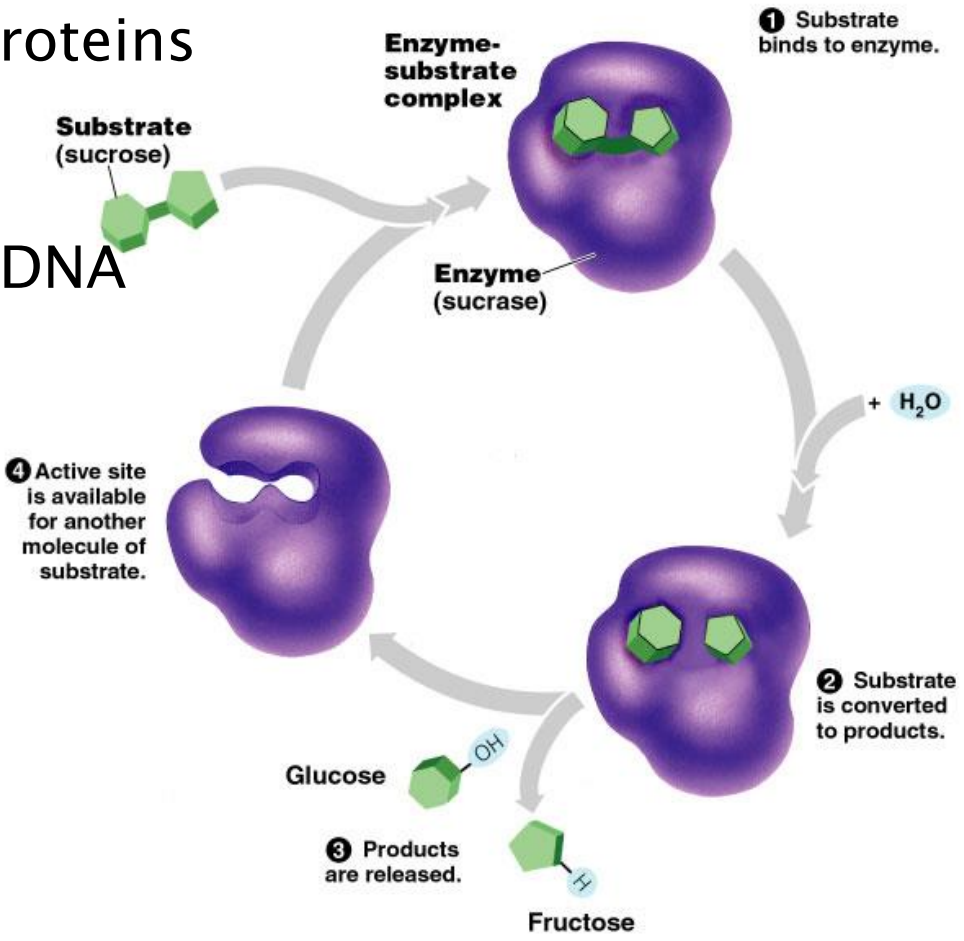
Factors affecting enzyme function

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



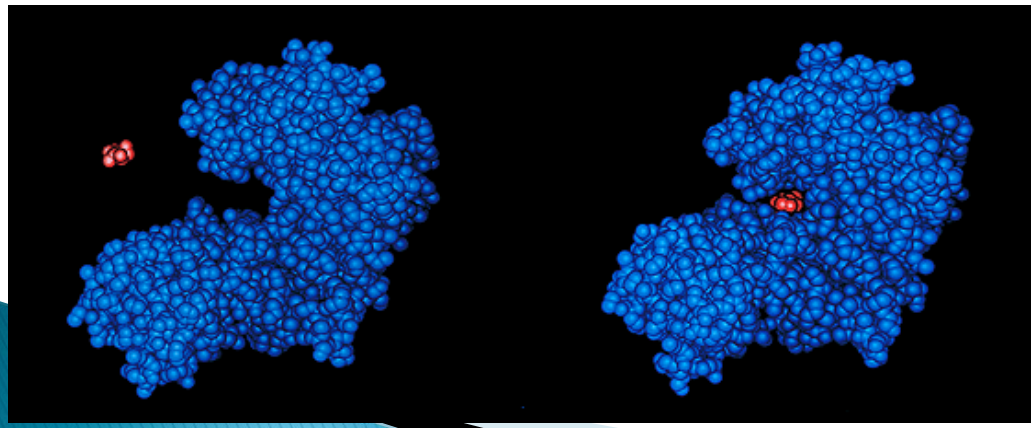
Naming conventions

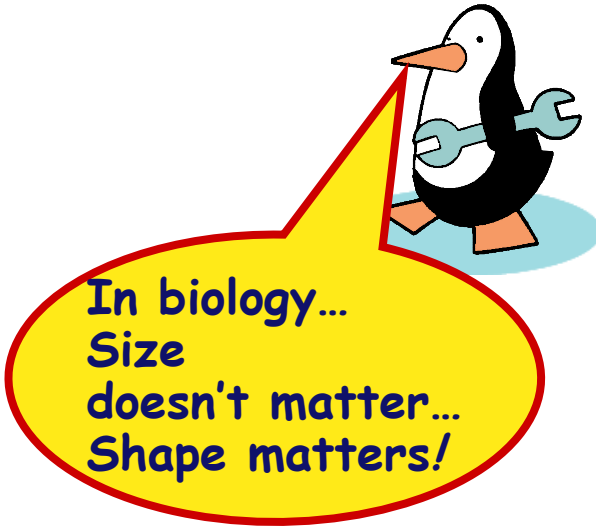
- Enzymes named for reaction they catalyze
  - sucrase breaks down sucrose
  - proteases break down proteins
  - lipases break down lipids
  - DNA polymerase builds DNA
    - adds nucleotides to DNA strand
  - pepsin breaks down proteins (polypeptides)

