

Physics 177: Molecular Biophysics. Spring 2008.

Problem Set 4 - Fluorescence

Assigned April 2. (Updated Version) Due April 11.

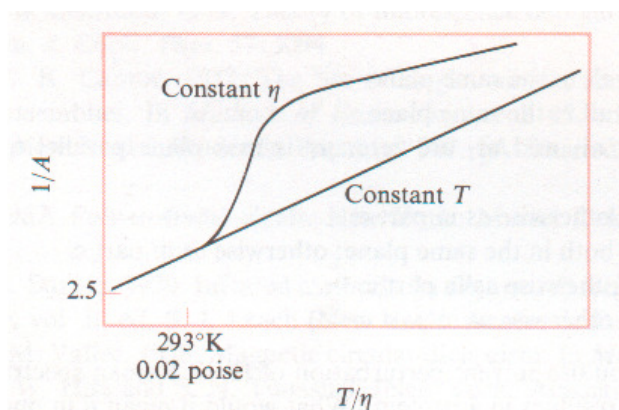
1. In a fluorescence lifetime experiment a very short pulse of light is used to excite a sample. Then the intensity of the fluorescence is recorded as a function of time. Suppose we study a dansyl fluorophore. The intensity of the fluorescent light versus time is given below.

Fluorescence intensity	Time (ns)
1.1×10^4	0
4.9×10^3	5
2.3×10^3	10
1.3×10^3	15
5.6×10^2	20

- a) What is the observed fluorescence lifetime?
- b) The fluorescence quantum yield under the same conditions was determined to be 0.7. What is the intrinsic rate constant for radiative fluorescence decay?
- c) It has been determined separately that when the dansyl chromophore is 20 Å from the 11-cis retinal chromophore in rhodopsin, the efficiency of fluorescence energy transfer is 50%. In a second experiment rhodopsin is covalently labeled with a dansyl chromophores and the observed fluorescence life-time for the dansyl-rhodopsin complex is 6 ns. What is the distance between the dansyl label and the retinal chromophore in rhodopsin?
2. The probe 1-dimethylamino-5-naphthalene sulfonic acid (DNS) binds to bovine serum albumin (BSA).
- a) Assume initially that the quantum yield of the probe is not affected by binding. Given the following data calculate the dissociation constant (K_d) of the DNS-BSA complex.
- Hint: express the anisotropy (A) of the solution as the weighted average of the contributions of the free and bound probe.

[BSA]	[DNS]	A
0	1×10^{-7} M	0.0149
2×10^{-5} M	1×10^{-7} M	0.2727
$\gg K_d$	1×10^{-7} M	0.3913

- b) Now assume that the quantum yield of DNS increases two-fold upon binding to BSA. Using the same values as above calculate the K_d that would explain this data.
- c) How would you determine whether the quantum yield of DNS changes upon binding to BSA?
- d) Predict the time-resolve decays of anisotropy for the solution with $[BSA] \gg K_d$, at 20°C. Assume $P_0 = 0.3913$ for DNS and that the rotational correlation time of DNS and BSA are well approximated by that predicted for an anhydrous sphere. The molecular weight of BSA is 64,000 g/mol. The viscosity of the medium is $0.894 \text{ (g cm}^{-1} \text{ sec}^{-1})$. The density of the protein can be assumed to be 1.3 g/cm^3 .
3. The fluorescence anisotropy of a dye labeled protein was measured first as a function of temperature at a fixed viscosity and then at a constant temperature but variable viscosity. See the Perrin plot below. (Cantor and Schimmel, II, Problem 8-2)



- a) Derive an expression that describes the dependence of the inverse of the anisotropy ($1/A$) at constant temperature but variable viscosity (η).
- b) Explain the data at constant viscosity but variable temperature. Specifically, why are the curves different for variable viscosity and variable temperature at high T/η and why are they the same for low T/η .
- c) Explain the significance of the different slopes at high T/η for these two experiments.
- d) Assuming that a fluorophore with lifetime 12 ns is attached to a 100 kDalton protein. Treat the protein as a sphere with average density 1.2 g/cm^3 and calculate the predicted slope of the Perrin plot.

4. Assume that you have isolated a protein which contains a single tryptophan residue, and which binds dinitrophenol (DNP) in the active site. The absorption spectrum of DNP overlaps with the emission spectrum of the tryptophan residue. Assume $R_0 = 50 \text{ \AA}$. DNP is not fluorescent. The fluorescence intensities of the tryptophan residues are 20.5 and 4.1 in the absence and presence of DNP, respectively.
- What is the transfer efficiency?
 - Assume that the unquenched lifetime is 5 ns. What is the expected life-time in the presence of DNP?
 - What is the transfer rate?
 - What is the distance between these groups?
 - Assume that the solution conditions change so that the distance between the DNP and the tryptophan is 20 \AA . What is the expected intensity for the tryptophan fluorescence?
 - For this same solution ($R = 20 \text{ \AA}$) what would be the effect on the fluorescence intensity of a 1% impurity of a second protein which did not bind DNP? Assume this second protein had the same life-time and quantum yield.
 - What lifetime would you expect for the sample which contains the impurity? Would this lifetime provide any indication of the presence of an impurity?