tion. To collect the remaining sulfonics individually, we then reinjected the sulfonics into the IC, which was equipped with a 10 m by 100 mm Phenomenex (Torrance, CA) star-ion anion exchange column, and eluted them with a 5.4 mM Na_2CO_3 + 5.1 mM $NaHCO_3$ buffer. We separated ESA from a small amount of co-eluting sulfate by reinjecting the two compounds and eluting them with a 3.2 mM Na_2CO_3 + 3.1 $NaHCO_3$ mM buffer. Baseline separation between MSA and ESA/sulfate was ~2 min; between ESA/sulfate and iPSA, it was ~9 min, and between ESA and sulfate, it was ~1 min. After removal of the carbonate buffer with HCl, each compound was then placed in a quartz tube and dried by rotary evaporation (maximum temperature ~ 50°C). Copper oxide was then placed in each tube, and high vacuum, with mild warming (<100°C), was used for complete drying. The samples were then sealed and combusted to sulfate, CO_2, and H_2O at 800°C. The CO_2 and H_2O were collected with a procedure similar to that of Epstein *et al.* (*13*). The H_2O was converted to H by the method of Coleman and Moore (*27*) with a zinc catalyze from J. M. Hayes of Indiana University. The CO_2 and H were then analyzed with 6-60-Nuclide and 3-60-HD-Nuclide mass spectrometers. The sulfate was extracted from each tube with hydrochloric acid and prepared for S isotopic analysis as in (*6*).

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24. Some reported CS reaction products are CS_2, C_2S, C_2S_2, C_3S, C_3S_2, CO, CO_2, OCS, S, S_2, SO, and SO_2. On the basis of infrared observations of low-temperature (solid phase) CS reactions, Steudel (*28*) suggested a reaction sequence where molecules of increasing carbon number are formed, leading to a polymer: $x\text{CS} \rightarrow CS_2, \text{CCS}, C_3S_2, C_yS_z \rightarrow$ polymer. Of the above molecules, C_xS_2 and S_2 (if searched for) have not been reported to occur in the interstellar medium. In addition to CS_2 (see text), OCS and S_x are also observed in carbonaceous meteorites (*25*, *26*).
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Discrete Intensity Jumps and Intramolecular Electronic Energy Transfer in the Spectroscopy of Single Conjugated Polymer Molecules

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Single-molecule fluorescence spectroscopy of a multichromophoric conjugated polymer (molecular weight ~20,000) revealed surprising single-step photobleaching kinetics and acute jumps in fluorescence intensity. These jumps were shown not to result from spectral diffusion and were attributed to fluctuations in the quantum yield of emission for the molecules. The data indicate efficient intramolecular electronic energy transfer along the polymer chain to a localized fluorescence-quenching polymer defect. The defects are created by reversible photochemistry of the polymer. These findings have implications for the use of conjugated polymers in light-emitting diode displays and sensors.

Recent developments in fluorescence microscopy have allowed for the detection, imaging, and spectroscopy of single molecules at room temperature (*1–12*). As a consequence, single-molecule spectroscopy (SMS) has moved from studies at cryogenic temperatures (*13*) to a broader range of environmental conditions. In many cases, direct information about the molecular distribution of physical quantities, rather than just the ensemble-averaged values obtained by bulk measurements, can now be obtained. Most room-temperature SMS studies have involved aromatic dye molecules that correspond to a single chromophore in a fluctuating environment and have revealed direct information on chemical and spectroscopic heterogeneity of single molecules (*4*, *14*), as well as on the dynamics of these inhomogeneities (*10*, *15*).

Here, we used SMS to study molecules that each comprise many similar chromophores, such as conjugated fluorescent polymers. Intramolecular migration of energy among these chromophores and the ultimate impact of the energy migration on photochemistry is of intense fundamental and practical interest. Conjugated polymers such as poly(*p*-phenylene vinylene) (PPV) and poly(*p*-pyridylene vinylene) (PPyV) have shown great promise for light-emitting device applications (*16*, *17*). We studied a derivatized PPV-PPyV copolymer compound with a molecular weight of ~20,000 (Fig. 1A).

