Quiz-I (BT 624: Fluorescence Techniques in Biotechnology)

Feb 14, 2023 | Maximum marks: 5 | Time: 20 minutes

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Instructions

1. Write your name and Roll No. on the answer sheet. A 0.5 mark penalty will be imposed for not doing that.
2. The question paper carries 2 questions that span 2 pages.
3. Answers/Solutions to be written in the space provided below the question.

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1. A fluorophore has a fluorescence lifetime of 5 ns. If the natural lifetime of the fluorophore is 25 ns, what is its quantum yield? [1 mark]

$$\tau = \frac{1}{\Gamma + K_{nr}} = 5\,ns$$

$$\tau_n = \frac{1}{\Gamma} = 25\,ns$$

$$\frac{\tau}{\tau_n} = \frac{\Gamma}{\Gamma + K_{nr}} = \frac{5}{25} = 0.2$$

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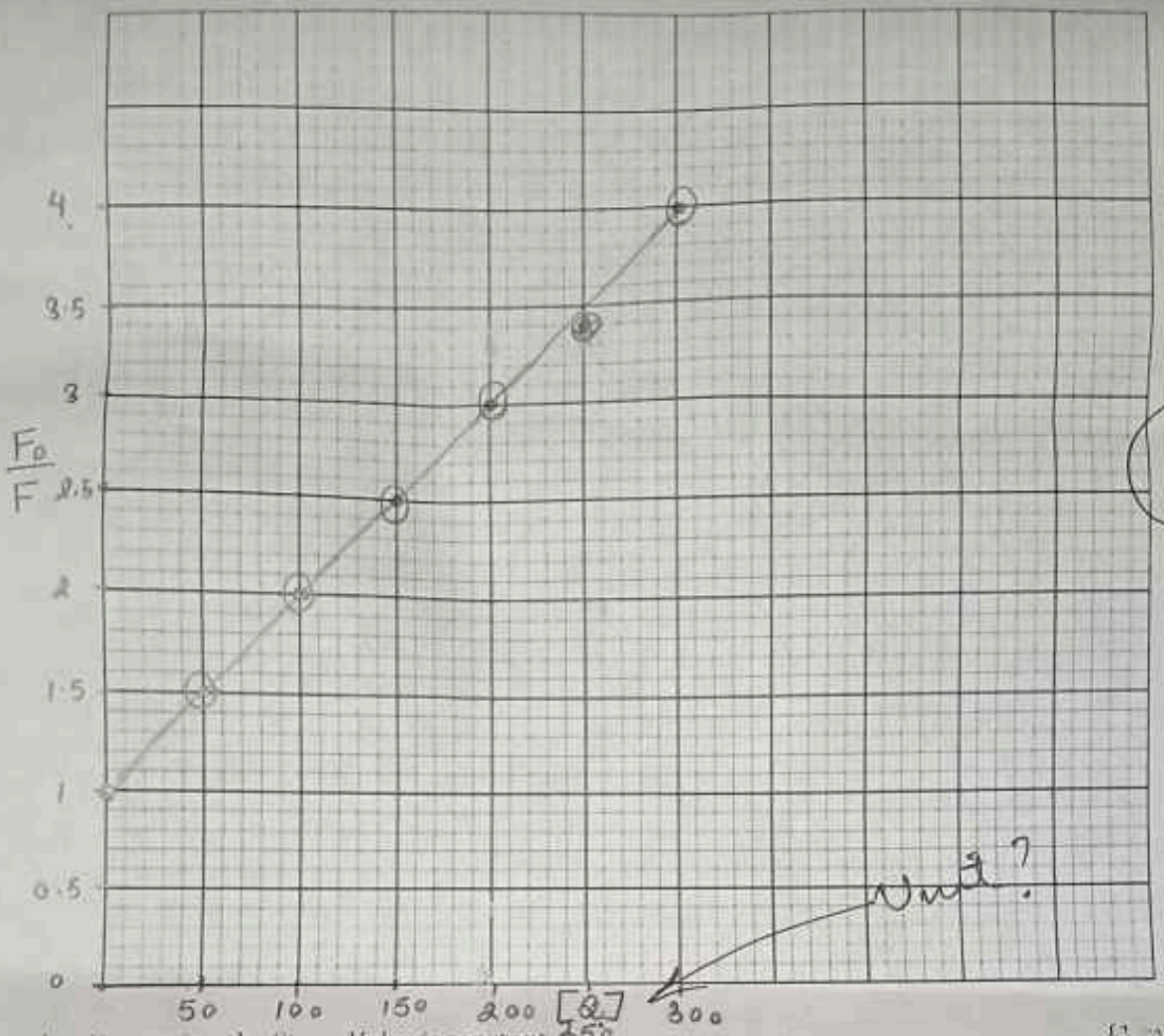
2. The tryptophan fluorescence intensity data for a peptide in the presence of aqueous quencher acrylamide is shown in the table:

Acrylamide concentration (mM)	Fluorescence intensity (Arbitrary units)	
0	100	
50	67	
100	50	
150	40	
200	33	
250	29	
300	25	

a. Draw a neat, labelled Stern-Volmer plot. (Graph paper given overleaf) [2 marks]

$$\frac{F_0}{F} = 1 + \frac{1}{Q}\left[\frac{F_0}{F} - 1\right] = K_{SV}$$

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



b. Determine the Stern-Volmer constant. {1 mark}

$k = \frac{2.5-2}{50} = 0.01\,M^{-1}$ (I know this is not the best way to get slope, but since the points are following $y=mx+c$ so religiously, slope will more or less be same. This assumption is feasible, don't need linear regression here)

c. Calculate the efficiency of quenching if the k_0 for the given system is $0.8 \times 10^{10}\ M^{-1}s^{-1}$, and the fluorescence lifetime of the unquenched fluorophore is 4 ns. {1 mark}

$K_0 =$

$K_q\tau_0 = K_{SV}$

$K_{SV} = K_0\tau$

$= 0.8\times10^{10} \times 4\times10^{-9}$

$= 3.2\times10$

$K_{SV} = 32$

$Q = \frac{\tau}{\tau_0 K_{SV}}$

$\simeq \frac{\tau}{K_{SV}} = \frac{4\times10^{-9}}{32} = 1.25\times10^{-10}$