Spectroscopic Studies of Chlorophyll a Complexes with Trinitro-Substituted Flourene Derivatives

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SPECTROSCOPIC STUDIES OF CHLOROPHYLL $a$ COMPLEXES WITH TRINITRO-SUBSTITUTED FLUORENE DERIVATIVES

A Thesis
Presented to
The Faculty of the Department of Chemistry
Western Kentucky University
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In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

By
Stephanie Rachelle Smalley
May 2004
SPECTROSCOPIC STUDIES OF CHLOROPHYLL \(a\) COMPLEXES WITH TRINITRO-SUBSTITUTED FLUORENE DERIVATIVES

Date Recommended \textit{May 07, 2004}

\textit{[Signatures]}
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I.</strong> INTRODUCTION</td>
<td></td>
</tr>
<tr>
<td>A. General Photosynthesis Background</td>
<td>1</td>
</tr>
<tr>
<td>B. Photosystems I and II</td>
<td>3</td>
</tr>
<tr>
<td>C. Z Scheme</td>
<td>3</td>
</tr>
<tr>
<td>D. Determination of the Ground State Equilibrium Constant, $K_a$, from UV-Vis Absorption Spectra</td>
<td>5</td>
</tr>
<tr>
<td>E. Fluorescence Quenching Analysis from Emission Spectra</td>
<td>7</td>
</tr>
<tr>
<td>F. Solvent Effects on Fluorescence Quenching</td>
<td>12</td>
</tr>
<tr>
<td>G. Light and Dark Reactions (<em>in vivo</em>)</td>
<td>15</td>
</tr>
<tr>
<td>H. Quenching Studies by Quinones and Nitroaromatics (<em>in vitro</em>)</td>
<td>18</td>
</tr>
<tr>
<td>I. Objective of this Study</td>
<td>22</td>
</tr>
</tbody>
</table>

| II. EXPERIMENTAL | |
| A. Materials | 24 |
| B. Solution Preparation | 25 |
| C. Instrumentation | 27 |
| D. Molecular Modeling | 30 |

| III. RESULTS AND DISCUSSION | |
| A. Absorption | 34 |
B. Fluorescence ................................................................. 45
C. Excited State Lifetime Studies ....................................... 55
D. Molecular Modeling .................................................... 59

IV. CONCLUSIONS ............................................................. 62
V. FUTURE WORK ............................................................ 63
VI. BIBLIOGRAPHY ........................................................... 64
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Binding Constants and Gibb's Energy of Chl a with Various Acceptor Molecules in CH$_3$CN and CH$_2$Cl$_2$ at 25°C</td>
<td>44</td>
</tr>
<tr>
<td>2. Stern-Volmer Constant, $K_{SV}$, for Chl a in CH$_3$CN and CH$_2$Cl$_2$ with Various Quenchers at 25°C</td>
<td>54</td>
</tr>
<tr>
<td>3. Summary of Excited State Lifetimes of Chl a Systems in CH$_3$CN</td>
<td>58</td>
</tr>
<tr>
<td>4. Calculated Electron Affinities of Quencher Molecules Using Gaussian W03 with B3LYP functional and 3-21G Basis Set</td>
<td>60</td>
</tr>
<tr>
<td>5. Calculated Interaction Energies Between Chl a and Acceptor Molecules, ($\pi-\pi$ Configuration) in the Gas Phase at 300K, using PC Model 7.5 Software Package with MMX Force Field</td>
<td>61</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Reaction Center</td>
<td>2</td>
</tr>
<tr>
<td>2. The Z-Scheme</td>
<td>4</td>
</tr>
<tr>
<td>3. Comparison of Dynamic versus Static Quenching</td>
<td>8</td>
</tr>
<tr>
<td>4. Possible Pathways of Excited Chlorophyll</td>
<td>17</td>
</tr>
<tr>
<td>5. Structures of (a) Chlorophyll $a$ and (b) Plastoquinone</td>
<td>19</td>
</tr>
<tr>
<td>6. Fluorene Derivatives and Model Structure of Chl $a$</td>
<td>21</td>
</tr>
<tr>
<td>7. Experimental Set-up for (a) Fluorescence and (b) Excited State Lifetime Measurements</td>
<td>28</td>
</tr>
<tr>
<td>8. Mercury Lamp Calibration</td>
<td>31</td>
</tr>
<tr>
<td>9. Optimized Model Structure of (a) Chl $a$, (b) Chl-$a$ Fluorene, (c) Chl $a$-TFPH, (d) Chl $a$-TFPR, (e) Chl $a$-ITNF</td>
<td>32</td>
</tr>
<tr>
<td>10. Opt. Model Structure of (a) Chl $a$-TNFO, (b) Chl $a$-PQ, (c) Chl $a$-TNF</td>
<td>33</td>
</tr>
<tr>
<td>11. Absorption Spectra of Chl $a$, TFPH, TFPR, and fluorene in CH$_2$Cl$_2$</td>
<td>37</td>
</tr>
<tr>
<td>12. Absorption Spectra of Chl $a$ with TFPH in CH$_3$CN</td>
<td>38</td>
</tr>
<tr>
<td>13. Nash Plot of Chl $a$ with TFPH in CH$_3$CN</td>
<td>38</td>
</tr>
<tr>
<td>14. Absorption Spectra of Chl $a$ with TFPH in CH$_2$Cl$_2$</td>
<td>39</td>
</tr>
<tr>
<td>15. Nash Plot of Chl $a$ with TFPH in CH$_2$Cl$_2$</td>
<td>39</td>
</tr>
<tr>
<td>16. Absorption Spectra of Chl $a$ with TFPR in CH$_2$Cl$_2$</td>
<td>40</td>
</tr>
</tbody>
</table>
17. Nash Plot of Chl $a$ with TFPR in CH$_2$Cl$_2$.................................40
18. Absorption Spectra of Chl $a$ with TFPR in CH$_3$CN..........................41
19. Nash Plot of Chl $a$ with TFPR in CH$_3$CN...................................41
20. Absorption Spectra of Chl $a$ with Fluorene in CH$_3$CN.......................42
21. Nash Plot of Chl $a$ with Fluorene in CH$_3$CN................................42
22. Absorption Spectra of Chl $a$ with Fluorene in CH$_2$Cl$_2$.....................43
23. Nash Plot of Chl $a$ with Fluorene in CH$_2$Cl$_2$...............................43
24. Jablonski Diagram..............................................................................46
25. Fluorescence Spectra of Chl $a$ with TFPH in CH$_3$CN.........................47
26. Stern-Volmer Plot of Chl $a$ with TFPH in CH$_3$CN..............................47
27. Fluorescence Spectra of Chl $a$ with TFPH in CH$_2$Cl$_2$.......................48
28. Stern-Volmer Plot of Chl $a$ with TFPH in CH$_2$Cl$_2$............................48
29. Fluorescence Spectra of Chl $a$ with TFPR in CH$_2$Cl$_2$.......................49
30. Stern-Volmer Plot of Chl $a$ with TFPR in CH$_2$Cl$_2$............................49
31. Fluorescence Spectra of Chl $a$ with ITNF in CH$_3$CN..............................50
32. Stern-Volmer Plot of Chl $a$ with ITNF in CH$_3$CN...............................50
33. Fluorescence Spectra of Chl $a$ with ITNF in CH$_2$Cl$_2$........................51
34. Stern-Volmer Plot of Chl $a$ with ITNF in CH$_2$Cl$_2$............................51
35. Fluorescence Spectra of Chl $a$ with Fluorene in CH$_2$Cl$_2$...................52
36. Stern-Volmer Plot of Chl $a$ with Fluorene in CH$_2$Cl$_2$........................52
37. Fluorescence Spectra of Chl $a$ with Fluorene in CH$_3$CN.......................53
38. Stern-Volmer Plot of Chl $a$ with Fluorene in CH$_3$CN............................53
39. Excited State Lifetime Plots for Chl $a$ with TFPR in CH$_3$CN..................56
40. Excited State Lifetime Plot for Chl $a$ with TFPH in CH$_3$CN

