Single-molecule spectroscopy: The road ahead

ARTICLE *in* THE JOURNAL OF CHEMICAL PHYSICS · DECEMBER 2002

Impact Factor: 2.95 · DOI: 10.1063/1.1521152

1 AUTHOR:

Michel Orrit
Leiden University
207 PUBLICATIONS **8,957** CITATIONS

Single-molecule spectroscopy: The road ahead

Michel Orrit[a)]
MoNOS, Huygens Laboratory, Universiteit Leiden, Postbus 9504, 2300 RA Leiden, The Netherlands

(Received 7 March 2002; accepted 18 September 2002)

The spectroscopy of single molecules in frozen matrices at liquid helium temperatures reveals very narrow lines, often with lifetime-limited widths. The sensitivity of such sharp lines provides a wealth of information about the molecules, their environment, and their interaction with electromagnetic fields. As compared to more conventional bulk investigations, single molecules reveal the full extent of inhomogeneity at small scales, in the static or structural sense as well as in the time-resolved or dynamical sense. A few examples from the recent literature, on single molecules as sources of single photons, as probes for molecular or electronic motion, or as beacons in structural studies of biological molecules, illustrate these general features of the technique. The author's best hopes for advances in the field include bridging between room and liquid helium temperatures, investigating structural problems on frozen biomolecules, using a single molecule as a tip for near-field optics, or as an input or output gate for quantum cryptography and quantum computing, and probing and exploiting the multiple possible interactions between single molecules. © *2002 American Institute of Physics.* [DOI: 10.1063/1.1521152]

I. INTRODUCTION

Twenty years ago, the worlds of optical microscopy and of optical spectroscopy were neatly separated. Apart from fluorescence microscopy and from a few applications such as the Raman microprobe for chemical imaging, spectroscopists were not much interested in small samples. Although the necessary equipment was available, and although coupling a microscope to lasers, spectrographs, and detectors is straightforward, the general attitude then was to work with ''well-defined'' macroscopic samples, where all molecules were supposed to behave in exactly the same way. Therefore, the emphasis was rather on sample quality than on sample size and sensitive detection. Modern science, however, is headed toward smaller and smaller scales, and toward more and more complex objects. The rigid concept of a ''well-defined'' sample loses much of its meaning when applied, for example, to a synapse between two nerve cells, or to a protein complex presenting a large number of different conformational states, or even to a small electronic circuit whose properties might depend critically on a few atomic impurities. For such problems, access to small scales requires one to couple optical spectroscopy with microscopy.

The attitude of optical spectroscopists changed dramatically in the late 1980s and early 1990s, when it became clear that despite its relatively poor spatial resolution, optical microscopy can, under suitable conditions, reach the ultimate sensitivity to a single molecule. Detecting single molecules optically was an old dream, already pursued by Jean Perrin at the beginning of the 20th century.[1] The first convincing detection of a single molecule was achieved in 1989 by Moerner and Kador in an absorption measurement.[2] Soon hereafter, fluorescence was shown to provide a much better signal-to-noise ratio, in cryogenic conditions[3] as well as at room temperature.[4,5] After 1990, single-molecule fluorescence has first been extended to a series of low-temperature experiments. A number of spectacular results have shown that much of the earlier spectroscopy of large populations could be repeated on single molecules, for example the detection of the magnetic resonance of single electronic or nuclear spins. Not only were single-molecule experiments easier to interpret and to confront to theory, they often provided surprising insights into inhomogeneity at small scales. The microscopy of single molecules at room temperature took off in 1993 with Betzig's detection of immobilized molecules on a solid surface by means of excitation with a near-field optical source.[6] The scope of the method expanded suddenly when several groups[7–9] showed that single molecules could be detected at ambient conditions with a simple confocal microscope. Single-molecule microscopy by fluorescence at room temperature has now become a versatile and general technique, opening investigations of the so-called nanoworld, i.e., of nanometer scales in condensed matter, for many kinds of problems about a broad variety of samples. Several reviews of the field appeared recently.[10–15] The present survey is restricted to the fine spectroscopy of single molecule in rigid samples at cryogenic temperatures, i.e., in conditions where most of the dynamics taking place at room temperature problems are frozen. This does not mean, however, that cryogenic single-molecule spectroscopy is irrelevant for room-temperature problems, for two reasons. First, very relevant structural information is still available at low temperature. Second, the deep insight which cryogenic experiments provide into the basic interactions and processes in the nanoworld is probably the best guide to interpret the more complex room-temperature observations. Before going deeper into these arguments, let me first summarize the experimental requirements for the detection of single-molecule fluorescence.

a)Electronic mail: orrit@molphys.leidenuniv.nl

Nearly all optical single molecule experiments to this day are based on one phenomenon: fluorescence or photoluminescence, i.e., the re-emission of a photon, generally at a later time and at a longer wavelength than the absorbed exciting photon. As compared to absorption, fluorescence has the big advantage that we can cut off the excitation light very efficiently with filters, thus allowing only fluorescence photons to reach the detector. Each detection event is therefore the signature of an absorption process. Since the fluorescence signal from a single molecule is fairly weak, it is important to achieve high collection and detection efficiencies.[10] Then, the signal has to overcome shot noise and accidental fluctuations arising from the background. Usual background sources are fluorescence counts from other impurities in the sample (which may have lower fluorescence yields, but often are much more numerous than the molecules under study), from optical elements, experimental imperfections in sources, filters, or detectors, and Raman scattering from the matrix or solvent, which cannot be separated spectrally from the molecular fluorescence. To obtain a given signal rate from a single molecule, one needs a given intensity (i.e., laser power per unit area). Obviously, for a given intensity, background is proportional to the excited volume (or to the detection volume, if it is smaller). Thus, in single-molecule experiments, the excited volume has to be reduced as much as possible. At low temperature, one usually excites the zero-phonon lines of single molecules, which are very narrow and intense. Their cross sections being comparable to a fraction of the squared wavelength of light, the excited volumes can be as large as hundreds or thousands of cubic microns. At room temperature, to the contrary, fluorescent molecules present only broad bands, with cross sections about five orders of magnitude lower than those of zero-phonon lines. Therefore, the excited volumes have to be reduced to 1 f1 (i.e., $1\mu m^3$) or less. Such small volumes, two to four orders of magnitude lower than excited volumes in low-temperature experiments, can only be achieved either with high-quality diffraction-limited microscope objectives, or with near-field optics. In order to reduce the background even further, one can play on the geometry of the sample, using for instance thin films,[16] or nanocrystals.[17]

