Energy transfer projects

Introduction

1.1 Energy transfer

Energy transfer in fluorescence spectroscopy has become an industry. The power of the method is the ability to determine distances at on a length scale from 5 to 100 Å based on an optical method. The basic concept is that a donor excited state can be transferred to an acceptor (while the donor returns to the ground state), such that the observed fluorescence is that of the acceptor rather than the donor. It is like quenching except that quenching involves a transfer to a non-radiative system, while fluorescent resonant energy transfer (FRET) involves transfer to a radiative system. This transfer can only occur if the two molecules are within a certain distance. In fact, the probability of occurrence is predictable for pairs of molecules that have been studied, such that it is a molecular scale ruler. For example, two labeled proteins that bind to each can be detected in the bound state if one has a FRET donor and the other an acceptor that are located at the correct distance in the bound state.

In this research project we present several basic FRET experiments based on the chemical literature. These are relatively simple in that they involve direct transfer from a donor (fluorescein) to an acceptor (rhodamine) in solution. One study involves using viscous solutions of ethylene glycol and ethanol to immobilize the donors and acceptors. This method has the advantage of removing diffusion from the analysis. A second study probes the pH dependence of the FRET process in aqueous solution. Fluorescein has a pH dependent fluorescence by itself. It is a pH sensor on its own. But the FRET pair of fluorescein with rhodamine has potential as a more sensitive pH sensor. Of course, our goal is not to build an expensive pH sensor, but to understand the basic physics behind the energy transfer process.

2.1 Fluorescence quenching and fluorescence energy transfer

The topic of resonance energy transfer include fluorescent resonant energy transfer, but has a number of other applications in quantum chemistry.[1] When applied to fluorophores we need to understand both absorption and emission spectra, including their line shapes. Since the fluorescence energy transfer rate is proportional to spectral overlap of the donor and acceptor, there is a further relationship between static and time-resolved spectroscopy. One can measure energy using both. The quantum yield gives the intrinsic fraction of the molecules that decay by emitting light.

As we consider the energy transfer pathway, we must always be aware that it competes with a number of other deactivation pathways:

1. Collisional quenching – molecular collisions in solution
2. Intersystem crossing – conversion from singlet to triplet
3. Electron transfer – $^1DA \rightarrow D^+A^-$
4. Energy transfer – emission is transferred to an acceptor

Fluorescence quenching can be both beneficial and a source of error in experiments. Since fluorescence is subject to quenching, one must be careful to account for any possible fluorescence quenching that may occur during an experiment. However, the intentional addition of quenchers can be used to monitor the accessibility of fluorophores, and is thus a useful method for determining the location of fluorophores in a cell. Collisional quenching is a function of concentration, and is found in all solutions. This type of quenching can be minimized by keeping the concentration of fluorophores low. Typically, it is best to work in the micromolar range or lower. Intersystem crossing occurs due to the "heavy atom effect". Halogens like bromine and iodine, metals, and other elements, including heavy noble gases such as Xenon can give rise to spin flips that change the singlet excited state to a triplet excited state. Since fluorescence is emission from the singlet excited state, the singlet→triplet conversion, known as intersystem crossing, causes a decrease the fluorescence quantum yield. Electron transfer can occur when electron acceptors are in solution. Studies of solutions reveal much general information useful for more localized and defined FRET measurements.[2] The loss of an electron from a molecular excited state leads to a reduction in the fluorescence quantum yield. Energy transfer is similar, but the energy transfer process can lead to fluorescence from another molecule. Energy transfer is very useful in biomolecular studies since it is a strong function of the distance between the donor and acceptor. Thus, energy transfer can be used to determine whether there are changes in the distance of the donor and acceptor on the nanometer length scale. It is a molecular ruler.

2.2 Fluorescence resonant energy transfer (FRET)

Fluorescence resonance energy transfer (FRET) is a distance-dependent interaction between the electronic excited states of two dye molecules in which excitation is transferred from a donor molecule to an acceptor molecule without emission of a photon. FRET is dependent on the inverse sixth power of the intermolecular separation, making it useful over distances comparable with the dimensions of biological macromolecules. Thus, FRET is an important technique for investigating a variety of biological phenomena that produce changes in molecular proximity.

