

※ 注意：請於試卷內之「非選擇題作答區」依序作答，並應註明作答之大題及小題題號。

一、 蛋白結構與酵素化學 (共 25 分)

(一) 選擇題 (1分/題; 共 7 分)

1. Enzymes act by
 - a. lowering the overall free energy change of the reaction.
 - b. decreasing the distance reactants must diffuse to find each other.
 - c. increasing the activation energy.
 - d. decreasing the activation energy.
 - e. Both b and d
2. A reaction in which the substrate glucose binds to the enzyme hexokinase and the configuration of both molecules changes is an example of
 - a. a lock-and-key mechanism.
 - b. an induced fit mechanism.
 - c. competitive inhibition.
 - d. allosteric inhibition.
 - e. bonding with an intermediate.
3. Coenzymes are
 - a. enzymes in the same pathway.
 - b. proteins that form dimeric enzymes.
 - c. small molecules that work with an enzyme to enhance reaction rate.
 - d. small molecules that allosterically regulate enzymes.
 - e. All of the above
4. In allosteric regulation, binding of a small regulatory molecule to an enzyme _____ the enzyme activity.
 - a. inhibits
 - b. stimulates
 - c. either stimulates or inhibits
 - d. neither stimulates nor inhibits
 - e. both stimulates and inhibits

Textbook Reference: The Central Role of Enzymes as Biological Catalysts, pp. 79–80
5. The free-energy change of a reaction is determined by the
 - a. intrinsic properties of the reactants and products.
 - b. concentrations of the reactants and products.
 - c. temperature of the reactants and products.
 - d. All of the above
 - e. None of the above
6. The lock-and-key and induced-fit models are two alternative models that can explain how enzyme and substrates interact. These two models can be distinguished from one another according to changes in

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- a. reaction solution viscosity as substrates and enzymes bind.
- b. substrate and enzyme shape as binding occurs.
- c. reaction solution temperature as a result of the endothermic cooling that occurs during substrate binding.
- d. reaction solution color during enzyme-substrate binding.

7. Which statement is true for protein structures

- a. interior of protein is hydrophobic
- b. the α helix has a dipole moment
- c. beta(β)sheets usually have their beta strands either parallel or antiparallel
- d. proline does not exist in α helix
- e. all of the above

(二) 簡答題 (共 18 分)

1. The structure of a protein is classified into primary, secondary, tertiary and quaternary levels. Please describe the attribute of each of them in proteins structure. (8 pts)
2. State the two main ways that cells regulate the activity of their enzymes, and provide one example of each. (4 pts)
3. Hemoglobin is a [$\alpha(2)$: $\beta(2)$] tetrameric hemeprotein found in erythrocytes where it is responsible for binding oxygen in the lung and transporting the bound oxygen. Please describe the allosteric oxygen binding nature of this protein in term of protein structure point of view. (6 pts)

二、以下問題請標明題號，詳細作答：

1. Explain the phenomenon that expression of the *Lac* operon in *E. coli* is regulated by both negative and positive control mechanisms. (6 分)
2. In prokaryotes, regulation of RNA transcription may be achieved by the following mechanisms. For each term, please explain and give an example. (每小題 4 分)
 - (1) Antitermination
 - (2) Attenuation
 - (3) Riboswitch

3. For each of the following cases, what would be its effect on gene expression? (每小題 1 分)
- (1) A mutation occurred in the promoter region.
 - (2) Two bases were inserted in the middle of an mRNA.
 - (3) An amber mutation was found in the middle of an open reading frame.
 - (4) The last base of the open reading frame was deleted from an mRNA.
4. DNA replication in *E. coli* requires the participation of DNA polymerase I, primase, helicase, and topoisomerase, etc. Why are primase and helicase important for this reaction? (3 分)

三、

1. 若有一 pathway 之中間產物為 A,B,C,D,E，設計方法建立其反應順序？若 $A \longrightarrow B$ 為其中之一反應，且其需要一 enzyme 來催化其反應，設計一方法來純化此酵素？酵素找到後設計一方法來找出其基因？基因找到後如何研究其調控？(16%)
2. 請畫出 citric acid cycle (7%)。
承上，請把下列代謝物與 citric acid cycle 串起來(7%)。
 - a. Glutamate
 - b. Arginine
 - c. Aspartate
 - d. Phosphoenolpyruvate
 - e. Serine
 - f. Porphyrines
 - g. sterols

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四、生化基本分析技術 (共20分)

1. Protein purification is an often and essential for biochemical analyses. Please briefly describe the basic principles of following techniques. (6 分)

a. Immunoprecipitation

b. Gel filtration

c. GST pull down

2. Protein-protein interaction is a fundamental concept in many cellular and biochemical processes. Please describe the basic principles of following techniques. (6 分)

a. Far-Western

b. FRET (fluorescence resonance energy transfer)

c. Co-immunoprecipitation

3. Viroids, exclusively bearing a circled, single-strand, non-coding RNA, are emerging pathogens to enable infecting a variety of plants. To study the viroid-host molecular interaction in host plants, several RNA-protein interactions are focusing, in particular, the identification of viroid RNA-binding proteins (from host/experimental plants). Please design an experiment to explore the possibility of *Citrus Exocortis viroid* (CEVd; one species of viroids enables infecting both of citron and tomato) interaction with host protein designed as X protein. (8 pts)

Note: The materials start from the CEVd infectious cDNA construct, 3-week old susceptible tomato seedlings with or without inoculated with CEVd, antibodies for X proteins or whatsoever, recombinant X protein, and all reagents for the following experiments.

a. *In vitro* transcription of RNA (with remove DNA template)

b. Mobility shift analysis

c. Sucrose gradient analysis

d. Mechanical inoculation of CEVd

e. UV cross-linking

f. Preparation of protein lysate from plant tissue(s)

g. Mass spectrum analysis

h. SDS-polyacrylamide gels electrophoresis

i. Reverse transcriptase-polymerase chain reaction (RT-PCR).

j. Specific (radio-)labelling of RNA at an internal site

k. Immunoprecipitation

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