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: Aadhithya Radhika Roll No. 224106002

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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.

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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 $Pa{\cdot}s$

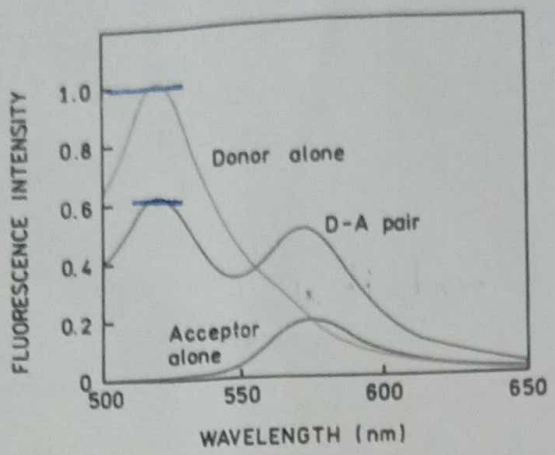
   Fluorescence lifetime: 6 ns

   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 $J{\cdot}mol^{-1}{\cdot}K^{-1}$ or 8.314 $Pa.m^3{\cdot}mol^{-1}{\cdot}K^{-1}$) and T is the temperature.

   {4 marks}

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)	$(\frac{\tau_0}{\tau}-1)\frac{1}{Q}$
0	1000	17.6	—
0.05	216	3.26	87.97
0.1	94	1.8	87.77
0.2	43	0.95	87.63
0.3	27	0.64	88.33
0.4	15	0.49	87.29
0.5	7	0.39	88.25

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}**

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APPENDIX

$$E = \frac{R_0^6}{R_0^6 + r^6} \qquad \frac{F_0}{F} = 1 + (K_D + K_S)[Q] + K_D K_S[Q]^2 \qquad \frac{\tau_0}{\tau} = 1 + \tau/\theta = 1 + 6D\tau$$

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

$y = A\sin(kx - \omega t)$

INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI

END-SEMESTER EXAMINATION, 2023

Course: BT624, Fluorescence Techniques in Biotechnology **Total marks: 50**

Date: May 11, 2023 **Time: 3 hours (2 –5 P.M.)**

Name: Adhithya. B **Roll No:** 224106002

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Instructions

1. Write your name and Roll No. on question paper as well as the answer sheet.
2. The question paper carries 6 questions that span 3 pages.
3. You will be given a Graph sheet. If you need more than one, please ask.
4. All questions are compulsory.

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1. Answer the following questions in not more than 4 sentences: **{2 × 7 = 14 marks}**
   a. What are quantum dots? Name any one advantage over conventional fluorophores.
   b. List any two limitations of lanthanides compared to conventional fluorophores.
   c. Briefly explain the role of tube lens in a fluorescence microscope?
   d. The data shown in Figure 1 obtained from an FCS experiment. What are the vertical and horizontal axes in this data?
   e. Look at the absorption and fluorescence emission spectrum shown in Figure 2 for quinine sulfate in 1 M H_2SO_4. Under these conditions, quinine sulfate exists as fully protonated species. The emission spectrum is not the mirror image of the absorption spectrum shown. Explain the reason behind this.
   f. You measure the anisotropy of a fluorophore. It turns out to be a negative value. How do you explain this observation?
   g. Name any two lens aberrations.

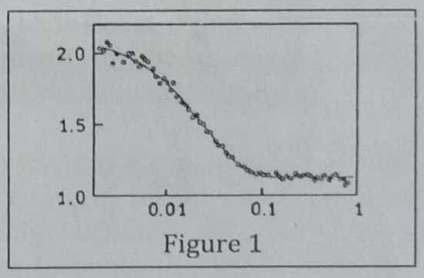

Figure 1

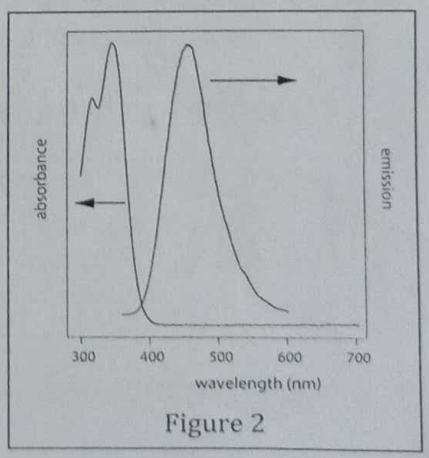

Figure 2

$\tau = 10.02\,ns$; $\tau_n = 13.361\,ns$

2. The fluorescence intensity decay data obtained after flash excitation of a fluorophore is given in the table 1 below. Calculate the intrinsic fluorescence lifetime τ_0 if the quantum yield is 0.75. **{5 marks}**
(Assume single exponential decay)

Table 1

t (nsec) = 0	2	6	14	30
I = 200	164	110	50	10

3. The binding of a small fluorescent dye with a protein is studied using fluorescence anisotropy. The data is given in table 2. Determine the dissociation constant (K_d) for the binding assuming that the bound and free dye has same quantum yield. **{5 marks}**

$f_1 = 0.2368$
$f_2 = 0.7631$

Table 2

[Fluorophore] M	[Protein] M	Observed anisotropy
1×10^{-7}	0	0.01
1×10^{-7}	2×10^{-5}	0.20
1×10^{-7}	>>K_d	0.30

$r_1 = 0.01$
$r_2 = 0.39$
$K_d = 3.1031\,M$

4. Water Raman Stokes peak is always located around 3500 cm^{-1} away from the excitation wavenumber.
   a. Convert the wavenumber (3500 cm^{-1}) to wavelength in nanometers. **{2 marks}**
   b. You record tryptophan (Trp) fluorescence emission spectrum in water. Draw a labelled qualitative fluorescence emission spectrum (from 290 – 400 nm) if the excitation wavelength is 280 nm. Label all the features of the spectrum. (Given: Raman peak lies between 3400-3600 cm^{-1} only, Trp emission maximum intensity is twice that of Raman peak and the peak width at base is 50 nm. **{4 marks}**

5. 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 diameter of 40 Å. What can you say about the shape of rhodopsin? **{2 marks}**

6. 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 lifetimes of the unquenched tryptophan are 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⁻¹s⁻¹~~ $M^{-1}s^{-1}$, determine the efficiency of quenching for both the cases. **{2 marks}**

Without Lipid	With Lipid
$K_D = 21.142\ M^{-1}$	$K_D = 4.325\ M^{-1}$
$K_{qr} = 7.047E9\ M^{-1}s^{-1}$	$K_{qr} = 1.458E9\ M^{-1}s^{-1}$
$f_q = 0.88$	$f_q = 0.182$