Single Molecule Chiroptical Spectroscopy: Fluorescence Excitation Circular Dichroism and Circular Polarized Luminescence of Bridged Triarylamine Helicenes

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SINGLE MOLECULE CHIROOPTICAL SPECTROSCOPY:
FLUORESCENCE EXCITATION CIRCULAR DICHROISM AND CIRCULAR POLARIZED LUMINESCENCE OF BRIDGED TRIARYLAMINE HELICENES

A Dissertation Presented

by

RUTHANNE HASSEY PARADISE

Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY

September 2009

Chemistry
SINGLE MOLECULE CHIROPTICAL SPECTROSCOPY:
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DEDICATION

I dedicate this work to Mrs. Regina Skudera, my high school chemistry teacher. She showed me the wonders and delights of exploring chemistry and inspired me to pursue it as a career.
ACKNOWLEDGMENTS

All of the work on this project would not have been possible without the help of many people. First and foremost, I would like to thank Dr. Michael Barnes, my professor and advisor, who has taught me a great deal about being a scientist. Dr. Nathan Hammer, a post-doctoral fellow in the lab, gave me assistance and guidance in performing experiments. Dr. Kevin McCarthy, a post-doctoral fellow in my lab as well, worked on the simulations of the defocused images. My lab mates Michael Odoi, Kevin Early, JinHong Kim, and Austin Cyphersmith gave me assistance, and I had many discussions with them about the research. Many undergraduates helped with the research over the past five years, most notably Ellen Swain, Tim Mortsolf, and Anna May Tilley. I would also like to thank our collaborators Dr. Dhandapani Venkataraman and Dipankar Basak, for their research on Bridged Triarlyamines and synthesis of the molecules. We had many conversations about the chiroptical properties of molecules with James Cheeseman, Bart Kahr, and Pat Vaccaro.
In this thesis, I describe the first exploratory experimental efforts probing light-matter interactions of chiral systems at the single molecule level. The dissymmetric single molecule chiroptical response in both excitation and emission polarization has been studied for different diastereomeric forms of bridged triarylamine helicenes. Fluorescence excitation circular dichroism (FECD), measuring the dissymmetric absorption with respect to excitation polarization, reports on the response to excitation polarization. The magnitude and distribution of chiroptical single molecule responses suggest both surface and orientation effects play a significant role. Computational modeling done to calculate the dissymmetry for specific orientations supports orientational dependence. Using a defocused imaging technique, which can be used to obtain orientation information for linear dipoles, emission patterns were obtained that lacked bilateral symmetry. These emission patterns were simulated using a semi-classical model that closely approximated the lack of bilateral symmetry. Refinement of the model and additional experiments using oriented molecules will allow for direct correlation of orientation and dissymmetry which is important for understanding the
heterogeneities in the single molecule responses. In addition, dissymmetry in emission polarization has been studied using a novel imaging technique resolving polarization components on a frame-by-frame basis. The research into the intersection of single molecule spectroscopy and chiroptics has given new insight into the role of solvation and local environment in chiroptical interactions and may be useful for understand chiral-based photonics and advancing new technologies.
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CHAPTER 1
INTRODUCTION

1.1 Background of chiroptics

For over two centuries, molecular chirality has been a topic of interest to scientists of all fields. Molecular chirality is key in the structure of amino acids, molecular recognition\textsuperscript{1-15}, biochemical synthesis\textsuperscript{16-20}, and self-assembly\textsuperscript{1, 21-39} processes in nature. Of particular interest is the way chiral systems interact with circularly polarized light. This is because chirality has applications in many areas ranging from investigating medicine\textsuperscript{40} to information storage, and display technologies.\textsuperscript{41, 42} The knowledge base, both theoretical and experimental, on light-matter interactions with chiral materials\textsuperscript{43-45} and assignment of absolute chiral configurations from spectroscopic data\textsuperscript{46-55} is very large and continues to increase.

The basic spectroscopic tools for interrogating chiroptical dissymmetries are Optical Rotary Dispersion \textsuperscript{56-60} (ORD), Circular Dichroism \textsuperscript{45, 61} (CD), Circularly Polarized Luminescence \textsuperscript{62-74} (CPL), and Fluorescence Detected Circular Dichroism \textsuperscript{13, 75-87} (FDCD). ORD is a non-resonant effect looking at the dissymmetric rotation of light, whereas CD and FDCD report on the resonant absorption of circular polarized light, and CPL reports on emission polarization. The use of these techniques have given us a great amount of knowledge into the dissymmetric chiroptical response of ensembles of structurally identical chiral systems. All of these techniques typically use ensembles because the dissymmetric response is so small and the experimentally observed dissymmetry is a function of concentration and path length. Due to the use of ensembles
the influence of local molecular environment and configurational fluctuations in the chiroptical response of isolated molecular systems, and the significant heterogeneities they might produce in those systems, is largely unexplored. In this dissertation, I describe the first experimental efforts designed to explore these influences using single molecule chiroptical probes.

![Figure 1: A system that has simultaneous nonorthogonal elements of translation ($\mathbf{k}$) and rotation ($\mathbf{S}$) the helicity (chirality) is defined by the sense of rotation relative to the direction of translation. In this case, for a photon, the angular momentum can be aligned parallel or antiparallel to the linear momentum giving rise to a left- or right-handed helix respectively.](image)

At the molecular level, chirality is seen as two molecules that are mirror images of each other, but cannot be superimposed on one another. They cannot have any improper rotation axis, such as centers of inversion, reflection planes, and rotation-reflection axes. A useful example of a chiral system displaying these properties is the photon. The spin angular momentum can be aligned either parallel or antiparallel to the linear momentum, causing right-handed or left-handed helicity. (See Figure 1) In molecular systems, the molecule’s electric dipole ($\mu$) plays a role analogous to linear momentum, and its magnetic dipole ($m$) is analogous to angular momentum (or rotation). The product of the electric and magnetic transition dipoles in chiral systems therefore has a right- or left-handed helicity, and thus interacts with left or right circular polarization.
dissymmetrically. This can be measured as either a differential absorbance (CD, a resonant interaction) or dispersion (ORD, a nonresonant interaction). Both the sign and magnitude of this response is captured by the dissymmetry parameter “g”, defined in terms of molecular parameters

\[ g = \frac{4R}{cD} \]  

(1)

The transition from \(|n\rangle\) the initial state to \(|j\rangle\) the final state, where R is the rotatory strength is defined as

\[ R_{n\leftrightarrow j} = \frac{1}{3} \omega_{n\leftrightarrow j} Re \left( \vec{\mu} \times \vec{\Theta} \right) - Im(\vec{\mu} \cdot \vec{m}), \]  

(2)

where \(\vec{\mu}\) represents the electric dipole vector

\[ \vec{\mu} = \sum_i e_i r_i \]  

(3)

where \(e_i\) and \(r_i\) are the charge and position vector of the \(i^{th}\) charge. The magnetic dipole vector \((\vec{m})\) is

\[ \vec{m} = \sum_i \frac{e_i}{2m_i} \times p \]  

(4)

where \(m_i\) and \(p\) are the mass and linear momentum of the \(i^{th}\) charge. \(\vec{\Theta}\) is the electric quadrupole tensor, D is the dipole strength

\[ D = \vec{\mu} \cdot \vec{\mu} \]  

(5)

and \(c\) is the speed of light. The interaction of the electric dipole with the quadrupole is symmetric, whereas the interaction of the electric dipole with the magnetic dipole is asymmetric. When the rotatory strength is observed along the z-axis, it can be expressed as
\[
R_z(j \leftarrow n) = \frac{1}{3} \omega_n \left[ Re(\langle n|\mu_z|j\rangle\langle j|\Theta_{yz}|n\rangle) - Re(\langle n|\mu_z|j\rangle\langle j|\Theta_{xz}|n\rangle) - Im(\langle n|\mu_x|j\rangle\langle j|m_x|n\rangle) - Im(\langle n|\mu_y|j\rangle\langle j|m_y|n\rangle) \right]
\] (6)

The expression for any arbitrary observation angle is

\[
\Gamma_{n0}(\theta, \phi) = \hat{n} \cdot \vec{R} \cdot \hat{n}
\]

\[
= \sin^2 \theta \left[ R_{xx}^{n0} \cos^2 \phi + R_{yy}^{n0} \sin^2 \phi + \frac{1}{2} (R_{xy}^{n0} + R_{yx}^{n0}) \sin 2\phi \right] + \sin 2\theta \left[ \frac{1}{2} (R_{xz}^{n0} + R_{zx}^{n0}) \cos \phi \right. \\
\left. + \frac{1}{2} (R_{yz}^{n0} + R_{zy}^{n0}) \sin \phi \right] \cos^2 \theta \frac{1}{2} R_{xz}^{n0}.
\] (7)

where \( \hat{n} \) is the observation angle and \( \theta \) and \( \phi \) are the polar angles. The dissymmetry parameter for a specific observation angle therefore becomes

\[
g(\theta, \phi) = \frac{4\Gamma(\theta, \phi)}{c D(\hat{n})}
\] (8)

where \( D \) is the dipole strength perpendicular to the observation angle, defined as

\[
D(\hat{n}) = (\vec{\mu} \cdot \vec{n} - (\hat{n} \cdot \vec{\mu})^2)
\] (9)

