ELIZADE UNIVERSITY, ILARA MOKIN, ONDO STATE
DEPARTMENT OF PHYSICAL AND CHEMICAL SCIENCES
2017/2018 SECOND SEMESTER B.Sc. DEGREE EXAMINATIONS

BCH 404: ADVANCED ENZYMOLOGY

Instructions: Answer any four (4) questions Time: 2 Hrs

1. (a) For a bisubstrate reaction mechanism which is "ordered sequential", derive the rate of reaction and then elaborate how the real kinetic parameters, Kia, K_m^A, K_m^B and Vmax will be obtained

$$E + A \longleftrightarrow EA + B \longleftrightarrow EAB \longleftrightarrow EPQ \longleftrightarrow P + EQ \longleftrightarrow E + Q$$ *(15marks)*

(b) Elaborate on the effects of pH and temperature on the rate of enzymatic catalysis *(10 marks)*

2. (a) According to Luisi, 1979, enzymes are regarded as macromolecules. Highlight six possible reasons for this phenomenon and discuss any TWO *(10 marks)*

(b) Rearrange the Arrhenius equation, $k = Ae^{-Ea/RT}$ to give the Arrhenius plot *(2 marks)*

(c) For a typical bisubstrate reaction mechanism that is "ping pong", determine how the real kinetic parameters (K_m^A, K_m^B and Vmax) will be obtained *(8 marks)*

(d) Describe the Theorell-Chance mechanism (5 marks)

3. (a) Using BOTH steady state AND equilibrium approach, derive from the first principles the reaction rate for a single substrate enzyme catalyzed reaction scheme indicated below:

$$E + S \longleftrightarrow ES \longrightarrow E + P$$ *(15 marks)*

(b) Using specific examples explain the phenomena of allostery and feedback inhibition *(4 marks)*

(c) Discuss the "induced fit" and the "lock and key" models of enzymatic catalysis. Use appropriate illustrations where possible. *(6 marks)*

4. (a) Derive the Hill equation for the binding reaction between hemoglobin and oxygen

$$Hb + nO_2 \longleftrightarrow Hb(O_2)n$$ *(15 marks)*

(b) Using an appropriate sketch, illustrate the difference between enzymes that obey Michealis-Menten kinetics and those that display positive and negative cooperativity. *(5 marks)*

(c) Explain the significance of the Hill coefficient at n=1, n >1 and n < 1 *(5 marks)*

5. (a) From the table below, obtain the value for the activation energy, E_a.

T (K)	Vmax (Units/mg protein)
300	15.5
308	30.8
318	43.5
328	60.9
338	72.3

($R = 8.314\ J\ mol^{-1}K^{-1}$) *(3 marks)*

(b) Using mathematical manipulation as well as the corresponding diagnostic plots, rearrange the simple Michealis-Menten's equation to obtain the Lineweaver-Burk, Eadie-Hofstee and Hanes-Woolf plots. *(12 marks)*

(c) Discuss the mechanism of action of either Ribonuclease A, Chymotrypsin or Lysozyme. *(10 marks)*

6. (a) Define protein engineering and the discuss briefly the approaches involved in engineering a desired protein. *(4 marks)*

(b) Describe very briefly **ALL** of the following:

i. enzyme assay *(2 marks)*

ii. analytical assays *(2 marks)*

ii. enzyme assay techniques *(2 marks)*

iii. criteria for purity *(2 marks)*

(c.) complete the typical purification table below

	Volume	Activity (U/ml)	Protein (mg/ml)	Total Activity (U)	Total Protein (mg)	Specific Activity (U/mg)	Yield	Purification Fold
Crude	12	75	1.2				100	1.0
50% Ammonium Sulphate Fractionation and Dialysis	5	56	1.1					
Gel-Filtration Chromatography on Sephadex G-100	20	35	0.05					

(3 marks)

d. Derive the rate of reaction for a competitive enzyme inhibition and show how the apparent K_m and V_{max} values can be obtained from the diagnostic plot. *(10 marks)*