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/cm^2 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_{max} values. Finally, I_{min} values were calculated using Eqn. 1 in the main text from the generated *M* and I_{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_{max} and I_{min} values generated above and well-reproduced the signal:noise ratio seen experimentally. Using these new I_{max} and I_{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_{max} and I_{min} values from the initial (noise-free) I_{max} and I_{min} values, i.e., $I_{\max}^{\text{quenching}} = I_{\max} * \alpha/(1 + \beta I_{\max})$ and

$I_{\min}^{\text{quenching}} = I_{\min} * \alpha/(1 + \beta I_{\min})$. α and β 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_{min} and I_{max} values after applying quenching and noise.

Supporting Figures

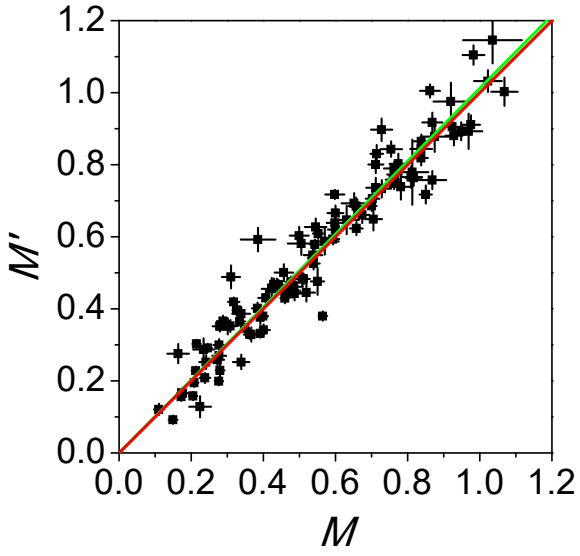

Figure S1. Scatter plot of *M* values of the molecules shown also in Fig. 3 in the main text measured twice at 50 W/cm^2, before and after the measurement at 200 W/cm^2. Error bars represent fit uncertainty. Red line is a guide to the eye with y = x. Green line is a linear fit to the data with slope = 1.01.

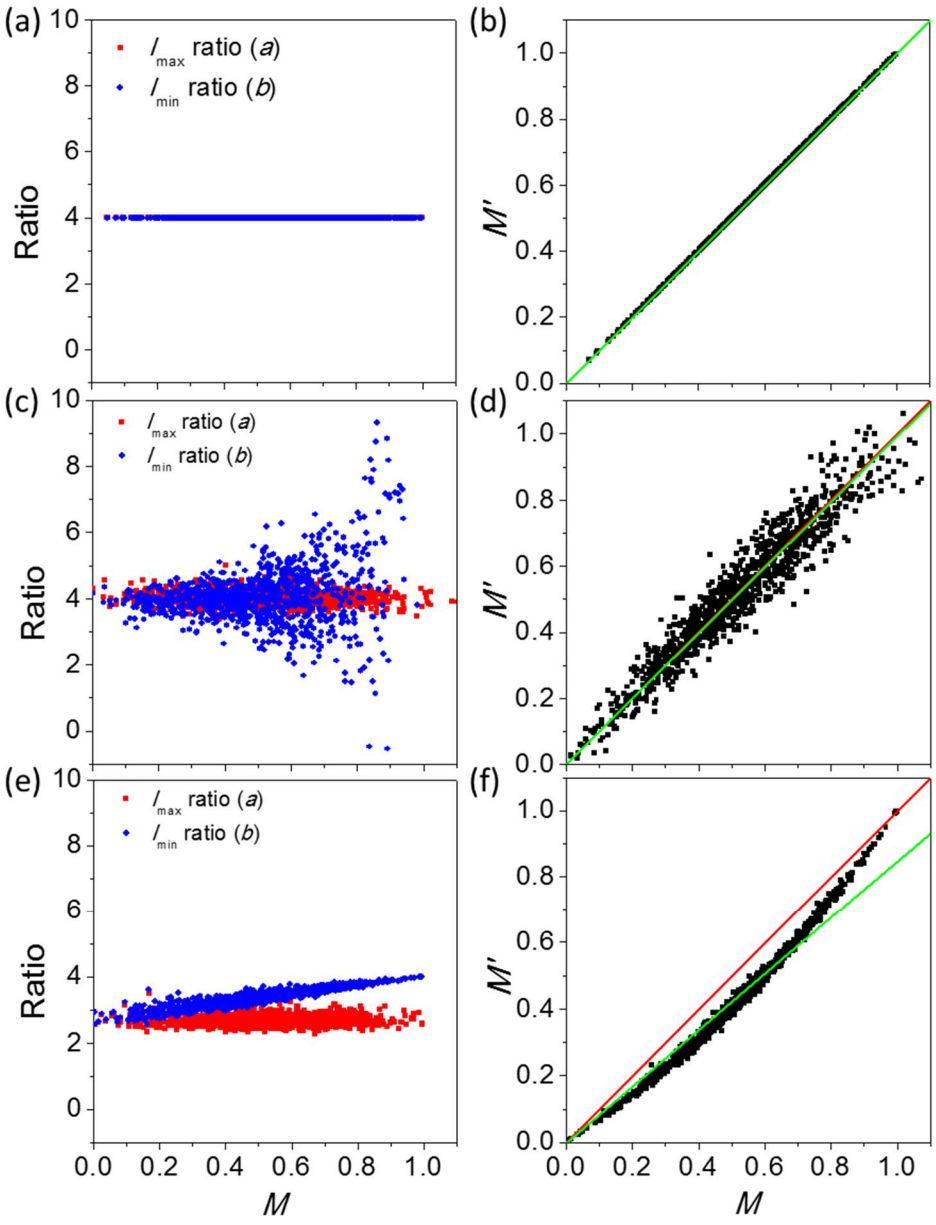

Figure S2. (a) Scatter plots of I_{max} ratio and I_{min} ratio as defined in Eqn. 6 of the main text vs. M for a simulation of 1000 molecules with neither noise nor quenching and an excitation intensity ratio of 4, as in the experiment. (b) *M'* vs. *M* for the simulation also depicted in (a). (c) Scatter plots of I_{max} ratio and I_{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_{max} ratio and I_{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

(1) Crocker, J.; Grier, D. Methods of Digital Video Microscopy for Colloidal Studies. *J. Colloid Interface Sci.* **1996**, *179*, 298–310.

(2) Park, H.; Hoang, D. T.; Paeng, K.; Yang, J.; Kaufman, L. J. Conformation-Dependent Photostability among and within Single Conjugated Polymers. *Nano Lett.* **2015**, *15*, 7604–7609.