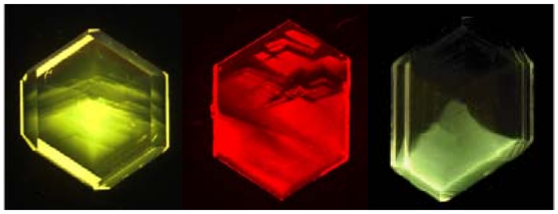
Winking, Blinking, and Odd: Single-Molecule Microscopy Studies of Dispersed Kinetics in Novel Environments



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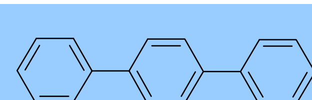
University of Washington, Seattle

Single-Molecule Spectroscopy in Mixed Crystals

- Pioneering work by Moerner and Orrit on pentacene in p-terphenyl

pentacene

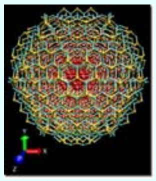


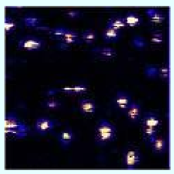
p-terphenyl

- Demonstrated the use of chromophores as “nanoreporters” of local environment.
- Consistent with principle of “isomorphism”.

Single Molecule Probes Come in All Sizes!

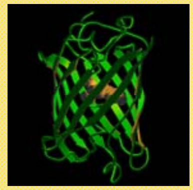
Quantum Dots
(CdS, CdSe, etc.)

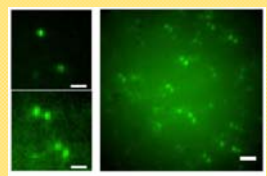




Bright and robust

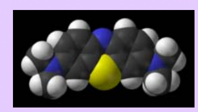
Green Fluorescent Protein

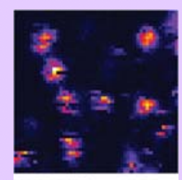




A “post-transcription” probe

Organic Molecules





Cheap and “non-toxic”

Relevance: Applicability, Photophysics, and Single-Photon Sources

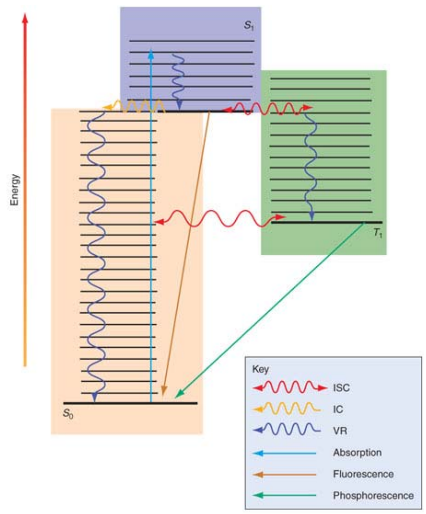
- The application of SM emitters to problems in chemistry and biology requires knowledge of the emission statistics from the emitters.

- Need emitters that are robust. Can analyze emission to learn about the photophysical processes that occur in single emitters (we’ll focus on this today).

- For applications such as quantum communications, a single-photon source is required that possess:

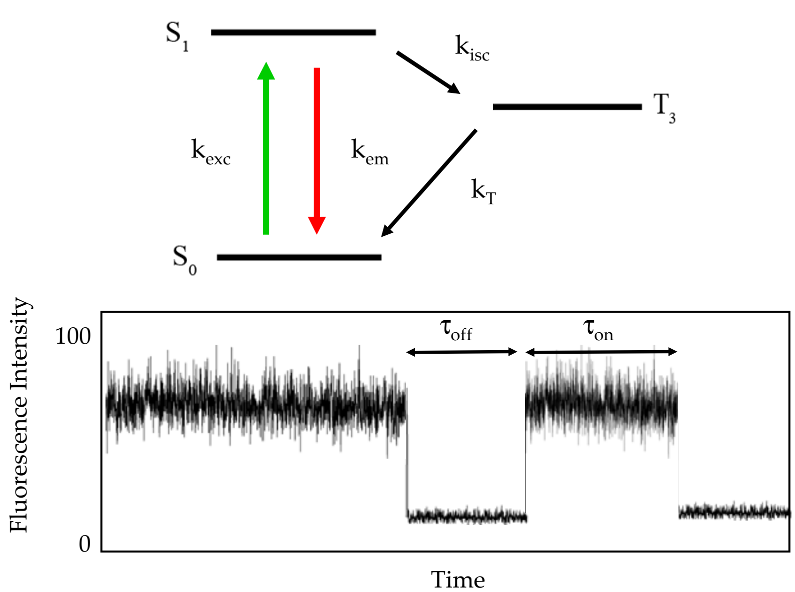
    Deterministic emission
    Very low probability of two-photon output
    Photostable
    Polarization control
    Easily fabricated

