Probing Single-Molecule Photophysics of Rhodamines on TiO2

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Probing Single-Molecule Photophysics of Rhodamines on TiO$_2$

A thesis submitted in partial fulfillment of the requirement
for the degree of Bachelor Science in Chemistry from
The College of William and Mary

By

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Accepted for (Honors)

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Abstract

Dye-sensitized solar cells (DSSCs) are promising solar devices to provide for future energy needs. Improvements in DSSC efficiency require an understanding of the complete distributions of electron injection and charge recombination. In this thesis, a series of rhodamine dyes is investigated to probe the impact of structure, driving force for photoinduced electron transfer, and adsorption affinities to TiO$_2$ on electron transfer (ET) dynamics. A combination of ensemble-averaged techniques, single-molecule spectroscopy, and modeling approaches are used to interpret the dispersive ET kinetics.

Ensemble-averaged measurements provided insight into aggregation effect on binding affinity to and differences in driving forces for electron injection and recombination. Absorbance and fluorescence measurements revealed that adsorption affinity to TiO$_2$ increases as follows: rhodamine 6G (R6G) < rhodamine 123 (R123) < rhodamine B (RB) < 5-carboxy-X-rhodamine (5-ROX). 5-ROX contains a para-substituted carboxyl group that is less sterically hindered for TiO$_2$ binding relative to the ortho-substituted carboxyl group in RB. Electrochemical measurements evaluated the range of driving forces for electron injection and recombination in all of the dyes, where RB had the smallest driving force for electron injection and the largest driving force for recombination. Overall, these ensemble-averaged characteristics demonstrated the diversity of driving forces and adsorption geometries exhibited by these series of rhodamine dyes.

Single-molecule blinking measurements were compiled from ~100 molecules of RB, R6G, R123, and 5-ROX. Blinking traces were separated into on-time and off-time distributions, which were fitted to various heavy-tailed exponential forms (i.e., power
law, log-normal, and Weibull) to establish the best fit with robust statistical tests. Our analysis reveals that although power law seems to be statistically significant for on-time distributions for all of the rhodamine dyes, the onset time of the power-law fit only describes a small portion of the data (i.e., less than 25%). Instead, we observe that log-normal distributions better capture the entire on-time distribution. Furthermore, the log-normal function also characterizes off-time distributions, which supports the Albery model of dispersive ET kinetics (i.e., ET rates will be log-normally distributed). The physical interpretation of the associated log-normal fit parameters was found with Monte Carlo (MC) simulations based on the Albery model. Changes to rates of injection and recombination resulted in changes to $\mu_{on/off}$, respectively, while the extent of energetic dispersion around the mean activation barrier was associated with $\sigma_{on/off}$. The single-molecule results for RB, R6G, R123, and 5-ROX, on TiO$_2$ are interpreted in the context of ensemble-averaged data and suggest that heterogeneity in electronic coupling and reorganization play a significant role in the observed dispersive kinetics.

Time-correlated single photon counting (TCSPC) measurements are implemented to study fluorescence in combination with single-molecule studies. Control experiments are taken to establish precision and accuracy in the TCSPC setup. The instrument response function (IRF) of the system is minimized to an appropriate full width at half maximum for lifetime measurements. Further work is needed on obtaining literature appropriate values for lifetime standards of common fluorophores. Once a complete series of lifetime standards are measured, fluorescence decay measurements from rhodamine dyes will enrich our model of the excited state in a dye-TiO$_2$ system.
TABLE OF CONTENTS

List of Figures .................................................................................................................................................. iii
List of Tables ...................................................................................................................................................... iv
Glossary ............................................................................................................................................................... v
Acknowledgements .............................................................................................................................................. vi

Chapter 1. Introduction ................................................................................................................................. 1
  1.1 Development of Efficient Solar Cells ................................................................................................. 1
  1.2 Single-Molecule Spectroscopy Studies of Electron Transfer ......................................................... 3
  1.3 Quantitative Analysis of Blinking Dynamics ..................................................................................... 5
  1.4 Outline .................................................................................................................................................... 7
  1.5 References ............................................................................................................................................. 10

  2.1 Introduction ........................................................................................................................................... 13
  2.2 Experimental ........................................................................................................................................ 15
    2.2.1 Bulk Characterization .................................................................................................................. 15
    2.2.2 Sample Preparation ..................................................................................................................... 16
    2.2.3 Single-Molecule Confocal Microscopy ....................................................................................... 17
    2.2.4 Blinking Analysis and Monte Carlo Simulations ....................................................................... 18
  2.3 Results and Discussion ......................................................................................................................... 19
    2.3.1 Adsorption Affinity and Driving Force for Photoinduced Electron Transfer ............................. 19
    2.3.2 Single-Molecule Photophysics: On-time and Off-time Distributions ......................................... 25
    2.3.3 Interpretation of the Log-normal Distribution with MC Simulations ..................................... 31
  2.4 Conclusion ............................................................................................................................................. 38
  2.5 References ............................................................................................................................................. 40

Chapter 3. Time-Correlated Single Photon Counting Studies of Dispersive Electron Transfer ......................... 44
  3.1 Introduction ........................................................................................................................................... 44
  3.2 Experimental ....................................................................................................................................... 46
    3.2.1 Sample Preparation .................................................................................................................... 46
    3.2.2 Confocal Microscopy and Time-Correlated Single Photon Counting ..................................... 46
  3.3 Results and Discussion ......................................................................................................................... 48
  3.4 Future Work ....................................................................................................................................... 51
  3.5 References ........................................................................................................................................... 53
**List of Figures**

<table>
<thead>
<tr>
<th>Figure Number</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1</td>
<td>Dye-sensitized solar cell schematic and energy level diagram</td>
<td>2</td>
</tr>
<tr>
<td>Figure 1.2</td>
<td>False-colored image of R6G molecules on TiO₂</td>
<td>5</td>
</tr>
<tr>
<td>Figure 1.3</td>
<td>Structures of RB, R6G, 5-ROX, and R123</td>
<td>8</td>
</tr>
<tr>
<td>Figure 2.1</td>
<td>Structures and absorbance spectra of rhodamine dyes</td>
<td>20</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Absorbance spectra of rhodamines in solution and TiO₂</td>
<td>21</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>Energy-level diagram of driving forces for rhodamines on TiO₂</td>
<td>23</td>
</tr>
<tr>
<td>Figure 2.4</td>
<td>On and off-time distributions of rhodamines on TiO₂</td>
<td>27</td>
</tr>
<tr>
<td>Figure 2.5</td>
<td>Power-law and log-normal fits for on-time distributions</td>
<td>29</td>
</tr>
<tr>
<td>Figure 2.6</td>
<td>Albery model for electron transfer</td>
<td>32</td>
</tr>
<tr>
<td>Figure 2.7</td>
<td>Adjustments to log-normal fit parameters</td>
<td>33</td>
</tr>
<tr>
<td>Figure 3.1</td>
<td>Fluorescence decay curve of RB in solution</td>
<td>49</td>
</tr>
</tbody>
</table>
List of Tables

Table Number

Table 2.1  Best-fit parameters for power-law and log-normal distributions ..................28
Table 2.2  Driving force interpreted with log-normal fit parameters ...........................35
Table 3.1  Multi-exponential lifetime components of RB ..........................................50
Glossary

5-ROX: 5-carboxy-X-rhodamine
APD: Avalanche photodiode detector
CCDF: Complementary-cumulative distribution function
CPD: Change-point detection
CV: Cyclic voltammetry
DSSC: Dye-sensitized solar cell
ET: Electron transfer
FWHM: Full width at half maximum
IRF: Instrument response function
KS: Kolmogorov-Smirnov
MC: Monte Carlo
MLE: Maximum likelihood estimation
R123: Rhodamine 123
R6G: Rhodamine 6G
RB: Rhodamine B
SMS: Single-molecule spectroscopy
TCSPC: Time-correlated single photon counting
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Chapter 1

Introduction

1.1 Development of Efficient Solar Cells

Global energy demands are projected to increase by ~30% within the next 30 years.\(^1\) As fossil fuels face rapid depletion, the solution for future energy consumption requires the development of sustainable energy sources. Renewable energy technologies (i.e., wind turbines, geothermal sources) are expected to overtake nonrenewable sources in meeting these energy demands, which will also reduce harmful greenhouse gas emissions.\(^1,2\) In particular, investment in solar energy technology is attractive because the amount of radiation that hits the Earth’s surface in one hour (i.e., \(4.3 \times 10^{20}\) J) provides more energy than humans consume in a year (i.e., \(4.1 \times 10^{20}\) J).\(^2\) Photovoltaic devices capture solar energy for electricity conversion based on the principle of charge separation at the interface of two materials with different conductivity.\(^3\) However, improving public access to solar cells precipitates the need for more cost-effective and efficient devices. In recent years, dye-sensitized solar cells (DSSCs) have emerged as an economically and technically viable alternative to traditional silicon-based solar cells with reduced production costs, tunable optical properties, and commercial integration potential.\(^3-6\)

Modern DSSCs are composed of dye sensitizers adsorbed onto nanocrystalline TiO\(_2\).\(^3,6-8\) Unfortunately, the efficiency of DSSCs has plateaued in recent years. Even with modifications to the dye sensitizer\(^9-11\) and metal-oxide semiconductor,\(^12,13\) the highest recorded device efficiency is only \(\sim 13\%\).\(^9,10\) Previous studies suggest that complex electron transfer (ET) dynamics at the dye-TiO\(_2\) interface can contribute to inefficient DSSC device performance.\(^14-24\) Although ET dynamics at this interface are poorly
understood, increased recognition of the importance of interfacial ET processes on DSSC efficiency has motivated studies to improve the understanding of these ET dynamics.\textsuperscript{14-24,28-31}

At the heart of the DSSC is photoinduced ET between the excited dye sensitizer and the TiO\textsubscript{2} semiconductor.\textsuperscript{3-6} In the DSSC, dye sensitizer molecules absorb sunlight, and electrons undergo photoexcitation (Figure 1). An electron is transferred to the conduction band of TiO\textsubscript{2} by electron injection to be converted into electricity at the electrode. Dye molecules are restored by electron donation from an electrolyte in order to repeat the solar energy conversion process. Occasionally, the electron can recombine with the dye from TiO\textsubscript{2} by charge recombination.\textsuperscript{14-16}

In an ideal DSSC system, electron injection should be maximized, while charge recombination is minimized.\textsuperscript{17} Thus, the efficiency of DSSCs is dependent upon the interfacial ET dynamics between the dye sensitizer and the semiconductor. Previous studies have shown that the ET kinetics at the dye-TiO\textsubscript{2} interface are complex and multiphasic due to the heterogeneous local environment of the dye molecule and variations in electronic coupling between the sensitizer and semiconductor.\textsuperscript{5,6,12-20} The heterogeneous ET kinetics are thought to contribute to inefficiency in DSSCs, but the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{(Left) DSSC schematic and (right) energy-level diagram of interfacial ET between a dye molecule and TiO\textsubscript{2}. ET dynamics are affected by the rates of photoexcitation ($k_{\text{exc}}$), injection ($k_{\text{inj}}$) into the conduction band (CB) of TiO\textsubscript{2}, and recombination of the electron from TiO\textsubscript{2} to the dye ($k_{\text{rec}}$).}
\end{figure}
photophysical processes underlying the complex kinetic behavior are not well understood.

While ensemble-averaged techniques provide averaged information about ET and a general characterization of the operating ET processes, these methods obscure the complexity of nanoscale behavior that occur at the dye-metal-oxide interface. Single-molecule spectroscopy (SMS) provides the ability to probe the impact of local environment and structure on ET behavior at the single-molecule level. This thesis will employ SMS to yield a complete characterization of the interfacial ET kinetics and its physical origins at the dye-TiO₂ interface in DSSCs.

1.2 Single-Molecule Spectroscopy Studies on Electron Transfer

Single-molecule spectroscopy (SMS) is a powerful tool that can resolve the full distribution of ET kinetics in DSSCs. The complexity and heterogeneity of the dye-TiO₂ environment makes it difficult for bulk characterization to fully analyze ET kinetics. Previous studies demonstrate that interfacial ET dynamics are better understood with SMS by determining the role of the sensitizer, surface adsorption sites, and binding orientation. For example, Jin et al. revealed that photocatalytic activity was dependent on the surfaces of the single crystal facets of TiO₂. Homogeneous polycrystalline TiO₂ films exhibited narrower distributions of ET rates relative to the heterogeneous nanoparticle films, which resulted in enhanced performance by the polycrystalline films. Since interfacial ET rates can vary between molecules, the ability to probe the connections between the local environment and ET kinetics with single-molecule resolution provides details hidden under ensemble-averaged measurements.
SMS can reveal the full distribution of ET rates in a heterogeneous environment rather than an average ET rate provided by ensemble-averaged measurements.

Single-molecule photophysics are often probed by measuring emissive (i.e., on state) and non-emissive events (i.e., off state) from single molecules.\textsuperscript{18-28} Specifically, molecules exhibit a phenomenon called blinking, which is characterized as the fluctuations in emissive intensities due to the population and depopulation of optically-bright and -dark vibronic states (Figure 1.2).\textsuperscript{18-28} Emissive events occur when a molecule undergoes fluorescence illustrated by pathway b in Figure 1.2 (i.e., emission of photons after relaxation to the ground state), and non-emissive events represent transfer into a dark state. When blinking occurs through a triplet state, the population and de-population processes of the triplet state follow first-order kinetics and are described by exponential functions. First-order rate constants for intersystem crossing into the triplet state and decay to the ground state can be extracted from the exponential fits.

However, several single-molecule studies report that the ET kinetics of dye-TiO\textsubscript{2} systems do not obey first-order kinetics. Instead, dispersive ET kinetics is observed, which indicates that the rate constants for population and de-population of the non-emissive state evolve over time.\textsuperscript{19-21,23-28} In the presence of TiO\textsubscript{2}, a fluorescing molecule that is producing on states can undergo electron injection (pathway c) to TiO\textsubscript{2}, which results in off states, or periods of non-emissive intensities. If the electron returns to the dye molecule through charge recombination (pathway d), the molecule can undergo fluorescence and produce on states once again. Therefore, the durations of the on states (i.e., on times) are associated with electron injection, and the durations of the off states (i.e., off times) correspond with charge recombination. Interpreting single-molecule
blinking dynamics will provide insight into the dispersive ET kinetics exhibited at the dye-TiO$_2$ interface.

**Figure 1.2** (Left) 7x7$\mu$m$^2$ false-colored fluorescent image of R6G molecules on TiO$_2$ are detected. Once a molecule is located (circled in red), it is continuously excited by a laser to produce blinking dynamics (right). The pathways an electron can undergo are: (a) photoexcitation, (b) fluorescence, (c) electron injection to TiO$_2$, or (d) charge recombination back to the ground state of the dye molecule. Intensity counts are photons detected from fluorescence. Threshold analysis (red line) determines the on and off states of each blinking trace.

### 1.3 Quantitative Analysis of Blinking Dynamics

The single-molecule blinking dynamics are quantified to connect changes in fluorescence to ET kinetics. The durations of on and off states (i.e., on times and off times, respectively) obtained from blinking dynamics are compiled into histograms and fit by probability functions.$^{20,25,27,28}$ The probability function that best fits the on- and off-time distributions can reveal information about electron injection and charge recombination, respectively. Heavy-tailed functions including power law, log-normal, and Weibull are able to model the dispersive ET kinetics in dye-TiO$_2$ systems.$^{20,27,28}$

Several studies observe that on and off-time distributions are best fit by the power-law model.$^{19,29,30}$ Although power laws are seen as a universal feature of blinking
and often used to interpret single-molecule photophysics, recent studies demonstrate that on- and off-time distributions, which appear power law on log-log axes, may not actually be characterized by power law.\textsuperscript{20,27,28,32} For example, Riley \textit{et al.} evaluated the validity of the power-law distribution describing two systems: single CdSe/CdS core-shell nanocrystals and single crystals of organic chromophore violamine R.\textsuperscript{27} A statistical approach was offered, which involved estimating the best fit parameters for a proposed function and employing a hypothesis test to determine the fit of the model to the experimental data.\textsuperscript{27,32} By using this robust analysis, it was discovered that power law did not describe these two systems as previously thought. Instead, alternatives to power law, such as the log-normal or Weibull distributions, were also tested for fitting with on and off-time distributions to characterize photophysical behavior. Additional studies demonstrated the importance of using robust statistical tests to interpret blinking measurements, since differences in fitting methods can produce varied interpretations of the same data.\textsuperscript{20,27,28}

Recently, Wong \textit{et al.} used single-molecule blinking measurements with rigorous statistical analyses to interpret the dispersive electron-transfer dynamics of rhodamine B (RB) and rhodamine 6G (R6G) on TiO\textsubscript{2}.\textsuperscript{20} Their analysis revealed that a mixture of heavy-tailed models described dispersive kinetics. Specifically, on-time distributions (i.e., electron injection) and off-time distributions (i.e., charge recombination) were best fit by power law and the log-normal function, respectively. By demonstrating that power laws are not a universal feature of all ET dynamics for single-molecules on TiO\textsubscript{2}, fit parameters associated with alternative models to power law can be used for ET dynamics interpretation. However, understanding the origins of these ET dynamics will require
modeling to provide greater insight into the kinetic models. Wong et al. determined that Monte Carlo (MC) simulations were capable of reproducing the functions describing dispersive kinetics behavior through artificial blinking distributions. In addition to clarifying the relationships between fit parameters and ET kinetics, MC simulations can also generate artificial blinking distributions outside of our experimental scope to investigate other hypotheses.

