11-2010

Electronic Properties Of N(5)-ethyl Flavinium Ion

Vincent Sichula

Pavel Kucheryavy

Renat Khatmullin

Ying Hu

Ekaterina Mirzakulova

See next page for additional authors

Follow this and additional works at: https://scholarworks.bgsu.edu/chem_pub

Part of the [Chemistry Commons](#)

How does access to this work benefit you? Let us know!

Repository Citation

Sichula, Vincent; Kucheryavy, Pavel; Khatmullin, Renat; Hu, Ying; Mirzakulova, Ekaterina; Vyas, Shubham; Manzer, Samuel F.; Hadad, Christopher M.; and Glusac, Ksenija D., "Electronic Properties Of N(5)-ethyl Flavinium Ion" (2010). *Chemistry Faculty Publications*. 159.
https://scholarworks.bgsu.edu/chem_pub/159

This Article is brought to you for free and open access by the Chemistry at ScholarWorks@BGSU. It has been accepted for inclusion in Chemistry Faculty Publications by an authorized administrator of ScholarWorks@BGSU.

Author(s)

Vincent Sichula, Pavel Kucheryavy, Renat Khatmullin, Ying Hu, Ekaterina Mirzakulova, Shubham Vyas, Samuel F. Manzer, Christopher M. Hadad, and Ksenija D. Glusac

Electronic Properties of N(5)-Ethyl Flavinium Ion

Vincent Sichula,[†] Pavel Kucheryavy,[†] Renat Khatmullin,[†] Ying Hu,[†] Ekaterina Mirzakulova,[†] Shubham Vyas,[‡] Samuel F. Manzer,[‡] Christopher M. Hadad,[‡] and Ksenija D. Glusac^{*†}

Department of Chemistry, Bowling Green State University, Bowling Green, Ohio 43403, United States, and
Department of Chemistry, The Ohio State University, 100 West 18th Avenue, Columbus, Ohio 43210, United States

Received: July 7, 2010; Revised Manuscript Received: October 7, 2010

We investigated the electronic properties of N(5)-ethyl flavinium perchlorate (Et-FI⁺) and compared them to those of its parent compound, 3-methylflavin (FI). Absorption and fluorescence spectra of FI and Et-FI⁺ exhibit similar spectral features, but the absorption energy of Et-FI⁺ is substantially lower than that of FI. We calculated the absorption signatures of FI and Et-FI⁺ using time-dependent density functional theory (TD-DFT) methods and found that the main absorption bands of FI and Et-FI⁺ are (π, π^*) transitions for the S₁ and S₃ excited states. Furthermore, calculations predict that the S₂ state has (n, π^*) character. Using cyclic voltammetry and a simplistic consideration of the orbital energies, we compared the HOMO/LUMO energies of FI and Et-FI⁺. We found that both HOMO and LUMO orbitals of Et-FI⁺ are stabilized relative to those in FI, although the stabilization of the LUMO level was more pronounced. Visible and mid-IR pump–probe experiments demonstrate that Et-FI⁺ exhibits a shorter excited-state lifetime (590 ps) relative to that of FI (several nanoseconds), possibly due to faster thermal deactivation in Et-FI⁺, as dictated by the energy gap law. Furthermore, we observed a fast (23–30 ps) S₂ → S₀ internal conversion in transient absorption spectra of both FI and Et-FI⁺ in experiments that utilized pump excitations with higher energy.

Introduction

Flavoproteins catalyze a wide range of biochemical transformations, such as dehydration, electron transfer, dehydrogenation, and other oxidation and reduction processes.¹ An interesting class of flavoproteins are flavin-dependent mono-oxygenases, which catalyze the incorporation of atomic oxygen into organic substrates.² The chemical transformations catalyzed by flavoprotein mono-oxygenases include hydroxylations, sulfoxidations, amine oxidations, epoxidations, and Baeyer–Villiger oxidations. The active catalyst in mono-oxygenases is the flavin cofactor in its reduced form. The mechanism of the catalytic oxidation involves an electron transfer from the reduced flavin to molecular oxygen. The subsequent spin inversion leads to the production of reactive C_{4a}-hydroperoxyflavin species.³ The flavin moiety in hydroperoxyflavin derivatives polarizes the O–O bond, enabling the release of atomic oxygen and formation of C_{4a}-hydroxyflavin. The recovery of the catalyst involves a release of water from C_{4a}-hydroxyflavin to produce flavin in the oxidized form, followed by the reduction of flavin to the active reduced state. A special case of this type of oxidation is observed in bacterial luciferases, where the catalytic oxidation produces the C_{4a}-hydroxyflavin in its excited state, causing bioluminescence.⁴

In synthetic organic chemistry, most of the catalysts used for introducing an oxygen atom into organic molecules are heavy-metal complexes.⁵ Although efficient catalysts, these complexes are expensive and are often toxic. To overcome these shortcomings, several groups have designed a fully organic mimic of mono-oxygenase flavoproteins and investigated its catalytic activity toward activation of oxygen and hydrogen peroxide.⁶

The synthetic analog used in these studies is N(5)-ethyl flavinium perchlorate^{7,8} (Et-FI⁺), which is shown in Scheme 1. The main difference between the naturally occurring flavin cofactors and Et-FI⁺ is the presence of the ethyl group at the N5 position. Alkylation at the N5 position stabilizes the C_{4a}-hydroperoxyflavin derivative, which enables the use of flavin-based catalysis outside the protein environment.⁷ Using Et-FI⁺, successful oxidations of amines to *N*-oxides,^{9–14} sulfides to sulfoxides,^{10,11,13–16} aldehydes to acids,^{7,15} hydroxylations of aromatic compounds,¹⁷ and Baeyer–Villiger oxidations of ketones^{18–20} have been demonstrated.

