Integration of Fundamental Research and CER: The Role of Authenticity in Developing Views on the Nature of Teaching, Learning, and Doing Science

2018

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ABSTRACT

This dissertation is an integration of fundamental research and chemical education. It begins with two nonlinear spectroscopic studies of compounds important to the study of brain chemistry. In Chapter 2, we present a novel method using quantum mechanics for modelling ligand docking and the potential of nonlinear circular dichroism for elucidating the mechanism of cannabinoids docking to their receptor, a contribution to studies of varying psychological effects of cannabinoids. Considering existent challenges with measuring this phenomenon, in Chapter 3, we evaluate two-photon absorption properties of Thioflavin T (ThT) in varying glycerol/water content solutions and discuss the enhancement of nonlinear absorption due to small micelle formation. Our results represent the potential to enhance the applications of ThT for imaging Amyloid beta plaques in vitro and ex vivo and its potential application in vivo. Next, we consider the benefits of incorporating modern research into the undergraduate curriculum. In Chapter 4, we describe the integration of nonlinear optics into the physical chemistry laboratory in a course-based undergraduate research experience and the effects on student learning and perceptions. In Chapter 5, we expand our impact to secondary students by describing the development and assessment of the Orlando Chemistry Training, Enrichment, and Tutoring (OCTET) camp and its success in conveying chemistry concepts and inspiring students to pursue chemistry. In Chapter 6, we combine the successes of the previous two studies and incorporate a research component into OCTET. We study the effect on participants’ views about science and show the impact on their practical knowledge about doing science. Finally, in Chapter 6, we extend the implementation of authentic learning to the classroom, present the implementation of active learning in physical chemistry, and describe students’ perceptions. The results presented in this dissertation
demonstrate successful integration of fundamental research into education and the powerful impact on all parties.
This dissertation is dedicated to the generations of women in my life who have instilled in me the values of hard work, commitment, and above all, passion. It is the influence of these women that provided me the motivation to persist in this endeavor.
ACKNOWLEDGMENTS

The substance of this dissertation is a representation of the support and guidance of many people. I would like to extend my deepest gratitude for them since without them, this document would never have been completed.

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I would also like to mention my high school chemistry teacher, Mrs. Joyce Miller, who encouraged me to do science research and then convinced me to study fluorescence (her idea) rather than the effect of herbs on the behaviors of birds (my idea) and my undergraduate advisor, Dr. Clayton Baum who supported my undergraduate career and research.

Finally, I will try to express the gratitude I have for my family during this process. In particular, my parents have always demonstrated unconditional love and support for me and all of my endeavors. I recognize that I would not be defending my dissertation now had they not cheered me on at science fair and every other extracurricular event I dragged them to as a child. Your cheers gave me the confidence to even attempt this and your high expectations gave me the confidence to finish. This brings me to the two most important people in my life now – Kevin and Nora. Kevin – I hope you know how much I love and appreciate you for providing me the financial, emotional, and logistical support to do this. I know you knew how important it was to me and I know you have made sacrifices to help me do it. Our daughter, Nora, is still the best thing that I have ever done and the best thing we have ever done together. This Ph.D. will be as much yours as mine.
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<td>American Chemical Society</td>
</tr>
<tr>
<td>ACT</td>
<td>American College Test</td>
</tr>
<tr>
<td>AP</td>
<td>Advanced placement</td>
</tr>
<tr>
<td>ARE</td>
<td>Authentic research experience</td>
</tr>
<tr>
<td>B3LYP</td>
<td>Becke 3-parameter Lee-Yang-Parr hybrid functional</td>
</tr>
<tr>
<td>BIOTEC</td>
<td>Biology Integrated Orlando Training and Enrichment Camp</td>
</tr>
<tr>
<td>CAM-B3LYP</td>
<td>Coulomb attenuating method B3LYP</td>
</tr>
<tr>
<td>c</td>
<td>Speed of light ($3 \times 10^8$ m/s)</td>
</tr>
<tr>
<td>CARS</td>
<td>Coherent Anti-Stokes Raman Scattering</td>
</tr>
<tr>
<td>CB1</td>
<td>Cannabinoid receptor 1</td>
</tr>
<tr>
<td>CBD</td>
<td>Cannabidiol</td>
</tr>
<tr>
<td>CBN</td>
<td>Cannabinol</td>
</tr>
<tr>
<td>CD</td>
<td>Circular dichroism</td>
</tr>
<tr>
<td>CER</td>
<td>Chemical education research</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>CT</td>
<td>Charge transfer</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>CURE</td>
<td>Course-based undergraduate research experience</td>
</tr>
<tr>
<td>DFT</td>
<td>Density functional theory</td>
</tr>
<tr>
<td>DFWM</td>
<td>Degenerate four-wave mixing</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic light scattering</td>
</tr>
<tr>
<td>$e$</td>
<td>Electron charge ($1.602 \times 10^{-19}$ Coulombs)</td>
</tr>
<tr>
<td>ECD</td>
<td>Electronic circular dichroism</td>
</tr>
<tr>
<td>$f$</td>
<td>Oscillator strength</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>$fs$</td>
<td>Femtosecond ($10^{-15}$ s)</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full width at half maximum</td>
</tr>
<tr>
<td>FROG</td>
<td>Frequency-resolved optical gaiting</td>
</tr>
<tr>
<td>$g(n\omega, \omega_0f, \Gamma)$</td>
<td>Normalized lineshape for an $n$-photon excitation</td>
</tr>
<tr>
<td>GM</td>
<td>Goppert-Mayer unit for the TPA and TPCD cross section ($1 \times 10^{-50}$ cm$^4$ s photon$^{-1}$ molecule$^{-1}$)</td>
</tr>
<tr>
<td>GPCR</td>
<td>G-protein coupled receptor</td>
</tr>
<tr>
<td>$h$</td>
<td>Planck’s constant ($6.626 \times 10^{-34}$ J/s)</td>
</tr>
<tr>
<td>He-Ne</td>
<td>Helium-Neon laser</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest occupied molecular orbital</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz (unit of frequency)</td>
</tr>
<tr>
<td>I\textsubscript{∥}</td>
<td>Parallel polarized incident light</td>
</tr>
<tr>
<td>I\textsubscript{⊥}</td>
<td>Perpendicular polarized incident light</td>
</tr>
<tr>
<td>IB</td>
<td>International Baccalaureate</td>
</tr>
<tr>
<td>ICT</td>
<td>Intramolecular charge transfer</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
</tr>
<tr>
<td>kJ</td>
<td>Kilojoules</td>
</tr>
<tr>
<td>LUMO</td>
<td>Lowest occupied molecular orbital</td>
</tr>
<tr>
<td>m</td>
<td>Mass of an electron ((9.109\times10^{-31} \text{ kg}))</td>
</tr>
<tr>
<td>M</td>
<td>Molar (unit of concentration)</td>
</tr>
<tr>
<td>M</td>
<td>Two-photon magnetic transition dipole matrix element</td>
</tr>
<tr>
<td>MCD</td>
<td>Magnetic circular dichroism</td>
</tr>
<tr>
<td>n\textsubscript{0}</td>
<td>Linear refractive index</td>
</tr>
<tr>
<td>n\textsubscript{2}</td>
<td>Nonlinear refractive index</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer ((10^{-9} \text{ m}))</td>
</tr>
<tr>
<td>NOS</td>
<td>Nature of science</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>NT</td>
<td>Normalized transmittance</td>
</tr>
<tr>
<td>OCTET</td>
<td>Orlando Chemistry Training, Enrichment, and Tutoring Camp</td>
</tr>
<tr>
<td>OPA</td>
<td>One-photon absorption</td>
</tr>
<tr>
<td>OPG</td>
<td>Optical parametric generator</td>
</tr>
<tr>
<td>ORD</td>
<td>Optical rotatory dispersion</td>
</tr>
<tr>
<td>P</td>
<td>Two-photon electric transition dipole moment</td>
</tr>
<tr>
<td>PISA</td>
<td>Programme for International Student Assessment</td>
</tr>
<tr>
<td>PCM</td>
<td>Polarizable continuum model</td>
</tr>
<tr>
<td>PDB</td>
<td>Protein data bank</td>
</tr>
<tr>
<td>PhYSICOS</td>
<td>Physics Youth Scholars Integrate Camp for Orlando Scientists</td>
</tr>
<tr>
<td>$q^d$</td>
<td>Dynamic component of the solvent reaction field</td>
</tr>
<tr>
<td>$q'$</td>
<td>Delayed component of the solvent reaction field</td>
</tr>
<tr>
<td>$R_{0f}^{ECD}$</td>
<td>Rotatory strength</td>
</tr>
<tr>
<td>RAILLS</td>
<td>Reversed and informal learning scheme and space</td>
</tr>
<tr>
<td>RCM</td>
<td>Receptor cutting method</td>
</tr>
<tr>
<td>REU</td>
<td>Research experience for undergraduates</td>
</tr>
<tr>
<td>ROA</td>
<td>Raman optical activity</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
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<td>--------</td>
<td>------------------------------------------------</td>
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<tr>
<td>$S_0$</td>
<td>Ground state, singlet</td>
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<td>$S_1$</td>
<td>First excited state, singlet</td>
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<td>$S_2$</td>
<td>Second excited state, singlet</td>
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<tr>
<td>$S_n$</td>
<td>$n^{th}$ excited state, singlet</td>
</tr>
<tr>
<td>$S_{0f}^{ij}$</td>
<td>Two-photon transition matrix elements</td>
</tr>
<tr>
<td>SAS</td>
<td>Solvent accessible surface</td>
</tr>
<tr>
<td>SCALE-UP</td>
<td>Student-centered active learning environment with upside-down pedagogies</td>
</tr>
<tr>
<td>SES</td>
<td>Solvent excluded surface</td>
</tr>
<tr>
<td>SFG</td>
<td>Sum-frequency generation</td>
</tr>
<tr>
<td>SHG</td>
<td>Second harmonic generation</td>
</tr>
<tr>
<td>SCRF</td>
<td>Self-consistent reaction field</td>
</tr>
<tr>
<td>STEM</td>
<td>Science, Technology, Engineering, and Mathematics</td>
</tr>
<tr>
<td>T</td>
<td>Two-photon electric transition quadrupole moment</td>
</tr>
<tr>
<td>TPA</td>
<td>Two-photon absorption</td>
</tr>
<tr>
<td>TPIF</td>
<td>Two-photon induced fluorescence</td>
</tr>
<tr>
<td>TD-DFT</td>
<td>Time dependent DFT</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
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<tr>
<td>THC</td>
<td>(-)-trans(\Delta^9)-tetrahydrocannabinol</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>ThT</td>
<td>Thioflavin T</td>
</tr>
<tr>
<td>URE</td>
<td>Undergraduate research experience</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
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<tr>
<td>VCD</td>
<td>Vibrational circular dichroism</td>
</tr>
<tr>
<td>VNOS</td>
<td>Views on the Nature of Science questionnaire</td>
</tr>
<tr>
<td>(w_0)</td>
<td>Beam radius at the focal plane</td>
</tr>
<tr>
<td>XCF</td>
<td>Exchange correlation functional</td>
</tr>
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<td>Rayleigh range</td>
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<td>Transition from a bonding (\pi) orbital to an antibonding (\pi) orbital ((\pi^*))</td>
</tr>
<tr>
<td>(\sigma \rightarrow \sigma^*)</td>
<td>Transition from a bonding (\sigma) orbital to an antibonding (\sigma) orbital ((\sigma^*))</td>
</tr>
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<td>(\alpha)</td>
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<tr>
<td>(\beta)</td>
<td>Two-photon absorption coefficient</td>
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<tr>
<td>(\gamma)</td>
<td>Three-photon absorption coefficient</td>
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<tr>
<td>(\Gamma)</td>
<td>Empirical linewidth parameter</td>
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<tr>
<td>(\delta^{TPA})</td>
<td>Two-photon absorption cross section</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
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<td>--------</td>
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</tr>
<tr>
<td>$\delta^{{TPA}}_L$</td>
<td>Two-photon absorption cross section for left circularly polarized light</td>
</tr>
<tr>
<td>$\delta^{{TPA}}_R$</td>
<td>Two-photon absorption cross section for right circularly polarized light</td>
</tr>
<tr>
<td>$\Delta\delta^{{TPCD}}$</td>
<td>Two-photon circular dichroism</td>
</tr>
<tr>
<td>$\Delta f$</td>
<td>Lippert’s function</td>
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<td>$\Delta\mu$</td>
<td>Difference between ground and excited state dipole moment</td>
</tr>
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<td>$\varepsilon$</td>
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<td>Dielectric constant</td>
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<td>Ellipticity</td>
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<tr>
<td>$\lambda_{\text{max}}$</td>
<td>Maximum absorption wavelength</td>
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<td>$\mu_{gf}$</td>
<td>Transition moment (ground to excited state)</td>
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<tr>
<td>$\mu_{E^+}$</td>
<td>Excited state permanent dipole moment</td>
</tr>
<tr>
<td>$\mu_G$</td>
<td>Ground state permanent dipole moment</td>
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<tr>
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<td>Absorption frequency</td>
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<tr>
<td>$\tilde{\nu}_F$</td>
<td>Emission frequency</td>
</tr>
<tr>
<td>$\Phi_f$</td>
<td>Fluorescence quantum efficiency</td>
</tr>
<tr>
<td>$\chi^{(n)}$</td>
<td>$n^{th}$ order nonlinear susceptibility tensor</td>
</tr>
<tr>
<td>$\Psi_0$</td>
<td>Ground state wavefunction</td>
</tr>
</tbody>
</table>
$\Psi_f$ \hspace{1cm} Excited (final) state wavefunction

$\omega$ \hspace{1cm} Circular frequency
CHAPTER 1 : INTRODUCTION

The investigation of the interaction between light and matter has been of interest to scientists for hundreds of years.\textsuperscript{1-2} Research that has its foundation in light-matter interaction has led to the development of culturally and societally pivotal technologies from the internet to imaging of biological tissue.\textsuperscript{3} Considering the relevance of this field, chemistry and physics educators emphasize the basic concepts of light-matter interaction in their courses.

The field primarily responsible for fundamental research in this area is that of photonics in which the generation, detection, and manipulation of photons is the focus of study.\textsuperscript{4-5} The advent of this discipline was accompanied by a myriad of spectroscopic techniques for studying these phenomena. Before the introduction of lasers in 1960, linear spectroscopic techniques, those taking advantage of single photon absorption, were the most commonly used in photonics. However, laser systems made it possible to study nonlinear phenomena, those in which the optical response depends nonlinearly on the incident irradiation. The lower energies used for excitation in multiphoton processes present many advantages over those energies used for single photon processes. Notably, reduced absorption in the excitation region, scattering, and photodamage to the sample in addition to increased penetration depth and spatial resolution.\textsuperscript{5-6} These benefits have led to the development of several new applications based on multiphoton processes including multiphoton bioimaging and photodynamic therapy,\textsuperscript{1, 3} optical data storage, microfabrication,\textsuperscript{7} microscopy,\textsuperscript{8} and optical limiting,\textsuperscript{9} among others. Exciting developments in photonics are only possible with continual fundamental research that furthers the development of new techniques for characterizing molecular systems.

Of particular biological importance are optically active molecules. Chirality is a geometric property of molecules whose mirror images are non-superimposable. These molecules alone
exhibit optical activity, or the ability to rotate the plane of polarization of light. Investigations in this field have implications in biological processes due to the chiral nature of biological systems. The most commonly explored phenomenon associated with optically active biomolecules such as proteins is electronic circular dichroism (ECD), the difference in absorption between left and right circularly polarized light. In order to overcome the existent challenges associated with linear absorption spectroscopic techniques mentioned previously, several nonlinear phenomena have been investigated. These include second harmonic generation, sum frequency generation, nonlinear optical activity, and multiphoton optical rotation. However, none of these phenomena are true nonlinear counterparts to the polarization dependent ECD.

In the 1970s, Tinoco and Power, theoretically predicted two-photon circular dichroism (TPCD), the true nonlinear counterpart to ECD. TPCD is the difference in two photon absorption between left and right circularly polarized light, the first experimental proof of which was presented in 1994 when Gunde and Richardson used fluorescence detection. Ten years later, TPCD was implemented computationally by Rizzo and co-workers. However, it was not until 2008 with the advent of the Double L-scan technique that TPCD could be experimentally measured reliably and reproducibly.

The Double L-scan technique, developed by Hernández and co-workers, allows the simultaneous measurement of polarization dependent two-photon absorption of left and right circularly polarized light. Several studies have been completed to show not only the excellent agreement between the theoretical predictions and experimental measurements, but the structure-property relationships of molecules with both helical and axial chirality. More recently, TPCD has been shown to be far more sensitive to differences between α-strand, β-helix, and Random coil
conformations of amino acid residues. Based on these recent developments, this dissertation begins with a conformational study of cannabinoids, a class of compounds found in marijuana. However, in order to understand the studies presented in each chapter, the fundamentals of the interaction between light and matter must first be described.

1.1 Fundamentals of Linear and Nonlinear Absorption and Emission

1.1.1. One-photon absorption

The simplest and most common form of light-matter interaction is one-photon absorption (OPA). This process involves an electronic transition between an initial (S₀) and final (Sₙ) excited state as shown in Figure 1.1. It is important to note that these transitions take place within the Franck-Condon principle, which refers to the transition between vibrational levels within each electronic state. A relaxation process known as *internal conversion* (represented by orange arrows in the figure) occurs very quickly (10⁻¹² s or less) so that the molecule ends up in the lowest vibrational level of the electronic state.

When relatively weak electromagnetic radiation arrives at a molecule, the molecule’s electron cloud is perturbed by the electric field. A resonance condition is established when the oscillation of the electric field matches one of the natural oscillation frequencies of the electron cloud. In this case, the incident photon is absorbed and a transition dipole moment (µ_ij) is established, promoting the molecule to an excited electronic state. The transition dipole moment is an oscillating dipole moment, the value of which gives the probability of the transition occurring.
Figure 1.1. Simplified Jablonski diagram. One- and two-photon absorption (OPA and TPA, respectively), which occur between the ground and excited states, depending on selection rules, and fluorescence and upconversion, which occur from the same excited state following internal conversion (orange arrows).

The relationship between the theoretical parameter, $(\mu_{ij})$ and the molar absorptivity ($\varepsilon$), expressed in Equation 1.1, allows access to the estimation of the probability of the transition.

$$\int \varepsilon d\tilde{\nu} = (2.512 \times 10^{19} \text{ l mol}^{-1} \text{ cm}^{-3}) \frac{\tilde{\nu}}{e^2} |\mu_{ij}|^2$$  \hspace{1cm} 1.1

Where $\tilde{\nu}$ is the frequency of light expressed in wavenumbers, $e$ is the charge of an electron, and $|\mu_{ij}|^2$ corresponds to the probability of transition.

In the case that this property is measured when a polar molecule of interest is dissolved in some solvent, the dielectric properties of the solvent can have a significant impact on the charge distribution in the ground, but especially, the excited states. This is critical because the energy gap between the ground and excited states is altered. This change can be described using the Lippert equation. \cite{31}
\[ \tilde{\nu}_A - \tilde{\nu}_F = \frac{2}{\hbar c} \left( \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \right) \left( \mu_E - \mu_G \right)^2 \frac{1}{a^3} \] 

where \( \tilde{\nu}_A \) and \( \tilde{\nu}_F \) are the frequencies of absorption and emission expressed in wavenumbers. The difference between these two values corresponds to the energy gap between the ground and excited states. Additionally, \( h \) is Planck’s constant, \( c \) is the speed of light in vacuum, \( \varepsilon \) is the dielectric constant of the solvent, \( n \) is refractive index, and \( a \) is the radius of the cavity in which the molecule is contained. Finally, \( \mu_E \) and \( \mu_G \) are the permanent dipole moments of the excited and ground states, respectively.

After absorption, there are several paths for the molecule to relax back to the ground electronic state. Of particular interest in this work is the radiative process known as fluorescence, depicted in Figure 1.1. In this case, a photon is emitted from the lowest vibrational state of the excited electronic state, resulting in luminescence.

In addition to the requirement that the energy of the incident radiation matches that of the energy gap between two electronic states, there are several rules derived from quantum mechanics that differentiate between “allowed” and “forbidden” transitions. Selection rules are derived from the transition dipole moment integral:

\[ \mu_{ij} = \int \Psi_i^* \mu \Psi_j d\tau \]

where \( \Psi_i \) and \( \Psi_j \) are the wavefunctions for the initial and final states involved in the transition. When the value of the transition dipole moment, \( \mu_{ij} \) reaches zero, the transition is forbidden and will not appear in the experimental spectrum. When \( \mu_{ij} \) does not disappear, the transition is allowed and the magnitude is given by Equation 1.1. Of course, as with most rules there are
exceptions. Even in the case that $\mu_{ij}$ is finite, it could be of such small magnitude as to not appear experimentally. On the other hand, if the selection rule is relaxed, a forbidden transition may be observed experimentally.

Two types of selection rules determine whether a transition will be allowed. First, spin selection rules assume that neither the electric dipole nor the quadrupole moments have a significant impact on spin. Consequently, if the spin of the two states involved in the transition are different, Equation 1.3 goes to zero and the transition is forbidden. The selection rule then is $\Delta S=0$, that both states must have the same spin for the transition to be allowed. Second, spatial symmetry selection rules refer to the requirement for both states to have equal parity. This is because the transition dipole moment can vanish with integration over the space coordinates of the electrons in the case that the integrand is not symmetric or containing symmetric components.\textsuperscript{32}

The preceding discussion of linear absorption is important to understanding the fundamentals of spectroscopy, but linear spectroscopy can present some challenges in various applications, such as scattering and absorption in the excitation region, which can be overcome by taking advantage of nonlinear absorption.

1.1.2. Nonlinear absorption

In 1931, Maria Göppert-Mayer predicted the phenomenon of two-photon absorption in her doctoral dissertation.\textsuperscript{33} The advent of lasers in 1960 allowed this phenomenon to be verified experimentally when two-photon induced fluorescence was measured in a europium-doped crystal.\textsuperscript{34} Two-photon absorption is the simultaneous absorption of two photons (Figure 1.1), which results in an excited state whose energy difference from the ground state is twice the energy of each individual photon. In particular, as Göppert-Mayer explicitly predicted, the process is made
possible by the existence of virtual states, which are located between real electronic states. This is depicted in Figure 1.1 as a dotted line.

As in the case of linear absorption, when an electromagnetic field interacts with a molecule, the electron cloud is perturbed. However, in the case that the incident irradiation is intense, such as a laser, the response deviates from linearity and the electronic oscillations are better described by an anharmonic oscillator. In order to describe this response, the polarization of the material can be expressed in terms of a Taylor series as a function of the electric field:

\[ P = \chi^{(1)} \cdot \vec{E} + \chi^{(2)} \cdot \vec{E}^2 + \chi^{(3)} \cdot \vec{E}^3 + \ldots + \chi^{(n)} \cdot \vec{E}^n \]  

where \( \chi^{(n)} \) are the susceptibility tensor quantities that describe the polarization of the medium. The first order, \( \chi^{(1)} \), describes the linear polarization and the second order, \( \chi^{(2)} \), describes the first nonlinear polarization. However, it is the third order susceptibility, \( \chi^{(3)} \), that refers to two-photon absorption and thus, will be the focus of this discussion. Specifically, the real part of the \( \chi^{(3)} \) tensor is related to the nonlinear refractive index of the medium, while the imaginary part is related to the two-photon absorption. For the former:

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

and

\[ \text{Re} \chi^{(3)} = \frac{4n_0^2 \varepsilon_0}{3} n_2 \]  

where \( n_0 \) is the linear refractive index of the material and \( \varepsilon_0 \) is the vacuum permittivity.

For the latter, the attenuation of the beam of light passing through the medium is given by:③⁵
\[ \frac{dI(z)}{dz} = -\alpha I - \beta I^2 - \gamma I^3 - \eta I^4 - \ldots \] 1.7

where \( I(z) \) is the incident irradiance and \( \alpha, \beta, \gamma, \) and \( \eta \) are the one-, two-, three-, and four-photon coefficients of the medium, respectively. While the imaginary part of the higher susceptibility terms are related to higher-order multiphoton absorption processes, the work presented in this dissertation is focused on two-photon absorption and, thus, the relationship between \( \text{Im}\chi^3 \) and \( \beta \),

\[ \text{Im}\chi^3 = \frac{n_0^2 \varepsilon_0 c^2}{\omega} - \beta \] 1.8

where \( c \) is the speed of light in vacuum and \( \omega \) is the frequency of light.

Connection of the real and imaginary parts of the susceptibility tensors can facilitate the calculation of the refractive index profile of the medium from its measured spectra and vice versa. This relationship for the \( \chi^3 \) tensor is described using the Kramer-Kronig (KK) relations:\(^36\)

\[ \text{Re}\{\chi^3(\omega)\} = \frac{2\omega'}{\pi} \frac{1}{\phi} \int_0^\infty \frac{\omega \text{Im}\{\chi^3(\omega)\}}{\omega^2 - \omega'^2} \, d\omega \] 1.9

and

\[ \text{Im}\{\chi^3(\omega)\} = -\frac{2\omega'}{\pi} \frac{1}{\phi} \int_0^\infty \frac{\omega \text{Re}\{\chi^3(\omega)\}}{\omega^2 - \omega'^2} \, d\omega \] 1.10

where \( \phi \) indicates that the integral should be evaluated using the Cauchy principle value.\(^37\)

TPA is also commonly expressed in terms of the cross-section, \( \sigma_{\text{TPA}} \), which is expressed as:\(^38\)

---

8
\[ \sigma_2 = \frac{\beta \hbar \omega}{N_0} \]  \hspace{1cm} 1.11

where \( \omega \) is the excitation frequency and \( N_0 \) is the number of molecules that can be excited with two photons.

The variation in selection rules for TPA from those for OPA arises from the presence of a virtual state involved in the two-photon transition. Thus, the selection rules for a centrosymmetric molecule are opposite for TPA: transitions may only occur between two states with equal parity. This changes the states that can be reached using two photons to states that could not be accessed using one photon.\(^{39-40}\)

The properties described up to this point could be present in any molecular system, but optical activity is a property associated only with molecules that exhibit chirality. The next subsections will focus on the counterparts of the aforementioned properties for chiral molecules.

### 1.1.3. Absorption properties of chiral molecules

As mentioned previously, chiral molecules rotate the plane polarization of light. It is this property that makes them unique and that can give rise to the study of interesting and important chemical properties. As such, it is important to review the fundamentals of polarized light so that the effect of chiral molecules on that polarization may be effectively discussed.

When the electric component of an electromagnetic wave is constant in time, the wave is linearly polarized. In order to analyze any other polarization states, it is important to consider two linearly polarized orthogonal waves propagating in time along the z-axis.\(^{41}\) The electric vector of these two waves is described by:
\[ E_x(z, t) = \hat{E}_{0x} \cos(kz - \omega t) \]  

and

\[ E_y(z, t) = \hat{E}_{0y} \cos(kz - \omega t + \varphi) \]

where \( k, \omega, \) and \( \varphi \) are the light wave vector, frequency of light, and relative phase difference, respectively and \( E_{0x} \) and \( E_{0y} \) are the amplitude of the electric field in the x and y direction. The combination of these two components is given by:

\[ \vec{E}(z, t) = \vec{E}_x(z, t) + \vec{E}_y(z, t) \]

or

\[ \vec{E} = (\hat{i}E_{0x} + \hat{j}E_{0y}) \cos(kz - \omega t) \]

when the two waves are in phase and \( \varphi = \pm n\pi \) and \( n = 0, 1, 2, 3... \) The wave that results from this combination has a constant amplitude, \((\hat{i}E_{0x} + \hat{j}E_{0y})\), so is said to be linearly polarized.

The combination of two linearly polarized waves does not necessarily result in a linearly polarized wave. For example, if the two waves have identical amplitude, \( E_{0x} = E_{0y} = E_0 \), but a quarter wave phase difference, \( \varphi = (-\pi/2) + (2m\pi) \) where \( m = 0, \pm1, \pm2... \) the behavior of \( \vec{E}_y(z, t) \) changes from cosinusoidal to sinusoidal and the combination of the waves is described by:

\[ \vec{E} = E_0[\hat{i}\cos(kz - \omega t) + \hat{j}\sin(kz - \omega t)] \]

which shows a constant amplitude, but a direction that rotates continuously in time around the z-axis. This wave is said to be circularly polarized. Since the terms in Equation 1.16 are added
together, this wave would be observed as rotating in a clockwise direction and considered right circularly polarized. On the other hand, if the two terms were subtracted, if the phase difference was \( \varphi = (\pi/2) + (2m\pi) \), the wave would be observed as rotating counterclockwise and considered left circularly polarized light.\(^{41}\)

**1.1.4. Electronic circular dichroism**

When linearly polarized light travels through a chiral medium, the circularly polarized components exhibit two responses. First is a difference in refractive index \( \eta_L \neq \eta_R \), termed circular birefringence which induces the rotation of the polarization of plane-polarized light. Second, is a difference in absorption coefficient, circular dichroism, which is observed in the preferential absorption of one-handed circularly polarized light over the other.

The preferential absorption of one-handed circularly polarized light over the other results in unequal decreases in the amplitudes of the electric field vectors. The resulting light, then, is elliptically polarized.\(^{42}\) The degree of ellipticity, \( \theta \), is calculated from the arctangent of the lower to the higher elliptical axis (Figure 1.2) and is related to absorption as follows:\(^{43}\)

\[
\theta = 32.988\Delta A
\]

where \( \Delta A = A_L - A_R \) is the circular dichroism since \( A_L \) and \( A_R \) are the absorbance of left and right circularly polarized light. Combining Equation 1.17 with Beer’s law\(^{43}\) yields an expression for the molar ellipticity:

\[
[\theta] = \frac{\theta}{c \cdot l} = \frac{32.988\Delta A}{c \cdot l} = 32.988\Delta \epsilon
\]
where $c$ is the molar concentration, $l$ is the path length in centimeters, and $\Delta \varepsilon$ is the circular dichroism in terms of molar absorptivity. Experimental circular dichroism is commonly reported as either $[\theta]$ or $\Delta \varepsilon$.

**Figure 1.2.** The addition of the electric components with different amplitudes (blue: right circularly polarized and red: left circularly polarized) after interaction with a chiral system. The resultant wave is elliptically polarized (purple). This schematic represents a complete oscillation ($2\pi$) of the electric field vector from the observer’s perspective.

The experimental measurement of circular dichroism is widely performed using a technique known as Electronic Circular Dichroism (ECD). ECD is very useful for assigning absolute configurations of chiral molecules and macromolecules and is very sensitive to conformational and structural changes of molecular and macromolecular systems. Thus, ECD has become the standard technique for the study of optically active biomolecules. However, for electronic transitions that occur via excitation below 200 nm, ECD presents challenges since there is an increase in the linear absorption of common solvents and buffers in this region as well as an increase in scattering at short wavelengths. Several experimental methods have been proposed to overcome these challenges including synchrotron radiation circular dichroism (SRCD),\textsuperscript{45}
magnetic circular dichroism (MCD),\textsuperscript{46} vibrational circular dichroism (VCD),\textsuperscript{47} and Raman optical activity (ROA).\textsuperscript{48} Unfortunately, none of these methods has successfully allowed access to the UV region where ECD is hindered. Recently, however, a polarization-dependent experimental technique based on Two-Photon Circular Dichroism (TPCD) has demonstrated true potential for gaining access to this region.

\subsection*{1.1.5. Nonlinear circular dichroism}

TPCD is the true nonlinear counterpart of ECD as depicted in Figure 1.3. It is defined as the difference between TPA cross-section of left and right circularly polarized light:\textsuperscript{20-21}

\[ \Delta \delta^{TPCD} (\lambda) = \Delta \delta_L^{TPA} (\lambda) - \Delta \delta_R^{TPA} (\lambda) \] \hspace{1cm} 1.19

TPCD combines the advantages of ECD and TPA. Namely, it is only exhibited by chiral systems, so enantiomers can be distinguished and it presents all the advantages described previously for TPA. Hence, it has become an excellent complimentary technique for ECD.\textsuperscript{49}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure13.png}
\caption{Comparative representation of one-photon absorption (OPA), electronic circular dichroism (ECD), two-photon absorption (TPA), and two-photon circular dichroism (TPCD).}
\end{figure}

1.2 Theoretical Framework for the Calculation of Linear and Nonlinear Spectra

The integration of computational and experimental physical chemistry has increased in the past thirty years since the computational costs associated with the prediction of a myriad of
properties has reduced in the same time. The study of various properties can be achieved by taking advantage of the synergy between these two approaches. Some relevant examples of studies that use both theoretical and experimental chemistry to predict, observe, and analyze properties of molecules include the recent investigations of the structure-property relationship of the TPCD of molecules with axial and helical chirality.