Molecular Photophysics: A One-Slide Primer

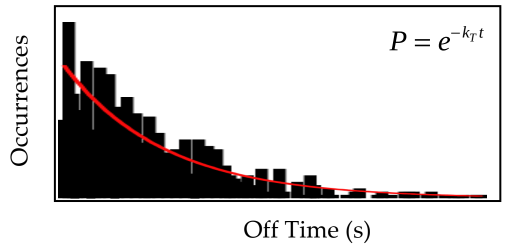


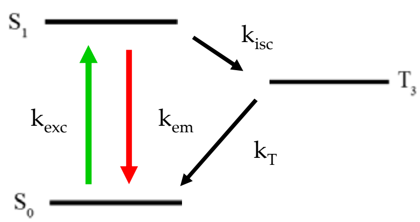
- In SM optical studies, the molecule absorbs a photon promoting the molecule from the ground electronic state (S_0) to an excited state (S_1).
- Once populating S_1, the molecule can return to the ground state through fluorescence, or populate another electronic excited state known as the triplet state (T_1).
- The molecule can not absorb another photon unless it is in S_0.

Studying Single-Molecule Photophysics: Blinking



Single-Molecule Emission Kinetics





$$S_o \xrightarrow{k_{exc}} S_1 \qquad P = e^{-k_{exc} t}$$

$$S_1 \xrightarrow{k_{em}} S_0 \qquad P = e^{-k_{em} t}$$

$$S_1 \xrightarrow{k_{isc}} T_3 \qquad P = e^{-k_{isc} t}$$

$$T_3 \xrightarrow{k_T} S_0 \qquad P = e^{-k_T t}$$

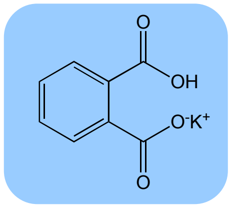
What is actually observed?

- Some SM emitters demonstrate blinking behavior that is well described by exponential kinetics, with dark periods defined by the rate constants for T_1 production and decay.

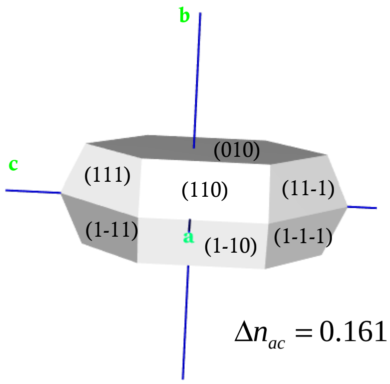
- Recently, many SM emitters (including quantum dots and the majority of organic molecules) demonstrate blinking behavior that is **not** consistent with simple exponential kinetics for dark-state production and decay.

- Current work in the area of “fluorescence intermittency” involves understanding the origin of this complex blinking behavior.

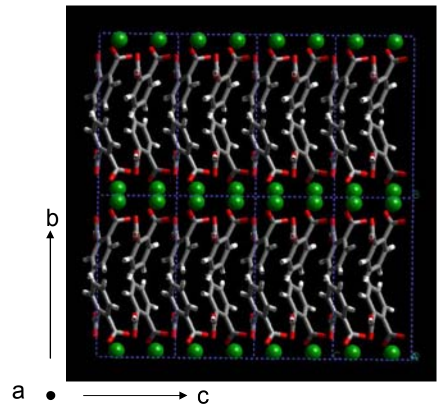
Crystal Host: Potassium Acid Phthalate (KAP)