SMS presents the opportunity to probe dispersive ET kinetics in a series of rhodamine dyes on TiO$_2$. RB, R6G, rhodamine 123 (R123), and 5-carboxy-X-rhodamine (5-ROX) are known to be favorable dye sensitizers for SMS studies due to their high extinction coefficients and quantum yields for fluorescence. These rhodamine dyes are structurally analogous with their xanthene backbones but exhibit different modes of attachment to TiO$_2$ (Figure 1.3). Specifically, RB and 5-ROX possess carboxylic acids, which are demonstrated to be optimal anchoring groups to TiO$_2$ relative to the ethyl ester and methyl ester groups on R6G and R123, respectively. Since 5-ROX contains an additional carboxylic acid that is para relative to the xanthene backbone, 5-ROX is less sterically hindered to bind to TiO$_2$ than the ortho-substituted carboxylic acid

![Figure 1.3 Structures of 5-ROX, RB, R123, and R6G. RB and 5-ROX contain carboxylic acids for attachment to TiO$_2$. The para-substituted carboxylic acid on 5-ROX is less sterically hindered for binding. R6G and R123 possess an ethyl ester and methyl ester functional group, respectively.](image-url)
on RB. The variations in dye structure are expected to impact the adsorption geometries to TiO$_2$ and photophysical behavior in the presence of TiO$_2$. In order to properly evaluate blinking data and the resulting on and off-time distributions, a rigorous statistical approach is needed to fit the experimental data. MC modeling is then used to interpret the observed photophysical behavior. This multilateral and robust approach enables us to investigate ET kinetics at the dye-TiO$_2$ interface in order to understand the photophysical origins of dispersive ET behavior.

1.4 Outline

In this thesis, we present SMS studies of the ET dynamics in a dye-TiO$_2$ system. In Chapter 2, SMS is used to probe the impact of varying dye structure, adsorption affinities, and driving force for photoinduced ET on the dispersive ET dynamics of RB, R6G, 5-ROX, and R123 on TiO$_2$. The SMS studies and MC simulations reveal that both on and off-time distributions for all dyes are best represented by the log-normal distribution, which suggests that power laws are not universal. The bulk characterization and SMS analysis of the rhodamine dyes indicate that other factors, such as electronic coupling and reorganization energy, play a decisive role in ET kinetics for this rhodamine series on TiO$_2$ and should be included in future experimental and modeling studies.

Chapter 3 illustrates the expansion of the current spectroscopic system by incorporating time-resolved measurements. Previous studies have reported that electron injection at the dye-TiO$_2$ interface can occur on the timescale of nanoseconds to femtoseconds, while recombination rates range from the milliseconds to microseconds timescale. Thus, investigating this vast range of time regimes will provide further
insight into ET kinetics. The current SMS setup has a 10-ms time resolution, where intensity counts for blinking traces are obtained by the number of photons collected during 10-ms time bins from the sample. This time resolution can obscure ET events occurring on a timescale faster than the millisecond regime. Accordingly, improvements to the experimental setup will focus on overcoming the time-resolved limits in studying single molecules. Time-correlated single photon counting (TCSPC) provides the opportunity to probe ultrafast ET events by detecting single photons at a picosecond time resolution.\textsuperscript{37} This chapter outlines the initial control experiments to integrate TCSPC with SMS studies for future investigations on dispersive ET kinetics in a dye-TiO\textsubscript{2} system.
1.5 References


Chapter 2

Impact of Structure and Driving Force on the Dispersive Electron-Transfer Kinetics of Rhodamines on TiO$_2$

2.1 Introduction

Dye-sensitized solar cells (DSSCs) are sustainable and inexpensive solar devices that represent a promising strategy to meet growing energy needs.$^1$ DSSCs contain nanocrystalline TiO$_2$ films decorated with chromophore sensitizers (i.e., organic and inorganic dyes) that inject electrons from the molecular excited state of the sensitizer into the conduction band of the semiconductor upon photoexcitation.$^1$-$^3$ Dyes are regenerated either through electron donation from an electrolyte or recombination from TiO$_2$.$^4$ Although interfacial electron transfer (ET) dynamics of dyes on nanocrystalline TiO$_2$ films have been explored, these systems report complex and multiphasic ET kinetics.$^4$-$^{10}$ Ensemble-averaged techniques obscure spatial and temporal factors that contribute to this heterogeneous behavior. Therefore, single-molecule spectroscopy (SMS) has been used to investigate interfacial ET in the DSSC system.$^4$-$^{17}$

Single-molecule studies have observed that interfacial ET kinetics are described by nonexponential functions.$^7$-$^{10}$ Specifically, emissive (“on”) and non-emissive (“off”) temporal durations are fit by power law, which is consistent with dispersive kinetics, where the rate constants for population and de-population of the dark state (i.e., injection and recombination) are distributed.$^{19}$ Although power law appears to be a universal feature of single-molecule photophysics, recent studies reveal that data, which are qualitatively fit by power laws on log-log axes, may not be power-law distributed.$^8$-$^9$,$^{18}$-$^{20}$ For example, we recently used a rigorous statistical approach based on maximum
likelihood estimation (MLE) and Kolmogorov-Smirnov (KS) tests to interpret interfacial ET dynamics of rhodamine B (RB) and rhodamine (6G) on TiO$_2$. This statistical approach revealed that only parts of on-time distributions are fit by power law, and off-time distributions are log-normally distributed. These observations were reproduced with Monte Carlo (MC) simulations based on the Albery model$^{19}$ for dispersive kinetics (i.e., activation barriers for ET are Gaussian distributed). By demonstrating that emissive and non-emissive events on TiO$_2$ are not completely described by power law, these results motivated additional questions about the log-normal distribution and its fit parameters. In particular, how are changes to the underlying ET kinetics revealed through the log-normal distributions?

To address this question, this study investigates the single-molecule interfacial ET kinetics of a series of rhodamine dyes with varying structure, driving forces for photoinduced ET, and adsorption affinities to TiO$_2$. Both 5-carboxy-X-rhodamine (5-ROX) and RB contain carboxyl groups for attachment to TiO$_2$, which is thought to improve potential binding to TiO$_2$ relative to dyes without carboxyl groups such as R6G and rhodamine (R123). Furthermore, 5-ROX possesses a carboxyl group in a para-substituted position relative to its backbone, which can increase access to TiO$_2$ relative to the ortho-substituted carboxyl group on RB. As a result, all the dyes are expected to exhibit different driving forces and adsorption geometries on TiO$_2$. Using a combination of ensemble-averaged studies with single-molecule blinking measurements, we can investigate the impact of chromophore structure, driving force, and adsorption to TiO$_2$ on the dispersive ET dynamics of RB, 5-ROX, R6G, and R123 on TiO$_2$. The role of the
underlying kinetics on log-normal fit parameters is investigated with MC simulations to provide insight into the origin of log-normal behavior.

2.2 Experimental

2.2.1 Bulk Characterization

Solution UV/vis and fluorescence measurements were performed on a Perkin Elmer Lambda 35 and Perkin Elmer LS-55 spectrophotometer, respectively. UV/vis reflectance measurements of dyes on anatase TiO₂ (Acros Organics, 98+/%) films on glass slides (Fisher) were performed on a Cary 60 spectrophotometer equipped with a fiber-optic coupler and diffuse reflectance probe. Titania films on glass were immersed in 10⁻⁴ M solutions of the dyes in acetonitrile for 12 hours and rinsed thoroughly with acetonitrile prior to absorbance measurements. The absorbance from dyed films was measured after repeated rinsing and soaking in acetonitrile over 72-hour periods in order to ensure that residual unbound dyes were removed from the films.

Electrochemical measurements (CH Instruments 620D) were performed in analytical grade acetonitrile with 0.1 M tetrabutylammonium hexafluorophosphate (TBAPF6) electrolyte in a standard three-electrode cell, with a standard calomel reference electrode (SCE). Platinum working and auxiliary electrodes were thoroughly polished with 0.5 µm alumina powder paste on a cloth-covered polishing pad and then rinsed with water and acetonitrile before each scan. Samples were degassed for 10 minutes with Ar prior to cyclic voltammetry (CV) measurements. Potentials were scanned between -1 and 2 V vs. SCE at a scan rate of 200 mV/s. Ferrocene was used as a reference standard.
2.2.2 Sample Preparation

RB (99+%), R6G (99%), and R123 (99+) were used as received from Acros Organics. 5-ROX (5-carboxy-X-rhodamine, triethylammonium salt) was obtained from ThermoFisher Scientific. Titanium isopropoxide (98+%), isopropanol, and hydrochloric acid were used as received from Sigma Aldrich. Deionized water (18.2 MΩ cm) was obtained using a water purification system (ThermoScientific, EasyPure II). Glass coverslips (Fisher Scientific, 12-545-102) were cleaned in a base bath for 24 hours, thoroughly rinsed with deionized water, and dried using clean dry air (McMaster Carr, filter 5163K17). Colloidal suspensions of anatase TiO$_2$ nanoparticles were synthesized by the hydrolysis of titanium isopropoxide.$^{17}$ Briefly, 2 mL of titanium isopropoxide in isopropanol was slowly injected into 20 mL of acidified water (pH ~1.5, adjusted with HCl). The resulting colloidal suspension exhibited an absorbance maximum at approximately 280 nm. All dye solutions were prepared in deionized water using base-treated glassware. For single-molecule measurements on bare glass, samples were prepared by spin-coating 35 μL of a 10$^{-9}$-10$^{-10}$ M dye solution onto a clean cover slip using a spin coater (Laurrell Technologies, WS-400-6NPP-LITE) operating at 3000 rpm. For single-molecule measurements on colloidal TiO$_2$, 100 μL of ~10$^{-9}$ M dye solution was diluted to a final concentration of ~10$^{-10}$ M with 900 μL of colloidal TiO$_2$ (~500 mg/L). A 35-μL aliquot of the resulting 10$^{-10}$ M dye/TiO$_2$ solution was spin-coated on a clean glass cover slip at 3000 rpm. The resulting samples were mounted in a custom designed flow cell for environmental control and flushed with dry N$_2$ throughout the single-molecule experiments.
2.2.3 Single-Molecule Confocal Microscopy

Samples for single-molecule studies were placed on a nanopositioning stage (Queensgate, NPS-XY-100B or Physik Instrumente LP E-545) atop an inverted confocal microscope (Nikon, TiU). Continuous laser excitation at 532 nm (Spectra Physics, Excelsior) was focused to a diffraction-limited spot using a high numerical aperture (NA) 100× oil-immersion objective (Nikon Plan Fluor, NA = 1.3). Excitation powers ($P_{exc}$) of ~1 µW and ~5 µW at the sample were used for single-molecule measurements on glass and TiO$_2$, respectively. Epifluorescence from the sample was collected through the objective, spectrally filtered using an edge filter (Semrock, LP03-532RS-2S), and focused onto an avalanche photodiode detector (APD) with a 50-µm aperture (MPD, PDM050CTB) to provide confocal resolution. A custom LabView program was used to control the nanopositioning stage in 100-nm steps and collect emission. A z-axis microscope lock (Applied Science Instruments, MFC-2000) was used to maintain the focal plane of the objective during raster scans. Single-molecule emission was established based on the observation of diffraction-limited spots, irreversible single-step photobleaching, and concentration dependence of the diffraction-limited spot density. The number density of molecules (i.e., ~10 molecules per 100 µm$^2$) was equivalent for 10$^{-10}$ M dye spun coat on TiO$_2$ as well as bare glass, demonstrating that single-molecule studies on TiO$_2$ probed the majority of molecules. Blinking dynamics were acquired using a 10-ms integration time for ~200 s or until the single-step photobleaching event occurred. The blinking dynamics of RB, R6G, R123, and 5-ROX on glass were measured and analyzed as a control. Consistent with previous work,$^8$ the on-time and off-time distributions for rhodamines on glass are modified with respect to the TiO$_2$ data in terms
of the probability distribution functions that best represent the data as well as corresponding fit parameters (Appendix A, Table A1).

2.2.4 Blinking Analysis and Monte Carlo Simulations

Blinking dynamics were analyzed using the change-point detection (CPD) method,\textsuperscript{15,16} which reports statistically-significant intensity change points as well as the number and temporal durations for up to 30 intensity levels. The first and last events were disregarded, since they are artificially set by the observation period. The lowest deconvolved intensity state is designated as non-emissive (off). Deconvolved states with intensities greater than one standard deviation above the rms noise (i.e., \(~\text{20\% of the maximum emission intensity}\)) are denoted as emissive (on). Throughout the manuscript, the temporal durations of statistically-significant emissive and non-emissive intensity levels are termed on times and off times, respectively. Consistent with previous studies,\textsuperscript{8,18} the experimental on-time and off-time distributions are converted into complementary cumulative distribution functions (CCDFs) that describe the probability of an event occurring in a time greater than or equal to \( t \) according to:

\[
CCDF = 1 - \frac{1}{N} \sum_{i} t_i \leq t.
\]

The CCDF is presented for better visualization of the data and corresponding fits since blinking events are most probable at short times. For clarity, we use the term probability distribution for CCDF throughout the manuscript. The fit parameters and corresponding goodness-of-fit between the experimental CCDFs and proposed functional forms (i.e., power law, log-normal, and Weibull) are quantified using the MLE and KS statistic (i.e., \( p \)-value) described later in the chapter.\textsuperscript{8,18,20} Standard errors in the fit
parameters are determined by calculating the inverse of the Hessian matrix (i.e., the second derivative of the log-likelihood with respect to the function).\textsuperscript{20}

Blinking dynamics were simulated by generating population trajectories. A random number is compared to the probability of leaving the occupied electronic state (i.e., \( P_l = \sum_j k_{ij} t \), where \( t \) is the 1-ns computational time step). In each blinking simulation, the molecule starts from the ground state and can proceed to the excited state if the probability of excitation (i.e., \( P_1 = k_{12} t \)) exceeds a random number. An emission event occurs if the random number exceeds the fluorescence quantum yield, \( \Phi_f = k_{21}/(k_{21} + k_{23}) \), in which case a count is added to the macroscopic (i.e., 10-ms) time bin. Simulated fluorescence intensity trajectories were analyzed using thresholding, consistent with previous work.\textsuperscript{8} All Monte Carlo simulations were performed in Matlab (version R2015b) with custom code (Appendix D).