The electronic absorption spectrum of Et-FI⁺ extends far into the visible range (Figure 1), which can be used to enable photocatalytic oxidations using Et-FI⁺ analogous to the ones outlined in the previous paragraph. For this reason, we investigated the excited state behavior of Et-FI⁺ and compared it to the well-known 3-methylflavin (FI in Scheme 1). Our group has previously studied the excited-state properties of a series of flavin cofactors in order to elucidate the mechanism of light-driven catalysis by another flavoprotein, DNA photolyase.^{21–23}

The present manuscript outlines the electronic properties of Et-FI⁺ determined using steady-state absorption and fluorescence spectroscopy, cyclic voltammetry, ultrafast UV–vis pump–probe and time-resolved IR spectroscopy (TRIR). The experimental results were interpreted using density functional theory (DFT)^{28,29} calculations. The ground states were characterized both in the gas-phase and with consideration of implicit solvation (acetonitrile). Difference density methods were utilized to interpret the character of the calculated electronically excited states. Furthermore, to evaluate the excited-state vibrational frequencies of FI and Et-FI⁺, the geometries of the S₁ states were optimized using time-dependent DFT.²⁴ We found that the electronic properties of FI and Et-FI⁺ are similar: (i) the main absorption bands of both compounds arise from S₁ and S₃ (π, π^*) excited states; (ii) both compounds exhibit two one-electron reduction

* To whom correspondence should be addressed. E-mail: kglusac@bgsu.edu.

[†] Bowling Green State University.

[‡] The Ohio State University.

SCHEME 1: Structures of 3-Methylflavin (Fl) and N(5)-Ethylflavinium Perchlorate (Et-Fl⁺)

peaks in their cyclic voltammograms to produce the corresponding semiquinone and hydroquinone derivatives. However, we find that the HOMO and LUMO energies of Et-Fl⁺ are stabilized relative to those of the parent compound, Fl. This stabilization leads to the pronounced red-shift in the absorption frequencies of Et-Fl⁺ and a decrease in its reduction potentials. Pump–probe measurements demonstrate that both compounds exhibit two excited-state deactivation channels: a fast, radiationless decay from the S₂ (n,π*) state and a slow radiative decay from the S₁ (π,π*) state.

Experiment and Theory

Materials. 3-Methylflavin (Fl) and N(5)-ethylflavinium perchlorate (Et-Fl⁺) were synthesized according to the published procedure.²⁵ Anhydrous acetonitrile was purchased from Sigma-Aldrich (water content was 0.2%). Steady-state absorption and fluorescence measurements and visible pump–probe experiments were done using acetonitrile as received. For cyclic voltammetry, acetonitrile was purified as discussed in the next section. TRIR measurements were performed in deuterated acetonitrile (Sigma Aldrich).

Steady-State Measurements. The absorption measurements were completed with a UV–visible spectrophotometer (Varian Cary 50 Bio). The photoluminescence spectra were obtained with a single photon counting spectrofluorimeter from Edinburgh Analytical Instruments (FL/FS 900). FTIR spectra were collected using FTIR-8400S (Shimadzu). Cyclic voltammograms were collected using a BASi Epsilon system. The electrodes used in our experiments were as follows: Pt working electrode, platinum wire as an auxiliary electrode, and nonaqueous AgNO₃/Ag electrode as the reference. The electrolyte was tetrabutylammonium perchlorate (TBAP), which was dried over vacuum. All electrochemical measurements were obtained under an argon atmosphere and in the dark at 25 °C. Anhydrous acetonitrile was purchased from Sigma-Aldrich (water content was 0.2%) and was used after distillation three times over CaH₂ until the temperature of the distillate reached 81 °C.

Laser Spectroscopy. Ultrafast UV–vis Pump–Probe Measurements. The spectra were collected using the instrument described previously.²⁶ Briefly, 800 nm laser pulses (fwhm = 110 fs) were produced at a 1 kHz repetition rate by a mode-locked Ti:Sapphire laser (Hurricane, Spectra-Physics). The pump beam of desired frequency was produced using optical parametric amplifier (Spectra Physics OPA800). The 2 mM solution of Fl (2 mm quartz cuvette, Spectrocell Inc.) was excited at 400 nm, and the 2 mM solution of Et-Fl⁺ was excited at 555 nm. The probe beam was produced using a CaF₂ crystal. Transient absorption spectra were collected using a CCD camera (Ocean Optics, S2000). Data were chirp-corrected and processed using a method described previously.²³

Ultrafast TRIR Measurements. The spectra were collected using the instrument described previously.^{21,27} The source of

femtosecond pulses at 800 nm (1 kHz repetition rate, ~90 fs pulse width) was a Ti:Sapphire oscillator/regenerative amplifier (Hurricane, Spectra Physics). The output from the amplifier was split into two beams, and the wavelengths for pump and probe beams were obtained using two TOPAS-C systems (Quantronix/Light Conversion). The 10 mM solutions of Fl were excited at 400 nm, while the 10 mM solutions of Et-Fl⁺ were excited at 500 nm. The sample solution flow was achieved using a fluid-metering RSHY lab pump (Scientific Support) through a demountable liquid flow cell with Swagelok fittings (DSC-S25, Harrick Scientific Products). The path length was 250 μm, created by a Teflon spacer between two round BaF₂ windows (25 × 2 mm, REFLEX Analytical Corporation). After passing through the sample cell, the probe and reference beams were directed to a Chromex Imaging Spectrograph (250 is/sm), and the signal was read by a 2 × 32 array of MCT detectors (Infrared Systems Development Corporation). Global analysis of the data was achieved using SPECFIT/32™ Global Analysis System software (Spectrum Software Associates, MA, USA). This program allows a decomposition of pump–probe data using kinetic models. The fitting process returns the predicted absorption spectra of individual colored species along with their decay profiles. The analysis is achieved by a global analysis method that uses singular value decomposition method to reduce the size of the fitted data.²⁸ In our study, we used the A → B → C kinetic model (A and B absorb, but C is not colored).