Space group: *Pca*2_1
Lattice Parameters
a = 9.614 Å
b = 13.330 Å
c = 6.479 Å

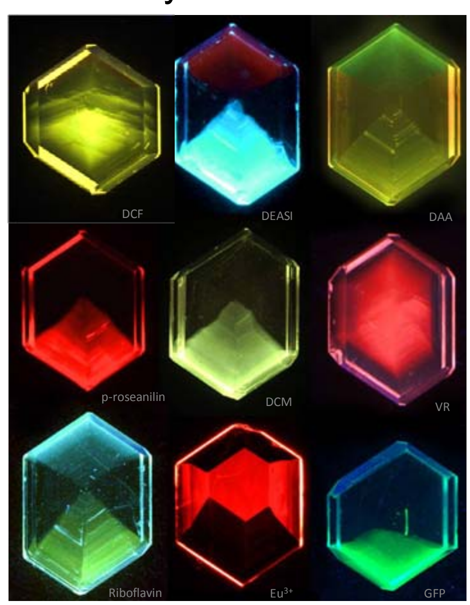


$\Delta n_{ac} = 0.161$

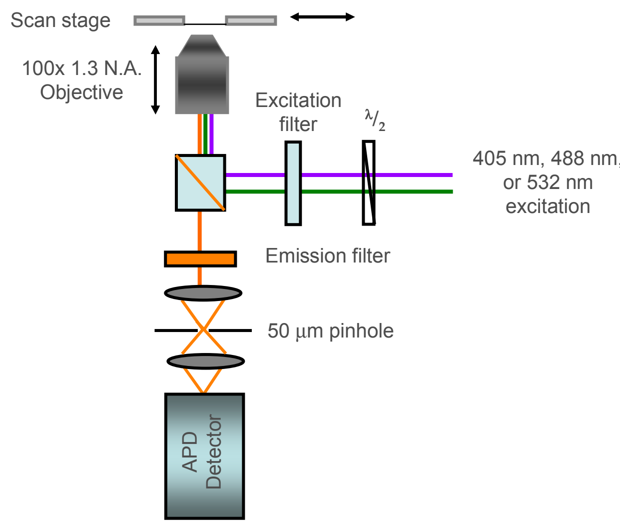


"Next Generation" Mixed Crystals

- "The essence of a recrystallization is purification" – Zubrick's "The Organic Chem Lab Survival Manual"
- Mitscherlich's principle of isomorphism states that for a mixed crystal, components must be similar in size and shape.
- However, the driving force of supersaturated solutions far from equilibrium overcomes the thermodynamic constraints of isomorphism.

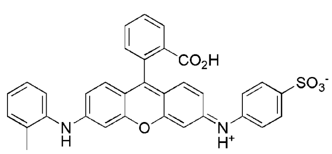


Experimental Setup: Confocal Microscope

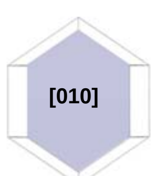


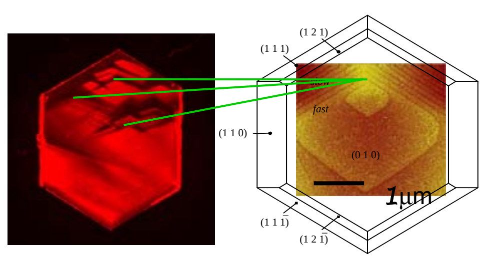
Why Study Violamine R Dyed KAP?

- ***Inter*-Sectoral Zoning** - Different crystallographic faces have different affinities for the impurities, trapping them in polyhedral sub-volumes of the crystal called growth sectors.
- ***Intra*-Sectoral Zoning** - Impurities may inhomogeneously deposit within a growth sector due to further polygonization of the surface.

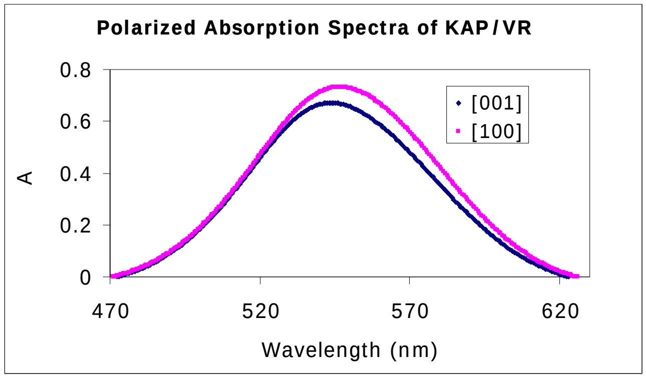


Violamine R (VR)





