Excited State Relaxation in Cyanine Dyes: A Remarkably Efficient Reverse Intersystem Crossing from Upper Triplet Levels

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The upper triplet states of Merocyanine 540 and related cyanine dyes have been generated by a two-color, two-pulse method. The decay of this state partitions between internal conversion to T_1 and remarkably efficient reverse intersystem crossing to the singlet manifold. Thus, UV or visible laser generation of the T_1 state (either directly or by a sensitized route) followed by excitation with a 640 nm pulse ultimately results in fluorescence and isomerization from the lowest excited singlet state (S_1), as evidenced by the production of the ground state photoisomer absorption and emission coincident with the second laser pulse. Since identical behavior (isomerization and fluorescence) is observed for direct excitation into the singlet manifold, we conclude that both the singlet and triplet states are of the same geometry and that isomerization does not occur directly from either T_1 or T_n.

Introduction

In recent years we have studied both the photophysical and photosensitizing properties of cyanine dyes, *e.g.*, dithia- and diselenacarbocyanines[1−3] and various derivatives based on the structure of merocyanine 540,[4] a compound of interest in photochemical bone marrow purging[5−9] and other photodynamic applications.[10,11] Cyanine dyes are also of interest in industry for applications such as photography,[12,13] and nonlinear optics and in laser operation.[14,15] The photophysical relaxation mechanisms that occur following excitation of these molecules are complex, and possible deactivation pathways include fluorescence and internal conversion in the singlet manifold, intersystem crossing to the triplet state, and photoisomerization around a double bond in the polymethine bridge. We have reported fluorescence (Φ_f) and triplet state (Φ_{isc}) quantum yields and lifetimes obtained from nanosecond laser flash photolysis studies.[1,3,4] We have also shown that cyanine dyes which lack heavy atom substitution decay predominantly via singlet mechanisms with the result that intersystem crossing has a very low efficiency in most of these compounds.[1,3,5] Substitution of sulfur atoms with selenium in the ring systems produces an order of magnitude increase in the intersystem crossing and subsequent singlet oxygen yields which dramatically improves the photosensitizing efficiency of these compounds in biomedical applications.[3,4]

There has been much discussion in the literature on the mechanism of deactivation of excited cyanine dyes, especially with respect to photoisomerization.[16−26] Generally, it has been proposed that isomerization occurs specifically from the excited singlet state.[17,19,20,22] The suggested mechanism entails excitation of the more stable isomer (usually *trans*) to the singlet state, bond torsion in the polymethine chain to give a 90° twisted geometry, and rapid deactivation to the twisted ground state

via nonradiative relaxation. The twisted ground state then partitions to the original ground state configuration or the photoisomer. The photoisomer and precursor conformations are separated by a relatively small rotational barrier (for cyanine dyes) with the result that the photoisomer reverts back to the original, more stable conformer by a thermally assisted process at room temperature. Our initial work also supported a singlet state mediated photoisomerization for dialkylthiacarbocyanine dyes, based on the absence of detectable photoisomer in triplet sensitization experiments.[1]

Recently, Serpone and Sayhun[27] reported the results of picosecond laser flash photolysis work and interpreted their observations as demonstrating a much greater involvement of the triplet state of dialkylthiacarbocyanine dyes in the relaxation of the excited dye. On the picosecond time scale they observed the absorption of the lowest excited singlet state and an additional band at 483 nm which was assigned to a triplet state obtained by torsional relaxation of the ground state structure. This initial triplet state, formed in high quantum yield, was proposed to decay rapidly (on the short nanosecond time scale) to give either the photoisomer in its ground state or the lowest excited triplet state of the original geometry, the latter process being subject to an energy barrier accounting for the relatively low overall quantum yield for this species.[1] Thus, Serpone and Sahyun[27] identified this low yield triplet state as the triplet state observed in our nanosecond laser flash photolysis experiments.[1,3]