Computational Methods

All computations were performed at the Ohio Supercomputer Center. Ground-state geometry optimizations were performed using Gaussian 03²⁹ at the B3LYP/6-31G* level of theory.^{30,31} All stationary points were confirmed to be energy minima using vibrational frequency calculations (B3LYP/6-31G*), which confirmed that all of the computed vibrational frequencies were real (i.e., no imaginary vibrational frequencies). Vertical excitations were then calculated with time-dependent DFT at the TD-B3LYP/6-31+G* level of theory in the gas phase as well as with consideration of implicit solvation of acetonitrile (using the polarization continuum model, PCM^{32–37}). Difference-density plots were generated from the computed electron densities of the ground and excited states (TD-B3LYP/6-31+G*) using Gaussian 09.³⁸ Excited state geometries were optimized at the TD-B3LYP level of theory with the SVP basis sets as implemented in the Turbomole-5.10.0 software suite.³⁹ These stationary points were verified to be minima by numerical vibrational frequency analysis (TD-B3LYP/SVP) using Gaussian 09. To compare these numbers with that of the ground state, we reoptimized the ground state geometries at the TD-B3LYP/SVP level of theory using Turbomole-5.10.0. Since a scaling factor is not available for the B3LYP/SVP level of theory, and SVP basis sets are considered to be comparable to 6-31G*,³⁷

Figure 1. (a) UV-vis absorption spectra of FI (blue) and Et-FI⁺ (red) in acetonitrile ($c = 2 \times 10^{-5}$ M). Bars represent calculated transition energies for FI (blue) and Et-FI⁺ (red); Calculations were performed using time-dependent DFT (TD-B3LYP/6-31+G**/B3LYP/6-31G*); (b) Emission spectra of FI (blue, $\lambda_{exc} = 430$ nm) and Et-FI⁺ (red, $\lambda_{exc} = 570$ nm) in acetonitrile; (c) a table of experimental (exp) and calculated (cal) absorption wavelengths for FI and Et-FI⁺. The calculated values were obtained in the gas phase and in acetonitrile (ACN) using the PCM solvation model.

we have utilized the B3LYP/6-31G* scaling factor of 0.9614⁴⁰ for our B3LYP/SVP calculations as described in this paper.

Results and Discussion

UV-vis Absorption and Fluorescence Spectra. Figure 1 presents the absorption and emission spectra of FI and Et-FI⁺ in acetonitrile. The absorption spectrum of FI consists of two bands centered at 338 and 443 nm, while the emission spectrum appears at 519 nm. For Et-FI⁺, the shapes of the absorption and emission spectra are similar to those of FI, but the maxima are shifted to lower energies, at 414 and 557 nm, while the emission band appears at 661 nm. To understand the origin of these absorption bands, we performed gas-phase TD-DFT (TD-B3LYP/6-31+G*) calculations on FI and Et-FI⁺, which are summarized in Figure 1; also, Table S1 in the Supporting Information lists the vertical excitations for the S₁–S₆ states of FI and Et-FI⁺. The gas-phase calculations predict that S₁ and S₃ states of Et-FI⁺ have high oscillator strengths and are located at 422 and 575 nm. On the other hand, the S₂ state has a near zero oscillator strength and is energetically close to the vertical S₁ state (523 nm for Et-FI⁺). The same types of S₁ and S₂ transitions were observed for FI, but the absorption energies are blue-shifted relative to Et-FI⁺. Contrary to Et-FI⁺, the S₃ state of FI carries a low oscillator strength and is assigned to an (n,π*) transition. Our assignment of FI excited states is in

the agreement with the previously published gas-phase TD-DFT calculations.⁴¹ The effect of solvent (acetonitrile) on these absorptions has been evaluated by performing analogous calculations using the PCM solvation model.^{32–37} We found that all three absorptions of Et-FI⁺ shift to higher energy in the presence of solvent, with the most dominant effect being for the S₂ state. A similar effect was observed for FI. This observation can be explained by the computed dipole moments of the singlet states. The dipole moment data (see Supporting Information, Table 6) suggests that the dipole moment for the S₂ and S₃ states are significantly lower than the S₀ state; thus, calculations predict a blue-shift in the absorption spectra upon increase in solvent polarity. On the other hand, the dipole moment of S₁ state is slightly higher than the S₀ state, which predicts that the absorption corresponding S₁ state would be red-shifted in polar solvents. Interestingly, with consideration of solvation, the S₃ state of FI has a high calculated oscillator strength, suggesting a (π,π*) state. This assignment is different from the gas-phase calculations, where the S₃ state has an (n,π*) character, and perhaps, the crossover occurs due to the destabilization of the (n,π*) state in the absence of polar solvent.⁴² Calculated PCM absorption energies for the first three states and the corresponding oscillator strengths are compared with the experimental absorption spectra in Figure 1, demonstrating good agreement between the calculated and experimental

Figure 2. Difference density plots calculated using time-dependent DFT methods (TD-B3LYP/6-31+G**/B3LYP/6-31G*). A red color indicates depletion of the electron density from the ground state, while the green color indicates accumulation of electron density in the excited state: (a) $S_1 - S_0$ of Et-Fl $^+$; (b) $S_2 - S_0$ of Et-Fl $^+$; (c) $S_1 - S_0$ of Fl; (d) $S_2 - S_0$ of Fl. The contour value is ± 0.001 au.

values. The character of the first two excited states can be elucidated from difference density plots (DDPs) as presented in Figure 2 (see Supporting Information, Figures S1 and S2). We recently applied DDPs in other photochemical systems^{42–45} as a tool to visualize the changes in the electron density upon vertical excitation. The $S_1 - S_0$ DDPs of both Fl and Et-Fl $^+$ exhibit a change in the electron density that alternates in sign along the conjugated system, which is characteristic of a (π, π^*) excited state. The same behavior was observed for the $S_3 - S_0$ DDPs of Et-Fl $^+$ and Fl in acetonitrile as a solvent. DDPs corresponding to the S_2 states of Fl and Et-Fl $^+$ exhibit depletion of the electron density from the lone pairs of the oxygen atoms located on the imide moieties, which is characteristic of an (n, π^*) excited state. Thus, we assign the two strong absorption bands of Fl and Et-Fl $^+$ to the S_1 and S_3 (π, π^*) transitions. We also identify the presence of an (n, π^*) S_2 state between S_1 and S_3 states with its characteristic low oscillator strength. The emission bands of Fl and Et-Fl $^+$ are assigned to fluorescence from the (π, π^*) S_1 excited state. Although the same transitions are responsible for the absorption bands of Fl and Et-Fl $^+$, the absorption maxima of Et-Fl $^+$ are shifted to lower wavelengths. As will be discussed in the next section, the cause for this shift is the stabilization of the LUMO orbital of Et-Fl $^+$.

