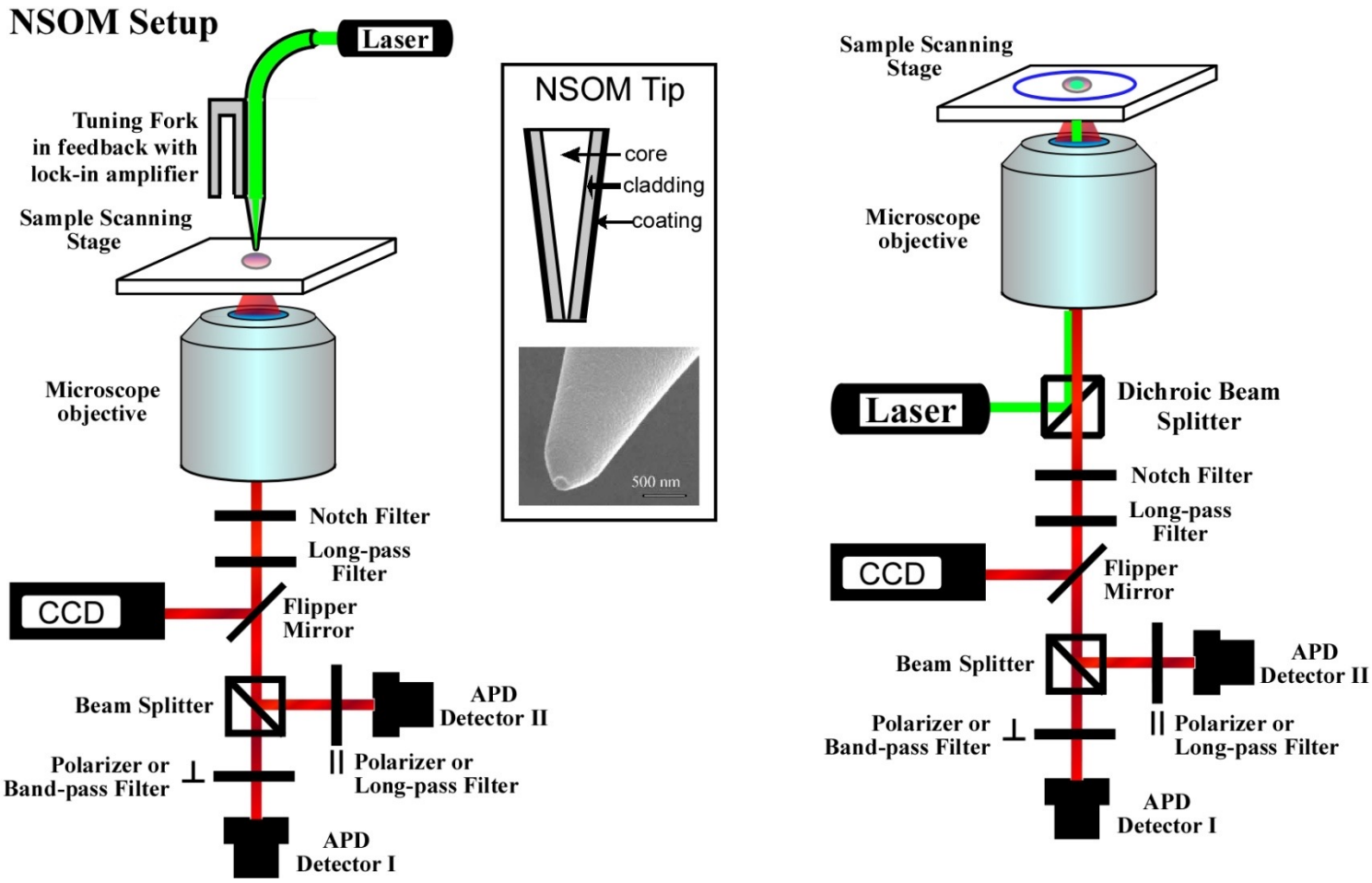
Lecture 20: SCM of single-molecule switching, rotation and diffusion, and beyond to nanoparticles

- Single-molecule switching and sensing ---depending on local environment.
- Single-molecule rotation.
- Single-molecule diffusion
- Extending of single-molecule imaging to single-particle imaging --- emission blinking of Q-dot, the nanomaterials behind QLED TV panel

SCM vs. NSOM for single molecule imaging

- SCM provides fast, real-time imaging, while maintain the high spatial resolution for single molecule imaging.
- SCM is more adaptable for diverse kinds of samples, from surface, to bulk phase (transparent to excitation beam), from solid to liquid.
- High intensity of photo-illumination generates sufficient emission for quick reliable detection --- high photon throughput removes the need of large bin time for photo-detector, so speed up the imaging scanning process.

NSOM vs. SCM


NSOM Setup
Laser
Tuning Fork
in feedback with
lock-in amplifier
Sample Scanning
Stage
Microscope
objective
Notch Filter
Long-pass
Filter
CCD
Flipper
Mirror
Beam Splitter
APD
Detector II
Polarizer or
Band-pass Filter
Polarizer or
Long-pass Filter
APD
Detector I
NSOM Tip
core
cladding
coating
500 nm
Sample Scanning
Stage
Microscope
objective
Laser
Dichroic Beam
Splitter
Notch Filter
Long-pass
Filter
CCD
Flipper
Mirror
Beam Splitter
APD
Detector II
Polarizer or
Band-pass Filter
Polarizer or
Long-pass Filter
APD
Detector I

Chem. Rev. **1999,** *99,* 2929–2956

Single Molecule Fluorescence Spectroscopy at Ambient Temperature

W. Patrick Ambrose,[†] Peter M. Goodwin,[†] James H. Jett,[‡] Alan Van Orden,[†,§] James H. Werner,[†] and Richard A. Keller[*,†]

Chemical Science and Technology Division and Life Sciences Division, MS M888, Los Alamos National Laboratory, Los Alamos, New Mexico 87545

910 *J. Phys. Chem. B* **2002,** *106,* 910−927

FEATURE ARTICLE

A Dozen Years of Single-Molecule Spectroscopy in Physics, Chemistry, and Biophysics

W. E. Moerner*

Department of Chemistry, Stanford University, Mail Code 5080, Stanford, California 94305-5080

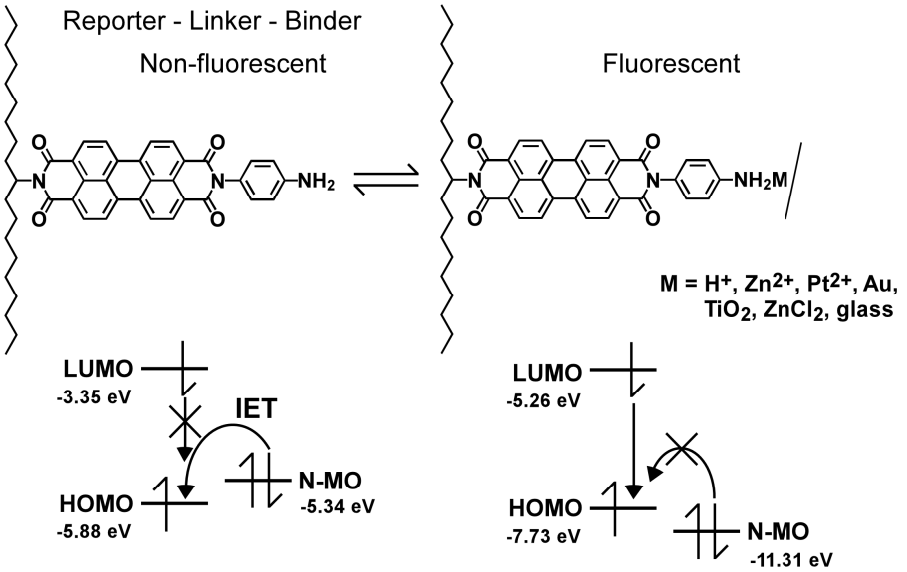
Received: August 2, 2001

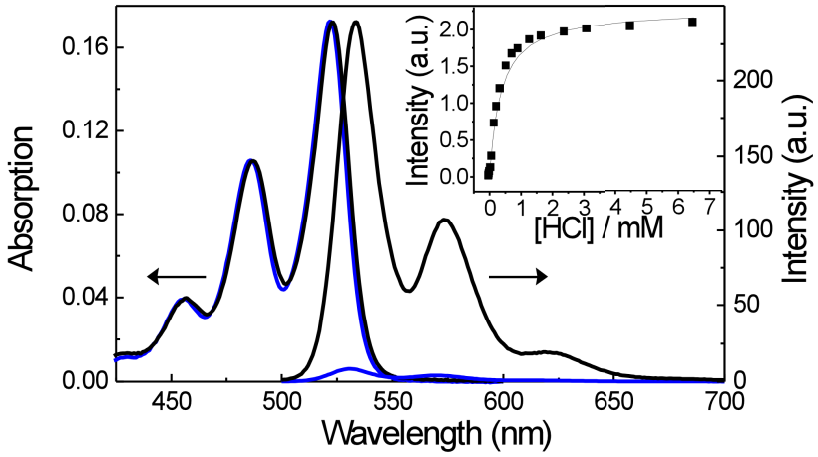
Topic 1: Single Molecule Switching & Sensing

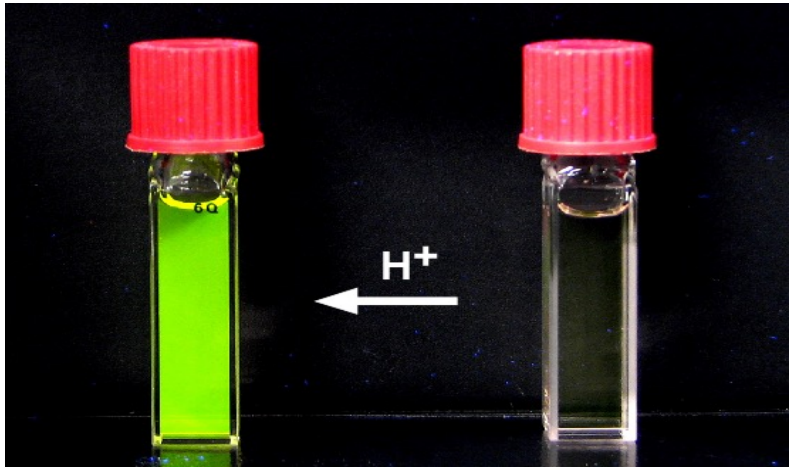
Why single molecule level?

- The ultimate degree of sensitivity for detecting local structure, dynamics, chemical reactions, and physical processes;
- Single molecule spectroscopy is powerful and unique for studying the behavior of individual molecules under ambient conditions;
- High selectivity with the fluorescence **switching** property;
- Revealing the rare processes, such as slow electron transfer, which can hardly be detected by ensemble lifetime measurements.

A Single Molecule Sensor Based on Intra-molecular Electron Transfer



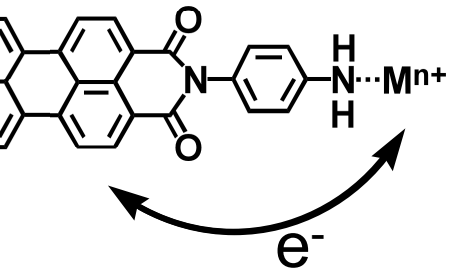


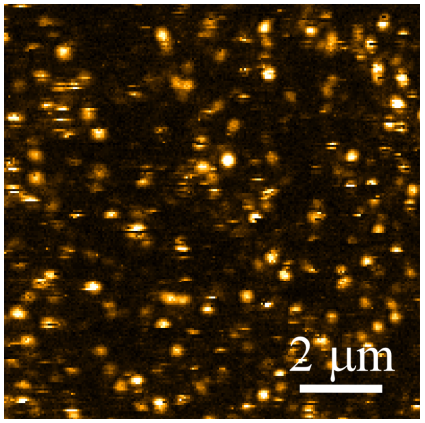


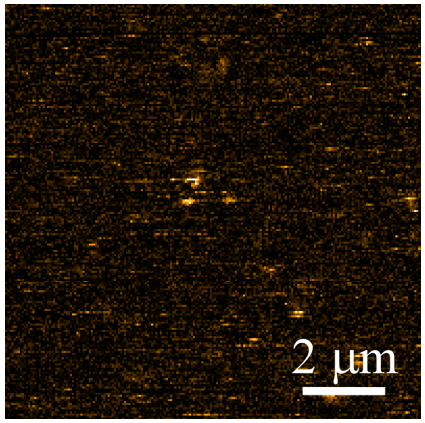
L. Zang et al. *J. Am. Chem. Soc.* **124** (2002) 10640-10641.

