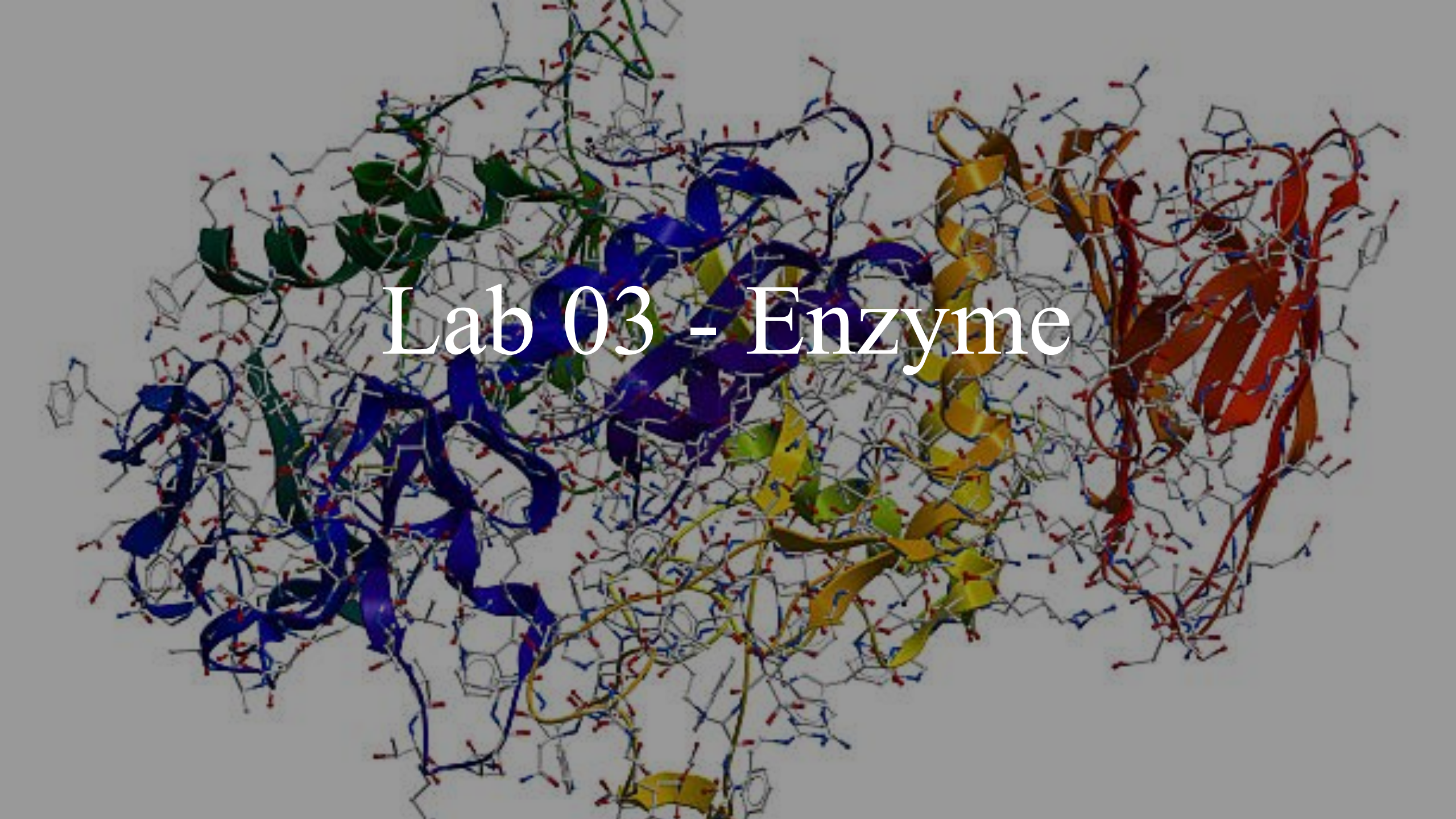


Lab 03 - Enzyme

Assignment tips

Formatting

- Turn in on Canvas as a .docx or .doc
- You can use the protocol as a template, just remove the actual protocol
- Everything should be typed. If I can’t read it, I can’t grade it
- When in doubt show your work
- If you don’t know how to do something, USE YOUR RESOURCES.

Safety

- Gloves, Goggles, Closed-toe shoes REQUIRED
- No food or drink in lab
- Work in groups of 4
- **All ONPG should be disposed of in the waste container on the side bench**
- ***E. coli* are an opportunistic pathogen**
  - **If you are immunocompromised, it is best that you step outside until the cells are lysed**
- Pipette tips are biohazard waste – dispose of them in the discard containers on your bench ONLY

Objectives

1) Measure enzyme activity.
2) Determine how changes in substrate and enzyme concentration affect enzyme activity.

Enzymes: An Overview

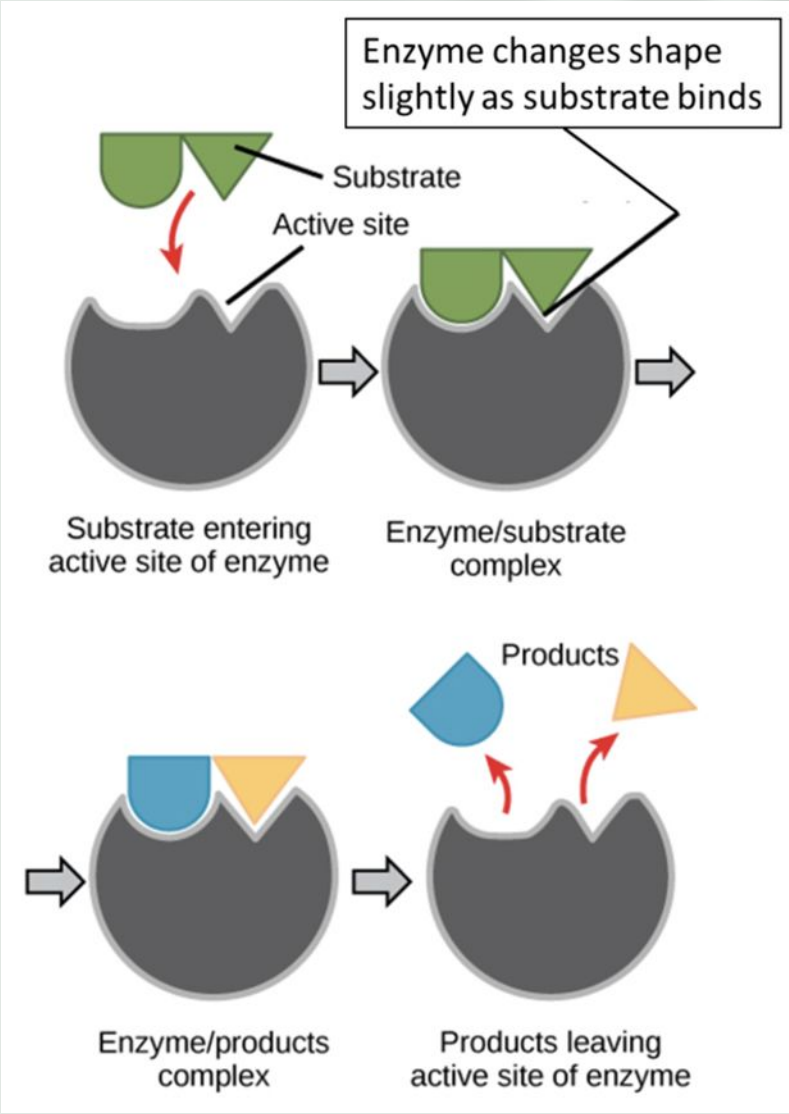
Enzymes are proteins that act as biological catalysts by accelerating chemical reactions.

The molecules upon which enzymes may act are called substrates, and the enzyme converts the substrates into different molecules known as products.

