Chapter 1 General Introduction

1.1 Luminescence

Luminescence is the ultraviolet, visible or infrared radiation emitted by the electronic excited molecule. There are two types of luminescence: fluorescence and phosphorescence, which is distinguished by the nature of the excited state. If the spin of the upper state and the lower state is the same (single-single or triplet-triplet), the radiatve transition occur between those two states is called fluorescence. On the other word, if spin of the upper and the lower state is different, the radiative transition occur between those two states is called phosphorescence. In most cases, the spin of the ground state is singlet, and the process of emitting a photon from singlet excited state to ground state is spin-allowed. The emission of fluorescence can occur rapidly, and the typical radiative lifetime is ranging from nanosecond (10^{-9}s) to microsecond (10^{-6}s) time scale. Once the molecule is excited, many other pathways like internal conversion, intersystem crossing, intramolecular conformational change and charge transfer….etc, will compete with the radiatve process. (Figure 1.1)

Therefore, the characteristic of fluorescence (spectrum, quantum yield, and lifetime) can provide us the information of the above-mentioned non-radiative process. In early years, because of the restriction of instruments, the fluorescence measurement was restricted on the steady state spectra, which can be thought as the average of the time-resolved spectra:

$$I_{ss} = \sum_{n=1}^{\infty} \int_{0}^{\infty} I_0 \times e^{-t/\tau_n} dt \qquad (1)$$

The multi-exponential decay reflects the processes that involved in the excited-state deactivation. However, much information about the molecules is lost during the time-averaging process. For that reason, to obtain the time-resolved fluorescence spectra become very important to investigate those non-radiative processes, and the time-resolved fluorescence technique will be introduced in the next section.

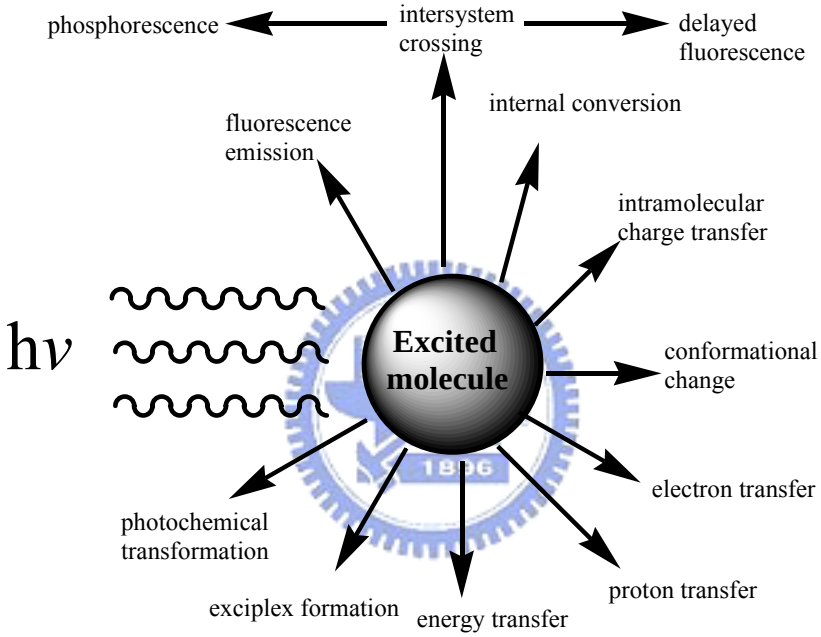

Figure 1.1: Possible non-radiative processes that compete with the fluorescence emission

1.2 Fluorescence lifetime

Fluorescence lifetime is the most important quantity for people who want to investigate the excited state dynamics by using time-resolved fluorescence spectroscopy. Since fluorescence is proportional to the excited state population of molecules, fluorescence lifetime can be defined as the reciprocal of the rate of depopulation of excited state molecules following optical excitation.[1] For radiative

[1] Lakowicz, J. R. *Topics in fluorescence spectroscopy vol. 1: Techniques*; Plenum Publishing

process, the decay of excited state population can be described with the following equation:

$$\frac{d[M^*]}{dt} = -\frac{[M^*]}{\tau_r} \quad (2)$$

In which, $[M^*]$ denotes the concentration of excited state molecules.

