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Photophysics of Squaraine Dyes: Role of Charge-Transfer in Singlet Oxygen Production and Removal

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The unique optical properties of squaraines render these molecules useful for applications that range from xerography to photodynamic therapy. In this regard, squaraines derived from the condensation of nitrogen-based heterocycles and squaric acid have many promising attributes. Key solution-phase photophysical properties of six such squaraines have been characterized in this study. One feature of these molecules is a pronounced absorption band in the region ∼600−720 nm that has significant spectral overlap with the fluorescence band (i.e., the Stokes shift is small). As such, effects of emission/reabsorption yield unique excitation wavelength dependent phenomena that are manifested in quantum yields of both fluorescence and sensitized singlet oxygen production. Comparatively small squaraine-sensitized yields of singlet oxygen production and, independently, large rate constants for squaraine-induced deactivation of singlet oxygen are consistent with a model in which there is appreciable intra- and intermolecular charge-transfer in the squaraine and squaraine−oxygen encounter complex, respectively. The results reported herein should be useful in the further development of these compounds for a range of oxygen-dependent applications.

1. Introduction

The condensation of electron-rich substrates and squaric acid yields a class of molecules called squaraines that are characterized by having an appreciable amount of intramolecular charge transfer (i.e., they are electron donor−acceptor−donor molecules).[1–4] These compounds have several desirable properties including pronounced absorption in the visible/near IR spectral region, good photostability and high photoconductivity.[5–7] As such, over the years, squaraines have been extensively investigated for a large number of technological applications including xerography,[6] data storage,[8] light emitting field-effect transistors,[9] solar cells,[10–12] and nonlinear optics.[13] It has recently been shown that squaraines are also efficient two-photon absorbers[14,15] and fluorescent histological probes,[16] and can be used in fluorescence patterning applications.[17]

Squaraines dissolved in solution have a characteristic and distinct absorption band in the region of ∼600−720 nm and, as such, have also been considered for use as sensitizers in photodynamic therapy (PDT).[15,18,19] Indeed, over the years, there has been a concerted effort to develop PDT sensitizers that absorb at comparatively long wavelengths where tissue is most transmissive.[20] To this end, it has been shown that selected squaraines are readily incorporated into a cell[15] and that, upon irradiation, cell death can ensue.[21]

With these applications, and particularly with respect to PDT, it is essential that, as new squaraines are developed, one includes a study of oxygen-dependent phenomena when characterizing photophysical and photochemical properties. Of particular interest is the efficiency with which a given squaraine will sensitize the production of singlet molecular oxygen, $O_2(a^1\Delta_g)$, and, independently, the efficiency with which the squaraine will deactivate/remove any singlet oxygen that has been formed. It is well-established that singlet oxygen can perturb cellular processes and ultimately cause cell death via apoptosis and/or necrosis.[22]

We recently reported the design and synthesis of several symmetric squaraine derivatives derived from π-excessive heterocycles, and considered their possible application as one- and two-photon singlet oxygen sensitizers pertinent to PDT.[15] Because of the demanding requirements in the development of modern PDT sensitizers and to help establish a foundation for the systematic design of viable molecules, we felt it important to further investigate the oxygen-dependent photophysical behavior of heterocycle-based π-extended squaraines. We report herein the synthesis, the photophysical and electrochemical characterization of selected members of two classes of such squaraines (Figure 1).

Through the first series of molecules, we wanted to examine the effects of (a) extending the π system by ring fusion in the terminal moieties (i.e., **1a** → **1b**), (b) adding sulfur to the chromophore in an attempt to facilitate S_1−T_1 intersystem crossing and, in turn, facilitate the triplet state sensitized production of singlet oxygen (i.e., **1a** → **1c**), and (c) making a nonsymmetric derivative to investigate how the presence of a static dipole moment influences interactions with molecular oxygen (i.e., **1a** → **1d**). Some aspects of molecules **2a** and **2b** were examined in our previous study[15] and, as such, we felt they would make a nice benchmark for the present work. However, as outlined herein, our more detailed current investigation revealed new information about these molecules that helps us develop a general framework through which the oxygen-dependent behavior of squaraines can be considered.

