Practice Exam I - Spectroscopy

Show your work for full credit. Be concise, but complete.
Avoid long rambling answers which indicate that you don't really understand the question.

1. (25 points) We have seen that quenching of fluorescence can depend on the concentration of the quenching agent. Assuming that quenching is first order with respect to the quencher Q (with a first order rate constant of k_q), derive an expression for the ratio of the fluorescence in the absence of quencher to that in the presence of quencher, F_o/F_Q, as a function of [Q], k_q, and τ_o (the lifetime of the excited state in the absence of quencher).

2. (25 points) In order to efficiently utilize the spectrum of electromagnetic radiation reaching the surface of the earth, nature has evolved large "particle in a box" π systems in order to fine tune electronic transitions to match this spectrum. In photosynthesis, such molecules are built around porphyrin rings (see your nearest Biochemistry text). This can be viewed very approximately as a two-dimensional "box" with equal sides of length about 8!Å. There are 24 electrons in the π system (this is important!).

The energy of the levels for an arbitrary two dimensional "box" is given by:

$$E = \frac{h^2}{8m}\left[\left(\frac{n_x}{a}\right)^2 + \left(\frac{n_y}{b}\right)^2\right] \quad \text{where } n_x = 1,2,3,\dots \ \& \ n_y = 1,2,3,\dots$$

Calculate the wavelength of light (nm) corresponding to the *lowest energy* electronic transition in this system (assume that all are allowed) and show that it indeed falls in the visible range of the electromagnetic spectrum.

3. (25 points) The chromophore involved in both bacteriorhodopsin and in the visual systems of higher organisms is retinal. This highly conjugated molecule can exist in a number of isomeric forms, depending on the *cis-trans* state of each of the carbon-carbon bonds. In the all-trans form, the methyl group at position 5 shows *a slight* steric-clash with the proton at position 8. However, in the 7-cis form, there is much more severe steric repulsion between 5–methyl and the 9–methyl groups.


all-*trans* retinal

7-*cis* retinal
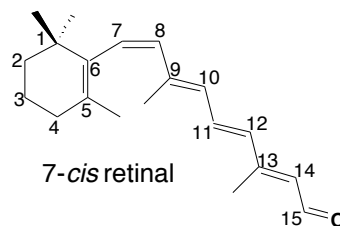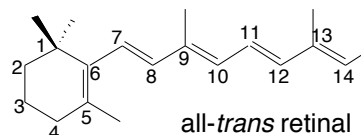
	Hexane	Ethanol
all-*trans*	368 nm	383 nm
7-*cis*	359 nm	377 nm

Explain the differences in the above absorption maxima, **both** in terms of differences **1)**!between 7-*cis* and all-*trans*, and **2)** between the solvents hexane and ethanol. Think about what the *steric clash* will do to the structure of the molecule and to the π system (time to brush up on your Gen Chem and Organic Chem).

4. (25 points) Someone has told you to do a
    FRET measurement, and has given you two
    molecules, but told you little else. Their
    fluorescence spectra are shown at right.

a) Which is the donor and which is the acceptor?

——— Excitation Spectra

------ Emission Spectra

Molecule A

$\lambda\longrightarrow$

Molecule B

$\lambda\longrightarrow$
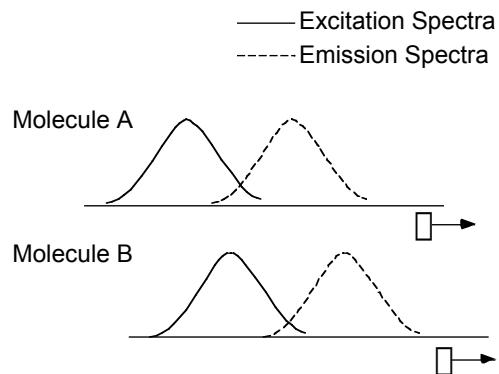
b) If you want to observe FRET by watching
    fluorescence from the acceptor, at what wavelength would you excite and at which would
    you observe? Show wavelengths by placing labeled arrows in the picture above.

c) For the cleanest interpretations, you want to be careful precisely where you excite and
    where you observe. **Explain that statement**.