This recent work[27] prompted us to reinvestigate some of the cyanine dyes we had previously studied, and we report here experiments designed to investigate the photophysics of the cyanine triplet states and possible interconversions between isomeric species. We have utilized direct excitation and triplet state sensitization to generate lowest triplet states of cyanine dyes which were then selectively excited using a second laser pulse of optimum wavelength. In this manner we are able to determine whether energy barriers exist between *cis* and *trans* excited triplet states of these types of compounds. Our results show that the major consequence of excitation to upper triplet states involves reverse intersystem crossing to the singlet state.
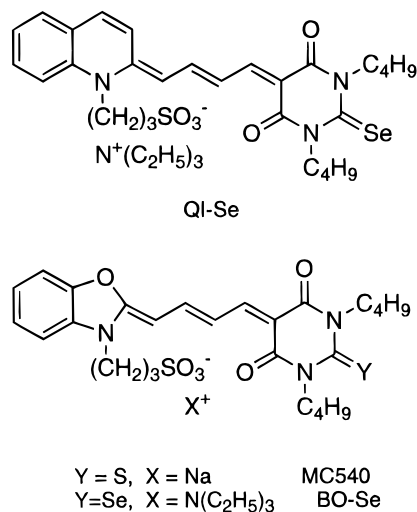
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Figure 1. Chemical structures of cyanine dyes used in this study.

Figure 2. Schematic diagram of the beam geometry used in laser flash photolysis measurements.

No evidence for photoisomerization in the triplet manifold was obtained.

TABLE 1: Photophysical Properties of Cyanine Triplet States[a]

	MC540	BO-Se	QI-Se
$10^{-4}\Delta\epsilon_T$ (M^{-1} cm^{-1})	7.1	6.8	4.0
Φ_{isc}	0.04	0.80	0.13
Φ_{bl}	0.74	0.15	0.58
$\Phi_{bl}/(1 - \Phi_{isc})$	0.77	0.75	0.68

[a] Errors in Φ_{bl} values estimated at 20%.

Experimental Section

meso-Tetraphenylporphyrin (TPP) and methanol (spectroscopic grade) were obtained from Aldrich. Merocyanine 540 was obtained from Sigma. The BO-Se and QI-Se dyes (see Figure 1) were synthesized according to the literature.[4] 2′-Acetonaphthone (2AN) was purchased from Aldrich and recrystallized from aqueous ethanol before use.

Ground state absorption spectra were recorded on a Hewlett-Packard 8541A UV−vis spectrophotometer. Laser flash photolysis experiments were carried out using the apparatus described previously.[28−30] Laser excitation was obtained from a Lambda Physik EMG MSC 103 or Lumonics EX510 XeCl excimer laser (8 ns pulse duration), a Quantel YG660A or Continuum Surelite Nd:YAG laser at 355 or 532 nm (8 ns duration), a Candela flashlamp-pumped dye laser (640 nm emission from Rhodamine 640 perchlorate (Exciton) with 350 ns pulse duration), or a Spectra Physics MOPO 710 system. In two-color, two-photon excitation experiments, the first pulse was absorbed by the precursor ground state to generate the triplet state which was subsequently excited selectively using a wavelength at which the triplet absorbs but the ground states or other transient species do not. The dye or OPO laser pulse used to excite the cyanine triplet state was synchronized to fire with a delay of a few microseconds with respect to the first pulse. In some experiments emission was detected from the sample using a gated, intensified, dual-diode array, optical multichannel analyzer (OMA, Princeton Instruments DIDA-512G detector, ST1000 controller, and PG-10 pulse generator).

