*J. Phys. Chem. A* **2007,** *111,* 1593−1597

# Characterization of Photoinduced Isomerization and Intersystem Crossing of the Cyanine Dye Cy3

**Ke Jia, Yan Wan, and Andong Xia***

*The State Key Laboratory of Molecular Reaction Dynamics, and Beijing National Laboratory for Molecular Sciences (BNLMS), Institute of Chemistry, Chinese Academy of Sciences, Beijing-100080, P. R. China*

**Shayu Li, Fangbin Gong, and Guoqiang Yang**

*CAS key Laboratory for Photochemistry, and Beijing National Laboratory for Molecular Sciences (BNLMS), Institute of Chemistry, Chinese Academy of Sciences, Beijing-100080, P. R. China*

*Received: November 27, 2006; In Final Form: January 7, 2007*

Several important photophysical properties of the cyanine dye Cy3 have been determined by laser flash photolysis. The triplet-state absorption and photoisomerization of Cy3 are distinguished by using the heavy-atom effects and oxygen-induced triplet → triplet energy transfer. Furthermore, the triplet-state extinction coefficient and quantum yield of Cy3 are also measured via triplet−triplet energy-transfer method and comparative actinometry, respectively. It is found that the triplet → triplet ($T_1 \rightarrow T_n$) absorptions of *trans*-Cy3 largely overlap the ground-state absorption of *cis*-Cy3. Unlike what occurred in Cy5, we have not observed the triplet-state $T_1 \rightarrow T_n$ absorption of *cis*-Cy3 and the phosphorescence from triplet state of *cis*-Cy3 following a singlet excitation ($S_0 - S_1$) of *trans*-Cy3, indicating the absence of a lowest cis-triplet state as an isomerization intermediate upon excitation in Cy3. The detailed spectra of Cy3 reported in this paper could help us interpret the complicated photophysics of cyanine dyes.

## Introduction

In the past 10 years, cyanine dyes have been intensively studied by various photophysical and photochemical means,[1−17] owing to the interest for their widespread applications such as spectral sensitizers in photography,[18,19] in biomedical application,[20−23] in nonlinear optics and laser physics,[24−25] and especially in single-molecule detection.[26−33] Although there has been much discussion in the literature on the mechanism of the deactivation of excited cyanine dyes, the underlying mechanisms about fluorescence, intersystem crossing, and the trans−cis photoisomerization remain unknown to a large extent.[15−17,30,31,34−36]

Recently, single-molecule fluorescence experiments have observed several important and unexpected photophysical phenomena of cyanine dyes (such as Cy3 and Cy5) related to the cis−trans isomerization and triplet-state formation.[4,15−17,26−28,30,34,36−38] For instance, in a single-molecule optical switch, it is unexpected that the conversion of Cy5 from dark state to fluorescence state is dramatically enhanced in the presence of Cy3.[34,37] The interaction between Cy3 and Cy5 exhibits a distance dependence much steeper than that of conventional Förster resonance energy transfer (FRET) and therefore acts as a short-range spectroscopic ruler for the study of biomolecules at the single-molecule level, where the mechanism about the enhanced conversion of Cy5 from dark state to fluorescence state in the presence of Cy3 remains unclear.[34,37] On the other hand, Cy3 has been reported as an adjacent reporter molecule to “sense” the presence of the dark intermediates of Cy5, where the dark states of Cy5 are still able to quench the fluorescence of the donor Cy3.[4,16,36] Further study indicated that a contact-induced reaction between an excited Cy3 and blinked Cy5 is necessary to bring back Cy5 from blinking. Such recovery occurs so quickly at short distances that blinking is not observed.[4,16,36,38] In addition, an alternative triplet-state quencher, Trolox, was shown to eliminate cyanine dye blinking, suggesting a link between triplet state and blinking. Unfortunately, it is still unclear whether this excited Cy3 quenched by dark Cy5[4,16,36,38] is also related to the enhanced switching behavior mentioned above.[34,37]

In our previous studies on the specific spectral properties of Cy5,[15−17] we have spectrally determined several specific photophysics of Cy5 by means of ensemble and single-molecule measurements. It was found that the triplet state ($T_1 \rightarrow T_n$) absorptions largely overlap the ground-state absorptions for either *trans*- or *cis*-Cy5, which results in several unexpected photophysical phenomena of Cy5.[4,15−17] Furthermore, an important isomerization pathway for Cy5 from the *trans*-$S_1$ state to the *cis*-$T_1$ state upon excitation is also identified from the observed delayed fluorescence and *cis*-$T_1$ → *cis*-$T_n$ absorption.[15,16] Since Cy3 and Cy5 are often used as a donor−acceptor pair for FRET measurement,[4,34,37] the complicated spectral behaviors of donor Cy3 could largely affect the Cy3/Cy5 FRET results.[4,16,34,36−38] Compared to the longer polymethine chain length for Cy5, cyanine dye Cy3 has a shorter chain length, which may lead to different competition behaviors between isomerization and intersystem crossing (ISC).

