THE EXCITED STATE BEHAVIOR OF IMINIUM DERIVATIVES AND THEIR REDUCED FORMS

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ABSTRACT

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Photoinduced heterolytic bond cleavage is at the heart of many photochemical researches in renewable energy and biocatalysis. This dissertation outlines studies of photochemical heterolytic reactions involving the –OH and \( H^- \) release from the iminium derivatives. In specific, the heterolytic properties of the iminium salt derivatives, such as N(5)-ethyl-4a-hydroxyflavin (Et-FlOH), 9-hydroxy-10-methyl-9-phenyl-9,10-dihydroacridine (AcrOH), and 10-methyl-9-phenyl-9,10-dihydroacridine (AcrH), were studied using transient absorption (TA) spectroscopy method.

In the first part, the heterolytic property of Et-FlOH is studied, which is also related to understanding the photocatalytic mechanism of the bacterial bioluminescence. The heterolysis is not observed from the TA study of Et-FlOH except a fast \( S_1 \rightarrow S_0 \) decay. Combining with the results of time-dependent density functional theory calculations of Et-FlOH excited-states, a \( S_1 \rightarrow S_0 \) conical intersection (CI) is approved as the most important role for this fast decay. This identification proves that a rigid bio-condition is required to inhibit the formation of CI for this photocatalytic bacterial bioluminescence.

In the second part, the excited state behavior of AcrOH is studied in different solvents via UV-vis TA spectroscopy. A fast heterolytic cleavage (\( \tau = 108 \) ps) coupled with a –OH release is observed in the protic solvent, while intersystem crossing is observed in the aprotic solvent. This photoinduced heterolytic behavior exhibits the characteristic required for pOH jump catalytic research, such as the conformational changes in DNA/RNA and the release of drugs from host molecules.
In the last part, a photoinduced hydride release from AcrH was investigated using TA spectroscopy. The hydride release is postulated to be a stepwise electron/H-atom transfer process from the triplet excited state of AcrH, and O₂ acts as an important electron acceptor in this process. These results show the high potential that, coupled with an inorganic catalyst, AcrH can be used as a photocatalyst in a bi-catalyst catalytic H₂ production system.
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CHAPTER I: INTRODUCTION

My Ph.D research is focused on the excited state behavior study of iminium derivatives in photochemistry and photophysics. Ultrafast transient absorption (TA) spectral analysis is the main technique in my research. In this chapter, the generation and decay of the excited state and the target compounds for the study of the excited state will be introduced.

1.1 Photochemistry

Photochemistry, a science studying light related chemistry, focuses on the production, structure characteristics, physical behavior, and chemical behavior of the electronic excited state of various materials.\textsuperscript{1,2} The electronic excited state of a molecule is normally achieved through a photon excitation process. The interaction of a photon with a molecule can be generally described in a very simple form:

\[ R + h\nu = R^* \]  \hspace{1cm} (1.1)

where $R$ is a ground state molecule, $h\nu$ is the energy of absorbed photon, and $R^*$ is the molecule in an electronically excited state. The laser is a very powerful tool which can be used to study the excited state behavior of molecules due to its two characteristics: (i) its spatial coherence allows it to be focused on a tight spot, and (ii) its temporal coherence allows it to be converted to units as short as a femtosecond (fs) pulses of light. The first characteristic is helpful to achieve a locally bigger excited state molecule to ground state molecule ratio. The second one allows us to study the early excited state behavior after excitation because the absorption spectrum cannot be collected during excitation. Ultrafast TA spectroscopy, the simplest approach and the most common laser technique of photochemical study, is an important method to investigate the excited state of molecules.
1.1.1 The Generation and Decay of The Excited State

The major difference between photochemistry and thermal chemistry is that the photochemical reaction is initiated or takes place where the molecule is in an excited state. When a molecule absorbs a photon, one electron of this molecule will be excited to a higher energy orbital, thereby causing an electronic structure change in this molecule. The state of this electronic structure change in this molecule is called the electronically excited state. The electron in the higher energy orbital is not stable and prefers a lower energy orbital; therefore, the excited state molecule will decay to a lower energy state. The decay of the molecule in the excited state comes through either a chemical or physical change. The chemical decay is normally coupled with a new product generation, and it shows up as an electron transfer (ET) process, or breaking a bond. The physical decay could result in nonradiative transition (heat release), radiative transition (lower energy light emitting process), and energy transfer. Nonradiative transition could result in vibrational relaxation, intersystem crossing, and internal conversion, radiative transition contains fluorescence and phosphorescence, and energy transfer is mainly an intermolecular process. The generation and decay of the excited state of a molecule can be well described in a Jablonski diagram. (Scheme 1.1)

Scheme 1.1. Jablonski Diagram Showing the Generation and Decay of the Excited State.
The basic concepts are presented in this basic photophysics module. \( S_0, S_1, \) and \( S_2 \) are singlet, and \( T_1 \) are triplet states. Radiative and nonradiative transitions are indicated by straight and squiggly arrows, respectively. IC: Internal Conversion, ISC: Intersystem Crossing (Spin-flip process), VR: Vibrational Relaxation.

1.1.2 Electron Transfer

The photoinduced ET is a fundamental excited state deactivation process. It is the transfer of an electron from a donor to an acceptor, and either the donor or the acceptor is in an excited state before the ET. Figure 1.1 shows the basics of the excited state ET process.

Figure 1.1. A schematic presentation of a photoinduced ET process. D (A) and D* (A*): ground and excited states electron donor (acceptor),
The free energy change of the photoinduced ET is given by eq 1.2. It can be used to calculate the energy change resulting from the electron donor to the excited electron acceptor,\(^3\)

\[
\Delta G^0 = e (E_{ox}^0 - E_{red}^0) \tag{1.2}
\]

where \(e\) is the elementary charge, \(E_{ox}^0\) is the one-electron oxidation potential of the donor, \(E_{red}^0\) is the one-electron reduction potential of the excited state acceptor, \(E_{red}^0\) equals the sum of the one-electron reduction potential of the ground state acceptor and the energy of the excitation wavelength. Experimentally, a linear free energy \((-\Delta G^0)\) relation relates the logarithm of a reaction rate constant or equilibrium constant for a reaction. This misleads one to thinking that the rate should increase as we increase \(-\Delta G^0\) for the reaction. This behavior is only true for a limited extent in \(\Delta G^0\).

\[\text{Figure 1.2.}\] The potential energy surfaces for the reactants (Red) and products (Dark blue) in an ET process. \(\Delta G^0\): Free energy for an ET, \(\Delta^\dagger G\): Activation energy, \(\lambda\): Reorganization energy, and \(q_R^0, q^*, q_P^0\): Reaction coordinates of reactants, transition state, and products.
In 1956, Rudolph Marcus explained the relationship between the rate of a reaction and the free energy for an ET process. (Figure 1.2) His theory states that the probability of an ET in a donor-acceptor system during a transition state will decrease with an increasing donor-acceptor distance ($R_{ed}$), and the rate constant of ET ($k_{ET}$) is controlled by the donor-acceptor distance, the activation energy ($\Delta \overline{G}$), and the reorganization energy ($\lambda$), as shown:

$$k_{ET} = \frac{2\pi h V_0^2}{\hbar^2} \exp(-\beta R_{ed}) \frac{1}{\sqrt{4\pi \lambda kT}} \exp \left[ \frac{-\Delta \overline{G}}{kT} \right]$$

(1.3)

where $\beta$ is the distance coefficient, $R_{ed}$ is the edge to edge donor-acceptor distance, $V_0$ is the orbital overlap integral when $R_{ed} = 0$, $k$ is the Boltzmann constant, and $T$ is the absolute temperature. The $\Delta \overline{G}$ is determined by the $\Delta G^0$ and the $\lambda$, as shown:

$$\Delta \overline{G} = \frac{(\Delta G^0 + \lambda)^2}{4\lambda}$$

(1.4)

The $\lambda$ is an energy change resulting from the molecular rearrangement that is composed of outer solvational and inner vibrational components. The detailed discussion on $\lambda$ can be found in Marcus’s later works. Equation 1.3 can be simplified by introducing a pre-exponential factor (A):

$$k_{ET} = A \exp \left[ \frac{-(\Delta G^0 + \lambda)^2}{4\lambda kT} \right]$$

(1.5)

Equation 1.5 expresses a nonlinear behavior expected for the dependence(s) of the $k_{ET}$ on the $-\Delta G^0$ or (and) $\lambda$ (Figure 1.3). As shown in Figure 1.3 A and C, when the $-\Delta G^0$ increases in the positive direction, and $\lambda$ remains a constant, the $k_{ET}$ accordingly increases in the initial region and gets to the maximum at $-\Delta G^0 = \lambda$. This region is called the normal region. After reaching the maximum, increasing $-\Delta G^0$ ($-\Delta G^0 > \lambda$) will result in a deceasing of the $k_{ET}$. 
This is the region called the inverted region. The same nonlinear relationship exists between $k_{ET}$ and $\lambda$ is obtained when $\Delta G^0$ is retained as a constant as Figure 1.3 B and C shown.

Figure 1.3. Generic Marcus theory diagram for an intermolecular ET. A and B: the relative position changes of potential energy surfaces of the reactant (red) and product (dark blue) when the free energy ($\Delta G^0$) or reorganization energy ($\lambda$) is retained as a constant and C: the plot of the rate constants ($k_{et}$) as a function of $\Delta G^0$ or $\lambda$ change.

1.1.3 Conical Intersection

Computational chemistry studies show an energy surface crossing (Conical intersection, CI), which is a main way for a nonradiative transition (internal conversion) of the excited state. This surface crossing functions as a funnel that permits transition from one energy surface (state) to another, as shown in Scheme 1.2. Through the CI, the excited reactant energy surface relaxes quickly to either the ground reactant energy surface (photophysical channel) or the ground product energy surface (photochemical channel) without luminescence.  

Scheme 1.2. A Schematic Representation of the Transition from the Excited State.
The CI plays an important role in the mechanistic photophysics and photochemistry.\textsuperscript{11,12} The most important characteristic of the CI is the ability to induce an ultrafast internal conversion. For example, the CI stabilizes DNA and RNA with respect to UV irradiation.\textsuperscript{13} The ultrafast non-radiative transitions of adenosine (0.29 ps), guanosine (0.46 ps), cytidine (0.54 ps), and thymidine (0.72 ps) in an aqueous solution\textsuperscript{14} and guanine (0.8 ps), cytosine (3.2 ps), uracil (2.4 ps), and thymine (6.4 ps) in the gas phase\textsuperscript{15} have been investigated, and it has been suggested that a CI is a possible way to produce this process. Later, Yamazaki and Kato did theoretical work which is focused on the solvent effects on the geometries and energies of excited and ground states and the CIs between them.\textsuperscript{16} They found that the structures of the CIs for these DNA and RNA bases are largely affected by the solvent. In an aqueous solvent, the energy surface of the excited state is close to the energy surface of the ground state, thereby largely enhancing the vibronic coupling between these states and creating more CIs in a wide area of the geometry space. This is, for these DNA/RNA bases, why the decay dynamics of the excited states in a polar solvent is much faster than that in the gas phase.

\subsection*{1.1.4 Bond Cleavage}
Chemically, the decay of an excited state is accomplished through a chemical reaction, which principally includes bond(s) cleavage and bond(s) forming. These processes proceed in two ways, i.e. either cleavage occurs in a singlet excited state or, after intersystem crossing, the bond cleavage occurs in the triplet excited state. Due to the short lifetime of the singlet excited state, CI normally has an important role in achieving the bond cleavage process from this state, and the multistep cleavage process is achieved from a triplet excited state, such as photoinduced bond cleavage reactions of ketones and their derivatives. As shown in Figure 1.4, a Norrish type II photochemical reaction of 2-pentanone represents a chemical excited state deactivation through a process of an intersystem crossing and a hydrogen atom abstraction.

**Figure 1.4.** A photoinduced Norrish type II hydrogen abstraction. The 2-pentanone is excited to the singlet excited state (S₁). Because the intersystem crossing process is significantly faster than the S₁ hydrogen abstraction process, most of the S₁ of 2-pentanone is converted to its triplet excited state (T₁), and the T₁ hydrogen abstraction becomes the dominant process.

**Figure 1.5.** Photoinduced homolytic and heterolytic bond cleavages.
There are two types of bond cleavage: homolytic and heterolytic cleavages (Figure 1.5). The homolytic cleavage coupled with the yielding of a pair of radicals is the main fission form for a covalent bond cleavage.\textsuperscript{26-28} The radicals generated from homolysis are important intermediates or initiators in organic synthesis, biological transformations, and polymer chemistry. Comparing to the homolytic cleavage, the heterolytic cleavage coupled with the generation of a cation and an anion (Figure 1.5) has barely attracted attention, although there are some examples, such as the photolysis of alkyl, vinyl, and phenyl halides in solution.\textsuperscript{27,29-32}

\textbf{Figure 1.6.} Heterolytic C-halide bond cleavage of alkyl, vinyl, and phenyl halides.

Solvents play an important role in decay of electronically excited molecules, especially in the excited state bond cleavage process.\textsuperscript{33-37} A combination of medium effects (polarity, polarizability, and protic ability) may be decisive in controlling the bond cleavage process. In the gas phase, the bond dissociation enthalpy of homolysis is far lower than heterolysis, so the homolysis is the dominant process, normally.\textsuperscript{30} However, heterolysis can also be achieved with solvent assistance. The ionic products in the solution derive much of their stability from solvation effects and are generated easily in more polar solvent.\textsuperscript{38} A well delocalized structure of
the product ion will decrease the electrophilicity or the nucleophilicity of itself, thereby stabilizing the product and decreasing the recombination rate. There are some examples that show the different solvent effect on the heterolysis process, such as the C-halide bond heterolysis of alkyl halides\textsuperscript{30,32} and the C-O bond heterolysis of 9-phenylxanthen-9-ol\textsuperscript{39} and 9-fluorenol (Figure 1.7).\textsuperscript{38,40}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{structures.png}
\caption{Structures of 9-fluorenol (1), 9-phenylxanthen-9-ol (2), diphenylmethyl halide (3), and their cations and radicals generated upon irradiation.}
\end{figure}

The heterolysis of the C-halide bond has been well studied and summarized by Bartl and Kropp.\textsuperscript{30,32} The solvation energy plays an important role in the C-halide bond cleavage process. The gas-phase bond dissociation enthalpies of Ph\textsubscript{2}CHCl are 64 kcal/mol (homolysis) and 150 kcal/mol (heterolysis), respectively. The solvation energy of the radical product is very small. Interestingly, the value for heterolysis of ground-state Ph\textsubscript{2}CH-Cl in ACN is only 7-17 kcal/mol, which is lower than 64 kcal/mol for homolysis, thereby making heterolysis more favored than homolysis. Later, the ps TA spectroscopy studies of this photoinduced homolysis and heterolysis in ACN clearly describe that both radical pairs and ion pairs are produced from the excited
singlet state. Finally, the fs TA spectroscopy studies on this compound well describe the solvent effect in this C-halide bond cleavage process. (Figure 1.8)

![Figure 1.8.](image)

The solvent (A: Cyclohexane, B: ACN) effect on C-Cl bond cleavage of diphenylmethylchloride (DPMC).

![Figure 1.9.](image)

Photolysis of 9-phenylxanthen-9-ol (9-PhX-OH) and 9-fluorenol (9-F-OH) in aprotic (A) and protic (B) solvents.

The systematic studies on the 9-phenylxanthen-9-ol were carried out by Minto (Figure 1.9). In protic solvents (water or methanol), the heterolytic cleavage of the C-O bond is the main pathway to generate an ion pair. The homolytic cleavage is the only pathway in aprotic and nonpolar solvents (cyclohexane or benzene). In a polar aprotic solvent (acetonitrile), heterolytic cleavage takes place but is a minor process. The similar studies on 9-fluorenol and its methyl ether form in different polarity and different protic ability solvents were carried out by Wan (Figure 1.9). Only the protic solvent can initialize the heterolysis process. For both cases, the increased yield of the corresponding cation with the increasing ratio of water in the solvent
mixture can be attributed to a lowering of the free energy necessary photosolvolytic reactivity in an aqueous solution.

### 1.2 Iminium Ions and Their Derivatives

Our group is focusing on the catalytic performance study of iminium ions and their derivatives. Iminium ion, which has the general structure as shown in Figure 1.10, is an important active intermediate in the catalytic cycle.\(^{43,44}\) It is normally synthesized by reaction of the primary or secondary amine with an aldehyde or ketone under an acidic condition.\(^{43,45}\) Due to its strong electrophilicity, the iminium ion can be converted to an amine under a neutral or nucleophilic condition, such as 1,2- and 1,4-additions shown in Figure 1.10.\(^{46,47}\)

![Figure 1.10](image.png)

**Figure 1.10.** Formation and nucleophilic 1,2- and 1,4-addition on iminium and enamine (\(R_2 = \text{alkenyl}\)) ions.

The new formed C-Nu covalent bond of the nucleophilic addition products, the amine or enamine shown in Figure 1.10, normally is highly polarized, so it shows different stabilities depending on the compound structures and solvent environments. A good understanding of the excited state behavior of the C-Nu bond will be very valuable to perfect the catalytic performance of iminium ions and their derivatives,\(^{43,44}\) so my Ph.D research is focused on the excited state behavior studies of iminium ions and their derivatives. TA spectroscopy will be the main technique used in my study. In the following chapters, the principle of TA spectroscopy
will be introduced, and the excited state behavior of three iminium derivatives will be investigated using TA spectroscopy technique. (Scheme 1.3)

**Scheme 1.3. The Structures of Iminium Derivatives:** 5-Ethyl-4a-hydroxy-3-methyl-4a,5-dihydrolumiflavin (Et-FlOH), 9-Hydroxy-10-methyl-9-phenyl-9,10-dihydroacridine (AcrOH), and 10-Methyl-9-phenyl-9, 10-dihydroacridine (AcrH).

**References:**


CHAPTER II: ULTRAFAST TRANSIENT ABSORPTION SPECTROSCOPY

2.1 Introduction

In chemistry, it is very important to understand fundamentals of the dynamics in any chemical reaction on a molecular level, such as the radiative decay, the rotational motion, and the vibrational motion. These dynamics mentioned above normally are ultrafast processes and can be studied using spectroscopy, so desired spectroscopy techniques are required to investigate these processes. The nanosecond (ns, $10^{-9}$ s) and picosecond (ps, $10^{-12}$ s) laser spectroscopy techniques were invented in 1960s and 1970s, respectively. These two techniques allow the researcher to study the radiative decay (ns scale) and the rotational motion (ps scale) in a chemical reaction. As the research on the fundamental processes of chemical reaction is further carried out, the requirement on the time resolution of laser spectroscopy needs to become faster. In order to understand the ultrafast vibrational motion processes in chemical reactions, such as bond cleavage, electron transfer, and energy transfer, researchers developed the laser system on a femtosecond (fs) scale in 1980s.\(^1\) The time resolution on fs scale allows us to “freeze” structures of reactants prior to their vibrational and rotational motions and observe a continuous process for a chemical reaction from reactants to transition states to products,\(^2\) thereby answering the pertinent questions about the dynamics of the chemical reaction.

Our group is interested in various ultrafast processes in chemical reactions, so we built our fs transient absorption (TA) laser system in our lab. The purpose of this chapter is to describe the principles of laser and fs laser pulse generation, ultrafast TA spectroscopy systems, and data processing and analysis.
2.2 Laser Basics and Femtosecond Laser Pulse Generation

For the application of fs laser photolysis in chemistry, it is important to know the basic principles of laser and ultra-short pulses generation. A better understanding of the laser theory can be found in Rulliere’s book.³

2.2.1 Basic Laser Principles

Lasers are devices that emit light (electromagnetic radiation) through a process of optical amplification based on the stimulated emission of photons. The photoinduced stimulated emission is shown in Figure 2.1A. One molecule is pumped to an excited state \( E_2 \) from the ground state \( E_1 \) by a certain wavelength photon. Another photon with the lower energy than the excited electron is applied to interact with the excited electron. Two photons will be emitted while the molecule is relaxing back to the ground state. These two photons have the same phase, frequency, polarization, and direction of travel as the incoming photon. Through this method, one photon is cloned to two photons. This stimulated emission process is the key role for the light amplification in the laser system.

