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Spectroscopy and photophysics of flavin-related compounds: 3-ethyl-lumiflavin

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Abstract

Electronic structure and singlet–singlet and triplet–triplet absorption spectra of 3-ethyl-lumiflavin were calculated using time-dependent density functional theory (TD-DFT) methods. The measured lower-energy transitions are well reproduced in calculations, which are limited by the neglect of the solvent interactions. All the observable singlet–singlet and triplet–triplet transitions have π–π^* character. Singlet oxygen production by the studied compound demonstrated that, similar to other lumiflavins, it is an efficient singlet oxygen sensitizer ($\phi_\Delta = 0.55$). Radiationless deactivation of the S_1 state in solutions was shown to result in the T_1 state formation.

Keywords: TD-DFT; 3-Ethyl-lumiflavin; Absorption

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1. Introduction

Flavins play an important role in living organisms. Flavin photochemistry has been the subject of intense research over the years. The parent molecule from which all other variants derive, e.g. vitamin B2 (riboflavin), flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), is lumiflavin (7,8,10-trimethyl-10*H*-benzo[*g*]pteridine-2,4-dione), see Fig. 1. Flavins possess the yellow chromophore characteristic of flavoproteins—enzymes occurring widely in animals and plants. Since the discovery of the old yellow enzyme some 70 years ago, it has become clear that flavoproteins are involved in a very wide range of biological processes. Studies of the photochemistry and photophysics of flavins were prompted in part by the proposed involvement of flavin excited states in a number of important photobiological and photochemical processes, such as phototropism, phototaxis, and photodynamic action. Flavoenzymes are among the most structurally and functionally diverse families of redox proteins. They catalyse an enormous range of biotransformations and electron transfer processes using a single redox unit, the flavin. These redox events can have either one- or two-electron mechanisms. Since the volume of data on ground and excited state properties of flavins is overwhelming, we will refer only to the symposium series, entitled Flavins and Flavoproteins [1], which illustrates both the wealth of the available information and the progress that has been made in the photochemistry, structure and functionality of flavins.

In our past studies we were interested in the effects of the nature and position of substituents and on the spectroscopy

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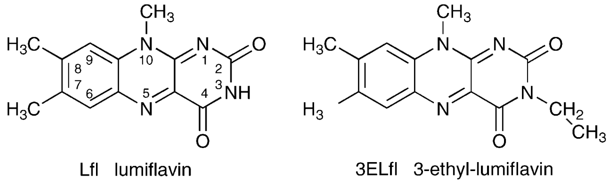

Fig. 1. Molecular structure of lumiflavin and 3-ethyl-lumiflavin.

and photophysics of alloxazines. However, recently we have begun studies of flavins also focused on their spectral and photophysical properties [2–4]. Our general aim is to characterise and reconcile the diverse photophysical and spectroscopic properties of flavins in solution, with special attention to singlet oxygen quantum yields with different flavins as photosensitizers. This paper describes steady-state and time-resolved studies of the ground and excited singlet states of 3-ethyl-lumiflavin. The present investigation was undertaken in order to supply an insight into the photophysics of 3-ethyl-lumiflavin in solution, and to correlate these with its electronic structure calculated based on time-dependent density functional theory (TD-DFT) [5]. To the best of our knowledge, there are no published TD-DFT predictions of electronic spectra for 3-ethyl-lumiflavin, and no data on the triplet–triplet absorption, while the information about its singlet excited states is also very limited. The molecular structures of 3-ethyl-lumiflavin together with its parent molecule, lumiflavin, are presented in Fig. 1.

2. Experimental

The solvents acetonitrile, 1,2-dichloroethane, dioxane and methanol, all from Aldrich, were used as received. The title compound was available from previous work [6].

UV–vis absorption spectra were recorded on a Varian Cary 5E spectrophotometer. Steady-state fluorescence excitation and emission spectra were recorded on a Jobin Yvon-Spex Fluorolog 3-11 spectrofluorometer, with correction for instrumental factors by means of a Rhodamine B quantum counter and correction files supplied by the manufacturer. Fluorescence quantum yields were measured relative to quinine sulphate in 0.1N H_2SO_4 ($\phi_F = 0.52$) as standard [7]. Fluorescence lifetime measurements were performed with an IBH (model 5000U) single-photon timing instrument using excitation at 355 nm. A hydrogen-filled nanosecond flashlamp has been used as an excitation source and the analysis software supplied by the manufacturer has been applied. Theoretical equations were fitted to experimental data by means of a nonlinear weighted least-squares routine based on the Marquardt algorithm. All experiments were carried out at room temperature.