\section*{2.3 Results and Discussion}

\subsection*{2.3.1 Adsorption Affinity and Driving Force for Photoinduced Electron Transfer}

Ensemble-averaged measurements by absorbance and fluorescence provided insight into the binding ability of the dyes to TiO\textsubscript{2} via different anchoring groups. Although these ensemble-averaged measurements do not reflect the complexity of photophysical behavior amongst these dyes, interpretation of single-molecule data is supplemented by ensemble-averaged data. For initial characterization of the rhodamine dyes, the absorbance spectra of R123, R6G, RB, and 5-ROX in aqueous solution demonstrate single maxima at 500 nm, 526 nm, 554 nm, and 578 nm, respectively (Figure 2.1). Corresponding fluorescence studies demonstrate the dyes exhibit Stokes
shifts of ~22 nm (Appendix A, Figure A1). The extent of molecular adsorption to TiO\(_2\) can be probed by the diffuse reflectance spectra of rhodamine dyes on titania films. Reflectance measurements are especially useful for measuring samples that are in powder or crystalline form rather than liquid media.\(^{21}\) For these studies, TiO\(_2\) films on glass were immersed in solutions containing 10\(^{-4}\) M dye and thoroughly rinsed with acetonitrile prior to measurement. The resulting absorbance spectra of R123, R6G, RB, and 5-ROX on anatase TiO\(_2\) display broadened maxima relative to solution form at approximately 515 nm, 520 nm, 520 nm, and 575 nm, respectively (Figure 2.2). R6G and R123 on TiO\(_2\) exhibit modest maximum absorbance (\(A_{max}\)) values of 0.10 and 0.17, respectively, indicating poor adsorption to TiO\(_2\) due to the absence of carboxyl groups that would promote binding.\(^{10,11}\) R123 exhibits slightly more adsorption to TiO\(_2\) relative to R6G, which may be related to steric considerations (i.e., the methyl ester group on R123 has less steric bulk than the ethyl ester group on R6G). In contrast, both carboxyl-containing RB and 5-ROX exhibit persistent absorbance on TiO\(_2\) after repeated rinsing, given by \(A_{max}\) values of 0.68 and 1.0, respectively. 5-ROX displays strong adsorption to TiO\(_2\) because 5-ROX has a para-substituted carboxyl group (relative to the xanthylum
backbone), which increases access for binding relative to the ortho-substituted carboxyl group in RB. Ultimately, the data in Figure 2.2 demonstrate that the relative adsorption to TiO$_2$ decreases as follows: 5-ROX > RB > R123 > R6G.

The absorbance spectra of 5-ROX and RB on TiO$_2$ also exhibit hypsochromic shifts (i.e., blue shift) and significant broadening relative to solution. For example, the absorbance spectra of 5-ROX in solution and on TiO$_2$ exhibit main absorbance peaks at approximately 575 nm, corresponding to full width at half-maximum (FWHM) values of 36 nm and 143 nm, respectively. The absorbance peak at 554 nm for RB in solution (FWHM = 31 nm) is blue-shifted by $>30$ nm and broadened upon adsorption to TiO$_2$ (i.e., FWHM = 93 nm). Although previous studies have shown that the photodegradation of RB can also result in hypsochromic shifts,$^{22}$ these shifts only occurred after exposure to continuous light. Here, the dye-sensitized films were prepared and stored in the dark. Previous studies on chalcogenorhodamine dyes have demonstrated the controlled formation of H-aggregates on TiO$_2$ by tuning the dye-surface orientation through structure modification and anchoring mode.$^{23-25}$ For example, chalcogenorhodamines with 3-thienyl-2-carboxy...
groups adsorbed in amorphous monolayers to TiO$_2$, due to the steric influence from the 2-carboxy group, which prevented coplanarity and H aggregation.$^{23}$ In contrast, 2-thienyl-5-carboxy-substituted dyes exhibited H aggregation in addition to monomers, since the 5-carboxy group has little steric impact on the xanthylium backbone and enables coplanarity of the dyes.$^{11,23,25}$ Therefore, the formation of H-aggregates (i.e., from plane-to-plane $\pi$ stacking) on TiO$_2$ represents a more plausible explanation for the observation of blue-shifted absorbance and broadening for 5-ROX and RB on TiO$_2$ relative to solution.$^{11,23-25}$ Although aggregation does not play a role in single-molecule photophysics, the preferential orientation of dyes on TiO$_2$ is expected to play a role in ET dynamics. The absorbance spectra of R123 and R6G are relatively unchanged upon adsorption to TiO$_2$, consistent with amorphous adsorption of dye monomers to TiO$_2$ and lack of aggregation.

To estimate the driving force of photoinduced electron transfer ($\Delta G$) for R123, R6G, RB, and 5-ROX on TiO$_2$, the redox potentials of the dyes in solution were measured using cyclic voltammetry (CV). R123, R6G, RB, and 5-ROX exhibit oxidation potentials at $1.05 \pm 0.03$, $1.03 \pm 0.01$, $1.22 \pm 0.03$, and $1.03 \pm 0.01$ V vs. SCE, respectively, with the error corresponding to the standard deviation from the mean, consistent with previous CV measurements of rhodamines by Park and Bard.$^{26}$ The driving force for electron injection is estimated using the equation for the Gibbs energy of photoinduced electron transfer:$^{27}$

$$\Delta G = E_{ox}(D/D^+) - E_{red}(A/A^-) - E_{00}.$$  \hspace{1cm} (1)
In Equation 1, $E_{ox}(D/D^*)$ is the oxidation potential of the donor, $E_{red}(A/A^-)$ is the reduction potential of the acceptor (i.e., -0.49 V vs. SCE for TiO$_2$)\textsuperscript{,28} and $E_{00}$ is the singlet energy of the fluorophore, which is obtained from the intersection of the normalized aqueous absorption and fluorescence spectra of the dye.\textsuperscript{27} Using this equation, the driving force of photoinduced electron injection for R123, R6G, RB, and 5-ROX to TiO$_2$ were found to be $-0.88 \pm 0.03$, $-0.78 \pm 0.01$, $-0.47 \pm 0.03$, and $-0.56 \pm 0.01$ eV, respectively. Corresponding driving forces for charge recombination from TiO$_2$ to the HOMO of R123, R6G, RB, and 5-ROX, are $-1.54 \pm 0.03$, $-1.52 \pm 0.01$, $-1.71 \pm 0.03$, and $-1.52 \pm 0.01$ eV, respectively.\textsuperscript{29} Figure 2.3 summarizes the estimated driving forces for electron injection and recombination in an energy level diagram.

![Energy Level Diagram](image)

**Figure 2.3** The energy level diagram summarizes the ensemble-averaged driving forces for electron injection and recombination for the series of rhodamine dyes. The driving forces were estimated from oxidation and reduction potentials along with the singlet energy for the fluorophore.

These driving forces are related to the capacity for the electrons in a dye to undergo electron injection or charge recombination.\textsuperscript{30-32} For example, RB exhibited a driving force of -0.47 eV for injection, which is one of the smallest driving forces and
suggests that RB will experience one of the faster rates of electron injection due to decreased energetic hindrance. Conversely, RB experiences the largest driving force for recombination, so recombination rates for RB may be slow relative to other dyes. Ensemble-averaged driving forces can provide initial assessment for rates of injection and recombination, but single-molecule studies will need to be done in combination to provide a complete picture of the various contributions to actual ET kinetics.

In summary, RB and 5-ROX exhibit strong adsorption to TiO$_2$ due to the presence of carboxyl groups. In particular, 5-ROX has a para-substituted carboxyl group that facilitates access to TiO$_2$ binding, which is evident in the large $A_{max}$. Although aggregation effects will not impact single-molecule photophysics, understanding preferential orientation at the aggregate level can help us determine physical mechanisms of attachment that will most likely occur at single-molecule concentrations. Estimating the driving forces of these dyes with ensemble measurements can also assist with predictions about single-molecule behavior. Ultimately, the ensemble-averaged characterization of R123, R6G, RB, and 5-ROX demonstrates that the dyes exhibit a range of driving forces for electron transfer as well as a distribution of adsorption geometries on TiO$_2$.

2.3.2 Single-Molecule Photophysics: On-time and off-time distributions

To probe the impact of chromophore structure, energetics, and surface adsorption on the dispersive electron-transfer kinetics of rhodamine dyes on colloidal TiO$_2$, the blinking dynamics of R123, R6G, RB, and 5-ROX single molecules were measured and compiled into on-time and off-time probability distributions. To identify the functional
forms of the blinking dynamics from the rhodamines, the on-time and off-time distributions were fit to several heavy-tailed probability distribution functions (PDFs) using the combined maximum likelihood estimation (MLE) and Kolmogorov-Smirnov (KS) statistic approach. The MLE/KS approach circumvents problems associated with the least-squares linear regression fitting and visual inspection with log-log plots. Specifically, the combined MLE/KS method is used to determine the best-fit parameters to several hypothetical heavy-tailed models: power law, Weibull, and log-normal distributions. Clauset et al. applies the MLE/KS approach on the power-law model due to its pervasiveness in literature, especially in ET kinetics studies. In this method, the power law (i.e., \( P(t) = At^{-\alpha} \)), is normalized with an onset time \( (t_{\text{min}}) \) to yield:

\[
P(t) = \frac{\alpha^{-1}}{t_{\text{min}}} \left( \frac{t}{t_{\text{min}}} \right)^{-\alpha}, \quad \alpha > 1. \tag{2}
\]

\( \alpha \) is the power-law exponent, and only data that is greater than \( t_{\text{min}} \) is described by the power law. Next, the power law is converted into a cumulative distribution function (CDF), which represents the probability of observing an on or off event duration less than or equal to \( t \).

\[
CDF = S(t)_{\text{fit}} = \int_{t_{\text{min}}}^{t} P(t') dt' \tag{2}
\]

Consequently, experimental histograms of on and off-time events are converted into CDFs via summation.

\[
S(t)_{\text{data}} = \frac{1}{N} \sum_{i} t_{i < t} \tag{3}
\]

In order to determine the best fit parameters, all possible time values within a data set are posited as the true potential \( t_{\text{min}} \) to determine the potential power-law exponent \( (\alpha) \). Power laws with \( \alpha \) and \( t_{\text{min}} \) values are each assigned a KS statistic \( (D) \),
which represents the maximum deviation between the CDFs of the raw data and functional fit:

\[ D = \max_{t \geq t_{\text{min}}} \left| S(t)_{\text{data}} - S(t)_{\text{fit}} \right| \] (4)

The power-law fit parameters that minimize \( D \) (i.e., \( D = 0 \)) indicate the best fit to the experimental distribution because that means there is little deviation between the data and the functional fit. Therefore, the \( \alpha \) and \( t_{\text{min}} \) with the lowest \( D \)-value best describes the on- or off-time distribution.

Once the best-fit parameters are determined, the validity of these parameters and the functional model chosen in representing the experimental data are tested. The corresponding goodness of fit is determined using a KS test, which quantifies the distance between the empirical data and hypothesized model in a \( p \)-value. To start calculating the \( p \)-value, the best-fit parameters are used to generate synthetic data sets, which are each given its own synthetic KS statistic (i.e., \( D_{\text{synth}} \)). Then the \( p \)-value is determined by comparing the \( D_{\text{synth}} \) with the original \( D \) value of the blinking data. Synthetic data sets with KS statistics (\( D_{\text{synth}} \)) greater than the KS statistic of the experimental data (\( D \)) are part of the \( p \)-value calculation, where \( N_s \) represents the number of synthetic data sets.

\[ p = \frac{\sum_{D \leq D_{\text{synth}}} \left| D_{\text{synth}} \right|}{N_s} \] (5)

If \( p = 0 \), the empirical data and the model are fundamentally different, since \( D \) is greater than \( D_{\text{synth}} \). A \( p \)-value of < 0.05 indicates that the model is not consistent with the data and is considered insignificant, since the model has less than a 5% probability of reproducibly fitting the data by chance.\(^{20}\) If \( p = 1 \), differences between the empirical data and the model are attributed to statistical fluctuations. Thus, the probability that the data is represented by the hypothetical model increases as the \( p \)-value approaches unity. This
MLE/KS analysis is also applied to interpretation of the log-normal and Weibull functions with their corresponding fit parameters.

**Figure 2.4** presents the resulting on-time and off-time probability distributions for 91, 141, 70, and 150 molecules of R123, R6G, RB, and 5-ROX, respectively, on TiO\(_2\). The distributions are given as the complementary CDF (CCDF = 1-CDF) for better visualization, since blinking events are more likely to occur at short times.\(^8\) We find that the CCDFs, from here on referred to as the probability distribution, are broad and distinct between fluorophores, indicating heterogeneity between molecules and dyes. For example, the on-time distribution for 5-ROX on TiO\(_2\) contains 555 events, with individual values ranging from 0.02 s to 42.36 s, and an average on time of 1.82 s (**Figure 2.4a**).

The corresponding off-time distribution for 5-ROX/TiO\(_2\) contains 327 events, with an average off time of 18.52 s and individual values ranging from 0.05 s to 138.26 s (**Figure 2.4b**).

**Table 2.1** presents the best-fit parameters and corresponding \(p\)-values for power-law and log-normal fits to the blinking data for RB, R6G, 5-ROX, and R123, on TiO\(_2\). Significant \(p\)-values (i.e., \(p \geq 0.05\)) are in bold. Corresponding MLE/KS analysis for
Weibull (i.e., stretched exponential) distributions yielded statistically-insignificant results (Table A2). The fitting results presented in Table 2.1 demonstrate that the off-time distributions for RB, R6G, and R123, on TiO₂ are well represented by log-normal distributions, consistent with previous results and the observation of statistically-significant p-values (i.e., ranging from 0.08 to 0.68).

However, interpretation of the on-time distributions as well as the off-time distribution for 5-ROX/TiO₂ is less straightforward, but provides a window into which we can assess the application of the power-law model. According to the p-values alone, the on-time distribution for 5-ROX/TiO₂ are power-law distributed with \( p = 0.53 \). The corresponding best fit to a log-normal distribution with \( \mu = -0.96 \pm 0.06 \) and \( \sigma = 1.48 \pm 0.05 \) demonstrates a relatively low p-value of 0.03.

<table>
<thead>
<tr>
<th></th>
<th>( t_{\text{min}} ) (s)</th>
<th>( \alpha )</th>
<th>( \rho )</th>
<th>( \mu )</th>
<th>( \sigma )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ON</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RB</td>
<td>0.66</td>
<td>2.53 ± 0.06</td>
<td><strong>0.16</strong></td>
<td>-1.90 ± 0.05</td>
<td>1.25 ± 0.03</td>
<td>0</td>
</tr>
<tr>
<td>R6G</td>
<td>0.76</td>
<td>2.22 ± 0.05</td>
<td><strong>0.23</strong></td>
<td>-1.52 ± 0.05</td>
<td>1.34 ± 0.04</td>
<td>0</td>
</tr>
<tr>
<td>5-ROX</td>
<td>1.83</td>
<td>2.25 ± 0.05</td>
<td><strong>0.53</strong></td>
<td>-0.96 ± 0.06</td>
<td>1.48 ± 0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>R123</td>
<td>0.63</td>
<td>2.21 ± 0.08</td>
<td><strong>0.24</strong></td>
<td>-1.18 ± 0.09</td>
<td>1.37 ± 0.06</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>OFF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RB</td>
<td>1.25</td>
<td>1.71 ± 0.06</td>
<td>0.04</td>
<td>0.70 ± 0.13</td>
<td>1.56 ± 0.09</td>
<td><strong>0.68</strong></td>
</tr>
<tr>
<td>R6G</td>
<td>14.14</td>
<td>2.78 ± 0.09</td>
<td>0.02</td>
<td>1.09 ± 0.07</td>
<td>1.45 ± 0.05</td>
<td><strong>0.25</strong></td>
</tr>
<tr>
<td>5-ROX</td>
<td>117.58</td>
<td>22 ± 1</td>
<td><strong>0.94</strong></td>
<td>1.7 ± 0.1</td>
<td>1.77 ± 0.07</td>
<td>0.01</td>
</tr>
<tr>
<td>R123</td>
<td>37.72</td>
<td>2.7 ± 0.1</td>
<td><strong>0.06</strong></td>
<td>2.2 ± 0.1</td>
<td>1.68 ± 0.07</td>
<td><strong>0.08</strong></td>
</tr>
</tbody>
</table>

Table 2.1 The best-fit parameters for power-law and lognormal distributions corresponding with the on and off-time distributions of RB, R6G, R123, and 5-ROX are presented. Statistically significant p-values are in bold. Errors represent one standard deviation.
Although the observation of significant $p$-values (i.e., ranging from 0.16 to 0.53) seems to support the power-law hypothesis for the on-time distributions, only a minor portion of the data are actually represented by power laws (Figure 2.5a). In particular, the power law is only operative after the onset time (i.e., $t_{min}$) that is determined using MLE/KS analysis. For 5-ROX/TiO$_2$, the $t_{min}$ value of 1.83 s means that the power law represents only 16% of the on-time distribution. Similarly, power laws are only operative for 14%, 19%, and 24% of the on-time distributions for RB, R6G, and R123, on TiO$_2$, respectively.