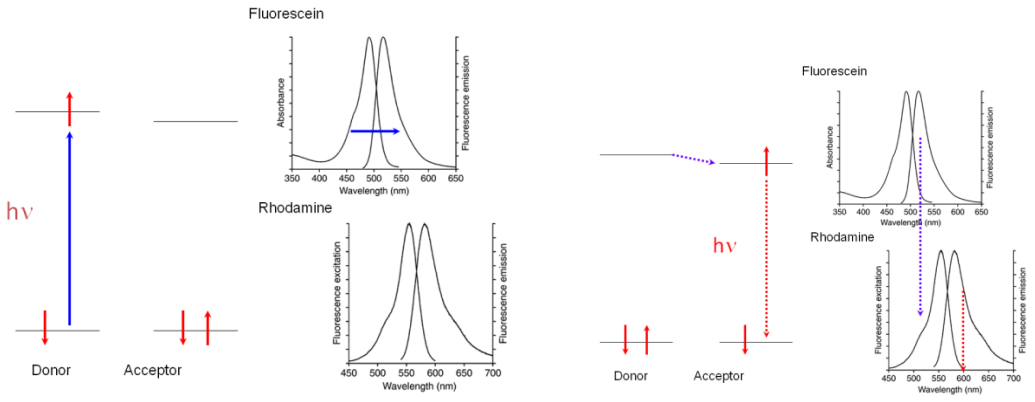

Figure 1. Energy transfer from one fluorophore (fluorescein) to another (rhodamine).

There are several requirements for energy transfer to occur. Because of the steep distance dependence, the donor and acceptor molecules must be in close proximity to one another (typically 10–100 Å). The absorption spectrum of the acceptor must overlap fluorescence emission spectrum of the donor. The donor and acceptor transition dipole orientations must be approximately parallel for maximum effect. The orientation dependence is $\cos^2\theta$, where θ is the angle between the transition dipole moments of the donor and acceptor.

In a fundamental sense the quantum yield for energy transfer is

$$E = \frac{k_{ET}}{k_f + k_{ET} + k_{nr} + k_Q[Q] + \cdots} \tag{2.1}$$

If no other quenchers are present then the normal k_{obs} of the monomer donor is $k_{obs} = k_f + k_{nr}$ as expected. Under these conditions we see that

$$E = \frac{k_{ET}}{k_{obs} + k_{ET}} = \frac{1}{1 + \frac{k_{obs}}{k_{ET}}} \tag{2.2}$$

Elsewhere the theory for dipole-dipole transitions shows a $1/R^6$ dependence ($1/R^3$ for each dipole) so that we can write the energy transfer efficiency is

$$E = \frac{1}{1 + \left(\frac{R}{R_0}\right)^6} \tag{2.3}$$

The rate constant for energy transfer can be written in a simple form that emphasizes the distance dependence,

$$k_{ET} = \frac{1}{\tau_{obs}}\left(\frac{R_0}{R}\right)^6 \tag{2.4}$$

The $1/R^6$ distance dependence arises from the fact that energy transfer is a dipole-dipole interaction. Speaking more precisely, it is the interaction of the transition dipole moment on the donor and the acceptor that gives rise to the distance dependence. In Eqn. 2.4, the constant R_0 represents the distance at which the energy transfer efficiency is 50%:

$$R_0 = \sqrt[1/6]{\frac{8x\ 10^{-28}\kappa^2\Phi_D J(\lambda)}{\tau_{obs} n^4}} \tag{2.5}$$

In Eqn. 2.5 the following quantities are defined as:

κ^2 - orientation factor (2/3 for an isotropic sample); n - index of refraction;

Φ_f - quantum yield of the donor

The spectral overlap integral is,

$$J(\lambda) = \int \varepsilon(\lambda) F_D(\lambda) \lambda^4 d\lambda \tag{2.6}$$

Where the quantity F_D is the normalized fluorescence. If the experimental fluorescence spectrum is represented by f_D then the two can be related by

$$\int \varepsilon F_D \lambda^4 d\lambda = \frac{\int \varepsilon f_D \lambda^4 d\lambda}{\int f_D \lambda^3 d\lambda} \tag{2.7}$$

We usually prefer to express spectral quantities in wave numbers, $\tilde{\nu}$.