![Figure 2.1](image.png)

**Figure 2.1.** Light emitting process in laser systems. A. The one photoinduced stimulated emission. B. The four-level laser system.
In reality, whether we can achieve the overall emission amplification depends on the number of molecules in the excited state vs. lower energy state in the laser media (Figure 2.1 B). The number of the excited molecules on the higher energy state has to be larger than the number on the lower energy state. This is what we call population inversion, which is easy to achieve in a four-level laser system. A four-level laser is the most practical laser system shown in Figure 2.1B. Electron excitation \((E_1 \rightarrow E_2)\) and radiationless transition \((E_2 \rightarrow E_3)\) are fast processes and can be achieved in 10 ns, so the electron population of \(E_3\) can be very large if the lifetime of \(E_3\) to \(E_4\) is significantly longer than 10 ns. The radiationless transition \((E_4 \rightarrow E_1)\) is also a very fast process, so the molecular population of \(E_4\) is close to zero.

2.2.2 Mode-locking

Mode-locking is the most important technique in the ultrafast laser generation. By using this technique, the ultrafast laser can be concordant to extremely short duration (fs) and high power pulses of light in a laser resonant cavity. After excitation, a laser medium simultaneously lases in many different longitudinal modes (the more, the better); however, the phases of these different modes are completely independent. This will produce random superposition in the intensity over time. By the mode-locking technique, a fixed phase relationship between the modes can be achieved, thereby making it possible to estimate when the maximum intensity will occur (Figure 2.2). There are two ways to mode-lock a laser in a cavity: (1) active mode-locking, which uses a physical device to modulate the phases of the cavity modes; (2) passive mode-locking, which uses an intensity dependent loss-mechanism to damp less intense radiation out and keep high intense pulse oscillating between two high reflection mirrors in the cavity. Kerr-lens mode-locking is the most successful passive mode-locking technique in the ultrafast laser field.
Figure 2.2. Mode-locking. Inset shows that different modes in laser resonant cavity. L: the cavity length, n: an integer known as the mode order, $\lambda_n$: wavelength. By tuning L, the modes can be chosen to be locked.

The optical Kerr effect (OKE) describes a nonlinear response of an optical medium (e.g. the Ti:sapphire laser crystal itself) to the electric field of an electromagnetic wave. This effect will allow us to selectively keep the higher intensity part of input beam as the only resident in the laser resonant cavity. The principle of the OKE will be discussed in section 2.3.2 (nonlinear optics). Through the OKE, a Kerr-lens can be used to achieve the mode-locking. By applying both the OKE and Mode-locking theories, the desired narrow range waves are focused, and the beam size becomes smaller, so the losses induced by the pinhole are decreased. After several round trips, the waves are amplified and more focused. Finally, the fs laser pulse can be sent out with very few losses.

2.2.3 Pulse Duration Measuring
It is generally difficult to measure a sample during the excitation, so it is usually necessary to wait until the end of the excitation to analyze the behavior of the excited state. Based on this rigorous requirement, the pulse duration has to be precise for an accurate measurement.

Mandelstam’s work points out that there is a limit to the information we can obtain from the energy, $E$, and time, $t$, variables of a system:

$$\Delta E \Delta t \geq \frac{\hbar}{2} \quad (2.1)$$

For photons, $\Delta E = \hbar \Delta \omega$

So,

$$\Delta \omega \Delta t \geq \frac{1}{2} \quad (2.2)$$

For a laser pulse, $\Delta \omega$ and $\Delta t$ describe the spectral bandwidth and the temporal duration of the pulse, respectively. Based on this relationship, there must be a broad spectrum for an fs laser pulse; however, a broad spectrum does not offer an ultra-short laser pulse time duration. Fourier analysis can be applied to this calculation to achieve a pulse limit:

$$\Delta \nu \Delta t \geq K \quad (2.3)$$

where $\Delta \nu$ and $\Delta t$ are defined as the spectral Full Width at Half Maximum (FWHM) and the temporal duration at half maximum of the pulse, respectively. $K$ is a constant number depending on the shape of the pulse: for a Gaussian shape pulse, $K = 0.441$; for a sech$^2$ shape pulse, $K = 0.315$. For a Gaussian shape spectrum of 12 nm FWHM centered at 800 nm, the limited pulse duration can be calculated as:

$$\frac{\Delta \nu}{\nu_0} = \frac{\Delta \lambda}{\lambda_0} \quad (2.4)$$
where $\Delta \lambda$ is defined as the spectral FWHM, and $\nu_0$ and $\lambda_0$ are the central frequency and wavelength. By uniting Equation 2.4 with Equation 2.3, the temporal duration at half maximum of this 800 nm pulse is calculated as around 80 fs.

The laser pulse duration can also be measured by a second-order autocorrelation technique, a dual-beam frequency-doubling process. The basic principle to operate an autocorrelator for a pulse duration measurement is explained as follows. The input beam is separated into two copies by a beam splitter, and then these copies are overlapped in a nonlinear crystal to interact based on some nonlinearity, provided by their pulses overlapping temporarily. As shown in Figure 2.3:

![Figure 2.3. Setup of an intensity autocorrelator. DL: Delay-line, BS: Beam-splitter, DT: Detector, NC: Nonlinear crystal, $\omega$: Input beam frequency, $2\omega$: SHG or SFG frequency, $k_1$ and $k_2$: Momentums of two input beams. Up-left inset shows a delay-line controlled pulse overlap in the nonlinear crystal.](image)

By tuning the delay-line, the overlap of two pulses can be regulated infinitely from zero to a maximum which corresponds to the intensity change of the SFG of two input beams in the nonlinear crystal. This intensity change gives a symmetrical autocorrelation trace. For instance,
the FWHM ($\Delta \tau$) of this autocorrelation trace can be converted to FWHM pulse duration ($\Delta t$) of a Gaussian shape pulse: \(^3\)

$$\Delta t = \Delta \tau / 1.414$$ \hspace{1cm} (2.5)

### 2.2.4 fs Pulse Amplification

If the generation of fs laser pulse is a milestone of fs laser technology, the chirped pulse amplification technique is the second milestone of this technology.\(^6,7\) The fs laser pulse generation can induce nonlinear phenomena, such as SHG or white light generation. It requires peak power on a GW/cm\(^2\) scale. However, the peak power of the original fs laser pulse from resonant cavity is only in MW/cm\(^2\), so the original fs laser pulse has to be amplified for experimental applications.

For an fs pulse amplification (Figure 2.4), a gain medium is used. It is pumped by an external pump source. The original fs pulse is used as a seed beam for a stimulated emission process in the pumped gain medium. The main difference between this pulse amplification resonant cavity and seed fs pulse resonant cavity is that the stimulated emission and oscillation are controlled by two Pockels cells and a broadband polarizer in the former. Both Pockels cells work as controllable quarter-wave plates and rotate the through beam 90 degrees for a round-trip when it is on.
Figure 2.4. Regenerative amplification. A) Injection of the seed pulse in the cavity: When the input Pockels cell is on, and acts as a quarter-wave plate, oscillation and amplification of the pulse are processing in the cavity. B) Amplified pulse dump: When the output Pockels cell is on and changes the polarization of the pulse, amplified pulse is sent out. Note when the input Pockels cell is off, output polarizer dumps the seed pulse out.

By this technique, the pulse energy can be amplified from nJ/pulse to mJ/pulse after tens of passes in the gain medium. If the fs pulse energy achieves mJ, the pulse power will be on the GW scale. This power will reach the damaging threshold of the gain medium crystal. To avoid this kind of damage, the fs seed pulse has to be stretched to hundreds of ns before it is sent into the amplifier. After amplification, the pulse duration can be compressed into fs scale but not as short as unstretched seed pulse due to the loss of parts of the input spectrum. For example, the stretching grating is usually too small to diffract the whole spectrum of the fs seed pulse; thus, its duration is increased.

2.3 Ultrafast Transient Absorption Spectroscopy Systems

The ultrafast TA laser systems used in our lab are shown in Figure 2.5. The 800 nm (130 fs, 1 KHz) Hurricane output beam is split into two beams for pump (OPA or Tripler) and probe (TOPAS or CaF₂) frequencies generation. The output from TOPAS is split to probe and
reference beams for mid-IR measurement. A CaF$_2$ crystal is used to generate a UV-vis probe beam for the 350-800 nm range measurement with a CCD camera as the detector. The detection of mid-IR probe beams is achieved by using 2 x 32 arrays of mercury cadmium telluride (MCT) elements.

**Figure 2.5.** fs TA system setup for the UV-vis and mid-IR region measurements. The inset shows the details of fs laser source (Hurricane).

### 2.3.1 Laser Source

A Ti:Sapphire oscillator/amplifier four-level laser system (Hurricane) provided by Spectra Physics is used as our laser source. It includes five parts: a Ti:Sapphire oscillator, a 527 nm pump source, a regenerative amplifier, a stretcher, and a compressor (Figure 2.5). This system generates 130 fs pulses at 800 nm, 1 kHz frequency, and 1 W power (1 mJ/pulse, 1K pulse/s). In order to generate ultrafast optical pulses, two titanium-sapphire (Ti:sapphire) lasing mediums are used in this system. They are sapphire crystals doped with titanium ions. One is in the oscillator and the other one is in the regenerative amplifier.

The Ti:sapphire crystal in the oscillator is pumped by a 532 nm laser. A diode laser pumped neodymium doped yttrium orthovanadate (ND:YVO$_4$) crystal is used to generate a 1064
nm laser, which is frequency doubled through a lithium triborate (LBO) crystal to 532 nm as the Ti:sapphire crystal pump source. Through a set of prisms, the Ti:sapphire emission beam is separated into different wavelength beams. A slit is used to block the undesired wavelength waves and to choose the ~800 nm wave of Ti:sapphire emission to mode-lock and send out from this cavity. After stretching (80 fs → >200 ns), this output beam (seed beam) will be amplified in the regenerative amplifier.

At the same time, in the 527 nm pump source, a diode laser pumped neodymium-doped yttrium lithium fluoride (Nd:YLF) Q-switched laser (1054 nm) is Q-switched in the optical resonator at 1 kHz, and then doubled to its SHG (527 nm) through a LBO crystal. Finally, a 6 W, 1kHz, 527 nm beam is sent out as a pump beam for the regenerative amplifier. In this system, Q-switch is a technique which can produce extremely high peak power laser pulses through an attenuator controlling the Q-factor of a laser resonator. The simple principle is that, when Q-switch is set at a low Q-factor, the emission from gain medium cannot come back to the gain medium to initiate stimulated emission, so the energy stored in the gain medium will increase under continuous pumping conditions, and this energy accumulation process will reach a maximum level due to some self-loss processes, such as spontaneous emission. At this point, the Q-switch is switched to a high Q-factor, and then the emission from gain medium comes back to initiate stimulated emission. Because there are large amounts of energy in the gain medium, the stimulated emission process will be very fast and generate a short laser pulse (hundreds ns) with high peak intensity.

The second Ti:sapphire resonant cavity is located in the regenerative amplifier. As discussed in chapter 2.2.4, the 527 nm pump beam excites the Ti:sapphire gain medium, and then the stretched 800 nm seed beam initiates a stimulated emission. By the Kerr-lens mode-locking
process, the strong laser pulse is regenerated, and its peak power is much stronger than the input seed beam. This laser pulse will be compressed to 130 fs duration and sent out from Hurricane.

2.3.2 Nonlinear Optics

The light induced polarization (P) is the interaction of light with matter. At low light intensity, this polarization depends linearly on the electric field (E); thus, it is called linear optics. In cgs, the polarization P is defined as:

$$\vec{P}(t) = \chi^{(1)} \vec{E}(t)$$ (2.6)

where $\vec{E}(t)$ is the fast oscillating electric field of the light and $\chi^{(1)}$ is the first-order susceptibility or linear susceptibility.

Nonlinear optics describes the nonlinear behaviour at high enough light intensity. The dielectric polarization responds nonlinearly to the electric field of the light. The polarization can be described as a power series of the electric field (Taylor series):

$$\vec{P}(t) = \chi^{(1)} \vec{E}(t) + \chi^{(2)} \vec{E}^2(t) + \chi^{(3)} \vec{E}^3(t) + \ldots$$ (2.7)

where $\chi^{(2)}$ and $\chi^{(3)}$ are the second- and third-order susceptibilities. If the electric field is very weak, the high order terms will be too small to influence the polarization appreciably; however, if the electric field is very strong, such as intense laser beam, the magnitude of the polarization will depend on their susceptibility. The non-linear term $\chi^{(2)} E^2(t)$ is responsible for the second harmonic generation (SHG), the sum frequency generation (SFG), and the difference frequency generation (DFG).

Using an incident field of the form:

$$\vec{E}(t, z) = E_1 \cos(\omega_1 t - k_1 z) + E_2 \cos(\omega_2 t - k_2 z)$$ (2.8)
where $k_1$ and $k_2$ are the wavevector components in the $z$ direction, and $\omega_1$ and $\omega_2$ are the angular frequencies. The second order polarization can be described ($z = 0$) as

$$\vec{E}^2(t) = E_1^2 \cos^2(\omega_1 t) + E_2^2 \cos^2(\omega_2 t) + 2E_1E_2 \cos(\omega_1 t) \cos(\omega_2 t). \quad (2.9)$$

Because $\cos^2 x = \frac{1}{2}(1 + \cos 2x)$ and $\cos x \cos y = \frac{1}{2}\cos(x + y) + \frac{1}{2}\cos(x - y)$, we derive

$$\vec{E}^2(t) = \frac{1}{2}(E_1^2 + E_2^2) + \frac{1}{2}E_1^2\cos(2\omega_1 t) + \frac{1}{2}E_2^2\cos(2\omega_2 t) + E_1E_2 \cos((\omega_1 + \omega_2)t) + E_1E_2 \cos((\omega_1 - \omega_2)t). \quad (2.10)$$

In Equation 2.7, when $\chi^{(2)} \neq 0$, the $2\omega_1$, $2\omega_2$, $\omega_1 + \omega_2$, and $\omega_1 - \omega_2$ of Equation 2.10 correspond to electromagnetic waves with double, sum, and difference frequencies of two incident lights, respectively; however, the high incident light intensity is necessary to generate these waves, but is insufficient since certain additional conditions, such as phase-matching, must also be satisfied. In the case of SFG ($\omega_3 = \omega_1 + \omega_2$), two input beams are plane waves, so the sum of two input beam wavevectors, $k_1$ and $k_2$, will be equal to the sum-frequency wavevector, $k_3$, in a phase-matching condition.$^{10,11}$ (Figure 2.6)

![Figure 2.6. Phase-matching condition - a proper phase relationship between the interacting waves.](image)

The wavevector $k$ is related to the frequency $\omega$:

$$k = n\omega/c \quad (2.11)$$

In a dispersive crystal, the refractive index of a wave is in proportion with its frequency.
However, a birefringent crystal can give different refractive indexes for a wave at different polarizations, so it offers other degrees of freedom to achieve the matching conditions \( n_1 = n_2 = n_3 \). In the birefringent crystal, for example, the linear polarization input wave will be split into an extraordinary wave and an ordinary wave. The extraordinary one will have a lower refractive index; therefore, the highest-frequency wave, \( \omega_3 \), has to be polarized in the direction of the extraordinary polarization of the birefringent crystal to achieve the same refractive index as the ordinary wave. For the very common collinear phase-matching in an ultrafast TA system, rotation of the birefringent crystal can fulfill the requirement for all three-wave mixing processes (SHG, SFG, and DFG).

In the Kerr medium, the even-order terms of Equation 2.7 are typically eliminated due to its inversion symmetry, and the third-order nonlinear term will be the dominant term. The third order nonlinear effect, typically an OKE, will finally cause the refractive index of the medium to be dependent on the field strength:

\[
n(I) = n_0 + n_2 I
\]  

(2.13)

where \( n_0 \) and \( n_2 \) are the linear and nonlinear refractive (Kerr) indexes of input beam, and \( I \) is the input beam intensity. For most of the materials, \( n_2 \) is positive, so the higher intensity part of the input beam will be focused more tightly by itself, and the introduction of a pinhole allows the high intensity beam as the only resident in the resonant cavity.

2.3.3 Optical Parametric Amplification
Excitation beams of different wavelengths are required for different samples. The frequency tunability of the fundamental laser source is limited to a narrow range, \( \sim 800 \text{ nm} \). An optical parametric amplifier (OPA), provided by Spectra Physics, is used to convert the fundamental (\( \sim 800 \text{ nm} \)) beam to an excitation beam of the proper wavelength. OPA is a parametric amplification system based on the second-order nonlinearity, the second-order term of the Equation 2.7 \(^{12} \) (White light is based on the higher-order term and discussed later). The principle of OPA is that a low frequency (\( \omega_s \)) and low intensity signal beam (seed beam) is amplified by a high frequency (\( \omega_p \)) and high intensity pump beam through a nonlinear crystal (type II BBO crystal); a third beam called an idler beam with a frequency \( \omega_I = \omega_p - \omega_S \) is generated. The signal beam (white light) generation will be explained later. In the optical parametric amplification process, the energy and the momentum (phase-matching) are conserved for an efficient interaction:

\[
\hbar \omega_p = \hbar \omega_s + \hbar \omega_I \tag{2.14}
\]

\[
\hbar k_p = \hbar k_s + \hbar k_I \tag{2.15}
\]

where \( k_p, k_s, \) and \( k_I \) are the wavevectors of pump, signal, and idler beams, respectively. The optical parametric amplification process is a combination of a difference frequency generation with a stimulated emission.

The frequency tunability of our OPA is 1100-3000 nm. By applying different nonlinear crystals (SHG, SFG, and DFG), the second and fourth harmonic signal and idler beams, the sum frequency of pump and signal or idler beams, and the difference frequency of signal and idler beams will extend the tuning range from UV to mid-IR (300-10000 nm). In our fs TA system, OPA is used to generate a pump beam of one different wavelength, and TOPAS (Travelling-
wave Optical Parametric Amplifier of Superfluorescence) is used to generate a mid-IR beam for one mid-IR TA spectra measurement.

2.3.4 White Light Generation

White light generation, which is also called supercontinuum generation, is a nonlinear physical phenomenon leading to a spectral broadening of high power laser pulses propagating through a nonlinear medium. It is mainly applied in two aspects in the ultrafast TA system: the seed beam generation in OPA or TOPAS and the probe beam generation for UV-vis TA measurement.\textsuperscript{13}

White light generation involves most of the classical nonlinear-optical effects, such as self-phase modulation, four-wave mixing, stimulated Raman scattering, soliton phenomena, and many others. For an fs laser pulse-generated white light, self-modulation plays a major role.\textsuperscript{14-16} Self-phase modulation is a third-order nonlinear process arising from the OKE. After a laser pulse propagates through a length of material, L, the total phase shift of the pulse is described by:

\[
\Phi(t) = \omega_0 t - \frac{[n_0 + n_2 I(t)] \omega_0 L}{c} \tag{2.16}
\]

where \(\omega_0\) is the carrier frequency, \(n_0 + n_2 I(t)\) is OKE caused refractive index, \(t\) is time, and \(c\) is the light speed in a vacuum. By taking the derivative of the total phase shift with respect to time, the instantaneous frequency of the pulse, \(\omega(t)\), can be determined by:

\[
\omega(t) = \omega_0 - \frac{n_2 \omega_0 L}{c} I'(t) \tag{2.17}
\]

The time-dependent frequency deviation is described as:

\[
\Delta \omega(t) = \frac{n_2 \omega_0 L}{c} I'(t) \tag{2.18}
\]
Equation 2.18 describes that the self-phase modulation depends not only on material parameters, but also on the intensity and duration of the pulse. The resulting spectral broadening of the pulse can be estimated by:

\[
\Delta \omega = \frac{n_2 \omega_0 I_0}{ct}
\]  

(2.19)

where \( I_0 \) and \( \tau \) are the intensity and duration of the pulse. From Equation 2.19, it is clearly shown that given strong enough intensity and short enough duration, spectral broadening can be achieved by interactions between light and matter.

**Figure 2.7.** Spectral broadening. Blue: A Gaussian pulse in time. Red: Modification of the carrier frequency as a function of time for self-phase modulation.