For the prediction of the optical properties presented in this work, Time-Dependent Density Functional Theory (TD-DFT) is the most commonly used method. It is important to consider the best combination of functional and basis set to most accurately describe the electronic structure of the molecule, thus yielding the best prediction of optical properties. In this work, the Becke’s Three-Parameter Yee, Lang, and Parr (B3LYP) exchange correlation functional is used for molecules with little intramolecular charge transfer (ICT). However, this method underestimates excitation energies for molecules with significant ICT, so the Coulomb Attenuated Method (CAM) B3LYP is used to correct for this deficiency.

The equations used in TD-DFT calculations of OPA, ECD, TPA, and TPCD spectra from molecular parameters are presented next.

1.2.1. One-photon absorption

OPA spectra are calculated from \( f_{0f} \), and reported in molar absorptivity \( (\varepsilon) \):

\[
\varepsilon^{OPA}(\omega) = \frac{4\pi^2\omega N_A}{3 \times 1000 \times \ln(10)(4\pi\varepsilon_0)\hbar c_0} \times \frac{3}{2\hbar} \sum_f g(\omega, \omega_{0f}, \Gamma) \frac{f_{0f}}{\omega_{0f}}
\]

\[
\varepsilon^{OPA}(\omega) \approx 1.05495 \times 10^3 \times \omega \sum_f g(\omega, \omega_{0f}, \Gamma) \frac{f_{0f}}{\omega_{0f}}
\]
where $f_{0f}$ is the oscillator strength which depends on the transition dipole moment, $\mu_{0f}$ as follows:

$$f_{0f} = \frac{2m_e \omega_0 \mu_{0f}^2}{3\hbar e^2}$$

1.22

Further, $\omega = 2\pi c_0 / \lambda$ is the circular frequency of the incident light where $c_0$ is the speed of light in vacuo, $N_A$ is Avogadro’s number, and $g(\omega, \omega_{0f}, \Gamma)$ is the line-broadening function centered at $\omega = \omega_{0f}$ with a full width half-maximum (FWHM), $\Gamma$. The latter is described either by a Lorentzian (Equation 1.23) or Gaussian (Equation 1.24) function:

$$g_L(\omega, \omega_{0f}, \Gamma) = \frac{1}{\pi} \frac{\Gamma}{(\omega_{0f} - n\omega)^2 + \Gamma^2}$$

1.23

$$g_G(\omega, \omega_{0f}, \Gamma) = \frac{1}{\Gamma \sqrt{2\pi}} e^{-\frac{(\omega_{0f} - n\omega)^2}{2\Gamma^2}}$$

1.24

The units of $\varepsilon^{OPA}(\omega)$ are L.mol$^{-1}$.cm$^{-1}$ when obtained from Equation 1.21.

1.2.2. Nonlinear absorption

Theoretical TPA spectra are obtained from:

$$\delta^{TPA}(\omega) = \frac{1}{30} \frac{(2\pi)^3 \omega^2}{c_0^2 (4\pi \varepsilon_0)^2} \times \sum_f g(2\omega, \omega_{0f}, \Gamma) \cdot \delta_{0f}^{TPA}(\omega_{0f})$$

1.25

$$\delta^{TPA}(\omega) \approx 8.35150 \times 10^{-4} \times \omega^2 \sum_f g(2\omega, \omega_{0f}, \Gamma) \cdot \delta_{0f}^{TPA}(\omega_{0f})$$

1.26

here $\delta_{0f}^{TPA}(\omega_{0f})$ is the orientationally averaged two-photon probability for the degenerate case and is defined in terms of the molecular parameters $A_1$ and $A_2$. 
\[ \delta_{0f}^{TPA}(\omega_0) = F \times A_1(\omega_0) + (G + H) \times A_2(\omega_0) \quad 1.27 \]

\[ A_1(\omega_0) = \sum_{\rho\sigma} S_{\rho\rho}^{0f}(\omega_0) S_{\sigma\sigma}^{0f,\ast}(\omega_0) \quad 1.28 \]

\[ A_2(\omega_0) = \sum_{\rho\sigma} S_{\rho\sigma}^{0f}(\omega_0) S_{\rho\sigma}^{0f,\ast}(\omega_0) \quad 1.29 \]

where \( S_{ii}^{0f} \) refers to the two-photon transition matrix elements and \( F, G \) and \( H \) are scalars that define the polarization of the excitation. For linearly polarized light \( F = G = H = 2 \).

Theoretical TPA spectra are reported in Göppert-Mayer units (1 GM = \( 10^{-50} \) cm\(^4\)s\(\cdot\)molecule\(^{-1}\)\(\cdot\)photon\(^{-1}\)) as long as the equation elements are introduced in atomic units.

1.2.3. Electronic circular dichroism

ECD spectra are calculated from the ECD rotatory strength, \( R_{0f}^{ECD} \), and reported in molar absorptivity difference (\( \Delta \varepsilon \)),\(^{59,61-62} \)

\[ \Delta \varepsilon^{ECD}(\omega) = \frac{64\pi^2 N_A \omega}{9 \times 1000 \times \ln(10) (4\pi \epsilon_0) \hbar c^2} \sum_f g(\omega, \omega_0, \Gamma) \cdot R_{0f}^{ECD} \quad 1.30 \]

\[ \Delta \varepsilon^{ECD}(\omega) \approx 2.73719 \times 10^1 \times \omega \sum_f g(\omega, \omega_0, \Gamma) \cdot R_{0f}^{ECD} \quad 1.31 \]

where \( R_{0f}^{ECD} = \frac{3}{4} \Im(\mu_{ij} \cdot m_{ij}) \).

ECD spectra have units of mol\(^{-1}\)cm\(^{-1}\), as long as all the elements in Equation 1.31 are introduced in atomic units.

1.2.4. Nonlinear circular dichroism

TPCD spectra are calculated using the equation:\(^{61,63-64} \)

\[ \text{TPCD} \]
\[
\Delta \delta^{TPCD}(\omega) = \frac{4}{15} \frac{(2\pi)^3 \omega^2}{c_0^3 (4\pi \epsilon_0)^2} \times \sum_f g(2\omega, \omega_{0f}, \Gamma) \cdot R_0^{TPCD}(\omega_{0f})
\]

\[
\Delta \delta^{TPCD}(\omega) \approx 4.87555 \times 10^{-5} \times \omega^2 \sum_f g(2\omega, \omega_{0f}, \Gamma) \cdot R_0^{TPCD}(\omega_{0f})
\]

where \( R_0^{TPCD}(\omega_{0f}) \) was obtained from:

\[
R_0^{TPCD}(\omega_{0f}) = -b_1 B_1(\omega_{0f}) - b_2 B_2(\omega_{0f}) - b_3 B_3(\omega_{0f})
\]

where \( b_1, b_2 \) and \( b_3 \) are scalars that depend on the geometry of the experiment. In the double L-scan technique, \( b_1 = 6 \) and \( b_2 = b_3 = 2 \) since excitation occurs via absorption of two right or left circularly polarized co-linear and co-propagating photons. The molecular parameters \( B_i(\omega_{0f}) \) were obtained from:

\[
B_1^{TI}(\omega_{0f}) = \frac{1}{\omega_3} \sum_{\rho \sigma} M_{p_\rho \sigma}^{p_\rho 0f}(\omega_{0f}) P_{p_\rho \sigma}^{p_\rho 0f}(\omega_{0f})
\]

\[
B_2^{TI}(\omega_{0f}) = \frac{1}{2\omega_3} \sum_{\rho \sigma} T_\rho^{+, 0f}(\omega_{0f}) P_{p_\rho \sigma}^{p_\rho 0f}(\omega_{0f})
\]

\[
B_3^{TI}(\omega_{0f}) = \frac{1}{\omega_3} \sum_{\rho \sigma} M_{p_\rho \sigma}^{p_\rho 0f}(\omega_{0f}) P_{p_\rho \sigma}^{p_\rho 0f}(\omega_{0f})
\]

where \( P_{p_\rho \sigma}^{p_\rho 0f} \) and \( T_\rho^{+, 0f} \) refer to the electric transition dipole and quadrupole matrix elements in the velocity formulation, respectively and \( M_{p_\rho \sigma}^{p_\rho 0f} \) refers to the magnetic transition dipole matrix element. TPCD spectra obtained from Equation 1.33 are reported in Göppert-Mayer units (1 GM = 10^{-50} \text{ cm}^4 \cdot \text{s} \cdot \text{molecule}^{-1} \cdot \text{photon}^{-1}) as long as the equation elements are introduced in atomic units.
1.2.5. Solvent Models

The geometry and the electronic structure of a chemical species can be significantly influenced by its environment (i.e. the solvent). In particular, excited states of a molecule are sensitive due to the increased polarization of charges in these states. Thus, the absorption properties of the molecule are best predicted when considering the solvent. Two main categories of methods used to model solvent effect are explicit and implicit models.66-67 These are graphically compared in Figure 1.4. The former considers the interaction of discrete solvent molecules with the solute and can be modeled using quantum mechanics, molecular mechanics, or a combination of the two.

The latter method is also referred to as a continuum model since the solvent environment is treated as a polarizable continuum and is characterized by its dielectric constant, \( \varepsilon \). The solute molecule is modeled within a cavity in the continuum and is described by quantum mechanics.67 Early work using this model considered a spherical or ellipsoidal cavity in which the solute molecule resides.68 However, a more modern method, the Polarizable Continuum Method (PCM)69-70 more accurately models the solute cavity in terms of its shape.

PCM yields a molecular-shaped cavity by generating a series of imaginary interlocking spheres with Van der Waals radii with each of the solute atoms.71 Important to consider are two distinct surfaces: the solvent excluded surface (SES) which defines the cavity and the smallest distance from the solute that the solvent can reach and the solvent accessible surface (SAS) which is obtained by addition of the solvent radius to the SES.
Figure 1.4. Comparative schematic of a) explicit and b) implicit solvent models. Explicit models consider discrete solvent molecules while implicit models treat the solvent as a continuum.

Using PCM, the solvation free energy is calculated as the sum of the electrostatic contribution and the cavitation energy, both obtained from the SES, and the dispersion-repulsion contribution, calculated from the SAS. The SES is segmented into small pieces of known area, each one considered a point charge, and used to calculate the surface charges’ localization and value.

On the other hand, when electronic transitions are simulated using PCM, sudden changes in the charge distribution of the solute result in non-instantaneous relaxation of the solvent molecules. Thus, non-equilibrium contributions must be considered. In order to do this, the reaction field is separated into two components. The first is associated with the electronic relaxation and is referred to as the dynamic component ($q^d$). $q^d$ is calculated from the optical or dynamic dielectric constant ($\varepsilon_{opt}$), which is the square of the solvent’s refractive index. The second is associated with the nuclear motion of the solvent molecules and is referred to as the delayed component ($q_i$) which is calculated from the static dielectric constant ($\varepsilon_{sta}$).
1.3 Experimental Measurements of Nonlinear Absorption and Circular Dichroism

Currently, there exist three main categories of methods for determining TPA coefficients (β) of molecular systems. These include wave-mixing techniques, indirect methods, and indirect methods.73 The most popular wave-mixing techniques include Coherent Anti-Stokes Raman Scattering (CARS),74-75 Optical Kerr effect,76-77 and Degenerate Four-Wave Mixing (DFWM).78-79 Since the experimental setup of these techniques requires careful control of the temporal and spatial overlapping of the beams,73 this has limited the use of these techniques, the challenges of which are overcome by using indirect methods, which require considerably simpler experimental set-ups. For example, Two-Photon Induced Fluorescence (TPIF)80-81 is the most common indirect method. However, this method presents its own challenges including the fact that any uncertainty in the molar absorptivity of the sample could lead to an inaccurate determination of β. Additionally, TPIF is limited to use in samples that exhibit detectable fluorescence emission. Direct methods, as the name implies, do not rely on the emission properties of the molecule, rather on their nonlinear absorption directly. The simplest, most reliable technique for measuring nonlinear absorption and refractive index was developed in 1989 by Sheik-Bahae, et al.82 and is called the Z-scan.

1.3.1. The Z-scan Technique

The experimental arrangement of the Z-scan technique is presented in Figure 1.5. A laser beam with a Gaussian profile and fixed energy is focused on a sample. The sample is mounted on a translational stage that can move the sample along the propagation axis (the z-axis) through the focal region.38 The intensity distribution is at a maximum in the focal plane, thus it is in this region where nonlinear processes are induced. These nonlinear processes modify both the intensity and
phase of the beam, allowing two separate properties to be measured using the technique (i.e. nonlinear absorption and refractive index, respectively). There is a slight difference in the experimental arrangement for the measurement of each property, thus the techniques are referred to as the open- and closed-aperture z-scan, respectively and will be discussed next.

In both cases, three important experimental parameters should be specified. First, the Rayleigh range ($z_0$) is defined as the confocal parameter of the beam and, for Gaussian beams, is equal to $\frac{\pi w_0^2}{\lambda}$ where $w_0$ is the beam waist. Second, the sample thickness ($L$) must be less than $z_0$ in order to avoid changes in the beam spatial distribution in the sample cell due to nonlinear absorption or diffraction. Third, a minimum scan length of $\pm 5z_0$ from the focal point must be used in order to guarantee proper evaluation of the nonlinear property.

The open-aperture z-scan is used to measure nonlinear absorption and is performed as depicted in Figure 1.5 allowing all transmitted light to reach the detector. Far from the focal region, the diameter of the beam is too large to produce an intensity that is adequate to induce an electronic transition associated with nonlinear absorption of multiple photons and the normalized transmittance (NT) is adjusted to one. Data from a typical open-aperture z-scan curve are presented
in Figure 1.6a. As the sample translates in the z-direction, the intensity of the beam increases and when the nonlinear absorption process begins, the NT decreases until a minimum is reached at the focal point. As the sample translates away from the focal region, the diameter of the beam increases, the intensity decreases, and the NT returns to its initial value (1). The magnitude of $\beta$ is determined by fitting the NT data to the following equation:

$$\Delta T(Z) \approx 1 + \frac{\beta I_0 L_{eff}}{2\sqrt{2}} \left( \frac{1}{1 + \frac{z^2}{z_0^2}} \right)$$

where $I_0$ is the peak irradiance at the focus, $L_{eff}$ is the effective sample length (the actual sample length can be used if the sample is transparent), $z$ is the position of the sample with respect to the focal point, and $z_0$ is the Rayleigh range ($n\pi w_0^2/\lambda$).

**Figure 1.6.** Data from typical a) open-aperture and b) closed-aperture z-scan curves.

On the other hand, the closed-aperture z-scan is used to measure nonlinear refractive index ($\eta_2$) and is performed in the same manner, but by placing a circular aperture concentrically with the beam in the far field so that light propagating close to the optical axis is transmitted through it. The sample acts as either a converging or diverging lens, self-focusing ($\eta_2 > 0$) or self-defocusing.
(\eta_2 < 0) depending on the sign of \eta_2. The propagating beam either converges or diverges depending on the aforementioned effects, increasing or decreasing the amount of light that is transmitted through the iris. This change is detected as a change in the sample transmittance. Data from a typical closed-aperture z-scan experiment are presented in Figure 1.6b. At the position where self-focusing and self-defocusing processes occur, the transmittance is the same for negative and positive \eta_2 as a result of the thin lens approximation. \eta_2 is obtained using:

\[
|\eta_2| = \left(\frac{\lambda}{2\pi}\right) \left(\frac{|\Delta \Phi_0|}{I_0 L}\right) \tag{1.39}
\]

Once again, \(I_0\) is the peak irradiance at the focal point. \(|\Delta \Phi_0|\) is the absolute value of the nonlinear pulse shift and is calculated from:

\[
|\Delta \Phi_0| = \frac{\Delta T_{pv}}{0.406(1 - S)^{0.25}} \tag{1.40}
\]

where \(\Delta T_{pv}\) is the separation between the peak and valley transmittance and \(S\) is the transmittance factor which is the fraction of transmitted light that travels through the iris and usually ranges between 0.1 and 0.5.

This technique is the most versatile and reliable technique for measuring nonlinear absorption and refractive index. However, the experimental approach does not allow for the polarization dependent measurement of multiphoton absorption. The most reliable method for the measurement of this phenomenon, the double-L scan is described next.

**1.3.2. The Double L-scan Technique**

The double L-scan is a modification of the open-aperture z-scan technique and is depicted in Figure 1.7. While in the z-scan, the sample is translated along the z-axis, in the double-L scan,
the sample is fixed and the foci of two “twin beams” are synchronously translated across the sample using focusing lenses. Both beams travel through the same optical elements and path, ensuring equal fluctuations due to change in spatial profile, energy distribution, and size of the beam. Hence, any difference in signal ($\Delta NT \neq 0$) is attributed to TPCD of the sample.

Figure 1.7. Double L-scan experimental arrangement. Mirrors (M1, M2, M3); half wave plates (HWP); quarter wave plates (QWP1, QWP2); polarizer (P); beamsplitters (BS1, BS2); convergent lenses (L1, L2, L3, L4, L5); detectors (D1, D2, D3); neutral density filters (DF1, DF2, DF3); translation stages (TS1, TS2); step motors (SM1, SM2); synchronization box (SB); sample (S); control box (CB). Since the introduction of the double L-scan, several studies have reported on the structure-property relationships of the phenomenon. Pertinent to this work, however, are the recent studies on the conformational sensitivities of TPCD in amino acids. This property has been demonstrated theoretically, but experimental measurements have proven challenging due to a photochemical reaction that samples such as natural amino acids undergo when exposed to prolonged radiation in the near to far-UV range.

1.4 The Integration of Research and Education

The phenomena, theoretical approaches, and experimental techniques presented so far have wide-ranging applications in science and technology including 3D optical data storage,
microfabrication, bioimaging, photodynamic therapy, and optical limiting. They also have foundations in the undergraduate physical chemistry course that traditionally covers quantum mechanics concepts including operators and matrix elements, particle-in-a-box, simple harmonic oscillators, rigid rotor and angular momentum, and computational chemistry. However, few, if any, of the concepts discussed here or in other advanced literature are integrated into the undergraduate curriculum. Incorporated at the appropriate level, these concepts and their applications could contribute to students’ growth into professionals in the workforce.

Some suggestions to meet this goal include involving students in the practices of science and adopting evidence-based practices and replacing traditional laboratory courses with discovery-based courses, among other strategies. Active learning has become a popular term that encompasses both of these approaches. While there is no single definition, active learning emphasizes higher order thinking and the involvement of students in the process of learning rather than passively listening. In the classroom, active learning can manifest as group problem-solving, the use of personal response systems and peer instruction, or studio course designs. In the laboratory, inquiry-style experiments can encourage student engagement in the learning process. In any case, an underlying theme that drives the use of any of these strategies is the need for authenticity.

Authenticity has different meanings in different contexts. In science education, we could consider authentic experiences as activities that represent the world about which we are trying to teach. This could be conceptualized as developing authentic assessments for a course (i.e. a portfolio instead of exams) or as an immersive research experience (i.e. research experiences for undergraduates). However, typical course activities in no way represent true scientific inquiry;
there is still a heavy reliance on highly structured laboratories and traditional lectures. These practices do nothing to inspire students in the discipline or foster meaningful learning of concepts.

An added challenge for chemistry educators is the perceived abstract nature of the concepts. Physical chemistry, in particular, is associated with negative student perceptions and low expectations for success for this reason. However, the studies that reveal these negative student perceptions often involve students who have taken a traditional lecture or laboratory course. On the other hand, incorporation of active learning has proven to increase positive perceptions of physical chemistry and authentic research experiences have demonstrated the ability to increase positive perceptions of the practice of science.

Contemplating the implications of the current goals for STEM education and the argument that meaningful learning occurs within authentic contexts, the goal of the remainder of the work presented in this dissertation was transformed. Our attention turned to the development, implementation, and evaluation of authentic research experiences and classroom teaching strategies that might influence students’ views about learning and doing science. Thus, the second part of this introduction will describe the pedagogies, learning theories, and research methodologies that might facilitate the integration of the concepts discussed thus far with the concepts taught in chemistry curricula and the study of students’ perceptions of these experiences.

1.5 Authenticity in Science and Chemistry

Authentic learning environments are difficult to define but usually are in some way comparable to the context that they are intended to teach. In 1995, Roth suggested that in order for activities in science education to be authentic, they “need to share key features with those worlds about which they teach”. He described five features of authentic activities including ill-defined
problems, the uncertainty that accompanies the social aspect of scientific inquiry, consideration of the learner’s prior knowledge, participation in the community of practice, and interaction with experts within the community. These characteristics align well with some of the nine critical characteristics of situated learning defined by Herrington and Oliver in 2000. They developed a framework for authentic learning across disciplines based on the most comprehensive review of the literature in this area. The nine characteristics that make up this framework are:

1) reflect on the way knowledge will be used in real life
2) involve relevant, ill-defined problems
3) access to thinking and modelling processes at varying levels of expertise
4) provide multiple perspectives and points of view
5) foster collaboration
6) foster reflection
7) offer opportunities to articulate or communicate knowledge
8) instructor acts as a coach and scaffolds support
9) use of complex and contextual assessments

1.5.1. A Framework for Authentic Learning

Authentic learning environments must reflect the way the knowledge will be used in real life. This indicates that simply communicating relevance or providing examples of real-life situations is not sufficient. True authentic experiences involve a problem that must be solved in a realistic way in the relevant physical environment with appropriate resources. Emphasis is placed on maintaining the complexity of the concepts rather than presenting them in a fragmented manner. Authentic activities usually involve relevant, ill-defined problems that involve tasks, which, being
complex and applicable across disciplines, can be defined by students in order to complete the activity. Students should have access to thinking and modelling processes at varying levels of expertise and the opportunity to observe the community from the periphery as real-life episodes occur. Multiple perspectives and points of view should be provided by means of collaboration. In fact, instruction that presents a single “correct” answer is considered inadequate whereas, examining the problem from the perspective of multiple stakeholders leads to a deeper understanding of the topic. Collaboration in small- or whole-groups should be fostered in order to construct knowledge in a social manner. This involves assigning tasks to the group that would not be possible to complete independently. Collaboration can also be applied to foster reflection so that the student has an opportunity to compare their understanding to experts’ and peers’ understanding. Opportunities to articulate or communicate the knowledge helps students to develop knowledge that is more meaningful. This could be accomplished using debates, presentations, or publication of information online, which provides a format for students to justify their work to their peers and others in the field. An instructor who acts more as a coach and scaffolds support is most effective. Rather than the traditional didactic role, instructors should be more involved in the development of students’ metacognitive skills. Students need to be assessed with multiple reliable and valid indicators using activities that are complex and contextual. Polished products of students’ learning should be seamlessly integrated and provide students an opportunity to be successful using their developed knowledge and skills. These elements are the compilation of an extensive review of the literature on authentic learning. However, it is not necessary that every instructor that implements authentic learning include all of these elements in their course.104-105
1.5.2. Manifestations of Authenticity in Chemistry Education

Recent studies in chemistry education that involve one or more elements of Herrington and Oliver’s framework are abundant in the literature but do not necessarily address authenticity explicitly. These studies range from the implementation of active learning strategies in the classroom to immersive and long-term research experiences. They demonstrate increased performance in STEM courses, understanding of the nature of science, and acquisition of research skills among countless other outcomes.

The flipped classroom has recently become popular in science and chemistry education since it makes time during class for group work and problem-solving. Most commonly, instructors deliver lectures outside of class so that in-class time can be used for activities that are more authentic. The activities that are usually implemented involve somewhat ill-defined problems (although the answer may already be apparent to the instructor), collaboration, and the opportunity to articulate knowledge in the form of presentations. In addition, the absence of lecture allows the instructor to act more as a coach during class time than as a conveyor of information. Results of studies on the flipped classroom indicate improved performance, increased conceptual understanding, and positive affect. Despite the myriad of benefits of implementing active learning strategies in the chemistry classroom, many would argue that it is not a truly authentic environment since students are taken out of the context in which real scientific knowledge is generated. An age-old approach to overcoming this challenge is the use of the laboratory-learning environment.

Traditionally, laboratory-learning environments have been used for students to replicate previously performed experiments in order to teach or support concepts being covered in the
lecture course. However, in recent years, inquiry- and problem-based methods have become increasingly popular since the emphasis is on the construction of knowledge rather than on the repetition of established experiments. Guided inquiry labs involve the posing of a question and then the scaffolding of knowledge and skills needed to develop a plan to answer the question. These involve somewhat ill-defined problems (once again, the answer is likely already known to the instructor), multiple perspectives since students must work in collaboration to develop a procedure, and the opportunity to reflect and articulate. In addition, the guided nature of the inquiry implies that the instructor acts as a coach and allows the opportunity to integrate authentic assessments. Inquiry labs have demonstrated better performance and development of conceptual understanding over traditional labs, increased positive affect about performing experiments, and changing perceptions of the purpose of laboratory courses as an environment to learn transferable skills like problem-solving rather than technical skills. However, they have met some criticisms when considering how they convey the nature of science. In fact, the results of one study show that inquiry labs have a negative impact on students’ views of the nature of science since they do not necessarily accurately reflect the way that scientists work.

Recently, the introduction of course-based undergraduate research experiences (CUREs) has provided a path to more authentic experiences that are embedded within laboratory courses. These are usually implemented in higher-level undergraduate labs, but recently have been described in a first-year course. Whole groups engage in the pursuit of answering a research question that is unknown to both the student and the instructor. This is perhaps the best example of a course-based authentic experience described thus far since it fosters all nine aspects of the framework for authentic learning and is the only example that presents a truly ill-defined problem.
Students that participate in CUREs claim that they have a better understanding of concepts, increased self-efficacy and attitudes towards science, and developed research skills.\textsuperscript{108-109, 121-122} However, there are still even more immersive experiences that place the student within the community of practice during the learning experience.

Research apprenticeships have a long tradition in science education. Modern implementations include undergraduate research, research experiences for undergraduates (REUs), and internships. In these cases, students are immersed into the laboratory as one of its members. Responsibilities vary, but students usually develop more accurate perceptions of scientific practice\textsuperscript{114, 123} and research skills\textsuperscript{124-125} from these experiences and are more likely to persist in STEM fields than students without the experience.\textsuperscript{114, 126-127} These experiences are not associated with a traditional course and usually involve the direct guidance of a faculty member. They are the best example of a truly authentic experience described in the literature.

The variation in authenticity involved in these experiences is large. However, students gain access to common advantages in all cases. Most notably, improved performance and persistence in STEM. The literature on the effect on learning is existent, but not as vast. Affectively, students gain more positive perceptions about doing science, increased self-efficacy and scientific identity, and developed skills in research. Having established the importance of authentic learning environments from the classroom to the laboratory and the important role that authenticity plays in developing student views about science, we next discuss the theoretical framework for the development and evaluation of the authentic experiences described in this dissertation.
1.6 Situated Learning Theory

Situated learning theory differs from other theories of learning that focus on the decontextualized individual in the learning process. It suggests that the activity in which information is conveyed is an integral part of what the student learns.\textsuperscript{128-129} In fact, learning cannot be meaningful if it separated from context. For example, research on vocabulary learning demonstrates that language acquisition is much more efficient when a person is conversing or reading than when asked to learn an abstract definition.\textsuperscript{130} It follows, then, that knowledge cannot be developed meaningfully without authentic context in the form of social and/or physical activity.\textsuperscript{128, 131} To provide context, it must be acknowledged that activity, concept, and culture are interdependent.

One major assumption of situated learning theory is that individual and their environments co-construct each other.\textsuperscript{132} The learning experience is considered a transaction between the learner and their environment. \textsuperscript{133-134} The result of each experience influences how the individual understands a concept, and their understanding of that concept influences future experiences.\textsuperscript{135} This iterative process results in the student becoming a member of the community of practice. Thus, any activity employed to teach a concept must reflect the culture of the community.

A community of practice is a group of people with socially constructed beliefs, values, and norms. Groups might be described by their profession or academic discipline and could be general (i.e. scientists) or specific (i.e. chemical education researchers). In classroom settings, it is common to ask students to learn about topics without knowing anything about the culture of these communities. However, in order to understand the concepts, they need to practice using the “tools”
of the community. Hence, learning is considered a process of enculturation and involves the student learning the vocabulary, behaviors, and practices of the community’s members.\textsuperscript{128}

Two main approaches to situated learning have been described. First, Brown and co-workers\textsuperscript{128} describe an approach similar to traditional craft apprenticeships called \textit{cognitive apprenticeship} in which students are enculturated into authentic practices of the discipline by being exposed to the modeling of practices by an expert in the community. In an undergraduate research experience, this could manifest as the interactions that the undergraduate member of a research group has with senior members. The practices and problem solving processes they observe the senior members using will influence how they carry out the same tasks in the future. Second, Lave and Wenger\textsuperscript{129} describe the process of moving from peripheral roles into more central roles as students gain knowledge and experience. The theory is referred to as \textit{legitimate peripheral participation}. For example, a new member of a research group may be responsible for simpler tasks than a senior member. With participation in more complex tasks (i.e. troubleshooting and decision-making) under the guidance of the senior member, the new member will become a central player in the group and have more complex responsibilities. Both approaches highlight the necessity to provide authentic learning experiences so that students might feel a part of the community and learn meaningfully.

Considering the interaction between the learner, their environment, and other people in their environment, it is important to focus research on these interactions and the role they play in learning. Research methods that help to describe students’ learning experiences can give insight to these interactions and are described next.
1.7 Research Methods

Discipline-based educational research methods span from quantitative to qualitative with a spectrum of a combination of the two in between. The studies presented in this dissertation began with the intention to describe the experience of students in authentic learning environments. In order to describe the lived experience of individuals accurately, a qualitative research methodology offers better opportunity to observe the phenomenon from a naturalistic setting. This allows the researcher to interpret the phenomenon in terms of the subject’s perspective.

1.7.1. Phenomenology

A qualitative research method that is specifically intended to produce a rich description of the common meaning of individuals who experience the same event or phenomenon is phenomenology. The purpose of this method is to reduce the individual experiences to a common description of the “essence” of the experience by describing what is experienced and how it is experienced. Some important perspectives to consider when using a phenomenological research method are i) the study should be performed without presuppositions about what is “real” and ii) that reality is an object of one’s consciousness. These assumptions indicate that a phenomenological study is not theory-driven. Rather the result is a description of the essence of the experience.

Phenomenological studies usually involve the following features. First, is an emphasis on the phenomenon being explored. In this dissertation, the phenomenon being explored is learning in authentic environments. Next, is an exploration of the phenomenon with a heterogeneous group of individuals who experienced the same event. Then, data collection is largely based on interviews, but since phenomenology can be considered as lying on a
“continuum” between qualitative and quantitative research, other sources of data such as observations and documents can be used as well. Finally, data analysis in phenomenological studies is inductive, moving from narrow units of analysis to broader ones that lead to detailed descriptions of the essence of the experience under study.

The specific observational protocols, documents, and interview protocols used in the studies in this dissertation are described in detail in the corresponding chapters. However, some notes are provided here about how each type of data contributed to these studies.

1.7.2. Instruments

Interviews

Interviews are the most common form of data collected in phenomenological studies. The interaction between the interviewer and interviewee is social and allows knowledge about the phenomenon to be constructed. However, it is important to use an interview protocol developed based on the characteristics of the participants in the study, the purpose of the study, and the research questions and subquestions to be answered. In order to develop interview protocols that produce data pertinent to the study, we started by identifying the data needed to address the research question(s). Once the data needed was identified, question types were chosen (i.e. direct, hypothetical, or scenario, etc.) and draft questions were written. From this blueprint, the final interview protocols (provided in Appendices J and L) were developed with main interview questions and prompts for eliciting further information. Prompts and elicitations were included in the protocol since semi-structured interviews were conducted. Semi-structured interviews offer the advantage of deeper exploration of unanticipated responses.
Observations

Observations are a powerful tool for collecting data on a phenomenon in the field. Observational protocols provide a guide for what subjects and behaviors to observe and record so that information pertaining to the focus of the study can be collected. The observational protocol used in this dissertation is a previously established instrument for the simple and discrete observation the engagement of large groups of students and is described in detail in Chapter 6.