For isotropic samples, quadrupole interactions are averaged out, and the rotatory strength therefore appears as the product of electric and magnetic dipole matrix elements alone. This reduces the dissymmetry parameter to the more familiar expression.

\[
g = \frac{2}{3c} Im(\mu_z m_z)
\] (10)

This approximation (Equation 10) of dissymmetry is valid for solution or un-oriented solid phases because all orientations are sampled, or rotational diffusion is fast compared to the fluorescence emission rate. In FDCD and CPL, there is a limit to the isotropic approximation of the rotatory strength. As the rotational diffusion of the
molecules becomes slower than the fluorescence lifetime, as in viscous solvents, the approximation fails.\textsuperscript{86, 87, 89} In such a case, the electric dipole/electric quadrupole interactions can strongly distort the measured dissymmetry. Typical experimental formats in crystals\textsuperscript{61, 90-93} and polymer-supported films used in single molecule spectroscopy have fixed orientation. Therefore, electric-magnetic dipole interaction, \textit{along with} the electric dipole-quadrupole interaction, contributes to the measured dissymmetry. The work of Kahr and co-workers is of particular note, and has application to our work\textsuperscript{94}. They work with anisotropic systems, and show that even achiral species (such as water) may show dissymmetric chiroptical responses, so long as they have well-defined orientations.\textsuperscript{95}

Molecular solvation and local environment are known to greatly modify the chiroptical properties of isolated molecules, yet remain poorly understood.\textsuperscript{96-98} Vaccaro and co-workers have used gas-phase spectroscopy and cavity-ring down polarimetry (CRDP) to interrogate the chiroptical properties of isolated molecules.\textsuperscript{59, 60, 99-103} They found that the solvation effects were drastically modified, and in some cases, the sign of the optical rotatory dispersion was even inverted. These experiments, along with the theoretical work of Kongstead\textsuperscript{104-110}, Crawford\textsuperscript{97, 111-116} and Autschbach\textsuperscript{56, 117-120}, have shown that changes in the solvation environment can notably change the chiroptical response by perturbing the electronic structure of the molecule. A large amount of information has been learned about orientational and solvent effects using ensemble averages. Single molecule spectroscopy gives direct access to environmental and orientational effects on individual molecules by studying the heterogeneities in the photophysics of isolated and oriented molecules.
1.2 Background of Single Molecule Spectroscopy

Single molecule spectroscopy (SMS) was first developed 20 years ago by W. E. Moerner and co-workers to investigate the nature of inhomogeneous broadening of dopant fluorophores in cryogenic organic crystals. Great advances have occurred in the development of such tools such as ultrahigh precision time-to-digital converters with femtosecond resolution, enhanced-sensitivity/high-speed charge-coupled device (CCD) imaging detectors, and photon-counting avalanche photodiodes. These advances allow exploration of single molecule behavior, structure, and the local environment using single molecule imaging techniques, such as time tagged time resolved measurements, spectral imaging, atomic force microscopy, spatial imaging, and polarization measurements. Specifically, fluorescence lifetime, blinking, spectral and spatial diffusion, and polarization anisotropy can be explored to learn how local environment affects the photophysics of the molecules. Additional methodologies in single molecule imaging that have arisen in recent years, which include sub-diffraction limit spatial imaging, fluorescence resonant energy transfer (FRET), integration of scanning probe microscopies with fluorescence probes, multi-photon imaging, and molecular orientation determined by defocused emission pattern measurements.

SMS requires a large number (10^5 – 10^8) of fluorescence photons from a single molecule. Efficient photon collection is done using a infinity-corrected aberration-corrected microscope platform. In addition, a high-sensitivity CCD camera or an avalanche photodiode (APD) is used to probe single molecule behavior on a time scale as short as 500 ps -100 µs, limited only by the detector and its electronics. APDs are
primarily used for high time-resolution measurements (ps-ns) such as fluorescence lifetimes or fluorescence correlation spectroscopy. The high time resolution of APDs generally comes at the expense of spatial information. In comparison, a CCD camera provides information on spatial resolution because it records time, photons counted per pixel, and spatial position.

Figure 2 shows the electronic state diagram for a molecule and the transition between them. In single molecule spectroscopy, the pump rate of the laser must be such that the transition to the excited state is faster than the spontaneous emission rate, so that the photon emission rate approaches the spontaneous emission rate. A few thousand fluorescence photons can be detected in ~100 µs, assuming overall photon detection efficiencies ≈ 50%. Current developments in technology for time-tagged/time-resolved measurements and pulsed lasers make it is possible to measure fluorescence lifetimes with only a few thousand photons.

**Figure 2: Electronic state diagram showing the transitions between states.**

The initial excitation requires a photon absorption driving the molecule from S₀ to S₁. From S₁, three different transitions are available to the molecule: (1) the molecule
can relax to the ground state ($S_0$) by emitting a photon, (2) it can undergo intersystem
crossing to the triplet state ($T_1$) and then relax nonradiatively from that state, or (3) it can
be further excited to the $S_2$ state. The transition from $S_1$ to $S_0$ is what is observed as
fluorescence. The transition from $S_1$ to $T_1$ and then to $S_0$ is called trapping because the
molecule is in the $T_1$ state for a relatively long time because the transition to the ground
state is nominally spin forbidden. At this point, the molecule is observed to be “dark,”
and may stay that way for tens to hundreds of microseconds (or 10s of seconds!). Finally
the molecule relaxes nonradiatively from $T_1$ to $S_0$. The combination of fluorescence and
trapping causes the phenomenon called “blinking” where the molecule is dark for a time
period and then fluoresces. In addition, absorption events in the excited state can lead to
irreversible photochemical bleaching, this typically occurs after $10^8 – 10^{10}$ emission
cycles of the molecules.

Single molecules are typically immobilized in some way to restrict diffusion, such
as a polymer-supported thin film, or a biotin-streptavidin linkage to a glass substrate.
This is necessary because it is easier to identify individual molecules if they are
immobilized. Immobilization can greatly reduce blinking, thus increasing short-time
photostability. It can also increase long-time photostability by isolating the molecules
from oxygen, reactions with which can cause photodestruction.

Because of the spectral constraints and detection limits of the camera, only
molecules that have a robust and stable emission in the visible spectrum can be analyzed.
The rate at which molecules blink should be as low as possible, and this rate can often be
controlled through environmental factors such as surface, atmosphere, and temperature.
Blinking can still be problematic because information content can occur on the same time
scales as the blinking. Also, the total integrated fluorescence signal that can be extracted from a single molecule stops once the molecule undergoes irreversible photobleaching, and is limited by the average number of excitation-emission cycles it undergoes beforehand. Considering all of this, a molecule designed as a chiroptical probe must have good short-time and long-time photostability because we are interested in the dissymmetries in FECD and CPL.

1.3 Plan for Dissertation

The research described in this dissertation was designed to address the following issues: We do not know if the single molecule chiroptical response is representative of the ensemble, or if there are wide variations that might be attributable to heterogeneities in molecular structure or local environment. We want to determine if there are any fluctuations in the chiroptical response of an individual molecule, and if so, what the time scales and mechanisms involved are. Also, we want to investigate if there is a correlation between excitation and emission polarization.

The solid state properties of bridged triarylamine helicene are characterized to determine the feasibility of single molecule chiroptical measurements. The heterogeneities in FECD are examined and characterized by looking at histograms of single molecule dissymmetries. When we look at the distributions of dissymmetries, we will see a broad range of responses, and the structure of the distributions will suggest specific orientations of the molecules on the surface. In addition, the changes in the distributions of dissymmetries with wavelength dependence are studied. Using CPL, the heterogeneities in emission dissymmetry are examined and characterized, and the
correlation between excitation and emission is explored. Using molecular modeling and experimental and simulated defocused imaging, we will look at the role of orientation and the contribution of the quadrupole in single molecule dissymmetries. Finally, we will conclude with a summary of the information that we learned, and of the questions raised about orientation and the role of the quadrupole. Potential methods to answer those questions are briefly described.
2.1 Bridged Triarylamine Helicenes

In 2003, Venkataraman and Riehl demonstrated the synthesis and bulk characterization of a new kind of fluorescent helicene molecule based on a bridged triarylamine structure. These are easily functionalized to build stable helical structures for electronic or optical applications. The right- (P) and left- (M) handed diastereomeric conformations are enforced by the presence of a camphane group at the indicated position (Figure 3). The camphane group only serves to maintain chirality and help with resolution of the two diastereomers. The camphane does not absorb or emit light at the wavelengths employed; therefore it is not expected to contribute to the chiroptical properties of the helicene molecules. The purity of the resolved M2 and P2 samples was verified by $^1$H NMR. In the Venkataraman and Riehl work, a small ensemble averaged dissymmetry ($g = \Delta \varepsilon / \varepsilon$, where $\Delta \varepsilon$ is the difference in absorption of right and left circularly polarized light and $\varepsilon$ is total absorption) in the circular polarized luminescence ($\Delta \varepsilon / \varepsilon \approx 0.001$) was observed from the solution phase samples of the pure M2 and P2 diastereomers, and was similar in magnitude to the circular dichroism at the same excitation wavelengths.
Our experimental approach here was to use ultra-dilute solutions of helicenes immobilized in a thin polymer film, and single-molecule fluorescence imaging techniques to probe dissymmetry in right and left circularly polarized absorption at different wavelengths. The single molecule spectroscopy (SMS) experiments were designed around the absorption and/or emission constraints in solid state.
Figure 4 shows the bulk solid state absorption and emission spectra of M2 and P2, also indicating the wavelengths of our laser excitation sources. The samples were prepared by drop casting a very concentrated sample on a slide and evaporating the solvent, leaving behind a solid thin-film. The thin film CD spectra indicated that the optical activity was preserved, and not greatly different from solution. The dissymmetries in Table 1 indicate circular dichroism in the solid state calculated at the wavelengths used for the single molecule measurements. Within the lowest electronic absorption band where the solid film CD for these molecules was observed, three different fixed-wavelength lasers were used as excitation sources in the fluorescence-excitation circular dichroism measurements. These spectral results showed no large change in spectral properties from solution to solid state, indicating M2 and P2 are good candidates for single molecule chiroptical studies.