After integration, we obtain:

$$[M^*] = [M^*]_0 \times e^{-t/\tau_r} \quad (3)$$

Where $[M^*]_0$ indicates the excited state concentration at time-zero. Equation 3 describes the simplest case of fluorescence decay, in which all excited molecules return to the ground state via radiative process. In this case, τ_r is called the radiative lifetime of the molecule, and radiative rate constant k_r is the reciprocal of τ_r. However, in most cases non-radiative processes will compete with the radiative pathway, and the observed fluorescence lifetime τ_f is the reciprocal of the sum of k_r and nonradiative rate constant k_{nr}:

$$\tau_f = \frac{1}{k_r + k_{nr}} \quad (4)$$

In general, the fluorescence lifetime of the interesting molecule will be measured under various conditions (different excitation and probing wavelengths, viscosity, temperature, etc). The nature of the nonradiative process can be investigated by comparing the fluorescence transients under those conditions, and verify how k_{nr} changes with those parameters

1.3 Phase-modulation and pulse excitation method

The first nanosecond lifetime was measured by E. Gaviola in 1926 by using phase

Corporation: New York, 1991; Vol. 1.

modulation method.[2] In phase-modulation method, the sample was excited by intensity-modulated light. Usually, the excitation light is modulated with sin wave. Once the molecule was excited by modulated light, the intensity of fluorescence was forced to respond at the same modulation frequency. However, the phase of emitted fluorescence would be shifted, and the extent of modulation would be decreased due to the delay of the fluorescence emission.

An alternative method is pulse-excitation method, which is the most popular method at present. In pulse-excitation method (Figure. 1.2), the sample is excited with a short pulse. The time-dependent fluorescence transient is detected following optical excitation, and the decay time is calculated from the slope of plot intensity versus time. Usually fluorescence lifetime τ is defined as the time of the intensity decrease to 1/e of the value at time-zero.

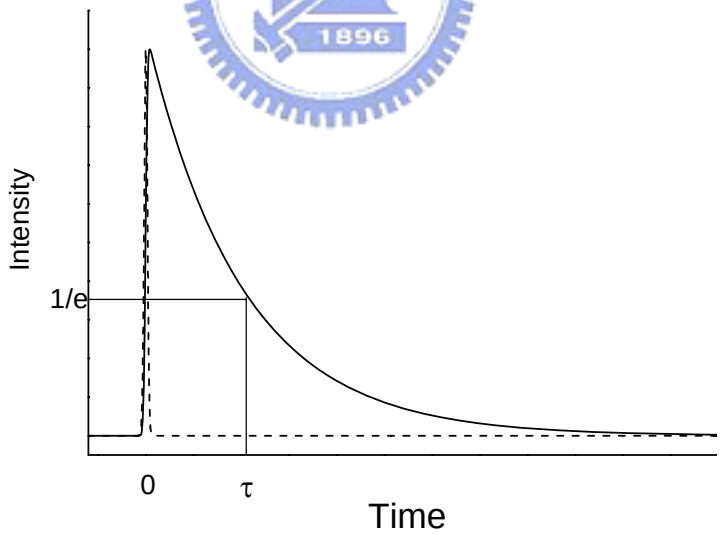

Figure 1.2: The principle of time-domain method for measuring fluorescence lifetime

1.4 Pulse excitation techniques

At present, time-correlated single photon counting (TCSPC) is widely used in

[2] Valeur, B. *Molecular fluorescence*; Weinheim; Wiley-VCH: New York, 2002.

time-domain lifetime measurement. This method was first developed by Bollinger and Thomas in 1961,[3] and the basic principle is that the probability of detecting a single photon at a delay time "t" is proportion to the fluorescence intensity at that time. Therefore, by histogramming the arrival time of individual photon over millions of excitation cycles, we can reconstruct the fluorescence decay profile. With the improvements of technology, the time resolution of TCSPC technique can reach tens of ps. Because of the importance of TCSPC method, the detail principle will be described in Chapter 2.

