THE JOURNAL OF PHYSICAL CHEMISTRY A

 VOLUME 104, NUMBER 1, JANUARY 13, 2000

FEATURE ARTICLE

Ten Years of Single-Molecule Spectroscopy

Ph. Tamarat, A. Maali, B. Lounis, and M. Orrit*

Centre de Physique Moléculaire Optique et Hertzienne, UMR 5798 CNRS et Université Bordeaux I, 351 Cours de la Libération, 33405 Talence, France

Received: July 20, 1999; In Final Form: October 19, 1999

Single-molecule spectroscopy (SMS) combines some of the advantages of local probe microscopies with those of optics. Since this field came into being 10 years ago, it has expanded at a breathtaking pace. From the first cryogenic experiments up to the recent studies of basic processes in molecular biology, single-molecule methods have found their way into an ever broadening range of applications. Their common feature is the complete elimination of ensemble averaging. By exposing individual variations as well as dynamical fluctuations, SMS provides new insights into any system with spatial or temporal inhomogeneity. The present article illustrates single molecule spectroscopic experiments at cryogenic temperatures, mainly from the authors' group. The results reviewed here range from molecular photophysics, to the dynamics of the solid matrix around the molecule, and to the interactions between a single molecule and electromagnetic fields, i.e., quantum optics. SMS is now ripe for a variety of applications in physical chemistry, such as, for example, surfaces, growth structures, catalysis, or porous media.

1. Introduction

All of us, physicists, chemists, and biologists, think and talk about single molecules when we discuss molecular processes to interpret our data. Yet, our experiments usually involve huge numbers of molecules, called ensembles, which we observe over long periods of time. How can we be sure that all molecules behave in exactly the same way, that such a concept as "the pathway of the reaction of molecule A with molecule B" makes any sense at all? In some cases, for example, the NMR line of a small molecule in a liquid solution, the assumption of an homogeneous system seems reasonable. In other cases, for example, for the adsorption of molecules in an inhomogeneous porous solid, we know that different molecules must behave differently, according to pore size, micro-environment, etc. The study of single molecules, which has only recently become possible, completely eliminates ensemble averaging, and therefore offers the most direct way of checking this homogeneity hypothesis. Single molecules give access, on one hand, to statistical distributions and correlations of microscopic parameters, on the other hand, to their temporal fluctuations, which are hidden in ensemble measurements.

To define the conditions under which single molecule studies can bring new information, let us introduce two time windows. The first corresponds to the time scale of the natural fluctuations of the investigated system and is comprised between t_f and T_f ($t_f \ll T_f$). The second one is the time window between t_m and T_m ($t_m \ll T_m$) and corresponds to the measurement method. Depending on the respective position of these time windows, single-molecule measurements will present different aspects.

(i) $T_f \ll t_m$. All system fluctuations are faster than the shortest response time of the measurement. For example, this would apply to the NMR lines of a small solute molecule in liquid solution. All molecules would appear identical, the measurement of a single molecule would reproduce the average spectrum of the ensemble. The system would appear *homogeneous and static*.

* Corresponding author.

10.1021/jp992505l CCC: $19.00
Published on Web 12/14/1999

(ii) $t_m \ll T_f \ll T_m$**.** Slow system fluctuations can be detected in the experimental time window. For example, if we could follow the C═O stretching frequency of a single ester molecule in an esterification equilibrium, we would see it change from ester to carboxylate frequencies. Single molecules would all appear statistically identical, but their parameters would seem to fluctuate. The system would appear *homogeneous, but fluctuating*. It is important to see that a single molecule measurement gives a direct appreciation of the fluctuations around the equilibrium, whereas an ensemble measurement would give only averaged, static information. To retrieve time-resolved information with an ensemble, a synchronization step is needed (for example adding the pure ester to an alkaline solution). This synchronization step may be difficult, if not downright impossible, to achieve, and it often induces deep perturbations on the investigated system.

(iii) $t_f \ll T_m \ll T_f$**.** Some of the system's fluctuations are very slow or frozen, while others can still be measured. For example, disordered solids such as polymers have extremely long relaxation times, but fast fluctuations also occur. The environments of single molecules in a solid polymer will appear different, even when the experimental time scale extends to minutes or days. The system appears to be *inhomogeneous and fluctuating*.

(iv) $T_m \ll t_f$**.** All fluctuations are slower than the longest experimental time. This is the case of optical spectra of single molecules in a crystal at superfluid helium temperatures. There are no long-term fluctuations observable in the crystal. Single-molecule measurements provide the extent of static disorder and the distributions of molecular parameters induced by the frozen-in disorder of the matrix. The system now appears *inhomogeneous and static*.

We thus see that, except for systems which appear homogeneous and static on average, single-molecule data will bring new information about the fluctuations and about the distribution of local environments. This will happen whenever the longest characteristic time T_f of the system extends beyond the shortest time resolution of the experimental technique t_m. Optical measurements of single molecules have reached time resolutions as short as microseconds, but even a modest time resolution of milliseconds or seconds can reveal new information on systems with long characteristic times and/or static disorder. These include many objects of physical chemistry, such as solids, gels, polymers, liquid crystals, critical systems, surfaces, solid−liquid interfaces, etc., but also all the many complex molecular structures of biology.

The dream of seeing and manipulating single molecules was born one century ago,[1] as soon as their existence came to be widely accepted. Physicists and chemists had to wait for the high-resolution electron microscopes to see the first images of atoms and molecules,[2] at the expense, however, of fast and irreversible damage to soft samples caused by the heavy irradiation and the high energies necessary for atomic resolution. In electron microscopy, as in other similar techniques, such as optical microscopy, images of a sample are reconstructed by analyzing the scattered waves or particles. A radical change in point of view took place in the early 1980s with the scanning tunneling microscope.[3] A sharp metal tip was scanned above the surface of a conducting sample, while the tunneling current between tip and surface was kept constant. In this way images of the sample's topography and electronic density of states can be obtained with a spatial resolution essentially limited by the sharpness of the tip. Very soon, the same technique was applied to other kinds of signals (atomic contact or magnetic forces, optical signals, etc.), and a whole new set of scanning probe microscopies were developed. Not only did these flourishing new microscopies show that imaging single atoms or molecules was well within reach of modern technology, they also broke a hidden psychological barrier. Following the success of local probe microscopies, there were several attempts to manipulate and detect single molecules by other methods, among them the optical techniques we discuss here.

The most practical method so far to detect and study a single molecule by optical means is to detect the laser-induced fluorescence of a small sample volume, in which at most one molecule can be excited by the incoming laser. By collecting the fluorescence from this illuminated volume, we observe a signal that necessarily arises from a single molecule. This method combines ideas from the optical or electron microscope in that only waves are sent to or collected from the sample, but also from the tunneling microscope, in that the probing of the sample is local and mediated by a small object, here the molecule itself instead of a sharp solid tip. Optical spectroscopy is one of the oldest and cheapest ways to investigate structure and dynamics of condensed matter.[4,5] Its main advantage is that it probes a sample "at a distance": Therefore, it is relatively noninvasive, it enables studies beyond the surface layer (while probe microscopies are restricted to the surface), and it works in a wide range of conditions (ambient atmosphere, liquid water, etc.). In addition, a whole toolbox of optical spectroscopic techniques has been developed in the past for bulk materials or ensembles: polarization or intensity modulation, infrared absorption, Rayleigh, Brillouin, and Raman scattering, nonlinear optical methods,[6] like multiphoton resonances, or methods involving intermolecular interactions such as Förster energy transfer,[5] etc. When applied to single molecules, these methods are brought down to a nanometer scale, and can be used to investigate spatial and temporal inhomogeneity in the structure and dynamics of matter.

The first optical studies of single quantum systems, ions or atoms, were done twenty years ago in the gas phase, with attenuated atomic beams[7] or with single ions in traps.[8,9] The detection of single molecules in condensed matter was slower to develop, because of two main obstacles. First, the surrounding matrix or solvent gives rise to background emission that easily drowns the single molecule's signal unless the illuminated volume is severely limited. Second, the total number of photons that a single molecule can emit is usually limited by photobleaching at room temperature, or by spectral jumps at low temperatures (whereas a single ion will "live" for as long as it remains in the trap). Large biological macromolecules marked with about one hundred fluorescent labels could be detected one by one as early as 1976.[10] Steady progress in the sensitivity of detectors and in optical parts led to the detection of single molecules in a liquid in 1990 by Keller and collaborators.[11,12] The dye molecules were detected via the short fluorescence bursts they emitted when they crossed the exciting laser beam in a capillary flow. But the first optical detection of a single molecule was done in a solid at low temperatures, by Moerner and Kador in 1989,[13] who used a sensitive doubly modulated absorption method. In 1990, Orrit and Bernard[14] showed that fluorescence excitation spectra enhance the signal-to-noise ratio of single molecule lines dramatically. The stronger signals, and the possibility to study a single molecule for extended periods of time, which had been impossible in liquid solution because of diffusion and flow, opened the way to many experiments which earlier had been done on large ensembles only. Experiments formerly done on spectral holes at low temperatures were

first to follow, for example, the external field effects measured by Wild et al.[15] In parallel, the detection and spectroscopy of single quantum systems was extended to solid-state physics, with self-organized quantum dots[16−19] obtained by molecular beam epitaxy, and later to semiconductor nanoparticles[20,21] and to colored centers in diamond.[22] The photophysical properties of these inorganic systems differ substantially from those of molecules, and therefore they will be left out of the present review.[23,24] In 1993, Betzig and Chichester[25] obtained the first room-temperature images of single molecules immobilized on a surface, with a scanning near-field optical microscope. This result suddenly broadened the scope and potential of single-molecule spectroscopy. From a nice but specific spectroscopic tool, largely restricted to some well-chosen host−guest systems with narrow lines at a few Kelvin, the optical study of single molecules became a general method capable of addressing the many systems and questions of physical chemistry and biology in ambient or even physiological conditions. The potential of the method was further increased when it was realized that the easier and more classical method of confocal microscopy could provide a signal-to-noise ratio as good as near-field optical microscopy.[26−29] Suddenly, single-molecule experiments had become accessible to many groups.

One of the fields to benefit most from this new optical spectroscopy is biophysics, because of the large variety of objects and processes going on in living beings, and because many of these processes involve only very small numbers of molecules, often single molecules. The scope and prospects of room-temperature experiments on biological systems are covered in an excellent review by Weiss.[30] Biological structures are traditionally investigated by optical microscopy with specific fluorescent markers for various cell parts and biomolecules. In the past few years, an increasingly wide variety of biological molecules in membranes,[31] proteins and enzymes,[32−34] molecular motors,[35] DNA,[36,37] etc., have been detected individually after labeling with fluorophores, or via their intrinsic fluorescence.[38,39] They relay first-hand information about their surroundings. The scope of single molecule methods in biology is very broad and expanding, but many problems of physical chemistry could also benefit from the unprecedented sensitivity and accuracy of optical single molecule methods, both at room and at cryogenic temperatures, as has been illustrated by recent work on polymers,[40] on Langmuir−Blodgett films and monolayers, gels,[41] molecular crystals,[42] or on surface-enhanced Raman scattering.[43,44] There are many inhomogeneous and fluctuating systems in physical chemistry, on which single molecule studies could bring new insights by removing ensemble averaging. Liquid crystals, surfaces and interfaces, heterogeneous systems, such as emulsions, nanoparticles and porous media, and electrochemical cells, are but a few examples.

Many different optical techniques have been applied to exploit the detailed information given by single molecules. The most direct information is simply the location of molecules in images. With a high number of detected photons per molecule, it is possible to obtain a much better accuracy on the molecular location than the spatial resolution of the image.[45−47] Accurate determination of molecular positions gives information about their translational diffusion or about the colocalization of various molecules labeled with different fluorophores.[48] Sudden fluctuations of the emission intensity (blinking) reflect variations in the fluorescence yield or in the absorption spectrum of the chromophore.[14,40,49,50] From the polarization of the absorption and emission, it is easy to determine the direction of the transition dipole moments in the focal plane of the microscope.[51,52] Several methods were proposed recently to determine the full 3-dimensional orientation of the molecules.[29,53−55] The dipole orientation in turn yields the rotational diffusion of the fluorophore. Polarization may also help determine the location[56,57] of a single molecule in a birefringent crystal. Intensity variations can be used for the same purpose.[58] Fluorescence resonance energy transfer (FRET) between two fluorophores, usually by Förster's mechanism, can be used at distances shorter than about 10 nm to measure distance fluctuations once the orientations are known, or if rotational diffusion is fast.[30,36]

In all room temperature studies, however, the amount of spectral information available from the data is limited by the large breadth of electronic spectra, due to thermal fluctuations and to the sensitivity of the molecular conjugated cloud. Therefore, only strong perturbations of the molecules, such as large distortions, changes in chemical bonds, etc., will clearly appear as shifts of these broad optical bands. The situation is quite different at low temperature, where lines are very narrow, and where much insight can be obtained from line position and shape. The present article is concerned with single molecule spectroscopy at low temperatures. So far, there are essentially three kinds of applications: (i) in molecular physics, where high-resolution information can be obtained from a single molecule more easily than for an ensemble, because inhomogeneous broadenings and ensemble averagings have been eliminated; (ii) in solid-state dynamics, because the molecule can act as a probe for motion in its surroundings; in many disordered systems, complex dynamics remain even at cryogenic temperatures. Single molecules give the opportunity to probe these dynamics along with their inhomogeneity, at a nanometer scale: (iii) in the interaction of the molecule with light, where nonlinear and quantum optical effects can be studied with single molecules.

Several reviews of this field have appeared since 1992.[59−67] Reference 65 presents particularly detailed accounts of the results published before 1996. The present article therefore gives a general overview of the field, but concentrates on the newest results, particularly from our group in Bordeaux. The paper is organized as follows. Section 2 describes the common general features that experimental setups must present to make single molecule detection possible at room or at low temperatures. Features specific to low temperature designs are then discussed in more detail. In section 3, we review some recent results. A summary and conclusion are given in section 4.