Quantum yields of reaction (Φ_{bl}) from the upper triplet states (T$_n$) of these molecules were measured using comparative actinometry as described below. The beam geometry used in these experiments was quasi-colinear for both laser pulses. The transient absorption changes are measured in the volume of sample in the first 2 mm from the incident surface, as shown in Figure 2.[31] In this way an essentially homogeneous distribution of excited states at its highest concentration was probed by the analyzing beam. The absorption of the transient species (A_t) across the 10 mm path length was then accurately measured by flash photolysis at the wavelength and time delay of the second laser pulse. When the two-laser sequence was used, the transient absorption changes (ΔA) induced by the second laser were monitored at another suitable wavelength, usually the absorption

maximum of the species of interest. The energy of the second laser pulse was varied and ΔA measured as a function of the second laser pulse energy. Due to the collinear excitation geometry and homogeneous transient distribution in the path of the analyzing beam, the actinometer used in these experiments was of the type used in simple one-laser flash photolysis experiments, where only the second laser pulse was used and the ground state absorption of the sample was set to equal that of the transient species in the two-laser experiment.[31] For these experiments the actinometer used was *meso*-tetraphenylporphyrin (TPP) excited at 640 nm where the relevant triplet state properties are a difference absorption coefficient ($\Delta\epsilon_T$) of 67 000 M^{-1} cm^{-1} at the detection wavelength of 440 nm[32] and a quantum yield of formation of 0.62.[32] Determination of the slopes of the ΔA vs laser pulse energy plots for both sample and actinometer allow the determination of the quantum yield of photoreaction of the upper triplet state from

$$\Phi_{bl}^S = (\text{slope}^S/\text{slope}^A)(\Delta\epsilon_T^A/\Delta\epsilon_T^S)\Phi_{isc}^A \qquad (1)$$

Results

The triplet states of the dyes were generated in deaerated methanol by direct excitation at 532 nm. The dyes studied were found to be very photostable in deaerated methanol solution, as judged by absorption spectroscopy. The quantum yield of intersystem crossing for MC540 is relatively low (see Table 1), and in some cases 355 nm excitation of a sensitizer, 2′-acetonaphthone (2AN) in solution containing 5×10^{-4} M dye, was also used to increase the yield and absorbance of the triplet state in solution (rate constant for energy transfer, $k = 8.5 \times 10^9$ M^{-1} s^{-1}). All the dyes studied exhibited strong T$_1$ absorption at $\lambda > 600$ nm, to the red of the respective ground state absorption spectra.[1,4] In all cases the excitation of T$_1$ to T$_n$ was accompanied by a substantial irreversible loss (bleaching) of the T$_1$ absorption, as exemplified by MC540 in Figure 3.

Almost complete bleaching of the T$_1$ absorption was achieved using laser pulse energies of >40 mJ/pulse. Irreversible photobleaching of the T$_1$ absorption denotes a mechanism for removal of molecules in the T$_n$ state which competes with internal conversion from T$_n$ back to T$_1$. This behavior can be observed in systems where photochemical processes, such as bond fission or photoionization, are responsible for loss of the T$_1$ state.[28,29,33−36] Such mechanisms are unlikely to operate in

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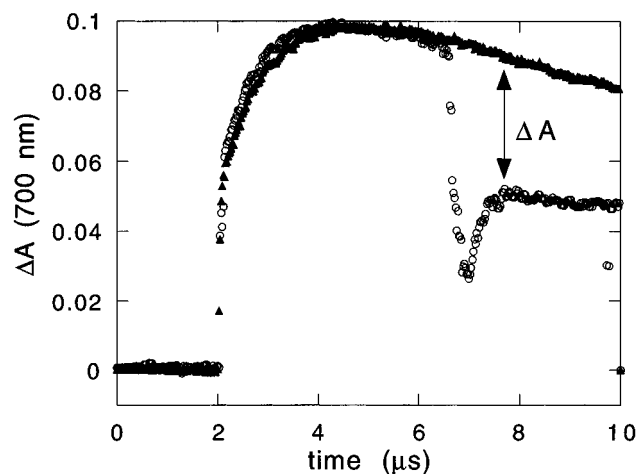
Figure 3. Transient absorbance changes at 700 nm produced by excitation of 2′-acetonaphthone in the presence of 0.5 mM MC540 in deaerated methanol with (▲) 355 nm and (○) 355 + 640 nm laser pulses.