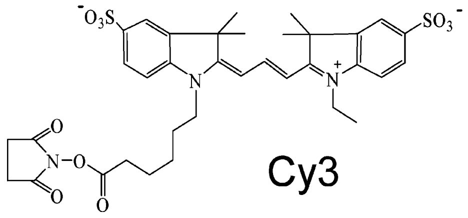
In this paper, we report the detailed spectral properties of the triplet-state formations of Cy3 structurally related to trans−cis photoisomerization by means of laser flash photolysis. The triplet-state absorption and photoisomerization of Cy3 are distinguished by using the heavy-atom effects and oxygen-induced triplet→triplet energy transfer. The triplet-state extinction coefficient and quantum yield of Cy3 are also measured

---

* To whom correspondence should be addressed. E-mail: andong@iccas.ac.cn.

10.1021/jp067843i CCC: $37.00

**CHART 1: Chemical Structure of Cy3**



via triplet−triplet energy-transfer method and comparative actinometry, respectively. The delayed fluorescence and phosphorescence of Cy3 are measured to see whether the lowest cis-triplet state is also involved in the formation of the isomer *cis*-Cy3 following the singlet $S_0 \rightarrow S_1$ excitation of *trans*-Cy3 as what occurred in Cy5.[15,16] Our results show that the triplet state ($T_1-T_n$) absorptions of *trans*-Cy3 largely overlap the ground-state absorption of *cis*-Cy3. Furthermore, we have not observed the triplet-state absorption of *cis*-Cy3 and the *cis*-phosphorescence from triplet state of *cis*-Cy3 following a singlet ($S_0 \rightarrow S_1$) excitation of *trans*-Cy3, indicating the absence of a lowest cis-triplet state as an isomerization intermediate upon excitation in Cy3. These corresponding results of Cy3 reported in this paper could help us interpret the complicated photophysics of cyanine dyes.[4,16,34,36−38]

## Materials and Methods

Cy3 was purchased from Amersham Biosciences. 2′-Acetonaphthone (2AN) and ethyl iodide were obtained from Aldrich. Methanol was of analysis grade.

Absorption spectra were recorded with a UV−vis spectrophotometer (Model U-3010, Shimadzu). Fluorescence spectra were measured with a fluorescence spectrophotometer (F4500, Hitachi).

The laser flash photolysis experiments were carried out using an Edinburgh LP920 spectrophotometer (Edinburgh Instruments). In this setup, samples were excited using either the frequency-doubled (532 nm) or triplet (355 nm) output from a pulse Nd:YAG laser (10 Hz, 8 ns) (continuum Surelite) as excitation sources. Samples were freshly prepared for each measurement and were adjusted to an absorbance of ∼0.3 in 10-mm path length quartz cuvettes at the laser wavelength used. Samples were deaerated by argon purging for about 30 min before the laser flash photolysis experiments unless otherwise stated. Data was analyzed by the online software of the LP920 spectrophotometer. The fitting quality was judged by weighted residuals and a reduced $\chi^2$ value.

Phosphorescence and delayed fluorescence were measured with a high-quality emission spectrophotometer (Jobin Yvon TRIAX 320) with highly sensitive LN-CCD as detector (Jobin Yvon, CCD-3000V) combined with a high-speed rotated sector wheel for chopping the laser and emission beams in turn.[15] The sector wheel was revolved at a speed to allow observation of all delayed fluorescence and phosphorescence with a lifetime greater than 0.5 ms. No other prompt fluorescence and stray light from laser scattering were detected during delayed fluorescence and phosphorescence measurements. Cy3 samples were excited at 532 nm with a CW laser (Verdi-V5, Coherent, United States) for phosphorescence and delayed fluorescence measurements. An exposure of about 10 s of the LN-CCD was used to accumulate the weak delayed fluorescence and phosphorescence.

The triplet-state extinction coefficient ($\epsilon_T$) of Cy3 was measured using the energy-transfer method. In this method, a solution of the donor molecule (2AN) with known $\epsilon_T$ (10 500 $M^{-1}$ $cm^{-1}$ at 430 nm)[39,40] was excited at 355 nm in the absence and presence of a sufficient concentration of Cy3 quencher. As a control experiment, it is found that there is no obvious ground-state absorption from Cy3 acceptor by directly exciting the Cy3 solution at 355 nm with a low laser pulse energy (about 1.8 mJ). Therefore, all the triplet states of 2AN donor molecules formed after exciting at 355 nm are quenched by triplet−triplet energy transfer to the Cy3 acceptor. Under these conditions, the triplet-state extinction coefficient ($\epsilon_T$) of Cy3 could be determined according to eq 1