2. Experimental Procedures

In this section, we briefly discuss the various experimental methods and arrangements used to isolate single molecules optically. The first successful detection of a single molecule used a sensitive measurement of its optical absorption.[13] All subsequent work, however, has been based on the detection of the fluorescence emitted by the molecule after excitation.[14] As the red-shifted fluorescence photons can be efficiently separated from stray laser photons scattered from the intense exciting beam, the weak single molecule signal appears on a very low background, with a high signal-to-noise ratio. Only fluorescence methods are discussed here.

Detecting a single molecule requires optimization of the fluorescence signal. For a given line width, the absorption cross section[68] of a transition increases with its oscillator strength, which favors strong dipole-allowed transitions. Even for line widths which are limited only by the radiative lifetime, weak transitions will lead to low fluorescence and counting rates. Therefore long accumulation times will be required to detect single molecule signals against background and dark counts from

the detector. For the same reason, bottleneck effects from the triplet or from other metastable states should be as small as possible, because they limit the average fluorescence rate. In particular, the molecule should be photochemically stable over long periods of time, since any laser-induced reaction will give products which, in general, possess neither the resonant absorption spectrum nor the high fluorescence yield of the original molecule. Finally, the fluorescence yield should be as high as possible, ideally close to unity.

The optical isolation of a single molecule requires that at most one molecule is in resonance with the laser within the illuminated spot. At room temperature, where spectra are very broad, this condition imposes that the average number of molecules in the laser focal spot be less than unity. The easiest way to reduce the number of resonant molecules is to have a much diluted bulk sample, in which the exciting laser essentially performs a spatial selection. The number concentration c of the fluorescing molecules should be less than $1/V_{sel}$, where V_{sel} is the selected volume, i.e., the volume of the part of the sample which is *at the same time* illuminated by the laser and imaged onto the detector by the collection optics. This spatial selection is most often done optically, but it can also be obtained by reducing the sample volume, e.g. by using a thin or ultrathin film,[69] a thin fiber, a single droplet,[70] a single quantum dot,[71] or nanoparticle,[23,72] etc.

If fluorescent molecules are immobilized in a solid or on a solid surface, and detected spatially, they appear as spots on the images obtained with an intensified camera[73] or a CCD camera[45] or by scanning the excitation spot across the sample.[25] If the molecules are mobile, either diffusing or moving together with the sample in a liquid flow, the signal from single molecules will appear in the time domain as a sudden fluorescence burst[12] when a molecule crosses the excitation spot.

We now consider cryogenic experiments in particular. At liquid helium temperatures, the zero-phonon lines[74,75] of the electronic component (0−0 vibronic line) of a single molecule can become extremely narrow (line width γ_{hom}), while the center frequencies of different molecules are still spread over a broad inhomogeneous band (bandwidth Γ_{inh}). The inhomogeneous broadening arises from the many defects in the solid matrix, which shift single-molecule lines at random. Therefore, for each particular laser frequency, resonance is achieved only for a very small fraction ($\gamma_{hom}/\Gamma_{inh}$) of the molecules in the sample.[76] In addition to the spatial selection described earlier, the laser thus selects molecules spectrally too, as is indicated schematically in Figure 1. To obtain single molecule excitation, the concentration can now be larger than at room temperature, since it can be as large as $\Gamma_{inh}/(\gamma_{hom}V_{sel})$. Cryogenic experiments present several drawbacks with respect to those at room temperature. They are more expensive and more difficult, and cryogenic conditions are often incompatible with those in which many interesting systems, such as biological structures, operate. Although cryogenic experiments at high spectral resolution are far from general, because only few well chosen host−guest couples will give narrow zero-phonon lines,[77] they present some specific advantages of their own: (i) The extremely narrow line leads to a maximum absorption cross section which is nearly a million times larger than the physical size of the molecule. It can reach 10^4 nm^2, whereas the absorption cross section at room temperature is of the order of a few 10^{-2} nm^2 only. The saturation intensity at low temperature is reduced by the same ratio, i.e., by several orders of magnitude. If bottleneck effects can be neglected, the saturation intensity is about 100 mW/cm^2 at low temperature[78] (while it can reach 100 kW/cm^2 at room temperature[26,80]). The resonance effect makes detection of the narrow zero-phonon line against background much easier. (ii) Because the molecule is held together by the solid cage, and because diffusion of small reactive molecules is frozen, photochemical processes are strongly reduced, and often completely absent. (iii) narrow lines are extremely sensitive to all kinds of perturbations, and relay first-hand information about subtle motion in the molecule's neighborhood.

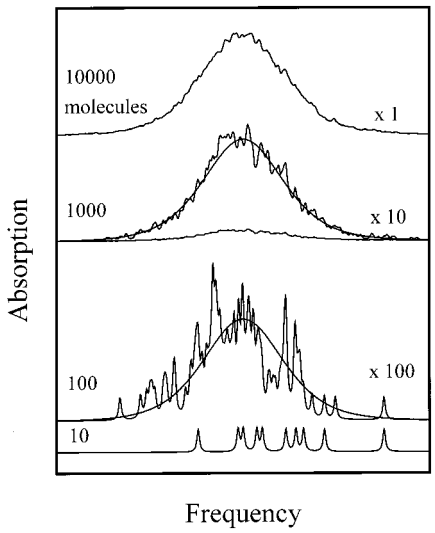

Figure 1. Simulations of an inhomogeneously broadened absorption band for various numbers of absorbers. Each molecule presents a narrow homogeneous absorption line. The single molecule regime is obtained in the lowest spectrum, or in the wings of the band for larger numbers of molecules.

The environment of single molecules in cryogenic experiments can be well controlled and fixed for long periods of time. Therefore, the comparison between experiment and theory can be pushed to a high degree of accuracy, making high-resolution spectroscopy of single molecules an excellent tool for fundamental studies of intermolecular interactions or of light-matter interaction.

The optical setup for fluorescence studies of single molecules includes the laser and optics for excitation, the collection optics, and the detection. As said earlier, either excitation or collection optical devices can operate the spatial selection of molecules, according to which one of them effectively limits the addressed sample volume. The various elements of the setup are discussed hereafter (see Figure 2).

(i) Excitation. Microscope objectives are ideal optical parts at room temperature.[26,31] They give diffraction-limited spot sizes; they can collect light over a wide solid angle (particularly immersion objectives). They are achromatic, which is important when fluorescence with a broad spectrum is sent to a small detector such as an avalanche photodiode (APD). Flat-field objectives can image an extended area of the sample on a CCD camera (flat-field is not necessary if the sample is scanned). Although some standard objectives can be used in liquid helium,[80] they are not optimized for these conditions. Moreover, immersion at a few Kelvin rules out the adjustment of the distance to the sample, since it requires a liquid medium with high refractive index between objective and sample. Therefore, other optical devices are often used in cryostats. In the first fluorescence experiment,[14] the excitation light passed through a single mode optical fiber (Figure 2). This very simple design has several advantages: it is very stable, particularly for studies as a function of temperature, no adjustment is needed, and isolation from stray laser light is easy to obtain. There are

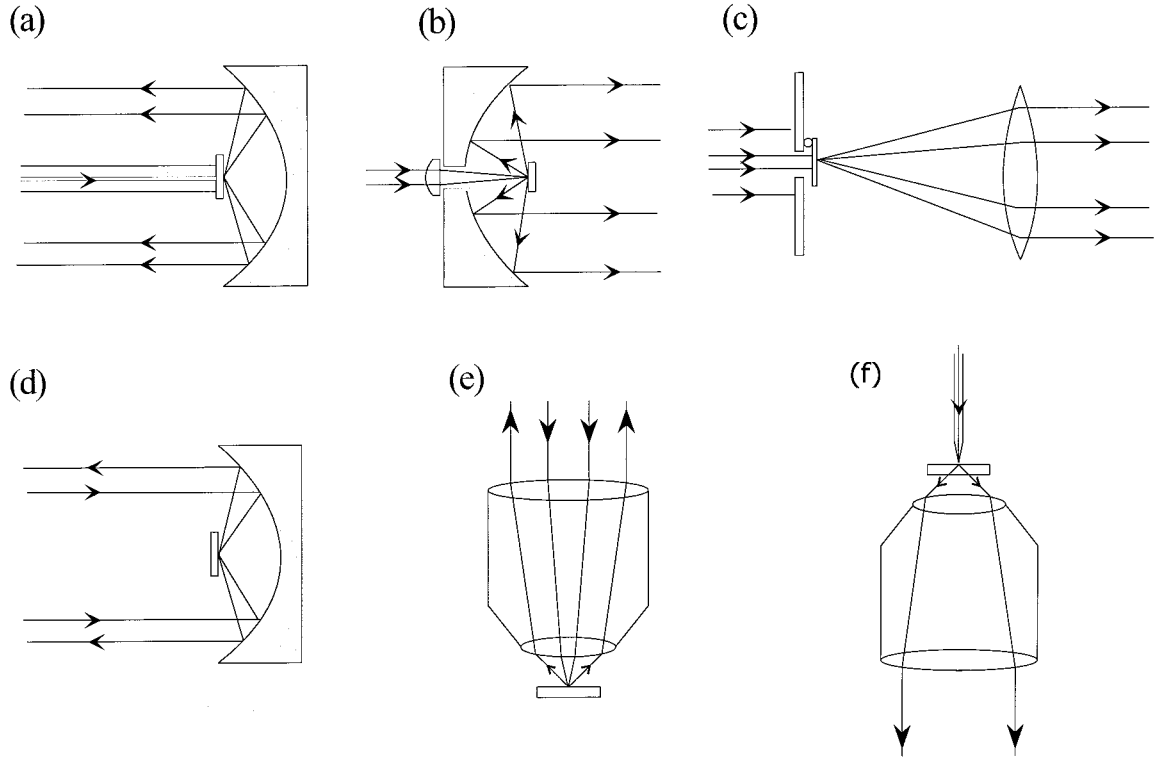

Figure 2. Various optical schemes for the excitation of single molecules and the collection of their fluorescence. (a) fiber-paraboloid,[14] (b) lens-paraboloid,[78] (c) pinhole-lens,[15] (d) confocal parabolic mirror,[81] (e) confocal microscope objective[26] (used in most room-temperature experiments), (f) near-field microscope with tapered fiber.[25]

drawbacks, however: contact between the fiber and the sample can induce defects upon cooling; the polarization of the incident light is difficult to control and to change continuously; it is impossible to move the beam with respect to the molecule, and therefore to ensure that all the molecules studied see the same light intensity. Several experimentalists have used a small lens[78] to focus a parallel laser beam onto the sample (which requires at least one adjustment), or a pinhole[15] to limit an incident laser beam. The polarization and beam position can be varied easily, but the mechanical mounting of the sample and lens should be very stable to avoid thermal drifts. Other optical parts can also be used to illuminate a small spot on the sample, such as parabolic mirrors,[81] gradient index lenses,[82] or specially designed objectives.[80]

Another possible way to excite an even smaller spot (smaller than the optical diffraction limit), is to excite through a small aperture, usually a tapered, metal-coated optical fiber. The tip is scanned at a distance of a few nm from the surface, while the distance is regulated by an atomic force signal. With such a setup, called a scanning near-field optical microscope (SNOM), Betzig and Chichester[25] imaged single molecules at room temperature. But operating a SNOM at cryogenic temperatures is difficult, and few attempts have been made so far.[83−85] In the long term, however, near-field imaging would nicely complement far-field observations, since it would provide correlation with an atomic force image, and enable direct mechanical or electrical action on the molecules.

(ii) Collection. Since fluorescence is emitted in all directions of space, it is necessary to collect it in a wide solid angle. Again, the immersion objective is a perfect solution at room temperature. At low temperatures, various wide-angle optics have been used: concave parabolic or elliptic[86] mirrors, standard or specially designed objectives, objective lens outside the cryostat, index gradient lens. When the same optical part (objective,[26] parabolic mirror,[81] and index gradient lens[82]) is used for excitation and, in the reverse pathway, for collection (sometimes with a pinhole to limit the active area of the detector), the setup is usually called a confocal arrangement. When excitation and detection optics are different, one of them often has a much worse resolution than the other, to make adjustments easier (for example, in the following combinations: fiber-paraboloid, lens-paraboloid, tip-objective in a SNOM, etc.).

(iii) Detection. A severe spectral filtering of the collected light is needed to eliminate laser photons. Convenient filters are colored glass, notch holographic filters, dichroic mirrors, and combinations thereof. For narrow emission lines, a monochromator can dramatically reduce the background.[19] The photon-counting detectors used to measure weak fluorescence signals should have a low rate of dark counts and a high quantum yield. The detection quantum efficiency can reach about 20% for a photomultiplier tube (PMT), or exceed 60% for an avalanche photodiode (APD). Single channel detectors such as PMTs or APDs have a high time resolution and can be used to measure fluorescence lifetimes and intensity correlation functions. Multichannel detectors such as CCD cameras have lower time resolution,[31] but the parallel imaging of many single molecules can be a big advantage for statistical studies.[87] In that case, the imaging quality and field size of the imaging system should be as high as possible (which rules out paraboloids, for instance, because of spherical aberrations).