What are the features of single molecules that make them important and promising objects? First, measurements on single molecules eliminate all kinds of averages that often obscure measurements on large populations. In general, individual molecules in a sample differ because their environments are different, or in the case of large and complex molecules such as proteins, because they present a number of different conformations. Not only do single-molecule studies give access to static inhomogeneities, revealing the extent of variations in a population, but they also show dynamical fluctuations, i.e., time-resolved changes in molecular properties.[18] Conventional kinetic experiments must synchronize all individuals in a large ensemble, as is done for instance in photochemistry or chemical kinetics. But many, if not most, of the interesting motions in complex systems are very difficult to address and to synchronize, particularly when they are steps or intermediates along complicated pathways, as is the case in protein folding, for example. By looking at a single molecule, we select only one state or one conformation at a given time. Therefore, we may in principle follow a single molecule at all times, even along an arbitrarily complex evolution path. Of course, the price to pay is that we will have to repeat the experiment on many single molecules to get statistically significant results. Another unique aspect of single molecules is their small sizes. They can be used as nearly point-like probes to monitor processes in the nanoworld, or as addressable local inputs for energy or information.

All of the above-mentioned considerations would apply to any microscopic technique. What is specific to optical experiments is the easy access they provide to many systems, and the possibility to probe molecules beyond the surface of a sample. More specifically, the low temperature single-molecule spectroscopy we discuss here offers very sharp lines, whose properties can be accurately compared to theory. One might think that physics and chemistry in the nanoworld should be directly understandable from first principles, but this is not obvious. We know that new behaviors can emerge at mesoscopic or nanometer levels. An often deceptively simple macroscopic picture may mask a wealth of complexity at smaller scales. Spectral diffusion, for example, the random spectral jumps of the optical line of a single molecule, is hidden by the inhomogeneous broadening of a large ensemble. Or the many complex and nonlinear conduction processes at nanoscales conspire and average out to produce good old Ohm's law at macroscopic scales. Single-molecule spectroscopy is a powerful tool to explore this new frontier, the intricate border between our macroscopic world and the nanoworld. In this survey article, I wish to present a brief review of some important results of cryogenic single-molecule spectroscopy, and to discuss personal ideas about its future development. In a way, single-molecule spectroscopy can be regarded as a standard technique, since one type of bright fluorophore, terrylene for example, suffices as a probe for a wide range of molecular systems. However, this technique still suffers from many limitations, both experimental and conceptual. Much remains to be done to broaden the scope of the method and to improve it.

II. MAIN ACCOMPLISHMENTS AND DIFFICULTIES

In this section, I try to illustrate with a few examples the main kinds of experiments that can be done with single molecules, and to pinpoint the limitations of the present state-of-the-art.

A. Molecular physics

A first use of single-molecule signals is to gather information about the photophysics within the molecule itself. Optical saturation, i.e., the saturation of the signal at high exciting powers, is difficult or impossible to observe on inhomogeneous bands or on spectral holes.[19] It is, however, straightforward to observe it directly on an isolated single-molecule line, by varying the laser power.[20] Saturation can arise within the electronic two-level system, because the populations of ground and excited states tend to equalize at high power, or within the three-level system[21] including a third metastable or bottleneck level (usually the triplet state

for organic molecules), where a significant part of the population can be stored. The nature of saturation is usually easy to recognize from a correlation function[3,10] of the fluorescence intensity, showing strong photon bunching in the latter case. Furthermore, transitions between triplet sublevels, which are nondegenerate even in zero magnetic field, can be investigated when microwaves are applied to the sample. By inducing transitions between the triplet sublevels, the microwave modifies their populations, and this can be detected optically (whence the acronym ODMR, for optically detected magnetic resonance), as a change in the fluorescence intensity of the molecule,[22,23] or in its correlation function.[24] In doing so, one measures the magnetic resonance of a single electronic spin. Going one step further, by applying an additional radio-frequency to the molecule, one can even detect the magnetic resonance [nuclear magnetic resonance (NMR)] of a single proton![25] These measurements of magnetic resonance go much beyond the sensitivity of conventional optically detected electron paramagnetic resonance and NMR, which require millions or billions of spins. However, single-molecule ODMR is restricted to the spin of the optical electron in a fluorescent aromatic, or to spins which are strongly coupled to this optical electron. An exciting perspective would be to extend the detection of resonance to spins further away from the optical electron, in the single molecule's environment. The optical electron spin of a single molecule could then be used in future experiments as a nanoprobe for local magnetic fields, or for exchange interactions with other spins in the surroundings.

Single-molecule spectroscopy is sensitive to molecular variations caused by the environment. For example, the shifts of single-molecule lines under such external perturbations as electric field,[26,27] pressure,[28–30] or magnetic field,[31] give information about the geometry of the molecule and sometimes of its first solvent shells. An advantage of these studies is that single-molecule lines remain narrow under arbitrary applied fields, whereas ensemble signals such as spectral holes not only shift but invariably broaden and can no longer be detected for high fields. Another application of single molecules to molecular physics is vibrational spectroscopy. A single molecule excited via its pure electronic zero-phonon line generates a high resolved fluorescence spectrum, displaying vibrational levels of the ground electronic state.[32–34] The frequencies, linewidths, and intensities of these vibrations can be compared to those obtained from bulk resonance Raman spectra, and yield information about the chemical environment of individual molecules. Such vibrational fingerprints of single molecules cannot be obtained directly at room temperature because fluorescence spectra are much too broad. Surface-enhanced Raman spectroscopy[35,36] provides vibrational fingerprints of single molecules, but this method does not apply to any aromatic molecule, and seems to require strong interactions with the metal particle or protrusion responsible for the enhancement. Obtaining highly resolved vibrational spectra of single fluorescent molecules at room temperature is an exacting challenge requiring nonlinear spectroscopy.

