

Homework 4 Solutions

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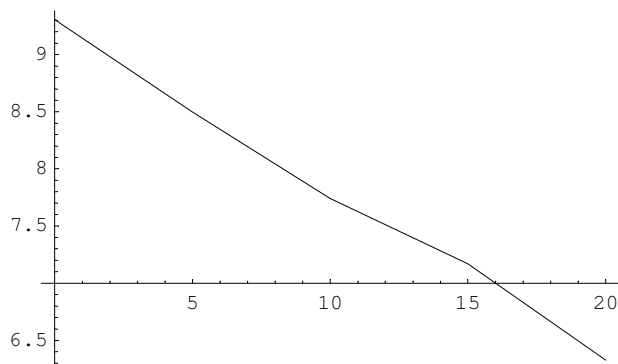
■ Problem 1

■ Part a (3 points)

The fluorescence intensity as a function of time is

```
intensity = {{0, Log[1.1 104]}, {5, Log[4.9 103]},  
            {10, Log[2.3 103]}, {15, Log[1.3 103]}, {20, Log[5.6 102]}};
```

```
ListPlot[intensity, PlotJoined → True];
```



where we have plotted the intensity on a semi-log plot. (Exponential decays are straight lines on such a plot!)

We can now fit a straight line to the semilogplot

```
Fit[intensity, {1, t}, t]
```

```
9.26473 - 0.145646 t
```

Thus, the life time of the fluorophore is $\frac{1}{0.146}$ ns ~ 6.85 ns

■ Part b (3 points)

Recall that the quantum yield, ϕ , is the ratio of the rate of fluorescence emission to the total rate at which the system can decay:

$$\phi = \frac{k_F}{k_F + k_{IC} + k_{IS} + k_Q}$$

and that the total rate at which the system can decay is the inverse of the fluorescence lifetime. Thus,

$$\frac{1}{\tau} = k_F + k_{IC} + k_{IS} + k_Q$$

Combining these two expressions yields

$$\phi = \tau k_F$$

Thus, the intrinsic rate of fluorescence is

$$k_F = \frac{\phi}{\tau} = \frac{0.7}{6.85 \text{ ns}} = 0.102 \text{ ns}^{-1} = 1.02 \times 10^8 \text{ s}^{-1}$$

■ Part c (3 points)

Recall that the efficiency of energy transfer is

$$E = \frac{R_0^6}{R^6 + R_0^6}$$

where R_0 is a constant. If we know that $E = 1/2$ when $R = 20 \text{ \AA}$, this implies that

$$E = \frac{1}{2} = \frac{R_0^6}{R^6 + R_0^6} \Rightarrow R_0^6 = R^6 \Rightarrow R_0 = R$$

thus, we know that the constant R_0 is 20 \AA .

Recall that the rate of energy transfer is

$$k_T = \frac{1}{\tau_{D,0}} \left(\frac{R_0}{R} \right)^6$$

where $\tau_{D,0}$ is the lifetime of the donor in the absence of the acceptor, and R_0 is a constant.

The lifetime of the donor in the presence of the acceptor can be written as

$$\frac{1}{\tau_D} = k_1 + k_T$$

where k_1 is the rate of fluorescence decay by all pathways (k_F , k_{IS} , k_{IC} , etc.), and from above $k_1 = \frac{1}{\tau_{D,0}}$. Combining these two expressions

$$\frac{1}{\tau_D} = \frac{1}{\tau_{D,0}} \left(1 + \left(\frac{R_0}{R} \right)^6 \right)$$

Thus, if we know the lifetime of the donor in the absence of the acceptor, the lifetime in the presence of an acceptor at a distance R , and the constant R_0 , we can calculate the distance R . Solving for R in the above expression

$$R = R_0 \left(\frac{\tau_{D,0}}{\tau_D} - 1 \right)^{-1/6} = 20 \text{ \AA} \left(\frac{6.85 \text{ ns}}{6 \text{ ns}} - 1 \right)^{-1/6} = 27.7 \text{ \AA}$$

■ Problem 2

■ Part a (3 points)

Because the individual anisotropy of the free DNA and the bound DNS add, we can express the final anisotropy a given solution as

$$A = p_B A_B + p_F A_F$$

where p_B and A_B are the probability of a molecule of DNA being bound to BSA and A_B is the anisotropy of DNS when bound to BSA, similarly p_F and A_F represent these values for free DNS.

Because a molecule of DNS is either bound or not bound to BSA, $p_B + p_F = 1$.

When $[BSA] = 0$, $p_B = 0$ and $p_F = 1$, thus, $A_F = 0.0149$.

Similarly when $[BSA] \gg K_d \gg [DNS]$, then all of the DNS will be bound, $p_F = 0$, and $A_B = 0.3913$.