Abs. & Em. Dichroism: Incorporation w/ Alignment



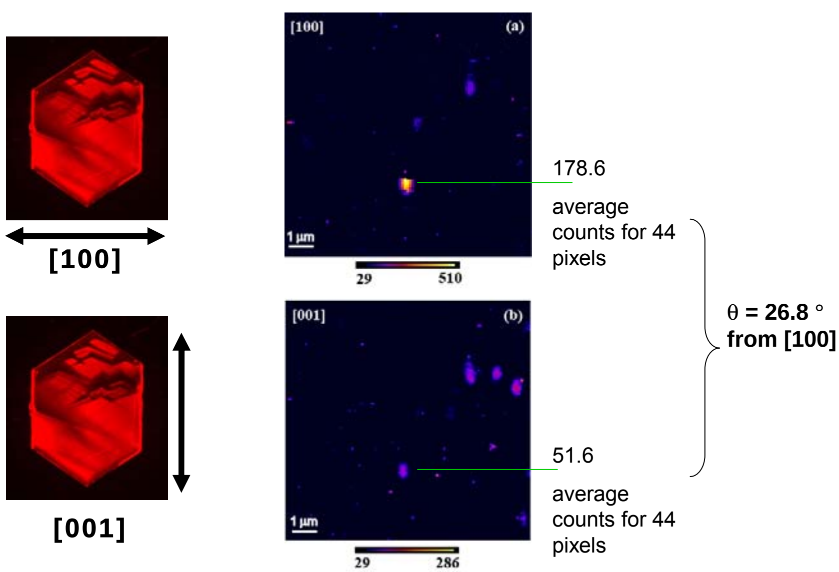
Absorption

$$\theta = \arctan\left[\sqrt{\frac{A_{\parallel}}{A_{\perp}}}\right] = 45.0^{\circ}$$

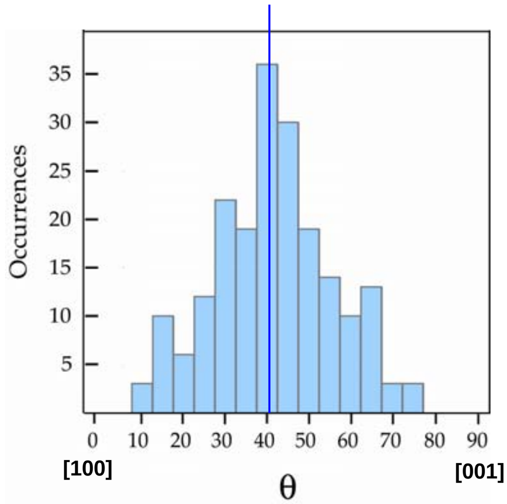
Fluorescence Excitation

$$\theta = \arctan\left[\sqrt{\frac{I_{\parallel}}{I_{\perp}}}\right] = 44.5^{\circ}$$

Excitation Dichroism of Single-Molecules in KAP/VR

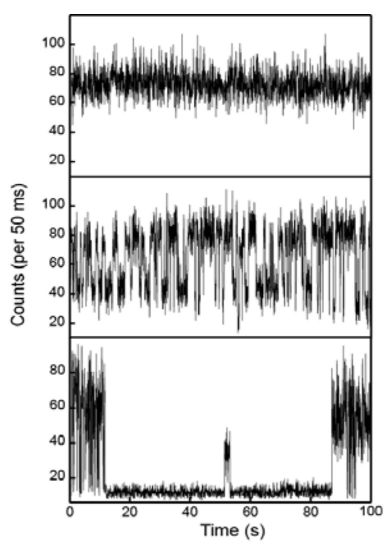


Orientational Distribution of VR in KAP



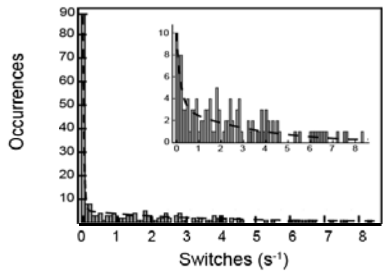
- 108 molecules
- Orientation:
  - Average = 42.3 ± 10.4°
  - Ensemble average = 45.0°
  - Range from 20.1° to 72.9°

