Mid-semester examination

BT 501: Biotechniques (July-Nov, 2022) **September 22, 2022**

Maximum marks: 60 (to be normalized to 30) **Time: 2 hours (9-11 A.M.)**

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Instructions:

1. **This question paper is divided into two parts: Part A and Part B.**
2. **There are 9 questions.**
3. **Attempt all the questions.**

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Part A

Q1. In a hypothetical experiment, you are given the task of determining the concentration of six different proteins. **Only** the following details are provided about those proteins: 5

(a) Short amino acid stretches of these proteins are known, and incidentally they resemble the names of few of your classmates.

P1. SANAWASTHI
P2. AARITHDAMIANDAVIS
P3. ISHITAMATHR
P4. SWATHYGPALN
P5. NIRMALSARKAR

(b) 2. Molar extinction coefficients of Tryptophan and Tyrosine at 280 nm are 5600 $M^{-1}cm^{-1}$ and 1400 $M^{-1}cm^{-1}$, respectively.

(c) All the proteins that absorb at 280 nm have a uniform Absorbance of 0.34 in a 1 cm path length cell.

Answer the following questions with this information

(a) Concentration of which all proteins can be accurately estimated with the information provided? Why?

(b) Estimate the concentrations of as many proteins possible with the information provided.

Q2. 8 M urea or 6 M guanidine hydrochloride are very commonly used to denature a protein. Assume that you only have an IR Spectrophotometer to monitor the denaturation of SH3 domain protein (PDB ID: 1SHG.pdb). Structure of the protein is given below. Design an experiment to monitor the completion of this experiment. Explain the rationale. 5

SH3 domain (PDB ID: 1SHG.pdb)

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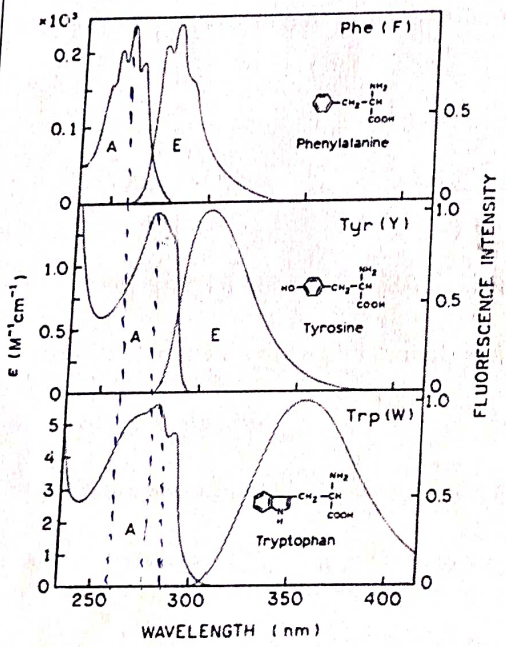
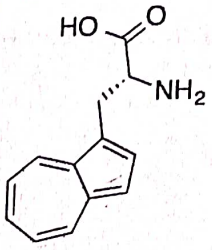
Q3. Absorption and emission spectra of three aromatic amino acids, F, Y and W are shown in the following figure 1A. Draw an approximate absorption spectrum of the two molecules in figure 1B and 1C, with x axis and y axis properly labeled. Are they (1B or 1C) likely to have any color? If YES, why?

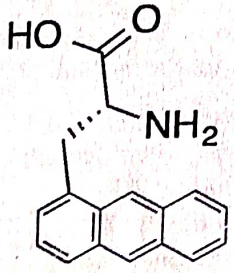
Figure 1A



1B



1C



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Q4. The vibration frequency of $^{2}H^{35}Cl$ is 2144 cm^{-1}. Which among the following molecules, $^{1}H^{35}Cl$, and $^{2}H^{37}Cl$, can be clearly distinguished from $^{2}H^{35}Cl$, in an IR spectrum recorded with identical parameters. Rationalize your answer after estimating the frequency for $^{1}H^{35}Cl$, and $^{2}H^{37}Cl$.

Notes: Isotopes can have different masses; IR spectrum is usually reported in wave numbers, with a unit of cm^{-1}.

Q5. The transmittance of an aqueous solution of $KMnO_4$ at a certain wavelength is 1 percent (0.01) for 10^{-3} M solution in 1 cm cell. What are (a) its absorbance and (b) the molar absorption coefficient.

