**Problem 8.1:** A molecule phosphoresces with a single peak wavelength of 550 nm. The single fluorescence peak is at 500 nm. The absorption peak is at 425 nm. Sketch a rough Jablonski diagram based on this information, labeling transitions and calculating differences in each energy state in nm and cm\(^{-1}\).

![Jablonski diagram](image)

**Problem 8.2:** You have been given a report on luminescence measurements for an important molecule in your biochemistry lab. The report describes the fluorescence of the molecule with a peak at 675 nm, absorption peak at 455 nm, and phosphorescence peak at 560 nm. What is wrong with this information?

The phosphorescence should occur at a longer wavelength (lower energy) than the fluorescence peak. Probably the fluorescence and phosphorescence peaks are switched.

**Problem 8.3:** The absorption and emission spectra for quinine are shown in Figure 8.3. Does quinine obey the mirror image rule? Based on your response, what can you conclude about whether there is absorption to states above \(S_1\)?

No, visually the absorption and emission spectra are not mirror images. This is because there are absorptions other than \(S_0 \rightarrow S_2\); the \(S_0 \rightarrow S_2\) absorption is specifically labelled on Figure 8.3.

**Problem 8.4:** Use the anthracene absorption and emission spectrum in Figure 8.5 to construct a combined Jablonski diagram/absorption/emission spectrum sketch, as shown in Figure 8.3. Label all states and calculate transition energies in cm\(^{-1}\).
Absorptions:
310 nm = 32260 cm\(^{-1}\) 325 nm = 30770 cm\(^{-1}\) 340 nm = 29410 cm\(^{-1}\)
355 nm = 28170 cm\(^{-1}\) 374 nm = 26740 cm\(^{-1}\)

Emissions:
380 nm = 26320 cm\(^{-1}\) 400 nm = 25000 cm\(^{-1}\) 423 nm = 23640 cm\(^{-1}\)
450 nm = 22222 cm\(^{-1}\)

**Problem 8.5:** Figure 6.5 shows the absorption spectra for anthracene and tetracene. Apply the mirror image rule and use the absorption and emission spectrum shown in Problem 8.4 as a guide to sketch an absorption and emission spectrum for tetracene. Then, construct a combined Jablonski diagram/absorption/emission spectrum sketch, as shown in Figure 8.4. Label all states and calculate transition energies in cm\(^{-1}\).

To be included (similar to Prob 8.4)

**Problem 8.6:** A molecule phosphoresces with a single peak wavelength of 550 nm. The single fluorescence peak is at 500 nm. The absorption peak is at 425 nm. Based on this information, roughly sketch the Jablonski diagram, labeling transitions and calculating differences in each energy state in nm and cm\(^{-1}\).

This is the same as Problem 8.1 – to be corrected
Problem 8.7: You have taken time resolved fluorescence measurements for two samples, A and B. The table shows your data. Determine the fluorescence lifetime for each sample. Do this by plotting the ln(intensity) versus time and using the best fit line to calculate $\tau$ for each sample.

<table>
<thead>
<tr>
<th>$t$ (ns)</th>
<th>Sample A</th>
<th>Sample A</th>
<th>Sample B</th>
<th>Sample B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ln(int)</td>
<td>ln(int)</td>
<td>ln(int)</td>
<td>ln(int)</td>
</tr>
<tr>
<td>0</td>
<td>10.01</td>
<td>2.304</td>
<td>4.03</td>
<td>1.394</td>
</tr>
<tr>
<td>1</td>
<td>6.76</td>
<td>1.911</td>
<td>2.86</td>
<td>1.051</td>
</tr>
<tr>
<td>2</td>
<td>4.55</td>
<td>1.515</td>
<td>2.01</td>
<td>0.698</td>
</tr>
<tr>
<td>3</td>
<td>3.06</td>
<td>1.118</td>
<td>1.47</td>
<td>0.385</td>
</tr>
<tr>
<td>4</td>
<td>2.04</td>
<td>0.713</td>
<td>1.04</td>
<td>0.039</td>
</tr>
<tr>
<td>5</td>
<td>1.38</td>
<td>0.322</td>
<td>0.75</td>
<td>-0.288</td>
</tr>
<tr>
<td>6</td>
<td>0.98</td>
<td>-0.020</td>
<td>0.53</td>
<td>-0.635</td>
</tr>
<tr>
<td>7</td>
<td>0.64</td>
<td>-0.446</td>
<td>0.37</td>
<td>-0.994</td>
</tr>
<tr>
<td>8</td>
<td>0.47</td>
<td>-0.755</td>
<td>0.21</td>
<td>-1.204</td>
</tr>
<tr>
<td>9</td>
<td>0.29</td>
<td>-1.238</td>
<td>0.21</td>
<td>-1.561</td>
</tr>
<tr>
<td>10</td>
<td>0.23</td>
<td>-1.470</td>
<td>0.16</td>
<td>-1.833</td>
</tr>
</tbody>
</table>