Probing Glass (*Corning*) Surface: *Local Structure*

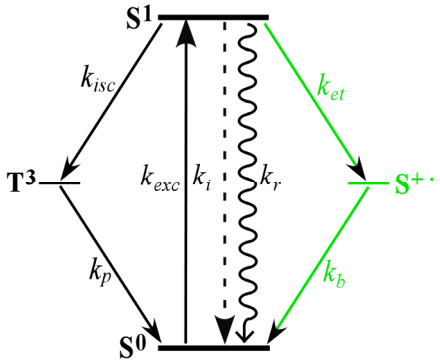
7% TiO_2; 10% ZnO; 3% Al_2O_3

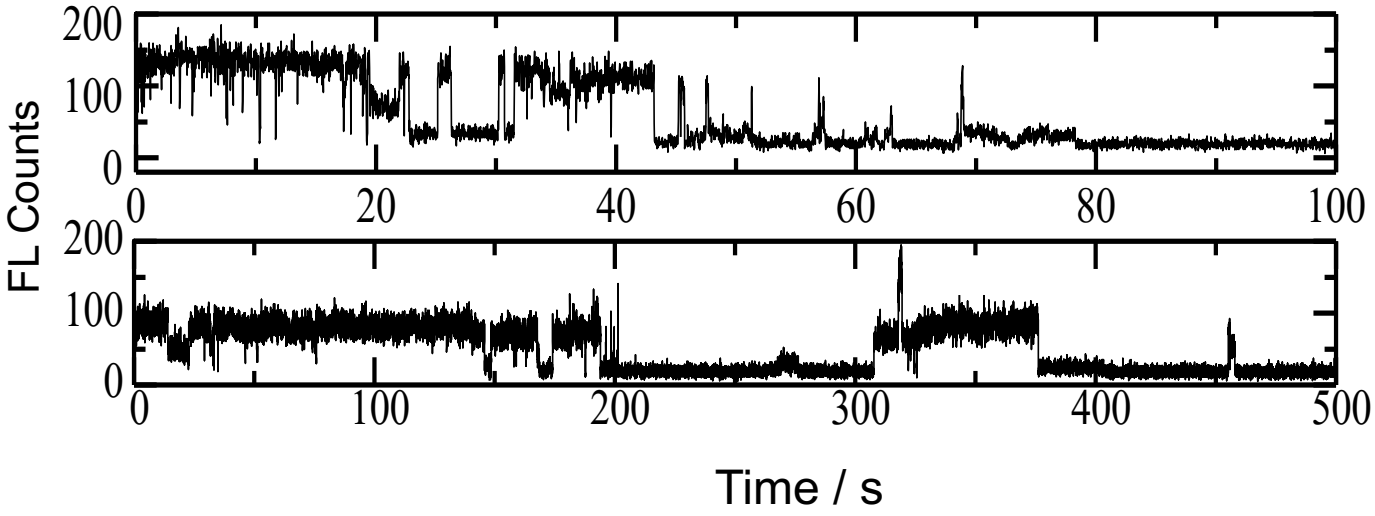






Quartz (99.999% SiO_2)

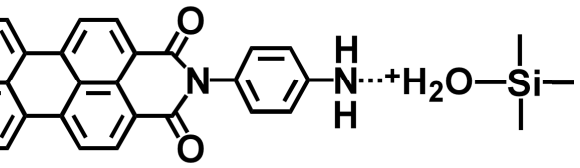


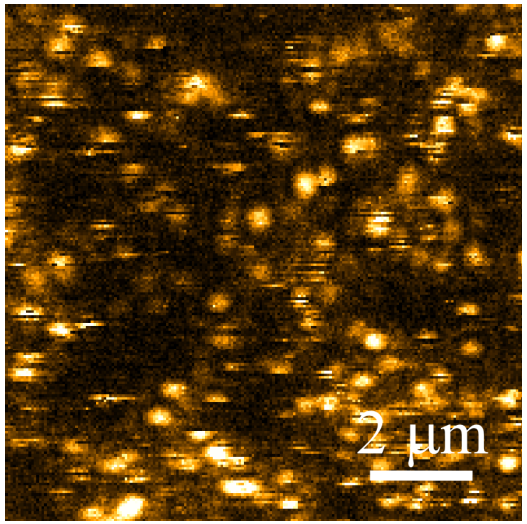


Long *off time* due to charge transfer state, $S^{+\bullet}$

Probing Local Protonated Sites (-OH_2^+) on Quartz: *Molecule Dynamics due to loose dangling hydrogen bond*

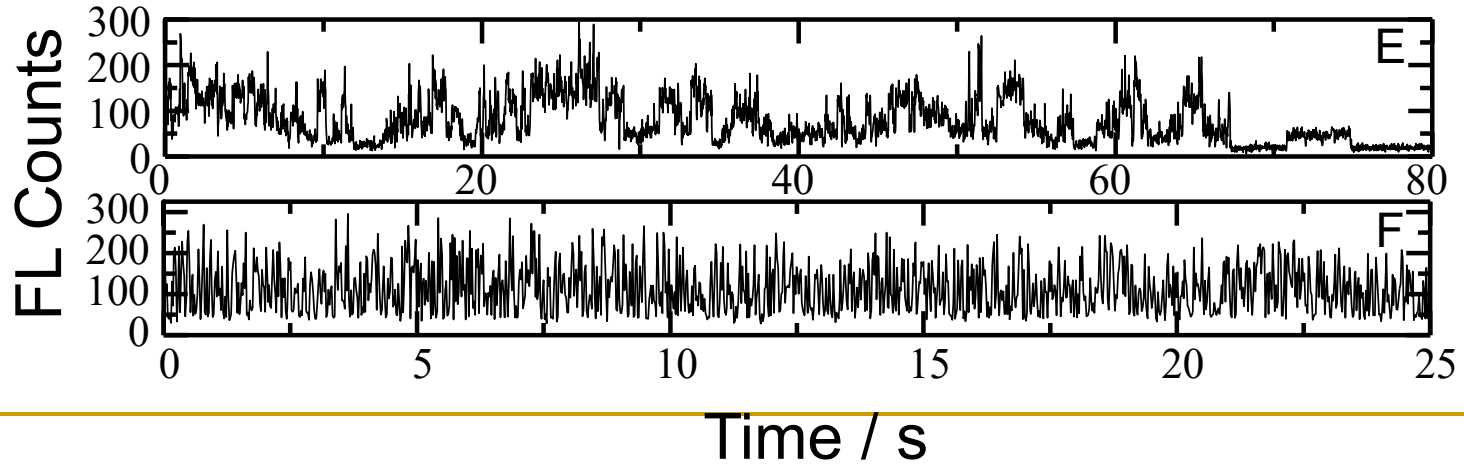
99.999% SiO_2



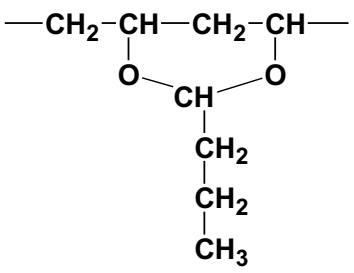


Spots appeared after exposed in humid air

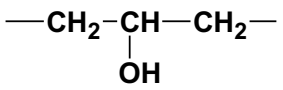
No spots came out for NaOH cleaned surface



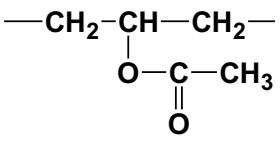
Monitoring Schiff Base Formation in PVB Film: molecules are now immobilized as embedded in the polymer matrix, thus showing constant emission intensity.



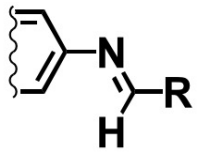
80%



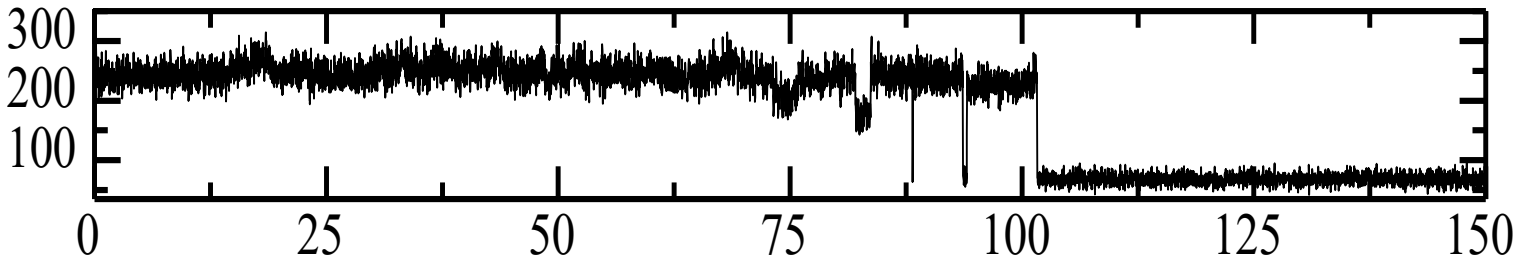
18%



2%

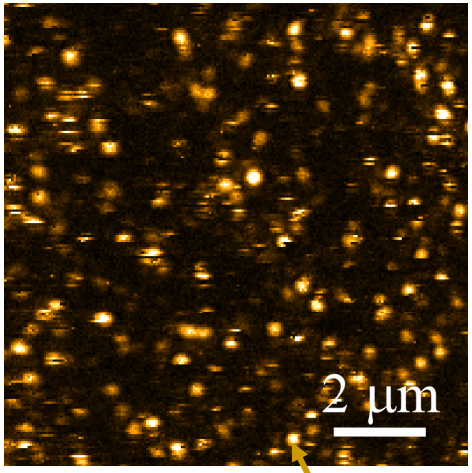


Schiff base formation turns on NDAPP

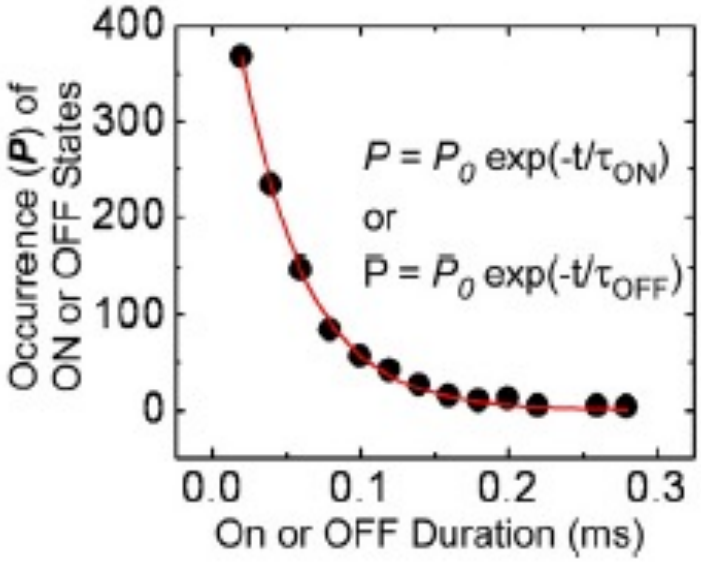


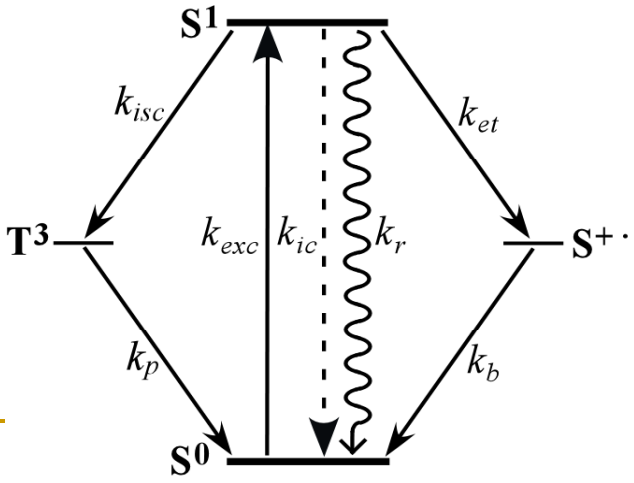
Much shorter *off time* due to triplet state, T^3

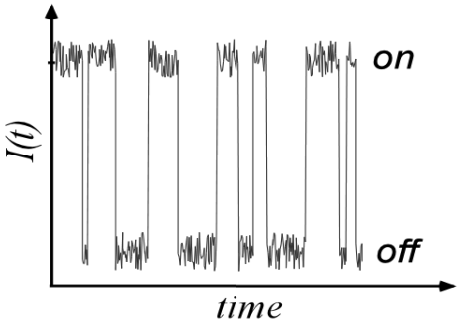
Single Molecule Imaging → dynamic/kinetic information