41. Excited State Lifetime Plot of Chl $a$ in CH$_3$CN

42. Excited State Lifetime Plot of $6 \times 10^{-4}$ M Chl $a$/Fluorene Complex in CH$_3$CN
Absorption, fluorescence, and excited-state lifetime studies of chlorophyll \( a \) (Chl \( a \)) with fluorene, 2-(2,4,7-Trinitro-fluoren-9-ylideneaminoxy)-propionic acid (TFPR), 2-(2,4,7-Trinitro-fluoren-9-ylidenemethyl)-phenol, (TFPH), and 9-Isopropylidene-2,4,7-trinitro-9H-fluorene (ITNF) have been investigated. These systems were studied in acetonitrile and dichloromethane at room temperature. Absorption data were analyzed with the Nash plot. Linearity of the Nash plots proved that a 1:1 complex was formed between the donor and acceptor. Ground state binding constants \( (K_a) \) of each system were also determined from the \( y \)-intercept of these plots. Fluorescence data were analyzed with a Stern-Volmer plot. The quenching constants \( (K_{SV}) \) were obtained from the slope of these plots. These results show that the quenching occurs dynamically, statically, or by electron transfer mechanisms. Molecular modeling was also performed on these systems, which provided thermodynamic parameters, dipole moments and electron affinities.
I. INTRODUCTION

A. General Photosynthesis Background

Photosynthesis is a vital photobiological reaction. Green plants, algae, and photosynthetic bacteria use photosynthesis to convert solar energy into chemical energy. The input of chemical energy (endergonic) allows photosynthetic organisms to carry out chemical reactions which result in the formation of oxygen (in green plants), sulfur (in bacteria), and carbohydrates.\(^1\),\(^2\)

There is a separation of positive and negative charges across a membrane during the central process of photosynthesis. This separation is induced by light and catalyzed by special types of membrane-bound pigment-protein complexes called photosynthetic reaction centers (RCs). This process is illustrated in Figure 1. The light-induced charge separation initiates at the primary donors. Chlorophyll (Chl) is the excited chromophore in green plants that serves as the primary electron donor.

Absorption of ultra-violet light from the sun by Chl molecules induces intermolecular electron transfer interactions in photosynthetic systems. The absorption of light in the visible region is a result of the highly conjugated porphyrin system. Electron transfer interactions between Chl and acceptor molecules, such as quinones\(^3\) and nitro aromatic compounds,\(^4\) have been studied extensively in literature to gain an understanding of the mechanisms involved in photosynthesis.\(^5\) However, a detailed spectroscopic and theoretical study on structural effects of these acceptor molecules has
Figure 1. Reaction center.
not yet been done. The focus of this research is on the interactions of Chl a molecules with nitroaromatic compounds.

B. Photosystems I and II

There are two different types of RCs – photosystem I (PSI) and photosystem II (PSII). Terminal acceptors are reduced upon trans-membrane electron transfer to the acceptor side, and these differ according to the type of RC. The acceptors are [Fe₄S₄] clusters in PSI and quinone molecules in PSII. It is understood that these acceptor molecules quench the fluorescence of Chl.³ Studies have also shown that nitro aromatic compounds can reduce the fluorescence of Chl in vitro.²

Both PSI and PSII are driven by light of 680 nm, however PSI is also driven by light of longer wavelength (700 nm). Each photosystem has its own photosynthetic unit, which contains 200-300 light-harvesting Chls and other pigments. The ratio of Chl a to chlorophyll b (Chl b) is far greater in PSI than in PSII. The RC in PSI contains dimeric Chl a molecules whose maximum absorbance is at 700 nm, hence this RC is called pigment 700 (P700). The primary source of electron reduction of CO₂ is PSI.¹ The RC in PSII contains monomeric Chl a molecules. They have an absorbance maximum at 680 nm. The name given to the PSII RC is P680.⁵ It is the PSII RC that produces the oxidation power required for the abstraction of an electron from H₂O.¹

C. Z Scheme

The relationship between PSI and PSII is shown in Figure 2, which is known as a Z scheme. The Z scheme begins with PSII. A photon is received at the RC, which causes the formation of the oxidant, D⁺, and the reductant, A⁻. The purpose of D⁺ is to oxidize water to molecular oxygen. The acceptor in this CT reaction is plastoquinone
Figure 2. The Z-scheme.\textsuperscript{5}
(Q). This reaction is an uphill one, which means that the electron is electrochemically boosted uphill from Chl to Q. Alternatively, the electron flow from Q to PSI is a downhill process. Cytochromes and plastocyanin are electron carriers along this downhill path.\(^5\)

In PSI, the immediate donor molecule can be cytochrome \(f\), plastocyanin or both. The acceptor molecules are [Fe\(_4\)S\(_4\)] clusters in PSI, and these are marked X in the Z scheme. The [Fe\(_4\)S\(_4\)] clusters are in a cubane structure and can also be annotated \(\text{Fe}_4(\mu_3-\text{S})_4\). Unlike PSII, the illumination of PSI results in a weak oxidant and a strong reductant. Lastly, there is another downhill flow of the electron from \(X\), the strong reductant, to NADP. The latter is reduced to NADPH.\(^5\)

D. Determination of the Ground State Equilibrium Constant, \(K_a\), from UV-Vis Absorption Spectra\(^1,7\)

Various methods\(^7\) have previously been developed for treating visible and ultraviolet spectral data to obtain equilibrium constants for complex formation. If the molar absorptivity for the donor is known, the quantitative analysis of the complex formed can be achieved.

The assumption is made that the chemical equilibrium is of the form,

\[
\text{Q} + \text{D} \rightleftharpoons \text{QD} \tag{1}
\]

where Q is the quencher substrate, and D is the donor molecule. It is also assumed that only D and QD absorb in the wavelength region of interest. The concentration of D is small in comparison to that of Q, therefore the equilibrium constant (K) of equation 1 can be given by

\[
K = \frac{[\text{QD}]}{[\text{Q}][\text{D}]} \tag{2}
\]
where \([QD]\) is the equilibrium concentration of the QD complex, \([Q]\) and \([D]\) are the final concentrations of \(Q\) and \(D\). It can be noted that the reaction in equation 1 forms a 1:1 complex. If both \(D\) and \(QD\) obey Beer’s Law \((A = \varepsilon bc)\) at a given wavelength, then the total absorbance \((A)\) per cm of path is given by,

\[
A_{QD} = \varepsilon_D[D] + \varepsilon_{QD}[QD]
\]  

where \(\varepsilon_D\) and \(\varepsilon_{QD}\) are the molar absorptivities of the donor molecule and the formed complex, respectively, and \(A_{QD}\) is the absorbance of the complex, formed from equation 1.

Without the complexing agent, \(Q\), the total absorbance per cm is given by,

\[
A_D = \varepsilon_D[D]_0
\]  

where \(A_D\) is the absorbance and \([D]_0\) is the initial concentration of the donor molecule in the absence of the complexing agent, \(Q\). Division of equation 3 by equation 4, and substitution for \([QD]\) from equation 2 yields,

\[
\frac{A_{QD}}{A_D} = \frac{([D]/[D]_0)\{1 + (\varepsilon_{QD}/\varepsilon_D)K[Q]\}}{1 + K[Q]}
\]  

Introduction of the conservation of species \(D\) gives,

\[
[D]_0 = [D] + [QD]
\]  

and using equation 2 again gives,

\[
[D]/[D]_0 = (K[Q] + 1)^{-1}
\]  

Substitution of equation 7 into equation 6 results in the following equation:

\[
\frac{A_{QD}}{A_D} = \frac{1 + (\varepsilon_{QD}/\varepsilon_D)K[Q]}{1 + K[Q]}/ \{1 + K[Q]\}
\]  

For mathematical simplicity let,

\[
Z = \frac{A_{QD}}{A_D}
\]  

\[
\alpha = (\varepsilon_{QD}/\varepsilon_D)K
\]
[Q] = 1/Y \quad [11]

This allows equation 8 to become,

\[ Z = \frac{[1 + (\alpha/Y)]}{[1 + (K/Y)]} \quad [12] \]

Solving for Y yields,

\[ Y = \frac{(KZ - \alpha)}{(1 - Z)} \quad [13] \]

If we let,

\[ X = \frac{1}{(1 - Z)} \quad [14] \]

then substituting equation 14 into equation 13 results in the following linear equation:

\[ Y = (K - \alpha)X - K \quad [15] \]

When [Q]$^{-1}$ (equation 11) is plotted against [A$_D$/(A$_D$-A$_Q$D)], a straight line should result (equation 15) if there has been the formation of a 1:1 complex. The intercept of this line is the negative of the equilibrium constant (K$_a$), and the slope is related to the molar absorptivity of the complex.

