Photophysics of Single Dye-molecules Isolated in Salt Crystals

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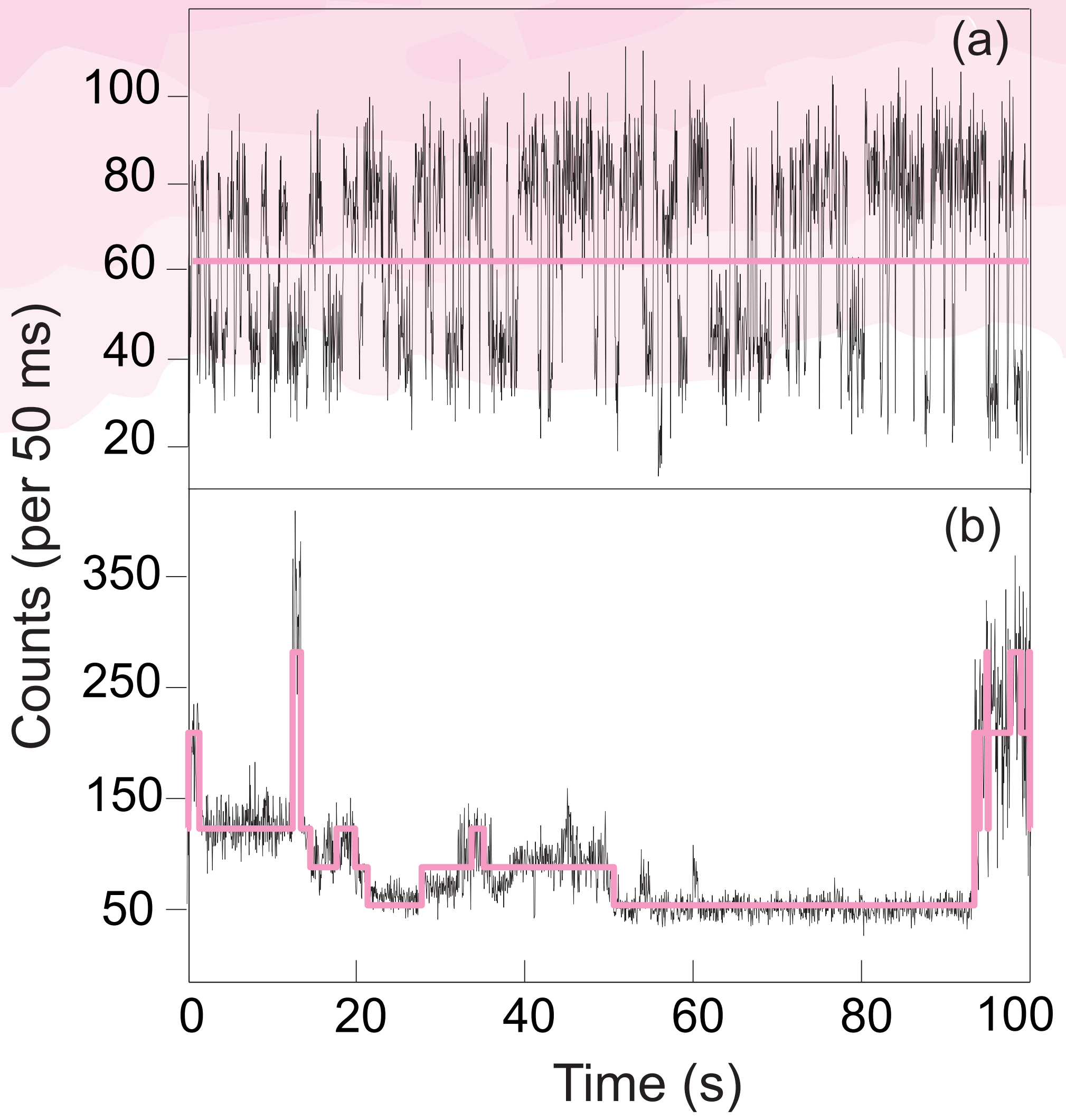
thrust 1 ♥ project 1.1