Transient absorption measurements were made using nanosecond laser flash photolysis systems available in Barcelona. The LKS60 instrument from Applied Photophysics was used: the third harmonic (355 nm) of a Q-switched Nd:YAG laser (Spectron Laser Systems, UK; pulse width ca. 9 ns) was employed for laser flash excitation.

Singlet oxygen luminescence experiments were carried out by excitation of the sample with the third harmonic (355 nm) of a Nd:YAG laser (Lumonics hyperYAG HY200, 8 mJ per pulse, ca. 8 ns FWHM). The excitation energy was attenuated using solutions of sodium nitrite in water. Emission at 1270 nm from singlet oxygen was measured with liquid-nitrogen-cooled *North Coast Scientific* EO-980P Ge photodiode detector. The luminescence was detected after passing through *Melles Griot* interference filter and data capture was with a 250MS/s digitising oscilloscope (Tektronix 2432A). Perinaphthenone (Aldrich) was used as a reference for singlet oxygen quantum yield, $\phi_\Delta = 0.95 \pm 0.05$ [8].

TD-DFT [5] calculations were carried out in order to predict electronic structure of 3-ethyl-lumiflavin using the B3LYP functional [9] in conjunction with a modest 6-31G(d) split-valence polarised basis set [10]. The optimised ground-state geometries have been used in calculations of excitation energies and transition intensities. Oscillator strengths were calculated in the dipole length representation. Calculations were performed using the Gaussian 98 package of ab initio programs [11]. Excitations from the lowest triplet state of 3-ethyl-lumiflavin were calculated using the unrestricted formalism (UB3LYP/6-31G(d)). The excitation energies computed using TD-DFT/6-31G(d) method are estimated to be accurate within 2000–3000 cm^{-1}, usually requiring a shift towards the red to reproduce experimental spectra. However, regarding the quality of our spectral predictions it should be noted that the difference in the experimental transition energies in 1,4-dioxane solution between lumiflavin and lumichrome (22,680 and 26,390 cm^{-1}) is reproduced in the calculations (24,500 and 27,800 cm^{-1}) to within 500 cm^{-1} [12], with the shift of predicted values as compared to the experimental ones to the blue by about 2000 cm^{-1}. The T–T excitation energies and transition intensities were determined for the optimised geometry of the lowest triplet state (T_1).

3. Results and discussion

Table 1 presents a summary of photophysical parameters of 3-ethyl-lumiflavin in methanol, including the wavelengths of the absorption and emission maxima, fluorescence quantum yields and lifetimes, and the radiative and non-radiative decay constants for the lowest excited singlet state calculated respectively as $k_r = \phi_F/\tau_F$, and $\sum k_{nr} = (1 - \phi_F)/\tau_F$. Here, k_r is the radiative decay rate constant of the excited species and $\sum k_{nr}$ is the sum of all first order and pseudo-first order rate constants for its non-radiative decay. The sum, $\sum k_{nr}$, may include contributions from the pseudo-first order concentration quenching and oxygen quenching of the excited species. 3-Ethyl-lumiflavin exhibits absorption spectra with several major bands in the UV–vis range, typical for flavins, with two characteristic bands at about 28,400 cm^{-1} (352 nm) and 22,400 cm^{-1} (446 nm) in methanol solutions, see Fig. 2.

Table 1
Spectroscopic and photophysical data for the singlet state of 3-ethyl-lumiflavin in different solvents

Solvent	λ_2 (nm)	λ_1 (nm)	λ_F (nm)	ϕ_F	τ_F (ns)	k_r (10^8 s^{-1})	Σk_{nr} (10^8 s^{-1})
Dioxane	334	443	527	0.14	–	–	–
1,2-Dichloroethane	344	448	531	0.12	6.5	0.18	1.4
Acetonitrile	341	445	530	0.14	7.0	0.20	1.2
Methanol	350	446	532	0.11	6.3	0.17	1.4

λ_1, λ_2 are the positions of the two lowest-energy bands in the absorption spectra, λ_F the fluorescence emission maximum, ϕ_F the fluorescence quantum yield, τ_F the fluorescence lifetime, k_r the radiative rate constant and $\sum k_{nr}$ the sum of nonradiative rate constants. The estimated relative error of ϕ_F and τ_F is 10%.

