Selective topics in physical chemistry
Introduction to Fluorescence

Luminescence is the emission of light from any substance, and occurs from electronically excited states. Luminescence is formally divided into two categories—fluorescence and phosphorescence—depending on the nature of the excited state. In excited singlet states, the electron in the excited orbital is paired (by opposite spin) to the second electron in the ground-state orbital. Consequently, return to the ground state is spin allowed and occurs rapidly by emission of a photon. The emission rates of fluorescence are typically 10^8 s^{-1}, so that a typical fluorescence lifetime is near 10 ns (10 x 10^{-9} s), the lifetime (τ) of a fluorophore is the average time between its excitation and return to the ground state. It is valuable to consider a 1-ns lifetime within the context of the speed of light.

Phosphorescence is emission of light from triplet excited states, in which the electron in the excited orbital has the same spin orientation as the ground-state electron. Transitions to the ground state are forbidden and the emission rates are slow (10^3 to 100 s^{-1}), so that phosphorescence lifetimes are typically milliseconds to seconds. Even longer lifetimes are possible. Following exposure to light, the phosphorescence substances glow for several minutes while the excited phosphors slowly return to the ground state. Phosphorescence is usually not seen in fluid solutions at room temperature. This is because there exist many deactivation processes that compete with emission, such as non-radiative decay and quenching processes. It should

be noted that the distinction between fluorescence and phosphorescence is not always clear. Transition metal–ligand complexes (MLCs), which contain a metal and one or more organic ligands, display mixed singlet–triplet states. These MLCs display intermediate lifetimes of hundreds of nanoseconds to several microseconds. Fluorescence typically occurs from aromatic molecules. Some typical fluorescent substances (fluorophores) are shown in Figure 1. One widely encountered fluorophore is quinine, which is present in tonic water. If one observes a glass of tonic water that is exposed to sunlight, a faint blue glow is frequently visible at the surface.

1,4-bis(5-phenyloxazol-2-yl) benzene (POPOP)

