Photodynamic therapy is a treatment in which the combination of a dye, light, and oxygen causes photochemically induced cell death. Observations of this effect occurred at least as early as the end of the last century. In 1900, Raab reported that the dye acridine rapidly killed paramecia when exposed to light, but had no effect in the dark. This observation quickly inspired attempts to use this effect to treat disease. Light and the dye eosin were combined to treat skin cancer in 1903; however, significant progress in applying photodynamic therapy to the treatment of cancer did not occur until the 1940s and 1950s, when it was discovered that porphyrin-based photosensitive dyes preferentially accumulated in malignant tissues. A sustained series of studies into the mechanisms and applications of photodynamic therapy for the treatment of a broad range of cancers was initiated by Dougherty in the 1970s. This work led the U.S. Food and Drug Administration in December 1995 to approve the treatment of advanced esophageal cancer using photodynamic therapy with Photofrin®, a porphyrin-based photosensitizer. In 1998 this approval was extended to cover the treatment of early-stage lung cancer. Other countries have also approved photodynamic therapy for the treatment of bladder, gastric, and cervical cancers. Several review articles have been published that provide an overview of the clinical results as well as the open questions about this therapy that require further research.

The photodynamic effect is a result of three primary processes. First, the ground state of the dye ($S_0$) is optically excited to produce the excited singlet state ($S_1$). Population from this excited state is transferred by intersystem crossing (a radiationless transition) to the dye’s lowest triplet state ($T_1$). Finally, collisional energy transfer from the triplet dye to ground-state molecular oxygen ($^{3}\text{O}_2$) produces highly reactive singlet oxygen ($^{1}\text{O}_2$) and returns the dye to its ground state:

$$S_0 + h\nu \rightarrow S_1$$
$$S_1 \rightarrow T_1$$
$$T_1 + ^{3}\text{O}_2 \rightarrow S_0 + ^{1}\text{O}_2.$$
Reverse intersystem crossing may also occur from higher-lying triplet states where intersystem crossing to the singlet manifold competes with direct internal conversion to the lowest triplet state. This process of reverse intersystem crossing from higher-lying triplets is responsible for two-step laser-induced fluorescence (TSLIF) observed in several dyes. The quantum yield of reverse intersystem crossing, $\Phi_{\text{risc}}$, can be quite small ($<10^{-5}$), but there are reports of exceptionally high yields ($\Phi_{\text{risc}} > 0.1$) in 9,10-dibromoanthracence, several merocyanine derivatives, tetraphenylporphyrin, erythrosin B, and rose bengal.

Several reports of reverse intersystem crossing in rose bengal (RB) have been published. Durán and Cilento describe observations of fluorescence following generation of RB triplets by energy transfer from excited triplet acetone. It was believed that higher-lying triplets were populated through triplet-triplet excitation transfer and subsequently relaxed to $S_1$ through reverse intersystem crossing. The magnitude of the emission was compared for a series of xanthene dyes (fluorescein, eosin, and rose bengal), which revealed that heavy-atom substitution enhanced the effect. This process was not associated with a particular triplet state, and no attempt was made to quantify its yield. Ketsle et al. investigated transient absorption changes following two-pulse excitation (532 nm + 694 nm) of various fluorescein derivatives, including rose bengal, incorporated in polymer hosts. Photobleaching of the $T_1$ absorption due to the second pulse was observed to have a component that was irreversible on the microsecond time scale. It was observed that the decrease in concentration of $T_1$ equaled the increase in concentration of $S_0$, providing evidence for a photophysical rather than photochemical process. Fluorescence emission was also observed coincident with the second pulse. A reverse intersystem crossing quantum yield of 0.72 was reported for $T_3$, the triplet state excited by red light. Most recently, the work of Reindl and Penzkofer reported an 80% quantum yield of reverse intersystem crossing for $T_4$, the state excited through absorption of green light by $T_1$. Using a model of the population dynamics, the yield was extracted from measurements of the pulse-to-pulse variation in fluorescence for a train of picosecond pulses.

The present work is the first study to identify and investigate the properties of $T_2$, a triplet state in rose bengal populated by near-infrared light ($\lambda = 1064$ nm). We have used laser flash photolysis and two-step laser-induced fluorescence measurements to determine the triplet-triplet absorption cross-section spectrum in the near infrared and the quantum yield of reverse intersystem crossing and lifetime of $T_2$. In addition, upper limits on the reverse intersystem crossing yield for $T_3$ are established.

**Experimental**

The foundation of the laser system is a mode-locked Nd:YAG laser that generates a train of pulses at 76 MHz. Every 400 ms a single pulse is selected using an electro-optic switch and amplified using a regenerative amplifier followed by a flashlamp-pumped two-pass amplifier (both Nd:YAG). The amplified pulses have a wavelength of 1064 nm, a pulse length of ~190 ps, and energies exceeding 2 mJ. The second harmonic is generated from this pulse using a KDP crystal, resulting in a pulse with a wavelength of 532 nm, a pulse length of ~134 ps, and an energy greater than 250 $\mu$J.