**Table 1: Dissymmetries for excitation wavelengths of 405, 437.5, and 457 nm from thin-film CD.**

<table>
<thead>
<tr>
<th>λ (nm)</th>
<th>g=Δε/ε</th>
<th>λ (nm)</th>
<th>g=Δε/ε</th>
</tr>
</thead>
<tbody>
<tr>
<td>405</td>
<td>0.0004</td>
<td>405</td>
<td>-0.0006</td>
</tr>
<tr>
<td>437.5</td>
<td>0.0006</td>
<td>437.5</td>
<td>-0.0009</td>
</tr>
<tr>
<td>457</td>
<td>0.0001</td>
<td>457</td>
<td>-0.0000</td>
</tr>
</tbody>
</table>

**2.2 Fluorescence Excitation Circular Dichroism**

Fluorescence Excitation Circular Dichroism (FECD) is a measurement that uses single molecule fluorescence intensity as a reporter for relative absorption cross-sections for right and left circular excitation polarization. It is similar in concept to fluorescence detected circular dichroism (FDCD), the difference being that FDCD is done on an isotropic solution. In FDCD, the samples are molecules in solution where the molecules
are rotating fast enough in the sample that the emission is also isotropic.\textsuperscript{45} Thus, FDCD reports on the diagonal elements of the rotatory strength, \( \overline{R} \); any off-diagonal contributions vanish due to rotational averaging. In applications of single molecule FECD, the molecules have a fixed orientation for absorption and emission. As mentioned in Equation (1), dissymmetry is a function of rotatory strength, which in turn is a function of observation/excitation vector shown in equation (8). Therefore, for a single molecule in a thin film, the off-diagonal elements of the rotatory strength tensor may contribute to the chiroptical signature because they are not averaged out as in an isotropic solution. As we measure the dissymmetry in absorption as a function of emission intensity of single molecules we relate dissymmetry back to rotatory strength and its orientational dependence.

This chapter on FECD describes the experimental details of the single molecule measurements done to study the chiroptical signature of an individual molecule, as well as the heterogeneity of response in absorption, and wavelength dependence. The results for the FECD measurements done at 405, 440 and 457 nm are shown and discussed in detail.

\textbf{2.2.1 Experimental Setup}

Figure 5 shows a schematic of the experimental apparatus for the FECD measurements. The design of the setup to perform these measurements was similar in concept to work by Bart Kahr’s group on chiroptical properties of enantiomorphous twinning in biaxial crystals of 1,8-dihydroxyanthraquinone.\textsuperscript{93} Our experimental design used an epi illumination configuration on a Nikon TE300 microscope with a 1.4 NA
objective. Right or left circularly polarized light from a CW Ar$^+$ ion laser (457 nm 100 µW-200 µW) was delivered to the sample by orienting a multi-order quarter wave plate (QWP) on a rotation stage at $\pm 45^\circ$ with respect to the (horizontal) input polarization axis. Appendix A give details of tuning and polarization characterization. For each orientation of the QWP, we acquired 10 sequential CCD camera exposures (Roper Scientific PhotonMax) of the fluorescence from the molecules; this fluorescence was collected and filtered through either a 510WB40 bandpass or a 480ALP long pass filter (Omega). Individual frame exposure times were set to 2 sec, which averaged out most of the short-time instabilities (blinking) in the fluorescence, and yielded a higher signal-to-noise ratio in the fluorescence image. The fluorescence intensity for a given molecule extracted from the fluorescence image versus time is called its intensity trajectory. For molecules with longer photochemical survival time, we were able to assess any cycle-to-cycle changes in the dissymmetry within the experimental observation time using the intensity trajectories.

Figure 5: Schematic for FECD. The laser polarization was periodically modulated between right and left circular polarized light. Fluorescence from single M2 or P2 molecules was collected in epi configuration with a 1.4 NA oil objective and light sensitive CCD camera.
In principle, FECD can be extremely sensitive to linear polarization artifacts. Therefore, we took care to minimize and characterize the ellipticity of the input polarization in order to have the purest polarization at the sample plane. By avoiding a tight focus of the laser at the sample, the polarization was not scrambled at the sample interface, which might otherwise occur in a total internal reflection or a confocal arrangement. A 2027DRLP dichroic (Omega), which reflects s- and p- polarizations uniformly, was used to reflect the light up to the sample plane. The typical circular polarization purity of circular polarization (probed by retroreflecting the beam at the sample plane) was determined to be >98% (See Appendix A for more details).

Figure 6: Portion of a typical in-focus fluorescence image captured by the CCD camera of a 30x30 µm area for FECD. The fluorescence from the molecules is indicated by high counts as shown by the color yellow.

Solutions of the two diastereomers were prepared by dissolving them in either semiconductor grade cyclohexane or methanol, and then diluted to concentrations ~10^{-11}M. Films of these samples were prepared by drop casting ~200 µL of the ultradilute solution onto a thin polycycloolefin (Zeonex) polymer film. These concentrations gave us a spot density of about 30-50 diffraction limited spots in a 30 x 30 µm area. In Figure 6, a portion of a typical in-focus scene shows the appearance of diffraction limited spots. We found that the photochemical stability of the helicenes was significantly enhanced by the use of a Zeonex supporting film over clean glass. This
stability allowed us to sample the same spots for multiple quarter wave plate (QWP) cycles. The power was also adjusted to optimized photochemical stability. (See Appendix B for details on sample preparation.)

**2.2.2 Results and Discussion**

In FECD experiments, the excitation polarization was changed every 10 frames (2 second exposure time). The dissymmetry parameter \( g \), defined for the fluorescence excitation experiments as \( g = 2[(I_{L}-I_{R})/(I_{L}+I_{R})] \) was extracted from intensity trajectories where \( I_{L}, I_{R} \) are the average measured fluorescence intensities in the half-cycle corresponding to left or right circularly polarized excitation. Single molecule FECD dissymmetry parameters were determined for each right/left circular polarization cycle, and only molecules with sufficient photochemical stability to follow intensity trajectories for at least 1.5 modulation cycles were used in analysis. (More details about data analysis are provided in Appendix C)

Figure 7 shows typical single molecule fluorescence trajectories from M2 and P2 molecules excited with right and left circularly polarized light. They show evidence of single molecule behavior such as blinking (M2-B) and photobleaching (M2-A and P2-C). For a single molecule, some variations in \( g \) are observed in a single trajectory, but the overall dissymmetry appears to be well defined during the photochemical lifetime. This suggests that the \( g \)-parameter in FECD (for a particular molecule) was determined by either a predominantly static orientation or local environment.
Figure 7: Fluorescence intensity trajectories from different selected M2 and P2 molecules during FECD measurements. In these examples, a 2 second exposure was used, rotating between right and left circular polarized excitation every 10 frames.

FECD measurements on the FluoSpheres® (505/515 invitrogen Molecular Probes) were performed in order to ensure experimental artifacts associated with linear polarization bias did not contribute to our results. FluoSpheres® are 20 nm beads doped with multiple dye molecules and have a nominally nonpolarized absorption and emission. The same protocol was used to take FECD measurements on the FluoSpheres®. The isolation of the beads was confirmed by correlating AFM with fluorescence. The histogram of dissymmetries from FluoSpheres® (20 nm dye doped polymer beads from Invitrogen) is shown with the black dotted line in Figure 8. The distribution of dissymmetries was symmetrically centered at about $g = 0$ with a width of $\sigma_g \approx 0.05$. Appendix D reports the results for the second control, DiIC$_{18}$. These results also show a narrow distribution and centered about zero.
Figure 8: Normalized histogram of FECD dissymmetry parameters determined from single molecule fluorescence measurements. Red with open circles represents data from M2 $\langle g \rangle = -0.168$. Blue with open triangles represent data from P2 $\langle g \rangle = 0.0691$. For comparison, results from our control experiment with FluoSpheres® (FS) (dye doped 20 nm polymer nanospheres) are shown black with crosses $\langle g \rangle = 0.04$. Data from approximately 500 single molecules of each type were used.

