Spectral Diffusion

We explored the role of spectral diffusion in the blinking dynamics of single DCF molecules in potassium acid phthalate (KAP) crystals. The emission from individual molecules was separated into spectral components using a dichroic mirror centered at 540 nm, then focused onto two avalanche photodiodes. This approach allowed us to monitor changes in emission energy from one molecule to another, as well as time-dependent spectral fluctuations (spectral diffusion) in a single molecule.

Intensity-Lifetime Analysis

Intensity-lifetime analysis was performed using time-resolved single-photon counting (T3R-TCSPC). Time-resolved intensity histograms were used to calculate the temporal probability distribution of energy drifts. Panel (c) was calculated by using the total number of photons emitted from each occurrence shown in (a). This distribution best corresponds to the ensemble fluorescence.

The ensemble fluorescence maximum of DCF differs by roughly 1300 cm⁻¹ between polar solvents and aqueous solutions where the anionic form is dominant and the same separation is evident in the segment-weighted energy-shift histogram. The ensemble fluorescence maximum was shown to be a function of both the detection and the time delay between each excitation pulse (τf), as well as the temporal delay between each excitation pulse (τe) and when an emissive photon is detected (τd).

The different protonation forms of DCF with partition coefficients in potassium acid phthalate (KAP) are shown in the following figure:

- **Lactone**: pKa1 = 4.00
- **Quinonoid**: pKa2 = 5.19
- **Anionic**: pKa3 = 5.25
- **Dianionic**: pKa4 = 4.28

The distribution of excited-state lifetimes for all 61 DCF molecules in KAP is shown in (a). The lifetimes span 0.6 to 7.8 ns, with an average of 1.92 ± 1.8 ns. (b) presents excited-state lifetimes for the molecules that exhibited single-exponential excited-state decay and (c) the lifetimes for molecules that exhibited stretched-exponential excited-state decay. Molecular lifetime distributions for molecules exhibiting single-exponential decay have a narrow distribution of lifetimes, and the corresponding distribution for molecules exhibiting stretched-exponential decay is significantly broader.

The combined intensity for each blinking trace was determined using a change-point detection algorithm, the spectral energy shifts were calculated for every detected segment based on the average intensity ratio between the detectors. Histograms of energy-shift occurrences are shown in (a). The energy distribution in (a) is unwrapped, but the probability is based on the segment duration to create a temporal-probability distribution of energy shifts. Panel (c) was calculated by using the total number of photons emitted from each occurrence shown in (a). This distribution best corresponds to the ensemble fluorescence.

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The correlation plot for all molecules demonstrates a high degree of dispersion, however decomposition of these data into molecules exhibiting single-exponential (f) and stretched-exponential (δf) reveals that the stretched-exponential population is largely responsible for this dispersion. These results show that intensity modification of SMs is complex, and could arise from changes in the protonation state of DCF in KAP.