A wealth of molecular properties can be explored at the individual level with single-molecule spectroscopy. That method could have been implemented 30 years ago already, and would then have been very helpful to explore the properties of molecular guests in crystals and glasses. If the interest for such bulk systems has to a certain extent waned in the past years, the unique features of single-molecule spectroscopy can now be exploited for nanoscience, to explore the variety and complexity of matter at molecular scales. The really new contributions of single molecules are direct evidence for environment's influence on the measured properties, and the capability to focus on single, local spots in a large sample. However, a weak point of cryogenic single-molecule spectroscopy is that it does not apply to any type of molecules.[11,37] Despite continued effort to generalize it to more host–guest systems, only a handful have provided both the narrow zero-phonon lines and the intense fluorescence signals required. Two strategies may help to investigate a larger number of host–guest systems in the future. On one hand, it is certainly possible to improve the detection efficiency by means of better optics at low temperature. On the other hand, the very demanding conditions needed for lifetime-limited widths could be relaxed to some extent. Broader lasers could be tuned over a wider range of frequencies, and open the search for new classes of systems. For example, recent spectroscopic investigations of single antenna complexes have revealed lines a few wavenumbers broad, i.e., a thousand times broader than zero-phonon lines in crystals.[38]

B. Quantum and nonlinear optics

A molecule is a complex multilevel system, absorbing laser photons via its pure electronic zero-phonon transition and emitting fluorescence photons toward the vibrational levels of its ground state. Yet, for many properties and notably for the fluorescence excitation zero-phonon line, a single molecule can be considered as a pure two-level system, because the lifetime of the vibrational levels is much shorter than all other characteristic times in the evolution of the coupled system. On the other side of the scale of times, transitions toward and from the triplet subspace are very slow, so that the triplet states can often be neglected, or be included in effective ways in the calculation of the populations of molecular states. If a molecule behaves as an electronic two-level system, its interaction with one or several electromagnetic waves can be described accurately by optical Bloch equations, just as atoms are treated in many of the ''classical'' nonlinear and quantum optical experiments. Indeed, several experiments have proved that this description holds both qualitatively and quantitatively. After the light-shift and Autler–Townes splitting,[39] multiphoton resonance (subharmonic resonances) were detected,[40] followed by rf Rabi resonances[41] between so-called dressed states of the molecule. If from a purely descriptive point of view these experiments do not teach us much new — we already knew that optical Bloch equations work well! — they challenge the commonly held view that large molecules are too complex for quantitative treatments. Under the proper circumstances, when inhomogeneity is eliminated, even such a complex system as a big molecule with more than thirty atoms can behave as simply as a two-level atom!

The strengthened confidence in the simple picture of the electronic two-level system allowed our group in Bordeaux to devise an experiment in which the quantum state of the molecule was manipulated by laser and applied electric fields.[42] This work was based on a simple thought experiment: How to transfer the whole population of the single molecule from the pure ground state to the pure excited state? A well-known solution in magnetic resonance is a rapid adiabatic passage, occurring when the molecular transition frequency is swept through resonance with the laser, slowly enough to keep the quantum state in line with the applied effective field, and at the same time fast enough to prevent relaxation during the passage time.[43] Driving the molecule into its excited state, with certainty, causes it to emit one and only one photon, with certainty. Such a triggered source of single photons would be very useful in quantum cryptography and for further experiments in quantum optics and quantum computing. Because of background and of technical limitations, the rate of production of single photons in our experiment was at best 70%. However, more recently, single terrylene molecules in a *p*-terphenyl crystal at room temperature were excited with a pulsed laser, and shown to deliver single photons after 80% of the pulses, with a negligible proportion of emissions of two photons at the same time.[44] The advantage of the crystal matrix is that molecules are protected form atmospheric oxygen, which would lead to photobleaching. Another appealing system is the N-V color center[45–47] in thin films or nanocrystals of diamond, which are very resistant to photobleaching. A number of fluorescing systems could be used as single photon sources, but an important challenge for the future is to improve the collection and detection efficiency in the whole chain from photon production to detection. There, apart from improvements in optics and electronics, coupling the molecules or centers to resonant cavities or photonic crystals could lead to important advances. With a proper development and engineering effort, single molecules or single centers might lead to practical devices for quantum communication or even quantum computing[48] in the near future.

On a more speculative note, the adiabatic passage experiment is a manipulation of the quantum state of a molecule. This brings to mind the possibility to use a single molecule as a quantum bit, for quantum computing. With the proper combination of laser and/or static electric fields, it is straightforward to prepare superpositions of states. But, even at low temperatures, these optical coherences are quickly destroyed by spontaneous emission and cannot live longer than tens of nanoseconds, times much too short for demonstration purposes, let alone for practical applications. This obstacle appears insurmountable since the strong spontaneous emission is necessary for single-molecule detection. However, the short-lived states of the strongly emissive optical electron in a single molecule can be coupled to long-lived states, for example triplet states, either in the same molecule, or in another molecule in the neighborhood. Alternatively, other long-living levels such as those of single electronic or nuclear spins could be used as material supports for the quantum bits. Coherence manipulation and computing could be done on the long-lived states, while input and output would be performed via the orbital degrees of freedom of the coupled optical electron.