Surveys

Document analysis can provide deeper insight into how people experience events. Documents included in the studies in this work are open-ended survey reflections of what was learned or what was experienced. One survey, the Views on the Nature of Science (VNOS) questionnaire\textsuperscript{140} is a previously developed and established instrument that was scored following the developer’s scoring guide. The other was a simple student perception survey that asked students to describe their favorite and least favorite aspects of a course. Responses to this survey were coded inductively as described in detail in Chapter 7.
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In the first chapter of this dissertation, we present my first study as a graduate student in which we investigated theoretically the nonlinear optical properties of (-)-trans-Δ⁹-tetrahydrocannabinol (Δ⁹-THC) and cannabidiol (CBD). These compounds are the most biologically interesting psychoactive and nonpsychoactive components of marijuana, respectively. While state-level legalization of medical and recreational marijuana is more widespread recently, the federal government maintains that it should undergo the same scientific scrutiny as any other drug on the market. This chapter is a contribution to the response to that call.

The exact mechanism of binding of THC to the cannabinoid receptor, CB1, is unknown. Conformational information of the cannabinoids may give insight to this mechanism and the elicited effects of consumption. Herein, we report on the theoretical conformational study of Δ⁹-THC and cannabidiol CBD using electronic circular dichroism (ECD) and two-photon circular dichroism (TPCD). The compounds were optimized in vacuo and in the receptor site using DFT and B3LYP with the 6-311G(d,p) basis set and spectra were calculated using the same level of theory, but with the 6-311++G(d,p) basis set. First, we present and discuss the comparison of experimental and theoretical ECD spectra of (-)-trans-Δ⁹-THC and CBD in methanol solution in order to corroborate our theoretical approach. Second, we show, theoretically, the enhanced sensitivity of TPCD compared with ECD to conformational changes of cannabinoids upon
docking, giving rise to a potential application in vivo. Finally, the comparative analysis of the theoretical TPCD spectra of Δ⁹-THC and CBD show distinct fingerprints in the far-UV region for two conformers of each molecule, which may help to understand the specific binding mechanisms of these species to the cannabinoid receptors and to describe the difference in psychological effects upon consumption. Our results show the complementarity of these two spectroscopic techniques and the potential of TPCD to determine the conformational changes of cannabinoids upon docking to the CB1 receptor.

2.1 Introduction

For centuries, marijuana has been used for medical purposes due to its properties as a pain reliever, appetite stimulant and antiemetic¹. More recently medical marijuana has been used in the treatment and prevention of several medical conditions including chemotherapy-induced nausea² and chronic pain and spasticity associated with multiple sclerosis³. With the onset of the legalization of medical and recreational marijuana in the United States, interest has increased in possible therapeutic uses of the drug. However, medical marijuana remains federally illegal in the U.S. since it has yet to go through the comprehensive studies required for Food and Drug Administration (FDA) approval.

Cannabinoids, a class of compounds unique to the cannabis sativa plant, are known to exhibit various physical and psychological effects upon consumption of marijuana. Remarkably, of the more than 80 cannabinoids present in cannabis sativa, (-)-trans-Δ⁹-tetrahydrocannabinol (THC) and cannabidiol (CBD) are considered the two most pharmacologically interesting. THC, the main psychoactive component of marijuana attributed with the euphoric effect⁴⁻⁵, is also reportedly responsible for the stimulation of appetite⁶⁻⁷ and reduction of pain¹ as well as increased anxiety, paranoia and impairment of memory⁸, among many other effects. While CBD is likewise
credited with appetite stimulation and pain relief\textsuperscript{9}, it has been shown to have opposing psychological effects\textsuperscript{10}. In fact, when administered together, CBD has been shown to reduce the psychotropic effects of THC\textsuperscript{11-13}, making it a potentially exceptionally useful therapeutic agent for psychological disorders.

The psychological effects of marijuana are specifically associated with the activation and blocking of cannabinoid 1 (CB1) receptors in the brain\textsuperscript{14}. While THC is a known weak agonist, CBD acts as an antagonist to CB1, partially explaining the opposing effects. In the pursuit of therapeutic drugs without the euphoric effect, it would be helpful to understand why these different cannabinoids interact differently with CB1. Certainly, the molecular mechanism of cannabinoid docking to CB1 can provide clues to the varying effects of cannabinoids, but this mechanism is yet unknown. In an attempt to answer this question, we propose the following two possible scenarios:

- **Scenario 1:** The receptor site of CB1 has a slightly different conformation in each area of the brain, which is responsible for controlling different functions. This may allow only certain ligands to bind in certain areas of the brain. In this case, it would be possible that CBD does not bind to CB1 in the area of the brain that produces the so-called “high”, but does bind in the area that controls, for example, appetite.

- **Scenario 2:** CB1 has the same conformation in all areas of the brain, which allows more than one type of ligand to bind. However, when the cannabinoids do bind, they induce different structural changes on the protein, which sends a different signal to the cell, causing different effects.

Of course, we are aware that interest in this mechanism is not novel. In 2001, a generic mechanism for G-protein coupled receptors (GPCRs), a larger class of proteins that encompasses
CB1, was theoretically predicted in which transmembrane helices of a GPCR rotate when the receptor is activated\textsuperscript{15}. Other theoretical studies focused on changes in the receptor site when a ligand was bound. In 2004, a toggle-switch mechanism was predicted in which a tryptophan residue undergoes a $\chi_1$ rotamer change upon activation of CB1\textsuperscript{16}. However, the changes that cannabinoids undergo upon binding to the receptor remain unexplored theoretically and experimentally. Finding new ways to study these changes will contribute to the understanding of the proposed mechanisms.

Taking advantage of the chirality present in THC and CBD one could consider using electronic circular dichroism (ECD) for investigating the changes that cannabinoids undergo upon binding to the receptor. It should be highlighted that circular dichroism has been the standard technique of excellence for the study of optically active biomolecules during the last two decades.\textsuperscript{17} While ECD has proven to be a reliable technique for studying large systems such as proteins, there are some limitations when working with small molecules, i.e. in the same spectral region where the signature of a small molecule is expected to appear, linear absorption of common solvents and buffers, and scattering at short wavelengths is observed. In order to overcome the limitations of ECD, Hernández and co-workers recently proposed to use two-photon circular dichroism (TPCD)\textsuperscript{18}, the nonlinear counterpart of ECD. TPCD, first proposed theoretically in the ‘70’s by Tinoco\textsuperscript{19} and Power\textsuperscript{20}, is defined as the difference between two-photon absorption (TPA) cross-sections using circularly polarized light of opposite handedness. This phenomenon was not able to be measured reliably until the development of the double-L scan technique in 2008.\textsuperscript{21}

Since two-photon absorption occurs at lower energies than one-photon absorption (OPA), there is negligible linear absorption in the excitation region and scattering is minimized. Additionally, depth penetration and spatial resolution are increased\textsuperscript{22} and photodamage to living
cells is diminished.\textsuperscript{23} Recently, TPCD has been used to investigate the structure of natural amino acids.\textsuperscript{24} Vesga and co-workers showed theoretically that TPCD is more sensitive than ECD to the differences between α-helix, β-strand and random-coil conformations of a tryptophan residue. Based on this, we consider TPCD as a potentially effective method of detection of minor conformational changes of cannabinoids upon binding to the receptor site of CB1.

Traditionally, theoretical docking studies are performed using molecular mechanics, but the advent of “quantum biology” has generated interest in how biological systems behave quantum mechanically.\textsuperscript{25} Therefore, to introduce ligand docking to the world of quantum biology, we proposed to use quantum mechanics in the study of the conformational changes of cannabinoids upon binding to CB1. However, CB1 is a seven transmembrane helix GPCR composed of more than 6000 atoms. Consequently, optimizing a ligand in the receptor site of CB1 using \textit{ab initio} methods poses a serious computational challenge; optimization of a system of this size using quantum mechanics is virtually impossible. Hence, in order to acquire a system that would be more manageable for \textit{ab initio} calculations, we developed a novel method, which we coined the \textit{receptor cutting method} (RCM).

In this chapter, we describe the development of RCM, its application for the quantum mechanical optimization of a ligand in the known binding site of a receptor, and the subsequent theoretical spectroscopic analysis. We report the experimental and theoretical OPA and ECD of THC and CBD, as well as the theoretical TPA and TPCD spectra of THC, CBD, (-)-\textit{cis}-Δ\textdegree{}9-THC ((-)-cis), and cannabinol (CBN) calculated both in gas phase and when bound to the receptor site. Our results demonstrate the potential of RCM to allow access to \textit{ab initio} conformational studies of ligand-receptor docking using linear and nonlinear spectroscopy, and even more specific the possibility of using TPCD for determining the mechanism of activation of CB1 induced by
cannabinoids. We expect RCM to pave the road to novel quantum mechanical studies of receptor-ligand interactions.

### 2.2 Experimental and Theoretical Methods

THC and CBD were purchased in methanol from Lipomed AG. OPA measurements were performed on a single-beam spectrophotometer (Agilent 8453 Diode Array UV-Vis) in a 1 cm cell in 20 μM solutions. ECD measurements were carried out on $10^{-4}$ M and $10^{-3}$ M solutions of THC and CBD respectively on an AVIV 215 CD Spectropolarimeter with a path length of 0.1 cm and a scan speed of 1 nm/s.

The alkyl chain attached to the aromatic ring of cannabinoids leads to a large degree of freedom of the molecule so for each cannabinoid, two conformers (Figure 2.1) were considered. The structures were first optimized in vacuo using density functional theory (DFT)\textsuperscript{26} and Becke’s three-parameter exchange, Lee, Yang and Parr correlation (B3LYP) functional\textsuperscript{27-29} using the 6-311G(d,p) basis set\textsuperscript{30-31} in Gaussian 09.\textsuperscript{32} Each of these conformers were then placed in the receptor site of CB1 to be re-optimized to the same level of theory.

![Chemical structure of (-)-trans-$\Delta^9$-tetrahydrocannabinol](image)

**Figure 2.1.** Chemical structure of (-)-trans-$\Delta^9$-tetrahydrocannabinol (THC) (top). Lowest energy conformations of THC (bottom). Optimizations were performed with DFT/B3LYP/6-311G(d,p) in gas phase using Gaussian 09.
OPA and ECD spectra were calculated using Equations 2.1 and 2.2 for the first 100 excited states of each conformer optimized in vacuo and in site using time-dependent DFT (TD-DFT) at the B3LYP/6-311++G(d,p) level of theory in Gaussian 09. The polarizable continuum model (PCM) was used to account for solvent (methanol) effects.

OPA spectra are reported in molar absorptivity,

\[ \varepsilon_{\text{OPA}}(\omega) \approx 1.05495 \times 10^3 \times \omega \sum_f g(\omega, \omega_{of}, \Gamma) \frac{f_{of}}{f_{0f}} \]

Here \( \omega \) is the circular frequency of the incident light, \( \omega_{of} \) is the excitation circular frequency for a \( 0 \rightarrow f \) transition, \( f_{of} \) is the oscillator strengths and \( g(\omega, \omega_{of}, \Gamma) \) is the Lorentzian lineshape function for the linear absorption case.

ECD spectra are reported in molar absorptivity difference from \( R_{0f}^{ECD} \),

\[ \Delta \varepsilon_{\text{ECD}}(\omega) \approx 2.73719 \times 10^1 \times \omega \sum_f g(\omega, \omega_{of}, \Gamma) R_{0f}^{ECD} \]

where \( R_{0f}^{ECD} \) is the velocity rotatory strengths.

Both have units of mol\(^{-1}\) cm\(^{-1}\) L as long as the elements in Equations 2.1 and 2.2 are introduced in atomic units.

TPA and TPCD spectra were calculated in gas phase for the first 50 excited states using TD-DFT at the B3LYP/6-311++G(d,p) level of theory in Dalton 2013.

TPA spectra were calculated using

\[ \delta_{0f}^{\text{TPA}}(\omega) \approx 1.25273 \times 10^{-2} \omega^2 \sum_f g(2\omega, \omega_{of}, \Gamma) \cdot \delta_{0f}^{\text{TPA}}(\omega_{of}). \]
where \( \delta_{0f}^{TPA}(\omega_{0f}) \) is the orientational two-photon probability for the degenerate case, \( g(2\omega, \omega_{0f}, \Gamma) \) is a normalized Lorentzian line shape function where \( \Gamma \) is the line width and \( \omega \) is the excitation frequency.

TPCD spectra were calculated as described by Rizzo et al. using the following equation:19, 35, 39

\[
\delta^{TPCD}(\omega) \approx 4.87555 \times 10^{-5} \times \omega^2 \sum_f g(2\omega, \omega_{0f}, \Gamma) \cdot R_{0f}^{TPCD}(\omega_{0f})
\]

where \( R_{0f}^{TPCD}(\omega_{0f}) \) is the TPCD rotatory strength which is obtained from:

\[
R_{0f}^{TPCD}(\omega_{0f}) = -b_1 B_1^{TI}(\omega_{0f}) - b_2 B_2^{TI}(\omega_{0f}) - b_3 B_3^{TI}(\omega_{0f})
\]

where \( b_1, b_2 \) and \( b_3 \) are scalars that depend on the geometry of the experiment. In this case, it is assumed that two left or right circularly polarized photons are collinear and co-propagating so that \( b_1 = 6, b_2 = 2 \) and \( b_3 = -1 \). \( B_1, B_2 \) and \( B_3 \) are molecular parameters all of which depend on the electric transition dipole moment. \( B_1 \) and \( B_3 \) also depend on the magnetic transition dipole moment and \( B_2 \) depends on the electric transition quadrupole moment. TPA and TPCD spectra obtained from Equations 2.3 and 2.4 are in units of Göppert-Mayer (GM) \((10^{-50} \text{ cm}^4 \cdot \text{s} \cdot \text{molecule}^{-1} \cdot \text{photon}^{-1})\) as long as the equation elements are introduced in atomic units.
2.3 The Receptor Cutting Method (RCM)

Figure 2.2. Graphical description of the Receptor Cutting Method (RCM). The general steps in RCM are: 1. Identification of receptor of interest. 2. Identification of binding site residues. 3. Elimination of all other residues. 4. Connection of residues with alkyl chains and immobilization of connecting atoms. 5. Introduction of ligand to site and optimization. 6. Extraction of ligand from site.

Next, we describe a remarkable method, which allows for modeling the docking of ligands to the receptor site using quantum mechanics. Figure 2.2 displays, in a sequential manner, the protocol for applying RCM to a receptor-ligand pair. RCM progresses in the following sequence:

1. Identification and acquisition of a model of the receptor of interest. Crystal structures of many receptors are known and models can be obtained via the protein data bank (PDB).
(2) Identification of amino acid residues in the binding site. It is imperative that an initial identification of the site is made so the ligand can be introduced in such a way that it is already close to the correct location for binding. **Note:** Ligand binding assays and theoretical docking studies are valuable resources for this information.

(3) Elimination of remaining residues. Since the remaining residues are not expected to directly interact with the binding ligand they are eliminated, isolating the receptor site residues.

(4) Connection of binding residues with alkyl chains and immobilization of connecting atoms. To avoid compromising the structural integrity of the receptor site, the residues involved in binding are reconnected. Alkyl chains provide connection between residues with a minimal number of atoms, reducing computational cost. Still, optimization of this system would most likely result in the residues moving as far apart as possible, only being limited by the length of the alkyl chains. To mimic the relatively restricted movement of the residues in the receptor site when they are attached to the entire protein, the alkyl chains are frozen prior to optimization. The binding residues, however maintain their degrees of freedom in order to arrange for docking.

(5) Introduction of the ligand to the receptor site and subsequent optimization. As already stated, the ligand must be introduced to the site in an orientation such that it will likely interact with the receptor as previously observed. The more manageable system is composed of considerably fewer atoms and is optimized using an *ab initio* method, i.e. Density Functional Theory.

(6) Extraction of ligand from site. Finally, the ligand that has been optimized, and molecularly transformed in order to bind, is extracted from the site so that its ground and excited state properties can be investigated without any further optimization.
2.4 Results and Discussion

2.4.1. Receptor Cutting Method

To evaluate RCM for the purposes of quantum mechanical studies of protein-ligand interactions, we docked various cannabinoids in the receptor site of CB1 and looked for interactions between cannabinoids and CB1 that have been described in traditional docking studies. Since, to date, no crystal structure of CB1 is available, we used the model based on antagonist docking that was recently developed by Liu and co-workers.\textsuperscript{40} The amino acid residues in the receptor site of CB1 were identified as described in ref. 41 and isolated from the rest of the protein. The binding site residues were stabilized using frozen alkyl chains before the cannabinoid was placed with the alkyl chain towards residues Ile-354, Cys-355, Trp-356, Leu-359 and Leu-360 and the tricyclic scaffold near residues Phe-177, Phe-189, Lys-192, Met-363, Phe-379 and Ser-383, oriented so that the phenolic hydrogen could feasibly form a hydrogen bond with Lys-192. The entire system was optimized using DFT/B3LYP/6-311G in Gaussian 09.\textsuperscript{32} After optimization we looked for (i) the formation of a hydrogen bond between the phenolic oxygen and the lysine residue, Lys-192\textsuperscript{41} and (ii) the toggle-switch mechanism previously described, in which Trp-357 undergoes a $\chi_1$ rotamer change.\textsuperscript{16} Indeed, for each conformer of THC, (-)-cis, CBD and CBN, there was a rotation of the tricyclic scaffold as well as the phenolic hydrogen to increase proximity to Lys-192, forming the hydrogen bond and a change in the dihedral angle of the alkyl chain in order to allow the tryptophan rotamer change.

In Figure 2.3 we present these changes for THC2 (similar representation of changes in the other three molecules can be found in Appendix E). The manifestation of these interactions corroborates the validity of RCM for feasibly modeling ligand binding to protein receptor sites using \textit{ab initio} methods. This approach is promising since if ligand-site interactions are known,
RCM has potential to expand on the accessible structural and mechanistic information obtained using traditional docking methods. Additionally, RCM opens a path to quantum mechanical methods to predict, more accurately, the transformations of and interactions between receptors and ligands during binding.

![Before Optimization](image1.png)

![After Optimization](image2.png)

**Figure 2.3.** THC2 in site before (upper left) and after (lower right) optimization using RCM. Highlighted regions indicate formation of hydrogen bond between Lys-192 and the phenolic hydrogen as well as the Trp-357 rotamer change. The molecule before and after is overlaid in the upper right. Before (red) and after (green) optimization.

To explore these latter aspects, we analyzed the changes in physical conformation of all four molecules after their optimization in the receptor site. The change in the dihedral angle between the aromatic ring and the alkyl chain (Δφ), the change in dihedral angle between the aromatic ring and the double bond of the neighboring ring (Δα) as well as the rotation of the phenolic hydrogen (θ) (as indicated in Figure 2.4) are reported in Table 2.1.

![Diagram](image3.png)
Figure 2.4. Alkyl chain dihedral angle, φ, cyclic scaffold dihedral angle, α, and the rotation, θ, of the phenolic hydrogen of each cannabinoid (shown for THC1) were recorded before and after optimization in the receptor site.

Table 2.1. Change in alkyl chain and cyclic scaffold dihedral angles (Δφ and Δα respectively) and hydrogen rotation (θ) of each conformer upon cannabinoids binding to CB1.

<table>
<thead>
<tr>
<th>Cannabinoid</th>
<th>Conformer</th>
<th>Δφ (°)</th>
<th>Δα (°)</th>
<th>θ (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-trans-Δ9-THC (THC)</td>
<td>1</td>
<td>2.86</td>
<td>0.58</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11.3</td>
<td>2.80</td>
<td>121</td>
</tr>
<tr>
<td>(-)-cis-Δ9-THC ((-)-cis)</td>
<td>1</td>
<td>1.00</td>
<td>-5.20</td>
<td>37.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-22.7</td>
<td>-0.60</td>
<td>32.2</td>
</tr>
<tr>
<td>Cannabidiol (CBD)</td>
<td>1</td>
<td>10.1</td>
<td>-48.7</td>
<td>57.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-34.6</td>
<td>-9.10</td>
<td>78.4</td>
</tr>
<tr>
<td>Cannabinol (CBN)</td>
<td>1</td>
<td>-21.5</td>
<td>-14.4</td>
<td>46.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.36</td>
<td>-29.9</td>
<td>116</td>
</tr>
</tbody>
</table>

First, THC underwent seemingly small conformational changes compared to the other cannabinoids. Since it is known that THC exhibits a relatively high potency, these results suggest that any conformation of THC would be more energetically favorable to bind without undergoing considerable physical changes. Second, it seems for (-)-cis that the ring moves significantly for conformer 1 and the chain moves significantly for 2. Since one part of the molecule moves while
the other remains essentially the same, we suggest that (-)-cis is restricted from significant
conformational changes, probably due to the steric effects imposed by the hydrogens on the back
side of the ring. When comparing the changes between the four molecules, CBD shows the most
significant changes overall which is most likely attributed to its large degree of freedom due to the
absence of the center ring in the scaffold. This might make binding favorable for CBD since it can
adopt so many different conformations in order to fit into the site. Last, CBN shows larger
conformational changes than expected due to the rigidity of the tricyclic scaffold. However, the
absence of the hydrogens on the outer ring probably allow for less restricted movement. An
important note is that this model of CB1 is based on antagonist docking. Of the four cannabinoids
docked, only CBD is an antagonist for CB1. The fact that it would dock to CB1 despite the
significant changes supports the idea of Scenario 1. Perhaps in a different conformation of CB1,
CBD would not be able to undergo the changes necessary to bind.

2.4.2. Validation of Theoretical Approach

In addition to exploring the physical changes made possible by RCM, we wanted to determine,
theoretically, whether we could differentiate between cannabinoids in vacuo and in site
spectroscopically. In order to initially validate our theoretical approach, the experimental OPA and
ECD spectra of THC and CBD in methanol and their corresponding theoretical spectra calculated
in methanol using B3LYP/6-311++G(d,p) are compared in Figure 2.5. Theoretical spectra were
slightly blue-shifted, i.e. -8 nm (THC) and -6 nm (CBD), to obtain a better theoretical-experimental
overlap (this is common practice in theoretical-experimental work). It should be noted that
while the ECD of cannabinoids has previously been reported, basic conditions were employed
during the measurements, significantly altering the spectra. For each cannabinoid, we calculated
OPA and ECD for two conformers (red and blue in the figure for conformer 1 and 2, respectively)
and averaged the resulting spectra (violet) for comparison with experimental results. We realize that eliminating the alkyl chain might yield spectra that more accurately represent a population of conformers, but the initial position of the chain is especially important during binding. Nevertheless, in both cases the average predicted spectra (violet) match very well with our experimental results. We only observed notable deviations in the ECD spectrum of CBD where the bands are present, but they are more negative than predicted (indicated with orange arrows in the figure). This minor deviation can be attributed to the larger degree of freedom associated with CBD due to the absence of the central ring that maintains the relative rigidity of THC.

![Comparative plots of experimental (black dots) and calculated (solid lines) OPA (left) and ECD (right) spectra of cannabinoids in solution. OPA and ECD response for the lowest 100 excited states of optimized structures were calculated with TD-DFT/B3LYP/6-311++G(d,p) in methanol using Gaussian 09. Spectra calculated for each conformer (red dotted line for conformer 1 and blue dashed line for conformer 2) were averaged (violet solid line) to match experimental, are only shown within the experimentally measureable range (190-260 nm) and have been shifted: THC (-8 nm) and CBD (-6 nm). Γ=0.35 eV (FWHM) was used for all calculated spectra. Excited states that significantly contribute to the spectral features observed in theoretical and experimental spectra are identified in Appendix G.](image-url)
2.4.3. Linear and Nonlinear Characterization

Having established that the theoretical approach is valid, we computed the OPA, ECD, TPA and TPCD spectra for both conformers of THC, (-)-cis and CBD in methanol when optimized in \textit{vacuo} and in site using B3LYP/6311++G(d,p) and $\Gamma=0.35\text{eV}$ (FWHM) for OPA and ECD and $\Gamma=0.2\text{eV}$ (FWHM) for TPA and TPCD. It should be noted that OPA and TPA were calculated for CBN as well, but as the molecule is achiral, ECD and TPCD were not calculated. Comparison of theoretical OPA and TPA spectra for each molecule optimized in \textit{vacuo} and in site revealed insignificant differences in all cases (These spectra can be found in Appendix F).

First, we compare the calculated ECD spectra of each conformer of THC. These spectra are presented on the left side of Figure 2.6. Differences in the ECD spectra in a region between 180-190 nm suggest that both THC conformers in \textit{vacuo} would be distinguishable from their counterparts in site using this technique. In reality, though, this region is experimentally unreliable due to scattering and linear absorption of the solvent. The calculated TPCD spectra comparing each conformer of THC in \textit{vacuo} and in site are presented on the right side of Figure 2.6. Regarding the first conformer, there is a fingerprint around 400 nm that would allow the conformer in site to be distinguished from the one in \textit{vacuo}. Additionally, the signals for THC1 optimized in \textit{vacuo} and in site are opposite in sign around 420 nm which would make them clearly distinguishable experimentally. The most dominant transitions in the 380-410 nm region, where we observe the appearance of the fingerprint after docking, change from 31-33 in \textit{vacuo} to 29 and 41 in site. We attribute these bands to high energy $\pi\rightarrow\pi^*$ transitions that are affected by the slight movement (less than 3° $\Delta\varphi$) of the side alkyl chain in order to avoid steric effects from the rotating tryptophan residue. For THC2, the differences in the TPCD spectra are less significant and occur in a different region than THC1. The signals for THC2 in \textit{vacuo} and in site are opposite in sign between 460
and 490 nm. The dominant transitions in this region change from 4 & 7 to 6 & 7 and also change from positive to negative. While the change is attributed to the tricyclic scaffold rotation to a higher degree for this conformer, the position of the bands allows us to assign these relatively lower energy transitions to $\pi\rightarrow\pi^\ast$.

**Figure 2.6.** Comparative plots of ECD (left) and TPCD (right) spectra of (-)-trans-Δ9-THC conformers. Dotted lines correspond to structures optimized in gas phase, solid lines correspond to structures optimized in the receptor site. ECD response for the first 100 excited states of optimized structures were modeled with TD-DFT/B3LYP/6-311++G(d,p) in methanol using Gaussian 09. TPCD response for the first 50 excited states were modeled with TD-DFT/B3LYP/6-311++G(d,p) in gas phase using Dalton 2013. Dominant transitions are labeled, colored arrows indicate regions of the corresponding spectra that could allow for clear identification of which conformer is present. Shaded region of ECD spectra is experimentally unreliable.

In addition to comparing the conformations in vacuo and in site, we performed the comparison of both conformers of THC in the receptor site to determine whether they could be differentiated experimentally. Examination of the TPCD spectra reveals fingerprints for conformer 1 in site at 400 and 440 nm and the signals are opposite in sign around 400 and 480 nm. This would allow for the experimental determination of the conformation of THC in site for one or a mixture of both conformers, which would not be possible using only ECD.
For comparison of the psychoactive component of marijuana (THC) with another naturally occurring optical isomer, we examined the ECD spectra of (-)-cis conformers before and after binding to CB1 (left side of Figure 2.7). The calculated ECD spectra of (-)-cis1 appear quite different in \textit{vacuo} and in site, but a spectral shift could cause obscurity. The spectra of conformer 2 are nearly identical in \textit{vacuo} and in site, so binding would be undetectable using ECD. Upon analysis of the TPCD spectra, we observed extreme similarities. With regards to (-)-cis1, there is a nearly identical band around 410 nm due to transitions 23, 25, 26 and 33 in \textit{vacuo} and 24, 26, 30 and 34 in site. However, the TPCD signal is opposite around 360 nm due primarily to transition 46 in \textit{vacuo} and 49 in site and the shoulder specific to the conformer in vacuo at 435 nm. These spectral differences would allow detection of binding even in the event of a spectral shift. On the other hand, the TPCD spectra of (-)-cis2 are nearly identical across the spectrum in \textit{vacuo} and in site. The bands in the 350-400 nm region are associated mostly with transitions 46 and 50 both in \textit{vacuo} and in site. The dominant transition in the region between 415 and 465 nm changes from 12 to 20 causing the 20 nm blue shift of that band. Similarly, there is a 20 nm blue shift from 485 to 470 nm due to the dominant transition shifting from 4 to 5 after optimization in the site. Analysis of the physical changes reveals the fact that the ring moves significantly, but the chain remains virtually unchanged for (-)-cis1 and vice versa for (-)-cis2. This, paired with the minor changes in the spectra suggest that the (-)-cis isomer is unlikely to undergo a significant conformational change in order to bind to CB1. This conjecture explains therefore, its low binding affinity and therefore potency. The spectra of the conformers in site are significantly different, when compared, though, proving that TPCD could be used again to detect the conformation of the isomer in site.
Figure 2.7. Comparative plots of ECD (left) and TPCD (right) spectra of (-)-cis-Δ9-THC conformers. Dotted lines correspond to the structure optimized in gas phase, solid lines correspond to the structure optimized in the receptor site. ECD response for the first 100 excited states were modeled with TD-DFT/B3LYP/6-311++G(d,p) in methanol using Gaussian 09. TPCD response for the first 50 excited states were modeled with TD-DFT/B3LYP/6-311++G(d,p) in gas phase using Dalton 2013. Colored arrows indicate regions of the corresponding spectra that could allow for clear identification of which conformer is present. Shaded region of ECD spectra is experimentally unreliable.

Comparison of the ECD spectra of both conformers of the reportedly non-psychoactive component of marijuana, CBD, reveal seemingly significant differences (Figure 2.8). Once again, however, if either spectrum were to undergo a spectral shift, the conformers would be experimentally indistinguishable. Furthermore, comparison of the conformers of each molecule in site confirms that while identification of the cannabinoid may be possible, conformation would be ambiguous. The main difference between the TPCD spectra of CBD and those of THC and (-)-cis is the increased amplitude of the signal, which is between 3 and 4 times greater for CBD. Additionally, recall that CBD undergoes the most significant physical changes upon binding; these properties can be attributed to the increased degree of freedom associated with the unrestricted cyclic scaffold on CBD which can explain why CBD has a higher binding affinity than the other
cannabinoids. It is likely energetically favored for this molecule to bind since it has a large degree of freedom.

**Figure 2.8.** Comparative plots of ECD (left) and TPCD (right) spectra of CBD conformers. Dotted lines correspond to the structure optimized in gas phase, solid lines correspond to the structure optimized in the receptor site. ECD response for the first 100 excited states were modeled with TD-DFT/B3LYP/6-311++G(d,p) in methanol using Gaussian 09. TPCD response for the first 50 excited states were modeled with TD-DFT/B3LYP/6-311++G(d,p) in gas phase using Dalton 2013. Dominant transitions are labeled, colored arrows indicate regions of the corresponding spectra that could allow for clear identification of which conformer is present. Shaded region of ECD spectra is experimentally unreliable.

The TPCD spectra for CBD1 have similar shapes in vacuo and in site. However, the signals are opposite in sign (around 430 nm) due to the negative contributions of transitions 18, 22 and 26 in vacuo and the positive contributions of transitions 20, 25 and 26 in site. These drastic changes, coupled with the fingerprints for conformer 1 around 400 and 460 nm and for conformer 2 around 430 nm would allow for experimental identification of the conformers. Similar to what was observed for THC, the intensification of signal around 400 nm and appearance of a positive band around 430 nm can be attributed to σ→σ* transitions in the alkyl chain and the appearance of bands between 460 and 540 nm indicate π→π* transitions associated with the rotation of the cyclic
scaffold. On the other hand, the TPCD spectra of CBD2 are similar in *vacuo* and in site and the conformers would be challenging to distinguish. However, the disappearance of the signal around 530 nm due to the change in dominant contributions from 3 to 1 would indicate the presence of this conformer in site and again could be attributed to low energy $\pi \rightarrow \pi^*$ transitions. There is also a slight red shift (about 10 nm) in the peak around 400 nm due to the transitions 34 and 37 in *vacuo* changing to 29 and 33 in site. This common fingerprint would allow the conformers to be distinguished from THC or (-)-cis, but since the signal for both conformers in site is positive across the spectrum, the conformation of CBD would be challenging to identify in site.

2.5 Conclusions

The *receptor cutting method* makes *ab initio* calculations on small portions of large proteins achievable. By using frozen alkyl chains to restrict the movement of amino acid residues in the receptor site, ligand binding can be accurately and reliably modeled using quantum mechanics. TPCD was confirmed to be more sensitive than ECD to the conformational changes of cannabinoids associated with binding to CB1. TPCD may act as a method for identifying not only which cannabinoid is bound, but also what conformation it is in. Our results have shown evidence to support the scenarios in which the varying effects of cannabinoids may be attributed to the conformational changes experienced by both the receptor and the ligand during binding. RCM is expected to contribute to the field of quantum biology and help in the fundamental understanding and modeling of the mechanisms of ligand docking to protein receptors.
2.6 References


40. Liu, H.; Patel, R. Y.; Doerksen, R. J., Structure of the cannabinoid receptor 1: homology modeling of its inactive state and enrichment study based on CB1 antagonist docking. MedChemComm 2014, 5 (9), 1297-1302.


