

Department of Biosciences and Bioengineering  
Indian Institute of Technology Guwahati

Quiz 2 (BT 501: Biotechniques)

Sep 16, 2022

Maximum marks: 10 (to be scaled to 5)

Time: 30 minutes

Instructions

- Write your name and Roll No. on the answer sheet. A 0.5 mark penalty will be imposed for not doing that.
- The question paper carries 7 questions that span 2 pages.

- Fluorescence lifetime of a fluorophore under some given conditions is 10 ns. If the quantum yield under the same conditions is 0.4, what is the natural lifetime? {1 mark}

$\tau = 10 \text{ ns}$

$Q = 0.4$

$\tau_n = \frac{\tau}{Q} = \frac{10}{0.4} = 25 \text{ ns}$

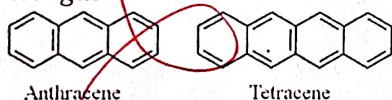
- What happens to the intensity of the unpolarised light when it passes through a linear polarizer? {1 mark}

The intensity of the unpolarized light decreases when it passes through a linear polarizer. (becomes  $\frac{1}{2}$ )

- Explain in precisely one sentence the circular birefringence. {1 mark}

Circular birefringence  $\rightarrow$  a phenomenon where the medium shows different refractive indices for left and right circularly polarized light.

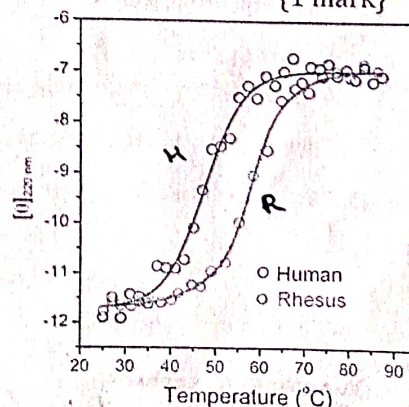
- The structures of anthracene and tetracene are given below. Which of them will emit at longer wavelength {1 mark}



- You study two similar proteins, one from human and the other one from Rhesus macaque. In an attempt to compare their thermodynamic stability, you carry out thermal denaturation and study it using CD spectroscopy. The data for the two proteins is shown here. Which of the two proteins, human or Rhesus macaque, is more thermostable? {1 mark}

~~Rhesus ma~~

Protein of Rhesus macaque is more thermostable than protein of human.





6. A protein with known structure and a single Trp residue gives a fluorescence lifetime of 3 ns for Trp fluorescence. The protein binds to a ligand that happens to be a resonance energy transfer acceptor with Trp as the donor. What will be the fluorescence lifetime of Trp in the ligand-bound form if the efficiency of energy transfer is 25%? {2 marks}

$$E = 0.25$$

~~$\tau_D = 3 \text{ ns}$~~

$$\tau_D = 3 \text{ ns}$$

$$E = 1 - \frac{\tau_{DA}}{\tau_D}$$

$$\Rightarrow 0.25 = 1 - \frac{\tau_{DA}}{3}$$

$$\Rightarrow 0.25 = 1 - \frac{\tau_{DA}}{3}$$

$$\Rightarrow \frac{\tau_{DA}}{3} = 0.75$$

$$\Rightarrow \tau_{DA} = 2.25 \text{ ns}$$

7. 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	$F_0/F$
0	1000	1
50	700	1.43
100	500	2
200	333	3.003
250	244	4.09
300	178	5.61

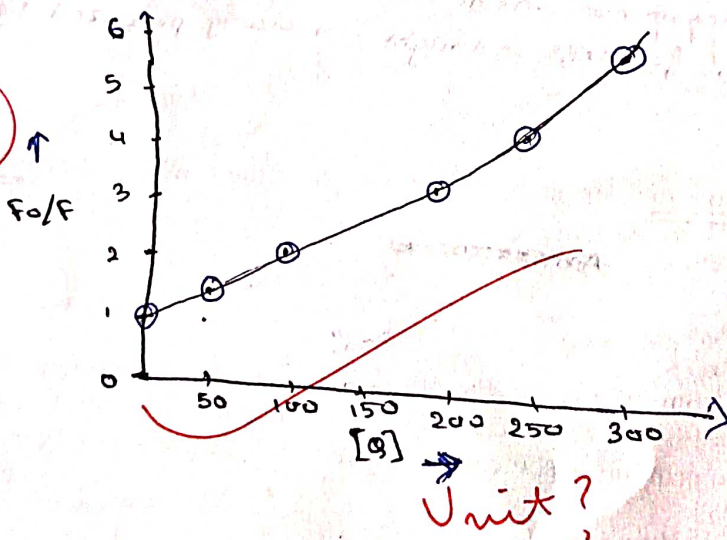
$$(y = c + mx)$$

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

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

- a. Draw a neat, labelled Stern-Volmer plot.

{2 marks}



- b. What can you say about the type of quenching?

Dynamic quenching

{1 mark}