We use Eq. 8.4 to calculate tau:

Sample A: $\text{slope} = -\frac{1}{\tau}$

$-0.3830 = -\frac{1}{\tau}$

$\tau = 2.61 \text{ ns}$

Sample B: $\text{slope} = -\frac{1}{\tau}$

$-0.3247 = -\frac{1}{\tau}$

$\tau = 3.08 \text{ ns}$

Problem 8.8: A sample of a fluorescent molecule (concentration of $1.0 \times 10^{-5}$ M) with no quenching agent has an emission intensity resulting in a 4.6 mV reading on the detector. An unknown amount of quenching agent is added to the solution and the fluorescence intensity is found to be 2.5 mV. Use the value of $K_q = 15.0 \text{ liter/mol}$ to determine the concentration of quenching agent.

We can use Eq. 8.9 to calculate the concentration of quencher, $[Q]$: 

$\frac{\phi_0}{\phi_Q} = 1 + K_q[Q]$ 

$\frac{4.6}{2.5} = 1 + (15.0 \text{ L/mol})[Q]$ 

$[Q] = 0.056 \text{ M}$
Problem 8.9: The data table shows the ratio $F_0/F$ for tryptophan as a function of the concentration of acrylamide quencher. What conclusion can you draw about dynamic and static quenching?

<table>
<thead>
<tr>
<th>CONCENTRATION (mmol/L)</th>
<th>$F_0/F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>50</td>
<td>1.7</td>
</tr>
<tr>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>150</td>
<td>3</td>
</tr>
<tr>
<td>200</td>
<td>4.1</td>
</tr>
<tr>
<td>250</td>
<td>5.6</td>
</tr>
</tbody>
</table>

The fact that $F_0/F$ is not linear with respect to quencher concentration implies that the quenching is either static or a combination of static and dynamic.

Problem 8.10: For this question, consider benzene and naphthalene.

(a) Which molecule would you expect to have a larger quantum yield?

(b) Which molecule would you expect to have a shorter peak wavelength for fluorescence?

(a) We would expect naphthalene to have a higher quantum yield – the greater conjugation lowers the energy of the $\pi \rightarrow \pi^*$ transition, allowing the $\pi^*$ orbital to be populated more efficiently.

(b) For essentially the same reason as given in (a), benzene should have a shorter fluorescence wavelength because its energy gap would be larger.

Problem 8.11: You are setting up a fluorescence measurement using a high power Nd:YAG laser with excitation at 355 nm. Your wavelength range of interest is 430-730 nm. You are using a grating monochromator to scan through the wavelength range onto a PMT. Should you be concerned about the possibility of secondary diffraction peaks? Justify your answer.

Yes – you would expect a 2$\text{nd}$ order diffraction at 710 nm (2x355), which is within the range you are scanning for emission.

Problem 8.12: You are using a Si CCD detector to study steady-state fluorescence in a homemade spectrometer. You measured two emission peaks at 700 nm and 800 nm that have roughly the same intensity. You have not made any correction for the detector. Roughly estimate the actual ratio of the two peaks based on the type of detector used.
From Figure 8.13, we can see that the quantum efficiency of the Si CCD is about 0.24 at 700 nm and about 0.12 at 800 nm. Since the peak intensities are the same, we can estimate that the peak at 800 nm is roughly twice the intensity of the one at 700 nm.

**Problem 8.13:** When discussing UV-vis absorption spectroscopy (Chapter 6), we did not discuss correcting for the spectral response of the detector. Why is this correction needed when measuring an excitation spectrum in a fluorometer but not needed when measuring UV-vis absorption?