E. Fluorescence Quenching Analysis from Emission Spectra

Fluorescence quenching refers to any process that decreases the fluorescence intensity of a sample. This quenching can result from a variety of molecular interactions, which include excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation, and dynamic quenching. Quenching of Chl a by electron acceptors occurs either dynamically, statically, or by electron transfer mechanisms. (Figure 3)$^8$

E.1. Dynamic Quenching (Derivation of the Stern-Volmer Equation)

Dynamic (collisional) quenching requires molecular contact between the donor and acceptor molecules. It is a diffusion-controlled mechanism. The acceptor must
Collisional Quenching

\[ f(t) \xrightarrow{Q} (F^\ast) \xrightarrow{k_q [Q]} Q \]

Static Quenching

\[ F^\ast + Q \rightleftharpoons (F \cdot Q)^\ast \]

\[ h\nu \rightarrow F + Q \xrightarrow{K} F \cdot Q \]

Figure 3. Comparison of dynamic versus static quenching.
diffuse to the donor during the lifetime of the excited state. Once contact occurs, the donor returns to the ground state without emitting a photon. Quenching occurs without any permanent change in the molecules. It can be distinguished by its differing dependence upon temperature and viscosity. Lower viscosities result in faster diffusion and hence, larger amounts of dynamic quenching. This increase in dynamic quenching can be seen by performing excited-state lifetime measurements.\textsuperscript{8} Therefore, quenching studies can be used to reveal diffusion rates of quenchers. Dynamic quenching of fluorescence is described by the Stern-Volmer equation,

\begin{equation}
\frac{I_0}{I} = 1 + k_q \tau_0 [Q] = 1 + K_{SV} [Q] \tag{16}
\end{equation}

In this equation, $I_0$ and $I$ are the fluorescence intensities in the absence and presence of the quencher, respectively, $k_q$ is the bimolecular quenching constant, $\tau_0$ is the lifetime of the fluorophore in the absence of the quencher, and $[Q]$ is the concentration of the quencher. The Stern-Volmer quenching constant is given by $k_q \tau_0$ or $K_{SV}$.\textsuperscript{8}

Quenching data are usually presented as plots of $I_0/I$ vs. $[Q]$. This plot yields a line with a $y$-intercept of 1 and a slope equal to the Stern-Volmer constant, $K_{SV}$. The magnitude of $K_{SV}$ scales with the strength of the quencher. There is a linear dependence of $I_0/I$ upon the concentration of the quencher. Linearity of the Stern-Volmer plot generally indicates that dynamic quenching has occurred. Most systems behave in a linear fashion at lower concentrations and nonlinearly at higher concentrations of quencher. The nonlinear plot indicates that either aggregates with respect to the Chl populations are present in the solution, or more than one quenching mechanism is involved in the system.
The fluorescence intensity, \([I]\), of a fluorophore is directly proportional to the population of excited molecules. A fixed population of fluorophore can be established under continuous illumination, which results in \(d[I^*]/dt = 0\). In the presence of a quencher, the following differential equation can be used to describe \([I^*]\):

\[
1 = d[I^*]/dt = f(t) - \gamma[I^*]_0 = 0
\]  \[17\]

and in the absence of a quencher:

\[
I_0 = d[I^*]/dt = f(t) - (\gamma + k_q[Q])[I^*]_0 = 0
\]  \[18\]

where \(f(t)\) is the constant excitation function, and \(\gamma = \tau_0^{-1}\) is the decay rate of the fluorophore without the quencher. Division of Eq. [3] by Eq. [2] yields

\[
I_0/I = \frac{y + U}{\gamma} = 1 + k_q \tau_0[Q]
\]  \[19\]

which is the Stern-Volmer equation.

Dynamic quenching is a rate process that depopulates the excited state. The excited-state lifetimes in the absence (\(\tau_0\)) and presence (\(\tau\)) of a quencher are given by:

\[
\tau_0 = \gamma^{-1}
\]  \[20\]

\[
\tau = (\gamma + k_q[Q])^{-1}
\]  \[21\]

and therefore:

\[
\tau_0/\tau = 1 + k_q \tau_0[Q]
\]  \[22\]

This equation shows that there is an equivalent decrease in fluorescence intensity and in lifetime that is, for dynamic quenching,

\[
I_0/I = \tau_0/\tau
\]  \[23\]

There is a decrease in lifetime because quenching is an additional rate process that depopulates the excited state. Quenching depopulates the excited state without fluorescence emission, and that is what accounts for the decrease in yield.\(^8\)
E.2. **Static Quenching**

Static quenching can occur as a result of the formation of a nonfluorescent complex between the fluorophore and the quencher. The molecules do not have to move around but are positioned close enough for the reaction to occur. The excited-state lifetime is not decreased by static quenching because only the fluorescent molecules are observed. The uncomplexed fluorophores have the unquenched lifetime $\tau_0$. When the complex absorbs light, it immediately returns to the ground state without emitting a photon.$^{1,8}$

E.3. **Comparison of Dynamic and Static Quenching**

The measurement of fluorescence lifetimes is the most definitive method to distinguish between static and dynamic quenching. Static quenching removes a fraction of the fluorophores from observation. The complexed fluorophores are nonfluorescent, and the only observed fluorescence is from the uncomplexed fluorophores. The uncomplexed fraction is unperturbed hence the lifetime is $\tau_0$. Therefore, for static quenching, $\tau_0/\tau = 1$, whereas for dynamic quenching, $\tau_0/\tau = I_0/I$.\(^8\)

Temperature dependence studies can also be used to distinguish between static and dynamic quenching. Higher temperatures result in the dissociation of weakly bound complexes, and hence smaller amounts of static quenching. The following equation gives the relationship of the diffusion coefficient (D), temperature (T) and viscosity of the solvent ($\eta$):\(^1,8\)

$$D = (kT)/(6\pi\eta R)$$  \[24\]

where $k$ is the Boltzmann constant and $R$ is the collision radius. This equation is known as the Stokes-Einstein equation. An increase in temperature results in a decrease in static
quenching constants due to the decrease in the stability of the formed complex. The diffusion coefficient is directly proportional to temperature; hence increasing the temperature will result in a higher diffusion constant.

E.4. Electron-transfer Quenching

Electron-transfer quenching forms a very short-lived ion pair intermediate from the excited singlet state interaction with the acceptor. The ion-pair undergoes rapid spin-allowed back electron transfer to yield ground state reactants. The same process for the ion-pair produced from the excited triplet state of Chl is spin-forbidden and only occurs as a consequence of intersystem crossing. The intersystem crossing is induced by spin mixing of the excited state. When the donor and acceptor molecules undergo complexation, a charge transfer occurs. This CT complex is assumed to decay to the ground state before separation of the ion pair can occur. For this process to occur, there has to be spectral overlap between the spectrum of the donor and that of the acceptor molecules. The latter will facilitate electron transfer between energy levels that are in close proximity.

F. Solvent Effects on Fluorescence Quenching

The nature of the solvent plays a large role in the fluorescence spectra of organic molecules. The Frank-Condon principle explains why this is true. The principle emphasizes that electronic transitions occur much faster than nuclear motions. Electronic charge distributions in excited state organic molecules vary from those in the ground-state due to the differences in the equilibrium arrangement of solvent molecules about an excited solute than from a ground-state solute. Solvent reorientation occurs after the electronic excitation takes place. The time scale for solvent reorientation is on the order
of picoseconds. Femtosecond lasers can be used to measure solvent relaxation processes. It is possible that an electronic excited state can occur without solvent reorientation. This transitory excited-state is called a “Frank-Condon” state. After the solvent has reoriented an “equilibrium excited state” is present. Fluorescence originates in room-temperature solutions in this equilibrium excited-state. Fluorescence excited lifetimes are on the order of $10^{-9}$ to $10^{-7}$ seconds.