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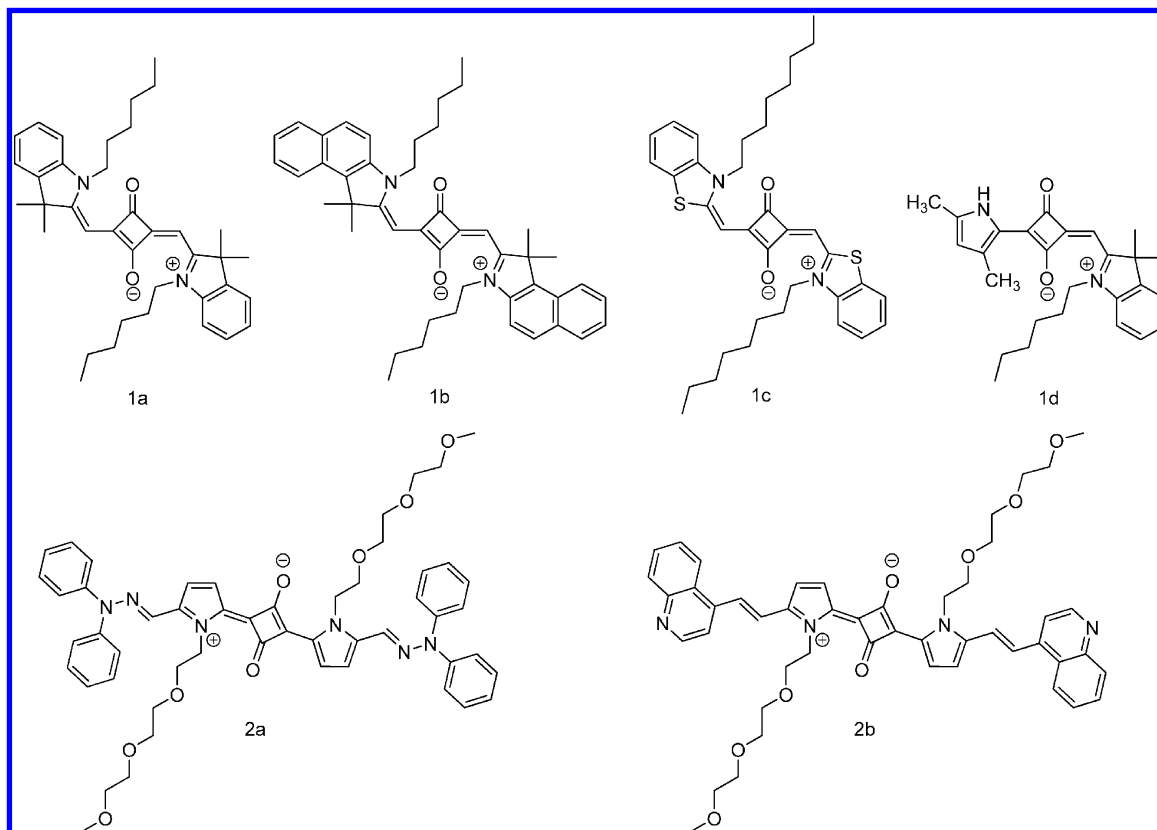[‡] Aarhus University.

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Figure 1. Structures of the squaraine dyes studied.

TABLE 1: Spectroscopic and Photophysical Properties of the Squaraines in Air-Equilibrated Solvents

compound	solvent	$\lambda_{abs,max}{}^{a}$ /nm (ε^{b})	$\lambda_{em,max}{}^{c}$ /nm	Stokes shift /nm (/cm^{-1})^{d}	$E_{0,0}{}^{e}$ /kJ mol^{-1}	τ_{F} /ns	"Apparent" Φ_{F}
1a	toluene	641 (2.50)	649	8 (192)	185.5 ± 0.4	2.84 ± 0.02	0.29 ± 0.06^{g} (0.048 ± 0.002^{h})
	acetonitrile	634 (2.35)	643	9 (221)	187.4 ± 0.4		0.068 ± 0.018^{g}
1b	toluene	672 (2.75)	678	6 (132)	177.2 ± 0.4	4.22 ± 0.03	0.51 ± 0.06^{i} (0.063 ± 0.002^{h})
	acetonitrile	^{f}	^{f}	^{f}	^{f}	^{f}	^{f}
1c	toluene	682 (2.95)	705	23 (478)	172.5 ± 0.4	6.54 ± 0.07	0.85 ± 0.10^{j} (0.081 ± 0.005^{h})
	acetonitrile	663 (2.80)	675	12 (268)	178.8 ± 0.4		0.21 ± 0.02^{j}
1d	toluene	605 (2.30)	612	7 (189)	196.6 ± 0.4	1.55 ± 0.01	0.13 ± 0.04^{k}
	acetonitrile	593 (1.95)	602	9 (252)	200.2 ± 0.4		0.022 ± 0.007^{k}
2a	toluene	729 (3.00)	750	21 (384)	161.8 ± 0.4	1.04 ± 0.02	0.054 ± 0.010^{l}
	acetonitrile	719 (2.80)	748	29 (539)	163.1 ± 0.4		<0.005^{l}
2b	toluene	707 (2.86)	728	21 (408)	166.7 ± 0.4	<1	0.06 ± 0.02^{m}
	acetonitrile	683 (2.64)	713	30 (616)	171.4 ± 0.4		<0.005^{m}

^{a} Wavelength of absorption band maximum. Error of ± 0.5 nm. ^{b} Molar extinction coefficient, × 10^{-5} L mol^{-1} cm^{-1}, estimated error ± 10%. ^{c} Wavelength of emission band maximum. Error of ± 0.5 nm. ^{d} Stokes shift in both nm and cm^{-1}. The latter has an error of ± 30 cm^{-1}. ^{e} S$_0$-S$_1$ energy. ^{f} Pronounced ground state aggregation. ^{g} Irradiated at 640 nm. ^{h} Irradiated in the UV at 380 nm. ^{i} Irradiated at 672 nm. ^{j} Irradiated at 682 nm. ^{k} Irradiated at 600 nm. ^{l} Irradiated at 730 nm. ^{m} Irradiated at 700 nm.

2. Results and Discussion

2.1. Absorption and Emission Properties. Spectra of all compounds were recorded in both toluene and in acetonitrile, with the exception of **1b** which aggregated in acetonitrile. Pertinent data are summarized in Table 1, and representative spectra are shown in Figure 2 (spectra of all compounds can be found in the Supporting Information).