Cyclic Voltammetry. To further investigate the electronic properties, we obtained cyclic voltammograms of Fl and Et-Fl $^+$ in acetonitrile solution (Figure 3). The voltammogram of Fl contains two reversible reduction peaks at -0.95 and -1.78 V. The first reduction peak is assigned to the reduction of Fl to flavin-semiquinone radical anion Fl $^{\cdot-}$, while the second reduction produces the Fl $^{2-}$ dianion. Electrochemistry of flavin derivatives is well-documented and is different in protic^{46,47} and aprotic solvents.^{48,49} In protic solvents, electron transfer is accompanied by a proton transfer to produce the neutral semiquinone radical, FlH $^{\cdot}$. This proton-coupled electron transfer leads to charge neutralization, and the reduction potential of FlH $^{\cdot}$ becomes less negative than the reduction potential of Fl,

Figure 3. Cyclic voltammograms of argon-purged 2 mM Fl (blue) and 2 mM Et-Fl $^+$ (red) in acetonitrile using tetrabutylammonium perchlorate (TBAP) as a supporting electrolyte. Sweep rate: 100 mV/s.

thus leading to the appearance of only one two-electron reduction peak. On the contrary, proton donation cannot occur in aprotic solvents, which causes the reduction potential of anionic Fl $^{\cdot-}$ to appear at a more negative potential than Fl. This leads to the presence of two resolved reduction peaks in the voltammogram, as shown in Figure 3.

The cyclic voltammogram of Et-Fl $^+$ exhibits two analogous reduction peaks at $+0.17$ and -0.52 V, which we assign to the one-electron and two-electron transfer processes to form the neutral semiquinone radical Et-Fl $^{\cdot}$ and reduced anionic Et-Fl $^{\cdot-}$. The potentials at which these reduction processes occur are less negative than the corresponding potentials for Fl, which is to be expected due to the positive charge present in Et-Fl $^+$. This result demonstrates that the LUMO orbital of Et-Fl $^+$ is stabilized relative to the LUMO of Fl. In addition to these two reduction

SCHEME 2: HOMO and LUMO Energies and Orbitals of FI (Blue) and Et-FI⁺ (Red)^a

^a (a) Energies estimated from cyclic voltammetry ($E^{\text{abs}} = E^{\circ} + 4.44$ V); (b) Energies calculated at the B3LYP/6-311+G**//B3LYP/6-31G* level of theory in acetonitrile as a solvent (PCM model); (c) HOMO and LUMO orbitals of Et-FI⁺.

Figure 4. (a) Upper panel: ground state absorption (black) and fluorescence (red) spectra of FI in acetonitrile ($c = 2 \times 10^{-5}$ M); lower panel: transient absorption spectra of 2 mM FI in acetonitrile collected 1 ps (black), 183 ps (red) and 1.1 ns (green) after the 400 nm excitation pulse; (b) decays of transient signals collected at 379 nm (black dots) and 444 nm (red dots). Solid curves represent fits obtained using a biexponential decay function with lifetimes of $\tau_1 = 53$ ps and $\tau_2 \sim 8$ ns.

peaks, both FI and Et-FI⁺ exhibit irreversible oxidation peaks at +1.95 and +2.14 V. Assuming that the first reduction and oxidation peaks of FI and Et-FI⁺ correlate well with the energies of the corresponding HOMO and LUMO orbitals, we used experimental reduction potentials to calculate the HOMO–LUMO energies for FI and Et-FI⁺ ($E_{\text{HOMO/LUMO}} = E^{\circ}_{\text{FI(OX/RED)}} + E^{\circ}_{\text{SHE}}$, where $E^{\circ}_{\text{SHE}} = 4.44$ V,⁵⁰ Scheme 2). These experimental values were then compared to the calculated HOMO–LUMO energies at the B3LYP/6-311+G**//B3LYP/6-31G* level of theory, including consideration of solvation with the PCM model of acetonitrile. As can be seen from Scheme 2, experiment and calculation are in good qualitative agreement. Moreover, the calculated singly occupied molecular orbitals (SOMOs) for the vertical radical anions of FI and Et-FI⁺ are identical to the LUMO orbitals, respectively (see Supporting Information, Figure S4). The experimental and computational efforts suggest that the HOMO and LUMO orbitals of Et-FI⁺ are stabilized relative to those of FI. However, the stabilization effect is much stronger for the Et-FI⁺ LUMO orbital. The reason for the larger stabilization of the LUMO orbital can be understood by analyzing the HOMO and LUMO orbitals of Et-FI⁺, as presented in Scheme 2. Although the HOMO orbital of Et-FI⁺ does not involve the ethyl group, the LUMO orbital “leaks” into the N5-ethyl group, which gives rise to the observed

additional stabilization of the Et-FI⁺ LUMO orbital. This unequal stabilization of HOMO and LUMO orbitals explains the red-shift of the absorption and fluorescence bands of Et-FI⁺.

UV–vis Pump–Probe Experiments. We investigated the excited-state behavior of FI and Et-FI⁺ using ultrafast UV–vis pump–probe spectroscopy. Figure 4 shows the transient absorption spectra of FI obtained at several time delays after the 400 nm excitation pulse. Spectra consist of three major bands: (i) excited-state absorption band with a maximum at 379 nm; (ii) ground-state bleach at 444 nm, and (iii) stimulated emission observed at 550 nm. The decays at 379 and 444 nm consist of two time components, with lifetimes of $\tau_1 = 53$ ps and $\tau_2 =$ several nanoseconds (the lifetime of the longer component is much larger than our experimental time window of 1.2 ns). The long decay component of FI is assigned to the decay of the S_1 (π, π^*) state due to intersystem crossing to the triplet T_1 (π, π^*) state of FI. This assignment is consistent with the previously reported 8.4 ns lifetime obtained from time-resolved fluorescence measurements.⁴¹ In addition, previous nanosecond transient absorption experiments noted that the S_1 state deactivates due to intersystem crossing to the T_1 state.⁵¹

The short 53 ps component could be explained by several nonradiative processes: (i) relaxation of the Franck–Condon

Figure 5. (a) upper panel: ground state absorption (black) and fluorescence (red) spectra of Et-FI⁺ in acetonitrile ($c = 2 \times 10^{-5}$ M); lower panel: transient absorption spectra of 2 mM Et-FI⁺ in acetonitrile collected 1 ps (black), 183 ps (red) and 1.1 ns (green) after the 555 nm excitation pulse; (b) decays of transient signals collected at 350 nm (black dots) and 417 nm (red dots). Solid curves represent fits obtained using exponential decay function with lifetime $\tau = 590$ ps.