Although off-time distribution for 5-ROX/TiO$_2$ is best fit to a power law according to its $p$-value of 0.94, the power law represents only 2% of off times. The MLE/KS analysis demonstrates that the on-time distributions are only power-law distributed above onset times of ~1 s or longer, but for a majority of the data the power-law hypothesis is rejected. Therefore, statistical tests reveal that the significance of the power-law fit by the $p$-value must be taken into context with the onset time of power-law behavior. Since MLE/KS analysis revealed that the power law does not describe the
entire on-time distribution, alternatives to the power-law model may better describe these empirical distributions of blinking data.

Previous single-molecule studies of xanthylum chromophores in crystal and semiconductor environments have shown that log-normal distributions are a viable alternative to the power-law model for blinking. The log-normal distribution occurs when the logarithm of the sampled variable is normally distributed according to:

$$P(t) = \frac{1}{\sqrt{2\pi}\sigma t} e^{-\left[\frac{\log(t) - \mu}{2\sigma^2}\right]^2}$$

(6)

where the fit parameters $\mu$ and $\sigma$ correspond to the geometric mean and standard deviation of the variable’s natural logarithm, respectively. Indeed, the best-fit parameters and associated $p$-values presented in Table 2.1 demonstrate that log-normal distributions most closely represent the complete on-time distributions for RB, R6G, 5-ROX, and R123, on TiO$_2$ (Figure 2.5b) given by several $p$-values $\geq 0.01$. Although these $p$-values are insignificant, they indicate a small possibility of fitting to the experimental data in contrast to the $p$-values for Weibull fits to the on-time distributions, which are all equal to zero (Table A2). With this condition in mind, the off-time distribution for 5-ROX/TiO$_2$ is best fit to a log-normal distribution (i.e., $\mu = 1.7 \pm 0.1$, $\sigma = 1.77 \pm 0.07$, and $p = 0.01$), consistent with the fitting results for RB, R6G, and R123, on TiO$_2$.

Furthermore, the log-normal fit parameters are sensitive to changes in environment (i.e., on glass substrates, Table A1) and sensitizing molecule. For example, the geometric mean of the log-normal distribution of on times ($\mu_{on}$) is modified with sensitizing molecule (Table 2.1). The corresponding $\sigma$ values (i.e., $\sigma_{on}$) range from $1.25 \pm 0.03$ for RB to $1.48 \pm 0.05$ for 5-ROX. Accordingly, the geometric mean of the off-time distribution ($\mu_{off}$) is also modified with sensitizing molecule (Table 2.1) and
\(\sigma_{\text{off}}\) values range from 1.45 ± 0.05 for R6G to 1.77 ± 0.07 for 5-ROX. The log-normal fit parameters are distinct for each molecule, which will allow us to interpret physical meaning between changes to the sensitizer and subsequent ET dynamics. Altogether, the MLE/KS analysis reveals that log-normal distributions, not power laws, most closely represent the entire on-time and off-time distributions for RB, R6G, R123, and 5-ROX, on TiO\(_2\). In order to determine the significance of the log-normal distribution, modeling will be used to address the impact of underlying kinetics on the log-normal fit parameters.

**2.3.3 Interpretation of the Log-normal Distribution with MC Simulations**

Monte Carlo (MC) simulations bridge theory and experiment to gain insight into DSSC mechanisms. Previous studies demonstrated that modeling the energetic dispersion, trap sites, and morphology in a heterogeneous DSSC system reproduces the dispersive kinetic behavior for further insight into ET dynamics.\(^{33-35}\) More recently, Monte Carlo simulations based on the Albery model\(^8,19\) for dispersive electron-transfer kinetics reproduced the power-law (i.e., after an onset time) and log-normal distributions for RB and R6G on TiO\(_2\).\(^8\) In order to understand the physical meaning of the changes in the log-normal fit parameters for on and off-time distributions, Monte Carlo simulations can provide more context about the physical system of the underlying ET kinetics.

These simulations are based upon a three-level system that includes a singlet ground state (1), a singlet excited state (2), and a non-emissive state corresponding to electron transfer to the semiconductor (3). The photoexcitation (\(k_{12}\)) and fluorescence (\(k_{21}\)) rate constants were set using the experimental laser power, absorption cross-section of the dye, and the reported fluorescence lifetime of R6G on TiO\(_2\).\(^{36}\) The rate constants
for dark-state population \((k_{23})\) and depopulation \((k_{31})\) were approximated using the Albery\(^{19}\) model for dispersive electron-transfer kinetics (Figure 2.6).

**Fig. 2.6** Kinetic model used in MC simulations. The three electronic levels correspond to the ground state (1), excited state (2), and the non-emissive state (3). \(k_{12}\) and \(k_{21}\) are the rates of excitation and fluorescence, which are set by experimental conditions. \(k_{23}\) and \(k_{31}\) correspond with dark-state population (i.e., FET) and de-population, (i.e., BET) and are log-normally distributed (dashed arrows), which is consistent with a Gaussian distribution of energy barriers.

In this model, electron transfer proceeds along a Gaussian distribution of activation energies \((\Delta G_{ij}^\ddagger)\) according to:

\[
\Delta G_{ij}^\ddagger = (\Delta G_{0}^\ddagger)_{ij} + \gamma x k_B T. \tag{7}
\]

Here, \(i\) and \(j\) refer to the initial and final states, respectively, \(\Delta G_{0}^\ddagger\) is the average activation energy, \(\gamma\) determines the magnitude of the energetic dispersion about the mean activation energy \((\Delta G_{0}^\ddagger)\), \(x\) is a random number selected from a Gaussian distribution, and \(k_B\) is the Boltzmann constant. Inserting the Gaussian distribution into the Arrhenius equation yields a log-normally distributed rate constant expression:

\[
k_{ij} = \kappa_{ij} e^{-\gamma x} \tag{8}
\]

where \(\kappa_{ij}\) is an average, first-order rate constant corresponding to the mean activation barrier (i.e., \(\kappa_{ij} = e^{-(\Delta G_{0}^\ddagger)_{ij}/k_B T}\)). Thus, the Albery model predicts that the rate constants for dark-state population and depopulation (i.e., injection and recombination, respectively) are log-normally distributed.
We varied the input parameters for injection (i.e., $\kappa_{23}$), recombination (i.e., $\kappa_{31}$), and the energetic dispersion (i.e., $\gamma$) to reproduce experimental on and off-time distributions. Once the simulated distributions were generated, they were analyzed by MLE/KS analysis to determine the best fit to power-law, log-normal and Weibull distributions. The simulations revealed the impact of the input parameters (i.e., $\kappa_{23}, \kappa_{31}, \gamma$) on the log-normal distributions. Moreover, the log-normal fit parameters (i.e., $\mu$ and $\sigma$) were interpreted in the context of ET rates and energetic dispersion, giving credence to the use of the log-normal model to characterize on and off-time distributions.

The measured on-time and off-time distributions for RB, R6G, 5-ROX, and R123, on TiO$_2$, are best represented by log-normal distributions (Figure 2.5). To understand the observed changes in $\mu_{on/off}$ and $\sigma_{on/off}$ with sensitizing molecule, we performed a series of Monte Carlo simulations to investigate the impact of the underlying physical processes, represented by the input parameters $\kappa_{23}$, $\kappa_{31}$ and $\gamma$, on the resulting log-normal fit parameters (Figure 2.7). The log-normal distribution was reproducible for on and off-times blinking data using MC simulations. For these simulations, $\kappa_{23}$ and $\kappa_{31}$ were set to values that are consistent with the reported

![Probability vs. On Time (s)](a)

![Probability vs. Off Time (s)](b)

![Probability vs. On Time (s)](c)

**Fig. 2.7** Adjustments to log-normal fit parameters reveal that increases in (a) $\kappa_{23}$, (b) $\kappa_{31}$, and (c) $\gamma$ corresponds with changes in the on times, off times, and energetic dispersion, respectively.
ensemble-averaged injection and recombination rate constants of xanthylum dyes on TiO$_2$.\textsuperscript{37,38} An increase in $\kappa_{23}$ (i.e., the average rate constant for electron injection) is expected to decrease the average on time ($\langle t_{on} \rangle$) and the associated location parameter of the on-time distribution ($\mu_{on}$), since on times are dependent on the excitation rate constant and quantum yield for injection according to:

$$t_{on} = \left[ \frac{k_{23}}{(k_{23} + k_{21}) k_{12}} \right]^{-1}. \quad (9)$$

Figure 2.7a demonstrates as $\kappa_{23}$ is increased from $10^{10}$ s$^{-1}$ to $10^{12}$ s$^{-1}$, with $\kappa_{31}$ and $\gamma$ set to $10^6$ s$^{-1}$ and 16, respectively, the on-time distribution is shifted to shorter times and $\mu_{on}$ is decreased (i.e., from $-2.3 \pm 0.4$ to $-2.9 \pm 0.3$). Similarly, as $\kappa_{31}$ is increased from $10^6$ s$^{-1}$ to $10^9$ s$^{-1}$, with $\kappa_{23}$ and $\gamma$ fixed at $10^{12}$ s$^{-1}$ and 3, respectively, $\mu_{off}$ is decreased from $0.9 \pm 0.1$ to $-2.52 \pm 0.02$ (Figure 2.7b), consistent with the inverse relationship between off-state lifetime and the rate constant for charge recombination (i.e., $t_{off} = k_{31}^{-1}$). According to the Albery model, the input parameter $\gamma$ determines the extent of energetic dispersion about the mean activation barrier. Therefore, an increase in $\gamma$ is expected to generate a concomitant increase in the standard deviation of ln$t_{on/off}$ (i.e., $\sigma_{on/off}$). Figure 2.7c demonstrates that increasing the energetic dispersion from 5 to 10, with $\kappa_{23} = 10^{12}$ s$^{-1}$ and $\kappa_{31} = 10^6$ s$^{-1}$, is correlated to an increase in the spread of the on-time distribution (i.e., $\sigma_{on}$ is modified from $1.08 \pm 0.03$ to $2.8 \pm 0.2$). Attempts to find the absolute rates of injection and recombination using these conditions are expanded upon in Appendix B.
<table>
<thead>
<tr>
<th></th>
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<td>1.34</td>
</tr>
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<td>R6G</td>
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</tr>
<tr>
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<tr>
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Table 2.2 Driving force for photo-induced electron transfer are given with the log-normal fit parameters for on-time and off-time distributions to provide context for single-molecule photophysics.

Relative rates of injection and recombination can be predicted, based on the simulation results presented in Figure 2.7, which demonstrate the influence of the average rate constant for injection and recombination as well as the extent of energetic dispersion on $\mu_{\text{on/off}}$ and $\sigma_{\text{on/off}}$. In short, $-\mu_{\text{on}}$ and $-\mu_{\text{off}}$ are proportional to the average rate constants for injection and recombination, respectively (i.e., $-\mu_{\text{on/off}} = \langle \ln(k_{\text{on/off}}) \rangle$). Values for $\sigma_{\text{on/off}}$ are proportional to the extent of energetic dispersion about the mean activation barrier. Table 2.2 summarizes the $-\mu_{\text{on/off}}$ and $\sigma_{\text{on/off}}$ values obtained from single-molecule measurements, showing that the average injection rate constant decreases as follows: RB > R6G > R123 > 5-ROX (i.e., Table 2.1, $-\mu_{\text{on}}$ is equal to 1.90 ± 0.05 for RB, 1.52 ± 0.05 for R6G, 1.18 ± 0.09 for R123, and 0.96 ± 0.06 for 5-ROX). The average rate constant for recombination decreases according to: RB > R6G > 5-ROX > R123 (i.e., $-\mu_{\text{off}}$ is 0.70 ± 0.13 for RB, 1.09 ± 0.07 for
R6G, $-1.7 \pm 0.1$ for 5-ROX, and $-2.2 \pm 0.1$ for R123). Comparison of the $\sigma$ values in Table 2.1 demonstrates that the energetic dispersion is largest for 5-ROX and smallest for RB on times and for R6G off times. What is the physical interpretation of these results?

According to Marcus theory, the rate constant for electron transfer is dependent on the driving force of the reaction ($\Delta G$) and the reorganization energy ($\lambda$) between reactant and product states:

$$k = A e^{-\frac{\left(-\Delta G + \lambda\right)^2}{4k_BT}} = A e^{-\frac{-\Delta G^\ddagger}{k_BT}}$$

(10)

where $A$ is a pre-exponential factor that depends on the electronic coupling between initial and final states as well as the density of unoccupied acceptor states.$^{39}$ In other words, the average activation energy for electron transfer ($\Delta G^\ddagger$) can be expressed in terms of $\Delta G$ and $\lambda$ (i.e., $\Delta G^\ddagger = \frac{-(\Delta G + \lambda)^2}{4\lambda}$). Table 2.2 summarizes the ensemble-averaged driving forces for electron injection and recombination alongside the $-\mu_{on/off}$ and $\sigma_{on/off}$ values obtained from single-molecule measurements. Comparison of $\Delta G$ and $-\mu_{on/off}$ values in Table 2.2 suggests that differences in the electronic coupling and reorganization energy play a decisive role in the electron-transfer kinetics for these rhodamines on TiO$_2$. For example, although RB demonstrates the smallest driving force for electron injection (i.e., $\Delta G_{inj} = -0.47$ eV), single-molecule data reveal that RB/TiO$_2$ exhibits the largest average rate constant for injection (i.e., $-\mu_{on} = \langle \ln(k_{on}) \rangle = 1.90$).

Corresponding single-molecule studies of RB, R6G, 5-ROX, and R123 on glass demonstrate that the average injection rate, obtained from relative $-\mu_{on}$ value, is smaller for molecules on glass relative to TiO$_2$ (Table A1), consistent with a decrease in driving
force for electron injection to glass as compared to TiO₂. Although estimated driving force for charge recombination is equivalent within error for R6G, 5-ROX, and R123, the corresponding charge recombination kinetics are distinct (Table 2.2), which suggests that recombination is not dependent on driving force.⁴⁰-⁴¹

The hypothesis that electronic coupling and reorganization energy are different for these rhodamine dyes on TiO₂ is consistent with the observation that 5-ROX and RB adsorb appreciably to TiO₂, while R6G and R123 do not (Figure 2.2). For example, previous studies demonstrated that introducing spacers between the anchoring groups (i.e., carboxyl group) and dye sensitizer results in a dramatic decrease in the injection and recombination rate constants, consistent with a reduction in electronic coupling.⁴²-⁴⁵ Furthermore, reorganization energy is dependent on the distance between the distance between the donor and acceptor, as well as the binding motif.⁴⁶-⁴⁷ These observations suggest that improvements to MC simulations should include the addition of driving force, electronic coupling, and reorganization energy to accurately model the single-molecule photophysics of rhodamines on TiO₂.

The dispersion in ET kinetics is modified by sensitizing molecule (Table 2.1), and the differences are manifested in the σ_on/off values and ensemble-averaged measurements. For example, 5-ROX/TiO₂ exhibits the most dispersion in ET rates, consistent with the largest σ_on/off values (i.e., σ_{on} = 1.48 and σ_{off} = 1.77). Ensemble-averaged measurements indicate that kinetic dispersion is related to the opportunities for dye-surface attachment (i.e., ortho- or para-carboxyl groups). Since 5-ROX has two carboxyl groups for potential binding to TiO₂, whereas RB possesses only one carboxyl group, RB is expected to exhibit less adsorption heterogeneity than 5-ROX, which shows
in smaller observed $\sigma_{on/off}$ values for RB/TiO$_2$. Therefore, 5-ROX/TiO$_2$ exhibits greater kinetic dispersion due to the possibility of adsorbing to TiO$_2$ through the para- or ortho-carboxyl group, which may result in a variety of adsorption orientation and geometry. Interestingly, R6G and R123 exhibit intermediate values for $\sigma_{on/off}$, demonstrating the contribution to kinetic dispersion due to unspecific adsorption to TiO$_2$. Altogether, the combination of single-molecule studies with robust MLE/KS analysis, Monte Carlo simulations, and ensemble-averaged measurements demonstrate that the log-normal distributions, which report on the dispersive injection and recombination dynamics on TiO$_2$, are dependent on chromophore structure, driving force, and adsorption affinity the semiconductor surface.