All compounds are characterized by a pronounced absorption band in the spectral region ~600–730 nm and emission with a very small Stokes shift. These spectral features are not particularly sensitive to the change in solvent and, as such, suggest that the excited state initially populated as well as the excited state that emits do not contain a significant amount of charge-transfer (CT) character (or rather, that the extent of CT
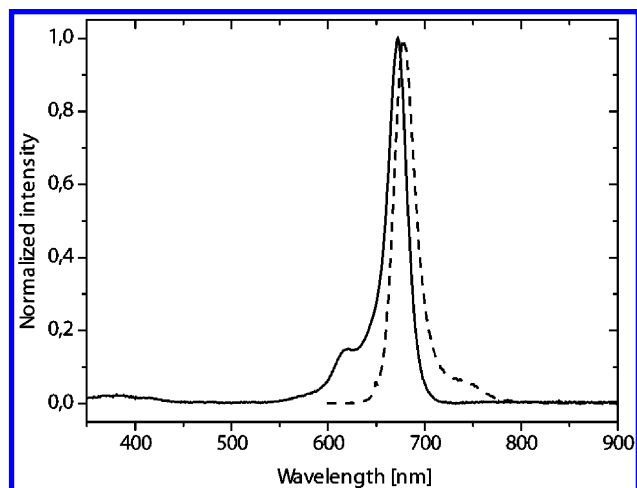
Figure 2. One-photon absorption (solid line) and fluorescence (dashed line) spectra of **1b** in toluene.

Figure 3. The lifetime of the fluorescence state, τ_F, plotted against the fluorescence quantum yield, Φ_F, for the squaraines dissolved in air-saturated toluene.

in these excited states is similar to that in the ground state). Relative to the data from **1a**, the shifts to longer wavelength observed in both the absorption and emission spectra of **1b** are consistent with the extension of the π-conjugated system. Again relative to the data from **1a**, the shifts to longer wavelength observed in the absorption and emission spectra of **1c** reflect the stabilizing effect of the electron-rich sulfur atoms (i.e., lone-pair donating) on the positive charge delocalized along the squaraine backbone, an observation that is consistent with data recorded from similar compounds.[6] The data recorded for the pyrrole derivatives **2a** and **2b** are consistent with the further introduction of mild electron donating and electron withdrawing groups (hydrazone and quinoline moieties, respectively) on the ends of the molecules. The shifts to a shorter wavelength observed for the data from **1d** are consistent with the loss of symmetry in the system, as previously described.[7]

The extensive spectral overlap between the absorption and emission profiles indicates that these molecules are susceptible to the effects of reabsorption (i.e., the so-called inner-filter effect) where a fraction of the emitted light will be reabsorbed by the sample.[23] This phenomenon can significantly influence attempts to measure emission quantum yields (vide infra).

2.2. Fluorescence Yields and Lifetimes. Given the spectral overlap between the pronounced absorption band in the visible region of the spectrum and the fluorescence band, we chose to monitor key photophysical properties upon excitation of the squaraine in the UV ($\sim$380–400 nm) and, independently, upon excitation into the distinct visible band ($\sim$600–730 nm). However, with the comparatively small molar absorption coefficient in the UV (e.g., see Figure 2), it was necessary to perform the UV experiments using a higher concentration of the squaraine to obtain an appreciable absorbance. It is important to note that, in these experiments, although the normalized emission intensities thus recorded depended on the excitation wavelength (vide infra), the emission spectrum was unaffected. Thus, irrespective of the excitation wavelength, emission originates from the lowest vibrationally relaxed singlet state (i.e., we observe no deviation from Kasha's rule).

Not surprisingly, the fluorescence quantum yield (Φ_F) obtained upon UV irradiation was significantly smaller than that obtained upon visible irradiation of the same squaraine (Table 1). This reflects the fact that, as a consequence of the small Stokes shift, emitted light will be reabsorbed by surrounding ground-state molecules and that such reabsorption will be more pronounced for the system with the higher squaraine concentra-
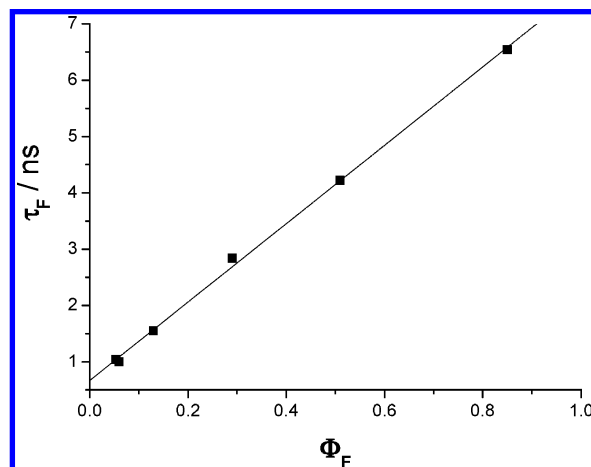
tion (i.e., the UV experiments). Under these latter conditions, less fluorescent light will escape the sample cuvette to be detected by the spectrometer. On this basis, the more accurate Φ_F data are obtained following excitation of the squaraine in the visible region of the spectrum.

It follows from this discussion that it is more appropriate to refer to an "apparent" fluorescence quantum yield. Even upon irradiation in the visible region of the spectrum, some of the emitted light will be reabsorbed and the measured value of Φ_F will be artificially low.

The Φ_F data also depend significantly on the solvent (Table 1). Specifically, despite minor solvent-dependent changes in the spectra (vide supra), the fluorescence quantum yields decrease substantially when the solvent is changed from toluene to acetonitrile. Such data are consistent with a model in which (1) the Franck–Condon excited state initially populated upon light absorption can relax to produce an emitting state as well as a lower-energy "dark state" (i.e., one that does not emit), (2) the dark state has appreciable CT character which, in more stabilizing polar solvents, can better facilitate nonradiative deactivation to the ground state, and (3) the dark state plays an increasingly greater role as the solvent becomes more polar.[24] It is reasonable to associate this dark state with a "twisted internal charge transfer" state (so-called TICT state).[5,25] The increase in Φ_F for the series **1a** → **1b** → **1c** may reflect an increased structural rigidity that could inhibit the relaxation process that populates the dark CT state.