For the fluorescence decay within the range between picosecond to subpicosecond time scale, fluorescence up-conversion method has been widely used. The basic idea is to pass the fluorescence signal through a nonlinear crystal (LBO or BBO) and gated by another femto or picosecond light pulse.[4] Once phase-matching angle and optical delay between fluorescence and gate pulse are properly adjusted, the up-converted signal will appear. The wavelength of the up-converted signal can be calculated using the following equation:

$$\frac{1}{\lambda_{\text{signal}}} = \frac{1}{\lambda_{\text{fluorescence}}} + \frac{1}{\lambda_{\text{gate}}} \tag{5}$$

The time-resolved transient is obtained by measuring the intensity of up-converted signal as the delay time is changed. (Figure. 1.3) The superior time resolution (<150 fs, depending on the pulse duration) of this method makes the investigation of the ultrafast elementary molecular-relaxation processes become possible. However, this method is difficult to obtain the fluorescence in nanosecond timescale, because the

[3] Bollinger, L. M.; Thomas, G. E. *Rev. Sci. Instrum.* **1961**, *32*, 1044.
[4] Kahlow, M. A.; Jarzeba, W.; DuBruil, T. P.; Barbara, P. F. *Rev. Sci. Instrum.* **1988**, *59*, 1098.

delay line will become too long (1 ns is corresponding to 30 cm optical delay), and the alignment of the delay line will be too difficult to maintain as the time delay is changed.

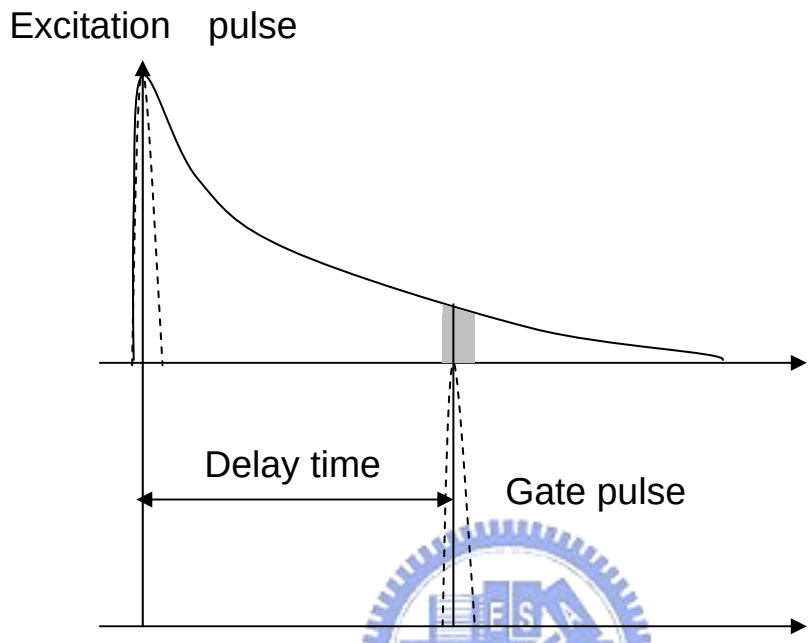

Figure 1.3: Scheme layout of the principle of fluorescence up-conversion

1.5 Summary

In summary, by using the above-mentioned time-resolved fluorescence spectroscopy, my works are focused on the excited-state dynamics of organic nanoparticles and 3,6-bis(1-methyl-4-vinylpyridinium) carbazole diiodide (BMVC), and I will give a brief introduction in the following paragraph.