The elimination of background sources is the most critical step in single molecule studies. Background may arise from experimental imperfections, like emission of the laser in the spectral range of the detected fluorescence, residual transmission of laser light through the filters, or fluorescence from optical parts, notably cutoff filters. Intrinsic emission from the sample itself is more difficult to eliminate. It can be Raman scattering or, more often, residual fluorescence from impurities, from out-of-focus fluorophores or, in high-resolution experiments, from the phonon sidebands of out-of-resonance molecules. A careful

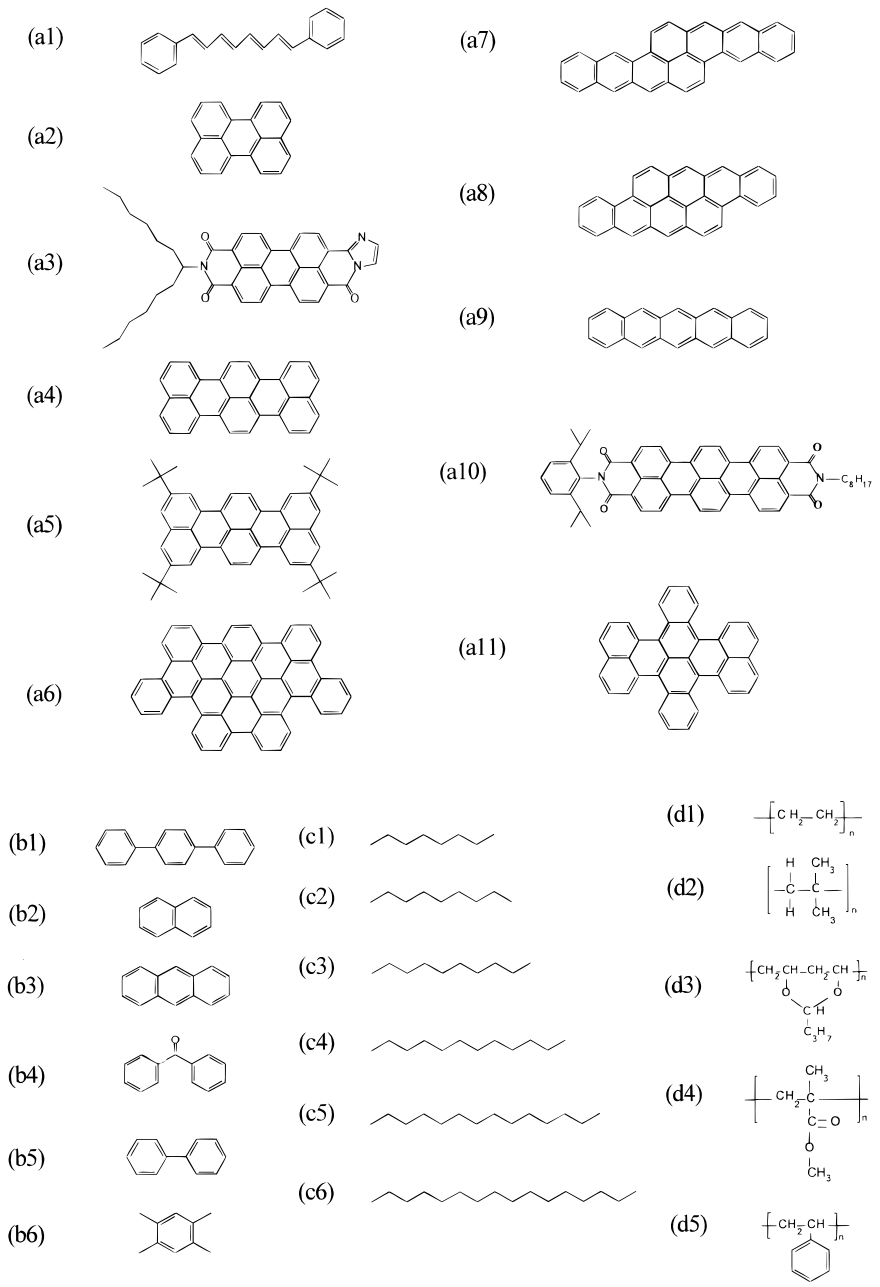

Figure 3. Structures of the main aromatic molecules and their matrixes used in single molecule experiments at cryogenic temperatures. Not all host−guest combinations will give intense and sharp single molecule signals. Colored molecules: (a1) diphenyl-octatetraene,[187] (a2) perylene,[158] (a3) peryleneamidinimide,[204] (a4) terrylene,[136] (a5) tetra-tertbutyl-terrylene,[69] (a6) benzo-diphenanthro-bisanthene,[205] (a7) dinaphtho-pyrene,[206] (a8) dibenzo-anthanthrene,[117] (a9) pentacene,[13] (a10) terrylenediimide,[116] (a11) dibenzo-terrylene.[105] Matrixes: (b1) *p*-terphenyl, (b2) naphthalene, (b3) anthracene, (b4) benzophenone, (b5) biphenyl, (b6) durene, (c1) octane, (c2) nonane, (c3) decane, (c4) dodecane, (c5) tetradecane, (c6) hexadecane,[207] (d1) polyethylene (linear), (d2) polyisobutylene, (d3) polyvinylbutyral, (d4) poly(methyl methacrylate), (d5) polystyrene.

and clean preparation of the samples is therefore extremely important. A single-molecule experiment starts with a good knowledge of the spectra and of the photophysical properties of the bulk, more concentrated solution. In the absence of any strong background source, single molecule fluorescence in "good" systems is usually intense enough to give at least several thousands counts per second and per molecule, and is fairly easy to detect.

At least two classes of molecules fulfill the requirements for optical detection by fluorescence: planar polycyclic aromatic hydrocarbons, many of which are highly fluorescent and stable,[88,89] and laser or marker dyes, which have been synthesized and selected for their high fluorescence yield and stability.[90] Many chemical variants of these marker dyes are used routinely in fluorescence microscopy of biological structures. All of the single molecule experiments at cryogenic temperatures[91] have been done with aromatic impurities (guests) or closely related molecules, dispersed or dissolved in various matrixes (hosts): molecular crystals, Shpol'skii matrixes[92] or polymers.[93] Figure 3 shows the chemical structures of some host and guest molecules used in experiments at low temperatures.

3. Single Molecule Experiments at Cryogenic Temperatures

The dynamics and absorption line shape of an electronic system in a solid is complicated because it is coupled to many vibrational modes. For a molecule, both the intramolecular

vibrations and the lattice phonons are coupled to the optical electron. If, for instance, we look at an electronic system coupled to a single harmonic oscillator, e.g., an intramolecular vibration, the electronic transition can create or destroy vibrational quanta. At zero temperature, the absorption transition with the lowest energy will connect the ground vibrational levels of the ground and excited electronic states, and is called the 0−0 transition. When the molecule is imbedded in a solid, the many phonon modes of the solid are coupled to the optical electron. The problem of an electronic transition coupled to many harmonic oscillators is analogous to the Debye−Waller problem in X-ray diffraction or to the Mössbauer problem in gamma-ray spectroscopy. It can be shown[74] that a narrow line called the zero-phonon line (ZPL) arises, corresponding to transitions where the number of phonons does not change. Since the initial and final levels are long lived, the ZPL is very narrow. The intensity of the ZPL decreases sharply (exponentially) with temperature, so that the ZPL vanishes for temperatures comparable to the Debye temperature, i.e., when the strongly coupled optical modes start to be activated. Most molecular systems are held together by weak van der Waals forces between molecules, so that they are relatively soft. This explains why ZPLs cannot usually be observed at temperatures higher than 50 K in these materials. Since the broadening of the ZPL increases exponentially with temperature, we restrict our present discussion to liquid helium temperatures. We have seen in section 2 which particular features of ZPLs can be used for single molecule investigations. Let us stress again the extreme sensitivity of optical electrons to their environment and two consequences thereof.

(i) Real solids, even crystals, contain numerous defects (if only those produced by the guest molecules themselves, or by isotopic substitutions). These defects are mostly frozen at low temperatures, and give rise to a random shift of the guest transition frequency, i.e., to inhomogeneous broadening of the ZPL component of the absorption spectrum. The inhomogeneous width of an optical line reflects the amount of short-range disorder in the solid. It can be smaller than 1 GHz in the best crystals, strain-free single-crystalline sublimation flakes,[94,95] but it can exceed 10 THz (300 cm^{-1}) in polymers. The concentration of defects may also depend on the distance to an interface.[96]

(ii) Even at low temperatures, residual movements still exist in many systems, particularly when they are disordered. These dynamics lead to a broadening of the ZPL. Many broadening effects are conveniently interpreted with the following simple image. Small fluctuations of molecular positions in the surrounding matrix give rise to fluctuations of the electron cloud of the molecule, i.e., to small changes in dipole moments and energies of states. Changes of a few parts in a thousand of a dipole moment are unobservable and do not lead to any significant effect, at least for an allowed transition, but such small relative changes are extremely important for the position and width of a narrow ZPL. Taking into account only the fluctuations of the transition frequency results in a semiclassical model in which nuclear degrees of freedom of the matrix modulate the transition frequency[97] (it is easy to write a quantum-mechanical version of this model by quantizing matrix movements such as phonons, or two-level systems[98]). The effect of the perturbation on the line shape depends on the amplitude of the frequency fluctuation $\delta\omega$ and on its correlation time τ_c. For $\delta\omega\tau_c \ll 1$, e.g. for thermal acoustic phonons or for a short-lived quasilocal mode, the Heisenberg relation shows that it has no meaning to define a frequency fluctuation $\delta\omega$ during the short correlation time τ_c. It can be shown[97] that fast fluctuations

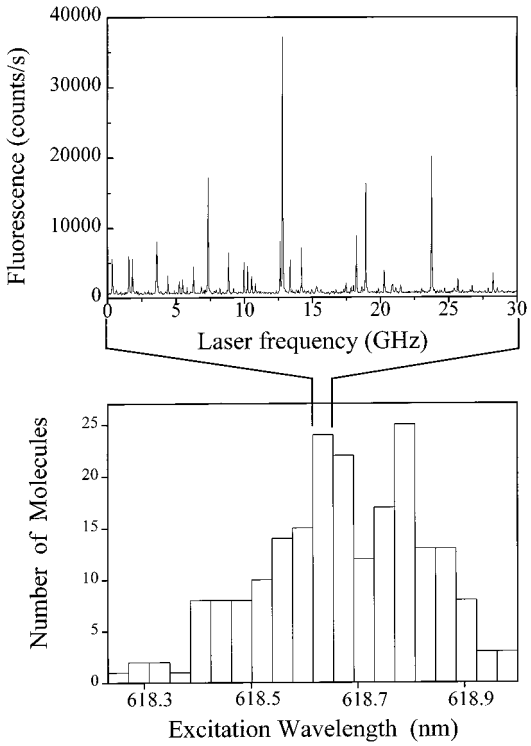

Figure 4. Top: Example of a fluorescence excitation spectrum for dibenzanthanthrene in naphthalene. Bottom: histogram of the number of molecules as a function of wavelength.

of the matrix are motionally narrowed, and lead to a loss of optical phase (pure dephasing process), with a broadening of about $\delta\omega^2\tau_c$, which decreases when τ_c shortens. For $\delta\omega\tau_c \gg 1$, a classical frequency may be defined with a precision better than $\delta\omega$ during the slow fluctuations of the matrix. It is thus possible, in principle, to follow the optical line in the spectrum as a function of time. The corresponding regime is called spectral diffusion, and some examples will be presented in section 3.2. Let us briefly comment here on the close analogy between spectral diffusion and the more familiar spatial diffusion, e.g., of dye molecules in a liquid. Just as a packet of diffusing chromophores spreads as a function of time, the frequency width of a packet of resonant molecules in a hole-burning experiment will increase as time goes.[99] On the other hand, the frequency of a single molecule is well-defined at any time, just as the position of a single molecule is well-defined in space. There may be differences in the detailed mechanism of diffusion, which is continuous for spatial diffusion in a liquid, but usually shows discrete jumps for spectral diffusion at low temperatures.[100]

At a sufficiently low temperature, which depends on the system at hand, and after a sufficiently long relaxation time, thermal fluctuations disappear, and only inhomogeneous broadening is left. Figure 4 shows an example of a fluorescence excitation spectrum with single molecular ZPLs. The narrow lines at fixed frequencies in the spectrum give a wealth of information about physics within the molecule as well as in its neighborhood, and they allow accurate tests of light−matter interactions. These three subfields of single molecule studies have been explored in the past few years and are discussed hereafter.

3.1. Molecular Physics. Single-molecule spectroscopy offers the opportunity to measure molecular states and transitions with high precision in well-chosen systems, where the lines are very narrow and the structure is stable enough for very long accumulation times. Moreover, because the measurement can

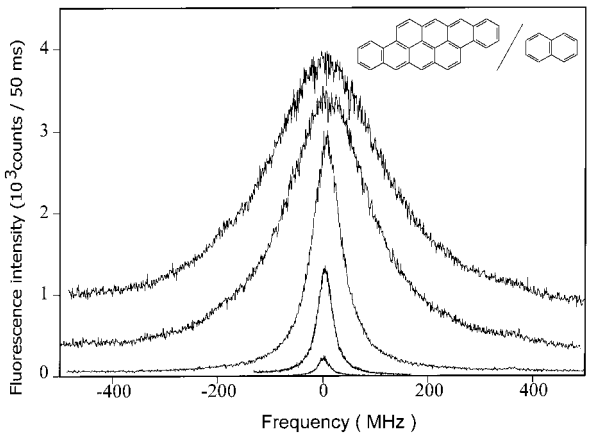

Figure 5. Optical saturation of a single molecule line (here for DBATT in naphthalene). Note the saturation of the line intensity and its broadening as the laser power increases.

be done molecule by molecule, it is straightforward to study the influence of the environment on molecular parameters, such as the rates of intersystem crossing between singlet and triplet states.[101,95] Such statistical studies of the distributions of molecular quantities, and of the statistical correlations between various quantities go far beyond the usual measurement of average values under various conditions. Just as for the very complex systems studied in other fields of science (e.g., in astronomy, medicine, or social sciences), statistical information can be crucial to discriminate between different theoretical models. So far, statistical studies have been done on fairly small samples of a few hundreds of molecules at most. One of the long-term goals of single molecule science will be to automatize measurement procedures,[87,102] in order to gather statistics with many thousands of molecules.