Figure 2.7 illustrates a Gaussian intensity curve (blue) as well as \( \Delta \omega \) (red) as a function of time. \( \Delta \omega \) is the change of the carrier frequency, \( \omega_0 \), caused by self-phase modulation. \( \Delta \omega < 0 \) corresponds to a red-shift of the pulse wavelength, and \( \Delta \omega > 0 \) corresponds to a blue-shift. For a Gaussian pulse, the front (right side) of the pulse is red-shifted while the back (left side) is blue-shifted. In our ultrafast TA system, two sapphire crystals are used to generate white light as seed beam in OPA and TOPAS, respectively. A calcium fluoride crystal is used to generate white light as a probe beam for UV-vis TA spectra collection.

### 2.3.5 Computer Controlled Delay-line, Chopper, CCD Camera, and MCT Detector
To study the excited state dynamic of a sample in an fs TA experiment, a delay-line is used to generate a probe delay, thereby achieving a continuous dynamic observation. A C-995 optical chopper provided by Terahertz Technologies Inc. is used to block the pump beam for the ground state absorption spectrum collection, thereby achieving difference spectrum collection of excited and ground states. The ground and excited states spectra of the sample for UV-vis TA measurement were obtained from a USB2000 fiber optical spectrometer provided by Ocean Optics Inc. An fs pulse acquisition spectrometer (MCT-14-2x32) provided by Infrared Systems Development Corporation is used to collect ground and excited states spectra of the sample for a mid-IR TA measurement. All four devices are controlled by computers.

A high performance 250 mm travel linear stage with a 0.5 μm resolution is used as the delay-line in our TA system and can generate a 1666 ps delay range with a 3.3 fs minimum step size. A 1-axis motion controller/driver is used to link the delay-line to the computer. First, the controller receives motion commands from a computer through a USB cable, verifies the real delay-line position, and generates the necessary control signals, and then the control signals are converted by the driver to the right format and power necessary to drive the delay-line motor through a cable with 25-pin Sub-D connectors.

For the UV-vis TA system, the detector of spectrometer is a charge-coupled device (CCD), so this spectrometer is also called a CCD camera. Because the minimum acquisition time of CCD camera is 2 ms, and 1 ms is required to close the shutter, the working frequency of CCD camera is set on 200 Hz. To fit well with the CCD camera, the working frequency of the chopper is set on 100 Hz. It means that the original trigger signal (1000 Hz) from the laser source cannot be directly used to trigger the chopper and the CCD camera. A PCI data acquisition (DAQ) card with a shielded BNC connector block is needed for the trigger signal frequency conversion. The
DAQ card receives a 1000 Hz original trigger signal from laser source through the BNC connector and transfers it to the computer. In the computer, the 1000 Hz trigger signal is converted to 100 Hz and sent back to the chopper through the DAQ card. Through the chopper control box, the trigger signal is separated to two parts. One is sent back to the BNC connector, and another one is sent through a doubler, which doubles the trigger signal frequency to 200 Hz, to trigger the CCD camera. The 200 Hz trigger signal is also sent back to the BNC connector after the CCD camera. The returned trigger signals from the chopper and the CCD camera will be used by the computer to separate the ground and the excited states spectra. By applying the Equation 2.20, the difference between ground and excited states absorption spectra, \( \Delta A \), will be obtained.

\[
\Delta A = \log\frac{l_0}{l_e} - \log\frac{l_0}{l_g}
\]  

(2.20)

where \( l_0 \) is the probe beam intensity before the sample, and \( l_g \) and \( l_e \) are the probe beam intensities after unexcited and excited samples, respectively. The relation between laser pulse patterns and corresponding trigger signals is shown in Figure 2.8. If every 5 successive pulses are set as a group, the chopper will cut the second group of every 2 successive groups. Because the acquisition time of the CCD camera is set at 2 ms, the CCD camera will collect two pulses each time. Because the CCD camera is synchronized with the chopper, these two pulses will come from each individual group.
**Figure 2.8.** The relation between laser pulse patterns and corresponding trigger signals. The blue bars are laser pulses with 130 fs duration and 1000 Hz frequency, the orange bars are the chopper trigger signals with 100 Hz frequency, and the red dots are CCD camera trigger signals with 200 Hz frequency.

Through a moving delay-line to collect a series of absorption spectra at different time delays, a set of three dimension (wavelength, time, and intensity) TA spectra will be obtained and saved in the computer for data processing and analysis.

For the mid-IR TA system, the fs pulse acquisition spectrometer can be set on 1000 Hz frequency to collect each individual pulse. The chopper trigger frequency is set on 500 Hz through a two-to-one frequency divider. The relation between laser pulse patterns and corresponding trigger signals is similar to UV-vis TA setup except that the group size is decreased from 5 pulses to 1 pulse. Compared with the efficiency of operations of CCD camera, the advantage of the fs pulse acquisition spectrometer is that it will collect more spectra in the same time period, thereby speeding up the whole mid-IR TA experiment process.

In all the processes mentioned above, the original trigger signal conversion, the synchronization between chopper and CCD camera, the coordination between spectra collection and delay-line moving, and data saving, are achieved through a self-developed data acquisition program compiled in National Instruments LabView 7.1.

### 2.4 Data Processing and Analysis

The white light used as the probe beam in UV-vis TA system contains the light from 450-800 nm (sapphire) or 350-800 nm (CaF$_2$). When this broadband probe beam passes through a glass lens or window, the longer wavelength portions of the probe beam travel faster than the shorter wavelength portions in the material due to its lower refractive index ($v = \frac{c}{n}$), thereby
causing the different arrival times of different wavelengths of white light (Figure 2.9). This phenomenon is called the group velocity dispersion (GVD) and known as ‘chirp’, which may greatly affect a TA measurement data collection, such as if, in experiments, the chirp is approximately 4 ps across the white light, TA spectra will be severely distorted for measurements with delay times less than 4 ps; therefore, UV-vis (broadband) TA spectroscopy requires chirp correction.

Figure 2.9. Example white light chirp delays corresponding to wavelengths

Noise is also a serious restriction to UV-vis TA measurement. One kind of noise is from array detectors of CCD camera limiting the use of electronic noise suppression. The primary limit in using a CCD camera is the noise generated by the camera itself, such as thermal noise. Another kind of noise is from the laser and the sample. The external factors cause the laser intensity change, the sample concentration change, etc. To obtain better view spectra and clear decays, the noise mentioned needs to be reduced.

2.4.1 Chirp Correction

The chirp (GVD) of the probe beam can be determined and corrected by nonresonant OKE measurements. The OKE contains an instantaneous electronic response, which is an external electric field (pump beam) induced dipole in the molecules. This dipole finally causes a
birefringent behavior in the molecules, thereby changing the polarization of the passing probe beam. This instantaneous polarization change corresponds to the transient OKE signal of molecules, which has a peak at the zero time delay. With this method, the "zero-time" pulse position of a single wavelength of the probe beam relative to that of the excitation beam for white light generation and the difference in pulse delay between two different wavelengths of the probe beam can be determined.

In the practical application, the excitation beam is focused on a quartz cell containing carbon tetrachloride. The sample cell is placed between a pair of crossed polarizers, through which the excitation beam is sent. Before the sample cell, the relative polarization between the pump and the probe beams is set at 45°. The induced OKE signal is measured at various time delays. A program written with Matlab 7.1 software is used to extract a chirp correction file from the collected TA chirp data. In this process, the OKE signal at every wavelength is fitted with a Gaussian function, and then the values for the all peak maxima obtained from the fitting are constructed to a zero-time curve. Finally, the time arrays from the TA data are chirp-corrected by subtracting the value of zero-time at every corresponding wavelength.

2.4.2 Noise reduction

The noises from laser, sample, and CCD camera can be reduced in two ways. The first one is a commonly used method, in which the spectra are repetitively collected at the same time delay position, and the averaged spectra are saved in the data file. After applying the commonly used method to reduce noise, there is some noise contained in the data file. The second method, which is a singular value decomposition (SVD) method (Matlab 7.1), is used to reduce the noise left in the data file. By using the SVD method, the data matrix (rectangular) \( \Delta A = m \times n \) will be decomposed into a product of three matrices according to Equation:
\[ \Delta A = USV \]  \hspace{1cm} (2.21)

where matrices \( U \) and \( V \) are \( m \times m \) and \( n \times n \) orthogonal matrices (square), and matrix \( S \) is a diagonal matrix (rectangular) with the singular values as entries. Through visual inspection of the SVD components, a new matrix \( S_{NR} \) is reconstructed by keeping only the relevant singular values (usually the first 2–3 diagonal elements), and the rest of the diagonal elements, which mainly represent the noise, are set to zero. Now, the noise reduced data \( \Delta A_{NR} \) is reconstructed according to Equation:

\[ \Delta A_{NR} = US_{NR}V \]  \hspace{1cm} (2.22)

The decays of the noise-reduced data \( \Delta A_{NR} \) are compared with that of the original data \( \Delta A \) at several wavelengths to ensure that the kinetic profiles are not altered.

2.4.3 Data Analysis

TA data is analyzed by using the SPECFIT/32TM Global Analysis System (Spectrum Software Associates, MA, USA). This program allows a decomposition of TA data by using kinetic models. The fitting process returns the predicted absorption spectra of individual colored species involved in the photochemical process along with their decay profiles. The analysis is achieved by a global analysis method that uses the SVD method to reduce the size of the fitted data.\(^{22}\)

References


(2) Zewail, A. H. Femtochemistry - Untrafast Dynamics of the Chemical Bond; World Scientific Pub Co Inc: Singapore, 1994; Vol. I & II.


CHAPTER III: THE EXCITED STATE BEHAVIOR OF Et-FI^+ AND Et-FIOH

3.1 Introduction

3.1.1 Flavoprotein Mononucleotide and Its Function in Bacterial Luminescence

Flavoproteins have been recognized as important catalyst groups. A variety of biochemical transformations can be catalyzed by flavoproteins. Flavin mononucleotide (FMN) is a strong oxidizing agent and can participate in both particularly useful one and two electron transfers. Due to the oxidizing character of FMN, it is involved in some biological processes, such as bioluminescence and NADH dehydrogenation.

Many bacteria found in seas and oceans are known to cause bioluminescence, which is catalyzed by flavoprotein luciferases. While isolated bacteria do not express the genes necessary for bioluminescence, high densities of bacteria produce luminescence that is detectable even by satellites. This cooperative bioluminescence by bacteria is known as quorum sensing. Due to their light-emitting properties, bacteria can form symbiosis with fish and squids. In this symbiotic relationship, the host uses bacterial luminescence to confuse predators and attract prey, while bacteria benefit from available sources of nutrients and oxygen. The reason for bacterial luminescence is still not known, but there is some evidence that the emitted light allows bacteria to perform a light-driven DNA repair in a dark environment.

The bacterial luminescence reaction involves the oxidation of a long-chain aldehyde to the carboxylic acid by molecular oxygen. The sequence of steps that is thought to occur during the catalytic cycle is as follows (Scheme 3.1): (i) the reaction of a flavoprotein cofactor FMNH_2 with molecular oxygen creates an unusually stable hydroperoxide FMNHOOH, which has been characterized by NMR spectroscopy; (ii) the reaction of FMNHOOH with the substrate (a long-
chain aldehyde) is thought to lead to the formation of a peroxyhemiacetal;\(^9\) (iii) intramolecular electron transfer leads to the fragmentation of the peroxyhemiacetal and creates a carboxylic acid and 4a-hydroxyflavin FMNHOH in its excited state;\(^{10}\) (iv) the excited FMNHOH returns to the ground state with the emission of blue light with \(\lambda_{\text{max}}=490\) nm;\(^{11}\) (v) once in the ground state, FMNHOH spontaneously eliminates water and converts to FMN; (vi) reduction of FMN to FMNH\(_2\) recovers the catalyst.

Scheme 3.1. Steps and Intermediates for a Bacterial Luminescence Reaction Occur During the Catalytic Cycle

3.1.2 N(5)-alkylation of the Flavin Derivative and Its Catalytic Activity

Even though it is generally accepted that the emitter in bacterial bioluminescence is FMNHOH, this compound has not been characterized due to its tendency to release water and produce thermodynamically stable FMN. The lifetime of FMNHOH in the luciferase is only 7 minutes at 9 °C,\(^{12}\) while it has never been prepared in solution. Due to its short lifetime, only the absorption and emission spectra of FMNHOH have been obtained.\(^{11,12}\) On the contrary, N(5)-alkylation of the flavin derivative stabilizes its 4a-hydroxyl form (pseudobase) relative to parent iminium cations. Stable N(5)-alkylated iminium salts, their 4a-hydroperoxides and pseudobases have been synthesized and investigated by Bruice\(^ {13-19}\) and Tu.\(^ {20-23}\) It was demonstrated that 4a-
hydroperoxides produce chemiluminescence in the presence of aldehydes,\textsuperscript{15-17} which makes these derivatives suitable model compounds for mechanistic studies of bacterial bioluminescence. Pulse radiolysis\textsuperscript{20,24} and cyclic voltammetry\textsuperscript{13,14,21} experiments suggested that the pseudobase is the emitter responsible for chemiluminescence; however, further analysis of the chemiluminescence mechanism is complicated by the fact that pseudobases derived from N(5)-alkylated flavinium salts have extremely low fluorescence quantum efficiencies (less than \(10^{-5}\) at room temperature in aqueous solution\textsuperscript{14}). The fluorescence spectrum of the pseudobase was obtained either in glassy solvent at 77 K\textsuperscript{15} or by inserting the pseudobase in the luciferase active site.\textsuperscript{22,23}

The synthetic analog used in these studies is N(5)-ethyl flavinium perchlorate\textsuperscript{15,25} (Et-Fl\textsuperscript{+}) and its derivative N(5)-ethyl-4a-hydroxyflavin\textsuperscript{26} (Et-FIOH) presented in Scheme 3.2. The main difference between the naturally occurring flavin cofactors and Et-Fl\textsuperscript{+} is the presence of ethyl group at N5 position, which enables the use of flavin-based catalysis outside the protein environment.

**Scheme 3.2. Structures of 3-Methyllumiflavin (Fl), N(5)-ethylflavinium Perchlorate (Et-Fl\textsuperscript{+}), N(5)-ethyl-4a-hydroxyflavin (Et-FIOH), and (5)-Ethyl-4a-methoxyflavin (Et-FIOMe)**

To understand the mechanism of FMN based bacterial luminescence reaction (Scheme 3.1), especially possible origins of fast excited-state decay of flavin pseudobases, our group
investigated the excited state behavior of Et-Fl\(^+\) and Et-FIOH, and compared the excited state behavior of Et-Fl\(^+\) with the well-known 3-methylllumiflavin (Fl in Scheme 3.2). Our experimental data show that both Et-Fl\(^+\) and Fl exhibit two excited-state deactivation channels: a fast, radiationless decay from the \(S_2\) (\(n,\pi^*\)) state and a slow radiative decay from the \(S_1\) (\(\pi,\pi^*\)) state, and Et-FIOH exhibits complex excited-state behavior, with the most dominant decay component exhibiting a 0.5 ps lifetime. For Et-FIOH, it is investigated several possible origins of such fast excited-state behavior: (a) excited-state tautomerization to a keto-amine; (b) excited-state release of OH\(^-\) ion; (c) deactivation by conical intersection (CI) involving a dark \(1n,\pi^*\) state; and (d) deactivation by a CI involving excited-state planarization of \(N(5)\)-atom and a distortion of the imide ring of Et-FIOH. Based on our studies with model compounds Et-FIOMe (Scheme 3.2) and Et-Fl\(^+\) and time-dependent density functional theory (TD-DFT) calculations, we conclude that the mechanism (d) can be used to interpret the experimental findings.

3.2 Results and Discussion on Fl and Et-Fl\(^+\)

3.2.1 Absorption and emission spectra of Fl and Et-Fl\(^+\)

![Figure 3.1](image)

**Figure 3.1.** (a) UV-vis absorption spectra of Fl (blue) and Et-Fl\(^+\) (red) in ACN. Bars represent calculated transition energies for Fl (blue) and Et-Fl\(^+\) (red); (b) Emission spectra of Fl (blue, \(\lambda_{\text{EXC}}=430\) nm) and Et-Fl\(^+\) (red, \(\lambda_{\text{EXC}}=570\) nm) in ACN.

The absorption and emission spectra of Fl and Et-Fl\(^+\) in ACN solution were collected (Figure 3.1). There are two bands centered at 338 and 443 nm in the absorption spectrum of Fl
and one band centered at 519 nm in the emission spectrum. There are similar absorption and emission spectra for Et-Fl⁺ to those of Fl, but with red shifts: absorption bands appear at 415 and 555 nm, while the emission band appears at 660 nm. TD-DFT (B3LYP functional, 6-31+G* basis set, gas phase) calculations were done on FL and Et-Fl⁺ to understand the origin of their absorption bands. The calculated absorption energies for the first three states and the corresponding oscillator strengths agree well with the experimental absorption spectra in Figure 3.1.

Figure 3.2. DDPs calculated using TD-DFT method: (a) S₁-S₀ of Et-Fl⁺; (b) S₂-S₀ of Et-Fl⁺; (c) S₁-S₀ of Fl; (d) S₂-S₀ of Fl. Red indicates depletion of the charge in the excited state, while the green indicates accumulation of charge in the excited state.

Difference density plots (DDPs) were recently applied in other photochemical systems as a tool to visualize the electron density changes upon vertical excitation. Figure 3.2 presents the first two excited states elucidated from DDPs. For S₁-S₀ DDPs of Fl and Et-Fl⁺, a change in the electron density of the conjugated system is a characteristic of the π, π* excited state. For S₂-S₀ DDPs, the depletion of the electron density from the n-orbitals of oxygen atoms and nitrogen
atoms is the characteristic of the n, π* excited state. Thus, the two absorption bands of Fl and Et-Fl⁺ are assigned to the π, π*(S₁) and n, π* (S₂) transitions, and the emission bands are assigned to the fluorescence from the ¹π, π* excited state.

3.2.2 Pump-Probe Data on Fl and Et-Fl⁺

![Image](image.png)

**Figure 3.3.** (a) upper panel: ground state absorption (black) and fluorescence (red) spectra of Fl in ACN; lower panel: TA spectra of 2 mM Fl in ACN 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) and 444 nm (red). Solid curves represent fits obtained using a biexponential decay function with lifetimes of τ₁ = 53 ps and τ₂ = ~8 ns.

**UV-vis Pump-Probe Experiments.** The excited-state behavior of Fl and Et-Fl⁺ are studied by ultrafast visible pump-probe spectroscopy. Figure 3.3 shows the TA spectra of Fl. Compared with its ground state UV-vis absorption and emission spectra, the three TA bands can be attributed to: (i) 379 nm excited-state absorption band; (ii) 444 nm ground-state bleach band, and (iii) 550 nm stimulated emission band. The fitting of decays at 379 nm and 444 nm gives two lifetime components of τ₁ = 53 ps and τ₂ = several ns. The longer one is assigned to the intersystem crossing of S₁ (π,π*) state to T₁ (π,π*) state of Fl. This assignment is supported by
the previously reported 8.4 ns fluorescence lifetime. In addition, previous ns TA experiments note this as an intersystem crossing process.

There could be several nonradiative processes to explain the shorter one (53 ps): (i) $S_1$ state vibrational cooling; (ii) $S_2 \rightarrow S_1$ internal conversion (IC), and (iii) $S_2 \rightarrow S_0$ IC. Two arguments can be used to rule out hypothesis (i). First, for these medium-sized molecules, the vibrational cooling process is usually shorter than 10 ps. In riboflavin, this process is done with a 4 ps lifetime. Second, no blue shift, which is the characteristic of a vibrational cooling process, is obtained from TRIR experiments (see next section). Hypothesis (ii) is ruled out by two arguments. First, 53 ps is too long for a $S_2 \rightarrow S_1$ IC process. For example, $S_2 \rightarrow S_1$ IC in porphyrins and diphenylpolyenes occurs within 1 ps. Second, the bleach recovery at 444 nm also shows a 53 ps component (Figure 3.3), which is not expected with the $S_2 \rightarrow S_1$ IC process. Hypothesis (iii) is most likely to occur. As shown in Figure 3.1a, using the 400 nm pump beam, it is possible to produce both the $S_1 (\pi,\pi^*)$ and $S_2 (n,\pi^*)$ states of Fl; This behavior is supported by our TD-B3LYP calculations. Thus, this 53 ps component is assigned to the $S_2 \rightarrow S_0$ IC of Fl. Furthermore, $^1n,\pi^*$ state nonradiative decays of other organic systems were found with similar lifetimes. Hence, it is concluded that the 400 nm excitation produces radiative $S_1 (\pi,\pi^*)$ and nonradiative $S_2 (n,\pi^*)$ states of Fl, which decay to the ground state via two separate channels.
Figure 3.4. (a) upper panel: ground state absorption (black) and fluorescence (red) spectra of Et-Fl\(^+\) in ACN; lower panel: TA spectra of 2 mM Et-Fl\(^+\) in ACN collected 1 ps (black), 183 ps (red) and 1.1 ns (green) after the 550 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.