VR "Blinking" in KAP



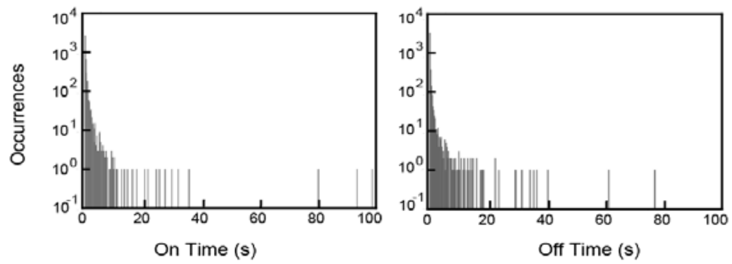
- Continual luminescence (no blinking)
- Periodic toggling between "on" and "off" states
- Two blinking timescales observed (i.e. fast and slow)

Photophysics in VR Dyed KAP



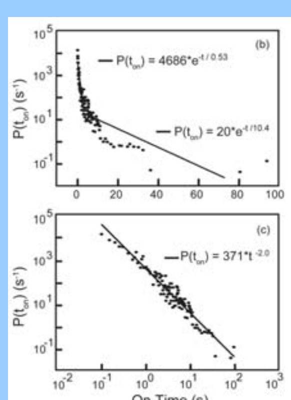
Switching behavior:

- Roughly 40% continually emit
- Remainder (inset) exhibit a distribution of switching rates

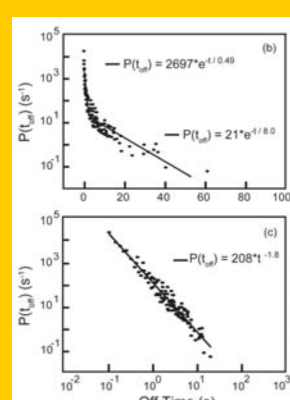


VR/KAP: Not Exponential…Power Law!

On Time

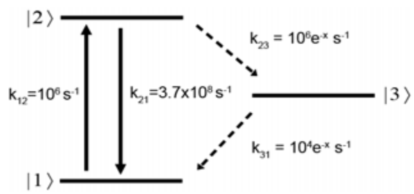


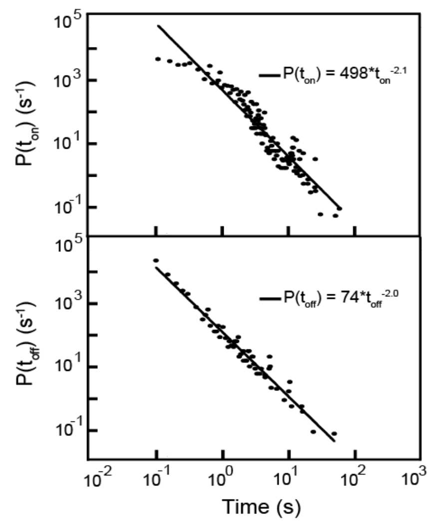
Off Time



Power-law behavior is evidence for “dispersed” kinetic behavior

Monte Carlo Results





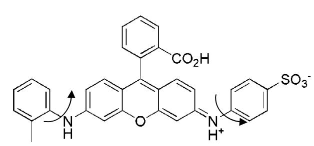
k_{12} and k_{21} defined by experiment.

k_{23} ~ 10^6 s^{-1} and k_{31} ~ 10^4 s^{-1}

Power law behavior (α = 2) is reproduced.

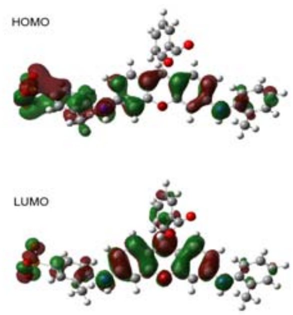
Possible Origins of Power-Law Kinetics

- Population and depopulation of a triplet state
- Conformational changes to a metastable non-emissive state



- Formation of non-emissive radicals through intermolecular electron transfer
- Spectral diffusion