When spectral diffusion is absent, the absorption line profile is Lorentzian and characterized by its intensity and width. One of the most straightforward measurements on a single molecule is to study the intensity and width of the fluorescence excitation line as functions of laser intensity, i.e., to perform a so-called saturation study.[78,26] The broadening and saturation of the intensity of a single molecule line provides a very direct test of the theory of optical two-level systems in condensed matter. Earlier measurements by persistent spectral hole-burning on large ensembles were difficult to exploit and interpret, because of the nonlinearity of the burning process.[103,104] Figure 5 shows an example of optical saturation for a single dibenzanthanthrene (DBATT) molecule in naphthalene.[105] Saturation in a two-level system occurs mainly from the finite lifetime of the excited state, which can be measured on single molecules via time-correlated single photon counting.[106] But the saturation can be deeply influenced by the bottleneck effect of metastable states such as the triplet manifold.[78,101] For a single molecule, the bottleneck effect appears as on- and off-times in the fluorescence signal, a phenomenon known as photon bunching. The fluorescence intensity is strong when the molecule is in the singlet space, nil in the triplet. Sudden quantum jumps of the molecule between singlet and triplet states give rise to sudden intensity fluctuations, known as "blinking" or "flickering" in the more recent literature.[49] Bunched light from a single molecule is quite different from light from a coherent source (which has a Poissonian photon distribution in a given time interval), or from light from a thermal source where the field is a sum of the fields of many independent emitters.[107] Photon bunches can be seen directly in the fluorescence signal as a function of time,[108] and the statistics of on- and off-times can be recorded for suitable

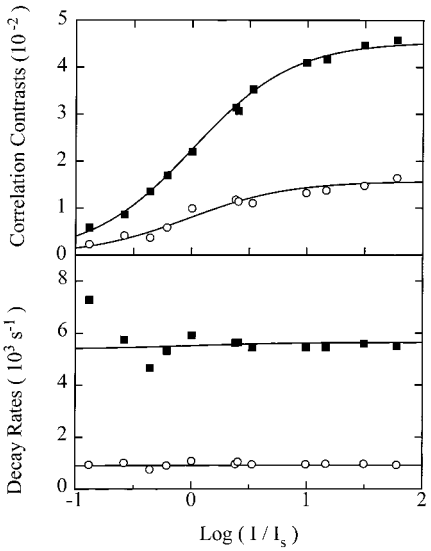

Figure 6. Decay rates (bottom) and amplitudes (top) of the two exponential components in the fluorescence autocorrelation function of a single molecule (dibenzanthanthrene in naphthalene). The two components point to two effective triplet sublevels.

systems,[108−110] from a long file where all the photon events have been stored.[111] A convenient and more traditional way to study bunching is to record the intensity autocorrelation function.[112,113] An electronic correlator keeps track of all photon pairs in a long time interval (typically a few minutes) and plots a histogramme of the number of photon pairs as a function of delay. The contrast and decay rate of a single-exponential correlation function give the average durations of on- and off-times. Careful measurements of the fluorescence correlation function of single aromatic molecules at superfluid helium temperature have revealed two exponential components in most cases (for pentacene,[114] terrylene,[115] terrylene-diimide,[116] DBATT,[117,105] etc., in various matrixes). The decay components can be ascribed to the different dwell times in the three sublevels of the triplet. Neglecting spin−lattice relaxation, we expect three exponential components, one for each sublevel, but two of them are too close to be resolved. The variations of the intensity and decay rate of these two components as functions of laser power give the population and decay rates for two effective triplet sublevels, as shown in Figure 6 for DBATT in naphthalene. Such measurements, which are very easy with cw excitation on a single molecule, require a pulsed laser for an ensemble of molecules.[118]

While the molecule has been brought into its triplet state by optical excitation, it is possible to flip the spin from one sublevel to another by applying a resonant microwave, i.e., to induce electron spin resonance (ESR) in zero applied magnetic field. Because population rates and lifetimes of triplet sublevels are different, the resonance can be detected as a change of the average fluorescence signal of a single molecule,[119,120] as in the well-known optically detected magnetic resonance (ODMR) on ensembles.[121] It is also possible to measure the correlation function[114] or the distribution of on- and off-times as functions of microwave frequency. The latter method has recently been applied to terrylene,[111] a molecule whose ODMR effect is too weak to detect directly. Transient experiments with microwave pulses have also been carried out on the triplet spin.[122,111] The blinking effect gives a simple way to know when the molecule is in the triplet or singlet states. It is then possible to synchronize microwave pulses with fluorescence drops and to achieve sublevel populations larger than with cw excitation.[122] The ESR

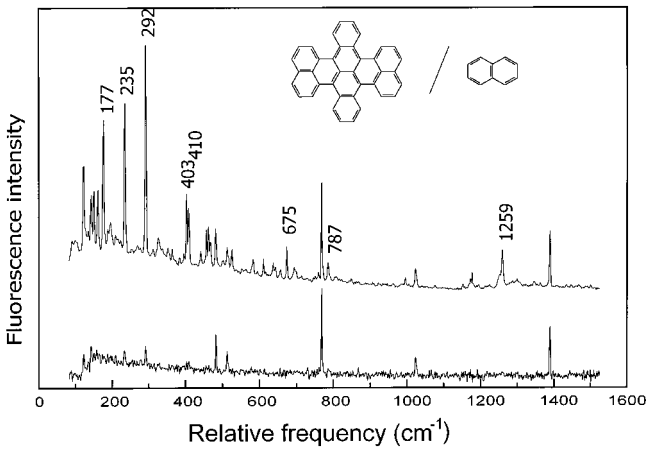

Figure 7. Dispersed fluorescence spectrum of a single dibenzoterrylene molecule in naphthalene. The lower spectrum shows the background from the host crystal, with naphthalene Raman lines.

line of a single molecule is broadened by hyperfine coupling to the nuclear spins (essentially the protons) within the molecule and in the surrounding matrix. The nuclear magnetic dipole moment of the deuteron is much smaller than that of the proton, and the ODMR lines of single deuterated pentacene molecules are much narrower than those of protonated molecules.[123,124] The ODMR effect on various isotopomers of pentacene (with one or two ^{1}H atoms in a deuterated molecule,[125] or with one ^{13}C atom[125,126,95]) gives rise to lines instead of the broad profile of the protonated molecule. The shift and width of these lines have been investigated in zero field and under an externally applied magnetic field.[127] It has even been possible to flip a single proton spin in a deuterated molecule with two protons via a carefully optimized sequence of RF pulses, in effect achieving nuclear magnetic resonance of a single proton.[128]

The fluorescence photons emitted by a single molecule within a few to up to tens of minutes can be dispersed in a spectrograph and accumulated in a CCD camera.[129] Thanks to the high detection sensitivity, even weak lines appear in the spectrum.[130,81] Dispersed fluorescence spectra yield the frequencies and intensities of intramolecular vibrations in the ground electronic state. Their analysis can be related to conformations and distortions of the molecules in different sites in the polymer matrix[129,131] or in the host crystal, even when the electronic transition frequency of the molecule is well outside the site distribution.[81] Fluorescence spectra have been recorded for several aromatic molecules (pentacene,[81] terrylene[129,132,133], DBATT,[105] etc.) in various matrixes. Figure 7 shows the fluorescence spectrum of dibenzoterrylene in naphthalene.[134] The vibrational frequencies and intensities can be used as a fingerprint to identify an individual molecule (for example an unknown impurity[135]) or to investigate molecular potentials by comparing electronic and vibrational spectra for isotopomers of a molecule, as was done for ODMR spectra.[95]

Narrow ZPLs of single molecules are extremely sensitive to any perturbing field applied on the samples. One of the first experiments done on single molecules was to shift their transition frequencies with an external electric field (Stark effect[15,136]). In spectral hole burning spectroscopy, the hole signal arises from a large number of molecules (the isochromat), whose parameters are slightly different. Therefore, spectral holes invariably broaden in external fields,[137,138] which makes their detection difficult for very high fields. In contrast, single molecule lines only shift or jump, and can be detected as easily in high fields as in low fields. Even for centrosymmetric molecules, the Stark effect on a single molecule line has linear

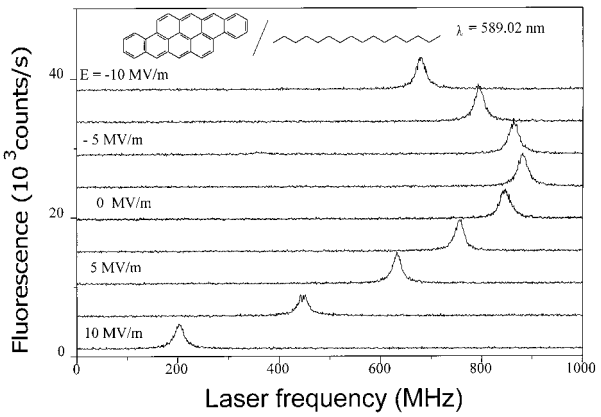

Figure 8. Stark effect of a single dibenzanthanthrene molecule in *n*-hexadecane, showing linear and quadratic contributions.

and quadratic components because the molecular symmetry is broken by defects.[60] Figure 8 shows the example of the Stark shift of a DBATT molecule in hexadecane. The linear and quadratic Stark effects are connected to the changes in dipole moment $\delta\mu$ and in polarizability $\delta\alpha$ upon excitation, and their strengths depend on the local symmetry and on the range of the fields applied. For systems, such as pentacene,[15] terrylene[139,140] in *p*-terphenyl, perylene in *n*-nonane,[141] or DBATT in naphthalene,[142] the dipole moment change $\delta\mu$ is less than a few millidebye, indicating that the insertion site is centrosymmetric. In strongly disordered matrixes such as polymers, $\delta\mu$ can be as large as 1 D,[136] but it is smaller than 0.3 D in a Shpol'skii matrix like hexadecane. In a recent study of DBATT in hexadecane,[142] a few molecules presented a cubic component in their Stark shift. This was attributed to strongly distorted molecules, where the applied field can lead to significant changes in the geometry of the molecule or of its surroundings. Single-molecule lines can also be shifted by hydrostatic pressure.[143−145] The shifts are found to be linear in the range of pressures used (up to a few kbar), but the slopes depend on the individual molecules. The pressure shift gives information about the local compressibility of the matrix and the environment of the molecule.[146]

3.2. Solid-State Dynamics. Many inter- and intramolecular movements, with widely different time scales and natures, occur at room temperature in molecular systems. One possible strategy to understand them better is to start investigating dynamics at the lowest temperatures. Various dynamical processes can still be active in a solid matrix at cryogenic temperatures. They cause a broadening of the zero phonon line. At low enough temperatures, the ZPL becomes very narrow and the homogeneous line width should reach the natural, lifetime-limited line width in a system in thermal equilibrium. Whether and how the natural line width limit is reached is an old problem of solid-state spectroscopy. The quasi-line spectra of Shpol'skii[60,77,91,92] were a big step forward in resolution, but they still suffered from inhomogeneous broadening. In the 1970s, selective spectroscopic methods such as fluorescence line-narrowing[77] and persistent spectral hole-burning showed that the lifetime limit could easily be reached in crystals, but not in disordered systems.[147,148] As discussed at the beginning of section 3, fast fluctuations, for example, phonons in crystals, broaden the homogeneous width via dephasing. In many systems, slow fluctuations are also present, and they give rise to spectral diffusion. Evidence for spectral diffusion was provided by monitoring spectral holes on a broad range of times after burning, first over days and weeks,[99] later on time scales as short as milliseconds or microseconds.[149,150] Spectral diffusion

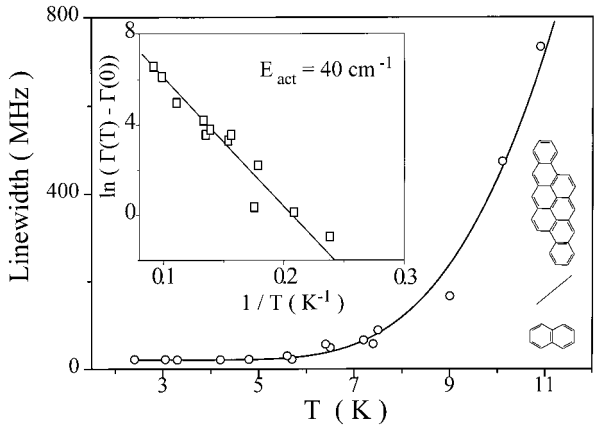

Figure 9. Dependence of the line width of a single dibenzanthanthrene molecule in a naphthalene crystal with temperature. The data are well fit by an Arrhenius plot. The activation energy corresponds to a local mode in the fluorescence spectrum.

can be studied on even shorter time scales via stimulated photon echoes,[151−153] which amounts to burn a sine-shaped hole (or population grating) with a pair of pulses, and read it at a later time with a third pulse. The slow dynamics which account for the time-dependent properties of many amorphous solids[154] came to be accepted as the origin for spectral diffusion of the optical lines. For all their sensitivity, however, hole-burning and photon echoes experiments probe large ensembles of molecules. Single-molecule methods, although their time resolution is fairly limited so far, can bring a very local view of the dynamical processes responsible for spectral diffusion. The spectral shape[155,156] and saturation[157] of single molecule lines make it possible to distinguish between broadenings by dephasing and by spectral diffusion. The first examples of spectral diffusion of a single molecule were found in a crystal,[78] but shortly afterward it was also observed in polymers.[158,159] These experiments gave direct evidence for the microscopic processes which until then had been empirically postulated to explain the low-temperature dynamics of disordered solids.[160,161]

In simple crystals of small, rigid molecules, the only degrees of freedom activated at low temperature are acoustic and optical phonons. The correlation time of perturbation by acoustic phonons is of the order of k_BT (below the Debye temperature), and for a dispersionless optical mode, it is of the order of the phonon lifetime. Since these times are shorter than the inverse of the frequency jitter caused by phonons (or much shorter than T_2, the coherence lifetime), perturbations by phonons are motionally narrowed and give rise to dephasing. Dephasing arises from elastic interactions with the phonon bath, and requires one phonon absorption and one phonon emission at the lowest order.[162,163] Therefore, dephasing varies as $n(n+1)$, where n is the average phonon number at temperature T. At low temperature, and for a single optical mode, this law is very similar to the Arrhenius dependence of n.[133] Figure 9 shows an example for DBATT in naphthalene, where the activation energy of 40 cm^{-1} corresponds to a quasilocal mode which appears in the fluorescence spectrum.[105] For broader temperature ranges, the full expression should be used.