S_1 state to the vibrationally “cold” S_1 state; (ii) $S_2 \rightarrow S_1$ internal conversion (IC), and (iii) $S_2 \rightarrow S_0$ IC. Assignment of this shorter time component to vibrational relaxation can be discounted for two reasons. First, the vibrational relaxation of medium-sized molecules usually occurs on a faster time scale, with the lifetime of <10 ps.^{52,53} In riboflavin, the vibrational relaxation occurs with a 4 ps lifetime, as determined using pump–probe spectroscopy.⁵⁴ Second, our data from TRIR experiments (see next section) do not show the characteristic blue shift of the vibrational bands, which is characteristic of vibrational relaxation.⁵⁵ IC from the S_2 state to the S_0 ground state is more likely to occur, since the 400 nm pump beam covers the blue part of the S_1 absorption band of FI (Figure 4) and the excitation pulse is expected to produce both the S_1 (π, π^*) and S_2 (n, π^*) states of FI, which is in good agreement with our TD-B3LYP calculations (Figure 1). The $S_2 \rightarrow S_1$ IC is expected to occur on a faster time scale than the observed 53 ps component. For example, $S_2 \rightarrow S_1$ IC in porphyrins and diphenylpolyenes occurs with lifetimes in the 400–700 fs range.^{56,57} The rate of IC is controlled by the energy gap law, as developed by Jortner.⁵⁸ On the basis of Jortner’s expression, the rate of IC depends on several factors, one of which is the energy gap between the two states involved in a transition. On the basis of our TD-DFT calculations (Figure 1), the S_2 – S_1 energy gap in FI is 3,815 cm^{-1} . A similar S_2 – S_1 gap exists in diphenylpolyenes (2000–6000 cm^{-1}), which were found to undergo S_2 – S_1 IC with lifetimes in the range of only 400–610 fs.⁵⁶ On the basis of this result, it seems unlikely that the 53 ps component arises due to S_2 – S_1 IC. Furthermore, the bleach recovery at 444 nm (Figure 4) exhibits the same 53 ps component, which is not consistent with the S_2 – S_1 IC process. On the other hand, the presence of the 53 ps component at the bleach wavelengths suggests that S_2 – S_0 IC occurs. Thus, we assign this short (53 ps) component to the internal conversion of the S_2 (n, π^*) state to the S_0 state of FI. The larger S_2 – S_0 gap can explain the longer 53 ps lifetime on the basis of Jortner’s energy gap law. Furthermore, (n, π^*) states of other organic systems, such as pyrimidine DNA bases, were found to decay nonradiatively to the ground state with similar lifetimes.^{59,60} Hence, we conclude

that the 400 nm excitation produces S_1 (π, π^*) and S_2 (n, π^*) states of FI, which decay via two separate channels to the ground state.

Transient absorption spectra of Et-FI⁺ obtained at several delay times after the 555 nm excitation pulse (Figure 5) consist of: (i) excited-state absorption bands at 350, 470, and 583 nm, (ii) a ground-state bleach at 417 nm, and (iii) a stimulated emission signal at wavelengths above 650 nm. Signals at all absorption wavelengths decay as a single exponential function, with the lifetime of 590 ps. We assign this state to the S_1 (π, π^*) state of Et-FI⁺ that decays to the S_0 ground state with the 590 ps lifetime. This observation is also supported by our calculations, which predict that only the S_1 state is accessible with 555 nm excitation (Figure 1). The excited-state lifetime of Et-FI⁺ is substantially shorter than that of FI, which we attribute to the lower HOMO–LUMO energy gap in Et-FI⁺. The lowering of the gap can lead to the faster excited-state deactivation, as dictated by the energy gap law.⁵⁸ Another difference between FI and Et-FI⁺ transient absorption spectra is the lack of S_2 absorption in Et-FI⁺. This is due to usage of different excitation wavelength positions relative to the absorption spectra of FI and Et-FI⁺. Whereas FI was excited between S_1 and S_3 absorption bands, Et-FI⁺ was excited at the maximum of the S_1 band. In the next section, we will show that the excitation of Et-FI⁺ using 500 nm (instead of 555 nm used here) creates both S_1 and S_2 states analogous to those observed for FI.

Time-Resolved IR Measurements. Figure 6 represents the ground-state FTIR spectra of FI and Et-FI⁺ in the 1500–1800 cm^{-1} range. Both compounds exhibit similar vibrational signatures that were assigned using computed vibrational frequencies at the B3LYP/SVP level of theory and are listed in Figure 6b (also, see Supporting Information, Tables S2 and S4). The vibrational modes responsible for the absorption bands of FI and Et-FI⁺ are as follows: (i) symmetric stretching of the two C=N bonds coupled with C=C stretching of the aromatic ring; (ii) asymmetric stretching of the C=N bonds coupled with C=C aromatic stretching; (iii) asymmetric stretching of the two C=O bonds, and (iv) symmetric stretching of C=O bonds. The

Figure 6. (a) Ground-state FTIR spectra of Fl (blue) and Et-Fl $^{+}$ (red) in acetonitrile. (b) Table of experimental and calculated vibrational frequencies for Fl and Et-Fl $^{+}$. Calculations were done using at the B3LYP/SVP level of theory. The calculated harmonic frequencies were scaled by a factor of 0.9614 (see text).

Figure 7. (a) Upper panel: ground-state FTIR spectrum of Fl in acetonitrile. Middle panel: transient absorption IR spectra of 10 mM Fl in acetonitrile collected 10 ps (black), 1 ps (blue), 500 ps (red), and 1 ns (green) after the 400 nm excitation pulse. Lower panel: transient absorption IR spectra of S_1 and S_2 states of Fl obtained from spectral deconvolution. (b) Decays of transient signals collected at 1610 (red), 1572 (blue), 1656 (black), 1587 (green), and 1553 (cyan) cm^{-1} . Solid curves represent fits obtained using biexponential decay functions with lifetimes $\tau_1 = 30$ ps and $\tau_2 = 4$ ns.

frequencies of Fl are consistent with the previously assigned FTIR spectra of riboflavin,^{54,61} flavin-mononucleotide (FMN),⁶² and flavin dinucleotide (FAD).⁶³

