INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI

MID-SEMESTER EXAMINATION, 2024

BT 624: Fluorescence Techniques in Biotechnology

Date: Feb 28, 2024 Time: 2 – 4 P.M. Maximum marks: 30

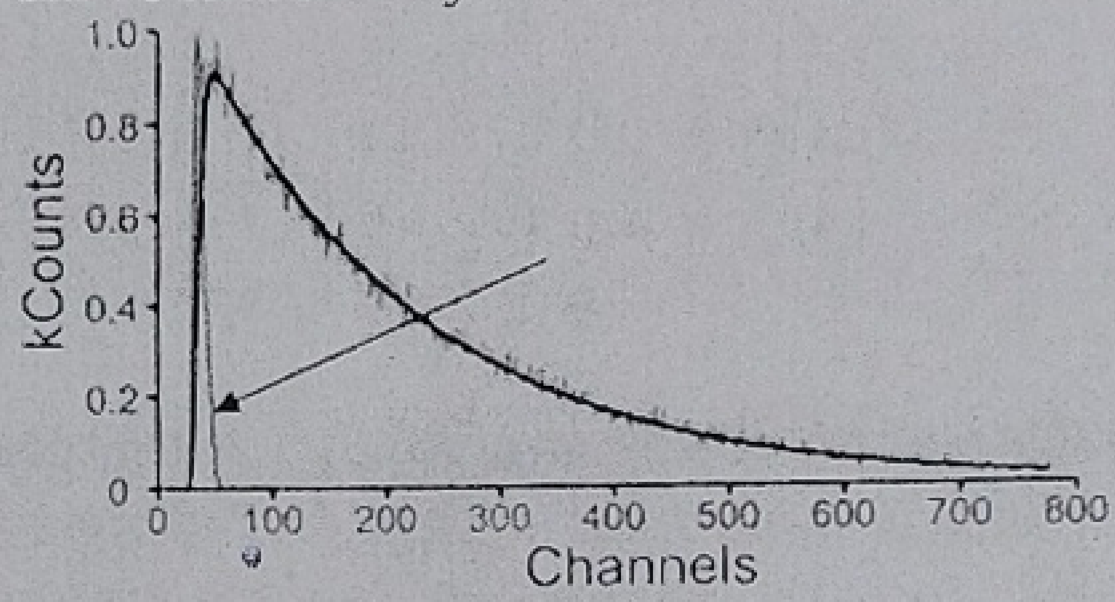
Name: .... Roll No.

Instructions

1. Write your name and Roll No. on question paper as well as on the answer sheet.
2. The question paper carries 10 questions that span 2 pages.
3. Some formulas are given in the Appendix. You can use them if required, otherwise ignore them.
4. You will be given a Graph sheet. If you need more than one, please free to ask.

Attempt all questions

1. The fluorescence emission intensity decay upon a picosecond excitation (fitted exponentially-decaying line) is shown below. What is the curve marked by the arrow? {1 mark}



2. Define the following in not more than 2 sentences for each part {1×4=4 marks}
   a. Internal conversion
   b. Photoselection upon excitation with polarized light
   c. G-factor in measuring fluorescence anisotropy
   d. Inner filter effect

3. Does fluorescence anisotropy depend on wavelength? (Answer in Yes/No) {1 mark}

4. Name any one technique that is used to measure fluorescence lifetime. {1 mark}

5. Name any two mechanisms of fluorescence quenching. {2 marks}

6. What is the maximum theoretical value of fluorescence anisotropy for a fluorophore upon single photon excitation? You recorded steady-state fluorescence anisotropy of an isotropic solution of a fluorophore. The observed anisotropy is 0.8. Explain this observation. {1+1 mark}