To complement the Φ_F data, we recorded lifetimes of the fluorescent state, τ_F, for the respective squaraines (Table 1). In all cases, the kinetic traces could be accurately modeled with a single exponential decay function. For the squaraines in toluene, the fluorescence lifetimes follow the same trend as the fluorescence quantum yield. Indeed, a plot of τ_F against Φ_F yields a straight line (Figure 3). These data indicate that, despite the structural differences, the rate constant for radiative deactivation of the emitting state, k_r, is similar in each of these molecules (i.e., $k_r = \Phi_F/\tau_F$).

Attempts to record values of τ_F in acetonitrile were hampered by (1) weak signal intensities and (2) comparatively fast decay kinetics. This is fully consistent with the solvent-dependent increase in the extent to which the CT-dominated "dark" state will play a role in the overall relaxation dynamics of these squaraines (vide supra).

Thus far, we have focused on the role that an intramolecular CT state plays in unimolecular excited state relaxation dynamics.

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TABLE 2: Sensitized Singlet Oxygen Yield, Φ_Δ(air), Squaraine Triplet State Yield in the Absence, $\Phi_T(N_2)$, and Presence of Air, Φ_T(air), and the Fraction of Triplet States Quenched by Oxygen That Yield Singlet Oxygen, S_Δ^T, in Solutions of Toluene

compound	Φ_Δ(air)	$\Phi_T(N_2)$	Φ_T(air)	S_Δ^T
1a	0.018 ± 0.004 (UV)[a] 0.010 ± 0.004 (Vis)[b]	0.022 ± 0.008	0.045 ± 0.016	0.40 ± 0.23
1b	0.021 ± 0.008 (UV)[a] 0.006 ± 0.001 (Vis)[b]	0.024 ± 0.009	0.06 ± 0.03	0.35 ± 0.30
1c	0.05 ± 0.02 (UV)[a] 0.012 ± 0.004 (Vis)[b]	0.020 ± 0.004	0.13 ± 0.03	0.38 ± 0.24
1d	<0.005[c]			
2a	<0.005[b]			
2b	<0.005[b]			

[a] Φ_Δ data obtained upon irradiation at 395 nm, whereas Φ_T data obtained upon 355 nm irradiation. [b] Irradiated at 655 nm. [c] Irradiated at 592 nm.

However, the magnitude of the τ_F values recorded in air-saturated toluene clearly indicates that a non-negligible amount of squaraine singlet state deactivation proceeds by a bimolecular oxygen-induced channel (i.e., in the absence of air, τ_F is sufficiently long and, upon the introduction of air, quenching by oxygen will occur to yield the values shown in Table 1). If we make the reasonable assumption that proclivity to form an intramolecular CT state will likewise be reflected in the proclivity to form an intermolecular CT state with oxygen, then our discussion is readily extended to corresponding transitions in the oxygen-squaraine complex.[26–28] This point is amplified below.

2.3. Singlet Oxygen Quantum Yields (Φ_Δ). The quantum yield of photosensitized singlet oxygen production, Φ_Δ, can be expressed as a sum of two terms: (1) a term that reflects the amount of singlet oxygen produced upon quenching of the sensitizer S_1 state by ground state oxygen, and (2) a term that reflects the amount produced upon quenching of the sensitizer T_1 state by ground state oxygen (eq 1).

$$\Phi_\Delta = S_1 \text{ quenching} + T_1 \text{ quenching} \quad (1)$$

Here, S_1 denotes the lowest-energy excited state with singlet spin multiplicity (i.e., generally the fluorescent state in organic molecules), and T_1 denotes the lowest energy state with triplet spin multiplicity (i.e., the phosphorescent state).

The quenching of S_1 by oxygen generally results in inter-system crossing to produce the T_1 state.[29–31] If the $S_1–T_1$ energy gap is greater than the excitation energy of singlet oxygen, then electronic energy transfer to oxygen can occur.[32] For some molecules, oxygen-induced $S_1–S_0$ internal conversion can also result in singlet oxygen production.[33]

Each of the terms in eq 1 can be expressed as the product of three parameters: (1) the quantum yield for the production of that respective state, Φ, (2) the fraction of those states quenched by oxygen, f, and (3) the fraction of these latter quenching events that lead to singlet oxygen production, S_Δ. In this way, we can rewrite eq 1 to yield eq 2.

$$\Phi_\Delta = \Phi_S f_S S_\Delta^S + \Phi_T f_T S_\Delta^T \quad (2)$$

From this description, it should be clear that, among other things, Φ_Δ will depend on the oxygen concentration in the given system being examined.

Experiments were performed in air-equilibrated toluene using UV and, independently, visible light for excitation. The Φ_Δ values for squaraine-sensitized singlet oxygen production thus obtained are shown in Table 2 and, again, as with the Φ_F data,

it should be clear that we imply an "apparent" quantum yield. For molecules **1d**, **2a**, and **2b** the singlet oxygen phosphorescence signals were very weak, yet detectable, and the estimated upper limit of Φ_Δ for these compounds is 0.005 in toluene. In acetonitrile, all compounds gave $\Phi_\Delta < 0.005$ [In acetonitrile, Φ_Δ data were not recorded for **1b**, which aggregated, and **1c**, which was not photochemically stable (see Supporting Information)].