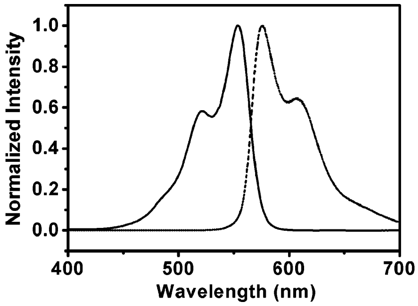


**Figure 1.** Normalized absorption (left) and fluorescence spectra (right) of Cy3 solution dissolved in methanol ($6.0 \times 10^{-6}$ M). ($\lambda_{exc} = 550$ nm.)

$$\frac{\epsilon_T(\mathrm{Cy3})}{\epsilon_T(\mathrm{2AN})} = \frac{\Delta \mathrm{OD(Cy3)}}{\Delta \mathrm{OD(2AN)}} \tag{1}$$

where $\epsilon_T$ (2AN) = 10 500 $M^{-1}$ $cm^{-1}$ for 2AN at 430 nm,[39.40] ΔOD(2AN) is the maximum optical density of the donor 2AN triplet state in the absence of acceptor, and OD(Cy3) is the maximum optical density of the acceptor Cy3 triplet when both donor and acceptor were present.

Furthermore, the quantum yields of triplet formation ($\Phi_T$) of Cy3 were obtained by comparative actinometry with 2AN as a reference ($\Phi_T = 0.84$, $\epsilon_T = 10\,500$ $M^{-1}$ $cm^{-1}$).[39,40] Cy3 or 2AN was irradiated at 355 nm in argon-saturated methanol. The laser pulse energy-dependent triplet-state absorbance at the respective peaks for Cy3 (monitored at 580 nm) and 2AN (monitored at 430 nm) was measured with high laser pulse energy. With the determined $\epsilon_T$ mentioned above, the quantum yields of triplet formation ($\Phi_T$) of Cy3 could be estimated from the relative slope of energy-dependence plots according to eq 2:

$$\frac{\Phi_T(\mathrm{Cy3})}{\Phi_T(\mathrm{2AN})} = \frac{\mathrm{slope(Cy3)}}{\mathrm{slope(2AN)}} \frac{\epsilon_T(\mathrm{2AN})}{\epsilon_T(\mathrm{Cy3})} \tag{2}$$

## Results and Discussion

Cy3 is believed to adopt an all-trans configuration in its ground state as its thermodynamically stable conformation.[26−28] The molecular structure of Cy3 is shown in Chart 1. Figure 2 shows the absorption and fluorescence spectra of *trans*-Cy3 solution in methanol. There is a broad ground absorption ranging from 500 to 560 nm with the maximum at about 550 nm. The prompt fluorescence peak is around 570 nm.

Figure 2 shows the transient absorption spectra of the Cy3 ($1.0 \times 10^{-4}$ M) solution in methanol after laser flash photolysis at 532 nm. It is found that there is a broad positive absorption band ranging from 560 to 600 nm and a broad negative absorption band ranging from 450 to 540 nm. The negative absorption band is mainly the fraction of Cy3 that is in the ground state following the initial laser flash. The time evolutions of the positive transient absorption at 580 nm after the 532 nm laser flash are shown in the inset of Figure 2.

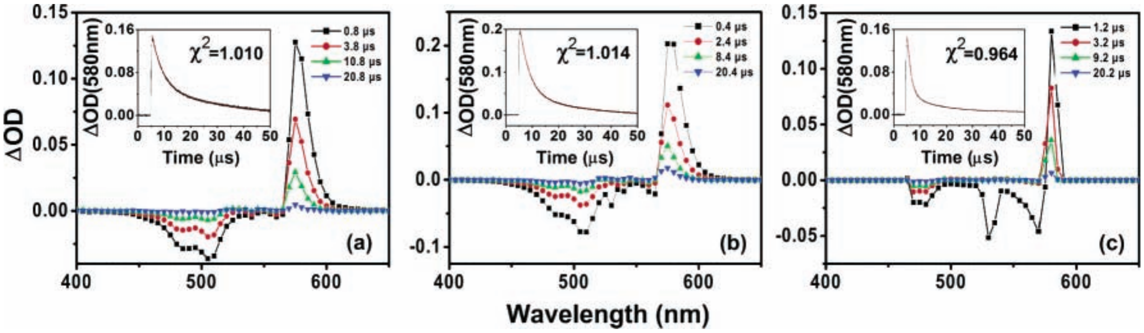


**Figure 2.** Spectra observed by laser flash photolysis of Cy3 ($1.0 \times 10^{-4}$ M) in argon-saturated methanol solutions (a), argon-saturated methanol solutions after adding ethyl iodide (b), and oxygen-saturated methanol solutions (c). Decay dynamics at 580 nm are shown in insets, respectively. The value of $\chi^2$ for each fitting was in the range of 0.964−1.014.