Figure 4. Plot of dependence of absorbance change (ΔA) on 640 nm laser pulse energy for bleaching of MC540 triplet state (△) in deaerated methanol at 700 nm and generation of triplet state of TPP (●) in deaerated benzene at 440 nm.

this case as the amount of energy absorbed by the molecule is fairly low (equal to the T_1 energy content of the cyanine plus the energy from the second photon) and is indeed less than that resulting from 308 nm excitation of the ground state where photodegradation of these dyes does not appear to occur. No transient absorption due to formation of the solvated electron was observed following the second laser pulse which confirms a lack of photoionization under these excitation conditions. The solvated electron in methanol has a strong absorption located in the vis−near-IR region with maximum around 630 nm.[37]

Chemical reaction from upper T_n states was further probed by monitoring the loss or change of ground state absorbance of these dyes as a function of irradiation conditions. The photodegradation of these compounds was identical on irradiation with either a 532 nm single pulse or with the 532 + 640 nm two-pulse irradiation schemes; i.e., excitation of T_1 to the T_n state did not enhance the overall photodegradation rate. The lack of observed chemistry (no change in absorption spectrum) suggests a physical mechanism of relaxation for the T_n state.

Quantum yields for photobleaching (Φ_{bl}) of the T_1 states were measured for all dyes studied using the approach outlined in the Experimental Section. Slopes of the plots of the dependence of bleached T_1 absorbance and actinometer triplet absorption on dye laser pulse energy were determined and inserted into eq 1. Figure 4 shows the example for the case of ^{3}MC540 and TPP in deaerated methanol excited at 640 nm. The resulting values are given in Table 1, along with T_1 absorption coefficients[4] ($\Delta\epsilon_T$) for the respective dyes at the detection wavelengths used in bleaching experiments, which are required in the determination of Φ_{bl}. Also given are the quantum yields of intersystem crossing (Φ_{isc}) for these compounds, measured in a previous study.[4] A large variation in the Φ_{bl} value is observed within the compounds studied.

Quantum yields of photobleaching (Φ_{bl}) were measured in the wavelength range where only the T_1 state absorbs ($\lambda > 620$ nm). Throughout this range the fraction of T_1 absorption bleached at each detection wavelength was constant, as expected. For MC540 at lower wavelengths, where ground state and possibly the photoisomer absorb, the fraction of T_1 bleached was dependent on wavelength. In direct excitation of MC540 this is understandable as a significant product from direct excitation into the singlet manifold is the long-lived photoisomer which absorbs in the range $\lambda < 620$ nm. Thus, the transient absorption observed is a composite of triplet and photoisomer, and as the underlying photoisomer contribution is not affected
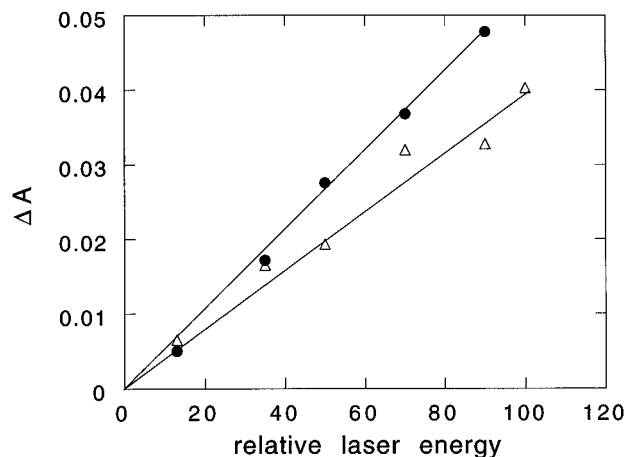
by the second pulse, only the absorption due to T_1 is reduced. BO-Se and QI-Se do not generate photoisomers on direct excitation at 532 nm[4] and therefore do not show this behavior. Only triplet state photobleaching was observed at all wavelengths, and no additional transient species were observed for these two dyes.