Considering the existent challenges with measuring the TPA and TPCD of cannabinoids, we decided to embark on the study of a different compound that is highly involved in the chemistry of the human brain. Thioflavin T (ThT) is a commonly used fluorophore for imaging of amyloid fibrils associated with Alzheimer and Parkinson’s diseases. However, it has yet to be used \textit{in vivo} since it is excited at \(~340\) nm, a wavelength than cannot penetrate living tissue. Nonetheless, in addition to the attributes that make it an excellent dye for \textit{ex vivo} and \textit{in vitro} bioimaging, ThT has substantial \(\pi\)-electron delocalization and intramolecular charge transfer architecture, making it an excellent candidate for multiphoton imaging. This could introduce the potential of ThT as an \textit{in vivo} bioimaging dye.

In this chapter, we report the systematic characterization of the two-photon absorption of ThT in different mixtures of glycerol/water solution. The relationships of TPA peak position and amplitude revealed a dependence on particle size suggesting that the curious trend observed in TPA with changing glycerol content could be attributed to the presence of micelle-type aggregates. Consequently, the relatively strong TPA cross-section \((\delta_{\text{TPA}} = 300\text{GM})\) obtained in \(8.75\%\) glycerol/water solutions could be attributed to the immobilization of dye molecules and the strong coupling of the molecular transition dipoles in micelle-type aggregates. This enhancement of TPA, in addition to the already reported significant fluorescence quantum yield of ThT attached to brain
tissue, is expected to boost the application of this compound for *in vitro* and perhaps *in vivo* high-resolution multiphoton bioimaging of amyloids in brain tissue.

### 3.1 Introduction

Thioflavin T (ThT) has become a commonly used dye for *in vitro* and *ex vivo* fluorescence imaging of fibrils associated with Alzheimer’s and Parkinson’s diseases since its first study and application completed by Vassar and Culling in 1959. In their initial work, these authors described the dramatic increase and red shift in fluorescence emission of ThT upon selective binding to amyloid fibers. The fluorescence quantum yield ($\Phi$) of ThT increases from nearly zero in aqueous solution to approximately 0.40 in amyloid fibril. The mechanism of this enhancement has been investigated and largely attributed to the restriction of torsional motion between the benzothiazole and aminobenzene rings when the dye binds to the amyloid. Several other effects on fluorescence enhancement have also been proposed including morphological differences in fibrils, stabilization of charge on the benzothiazole ring and aggregation of the dye molecules, among others.

The selectivity of the dye to bind to amyloids, ease of administration and its non-toxic nature, in addition to its extended $\pi$-electron delocalization and intramolecular charge transfer architecture, make ThT an excellent candidate for multiphoton imaging of amyloid fibrils, which could introduce additional advantages. As it is already well known, two-photon induced fluorescence bioimaging allows for the use of lower excitation energies (longer wavelengths) than for linear processes, making it possible to induce the excitation of the fluorophore in a region where biological tissues are transparent. Consequently, the negligible linear absorption and the minimization of scattering in this spectral region opens a path to greater penetration depth.
Additionally, the quadratic dependence of TPA cross section on incident irradiance yields increased spatial resolution\textsuperscript{16}, and using longer wavelengths in bioimaging reduces photobleaching of the fluorophore and photodamage to living cells.\textsuperscript{17} These advantages have already found a variety of practical applications in the fields of biology\textsuperscript{17-18} and medicine.\textsuperscript{19}

In an effort to expand the application of multiphoton bioimaging in the understanding and diagnosis of Alzheimer’s and Parkinson’s diseases, herein we report on the systematic experimental analysis of the TPA and fluorescence upconversion of Thioflavin T in glycerol/water solutions. Our results show the potential of ThT as a two-photon fluorescent probe for in vitro multiphoton bioimaging of amyloids in brain tissue and may lead to its application in vivo.

3.2 Experimental Methods

Thioflavin T (ThT) (97\%) was purchased from AnaSpec and used without any further purification. The linear absorption spectra were measured in aqueous solutions at various concentrations ranging from 9.3 µM to 1 mM and different glycerol content (0-35\%), between 190 and 1100 nm on an Agilent 8453 Diode Array UV-Vis single-beam spectrophotometer in a 1 cm quartz cuvette. Contributions from the solvents and the cell were subtracted from the spectra. The fluorescence excitation and emission spectra were measured in the same solutions on an FLS980 spectrofluorimeter (Edinburgh Instruments Ltd), using a 1 cm path length spectrofluorimetric quartz cell. The excitation anisotropy spectrum of ThT was obtained upon emission at 420 nm in a 0.1 mM solution in ethylene glycol to inhibit molecular rotational relaxations. The emission intensity was measured through a polarizer oriented parallel (I\parallel) and perpendicular (I\perp) to the direction of the polarized excitation.\textsuperscript{20} Fluorescence quantum yield measurements were made at 25°C via the comparative method using lucigenin (\(\Phi_f = 0.67\)) as a reference.\textsuperscript{21} Lucigenin was chosen as a
standard since its quantum yield is known and it has a similar excitation wavelength (368 nm) to ThT. Particle sizes were obtained using the Zetasizer nano ZS90, utilizing the He-Ne laser (633 nm) light source, in a solution with concentration of 1 mM and using a 1 cm glass cuvette. The solvents were filtered with syringe filters in order to remove macro-size particles. The glycerol solvent was filtered with a 2 μm Whatman syringe filter, and water was filtered with a 0.2 μm Whatman syringe filter before adding the ThT solute. Then, the solution was sonicated until there was no evidence of large particles in solution.

The open aperture Z-scan method\textsuperscript{22} was used to perform the nonlinear two-photon absorption (TPA) measurements at room temperature. Samples were prepared in water and glycerol/water mixtures at a concentration of 1 mM. Two-photon excitation was induced with a computer-controlled femtosecond optical parametric amplifier (OPerA Solo) pumped by an amplified laser system from Coherent, Inc. The whole system is capable of generating 90 fs (FWHM) pulses over a wavelength range that spans from 290 nm to 2.6 μm, with pulse energies of up to 4 mJ and a variable repetition rate ranging from 1 Hz to 1 kHz. Experiments were performed between approximately 450 to 850 nm, with typical input energies ranging from 7 to 45 μJ/pulse at a 50 Hz repetition rate to avoid any contribution from accumulative effects. The pulse width was determined using a single-shot autocorrelator from Coherent, Inc. and a frequency-resolved optical gating (FROG) from Swamp Optics LLC.

3.3 Theoretical and Computational Methods

The molecular structure of ThT was optimized from an initial planar geometry using Density Functional Theory (DFT)\textsuperscript{23} in GAUSSIAN 09\textsuperscript{24} using the Becke’s three-parameter exchange, Lee, Yang and Parr correlation (B3LYP) hybrid functional\textsuperscript{25-27} and the 6-311++G(d,p)
The first 100 excited states and molecular orbitals were performed using Time-Dependent DFT (TD-DFT)\cite{23} with the Coulomb-attenuating method (CAM)-B3LYP exchange correlation functional (XCF)\cite{30} and the same basis set as for the optimization. The CAM-B3LYP XCF was used because it has been proven more reliable than B3LYP in molecules with significant intramolecular charge transfer (ICT).\cite{30,31,32} Solvent effects for water were considered under the polarizable continuum model (PCM).\cite{33}

The TPA response (degenerate case) for the lowest 20 electronic excited states of the optimized structure \textit{in vacuo} was computed with Dalton 2013\cite{34}, using TD-DFT at the CAM-B3LYP/6-311++G(d,p) level of theory. TPA spectra were calculated using\cite{35,36}

\[
\delta_{0f}^{\text{TPA}}(\omega) = \frac{4\pi^3 a_0^5}{c} \sum_f (\hbar\omega_f)^2 \delta_{0f}^{\text{TPA}}(\omega_{0f}) g(2\omega, \omega_{0f}, \Gamma),
\]

\[
\delta_{0f}^{\text{TPA}}(\omega) \approx 1.25273 \times 10^{-2} \times \omega^2 \sum_f g(2\omega, \omega_{0f}, \Gamma) \cdot \delta_{0f}^{\text{TPA}}(\omega_{0f})
\]

Here $c$ is the speed of light in vacuum, $a_0$ is the Bohr’s radius, $\alpha$ is the fine structure constant, $E = \hbar\omega$ is the photon energy (half of the transition energy for the degenerate case), $\delta_{0f}^{\text{TPA}}(\omega_{0f})$ is the orientational averaged two-photon probability for the degenerate case, and $\omega$ is the excitation frequency. A normalized Lorentzian lineshape function ($g(2\omega, \omega_{0f}, \Gamma)$) was employed to broaden electronic transitions (Equation 3.3).
where $\Gamma$ is the FWHM linewidth for all transitions, in this specific case, 0.12 eV to best fit experimental spectra. The TPA calculated spectra are in Göppert-Mayer units (GM), i.e. $10^{-50} \text{cm}^4 \cdot \text{s.molec}^{-1} \cdot \text{photon}^{-1}$ when atomic units are used for the elements of Eqs. 3.1 and 3.2.

3.4 Results and Discussion

Figure 3.1 displays the normalized excitation, absorption and emission spectra of an aqueous solution of ThT at a concentration of 0.625 mM. It also shows the excitation anisotropy (ThT/ethylene glycol) and the calculated one-photon absorption (OPA) spectrum. These results are in agreement with the linear optical properties of ThT described in the literature$^9$, where the excitation, absorption and emission maxima are located at 340, 410 and 460 nm, respectively. In the fluorescence anisotropy spectrum, one can identify three different spectral regions corresponding to $S_0 \rightarrow S_1$, $S_0 \rightarrow S_2$ and $S_0 \rightarrow S_n$ at 350-370 nm, 300-320 nm and 260-290 nm, respectively. According to this outcome, one could anticipate a strong TPA in the 300-320 nm range, a region that corresponds to a transition between states of equal parity ($S_0 \rightarrow S_2$). Although ThT is not a centrosymmetric molecule, and it is known that selection rules are more relaxed in such a scenario, considering parity restriction is still essential.
Figure 3.1. Normalized excitation (purple dash-dotted line), one-photon absorption (blue dashed line), and emission (green dotted line) spectra of ThT in water. Theoretical one-photon absorption (blue solid line) and excitation anisotropy (red solid line) for ThT in ethylene glycol. The theoretical spectrum was calculated using TD-DFT/CAM-B3LYP/6-311++G(d,p) in water using PCM for the first 100 electronic excited states of the optimized structure. The Lorentzian convolution was obtained using a linewidth of 0.35 eV (FWHM) to best fit the experimental spectrum. The inset exhibits the molecular structure of ThT.

Interesting to note is the poor overlap between excitation and absorption. Chen and co-workers attribute this effect to a degradation of pure ThT into three kinds of derivatives (dThT-1, dThT-2, and dThT-3) after storage or by UV irradiation. Specifically, dThT-1 is responsible for the emission observed at approximately 450 nm when excited at 340 nm. Emission is not observed in pure ThT, dThT-2, and dThT-3 unless the molecules are bound to amyloid fibrils. This suggests an additional advantage for using ThT as a TPA biomarker; since UV light is not used for excitation, one would only expect to see fluorescence from bound molecules.
Next, in order to truly assess the potential application of ThT for two-photon bio-imaging, we measured the full TPA spectrum in a 1 mM aqueous ThT solution. In Figure 3.2 we present the experimental and theoretical TPA spectra along with the associated transitions. First, it is important to point out the remarkable spectral match between theory and experiment. The theoretical spectrum was slightly red-shifted (+30 nm) to obtain the best theoretical-experimental overlap (this is common practice in theoretical-experimental works).\textsuperscript{31, 38-41} Interesting to highlight is the fact that most of the TPA spectrum, within the regions of highest measurable TPA cross section, is determined by the transitions to the first and fourth excited states. Second, two of the three prominent bands observed in the theoretical spectrum (760 nm and 540 nm) can be distinguished in the experimental spectrum. However, because of the occurrence of linear absorption below 480 nm the third theoretical band centered at 410 nm cannot be accessed experimentally. The presence of the band at approximately 540 nm (equivalent to a 270 nm excitation by one-photon) was anticipated based on excitation anisotropy (see Figure 3.1). Finally, the presence of the two relatively important TPA bands is ascribed to the significant intramolecular charge transfer (ICT) associated with transitions to the first and fourth excited states. It is well-known that a large charge transfer within a molecule can be associated with a large electric transition dipole moment, which favors TPA absorption.\textsuperscript{42} Our hypothesis can be corroborated by a close inspection of the molecular orbitals involved in the dominant transitions, those to the first and fourth excited states (Figure 3.3). While the former mainly involves a transition between HOMO and LUMO, the latter is dominated by the following two transitions: HOMO-3 $\rightarrow$ LUMO (21%) and HOMO $\rightarrow$ LUMO +2 (55%). In both cases, one can observe a relatively strong ICT. This effect is more pronounced between HOMO-3 and LUMO where a significant charge transfer occurs from the benzothiazole
to the aminobenzene ring, and between HOMO and LUMO +2 where the charge transfer looks significant but in the opposite direction within the molecule.

**Figure 3.2** Theoretical and experimental TPA of ThT. Experimental TPA was obtained in a 1 mM aqueous solution. The theoretical spectrum was computed for the first 20 electronic excited states with TD-DFT/CAM-B3LYP/6-311++G(d,p) in vacuo using Dalton 2013 and was spectrally shifted (+30 nm). The Lorentzian convolution was obtained using a linewidth of 0.12 eV (FWHM) to best fit the experimental spectrum. Transitions 1 and 4 are indicated as the main contributors to the measurable bands around 760 nm and 540 nm. The inset presents a closer view of the TPA spectrum from 460 to 820 nm.
Figure 3.3. Molecular orbitals (MOs) of ThT modeled using GAUSSIAN 09 at the CAM-B3LYP/6-311++G(d,p) level of theory in water. Arrows represent the first (95% HOMO → LUMO) and fourth (21% HOMO -3 → LUMO and 55% HOMO → LUMO +2) two-photon excited states at their corresponding maximum absorption.

Although the initial measurements of the TPA spectrum on ThT in aqueous solutions seem promising, a maximum cross section of 60 GM at 540 nm is still low for practical applications in nonlinear bioimaging. However, considering the restrictive environment available for ThT in amyloid plaques one could, in principle, anticipate a larger TPA cross-section in addition to the greater fluorescence quantum yield for attached dye molecules. While the latter has already been proven by Turoverov et al, TPA has never been attempted under these conditions.
In order to prove this point, we started by measuring the quantum yield of ThT in glycerol/water solutions of varying composition. As shown in Figure 3.4, the quantum yield of ThT increased linearly from 0.006 in water to 0.068 in a 35% glycerol aqueous solution. This result is not totally unanticipated considering previous results\(^8\) and the fact that as the glycerol content is increased, the viscosity of the mixture increases, restricting the degree of freedom of ThT in solution.

![Quantum yield vs Glycerol content](image)

**Figure 3.4.** Quantum yield was determined at 25°C via the comparative method using lucigenin as the reference. Lucigenin has a quantum yield of 0.67 and was chosen since it has a similar excitation (368 nm) to ThT.\(^{43}\)

Next, we measured the TPA spectra of ThT in similar glycerol/water solutions of varying composition. Figure 3.5 displays the TPA spectra of ThT in these solutions. At first, one cannot identify any obvious dependence of the TPA cross-section with the content of glycerol in the solvent mixture. It is clear that the TPA cross section does, indeed, change with glycerol content, but in a not linear fashion. Interesting to highlight, is the fact that the TPA cross section is the
highest in a solution with 8.75% glycerol. Additionally, the peak position corresponding to the second transition also varies with glycerol content. This seems to indicate that TPA also presents a dependence with viscosity.

Figure 3.5. Two-photon absorption spectra of ThT ($\delta_{\text{TPA}}$) in varying glycerol content. TPA was measured using the open-aperture z-scan method with an amplified femtosecond laser (Coherent, Inc.).

In 2014, Manners and co-authors presented multicompartment micelles in which the emission of each segment was controlled to produce any color in the visible range. Additionally, Krishnamoorthy et al reported that ThT preferred to reside inside anionic micelles resulting in an enhancement in the fluorescence emission by more than an order of magnitude. Furthermore, Singh and co-workers showed that the ThT forms micelles in aqueous solutions due to the combination of polar and hydrophobic regions within the molecule. These findings, in combination with the observed enhancement of the TPA cross section, led us to reconsider our first hypothesis based solely on viscosity and to contemplate the possibility of formation of aggregates, which could indeed affect both properties. In order to examine this possibility, we measured particle size using dynamic light scattering (DLS) in the solutions used to obtain the
TPA spectra. In fact, in the aqueous solution, particles with diameter of approximately 620 nm were detected, confirming the presence of micellar-type aggregates. An interesting relationship between micelle size and glycerol content is reported in Figure 3.7. The smallest particle size was measured in the 8.75% glycerol solution, which corresponds to the solution exhibiting the highest TPA cross section. This outcome suggests that the curvature of the micelles formed in this specific solution induce an optimal intermolecular distance for a strong coupling of the molecular transition dipole moments due to the dihedral angle afforded (approximately $37^\circ$) within each molecule that is optimal for ICT.\textsuperscript{10} Also, important to note is the similar particle size for solutions with 5 and 12% glycerol content as well as those with 17.5 and 35%. Both, the amplitude and peak positions of the most intense measureable TPA bands are comparable within these sets of glycerol/water solutions (see Figure 3.8). The average micelle size for the 5% and 12% glycerol aqueous solutions were 355 and 320 nm, respectively. Their peak positions were 580 nm and 590 nm, correspondingly and the cross sections of 63 and 68 GM, respectively. This relationship is not clear for the 17.5% and 35% set since the two peaks are not clearly distinguishable in the spectrum of the 35% glycerol solution. In an effort to clarify this point, we attempted to separate the contributions of the different bands upon deconvolution. We were able to establish that the peak position and amplitude of the bands of the 35% solution are more noticeably comparable to that of the 17.5% band (the deconvoluted spectrum is presented in Figure 3.6).
Figure 3.6. Deconvoluted TPA spectrum of ThT in 35% glycerol. The raw spectrum (solid blue line) was deconvoluted to give Peak 1 (blue dotted line) and Peak 2 (solid green line). The peak position and amplitude from Peak 2 were used to compare with 17.5%.

Interesting to highlight is the fact that for both solutions the average particle size was approximately 420 nm, the peak position 560 nm, and the TPA cross-sections were 51 and 66 GM for the 17.5 and 35% solutions, respectively. These results further support the notion that micelle formation is responsible for the change in TPA spectrum and that different glycerol/water ratios cause micelle formation with different sizes, thus affecting the corresponding dihedral angles and intermolecular distances between molecules. Since the ICT and intermolecular coupling of ThT molecules in these solutions (17.5% and 35% glycerol/water solutions) is similar, the TPA peak position and amplitude are also similar.
Figure 3.7. Average particle size as a function of glycerol content.

Finally, the results displayed in Figure 3.8 revealed an inverse relationship between micelle size and peak amplitude, and a blue shift of the peak associated with the $S_0 \rightarrow S_2$ transition as micelle size become larger. These effects are attributed to differences in the dihedral angle between the benzothiazole and aminobenzene ring when micelles form in different aqueous solution with varied glycerol content. As a result, a change in ICT takes place as predicted theoretically in reference 10. In a purely aqueous solution, dihedral angles would not be restricted while in more viscous solutions, the larger size of the ThT micelles are more likely to contain molecules with varying dihedral angles. Based on our results, we may consider that ThT molecules form micelles in 8.75% glycerol/water solution immobilized nearest the $37^\circ$ dihedral angle, the optimum dihedral angle for strong ICT.$^{10}$
Figure 3.8. TPA peak position (a) and cross section (b) as a function of micelle size.

3.5 Conclusion

We have shown, for the first time to our knowledge, the full theoretical and experimental TPA spectra of ThT in pure aqueous solution and in glycerol/water solutions of different compositions. We demonstrated that while viscosity enhances the fluorescence quantum yield of ThT in solution, the formation of relatively small micelle-type aggregates could be responsible for the observed enhanced TPA in glycerol/water solutions. This is due to the strong coupling of the molecular transition dipoles of ThT molecules in that arrangement. The combination of the relatively high TPA cross-section ($\delta^{TPA} = 300 \text{ GM}$) at 540 nm in an 8.75% glycerol/water solution and the already reported fluorescence quantum yield (0.4) of ThT bound to amyloid plaques,\(^8\) may
lead to potential application of ThT as a two-photon biomarker for \textit{in vitro}, and perhaps, \textit{in vivo} multiphoton bioimaging of amyloids in brain tissue.
3.6 References


CHAPTER 4: TWO-PHOTON ABSORPTION SPECTROSCOPY ON CURCUMIN IN SOLUTION: A STATE-OF-THE-ART PHYSICAL CHEMISTRY EXPERIMENT


After completing these two studies in nonlinear optics, I realized I had been exposed to phenomena, instrumentation, and theoretical methods I had little or no knowledge of before entering graduate school. So far, my experience had transformed the way I thought about what it means to *do* science. With a passion for teaching, I wanted to bring these state-of-the-art and modern scientific practices to the undergraduates in the Physical Chemistry lab I was teaching so they might experience some part of what I had experienced. I thought that by teaching them these higher-level concepts and introducing them to state-of-the-art instrumentation I could not only better prepare them as future scientists, but could give them an idea about what it was like to do research and get them excited about it.

In this chapter, we report on the implementation of the open aperture Z-scan method for measuring two-photon absorption (TPA) as a laboratory experiment for the undergraduate Physical Chemistry Lab. The objectives of this experiment are to: i) expose undergraduates to the concept of TPA, ii) to teach the principles of the Z-scan technique and iii) to allow students the opportunity to measure TPA cross section of a naturally occurring biomarker, curcumin, using open-aperture Z-scan. After completion of the experiment, students can compare and contrast the processes of one- and two- photon absorption as well as fluorescence and upconversion. In addition, they can explain the basic experimental “apparatus” (experimental arrangement) and procedure for
performing the open-aperture Z-scan. Assessment of student perception showed that students appreciated the opportunity to be exposed to techniques that build on what they learn in the classroom and have applications in research labs as well as practical settings.

4.1 Introduction

In an increasingly competitive global economy, the United States has been encouraging growth in the number and quality of STEM graduates for over 50 years. However, with the current technological growth of society, undergraduate chemistry students are challenged to learn not only traditional concepts and instrumentation covered in their coursework, but techniques commonly used in research and industry as well. In fact, faculty teaching upper division chemistry courses (i.e. analytical and physical) identified Research Experience as a goal for the laboratory course in a recently developed survey. Students in these courses are generally majors who will be required to be familiar with modern techniques used in a professional research setting.

Spectroscopy experiments are common in physical chemistry laboratories for teaching the quantum mechanical concepts covered in traditional lecture courses (i.e. in Ref. 6) and introducing upper division students to instrumentation (i.e. in Ref. 7). However, experiments such as these generally use techniques that are already covered thoroughly in undergraduate lectures and laboratories (i.e. NMR, UV-Vis and Raman) and few expand to more state-of-the-art methods and instrumentation.

In an attempt to expand and introduce modern instrumentation and methods into the physical chemistry lab, we developed and implemented a two-photon absorption lab using an amplified femtosecond laser system with the objectives to i) teach undergraduates principles and potential applications of multi-photon absorption, ii) teach the principles of the Z-scan technique,
the most reliable and simple experimental method to measure the two-photon absorption cross-section of organic molecules and iii) to give students an opportunity to measure the two-photon absorption cross-section of curcumin using an amplified femtosecond laser. Our hope in incorporating this technique into an undergraduate course is to engage students in an experiment that is interesting and inspiring while promoting critical thinking.

4.2 Theory of the Experiment

Several primary\textsuperscript{8-10} sources of basic concepts as well as supplementary references\textsuperscript{11-13} are provided to the students prior to lab. They are expected to read at least the primary references and understand as much as possible before coming to lab. However, we offer a 30-minute lecture when they arrive to solidify key ideas and clear any misconceptions. The following information is emphasized during this lecture.

4.2.1. Two-photon absorption and upconversion

Two-photon absorption (TPA) is the simultaneous absorption of two photons of equal energy (in the degenerate case) in order to excite a molecule to a higher energy level. It is a third-order nonlinear process that differs from one-photon absorption (OPA) in that the probability of a transition does not depend on the incident intensity linearly, but quadratically. Whereas in OPA, the energy of the photon must be equal to the energy difference between the ground and excited states ($\Delta E = h\nu$), in TPA, the sum of the energies of the photons must add up to the difference in
energy between the two states ($\Delta E = 2h\nu$ in the degenerate case). This comparison is depicted in Figure 4.1.

![Figure 4.1. Comparative schematic between OPA, Fluorescence and TPA and upconversion fluorescence.]

Since OPA and TPA are dependent on the first- and third-order susceptibilities, respectively, they follow different sets of selection rules for transitions between states. Students may already be familiar with this concept if IR and Raman spectroscopies have been previously discussed. For example, in a centrosymmetric molecule, electronic transitions are mutually exclusive. In OPA, a transition must occur between states of opposite parity (i.e. gerade ($g$) to ungerade ($u$) or vice versa) while in TPA, a transition must occur between two states with the same parity (i.e. $g$ to $g$ or $u$ to $u$).\textsuperscript{14-15} Therefore, in the case of a centrosymmetric molecule, if a one-photon transition is allowed between two states, a two-photon transition will not be.

Although OPA and TPA occur to different excited states, the relaxation process may be the same. In OPA, after vibrational relaxation, the molecule can return to the ground state via fluorescence or some other non-radiative process. On the other hand, in the case of TPA, the molecule may be excited to a higher energy level (i.e. $S_2$), but after relaxation to $S_1$ may return to $S_0$ by the same process. If the molecule relaxes via fluorescence after TPA, the process is referred
to as *upconversion*. This term is especially important to point out to students since in the case of two-photon excitation the energy of the individual photons involved in the transition is lower than that of the photon that is emitted (i.e. $E_{ex} < E_{em}$). This disobeys the phenomenon they may already be familiar with: the Stokes shift in which $E_{ex} > E_{em}$.

### 4.2.2. Z-scan Method

The requirement for high incident intensity and necessity to measure the TPA at different wavelengths indicates that a tunable, pulsed laser source is needed to measure the TPA. The simplest and most reliable method is the open-aperture Z-scan.\textsuperscript{12-13} The experimental arrangement of the open-aperture Z-scan is shown in Figure 4.2. The beam is passed first through a beamsplitter so that part of the beam is sent to a reference detector in order to account for fluctuations in the intensity of the beam between pulses. The remainder of the beam is directed through a focusing lens and the sample is translated across its focal region. The beam is then fully collected and the change in transmittance with the sample position is attributed to multi-photon absorption.

![Figure 4.2. Experimental setup of the open aperture Z-scan.](image)

The normalized transmittance data is fit to the following equation:

$$T_0(z) = \frac{T(z)}{T_{ref}}$$

The normalized transmittance data is fit to the following equation:
\[ \Delta T(Z) \approx 1 + \frac{\beta I_0 L_{\text{eff}}}{2 \sqrt{2}} \left( \frac{1}{1 + \frac{Z^2}{Z_0^2}} \right) \]

where \( \beta \) is the two-photon absorption coefficient, \( I_0 \) is the peak irradiance at the focus, \( L_{\text{eff}} \) is the effective sample length (the actual sample length can be used if the sample is transparent), \( Z \) is the position of the sample with respect to the focal point and \( Z_0 \) is the Rayleigh range \((n \pi w_0^2/\lambda)\) which can be determined by performing a closed aperture Z-scan\(^1\). The TPA molecular cross section (\( \delta \)) is then calculated, in cm\(^4\)/photon/s, by its relationship to \( \beta \) by the concentration of the sample, i.e. \( \delta = \frac{h \nu \beta N A d_0 \cdot 10^{-3}}{1} \). TPA cross section has units of Göppert-Mayer (GM), where 1 GM = 1\(0^{-50} \) cm\(^4\) s photon\(^{-1}\) molecule\(^{-1}\). Further emphasis is put on the low probability of occurrence of transition by pointing out the units of \( \delta \). The units are a result of the product of two areas (one for each photon) and the time in which both must arrive to be considered “simultaneous”. The large scaling factor is a result of the use of the molecular cross-section as opposed to \( \beta \), which is typically used to describe bulk materials.

**4.2.3. Advantages and Applications**

TPA offers many advantages over OPA. Since absorption only occurs at the focal point of a laser beam, spatial resolution and depth penetration are improved. This opens applications in

---

\(^1\) The phase shift of the beam is measured by introducing an iris before the detector. Any variation in the amount of light falling on the detector will be caused by the change in nonlinear refractive index as the sample is translated through the beam.
microfabrication, data storage and optical limiting. In addition, since lower energies are used for excitation, biological applications, such as bioimaging can take advantage of TPA as well.

4.3 Experimental Procedure

For linear characterization, students prepared a $3 \times 10^{-5}$ M solution of curcumin in tetrahydrofuran (THF). Linear absorption was measured on an Agilent 8453 Diode Array UV-Vis and fluorescence was measured using a USB-2000 Ocean Optics spectrometer in a perpendicular arrangement.

A second solution of curcumin in THF (0.025 M) was prepared for nonlinear characterization. The two-photon absorption cross section of this solution was measured using the open aperture Z-scan method. Two-photon excitation was induced using a computer-controlled femtosecond optical parametric amplifier (OPerA Solo) which is pumped by an amplified laser system from Coherent, Inc. The experiment was performed at 740 nm using 90 fs (FWHM) pulses with energies approximately 0.096 $\mu$J per pulse at a 50 Hz repetition rate. Parameters are chosen to obtain no more than 20% absorption. The sample is translated through the beam several times and the transmittance data is averaged. While the sample is translating, students are escorted into the lab in groups of four to observe the measurement. They get a short (approximately 20 minutes) tour of the lab and introduction to the equipment before observing the translation of the sample through the beam. The change in intensity of fluorescence is emphasized during this observation to illustrate the importance of the focal point. The upconversion spectra were obtained using a USB-2000 Ocean Optics spectrometer, pumped at 740 nm.
4.3.1. Hazards

Tetrahydrofuran is a skin and eye irritant, toxic upon ingestion or inhalation and is flammable. Students are required to wear a lab coat, safety goggles and gloves while preparing and handling the solutions.

Laser radiation poses serious risk of injury to the eye, the severity of which depends on the wavelength and output power. When students enter the femtosecond laboratory they must wear laser safety goggles at all times, maintain their eyes above the height of the beam and are prohibited from attempting to look directly into the line of the beam.

4.4 Results and Discussion

First, students are asked to plot their data from the z-scan at 740 nm and discuss the shape of the curve. As shown in Figure 4.3, a typical Z-scan curve appears as a “V” shape peak centered at the focal point with two “wings” on either side. As the sample translates through the beam toward the focal point, TPA increases, causing the decrease in transmittance.

![Figure 4.3. Open aperture Z-scan curve measured for curcumin in THF at λex=740 nm.](image)

Students record experimental parameters and the cross section is calculated using software (i.e. National Instruments LabVIEW) which fits the data to Equation 4.1. Using a curve like that
shown in Figure 4.3, students usually obtain a cross section for curcumin, $\delta \approx 90$ GM at 740 nm. In practice, the rest of the spectrum would be measured simply by tuning the wavelength of the laser using the OPerA Solo. However, this is not feasible for 24 students to complete in a normal 6-hour lab time, so the remainder of the TPA spectrum is provided.

Most important for the students to discuss is the comparison between the one- and two-photon processes and spectra. In order to compare the nonlinear process of TPA with OPA, students first measured OPA and fluorescence of curcumin. These are shown in Figure 4.4. Students can see that, as expected, curcumin shows OPA at 422 nm and fluorescence emission around 500 nm. This relationship between excitation and emission energy ($E_{\text{ex}} > E_{\text{em}}$) is known as the Stokes shift and students may already be familiar with the concept from previous discussions of fluorescence.

![Normalized OPA, fluorescence, TPA and upconversion fluorescence spectra of curcumin in THF solution. Linear and nonlinear spectra were obtained using solutions with concentrations $3 \times 10^{-5}$ M and 0.025M, respectively.](image)

**Figure 4.4.** Normalized OPA, fluorescence, TPA and upconversion fluorescence spectra of curcumin in THF solution. Linear and nonlinear spectra were obtained using solutions with concentrations $3 \times 10^{-5}$ M and 0.025M, respectively.

