

YOUR NAME: \_\_\_\_\_

Key

NOTES:

1. where appropriate please show work - if in doubt show it anyway.
2. pace yourself - you may want to do the easier questions first.
3. please note the point value of questions - adjust your answers and effort accordingly.
4. some questions may have more data than you need.
5. please be brief - unfocused, rambling answers won't receive as much credit as a few short appropriate phrases.
6. Please write CLEARLY - if I cannot read it - it is wrong.
7. Good luck

correct answer 2pt (set up = 1 pt)

Question 1. (16 pts.) Short problems. Show work, but most credit goes to the correct numerical answer.

- a. an enzyme has a  $V_{max}$  of  $0.2 \mu\text{mol}/\text{min}$  and a rate of  $0.03 \mu\text{mol}/\text{min}$  at  $10 \mu\text{M}$  substrate. What is the  $K_m$  for the substrate

$K_m = \underline{\text{56.666}} \text{ M}$

$v = \frac{V_{max} \cdot S}{K_m + S}$

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

$K_m = \left( \frac{V_{max}}{v} - 1 \right) [S]$

$= \left( \frac{0.2 \mu\text{mol}/\text{min}}{0.03} - 1 \right) (10 \mu\text{M})$

$\text{56.666 } \mu\text{M}$

3

- b. In "a" above the molecular weight of the enzyme was  $25,000 \text{ g/mol}$  and the amount used  $10 \mu\text{g}$ . What is the maximal turnover number

$\text{TN} = \underline{500} / \text{min}$

$\frac{0.2 \times 10^{-6} \text{ mol}/\text{min}}{10 \times 10^{-6} \text{ g} / 25000 \text{ g/mol}} = 500 / \text{min}$

3

- c. A competitive inhibitor of an enzyme is present at  $6 \text{ mM}$  and shows a  $K_i$  of  $1 \text{ mM}$ . If the  $K_m$  of an enzyme for its substrate is  $12 \text{ mM}$  what is the apparent  $K_m$  with the inhibitor present?

$K_m = \underline{84} \text{ mM}$

$K_{m, \text{apparent}} = K_m \left( 1 + \frac{I}{K_i} \right)$

$= 12 \left( 1 + \frac{6 \text{ mM}}{1 \text{ mM}} \right) = 12 \times 7$

3

- d. A single subunit (monomeric) oxygen binding protein shows a  $K_p$  of  $1 \text{ mm}$  what is the fractional saturation at  $2 \text{ mm}$  partial pressure of oxygen?

Fractional saturation  $\underline{0.67}$

$\text{Fractional saturation} = \frac{[O_2]_p}{K_p + pO_2}$

$= \frac{2 \text{ mm}}{(1 + 2) \text{ mm}} = \frac{2}{3}$

e. The equilibrium constant for the reaction:



$$K = \frac{[\text{glucose-6P}][\text{ADP}]}{[\text{ATP}][\text{glucose}]}$$

Is 935 calculated from the standard free energy of the reaction.

In the cell, the concentrations were measured as:

ATP = 10 mM; ADP = 1 mM; glucose = 1 mM; Glucose-6P = 0.5 mM

Calculate the value of the equilibrium constant from these values:

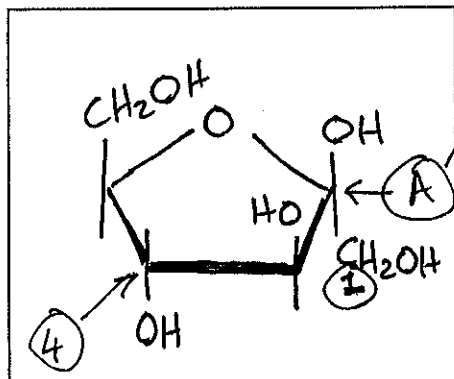
$$K = \underline{0.05} \quad (2)$$

1 set up

$$K = \frac{(0.5 \text{ mM})(1 \text{ mM})}{(10 \text{ mM})(1 \text{ mM})} = \frac{.5}{10} = .05$$