Figure 8 shows the histograms of the dissymmetry values calculated from trajectories such as shown for M2 (red) and P2 (blue) in Figure 7. The structure of these histograms is striking in several aspects. First, they are mirror images of each other, which is expected because diastereomers have equal and opposite responses to circular polarized light. Second, each distribution spans a significant range of both positive and negative dissymmetries. Approximately 98% of the probability density is contained between ±1 and about 85% is between ±0.7. One possible reason for the high dissymmetries is that the experiment was biased towards molecules with higher photostability, and this could have biased the dissymmetries to higher absolute values.
Due to the breadth of response, we considered the possibility that despite the fact that M2 and P2 were isolated as diastereomers whose purity was verified by $^1$H NMR the other diastereomer was present in the single molecule FECD measurements. We speculated that the unexpected breadth could be due to formation of the opposite diastereomer by partial racemization in the methanol solution through trans-esterification, or reaction with condensed water vapor when the film was formed. We tested this by starting with pure anhydrous M2 and P2 and using cyclohexane as the solvent instead of methanol. The histograms in Figure 9 show that the distributions of responses remain approximately the same despite the solubility of the Zeonex in cyclohexane. This solubility could have affected the distribution and stabilization of different orientations, causing the observed differences in the wings of the distributions. Therefore, we concluded that the breadth of response is a photophysical property of the molecular system and not due to degradation of the sample.

Figure 9: Comparison of normalized dissymmetry parameter histograms for M2 (red) and P2 (blue) dispersed from cyclohexane (dashed lines) and methanol (solid lines).
Examination of the structure of the M2 and P2 dissymmetry value distributions suggests three distinct components, with similar amplitudes but opposite signs for the two diastereomers. We propose that these components (illustrated with dotted Gaussian curves as seen in the M2 distribution in Figure 10) are associated with three distinct stable orientations at the surface. The suggested orientations are shown in Figure 10: camphaneate down, camphaneate up, and “tripod” (chiral axis perpendicular to the optical axis). These proposed orientations are supported by molecular dynamic (MD) simulations by B.G. Sumpter and co-workers in the Computation Science and Mathematics Division at Oak Ridge National Laboratory, corresponding to the helicene frames parallel or perpendicular to the surface. The two in-plane orientations
(camphanate up and camphanate down) may be distinguishable although unclear to what extent, since for the camphanate up orientation, the frame of the molecule is in contact with (or solvated by) the polymer film, whereas the camphanate down orientation prevents the same sort of contact.

Figure 11: Normalized dissymmetry parameter histograms determined from single molecule fluorescence measurements at different excitation wavelengths (M2 red, P2 blue)

In order to gain more insight into the effect of orientation on dissymmetry more closely, we looked at excitation wavelength dependence using 457 nm and 405 nm both at the edges of the electronic transition and in the heart of the transition and 440 nm. The normalized P(g) determined from single molecule fluorescence measurements of M2(red) and P2(blue) using 405 nm, 440 nm, and 457 nm excitation wavelengths are shown in Figure 11. The shapes of the distributions were slightly different at each of the excitation wavelengths. At 405 nm and 457 nm, the first moment of the distributions were \(<g>_{405\text{ nm}} = -0.03(M2), 0.17(P2)\) and \(<g>_{457\text{ nm}} = -0.18(M2), 0.06(P2)\), which are consistent with the sign of the bulk CD/CPL measurements, except much larger. However, with 440 nm excitation (where the largest dissymmetry was measured at the bulk level) an inversion of the sign of the first moment of the single molecule distribution was measured with respect to the bulk. At 440 nm, the first moment of the distribution was \(<g>_{440\text{ nm}} =
0.03 for M2 and -0.01 for P2. In comparison the bulk solid state measurements, seen in Table 1 the values for M2 was -0.009 and P2 was 0.0006.

At this juncture, we are uncertain what is causing the inversion of dissymmetry at 440 nm. It may be due to the effective absorption at 440 nm causing a larger than normal contribution from the quadrupole. In addition, the orientation and solvation of the molecule may be affecting the rotatory strength of the molecule and the off diagonal elements of the tensor are contributing to the measured dissymmetry due to orientation.

Our work thus far has demonstrated the feasibility of interrogating the fundamental nature of the interaction of light with chiral molecules at the single quantum system level in absorption. In the single molecule FECD measurements we observed a wide breadth (heterogeneity) in the single molecule chiroptical response as seen in the dissymmetry histograms, which we believe derives from different orientations of the molecules on the surface. In the following sections, I discuss further experiment and computational modeling designed to illuminate the connection between molecular orientation and chiroptical response.

2.3 Simulation of Orientation Effects on Single Molecule Chiroptical Spectroscopy

In order to further understand the role that orientation plays in the dissymmetry of an individual molecule, we calculated what the observed dissymmetry would be for M2 in a variety of orientations using computer simulations. Starting with the crystal structure of M2 and substituting an acetyl group for the camphanate group for computational convenience, we optimized the geometry of the structure using density function theory (DFT) by using the B3LYP exchange-correlation functional and the 6-31g* basis set in
Gaussian 03. \cite{216, 217} Time dependent DFT (TDDFT) \cite{218} was used to study the excited state characteristics (excitation energies, $\Delta E$, oscillator strength, $f$, and rotatory strengths, $R$).

Using the rotatory strength tensors for the lowest energy electronic states involved in optical transitions, we computed angle-integrated dissymmetry values for a particular molecular orientation, assuming a high numerical aperture for collection of radiation. For a specific $k$-vector (observation direction) aligned along an arbitrary direction $\hat{n}$ relative to the chiral axis of the molecule, the dissymmetry in circular dichroism is given by the scalar product:

$$
\Gamma_{n0}(\theta, \phi) = \hat{n} \cdot \vec{R} \cdot \hat{n}
$$

$$
= \sin^2 \theta \left[ R_{yy}^{n0} \cos^2 \phi + R_{xx}^{n0} \sin^2 \phi + \frac{1}{2} (R_{xy}^{n0} + R_{yx}^{n0}) \sin 2\phi \right] 
$$

$$
+ \sin 2\theta \left[ \frac{1}{2} (R_{xx}^{n0} + R_{yy}^{n0}) \cos \phi \right] 
$$

$$
+ \frac{1}{2} \left( R_{yz}^{n0} + R_{zy}^{n0} \right) \sin \phi \cos^2 \theta \frac{1}{2} R_{zz}^{n0}.
$$

(11)

In the preceding, $\theta$ and $\phi$ are spherical polar angles for $\hat{n}$ in the molecular frame and the $R_{ij}$ values are the elements of the rotatory strength tensor \cite{219}. To calculate the dissymmetry values, the rotatory strength tensor was rotated around the x-axis and the y-axis of the lab frame (shown in Figure 12 A) in order to sample all the basic orientations.
Figure 12: (A) Geometry of the lab frame with respect to the 1.4 NA objective. The objective is located at $-z$. $\gamma$ ($\approx 67^\circ$) describes the solid angle of collection. $\alpha$ and $\beta$ correspond to counter-clockwise rotations of the molecule and $\Gamma(\theta, \phi)$ about the $x$ and $y$ axis respectively. (B) M2 overlaid with $\Gamma(\theta, \phi)$ in the lab frame with $\alpha = 0$ and $\beta = 0$. This orientation corresponds to camphanate up. (C) M2 and $\Gamma(\theta, \phi)$ have been rotated by $\beta = 90^\circ$ corresponds to the tripod orientation. The green and red in the plots correspond to positive and negative values respectively.

The scalar product $\Gamma_{n,0}(\theta, \phi)$ is pictorially displayed in Figure 12(B,C) using the green/red spherical plot representing positive and negative values, respectively and the distance from the origin is the magnitude of the scalar product. The total dissymmetry, calculated by integrating over $4\pi$ steradians, was -0.0016, in agreement with the ensemble value in solution of -0.0010. The orientations seen in Figure 12 B and C correspond to the camphanate up and tripod orientations of M2 in the lab frame (The molecule shown has an acetyl group substituted for the camphanate). Figure 13 shows the traces of the integrated dissymmetry for the solid angle of observation as the rotatory strength tensor is rotated $360^\circ$ through $\alpha$ and $\beta$. In Figure 13 (A) the red rectangle corresponds to camph down and the gray camph up. The traces in A and B clearly show
the oscillation of the observed dissymmetry as dependent on observation angle. Focusing on the red rectangle we see the dissymmetries range from 0.003 to -0.003 and that this change occurs within a rotation of 100 degrees of the chiral axis.

![Figure 13](image)

**Figure 13:** Trace of integrated dissymmetry for the solid angle of observation as the rotatory strength tensor is rotated 360° through α and β. (A) The gray rectangles indicate camph up and the red camph down as the molecule is rotated through α. (B) The blue rectangle indicates tripod as the molecule is rotated through β.

Table 2 displays the dissymmetry values for these and other orientations of M2 in the lab frame. These dissymmetry values were much smaller than those measured experimentally. We think this difference in magnitude was due to environmental effects on the multipolar transition matrix elements. As the molecules are on the surface of the Zeonex, camph-up and camph-down present slightly different electronic interactions. For camph up the electron π cloud of the molecular frame is in contact with the Zeonex. In comparison in the camph down orientation the camphanate group prevents the intimate contact of the π cloud of the molecular frame with the surface. These differences could have a profound effect on the magnitude of the dissymmetries by affecting the magnitude and interactions of the electric dipole, magnetic dipole, or quadrupole.
Table 2: Computed dissymmetry values obtained for M2 by numerical integration of the scalar product of $\Gamma_{n,o}(\theta, \phi)$ over a solid angle with a half angle of $\sim 67^\circ$ corresponding to the collection angle of the 1.4 NA objective.

