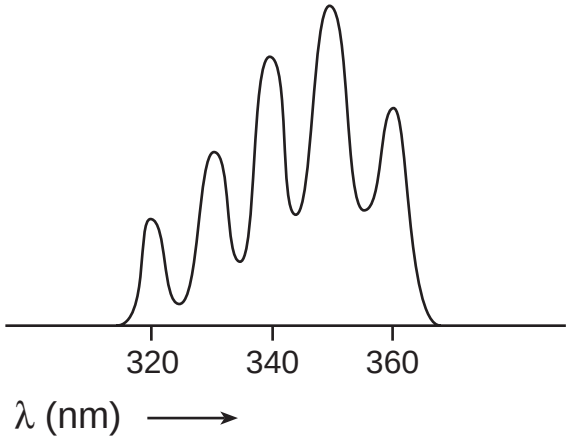
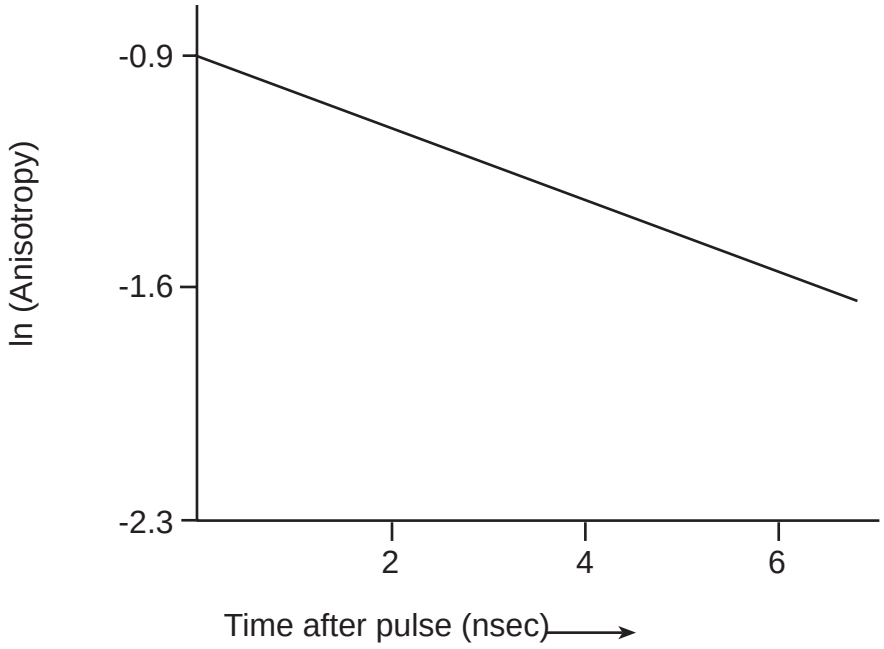
Study Problems – Fluorescence

1. Below is shown the excitation spectrum for a fluorescent polycyclic aromatic hydrocarbon in a membrane:



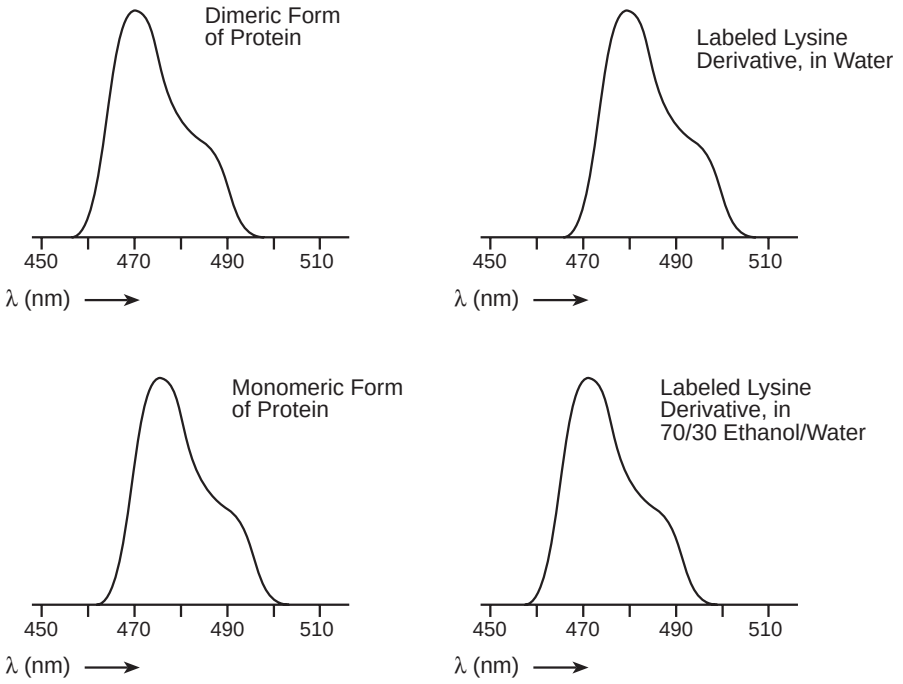
Calculate the energy separation (in kJ mol^{-1}) between the lowest energy levels of 'the' ground and 'the' excited states. Define what 'the' ground and 'the' excited states represent in this case.

2. A roughly spherical protein of molecular weight 18,900 Da and v_p = 0.72 cm^3 g^{-1} contains a single tryptophan residue. Fluorescence-anisotropy measurements on the native protein give these results (T = 25°C, η_{solv} = 0.011 poise):



From these data, estimate the rotational correlation time for the motion of the tryptophan residue. Does this tryptophan residue tumble at the rate that the whole protein does, or does it move much faster (as might be the case if for example it is found in a surface loop of the protein that **locally** moves [twists, turns, etc.] much faster than does the protein as a whole).

3. Below are shown fluorescence emission spectra obtained under two different conditions for a protein labeled with a fluorescent group **X** on the side chain of a specific lysine residue. Spectra are also shown for an **X**-labeled lysine derivative (α-N-acetyl-ε-**X**-lysine amide) which serves as a simple reference compound:



Assume that the labeled lysine residues do not exhibit 'cross-talk' (i.e., affect each other's spectra) between the two monomers in the dimeric form of the protein.

Propose a plausible explanation for the relative positions of the emission spectra for the monomeric vs. the dimeric form of the labeled protein, based on the spectra for these forms of the protein and for the labeled reference species.

4. Two different fluorescent groups, **A** (λ_{ex} = 388 nm, λ_{em} = 475 nm) and **B** (λ_{ex} = 483 nm, λ_{em} = 538 nm) are attached to distinct sites on a monomeric protein. The following data are obtained:

Fluorescence of 10 pmol of protein labeled with **A** only: 160.1 fluorescence units
Fluorescence of 10 pmol protein labeled with **A** and **B**: 4.0 fluorescence units

The molecular weight of the protein is 70,000 and the partial specific volume v_p is 0.73 $cm^3\ g^{-1}$. The Förster length for the **A** – **B** pair is 64 Å.

From these data, what can you conclude (if anything) about the shape of the protein? You may not be able to give a unique answer. If not, propose <u>one</u> possible model..