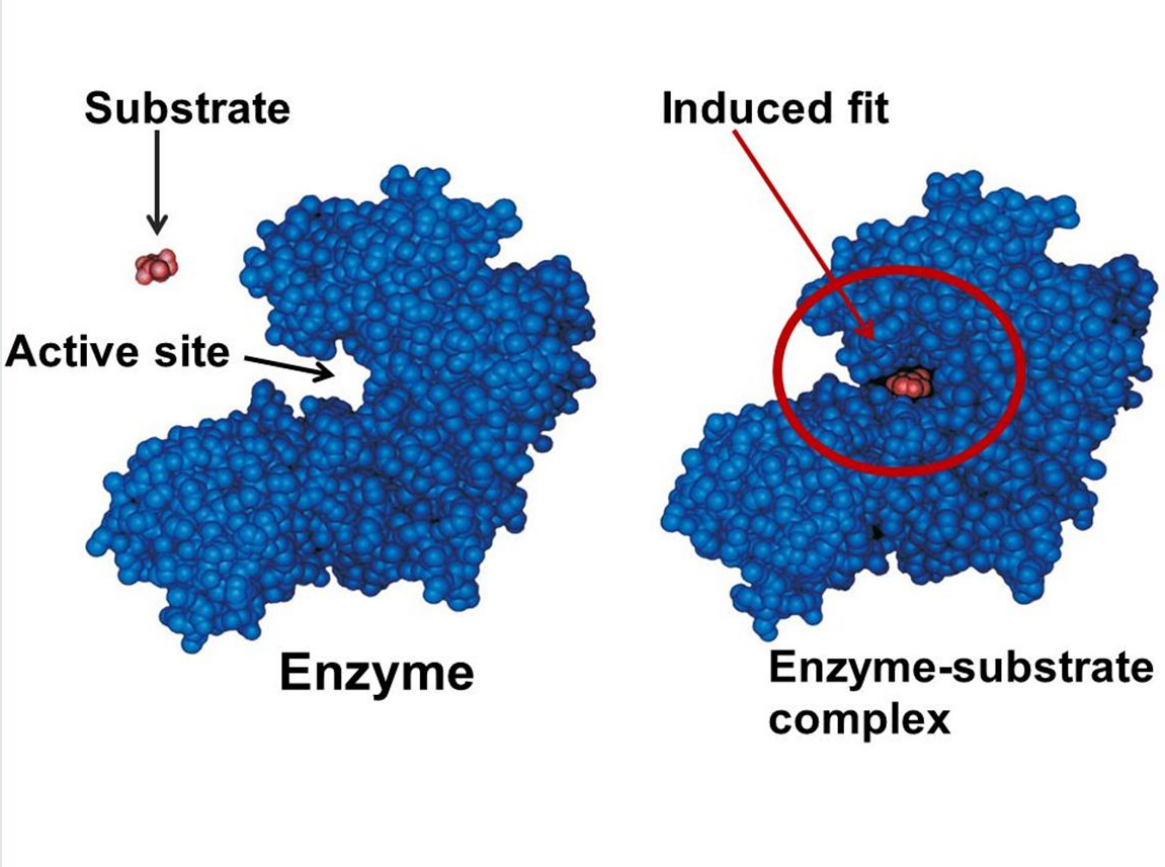
As the enzyme and substrate come together, their interaction causes a mild shift in the enzyme's structure that confirms an ideal binding arrangement between the enzyme and the transition state of the substrate.

When an enzyme binds its substrate, an enzyme-substrate complex is formed. This complex lowers the activation energy of the reaction and promotes its rapid progression.

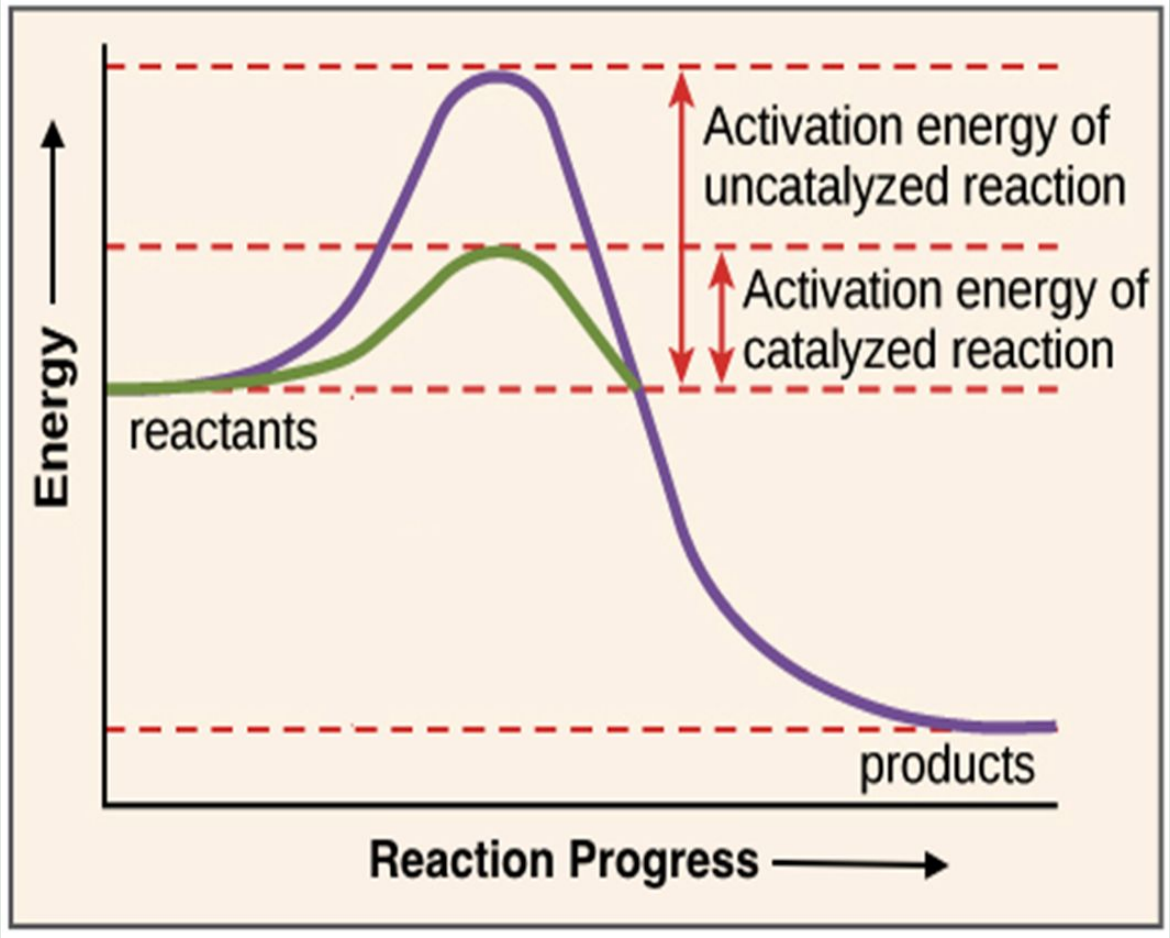
One of the hallmark properties of enzymes is that they remain ultimately unchanged by the reactions they catalyze. After an enzyme is done catalyzing a reaction, it releases its product(s).