Students are asked to plot the linear spectra along with the complete TPA spectrum as well as the upconversion spectrum of curcumin pumped at 740 nm. As shown in the figure, TPA occurs
at a longer wavelength than fluorescence upconversion (i.e. $E_{ex} < E_{em}$). This is most likely the first time the students have observed this phenomenon.

Upon comparison of the linear and nonlinear spectra, students make two important observations. First, OPA occurs at a shorter wavelength than TPA, but fluorescence via one- and two-photon absorption occur at the same wavelength. This is due to the fact that the fluorescence is a result of both absorption processes and occurs from the same excited state. Second, the highest TPA peak does not occur at exactly double the wavelength of the OPA peak. While selection rules apply, they are not a perfect indication of TPA peak positions, especially since curcumin is not centrosymmetric.

4.5 Student Learning Outcomes and Perception

The TPA lab was implemented with two groups of students enrolled in the physical chemistry lab at the University of Central Florida in the Spring 2016 semester (40 students total). After performing the experiment, students were able to discuss on a post-lab quiz the fundamentals and objective of the open aperture Z-scan method, selection rules and applications of TPA such as bioimaging.

Twenty-four students responded to a survey and agreed that they were challenged to learn more than they expected (an average response of 4.1 out of 5 on a Likert scale with 5 representing “Strongly Agree”) and that what they were asked to learn was important (an average of 4.0 out of 5).

4.6 Conclusions

We have successfully incorporated the z-scan method for measuring the TPA cross section into an undergraduate laboratory course. Students were able to learn important concepts not
covered in a traditional Physical Chemistry course and understand them in the context of real-world applications. Although the instrumentation used in this lab may not be readily available in many universities, the measurements can be made with pico- or nanosecond pulses as long as the possible implications of this difference (i.e. possible excited state absorption) are discussed with the students beforehand. Additionally, even if this particular experiment cannot be implemented due to cost, the content may be conveyed in the lecture course or as a supplement to a linear spectroscopy experiment as an introduction to non-linear spectroscopy. We also hope that its success will inspire others to implement more advanced experiments in undergraduate laboratories in order to foster the learning and engagement of this new generation of chemistry students.
3.7 References

1. Memorandum of Conference with President Eisenhower After Sputnik, October 8, 1957.
CHAPTER 5 : OCTET AND BIOTEC: A MODEL OF A SUMMER INTENSIVE CAMP DESIGNED TO CULTIVATE THE FUTURE GENERATION OF YOUNG LEADERS IN STEM

Reproduced with permission from Donnelly, J.; Diaz, C.; Hernandez, F. E., OCTET and BIOTEC: a model of a summer intensive camp designed to cultivate the future generation of young leaders in STEM. Journal of Chemical Education 2016, 93 (4), 619-625. Copyright 2016 American Chemical Society

Having successfully taught undergraduate fundamentals of two-photon absorption and nonlinear spectroscopy as well as piquing their curiosity about physical chemistry research, we considered the fact that inspiring students in STEM often occurs at an earlier stage. Thus, we decided to develop an outreach activity for students in high school in order to inspire them to pursue science careers.

In this chapter, we describe an effective and tested model of a week-long summer science intensive program for high school students that aimed to i) elaborate on concepts covered in a high school chemistry or biology course and ii) provide high school students an opportunity to learn about studying and pursuing careers in the sciences. The program was developed to combine instructional sessions, hands-on activities and laboratory tours in order to review and build upon what students learn in high school. Student assessment and feedback confirms the effectiveness of this student-run program to teach and inspire high school students to pursue the sciences in their academic career.

5.1 Introduction

There is abundant evidence that American students do not perform at a proficient level in science to be promising for the future of the United States as the world leader in science and engineering. For example, The Programme for International Student Assessment (PISA) reported
in 2014 that 15-year-old Americans continue to score lower than students in 22 other countries in scientific literacy on the PISA science test. Likewise, the results in the National Condition of STEM 2014 Report, published by ACT, indicated only 37% of college-bound seniors are prepared for college-level science courses. In addition to poor performance, while interest in STEM remains high, most of the growth is occurring in the engineering and technology area, not in the pure sciences.

Several outreach activities are described in the literature that aim to overcome the aforementioned limitations of American students in science. For example, Science in Motion is a 15-year-old outreach program that brings modern equipment and instrumentation to high school students. Thomas assessed the program in 2012 and showed that it successfully improved student learning. However, while these types of programs may increase scientific literacy they do not necessarily emphasize promotion of STEM.

In an effort to promote interest, many outreach activities have been developed to engage elementary and middle school students in STEM. These activities have been successful, especially when employing university students in the implementation. However, in these instances, the outreach was not focused on students that would soon decide on a college major. Recently, Pluth and co-workers found that employing university students in outreach programs to high schools was very beneficial since undergraduate and graduate students were considered “near-peer” mentors to high school students.

Despite the clearly positive impact that these programs have had, the results of the ACT report lead us to believe that perhaps the goal of a more impactful outreach activity should be to both increase scientific literacy of college-bound secondary students while simultaneously
inspiring them to pursue the pure sciences in their academic careers. With the intention to develop a program that could achieve this goal, we considered combining the training aspect with the enrichment aspect of the outreach programs previously described. We aimed to offer an opportunity to high performing secondary students to expand on the information and opportunities provided in a high school chemistry or biology course. To meet this aim, the Orlando Chemistry Tutoring, Enrichment and Training (OCTET) Camp and the Biology Integrated Orlando Training and Enrichment Camp (BIOTEC) were designed as weeklong summer intensives to cultivate the future generation of young leaders in chemistry and biology. The camps consisted, as explained next, of a series of instructional sessions and enrichment activities facilitated by chemistry and biology graduate students at the University of Central Florida (UCF).

The shared emphases of these programs were training and enrichment. Therefore, our goals for OCTET and BIOTEC were to elaborate on the information covered in a traditional high school classroom by doing problem solving and hands-on activities, and to foster the curiosity of high school students in STEM fields by providing research laboratory tours and opportunities to interact with high school, undergraduate, graduate and faculty researchers.

Herein, we describe the OCTET and BIOTEC programs, which can easily be implemented at other universities by working primarily with graduate and undergraduate students under the guidance of a faculty advisor. The model can be adapted to any discipline. In fact, the Physics Youth Scholastic and Instructing Camp for Orlando Scientists (PhYSICOS) is also currently being developed using the same model.
5.2 History of OCTET

The first OCTET camp was run in 2011 and again in 2012. In these instances, the program was held on Saturdays over a period of seven months during the academic year. In this implementation of the camp, retention rates were rather low over the entire course (about 70%). In 2015 OCTET was redesigned as an intensive program in which students attended sessions from 8:30 a.m. to 4:00 p.m. for one week (Monday through Saturday) during the summer. In addition to OCTET, in 2015 BIOTEC was also introduced following the same intensive approach. Using this approach, we were able to increase the retention rate to over 90%. Photos of participants of OCTET and BIOTEC 2015 are presented in Figure 5.1.

Figure 5.1. OCTET and BIOTEC 2015 participants. Activities included determination of acetic acid content in vinegar using titration (top left, OCTET), indication of pH using red cabbage extract (top right, OCTET), virtual experiments performed using laptops (bottom left, BIOTEC) and dissection of a preserved cat (bottom right, BIOTEC).
5.3 Program Description

One emphasis of OCTET and BIOTEC was training. The week-long intensive schedule of instructional sessions is outlined in Figure 5.2 along with the general topics covered in each camp.

The first day of camp consisted of a morning session during which students were welcomed by the faculty coordinator, engaged in a “get-to-know-you” activity with the graduate student instructors and then took a pretest modeled after the ACS Olympiad and USA Biology Olympiad exams. The afternoon session and days 2-5 consisted of instructional sessions during which many

Figure 5.2. Schematic of the daily schedule of topics covered in OCTET and BIOTEC.
methods of instruction were employed. Instructors were encouraged to use their choice of pedagogy when planning their sessions and were prepared to improvise when students needed more explanation than what was provided during the planned lesson. This was often necessary since the participants in 2015 were a mixture of students that had taken regular and Advanced Placement-level chemistry or biology. Activities used during instructional sessions of OCTET and BIOTEC 2015 include estimation of pH using cabbage extract, explosion of a hydrogen balloon (performed as a demonstration), dissection of a preserved cat, culturing bacteria from the students’ mouths as well as problem solving in groups and Socrative© clicker questioning. The aim of combining hands-on activities with conceptual activities was to help students grasp concepts in more depth while giving them an experience that they might not get in a conventional high school setting.

During each camp, students were introduced to the laboratory to review laboratory techniques and perform challenging experiments. The OCTET experiment, for example, was modeled after laboratory practicals on the ACS Chemistry Olympiad National Exams; students performed an inquiry-style experiment in which they were required to develop a procedure to determine the concentration of acetic acid in store-bought vinegar.

According to the preliminary surveys, students were also very interested in seeing research labs and interacting with students and faculty at the university. To meet their expectations, lab tours were given on the last morning of BIOTEC and throughout the week of OCTET. In addition to interacting with the graduate student instructors each day, participants had a chance to meet and speak with undergraduate interns, graduate students, post-doctoral researchers and faculty during the lab tours. Additionally, about an hour during one of the instructional sessions of each camp
was dedicated to speakers. The students networked with high school, undergraduate and graduate student researchers working at UCF. They indicated to instructors that these opportunities exposed them to options they had never considered (i.e., participating in university research in high school and doing research that spans different disciplines).

To expand on the enrichment aspect, during a 90 minute lunch period, undergraduate student volunteers engaged participants in educational games and activities that tested basic scientific knowledge and promoted camaraderie. A lead undergraduate student was appointed to organize volunteers and order and purchase food, which also represented a good opportunity to build leadership and organizational skills.

### 5.4 Timeline and Planning

Planning began approximately twelve months prior to the start of the camp. The first step in planning the summer camps was to secure funding. OCTET and BIOTEC are relatively inexpensive programs and were offered at no cost to students to promote inclusiveness. Lunch and basic school supplies were provided to the students and t-shirts bearing the camp logo helped to establish a sense of community among participants and volunteers. To cover these costs, the faculty and graduate student coordinators secured funding from the ACS, NSF, local companies, Pittcon, and the chemistry and biology departments at UCF. However, it should be noted that if students are asked to provide their own lunches and bring their own classroom supplies, the camp could conceivably be implemented with virtually no budget.

Next, volunteers were recruited to facilitate the camp. Members of the chemistry and biology graduate student associations at UCF were enlisted to volunteer as instructors, and undergraduate members of the ACS Student Chapter volunteered to oversee educational games
and activities following lunches. Volunteers were recruited based mainly on their enthusiasm in participating in the camp along with their communications skills. Diversity is also a goal in forming the teams of volunteers. Five to ten graduate student volunteers coordinated to decide who would teach each topic, and a lead undergraduate volunteer coordinated two or three students to facilitate lunch activities each day. The faculty and graduate student coordinators obtained IRB approval for human subject research.

Detailed organization of the schedule and lesson planning by graduate and undergraduate student volunteers began seven to eight months prior to the beginning of the camps. Instructors met monthly to discuss potential activities and develop the pre- and posttests. Instructors were given freedom regarding which topics to focus on and tried to develop and implement activities that would help students understand concepts in more depth and exercise problem-solving skills. Flexibility was maintained, though, to allow the instructors to modify the difficulty and length of the activities before and during the camp. Graduate student instructors were also teaching assistants in the chemistry and biology departments so were required to complete safety as well as teaching assistantship training at the university as part of that responsibility.

Invitations for applications were distributed via science resource teachers in the surrounding counties six months prior. Teachers nominating prospective participants returned applications five months prior via e-mail to the faculty coordinator. Students could apply and be accepted to one or both camps since they are held during different weeks.

Participants were selected by the faculty and graduate student coordinators based on their performance in chemistry and biology courses. AP-level students took priority, but the camp was also offered to honors students as a preparation for AP chemistry. Participants were informed via
e-mail that they had been selected and were asked to return consent forms for participation. The planning timeline is outlined in Figure 5.3.

Figure 5.3. Summary of preliminary planning activities beginning up to one calendar year before the summer intensives.

5.5 Program Assessment

To gauge the success of the training emphasis, a pre- and posttest was administered for each camp. Both were developed by graduate student instructors and were modeled after questions on standardized chemistry and biology exams (i.e. Advanced Placement exams, Scholastic Aptitude Test subject area exams, the American Chemical Society Chemistry and USA Biology Olympiad Exams, and the American Chemical Society general chemistry exam). The reference sheet provided for the ACS Olympiad Exam was provided to participants during the pre- and
posttests for OCTET. A sample of the OCTET pretest can be found in Appendix I; posttests covered the same concepts. The OCTET pre- and posttests consisted of 18 and 17 questions respectively, with about half knowledge-comprehension level questions and half application-analysis level problems. As shown in Figure 5.4, only one student answered fewer questions correctly on the posttest, the other twelve students improved between 27 and 191%. The BIOTEC pre- and posttests consisted of 40 and 39 questions, respectively, and were focused mostly on recall knowledge and comprehension. In that case, all 16 participants improved on the posttest, with percentage improvements ranging 3–47%. To establish that the difference in percentages between the pre- and posttest were statistically different, we performed a paired t-test on the differences for each camp and found that $p = 10^{-6}$ for BIOTEC and $p = 10^{-5}$ for OCTET. Both results are significant within a 95% confidence level. This is indisputable evidence that the summer intensives were effective in improving students’ content knowledge and test-taking skills.

Upon itemized analysis of the test scores, we found that in OCTET, students initially had a significant weakness in the applied thermochemistry question. While zero students answered this question correctly on the pretest, 46% of the students answered it correctly on the posttest, indicating that OCTET was successful in increasing student understanding of difficult topics which they may not have time to fully digest in a traditional classroom. Students also improved
significantly in conceptual comprehension of topics such as periodic trends, dynamic equilibrium and most notably kinetics. There was a 156% increase in the number of students who correctly answered the question about reaction mechanisms. Students improved on application type questions as well. The most improvement on the entire posttest was on the reaction stoichiometry question. There was a 305% increase in correct responses among the students for this question. However, students struggled the most with Raoult’s law. A percentage decrease in the number of correct responses to this question might be attributed to the increased difficulty of the question on the posttest, as shown in Table 5.1.

Table 5.1. Comparison of an example pretest and posttest question

<table>
<thead>
<tr>
<th>Pretest Question</th>
<th>Posttest Question</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene and toluene form an ideal solution. The vapor pressure of benzene at 55 °C is 400 mm Hg while the vapor pressure of toluene at 55 °C is 130 mm Hg. What is the vapor pressure of a solution consisting of 0.5 mole fraction of benzene and 0.5 mole fraction of toluene at 55 °C?</td>
<td>Pentane (C₅H₁₂, M = 72) and hexane (C₆H₁₄, M = 86) form an ideal solution when mixed. What is the vapor pressure of a solution prepared by mixing equal masses of pentane and hexane? (The vapor pressures of pentane and hexane are 511 mm Hg and 150 mm Hg, respectively.)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Answer Options</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Lower than 130 mm Hg</td>
<td>150 mm Hg</td>
</tr>
<tr>
<td>(b) Between 130 and 400 mm Hg</td>
<td>330 mm Hg</td>
</tr>
<tr>
<td>(c) Exactly 400 mm Hg</td>
<td>345 mm Hg</td>
</tr>
<tr>
<td>(d) Greater than 400 mm Hg</td>
<td>511 mm Hg</td>
</tr>
</tbody>
</table>

The students may have been confused by the fact that there were two equal masses rather than equal mole fractions provided in the question on the posttest. Next year, perhaps the instructors should place more emphasis on how to recognize and approach indirect or multistep problems in standardized test questions.

In BIOTEC, students showed initial weakness in the microbiology as well as animal anatomy topics. The most notable improvement was observed in one of these topics. On average, participants answered 54 and 44% more items correctly for microbiology and plant anatomy and physiology, respectively. We did not see a decrease in the average number of correct responses for
any general topic, but the least improved were the animal anatomy and ecology topics. This itemized analysis also gives instructors good insight into what types of activities they should implement in future camps. For example, students answered the question about villi in the small intestine correctly 23% more frequently on the posttest. The activity that covered this information was highly interactive: students built a giant small intestine in the classroom and crawled through it. Perhaps this type of activity should be a model for the camps next year because it shows considerable improvement of conceptual understanding in an area where students show a weakness.

Participant perception of instructional sessions was also excellent; 96% of respondents agreed or strongly agreed that the sessions were informative and fun.

Survey responses gave significant insight regarding the effectiveness of the enrichment focus of OCTET and BIOTEC. The surveys asked specifically about three aspects of the enrichment focus of the camps: (i) lab practicals; (ii) science-related lunch games; and (iii) lab tours. As is shown in Table 5.2, these aspects of the camps were rated very highly. The average response referring to the games during lunch was relatively low, however, with scores of 3.5 and 3.9 out of 5 for OCTET and BIOTEC, respectively. The instructors noted that some of the activities they observed during lunch could have been more interactive to allow the students to be more active and take a break from instruction.
Table 5.2. Distribution of student responses to survey questions regarding enrichment aspects of OCTET and BIOTEC.

<table>
<thead>
<tr>
<th>Statements for Student Response</th>
<th>Program</th>
<th>Score Distribution&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>I found the lab practical to be informative and fun.</td>
<td>OCTET&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 0 0 6 7</td>
</tr>
<tr>
<td></td>
<td>BIOTEC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0 0 0 6 10</td>
</tr>
<tr>
<td>The science-related games during lunch were fun and enriching to my OCTET experience.</td>
<td>OCTET&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 3 4 4 2</td>
</tr>
<tr>
<td></td>
<td>BIOTEC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1 1 2 6 6</td>
</tr>
<tr>
<td>The lab tours were interesting and fun.</td>
<td>OCTET&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 0 0 2 11</td>
</tr>
<tr>
<td></td>
<td>BIOTEC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0 0 1 1 14</td>
</tr>
</tbody>
</table>

<sup>a</sup>For the OCTET program, N = 13. <sup>b</sup>For the BIOTEC program, N = 16. <sup>c</sup>The scale for response ranges from 1 (strongly disagree) to 5 (strongly agree).

Perhaps the most useful in assessing the effectiveness of the enrichment aspect were the comments on the surveys. Students mostly commented on the activities they participated in that are not offered in a traditional classroom, and activities that made them aware of what a career in science is really like.

*I was able to learn information and complete activities, like the cat dissection, that aren’t covered at school… I genuinely found this program to be an enriching experience that gave high school students like me a very realistic look into biology that isn’t restricted to a classroom setting.*

*It was helpful in influencing my future career choices and seeing what would/is available to me… OCTET was a great opportunity for me to see just how many different things someone can do in science…*

The comments from multiple students from both camps suggested that the instructors had a superbly meaningful impact.

*YOU GUYS ROCK! … Biology became so much more interesting when learning from people who really love it… …You guys have truly inspired… …The enthusiasm of the teachers made it easier to learn…*
Clearly, having graduate students act as instructors was significant to the enrichment aspect as the participants were very interested to know what the “day in the life of a scientist” was like. Likewise, the graduate students rated their experiences very high; all respondents to the instructor survey said they felt their work was very or extremely meaningful and that they would volunteer as an instructor again.

Figure 5.5 makes the impact of OCTET and BIOTEC clear. Overall, students reported that they felt more prepared to take standardized biology/chemistry exams, are more likely to consider studying the sciences in college and are more likely to consider pursuing these disciplines as careers. While the average response for the third statement rated somewhat lower on average than the other two, at least 50% of the participants from each camp agreed or strongly agreed that they would be more likely to pursue a career in science.

**Figure 5.5.** Attitude of students toward STEM after participating in BIOTEC (blue) and OCTET (red). Statement 1: I feel more prepared to take standardized exams. 2: I am more likely to consider studying biology/chemistry. 3: I am more likely to pursue chemistry/biology as a career.
5.6 Future Goals

In 2016, we plan to implement PhYSICOS using the model described here. In fact, one OCTET student commented “I would love if there was also a physics camp!” Additionally, we would like to follow up with the participants to determine how successful they are in AP courses and on standardized exams, including the ACS and USA Biology Olympiad Exams. Finally, we hope to work with surrounding universities to extend the impact of this activity beyond our own campus.

5.7 Conclusion

Based on notable improvement on assessments, and exceptionally positive feedback from both participants and volunteers, it is clear that OCTET and BIOTECH were highly effective outreach programs for training and enriching the science experience of high school students in central Florida. After participating, students have a better grasp on science concepts and are more interested in pursuing the pure sciences. We hope that OCTET and BIOTECH will serve as pilot outreach programs in the country.
5.8 References


CHAPTER 6: PRE-CURE: THE EFFECT OF A WEEK-LONG AUTHENTIC RESEARCH EXPERIENCE ON HIGH SCHOOL STUDENTS’ VIEWS OF SCIENCE

In the previous chapter, we described the development and implementation of a week-long summer chemistry intensive camp for high school students that both elaborates on concepts learned in a high school classroom and offers opportunities to learn about studying and pursuing careers in the sciences. Based on the results of the evaluation of this program and the success of course-based undergraduate research experiences (CUREs) we have implemented in undergraduate labs, in the 2017 implementation, we incorporated an authentic research experience, a pre-CURE. Participants spent 2-4 hours per day for five days in a research lab completing a simple, yet authentic research project under the guidance of graduate students. On the sixth and final day of the camp, we held a poster session in which participants shared their research with each other, their parents, and faculty members from the department. The purpose of this study was to understand the effect of this pre-CURE on participants’ views about science. In order to explore this effect, we administered the Views on the Nature of Science survey and followed up with semi-structured interviews. Results of the VNOS and interviews revealed some impact on participants’ views about the empirical and creative nature of science. A more significant impact was discovered considering participants’ practical views about what it is like to do science in an authentic research lab. Implications for practice and research on authentic research experiences for secondary students are discussed.

6.1 Introduction

As part of the ongoing effort to increase recruitment efforts into STEM disciplines, we recently described the development, implementation, and evaluation of the Orlando Chemistry Training, Enrichment, and Tutoring (OCTET) Camp. Namely, we have demonstrated the
effectiveness of OCTET in increasing high school students’ performance on standardized-inspired chemistry assessments and interest in pursuing the sciences\(^1\).

Based on our results and the National Research Council’s 2011 Report that students who had research experiences in high school are more likely to complete a STEM major,\(^2\) we incorporated a research experience into our summer camp. Our goal was to offer participants an authentic research experience (ARE) during the summer camp and to evaluate how it influenced participants’ views about science.

### 6.2 Background

#### 6.2.1. Views about Science

Secondary students and teachers alike have less than informed views about the nature of science (NOS).\(^3\) For example, novices are unlikely to recognize the role of creativity and imagination in the interpretation of results. Rather, they believe that scientists are totally objective in their analyses. Likewise, they are likely to rely solely on direct observations as sources of “proof” rather than to recognize the value of inferences and the importance of evidence. Consequently, it is of considerable importance to teach and assess students’ views of the NOS, especially if they are perspective scientists or science teachers.

Most commonly, the instruction of the NOS as reported in the literature is accomplished using an explicit/reflective method.\(^4\) This method is most often used in a classroom setting and less often accompanying an ARE and involves students in questioning, discussing, and reflecting upon NOS aspects as they are manifested in inquiry experiences, activities, or cases.\(^5\) On the other hand, implicit instruction of the NOS has been less than effective, but most of these studies have taken place in traditional classroom settings.\(^5\) In fact, there is some contention in the literature
about whether the NOS can be taught implicitly. Some argue that even high levels of inquiry experiences have limited impacts on students’ views of the NOS without explicit instruction. Others argue that these types of experiences are not truly authentic and that simply engaging in the authentic practice of science can be an explicit approach to teaching the NOS.6

In addition to understanding the NOS, the goal of many laboratory courses is to deepen understanding of content, reproduce experiments, become familiar with scientific equipment and techniques, and acquire the technical skills required of a member of the 21st-century workforce.7-8 AREs have demonstrated superior strengths in developing these valuable skills. They offer an opportunity for students to learn lab techniques, gain confidence in doing research, feel like a part of the scientific community, and acquire soft skills such as presenting and writing. In addition, the experiences are correlated with increased persistence in STEM.7, 9-12

A recent study of the long-term effects of an undergraduate research experience (URE) showed that participants of the research program felt like they had learned more about doing science and gained a better understanding of scientific research than did students in a traditional laboratory-learning environment. In addition, they were more likely to feel like they had been creative during their research experience.7

The results of studies in this area point to the importance of AREs both in developing students’ views on the NOS and the practices of science.

6.2.2. Research Experiences

The literature on the efficacy of UREs is growing with the increased emphasis on their potential for increasing students’ research skills.13-16 Undergraduates who participate in internships, apprenticeships, research experiences for undergraduates (REUs), and the myriad of
other varieties of research experiences report experiencing several benefits such as learning scientific concepts\textsuperscript{17-18} and logical and critical thinking skills\textsuperscript{11, 19} to being more intellectually curious.\textsuperscript{20} Results of this area of research, though primarily based on self-report data, suggest that undergraduates gain valuable knowledge about the reality of practicing science.\textsuperscript{7, 14, 18} For example, undergraduate participants of a 3-week summer research experience report increased positive perceptions of the practice of science as a result of their involvement in diverse laboratory and field research.\textsuperscript{14}

Recently, research experiences have expanded into undergraduate laboratory courses both in response to calls to replace standard laboratory courses with discovery-based courses\textsuperscript{21} and in an attempt to reduce barriers to AREs with few resources.\textsuperscript{17, 22-24} Indeed, we have integrated our own research into the undergraduate physical chemistry lab in a course-based undergraduate research experience (CURE)-style experiment and found positive student outcomes in terms of learning and perception of advanced physical chemistry concepts.\textsuperscript{25} CUREs are distinguished from traditional and inquiry-based laboratory courses since they involve the integration of scientific practices, discovery, broadly relevant or important work, collaboration, \textit{and} iteration. They usually involve students enrolled in a laboratory course addressing a problem with an outcome that is unknown not only to them but to the instructor.\textsuperscript{22, 26-28} Initial assessments of CURES were based predominantly on student perceptions of their gains in content knowledge, research skills, and intent to persist in science.\textsuperscript{17, 22-23} Comparison of CUREs with other types of research experiences showed similar results in student perceptions of the aforementioned gains.\textsuperscript{22} Further, when compared with other types of laboratory courses (i.e. inquiry), CURE students show superior progress in their views of the NOS as creative and process-based.\textsuperscript{29} These variations appear to be enduring over the long-term.\textsuperscript{7}
Until recently, most AREs have not been offered or available until undergraduates’ third or fourth years. With the emphasis on the importance of AREs and the incorporation of research into courses, more opportunities have now become available for first- and second-year undergraduates. However, as early as 1999, educators recognized high school students’ lack of knowledge about how scientific research is done and attributed this to their lack of exposure to authentic research environments. In addition, a recent survey of alumni of a summer science internship program for high school students showed that hands-on projects, including research experiences, had a significant influence on their decision to choose and persist in STEM. In particular, participants reported gaining a better understanding of scientific research.

Considering these results and that one of the main goals of science education is to teach students about scientific practices, research experiences earlier in the academic career may be justified. In fact, some studies show the value of AREs for high school students in developing their views on the nature of science (NOS). A recent study of a seven-week residential summer research experience for high school students compared the impact on participants’ views on the NOS when they were exposed to explicit, reflective, and implicit instruction on the NOS. The authors modified the Views on the Nature of Science (VNOS) questionnaire, administered the survey and did several interviews at the beginning and end of the experience. While their results demonstrate the strength of explicit over implicit approaches to teaching NOS, even students without explicit instruction showed gains in their views by virtue of their ARE.

In 2013, Burgin and Sadler summarized numerous summer ARE programs for secondary students. The programs range from 3-8 weeks long and vary in cost from $0-5000. Most take place over the summer with faculty acting as mentors.
A review of the research on secondary AREs reveals the heavy reliance on self-report measures of efficacy, similar to the limitation in the area of undergraduate research evaluation. Nonetheless, secondary students also report deepened understanding of science content and nature of science, fostered career aspirations in science, and increased confidence to engage in science as a result of participation in AREs.\textsuperscript{34,36} The limited number of studies in this area warrants a deeper investigation into the results of secondary AREs.

6.3 Theoretical Framework

The theoretical framework used for this study was situated learning theory and is described in depth in Chapter 1.\textsuperscript{37-38} Briefly, situated learning theory posits that there is a barrier between \textit{learning} and \textit{using} knowledge (i.e. knowing \textit{what} and knowing \textit{how}) and that the barrier is context. Brown, et al.\textsuperscript{37} emphasize the importance of authentic activities in the process of enculturation since they allow students to develop “tools” which “can only be fully understood through use, and using them entails both changing the user’s view of the world and adopting the belief system of the culture in which they are used.”

Research experiences at both the undergraduate and secondary level offer students an opportunity to participate in the community of practicing scientists. In this way, novice scientists can learn the customs and norms of the community, deepening their understanding of the nature and practice of science.

6.4 Context of the Study

In 2011, we developed a free summer camp for high school (grades 9-12) students called the Orlando Chemistry Tutoring, Enrichment, and Training (OCTET) Camp with the goals of increasing interest in and understanding of chemistry. The development, implementation, and
evaluation of the OCTET camp have been described previously. Briefly, participants spend six consecutive days at the university participating in instructional sessions, hands-on activities and experiments, lab tours, and lectures about current research at no cost to them. The camp is planned and implemented entirely by graduate students with mentorship from a faculty member.

In our initial evaluation of OCTET, we demonstrated using modified standardized exams and self-report surveys that OCTET was effective in increasing student performance on the developed chemistry assessments and increasing interest in the pure sciences. However, with the motivation to develop students’ views about the nature and practices of science and thus, the intent to pursue and persist in scientific careers, we decided to incorporate an ARE as part of the pre-existing summer camp. As the research experience would be part of a summer camp intended to teach fundamental concepts in chemistry, we coined the term pre-CURE to emphasize the use of the CURE model at the secondary level.

With the goal of describing the impact of the camp with the incorporation of a research component, we sought a rich description of the participants’ experience. Thus, we present our findings of the impact of the OCTET research component both on participants’ views of the nature and practice of science.

6.5 Research Question

Considering that our previously established program increases participants’ performance on standardized exam-inspired assessments and their intent to pursue chemistry as an academic major, we decided to focus this study on the impact that the experience might have on their views on the nature of science and what it is like to do science. Thus, two research questions guided this study: 1) How does a week-long authentic research experience influence high school students’
views about the nature of science? and 2) How does a week-long authentic research experience influence high school students’ views about the practice of science?

6.6 Methods

6.6.1. Setting and Participants

The Orlando Chemistry Training, Enrichment, and Tutoring (OCTET) Camp is hosted by graduate students at the University of Central Florida (UCF) during one week of the summer each year. This study was conducted during the 2017 implementation of the camp, with an emphasis on the research component. The invitation, application, and selection process were completed as described previously.¹ High school students (grades 9-12) were invited via e-mails to the county science resource teachers and individual science teachers. Graduate student coordinators collected contact information for these individuals in seven counties in the area surrounding UCF. Teachers returned applications for students who were interested and students, parents, and teachers were notified via e-mail of acceptance. Thirty-three students applied to participate in the camp and were accepted. Twenty-five of those had taken honors, Advanced Placement (AP), or International Baccalaureate (IB) Chemistry before. The other eight had never taken a chemistry course.

In order to participate in the survey portion of the study, students had to bring an Informed Consent form approved by the university’s Institutional Review Board (IRB) signed by their parents to the first day of camp. During the Welcome session, which parents were encouraged to attend if they had questions about the camp or research, the first author described the study and explained that participation in the camp was not contingent upon participation in the study.

Following completion of the camp, students, parents, and teachers were e-mailed to recruit participants to do interviews with the first author. Four students volunteered to sit for an interview.
In order to participate, they had to return a separate parental Informed Consent form, which explained the purpose of the interview and the fact that the participant would be audio recorded.

### 6.6.2. Incorporation of the Research Component

In order to develop the pre-CURE, we added time for the research project following the afternoon instructional session each day of the camp beginning on the first day. In addition, we rearranged the schedule of topics in order to add a research session on Friday. The purpose of this session was for participants to either complete data collection or begin working on their poster for the poster session on Saturday.

After taking the survey on the first day of camp, each graduate student who was willing to take on participants as mentees gave a brief (less than five minutes) description of the project they would be working on in their lab that week. These presentations mostly focused on which branch of chemistry the graduate student worked in, the applications of their research, and the methods or instrumentation they use. Ten graduate students presented and the participants were asked to write down their top three choices. The graduate student coordinator organized the participants into their labs considering their choices and announced the placements during lunch on the first day.

