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.
   1. 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.
   2. 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 (Kd = 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:
   1. 5 nM protein dimer (saturating cAMP]
   2. 5 nM dimer + cAMP after addition of 100 nM DNA (100 base-pair oligo with specific dimer site)
   3. Diffusion of 1 nM dimer (saturating cAMP)
   4. 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 κ2 = 2/3. Draw fluorescence decay curves (log of intensity vs time) for the following situations (label and provide explanation):

1) D to A distance = R0; 2) D to A distance >> R0; 3) D to A distance << R0