① Why are the numbers different? This reaction is not at equilibrium in the cell

Question 2 (5 pts) Regarding the monosaccharide shown to the left



- Label the anomeric carbon with an A
- This molecule is in the  $\alpha$ - or  $\beta$ - configuration  $\beta$
- This molecule is: aldose ketose cannot say
- Label carbon atoms 1 and 4

ketose

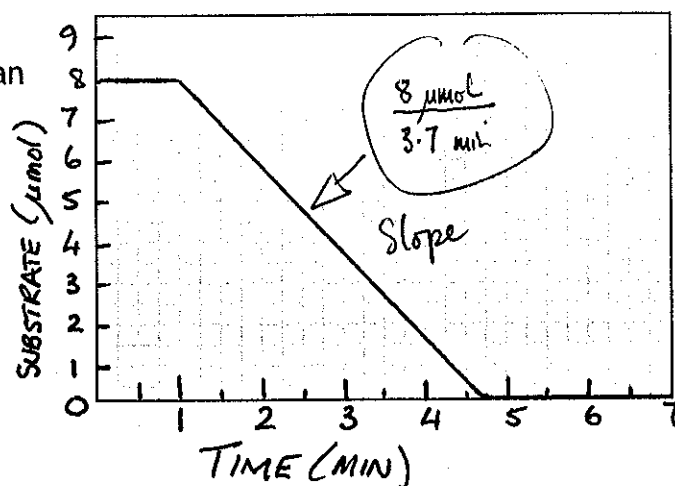
A or B Question 3 (3 pts.) Which is the most appropriate answer?

- the rates of enzyme catalyzed reactions are usually limited by the rate of substrate binding
- the Michaelis complex is never covalent
- the  $K_m$  is equal to  $V_{max}/2$  for an enzyme-catalyzed reaction
- mechanism-based inhibitors do not bind to their target enzymes
- all of the above are false

C Question 4 (3 pts.) Which is the most appropriate answer. At constant  $E_T$ , if the rate of an enzyme assay almost doubled when the substrate concentration doubled:

- the enzyme is saturated
- the substrate concentration is well above the  $K_m$
- the substrate concentration is well below the  $K_m$
- more enzyme is needed
- all of the above are false

Question 5 (10 pts.) The graph to the right shows an enzyme assay converting a single substrate into a single product ( $S \rightarrow P$ ). It was started at time 1 minute by the addition of 20 micrograms of enzyme to a solution of 1 mL of substrate containing the amount of substrate shown in the graph. The pH was 7.5 at 25 °C.



Answer the following questions - there is more information than you need.

- Calculate the rate of the enzyme assay 2.2 (2.0-2.5) micromole substrate/min (2)
- What is the rate in the absence of enzyme? 0 micromoles/min (1)
- The enzyme is saturated with substrate over most of the assay - circle the appropriate:
 

<u>YES</u>	NO	Cannot say	(1)
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- the enzyme is operating at  $V_{max}$  over most of the assay - circle the most appropriate
 

<u>YES</u>	NO	Cannot say	(1)
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- the product (P) is a powerful competitive inhibitor of the enzyme - circle the most appropriate
 

YES	<u>NO</u>	Cannot say	(1)
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- the equilibrium constant for the conversion of  $S \rightarrow P$  is greater than 10
 

<u>YES</u>	NO	Cannot say	(1)
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- what is the concentration of substrate in the assay before the addition of enzyme
 

8 μmol in 1 mL	$\frac{8 \times 10^{-6}}{.001 L}$	$\frac{8 \times 10^{-3}}{(8 mM)} [M]$	(2)
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Question 6 (4 pts.) You take equal volumes of blood from a sickle cell patient and a normal individual and mix them carefully and then remove oxygen from the blood sample.