Besides phonons, slower dynamics can also take place in some crystals. Unexpectedly, spectral diffusion of single molecules was first observed in pentacene in para-terphenyl, a crystalline system. More generally, if a system presents multiple ground states with nearly degenerate energies, long dwell times in each metastable state at low temperatures will lead to long memory times and to spectral diffusion. Spectral diffusion of single molecules often appears as sudden jumps of their transition frequency. Spectral jumps can be followed on a spectral trail,[164,165] i.e., on a collection of fluorescence excitation spectra recorded as a function of time. It is also possible to deduce a spectral trajectory of a single molecule when the spectrum is simple enough, and the number of molecules very small.[69,78,166] In the case of pentacene in *p*-terphenyl, Ambrose et al.[78] found that jumping did not depend on laser power (i.e., spectral diffusion was spontaneous, or thermally induced). Different molecules presented different kinds of trajectories, each trajectory with a large number of various jump amplitudes. Reilly and Skinner[167] explained the many jump amplitudes by coupling the molecule to a large number of uncorrelated two-level systems (TLSs). The TLSs cannot be related to flips of the central phenyl rings of *p*-terphenyl molecules in the bulk of the crystal, because the activation barrier (approximately equal to the temperature of the order−disorder transition of the crystal) would be too high. But they could be related to p-terphenyl molecules in domain walls, i.e., in regions where the molecules hesitate between two degenerate, but different crystal structures related by a symmetry. A simple model of a molecule coupled to a 2-dimensional lattice of TLSs reproduced the distribution of frequency jumps and the temperature dependence of spectral diffusion with essentially three parameters: the coupling constant, the activation barrier of the TLSs, and the distance of the molecule to the lattice. Spectral diffusion can also occur in other crystals such as Shpol'skii matrixes,[164,168] which present a high concentration of defects due to their preparation mode.

Single molecules have been detected and investigated in a number of polymers. Compared to crystals, polymers are complex systems, because they present a broad distribution of local environments, of tunneling barriers, and therefore a very broad spread of jumping rates, over many orders of magnitude (from picoseconds to years and beyond). When large ensembles of solute molecules are studied, the broad spread of time constants leads to quasi-logarithmic kinetic laws.[103] A single molecule probes its immediate surrounding (within a few tens of nanometers), and the spectral diffusion of each molecule thus reflects the specific kinetic features of its neighborhood. Therefore, there will be different line widths and line shapes,[155] different amounts of dephasing and of spectral diffusion for different individual molecules.[166,169,170] The shorter jumping times (shorter than T_2) will give rise to dephasing, i.e., to a broadening of the homogeneous line. Jumping times longer than T_2 will give rise to spectral diffusion, which can be studied by the method of the spectral trail if it is not too fast (i.e., for correlation times longer than one second, a typical scanning period). Shorter correlation times (between seconds and microseconds) can be studied by the autocorrelation function of the fluorescence intensity.[171] Histogrammes of spectral widths of single molecules have been plotted for terrylene in various polymers, polyethylene (PE),[155] polyvinylbutyral, polystyrene, poly(methyl methacrylate),[93] and polyisobutylene (PIB).[69] The specific dynamical properties of glasses at very low temperatures are usually explained within the standard model of two-level systems (TLSs) which states that local domains in the glass may jump between two configurations by tunneling. A single molecule is coupled to TLSs by strain or electric fields, and its transition frequency is modulated by the jumps of surrounding TLSs as illustrated in Figure 10. The general shape of the histogramme was found to be consistent with the standard TLS model,[155,172] assuming that anomalous glass dynamics is produced by a random distribution of local defects tunneling between two positions. However, since the histogramme is

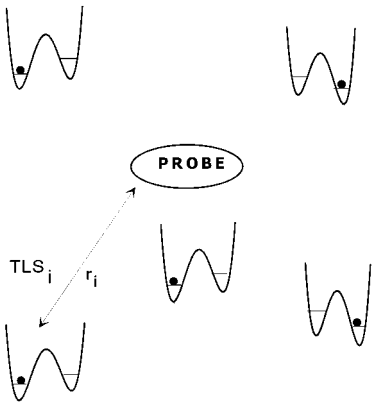

Figure 10. Schematic representation of a probe molecule in a glass at low temperature. The molecule is surrounded by a "sea" of two-level systems, each with different asymmetries, barrier heights, and widths.

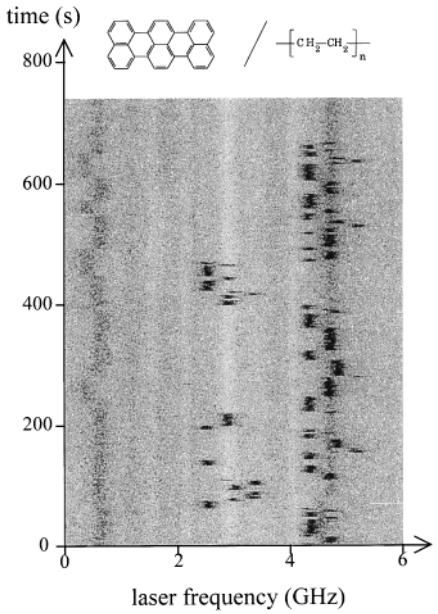

Figure 11. Example of the frequency trail of a single molecule coupled to three independent two-level systems. The molecule jumped away at 700 s. Such a trail is compatible with the standard model of glasses at low temperatures.

integrated over many single molecules, its general shape is not expected to be very sensitive to details of the model. Individual data are more likely to show deviations from the TLS model. For example, while many intensity correlation functions of single molecules were found to agree with a model of a molecule coupled to one or two two-level systems in its close neighborhood, some functions were clearly more complicated and showed a broad distribution of characteristic times, instead of the few times expected from the known concentration of two-level systems. Unfortunately, the correlation function is not very sensitive to large frequency excursions of the molecules. The most sensitive test of the coupling of single molecules to TLSs is the spectral trajectory,[173] or rather the spectral trail,[165] obtained by recording many fast spectra. For terrylene in PIB and PE, a majority of trails was compatible with the standard TLS model, as shown in Figure 11 for a single molecule coupled to three independent TLSs. However, a significant fraction of the trails presented deviations which cannot be reconciled with the standard model. In the standard model, TLS are supposed to be constant entities, which just carry out tunneling jumps between two fixed conformations. Figure 12 illustrates a particularly striking deviation from this behavior: this molecule

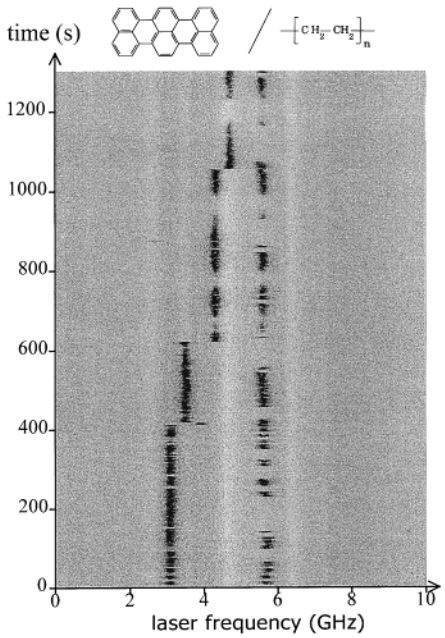

Figure 12. Spectral trail of a molecule coupled to a two-level system, whose characteristic parameters seem to suddenly change with time. In the standard model, two-level systems do not evolve with time.

is coupled to a fast TLS whose two wells seem to converge after a small number of jump-like events.[165] One big disadvantage of spectral trails is the time required for scanning the laser and acquiring fluorescence excitation spectra. With shorter scans and massive data treatment such as the frequency correlation proposed by Plakhotnik and colleagues,[171,174] access to shorter time scales should be possible. An important issue is the nature of the jumping mechanism. Some jumps are found to be spontaneous, but many of them are clearly photoinduced, since the jump rate increases with laser power. At least some of the deviations from the standard model could be explained by considering photoinduced jumping. For example, photoinduced flipping of slow TLSs surrounding the probe molecule could increase the effective TLS density.

Apart from the naturally occurring TLSs in disordered media, it is interesting to introduce known and controlled TLSs. This was done in a Shpol'skii matrix using the triplet state of deuterated triphenylene, which was produced by UV excitation.[175] The average duration of the spectral jumps corresponded exactly to the lifetime of the triplet state of triphenylene, about 20 s. More recently, Bach et al.[176] extended this idea to a naphthalene crystal, which served as a matrix for terrylene molecules. This time, the spectral jumping had a much longer lifetime than the triplet lifetime. By a combination of triplet exciton migration, trapping and annihilation, the exciton number around the probe molecule remains constant for up to three to four times the triplet lifetime. These preliminary experiments show that single molecules enable a much more accurate study of migration kinetics than former experiments on large ensembles of molecules.[177]

3.3. Nonlinear and Quantum Optics. Single molecules are excellent test objects for nonlinear and quantum optics. For all single molecules studied so far, except the N−V center in diamond, the level diagram is fairly simple, with singlet ground and excited states, and an intermediate manifold of triplet states. Since inhomogeneous broadening is eliminated by the selection of single molecules, there is no need to average the results over angles, transition frequencies, or other parameters, as was the case in former experiments, for example by spectral hole

burning. For large molecules in condensed matter, the following approximations are usually valid: the rates of relaxation to the lowest vibrational level of all electronic states are very high, of the order of 10^{12} s^{-1}. In other words, heat dissipation in the surrounding solid is very fast. On the other hand, the rate of intersystem crossing from the excited singlet state to the triplet manifold is very low. It is therefore possible to describe the dynamics of the molecule-laser field coupled system by means of optical Bloch equations, and correcting the results for the influence of the triplet. For example, the excited-state population p of a pure two-level system is modified by the triplet state into the new population P according to[156]

$$P = p/(1 + p \sum_i a_i \tau_i)$$

a_i and τ_i being the population rate and the lifetime of each triplet sublevel. Of course, this modeling of a complex molecule by a simple two-level system is an oversimplification which is only valid for resonant properties, such as fluorescence excitation spectra close to the resonance. For other properties, such as fluorescence spectrum, the full vibrational structure of the molecule must be taken into account (section 3.1).

One of the first quantum effects to be measured on single molecules was antibunching,[178,179] after it had been observed in dilute atom beams[7] and on trapped ions.[9] Fluorescence photons from a two-level electronic system are emitted one at a time. The observation of a fluorescence photon "projects" the molecule into its ground state (in the quantum-mechanical sense), whence a second photon cannot be emitted immediately. The dead time between two photon emissions corresponds to the delay needed for re-excitation. It can be pictured as arising from an "inertia" of the coupled electron-laser system. The time distribution of the emitted photons cannot be measured directly with a single detector because of experimental dead times. It can be measured by means of a Hanbury-Brown and Twiss setup,[180] where the fluorescence beam is split and sent to two detectors which measure the delay. Antibunching appears as a dip at short times, whose width depends on T_1, T_2, and on the Rabi frequency (i.e., on the laser field amplitude). At low power, the dip's width is approximately T_1, i.e., the time the molecule stays in the excited state. At high power, oscillations appear due to a transient motion of Bloch vector during the coherence damping time T_2 (these oscillations cannot be seen at room temperature, because T_2 is much too short[181,182]). It should be stressed that antibunching is a typical quantum phenomenon, without any equivalent for a classical light source, i.e., a source emitting a classical field as a function of time. The light emitted by a single molecule has a basically quantum nature, which can be exploited in quantum optical experiments.

One of the most interesting nonlinear optical effects is the modification of optical properties of matter by light. For example, a molecular transition frequency can be shifted by the electric field of a laser beam. The field from a continuous wave source is very weak, but its effect can be enhanced by resonance. This shift arises from an ac-Stark effect. It is often called light-shift,[183] and was observed a long time ago on atoms or molecules in the gas phase.[184,185] The observation of the light shift in solids was usually obscured by inhomogeneous broadening, although it has been detected with short pulses on excitons in semiconductors. Tamarat et al.[156] observed the light shift at high resolution in a solid for the first time, by shifting a single molecule's transition with a near-resonant pump laser. Far from resonance, the shift follows second-order perturbation theory, but strong deviations appear when the pump beam is close to molecular resonance.[186] Multiphoton resonances can be induced by a strong pump. The hyper-Raman resonance involves the absorption of two pump photons and the stimulated emission of one probe photon. It would appear as an amplification of the probe beam,[184] but was observed[186] as an increase of the fluorescence intensity when the probe frequency is symmetrical to the shifted molecular frequency with respect to pump frequency. The line shape and the amplitude of the effect are in excellent agreement with a calculation from optical Bloch equations where all parameters have been determined from optical saturation. Finally, even more complicated multiphoton processes arise when the probe beam is also strong, i.e., when a strong bichromatic field is applied to the molecule.[186] The ensuing dynamics is complicated, but can be grasped with a simple physical picture. An optical two-level system in a strong beating field is saturated most of the time, except when the two fields cancel each other. After each cancelation, the Bloch vector tries to follow the applied field, giving birth to a transient movement. Interference between this transient movement (oscillating at the Rabi frequency) and the beating field translates into oscillations of the average population of the excited state when the pump−probe detuning is varied. The movement is similar to that of a periodically kicked swing. Again, the agreement with optical Bloch equations was very good, without any fit parameter.

Single molecules can be used as nonlinear components to mix different frequencies. For example, the blue fluorescence of a single molecule can be excited by the joint absorption of two infrared photons.[187−189] Because the exciting wavelength is in a quite different wavelength range from the fluorescence, the laser light can be easily filtered out, giving a better signal-to-background ratio. The widths and shapes of single molecule lines can be compared for one-photon and two-photon excitation.[188] Similar experiments have been done at room temperature on dye molecules.[190,191] Another recent experiment consisted in mixing radio frequency (RF) and laser photons with a single molecule.[192] This is an electrooptical effect, which is strong for single molecules with a high linear Stark coefficient, i.e., a change in dipole moment between ground and excited state (see section 3.1). To the lowest order in laser and RF fields, a combined transition occurs when the molecular frequency and the laser frequency differ by the RF frequency. For weak laser field, the laser probes a molecular transition sinusoidally shifted in time by the RF. Applying the classical picture of frequency modulation, one expects absorption by the carrier and by lateral bands, whose intensity is given by Bessel functions of the modulation parameter. An equivalent way of seeing the sidebands is to dress the molecule with RF photons, similar to the Franck−Condon dressing of molecular states by vibrations. The change in dipole moment of the molecule plays the part of the molecular distortion. For weak RF and strong laser, the molecule is dressed by laser photons. The transition now occurs when the RF frequency connects the laser-dressed molecular states, i.e., at frequencies different from those for a weak laser. When both RF and laser are strong, none of the dressing pictures is convenient. The molecule undergoes a complicated dynamics, analogous to that in the bichromatic field mentioned earlier, and where the RF frequency plays the role of the beating frequency. The good agreement of experiment with Bloch equations (see Figure 13) shows that the two-level sytem picture accounts quantitatively for the observed spectra, and that the single molecule frequency can be shifted at high rates.