TA spectra of Et-Fl\(^+\) exhibit similar spectral features with Fl (Figure 3.4). Compared with the ground state UV-vis absorption and emission spectra, the five TA bands can be attributed to: (i) 350, 470 and 583 nm excited-state absorption bands, (ii) a 417 nm ground-state bleach band and (iii) an above 650 nm stimulated emission band. All five bands give a same single exponential decay with a 590 ps lifetime, which is assigned to the \(S_1 (\pi, \pi^*) \rightarrow S_0\) IC process of Et-Fl\(^+\). One difference between Fl and Et-Fl\(^+\) TA spectra is the lack of the \(S_2 \rightarrow S_0\) IC in Et-Fl\(^+\). While Fl was excited between \(S_1\) (443 nm) and \(S_2\) (397 nm) absorption bands, Et-Fl\(^+\) was excited at the maximum of the \(S_1\) (557 nm) band. In the TRIR section, 500 nm (instead of 550 nm used here) is used to generate both \(S_1\) and \(S_2\) (528 nm) states of Et-Fl\(^+\).

**Time-Resolved IR (TRIR) Measurements.** The ground-state FTIR spectra of Fl and Et-Fl\(^+\) were collected in the 1500−1800 cm\(^{-1}\) range (Figure 3.5a). The vibrational signatures of both compounds were assigned using computed vibrational frequencies at the B3LYP/SVP level of theory (Figure 3.5b). Frequency assignments of Fl are supported by the previously assigned FTIR spectra of riboflavin,\(^{37}\) flavin-mononucleotide (FMN),\(^{41}\) and flavin dinucleotide (FAD).\(^{42}\)
Figure 3.5. (a) Ground-state FTIR spectra of Fl (blue) and Et-Fl$^+$ (red) in d$_3$-ACN. (b) Table of experimental and calculated vibrational frequencies for Fl and Et-Fl$^+$, (i) C═N bonds symmetric stretching coupled with C═C stretching of the aromatic ring; (ii) C═N bonds asymmetric stretching coupled with C═C aromatic stretching; (iii) C═O bonds asymmetric stretching, and (iv) C═O bonds symmetric stretching.

<table>
<thead>
<tr>
<th></th>
<th>$\nu_{\text{exp}}$ / cm$^{-1}$</th>
<th>$\nu_{\text{calc}}$ / cm$^{-1}$</th>
<th>$\nu_{\text{exp}}$ / cm$^{-1}$</th>
<th>$\nu_{\text{calc}}$ / cm$^{-1}$</th>
<th>Assignment</th>
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<td>1555</td>
<td>1559</td>
<td>1556</td>
<td>C=C arom. and C=N sym.</td>
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<tr>
<td>Et-Fl$^+$</td>
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<td>1590</td>
<td>1599</td>
<td>1588</td>
<td>C=C arom. and C=N asym.</td>
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<td>1748</td>
<td>1714</td>
<td>1758</td>
<td>C=O sym.</td>
</tr>
</tbody>
</table>

Figure 3.6. (a) Upper panel: ground-state FTIR spectrum of Fl in ACN. Middle panel: TRIR spectra of 10 mM Fl in ACN collected after the 400 nm excitation pulse. Lower panel: TRIR spectra of $S_1$ and $S_2$ states of Fl obtained from spectral deconvolution. (b) Decays of transient signals with lifetimes $\tau_1 = 30$ ps and $\tau_2 = 4$ ns.

TRIR spectra of Fl at different time delays were obtained after the 400 nm excitation pulse (Figure 3.6). The 1 ps spectrum contains ground state bleach bands at frequencies 1553,
1587, 1656, and 1706 cm\(^{-1}\) and a clear excited-state absorption band at 1610 cm\(^{-1}\) (two weak absorption bands at 1535 and 1640 cm\(^{-1}\)). Two lifetimes (\(\tau_1 = 30\) ps and \(\tau_2 = 4\) ns) are observed from the fitting of all decays (Figure 3.6b). As the analysis of the UV-vis TA data of Fl shows, these two components are assigned to S\(_2\) → S\(_0\) IC (30 ps) and S\(_1\) → T\(_1\) intersystem crossing (4 ns). The lifetime for the S\(_2\) → S\(_0\) conversion in TRIR is shorter than the one in UV-vis TA, which suggests a lowered energy of \(^1\)n,\(\pi^*\) state. It is possible that the high sample concentration for the TRIR experiment stabilizes the intramolecular interaction. The lowest panel of Figure 3.6a presents the TRIR spectra of S\(_2\) and S\(_1\) states obtained through a global analysis of the data with a biexponential decay function (see experimental section). The spectrum of the S\(_2\) state contains three excited state absorption bands at 1535, 1605, and 1640 cm\(^{-1}\), while the S\(_1\) state contains one at 1610 cm\(^{-1}\).

**Figure 3.7.** (a) Upper panel: ground-state FTIR spectrum of Et-Fl\(^+\) in ACN. Middle panel: TRIR spectra of 10 mM Et-Fl\(^+\) in ACN collected at different time delays, \(\lambda_{\text{ext}}=500\) nm. Lower panel: TRIR spectra of S\(_1\) and S\(_2\) states of Et-Fl\(^+\) obtained after spectral deconvolution. (b) Decays of transient signals with lifetimes \(\tau_1 = 28\) ps and \(\tau_2 = 590\) ps.

Figure 3.7a presents TRIR spectra of Et-Fl\(^+\) after the 500 nm excitation pulse. The 1 ps spectrum consists of four ground state bleaches at frequencies, 1552, 1595, 1672, and 1710 cm\(^{-1}\).
and three excited-state absorption bands at 1539, 1627, and 1653 cm$^{-1}$. Two lifetimes (28 and 590 ps) are observed from the fitting of all decays. The 28 ps component was not observed in the UV-vis TA experiment ($\lambda_{\text{ext}}=550$ nm). This 28 ps component is consistent with the $S_2 \rightarrow S_0$ IC, which is due to the excitation pulse with higher energy (550 nm $\rightarrow$ 500 nm). The 590 ps component is assigned to the decay of the $S_1 (\pi,\pi^*)$ state. TRIR spectra of the $S_1 (\pi,\pi^*)$ and $S_2 (n,\pi^*)$ states of Et-Fl$^+$ are obtained through spectral deconvolution, as shown in the lower panel of Figure 3.7a. The 1627 cm$^{-1}$ C═O stretching band is purely due to vibrational modes of the $S_1$ state although the 1529 and 1651 cm$^{-1}$ bands arise from both $S_1$ and $S_2$ states.

3.3 Results and Discussion on Et-FIOH and EtFIOMe

3.3.1 Absorption and emission spectra of Et-FIOH and EtFIOMe

Figure 3.8. Normalized absorption and emission spectra of N(5)-ethyl-4a-hydroxyflavin (Et-FIOH) in ACN.

Figure 3.8 presents the absorption and fluorescence spectra of Et-FIOH in ACN. The absorption maximum appears at 348 nm, and we assign this band to the $^1\pi, \pi^*$ transition (based on the TD-DFT calculation presented below). The fluorescence spectrum exhibits a large Stokes shift ($\lambda_{\text{MAX}}=496$ nm), indicating significant nuclear rearrangements in the excited state. The quantum yield of Et-FIOH fluorescence is only $3 \cdot 10^{-3}$, suggesting that fast $S_1 \rightarrow S_0$ thermal deactivation or a photochemical reaction occurs in Et-FIOH. A previous report by Tu and
coworkers$^{23}$ shows that the insertion of Et-FIOH into the luciferase active site leads to a strong increase in its fluorescence intensity, and the decrease in the Stokes shift (fluorescence occurs with a maximum at 440 nm). These results demonstrate that the photochemical (photophysical) reactivity that leads to a decrease in emission quantum yields of Et-FIOH can be controlled by appropriate host-guest complexation. The experiments presented in the rest of this manuscript aim to investigate the origins of this photochemical/photophysical reactivity.

3.3.2 Pump-Probe Data on Et-FIOH and Et-FIOMe

![Figure 3.9](image)

**Figure 3.9.** (a) Transient absorption spectra of 0.5 mM Et-FIOH in ACN collected −3 (black), 0 (purple), 0.2 (blue), and 10 ps (green) after the 350 nm excitations pulse. (b) Decay dynamics of Et-FIOH transient absorption signal collected at 410 nm (dots). Solid line represents a fit obtained using a triexponential decay function convolved with the instrument response function. Dashed line represents instrument response function.

TA spectra of Et-FIOH obtained at several time delays exhibit significantly different spectral features (Figure 3.9a). At t=0 ps, the chirp-corrected spectrum consists of an excited-state absorption band at 406 nm and a stimulated emission band at 478 nm. The stimulated emission band appears to red-shift at longer time delays, reaching the value of 585 nm at t=10 ps. The complexity of Et-FIOH excited-state dynamics can also be observed from the 410 nm decay (Figure 3.9b), which was fit to a multiexponential decay function convolved with the instrument
response function. The following lifetimes were obtained: $\tau_1=0.14$ ps ($A_1=-0.2$), $\tau_2=0.50$ ps ($A_2=1.1$), $\tau_3=15$ ps ($A_3=0.3$). These results demonstrate that the non-emissive deactivation of Et-FlOH occurs at a very fast timescale, with the most dominant decay component having a 500 fs lifetime. Furthermore, the non-emissive deactivation occurs via several emissive transient species, as illustrated by a progressive red-shift in the stimulated emission signal of Et-FlOH as a function of probe delay.

The red-shift of the stimulated emission signal is more clearly observed in the absorption spectra of the components obtained using spectral deconvolution software. Component analysis of Et-FlOH TA data was obtained using $S_1^a \rightarrow S_1^b \rightarrow S_1^c \rightarrow S_0$ model ($S_1^a$ and $S_1^b$ had initial concentration at $t=0$). De-convolved TA spectra of $S_1^a$, $S_1^b$ and $S_1^c$ and their lifetimes are shown in Figure 3.10. We can see that the initially produced $S_1^a$ state exhibits stimulated emission signal at 411 nm and a very short lifetime (140 fs). The $S_1^b$ and $S_1^c$ states exhibit red-shifted stimulated emission maxima (538 and 575 nm) as well as longer lifetimes (660 fs and 24 ps).

![Figure 3.10](image)

**Figure 3.10.** Absorption spectra and lifetimes of three components obtained from analysis of Et-FlOH transient absorption data using Specfit/32 software.

**Scheme 3.3. Possible Excited-state Tautomerization in Et-FlOH**
One possible explanation of complex Et-FIOH dynamics is the excited state
deprotonation of the –OH proton. The pKa value of Et-FIOH in the ground state is 9.2,43 and it is
possible that the excited-state acidity increases due to the coupling of the OH-group with the
flavin chromophore. Similar excited-state deprotonations are observed in alcohols and amines
that are strongly coupled to the aromatic chromophores, such as 2-naphthol,44 azaindole45 or
pyranine.46 If the proton transfer in Et-FIOH is intramolecular, we could observe excited-state
tautomerization from Et-FIOH hemiaminal form to the corresponding amino-ketone isomer
(Scheme 3.3). Similar excited-state tautomerizations are known to occur in many alcohols and
amines.47-52 In most cases, the intramolecular tautomerization occurs via a 6-membered ring
intermediate (salicylic acid,47 hydroxybenzothiazole50 hyperycin51), but in several cases the 4-
membered (cytosine48) and 5-membered (pyridil-pyrrole,49 hydroxyflavone52) tautomerizations
were observed.

**Figure 3.11.** (a) Ground state absorption and fluorescence of 35 μM Et-FIOMe in ACN
(fluorescence quantum yield: 4.9 × 10⁻³). (b) TA spectra of 1.5 mM Et-FIOMe in ACN obtained
after the 350 nm excitation pulse. (c) Decay dynamics of Et-FIOMe TA signal collected at 413
nm (dots). Solid line represents a fit obtained using a triexponential decay (τ₁ = 0.2 ps, A₁ =
$-0.15, \tau_2 = 2.8 \text{ ps}, A_2 = 0.02, \tau_3 = 91 \text{ ps}, A_3 = 0.005)$ function convolved with an instrument response function.

To investigate this possibility, we synthesized 4a-methoxy-N(5)-ethylflavin derivative (Et-FLOMe) which lacks a hydroxylic proton and thus cannot tautomerize. TA spectra of Et-FLOMe were obtained at different time delays after the 350 nm excitation pulse (Figure 3.11). Similarly to Et-FLOH, stimulated emission spectra of Et-FLOMe shift to the lower energy at longer time delays: from 482 nm at 0 fs to 574 nm at 4.2 ps. Figure 3.11c shows the decay dynamics collected at 413 nm and a fit using a tri-exponential function ($\tau_1=200 \text{ fs}, \tau_2=2.8 \text{ ps and } \tau_3=91 \text{ ps}$). The lifetimes obtained for Et-FLOMe are longer than those obtained for Et-FLOH, possibly due to increased steric effects of $\text{–OCH}_3$ group in the structural rearrangements of excited Et-FLOMe. However, the spectra and lifetimes of Et-FLOMe are qualitatively similar to those of Et-FLOH.

![Figure 3.12. Absorption spectra and lifetimes of three components obtained from analysis of Et-FLOMe TA data using Specfit/32 software.](image)

The spectral similarity can also be observed from the component analysis of Et-FLOMe TA data (Figure 3.12) using $S_1^a \rightarrow S_1^b \rightarrow S_1^c \rightarrow S_0$ model. The de-convolved spectra of Et-FLOMe are similar to the behavior as Et-FLOH: (i) we observed three decay components with similar red-
shift of the stimulated emission; (ii) the lifetimes of Et-FIOMe components are longer than, but comparable to the corresponding Et-FIOH lifetimes. Based on these findings, we exclude excited-state tautomerization as a possible origin of fast Et-FIOH excited-state decay.

Figure 3.13. Schematic representation of Förster cycle used to estimation the pseudobase $pK_a^{*}$ of Et-FIOH. The absorption wavelengths for Et-FIOH and Et-Fi$^+$ were obtained from the UV/vis absorption spectra in ACN.

Another possible deactivation pathway for Et-FIOH excited state involves a release of OH$^-$ ion from photoexcited Et-FIOH ($\text{Et-FIOH} + h\nu \rightarrow \text{Et-Fi}^+ + \text{OH}^-$). The light-induced heterolytic bond cleavage is frequently observed in systems where the cation is stabilized by resonance, such as arylmethyl alcohols.$^{53,54}$ Pseudobase derivatives are also known to undergo photochemical OH$^-$ ion release, as was demonstrated in the case of a pseudobase derived from acridinium ions.$^{55}$ The Et-FIOH/Et-Fi$^+$ equilibrium occurs in the ground state with a pseudobase pKa value of 3.6,$^{26}$ suggesting that the ground-state release of OH$^-$ is thermodynamically unfavorable by 0.61 eV (Figure 3.13). To estimate whether the release of OH$^-$ from photoexcited Et-FIOH is a thermodynamically favored process, we used the Förster cycle$^{56}$ presented in Figure 3.13. Based on the UV-vis absorption spectra of Et-FIOH ($\lambda_{\text{ABS}}=350 \text{ nm}$)$^{26}$ and Et-Fi$^+$ ($\lambda_{\text{ABS}}=557 \text{ nm}$),$^{57}$ and on the ground-state pseudobase pKa value of Et-FIOH (pKa=3.6),$^{26}$ we find that the
Et-FlOH pseudobase pKa value changes drastically in the excited state ($pK_a^* = 14$, $\Delta G^* = -0.71$ eV).

Based on the results of this simple analysis, we investigated the photobasic properties of Et-FlOH. Since the photorelease of OH$^-$ leads to the formation of Et-Fl$^+$ in its ground $S_0$ and/or excited $S_1$ state, we first identified the absorption bands of these two states. The $S_0$ state of Et-Fl$^+$ exhibits two absorption bands at 420 and 550 nm (black curve in Figure 3.4a upper), while the $S_1$ state is characterized by a broad absorption band in the 350-650 nm range and a stimulated emission in the 650-750 nm range (black curve in Figure 3.4a lower). By comparing these Et-Fl$^+$ signature peaks with the spectral features of the transients obtained in the pump-probe experiment using Et-FlOH sample (Figure 3.4a 9 and 10), we conclude that the formation of Et-Fl$^+$ does not occur upon excitation of Et-FlOH. Even though it is a thermodynamically favorable process, the release of OH$^-$ from photoexcited Et-FlOH does not occur.

### 3.3.3 TD-DFT Calculations

Another possible mechanism of fast Et-FlOH excited-state decay involves internal conversion via the low-lying $^1n,\pi^*$ state. The similar mechanism was found to occur in pyrimidine DNA bases, where excited-state decay occurs via two conical intersections involving initially excited $^1\pi,\pi^*$ state, subsequently populated dark $^1n,\pi^*$ state and the ground state of pyrimidine bases. To investigate whether $^1n,\pi^*$ states are involved in the fast excited-state deactivation of Et-FlOH, we studied the excited-states of Et-FlOH using TD B3LYP/6-31+G* methodology. In all calculations presented here, we considered only the S-stereoisomer of Et-FlOH, as we do not expect stereochemistry to play a role in the excited-state behavior of Et-FlOH. We considered implicit solvation of ACN using the PCM model. Frequency calculation was conducted to confirm the absence of imaginary frequencies. The front and side views of the
optimized Et-FIOH ground-state geometry are shown in Figure 3.16a (Conformer 1). The computed optimized structure suggests that the central ring of the isoalloxazine ring is not planar due to the sp$^3$-type geometry of N5 atom. On the other hand, phenyl and imide rings of the isoalloxazine ring maintain their planarity.

**Figure 3.14.** Black curve represents normalized absorption spectrum of Et-FIOH in ACN. Red lines represent vertical excitation energies of Et-FIOH calculated using TD-B3LYP/6-31+G* level of theory (ACN as a solvent using PCM model).

Using the optimized Et-FIOH structure, we performed TD-DFT calculations to estimate vertical excitation energies and oscillator strengths for the first four singlet excited states of Et-FIOH. The results of our calculations are compared with the experimental absorption spectrum of Et-FIOH in Figure 3.14. The computed S$_1$ energy underestimates the experimental value by 0.32 eV, which is well within the acceptable accuracy of TD-DFT method (typical error is in the 0.1 – 0.5 eV range$^{60}$). The presence of $^1n,\pi^*$ state is usually revealed by the existence of low oscillator strength transition. In the case of Et-FIOH, the first excited state with low oscillator strength is S$_4$ state ($\lambda_{\text{ABS}}$=268 nm), suggesting this state could have $^1n,\pi^*$ character.
To evaluate the characteristics of calculated excited states, we plotted their DDPs\textsuperscript{27,57} in Figure 3.15. Each plot represents a difference in the electronic density between the excited state of interest and the ground state (S\textsubscript{n}-S\textsubscript{0}). DDPs of the first three Et-FIOH excited states are qualitatively similar and involve density changes across the entire π system of the isoalloxazine ring, with the charge depletion from the phenyl ring (red color) and accumulation of electronic density on the imide moiety of the isoalloxazine framework (green). We assign these three state to \textit{1}\textsubscript{π,π}\textsuperscript{*} states with a charge transfer character. The first state with \textit{1}n,π\textsuperscript{*} character state is the S\textsubscript{4} state, as can be visualized in Figure 3.15 as decrease of electronic density from the two imide oxygen atoms and increase in the electron density at the imide ring. Since this state has very high excitation energy (4.6 eV), we conclude that the \textit{1}n,π\textsuperscript{*} state is probably not involved in fast internal conversion of Et-FIOH. In addition, energies of S\textsubscript{2} and S\textsubscript{3} states are also significantly higher than that of S\textsubscript{1} state, and as such are not likely to be involved in internal conversion of Et-FIOH. These results suggest that a single S\textsubscript{1}-S\textsubscript{0} conical intersection causes the excited-state deactivation of Et-FIOH, without the mediation by higher excited states.
3.3.4 DFT calculations on Et-FIOH conformers

The fact that none of the above proposed mechanisms accounts for the fast excited-state decay in Et-FIOH led us to postulate that the decay mechanism involves $S_1 \rightarrow S_0$ conical intersection. To identify the nuclear coordinates responsible for deactivation, we investigated $S_0$ and $S_1$ energies of several different Et-FIOH conformers. Using B3LYP/6-31+G* methodology, we optimized $S_0$ state of Et-FIOH starting from a range of different input geometries. The optimization of all input structures produced one of the three local minima presented in Figure 3.16. The lowest-energy structure (conformer 1) has two planar rings (phenyl and imide rings), while the central ring of the isoalloxazine structure is bent (Figure 3.16a). In conformers 2 and 3, we observe increased planarity of the central ring and increased bending of the imide ring (Figure 3.16b and c).

