Department of Biochemical Engineering and Biotechnology

I SEMESTER 2016 – 2017
BEL714 – PROTEIN SCIENCE AND ENGINEERING

MAJOR TEST | **Date** | **Nov 19, 2016**

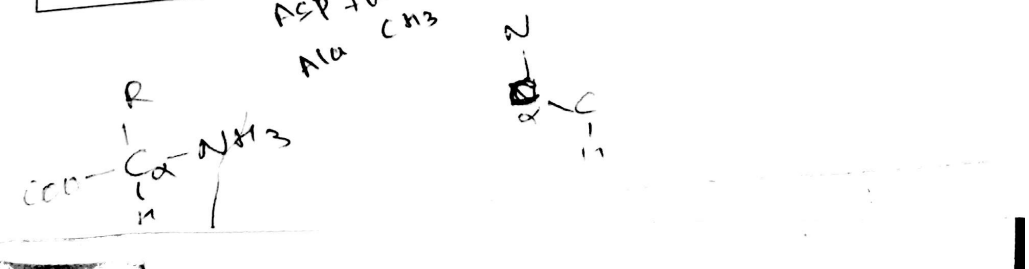
Time = 2 hours (3:30 – 5:30 PM) **(Venue - LH-512)** **Max. Marks = 40**

Instructions

1) **The question paper contains two Sections – A and B**
2) **Please use separate Major Test Answer Book for answering the questions in Section A and B respectively**

SECTION – A (30 marks)

Question #	Question	Marks
1	Describe the amphipathic (hydrophobic/hydrophilic) patterns found in alpha helices and beta strands in proteins. Explain why they exist.	(2)
2	Describe the contributions to protein stability of the following: (i) Hydrogen bonding (ii) the hydrophobic effect (iii) conformational entropy.	(3)
3	Protein engineering using directed evolution is a common strategy for improving the catalytic properties of enzymes. With examples, describe any two methods of directed evolution that could be applied and comment on its advantages and limitations.	(4)
4	Briefly describe the function of each of the following, employed in creating an incremental truncation protein fragment library: (a) Exonuclease III (b) Mungbean nuclease (c) Klenow	(1)
5	Employing an ITCHY strategy, describe how you will obtain a non-associating heterodimeric methyltransferase as a platform for developing site-specific methyltransferases. Include a detailed schematic diagram to explain various steps of protein fragment complementation and assisted re-assembly.	(4)
6	In most protein structures, a very large fraction of the residues are found either in alpha helices or in beta-sheets. a) List the key structural features of an alpha helix (hydrogen bonding pattern, direction, in which side chains point, etc.) b) Large aromatic side-chains prefer to reside in beta-sheets rather than alpha- helices. Why?	(3)
7	Using high resolution structural data on your enzyme of interest, you select a buried aspartate residue for substitution with alanine. You have reason to believe that the aspartate residue is important for substrate binding. What effect would you predict this substitution to have on the stability and activity of the enzyme? Explain your answer.	(3)



Question #	Question	Marks
8	A single-chain enzyme of relative molecular mass 15,000 has been chosen for use in an industrial process which will take place at a temperature slightly above its melting temperature, Tm. To increase the operational half-life of the enzyme at the process temperature, it has been decided to attempt to increase its stability by introducing a disulfide bridge. The enzyme has been cloned but its three-dimensional structure is not available. However, both the primary and high resolution crystal structures are available for a highly homologous enzyme from the same family. a) Explain how the primary and tertiary structural information that is available can be used to generate a homology model of the target enzyme. b) How you might use the homology model to help decide which residue(s) to mutate in order to introduce a disulfide bridge? In what situations would you choose to do Fold recognition/threading? c) Introducing a disulfide bridge is one means by which protein stability may be enhanced. Provide rationalizations for TWO other approaches which may be employed.	(2) (2) (2)
9	In class we discussed the advantages of using sequence motifs over consensus sequences. However, there are some features that sequence motifs do not capture. Carefully examine the sequences below, which are a representative part of a much larger data set. Identify a sequence pattern that is not captured by a sequence motif built from these sequences (even if you include pseudocounts). In other words, what types of sequences are unlikely to ever occur in this full dataset but would be scored well by a motif built on these data. (You do not need to compute the motif to answer this question).	(4)

1	2	3	4	5	6	7	8	9	10	11	12
A	C	T	A	T	T	C	G	T	A	G	T
G	G	C	A	T	T	C	G	T	G	C	C
A	G	A	A	T	T	C	G	T	T	C	T
G	G	T	A	T	T	C	G	T	A	C	C
C	C	G	T	T	T	C	G	A	C	G	G
C	T	G	C	T	T	C	G	G	C	A	G
A	A	G	C	T	T	C	G	G	C	T	T
C	G	A	C	T	T	C	G	G	T	C	G
C	A	C	G	T	T	C	G	C	G	T	G
C	T	A	G	T	T	C	G	C	T	A	G
C	G	C	G	T	T	C	G	C	G	C	G
A	A	G	A	T	T	C	G	T	C	T	T
A	A	C	T	T	T	C	G	A	G	T	T
T	G	G	T	T	T	C	G	A	C	C	A
T	A	C	T	T	T	C	G	A	G	T	A

SECTION – B (10 marks)

Question #	Question	Marks
10	a) While incorporating non-natural amino acids in the peptide chain, non-enzymatic acylation was done of yeast tRNA. Show the steps involved in non-enzymatic acylation. Why was not an *E. coli* tRNA chosen for the purpose?	(2)
	b) What were the experimental observations in the literature that led to the hypothesis that unnatural amino acids can be incorporated in the proteins? What experiments would you design to show that indeed the unnatural amino acid has been incorporated in the protein in *in vitro* experiments?	(2)
	c) Examine a part of the data generated through *in vitro* translation of β–lactamase gene. How do you determine the amount of β–lactamase synthesized in the *in vitro* reaction? What was the efficiency of incorporation of Phe using suppressor tRNA? Is it similar or dissimilar to what was observed with other modified amino acid. Comment.	(2)
	Amino acid / Suppressor / Enzyme synthesized (µg/ml) / km / kcat Phe / - / 26.0 ± 3.8 / 55 ± 5 / 880 ± 10 Phe / Phe-tRNA$_{CUA}$ / 2.9 ± 0.9 / 59 ± 6 / 870 p-FPhe / p-FPhe-tRNA $_{CUA}$ / 2.1 ± 0.9 / 59 ± 2 / 1120 ± 290	
11	a) What are the chemical constituents in inclusion bodies and is it good or bad to have your proteins precipitated in the form of inclusion bodies? Explain.	(2)
	b) Describe how the circular dichroism method can be used to determine the structural integrity of proteins. What would the CD data look like if the protein is fully denatured.	(2)