C. Solid state dynamics

A central feature of the narrow optical lines of single molecules in cryogenic conditions is the sensitivity of their frequencies to the environment. Because optical resonances have a very high quality factor (often larger than10^7), they are exquisitely sensitive probes of the matrix around them. The static part of the frequency shift, responsible for the inhomogeneous broadening in large populations, results from the precise configuration of all molecules around the probe, and hardly provides any exploitable information. The time-dependent part of the shift, however, gives information about the dynamics of motion in the molecular surrounding, namely about the couplings and the characteristic times of these movements.[49] A simple random time-dependent perturbation of the optical frequency can be characterized by two parameters; the amplitude $\Delta\nu$ of the frequency shifts, and the characteristic time τ of the change. According to the product $\Delta\nu\cdot\tau$ of these two parameters, we have two different regimes of perturbation for the line of the single molecule.[10] In the case of low-amplitude or fast movements, $\Delta\nu\cdot\tau\ll 1$, the frequency fluctuations are narrowed by motion and they only cause a broadening of the line, by a process called dephasing. Examples of such processes are fluctuations induced by the phonon bath. For large-amplitude or slow movements, $\Delta\nu\cdot\tau\gg 1$, the optical line can be followed in the spectrum, and motion leads to spectral drifts or jumps of the line. Accordingly, this process is called spectral diffusion, and can be caused by any kind of slow movement in a solid. Prior to single-molecule experiments, hole-burning studies in doped glasses had showed that additional degrees of freedom in amorphous solids were responsible for a time-dependent spectral broadening of optical lines, i.e., spectral diffusion. These additional degrees of freedom of glasses had been modeled in the 1970s and 1980s as two-level systems (TLS),[50] but many questions remained: Do TLSs really exist and what is their lifetime? Are they the only excitations left at low temperature? Are they coupled? What is the physical nature of TLS movement? Studies of single molecules in amorphous polymers have provided direct evidence for single TLSs[51] as well as answers to some of the above-posed questions. Much, but not all, of the observations are consistent with the standard TLS model. In a significant number of cases, coupling between TLSs, and more complex behaviors were found.[52] The optical study of single molecules also showed the importance of photo-induced jumps, i.e., the switching of TLSs caused by the optical excitation of the probe molecules. Further, the study of single molecules has brought evidence that spectral diffusion can also be found in media where it was unexpected, in such crystalline solids as *p*-terphenyl crystals or Shpol'skii matrices. The spectral diffusion of pentacene in *p*-terphenyl crystals[20] has been attributed by Skinner and co-workers[53] to flips of the central phenyl rings of *p*-terphenyl molecules in the walls between the domains where these rings have well-defined orientations. Another spectacular example is the recent elucidation of the optical switching mechanism of single terrylene molecules,

again in *p*-terphenyl crystals.[54,55] Molecular dynamics and mechanics simulations[56] have shown that a flip of a phenyl ring in the second solvent shell is responsible for the very reproducible photoinduced spectral jumps of terrylene molecules observed in high-quality crystals. The terrylene guest molecule replaces one *p*-terphenyl molecule, but is significantly bulkier. As a consequence, there are strong distortions of the host molecules up to the second solvent shell. Each one of two molecules in the second shell thus presents two configurations, differing by the central phenyl ring's flip angle. This gives rise to four different configurations corresponding to three apparent spectral positions of the optical line, between which photoinduced transitions can take place.

Single-molecule studies have helped to clarify the nature of optical spectral diffusion in low temperature solids. In all the cases investigated so far, a relatively bulky group of atoms (either a two-level system in a polymer or a phenyl ring in a molecular crystal) switches from one to another conformation. Several other nuclear motions do take place at low temperature, and could be studied in a similar way on a nanometer scale with single molecules. For example, some crystals such as biphenyl present incommensurate phases. In biphenyl, this incommensurate phase persists down to zero, or at least sub-Kelvin temperatures. A local single-molecule study[57] of the incommensurate modulation could shed light on the interaction or pinning of the modulation wave with defects and impurities. Another interesting example would be the tunneling of methyl groups of isolated molecules in solids. The tunneling barrier is notoriously sensitive to slight perturbations by defects and other impurities. The tunneling of single-methyl groups could reveal the extent of these perturbations, and the correlation of the tunneling splitting with other spectroscopic observables could perhaps lead to clarify the effects of variations in barrier width and height. More generally, single molecules can be applied to study materials presenting structural changes, phase transitions such as the ferroelastic transition of *p*-terphenyl, glassy transitions,[58] or any dynamics arising from atomic movements. Since temperature is clearly a crucial parameter for these problems, the widely used cryogenic conditions of spectroscopy at lifetime-limited widths will have to be relaxed. Single-molecule spectroscopy at variable temperatures, although much less resolved than at liquid helium temperature, could yet provide much more spectral information than broad bands at room temperature.

The atomic degrees of freedom are not the only ones potentially active at low temperatures. In conducting and semiconducting materials, electrons and holes are light and mobile and therefore prone to tunneling. Interactions between a localized electron in a molecular impurity and charge carriers in a conducting material could transform the optically accessible electron into a nanoinstrument, to probe conduction processes in materials or circuits at nanometer scales. This question has barely been touched in the recent literature.[59] In recent exploratory experiments on single molecules on semiconductor surfaces, we have found evidence for such interactions and, in addition to that, for a number of unexpected effects.[60] On a poorly conducting zinc oxide film, single molecules experience very inhomogeneous environments. Most molecules are sitting far from local conduction paths, and do not feel any effect of an applied current in the macroscopic sample. A small fraction of the molecules, however, sit on ''hot spots.'' They display spectacular shifts and broadenings of their optical lines, which can be attributed to very local Joule heating. This shows that conduction in the film is highly heterogeneous, and suggests that optical microscopy on the molecules would help reconstruct the conduction paths in the film. On thin films of a good conductor, indium-doped tin oxide, a majority of molecules showed very interesting and puzzling effects. Not only did the lines shift strongly when a static voltage was applied to the sample, but this shift itself resonated at low frequencies when an alternating voltage was applied. Although the coupling mechanisms leading to those shifts and low-frequency resonances remain unclear, the very existence of a coupling demonstrates the sensitivity of single molecules to charge carriers in a neighboring material. Extending these experiments to better defined samples, for example metal-coated and doped molecular crystals,[61] would open novel ways to probe the dynamics of electron gases on a local scale, and to approach phase transitions in these gases from a microscopic point of view.