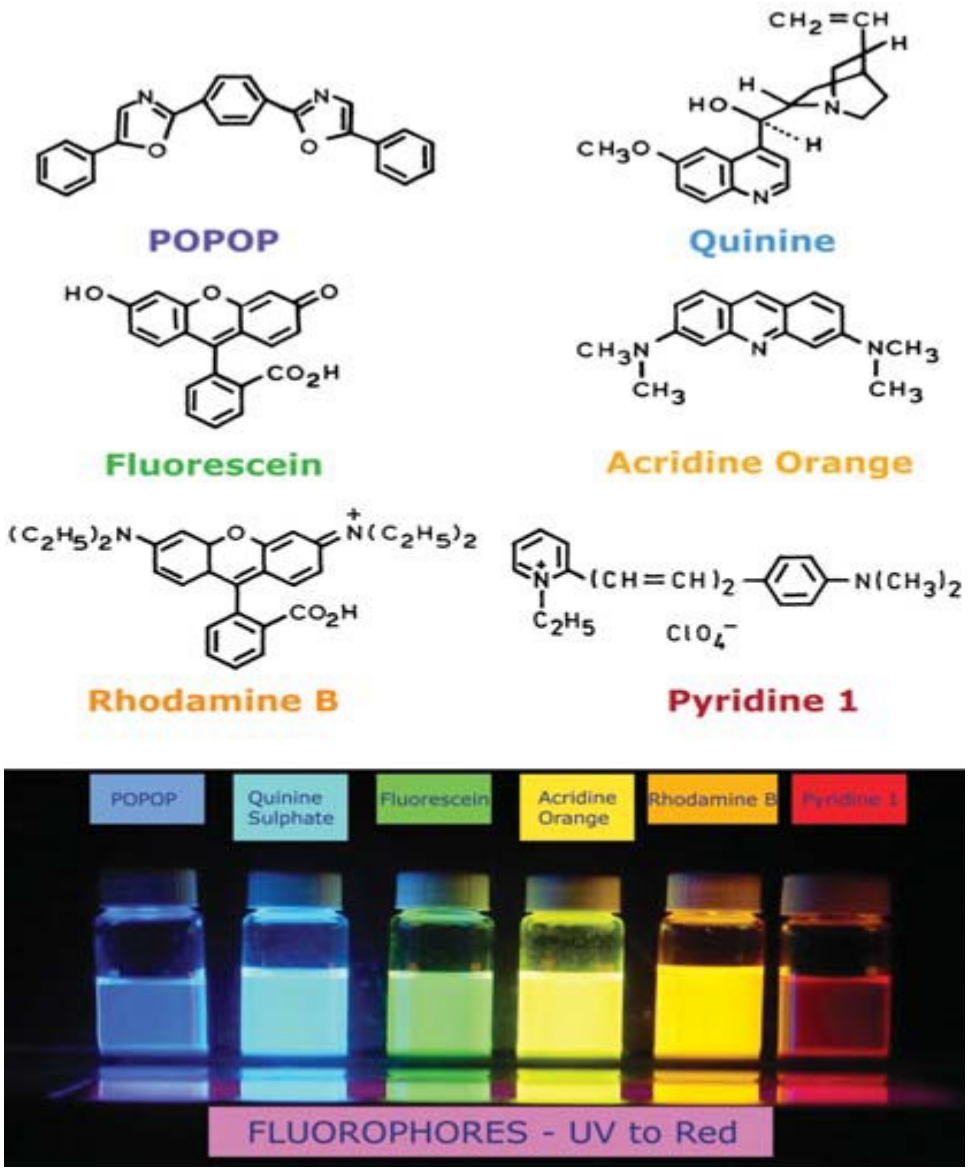

Figure 1. Structures of typical fluorescent substances

This glow is most apparent when the glass is observed at a right angle relative to the direction of the sunlight, and when the dielectric constant is decreased by adding less polar solvents like alcohols. The quinine in tonic water is excited by the ultraviolet light from the sun. Upon return to the ground state the quinine emits blue light with a wavelength near 450 nm. The first observation of fluorescence from a quinine solution in sunlight was reported by Sir John Frederick William Herschel in 1845.

Sir John Fredrich William

Fluorescence spectral data are generally presented as emission spectra. A fluorescence emission spectrum is a plot of the fluorescence intensity versus wavelength (nanometers) or wavenumber (cm^{-1}). Two typical fluorescence emission spectra are shown in Figure 2. Emission spectra vary widely and are dependent upon the chemical structure of the fluorophore and the solvent in which it is dissolved. The spectra of some compounds,

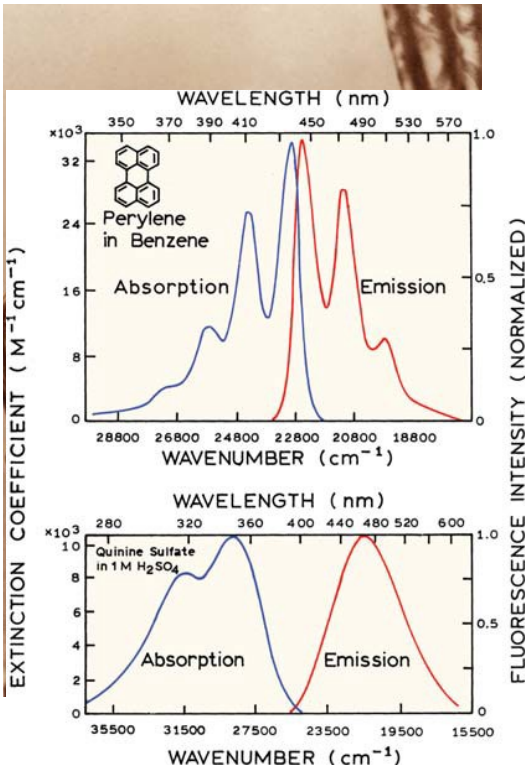

Figure 2. Absorption and fluorescence emission spectra of perylene and quinine. Emission spectra cannot be correctly presented on both the wavelength and wavenumber scales

such as perylene, show significant structure due to the individual vibrational energy levels of the ground and excited states. Other compounds, such as quinine, show spectra devoid of vibrational structure.

JABLONSKI DIAGRAM

The processes that occur between the absorption and emission of light are usually illustrated by the Jablonski diagram. Jablonski diagrams are often used as the starting point for discussing light absorption and emission. Jablonski diagrams are used in a variety of forms, to illustrate various molecular

processes that can occur in excited states. These diagrams are named after Professor Alexander Jablonski , who is regarded as the father of fluorescence spectroscopy because of his many accomplishments, including descriptions of concentration depolarization and defining the term "anisotropy" to describe the polarized emission from solutions.

A typical Jablonski diagram is shown in Figure 3. The singlet ground, first, and second electronic states are depicted by S_0, S_1, and S_2, respectively. At each of these electronic energy levels the fluorophores can exist in a number of vibrational energy levels, depicted by 0, 1, 2, etc. In this Jablonski diagram we excluded a number of interactions, such as quenching, energy transfer, and solvent interactions. The transitions between states are depicted as vertical lines to illustrate the instantaneous nature of light absorption. Transitions occur in about 10^{-15} s, a time too short for significant displacement of nuclei. This is the Franck-Condon principle.