2.4 Conclusion

Through SMS and MLE/KS analysis, we can probe the interfacial ET dynamics on series of rhodamine dyes on TiO$_2$ with varying structure, driving force, and adsorption affinities to TiO$_2$. In combination with MC simulations and ensemble-averaged studies, these results reveal several new insights about dispersive ET kinetics for single molecules on TiO$_2$. First, both on-time and off-time distributions of RB, R6G, R123, and 5-ROX on TiO$_2$ are best represented by log-normal distributions. Although $p$-values indicate power-law fit significance for on-time distributions, the onset time of power-law behavior (i.e., $t_{min}$) reveals that less than 25% of the data for on-time events are described by power-law. Furthermore, the associated log-normal fit parameters are dependent on sensitizing molecule, which can be probed through MC simulations.
MC simulations based on the Albery model demonstrate that average rates of injection and recombination are proportional to \(-\mu_{on/off}\), respectively, and the \(\sigma_{on/off}\) values are proportional to the extent of energetic dispersion around the mean activation barrier. Altogether, these results show that the average injection rate constant decreases as follows: RB > R6G > R123 > 5-ROX, while the average rate constant for recombination decreases according to: RB > R6G > 5-ROX > R123. The energetic dispersion is largest for 5-ROX and smallest for RB. Our results suggest that dispersion from electronic coupling and reorganization energy play an important role in the ET kinetics of these series of rhodamine dyes on TiO\(_2\). These observations motivate further experimental and MC simulation studies in order to investigate the impact of dispersive electronic coupling and reorganization energy on injection and recombination. By incorporating these factors into experimental and simulation studies, our understanding of the ET kinetics at the dye-TiO\(_2\) interface in a model DSSC will be improved.
2.5 References

1. Gratzel, M. Conversion of Sunlight to Electrical Power by nanocrystalline dye-


3. Listorti, A.; O’Regan, B.; Durrant, J. Electron Transfer Dynamics in Dye-


Chapter 3

Time-Correlated Single Photon Counting Studies of Dispersive Electron Transfer

3.1 Introduction

Dye-sensitized solar cells (DSSCs) are an inexpensive alternative to inorganic silicon-based solar cells but have plateaued at efficiencies of ~13\%.\textsuperscript{1-3} As described in Chapter 2, device performance of DSSCs is related to the interfacial electron transfer (ET) kinetics between the dye sensitizer and semiconductor. Extensive studies of the ET kinetics at the excited state demonstrate that electron injection of dye-TiO\textsubscript{2} systems occur at subpicosecond timescales, which are 2-5 orders of magnitude faster than radiative decay from the excited state to the ground state.\textsuperscript{4-5} Thus, electron injection is generally not considered a limiting factor for device efficiency. Yet, studies find that interfacial ET kinetics are complex and multiphasic,\textsuperscript{6-12} with recent studies reporting that electron injection can actually occur on a range of timescales much slower than subpicoseconds.\textsuperscript{6-10} This vast range of timescales can be problematic for efficient electron injection because of introduced competition between different kinetic processes. According to equation 11, the lifetime of the excited state ($\tau_{on}$) is dependent upon the excitation rate constant ($k_{12}$), dark-state population ($k_{23}$), and emission dynamics ($k_{21}$):

$$\tau_{on} = \left[ \left( \frac{k_{23}}{k_{23}+k_{21}} \right) k_{12} \right]^{-1} \quad (11).$$

In a dye-TiO\textsubscript{2} system, $k_{23}$ and $k_{21}$ represent electron injection and fluorescence, respectively. Thus, the excited-state is impacted by the interaction of several complex kinetic processes. In an ideal system, electron injection should occur only on the ultrafast
timescales (e.g., femtoseconds - picoseconds), while fluorescence operates on the nanosecond timescale. If electron injection occurs much more slowly, it can operate in the same time regime as fluorescence, which introduces kinetic competition between the two processes at the excited state. This competition can decrease the efficiency of electron injection into the conduction band of the semiconductor, which reduces the productivity of a DSSC. Therefore, investigating the excited state in a dye-TiO$_2$ system will provide further insight into the dispersive ET kinetics.

Probing fluorescence offers a wealth of information about the excited state. The fluorescence lifetime ($\tau_{fl}$) is a convolution of fluorescence ($k_{fl}$) and injection ($k_{inj}$) dynamics along with non-radiative processes ($k_{nr}$) according to:

$$\tau_{fl} = \left[ \frac{k_{fl}}{k_{fl} + k_{inj} + k_{nr}} \right]^{-1} \quad (12).$$

Obtaining measurements on fluorescence lifetimes can yield insight into the complexities of the interactions of the kinetic processes in a dye-TiO$_2$ system. In particular, time-correlated single photon counting (TCSPC) is a well-established technique to exploring molecular photophysics by measuring and analyzing fluorescence decay curves.$^{13-17}$ Several properties of a fluorophore can be probed by TCSPC including absorption and fluorescence spectral properties, fluorescence quantum yield, fluorescence lifetime, and anisotropy.$^{17}$ TCSPC is based upon the detection of single photons – the arrival time of these fluorescent photons with respect to an exciting laser pulse is recorded by spectrally resolved and polarized detection channels.$^{15,17}$ By generating histograms of the relative times of photon arrival, the fluorescence kinetics decay curve is constructed.$^{13-17}$ TCSPC is an ideal technique for capturing multicomponent decays and ultrafast processes
because of its high sensitivity, fast response, and dynamic range.\textsuperscript{14-16} Moreover, when light levels are low, such as in single-molecule experiments, TCSPC is a valuable method to those studies since it requires very low photon counts.\textsuperscript{14} The next phase of studies TCSPC measurements will be taken on single molecules of rhodamine dyes on TiO\textsubscript{2} in order to increase our understanding of the interfacial ET kinetics in a dye-TiO\textsubscript{2} system.\textsuperscript{12,18}

This chapter focuses on efforts to construct an accurate SM-TCSPC setup by minimizing the instrument response function (IRF) and measuring the lifetimes of established lifetime standards. Obtaining accurate TCSPC measurements requires a short IRF, which is a measure of the instrument effect on fluorescence intensity measurements.\textsuperscript{13-15} Once the TCSPC setup has been rigorously evaluated for any inconsistencies, the technique can be used in combination with SMS to quantify the ET kinetics in model DSSCs.

3.2 Experimental

3.2.1 Sample Preparation

FluoSpheres, carboxylate-modified, 1.0 μm were used as received from Thermo Fisher Scientific. RB (99+%) was used as received from Acros Organics. Deionized water (18.2 MΩ cm) was obtained using a water purification system (ThermoScientific, EasyPure II). Glass coverslips (Fisher Scientific, 12-545-102) were cleaned in a base bath for 24 hours, thoroughly rinsed with deionized water, and dried using clean dry air (McMaster Carr, filter 5163K17). All dye solutions and fluorescent bead samples were prepared in deionized water using base-treated glassware. The fluorescent bead sample was prepared
by baking a 100 μL of 10^{-10} M FluoSpheres at 100°C for ~45 minutes in an oven. For lifetime-standard measurements deposited on glass, dye samples were prepared by spin-coating 35 μL of a 10^{-4}-10^{-6} M dye solution onto a clean cover slip using a spin coater (Laurell Tecnologies, WS-400-6NPP-LITE) operating at 3000 rpm. The resulting samples were mounted in a custom designed flow cell for environmental control and flushed with dry N\textsubscript{2} throughout the experiments.

3.2.2 Confocal Microscopy and Time-Correlated Single Photon Counting

Samples for single-molecule resolution and TCSPC studies were placed on a nanopositioning stage (Physik Instrumente LP E-545) atop an inverted confocal microscope (Nikon, TiU). Pulsed laser excitation at 470 nm at a 40 MHz repetition rate (PicoQuant, PDL 800-D) was focused to a diffraction-limited spot using a high numerical aperture (NA) 100× oil-immersion objective (Nikon Plan Fluor, NA = 1.3). Excitation powers \( P_{\text{exc}} \) of ~0.01 μW and ~1 μW at the sample were used for single-molecule measurements for the sample of fluorescent beads and dyes sample, respectively. Rayleigh scattering from the laser was eliminated by an excitation filter (Semrock, FF01-475/28-25), Epifluorescence from the sample was collected through the objective, spectrally filtered with a dichroic beamsplitter (Semrock, Di02-R488-25x36) and an edge filter (Semrock, BLP01-488R-25), and focused onto an avalanche photodiode detector (APD) with a 50-μm aperture (MPD, PDM050CTB) to provide confocal resolution. A custom LabView program was used to control the nanopositioning stage in 100-nm steps and collect emission. A z-axis microscope lock (Applied Science Instruments, MFC-2000) was used to maintain the focal plane of the objective during raster scans.
Once false-colored images of the fluorescent beads were obtained to ensure proper detector alignment (Appendix C), IRFs were measured on bare glass. After the IRF measurement, dye samples were examined as fluorescent lifetime standards with TCSPC instruments and hardware. Signals from the detector were sent to the TCSPC module (PicoQuant, PicoHarp 300). To ensure single photon counting statistics, the detected photon count rate is kept below 0.1% of the excitation pulse rate. Fitting of the fluorescent decay curves was accomplished with Fluofit software version 4.6 from PicoQuant for global fluorescence decay data analysis. The goodness of fit was determined by a reduced $\chi^2$ parameter and visual inspection of the weighted residuals.

### 3.3 Results and Discussion

The full width at half-maximum (FWHM) of the IRF was measured with a 470 nm laser at 40 MHz repetition rate in order to characterize the time resolution of the fluorescence decay measurements. **Figure 3.1** exhibits an IRF (red) obtained from blank glass (FWHM = 136 ps) fitted to a single exponential function. In addition to the short FWHM, **Figure 3.1** demonstrates that there is no secondary peak or long tail, which can obscure longer lifetime components from the fluorophore. Although APDs can attain IRFs using instruments with FWHMs as small as 35 ps, TCSPC measurements have been obtained for IRFs as large as 300 ps. In essence, IRFs can vary between studies as long as the fluorophore of interest has a lifetime range longer than the IRF. For R6G and RB, their measured lifetimes are in the nanosecond regime; therefore, the measured IRF in **Figure 3.1** is sufficient to use in future TCSPC measurements.
Fluorophores with known lifetimes are necessary for testing any systematic errors and calibration requirements. Lifetime standards for controls follow several criteria: i) exhibit single-exponential decay kinetics that are independent of excitation and emission wavelength, ii) large Stokes shift and quantum yield, and iii) chemical stability and photostability during measurements. Additionally, having a variety of fluorophores with lifetimes from nanoseconds to picoseconds will be important in order to test those timescales. Fluorophores including erythrosine B, coumarin 153, and RB are frequently used as lifetime standards.
Figure 3.1 depicts the fluorescence decay curve (blue) of $10^{-4}$ M RB in solution on glass. The fluorescence decay curve of RB is obtained with an IRF of 136 ps. Analysis with Fluofit software indicated this sample is best fit to a multi-exponential function with three lifetime exponential components (Table 3.1). The majority of the photon counts is fit by the shortest lifetime component (i.e., $\tau_1=2.45$ ns). The goodness-of-fit is indicated by the reduced $\chi^2$ value,$^{14,15,22}$ which is described by:

$$\chi^2 = \frac{1}{N-m-1} \sum_{i=1}^{N} \left[ \frac{(D_i-M_i)^2}{M_i} \right]. \quad (11)$$

Here, $D_i$ represents the experimental data point while $M_i$ represents a model prediction. $N$ is the total number of data points, and $m$ total number of fitting parameters. In other words, the reduced $\chi^2$ value is the sum of the squared residual errors divided by the reduction term ($N-m-1$). The goodness-of-fit is also evaluated by the weighted residuals (Figure 3.1), which are essentially deviation plots that show where a misfit of a data point occurs. Ideally, the weighted residuals should be randomly distributed around zero for a good fit.$^{15,22}$ However, additional goodness-of-fits tests should be conducted to determine if the fit is actually meaningful, since the $\chi^2$ value is sensitive to the summed deviations.$^{14,15,22}$

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<th>$\tau_3$ (ns)</th>
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<td>1.04 (7.99%)</td>
<td>0.08 (2.80%)</td>
<td>0.96</td>
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</table>

Table 3.1 Multi-exponential lifetime components of RB in solution. 89.21% of the photon counts are described by the first lifetime component, $\tau_1 = 2.45$ ns.

Previous TCSPC studies have shown that a $10^{-4}$ M solution of RB should exhibit a single exponential lifetime component of $1.72 \pm 0.04$ ns ($\lambda_{exc} = 488-575$ nm, and $\lambda_{em} =$
The observed discrepancy between experimental and literature results can be attributed to differences in the environment. Fluorescence is sensitive to environmental factors such as solvent polarity, proximity and concentration of quenching species, and pH of the aqueous medium. These environmental parameters can influence the activity of the fluorophore in the excited state. For example, the dipole moment of the solvent molecules can interact with the dipole moment of the fluorophore to yield an ordered distribution of the solvent around the fluorophore. When the fluorophore is excited and relaxes to the lowest vibrational energy level of the excited state, these solvent molecules can assist in stabilizing and further decreasing the energy level of the excited state by re-orienting around the fluorophore. Likewise, proximity of other fluorophore molecules can impact fluorescence lifetime by inducing the probability of other pathways (i.e., nonradiative) or even quenching of fluorophore molecules. Therefore, modification of the sample conditions may help change the environment of the lifetime standard to yield literature-appropriate values.

3.4 Future Work

Dispersive excited-state dynamics can be probed through fluorescence decay measurements using techniques such as TCSPC. This thesis demonstrates control over conditions to minimize the IRF in the TCSPC setup, with a minimized FWHM of 136 ps. Measurements of lifetime standards (i.e., RB) encounter disparities with literature values due to variations in sample setup. Once the environment of the standard is established according to literature precedents, a series of well-known fluorophores with single-exponential lifetimes will be measured to systematically test the TCSPC setup and
instruments. Establishing these standard measurements will ensure that TCSPC results from future studies on electron injection kinetics in dye-TiO$_2$ systems are reliable and accurate.

Time-resolved measurements from TCSPC can provide the desired insight into the complexity of the excited state. Future experimental studies will use TCSPC to focus on fluorescence decay measurements from the series of rhodamine dyes previously investigated in Chapter 2 (i.e., RB, 5-ROX, R6G, and R123). The combined single-molecule and TCSPC results will further elaborate on the following factors on ET kinetics: i) impact of substrate (i.e., glass and TiO$_2$), ii) varying structure, iii) adsorption affinity, and iv) driving forces. The lifetimes of these rhodamine dyes will be interpreted in context with SMS results to augment our understanding of interfacial kinetics in the DSSC model.

Additionally, MC simulations will be modified to include reorganization energy and electronic coupling in the calculation of the average rate constants for injection and recombination. Additional changes to the simulations will be adapted for consistency with observations from SMS and TCSPC results. Ultimately, the combination of single-molecule and temporally-resolved measurements will construct a highly refined model for dispersive ET kinetics for dyes on TiO$_2$. The increased understanding from this model will lend to efficient device design for DSSCs in the future.
3.5 References


Appendix A: Supplementary Information for Chapter 2

<table>
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<th>Weibull: $\frac{A}{B} \left(\frac{t}{B}\right)^{A-1} e^{-\left(\frac{t}{B}\right)^A}$</th>
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<td>$p$</td>
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</table>

Table A1. Best-fit parameters and $p$-values for 78, 85, 76, and 112 molecules of RB, R6G, 5-ROX, and R123 on bare glass for comparison to TiO$_2$. Errors represent one standard deviation. On-time distributions are not described well by any of the functions, and off-times are log-normally distributed.
**Figure. A1** Normalized fluorescence spectra of R123 (green), R6G (black), RB (red), and 5-ROX (blue) in deionized water (solid lines) and TiO$_2$ (dashed lines). Spectral shifts are seen for RB and 5-ROX in solution relative to TiO$_2$, most likely due to the carboxylic acid groups. RB demonstrates a fluorescence maximum at 575 nm and 580 nm in deionized water and TiO$_2$, respectively. 5-ROX demonstrates a fluorescence maximum at 598 nm and 611 nm in deionized water and TiO$_2$, respectively.
Weibull fit parameters and corresponding $p$-values are given for on and off-time distributions of RB, R6G, 5-ROX and R123 all on TiO$_2$. Fits are insignificant for all on times.

