Assignment-6

1. Perrin plot \( \left( \frac{1}{r_{SS}} \text{ vs } T/\eta \right) \) of a globular protein in water gives a straight line with a slope of \( 4.07 \times 10^{18} \text{ kg.m}^{-1}.\text{s}^{-1}.\text{K}^{-1} \) and intercept 2.5. Lifetime of the protein is 4 ns. The molar volume of the globular protein is
(a) 10000 Å³ mol⁻¹
(b) 20000 Å³ mol⁻¹
(c) 30000 Å³ mol⁻¹
(d) 40000 Å³ mol⁻¹

2. Consider a molecule X whose \( S_0-S_1 \) transition moment and \( S_0-S_2 \) transition moment makes an angle of 30° with each other. The \( S_0-S_1 \) energy gap is 500 nm while the same for \( S_0-S_2 \) is 400 nm and assume this molecule obeys Kasha’s rule. Now if we excite the molecule with 400 nm light, the fundamental anisotropy \( (r_0) \) for this molecule will be
(a) -0.05
(b) 0
(c) 0.25
(d) 0.4

3. Perrin plots \( \left( \frac{1}{r_{SS}} \text{ vs } T/\eta \right) \) for two proteins X and Y in water give straight lines with intercepts 5 and 2.5 and slopes \( m_X \) and \( m_Y \), respectively. If \( m_y/m_x = 1.2 \) then the ratio of the volumes of protein X and Y \( (V_X/V_Y) \) is

(Consider the fluorescence lifetime of the proteins X and Y are exactly same.)
(a) 0.6
(b) 1.6
(c) 2.6
(d) 3.6

4. Human serum albumin (HAS) is a big multidomain protein, which contains a single cysteine residue at the 34th position. Tetramethylrhodamine-5-maleimide (TMR) is a thiol specific dye and can be attached selectively to cys-34 residue of HSA. Consider you have two unknown solutions, out of which one is TMR
in water and the other one is TMR labeled HSA in water. How could you identify these two solutions by anisotropy measurement?

(a) It is not possible only by measuring anisotropy of these two solutions.

(b) The orientational relaxation time of the TMR tagged HSA in water will be much smaller than that of TMR in water.

(c) The fluorescence anisotropy of TMR in water will be much smaller compared to that of TMR tagged HSA in water.

(d) The fluorescence anisotropy of TMR in water will be much higher compared to that of TMR tagged HSA in water.

5. In an isotropic solution, the fluorophores are oriented randomly. Upon excitation, one preferentially excites those molecules whose absorption transition moments are parallel to the electric vector of excitation light. Now, consider that the molecule is being excited with vertically polarized light. From these excited fluorophores, we get polarized emission. The horizontally polarized emission is called $I_{\perp}$ and the vertically polarized emission is called $I_{||}$. Which of the following relationship between $I_{||}$ and $I_{\perp}$ is NOT possible?

(a) $I_{||} > I_{\perp}$

(b) $I_{||} < I_{\perp}$

(c) $I_{||} = I_{\perp}$

(d) $\frac{I_{||} - I_{\perp}}{I_{||} + 2I_{\perp}} = 0.21$

6. The G value in fluorescence anisotropy is a measure of the

(a) ratio between ground state and excited state dipole moment of the molecule.

(b) ratio between the populations of molecules oriented vertically and horizontally in solution.

(c) ratio of the excitation light intensity in the vertical and horizontal direction.

(d) ratio of the sensitivity of the monochromator and detector for the vertically and horizontally polarized light.

7. The steady state fluorescence anisotropy of a molecule in water is 0.04 and in glycerol is 0.3. The difference in the anisotropy value can be attributed to
(a) **high viscosity of glycerol**
(b) low dielectric constant of glycerol
(c) high refractive index of glycerol
(d) high dipole moment of glycerol

8. The fundamental anisotropy value \( r_0 \) of two molecules, X and Y, are 0.4 and -0.2, respectively. The reason for these different values of fundamental anisotropy for X and Y is

(a) **absorption and emission transition moment are collinear for X and perpendicular for Y.**
(b) slower rotational movement of the molecule, X as compared to that of Y.
(c) slower rotational movement of the molecule, Y as compared to that of X.
(d) absorption and emission transition moment are collinear for Y and perpendicular for X.

9. During the measurement of fluorescence lifetime of a molecule, the emission is collected at the magic angle polarization

(a) to remove the polarity effect.

(b) **to remove the rotational relaxation time.**
(c) to remove the quenching effect by dissolved oxygen.
(d) none of the above is correct.

10. We know that to measure the pure fluorescence lifetime, you need to rotate the emission polarizer at magic angle (54.75°) polarization from the excitation polarization. Consider a case where we have a set-up where the emission polarizer can be set at only two angle 0° and 90°. For measuring proper lifetime of a fluorophore in this set-up

(a) **we have to record** \( I_\parallel + 2I_\perp \) **in order to remove the rotational relaxation time.**
(b) we have to record \( I_\parallel - I_\perp \) in order to remove the rotational relaxation time.
(c) we have to record \( I_\parallel - 2I_\perp \) in order to remove the rotational relaxation time.
(d) there is no way out.