(c) There are two sites for attachment of fluorescent labels in a protein X. A pair is used for which R_0 is $23A^0$. The energy transfer efficiency is 1.5%. Estimate the distance between the labels.

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

7. (a) Define the terms in the elastic scattering equation $I_\theta / I_0 \approx R_\theta = P_\theta$ X K X cXM. What are its assumptions. What is the corrected Debye scattering equation for non-ideal solutions? Define the distinguishing parameters in the non-ideal case.

(b) (i) Qualitatively explain how wavelength analysis of scattered light (part of dynamic light scattering) allows to explain transport properties of biological macromolecules.

(ii) Use the Stokes-Einstein equation to define the diffusion coefficients D and D_0 and the frictional coefficient ratio f/f_0. Explain the characteristics of the hydrodynamic radius.

(c) How have photobleaching experiments for measuring diffusion of small DNA molecules in cells been used for gene delivery/antisense therapy applications?

(1 × 4) + (1 + 2 + 2) + 3 = 12

Group – E

8. (a) Use a diagram to depict the operating principles and instrumentation of total internal reflectance microscopy (TIRF). Explain how the evanescent wave technique gives better optical resolution than confocal methods. To what biological samples has this technique been most extensively applied?

(b) Explain the technique of Immunofluorescent microscopy. What are the typical labels used and why? Outline its applications with specific reference to cell biology and bacteriology.

(c) Pointwise elaborate the technique and sample preparation protocol in cryo-electron microscopy. Distinguish between low-dose and high-dose sample imaging techniques in cryo-electron microscopy. What are some of the biological samples whose structure determination by cryo-EM have made the technique a viable one for protein structure prediction?

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

9. (a) Use a labelled information flow diagram to represent the functioning of a laser scanning confocal microscope (LSCM). What is the powerful operational detail in the LSCM approach? Outline two major advantages of the LSCM approach compared with conventional epifluorescence microscopy.

(b) Draw a schematic diagram of an atomic force microscope (AFM). Briefly describe the *tapping* mode of operation of an AFM instrument. How has AFM been used in single molecule studies of active transcription by RNA polymerase? Use a sketch of a force-distance curve to represent the unfolding of the protein titin with AFM.

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

PHYSICOCHEMICAL TECHNIQUES IN BIOTECHNOLOGY
(BIOT 5102)

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) Which of the following is NOT correct about the stabilizing forces in a protein molecule?
(a) Protein structures are maintained by noncovalent forces
(b) Interactions within a protein molecule include electrostatic interactions but not van der Waals forces, and hydrogen bonding
(c) Electrostatic interaction is a stabilizing force in a protein structure
(d) Hydrophobic interaction occurs between non polar amino acid residues.

(ii) Which amino acid possesses a net negative charge at pH 7.0?
(a) Lysine (b) Phenylalanine
(c) Glutamic acid (d) Leucine.

(iii) The fluorescence excitation decay is given by which one of the following choices?
(a) $-dN(t)/dt = N(t)k$ (b) $N(t) = N(0)\, e^{-kt^2}$
(c) $-dN/dt = N(t)k^2$ (d) $N(t) = N(0)\, /e^2$

(iv) The FRET effect is given by which of the following expressions assuming R_0 is the distance between donor and acceptor chromophores?
(a) FRET$\propto 1/R_0^6$ (b) FRET$\propto R_0^6$
(c) FRET $\propto K_a R_0$ (d) FRET $1/R_0^4$.

(v) Using homonuclear and heteronuclear NMR respectively, the number of optimal restraints that will produce a NMR structure of a protein with 0.2 nm resolution are
(a) 15/25 (b) 10/20 (c) 5/10 (d) 20/30.

(vi) Degree of scattering in transmission electron microscope is a function of
(a) wavelength of electron beam used
(b) number of atoms that lie in the electron path
(c) number and mass of atoms that lie in the electron path
(d) mass of atoms that lie in the electron path.

