Temperature Dependent Single Molecule Fluorescence Intermittency

Chris Bingham Erin A. Riley Dan Sluss Eric Bott Philip J. Reid

Box 351700, Department of Chemistry, University of Washington, Seattle WA 98195

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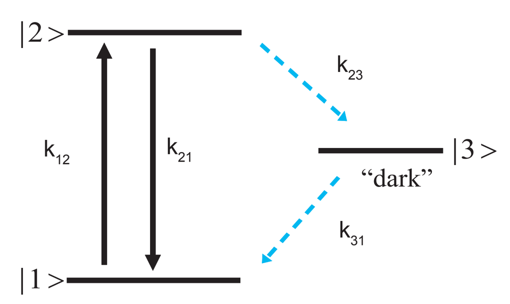
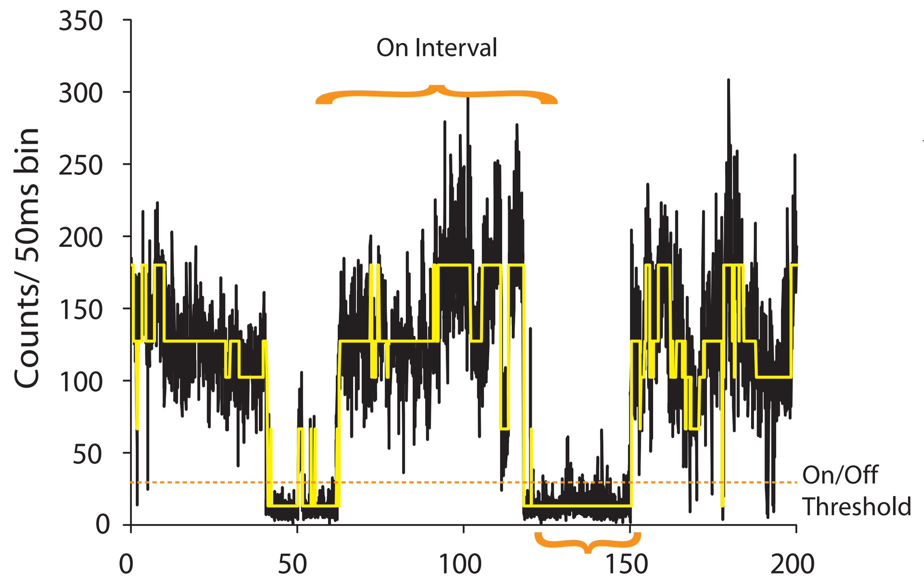
Introduction

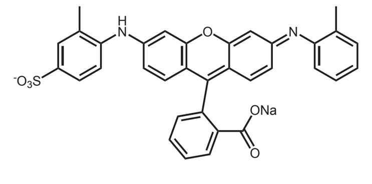
An important issue limiting the wide-spread use of organic electro-optical materials is photodecomposition. Photo-oxidation is one pathway that results in photodecomposition, however, bulk photostability studies have shown that other pathways are operative that do not involve oxygen. We are interested in identifying the intermediate, or optically "dark", states that result in non-oxidative photodecomposition. Identification of these dark states as well as the mechanism for population and decay of these states will lead to advances in material photostability.

Single molecule (SM) techniques allow for the direct observation of dark state population and recovery by recording the fluorescence as a function of time (see figure below). We study the transitions to and from emissive and dark states of single molecules and quantify the duration of "on-" and "off-" intervals.

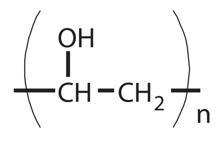


The kinetic processes responsible for blinking can be investigated by constructing probability histograms of on- and off- interval durations. These are distributed according to the underlying rate constants for dark state population and recovery. For SM in polymers these plots are best fit power-law functions of the form $P_{on/off} \propto t^{-m}$ where m is the power exponent. Power-law functions indicate that the rate constants giving rise to blinking are not single valued, but distributed in time. It has been proposed that time dependent fluctuations in the SM environment serve to promote dark state population. This poster presents temperature dependent SM blinking of violamine R (VR) in thin films of poly(vinyl alcohol) (PVA), from room temperature through the glass transition temperature (T_g) of PVA.[1] In this range PVA undergoes drastic changes in viscosity, providing a perturbation of environment without changing chemical species.





