Lecture 21: Single-molecule (particle) spectroscopy via SCM

- Advantages of SCM for single-molecule measurement.
- Single-molecule sensing and switching ---environment effect.
- Revealing rare but critical electron transfer processes.
- Extending of single-molecule imaging to singleparticle imaging.

Emerging of SCM based on NSOM observation

- High spatial resolution of NSOM --- critical for studying nanostructured materials, but not necessary for single-molecule imaging, because the molecules can be dispersed such that they are far enough apart (> 300 nm) that diffraction limited optics can resolve individual ones.
- Historically, detection of the fluorescence from single molecules on a surface at room temperature was originally achieved with NSOM.
- Soon after this discovery, confocal microscopy had emerged as a more popular technique to detect single molecules. Although confocal microscopy is not capable of sub-wavelength resolution imaging, it has a number of advantages over NSOM.

NSOM vs. SCM



Optics alignment of NSOM detector system



Advantages of SCM over NSOM

- Very high optical excitation powers are possible, whereas with NSOM, the upper limit of the excitation power is severely limited. The higher fluorescence intensities that accompany higher excitation powers raises the S/N and reduces the time integration needed for any given experiment. This is important for faster imaging rates and for looking at fluorescence signals which fluctuate on short (millisecond) time scales.
- 2. SCM is relatively easy to set up in comparison to NSOM, since no feedback system to be maintained. Setting up and optimizing shear force distance regulation is the most time consuming step in NSOM.
- 3. For NSOM, the position of the APD needs to be adjusted each time a new tip is used, and should also be optimized, or at least checked for optimization, each time a new region of the sample is imaged (i.e. when the course position of the sample is adjusted). This is because the tip, each time replaced or moved slightly, is not aligned confocal with the detector. In contrast to this, the focal spot of SCM objective does not change. Multiple samples can be imaged over days, and probably weeks, without any drifts substantial enough to require adjusting the position of the APD --- high throughput screening.
- 4. The excitation power of SCM is more easily measured. SCM is capable of power dependence studies, which simply cannot be done with NSOM because the high power regime is inaccessible. For NSOM, the differences in the throughput of each tip and differences in the efficiency of coupling junctions (typically, one which couples light from a free space laser beam to the single mode fiber, and another fiber-to-fiber splice to couple into the NSOM probe) prevent simply measuring the power of the input beam.

Annu. Rev. Phys. Chem. 1998. 49:441–80 Copyright © 1998 by Annual Reviews. All rights reserved

OPTICAL STUDIES OF SINGLE MOLECULES AT ROOM TEMPERATURE

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Jay K. Trautman SEQ Ltd, 17 Princess Road, Lawrenceville, New Jersey 08648; e-mail: jtrautman@seqltd.com Annu. Rev. Phys. Chem. 2004, 55:457–507 doi: 10.1146/annurev.physchem.55.111803.143246 Copyright © 2004 by Annual Reviews. All rights reserved First published online as a Review in Advance on February 6, 2004

THEORY OF SINGLE-MOLECULE SPECTROSCOPY: Beyond the Ensemble Average

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Robert Silbey Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; email: silbey@mtl.edu Chem. Rev. 1999, 99, 2929–2956

Single Molecule Fluorescence Spectroscopy at Ambient Temperature

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910

J. Phys. Chem. B 2002, 106, 910-927

FEATURE ARTICLE

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

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July 2005 Volume 38 Number 7

A Publication of the American Chemical Society

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ACCOUNT'S of CEEEVEICAL RESEARCE

Social issue on Single-Molecule Spectroscopy

Vol. 38, Issue 07 July 19, 2005 Cover

The Power of One.

Major researches of single-molecule spectroscopy

- 1. Single-molecule sensing and switching --- the ultimate degree of sensitivity.
- 2. Single-molecule fluorescence probing --- particularly for protein systems. (next lecture)
- 3. Single-molecule (particle) optoelectronic calibration.

Single Molecule Sensing and Switching

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% TiO₂; 10% ZnO; 3% Al₂O₃



Long off time due to charge transfer state, S**

Probing Local Protonated Sites (-OH₂⁺) on Quartz: *Molecule Dynamics* due to loose dangling hydrogen bond

99.999% SiO₂





Spots appeared after exposed in humid air

No spots came out for <u>NaOH</u> cleaned surface



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





Much shorter off time due to triplet state, T³



Zang & Adams et al. J. Am. Chem. Soc. 126 (2004) 16126 -16133.

Single Molecule Imaging: revealing rare events.



Zang & Adams et al. J. Am. Chem. Soc. 126 (2004) 16126 -16133.