**TABLE 1: Fitting Parameters of Time Evolutions of the Transient Absorption Band of Cy3 in Different Experimental Conditions at 580 nm**

| solvent | $\tau_1$ ($\mu$s) | $A_1$ | $\tau_2$ ($\mu$s) | $A_2$ |
|---|---|---|---|---|
| argon + methanol | 3.91 | 35% | 13.58 | 65% |
| ethyl iodide + methanol | 3.68 | 56% | 13.96 | 44% |
| oxygen + methanol | 0.94 | 39% | 13.50 | 61% |
| argon + glycerol | 3.65 | 32% | 35.18 | 68% |
| ethyl iodide + glycerol | 3.63 | 53% | 38.52 | 47% |

As shown in Figure 2a, the decay of the 580-nm species of Cy3 in argon-saturated solution is fitted well with a double-exponential function about 3.91 $\mu$s and about 13.58 $\mu$s with the respective normalized amplitudes 35% and 65%. Table 1 lists the fitting parameters for the time evolutions of these two decay components. These two processes are expected to be the triplet-state absorption or the cis-isomer ground-state absorption, alternatively.

To distinguish these two components, ethyl iodide is added to the Cy3 solution in argon-saturated methanol. For ethyl iodide, because of the heavy-atom effect, it is expected that the intersystem crossing could become more significant and lead to the increase of triplet-state yield.[26,41,42]

Figure 2b shows the transient absorption spectra of the Cy3 ($1.0 \times 10^{-4}$ M) solution in argon-saturated methanol after adding ethyl iodide upon laser flash photolysis at 532 nm. It is found that the broad absorption with a maximum about 580 nm was enhanced compared to that of ethyl iodide-free Cy3 solution. This indicates a promoted intersystem crossing via spin−orbit coupling through heavy-atom effects. The enhanced absorption at 580 nm is mainly contributed from the increased $T_1 \rightarrow T_n$ triplet-state absorption of *trans*-Cy3 after adding ethyl iodide. As shown in the inset of Figure 2b, the decay of the 580-nm species is also fitted well with a double-exponential function about 3.68 $\mu$s and about 13.96 $\mu$s with the respective normalized amplitudes 56% and 44%. Compared to a small amplitude of about 35% of iodide-free Cy3 solution, the increased amplitude of the fast decay component of about 56% implies that the population of the Cy3 triplet state is promoted after adding ethyl iodide in Cy3 solution, and we thus attribute the fast decay component with time constant about 3.91 $\mu$s to be the triplet-state ($T_1 \rightarrow T_n$) absorption of *trans*-Cy3.

To further prove whether this fast decay component is really attributed to the triplet-state ($T_1 \rightarrow T_n$) absorption of Cy3, we perform the laser photolysis experiments of Cy3 solution in oxygen-saturated methanol excitation at 532 nm. It is known that in most cases, oxygen can also induce intersystem crossing between the singlet and triplet states of the molecule because of its paramagnetic ground state.[43] Furthermore, oxygen as a good electron acceptor of triplet state can react with the electron of the triplet state of Cy3 and consequently shorten the lifetime of triplet absorption.[43−46] As shown in Figure 3a and c, the time constant of the fast decay component decreases dramatically from 3.91 $\mu$s for Cy3 solution in oxygen-free methanol to about 0.94 $\mu$s for Cy3 in oxygen-saturated solution. Meanwhile, the transient absorption band at 580 nm becomes narrower significantly in oxygen-saturated solution compared to that of Cy3 solution in oxygen-free methanol. These results indicate that the resulting triplet state of Cy3 is efficiently quenched by oxygen through electron transfer.

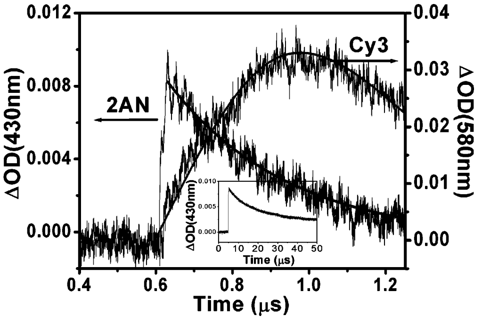


**Figure 3.** Matched dynamical decay of 2AN triplet absorption monitored at 430 nm in the presence of Cy3 in argon-saturated methanol and dynamical growth of Cy3 triplet absorption monitored at 580 nm during triplet−triplet energy-transfer measurements. Inset shows the dynamical decay of 2AN triplet absorption in the absence of Cy3 in argon-saturated methanol with the decay time constant about 26.2 s at 430 nm. The time constants of the rise time (about 0.25 $\mu$s) of Cy3 and the decay time (about 0.28 $\mu$s) of 2AN are obtained.