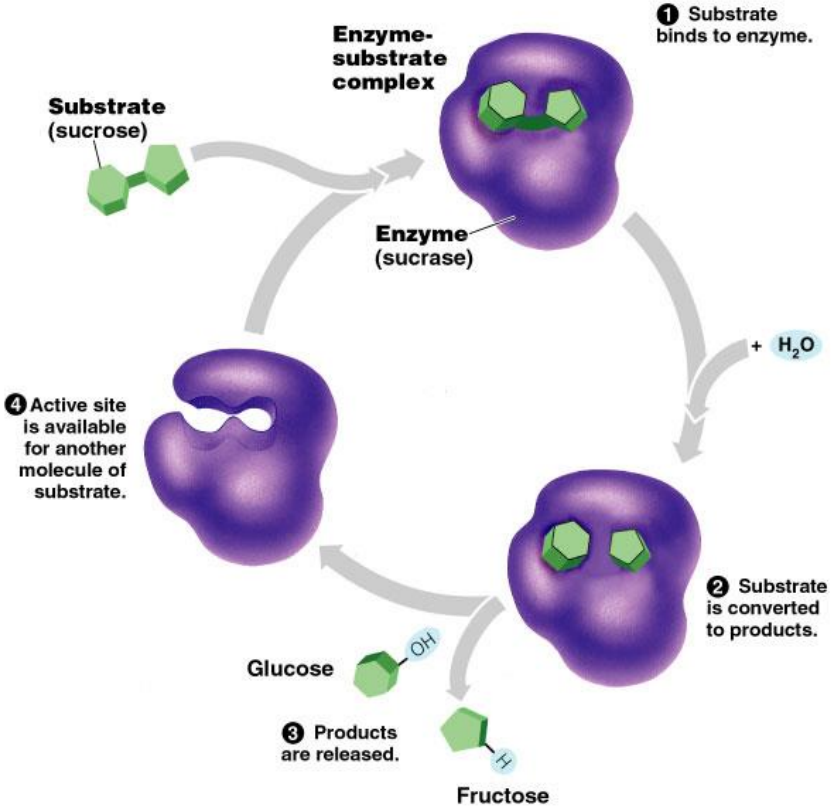


Lock and Key model

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

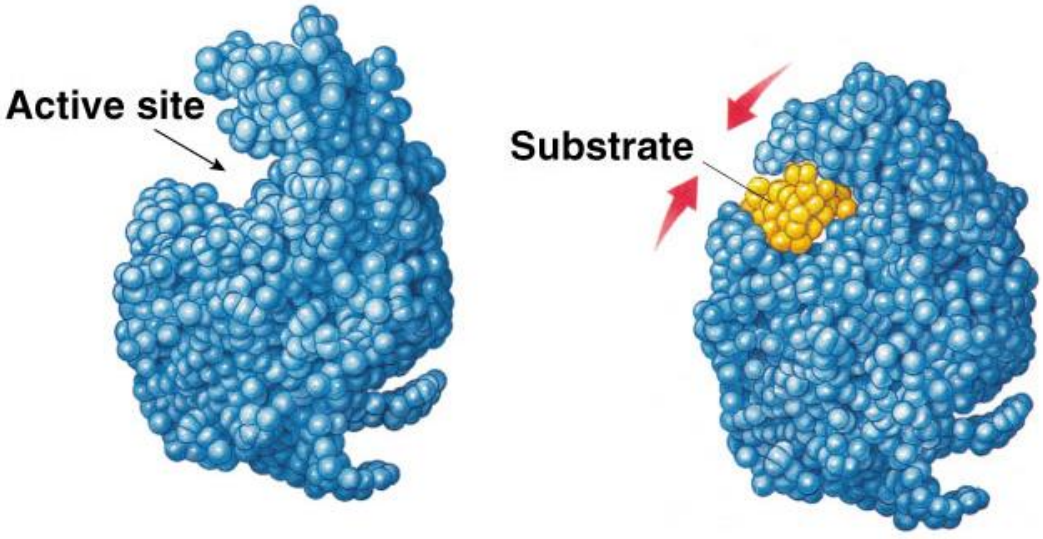






Induced fit model

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



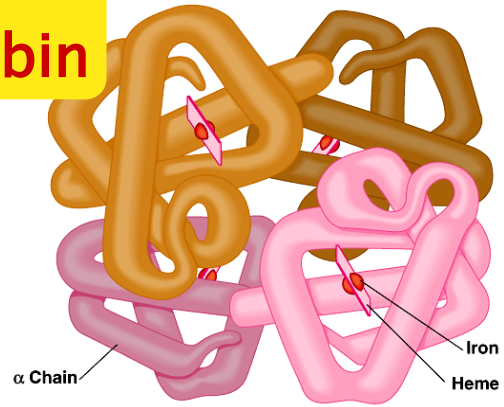
How does it work?

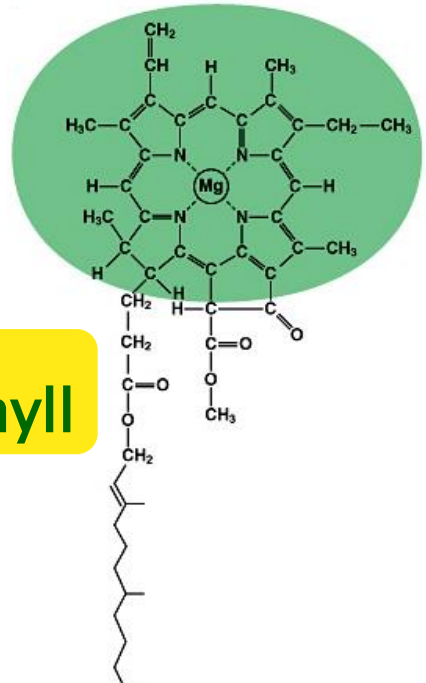
- Variety of mechanisms to lower activation energy & speed up reaction
  - synthesis
    - active site orients substrates in correct position for reaction
      - enzyme brings substrate closer together
  - digestion
    - active site binds substrate & puts stress on bonds that must be broken, making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

Compounds which help enzymes

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

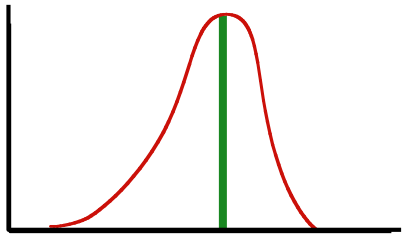
Fe in hemoglobin





Mg in chlorophyll

Factors affecting enzyme function



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

MR.W ENZYMES