Activation energy

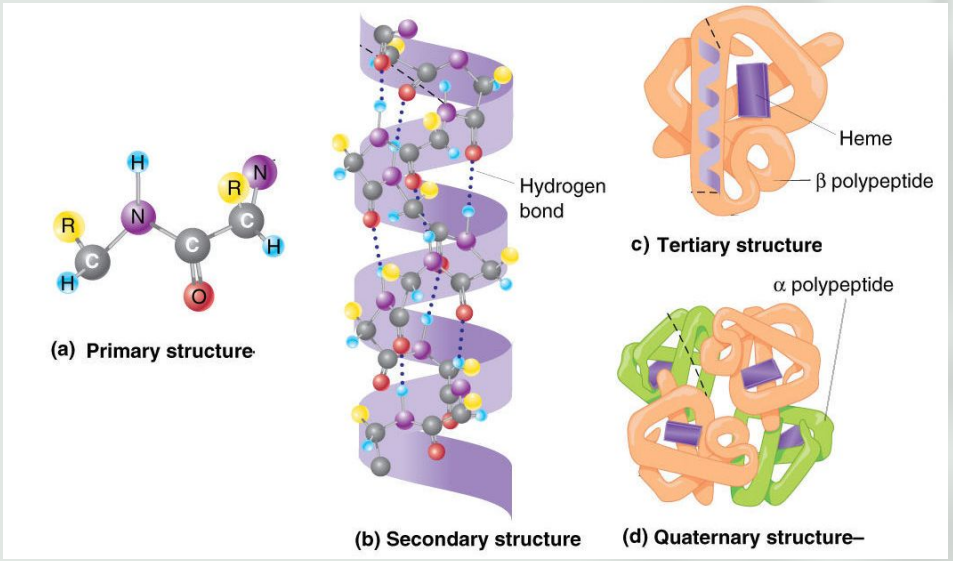


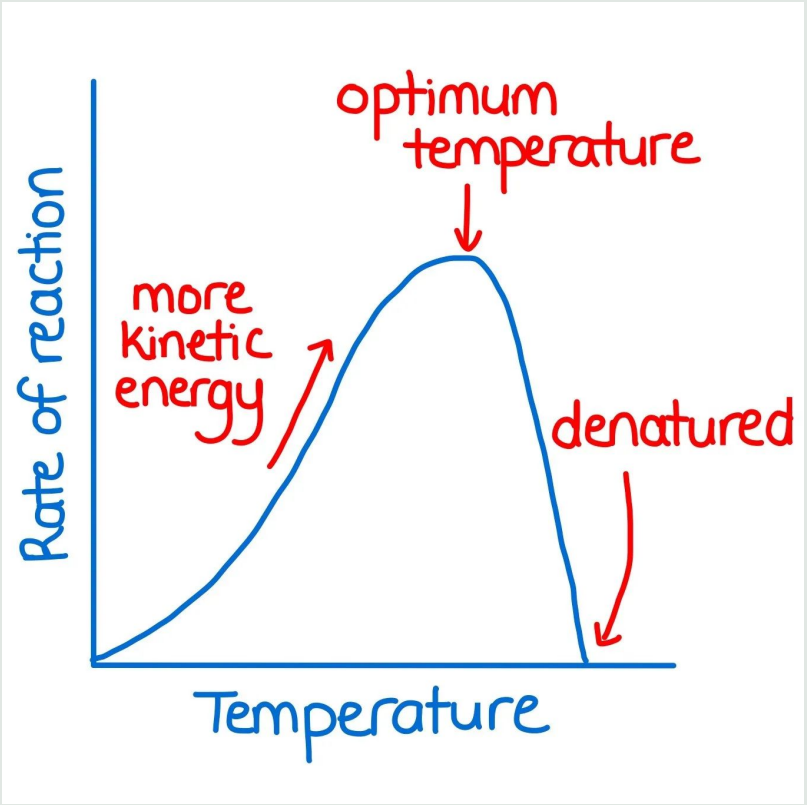
Activation energy

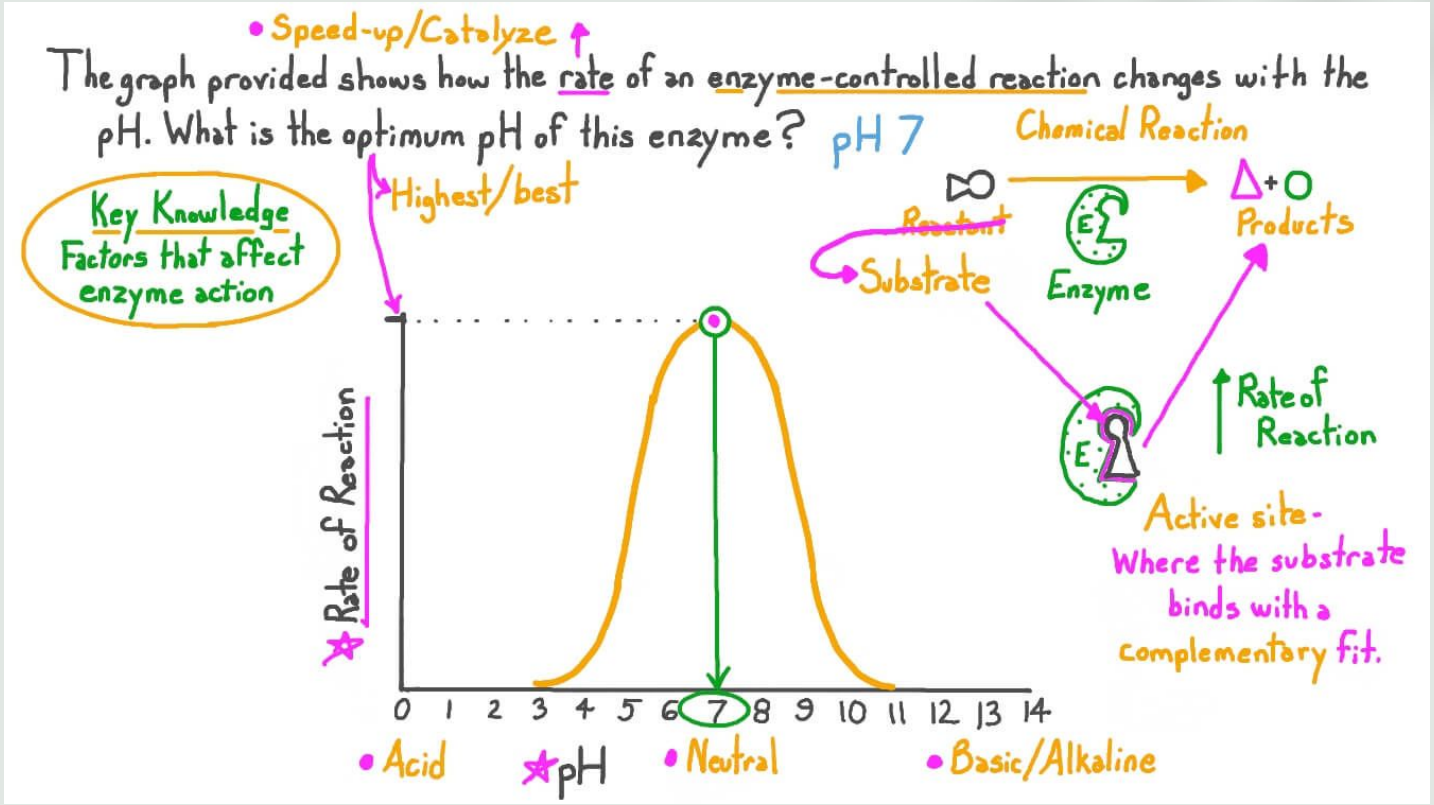


<u>Denaturing</u> = an enzyme or unfolding it causes the enzyme to lose functionality.

Extreme conditions (**pH** and **temperature**) can affect the structure of an enzyme.

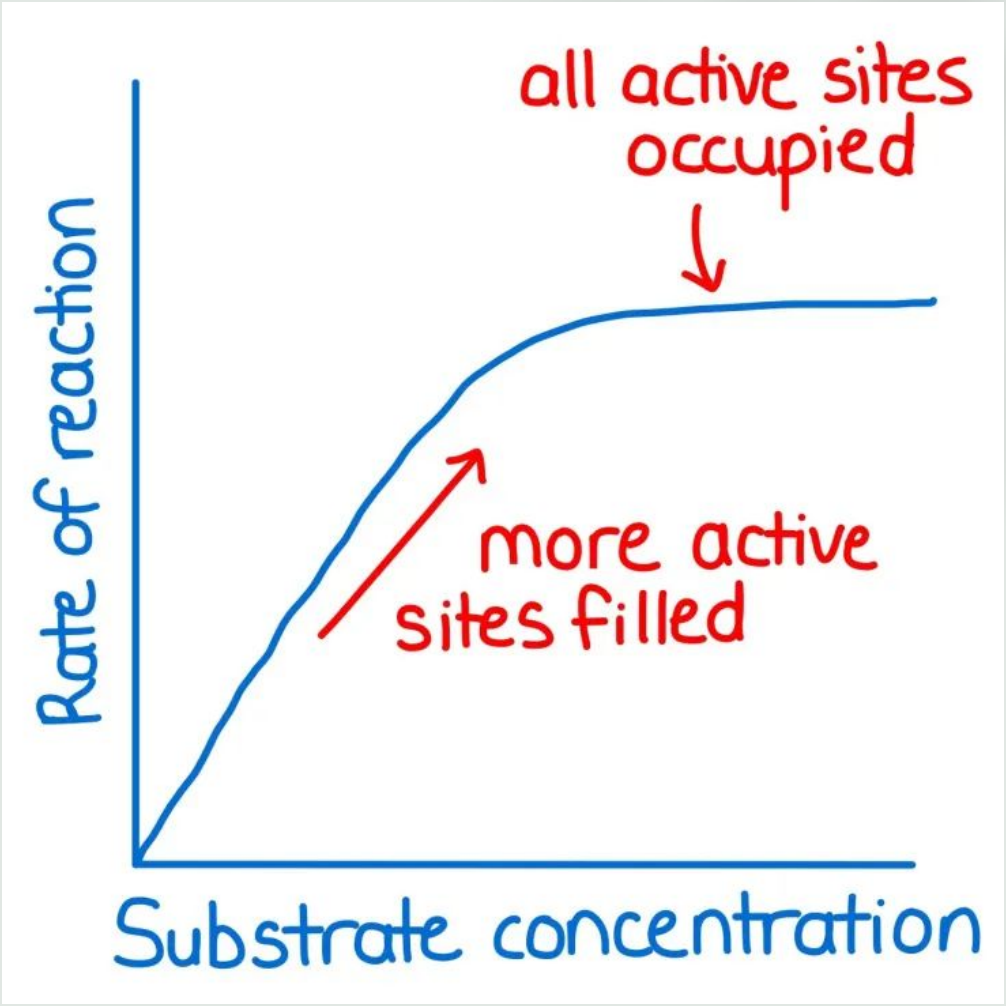






Lab Activity 1: What are the effects of changing <u>substrate concentration</u> on enzyme activity?