Figure 7a represents time-resolved IR (TRIR) spectra of Fl at different time delays after the 400 nm excitation pulse. The spectrum obtained 1 ps after the excitation pulse contains bleach bands at frequencies where the ground-state absorption occurs. In addition, we observed the excited-state absorption at 1610 cm^{-1} , and two weak absorption bands at ~ 1535 and 1640 cm^{-1} . Decays at all frequencies can be fitted to a biexponential decay function with lifetimes of $\tau_1 = 30$ ps and $\tau_2 =$ several nanoseconds (Figure 7b). In analogy to the visible pump–probe

data on Fl, we assign these two components to $S_2 \rightarrow S_0$ IC (30 ps) and $S_1 \rightarrow T_1$ intersystem crossing (several nanoseconds). The lifetime for the short $S_2 \rightarrow S_0$ component is somewhat different in visible (53 ps) and TRIR (30 ps) experiments. The shorter lifetime in TRIR experiments suggests that the energy of (n, π^*) state is lowered, possibly due to stabilizing intramolecular interactions at high sample concentrations used for TRIR experiments. The 30 ps component could also be assigned to the vibrational cooling of the initially formed Franck–Condon S_1 state,⁵⁵ which usually leads to a blue-shift of excited-state absorption bands analogous to the shift of the 1610 cm^{-1} band, as presented in Figure 7a. We rule out vibrational cooling for

TABLE 1: Experimental (Acetonitrile) and Calculated Gas-Phase (B3LYP/SVP^a) Frequencies (in cm⁻¹) for the S₁ States of FI and Et-FI⁺

FI			Et-FI ⁺		
ν_{calc}	ν_{exp}	assignment	ν_{calc}	ν_{exp}	assignment
1495		C=C _{arom} /C=N sym.	1524	1539	C=C _{arom} /C=N sym.
1540		C=C _{arom} /C=N sym.	1670	1637	C=C _{arom} /C=N/C=O
1582	1610	C=C _{arom} /C=N asym.	1724	1663	C=C _{arom} /C=N/C=O
1590		C=C _{arom} /C=N asym.			
1730		C=O			

^a The calculated harmonic frequencies were scaled by a factor of 0.9614.

two reasons: (i) vibrational relaxation in medium-sized molecules usually occurs at a faster time scale (<10 ps),^{52,53} and (ii) TRIR spectra of Et-FI⁺ (next section) exhibit a similar short-lived component that is not associated with the blue-shift of absorption bands.

The lowest panel of Figure 7a shows the transient absorption spectra of S₂ and S₁ states obtained using a global analysis of the data with a biexponential decay function (see experimental section). The spectrum of the short-lived S₂ state exhibits absorption bands at 1535, 1605, and 1640 cm⁻¹, while the long-lived S₁ state exhibits absorption band at 1610 cm⁻¹. To assign the 1610 cm⁻¹ frequency, we optimized the S₁ state of FI using the TD-B3LYP/SVP level of theory and calculated its vibrational frequencies (Table 1; also see Supporting Information, Tables S2 and S3 for all calculated frequencies). The optimized geometry of the excited state and the ground state are compared in Figure S3 (Supporting Information). The calculations indicated that the S₁ excited state of FI would exhibit intense vibrational modes at 1582 and 1590 cm⁻¹, which are in good agreement with experimental observation of 1610 cm⁻¹.

By comparing the S₀ (Figure 1) and S₁ (Table 1) frequencies of FI, we can see that the electronic transition causes significant difference in the normal modes of the molecule. For example, the S₀ state of FI exhibits two calculated C=O stretching bands at 1714 and 1748 cm⁻¹, whereas the S₁ state exhibits only one C=O stretching band at 1730 cm⁻¹. In the S₁ excited state, one of the C=O bonds remained the same while the other C=O bond lengthened by 0.09 Å in the S₁ state upon relaxation (Figure S3, Supporting Information). It is interesting to note that, contrary to intuitive expectation, the C=O stretching mode of S₁ state appears at a higher frequency than the corresponding S₀ state. While we did not observe the absorption due to the C=O stretch of the FI S₁ state in our TRIR experiment, TRIR

measurements on riboflavin report a blue-shifted C=O absorption band,⁵⁴ which is consistent with our calculations. In addition to absorption bands due to C=O stretches, the calculation predicts four additional absorption bands due to a combination of aromatic C=C stretch and C=N symmetric and asymmetric modes. One of these frequencies appears at 1590 cm⁻¹, which is in good agreement with the experimentally observed 1610 cm⁻¹ frequency of the FI S₁ state (Table 1). Thus, we assign the 1610 cm⁻¹ absorption band to the combination of aromatic C=C and asymmetric C=N stretching modes of the S₁ state of FI.

TRIR spectra of Et-FI⁺ at several time delays after the 500 nm excitation pulse are presented in Figure 8a. The spectrum obtained 1 ps after the excitation pulse consists of bleaches at four frequencies of the ground state and three excited-state absorption bands at 1539, 1637, and 1663 cm⁻¹. Decays at all frequencies can be fit using a biexponential decay function with lifetimes of 23 and 590 ps. The short-lived 23 ps component was not observed in the visible pump-probe experiment (excitation at 550 nm), but it is present in TRIR data (excitation at 500 nm). This is consistent with the S₂ → S₀ internal conversion, which will be more apparent as the energy of the excitation pulse is higher. As previously discussed, another possible assignment of the 23 ps component is the vibrational relaxation of the S₁ state. We exclude this possibility based on the lack of blue-shifted absorption bands characteristic of vibrational relaxation. Within the initial 23 ps, the two bands at 1637 and 1663 cm⁻¹ undergo changes in their intensities; while the 1663 cm⁻¹ decreases in intensity, the band at 1637 cm⁻¹ grows stronger. Since this red shift is not consistent with vibrational relaxation, we assign this process to the S₂ → S₀ internal conversion. The second decay component has a lifetime of 590 ps and is assigned to the decay of the S₁ (π,π*) state. Using spectral deconvolution, we obtained transient absorption spectra of the S₁ (π,π*) and S₂ (n,π*) states of Et-FI⁺, as shown in the last panel of Figure 8a. Although the 1539 and 1663 cm⁻¹ bands arise from both S₁ and S₂ states, the C=O stretching band at 1637 cm⁻¹ is purely due to vibrational modes of the S₁ state. In order to assign the S₁ bands of Et-FI⁺ to specific vibrational modes, we optimized the S₁ state of Et-FI⁺ using the TD-B3LYP/SVP method and calculated its vibrational frequencies (Table 1; also, see Supporting Information, Tables S4 and S5 for all calculated frequencies). Theory predicts three absorption bands in the spectral region of interest; the 1524 cm⁻¹ band is a combination of aromatic C=C and symmetric C=N stretching modes, while the 1670 and 1724 cm⁻¹ bands are combinations