1.1

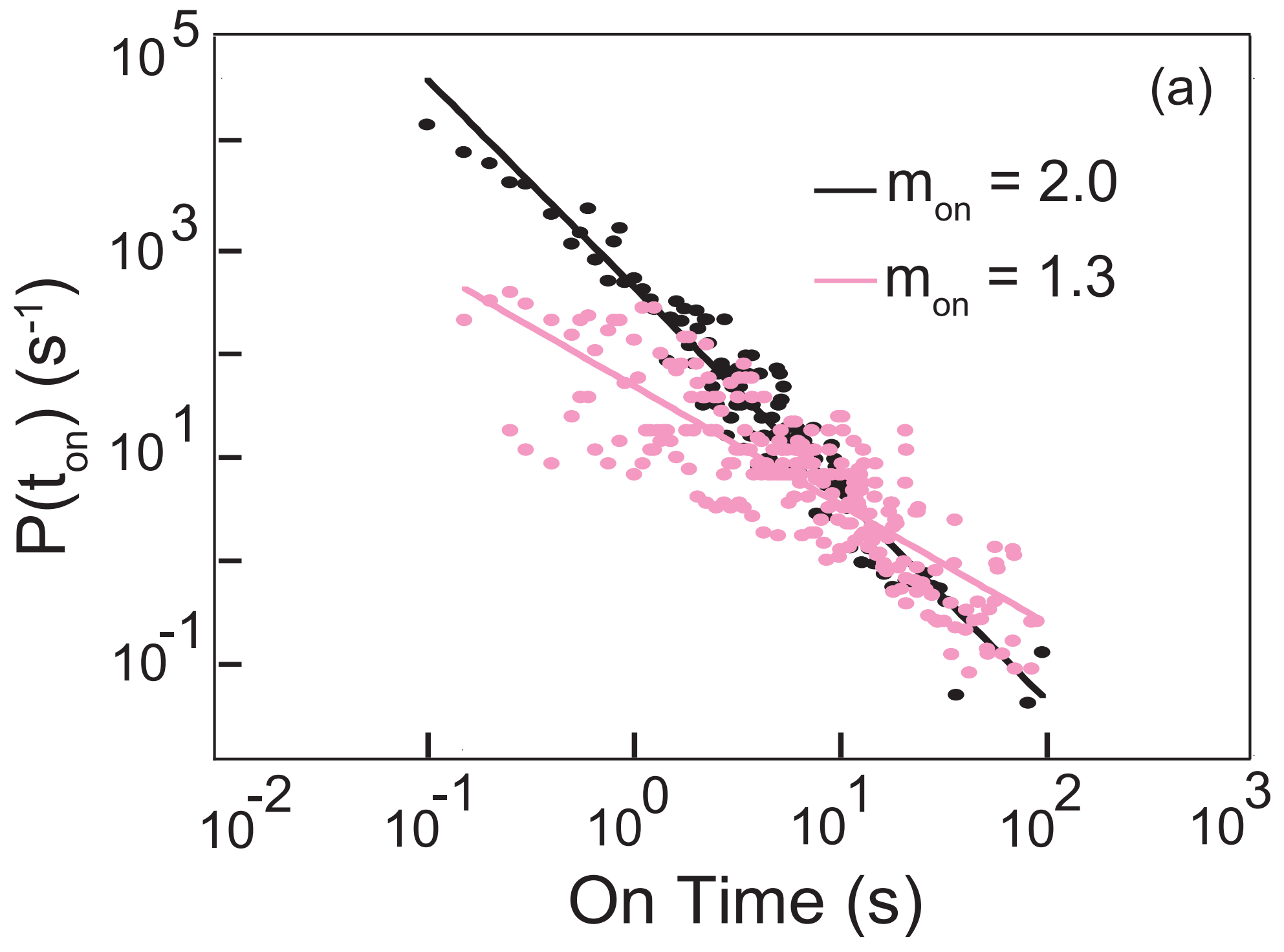
change-point detection



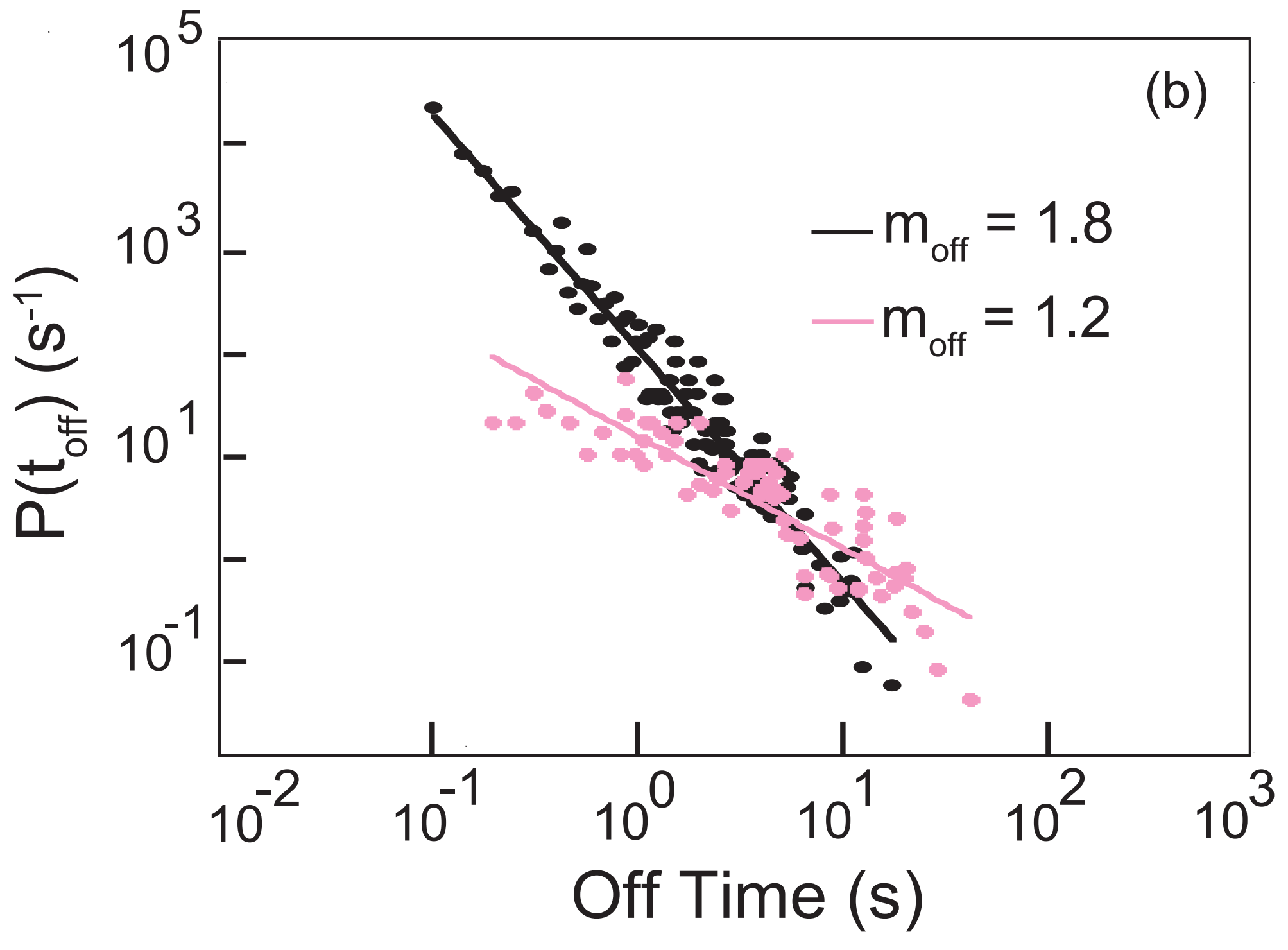
By visual inspection, many SM emission traces appear to undergo transitions between two states, *on* and *off*, which were analyzed previously using a hard threshold (a). Spurious short-time events generate artificial change points and alter statistics.

A more rigorous method, originally developed by Haw Yang et al [1], employs information theory to locate temporal change points and deconvolve intensity levels (b). With this new change point detection (CPD) algorithm, we are able to fully explore the complex photophysical behavior of single molecules in complex environments.

