** This examination is open book, but is to be worked on ***independently***. You may not discuss or otherwise communicate *any* aspect of the exam with *anyone* other than C. Martin. This includes any discussions with anyone after you are done with the exam, but before the exam's due date and time. This is ***very important***.

Due in class, 9:30am, Tuesday, March 5

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).

$$\phi_o = \frac{k_f}{k_f + k_{ic} + k_{is}} \qquad \phi_Q = \frac{k_f}{k_f + k_{ic} + k_{is} + k_Q Q}$$

$$\frac{F_o}{F_Q} = \frac{\phi_o}{\phi_Q} = \frac{k_f}{k_f + k_{ic} + k_{is}} \frac{k_f + k_{ic} + k_{is} + k_Q Q}{k_f}$$

$$\frac{F_o}{F_Q} = \frac{k_f + k_{ic} + k_{is} + k_Q Q}{k_f + k_{ic} + k_{is}} = \frac{k_f + k_{ic} + k_{is}}{k_f + k_{ic} + k_{is}} + \frac{k_Q Q}{k_f + k_{ic} + k_{is}}$$

$$\frac{F_o}{F_Q} = 1 + \frac{k_Q Q}{k_f + k_{ic} + k_{is}} \qquad but \qquad \tau_o = \frac{1}{k_f + k_{ic} + k_{is}}$$

therefore

$$\frac{F_o}{F_Q} = 1 + k_Q \tau_o Q$$

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" 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!).

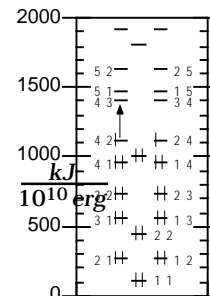The energy of the levels for an arbitrary two dimensional "box" is given by:

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

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.

The figure at right shows the lowest lying energy levels for this 2D "box" and how the 24 electrons will fill the energy levels. The lowest energy transition is then from (n_x=4,n_y=2) to (n_x=4,n_y=3) (with a similar degenerate transition from 2/4 to 3/4).

$$E = \frac{\left(6.63 \times 10^{-27}\, erg\, s\right)^2}{8\left(9.1096 \times 10^{-28}\, g\right)} \left[\left(\frac{n_x}{8\,\text{Å}}\right)^2 + \left(\frac{n_y}{8\,\text{Å}}\right)^2\right] \left(\frac{10^8\,\text{Å}}{cm}\right)^2 \frac{g\ cm^2\ s^{-2}}{erg} \frac{6.02 \times 10^{23}}{mole} \frac{kJ}{10^{10}\, erg}$$

$$E = 56.7\left[n_x{}^2 + n_y{}^2\right] \frac{kJ}{mole}$$

From the expression above, the ground and excited state energies are 1134 kJ/mole and 1418 kJ/mole, respectively, leading to a transition energy of 284 kJ/mole. Finally:

$$\lambda = \frac{hc}{E} = \frac{\left(6.63 \times 10^{-27}\, erg\, s\right)\left(3 \times 10^{10}\, cm\,/\,s\right)}{\left(284 \times 10^3\, J\,/\,mole\right)} \frac{\left(6.02 \times 10^{23}\,/\,mole\right)\left(10^7\, nm\,/\,cm\right)}{\left(10^7\, erg\,/\,J\right)} = 422\ nm$$

It's in the visible region of the 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
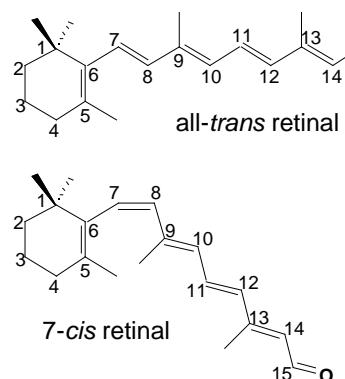
	Hexane	Ethanol
all-*trans*	368 nm	383 nm
7-*cis*	359 nm	377 nm

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   system (time to brush up on your Gen Chem and Organic Chem).

The severe steric clash present in the *cis* form will force the linear chain and double bond in the ring to rotate out of plane with respect to one another. This will partially break the   bond system, leading to a reduction in the size of the box. Consequently in the *cis* form, the box will be smaller, the energy separations bigger, and the energy of the transition higher (the wavelength lower).

The differences in $_{max}$ in the different solvents is explained as we saw in class. The more polar antibonding orbital is stabilized more by a polar solvent than is the ground state. Consequently, the energy difference for   * is lessened for a more polar solvent (ethanol is more polar than hexane).

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The differences in λ_{max} in the different solvents is explained as we saw in class. The more polar antibonding orbital is stabilized more by a polar solvent than is the ground state. Consequently, the energy difference for π^* is lessened for a more polar solvent (ethanol is more polar than hexane).

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

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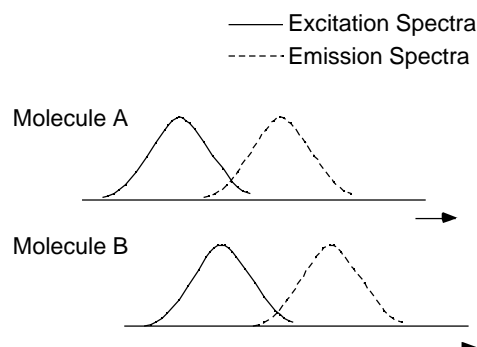
b) If you want to observe FRET by watching fluorescence from the acceptor, at what wavelength would you excite and at which would you observe? Show wavelengths by placing labeled arrows in the picture above.

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