Spectroscopic studies on bulk polymer materials suggest that conjugated polymer emission is often dominated by energy migration to local minima in their optical band structure (*18*). For example, anthracene-derivatized poly(*p*-phenylene-ethynylene)s in solution have been shown to selectively emit from states associated with the anthracene end groups (*19*). Although excitation diffusion along the chains in conjugated polymers is efficient, the actual optical excitation (exciton) may be localized and may have a coherence length of only a few monomer units (*20*). Thus, spectroscopic evidence of conjugated polymers suggests that their broad absorption spectra are the result of overlapping absorption bands from different segments along the polymer chain, and that their emission is strongly affected by energy migrations, often to sites remote from the absorption (*18*). This hypothesis can be

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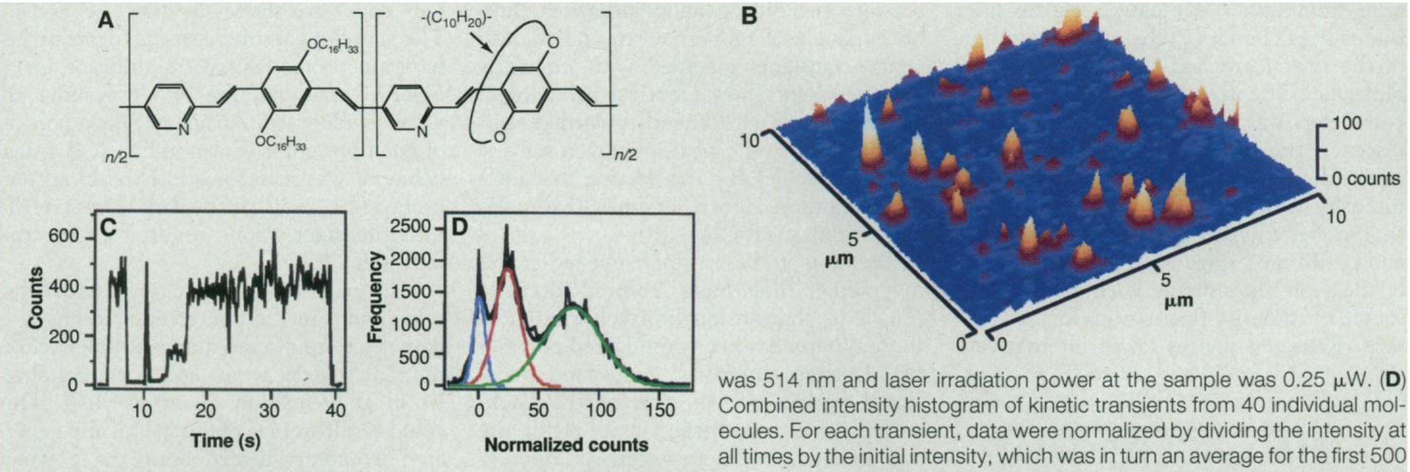

Fig. 1. (**A**) Molecular structure of the conjugated polymer molecules studied. (**B**) Fluorescence scanning confocal image of the polymer single molecules. (**C**) Kinetic transient for the emission intensity, where the intensity is measured in counts per 100-ms sampling window. The excitation wavelength was 514 nm and laser irradiation power at the sample was 0.25 μW. (**D**) Combined intensity histogram of kinetic transients from 40 individual molecules. For each transient, data were normalized by dividing the intensity at all times by the initial intensity, which was in turn an average for the first 500 ms of irradiation. Each transient was further scaled by the average initial intensity for the entire ensemble (100 counts). Colored curves are Gaussian fits to the dark levels (blue), intermediate levels (red), and bright levels (green).

tested directly by SMS because multichromophoric systems with energy transfer will show results markedly different from those without energy transfer. We show that in the SMS of this PPV-PPyV derivative, intramolecular energy migration coupled to nonradiative processes has remarkable qualitative and quantitative manifestations, including discrete jumps in the fluorescence intensity and discrete single-step photobleaching.