<table>
<thead>
<tr>
<th>Orientation</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>Dissymmetry (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camph up</td>
<td>300°</td>
<td>0°</td>
<td>+0.0021</td>
</tr>
<tr>
<td>Camph up</td>
<td>315°</td>
<td>0°</td>
<td>+0.0034</td>
</tr>
<tr>
<td>Camph up</td>
<td>0°</td>
<td>0°</td>
<td>+0.0022</td>
</tr>
<tr>
<td>Camph up</td>
<td>45°</td>
<td>0°</td>
<td>-0.0026</td>
</tr>
<tr>
<td>Tripod</td>
<td>0°</td>
<td>90°</td>
<td>-0.0025</td>
</tr>
<tr>
<td>Camph down</td>
<td>120°</td>
<td>0°</td>
<td>+0.0021</td>
</tr>
<tr>
<td>Camph down</td>
<td>135°</td>
<td>0°</td>
<td>+0.0034</td>
</tr>
<tr>
<td>Camph down</td>
<td>180°</td>
<td>0°</td>
<td>+0.0022</td>
</tr>
<tr>
<td>Camph down</td>
<td>225°</td>
<td>0°</td>
<td>-0.0026</td>
</tr>
</tbody>
</table>

Table 2, shows that the dissymmetry values were identical for camphanate up (300°) and camphanate down (120°) for the same molecular frame orientation relative to the objective. The same similarity was seen for the other chiral axis orientations. When we first looked at the spread of dissymmetries in fluorescence excitation, we speculated we would be able differentiate between camphanate up and camphanate down. From these calculations it initially appeared that it would not be possible to distinguish the two orientations because a helix has the same chirality whether it is right side up or upside down. A molecule with a specific chiral axis orientation is indistinguishable from one rotated 180°. But after considering that for camphanate up, the molecular frame will be almost flat on the surface with its chiral axis perpendicular to the surface. For the camphanate down orientation, the camphanate prevents the molecular frame from being flat on the surface changing the surface interactions, and the chiral axis is at a different angle to the surface. These differences in orientation and surface interactions may allow for differentiation between camphanate up and down.
The dissymmetries are similar for different chiral axis orientations and thus make identification of molecular orientation difficult. Using spatial filtering of the excitation laser we may be able to accurately identify chiral axis orientation. The spatial filter, filters the beam so either the interior or the exterior of the beam is used for excitation. This is beneficial because the design of the objective causes the exterior of the beam to be transmitted with a higher angle as shown in Figure 14. Spatially filtering the excitation, as shown in Figure 14 B and C, allows for a narrow selection of excitation angle of the electric field. Using both the high and lower angle excitation angle different portion of the rotatory strength tensor are accessed. Taking the ratio of these two dissymmetries would give a more precise identification of chiral axis orientation.

![Figure 14: (A) Schematic of transmitted light for a 1.4 NA objective. (B) Light transmitted for the outer edge of the beam indicated by the black ring. (C) Light transmitted for inner excitation indicated by the black circle.](image)

In order to determine the feasibility of identifying different orientations by this method, we did some calculations to determine the ratio of exterior to interior dissymmetries based on the angles the molecules would be excited from using the spatial filtering. These calculations were done for a variety of orientations for camphanate up and camphanate down. The results are shown in Table 3. These results demonstrate that the differences in orientation could be determined using excitation from low and high...
angles. Though, when using this method, determination of camphanate up or camphanate down still does not appear to be possible unless some external factors play a role such as surface interactions, but could, in principle distinguish other orientations.

Table 3: Calculated dissymmetries for inner and outer rings of excitation and the ratio of outer to inner.

<table>
<thead>
<tr>
<th>Orientation</th>
<th>α</th>
<th>Inner g</th>
<th>Outer g</th>
<th>ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camph up</td>
<td>0°</td>
<td>6.76E-39</td>
<td>-2.2E-39</td>
<td>-0.32621</td>
</tr>
<tr>
<td>Camph up</td>
<td>20°</td>
<td>6.81E-40</td>
<td>-6.1E-40</td>
<td>-0.89405</td>
</tr>
<tr>
<td>Camph up</td>
<td>38°</td>
<td>-4.3E-39</td>
<td>6.93E-40</td>
<td>-0.16209</td>
</tr>
<tr>
<td>Camph down</td>
<td>180°</td>
<td>6.76E-39</td>
<td>-2.2E-39</td>
<td>-0.32621</td>
</tr>
<tr>
<td>Camph down</td>
<td>200°</td>
<td>6.81E-40</td>
<td>-6.1E-40</td>
<td>-0.89405</td>
</tr>
<tr>
<td>Camph down</td>
<td>218°</td>
<td>-4.3E-39</td>
<td>6.93E-40</td>
<td>-0.16209</td>
</tr>
</tbody>
</table>

The dissymmetry values calculated for all the different orientations support the idea of a discrete set of orientations contributing to the single molecule distribution. Such a multimodal distribution is characteristic of molecules with fixed orientations, and unlike isotropic samples where all orientations are averaged together (such as solution), reflects rotatory strength contributions from not just the electric and magnetic dipole, but also the electric quadrupolar properties of the electronic transition. In addition, using the method of inner and outer excitation appears to be a feasible way to start determining the orientation of molecules on a surface.

2.4 Emission Pattern Imaging of Single Helicenes

Defocusing samples to obtain spatial intensity patterns is a well-established tool in single molecule spectroscopy for probing molecular orientation in condensed phase. For example, the transition electric dipole of coumarin 6 causes it to behave like a linear dipole. Linear dipoles typically emit with a cosine-squared distribution.
relative to dipole orientation. So for a dipole perpendicular to the interface, light is only collected at high angles, whereas a dipole parallel to the surface emits light directly into the plane and therefore this light is collected at shallow angles. The differences in the refractive indices of the glass and oil causes there to be angle dependence to the light collected. The light collected at higher angles will have a longer distance to travel to the image plane than those collected at shallow angles. This optical path difference causes the light collected at different angles to be out of phase with each other and cause interference. These slightly aberrated images can be simulated by taking into account the optical path difference experienced by a single molecule’s emission as a function of collection angle. For a dipole perpendicular to the surface, the emission pattern looks like a “doughnut”; and for a dipole parallel to the surface, the emission pattern has a distinct pattern that depends sensitively on degree of defocusing. See Figure 15 for images of these defocused images. In contrast to these defocused images, multi-chromophoric sources such as dye-doped spheres yield circularly symmetric Airy spatial intensity patterns.

![Figure 15: (Top) Linear dipoles oriented on a dielectric surface. (Bottom) Corresponding defocused images. Left parallel dipole with “wings”. Right, a perpendicular dipole is a “doughnut”.](image-url)
For our experiment, the samples were prepared in the same manner as for FECD and CPL. A detailed description can be seen in Appendix B. The M2 molecules on the polymer film were excited with right circularly polarized 457 nm light in epi configuration. The fluorescence emission patterns were collected using the 1.4 NA 100x objective. The fluorescence was then filtered using a 2027DRLP dichroic and a 510/40 bandpass (Omega) and imaged on the CCD camera (PhotonMax). As shown in Figure 16. Switching between the in-focus and out-of-focus images was performed by defocusing the microscope objective by ~200 nm using the z-control of the microscope in steps of 100 nm.

Figure 16: Schematic of experimental setup for imaging single molecule orientations. Fluorescence from single M2 or P2 molecules was collected in epi configuration with a 1.4 NA oil objective and light sensitive CCD camera. θ and φ of the molecular orientation are described relative to the surface of the slide.

Figure 17 (panels A-F) shows defocused helicene images collected using a defocused depth of ~200 nm. Comparison of these images with the defocused images of 1-D dipoles in Figure 15 shows similarities, but there are some important differences in
the symmetry of the images. Theory predicts the emission pattern from a linear 1-d dipole will always possess a line of bilateral symmetry.\textsuperscript{222} The experimental images in panels A-F of Figure 17 showed a pronounced breaking of this bilateral symmetry. We want to understand this lack of symmetry. Therefore we need to modify a model of emission for a 1-D dipole at an interface in order to correlate orientation with emission pattern for the helicenes.

Figure 17: Panels A–F: Defocused fluorescence images from (different) single M2 molecules using right circular excitation. The contours on E emphasize the asymmetry of the emission pattern. Panels G and H depict simulated defocused images of two non-coplanar dipoles with a relative phase difference of $\pi/2$ and magnitudes of $\mu_2 = \mu_1/5$ and a defocus depth of 600 nm, illustrating the effects of a slight elliptical polarization of the fluorescence emission.

2.5 Simulations

The chiroptical properties of molecules have been modeled with quantum chemical models\textsuperscript{223, 224} and with classical models\textsuperscript{225-228} where the molecules are considered as a series of coupled oscillators. Our goal was to modify existing “dipole-at-an-interface” models to incorporate additional electro-magnetic terms to simulate
emission patterns for determining the correlation between orientation and emission patterns.

The models currently used to simulate emission patterns are based on modeling a linear dipole at an interface. Therefore one of the most applicable models of chiroptical properties is the Kirkwood model of optical activity; it is based on the semi-classical picture of a chiral molecule as a pair of non-coplaner, coherently coupled oscillators (see Figure 18). This model approximates a chiral system by treating the electric moments induced in the subunits of a molecule as if they were localized at the centers of gravity of the subunits, oriented relative to the surface. The phase and magnitude of the two electric dipoles were left adjustable in the simulation which adapts the Kirkwood model.

