

**Part A – Answer any five questions (2-4 sentences) from the following set (5 points each)**

(A.1) What is the hydrophobic effect? Is this an enthalpic or entropic effect?

(A.2) What is an abzyme? Why are these not as efficient as “real” enzymes?

(A.3) Describe the mechanism of action of the HSP60 family of chaperones. What role does the rate of ATP hydrolysis play in their activity?

(A.4) What is the dielectric constant and how does it affect forces between atoms. What is the typical value of this constant in the interior of a protein ?

(A.5) Describe the mechanism used by DNA polymerases to enhance their specificity. Is this mechanism also used by RNA polymerases?

(A.6) Why do most proteins denature when exposed to very high/low pH ? Is this mechanism the same as protein denaturation by chemical agents such as high concentrations of Urea or Guanidinium chloride ?

**Part B – Answer the following questions in 1-2 sentences (Answer any five; 2 points each)**

(B.1) What is the “trigger factor” ?

(B.2) What is a molten globule ?

(B.3) Why are proteins only marginally stable?

(B.4) Is cold denaturation of proteins an enthalpic or entropic effect? Explain your answer.

(B.5) What is the time scale for folding of proteins ?

(B.6) What is Levinthal’s paradox? Is this true?

**Part C – Answer any four of the following questions in 1-2 sentences (2.5 points each)**

**You have discovered a novel bacterial protein, BmbA, and would like produce it using the T7 over-expression system in *E. coli*.**

(C.1) Name and describe in a sentence, two different affinity tags that you could use to purify BmbA.

(C.2) Your initial over-expression tests show that BmbA is prone to aggregation and inclusion body formation. Name and describe in a sentence, a solubility “tag” that could be tried to improve the expression of soluble BmbA.

(C.3) From its sequence, you compute that BmbA has a pI of 4.9. Which type of ion exchange column can be used to purify BmbA. What pH buffer would you use for this purification? (explain your answer).

(C.4) After purification, you get reasonable pure BmbA. However, this protein behaves anomalously on a SDS gel and you are not sure if you have expressed the full protein. Describe one method that can be used to check if the protein that you expressed and purified is indeed BmbA.

(C.5) You suspect that BmbA is a very stable protein. Describe a technique to measure the  $T_m$  for BmbA, and to estimate the  $\Delta H$  for its denaturation.