$$J = 10^{28} \int \frac{\varepsilon f_D}{\tilde{\nu}^4} d\tilde{\nu} = \int \varepsilon F_D \lambda^4 d\lambda \tag{2.8}$$

Thus, we replace $J(\lambda)$ with the same expression using wave numbers

$$\frac{10^{28} \int \frac{\varepsilon f_D}{\tilde{\nu}^4} d\tilde{\nu}}{\int \frac{f_D}{\tilde{\nu}^3} d\tilde{\nu}}$$

The combination of orientation factors and indices of refraction gives a factor of (we assume water where n = 1.33) and κ^2 = 2/3.

$$\frac{\kappa^2 \Phi_D}{n^4} = 0.231 \tag{2.9}$$

Assuming

$$\Phi_D = 1$$

The FRET radius is

$$R_0 = \sqrt[1/6]{\frac{1.84 \int \frac{\varepsilon f_D}{\tilde{\nu}^4} d\tilde{\nu}}{\tau_{obs} \int \frac{f_D}{\tilde{\nu}^3} d\tilde{\nu}}} \tag{2.10}$$

From the Excel worksheet (see Excel for Spectral Overlaps on the website), for R6G-Fl the integral is 2.172. Substituting this value into the expression and using the fact that the observed lifetime for fluorescein (or uranine) is ~4 ns, we have

$$R_0 = \sqrt[1/6]{\frac{1.84(2.172)}{4\ x\ 10^{-9}}} \tag{2.11}$$

Based on the spectral overlap model the FRET radius for fluorescence and rhodamine 6G (donor and acceptor) is

$$R_0 = 31 \text{ Å} \tag{2.12}$$

Two examples of so-called FRET pairs are given in Table 1. These distances may be solvent dependent since spectra can change in different solvents, the donor lifetime may change and the solvent index of refraction enters the equation to the fourth power.

Table 1 Example of FRET pairs, including the distance of 50% FRET efficiency, R_o.

Donor	Acceptor	R_o (Å)
Fluorescein	Tetramethylrhodamine	55
IAEDANS	Fluorescein	46

2.2 Relating R_0 to concentration

The experiments in this project use solutions, which have a statistical distribution of molecules in a volume. Taking concentration c, the volume per molecule is v = 1.66/c. The average separation is usually considered to be the cube root of this value.

$$R = \sqrt[1/3]{1.66/c}$$

We can also calculate the equivalent concentration corresponding to FRET distances. In order to calculate the concentration in proper units we multiply the right hand side by Avagadro's number, N_A

$$c_0 = N_A \frac{1.66}{R_0^3}$$

Which leads to

$$c_0 = \frac{6.022\ x\ 10^{23}}{10^{-24}} \frac{1.66}{R_0^3}$$

The value of 10^{-24} is the conversion from Å to cm

$$c_0 = \frac{100}{R_0^3}$$

With c_0 in molar and R_0 in Å. Using the result obtained above for the fluorescein – rhodamine 6G FRET pair

$$c_0 = \frac{100}{45.5^3} \approx 1\ mM$$

This is a higher concentration than we will probably use, which means of course that the FRET yield is expected lower than 50%. This is why corrections for absorbance were used in analysis of fluorescence data, as discussed in the subsequent sections.

2.3 Measurement of FRET

As is the case in studies of fluorescence quantum yield and kinetics experiments complement each other.

2.3.1 Measurement based on the donor quantum yield reduction

The energy transfer yield can be calculated from the experimental ratio of donor emission in the presence of acceptor relative to the donor emission alone as given by Eqn. 2.13

$$E = 1 - \frac{\Phi_{AD}}{\Phi_D} \tag{2.13}$$

This equation is of limited value since it is often not possible to define a region of the spectrum that unique belongs to the donor for the Φ_{AD} calculation. Thus, in practice this is done at a single wavelength that is sufficiently far from the acceptor that it can be approximately set equal to relative donor population. In this case, we need only measure the intensities at that wavelength.

$$E = 1 - \frac{\mathrm{I}_{AD}}{\mathrm{I}_D} \tag{2.14}$$

Since concentrations of the donor can vary slightly from sample to sample, the equation can be corrected using absorbance data.

$$E = 1 - \frac{\mathrm{A}_D}{\mathrm{A}_{AD}} \frac{\mathrm{I}_{AD}}{\mathrm{I}_D} \tag{2.15}$$

where A_{D} and A_{DA} are the donor absorbances at the excitation wavelength.