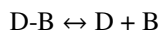
We can solve the above expression for p_B given the individual values of the bound and free anisotropies,

$$p_B = \frac{A - A_F}{A_B - A_F}$$

thus when $[BSA] = 2 \times 10^{-5} M$, $A = 0.2727$, and the probability of being bound is

$$p_B = \frac{A - A_F}{A_B - A_F} = \frac{0.2727 - 0.0149}{0.3913 - 0.0149} = 0.685$$

Now to relate this to the K_d , recall that the K_d is the equilibrium constant for the dissociation of DNS from BSA.



$$K_d = \frac{[B][D]}{[D-B]}$$

and the the probability of DNS being bound to BSA is simply the concentration of bound DNS over the total concentration of DNS, bound and unbound:

$$p_B = \frac{[D-B]}{[D] + [D-B]}$$

Combining these two equations relates K_d to p_B and the BSA concentration $[B]$ via

$$K_d = \frac{1 - p_B}{p_B} [B] = \frac{1 - 0.685}{0.685} (2 \times 10^{-5} M) = 9.2 \times 10^{-6} M$$

Note that this value of K_d is below the concentration of $[B]$ that we just considered, consistent with the fact that the probability of being bound is greater than 0.5!

■ Part b (3 points)

The relative probability of being bound can be shown in this case to be

$$p_B = \frac{A - A_F}{A_B - A_F + (g-1)(A_B - A)}$$

where g is the ratio of the quantum yield in the bound form to the free form. This yields a probability of being bound of

$$p_B = \frac{A - A_F}{A_B - A_F + (g-1)(A_B - A)} = \frac{0.2727 - 0.0149}{0.3913 - 0.0149 + (2-1)(0.3913 - 0.2727)} = 0.521$$

The relationship between the probability of being bound and the K_d is unchanged, thus the new K_d is

$$K_d = \frac{1 - p_B}{p_B} [B] = \frac{1 - 0.521}{0.521} (2 \times 10^{-5} M) = 1.84 \times 10^{-5} M$$

■ Part c (2 points)

If there is a change in the quantum yield upon binding of DNS to BSA, the total intensity of the fluorescence when $[BSA] = 0$ and when $[BSA] \gg K_d$ will be different. The ratio of these two intensities is the ratio of the quantum yields!

■ Part d (3 points)

Recall that the correlation time for anisotropy, τ_c , is related to the hydrated volume of a protein (assuming its spherical), the viscosity of the solution, η , and the thermal energy by

$$\tau_c = \frac{V_h \eta}{k_B T}$$

with a molecular weight, and an average density we can calculate V_h

$$V_h = \frac{M}{\rho} = \frac{(64000 \frac{g}{mol}) (\frac{mol}{6.022 \times 10^{23} \text{ molecules}})}{(1.3 \text{ g/cm}^3)} = 8.17 \times 10^{-20} \text{ cm}^3$$

Thus, the correlation time is

$$\tau_c = \frac{V_h \eta}{k_B T} = \frac{(8.17 \times 10^{-20} \text{ cm}^3) (0.894 \frac{g}{\text{cm} \cdot \text{s}})}{1.38 \times 10^{-16} \text{ g} \frac{\text{cm}^2}{\text{s}^2 \text{ K}} (293 \text{ K})} = 1.8 \times 10^{-6} \text{ s} = 1.8 \mu\text{s}$$

This incredibly large result stems from a typo in the HW. The viscosity for water is 100 times smaller, 0.00894 poise (g/cm s) at 25 C. Using this viscosity we find a correlation time 100 times smaller, or 18 ns. This is a much more typical value.

■ Problem 3

■ Part a (2 points)

The Perrin relation states that the average anisotropy measured for a sample, \bar{A} , is related to the correlation time and the fluorescence lifetime via the expression

$$\frac{1}{\bar{A}} = \frac{1}{\bar{A}_0} \left(1 + \frac{\tau_F}{\tau_C} \right)$$

where \bar{A}_0 is the anisotropy when the system is not free to diffuse.

Using the relation that $\tau_C = \frac{V_h \eta}{k_B T}$, the above expression becomes

$$\frac{1}{\bar{A}} = \frac{1}{\bar{A}_0} \left(1 + \tau_F \frac{k_B}{V_h} \frac{T}{\eta} \right)$$

At constant temperature, the volume of the protein will not change significantly with changes in the viscosity of the solvent; thus, this is the final relation between anisotropy and η . For a challenge, derive a similar expression as a function of T when allowing for the fact that with increasing temperature the protein will denature, changing its effective volume!

■ Part b (2 points)

The two curves are different when T is held constant but η is changed or when η is held constant and T is changed because temperature can denature the protein, changing its volume, while changes in the viscosity tend not to change the shape of a protein significantly. At low values of T/η , the protein is still properly folded, so changes in the viscosity or the temperature have the same effect on the rotational correlation time. However, above a certain value of T , the protein unfolds changing its effective volume.

■ Part c (2 points)

Since the slope of the line is inversely proportional to the volume, the smaller slope for the experiment at constant η , implies that the denatured protein occupies a larger volume than the folded protein.