Focus on one



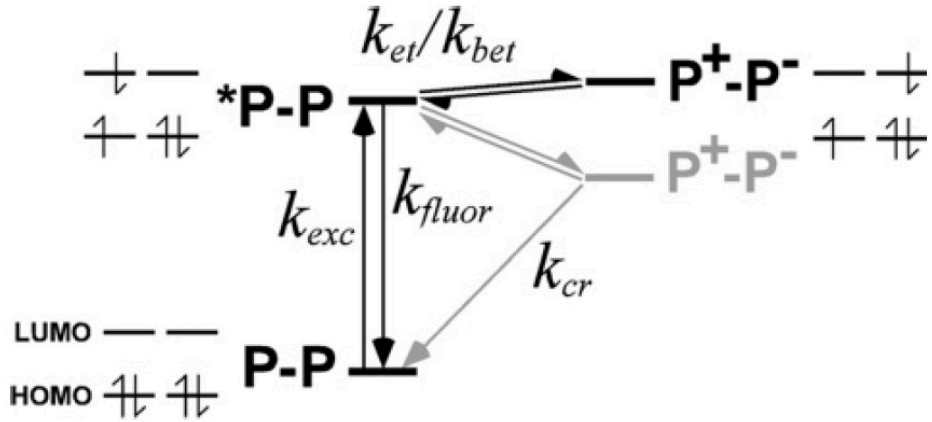




For PTCDI molecules, $\Phi(T^3) \sim 0$ and $\tau(T^3) < 1$ ms; "*off*" time is due to charge transfer state.

$$\frac{1}{\tau_{on}} = k_{exc}\frac{k_{ic}+k_{isc}+k_{et}}{k_r+k_{ic}+k_{isc}+k_{et}}; \quad \frac{1}{\tau_{off}} = k_b$$

Sensing Local Environment in Polymer Films



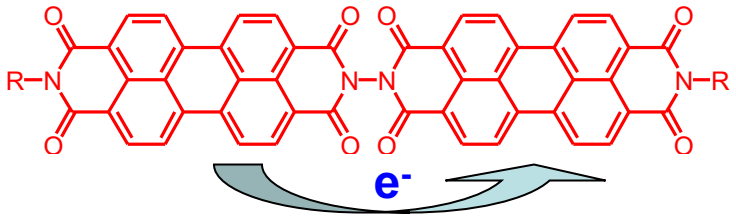
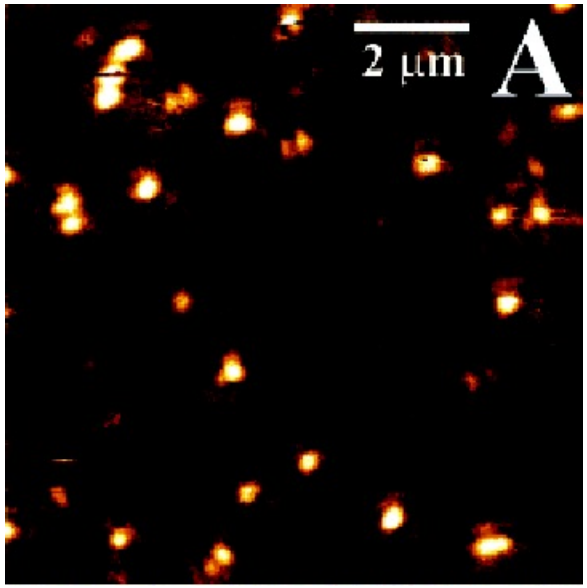
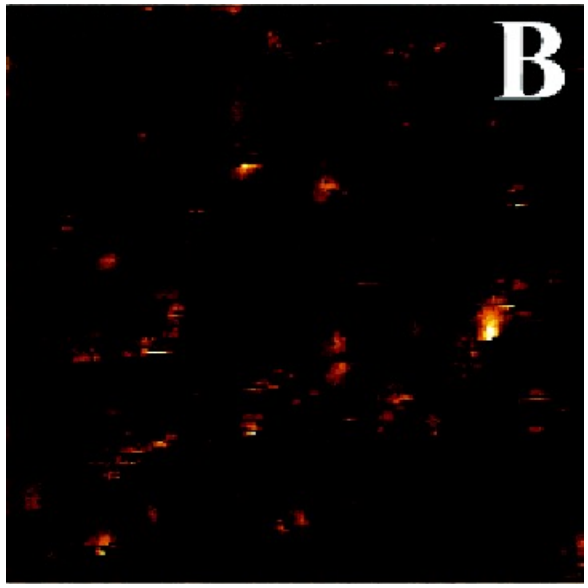


Figure 2. Energy levels and kinetic diagram for perylenebisimide dimers in nonpolar (black) and polar (gray) solvents.



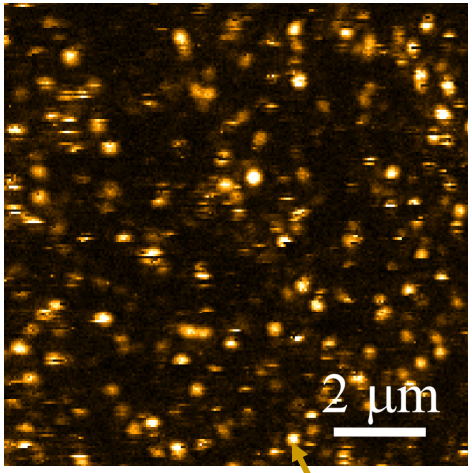
poly(vinyl acetate)
--- non-polar



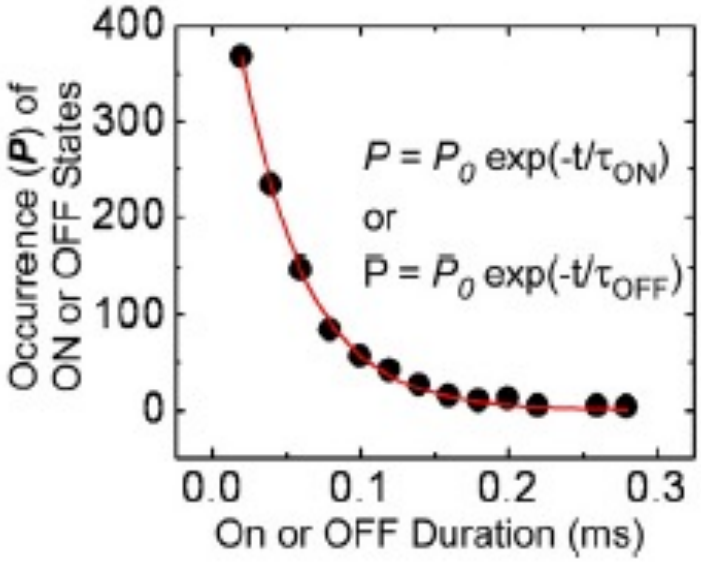
poly(vinyl alcohol)
--- polar

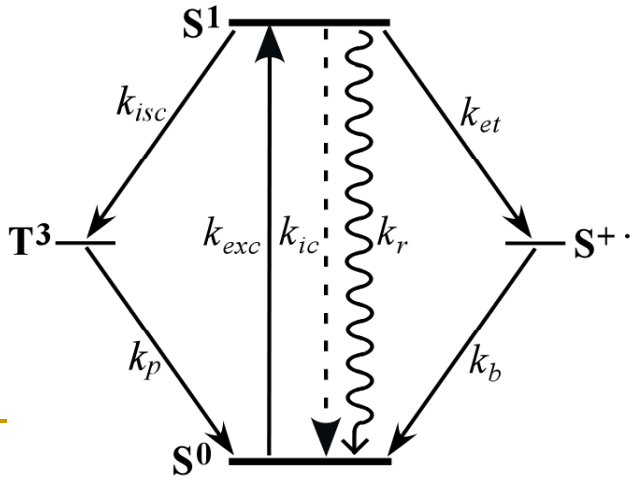
Zang, Adams et al. ***J. Am. Chem. Soc.*** 2004, vol. 126, 16126 -16133 .

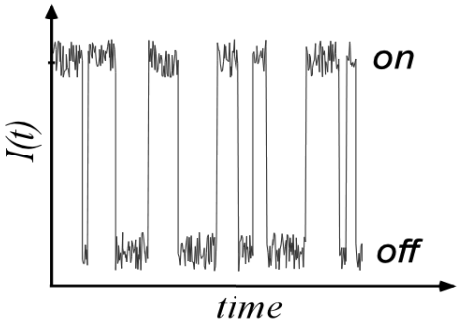
Single Molecule Imaging → Excited state kinetics



Focus on one







For PTCDI molecules, $\Phi(T^3) \sim 0$ and $\tau(T^3) < 1$ ms; "*off*" time is due to charge transfer state.

$$\frac{1}{\tau_{on}} = k_{exc}\frac{k_{ic}+k_{isc}+k_{et}}{k_r+k_{ic}+k_{isc}+k_{et}}; \quad \frac{1}{\tau_{off}} = k_b$$

Molecule properties measured at single-molecule level

- Optical properties: excited states (formation and decay), fluorescence wavelength and quantum yield and dependence on environment (sensor); energy transfer (dependent on distance --- for protein conformation detection).
- Electronic properties: electron transfer (electronic devices) and separation (photovoltaic), binding recognition (changing fluorescence properties via redox processes).
- **Molecule dynamics: rotation and diffusion**.

1. Critical for understanding and justifying the application in probing of surface properties and protein dynamic structures --- for any probing, the behavior of the probe itself should be characterized and controlled first.
2. Compared to particles (like pollen grains), **diffusion** of molecules are quite difficult to be measured at single-molecule level (mainly due to the small signal with respect to the fast motion), although Brownian motion has been known for long time. Fortunately, high sensitivity of fluorescence helps to image such a motion with confocal microscopy.
3. However, molecular **rotation** cannot be simply detected by fluorescence measurement. It should employ **polarization technique** --- since only polarized fluorescence depends on molecule dipole, which in turn depends on rotation. Such orientation dependence can only revealed at single-molecule imaging level. Why?

Topic 2: imaging molecular rotation

VOLUME 77, NUMBER 19 PHYSICAL REVIEW LETTERS 4 NOVEMBER 1996

Single Molecule Dynamics Studied by Polarization Modulation

T. Ha, Th. Enderle, and D. S. Chemla

Physics Department, University of California at Berkeley, Berkeley, California 94720 and Molecular Design Institute, Materials Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, California 94720

P. R. Selvin

Life Science Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, California 94720

S. Weiss

Molecular Design Institute, Materials Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, California 94720

Polarized emission

- Fixed polarization: the angle of polarizer is fixed, so the excitation polarization is constant. Only the molecules with dipole parallel to the polarization can be excited, whereas the molecule with dipole perpendicular to the polarization cannot be excited. If a molecule is immobilized, the emission intensity will be somewhere between zero and the maximum (parallel). By measuring large number of molecules, one can reveal the distribution of molecule orientation. This cannot be done by the ensemble measurement, which gives only averaged result that does not depend on the polarization angle. Draw a scheme.

- Modulated polarization: the polarization angle changes with time (here milliseconds).

1. If the molecule is immobilized, the intensity will be modulated with the polarization following, $I = a \cos^2(x-b) + c$, where a is the maximum intensity, x is the polarization angle, b is the in plane dipole angle, is the background correction.
2. If the molecule is mobile --- rotating: the intensity will show a irregular behavior.

Molecule under imaging: DNA (20 base pairs)---C6 linker---fluorophore (Texas red).

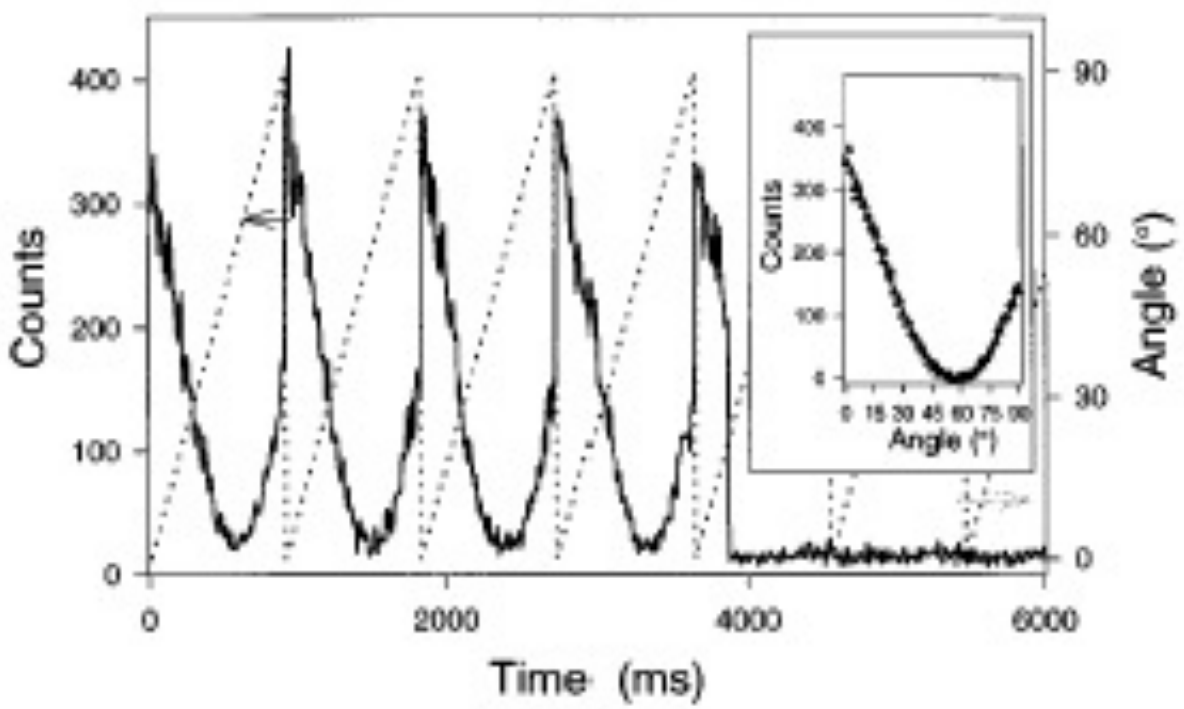


FIG. 1. Fluorescence time trace of a single TR molecule (solid line) and instantaneous excitation polarization angle (dotted line). Inset shows the averaged signal of the first four periods (filled triangles), and a fit to $a\cos^2(x-b)+c$ (solid gray line).