These Φ_Δ data are consistent with the Φ_F data presented in the previous section, and can likewise be interpreted in the context of (1) fluorescence emission/reabsorption and (2) a solvent-dependent "dark" state with substantial CT character. Here, we find that Φ_Δ obtained upon UV excitation is appreciably larger than that obtained upon excitation in the visible region of the spectrum. This is the expected result when fluorescent photons can be reabsorbed to produce additional singlet oxygen precursors. Indeed, note that the molecule with the largest Φ_F, **1c**, also produces singlet oxygen with the greatest efficiency upon UV irradiation. As such, it appears that there is a self-promoting amplification of singlet oxygen generation that derives from the inner filter effect. In the least, these wavelength-dependent values of Φ_Δ do not appear to reflect the effects of a more fundamental molecular phenomenon such as the wavelength dependent population of different states that, in turn, produce singlet oxygen with a different efficiency.[34,35] Also, and as expected, the Φ_Δ values are systematically lower in acetonitrile, a solvent in which CT-mediated excited state deactivation will kinetically compete with energy transfer to ground state oxygen and, as such, adversely effect singlet oxygen production.[26,27]

The Φ_Δ data indicate that these squaraine dyes are poor singlet oxygen photosensitizers. This is a reasonable, indeed expected, observation when the photosensitizer and/or the sensitizer-oxygen complex are characterized by appreciable CT character (i.e., intra- and intermolecular CT, respectively).[26,27,32,36–39]

Singlet oxygen quantum yields have previously been reported for two of the squaraines examined in the present study (**2a** and **2b**)[15] and for a subtle variant of a third (**1c**).[19,40] In the latter case, the molecule previously studied had *n*-hexyl appendages on the nitrogen atom, whereas our molecule, **1c**, has *n*-octyl appendages. We expect that these structural differences will be of no consequence. The previous experiments were performed in different air-equilibrated solvents using different experimental procedures, and Φ_Δ values of 0.26 ± 0.02 for the **1c** variant in CH_2Cl_2,[19,40] 0.16 ± 0.04 for **2a** in CS_2,[15] and 0.33 ± 0.06 for **2b** in CS_2[15] were reported. Clearly, these numbers are significantly larger than what we report in Table 2 for our time-resolved experiments performed in toluene.

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To help ascertain the origin of the differences in these Φ_Δ values, we performed selected experiments under conditions that better approximated those used in the earlier studies. This included using different excitation laser systems (i.e., nanosecond vs femtosecond pulses) and different near IR detectors to monitor singlet oxygen phosphorescence (i.e., Ge semiconductor detector vs PMT, see the Experimental Section). Under all conditions, we were unable to reproduce the previously published data. Rather, our results were always consistent with those reported in Table 2. A key feature of these earlier studies was that irradiation was always in the UV region of the spectrum, not in the visible region where the squaraines have a prominent absorption band. Given the available information about these published studies, we can only surmise that unreasonably high concentrations of the squaraines were used in order to obtain an appreciable absorbance at the excitation wavelength. In turn, one could then be adversely influenced in a number of ways: First, by sample fluorescence and light reabsorption which, as we have already described, will give an abnormally large signal and, at the same time, by appreciable quenching of singlet oxygen by the squaraine itself (vide infra, Section 2.6). Moreover, for some of our experiments performed under these latter conditions of a high squaraine concentration, our luminescence detectors saturated (either from intense fluorescence or scattered excitation light) giving a false signal that one might incorrectly assign to singlet oxygen. In conclusion, we believe that the previously published Φ_Δ data are characterized by artifacts and we stand by the accuracy of our present results.

2.4. Triplet State Yields. To further elucidate details of the squaraine sensitized production of singlet oxygen, we used a method developed by Wilkinson and co-workers[41,42] by which triplet state yields can be quantified. This approach is based on the fact that many quenchers of the S_1 state specifically induce intersystem crossing to yield the T_1 state. By monitoring quencher-dependent changes in the fluorescence intensity along with corresponding quencher-dependent changes in the amount of triplet state formed using, for example, time-resolved absorption techniques, one can obtain quantum yields for triplet state production.

From our present perspective, it is important to recognize that one efficient quencher of the S_1 state is oxygen. Furthermore, oxygen-induced deactivation of the S_1 state generally proceeds via intersystem crossing to produce the T_1 state.[29–31] Admittedly, it has recently been shown that, for some molecules, oxygen-induced S_1 deactivation can proceed via $S_1 \rightarrow S_0$ internal conversion.[33] Nevertheless, given the latter caveat, it is still instructive to proceed with the Wilkinson approach for the present work on the squaraines.

Triplet state quantum yields, Φ_T, thus obtained are shown in Table 2 (pertinent data are shown in the Supporting Information). Given the method employed, the assumptions made, and the fact that the signals monitored were comparatively weak, the data are characterized by large error bars. A clear and unambiguous conclusion can nevertheless be drawn; for the three pertinent squaraines, the triplet yield is not large. Moreover, the relative magnitude of the singlet oxygen quantum yield, determined in an independent experiment under air-saturated conditions (vide supra), is consistent with the corresponding value of the triplet state quantum yield (i.e., $\Phi_\Delta < \Phi_T$).

From the time-resolved triplet state absorption experiments, we ascertained that, for the three squaraines examined, T_1 is quantitatively quenched by ground state oxygen in an air-saturated solution (i.e., $f_T = 1$ in eq 2). If we make the reasonable assumption that the only source of singlet oxygen in this system
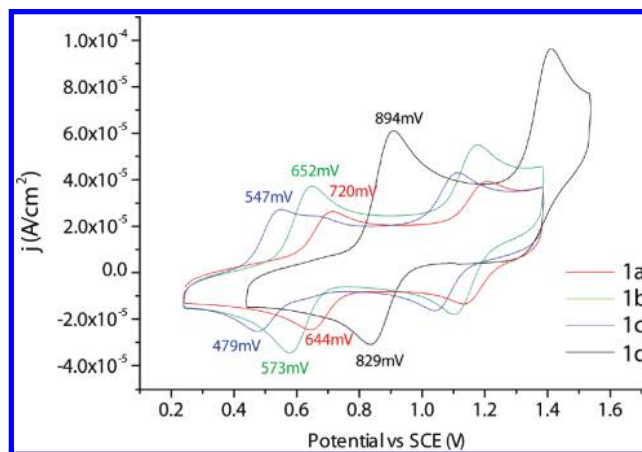