<table>
<thead>
<tr>
<th></th>
<th>$A$</th>
<th>$B$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ON</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RB</td>
<td>0.66</td>
<td>0.86</td>
<td>0</td>
</tr>
<tr>
<td>R6G</td>
<td>0.70</td>
<td>0.45</td>
<td>0</td>
</tr>
<tr>
<td>5-ROX</td>
<td>0.68</td>
<td>0.85</td>
<td>0</td>
</tr>
<tr>
<td>R123</td>
<td>0.72</td>
<td>0.62</td>
<td>0</td>
</tr>
<tr>
<td><strong>OFF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RB</td>
<td>0.68</td>
<td>4.37</td>
<td>0.056</td>
</tr>
<tr>
<td>R6G</td>
<td>0.71</td>
<td>5.99</td>
<td>0</td>
</tr>
<tr>
<td>5-ROX</td>
<td>0.58</td>
<td>13.13</td>
<td>0.008</td>
</tr>
<tr>
<td>R123</td>
<td>0.62</td>
<td>21.15</td>
<td>0.019</td>
</tr>
</tbody>
</table>

**Table A2.** Weibull fit parameters and corresponding $p$-values are given for on and off-time distributions of RB, R6G, 5-ROX and R123 all on TiO$_2$. Fits are insignificant for all on times.
Appendix B: Monte Carlo Simulations of Blinking Dynamics

It is expected that changes to $\kappa_{23}$ and $\kappa_{31}$ should affect only average rate constants for electron injection and recombination, respectively, which are manifested in the log-normal fit parameters $\mu_{on/off}$. Meanwhile, changes to the extent of energetic dispersion around the mean rate constant of the distributions for injection and recombination should only correspond with $\gamma$. However, changes in each log-normal fit parameter would also affect non-corresponding photophysical characteristics. For example, changes to $\gamma$ were of great interest because of its ability to impact both $\mu_{on/off}$ in addition to $\sigma_{on/off}$. Table B1 presents a serial examination of the impact of $\gamma$ on log-normal fit parameters for both on-time and off-time distributions as $\kappa_{23}$ and $\kappa_{31}$ were kept constant. As expected, since $\gamma$ is corresponds with the energetic dispersion (i.e., $\sigma_{on/off}$ for the log-normal fit parameter), an increase in $\gamma$ results in an increase for both $\sigma_{on/off}$. MC simulations demonstrated an inverse relationship between $\gamma$ and $\mu_{off}$, where the smallest $\gamma$ (i.e., 4) gives the largest $\mu_{off}$ (i.e., 1.87) and the largest $\gamma$ (i.e., 16) resulted in a small $\mu_{off}$ (i.e., -0.08).

The relationship between $\gamma$ and $\mu_{on}$ is not straightforward, but the overall trend indicates that they are proportional to one another: as $\gamma$ increases, $\mu_{on}$ also increases (i.e., $-4.38 \pm 0.02$ to $-2.86 \pm 0.27$). How can $\gamma$ change multiple variables? Previous work$^1$ with MC simulations revealed that due to our experimental time bin of 10 ms, on and off times shorter than 10 ms are being averaged together. Consequently, the blinking data is skewed to represent long time events more accurately than short time events, which means we are overlooking the femtosecond –millisecond blinking events, and input parameters appear to influence other factors when they actually do not (i.e., $\gamma$ seems to
influence $\mu_{on/off}$). Even though a limited time resolution suggests that we cannot find the absolute ET rates of injection and recombination, we can attempt to find the relative rates of injection and recombination for each rhodamine dye. To guide us through the process of determining the appropriate kinetics that yield the experimental log-normal fit parameters, we will use our knowledge of the relationships between input parameters, especially $\gamma$ due to its influence on both $\mu_{on/off}$ and $\sigma_{on/off}$.

<table>
<thead>
<tr>
<th>$\gamma$</th>
<th>$\mu_{on}$</th>
<th>$\sigma_{on}$</th>
<th>$\mu_{off}$</th>
<th>$\sigma_{off}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>-4.10 ± 0.05</td>
<td>1.08 ± 0.03</td>
<td>1.16 ± 0.10</td>
<td>2.13 ± 0.07</td>
</tr>
<tr>
<td>6</td>
<td>-3.82 ± 0.06</td>
<td>1.18 ± 0.04</td>
<td>0.87 ± 0.13</td>
<td>2.34 ± 0.10</td>
</tr>
<tr>
<td>7</td>
<td>-3.48 ± 0.09</td>
<td>1.54 ± 0.06</td>
<td>0.48 ± 0.19</td>
<td>2.70 ± 0.14</td>
</tr>
<tr>
<td>8</td>
<td>-3.92 ± 0.12</td>
<td>1.51 ± 0.09</td>
<td>0.64 ± 0.21</td>
<td>2.62 ± 0.15</td>
</tr>
<tr>
<td>10</td>
<td>-2.26 ± 0.21</td>
<td>2.70 ± 0.15</td>
<td>0.61 ± 0.27</td>
<td>2.59 ± 0.19</td>
</tr>
<tr>
<td>12</td>
<td>-2.31 ± 0.28</td>
<td>2.76 ± 0.20</td>
<td>0.02 ± 0.39</td>
<td>2.89 ± 0.28</td>
</tr>
<tr>
<td>16</td>
<td>-2.86 ± 0.27</td>
<td>2.53 ± 0.19</td>
<td>-0.08 ± 0.44</td>
<td>2.73 ± 0.31</td>
</tr>
</tbody>
</table>

**Table B1** Impact of $\gamma$ on log-normal fit parameters for on and off-time distributions. $\kappa_{23}$ and $\kappa_{31}$ were set to $1 \times 10^{12}$ s$^{-1}$ and $1 \times 10^{6}$ s$^{-1}$, respectively.

### 3.3.2 Reproducing Blinking Distributions with MC Simulations

Realizing the impact of $\kappa_{23}$, $\kappa_{31}$, and $\gamma$ on blinking distributions will facilitate the reproduction of experimental log-normal distributions of rhodamines on TiO$_2$ by MC
simulations. Current experimental time resolution will limit studies to determining relative rates of injection and recombination. Previous MC simulations were able to reproduce log-normal fit parameters with relative rates of injection and recombination calculated from the experimental average on and off times (Table B2 and Table B4). Since the average on and off times are skewed by the 10 ms time bin, the relative ET rates were much slower than literature-obtained values (“slow”). Figure B2 illustrates simulated blinking traces for R6G on TiO₂ with literature-appropriate ET rates (“fast”). The off-time distributions (Fig. B2a) are easily reproducible, where both slow (red) and fast (blue) input ET rates for MC simulations reflect the experimental distribution (black). In fact, the simulated data with fast kinetics are a better match to the experimental data within error, which indicates the use of more literature-appropriate kinetics (Table B3).

Examining the on-time distributions reveals a different story, where the distribution simulated with fast kinetics does not match the experimental on-time

Fig. B2 (a) Off-times and (b) on-times for R6G on TiO₂ are given where the experimental (black), slow kinetics (red), and fast kinetics (blue) distributions are compared.
distribution at all (Table B3). While the experimental on-time distribution has some semblance to a log-normal fit, the fast kinetics on-time distribution is distinctly power law and heavily skewed toward shorter on-time durations (Fig. B2b). The time bin, as discussed before, can be problematic and give the appearances of power -law fit when in reality distributions are not power law. Another problem to consider is the lack of significant $p$-values for the off-time distributions simulated by slow and fast kinetics. Encountering these difficulties when attempting to simulate blinking distributions with literature-appropriate kinetics has guided us in implementing improvements for future work: i) add complexity to the MC model by considering other factors that can contribute to energetic dispersion, and ii) since on-times are affected by more parameters (i.e., $\kappa_{12}$, $\kappa_{21}$, $\kappa_{23}$), we need to not only improve the MC model to reflect complexity in the excited state, but we must also probe the excited state experimentally with improved spectroscopic technique.

<table>
<thead>
<tr>
<th>Input Parameters</th>
<th>Log-normal Off</th>
<th>Log-normal on</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{23}$</td>
<td>$K_{31}$</td>
<td>$\gamma$</td>
</tr>
<tr>
<td>Exp</td>
<td>1.09 ± 0.07</td>
<td>1.45 ± 0.5</td>
</tr>
<tr>
<td>Slow</td>
<td>$8 \times 10^2$</td>
<td>0.13</td>
</tr>
<tr>
<td>Fast</td>
<td>$7.5 \times 10^{11}$</td>
<td>$5 \times 10^6$</td>
</tr>
</tbody>
</table>

Table B3 Comparison of log-normal fit parameters for MC-simulated on and off-time distributions to experimental blinking distributions. Obtaining a significant $p$-value is difficult for both on and off-time distributions.
<table>
<thead>
<tr>
<th></th>
<th>$&lt;t_{\text{on}}&gt;$</th>
<th>$t_{\text{min on}}$</th>
<th>$t_{\text{max on}}$</th>
<th>$&lt;t_{\text{off}}&gt;$</th>
<th>$t_{\text{min off}}$</th>
<th>$t_{\text{max off}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RB/GLASS</strong></td>
<td>0.72</td>
<td>0.02</td>
<td>42.78</td>
<td>5.78</td>
<td>0.05</td>
<td>48.53</td>
</tr>
<tr>
<td><strong>R6G/GLASS</strong></td>
<td>0.56</td>
<td>0.02</td>
<td>10.69</td>
<td>4.95</td>
<td>0.05</td>
<td>43.81</td>
</tr>
<tr>
<td><strong>5ROX/GLASS</strong></td>
<td>1.29</td>
<td>0.02</td>
<td>51.65</td>
<td>12.29</td>
<td>0.06</td>
<td>137.01</td>
</tr>
<tr>
<td><strong>R123/GLASS</strong></td>
<td>1.27</td>
<td>0.02</td>
<td>23.75</td>
<td>15.84</td>
<td>0.09</td>
<td>156.7</td>
</tr>
<tr>
<td><strong>RB/TiO$_2$</strong></td>
<td>0.37</td>
<td>0.02</td>
<td>15.71</td>
<td>5.66</td>
<td>0.05</td>
<td>37.42</td>
</tr>
<tr>
<td><strong>R6G/TiO$_2$</strong></td>
<td>0.64</td>
<td>0.02</td>
<td>25.31</td>
<td>7.39</td>
<td>0.06</td>
<td>64.37</td>
</tr>
<tr>
<td><strong>5ROX/TiO$_2$</strong></td>
<td>1.26</td>
<td>0.02</td>
<td>42.36</td>
<td>18.52</td>
<td>0.05</td>
<td>138.26</td>
</tr>
<tr>
<td><strong>R123/TiO$_2$</strong></td>
<td>0.82</td>
<td>0.02</td>
<td>21.77</td>
<td>24.79</td>
<td>0.03</td>
<td>184.92</td>
</tr>
</tbody>
</table>

Table B2. Average on and off times of experimental blinking distributions for RB, R6G, R123, and 5-ROX on glass and TiO$_2$. Average times were used to back calculate average rates of injection and recombination on TiO$_2$. 
Table B4. Slow input parameters from previous work with MC simulations, where $\kappa_{12} = 2.1 \times 10^6$ and $\kappa_{21} = 9.1 \times 10^8$ according to set experimental conditions. Rates for injection and recombination were calculated from average on and off times in Table A3, and adjusted for the appropriate gamma. Most simulated log-normal fit parameters match the experimental log-normal fit parameters.

<table>
<thead>
<tr>
<th></th>
<th>$K_{23}$</th>
<th>$K_{31}$</th>
<th>$\gamma$</th>
<th>$\mu$</th>
<th>$\sigma$</th>
<th>$\mu$</th>
<th>$\sigma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB/TiO$_2$</td>
<td>$1.9 \times 10^3$</td>
<td>0.24</td>
<td>1.1</td>
<td>$-1.86 \pm 0.04$</td>
<td>$1.41 \pm 0.03$</td>
<td>$0.77 \pm 0.04$</td>
<td>$1.58 \pm 0.03$</td>
</tr>
<tr>
<td>R6G/TiO$_2$</td>
<td>$8 \times 10^2$</td>
<td>0.13</td>
<td>1.0</td>
<td>$-1.04 \pm 0.05$</td>
<td>$1.43 \pm 0.03$</td>
<td>$1.24 \pm 0.05$</td>
<td>$1.51 \pm 0.04$</td>
</tr>
<tr>
<td>5-ROX/TiO$_2$</td>
<td>$8 \times 10^2$</td>
<td>0.05</td>
<td>1.5</td>
<td>$-0.84 \pm 0.08$</td>
<td>$1.65 \pm 0.06$</td>
<td>$1.56 \pm 0.10$</td>
<td>$1.63 \pm 0.07$</td>
</tr>
<tr>
<td>R123/TiO$_2$</td>
<td>$1.8 \times 10^3$</td>
<td>0.01</td>
<td>2.0</td>
<td>$-1.38 \pm 0.11$</td>
<td>$1.59 \pm 0.08$</td>
<td>$2.40 \pm 0.18$</td>
<td>$1.61 \pm 0.13$</td>
</tr>
</tbody>
</table>

References

Figure C1. 3x3 μm² false-colored image of 1-μm fluorescent beads on glass obtained from a pulsed 470 nm laser at 40 MHz. Fluorescent beads are used to achieve optimal detector alignment to prepare for SMS studies.
Appendix D: Customized Matlab Codes

D1. CPD Analysis

function CPD4(targetfile, threshold)
%modified by Alana Ogata 10/17/13 to include display that the file is "bad"
%if the threshold shotnoise is below the calculated value (from control experiments) and there is atleast 1 on segment found (code is paired with %blinks2)

%SINGLE molecule intensity change point identification code, first
%developed by Haw Yang (J Phys Chem v109 p617).
%adapted for matlab
%
%section cp_main.m opens and manipulates the time trace and
%manages the subroutines
%
%by Eric Bott 08-2007
%
%Updated 05-2008 by EB for use with new data types and more than 5 intenstiy points
%and protection for when the # of CPs are less than the max # of intensity levels
%(renamed CPD2.m)
%
%Adapted for input data of form (time, intensity) for W&M data(renamed CPD3.m)
%The output is a CPD3targetfile.txt with 1st column as intensities, 2nd column
%as corresponding times, 3rd is on intervals and 4th if off intervals. The latter
%are established using a threshold of one standard deviation above shot noise.
%The plot is saved as CPD3figure1.jpg - needs to be renamed to save permanently.
%
%by Kristin Wustholz 08-2011, 02-2012

clearvars -except targetfile and threshold; %clear the memory
close all; %close all open plots

trace1d = 0; %the temporal photon trace to be analyzed
max_state = 0; %an initial guess as to the number of states in the trace, used for the intensity classification analysis after the location of the change points
trace_seg = 0; %segment of trace sent to cd_identify for CP analysis
begin_seg = 0; %beginning (index, not temporal) of trace_seg within the full trace
end_seg = 0; %end (index, not temporal) of trace_seg within the full trace
C
tau = 0;  % type I error array (imported from
tau_prime = 0;  % the confidence region array (imported
file, calculated numerically)
cp_array = 0;  % array to hold the locations and
 uncertainties of the change points
I_array = 0;  % array to hold the photon numbers and
time turnations of the segments once the change points have been
located
I_hats = 0;  % array for average intensity for the
group assignments
G_array = 0;  % keeps track of the state assignments
for different possible number of states
I_AHG = 0;  % list of intensitiy levels that are most
likely to have arisen from the same emissive state of the trace, from
the agglomerative hierarchical grouping method
L_em = 0;  % the log-likelihood function for the
intensities
j_min = 0;  % row location of the M_jm_min value
m_min = 0;  % column location of the M_jm_min value
CPs = [];  % array for the locations of the change
points, in 'units' of array position, not time
number_o_segments = 0;  % the number of segments the trace has
been divided into, so the routine knows how many iterations to run
pt_array = 0;  % array to hold the edges of the change
points, for dividing the array in the 'reverse binary segmentation'
scheme
done = 0;  % boolean term, returned if the routine
could find no statistically significant change points
done_ct = 0;  % the number of trace segments that do
not contain any statistically significant change points
i = 0;  % just a counter, please be nice to it
j = 0;  % another counter
p_mj = 0;  % probability matrix of assigning the
the j-th intensity level to the m-th trace segment
BIC_state_number = 0;  % number of states as determined by the
BIC routine
tau = 0;
tau_prime = 0;
cp_array = 0;
I_array = 0;
I_hats = 0;
G_array = 0;
I_AHG = 0;
L_em = 0;
j_min = 0;
m_min = 0;
CPs = [];
number_o_segments = 0;
pt_array = 0;
done = 0;
done_ct = 0;
i = 0;
j = 0;
p_mj = 0;
BIC_state_number = 0;
TAUFILENAME = 'Henderson_tau90.asc';
% For now, just open the 90% likelihood parameters
fid = fopen(TAUFILENAME);
tau = fscanf(fid, '%*c %f', [1 inf]);
tau = tau';
fclose(fid);