The scanning confocal fluorescence microscopy image of a spin-cast dilute film of the PPV-PPyV polymer embedded in polystyrene (*21*) is shown in Fig. 1B. This image was recorded by a sample-scanning confocal microscope similar to those previously described (*22*), and it exhibits diffraction-limited (~300 nm) spots resulting from individual fluorescent polymer molecules. The spots in the image are identified as polymer single molecules by the following standard criteria (*1*): (i) The areal density of the features is within a factor of 5 of the expected density, and the density varies linearly with concentrations of polymer molecules in the spin-cast solution (*23*). (ii) Polarized excitation and emission images show the molecules have fixed absorption and emission dipoles. (iii) The molecules show discrete photochemistry.

This last criterion is an obvious one for a single-chromophore system but is not necessarily true for a multichromophore molecule. If the polymer has many chromophores that are uncoupled from one another, it should photobleach in a stepwise fashion in much the same way as a small cluster of well-separated chromophores. However, if the chromophores do communicate, the entire polymer should behave like one large single molecule. Discrete single-step photobleaching was seen for every one of the hundreds of individual polymer molecules studied. This result is quite remarkable, considering the large number of chromophores each polymer chain contains. The single-step photobleaching shows that there is strong communication along the polymer chain. Similar photobleaching has been observed in another multichromophore single-molecule system and has been attributed to the entire system behaving as a single quantum system (*24*). However, the broad nature of both the excitation and emission spectrum of the polymer molecules (Fig. 2A) argues against the entire polymer being one large delocalized quantum system (*25*). Therefore, the discrete single-step photobleaching must result from the creation of a permanent photochemical "defect" (perhaps attributable to oxidation) that quenches excitations along the entire chain. Indeed, photo-oxidation in bulk films of PPV has been seen to create carbonyl defects that strongly affect the polymer's emission quantum yield, even at low concentrations (*26*).

The shapes of the fluorescence spots in Fig. 1B are irregular because of fluorescence intensity fluctuations of the individual molecules. This latter effect is demonstrated by the intensity transient in Fig. 1C. The transient was recorded by fixing the laser focus on top of a molecule previously located in an image. Immediately after the excitation laser was switched on, the observed intensity jumped to 4000 counts per second (cps). At ~40 s, the intensity abruptly and permanently jumped from ~4000 cps to the background level (50 cps) as a result of permanent photochemistry (photobleaching). Before that occurred, the molecule showed intensity fluctuations at various times, manifesting discrete intensity jumps, including periods of time when the intensity was indistinguishable from the background level. These periods are denoted as "dark levels." The dark levels are ascribed to polymer molecules that have a photogenerated fluorescence quencher that can reversibly convert back to a fluorescent state.

We investigated hundreds of individual polymer molecules and observed similar fluctuations of the fluorescence intensity, including reversible formation of dark levels. The fluorescence intensity jumped between numerous levels, but it did not sample all intensities continuously. A histogram of the normalized intensity levels for a collection of single-molecule transients is shown in Fig. 1D. The histogram shows three distinct peaks. Almost all of the molecules show the same three intensity peaks in histograms of their fluorescence transients, and none of the molecules show a continuous distribution of intensities. A fluorescence transient of a particularly long-lived molecule is shown in Fig. 2D. It shows the same behavior as the transient in Fig. 1C, with a large number of intensity jumps and dark levels of various duration times.

The fluorescence intensity observed from a single molecule is proportional to the product of the probability that the molecule absorbs a photon and the probability that it emits one. These can be broken down to the following factors: the excitation power, the relative orientation of the molecule's transition moment and the laser polarization, the molecule's absorption cross section, and its fluorescence quantum yield. Fluctuations in any of these could be responsible for the intensity jumps observed for individual polymers. To identify the source of the fluorescence intensity jumps, we addressed individually each factor responsible for the fluorescence signal. First, fluctuation in the excitation power could arise from either fluctuation of the laser intensity or changes in the relative position of the laser focus and the position of the molecule. The stability of both of these parameters was verified by recording fluorescence transients of 20-nm dye-labeled latex spheres. The sphere transients showed intensity variations only as a result of the shot noise, indicating that both the laser and positioning stage were stable. Rotation of the molecule's dipole is eliminated as a source of intensity fluctuations for two reasons. Repeated images taken alternatively between two orthogonal polarizations show no evidence of reorientation, and fluorescence transients recorded with circularly polarized light show identical behavior to those recorded with linearly polarized excitation. This is not surprising, given the size of the molecules and considering that little or no rotation is seen for small dye molecules on glass substrates (*3*).