Figure 4. Cyclic voltammograms for squaraines **1a–d** recorded in CH_2Cl_2.

is the squaraine triplet state (i.e., oxygen quenching of the S_1 state does not produce singlet oxygen), then we can comment further on the efficiency of singlet oxygen production, S_Δ^T, in the interaction between T_1 and $O_2(X^3\Sigma_g^-)$. Specifically, S_Δ^T is easily obtained using the expression $S_\Delta^T = \Phi_\Delta/\Phi_T$ (eq 2). The results of this latter exercise (Table 2) clearly demonstrate that the squaraine triplet states do not produce singlet oxygen with high efficiency. When considering these S_Δ^T values, it is important to recognize that they were obtained from experiments with UV excitation. We assume that the amplification effects ascribed to emission/reabsorption (vide supra) equally apply to both Φ_Δ and Φ_T and, thus, should cancel in the ratio Φ_Δ/Φ_T. (If some singlet oxygen were actually produced by the S_1 state, then the S_Δ^T values shown would only be an upper limit to the efficiency of singlet oxygen production from the triplet state.)

In conclusion, the results of these Φ_T studies are entirely consistent with our Φ_Δ data and with our model that points to a squaraine-oxygen CT state that adversely affects the squaraine-sensitized production of singlet oxygen.

2.5. Electrochemical Characterization. If there is indeed an appreciable amount of CT character in a given squaraine-oxygen complex, it is incumbent upon us to confirm that a given squaraine can readily donate an electron or appreciable charge to oxygen. To this end, we set out to examine oxidation potentials for these molecules. Pertinent data have been published for **2a** and **2b**,[15] but it was necessary to record cyclic voltammograms for **1a–1d** (Figure 4). The data thus obtained are shown in Table 3.

Squaraines **1a–d** show two redox waves at positive potentials. For **1a–c**, these correspond to the oxidation of the enaminic moieties, whereas for **1d** they correspond to the oxidation of the enaminic moiety and, independently, of the pyrrole moiety. Likewise, the cyclic voltammograms of **2a** show two positive potential waves related to the oxidations of the two hydrazonic moieties, while compound **2b** shows an irreversible wave derived from the oxidation of the pyrrole moiety.[15]

The most important and pertinent feature of the data shown in Table 3 is that, for all compounds, the first oxidation potential is comparatively low. Thus, the squaraine-oxygen CT state formed as a consequence of electron/charge transfer from the squaraine to oxygen should be comparatively low in energy and, as such, contribute appreciably to the photophysics of this system. In short, it is indeed reasonable to conclude that both intra- and intermolecular CT-mediated excited state deactivation kinetically competes with energy transfer from the squaraine to produce singlet oxygen.

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TABLE 3: Half-Wave Potentials ($E_{1/2}$), Associated Peak Separation Values (ΔEp), and the Peak Separation between the First and the Second Redox Processes (ΔE), for the Squaraines Studied

compound[a]	$E_{1/2}(1)$/V	$\Delta Ep(1)$/mV	$E_{1/2}(2)$/V	$\Delta Ep(2)$/mV	ΔE/V
1a	0.68 ± 0.01	76	1.17 ± 0.01	71	0.49
1b	0.61 ± 0.01	79	1.14 ± 0.01	70	0.53
1c	0.51 ± 0.01	68	1.08 ± 0.01	69	0.57
1d	0.86 ± 0.01	65	1.41 ± 0.01	irreversible process	0.55
2a	0.18 ± 0.01	65	0.46 ± 0.01	60	0.28
2b	0.55 ± 0.01				

[a] Data for **1a**−**d** were recorded in CH_2Cl_2 with Tetrabutylammonium hexafluorophosphate as the supporting electrolyte. Data for **2a**−**b** were recorded previously in CH_3CN with Tetrabutylammonium *p*-toluenesulphonate as the supporting electrolyte.[15]

2.6. Singlet Oxygen Quenching by the Squaraine Ground Electronic State. It is well established that molecules with a comparatively low oxidation potential are generally excellent quenchers of singlet oxygen, and that the quenching mechanism involves deactivation mediated by an intermolecular CT state between oxygen and the given molecule (i.e., $Q^{\bullet+} \cdots O_2^{\bullet-}$).[32,43–46] On the basis of our preceding discussion, one might thus expect that these squaraines would be effective quenchers of singlet oxygen. To this end, and to further substantiate our model of oxygen-dependent squaraine photophysics, we set out to quantify rate constants for singlet oxygen removal, k_q, by our squaraines.

In these experiments, singlet oxygen was produced by irradiating an independent photosensitizer, perinaphthenone, at a wavelength and with solute concentrations such that the incident light would not be absorbed by the squaraine. Using the time-resolved 1270 nm phosphorescence of singlet oxygen as a probe, singlet oxygen lifetimes, τ_Δ^Q, were then monitored as a function of the squaraine concentration. The first-order rate constants thus obtained (i.e., $1/\tau_\Delta^Q$) were plotted against the concentration of added squaraine to yield the bimolecular rate constant for singlet oxygen removal (Figure 5, Table 4).