- Given enzymes are unchanged by the reactions they catalyze
  - Repeated hydrolysis of substrate at a distinct rate
  - Dependent on enzyme and substrate coming together (Diffusion)
  - Concentration increases the probability of enzyme and substrate coming together
- After hydrolysis substrate is permanently changed
  - Therefore, substrate is limited
  - Reactions times must be limited before substrate is fully consumed.



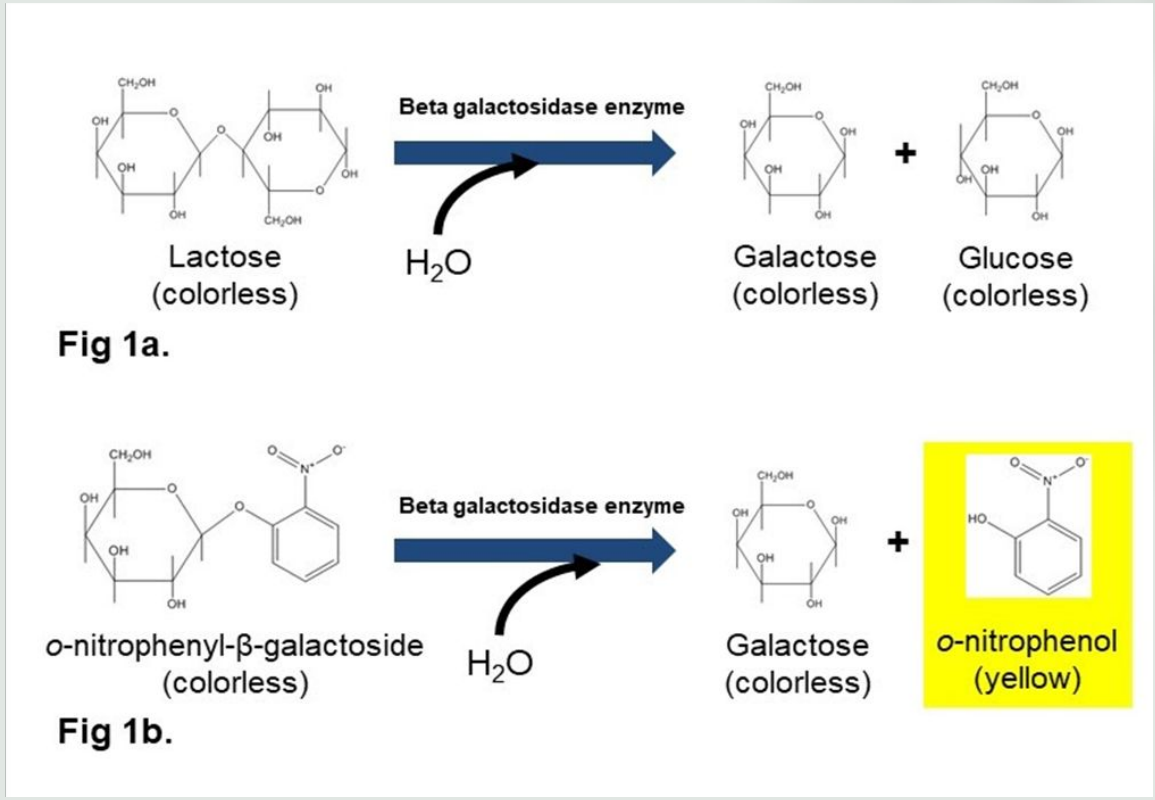
Our Experiment

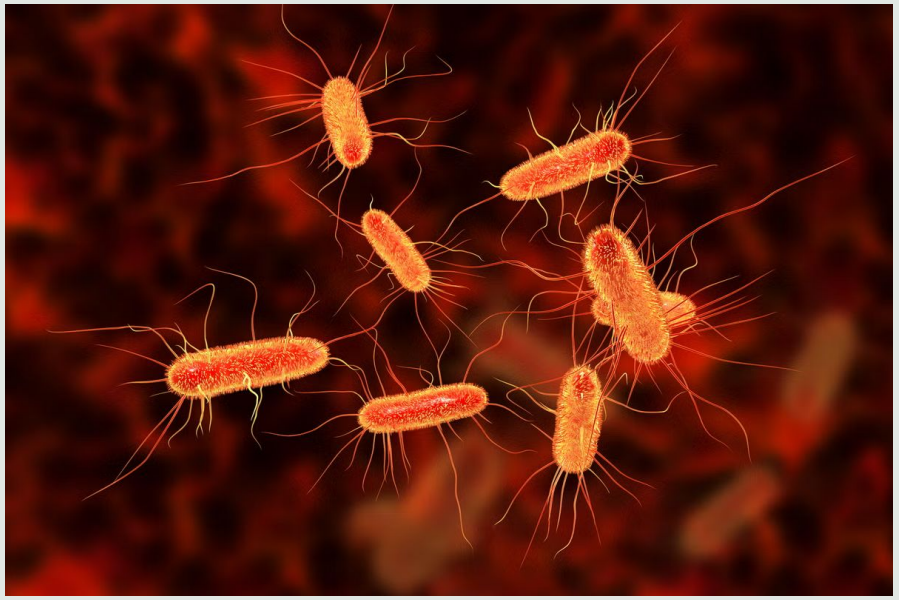
β-Galactosidase (β-Gal):
is an enzyme produced by Escherichia coli (E. coli) that catalyzes the hydrolysis of the disaccharide lactose to the monosaccharide's galactose and glucose (metabolized by cells).

Although lactose is the natural substrate for β-gal, β-gal will hydrolyze other molecules that have the relevant bond in the same place as lactose (other β-galactosides).

Ortho-nitrophenyl-β-galactoside (ONPG), is cleaved by β-gal into galactose and ortho-nitrophenol.

ONPG and galactose are colorless, but **ortho-nitrophenol is a bright yellow with an absorption wavelength of 420 nm.**

