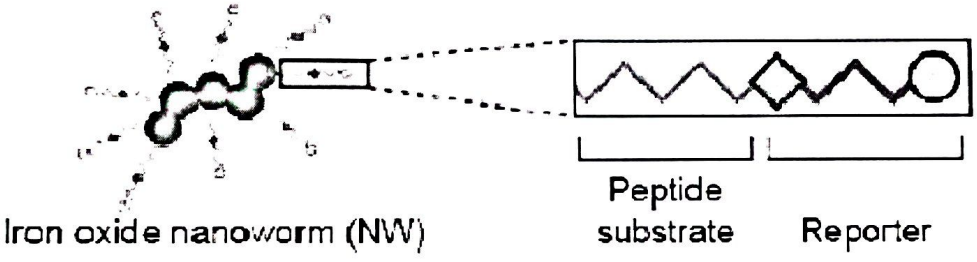
Department of Biochemical Engineering & Biotechnology
BEL 433: Enzyme Science & Engineering
Major Test, 2018

M.M.: 20 **Time: 90 Mins**

1. How droplet microfluidic is used in enzyme screening? Explain how single cell encapsulation per droplet is achieved in this system?
2. What is directed evolution? How can one engineer protein with desired specificity to a particular substrate?
3. Sangeeta Bhatia group in harvard designed this novel peptides as non-invasive diagnostic tool for cancer detection. Explain why such a method can be useful compared to existing systems. Principle behind this assay.



4. Write short note on Kinetic resolution of alchols. Why this is useful in pharmaceutical industry.
5. Write short note on Co-factor regeneration in reactor.
6. How can you improve temperature stability of enzyme using protein engineering?
7. Write short note on L-DOPA synthesis by enzymatic route. How this process is one most economical compared to chemical synthesis?
8. Explain the role of Lipase enzyme in biodiesel production.
9. List different enzyme used in conversion of cellulose to ethanol and their use.
10. What are the advantage of using enzymatic reaction in non-aqueous media? Give an example.

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Laboratory Quiz – 10 marks

1. A glucoamylase producer claims that its commercial preparation has an activity of 0.033 katals/Land you have to check the accuracy of such information. To do so,you will follow the procedure recommended by the enzyme producer, which determinesthe glucoamylase activity using maltose as a substrate (maltose + H_2O → 2glucose) and an enzymatic kit (Glucostat) that quantifies glucose according to:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{Glucose 6-Pi} + \text{ADP}$$

$$\text{Glucose 6-Pi} + \text{NAD}^+ \xrightarrow{\text{glucose 6-Pi dehydrogenase}} \text{6-Pi gluconate} + \text{NADH} + \text{H}^+$$

TheNADHformed can conveniently bemeasured spectrophotometrically by recordingoptical density (OD) at 340nm. The extinction coefficient of NADH is90mL/cm/mmol.According to the stoichiometry, 1mol of glucose reacted yields1mol of NADH.The following results have been obtained with the properly diluted glucoamylasepreparation by putting it in contact with a saturated maltose solution under an excess of ATP, NAD+ and coupling enzyme activities at the conditions recommended by the enzyme producer:

Reaction time (minutes)	OD_{340}(1:7500 dilution)
0	0.005
1	0.0563
2	0.107
3	0.159
4	0.210
5	0.261

2. Working with a purified L-malic enzyme (L-malate:TPNoxidoreductasedecarboxylating,EC(1.1.1.40) from Escherichia coli, a K_M of 0.15mM and a V_{max}of5µmol/sec/mgwere obtained. The strain was subjected to directedevolution to obtain a mutant L-malic enzyme whose affinity for L-malate wasdoubled, but the reactivity was reduced by 70% with respect to the native enzyme.The enzyme catalyzes the reaction:

$$\text{(S)-malate} + \text{NADP}^+ \rightarrow \text{pyruvate} + \text{NADPH} + \text{CO}_2$$

In one kinetic experiment, 1 mg of native enzyme and 1 mg of mutant enzymewere added to an L-malate solution and the initial reaction rate obtained was385 µmoles pyruvate permin. What was the (S)-malate concentration in thesolution?

3. State beer-lamberts law. Why an OD above 1 is not taken? Which dye is used in Bradford method, and how does it work? What is a standard curve, and which protein is used to make standard curve in Bradford method?

4. Explain the polymerization mechanism of alginate.

5. The hexokinase enzyme that uses glucose as substrate was characterized in the presence of 2 inhibitors I_1(triangle) and I_2(x). The initial rates of the reaction were determined in the presence and absence of the inhibitors as shown below.Identify the type of inhibition by I_1 and I_2. Determine all the kinetic parameters i.e., V_{max}, K_m, K_{I1}, K_{I2}.