![Figure 3.16. Optimized geometries for conformers 1–3 (B3LYP/6-31+G* and a PCM model of ACN). Color code: gray, carbon; blue, nitrogen; red, oxygen. H-atoms are excluded for clarity.](image)

The $S_0$ energy of these conformers strongly depends on the geometry of the N(5) atom: the closer the C4a—N5—C5a angle is to the sp$^3$-type tetrahedral geometry, the lower is the $S_0$ energy of Et-FIOH conformer. This trend can be clearly observed as changes in C4a-N5-C5a and N1-C10a-C4a-C4 angles (Figure 3.17). We further performed TD-B3LYP/6-31+G* calculations to estimate energies of $S_1$ states in these three conformers (Figure 3.17). In contrast to ground-
state energies, $S_1$ energies decrease with increasing C4a—N5—C5a angle. Thus, increased planarity of N5-atom leads to $S_0$ state destabilization and $S_1$ state stabilization (Table 1).

**Figure 3.17.** Structures and $S_1$ and $S_0$ energies of three conformers of Et-FIOH obtained using B3LYP/6-31+G* methodology.

**Table 1. Calculated $S_0$ and $S_1$ Energies for Three Et-FIOH Conformers**

<table>
<thead>
<tr>
<th>Conformer</th>
<th>C4a—N5—C5a angle (deg)</th>
<th>$S_1$ energy (Hartrees)</th>
<th>relative $S_0$ energy (kcal/mol)</th>
<th>$\lambda_{\text{AB}}$ (nm)</th>
<th>orbitals involved</th>
<th>relative $S_1$ energy (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>112.7</td>
<td>1105.8248</td>
<td>0</td>
<td>382</td>
<td>HOMO $\rightarrow$ LUMO</td>
<td>74.85</td>
</tr>
<tr>
<td>2</td>
<td>118.2</td>
<td>1105.8197</td>
<td>3.75</td>
<td>407</td>
<td>HOMO $\rightarrow$ LUMO</td>
<td>74.00</td>
</tr>
<tr>
<td>3</td>
<td>120.9</td>
<td>1105.8176</td>
<td>5.05</td>
<td>434</td>
<td>HOMO $\rightarrow$ LUMO</td>
<td>70.93</td>
</tr>
</tbody>
</table>

*Calculations were performed using TD-B3LYP/6-31+G* and a PCM model of ACN.

Given the high sensitivity of $S_0$ and $S_1$ energies to the degree of hybridization on N(5) atom, we postulate that the cause of fast excited-state deactivation in Et-FIOH is $S_1$$\rightarrow$$S_0$ conical intersection reached by C4a—N5—C5a angle coordinate. It is interesting to note that the increased planarity of the central isoalloxazine ring (observed as an increase C4a—N5—C5a angle) leads to the decrease in planarity of the imide ring (observed as an increase in the N1—
C10a—C4a—C4 dihedral angle). A similar distortion was found to cause the \( S_1 \rightarrow S_0 \) conical intersection in cytosine, which is structurally akin to the imide ring moiety of Et-FIOH.\(^{59,61,62}\)

### 3.4 Conclusions

In summary, we studied the excited state behaviour of Et-Fl\(^+\) and the mechanism of fast excited-state decay in Et-FIOH, two model compound for bacterial bioluminescence. We investigated the electronic properties of the flavinium salt Et-Fl\(^+\) and compared them with 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 \( S_1 \) and \( S_3 (\pi,\pi^*) \) excited states, while the \( S_2 \) state is an \( (n,\pi^*) \) state. Visible and mid-IR TA 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.\(^63\) Furthermore, we observed a fast (23–30 ps) \( S_2 \rightarrow S_0 \) internal conversion in TA spectra of both Fl and Et-Fl\(^+\) in experiments that utilized higher energy pump beams.

We found that Et-FIOH exhibits complex excited-state decay dynamics with the highest amplitude decay component exhibiting 500 fs lifetime. We investigated several possible mechanisms of Et-FIOH excited-state deactivation: (i) excited state tautomerization; (ii) deactivation via a dark \(^1n, \pi^*\) state; (iii) excited-state release of hydroxide ion, and (iv) thermal deactivation due to \( S_1 \rightarrow S_0 \) conical intersection. Based on our pump-probe data and TD-DFT calculations, we postulate that the \( S_1 \rightarrow S_0 \) conical intersection along the C4a—N5—C5a // N1—C10a—C4a—C4 coordinates is the reason for the low fluorescence/chemiluminescence
efficiency of Et-FlOH. We expect this work to be useful for the development of improved models for bacterial bioluminescence. Furthermore, we\textsuperscript{64} and others\textsuperscript{65} previously postulated that similar nitrogen inversion in isoalloxazine ring causes fast excited-state deactivation in reduced flavin cofactors. Thus, the study presented here could be extended to the excited-state behavior of a broad range of reduced flavins.

3.5 Experiments and Theory

3.5.1 Synthesis

3-Methyl-lumiflavin (Fl), N(5)-ethylflavinium perchlorate (Et-Fl\textsuperscript{+}), N(5)-ethyl-4a-hydroxyflavin (Et-FlOH) and N(5)-ethyl-4a-methoxyflavin (Et-FlOMe) were synthesized according to the published procedure.\textsuperscript{26}

3.5.2 Steady-State Spectroscopy

UV-vis absorption spectra were recorded on Agilent 8453UV spectrometer in a 1 cm quartz cell. Emission spectra were recorded using QM-4/2006SE spectrometer (Photon Technologies Incorporated) in a 1 cm quartz cell. The sample solutions for fluorescence measurements had absorption of 0.1-0.15 at the wavelength of excitation. Fluorescence quantum yield for Et-FlOH was determined using an argon-purged solution of [Ru(bpy)\textsubscript{3}](CF\textsubscript{3}SO\textsubscript{3})\textsubscript{2} in water as a standard (quantum yield is 0.042).\textsuperscript{66} FTIR spectra were collected using FTIR-8400S (Shimadzu).

3.5.3 Femtosecond Transient Absorption (TA) Experiment

Ultrafast UV-vis TA measurements. The femtosecond (~130 fs) laser system for the ultrafast TA measurement was described in chapter II. Briefly, the 800 nm laser pulses were produced at a 1 kHz repetition rate by a mode-locked Ti:Sapphire laser (Hurricane, Spectra-Physics). The output from a Hurricane was split into pump (85 %) and probe (8 %) beams. The
pump beam (800 nm) was sent into an optical paramagnetic amplifier (OPA-800C, Spectra Physics) to obtain a 350 nm excitation source. The energy of the pump beam was \( \sim 3 \mu J/pulse \).

The probe beam (800 nm) was delayed by a delay stage (MM 4000, Newport) and then focused into a rotating CaF\(_2\) crystal for white light continuum generation between 350 and 800 nm. After passing through the cell, the continuum was coupled into an optical fiber and input into a CCD spectrograph (Ocean Optics, S2000). The data acquisition was achieved using in-house LabVIEW (National Instruments) software routines. The group velocity dispersion of the probing pulse was determined using nonresonant optical Kerr effect (OKE) measurements.\(^6\) The excitation beam was focused on a sample cell containing carbon tetrachloride (Aldrich Chemical Company). The sample cell was placed between a pair of crossed polarizers through which the probe beam was sent. The relative polarization between the incoming pump and probe beams was set at 45\(^o\). The induced OKE signal was measured at various time delays. This measurement was used to perform a chirp correction in the collected TA data by a code written with Matlab 7.1 software. First, the temporal evolution of the OKE signal at every wavelength was fitted to a Gaussian function. The value for the peak maximum obtained from the fit was used to construct the wavelength-dependent zero-times. Then, the time arrays from the TA data were chirp-corrected by subtracting the value of zero-time at every wavelength.

**Ultrafast Time-Resolved IR measurements.** The spectra were collected using the instrument described in chapter II. The source of fs pulses at 800 nm (1 kHz repetition rate, \(~130 \) 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 Fl solution was excited at 400 nm, while the Et-Fl\(^+\) solution was excited at 500 nm. The sample
solution flow was achieved using fluid metering RHSY lab pump (Scientific Support Inc.) through a demountable liquid flow cell with Swagelok fittings (DSC-S25, Harrick Scientific Product Inc.). The path length was 80 µm created by Teflon spacer between two round CaF₂ 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 x 32 array of MCT detectors (Infrared Systems Development Corporation).

3.5.4 Data Analysis

TA data were analyzed using SPECFIT/32™ Global Analysis System (Spectrum Software Associates, MA, USA). This program allows a decomposition of TA data using kinetic models. The fitting process returns the predicted absorption spectra of individual colored species involved in the photochemical process 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.68

3.5.5 Computational Methods

All calculations were performed at the Ohio Supercomputer Center. For Et-Fl⁺ and Fl, geometry optimizations were performed using Gaussian 0369 at the B3LYP70,71/6-31G* level of theory. All stationary points were confirmed to be energy minima using vibrational frequency calculations (B3LYP/6-31G*), which showed that all of the computed vibrational frequencies were non-imaginary. Vertical transition energies for the optimized structures were then calculated at the TD-B3LYP/6-31+G* level of theory. Difference-density plots were generated from vertical transitions (TD-B3LYP/6-31+G*) computed using Gaussian 09.72 For Et-FIOH and Et-FIOMe, ground-state geometry optimizations of Et-FIOH were performed starting from
several input geometries using Gaussian 09 at the B3LYP/6-31+G* level of theory and with consideration of implicit solvation of acetonitrile (ACN) (using the polarization continuum model, PCM). Tight optimization criteria were used in all calculations. All stationary points were confirmed to be energy minima using vibrational frequency calculations (B3LYP/6-31+G*), 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 with consideration of implicit solvation of ACN using the PCM. Difference-density plots were generated from the computed electron densities of the ground and excited states (TD-B3LYP/6-31+G*) using Gaussian 09.

References:


(41) Abe, M.; Kyogoku, Y.; Kitagawa, T.; Kawano, K.; Ohishi, N.; Takai-Suzuki, A.; Yagi,

(42) Kondo, M.; Nappa, J. r. m.; Ronayne, K. L.; Stelling, A. L.; Tonge, P. J.; Meech, S. R. J.


2009, 131, 16939-16943.

(49) Kijak, M.; Nosenko, Y.; Singh, A.; Thummel, R. P.; Waluk, J. J. Am. Chem. Soc. 2007,
129, 2738-2739.


(56) Forster, T. Z. Elektrochem. 1950, 54, 42.


Farkas, O., Foresman, J. B., Ortiz, J. V., Cioslowski, J., and Fox, D. J. Gaussian, Inc., 2009, Wallingford CT.


(77) Barone, V.; Cossi, M.; Tomasi, J. Journal of Chemical Physics 1997, 107, 3210-3221.

CHAPTER IV: MECHANISTIC STUDY OF THE PHOTOCHEMICAL HYDROXIDE ION RELEASE FROM AcrOH

4.1 Introduction

Photohydroxide emitters are a class of compounds that release hydroxide anions (\(\cdot\)OH) to induce a pOH jump upon UV-vis irradiation. This process is similar to the pH jump methods achieved using various photoacids.\(^1\)-\(^4\) The pOH jump is generally achieved through heterolytic cleavage of the C–O bond, as is exhibited by compounds such as 9-fluorenol,\(^5\)-\(^6\) 9-phenylxanthen-9-ol,\(^7\) and triphenylmethane hydroxide,\(^8\),\(^9\) whose resonance structures stabilize the resulting carbocations. The application of these compounds in driving a photochemical pOH jump is mostly limited by the fast recombination of hydroxide and carbocation.\(^5\)-\(^7\) Additionally, homolytic C–O bond cleavage can complicate the chemistry with the formation of hydroxyl radical (\(\cdot\)OH).\(^5\),\(^7\),\(^10\)

Malachite green carbinol base (MGCB) has been suitable for photochemical pOH jump\(^9\) because the lifetime of \(\cdot\)OH, which is generated in 300 ps,\(^8\) is several minutes, and \(\cdot\)OH is not formed. Upon excitation, MGCB was found to induce a jump from pH=6 to 11, making it a possible agent for studies of conformational changes in DNA/RNA\(^1\),\(^12\) and also for the release of drugs from host molecules.\(^13\)

Recently, Fréchet and coworkers discovered that 9-hydroxy/methoxy-10-methyl-9-phenyl-9,10-dihydroacridine (AcrOR, R=H or Me) exhibits somewhat similar photochemical behavior to that of MGCB.\(^14\) Upon irradiation in H\(_2\)O/acetonitrile (ACN) mixture, AcrOH releases 10-methyl-9-phenylacridinium (Acr\(^+\)) and \(\cdot\)OH, resulting in a pOH jump, which is longer lasting than that of MGCB. However, the photochemical mechanism of AcrOH was not
studied until now. This study presents a detailed investigation of the excited-state dynamics of AcrOR via femtosecond (fs) and nanosecond (ns) transient absorption (TA) spectroscopy (Scheme 4.1), including the varying solvent-facilitated pathways of Acr$^+$ generation. Generation of Acr$^+$ is obvious in protic solvation (methanol, MeOH), while an intersystem crossing (ISC) process is observed in aprotic solvation (ACN and benzene).

**Scheme 4.1. Excited State Behavior of AcrOH in Different Solvents: MeOH (blue) and ACN or Benzene (red).** For AcrOMe, $\tau_{\text{hetero}} = 83$ ps and $\tau_{\text{ISC}} = \sim 550$ ps.

### 4.2 Results and Discussions

#### 4.2.1 Ground State Spectra and Förster Energy Diagram

To evaluate the driving force for the excited-state heterolytic and homolytic C–O bond cleavage in AcrOH, a Förster energy diagram$^{15}$ was constructed from pH-dependent absorption and emission measurements along with the previously known reduction and oxidation potentials of Acr$^+$ and $\ce{OH^-}$ (Figure 4.1). Using pH-dependent absorption measurements, the ground-state pseudobase pKₐ value of AcrOH was determined to be 11.11, which is consistent with the literature.$^{16}$ Based on the Förster energy diagram, the driving force for the excited-state hetero
and homolytic C–O bond cleavage was evaluated as $\Delta^1 G_{\text{hetero}}^* = -1.048 \text{ eV}$, $\Delta^3 G_{\text{hetero}}^* = -0.263 \text{ eV}$, $\Delta^1 G_{\text{homo}}^* = -1.513$, and $\Delta^3 G_{\text{homo}}^* = -0.728 \text{ eV}$, indicating that both heterolytic and homolytic cleavage are possible in the excited state.

**Figure 4.1.** Upper: pH dependent UV-vis absorption and emission spectra of Acr$^+$ (0.02 mM, H$_2$O:ACN 1000:1, $\lambda_{\text{ex}} = 290 \text{ nm}$) used to estimate the pseudobase pK$_a$ of AcrOH; Lower: Schematic representation of Förster cycle used to estimate the $\Delta G^*$ for excited state bond cleavage of AcrOH.

**Description of Figure 4.1:** To evaluate the ground state ($\Delta G$) and excited state ($\Delta G^*$) Gibbs free energy for the heterolytic C-O bond breaking in AcrOH, the pH-dependent absorption and emission spectra of 0.2 mM Acr$^+$ in 1000:1 H$_2$O:ACN are collected and presented along with a Förster cycle$^{15}$ in Figure 4.1. As the solution pH increases, the decrease of Acr$^+$ absorption at 427 nm is coupled with the increase of AcrOH absorption at 287 nm. The pH at which the Acr$^+$ 427 nm absorptivity decreased to 50% (pH 11.11) was used to evaluate $K_{\text{hetero}}$, as follows:
At pH 11.11, the concentrations of Acr\(^+\) and AcrOH are equivalent: \([Acr^+] = [AcrOH]\).

So, \(K_{\text{hetero}} = \frac{[OH^-]}{[AcrOH]}\) = \(10^{-14} / 10^{-11.11} = 10^{-2.89}\)

\(\Delta G_{\text{hetero}}\) was determined by thermodynamically relating the equilibrium constant \((K)\) of AcrOH dissociation to the change in Gibbs free energy for the process.

\[
\Delta G_{\text{hetero}} = -RT \ln K_{\text{hetero}}
\]

\[
= 0.172 \text{ eV}
\]

The \(S_1\) and \(T_1\) state \(\Delta G^*\)s for heterolytic and homolytic bond cleavages were evaluated using the Förster cycle, as follows:

\[
\Delta^1 G^*_{\text{hetero}} = h\nu_{320\ \text{nm}} - \Delta G_{\text{hetero}} - h\nu_{467\ \text{nm}}  \tag{1}
\]

\[
\Delta^1 G^*_{\text{homo}} = h\nu_{320\ \text{nm}} - \Delta G_{\text{hetero}} - \Delta G_{\text{ET}}  \tag{2}
\]

\[
\Delta^3 G^*_{\text{hetero}} = h\nu_{401\ \text{nm}} - \Delta G_{\text{hetero}} - h\nu_{467\ \text{nm}}  \tag{3}
\]

\[
\Delta^3 G^*_{\text{homo}} = h\nu_{401\ \text{nm}} - \Delta G_{\text{hetero}} - \Delta G_{\text{ET}}  \tag{4}
\]

The energy gaps between the ground state and the excited state of AcrOH and Acr\(^+\) are evaluated by the mean values of the first absorption and emission peaks of both compounds (Figure 4.1 and 2), respectively (\(^1\)AcrOH\(^*\): 320 nm, \(^3\)AcrOH\(^*\): 401 nm, and \(^1\)Acr\(^+\): 467 nm). The energy \((\Delta G_{\text{ET}} = E_{\text{ox}}^-(\text{OH}) - E_{\text{red}}(\text{Acr}^+) = 2.19 \text{ eV})\) needed for one electron transfer from \(^-\text{OH}\) to Acr\(^+\) is obtained from CV measurements and previous literature. The standard reduction potential of Acr\(^+\), which was measured by our group, is \(E^0_{\text{red}} = -0.3 \text{ V vs SHE}\),\(^{17}\) and the reported standard oxidation potential of \(^-\text{OH}\) is \(E^0_{\text{ox}} = 1.89 \text{ V vs SHE}\).\(^{18}\) Equations 1 and 2, obtained from the Förster energy diagram, are used to calculate the energy needed for excited state C-O bond cleavage.
Based on the calculations, both heterolytic (AcrOH + hv → Acr\(^{+}\) + •−OH) and homolytic (AcrOH + hv → Acr\(^{•}\) + •OH) bond cleavages were estimated to be thermodynamically possible in the excited state (Δ\(^{1}\)G\(^*_\)\(_{\text{hetero}}\) = −1.048 eV, Δ\(^{3}\)G\(^*_\)\(_{\text{hetero}}\) = −0.728 eV, Δ\(^{1}\)G\(^*_\)\(_{\text{homo}}\) = −1.513 eV and Δ\(^{3}\)G\(^*_\)\(_{\text{homo}}\) = −0.263 eV); however, the experimental results have a little variation. As shown in the Figure 4.1 lower, S\(_{1}\)→T\(_{1}\)→S\(_{0}\) of AcrOH process happens in aprotic solvent (red color), and \(^{1}\)AcrOH\(^{•}\)→Acr\(^{+}\) happens in protic solvent.