2.3.2 Measurement based on the donor lifetime

The energy transfer yield can be calculated from the experimental ratio of donor emission

$$E = 1 - \frac{\tau_{AD}}{\tau_D} \tag{2.16}$$

The lifetime is shortened by the energy transfer process so that the ratio τ_{AD}/τ_D.

2.3.3 Measurement based on the acceptor fluorescence yield

The energy transfer yield can be calculated from the experimental ratio of acceptor emission, assuming that the a single wavelength intensity can be found the is exclusively indicative of the acceptor.

$$E = \frac{I_{AD}A_{AA} - I_{AA}A_{AD}}{I_{AA}A_{DD}} \quad (2.17)$$

Where the terms A_{AA} represent the absorbance at the same wavelength. This expression can also be expressed in terms of the molar absorptivities.

$$E = \frac{I_{AD}\varepsilon_{AA} - I_{AA}\varepsilon_{AD}}{I_{AA}\varepsilon_{DD}} \quad (2.18)$$

2.4 Polarization and Anisotropy

Anisotropy is induced by excitation using a polarized source. The excited transition dipole moments form a cone formed by the average of $\cos^2\theta$. Starting from this selection there molecules in the sample may rotate on the time scale of the measurement, permitting measurement of the rotational correlation time using polarization.

2.4.1 Experimental configuration

Emitted light can have two polarization components, parallel or perpendicular to the incident polarization. These polarizations are detected using an analyzing polarizer in front of the entrance slit of the spectrograph. The fluorescence polarization is an important experimental observable and can give information on molecular orientation. Specifically, one can measure the reorientation of molecules after excitation by monitoring changes in the anisotropy. One can excited using a parallel polarized laser and then detect using a polarizer. One makes two measurements, polarizer parallel or polarizer perpendicular. By determining the change in the emitted light in these two channels one can determine the rotational correlation time of a fluorescent molecule. This is of value in determining how mobile molecules are in solution. For example, in a viscous solution of glycerol and ethanol one would expect a significant slowing of the molecular rotation. This also indicates a slowing of diffusion.

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2.4.2 The definition of anisotropy

For Z-polarized incident radiation we can detect scattered light using a 90 degree geometry shown below. The incident light propagates along the X-direction and the scattered light along the Y-direction. The uppercase letters here refer to the laboratory coordinate system.

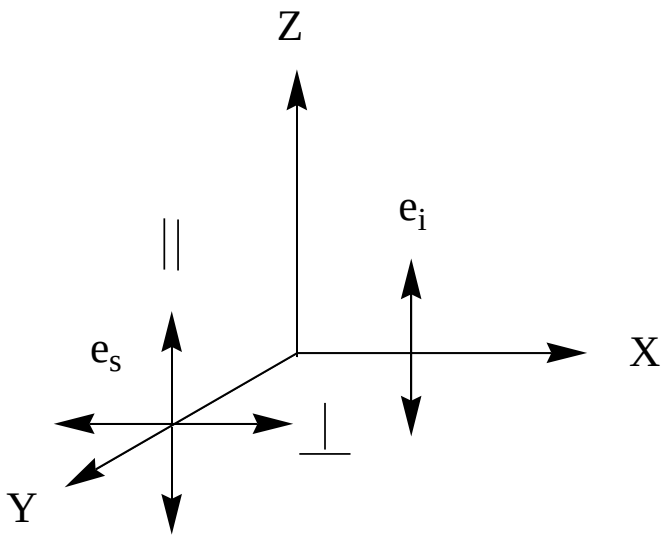

Figure 2. Definition of Raman polarization.

A polarization analyzer between the sample and the detector can be used to distinguish the polarization of emitted light. If the polarization in the emittred light is the same as the incident we refer to this as $I_{\parallel}$, I_{ZZ} or I_{pol}. If the polarization in the emitted light is perpendicular to the incident we refer to this as $I_{\perp}$, I_{XZ} or I_{dep}. Using these definitions, the anisotropy ratio is defined as

$$\alpha = \frac{I_{\parallel} - I_{\perp}}{2I_{\perp} + I_{\parallel}} \tag{2.20}$$

3. Reference literature for this experiment

Presented here are the abstracts or other information on several references that can be used to plan this experiment. The planning and protocol will be done with Dr. Franzen to ensure a feasibly and successful experiment.