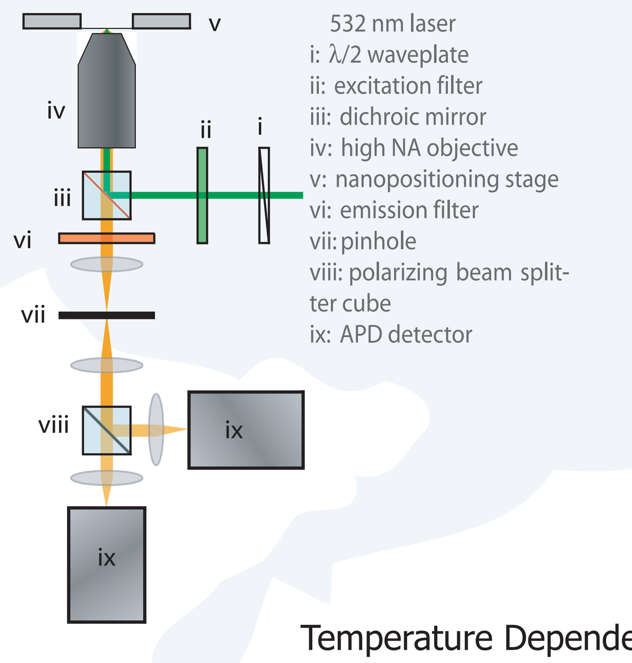
violamine R (VR)



polyvinyl alcohol (PVA)

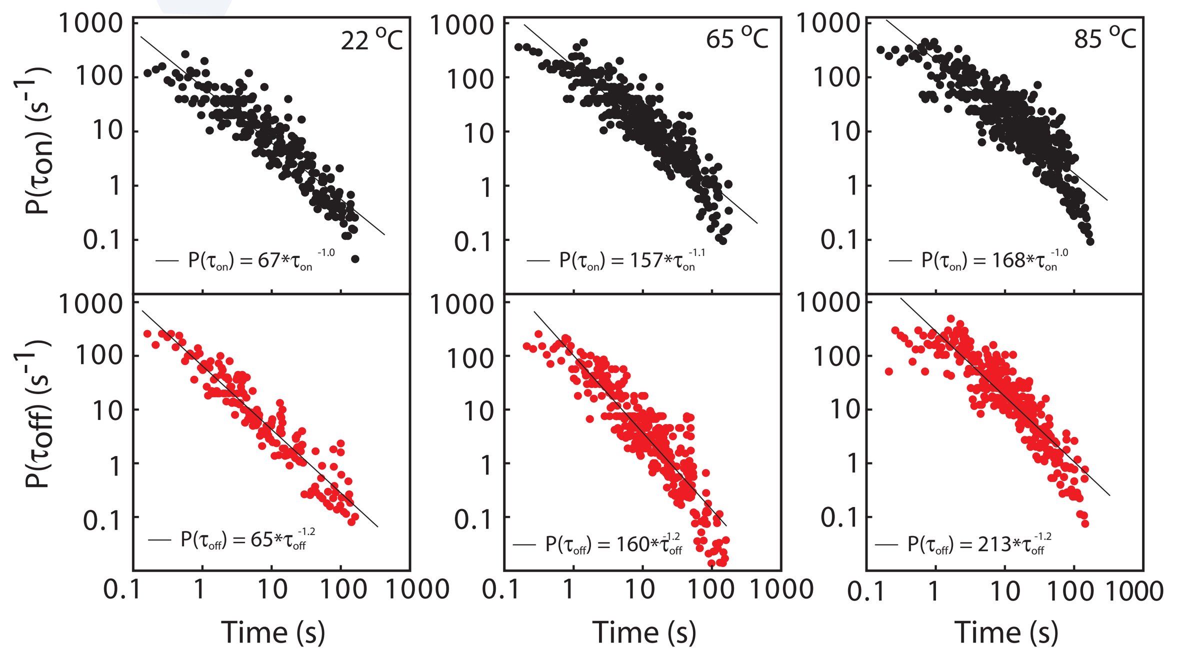
Above is a representative blinking time trace, shown with intensity levels determined by change point detection overlaid. The orange dashed line is the threshold separating the on and off states. The brackets indicate a single on and off interval. To the right of the trace the structures of VR and