Fluorescence intensity modulates consistently with the polarizer: molecule is fixed

-42.8 °

-41.3 °

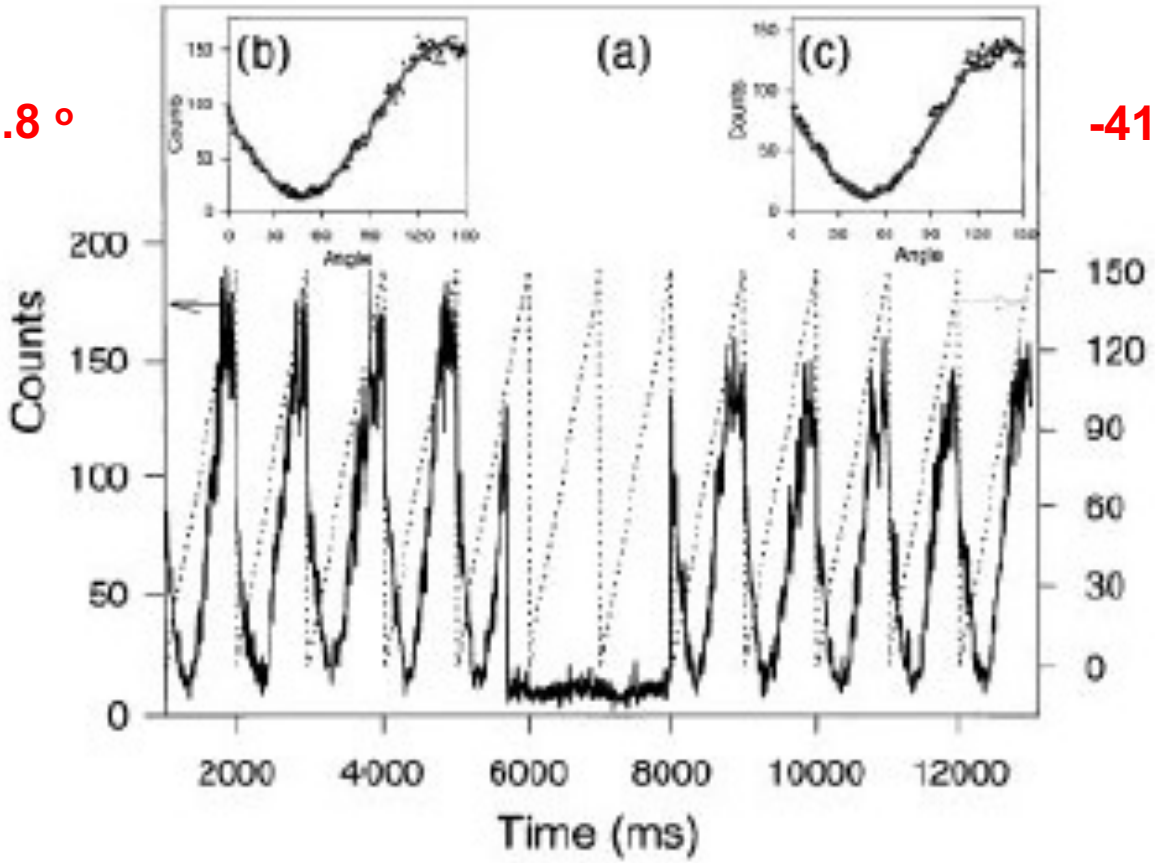


FIG. 2. (a) A transition to a dark state (2.3 s long) of a TMR molecule. The molecule photobleached after 51 s (not shown). The molecule maintained the same dipole orientation [from fits in insets (b) and (c)] throughout the transition to the dark state.

Molecule “died” (non-fluorescent) in middle, but back on in the same orientation as seen from the recovered fluorescence-polarization modulation profile.

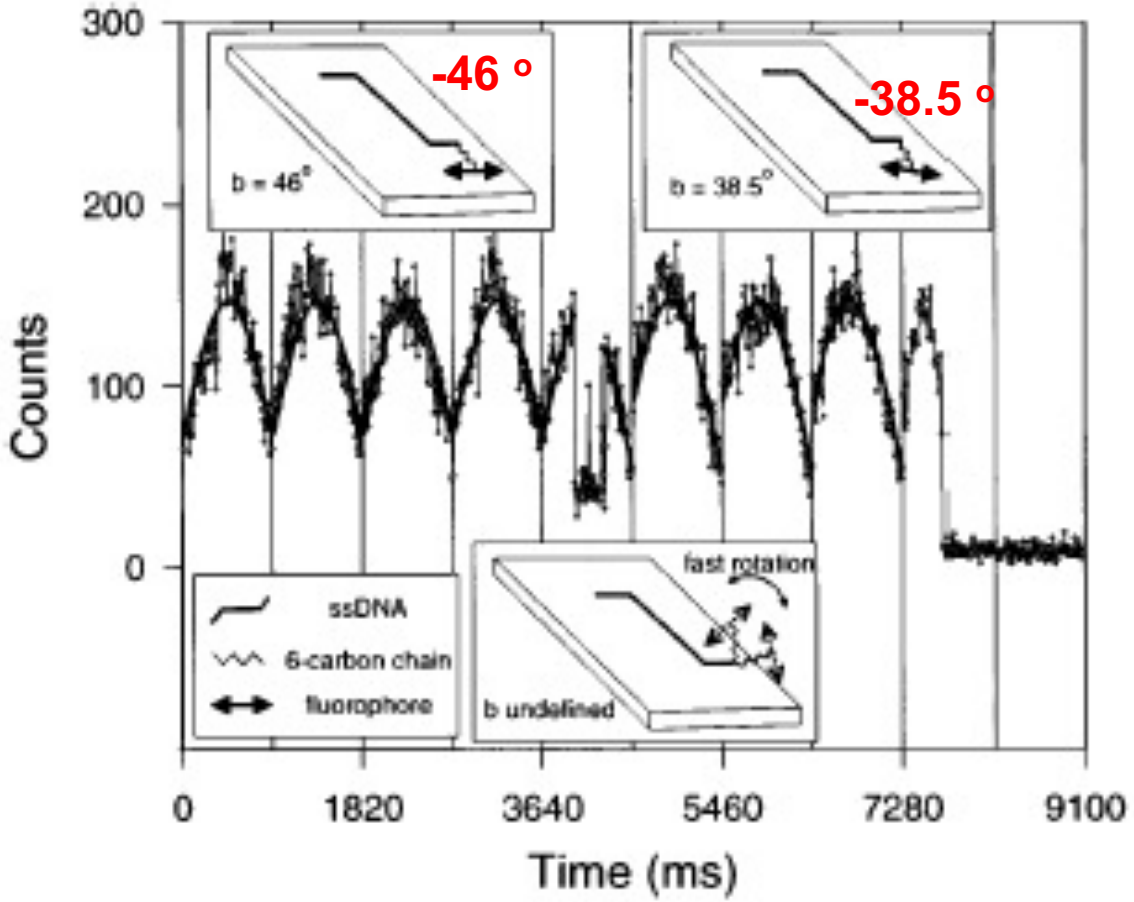


FIG. 4. A 7.5° in-plane rotational jump of a TR molecule. Solid line is the fit to the data before and after the interruption. Vertical lines separate each modulation period from 0 to 90 degrees. Insets show a model for the rotational jump.

Molecule becomes “crazy” (rotation) in middle, and returns to the normal fluorescence-polarization modulation profile, though in different orientation as indicated by the different modulation profile.

Single-molecule rotation imaged as movie clips in various time intervals

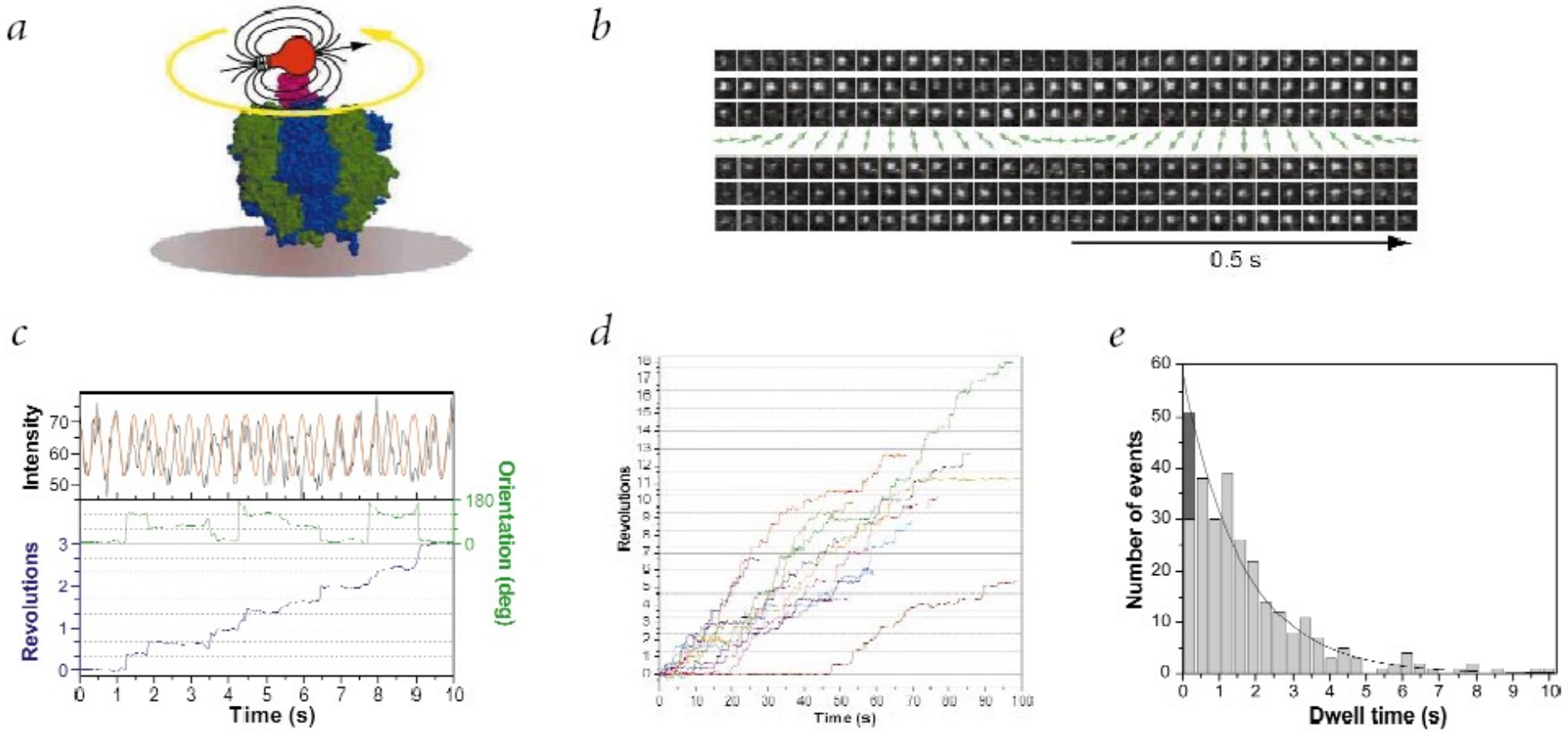


Fig. 4 Single molecule dipole rotation. ***a***, A cartoon of the F_1-ATPase motor with a single fluorescent probe attached to its rotor. ***b***, Two sequential fluorescence images (33 ms intervals) of single Cy3-F_1-ATPase molecules. The direction of the modulated excitation polarization is shown by green arrows. ***c***, Time trajectories of the fluorescence intensity (black, top) and calculated fluorophore angle between 0° and 180° (green, bottom). The accumulated rotation angle (blue, bottom) was obtained by assuming that all steps were counterclockwise. ***d***, Several time trajectories showing stepwise rotation of the rotor. Different lines represent trajectories of different fluorophores. ***e***, Distribution of dwell times between steps. (Figure modified from ref. 48 with permission.)