A general difficulty in all these experiments in which the molecule is used as a nanoprobe is that the molecule is fixed with respect to the sample. Fascinating prospects would open if the molecule could be used as a tip in a scanning probe microscope. The concentration of the optical field in a molecular excitation would go far beyond what is currently achieved with metal or metal-coated tips in near-field optics. A single-molecule tip would immediately improve the spatial resolution of near-field optics by two or three orders of magnitude. Moreover, the narrow lines of single-molecule spectroscopy would make it possible to combine high spatial with high spectral resolutions. No principle opposes this combination, which could be attained with molecules close to surfaces or molecules in nanocrystals. The lines of such molecules are not expected to be particularly broadened, as was found in a study of dibenzanthanthrene in naphthalene crystals.[62] Although sublimation of the host during the preparation had enriched the sample in surface guest molecules, interface effects could even lead to narrower lines than for bulk molecules. First steps in the direction of placing a nanocrystal at the end of a tip were taken by Sandoghdar and his group.[17,63] For molecules close to the crystal surface, such a tip would present unprecedented sensitivity. The fabrication of the tip is still very difficult, because the technologies for preparing and manipulating nanocrystals, and for attaching them to the end of a scanning tip are still in their infancy. I have little doubt, however, that this goal will be reached eventually, and that it will provide a wonderful tool for material science and nanoscale physics.

D. Structural information about biomolecules

Since molecule microscopy is now widely applied to fascinating problems in molecular biology. The main appeal of the method is that basic processes can be studied at the molecular level in natural, i.e., ambient conditions. Freezing a sample down to cryogenic temperatures dramatically blocks

all molecular interactions and processes relevant to biology. But since the structure of a sample is often conserved on freezing, cryogenic investigations can still provide very direct structural information about each individual molecule. This information may be difficult or impossible to get by other methods. A nice example of such an application is the recent study of single bacterial antenna complexes by the groups of Schmidt, Aartsma, and Köhler.[38] Photosynthetic purple bacteria use these colored protein complexes to harvest photons under low light levels and concentrate solar energy toward their reaction centers. From the spectroscopic point of view, the complex structure of the antenna complex, known from x-ray diffraction, mainly presents two different coaxial rings of bacteriochlorophyll-a (BChla) molecules, both with C_9 symmetry in the case of *Rhodopseudomonas acidophila.* One ring has 9 BChla molecules lying nearly flat on the ring plane (the so-called B800 system), while the other ring comprises 18 molecules nearly perpendicular to the ring plane, arranged like the blades of a turbine (the so-called B850 system). From the structure and from earlier spectroscopic experiments, no strong interaction was expected between BChla molecules in the B800 system, but it was unclear how strong this interaction was within the B850 system. Spectroscopic studies of single complexes at 2 K have confirmed that BChla molecules behave nearly independently in the B800 ring, but they indicated that the strong interactions in the B850 ring generate a fully delocalized exciton, at least at low temperature. Single complexes also provided better insight into the parts played by interactions and disorder, and highlighted the importance of complex-to-complex heterogeneity (intercomplex disorder). Neglecting intercomplex disorder leads to overestimating the actual spread of resonance energies from the disorder in each ring, and therefore to underestimating exciton delocalization. Finally, the low-temperature data provided clear evidence for deformation of the ring, which had not been detected with x-rays. It is not yet clear whether this is a cooling-induced deformation or not, although single molecule experiments at room temperature[64] confirm that the C_9 symmetry is also broken at room temperature. This example forcefully demonstrates the power of single-molecule investigations to elucidate relevant questions about structure in biology, even under cryogenic conditions.

More generally, spectroscopic studies of single biomolecules at low temperatures may provide rich knowledge about their structure: their various possible conformations, their colocalization with other labeled molecules, their orientation and position about cellular structures and organelles. By cycling the molecules to higher and higher temperatures, their potential energy landscape could be mapped, in principle, for each individual molecule, which would clarify the fast dynamics that are crucial for their function. This technique could even be extended to room temperatures. This would yield structural snapshots of the pathway of functional conformation changes. However, the molecule must be cooled and heated fast enough to conserve its structure, in particular water crystallization must be avoided. Single molecules offer an appealing solution to this problem, because heating and cooling of micron-sized volumes can be completed in microseconds. A similar problem has been solved in cryo-electron microscopy by using quick cooling of small droplets. Spectroscopic studies of frozen biomolecules would make long times available for the acquisition of high-precision structural data, while photobleaching would be strongly reduced.