- (2) The RATE of sickling will: INCREASE DECREASE STAY THE SAME CANNOT PREDICT

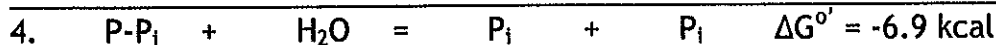
Now in one line explain your answer:

(1) Because hemoglobin is in red blood cells, no dilution of  $Hb_S$  by  $Hb_A$

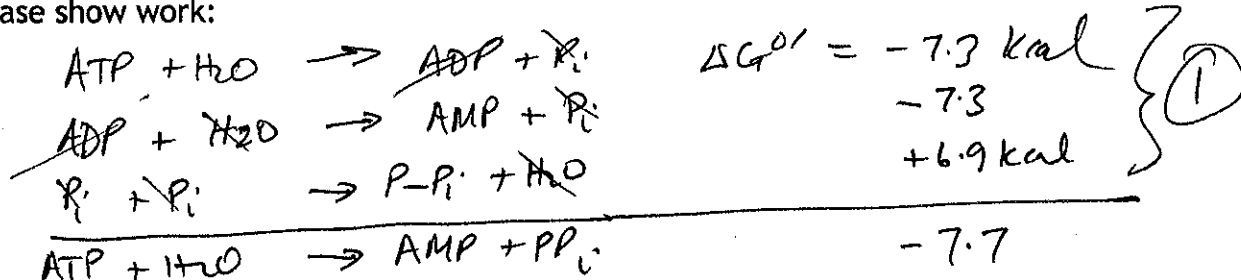
Question 7 (6 pts.) Given the following calculate  $\Delta G^{\circ'}$  for equation 1:



$\Delta G^{\circ'} = -7.7 \text{ kcal}$



Please show work:



Pyrophosphatase catalyzes reaction 4. The addition of this enzyme to equation 1 will (circle all answers that are appropriate)

a. make reaction more exergonic

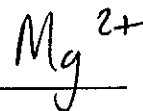
b. drive the reaction to the right

c. drive the reaction to the left

d. have no effect on the equilibrium position incorrect

e. insufficient information to make choices

What metal ion would you expect to be involved in pyrophosphatase action?



or a divalent ...

Question 8 (15 pts.) Short answers (3 pts each) - a few phrases or a labelled diagram is sufficient.

a. Describe FRAP Fluorescence recovery after photobleaching.

Membrane / bilayer treated with a fluorescent-labelled phospholipid. Small area illuminated w/ intense light (laser) beam. Fluorescent molecules are destroyed in area. ~~area~~ other non-photobleached molecules rapidly diffuse to fill "hole".

b. Why is the affinity of oxygen for normal hemoglobin dependent on  $[\text{H}^+]$ ?

Because transition from  $T \rightleftharpoons R$  involves release of a proton (or part)  
Several ionizing groups have different pKs between T & R so:  
 $T \rightleftharpoons R + \text{H}^+$  so  $[\text{H}^+] \uparrow$  affects  $\text{O}_2$  affinity

- c. Vitamin B12 is a large, polar, molecule that is concentrated from the environment into certain microbial cells. What type of process would be involved and why?

Concentration against a gradient would require active transport  
e.g. via expenditure of ATP

- d. Glycogen and starch are stored within cells as granules. Why not store energy in the form glucose instead?

If a glycogen molecule were hydrolyzed completely the resulting osmotic pressure would be  $\uparrow$  by factor of ~1000-fold  
Osmotic pressure is a COLLIGATIVE PROP. (Chemistry...)

- e. You hear of an enzyme whose kinetic constants were reported to be  $k_{cat} = 1000 / \text{sec}$  and  $K_m = 0.1 \mu\text{M}$ . Why should you be suspicious?

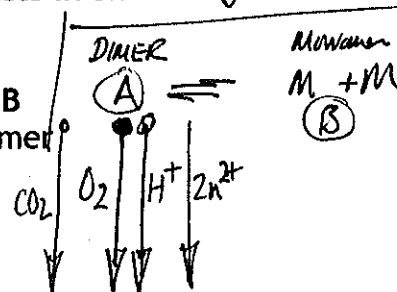
Because  $\frac{k_{cat}}{K_m} = \frac{1000 / \text{sec}}{0.1 \times 10^{-6} \text{ M}} = 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ . TOO FAST for

diffusion of substrate to enzyme. SO IMPOSSIBLE must be wrong!

Question 9 (6 pts.) An oxygen binding protein (OBP) from Europa exists in two states A and B that are in equilibrium.

