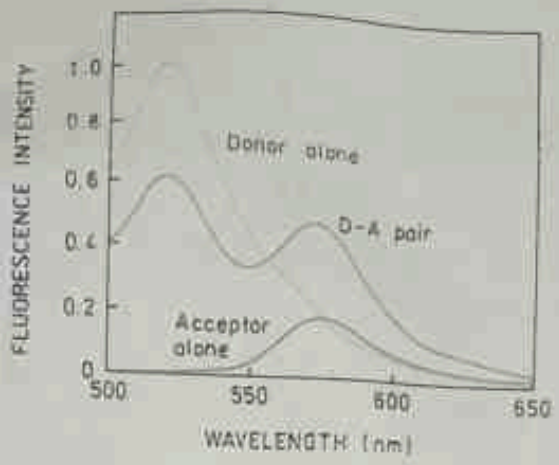
5. Calculate the distance between the donor and acceptor molecules from the given Förster resonance energy transfer data if the Förster distance for the given FRET pair is 65 Å.

{4 marks}



6. You are studying the fluorescence quenching of a peptide containing a single Trp. The acrylamide quenching data recorded at emission maximum (λ_{ex} = 295 nm) is shown below.

Concentration of acrylamide (M)	Fluorescence intensity (AU)	Lifetime (ns)
0	1000	17.6
0.05	216	3.26
0.1	94	1.8
0.2	43	0.95
0.3	27	0.64
0.4	15	0.49
0.5	7	0.39

a. Trp absorption maximum is near 280 nm, what could be the reason of using 295 nm excitation wavelength? **{1 mark}**

b. Draw a neat Stern-Volmer plot. **{3 marks}**

c. Is the quenching static or dynamic or both? **{1 mark}**

d. Determine the quenching contant(s) (K_D, K_S, or both: depends on your answer in part 'b') **{3 marks}**

APPENDIX

$$E = \frac{R_0^6}{R_0^6 + r^6} \qquad \frac{F_0}{F} = 1 + (K_D + K_S)[Q] + K_D K_S [Q]^2$$

$$\frac{\tau_0}{\tau} = 1 + \tau k_Q = 1 + 6D\tau \qquad r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} \qquad I(t) = I_0 e^{-t/\tau}$$

INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI

MID-SEMESTER EXAMINATION, 2023

BT 624: Fluorescence Techniques in Biotechnology

Date: March 03, 2023 Time: 2 – 4 P.M. Maximum marks: 30

Name: Bhargav Gudlaka Roll No. 200160020

Instructions

1. Write your name and Roll No. on the answer sheet.
2. The question paper carries 6 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 ask.

Attempt all questions

1. a. Draw a labeled Jablonski diagram showing absorption, internal conversion, fluorescence, and phosphorescence. **{3 marks}**

   b. Arrange the processes mentioned in part (a) with decreasing rates (increasing lifetime). **{1 mark}**

2. Explain briefly the following terms: **{6 marks}**
   a. Fluorescence
   b. Phosphorescence
   c. Singlet state
   d. Triplet state
   e. Quantum yield
   f. Intersystem crossing

3. Using an appropriate diagram, explain the Franck-Condon principle and mirror image rule for fluorescence emission. **{4 marks}**

4. The intrinsic fluorescence anisotropy, r_0 of a fluorophore is 0.36. Calculate the hydrodynamic volume of the fluorophore from the given data if the observed anisotropy in water at 20 °C is 0.1.

   Coefficient of viscosity of water at 20 °C = 0.1 *Pa·s*

   Fluorescence lifetime: 6 *ns*

   Rotational correlation time $= \frac{\eta V}{RT}$,

   where, η is the coefficient of viscosity, V is the hydrodynamic volume of the rotating unit, R is the Universal gas constant (8.314 $J\,mol^{-1}K^{-1}$ or 8.314 $Pa.m^3.mol^{-1}K^{-1}$) and T is the temperature. **{4 marks}**