III. PERSPECTIVES: BEST HOPES

In contrast to other nano-objects such as semiconductor nanocrystals, metal nanoparticles, or scanning microscope tips, molecules possess well-defined chemical structures and relatively simple properties. They have been widely studied and are well characterized and understood. Therefore, single molecules can be used as well-defined nano-probes to relay fundamental information about complex systems, in biophysics or biochemistry, or about materials and microscopic interactions in solids or in soft matter. In virtually all fields of condensed matter science, a point probe may provide deeper microscopic insight into the structure and dynamics of a sample. This new viewpoint will not replace the more macroscopic or mesoscopic points of view of statistical physics, but it may nicely complement it. Local insights not only help understand the sometimes subtle mechanisms at work in the nanoworld, they also give irreplaceable access to heterogeneity and fluctuations in a direct and reliable way. In our brief survey of some of the recent results, we have seen that single molecules can act as reporters for a broad variety of dynamical degrees of freedom. I expect that similar fine probing will extend in the near future to conditions ranging from solids in liquid helium to fluids at ambient temperature. For example, using the sensitivity of molecular electronic states and energies to charges in the molecular neighborhood, the properties of charge carriers and mechanisms of electrical conduction could be explored. Several unexpected phenomena have been observed already, opening a window on local aspects of the complex phenomena taking place in dense electron systems. Another exciting perspective is to use the spin of a single molecule as a probe for local magnetization or for other spins in the environment, opening the way for EPR and NMR investigations at the single-molecule level. Similar nano-probing ideas can be applied to obtain structural information about biological systems, by means of the very sensitive methods of low-temperature spectroscopy, if the system under study can be rapidly quenched without significant structural changes. Besides these uses of single molecules which appear well within reach of present day possibilities, completely new fields of application have barely been touched yet. Here are a few ideas.

It would be very appealing for the high-resolution methods to bridge the gap between room temperature microscopy where bands are very broad, and the sharp and sensitive lines observed in some systems at liquid helium temperatures. In this intermediate temperature range, lines would certainly be much broader than the lifetime limit, but still convey much more information than the broad bands of room temperature. Their study would be very important to probe the potential energy landscape of complex systems, in particular that of proteins.

A general problem in single-molecule spectroscopy and microscopy is the statistical significance of the data and the importance of bias. The number of molecules sampled has to be systematically increased, by automatic and parallel recordings of many molecules in large samples. The analysis of the large flow of data requires better software, and in-depth analysis[65] to understand the sources of possible biases.

So far, most of the results in single object fluorescence have been obtained on a very small class of organic aromatic molecules. Other classes of molecules, and other fluorophores should be tried: there are good fluorophores among organic radicals, inorganic ions, color centers, vacancy-related defects, which could be detected individually. They could be placed in a variety of new matrices and thus extend considerably the possibilities for probing processes at nanometer scales with localized optical electrons.

In the vast majority of experiments published to this day, spatial and spectral selection requirements restrict single molecule investigations to very dilute samples, in which interactions between individual molecules are very unlikely. Interactions between single molecules could be introduced by increasing the concentration and decreasing the size of the sample much below the optical spot size, e.g., with single nanoparticles, or by decreasing the spot size with near-field optical excitation, or by attaching one or more fluorophores covalently to the same chemical structure, which could then be diluted at will. Interacting single molecules would open a broad variety of new experiments. Possible interaction mechanisms are exciton coupling, energy transfer between donor and acceptor, energy level shifts induced by optical excitation or nuclear rearrangements. Various nonlinear optical effects can be expected when two or more molecules are excited at the same time (multi-excitation). These complex interactions have been studied so far in big ensembles, subject to inhomogeneities with a dispersion of parameters. As the example of the antenna complex has shown,[38] it is much easier to unravel the true nature of coupling on a single pair or on a single multichromophoric assembly. The additional handles in interacting systems of single molecules could provide nice new ways to build devices for the quantum storage and treatment of information.

A single fluorescing molecule at the end of a tip in a scanning microscope would be the ultimate scanning near-field optical probe. At a scale of a few nanometers, a molecule is, to a very good approximation, a point dipole, generating the simplest optical near field. This point dipole can not only probe the optical electric field of any scattering or absorbing object with unprecedented resolution, it could also feel the dielectric or optical properties of a sample with the same resolution. A general problem with the design of scanning near-field microscopes is that they rely on the specific optical properties of metals for spatial resolution and for the confinement of light. But metals are not ideal loss-free absorbers in the visible, and their finite skin depth limits the resolution of tips as well as of metal-coated light guides. A single molecule, by contrast, concentrates the whole energy of one photon in a volume smaller than a cubic nanometer when it is excited. This high concentration factor, with virtually no leakage in dielectric environments, makes molecular resolutions possible. Combining high spatial resolution with the high spectral resolution of low-temperature spectroscopy would open many fascinating possibilities for sensitive material analysis at the nanometer scale.

Beyond their use as an analysis tool in nanoscience problems, I think that optical single-molecule techniques are of great potential interest for nanotechnology as well. Nanotechnology aims at building devices or machines from single molecules. Photons are not necessarily convenient for that purpose, and there are many more interactions at the molecular level than those mediated by optical photons, for example electrostatic, contact interactions, electron exchange, etc. Many of these processes can be used to manipulate and assemble molecules, then to let them interact. But optical methods have the advantage that they may act from the macroscopic world with minimal disruption of the system addressed. The optical interface can be used for diagnostics, but also as an input and output channel to feed information or energy into a nanomachine or extract information from it. Single molecules could not only be used as doorways for nanomachines, but also as elements for the treatment and storage of information. We have seen that single molecules interacting with light can often be seen as well-defined two-level systems, and therefore that they could be used as quantum bits. Single molecules can serve as sources of single photons for quantum cryptography, but with longer-lived states, the field of quantum computation would become accessible.

IV. CONCLUSION

In the past few years, optical studies of single molecules have raised great hopes in bioscience, but also in physical chemistry and material science, because they provide unaveraged, microscopic information, with minimal contact or perturbation of the system under study. Most of the applications of single-molecules methods will be at room temperature, because dynamics and interactions of proteins, interface phenomena between solid or liquid substances, etc., all take place at room temperature. Many of these questions can no longer be addressed at low temperatures, when systems are frozen to a standstill. Is this to say that there is hardly any room left for cryogenic experiments? That having fulfilled their part in demonstrating the feasibility of the optical detection of single molecules, cryogenic experiments now belong to history and should fade into the background of nice academic research? I would disagree with this view, for the following reasons.

First of all, the structural information obtained from low-temperature experiments can be highly relevant for processes at room temperature, as long as structure is conserved, i.e., if the cooling is performed under the proper conditions. The structure may even become clearer at low temperature, because movements potentially blurring it are eliminated. This is particularly true when structural changes are very fast, in view of the long acquisition times required by fluorescence.