In the past few years, organic nanostructures has became more and more important because of its large variety and the interesting properties.[5,6,7]. In many cases, the fluorescence property of organic molecules and its nanostructure are very

[5] Horn, D.; Rieger, J. *Angew. Chem. Int. Ed.* **2001**, *40*, 4330.
[6] Kasai, H.; Kamatani, H.; Okada, S.; Oikawa, H.; Matsuda, H.; Nakanishi, H. *Jpn. J. Appl. Phys.* **1996**, *35*, L221
[7] Yoshikava, H.; Masuhara, H. *J. Photochem. Photobiol., C* **2000**, 57.

different.[8,9,10,11] For 1,4-distyrylbenzene (DSB)[12] or DPST[13] , the formation of nanoparticles will decrease the fluorescence. However, for CN-MBE, the formation of the nanoparticle increases the fluorescence.[13] In order to understand the crucial factor in determining the fluorescence change of nanoparticles, we investigated the excited state dynamics of 1,4-di[(E)-2-phenyl-1-propenyl]benzene (PPB), and CNDSB. Chapter 3 is focused on PPB nanoparticles. In PPB, we observed the enhancement of the fluorescence due to the formation of nanoparticles. Our results reveal that the fluorescence enhancement is due to not only the geometry restriction of the molecules, but also the combined effects of the conformational planarization and the herringbone-type aggregate formation. In Chapter 4, I extend the work to CNDSB, which forms nanobelts in solution, and we also observe similar fluorescence enhancement due to the formation of nanostructures. However, a unique ultrafast energy transfer is observed in this case, which might be caused by the one-dimensional nanostructure of the nanobelts.

The second series of studies is about the 3,6-bis(1-methyl-4-vinylpyridinium) carbazole diiodide (BMVC), which can recognize the quadruplex structure of DNA. Recently, the interaction between telomerase and telomere has attracted lots of attention because of its importance in the abnormal overgrowth of cancer cells.[14,15,16]

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[14] Greider, C. W. and Blackburn, E. H. *Cell* **1987**, *51*, 887.
[15] Feng, J., Funk, W. D., Wang, S.-S., Weinrich, S. L., Avilion, A. A., Chiu, C.-P., Adams, R. R., Chang,

some studies pointed out that the formation of quadruplex structure in telomere can inhibit the activity of telomerase,[17,18] and therefore the studies about the quadruplex structure DNA become more and more important. In 2003, Ta-Chau Chang's group synthesized a new carbazole dye: BMVC, which can be used to distinguish the duplex and quadruplex structure of DNA, and can be used to label the cancer cell. [19] According to their result, the fluorescence of BMVC is weak in tris-buffer.[19] However, as BMVC interacted with liner duplex (LD) and quadruplex (Hum24) DNA, they observed a dramatic enhancement of the fluorescence. The emission spectrum of BMVC/LD and BMVC/Hum24 is central at ~560 nm and ~580 nm, respectively. Therefore it can be used to recognize the quadruplex structure of DNA. However, the information about the excited state dynamics of BMVC is still absent. In Chapter 5, the excited state dynamics of BMVC in tris-buffer and glycerol is reported. By comparing the fluorescence transient of BMVC in tris-buffer and glycerol, we found that both rotational induced internal conversion and vibrational relaxation process are involved in the excited-state dynamics of the molecules. In glycerol, because of the high viscosity of glycerol, the intramolecular motion is suppressed, therefore we observed the increase of fluorescence. By reconstructing the time-resolved emission spectra, the environment sensitivity of the solvation dynamics were tested in tris-buffer and glycerol, and the results indicated that it is sensitive to the local

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E., Allsopp, R. C., Yu, J., Le, S., West, M. D., Harley, C. B., Andrews, W. H., Greider, C. W. and Villeponteau, B. *Science* **1995**, *269*, 1236.

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environment. In Chapter 6, we focused on the interaction between BMVC and DNA in BMVC/DNA (LD or Hum24) complexes. When BMVC interacted with linear duplex DNA (LD) or quadruplex DNA (Hum24), the fluorescence intensity and lifetime increase dramatically. The results indicated that the interaction between BMVC and DNA molecule can suppress the intramolecular motion of the molecule. The solvation dynamics reveals that for BMVC in LD and Hum24, the solvation process is contributed from both bulk like water and weakly bound water. The relative amplitude of the salvation caused by those two kinds of water is different in LD and Hum24. It can be used as the alternative method to obtain the information about the bind site of BMVC in LD and Hum24 DNA.