- A binds oxygen tighter than B
- A has a higher pI than B
- A binds  $\text{CO}_2$  tighter than B

- A binds  $\text{Zn}^{2+}$  tighter than B
- A is a dimer, B is a monomer



- a. Increasing  $\text{CO}_2$  causes oxygen affinity of OBP to (circle):

increase decrease stays unchanged cannot predict

- b. Increasing Zn causes oxygen affinity of OBP to (circle):

increase decrease stays unchanged cannot predict

- c. Increasing total OBP concentration causes oxygen binding of OBP to (circle):

increase decrease stays unchanged cannot predict

- d. Increasing total OBP concentration causes  $\text{CO}_2$  binding of OBP to (circle):

increase decrease stays unchanged cannot predict

- e. Lowering the pH causes oxygen binding of OBP to (circle):

increase decrease stays unchanged cannot predict

- f. Lowering the pH causes the percentage of monomeric OBP to (circle):

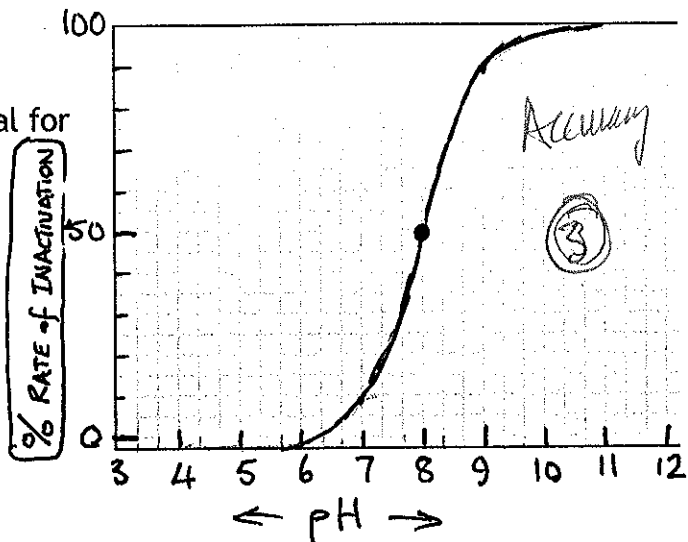
increase decrease stays unchanged cannot predict

Question 10 (7 pts.) An enzyme has a single cysteine side chain ( $-\text{CH}_2\text{-SH}$ ;  $\text{pK} = 8$ ) which is essential for activity. The enzyme is inactivated by reacting with iodoacetate.

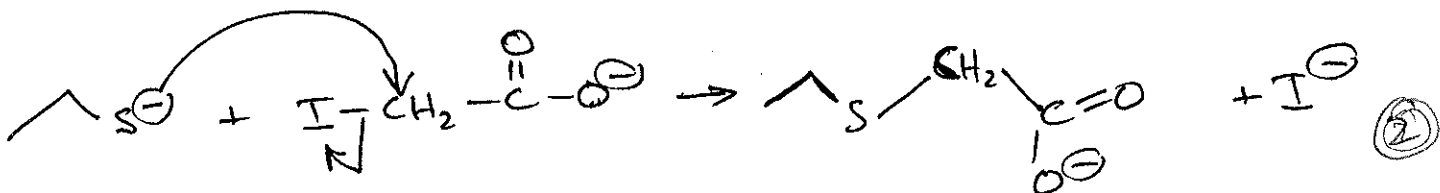
a. iodoacetate is what type of inhibitor?

covalent / irreversible (1)