Then, room temperature experiments on single fluorophores are strongly limited by photobleaching processes. These processes are usually blocked at low temperatures. Then, spectral diffusion becomes the main limitation, but it

may be overcome by broad and fast frequency scanning. In crystals where there is no spectral diffusion, single molecules can be investigated over very long times, whereas these times are limited at room temperature because of oxygen diffusion into the crystal lattice. So far, there has been no systematic study of photobleaching at low temperature, i.e., studies with a wide-band or vibronic excitation such that the signal is insensitive to spectral diffusion. It is very likely that cryogenic temperatures will severely reduce photobleaching, if they do not suppress it altogether. Low temperature spectroscopy would thus be the only way to obtain the long accumulation times and large numbers of photons required in many single-molecule investigations.

Last but not least, fundamental interactions and processes are much easier to understand at low temperature. It could be argued naïvely that all fundamental principles (electromagnetism, quantum mechanics, etc.) being known at atomic scales, single molecules could not teach us anything new. This is of course a strongly reductionist view, which ignores the emerging and cooperative properties appearing at scales larger than molecules. These new phenomena are often difficult to predict, and sometimes even to understand, from first principles. As examples, we may think of all the complex many-body effects displayed by dense electron gases at mesoscopic scales, most of which were observed before being understood or predicted. Behaviors which appear simple at the macroscopic scale surprisingly reveal a rich and complex range of new effects at submicron scales. For example Ohm's law in a disordered semiconductor resolves into strange phenomena when probed at nanoscales by single molecules. Similarly, the broad and boring inhomogeneous optical spectra reveal rich spectral diffusion patterns on closer inspection, when spectral hole-burning or single-molecule spectroscopies are applied. Time and again, we find that Nature's imagination greatly surpasses our own. My bet is that the terra incognita of the nanoworld still holds surprises for many years to come, and that optical studies of single molecules are natural doorways to this new territory.