The solvent can affect fluorescence spectra in several ways. These are:

F.1. **Polarization shifts**

Polarization shifts involve a shift of energy of the fluorescence maximum from higher or lower energy as a function of the solvent polarity or polarizability. In the absence of specific solute-solvent interactions, such as hydrogen bonding, the changes in fluorescence energy with solvent can often be related to the dielectric constant of the solvent. These energy changes are termed “polarization shifts” and are credited to polarization of solvent molecules induced by the transition dipole of the solute. In addition to induced dipole interactions, permanent dipole-dipole forces may be of importance. Previous studies have observed electrostatic effects upon fluorescence energies. These studies indicate that many organic molecules become considerably more polar when electronically excited. Studies have shown that the magnitude of the frequency shifts observed upon changing the solvent can be used to calculate dipole moments of excited organic molecules.\(^{10}\)

F.2. **Hydrogen Bonding**

Fluorescence is affected if the solute and solvent can engage in intermolecular hydrogen bonding. The effects of hydrogen bonding upon fluorescence spectra are
frequently difficult to predict in advance. It is best to avoid hydrogen bonding situations wherever possible. Hydrogen bonding can cause either red or blue fluorescence shifts. Prediction of the type of hydrogen bonding that will predominate in the fluorescence spectrum is difficult, since hydrogen-bonding abilities of organic solutes are often strikingly different in electronically excited states and in the ground state. It is often possible to predict that hydrogen-bonding effects will occur in a given system, but it is considerably more difficult to predict the magnitude of the wavelength shifts.21

It has been shown that hydrogen bonding of a fluorescent solute with the solvent (or another solute) can cause significant changes in the emission efficiency of the solute. It seems that hydrogen bonding produces fluorescence quenching if the hydrogen bond is closely related with the π-electron system of the proton donor, and the π-electron systems of the donor and acceptor are conjugated. However, if the hydrogen bond is isolated from the π-electron system of the proton donor by a sigma bond, fluorescence is enhanced by hydrogen bonding. With some proton donor-acceptor pairs, it seems that dipole interactions are more important than hydrogen bonding action. For a given solute whose fluorescence is observed in a variety of solvents, there may be no correlation between the fluorescence efficiency of the solute and the hydrogen bonding ability of the solvent, even if all the solvents are capable of hydrogen bonding. However, it is usually preferable, if possible, to avoid hydrogen bonding in analytical applications of fluorescence.10

F.3. Solvent Quenching

Certain solvents can quench the fluorescence of a solute. The fluorescence efficiencies of organic compounds are usually greater in the vapor phase than in liquid
solution. The efficiency in liquid solution is a function of the particular solvent used, and
some solvents exhibit unusual quenching effects. Solvent quenching occurs to a
considerable extent even in nonpolar solvents, but it becomes much more severe for polar
solute-solvent pairs. The stronger the interaction between the solvent and excited solute,
the greater the energy lost through solvent quenching. This interaction should be a
consideration when choosing solvents for fluorometric analysis.¹⁰

F.4. Temperature Effects

Temperature variations affect the efficiency and energy of fluorescence.
Fluorescence quantum efficiency in liquid solution is generally a function of temperature.
As the temperature is decreased, the fluorescence efficiency of an organic compound
usually increases. This increase in fluorescence can be either sharp (internal conversion)
or gradual (intersystem crossing). Temperature effects can be useful in the study of
radiationless processes.

Frequency shifts can also occur when the temperature of a fluorescent
solution is changed. Fluorescence maxima shift to lower frequency (longer wavelength)
as the temperature is raised from liquid nitrogen temperature to room temperature. The
frequency shift is related to solvent reorientation processes.¹⁰

G. Light and Dark Reactions (in vivo)

The photosynthetic process can be divided into two main reactions – light and
dark reactions. Light reactions involve the absorption of light energy by the
photosynthetic pigments (Chl a), the stabilization of energy by charge separation, and the
transfer of energy among the pigment molecules.¹¹ Dark reactions occur in the absence
of light and involve carbon dioxide fixation and its conversion to carbohydrates.
Chlorophyll $a$ is one of the major light-capturing pigments in the light reaction. It is also referred to as the primary photoacceptor or the principal energy-transfer agent. In the ground-state, the Chl $a$ molecule absorbs a quantum of light (solar energy) and an electron jumps to a higher energy level, thus accounting for the formation of the excited-state of Chl $a$. The excess energy loss of Chl $a$ can be accounted for in one of four ways, as shown in Figure 4:

1. Fluorescence (emitted light)
2. Photochemistry (occurs through an oxidation-reduction reaction)
3. Heat (Radiationless de-excitation)
4. Resonance Energy Transfer (occurs through the transfer of energy from an excited molecule to another molecule in the ground-state)

Chlorophyll $a$ does not undergo de-excitation and emit energy as fluorescence, in vivo. Rather, it donates an electron to plastoquinone (photochemistry) or goes through a resonance energy transfer. Molecules in an excited singlet state may return to the ground state without the emission of a photon, in which all the excitation energy is converted into heat. This process is called internal conversion, and its efficiency is difficult to measure. The main photochemical reaction requires an electron transfer from Chl $a$ to an appropriate acceptor ($A$), which is plastoquinone in green plants.
Figure 4. Possible pathways of excited chlorophyll.\textsuperscript{13}
The products Chl $a^+$ and A$^-$ serve as starting points for the successive electron transfer processes that produce chemical free energy stored in the form of adenosine triphosphate, (ATP) and reduced nicotinamide adenine dinucleotide phosphate, (NADPH). At this point, A$^-$ serves as a secondary donor, and it donates its electron to Chl $a^+$ (ground-state), and the cycle begins again.

Dark reactions occur in the absence of light and involve CO$_2$ fixation and its conversion to carbohydrates. ATP and NADPH drive these reactions. The fixation of CO$_2$ requires that both PSI and PSII of light reactions function in a cooperative manner to give a continuous source of ATP and NADPH. All photosynthetic organisms use the Calvin cycle to reduce and process fixed carbon into carbohydrates.

In the transfer of resonance energy, the excited molecule transfers energy to a ground-state molecule (photochemistry is not involved). There must be some overlap with the absorption spectrum of the excited molecule and the ground-state molecule. They must be physically close to each other since the efficiency of this energy transfer decreases as the distance between the molecules increases.

H. Quenching Studies by Quinones and Nitroaromatics (in vitro)

Quinones are present in photosynthetic membranes. Plastoquinones (Figure 5) operate as key mobile electron carriers in thylakoids of higher plants by functionally connecting PSII and PSI. Plastoquinones serve a dual purpose as both photochemical and non-photochemical quenchers of energy in PSII, in vivo. They are involved in photosynthetic electron transport and contribute to non-photochemical control of excitation energy dynamics in light-harvesting antenna.
Figure 5. Structures of (a) Chlorophyll $a^6$ and (b) Plastoquinone 9.\textsuperscript{16}
Artificial quinones are efficient quenchers of Chl fluorescence in organic solutions. The degree of Chl fluorescence quenching is highly variable (K_{SV} values range from 1.6 \times 10^2 - 2.9 \times 10^4 M^{-1}) and depends on quinone structure. The quenching by artificial quinones is a result of both non-photochemical mechanisms due to direct interaction between the added quinones and the excited antenna Chls, and photochemical events owing to competition between artificial and natural quinones for electrons introduced by PSII.\textsuperscript{3,9,15}

Nitro aromatics (Figure 6) are strong electron acceptors. Previous studies have shown that Chl \textit{a} forms 1:1 complexes with the following nitro aromatic acceptors: sym-trinitrobenzene (TNB)\textsuperscript{17}, 2,4,7-trinitrofluorenone (TNFO, K_{SV} = 2.6 \times 10^2 M^{-1} in CH_2Cl_2)\textsuperscript{18}, and 2-(2,4,7-trinitro-fluoren-9-ylidene)-malonitrile (TNFM, K_{SV} = 1.8 \times 10^3 M^{-1} in CH_2Cl_2)\textsuperscript{4}. The quenching efficiency is directly proportional to the number of nitro group substituents – fewer nitro groups yield the lowest quenching capabilities (i.e. nitrobenzene < dinitrobenzene < trinitrobenzene). The direct correlation between fluorescence quenching to the number of nitro groups can be partially attributed to increased static quenching.\textsuperscript{19}

The intermolecular interactions of Chl \textit{a} with TNFO have been investigated. The properties of complexation have been studied in both the ground and excited states. These investigations allow for an understanding of possible mechanistic routes for electron transfer from the excited singlet state of Chl \textit{a} to the quencher in the ground state and for the modes of excited-state energy transfer. TNFO is a good \pi acceptor and is endowed with a keto-carbonyl group that can interact coordinatively with metal ions providing exciting situations for study.\textsuperscript{18}
Figure 6. Fluorene derivatives (I-VIII) and the model structure of Chl a (VII) used in the Gaussian 03 DFT calculations. Hydrogen atoms are not shown for simplicity. The interactions of fluorene, ITNF, TFPR and TFPH with Chl a were studied in this work.
Steady-state fluorescence quenching and lifetime measurements of TNFM with Chl a in acetonitrile (CH$_3$CN) and dichloromethane (CH$_2$Cl$_2$) have been measured in a previous study. The results indicated that TNFM is a very strong electron acceptor. It has a higher electron affinity than TNFO, which is due to the presence of cyano groups. It was concluded that the quenching was dynamic in nature by collisional interaction with the uncomplexed fluorophore.