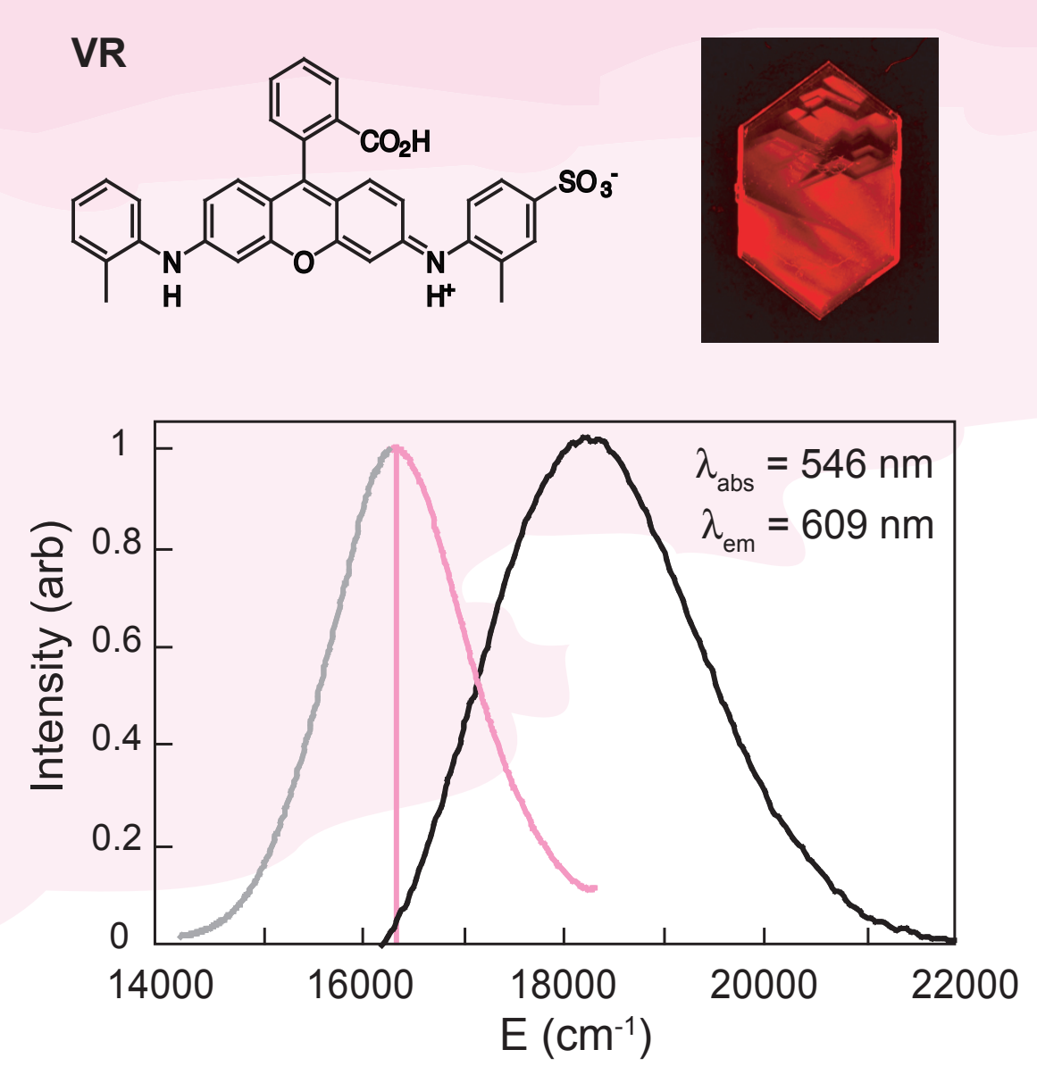
The CPD algorithm was applied to 40 SM emission traces previously analyzed with a hard threshold. The results are still power law in nature, but the fit exponent has changed from ~2 (black) for the on and off time probability histograms to ~1.5 (pink). The power-law exponents are reduced due to a decrease in the number of short-time events since the CPD method removes spurious change points associated with Poissonian noise.

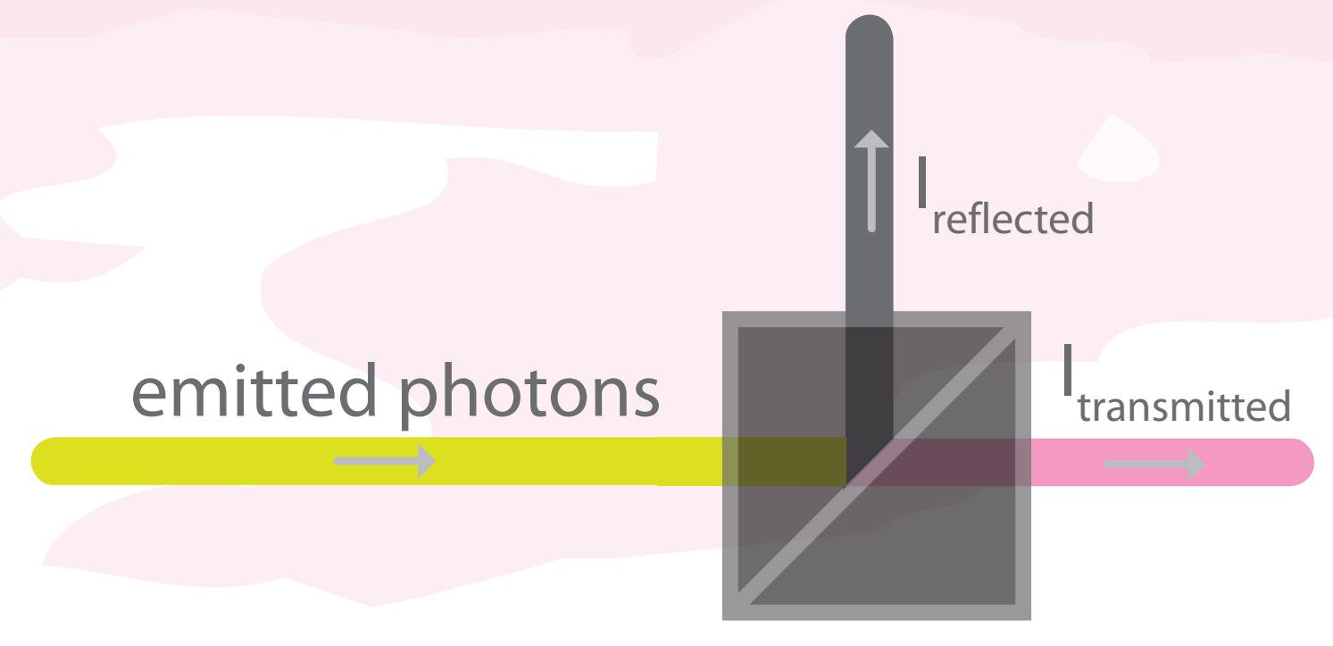


Power-law exponents of ~1.5 are consistent with electron tunneling and photoinduced spectral diffusion processes. It is possible that one of these processes is responsible for the intermittent fluorescence. To ascertain which of these processes is operative, we have explored the role that spectral diffusion plays in single-molecule blinking.



