NONBINARY SINGLE-MOLECULE BLINKING IN NILE RED REVEALS TIME-CORRELATED PHOTOPHYSICAL DYNAMICS

Ben Steinman, Chloe Autry, John Cooley, Claire Cook, Kristina Knauss, Alisha Khodabocus, Jonathan Stashenko, Walker Knapp, Dr. Eric Walter, Dr. Kristin Wustholz
College of William & Mary Advisor: Prof. Kristin Wustholz William & Mary, Department of Chemistry, P.O. Box 8795, Williamsburg, VA 23187

ABSTRACT: Single-molecule fluorescence intermittency ("blinking") is typically modeled as binary switching between emissive ("on") and non-emissive ("off") states. However, Nile Red (NR) exhibits characteristic emission behavior that challenges this framework. Here, we combine single-molecule fluorescence measurements, statistical analysis of blinking dynamics, and stochastic kinetic modeling to investigate the photophysical origins of this behavior. Change point detection (CPD) reveals multi-level emission and heavy-tailed "on"/"off" dwell-time distributions that are not well described by single-rate kinetics, suggesting dynamic disorder in the underlying transition rates. Ensemble spectroscopy and (TD-)DFT calculations indicate that charge-transfer-mediated relaxation pathways, consistent with twisted intramolecular charge transfer (TICT), are accessible in NR. To connect these observations, we develop a Monte Carlo (MC) kinetic model incorporating time-correlated fluctuations in transition rates. Stochastic simulations reproduce key experimental features. These results demonstrate that NR blinking can be explained via an environmentally modulated, time-correlated photophysical model and establish fluorescence intermittency as an information-rich probe of nanoscale environments.