Typical fluorescence emission spectra of 3-ethyl-lumiflavin excited at 355 nm show a single broad band, the exact position depending slightly on the solvent. For example, the fluorescence maxima of 3-ethyl-lumiflavin are located at about 18,900 cm^{-1} (529 nm) and 18,800 cm^{-1} (532 nm) in acetonitrile and in methanol, respectively.

The absorption and the corrected fluorescence excitation spectra agree well with one another. The fluorescence decays are modelled well by single-exponential functions, as shown by the usual statistical goodness-of-fit criteria.

Spectral and photophysical properties of 3-ethyl-lumiflavin presented in Table 1 and Fig. 2 show essentially the same characteristics as lumiflavin and 3-methyl-lumiflavin [2,12]. Many studies have shown that hydrogen bonding by solvent to N(3)–H perturbs the electronic structure of the lumiflavin. For example, it has been shown that hydrogen bonding to N(3)–H can modulate the reduction potential for the reduction of flavin to the radical anion [13]. Methyl or ethyl group at this position prevents such an interaction, additionally improving the solubility in organic solvents.

The absorption spectrum of 3-ethyl-lumiflavin shows two characteristic bands at about 28,400 cm^{-1} and 22,400 cm^{-1} in methanol solution. The corresponding spectra of lumiflavin and 3-methyl-lumiflavin are practically identical. According to the calculations presented in Table 2, all peaks appearing in the absorption spectrum of 3-ethyl-lumiflavin can be attributed to π–π^* transitions, see also Fig. 2.

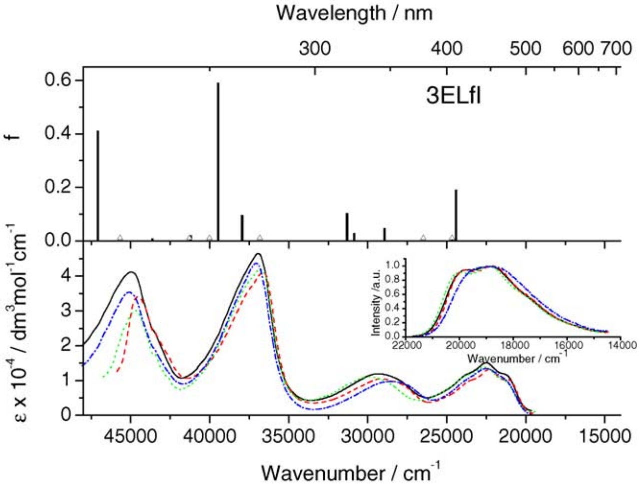

Fig. 2. Predicted lowest-energy singlet–singlet transitions of 3-ethyl-lumiflavin compared to the experimental spectra. Triangles mark locations of weak n,π^* transitions. The experimental ground-state absorption spectra together with the fluorescence spectra refer to 3-ethyl-lumiflavin in acetonitrile (—),1,2-dichloroethane (- - - -), dioxane (. . . . . .), and methanol (- · -). Inset: fluorescence emission spectra of 3-ethyl-lumiflavin in the same solvents.

Numerous quantum chemical calculations on flavins have been reported in literature. TD-DFT calculations have already been used to predict various spectral, photophysical and photochemical properties of lumiflavin [2,12,14–19]. Recently, similar TD-DFT calculations have been made for the singlet and triplet absorption spectra of uracil and lumiflavin and some of its derivatives [2,12,18,20]. However, there still exist a limited number of TD-DFT calculations of the UV–vis singlet–singlet and triplet–triplet electronic spectra of flavins. Recent TD-DFT calculations performed to predict singlet and triplet absorption spectra of lumiflavin [15–18,20] demonstrated some very encouraging improvements as compared to previous semi-empirical and ab initio calculations [21,22], in that they succeeded in reproducing the correct order of the observed singlet excited states and oscillator strengths of the respective transitions.