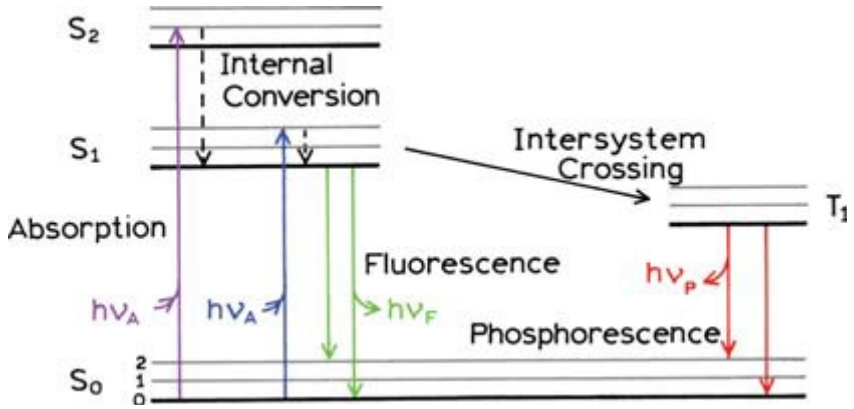

Figure 3. One form of a Jablonski diagram.

Following light absorption, several processes usually occur. A fluorophore is usually excited to some higher vibrational level of either S_1 or S_2. With a few rare exceptions, molecules in condensed phases rapidly relax to the lowest vibrational level of S_1. This process is called internal conversion and generally occurs within 10^{-12} s or less. Since fluorescence lifetimes are typically near 10^{-8} s, internal conversion is generally complete prior to emission. Hence, fluorescence emission generally results from a thermally equilibrated excited state, that is, the lowest energy vibrational state of S_1.

Return to the ground state typically occurs to a higher excited vibrational ground state level, which then quickly (10^{-12} s) reaches thermal equilibrium (Figure 3). Return to an excited vibrational state at the level of the S_0 state is the reason for the vibrational structure in the emission spectrum of perylene. An interesting consequence of emission to higher vibrational ground states is that the emission spectrum is typically a mirror image of the absorption spectrum of the $S_0 \rightarrow S_1$ transition. This similarity occurs because electronic excitation does not greatly alter the nuclear geometry. Hence the spacing of the vibrational

energy levels of the excited states is similar to that of the ground state.

As a result, the vibrational structures seen in the absorption and the emission spectra are similar.

Molecules in the S_1 state can also undergo a spin conversion to the first triplet state T_1. Emission from T_1 is termed phosphorescence, and is generally shifted to longer wavelengths (lower energy) relative to the fluorescence. Conversion of S_1 to T_1 is called intersystem crossing. Transition from T_1 to the singlet ground state is forbidden, and as a result the rate constants for triplet emission are several orders of magnitude smaller than those for fluorescence. Molecules containing heavy atoms such as bromine and iodine are frequently phosphorescent. The heavy atoms facilitate intersystem crossing and thus enhance phosphorescence quantum yields.

The Stokes Shift

Examination of the Jablonski diagram reveals that the energy of the emission is typically less than that of absorption. Fluorescence typically occurs at lower energies or longer wavelengths. This phenomenon was first observed by Sir. G. G. Stokes in 1852. The source of ultraviolet excitation was provided by sunlight and a blue glass filter, which was part of a stained glass window. This filter selectively transmitted light below 400 nm, which was absorbed by quinine. The incident light was prevented from reaching the detector (eye) by a yellow glass filter. Quinine fluorescence occurs near 450 nm

and is therefore easily visible. Energy losses between excitation and emission are observed universally for fluorescent molecules in solution. One common cause of the Stokes shift is the rapid decay to the lowest vibrational level of S_1. Furthermore, fluorophores generally decay to higher vibrational levels of S_0, resulting in further loss of excitation energy by thermalization of the excess vibrational energy. In addition to these effects, fluorophores can display further Stokes shifts due to solvent effects, excited-state reactions, complex formation, and/or energy transfer.

It is interesting to ask why perylene follows the mirror image rule, but quinine emission lacks the two peaks seen in its excitation spectrum at 315 and 340 nm (Figure 2). In the case of quinine, the shorter wavelength absorption peak is due to excitation to the second excited state (S_2), which relaxes rapidly to S_1. Emission occurs predominantly from the lowest singlet state (S_1), so emission from S_2 is not observed. The emission spectrum of quinine is the mirror image of the $S_0 \rightarrow S_1$ absorption of quinine, not of its total absorption spectrum. This is true for most fluorophores: the emission is the mirror image of $S_0 \rightarrow S_1$ absorption, not of the total absorption spectrum.