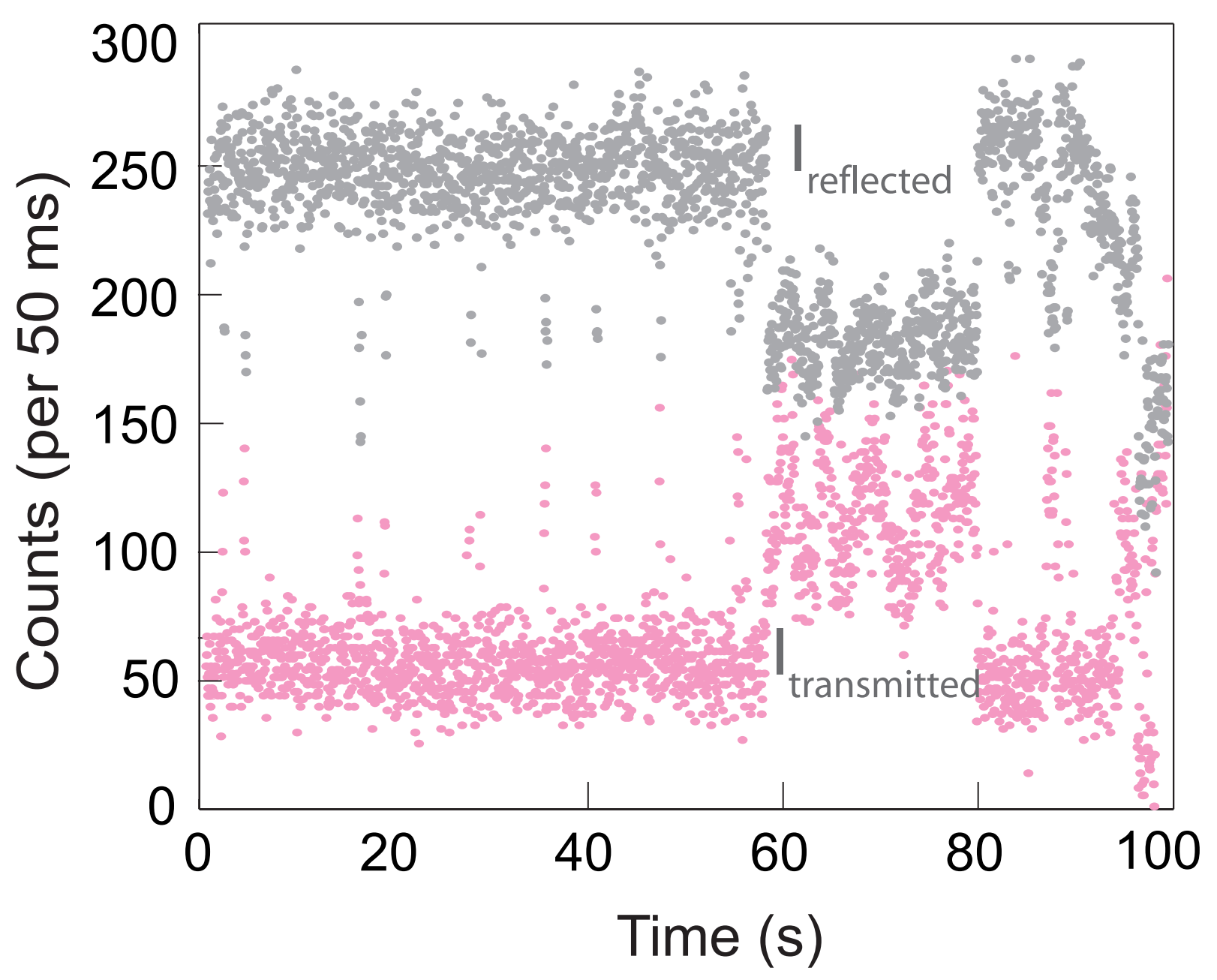
spectral diffusion

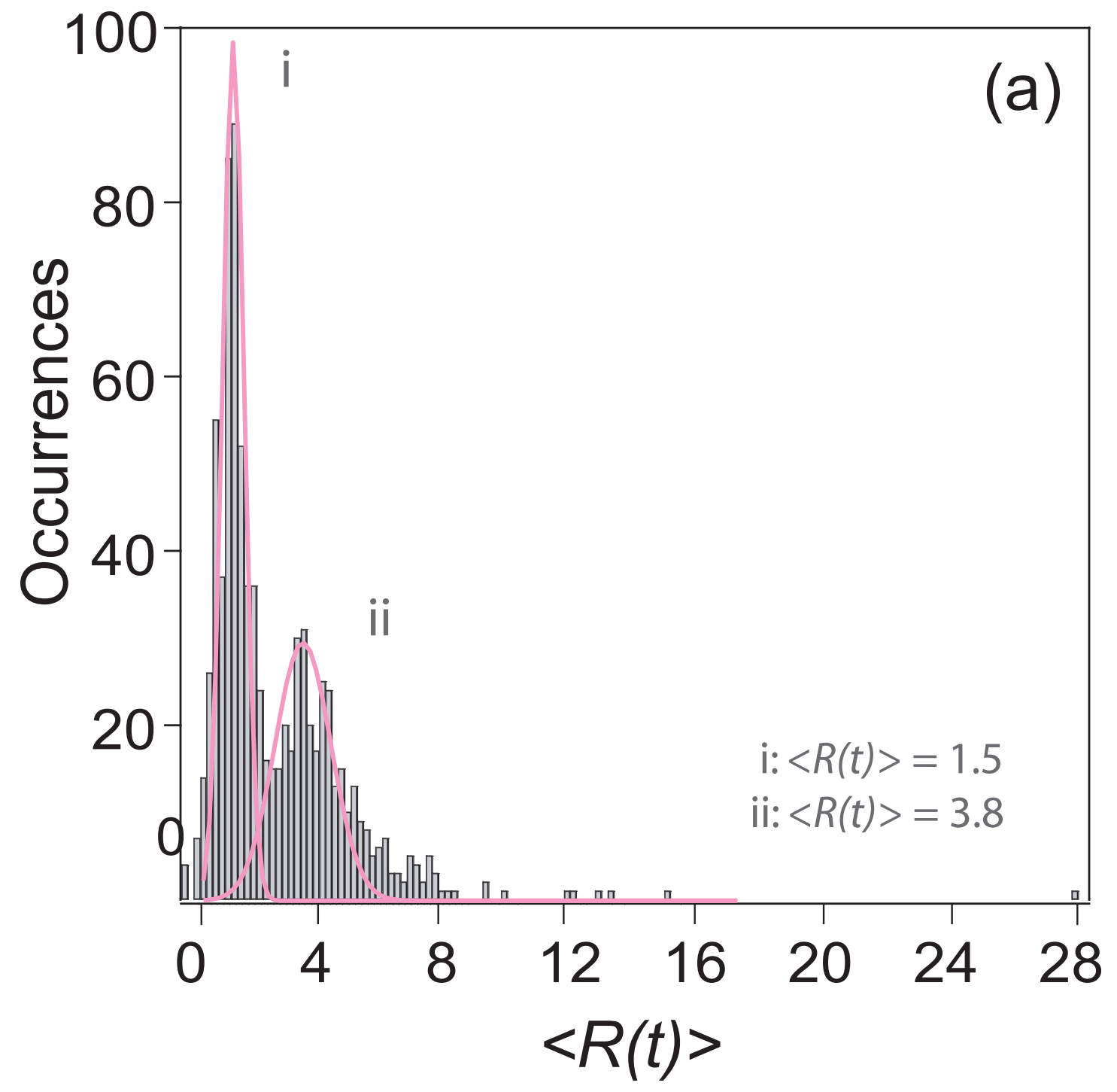




$$\langle R(t)\rangle = \langle I_{reflected} / I_{transmitted}\rangle$$

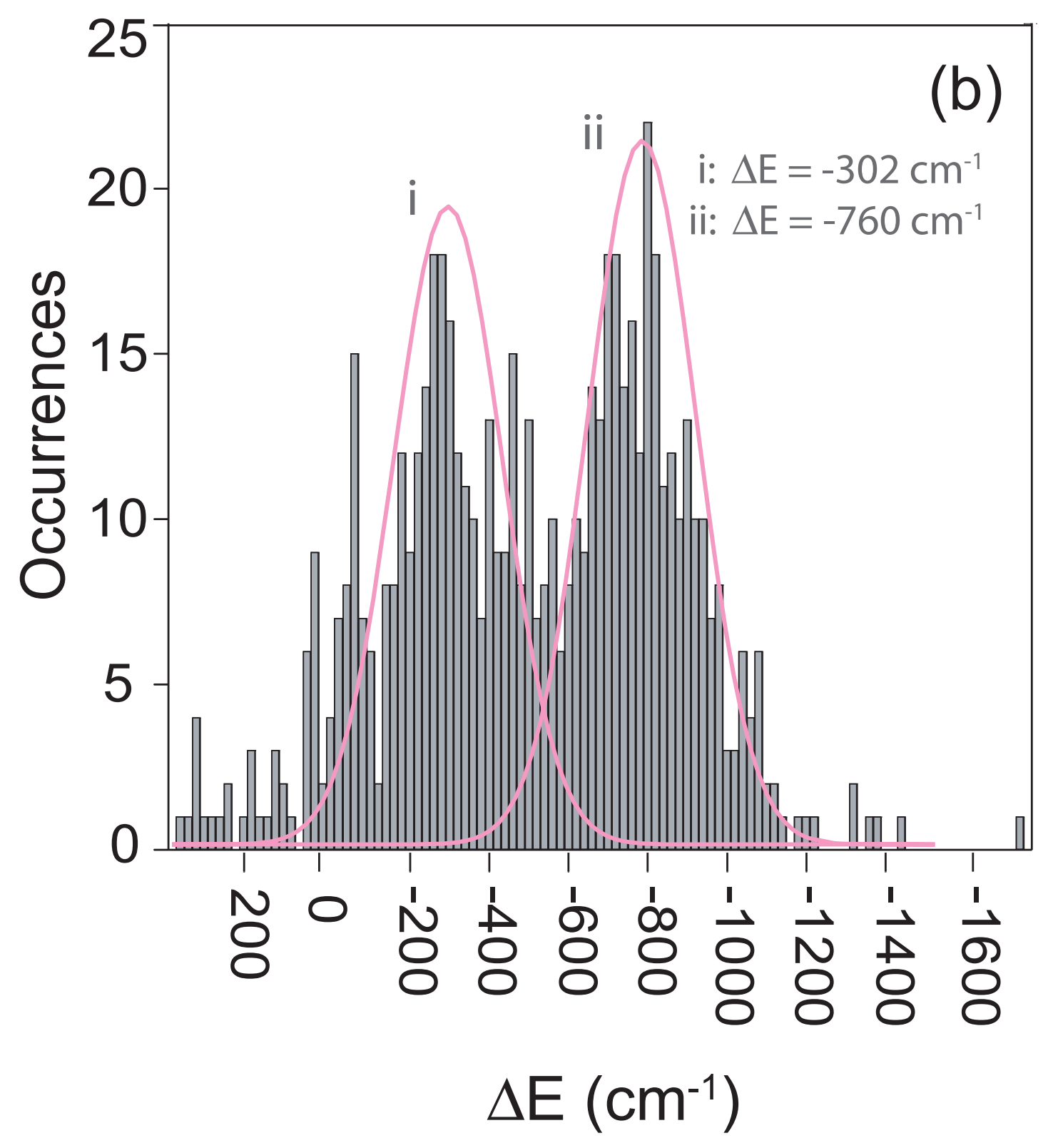
We explored the role of spectral diffusion in the blinking dynamics of single violamine R (VR) molecules in potassium acid phthalate (KAP). The emission from individual molecules was separated into spectral components using a dichroic mirror centered at 600 nm, then focused onto two avalanche photodiodes. This approach allows us to monitor changes in emission energy from one molecule to another, as well as time-dependent spectral fluctuations associated with spectral diffusion. A representative single-molecule emission time trace with the emission separated in terms of reflected and transmitted intensity from the dichroic is presented below.