I. **Objective of this Study**

Fluorescence quenching studies of Chl have been performed in an attempt to investigate the primary role in the process of photosynthesis. Our research is focused mainly on the investigation of fluorescence quenching of Chl a by nitro aromatics, specifically tri-nitro substituted fluorene derivatives (electron acceptors). The effect of the various substituents on these tri-nitro fluorene compounds can be studied by changing the functional group at position nine of each fluorene moiety. Position nine was selected since substitution of electron withdrawing functional groups at this position will reduce the electron density in the $\pi$ system of the adjacent benzene rings, hence favor complexation. The excited Chl molecule will donate electrons to the ground state acceptor molecules (nitroaromatics).

The chemistry of Chl a with fluorene and the following three compounds were investigated: 2-(2,4,7-Trinitro-fluoren-9-ylideneaminoxy)-propionic acid [TFPR], 2-(2,4,7-Trinitro-fluoren-9-ylidenemethyl)-phenol [TFPH], and 9-Isopropylidene-2,4,7-trinitro-9H-fluorene, [ITNF]. The solvents used in this study were acetonitrile and dichloromethane, CH$_3$CN and CH$_2$Cl$_2$, respectively. Absorption, emission, and excited-state lifetimes of these systems were measured using a UV-Vis spectrophotometer and
laser induced fluorescence (LIF) spectroscopy. It is anticipated that this research will provide a better understanding of the electron transfer mechanisms that occur in PSII, and these results will contribute to artificial photosynthesis.
II. EXPERIMENTAL

A. Materials

*The following reagents were obtained from Aldrich Chemical Company:*

1. **Chlorophyll a, (Chl a), from spinach** – Chlorophyll a (C_{53}H_{72}MgN_{4}O_{5}) has a formula weight of 893.48 amu and a melting point in the range of 117-120°C. It is microcrystalline in form with a waxy blue-black appearance. The literature values for the extinction coefficients (ε) of Chl a dissolved in ether are: $\varepsilon_{662\text{ nm}} = 8.63 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ and $\varepsilon_{428\text{ nm}} = 11.2 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$, and the observed values by Sigma are: $\varepsilon_{659\text{ nm}} = 8.63 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ and $\varepsilon_{427\text{ nm}} = 10.6 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$. It is freely soluble in several solvents, including: ether, acetonitrile, and dichloromethane. When Chl a was not in use, it was stored in the freezer (0°C) as recommended by Aldrich Company.

2. **Fluorene, zone-refined, 99%** - Fluorene has a formula weight of 166.21 amu, with a melting point in the range of 114-116°C and a boiling point of 298°C. The density is 1.202g/mL. Fluorene appears as dazzling white leaflets or flakes from alcohol and sublimes easily in high vacuum.

3. **2-(2,4,7-Trinitro-fluoren-9-ylideneaminoxy)-propionic acid (TFPR)** – TFPR (C_{16}H_{10}N_{4}O_{9}) has a formula weight of 402.28 amu.

4. **2-(2,4,7-Trinitro-fluoren-9-ylidenemethyl)-phenol (TFPH)** – TFPH (C_{20}H_{11}N_{3}O_{7}) has a formula weight of 405.32 amu.

5. **9-Isopropylidene-2,4,7-trinitro-9H-fluorene (ITNF)** – ITNF
(C_{16}H_{11}N_{3}O_{6}) has a formula weight of 341.28 amu

6. **Acetonitrile, 99.5%, spectrophotometric grade** – Acetonitrile (CH$_3$CN) formula weight of 41.05 amu, with a melting point of -45°C and a boiling point of 81.6°C. The dielectric constant for CH$_3$CN 20°C is 38.8. The dipole moment is 3.94 D for CH$_3$CN.

*The following reagent was obtained from ACROS Organics:*

1. **Dichloromethane, stabilized, spectrophotometric grade, 99.8%** – Dichloromethane (CH$_2$Cl$_2$) has a formula weight of 84.93 amu with a melting point of -97°C and a boiling point of 39-40°C. The density of CH$_2$Cl$_2$ is 1.3200 g/mL. The dielectric constant is 9.1 at 20°C. The dipole moment for CH$_2$Cl$_2$ is 1.57 D.

*All compounds (Chl a, fluorene, TFPR, TFPH, and ITNF) are soluble in CH$_3$CN and CH$_2$Cl$_2$.*

B. **Solution Preparation**

B.1. **Absorption Measurements**

A stock solution of Chl $a$ was prepared by dissolving a 1 mg sample of Chl $a$ into a 10 mL volumetric flask and filled to the mark with either CH$_3$CN or CH$_2$Cl$_2$. This produced a solution of $\sim$1.1x10$^{-4}$ M. Stoichiometric calculations were used to determine an approximate value for the concentration. The absorbance of the prepared stock solution was measured using the UV-VIS spectrophotometer. Beer’s Law was used to determine the concentration of the Chl $a$ stock solution in each solvent ($\varepsilon_{\text{CH}_{3}\text{CN}} = 7x10^4$ M$^{-1}$cm$^{-1}$).

A Chl $a$ stock solution of 1x10$^{-5}$ M was prepared. This concentration was low enough to ensure that Chl $a$ existed in the monomeric state.
A volume of 3.4 mL of the $1 \times 10^{-5}$ M Chl $a$ (donor molecule) solution was measured into a quartz cuvette. The acceptor molecule (ITNF, TFPR, TFPH, or fluorene) was introduced to this volume, with constant stirring, in 1 mg increments up to 6 mg. The absorption spectrum was collected after each addition.

**B.2. Fluorescence Measurements**

A solution of $3 \times 10^{-6}$ M Chl $a$ in a chosen solvent was prepared from the initial stock solution. The concentration was confirmed by measuring the absorbance spectrum. A stock solution of the acceptor molecule was also prepared at a concentration of $2 \times 10^{-3}$ M. From this concentration, diluted solutions of the acceptor molecule at varying concentrations were prepared. These solutions were then complexed (1:1, by volume) with the fixed concentration, $3 \times 10^{-6}$ M, of Chl $a$ solution, and the fluorescence spectra of the uncomplexed Chl $a$ solution and its donor-acceptor complexes were acquired. The variation of the Chl $a$ fluorescence intensities was monitored as a function of the acceptor’s concentration in a given environment at constant temperature, 25°C. The concentration of the donor was kept constant at low concentrations ($10^{-6}$ M) to minimize the formation of aggregates, such as dimers.

**B.3. Excited State Lifetime Measurements**

Time resolved fluorescence decay of the Chl $a$ molecule was monitored as a function of the acceptor concentration in a given medium. Solutions were aerated with nitrogen gas in a quartz cuvette for several minutes to eliminate/minimize the presence of oxygen. Triplet oxygen is known to quench fluorescence of fluorophores. The N$_2$-pumped dye laser system was used to excite the Soret band ($\lambda = 430$nm) of the Chl $a$ or its complex.
The following instruments (with indicated operational settings) were used for physical measurements:

C. Instrumentation

C.1. Absorption

A Shimadzu UV-2101 PC (UV-VIS) spectrophotometer was used to perform absorbance measurements. The wavelength range was set in the visible region from 400-700 nm, and the slit width was 2.0 nm.

C.2. Laser Induced Fluorescence (LIF)

An experimental set-up for the LIF instrument is shown in Figure 7 a & b.

C.2.1. N₂-pumped dye laser system

The nitrogen laser produces light at 337.1 nm. The pulsewidth is 600 ps, which is nominal. The repetition rate was set at 10 Hz. The energy/pulse at this rep rate was 100 μJ (λ = 430 nm) with a stability of ± 5%. The peak power was greater than 2 MW. The power density was greater than 10 MW/sq. cm at the exit, and the beam dimensions were 3x6 mm at the exit. The beam divergence was 3x7 mrad. The triggering was internal. The trigger out had a power of 25 V and occurred 1 μs before lasing. Nitrogen gas was used (> 99.995% purity), with a consumption rate of 5L/min at 10 Hz.