Based on discussions at monthly meetings leading up to the camp, the graduate mentors had developed projects for the participants to work on throughout the week, helped the students collect data, and helped them make a poster to present their findings. Projects ranged from reproductions of previous experiments to contributions to ongoing work. Nonetheless, all projects were completed in a research lab and related to ongoing research in the lab. For example, one group attempted to reproduce nanoparticle solutions and analyzed the absorption spectra of the solutions in order to determine size and shape of the particles. Then they used those solutions to
make nanoparticle films in various solvents and discussed the potential applications of this line of research. Another group completed a fluorescence characterization (excitation, emission, anisotropy, and lifetime) of a chiral dye the graduate student was using for a larger nonlinear optical study. She told the participants about the applications of her work in optics, sensors, and bioimaging and that they would be using a spectrofluorometer to make measurements.

We coined the experience a pre-CURE since it incorporates all five characteristics of CUREs, but is part of a secondary summer science camp rather than a standalone research experience. First, all projects involved the use of scientific practices. While the level and frequency varied between groups, all projects involved the scientific practices outlined by Auchincloss, et al.,22 in particular asking questions, proposing hypotheses, using the tools of science, gathering and analysing data, navigating the messiness of real-world data, developing and critiquing interpretations, and communicating findings. Second, the projects were based on discovery. While some groups repeated experiments that the graduate mentor had done previously, the results were not always anticipated, even by the graduate student (as described in the interview findings). This led to a need for creative interpretation of data. Third, the work was always broadly relevant or important. Graduate mentors were encouraged to discuss the larger motivation of the study with the participants. One group, in particular, was studying the Zika virus and discussed how their findings could impact the medical industry (incidentally, this was one of the most frequently requested topics). Fourth, participants mostly worked in groups. Each participant was placed in a group of 1-4 of their peers led by a graduate student mentor (one undergraduate mentor also led a group) based on their preferences. Only two participants worked independently with a graduate student, but the graduate students were working in the same lab so the participants had contact with one another. Most participants had to work as part of a group to collect and analyze data and
present their findings. This aspect proved to be very important to the participants. Finally, participants experienced the iterative nature of scientific research. Some of them repeated experiments previously done in the literature while some of them repeated their own experiments with new parameters each time to optimize results.

Graduate students, faculty, and parents of the participants attended the poster session. Participants took turns standing at their posters to explain their results while their group members circulated to learn about others’ projects. Feedback forms were distributed that asked: *what did you like best about this poster?* and *what suggestions would you make for improving this poster?* The completed forms were provided to the participants to take home.

6.6.3. Data Collection

Sixteen participants completed the Views on the Nature of Science (VNOS) questionnaire\(^3\) (described below) both on the first and last day of the camp. On the first day of camp, the survey was administered during the welcome session. Participants were given as much time as they needed to complete the survey (the developers recommend sixty minutes), but most finished in approximately thirty minutes. The same questionnaire was administered following the experiments session, but before lunch on the final day. Again, participants were given as much time as needed to complete the questionnaire, but most completed it within thirty minutes. We did not analyze any surveys of participants who completed the questionnaire on the first day, but not on the last day since the goal of the study was to determine if there was any impact of the experience on the participants’ views about science. There was a decrease in respondents on the last day due to absences on Saturday and some choosing not to complete the survey or only completing part of it.
6.6.4. Instrument and Data Analysis

The VNOS-D+ questionnaire is intended to elucidate high school students’ beliefs and attitudes about the nature of science. The instrument consists of ten open-ended questions that target seven aspects of the NOS (listed below). While each question targets one or more specific aspects of NOS, it is possible that students will refer to other aspects in their responses. Responses were scored according to the scoring rubric downloaded from PhysPort at www.physport.org/key/VNOS and the process is described briefly next.

First, the responses were categorized as one of the seven aspects of the nature of science (NOS) targeted by the questionnaire:

- Distinction between observations and inferences
- Empirical
- Creative and imaginative
- Subjective
- Social and culture embeddedness
- Tentative
- Distinction between scientific laws and theories

Next, each response was categorized as naïve (not at all consistent with NOS aspect), transitional (consistent with some, but not all, parts of aspect), or informed (consistent and addresses all parts of aspect). Each aspect is described fully in the scoring rubric. For example, in response to the following question:

*Scientists try to find answers to their questions by doing investigations/experiments. Do you think that scientists use their imaginations and creativity when they do these investigations/experiments?*

*If NO, explain why.*
If YES, in what part(s) of their investigations (planning, experimenting, making observations, interpretation, reporting results, etc.) do you think they use their imagination and creativity? Give examples if you can.

Several participants’ responses were categorized under the “creative and imaginative” NOS aspect which is described in the scoring rubric as “Scientific knowledge involves human imagination and creativity. Science involves the invention of explanations and this requires a great deal of creativity by scientists”. Examples of naïve, transitional, and informed responses are presented in Table 6.1.

In order to establish reliability of the data, two raters scored the questionnaires. Initially, the raters reviewed both the questionnaire and scoring rubric individually and then together. Then they discussed each aspect of the NOS, the aspect targeted by each question on the questionnaire and what other possible aspects might arise in the responses. Before scoring the questionnaires individually, the raters scored one collaboratively. An inter-rater reliability of 80% was achieved on all of the student responses, discrepancies were discussed, and 100% consensus was reached.

The developers of this instrument emphasize the need for follow-up interviews in order to explore the meaning of students’ responses. Thus, we decided to interview participants after completion of the camp in order to determine what impact the experience had on their views about science. Due to time constraints, we only performed interviews after completion of the camp. Interview questions, rather than being directly from the questionnaire, targeted various aspects within the questionnaire but were asked in such a way that the participant would think about the pre-CURE in particular and how the experience influenced their views. Four of the sixteen participants (25%) volunteered to participate in interviews. They were asked two questions about aspects targeted by the questionnaire, one question that targeted the discovery (and more

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<table>
<thead>
<tr>
<th>NOS Aspect</th>
<th>Naïve Example</th>
<th>Transitional Example</th>
<th>Informed Example</th>
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<tbody>
<tr>
<td>Distinction between observation and inference</td>
<td>Not exactly, being the Earth is always changing little by little, but the main idea is about right. (Pre-Q6)</td>
<td>No, because a model is used to help scientists understand something in a visual way, not depict it exactly. (Pre-Q6)</td>
<td>No, since it is based on geographical, topological, and geological evidence and observations. However, we can’t exactly see all the layers of earth to know exactly how they look. (Pre-Q6)</td>
</tr>
<tr>
<td>Empirical</td>
<td>It is based off of only fact, hypothesis, and experimentation (Pre-Q2)</td>
<td>Fossils and other paleontologic data provides evidence that such creatures existed. (Post-Q4a)</td>
<td>Science is the study of the natural world through observation and experimentation (Post-Q1)</td>
</tr>
<tr>
<td>Creativity and Imagination</td>
<td>No because that can lead to crazy results. You have to stick to scientific methods. (Post-Q7a)</td>
<td>Yes; planning and experimenting involves creativity because they must have an original answer and experiment to support their new finding. (Post-Q7b)</td>
<td>Yes, society can dictate scientific advancements. Da Vinci was forbidden from dissecting cadavers, but his contributions laid the foundation for anatomy. (Pre-Q10)</td>
</tr>
<tr>
<td>Subjectivity</td>
<td>It is an objective analysis of fact with a mathematical basis; other subjects are dependent upon interpretation. (Pre-Q2)</td>
<td>...they don’t have all of the information about what happened and they interpret the information differently (Pre-Q4c)</td>
<td>When it comes to interpretation, it all comes to ways to understand the data based on previous knowledge and ideas, like the interpretations of quantum mechanics. (Pre-Q7b)</td>
</tr>
<tr>
<td>Social and cultural embeddedness</td>
<td>No, I don’t think so because cultural values may be incorrect (Pre-Q10)</td>
<td>Yes-science can influence society in important ways. Science can help us understand the world and create new things-like cars and computers-that influence our society and culture. (Pre-Q10)</td>
<td>Yes, society can dictate scientific advancements. Da Vinci was forbidden from dissecting cadavers, but his contributions laid the foundation for anatomy. (Pre-Q10)</td>
</tr>
<tr>
<td>Tentative</td>
<td>Nature can act weird. (Pre-Q5b)</td>
<td>Yes, models are constantly improving and new information is being discovered (Post-Q3)</td>
<td>Yes, science is always evolving when new things in nature are presented such as mutations. The circumstances in nature may change, or new information may be acquired causing hypothesis to be altered. (Pre-Q3)</td>
</tr>
<tr>
<td>Distinction between theory and law</td>
<td>Yes, a theory is still a theory, (not fully proven), not fact, while a law is a fact (ex: the theory of evolution – not enough proof yet vs. the law of gravity –enough proof) (Pre-Q8)</td>
<td>Yes. Theories answer why? and laws answer what? Laws are more confirmed than theories. (Pre-Q8)</td>
<td>Yes, a law tells us what happens, it describes a natural pattern, while a theory tells or describes why something happens. For instance, Newton’s law of gravity describes the relation of the force of gravity between objects based on their mass and distance between each other, while Einstein’s Theory of General Relativity describes the space-time curvature as gravity. (Pre-Q8)</td>
</tr>
</tbody>
</table>
importantly, frustration) dimension of CUREs, and one general question about their experience. The complete interview protocol is presented in the Appendix J. Interviews were conducted by the first author either in person or via Skype. An undergraduate researcher was present for one of the interviews and participated in the transcription following. Each interview lasted about thirty minutes.

Interviews were transcribed immediately following the interviews by the first author. An alternative to verbatim transcriptions was used that emphasizes the use of written field notes which better capture the researchers thought and reflections during the interview (descriptive transcription). While audiotaping each interview, the interviewer took notes and, at the end of the interview, reviewed her field notes with the interviewee to ensure validity of her reflections and interpretations and that the participant had said everything they wanted to say (member checking). Immediately following the interview, the interviewer expanded on her notes from memory, being sure to include all major ideas (reflective journalizing). After reflective journalizing, the interviewer listened to the audiotape and revised the field notes accordingly, adding verbatim transcriptions where appropriate. Interview responses that supported the results of the VNOS or indicated interesting potential areas of investigation were identified and agreed upon by the first and corresponding author based on the transcriptions.

6.7 Findings

6.7.1. VNOS Responses

First, we briefly present the results of the written VNOS questionnaire, but avoid extensive interpretation since the questionnaire is intended to be scored without inference. Rather, interviews with participants should be conducted to extract meaning from the results of the written survey.
Raw frequencies of naïve, transitional, and informed responses by aspect on the pre- and post-survey are presented in Appendix K.

To begin, it is important to highlight participants’ understanding of the NOS aspects at the beginning and end of the camp. The most informed understanding at the beginning of the camp was the tentative NOS with 12 responses within this aspect coded as “informed”. The least informed understanding was the creative and imaginative nature with 7 responses being categorized as “naïve”. Interestingly, this aspect saw a noteworthy decrease in responses within the “naïve” category after the pre-CURE, reducing to 3. The responses seemingly developed into the “transitional” category (since there was no increase in the number of “informed” responses). At the conclusion of the pre-CURE, students’ understandings of the tentative nature were still the most well informed among the aspects (8 responses), although there was a decrease in the number of informed responses. Students understandings of the difference between scientific laws and theories were the least informed after (10 naïve responses).

Next, we present the changes in NOS understandings by aspect as presented in Table 6.2. Two important points about these results should be taken into consideration. First, unknown ratings are not reported. That is, there were 21 responses in which an aspect could not be identified so they are not included. For example, in response to the question about how science is different from other subjects/disciplines, one student wrote, “science relates all topics together into one.” In addition, if the aspect in the response to the question was not the same on the pre- and post-survey, the change was not tabulated. Second, the degree of change is ambiguous. For instance, positive changes include movement from naïve to transitional, transitional to informed, and naïve to informed. This happened in only three cases, so the occurrence is not considered significant.
Table 6.2. Frequencies in changes of NOS understandings by aspect as reveal through written VNOS. Positive, no change, and negative change are indicated with +, 0, -, respectively.

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Distinction between observation and inference</td>
<td>1</td>
</tr>
<tr>
<td>Empirical</td>
<td>8</td>
</tr>
<tr>
<td>Creativity and imagination</td>
<td>4</td>
</tr>
<tr>
<td>Subjectivity</td>
<td>0</td>
</tr>
<tr>
<td>Social and cultural embeddedness</td>
<td>2</td>
</tr>
<tr>
<td>Tentative</td>
<td>7</td>
</tr>
<tr>
<td>Distinction between theory and law</td>
<td>0</td>
</tr>
</tbody>
</table>

Positive change occurred most frequently in the empirical and tentative NOS aspects and not at all in the subjective nature or the difference between laws and theories. The subjective nature also saw no negative changes in understanding, but the difference between laws and theories saw five negative changes. Overall, most responses were categorized at the same level before and after the camp. Since no explicit instruction was offered, this is not unexpected. However, the possibility that understandings of some aspects (i.e. empirical and creative) evolved during the pre-CURE is intriguing and we pursued this in the interviews. In addition, since the creative aspect saw the most significant decrease in naïve responses after the pre-CURE, we decided to target it as well.

6.7.2. Interviews

The interview questions (provided in Appendix J) targeted four main ideas: participants’ views about evidence, creativity, the role of making predictions, and the overall experience. Multiple examples of each main idea were identified in the participants’ responses. Students did not necessarily give an example pertaining to the main idea targeted by the interview question. The main ideas and examples are shown in Figure 6.1.
Four participants volunteered to do interviews with the first author and their names have been changed to protect their privacy. Characteristics of the participants that pertain to the study are presented in Table 6.2. First, the grade they were in when they applied to OCTET is presented. When interviews were conducted, the following school year had begun so the participants were in the next grade. This is important since three of them had started the process of applying to college at the time of the interview. Second, the sub-discipline of their research during the pre-CURE is reported. Important to note is that Nora and Lindsay were in the same group. Finally, any experience that they had between the pre-CURE and the interview that could have influenced their responses is reported. This characteristic proved only to significantly influence Kevin’s responses since he had been working as part of a research group at the university since the pre-CURE. Since this opportunity was an extension of his experience, we present those responses here.
Next, we discuss the three main ideas discussed during the interviews. The first two main ideas provide more information about how participants experienced the NOS during the pre-CURE. The second main idea, the role of making predictions, revealed the resiliency of the participants in the face of unexpected outcomes. The third main idea provided information about which aspects of the pre-CURE participants found particularly impactful.

*The Role of Evidence*

First, participants recognized the role of evidence in planning and preparation, carrying out an experiment, and disseminating information. Lindsay referred to the role of evidence in the preparation of the nanoparticle solutions. She said that the graduate mentor provided them “examples” to which they could visually compare their solutions. She cited the comparison with these examples and visual changes in the solutions as forms of evidence. Jenny spoke about the role of evidence in determining the parameters needed to carry out her experiment successfully. She said that out of the five days they had in the lab they spent four running experiments to determine which parameters were right for the experiment. She said that they had to have a lot of evidence to support each choice. She was surprised by this because she “thought it would be more time finding the actual results”. Kevin referred to the dissemination of information when asked
about the role of evidence. He said that he now recognizes the importance of evidence when writing papers because he sees his lab mates doing that now. He said, “Evidence is how other researchers will know that the information is valid”. Nora mentioned taking images of the nanoparticles using a nano-microscope as an example of the evidence she used.

Each of the participants recognized the role of evidence in different parts of their project. This could be attributed to the differences in the nature of their experience. For instance, Lindsay participated in a project that was a reproduction of something the graduate student had recently done. Thus, she saw the role of evidence in sample preparation since that was what most of her time was spent on. On the other hand, Jenny’s project consisted of determining best parameters for an experiment, something that Lindsay did not experience.

Next, pre-CURE participants were not surprised by the role of evidence in their projects. When asked whether she was surprised about the importance of evidence, Nora simply said, “no”. However, while Kevin and Jenny were not surprised, they reflected on the role of evidence. Kevin said that he had known about the role of evidence before, but that now the idea is “magnified” because he sees how important it is when his lab mates are writing papers to be published. Jenny said she had had the view about the importance of evidence before the pre-CURE, but “never thought about it”. The pre-CURE gave them an opportunity to experience the NOS rather than simply gain knowledge about it.

Finally, it was interesting that two of the participants indirectly discussed the difference between observation and inference in response to the question about evidence. In Lindsay’s group, the graduate mentor had previously made solutions with which the participants could visually compare their samples, but she was quick to add that they were also “able to see that a chemical
reaction was going on”. She was making an inference when deciding whether her solution was right by comparing it to the standard, but a direct observation when she watched the visible change in the solution. What is unclear is whether she considered her direct observation of the change in the appearance of the solution a direct observation of a chemical reaction. Nora addressed this more directly. When explaining how the nano-microscope she used worked, she said, “it isn’t really a picture…because it’s so small, you get an image bounced back than (sic) actually seeing it”. She recognized that the images of the nanoparticles were not direct observations, but inferences based on what she could directly observe. Once again, both of these students were exposed to a situation where they needed to differentiate between observation and inference, even if they were not explicitly instructed to do so. In fact, Nora’s response would be coded as “informed” within the observation and inference distinction aspect. Interesting to consider is that both of these students were in the same group and obviously had different recollections about when this difference was important. This provides evidence in support of the implicit instruction of the NOS.6

While the pre-CURE may not have explicitly taught these students about the empirical NOS, it gave them an opportunity to experience it. They became more aware of the importance of this aspect in multiple components of their project and differentiated between direct observations and inferences.

The Role of Creativity

Similar examples emerged when the participants were asked about the role of creativity in their project. First, the students recognized the role of creativity in planning, making predictions, interpreting results, and dissemination of information. Lindsay said that even though she was not
involved in the development of the experiment, she knew her graduate mentor had to use his imagination to do so. Kevin referred to the role of creativity in making predictions asking rhetorically, “What will happen if we change this? Do that?” The results of Jenny’s experiment were not as she expected. She said she had to use her imagination to explain why, referring to the interpretation of her results. This is representative of an informed view of the NOS which seems to have been influenced by her pre-CURE experience since she also expressed that she was surprised she had to do this. Finally, when Nora was asked about the role of creativity, she did not hesitate to describe making the poster for the poster session. She discussed trying to decide physical placement of figures and text and what information she wanted to include on the poster in order to tell her peers about the project. While this is not explicitly referred to in the VNOS developer’s description of this NOS, it does represent the development of a soft skill that results from AREs.

Some participants were surprised by the role of creativity in their project and some were not, but they did reflect on it. Kevin and Lindsay were not surprised that creativity played a role in their project. Lindsay mentioned that she does many inquiry labs in her science classes and has to be creative enough to come up with procedures and interpret her results. Kevin was not surprised because “that’s how ingenuity happens. You have to think outside the box.” On the other hand, Jenny was surprised she had to use her imagination so much to interpret her results. She said she thought the process would be “simpler and less difficult”. These responses were similar to the corresponding examples under the evidence topic. The participants claimed to already know that creativity and imagination play roles in scientific research, but the pre-CURE gave them an opportunity to experience it in practice.
Once again, the pre-CURE did not seem to drastically change students’ views about the NOS. However, it gave them an opportunity to use their imaginations to carry out research and experience the NOS.

The Role of Making Predictions

Our question about whether students made predictions during their research experience was usually answered with “yes” and an example. These examples, once again, reflected the role of predicting in various parts of the process. The question was always followed up with, “were your predictions always correct?” All four participants answered “no”, usually accompanied by laughter. We explored whether that was frustrating to them.

Nora referred to making predictions about what her nanoparticle solutions would look like when the nanoparticles formed. She said that her predictions were based on what her mentor taught her group about making nanorods and the properties of the resulting solutions. While Nora was making predictions during the data collection and initial analysis steps of the scientific method, Jenny was using predictions during the planning stage. She mentioned making predictions about which parameters would be best to use to run the experiment and get good results. Kevin said that he did not make many predictions during the pre-CURE, but that in his current role as a member of a research group in the chemistry department he is “constantly making predictions”. He also referred to changing parameters (i.e. pH) based on what he thought would work best in the experiment. Like the role of evidence and creativity, the realization of the role of predictions in different parts of the project is likely by virtue of the nature of the particular experience. This further demonstrates that even if a particular outcome was anticipated based on previous results,
being in the authentic environment gave participants an opportunity to experience this reality of practicing science.

The participants we interviewed proved to be exceptionally resilient. While none of them necessarily got results they were expecting, they were not deterred. Jenny said that it did not make her feel differently about doing research because “it’s just another challenge. If you avoid any difficulties then you would never really be able to do anything.” Kevin even said it was “good” because it was interesting for him to see that, “scientists don’t always get the answers right the first time.” Lindsay had a similar perception. She thought it was “cool” to see what happened when a solution did not look the way it was predicted to and that even her graduate mentor was interested to know how the participants made the solution that way. She said, “If anything it piqued my interest even more.” We do not claim that these would be representative reactions of all high school students. We recognize that these are relatively high-performing and motivated students who previously had some interest in science. While we admire their resilience in the face of failure, we wonder what the reactions of different populations of students would be.

The Lab Experience

In addition to targeting specific aspects of the NOS, we also asked the participants to describe their experience. Usually, they started by describing the topic and purpose of their project. We probed by asking what specific tasks they got to do, which tasks they were assigned, which steps they participated in, and whether the experience had an impact on their career goals. Students gave three examples of this main idea. First, collaboration with their peers (and in one case the graduate student) was one of the most highly regarded features of the camp. Kevin said that one of his favorite parts of the camp was working with his classmates. He and his group members even
engaged in a friendly competition to see “who could make the best nanoparticles”. Nora’s first response when we asked her to describe the experience was that she was able to meet new people with similar interests. She has since connected with them on social media and they traded phone numbers to stay in touch. She said that some of them even go to her school, but since there are so many students, she did not know them before the pre-CURE. Lindsay’s initial response to this prompt addressed the same topic. She said that the pre-CURE was her favorite part of OCTET because she “could really work with the other kids and get to meet them”. Her favorite part of the research component was learning about the research from the graduate student and getting to experience what he does on a daily basis. Kevin, Nora, and Lindsay placed a high value on the interaction they had with their peers during the pre-CURE. This sentiment is in agreement with previous comparisons of collaborative and apprenticed research experiences. The results of this study suggest that collaborative experiences are equally as positive as apprenticeships, but our results suggest that the opportunity to collaborate made the experience even more positive. Thus, the collaborative aspect of the pre-CURE is not only more efficient in terms of conserving resources, but a benefit to the experience and a possible mechanism for increasing persistence in STEM.

Lindsay went further to share the impact that working with the graduate student had on her. Her discussion about what the graduate student “experience” is like leads to the next topic that the participants discussed. The participants described the opportunity to do science in the research lab in very positive terms. Commonly used words to describe the pre-CURE were “cool” and “awesome”. Kevin was general in his description saying that it let him see “what a lab experience really looks like” and that it was “cooler” than he imagined. When asked for specifics, he said that the level of the material they were covering during the pre-CURE was “engaging”. Nora and Jenny
were more specific about what it was they enjoyed about doing science in the research lab. Multiple times during the interview, Nora mentioned how much she enjoyed using micropipettes. She also enjoyed performing calculations to determine how much of each component she needed to make nanoparticles. A seemingly minor part of the experience, this is a skill in which students in an introductory summer research experience also reported feeling more confident. Another of her comments reflected the fundamentally interesting nature of the experience, she said, “Having it react before your eyes and saying, ‘I did that!’ That’s cool.” Jenny mentioned micropipettes as well, along with other lab equipment and instrumentation that was “awesome” and said she was “excited to learn how to use them”. These responses suggest that having the experience of being in a research lab for only a week might give high schools students an idea of what it is really like to do science. Rather than repeating what has already been done, they get to use modern equipment, instrumentation, and techniques to explore their interests – not without the risk of failure.

The final topic that participants discussed with regards to the lab experience was the impact that the experience had on the participants. Students are nominated by their teachers to participate, but they are usually students who were previously interested and high-performing in their science courses (although some past OCTET participants did not necessarily have STEM career goals). We have found in the past that participating in OCTET gives students an opportunity to better define what it is they want to do in science, rather than actually changing career goals. This was mostly true for our current participants as well. Kevin wanted to be a neuroscientist before the pre-CURE, but says now he is “more excited about it”. Further, he said that if someone had asked him before if he wanted to do research, he would have said “sure”, but now it is an emphatic “yes!” Nora had heard about nanoengineering before, but between her experience in the lab and listening to a faculty member presentation on his nanomaterials research, she said she is considering it as a
career now. She elaborated by saying that she liked “going into an actual lab” and that she “wouldn’t mind working in a lab like that.” The pre-CURE had a significant impact on Jenny. She said that the research was “really cool” and gave her a better idea of what she wanted to do. In fact, before the pre-CURE she was torn between “being an engineer and…not being one. I don’t really know what I would be if I wasn’t an engineer.” However, on her college applications, she is specifying biochemistry as her intended major and is interested in doing research. These results align well with assessments of the influence of CUREs on students’ career plans. CURE students have reported sustained or increased interest in postgraduate education rather than drastic changes in career plans.40-42

6.8 Conclusions

Results of the VNOS indicate that the pre-CURE does not have a significant impact on participants’ formal views of the NOS. However, interview responses from pre-CURE participants suggest that the experience may have an important influence on how they view the practices of science. These participants reflected on the role of evidence and creativity and recognized the roles of these aspects in various steps of their projects. They experienced failure, which could be frustrating, but demonstrated resilience and some even became more intellectually curious about what they were doing. They enjoyed interacting with their peers, focusing on the common interests of the people they met. Finally, doing science in a real research lab gave them an authentic experience, which they recognized, and had an impact on what careers they plan to pursue. Rather than drastically transforming their views about the NOS, the camp allowed these participants to see how the NOS is manifested in practice.
6.9 Implications

There is ample research about the beneficial effects of participation in undergraduate research. However, there is substantially less evidence for these effects for secondary students. Our results demonstrate that participants may deepen their understanding of how the NOS is manifested and how scientific research is practiced from being immersed in an ARE with a graduate student mentor. These participants also appreciated the opportunity to practice tasks that professional scientists might find routine (i.e. using micropipettes and performing calculations to make solutions). Involving high school students in AREs can be beneficial to all parties. High school participants learn the intricacies of practicing science (the role of evidence and creativity in various steps of the process, the iterative nature that is often accompanied by a level of frustration) and graduate students have an opportunity to mentor. In addition, a weeklong experience like the one we have described may, as in Kevin’s case, result in a new member of the research group, which could lead to more productivity.30

6.10 Limitations

The primary limitation of this study is the sample size. Only about half of our participants responded to the VNOS questionnaire both on the first and last day of the camp. This was largely due to the number of students who could not attend the last day of camp. In addition, some students only partially filled out the survey on the final day since we presume, they turned in whatever they had completed by the time their peers were finished. Further, only four students volunteered to do interviews.

The characteristics of the participants of this study are another limitation. While the results of our study are mostly in agreement with the outcomes of research experiences for
undergraduates, all of our participants are motivated, high-performing students who were already interested in pursuing STEM careers. While this experience gave them a better idea of exactly what they wanted to do in their academic careers, it would be useful to investigate how students with less access to these types of experiences are impacted by participation. For instance, we have not been able to provide transportation or lodging to our participants, which places limitations on students whose parents cannot drop them off and pick them up every day.
6.11 References


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32. Hapkiewicz, A., Authentic research within the grasp of high school students. *Journal of Chemical Education* 1999, 76 (9), 1212.


While we acknowledged our success in changing students’ views about science using authentic research experiences, we considered the fact that more of our instructional time is spent in the classroom setting. Thus, we developed the goal to create an authentic learning environment in our Physical Chemistry lecture course and evaluate students’ perceptions of the updated and evidence-based instructional style.

Physical chemistry students often have negative perceptions and low expectations for success in physical chemistry, attitudes that likely affect their performance in the course. Despite the results of several studies indicating increased positive perception of physical chemistry when active learning strategies are used, a recent survey of faculty in the U.S. revealed the continued prevalence of instructor-centered approaches in physical chemistry. In order to reveal a deeper understanding of student experiences in an active learning physical chemistry course, we present a phenomenological study of students’ perceptions of physical chemistry when the course is completely redesigned using active learning strategies. Using the flipped classroom, an active learning space, cooperative learning and alternative assessments, we emphasized fundamental concepts and encouraged students to take responsibility for their learning. Based on open-ended surveys and interviews with students, we found that students struggled with the transition, but had some significant positive perceptions of the approach. This is in agreement with previous studies
Introduction

The topics covered in a Physical Chemistry course are fundamental to the understanding of chemistry, physics, engineering and many other related fields. Due to prerequisite skills acquired in calculus and physics courses, Physical Chemistry is often integrated into the curriculum as an upper-level undergraduate course for students in the major. It is well known by practitioners, but also demonstrated by survey results and interviews, that chemistry students often enter Physical Chemistry with negative perceptions and low expectations for success. Specifically, lecturers and students alike have identified the abstract nature and mathematical content of thermodynamics as a source of student difficulties. In fact, many alternative conceptions and student difficulty with math transfer have been identified in the chemical education literature. Much effort has been placed on identifying factors of success for physical chemistry students, but less emphasis has been placed on student perceptions of the course. As early as 1989 and more recently student attitudes and self-concept have been identified as predictors of success in introductory chemistry courses. Logically, practitioners may consider that increasing student perceptions of physical chemistry may result in increased performance.

The term active learning refers to a myriad of constructivist course designs that can range in the degree and execution of strategies such as group problem solving, the use of classroom response systems, or worksheets completed during class. According to Freeman, et al. “active
learning engages students in the process of learning through activities and/or discussion in class, as opposed to passively listening to an expert. It emphasizes higher order thinking and often involves group work.” Several studies show increased positive perception of physical chemistry when active learning strategies are incorporated.10-12 Conversely, other studies show that students experience discomfort in the transition.13 In any case, there is an abundance of evidence in support of the effectiveness of any version of active learning in STEM courses in terms of performance.9

Despite the evidence in support of active learning, a recent survey revealed that a majority of physical chemistry faculty (79% of those surveyed) continue to teach using instructor-centered approaches like lecture to deliver content.14 This was even more common among faculty at large institutions similar to the institution at which this study took place. Of the few notable exceptions reported in the literature, faculty, for example, modified the lecture to include frequent breaks and collaborative problem-solving12 or completely redesigned the course using cooperative learning13,15 or process-oriented guided inquiry.10

Considering that student perceptions of a course can affect their emotions, motivation, and thus learning and performance, we decided to embark on the investigation of student perceptions of a physical chemistry course taught using active learning strategies. The design of the course was rooted in two currently emerging popular pedagogical approaches in chemical education: the flipped classroom16 and active learning spaces inspired by the SCALE-UP learning environments introduced at North Carolina State University.17-18 Use of these approaches offered significant opportunity for less formal assessment than the traditional chapter exam, so exams were eliminated in an effort to reduce test anxiety and increase focus on deep conceptual understanding rather than rote learning. The effect of implementing these active learning strategies was studied using a
phenomenological approach. As part of a larger study of the effect of using active learning strategies in physical chemistry, the research question that guided this part of the study was: *What are student perceptions of active learning in a physical chemistry course?*

### 7.1.1. Student Perceptions of Physical Chemistry

Two noteworthy studies have explored student perceptions of physical chemistry in a traditionally taught course. Nicoll and Francisco⁵ identified students’ anticipation of the difficulty of the course and the considerable amount of time they would be required to devote to studying. Later, Sözbilir⁴ investigated perceived difficulties in more detail. The factors influencing students’ perceptions were categorized as *student*, *course*, and *staff*. The largest contribution to the *student* category was lack of student motivation or interest in the material. On the other hand, the largest contribution to the *staff* category was teacher-centered learning. This leads us to think that a move away from teacher-centered learning might increase student motivation and interest. In fact, students’ suggestions for reducing these difficulties included faculty promotion of group work and discussions. Students attributed difficulties associated with the *course* to abstract concepts, overloading of course content, and superficial understanding of concepts among other factors. As shown next, these factors have been mitigated in other courses using various active learning strategies.

In 2000, Pentecost and James¹³ used a flipped classroom approach facilitated by instructor-provided guided reading materials in order to give students more responsibility for learning. Interviews with their students over three implementations of the course revealed mixed emotions about the change in teaching style. Their results indicated that while students may value the goals
of active learning strategies in theory, the drastic change might initially lead to negative perceptions.

