M.TECH/BT/1st SEM/BIOT 5103/2017
PHYSICOCHEMICAL TECHNIQUES IN BIOTECHNOLOGY
(BIOT 5103)

Time Allotted: 3 hrs **Full Marks: 70**

Figures out of the right margin indicate full marks.

Candidates are required to answer Group A and
Any 5 (five) from Group B to E, taking at least one from each group.

Candidates are required to give answer in their own words as far as practicable.

Group – A
(Multiple Choice Type Questions)

1. Choose the correct alternative for the following: **10 × 1=10**

(i) Pair of amino acid residues that can form salt bridge are
(a) Lys and Arg (b) Lys and Glu
(c) Glu and Asp (d) Glu and Glu

(ii) When the power of ocular lens is 10X and objective lens is 20X, the magnification is
(a) 30 times (b) 20 times (c) 200 times (d) 2000 times

(iii) 310 helix and π helix are
(a) less stable and less common than α helix
(b) more stable but less common than α helix
(c) less stable but more common than α helix
(d) as stable and as common as α helix

(iv) The interaction that is NOT an weak interaction is
(a) hydrogen bond (b) hydrophobic interaction
(c) disulfide bond (d) electrostatic bond

(v) Which of the following is best suited to get the surface view of an object?
(a) SEM (b) TEM
(c) both (a) and (b) (d) Compound microscope

(vi) Singular value decomposition (SVD) is a mathematical theorem that is used in data deconvolution of several biophysical techniques of which one is
(a) NMR (b) EPR (c) MRI (d) CD

(vii) The shift of absorption toward a longer wavelength due to addition of a group is called
(a) bathochromic shift (b) hypsochromic shift
(c) hypochromic shift (d) hyperchromic shift

(viii) In which of the following wavelength ranges the absorption of UV light by nucleic acids occurs?
(a) 190-210 nm (b) 280-310 nm
(c) 260-275 nm (d) 340-438 nm

(ix) *Lippert* equation predicts which of the following?
(a) dynamic quenching effect
(b) effects of bathochromic shift
(c) fluorescence energy shift for general solvent effects
(d) fluorescence energy shift for specific solvent effects

(x) In atomic force microscopy (AFM) the most common mode of operation is
(a) tapping mode (b) non contact mode
(c) contact mode (d) dynamic force mode

Group - B

2. (a) Phenylalanine is a hydrophobic amino acid residue. However, sometimes it is found on the surface of a protein. Explain the observation.

(b) State the likely positions (on the surface or in the core) of the following amino acid residues in a protein molecule. State the reason.
i)Glu, ii) Arg, iii) Val, iv) Trp, v) Ile, vi) Asn, vii) Lys, viii) Ser, ix) Thr

3 + 9 = 12

3. (a) A specific protein has an 18-residue α helix with the following sequence.
Glu-Asn-Gln-Tyr-Lys-Glu-Glu-Leu-Asp-Thr-Arg-Tyr-Arg-Asn-Ala-Leu-Gln-Pro
i) How many full turns are there in the α helix?
ii) What is the length of the helix?
iii) How many hydrogen bonds will be present in the backbone of the helix? Explain your answer.

(b) In the above sequence, mark three possible pairs of amino acid residues that can form a salt-bridge.

(2+2+5) + 3 = 12

Group - C

4. (a) For the peptide Met-Ala-Met-Val-ser-Glu-Phe-Leu-Lys-Gln-Ala-trp-Phe-Ile-Glu-Asn-Glu-Glu-Gln-Glu-Tyr-Val-Lys-Ser-Ser-Lys-Gly-Gly-Pro-Gly-Ser-Ala-Val-Ser-Pro-Tyr-Pro-Thr-Phe-Asn-Pro-Ser-Ser in water, estimate (a) the molar extinction coefficient at 280 nm. (with ϵ for Cys_2 as 120, for Trp as 5690 and for Tyr as 1280).

   (b) How would you spectrophotometrically find the concentration of an aqueous solution of a DNA sample? What does the ratio A_{260}/A_{280} mean?

   (c) Why is ϵ (190 nm) important for protein concentration measurement? What are the precautions necessary for such measurements?

   (d) What is solvent perturbation in UV spectroscopy? What is it useful for?

3 × 4 = 12

5. (a) Draw the normal vibrational modes for CO_2 with assignments for the bands.

   (b) Phenacetin was the first synthetic anti-pyretic pharmaceutical to be commercialized. Draw the signature regions of its FT-IR spectrum. Based on its chemical structure, assign the particular signature bands to the respective bonds in the structure

   (c) Give a tabular representation of NMR derived structural parameters of molecules. Briefly itemize how NMR can be used to determine the solution structure of proteins and peptides.

3 + (2 + 2) +(3 + 2) = 12

Group - D

6. (a) What is the expression for fluorescence quantum yield (ϕ)? What does it measure? How is it evaluated in practice for biochemical systems?

   (b) Write out the Stern-Volmer equation for collisional (dynamic) quenching property defining all the terms.

   (c) Iodide quenching or tryptophan fluorescence can be used to determine whether tryptophans are exposed to the solvent. If a protein is known to contain only one tryptophan and iodide fails to quench, what possible explanations might be given to account for the lack of quenching? If the protein contains eight tryptophans and iodide quenches 25% of the fluorescence, it is tempting to assume that two

tryptophans are accessible to the solvent. State several factors that would make this conclusion invalid.

(2 + 1 + 1) + 3 + (2 + 3) = 12

7. (a) If a molecule emitted light on excitation, what experiment would you do to tell if the process was fluorescence or phosphorescence ?

   (b) Quinine has a fluorescence quantum yield approximately equal to 1. Light at 10^{15} photons s^{-1} is incident on a solution of quinine in a 1 cm cell, and the concentration of quinine is 1.0 X 10^{-2} M. The wavelength is 300 nm, and the extinction coefficient is 1.0 X 10^4 at this wavelength. How many photons are fluoresced per second?

   (c) Why is a fluorescence spectrum independent of the wavelength of excitation? What are the types of fluorescence measurements that should be done to distinguish between static and dynamic quenching? How can FRET be applied for DNA sequencing applications?

3 + 3 + (2 + 2 + 2) = 12

Group - E

8. (a) Write down names of three different fluorophore. What is immunofluorescence?

   (b) When electrons interact with matter what are the different processes that may happen? Write down the working principle of an atomic force microscope.

(3 + 2) + (2 + 5) = 12

9. (a) Using essential component diagrams, differentiate between scanning electron and transmission electron microscopies (viz. SEM and TEM)

   (b) Explain using *appropriate parameters*, how would you calculate the *resolution limit expression* of a transmission electron microscope? Cite two biophysical applications of a TEM for large protein complexes.

   (c) Briefly explain EITHER
   (i) phase contrast microscopy OR
   (ii) epifluorescence light microscopy .

4 + (3 + 2) + 3 = 12