1. The decay of fluorescence of dansyl is measured by exciting the fluorescence with a very fast light pulse and then measuring the dansyl fluorescence over time. A plot of the natural log of the fluorescence intensity versus time is plotted at right.

\[ I = I_0 e^{-k_d t} \]

so \[ \ln I = \ln I_0 - k_d t \]

\[ \text{slope of } \ln I \text{ vs. } t \text{ plot} \]

\[ \text{slope} = \frac{\Delta y}{\Delta x} = \frac{6.4 - 9.3}{20 \text{nsec} - 0 \text{nsec}} = -0.145 \frac{1}{\text{nsec}} \]

\[ k_d = 1.45 \times 10^8 \frac{1}{\text{sec}} \]

b) What is the fluorescence lifetime of dansyl?

\[ \tau = \frac{1}{k_d} = \frac{1}{1.45 \times 10^8 \text{ sec}^{-1}} = 6.9 \times 10^{-9} \text{ sec} = 6.90 \text{ nsec} \]

c) The fluorescence quantum efficiency for dansyl under the same conditions as in a) is 0.7. What is the intrinsic radiative fluorescence decay rate constant \( k_r \) for dansyl?

\[ \phi_r = \frac{k_r}{k_d} \]

so \[ k_r = \phi_r k_d = (0.7) \left( 1.45 \times 10^8 \frac{1}{\text{sec}} \right) = 1.02 \times 10^8 \frac{1}{\text{sec}} \]

d) It has been determined separately that when dansyl is 2 nm away from the 11-cis-retinal chromophore in rhodopsin, the efficiency of Förster energy transfer from dansyl to retinal is 50%. Suppose rhodopsin is covalently labeled with dansyl, and the fluorescence lifetime of this bound dansyl is found to be 6 nsec. What is the distance between the dansyl label and the retinal chromophore in rhodopsin?

\[ E_{\text{FRET}} = \frac{r_0^6}{r_0^6 + (2 \text{nm})^6} \]

\[ 0.5 = \frac{r_0^6}{r_0^6 + (2 \text{nm})^6} \]

so \[ r_0 = 2 \text{ nm} \]

Also, \[ E_{\text{FRET}} = \frac{r_0^6}{r_0^6 + (2 \text{nm})^6} \]

so \[ \phi_r = \frac{r_0^6}{r_0^6 + (2 \text{nm})^6} \]

or \[ r = 2.75 \text{ nm} \]
2. The proton magnetic resonance spectrum of partially deuterated methyl ethyl ether (as shown) was measured in a 100 MHz spectrometer.

\[
\text{CHD}_2\text{CH}_2\text{OCHD}_2
\]

\[
\begin{array}{cccccc}
\text{I}_1 & \text{I}_2 & \text{I}_3 & \text{I}_4 & \text{I}_5 & \text{I}_6 \\
\text{δ (ppm)}
\end{array}
\]

a) Which line or lines (I₁ to I₆) can be assigned to proton a?
Proton a will be split into a triplet by neighbor protons b, so I₂, I₄, and I₆ are from proton a. The splitting of b will be the same for protons a and b, so the splitting is also 0.2 ppm.

b) If the distance between lines I₂ and I₃ is 0.2 ppm, what is the distance between lines I₄ and I₅?
I₂ and I₃ are for protons b, split by proton a. The splitting Jₙb will be the same for protons a and b, so the splitting is also 0.2 ppm.

c) Suppose the sample is measured in a 500 MHz spectrometer. What will be the distance between lines I₂ and I₃ in this spectrometer?
The magnetic field strength in a spectrometer is proportional to the spectrometer frequency. Since \( \Delta \delta \propto \frac{1}{f} \), the chemical shifts are 5x larger in the 500 MHz spectrometer. However, the spin-spin coupling splittings do not depend on \( B_0 \), so the I₂-I₃ distance is unchanged.

d) Sketch the proton NMR spectrum for CD₃CH₂OCD₃ that is measured in a 100 MHz spectrometer. Indicate on the spectrum the positions of lines I₁ to I₆ from the spectrum of CHD₂CH₂OCHD₂.

Deuterium does not have a magnetic moment, so none of the Ds will have an NMR signal. Only the "b" protons from the original compound will show up, and their previous signal was the I₂-I₃ doublet.
The remaining "b" protons will not be split since they are in the same chemical environment.