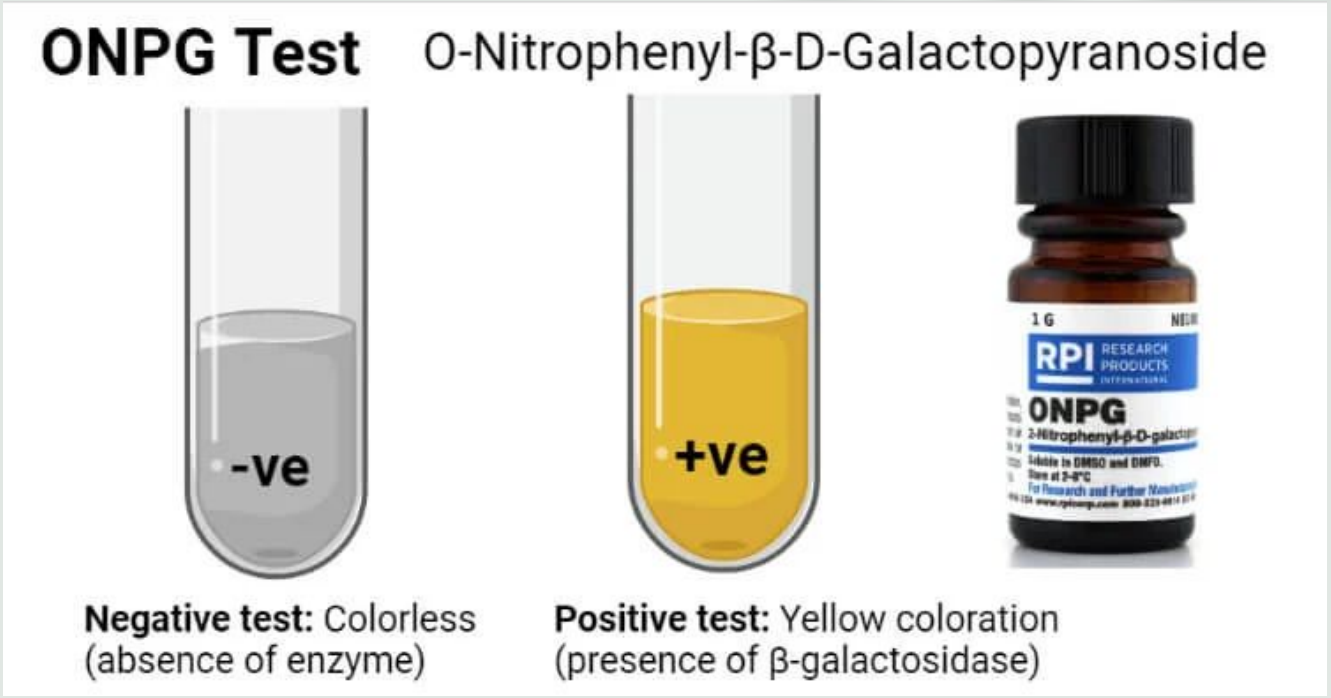




E.Coli

Lysis buffer

Z buffer



Homework Help

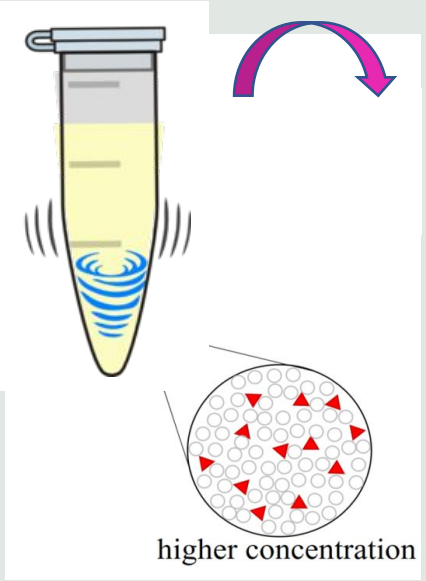
Rxn #	ONPG in Rxn	Volume of 4mg/mL ONPG Stock	Enzyme (E. coli) Volume	Lysis Reagent	Z Buffer to bring to 1mL Total	Concentration of ONPG in Tube (M)
1	.1mg	25uL	30uL	10uL	935uL	3.3 x10-4 M
2	.2mg	50uL	30uL	10uL	910uL	
3	.4mg	100uL	30uL	10uL	860uL	
4	.8mg	200uL	30uL	10uL	760uL	
5	1.2mg	300uL	30uL	10uL	660uL	
Blank	.4mg	100uL	30uL (water)	10uL	860uL	

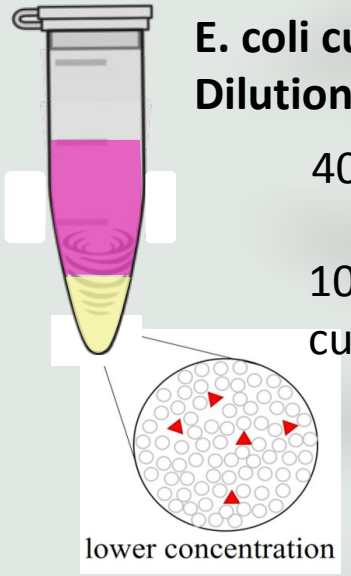
Molar Concentration Calculations:

1. Convert mass units from mg to g (divide by 1000mg)
2. Divide converted mass by molecular weight of 301 g/mol (to get moles)
3. Divide moles by reaction volume (1.0 mL=0.001L) to get molar concentration

Dilution

- Dilute the E. coli culture 1:5 by pipetting 100 µL of E. coli culture into a microcentrifuge tube. Add 400 µL of water, close the cap and mix well.
- Spectrophotometer

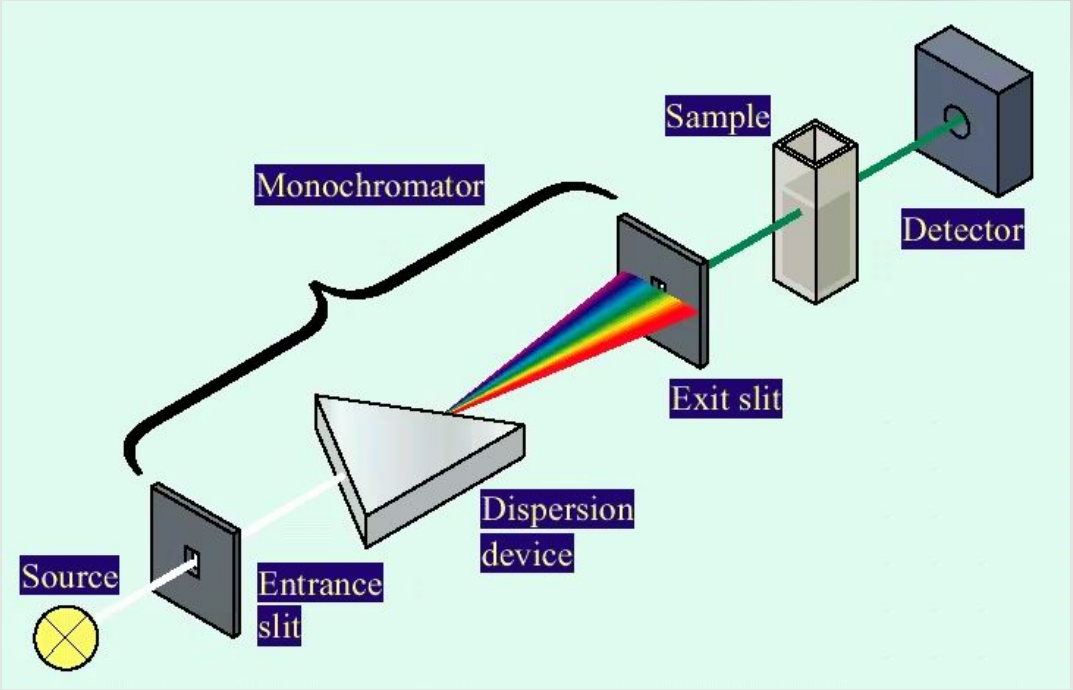




E. coli culture 1:5 Dilution

400 µL of water

100 µL of E. coli culture



Experimental setup

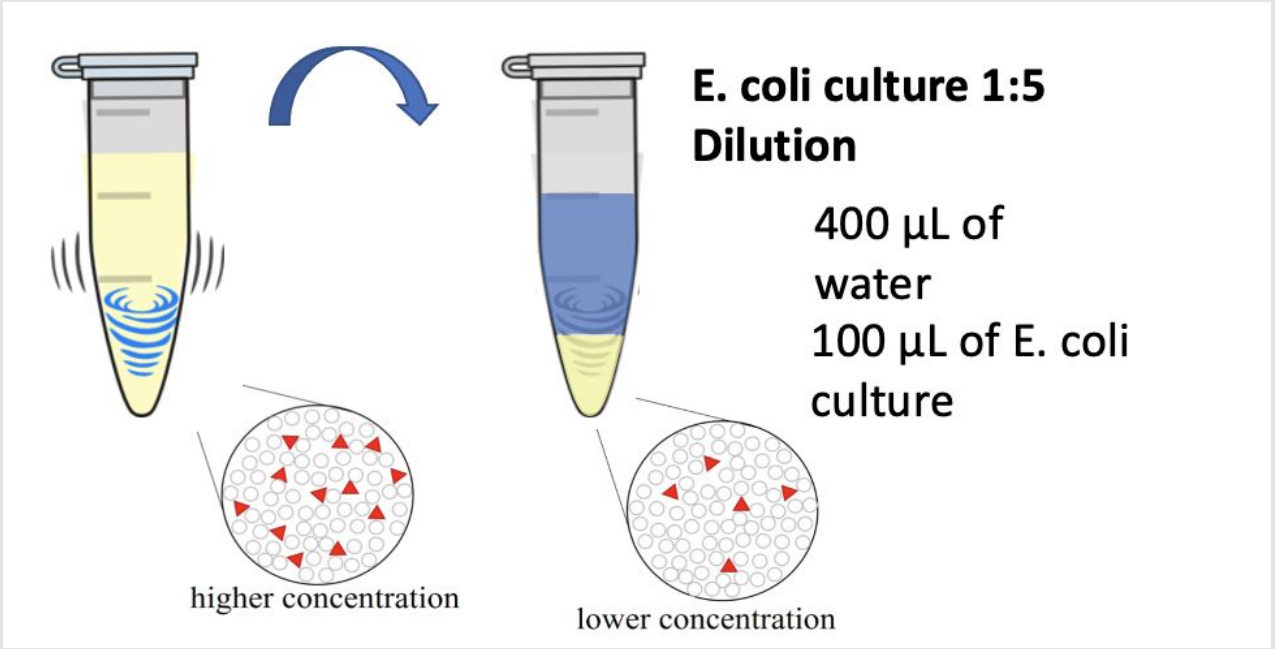
1) E. coli is diluted (100uL of E. coli & 400uL of H20)
2) By Table 1 – add E. coli into each (6) microcentrifuge tubes, then add lysis reagent to each. Wait 10 min for lysis – then place on ice.