Suppose the absorption spectrum of a fluorophore shows distinct peaks due to the vibrational energy levels. Such peaks are seen for anthracene in Figure 4. These peaks are due to transitions from the lowest vibrational level of the S_0 state to higher vibrational levels of the S_1 state. Upon return to the S_0 state the fluorophore can return to any of the ground state

vibrational levels. These vibrational energy levels have similar spacing to those in the S_1 state. The emission spectrum shows the same vibrational energy spacing as the absorption spectrum.

According to the Franck-Condon principle, all electronic transitions are vertical, that is, they occur without change in the position of the nuclei. As a result, if a particular transition probability (Franck-Condon factor) between the 0th and 1st vibrational levels is largest in absorption, the reciprocal transition is also most probable in emission (Figure 4).

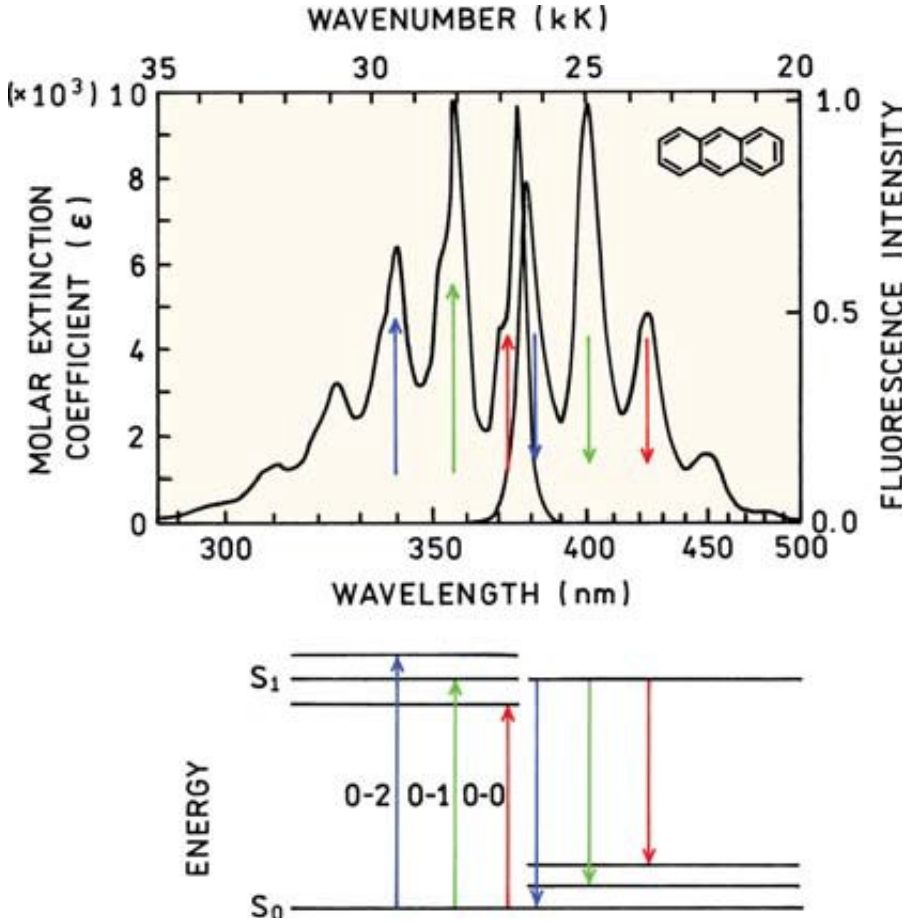

Figure 4. Mirror-image rule and Franck-Condon factors. The absorption and emission spectra are for anthracene. The numbers 0, 1, and 2 refer to vibrational energy levels.

FLUORESCENCE LIFETIMES AND QUANTUM YIELDS

The fluorescence lifetime and quantum yield are perhaps the most important characteristics of a fluorophore. Quantum yield is the number of emitted photons relative to the number of absorbed photons. Substances with the largest quantum yields, approaching unity, such as rhodamines, display the brightest emissions. The lifetime is also important, as it determines the time available for the fluorophore to interact with or diffuse in its environment, and hence the information available from its emission.

The fluorescence quantum yield is the ratio of the number of photons emitted to the number absorbed. The rate constants Γ and *k*nr both depopulate the excited state. The fraction of fluorophores that decay through emission, and hence the quantum yield, is given by

$$Q=\frac{\Gamma}{\Gamma+K_{nr}} \qquad 1$$

The quantum yield can be close to unity if the radiationless decay rate is much smaller than the rate of radiative decay, that is *k*nr < Γ . We note that the energy yield of fluorescence is always less than unity because of Stokes losses.