Furthermore, there is no significant change of the slow decay component with time constant around 13.6 $\mu$s when we perform the laser photolysis measurements in all the experimental conditions (e.g., Cy3 in oxygen-free or ethyl iodide-added or oxygen-saturated solutions). Therefore, the slow decay component about 13.6 $\mu$s is assigned to the ground-state absorption of the photoisomer (*cis*-Cy3) of the cyanine dye formed following the *trans*-$S_0 \rightarrow$ *trans*-$S_1$ excitation with 532-nm laser pulse. The time constant about 13.6 $\mu$s represents the back isomerization time through thermal deactivation from *cis*-$S_0$ to *trans*-$S_0$ isomerization. Moreover, to test whether this slow process is really accounted for in this isomerization process, we introduce a solvent viscosity-dependent laser flash photolysis measurement of Cy3 in glycerol, where the trans−cis isomerization is hindered significantly with increased viscosity.[26] As shown in Table 1, it is found that the time constant of the slow component about 13.6 $\mu$s in glycerol-free methanol solution increases to 35−38 $\mu$s in a high viscous solvent (methanol/glycerol mixture with volume ratio about 3/1), where no significant change was observed for time constant of fast component. These results suggest that the slow process results from the isomerization of Cy3.

The observation of two decay species of Cy3 with a broad absorption band around 580 nm indicates that the triplet-state absorption of *trans*-Cy3 and the ground-state absorption of the photoisomer *cis*-Cy3 are largely overlapped at 580 nm after laser photolysis at 532 nm. Since the photoisomerization and triplet-state absorption of the Cy3 are the competing processes originating from the excited singlet manifold, it is difficult to determine quantitative information on triplet-state properties through direct excitation experiments because of the large overlap with ground-state absorption of *cis*-Cy3 by means of 532-nm laser flash photolysis. Fortunately, the triplet-state photophysical properties could be attained through triplet−triplet energy-transfer measurements.[21,47]

Here, we turn to determine the quantitative information on triplet-state properties of Cy3 with a well-studied 2AN as the triplet-state donor,[39,40] where the 2AN can be excited selectively at 355 nm. Since Cy3 shows no obvious absorption at this excitation wavelength with low excitation power (1.8 mJ), the affects from photoisomer absorption of Cy3 could be avoided during measurements. Figure 3 shows the dynamics of the triplet−triplet ($T_1-T_n$) absorption of donor 2AN monitored at 430 nm in the absence and presence of Cy3 in argon-saturated methanol solution. It is found that the triplet state of 2AN shows a single-exponential function with time constant ($\tau_T$) about 26.2 $\mu$s in the absence of Cy3 as shown in the inset of Figure 3. Upon adding sufficient Cy3 ($5.0 \times 10^{-4}$ M) into the 2AN solution in argon-saturated methanol, 2AN triplet state at 430 nm was quenched efficiently with time constant down to 0.28 $\mu$s, whereas a growth with time constant about 0.25 $\mu$s for Cy3 triplet-state absorption was observed at 580 nm. The agreement in the time constants of the rise time (about 0.25 $\mu$s) of Cy3 and the decay time (about 0.28 $\mu$s) of 2AN strongly indicates the efficient triplet-state energy transfer between 2AN and Cy3, where the rate of formation of acceptor Cy3 triplet state is identical to the rate of decay of donor triplet state. The decay dynamics of the Cy3 triplet state in the presence of 2AN excited at 355 nm has no significant changes with time constant about 3.9 $\mu$s during measurements.

By taking $\epsilon_T$ (2AN) = 10 500 $M^{-1}$ $cm^{-1}$,[39,40] $\Delta$OD(2AN) and $\Delta$OD(Cy3) are estimated from Figure 3 as 0.0083 and 0.0320, respectively. Therefore, according to eq 1, the triplet-state extinction coefficient of Cy3 ($\epsilon_T$(Cy3)) is calculated to be equal to 40 770 $M^{-1}$ $cm^{-1}$.

To further estimate the quantum yield $\Phi_T$ (Cy3) of triplet formation, we perform the laser pulse energy-dependent triplet-state absorption measurements for Cy3.[39,40] Since 355-nm excitation at high laser pulse energy can simultaneously produce both the triplet state and isomer of Cy3, we estimate the value of $\Delta$OD(Cy3) by a double-exponential function fitting of the observed dynamics (monitored at 580 nm) at higher laser pulse energy as shown in Figure 4, where the amplitude of fast decay component was taken as the value of $\Delta$OD(Cy3). Figure 4 shows the laser pulse energy-dependent triplet-state absorbance ($\Delta$OD(Cy3)) of Cy3 and 2AN in argon-saturated methanol with the excitation at 355 nm. The relative slopes from the energy-dependence plots are thus obtained as 0.0021 for 2AN and 0.0006 for Cy3 from Figure 4. Therefore, the quantum yield of triplet-state formation ($\Phi_T$) of Cy3 can then be estimated by comparative actinometry methods.[39,40] Using the above determined $\epsilon_T$(Cy3) about 40 770 $M^{-1}$ $cm^{-1}$, and taking $\Phi_T$ (2AN) = 0.84 and $\epsilon_T$ (2AN) = 10 500 $M^{-1}$ $cm^{-1}$,[40] the quantum yield of triplet formation $\Phi_T$ (Cy3) is calculated from eq 2 to be equal to 0.03 in argon-saturated methanol solution.