The stilbene 420 laser dye (1.8×10⁻³ M in methanol) was excited by the nitrogen laser (337nm) to produce light at 430nm. This energy was used for excitation.
Figure 7. Experimental set-up for (a) fluorescence and (b) excited state lifetime measurements.
C.2.2. Oriel MS 260i Monochromator with ICCD Camera Detector

A controller card for the ICCD camera and monochromator was inserted on the motherboard of the computer to interface the instrument with the computer. A filter was placed prior to the entrance slit of the spectrograph to block the excitation wavelength. The monochromator, coupled to the ICCD camera, was used to select an operational wavelength range (i.e. 500-800 nm). Andor Istar software package was used to set the experimental parameters.

For calibration, the mode of acquisition was set to real time. The trigger mode was internal, and the exposure time was 0.021 s. The gain level was 100, and the gate width was $1 \times 10^7$ ns. A mercury (Hg) lamp was used for calibration. Experimentally acquired transitions were compared with literature values (Figure 8).

For experimental measurements, the acquisition mode was set to accumulate (100 accumulations), and the trigger was external. The exposure time was 0.067 s. The gain level was 60, and the gate width was $1 \times 10^5$ ns.

For calibration and data acquisition, the data were recorded in counts. The delay was 0.021 s, with a pixel readout time of 16 µs. The readout mode was full vertical binning, and the insertion delay was normal.

C.3. Excited State Lifetimes

Measurements were obtained using the N$_2$-pumped dye laser system* (see above for specifications). The time resolved fluorescence decay signal detection was facilitated with a single wavelength selector monochromator, Hamamatsu R928 photomultiplier tube (PMT), and a 400MHz oscilloscope. The oscilloscope was triggered with laser light sent to a photodiode. The signal from the PMT was also sent to the oscilloscope. No
signal was detected by the PMT when there was no sample in the cuvette since the excitation light was eliminated with a red filter with a cut-on wavelength of 600nm.

Communication between the oscilloscope and a PC computer was facilitated by a RS-232 connection. Wavestar software package was used for data acquisition, and the files could be converted into excel spreadsheets.

D. Molecular Modeling

All structures presented in Figure 6 were optimized at the density functional theory (DFT) level, and their minimized energies plus dipole moments are listed in Table 4. The model structure of Chl $\alpha$ has a hydrogen atom replacing the phytlyl group to reduce the computational cost. Previous studies indicate that the phytlyl group does not participate in the complexation. Our calculated electron affinity results compared favorably (within 1% error) with previously investigated systems of fluorene and its anion analog.

Enthalpies of reaction for the formation of the 1:1 complex (Equation 1) were computed with PCModel software, version 7.5. Heats of formation of donor (D), quencher (Q), and the complex (DQ) were computed individually. The $\Delta H_{\text{rxn}}$ was determined from:

$$\Delta H_{\text{rxn}} = \Sigma n \Delta H_f(\text{products}) - \Sigma n \Delta H_f(\text{reactants})$$  \[25\]

These values are summarized in the results section and their corresponding 1:1 structures are presented in Figures 9 and 10.

Combining spectroscopic methods with theory will facilitate in understanding the processes involved in the charge transfer mechanism of the proposed systems.
Figure 8. Mercury lamp calibration: Black spectrum is from the Hg lamp. The tick marks in red are literature values for Hg lines.
Figure 9. Optimized model structure of (a) Chl $a$, (b) Chl $a$-Fluorene, (c) Chl $a$-TFPH, (d) Chl $a$-TFPR, (e) Chl $a$-ITNF.
Figure 10. Optimized model structure of (a) Chl α-TNFO, (b) Chl α-Plastoquinone (PQ), (c) Chl α-TNF.
III. RESULTS AND DISCUSSION

A. Absorption

Electronic transitions are involved in both visible (Vis) and ultraviolet (UV) spectra. Singlet-singlet or triplet-triplet transitions are much more favored, energetically. Singlet-triplet transitions are forbidden by formal spectroscopic selection rules. Absorption of UV-Vis by Chl $a$ molecules causes the complex to undergo a charge transfer (CT). A special type of electronic spectra arises from CT interaction between a pair of molecules (see introduction for more detailed explanation of CT reactions). The extent of CT that occurs in the system depends on the energy separation between the Chl $a$ and the acceptor molecule. The energy separation between two electronic states is taken as the difference between the zeroth vibrational levels.

Occasionally, a solution will contain two absorbing substances in equilibrium whose bands overlap. The molar absorptivities of the two species may be equal at some wavelength over the overlapped region. If the sum of the concentrations of these two compounds in solution is held constant, there will be no change in absorbance at this wavelength as the ratio of these two compounds is varied. This invariant point is called the isosbestic point. An isosbestic point was observed for the Chl-TFPR complex in dichloromethane ($\text{CH}_2\text{Cl}_2$) and acetonitrile ($\text{CH}_3\text{CN}$). Isosbestic points usually indicate that only two absorbing species are present (donor and complex). They also eliminate the possibility that the acceptor molecules are absorbing in the range of interest. However,
isosbestic points will not necessarily be present in all systems that only have two absorbing species in the region of interest.\textsuperscript{1,5}

Absorption spectra result in broad bands because the transition between two electronic states occurs over a range of wavelengths. The intensity of an electronic absorption band is a measure of the probability of an electronic transition between the ground and excited states. The intensity of an electronic transition is influenced by the following:\textsuperscript{10}

1. The multiplicities of the ground state and the excited state (spin-forbidden versus spin-allowed transitions). That is, $\Delta S = 0$ transitions are allowed and transitions that change multiplicity are very weak.

2. The degree of overlap between the wavefunctions of the ground and excited electronic states (Franck-Condon Principle).

3. The symmetries of the wave functions of the ground state and the excited state, gerade (g) versus ungarade (u). That is, $g \leftrightarrow u$ is allowed for centrosymmetric molecules.

Absorption measurements were made to determine the ground-state complexation properties between chlorophyll and the acceptor molecules. There is an inverse relationship between the absorbance of the complex and the concentration of the acceptor molecule. The absorbance of the complex decreased as the concentration of the acceptor molecule was increased. The acceptor concentration used to decrease the absorbance of the donor in the ground state was at least one to two orders of magnitude higher than the concentration of Chl (donor molecule).

Linearity of the Nash plots indicates a 1:1 formation of the complex. The binding
constants ($K_a$) are obtained from the y-intercept of these plots and are summarized in Table 1. All measurements were made at room temperature, and the constants are within 10% limit due to experimental error. Figures 11-23 show the absorption spectra and Nash Plots generated.
Figure 11. Absorption spectra of chlorophyll $\alpha$ from spinach, fluorene, 9-isopropylidene-2,4,7-trinitro-9H-fluorene (ITNF), 2-(2,4,7-trinitro-fluoren-9-ylideneaminoxy)-propionic acid (TFPR), and 2-(2,4,7-trinitro-fluoren-9-ylidenemethyl)-phenol (TFPH) in dichloromethane.
Figure 12. Absorption spectra of Chl $a$ with TFPH in CH$_3$CN.

Figure 13. Nash plot of Chl $a$ with TFPH in CH$_3$CN.
Figure 14. Absorption spectra of Chl $a$ with TFPH in CH$_2$Cl$_2$.

Figure 15. Nash plot of Chl $a$ with TFPH in CH$_2$Cl$_2$. 
Figure 16. Absorption spectra of Chl $a$ with TFPR in CH$_2$Cl$_2$.

Figure 17. Nash plot of Chl $a$ with TFPR in CH$_2$Cl$_2$. 
Figure 18. Absorption spectra of Chl a with TFPR in CH$_3$CN.

Figure 19. Nash plot of Chl a with TFPR in CH$_3$CN.
Figure 20. Absorption spectra of Chl $a$ with fluorene in CH$_3$CN.

Figure 21. Nash plot of Chl $a$ with fluorene in CH$_3$CN.
Figure 22. Absorption spectra of Chl $a$ with fluorene in CH$_2$Cl$_2$.

Figure 23. Nash plot of Chl $a$ with fluorene in CH$_2$Cl$_2$. 

$y = 10.902x - 20.291$ 
$R^2 = 0.9989$
Table 1. Binding Constants and Gibb’s Energy of Chl a with Various Acceptor Molecules in CH$_3$CN and CH$_2$Cl$_2$ at 25°C.