The ab initio results are presented in Table 2 and Figs. 2 and 3. Similar to isoalloxazines, recently examined by us [2,3,12], 3-ethyl-lumiflavin possesses close-neighbouring n,π^* and π,π^* singlet excited states (calculated $\Delta E < 300$ cm^{-1}), with the lowest excited singlet state of π,π^* character. For comparison, in lumiflavin, 3-methyllumiflavin

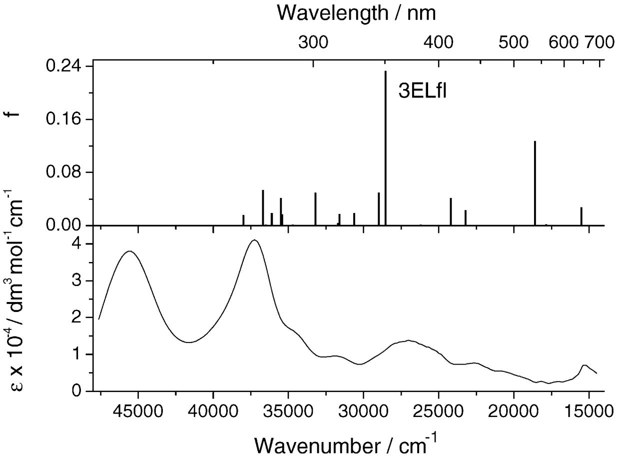

Fig. 3. Predicted T–T transitions of 3-ethyl-lumiflavin obtained using the DFT method compared to the experiment. The experimental transient absorption spectrum refers to 3-ethyl-lumiflavin in methanol excited at 355 nm.

Table 2
Predicted (B3LYP/6-31G(d)) singlet ($S_0 \rightarrow S_i$) and triplet ($S_0 \rightarrow T_i$) excitation energies starting from the ground state and calculated (UB3LYP/6-31G(d)) triplet ($T_1 \rightarrow T_i$) excitation energies starting from the lowest triplet state of 3-ethyl-lumiflavin, with their corresponding oscillator strengths, *f*

$S_0 \rightarrow S_i$	$E \times 10^{-3}$/cm^{-1}	*f*	$S_0 \rightarrow T_i$	$E \times 10^{-3}$/cm^{-1}	*f*	$T_1 \rightarrow T_i$	$E \times 10^{-3}$/cm^{-1}	*f*
$^1(\pi,\pi^*)$	24.4	0.189	$^3(\pi,\pi^*)$	16.6	0	$\rightarrow T_2$	7.75	<0.001
	22.4							
$^1(n,\pi^*)$	24.7	0.005	$^3(n,\pi^*)$	22.0	0	$\rightarrow T_3$	8.18	0.005
$^1(n,\pi^*)$	26.5	<0.001	$^3(\pi,\pi^*)$	23.0	0	$\rightarrow T_4$	8.24	<0.001
$^1(\pi,\pi^*)$	28.9	0.046	$^3(n,\pi*)$	23.7	0	$\rightarrow T_5$	10.5	0.004
	28.7							
$^1(n,\pi^*)$ 88%	30.9	0.027	$^3(\pi,\pi^*)$	24.8	0	$\rightarrow T_6$	13.3	0.001
$^1(\pi,\pi^*)$	31.3	0.101	$^3(n,\pi^*)$	28.8	0	$\rightarrow T_7$	15.5	0.027
$^1(n,\pi^*)$	36.8	<0.001				$\rightarrow T_8$	17.8	0.001
$^1(\pi,\pi^*)$	38.0	0.094				$\rightarrow T_9$	18.6	0.127
$^1(\pi,\pi^*)$	39.5	0.589						
	37.0					$\rightarrow T_{10}$	23.2	0.023
$^1(n,\pi^*)$	40.0	<0.001				$\rightarrow T_{11}$	24.2	0.041
$^1(\pi,\pi^*)$	41.2	0.018				$\rightarrow T_{12}$	26.2	<0.001
$^1(n,\pi^*)$	41.3	<0.001				$\rightarrow T_{13}$	28.5	0.233
$^1(\pi,\pi^*)$	43.6	0.008				$\rightarrow T_{14}$	29.0	0.049
$^1(n,\pi^*)$	45.7	0.001				$\rightarrow T_{15}$	29.0	0.011
$^1(\pi,\pi^*)$	47.1	0.409				$\rightarrow T_{16}$	30.6	0.019
	45.0							

Energy of the first triplet state calculated using the unrestricted formalism (UB3LYP/6-31G(d)) is 15.7×10^3 cm^{-1}. Experimental values taken in methanol are listed in bold type for comparison.