The large values of k_q obtained along with the associated toluene/acetonitrile solvent effect are indeed consistent with our expectation, and provide further support for our model of a squaraine-oxygen complex characterized by appreciable CT character. Our data are also consistent with k_q values reported for other aromatic amines and enamines for which a CT-based quenching mechanism has likewise been proposed.[46]

3. Experimental Section

3.1. Spectroscopic and Photophysical Measurements. All steady-state UV/vis absorption spectra were recorded using either a Hewlett-Packard/Agilent diode array HP8453 spectrometer or a Shimadzu UV3600 spectrophotometer. All of the steady-state emission spectra were recorded using a Horiba Jobin Yvon fluorimeter (Fluoromax P).

Fluorescence quantum yields (Φ_F) obtained upon irradiation in the visible region of the spectrum were determined in steady-state experiments relative to a standard, zinc-phthalocyanine in pyridine, for which $\Phi_F = 0.30 \pm 0.03$.[47] For experiments performed upon UV irradiation, an air-saturated solution of 9,10-dicyanoanthracene in toluene was used as the reference ($\Phi_F = 0.69 \pm 0.05$[48]). Using standard procedures,[23] the integrated fluorescence intensities were recorded as a function of sample absorbance, and we accounted for differences in the light collection efficiency that arise from differences in the solvent refractive index.[49] The spectral bandwidth of the excitation wavelength was ~2 nm fwhm, reflecting the slit widths of spectrometer. This latter parameter is particularly important in this study considering the spectral overlap between the emission and absorption profiles.
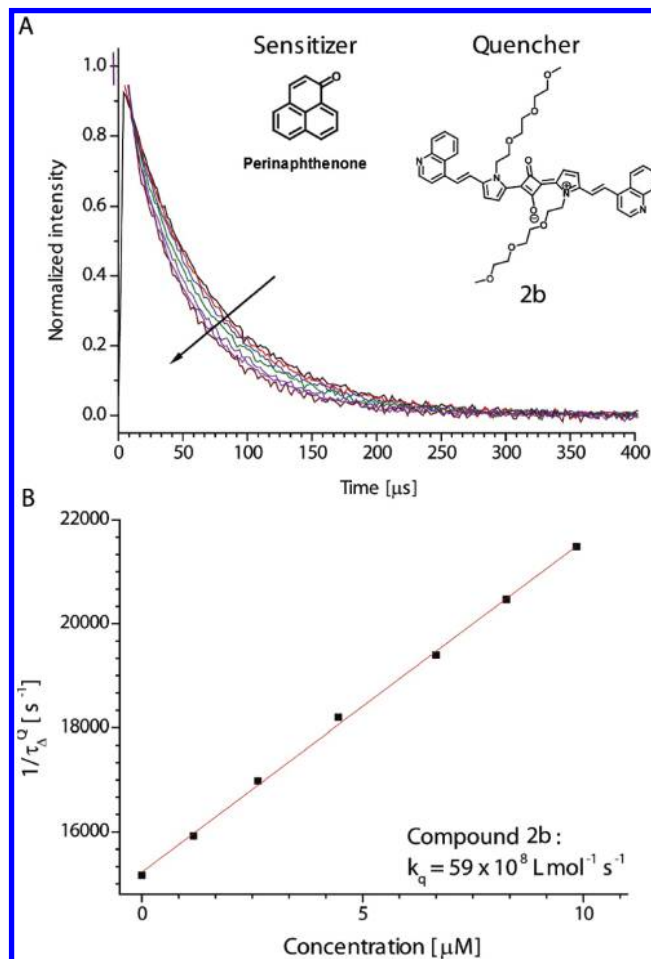

Figure 5. (A) Representative time-resolved decay traces of singlet oxygen phosphorescence upon 355 nm irradiation of an air-equilibrated acetonitrile solution of perinaphthenone with different concentrations of the squaraine **2b**. The superimposed arrow indicates an increase in the concentration of added **2b**. (B) Representative plot of the reciprocal singlet oxygen lifetime in the presence of the squaraine, $1/\tau_\Delta^Q$, against the concentration of added squaraine.

For fluorescence lifetime experiments, the 790 nm output of a femtosecond Ti:sapphire laser (Spectra Physics, Tsunami 3941) was amplified by a regenerative amplifier (Spectra Physics Spitfire pumped by a Spectra Physics Evolution Nd:YLF laser) and frequency-doubled to 395 nm by a BBO crystal for sample irradiation. The luminescence from the sample, collected at 90° to the excitation direction, was passed through a suitable interference filter positioned in front of a fast photodiode (Newport model 818-BB-21, 300 ps rise time) connected to a digital oscilloscope (Tektronix TDS754A). The collection efficiency was enhanced by a parabolic mirror placed on the backside of the cuvette.

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TABLE 4: Rate Constants for the Quenching of Singlet Oxygen, k_q, by the Squaraine Dyes in Air-Saturated Toluene and Acetonitrile

solvent	compound[a]					
	1a	**1b**	**1c**	**1d**	**2a**	**2b**
toluene	5.0	4.0	3.0	5.9	35	37
acetonitrile	8.1	[b]	[c]	21	52	59

[a] Reported as $\times~10^8$ L mol^{-1} s^{-1} with an error of $\pm$ 10%. [b] Pronounced ground-state aggregation of the squaraine. [c] System not stable upon irradiation (see Supporting Information).