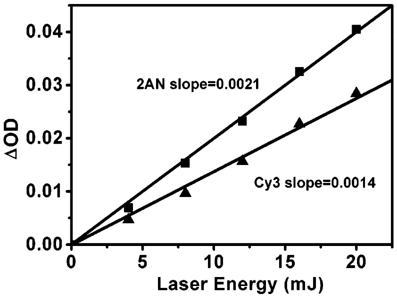


**Figure 4.** The laser pulse energy-dependent triplet-state absorbance of Cy3 (580 nm) and 2AN (430 nm) in argon-saturated methanol with the excitation at 355 nm.

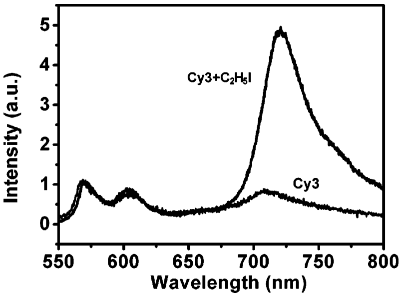


**Figure 5.** Phosphorescence and delayed fluorescence of Cy3 methanol solution in the absence and presence of ethyl iodide excited at 532 nm.

Furthermore, the $T_1-T_n$ absorptions largely overlap the ground-state absorptions for either *trans*-Cy5 or *cis*-Cy5 as reported in our previous studies.[16] The delayed fluorescence and phosphorescence measurements further displayed that the lowest cis-triplet state is also involved in the formation of the isomer (*cis*-Cy5) following the *trans*-$S_0 \rightarrow$*trans*-$S_1$ excitation as what occurred in Cy5.[15,16] In comparison to Cy5, the cyanine dye Cy3 contains three carbons in conjugated chain between the two head groups instead of the five carbons of the Cy5 molecules. We found that the decay time constants for both triplet-state absorption and isomerization of Cy3 are faster than those of Cy5. For instance, the time constant of isomerization is about 13.6 $\mu$s for Cy3 and about 150 $\mu$s for Cy5,[16] indicating a low-barrier back-isomerization with a shorter length of conjugated hydrocarbon chain for Cy3. Furthermore, as shown in Figure 5, the phosphorescence spectrum of the Cy3 solution at low temperature (77 K) excited at 532 nm only shows a broad *trans*-phosphorescence peak around 722 nm from trans-triplet state of Cy3 besides a weak delayed fluorescence. We did not observe the cis-phosphorescence of Cy3 from cis-triplet state even after adding the heavy atomic ethyl iodide as what appeared in Cy5.[15] This suggests that isomerization in Cy3 occurs from excited trans-singlet state to cis-ground state of Cy3 following a *trans*-$S_0 \rightarrow S_1$ excitation, whereas the cis-triplet state is not involved during isomerization for the short-chain cyanine dye Cy3.

Finally, it should be useful to explain tentatively the mechanism by which Cy3 facilitates dramatically the photoinduced recovery of Cy5.[34,37] In our previous works,[15,17] it was found that cis-triplet state is the intermediate state during the isomerization from *trans*-$S_1$ state following a trans-$S_0 \rightarrow$*trans*-$S_1$ excitation of Cy5, whereas $T_1 \rightarrow T_n$ absorptions largely overlap the ground-state absorptions for either *trans*- or *cis*-Cy5. This could be the important point for us to tentatively interpret the unusual enhancement of the conversion of Cy5 from the dark to fluorescence state in the presence of the secondary chromophore of Cy3.[34,37] Since the energy about 1.98 eV (625 nm) of triplet−triplet absorption of *trans*-Cy5 and the energy about 1.80 eV (690 nm) of triplet−triplet absorption of *cis*-Cy5 are higher than the energy about 1.72 eV (722 nm estimated from the phosphorescence spectrum of Cy3) of trans-triplet state of Cy3,[15,16] it is believed that the reverse triplet−triplet energy transfer from Cy5 to Cy3 could be expected by which a proximal Cy3 facilitates the unexpected photoinduced recovery of Cy5, where the reverse energy transfer results from the higher excited $\mathrm{T}_n$ state of Cy5 (initiated from *trans*-$\mathrm{S}_1$ to $\mathrm{T}_1$ state upon excitation with red light only for Cy5) to trans-triplet state of Cy3. Because of the large spectral overlap between the $\mathrm{T}_1 \rightarrow \mathrm{T}_n$ absorptions and the ground-state absorptions for either *trans*- or *cis*-Cy5, the excitation of $\mathrm{T}_1-\mathrm{T}_n$ is expected to be highly efficient following a *trans*-$\mathrm{S}_0 \rightarrow$ *trans*-$\mathrm{S}_1$ excitation of *trans*-Cy5, leading to the efficient reverse triplet−triplet energy transfer from the upper $\mathrm{T}_n$ state of Cy5 to $\mathrm{T}_1$ state of Cy3. In addition, the fact that an alternative triplet state quencher, Trolox, was reported to eliminate Cy5 dye blinking[38] strongly indicates a link between triplet states and blinking, which correspond to the enhanced conversion of Cy5 from dark state to fluorescence state in the presence of Cy3. Furthermore, the lifetime for *trans*-$\mathrm{T}_1$ of Cy5 is about 35 $\mu$s,[16] and *trans*-$\mathrm{T}_1$ of Cy3 is about 3.9 $\mu$s, thus an almost 9 times faster decay could be achieved for Cy5 when the reverse triplet−triplet energy transfer occurs from the upper $\mathrm{T}_n$ state of Cy5 to triplet state of Cy3; this is in agreement with the single-molecule results.[34,37] The detailed model and experiments based on this point concerning the unexpected reverse triplet−triplet energy transfer from $\mathrm{T}_n$ of Cy5 to $\mathrm{T}_1$ of Cy3 will be reported elsewhere soon.