TAUFILENAME = 'Henderson_tauprime90.asc';
fid = fopen(TAUFILENAME);
tau_prime = fscanf(fid, '%*c %f', [1 inf]);
tau_prime = tau_prime';
fclose(fid);

SIGFILENAME = targetfile;
fid = fopen(SIGFILENAME);
trace1d = fscanf(fid,'%g %g',[2 inf]);
trace1d = trace1d';
fclose(fid);
trace1d=trace1d+1;    %KW: The program doesn't like zeroes... so adding 1 photon to each point, which shouldn't impact kinetics.

disp(['you are using convergence test file ' num2str(TAUFILENAME,2) ' and confidence interval file ' num2str(TAUPFILENAME,2)]);
disp(['with file: ' num2str(SIGFILENAME,2)]);

sizer = size(trace1d);
trace1d = trace1d(2:sizer(1,1),:);
%KW: Yes, this gets rid of first data point, not sure why but it's needed - otherwise deconvolved lines is translated on plot.
sizer = size(trace1d);
bintime = input('Integration time in seconds?');
%KW: Input bin time for trace in seconds
if isempty(bintime)
%KW: Default is 10 ms
    bintime = .01;
    disp('default integration time = 10 ms')
end
total_int = trace1d(:,2);
%KW: Intensity is just second column of WM data (one detector)
timeaxis = ( 1:sizer(1,1) )'*bintime;
%KW: Keep bintime in there as time axis has been decreased by one
trace2d = [ timeaxis total_int ];

max_state = input('Maximum number of states in the trace?');
%KW: Max states default 12 - per more recent Reid paper
if isempty(max_state)
    max_state = 12;
end
disp(['with a maximum of ' num2str(max_state,2) ' intensity groups']);

pt_array = [1, sizer(1,1) ];
%automatically include the beginning and end points of the time trace
for the pt_array
number_o_segments = max(size(pt_array))/2;
%the number of segments is the size of the pt_array over two, because each CP is defined by a left and right error point, not just the center max position
%
%=================================================================================%
% ** REVERSE BINARY SEGMENTATION ALGORITHM FOR CHANGE POINT DETECTION THROUGHOUT THE TRACE ** %
Starting Reverse Binary Segmentation Algorithm for change point detection;

while number_o_segments > done_ct
    disp('busy...');
    begin_seg = pt_array(1,(done_ct*2+1))+1;
    end_seg = pt_array(1,(done_ct*2+2))-
    1;
    if (end_seg - begin_seg) >= 1
        trace_seg = trace2d(begin_seg:end_seg,:);
        cp_array = cp_identify(trace_seg,tau,tau_prime,bintime,begin_seg);
        done = cp_array(1,4);
    else
        done = 1;
    end

    if done == 0
        if cp_array(1,2) == 1
            cp_array(1,2) = 2;
        end
    end

    pt_array = [pt_array, cp_array(1,2), cp_array(1,3)]:
    pt_array = sort(pt_array);
    CPs = [CPs, cp_array(1,1)];

    elseif done == 1
        done_ct = done_ct + 1;
    else
        disp('really? the boolean term is not 0 or 1? mu?');
    end

    number_o_segments = max(size(pt_array))/2;
end

CPs = sort(CPs);

% *** SEGMENT CALCULATION FOR DETERMINATION OF THE NUMBER OF STATES AND
% INTENSITY LEVELS ***

CPs_temp = [ 1, CPs, sizer(1,1) ];
sizeCPs_temp = size(CPs_temp);
for ii = 1:(sizeCPs_temp(1,2)-1)
    %in this loop we'll make the array which holds information about the
different trace segments that is needed for the determination of
intensity levels
index_start = CPs_temp(1,ii);
%the segments are defined by the change points themselves
index_end = CPs_temp(1,ii + 1);
%same with the end of the segments (see above comment)
I_array(ii,1) = ii;
%include column that will hold the j value for the individual I_j
intensity levels. initially there are am many supposed intensity levels
as there are teace segments
I_array(ii,2) = index_start;
%start position of the segment
I_array(ii,3) = index_end;
%end position of the segment
I_array(ii,4) = sum( trace2d( index_start:index_end,2) );
%calc the total number of photons included in each segment
I_array(ii,5) = ( index_end - index_start + 1 )*bintime;
%calc the time lengths of the segments (+ 1 to count the edge, or else
segments with single index widths are too short by one bintime... as
are all others, but it changes their stats very little)
I_array(ii,6) = 1;
%KW: Placeholder to keep EB's indexing the same - pain to fix - to do
end

I_array = [ I_array(:,1:5), (I_array(:,4)*bintime)./I_array(:,5),
I_array(:,6) ];
I_array_old = I_array;
%archive origonal I_array for later use after BIC analysis

% *** CREATE INITIAL STATE ASSIGNMENTS USING AHG METHOD FOR ALL
POSSIBLE NUMBER OF STATES G >= Gmax ***

state_adj = 0;
if sizeCPs_temp(1,2)-1 < max_state
%this little part is for protecning for traces which have less CPs than
the max I-levels, which will crash the program. and for ones with only
1 CP, which would never get into the next while-loop and make a real
entry to the G_array
max_state = sizeCPs_temp(1,2)-1;
state_adj = 1;
%make note that the max number of states tested for has been changed.
this is needed later
if max(I_array(:,1)) == 2
    G_array = I_array(:,1)';
end
end

disp('----------------');
disp('Starting Agglomerative Hierarchical Grouping Method for initial
intensity level analysis');
G_array = zeros(max_state-1,(sizeCPs_temp(1,2)-1));
%Setup the G_array for the right size. # rows = number of possible
state assingments (AHG assings for the max number of states,
"max_state", down to the minimum number, two states) and # columns =
number of segments
I_hats = zeros(size(I_array));
i = 1;

%disp(I_array);

while max(I_array(:,1)) > 2;
    %consolidate the levels using the AHG method through the max amount the
    %user guessed at from the beginning
    I_array = cp_AHG(I_array,bintime);
    if state_adj == 1;
        %if the number of tested states has been decreased, the first entry for
        %the G_array is the most basic list from the initial (simply counting
        %up for each segment)
        G_array(i,:) = I_array(:,1)';
        %if you do not do this, the code combines two of the states first, thus
        %eliminating one possible state and deconvolving for one less than you
        %think you are... lame.
        state_adj = 0;
        i = i + 1;
    end
    if max(I_array(:,1)) <= max_state;
        %when the states have been consolidated down to the number of
        %max_state's, begin to record the assignments down to 2 states
        G_array(i,:) = I_array(:,1)';
        i = i + 1;
    end
end

%disp(G_array)

% *** SOLIDIFY STATE ASSIGMENTS USING EMC METHOD USING AHG ASSIGMENTS
% AS INITIAL GUESSES ***

disp('----------------');
disp('Starting Expectation-Maximization Clustering for further
refinement of intensity level grouping');
size_G = size(G_array);
L_em = zeros(size_G(1,1),1);
%setup the L_em array to the number of possible G <= Gmax groupings

%disp('there');

pp_holder = zeros(max_state -1,size_G(1,2));

%disp('here');
for i = 1:size_G(1,1)
    p_mj = zeros(max(G_array(i,:)),size_G(1,2));
    for j = 1:size_G(1,2)
        p_mj(G_array(i,j),j) = 1;
    end
    tempout = cp_EMC(p_mj,I_array,bintime);
    %call the cp_EMC.m routine to calculate the log-likelihood function for
    %the intensities
    L_em(i,1) = tempout(1,1);
    tempout(:,1) = [];
    %delete the first column of tempout to leave just the pp_mj matrix
size_pp = size(tempout);
for j2 = 1:size_pp(1,2) %coulmns
    p_max = 0;
    i_max = 0;
    j_max = 0;
    for i2 = 1:size_pp(1,1) %rows
        if tempout(i2,j2) > p_max
            p_max = tempout(i2,j2);
            i_max = i2;
            j_max = j2;
        end
    end
    pp_holder(size_pp(1,1)-1,j2) = i_max;
end

size_p = size(p_mj);
L_em(i,2) = size_p(1,1);
end

% *** FIND MOST LIKELY NUMBER OF INTENSITY STATES USING BIC CRITERION ***

disp('------------------');
disp('Starting Bayesian Infomation Criterion to find minimum number of intenstiy levels required to accurately fit the data');
BIC = zeros(size_G(1,1),1);
BIC_max = -Inf;
for i = 1:size_G(1,1)
    BIC(i) = cp_BIC(L_em,I_array,i);
    if BIC(i) > BIC_max
        BIC_max = BIC(i);
        BIC_state_number = L_em(i,2);
    end
end

%-------------------------------------------------------------%
% *** PROCESS AND ORGANIZE THE CHANGE POINT LOCATION DATA *** %
%-------------------------------------------------------------%

I_array_old(:,1) = pp_holder(BIC_state_number-1,:);'
sizest = size(I_array_old);
for j = 1:BIC_state_number
    totalN = 0;
    totalT = 0;
    for i = 1:sizest(1,1)
        if I_array_old(i,1) == j
            totalN = totalN + I_array_old(i,4);
            totalT = totalT + I_array_old(i,5);
        end
    end
    for i = 1:sizest(1,1)
        if I_array_old(i,1) == j
            I_array_old(i,6) = (totalN/totalT)*bintime;
        end
    end
end
disp('--------------------');
disp(['Done CPD.  Found ' num2str(BIC_state_number,2) ' intensity groups...removing unbound segments...']);

for j = 1:sizer(1,1)
    %setup the error_plot array to be the same size as the original trace
    error_plot(j) = 0;
end

for i = 1:number_o_segments-1
    %setup the visual data output arrays some more
    error_plot(pt_array(1,2*i):pt_array(1,2*i+1)) = max(trace2d(:,2))/2;
    cp_plot(i,2) = (max(trace2d(:,2))/2)+.01*(max(trace2d(:,2))/2);
    cp_plot(i,1) = timeaxis(CPs(1,i));
end

error_plot = [ timeaxis, error_plot' ];
%build the error_plot for graphical data output

keepitup = 1;
%delete the parts of the error_plot that are zero... so there is not a line of dots at the bottom of the plot
i = 1;
while keepitup == 1
    if error_plot(i,2) == 0
        error_plot(i,:) = [];
    else
        i = i + 1;
    end
    if i > max(size(error_plot))
        keepitup = 0;
    end
end

close all;
%close all open plots
hold on
%this is all plot stuff
colordef white
%KW: hide error plot or plot(error_plot(:,1), error_plot(:,2),'r.');
plot(trace2d(:,1), trace2d(:,2), 'k');
%KW: plot original blinking trace - black line
plot(cp_plot(:,1), cp_plot(:,2), 'r+');
%KW: plot change points - red hatch marks

deconvolve_plot = [];
for i = 1:(sizeCPs_temp(1,2)-1)
    deconvolve_plot = [deconvolve_plot ; I_array_old(i,6), CPs_temp(1,i)*bintime ; I_array_old(i,6), CPs_temp(1,i+1)*bintime ];
end
plot(deconvolve_plot(:,2),deconvolve_plot(:,1), 'g', 'Linewidth', 2);
%KW: plot deconvolved intensity levels vs. time - green line
outter = [I_array_old(:,1),I_array_old(:,5),I_array_old(:,6),I_array_old(:,7)];
done = 0;
i = 2;
while done == 0
    % this routine combines the intensity groups of the same level which are
    % right next to each other
    if i <= max(size(outter(:,1)))
        if outter(i,1) == outter(i-1,1)
            outter(i-1,2) = outter(i,2) + outter(i-1,2);
            outter(i-1,4) = (outter(i,4) + outter(i-1,4))/2;
        else
            i = i + 1;
        end
    else
        done = 1;
    end
end

outter2 = [outter(:,3),outter(:,2)];
outter2 = [outter2 ; 0,0];
% KW: This puts zeros after molecule to aid many molecule analysis
% u = 0;
% u = size(outter2,1);
% outter2(1,:)=[];
% KW: Removes the last (unbound) segment - artificially set by experiment
% outter2(1,:)=[];
% KW: Removes the first (unbound) segment - artificially set by experiment
%
% FINAL SAVING AND PRINTING
output = blinks2(outter2, threshold);
filer2 = ['CPD4' targetfile];
fid = fopen([filer2], 'a');
fprintf(fid, '%12.8f %12.8f %12.8f %12.8f %12.8f %12.8f\n', output);
% KW: Note - for comma-delimited output put comma between 12.8's
print -f1 -r600 -djpeg CPD3figure1
% KW: Save figure as jpg for quick reference
%print -f1 -r600 -depsc CPD3figure1
fclose(fid);
disp(SIGFILENAME);
D2. MLE Analysis for Log-normal Fits

function [A, B]=lognormfit(x, varargin)
% LOGNORM fits a weibull distributional model to data
% Lognormfit(x) estimates A (alpha) and B(beta) according to maximum
% likelihood estimation (MLE)
% Using the log-likelihood gives two equations for A and B.
% this code is only applicable to discrete data sets

% Note that this procedure gives no estimate of the uncertainty of the
% fitted parameters, nor of the validity of the fit.

vec     = [];
sample  = [];
xminx   = [];
limit   = [];
finite  = false;
nosmall = false;
nowarn  = false;

% parse command-line parameters; trap for bad input
i=1;
while i<=length(varargin),
    argok = 1;
    if ischar(varargin{i}),
        switch varargin{i},
        case 'range',        vec     = varargin{i+1}; i = i + 1;
        case 'sample',       sample  = varargin{i+1}; i = i + 1;
        case 'limit',        limit   = varargin{i+1}; i = i + 1;
        case 'xmin',         xminx   = varargin{i+1}; i = i + 1;
        case 'finite',       finite  = true;
        case 'nowarn',       nowarn  = true;
        case 'nosmall',      nosmall = true;
        otherwise, argok=0;
        end
    end
    if ~argok,
        disp(['(PLFIT) Ignoring invalid argument #' num2str(i+1)]);
    end
    i = i+1;
end
if ~isempty(vec) && (~isvector(vec) || min(vec)<=1),
    fprintf('(PLFIT) Error: ''range'' argument must contain a vector;
using default.
');
    vec = [];
end;
if ~isempty(sample) && (~isscalar(sample) || sample<2),
    fprintf('(PLFIT) Error: ''sample'' argument must be a positive
integer > 1; using default.
');
    sample = [];
end;
if ~isempty(limit) && (~isscalar(limit) || limit<min(x)),
fprintf('(PLFIT) Error: ''limit'' argument must be a positive value 
>= 1; using default.\n');
limit = [];
end;
if ~isempty(xminx) && (~isscalar(xminx) || xminx>=max(x)),
fprintf('(PLFIT) Error: ''xmin'' argument must be a positive value < max(x); using default behavior.\n');
xminx = [];
end;

% reshape input vector
x = reshape(x,numel(x),1);

% select method (discrete or continuous) for fitting
if isempty(setdiff(x,floor(x))), f_dattype = 'INTS';
elseif isreal(x), f_dattype = 'REAL';
else
f_dattype = 'UNKN';
end;
if strcmp(f_dattype,'INTS') && min(x) > 1000 && length(x)>100,
f_dattype = 'REAL';
end;

%Code of after this point was rewritten by Alana Ogata, July 2012 based
%off of Clauset Code PLFIT
% Estimating Alpha and Beta parameters for log-normal function
A1 = [];
A2 = [];
A = [];
B = [];
b = [];
j = 1;
H = [];
k = 0;
k1 = 0;
t = 1;
flag = 0;
n = length(x);

% Code after this point was written by Alana Ogata, July 2012
% based off of Clauset code PLFIT

A(j)=(sum(log(x)))/n;  %mew(u) from MLE calculations
B(j)=(sum(((log(x)-A(j)).^2)))/n;  %sigma squared (r^2) from MLE
R=(sum(((log(x)-A(j)).^2)));

AE=sqrt((B)/n)  %standard error of A
%after loop is done, there are 98 values of A with corresponding value
%of B
%and Dvalue. D-value represents deviation of fit from the data, so the
%parameter pair with the smallest D value means it has the least
%deviation
%and is the best fit
A = \text{A(j)};