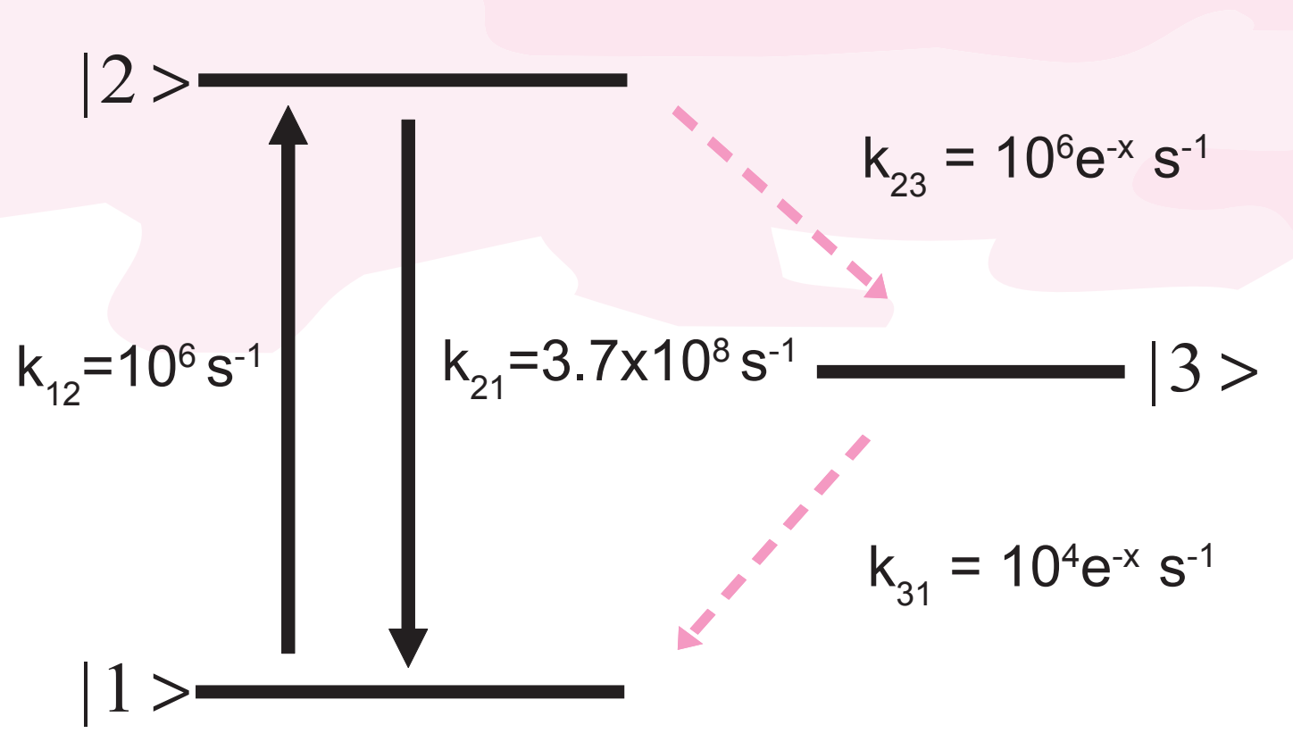


Analysis of 61 KAP/VR molecules using the CPD method with the distribution of *<R(t)>* values best fit to two Gaussian functions shown right, (a).

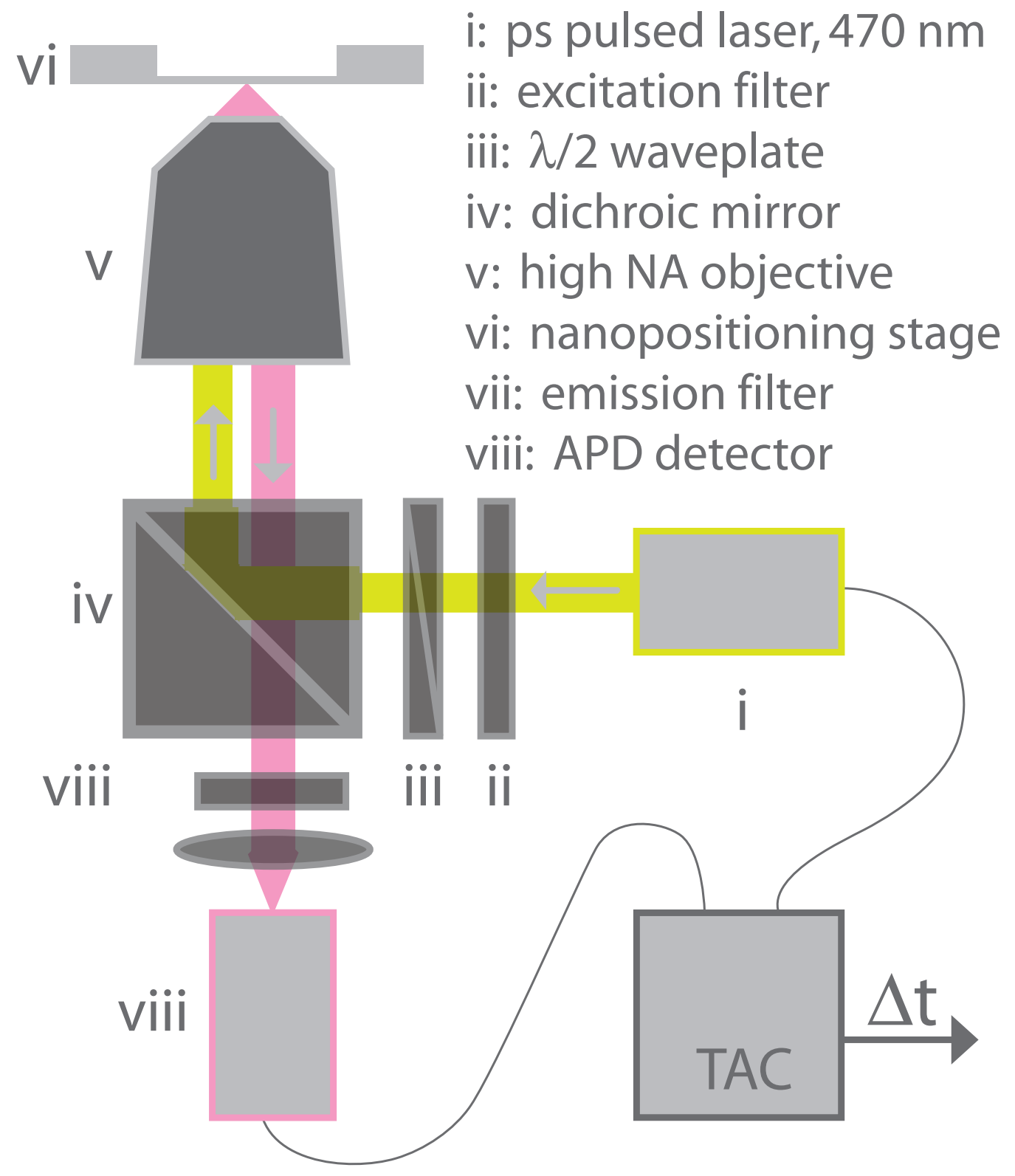
Values of <R(t)> were converted to energy shifts (ΔE) from the bulk emission spectrum maximum by convolving it with the transmission spectrum of the dichroic mirror. The resulting distribution is shown at right (b), with best fit Gaussians corresponding to two emission subpopulations at 620 nm and 639 nm. The broad distribution of emission energies suggest that molecules experience a distribution of local dielectric environments.