   Add Z buffer into 6 cuvettes(by table 1)

   Add contents of lysis tubes to corresponding cuvettes.

3) Add ONPG (by table 1) to one tube at a time, mix, and record start time (counting). Complete for all tubes.
4) When color matches yellow post-it note, record stop time and add 500uL 1M sodium carbonate. Mix.
5) When all tubes are finished, measure A420 on spectrophotometer.

Visual Protocol

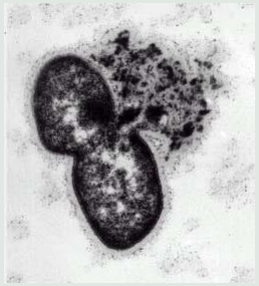


10 min

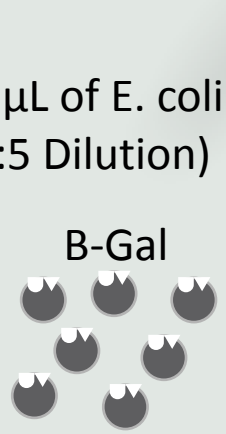
Lysis of E.coli

10 µL of Lysis Reagent

30 µL of E. coli (1:5 Dilution)



B-Gal

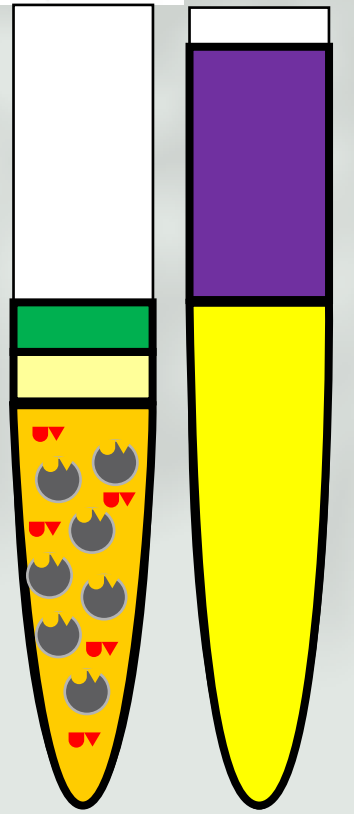


Lysis of a dividing pair of E. coli cells in the presence of an antibiotic.

