Supporting Information

Complex Photophysical Behaviors Affect Single Conjugated Molecule Optical Anisotropy Measurements

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1. Data Analysis

Collected movies were analyzed using a custom-made program in Python. Feature finding was done using the Crocker-Grier algorithm via Trackpy Python package.¹ Features were excluded from M analysis if they exhibited significant photoblinking during a given M measurement or showed altered PL intensity at 50 W/cm² before and after high intensity measurements (Fig. S1). Feature intensity was defined as the mean value of the five brightest pixels of 49 pixels (7 x 7) around each identified feature after background subtraction. More details on feature identification, feature intensity, and background subtraction can be found in Ref. 2

2. Simulations

An M distribution similar to the experimental distribution (Fig. 3b) was first generated: a Gaussian distribution of 1000 random numbers centered at 0.52 with a standard deviation of 0.2 was generated. The lower and upper bounds of the distribution were set to 0 and 1, respectively. Then, 1000 random numbers from a Gaussian distribution with mean value of 1 and standard deviation of 0.2 were generated, with these values representing the $I_{\text{max}}$ values. Finally, $I_{\text{min}}$ values were calculated using Eqn. 1 in the main text from the generated M and $I_{\text{max}}$ values. These values were used for the simulation without noise. With no noise or quenching, the coefficients $a$ and $b$ in Eqn. 6 in the main text are simply equal to 4. Therefore, the M distribution at high excitation power is identical to that initially generated (Fig. S2,b).

Noise was introduced in the simulation by starting with a Gaussian distribution of 1000 random numbers with mean value of 0 and standard deviation of 0.04 and 0.08 for low and high excitation intensity, respectively. These random numbers were added to the original $I_{\text{max}}$ and $I_{\text{min}}$ values generated above and well-reproduced the signal:noise ratio seen experimentally. Using these new $I_{\text{max}}$ and $I_{\text{min}}$ values, the new M distributions were calculated using Eqn. 1 for both excitation intensities. The results are shown in Fig. S2,c and d.

To include quenching in the simulation, Eqn. 3 in the main text was used to calculate $I_{\text{max}}$ and $I_{\text{min}}$ values from the initial (noise-free) $I_{\text{max}}$ and $I_{\text{min}}$ values, i.e., $I_{\text{max}}^{\text{quenching}} = I_{\text{max}} * \alpha/(1 + \beta I_{\text{max}})$ and...
\[ I_{\text{min}}^{\text{quenching}} = I_{\text{min}} \times \alpha/(1 + \beta I_{\text{min}}). \] \( \alpha \) and \( \beta \) values of 1 and 0.2 were used, respectively, as these values well-reproduced the experimental data. New \( M \) and \( M' \) values were then calculated following Eqn. 1 at both low and high excitation powers. These results are shown in Fig. S2,e and f. Finally, Fig. 5 in the main text was produced by adding both quenching and noise into the simulation in that order. New \( M \) and \( M' \) values were then calculated following Eqn. 1 at both low and high excitation powers using the newly calculated \( I_{\text{min}} \) and \( I_{\text{max}} \) values after applying quenching and noise.
Supporting Figures

Figure S1. Scatter plot of $M$ values of the molecules shown also in Fig. 3 in the main text measured twice at 50 W/cm$^2$, before and after the measurement at 200 W/cm$^2$. Error bars represent fit uncertainty. Red line is a guide to the eye with $y = x$. Green line is a linear fit to the data with slope = 1.01.
Figure S2. (a) Scatter plots of $I_{\text{max}}$ ratio and $I_{\text{min}}$ ratio as defined in Eqn. 6 of the main text vs. $M$ for a simulation of 1000 molecules with neither noise nor quenching and an excitation intensity ratio of 4, as in the experiment. (b) $M'$ vs. $M$ for the simulation also depicted in (a). (c) Scatter plots of $I_{\text{max}}$ ratio and $I_{\text{min}}$ ratio as defined in Eqn. 6 of the main text vs. $M$ for a simulation of 1000 molecules with noise as described in the Supporting Text but no quenching and an excitation intensity ratio of 4, as in the experiment. (d) $M'$ vs. $M$ for the simulation also depicted in (c). Red line is a guide to the eye, showing $y = x$. Green line is a linear fit to the data with slope = 0.99. (e) Scatter plots of $I_{\text{max}}$ ratio and $I_{\text{min}}$ ratio as defined in Eqn. 6 of the main text vs. $M$ for a simulation of 1000 molecules with quenching as described in the Supporting Text but no noise and an excitation intensity ratio of 4, as in the experiment. (f) $M'$ vs. $M$ for the simulation also depicted in (e). Red line is a guide to the eye, showing $y = x$. Green line is a linear fit to the data with slope = 0.85.
References