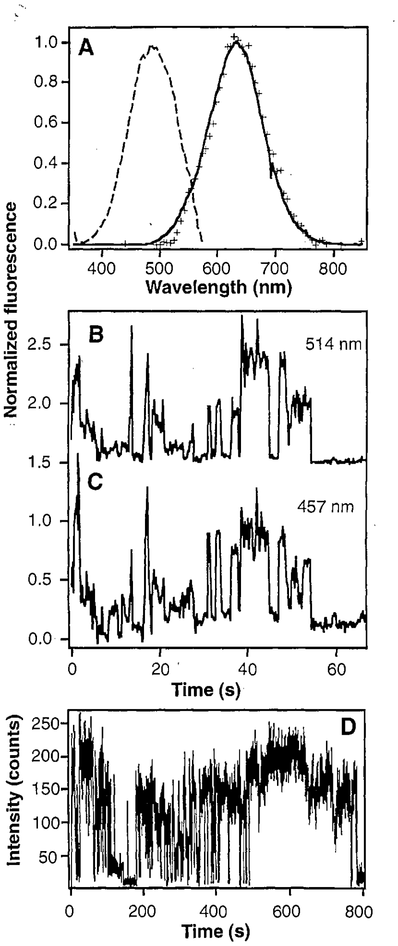

Fig. 2. (**A**) Fluorescence spectroscopy of the conjugated polymer molecules. The dashed line is the excitation spectrum for an ensemble of single polymer molecules collected form a concentrated single-molecule sample. The solid line in the plot is the emission spectrum for the same concentrated sample. The crosses overlaid on the emission spectrum represent the spectrum of a typical single molecule. (**B** and **C**) Portions of two simultaneous fluorescence transients recorded at two excitation wavelengths, 457 nm (blue edge) and 514 nm (red edge). The two transients are virtually identical, indicating that neither the small-scale nor large-scale intensity jumps are the result of spectral diffusion. (**D**) A particularly long fluorescence transient of an individual polymer molecule (514-nm excitation, 500 W cm^{-2}, and 50-ms dwell).

Previous studies of single dye molecules in polymer films have reported intensity transients that are qualitatively similar to those observed for the conjugated polymer (*8*). Discrete intensity jumps and occasional periods of emission at the background level have been observed for the dye molecules. These intensity fluctuations have been assigned in many cases to spectral diffusion of the absorption spectrum, that is, time-dependent variations in the shape or position of the single molecule's spectrum. Spectral diffusion has been probed in single dye systems by monitoring the time evolution of the mean frequency of the emission spectrum. Such measurements are difficult to make with high time resolution, particularly for a system with a lower quantum yield, because the fluorescence photons are dispersed in wavelength before being detected. We now report an alternative experimental approach, in which spectral diffusion is probed with greater time resolution by monitoring changes in fluorescence transients taken at two wavelengths by rapidly alternating between excitation on the red and blue edges of the excitation spectrum (*27*). If the molecule's absorption spectrum is shifting in time, then the molecule's cross section at the excitation frequencies will change. A blue shift of the molecule's absorption band would cause a simultaneous increase in emission for the blue edge excitation and a decrease in the red edge excitation. A red shift would show the corresponding opposite behavior. An estimate of the excitation spectrum for the single molecules can be obtained from an ensemble spectrum and images of single molecules recorded at various wavelengths.

The excitation spectrum of an ensemble of single polymer molecules (*28*) is seen in Fig. 2A. Images of single molecules were taken at three wavelengths that span the ensemble spectrum (457, 488, and 514 nm) to ensure that the single molecules had similar spectra. The images show all of the same single molecules, indicating that the individual molecules must have excitation spectra with widths similar to that for the ensemble.