The experimental setup for the laser flash photolysis measurements is shown in Fig. 77.33 using the symbols defined in Table 77.II. In this configuration the second-harmonic pulse ($\lambda = 532$ nm) is separated from the fundamental by a dichroic mirror (DM) with any residual light at the fundamental wavelength ($\lambda = 1064$ nm) further attenuated by a filter (F). The majority of this frequency-doubled pump pulse is focused by a cylindrical lens (CL) onto the masked sample cuvette, exciting a 2-mm by 1-cm cross-sectional area. A small fraction of the pump pulse is reflected by a glass plate (BS) prior to the cylindrical lens, attenuated by neutral density filters, and then detected by silicon photodiode PD1. This signal is captured by gated integrator GI1 and transferred to a computer. By removing the sample cuvette and placing an energy meter behind the beam mask, the pump pulse monitor signal measured by PD1 can be calibrated with respect to the energy reaching the...
Table 77.11: Equipment used in the laser flash photolysis and two-step laser-induced fluorescence experiments.

<table>
<thead>
<tr>
<th>Description</th>
<th>Manufacturer, Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Fast amplifier</td>
</tr>
<tr>
<td>BS</td>
<td>Glass plate (microscope slide)</td>
</tr>
<tr>
<td>CL</td>
<td>Cylindrical lens</td>
</tr>
<tr>
<td>DM</td>
<td>Dichroic mirror, R@532 nm, T@1064 nm</td>
</tr>
<tr>
<td>F</td>
<td>Short-pass filter</td>
</tr>
<tr>
<td>GI1–4</td>
<td>Gated integrator</td>
</tr>
<tr>
<td>L1–3</td>
<td>Lenses</td>
</tr>
<tr>
<td>P</td>
<td>Prism</td>
</tr>
<tr>
<td>PD1–2</td>
<td>Silicon photodiodes</td>
</tr>
<tr>
<td>PL</td>
<td>Mercury lamp</td>
</tr>
<tr>
<td></td>
<td>Fast shutter</td>
</tr>
<tr>
<td></td>
<td>Long-pass filter</td>
</tr>
<tr>
<td>PMT</td>
<td>Photomultiplier tube</td>
</tr>
<tr>
<td>PS</td>
<td>Polarizing beam splitter</td>
</tr>
<tr>
<td>S</td>
<td>Sample cuvette and beam mask</td>
</tr>
<tr>
<td>SHG</td>
<td>Second-harmonic generator (KDP crystal)</td>
</tr>
<tr>
<td>SP1</td>
<td>Monochromator, bandwidth ~13 nm</td>
</tr>
<tr>
<td>SP2</td>
<td>Monochromator, bandwidth ~4 nm</td>
</tr>
<tr>
<td>TD</td>
<td>Digitizing oscilloscope</td>
</tr>
<tr>
<td>WP</td>
<td>Half-wave plate (λ = 1064 nm)</td>
</tr>
</tbody>
</table>

sample. Transient absorption changes are probed by a broadband light beam traveling along the length of the irradiated zone (perpendicular to the pump pulse). The probe pulse has a 20-ms duration and is produced by a mercury lamp followed by a long-pass filter and a fast mechanical shutter. This collection of elements is represented by PL in Fig. 77.33. The probe pulse passes through monochromator SP1 before being detected by silicon photodiode PD2. The photodiode signal is increased by multistage amplifier A and then recorded by digital oscilloscope TD. The average signal from 64 shots at 9-bit resolution is then transferred to a computer for analysis.

The two-step laser-induced fluorescence measurements probing T2 are made with the optical layout shown in Fig. 77.34. To achieve a high degree of spectral separation between the fundamental and second-harmonic pulses, prism P is used to spatially disperse the two beams. The first pump pulse (P1) has a wavelength of 532 nm, and the second pump pulse (P2) has a wavelength of 1064 nm. P2 is delayed by 34 ns relative to P1 by traversal of a greater optical path length. The delay path includes a half-wave plate followed by a polarizing beam splitter, allowing for continuous variation of the second pump pulse energy. The pump pulses, P1 and P2, are recombined spatially at dichroic mirror DM. The pulses pass through two pinholes, ensuring collimation, before irradiating a 2-mm-diam spot at the sample cuvette. As in the laser flash photolysis layout, a small fraction of the excitation light is reflected by a glass plate to a silicon photodiode. This signal is split before sampling by two gated integrators, GI1 and GI2, which distinguish between the P1 and P2 signals. The pump-pulse signals are calibrated individually using an energy meter. Emission from the excited sample is collected, spectrally resolved using monochromator SP2, and detected by a photomultiplier tube. The signal from the PMT is split and sampled by the gated integrators GI3 and GI4. The temporal gate of GI3 is centered on the fluorescence excited by P1. The center of the GI4 temporal gate is set to be 34 ns later than the center of the GI3 gate, corresponding to the time delay between the pump pulses. Both gates are 20 ns wide. The values of all four gated integrators are recorded by a computer for each shot.
Two-step laser-induced fluorescence measurements probing $T_3$ are made using a similar setup. In this case $P_2$, the 1064-nm pump pulse, is replaced by a 632-nm-wavelength pump pulse, while the first pump pulse remains at 532 nm. The 632-nm light is generated by Raman scattering of the Nd:YAG second harmonic in an 18-cm ethanol cell, resulting in $60\,\mu$J/pulse with a pulse length of approximately 80 ps. The 632-nm light is separated from the 532-nm light by a pair of prisms before $P_2$ enters the delay line. The pulses are spatially recombined at the dichroic mirror DM, and from this point the system is identical to the previously described two-step, laser-induced fluorescence apparatus.