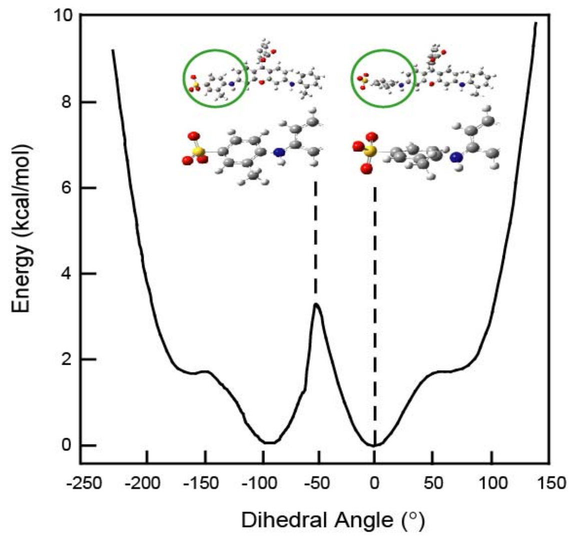
Exploring Conformational Dependence of Emission



•TD-DFT (B3LYP) and TD-HF (6-31G*)

•λ_{exc} ~ 541 nm

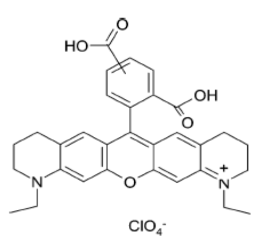
•f = 0.81 w/ ~10% change with conformation.



w/ C. Isborn and X. Li

Electron-Transfer Hypothesis

- Single molecules of Atto 565 spun coat on glass and rhodamine 6G dispersed in a poly(vinyl alcohol) film exhibited power-law kinetics.
- Distributed kinetics are attributed to the formation of non-emissive radicals through electron transfer between the molecule and the disordered surroundings.

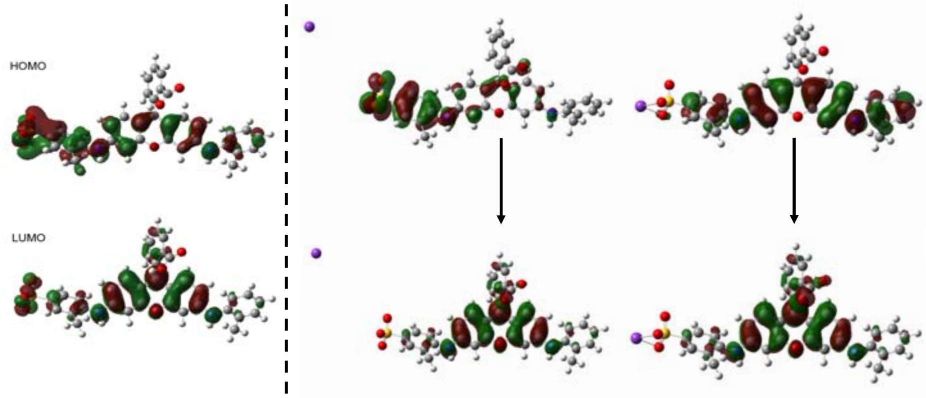


Atto 565

Yeow, E. K. L.; Melnikov, S. M.; Bell, T. D. M.; De Schryver, F. C.; Hofkens, J. *Journal Of Physical Chemistry A* 2006, *110*, 1726.

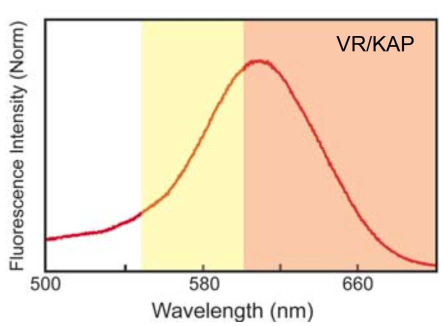
Spectral Diffusion in KAP

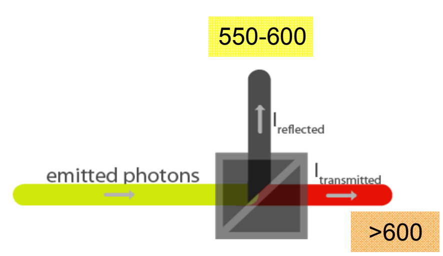
Isolated VR | Potassium cation ~8 Å from carboxylate | Potassium cation ~1 Å from sulfonate



