MANIPAL INSTITUTE OF TECHNOLOGY

MANIPAL

(A constituent unit of MAHE, Manipal)

SEMESTER B.TECH END SEMESTER EXAMINATIONS

NOVEMBER/DECEMBER 2019

SUBJECT: Protein Engineering [BIO 4008]

Date of Exam: **28-11-2019** Time of Exam: **2-5 PM** Max. Marks: **50**

Instructions to Candidates:

- Answer ALL the questions & missing data may be suitable assumed

1A.	It is a common observation that antiparallel strands in a β sheet are connected by short loops, but that parallel strands are connected by α helices. Why do you think this is?	**2**
1B.	Explain the type of amino acid, interactions and packing in coiled-coil protein structure.	**4**
1C	A specific protein has an 18-residue long α-helix with the following sequence: PENQWKQELDTRYRNALQ ***i.*** How many full turns are in this α-helix? ***ii.*** How many hydrogen bonds between the backbone atoms are in this helix? Explain your reasoning. ***iii.*** Identify all residues that are involved in the formation of the hydrophobic core and hydrophilic edges of the protein in aqueous environment. ***iv.*** In addition to hydrogen bonding, what other interactions could contribute to the stabilization of this helix?	**4**
2A.	Outline the steps involved in GroEL-GroES assisted proteins folding. How does this response benefit the cell? How do you suppose the chaperones themselves manage to fold correctly?	**5**
2B.	Explain the importance of amino acid proline in protein folding?	**3**
2C.	GPCRs activate G proteins by reducing the strength of GDP binding, allowing GDP to dissociate and GTP, which is present at much higher concentrations, to bind. How do you suppose the activity of a G protein would be affected by a mutation that caused its affinity for GDP to be reduced without significantly changing its affinity for GTP?	**2**
3A.	Explain the entire events of conformational changes in protein kinase for cell cycle regulation?	**4**
3B.	Why do you suppose formation of hydrophobic patches serve as critical signals for the identification of diseased and normal protein? Explain with an example?	**4**
3C.	Why do you determine protein structure?	**2**
4A.	A peptide was digested using trypsin and chymotrypsin. Sequencing results of the cleaved product are. Trypsin treatment: YLDR, GSAK, WGSM. Pepsin treatment: YLDR, GSAK, WGSM. ***i.*** What is the sequence of your peptide? ***ii.*** Explain why neither of these steps alone is sufficient to unambiguously determine the sequence of your peptide?	**4**

	iii. Can you use any of the other three cleavage agents listed in the table in order to unambiguously determine the sequence? Explain your answer. ***iv.*** Can you use peptide hydrolysis with 6M HCl to solve the problem? Explain your answer.	
4B.	For separation of proteins by two-dimensional polyacrylamide-gel electrophoresis, what are the two types of electrophoresis that are used in each dimension? Does that makes any difference which electrophoretic method is applied first? Why or why not?	**3**
4C.	Tropomyosin 93 kd and haemoglobin 65-kd is centrifuged. Explain the order of protein sedimentation. Can you think of an analogy from everyday experience that might help you with this sedimentation?	**3**
5A.	Engineering a protein can be achieved by DNA shuffling. Outline the steps involved in the method.	**3**
5B.	Protease added in laundry detergent becomes inactivated by temperature and bleach. Discuss protein engineering principles to improve protease efficiency.	**2**
5C.	Uropathogen detection and chemical screening has great benefits for the diagnosis and treatment of urinary tract infections. Develop a portable and inexpensive analytical device for detecting the presence of pathogen and rapidly testing for nitrite on the same device.	**5**

Supplementary table

Amino acid	alanine	arginine	asparagine	aspartic acid	asparagine or aspartic acid	cysteine	glutamic acid	glutamine
One letter code	A	R	N	D	B	C	E	Q
Amino acid	glutamine or glutamic acid	glycine	histidine	isoleucine	leucine	lysine	methionine	phenylalanine
One letter code	Z	G	H	I	L	K	M	F
Amino acid	proline	serine	threonine	tryptophan	tyrosine	valine		
One letter code	P	S	T	W	Y	V		