Rose bengal was purchased from Sigma (St. Louis) and used without further purification. All experiments were carried out in phosphate-buffered saline with a pH of 7. Effects of photobleaching were minimized by continuously stirring all samples with a micro-stirbar during irradiation. Photobleaching was monitored by measuring the decrease in fluorescence as a function of the number of excitation pulses. There was a less-than-5% decrease in fluorescence after more than 3700 two-step excitations. Samples had a concentration of approximately 20 µM and were stored in the dark prior to use.

**Analysis**

Several photophysical parameters associated with an upper triplet state can be determined from two-step laser-induced fluorescence (TSLIF) measurements collected over a range of second pump-pulse ($P_2$) fluences. These measurements are sensitive to the lifetime of the upper triplet state excited by $P_2$, the quantum yield of intersystem crossing from this state back to the singlet manifold, and its thermalization rate. The upper triplet photophysical parameters are determined by fitting a model of the two-step laser-induced fluorescence process to the fluence-dependent TSLIF data.

The kinetic model used to analyze the TSLIF experiments is shown in Fig. 77.35(a). The rate equations describing this model are

\[
\begin{align*}
\frac{dp_s}{dt} &= -\sigma_{S_0S_1}(p_{S_0} - p_{S_1})I_1(t) + (1 - \Phi_{isc})\tau_{S_1}^{-1}p_{S_1} + \tau_{T_1}^{-1}p_{T_1}, \\
\frac{dp_{T_1}}{dt} &= \Phi_{isc}\tau_{S_1}^{-1}p_{S_1} - \tau_{T_1}^{-1}p_{T_1} - \sigma_{T_1T_4}(p_{T_1} - p_{T_4})I_1(t) - \sigma_{T_1T_n}(p_{T_1} - p_{T_n})I_2(t) + (1 - \Phi_{isc,T_4})\tau_{T_4}^{-1}p_{T_4} + (1 - \Phi_{isc,T_n})\tau_{T_n}^{-1}p_{T_n}, \\
\frac{dp_{S_1}}{dt} &= k_r p_{S_1} - \tau_{S_1}^{-1}p_{S_1}, \\
\frac{dp_{S_4}}{dt} &= \sigma_{S_0S_1}(p_{S_0} - p_{S_1})I_1(t) - k_r p_{S_4}, \\
\frac{dp_{T_4}}{dt} &= \sigma_{T_1T_4}(p_{T_1} - p_{T_4})I_2(t) - k_r p_{T_4}, \\
\frac{dp_{T_n}}{dt} &= \sigma_{T_1T_n}(p_{T_1} - p_{T_n})I_2(t) - k_r p_{T_n}.
\end{align*}
\]
where the $p_i$’s are the populations of $S_0$, $T_1$, $S_1$, $S_1'$, $T_n$, $T_n'$, and $T_4$ (arranged in order of increasing energy), where $n = 2$ or $3$. Table 77.III lists the definitions and values of the photophysical parameters. The unknown parameters are $\Phi_{\text{risc}, T_n}$, $\tau_{T_n}$, and $k_{r, T_n}$. The pump pulses P1 and P2 have a Gaussian temporal profile such that

$$I_1(t) = \frac{F_1}{\sqrt{2\pi \delta_1^2}} \exp\left[-\frac{(t + \Delta/2)^2}{(2\delta_1^2)}\right]$$

and

$$I_2(t) = \frac{F_2}{\sqrt{2\pi \delta_2^2}} \exp\left[-\frac{(t - \Delta/2)^2}{(2\delta_2^2)}\right],$$

where $F_1$ and $F_2$ are the fluences, $\delta_1$ and $\delta_2$ are related to the full-width-at-half-maximum pulse lengths by FWHM $= \sqrt{8 \ln 2} \delta$, and $\Delta$ is the time delay between the peaks of P1 and P2.

Excited-state absorption from states other than $T_1$ has been neglected. Previous experiments have found no evidence for absorption of 532-nm light by $S_1$. The state $T_4$ may absorb 532-nm light and thus populate an even higher-lying state, but we assume with Reindl and Penzkofer that any such extremely high-lying state will relax back to $T_4$ immediately. This process would affect transmission measurements, but since the present studies are concerned only with emission, it appears reasonable to omit it in this case. Finally, absorption by $T_n$ is also not included in this model. The validity of this assumption will be discussed in the Results section.

As a result of the large time delay between the pump pulses ($\Delta = 34$ ns), it is possible to separate the system of rate equations [(Eq. (1)] into two subsets. The set of equations describing the effects of the first pump pulse is

$$\begin{align*}
\sigma_{T_1T_1} & = k_{\text{isc}} + k_{r, T_1} + k_{risc, T_1} \\
\sigma_{S_0S_1} & = k_{r, S_0} + k_{risc, S_0} \\
\sigma_{S_1S_0} & = k_{r, S_1} + k_{risc, S_1} \\
\sigma_{T_nT_n} & = k_{\text{isc}} + k_{r, T_n} + k_{risc, T_n}
\end{align*}$$