Adachi, K. *et al. Proc. Natl. Acad. Sci. USA* **97**, 7243–7247 (2000).

Topic 3: single-molecule diffusion

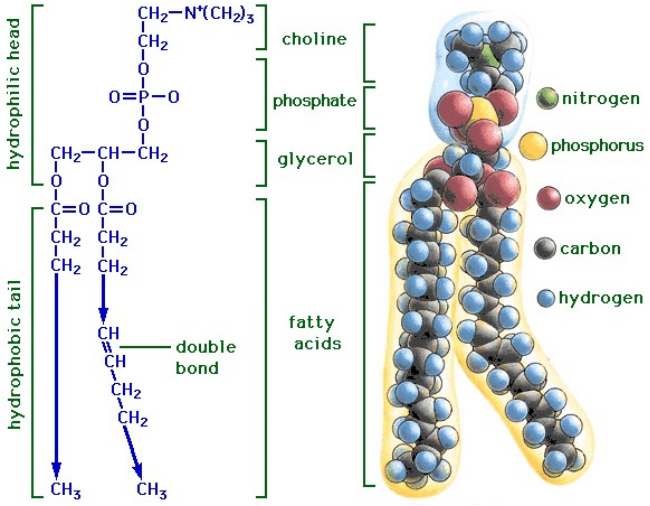
Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 2926–2929, April 1996
Biophysics

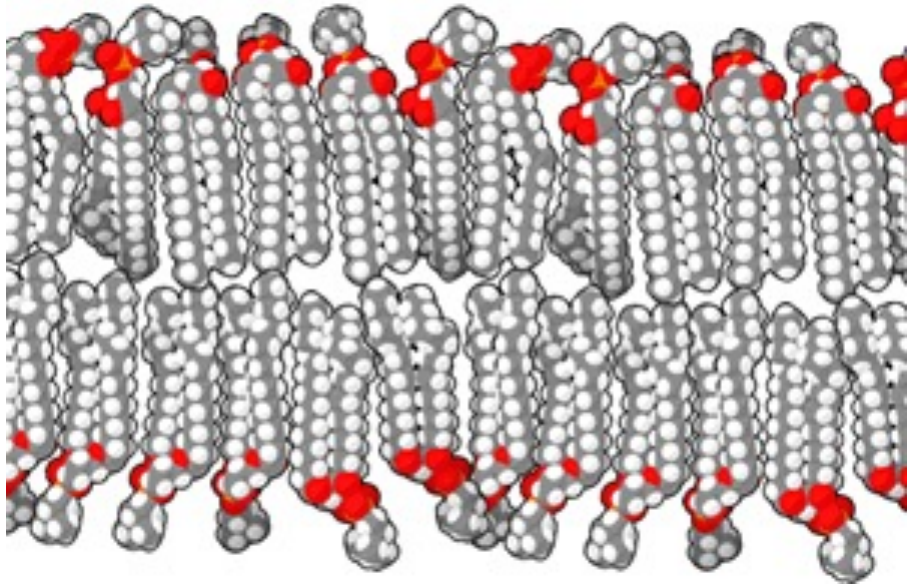
Imaging of single molecule diffusion

(fluorescence microscopy/single dye detection/time-resolved imaging/quantal fluorescence/lipid bilayers)

TH. SCHMIDT, G. J. SCHÜTZ, W. BAUMGARTNER, H. J. GRUBER, AND H. SCHINDLER

Institute for Biophysics, University of Linz, 4040 Linz, Austria





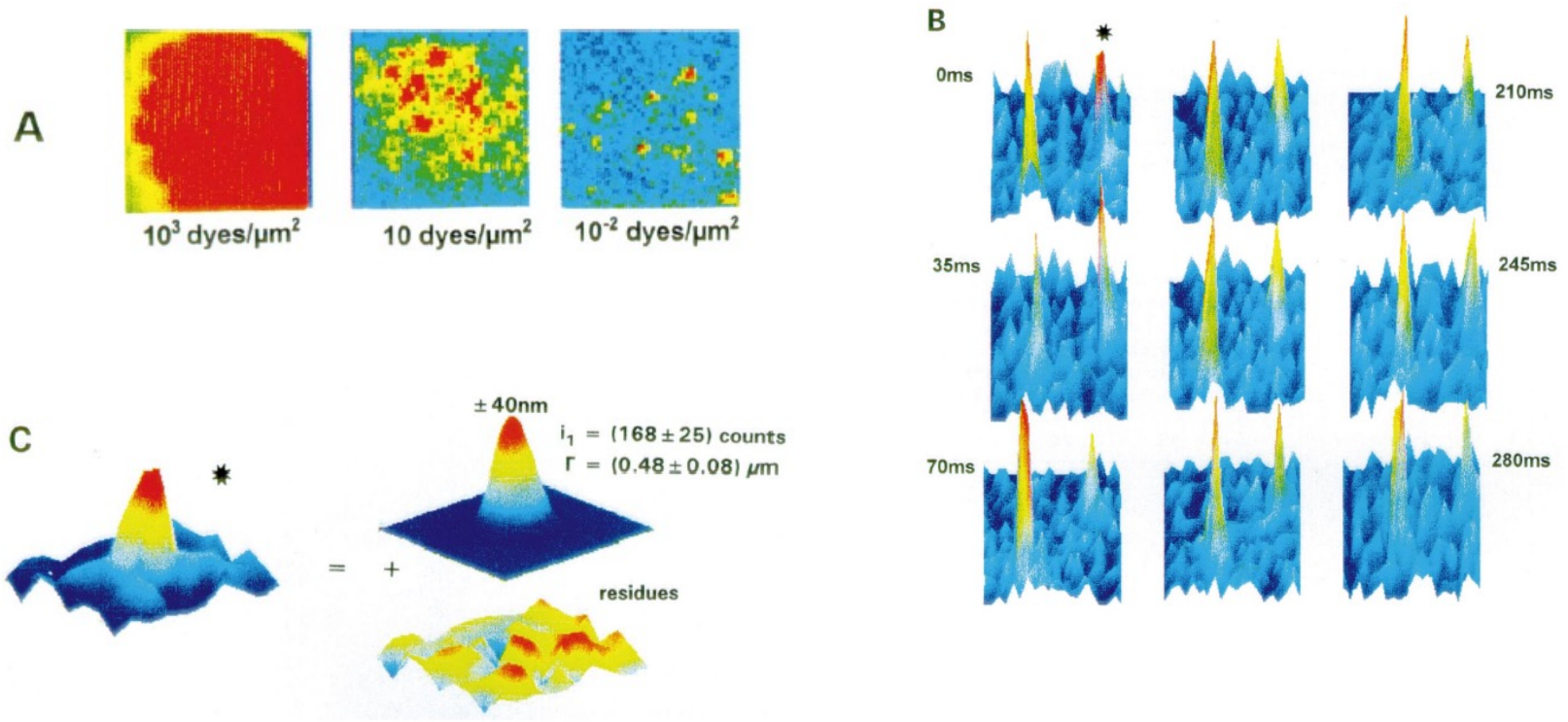


FIG. 1. Images of fluorescence-labeled lipids in a lipid membrane. The samples were illuminated for 5 ms with a Gaussian-shaped laser beam of 6.1 μm width and 57 kW/cm^2 mean excitation intensity. (*A*) Fluorescence images for three surface densities of labeled lipid. The images show a membrane area of 6.8 $\times$ 6.8 μm^2. Color scaling (blue = 0; red = 60 counts) is identical for the three images presented. Image intensity at 10^3 dyes/μm^2 was divided by 60. (*B*) Sequence of nine images of two labeled lipids observed at lowest surface density in a 5.4 $\times$ 5.4 μm^2 membrane area, taken every 35 ms. (*C*) Subimage of a 2.4 $\times$ 2.4 μm^2 membrane area showing the peak marked by $*$ in *B*. The nonlinear least-squares fit of a two-dimensional Gaussian profile (18) to the peak yielded the indicated values for fluorescence intensity $\overline{i_1}$, width and two-dimensional position with their respective confidence limits (see *Materials and Methods*). Residues of the fit compare with background noise.

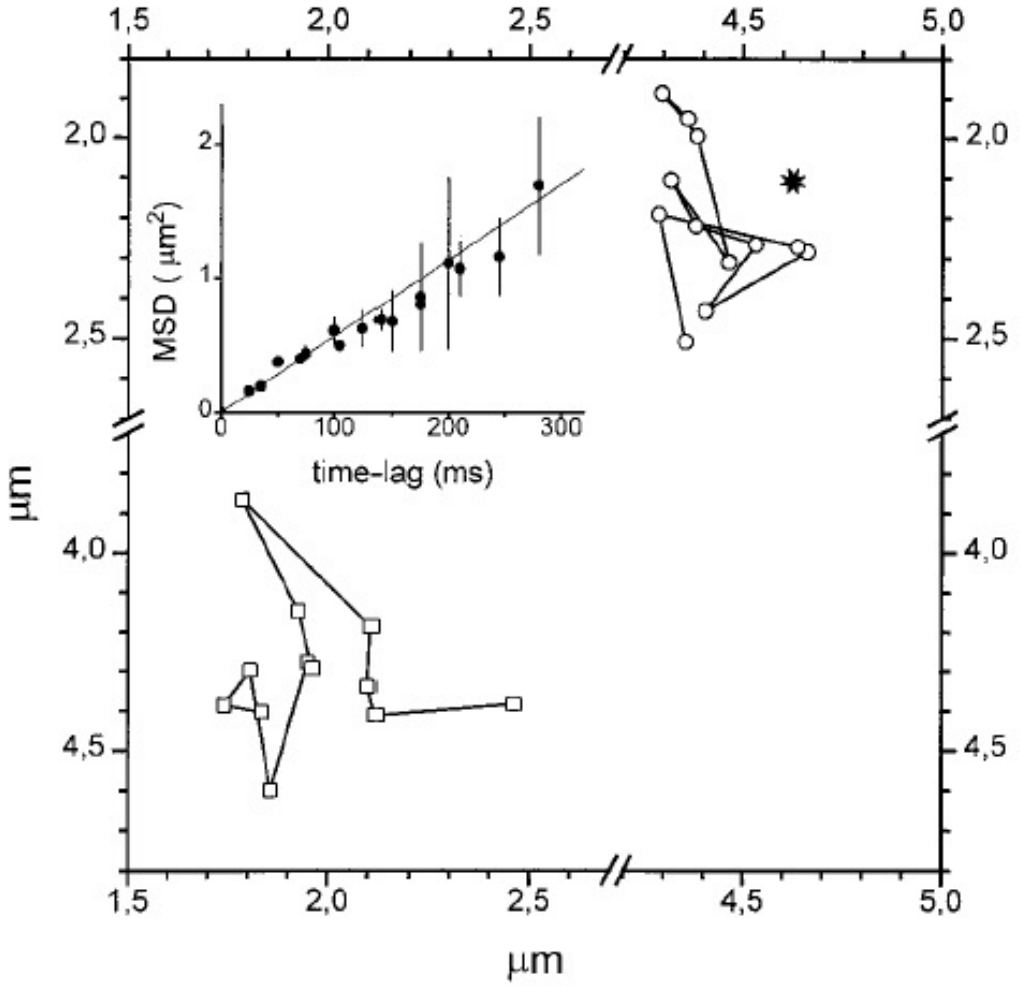


FIG. 3. Diffusion of single lipid molecules. Trajectories of the two-dimensional diffusion for the two lipid molecules in Fig. 1*B* determined from 12 successive images. (*Inset*) MSD versus time lag calculated for 531 trajectories. Observed displacements are consistent with two-dimensional Brownian diffusion of the lipids, since the data follow a linear relationship (solid line) well within their variance.

MSD: mean-square displacement

$$r = \sqrt{6\,Dt}$$

MSE 5034: Kinetics

$$r^2 = 6\,D\,t$$

D: diffusion coefficient

t: time

r^2 :

mean-square displacement (MSD)

D =

$1.42 \pm 0.13 \times 10^{-8}$ cm^2/s

Topic 4:
Extending Single-molecule imaging to single-particle imaging

- Characteristics and applications of nanocrystals.
- Rationale and necessities of single-particle investigation.
- Static and dynamic modes for single-particle imaging.

Characteristics of semiconductor nanocrystals

- Size: tunable (1.5 – 10 nm), narrow distribution (< 5% rms, root-means-squared). In this size range, the nanocrystals are smaller than the diameter of the bulk Bohr exciton (11.2 nm for CdSe). As a result, the electronic structure is dominated by quantum confinement effects in all three dimensions, making these nanocrystals truly zero-dimensional structures. --- usually nicknamed **quantum dots, QDs**.

  the narrow size distribution offers feasibility for solution based investigations, but a uniform size does not mean a uniform particle system, which includes some other parameters such as local environments (when spread on surface, or dispersed in solution for probing), the surface chemistry (binding, charges, defects), or the interparticle interactions (2 particles vs. three particles). These cases require single-particle investigations. --- see next slide.