Two simultaneous fluorescence transients for an individual polymer molecule taken at 457 nm (blue edge) and 514 nm (red edge) are shown in Fig. 2, B and C. The simultaneous increase and decrease behavior expected for spectral diffusion is not observed. Instead, the two transients are virtually identical. Although only a portion of one transient is shown in Fig. 2, identical behavior was seen for all of the molecules probed (*29*). Neither the dark levels nor the intensity fluctuations result from spectral diffusion.

The remaining explanation for the discrete jumps in the fluorescence intensity is that there are discrete jumps in the exciton nonradiative decay rate and, correspondingly, in the emission quantum yield. This could result from a photochemically generated temporary defect along the polymer chain, such as a pair of separated charges, that is, a radical cation and radical anion. Radical cations have been observed to be efficient quenchers of fluorescent excitons in conjugated multichromophoric molecules (*30*). The discrete jumps in the fluorescence intensity suggest that exciton migration is sufficiently rapid and efficient that the excitons can collide with the quenching defects on a time scale much shorter than their excited-state lifetime. Furthermore, the numerous discrete jumps in intensity from the background level to the average intensity level indicate that the quenching defects can revert back to an "undefected" state (such as the reverse electron transfer of the radical cation and radical anion). The reversible formation of charge-separated species has been observed in bulk conjugated polymers at low temperature (*31*).

To definitively show that the quenched states are photochemically created, we studied in more detail the rate of transition from the emissive to the non-emissive form of the molecule, defined as follows: In the non-emissive state ("off"), the molecule's emission intensity is within 3 standard deviations of the background intensity level. In the emissive state ("on"), the emission is at all higher intensities. If the changes in the molecule are spontaneous (environmental), then the rate of on → off transitions should show no changes with excitation power. However if the non-emissive states are photochemically created, then the rate should increase with excitation power. Both types of behavior have been seen to be responsible for spectral diffusion in the single chromophore dyes (*15*). The rate of the transition can be measured by compiling histograms of the number of times a molecule is either on or off for a given length of time. If we use this simple model, the histogram should show a single-exponential decay that reflects the rate of the transition (*32*).

Histograms of the frequency of on and

off periods of the polymer single molecules are shown in Fig. 3. The histograms in Fig. 3, A to D, are for an ensemble of molecules measured individually. They have been combined because most molecules do not survive long enough to give a large number of transitions. Comparison of the rate of on → off transitions at two powers (300 and 1500 W cm^{-2}) shows that the rate changes nearly linearly with laser power, indicating that non-emissive off states are photochemically generated. Conversely, the off → on transitions show no observable power dependence. Therefore, the relaxation of the off states is spontaneous. A few of the molecules observed lasted long enough to give transients with many transitions (Fig. 2D). The on → off and off → on histograms of one such molecule are seen in Fig. 3, E and F. Both of the rates are consistent with the ensemble measurements. Additionally, the long transients show times when the molecule is on or off for many seconds. Similar behavior has recently been observed for single semiconductor nanocrystals where long-lived ionized states quench the emission of the entire nanocrystal (*33*).

These SMS studies of individual polymer molecules reveal that strong communication occurs between the localized segments of the polymer. This results in single-step photobleaching and in discrete jumps in the emission intensity of single polymer molecules. The same behavior is seen in both high and low molecular weight fractions of the polymer (*34*), and the same qualitative behavior is also seen in SMS recently begun on PPyV (*35*). In contrast to SMS studies of single chromophore dye molecules, the intensity jumps in the polymer are not the result of spectral diffusion. The creation of dark states as a result of changes in the nonradiative rate of the single molecules may be important for both multichromophore and single-chromophore systems. The dark states of the polymer chains indicate that only a few, and possibly only one, photochemical defect can quench all the emission from a single polymer chain. In polymer films, there is the additional possibility that the photochemically generated quenching sites could also reduce the quantum yield of neighboring chains. This implies that the efficiency of light emission in bulk polymer films such as those used in light-emitting devices could be markedly reduced by the presence of a small number of chemical defects.