Single-Molecule Blinking



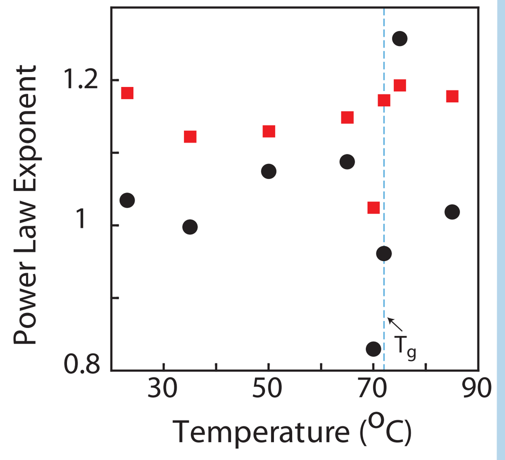
Single molecule emission was observed on a confocal microscope (left). Samples consisted of 1.5 x 10^{-8} M VR in 10 wt% PVA/$H_2$0 solution spin coated on glass coverslips and dried under vacuum. T_g of resulting films was measured to be 72 °C. SM emission traces were collected at 50 ms bin times for 200 s and on- and off- intervals were determined using CPD analysis with an emissive threshold of 20% the maximum deconvolved intensity state. Traces were collected at 23 °C, 35 °C, 50 °C, 65 °C, 70 °C, 72 °C, 75 °C, and 85 °C.

Temperature Dependence of Power Law



Above are probability histograms for on- and off- interval durations. The data are best fit by power-law functions, indicative of dispersed kinetics for dark state population and ground state recovery. As the temperature is increased through T_g only modest changes in the power law exponent are observed. The shapes of the histograms indicate that as the temperature increases, the data become increasingly single exponential. A plot of power-law exponents for all temperatures is shown below.

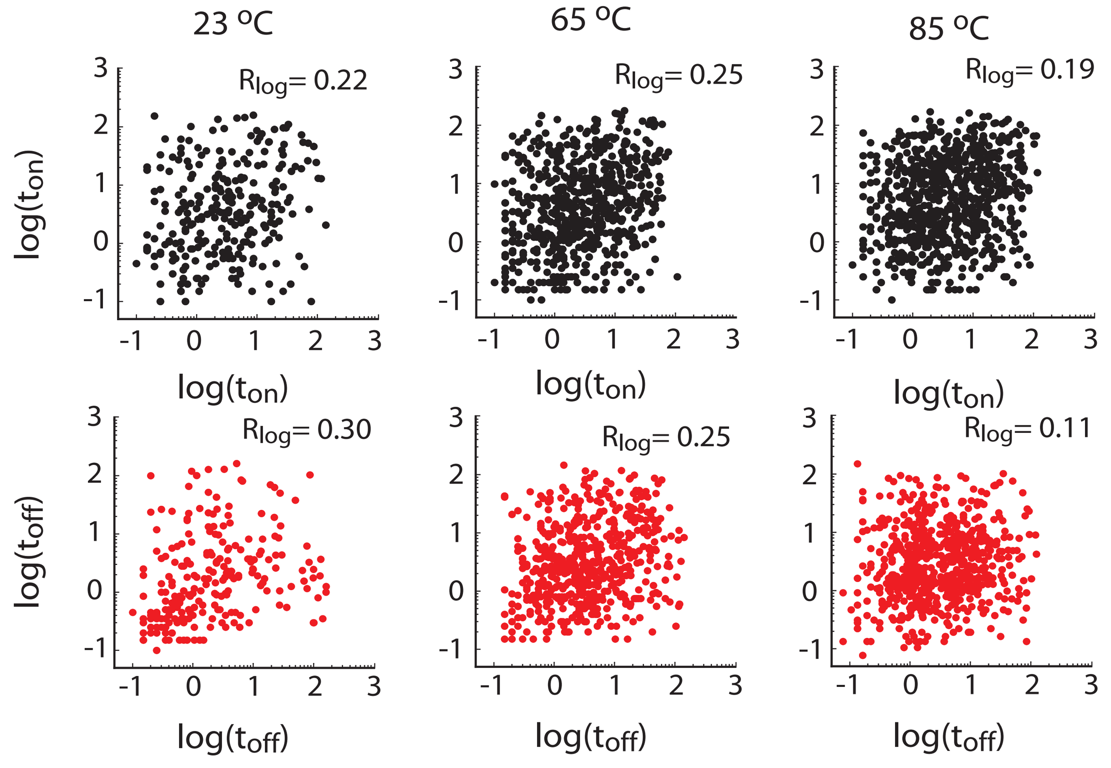
It is surprising that there is little variation between the power-law exponents, despite drastic changes in the polymer viscosity. This may mean that local structure of polymer domains changes little during the phase change, such that the SM environment is unperturbed. This is in agreement with work by Orrit and coworkers[2] in which they used rotational dynamics to probe local domain heterogeneity. They found that the local domains exchange slowly, even above T_g. Modest changes in the shape of the histograms may be due to an increase in rate constants for reaction over a thermally accessible barrier.