StopWatch Counts up

1.0 mL sodium carbonate

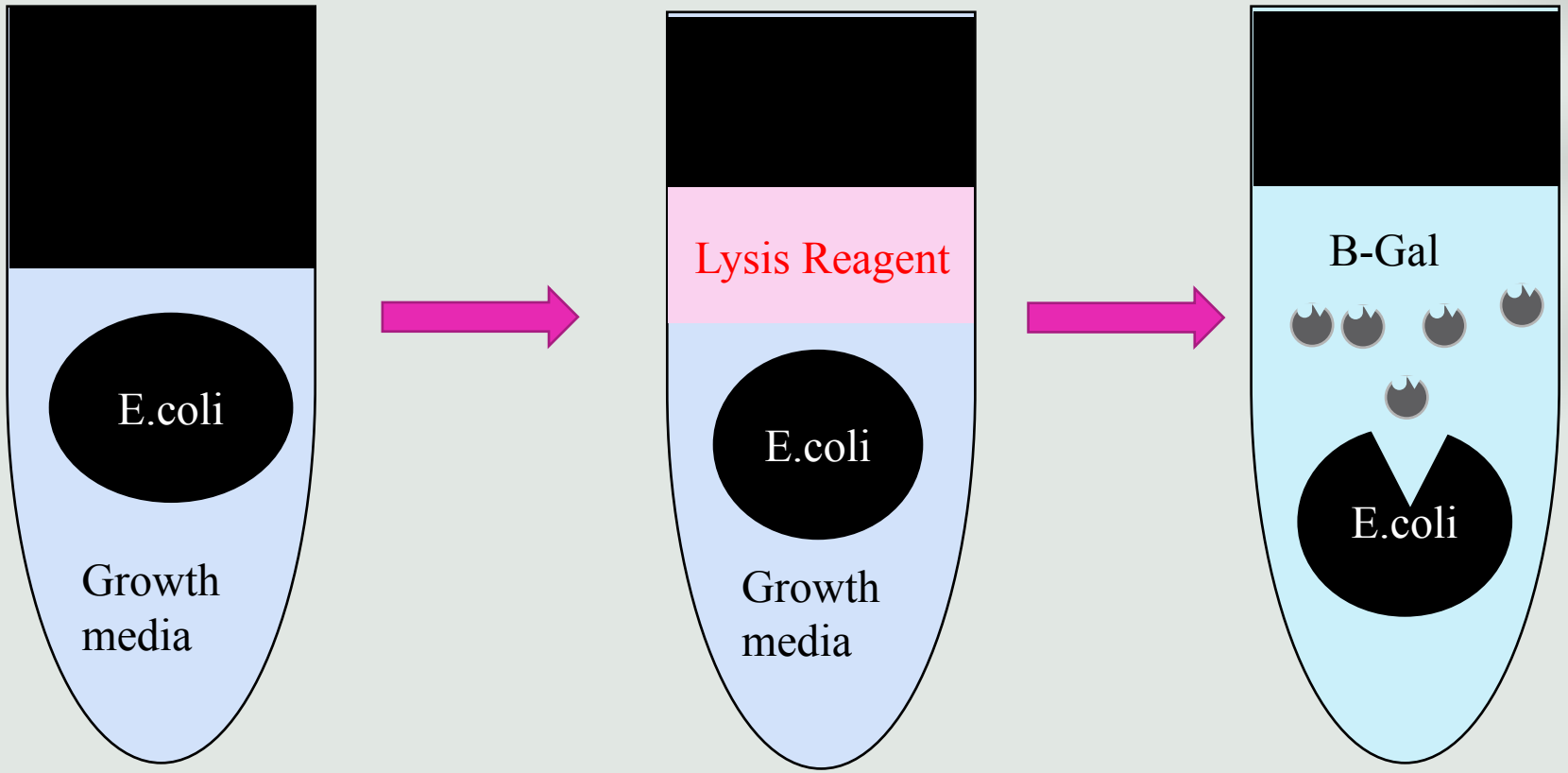
Add ONPG



Z Buffer
Lysed E. coli
ONPG

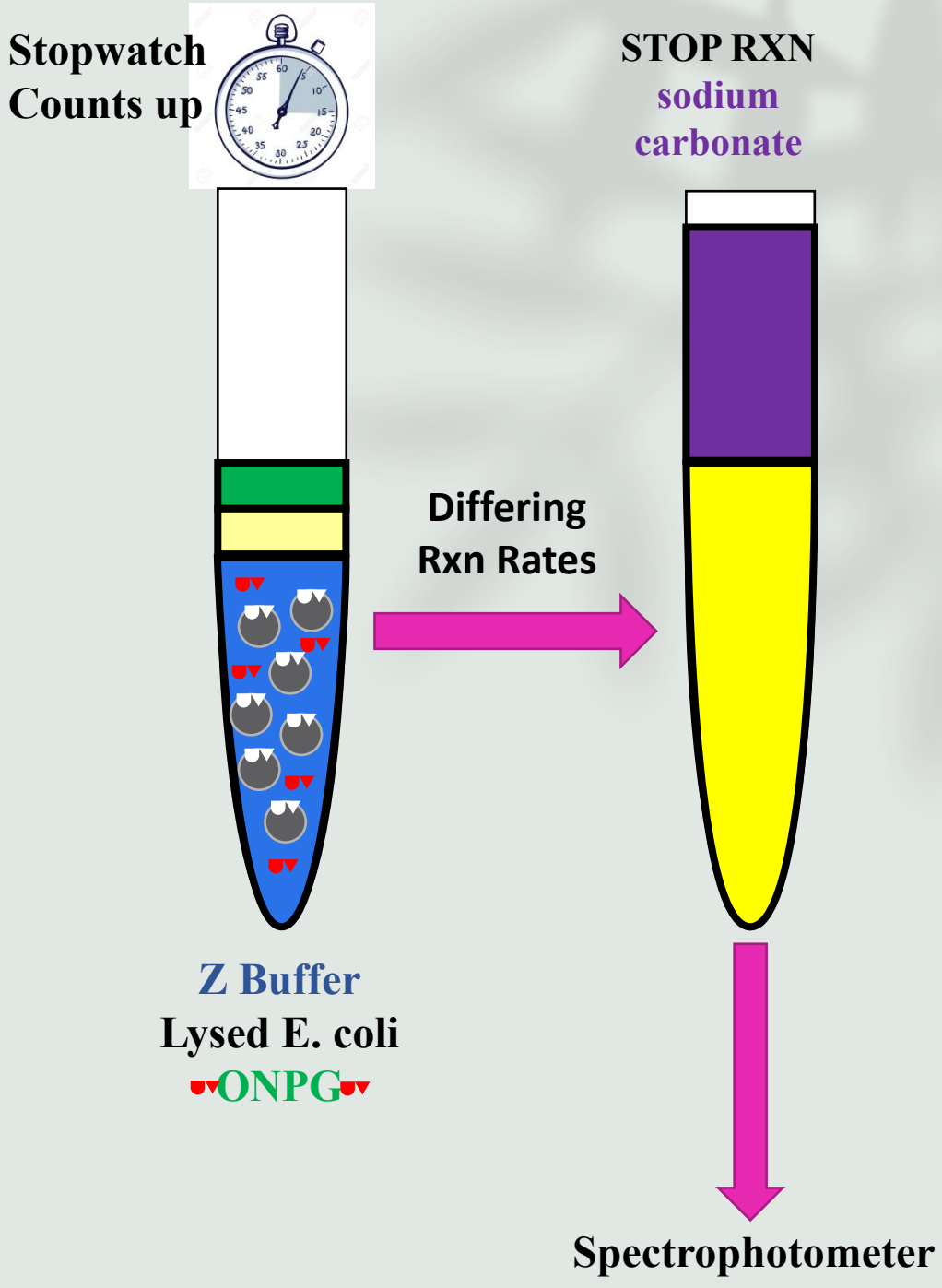
Spectrophotometer

Lysis of *E.coli*



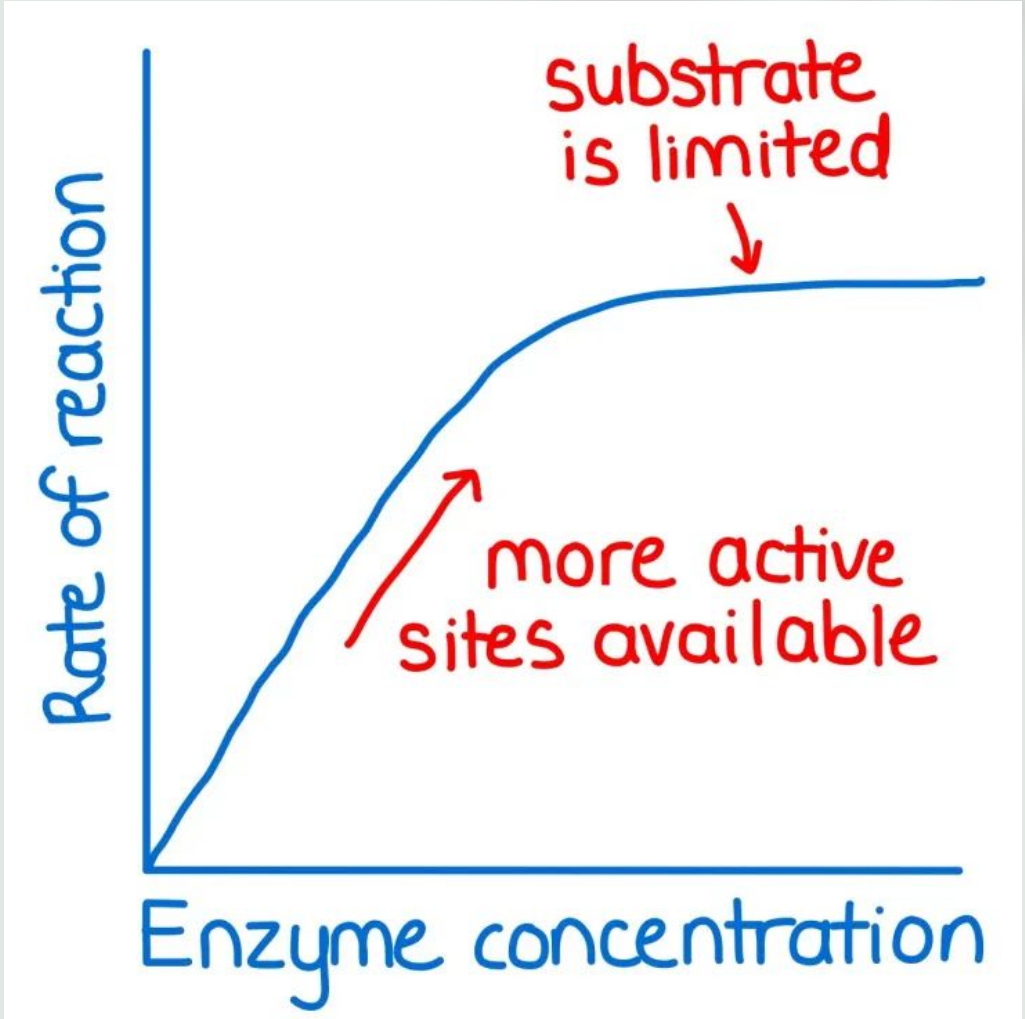
Calculating Enzyme Activity

- Given the differing enzyme and substrate concentrations you must consider the **time** it takes for the reaction (Rxn) to turn yellow
  - More enzyme that faster the rate
  - More substrate the faster the rate
- Absorbance changes over time
  - Perfect scenario, all reactions are stopped at the same absorbance (shade of yellow)
- Enzyme activity = (Absorbance/ Elapsed Time in seconds) x 1000
- Elapsed time – (time stopped-time started) and Make sure to convert to seconds



Lab Activity 2: What are the effects of changing <u>enzyme concentration</u> on enzyme activity and product formation?

- Given enzymes are unchanged by the reactions they catalyze
  - Repeated hydrolysis of substrate at a distinct rate
  - Dependent on enzyme and substrate coming together (Diffusion)
  - Concentration increases the probability of enzyme and substrate coming together
- After hydrolysis substrate is permanently changed
  - Therefore, substrate is limited
  - Reactions times must be limited before substrate is fully consumed.



Experimental setup

- Activity 2
  - Repeat procedure from Activity 1, varying concentration of enzyme (E.coli volume) instead of substrate concentration.
  - Use 200 uL ONPG for each reaction. **CHANGE TIPS BETWEEN EACH TRANSFER**.
  - Use 20 uL lysis reagent for each reaction. **CHANGE TIPS BETWEEN EACH TRANSFER**.
  - Use 6 uL, 20 uL, 60 uL, 200 uL E.coli, and 60 uL media (blank).

Table 3

Rxn #	ONPG In Rxn	Vol. 4 mg/mL ONPG stock	Enzyme (culture volume)	Lysis Reagent	Z buffer to bring to 1 mL total
1	0.8 mg	200µL	3 µL	10 µL	787µL
2	0.8 mg	200µL	10 µL	10 µL	780µL
3	0.8 mg	200µL	30 µL	10 µL	760µL
4	0.8 mg	200µL	100 µL	10 µL	690µL
Blank	0.8 mg	200µL	30 µL media	10 µL	760µL

Note - enzyme volume (E. coli volume) going to be varied; substrate volume (ONPG) going to be same.