When 2AN-photosensitized generation of the T_1 state of MC540 cyanines was employed, the photoisomer or singlet state in the cyanine induced by the first laser pulse at 355 nm does not complicate the measurements. When the sensitized T_1 state was excited with a 640 nm laser pulse, the same bleaching process and wavelength-dependent photobleaching spectrum was observed as upon direct excitation. At $\lambda > 620$ nm only T_1 absorbs, and a constant fractional bleaching was observed. At lower wavelengths the kinetic behavior was different, and a longer-lived species, which was not the triplet state, was observed following 640 nm dye laser excitation of T_1. Figure 5A shows a comparison of transient absorption spectra resulting from 355 nm sensitized formation of the T_1 state in the presence and absence of a 640 nm pulse to excite T_1 to T_n. In the presence of the second pulse, excitation of T_1 state gives rise to a long-lived species (Figure 5B) which shows a small peak at 585 nm and is not observed in the absence of the second pulse, where only T_1 absorbance is observed. These spectral and kinetic characteristics allow this species to be readily identified as a photoisomer of the starting material.[4] Thus, excitation of T_1 to T_n results in the formation of photoisomer. The lower fractional photobleaching observed at $\lambda < 620$ nm is due to formation of photoisomer which possesses significant absorption in the region where the ground state also absorbs. Additionally, under higher pulse energy conditions, while complete photobleaching of the T_1 state was observed at $\lambda > 620$ nm an accompanying complete recovery of ground state absorption in the difference spectrum at $\lambda < 620$ nm is not observed as the isomer formed at the expense of the ground state does not absorb as strongly, and a net reduction in absorption is observed in this region.

The two-color, two-pulse experiments provided additional information on the mechanism of deactivation of the T_n states. Upon photolysis of the T_1 state by a 640 nm laser pulse, a coincident luminescence was observed. This emission was only detected in two-color, two-pulse experiments and was not present when the 640 nm pulse alone was incident on the sample. Stray or scattered laser light from the 640 nm dye pulse can therefore be ruled out as the source of this emission. This emission was not detected when the triggering of the lasers was
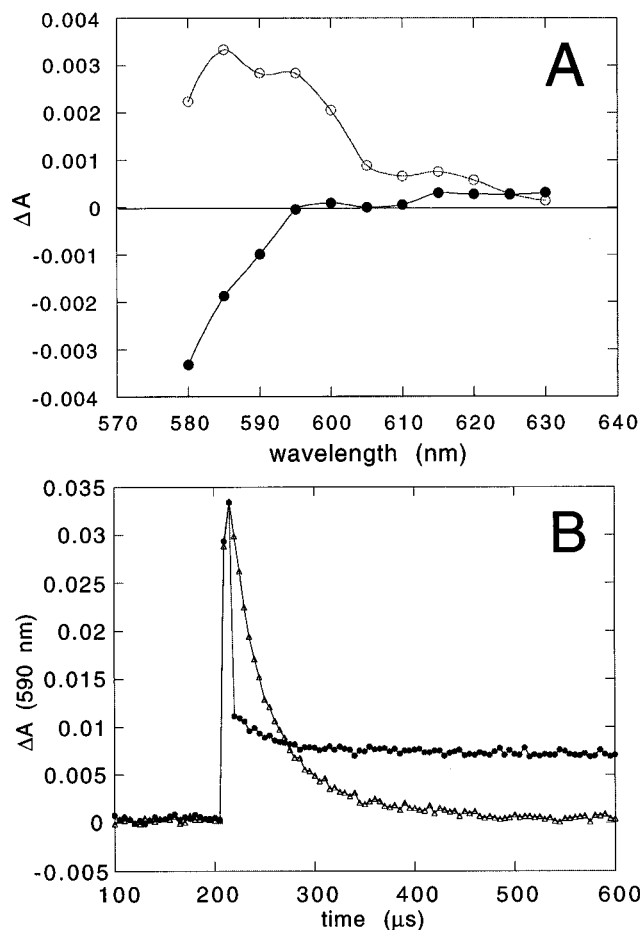
Figure 5. (A) Transient absorption spectra recorded 200 μs after 355 nm excitation of 2AN + MC540 in deaerated methanol with (○) and without (●) 640 nm excitation of the triplet state at a delay of 10 μs after the 355 nm pulse. (B) Kinetic signal observed at 590 nm after 355 nm excitation of 2AN + MC540 in deaerated methanol with (●) and without (△) 640 nm excitation of the triplet state at a delay of 10 μs after the 355 nm pulse.
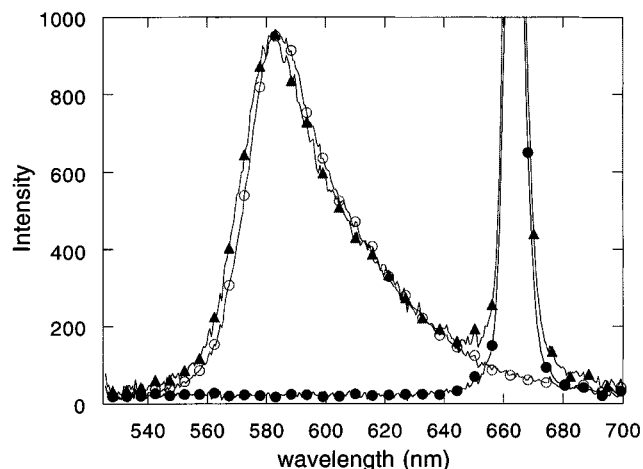
Figure 6. Emission spectrum detected from MC540 on direct excitation with 520 (○), 660 (●), and (355 + 660) nm (▲) light.