The differential detector response is accounted for in absorption spectroscopy in the calculation of transmission. At each wavelength, $T = P/P_0$ is determined (in order to find $A = -\log(T)$). In fluorescence spectroscopy, we are not referencing the response against another signal—in order to relate the relative intensity of peaks at different wavelengths, we must know how the detector responds differentially at each wavelength.

**Problem 8.14:** What are the extreme ranges of possible values for degree of polarization, $P$? Explain what is occurring at the lowest and highest values of $P$.

Assuming the maximum intensity at either polarization is 1, the value $P$ ranges from -1 to +1:

- \[ F_{\parallel} = 0, F_{\perp} = 1 \quad P = \frac{F_{\parallel} - F_{\perp}}{F_{\parallel} + F_{\perp}} = \frac{0 - 1}{0 + 1} = -1 \]
  
  this would indicate that the analyte did not rotate the polarized light at all; the sample would have to be rigid, perhaps solidified or captured in a solid or gel matrix.

- \[ F_{\parallel} = 1, F_{\perp} = 0 \quad P = \frac{F_{\parallel} - F_{\perp}}{F_{\parallel} + F_{\perp}} = \frac{1 - 0}{1 + 0} = +1 \]
  
  This would indicate that the analyte rotated 100% of the light by 90°. This is a very unlikely scenario, but it would imply that the time required for 90° rotation exactly matched the lifetime of the excited state.

**Problem 8.15:** In RET spectroscopy, a particular donor, CFP, and a particular acceptor, GFP, have a Forster distance of 4.8 nm. The fluorescence lifetime of CFP is 2.1 ns without the presence of GFP protein. Calculate the rate constant for a donor–acceptor distance of 6 nm.

\[
K_T = \frac{1}{\tau_D} \left( \frac{R_0}{r} \right)^6 = \frac{1}{2.1 \times 10^{-9} s} \left( \frac{4.8 \text{ nm}}{6 \text{ nm}} \right)^6 = 1.2 \times 10^8 \text{ s}^{-1}
\]

**Problem 8.16:** One advantage of multiphoton absorption is the ability to penetrate more deeply into certain types of samples—for example, living biological systems. In a particular system, a 300 nm photon is absorbed by one-photon absorption. What wavelength photon would result in this same transition as a two-photon process? How about for three photons? Sketch each process on a Jablonski diagram.

For the 2-photon process: two photons of wavelength 600 nm could excite the system as would a single 300 nm photon, and three photons of wavelength 900 nm would do the same:
Exercise 8.1: In Chapter 5, we introduced the Boltzmann distribution as we discussed the role thermal energy plays in potentially populating excited states.

(a) Use the anthracene fluorescence spectrum to estimate the energy state difference between the vibrational ground state of $S_0$ and the first excited vibrational state of $S_0$.

(b) We assumed that the first excited vibrational state was not sufficiently populated to result in absorption to $S_1$ (in Figure 8.4, for example, all absorption transitions originate in the ground vibrational state). Use the Boltzmann distribution to calculate the ratio of populations in these states at 273K (room temperature) and at 350K.

(a) The Anthracene spectrum is given in Figure 8.5. The wavelength of the $S_1 v_1 \rightarrow S_0 v_1$ transition is about 400 nm; we can estimate the energy as

$$E = \frac{hc}{\lambda} = \frac{(6.626 \times 10^{-34} J s)(2.998 \times 10^8 m/s)}{400 \times 10^{-9} m} = 4.9662 \times 10^{-19} J$$

Similarly, the wavelength of the $S_1 v_2 \rightarrow S_0 v_1$ transition is about 380 nm, giving an energy of

$$E = \frac{hc}{\lambda} = \frac{(6.626 \times 10^{-34} J s)(2.998 \times 10^8 m/s)}{380 \times 10^{-9} m} = 5.2276 \times 10^{-19} J$$

So we can estimate the energy difference between $v_1$ and $v_2$ as $2.614 \times 10^{-20} J$.

