Luminescence Virtual Course

Luminescence is a general phenomenon, which has received wide use in science. From cell biology to engineering, researchers have used the phenomena of fluorescence and phosphorescence combined with quenching and energy transfer to label proteins, determine solution pH, polarity or immobilization of a dye. These applications are based measurement of the quantum yield. The quantum yield is a measure of the tendency of molecular excited state to return to the ground state by emission of a photon. It is a number between zero and one that tells us the ratio of excited state molecules that emit light. The other alternative is a non-radiative pathway back to the ground state. In addition to intrinsic or molecular non-radiative pathways, a molecule may lose energy by means of energy transfer, which may lead to quenching or radiation by a different molecule. Because there is a distance dependence to energy transfer the phenomenon has been developed as a means of determining distance on a molecular scale.

The discussion will focus mainly on fluorescence. However, phosphorescence follows similar principles.

Factors that affect the fluorescence quantum yield

The quantum yield of the fluorescence is given by,

$$\Phi_{\mathrm{f}} = \frac{k_f}{k_f + k_{nr}}$$

Where k_f and k_{nr} are the intrinsic fluorescence and non-radiative rate constants, respectively. It is important to understand that a time-resolved fluorescence measures an observed rate constant, k_{obs} which is the sum of the two intrinsic rate constants,

$$k_{obs} = k_f + k_{nr}$$

Thus, we see that by measurement of both Φ_{f} and k_{obs} we can obtain the intrinsic rate constants.

$$k_f = \Phi_{\mathrm{f}} k_{obs}$$

$$k_{nr} = k_{obs} - k_f$$

When quenching is also involved the quantum yield becomes

$$\Phi_{\mathrm{q}} = \frac{k_f}{k_f + k_{nr} + k_q[Q]}$$

and

$$k_{obs,q} = k_f + k_{nr} + k_q[Q]$$

The quenching rate in solution differs from quenching in membranes or immobilized systems. Thus, quenching can be used to determine the environment of a fluorophore.

Solvent polarity and pH can also affect the fluorescence quantum yield. The origin of these effects is more complicated. In some molecules, the fluorescence rate constant itself is altered. The fluorescence rate constant is related to the absorption rate constant (see Einstein relations). Thus, if the absorption spectrum is reduced in intensity because of solvent effects, then we expect that the fluorescence associated with the transition to that state will also be reduced. Increased solvent polarity can also increase the charge transfer character of the excited state. This tends to increase the non-radiative rate constant, k_{nr} and decrease the quantum yield.

Measuring time-resolved fluorescence

We have shown that two measurements are necessary to obtain the fundamental parameters, k_f and k_{nr}. These two rate constants, in their turn, are needed to conduct an analysis of fluorescence quenching using the Stern-Volmer method. Time-resolved measurements are difficult on the nanosecond time scale because the response time of electronic circuits is often several nanoseconds. The response time of a circuit is determined by the RC time constant. A typical resistor of 1 kiloohms combined with stray capacity of 1 picofarad corresponds to a time constant of about 6 nanoseconds. Often these values are difficult to achieve and the time resolution is worse. While direct measurement is possible, it is difficult and not often reported for that reason.

There are two main methods to obtain time-resolved data on the nanosecond time scale, (1) time-correlated single photon counting (TCSPC) and (2) phase sensitive emission spectroscopy.

There is a lecture available in powerpoint form. Edinburgh Instruments has some of the best material and excellent instrumentation for measuring time-resolved spectra using TCSPC

https://www.edinst.com/blog/what-is-tcspc/

Review

Please read the luminescence background documentation written for this lab. Check out the wiki-lecture at:

https://www.wikilectures.eu/w/Fluorescence_quenching

Please Answer the Review Questions

Assignment: Use the data provided to determine the Stern-Volmer quenching of uranine by iodide. Write a laboratory report based on this analysis.