reversed, i.e., 640 nm first and 355 nm second, showing that the emission is a direct result of excitation of the T_1 state. The spectrum of this emission was obtained using the diode array detection system of the OMA (described above) with the detector gate width set to enclose only the 660 nm laser pulse.[38] The spectrum thus obtained is shown in Figure 6 and exhibits emission in the 550−650 nm range, with a maximum around 580 nm. This spectrum is compared to the normal fluorescence
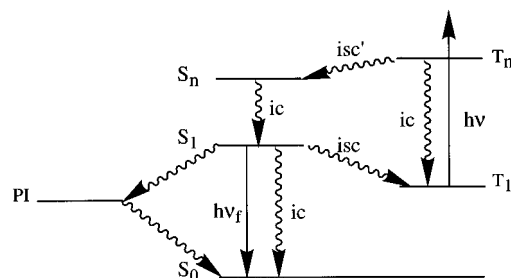
Figure 7. Energy level diagram depicting relaxation processes following $T_1 - T_n$ excitation.

emission spectrum obtained on direct excitation of MC540 with a pulse from the OPO at 520 nm.[39]

Discussion

This study has demonstrated a very efficient photophysical deactivation pathway from the upper excited triplet states of cyanine dyes. Although efficient irreversible photobleaching of the T_1 state was observed on selective excitation of the lowest triplet states with visible photons of $\lambda > 600$ nm, we found no evidence for *chemical* change in the dye. The nature of the photobleaching was determined to be physical in nature as there was no discernible increase in photodegradation on comparison of one- and two-laser pulse excitation. The photobleaching reaction was accompanied by emission of light in the 550-650 nm range and the generation of a long-lived species identified as the photoisomer (PI) formed on direct photolysis of the dye with visible excitation. The spectrum of the emission concurrent with selective excitation of the T_1 state light is identical to that of the normal fluorescence emission spectrum of the respective dyes, indicating a repopulation of the excited singlet state (S_1) of the dye.

