FINAL EXAMINATION - ANSWERS

1. What you understand by the following terms?
   a) Enzyme - Enzymes are biological molecules (typically proteins) that significantly speed up the rate of virtually all of the chemical reactions that take place within cells. They are vital for life and serve a wide range of important functions in the body, such as aiding in digestion and metabolism. In other words, Enzymes are proteins that act as biological catalysts (biocatalysts). Catalysts accelerate chemical reactions.
   b) Protein folding- this a process in which a polypeptide folds into a specific, stable, functional three-dimensional structure. It is the process by which a protein structure assumes its functional shape or conformation.
   c) Molecular chaperones – These are proteins that assist the covalent folding and the assembly or disassembly of other macromolecular structures. They are present when the macromolecules perform their normal biological functions and have correctly completed the process of folding and/ or assembly.
   d) Chaperonins: these are proteins that provide favorable conditions for the correct folding of other proteins thus preventing aggregation. Newly formed proteins must fold from a linear chain of amino acids into a three-dimensional form. They belong to a large class of molecules that assist protein folding called molecular chaperones. The energy to fold is supplied by the adenosine triphosphate.
   e) Immobilization of enzymes – The term immobilized enzymes refer to enzymes physically confined or localized in a certain way and in a certain

defined region of space with retention of their catalytic activities and can be used repeatedly and continuously.

2. One of the major functions of molecular chaperones is to prevent both newly synthesized polypeptide chains and assembled subunits from aggregating into non-functional structures. List five properties of molecular chaperones.
   i. Molecular chaperones interact with unfolded or partially folded protein subunits e.g., nascent chains emerging from the ribosome, or extended chains being translocated across subcellular membranes.
   ii. They stabilize non-reactive conformation and facilitate correct folding of protein subunits.
   iii. They do not interact with native proteins nor do they form part of the final folded structures.
   iv. Some chaperones are non-specific and interact with a wide variety of polypeptide chains but others are restricted to specific targets.
   v. They often coupe ATP binding/hydrolysis to the folding process.
   vi. Essential for viability, their expression is often increased by cellular stress.
   vii. They prevent inappropriate association or aggregation of exposed hydrophobic surfaces and direct their substrates into productive folding, transport or degradation pathways.

3. Protein stability is one of the common problems in protein expression. During this process, protein sequences cannot be deleted or mutated thus the

need for strategies for improving protein stability for these proteins. Discuss five of these strategies that improve protein stability.

i. Performing expressions in special media containing trace metals, minerals and vitamins. These chemicals may not be needed for host cell growth but they may serve as co-factor prosthetic groups or ligands for recombinant proteins. Therefore, they may be critical for correct protein folding and stability.

ii. Induce the protein at lower temperature for shorter induction time

iii. Fuse the protein with a tag or fusion partner. A tag can change the N-terminal sequence of the protein and therefore increase the yield and stability. In addition to the N-terminal sequence, a relatively large fusion partner can further stabilize the protein as compared with the protein that was expressed alone or with a small tag.

iv. Design the protein construct with intact domain or structure. – A full-length protein, a part of a protein with intact domains, or an intact domain of a protein can be stably expressed. An integral folding unit of a protein cannot be truncated. A domain is often a folding unit.

v. Change the host cell strain. – some cell strains are deficient in some proteases. For example, BL21 lacks cytoplasmic ion and periplasmic omtp proteases. Using such cell strains as these will lead to enhanced protein stability. Sometimes, simply just changing a host strain will increase recombinant protein stability.

vi. Change the location of expression. Some proteins are not stable if they are expressed in cytoplasm. When they are expressed in the periplasmic region, it becomes stable. Periplasmic expression may lead to correct folding of a protein. Periplasmic region may also lack the protease to degrade the protein.

vii. Express the protein in cell strains containing molecular chaperones. Molecular chaperones may facilitate protein folding and increase its stability.

4. There are several methods of enzyme immobilization. State FIVE below and briefly state their individual advantages and disadvantages
   i. Physical absorption – weak bonds are involved here through van der waals or ionic interractions.
      Advantages: simple and cheap and little conformational change of the enzyme.
      Disadvantage: Desorption and nonspecific adsorption.
   ii. Affinity – this is the affinity bonds between two affinity partners.
      Advantages: Simple and oriented immobilization and remarkable selectivity.
      Disadvantage: High cost.
   iii. Covalent binding – This is the chemical binding between functional groups of the enzyme and support.
      Advantage: No enzyme leakage. Potential for enzyme stabilization.
      Disadvantage: Matrix and enzyme are not regenerable and there is major loss of activity.
   iv. Entrapment – This is the occlusion of an enzyme within a polymeric network.
      Advantage: Wide applicability
      Disadvantage: Mass transfer limitations and enzyme leakage
   v. Cross-linking – Enzyme's molecules are cross-linked by a functional reactant
      Advantages: Bio- catalyst stabilization

Disadvantages: Cross-linked bio- catalysts are less useful for packed beds. They also are susceptible to mass transfer limitations and loss of activity.

5. a) The Genome is basically an organism's complete set of genetic instructions. Expand.

   In the fields of molecular biology and genetics, a genome is all genetic material of an organism. Each genome contains all of the information needed to build that organism and allow it to grow and develop. It consists of DNA (or RNA in RNA viruses). The genome includes both the genes (the coding regions) and the noncoding DNA, as well as mitochondrial DNA and chloroplast DNA.

   b) What is a proteome?

   This is the entire set of proteins that is, or can be, expressed by a genome, cell, tissue, or organism at a certain time. It is the set of expressed proteins in a given type of cell or organism, at a given time, under defined conditions. Proteomics is the study of the proteome.

   c) What are Transposable elements in genome study and practice?

   Transposable elements (TEs) are sequences of DNA with a defined structure that are able to change their location in the genome. Transposable elements are categorized as either as a mechanism that replicates by copy-and-paste or as a mechanism that can be excised from the genome and inserted at a new location In the human genome.

   d) What are Polypeptides?

   Polypeptides are chains of amino acids. Proteins are made up of one or more polypeptide molecules. The amino acids are linked covalently by peptide

bonds. A polypeptide is a longer, continuous, unbranched peptide chain of up to approximately fifty amino acids. Hence, peptides fall under the broad chemical classes of biological polymers and oligomers, alongside nucleic acids, oligosaccharides, polysaccharides, and others. A polypeptide that contains more than approximately fifty amino acids is known as a protein.

e) What is Metabolic engineering?

This is the practice of optimizing genetic and regulatory processes within cells to increase the cell's production of a certain substance. These processes are chemical networks that use a series of biochemical reactions and enzymes that allow cells to convert raw materials into molecules necessary for the cell's survival. Metabolic engineering specifically seeks to mathematically model these networks, calculate a yield of useful products, and pin point parts of the network that constrain the production of these products. Genetic engineering techniques can then be used to modify the network in order to relieve these constraints