Other studies revealed largely positive student perceptions.\textsuperscript{11-12} These results may lend more encouragement to faculty to incorporate active learning strategies into upper-level chemistry courses. Introduction of different degrees of cooperative learning into two physical chemistry courses received positive student feedback.\textsuperscript{11} Students said that the cooperative learning activities led to a deeper understanding of concepts. These students felt motivated by the flexibility of the course structure, the pressure to contribute to group work, and the experience of relating to their peers (i.e. realizing that others have the same conceptual difficulties). More recently, Partanen\textsuperscript{12} incorporated student-centered strategies including exam “cheat sheets” and interactive lectures with breaks and activating tasks like example problems. Her students also responded well to the incorporation of these strategies, especially group work since they felt more comfortable communicating their misconceptions to their peers. An especially interesting result of her interviews with students was in regards to the origins of the negative perceptions coming into the course. Their negative impressions were triggered by students who had taken the course previously and been unsuccessful in passing. This indicates that negative perceptions can be mitigated early on if students see that passing the class is an obtainable reality.

Taken together, the results of these studies suggest that with thoughtful planning, active learning might increase positive affect and student perception of physical chemistry, thus increasing student performance, and, more importantly, conceptual understanding of this fundamental field of chemistry.
7.2 Methods

7.2.1. Research Design

Considering the emphasis of the research question on student experiences, we decided to use a phenomenological research design in order to elicit rich descriptions of student perspectives and be able to understand more deeply their experience in an upper-level course that uses active learning. Phenomenological research studies are designed to describe the common lived experiences of a group of individuals experiencing the same phenomenon. They describe the “what” and the “how” of the experience largely through interviews. However, phenomenology has been said to lie on the line between qualitative and quantitative research so data such as observations can be used to help describe the experience as well.

Exploring student perspectives of Physical Chemistry is not in itself a novel research endeavor, but in this course design, students were expected to take most of the responsibility for their learning with the instructors acting as coaches. That level of active learning has not yet been described in the literature in terms of student experiences. This information could have implications for upper-level chemistry instructors who expect their students to have the skills necessary to be successful in this type of learning environment and inform the practitioner about how to implement more effectively active learning strategies.

7.2.2. Setting and Participants

This study was conducted at a public research university with a total undergraduate enrollment of roughly 56,000. Sixty-three students enrolled in Physical Chemistry I in the Fall 2016 semester and agreed to participate in the study. In order to participate in the study, students had to be present on the first day of class to receive the Explanation of Research with the course
syllabus and to take a pre-instruction assessment. The Explanation of Research described the goals and procedures of the research and explained that the students could choose not to participate without detriment to their course grade. This protocol (#SBE-16-12307) was approved by the university’s Institutional Review Board (IRB) after an expedited review. Their class standing, academic program, gender, and race/ethnicity distribution are presented in Figure 7.1.

![Class demographic data](image)

**Figure 7.1.** Class demographic data.

The course was an upper-level Physical Chemistry I course which focuses heavily on chemical thermodynamics and basic kinetics and is required for all chemistry majors to take in most university chemistry programs. The class met four days a week for 50 minutes. The corresponding author served as the professor for the course. He had taught this course six times before, the graduate chemical thermodynamics course eight times and the undergraduate physical chemistry laboratory eleven times. He facilitated the whole group discussions, chose homework and class activity problems, and wrote the questions for the conceptual assessments. The primary author acted as the teaching assistant, providing support to students during group activities and facilitating class on occasion.
7.2.3. Instructional Approach

Figure 7.2 describes schematically the instructional approach used based on the implications of findings from previously discussed studies of factors that influence student success in physical chemistry. Two main strategies served as the foundation of the course structure: the reversed learning scheme (or flipped classroom) and the informal learning space, (or active learning space). Because of the substantial opportunity for assessment using these approaches, we decided to eliminate the traditional chapter and final exams. Instead, students were evaluated solely on weekly homework assignments, in-class group activities, and participation in class discussions. Students received weekly feedback on homework problems and daily feedback via posting of correct answers to in-class activities on the online learning management system. In addition, pre-instruction, midterm semester, and post-instruction assessments were administered. These assessments were administered as part of the evaluation of the effect of this course structure on student conceptual understanding, but interview findings revealed that students might have used these ungraded assessments as a measure of their own growth.
The first main strategy used was an adaptation of the flipped classroom approach in order to establish a culture of inquiry before students arrived to class. Whereas in most flipped classrooms, students watch video lectures prior to class,\textsuperscript{16} we asked our students to read one chapter and complete two comprehensive questions from the text. These homework assignments were graded based on correctness with partial credit by the instructor. This was similar to the approach taken by Pentecost & James,\textsuperscript{13} but by assigning comprehensive problems in place of a guided reading activity. In order to ease the transition, we dedicated the first class meeting to modeling how one would extract concepts from the text. The instructor read main headings and subheadings from the text and engaged the class in a questioning-predicting exercise for the first half of class. Second, although the furnishings associated with current “active learning” classrooms based on the SCALE-UP classroom design\textsuperscript{17} were not available for us to use with this class size, we considered...
the space an “active learning space”. Students were able to move their desks, work independently or in groups, and utilize any technology they had available.

Class time most often began with a whole group discussion and ended with a small group activity. The instructor facilitated the whole group discussion, emphasizing main ideas from the chapter and key points that may often be overlooked or misunderstood. The whole group discussion was purposefully utilized as an attempt to correct alternative conceptions students usually have in physical chemistry. We thought directly addressing some of these concepts (i.e. equilibrium) may help correct misconceptions. Small groups (4-5 students) were alternately chosen by the students or assigned by the instructors based on performance on the homework for that week.

Class activities were tentatively assigned at the beginning of the semester (students received a tentative schedule with the syllabus with homework and class activity assignments). Following whole group discussion, considering the concepts covered during the whole group discussion and the time remaining in class, the class activity was assigned. Most commonly, the activity was a problem directly from the text. For example, on the day that temperature and the zeroth law of thermodynamics were discussed, very little time was left (about five minutes) to answer the two assigned conceptual problems for that day. Based on this, only one question (Can you think of any property of a living system that could be used as a thermometer?) was assigned as an individual exit slip type of activity, which students completed in the last 2-3 minutes of class. When more time was left, students might be posed with a more involved problem. For example, when covering equilibrium constants, one class activity was as follows:
The equilibrium vapor pressure of water over $K_4Fe(CN)_6\cdot3H_2O(s)$ plus $K_4Fe(CN)_6(s)$ has been reported as 10.0 torr at 25.0°C and 7.20 torr at 20.0°C.

- Compute $K$ at 20.0°C and 25.0°C for the reaction
- $K_4Fe(CN)_6\cdot3H_2O(s) \rightleftharpoons K_4Fe(CN)_6(s) + 3H_2O(g)$
- Compute $\Delta G^\circ_{rxn}$ at 25.0°C for the reaction.
- Compute $\Delta H^\circ_{rxn}$.
- Compute $\Delta S^\circ_{rxn}$.

Class activities were to be completed by the end of the class period during which time the instructor and TA would circulate to answer questions and guide students. The assignments were graded based on correctness with partial credit by the TA.

In order to monitor student progress, a pre-instruction, midterm semester, and post-instruction assessment consisting of conceptual questions written by the instructor were administered. The pre-instruction assessment was administered on the first day, the midterm halfway through the semester, and the post-instruction assessment on the day that the university schedules a final exam for the course. The results of these assessments were used to study student conceptual change as a part of a larger study, but they were not used to provide feedback to the students or the instructors and they were not graded assessments. Homework and class activities served those purposes.

**7.2.4. Data Collection**

In order to assess student engagement, we monitored attendance using class activities and asked our colleagues to observe the class. The Behavioral Engagement Related to Instruction Protocol was used since this was a relatively large enrollment class. Graduate students interested in physics and chemistry education were asked to observe the class. Observers were provided with a list of engaged and disengaged behaviors as described by Lane and Harris. They were asked enter the room inconspicuously and choose ten students within their range of view to observe. In two-minute
increments, they counted the number of students in that group exhibiting behaviors on the “engaged” list and took notes about student activities and behaviors during the class period. In addition, they took notes about what topics were being covered, what instructional strategies were being used, and where the students were sitting in the room relative to the instructor.

Following the post-instruction assessment, students were asked to respond to three demographic and one open-ended question: *What did you like the most about this teaching style? The least? Please be specific.* Students responded to the survey question using Socrative, a free classroom response system that students can access using their smart device or computer. Instructions for accessing the survey were included with the post-instruction assessment.

Semi-structured individual student interviews were conducted in the semester following completion of the course. All students who participated in the study were contacted via e-mail and asked to volunteer to participate in an interview. Six students volunteered to discuss their perceptions of the course and some thermodynamics concepts with the first author. Interviews were conducted with only the participant and the first author and lasted 30-45 minutes. The semi-structured interviews began with a question about the students’ general perceptions of the course. This was followed up by asking students to compare their experience in Physical Chemistry 1 to another traditionally taught course. Students were also asked about the dynamics between group members during class activities and whether they understood any specific topic better at the end of the course as a result of something that occurred during the semester. These questions were adapted from those used by Towns & Grant when they incorporated cooperative learning into their Physical Chemistry course. The complete interview protocol is presented in Appendix L.
7.2.5. Data Analysis

Sixty students responded to the survey and their responses to the open-ended survey question were coded by inductive content analysis. After choosing the unit of analysis (in this case, survey responses) the next step in inductive content analysis is for the researcher to become “immersed” in the data. This involves reading through the data several times in order to become familiar with it. Next, the researcher organizes the qualitative data using open coding and by creating categories. The categories are grouped with similar data until all of the codes contained in one heading are similar and “belong” to that group. Thus, themes arose from student responses rather than from any specific theoretical framework. Four major themes were identified and aligned fairly well with the strategies incorporated into the course redesign: homework assignments/flipped classroom, whole group discussions, group work, and alternative assessments. Notes on how comments were coded and sample responses for each code are provided in Table 7.1.

Interviews were audio recorded with the participants’ verbal permission, transcribed verbatim by the first author, and analyzed according to the four major themes that arose from the survey responses.
Table 7.1. Themes identified in survey responses. Similar themes were identified and analyzed in interview transcripts.

<table>
<thead>
<tr>
<th>Theme</th>
<th>Note</th>
<th>Frequency</th>
<th>Sample Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternative assessment</td>
<td>Students were not evaluated using traditional quizzes, tests or exams. This code was used when students referred to not having tests, exams or having their “only grades” based on other assessments.</td>
<td>19</td>
<td>(Negative example) I did not like having the only grades being homework and classwork because if you don’t understand the concepts on your own you don’t do well on them. Having no tests also minimized the motivation for me to learn.</td>
</tr>
<tr>
<td>Discussion</td>
<td>The beginning of most class periods consisted of a whole group discussion. This code was used when students referred to the discussions explicitly or to “lectures.”</td>
<td>18</td>
<td>(Positive example) I enjoyed how interactive the lectures were. I found it helpful.</td>
</tr>
<tr>
<td>Flipped classroom</td>
<td>Students were asked to read one chapter per week and complete a homework assignment consisting of two problems, usually assigned from the textbook. This code was used if students referred explicitly to the flipped classroom, homework or learning material on their own.</td>
<td>17</td>
<td>(Negative example) It's hard to complete the homework assignments ahead of the lectures. OR Teaching one self for this class is challenging.</td>
</tr>
<tr>
<td>Group work</td>
<td>Following the whole group discussion, students would either be asked to choose or were assigned groups to work with. If students referred explicitly to group work, this code was used.</td>
<td>8</td>
<td>(Positive example) The class assignments I liked, we were able to work in groups and it helped me learn the material from other people's perspective.</td>
</tr>
</tbody>
</table>

7.3 Results and Discussion

7.3.1. Attendance

Since high attendance has been correlated with high performance, and may give insight to student engagement, our first measure of student engagement with the course was attendance. It is important to note that, while attendance was not technically required, class activities accounted for 30% of the final grade. These activities could not be made up in the event of an absence, providing some extrinsic motivation for students to attend class. However, we did have a policy, which allowed only about 90% of the class activities to be graded so that any unanticipated
absences or unusually poor performance on assignments would not be detrimental to the course grade. Using class activity participation as a measure of attendance, we determined that students attended, on average, 85% of scheduled classes. We have no available records of attendance for this course prior to Fall 2016, but according to Credé and co-workers, this would be considered a high rate of attendance, indicating high student motivation, at least to attend class. A second interesting observation is that the retention rate from the first day of class to the last day of class was 70%, a good indication of persistence. In addition, there were no withdrawals or D’s. Only one student received an F because they inexplicably stopped attending class early in the semester.

**Figure 7.3.** Observation data showing number of students engaged during every 2-minute interval.
7.3.2. Observations

Four representative observations are displayed in Figure 7.3 with the date of the observation in the lower right corner of each plot of student engagement versus time. Important to remember is that each plot represents the engagement of a group of 10 students in the class. Student engagement is reported as a fraction since there were some cases in which groups of 11 were observed if a student came in late and joined a group already being observed. Points representing engagement during an activity not related to course material (i.e. grading policies or moving into groups) are not shown.

As shown in the plots, with only three exceptions, at least 6/10 students in the observed groups were engaged at any given two-minute interval. Further analysis reveals that average engagement was 0.75 or above. These values are reported for each class period on the respective plots. It is especially interesting to compare the top two plots, which represent the typical class in which half was dedicated to whole group discussion and half was dedicated to group work, with the bottom two in which the majority of the class time is spent on only one activity. Surprisingly, engagement was slightly higher on average in the latter case. Specifically, the third observation was done during a class in which we asked the students to perform a “lab”. They were provided with cooling curves from a cryoscopic determination of molar mass experiment and asked to calculate the molar mass of an unknown and to identify it based on their results (they were provided with a list of possible substances).

Exploration of some specific points reveals interesting information. First, it is not surprising to see engagement alternate and then decrease toward the end of the discussion in the first observation, but engagement is increased immediately upon starting the class activity. In the
second observation, engagement drops around 12 minutes into class. At 18 minutes, engagement had not increased significantly so the instructor asked a question to help stimulate the discussion. During the group activity, engagement was relatively low at the beginning (0.6). The observer noted that three students were talking about something unrelated and one was using their phone. This is simply attributed to the transition to the new activity since engagement increases for most of the rest of the activity. The exception occurs around 6 minutes before the end of class when the observer noted that students had begun to do other work and pack up either indicating that they were finished with the problem or relying on a group member to finish.

7.3.3. Survey Responses

After the post-instruction assessment was administered, we asked students to respond to one open-ended survey question: Sixty students responded to the question. Several themes including, but not limited to, those in Table 1, were identified in their responses. Other topics included the conceptual emphasis, textbook, instructor, participation requirement, and the informal learning environment. However, these topics were mentioned very infrequently, and thus are not included in the table. The complete set of themes is presented in Appendix L. Here, the four most frequently discussed themes, alternative assessments, discussions, flipped classroom, and group work will be analyzed since they were also common themes addressed in the interviews and provided the framework for the structure of the course. All responses to the survey are reported verbatim.

The first topic was alternative assessments. Nineteen students discussed this topic on the surveys. Twelve of the nineteen responses referred to the alternative assessment approach as a positive attribute of the course. Eleven of those cited reduced stress as a reason for liking this
approach. One wrote, “I enjoyed the class ideology, tests are stressful and do not assist in learning, only memorizing information.” and another, “I liked that I didn’t have to worry as much about test and could focus on actual learning. It was a nice change from having to memorize material.” Avoiding rote memorization and reducing test anxiety were precisely the goals we had for eliminating exams. This approach had other surprising and significant effects, though. Some students considered the fact that even without exams they still had to study. For example, one said, “I also liked no test even though each class was a "test" because if you didn't study ahead or read the material you would do poorly similar to an exam. It was a lot less stressful and I definitely learned a ton!” However, some students did not share this perspective, but had mixed emotions. Six of the nineteen responses referring to the alternative assessment approach indicated that students enjoyed not having tests, but four of those felt that this caused them to lack motivation, “It was nice not having tests but I think I would have put in more effort if there had been.” Only one student had a negative perception of the lack of tests and they cited motivation as the reasoning.

The second most common topic was the whole group discussion. Eighteen students mentioned this on the survey, seventeen of whom considered it a positive aspect. Six students explained that they like the informal nature of the lectures and the conversational, rather than traditional “lecture”, style. In fact, one student responded, “I felt like I was having a conversation about the topics rather than just being talked at for an hour.” Two students said they felt like the possibility that they would be asked to participate in the discussion motivated them to prepare for class each day. One student wrote, “The set-up of the class also helped since it was a group discussion and you would be randomly called on so you would have to be quick on your feet and actually know the material, which gave more incentive to learn the material.” Two students also mentioned that they enjoyed discussions because it gave them other students’ perspectives of the
material. One wrote, “I enjoyed the participation from classmates as well, it helped me to pay more attention and to see where my mistakes and theirs’ lied.” These were common themes discussed during the interviews as well.

The third most discussed topic was also the strategy with the most negative perception. Seventeen students mentioned the flipped classroom approach with most emphasis on the difficulty of the homework. Fifteen students cited this as a reason they had a negative perception of this aspect. Most commonly, they expressed the belief that they were required to master concepts before they were taught in class: “…but I did not like the flipped classroom teaching style. We should not have been expected to do the homework without any instruction on the chapter.” and, “However, it was especially stressful to complete homework without being taught the material beforehand, and I believe it is not an efficient way for students to learn.” Two students mentioned the amount of time they had to devote to the homework outside of class. For example, “The homeworks required a lot of time, between reading and trying to understand the chapter and then trying to actually solve the problems it would take me at least 12-15 hours, sometimes more.” One student even said, “I feel like this entire course was essentially "figure it out on your own", which is not what I’m paying tuition for.” which is a similar sentiment made by a student in Pentecost’s study. One student had a neutral perception, recognizing the skills they developed by using this method, “I didn’t like how the math and practical problems I would usually have to figure out by myself through the readings and homework. Although in the long run I felt like it helped me more than traditional teaching methods because I would probably forget it all within a semester.” Only one student mentioned the flipped classroom in a positive light, “the homework builds knowledge before the subject is covered.”
Group work was less frequently discussed, but a very important component of the course and discussed at length in interviews. Eight students mentioned this component on the survey. Five of these considered group work a positive aspect and either expressed that they like to work with their peers or that they like getting their peers’ perspectives. One of these students added a contingency, though, “I enjoyed doing group work, because I work better with other people (when everyone participates and actually tries).” This was another student’s reason for disliking group work, “The least aspect I liked about this course is the fact that when placed in group discussions some people do not put in the same amount of effort as others.” One student did not like “being forced into specific groups.”

Three other themes we identified were the conceptual emphasis in the course, the instructor, and the textbook. Seven students expressed that they particularly liked the conceptual emphasis, but one requested, “I wish we would go over more math based problem (sic) really helps with concepts.” Five students had strong negative opinions about the textbook, which, as a side note, was chosen by the instructor specifically because of its emphasis on concepts. Finally, all five mentions of the instructor were positive and mentioned his passion and enthusiasm for teaching.

7.3.4. Interviews

The purpose of the interviews was to explore the four major themes discussed in the surveys (alternative assessments, discussion, flipped classroom, and group work) in more detail. These discussions with students also revealed some interesting trends in student engagement and motivation throughout the course.
**Alternative Assessments**

Many students cited reduced anxiety on the survey as a reason for liking the “no tests” approach. During the interviews, Student 6 agreed with this view. She expressed that she was “not very good at taking tests” and that while there was still pressure to complete homework assignments, she felt like she could focus more on learning and understanding, rather than memorizing and regurgitating when she did not have the pressure of an exam:

**Student 6:** *I mean the fact that I...that class put absolutely no pressure on, I mean there was pressure to get the homework done because it was, like, graded, but at the same time there was no pressure in terms of memorization and regurgitation in the class. It’s like he sat down and made sure that, hey, you know, this is how you do a problem. This is why it’s like this in the book.*

Student 4 explained that not having tests did not necessarily make the course any easier. When asked what she thought about not having exams, she said, “I liked it too, but like the homeworks, they were hard. It makes you put a lot of time on P. Chem.” These responses are interesting to compare with Student 3’s response:

**Student 3:** *Um...I really enjoyed the way the class was taught. I didn’t really like the no test method, though ’cause as a student I feel like a test is a way of...pressures you to learn. And without tests you lose that stress and that pressure...that pressure to learn, learn the material so um...I don’t like the no test method. I thought like if the class was exactly the same, structured the same just with three tests throughout and a final, that probably would, I mean you’d learn a lot more P. Chem. 1.*
On the other hand, Student 2 missed having exams because she felt like she could better track her progress with periodic formal assessments. She said, “I like having exams. It kind of, um, lets you track your progress and if you’re retaining anything.” Student 6 had an opposing argument, though:

**Interviewer:** So do you feel like taking the pre, mid and post kind of helped you reflect on what you had learned and see...?

**Student 6:** My progression in the class, most definitely. It definitely, um, opened up my eyes and it like got me excited. The fact that I was retaining the information and not just memorizing, regurgitation and, you know, and that’s a thing where, ‘cause that’s a thing where from my...my pretest, my midterm and then my posttest, like none of my answers are probably the same exact, word for word, this is what’s going on in terms of things, you know. And I thought that was really exciting - actually going into a test and actually knowing, you know, what’s going on in the problem and not just, you know, “Oh. This is what you said. I’m pretty sure this is what the answer is.”

All four students refer in some way to anxiety, stress or pressure to perform. However, Student 6 appreciated the reduced text anxiety because it allowed her to focus on learning. Student 4 still experienced some pressure due to the challenging nature of the homework assignment while Student 3 expressed feeling little pressure to “learn” the concepts because he never had to take a test or exam. Later in the interview, Student 3 was discussing strategies he and his classmates used to complete the homework assignments and said, “…like I did study a little bit, just not a lot. Not what the course demanded.” It became apparent that Student 3 held some previous perceptions of what a Physical Chemistry course should require in line with what Sözbilir (2004) found in his study. Interestingly, Student 3 received an A in the course regardless of his perceptions of the
assessments method. According to Nicoll’s findings, attitudes about physical chemistry do not play a significant role in student performance. However, we wonder whether Student 3 would have performed better on the post-instruction assessment had he had Student 6’s perception of the lack of exams. These results highlight the different perspectives and responses students have to certain strategies. Student 6 appreciates the reduction of test anxiety which lets her focus on understanding, but can still track her growth using alternative assessments. On the other hand, Student 3 considers test anxiety a motivation to learn and does not initially consider working on homework assignments as “studying.”

Discussions

Two students on the surveys expressed that the discussions made them more accountable for knowing the concepts covered in class each day. Students 1 and 5 shared this view:

**Student 1:** Um...by far my favorite was when Dr. Hernandez would randomly call on people to answer questions. Because it forced...like I have to know what’s going on right now and just be prepared.

Student 5 was more explicit about his underlying motivation during the discussions:

**Student 5:** Yeah and when he would call on people at random and put them on the spot. That would be the ultimate motivation – fear of public humiliation.

**Interviewer:** So that was motivating, not demotivating for you?

**Student 5:** Yes. ‘Cause you wouldn’t want that to happen again.

These comments agree well with some of the survey comments about the responsibility for knowing material and may account for the high engagement observed during discussion. Even
when students had a negative perception of this pressure, they recognized its value. Student 3 explained that contributing to discussions was stressful, but he “liked” it. However, he felt that most students (not referring explicitly to himself) were not prepared, “so it didn’t work.” Interestingly, he blamed the characteristics of the students at this particular institution, but acknowledged the value of the strategy itself.

*Homework/flipped classroom*

The flipped classroom approach was the least popular topic on the surveys. Student 1 felt like the assigned homework problems were too complex.

**Student 1:** *I think it would have been better to have more questions that were a simpler level ‘cause the homework was a little over...like over my head sometimes. And so then I would just end up getting confused and copying something down and I don’t know if I necessarily learned as much as I should have from it...”*

The fact that the material was not taught in class before students were required to complete the homework was the major reason for students’ low perceptions according to the surveys. In fact, during the interview, Student 2 said that she was skeptical “that I can, um, read a difficult material and understand it basically on my own.” This largely negative perception is not necessarily surprising. O’Flaherty & Phillips acknowledge the “significant minority” of courses in which students have negative perceptions of the flipped classroom. For example, students in an inverted statistics course were “unsettled” with the lack of classroom structure and had difficulties orienting to class activities. Additionally, most research on flipped classroom in chemistry is in introductory courses (i.e. general and organic). Physical chemistry is often associated with
abstract concepts,\textsuperscript{3} so student resistance to taking the responsibility for learning is not surprising. However, further analysis of the interview responses gives insight to overcoming this challenge.

All students interviewed shared some of their strategies for completing the homework. The approach we, as instructors, like to think all students take was initially described by Student 4. She explained that she would read the chapter before Friday so she could go to office hours to ask questions and then complete the homework before class on Monday. Although, this is not the approach she took from the beginning. She explained earlier in her interview that she “thought it’s like the other homeworks for the other classes: you spend three-four hours and then you’re done. So I left it to the last minute.” After the first homework, she realized that this approach was not sustainable and devised a new plan. She explained that this new approach made her more responsible since the material was not taught in lecture beforehand. She also learned to be resourceful and said, “It made me really work.”

Student 6 also felt like she gained valuable study skills from learning using this approach. In fact, she stated that she was currently taking a different approach in her Calculus class than she might have before. She pointed out that since the homework in her Calculus class had a relatively low impact on her grade, she had little motivation to complete it.

**Student 6:** “Um… I would say that taking P. Chem. I actually helped me transition over into calculus and use that in order to study for… you know, my math, you know, tests and stuff like that because, sure, the homework is required but it’s only a small fraction of, like, the grade so…”

More commonly, students expressed during the interviews that they read the homework assignment and then looked through the book or online specifically for information to help them
answer the question rather than reading the chapter, trying to understand the concepts, and then approaching the homework. Student 5 discussed how he approached the homework:

**Student 5:** Read the question. Skim the chapter looking for a similar problem you could draw upon. If that didn’t work, type the question directly into Google. See if that gives you an answer, but the book we used was so obscure that that never worked, really. Then failing that would be looking at lecture notes from other P. Chem. classes, going through those quick, but then most of the time I could get some idea of what I needed to do...

Interestingly, continuing his discussion, he revealed that this search, while not always fruitful in terms of what he needed to complete the assignment, always left him with some understanding of various concepts he came across in his search.

On the other hand, while Student 3 had a similar approach, he discussed the fact that searching for specific information often left him with a shallow understanding of the concept. When asked whether he looked at the homework questions first then found the information needed to answer:

**Student 3:** Those specific questions, not the concepts, like I knew I had to find an equation specifically talking about, you know, I don’t know, cell potential at this exact, like, temperature. So I’m just going to find one dealing with cell potential at varying temperatures and that’s it. That’s all I care about. I didn’t care about how the temperature affected it or anything like that. I just had to get done, so that’s what I was thinking.

Student 3 admittedly did not necessarily understand the concepts after completing the homework. Rather, in agreement with findings from other studies, he was able to succeed by virtue
of his perceived mathematical abilities and logical thinking skills. Regardless of the fact that teaching approaches meant to give the student more responsibility for learning were employed, some students were still able to perform well without really obtaining a deep conceptual understanding of the material. In his study, Hahn posits that homework completion is a good indication of study skills, thus predicts performance. However, Student 3 admittedly did not reflect on concepts when completing homework assignments, but was still able to achieve an A in the course. His account gives more details about how homework is completed. It shows that even if a student completes homework assignments, they may not necessarily grasp chemical concepts depending on the strategies they use. These findings are in agreement with those of students in a quantum mechanics course in which alternative conceptions of concepts stemmed from non-productive strategies used by students when doing homework. These students, as well as ours, exhibited what Gardner refers to as the “problem-solving mindset”: their expectation is simply to solve problems, which influences their approaches to answering homework questions. Gardner’s students and ours used similar strategies including scanning the book for equations and following worked example problems.

Group work

On the surveys, a handful of students complained that during group work, effort was not put forth equally by all group members. This was also a finding in Pentecost’s study, but was in contrast to students in Partanen’s study who actually felt like responsibility to their peers motivated them to prepare before class. During the interviews, Students 1, 2, and 5 shared the inequality complaint. For instance, Student 1 said, “…there was one week where half my group members came late, the other half didn’t know what was going on and I had to end up doing the whole assignment on my own, which was fine, but we would end up losing points because we...
wouldn’t have it completed because I couldn’t get everything done.” Student 2 said that she usually took charge in the group, but that “there would always be a couple people who just kind of linger, who don’t do anything.” Student 5 also mentioned, “…there would inevitably be about two people that wouldn’t know anything. They’re just sitting there and they’ll say yes to anything you suggest…” These three students felt like they were the strongest in the group, and thus had to carry the weight of the rest of their classmates. These student perceptions do not seem to be in agreement with observational data that shows high engagement during group activities. However, one observer noted the emergence of one student in each group as a leader. We acknowledge the possibility that even though it seemed as though their classmates were not engaged, they might simply not have been contributing in the way the leader expected. One observer even recorded 9/10 for one data point during group work, but then crossed it out and wrote 10/10. He noted that the student seemed to be disengaged, but when a group member asked them a question, they answered in such a way that indicated they had, in fact, been engaged during the small group’s discussion.

Student 3 recognized that when groups were assigned, he was often the highest performing student, but he did not believe he had the ability to carry the weight of the group:

**Student 3:**… and then we were put in groups and I always had an A so when you started putting us in groups, I think with percentiles I was always the top quartile, but like I definitely did not know P. Chem. so I was always with three people who knew even less than me...

Most of these students felt differently when they were allowed to choose their own groups. Students 1 and 3 described clear strategies in which they would divide the work or problems based on the abilities in their groups. They expressed their comfort discussing the concept and their
reasoning for their answers with their chosen peers. Student 2, however, said the dynamic between members when she was allowed to choose her group was “About the same. Because I know that this person can be charged to like, write it down, you know, that we can talk about it and there would still be someone who just lingers.” Student 5 had an opposing view. He discussed the fact that when students were allowed to choose their own groups “the people that knew what they were doing, they would usually congregate in their own group and everybody else is just shut out of that. So they’re not exactly sharing their wealth of knowledge with anybody else.”

Other students recalled group activities differently. Student 6 began by saying, “In class when we were presented with an activity, between group members you would see, um, pretty much others that were trying to help, um, the people in the group that didn’t understand the information.” She also expressed that working in assigned groups “wasn’t catastrophic, it was still a good experience to know that everybody…it’s not just you in the class that didn’t understand it, there’s other people that didn’t understand it or you know, there’s other people that could show you how to do it in other ways…” This is a similar sentiment to several students on the survey as well as students in Pentecost’s study. Student 4 described a similar strategy for group work to Students 1 and 3, however, she seemed to feel more like Student 6 in terms of the group dynamics:

**Student 4:** *I mean, what I was trying to tell my friends when I was doing it, like each one do something and then we collect everything and, like, if you have a question, we can work on it, like, something hard – that’s how I was trying to do it.*

One important note to make is that students often felt rushed to complete the class activities. It was a common concern over the course of the semester, so the instructor made a practice to stay in the hallway outside of the classroom after the class time was up in order to give students time
to finish writing down their answers. Students reacted differently to this time crunch. For example, Student 4 liked the challenge and thought it compelled her to problem solve:

**Student 4:** *When we were doing the homework from the book and talking to each other in groups. It was, like, a little bit challenging – the no time and we were running and fighting, you know, one looking left, one looking right, you know what I mean? I like that. Because it makes you like, I don’t know, talk to your friends and then try to solve in groups. That’s what I liked a lot.*

However, Students 1, 3, and 5 felt that they were pressured to “put something on paper” and did not really learn the concept from the activity. In his discussion of working with assigned group members, Student 1 indicated that understanding and explaining the concepts was not his priority when there was a time crunch for finishing the assignments:

**Student 1:** *…but then there were a couple times where I don’t think they, I don’t think my group mates would grasp the idea that we had five minutes to finish this, we need to get this done and they’d be like, “Wait. Can you explain it?”*

**Interviewer:** And so did you explain it when somebody had a question?

**Student 1:** *If we had time. If not, I told them to leave it for later because I needed to get the assignment done.*

Student 3 tended to feel stressed:

**Student 3:** *…and I didn’t know a lot to begin with so we’re all just like, “Alright we have 2 problems in 10 minutes and we have no idea what to do.” And it was always like, “All right let’s put something on paper” so that was always pretty stressful too…*
Student 5 said that the lack of time would force them to find a superficial strategy to solve the problem and if a group member found an answer, they did not all necessarily understand and agree on the approach, rather, “It was all in on one answer.”

