09/10

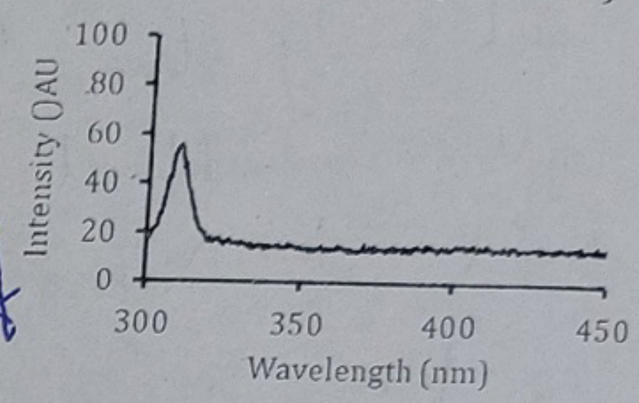
DEPARTMENT OF BIOSCIENCES AND BIOENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
BT 624: FLUORESCENCE TECHNIQUES IN BIOTECHNOLOGY
Quiz-I (Feb 02, 2024)

Total marks = 10 **Time = 30 minutes**

Name: [redacted] Roll No.: [redacted]

1. You want to study the fluorescence of a protein dissolved in water by exciting at 280 nm. You record the fluorescence emission spectrum of water as blank. The spectrum for blank is shown below. Explain the origin of the peak around 310 nm. {1 mark}

This ~~[illegible]~~ peak ~~[illegible]~~ is due to the Raman scattering ~~[illegible]~~ of water.



2. 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? {2 marks}

$\tau = 10$ ns

$Q = 0.4$

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

3. Explain briefly (not more than 2 sentences) the following: {2 marks}

a. Phosphorescence

Phosphorescence is the emission of a photon when an electron in the triplet state de-excites to the ground state. Lifetime ~~[illegible]~~ in ms to minute range.

b. Kasha's rule

Kasha's rule states that the emission spectrum does not depend on the absorption wavelength (emission occurs from the lowest vibrational state of S_1 (v_0')).