Figure 8. (a) Upper panel: ground-state FTIR spectrum of Et-FI⁺ in acetonitrile. Middle panel: transient absorption spectra of 10 mM Et-FI⁺ in acetonitrile collected at different time delays after the 500 nm excitation pulse. Lower panel: transient absorption spectra of S₁ and S₂ states of Et-FI⁺ obtained after spectral deconvolution. (b) Decays of transient signals collected at 1636 (green), 1661 (red), 1682 (black) and 1562 (blue) cm⁻¹. Solid curves represent fits obtained using biexponential decay functions with lifetimes $\tau_1 = 28$ ps and $\tau_2 = 590$ ps.

of C=C/C=N and C=O stretching modes. As predicted by theory, the spectrum of the Et-Fl⁺ S₁ state obtained using TRIR data consists of three absorption bands at similar frequencies. Thus, we assign these absorption bands accordingly (Table 1).

Conclusions

We investigated the electronic properties of the flavinium salt Et-Fl⁺ and compared them to those of its parent compound Fl. Steady-state UV-vis absorption and fluorescence measurements demonstrate that both Fl and Et-Fl⁺ exhibit similar spectral features, but the absorption energy of Et-Fl⁺ is substantially lower than that of Fl. We compared the experimental absorption spectrum with those calculated using time-dependent DFT methods and found that the absorption bands of Fl and Et-Fl⁺ originate from transitions to produce S₁ and S₃ (π, π^*) excited states, while the S₂ state is an (n, π^*) state. Cyclic voltammograms of Fl and Et-Fl⁺ allowed us to compare the energies of their HOMO and LUMO levels, and we found that both HOMO and LUMO orbitals of Et-Fl⁺ are stabilized relative to those in Fl, with the stabilization of the LUMO level being more pronounced. Visible and mid-IR pump-probe experiments demonstrate that Et-Fl⁺ exhibits a shorter excited state lifetime (590 ps) relative to that of Fl (several nanoseconds), possibly due to faster thermal deactivation in Et-Fl⁺ as dictated by the energy gap law.⁵⁸ Furthermore, we observed a fast (23–30 ps) S₂ → S₀ internal conversion in transient absorption spectra of both Fl and Et-Fl⁺ in experiments that utilized higher energy pump beams. Computed excited state vibrational frequencies are in good agreement with the experiments and provided some insight into the vibrational motions associated with those frequencies.

Acknowledgment. The ultrafast pump-probe experiments were done in the Ohio Laboratory for Kinetic Spectrometry at BGSU. C. M. H. thanks the Ohio Supercomputer Center and the National Science Foundation for computational and financial support. S. V. acknowledges an Ohio State University Presidential Fellowship.

Supporting Information Available: Calculated vertical excitation energies, vibrational frequencies, dipole moments, difference density plots, and optimized geometries for Fl and Et-Fl⁺. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- Ghisla, S. K., P.; Macheroux, P.; Sund, H. *Flavins and Flavoproteins* Rudolf Weber: 1999.
- Van Berkel, W. J. H.; Kamerbeek, N. M.; Fraaije, M. W. *J. Biotechnol.* **2006**, *124*, 670–689.
- Hastings, J. W.; Balny, C.; Lepeuch, C.; Douzou, P. *Proc. Nat. Acad. Sci.* **1973**, *70*, 3468–3472.
- Meighen, E. A.; Dunlap, P. V. *Physiological, Biochemical and Genetic Control of Bacterial Bioluminescence*, in *Advances in Microbial Physiology*; Academic Press: London, 1993; pp 1–67.
- Punniyamurthy, T.; Velusamy, S.; Iqbal, J. *Chem. Rev.* **2005**, *105*, 2329–2363.
- Gelalcha, F. G. *Chem. Rev.* **2007**, *107*, 3338–3361.
- Kemal, C.; Bruice, T. C. *Proc. Nat. Acad. Sci.* **1976**, *73*, 995–999.
- Bruice, T. C.; Noar, J. B.; Ball, S. S.; Venkataram, U. V. *J. Am. Chem. Soc.* **1983**, *105*, 2452–2463.
- Ball, S.; Bruice, T. C. *J. Am. Chem. Soc.* **1979**, *101*, 4017–4019.
- Murahashi, S. I.; Oda, T.; Masui, Y. *J. Am. Chem. Soc.* **1989**, *111*, 5002–5003.
- Imada, Y.; Iida, H.; Ono, S.; Murahashi, S. I. *J. Am. Chem. Soc.* **2003**, *125*, 2868–2869.
- Imada, Y.; Iida, H.; Naota, T. *J. Am. Chem. Soc.* **2005**, *127*, 14544–14545.
- Minidis, A. B. E.; Backvall, J. E. *Chem.—Eur. J.* **2001**, *7*, 297–302.
- Linden, A. A.; Kruger, L.; Backvall, J. E. *J. Org. Chem.* **2003**, *68*, 5890–5896.
- Kemal, C.; Chan, T. W.; Bruice, T. C. *Proc. Nat. Acad. Sci.* **1977**, *74*, 405–409.
- Bruice, T. C. *J. Chem. Soc. Chem. Comm.* **1983**, *14*, 15.
- Mager, H. I. X.; Tu, S. C. *Tetrahedron* **1994**, *50*, 6759–6766.
- Mazzini, C.; Lebreton, J.; Furstoss, R. *J. Org. Chem.* **1996**, *61*, 8–9.
- Murahashi, S. I.; Ono, S.; Imada, Y. *Angew. Chem., Int. Ed.* **2002**, *41*, 2366–2368.
- Imada, Y.; Iida, H.; Murahashi, S. I.; Naota, T. *Angew. Chem. Int. Ed.* **2005**, *44*, 1704–1706.
- Li, G.; Glusac, K. D. *J. Phys. Chem. B* **2009**, *113*, 9059–9061.
- Li, G.; Sichula, V.; Glusac, K. D. *J. Phys. Chem. B* **2008**, *112*, 10758–10764.
- Li, G.; Glusac, K. D. *J. Phys. Chem. A* **2008**, *112*, 4573–4583.
- Furche, F.; Ahlrichs, R. *J. Chem. Phys.* **2004**, *121*, 12772–12773.
- Sichula, V.; Hu, Y.; Mirzakulova, E.; Manzer, S. F.; Vyas, S.; Hadad, C. M.; Glusac, K. D. *J. Phys. Chem. B* **2010**, DOI: 10.1021/jp104443y.
- Nikolaichik, A. V.; Korth, O.; Rodgers, M. A. J. *J. Phys. Chem. A* **1999**, *103*, 7587–7596.
- Li, G.; Parimal, K.; Vyas, S.; Hadad, C. M.; Flood, A. H.; Glusac, K. D. *J. Am. Chem. Soc.* **2009**, *131*, 11656–11657.
- Gampp, H.; Maeder, M.; Meyer, C. J.; Zuberbühler, A. D. *Talanta* **1985**, *32*, 95–101.
- Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, Revision C.02; Gaussian, Inc.: Wallingford, CT, 2004.
- Lee, C. T.; Yang, W. T.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785–789.
- Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648–5652.
- Tomasi, J.; Persico, M. *Chem. Rev.* **1994**, *94*, 2027–2094.
- Cossi, M.; Barone, V.; Cammi, R.; Tomasi, J. *Chem. Phys. Lett.* **1996**, *255*, 327–335.
- Cossi, M.; Barone, V. *J. Chem. Phys.* **1998**, *109*, 6246–6254.
- Barone, V.; Cossi, M.; Tomasi, J. *J. Chem. Phys.* **1997**, *107*, 3210–3221.
- Barone, V.; Cossi, M.; Tomasi, J. *J. Comput. Chem.* **1998**, *19*, 404–417.
- Cramer, C. J.; Truhlar, D. G. *Chem. Rev.* **1999**, *99*, 2161–2200.
- Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R. E.; Stratmann, O.; Yazyev, A. J.; Austin, R.; Cammi, C.; Pomelli, J. W.; Ochterski, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09*; Gaussian, Inc.: Wallingford, CT, 2009.
- Ahlrichs, R.; Bar, M.; Haser, M.; Horn, H.; Kolmel, C. *Chem. Phys. Lett.* **1989**, *162*, 165–169.
- Scott, A. P.; Radom, L. *J. Phys. Chem.* **1996**, *100*, 16502–16513.
- Sikorski, E.; Khmelinskii, I. V.; Prukala, W.; Williams, S. L.; Patel, M.; Worrall, D. R.; Bourdelande, J. L.; Koput, J.; Sikorski, M. *J. Phys. Chem. A* **2004**, *108*, 1501–1508.
- Kucheryavy, P.; Li, G. F.; Vyas, S.; Hadad, C.; Glusac, K. D. *J. Phys. Chem. A* **2009**, *113*, 6453–6461.
- Vyas, S.; Hadad, C. M.; Modarelli, D. A. *J. Phys. Chem. A* **2008**, *112*, 6533–6549.