and 3,7,10-trimethyl-isoalloxazine, the difference between the lowest n,π* and π,π* states is about 200–300 cm^{-1}, with the lowest-energy transitions of the π–π* character [2,3,12]. Isoalloxazines represent a class of nitrogen-containing heterocyclic compounds with active centres at N(10), N(5), N(3) and N(1), and at both carbonyl oxygens. It is well known that many other nitrogen heterocycles also possess a close proximity of the two lowest n,π* and π,π* singlet states. The *proximity effect*, believed to be a consequence of vibronic interaction between close-lying n,π* and π,π* singlet states, leads to the potential energy surface distortion and displacement along the vibronically active out-of-plane bending modes [23–25]. Comparison of quantum chemical results to experimental spectral and photophysical properties of these compounds indicates that these properties may be rationalised on the basis of the proximity effect. These predictions of the DFT calculations have their confirmation in the experimental results presented in the Table 1, and are especially well perceived if compared to data of other isoalloxazines examined in Ref. [2,3,12]. Specifically, the nonradiative decay rates were noted to vary systematically with the solvent polarity, which alters the distance between the lowest n,π* and π,π* singlet states and consequently the strength of the proximity effect.

Fig. 4 depicts the structures of the lowest unoccupied and highest occupied (frontier) molecular orbitals, mainly involved in the transitions to the low-lying excited states. TD-DFT calculations predict the nature of the molecular orbitals involved in the predominant excitations. For example, the $S_0 \rightarrow S_1$ transition has a dominant contribution from the HOMO → LUMO excitation, and can be interpreted as an allowed π → π* transition (see Table 2). With the use of the TD-DFT method, the vertical excitation energy from the ground-state to the π,π* state was calculated to be 24,400 cm^{-1}, which is consistent with the experimentally determined of $S_0 \rightarrow S_1$ absorption band at about 22,400 cm^{-1} for the first π,π* singlet excited state.

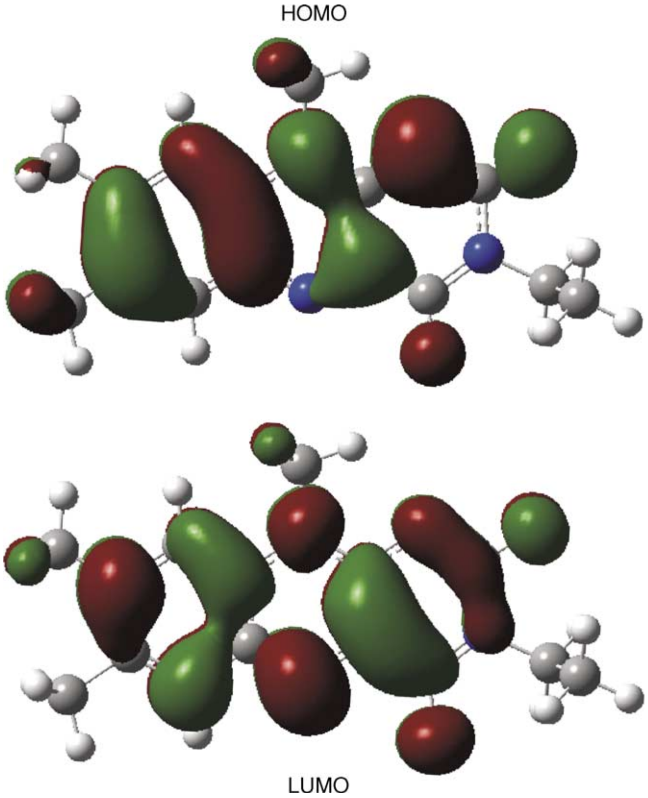

Fig. 4. The shape of the highest occupied (HOMO) and the lowest unoccupied (LUMO) molecular orbitals mainly involved in the lowest energy transitions. The isosurfaces correspond to the value of ±0.02.