Since the transition frequency of a single molecule can be driven by an RF electric field, the molecule can be brought in

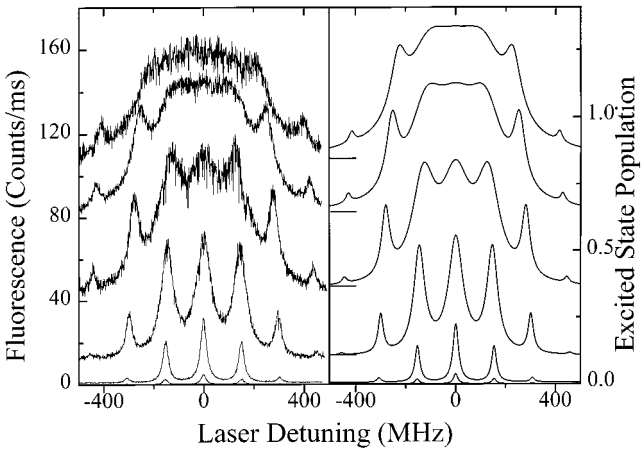

Figure 13. Fluorescence excitation spectrum of a single molecule coupled to a strong RF field and to a strong laser field (left part). The laser power increases from bottom to top. The complex spectral shape is well reproduced by a calculation based on optical Bloch equations (right part).

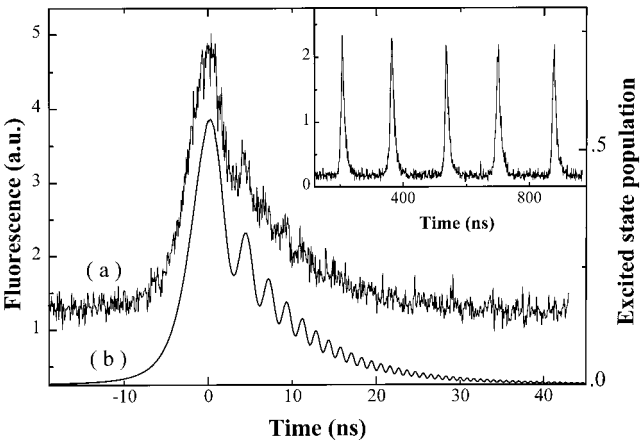

Figure 14. Averaged time-dependence of the fluorescence burst of a single molecule driven in to and out of resonance with a laser at a fixed frequency by an RF field. If the conditions for rapid adiabatic passage were fulfilled, no oscillation would appear in the decay.

to and out of resonance with a fixed laser, as was done to observe coherent transients on molecular vapors.[193] Our aim here was to prepare the molecule in its excited state with certainty, so that it emits one and only one photon and behaves as a triggered source of single photons.[194,195] The method chosen was a rapid adiabatic passage (or adiabatic following[6]). When the Rabi frequency is large enough, the Bloch vector representing the density matrix of the molecule accompanies the effective magnetic field from downward to upward pointing when the transition frequency is swept through resonance with the laser. The passage must be slow enough to enable adiabatic following, but fast enough to eliminate relaxation processes for coherence and population. A sinusoidal RF voltage was applied to electrodes on either side of the molecule, shifting it through the resonance. After each passage, the molecule has been excited and emits a fluorescence photon. Figure 14 shows the averaged time dependence of the emission, with triggering by the applied RF, and the detailed shape of an emission burst. For perfect adiabatic following, we expect a fast rise-time and an exponential decay of population, since no coherence has been prepared. In the case shown, the overall decay time of the burst is in good agreement with the fluorescence lifetime (8 ns). However, there are clear oscillations, which compare very well to a quantum Monte Carlo simulation. Therefore, the passage

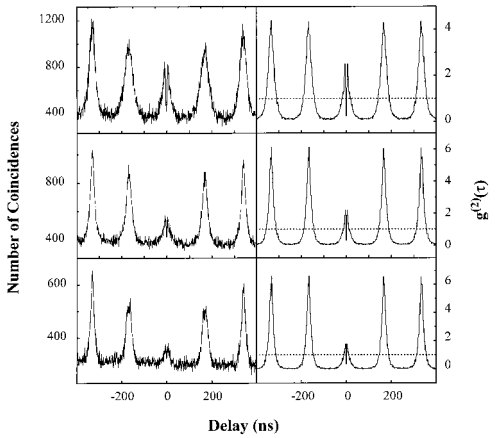

Figure 15. Histogramme of the delays between pairs of photons from the single molecule source. The depleted structure at the center shows that the molecule operates as a single photon source in up to 70% of the scans. The right part shows a comparison to a quantum Monte Carlo simulation using Bloch equations.

was too fast to be completely adiabatic. Having checked that the photons are emitted only after resonance, we wanted to know how many photons are emitted in each sweep. The probability of zero-photon and one-photon passages cannot be determined accurately because absolute yields (here, about 10^{-2} at best) are difficult to measure. Instead, we decided to measure the number of photon pairs emitted in the same burst, with a Hanbury−Brown and Twiss setup. Figure 15 shows the histogramme of time delays between photons, showing the general character of a pulsed source, with peaks at half-periods of the RF. By comparing the intensity of the central peak to that of the lateral ones, we can estimate the probability of two-photon events. Note that the central peak has a dip in the middle because of antibunching. This effect is not essential here, but confirms that our signal arises from a single molecule. The excellent agreement (see Figure 15) with simulations of the histogramme by quantum Monte Carlo simulations of Bloch equations enables us to deduce the number of sweeps leading to zero-, one- and two-photons. In the first experiment (passage time 0.7 ns), the passage is too slow, the molecule is often excited twice during its passage. The probability of a one-photon sweep is 0.56, that of a two-photon sweep is 0.31. In the third experiment, the passage is too fast, and there are too many zero-photon passages (probability 0.22). In the second experiment, the probability of a one-photon passage is optimal for our Rabi frequency. The probability is about 0.68, which ought to be compared to the optimal probability of one-photon events for an attenuated laser pulse, 0.37. To improve our single photon source, we could increase the laser intensity, but we are limited by the background. It would be preferable to use other methods to prepare the excited states, by using a laser π-pulse, for example, or by exciting a vibronic state to prepare the excited state by fast relaxation.

4. Summary and Outlook

Ten years have elapsed since the first successful optical detection of a single molecule. There is now a whole set of new optical techniques to select and investigate single molecules in condensed matter, under a broad range of different conditions. This quick methodological development, together with steady progress in selection and imaging devices, and in the signal analysis, have made single molecule observations easy and affordable to many laboratories. Just as today's developments would have been hard to predict in 1989, it is difficult to foresee

the next advances. While many of the newest applications concern biological systems and problems, the potential of single molecule methods for basic investigations of physical and chemical systems is far from being exhausted.

The present article has reviewed low-temperature spectroscopic investigations. These experiments exploit the large absorption cross sections, the high sensitivity to perturbations, and the high photostability of the sharp lines of single molecules, when they are included in suitable rigid matrixes. We have seen that individual molecules produce new results in molecular spectroscopy, about triplet states, fluorescence spectra, magnetic resonances, the spectroscopic influence of isotopic substitutions and of insertion sites. Detailed results can be compared to theoretical models, which facilitates the attribution of spectroscopic sites. But one of the main assets of the method is that all these spectroscopic experiments can be performed on the same individuals, so that their fluctuations can be correlated from molecule to molecule.

Single molecules can also be used as probes of the dynamics of the surrounding solid. Experiments of this kind obviously suffer from the general drawback of the back-action of the probe on the system. The probe molecule itself may alter the dynamics in its neighborhood, or its optical excitation can trigger unwanted photoinduced processes. Nevertheless, they may be the only practical methods to access dynamical properties at nanometer scales. We have seen the example of the specific dynamics of glasses, for which the two-level system hypothesis can be tested in a detailed manner, and the example of specific dynamics in disordered crystals such as *p*-terphenyl, where domain walls are thought to be the main source of fluctuations at low temperatures. Other crystalline systems could be studied in the same way, for example the incommensurate phase of biphenyl,[196] crystals where methyl groups can tunnel between different rotational-spin states,[197] or systems in which other dynamical degrees of freedom, e.g., electronic,[198] are active.

Third, many test experiments have been done these last years in quantum optics and nonlinear optics. Although much of their results have been obtained earlier with atoms, single molecules offer the opportunity of solid-state components and devices which would be more compact. An example is the use of a molecule as a single photon source. New experiments can now be considered in the field of the treatment of quantum information,[199] in multifrequency excitation or by coupling molecules to a resonant cavity.[200]

So far, low temperature studies have concentrated on isolated single molecules, i.e., far from other resonant molecules. It is a very exciting challenge to introduce interactions between controlled single molecules. This would open up many problems, such as the mechanism of incoherent energy transfer, excitonic coupling and the build-up of exciton coherence in dimers and higher-order oligomers. A particularly appealing example is the coherent exciton recently investigated in single bacterial antenna complexes[201]). Another fascinating topic would be the nonlinear effects appearing when two or more molecules are excited at the same time. Several methods could be used to study interactions between single molecules. One is the chemical synthesis or the self-assembly of bichromophoric molecules, which could then be studied one by one. It would also be possible to isolate single molecules in more concentrated samples, provided the excited volume is small enough. This could be achieved by using single nanoparticles containing several molecules, or by near-field optical techniques.

Most of the single molecule experiments are done and will continue to be done with far-field confocal microscopes. However, the higher spatial resolution of optical near-field microscopes, and the possibility to correlate optical output to signals and actions from a tip are highly desirable in the long run. Besides the first original designs based on tapered fibers,[25] new apertureless designs use the enhancement of the optical field by a metal tip[202,203] for linear or nonlinear spectroscopy.

Finally, the potential of single molecule investigations in ambient conditions is obviously far-reaching. In particular, single molecules give first rate information in biochemistry and biophysics, where many processes often occur at the single molecule level. But single molecules can also be used as point probes in many experiments in physical chemistry, to study surfaces and interfaces, electrochemistry, wetting, growth, diffusion in heterogeneous media, photochemistry, chemical reactions. The authors expect that single molecule spectroscopy will continue to spread and develop in the next 10 years, to become a routine tool in the hands of physical chemists.

Acknowledgment. We acknowledge financial support of our research projects by C.N.R.S. (Program Ultimatech) and by Conseil Régional d'Aquitaine. Some of the results presented here were obtained by Fedor Jelezko, Anne-Marie Boiron, and Christian Brunel.

References and Notes

(1) Perrin, J. *An. Phys.* **1918**, *9*, 133.
(2) Spence, J. C. H. *Experimental High-Resolution Electron Microscopy*; Clarendon Press: Oxford, 1981.
(3) Binnig, G.; Rohrer, H.; Gerber, Ch.; Weibel, E. *Phys. Rev. Lett.* **1983**, *50*, 120.
(4) Kuzmany, H. *Solid State Spectroscopy, An Introduction*; Springer; Berlin, 1995.
(5) Haken, H.; Wolf, H. C. *Molecular Physics and Elements of Quantum Chemistry*; Springer: Berlin, 1995.
(6) Shen, Y. R. *The Principles of Nonlinear Optics*; Wiley: New York, 1984.
(7) Kimble, H. J., Dagenais, M., and Mandel, L. *Phys. Rev. Lett*. **1977**, *39*, 691.
(8) Neuhauser, W., Hohenstatt, M., Toschek, P., and Dehmelt, H. *Phys. Rev. A* **1980**, *22*, 1137.
(9) Diedrich, F.; Walther, H. *Phys. Rev. Lett.* **1987**, *58*, 203.
(10) Hirschfeld, T. *Appl. Opt.* **1976**, *15*, 2965.
(11) Nguyen, D. C.; Keller, R. A.; Jett, J. H.; Martin, J. C. *Anal. Chem.* **1987**, *59*, 2158.
(12) Shera, E. B.; Seitzinger, N. K.; Davis, L.; Keller, R. A.; Soper, S. A. *Chem. Phys. Lett.* **1990**, *174*, 553.
(13) Moerner, W. E.; Kador, L. *Phys. Rev. Lett.* **1989**, *62*, 2535.
(14) Orrit, M.; Bernard, J. *Phys. Rev. Lett.* **1990**, *65*, 2716.
(15) Wild, U. P.; Güttler, F.; Pirotta, M.; Renn, A. *Chem. Phys. Lett*. **1992**, *193*, 451.
(16) Brunner, K.; Bockelmann, U.; Abstreiter, G., Walther, M., Böhm, G., Tränkle, G.; Wiemann, G. *Phys. Rev. Lett.* **1992**, *69*, 3216.
(17) Birotheau, L.; Izrael, A.; Marzin, J.-Y.; Azoulay, R.; Thierry-Mieg, V.; Ladan, F. R. *Appl. Phys. Lett.* **1992**, *61*, 3023.
(18) Marzin, J.-Y.; Gérard, J.-M.; Izraël, A.; Barrier, D.; Bastard, G. *Phys. Rev. Lett.* **1994**, *73*, 716.
(19) Gammon, D.; Snow, E. S.; Shanabrook, B. V.; Katzer, D. S.; Park, D.; *Science* **1996**, *273*, 87.
(20) Nirmal, M.; Dabbousi, B. O.; Bawendi, M. G.; Macklin, J. J.; Trautman, J. K.; Harris, T. D.; Brus, L. E. *Nature* **1996**, *383*, 802.
(21) Empedocles, S. A.; Norris, D. J.; Bawendi, M. G. *Phys. Rev. Lett.* **1996**, *77*, 3873.
(22) Gruber, A.; Dräbenstedt, A.; Tietz, C.; Fleury, L.; Wrachtrup, J.; von Borczyskowski, C. *Science* **1997**, *276*, 2012.
(23) Alivisatos, A. P. *Science* **1996**, *271*, 933.
(24) Gammon, D.; Brown, S. W.; Snow, E. S.; Kennedy, T. A.; Katzer, D. S.; Park, D. *Science* **1997**, *277*, 85.
(25) Betzig, E.; Chichester, R. J. *Science* **1993**, *262*, 1422.
(26) Nie, S.; Chiu, D. T.; Zare, R. N. *Science* **1994**, *266*, 1018.
(27) Trautman, J. K.; Macklin, J. J. *Chem. Phys.* **1996**, *205*, 221.
(28) Funatsu, T.; Hanada, Y. Tokunaga, M.; Saito, K.; Yanagida, T. *Nature* **1995**, *374*, 555.
(29) Macklin, J. J.; Trautman, J. K.; Harris, T. D.; Brus, L. E. *Science* **1996**, *272*, 255.
(30) Weiss, S. *Science* **1999**, *283*, 1676.