The geometry of the AcrOH ground state was optimized and the character of the AcrOH excited states was evaluated using density functional theory (DFT) and time-dependent DFT calculations, respectively (Figure 4.2). Difference density plots\(^{19,20}\) shown in Figure 4.2 indicate that the S\(_{0}\)→S\(_{1}\) transition exhibits π–π\(^{*}\) character within the acridine framework, while the S\(_{0}\)→S\(_{2}\) transition generates a π–π\(^{*}\) state in which an intramolecular electron transfer from the acridine framework to the phenyl moiety occurs. The laser measurements shown in this work were performed using a 310 nm excitation pulse; when compared to the calculated vertical excitations (Figure 4.2), this laser excitation should generate only the singlet excited (S\(_{1}\)) state with π–π\(^{*}\) character.
**Figure 4.2. Upper:** Normalized ground state absorption (red) and fluorescence (blue, $\lambda_{\text{ex}} = 310$ nm) spectra of 0.080/0.041 mM AcrOH/AcrOMe in acetonitrile (Extinction Coefficients (M$^{-1}$cm$^{-1}$): 5030/9654 (310 nm), 6154/8196 (266 nm), Fluorescence Quantum Yields: 0.23/0.11 respectively). Black lines represent vertical excitation energies of AcrOH/AcrOMe calculated using TD-B3LYP/6-31++G* level of theory, PCM acetonitrile. **Inset:** 77K fluorescence and phosphorescence ($\lambda_{\text{ex}} = 310$ nm) of AcrOH in ACN. **Lower:** DD plots for the first two singlet excited states of AcrOH/AcrOMe. Isovalue: $\pm 0.0004$. Red color represents depletion of electron density in the ground state, while the green contours represent accumulation of electronic density in the specific excited state.

**Description of Figure 4.2:** Figure 4.2 shows the experimental absorption and emission spectra of AcrOR in ACN, as well as calculated transition energies for AcrOH obtained using time-dependent density functional theory (TD-DFT) calculations. For both AcrOH and AcrOMe, the calculated frequencies match well with the experimental absorption bands. The lower panel of Figure 4.2 shows the electron difference density (DD) plots$^{19}$ for $S_1-S_0$ and $S_2-S_0$ states of
AcrOR. DD plots of the $S_0$–$S_1$ transition shows a change in the electron density along the acridine moiety, which is consistent with the $\pi$–$\pi^*$ state localized on the acridine rings. On the other hand, the $S_0$–$S_2$ transition generates a $\pi$–$\pi^*$ state in which an intramolecular electron transfer from the acridine framework to the phenyl moiety occurs. Both above results indicate that the 310 nm wavelength, which was used in laser flash measurements, only can excite an $S_1$ state with the $\pi$–$\pi^*$ character.

4.2.3 Excited State Spectra

![Figure 4.3](image)

**Figure 4.3.** (a) fs TA spectra, time delays: -4.5 ps (▬), 0 ps (▬), 11 ps (▬), 310 ps (▬), 570 ps (▬), 1150 ps (▬), and 1574 ps (▬), and (b) kinetics of 1 mM AcrOH in ACN, $\lambda_{EX} = 310$ nm; (c) UV-vis spectroelectrochemistry spectra of 1 mM Acr$^+$ in ACN at -0.56 V vs SHE, time delays: 0 s (▬), 7 s (▬), 14 s (▬), 21 s (▬), 49 s (▬); UV-vis absorption of Acr$^+$ (▬) (d) Cyclic voltammogram of 3 mM Acr$^+$ in anaerobic ACN containing 0.1 M tetrabutylammonium perchlorate, $E_{\text{red}} = -0.3$ V vs SHE.

The photochemical behavior of AcrOH in different solvents (ACN, benzene, and MeOH) was investigated using the fs TA technique. In ACN, the $t = 0$ TA spectrum of AcrOH exhibits a broad absorbance from 350 to 800 nm with $\lambda_{\text{max}}$ at 780 nm that decays with a lifetime of 1.4 ns
(Figure 4.3). This component is assigned to the $S_1$ state of AcrOH. The decay of the $S_1$ state is accompanied by the growth of a new band at 550 nm that does not decay within 1.5 ns (the time limit of fs TA detection). The excited-state behavior of AcrOH and AcrOMe in other aprotic solvents, such as benzene (Figure 4.4), is similar to that shown in Figure 4.3.

**Figure 4.4.** fs TA spectra of AcrOR at varying time delays after the 310 nm excitations pulse.

Upper: Singlet excited state lifetimes of AcrOH/AcrOMe collected at 781 nm using the fs UV-vis TA setup. The lifetime of $S_1$ state in AcrOH is

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\tau_{S1}$ (ns)</th>
<th>$\tau_{\text{isc}}$ (ps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACN</td>
<td>1.4</td>
<td>544</td>
</tr>
<tr>
<td>Benzene</td>
<td>1.3</td>
<td>571</td>
</tr>
</tbody>
</table>

**Description of Figure 4.4:** Upper table shows the singlet excited state lifetimes of AcrOH and AcrOMe collected at 781 nm using the fs UV-vis TA setup. The lifetime of $S_1$ state in AcrOH is
\( \tau_{S1} = 1.4 \text{ ns in ACN and Benzene, which is slower than AcrOMe (} \tau_{S1} = 550 \text{ ps) under the same conditions. Lower Figure 4. presents the fs TA spectra of AcrOH and AcrOMe in ACN and benzene, obtained upon excitation at 310 nm pulses. All spectra show that a } \sim 550 \text{ nm absorbance generation (} T_1 \text{ state) is coupled with a } \sim 780 \text{ nm absorbance decay (} S_1 \text{ state). In fs TA spectra of AcrOMe in ACN, the formation of 550 nm absorbance (} T_1 \text{ state) is also coupled with a } 427 \text{ nm absorbance generation, due to the formation of the ground state Acr}^+. \) This data indicates that the hydrogen bonding of polar protic solvation is not necessary for heterolytic cleavage, as even in polar aprotic solvation, heterolytic cleavage of C-O bond is still observed, if this bond is sufficiently weak.

If the excited-state heterolytic C–O bond cleavage occurred, the 550 nm signal could be assigned to the formation of either \( \text{Acr}^+ \) (nonadiabatic process) or \( \text{Acr}^{+*} \) (adiabatic process). However, comparison of the absorption spectra of \( \text{Acr}^+ (\lambda_{\text{max}} = 425 \text{ nm; Figure 4.3c}) \) and \( \text{Acr}^{+*} (\lambda_{\text{max}} = 390 \text{ and } 470 \text{ nm; Figure 4.5}) \) to this 550 nm band shows that the heterolytic C–O bond cleavage does not occur from the \( S_1 \) state of AcrOH.

**Figure 4.5.** fs TA spectra of Acr\(^+\) in ACN, 1 mM, \( \lambda_{\text{ex}} = 310 \text{ nm.} \)

**Description of Figure 4.5:** The TA spectrum of Acr\(^+\) was obtained in order to determine whether the excitation of AcrOH leads to an adiabatic release of \( \text{–OH} \) ions and the generation of the \( S_1 \)
state of Acr\(^+\). Figure 4.5 presents the fs TA spectra of 1 mM Acr\(^+\) in ACN excited by 310 nm pulses, at varying intervals of time after excitation. The excited state Acr\(^+\) absorbance (350 – 500 nm) is overlapped with the ground state bleach of Acr\(^+\) at 427 nm, while the stimulated emission occurs at 540 nm. Thus, if Acr\(^+\) is generated during the excitation of AcrOR, one would expect the absorption in the 350-500 nm range and a stimulated emission at 540 nm. As can be seen from Figure 4.4, these signature peaks are not observed, thus ruling out the possibility that Acr\(^{+}\ast\) is generated upon excitation of AcrOR.

On the basis of previous literature,\(^7\) this 550 nm peak could be postulated to arise from the acridine radical (Acr\(^\ast\)), which would be formed by homolytic C–O bond cleavage (AcrOR + hv → Acr\(^\ast\) + \(\cdot\)OR). The absorption spectrum of Acr\(^\ast\) was obtained by a one-electron reduction of Acr\(^+\) (see the cyclic voltammogram in Figure 4.3d). The UV–vis spectroelectrochemical results shown in Figure 4.3c demonstrate that Acr\(^\ast\) absorbs at 500 nm, which matches well with the previously reported Acr\(^\ast\) spectrum obtained using chemical reduction.\(^21\) Given the large mismatch between the 550 nm band obtained in the TA spectra and the Acr\(^\ast\) signature band, we conclude that homolytic C–O bond cleavage from AcrOH\(^\ast\) does not occur. The experiments performed on the ns time scale show that the 550 nm transient is long-lived (\(\tau_{550\ nm} = 277\) ns; Figure 4.6) and readily quenched by molecular oxygen. Thus, this intermediate is assigned to the triplet excited (T\(_1\)) state of AcrOH.
Figure 4.6. ns TA spectra of AcrOH/AcrOMe after the 266 nm excitation pulse. (a) AcrOH in ACN, 0.17 mM, Ar-degassed. (b) AcrOH in ACN, 0.17 mM, O$_2$-saturated. (c) AcrOMe in ACN, 0.12 mM, Ar-degassed. (d) AcrOMe in ACN, 0.12 mM, O$_2$-saturated.

Description of Figure 4.6: Figure 4.6 presents the ns TA spectra of AcrOH and AcrOMe in ACN, excited by 266 nm pulses. For AcrOH, in Ar-degassed ACN (Figure 4.6a), the 550 nm absorbance is generated within 20 ns laser pulse with a 277 ns lifetime, while no signal is observed in O$_2$-saturated solution (Figure 4.6b). The AcrOMe spectra (Figure 4.6c) show the absorption at 550 nm due to triplet formation and the 427 nm absorption due to Acr$^+$. As expected, the ~550 nm absorbance is affected by O$_2$, while the Acr$^+$ absorbance is clearly observed in O$_2$-saturated solution. The Acr$^+$ generation in ns TA of AcrOMe in ACN proves that heterolytic cleavage is possible in the polar aprotic solvent if the C-OR bond is sufficiently weak.

On the other hand, no 500 nm Acr$^*$ absorption is observed in all ns TA measurements, which proves the homolytic cleavage of the C-OR bond does not occur from neither $S_1$ nor $T_1$ states of AcrOR. The lack of Acr$^*$ formation from the $T_1$ state of AcrOR in the ns TA measurements of AcrOR in different solvents is possibly due to lower energy of the $T_1$ state than the energy required to homolytically break the AcrOR.
These results suggest that even though the homolytic bond cleavage is thermodynamically favorable (Figure 4.1) and previous reports on similar systems\textsuperscript{7} demonstrate the formation of the homolytic products, the S\textsubscript{1} state of AcrOH does not undergo homolytic C–O bond breaking. This is possibly due to a competing deactivation channel of the S\textsubscript{1} state via ISC to form the T\textsubscript{1} state. Additionally, formation of the Acr\textsuperscript{'} absorbance at 500 nm was not observed in the ns TA measurements, showing that homolytic C–O bond cleavage on the T\textsubscript{1} state surface does not lead to separated Acr\textsuperscript{'} and \textsuperscript{'}OH radicals, even though the evaluated AcrOH T\textsubscript{1} energy is higher than the energy needed for the C–O bond homolysis (Figure 4.1). A possible scenario that can explain this absence of observable homolytic products is one in which a conical intersection between the T\textsubscript{1} and S\textsubscript{0} states exists along the C–O bond coordinate. Since the T\textsubscript{1} lifetime of AcrOH (277 ns) is shorter than the T\textsubscript{1} lifetimes of most organic compounds (usually in the \textmu s to ms range\textsuperscript{22}), it is possible that such a mechanism operates in this case. Indeed, DFT calculations suggested that homolytic C–O bond cleavage in the T\textsubscript{1} state occurs at a C–O bond distance of 1.8 Å. This triplet transition state would lead to a pair of separated radicals at a distance of 3.0 Å. However, along the C–O distance, the ground-state singlet surface and the triplet surface cross each other at a separation of 2.8 Å. Thus, we suggest that homolytic C–O bond cleavage in the triplet state leads to a conical intersection between the S\textsubscript{0} and T\textsubscript{1} states, resulting in the recovery of AcrOH in the ground state (Figures 4.7–4.9).
Figure 4.7. Jablonski diagram for calculated T₁ and S₁ energies of AcrOH as a function of C-O bond lengths.

Description of Figure 4.7: Figure 4.7 shows the T₁ energy profile of AcrOH as a function of the C-O bond length calculated using DFT methodology (B3LYP/6-31G*). The increase in the C–O bond length leads to an increase in the T₁ energy until the transition state (TS) is reached at 9.8 kcal/mol above the energy of the relaxed T₁ state. Given that the Frank-Condon S₁ state (95 kcal/mol above S₀ state) is sufficiently above the T₁ transition state (84.8 kcal/mol), the generated AcrOH is expected to have sufficient energy to cross the barrier and lead to the homolytic bond breaking products. However, comparison of the calculated S₀ and T₁ radical pair energies show that the T₁ Acr⁺ + ·OH radical pair exhibits lower energy than the S₀ radical pair (by 8.4 kcal/mol). Given this reversal in S₀ and T₁ energies along the C-O bond coordinate, it is very likely that the T₁–S₀ conical intersection exists along this reaction coordinate, and that it prevents the formation of separated ion pairs. Instead, the system returns back to the AcrOH ground state. Given that this T₁–S₀ conical intersection requires the conversion from the open-shell T₁ system into the closed-shell S₀ state, it is very likely that the crossover from T₁ to S₀ state occurs by an intramolecular electron transfer from the Acr⁺ moiety to the ·OH moiety of AcrOH.
Similar polar effects were observed previously by the Hadad team in their study of the reactions of hydroxyl radical with aromatic hydrocarbons.\textsuperscript{23}

**Figure 4.8.** Intrinsic Reaction Coordinate (IRC) starting from the transition state (C–O distance = 1.76 Å) towards formation of the Acr$^*$ + *OH radical pair (C–O distance =2.98 Å) as calculated at the B3LYP/6-31G* level on the T$_1$ surface.

**Description of Figure 4.8:** Figure 4.8 shows the T$_1$ energy profile along the IRC path starting from the transition state (C–O distance = 1.76 Å) towards the Acr$^*$ + *OH radical pair (C–O distance =2.98 Å) calculated at the B3LYP/6-31G* level of theory. The corresponding S$_0$ energy was computed at the B3LYP/6-31G* level using the triplet optimized geometries along the IRC path. There is a potential crossing between S$_0$ and T$_1$ states when the C–O bond length is $\sim$ 2.8 Å.
Figure 4.9. Natural population analysis on geometries along the triplet IRC path on the triplet surface and singlet surface at the B3LYP/6-31G* level of theory.

Description of Figure 4.9: Figure 4.9 shows the natural population analysis of geometries along the IRC pathway described in Figure 4.8. Both the charge on the OH fragment (close to 0 e) and the spin density on OH (close to 1) for the triplet state shows that the OH remains as a radical throughout the C–OH breaking process, while on the singlet surface, the OH species would be more like an hydroxide ion.

On the other hand, the behavior of AcrOH in protic solvents is different (Figure 4.10). The initially formed $S_1$ state decays within 108 ps to generate a product that absorbs at 425 nm. This product is assigned to Acr$^+$ by comparison to the ground-state absorption spectrum (Figure 4.10a inset). The protic solvation clearly has a strong effect on the energetics of the heterolysis, as schematized in Figure 4.1. As previously discussed by Steen,$^{24}$ the protic solvation stabilizes $^-$OH by promoting the migration of the negative charge along the hydrogen-bonded solvent.
network. The formation of Acr$^+$ occurs without any observable intermediate steps. To be specific, the formation of the intermediate Acr$^+$ via adiabatic heterolytic bond breaking along the S$_1$ surface of AcrOH was not observed in the TA spectra. The steady-state emission measurements also confirmed that Acr$^+$ is not formed as an intermediate during heterolytic bond cleavage (Figures 4.11), since no Acr$^+$ emission (450 to 650 nm) was observed in the emission measurements of AcrOH or AcrOMe in ACN or water.

**Figure 4.10.** (a) fs TA spectra (inset: UV-vis absorption spectrum of Acr$^+$), time delays: -6.3 ps ( ), 0 ps ( ), 30 ps ( ), 80 ps ( ), 200 ps ( ), 400 ps ( ), and 1487 ps ( ), and (b) kinetics of 1 mM AcrOH in MeOH, $\lambda_{EX} = 310$ nm.; (c) ns TA spectra, time delays: 60 ns ( ) and 510 ns ( ), and (d) kinetic of 0.17 mM AcrOH in MeOH, $\lambda_{EX} = 266$ nm, O$_2$ saturated.
**Figure 4.11.** UV-vis absorption (red) and emission (blue) of AcrOH (0.015mM) and AcrOMe (0.01 mM) in pH=12.31 water, and in the presence of 16 mM dodecyltrimethylammonium bromide, $\lambda_{ex} = 310$ nm.

**Description of Figure 4.11:** The steady-state emission spectra of AcrOR were collected to evaluate whether the Acr$^+$ fluorescence is observed upon excitation of AcrOR. The presence of Acr$^+$ fluorescence at 500 nm (Figure 4.1) would indicate the adiabatic excited-state C-O bond cleavage from AcrOR. The UV-vis absorption and emission spectra of AcrOR were collected in pH 12.31 water with surfactant (dodecyltrimethylammonium bromide, 16 mM) added to improve the solubility. No 500 nm emission was observed.

The TA experiments on the ns time scale showed that the Acr$^+$ signal at 425 nm is long-lived, and no signal decay was observed within 100 μs (Figure 4.10d). The long-lived charge separation is most probably due to the high stability of the fully aromatic Acr$^+$ ion that is formed upon heterolysis. For example, a previous study showed an increase in the recombination lifetimes for more stable cations formed upon heterolysis. The observed long lifetime of Acr$^+$ is a very encouraging result, since it demonstrates that the OH generated in the photochemical process can be utilized to drive pOH jump experiments. Similar behavior was observed for AcrOMe; however, some differences are also worth mentioning.
Figure 4.12. (a) fs TA spectra (The inset is the UV-vis absorption spectrum of Acr\(^{+}\)) and (b) kinetics of 0.5 mM AcrOMe in MeOH, \(\lambda_{\text{EX}} = 310\) nm (c) ns TA spectra of 0.1 mM AcrOMe in MeOH, \(\lambda_{\text{EX}} = 266\) nm, Ar-degassed and (d) O\(_2\)-saturated.

**Description of Figure 4.12:** Figure 4.12a presents the fs TA spectra of 0.5 mM AcrOMe in MeOH. The decay of the initially formed \(S_1\) state decay of AcrOMe at 781 nm is accompanied with the formation of Acr\(^{+}\), with absorption at 427 nm, and the formation of small amount of \(T_1\) state, with the absorption at 540 nm. The C-OMe heterolytic bond cleavage in AcrOMe (83 ps, Figure 4.12b) is faster than the C-OH cleavage in AcrOH (108 ps, Figure 4.10). Figure 4.12c shows the ns TA spectra of 0.1 AcrOMe, where the 427 nm absorption due to Acr\(^{+}\) is observed, however, no clear \(\sim 540\) nm \(T_1\) absorption is observed due to the weak \(T_1\) content. The signal is not sensitive to \(O_2\), as shown from the \(O_2\)-saturated conditions, shown in Figure 4.12d.

In the fs TA spectra of AcrOMe in MeOH (Figure 4.12), most of the AcrOMe was converted to Acr\(^{+}\) after the laser flash, though there was still some generation of the \(T_1\) state of AcrOMe (540 nm). These results reflect the fact that ISC is faster for AcrOMe. Comparison of the lifetime of the C–OH bond cleavage (108 ps) and the C–OMe bond cleavage (83 ps) shows...
that the C–OMe bond is weaker than the C–OH bond in MeOH. This conclusion is further supported by the known bond strengths of PhCH₂–OH and PhCH₂–OMe (78 and 68–70 kcal/mol, respectively). The lower bond energy of C–OMe also reflects the different reactivities of AcrOH and AcrOMe in aprotic solvents: while AcrOH does not generate Acr⁺ in aprotic solvents, the TA spectra of AcrOMe in ACN (Figures 4.4 and 4.6) show the formation of Acr⁺ from the singlet excited state.

### 4.3 Conclusions

In conclusion, photochemical excitation of AcrOH has been shown to result solely in heterolytic cleavage in protic solvents. Since the heterolysis is fast (108 ps) and followed by slow recombination of the ions (time scale of hours), the acridine derivatives such as AcrOH show promise for use in producing fast, long-lived pOH jumps.