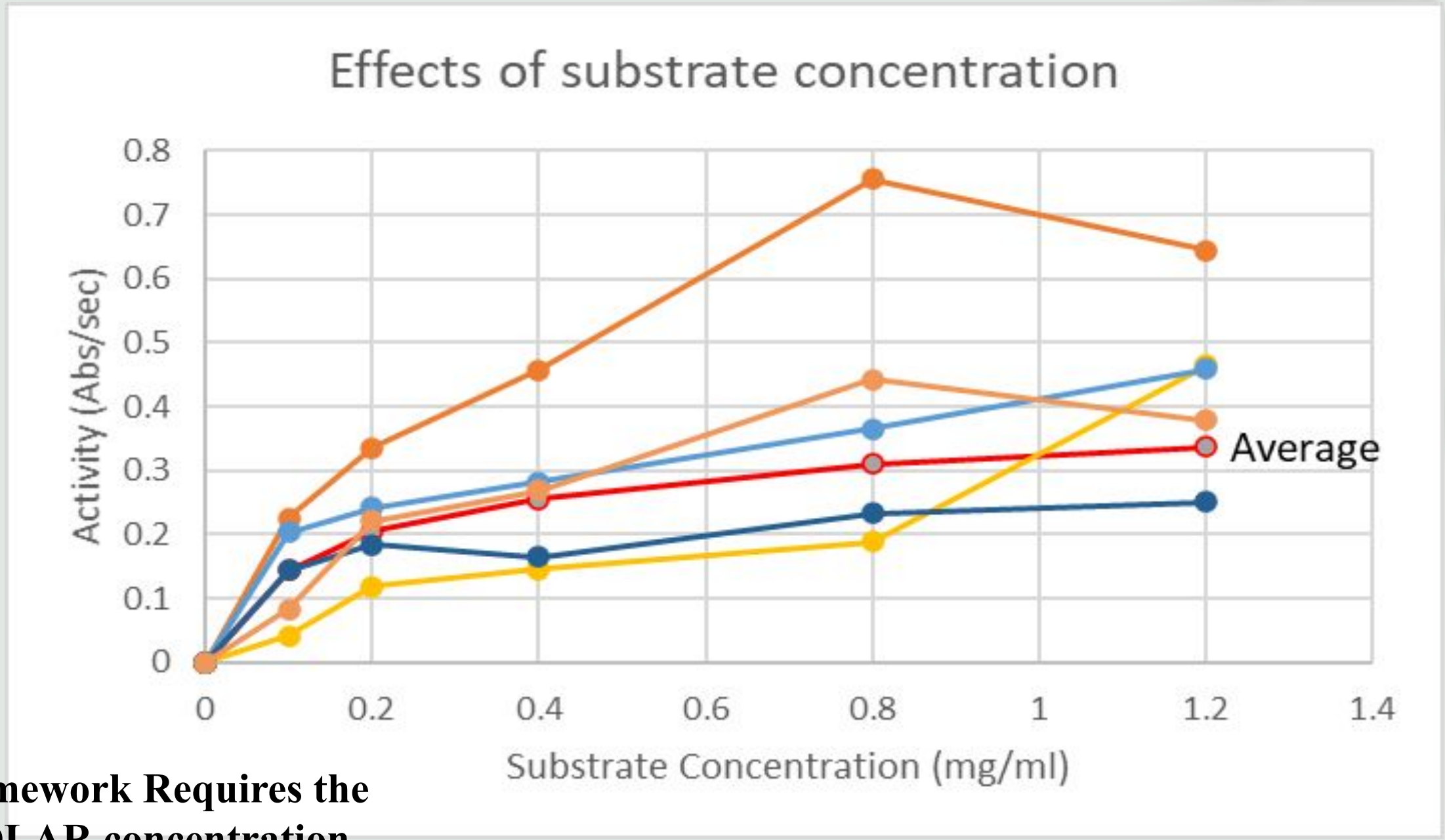
Summary

Enzyme Activity Calculations:

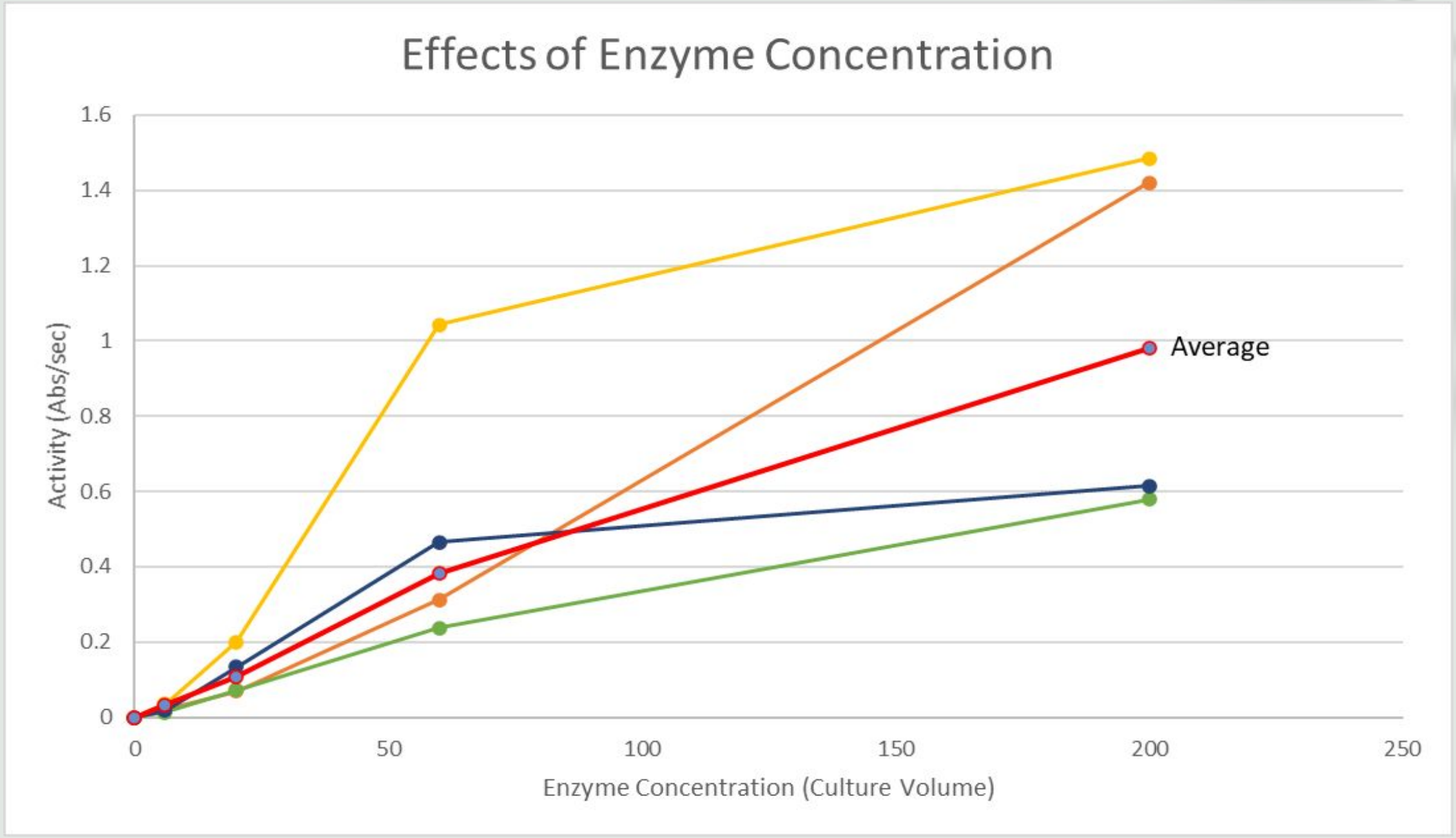
- (Absorbance / Time) x 1000
- Time should be in SECONDS: (minutes x 60) seconds
- What is the effect of substrate concentration on enzyme activity?
- What is the effect of enzyme concentration on enzyme activity?

Molar Concentration Calculations:

- Convert mass units from mg to g (divide by 1000)
- Divide converted mass by molecular weight of 301 g/mol (to get moles)
- Divide moles by reaction volume (1.0 mL) to get molar concentration
- Does this experimental setup have any problems for getting good results?
- Compare shared results from before.
- How did we get our enzyme? Is that consistent/replicable?



Homework Requires the MOLAR concentration


Effects of Enzyme Concentration
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Activity (Abs/sec)
0
50
100
150
200
250
Enzyme Concentration (Culture Volume)
Average