<table>
<thead>
<tr>
<th>System</th>
<th>Solvent</th>
<th>$\lambda_{\text{abs}}^\text{max}$ (nm)</th>
<th>$K_a$ (M$^{-1}$)</th>
<th>$\Delta G$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl a only</td>
<td>CH$_3$CN</td>
<td>660</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>CH$_2$Cl$_2$</td>
<td>663</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chl a -Fluorene</td>
<td>CH$_3$CN</td>
<td>660</td>
<td>60</td>
<td>-10.1</td>
</tr>
<tr>
<td></td>
<td>CH$_2$Cl$_2$</td>
<td>663</td>
<td>20</td>
<td>-7.42</td>
</tr>
<tr>
<td>Chl a -TFPR</td>
<td>CH$_3$CN</td>
<td>661</td>
<td>2370</td>
<td>-19.2</td>
</tr>
<tr>
<td></td>
<td>CH$_2$Cl$_2$</td>
<td>664</td>
<td>450</td>
<td>-15.1</td>
</tr>
<tr>
<td>Chl a -TFPH</td>
<td>CH$_3$CN</td>
<td>661</td>
<td>300</td>
<td>-14.1</td>
</tr>
<tr>
<td></td>
<td>CH$_2$Cl$_2$</td>
<td>662</td>
<td>2620</td>
<td>-19.5</td>
</tr>
<tr>
<td>Chl a -TNFM$^1$</td>
<td>CH$_3$CN</td>
<td>660</td>
<td>3240</td>
<td>-20.0</td>
</tr>
<tr>
<td></td>
<td>CH$_2$Cl$_2$</td>
<td>660</td>
<td>2200</td>
<td>-19.1</td>
</tr>
<tr>
<td>Chl a -TNFO$^2$</td>
<td>CH$_2$Cl$_2$</td>
<td>662</td>
<td>103</td>
<td>-11.5</td>
</tr>
</tbody>
</table>

Association constants and free energies are not applicable for Chl a only system.

$^1$Refer to No. 4 in Bibliography Section.

$^2$Refer to No. 18 in Bibliography Section.
B. Fluorescence

Fluorescence measurements are made to detect photon emission that occurs when a molecule in the excited state returns to the ground state without any change in multiplicity, as shown in Figure 24. The initial absorption is from the ground state singlet $S_0$ to the first excited singlet $S_1$. Fluorescence may appear to be exactly the reverse process of absorption, which is true on the atomic level. However, when the absorption and emission spectra of most molecules are compared, they are actually mirror images of each other. This observation implies that the geometries of the molecules in the ground state are similar to those in the excited-state. The emission spectrum is displaced toward the longer wavelength. Most of the energy is dissipated to the surroundings as heat.

A quantum of radiation is emitted in fluorescence that will be of lower energy on average than the quantum absorbed by the molecule. This emission is due to vibrational relaxation. The change in photon energy results in a shift of the fluorescence spectrum to longer wavelengths, relative to the absorption spectrum. This change in photon energy is known as a Stokes Shift ($\Delta \nu$), and these values are tabulated in Table 2. The Stokes Shift energy ($\Delta \mu$) represents the magnitude of vibrational relaxation in each system.

The fluorescence of the chlorophyll complexes was measured using a N$_2$-pumped dye laser system. All spectra showed an inverse relationship between the fluorescence intensity and the concentration of the acceptor. The intensities decreased as the concentration of the acceptor molecule increased. Figures 25-38 show the fluorescence spectra and the Stern-Volmer Plots generated. The Stern-Volmer constants are listed on Table 2.
Figure 24. Jablonski diagram.$^{20}$
Figure 25. Fluorescence spectra of Chl a with TFPH in CH$_3$CN.

Figure 26. Stern-Volmer plot of Chl a with TFPH in CH$_3$CN.
Figure 27. Fluorescence spectra of Chl $a$ with TFPH in CH$_2$Cl$_2$.

Figure 28. Stern-Volmer plot of Chl $a$ with TFPH in CH$_2$Cl$_2$. 

$y = 28.786x + 0.9429$

$R^2 = 0.9864$
Figure 29. Fluorescence spectrum of Chl a with TFPR in CH$_2$Cl$_2$.

Figure 30. Stern-Volmer plot of Chl a with TFPR in CH$_2$Cl$_2$.
Figure 31. Fluorescence spectra of Chl a with ITNF in CH$_3$CN.

Figure 32. Stern-Volmer plot of Chl a with ITNF in CH$_3$CN.
Figure 33. Fluorescence spectra of Chl $\alpha$ with ITNF in CH$_2$Cl$_2$.

Figure 34. Stern-Volmer plot of Chl $\alpha$ with ITNF in CH$_2$Cl$_2$. 

$y = 209.39x + 0.9727$

$R^2 = 0.9487$
Figure 35. Fluorescence spectra of Chl a with fluorene in CH$_2$Cl$_2$.

Figure 36. Stern-Volmer plot of Chl a with fluorene in CH$_2$Cl$_2$.
Figure 37. Fluorescence spectra of Chl a with fluorene in CH$_3$CN.

Figure 38. Stern-Volmer plot of Chl a with fluorene in CH$_3$CN.
Table 2. Stern-Volmer Constant, $K_{SV}$, for Chl a in CH$_3$CN and CH$_2$Cl$_2$ with Various Quenchers, at 25 °C.

<table>
<thead>
<tr>
<th>System</th>
<th>Solvent</th>
<th>$\lambda_{abs\ max}$ (nm)</th>
<th>$\lambda_{em\ max}$ (nm)</th>
<th>Stokes Shift (cm$^{-1}$)</th>
<th>$K_{SV}$ (mM)</th>
<th>$Q_{1/2}$ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl a only</td>
<td>CH$_3$CN</td>
<td>660</td>
<td>670</td>
<td>226.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>CH$_2$Cl$_2$</td>
<td>663</td>
<td>672</td>
<td>202.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chl a – Fluorene</td>
<td>CH$_3$CN</td>
<td>660</td>
<td>670</td>
<td>226.1</td>
<td>240</td>
<td>4.17</td>
</tr>
<tr>
<td></td>
<td>CH$_2$Cl$_2$</td>
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<td>673</td>
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<td>510</td>
<td>1.96</td>
</tr>
<tr>
<td>Chl a – TFPR</td>
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<td>669</td>
<td>180.9</td>
<td>2070</td>
<td>4.83</td>
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<tr>
<td></td>
<td>CH$_2$Cl$_2$</td>
<td>664</td>
<td>672</td>
<td>224.8</td>
<td>160</td>
<td>6.25</td>
</tr>
<tr>
<td>Chl a – TFPH</td>
<td>CH$_3$CN</td>
<td>661</td>
<td>670</td>
<td>203.2</td>
<td>8650</td>
<td>0.114</td>
</tr>
<tr>
<td></td>
<td>CH$_2$Cl$_2$</td>
<td>662</td>
<td>670</td>
<td>180.4</td>
<td>28,790</td>
<td>0.0695</td>
</tr>
<tr>
<td>Chl a – ITNF$^*$</td>
<td>CH$_3$CN</td>
<td>670</td>
<td>670</td>
<td>480</td>
<td>2.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CH$_2$Cl$_2$</td>
<td>670</td>
<td>670</td>
<td>220</td>
<td>4.50</td>
<td></td>
</tr>
<tr>
<td>Chl a – TNFM$^1$</td>
<td>CH$_3$CN</td>
<td>660</td>
<td>669</td>
<td>203.8</td>
<td>3230</td>
<td>0.310</td>
</tr>
<tr>
<td></td>
<td>CH$_2$Cl$_2$</td>
<td>660</td>
<td>670</td>
<td>226.1</td>
<td>1850</td>
<td>0.541</td>
</tr>
<tr>
<td>Chl a – TNFO$^2$</td>
<td>CH$_2$Cl$_2$</td>
<td>662</td>
<td>669</td>
<td>158.1</td>
<td>260</td>
<td>3.85</td>
</tr>
</tbody>
</table>

$^*$Stokes Shifts were not determined for ITNF system.

$^1$Refer to No. 4 in Bibliography Section.

$^2$Refer to No. 18 in Bibliography Section.
C. Excited State Lifetime Studies

The lifetime of an excited singlet state is approximately $10^{-9}$ to $10^{-7}$ s. The fluorescence decay time is of the same order of magnitude. Increasing the lifetime of an excited singlet state should increase the fraction of excited molecules which undergo intersystem crossing, compared with those that fluoresce, and therefore should decrease the quantum efficiency of fluorescence.