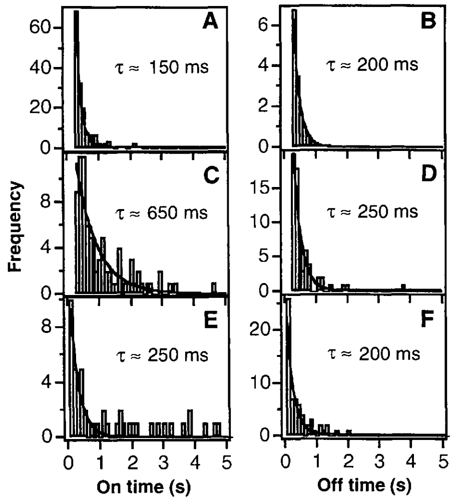

Fig. 3. (**A** and **B**) Histograms of the duration of the on times (bright levels) and off times (dark levels) taken at high excitation power (1500 W cm^{-2}) for an ensemble of single polymer molecules. (**C** and **D**) The same histograms taken at 300 W cm^{-2}. The on → off rate changes by approximately a factor of 5, whereas the off → on rate is essentially the same. This result indicates that the dark states of the molecule are photochemically created. (**E** and **F**) The same histogram for an individual molecule, showing consistency with the rates measured from the ensemble.

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21. The dispersed single-molecule sample is spin-cast from a drop of toluene solution that has 5 × 10^{-9} g ml^{-1} of the conjugated polymer and 3 × 10^{-3} g ml^{-1} of the polystyrene (molecular weight ~50,000). The resulting film is ~20 nm thick.
22. The sample scanning confocal microscope consisted of an inverted Zeiss optical microscope (Axiovert 135TV) and an X-Y sample scanning stage (Newport 462) driven by electrostrictive actuator micrometers (Newport). The laser excitation was delivered to the epi-illumination port of the microscope with a single-mode optical fiber and collimating lens and reflected up to the sample by a dichroic beam splitter (Omega). The size of the beam was chosen to slightly overfill the back focal plane of the objective (Zeiss, Achrostigmat 100× oil immersion 1.25 N.A.). The fluorescence signal was taken out of the bottom port of the microscope and focused onto a 200-μm aperture in the first image plane. The fluorescence spot was reimaged onto a 100-μm pinhole and then onto an avalanche photodiode detector (EG&G Canada SPCM). Between the aperture and pinhole, a notch filter at the excitation wavelength (Kaiser Notch-plus) and a colored-glass long-pass filter (OG530) removed residual excitation light. Spectra were taken by reimaging the fluorescence spot after the initial aperture onto the 100-μm slits of a polychrometer (Acton Spectrapro 150) equipped with a front-illuminated liquid nitrogen–cooled charge-coupled device camera (Princeton Instruments).
23. The expected areal density for the film is ~1.5 molecules μm^{-2}. The measured density is ~0.5 molecule μm^{-2}. The density is seen to vary linearly with the concentration of the original solution over two orders of magnitude.
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27. Simultaneous transients at two wavelengths were recorded by coupling two laser wavelengths into the optical fiber source of the microscope. The sample was alternately illuminated for 10 ms by each color. The two beams were modulated with two acousto-optic modulators (IntraAction). The final 100-ms dwell transients were reconstructed by binning five points for each excitation wavelength. Because of chromatic aberrations in the optical path, the beams were collimated to a smaller diameter and focused to a larger spot size (500 μm). This procedure caused a slight increase in the background, but it ensured that both wavelengths would focus in the same spot on the sample.
28. The concentrated single-molecule sample is made from a toluene solution with 5 × 10^{-5} g ml^{-1} of the conjugated polymer and 3 × 10^{-3} g ml^{-1} of the polystyrene. Several layers of the film were cast on top of each other to make a sample ~1 μm thick.
29. At long times, the 457-nm transients occasionally showed intensity, whereas the 514-nm transients remained at the background. This result indicates that there may be some chemistry that was driving the spectrum of the polymer to the blue at long times. Apparent red shifts in the spectrum were never seen.
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35. D. A. Vanden Bout, W.-T. Yip, D. Hu, D.-K. Fu, T. A. Swager, P. F. Barbara, unpublished results.
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