- (44) Wang, J.; Kubicki, J.; Burdzinski, G.; Hackett, J. C.; Gustafson, T. L.; Hadad, C. M.; Platz, M. S. *J. Org. Chem.* **2007**, *72*, 7581–7586.
- (45) Burdzinski, G.; Hackett, J. C.; Wang, J.; Gustafson, T. L.; Hadad, C. M.; Platz, M. S. *J. Am. Chem. Soc.* **2006**, *128*, 13402–13411.
- (46) Ksenzhek, O. S.; Petrova, S. A. *Bioelectrochem. Bioenerg.* **1983**, *11*, 105–127.
- (47) Dryhurst, G. *Electrochemistry of Biological Molecules*, in *Electrochemistry of Biological Molecules*; Academic Press: London, 1977; pp 365–391.
- (48) Niemz, A.; Imbriglio, J.; Rotello, V. M. *J. Am. Chem. Soc.* **1997**, *119*, 887–892.
- (49) Tatwawad, S.; Santhana, S.; Bard, A. J. *J. Electroanal. Chem.* **1968**, *17*, 411.
- (50) Trasatti, S. *Pure Appl. Chem.* **1986**, *58*, 955–966.
- (51) Hemmerich, P.; Knappe, W. R.; Kramer, H. E. A.; Traber, R. *Eur. J. Biochem.* **1980**, *104*, 511–520.
- (52) Sension, R. J.; Repinec, S. T.; Szarka, A. Z.; Hochstrasser, R. M. *J. Chem. Phys.* **1993**, *98*, 6291–6315.
- (53) Lenz, K.; Pfeiffer, M.; Lau, A.; Elsaesser, T. *Chem. Phys. Lett.* **1994**, *229*, 340–346.
- (54) Wolf, M. M. N.; Schumann, C.; Gross, R.; Domratcheva, T.; Diller, R. *J. Phys. Chem. B* **2008**, *112*, 13424–13432.
- (55) Hamm, P.; Ohline, S. M.; Zinth, W. *J. Chem. Phys.* **1997**, *106*, 519–529.
- (56) Hirata, Y.; Mashima, K.; Fukumoto, H.; Tani, K.; Okada, T. *Chem. Phys. Lett.* **1999**, *308*, 176–180.
- (57) Sytina, O. A.; van Stokkum, I. H. M.; Heyes, D. J.; Hunter, C. N.; van Grondelle, R.; Groot, M. L. *J. Phys. Chem. B* **2010**, *114*, 4335–4344.
- (58) Englman, R.; Jortner, J. *Mol. Phys.* **1970**, *18*, 145–&.
- (59) Hare, P. M.; Crespo-Hernandez, C. E.; Kohler, B. *Proc. Nat. Acad. Sci.* **2007**, *104*, 435–440.
- (60) Hare, P. M.; Crespo-Hernandez, C. E.; Kohler, B. *J. Phys. Chem. B* **2006**, *110*, 18641–18650.
- (61) Abe, M.; Kyogoku, Y. *Spectrochim. Acta A* **1987**, *43*, 1027–1037.
- (62) Abe, M.; Kyogoku, Y.; Kitagawa, T.; Kawano, K.; Ohishi, N.; Takaisuzuki, A.; Yagi, K. *Spectrochim. Acta A* **1986**, *42*, 1059–1068.
- (63) Kondo, M.; Nappa, J.; Ronayne, K. L.; Stelling, A. L.; Tonge, P. J.; Meech, S. R. *J. Phys. Chem. B* **2006**, *110*, 20107–20110.

JP106288S