(31) Schmidt, Th.; Schütz, G. J.; Baumgartner, W.; Gruber, H. J.; Schindler, H. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 2926.
(32) Schmidt, Th.; Schütz, G. J.; Gruber, H. J.; Schindler, H. *Anal. Chem.* **1996**, *68*, 4397.
(33) Lu, H. P.; Xun, L.; Xie, X. S. *Science* **1998**, *282*, 1877.
(34) Ha, T.; Ting, A. Y.; Liang, J.; Caldwell, W. B.; Deniz, A. A.; Chemla, D. S.; Schultz, P. G.; Weiss, S. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 893.
(35) Yokota, H.; Saito, K.; Yanagida, T. *Phys. Rev. Lett.* **1998**, *80*, 4606.
(36) Ha, T.; Enderle, Th.; Ogletree, D F.; Chemla, D. S.; Selvin, P. R.; Weiss, S. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 6264.
(37) Eggeling, C.; Fries, J. R.; Brand, L.; Günther, R.; Seidel, C. A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 1556.
(38) Dickson, R. M.; Cubitt, A. B.; Tsien, R. Y.; Moerner, W. E. *Nature* **1997**, *388*, 355.
(39) Jung, G.; Wiehler, J.; Göhde, W.; Tittel, J.; Basché, Th.; Steipe, B.; Bräuchle, C. *Bioimaging* **1998**, *6*, 54.
(40) Lu, H. P.; Xie, X. S. *Nature* **1997**, *385*, 143.
(41) Dickson, R. M.; Norris, D. J.; Tzeng, Y. L.; Moerner, W. E. *Science* **1996**, *274*, 966.
(42) Fleury, L.; Sick, B.; Zumofen, G.; Hecht, B.; Wild, U. P. *Mol. Phys.* **1998**, *95*, 1333.
(43) Kneipp, K.; Yang, Wang, Kneipp, H.; Perelman, L. T.; Itzkan, I.; Dasari, R. R.; Feld, M. S. *Phys. Rev. Lett.* **1997**, *78*, 1667.
(44) Nie, S.; Emory, S. R. *Science* **1997**, *275*, 1102.
(45) Schmidt, Th.; Schütz, G. J.; Baumgartner, W.; Gruber, H. J.; Schindler, H. *J. Phys. Chem.* **1995**, *99*, 17662.
(46) van Oijen, A. M.; Köhler, J.; Schmidt, J.; Müller, M.; Brakenhoff, G. J. *J. Opt. Soc. Am. A* **1999**, *99*, 909.
(47) Bopp, M. A.; Tarrach, G.; Lieb, M. A.; Meixner, A. J. *J. Vac. Sci. Technol*. **1997**, *A15*, 1423.
(48) Schütz, G. J.; Trabesinger, W.; Schmidt, Th. *Biophys. J.* **1998**, *74*, 2223.
(49) Moerner, W. E. *Science* **1997**, *277*, 1059.
(50) Basché, Th. *J. Lumin.* **1998**, *76&77*, 263.
(51) Ha, T.; Enderle, Th.; Chemla, D. S.; Selvin, P. R.; Weiss, S. *Phys. Rev. Lett.* **1996**, *77*, 3979.
(52) Ha, T.; Glass, J.; Enderle, Th.; Chemla, D. S.; Weiss, S. *Phys. Rev. Lett.* **1998**, *80*, 2093.
(53) Bartko, A. P.; Dickson, R. M. *J. Phys. Chem. B* **1999**, *103*, 3053.
(54) Empedocles, S. A.; Neuhauser, R.; Bawendi, M. G. *Nature* **1999**, *399*, 126.
(55) Jasny, J.; Sepiol, J. *Chem. Phys. Lett.* **1997**, *273*, 439.
(56) Güttler, F.; Croci, M.; Renn, A.; Wild, U. P. *Chem. Phys.* **1996**, *211*, 421.
(57) Croci, M.; Palm, V.; Wild, U. P. *Mol. Cryst. Liq. Cryst.* **1996**, *283*, 137.
(58) Michaelis, J.; Hettich, C.; Zayats, A.; Eiermann, B.; Mlynek, J.; Sandoghdar, V. *Opt. Lett*. **1999**, *24*, 581.
(59) Moerner, W. E.; Basché, Th. *Angew. Chem., Int. Ed. Engl*. **1993**, *32*, 457.
(60) Orrit, M.; Bernard, J.; Personov, R. I. *J. Phys. Chem.* **1993**, *97*, 10256.
(61) Moerner, W. E. *Science* **1994**, *265*, 46.
(62) Moerner, W. E. *Acc. Chem. Res.* **1996**, *29*, 563.
(63) Kador, L. *Phys. Stat. Sol.* (b) **1995**, *189*, 11.
(64) Orrit, M.; Bernard, J.; Brown, R.; Lounis, B. In *Progress in Optics XXXV*; Wolf, E., Ed.; Elsevier: Amsterdam, 1996; p 61.
(65) Basché, Th.; Moerner, W. E.; Orrit, M.; Wild, U. P., Eds. *Single Molecule Optical Detection, Imaging and Spectroscopy*; VCH: Weinheim, 1997.
(66) Moerner, W. E.; Orrit, M. *Science* **1999**, *283*, 1670.
(67) Skinner, J. L.; Moerner, W. E. *J. Phys. Chem.* **1996**, *100*, 13251.
(68) Jackson, J. D. *Classical Electrodynamics*; Wiley: New York, 1975; p 803.
(69) Kettner, R.; Tittel, J.; Basché, Th.; Bräuchle, C. *J. Phys. Chem.* **1994**, *98*, 6671.
(70) Hill, S. C.; Salaheen, H. I.; Barnes, M. D.; Whitten, W. B.; Ramsey, J. M. *Appl. Opt*. **1996**, *35*, 6278.
(71) Bockelmann, U.; Roussignol, Ph.; Filoramo, A.; Weller, H.; Abstreiter, G.; Brunner, K.; Böhm, G.; Weiman, G. *Phys. Rev. Lett*. **1996**, *76*, 3622.
(72) Bruchez, M.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998**, *281*, 2013.
(73) Güttler, F.; Irngartinger, Th.; Plakhotnik, T.; Renn, A.; Wild, U. P. *Chem. Phys. Lett.* **1994**, *217*, 393.
(74) Rebane, K. K. *Impurity Spectra of Solids*; Plenum Press: New York, 1970.
(75) Sild, O.; Haller, K. Eds., *Zero-Phonon Lines*; Springer: Berlin, 1988.
(76) Moerner, W. E.; Carter, T. P. *Phys. Rev. Lett.* **1987**, *59*, 2705.
(77) Personov, R. I. In *Spectroscopy and Excitation Dynamics of Condensed Molecular Systems*; Eds. Agranovich, V. M., Hochstrasser, R. M. Eds.; North-Holland; Amsterdam, 1983; Chapter 10.
(78) Ambrose, W. P.; Basché, Th.; Moerner, W. E. *J. Chem. Phys.* **1991**, *95*, 7150.
(79) Brand, L.; Eggeling, C.; Zander, C.; Drexhage, K. H.; Seidel, C. A. M. *J. Phys. Chem. A* **1997**, *101*, 4313.
(80) Jasny, J.; Sepiol, J.; Irngartinger, Th.; Traber, M.; Renn, A.; Wild, U. P. *Rev. Sci. Instrum.* **1996**, *67*, 1425.
(81) Fleury, L.; Tamarat, Ph.; Lounis, B.; Bernard, J.; Orrit, M. *Chem. Phys. Lett.* **1995**, *236*, 87.
(82) Vacha, M.; Yokoyama, H.; Tokizaki, T.; Furuki, M.; Tani, T. *Rev. Sci. Instr.* **1999**, *70*, 2041.
(83) Hess, H. F.; Betzig, E.; Harris, T. D.; Pfeiffer, L. N.; West, K. N. *Science* **1994**, *264*, 1470.
(84) Moerner, W. E.; Plakhotnik, T.; Irngartinger, T.; Wild, U. P.; Pohl, D.; Hecht, B. *Phys. Rev. Lett.* **1994**, *73*, 2764.
(85) Göhde, W.; Tittel, J.; Basché, Th.; Bräuchle, C.; Fischer, U. C.; Fuchs, H. *Rev. Sci. Instrum*. **1997**, *68*, 2466.
(86) Heinecke, E.; Donovang, H.; Müller, C.; Hese, A. *Rev. Sci. Instrum.* **1997**, *68*, 2990.
(87) Bach, H.; Donley, E. A.; Traber, M.; Renn, A.; Wild, U. P. *Opt. Mater*. **1998**, *9*, 376.
(88) Clar, E. *Polycyclic Aromatic Hydrocarbons*; Academic Press: New York, 1964.
(89) Nakhimovsky, I.; Lamotte, M.; Joussot-Dubien, J. *Handbook of Low-Temperature Electronic Spectra of Polycyclic Aromatic Hydrocarbons*; Physical Sciences Data 40; Elsevier: Amsterdam, 1989.
(90) Eggeling, C.; Widengren, J.; Rigler, R.; Seidel, C. A. M. In *Applied Fluorescence in Chemistry, Biology and Medicine*, Rettig, W., Ed.; Springer: Berlin, 1998.
(91) Tamarat, Ph.; Jelezko, F.; Lounis, B.; Orrit, M. In *Shpol'skii Spectroscopy and Other Site Selective Methods*; Gooijer, C., Ariese, F., Hofstraat, H., Eds.; Wiley: New York, 1999; in press.
(92) Gooijer, C.; Ariese, F.; Hofstraat, H. Eds. *Shpol'skii Spectroscopy and Other Site Selective Methods*; Wiley: New York, 1999; in press.
(93) Kozankiewicz, B.; Bernard, J.; Orrit, M. *J. Chem. Phys.* **1994**, *101*, 9377.
(94) Basché, Th.; Kummer, S.; Bräuchle, C. *Chem. Phys. Lett.* **1994**, *225*, 116.
(95) Brouwer, A. C. J.; Köhler, J.; van Oijen, A. M.; Groenen, E. J. J.; Schmidt, J. *J. Chem. Phys.* **1999**, *110*, 9151.
(96) Fleury, L.; Gruber, A.; Dräbenstedt, A.; Wrachtrup, J.; von Borczyskowski, C. *J. Phys. Chem. B* **1997**, *101*, 7933.
(97) Anderson, P. W. *J. Phys. Soc. Jpn.* **1954**, *9*, 316. Kubo R. In *Fluctuations, Relaxation and Resonance in Magnetic Systems*; ter Haar, D., Eds.; Oliver and Boyd: Edinburgh, 1962.
(98) Reineker, P.; Kassner, K. In *Optical Spectroscopy of Glasses*; Zschokke, I., Ed.; D. Reidel: Dordrecht, 1986.
(99) Friedrich, J.; Wolfrum, H.; Haarer, D. *J. Chem. Phys.* **1982**, *77*, 2309.
(100) Brown, R.; Orrit, M. In *Single Molecule Optical Detection, Imaging and Spectroscopy*; Basché, Th, Moerner, W. E., Orrit, M., Wild, U. P., Eds., VCH: Weinheim, 1997, Chapter 1.4.
(101) Bernard, J.; Fleury, L.; Talon, H.; Orrit, M. *J. Chem. Phys.* **1993**, *98*, 850.
(102) Ha, T.; Chemla, D. S.; Enderle, Th.; Weiss, S. *Appl. Phys. Lett.* **1997**, *70*, 782.
(103) Friedrich, J.; Haarer, D. *Angew. Chem., Int. Ed. Engl*. **1984**, *70*, 113.
(104) Talon, H.; Orrit, M.; Bernard, J. *Chem. Phys*. **1990**, *140*, 177.
(105) Jelezko, F.; Lounis, B.; Orrit, M. *J. Chem. Phys.* **1997**, *107*, 1692.
(106) Pirotta, M.; Güttler, F.; Gygax, H.; Renn, A.; Wild, U. P. *Chem. Phys. Lett.* **1993**, *208*, 379.
(107) Loudon, R. *The Quantum Theory of Light*; Oxford University Press: London, 1973; Chapters 5 and 9.
(108) Basché, Th.; Kummer, S.; Bräuchle, Ch. *Nature* **1995**, *373*, 132.
(109) Vogel, M.; Gruber, A.; Wrachtrup, J.; von Borczyskowski, C. *J. Phys. Chem.* **1995**, *99*, 14915.
(110) Yip, W. T.; Hu, D.; Yu, J.; Vandenbout, D. A.; Barbara, P. F. *J. Phys. Chem. A* **1998**, *102*, 7564.
(111) Brouwer, A. C. J.; Groenen, E. J. J.; Schmidt, J. *Phys. Rev. Lett.* **1998**, *80*, 3944.
(112) Cummins, H. Z.; Pike, E. R., Eds. *Photon Correlation and Light Beating Spectroscopy;* Plenum, New York, 1974.
(113) Rigler, R.; Widengren J.; Mets, U. In *Fluorescence Spectroscopy*; Wolfbeis, E., Ed.; Springer: Berlin, 1992; p 13.
(114) Brown, R.; Wrachtrup, J.; Orrit, M.; Bernard, J. and von Borczyskowski, C. *J. Chem. Phys.* **1994**, *100*, 7182.
(115) Kummer, S.; Basché, Th.; Bräuchle, C. *Chem. Phys. Lett.* **1994**, *229*, 309.
(116) Mais, S.; Tittel, J.; Basché, Th.; Bräuchle, C.; Göhde, W.; Fuchs, H.; Müller, G.; Müllen, K. *J. Phys. Chem. A* **1997**, *101*, 8435.