Singlet oxygen quantum yields, Φ_Δ, were determined using a general approach that has previously been described.[50,51] However, in this case, experiments were performed using an fs laser system that has likewise been previously described.[52,53] Briefly, air-equilibrated solutions containing the sensitizers were irradiated using an fs laser, the output wavelength of which was tuned to the desired excitation wavelength. For experiments performed in the visible region of the spectrum, 2,3,7,8,12,13,17,18-octaethyl-porphyrin in toluene ($\Phi_\Delta = 0.75 \pm 0.05$), 5,10,15,20-tetraphenyl-21H,23H-porphine in toluene ($\Phi_\Delta = 0.69 \pm 0.05$), or zinc-phthalocyanine in pyridine ($\Phi_\Delta = 0.50 \pm 0.05$) were used as the reference standards.[54] For experiments performed with UV excitation, perinaphthenone dissolved in toluene, $\Phi_\Delta = 0.95 \pm 0.05$,[55] was the standard. The 1270 nm singlet oxygen phosphorescence emitted from the sample was isolated by an interference filter and detected by a liquid nitrogen cooled near-IR photomultiplier tube (Hamamatsu model R5509−42) used in a time-resolved photon counting mode. In all experiments, the singlet oxygen lifetimes thus obtained were consistent with expectation (e.g., ~30 μs in toluene and ~70 μs in acetonitrile[56]).

Rate constants for singlet oxygen quenching (k_q) were obtained upon irradiation of the sensitizer, perinaphthenone, using the 355 nm third harmonic of an ns Nd:YAG laser (Quanta-Ray GCR 230). The singlet oxygen phosphorescence emitted was detected by a liquid nitrogen cooled germanium detector (North Coast EO-817P) positioned at a 90° angle to the laser beam. To enhance the signal collection efficiency, a parabolic mirror was positioned at the backside of the cuvette. To spectrally isolate the desired phosphorescence signal, an Si-filter (CVI optics) and a 1270 nm bandpass filter (50 nm fwhm) were placed in front of the detector.

Squaraine triplet state kinetics and yields were studied in a pump−probe experiment using instruments and an approach that have likewise been previously described.[50,52] Briefly, a 5×10^{-5} M solution of the squaraine was irradiated at 355 nm. The absorption of the resultant triplet state was probed using the output of a steady-state 200W xenon lamp that had been passed through a water filter to remove the IR-components. The light from the xenon lamp transmitted through the sample was dispersed by a grating onto a PMT (Hamamatsu R928). Additionally, a filter was inserted to remove residual scattered laser light. The resulting time-resolved signal was digitized and monitored with an oscilloscope (Tektronix TDS5032B). Changes in the amount of transmitted light were monitored at 530 nm where the squaraine triplet state absorbance is strong. Samples for measurements performed in the absence of oxygen were prepared by gently bubbling nitrogen through the solution for ~30 min.

3.2. Electrochemical Measurements. Cyclic voltammograms of **1a−d** were recorded in CH_2Cl_2 using a squaraine concentration of 10^{-4} M with 0.1 M tetrabutylammonium hexafluorophosphate as the supporting electrolyte. Experiments were performed at a scan rate of 50 mV/s. Data were recorded using the ferrocene/ferricenium ion couple as a standard, and then converted to the SCE scale. Although the peak separation values, ΔEp, are slightly larger than the 60 mV expected for a reversible process, these ΔEp values could reflect the unique ohmic drop in CH_2Cl_2. In any event, values of $E_{1/2}$ were measured as a midpoint between the peak potentials.

3.3. Synthesis and Materials. Toluene and acetonitrile (Sigma-Aldrich, spectroscopic grade) were used as received.

Squaraines **1a−b** were obtained by microwave-assisted condensation starting from the appropriate heteroaryl anhydro-base precursor, imidazole, and squaric acid in a *i*-PrOH/toluene mixture for 2−4 h. Squaraines **1c** and **1d** were prepared following the methods described by Santos et al.[40] and by Treibs et al.,[57] respectively. Derivatives **2a−b** were obtained according to a published procedure from our laboratory.[15] Pertinent details are provided in the Supporting Information.

4. Conclusions

We have examined the photophysics of selected squaraines with respect to the squaraine-sensitized production of singlet oxygen and, independently, the squaraine-induced deactivation of singlet oxygen. We find that these molecules are poor singlet oxygen sensitizers, but effective singlet oxygen quenchers. These results are consistent with an appreciable amount of (1) intramolecular CT character in the squaraine and (2) intermolecular CT character in the squaraine-oxygen complex. Specifically, the behavior of these squaraines conforms to an established model in which a CT state facilitates excited state deactivation. In one case, this adversely affects the energy transfer process from the excited state squaraine to produce singlet oxygen. In the other case, such CT character promotes the squaraine-dependent deactivation of singlet oxygen.

Moreover, we have provided evidence for a photophysical phenomenon which, to our knowledge, has not previously been demonstrated in a singlet oxygen system. Specifically, we find that the squaraine-sensitized yield of singlet oxygen depends on the irradiation wavelength, and that this effect reflects light emission and reabsorption by the squaraine. In short, there is a self-promoting amplification of singlet oxygen generation that derives from the inner filter effect. Upon careful design and/or modification of a given sensitizer system, this phenomenon could be used to one's advantage in a number of applications.

Finally, we have demonstrated that these selected squaraines are not likely to be useful as PDT agents whose behavior derives from the effects of singlet oxygen. Rather, if such squaraines can indeed induce oxygen-dependent cell death upon irradiation, the mechanism of such cell death will more likely be a result of an electron transfer reaction.

In conclusion, we have demonstrated that squaraines provide a photosystem that have unique behavior with respect to the sensitized production and subsequent deactivation of singlet oxygen. We anticipate that, with forethought, these features can be exploited in the design of a new generation of oxygen-responsive molecules.

Supporting Information Available: Synthesis and characterization of squaraines **1a−d**, absorption and fluorescence spectra of all squaraines, time-resolved fluorescence data used to evaluate singlet state lifetimes, representative data used to

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quantify triplet state yields, and data illustrating the photoin-stability of **1c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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