Sensing Local Environment in Polymer Films



poly(vinyl acetate) --- non-polar



poly(vinyl alcohol)
--- polar

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



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

Extending to a trimer system:

PRL 93, 236404 (2004)

PHYSICAL REVIEW LETTERS

week ending 3 DECEMBER 2004

Single Molecule Photobleaching Probes the Exciton Wave Function in a Multichromophoric System

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FIG. 2 (color online). (a)–(c) Fluorescence intensity (I), polarization (P), and lifetime (τ) trajectories recorded for a single TPD molecule (molecule 1). Dashed lines separate trimer, dimer, and monomer levels. P is defined as $P = (I_x - I_y)/(I_x + I_y)$, where I_x and I_y are the emission components detected in two orthogonal polarization directions. Each τ value derives from a monoexponential fitting to the photon arrival time decay built every 600 ms. (d) Experimental (symbols) and fitted (lines) second-order correlation function for the trimer level of a TPD molecule. $\tau = 3.7$ ns is recovered from the fitting. (e) Emission spectra of the trimer (dashed line), dimer (solid line), and monomer (dotted line) levels of molecule 1.



FIG. 3. (a)–(c) Fluorescence lifetime distributions measured for the trimer, dimer, and monomer levels of 121 TPD molecules, respectively. Each τ value determined has an accuracy of 0.1 ns. (d) Histogram of the $\tau_{\text{monomer}}/\tau_{\text{trimer}}$ ratio.



FIG. 4. (a) Sketch of the two bleaching pathways for TPD. Empty and full circles stand for intact and bleached dye units. (b) $\tau_{\text{monomer}}/\tau_{\text{dimer}}$ vs $\tau_{\text{dimer}}/\tau_{\text{trimer}}$ correlation plot for 121 TPD aggregates. Molecules with low coupling strength are shown as empty circles ($\tau_{\text{monomer}}/\tau_{\text{trimer}} < 1.5$). Two distinct sets of molecules appear visible for trimers with stronger interaction ($\tau_{\text{monomer}}/\tau_{\text{trimer}} > 1.5$), which are associated with bleaching pathways 1 (gray circles) and 2 (black circles). (c) Lifetime distributions corresponding to the dimer levels originated from pathways 1 (crossed bars) and 2 (full bars).

Single-Molecule Electron Transfer

Reversible Intramolecular Electron Transfer at the Single-Molecule Level**

Roel Gronheid, Alina Stefan, Mircea Cotlet, Johan Hofkens, Jianqiag Qu, Klaus Müllen, Mark Van der Auweraer, Jan W. Verhoeven, and Frans C. De Schryver*

Angew. Chem. Int. Ed. 2003, 42, 4209-4214







Table 1: Fluorescence quantum yields of 1 and 2 (λ_{exc} =543 nm) in degassed methylcyclohexane (MCH), toluene, and ethyl acetate (EtAc). For toluene the quantum yields under aerated conditions are given in parentheses.

Solvent	1	2
мсн	0.91	0.99
Toluene	0.65 (0.51)	0.95 (0.91)
EtAc	0.036	0.81



Long lifetimes

due to back electron transfer, which is slow and rarely occurring. **Figure 3.** Histograms of the decay times τ of individual molecules of 1 and 2 in Zeonex (A and B, respectively) and in PS (C and D, respectively). For each compound-polymer combination, about 200 molecules were studied. In both matrices, 1 shows a multimodal distribution of decay times, whereas for 2 it is monomodal. The combination of 1 with PS has the only histogram displaying long (>10 ns) decay times indicative of the electron-transfer process. All molecules were analyzed with a monoexponential model, except for some of the molecules of C (especially those with longer decay times), which had to be fitted biexponentially. In this case, only the long decay times are included in the histogram.

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.</p>

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

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



(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
- 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:
- 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. Draw a scheme
- 2. Thermal catalysis: like fuel cell, see the review shown in the slide.

Hybrid Molecular Organic/Inorganic Nanocrystal LED Devices



Taking advantages of the narrow emission band of semiconductor nanocrystals.

CdSe based hybrid solar cells



From nanorods to printed cell

Solexant

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.

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.

A. Wokaun et al.

Reviews

Nanotechnology

Nanoparticles in Energy Technology: Examples from Electrochemistry and Catalysis

Fabio Raimondi, Günther G. Scherer, Rüdiger Kötz, and Alexander Wokaun*



static single-particle imaging

Revealing spectral dependence on irradiation intensity: this cannot be done through ensemble measurement due to the heterogeneity of particle systems, which normally does not keep the synchronization of dependence on irradiation intensity. See next s*lide*

Spectral diffusion is the result of changing local electric fields around individual nanocrystals, which result from charges trapped on or near the surface of the nanocrystal. The role of the released **excess** excitation energy may be to facilitate movement of these charges between local trap sites, resulting in fluctuations in the local electric field which produce the observed spectral diffusion shifts. The surface charge also affects the fluorescence fluctuation as discusses later.

