ELECTRONIC SPECTROSCOPY HOMEWORK QUESTIONS

Instructions: open book, work by yourself, show all calculations. Problems due Feb. 18, 2019

1. 2-Aminopurine (2AP) in a pH 7, 0.1M KCl solution has a 303-nm molar extinction coefficient of 6,000 M^{-1} cm^{-1}. What is the absorbance of 1 mM and 0.1 mM 2AP when measured in a cell with a 1-mm pathlength?

2. Describe a way (either steady-state or time-resolved) to distinguish Raman scatter from fluorescence.

3. Using an excitation wavelength of 295 nm, you measure the fluorescence emission spectrum of a protein, which binds to DNA depending upon the concentration of the small nucleotide cyclic adenosine monophosphate (cAMP). The protein has a single tryptophan and one tyrosine and forms a dimer at saturating concentrations of cAMP.

    a. Please explain the following observations: 1) in the native, ligand-free state (i.e., no cAMP and no DNA) the fluorescence emission has a maximum at 330 nm; 2) when cAMP is bound, the emission maximum shifts to 325 nm; and 3) when the protein is unfolded in 8 M urea, the emission maximum shifts to 350 nm.

    b. Suggest one or two experiments to test your explanations.

4. The cAMP-binding protein in the presence of saturating concentrations of cAMP, forms a dimer that can bind to a specific site on a 100 base-pair DNA oligo (K_d = 2 nM). You decide to study the protein-DNA interaction using fluorescence correlation spectroscopy (FCS). You generate a single Cys mutant and label it with Alexa-488, a small fluorophore excited by 488-nm light. Draw and label FCS curves appropriately scaled for the following situations:

    a. 5 nM protein dimer (saturating cAMP]

    b. 5 nM dimer + cAMP after addition of 100 nM DNA (100 base-pair oligo with specific dimer site)

    c. Diffusion of 1 nM dimer (saturating cAMP)

    d. 1 nM dimer + cAMP after addition of 100 nM DNA (100 base-pair oligo with specific dimer binding site)

5. FRET relationships: Assume that a FRET donor D has a single exponential decay in the absence of acceptor A and that the orientation factor $\kappa^2 = 2/3$. Draw fluorescence decay curves (log of intensity vs time) for the following situations (label and provide explanation):

    1) D to A distance = R_0; 2) D to A distance >> R_0; 3) D to A distance << R_0