- Discrete levels of excited states in nanocrystals: the width of transition lines (emission) should be narrow, but it appears significantly broader than expected when detected in solutions --- surface effects due to the large ratio of surface-to-bulk atoms.

  taking the same concept of single-molecule spectroscopy, Single-particle measurement targets one particle at one time, thus avoiding the mixture or overlap between multiple particles.

- Control of composite: single-domain (CdSe) and overcoated nanocrystals (CdSe/ZnS core shelled). The addition of a ZnS capping layer has been found to have many effects on the physical characteristics of these nanocrystallites, the most apparent of which is an increase in the fluorescence quantum yield, reported as high as 50% at room temperature.

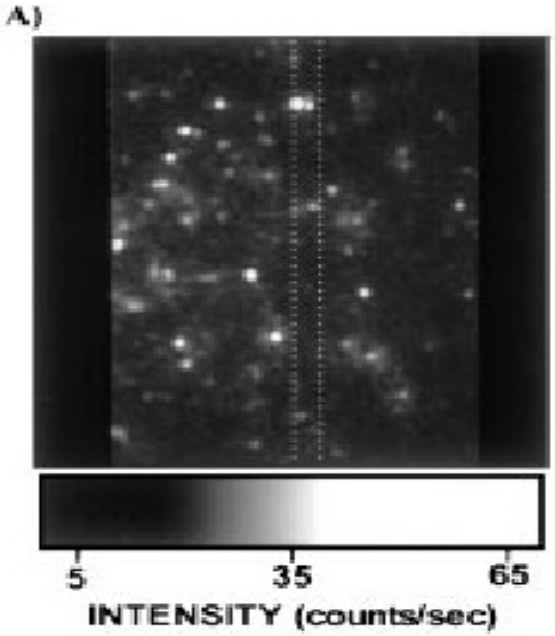
Single-particle imaging: remove the ensemble average, reveal the individual behavior

Phys. Rev. Lett. 1996, 77, 3873–3876

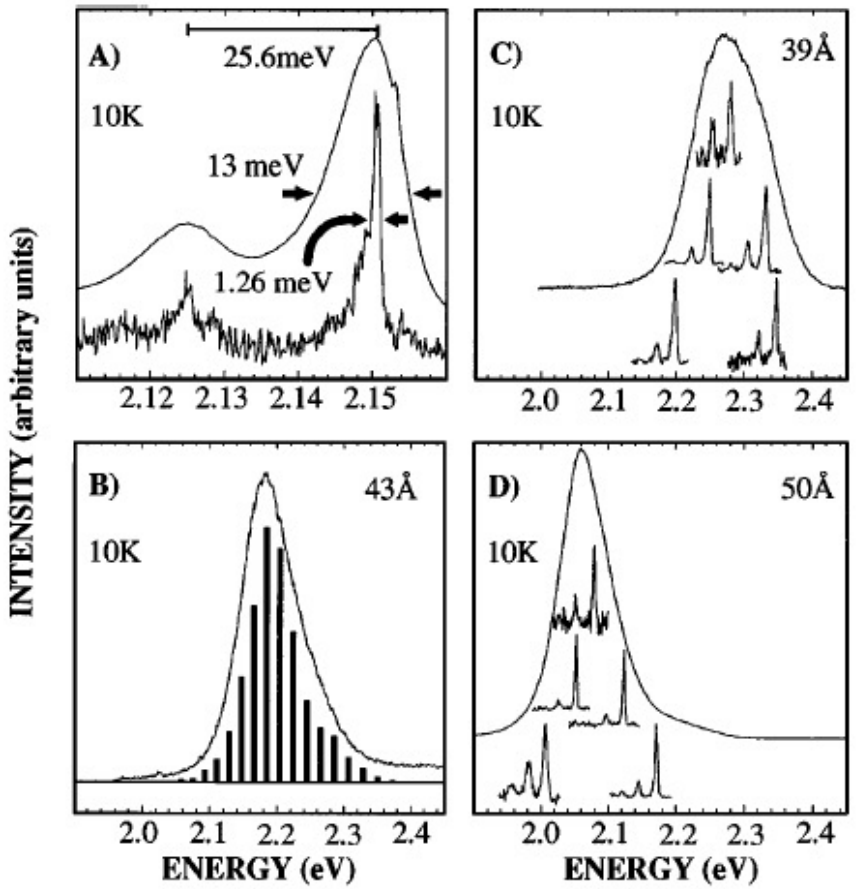
Photoluminescence Spectroscopy of Single CdSe Nanocrystallite Quantum Dots

S. A. Empedocles, D. J. Norris, and M. G. Bawendi
Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Comparison of emission spectra obtained by ensemble and single-particle measurements



Uniform CdSe particles show various emission intensities and dynamics on surface.

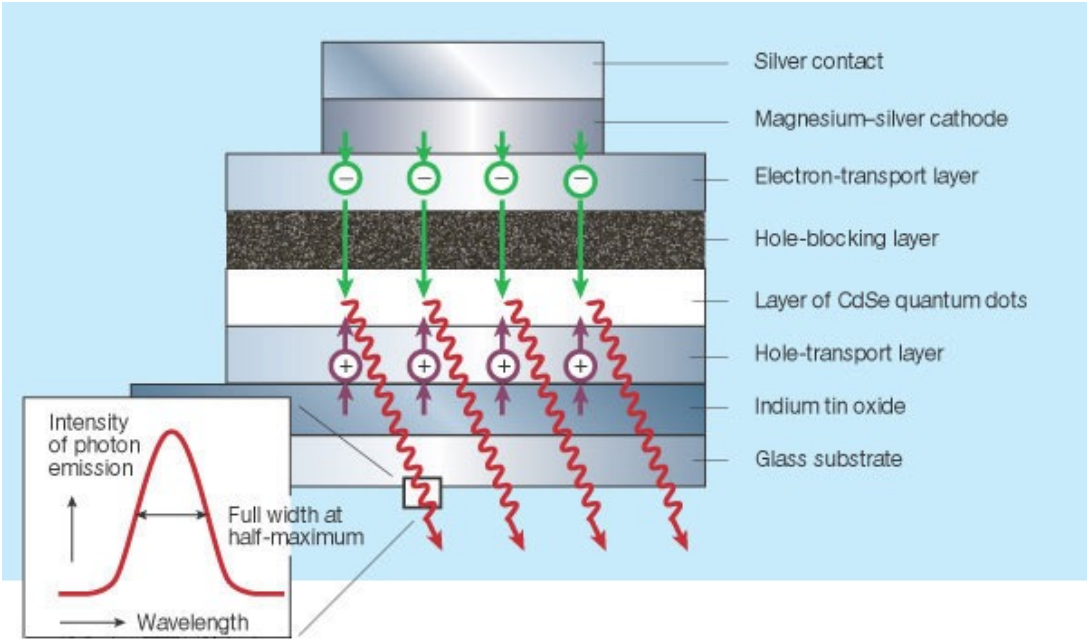


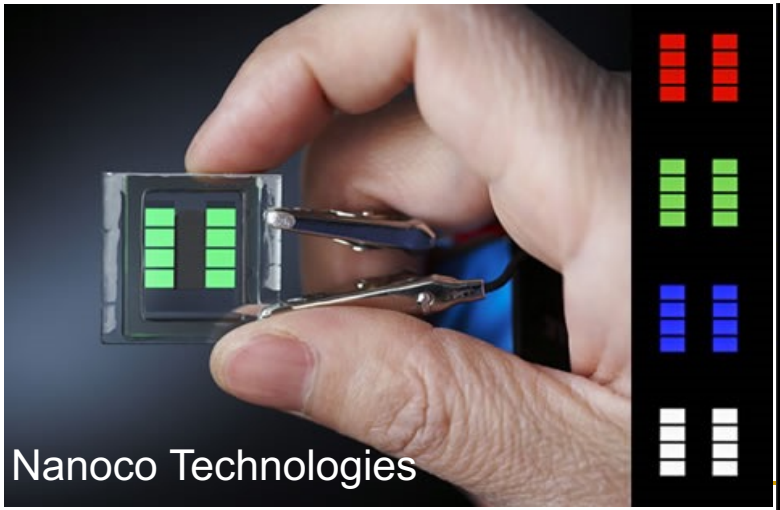
(a) Comparison of a single dot luminescence (SDL) spectrum from the 45 Å standard dot sample taken at 2.5 kW/cm2 (bottom) vs fluorescence line narrowing (FLN) spectrum of a sample of comparable size (top). The small peak on the blue edge of the FLN spectrum is scattered excitation light. (b)–(d) Ensemble spectra from three different size distributions (**43 Å overcoated dots** and **39 Å and 50 Å standard dots**, respectively) with corresponding SDL spectral information. (b) Ensemble spectrum with histogram of energies of 513 SDL spectra obtained from that sample. The histogram includes the scaled contribution of zero, one, and two phonon lines from each dot. (c),(d) Ensemble spectra with a representative set of SDL spectra obtained from these samples. All SDL spectra were taken with a 60 sec integration time.

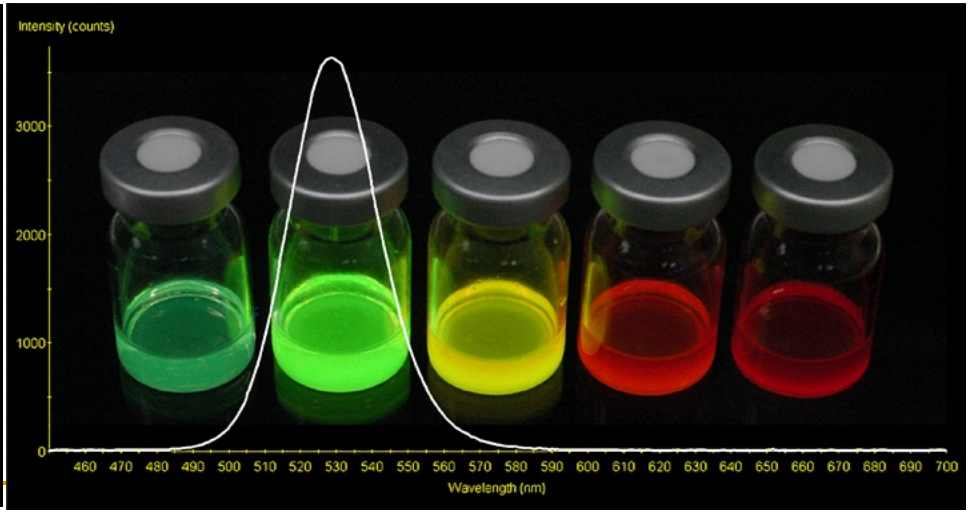
Device fabrication with semiconductor nanocrystals

- Light emitting diode (LED): improvement of efficiency (~ 20X) over the organic based devices, narrow emission band of the nanocrystals (CdSe). slide

Hybrid Molecular Organic/Inorganic Nanocrystal LED Devices







Taking advantages of the narrow emission band of semiconductor nanocrystals.