[1] J. Perrin, Ann. Phys. (Paris) **9**, 133 (1918).
[2] W. E. Moerner and L. Kador, Phys. Rev. Lett. **62**, 2535 (1989).
[3] M. Orrit and J. Bernard, Phys. Rev. Lett. **65**, 2716 (1990).
[4] E. B. Shera, N. K. Seitzinger, L. Davis, R. A. Keller, and S. A. Soper, Chem. Phys. Lett. **174**, 553 (1990).
[5] R. Rigler, J. Widengren, and U. Mets, in *Fluorescence Spectroscopy*, edited by E. Wolfbeis (Springer, Berlin, 1992), p. 13.
[6] E. Betzig and R. J. Chichester, Science **262**, 1422 (1993).
[7] S. Nie, D. T. Chiu, and R. N. Zare, Science **266**, 1018 (1994).
[8] J. K. Trautman and J. J. Macklin, Chem. Phys. **205**, 221 (1996).
[9] T. Funatsu, Y. Hanada, M. Tokunaga, K. Saito, and T. Yanagida, Nature (London) **374**, 555 (1995).
[10] M. Orrit, J. Bernard, R. Brown, and B. Lounis, in *Progress in Optics XXXV*, edited by E. Wolf (Amsterdam Elsevier 1996), p. 61.
[11] *Single Molecule Optical Detection, Imaging and Spectroscopy*, edited by Th. Basché, W. E. Moerner, M. Orrit, and U. P. Wild (VCH, Weinheim, 1997).
[12] T. Plakhotnik, E. A. Donley, and U. P. Wild, Annu. Rev. Phys. Chem. **48**, 181(1997).
[13] W. E. Moerner and M. Orrit, Science **283**, 1670 (1999).
[14] S. Weiss, Science **283**, 1676 (1999).
[15] Ph. Tamarat, A. Maali, B. Lounis, and M. Orrit, J. Phys. Chem. A. **104**, 1 (2000).
[16] R. Kettner, J. Tittel, Th. Basché, and Ch. Bräuchle, J. Phys. Chem. **98**, 6671 (1994).
[17] J. Michaelis, C. Hettich, J. Mlynek, and V. Sandoghdar, Nature (London) **495**, 325 (2000).
[18] W. E. Moerner, Science **277**, 1059 (1997).
[19] J. Friedrich and D. Haarer, Angew. Chem. Int. Ed. Engl. **70**, 113 (1984).
[20] W. P. Ambrose, Th. Basché, and W. E. Moerner, J. Chem. Phys. **95**, 7150 (1991).
[21] J. Bernard, L. Fleury, H. Talon, and M. Orrit, J. Chem. Phys. **98**, 850 (1993).
[22] J. Köhler, J. A. J. M. Disselhorst, M. C. J. M. Donckers, E. J. J. Groenen, J. Schmidt, and W. E. Moerner, Nature (London) **363**, 242 (1993).
[23] J. Wrachtrup, C. von Borczyskowski, J. Bernard, M. Orrit, and R. Brown, Nature (London) **363**, 244 (1993).
[24] A. C. J. Brouwer, E. J. J. Groenen, and J. Schmidt, Phys. Rev. Lett. **80**, 3944 (1998).
[25] J. Wrachtrup, A. Gruber, L. Fleury, and C. von Borczyskowski, Chem. Phys. Lett. **267**, 179 (1997).
[26] U. P. Wild, F. Güttler, M. Pirotta, and A. Renn, Chem. Phys. Lett. **193**, 451 (1992).
[27] M. Orrit, J. Bernard, A. Zumbusch, and R. I. Personov, Chem. Phys. Lett. **196**, 595 (1992); **199**, 408 (1992).
[28] M. Croci, H. J. Müschenborn, F. Güttler, A. Renn, and U. P. Wild, Chem. Phys. Lett. **212**, 71 (1993).
[29] A. Müller, W. Richter, and L. Kador, Chem. Phys. Lett. **241**, 547 (1995).
[30] T. Iwamoto, A. Kurita, and T. Kushida, Chem. Phys. Lett. **284**, 147 (1998).
[31] M. Bauer and L. Kador, posters presented at the Les Houches Spring School, May 2001, and at the International Conference on Hole-burning and Single Molecule Spectroscopie, Taipei, November 2001 (unpublished).
[32] P. Tchénio, A. B. Myers, and W. E. Moerner, J. Phys. Chem. **97**, 2491 (1993).
[33] P. Tchénio, A. B. Myers, and W. E. Moerner, Chem. Phys. Lett. **213**, 325 (1993).
[34] L. Fleury, Ph. Tamarat, B. Lounis, J. Bernard, and M. Orrit, Chem. Phys. Lett. **236**, 87 (1995).
[35] K. Kneipp, Y. Wang, H. Kneipp L. T. Perelman, I. Itzkan, R. R. Dasari, and M. S. Feld, Phys. Rev. Lett. **78**, 1667 (1997).
[36] S. Nie and S. R. Emory, Science **275**, 1102 (1997).
[37] Ph. Tamarat, F. Jelezko, B. Lounis, and M. Orrit, In *Shpol'skii Spectroscopy and Other Site Selective Methods*, edited by C. Gooijer, F. Ariese, and H. Hofstraat (Wiley, New York, 2000).
[38] A. M. van Oijen, M. Ketelaars, J. Köhler, T. J. Aartsma, and J. Schmidt, J. Phys. Chem. B **102**, 9363 (1998); Science **285**, 400 (1999); M. Orrit, *ibid.* **285**, 349 (1999).
[39] Ph. Tamarat, B. Lounis, J. Bernard, M. Orrit, S. Kummer, R. Kettner, S. Mais, and Th. Basché, Phys. Rev. Lett. **75**, 1514 (1995).
[40] B. Lounis, F. Jelezko, and M. Orrit, Phys. Rev. Lett. **78**, 3673 (1997).
[41] Ch. Brunel, B. Lounis, Ph. Tamarat, and M. Orrit, Phys. Rev. Lett. **81**, 2679 (1998).
[42] Ch. Brunel, Ph. Tamarat, B. Lounis, and M. Orrit, Phys. Rev. Lett. **83**, 2722 (1999).
[43] Ch. Brunel, Ph. Tamarat, B. Lounis, and M. Orrit, in *Single Molecule Spectroscopy*, Nobel Conference Lectures, edited by R. Rigler, M. Orrit, and Th. Basché (Springer, Berlin 2001).
[44] B. Lounis and W. E. Moerner, Nature (London) **407**, 491 (2000).
[45] A. Gruber, A. Dräbenstedt, C. Tietz, L. Fleury, J. Wrachtrup, and C. von Borczyskowski, Science **276**, 2012 (1997).
[46] Ch. Kurtsiefer, S. Mayer, P. Zarda, and H. Weinfurter, Phys. Rev. Lett. **85**, 290 (2000).
[47] A. Beveratos, R. Brouri, T. Gacoin, J. P. Poizat, and Ph. Grangier, Phys. Rev. A **64**, 061802 (2001).
[48] E. Knill, R. Laflamme, and G. J. Milburn, Nature (London) **409**, 46 (2001).
[49] R. Brown and M. Orrit, in *Single Molecule Optical Detection, Imaging and Spectroscopy*, edited by Th. Basché, W. E. Moerner, M. Orrit, and U. P. Wild (VCH, Weinheim, 1997), Chap. 1.4.
[50] W. A. Phillips, *Amorphous Solids: Low Temperature Properties* (Springer, Berlin, 1981).
[51] A. Zumbusch, L. Fleury, R. Brown, J. Bernard, and M. Orrit, Phys. Rev. Lett. **70**, 3584 (1993).
[52] A.-M. Boiron, Ph. Tamarat, B. Lounis, R. Brown, and M. Orrit, Chem. Phys. **247**, 119 (1999).
[53] Ph. D. Reilly and J. L. Skinner, J. Chem. Phys. **102**, 1540 (1995).

[54] F. Kulzer, S. Kummer, R. Matzke, C. Bräuchle, and Th. Basché, Nature (London) **387**, 688 (1997).
[55] F. Kulzer, R. Matzke, C. Bräuchle, and Th. Basché, J. Phys. Chem. A **103**, 2408 (1999).
[56] P. Bordat and R. Brown, Chem. Phys. Lett. **331**, 439 (2000).
[57] P. J. Walla, F. Jelezko, Ph. Tamarat, and M. Orrit, Chem. Phys. **233**, 117 (1998).
[58] L. A. Deschenes and D. A. Vanden Bout, Science **292**, 255 (2001).
[59] H. P. Lu and X. S. Xie, J. Phys. Chem. B **101**, 2753 (1997).
[60] J.-M. Caruge and M. Orrit, Phys. Rev. B **64**, 205202 (2001).
[61] J. H. Schön, Ch. Kloc, H. Y. Hwang, and B. Batlogg, Science **292**, 252 (2001). Although doubt was recently cast on the validity of the experimental results in this paper, molecular materials as ''tunable'' semiconductors do present unique features, which warrant further work, particularly at the local level of single molecules.
[62] A.-M. Boiron, B. Lounis, and M. Orrit, J. Chem. Phys. **105**, 3969 (1996).
[63] J. Michaelis, C. Hettich, A. Zayats, B. Eiermann, J. Mlynek, and V. Sandoghdar, Opt. Lett. **24**, 581 (1999).
[64] M. A. Bopp, A. Sytnik, T. D. Howard, R. J. Cogdell, and R. M. Hochstrasser, Proc. Natl. Acad. Sci. U.S.A. **96**, 11271 (1999).
[65] E. Donley and T. Plakhotnik, Single Mol. **2** 23 (2001).