Introduction

Current interest in single-molecule emission involves understanding the photophysics responsible for fluorescence intermittency or "blinking". We are particularly interested in identifying the nature of the non-emissive states that are populated during blinking since these state may be responsible for irreversible photodecomposition. Identifying the nature of these dark states ind developing strategies for limiting their production provides a rational pathway towards the elopment of more robust organic materials.

In this poster, we present recent studies of blinking for molecules isolated in organic crystals. This isolation technique provides us with the chemical technology to test potential mechanisms of dark-state formation. We have directly measured the decay kinetics of the optically-prepared excited state of 2',7'-dichlorofluorecin (DCF) embedded in crystals of potassium acid phthalate (KAP), and have found that the depopulation kinetics are dispersed consistent with earlier blinking studies. This behavior demonstrates that the apparent rate constant for excited-state depopulation varies with time. We have reproduced this behavior using Monte-Carlo similations which suggest that variation in the local environment of the chromophore provides for dispersed kinetic behavior. Future studies designed to correlate behaviors such as blinking and spectral diffusion are outlined

Previous Studies: Blinking of Single Molecules in KAP



[Top left: Violamine R (VR) dyed KAP. Bottom left: fluorecence transient of VR KAP. Right: log-log histograms of on- and off- times collected for 40 VR molecules.]

ingle molecules embedded in KAP crystals demonstrate fluorescence intermittency or "blinking". Histograms of the duration of emissive (on) and non-emissive (off) periods are used to measure the kintiecs of dark state population and depopulation. The on and off probabilities are power-law distributed consistent with the rate constants for dark-state population and depopulation varying in time (i.e., distributed kinetics). These studies report on kinetic behavior on the ~ms timescale. We have extended our studies to the ns timescale by measuring fluorescence lifetimes.

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[Top left: TCSPC set up. Top right: single molecule decay histogram DCF KAP β =0.57. Bottom right: histogram of β values for 56 DCF KAP]

Excited-state decay kinetics for single DCF molecules in KAP were measured using time correlated single-photon counting (TCSPC) microscopy. Both single and stretchedexponential fluorescence lifetimes were observed. Stretched-exponential behavior indicates that the excited-state depopulation kinetics are distributed.

Monte Carlo Calculations



[Left: Marcus potentials, λ reorganization energy, ΔG free energy of reaction, E₂ activation energy, k₂₃ non-radiative rate, k₂₁ radiative rate. Right: Energy distribution of 61 single VR moleucles with respect to emission maximum]

Blinking has been correlated to spectral shifts (spectral diffusion) of individual molecules. Dispersed rate constants may be caused by fluctuations in the surrounding environment. SMs of VR in KAP demonstrate spectral diffusion. The previously measured energy distribution of VR SMs was used to simulate SM lifetimes with Monte Carlo methods employing a Marcus formalism.



Single-Molecule TCSPC Studies of Dispersed Kinetics on Fast Timescales

Monte Carlo Results







[Left: Monte Carlo results β vs average k_{23} rate. $\Delta G = \lambda/4$ (cross), $\Delta G = 0$ (square), $\Delta G = -\lambda/4$ (circle), $\Delta G = -\lambda/2$ (diamond), and $\Delta G = -\lambda$ (triangle). Right: example energy well configurations used in simulations]

Monte Carlo simulations were performed by randomly selecting an energy shift δ to stochaistically modify the free energy of reaction ($\Delta G + \delta$), and k₂₃ was then calculated. The excited-state decay probability was computed iteratively, and the decay pathway chosen using the fluorescence quantum yield. Histograms of photon arrival times were fit to the stretched exponential function. The experimentally relevant energy distribution was able to reproduce the spread in β values from the TCSPC experiment.



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To correlate lifetimes, blinking, and spectral diffusion, time-tagged time-resolved TCSPC (T3R-TCSPC) techniques will be used. Initial results on DCF KAP (shown above) demonstrate that many intensity states exhibit single exponential lifetimes, while the overall TCSPC equivalent histogram is stretched ($\beta \sim 0.4$). Multi-parameter fluorescence experiments of this kind will be used to explain the nature of distributed kinetics at all timescales for SMs in dyed crystals.

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