Preliminary measurements of the excited state lifetimes of Chl $a$ alone and with TFPR, TFPH, and fluorene have been taken in CH$_3$CN. The time response of our laser is approximately 10 ns, which implies that we cannot accurately obtain lifetimes of processes occurring faster than 10 ns. The literature value for the excited state lifetime of Chl $a$ in CH$_3$CN is 5.7 ns. Therefore, due to instrument limitations, only approximations of lifetimes are presented in this work. However, the results do give a good indication of the type of quenching mechanism occurring with each complex. The complex of Chl $a$ with fluorene and TFPH both demonstrated dynamic quenching, while the Chl $a$/TFPR complex exhibited static quenching. Figures 39-42 show these results, which are summarized in Table 3. The lifetime data are fitted to the following equation:

$$\frac{I}{I_0} = e^{\frac{t}{\tau}}$$

where $I$ is the fluorescence intensity of the complex, $I_0$ is the fluorescence intensity of the Chl $a$ in the absence of the quencher, $t$ is the decay time, and $\tau$ is the excited state lifetime.

In diffusion-controlled systems (dynamic quenching) the excited state lifetimes of the complex are much longer than the exited lifetime of Chl $a$. This is because of reduced flexibility of the Chl $a$ molecule in the bound configuration.
Figure 39. Excited State Lifetime Plots for Chl a with TFPR in CH$_3$CN$^*$

Figure 40. Excited State Lifetime Plot of Chl a with TFPH in CH$_3$CN$^*$
Figure 41. Excited State Lifetime Plot of Chl $\alpha$ in CH$_3$CN$^*$

Figure 42. Excited State Lifetime Plot of $6 \times 10^{-4}$ M Chl $\alpha$/Fluorene Complex in CH$_3$CN$^*$

$^*$Symbols represent experimental data and the curves are a fit to equation 26.
<table>
<thead>
<tr>
<th>System</th>
<th>$\tau$ (ns)</th>
<th>Quenching Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl a only</td>
<td>5.7$^4$</td>
<td></td>
</tr>
<tr>
<td>Chl a - Fluorene</td>
<td>(10)10</td>
<td>Static</td>
</tr>
<tr>
<td>Chl a - TFPR</td>
<td>(5.0)5.0</td>
<td>Static</td>
</tr>
<tr>
<td>Chl a - TFPH</td>
<td>(5.6)17</td>
<td>Dynamic</td>
</tr>
</tbody>
</table>

The values in parenthesis are for Chl a only.
D. Molecular Modeling

In Table 4, the dipole moment scales with the free energy values. The TFPR and TNFM have approximately the same calculated dipole moments, however the differences in their free energy values can be accounted for by the larger electron affinity of TNFM.

Table 5 summarizes PC Model calculations done at molecular mechanics level using MMX force field. The ΔH values are calculated with equation 25, and the Van der Waals (VDW) forces are the intermolecular interaction energies between Chl.a and the quencher. The calculated VDW forces follow the same trend as experiment except TFPR and TNFO are switched.

The listed dipole moments are for the minimized structure of the complex. Future calculations will involve simulations of stacked π-π interactions between Chl.a and the quencher molecules. In addition, calculations for entropy will be performed. The latter results will be used to compute ΔG values using the expression, ΔG = ΔH – TΔS. The calculated enthalpies cannot be compared with ΔG values since they do not measure the same properties.
Table 4. Calculated Electron Affinities of Quencher Molecules Using Gaussian W03 with B3LYP functional and 3-21G basis set.

<table>
<thead>
<tr>
<th>Quencher’s Electron Affinity (EA) eV</th>
<th>$E_{A_{\text{calc}}}^1$ (eV)</th>
<th>$E_{A_{\text{exp}}}^2$ (eV)</th>
<th>Dipole Moment (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorene</td>
<td>-0.84 (-0.68)</td>
<td>0.700</td>
<td>0.520(0)$^3$</td>
</tr>
<tr>
<td>TNF</td>
<td>2.37 (2.42)</td>
<td>2.61</td>
<td></td>
</tr>
<tr>
<td>ITNF</td>
<td>2.36 (2.40)</td>
<td>4.48</td>
<td></td>
</tr>
<tr>
<td>TFPR</td>
<td>2.51 (2.56)</td>
<td>2.56</td>
<td></td>
</tr>
<tr>
<td>TNFO</td>
<td>2.78 (2.8)</td>
<td>2.05</td>
<td>0.982</td>
</tr>
<tr>
<td>TNFM</td>
<td>3.21 (3.25)</td>
<td>2.45</td>
<td>2.54</td>
</tr>
</tbody>
</table>

$^1(E_{A_{\text{molecule}}} = E_{\text{neutral}} - E_{\text{anion}})$ Electron affinities in parenthesis are zero-point energy corrected. The usual scale factor of 0.9804 was used for the zero-point energy correction.

$^2$Refer to 21 in Bibliography.

$^3$Refer to 22 in Bibliography.

$^4$Donor-Acceptor Complex in dichloromethane at 25 °C, $\Delta G^0 = -RT\ln K_a$. 
Table 5. Calculated Interaction Energies Between Chl $\alpha$ and Acceptor Molecules, ($\pi-\pi$ Configuration) in the gas phase at 300K, using PC Model 7.5 software package with MMX Force Field

<table>
<thead>
<tr>
<th>Donor-Acceptor Complex</th>
<th>$-\Delta H_{\text{calc}}$ (kcal/mole)</th>
<th>VDW (kcal/mole)</th>
<th>Distance (Å) Mg – C$_9^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl $\alpha$ – Fluorene</td>
<td>14.2</td>
<td>-12.8</td>
<td>3.39</td>
</tr>
<tr>
<td>Chl $\alpha$ – TNFO</td>
<td>66.6</td>
<td>1.98</td>
<td>4.02</td>
</tr>
<tr>
<td>Chl $\alpha$ – TNF</td>
<td>58.3 (19.4$^t$)</td>
<td>0.790</td>
<td>4.45</td>
</tr>
<tr>
<td>Chl $\alpha$ – TFPH</td>
<td>49.5</td>
<td>6.12</td>
<td>4.42</td>
</tr>
<tr>
<td>Chl $\alpha$ – TFPR</td>
<td>60.4</td>
<td>0.470</td>
<td>4.17</td>
</tr>
<tr>
<td>Chl $\alpha$ – ITNF</td>
<td>50.8</td>
<td>2.82</td>
<td>4.36</td>
</tr>
<tr>
<td>Chl $\alpha$ – PQ</td>
<td>36.9</td>
<td>-13.6</td>
<td>3.12$^{**}$</td>
</tr>
<tr>
<td>Chl $\alpha$ – TNFM</td>
<td>59.0(5.02)$^t$</td>
<td>4.73</td>
<td>4.11</td>
</tr>
</tbody>
</table>

*Distance between Mg and 9-carbon atom. ** Distance between Mg and carbon on the aromatic ring attached to the hydrocarbon chain.
IV. CONCLUSION

The following conclusions were made, based upon the data obtained from this research:

A. Absorption measurements showed that a 1:1 complex was formed between the donor and acceptor molecules in room temperature solution, and Nash plots revealed the binding constants of these complexes. The formation of an isosbestic point for the Chl a/TFPR system provided further support of the formation of a 1:1 complex.

B. Increasing the concentration of the quencher yielded a decrease in the fluorescence intensity of the complexes. The Stern-Volmer constants ($K_{SV}$) aided in determining the strength of the quencher. The results showed that TFPR, in CH$_2$Cl$_2$, had the weakest quenching capabilities ($K_{SV} = 1.6 \times 10^2$), while TFPH, in the same solvent, appears to have the strongest quenching effect ($K_{SV} = 2.9 \times 10^4$). However, this high $K_{SV}$ value could be a result of other things occurring in the system, such as a slight overlap in the Soret region (430 nm) between the Chl $a$ and TFPH.

C. Excited state lifetime studies assisted in distinguishing the type of quenching interaction occurring between Chl $a$ and the acceptor molecule. Dynamic quenching is occurring with the Chl $a$/TFPH complex, however, the fluorescence of the Chl $a$/TFPR and Chl $a$/fluorene complexes exhibit static quenching mechanisms.
V. FUTURE WORK

A. Excited state lifetime measurements will be performed on Chl \textit{a} with ITNF in CH$_3$CN.

B. Excited state lifetime measurements will be performed on Chl \textit{a} with fluorene, TFPR, TFPH, and ITNF in CH$_2$Cl$_2$.

C. The information gained from these studies can be used as a guide when choosing other fluorescence quenchers to research.

D. Molecular modeling will continue to be used to make predictions of the efficiency of the quencher.
VI. BIBLIOGRAPHY


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