Figure 77.35
Energy-level scheme for description of two-color excitation dynamics. (a) Complete two-step model, (b) P1 subset of model, (c) P2 subset of model. See Table 77.III for parameter descriptions and values.
Table 77.11: Parameters used in the two-step laser-induced fluorescence model for rose bengal.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma_{S_0S_1}$</td>
<td>Ground-state absorption cross section at 532 nm ($S_0 + h\omega \rightarrow S_1'$)</td>
<td>$1.8 \times 10^{-16}$ cm$^2$</td>
<td>16</td>
</tr>
<tr>
<td>$\sigma_{T_1T_2}$</td>
<td>Triplet absorption cross section ($T_1 + h\omega \rightarrow T_2'$)</td>
<td>$(1.1 \pm 0.1) \times 10^{-16}$ cm$^2$</td>
<td>This work</td>
</tr>
<tr>
<td>$\sigma_{T_1T_4}$</td>
<td>Triplet absorption cross section at 532 nm ($T_1 + h\omega \rightarrow T_4'$)</td>
<td>$7.4 \times 10^{-17}$ cm$^2$</td>
<td>16</td>
</tr>
<tr>
<td>$\tau_{S_1}$</td>
<td>$S_1$ lifetime</td>
<td>89 ps</td>
<td>16–18</td>
</tr>
<tr>
<td>$\tau_{T_1}$</td>
<td>Lifetime of $T_1$ (includes both phosphorescence and oxygen quenching)</td>
<td>3 $\mu$s</td>
<td>19</td>
</tr>
<tr>
<td>$\tau_{T_4}$</td>
<td>Lifetime of $T_4$</td>
<td>50 fs</td>
<td>16</td>
</tr>
<tr>
<td>$\tau_{T_n}$</td>
<td>Lifetime of $T_n$</td>
<td>fitting parameter</td>
<td></td>
</tr>
<tr>
<td>$\Phi_{isc}$</td>
<td>Intersystem crossing yield ($S_1 \rightarrow T_1$)</td>
<td>0.98</td>
<td>18,19</td>
</tr>
<tr>
<td>$\Phi_{risc,T_4}$</td>
<td>Reverse intersystem crossing yield ($T_4 \rightarrow S_1'$)</td>
<td>0.8</td>
<td>13</td>
</tr>
<tr>
<td>$\Phi_{risc,T_n}$</td>
<td>Reverse intersystem crossing yield ($T_n \rightarrow S_1'$)</td>
<td>fitting parameter</td>
<td></td>
</tr>
<tr>
<td>$F_1$</td>
<td>First pump-pulse fluence ($\lambda = 532$ nm)</td>
<td>$(8.8 \pm 0.5) \times 10^{15}$ photons/cm$^2$</td>
<td></td>
</tr>
<tr>
<td>$F_2$</td>
<td>Second pump-pulse fluence ($\lambda = 1064$ nm)</td>
<td>varied</td>
<td></td>
</tr>
<tr>
<td>$k_{ic,T_4}$</td>
<td>Internal conversion rate ($T_4 \rightarrow T_1$)</td>
<td>$\left(1 - \Phi_{risc,T_4}\right)/\tau_{T_4}$</td>
<td></td>
</tr>
<tr>
<td>$k_{ic,T_n}$</td>
<td>Internal conversion rate ($T_n \rightarrow T_1$)</td>
<td>$\left(1 - \Phi_{risc,T_n}\right)/\tau_{T_n}$</td>
<td></td>
</tr>
<tr>
<td>$k_{isc}$</td>
<td>Intersystem crossing rate</td>
<td>$\Phi_{isc}/\tau_{S_1}$</td>
<td></td>
</tr>
<tr>
<td>$k_{r,T_n}$</td>
<td>Thermalization rate ($T_n' \rightarrow T_n$)</td>
<td>fitting parameter</td>
<td></td>
</tr>
<tr>
<td>$k_r$</td>
<td>Thermalization rate ($S_1' \rightarrow S_1$)</td>
<td>$10^{12}$ s$^{-1}$</td>
<td>16</td>
</tr>
<tr>
<td>$k_{risc,T_4}$</td>
<td>Reverse intersystem crossing rate ($T_4 \rightarrow S_1'$)</td>
<td>$\Phi_{risc,T_4}/\tau_{T_4}$</td>
<td></td>
</tr>
<tr>
<td>$k_{risc,T_n}$</td>
<td>Reverse intersystem crossing rate ($T_n \rightarrow S_1'$)</td>
<td>$\Phi_{risc,T_n}/\tau_{T_n}$</td>
<td></td>
</tr>
<tr>
<td>$k_{T_1}$</td>
<td>$T_1$ relaxation rate</td>
<td>$1/\tau_{T_1}$</td>
<td></td>
</tr>
</tbody>
</table>
Reverse Intersystem Crossing from a Triplet State of Rose Bengal

\[ \frac{dp_{S_{0}}}{dt} = -\sigma_{S_{0}S_{1}}(p_{S_{0}} - p_{S_{1}})I_{1}(t) + (1 - \Phi_{\text{isc}})\tau_{S_{1}}^{-1}p_{S_{1}} + \tau_{T_{1}}^{-1}p_{T_{1}}, \]

\[ \frac{dp_{T_{1}}}{dt} = \Phi_{\text{isc}}\tau_{S_{1}}^{-1}p_{S_{1}} - \tau_{T_{1}}^{-1}p_{T_{1}} - \sigma_{T_{1}T_{4}}(p_{T_{1}} - p_{T_{4}})I_{1}(t) + (1 - \Phi_{\text{isc},T_{4}})\tau_{T_{4}}^{-1}p_{T_{4}}, \]

\[ \frac{dp_{S_{1}^{-}}}{dt} = k_{r}p_{S_{1}^{-}} - \tau_{S_{1}}^{-1}p_{S_{1}} \]