single-molecule lifetimes

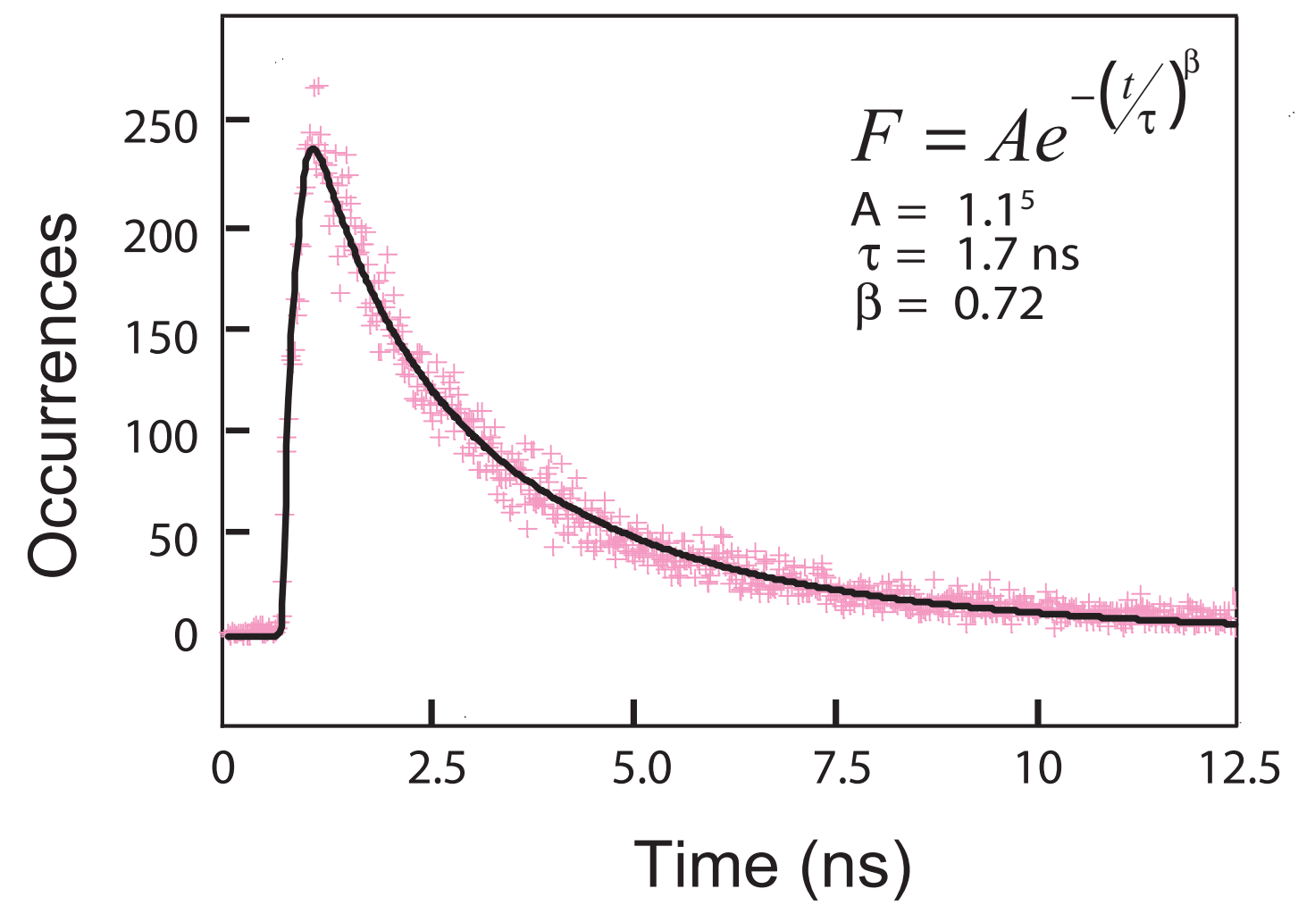


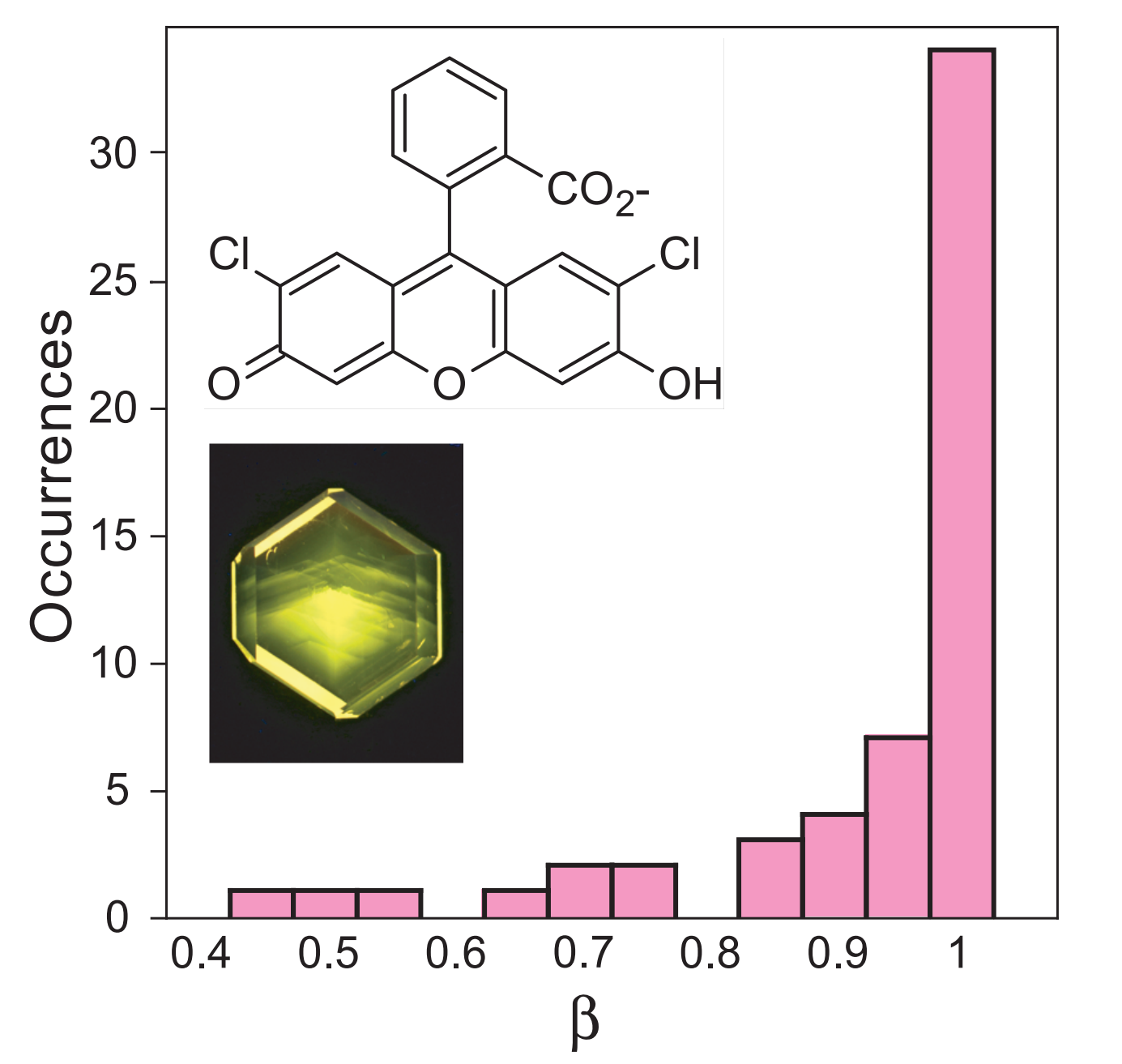
Previous Monte Carlo modeling of a three-level electronic system reproduced the power-law blinking dynamics with distributed rate constants for population and depopulation of the *dark* state [2]. This result suggests that the rate constants for depopulation of the optically-prepared excited state should be distributed. We have explored this idea using single molecule time-correlated single-photon counting (TCSPC).