TD-DFT calculations predict the nature of the molecular orbitals involved in the predominant excitations ($S_0 \rightarrow T_i$), and the symmetry of the corresponding T_i states, indicating the first one to be of $^3(\pi,\pi^*)$. The dominant contribution in this case is from the HOMO → LUMO excitation, and can be interpreted as an allowed $\pi \rightarrow \pi^*$ transition (see Table 2). The corresponding electronic structure of 3-ethyl-lumiflavin in its triplet state was predicted using the DFT method and compared to experimental T–T absorption spectra. Corresponding T–T excitation energies and transition intensities were determined for the optimised geometry of the lowest triplet state (T_1), and the results are shown in Table 2 and Fig. 3. Detectable transitions in 3-ethyl-lumiflavin are shown in the Fig. 3, and are located at about 15,500, 18,600, 23,200, 24,190 and 28,500 cm^{-1}, and some at still higher wavenumbers. Experimental absorption spectrum of 3-ethyl-lumiflavin in methanol is also shown in Fig. 3, exhibiting bands around 15,150, 19,600, 26,300, and 34,500 cm^{-1}. The bands predicted in the DFT calculations are blue-shifted relative to the corresponding experimental bands. However, it should be kept in mind that the comparison is made between an experimental spectrum in solution and calculations referring to isolated gas-phase molecules. The solvent effect has been recently elucidated by comparing gas-phase uracil spectrum to that in aqueous solution [20]; however, there are no known absorption spectra of *iso*- or alloxazines in the gas phase. Noting, however, that the uracil gas-phase absorption bands are blue-shifted by about 1200–2500 cm^{-1} relative to the aqueous solution spectrum, we consider that the agreement between theory and experiment is relatively good. Note that there are several lower-energy transitions (see Table 2), which could not be observed in the experimental spectrum because of the current limitations of the flash-photolysis setup.

Recently our interest in the photochemistry of alloxazines and flavins has focused on the study of the interactions, which occur between those molecules and oxygen in the presence of light. The driving force to determine quantum yield of singlet oxygen production by flavins and related compounds is the very limited amount of data of this type, see for example the review entitled “A compilation of singlet oxygen yields from biologically relevant molecules” where only a few data for the flavin class of compounds are reported [26]. Presently, we measured the emission at 1270 nm, which is highly specific to the $O_2(^1\Delta_g) \rightarrow O_2(^3\sum_g{}^-)$ transition, under laser excitation at 355 nm of the 3-ethyl-lumiflavin in air-equilibrated methanol. The emission intensity at 1270 nm increased in samples with higher oxygen concentrations and was extinguished by bubbling N_2 through the solution for a few minutes. In the present work the quantum yield ($\phi_\Delta = 0.55$) and lifetime of the singlet oxygen ($\tau_\Delta = 10\ \mu s$) formed by triplet photosensitization, were determined by exciting air-saturated 3-ethyl-lumiflavin solutions. The emission lifetime values ($\tau_\Delta = 10\ \mu s$) presented in Table 3 are typical for singlet oxygen in methanol [27]. All these observations confirm that 3-ethyl-lumiflavin acts as a photosensitizer for the singlet oxygen, and that $O_2(^1\Delta_g)$ is responsible for the emission at 1270 nm. The corresponding values for lumiflavin and 3-methyl-lumiflavin are also presented in Table 3 for comparison. It is interesting to note that while it has been shown that hydrogen bonding to N(3)–H can modulate the reduction potential for the reduction of flavin to the radical anion [13], there is little difference in singlet oxygen quantum yields between the parent flavin and the 3-alkyl substituted derivatives. These measurements were conducted in methanol solution, where hydrogen bonding interactions are likely to be important. It is therefore concluded that N(3)-alkyl substitution does not affect significantly the interaction between the flavins and oxygen. This observation suggests also that the triplet quantum yields are also equal for all three compounds, since the only other explanation is a variation in ϕ_T being exactly compensated by an opposite variation in f_Δ^T, the efficiency of singlet oxygen production from triplet state quenching. Note that the triplet state lifetimes are sufficiently long that in methanol solution, the fraction of triplet state quenched by oxygen f_Q^T can be taken as unity and there is no dependence of the singlet oxygen quantum yield on the concentration of dissolved oxygen. Hence, these flavins have been shown to be efficient singlet oxygen producers, with N(3)-alkyl substitution not modulating this efficiency.

Table 3
Triplet state lifetimes, τ_T, quantum yields of photosensitized production of singlet oxygen, ϕ_Δ, and the singlet oxygen lifetimes, τ_Δ, for selected lumiflavin derivatives in methanol solutions

Compound	τ_T/µs	ϕ_Δ	τ_Δ/µs
Lumiflavin	16.6	0.48*	10*
3-Methyl-lumiflavin		0.53	10
3-Ethyl-lumiflavin	9.5	0.55*	10*

* Data from Ref. [12].

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