b. Draw an accurate curve to represent the rate of inactivation with pH



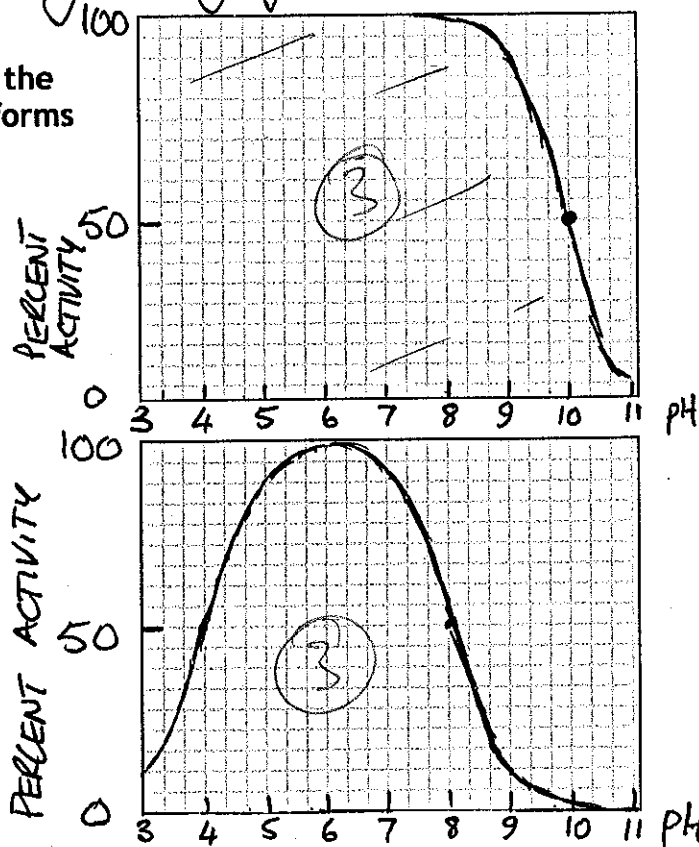
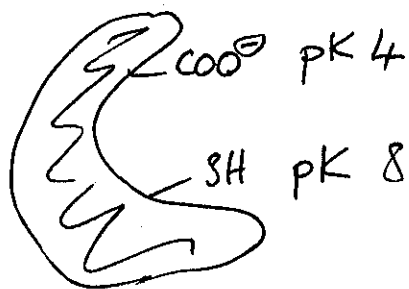
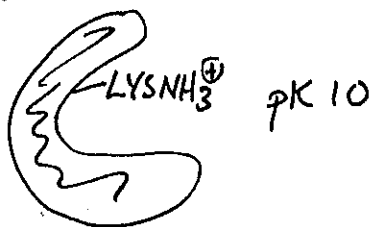
c. Draw an accurate representation of the chemistry of this inactivation reaction

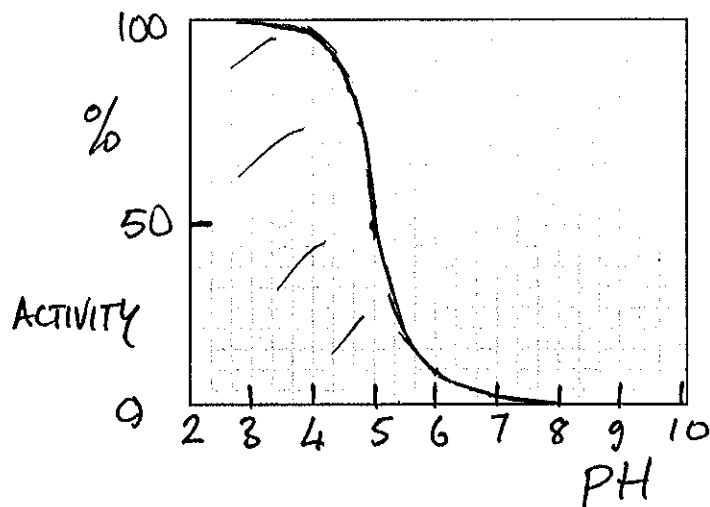


d. Inactivation of the enzyme was slowed 100-fold by the substrate of the enzyme. Suggest an explanation for this (one sentence)

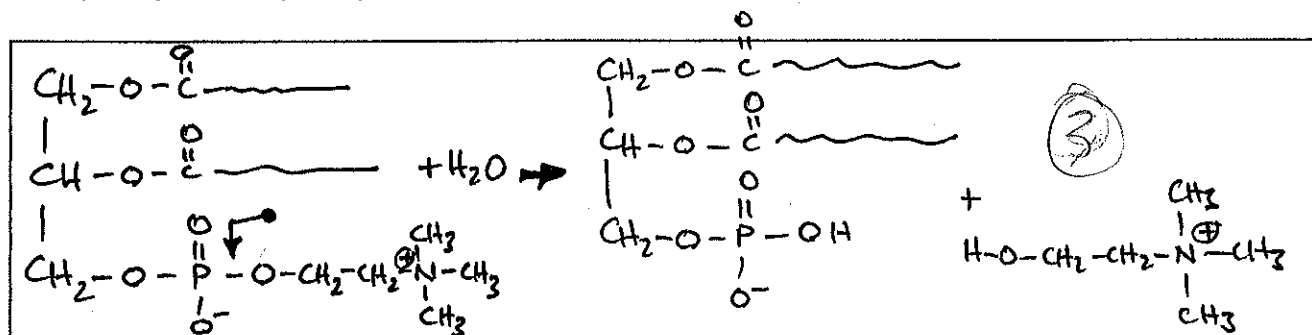
the substrate may physically block binding/docking of iodoacetate (1)

Question (9 pts.) Draw the pH activity curves for the enzymes shown to the left. Only these protonic forms are active.