1. A time-resolved study of forward and reverse excitation energy transport in a disordered two-component system of Na-fluorescein donor and rhodamine 6G acceptor in glycerol–

ethanol solution is reported. The experimental results are compared with those of the hopping model, accounting for the effect of reverse energy transfer. Quantitative agreement between the experiment and the theory is found for critical parameters close to those obtained from independent spectral measurements. Simultaneously, disagreement between experimental data and no-reverse-transport hopping model theory was found for this system.[1]

2. Effect of diffusion on excitation energy transfer and migration in a dye pair sodium fluorescein (donor) and Rhodamine-6G (acceptor) has been studied for different viscosities by both steady state and time domain fluorescence spectroscopic measurements. The donor-donor interaction appears to be weaker as compared to donor-acceptor interaction and thus favors direct Förster-type energy transfer. Interestingly, at low viscosity (water in this case) transfer appears to be controlled by material diffusion/energy migration. Further, acceptor dynamics reveals the fact that direct Förster transfer dominates in viscous media. [2]
3. Fluorescence Resonance Energy Transfer between two organic dyes Fluorescein and rhodamine 6G was investigated in aqueous solution in presence and absence of synthetic clay laponite. Spectroscopic studies suggest that both the dyes were present mainly as monomer in solution. Fluorescence Resonance Energy Transfer occurred from Fluorescein to Rhodamine 6G in solutions. Energy transfer efficiency increases in presence of laponite and the maximum efficiency was 72.00% in aqueous laponite dispersion. Energy transfer efficiency was found to be pH sensitive. It has been demonstrated that with proper calibration it is possible to use the present system under investigation to sense pH over a wide range from 1.5 to 8.0.[3]
4. The dye concentration dependence gain spectra for disodium fluorescein (FDS) cresyl violet (CV) and a dye mixture [Rh 590 (C1) + CV] dissolved in methanol have been studied by amplified spontaneous emission (ASE) technique under pulsed N_2-1aser excitation. On comparing the efficiency curves, the dominant role of photoquenching (excited state absorption) was clearly observed in the non-rigid dye FDS, whereas it is non-existent in rigid dye CV at the same concentration. In case of a dye mixture, the energy transfer excitation was found to overcome the inner filter effect and other losses. The various energy transfer mechanisms in these dyes have also been discussed.[4]
5. The values of R_0 obtained from quenching experiments are frequently somewhat larger than calculated from spectral data. One reason for such a discrepancy is the effect of diffusion on the efficiency of energy transfer. The Forster relationships are derived on the assumption that the donor and acceptor molecules do not diffuse together during the lifetime of the excited donor. This is a good approximation for short-lived excited states and viscous solvents. However, for longer-lived excited states and/or less viscous solvents, as the nearest excited donor/acceptor pairs are depleted, more pairs diffuse together leading to more efficient energy transfer, and hence a larger effective value of Rq. [5]
6. Here we present a new undergraduate laboratory that will introduce the concepts of time-resolved spectroscopy and provide insight into the natural time scales on which chemical dynamics occur through direct measurement. A quantitative treatment of the acquired

data will provide a deeper understanding of the role of quantum mechanics and various phenomenological expressions in predicting kinetic rates for fluorescence, phosphorescence, and nonradiative decay mechanisms. This laboratory framework focuses specifically on spectroscopy in the nanosecond regime assisted by various steady-state spectroscopic techniques in order to fully characterize the electronic structure and the picosecond-to-microsecond dynamics of the dye eosin B. There is great flexibility in both the recommended lab duration (1 week to several months) and course level (upper-division to graduate) due to the numerous additional experiments that may be performed at varying levels of difficulty. The necessary components include pump and probe light sources, photodiode detectors, a programmable signal delay generator, and an oscilloscope for measurements with requisite resolution. The cost of building this experiment from scratch is less than $20,000 at the time of publication, but costs are expected to decrease over time and alternate excitation sources are available. Although this lab requires some expertise with optical spectroscopy to initially build and troubleshoot, use by students has been a straightforward and valuable experience. [6]

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