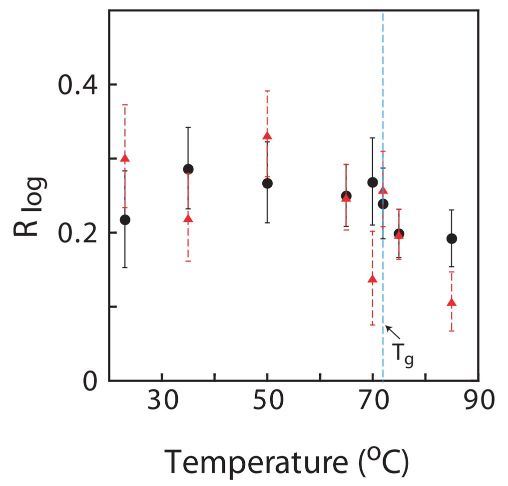


Plot of m_{on} (black circles) and m_{off} (red squares) as a function of temperature.

Memory

To see if alterations in the local environment are indeed slower than the relevant blinking timescale, correlations between on interval and off interval durations were investigated. If correlations exist, then the relative state energetics and couplings giving rise to blinking remain relatively constant over the time scale of the experiment. This is referred to as "memory". Below are scatter plots of the logarithm of successive interval durations at select temperature points. Correlation is observed as augmentation of point density along the diagonal. The correlation coefficient, R_{log}, was calculated to quantify the degree of correlation and varies between -1 and +1 with zero being no correlation. The data shows a slight decrease in correlation with increase in temperature, as expected. The bottom figure shows the correlation coefficient for all temperatures.





Conclusions and Future Work

We have shown that the emissive character of VR in PVA changes little with temperature, despite the phase change of PVA. This is likely due to the local stability and slow interconversion of polymer domains. However, dispersed kinetics are still manifest in power-law distributed on and off interval histograms, and modest changes in the shape of these histograms at elevated temperatures suggest that rate constants may still be modified. To further explore the effect that local environment has on dark state population and depopulation, other polymers will be investigated to establish the role of host dynamics in dark state formation and decay.

References:
1. Sluss, D.; Bingham, C.; Burr, M.; Bott, E. D.; Riley, E. A.; Reid, P. J. *J. Mater. Chem.*, **2009**, *19*, 7561
2. Zondervan, R.; Kulzer, F.; Berkhout, G. C. G.; Orrit, M. *PNAS*, **2007**, *104*, 12628

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