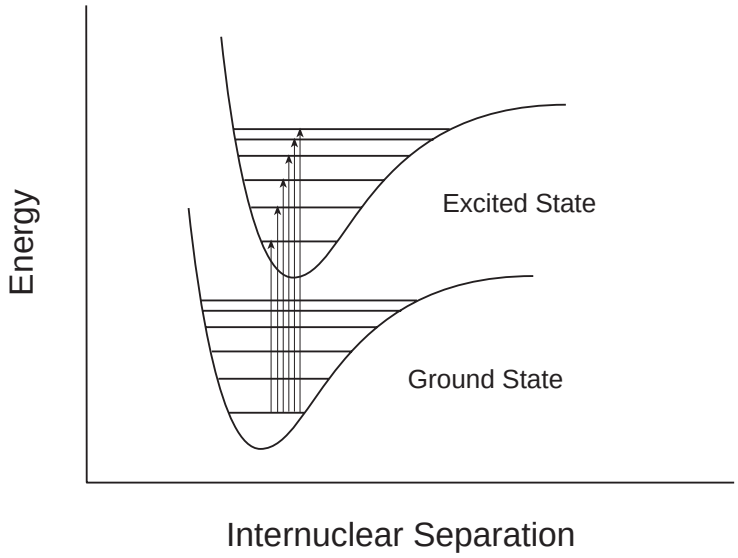
Answers – Study Problems in Fluorescence

1. Note that individual 'vibronic bands' can be seen in the spectrum. These correspond to transitions from the electronic ground state to different vibrational substates of the excited electronic state:



Now recall that the energy of an absorption transition is inversely proportional to the wavelength of a photon absorbed in that transition. The longest-wavelength vibronic band (the one peaking at 360 nm) is thus the one that corresponds to the lowest-energy transition above (shortest arrow), which is the transition from the lowest vibrational-energy level of the ground state to the lowest vibrational-energy level of the excited state. The energy of this transition is thus the energy separation between these two states, which can be calculated from the energy of a photon absorbed in this transition: $\Delta E = E_{photon} = (hc/\lambda)$. Plugging in the values for the speed of light (2.998×10^{10} cm sec^{-1}) and Planck's constant (6.63×10^{-27} erg sec^{-1}), and noting that 1 nm = 10^{-7} cm, we calculate that $\Delta E = 5.52 \times 10^{-12}$ erg $(molecule)^{-1}$. Multiplying this quantity by Avogrado's number and noting that 1 kJ = 10^{10} ergs, we calculate finally that **$\Delta E = 3.32 \times 10^{2}$ kJ mol^{-1}**.

'The' ground and excited states in this problem refer to the electronic ground and excited states, respectively, which as discussed before each have multiple vibrational and rotational substates.

2. To interpret the data, we need to predict the rotational correlation time for the tumbling of the protein as a whole. This requires these two formulas:

$$\tau_c = \frac{V_h \eta}{kT} \quad and \quad V_h = \frac{M\left(v_p + \delta_w/\rho_w\right)}{N_o}$$

From the graph we can calculate that for the slow component, **τ_c = 9.4 nsec** (I leave it to you to work this out – your actual calculated value might be slightly different). From the above two equations we can calculate that for a spherical protein of this size τ_c = 9.4 nsec

(assuming a hydration factor δ_w of 0.40 - if you use a smaller value for δ_w you will get a similar but slightly smaller estimate of τ_c). The (slow) motion detected in the anisotropy-decay curve thus represents whole-body rotation of the protein. We can conclude that the tryptophan residue undergoes a rapid but limited **local** rotational motion (which might represent for example motion of the tryptophan side chain or of a small local segment of the protein), and a larger-amplitude but slower rotational motion corresponding to whole-body motion of the protein.

3. For the emission spectra shown I determined the following peak wavelengths (your values could be *slightly* different):

Dimeric form of protein:	470 nm
Monomeric form of protein:	476 nm
Labeled lysine derivative in water:	480 nm
Labeled lysine derivative in 70/30 EtOH/water:	471 nm

Assuming that the labeled lysine derivative is a good model for an **X**-labeled lysine in the protein, we conclude from the latter two bits of data that **increasing environmental polarity shifts the emission peak wavelength to longer values** (ethanol is a less polar solvent than water). Applying this result to the spectral data shown for the labeled protein, we can conclude that the probe group X is present in a less polar environment in the dimeric form of the protein. One possible model for this result is that the group **X** becomes buried at the interface between the two subunits of the dimer. (You could also propose a more generic 'conformational change' in the dimeric protein that leads to **X** becoming more buried in the protein interior.) Note that even in the monomeric form of the protein the local environment of the probe is less polar than that in water, suggesting that even in the monomer the probe is not simply projecting out into the aqueous phase.

4. Using the data provided we can calculate that the efficiency of resonance energy transfer (ε) is 0.025, corresponding to $\mathbf{(R/R_o) = 1.842}$ or a separation between the two probes of **11.8 nm**. If we calculate the hydrated radius (r_h) of the protein, we obtain a value of 3.15 nm. It is thus clear that the protein must be significantly **asymmetric** (nonspherical) in order to allow to allow two groups to be separared within the same molecule by a distance much more than $2r_h$. This result alone does not tell us what the exact shape of the molecule might be – prolate or oblate, for example.