(vii) On what factors do the intensity of secondary electrons depends upon?
(a) Shape of the irradiated object
(b) Chemical composition of the irradiated object
(c) Number of electrons ejected
(d) Size and chemical composition of the irradiated object, number of electrons ejected and on the number of electrons reabsorbed by surrounding matrix.

(viii) In the two-state model representing structural transitions in biopolymers, which of the following models is most representative of what happens in practice in such systems?
(a) All-or-none (b) Non-cooperative
(c) Zipper (d) None of the above.

(ix) 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.

(x) Which of the following light is suitable for getting maximum resolution?
(a) Red (b) Green (c) Blue (d) Orange.

Group – B

2. (a) State whether the following statements are True or False. Justify your answer.
(i) Serine can be found only on the surface of a protein
(ii) Proline is a helix breaker
(iii) Addition of ethanol alters the three dimensional structure of a protein.

(b) An alpha helical segment in a protein has molecular weight of 2750. Calculate the number of amino acid residues and number of full turn present in that segment. (Given average molecular weight an amino acid residue in a protein structure is 110.

(3 × 3) + (1 + 2) = 12

3. (a) Some proteins can regain their native structure after removal of the denaturing agent, but some proteins cannot. Explain the above phenomenon with the energy diagram.

(b) Melting of an alpha helix is a cooperative process — Explain. Derive the relation between melting point (Tm) and ΔH for melting of an alpha helix.

5 + (4 + 3) = 12

Group – C

4. (a) How would you determine the concentration of an aqueous solution of a DNA sample using UV spectroscopy? Explain the calculations. Using an appropriate spectrum, enumerate the distinct features of a UV difference spectra compared to absolute spectra. Name four common applications of difference UV spectroscopy.

(b) Outline the technology and procedure respectively behind the optics and sample preparation for IR spectroscopy. Comment briefly on sensitivity and

resolution of the IR technique as applied to biological macromolecules. What are the wavenumbers for IR absorption bands for SSE's (secondary structural elements) in proteins? How are exchangeable hydrogen bonds detected by IR?

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

5. (a) The following data list the CD maxima for β-lactoglobulin A under two different conditions.

$\Lambda_{max,min}$(nm)	Δ€
In aqueous buffer, pH 5	
215	-1.7
196	+2.6
In 99% ethanol,0.01M HCl	
220	-7.0
208	-8.0
192	+15.5

Describe stepwise qualitatively what happens to β-lactoglobulin when it is transferred into *acidic* ethanol solution based on the above CD data. Explain your answer.

(b) A ligand L binds to a protein, and in so doing, affects the protein's 100 MHz NMR spectrum. In particular, proton resonances in the range of -80 to -160 Hz (relative to a DSS standard) are affected most. An affinity label analog L" of the ligand L is prepared. This label binds (covalently) to the protein. The attachment site proves to correspond to a tyrosine residue. Are the affinity labelling results consistent with the NMR results? If you were permitted to run one more NMR experiment to check your conclusion what would that experiment be? Explain your answers.

(c) What are the operating principles for atomic absorption spectroscopy (AAS)? Cite two of its major applications.

4 + (2.5 × 2) + (2 + 1) = 12

Group – D

6. (a) Explain the difference between fluorescence and phosphorescence. Why is a fluorescence spectrum independent of the wavelength of excitation?
Using the Einstein coefficient for spontaneous fluorescence to be A = 1.1 X 10^8 molecules sec^{-1}, calculate the intrinsic fluorescence lifetime of Tryptophan, τ_0. If the quantum yield q for in a protein is 0.3, what will be the corresponding lifetime?

(b) Write and define the parameters in the Stern-Volmer equation for dynamic (collisional) quenching. What is the corresponding equation for static quenching? What measurements would you undertake to distinguish between the two quenching processes? Iodide quenching decreases fluorescence intensity. Would you expect there to be a change also in the shape of the excitation or emission spectrum? Explain your answer.