## Conclusions

We report here the detailed photophysical properties of Cy3 concerning the properties of triplet state and isomerization by means of laser flash photolysis measurements. It is found that the triplet-state absorption ($\mathrm{T}_1-\mathrm{T}_n$) of *trans*-Cy3 and the ground-state absorption of the photoisomer (*cis*-Cy3) of Cy3 largely overlap each other around 580 nm with broad absorption region. Furthermore, the triplet-state extinction coefficient and quantum yield of triplet formation of Cy3 are estimated. The results presented in this article are of fundamental significance not only for general triplet states and trans−cis isomerization properties but also for the performance of the cyanine dyes in all applications.

**Acknowledgment.** We thank referees for their valuable suggestions for revising the manuscript. This work was financially supported by NSFC (90306013), Chinese Academy of Sciences, and State Key Project for Fundamental Research (2006CB806000).

## References and Notes

(1) Wabuyele, M. B.; Farquar, H.; Stryjewski, W.; Hammer, R. P.; Soper, S. A.; Cheng, Y. W.; Barany, F. *J. Am. Chem. Soc.* **2003**, *125*, 6937−6945.

(2) Kanony, C.; Akerman, B.; Tuite, E. *J. Am. Chem. Soc.* **2001**, *123*, 7985−7995.

(3) Armitage, B.; O'Brien, D. F. *J. Am. Chem. Soc.* **1992**, *114*, 7396−7403.

(4) Heilemann, M.; Margeat, E.; Kasper, R.; Sauer, M.; Tinnefeld, P. *J. Am. Chem. Soc.* **2005**, *127*, 3801−3806.

(5) Alexander, K. C.; Semen, V. S.; Helmut, G. *J. Photochem. Photobiol., A* **2001**, *141*, 39−45.

(6) DiPaolo, R. E.; Scaffardi, L. B.; Duchowicz, R.; Bilmes, G. M. *J. Phys. Chem.* **1995**, *99*, 13796−13799.

(7) Sanchez-Galvez, A.; Hunt, P.; Robb, M. A.; Olivucci, M.; Vreven, T.; Schlegel, H. B. *J. Am. Chem. Soc.* **2000**, *122*, 2911−2924.

(8) Ghelli, S.; Ponterini, G. *J. Mol. Struct.* **1995**, *355*, 193−200.

(9) Khimenko, V.; Chibisov, A. K.; Görner, H. *J. Phys. Chem. A* **1997**, *101*, 7304−7310.

(10) Soper, S. A.; Mattingly, Q. L. *J. Am. Chem. Soc.* **1994**, *116*, 3744−3752.

(11) Rodriguez, J.; Scherlis, D.; Estrin, D.; Aramendia, P. F.; Negri, R. M. *J. Phys. Chem. A* **1997**, *101*, 6998−7006.

(12) Ponterini, G.; Momicchioli, F. *Chem. Phys.* **1991**, *151*, 111−126.

(13) Vaveliuk, P.; Scaffardi, L. B.; Duchowicz, R. *J. Phys. Chem.* **1996**, *100*, 11630−11635.

(14) Smith, J. O.; Olson, D. A.; Armitage, B. A. *J. Am. Chem. Soc.* **1999**, *121*, 2686−2695.

(15) Huang, Z. X.; Ji, D. M.; Xia, A. D.; Koberling, F.; Patting, M.; Erdmann, R. *J. Am. Chem. Soc.* **2005**, *127*, 8064−8066.