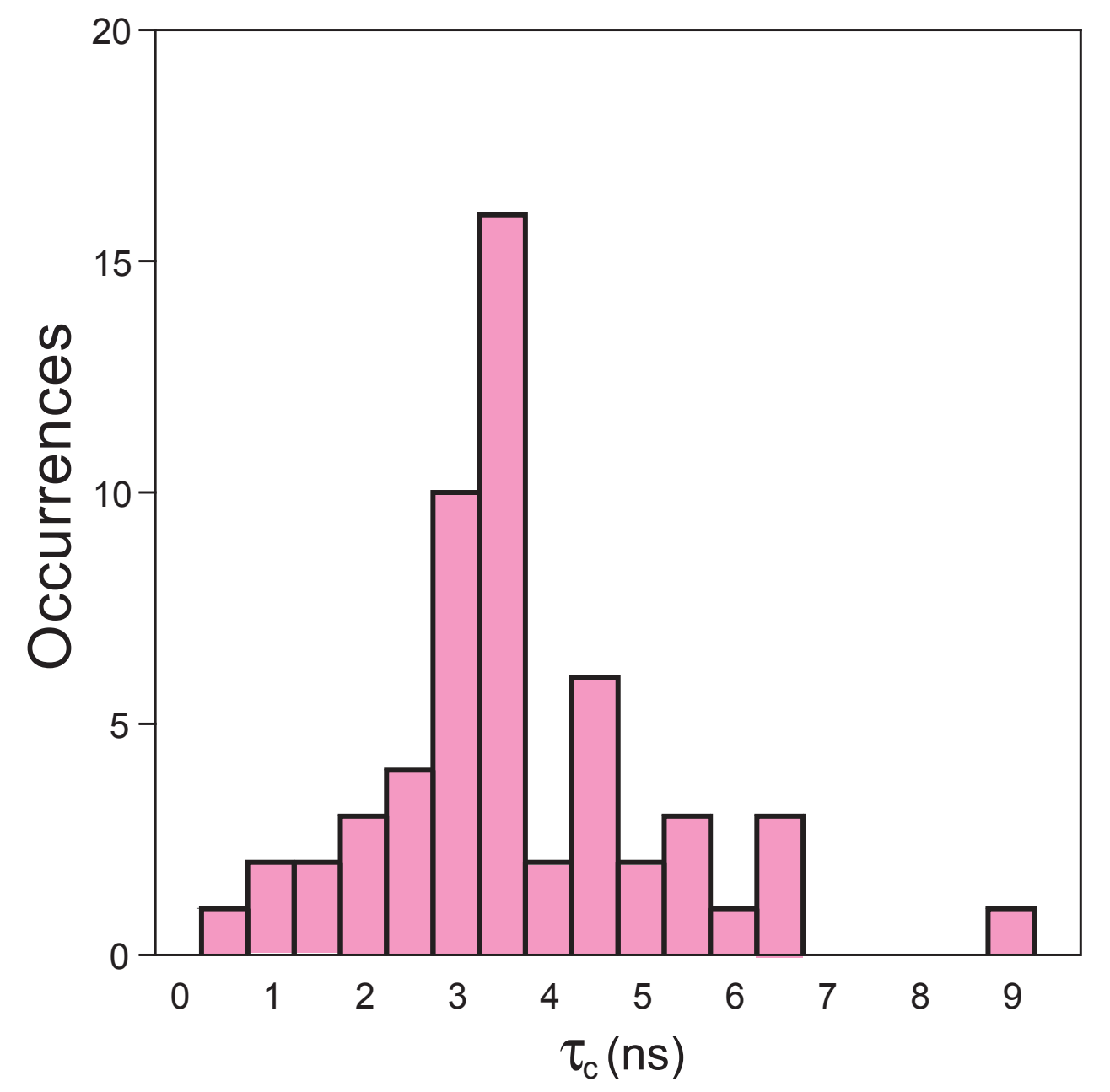


i: ps pulsed laser, 470 nm
ii: excitation filter
iii: λ/2 waveplate
iv: dichroic mirror
v: high NA objective
vi: nanopositioning stage
vii: emission filter
viii: APD detector

The TCSPC setup is shown at left. The system bins up histograms of time-delays between excitation and emission events to determine single molecule fluorescence lifetimes.







Above are histograms of the stretched exponential fits from 56 dichlorofluorescein (see inset above) molecules in KAP. Many molecules exhibit pure single exponential behavior, but many are better fit by stretched exponential functions with β values down to 0.46. Also, the τ_c values vary greatly from molecule to molecule. No clear trend between lifetime and β was found. This method integrates the individual photon delays into the same histogram, eliminating correlations between the photon delays and the blinking dynamics. Future work will focus on correlating these two factors.

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[1]: Watkins, L. P.; Yang, H. *J. Phys. Chem. B* 2005, 109, 617 [2]: Wustholz, K. L.; Bott, E. D.; Isborn, C. M.; Li, X.; Kahr, B.; Reid, P. J. J. Phys. Chem. C 2007, 111, 9146.