B = \sqrt{\text{B(j)}};
BE = \sqrt{\left( \frac{-\text{B}^4}{(n \times \text{B}^2) - 3R} \right)} \ \text{%standard error of B}
D3. KS test \((p\text{-value})\) for log-normal fits

```matlab
function [p,gof]=lognormpva(x, A,B, varargin)
% LOGNORMPVA calculates the p-value for the given Log-normal fit to some data.
% synthetic data sets are made through random number generator
% each synthetic data set goes through lognormfit calculations to calculate new
% Dsynth value
% tmin-lowest value in the original data set
% Notes: (based on Clauset plpva code, but still applicable to lognormpva)
% 1. In order to implement the integer-based methods in Matlab, the numeric
   maximization of the log-likelihood function was used. This requires
   that we specify the range of scaling parameters considered. We set this range to be \([1.50 : 0.01 : 3.50]\) by default. This vector can be
   set by the user like so,
   
   \[
p = \text{plpva}(x, 1, 'range',\,[1.001:0.001:5.001]);
\]
% 2. PLPVA can be told to limit the range of values considered as estimates
   for xmin in two ways. First, it can be instructed to sample these possible values like so,
   
   \[
a = \text{plpva}(x,1, 'sample',100);
\]
   which uses 100 uniformly distributed values on the sorted list of unique values in the data set. Second, it can simply omit all candidates above a hard limit, like so
   
   \[
a = \text{plpva}(x,1, 'limit',3.4);
\]
   Finally, it can be forced to use a fixed value, like so
   
   \[
a = \text{plpva}(x,1, 'xmin',1);
\]
   In the case of discrete data, it rounds the limit to the nearest integer.
% 3. The default number of semiparametric repetitions of the fitting procedure is 1000. This number can be changed like so
   
   \[
p = \text{plvar}(x, 1, 'reps',10000);
\]
% 4. To silence the textual output to the screen, do this
   
   \[
p = \text{plpva}(x, 1, 'reps',10000,'silent');
\]
```
vec = [];
sample = [];
limit = [];
xminx = [];
Bt = [];
quiet = false;
persistent rand_state;

% parse command-line parameters; trap for bad input
i=1;
while i<=length(varargin),
    argok = 1;
    if ischar(varargin{i}),
        switch varargin{i},
            case 'range', vec = varargin{i+1}; i = i + 1;
            case 'sample', sample = varargin{i+1}; i = i + 1;
            case 'limit', limit = varargin{i+1}; i = i + 1;
            case 'xmin', xminx = varargin{i+1}; i = i + 1;
            case 'reps', Bt = varargin{i+1}; i = i + 1;
            case 'silent', quiet = true;
            otherwise, argok=0;
        end
    end
    if ~argok,
        disp(['(PLPVA) Ignoring invalid argument #', num2str(i+1)]);
    end
    i = i + 1;
end
if ~isempty(vec) && (~isvector(vec) || min(vec)<=1),
    fprintf('(PLPVA) Error: ''range'' argument must contain a vector;
            using default.
        ');
    vec = [];
end;
if ~isempty(sample) && (~isscalar(sample) || sample<2),
    fprintf('(PLPVA) Error: ''sample'' argument must be a positive
            integer > 1; using default.
        ');
    sample = [];
end;
if ~isempty(limit) && (~isscalar(limit) || limit<1),
    fprintf('(PLPVA) Error: ''limit'' argument must be a positive value
            >= 1; using default.
        ');
    limit = [];
end;
if ~isempty(Bt) && (~isscalar(Bt) || Bt<2),
    fprintf('(PLPVA) Error: ''reps'' argument must be a positive value
            > 1; using default.
        ');
    Bt = [];
end;
if ~isempty(xminx) && (~isscalar(xminx) || xminx>=max(x)),
    fprintf('(PLPVA) Error: ''xmin'' argument must be a positive value
            < max(x); using default behavior.
        ');
    xminx = [];
end;
% reshape input vector
x = reshape(x,numel(x),1);
% select method (discrete or continuous) for fitting
if ~isempty(setdiff(x,floor(x))), f_dattype = 'INTS'
el elseif isreal(x), f_dattype = 'REAL'
else f_dattype = 'UNKN'
end;
if strcmp(f_dattype,'INTS') && min(x) > 1000 && length(x)>100,
    f_dattype = 'REAL'
end;
N = length(x);
x = reshape(x,N,1); % guarantee x is a column vector
if isempty(rand_state)
    rand_state = cputime;
    rand(’twister’,sum(100*clock));
end;
if isempty(Bt), Bt = 1000; end;
nof = zeros(Bt,1);
if ~quiet,
    fprintf(’Power-law Distribution, p-value calculation\n’);
    fprintf(’   Copyright 2007-2010 Aaron Clauset\n’);
    fprintf(’   Warning: This can be a slow calculation; please be
patient.\n’);
    fprintf(’   n    = %i \n   xmin = %6.4f \n   reps = %i\n’,length(x),length(nof));
end;
tic;
switch f_dattype,
    case ’REAL’,
        %Code of after this point was rewritten by Alana Ogata, July
2012 based off
        %of Clauset Code PLFIT
        %D-value for original data set is recalculated
        n=length(x);
        tmin=min(x);
        c=(0:n-1)’./n;
        %cf=logncdf(x,A,B); %eq.14 Riley CDF using integration of
        %normal probability distribution
        cf=(.5*(1+erf((log(x)-A)/(B*sqrt(2)))))
        gof=max(abs(c-cf));
        pz=1;

        % compute distribution of gofs from semi-parametric bootstrap
        % of entire data set with fit
        for C=1:length(nof);
            Q =[];
            h = 1;
                %loop to make sure all synthetic data sets have same tmin
            as
                %original data
                while h<=length(x);
D=rand;
%t=(erfc(x))*(erfcinv(x))

Q(h)=exp(((erfinv(1-(2*D)))*(B*sqrt(2)))+A); %using CDF formula from Wolfram
% if Q(h)>=tmin
  % if Q(h)<=max(x) %making sure data is inbetween tmin and 100s
  h=h+1;
  %else Q(h) = [];
  % end
%end

end
U=sort(Q)';
q=U;%synthesized data set

% Estimating Alpha and Beta parameters for Weibull function
A1 = [];
A2 = [];
b = [];
j = 1;
k = 0;
k1 = 0;
t = 1;
flag = 0;
n = length(x);
%First Alpha must be estimated using Maximum Likelihood Estimation,
%Derivative of Likelihood of log-normal function is taken and set equal to 0,
%equations for Alpha and Beta are solved for.

%Array that contains initial guesses for A (denoted A1) for Newtown-Raphson
%iteration to A2, depending on what your initial guess for Alpha is affects
%the final value of Alpha, so it is necessary to check Alpha based off of
%different initial guesses
AA=(sum(log(q)))/n;

BB=(sum(((log(q)-AA).^2))/n;
Bb=sqrt(BB);
dat = zeros(size(q));
for qm=1:length(q);
cq = (0:n-1)'/n;  %CDF as summation directly from data
%cf=logncdf(x,AA,BB);  %Riley eq.14

cff=(.5*(1+erf((log(q)-AA)/(Bb*sqrt(2)))))
dat(qm) = max( abs(cq-cff) );  % KS Statistic (D-value)
end;
if ~quiet,
    fprintf('[%i]tp = %6.4f t[%4.2fm]
',C,sum(nof(1:C)>=gof)./C,toc/60);
end;  % store distribution of estimated gof values
nof(C) = min(dat);
end;
p = sum(nof>=gof)./length(nof);  % eq.13 Riley- P-value equation

case 'INTS',
    if isempty(vec),
        vec = (1.50:0.01:3.50);  % covers range of most practical
    end;  % scaling parameters
zvec = zeta(vec);

% compute D for the empirical distribution
z = x(x>=xmin); nz = length(z);  xmax = max(z);
y = x(x<xmin);  ny = length(y);

L = -Inf*ones(size(vec));
for k=1:length(vec)
    L(k) = -vec(k)*sum(log(z)) - nz*log(zvec(k) - sum((1:xmin-1).^(-vec(k))));
end
[Y,I] = max(L);
alpha = vec(I);

fit = cumsum(((xmin:xmax).^(-alpha))./ (zvec(I) - sum((1:xmin-1).^(-alpha))));
cdi = cumsum(hist(z,(xmin:xmax))./nz);
gof = max(abs( fit - cdi ));
pz = nz/N;

mmax = 20*xmax;
pdf = [zeros(xmin-1,1); ((xmin:mmax).^(-alpha))'./ (zvec(I) - sum((1:xmin-1).^(-alpha)))];
cdf = [(1:mmax+1)' [cumsum(pdf); 1]];

% compute distribution of gofs from semi-parametric bootstrap
% of entire data set with fit
for B=1:length(nof)
    % semi-parametric bootstrap of data
    n1 = sum(rand(N,1)>pz);
    q1 = y(ceil(ny.*rand(n1,1)));

n2 = N-n1;

% simple discrete zeta generator
r2 = sort(rand(n2,1));  c = 1;
q2 = zeros(n2,1);        k = 1;
for i=xmin:mmax+1
    while c<=length(r2) && r2(c)<=cdf(i,2), c=c+1; end;
    q2(k:c-1) = i;
    k = c;
    if k>n2, break; end;
end;
q = [q1; q2];

% estimate xmin and alpha via GoF-method
qmins = unique(q);
qmins = qmins(1:end-1);
if ~isempty(xminx),
    qmins = qmins(find(qmins>=xminx,1,'first'));
end;
if ~isempty(limit),
    qmins(qmins>limit) = [];
    if isempty(qmins), qmins = min(q); end;
end;
if ~isempty(sample),
    qmins = qmins(unique(round(linspace(1,length(qmins),sample))));
end;
dat   = zeros(size(qmins));
qmax  = max(q); zq = q;
for qm=1:length(qmins)
    qmin = qmins(qm);
    zq   = zq(zq>=qmin);
    nq   = length(zq);
    if nq>1
        try
            zdiff = sum( repmat((1:qmin-1)',1,length(vec)).^-
                         repmat(vec,qmin-1,1) ,1);
            L = -vec.*sum(log(zq)) - nq.*log(zvec - zdiff);
        catch
            % iterative version (more memory efficient, but slower)
            L = -Inf*ones(size(vec));
            slogzq = sum(log(zq));
            qminvec = (1:qmin-1);
            for k=1:length(vec)
                L(k) = -vec(k)*slogzq - nq*log(zvec(k) -
                       sum(qminvec.^-vec(k))));
            end;
        [Y,I] = max(L);
        fit = cumsum(((qmin:qmax).^-'vec(I)))/ (zvec(I) -
                      sum((1:qmin-1).^-'vec(I))));
        cdi = cumsum(hist(zq,(qmin:qmax))./nq);
        dat(qm) = max(abs( fit - cdi ));
        end;
end;
else
    dat(qm) = -Inf;
end;

end
if ~quiet,
    fprintf('%i\tp =
%6.4f\t%4.2fm
',B,sum(nof(1:B)>=gof)./B,toc/60);
end;
% -- store distribution of estimated gof values
nof(B) = min(dat);
end;
p = sum(nof>=gof)./length(nof);

otherwise,
    fprintf('(PLPVA) Error: x must contain only reals or only integers.\n');
p = [];
gof = [];
    return;
end;
D4. Monte Carlo Simulations

function [MCtracelnb]=MC3LSlnb(rate12, rate21, rate23, rate31, step, bintime, length, gamma, sigma)

% Monte Carlo Simulations based on Mc_groundtun (Perl) from KLW
% Dissertation

% Three level system comprised of a ground state (1), excited state (2),
% and dark state (3). Rate constants for excitation and emission are set by
% the experiment. Rate constants to/from the dark state occur via
% tunneling. All rate constants and coefficients in Hz. Step, bintime,
% length in seconds. Step is computational step (~1ns), bintime is
% experimental bin time (~0.01 s), length is experimental (~100 s).
% Gaussian distribution of barriers heights (or ln k's) consistent with a
% log-normal CDF. gamma is set to 1 in initial version, saying there is some
% displacement about deltaG(zero) - but not dictating the magnitude of that
% displacement at this point. Sigma is variance in Gaussian distribution -
% see results from checknormrnd.

% Kristin Wustholz, 11/18/12

i = 1;
j = 1;
k = 1;

% Setting all state populations to zero

state = [0;0;0];

% Start simulation in ground state

state(2)=1;

% Setting the probability, rate and photon matrices to zero

rate = zeros(3,3);
prob = zeros(3,3);

bins = length/bintime; %Calculate how many macro bins per simulation (e.g., 100 s length / 0.01 s bintime = 10,000 bins)

while i<=bins
    photon(i) = 0;
    i = i + 1;
end
i = 1;
photon=photon';
% Setting up the time axis for final output

x = [1:1:bins];
x = (x.'*bintime')';

% Input values are placed in the rate matrix

rate(1,2) = rate12;
rate(2,1) = rate21;
r(2,3) = rate23;
r(3,1) = rate31;
%rate11 = rate(1,1);
%rate22 = rate(2,2);
%rate33 = rate(3,3);

% Probability matrix is set up prob(m,n) is the transition prob. from m to n
prob(1,2) = rate12*step;
prob(2,1) = rate21*step;
prob(2,3) = (rate21*step) + (rate23*step);
prob(3,1) = rate31*step;
prob(1,1) = 0;  %Molecule must be excited
prob(1,3) = 0;  %Not allowed
prob(2,2) = 0;  %Quantum yield drives whether it emits, stays or populates dark state
prob(3,2) = 0;  %Not allowed
prob(3,3) = 0;  %k31 drives whether it depopulates or stays in dark state

looper = bintime/step;  % calculate a micro bintime for simulation (e.g., ms bin / ns step is 10^6)

% Set coefficients as unchanging prefactors

prefactor23 = rate23;
prefactor31 = rate31;

% Compute dark-state population and depopulation rates, where x is a random number from a GAUSSIAN Distribution - consistent w/ log normal CDF

x23 = normrnd(0,sigma);
rate23 = prefactor23*exp(-(gamma*x23));

x31 = normrnd(0,sigma);
rate31 = prefactor31*exp(-(gamma*x31));

% Reset the probability matrix

prob(2,3) = prob(2,1)+(rate23*step);
prob(3,1) = rate31*step;

% Set start parameters
point = 1; %What state you're in (start overall simulation in ground state)
breakout_k = 1; %Where you went
counter = 0;
compare = 0;
flag = 1; %Flag: a transition happened
i = 1;

for i=1:bins %Loop that iterates the macroscopic (experimental) bins
counter = 0;

    for j = 1:looper %Loop that iterates the computational steps within 1 macro bin
        compare = rand;

            for k = 1:3 %Loop that checks where the molecule is
                if state(k) == 1
                    point = k;
                end
                k = k + 1;
            end

            flag = 1;

            if point == 1 && flag == 1 && compare<prob(1,2) %If the excitation probability is greater than a random #, it goes to state 2.
                state(1) = 0;
                state(2) = 1;
                state(3) = 0;
                breakout_k = 2;
                flag = 1;
                disp('Excited state!')
            end

            if point == 2 && flag == 1 && compare<prob(2,3) %If the dark-state population probability is greater than a random #, it checks against the FL quantum yield
                compare2 = rand;
                phi = rate21/(rate23+rate21);
                if compare2<phi %If the FL quantum yield is bigger than a new random #, it will emit.
                    state(1) = 1;
                    state(2) = 0;
                    state(3) = 0;
                    breakout_k = 1;
                    flag = 1;
                    elseif compare2>=phi %Otherwise, it will go to the dark state. There's not a c
                        state(3) = 1;
                        flag = 1;
                        breakout_k = 3;
                end

        end
    end
end
if point == 3 && flag == 1 && compare<prob(3,1) %If the dark-state depopulation probability is greater than a random #, it will go to state 1.
    state(1) = 1;
    state(2) = 0;
    state(3) = 0;
    breakout_k = 1;
    flag = 1;
end

%If a transition to/from the dark state occurred, this loop recalculates the rate constants for dark-state population and depopulation using a new random #.

if point == 3 && breakout_k == 1
    x23 = normrnd(0,sigma);
    rate23 = prefactor23*exp(-(gamma*x23)); %k=kappa*exp^-(gamma*x)
    prob(2,3) = prob(2,1)+(rate23*step);
end

if point == 2 && breakout_k == 3
    x31 = normrnd(0,sigma);
    rate31 = prefactor31*exp(-(gamma*x31));
    prob(3,1) = rate31*step;
end

if point == 2 && breakout_k == 1
    counter = counter + 1;
end

j = j + 1;
end

photon(i) = counter;
i = i+1;
end

format long
MCtracelnb = [x, photon];
%filename = ['MCtracelnb'];
%dlmwrite(filename, MCtracelnb, 'delimiter', '\t', 'precision', '%.9f')