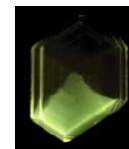
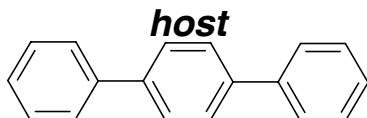
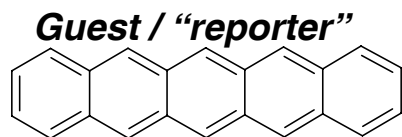


Single-Molecule Nanoreporters in Dyed Salt Crystals



Kristin Wustholz, IGERT Fellow
Chemistry: Reid and Kahr
3/7/06

We use dyes as single-molecule “nanoreporters” in transparent crystal hosts in order to study the molecular properties of included dyes as they relate to local environmental heterogeneity.



*Hundreds of combinations of dyed
organic and inorganic crystals
demonstrated by Kahr, et. al.*



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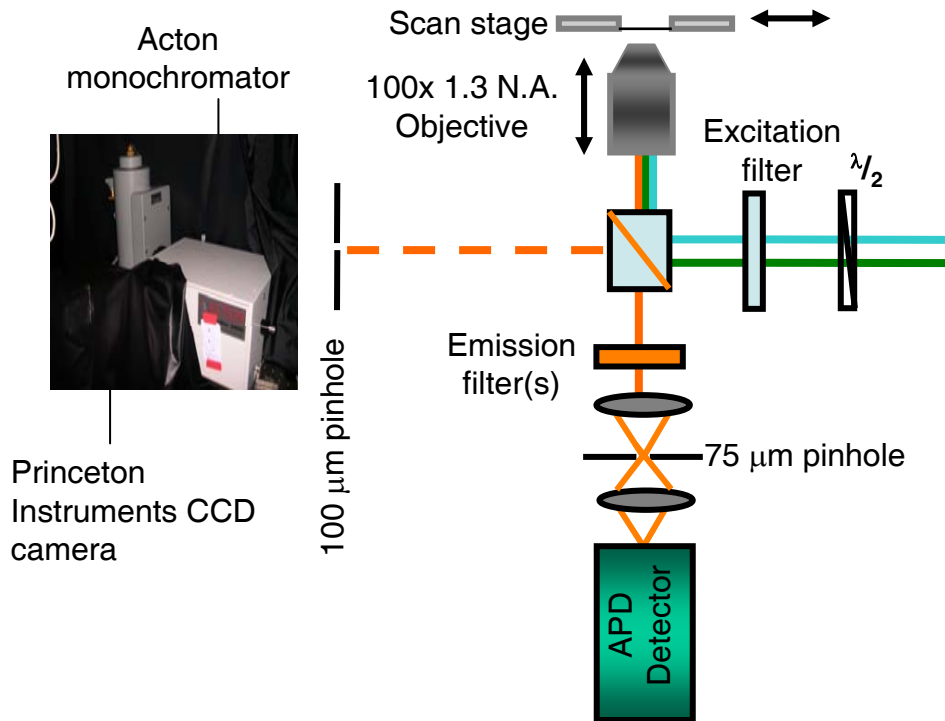




Single-Molecule Nanoreporters in Dyed Salt Crystals



Confocal Fluorescence Microscopy



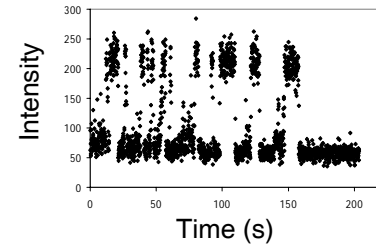
Orientations:

- polarized excitation along a crystallographic direction (a, c)
- measure total emission intensity

- compute angle using: $\theta = \arctan \left[\sqrt{\frac{I_c}{I_a}} \right]$

Photophysics:

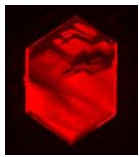
- record emission intensity for one molecule over time; "blinking dynamics"



Emission Spectra:

- record emission intensity through monochromator and CCD camera

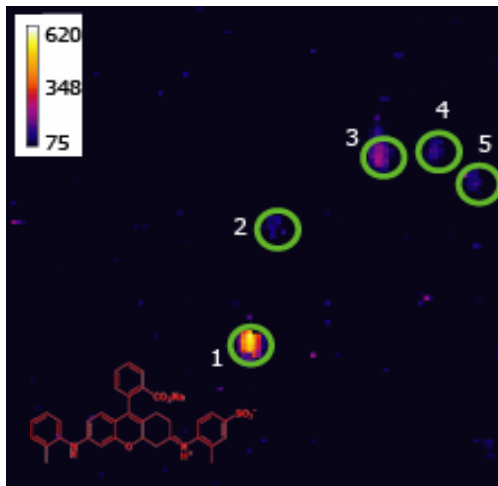




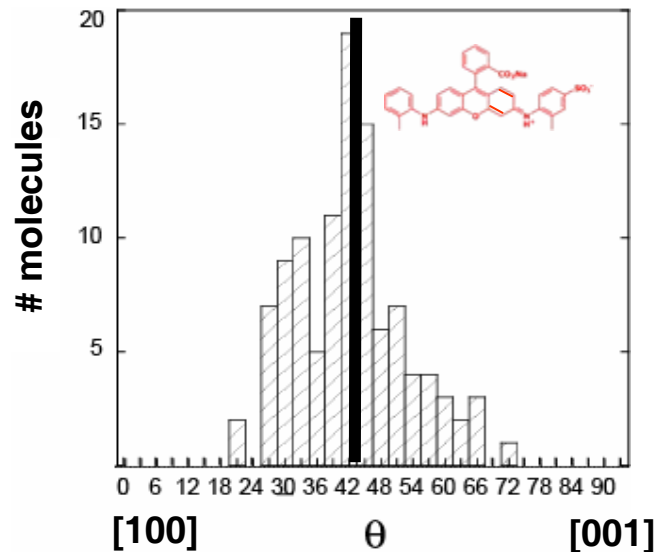
Single-Molecule Nanoreporters in Dyed Salt Crystals



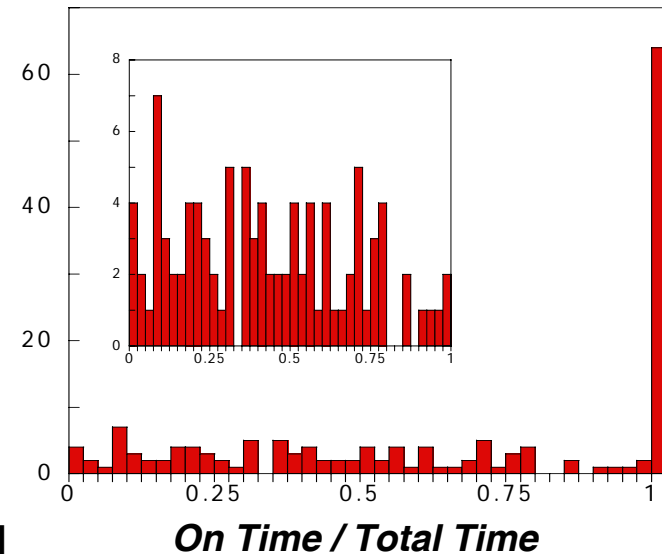
**Fluorescence image from
Violine R dyed KAP**



Orientational Histogram



Photophysics



- The average orientation of 108 single Violine R molecules in KAP ($42.3 \pm 10.4^\circ$) is in good agreement with ensemble measurements (42°), but our measurements show a wide arrange of orientations exist.
- The blinking dynamics of 161 single Violine R molecules in KAP were quantified, $\sim 40\%$ are “on”, while the remaining molecules exhibit a variety of photophysical behaviors – suggesting two subpopulations.
- Next step: the bigger picture, how do orientations, photophysics *and* emission spectra relate??