(16) Huang, Z. X.; Ji, D. M.; Wang, S. F.; Xia, A. D.; Koberling, F.; Patting, M.; Erdmann, R. *J. Phys. Chem. A* **2006**, *110*, 45−50.

(17) Huang, Z. X.; Ji, D. M.; Xia, A. D. *Colloids Surf., A* **2005**, *257−258*, 203−209.

(18) Hertz, A. H. *Adv. Colloid Interface Sci.* **1977**, *8*, 237−298.

(19) Chatterjee, S.; Gottschalk, P.; Davis, P. D.; Schuster, G. B. *J. Am. Chem. Soc.* **1988**, *110*, 2326−2328.

(20) Dragsten, P. R.; Webb, W. W. *Biochemistry* **1978**, *17*, 5228−5240.

(21) Krieg, M.; Redmond, R. W. *Photochem. Photobiol.* **1993**, *57*, 472−479.

(22) Benniston, A. C.; Harriman, A. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 1841−1847.

(23) Benniston, A. C.; Harriman, A.; Mcavoy, C. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 519−525.

(24) Shank, C. V.; Ippen, E. P. *Dye Lasers*; Springer-Verlag: Heidelberg, Germany, 1973.

(25) Maeda, M. *Laser Dyes*; Academic Press: Tokyo, 1984.

(26) Widengren, J.; Schwille, P. *J. Phys. Chem. A* **2000**, *104*, 6416−6428.

(27) Köhn, F.; Hofkens, J.; Gronheid, R.; Van der Auweraer, M.; De Schryver, F. C. *J. Phys. Chem. A* **2002**, *106*, 4808−4814.

(28) Weston, K. D.; Carson, P. J.; Metiu, H.; Buratto, S. K. *J. Chem. Phys.* **1998**, *109*, 7474−7485.

(29) Widengren, J.; Seidel, C. A. M. *Phys. Chem. Chem. Phys.* **2000**, *2*, 3435−3441.

(30) Tinnefeld, P.; Herten, D. P.; Sauer, M. *J. Phys. Chem. A* **2001**, *105*, 7989−8003.

(31) Tinnefeld, P.; Buschmann, V.; Weston, K. D.; Sauer, M. *J. Phys. Chem. A* **2003**, *107*, 323−327.

(32) Sahyun, M. R. V.; Serpone, N. *J. Phys. Chem. A* **1997**, *101*, 9877−9883.

(33) Redmond, R. W.; Kochevar, I. E.; Krieg, M.; Smith, G.; McGimpsey, W. G. *J. Phys. Chem. A* **1997**, *101*, 2773−2777.

(34) Mark, B.; Timothy, R. B.; Zhuang, X. W. *Phys. Rev. Lett.* **2005**, *94*, 108101.

(35) English, D. S.; Harbron, E. J.; Barbara, P. F. *J. Phys. Chem. A* **2000**, *104*, 9057−9061.

(36) Ha, T.; Xu, J. *Phys. Rev. Lett.* **2003**, *90*, 223002.

(37) Sabanayagam, C. R.; Eid, J. S.; Meller, A. *J. Chem. Phys.* **2005**, *123*, 224708.

(38) Rasnik, I.; McKinney, S. A.; Ha, T. *Nat. Methods* **2006**, *3*, 891−893.

(39) Bensasson, R. V.; Land, E. J. *Trans. Faraday Soc.* **1971**, *67*, 1904−1915.

(40) Gorman, A. A.; Hamblett, I.; Rodgers, M. A. J. *J. Am. Chem. Soc.* **1984**, *106*, 4679−4682.

(41) Basché, T.; Kummer, S.; Brauchle, C. *Nature* **1995**, *373*, 132−134.

(42) Tamarat, P.; Maali, A.; Lounis, B.; Orrit, M. *J. Phys. Chem. A* **2000**, *104*, 1−16.

(43) Wilkinson, F.; McGarvey, D. J.; Olea, A. F. *J. Am. Chem. Soc.* **1993**, *115*, 12144−12151.

(44) *Singlet Oxygen*; Frimer, A. A., Ed.; CRC Press: Boca Raton, FL, 1985; Vols. I−IV.

(45) Fukuzumi, S.; Imahori, H.; Yamada, H.; El-Khouly, M. E.; Fujitsuka, M.; Ito, O.; Guldi, D. M. *J. Am. Chem. Soc.* **2001**, *123*, 2571−2575.

(46) Smirnov, S.; Vlassiouk, I.; Kutzki, O.; Wedel, M.; Montforts, F.-P. *J. Am. Chem. Soc*. **2002**, *124*, 4212−4213.

(47) Redmond, R. W.; Srichai, M. B.; Bilitz, J. M.; Schlomer, D. D.; Krieg, M. *Photochem. Photobiol.* **1994**, *60*, 348−355.