(117) Boiron, A.-M.; Lounis, B.; Orrit, M. *J. Chem. Phys.* **1996**, *105*, 3969.
(118) de Vries, H.; Wiersma, D. A. *J. Chem. Phys.* **1979**, *70*, 5807.
(119) Köhler, J.; Disselhorst, J. A. J. M.; Donckers, M. C. J. M.; Groenen, E. J. J.; Schmidt, J.; Moerner, W. E. *Nature* **1993**, *363*, 242.
(120) Wrachtrup, J.; von Borczyskowski, C.; Bernard, J.; Orrit, M.; Brown, R. *Nature* **1993**, *363*, 244.
(121) Schmidt, J.; van der Waals, J. *Chem. Phys. Lett.* **1968**, *2*, 640.
(122) Wrachtrup, J.; von Borczyskowski, C.; Bernard, J.; Orrit, M.; Brown, R. *Phys. Rev. Lett.* **1993**, *71*, 3565.
(123) Köhler, J.; Brouwer, A. C. J.; Groenen, E. J. J.; Schmidt, J. *Chem. Phys. Lett.* **1994**, *228*, 47.
(124) Köhler, J.; Brouwer, A. C. J.; Groenen, E. J. J.; Schmidt, J. *Science* **1995**, *268*, 1457.
(125) Köhler, J.; Brouwer, A. C. J.; Groenen, E. J. J.; Schmidt, J. *J. Am. Chem. Soc.* **1998**, *120*, 1900.
(126) Brouwer, A. C. J.; Köhler, J.; Groenen, E. J. J.; Schmidt, J. *J. Chem. Phys.* **1996**, *105*, 2212.
(127) Gruber, A.; Vogel, M.; Wrachtrup, J.; von Borczyskowski, C. *Chem. Phys. Lett.* **1995**, *242*, 465.
(128) Wrachtrup, J.; Gruber, A.; Fleury, L.; von Borczyskowski, C. *Chem. Phys. Lett.* **1997**, *267*, 179.
(129) Tchénio, P.; Myers, A. B.; Moerner, W. E. *J. Phys. Chem.* **1993**, *97*, 2491.
(130) Tchénio, P.; Myers, A. B.; Moerner, W. E. *Chem. Phys. Lett.* **1993**, *213*, 325.
(131) Tchénio, P.; Myers, A. B.; Moerner, W. E. *J. Lumin.* **1993**, *56*, 1.
(132) Kulzer, F.; Kummer, S.; Matzke, R.; Bräuchle, C.; Basché, Th. *Nature* **1997**, *387*, 688.
(133) Kummer, S.; Kulzer, F.; Kettner, R.; Basché, Th.; Tietz, C.; Glowatz, C.; Kryschi, C. *J. Chem. Phys.* **1997**, *107*, 7673.
(134) Jelezko, F.; Lounis, B.; Orrit, M. *J. Phys. Chem.* **1996**, *100*, 13892.
(135) Fleury, L.; Tamarat, Ph.; Kozankiewicz, B.; Orrit, M.; Lapouyade, R.; Bernard, J. *Mol. Cryst. Liq. Cryst.* **1996**, *283*, 81.
(136) Orrit, M.; Bernard, J.; Zumbusch, A.; Personov, R. I. *Chem. Phys. Lett.* **1992**, *196*, 595; **1992**, *199*, 408.
(137) Kador, L.; Haarer, D.; Personov, R. I. *J. Chem. Phys.* **1987**, *86*, 5300.
(138) Altmann, R. B.; Renge, I.; Kador, L.; Haarer, D. *J. Chem. Phys.* **1992**, *97*, 5396.
(139) Kador, L.; Müller, A.; Richter, W. *Mol. Cryst. Liq. Cryst.* **1996**, *291*, 23.
(140) Kulzer, F.; Matzke, R.; Bräuchle, C.; Basché, Th. *J. Phys. Chem. A* **1999**, *103*, 2408.
(141) Pirotta, M.; Renn, A.; Wild, U. P. *Helv. Phys. Acta* **1996**, *69*, 7.
(142) Brunel, Ch.; Tamarat, Ph.; Lounis, B.; Orrit, M. *J. Phys. Chem.* **1999**, *103*, 2429.
(143) Croci, M.; Müschenborn, H. J.; Güttler, F.; Renn, A.; Wild, U. P. *Chem. Phys. Lett.* **1993**, *212*, 71.
(144) Müller, A.; Richter, W.; Kador, L. *Chem. Phys. Lett.* **1995**, *241*, 547.
(145) Iwamoto, T.; Kurita, A.; Kushida, T. *Chem. Phys. Lett.* **1998**, *284*, 147.
(146) Sesselmann, Th.; Richter, W.; Haarer, D.; Morawitz, H. *Phys. Rev. B*, **1987**, *36*, 7601.
(147) Moerner, W. E. Ed. *Persistent Spectral Hole Burning: Science and Applications*; Springer: Berlin, 1988.
(148) Zschokke, I. Ed. *Optical Spectroscopy of Glasses*; D. Reidel: Dordrecht, 1986.
(149) Berg, M.; Walsh, C. A.; Narasimhan, L. R.; Littau, K. A.; Fayer, M. R. *J. Chem. Phys.* **1988**, *101*, 1564.
(150) Wannemacher, R.; Smorenburg, H. F.; Schmidt, Th.; Völker, S. *J. Lumin.* **1992**, *53*, 266.
(151) Meijers, H. C.; Wiersma, D. A. *Phys. Rev. Lett.* **1992**, *68*, 381.
(152) Vainer, Yu. G.; Plakhotnik, T.; Personov, R. I. *Chem. Phys.* **1996**, *209*, 101.
(153) Zilker, S.; Kador, L.; Friebel, J.; Vainer, Yu. G.; Kol'chenko, M. A.; Personov, R. I. *J. Chem. Phys.* **1998**, *109*, 6780.
(154) Phillips, W. A. *Amorphous Solids: Low-Temperature Properties*; Springer, Berlin, 1981.
(155) Fleury, L.; Zumbusch, A.; Orrit, M.; Brown, R.; Bernard, J. *J. Lumin.* **1993**, *56*, 15.
(156) Tamarat, Ph.; Lounis, B.; Bernard, J.; Orrit, M.; Kummer, S.; Kettner, R.; Mais, S.; Basché, Th. *Phys. Rev. Lett.* **1995**, *75*, 1514.
(157) Plakhotnik T. *Opt. Commun.* **1996**, *123*, 105.
(158) Basché, Th.; Moerner, W. E. *Nature* **1992**, *355*, 335.
(159) Basché, Th.; Ambrose, W. P.; Moerner, W. E. *J. Opt. Soc. Am. B* **1992**, *9*, 829.
(160) Anderson, P. W.; Halperin, B. I.; Varma, C. M. *Philos. Mag.* **1972**, *25*, 1.
(161) Phillips, W. A. *J. Low Temp. Phys.* **1972**, *7*, 351.
(162) Jackson, B.; Silbey, R. *Chem. Phys. Lett.* **1983**, *99*, 381.
(163) Silbey, R. M.; Kassner, K. *J. Lumin.* **1987**, *36*, 283.
(164) Moerner, W. E.; Plakhotnik, T.; Irngartinger, T.; Croci, M.; Palm, V.; Wild, U. P. *J. Phys. Chem.* **1994**, *98*, 7382.
(165) Boiron, A.-M.; Tamarat, Ph.; Lounis, B.; Brown, R.; Orrit, M. *Chem. Phys.* **1999**, *247*, 119.
(166) Tittel, J.; Kettner, R.; Basché, Th.; Bräuchle, C.; Quante, H.; Müllen, K. *J. Lumin.* **1995**, *64*, 1.
(167) Reilly, Ph.D.; Skinner, J. L. *J. Chem. Phys.* **1995**, *102*, 1540.
(168) Pirotta, M.; Renn, A.; Werts, M. H. V.; Wild, U. P. *Chem. Phys. Lett.* **1996**, *250*, 576.
(169) Zumbusch, A.; Fleury, L.; Brown, R.; Bernard, J.; Orrit, M. *Phys. Rev. Lett.* **1993**, *70*, 3584.
(170) Zilker, S.; Haarer, D.; Vainer, Yu., G.; Personov, R. I. *J. Lumin.* **1998**, *76*−*77*, 157.
(171) Plakhotnik, T.; Walser, D. *Phys. Rev. Lett.* **1998**, *80*, 4064.
(172) Geva, E.; Skinner, J. L. *J. Phys. Chem. B* **1997**, *101*, 8920.
(173) Geva, E.; Skinner, J. L. *Chem. Phys. Lett.* **1998**, *287*, 125.
(174) Plakhotnik, T. *Phys. Rev. B* **1999**, *59*, 4658.
(175) Bach, H.; Renn, A.; Wild, U. P. *Chem. Phys. Lett.* **1997**, *266*, 317.
(176) Bach, H.; Renn, A.; Zumofen, G.; Wild, U. P. *Phys. Rev. Lett.* **1999**, *82*, 2195.
(177) Argyrakis, P.; Kopelman, R. *J. Chem. Phys.* **1985**, *83*, 3099.
(178) Basché, Th.; Moerner, W. E.; Orrit, M.; Talon, H. *Phys. Rev. Lett.* **1992**, *69*, 1516.
(179) Kummer, S.; Mais, S.; Basché, Th. *J. Phys. Chem.* **1995**, *99*, 17078.
(180) Hanbury-Brown, R.; Twiss, R. Q. *Nature* **1956**, *177*, 27.
(181) Ambrose, W. P.; Goodwin, P. M.; Enderlein, J.; Semin, D. J.; Martin, J. C.; Keller, R. A. *Chem. Phys. Lett.* **1997**, *269*, 365.
(182) Mets, U.; Widengren, J.; Rigler, R. *Chem. Phys.* **1997**, *218*, 191.
(183) Cohen-Tannoudji, C.; Dupont-Roc, J.; Grynberg, G. *Atom-Photon Interactions*; Wiley: New York, 1992.
(184) Wu, F. Y.; Ezekiel, S.; Ducloy, M.; Mollow, B. R. *Phys. Rev. Lett.* **1977**, *38*, 1077.
(185) Girard, B.; Sitz, G. O.; Zare, R. N.; Billy, N.; Vigué, J. *J. Chem. Phys.* **1992**, *97*, 26.
(186) Lounis, B.; Jelezko, F.; Orrit, M. *Phys. Rev. Lett.* **1997**, *78*, 3673.
(187) Plakhotnik, T.; Walser, D.; Pirotta, M.; Renn, A.; Wild, U. P. *Science* **1996**, *271*, 1703.
(188) Plakhotnik, T.; Walser, D.; Renn, A.; Wild, U. P. *Phys. Rev. Lett.* **1996**, *77*, 5365.
(189) Walser, D.; Plakhotnik, T.; Renn, A.; Wild, U. P. *Chem. Phys. Lett.* **1997**, *270*, 16.
(190) Sanchez, E. J.; Novotny, L.; Holtom, G. R.; Xie, X. S. *J. Phys. Chem. A* **1997**, *101*, 7019.
(191) Mertz, J.; Xu, C.; Webb, W. W. *Opt. Lett.* **1995**, *20*, 2532.
(192) Brunel, Ch.; Lounis, B.; Tamarat, Ph.; Orrit, M. *Phys. Rev. Lett.* **1998**, *81*, 2679.
(193) Levy, J. M.; Wang, J. H. S.; Kukolick, S. G.; Steinfeld, J. I. *Phys. Rev. Lett.* **1972**, *29*, 395.
(194) Brunel, Ch.; Tamarat, Ph.; Lounis, B.; Plantard J.; Orrit M.; C. R. *Acad. Sci. Paris* **1998**, *326*, 911.
(195) Brunel, Ch.; Tamarat, Ph.; Lounis, B.; Orrit, M. *Phys. Rev. Lett.* **1999**, *83*, 2722.
(196) Walla, P. J.; Jelezko, F.; Tamarat, Ph.; Lounis, B.; Orrit, M. *Chem. Phys.* **1998**, *233*, 117.
(197) Johnson, M.; Orth, K.; Friedrich, J.; Trommsdorff, H. P. *J. Chem. Phys.* **1996**, *105*, 9762.
(198) Lu, H. P.; Xie, X. S. *J. Phys. Chem. B* **1997**, *101*, 2753.
(199) Kilin, S. Y.; Maevskaya, T. M.; Nizotsev, A. P.; Shatokhin, V. N.; Berman, P. R.; von Borczyskowski, C.; Wrachtrup, J.; Fleury, L. *J. Lumin.* **1998**, *76*−*77*, 288.
(200) Norris, D. J.; Kuwata-Gonokami, M.; Moerner, W. E. *Appl. Phys. Lett.* **1997**, *71*, 297.
(201) van Oijen, A. M.; Ketelaars, M.; Köhler, J.; Aartsma, T. J.; Schmidt, J. *J. Phys. Chem. B* **1998**, *102*, 9363; *Science* **1999**, *285*, 400. Orrit, M. *Science* **1999**, *285*, 349.
(202) Zenhausern, F.; Martin, Y.; Wickramasinghe, H. K. *Science* **1995**, *269*, 1083.
(203) Sanchez, E. J.; Novotny, L.; Xi, X. S. *Phys. Rev. Lett.* **1999**, *82*, 4014.
(204) Kettner, R.; Tittel, J.; Mais, S.; Basché Th., Single Molecule Spectroscopy: New Systems and Methods colloquium, Presentation at the Ascona, Switzerland, March 1996.
(205) Vacha, M.; Tani, T. *J. Phys. Chem. A* **1997**, *101*, 5027.
(206) Tamarat, Ph.; Jelezko, F.; Lounis, B.; Orrit, M. 1997. Unpublished results.
(207) Vacha, M.; Liu, Y.; Nakatsuka, H.; Tani, T. *J. Chem. Phys.* **1997**, *106*, 8324.