■ Part d (2 points)

The volume of a 100 kDalton protein is

$$V = \frac{M}{\rho} = \frac{100 \times 10^3 \text{ g/mol}}{6.022 \times 10^{23} \frac{\text{molecules}}{\text{mol}}} \frac{1}{1.2 \text{ g/cm}^3} = 1.38 \times 10^{-19} \text{ cm}^3$$

From the above expression, the slope of the Perrin plot is

$$\frac{1}{\bar{A}_0} \tau_F \frac{k_B}{V_h}$$

Assuming that the dye is rigidly attached to the molecule and that the transition and emission dipole moments point in the same direction, then $\bar{A}_0 = 2/5$. Plugging in the volume above, we find that the slope is

$$\frac{1}{\bar{A}_0} \tau_F \frac{k_B}{V_h} = \frac{5}{2} (12 \times 10^{-9} \text{ s}) \frac{(1.38 \times 10^{-16} \frac{\text{g cm}^2}{\text{s}^2 \text{K}})}{1.38 \times 10^{-19} \text{ cm}^3} = 3 \times 10^{-5} \frac{\text{g}}{\text{cm s K}}$$

■ Problem 4**■ Part a (2 points)**

If the relative intensity of the tryptophan residues in the presence and absence of the quencher, DNP, is $4.1/20.5 = 0.20$, then the relative quantum efficiency between the presence and the absence of the quencher molecule is also 0.20. This is because the total intensity is proportional to the excitation intensity and the quantum yield since the excitation intensity is the same, the change in fluorescence intensity comes directly from the change in quantum yield.

Recall that the transfer efficiency is

$$E = 1 - \frac{\phi_Q}{\phi}$$

where ϕ is the quantum yield in the absence of the quencher and ϕ_Q is the quantum yield in the presence of the quencher.

Thus the transfer efficiency is

$$E = 1 - 0.2 = 0.8$$

■ Part b (2 points)

To calculate the unquenched lifetime recall that the ratio of the quantum yields is also equal to the ratio of the lifetimes of the quenched, τ_Q , and unquenched state, τ , i.e.

$$\frac{\phi_Q}{\phi} = \frac{\tau}{\tau_Q}$$

Thus,

$$\tau = \frac{\phi_Q}{\phi} \tau_Q = (0.2)(5 \text{ ns}) = 1.0 \text{ ns}$$

■ Part c (2 points)

From the notes, the transfer rate is related to the efficiency and the lifetime of the unquenched donor via

$$E = \frac{k_T}{k_T + 1/\tau}$$

Solving for k_T yields

$$k_T = \frac{1}{\tau} \frac{E}{1-E} = \frac{1}{5 \text{ ns}} \frac{0.8}{1-0.8} = 0.8 \text{ ns}^{-1} = 8.0 \times 10^8 \text{ s}^{-1}$$

■ Part d (2 points)

Recall that the transfer rate is related to the distance between the two groups via

$$k_T = \frac{1}{\tau} \left(\frac{R_0}{R} \right)^6$$

where τ is the lifetime of the unquenched molecule and R_0 is a constant. Solving for R

$$R = (k_T \tau)^{-1/6} R_0 = \left(\frac{0.8}{\text{ns}} 5 \text{ ns} \right)^{-1/6} (50 \text{ \AA}) = 39.7 \text{ \AA}$$

■ Part e (2 points)

If the distance changes, we can calculate the expected transfer efficiency, E , and from that the ratio the unquenched quantum yield to the quenched quantum yield. Starting with the transfer efficiency

$$E = \frac{R_0^6}{R^6 + R_0^6} = \frac{(50 \text{ \AA})^6}{(20 \text{ \AA})^6 + (50 \text{ \AA})^6} = 0.996$$

Thus, the ratio of the quenched quantum yield to the unquenched quantum yield and thus the ratio of the fluorescence intensity of the unquenched molecule to the quenched molecule will be

$$\frac{\phi_Q}{\phi} = \frac{I_Q}{I} = 1 - E$$

This implies that the new fluorescence intensity will be

$$I_Q = I(1 - E) = 20.5(1 - 0.996) = 0.082$$

■ Part f (2 points)

If 1% of the protein does not bind the quenching molecule, then 99% of the fluorescent signal will be from the intensity calculated above and 1% will be from the intensity of the unquenched tryptophans. Thus, the final observed intensity will be

$$I = 0.99(0.082) + 0.01(20.5) = 0.286$$

This is nearly a factor of 3.5 times larger than the signal one would get if all of the protein bound the DNP and was properly quenched. Thus, it is important that in experiments like this one that the protein is very pure!

■ Part g (2 points)

The expected lifetime of the 1% impurity, the protein that doesn't bind the DNP, should have a lifetime of the unquenched tryptophan, 5 ns. In fluorescence lifetime experiments, you would measure a fluorescence decay that is described by the sum of two exponentials, one with a fast component due to the 99% of the protein that has bound the dye, and thus has a reduced lifetime, and a second slow component, with a lifetime corresponding to the 1% impurity.