\[ \frac{dp_{S_{1}^{+}}}{dt} = \sigma_{S_{0}S_{1}}(p_{S_{0}} - p_{S_{1}^{+}})I_{1}(t) - k_{r}p_{S_{1}^{+}} + \Phi_{\text{isc},T_{4}}\tau_{T_{4}}^{-1}p_{T_{4}}, \]

\[ \frac{dp_{T_{4}}}{dt} = \sigma_{T_{1}T_{4}}(p_{T_{1}} - p_{T_{4}})I_{1}(t) - \tau_{T_{4}}^{-1}p_{T_{4}}, \]

which are used for \( t = -\infty \) to \( t = 0 \). The time \( t = 0 \) is midway between the peaks of P1 and P2, which are separated by a delay much greater than their pulse lengths and the lifetimes of all excited states except \( T_{1} \). The processes included in this first segment, where only the effects of P1 are relevant, are shown in Fig. 77.35(b). This set of equations describing the effects of P1 neglects all terms containing \( I_{2} \). Since \( T_{n} \) and \( T_{n}' \) are only populated by P2, corresponding terms can be eliminated from Eq. (1) since \( p_{T_{n}} = p_{T_{n}'} = 0 \). The process of reverse intersystem crossing is included in this model of the interaction of P1 with the sample. The first pump pulse may be absorbed by both \( S_{0} \) and by any \( T_{1} \) population created by preceding parts of the same pulse. Absorption of P1 light by the \( T_{1} \) state populates the \( T_{4} \) state, which has been shown to have a high yield of reverse intersystem crossing.\(^{13}\) It is necessary to include this process for pulses longer than the \( S_{1} \rightarrow T_{1} \) intersystem crossing time since it can lead to an apparent enhancement of the fluorescence yield, particularly at fluences resulting in depletion of the ground state. At the P1 fluence used experimentally, the solution of Eq. (4), which includes the reverse intersystem crossing process, led to a 29%-greater integrated fluorescence compared to an otherwise identical set of equations that neglected this process. It is important to emphasize that the reverse intersystem crossing described above occurs from the triplet state populated by secondary absorption of the first pump pulse and is easily distinguished temporally from the process this experiment is designed to measure: reverse intersystem crossing from the triplet state populated by the second pump pulse.

The effects of P1 and P2 can be separated cleanly since the system has relaxed such that only \( S_{0} \) and \( T_{1} \) are populated at \( t = 0 \). The effects of only the second pump pulse are considered from this time to \( t = +\infty \). The model of this second excitation step is shown in Fig. 77.35(c). The equations describing this segment are

\[ \frac{dp_{S_{0}}}{dt} = (1 - \Phi_{\text{isc}})\tau_{S_{1}}^{-1}p_{S_{1}} + \tau_{T_{1}}^{-1}p_{T_{1}}, \]

\[ \frac{dp_{T_{1}}}{dt} = \Phi_{\text{isc}}\tau_{S_{1}}^{-1}p_{S_{1}} - \tau_{T_{1}}^{-1}p_{T_{1}} - \sigma_{T_{1}T_{n}}(p_{T_{1}} - p_{T_{n}})I_{2}(t) + (1 - \Phi_{\text{isc},T_{n}})\tau_{T_{n}}^{-1}p_{T_{n}}, \]

\[ \frac{dp_{S_{1}^{-}}}{dt} = k_{r}p_{S_{1}^{-}} - \tau_{S_{1}}^{-1}p_{S_{1}} \]

\[ \frac{dp_{S_{1}^{+}}}{dt} = -k_{r}p_{S_{1}^{+}} + \Phi_{\text{isc},T_{n}}\tau_{T_{n}}^{-1}p_{T_{n}}, \]

\[ \frac{dp_{T_{n}}}{dt} = k_{r}p_{T_{n}} - \tau_{T_{n}}^{-1}p_{T_{n}}, \]

\[ \frac{dp_{T_{n}'}}{dt} = \sigma_{T_{1}T_{n}}(p_{T_{1}} - p_{T_{n}'})I_{2}(t) - k_{r}p_{T_{n}}p_{T_{n}'} \]

In this segment all terms containing \( I_{1} \) and \( p_{T_{n}}' \), the population of \( T_{4} \), are dropped from Eq. (1).

The fluorescence due to the two pulses is proportional to the population of \( S_{1} \) such that

\[ f_{1} = \Phi_{f} \int_{0}^{\tau_{S_{1}}} p_{S_{1}}(t) dt \]

and

\[ f_{2} = \Phi_{f} \int_{0}^{\infty} p_{S_{1}}(t) dt \].
where $\Phi_f$ is the fluorescence yield. The two-step, laser-induced fluorescence ratio $f_R$ is defined by

$$f_R \equiv \frac{f_R}{f_1}.$$ (8)

This is a convenient quantity to compare with experimental results since fluorescence yield, collection, and detection efficiency factors are eliminated.

The $T_n$ photophysical parameters are determined by fitting this model of the two-step, laser-induced fluorescence process to the fluence-dependent $f_R$ obtained experimentally. As will be discussed later, extraction of the parameters requires $f_R$ measurements over a range of $P_2$ fluences, which, at the upper limit, are sufficient to partially deplete the lowest triplet state. In addition, the length of the second pump pulse must exceed the lifetime of $T_n$. A numerical approach is required since under these conditions analytical solutions cannot be obtained easily. The numerical analysis consists of three major components: (a) a calculation of the fluence-dependent $f_R$ for a given set of $T_n$ photophysical parameters, (b) an algorithm that optimizes these parameters to provide the best fit to the experimental data, and (c) an estimate of the precision to which the extracted parameters are known based on a randomization and re-optimization technique.

