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

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Single-Molecule Spectroscopy in Mixed Crystals

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

  \[
  \text{pentacene} \quad \text{p-terphenyl}
  \]

- 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

S_0 \quad \quad k_{isc} \quad \quad S_1

\quad k_{em} \quad \quad k_{exc} \quad \quad T_1

\quad \quad k_T

Fluorescence Intensity

\begin{align*}
\text{Time} & \quad 0 \quad 100 \quad \tau_{on} \quad \tau_{off}
\end{align*}
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 T₁ 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: Pca2₁
Lattice Parameters
\[ a = 9.614 \text{ Å} \]
\[ b = 13.330 \text{ Å} \]
\[ c = 6.479 \text{ Å} \]

\[ \Delta n_{ac} = 0.161 \]

“Next Generation” Mixed Crystals


- 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

- Scan stage
- 100x 1.3 N.A. Objective
- 405 nm, 488 nm, or 532 nm excitation
- 50 μm pinhole
- APD Detector
- λ/2
- Emission filter

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)](image)
Abs. & Em. Dichroism: Incorporation w/ Alignment

### Polarized Absorption Spectra of KAP/VR

#### Absorption

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

#### Fluorescence Excitation

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

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Excitation Dichroism of Single-Molecules in KAP/VR

#### [100]

- 178.6 average counts for 44 pixels

- \( \theta = 26.8^\circ \) from [100]

#### [001]

- 51.6 average counts for 44 pixels
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!

Power-law behavior is evidence for "dispersed" kinetic behavior
Monte Carlo Results

- $k_{12}$ and $k_{21}$ defined by experiment.
- $k_{23} \sim 10^6 \text{ s}^{-1}$ and $k_{31} \sim 10^4 \text{ s}^{-1}$
- Power law behavior ($\alpha = 2$) 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*)
- $\lambda_{\text{exc}} \approx 541$ nm
- $f = 0.81$ w/ $\sim 10\%$ change with conformation.

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.

Spectral Diffusion in KAP

Isolated VR

Potassium cation ~8 Å from carboxylate

Potassium cation ~1 Å from sulfonate

\[ \lambda_{\text{exc}} = 541 \text{ nm} \]

\[ \lambda_{\text{exc}} = 614 \text{ nm} \]

\[ \lambda_{\text{exc}} = 462 \text{ nm} \]

Spectral Imaging of VR/KAP

\[ \langle R(t) \rangle = \frac{I_{\text{reflected}}}{I_{\text{transmitted}}} \]
Spectral Imaging of VR/KAP

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

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](image1)

![p-terphenyl](image2)

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?