### 4.4 Experiments and Theory

#### 4.4.1 Synthesis

AcrOMe, Acr⁺ and AcrOH were synthesized according to the modified procedures as described in Scheme 4.2. The synthesis of 9-methoxy-10-methyl-9-phenyl-9,10-dihydroacridine (AcrOMe) was achieved by a Grignard synthesis of phenylmagnesium bromide to 10-methyl-9(10H)-acridone (1) in THF. Acidification of AcrOMe with perchloric acid generated 10-methyl-9-phenylacridinium perchlorate, Acr⁺. The nucleophilic addition of–OH to Acr⁺ is used to produce 9-hydroxy-10-methyl-9-phenyl-9,10-dihydroacridine (AcrOH).

**Scheme 4.2: Synthetic Procedure for the Preparation of AcrOMe, Acr⁺, and AcrOH**
General Methods: All chemicals were purchased from commercial suppliers and used without further purification. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker Avance 300 MHz system. GC-MS spectra were measured on Shimadzu GC-MS-Q5050A spectrometer.

9-Methoxy-10-methyl-9-phenyl-9,10-dihydroacridine (AcrOMe): 10-Methyl-9(10H)-acridone (1) (1.02 g 5 mmol) was added to 10 mL of 1M phenylmagnesium bromide (10 mmol) solution in THF. The mixture was stirred at room temperature for 24 h, then poured into 200 mL of saturated aqueous solution of ammonium chloride and the pH was raised to 10 with 20 % NaOH aqueous solution. The solution was extracted three times with 100 mL portions of dichloromethane. Solvent was evaporated using rotary evaporator and the residue was recrystallized from 100 mL of methanol to yield 762 mg of AcrOMe (52 %). MS-EI: m/z 301 [M$^+$ calculated for C$_{21}$H$_{19}$ON]. $^1$H NMR (300 MHz, DMSO): 7.33 – 7.27 (m, 2H), 7.22 – 7.20 (m, 8H), 7.18 – 7.17 (m, 1H), 6.94 – 6.89 (m, 2H), 3.49 (s, 3H), 2.88 (s, 3H). $^{13}$C NMR (75 MHz, DMSO): 150.5, 140.9, 130.0, 128.9, 128.3, 126.6, 125.9, 124.1, 120.5, 113.3, 78.3, 51.1, 33.7.

10-Methyl-9-phenylacridinium perchlorate (Acr$^+$): AcrOMe (720 mg, 2.39 mmol) was suspended in 100 mL of water and 5 mL of 70 % HClO$_4$ was added. The mixture was heated to the boiling point and then cooled slowly to 0°C. The yellow needles were collected and dried to give 535 mg of Acr$^+$ (60 %), MS-EI: m/z 270 [M$^+$ calculated for C$_{20}$H$_{16}$N] $^1$H NMR (300 MHz, DMSO): 8.88, (d, J = 9.3, 2H, 1-H, 9-H), 8.49 – 8.44 (m, 2H, 2-H, 8-H), 7.95 – 7.93 (m,5H),
7.81 – 7.77 (m, 3H, o- and p- phenyl), 7.61 – 7.56 (m, 2H, m-phenyl). $^{13}$C NMR (75 MHz, DMSO): 160.9, 141.7, 138.9, 133.6, 130.6, 130.3, 130.0, 129.4, 128.4, 126.0, 119.7.

9-Hydroxy-10-methyl-9-phenyl-9,10-dihydroacridine (AcrOH): Acr$^+$ (225 mg, 0.6 mmol) was added to 100 mL of 1 M NaHCO$_3$. Acetonitrile (~25 mL) was added to the reaction mixture to dissolve all Acr$^+$. The pH was raised to 12.3 using 30 % NaOH. The Acr$^+$ disappearance was monitored by observing the 427 nm absorption band with UV-vis spectroscopy. When all Acr$^+$ converted to AcrOH, the reaction mixture was stirred for 15 minutes under dark conditions. The acetonitrile was evaporated in vacuo at room temperature, and the product was extracted with dichloromethane (3 x 100 mL). The organic layer was dried over anhydrous calcium chloride. Dichloromethane was evaporated and, excess of cyclohexane was added to precipitate any unreacted Acr$^+$. The precipitate was filtered-out and the filtrate was evaporated to give 95 mg of AcrOH (54 %). $^1$H NMR (300 MHz, DMSO): 7.59 (dd, $J = 7.7, 1.6$ Hz, 2H), 7.41 – 6.79 (m, 10H), 6.40 (s, 2H), 3.43 (s, 3H). $^{13}$C NMR (75 MHz, DMSO): 148.81, 139.62, 129.82, 127.67, 127.57, 126.59, 126.11, 125.41, 119.94, 112.10, 71.35, 33.14. MS-MALDI: m/z 287 [M$^+$ for C$_{20}$H$_{17}$NO].

4.4.2 Steady State UV-vis Absorption Experiments

Absorption spectra were recorded on an Agilent 8453 UV Spectrophotometer in a 1 cm quartz cell. Fluorescence spectra were recorded on an Edinburg Instruments fluorometer equipped with a Xe 900 lamp in the same 1 cm quartz cell. Solutions prepared for fluorescence measurements had absorption of 0.05-0.10 at the excitation wavelength. Fluorescence quantum yields were obtained using napthalene as a standard. AcrOH was excited at 279 nm whilst AcrOMe was excited at 280 nm, both in acetonitrile.

4.4.3 Femtosecond Transient Absorption (fs TA) Experiments
The laser system for the fs TA measurements was described in chapter II. Briefly, the 800 nm laser pulses were produced at a 1 kHz repetition rate (fwhm = 110 fs) by a mode-locked Ti:sapphire laser (Hurricane, Spectra-Physics). The output from the Hurricane was split into pump (85 %) and probe (10 %) beams. The pump beam (800 nm) was sent into an optical paramagnetic amplifier (OPA-800C, Spectra Physics) to obtain 310 nm excitation sources (E < 1 \(\mu\)J/pulse). The probe beam was focused into a horizontally moving CaF\(_2\) crystal for white light continuum generation between 350 and 800 nm. The flow cell (Starna Cell Inc. 45-Q-2, 0.9 mL volume with 2 mm pathlength), pumped by a Fluid Metering RHSY Lab pump (Scientific Support Inc.), was used to prevent photodegradation of the sample. After passing through the cell, the continuum was coupled into an optical fiber and input into a CCD spectrograph (Ocean Optics, S2000). The data acquisition was achieved using in-house LabVIEW (National Instruments) software routines. The group velocity dispersion of the probing pulse was determined using nonresonant optical Kerr effect (OKE) measurements. Sample solutions were prepared at a concentration needed to have absorbance of \(A \sim 0.6-1.0\) at the excitation wavelength.

**4.4.4 Nanosecond Transient Absorption (ns TA) Experiments**

The nanosecond laser flash photolysis experimental setup utilized for measurements in this paper is described in detail elsewhere. Briefly, a Nd:YAG laser (Spectra Physics LAB-150-10) was used as the excitation source with the excitation wavelength of 266 nm. All of the solutions utilized in these experiments were made such that the absorptivity at 266 nm had absorption of 1. Transient absorption spectra were recorded using a Roper ICCD-Max 512T digital intensified CCD camera with up to 2 ns temporal resolution. The single wavelength kinetic measurements were recorded using a PMT connected to an oscilloscope, which was directly connected to a computer that runs a custom LabView control and acquisition program.
4.4.5 Electrochemistry Experiments

All electrochemical measurements were done in acetonitrile using tetrabutylammonium perchlorate (TBAP) as the supporting electrolyte. Acetonitrile was purchased from Sigma-Aldrich (anhydrous, 99.8 %), and dried by reflux over CaH₂ for 8 h, followed by distillation. TBAP was purchased from Sigma Aldrich, recrystallized from methanol, and dried under vacuum. Cyclic voltammetry was performed using an EC Epsilon potentiostat in a VC-2 voltammetry cell (Bioanalytical Systems) using a glassy carbon working electrode (1.6 mm diameter, MF-2013, Bioanalytical Systems), platinum wire auxiliary electrode (MW-4130, Bioanalytical Systems), and nonaqueous Ag/Ag⁺ reference electrode (MF-2062, Bioanalytical Systems).

4.4.6 UV-vis Spectroelectrochemistry Experiments

Spectroelectrochemistry was done using a Pt mesh working electrode, non-aqueous Ag/Ag⁺ reference electrode and Pt wire as an auxiliary electrode. The absorption spectra were recorded on an HP 8453 UV-vis spectrophotometer. The details of the cell/spectrophotometer system were described previously.³⁰ Solution of 1 mM Acr⁺ in acetonitrile containing 0.1 M TBAP was degassed with argon (Ar) prior to each experiment. The changes in the absorption were monitored in 7 s intervals after applying a potential of −1.1 V vs. Ag/Ag⁺.

4.4.7 Computational Methods

All calculations were performed at the Ohio Supercomputer Center. Ground-state geometry optimizations of Acr⁺, AcrOH, and AcrOMe were performed using Gaussian 09 at the B3LYP/6-31++G* level of theory³²,³³ and with consideration of implicit solvation of acetonitrile (using the polarizable continuum model, PCM³⁴,³⁵). Tight optimization criteria were used in all calculations. All stationary points were confirmed to be energy minima using
vibrational frequency calculations (B3LYP/6-31++G*), which confirmed that all of the computed vibrational frequencies were real (i.e., no imaginary vibrational frequencies).

Electronic excitations were then calculated with time-dependent DFT at the TD-B3LYP/6-31++G* level of theory with consideration of implicit solvation of acetonitrile using the PCM method. Difference density plots were generated from the computed electron densities of the ground and excited states (TD-B3LYP/6-31++G*) using Gaussian 09.

In order to investigate the possibility of C–OH bond breaking on the triplet surface, we evaluated the potential energy surface on the \( T_1 \) and \( S_0 \) states as calculated at the unrestricted and restricted B3LYP/6-31G* levels of theory, respectively. The transition state for C–O bond breaking from the \( T_1 \) state was located at 9.8 kcal/mol above the optimized \( T_1 \) state (Figure 4.7).

Furthermore, the optimization of the Acr' + 'OH radical pair in the \( S_0 \) and \( T_1 \) state was achieved by optimizing the \( T_1 \) state and performing single-point B3LYP/6-31G* calculation to evaluate the energy of the \( S_0 \) state at this geometry. The results show that the \( T_1 \) state energy is lower than the \( S_0 \) state energy for a radical pair (Jablonski diagram in Figure 4.7). An intrinsic reaction coordinate (IRC)\(^{36,37} \) was performed from the transition state (located at a C–O bond length of 1.76 Å) to form the Acr' + 'OH radical pair (C–O distance = 2.98 Å). A number of geometries along this IRC path were used, and single-point B3LYP/6-31G* calculation were performed to evaluate the energy of the \( S_0 \) state at these geometries. These results are shown in Figure 4.8.

Natural population analyses\(^{38,39} \) were performed on both the triplet and singlet states for these geometries in order to evaluate the charge densities (singlet and triplet) and the spin densities (triplet). The sum of the charges on the OH fragment for both triplet and singlet states as well as the spin densities on the triplet state are shown in Figure 4.9.

References


CHAPTER V: PHOTOCHEMICAL HYDROGEN PRODUCTION: A MULTIPLE STEPS HYDRIDE RELEASE FROM 10-METHYL-9-PHENYL-9,10-DIHYDROACRIDINE

5.1 Introduction

Photochemical water reduction to produce H\(_2\) is of great importance for solar energy conversion and renewable energy resource development. The stoichiometric photoreduction was first achieved by reduced metal ions, such as Ce\(^{3+}\), \(\text{Cr}^{2+}\) and \(\text{Fe}^{2+}\). The catalytic cycle was first accomplished by an electron donor, sensitizer, and Ru(bpy)_3\(^{2+}\) complex set, and the most efficient H\(_2\) evolution catalyst is Pt nanoparticles because of its low overpotential for this process (\(\text{H}^+ + e^{-} \rightarrow \frac{1}{2}\text{H}_2\)). The catalytic H\(_2\) production using inorganic metal complexes is still an active research field, due to its potential applications in development of renewable energy sources. However, due to the high cost and limited supply of low earth abundant precious metals, earth-abundant metal centered complexes were recently designed and investigated towards catalytic H\(_2\) production, and organic sensitizers are also used to replace inorganic metal complex sensitizers. Nicotinamide adenine dinucleotide (NADH), as a ground state hydride donor, is an extremely important reductase in biological systems and has been efficiently applied in H\(_2\) productions. 10-Methyl-9-phenyl-9,10-dihydroacridine (AcrH, Scheme 5.1), a NADH analogue, has been investigated as a hydride donor in the last three decades, and its ground state hydride release process has been clearly identified, which occurs by an electron-proton-electron (e-p-e) transfers. Recently, the excited state behaviour
of 9-hydroxy-10-methyl-9-phenyl-9,10-dihydroacridine (AcrOH, Scheme 4.1) had been investigated, which releases a \( -\text{OH} \) from its \( S_1 \) state very quickly (\( \tau = 108 \) ps). So, AcrH is potentially treated as an excited state hydride donor in protic solvent. In this present study, we report a detailed study of pH dependent AcrH hydride release for \( \text{H}_2 \) production under stoichiometric conditions by the transient absorption (TA) spectroscopy technique, in which, an electron-‘hydrogen atom’ (e-H) two steps hydride release process is observed. (Scheme 5.1)

**Scheme 5.1. Excited State Hydride Release Process of AcrH**

![Scheme 5.1](image)

### 5.2 Results and Discussions

#### 5.2.1 Ground State Spectra of AcrH

The photochemical behavior of AcrH is affected by the molecular oxygen and by the pH of the solution. In the presence of oxygen, efficient photooxidation of AcrH to Acr\(^{+} \) was observed using UV-vis absorption spectroscopy (Figure 5.1), and similar results were reported previously for acridine\(^{25,26} \) and other NADH derivatives.\(^{27-29} \) While \( \text{H}_2\text{O}_2 \) is the likely coproduct in this reaction, the iodide test detected only minor amounts of \( \text{H}_2\text{O}_2 \) (Figure 5.1c), possibly due to the reaction of AcrH with triiodide.\(^{30} \)
Figure 5.1. UV-vis absorption changes of 0.08 mM AcrH upon irradiation (λex= 300-350 nm) in the presence of O₂. (a) at pH 0.65 (b) at pH 6. Times of spectral collection: (▬) 0 min. (▬) 2 min. (▬) 5 min. (▬) 10 min. (▬) 15 min. (c) Growth of I₃⁻ absorption at 290 nm and 365 nm after adding excess of I⁻ to the irradiated AcrH sample.

Supporting Information of Figure 5.1: The photochemical behavior of AcrH in the presence of molecular oxygen was studied at pH 0.65 and pH 6 followed by the detection of H₂O₂ which could be the possible co-product. Figure 5.1a and b show the changes in the UV-vis absorption spectra during the course of 300 nm irradiation of aerated AcrH in ACN:H₂O mixtures. The decrease of AcrH (λabs=287 nm) is accompanied with the growth of Acr⁺ (λabs=365 and 424 nm), suggesting that the efficient photo-oxidation of AcrH takes place. The conversion efficiencies of AcrH to Acr⁺ at both pH values are ~ 65%. Previous studies of organic hydrides reported similar oxygen reduction behavior. The triiodide method was used to test for the formation of H₂O₂, by monitoring the oxidation of iodide to triiodide, which absorbs at 290 nm and 365 nm. The detected amount of I₃⁻ was significantly lower (~6 %) than expected based on the amount of Acr⁺ formed (65%). One possible reason for the low yield of triiodide could be the reaction of I₃⁻ with the remaining AcrH to form Acr⁺ and I⁻ as shown below:

(i) AcrH + O₂ + H⁺ → Acr⁺ + H₂O₂

(ii) H₂O₂ + 3I⁻ +2H⁺ → 2H₂O + I₃⁻
(iii) $I^- + AcrH \rightarrow 3I^- + H^+ + Acr^+$

In the absence of oxygen and at neutral pH, photooxidation of AcrH to Acr$^+$ did not occur. Instead, other photoproducts are formed (Figure 5.2), possibly dimers formed upon bimolecular coupling of Acr$^-$ radicals formed upon irradiation (Figure 5.2a).

**Figure 5.2.** Photolysis of AcrH in ACN: water mixtures in the absence of O$_2$ at pH = 6 (a) UV-vis absorption changes of 0.08 mM AcrH upon irradiation ($\lambda_{ex}$=220 -350 nm). Times of spectral collection: (─) 0 min. (▬) 2 min. (▬) 5 min. (▬) 10 min. (▬) 17 min. (▬) 30 min. (▬) 45 min. (▬) 60 min. (b) GC-MS (DIP) result of the irradiated product analysis.

**Supporting Information for Figure 5.2:** The support for the dimer formation comes from the fact that the MS analysis of the reaction mixture gives a signal at m/z = 540, which corresponds to the mass of the Acr-Acr dimer (Figure 5.2b). Furthermore, the NMR study of the irradiated solution shows that the ratio of integrated peaks between aromatic hydrogens and the hydrogen at C-9 position is increasing with prolonged irradiation, suggesting a loss of hydrogen at C-9 position which ultimately leads to a dimer formation via the same carbon center. In addition, photochemical dimerization of other acridine derivatives was shown to generate dimeric species.$^{20}$
Figure 5.3. UV-vis absorption changes upon photo irradiation of 0.1 mM AcrH in ACN:different pH H₂O (v:v= 1:1) mixtures under Ar purged conditions.

Importantly, a decrease of the solution pH in the absence of oxygen leads to the formation of increasing amounts of Acr⁺ (Figure 5.3). As discussed in the previous section, the irradiation of an oxygen-free solution of AcrH at neutral pH does not generate Acr⁺; however, as the solution pH is decreased, the formation of Acr⁺ was observed (Figure 5.3). The UV-vis absorption spectra of irradiated AcrH in the pH= 2-5 range exhibit broad absorption throughout the visible range due to the formation of aggregates of varying size; however, at low pH values (pH<2), the formation of Acr⁺ is observed, with its characteristic absorption bands at 365 and 424 nm. These results clearly show that the overall hydride release occurs from the excited AcrH at low solution pH.
Figure 5.4. Photo-irradiation of 0.1 mM AcrH in ACN and pH 0.65 H2O mixture (V:V=1:1), in the absence of O2, λ<sub>exc</sub> = 220 - 350 nm. (a) UV-vis absorption changes upon irradiation. Times of spectral collection: 0 (▬), 2 (▬), 5 (▬), 10 (▬), 17 (▬), 30 (▬), 45 (▬) and 60 min. (▬); (b) Yield percentage of H2, detected using GC as a function of pH. (Inset in Figure 5.4b shows the GC signals for H2 in the absence (▬) and in the presence of 0.25 mM AcrH (▬).)

Figure 5.4 shows an example of such behavior recorded at pH=0.65, showing that 52% of Acr<sup>+</sup> is formed at the end of photoirradiation. Head space analysis via gas chromatography of the irradiated solution shows that the hydrogen is formed in this process, as one would expect for the photoreduction of water by AcrH. However, the quantitative analysis revealed that the yield of hydrogen relative to the starting AcrH is only 2.5% at pH=0.65 (Figure 5.4b), suggesting that the photoreduction by AcrH involves mostly reduction of the co-solvent ACN, possibly generating protonated ethyl amine, which is known to be formed upon reduction of ACN either chemically by molecular hydrogen and hydrides<sup>34</sup> or electrochemically.<sup>35</sup> Unfortunately, the measurements could not be achieved in a purely aqueous solution, due to low solubility of AcrH in water. Our attempts to replace ACN with other co-solvents, such as tetrahydrofuran, were not successful (the irradiation of the sample generated >50% of hydrogen, but the AcrH converted to a product...
that was not Acr\textsuperscript{+}). This setback can most likely be avoided in the future by the use of water-soluble photohydrides.