(b) this should refer to Figure 8.5, not Fig 8.4. We will do this calculation for the $v_2 / v_1$ states, assuming the degeneracy ratio is unity ($g_2 / g_1 = 1$):

$$\frac{N_2}{N_1} = \frac{g_2}{g_1} \cdot e^{-\frac{\Delta E}{kT}} = (1)e^{-\frac{(2.614 \times 10^{-20} J)}{(1.38 \times 10^{-23} J/K)(273 K)}} = 9.71 \times 10^{-4}, \text{ or about 0.1% in the excited state at 273 K.}$$

$$\frac{N_2}{N_1} = \frac{g_2}{g_1} \cdot e^{-\frac{\Delta E}{kT}} = (1)e^{-\frac{(2.614 \times 10^{-20} J)}{(1.38 \times 10^{-23} J/K)(350 K)}} = 4.5 \times 10^{-3}, \text{ or about 0.45% in the excited state at 350 K.}$$

Exercise 8.2: A strong pulsed laser, $\lambda = 266$ nm, is being used to excite a sample. You find two peaks in your emission spectrum, one at 505 nm and another at 532 nm. You expected to find the 505 nm peak from your literature search. The 532 nm peak is not reported in any papers. What might be the cause? How could you test your hypothesis?

Since 532 is $(2 \times 266)$, we would suspect that peak of being a 2nd order diffraction of the excitation wavelength. You could to a time resolved study. The 505 peak would expect to have a decay profile (an
excited state lifetime), whereas the 532 peak will exist as long as the source is being used but will not decay over time.

**Exercise 8.3:** You are conducting a fluorescence polarization experiment, and you have determined that the degree of polarization for your sample at room temperature to be 0.5. Would you expect this to increase, decrease, or stay the same if you did the polarization measurements at higher temperature?

In general, we would expect the degree of polarization to increase with temperature, because the average rate of rotation should increase while the lifetime of the excited state would remain relatively unchanged.

**Exercise 8.4:** In our discussion of the factors that affect $F_0$, the intensity of fluorescence, we used the Taylor series for $10^{-x}$ to go from Equation 8.5 to 8.6, although we skipped all the details. The Taylor expansion for $10^{-x}$ is:

$$10^{-x} = 1 - \log(10)x + \frac{1}{2}[\log(10)]^2x^2 - \frac{1}{6}[\log(10)]^3x^3 + \frac{1}{24}[\log(10)]^4x^4 + ...$$

Use the Taylor series to derive Equation 8.6.

To be included

**Exercise 8.5:** You are working on a quenching experiment and have found that the ratio of fluorescence without quenching to fluorescence with quenching increases linearly with the concentration of the quencher. You find that the ratio of lifetime without quenching to lifetime with quenching does not change as you increase the quenching concentration. What type of quenching is occurring?

These data indicate that dynamic or collisional quenching is taking place.

**Exercise 8.6:** A particular fluorophore in a quenching solution has a $K_q$ of 8 L/M. The fluorescence intensity is 35 mV in the absence of the quenching agents. Plot the intensity of fluorescence as a function of [Q] for concentrations from 0 to 0.5 M/L.
Exercise 8.7: You have a set of fluorescence data for a high-absorbance sample and want to decide if the inner filter effect is resulting in more than a 20% error in your measured intensities. The optical density (OD) at the excitation wavelength is 0.1 and the OD at the emission wavelength is 0.15.

To be included

Exercise 8.8: Review flash photolysis in Section 9 of Chapter 6 and compare and contrast flash photolysis with time-resolved fluorescence spectroscopy. Be mindful to point out the similarities and differences in the instrumental components and the similarities and differences in the acquisition of the signal.

To be included

Exercise 8.9: What is the typical lifetime range of a fluorescence event? What is the typical lifetime range of a phosphorescent event?

Fluorescence: nanoseconds to microseconds
Phosphorescence: milliseconds to seconds

Exercise 8.10: For this question, consider anthracene and tetracene.
(a) Which molecule would you expect to have a larger quantum yield?
(b) Which molecule would you expect to have a shorter peak wavelength for fluorescence?

(a) We would expect tetracene to have a higher quantum yield – the greater conjugation lowers the energy of the $\pi \rightarrow \pi^*$ transition, allowing the $\pi^*$ orbital to be populated more efficiently.
(b) For essentially the same reason as given in (a), anthracene should have a shorter fluorescence wavelength because its energy gap would be larger.
Exercise 8.11: A molecule phosphoresces with a single peak wavelength of 700 nm. The single fluorescence peak is at 590 nm. The absorption peak is at 490 nm. Based on this information, roughly sketch the Jablonski diagram, labeling transitions and calculating differences in each energy state in nm and cm$^{-1}$. 

![Jablonski diagram](image-url)