Calculation of the fluence-dependent, two-step laser-induced fluorescence ratio was based on the sequential solution of the rate equations given in Eqs. (4) and (5). These rate equations were solved using Runge–Kutta numerical integration. The agreement between the solutions obtained from this model and the experimental data can be quantified by the $\chi^2$ statistic, which is summed over the set $F_1, F_2$ for which experimental measurements of the TSLIF ratio, $f_R, \text{exp}$, were made. The standard deviations of those measurements are given by $\sigma_{R, \text{exp}}$. The next step is to search parameter space in order to find the values of $k_r, T_n$, $\tau_{T_n}$, and $\Phi_{\text{isc}}, T_n$ that minimize $\chi^2$. The optimization algorithm used is the downhill simplex method.\(^\text{20}\)

The downhill simplex method will find the set of parameters that minimizes $\chi^2$, but it does not report the precision with which these parameters are known given the uncertainties in the experimental measurements. This precision was estimated by running the optimization routine on sets of TSLIF ratio measurements, $f_{R, \text{mix}}$, calculated from

$$f_{R, \text{mix}}(F_1, F_2) = f_{R, \text{exp}}(F_1, F_2) + r\sigma_{R, \text{exp}}(F_1, F_2),$$ (9)

where $r$ is a uniformly distributed random number between $-1$ and $1$. The standard deviations of the parameters found in minimizing ten such data sets provide the estimated precision to which the parameters are known.

An analytical model of two-step laser-induced fluorescence that is limited to low-intensity and low-fluence conditions can be developed. In this regime the fluorescence signals are given by

$$f_1 = a\sigma_{S_0S_1} F_1$$ (10)

and

$$f_2 = a\Phi_{\text{isc}} \Phi_{\text{isc}, T_n} \sigma_{S_0S_1} F_1 \sigma_{T_1T_n} F_2,$$ (11)

where $a$ includes fluorescence yield, collection, and detection factors. Calculating the two-step laser-induced fluorescence ratio from Eqs. (10) and (11) gives

$$f_R = \Phi_{\text{isc}} \Phi_{\text{isc}, T_n} \sigma_{T_1T_n} F_2.$$ (12)

Although this expression cannot be used to determine $\tau_{T_n}$ and $k_r, T_n$, it is useful for estimating upper limits on $\Phi_{\text{isc}, T_n}$ when there is an undetectable two-step laser-induced fluorescence signal.

**Results**

The triplet-triplet absorption spectrum of rose bengal in the near infrared (Fig. 77.36) is derived from a series of transient absorption measurements acquired by laser flash photolysis. There is no measurable ground-state absorption in this region. Detector insensitivity prevented the extension of this spectrum beyond 1100 nm. Using the intensity variation method\(^\text{21}\) it was found that the absorption has a peak between 1050 and 1075 nm with a cross section $\sigma_{T_1T_2} = (1.1\pm0.1) \times 10^{-16}$ cm\(^2\).

Figure 77.37 shows two-step laser-induced fluorescence results for $T_2$. The ratio of two-step to one-step fluorescence $f_{R, \text{exp}}$ versus the fluence of the second pump pulse ($\lambda_2 = 1064$ nm) is plotted. The first pump-pulse fluence ($\lambda_1 = 532$ nm) was held approximately constant at $3.3\pm0.2$ mJ/cm\(^2\), and the resulting one-step fluorescence varied by less than 2%. No emission following $P_2$ was detected when $P_1$ was blocked. Each point in this plot represents the average of from 26 to 370 double-pulse excitations. The error bars indicate the corresponding standard deviations. In addition, the spectrum of the 532-nm + 1064-nm excited emission was measured and found to be the same as the $S_1\rightarrow S_0$ fluorescence spectrum,
confirming that the TSLIF results from repopulation of S₁. The parameters Φ_{risc,T₂}, τ_{T₂}, and k_{r,T₂} can be determined by analyzing the nonlinear dependence of f_R on F₂ using the multistate kinetic model described in the Analysis section. This analysis of the data shown in Fig. 77.37 gives Φ_{risc,T₂} = 0.0142 ± 0.0003, τ_{T₂} = 5.8 ± 1.6 ps, and k_{r,T₂} = 1.3 ± 0.2 ps⁻¹ with χ² = 0.011.

Similar measurements probing T₃ (λ₁ = 532 nm, λ₂ = 632 nm) failed to detect any two-step laser-induced fluorescence. Based on the fluorescence detection limits, the quantum yield of reverse intersystem crossing from T₃ can be constrained to Φ_{risc,T₃} < 0.06 using Eq. (12) with σ_{T₁,T₃} determined from Ref. 22 and f_R set equal to the uncertainty in the TSLIF measurement.

