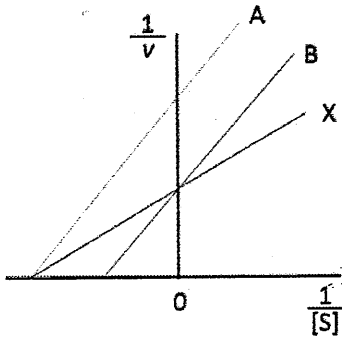


※ 考生請注意：本試題不可使用計算機。請於答案卷(卡)作答，於本試題紙上作答者，不予計分。

Part I: (Total 35 points)

1. Please use Hanes-Woolf plot to show how to calculate V_{max} and K_m . (4 points)
2. How do you define the catalytic efficiency of an enzyme and why? (hint: using V_{max} , K_m , or k_{cat}) (4 points)
3. The Lineweaver-Burt plot below showing line A for enzyme X with inhibitor A, line B for enzyme X with inhibitor B, and line X for enzyme X only.
 - (A) The inhibition of which inhibitor could be reversed when we add more substrates to the reaction? (1 point)
 - (B) Which inhibitor would lower V_{max} ? (1 point)



4. We are going to do site-directed mutagenesis to alter some amino acid residues on chymotrypsin. Please describe the influences (activation or inhibition or no influence) of changing Ser195 to be Gly in the active site. Why? (4 points)
5. If you find an unknown protein X and analyze the protein sequence, you find a potential phosphorylation site "LARKKGSIH" on protein X. Which kinase in the following table could phosphorylate protein X? (2 points)

Protein Kinase Class	Target Sequence*	Activators
I. Ser/Thr protein kinases		
A. Cyclic nucleotide-dependent cAMP-dependent (PKA) cGMP-dependent	—R(R/K)X(S*/T*)— —(R/K)KKX(S*/T*)—	cAMP cGMP
B. Ca ²⁺ -calmodulin (CaM)-dependent Phosphorylase kinase (PhK) Myosin light-chain kinase (MLCK)	—KRKQIS*VRGL— —KKRPQRATS*NV—	Phosphorylation by PKA Ca ²⁺ -CaM
C. Protein kinase C (PKC)	—PXX(S*/T*)P—	Ca ²⁺ , diacylglycerol
D. Mitogen-activated protein kinases (MAP kinases)	—PXX(S*/T*)P—	Phosphorylation by MAPK kinase
E. G-protein-coupled receptors β-Adrenergic receptor kinase (BARK) Rhodopsin kinase		
II. Ser/Thr/Tyr protein kinases		
MAP kinase kinase (MAPK kinase)	—TEY—	Phosphorylation by Raf (a protein kinase)

(Adapted from Garrett Biochemistry)

6. Please describe the influences (activation or inhibition or no influence) of the following molecules on glycogen phosphorylase activity.
- (A) AMP (1 point)
- (B) Glucose-6-phosphate (1 point)
- (C) Phosphorylase kinase (1 point)
7. What is the function of hypoxia inducible factor (HIF)? (4 points)
8. What are the differences between glyoxylate cycle and TCA cycle? (4 points)
9. What are uncouplers of oxidative phosphorylation? (4 points)
10. Please compare the difference using glycerol-phosphate shuttle and malate-aspartate shuttle for the yield of ATP from glucose oxidation. (4 points)

Part II (Total 25 points)

11. (a) When Arthur Kornberg discovered DNA polymerase I, why scientists think it's not the right DNA polymerase for replication of *E. coli* genome? *E. coli* genome is about 5 Mbp. (5 points)
- (b) what is the function of 5' → 3' exonuclease? (5 points)
- (c) what is the function of 3' → 5' exonuclease? (5 points)

TABLE 28.1 Properties of the DNA Polymerases of *E. coli*

Property	Pol I	Pol II	Pol III (core)*
Mass (kD)	103	88	130(α), 27.5(ϵ), 8.6(θ)
Molecules/cell	400		40
Turnover number [†]	20	40	1000
Polymerization 5' → 3'	Yes	Yes	Yes
Exonuclease 3' → 5'	Yes	Yes	Yes
Exonuclease 5' → 3'	Yes	No	No

* α -, ϵ -, and θ -subunits.[†]Nucleotides polymerized at 37°C/second/molecule of enzyme.

12. How eukaryotic cells replicate their DNA ends? (5 points)
13. In Sanger's method of DNA sequencing, what nucleotides were invented? (5 points)

Part III (Total 40 points)

14. What is the function of σ subunit of *E. coli* RNA polymerase during transcription? (3 points)
15. What is the committed step in the synthesis of fatty acids? How is it regulated? (5 points)
16. Use lac operon as an example to describe negative and positive gene expression control systems. (5 points)

17. Describe three kinds of post-translational modifications of histone that are important for gene expression in eukaryocytes. (3 points)
18. Describe how the 5' splice site and branch point are determined during RNA splicing. (5 points)
19. What are the functions of 16S rRNA and 23S rRNA during protein translation? (4 points)
20. AMPK plays an important role in cellular energy regulation. How is AMPK regulated? (5points)
21. Describe the ubiquitin-proteasome system in protein degradation. (6 points)
22. Calcium is an important secondary messenger in signal transduction. How is intracellular calcium concentration regulated? (4 points)