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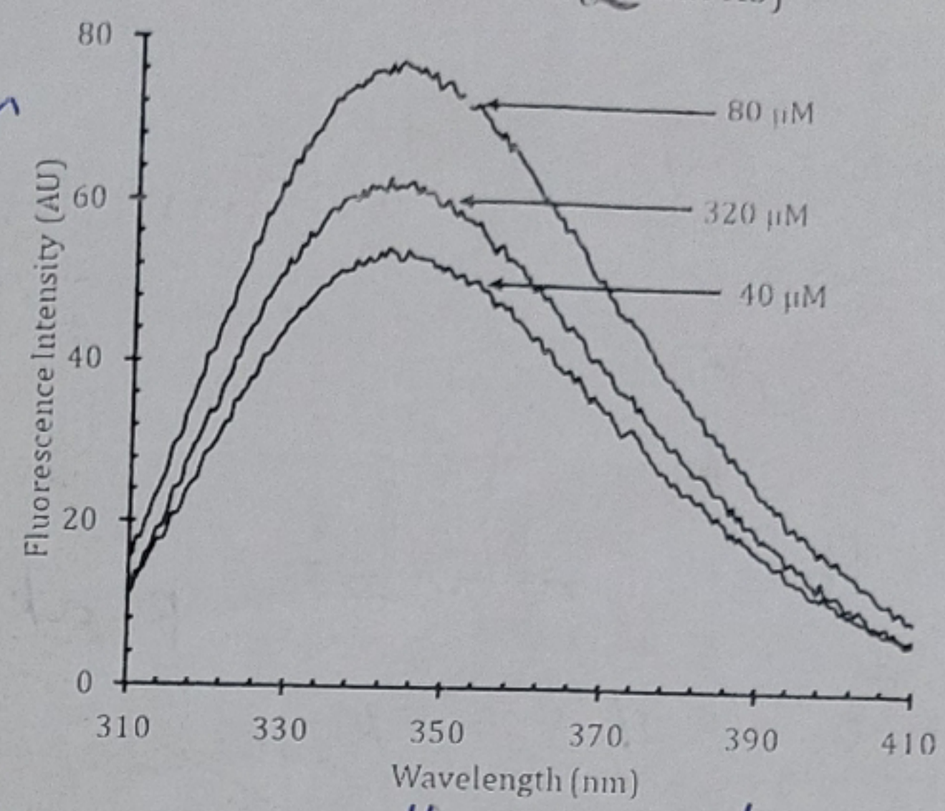
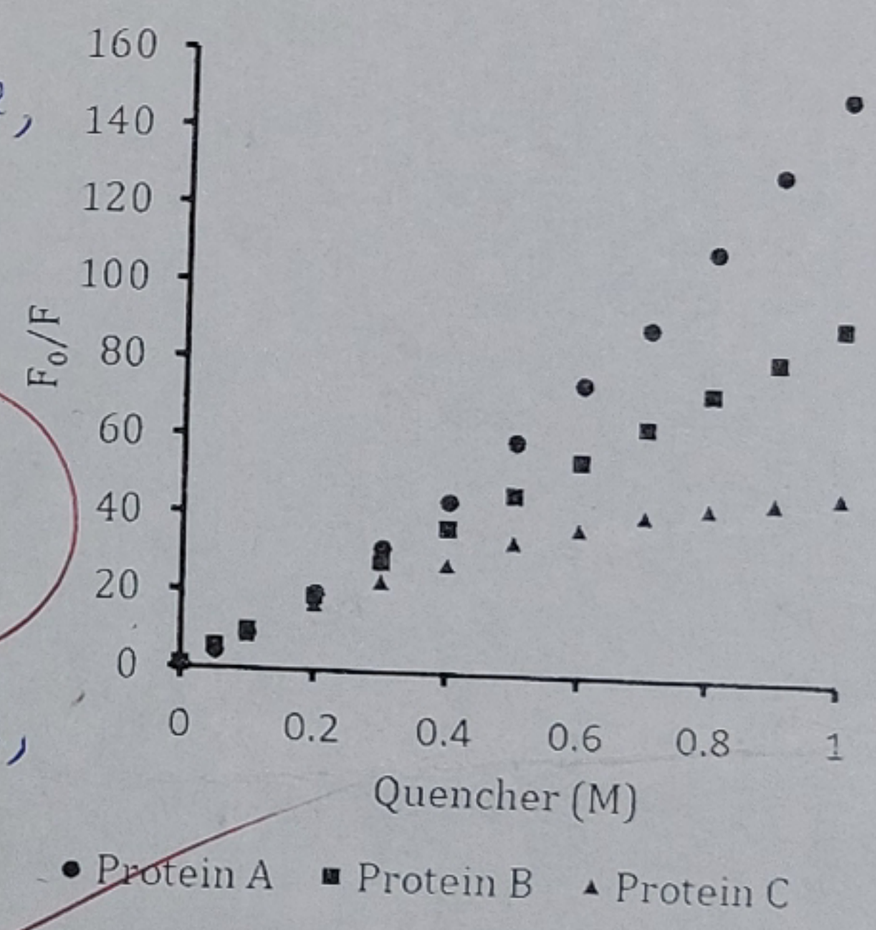
4. The fluorescence emission spectra of NATA (N-acetyl L-tryptophanamide) in methanol are recorded at different concentrations (shown below). Carefully analyze the spectra recorded at three different concentrations and explain your observations {2 marks}



As concentration increases from 80 μM to 320 μM, ~~[illegible]~~ we see a decrease in fluorescence intensity, due to the inner filter effect. Much of the light is absorbed by molecules near the surface, therefore molecules towards the bulk don't show fluorescence, therefore intensity is reduced. At 40 μM, there are less molecules in the solution for fluorescene, therefore there a decrease in intensity

2

5. You have studied tryptophan fluorescence quenching in three proteins with a known dynamic quencher acrylamide. The Stern-Volmer plots are shown below. Explain the reasons behind the shapes of these plots. {3 marks}



Protein ■ : Plot is a straight line, therefore it is dynamically quenched.

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

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Protein ● : Plot is curving upward, therefore it is best fitted by a parabola. It is quenched dynamically as well as by static quenching.

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

Protein ▲ : Plot is curving downward. Therefore, quenching is done by sphere of action.

equation: $\frac{F_0}{F} = (1 + K_D[Q])e^{-1}$.