Discussion

Although no analytical expression can be given for f_R(F₁,F₂) that is applicable for the high fluences used in these experiments, it is possible to explain qualitatively the shape of the f_R versus F₂ curve shown in Fig. 77.37. This explanation also provides some justification for why the kinetic model analysis is sensitive to the lifetime and thermalization rate of the upper triplet state. Under low-fluence and low-intensity conditions, Eq. (12) predicts that f_R will increase linearly with F₂. Deviations from the predicted linear response are expected to occur for P2 with sufficiently high intensity or fluence. Under conditions where the pulse length is shorter than the lifetime of T₂, the saturation fluence F_{sat} = (σ_{T₁,T₂})⁻¹ for T₁→T₂ excitation is 9 × 10¹⁵ photons/cm² (1.7 mJ/cm²). Multiple excitations are possible, however, for pulses that are longer than the lifetime of T₂. This allows the two-step laser-induced fluorescence ratio to continue to grow beyond the short-pulse saturation fluence limit. Limits on the growth of the two-step laser-induced fluorescence are not solely fluence dependent. The maximum rate at which population can be excited to the upper triplet state is limited by the thermalization rate k_{r,T₂}. In addition, the maximum number of excitation cycles that can be achieved during a pulse is limited by the upper triplet lifetime τ_{T₂} and the length of the second pump pulse δ₂. Since the nonlinear portion of the f_R curve is dependent on the upper triplet lifetime and its thermalization rate, it is possible to extract these parameters from a fit of the kinetic model to data obtained under high-intensity and high-fluence conditions where the deviation from linearity becomes significant.

The multistate kinetic model described in Eq. (1) and Fig. 77.35 is not the only possible explanation for fluorescence following 532-nm + 1064-nm excitation. An alternative
model that deserves consideration includes absorption of 1064-nm light by \( T_2 \) to populate \( T_4 \), a state already known to have a high reverse intersystem crossing yield.\(^\text{13}\) On the basis of energetic considerations, the \( T_2 \rightarrow T_4 \) absorption process appears to be plausible, although restrictions such as those based on parity may disallow this transition. If reverse intersystem crossing were to occur predominantly from \( T_4 \), then the expression for \( f_R \) given in Eq. (12) should be modified to give

\[
f_R = \Phi_{\text{isc}} \Phi_{\text{risc}, T_4} \sigma_{T_1 T_2} \sigma_{T_2 T_4} F_2^2 . \tag{13}
\]

According to this model, \( f_R \) increases quadratically rather than linearly in \( F_2 \) since population of \( T_4 \) from \( T_1 \) requires the sequential absorption of two 1064-nm photons. In addition, \( f_R \) is expected to saturate at a value greater than \( \Phi_{\text{isc}} \Phi_{\text{risc}, T_4} = 0.78 \). The experimental data shown in Fig. 77.37 do not exhibit this behavior, which justifies our elimination of this alternative model.

No two-step laser-induced fluorescence was detected in the 532-nm + 632-nm experiment. This experiment was performed under conditions much less favorable than for the 532-nm + 1064-nm experiment. Both the maximum P2 fluence and the triplet-triplet absorption cross section were significantly less at 632 nm compared to 1064 nm. Even with these limitations, however, the value of \( \Phi_{\text{risc}, T_3} \) can be determined to be less than 0.06. This result disagrees with a yield of 0.72 for this state reported previously.\(^\text{15}\)

Ketsle \textit{et al.} attempted to measure the yield of reverse intersystem crossing through measurements of the change in \( T_1 \) absorption (and therefore, concentration) in a two-step excitation experiment.\(^\text{15}\) Immediately following P2, a decrease in the concentration \( \Delta C_{ab} \) of \( T_1 \) was observed, which was followed by a partial recovery \( \Delta C_{ac} \), as shown in Fig. 77.38, which is a sketch showing the key features in the transient signal plotted in Fig. I of Ref. 15. The points \( a, b, \) and \( c \) in Fig. 77.38 represent the times immediately prior to P2, immediately following P2, and after the fast recovery of the transient bleaching, respectively. The lack of complete recovery is due to reverse intersystem crossing from the higher-lying triplet populated by P2. From these concentration changes Ketsle \textit{et al.} calculated the reverse intersystem crossing yield using the formula

\[
\frac{\Delta C_{ac}}{\Delta C_{ab}} = \Phi_{\text{risc}, T_3} \left( 1 - \Phi_{\text{isc}} \right) . \tag{14}
\]

Indeed, this latter interpretation of the bleaching fraction agrees with that used by Redmond \textit{et al.}\(^\text{12}\) Recalculating a yield based on Eq. (15) using the Ketsle \textit{et al.} bleaching fraction data found in Table I of Ref. 15 gives \( \Phi_{\text{risc}, T_3} >> 1 \). Since this quantum yield cannot exceed unity, it appears that their experimental data was obtained under conditions in which the assumptions used to derive these equations do not apply. In particular, these equations are valid only under conditions in which \( \Delta C_{ab} \) is proportional to the number of photons absorbed by \( T_1 \). This can occur only when the length of the exciting pulse is shorter than the lifetime of the upper triplet state \( \delta_2 \ll \tau_{T_1} \) and when the transient absorption detection system is capable of responding on this same time scale. Ketsle \textit{et al.} do not report the length of their second pump pulse but state only that it is from a ruby laser. It appears likely that their excitation pulse is longer than several nanoseconds, which is much greater than the expected upper triplet lifetime of picoseconds or less. In addition, the time response of their transient absorption detection system is not reported. The use of long pulses or slow detection systems with this transient

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\[ \text{Figure 77.38} \]

Schematic of transient changes in \( T_1 \) concentration (based on Fig. I in Ref. 15). The points \( a, b, \) and \( c \) in the figure represent the times immediately prior to P2, immediately following P2, and after the fast recovery of the transient bleaching, respectively.