λ_{exc} = 541 nm | λ_{exc} = 614 nm | λ_{exc} = 462 nm

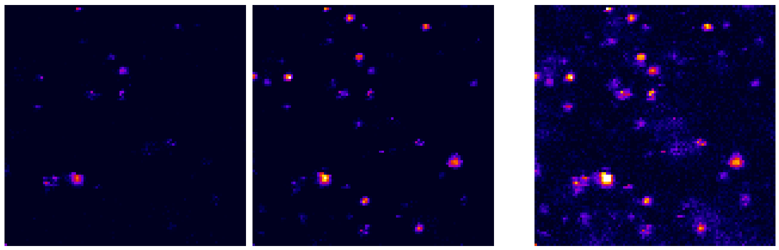
Spectral Imaging of VR/KAP





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

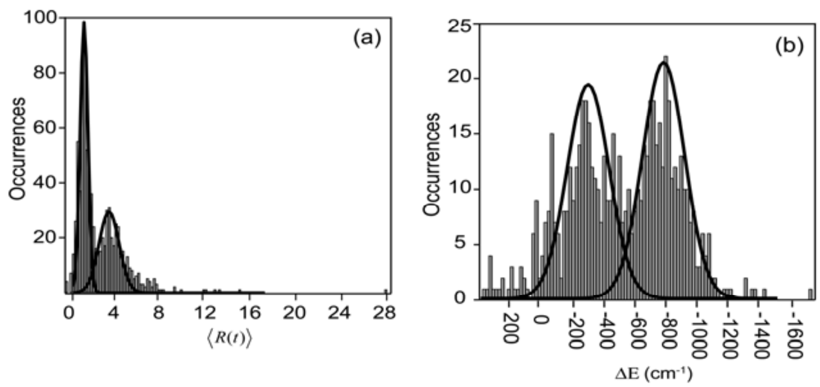
Spectral Imaging of VR/KAP



(550 – 600 nm)   >600 nm   Total Intensity

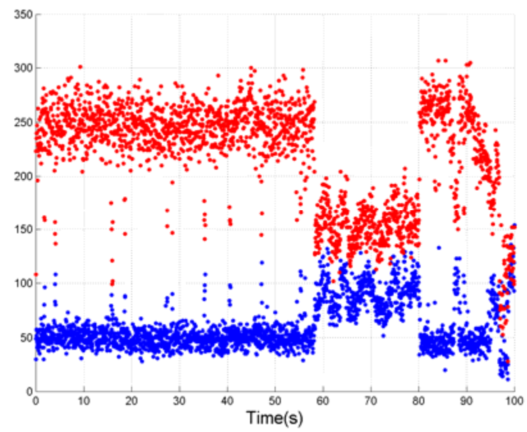
Intensity differences reveal distribution of dielectric environments.

Spectral Imaging of KAP/VR



- Spectral ratio provides a measure of the dielectric distribution.
- Two subpopulations are present at 620 nm and 639 nm.

Evidence for Spectral Diffusion in KAP



T = 550-600 nm
R = > 600 nm

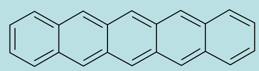
Evolution in spectrum demonstrates that local environment of molecule fluctuates in time.

Summary

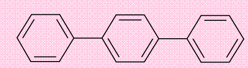
- Blinking dynamics in VR/KAP demonstrate power-law behavior.
- Results are modeled using Monte Carlo simulations employing exponentially-distributed kinetics to and from a "dark" state.
- Computations and experiments suggest that conformational flexibility is not responsible for the distributed kinetics. Importance of electron transfer is unclear at present.
- Instead, spectral diffusion is the current working hypothesis. Both time-dependent spectral shift measurements and blinking "memory" support this hypothesis.
- To test this hypothesis, measurements of single-molecule fluorescence spectra are underway.

Summary

1990:

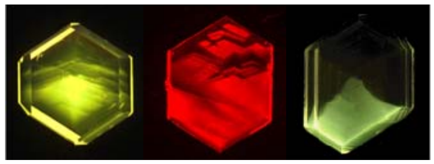


pentacene



p-terphenyl

2010:



Why do dyed crystals contain such diverse local environments?

What is the nature of the dark state?

Can we alter molecular structure or environment to affect blinking?

What happens in a “simpler” system?