![Figure 18: Illustration of the semi-classical Kirkwood model used to simulate emission patterns from chiral fluorophores. 4 angles (referenced to surface normal) are used to specify the relative orientations of 2 coherently coupled dipoles.](image)

Using this approximation of a chiral system as two coupled oscillators, we started with the work of Bohmer and Enderlein on modeling linear dipoles at a surface. Their work models a single electric dipole at an interface using wave-optical modeling. We extended their work by coherently incorporating the field of an additional electric dipole.
The phase lag of the second dipole was included to approximate the fields radiated by the effective magnetic dipole and electric quadrupole of the radiating system. The magnitude of the second dipole was 1/5 the first one in order to approximate our experimental results. The electric and magnetic fields used in this adaption of Bohmer and Enderlein’s work are as follows:

\[
\vec{E}_{total} = \vec{E}_1^\perp \cos \theta_1 + \vec{E}_1^\parallel \sin \theta_1 + e^{i\pi/2} \left( \vec{E}_2^\perp \cos \theta_2 + \vec{E}_2^\parallel \sin \theta_2 \right) \quad (12)
\]

\[
\vec{B}_{total} = \vec{B}_1^\perp \cos \theta_1 + \vec{B}_1^\parallel \sin \theta_1 + e^{i\pi/2} \left( \vec{B}_2^\perp \cos \theta_2 + \vec{B}_2^\parallel \sin \theta_2 \right) \quad (13)
\]

In the previous equations \(\theta_1\) and \(\theta_2\) refer to the angle of inclination of the two dipoles toward the surface. The position dependent detectable light intensity on the CCD camera is given by the z component of the Poynting vector:

\[
S = (c/8\pi)e_z \cdot (\vec{E} \times \vec{B}^\ast).
\]

Panels G and H in Figure 17 illustrate the results of these simulations. They produced defocused images that lack the clear bilateral symmetry of a 1-D dipole and have a similar shape to the experimental images.

These simulations were intended as an approximation of a higher order multipole source, and were motivated by the large dissymmetry values observed in the individual helicene measurements. We speculate that the complexities of the local molecular environment may serve to enhance the higher multipole transition matrix elements, resulting in larger dissymmetries and a somewhat distorted radiation pattern. Ongoing experimental and theoretical investigations extending the work of Hellen and Axelrod to incorporate the quadrupole transition seek to explore these possibilities.
2.6 Circular Polarized Luminescence

Circular polarized luminescence (CPL) is a complementary technique to FECD for probing the chiroptical properties of single molecules. FECD probes the transition of the molecules from the ground state to excited states, whereas CPL probes the polarization composition of the fluorescence photons. CPL typically reports on the polarization of the transition from the lowest excited singlet and/or triplet state to the ground state. Ultimately we want to correlate excitation polarization and emission polarization to learn if the molecule emits from the different excited states based on the excitation polarization.

2.6.1 Experimental Setup

Figure 19 shows the schematic of the experimental apparatus for the CPL measurements. Our experimental design used a configuration where the excitation beam came from the top on a Nikon TE300 microscope directed down at the 1.4 numerical aperture (NA) objective. This enabled us to deliver right- or left- circularly polarized light from a CW Ar+ ion laser (457 nm 100 µW-200 µW) to the sample by orienting a quarter wave plate (QWP) on a rotation stage at +\(- 45^\circ\) with respect to the input polarization axis without the use of a dichroic. The excitation laser light is blocked by a holographic notch filter at 457 nm with an OD >6 in conjunction with a 510WB40 (Omega). The fluorescent light is then transmitted through a QWP with an orientation fixed relative to the Wollaston prism. The QWP converts elliptical light into horizontal and vertical components which the Wollaston prism separates spatially, and is imaged on
the CCD camera (Roper Scientific PhotonMax) (As seen in Figure 20). Appendix E explains the correlation between the sides of the camera image and polarization.

**Figure 19: Schematic for CPL.** Laser light in a “from the top” configuration was circularly polarized using a QWP. Fluorescence from the M2 or P2 molecules on the slide was collected with a 1.4 NA oil objective and light sensitive CCD camera. The fluorescence was first filtered by a notch filter and bandpass to filter out laser light, and then analyzed with a fixed QWP and Wollaston prism to determine the emission polarization. The Wollaston spatially separates the vertical and horizontal polarization.

This setup allows us to determine the single-molecule dissymmetry in emission on a frame-by-frame basis using a single camera with no moving parts in the detection optics. The dissymmetry parameter ($g$) is defined for circular polarized luminescence experiments as $g = 2[(I_L-I_R)/(I_L+I_R)]$, where $I_L$ and $I_R$ are the measured fluorescence intensities in a frame. In each frame, the dissymmetry parameter was extracted from the intensity trajectories corresponding to left or right circularly polarized emission for a given spot. A more detailed explanation of data analysis is given in Appendix C.
This setup was designed to reduce the potential introduction of linear polarization artifacts that could be introduced into the excitation or emission polarization by dichroic reflective optics. While this effect is negligible for excitation, it is presumably much more severe for transmission near the dichroic band edge. In addition, using a fixed QWP in conjunction with a Wollaston prism eliminates any systematic error induced by mechanical rotation of the QWP. This setup increases data throughput because information on both right and left circular polarized luminescence are collected simultaneously. This setup is a better alternative to using a polarizing beam splitter and two cameras because a single camera is used and the noise and efficiency on only a single camera need to be taken into account. The samples for these CPL measurements were prepared in the same way as for FECD, and a description of the optics testing and alignment is described in detail in Appendix A.
2.6.2 Results and Discussion

As one of our controls to test the CPL setup we used FluoSpheres®, 20 nm dye doped polystyrene beads. In Figure 21 (A), the correlation between the two channels is clearly seen. The fluorescence trajectories in Figure 21 (B) do not show blinking and only slight fluctuations. The right (red) and left (blue) channels are the same intensity because the emission from these FluoSpheres® is nonpolarized. Figure 21 (C) shows the distribution of dissymmetries for this particle. The distribution is very narrow with a full width at half max of 0.19 and centered about zero. This indicates the breadth of response for a nonpolarized source.

![Figure 21](image_url)

Figure 21: FluoSphere® results using right circular polarized excitation. (A) A scene with one spot identified with a red square in both the right and left channels. (B) The fluorescence trajectories (top) and frame-by-frame dissymmetry (bottom) from the identified spot. Red and blue are the right and left channels respectively. (C) A histogram of the dissymmetries from the identified spot.

The dissymmetries from multiple FluoSpheres® are shown in Figure 22. The two traces correspond to right and left circular polarized excitation. They are centered within a bin width around zero, and their average dissymmetries are -0.02 and -0.03 for left and
right circular polarized excitation respectively. The distribution of dissymmetries for these FluoSpheres® is very narrow and centered around zero, with 98% of the probability density between ±1 and ~90% of the probability density located between ±0.5. These results indicate that the experiment does not have any artifacts relating to excitation polarization.

Figure 22: A histogram of the dissymmetries from a number of FluoSpheres® using right (red) and left (blue) circular polarized excitation.

We also examined CdSe/ZnS-capped quantum dots (EviDot 580) with a wurtzite structure. This system was examined because they are single quantum emitters as shown by single photon correlation done in our lab, as opposed to the FluoSpheres® which have multiple emitters. Figure 23 (A) shows a typical image of quantum dots captured during a CPL measurement using right and left circular polarized excitation for the same scene. The correlated fluorescence trajectories and the frame-by-frame dissymmetries for the indicated spot are shown in Figure 23 (B and C). This experiment was done with both right and left circular polarized excitation. The histograms in Figure 23 (B and C) show the histogram of the dissymmetries for the spots identified in Figure 23 (A). The narrow breadth of the histograms in Figure 23 (B) is striking as compared with the breadth for FluoSpheres®. The full width half max is ~0.08. The average dissymmetry
is 0.17 with left excitation and 0.25 for right excitation. This shift in average
dissymmetry as related to excitation polarization demonstrates a correlation exists
between excitation and emission polarization. This correlation varies from quantum dot
to quantum dot, as illustrated by difference in Figure 23 B and C. The average
dissymmetry shifts to emit more right handed light in Figure 23 (B) when changing from
left excitation and in Figure 23 (C) the shift is to emit more left handed light with right
circular polarized excitation.

Figure 23: Quantum dot (CdSe) results. In the intensity trajectories the red and
blue traces are the right and left channels respectively, and in the histogram of
dissymmetries red and blue correspond to right and left circular polarized excitation. (A) A scene with two spots correlated between right and left channels and right and left circular polarized excitation. (B) The fluorescence trajectories (top) and frame-by-frame dissymmetry (bottom) from the spot identified with a red box for right and left excitation, and the histograms of the dissymmetries from this spot. (C) The fluorescence trajectories (top) and frame-by-frame dissymmetry (bottom) from the spot identified with a yellow box for right and left excitation, and the histograms of the dissymmetries from this spot.
Figure 24 shows preliminary results for P2 and M2. Shown are typical CPL scenes for both M2 and P2. A single spot is identified in each of the scenes. Below the images are the fluorescence trajectories from the identified spot. The fluorescence trajectory for P2 is typical for both M2 and P2. The fluorescence trajectory for M2 is atypical, over time the dissymmetry in emission changes. From 40-80 seconds, right circular emission dominates, whereas from 120-180 seconds, left circular polarized emission dominates. This change in emission polarization could be due to the affect of excitation polarization. The change in dissymmetry could also be related to which excited state the molecule is emitting from. If the molecule is not well immobilized on the surface the change in dissymmetry could be cause change in orientation of the molecules chiral axis relative to the optical axis. Currently the experiment is still being optimized for M2 and P2, but the preliminary results demonstrate the feasibility of measuring CPL from single molecules of M2 and P2.
To conclude, single molecule CPL has been demonstrated for quantum dots and helicenes. The FluoSpheres® showed that there was no linear bias in the experiment when right or left circular excitation was used. The quantum dots demonstrated a very distinctive response in absorption and emission to right and left circular polarized excitation. There appears to be a correlation between excitation and emission in quantum dots. How this correlation arises between excitation and emission polarization is yet to be understood. Any parameters such as orientation and environment affecting the correlation have yet to be explored. Based on these results from quantum dots, the next step will be to study the correlation between excitation and emission for M2 and P2 at the single molecule level.
CHAPTER 3

CONCLUSION AND FUTURE WORK

The chiroptical properties of individual bridged triarylamine helicenes immobilized in polymer-supported films was investigated using single molecule spectroscopy. The feasibility of studying the chiroptical properties of single molecules was demonstrated. Questions about the heterogeneity of the chiroptical response in the absorption and emission of single molecules were addressed. The experiments gave insight into the role of orientation, the contribution of off diagonal elements in the rotatory strength tensor, and the relation of the transition quadrupole to the chiroptical properties of single molecules.

SM FECD investigations addressed the question of heterogeneity in the dissymmetric absorption monitored by single molecule fluorescence intensity. From extracted fluorescence intensity trajectories, we observed blinking and discrete photobleaching providing evidence that single molecules were indeed being interrogated. In addition, defocused images of the helicenes lack the bilateral symmetry of linear dipoles. The magnitude and distribution of dissymmetries from the M2 and P2 measurements suggest both surface and orientation effects play a significant role. We postulated that the breadth and structure was due to a distribution of orientations of the molecules on the surface. To examine the correlation of excitation wavelength with the distribution of dissymmetries, we used three different excitation wavelengths. These measurements showed the structure of the distributions changes at the different wavelengths. Also, the sign of first moment of the dissymmetries for 405 nm and 457 nm excitation were the same as for the bulk measurements, but an inverted sign was observed
for the 440 nm excitation. We hypothesize this inversion at 440 nm is due to the efficiency of absorption in the heart of the electronic transition. We believe the electronic quadrupole and orientation play a larger role than was originally thought because of this difference in absorption.

Several molecular modeling calculations were performed to understand the role orientation plays in chiroptical properties of single molecules. They showed the observed dissymmetry value varied for different orientations of the chiral axis relative to the surface. When the chiral axis was oriented to the same direction for camphanate up and camphanate down, the dissymmetry was exactly the same as expected in 1st order approximations. But, because the probable orientations of camphanate up and camphanate down have slightly different chiral axis orientations and surface interactions, we may be able to differentiate between them. These simulations support the idea of three distinct orientations, because the dissymmetry value changes (and even changes sign, in some cases) as the orientation changes.

Additional computation modeling will likely provide more understanding of the role the electric quadrupole, the magnetic dipole, and the electric dipole in the chiroptical properties of oriented single molecules. In addition, studying other molecules could give insight into how the physical structure plays a role in orientational dependence and viability for single molecule studies and device application. In the future, perhaps the chiroptical properties of a single molecule oriented on a surface will be modeled using molecular modeling, taking into account all the surface, orientational, and conformational effects.
The final method in these studies used defocused imaging to look at the correlation between orientation and emission patterns. The experimentally acquired defocused images of the helicenes lacked the bilateral symmetry typically found in linear dipoles. In order to correlate orientation with emission pattern, we simulated the defocused images. We adapted Bohmer and Enderlein’s work\textsuperscript{230} to Kirkwood’s model of optical activity\textsuperscript{228} to simulate emission patterns using two coherently added electric dipoles having a relative phase shift and different magnitudes. The emission patterns generated using this simulation approximated the experiment. Additional simulations adapting Hellen and Axelrod’s work\textsuperscript{222} to include accurate quadrupole and magnetic dipole contributions have been started, and may be amenable to correlating image and orientation.

Ultimately, meaningful comparison with theory is going to require both dissymmetry and orientation information. We want to learn from the defocused images of helicenes the same kind of orientation information as is learned from linear dipoles. The difficulty is we need to know the approximate orientation of the molecules on the surface in order to calibrate defocused image analysis. Therefore measurements with molecules whose orientation is already known would provide a useful reference. Currently, Venkataraman’s group has proposed a variety of molecules designed to form a self assembled monolayer with helicenes attached to them in a variety of different known orientations. Using the defocused images from these oriented molecules would provide a standard for comparison with the simulations of defocused images. In addition, measuring FECD and CPL from such oriented systems would give us more insight into the correlation between orientation and dissymmetry.
We also looked at circular polarized luminescence (CPL) in order to probe the
dissymmetry in the emission from single molecules, and the extent to which CPL
dissymmetry is correlated with excitation polarization. The single quantum dot CPL
dissymmetries had a very narrow distribution of responses, with a correlation between
excitation and the average dissymmetry in emission. This correlation varies from
quantum dot to quantum dot, the average dissymmetry sometimes shifts to emit more of
the same polarization, sometimes more of the opposite handed polarization. For example
if the excitation is right circular polarization and the average dissymmetry is 0.3 changing
to left circular polarization could either shift the average dissymmetry to 0.1 or 0.5 for
different quantum dots. Since this differs from quantum dot to quantum dot an
overarching correlation is not yet understood. Future work will look at parameters such
as orientation and environment that could be affecting the correlation.

In summary, there is a wide breadth (heterogeneity) and structure in the single
crystal molecule chiroptical response of helicenes, as seen in the dissymmetry histograms. We
believe this structure is due to three distinct orientations of the molecules on the surface.
This is supported by computation modeling which showed the observed dissymmetry
changes for different observation angles. Depending on the orientation of the molecule
on the surface, the surface interactions can change significantly, and potentially affect
dissymmetry values. Because the average single molecule dissymmetry at 440 nm was
inverted from the bulk, the transition quadrupole appears to be playing a larger role than
expected due to more efficient absorption at that wavelength. In order to correlate
orientation to dissymmetry, we looked at defocused images and observed that the
emission patterns are not from 1-D dipoles. Simulations of emission patterns adapted
from the Kirkwood model of two coupled oscillators have reasonably approximated the emission patterns of the helicenes. To confirm the accuracy of the simulations, we need molecules of known orientation to correlate simulated emission patterns to emission patterns from specific orientations. In addition, there appears to be some relationship between excitation and emission polarization that needs to be studied further. Our work thus far has demonstrated the feasibility of interrogating the fundamental nature of the interaction of light with chiral molecules at the single quantum system level, and provided useful insights into the photophysics of chiral fluorophores. These results create opportunities for the future development and control of molecules used in new materials involving efficient polarized light-emitting diodes (POLEDs) in next-generation display technologies.\textsuperscript{232}
APPENDIX A

OPTICAL ALIGNMENT AND CHARACTERIZATION

A number of different optics are used to prepare the excitation polarization and analyze the emission polarization. The optics used for polarization are a linear polarizer, a quarter waveplate, a dichroic, a depolarizer, and a Wollaston prism. The optics are oriented and characterized by the intensity of light transmitted through them or reflected off of them.

Figure 25 shows the setup to align a linear polarizer. Laser light is sent through the optic to a detector and the intensity is measured and maximized. The minimum intensity can also be found in order to determine the extinction ratio.

![Figure 25: Setup to align linear polarizer (black) using a detector (triangle)](image)

A depolarizer is used when an unpolarized excitation source is needed. The depolarizer (OFR DPU-15) is placed in the beam, and the linear polarizer is used to check the uniformity of intensity in all angles. See Figure 26 for the order of optics. The depolarizer is adjusted until a uniform intensity is achieved.

![Figure 26: Setup to align depolarizer (purple) with a linear polarizer (black).](image)

Figure 27 shows the basic setup to align a quarter waveplate. Figure 27A shows a linear polarizer followed by a quarter waveplate (multi-order broadband Melles Griot or achromatic Edmund Optics) and another linear polarizer. The first polarizer stays stationary and aligned with the laser polarization. The quarter waveplate is oriented at
45° to the linear polarization. The second linear polarizer is used to find the maximum and minimum intensities. The degree of circular polarization is the ratio of minimum over maximum intensity. The second method for testing the degree of circular polarization is based on a property of circular polarized light; when it is reflected off a surface it reverses its polarization. If the initial input into the quarter waveplate is vertical after retroreflection and passage back through the quarter waveplate again, the polarization of the light will be horizontal. The light’s polarization is then perpendicular to the linear polarizer, and therefore no signal is detected by the detector. The degree of circular polarization is measured by \((\text{max}-\text{min})/(\text{max}+\text{min})\). The setup for this alignment is shown in Figure 27B.

![Alignment of a quarter waveplate](image)

**Figure 27: Alignment of a quarter waveplate.** Linear polarizer (black), quarter waveplate (white), beam splitter (grey), mirror (blue), triangle detector

The effect of the dichroic (2027DRLP Omega) on circular polarization is determined in a similar fashion to aligning the quarter waveplate. Figure 28 shows how the dichroic is included in the optical train for testing. This can be done either in the microscope or in free space. First the quarter waveplate is aligned as described above, and then the dichroic is inserted into the optical train. The effect of reflecting off the dichroic is then determined. For the testing method in Figure 28A, the degree of circular polarization is the ratio of min/max intensity. For the testing method in Figure 28B, the degree of circular polarization is measured by \((\text{max}-\text{min})/(\text{max}+\text{min})\).
Figure 28: Determination of the effect of a dichroic on polarization. Linear polarizer (black), quarter waveplate (white), beam splitter (grey), dichroic (pink), mirror (blue), and detector (triangle)

A Wollaston prism (Thorlabs) separates horizontal and vertical components of polarized light. The alignment of this optic is done in order to determine 1) how it separates linear polarized light and 2) how it separates circular polarized light. First, to determine which channel corresponds to horizontal and vertical polarization, the Wollaston prism is placed in the beam, making sure that the transmitted beams of light are level (Figure 29A). Next, a linear polarizer is placed in the beam to determine which channel corresponds to horizontal and vertical polarization (Figure 29B). The second part of this alignment determines which transmitted beam corresponds to right or left circular polarization. This is done by first aligning two quarter waveplates by equalizing the horizontal and vertical intensities to generate circular polarization (Figure 29C). Finally, to see which channel corresponds to left and right circular polarization, both quarter waveplates with known orientation are used to determine which channel shows either right or left circular polarization (Figure 29D). The first quarter waveplate is adjusted to generate left or right circular polarized light while the second quarter waveplate remains fixed.
Figure 29: Wollaston Prism Alignment (A) Wollaston (B) Wollaston and Linear polarizer (C) Wollaston Linear polarizer and quarter waveplate (D) Linear polarizer and two quarter waveplates.
APPENDIX B

SAMPLE PREPARATION

Great care needs to be taken in preparing the samples. The samples are diluted to \( \sim 10^{-10}\text{ to } 10^{-12} \text{M} \) so any impurities in the sample, the solvent, the substrate, or on the glassware may result in artifacts. The purity of the samples is checked by the synthetic chemists using a variety of means such as NMR, CD, TEM, or breadth of emission spectra. The solvents used are of high purity, typically 99% or higher of semiconductor or HPLC grade. In order to prevent contamination, the glassware, bottles, capillaries, and slides are plasma cleaned. If the substrate is glass, it is plasma cleaned. If instead a polymer film is coated on the glass, the polymer film is prepared by dissolving the polymer in a clean solvent, and once the film is made the remaining impurities in the film are characterized. The method to form the films is the drop and swipe method shown in Figure 30. A capillary with \( \sim 90 \mu\text{L} \) of polymer solution is dropped on a slide and the capillary is used to swipe it into a thin film. The slide is tilted back and forth to evenly disperse the film until it dries.

![Figure 30: The drop and swipe method](image)

All of the samples are prepared in 20 mL vials that have been plasma cleaned. Figure 31 shows how a FluoSpheres® bead sample (one of our controls) is prepared. Approximately 30 µL of stock solution is diluted in 10 mL of water followed by serial
dilutions in water and methanol. All other samples are prepared using serial dilutions like these. The polymers are prepared differently. For example, to make a Zeonex® solution, five pellets of Zeonex are dissolved in 10 mL of cyclohexane using sonication. This method produces a clean polymer solution.

Figure 31: FluoSphere® preparation
APPENDIX C

DATA ANALYSIS

The raw data we work with is a movie file of the collected fluorescent intensities that are resolved spatially and in time. Each frame has an area of 30x30 µm area, and a typical exposure is 2 seconds. 30 to 40 fluorescence intensity spots are identifiable in a typical scene. The spatial coordinates of each fluorescent spot were identified, and in the case of CPL, the spots are correlated between the right and left side of the image shown in the first step of Figure 32.

![Diagram of data analysis process]

**Figure 32: Extraction of dissymmetries from raw camera data**

In the second step, the background was subtracted. Figure 33 illustrates the identification of background for an image. Either a general background is determined from an identified area such as in box A in Figure 33, or a local background is taken from the immediate surroundings as identified by box B in Figure 33. Once the background...
level is determined and subtracted, the fluorescence trajectories are extracted for the identified spots (step 3 in Figure 32).

![Figure 33: Illustrates the identification of background. (A) would be used as a background template to be subtracted from entire scene. (B) illustrates the location of background for local background identification and subtraction.]

Once the intensity trajectories are extracted, it is a simple matter to calculate the dissymmetries. The dissymmetry parameter, $g$, for FECD and CPL is defined as $g = 2(I_L-I_R)/(I_L+I_R)$, where $I_L$ is proportional to the number of photons when left circular polarized light is either absorbed (FECD) or emitted (CPL) and $I_R$ corresponds to when right circular polarized light is either absorbed (FECD) or emitted (CPL). Step 4 of Figure 32 depicts determining if the molecule is fluorescing based on whether the fluorescence intensity is greater than the $2\sigma$ above the noise in the background. If the molecule is not fluorescing, we also confirm no over-subtraction occurred and the intensity is still positive.

Based when the molecule is fluorescing the dissymmetries are calculated for the molecules, in step 5 of Figure 32. In FECD a molecule’s fluorescent trajectory is only used if it does fluoresce for at least two half cycles out of 1.5 cycles. This is because the molecule may have bleached when the excitation changed from left to right, or the absorption cross-section could be low. The difference between these two possibilities is not discernable without fluorescence in two half cycles out of 1.5. In addition, if the
trajectory can be used for dissymmetries but one of the half cycles has blinking or photobleaching occurring, that half cycle cannot be used because the intensity for that half cycle would be artificially lowered. In contrast to FECD, our experimental design for SM-CPL measurements give $g$ on a shot-by-shot basis, so we are able to see what the dissymmetry is in every frame as opposed to averaging a number of frames together as is done for FECD.

The final step is to determine the distribution of dissymmetries from single molecules. This is done by histogramming all the dissymmetries from single molecules. The average dissymmetry is found and compared to the bulk measurements. This whole process of analysis is continually being streamlined. More details on the improvements that are being done in IGOR can be found in Appendix F.
APPENDIX D

DiIC$_{18}$

Figure 34 shows the results the other control, DiIC$_{18}$. DiIC$_{18}$ was use because it is a molecule with a linear dipole and absorbs and emits linearly polarized light. This control was to test for any linear artifacts. The average dissymmetry is -0.049. The distribution is narrow, with 98% of the probability density between ±1 and 91% between ±0.75. These results are similar to the FluoSpheres®. This demonstrates there are no linear artifacts in the FECD measurements.

![Normalized Histogram of FECD Dissymmetry Parameters](image)

**Figure 34:** DiIC$_{18}$ normalized histogram of FECD dissymmetry parameters determined from single molecule fluorescence measurements. Data from DiIC$_{18}$ $<g> = -0.049$. 

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APPENDIX E

CPL CAMERA CORRELATION

In order to understand why a given side of the camera corresponds to left or right circular polarization, the path of the light and its polarization needs to be traced through the optic train of Figure 19. Starting with right circular polarization at the sample, the light passes through the notch filter unaffected and is transmitted through the microscope. The light is still right circularly polarized when it comes out of the microscope. Next, the light passes through a $\lambda/4$ waveplate, with its fast axis oriented at 45° to the left of vertical as the light propagates towards the observer. As the light passes through the $\lambda/4$ waveplate, the circular polarization is converted to linear polarization. In the case of right circular polarization, it is converted to horizontally polarized light. Looking at the front of the camera, as the light passes through the Wollaston prism, the vertical light is separated to the left side and the horizontal to the right side of the camera. When pure right-handed light is started with, it will appear in the horizontal channel, and pure left-handed light will appear in the vertical channel. If instead elliptically polarized light is emitted, the quarter waveplate decomposes the elliptical light into the relative components of left and right circular polarization, which are reported in the vertical and horizontal channels respectively. The ratio of the relative intensities $I_L$ for the vertical channel and $I_R$ for the horizontal channel will be used in $g = 2(I_L - I_R)/(I_L + I_R)$ to report the dissymmetry, the degree of circular polarization in emission.
Currently we are trying to improve the quality, consistency, and rate of throughout for our data analysis. A program is being developed to automate the analysis process by which spots are identified and extracted. Figure 35 shows how the data flows through IGOR. The raw data is loaded into the program and it is summed in order to first identify the location of good background regions of interest (ROI)s so that no spot intensity is included in the subtraction process. The background is removed from this summed image and the particles coordinates and ROIs are identified. We then return to the initial raw data and split the movie into separate frames. Using the background locations from the summed image, the background is removed. The fluorescence trajectories were then extracted from the recombined images, using the identified coordinates and ROIs.

Figure 35: Data flow for extraction of intensity trajectories from raw camera data


77. Muller G, Muller FC, Maupin CL, Riehl JP. The measurement of the fluorescence detected circular dichroism (FDCD) from a chiral Eu(III) system. *Chemical Communications* 2005(28):3615-3617.


81. Lamos ML, Walker GT, Krugh TR, Turner DH. Fluorescence-Detected Circular-Dichroism of Ethidium Bound to Poly(Dg-Dc) and Poly(Dg-M5dc) under B-Form and Z-Form Conditions. *Biochemistry* 1986;25(3):687-691.