Equation (14) attributes the bleaching to the entire fraction undergoing reverse intersystem crossing. It is more appropriate, however, to interpret bleaching as due to the fraction that undergoes reverse intersystem crossing and in addition does not repopulate the triplet manifold through \( S_1 \rightarrow T_1 \) transfer, implying

\[
\frac{\Delta C_{ac}}{\Delta C_{ab}} = \Phi_{\text{risc}, T_3} \left( 1 - \Phi_{\text{isc}} \right) . \tag{15}
\]
Absorption technique will lead to an underestimate of the number of absorbed photons, thus leading to values of \( \Phi_{\text{risc}, T_i} \) that exceed unity. The equipment requirements are not as demanding for fluorescence methods of measuring reverse intersystem crossing.

Reverse intersystem crossing yields have been calculated for a growing number of molecules. An aspect of this study that makes it of particular interest is that these yields have now been measured for several triplet states of rose bengal. Previous workers in this field have suggested that population excited to \( T_n \) relaxes rapidly to the next-lowest triplet state, and that the triplet-singlet transfer is predominantly due to reverse intersystem crossing from this less-energetic state.\(^{13,23}\) According to this model, the reverse intersystem crossing yield should be independent of the high-lying triplet state initially excited; experimental measurements clearly contradict this prediction with high yields of \( \Phi_{\text{risc}, T_4} = 0.80 \) if \( T_4 \) is initially excited,\(^{13}\) to much lower yields of \( \Phi_{\text{risc}, T_2} = 0.0142 \) for the case of direct population of \( T_2 \) (measured in this work).

To understand these results it is instructive to consider the energies of the relevant triplet and singlet states. The energies of the singlet states can be estimated from the peaks of the ground-state absorption spectrum. Similarly, the energies of the triplet states relative to \( T_1 \) can be estimated from triplet-triplet absorption spectra (the present work and Refs. 8 and 24). The energy of \( T_1 \) in methanol is 1.75 eV.\(^{25}\) These results have been compiled in Table 77.IV. The energy gaps between the excited triplet states and the nearest less-energetic singlet state are \( \Delta E (T_2 - S_2) = 0.51 \) eV, \( \Delta E (T_3 - S_3) = 0.35 \) eV, and \( \Delta E (T_4 - S_4) = 0.08 \) eV. Thus we find that the transition with the smallest energy gap exhibits the greatest reverse intersystem crossing yield (\( \Phi_{\text{risc}, T_2} = 0.0142 \), \( \Phi_{\text{risc}, T_1} < 0.06 \), \( \Phi_{\text{risc}, T_4} = 0.80 \)). Although this ordering is consistent with a simple interpretation of the energy gap law for nonradiative transitions, which states that reverse intersystem crossing is likely to be most favorable when there is a small energy gap between the triplet state and a nearby singlet, such an interpretation must be considered critically. As developed by Englman and Jortner,\(^{26}\) the energy gap law applies to a particular triplet-singlet pair, whereas here we are considering three such pairs. The strength of the spin-orbit coupling between different states may vary by several orders of magnitude. Since we do not know the values of the coupling parameters for the three transitions under consideration, it is impossible to definitively attribute the entire variation in reverse intersystem crossing yield to differences in the energy gap.

### Table 77.IV: Energies of rose bengal excited states. Singlet-state energies are estimated from the ground-state absorption spectrum. Triplet-state energies are estimated from the \( T_1 \) absorption spectrum.

<table>
<thead>
<tr>
<th>State</th>
<th>Energy (eV)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>( S_1 )</td>
<td>2.10</td>
<td>This work</td>
</tr>
<tr>
<td>( S_2 )</td>
<td>2.41</td>
<td>This work</td>
</tr>
<tr>
<td>( S_3 )</td>
<td>3.51</td>
<td>This work</td>
</tr>
<tr>
<td>( S_4 )</td>
<td>3.95</td>
<td>This work</td>
</tr>
<tr>
<td>( T_1 )</td>
<td>1.75</td>
<td>25</td>
</tr>
<tr>
<td>( T_2 )</td>
<td>2.92</td>
<td>This work</td>
</tr>
<tr>
<td>( T_3 )</td>
<td>3.86</td>
<td>8</td>
</tr>
<tr>
<td>( T_4 )</td>
<td>4.03</td>
<td>24</td>
</tr>
</tbody>
</table>

### Conclusion

We have presented what we believe to be the first study of a triplet state of rose bengal that is produced by 1064-nm excitation of \( T_1 \). The triplet-triplet absorption cross section was measured between 825 nm and 1100 nm. This state was further characterized using two-step laser-induced fluorescence to determine its thermalization rate, lifetime, and quantum yield of reverse intersystem crossing. Similar two-step laser-induced fluorescence measurements were made of the triplet excited by 632-nm light.

In earlier work, the reverse intersystem crossing yield was predicted to be independent of which higher-lying triplet state was initially excited. The present work finds that the yields for triplets excited by red and near-infrared light, \( T_3 \) and \( T_2 \), are much less than those reported earlier for the more-energetic state \( T_4 \), which is populated by green light.\(^{13}\) An analysis of the triplet-triplet absorption spectrum and the ground-state absorption spectrum shows that \( T_4 \) is energetically close to a state in the singlet manifold, whereas the corresponding gaps are significantly greater for \( T_2 \) and \( T_3 \).

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REFERENCES