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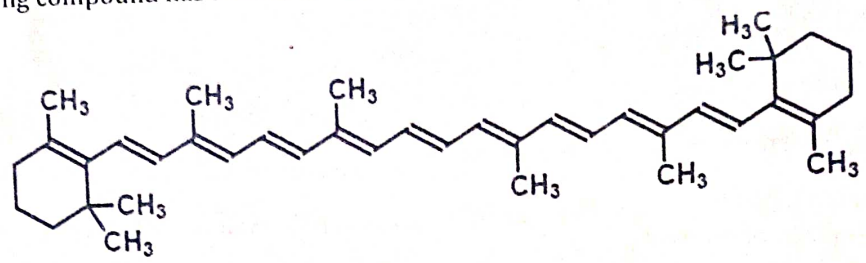
Q6. The following compound has a λmax of 453 nm.



What is the color of this compound?

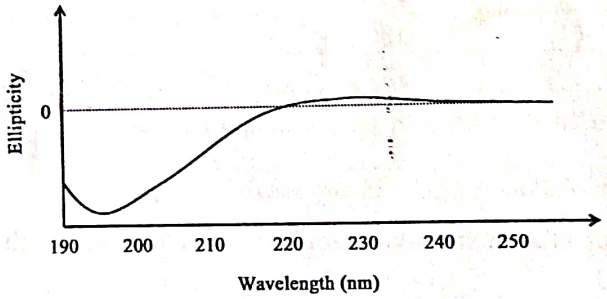
The extinction coefficient of this molecule is 139,500 $cm^{-1}M^{-1}$. Write all your inferences with regard to allowed and forbidden transitions at λ_{max} 453 in the absorption spectrum of the above molecule.

Part B

{2 × 5 = 10 marks}

Q.7 Answer the following questions:

(a) The far-UV CD spectrum recorded for a protein in water is shown below. What can you say about the protein structure from the spectrum? (mention only the most predominant conformation).



(b) Arrange the following processes with decreasing rate constants (increasing lifetime): *Fluorescence, internal conversion, absorption, phosphorescence*

(c) How is circularly polarized light generated using plane polarized light?

(d) What is the difference between circular dichroism and circular birefringence?

(e) Folding/unfolding of a protein can be studied using circular dichroism spectroscopy. Why such experiments are usually carried out at 220 nm?

Q.8 Bovine rhodopsin is a photoreceptor protein present in retinal rod cells and plays a key role in vision. It has a tightly bound 11-*cis*-retinal that has a strong absorbance at about 500 nm. The protein has a molecular weight of around 35 kDa. Three sites (sites A, B, and C) were labeled on the protein with fluorescent probes, A, B, and C, respectively. Resonance energy transfer was measured for each pair *i.e.* from A→11-*cis*-retinal, B→11-*cis*-retinal, and C→11-*cis*-retinal, A→B, A→C, B→C. The results of the experiments are summarized here:

Energy donor	Energy acceptor	Transfer Efficiency	R_0 (Å)
A	11-*cis*-retinal	0.09	51
B	11-*cis*-retinal	0.36	52
C	11-*cis*-retinal	0.12	33
A	B	0.90	51
A	C	0.92	48
B	C	0.92	47

(a) Calculate the distances between these six sites. {6 marks}

(b) A protein of molecular weight 35 kDa that is spherical has a radius of 40 Å. What can you say about the shape of rhodopsin? {2 marks}

Q.9 An amphipathic helical peptide contains a tryptophan residue on its hydrophobic face. The peptide binds to the lipid vesicles. The tryptophan fluorescence quenching data by the aqueous dynamic quencher acrylamide for the peptide is given below:

Acrylamide (M)	Fluorescence intensity in buffer without lipid vesicles	Fluorescence intensity in the buffer containing lipid vesicles
0	114	114
0.02	78	103
0.04	60	95
0.06	50	90
0.08	45	83
0.1	38	80
0.12	33	75
0.14	29	72
0.16	27	69

(a) Construct a neat, labeled Stern-Volmer plot for both the data (both the data plotted together on same graph). {6 marks}

(b) Determine the Stern-Volmer constant for both the cases. {2 marks}

(c) If fluorescence lifetime of the unquenched tryptophan is 3 ns for both free and lipid-bound peptide, determine the bimolecular quenching constants (k_q) for both the cases. {2 marks}

(d) If k_0 for tryptophan-acrylamide fluorophore-quencher pair is 0.8×10^{10} $M^{-1}cm^{-1}$, determine the efficiency of quenching for both the cases. {2 marks}