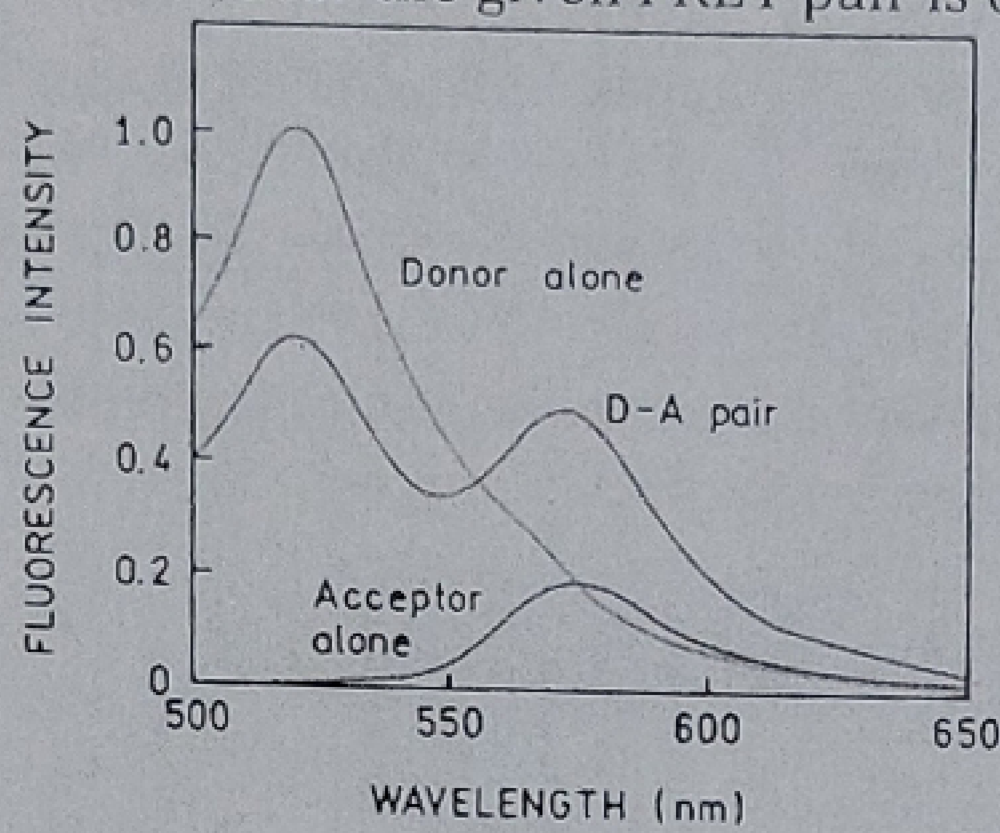
7. Pyrene is a fluorescent probe used for labeling sulfhydryl residues on proteins. The fluorescence lifetime of pyrene on a protein sulfhydryl was measured by determining the relative fluorescence intensity after excitation of pyrene with a flash lamp. Typical data are given in the table overleaf.

Relative Fluorescence intensity	Time (ns)
0.716	20
0.513	40
0.264	80
0.189	100

   a. Assuming single exponential decay, determine the fluorescence lifetime. {3 marks}
   b. If the quantum yield is 0.7, what is the natural (intrinsic) lifetime? {1 mark}
   c. When a drug molecule binds to the protein, the fluorescence lifetime becomes 10% shorter. What might be the cause of this change? {1 mark}

8. What is orientation factor in Förster resonance energy transfer? What is the maximum value that orientation factor can take? Which orientation gives this (the maximum) value? {3 marks}

9. Calculate the distance between the donor and acceptor molecules from the given Förster resonance energy transfer data if the Förster distance for the given FRET pair is 65 Å. {3 marks}



10. Iodide is a known fluorescence quencher. You are studying the tryptophan fluorescence quenching of a protein using potassium iodide. The protein is excited at 295 nm and fluorescence emission intensities (at different potassium iodide concentrations) are recorded at emission maximum wavelength. The fluorescence emission intensity data are shown below.

Concentration of KI (M)	Concentration of KNO_2 (M)	Fluorescence intensity (AU)
0	0.5	1000
0.05	0.45	200
0.1	0.4	100
0.2	0.3	43
0.3	0.2	25
0.4	0.1	15
0.5	0	9

a. Why is KNO_2 used in this experiment? {1 mark}
b. Trp absorption maximum is near 280 nm, what could be the reason behind using 295 nm excitation wavelength? {1 mark}
c. Draw a neat, labelled Stern-Volmer plot. {2 marks}
d. Is the quenching static or dynamic or both? {1 mark}
e. Determine the quenching constant(s) (K_D, K_S, or both: depends on your answer in part 'c'); *Given: fluorescence lifetime in the absence of quencher is 10 ns.* {3 marks}

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APPENDIX

$$\frac{F_0}{F} = (1 + K_D[Q]) \exp([Q]VN/1000)$$

$$r_0 = \frac{2}{5}\left(\frac{3\cos^2\beta - 1}{2}\right)$$

$$E = \frac{R_0^6}{R_0^6 + r^6}$$

$$k_T(r) = \frac{1}{\tau_D}\left(\frac{R_0}{r}\right)^6$$

$$\frac{F_0}{F} = 1 + (K_D + K_S)[Q] + K_D K_S [Q]^2$$

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}}$$

$$\frac{F_0}{F} = 1 + K_{app}[Q]$$

$$R_0^6 = \frac{9000(\ln 10)\kappa^2 Q_D}{128\pi^5 N n^4}\int_0^{\infty} F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda$$

$$I(t) = I_0 e^{-t/\tau}$$

$$\frac{r_0}{r} = 1 + \tau/\theta = 1 + 6D\tau$$

$$\frac{1}{r} = \frac{1}{r_0} + \frac{\tau RT}{r_0 \eta V}$$

$$\frac{F_0}{F} = 1 + k_q\tau_0[Q] = 1 + K_D[Q]$$