Other trends

Interestingly, a look at less frequent themes on the surveys reveals the effectiveness and enthusiasm of the instructor, reminding us that regardless of pedagogy, instructor beliefs and attitudes have a significant impact on students. In fact, during the interviews, students were also asked whether there was a particular concept they felt they understood better because of the teaching method. This was meant to probe corrected or lingering alternative conceptions, but interview results revealed that regardless of the concept the student mentioned, they felt they had a better understanding of it because of how it was explained by the instructor. Every student interviewed shared this view as shown below:

Student 1: Like when he would do it on the board in lecture, I would be like, “Oh my gosh. That just like…it clicked.”

Student 2: Well. I feel like I maybe got a better understanding of the equilibrium concept um…due to the way it was explained in class.

Student 3: I think dynamic equilibrium was one of them he explained that a lot better than I’ve learned it before and that made a lot of sense.
Interviewer: Ok and was there like a specific activity that we did that helped you understand that or was it just the way Dr. Hernandez was teaching?

Student 4: It was teaching. Like the energy one? Yeah, he was teaching it.

Interviewer: Was there anything that we did in the course that helped you really understand that?

Student 5: Explaining it in a way outside of mathematics.

Student 6: You know and the fact that he explained it in such detail...

7.4 Limitations

Phenomenological studies aim to reveal how students experience certain events. They do not necessarily reveal the “truth”. Thus, we have presented our best explanation of how these students experienced active learning in a Physical Chemistry I course covering thermodynamics and kinetics concepts. Students at different universities or with different professors may describe their experiences differently.

The observations were performed on only a group of ten students at a time so that a large enrollment course could be observed. However, it is important to remember that each observation does not necessarily represent what the students in every other group were doing during the class period. In addition, observers collected data on different groups of students, making it difficult to establish reliability using inter-rater reliability methods. However, these data merely corroborate the other evidence presented. They are not meant as standalone evidence of engagement.
The survey responses were coded using an inductive coding scheme which involves looking for themes without previously defining codes. However, both the first and corresponding author participated in the development and facilitation of the course, making coding impossible to do *tabula rasa*. In order to address any concerns about this coding procedure, we first express our belief that no research can truly be conducted *tabula rasa*. Second, we have presented the complete results of coding in Appendix L so that the reader can be assured that themes other than those corresponding to the course design were identified. We presented the chosen themes in this chapter since they gave the most information about how students experienced the active learning strategies we applied.

Finally, students were asked via e-mail to volunteer to participate in interviews. The six students that volunteered all obtained A’s in the course. Their perceptions may differ from students who did not receive an A or A- in the course. However, the diverse views expressed by these students can provide much insight into how students respond in an active learning environment.

### 7.5 Implications

The results presented herein can contribute greatly to the movement toward the implementation of active learning in upper-level chemistry courses. In particular, these results provide a useful resource for faculty, instructors, graduate teaching assistants, and other university educators when considering implementing active learning in the classroom. Obviously, students respond to these changes and their responses are important to keep in mind when developing an active learning course. Our students shared several characteristics with students from previous studies including increased feeling of responsibility for knowing concepts, manifestation of the “problem-solving mindset” which influenced their study strategies and mixed emotions about
group work (i.e. appreciation for different perspectives and resentment for lackluster group members). This provides validity to our findings and in tandem with our additional results (i.e. the combination of the strategies with an exam-free approach), provides implications for effectively implementing active learning in physical chemistry, improving this particular implementation and minimizing negative responses from students. These implications will be discussed in terms of each of the active learning strategies employed.

7.5.1. Alternative Assessments

The dualistic response to alternative assessments suggests that a minor change in how we refer to assessments could both keep test anxiety low while maximizing motivation to perform. Rather than referring to out-of-class assignments as “homeworks”, we could refer to them as “take-home exams”. Assigning several take-home exams after the material has been covered in class may provide extrinsic motivation for the students to study, but reduce the anxiety associated with the lack of time and resources available on a traditional in-class exam.

7.5.2. Discussions

The students’ perception of discussions indicated that they felt accountable for understanding the information before class and that they learned from hearing their classmates’ perspectives. Survey and interview responses clearly show that students recognize the value in this exercise. In addition, random calling on students during the whole-group discussions was one strategy that allowed the instructor to ensure individual accountability in a course with no exams. Any negative responses to this strategy (i.e. anxiety about being called on), can be alleviated, but not likely eliminated, by clearly communicating the expectation that students participate in the discussion.
7.5.3. Homework/flipped classroom

There are a myriad of descriptions in the literature about flipping the classroom. The common underlying theme is that content delivery occurs outside of class and there is some sort of practice or application opportunity for students during class. However, the specific manifestation of each of these components is varied. For instance, as instructors, we wanted our students to read the material rather than to watch videos. The reason for this was two-fold. First, we prefer the presentation of the material in the manner it is presented in the textbook we chose. Second, we wanted to guide our students in learning by actively reading rather than watching videos in order to help them develop this skill. However, we do not believe that our results implicate one way or another that reading should be used as content delivery over videos. This was simply our preference and the same argument can be made for having in-class discussions followed by problem solving in groups during class. This aside, it has been established that this kind of instruction requires scaffolding. For this reason, the instructor modeled some active reading strategies on the first day of class and continued in-class discussions to help students focus on main concepts. Despite this, students expressed feeling overwhelmed by the reading.

7.5.4. Group work

A common student complaint about group work, and one which some of our students expressed, was that not all group members “pulled their own weight”. It is important that the instructor ensure individual accountability during group work to reduce these experiences and encourage learning among all group members. For this reason, we reassigned groups frequently (every week or every two weeks depending on how effectively we thought the groups were working together). Most often groups were assigned based on course performance. Seldom, students were allowed to choose their own groups. Assigning groups based on performance, but
reassigning them frequently was intended to prevent students from relying on any single group member throughout the semester.
7.6 References


CHAPTER 8: FUTURE WORK

The research that constitutes this dissertation represents the deliberate effort to integrate fundamental science research into education and to evaluate the effect of this integration on student’s views of the nature of learning and doing science. The journey began with two investigations of the potential application of nonlinear optics in studying i) the mechanism of binding of cannabinoids and ii) the capabilities of Thioflavin T as a multiphoton bioimaging dye. The future of the former involves overcoming the challenge of experimentally measuring TPCD in a solution containing a large biomolecule like CB1 which limits the obtainable concentration of cannabinoids we are interested in measuring. The latter will continue when TPA of ThT is measured in amyloid plaques in vitro or ex vivo.

Following completion of these two studies, it became apparent that the concepts involved, having a foundation in undergraduate physical chemistry, were essentially out of reach for chemistry students. However, recent efforts to offer authentic research experiences inspired the development of a nonlinear spectroscopy course-based undergraduate research experience (CURE). Seeing the positive student perceptions that resulted from this experience, we decided to develop a pre-CURE for high school students participating in a summer camp and redesign a physical chemistry course to incorporate authentic learning experiences for chemistry students. Results of these studies demonstrated the effect on students’ views of the nature of practicing and learning science.

The current evaluation of CUREs is based largely on student self-reporting of skills and knowledge gained and their increase in positive attitudes toward science. However, it is important to develop measures for the skills students actually develop and use in future courses and other experiences (research, professional, etc.). This could be done qualitatively by
observing and interviewing CURE students in these environments and identifying what skills they are using successfully. In particular, we were interested in students’ experiences when using scientific practices such as those identified by the National Research Council in their Framework for K-12 Science Education. These practices include 1) asking questions, 2) developing and using models, 3) planning and carrying out investigations, 4) analyzing and interpreting data, 5) using mathematics and computational thinking, 6) constructing explanations, 7) engaging in argument from evidence, and 8) obtaining, evaluating, and communicating information. While the framework was developed for K-12 education, it can be applied to higher education. Considering the lack of change in participants’ formal views of the NOS, this might be a useful framework for analysis of the pre-CURE as well. In addition, student learning of concepts as a result of CUREs needs to be more accurately measured to provide better evidence in support of the practical use of these experiences.

In this dissertation, we focused on student views. However, using an active learning course redesign like that described in Chapter 7 obviously effects not only student perceptions of learning, but what and how they learn. For instance, a study consisting of the development of critical thinking skills in this setting would add to the literature based on the efficacy of active learning strategies on conceptual understanding. While it is clear from the literature that active learning increases performance, the evidence in support of learning is not robust yet. In addition, these two areas could be studied as they relate to each other. For instance, how do physical chemistry students’ perceptions about active learning correlate with what they learn and how they learn it?
The studies presented here are representative of very specific populations (i.e. high-performing secondary students and chemistry majors). However, expanding the STEM workforce and the scientific literacy of citizens of society requires an expansion of the populations involved in these types of studies. For this reason, a logical next step would be to evaluate the effect of authentic experiences on, for example, underrepresented minorities, women, disadvantaged youth, and non-chemistry majors. While the focus may remain on student views of the nature of science, the practices of science, and the learning process, other constructs might be more interesting for these different populations. For example, scientific identity and self-efficacy are enhanced particularly for underrepresented minorities and women during undergraduate research experiences. It would be useful to determine the effect of OCTET on these populations so that similar programs could be implemented around the country to recruit and increase the probability of retention of these groups in STEM.
8.1 References


APPENDIX A: PUBLICATIONS FROM DISSERTATION WORK


APPENDIX C: IRB OUTCOME LETTERS
Approval of Exempt Human Research

From: UCF Institutional Review Board #1
FWA00000351, IRB00001138

To: Julie Gama Donnelly

Date: December 18, 2015

Dear Researcher:

On 12/18/2015, the IRB approved the following activity as human participant research that is exempt from regulation:

Type of Review: Exempt Determination
Project Title: Evaluation of two novel Physical Chemistry Laboratory experiments
Investigator: Julie Gama Donnelly
IRB Number: SBE-15-11899
Funding Agency: 
Grant Title: 
Research ID: n/a

This determination applies only to the activities described in the IRB submission and does not apply should any changes be made. If changes are made and there are questions about whether these changes affect the exempt status of the human research, please contact the IRB. When you have completed your research, please submit a Study Closure request in IRIS so that IRB records will be accurate.

In the conduct of this research, you are responsible to follow the requirements of the Investigator Manual.

On behalf of Sophia Dziesiewski, Ph.D., L.C.S.W., UCF IRB Chair, this letter is signed by:

[Signature]

Signature applied by Joanne Muratori on 12/18/2015 03:28:34 PM EST

IRB Manager
Approval of Human Research

From:        UCF Institutional Review Board 
             FWA00000351, IRB00001138
To:          Julie Gama Donnelly and Co-PI Maryam S Hinsoul
Date:        December 04, 2017

Dear Researcher:

On 12/04/2017 the IRB approved the following modifications to human participant research until 05/04/2018 inclusive:

Type of Review: IRB Addendum and Modification Request Form
Modification Type: Updated protocol and consent to include an interview. Uploaded interview questions and new recruitment materials.
Project Title: OCETI & BIOTEC: Evaluation of a Summer Intensive Designed to Cultivate the Future Generation of Young Leaders in STEM.
Investigator: Julie Gama Donnelly
IRB Number: SBE-15-11344
Funding Agency: National Science Foundation
Grant Title: 
Research ID: 1056827

The scientific merit of the research was considered during the IRB review. The Continuing Review Application must be submitted 30 days prior to the expiration date for studies that were previously expedited, and 60 days prior to the expiration date for research that was previously reviewed at a convened meeting. Do not make changes to the study (i.e., protocol, methodology, consent form, personnel, site, etc.) before obtaining IRB approval. A Modification Form cannot be used to extend the approval period of a study. All forms may be completed and submitted online at https://iris.research.ucf.edu.

If continuing review approval is not granted before the expiration date of 05/04/2018, approval of this research expires on that date. When you have completed your research, please submit a Study Closure request in IRIS so that IRB records will be accurate.

Use of the approved stamped consent document(s) is required. The new form supersedes all previous versions, which are now invalid for further use. Only approved investigators (or other approved key study personnel) may solicit consent for research participation. Participants or their representatives must receive a copy of the consent form(s).

All data, including signed consent forms if applicable, must be retained and secured per protocol for a minimum of five years (six if HIPAA applies) past the completion of this research. Any links to the identification of participants should be maintained and secured per protocol. Additional requirements may be imposed by your funding agency, your department, or other entities. Access to data is limited to authorized individuals listed as key study personnel.

In the conduct of this research, you are responsible to follow the requirements of the Investigator Manual.

This letter is signed by:
Signature applied by Renea C. Carver on 12/04/2017 01:04:16 PM EST

Designated Reviewer
Approval of Exempt Human Research

From: UCF Institutional Review Board #1
FWA00000351, IRB00001138

To: Julie Gama Donnelly and Co-PI Florencio E. Hernandez

Date: April 20, 2017

Dear Researcher:

On 04/20/2017, the IRB approved the following activity as human participant research that is exempt from regulation:

Type of Review: Exempt Determination
Modification Type: Interview procedures added, updated protocol and consent
Project Title: SoTL in a senior level undergraduate STEM course fusing a Reversed Learning Scheme with an Informal Learning Space: Reaching a new generation!
Investigator: Julie Gama Donnelly
IRB Number: SBB-16-12307
Funding Agency: University of Central Florida (UCF)
Grant Title: n/a
Research ID: n/a

This determination applies only to the activities described in the IRB submission and does not apply should any changes be made. If changes are made and there are questions about whether these changes affect the exempt status of the human research, please contact the IRB. When you have completed your research, please submit a Study Closure request in iIRB so that IRB records will be accurate.

In the conduct of this research, you are responsible to follow the requirements of the Investigator Manual.

On behalf of Sophia Dziegielewski, Ph.D., L.C.S.W., UCF IRB Chair, this letter is signed by:

Signature applied by Renée C Carver on 04/20/2017 01:58:47 PM EDT

IRB Coordinator
APPENDIX D: AUTHORIZATIONS FROM EDITORIAL OFFICES FOR USE OF COPYRIGHTED MATERIAL
Title: Enhanced two-photon absorption and fluorescence upconversion in Thioflavin T micelle-type aggregates in glycerol/water solution

Author: Julie Donnelly, Yuly Vesga, Florincio E. Hernandez

Publication: Chemical Physics

Publisher: Elsevier

Date: 30 September 2016

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FW: Permission Request Form: Julie Donnelly

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Fri 3/2/2018 8:56 AM

To: Julie Donnelly <jdonnelly319@knights.ucf.edu>

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Sent: 28 February 2018 16:25
To: CONTRACTS-COPYRIGHT (shared) <Contracts-Copyright@rsc.org>
Cc: jdonnelly319@knights.ucf.edu
Subject: Permission Request Form: Julie Donnelly

Name : Julie Donnelly
Address :

3509 Roc Oak Tr

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APPENDIX E: IMAGES OF CANNABINOIDS IN RECEPTOR SITE
Figure E-1. (-)-trans-THC conformer 1 in site before (upper left) and after (lower right) optimization. The molecule before and after is overlaid in the upper right. Highlighted regions indicate formation of hydrogen bond between Lys-192 and the phenolic hydrogen as well as the Trp-357 rotamer change.
Figure E-2. (-)-cis-THC conformers 1 and 2 in site before and after optimization.
Figure E-3. CBD conformers 1 (top) and 2 (bottom) in site before and after optimization.
Figure E-4. CBN conformers 1 (top) and 2 (bottom) in site before and after optimization.
APPENDIX F: OPA AND TPA SPECTA OF (-)-TRANS-$\Delta^9$-THC, (-)-CIS-$\Delta^9$-THC, CBD AND CBN
Figure F-1. Comparative plots of OPA (left) and TPA (right) spectra of (-)-trans-$\Delta^9$-THC conformers. Dotted lines correspond to structures optimized in gas phase, solid lines correspond to structures optimized in the receptor site.

OPA response for the first 100 excited states of optimized structures were modeled with TD-DFT/B3LYP/6-311++G** in methanol using Gaussian 09. TPA response for the first 50 excited states were modeled with TD-DFT/B3LYP/6-311++G** in gas phase using Dalton 2013.
Figure F-2. Comparative plots of OPA (left) and TPA (right) spectra of (-)-cis-Δ^9-THC conformers. Dotted lines correspond to structures optimized in gas phase, solid lines correspond to structures optimized in the receptor site. OPA response for the first 100 excited states of optimized structures were modeled with TD-DFT/B3LYP/6-311++G** in methanol using Gaussian 09. TPA response for the first 50 excited states were modeled with TD-DFT/B3LYP/6-311++G** in gas phase using Dalton 2013.
Figure F-3. Comparative plots of OPA (left) and TPA (right) spectra of cannabidiol (CBD) conformers. Dotted lines correspond to structures optimized in gas phase, solid lines correspond to structures optimized in the receptor site. OPA response for the first 100 excited states of optimized structures were modeled with TD-DFT/B3LYP/6-311++G** in methanol using Gaussian 09. TPA response for the first 50 excited states were modeled with TD-DFT/B3LYP/6-311++G** in gas phase using Dalton 2013.
Figure F-4. Comparative plots of OPA (left) and TPA (right) spectra of cannabinol (CBN) conformers. Dotted lines correspond to structures optimized in gas phase, solid lines correspond to structures optimized in the receptor site. OPA response for the first 100 excited states of optimized structures were modeled with TD-DFT/B3LYP/6-311++G** in methanol using Gaussian 09. TPA response for the first 50 excited states were modeled with TD-DFT/B3LYP/6-311++G** in gas phase using Dalton 2013.
APPENDIX G: ASSIGNMENT OF THEORETICAL TRANSITIONS TO
EXPERIMENTAL BANDS FOR THC AND CBD SPECTRA
Assignment of theoretical excited states to experimental (Exp.) bands of OPA and ECD spectra for THC and CBD. Excited states contributing to 30% or more of the intensity of the corresponding bands were assigned. The theoretical wavelengths are reported with the spectral shift used to match the experimental.

\[ \lambda_{\text{max}} = \text{OPA wavelength of maximum absorption} \]

\[ \varepsilon = \text{molar absorptivity (l.mol}^{-1}.\text{cm}^{-1}) \]

\[ f = \text{oscillator strength} \]

E.S. = excited state number

\[ \Delta\varepsilon = \text{difference in molar absorptivity (l.mol}^{-1}.\text{cm}^{-1}) \]

\[ R^{\text{ECD}} = \text{rotatory strength (erg.esu.cm.Gauss}^{-1}) \]
Figure G-1. Theoretical OPA (left) and ECD (right) spectra of THC (top) and CBD (bottom). In each case, red is conformer 1, blue is conformer 2 and violet is the average. Colored symbols represent the oscillator strengths for each molecule. OPA and ECD for the lowest 100 electronic excited states were computed at the B3LYP/6-311++G(d,p) level of theory using Gaussian 09 in methanol. $\Gamma=0.35$ eV (FWHM) was used for all the spectra for best fit to experimentally observed spectra.
<table>
<thead>
<tr>
<th>Exp.</th>
<th>THC1 (shifted -1.5 nm)</th>
<th>THC2 (shifted -1.5 nm)</th>
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<tbody>
<tr>
<td>232</td>
<td>257 (1,382) 1 (0.02)</td>
<td>257 (1,041) 1 (0.02)</td>
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<tr>
<td>209</td>
<td>209 (59,014) 9 (0.11)</td>
<td>209 (59,620) 15 (0.26)</td>
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<tr>
<td></td>
<td>12 (0.11)         19 (0.29)</td>
<td>16 (0.11) 28 (0.19)</td>
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<tr>
<td></td>
<td>24 (0.14)         25 (0.15)</td>
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<tr>
<td></td>
<td>176 (26,482) 49 (0.06)</td>
<td>174 (27,139) 56 (0.07)</td>
</tr>
<tr>
<td></td>
<td>52 (0.07)         55 (0.06)</td>
<td>56 (0.05) 72 (0.10)</td>
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<tr>
<td></td>
<td>56 (0.05)         57 (0.07)</td>
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<tr>
<td></td>
<td>165 (17,532) 79 (0.03)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80 (0.02)         85 (0.03)</td>
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<tr>
<td></td>
<td>93 (0.03)</td>
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<table>
<thead>
<tr>
<th>Exp.</th>
<th>THC1 (shifted -10 nm)</th>
<th>THC2 (shifted -10 nm)</th>
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<tr>
<td>222</td>
<td>229 (3.33) 5 (6.08)</td>
<td>229 (4.37) 2 (3.10)</td>
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<tr>
<td>198</td>
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<td>198</td>
<td></td>
<td>165 (6.50) 56 (19.0)</td>
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<td></td>
<td>160 (61.1) 79 (17.1)</td>
<td>209 (-58.8) 8 (19.0)</td>
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<tr>
<td></td>
<td>74 (28.5)</td>
<td>58 (15.2)</td>
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<tr>
<td>209</td>
<td>210 (-37.8) 8 (-16.6)</td>
<td>209 (-58.8) 8 (-13.9)</td>
</tr>
<tr>
<td></td>
<td>8 (-19.0)</td>
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<td></td>
<td></td>
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<td></td>
<td>15 (-27.2)</td>
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<tr>
<td></td>
<td>188 (-40.4) 28 (-56.5)</td>
<td>190 (-30.58) 28 (-54.1)</td>
</tr>
<tr>
<td></td>
<td>34 (-27.2)</td>
<td>46 (-16.2)</td>
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<td>49 (-14.3)</td>
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<td>50 (-12.2)</td>
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<td>51 (-21.4)</td>
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<tr>
<td>172</td>
<td>172 (-38.2) 52 (-42.2)</td>
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<td></td>
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<td>51 (-21.4)</td>
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<td></td>
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<tr>
<td>151</td>
<td>151 (-13.0) 93 (-22.3)</td>
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<tr>
<td></td>
<td></td>
<td>153 (-25.4) 93 (-33.3)</td>
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<tr>
<td>CBD (in methanol)</td>
<td>Exp.</td>
<td>CBD1 (shifted -1.5 nm)</td>
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<td>-------------------</td>
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<td>------------------------</td>
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<tr>
<td></td>
<td>λ&lt;sub&gt;max&lt;/sub&gt; (ε)</td>
<td>λ&lt;sub&gt;max&lt;/sub&gt; (ε)</td>
</tr>
<tr>
<td>CBD</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>274</td>
<td>254 (1,805)</td>
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<td></td>
<td>231</td>
<td>232 (11,728)</td>
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<td>OPA Bands</td>
<td>208</td>
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<td>26 (0.09)</td>
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<td>35 (0.16)</td>
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<td>180 (20,150)</td>
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<td>54 (0.08)</td>
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<td>171</td>
<td>83 (0.04)</td>
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<td></td>
<td></td>
<td>15 (11,14)</td>
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<tr>
<td></td>
<td></td>
<td>83 (0.04)</td>
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</table>
Figure H-1. DLS size distribution based on intensity for 0%, 8.75%, 17.5%, and 35% glycerol content solutions. The light source used in DLS measurements was a He-Ne Laser centered at 633 nm.
APPENDIX I: OCTET PRETEST
1. Benzene and toluene form an ideal solution. The vapor pressure of benzene at 55°C is 400 mm Hg while the vapor pressure of toluene at 55°C is 130 mm Hg. What is the vapor pressure of a solution consisting of 0.5 mole fraction of benzene and 0.5 mole fraction of toluene at 55°C?
   a. lower than 130 mm Hg
   b. between 130 and 400 mm Hg
   c. exactly 400 mm Hg
   d. greater than 400 mm Hg

2. The value of which concentration unit for a solution changes with temperature?
   a. Molarity
   b. Molality
   c. Mole fraction
   d. Mass percentage

3. Which of the following are NOT listed in order of increasing ionic radius?
   a. Mg²⁺<Na⁺<Ne
   b. Ca²⁺<S²⁻<S⁻
   c. Al⁺⁺<Mg⁺⁺<Na⁺
   d. O²⁻<F⁻<Ne

4. Which element is a liquid at 25°C and 1 atm?
   a. Phosphorus
   b. Chlorine
   c. Bromine
   d. Iodine

5. The active ingredient in commercial bleach is sodium hypochlorite, NaOCl, which can be determined by iodometric analysis as indicated in these equations:
   \[ \text{OCl}^- + 2\text{H}^+ + 2\text{I}^- \rightarrow \text{I}_2 + \text{Cl}^- + \text{H}_2\text{O} \]
   \[ \text{I}_2 + 2\text{S}_2\text{O}_3^{2-} \rightarrow \text{S}_4\text{O}_6^{2-} + 2\text{I}^- \]
   If 1.356 g of a bleach sample requires 3 mL of 0.650 M Na₂S₂O₃ solution, what is the percentage by mass of NaOCl in the bleach?
   a. 2.68%
   b. 3.70%
   c. 5.35%
   d. 10.7%

6. Which reaction forms a product with a trigonal planar geometry?
   a. \( \text{N}_2 + 2\text{H}_2 \rightarrow \)
   b. \( 2\text{CO} + \text{O}_2 \rightarrow \)
   c. \( \text{PCl}_3 + \text{Cl}_2 \rightarrow \)
   d. \( 2\text{SO}_2 + \text{O}_2 \rightarrow \)

7. Which species has the largest bond angle?
   a. \( \text{NO}_2^+ \)
   b. \( \text{NO}_3^- \)
   c. \( \text{NO}_2^- \)
   d. \( \text{NO}_3^- \)

8. A 1.00 L sample of buffer contains 0.300 mol of CH₃COOH and 0.300 mol of CH₃COONa. Calculate the pH of this solution after 0.020 mol of NaOH is added. (Ka, acetic acid = 1.8 x 10⁻⁵)
   a. 4.83
   b. 4.80
   c. 4.75
   d. 4.70
9. Which statement(s) characterize(s) a chemical system at equilibrium?
   i. The rate of the forward reaction is equal to the rate of the reverse reaction.
   ii. The concentrations of the reactants and products are equal.
   a. i only
   b. ii only
   c. both i and ii
   d. neither i or ii

10. For the reaction $2A + B \rightarrow 2C$, the rate law is rate $= k[A][B]$. Which of the following mechanisms would be consistent with this information?
   a. $A + B \rightarrow C + M$ (fast)
      $A + M \rightarrow C$ (slow)
   b. $A + B \rightarrow C + M$ (slow)
      $A + M \rightarrow C$ (fast)
   c. $B \rightarrow M$ (slow)
      $2A + 2M \rightarrow 2C$ (fast)
   d. $B \rightarrow M$ (fast)
      $M + A \rightarrow N$ (slow)
      $N + A \rightarrow C + D$ (fast)

11. When the concentration of a particular reactant is tripled and the initial rate of the reaction increases by a factor of nine, what is the order of the reaction with respect to this reactant?
   a. Zero
   b. One
   c. Two
   d. Three

12. A piece of iron metal at an unknown temperature is added to 15.0 g of liquid water at 21.5 °C. The final temperature of the 18.2 g equilibrated mixture is 22.1°C. What was the original temperature of the iron? ($C_i$, iron = 0.449 J/g °C)
   a. 22.4 °C
   b. 23.3 °C
   c. 47.7 °C
   d. 48.3 °C

13. Which process is exothermic?
   a. Condensation
   b. Melting
   c. Sublimation
   d. Vaporization

14. What is the percentage ionization of HCOOH molecules in a 0.10 M solution?
   $[K_a = 1.8 \times 10^{-5}]$
   a. 4.2%
   b. 2.7%
   c. 1.8%
   d. 1.3%

15. Br$_2$(I) + 2e$^- \rightarrow 2$Br$^-$(aq) $E^\circ$=1.09 V
    I$_2$(s) + 2e$^- \rightarrow 2$I$^-$(aq) $E^\circ$=0.54 V
   Use the equations above to calculate the value of $\Delta G^\circ$ (in kJ/mol) for the reaction:
   I$_2$(s) + 2Br$^-$(aq) $\rightarrow 2$I$^-$(aq) + Br$_2$(l)
   a. 314 kJ
   b. -314 kJ
   c. -106 kJ
   d. 106 kJ

16. All of the reactions below represent oxidation-reduction processes EXCEPT the
   b. Decomposition of potassium chlorate.
   c. Neutralization of sodium hydroxide.
   d. Reaction of magnesium with hydrochloric acid.

17. Oxidation of a secondary alcohol with pyridinium chlorochromate (PCC) dissolved in dichloromethane gives a product with which functional group?
   a. aldehyde
   b. carboxylic acid
   c. ester
   d. ketone

18. The classification of a fat as saturated or unsaturated is based on whether
   a. It can be metabolized by humans.
   b. It contains carbon-carbon double bonds.
   c. It has twenty or more carbon atoms.
   d. It is of animal origin.
Semi-structured interview guide

The purpose of this phenomenological study is to determine the impact of an authentic research experience on high school students’ views on the nature of science.

<table>
<thead>
<tr>
<th>Data</th>
<th>Question</th>
<th>Prompts &amp; elicitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>To break the ice and provide some background.</td>
<td>Tell me about yourself why you wanted to participate in OCTET.</td>
<td>Motivation, a person of influence, career goals</td>
</tr>
<tr>
<td>To identify other experiences that could have had an impact.</td>
<td>Have you taken any science courses or participated in any activities in science since OCTET? Tell me about them.</td>
<td>Subjects, camps, specific tasks, experiences</td>
</tr>
<tr>
<td>Reflections on research experience.</td>
<td>Tell me about your experience during OCTET (specifically, the research component).</td>
<td>Favorite part, what did you learn, what tasks did you do, which parts of the research did you participate in (design, data collection, analysis), did your experience influence your career goals?</td>
</tr>
<tr>
<td>Views on the role of scientific evidence, empirical nature, observations vs. inferences</td>
<td>What was the role of evidence in your research project? How did this view change after OCTET?</td>
<td>In which aspects of your research did you need evidence? What surprised you?</td>
</tr>
<tr>
<td>The role of imagination and creativity in doing science.</td>
<td>How did you (or your mentor) use your imagination during the project? Did this surprise you?</td>
<td>Creative in planning, analysis, interpretation etc.</td>
</tr>
<tr>
<td>Views on the role of iteration.</td>
<td>Tell me about any predictions you made in your project. Were your predictions correct? If not, why? What did you do about that?</td>
<td>Formal (hypothesis), informal (planning), did you always get what you expected?</td>
</tr>
<tr>
<td>Member-checking.</td>
<td>Paraphrase what I heard about the main data: Background information Research experience Evidence Predictions Creativity</td>
<td>Anything the interviewee would like to add or clarify</td>
</tr>
</tbody>
</table>
APPENDIX K: VNOS RESPONSE FREQUENCIES
Raw frequencies of responses by aspect (defined below tables) and category (naïve, transitional, and informed).

<table>
<thead>
<tr>
<th>Pre-Survey</th>
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<th>2</th>
<th>3</th>
<th>4</th>
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1 = Distinction between observation and inference, 2 = Empirical, 3 = Creativity and imagination, 4 = Subjectivity, 5 = Social and cultural embeddedness, 6 = Tentative, 7 = Distinction between Laws and Theories
APPENDIX L: INTERVIEW PROTOCOL AND CODING SCHEME FOR
ACTIVE LEARNING IN PHYSICAL CHEMISTRY
<table>
<thead>
<tr>
<th>Data</th>
<th>Question</th>
<th>Prompts/elicitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ice breaker</td>
<td>As you look back over the semester, what are your perceptions of the course?</td>
<td>Compare to other courses, what they liked/didn’t like</td>
</tr>
<tr>
<td>Perception of learning gains and what strategies they think helped with that</td>
<td>Can you think of a topic in the course that you understand better now than you did at the beginning? What occurred within the course or outside of the course that helped you increase your understanding?</td>
<td>If everything”, ask for something in particular. Tell me about a time when something “clicked”. Another… Flipped classroom? Homeworks? Group work? Discussions?</td>
</tr>
<tr>
<td>Group dynamics</td>
<td>Imagine that I am part of your group during a class activity. What would I see happening in the group as we try to complete the activity?</td>
<td>Is someone “in charge”? Are roles/tasks assigned?</td>
</tr>
<tr>
<td>Summary</td>
<td>Summarize and ask if they have anything else to say.</td>
<td></td>
</tr>
</tbody>
</table>
Coding categories

After organizing and becoming familiar with all of the survey responses, we freely developed several categories by making notes on the surveys to describe the content in as much detail as possible. We then grouped these categories under higher order headings, reducing the number of main categories as much as possible. The main category we report in the chapter is “instructional strategies”. However, other main categories included “classroom environment”, “course focus” and “learning tools”. We chose to focus on the first category for two reasons. First, the sub-categories (alternative assessments, discussions, flipped classroom and group work) do align well with the structure of the course and, thus, would help us answer our research question. Second, the majority of responses fit in this category, so we chose to focus our analysis there. All coding categories are shown in the figure below.