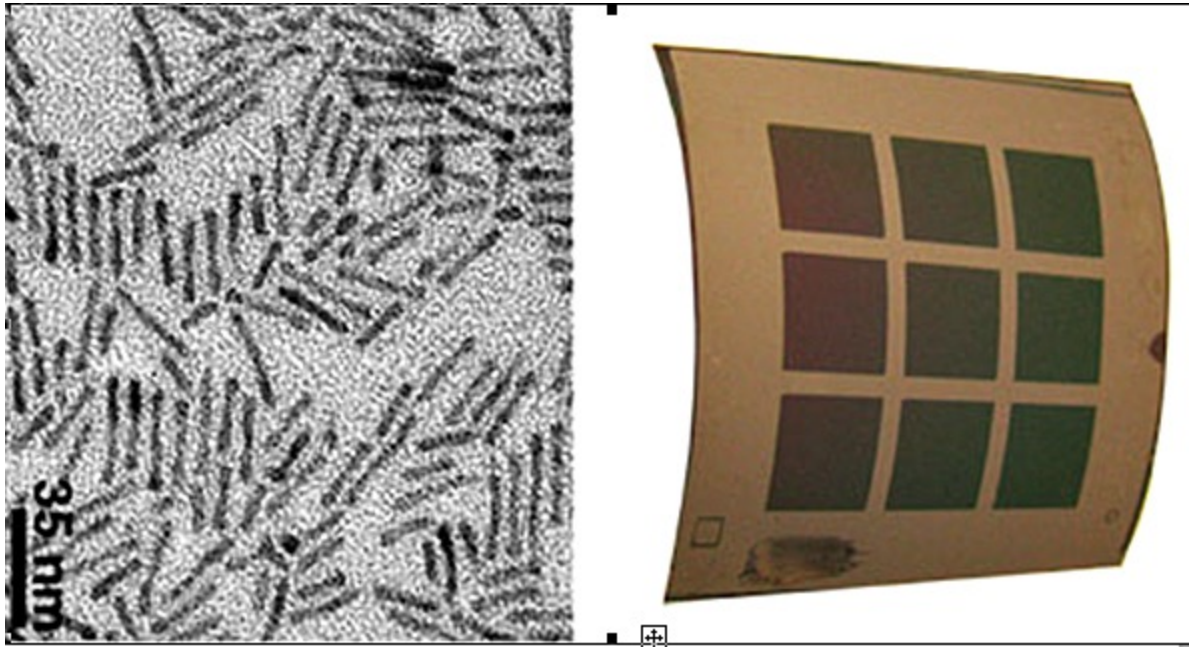
Quantum Dots → QLED TV

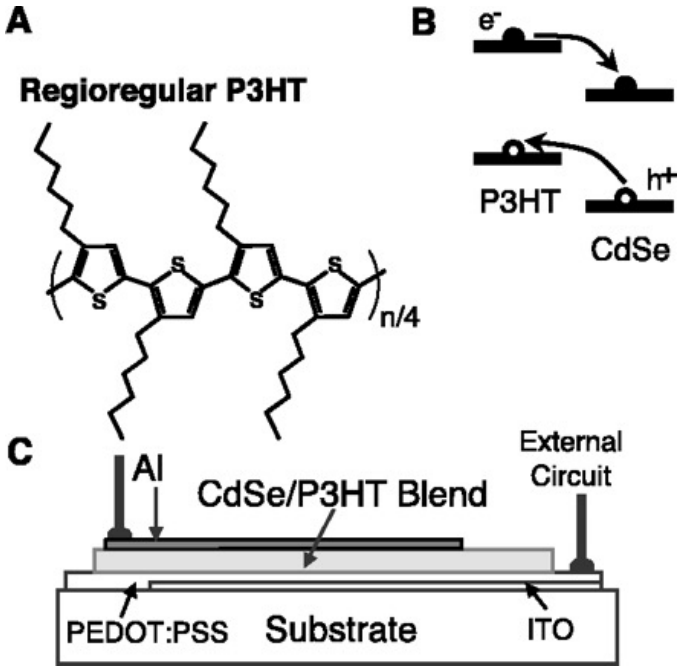
Device fabrication with semiconductor nanocrystals

- Light emitting diode (LED): improvement of efficiency (~ 20X) over the organic based devices, narrow emission band of the nanocrystals (CdSe). slide

- Solar cells composed of CdSe nanocrystals and conducting polymers: improved efficiency. slide

CdSe based hybrid solar cells





From nanorods to printed cell

Solexant

Alivisatos, SCIENCE, 2002, 295, p2425

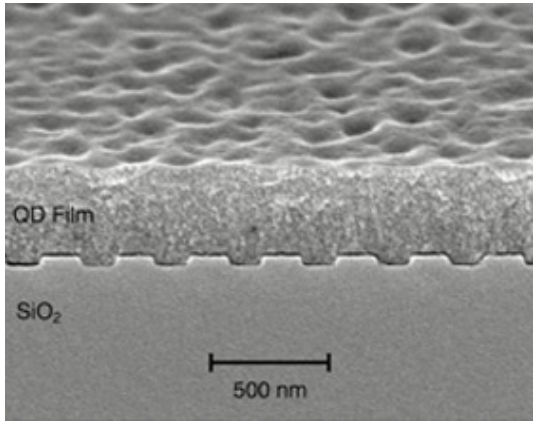
Device fabrication with semiconductor nanocrystals

- Light emitting diode (LED): improvement of efficiency (~ 20X) over the organic based devices, narrow emission band of the nanocrystals (CdSe). slide

- Solar cells composed of CdSe nanocrystals and conducting polymers: improved efficiency. slide

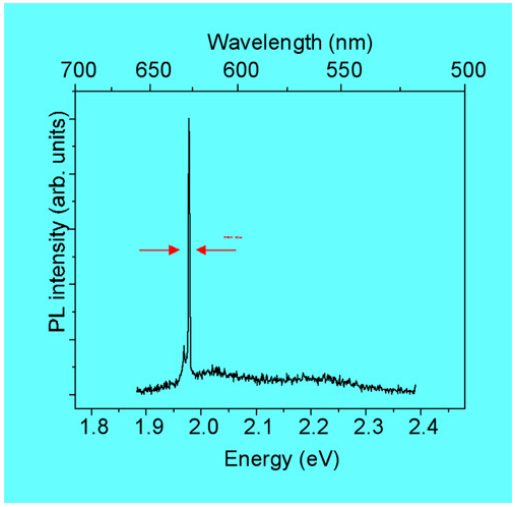
- Nanocrystal laser: enhanced narrow bands and tunable color by size. Slide

CdSe laser



A nanocrystal DFB laser structure:

A CdSe nanocrystal / titania waveguide coats a DFB grating etched in silica. DFB: Distributed feedback

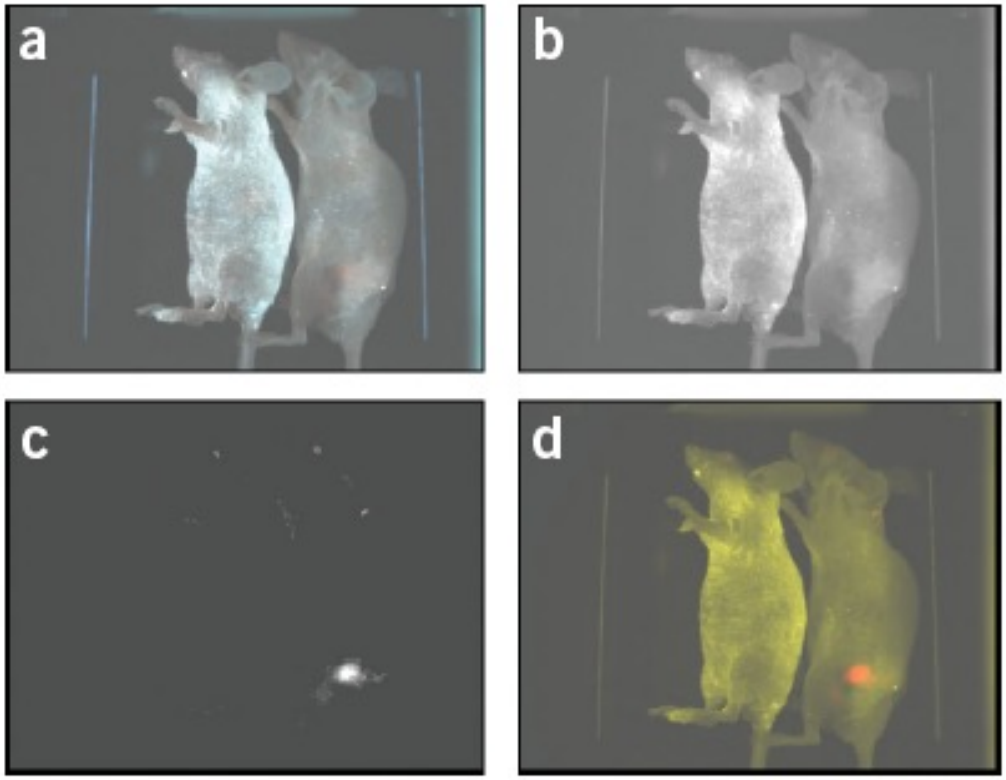


Room temperature spectrum of a CdSe nanocrystal laser above the threshold.

Device fabrication with semiconductor nanocrystals

- Light emitting diode (LED): improvement of efficiency (~ 20X) over the organic based devices, narrow emission band of the nanocrystals (CdSe). slide
- Solar cells composed of CdSe nanocrystals and conducting polymers: improved efficiency. slide
- Nanocrystal laser: enhanced narrow bands and tunable color by size. Slide
- Biological labeling: targeting cancer cells or tumors, living cell imaging, DNA recognition.

In vivo tumor labeling



Spectral imaging of QD-PSMA Ab conjugates in live animals harboring C4-2 tumor xenografts. Orange-red fluorescence signals indicate a prostate tumor growing in a live mouse (right). Control studies using a healthy mouse (no tumor) and the same amount of QD injection showed no localized fluorescence signals (left). (**a**) Original image; (**b**) unmixed autofluorescence image; (**c**) unmixed QD image; and (**d**) super-imposed image. After *in vivo* imaging, histological and immunocytochemical examinations confirmed that the QD signals came from an underlying tumor. Note that QDs in deep organs such as liver and spleen were not detected because of the limited penetration depth of visible light.

Shuming Nie, NATURE BIOTECHNOLOGY, 2004, 22, p969.

Device fabrication with semiconductor nanocrystals

- Light emitting diode (LED): improvement of efficiency (~ 20X) over the organic based devices, narrow emission band of the nanocrystals (CdSe). slide

- Solar cells composed of CdSe nanocrystals and conducting polymers: improved efficiency. slide

- Nanocrystal laser: enhanced narrow bands and tunable color by size. Slide

- Biological labeling: targeting cancer cells or tumors, living cell imaging, DNA recognition.

- Applications in energy:

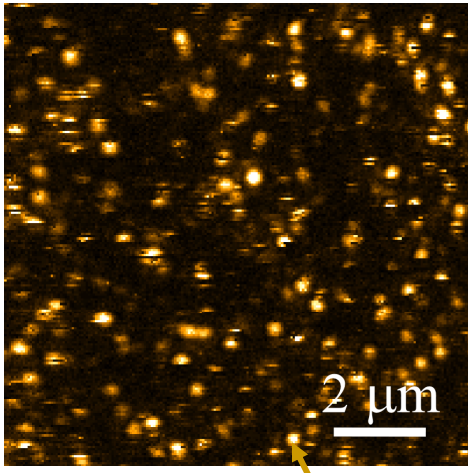
1. photocatalysis --- cartoon for the scheme, CB electron for reductions of proton (hydrogen production) and nitrogen (fixation), VB holes for oxidation of water (producing oxygen) or OH radical for degradation. and thermal catalysis.
2. Thermal catalysis: like fuel cell, see the review shown in the slide.

Dynamic single-particle imaging

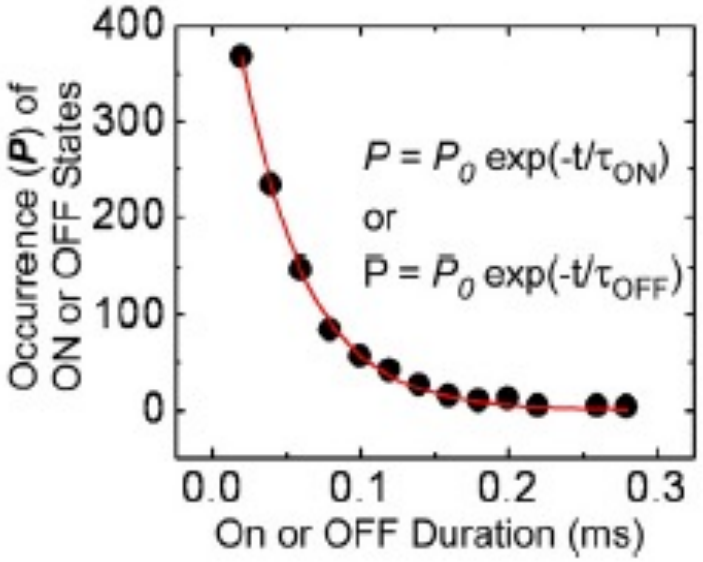
- Single-particle imaging reveals dynamics: both image and fluorescence-time profile show the **blinking** between on and off. This blinking is normally missed in ensemble measurements due to the average to zero. *Slide*
- Effects of excitation intensity: the "on" period scales inversely with excitation intensity, while the "off" period appears to be intensity independent. This suggests that the nonemissive state is created via the nanocrystal excited state. The emitting state is however recreated by a spontaneous thermal process from the nonemissive state. slide
- Coating effect of ZnS: Both the average “on” and “off” times increase dramatically in the ZnS-overcoated nanocrystal, compared to the bare CdSe nanocrystals. Slide
- **Auger mechanism** for the blinking:

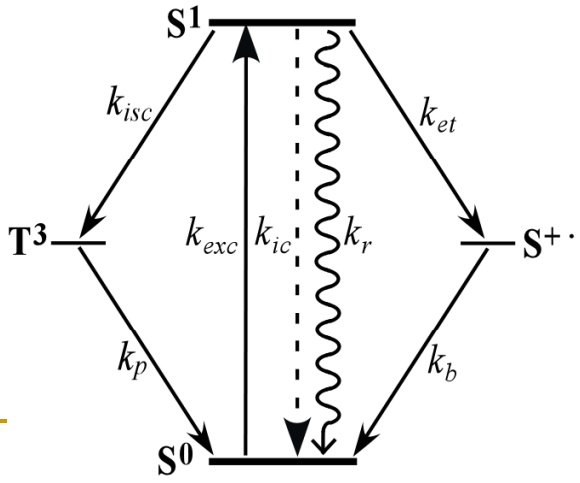
1. At an excitation intensities (~0.5 kW/cm2), typically one crystal gets excited every 10^{-5} s, and the nanocrystals decay in about 10^{-8} s. Only very rarely can two electron-hole pairs be simultaneously excited.
2. However, if there are two pairs such nanocrystals, the ca. 2 eV energy released from the recombination of one electron-hole pair may be transferred to the remaining carriers, one of which can then be ejected into the matrix --- Auger process. The resulting ionized nanocrystal is thus non-emitting upon excited.
3. Eventually the nanocrystal is neutralized via a second photoionization event or the return of the ejected carrier. Neutralization restores the emission. Since the “on” time is determined by the ease of ionization across the interface, and the “off” period by the time it takes for the ejected carrier to tunnel back through the same interface, ZnS-overcoated nanocrystals exhibit longer on/off times as expected from this model.