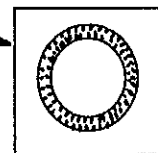




Question (10 pts). A schematic structure of a phospholipids is shown in the box. Phospholipase D hydrolyzes the bond shown. A. Complete the structure of the equation.



This phospholipid can form either vesicles (self-sealed spherical bilayers) or micelles.



Phospholipase D is added to either a vesicles or a micelle preparation of the phospholipids. The extent of hydrolysis is shown in the graph.

B. Explain the behavior in region I

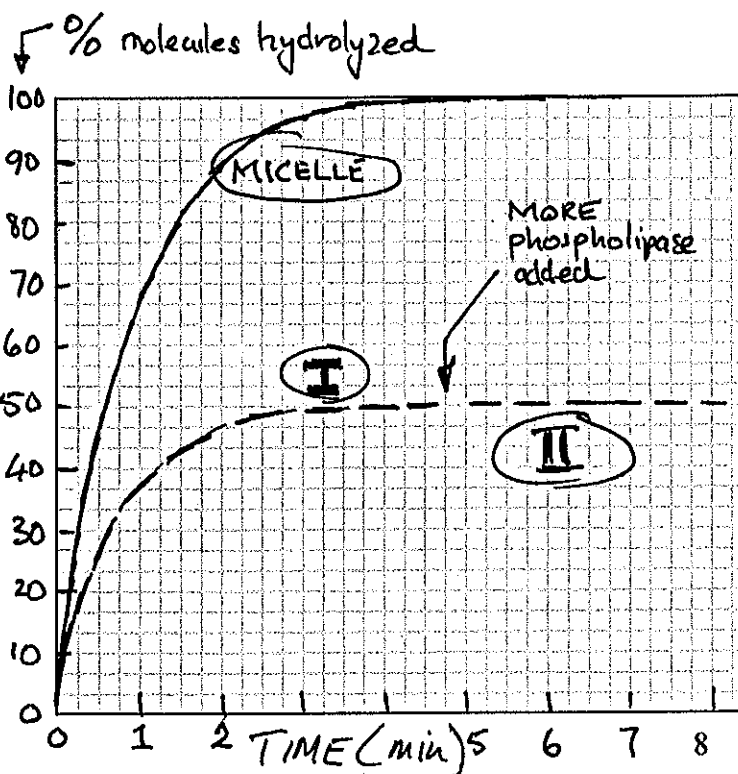
Phospholipase can only reach the outer leaflet of bilayer & hence hydrolyzes just  $\frac{1}{2}$  of total lipid. Flipping between layers is extremely slow

C. Explain the behavior in region II

The enzyme in I is not inhibited - a further addition makes no further "dent" in substrate -  $\frac{1}{2}$  unavailable (still)

D. Explain the behavior for the micelle

all phospholipid is available because they have hydrophobic CORE





Question 10 (pts.) Fill in the blanks with not more than 3 legible words.

a. Name an irreversible inhibitor of an enzyme

Sarin, e.g. ....

b. and the enzyme that is the target of your answer in "a"

MANY

COMBO'S

cholinesterase

c. name the glycosidic bond formed in cellulose

$\beta(1-4)$

d. name the glycosidic bond at the branch points in amylopectin

$\alpha(1-6)$

e. these enzymes do not follow Michaelis Menten Kinetics

ALLOSTERIC

f. name a non-saponifiable lipid

(many)

cholesterol

g. another name for biosynthesis

ANABOLISM

h. a small molecule binding to the central intersubunit cavity in hemoglobin

DPG

zz. the word that best describes this exam

easy

the end