We propose a scheme that accounts for the observed emission and transient absorption changes as depicted in Figure 7. Excitation of the T_1 state ultimately results in generation of the singlet state from which fluorescence and photoisomerization results. Thus, a reverse intersystem crossing from the T_n state back to the singlet manifold is required. Further evidence for a common pathway for the cyanine dyes arises when one considers that the observed photobleaching quantum yields are inversely proportional to the intersystem crossing yields for the compounds studied. This would be expected for a situation such as proposed here; reverse intersystem crossing would generate the singlet state (S_1) from which the normal partitioning between internal conversion, fluorescence, intersystem crossing, and possibly photoisomerization would occur. Thus, cyanine dyes that possess relatively high Φ_{isc} values exhibit low Φ_{bl} since once reverse intersystem crossing occurs from T_n to give the S_1 state, the S_1 state has a high probability of regenerating the T_1 state through intersystem crossing and a lower T_1 photobleaching is the result. The efficiency of the reverse intersystem crossing process can be estimated from of the ratio of observed Φ_{bl} (removal of T_1 state) to $(1 - \Phi_{isc})$. This ratio represents the observed photobleaching efficiency taking into consideration the repopulation of the T_1 state by intersystem crossing from S_1. Table 1 lists the high values of this ratio (∼0.7) for all cyanines studied, indicative of only 30% internal conversion from T_n back to T_1. On the basis of the few values for intersystem crossing yields to be found in the literature,[33,34] these values for the cyanines must be regarded as substantial and indicate that reverse intersystem crossing competes efficiently with internal conversion. The origin of this behavior may be found in both a large $T_n - T_1$ energy gap and the effect of heavy atom substitution (S, Se). The influence of the $T_n - T_1$

energy gap is seen for anthracene and meso-substituted anthracenes where the reverse intersystem crossing yields range from 10^{-5} for the unsubstituted (a thermally activated process since T_2 lies lower in energy than S_1) to 0.18 for 9,10-dibromoanthracene.[33,34] The effect of heavy atom substitution (the largest effect was observed for the bromo-substituted molecules) was also suggested to account for the observation of reverse intersystem crossing in these compounds.

By use of higher laser pulse energies at 640 nm ($>$40 mJ), almost complete T_1 photobleaching was achieved. As the laser pulse duration (OPO or dye laser) is much longer than S_1 and T_n lifetimes, the system can continuously cycle all triplet state until it is effectively removed by fluorescence, internal conversion, and isomerization from the S_1 state if sufficient intensities are available. With this scheme the formation of a photoisomer (PI) on excitation of T_1 can also be rationalized, even when the T_1 state of the cyanine is sensitized. The photoisomerization reaction in the case of MC540 is a major pathway in the deactivation of the S_1 state, and reverse intersystem crossing from T_n will generate the photoisomer, which is indeed detected following excitation of the T_1 state.

Two-laser, two-color generation of a singlet state that has the same properties as that produced by direct one-photon excitation implies that, for the merocyanine dyes studied here, the singlet and triplet states are of the same geometry. No detectable isomerization occurred directly from the upper T_n state. Rather, an efficient reverse intersystem crossing takes place to form the S_1 state from which photoisomerization takes place. As the cyanine dyes studied are different in each case, we cannot speculate as to the origin of the 483 nm absorbing species in the reported picosecond work,[27] but it would appear in the present study that the lack of isomerization in the triplet manifold is not simply due to the presence of energy barriers on the potential surface. These results confirm the singlet state rather than the triplet state as the active state in the process of isomerization in these molecules.

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(38) Excitation at 660 nm, rather than 640 nm, was used in these experiments to better separate the emission from the scattered laser light, as shown in Figure 6.

(39) Small differences between the emission spectra are due to differing amounts of ground state self-absorption. These are caused by the varying ground state concentrations necessitated by the different excitation conditions used.