- By decreasing the excitation intensity, it is possible to reduce spectral diffusion and line widths in nanocrystals. Line widths as narrow as 120 eV have been observed at 10 K. These peaks are ~600x narrower than the full ensemble fluorescence. slide
- To further investigate/confirm the local electric field effect, emission was observed at different applied electric field: Stark shift. See the following slide.

part C: the same linear correlation between phonon coupling and Stark shift or / spectral shift \rightarrow the spectral diffusion observed above is due to changes in the local electric field.

Alternated electric field: proves again the correlation between Stark shif and spectral shift, which are both dependent on local electric field. Stark shift can be seen accompanying spectral diffusion shifts. Following a spectral diffusion shift in the sixth frame, there is an increase in the magnitude of subsequent Stark shifts.

Spectral diffusion with time



(A) Spectrum of a single 56.5 Å overcoated nanocrystallite with a 10 s integration time at 10 K. (B) A representative sample of six spectra from the same nanocrystallite as in (A), with a 0.1 s integration time. Of the 150 spectra taken in rapid succession, frames 1-6 indicate spectrum numbers 1, 16, 35, 59, 84, and 150, respectively. (C) Histogram of peak positions from each of the 150 spectra described in (B). The excitation intensity for all spectra in Figure 3 was 200 W/cm2.

Zero longitudinal-optical (LO) phonon line

Bawendi, M. G. J. Phys. Chem. B 1999, 103, 1826

Emission line width at different irradiation intensities



314, 150, and 65 W/cm²

(A-C) Three spectra of the same single 45 Å nonovercoated nanocrystallite with 314, 150, and 65 W/cm2 excitation intensity, respectively, and 1 min integration time. (D) Average single-nanocrystallite line width as a function of excitation intensity for a sample of 34 overcoated nanocrystallites (56.5 Å) with an integration time of 30 s. (E) Average single-nanocrystallite line width as a function of integration time for 40 nanocrystallites from the same sample as in (d) with 85 W/cm2 excitation intensity. The top axes of (D) and (E) indicate the excitation energy density (time × intensity) at each point to allow a direct comparison of the effects of time and intensity on the average single-nanocrystallite line width.

Bawendi, M. G. J. Phys. Chem. B 1999, 103, 1826

Low irradiation intensity leads to narrow emission lines



(A-C) High-resolution spectra of ultranarrow zero longitudinual optical phonon line from three different single nanocrystallites from a 43 Å overcoated sample. Spectra were taken with a 1 min integration time and an excitation intensity of 25 W/cm2.

Bawendi, Phys. Rev. Lett. 1996, 77, 3873-3876

Effect of applied electric field

kV/cm



- (A) Seventeen spectra of the same single nanocrystallite (75 Å overcoated) under a range of electric fields.
 Spectra were taken with an excitation intensity of 25 W/cm2 and an integration time of 30 s. Inset numbers indicate the applied field in kV/cm and magnification of the *y*-axis.
- (B) Plot of Stark shift versus electric field for the single nanocrystallite in (A) with fit to the sum of a linear and quadratic function of the applied field.
- (C) Phonon coupling versus Stark shift for a single nanocrystallite in the presence of an applied electric field (closed circles) and versus spectral diffusion shift in the absence of an applied field (open triangles). Phonon coupling is measured as the ratio of the integrated intensity of the one LO phonon line to the ZPL. Stark and spectral diffusion data in (C) were taken from different nanocrystallites.



(A) Eleven consecutive 30 s emission spectra from a single nanocrystallite (75 Å overcoated) in the presence of a repeating sequence of electric fields: negative field, zero field, positive field, zero field. Inset numbers and symbols represent the frame number and applied field, respectively. (-), (0), and (+) represent -350, 0, and +350 kV/cm applied fields, respectively. A spectral diffusion shift is indicated in frame 6. (B) Summary of 100 consecutive 30 s spectra of the same nanocrystallite under the field conditions described above. Data are plotted as a function of time, peak energy, and electric field. Open triangles, closed circles, and open circles indicate fields of -350, 0, and +350 kV/cm, respectively. The excitation intensity for all spectra in Figure 8 was 25 W/cm2.

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 ZnSovercoated nanocrystal, compared to the bare CdSe nanocrystals. Slide
- Auger mechanism for the blinking:
- 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 recombinatiob 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.

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



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

SMS measurement based on intensity fluctuation and wavelength shift



(*top panel*) Fluorescence fluctuation monitored with photon counting (left) and spectra recording (right). (*bottom panel*) A scheme showing the basic method to get the statistic mean value of ON and OFF times.