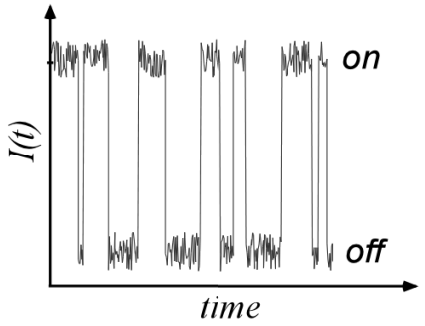
Single Molecule Imaging → dynamic/kinetic information



Focus on one



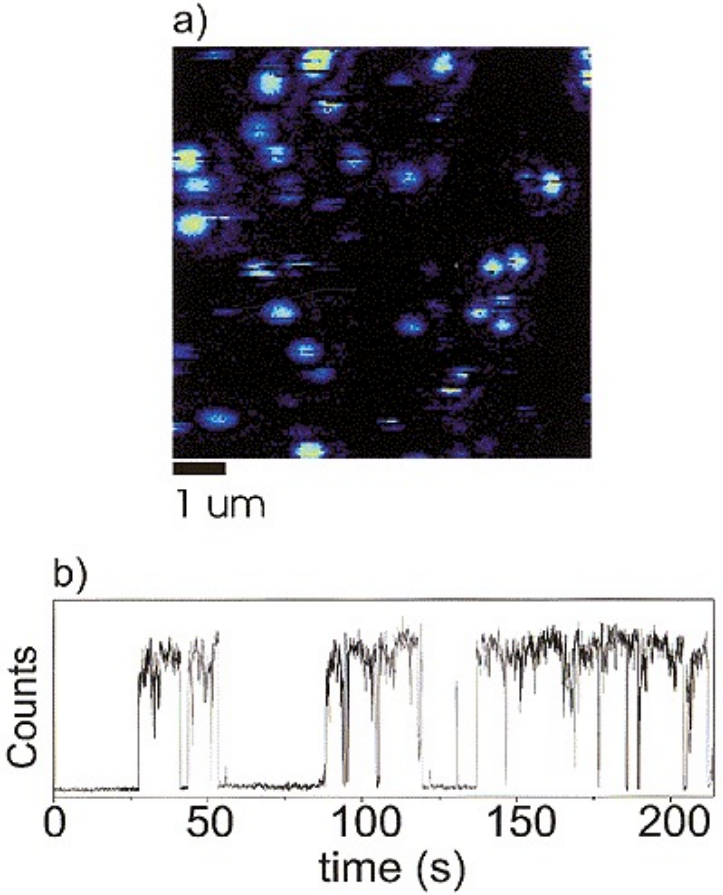




For PTCDI molecules, $\Phi(T^3) \sim 0$ and $\tau(T^3) < 1$ ms; "*off*" time is due to charge transfer state.

$$\frac{1}{\tau_{on}} = k_{exc}\frac{k_{ic}+k_{isc}+k_{et}}{k_r+k_{ic}+k_{isc}+k_{et}}; \quad \frac{1}{\tau_{off}} = k_b$$

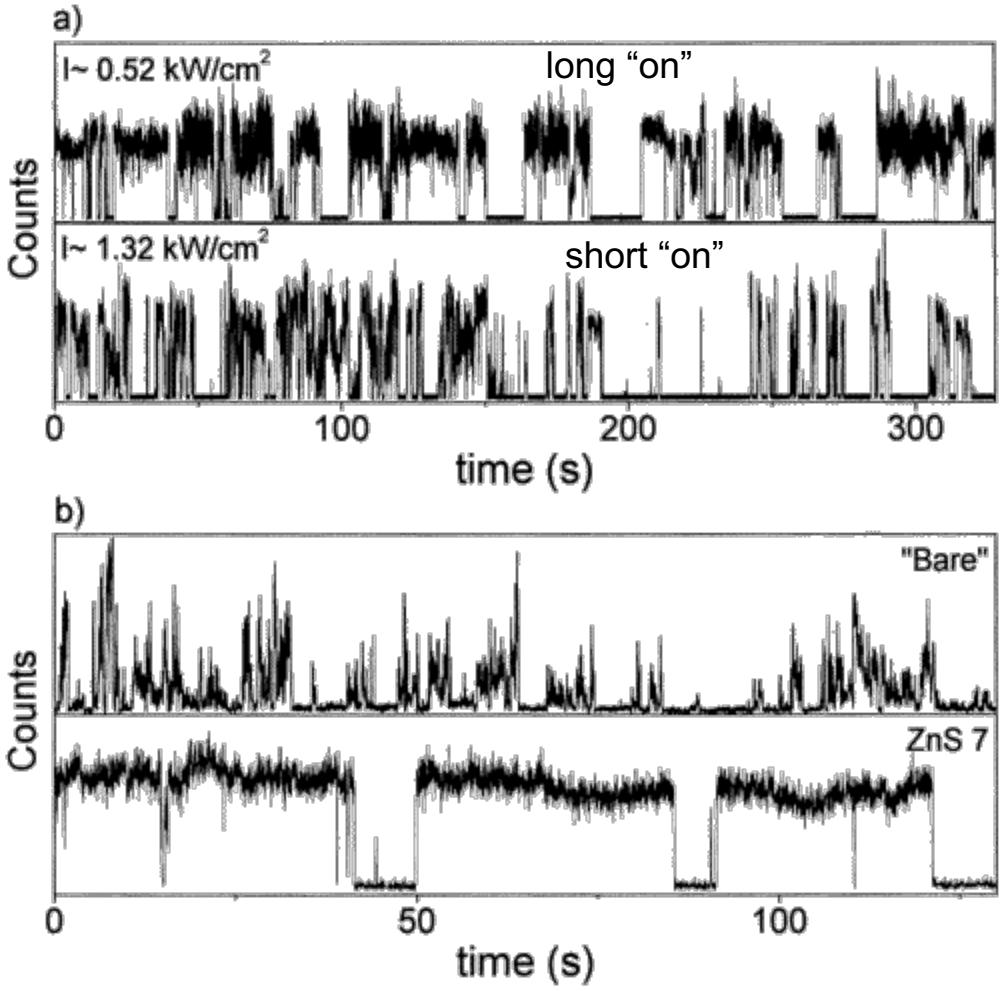
Emission blinking of a CdSe/ZnS core shell nanoparticle



(a) Image of a random field of single 21 Å radius CdSe nanocrystals with ~4 monolayers of ZnS on the surface, acquired by raster scanning the sample across a diffraction-limited laser spot (= 532 nm, fwhm 0.38 m) and collecting the red-shifted fluorescence onto an avalanche photodiode in an epi-illumination confocal geometry. (b) Fluorescence intensity versus time trace of a single 21 Å radius CdSe nanocrystal with a 40 ms sampling interval and an excitation intensity of ~0.52 kW/cm2

Brus, *Nature* **1996**, *383*, 802-804

Effect of irradiation intensity on blinking dynamics

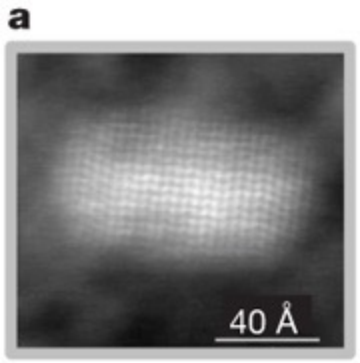


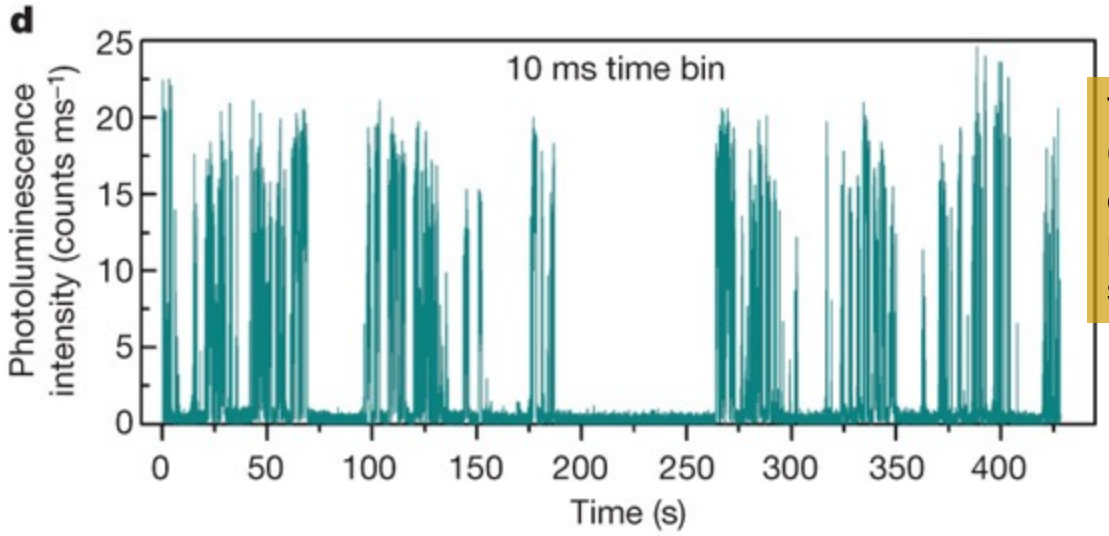
(a) Comparison of fluorescence intensity versus time traces at ~0.52 kW/cm2 and at ~1.32 kW/cm2 with a sampling interval of 10 ms.

(b) Fluorescence intensity versus time traces of a "bare", TOPO/TOPSe-passivated nanocrystal compared with that of a ZnS-overcoated one with a shell thickness of ~7 monolayers at the same excitation intensity.

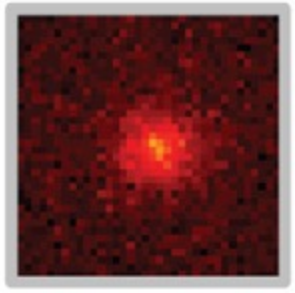
Brus, *Nature* **1996**, *383*, 802-804

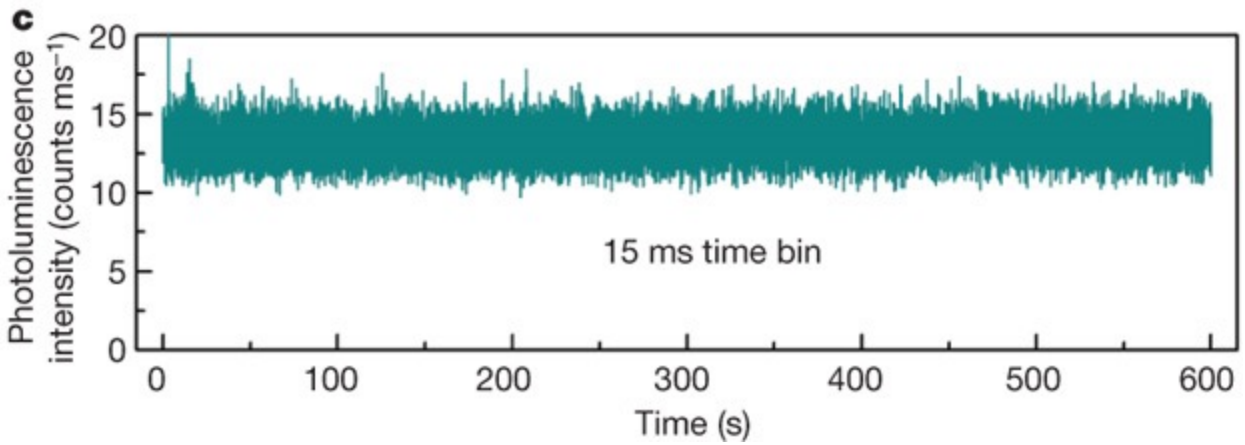
Ternary core/shell CdZnSe/ZnSe **nanocrystal stops blinking**





Typical CdSe/ZnS core-shell nanocrystal shows blinking





Todd D. Krauss, *Nature* **2009**, *459*, 686-689.