### 5.2.2 Excited State Spectra of AcrH

To investigate the mechanism of photoreduction by AcrH, femtosecond (fs) and nanosecond (ns) UV-vis TA experiments were collected. The initially formed transient is assigned to the singlet excited state of AcrH and exhibits a broad absorption in the visible range (0 ps spectrum in Figure 5.5a). The S\textsubscript{1} state exhibits a single-component decay with lifetime $\tau=2.5$ ns (Figure 5.5b). The 2.5 ns decay is accompanied by a growth of the new transient with $\lambda_{\text{max}}=550$ nm (2.25 eV). A similar species was observed in the TA spectra of AcrOH in aprotic solvation\textsuperscript{24} and was assigned to the triplet excited state. The intersystem-crossing ($\tau=2.5$ns for AcrH and 1.4 ns for AcrOH\textsuperscript{24}) is faster than expected for a molecule that lacks heavy atoms. However, similar findings were reported previously, where the relatively fast intersystem crossing was explained by transitions between states with different electronic configurations (El-Sayed rule).\textsuperscript{36} It is likely that the similar mechanism operates in the case of AcrH.

![Figure 5.5](image.png)

**Figure 5.5.** (a) fs TA spectra, time delays: -3.5 ps (---), 0 ps (----), 77 ps (-----), 329 ps (--), 898 ps (---), and 1521 ps (-----), and (b) kinetics of 1 mM AcrH in ACN-H\textsubscript{2}O (V/V : 1/1, pH 7), $\tau = 2.5$ ns, $\lambda_{\text{EX}} = 310$ nm;
Figure 5.6. Calculated (red line) and experimental (black line) absorption spectra of different AcrH species. (The calculations were performed using TD-DFT/TDA with B3LYP/6-31+G*, except for the T₁ absorption which was computed using BNL/6-31+G*).

Additional support for the assignment of 550 nm (2.25 eV) intermediate to the T₁ state of AcrH is provided by time-dependent density functional theory (TD-DFT) calculations (Figure 5.6). The computed maximum in the T₁ absorption spectrum is at 2.13 eV, which is slightly red-shifted (by 0.12 eV) relative to the experimental maximum. This is within error bars of the theoretical method employed, as confirmed by the differences between computed and observed
absorption maxima of other species (Table 5.1). These results provide additional support for the assignment of the 550 nm band to the T1 state of AcrH.

Table 5.1. Positions of the First Peak (eV/nm) in the Calculated and Experimental Spectra of Different AcrH Species

<table>
<thead>
<tr>
<th>Species</th>
<th>Calculation</th>
<th>Experiment</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhAcrH (cis2)</td>
<td>4.62/268</td>
<td>4.29/289</td>
<td>0.33</td>
</tr>
<tr>
<td>PhAcr+</td>
<td>3.20/388</td>
<td>2.91/426</td>
<td>0.29</td>
</tr>
<tr>
<td>PhAcr-</td>
<td>2.74/453</td>
<td>2.48/500</td>
<td>0.26</td>
</tr>
<tr>
<td>PhAcrH+</td>
<td>2.26/549</td>
<td>1.89/655</td>
<td>0.37</td>
</tr>
<tr>
<td>-[AcrH]2</td>
<td>2.13/583</td>
<td>2.25/550</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Figure 5.7. fs TA spectra (λEXC=310 nm) of 1 mM AcrH in ACN:H2O (1:1) mixture (the water pH was varied as shown).
In addition to the formation of T₁ state at 550 nm, another transient is formed at 420 nm (2.88 eV). The formation of this intermediate competes with intersystem crossing and is pH-dependent. fs TA spectra of AcrH in ACN:water mixtures were obtained at several pH values (Figure 5.7). At pH=7, the initially formed S₁ state decays with a concurrent formation of an intermediate with absorption at 550 nm, which is assigned to the T₁ state of AcrH. As the solution pH is lowered, another process competes with intersystem crossing. This process leads to a decrease in the absorption at 550 nm and the formation of a new transient with absorption in the 400-430 nm range. The possibility of excited-state protonation of AcrH S₁ state was excluded, since TD-DFT calculations predict the absorption of protonated AcrH₂⁺ to appear at 254 nm, which is below the spectral range covered in our TA experiment. We postulate that this intermediate is an excimer of AcrH, consistent with the previous studies of excimers formed from aromatic compounds.37,38 The AcrH excimer eventually decays to the ground monomeric state and no photochemical hydride release occurs via this pathway.
The T<sub>1</sub> state of AcrH transfers an electron to the solvent with a τ<sub>1</sub>=0.7 μs lifetime (Figure 5.8c) to generate AcrH<sup>+</sup>, with its characteristic absorption in the 500-800 nm range. The dynamics of AcrH<sup>+</sup> exhibit two components: (i) decay with a τ<sub>2</sub>=11 μs lifetime, possibly due to the electron recombination to generate AcrH in the ground state; (ii) decay with τ<sub>3</sub>=227 μs, which is assigned to either a single H-atom transfer or a slow H<sup>+</sup> transfer followed by a fast electron transfer. The assignment of this decay is based on the following findings: (i) the AcrH<sup>+</sup> decay is coupled with the growth of a band at 425 nm (Figure 5.8a), which is assigned to Acr<sup>+</sup> (absorbs at 425 nm, inset spectrum in Figure 5.8a); (ii) the kinetic isotope effects were observed for all lifetimes, τ<sub>1</sub>=1.3 μs, τ<sub>2</sub>=15 μs, and τ<sub>3</sub>=810 μs, when the deuterated AcrD sample was investigated (Figure 5.8b, 8c, and 8d), with k<sub>H</sub>/k<sub>D</sub> ≈ 1.5-1.9 for τ<sub>1</sub> and τ<sub>2</sub>, which is indicative of a second order isotope effect for an electron transfer process. k<sub>H</sub>/k<sub>D</sub> ≈ 4 for τ<sub>3</sub> indicates a first order isotope effect for a bond cleavage process. Based on the previous report, τ<sub>3</sub> is attributed to either a H-atom release from AcrH<sup>+</sup> or a slow H<sup>+</sup> release from AcrH<sup>+</sup>. The TA spectra of radical product, Acr<sup>-</sup> (500 nm), is not obtained in ns data, possibly due to the fact that the electron release process is too fast to detect in ns TA scan.

The acceptor of the ‘hydrogen atom’ released from AcrH<sup>+</sup> is either the solvent or another molecule of AcrH<sup>+</sup>. To determine the nature of the accepting species, the kinetics of AcrH<sup>+</sup> were investigated as a function of AcrH concentration. The lifetime τ<sub>3</sub> of AcrH<sup>+</sup> at 580 nm decreases as AcrH concentrations increases (the lifetime decreases from 264 μs at low concentrations to 93 μs at high concentration). This behavior is indicative of a bimolecular process, in which the
overall ‘hydrogen atom’ from AcrH$^+$ is released by an electron transfer to another AcrH$^+$
coupled with a release of the proton to the solvent (second equation presented below).

Based on these experimental findings, we propose that the photochemical oxidation of
AcrH to Acr$^+$ occurs in the following sequence of steps:

\[ \text{AcrH} + h\nu \rightarrow \text{AcrH}^+ + e^- \]
\[ 2\text{AcrH}^+ \rightarrow \text{Acr}^+ + \text{AcrH} + \text{H}^+ \]
\[ 5\text{H}^+ + 4e^- + \text{CH}_3\text{CN} \rightarrow \text{CH}_3\text{CH}_2\text{NH}_3^+ \]
\[ 2\text{H}^+ + 2e^- \rightarrow \text{H}_2 \]

5.2.3 Forster Cycle of Photochemical Processes of AcrH

It is interesting to compare the photochemical behavior of AcrH with the previously
reported photochemistry of AcrOH.\textsuperscript{24} Despite similar electronic structures, the S$_1$ state of AcrOH
releases \`OH in a single fast step, while AcrH releases a hydride ion from its T$_1$ state by a
stepwise mechanism. To evaluate the driving force for each of these photochemical events, we
employ a simple Forster cycle,\textsuperscript{44} which is frequently applied to evaluations of excited-state
acidities of aromatic alcohols.\textsuperscript{45} Using the excitation energies of AcrH/AcrOH and Acr$^+$, as well
as evaluated $\Delta G$ values for the corresponding ground state reactions, one finds that the Gibbs
free energy change for the excited state release of OH$^-$ ion from AcrOH is $\Delta G_{\text{OH}}^* = -24.2$
kcal/mol, while this value is $\Delta G_{\text{H}}^* = -1.93$ kcal/mol for the one-step proton reduction by excited
AcrH (Scheme 5.2). Both processes are thermodynamically favored, while only the concerted
hydroxide release from excited AcrOH was observed experimentally. In contrast, the one-step
proton reduction to hydrogen by excited AcrH does not occur; instead the reaction proceeds via a
stepwise process. The difference in behavior between AcrOH and AcrH likely arises due to
different barriers for the two photochemical processes. In the case of AcrOH, the hydrogen
bonding between the —OH group of AcrOH and the protic solvent (methanol) facilitates the heterolytic C-OH bond cleavage. The lack of such interaction between the C—H group of AcrH and the solvent makes the one-step hydride transfer less likely to occur. Scheme 5.2 presents estimated energies for different photochemical pathways of AcrH in ACN solution.

Scheme 5.2. Gibbs Free Energy Profile for Photochemical Processes of AcrH in the Presence of a Proton in ACN Solution.

The blue arrow shows AcrH excitation (310 nm). The red arrows show a hydride release process supported by TA spectra. The grey arrows show a concern electron coupled proton release process.

Estimation of Gibbs Free Energies from Experimental Values

(i) $S_1$ energy (81.23 kcal/mol) was obtained from emission peak of AcrH ($^1$AcrH*: 352 nm).
(ii) $T_1$ energy (69.23 kcal/mol) was obtained from emission peak of AcrH ($^3$AcrH*: 413 nm).
(iii) $S_1$ energy (57.41 kcal/mol) was obtained from emission peak of Acr$^+$ ($^1$Acr$^{+*}$: 498 nm).
(iv) The energy for the reaction AcrH + H$^+$ $\rightarrow$ AcrH$^+$ + H ($\Delta G = 68.00$ kcal/mol) was obtained as

$$\Delta G = -\Delta G_1 + \Delta G_2,$$

where:
The value for $\Delta G_1$ was obtained using the reported standard reduction potential $E^\circ = 0.94 \, V \, vs \, SCE^{20}$ (1.184 \, V \, vs. \, SHE^{46}). To obtain the absolute potential, we used $E_{\text{NHE}} = 4.281 \, V$.\textsuperscript{47} The value for $\Delta G_2$ was obtained using the standard reduction potential in water $E^\circ = -1.77 \, V \, vs \, SHE$.\textsuperscript{48}

(v) The energy for the reaction $\text{AcrH} \rightarrow \text{Acr}^- + \text{H} \ (\Delta G = 69.6\pm1.9 \, \text{kcal/mol})$ was estimated to be $\Delta G = -\Delta G_1 + \Delta G_2 + \Delta G_3$, where:

\[
\text{AcrH}^+ + e^- \rightarrow \text{AcrH} \quad \Delta G_1 \\
\text{AcrH}^- \rightarrow \text{Acr}^- + \text{H}^+ \quad \Delta G_2 \\
\text{H}^+ + e^- \rightarrow \text{H} \quad \Delta G_3
\]

The value for $\Delta G_2$ was obtained from the $pK_a$ value of $\text{AcrH}^+$. The reported $pK_a$ value in acetonitrile is 1.2\pm1.4 (1.64\pm1.9).\textsuperscript{20}

(vi) The energy for the reaction $\text{AcrH} + \text{H}^+ \rightarrow \text{Acr}^+ + \text{H}_2 \ (21.89\text{kcal/mol})$ was obtained as $\Delta G = \Delta G_1 - \Delta G_2 + 2\Delta G_3 - \Delta G_4$, where:

\[
\text{AcrH} \rightarrow \text{Acr}^- + \text{H} \quad \Delta G_1 \\
\text{Acr}^+ + e^- \rightarrow \text{Acr}^- \quad \Delta G_2 \\
\text{H}^+ + e^- \rightarrow \frac{1}{2} \text{H}_2 \quad \Delta G_3 \\
\text{H}^+ + e^- \rightarrow \text{H} \quad \Delta G_4
\]

The value for $\Delta G_2$ was obtained from the reported reduction potential ($E = -0.55 \, V \, vs \, SCE$).\textsuperscript{20} The value for $\Delta G_3$ was obtained from the absolute potential for the normal hydrogen electrode ($E_{\text{NHE}} = 4.281 \, V$).\textsuperscript{47} The same expressions were used for the thermodynamic parameters in
acetonitrile (Table 5.1). However, the aqueous $\Delta G$ values were replaced by the corresponding parameters in acetonitrile.$^{20,48}$

Hydride release via stepwise mechanisms is not desirable for the following reasons: (i) radicals generated after each step are reactive and can undergo unwanted chemistry; (ii) the stepwise processes are more energy demanding, making it unlikely to drive such photochemistry using low-energy visible photons. Thus, further research works are required to overcome these bottlenecks, such as tuning the electronic properties of organic hydride donors to enable the concerted process or using a metal nanoparticle as a cocatalyst to collect electrons for proton reduction. For the electronic property tuning, the first step is the development of chemical systems that exhibit thermodynamically favorable excited-state reduction of protons, while the $\Delta G$ values for the excited-state electron and hydrogen-atom transfer should be positive. While this thermodynamic condition does not ensure that the photoreduction will take place, it does increase its likelihood. For the metal nanoparticle cocatalyst, polyvinylpyrrolidone stabilized platinum nanoparticles have been used in a photocatalytic hydrogen reduction system, in which NADH is as a ground state hydride donor, and 2-phenyl-4-(1-naphthyl)-quinolinium ion is as a photocatalyst.$^{18}$

5.3 Conclusion

This manuscript describes a study of the photochemical hydride release from an organic hydride, AcrH. Using TA spectroscopy, we find that the hydride release occurs via a stepwise electron-‘hydrogen atom’ transfer process. The photoinduced concerted hydride transfer was not observed, even though it is thermodynamically favored.

5.4 Experiments and Theory

5.4.1 Synthesis
AcrH(D) was synthesized according to the literature\textsuperscript{23} under modified conditions as described in Scheme 5.3.

**Scheme 5.3: Synthesis of AcrH**

10-methyl-9-phenyl-9, 10-dihydroacridine (AcrH(D)): Acr\textsuperscript{+} (450 mg, 1.2 mmol) was suspended in 20 mL of ethanol. Sodium borohydride (NaBH\textsubscript{4}) (90 mg, 2.4 mmol) in ethanol was added drop-wise over a period of 5 mins. The resulting colorless solution was refluxed for 2 hours. Ethanol was evaporated in vacuo at room temperature, and the crude product was extracted with dichloromethane (3 x 40 mL). The organic layer was dried over CaCl\textsubscript{2} and recrystallized from ethanol to give AcrH as white crystals. (276 mg, 85%). \textsuperscript{1}H- NMR (300 MHz, CD\textsubscript{3}CN): 3.38 (s, 3 H), 5.23 (s, 1 H), 6.9-7.3 (m, 13 H). The deuterated compound (AcrD) was prepared by the same procedure with NaBD\textsubscript{4} and purified by recrystallization. \textsuperscript{1}H- NMR (300 MHz, CD\textsubscript{3}CN): 3.36 (s, 3 H), 6.8-7.4 (m, 13 H).

**5.4.2 Steady State UV-vis Absorption Experiment**

Solutions of \(~0.1\) mM AcrH were prepared in an ACN:H\textsubscript{2}O (1:1) mixture at different pH values. For oxygen-free experiments, the samples were degassed for 45 minutes with argon prior to the experiments without oxygen. For the experiments in the presence of oxygen, the samples were prepared under atmospheric conditions. As the irradiation source, a medium pressure Hg arc lamp (Hanovia PC 451050) was used and the excitation wavelengths were controlled below
350 nm using a short pass filter. (Asahi spectra USA inc.) All irradiations were performed at room temperature. The conversion of AcrH to Acr$^+$ was monitored using UV-vis spectrophotometry at different time intervals.

### 5.4.3 GC Hydrogen Detection

Shimadzu GC-8A was operated with ultra-high purity argon as carrier gas and 5 Å molecular sieves column (Restek) to separate gas mixtures. This GC was customized with two injection ports, the first for syringe injections, and the second for automatic injections from a 500 µL sample loop directly linked to a Schlenk line. The detector was calibrated against known amounts of H$_2$ gas. In principle, 500 µL of 10 % H$_2$ balanced Ar certified gas standard (Praxair) was injected at different pressures using a home-built Schlenk line linked to a pressure gauge. The area under the hydrogen peak was then plotted against the calculated number of the moles of hydrogen injected to get the calibration constant of the detector. This constant was then verified by syringe injections of different volumes of 25% H$_2$ balanced Ar certified gas standard (Praxair), equilibrated to atmospheric pressure and placed in an airtight vial. The calibration was performed routinely with variation typically below 5 % at a certain Ar flow rate.

A solution of 0.25 mM AcrH in ACN and pH 0.50 water (1:1) mixture was prepared in a custom built quartz reactor capped with a PTFE septum. (Solution volume was 54 mL; headspace volume was 18 mL). After irradiation, a 100 µl sample from the mixture headspace was injected to the GC using a Hamilton airtight syringe and the amount of hydrogen was quantified. The baseline hydrogen detection was done with the exact same conditions and components, but in the absence of AcrH.

### 5.4.4 Triiodide Method of H$_2$O$_2$ Detection
Hydrogen peroxide was detected using the triiodide method. A solution of 0.08 mM AcrH was prepared in ACN: water (1:1) mixture and irradiated (vide supra) for 12 minutes. The irradiated sample was treated with a solution of excess NaI and the formation of I$_3^-$ was monitored based on UV-vis absorption ($\varepsilon_{290\text{ nm}} = 5.2 \times 10^3$).

5.4.5 Femtosecond TA Experiments

The laser system for the fs TA measurements was described in chapter II. Briefly, the 800 nm laser pulses were produced at a 1 kHz repetition rate (fwhm = 110 fs) by a mode-locked Ti:sapphire laser (Hurricane, Spectra-Physics). The output from the Hurricane was split into pump (85 %) and probe (10 %) beams. The pump beam (800 nm) was sent into an optical paramagnetic amplifier (OPA-800C, Spectra Physics) to obtain 310 nm excitation sources (E < 1 $\mu$J/pulse). The probe beam was focused into a horizontally moving CaF$_2$ crystal for white light continuum generation between 350 and 800 nm. The flow cell (Starna Cell Inc. 45-Q-2, 0.9 mL volume with 2 mm pathlength), pumped by a Fluid Metering RHSY Lab pump (Scientific Support Inc.), was used to prevent photodegradation of the sample. After passing through the cell, the continuum was coupled into an optical fiber and input into a CCD spectrograph (Ocean Optics, S2000). The data acquisition was achieved using in-house LabVIEW (National Instruments) software routines. The group velocity dispersion of the probing pulse was determined using nonresonant optical Kerr effect (OKE) measurements.$^{50}$ Sample solutions were prepared at a concentration needed to have absorbance of A~0.6-1.0 at the excitation wavelength.

5.4.6 Nanosecond (ns) TA Experiments

The nanosecond laser flash photolysis experimental setup utilized for measurements in this paper is described in detail elsewhere.$^{51}$ Briefly, a Nd:YAG laser (Spectra Physics LAB-150-10) was used as the excitation source with the excitation wavelength of 266 nm. All of the
solutions utilized in these experiments were made such that the absorptivity at 266 nm had absorption of 1. TA spectra were recorded using a Roper ICCD-Max 512T digital intensified CCD camera with up to 2 ns temporal resolution. The single wavelength kinetic measurements were recorded using a PMT connected to an oscilloscope, which was directly connected to a computer that runs a custom LabView control and acquisition program.

5.4.7 UV-vis Spectroelectrochemistry Experiments

Spectroelectrochemistry was done using a Pt mesh working electrode, non-aqueous Ag/Ag⁺ reference electrode and Pt wire as an auxiliary electrode. The absorption spectra were recorded on an HP 8453 UV-vis spectrophotometer. The details of the cell/spectrophotometer system were described previously. Solution of 1 mM Acr⁺ in ACN containing 0.1 M TBAP was degassed with argon (Ar) prior to each experiment. The changes in the absorption were monitored in 7 s intervals after applying a potential of −1.1 V vs. Ag/Ag⁺.

5.4.8 Computational Methods

All thermochemical calculations were performed using wB97X-D functional. The solvation free energies were computed using CPCM model. Excited-sate calculations were performed using TDDFT/TDA with various functionals. All calculations were performed using the Q-Chem electronic structure package.

References:


