INVESTIGATION OF PHOTOPHYSICAL AND PHOTOCHEMICAL PROCESSES IN CONJUGATED POLYMER NANOPARTICLES BY SINGLE PARTICLE AND ENSEMBLE SPECTROSCOPY

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INVESTIGATION OF PHOTOPHYSICAL AND PHOTOCHEMICAL PROCESSES
IN CONJUGATED POLYMER NANOPARTICLES BY SINGLE PARTICLE
AND ENSEMBLE SPECTROSCOPY

A Dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Chemistry

by
Craig Szymanski
May 2009

Accepted by:
Jason McNeill, Committee Chair
Dvora Perahia
George Chumanov
Stephen Creager
ABSTRACT

Single molecule imaging has emerged as a powerful tool in a range of applications, but the field is limited by a lack of fluorescent probes with sufficient brightness and photostability for many demanding applications such as tracking of single biomolecules in cells and tissues at video rate with 1 nm spatial resolution. Conjugated polymers hold great promise as a solution to these issues, with a high density of π electrons and variety of chemistries, allowing efficient and tunable absorption of light. This dissertation describes the development and characterization of a novel type of nanoparticle composed of conjugated polymer called CPdots. CPdots retain the high brightness of conjugated polymers in solution and in films, but can be dispersed in water, making them suitable for many biological applications. These CPdots have been shown to have one-photon absorptivities that range from $10^6 - 10^7$ M$^{-1}$ cm$^{-1}$ (2-3 orders of magnitude higher than most other fluorescent dyes), and two-photon cross sections as high as $2 \times 10^5$ G.M. units (the highest reported value to date for a nanoparticle). A variety of complex photophysical phenomena were observed in CPdots, including complex photobleaching kinetics, reversible photobleaching, complex picosecond fluorescence kinetics, and collective excitation effects in single nanoparticles. A novel theoretical model describing the interactions between excitons and polarons in CPdots was developed. The model results predict complex photobleaching kinetics and complex picosecond fluorescence kinetics, in close agreement with experimental data. The model is also in qualitative agreement with many phenomena observed in fluorescence experiments performed on single nanoparticles. Gelation thermodynamics and kinetics
of the conjugated polymer poly(2,5-dinonyl-paraphenylene ethynylene), which are important in film casting techniques, were investigated allowing the design of film casting methods that will yield specific energy transfer efficiencies. These investigations provide a thorough understanding of CPdot photophysics, necessary for the rational design of improved fluorescent probes. It is also hoped that the results of these investigations could help in understanding key processes that could limit efficiency of organic optoelectronic devices such as polymer-based light-emitting displays and polymer-based photovoltaic devices, and thus help in the development of strategies aimed at improving device efficiencies.
ACKNOWLEDGMENTS

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CHAPTER 1

OVERVIEW

1.1 Introduction

Improvements in fluorescent probes for use in biological systems for imaging and tracking have enabled a new level of understanding in such systems. This endeavor is particularly problematic in living cells, which are far from the idealized environments of \textit{in vitro} studies, as they include organelles, cell membranes, and other types of inhomogeneity that interfere with fluorescence or blur the image if insufficient signal is obtained from the fluorescent probe. Further improvements in the area of single-molecule tracking requires fluorescent probes with improved brightness and stability to enable higher degrees of tracking accuracy, temporal resolution, and increased tracking time. Conjugated polymer-based probes provide an avenue to advance the state of the art of biological single molecule tracking probes, by improving on these figures of merit.

The following chapters focus on a variety of research topics related to conjugated polymers. Chapter 2 lays out the experimental techniques used, both of commercial and custom built instruments, and outlines their utilization in the research in this dissertation. Chapter 3 describes the development and near field scanning optical microscopy of conjugated polymer nanoparticles (CPdots). Chapter 4 focuses on the kinetics and thermodynamics of gelation of the polymer poly 2,5-dinonylparaphenylene ethynylene and their implications for its gel structure. Chapter 5 demonstrates the suitability of CPdots in two-photon spectroscopy. Chapters 6 and 7 concern the excited state processes
taking place in CPdots with both the experimental data and theoretical considerations, in each chapter respectively. Chapter 8 shows preliminary single molecule spectroscopy data and explains some of the phenomena that are observed.

1.2 Conjugated Polymers

1.2.1 Discovery and Applications

Since conjugated polymers were first recognized for their semiconducting properties in 1977 by Heeger, MacDiarmid, and Shirakawa, they have attracted great interest from the scientific community for their fluorescent and semiconducting properties combined with synthesis of a wide variety of structures and the possibility of low cost electro-optic devices such as displays and photovoltaic cells. Early studies focused on the simplest conjugated polymer, polyacetylene, however this polymer is almost completely insoluble in all solvents, limiting its usefulness. The development of soluble poly phenylene vinylene derivatives and the demonstration of polymer-based light-emitting devices based on such polymers led to renewed interest in the field.

1.2.2 Structure and Properties

Conjugated polymers contain alternating single and double bonds along the length of the polymer backbone which allow electrons to be delocalized along the length of the structure. Four commonly used conjugated polymer backbones are poly phenyl ethynlenes (PPEs), poly phenylene vinylenes (PPVs), polyfluorenes, and polythiophenes (PTs), as shown in Fig. 1.1.
Delocalization of the $\pi$ and $\pi^*$ energy levels and the moderate energy gap gives rise to semiconducting characteristics as well as efficient absorption of photons in the visible, near UV, and near IR spectral ranges. Variation of sidechain chemistry or the inclusion of heteroatoms, such as O, N, or S in either the backbone or the sidechains, allow tuning of photophysical and chemical properties.\textsuperscript{1, 10, 13, 14} While, in principle, the electronic states of a conjugated polymer molecule would be delocalized over the entire polymer molecule (for a perfectly ordered structure at absolute zero), under typical conditions structural features such as kinks, chemical defects, and bends in the polymer chain lead to lengths of 4-10 repeating units over which conjugation is unbroken.\textsuperscript{15, 16} Conjugation length plays an important role in determining the photophysical properties of the polymer, as the energy level of an electronic state is a function of the space available for delocalization, as explained by Hückel theory. Longer conjugation lengths have lower energy electronic states leading to red shifted absorption and emission wavelengths; conversely, short conjugation lengths lead to blue shifted spectra. This is especially important to consider when designing devices, as solution and film processing each have a dramatic impact on conjugation length and therefore spectral characteristics.\textsuperscript{17}
1.3 Frenkel Excitons and Exciton Dynamics

1.3.1 Frenkel Excitons

When a photon is absorbed by a conjugated polymer, an electron undergoes excitation from the $\pi$ to the $\pi^*$ band, creating a neutrally charged local excitation and polarization or distortion of the polymer matrix. This unit of excitation is called a Frenkel exciton (or molecular exciton). The exciton is generally contained in a segment of the polymer matrix with unbroken conjugation which comprises a chromophore. In the related system of crystalline organic semiconductors, the term “exciton” typically refers to collective excitations involving several chromophores (molecules), though in conjugated polymers, the spatial extent of the chromophore is somewhat ambiguous, and the degree of coupling between chromophore units is also difficult to quantify and currently the subject of some debate. Nevertheless, there are many similarities between the behavior of excitons observed in crystalline semiconductors and the exciton-like states of conjugated polymers, so the term “exciton” is commonly used to refer to both. Excitons are able to undergo either radiative or nonradiative decay, move to another chromophore via either a Dexter or Förster mechanism, or lose an electron to leave behind a hole polaron (discussed below). Exciton mobility in the polymer occurs through a combination of Dexter electron transfer and Förster energy transfer. As the emission spectrum from the donor chromophore needs to overlap with the absorption spectrum of the acceptor chromophore, there tends to be a red shift in the energy of an exciton as it diffuses through the particle, observable in the emission spectrum.
1.3.2 Polarons

Polarons are either electrons or holes (electron vacancies) that exist in an organic semiconductor\textsuperscript{8, 18}. They can be introduced by; chemical doping (p-type or n-type) by introduction of electron rich or electron deficient heteroatoms or structures into the polymer; electrical oxidation or reduction by inducing a charge and allowing the incorporation of electrolyte; by direct oxidation or reduction by an electrode; or by optical excitation followed by exciton dissociation. Any of these processes can lead to the oxidation or reduction of the semiconductor which creates a positive or negative charge that becomes localized to a segment of the material and distorts the nearby structure, creating a locally polarized volume. Polarons able to move to different locations in the material, acting as charge carriers in such devices as organic field effect transistors and solar cells.

Hole polarons in conjugated polymers are typically created when an electron is lost from the exciton, leaving behind a positively charged hole and a polarization of the polymer matrix. The absorption spectrum of a hole polaron is typically red-shifted as compared to the neutral polymer, resulting in an absorption spectrum with a high degree of overlap with the emission of the neutral polymer, which in turn results in highly efficient energy transfer to hole polarons, characterized by a large energy transfer radius (4-8 nm), enabling a small number of polarons to cause a large number of excitons in the vicinity to undergo FRET. As emission from polarons is typically red shifted and has a low quantum yield, thus a relatively small hole polaron density (~$10^{17}$ per cm$^3$, roughly 1 in 1000 chromophores) can nearly completely quench fluorescence from the conjugated
This effect is largely responsible for the difficulty of developing laser diodes based on conjugated polymers, despite numerous attempts. Since there are a range of ways in which hole polarons may be created and they can have such a dramatic effect on device performance (quenching the species that make them function), there is considerable interest in understanding and perhaps controlling the various processes of creation and elimination of these species, as well as the processes of energy transfer to the quencher species.

1.4 Interchain Interactions in Conjugated Polymers

As molecular materials, the photophysical properties of a conjugated polymer sample are strongly influenced by the structure of the polymer (whether in solution or in a film). In a good solvent, such as a molecular solution, excited state species are confined to a single chain (intrachain species), whereas in poor solvent conditions or in films, the π bands of neighboring polymer chains may overlap, leading to interchain species, such as aggregates. In this context, “aggregates” refers to a variety of species exhibiting spectroscopic characteristics associated with a range of phenomena associated with close-contact between two or more molecules, such as pi-stacking, interactions between transition dipoles (the H and J aggregates), excimers, and exciplexes. Aggregate species play an important role in determining photophysical properties of conjugated polymers. Often, these excited state species are delocalized over a larger number of atoms or chromophores, leading to a red shifted emission. Furthermore, due to the flexibility of these polymers, it is possible to form aggregates both between
different polymer chains (interchain aggregates)\textsuperscript{3, 24} and between different overlapping segments of the same polymer chain (intrachain aggregates)\textsuperscript{25, 26}.

The nature of the effects of various solvent conditions (quality and temperature) and their effects on optical properties have been studied for a range of conditions. The dipole interactions from nearby chromophores also makes it possible for FRET to take place between polymer chains, and the packing efficiency, which affects inter-chromophore (interchain) distance, determines the energy transfer rate. Photophysical properties including carrier mobility, quantum yield, and emission wavelength are therefore a product of casting properties in films or concentration in a solution or gel phase.\textsuperscript{3, 17, 27} It is known that the properties of these films are a product of solvent quality, temperature, and concentration, as well as the chemical properties of the polymer itself, such as backbone type, sidechains, heteroatoms, and heterogeneity. Solvent quality is dictated by a combination of solvent polarity, temperature, and polymer concentration, with good solvent leading to an open structure in the polymer with less efficient energy transfer and poor solvent quality can lead to gelation and interchain aggregation.

1.5 Fluorescence

Much of the work presented in this thesis involves fluorescence measurements, such as fluorescence spectroscopy, fluorescence quantum yield determination, and fluorescence lifetime measurements. A brief discussion of the relevant phenomena is given here. Absorption of a photon increases the energy in the molecule and takes it from the ground state to an excited state. For a molecule with a ground state \( S_0 \), the
photon can excite the molecule to the first excited singlet state (S\textsubscript{1}) as shown in Fig. 1.2.

The molecule may also have a triplet state T\textsubscript{1}.

Figure 1.2: The Jablonski diagram showing the interactions of singlet and triplet states in a fluorophore.

Fig. 1.2 describes the relationship between the several states (S\textsubscript{0}, S\textsubscript{1}, T\textsubscript{1}) of a sample fluorophore along with the rate constants for interchange between the states and for energy transfer from a donor molecule to an acceptor molecule (described in detail later).\textsuperscript{28}

The various rate constants are:

\[k_{abs}\] = Excitation of the molecule through the absorption of a photon

\[k_{IC}\] = Internal conversion, or vibrational relaxation

\[k_{R}\] = Radiative decay (Fluorescence)

\[k_{NR}\] = Non-radiative decay

\[k_{PB}\] = Irreversible Photobleaching

\[k_{ISC}\] = Intersystem crossing to the triplet state

\[k_{P}\] = Phosphorescence
Internal conversion is the relaxation from higher vibrational states to the ground vibrational state of the $S_1$ state, with a time constant on the order of $10^{-12}$ s, ensuring that in most cases transitions from the $S_1$ state (to the ground state or the triplet state) start from its lowest vibrational state.

After the molecule is excited, it spends a certain time in the $S_1$ state before it decays via one of several possible pathways. The time constant for residence in the excited state is called the fluorescence lifetime, $\tau_F$, and results from the combination of processes depopulating it (Eq. 1.1):

$$\tau_F = \frac{1}{k_R + k_{NR} + k_{ISC} + k_{PB}}$$  \hspace{1cm} 1.1

The fluorescence quantum yield is the probability that the absorption of a photon will result in the emission of a fluorescence photon. The expression for quantum yield, $\phi_F$, is the ratio of the radiative rate constant to all excited state depletion rate constants (Eq. 1.2):

$$\phi_F = \frac{k_R}{k_R + k_{NR} + k_{ISC} + k_{PB}}$$  \hspace{1cm} 1.2

The parameter that determines signal levels that can be obtained and that it in many ways the most important for defining the capabilities of a probe is brightness, defined as the product of the extinction coefficient and the quantum yield. It is also worth noting that in most cases $k_R$ and $k_{NR}$ are much larger than $k_{ISC}$ and $k_{PB}$ so lifetimes and quantum yields mostly depend on $k_R$ and $k_{NR}$.

### 1.5.1 Förster Resonance Energy Transfer
It is possible for many photophysical systems to transfer energy, such as in the light harvesting complex,\textsuperscript{29} or from one species to another such as in DNA molecular beacons.\textsuperscript{30} This radiationless transfer from donor to acceptor is accomplished through either the electron transfer mechanism known as Dexter energy transfer, or through Förster resonance energy transfer. The Dexter energy transfer requires wavefunction overlap between donor and acceptor and is a short range interaction (< 2 nm) and requires spin conservation.\textsuperscript{28} Förster resonance energy transfer involves the interaction of transition dipoles of donor and acceptor and is a longer range interaction (up to 10 nm). Förster theory is based on the assumption of a weak interaction between the transition dipoles of the donor and acceptor, with the application of Fermi’s Golden Rule to estimate the transition rate. The theory predicts that the energy transfer rate depends on intermolecular distance to the inverse sixth power,

\[
k_{\text{ET}}(R) = \frac{1}{\tau_D} \left( \frac{R_0}{R} \right)^6
\]

where \( \tau_D \) is the donor fluorescence lifetime in the absence of the acceptor, \( R \) is the donor-acceptor distance, and \( R_0 \) is the energy transfer radius or Förster radius, defined as the distance at which the energy transfer efficiency is 50%. The energy transfer rate constant (Eq. 1.3), being highly distance dependant, can be used a ‘molecular ruler’ by attaching the donor and acceptor to the same molecule and observing the degree of energy transfer.\textsuperscript{31} Based on the aforementioned assumption of weakly interacting transition dipoles and a number of other assumptions, Förster developed an expression for the energy transfer radius in terms of readily determined spectroscopic properties:
where $\phi_D$ is the quantum yield of the donor in the absence of acceptor; $\kappa^2$ is the configurational factor (accounts for relative orientation of the donor and acceptor dipoles, usually 2/3 in solution); $n$ is the index of refraction of the medium; $F_D(\lambda)$ is the pure donor emission spectrum, normalized for the area under the curve; $e_A$ is the pure acceptor emission spectrum; and $\lambda$ is wavelength. If energy transfer occurs, a $k_{ET}$ term is included in the denominator of the right sides of equations 1.1 and 1.2, reducing both the fluorescence quantum yield and lifetime of the donor. If the assumption is made that $k_{ISC}$ and $k_{PB}$ are much smaller than the other depletion mechanisms from the $S_1$ energy level, then the energy transfer efficiency can be calculated by (Eq. 1.5):

$$E = \frac{k_{ET}}{k_R + k_{NR} + k_{ET}}$$

As the population of the excited state is the same for all downward rates, the rate constants are directly proportional to the photon emission rates. The energy transfer can be determined by taking the ratio of the unquenched fluorescence intensity of the donor with acceptor to the intensity in the absence of an acceptor.

1.5.2 Multi-Photon Excited Fluorescence

Multiphoton excited fluorescence involves the absorption of two or more photons simultaneously, each with roughly a whole number fraction of the band gap for excitation, as depicted in Fig. 1.3:

$$R_0^e = \frac{9000 (\ln 10) \kappa^2 Q_D}{128 \pi^3 N_A n^4} \int_0^\infty F_D(\lambda) e_A(\lambda) \lambda^4 d\lambda$$ 1.4
Figure 1.3: Left: Energy level diagram showing single-, two-, and three-photon excitation. Each of the three excitation mechanisms leads to the emission of a photon with the same energy. Right: Single and multiphoton intensity dependence. The emission from single photon excitation occurs throughout the illuminated (dotted) area, while the multiphoton emission occurs only very near the focus of the beam.

This multiphoton absorption is accomplished without intermediate states, as the process takes place in one step. The absorption coefficient is a product of the square (for 2-hν) or cube (for 3-hν) of the intensity, making absorption very inefficient except at very high instantaneous power. This requirement of high instantaneous power leads to the use of tightly focused excitation using the mode-locked lasers, such as a Ti-Sapphire laser. Multiphoton experiments require well focused pulsed laser sources in order to achieve any excitation. Absorption coefficients for 2-hν absorption, the more commonly used type of multiphoton spectroscopy, are measured in Goppert-Mayer units (GM) where 1 GM = 10⁻⁵⁰ cm⁴ s photons⁻¹ and may vary considerably from one photon cross sections due to different selection rules. Once the molecule is excited to the S₁ state,
any other process may occur including fluorescence, energy transfer, etc, and to date, all emission spectra from multiphoton excitation are identical to those observed after single photon excitation.\textsuperscript{28}

Multiphoton microscopy, first demonstrated in 1990 by W. W. Webb and coworkers,\textsuperscript{35} takes advantage of the apparent limitation of requiring high power by tightly focusing the beam causing excitation in very small volume with no emission from sample that is out the focus. This has been used effectively in scanning microscopy of cells,\textsuperscript{36,37} deep tissue,\textsuperscript{38} and skin imaging,\textsuperscript{39} obtaining high resolution, low background images (molecules outside of the focus are not excited) at a long working distance (500 $\mu$m).\textsuperscript{36} There is also reduced damage to biological structures and decreased photobleaching rates for fluorophores, as molecules that are outside the focal plane do not absorb and therefore do not undergo the photon induced reactions that can occur in one photon microscopy.\textsuperscript{40} The experimental details involved with two-photon spectroscopy and microscopy will be described in Chapter 2.

1.5.3 Time Resolved Fluorescence Spectroscopy

In order to obtain information about the rates of various processes occurring in the excited state, such as collective (excitonic) effects, radiative and non-radiative relaxation rates, and rates of energy transfer processes, various time-domain spectroscopic methods are used. The fast time scale for fluorescence dynamics, on the order of picoseconds to nanoseconds, requires specialized tools to analyze. The two primary strategies for measuring fluorescence lifetime in use today are time-domain and frequency-domain (phase-shift) based measurements. The technique selected for all picosecond time
resolved experiments described later was time-correlated single photon counting (TCSPC), which was selected because of the high time resolution and its suitability to analyzing complex dynamics.\textsuperscript{28} Other time-domain methods include optical gating methods (such as fluorescence upconversion and Kerr gating) and streak-camera based methods. These are less widely used, due to the complexity and cost involved, but can provide subpicosecond time resolution.

TCSPC is a time domain technique that uses a mode-locked high repetition rate femtosecond or picosecond laser, such as a titanium sapphire (Ti:Sapphire) laser for sample excitation. The laser pulse is used as a start pulse for timing electronics and a fluorescence photon from the sample is used as the stop pulse. The delay times between the arrival of the laser pulse photon and the fluorescence photon are histogrammed to obtain the fluorescence decay curve, which also contains some unavoidable temporal ‘smearing’ due to the bandwidth limitations of the detector and electronics. Further details of the experimental considerations, along with optics and electronics used will be presented in Chapter 2.

\subsection*{1.6 Single Molecule Spectroscopy}

Single molecule spectroscopy was first demonstrated with doped crystals at low temperatures\textsuperscript{41} and later at room temperature with near field scanning optical microscopy (NSOM).\textsuperscript{42} Currently, much single molecule research utilizes confocal and wide field microscopy arrangements. While microscopy in the far field, as opposed to the near field, is limited by diffraction giving decreased resolution, this disadvantage is often more
than offset by advantages in signal level, flexibility, and ease of use. Single molecule microscopy has been used to investigate diffusion inside cells,\textsuperscript{43} probe enzyme kinetics,\textsuperscript{44} perform molecule tracking with 1 nm accuracy,\textsuperscript{45} monitor the configuration of single proteins,\textsuperscript{44, 46} and to probe the structure and photophysics of multichromophoric systems such as light-harvesting complexes,\textsuperscript{47} and conjugated polymers.\textsuperscript{26, 48}

1.6.1 Single Molecule Excitation

The signal level in single molecule fluorescence measurements depends on a number of factors, including the excitation intensity, optical cross-section and fluorescence quantum yield of the fluorescent molecule, collection efficiency, and detector quantum efficiency. The excitation rate of a single molecule can be estimated by analogy with the Beer-Lambert Law,

\[
A = \varepsilon \ c \ l
\]

where \(A\) is absorbance, \(\varepsilon\) is the molar absorptivity (wavelength dependent, usually in units of \(\text{M}^{-1}\text{cm}^{-1}\)), \(c\) is concentration (M), and \(l\) is the pathlength of sample (cm). While the molar absorptivity (\(\varepsilon\)) parameter is most often used to describe macro-scale experiments, the parameter used far more often at the single molecule level is absorption cross section (Eq. 1.7):

\[
\sigma = \frac{2.303 \ \varepsilon}{N_A}
\]

where \(\sigma\) is the absorption cross section (\(\text{cm}^2\)) and \(N_A\) is Avogadro’s number. This cross section defines the effective area that a single molecule absorbs at a particular wavelength of light. In optical configurations typically employed for single molecule
measurements, such as laser epi illumination or near-field illumination, intensities on the order of $10^6$ W/cm$^2$ are readily obtained. Typical extinction coefficients of 10,000 – 100,000 correspond to per-molecule optical cross-sections of roughly $4 \times 10^{-20} – 4 \times 10^{-19}$ cm$^2$. The excitation rate on a per-molecule basis is given by the product of the intensity (in photons per unit area per second) and the per-molecule optical cross-sections. For wavelengths in the visible range, excitation rates ranging from 1 kHz to several MHz are typically obtained.

1.6.2 Near Field Scanning Optical Microscopy

Near field scanning microscopy is a method of scanning microscopy that achieves better than diffraction limited optical resolution while simultaneously acquiring spatially synchronized topographic information.$^{42, 49}$ The instrument uses a sharpened, metal coated optical fiber with an aperture at the tip as the excitation, and in some configurations, the collection optic. Laser light coupled into the other end of the fiber passes through the fiber and out through the aperture at the tip. The configuration used here (Fig. 1.4) positions the detection optics below the sample and they are similar to those used in the sample scanning confocal microscope described above. As the sample is scanned in front of the objective, a feedback and sensing system (which measures the interaction forces between tip and sample) keeps the tip within a specified distance of the sample (usually a few angstroms) that is less than the wavelength of the excitation light, and this height information, needed to keep a constant distance, provides the topographic information. Fluorescence excitation is achieved through an uncoated section of the tip through which the sample may be excited by the evanescent field, a very short range.
effect that is not limited by the diffraction limit, allowing excitation areas only about 50 nm across (as opposed to the >250 nm for far field confocal measurements).

Figure 1.4: Schematic representation of a near field scanning optical microscope.

A high numerical aperture objective collects the fluorescence emission, filters remove remaining excitation light, and the signal is focused onto either an APD or a spectrograph and CCD. Though NSOM has the advantage of achieving spatially and spectrally correlated information with resolution below the diffraction limit, it has been used less frequently in recent years, as the resolution advantage is often not sufficient to offset the low signals and difficulty of this technique. In addition, optical microscopy techniques
based on $4\pi$ microscopy, and microscopy methods based on single molecule tracking such as STORM, can achieve similar resolution.

### 1.6.3 Sample Scanning Confocal Microscopy

One technique that is frequently used for single molecule spectroscopic studies is sample scanning confocal microscopy, shown in Figure 1.5 (left). This method, pioneered by Zare, images laser light on the back focal plane of a high numerical aperture (filling most of the rear aperture of the objective) objective to focus the light down to a diffraction limited spot in front of the objective. Excitation occurs in a small enough volume that it is straightforward to dilute a liquid sample sufficiently that there are typically one or zero molecules in the focal volume at a time; a $5.3 \times 10^{-8}$ M solution corresponds to an average of 1 molecule in a 250 x 250 x 500 nm focal volume. Excitation may also be performed in the wide field, in which the incoming light is
focused on the rear aperture of the objective (entering the objective as a small spot) and exits the top of the objective as a wide, defocused beam illuminating a large area. The fluorescence from many fluorophores in a large may be observed, such as tagged proteins diffusing in a cell. Naturally, APDs and PMTs are unsuitable for such an experiment and a CCD (or some other spatially correlated) detector must be used, as no scanning is taking place. The difference in illumination at the objective can be seen in Fig. 1.5 (center).

Fluorescence is collected through the objective and separated from scattered excitation light by a dichroic mirror, and is then detected either on a charged coupled device (CCD) camera, for spectral information or an avalanche photodiode (APD), for dynamical information. The collection efficiency of fluorescence is a function of the numerical aperture (NA) of the objective. Numerical aperture is defined as:

\[ NA = n \cdot \sin \theta \]  

Where \( n \) is the index of refraction of the oil/glass interface and \( \theta \) is the half angle of the light cone that is collected by the objective. This can be applied to the geometric picture of an objective to obtain an estimate of the efficiency of collection (\( \eta \)) given by:

\[ \eta = \frac{\Omega}{4\pi} = \frac{2\pi (1 - \cos \theta)}{4\pi} = \frac{(1 - \cos \theta)}{2} \]  

where \( \Omega \) is the solid angle of collected light (in steradians) out of \( 4\pi \) steradians for a complete sphere. For an objective with \( NA = 1.25 \) and oil/glass interface with \( n = 1.515 \), the collection efficiency is 22\%. While the loss of emitting light at the objective is the largest single loss for the system, there are other losses at each of the filters, lenses, and
mirrors in the optical pathway, usually with about 1-5% signal loss per optic. The detector is also imperfect, although detection efficiencies from 65-90% for avalanche photodiodes and intensified CCDs are typical. The overall detection efficiency of a typical single molecule microscope is between 1-10%, with ours being estimated at ~5%.

Many instruments include a pinhole before the APD or photomultiplier tube (PMT), in order to block out of focus from above or below the sample focal plane. This is unnecessary if the detector has a small detection element and is located at the focus of the fluorescence signal, as fluorescence from outside of the focal plane would only have a small effect on APD signal. The confocal arrangement has an advantage over wide field techniques because it focuses on a small area, which reduces background fluorescence. Scanning mode also allows the use single element detectors such as avalanche photodiodes and photomultiplier tubes (with the addition of a pinhole) which offer higher sensitivity and low detection limits than many CCD detectors, thought this advantage is less significant with newer deep-cooled, intensified CCD cameras. Scanning, however, requires more complex instrumentation including a high precision scanning stage and high synchronization between the detector and the stage movement. Wide field microscopy, however, frees experiments from the requirements of scanning, enabling images of entire cells and simultaneous tracking of multiple probes.

Ultimately, the success or failure of a single molecule experiment comes down to whether or not desired signal to noise levels can be achieved. The primary source of noise in an experiment in a single molecule experiment arises the influence of Poisson statistics on emitted photons. For a stochastic process in which events occur
independently of each other, the standard deviation of the number of events occurring within a given time interval is expected to be equal to $\sqrt{N}$ where $N$ is equal to the average number of events occurring in the time interval. Other sources of noise include background fluorescence, dark current in the detector itself (when there is no signal), and readout noise (occurs only when signal is read from detector, does not scale with time or intensity). The overall noise in the system can then be calculated by,

$$ SNR = \frac{\eta \phi_F \left( \frac{\sigma}{A} \right) \left( \frac{P_0}{h\nu} \right) t}{\sqrt{\eta \phi_F \left( \frac{\sigma}{A} \right) \left( \frac{P_0}{h\nu} \right) t + C_B t + C_D t + C_{read}^2}} $$

where $\phi_F$ is the quantum yield of the fluorophore, $\sigma$ is the absorption cross section of the fluorophore, $A$ is the illuminated area, $P_0$ is the illumination power, $h\nu$ is the energy of an incident photon, $t$ is the observation time, $C_B$ is the number of background counts detected, and $C_D$ is the number of dark counts inherent in the detector, and $C_{read}$ is the readout noise. A rhodamine 6G molecule with a $4 \times 10^{-16}$ cm$^2$ absorbance cross section, quantum yield approaching unity, excited with 1 $\mu$W of 488 nm excitation light, in a diffraction limited spot (~250 nm diameter), the expected signal (top term in fraction), is $2.5 \times 10^4$ Hz. The bottom terms are 25 000 Hz (first term equal to top), $C_B = 100$ Hz, $C_D = 250$ Hz, and $C_{read} = 0$ (for an APD), which gives a standard deviation of 159 Hz, or an SNR of 157, sufficient for single molecule detection. In this case the major contributor of noise is the first term which arises from Poisson noise, which accounts for 158 Hz of noise, indicating that while it is important to minimize background, dark, and readout noise, the greatest improvement in SNR can be obtained by using a brighter fluorophore.
1.6.4 Single Molecule Phenomena

Single molecule phenomena pertain to the observation of particle-to-particle and temporal heterogeneity. Particles often display heterogeneity in terms of kinetics, conformation, local viscosity, and emission spectrum. A large collection of particles is likely to contain every possible configuration and local environment available to the system and if they are observed simultaneously, all of the states are averaged together in the measurement.

Certain phenomena are observable at the single molecule (or few molecules) level, as their effects tend to be averaged out and are therefore are not typically observed directly in bulk measurements. One such example is the triplet blinking described as follows.\textsuperscript{53, 54} If a molecule is excited to the $S_1$ state, then undergoes intersystem crossing to the $T_1$ state (a spin forbidden, slow rate process), it may remain in the $T_1$ state for an extended period of time. Residence time in the $S_1$ state is given by the fluorescence lifetime, typically measured in picoseconds or nanoseconds, whereas residence time in the $T_1$ state is quite long, typically microseconds to seconds, owing to the fact that phosphorescence is also a spin forbidden process. During the time a molecule is in the $T_1$ state it is unable to undergo fluorescence and goes dark until it returns to the $S_0$ state. The on-off dynamics of single molecules can be observed as sharp (stepwise) changes in the fluorescence, as seen in simulated data in Fig. 1.5 (right).

Conjugated polymers have been studied extensively using single molecule techniques. An examination of the spectra and polarization in individual molecules of MEH-PPV in a polystyrene film using sample scanning confocal microscopy showed that
both characteristics were in agreement with bulk measurements in toluene.\textsuperscript{55} It was also found that the energy diffusion was extraordinarily fast at the single molecule level, possibly due to directed energy migration by an energy “funnel.” The slight blue shift in the emission of single molecules also suggests that defects in the conjugated polymer break up conjugation decreasing chromophore length and increasing average chromophore energy. Similar experiments with MEH-PPV examining the distribution of vibronic states in individual molecules found that while the summation of the individual spectra matched the bulk results, the individual spectra showed that the molecules followed a bimodal distribution.\textsuperscript{48} Several studies have shown the dependence of photophysics on the structural conformation of the chains. One such study directly showed that energy transfer rates depend on the conformation.\textsuperscript{26} In this case packing efficiency (and by inference energy transfer) was measured by the anisotropy of the molecule, since each energy transfer step would lead to more randomness in the polarization of the emitted light. In this case higher anisotropy was observed from MEH-PPV that was in a poorer solvent such as toluene (efficient packing to reduce surface area) than when it was in a good solvent such as chloroform (less efficient packing because of less dominant surface effects). Conjugated polymer with fluorescent end caps were observed with single molecule methods, probing energy transfer processes.\textsuperscript{56}

1.6.5 Characterization of Single Molecule Kinetics

The sharp transitions observed in single molecule dynamics are characterized by changes in intensity that take place in less than one dwell time (integration time). A simulated blinking data set for a two level system is shown in Fig. 1.6:
The on and off events are associated with the time constants $\tau_{on}$ and $\tau_{off}$, respectively. Determining these values and how they vary with excitation intensity and other factors is the primary objective in analyzing such single molecule “blinking” trajectories.

There are three principal methods of analysis for these single molecule dynamics which extract varying amounts of information. The first method is autocorrelation analysis. The autocorrelation function (in this case, the second order intensity autocorrelation function) is a statistical measure of the degree to which the timing of events in a data set are correlated to the timing of other events in the data set, and can be defined as:

$$G(\tau) = \frac{\langle I(t)I(t + \tau) \rangle}{\langle I(t) \rangle^2}$$  \hspace{1cm} 1.11
where $I$ is the intensity at time $t$, and $\tau$ is the correlation time. The correlation function for a set of stochastic on-off events (such as blinking) corresponding to a Poisson process is described by:

$$G(\tau) = 1 - \exp\left(-\left(k_{\text{on}} + k_{\text{off}}\right) \cdot \tau\right)$$

1.12

where $k_{\text{on}}$ and $k_{\text{off}}$ are the rates for the on and off processes (equal to $1/\tau_{\text{on}}$ and $1/\tau_{\text{off}}$), respectively. The time constant for the “on” events, $\tau_{\text{on}}$, then is representative of the time spent in the “off” state, before an “on” event occurs (and vice versa). Since the two rate constants are combined into one (overall) rate constant in the autocorrelation function (Eq. 1.12), it is not possible to directly determine two different rate constants from the (second order) autocorrelation function. This can be demonstrated by simulating a blinking data set for which $k_{\text{off}} = 0.20$ and $k_{\text{on}} = 0.05$ (the rate constants used to generate Fig. 1.6). When an autocorrelation is performed, the two are combined into one overall rate constant equal to 0.25 (Fig. 1.7). However, higher order correlation analysis can in some cases be used to extract such data.\textsuperscript{59}
Another analysis technique is that of thresholding to determine the duration of individual “on” and “off” periods, followed by analysis of histograms of the durations. In this technique, a threshold intensity is chosen, and intensities above that value are considered “on,” and intensities below that value are considered “off.” The results of thresholding on a trajectory with multiple rate constants can be seen in Fig. 1.8
Figure 1.8: Example of analysis of blinking data by thresholding. An intensity is chosen that is between most of the “on” intensities and most of the “off” intensities (left). Analysis of the duration of the \( t_{\text{on}} \) (blue) and \( t_{\text{off}} \) (red) times yields two separate exponentially decaying histograms (right). Simulated time constants are \( \tau_{\text{on}} = 20 \) ms and \( \tau_{\text{off}} = 5 \) ms.

Thresholding is useful for determining multiple rate constants, but only when the intensity of the on and off states remain at similar intensity throughout the analysis time. This method can be readily adapted for use in multilevel systems.
2.1 Nanoparticle Preparation

Conjugated polymer nanoparticles (CPdots) used in these studies were prepared from poly[2-methoxy-5-(2'-ethyl-hexyloxy)-1,4-phenylene vinylene] (MEH-PPV, average MW 200,000, polydispersity 4.0) and poly[{9,9-dioctyl-2,7-divinylene-fluorenylene}-alt-co-{2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylene}] (PFPV, MW 270,000, polydispersity 2.7). These polymers were purchased from ADS Dyes, Inc. (Quebec, Canada). The solvents tetrahydrofuran (THF, anhydrous, 99.9%) and toluene (HPLC grade 99.7%) were purchased from Sigma-Aldrich (Milwaukee, WI). The polymer poly(2,5-dinonylparaphenylene ethynylene) (dn-PPE, MW 30,000, polydispersity 3.1)\(^{13}\) was obtained from Uwe H. F. Bunz (Georgia Institute of Technology, Atlanta, Georgia). All chemicals were used without further purification. Chemical structures are shown in Fig. 2.1.
Fig. 2.1: Conjugated polymers used in these experiments

The procedure for nanoparticle preparation is modified from methods developed by Kurokawa and co-workers for the preparation of organic nanocrystals. The method involves reprecipitation of conjugated polymer from a water miscible organic solvent in deionized water, with sonication.

A 1000 ppm solution of polymer was dissolved by stirring overnight in HPLC grade THF. The solution was then further diluted to a concentration of 20 ppm. A 2 mL quantity of the MEH-PPV/THF solution was added quickly to 8 mL of deionized water while the mixture was sonicated. The THF was removed by evaporation under vacuum overnight, followed by filtration through a 0.1 µm filter. The resulting suspensions were clear (not turbid). CPdots prepared are typically between 5-30 nm in diameter, depending on the concentration of the solution injected into deionized water, verified by atomic force microscopy (AFM). Since high concentrations favor second order kinetics (aggregation) and low concentrations favor first order kinetics (chain collapse), it is possible to tune CPdot size by altering the concentration of the polymer/THF injected into deionized water. The suspensions were found to be stable for weeks at a time, with no evidence of aggregation. The suspensions were also stable upon concentration by water evaporation. Complete removal of water resulted in a film that was insoluble in water, perhaps due to coalescence as observed in previous reports of films cast from aqueous suspensions of larger (0.1 micron) conjugated polymer particles.

Reprecipitation of conjugated polymer occurs because the hydrophobic interactions combined with sonication at low polymer concentration cause higher rates of
chain collapse (CPdot formation) than aggregation. Sonication increases mixing rates to increase the rate of chain collapse and low polymer concentration ensures that competition between the second order kinetics of aggregation between polymer chains and the first order kinetics of individual conjugated polymer molecule chain collapse, favor chain collapse.

2.2 CPdot Characterization Methods

2.2.1 Sample Preparation for AFM and Single Molecule Spectroscopy

Glass coverslips were used as substrates for AFM and single molecule spectroscopy (SMS) measurements, and were cleaned with KOH/isopropanol, then with HCl to reprotonate surface and remove salts, and finally rinsed with deionized water and dried. After that a $1 \times 10^{-5}$ M aminopropyl silane in ethanol solution was dropped onto the surface and left for 3 minutes then rinsed off with DI water and dried. This prepares the surface with amine groups that the CPdots can attach to without aggregation. CPdots at 10 ppm (2.5 ppm for SMS) are then dropped onto the surface and left for 30 minutes to attach to the amine sites on the glass surface. Excess CPdots solution is the rinsed off with DI water and surface left to dry under vacuum.

2.2.2 Atomic Force Microscopy

CPdot size and morphology were determined on an Ambios Q250 multimode atomic force microscope (AFM) in intermittent contact mode (also called ‘tapping’ mode). In intermittent contact mode, the AFM tip is driven near its resonant frequency by a piezoelectric element (typically 70 – 200 kHz), and its oscillation is dampened by
interaction with the surface as it nears the sample. AFM cantilever oscillation (and dampng) is measured by the reflection of a laser beam, reflected off of the back of the AFM cantilever. The oscillation changes the angle of reflection of the laser beam and therefore the position of the beam on the detector. In constant force mode (more commonly used than constant height mode), the dampng of the tip oscillation by the surface is maintained at a constant value by altering the Z position of the tip relative to the surface in a feedback loop. The tip is raster scanned in feedback mode across the sample and the changes in Z position needed to maintain constant dampng are correlated to X-Y position to reconstruct the height image.\textsuperscript{63, 64}

For isolated particles, lateral resolution (X-Y direction) is determined by scanning particles of a known size, resulting in an image in which the observed size of the particles is actually the convolution of the tip with the particle size; tips are usually smaller than 10 nm across. Vertical resolution (Z direction) is determined by measuring the oscillation of the tip without touching a surface (in accordance with procedures in the manual). The vertical axis standard deviation was measured as 0.75 Å. While there are techniques to improve lateral resolution in certain types of close packed samples, the fact remains that for isolated particles the Z resolution is often much higher than the X-Y resolution, and so particle height is frequently used as a measure of particle size; an accurate assumption if the particle is roughly spherical and is not easily deformed by tip force.\textsuperscript{63, 64} Scans on CPdots were performed on a 2 µm x 2µm scan area, with 0.5 Hz scan rate (per line), and 500 lines scan resolution.
2.2.3 Transmission Electron Microscopy

TEM samples were scanned with a Hitachi H-7600 microscope operated at 120 kV. Samples were prepared by dropcasting on copper grids followed by drying at room temperature.

2.2.4 UV-Vis and Fluorescence

A Shimadzu UV-2101PC scanning spectrophotometer was used to verify CPdot solution concentration and the locations of absorption peaks. Samples were diluted to absorbance values between 0.1 and 1.0 AU and were measured in a 1 cm quartz cuvette. Fluorescence measurements were done in a Quantamaster, PTI Inc. commercial fluorimeter. Excitation powers were measured with a calibrated power meter (Newport 818-SL) and slit widths and scan speeds were set as specified for the particular experiment. Solutions were diluted to an absorbance no higher than 0.1 AU and were measured in a magnetically stirred quartz cuvette. Photobleaching measurements were performed using the ‘timebased’ mode in the software at a set excitation and emission wavelength that were fixed during the course of the experiment. Photobleaching measurements were performed for 2 hours while other types of kinetics experiments were collected for as long as indicated in the data. Fluorescence spectral kinetics were collected by repeated scans at the same settings for the entire course of the experiment with the excitation power blocked during between successive scans to reduce photobleaching.
2.2.5 Fluorescence Lifetime Measurements

Fluorescence lifetimes of samples were measured by time correlated single photon counting (TCSPC; optics in Fig. 2.2). The home built system incorporates a mode-locked Ti:Sapphire laser (Coherent Mira 9000) focused through a frequency doubling crystal (BBO, beta barium borate, 100 µm, AR-coated, cut for type 1 SHG) to produce a 400 nm beam of ~100 fs pulses with 76 MHz repetition rate. The start pulse is collected from scattered laser light on a fast PIN diode (Thorlabs, DET210). Two dichroic mirrors filter out undoubled 800 nm light and a bandpass filter (Thorlabs 400 nm bandpass) eliminates any remaining 800 nm light before focusing on the sample. Incoming laser light is focused by a 75 mm focal length lens onto a magnetically stirred 1 cm quartz cuvette. Fluorescence from the sample is collected with a 75 mm focal length achromatic lens, filtered with three 500 nm longpass filters, and focused onto an avalanche photodiode (id Quantique Model id00-50, Ultra-low noise). All optics and beams after the 400 nm bandpass filter are within two dark boxes, there is a third box inside of the others to prevent scattered light from the laser beam from reaching the APD around the longpass filters.
Figure 2.2: TCSPC optics (top) and electronics (bottom) schematic.
Electronic pulses from the PIN diode are amplified with a GHz bandwidth amplifier to reach the threshold for detection at the constant fraction discriminator (CFD, Philips Model 715). Pulses from the APD are inverted and attenuated for introduction into the CFD. The CFD operates by first splitting an incoming pulse, then time shifting, inverting, and attenuating one of the pulses, and recombining them so that the results resemble the derivative of the original pulse. The CFD outputs a -1 V (NIM level) timing pulse when the resulting waveform crosses zero volts. The time where the resulting wave passes zero volts remains very constant, therefore the CFD effectively “cleans up” the input pulses, reducing timing jitter arising from changes in intensity or shape of the pulses. Start and stop output pulses from the CFDs are then fed into the time to amplitude converter (TAC, Canberra Model 2145). When the TAC receives the start pulse, it quickly ramps up voltage until it receives a stop pulse, thus generating an output voltage that is proportional to the time between the start and the stop pulses. The voltage from the TAC is recorded by a multichannel analyzer (MCA, Fastcomtec Model MCA-3 Series P7882 Philips Model 715) in a desktop computer, which records the voltage using a fast 16 bit DAC to produce a histogram of arrival times. The TAC employed can only count one photon per start pulse. This results in a nonlinearity at high count rates. Because of this limitation, the count rate is set to no greater than 1 / 1000 of the laser repetition rate (by adjusting the variable circular attenuator).

The result is a histogram of number of arriving pulses with a particular voltage versus bin number. Since voltage itself is not time, it is necessary to calibrate each bin to a specific time delay (between start and stop). This is done by determining the number of
bins between peaks on the histogram and knowing the time between pulses, which is 1 / 76 MHz or about 13 ns. This allows easy determination of the ns / bin conversion factor. The resulting data set is the exponential decay convolved with the instrument response function (IRF; signal broadening inherent to the instrument). To compensate for the IRF in the signal, a lifetime reading is taken using a non-fluorescent latex sphere sample before each fluorescent sample lifetime reading. The known IRF can then be convolved with a guess exponential with particular parameters (e.g. time constant, amplitude) to determine how well the convolved guess fits the original data. The set of parameters which produces an exponential curve that when convolved with the IRF best fits the original data, is the ‘best fit.’ A typical IRF for our instrument is has a FWHM of 60-80 ps.

Data treatment to obtain the fluorescence lifetime requires deconvolution of the IRF information from the lifetime information. A convolution is a calculation of the overlap of one function as another function is scanned over it, the result being a blending of the two functions. The convolution of two functions $f$ and $g$ is defined as:

$$f \otimes g = \int_{-\infty}^{\infty} f(\tau) \cdot g(t-\tau) d\tau$$ \hspace{1cm} (1.1)$$

where $f$ and $g$ are the IRF data and the guessed decay function, $t$ is time, and $\tau$ is the time delay between the two functions as the convolution is evaluated.

Due to numerical instabilities, it is typically not possible to directly deconvolve the signal to retrieve the underlying kinetics signal. Instead, the general method is to guess at the time scale for the lifetime decay exponential, convolve the guessed lifetime curve with
the IRF, the compare the result to the actual data, and obtain the squared error. A range of attempted lifetimes can then be attempted with the result with the lowest error assumed to be the best fit. This method can also be applied to biexponential or stretched exponential kinetics (both of these kinetics schemes are addressed in detail in Chapter 6).

2.3 Single Molecule Spectroscopy

2.3.1 Sample Scanning Confocal Microscopy

![Figure 2.3: Schematic of a scanning confocal fluorescence microscope with excitation by argon-ion laser.](image)

Single molecule studies were performed on a home built confocal sample scanning fluorescence microscope, seen in Fig. 2.3. There were two separate excitation sources; a continuous wave (non-pulsed) 6 mW argon ion laser (Ion Laser Technology,
Model 5490) and a 800 nm, mode-locked Ti:Sapphire laser (Coherent Mira 9000) operating at 76 MHz with ~100 fs pulse width. Each laser has a substantially different optical pathway to the sample, but collection optics for the fluorescence are nearly identical.

Excitation with the argon-ion laser requires separation of the several laser lines emitted from the laser head by means of an equilateral triangular prism which is reflected onto a movable mirror for convenient wavelength selection. The laser line of choice then reflects off of two alignment mirrors and into a fiber coupler, which uses a small, high quality aspherical lens to focus the beam into the fiber core (~3 µm diameter core). Fiber transmission has the advantage of not only conveniently moving the beam from one part of the optical setup to another, but also emitting a single mode beam. Fiber coupling and transmission losses are typically less than 50%. The output coupler is inside the microscope dark box. Precise alignment and focusing of the laser beam into the microscope objective (Olympus 1.25 NA, infinity corrected, 100x mag.) is achieved by adjustment of the micrometer screws on the output fiber coupler and the first mirror after it. For confocal experiments the excitation laser light is focused such that the beam is very gradually expanding and fills the rear focal plane of the microscope objective, and aligned such that it passes vertically through the center of the objective. A 500 nm Olympus longpass dichroic mirror is used both to reflect the laser light towards the objective and to filter the majority of scattered laser light out from the fluorescence emission.
The second laser source (Fig. 2.4) is a 800 nm, mode-locked Ti:Sapphire laser (Coherent Mira 9000) operating at 76 MHz with \( \sim 100 \) fs pulse width, which is suitable for both single fluorescence lifetime measurements and two-photon single molecule spectroscopy (as well as being a 400 nm light source for other applications after doubling). This beam is focused onto a doubling crystal (BBO, beta barium borate, 100 \( \mu m \), AR-coated, cut for type 1 single harmonic generation), which doubles the frequency to obtain 400 nm light. The light is then collimated and introduced into a pair of 150 mm PCX UV lenses for proper focusing into the microscope. The first lens focuses the beam at a focal length of 150 mm (since the incoming beam is nearly collimated) so that the second lens can properly expand the beam onto the back focal plane of the microscope.
objective. The dichroic mirror used for experiments with experiments involving 400 nm pulsed light is a 420 nm longpass mirror (Chroma 420 DCXR).

Two photon experiments were performed using the Ti-Sapphire mode-locked laser emitting at 800 nm. The optics leading to the objective are identical except for the removal of the doubling crystal and replacement of the dichroic mirror in the microscope with a 675 nm shortpass filter (Chroma 675 DCSP). Scattering filters after the sample were 700 nm shortpass filters from (Thorlabs).

The objective, dichroic mirror, and X-Y scanning stage are all situated in or attached to an Olympus IX-71 epifluorescence microscope. Fluorescence collection is performed through the same excitation objective in an epifluorescence geometry.

Additional laser filters may be used outside the microscope and are typically 500 nm longpass filters, though these filters may be removed when spectra are obtained with the CCD camera. One remaining lens outside of the microscope focuses the fluorescence emission either onto an avalanche photodiode (Perkin Elmer SPCM-AQR-13), or a spectrograph and CCD camera (Roper Scientific model 7433-0006), with source selection performed by a flipper (removable) mirror.

Scanning of the sample was performed by means of a nanometer accuracy X-Y translation stage (Princeton Instruments P-517.3CL Stage with E-501.00 stage 3-channel controller), controlled with a custom written program in the LabView programming environment (National Instruments). It was possible to scan with varying rates, resolutions, and areas as well as obtain both single molecule kinetics and single molecule spectra within the software. Analysis and plotting of confocal scans, kinetics
information, and spectra were performed with custom scripts and functions within the MATLAB (MathWorks) programming environment.

2.3.2 Near Field Scanning Optical Microscopy

The fluorescence spectra of thin film and solution samples were recorded using a deep cooled CCD spectrograph (488 nm excitation). Thin film samples were placed at an angle of approximately 45° with respect to the excitation beam. Additional fluorescence spectra were recorded on a commercial fluorimeter system and yielded similar results. TEM images of nanoparticles drop-cast onto metal grids were obtained. Thin films cast from THF were obtained by spin-coating the 0.1 weight percent solution onto cleaned microscope cover glass. Partial layers of nanoparticles for NSOM studies were prepared by drop-casting the aqueous suspension onto cleaned microscope cover glass and drying under a nitrogen flow. Near-field fluorescence and transmission microscopy of the nanoparticles was performed on a modified Aurora 2 NSOM microscope. Excitation was provided by the 488 nm line of an argon ion laser, coupled to the optical fiber probe. Near-field transmission and fluorescence light were collected with a 50X objective mounted underneath the sample and focused onto a single-photon-counting avalanche diode. For fluorescence images, the collected light was passed through a 550 nm long-pass filter to remove the excitation light.

2.4 Gelation Kinetics and Thermodynamics

The conjugated polymer poly(2,5-dinonyl paraphenylene ethynylene) (dn-PPE, Figure 1, left) was synthesized as reported previously. Gel permeation chromatography
yielded a molecular weight of ~30,000 Daltons (approximately 90 repeat units) and a polydispersity of 3.1. The polymer was dissolved in spectroscopic grade toluene to a concentration of 1 wt. % with gentle heating to speed dissolution.

Absorbance measurements were performed on a Shimadzu UV-2101PC on dilute solutions of the polymer (0.01 wt. % in toluene) using a 1 mm pathlength cell at room temperature and 1 nm resolution. Fluorescence spectra were recorded in a conventional fluorimeter (Quantamaster, Photon Technology International, Inc.) equipped with a temperature-controlled cell holder. Due to the high optical density of the 1 wt. % dn-PPE solutions, a front-face geometry was employed to reduce self-absorption (the inner-filter effect). The excitation light (372 nm) was incident at 45° to the front face of a triangular cuvette, and emission was collected from the same face. In this geometry, the effective path length is \(1/\varepsilon c\), where \(\varepsilon\) is molar absorptivity at the excitation wavelength and \(c\) is polymer concentration, which for the 1 wt. % dinonyl PPE solution at 372 nm excitation is approximately 10 microns. For relatively well-separated absorption and emission, the short effective path length minimizes the effect of self-absorption on the relative intensities of fluorescence features. Based on the effective path length and the extinction coefficient at the emission wavelength, we estimated that the inner-filter effect would reduce the primary fluorescence peak intensity by less than 10 % in the front-face geometry. In order to verify that self-absorption did not significantly distort the fluorescence emission spectrum, a spectrum acquired in the front-face geometry (above the gelation temperature) was compared to a spectrum of a highly dilute (0.01 wt. %)
solution acquired using the conventional 90° geometry. The results indicated that self-absorption had only a negligible effect on the relative peak intensities.

The gel → sol transition was monitored by fluorescence spectroscopy. Due to the slow kinetics of gellation, it is preferable to start with a sample initially in the gel phase (at low temperature) and record spectra as the temperature of the gel is slowly increased through the transition. A 1 wt. % solution of polymer was refrigerated overnight at 2°C in order to ensure relatively complete gelation. Fluorescence spectra were obtained at several temperatures over the range of 4-60°C. The sample temperature was allowed to stabilize for several minutes between readings in order to ensure thermal and chemical equilibration. In a separate experiment, aggregation kinetics were determined by using fluorescence spectroscopy to monitor changes in the concentration of the free (unaggregated) polymer after a sudden reduction in temperature. A 1 wt. % solution was maintained at 60°C for 30 minutes in the temperature-controlled cell in order to completely dissociate any aggregate species. The temperature was then set to 4°C, and after the temperature of the cell holder and sample stabilized at the set point (approx. 7 minutes), a series of spectra were obtained. The spectra were collected every 5 minutes for 60 minutes and then every 10 minutes for another 80 minutes.
In this chapter, we present a straightforward method for producing a stable, aqueous suspension of hydrophobic, fluorescent π-conjugated polymer nanoparticles consisting primarily of individual conjugated polymer molecules. Features of the method are the facile preparation, purity, unique optical properties, and small size (~5-10 nm) of the resulting nanoparticles. The results of TEM, scanning force microscopy, and near-field scanning optical microscopy of particles cast from the suspension indicate that the particles are single conjugated polymer molecules. The NSOM results yield estimates of the optical cross-sections of individual conjugated polymer molecules. The UV-vis absorption spectra of the nanoparticle suspensions indicate a reduction in conjugation length attributed to deformations of the polymer backbone. Fluorescence spectra of the aqueous nanoparticle suspensions indicate interactions between segments of the polymer chain and intramolecular energy transfer. Some of the results presented here were previously published. 67
3.1 Introduction

Fluorescent conjugated polymers have been demonstrated in a wide range of electrical device applications.\(^1\), \(^{11}\), \(^{68}\) There is considerable current interest in improving control of nanoscale composition in conjugated polymer blends to improve the efficiency of polymer LEDs and photovoltaics.\(^{69}\)\(^{-}\)\(^{71}\) A mini-emulsion method was employed to prepare submicron sized conjugated polymer particles (\(~0.1\ \mu\text{m}\) diameter), and device layers were fabricated from aqueous suspensions of the conjugated polymer particles as a strategy for obtaining improved control over composition and nanostructure in device films.\(^{62}\), \(^{72}\), \(^{73}\) Fluorescent nanoparticles are also of interest for biomolecule labeling and sensing owing to the high brightness and photostability of fluorescent nanoparticles as compared to conventional fluorescent dyes.\(^{74}\), \(^{75}\) Most fluorescent nanoparticles to date are based on inorganic semiconductors\(^{74}\), \(^{75}\) or dye-loaded beads.\(^{76}\) Here, we present a novel, facile method for preparing aqueous dispersions of hydrophobic, fluorescent conjugated polymer nanoparticles consisting primarily of single conjugated polymer molecules. Possible applications for these nanoparticles include nanocomposite electrooptical devices and fluorescent labels. Conjugated polymer nanoparticles possess several advantageous properties for fluorescence labeling applications such as a short excited state lifetime (which minimizes saturation effects), quantum yields approaching unity,\(^{77}\) and a large absorption cross-section per particle. Additionally, conjugated polymers are of particular interest for fluorescence-based sensing due to the high fluorescence quenching efficiency associated with multiple energy transfer between segments of the polymer chain.\(^{78}\)\(^{-}\)\(^{80}\) For example, sensitive solution-based fluorescent
DNA probes based on multiple energy transfer in ionic conjugated polymers have been demonstrated.\textsuperscript{79}

There is a complex relationship between molecular conformation and the optical and electrical properties of conjugated polymers. Conjugated polymer conformation determines the interactions between segments of polymer chains. These interactions give rise to interchain species such as dimers, excimers, and exciplexes. The effects of conjugated polymer conformation and interchain interactions on the optical properties of solutions and films have been probed using a variety of optical techniques.\textsuperscript{77, 81-88} Near-field scanning optical microscopy (NSOM) and single molecule spectroscopy have emerged as powerful tools for determining how structure governs optical properties in conjugated polymers and polymer blends.\textsuperscript{8, 19, 26, 55, 89-98}

The typical red shift in the absorption and fluorescence of the film compared to that in solution is attributed to interchain interactions and rapid energy transfer to a small number of red-shifted chromophores. The ability to probe the photophysics of molecules of conjugated polymers in aqueous suspension provides a unique point for comparison to studies of the optical properties of conjugated polymers in various organic solvents and in bulk films. For a dilute aqueous suspension of hydrophobic polymer molecules, the interaction between molecules is miniscule, but the interactions between different segments of a given polymer chain increase. The aqueous suspension thus provides a way to eliminate interchain effects and determine the optical properties associated with intrachain interactions in individual collapsed polymer chains.
Here we demonstrate for the first time a simple, general method for preparing a pure, surfactant-free, stable, aqueous dispersion of fluorescent, conjugated polymer nanoparticles, starting from commercially available device-grade conjugated polymers. The well-studied conjugated polymer poly[2-meth-oxy-5-((2-ethylhexyl)oxy)-p-phenylenevinylene] (MEH-PPV) was chosen for the experiments reported here. The method is based on addition of the conjugated polymer dissolved in an organic, water-miscible solvent to water, followed by rapid mixing. Preparation does not involve emulsion polymerization or surfactants and can be applied to a wide variety of conjugated polymers that are soluble in organic solvents. In addition, the nanoparticles produced are typically 5-10 nm in diameter, consistent with single polymer molecules and an order of magnitude smaller than particles produced by other methods. Fluorescence and absorbance spectra provide evidence of significant interactions between segments of the polymer chain, intraparticle energy transfer, and decreased mean conjugation length.
Figure 3.1: TEM image (a) of a dense island of nanoparticles. Correlated scanning force (b) and fluorescence NSOM (c) of a sparse nanoparticle film.

3.2 Results and Discussion

Conjugated polymer nanoparticles used for analysis were prepared from a 0.005 wt % MEH-PPV in THF stock solution, as described in detail in Chapter 2. TEM was used to determine particle size and morphology (Figure 3.1), as described in Chapter 2.
The drop-cast films formed large islands of roughly spherical particles with diameters ranging from a few nanometers to 30 nm. The islands were similar in overall appearance to results obtained by Landfester et al. for larger conjugated polymer particles. In the interior of the islands, the particles appear to coalesce to form an interconnected network. This is consistent with our observations that evaporated films are insoluble in water. Such high surface-area networks are considered ideal for polymer photovoltaic applications. Further characterization of well-separated particles on a glass substrate was performed using NSOM, an ultraresolution microscopy technique that combines the nanoscale resolution of scanning probe microscopy with the chemical specificity of optical spectroscopy (as described in Chapter 2). NSOM was employed to measure the size distribution of nanoparticles as well as for obtaining fluorescence and absorbance images of individual nanoparticles. The resulting images (Figures 3.1b,c and 3.2a,b) indicate the presence of primarily isolated nanoparticles. In contrast with the TEM results, few large islands were observed on the films drop-cast onto glass, likely due to differences in casting conditions and substrate.
Figure 3.2: (a) Topography and (b) transmission NSOM images of a nanoparticle film, 2 x 2 \( \mu \text{m} \) area. (c) NSOM absorbance versus particle diameter (circles), compared to the estimate based on known absorptivity of MEH-PPV in solution (line). The error bars represent photon counting noise.
Figure 3.3: (left) Normalized absorption spectra (solid line, THF; dotted line, water) and (right) fluorescence spectra comparing MEH-PPV suspended in water (solid line) and dissolved in THF (dotted line). The dashed fluorescence curve is the thin film fluorescence. The inset is a comparison of the fluorescence intensities of MEH-PPV dissolved in THF (solid line) versus nanoparticles suspended in water (dashed line), multiplied by a factor of 25.

Transmission NSOM images were analyzed to obtain a direct measure of the optical cross-section of individual conjugated polymer nanoparticles as a function of particle diameter (Figure 3.2c). On the basis of the UV-vis absorption of the solution and the average molecular weight of 200 000, the optical cross-section of an MEH-PPV molecule is approximately 4 nm$^2$. If we assume the NSOM probe acts as a simple aperture, the fraction of photons absorbed by a particle within the near field aperture is given by the ratio of the optical cross section to the illuminated area. The measured diameters (estimated from the particle heights) ranged from 5 to 30 nm with the majority between 5 and 10 nm. The measured absorbance NSOM versus particle diameter approximately
follows the predictions of the simple aperture model assuming an NSOM aperture diameter of 50 nm and assuming the optical cross-section scales linearly with particle volume. To our knowledge, these results represent the first application of NSOM to estimate the optical absorption cross-sections of individual molecules. The scatter in the results can be ascribed to particle aggregates, inhomogeneous photobleaching, uncertainties in the measured particle diameters (including deviations from the assumed spherical shape), and near-field effects. The small diameters (5-10 nm) are evidence that the majority of the nanoparticles consist of a single molecule of polymer with an average molecular weight of 200 000. The small fraction of larger particles observed may be due to aggregates or represent the tail of the molecular weight distribution of the polymer. It should be noted that though the size range of the nanoparticles is roughly consistent with single polymer molecules, it is not possible, given the available data, to determine whether a given nanoparticle is a single molecule or an aggregate of smaller molecules, due to the large polydispersity of the polymer sample.

In Figure 3.3, the UV-vis absorption spectrum of the aqueous suspension of MEH-PPV nanoparticles is compared to the spectrum of MEH-PPV dissolved in THF. The absorption peak of the polymer nanoparticles is slightly blue-shifted and the spectrum is broadened as compared to that of the polymer dissolved in THF. The observed blue shift of the absorption spectrum of the nanoparticles is consistent with an overall decrease in the conjugation length. Because the nanoparticles possess a compact, approximately spherical structure, the reduced conjugation length can be attributed to bending or kinking of the polymer backbone. The results of electronic
structure calculations suggest that PPV-type polymers are more flexible than is generally believed, with a low barrier to spontaneous formation of tetrahedral defects. Such defects exhibit a blue-shifted spectrum, consistent with our conclusions. In contrast, the absorption spectra of thin films (and aggregates) are typically red-shifted as compared to that of the solution due to reduced bending of the polymer backbone and increased interchain interactions. The nanoparticle absorption spectrum also exhibits a long red tail, indicating some red-shifted chromophores, likely due to intrachain interactions in the collapsed, folded polymer structure.

The conformation of stiff, rodlike polymers under various conditions is a subject of intense practical and fundamental interest. Theoretical results indicate that a wide variety of conformations can be obtained, depending on the rigidity of the backbone and other factors. Although the results presented here indicate a roughly spherical shape, this is likely a quenched, metastable conformation produced by the rapid mixing process. Further studies of the structure of the single conjugated polymer nanoparticles are ongoing.

In Figure 3.3, the fluorescence spectrum of the colloidal suspension of nanoparticles is compared to that of MEH-PPV dissolved in THF and that of the thin film. The fluorescence spectra of the thin film and the nanoparticles are quite similar, with a large Stokes shift. The large Stokes shift for films is attributed to energy transfer to low-energy chromophores and weakly fluorescent aggregates. The fluorescence yield of the aqueous suspension was also reduced as compared to that of the solution, in agreement with reports of decreased, red-shifted fluorescence of MEH-PPV in solvent
mixtures containing methanol, attributed to energy transfer to weakly fluorescent intrachain aggregates.\(^{88}\)

The absorption spectrum of the nanoparticles suggests that the fraction of intrachain aggregates is small, yet the majority of the fluorescence signal is attributed to intrachain aggregates. Therefore it is appropriate to consider whether energy transfer to intrachain aggregates is expected to be sufficiently efficient to partly account for the red-shifted fluorescence spectra. The measured exciton diffusion length of MEH-PPV is approximately 20 nm,\(^ {107}\) larger than the average particle size and sufficiently large that a small number of red-shifted aggregates per particle can act as energy acceptors for a large majority of chromophores. This conclusion is also supported by reports of efficient quenching of conjugated polymer fluorescence by a variety of quencher molecules\(^ {78-80}\) and polarons,\(^ {19, 20}\) and by reports of intermittent fluorescence in individual tightly coiled conjugated polymer molecules.\(^ {8, 26, 55, 96-98}\)

### 3.3 Conclusions

We have demonstrated a facile method for the preparation of an aqueous suspension of single conjugated polymer molecule nanoparticles. The particle diameters, determined by TEM and NSOM, and optical properties, determined by NSOM, UV-vis, and fluorescence spectroscopy, are evidence that the nanoparticles consist primarily of single molecules of conjugate polymer. The NSOM results also provide a measure of the optical cross-section of single conjugated polymer molecules. The spectroscopy of the suspended nanoparticles reveals a reduction in the mean conjugation length associated
with bending or kinking of the polymer backbone as well as energy transfer to intrachain aggregate states. Intrachain energy transfer is important for possible nanoparticle applications such as sensors based on fluorescence quenching.
Previously, it was determined that solutions of derivatives of the conjugated polymer poly phenylene-ethynylene form weak, thermoreversible gels. Here we present the results of investigations into aggregation and gel formation in solutions of the conjugated polymer dinonyl PPE using fluorescence spectroscopy. The results described in this chapter were obtained in collaboration with Yunfei Jiang, Uwe Bunz, and Dvora Perahia. As the temperature of a solution of the polymer is lowered below the gel transition temperature, there is a reduction in overall fluorescence intensity and the appearance of weak, red-shifted band characteristic of conjugated polymer aggregates. The temperature-dependence and dynamics of the aggregate spectral features were determined. The initial kinetics of the disappearance of the spectral features associated with the free polymer are consistent with a second-order process—a strong indication that the aggregate spectral features are associated with the formation of inter-chain aggregates. The thermodynamics and kinetics results are consistent with previous structural results indicating that gelation proceeds via the formation of ribbon-like or plate-like aggregates via $\pi$-stacking interactions. Over time, the aggregates grow in size until interactions between aggregates lead to arrest and the formation of a gel phase. The unique optical properties and nanoscale morphology of conjugated polymer gels are of possible interest for electro-optic device applications.
4.1 Introduction

Conjugated polymers possess electronic and optical properties similar to those of conventional semiconductors as well as the attractive materials properties of polymers such as flexibility.\(^1\) As molecular materials, their device properties (e.g. carrier mobility, luminescence efficiency, and luminescence wavelength) are determined by electronic interactions between polymer chains which in turn are determined by the nanoscale structure of polymer films.\(^9, 24, 84, 108\) For example, spectroscopic comparisons between solutions and the films cast from the solution, for a range of solvent conditions (solvent quality and concentration) have shown that aggregates and conformations present in solution persist in solution-cast films and affect optical properties and carrier mobilities.\(^17, 27, 94\)

The importance of solution structure in determining device properties has led to increased interest in the structure of conjugated polymers in solution and the various factors that determine conformation and aggregation in solution, such as the structure of the polymer backbone, the structure of side chains, polymer conformation, solvent interactions, and interactions between polymer chains. For semi-rigid polymers such as conjugated polymers, a good solvent leads to an open, extended structure, whereas a poor solvent leads to a more compact structure and possibly the formation of aggregates.\(^{109}\) Aggregate species observed in the fluorescence spectra of concentrated solutions of conjugated polymer are usually attributed to *inter*-chain aggregates.\(^3, 24\) However, in some cases, interactions between segments of a given polymer chain can result in the
formation of intra-chain aggregate species, as noted in single molecule spectroscopy experiments.\textsuperscript{25, 26}

Derivatives of the conjugated polymer poly phenylene ethynylene (PPE) have recently attracted interest for applications such as OLEDs\textsuperscript{4}, molecular wires\textsuperscript{110}, and plastic lasers.\textsuperscript{111, 112} The photophysics of aggregates and pristine and annealed films of alkyl PPEs have been probed using fluorescence spectroscopy\textsuperscript{7, 9, 10, 77} as well as time-resolved fluorescence spectroscopy and NSOM.\textsuperscript{7} While the solution phase fluorescence spectrum is typically characterized by a vibronic progression of well-separated peaks, films typically exhibit a broad, red-shifted, and featureless emission with one or more weak shoulders, and are characterized as excimer-like. Suspensions containing polymer aggregates were also observed to exhibit broad, red-shifted features.\textsuperscript{77}

It was previously determined by Perahia et al. that dilute solutions of the conjugated polymer poly(2,5-dinonylparaphenylenylene ethynylene) form a weak, thermoreversible gel.\textsuperscript{113, 114} The results of neutron scattering experiments on the gels indicated the formation of plate-like aggregates at \textasciitilde30°C which thicken as the temperature is reduced further, coincident with increased viscosity. The results indicate that the transition from molecular solution to gel occurs between over the range of 25-40°C for a 1 wt. % solution.\textsuperscript{113} NMR studies on 1 wt. % solutions indicated a monotonic decrease in polymer and solvent mobility as temperature is decreased over the range of 40-25°C, indicating increasing inter-chain contact between PPE molecules and increased confinement of the solvent, phenomena which are characteristic of gel transitions.\textsuperscript{114}
The morphology of bulk and thin film phases of a variety of derivatives of PPE\textsuperscript{115} has been investigated using a variety of techniques such as X-ray diffraction, scanning tunneling microscopy, atomic force microscopy, small angle neutron scattering, and calorimetry. These studies indicate that, in many cases, the structure of dialkyl PPEs in the bulk and in thin films is dominated by $\pi$-stacking interactions which lead to intermolecular separation distances of about 3.8 Å and the formation of sheets or ribbons which stack with a spacing determined by the length and geometry of the side chains.\textsuperscript{114, 116} Similar structural features have been observed in liquid crystalline phases of dialkyl PPEs.\textsuperscript{116} It should be noted that the concentrations employed in the present study are well below the concentrations required for the formation of a liquid crystalline phase. STM and AFM images of thin films indicate the presence of a variety of aligned ribbon-like or rod-like structural features.\textsuperscript{115, 117} In many cases, the periodicity of the various topographical features can be related to the bulk structure.

Here, we report the temperature-dependent fluorescence spectroscopy of poly 2,5-dinonylparaphenylene ethynylene (dinonyl PPE) in solution. The advantages of fluorescence methods for probing conjugated polymer gels include the ability to detect the presence of aggregate species, as well as the ability to obtain information about the kinetics of the gelation process. The dinonyl PPE gels are found to possess spectra unlike those of cast films or dilute solution. The differences in the spectra likely arise from differences in nanoscale organization for the gel as compared to the bulk. The kinetic and thermodynamic data obtained from fluorescence measurements are analyzed to yield information about the nature of the polymer association process and the gel structure.
4.2 Results and Discussion

The conjugated polymer dn-PPE was obtained from Uwe H. F. Bunz (Georgia Institute of Technology, Atlanta, Georgia) and was used without further purification. Absorbance measurements were performed on a 0.01 wt. % dn-PPE solution in toluene in a 1 mm pathlength quartz cuvette according to the procedure described in Chapter 2. In order to determine the spectroscopic properties of the dn-PPE thermoreversible gels, as well as the thermodynamics of gelation, a series of fluorescence spectra of a 1 wt. % solution of dn-PPE in toluene were acquired. Due to the high optical density of the gels, a “front face” geometry (in which the emission is collected from the same face as the incident excitation, at an angle of 45°) was employed in order to minimize self-absorption or the “inner-filter” effect. According to our estimates based on the optical density of the polymer at the absorption and emission wavelengths, self-absorption is negligible using this geometry. Excitation was performed at 372 nm and spectra were obtained from 380 nm to 550 nm.

The gel → sol transition was monitored for a 1 wt. % dn-PPE solution in toluene starting with a completely gel phase sample, cooled over night at 2°C. The fluorimeter was cooled to 4°C with slow dry nitrogen flow in the sample compartment before the experiment (to prevent condensation), with nitrogen flow maintained during the experiment. Fluorescence spectra were acquired for a range of temperatures starting with 4°C and increasing in 1°C steps up to 10°C and 2°C steps thereafter up to 60°C to ensure complete sol phase was reached, with several minutes before the scan at each step to
allow for temperature equilibration. Kinetics of the sol \(\rightarrow\) gel transition were determined in a separate experiment starting again with a 1 wt. \% dn-PPE solution in toluene warmed to 60ºC for 30 minutes in the fluorimeter. The thermostat in the fluorimeter was rapidly changed to 4ºC (in the space of several seconds) and the sample completely cooled in approximately 7 minutes, during which time the gelation process began. Data acquisition began \textit{after} the sample had reached thermal equilibrium (data in Fig. 4.3 does not include any cooling time data), ensuring that any observed trends were due to gelation processes and not temperature changes. Spectra were obtained out to 80 minutes. Further experimental details are given in Chapter 2.

The fluorescence spectra of 1 wt. \% solutions of dn-PPE acquired at temperatures below the transition temperature and above the transition temperature are markedly different, as shown in Figure 4.1. The spectrum of the free polymer in solution is characterized by a prominent feature at 425 nm and other, weaker vibronic features at 450 nm and 490 nm. The fluorescence spectrum of the gel phase also has a significant, though smaller 425 nm feature as well as a set of broad, overlapping features ranging from 460 nm to 525 nm that are difficult to resolve. Features similar to those observed in the spectrum of the gel phase have been reported previously for similar PPE derivatives and are described as indicative of the presence of aggregates.\textsuperscript{77} For a large number of conjugated systems, the appearance of a broad, red-shifted fluorescence is the signature of aggregate formation,\textsuperscript{17, 24, 86} however, in some cases, blue-shifted H aggregates are known to form.\textsuperscript{118} The fluorescence emission spectra of thin films of alkyl PPE derivatives\textsuperscript{7} are notably more highly red-shifted than the gels, most likely due to
differences in nanoscale organization of the polymer molecules. The 425 nm band is attributable to free, unaggregated polymer, and in the remainder of the discussion, this feature is designated as $F$. The series of features from 460 nm to 525 nm are ascribed to dimers or aggregates and are denoted by $D$.

Figure 4.1: Left: Structure of poly(2,5-dinonyl paraphenylene ethynylene). Right: Absorbance spectrum of 0.01 wt % polymer and emission spectra of aggregate and free polymer molecules. Right Inset: A comparison of 1 wt % polymer concentration in a first surface fluorescence cuvette (blue, left peak), and 0.01 wt % polymer concentration in a square 1 cm cuvette (red, right peak).
Figure 4.2: Aggregation dependence on temperature in the transition region (12°C to 34°C). Arrows show decreasing temperature and increasing aggregation. Inset: Result of a second order thermodynamic fit based on a van’t Hoff model. The original data (squares) and the fit (line) are shown.
Figure 4.2 illustrates the progression of the fluorescence spectra of the gel phase of 1 wt. % dinonyl PPE/toluene as the temperature is raised from 12°C to 34°C in steps of 2°C. As the temperature is raised, there is a reduction in the intensity of D and a concomitant increase in the intensity of F. Only slight changes in the relative intensities of the spectral features occur below 12°C and above 34°C, indicating no discernable change in the relative amounts of free and aggregated polymer outside this temperature range. The intensity of F is used as an approximate measure of the fraction of free polymer in solution. While in other systems such as J aggregates, there are discernable features indicating progressively larger aggregates, in the present case there is little evidence of distinguishable features that could be uniquely assigned to dimers, tri-mers, or other, larger species. Therefore no reliable estimate of the aggregate size distribution can be obtained from the fluorescence spectra. A plot of the fraction of free species as a function of temperature is shown in Figure 4.2 (inset). The F feature is persistent well below the aggregation temperature, which can be taken as evidence of the persistence of free molecules or segments. The nature of the persistent feature is discussed later.

The temperature dependence of the fraction of free polymer was analyzed as follows. Free molecule concentration $f$, was estimated from the intensity of the F feature using the equation,
\[ f = \frac{I - I_{\text{min}}}{I_{\text{max}} - I_{\text{min}}} \]  

where \( I \) is the intensity of \( F \) at a given temperature, \( I_{\text{min}} \) is the minimum measured \( F \) feature fluorescence intensity (at 4°C), and \( I_{\text{max}} \) is the maximum measured fluorescence intensity (at 34°C). This expression subtracts out contributions to the fluorescence signal from a small fraction of the polymer chains that do not appear to aggregate, even at temperatures well below the aggregation temperature. The equilibrium constant, \( K \) was calculated for a second order reaction \( 2M \rightarrow D \) where \( M \) is the free molecule (monomer) and \( D \) is the dimer. The equilibrium constant for dimer formation can be expressed as,

\[ K = \frac{1 - f}{2c_0 f^2} \]

where \( c_0 \) is the initial concentration of polymer molecules in the solution (3.15*10^{-4} \text{ mol L}^{-1}). It should be noted that this expression does not include the effects of larger aggregates associating with the free polymer.

The temperature-dependence of the equilibrium constant was fit to the equation,\textsuperscript{120}

\[ \ln K = -\Delta H_{vH} / RT + \Delta S_{vH} / R \]

where \( T \) is the temperature of the system, \( R \) is the universal gas constant, and \( \Delta H_{vH} \) and \( \Delta S_{vH} \) are the van’t Hoff enthalpy and entropy, respectively. The resulting fit is shown in Figure 2, inset, which yielded \( \Delta H_{vH} = -470 \text{ kJ mol}^{-1} \) and \( \Delta S_{vH} = -1.5 \text{ kJ mol}^{-1} \text{ K}^{-1} \). Previous DSC results indicated a calorimetric enthalpy (\( \Delta H_{\text{cal}} \)) of -630 kJ mol\(^{-1}\) for the
The ratio of the two numbers is related to the coordination number of the polymer in the gel phase, discussed below.

The kinetics of association provides additional insight into the association process. A molecular solution at 55°C was quickly cooled to 4°C, and fluorescence spectra of the supercooled solution were collected during aggregation and concomitant reduction in the amount of free polymer. The concentration of the free polymer is estimated using the expression \( c(t) = c_0 f(t)/f_0 \). The initial dynamics are consistent with a second-order rate law (Figure 4.3), suggesting that the rate-limiting step for aggregation is the association of free polymer chains to form dimers. Attempts to model the kinetics assuming a first-order rate law resulted in a poor fit to the data. A second order rate constant of \( k = 3.75 \text{ mol L}^{-1} \text{ s}^{-1} \) was determined from a fit to the data. Therefore the kinetics results are strong evidence that under these conditions, the red-shifted features in the fluorescence spectrum are due to two or more associated polymers (dimers and larger aggregates), and not due to the presence of intra-chain aggregate species associated with polymer collapse.

A wide range of aggregation and gelation processes follow Smoluchowski aggregation kinetics.\(^\text{121, 122}\) According to the model, the population dynamics are given by the expression,

\[
n_i(\tau) = \frac{\tau^{-1}}{(1 + \tau)^{i+1}}
\]

where, \( n_i \) is the fraction of aggregate species containing \( i \) molecules, \( \tau = \gamma_s t \) is the reduced time, and \( t \) is the time since the onset of aggregation. The Smoluchowski diffusion-limited collision rate constant is given by \( \gamma_s = 8\pi D_0 R_0 c_0 \varepsilon \), where \( D_0 \) is the free...
molecule diffusion constant, \( R_0 \) is the hydrodynamic radius, \( c_0 \) is the initial concentration, and \( \varepsilon \) is the probability of a collision resulting in irreversible binding. At early times, the Smoluchowski rate constant is related to the second order rate constant by the expression, 
\[ k = \frac{\gamma_S}{c_0}. \]
A fit the kinetics data to Equation 4.4 for \( i = 1 \) (corresponding to free polymer) yields \( \gamma_S = 2.9 \times 10^{-4} \, \text{s}^{-1} \) and \( k = 0.92 \, \text{L mol}^{-1} \, \text{s}^{-1} \). Agreement with the diffusion-limited aggregation model is slightly better than agreement with the dimerization model at long times, which is evidence that the diffusion-limited aggregation picture is qualitatively correct for this system. Assuming spherical particles and applying the Einstein-Stokes relation yields the expression \( \gamma_S = 4RTc_0\varepsilon / 3\eta \), from which an estimated sticking probability of \( \varepsilon = 1.2 \times 10^{-10} \) at 25 °C is obtained. Sticking coefficients below unity indicate the presence of effects which reduce the probability of successful reaction given a collision between two molecules. These can include effects related to the geometry of the reactant species and its effect on the diffusivity and effective radius of interaction, as well as additional geometrical constraints on the reaction related to the size and geometry of reactive sites.\(^{123}\) In addition, there is an activation barrier. Based on the geometry of the polymer molecules, it is likely that the geometric factor is in the range of \( 10^{-2} \) to 1, consistent with an activation barrier of 40-50 kJ/mol. While the nature of the activation barrier, if indeed there is an activation barrier, is not known, it is likely that steric hindrance related to the presence of the alkane side chains is involved.

Based on the thermodynamics and kinetics data, the following picture of the association process emerges. Key parameters in determining the structure of the aggregates are the coordination number and geometry of the associating species. The
association of conjugated polymers chains is typically dominated by $\pi-\pi$ interactions along either side of the polymer backbone, resulting in an effective coordination number of 2. This picture is supported by previous structural data on PPE derivatives in the solid phase which indicate interchain distances of $\sim4\ \text{Å}$. Geometric factors, such as the rigid rod structure of the polymer molecules, together with $\pi-\pi$ interactions on both sides of the polymer backbone, would likely lead to the formation of the ribbon or sheet structures previously observed in AFM and STM studies. Stacking of ribbons or sheets would result in the formation of thicker plates. The distance between sheets is determined by the packing geometry of the alkyl sidechains. Fully extended sidechains meeting end-on would lead to interchain distances along the direction perpendicular to the $\pi-\pi$ stacking axis of around 25 Å. However, previous structural data indicate a spacing closer to 18 Å, consistent with a chain angle of about 40º. As the plate-like aggregates grow in size and interact, arrest occurs, resulting in the formation of a weak, thermoreversible gel. The ratio of the enthalpy determined from DSC (which includes the enthalpy due to all polymer-polymer interactions) and the van’t Hoff enthalpy (which only includes the enthalpy of dimer formation) provides an estimate of the coordination number of the system. In this case, 631 kJ mol$^{-1}$/474 kJ mol$^{-1}$ gives a coordination number of 1.34. This indicates that the actual gelation enthalpy is somewhat less than what is expected from geometrical considerations and the enthalpy of dimer formation. This discrepancy can be attributed to steric effects that lead to anti-cooperative binding, i.e., the binding enthalpy of a second molecule to a given polymer molecule is lower than that of the first.
One puzzling observation from the fluorescence results is the persistence of a fraction of the feature associated with the free, unaggregated polymer at temperatures well below the gelation temperature. Here we consider possible explanations for the persistent feature. First, there is some possibility that fully aggregated polymer chains (i.e., in the absence of solvent) exhibit substantial fluorescence at the wavelength of $F$, either due to chemical defects or conformations that break the conjugation, giving rise to oligomer-like spectral features similar to those of the free polymer. However, it is expected that efficient energy transfer would largely extinguish such features in the fluorescence spectrum of fully aggregated polymer chains. Indeed, fluorescence spectra of thin films of dn-PPE (after solvent removal) indicate that $F$ is completely extinguished, suggesting that the persistent feature is associated with free or nearly-free polymer. Another possible explanation for the persistence of the $F$ feature is that the aggregates could have dangling chain segments that are completely solvated and possess the spectral characteristics of free polymer chains. Finally, there is the possibility of the presence of some free oligomers in the solution. Oligomers of sufficient length would exhibit a fluorescence spectrum similar to that of the free polymer and would also have a much lower enthalpy of association. Such oligomers would remain free over the temperature range of the experiment and only form aggregates at much lower temperatures or when the solvent is removed, such as for cast films. There is also a recent report of a persistent free-molecule-like feature in highly ordered films of a dodecyl PPE derivative that is ascribed to the presence of an ordered phase that minimizes contact between polymer backbones. However, this result is not typical of other alkyl PPE derivatives. We
therefore conclude that either dangling chain segments or oligomers are present in the gel phase, giving rise to a persistent free molecule-like feature in the fluorescence spectrum of the gel phase. Additional experiments are required to determine whether the persistent feature is due to dangling chain segments or oligomers.

### 4.3 Conclusions

Dilute solutions (1 wt. %, in toluene) of the conjugated polymer dinonyl PPE are observed to form a weak, thermoreversible gel phase at temperatures below about 12 °C. Fluorescence spectra of the gel phase indicate the presence interchain interactions consistent with the formation of aggregate species. The fluorescence spectra of the gel phase differ substantially from those of both the dilute solution and the thin film, which possibly indicates that the nanoscale structure of the gel phase is rather different from those of the thin film and free molecule in solution. The temperature-dependence of the fluorescence spectra obtained during the gel→sol transition were analyzed to yield thermodynamic parameters describing the aggregation and dissolution processes. The kinetics of aggregation were determined to be consistent with a second-order polymer association process as early times and diffusion-limited aggregation at later times. The kinetics and thermodynamics of aggregation are consistent with previous results indicating the presence of nanoscale plate-like aggregates in the gel phase. There are a number of possible device applications for conjugated polymer gels, particularly applications for which the highly porous, nanoscale interconnected networks that are characteristic of the gel phase could be of advantage, such as for improved charge
separation efficiency in photovoltaic cells, for improved mobility and energy transfer in light-emitting devices, or improvements in the efficiency of light-emitting electrochemical cells.

Finally, it is worth noting that many conjugated polymers of interest for device applications are similar to the dinonyl PPE in that they consist of a non-polar, pi-conjugated backbone and possess alkyl side chains. It is likely that, under some solution conditions, other conjugated polymers such as polyphenylenevinylene and polyfluorene derivatives could also form gel phases. There is also the possibility that in some cases, transient gel phases of conjugated polymers occur during the film casting and drying process. Such transient gel phases, if they occur, could have a large impact on the resulting film morphology as well as electrical and optical properties.
5.1 Introduction

In this chapter, we will discuss evaluation of conjugated polymer nanoparticles as multiphoton fluorescence labels. These results have been published. The results are also the result of a collaboration involving Zachary Cain and Changfeng Wu. Multiphoton fluorescence microscopy has recently emerged as a powerful technique for three-dimensional imaging in biological systems. The nonlinear dependence of excitation probability on light intensity results in a highly localized excitation and improved spatial resolution. The small effective excitation volume also reduces background signal due to autofluorescence and the fluorescence of dye molecules outside of the laser focal volume, while the ability to employ near-IR wavelengths for excitation can reduce photodamage to the sample as well as facilitate imaging of biological specimens due to the near-transparency of many tissues in this spectral range. The widespread adoption of multiphoton fluorescence imaging and microscopy has been hindered by the bulky and expensive pulsed laser sources typically required for excitation due to the relatively low multiphoton excitation cross sections of available dyes. Interest in the development of brighter probes has led to the design and synthesis of dyes with two-photon action cross sections larger than 1000 Göppert-Mayer units (GM).
Gold nanorods have also been demonstrated as contrast agent for in vitro and in vivo two-photon luminescence imaging, and the two-photon action cross sections were determined to be ~2000 GM.\textsuperscript{128} Colloidal CdSe quantum dots appear to be very promising probes for two-photon microscopy due to their excellent photostability and large two-photon action cross sections (from 2000 to 47 000 GM),\textsuperscript{129} although single particle blinking and a significant fraction of “dark” nanoparticles are drawbacks for some applications.\textsuperscript{130}

Conjugated polymers are known to possess high absorption coefficients and high fluorescence efficiency, which have led to a wide range of applications in optoelectronic thin film devices.\textsuperscript{68, 131} The extraordinary light-gathering power of conjugated polymers is evidenced by the first reported direct determination of the optical absorption cross section of a single molecule at room temperature, in which an molar absorptivity of the conjugated polymer MEH-PPV in the vicinity of $10^7 \text{ M}^{-1} \text{ cm}^{-1}$ was determined.\textsuperscript{67} We have recently developed and characterized highly fluorescent nanoparticles consisting of one or more hydrophobic conjugated polymers.\textsuperscript{132, 133} These “CPdot” nanoparticles represent a new class of highly fluorescent probes with potential applications for biosensing and imaging. In this chapter, we determine the two-photon excited fluorescence properties of CPdots and explore their application for multiphoton fluorescence microscopy. Our measurements of the nonlinear optical properties of the CPdots indicate extraordinarily high two-photon action cross sections in one case as high as $2.0 \times 10^5 \text{ GM}$, the highest reported to date for a particle of comparable size. Detection of two-photon excited fluorescence of single nanoparticles was demonstrated using
relatively low laser power, demonstrating the great potential of these CPdots for multiphoton fluorescence imaging applications.

5.2 Results and Discussion

Figure 5.1: (a) Chemical structures of the conjugated polymers. (b) Photograph of the fluorescence from aqueous CPdot dispersions under two-photon excitation of an 800 nm mode-locked Ti:sapphire laser. (c) A typical AFM images of the PFPV dots on a silicon substrate. (d) Semilog plot of two-photon action cross sections ($\sigma_{2p}$) versus the excitation wavelength for CPdots and rhodamine B (reference compound).

The conjugated polymers employed in this study (Figure 5.1a) are the polyfluorene derivative poly(9,9-dihexylfluorenyl-2,7-diyl) (PDHF, average MW 55 000), the copolymer poly[{9,9-dioctyl-2,7-divinylenefluorenylene}-alt-co-{2-methoxy-5-(2-
ethylhexyloxy)-1,4-phenylene} (PFPV, average MW 270 000), and the polyphenylene-vinylene derivative poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene] (MEH-PPV, average MW 200 000). The polymers were purchased from ADS Dyes, Inc. and used without further purification. Stable, aqueous dispersions of CPdots were prepared using a reprecipitation method described previously.¹³²,¹³³ The nanoparticle size can be controlled over the range of 5-50 nm by varying the concentration of the precursor solution, with two such size distributions shown in Fig. 5.2. Unlike inorganic semiconductor nanoparticles, the particle size does not affect the shape of the absorption and fluorescence spectra of CPdots. UV-Vis absorption spectra and fluorescence spectra of particles prepared with different diameters (10 nm and 20 nm) are nearly identical (Figure 5.3), indicating that particle diameter has a negligible impact on the spectroscopic properties of the nanoparticles. Instead, an increase in particle size largely results in an increase in the optical cross section per particle.¹³²,¹³³
Figure 5.2: (a) A typical AFM image of PFPV dots prepared from 10 ppm precursor solution. Histogram of the particle height is shown in the bottom. (b) A typical AFM image of PFPV dots prepared from 20 ppm precursor solution. Histogram of the particle height is shown below each image.
The CPdots exhibit one-photon absorption in the wavelength range of 350-500 nm. However, when the output of a mode-locked Ti:sapphire laser (100 mW, 800 nm, 100 fs) is focused into aqueous dispersions of PFPV and MEH-PPV dots (8 ppm, 3 nM), strong fluorescence in the vicinity of the focus is clearly visible (Figure 5.1b). Power-dependent excitation efficiencies provide further evidence for the two-photon excited fluorescence. At a given laser intensity, \( I \), two-photon fluorescence intensity is proportional to \( \sigma_{2p} \phi I^2 \), where \( \sigma_{2p} \) is the two-photon absorption cross section and \( \phi \) is the fluorescence quantum yield. A convenient measure of the two-photon fluorescence brightness is the two-photon action cross section, \( \sigma_{2p} \phi \).\(^{36, 129}\) A custom-built photon-counting spectrometer was used to determine the two-photon action cross sections as a function of excitation wavelength. The measurements were performed on CPdots of ~20 nm diameter (Figure 5.1c), consisting of PDHF, PFPV, and MEH-PPV, respectively.
The experimentally determined two-photon action cross sections \((\lambda_{\text{ex}} = 770 \text{ nm})\) are \(1.4 \times 10^4\), \(5.5 \times 10^4\), and \(2.0 \times 10^5\) GM for PDHF, MEH-PPV, and PFPV dots, respectively (Figure 5.1d).

Two-photon action cross-sections were obtained from the fluorescence data as follows. The time-averaged detected fluorescence photon flux \(F(t)\) can be expressed as

\[
\langle F(t) \rangle \approx \frac{1}{2} \eta C \sigma^*_2 \frac{g_p}{f \tau} \frac{8n \langle P(t) \rangle^2}{\pi \lambda}
\]

where \(\eta\) is the fluorescence collection efficiency of the instrument; \(C\) is the fluorophore concentration; \(\sigma^*_2\) is two-photon action cross section of the probe; \(g_p\) is a unitless factor related to pulse shape (0.66 for Gaussian laser pulse); \(f\) is the laser repetition rate; \(\tau\) is the width (FWHM) of the laser pulse; \(n\) is the refractive index of the lens focusing the laser beam; \(\lambda\) is the laser wavelength; and \(\langle P(t) \rangle\) is the average power of the laser.\(^{126}\) The factor \(g_p/(f \tau)\) is approximately \(10^5\) for a Ti:sapphire laser with a 76 MHz repetition rate and 100 fs pulses. Only \(C\) and \(\sigma^*_2\) are related to the sample, and all other parameters are constants, determined by the measurement system. Varying the laser power \(\langle P(t) \rangle\) and recording the fluorescence intensity \(\langle P(t) \rangle\) yields a quadratic dependence of fluorescence intensity on laser power. Plotting \(\ln \langle F(t) \rangle\) vs \(\ln \langle P(t) \rangle\) results in a straight line with slope of 2 and an intercept \(b\), given by the expression,

\[
b = \ln \left( \frac{1}{2} \eta C \sigma^*_2 \frac{g_p}{f \tau} \frac{8n}{\pi \lambda} \right)
\]
Provided that a standard dye with known two-photon action cross-section is available, a relative determination of the two-photon action cross section of the species of interest is given by the expression,

\[
\sigma_{2p}^* = \sigma_{2p,0}^* \frac{C_0}{C} \exp(b - b_0)
\]

where \(b\) and \(b_0\) are obtained from log-log plots of laser intensity versus fluorescence intensity for the fluorophore of interest and the standard, respectively, and \(\sigma_{2p,0}^*\) is the two-photon action cross-section of the standard. The above method was validated by determining the two photon action spectrum of rhodamine B using Lucifer yellow as a standard. The determined two photon action spectrum for rhodamine B is consistent with literature results for the absolute two-photon action cross-section.\(^{126}\)

Figure 5.4 shows the log-log plot of the fluorescence intensities of the CPdots and rhodamine B solutions vs. the laser power at 800 nm wavelength. Fits to the experimental data yield a slope of 2.0±0.1, consistent with two-photon excited fluorescence. The sample concentrations were determined by UV-Vis spectrometry. The molar extinction coefficient used in determining the nanoparticle concentration is estimated using a previously described method based on AFM particle size measurements.\(^{67,132}\)
Figure 5.4: Two-photon fluorescence intensities vs. the excitation power for the rhodamine B and the CPdots. The sample concentrations determined from UV-Vis absorption are rhodamine (1.00 µM), PFPV (1.48 nM), MEH-PPV (1.54 nM), and PDHF (1.51 nM), respectively.

The two-photon action cross section for MEH-PPV dots is about 1 order of magnitude larger than that of the molecular solution, consistent with the particle size results that indicate 10-20 molecules per particle. Significantly, the results show that, as two-photon fluorescent probes, the PFPV dots are 3-4 orders of magnitude brighter than conventional fluorescent dyes and an order of magnitude brighter than quantum dots. It is somewhat surprising that PFPV dots were determined to have the highest brightness of the three polymers, given that under one photon excitation PDHF dots are brighter than both PFPV and MEH-PPV dots. However, the higher two-photon cross sections of PFPV and MEH-PPV are consistent with theoretical and experimental results.
indicating that π-conjugated systems with alternating donor-π-donor structures exhibit relatively large two-photon absorption cross sections. The alkoxy side groups in PFPV and MEH-PPV act as electron donors, forming the donor-π-donor motif associated with relatively high two-photon cross sections, while PDHF does not possess alkoxy side groups.

Figure 5.5: (a) A 5 µm x 5 µm fluorescence image of single PFPV dots immobilized on a glass coverslip obtained using two-photon excitation (800 nm). (b) Photobleaching kinetics of single PFPV dots under two-photon excitation. No obvious blinking was observed for ~20 nm PFPV dots, while it is often observed for smaller particles (<10 nm).

To demonstrate the potential of the CPdots for multiphoton fluorescence imaging, single particles on a glass substrate were imaged using a custom-built confocal fluorescence microscope employing the attenuated output of a mode-locked Ti:sapphire laser (800 nm, 100 fs) for excitation. Figure 5.5a shows a 5 µm x 5 µm fluorescence image of the PFPV dots sparsely dispersed on a glass coverslip. Each bright spot in Fig. 5.5a corresponds to a single PFPV dot. The high per-particle brightness is evident in the relatively low average laser power (260 µW, at the sample) employed to obtain high
contrast images. Typically, pulsed laser light is required to generate sufficient two-photon fluorescence signal for single fluorophore detection. However, on the basis of these results, we estimate it should be possible to obtain two-photon fluorescence images of single CPdot nanoparticles using ~10 mW CW laser illumination provided by an inexpensive semiconductor diode laser. Indeed, focusing several tens of milliwatts of 800 nm CW laser light (Ti:sapphire laser operating in CW mode) onto a single layer of nanoparticles generated fluorescence that was readily visible to the unaided eye.

Single conjugated polymer molecules typically exhibit complex photophysics such as fluorescence intermittence (blinking) and photon antibunching. Single particle fluorescence kinetics traces (Figure 5.5b) indicated no observable blinking for 20 nm PFPV dots, while it was often observed in smaller particles (<10 nm), consistent with a recent single particle results. As fluorescent probes for imaging or single particle tracking, the relatively steady fluorescence of CPdots compares favorably to that of quantum dots, which typically exhibit pronounced blinking on time scales of milliseconds to hundreds of seconds. Analyses of single particle kinetics traces indicate that approximately ~10^6 photons per particle (~10 nm diameter) were detected prior to photobleaching. This is lower than the photostability under one-photon excitation (~10^7 photons detected), consistent with prior observations that single fluorophores exhibit lower photostability under two-photon excitation than under one-photon excitation. Silica encapsulation would likely improve photostability.
5.3 Conclusions

In summary, we report on a new class of two-photon fluorescent nanoparticles, CPdots, which exhibit the largest two-photon action cross sections reported to date for particles of comparable size. Demonstration of single particle imaging using relatively low laser excitation levels demonstrates the potential utility of CPdots for multiphoton fluorescence microscopy applications and raises the possibility of employing small, inexpensive near-infrared diode lasers for two-photon excited fluorescence imaging.
CHAPTER 6
EXCITON DYNAMICS IN CONJUGATED POLYMERS

6.1 Introduction

Conjugated polymers (CPs) have long been investigated for electronic and
electro-optic device applications such as polymer based light emitting devices,\textsuperscript{2-5} photovoltaics,\textsuperscript{139-141} and transistors.\textsuperscript{68, 142, 143} Due to their high density of \( \pi \) electrons, CPs
typically have high absorptivities in the UV-Vis region. Also, CPs employed in polymer-
based light-emitting display applications often have relatively high fluorescence quantum
yields, since fluorescence quantum yield typically correlates with efficiency of the
efficiency of such devices. In the McNeill laboratory, we have proposed a rather
different application - that CPs could serve as the primary component and active material
in nanoparticle-based fluorescent tags for biological imaging and sensing.\textsuperscript{67, 124, 144, 145}
Due to the high absorptivities and high fluorescence quantum yields of CPs,
nanoparticles composed of these polymers (we shall refer to them as “CPdots”) exhibit
extraordinarily high fluorescence brightness, which makes them potentially well suited
for use as fluorescent tags or labels in photon-starved applications such as fluorescence-
based imaging, ultrasensitive detection of pathogens, and single molecule tracking.\textsuperscript{144}
One major concern in the development of CPdots as fluorescent labels thus far has been
their relatively poor photostability\textsuperscript{144} compared to competing technologies such as
quantum dots.\textsuperscript{146} However, the brightness and photostability of CPdots are both typically
much better than those of conventional dyes, and are typically sufficient for a wide variety of proposed imaging and sensing applications.

Improvements to the brightness and longevity of CPdots largely depend on better understanding the relevant processes. Of particular interest are the interactions of the fluorescent excited state (often referred to as a molecular exciton, or, equivalently, a Frenkel exciton - see Chapter 1 for a more detailed discussion) with one or more photogenerated “quencher” species. Such interactions are likely to be important due to possibility that a single quencher can, in some cases, have a quenching radius of several nanometers, due to long-range energy transfer processes (e.g., Förster Transfer). Thus, a small density of quenchers can have a large effect on the fluorescence quantum yield. A possible photogenerated quencher species is the radical cation of the conjugated polymer, which, in organic semiconductor terminology, is typically referred to as a (hole) polaron.¹⁷, ¹⁴⁷ (See Chapter 1 for a more detailed discussion of polarons and polaron-exciton collisions). Polarons, which are the primary charge carriers in organic semiconductors, can be produced by a variety of processes, such as oxidation, exciton dissociation and photoinduced electron or proton transfer. Hole polarons are likely to be effective fluorescence quenchers due to their (typically) red-shifted absorption spectra and low fluorescence quantum yields. Based on this proposed picture, rational approach to improving CPdot properties will involve understanding the processes that can limit or reduce fluorescence (e.g., quenching by hole polarons) and evaluating possible approaches to reduce the rate of unfavorable processes relative to the rate of fluorescence (e.g., by reducing quenching efficiency and suppressing the quencher population).
This chapter focuses on presenting results and analysis of a series of experiments aimed at providing additional information about the nature of the various species involved in the photobleaching process, the various processes associated with the various species, and the rate constants associated with such processes. Results from bulk photobleaching and picosecond fluorescence lifetime measurements are presented here that exhibit complex photobleaching kinetics, “reversible” photobleaching (fluorescence recovery after photobleaching), complex picosecond exciton decay kinetics, and charge transfer phenomena involving redox-active species in solution. While this chapter is largely concerned with describing and quantifying various phenomena observed in experiments, a more comprehensive model is described in the following chapter.

6.2 Reversible Photobleaching (Quenching) of CP Dots

In this section, we will discuss photobleaching kinetics and “reversible photobleaching” phenomena occurring in conjugated polymer nanoparticles. Here we define photobleaching as referring to all processes that lead to a decrease in the fluorescence of a material. For small fluorescent dye molecules, this includes a variety of photoinduced chemical reactions such as excited state charge transfer or proton transfer (sometimes resulting in conversion of the fluorophore into a nonfluorescent species), reaction of carbon-carbon double bonds with molecular oxygen, and many other chemical reactions.\(^\text{148}\) In conjugated polymers, the situation is more complex due to the nanoscale interactions between the large number of chromophores that make up a polymer chain as well as the fact that the conjugated polymer is an organic semiconductor, and therefore a
variety of photogenerated species such as polarons can annihilate or quench excitons, which are the fluorescent excited state. Indeed, complex dynamics have been observed in the fluorescence of single conjugated polymer molecules, as reported in the literature.\textsuperscript{8, 25, 149} In the McNeill lab, we have also observed complex fluorescence kinetics in single conjugated polymer nanoparticles, as discussed here (see Chapter 8) and in prior publications.\textsuperscript{124}

Photobleaching kinetics were recorded in a aqueous solution of 2.66 ppm poly[2-methoxy-5-(2'‐ethyl-hexyloxy)-1,4-phenylene vinylene] (MEH-PPV) CPdots in a conventional fluorimeter under constant excitation power at 488 nm, signal was collected at the MEH-PPV emission peak at 589 nm, and the emission intensity was recorded at regular intervals over the course of the experiment. For experiments in which the excitation was varied over the course of the experiment, dimming of excitation power was performed either by insertion of a neutral density filter (usually with an optical density of 1, blocking 90% of incoming light), adjustment of the slit width on the excitation side, or closing the excitation side shutter completely. The excitation powers employed varied by experiment and are noted for each experiment, typically in the captions for each figure. Exposure times were typically one minute before reducing (attenuating or blocking) excitation power, but exact time scales vary somewhat and are indicated (along with their specific excitation powers) on their respective figures.

Several phenomena were observed over the course of these experiments. Photobleaching experiments showed complex kinetics, which can be described as
stretched exponential kinetics (shown in Fig. 6.1 and described in detail later), similar to the photobleaching kinetics widely reported in thin films of conjugated polymers.$^{150-153}$

Figure 6.1: Non-single exponential photobleaching and single exponential fluorescence recovery in a bulk MEH-PPV CPdot sample. When the intensity is reduced from 400 $\mu$W to 43 $\mu$W then increased again, the fluorescence recovers significantly. The recovery can be seen clearly on the magnified portion of the data.

The observed kinetics are substantially different from the first order, single exponential kinetics that typically result from photodestruction of the fluorophore via a low quantum yield process.$^{154}$ Another observed phenomenon is that, upon reduction of the excitation intensity after photobleaching for some period of time, partial or full recovery of the fluorescence intensity was observed, depending on conditions, as shown in Fig. 6.1. Here, this phenomenon is referred to as reversible photobleaching. Another phenomenon that is observed is an extraordinary sensitivity of the photobleaching rate to
the partial pressure of oxygen. Initial analysis of these phenomena proceeded by attempting to fit the observed kinetics to a variety of phenomenological rate models and determination of the relative quality of fit.

6.2.1 Rate models

Initial analysis of the complex photobleaching kinetics proceeded by evaluation of the quality of numerical fits obtained using a several phenomenological rate models. Here, a description of the various rate models is provided. In many cases, molecules exhibit photobleaching dynamics that follow simple first-order (single exponential) kinetics. Single exponential kinetics (Eq. 6.1) is the result of the spontaneous decay of a population of species; classic first order chemical reactions follow this trend. For many fluorescent species such as small fluorescent molecules, single exponential decay of fluorescence is observed as each molecule eventually undergoes a photochemical reaction that results in loss of fluorescence, either through reduction in the extinction coefficient, a substantial shift in the extinction spectrum, a substantial shift of the fluorescence spectrum, or a reduction of quantum yield. The mathematical function that describes the exponential kinetic decay of a population, \( n(t) \), is,

\[
n(t) = n_0 e^{-t/\tau},
\]

where \( n_0 \) is the initial population, \( t \) is the experiment time, and \( \tau \) is the time constant.

Biexponential kinetics (Eq. 6.1) are observed when two distinct subpopulations undergo decay, each with its own time constant, to generate the observed kinetics. (A related kinetics picture is the exponential rise-and-decay picture, which involves
exponential decay from a “dark” state into the state of interest, followed by decay to a third state such as the ground state.) The function that describes biexponential kinetics is,

\[ n(t) = n_{0,A}e^{-t/\tau_A} + n_{0,B}e^{-t/\tau_B}, \]

where \( n_{0,A}, n_{0,B} \) are the initial subpopulations, \( t \) is the experiment time, and \( \tau_A \) and \( \tau_B \) are the time constants associated with each subpopulation. In principal, the above expression is readily modified to account additional subpopulations by simply adding more exponential decay terms. However, in practice, three or more exponential decay terms are not commonly used in fitting experimental kinetics results, as the uncertainty associated with each fit parameter generally increases rapidly with the number of parameters.\(^{155}\)

A less well known phenomenological kinetics model is the stretched exponential (Eq. 6.3) model (also called Kohlrausch-Williams-Watts or KWW). The KWW model is an empirical model that successfully describes the relaxation processes of a wide variety of systems such as discharge from a capacitor,\(^{156}\) dielectric relaxation in polymers, and complex luminescence decay.\(^{150}\) KWW kinetics is often observed when a system has a broad range of environments, each giving rise to different decay or relaxation characteristics, resulting in an overlap of many decay rates.\(^{150, 157}\) The functional form for stretched exponential kinetics is,

\[ n(t) = n_0e^{-(t/\tau)^\beta}, \]

which is similar to a single exponential function with the addition of a stretching parameter, \( \beta \), which can have values ranging from 0 to 1. The stretching parameter can be considered as representative of the width of the distribution of the rates for the decay
of the observed populations; if the distribution of rates is narrow, the $\beta$ value is close to 1 (yielding kinetics similar to single-exponential kinetics), whereas a wide distribution of rates results in a much lower $\beta$ value (as low as 0.2 for some of the results presented below).

In some cases, complex kinetics are well-described by a power law. Power law behavior is observed in an astonishingly wide array of physical phenomena. The functional form for power law kinetics is given by,

$$n(t) = At^k + \varepsilon,$$

where $A$ is a scaling factor, $k$ is the scaling exponent, and $\varepsilon$ is the deviation term, which is the value the function asymptotically approaches at long times. The resulting scaling exponent is in some cases related to the “order” of the chemical reaction mechanism associated with the rate-determining step. In many cases, power law and stretched exponential kinetics strongly resemble each other since they both represent a range of processes occurring at different rates. The data in Fig. 6.2 were fit to the several exponential functions and the power law function and their sample variance ($\sigma_n^2$) calculated as indicated in Eq. 6.5:

$$\sigma_n^2 = \frac{\sum_{i=1}^{N} (y_i - f_i)^2}{N},$$

where $y_i$ is the $i$th data point, $f_i$ is the corresponding $i$th fit point, and $N$ is the total number of data points.
Figure 6.2: Bleaching curve for MEH-PPV CP dots (blue, solid) with exponential (green, dotted), biexponential (red, dot-dash), stretched exponential (light blue, solid line), and power law (magenta, dashed). Inset: Residuals (fluorescence – fitting result) with the same color trend, with all lines solid. The residuals are offset for clarity, with zero difference (perfect fit) indicated by a black horizontal line for each residual. Data after 1500 s is not shown, since each function had an almost equally good fit, except for the exponential fit which was substantially worse.

<table>
<thead>
<tr>
<th>Method</th>
<th>Exponential</th>
<th>Biexponential</th>
<th>Stretched Exponential</th>
<th>Power Law</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\tau$</td>
<td>1.16 x 10^3 s</td>
<td>$\tau_A$ 2.62 x 10^2 s</td>
<td>$\tau$ 2.30 x 10^3 s</td>
<td>$K$ 0.242</td>
</tr>
<tr>
<td>$\sigma^2_N$</td>
<td>42.5</td>
<td>$\sigma^2_N$ 7.26</td>
<td>$\sigma^2_N$ 8.72</td>
<td>$\sigma^2_N$ 13.0</td>
</tr>
</tbody>
</table>
Table 6.1: Comparison of data to several kinetics schemes with significant fitting parameters and their sample variance ($\sigma^2_N$) values.

A key question in understanding the observed complex photobleaching kinetics is which phenomenological rate laws appear to best describe the kinetics. A photobleaching kinetics transient was recorded with MEH-PPV CP dot solutions containing 2.66 ppm CPdots (absorbance = 0.12 AU, diameter = 11 ± 4 nm) and 0.01 M DABCO in water. 2 mL of solution in a 1 cm quartz cuvette was exposed to 130 $\mu$W of 488 nm light in a Quantamaster, PTI Inc. commercial fluorimeter (standard 90° geometry) with constant stirring and temperature control at 25 °C. Fluorescence signal was obtained at the emission peak at 589 nm for the duration of the kinetics experiments. Figure 6.2 and Table 6.1 summarize the results of fits to the various kinetic models. When the fitting functions above are applied to the photobleaching data, such as the data in Fig. 6.2, the biexponential function and KWW function fit almost equally well, and each are significantly better than the single exponential and power law functions, as seen in the $\sigma^2_N$ values in Table 6.1. This does not, by itself, rule out the possibility that the power law represents the kinetics taking place in the photobleaching studies. In fact, the continuum model proposed in Chapter 7 follows a power law relationship, which suggests there may be some relationship between photobleaching and power law kinetics. Power law kinetics are not, however, consistent with the (more detailed) stochastic model also proposed in Chapter 7. The question for data treatment then becomes which of the two well-fitting functions (biexponential or KWW) should be used to analyze the data. The
factor to consider is the number of fitting parameters (as well as the underlying physical picture implied by each model, which will be discussed later). Generally, the quality of a fit can be improved simply by adding more fitting parameters so that the fitting function can more accurately match random fluctuations present in the data. In general, if two functions fit a data set equally well, or nearly equally well, the one with fewer fitting parameters should be used, and in this case the biexponential function has 4 parameters compared to the 3 in the KWW function, and so KWW will be used in later fitting attempts. Since KWW describes highly heterogeneous systems, this suggests that the processes giving rise to the observed photobleaching kinetics are also highly heterogeneous.

6.2.2 Photobleaching Decay Curves and Reversibility

One additional feature observed is the ability of the CP dots to recover some of their fluorescence once they have been photobleached, as seen in Fig. 6.1. These dynamics were observed by exciting samples and then either blocking or attenuating (with partially silvered mirrors) the excitation power for some length of time, while observing the intensity of fluorescence from the sample.

If excitation is completely blocked there is almost complete recovery of fluorescence in less than a minute, but if the excitation power is instead cut by a factor of about 10 (as in Fig. 6.1), the recovery rate can be observed via the remaining fluorescence (Fig. 6.1, magnified portion). Some of the photobleaching appears to be irreversible however, as seen in the general downward trend in the peaks of each recovery section (Fig. 6.3).
Figure 6.3: Emission from CP dots through a series of high (400 µW) and low (42 µW) excitation power periods (showing high excitation power portions only). The first peak is significantly higher because subsequent bleaching curves have a limited recovery time whereas the sample was in the dark for an extended period before the first photobleaching curve.

Reversible photobleaching is often caused if the reduction in intensity is caused by a fluorophore entering a long-lived non-fluorescent triplet state, then leaving that state once the excitation source is removed, but recovery half-lives for this mechanism are typically much shorter than those observed in the CP dot system (tens of µs to 1 few ms vs. seconds observed for CP dots). There are also significant differences between the rate constants obtained for photobleaching and recovery--the bleaching time constant is $5.7 \pm 0.3$ s while the recovery time constant is $12.2 \pm 1.3$ s. This is not surprising, since
photobleaching is a photodriven process, while recovery is not. Analysis of recovery
dynamics fails to yield a definitive exponential fit. For a set of 15 recoveries, a \( \beta \) value of
0.80 ± 0.08 was obtained, though with the large standard deviation and large noise in the
recoveries prevent a definitive determination of the kinetics model they follow; either
stretched or single exponential kinetics, or a combination of the two are possible.

6.3 Effect of Oxygen and Redox-Active Species on Reversible
Photobleaching

Two well-known strategies for reducing photobleaching are the removal of oxygen (which is often involved in reactions involving the excited state of a fluorophore) and the addition of antifade or antioxidant agents. Antifade agents are often used to decrease photobleaching in fluorescent samples, many of which function by scavenging free radicals from solution. The antifade agent 1,4-diazabicyclo[2,2,2]octane (DABCO) is often used in fluorescence imaging to prolong fluorescence through singlet oxygen and triplet scavenging.\(^{159, 160}\) Antioxidant species on the other hand are readily oxidized species that act by undergoing oxidation more easily than the fluorescent species,\(^{161}\) so that in the event that various radicals are produced, they is more likely to oxidize an antioxidant (such as ascorbic acid) than the fluorophore. It may also be possible that an oxidized CPdot can react with an antioxidant in solution, oxidizing it, thereby regaining an electron and eliminating a hole (their net effect on CPdot photobleaching is similar to that of antifade agents but will not be investigated here). Here we explore the effect of oxygen removal and the effects of antifade agents on the photobleaching kinetics of
conjugated polymer nanoparticles. The goal of these investigations is to try to gain additional information about the mechanism of photobleaching as well as evaluation of strategies for prolonging the life of the nanoparticles for demanding fluorescence applications.

Figure 6.4: Alternation of high and low excitation powers in CP dot solution in air without DABCO (blue, lowest curve), after bubbling with nitrogen (green, middle curve), and with the addition of 0.01 M DABCO (red, highest curve). Reversible bleaching is almost completely suppressed in the nitrogen and DABCO samples, though there is some slow time scale irreversible bleaching. While reasonable steps were taken to reduce artifacts during filter addition
and removal, some artifacts are present due to the fluorimeter’s need to be open with imperfect blocking of room light during the experiment.

**6.3.1 DABCO**

Fluorescence photobleaching experiments were conducted with the anti-fade agent, DABCO with CP dots. MEH-PPV CP dot solutions contained 2.66 ppm CPdots (absorbance = 0.12 AU, diameter = 11 ± 4 nm) and 0.01M DABCO in water. 2 mL of solution in a 1 cm quartz cuvette was exposed to varying intensities of 488 nm light in a Quantamaster, PTI Inc. commercial fluorimeter (standard 90° geometry) with constant stirring and temperature control at 25 °C. For the experiments in air the solution was uncapped; for the nitrogen saturated experiments the solution was sealed with a cap with a septum.

As seen in Fig 6.4, the photobleaching rate decreases dramatically with the addition of DABCO. While the very slight reversible photobleaching that still occurs in the presence of DABCO recovers, over the course of many cycles the overall intensity gradually decreases, likely due to irreversible photodestruction of the polymer. A longer time scale experiment (1000 s experimental time, constant excitation of 350 µW; data not shown) showed that the degree of photobleaching decreased significantly from 62% in air to only 24% with 0.01 M DABCO.

**6.3.2 Nitrogen vs. Air**

Experiments were conducted to determine the changes in dynamics either due to the addition of 0.01 M DABCO or bubbling with nitrogen gas to remove oxygen (Fig. 6.4). The overall effect of either the addition of DABCO or the removal of oxygen is
almost complete suppression of reversible photobleaching (though a small amount of irreversible photobleaching does still take place). Over a long time scale experiment (1000 s experimental time, constant excitation of 350 µW; data not shown), the degree of photobleaching for a solution in air was 62%, compared to 41% for nitrogen. The degree of photobleaching prevention with the addition of nitrogen was difficult to reproduce, sometimes only halving the photobleaching reduction, sometimes decreasing the time constant by more than an order of magnitude. This variability is likely due to the difficulty in obtaining consistent purging. Compared to DABCO, some experiments show purging to be as effective as DABCO (e.g. Fig. 6.4), in suppressing photobleaching, while other experiments exhibit a diminished effect. While there are minor differences, the overall effect of DABCO and nitrogen purging on the photobleaching kinetics is similar, indicating that oxygen is likely involved in the photobleaching process. Meanwhile, the reversible photobleaching indicates that the photobleached species can readily convert back to fluorescent form. A likely candidate for the photobleached species is the hole polaron. Conjugated polymers are known to undergo reversible oxidation to form hole polarons. Furthermore, hole polarons are thought to be efficient fluorescent quenchers. The involvement of oxygen in the production of hole polarons suggests that oxygen may be acting as an electron acceptor, forming superoxide ion, $O_2^-$, when the polymer excited state reacts with oxygen. Superoxide is known to be formed in this way, and superoxide can also act as an electron donor in some cases,\textsuperscript{162} which could explain the reversible photobleaching observed. Based on the observed data, it is not possible to determine how DABCO suppresses photobleaching, but it is likely either by
tying up oxygen or by acting as an electron donor. Additional experimental tests of various aspects of this proposed photobleaching mechanism are described below, including investigations of fluorescence saturation phenomena and the effect of photobleaching on the lifetime of the fluorescent excited state.

### 6.4 Saturation of Polymer Fluorescence

A well known phenomenon in many fluorescent systems is saturation of fluorescence as the excitation power is increased. Saturation can be due to a number of factors such as photoinduced buildup of an appreciable population in the triplet state or other metastable “dark” state. This can be observed by measuring the emission intensity as a function of excitation power. At low excitation power, the emission typically increases linearly with excitation power, but at sufficiently high excitation power saturation occurs and is indicated by flattening of the emission intensity as a function of excitation intensity, typically asymptotically approaching a certain value. For a simple 3-level system consisting of a ground state, an excited state, and a metastable “dark” state, the intensity-dependence of the fluorescence follows the form,

\[
F = \frac{I_e/I_s}{1 + I_e/I_s} F_\infty
\]

where \( F \) is the emission intensity at a given power, \( I_e \) is the excitation power, \( I_s \) is the saturation power, and \( F_\infty \) is the saturated fluorescence intensity.\(^{163}\) Fitting a set of intensity-dependent fluorescence measurements to the function can be used to obtain values for \( I_s \) and \( F_\infty \), which can be further analyzed to yield information about the
various rate constants that describe the system. It should be noted that for the case of CPdots, the photophysics are expected to be substantially more complex than the 3-level system described by the above equation. A fit to this equation does however provide both some measure of the saturation and useful information about the limitations of CPdots for applications requiring high emission rates. A more detailed model which describes saturation-like phenomena while incorporating additional effects such as polaron quenching, exciton diffusion, and energy transfer will be proposed in Chapter 7.

The saturation experiments were carried out on a 2.66 ppm solution of MEH-PPV CPdots (11 ± 4 nm diameter, 0.12 absorbance) in an open 1 cm cuvette. Excitation was performed at the absorbance peak (488 nm) with excitation powers of 91, 183, 272, 360, 568, 766, 1166, and 1543 µW, as measured using the internal power reading in the instrument which was calibrated using a Newport Model 1815-C power meter with an 818-SL detector head. Collection was performed at 90° and at the emission peak (589 nm). The experiment was performed by stepping through the excitation powers from the lowest to the highest and allowing the system to reach steady state fluorescence at each power, which was taken as the emission power at each excitation power. The experiment was also done stepping down from the highest power, which gave similar results. For this set of experiments, a fit to the above equation yields a saturation power of 2.96x10³ µW and maximum emission intensity of 7.35x10⁶ counts, as indicated in Fig. 6.5. It should be pointed out that while no analytical standards were used for this experiment, which limits quantitative value of these figures, the qualitative indication of saturation is still valid.
Figure 6.5: A series of bulk fluorescence experiments on MEH-PPV CP dots indicating saturation. Fit parameters indicate emission of $7.35 \times 10^6$ counts at the saturation power of $2.96 \times 10^3$ $\mu$W. The dotted line represents a linear relationship without saturation.

### 6.5 Effect of Photobleaching on Fluorescence Lifetimes

Time-resolved fluorescence spectroscopy is a powerful tool for determining the rates associated with various processes occurring in the excited state, such as radiative and non-radiative relaxation and energy transfer, through their effect on the lifetime of the fluorescent excited state.\textsuperscript{28,163} We employed time-resolved fluorescence spectroscopy to investigate the nature of the reversible photobleaching process in the conjugated
polymer nanoparticles (Fig. 6.6). If the photobleaching process involves simple photodestruction of fluorophores, or a simple, metastable dark state, then photobleaching should have little or no effect on fluorescence lifetime, as any remaining (unreacted) fluorophores would be unchanged. On the other hand, if the reduction in fluorescence involves energy transfer from excited fluorophores to photogenerated quenching species, there should be a marked change in the excited state lifetime when the density of quenchers changes. Since it appears that photobleaching is (partially) reversible, hypothetically due to photogeneration of quencher species, then the fluorescence lifetime should depend on the excitation intensity. In addition, energy transfer to more or less randomly distributed quenchers should result in complex non-exponential kinetics, as will be discussed in more detail in Chapter 7.
Figure 6.6: Top: Fluorescence lifetime (black circles), IRF (black dotted line), and deconvolution results for exponential (blue), biexponential (green), and KWW (red) kinetics are shown. Top Inset: Applicable fitting parameters, standard deviations, and $\sigma_n^2$ values for the fitted kinetics. The populations indicated for the biexponential results are normalized. Bottom: Fit residuals (fit – data) are shown (all normalized to data) with the same colors (offset for clarity). Reduced chi
squared values for the residuals are: Exponential = 74.1, Biexponential = 3.56, KWW = 3.37, and the FWHM of the IRF is 68 ps.

Since the lifetime of the fluorescent excited state is typically tens of picoseconds to several nanoseconds, well beyond the timing resolution of typical detectors and data acquisition electronics, specialized experimental methods are required. For determining the lifetimes of the fluorescent excited states of the conjugated polymer nanoparticles, we employed the time correlated single photon counting (TCSPC) method. TCSPC is experimentally challenging as compared to other commercially available methods such as phase modulation, \(^{28}\) due to the requirement of short pulsed (typically laser-based) excitation sources and the complexity of the electronics. TCSPC was selected over other methods due to the superior timing resolution, as well as the additional information obtained from TCSPC that is useful for analyzing complex kinetics (e.g., exponential rise-and-decay, multiexponential, or stretched-exponential kinetics). In contrast, phase modulation methods are less well-suited for systems exhibiting complex kinetics. The details of the experimental apparatus are described in Chapter 2.

The TCSPC instrument yields a kinetics trace constructed from a histogram of photon arrival times relative to the laser pulse, representing the time-dependent population of the excited state. Analysis of the kinetics trace in order to extract rate constants is somewhat complicated due to the limited bandwidth (i.e., timing resolution) of the detector and electronics, which results in a broadening of the kinetics trace. The broadening is described by the instrument response function (IRF), which can be
measured by detecting the laser light scattered from a non-fluorescent sample (e.g., polystyrene beads). Any broadening observed in the IRF signal is therefore not due to fluorescence, but due to the timing uncertainty (jitter or walk) associated with the detector and the timing electronics. An instrument’s IRF can change over time, requiring that it be measured again before each new sample. Typical values for the width of the IRF in our TCSPC setup are in the range of 60 to 80 ps. In cases such as the present case, in which the timing resolution associated with the electronics is similar to the lifetime of the fluorescent species, the resulting data set represents the fluorescence lifetime convolved with the IRF, requiring numerical deconvolution methods to extract the kinetics information. This is performed by calculating the convolution of a trial kinetics function (single exponential, multiexponential, or KWW) with the IRF. The convolution of the trial kinetics function with the IRF is compared to the TCSPC kinetics trace, and the quality of fit is determined by calculating the square error. This process is iterated over a range of kinetics parameters and the optimum set of parameters is determined by nonlinear least-squares minimization.\textsuperscript{155}

Picosecond fluorescence lifetime experiments were carried out on a 2.66 ppm solution of MEH-PPV CPdots (11 ± 4 nm diameter, 0.12 absorbance) in either an open top 1 cm cuvette for the experiments in air, or in a screw cap 1 cm cuvette with a septum for nitrogen purged experiments. Excitation was performed with a mode-locked 76 MHz Ti-Sapphire laser (Coherent Mira 9000) tuned to 400 nm emission (excitation powers between 59.4 µW and 292 µW, as indicated in Fig. 6.7). Collection was performed with an id100-50 single photon counting module, with three 500 nm dichroic long pass filters
used to block scattered excitation light. Samples were measured with no higher than 10 KHz photons counts, in keeping with statistical considerations described in Chapter 2; the lifetime was measured at least until the signal reached 20 K events at the timing histogram peak (giving S/N of 140:1 at peak). An example of the data set and fitting results for three types of exponential fit are shown in Fig. 6.6. The fit to single exponential kinetics was found to be poor in all cases, ($\sigma^2_N = 74.1$, much higher than the other kinetics fits), while the biexponential and KWW fit results were much better ($\sigma^2_N = 3.56$ for biexponential kinetics and 3.37 for KWW). The closest fit, as indicated by $\sigma^2_N$ was to the KWW function, yielding $\beta$ fitting parameters indicating a high degree of heterogeneity ($\beta$ ranging from 0.38 to 0.46) for experiments performed at all powers, with and without oxygen. This high degree of heterogeneity in excited state lifetimes for the nanoparticles is consistent with a wide range of energy transfer rates, as would be expected for randomly distributed quencher species within the nanoparticle or substantial particle-to-particle variation in energy transfer rates.

Even though the CP dot system shows high heterogeneity (according to the low $\beta$ values obtained from the fits) in both the (picosecond) fluorescence lifetime results and the photobleaching kinetics, it should be noted that the two experiments (TCSPC and photobleaching) have radically different time scales and their discussions should remain distinct, even if the trends initially appear to be very similar or related and perhaps reflect the same underlying processes. Hence, it is possible, in principal, for a system to yield homogeneous picosecond fluorescence lifetime results while yielding heterogeneous photobleaching, and vice versa. However, Chapter 7 presents a model that describes the
heterogeneity observed both in the bulk photobleaching experiments and in the picosecond fluorescence lifetime experiments by the same photophysical mechanism.

Analysis of the picosecond fluorescence lifetime data requires a caveat. As indicated by the squared errors from Fig. 6.6, a KWW function does indeed fit the data sets obtained from TCSPC quite well; however, it is difficult to account properly for the effect of $\beta$ values when calculating overall time constants. This leads to an artificially wide distribution of time constants from repeated experiments. The result is that time constants calculated using the intensity weighted time constants of the biexponential fit (Eq. 6.2) give a much closer grouping; $\tau_{\text{Avg}}$, used to determine decay time trends is calculated as: $\tau_{\text{Avg}} = (A_1 \tau_1 + A_2 \tau_2) / (A_1 + A_2)$. This use of the biexponential fit is advantageous because each parameter in the biexponential function has a specific physical significance attached to it and can be included in a meaningful way to determine an averaged time constant ($\beta$, while it describes ‘heterogeneity’ does not tie directly back to an observable value such as lifetime or intensity).

6.5.1 Lifetime Trends

As stated previously, if the photobleaching is caused by the creation of quencher species and not simply by photodestruction of the polymer, the fluorescence lifetime would be expected to decrease with an increase in the number of quenchers. To investigate the impact of varying quencher population on excited species lifetimes, TCSPC experiments were performed at varying excitation powers, as excitation power is proportional to quencher population. Aqueous solutions of 2.66 ppm MEH-PPV CPdots (11 ± 4 nm diameter, 0.12 absorbance units) were prepared in a 1 cm cuvette either in air
or with nitrogen purging (uncapped cuvette for air, septum sealed cap for nitrogen purged experiments) and analyzed in the previously described TCSPC instrument (see Chapter 2 for details and a description of data fitting). The nitrogen purged samples were bubbled with nitrogen for several hours with stirring. Excitation was performed with a mode-locked Ti-Sapphire laser with excitation powers of 59, 162, 281 µW for the air samples and 62, 244 µW for the nitrogen saturated samples.

Fig. 6.7: Box and whisker plot of the fluorescence lifetimes at several powers for MEH-PPV CP dots. The line in the center of the box represents the median value for a sample set, while the upper and lower bounds of the boxes indicate the medians for the values above and below the median value; the whiskers above and below the boxes cover the full range of the data (with 3
outlier points at 158 µW in air). Lifetimes were calculated from the two time constants of a biexponential function weighted by the relative intensities associated with the two time constants. The top set are the nitrogen purged results (red, diagonal filled) while the lower set are the results in air (blue, unfilled boxes).

The data points represented by the boxes in Fig. 6.7 are each separate lifetime experiments, with their own IRF measurements. Though the experiments spanned several days, a full set of experiments (at the complete set of powers) with and without nitrogen purging were also performed on the same day to ensure that the trends indicated in Fig. 6.7 were not simply due to daily fluctuation in instrument behavior (the data set from each day matched the trends shown in the figure). Additionally, the Monte-Carlo method for determining uncertainty by fitting to synthetic data sets was employed. In this method, it is assumed that the fit to the data set represents the true underlying kinetics. The difference between the real data set and the fit result (the residual) is therefore assumed to representative of the noise inherent to the system (whether Poisson counting noise, instrument jitter etc.). New synthetic data sets can then be created by starting with the fit result curve and adding the same magnitude of noise as was in the original data set. Fitting can then be performed on the synthetic data sets and fitting parameters obtained. The variation in the resulting set of fitting parameters therefore reflects the uncertainty in the original fitting parameters. In this case it was found that standard deviation in the weighted average biexponential time constants was approximately 25 ps for all air measurements as well as the nitrogen purged measurement
at 244 µW, while the measurement of the nitrogen purged sample at 62 µW had a standard deviation of 50 ps. As these values are nearly the same as the inter-quartile distance (the height of the boxes in Fig. 6.7), this indicates that the distribution of values in Fig. 6.7 is primarily due to noise in each measurement (e.g. counting noise, timing jitter) and not day-to-day instrument variation or batch-to-batch differences in the solutions.

Each experiment yields three kinetics traces due to the 50 ns timing window of the time-to-amplitude (TAC) converter, and the ~13 ns time between mode-locked laser pulses, all representing the same lifetime information and each data point used to construct the box and whisker plot (details in the figure caption) below represents the average of all time constants acquired from the peaks for a particular experiment. It is expected that the lifetime will decrease as the quencher population increases because each exciton is more likely to appear near or diffuse near a quencher at shorter times. As seen in Fig. 6.7 (bottom data set), the fluorescence lifetime does indeed decrease with increasing excitation power, though a specific relationship cannot be determined from the amount of data collected. This is even more pronounced when comparing the low excitation power nitrogen purged sample to the high excitation power air saturated sample. This indicates that the photobleaching is indeed due to the creation of quencher species and not simply photodestruction (depletion) of the CPdots. Also, the clear difference in the lifetime observed for nitrogen-purged samples (which exhibit much less reversible photobleaching, consistent with lower quenching) versus the samples in air
provides additional confirmation that the reversible photobleaching phenomena are due to the photogenerated quencher species.

While previous research has focused on either energy transfer to dopants, or exciton diffusion, this work focuses in detail on the kinetics associated with energy transfer to (photogenerated) quencher species. Based on these results, it appears that photogenerated quenchers are responsible for the quenching and lifetime heterogeneity observed in the picosecond fluorescence lifetime results. In addition, it appears that oxygen is involved in the generation of the quenchers. Also, based on the observation of reversible fluorescence photobleaching, it appears that the quenchers are produced via a (mostly) reversible process (such as a reversible electron transfer process) and not due to an irreversible process (such as addition across carbon-carbon double bonds). It is widely known that conjugated polymers, as organic semiconductor materials, can undergo reversible charge transfer resulting in the production of a charged species, often referred to as electron polarons (negatively charged species containing an excess electron in the LUMO) or hole polarons (positively charged species with an electron vacancy in the HOMO). Furthermore, it has been suggested that hole polarons (the principal charge carrier in MEH-PPV, which is typically characterized as a p-type organic semiconductor) can act as efficient fluorescence quenchers. Thus the following tentative picture emerges that appears to qualitatively explain the observed complex photobleaching kinetics, reversible photobleaching, and complex picosecond dynamics of the fluorescent excited state: light excitation of the polymer occasionally results in the ejection of an electron to the surroundings, resulting in the formation of a
hole polaron, which acts as a fluorescence quencher. The presence of more or less randomly distributed photogenerated quenchers, combined with the processes of exciton diffusion and energy transfer to quenchers, results in a range of excited state lifetimes. As we shall see (in Chapter 7), this picture also results in complex photobleaching kinetics similar to the results presented earlier in this chapter. Molecular oxygen appears to be involved, perhaps acting as an electron acceptor or electron scavenger. Eventually, recombination of the hole and the electron leads to loss of the quencher/polaron. Any successful model of the exciton dynamics of CPdots should account for heterogeneity in the fluorescent lifetimes and photobleaching kinetics, fluorescence recovery, fluorescence saturation, and decreasing fluorescence lifetime with increasing excitation power. As mentioned in the introduction to this chapter, the investigations described in this chapter were motivated by a desire to test and refine the tentative photophysical picture that emerged from analysis of single nanoparticle fluorescence trajectories. Thus, our attempt to understand single CP dot fluorescence trajectories has, for the first time, yielded a compelling and detailed photophysical picture of combined exciton-polaron dynamics in conjugated polymers. In the following chapter, a detailed model is proposed, and the results described in this chapter are compared to both qualitative and quantitative model predictions.

### 6.6 Conclusions

The interactions between the photobleaching, recovery, anti-fade agents, nitrogen, and fluorescence lifetime trends, portrays a system with a complex interplay of processes.
The recurrence of stretched exponential kinetics in photobleaching and picosecond lifetime kinetics suggest high heterogeneity (as indicated by low $\beta$ values), the cause of which will be investigated in Chapter 7. The anti-fade agent, DABCO, and nitrogen prolong the photobleaching lifetime of CPdots, and two subtly different mechanisms are proposed, both involving suppression of the quencher population. Further investigations are necessary to refine this picture, particularly with respect to such questions as how DABCO suppresses the quencher population, whether or not superoxide ion is involved, and the mechanism for recombination of hole polarons with an electron. An increase in fluorescence lifetime was observed when oxygen was displaced with nitrogen, also consistent with the presence of photogenerated quencher species and their suppression by removal of oxygen or addition of DABCO. In contrast, photobleaching due to simple photodestruction of chromophores would not result in changes in the fluorescence lifetime. Additionally, the fluorescence saturation observed and the effect of changes in excitation power on fluorescence lifetimes are consistent with intensity-dependent changes in photogenerated quencher population. A more detailed picture will emerge in Chapter 7 with two models that account for the (reversible) photobleaching, saturation, and picosecond lifetime measurements.
CHAPTER 7

THEORETICAL MODELS OF EXCITON DYNAMICS IN CPDOTS

7.1 Introduction

Data presented in Chapter 6 included photobleaching decay curves, fluorescence recovery, influence of electron donating species, picosecond fluorescence lifetime, and saturation of fluorescence. Chapter 7 endeavors to develop one or more theoretical models consistent with the observed phenomena that provide a readily understood qualitative picture while providing quantitative predictions. Two main approaches to modeling the observed behavior of conjugated polymer nanoparticles (CPdots) are considered below: a continuum model and a stochastic simulation. The continuum model develops a picture of bulk solution dynamics by considering each of the rate processes that are expected to occur in the nanoparticles, while neglecting detailed consideration of nanoparticle structure and the length scales of exciton diffusion and energy transfer. The stochastic model takes a rather different and complementary approach by simulating the conditions in an individual CPdot and representing picosecond dynamics with respect to exciton generation and diffusion, energy transfer, and quencher (hole polaron) creation and elimination (these species and processes are described in detail in Chapter 1). As will be shown in this chapter, both models agree qualitatively with many of the observed phenomena, such as reversible photobleaching, KWW photobleaching dynamics, and fluorescence saturation. The (detailed) stochastic model also reproduces the KWW behavior observed in the picosecond dynamics of excited state decay, and is consistent
with literature values for the exciton diffusion length. The stochastic model is a significant achievement, in that it provides a single model that, for the first time, gives a detailed explanation and excellent quantitative agreement with a number of rather perplexing observations (non-exponential photobleaching, saturation phenomena, non-exponential excited state dynamics, complex blinking behavior in single molecule fluorescence trajectories, and anomalously low photostability in some cases) in conjugated polymers and related systems. These models have also formed the basis for the development of rational strategies towards the optimization of CPdot optical properties for a given application. In addition, the development of these models, and their refinement and testing by careful comparison to the results of properly designed experiments, could result in a more detailed and accurate understanding of the nanoscale exciton diffusion and energy transfer processes occurring in organic semiconductors. Such understanding could aid in the design and optimization of organic semiconductor-based optoelectronic devices such as polymer-based light-emitting displays and photovoltaic devices.

7.1.1 Previously Described Dynamics in CPdots

CPdot spectroscopic experiments were described in detail in Chapter 6, but will be briefly summarized here. In fluorescence photobleaching kinetics measurements of poly[2-methoxy-5-(2'-ethyl-hexyloxy)-1,4-phenylene vinylene] (MEH-PPV) CPdot solutions, it was discovered that if a sample is partially photobleached, followed by a reduction in the excitation power, then the fluorescence will recover (almost completely in some cases). A common feature in these experiments is the tendency for
photobleaching decay and picosecond fluorescence kinetics to follow a stretched exponential, or Kohlrausch-Williams-Watts (KWW) function, described in detail in Chapter 6, as opposed to a single exponential, indicating complex kinetics. It was not possible to definitively determine the kinetics of fluorescence recovery, due to low signal levels.

In the next set of experiments, two chemical species were added to the system to modify the fluorescence bleaching behavior of MEH-PPV CPdots. First, the antifade agent 1,4-diazabicyclo[2,2,2]octane (DABCO) was added, as it is a common species used to preserve fluorescence; second, oxygen was removed from the system by bubbling with nitrogen. Both of these produced a marked increase in photobleaching time constants and greatly reduced the amount of reversible photobleaching (Fig 6.4), confirming their preservative effect in the CPdots solutions. The mechanisms by which these species modify the fluorescence dynamics has not been explored thus far, but a hypothesis will be presented in this chapter.

Picosecond fluorescence lifetime experiments on MEHPPV CPdots in air and nitrogen both indicate that as excitation power is increased, fluorescence lifetime decreases, with the decay of the excited state following KWW kinetics. This is not consistent with conventional photobleaching, and indicates that the reduction in fluorescence is due to a dynamic quenching process occurring in the excited state, such as collisional quenching or energy transfer.\textsuperscript{28} Since we are unaware of any relevant collisional process, and energy transfer is widely observed in conjugated polymers (even
at very low quencher levels), energy transfer to a quencher (likely a photogenerated hole polaron) is the most likely explanation.

The above dynamics will be explained by two separate but consistent models with different levels of abstraction. The continuum model described in this chapter involves the modeling of the populations of two excited species (excitons and hole polarons), and the influence of rates of creation and elimination on their populations. This model does not involve direct simulations of CPdots or excited species, instead it only accounts for the populations of the excited species in them. The stochastic model takes the different and complementary approach of simulating excited species behavior inside of individual CPdots. During the simulations excitons diffuse through the CPdots and may undergo energy transfer to hole polarons, decay through fluorescence, or create a hole, producing simulated fluorescence dynamics which qualitatively resemble single molecule experiments. Even though these two approaches deal with exciton-hole polaron interaction at different levels, they produce photobleaching trends which are similar both to the experimental and to each other.

7.2 A Continuum Model for Exciton and Quencher Dynamics

7.2.1 Creation and Elimination of Quenchers

Before the rate picture for the continuum model is proposed it is important to discuss possible mechanisms for the observed phenomena. The term “exciton” here refers to a Frenkel type exciton, as described in Chapter 1. In organic semiconductors, such as conjugated polymers, an exciton can “hop” from one location to another either by
energy transfer through the Förster mechanism, which is a relatively long-range-through-space interaction,\textsuperscript{168} or by the Dexter mechanism, which is a much shorter range interaction involving the exchange of two electrons.\textsuperscript{169} When these processes take place in a CPdot they collectively give rise to what is known as “exciton diffusion” whereby the exciton moves in a random walk fashion through the semiconductor medium. Occasionally the CPdot becomes oxidized, leaving behind a positively charged cation in the CPdot (possible oxidation pathway suggested below). This cation and the local distortion of the polymer matrix due to the charge are collectively called a hole polaron (or simply hole). There is evidence that holes act as highly efficient fluorescence quenchers in many organic semiconductors, due to the red-shifted spectra of cations resulting in highly efficient energy transfer to holes and the very low fluorescence quantum yields of holes.\textsuperscript{93, 170} Indeed, hole quenching volumes on the order of 10-400 nm\textsuperscript{3} (which, significantly, are similar to the volumes of the CP dot nanoparticles) have been determined.\textsuperscript{8, 93} It is also suggested by the observed fluorescence recovery in photobleached CPdots that it is possible for some species in solution to transfer an electron back to the polaron, reducing it, eliminating the hole, and restoring fluorescence. Such reversible electron transfer is known to occur on organic semiconductors, and is involved in hole conductivity. Based on this proposed picture and the observed reversible photobleaching dynamics, the rate constants associated with hole polaron creation under typical fluorimeter excitation powers (10 – 1000 µW) are usually on the order of seconds to several minutes, while recovery takes place with similar time constants in the dark.
Of less relevance to the dynamics discussed here is irreversible bleaching. This process can be observed in the gradual lessening of fluorescence intensity with each successive recovery step (see Fig 6.3), and is probably caused by reactive species which create keto or other defects in the polymer that break backbone conjugation and reduce fluorescence irreversibly. The mechanisms that cause irreversible photobleaching are not explored here, as their impact on emission is significantly less than those of hole species for the time scales considered in chapter 6.

7.2.2 Origin of Reversibility and the Effects of Electron Donors on Bleaching and Reversibility

Experiments were conducted with 0.01 M DABCO in solution or with nitrogen bubbling. Both experiments yielded similar results: the almost complete suppression of reversible photobleaching (Fig. 6.4). A proposed reaction scheme is presented in Eqs. 7.1 - 7.3 (* indicates species that have undergone an irreversible reaction). The scheme begins when a species dissolved molecular oxygen oxidizes a CPdot (Eq. 7.1) then creates superoxide anion, which then either reacts irreversibly with a CPdot or another species in the system, thus effectively impeding reverse electron transfer back to the CPdot, or donates the electron back to the CP dot, eliminating a hole and restoring fluorescence. Reaction of superoxide to produce hydroxide ion and hydrogen peroxide is also likely, as in Eq. 7.2, generating other reactive species which may still react with and bleach CPdots. If an antioxidant is present (AO) it can protect the CPdot in two ways: by reacting with a reactive species before they can react with the CPdot, or by electron restoration (Eq. 7.3) by undergoing oxidation by the CPdot. Antioxidants in general are
easily oxidized species that are therefore more likely to undergo oxidation than other species in solution.\textsuperscript{161} One species frequently involved with photobleaching schemes and radical chemistry is the superoxide anion, but in the scheme in Eqs. 7.1 - 7.3, it could be another species and the antioxidant could be any suitable antioxidant or weakly reducing species.

\[
NP + h\nu + O_2 \rightarrow NP^+ + O_2^- \tag{7.1}
\]

\[
2O_2^- + 2H_2O \rightarrow O_2 + H_2O_2 + 2OH^- \tag{7.2}
\]

\[
AO + NP^+ \rightarrow NP + AO^+ \tag{7.3}
\]

There is also an effect on fluorescence lifetime as observed in Fig. 6.7. In the absence of dissolved molecular oxygen, the fluorescence lifetime (the excited state lifetime) increases. This could be from the reduced number of quenchers resulting in longer lived excitons, reduced quenching by molecular oxygen, or from some other subtle photophysical effect. As is the case in air, CPdots in nitrogen have a decreasing fluorescence lifetime as the excitation power is increased, though there is less of an effect in nitrogen than there is in air. The photobleaching and lifetime results described in Chapter 6 indicate that molecular oxygen plays a role in the reversible photobleaching and quenching results.

7.2.3 Continuum Model for Bulk Photobleaching Dynamics

We pursued this notion further by developing a continuum rate model and comparing the results of this model with the experimental photobleaching results described above. The model is described as follows: exciton dynamics within a CPdot can be viewed as a continuous process of exciton creation (absorbance), exciton
relaxation (fluorescence), non-radiative emission, quencher creation, quencher elimination, and energy transfer to quenchers. The combination of these processes can be described using a pair of coupled differential equations. A simplified continuum rate model describing the time-dependence of the exciton population is described by the differential equation,

\[ \frac{dn_{ex}}{dt} = k_{abs} - k_r n_{ex} - k_{nr} n_{ex} - k_{qgen} n_{ex} - k_Q n_{ex} n_Q \]

where \( n_{ex} \) is the number of excitons, \( n_Q \) is the number of quenchers, \( k_{abs} \) is the rate of absorption (excitation), \( k_r \) is the (first order) radiative rate constant, \( k_{qgen} \) is the quencher generation rate constant (assumed to be first-order in \( n_{ex} \)), and \( k_Q \) is the (second order) rate constant describing the (bimolecular) quenching process. For all simulations described below, the distinction of an exciton decay being radiative or non-radiative is irrelevant, so \( k_r \) is assumed to include both of these processes and \( k_{nr} \) is set to zero. It should also be noted that this model does not explicitly include a microscopic description of the energy transfer processes resulting in quenching—such details (e.g. particle and quencher size, exciton mobility) are represented implicitly in the various rate constants.

The detailed microscopic nature of energy transfer occurring within a CPdot is treated within the framework of a stochastic (random walk) formalism described later. Within the continuum rate picture, we also take into account the generation and elimination of quenchers by reversible electron transfer using the differential rate equation,

\[ \frac{dn_Q}{dt} = k_{qgen} n_{ex} - k_{qelim} n_Q \]
where $k_{Qelim}$ is the quencher elimination rate constant, thus the exciton dynamics are described using a pair of coupled rate equations. These rate equations can be integrated numerically using the “ode45” tool in MATLAB to simulate a photobleaching experiment (an example using the “ode45” function in MATLAB can be found in Appendix A). ODE45 utilizes an adaptive 4th and 5th order Runge-Kutta integration method to yield numerical solutions to differential equations, and readily handles coupled differential equations.

Fig. 7.1: Bulk photobleaching kinetics of CPdots (Blue) and fit to Continuum Model (Red).
Initial results show that the continuum model yields a fairly close match to the bulk experimental photobleaching kinetics data (Fig. 7.1) and yields a close fit to individual CPdot trajectories, as shown in Chapter 8. The deviation at long times is likely due to irreversible photobleaching, which is not accounted for in this model. While other research has shown some agreement between photobleaching kinetics and the stretched exponential function, the current model appears to yield an improved fit using the same number of parameters. In addition, our model is based on photophysics known to occur in conjugated polymers.

One consequence of the reversibility, that is if $k_{Q\text{elim}} > 0$, is that $n_{ex}$ reaches a constant, non-zero value. This equilibrium $n_{ex}$ population also changes as the system conditions, such as a change excitation power ($k_{abs}$), resulting in a concomitant shift in $n_Q$ until equilibrium is reestablished. Transient changes in the fluorescence intensity following a change in excitation intensity are observed (Fig. 7.2).

Fig. 7.2: Left: Variable intensity experiment showing recovery. The high excitation power is 400 μW and the low power excitation is 43 μW. The bleaching rate constant is 1.40 s$^{-1}$ and the recovery rate constant is 0.60 s$^{-1}$. Right: Results of the continuum model showing simulated blinking and recovery.
Additionally, the exciton population at equilibrium can be determined algebraically when a simplification is made. It can be assumed that for a particular very short time at the beginning of the experiment that no quenchers have been generated and the number of excitons that generate quenchers is much less than the number of excitons that fluoresce. It simplifies the derivations and maintains the accuracy of the model to add the radiative and non-radiative terms from Equation 7.5 together into one associated fluorescence term, \( k_f n_{ex} \). For \( n_{ex} = 0 \), and \( k_f \gg k_{Q\text{gen}} \), and applying steady state conditions, Equation 7.5 changes to:

\[
0 = k_{abs} - k_f n_{ex} \tag{7.6}
\]

which rearranges to the following for the number of excitons in the absence of quenchers:

\[
n_{ex}^0 = \frac{k_{abs}}{k_f} \tag{7.7}
\]

The steady state number of excitons at infinite time can be determined by solving Eqs. 7.4 - 7.5 with \( dn_{ex}/dt \) and \( dn_Q/dt \) both equal to zero and solving for \( n_Q \):

\[
n_Q = \frac{k_{Q\text{gen}}}{k_{Q\text{elim}}} n_{ex} \tag{7.8}
\]

and substituting into Equation 7.4 to obtain:

\[
\frac{dn_{ex}}{dt} = k_{abs} - k_f n_{ex} - k_{Q\text{gen}} n_{ex} - \frac{k_Q k_{Q\text{gen}}}{k_{Q\text{elim}}} n_{ex}^2 \tag{7.9}
\]

If it is assumed that the quencher generation rate is much smaller than the fluorescence rate, \( k_f \gg k_{Q\text{gen}} \) then at steady state, Equation 7.9 becomes:
which can be solved for the steady state number of excitons, $n_{ex}^{ss}$, at a given excitation rate by using the quadratic equation. It is therefore possible to determine the degree of quenching for a given excitation power, $Q_{eff} = 1 - n_{ex}^{ss} / n_{ex}^0$. Numerical solutions to Equations 7.4 and 7.5 (in the long time limit) agree with this steady-state solution with greater than 99% accuracy. This expression is also useful for predicting saturation behavior described below.

7.2.2 Prediction of Saturation in the Continuum Model

A well known phenomenon in many fluorescent systems is saturation of fluorescence as excitation power is increased. Saturation can be due to a number of factors such as photoinduced buildup of appreciable population in the triplet state or other “dark” state. Our continuum model indicates that saturation of the polymer is possible because of the second order rate constant for the energy transfer term that grows faster than the emission term, which is first order. This can be observed by increasing the excitation power and measuring the emission intensity. The fitting function used to determine where the maximum emission and the excitation where that occurs, is given by:

$$ F = \frac{I_e / I_s}{1 + I_e / I_s} F_{\infty} $$

where $F$ is the emission intensity at a given power, $I_e$ is the excitation power, $I_s$ is the saturation power, and $F_{\infty}$ is the fluorescence intensity at a power high enough to induce
This equation was previously derived for a simple three-level system with intersystem crossing to a non-fluorescent triplet state, and may not represent a more complex system like a CPdot. For this set of experiments, a fit to the above equation yields a saturation power of $2.96 \times 10^3 \mu W$ and maximum emission intensity of $7.35 \times 10^6$ counts, as indicated in Fig. 7.3.

Fig. 7.3: A series of bulk fluorescence experiments on MEH-PPV CPdots indicating the trend towards saturation. Fit parameters indicate emission of $7.35 \times 10^6$ counts at the saturation power of $2.96 \times 10^3 \mu W$. The dashed straight line represents the expected trend in a non-saturable system.
Agreement between the published three-level picture saturation equation above and results from the continuum rate model is not as good, as indicated in Fig. 7.4. To produce this data set, the parameters in the continuum model were set as follows: $k_s = k_{abs}/100$, $k_{gen} = 10^{-3}$, $k_{qelim} = 10^{-2}$, $k_Q = 0.6$, and $k_{abs} = \text{varies}$ (see below) while the starting population of excitons, $n_{ex}$, was 100 and the starting number of quenchers, $n_Q$, was zero. The final exciton population could then be compared to the photon absorption rate. For a wide range of values of absorbed photons, the nonlinear relationship between excitation and emission is observed but is a relatively poor fit to the model above. The apparent difference between the good fit above and the poor fit below for what is, according to the model, essentially the same system, could be explained by the relatively narrow range of excitation powers used in to generate Fig. 7.3, when compared to the Fig. 7.4; there are many basically curved functions that would fit such a small portion of curve as seen in Fig. 7.3. In conclusion, the continuum model and the triplet saturation model both appear to reproduce the observed saturation phenomena, and additional experiments are required to probe a sufficiently high range of excitation intensities in order to determine which model more accurately represents the underlying mechanism.
7.3 Exciton Lifetime Heterogeneity and the Stochastic Model

7.3.1 Stochastic Simulation Method

Picosecond fluorescence kinetics measurements, performed using time correlated single photon counting (TCSPC), can reveal information about various processes occurring in the excited state, in this case the rate and heterogeneity in the rate of energy transfer from exciton to polaron hole. The best fit for MEH-PPV CPdots fluorescence
lifetime data is a stretched exponential or Kohlrausch-Williams-Watts (KWW) function of the form

\[ I(t) = A \exp\left(\left(-t / \tau\right)^\beta\right) \]

where \( I \) is the fluorescence intensity at a particular time \( t \), \( A \) is a proportionality constant, \( \tau \) is the time constant, and \( \beta \) is the stretching parameter, which is typically in the range of about 0.2 to 1.0, and is representative of the degree of heterogeneity in the dynamics. This function results from the overlap of many single exponential functions with different time constants. A \( \beta \) value of close to 1.0 indicates relatively little heterogeneity (a narrow distribution of time constants), while a lower \( \beta \) value indicates higher heterogeneity (a wider distribution of time constants).

For MEH-PPV, the TCSPC data indicate that the \( \beta \) value is between 0.36 and 0.45, indicating a high degree of heterogeneity in the exciton lifetimes. There are several possible sources of local variations in lifetime, including particle-to-particle variations of particle sizes or number and spatial arrangement of quenchers, random starting positions of excitons, and heterogeneity in local polymer conformation. In order to determine the possible effect and relative importance of each of these parameters on the overall heterogeneity parameter \( \beta \), a series of simulations were performed in the MATLAB programming environment using the function diffusion_fun.m (see Appendix B). The function simulates exciton creation, diffusion, emission, and energy transfer within CPdots. The stochastic model is based on a 3D random walk with discrete time steps of duration \( \Delta t \) and a discrete 3D cubic lattice with a spacing \( \Delta \varepsilon \). Excitons are created at a random position in the CPdot, and over the course of a given time interval, an exciton is
assumed to undergo one of a number of processes: (1) the exciton can decay radiatively (fluorescence) or nonradiatively (treated the same in this model), (2) the exciton can “hop” to a neighboring site (exciton diffusion), (3) the exciton can undergo energy transfer to a quencher, or (4) the exciton can turn into a hole polaron (a very small influence on exciton population). For a Poisson process, the probability that a single event occurs during the time interval $\Delta t$ is given by $p = 1 - \exp(-k\Delta t)$, where $k$ is a rate constant. The rate constant, $k$, for radiative or nonradiative relaxation is the inverse of the fluorescence lifetime, $\tau_F$, of a pristine CPdot solution (taken from experimental data). The energy transfer rate for a given exciton-quencher pair is given by the Förster expression,

$$k_q = \frac{1}{\tau_f} \left( \frac{R_0}{R} \right)^6$$

where $R$ is the distance between each quencher and exciton, the fluorescence lifetime is $\tau_F$, and the Förster quenching radius is $R_0$, and the expression is summed over all exciton-quencher combinations. During a given step, the probabilities of the above events occurring are calculated according to the above probability expression, and compared to a random number in order to determine the fate of the exciton during that time step. In the event that no relaxation processes occur, the exciton continues along its trajectory and is moved one step in the x, y, or z direction, chosen at random.

To investigate single molecule dynamics, a series of stochastic simulations were performed with parameters closely matching the experimentally determined parameters.
The results of the individual stochastic simulations are qualitatively similar to single molecule trajectories, with stepwise reductions in fluorescence intensity and significant Poisson (photon counting-like) noise, as shown in Fig. 7.5. The simulated particles were 4 nm radius, with 2 or 4 nm quencher Förster radius (as indicated in caption), 8 nm exciton diffusion length, and fluorescence lifetime of 334 ps. Simulation conditions were 1000 excitons created per time step, 200 time steps, and exciton step size of 0.10 nm). It can be seen that the results vary from particle to particle and that even the degree of photobleaching from a quencher is not uniform, even though the Förster radius is the same for all quenchers.
Figure 7.5: Several stochastic simulations under that same conditions showing the fluorescence emission (blue) and incremental increases in quencher population (red). The Förster radii are 2 nm (top) and 4 nm (bottom).

When the stochastic simulations are summed over many particles, the results resemble the reversible photobleaching phenomena that are observed both experimentally and in the continuum model. However, there are clear differences, and the stochastic model provides much additional detail—information such as particle-to-particle variability arising from differences in the positions of the quenchers and the relative quenching efficiencies of successive quenchers that can be directly compared to single molecule and ensemble results to yield a better understanding of the complex photophysical phenomena occurring in CPdots. It should be noted that this model is, to date, the only single model which accurately reproduces the major features observed in picosecond fluorescence kinetics (heterogeneous KWW picosecond dynamics), bulk photobleaching kinetics (non-exponential or power law photobleaching kinetics), and single molecule fluorescence trajectories of conjugated polymers (pronounced, complex blinking phenomena). The model also accurately predicts energy transfer efficiencies in dye-doped CPdots and in blended CPdots.\textsuperscript{133,145}

7.3.2 Quenching and Heterogeneity in Stochastic Simulations

Stochastic simulations, since they simulate the behavior of excitons in individual CPdots, can show particle-to-particle variation due to range of conditions. This section investigates the effects of quencher placement and variable Förster radius on quenching
efficiency and heterogeneity of the resulting dynamics. Simulations were carried out in 5 nm particles with a single quencher located at a fixed distance from the particle center (0 – 5 nm) with a range of Förster radii (1 – 5 nm). The exciton diffusion length was set at 8 nm, fluorescence lifetime ($\tau_F$) at 334 ps, with a diffusion simulation step size ($\Delta\varepsilon$) of 0.1 nm, and the production of extra quenchers was disabled. The quenching efficiency was determined by calculating the ratio of excitons that undergo energy transfer to quenchers (as opposed to emitting a fluorescence photon) to the total number of generated excitons. The observed trend is that the quenching efficiency is highest when the quencher is close to the center of the particle and lowest when the quencher is at the edge (since a great deal of its quenching radius is outside of the particle), as seen in Fig. 7.6. This trend holds for a range of Förster radii down to 1 nm, in which case there is little variation of quenching efficiency with location, due to the fact that the quenched volume is small enough that there is little difference in overall quenching as the hole nears the edge of the CPdot.
The decay times (either through energy transfer to a quencher or fluorescence) for the above simulations were histogrammed as well (100,000 excitons per histogram), yielding the simulated picosecond fluorescence decay curves, and the results were fit to the KWW function. It would be expected that the $\beta$ would be at its maximum (corresponding to a relatively low level of heterogeneity) when the quencher is located at the edge of the particle (at radius of 5 nm), since fewer excitons would be within the quenching volume; indeed Fig. 7.7 shows that this is the case. It is somewhat surprising that $\beta$ is at a

Fig. 7.6: Relationship between quenching efficiency and quencher distance to particle center. Quencher Förster radii are indicated.
minimum for quenchers positioned roughly 3 nm from the center, followed by a rise as the quencher nears the center of the particle. This indicates that a high degree of quenching is not, by itself, sufficient to induce a highly heterogeneous system. Instead, high heterogeneity (and a low $\beta$) requires a large distribution of distances to initial quencher positions (and therefore energy transfer rates). The data indicates that this happens not when the quencher is centered or at the edge, but halfway in between. For a 5 nm particle and a 3 nm Förster radius quencher, the minimum $\beta$ observed was 0.46, which is similar to the 0.36 to 0.46 obtained from TCSPC experiments on suspensions of CPdots (lower $\beta$ values are observed in the simulations for larger numbers of quenchers, as shown below).
As we can see, the simple case of a single quencher at a fixed distance from the particle center can achieve $\beta$ much lower than one. This indicates that a system like this is inherently heterogeneous at the single particle level and that additional sources of heterogeneity, such as particle to particle variations in size, polymer conformation, and quenching radius, are not required in order to explain the observed level of heterogeneity. This is not to say that those factors do not play a role in increasing heterogeneity, it just means that the idealized model of a uniform CPdot with one or more quenchers is sufficient to reproduce most of the wide distribution of lifetimes observed for real excitons. Additionally, preliminary estimates and calculations (described below) indicate that these other sources of heterogeneity only make a small contribution to $\beta$. Further experiments are required to more fully resolve the question of the role of polymer heterogeneity, such as single molecule experiments, experiments involving controlled addition of quencher dyes (to provide a well-defined average number of quenchers and Förster radius), low temperature experiments (to freeze out polaron-assisted exciton diffusion), picosecond fluorescence polarization anisotropy decay (to provide more detailed information about energy transfer processes), and femtosecond pump-probe or fluorescence upconversion experiments.

7.3.3 Heterogeneity and Number of Quenchers
Two more issues merit investigation including the relationship between number of quenchers and the quantum efficiency for energy transfer to quenchers ("quenching efficiency"), as well as the relationship between number of quenchers and the apparent degree of heterogeneity in excited state lifetime (in terms of the $\beta$ value). While it is clear that an increase in the number of quenchers should yield an increase in the quenching efficiency, it also seems reasonable to expect that the amount of additional quenching provided by the addition of one more quencher should decrease as the number of quenchers increases (diminishing returns as more quenchers are added). Also, the relationship between number of quenchers and heterogeneity is not known, nor is it known whether the addition of quenchers (2 or more quenchers) should increase or decrease the degree of lifetime heterogeneity as compared to a single quencher. Knowledge of these relationships and a comparison between experimental results and the results of simulations is crucial for validating the model, and possibly for helping to obtain quantitative estimates of the number of quenchers per nanoparticle for a given experiment and the quenching efficiency per-quencher. To explore these relationships, simulations were conducted for up to 20 quenchers to obtain the trends for specific numbers of quenchers.

The relationship between quenching efficiency and $\beta$ gets more complex when the two quantities are correlated against each other, as in Fig. 7.7:
Fig. 7.8: The correlation between $\beta$ and quenching efficiency for 1, 2, 3, 4, and 5 nm quenching radii and varying numbers of quenchers. The data points are the $\beta$ and quenching efficiency values averaged for each particle (with correction for spherical geometry). The points are for 1 (diamond), 2 (square), 3 (triangle), and 10 (x) quenchers. The Förster radii are 1 nm (black), 2 nm (green), 3 nm (purple), 4 nm (red), and 5 nm (cyan), as also indicated by labels.

Fig. 7.8 demonstrates that while for a given particle the trends for quenching efficiency and $\beta$ are very different, the ratio falls on a fixed function for a wide range of number of quenchers and Förster radii. It might be expected that for very large numbers of quenchers that the energy transfer probability for the entire particle would even out, as the quenching efficiency would be very high for much of the particle. It appears, however, that the $\beta$ values even for large numbers of quenchers are low owing to the
steepness of the quenching efficiency functional surface near any one of the quenchers (like a sharp mountain range, all high altitude but with much variation, as opposed to a high altitude plateau). The main limitation here is that the function is unconstrained in that either the number of quenchers or the quenching radius must still be known to use the quenching efficiency to $\beta$ relationship, somewhat limiting its utility. For example, one set of experiments indicated a steady state quenching efficiency of 10% and a $\beta$ value of 0.83. Plotting this on Fig. 7.8 gives a quencher radius of about 1.5 nm if the particle only has one quencher, but only 1 nm if the particle has 3 quenchers.

While it has been shown that the presence of a single quencher can give rise to a high degree of heterogeneity in exciton lifetimes, it seems possible that the variable number of quenchers per particle would lead to higher overall heterogeneity. A Poisson distribution describes the distribution of occurrences in individual samples in a population, given an average number of occurrences per sample in the population. In this case, if the average number of quenchers is known, the distribution of quenchers in the CPdots can be predicted by the Poisson distribution. This variation in quencher number should lead to a distribution of energy transfer rates, with higher rates when there are more quenchers than average, and vice versa. This wider range of energy transfer rates would be observed in the photobleaching decay fit as a lower $\beta$ value.

To explore the possible influence of a Poissonian distribution of quenchers, an average number of quenchers per particle was assumed (such as 4.5 quenchers) and the distribution of the number of quenchers calculated around that average, as indicated by the Poisson distribution. The exponential curves for the uniform number of quenchers
can then be added up, with weighting as indicated by the Poisson distribution. The resulting summation of Poisson distributed exponential decays was then fit to the KWW function to determine the $\beta$ value. The results (not shown), indicate that for a Poissonian distribution of quenchers in CPdots, the $\beta$ value is almost identical to that of a specific number of quenchers equal to the average number of quenchers. That is, a Poissonian distribution of quenchers with an average of 5 quenchers per particle produces approximately the same degree of heterogeneity as a collection of particles that all have exactly 5 quenchers. This indicates that very little of the macroscale heterogeneity is due to poissonian particle-to-particle variation in the number of quenchers.

Since the primary effect of increased excitation power is a shift in the equilibrium between exciton population and quencher population, and therefore number of quenchers, it makes sense to compare the fluorescence lifetimes of several simulated data sets with different numbers of quenchers. The resulting trend from fitting stochastic simulations results is shown in Fig. 7.9 for a range of numbers of quenchers and quenching radii. There is a clear downward trend in the lifetime with increasing quenchers at a given quenching radius.
Fig. 7.9: Simulated fluorescence lifetimes for 1 (blue diamonds), 2 (purple squares), 3 (red triangles), and 10 (green x) quenchers in a 5 nm CPdot. At 0 nm Förster radius all simulations have the pure polymer fluorescence lifetime, 334 ps.

7.4 Conclusions

Both the continuum model and stochastic simulations produce data which resembles the behavior of CPdot excitation and photobleaching. The continuum model represents the behavior of the CPdots with only rate constants with only an implied (through the rate constants) usage of information about particle size, Förster radius of quenchers, fluorescence lifetimes, or exciton diffusion rates. The continuum model
produces trends that line up well with the stretched exponential kinetics and recovery observed in actual bulk spectroscopy experiments. The stochastic method, on the other hand, does simulate the behavior of individual excitons in a CPdot; this clearly demonstrates that heterogeneity can arise simply from randomly located exciton creation positions and that variation in either quencher or particle location or size are not needed to give rise to such complex kinetics. The stochastic method also reproduces the expected trends in quenching efficiency and exciton lifetime, with respect to quencher placement within the particle and quencher size. While both models reproduce dynamics similar to the experimental data, the stochastic method can provide information about how single molecule dynamics and fluorescence lifetime are influenced by the physics of the individual CPdots (the stochastic model is much more computationally expensive, however). Somewhat surprisingly, the simulations predict that the amount of lifetime heterogeneity should increase with the number of quenchers. The simulations of lifetime heterogeneity, in terms of $\beta$, are in reasonable quantitative agreement with the experiment. It was hoped that careful determination of quenching efficiency and $\beta$ values from the lifetime data could, by comparison with simulation results, yield information about the quenching efficiency per quencher and the mean number of quenchers per nanoparticle under given conditions. Unfortunately, comparison of simulation results for different Förster radii (corresponding to different quenching efficiencies per quencher) indicated that, according to the stochastic exciton diffusion model, for example, a quenching efficiency of 0.50 always yields a beta value of around 0.35, regardless of whether the quenching is due to a small number of highly efficient quenchers or a large
number of inefficient quenchers. Thus it is concluded that additional experiments are required in order to determine the average number of quenchers per nanoparticle (for a given set of conditions), and the quenching volume of a single polaron. It is proposed that a number of additional investigations, including single molecule spectroscopy, temperature-dependent fluorescence spectroscopy, experiments with dye-doped particles, fluorescence anisotropy decay, and ultrafast spectroscopy, could help to clarify the nature of fluorescence quenching by hole polarons in these nanoparticles.
CHAPTER 8

OBSERVATION OF SINGLE NANOPARTICLE DYNAMICS

8.1 Introduction

The photophysical phenomena reported in previous chapters, such as complex photobleaching kinetics and reversible photobleaching observed in ensemble (bulk) measurements, arise from the multitude of discrete events taking place in individual CPdots. In order to gain a better understanding of the processes taking place in CPdots, and for further tests and possible refinement of the models developed, the dynamics of individual CPdot nanoparticles were investigated. Single molecule spectroscopy is often characterized by phenomena such as discrete, stepwise fluctuations in fluorescence intensity, the stepwise irreversible bleaching of all fluorescence, fluctuations in emission spectra (spectral diffusion or quantum jumps), and molecule-to-molecule heterogeneity in the emission spectra. Such phenomena have been observed in single fluorescent dye molecules, fluorescent proteins, and isolated conjugated polymer molecules. These phenomena often cannot be observed with large numbers of molecules as their effects are averaged out with the (unsynchronized) processes taking place within the other particles being observed.
8.2 Results and Discussion

8.2.1 Example Single Molecule Trajectories

For the following experiments, a home built sample scanning confocal fluorescence microscope was used with either an avalanche photodiode detector or a deep cooled CCD camera for detection (setup described in detail in Chapter 2). Samples were drop cast on microscope coverslips yielding a distribution of areas where the CPdots formed a thick layer to areas where the CPdots were distributed as isolated particles (submonolayer), which were the areas that were used for single particle spectroscopy. AFM scans were not performed on the samples used in these experiments, but similarly prepared samples had particle diameters that were in the range of 5-20 nm. Excitation was performed at 488 nm excitation with powers ranging from 1-100 µm measured in the sample plane. A 530 nm dichroic mirror was used in the microscope with an additional 500 nm filter before the APD. No extra filters (besides the dichroic mirror) were placed before the CCD camera. Samples are typically scanned 100 lines with 10 x 10 µm scan area (corresponding to 100 nm/pixel) with 250-350 nm FWHM resolution. Kinetics scans were performed by first scanning the sample then returning to a particle location for an extended period (typically 200 s) to record kinetics (typically with 1 ms time resolution). A typical scan (Fig. 8.1) shows diffraction limited resolution of ~300 nm.
Figure 8.1: Typical scan of MEH-PPV CPdots with number of counts indicated on right side color bar. Excitation power was 1.26 µW, FWHM of focal spot ~350 nm. Background was 40 counts with 6600 peak counts (for the lower left particle).
Figure 8.2: Kinetics trajectories of four of the CPdots imaged in Fig. 8.1. Intensity axes are scaled for the individual trajectories, integration time is 100 ms per intensity reading.

Four representative single particle kinetics trajectories vary in emission intensity, time scale of dynamics, reversibility, and sharpness of transition are shown in Figure 8.2. Sharp, vertical transitions are observed, as well as multiple intensity levels, consistent with prior reports of blinking in conjugated polymers, which have been attributed to reversible generation of quencher species, likely hole polarons. In addition, there is typically a gradual decrease in fluorescence over the course of the kinetics scan, which may arise from photodestruction of the polymer via a process that does not create a
quenching species but reduces the absorption cross section. Each of the trajectories in Fig. 8.2 qualitatively resembles the results of stochastic simulations presented in Chapter 7, in that reversible quenching and a range of “quenching depths” are observed. In Fig. 8.2 (a) reversible transitions are observed at several times, such as at roughly 4, 22, 31, seconds. In Fig. 8.2 (b), an (apparently) irreversible transition can be seen at 9 s, and several small reversible transitions can been seen at around 12 and 27 s. In this case, the quenching depth associated with the first quenching transition is far larger than the second, a trend repeated in (c). This is likely caused by the tendency of diminishing returns in quenching efficiency with additional quenchers (hole polarons) as seen in the results of stochastic simulations in Chapter 7. A gradual decrease in intensity can be seen over much of the time span in Fig 8.2 (a) and appears exponential, likely corresponding to conventional photobleaching. Fig 8.2 (b) also shows a likely exponential decrease in intensity from 0-9 s. It also appears that when a particle has little emission it undergoes less photodestruction than a bright particle, as seen in Fig. 8.2 (d) and at 50-88 seconds on Fig. 8.2 (b) and 30-75 seconds on Fig. 8.2 (c).
A comparison of bulk photobleaching kinetics of CPdots to the summation of 63 individual single nanoparticle kinetics traces (Fig. 8.3) shows that they both have similar kinetics, in particular the stretching parameter is nearly identical. This strongly suggests that the complex kinetics observed in bulk photobleaching is a result of reversibly photogenerated quenching species (likely polarons) that possess high quenching efficiencies, ranging from a few percent to in excess of 90%. The high quenching efficiency and variability in quenching efficiency are also consistent with long-range energy transfer processes occurring in the nanoparticles.

8.2.2 Change Point Detection

Many single nanoparticle kinetics traces appear to exhibit multiple intensity levels, as shown in Figure 8.4. It is likely that each distinct intensity level indicates a different “state”. In principle, it should be possible to analyze the intensity fluctuations in such multi-state kinetics traces to extract information about the state-to-state rate constants. However, extracting such information requires a more sophisticated approach than the “thresholding” approach described in Chapter 1. One such approach is the change point method.\textsuperscript{175} In this method, a maximum likelihood, minimum entropy approach is employed to estimate the positions of “change points”, while simultaneously estimating the intensity levels on either side of the change point. Typically, “prior
information”, such as the number of different intensity levels, is also included in the estimation process. The method we have chosen to analyze multilevel blinking of CPdots proceeds as follows. A differencing method is used to obtain initial guesses about the positions of change points. Then the position of each change point is adjusted via least squares minimization. In some cases, the intensity over each interval is then averaged, and the resulting intensities are used to generate a histogram of intensities. If there are clear “peak” intensities, then the peak intensities are selected as representative of individual states, and an additional round of minimization is used to determine the state associated with each interval (by comparing the experimental intensity over a given interval with the list of “allowed” intensities obtained from the intensity histogram). This may be followed by an additional round of minimization to refine the positions of change points. Also, each interval can undergo further testing to determine if there may be additional change points that were missed in the initial analysis. Finally, the residence time in each state is determined.

Change point analysis using the above described algorithm was applied to several intensity trajectories of single CPdots (a representative fit is given in Figure 8.4). While the analysis results clearly indicated the presence of several intensity states, the trajectories were determined to be of insufficient duration for reliable extraction of state-to-state rate constants. Most states corresponded to only a few intervals, insufficient for reliable estimation of rate constants. In addition, the analysis was hindered by a slow, continuous photobleaching process that made it difficult to assign the state corresponding to a given intensity level. Based on these results, it appears feasible (but challenging) to
extract additional information from the single CPdot kinetics results. These results could provide further information about the processes involved in polaron generation, such as whether polaron generation is governed by exponential kinetics or a more complex power-law behavior previously observed in colloidal CdSe nanoparticles. Also, such results could provide information about how the quencher generation rate and per-quencher quenching depth depend on the number of existing quenchers (the stochastic model provides predictions about these questions). Further progress will require further refinement of the change point analysis routines, as well as further reduction of photobleaching, improved time resolution, the acquisition of much longer kinetics traces (with a target of at least 20 transitions per state), acquisition and analysis of perhaps hundreds of trajectories, and careful studies of the effects of particle size and excitation intensity on the resulting kinetics.
Figure 8.4: Example of change point detection results for a real single molecule data set. The sample was a MEH-PPV CPdot on a sample scanning confocal microscope under the experimental conditions described in section 8.2.

8.3 Conclusions

Single molecule trajectories exhibit a range of phenomena that are consistent with the trends suggested by both the stochastic and continuum models described in Chapter 7. Reversible stepwise bleaching and slow, continuous decay are observed in many trajectories suggesting the appearance of hole polarons and photodestruction of polymer. The quenching depths observed are consistent with the predictions of the stochastic energy transfer model. The combination of stepwise, reversible quenching and continuous photobleaching occurring over many particles produces trends that are consistent with photobleaching kinetics observed in bulk spectroscopic experiments, as confirmed by comparison of summed single particle kinetics traces with the results of bulk photobleaching kinetics experiments. Changepoint analysis was proposed as a way to gain more detailed information about the complex single nanoparticle kinetics, and proof-of-concept experiments and analysis were performed.

Much of the research presented in the previous chapters was performed to test the fundamental picture that began to emerge from analysis of preliminary single CPdot intensity trajectories. The fundamental picture involved reversible photogeneration of quencher species, likely polarons, that exhibit large quenching efficiencies, corresponding to a quenching radius of several nanometers--comparable to the size of the
nanoparticle. This hypothesis naturally led to several questions, including (1) whether such reversible photobleaching phenomena could be observed in properly designed bulk photobleaching experiments, (2) whether such a picture could explain complex, non-exponential photobleaching kinetics of conjugated polymers that had previously been reported but not adequately explained, and (3) whether energy transfer effects could be observed in picosecond time-resolved fluorescence measurements. These questions directly led to the series of bulk photobleaching and picosecond fluorescence lifetime experiments reported in previous chapters and the development of the stochastic energy transfer model. This model is quite successful in that it provides, for the first time, a unified microscopic description of several key processes occurring on conjugated polymers and CPdot nanoparticles, with accurate, quantitative predictions of observed phenomena occurring at the picosecond timescale (picosecond stretched exponential kinetics) and the timescale of seconds and minutes (complex photobleaching kinetics and reversible photobleaching).

The results presented in this thesis have also been helpful in the development of CPdots as fluorescent labels. The demonstration of record two-photon fluorescence cross-sections for a nanoparticle could help broaden the applicability of two-photon fluorescence microscopy to a wider range of systems. Understanding energy transfer processes occurring in conjugated polymer nanoparticles also led to the development of strategies for improving nanoparticle brightness and longevity based on energy transfer to dyes.
In terms of the broader impact, these results help explain some perplexing, longstanding problems, such as the difficulty of producing high-efficiency, high power organic LEDs or organic semiconductor-based lasers. In the context of the results presented here, it seems likely that, at high current densities, quenching by hole polarons could be a key factor in limiting device efficiency in OLEDs and inhibiting the population inversion required for lasing action to occur. At high current densities, hole polaron densities on the order of $10^{17}$ cm$^{-3}$ or higher are often attained, which should result in a large amount of polaron quenching, based on the results presented here. A number of possible approaches, such as nanostructured device layers employing energy transfer to help separate excitons from polarons, could help mitigate this device performance issue. Based on the results presented here, it is also likely that polaron quenching could limit the performance of polymer-based photovoltaics.

While the experimental and model results presented here provide a reasonably detailed picture of several photophysical processes occurring in conjugated polymers and CPdot nanoparticles, many questions remain that could provide the basis for additional research in the future. For instance, the majority of the results presented here involve the conjugated polymer MEH-PPV. Conjugated polymers are a diverse class of polymers, as discussed in Chapter 1. Additional studies on a variety of polymers could help elucidate how backbone structure, heteroatoms, and side chains affect processes related to polaron generation and energy transport processes. In particular, single molecule and single nanoparticle studies of the polymer PFBT could provide detailed information about polaron generation and energy transfer processes--the high photostability of this polymer
should result in single molecule trajectories that are many times longer than those obtained with MEH-PPV. Also, the application of CPdots to imaging of a variety of biological systems promises to yield improvement in sensitivity, as well as the ability to track single molecules with high precision.
Appendix A

Example of Continuum Model Using ode45 Function

This script shows a sample implementation of the continuum model discussed in Chapter 7. The script simulates 5000 s of high excitation power, followed by 5000 s of low excitation power. The resulting plot shows the changes in the populations of excitons and quenchers.

```matlab
%Begin 'bleachdiffrun.m' on the following line
global kabs kf kqgen kq kqelim
kabs = 1000;
kf = kabs/100;
kqgen = .0001;
kqelim = kqgen*10;
kq = 0.6;
y1_0 = kabs / kf; % initial exciton number
y2_0 = 0; % initial quencher number
y0=[y1_0 y2_0];
t0=0;
tf = 5000;
options=odeset('RelTol',1e-5,'AbsTol',1e-8);
[t1,y1] = ode45(@bleachdiffeq,[t0 tf],y0,options);
kabs=10;
kf = kabs/10;
kqgen = .0001;
kqelim = kqgen*10;
kq = 0.6;
y1_0 = y1(end,1);
y2_0 = y1(end,2);
y0=[y1_0 y2_0];
options=odeset('RelTol',1e-5,'AbsTol',1e-8);
[t2,y2] = ode45(@bleachdiffeq,[t0 tf],y0,options);
plot([t1; (t2+t1(end))], [y1; y2])
legend('Exciton Population','Quencher Population');
xlabel('Time (s)')
ylabel('Population')

%End 'bleachdiffrun.m' on the previous line
```
Appendix B

Example of Exciton Diffusion Simulation

Some parts of this specific implementation of this function are not enabled in the function
and script below and those sections are commented out.

%Begin 'diffscript.m' on the following line
% script for simulating energy diffusion and transfer in a
quenchable, bleachable system
rand('seed', sum(100*clock));
%incr=input('What number should incrementing start on?: ');
SystemParams.ParticleRadius = 5;
SystemParams.ForsterRadius = 2.29;
SystemParams.QuenForsterRadius = 5;
SystemParams.NumQuenStart = 1;
SystemParams.DiffLength = 8;
SystemParams.RealTau = 334;
SystemParams.NumDyes = 5;  %Superceded
by NumDyeList below
SystemParams.Qkon = 1/50;  %"On" for
a quencher is it turning its quenching on.
SystemParams.Qkoff = 1/25;
SystemParams.StepSize = 0.1;
SimParams.NumExc = 5000;
SimParams.NumBatches = 20;
%SimParams.GridRes = 0.2;  %does
nothing, left over from a previous version
SimParams.dt = 1;
SimParams.QuenLimit = 1;
SimParams.QuenLimit =
max(SimParams.QuenLimit,SystemParams.NumQuenStart);  %Makes
sure the number of allowed quenchers is at least the number
at the start
%SystemParams.BLChance = 0.0000;
%SystemParams.QMakeChance = 0.0001;

%Some switches. Set any of them to 0 to cancel out the
effect, regardless of anything above.
SimParams.CanDyeET = 0;  %Probably better to change
"NumDyeList=[0];" below. Must be 0 or 1.
SimParams.CanMakeQuen = 0; %.002; % Can be any value, 0 kills quencher creation, 1 is 'normal' probability
SimParams.CanBleach = 0; % 0 or higher. 0 is no bleaching at all.
SimParams.CanQuenBlink = 0;

%Results = diffusion_fun(SystemParams, SimParams);

NumDyeList=[0];
NumQuenList=[1];
NumAverages=1;
PosList=0:.5:5;
EffMat=zeros([length(NumDyeList) NumAverages]);

for I=1:length(PosList),
    times=[];
    disp(['Quencher Dist = ' num2str(PosList(I)) ]);
    for avcnt=incr:NumAverages+incr-1,
        disp([ 'Avg #' num2str(avcnt) ])
        %SystemParams.NumQuenStart = NumQuenList(I);
        SystemParams.XQuenStart=PosList(I)
        %SystemParams.QuenForsterRadius   =
        10*rand;
        %SystemParams.RealTau               =
        334*2*rand;
        eval([ 'Results' num2str(avcnt) ' =
            diffusion_fun(SystemParams, SimParams); ' ]);
        eval([ 'times=[times Results' num2str(avcnt) '.DecayTimes(:)];' ])
        eval([ 'save c:\data\simulations\Results'
             datestr(now,'mmddyy-HHMMSS') '-Qrad'
             num2str(SystemParams.QuenForsterRadius) '-Qdist'
             num2str(SystemParams.XQuenStart) '-Avg' num2str(avcnt)
             '.mat Results' num2str(avcnt) ' ]);
        eval([ 'clear Results' num2str(avcnt) ' ]);
        % DyeEff = sum(Results.numET)/SimParams.NumExc;
        % DyeEffMat(dyecnt,avcnt) = DyeEff;
        % if dyecnt==3,
        %
decaylist=cat(2,decaylist,Results.AllDecayTimes(:)');
        % end
    end
% eval([ 'times' num2str(quencnt) ' =times;' ])
%End 'diffscript.m' before this line

%Begin 'diffusion_fun.m' after this line
function Results=diffusion_fun(SystemParams, SimParams)
rand('seed', sum(100*clock));  %Set a unique random seed
for this run through

ParticleRadius = SystemParams.ParticleRadius;
ForsterRadius = SystemParams.ForsterRadius;
ForsterRadius6 = ForsterRadius^6;  %ForsterRadius^6 is
used many times, this saves a little calculation time.
QuenForsterRadius = SystemParams.QuenForsterRadius;
QuenForsterRadius6= QuenForsterRadius^6;
DiffLength = SystemParams.DiffLength;
RealTau = SystemParams.RealTau;
NumDyes =
    SystemParams.NumDyes*SimParams.CanDyeET;
NumQuenStart =
    SystemParams.NumQuenStart;
ChanceQoff =
    SystemParams.Qkon;
ChanceQon =
    SystemParams.Qkoff;
%BLChance =
    SystemParams.BLChance;
%QMakeChance =
    SystemParams.QMakeChance;
StepSize =
    SystemParams.StepSize;
NumExc =
    SimParams.NumExc;
NumBatches =
    SimParams.NumBatches;
dt =
    SimParams.dt;
QuenLimit= 
    SimParams.QuenLimit;

%Save the input parameters for later reference
Results.SystemParams=SystemParams;
Results.SimParams=SimParams;

ldsteps =DiffLength/StepSize;  % DiffLength, in terms of
number of
    % steps of length

<StepSize>.

tau=ldsteps^2; % effective exciton "lifetime" for random walk. Not
    % the real lifetime, but the number of time
steps.
InvTau =
    1/tau; %Used over and over,
save a little computation time.

BLChance = SimParams.CanBleach*(1/(NumExc * ldsteps));
QMakeChance = SimParams.CanMakeQuen*(1/(NumExc * ldsteps));
% Generate Random Dye Positions Within Sphere of radius 
<ParticleRadius>

cnt=1; % index counter
XDye=[];
YDye=[];
ZDye=[];
while cnt <= NumDyes,
    xtry = ParticleRadius*2*(rand(1)-.5);
ytry = ParticleRadius*2*(rand(1)-.5);
ztry = ParticleRadius*2*(rand(1)-.5);
    if (xtry*xtry + ytry*ytry + ztry*ztry) < (ParticleRadius^2),
        XDye=[XDye; xtry];
        YDye=[YDye; ytry];
        ZDye=[ZDye; ztry];
        cnt=cnt+1;
    end
end

XQuen=[SystemParams.XQuenStart];
YQuen=[zeros(size(XQuen))];
ZQuen=[zeros(size(XQuen))]; %Positions of the quenchers created during the experiment. This is NOT reset for each new batch.
cnt=1;
while cnt+length(SystemParams.XQuenStart) <= NumQuenStart,
    xtry = ParticleRadius*2*(rand(1)-.5);
ytry = ParticleRadius*2*(rand(1)-.5);
ztry = ParticleRadius*2*(rand(1)-.5);
    if (xtry*xtry + ytry*ytry + ztry*ztry) < (ParticleRadius^2),
        XQuen=[XQuen; xtry];
        YQuen=[YQuen; ytry];
        ZQuen=[ZQuen; ztry];
        cnt=cnt+1;
    end
end

numhere=length(XQuen)

ExpTime=0; %Overall simulated time
Results.ETTimes=[];
Results.DecayTimes=[];
Results.numBL=[];
Results.numET=[];
Results.numDEC=[];
Results.numQ=[];
Results.numQuenET=[];
Results.numQon=[];
Results.numExc=[];

AllLdiff=[];

Qon=ones(size(XQuen));
umBL=0;
totBL=0;

for I=1:NumBatches,
    disp(['Batch #' num2str(I)])
    totBL=totBL+numBL;
    numBL=0;    %Number of excitons that have
    numET=0;    %Number of excitons that have
    numDEC=0;    %Number of excitons that have
    numQ=length(XQuen);      %Number of times that quenchers have been created from excitons
    numQuenET=0;    %Number of times that excitons have been quenched (not by dyes)

    BleachFrac=(totBL*(StepSize^3))/(1.33333*3.14159*ParticleRadius^3);

    %First, determine the starting positions of the excitons
    cnt=1;
    CurrNumExc=round(NumExc*(1-BleachFrac));
    Results.numExc=[Results.numExc;CurrNumExc];
    Xpos=zeros([1 CurrNumExc]);
    Ypos=zeros([1 CurrNumExc]);
    Zpos=zeros([1 CurrNumExc]);
    while cnt <= CurrNumExc,
        xtry = ParticleRadius*2*(rand(1)-.5);
        ytry = ParticleRadius*2*(rand(1)-.5);
        ztry = ParticleRadius*2*(rand(1)-.5);
        if (xtry*xtry + ytry*ytry + ztry*ztry) <
            (ParticleRadius^2)
Xpos(cnt)=xtry;  
Ypos(cnt)=ytry;  
Zpos(cnt)=ztry;  
cnt=cnt+1;
end
end

Xi=Xpos; Yi=Ypos; Zi=Zpos;  %Store the starting positions of the excitons
alive=ones(size(Xpos));  %Set all of the particles to be alive
TmpETTimes=zeros(size(Xpos));
TmpDecayTimes=zeros(size(Xpos));
time=0;  %Setting the time for the current batch of excitons
%This part determines which of the quenchers are active. This only needs to run once per batch.
% if SimParams.CanQuenBlink==1,
  % randQ=rand(size(XQuen));  %Random quencher number to determine whether or not they turn on or off
  % Qoff = abs(Qon-1);  %Generates a list for which quenchers are turned off currently
  % justQoff= -1.*Qon.*(randQ < ChanceQoff);  %List of which ones just turned off
  % justQon = Qoff.*(randQ < ChanceQon);  %List of which ones just turned on
  % Qon = Qon + justQoff + justQon;  %New list of on quenchers
% Results.numQon=[Results.numQon -1 Qon];  %Gives an output for the quenchers that are on, separated by a -1 for parsing later. It is done this way because th length of Qon will change through the run
% end

while sum(alive)~=0,  %This while loop runs as long as an exciton is alive
  OtherChance = zeros(size(Xpos));
  %This counts up probabilities each time make sure that each exciton can only die once.
  randnums=rand(size(Xpos));  %Only generate random number for particle once per cycle, then use for each possible fate.
We need to determine if the particle energy transfers, fluoresces, quenches, bleaches, or diffuses.

This part determines the rate for each exciton to transfer to a dye molecule.

% rates = zeros(size(alive));
% if NumDyes == 0,
% for excidx=find(alive==1), %Does
calculation for each live exciton
% DX = XDye - Xpos(excidx);
% DY = YDye - Ypos(excidx);
% DZ = ZDye - Zpos(excidx);
% Rsq = DX.*DX + DY.*DY + DZ.*DZ + 0.000001;
% rates(excidx) = sum( InvTau .* ForsterRadius6 .* (1./(Rsq.*Rsq.*Rsq)) ); %ForsterRadius6 is _not_ a typo
% end
% ETChance = 1 - exp(-rates); %This is a small number (less than 0.01 typically)
% justET = alive.* (randnums < ETChance);
%Exciton undergoes energy transfer, considered dead
% alive=alive - justET;
% numET=numET + sum(justET);
% TmpDecayTimes=TmpDecayTimes + justET.*time;
% OtherChance=OtherChance + ETChance; %This makes sure that only one thing happens to each excition.
% end

This determines whether or not excitons ET to the added quenchers (instead of dyes)
rates = zeros(size(alive));
if numQ == 0,
  for Quenidx=find(alive==1), %Does
calculation for each live exciton
  DX = XQuen(find(Qon==1)) - Xpos(Quenidx);
  DY = YQuen(find(Qon==1)) - Ypos(Quenidx);
  DZ = ZQuen(find(Qon==1)) - Zpos(Quenidx);
  Rsq = DX.*DX + DY.*DY + DZ.*DZ + 0.000001;
rates(Quenidx) = sum( InvTau .* QuenForsterRadius6 .* (1./(Rsq.*Rsq.*Rsq)) );
%QuenForsterRadius6 is _not_ a typo
end
QuenChance = 1 - exp(-rates);
justQ = alive.* ((randnums > Other_chance) & (randnums < Other_chance + QuenChance)); %Exciton undergoes energy transfer, considered dead
alive=alive - justQ;
numQuenET=numQuenET + sum(justQ);
TmpETTimes=TmpETTimes + justQ.*time;
OtherChance=OtherChance+QuenChance;
end

%This part determines whether or not the exciton decays (fluoresces)
DECChance = 1 - exp(-InvTau);
justDEC = alive.*((randnums > OtherChance) & (randnums < OtherChance + DECChance));
alive = alive - justDEC;
umDEC = numDEC + sum(justDEC);
TmpDecayTimes=TmpDecayTimes + justDEC.*time;
OtherChance=OtherChance+DECChance;

%This determines whether or not the exciton causes bleaching
% if SimParams.CanBleach>0, %Checks if bleaching is allowed
% justBL = alive .* ((randnums > OtherChance) & (randnums < OtherChance + BLChance)); %for I=find(justBL==1), %This code can be added to a modified form of the overall script to save the locations of bleached chromophores
% live( Xpos(I), Ypos(I), Zpos(I) ) = 0;
% end
% alive = alive - justBL;
% numBL = numBL + sum(justBL);
% TmpDecayTimes=TmpDecayTimes + justBL.*time;
% OtherChance=OtherChance+BLChance;
% end

%This determines whether or not the exciton becomes a quencher.
% if SimParams.CanMakeQuen>0, %Checks if more quenchers are allowed
  % justQMake = alive .* ((randnums > OtherChance) & (randnums < OtherChance + QMakeChance));
  % for I=find(justQMake==1), %Runs the quencher creation loop only for excitons that just became quenchers
    % XQuen=[XQuen Xpos(I)]; %Stores the position of each exciton that just made a quencher and makes a quencher there
    % YQuen=[YQuen Ypos(I)];
    % ZQuen=[ZQuen Zpos(I)];
    % Qon=[Qon 1];
    % if length(Qon)>=QuenLimit, %Checks if the maximum number of quenchers has been reached
      % QMakeChance=0; %Prevents more quenchers from being made
    % end
  % end
  % numQ = length(XQuen);
  % alive = alive - justQMake;
  % TmpDecayTimes=TmpDecayTimes + justQMake.*time;
% end

%Move the excitons that are still alive
% use random numbers to jump in either +/- X, Y, or Z direction
  jumps = sign(rand(size(Xpos))-.5);
  % dir : 0 -> X hop, 1 -> Y hop, 2 -> Z hop,
  dir = floor(rand(size(Xpos))*3);
  Xpos = Xpos + (jumps .* (dir==0)) .* alive * StepSize;
  Ypos = Ypos + (jumps .* (dir==1)) .* alive * StepSize;
  Zpos = Zpos + (jumps .* (dir==2)) .* alive * StepSize;

  % TODO: check for excitons that have escaped (R>ParticleRadius).
  % If there is an escape, then back off one step.
escapelist = find((Xpos.*Xpos + Ypos.*Ypos + Zpos.*Zpos) > ParticleRadius^2);

Xpos(escapelist) = Xpos(escapelist) - sign(Xpos(escapelist))*StepSize;
Ypos(escapelist) = Ypos(escapelist) - sign(Ypos(escapelist))*StepSize;
Zpos(escapelist) = Zpos(escapelist) - sign(Zpos(escapelist))*StepSize;

time = time + dt;
end

Ldiff = sqrt((Xpos-Xi).^2 + (Ypos-Yi).^2 + (Zpos-Zi).^2);
AllLdiff = [AllLdiff; [Ldiff zeros(1,NumExc-length(Ldiff))]];
ExpTime = ExpTime + time;
Results.ETTimes = [Results.ETTimes; [TmpETTimes zeros(1,NumExc-length(TmpETTimes))]];
Results.DecayTimes = [Results.DecayTimes; [TmpDecayTimes zeros(1,NumExc-length(TmpDecayTimes))]];
Results.numBL = [Results.numBL; numBL];
Results.numET = [Results.numET; numET];
Results.numDEC = [Results.numDEC; numDEC];
Results.numQ = [Results.numQ; numQ - sum(Results.numQ)];
Results.numQuenET = [Results.numQuenET; numQuenET];
Results.DyeDist = sqrt(XDye.*XDye + YDye.*YDye + ZDye.*ZDye);
Results.QPos = [XQuen; YQuen; ZQuen]';
end

Results.ETTimes = Results.ETTimes * RealTau/tau; % Scales the decays to the size of a time step.
Results.DecayTimes = Results.DecayTimes * RealTau/tau; % Scales the decays to the size of a time step.
Results.AllLdiff = AllLdiff;

% End 'diffusion_fun.m' before this line


