chains, many introduced species become involved in more complex natural food webs, with species interactions more reminiscent of chess than tic-tac-toe.

The touchstone of every biological control project should be an extremely narrow host range for any species considered for introduction. The questions asked at the outset should include: Is the target organism really a substantial public problem? What are the alternatives to introduction of an alien species for controlling the pest? What kinds of mischief, both direct and indirect, could the proposed introductions cause? What is the balance of benefits and costs, ecological as well as economic, from the introduction (5)? Biological control is an important arrow in the quiver of pest management, perhaps the only arrow in some cases of pests of grave environmental concern. However, willy nilly biological control without regard for environmental costs could result in societal resistance so great as to prevent even the sensible application of biological control. The troubling case of *R. conicus* in North America highlights the need for ecology to be at the heart of both science and application of biological control.

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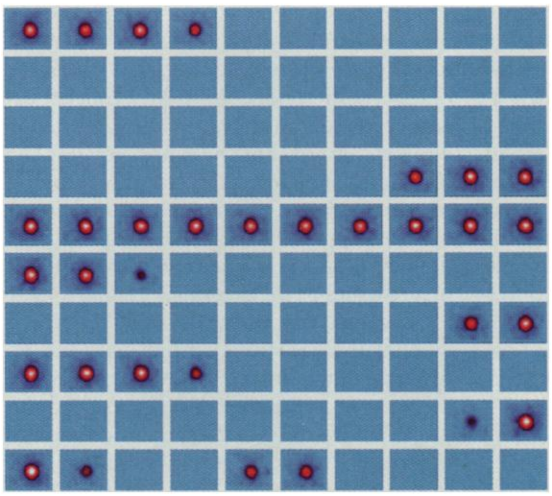
POLYMER LUMINESCENCE

Those Blinking Single Molecules

W. E. Moerner

A dramatic revolution has occurred in the study of matter on a nanometer scale. In the past few years, a variety of optical experiments have reached the regime of detecting and measuring the properties of an individual molecule in a condensed matter sample (*1*). To observe only one molecule, researchers typically use extremely small sample volumes (<1 μm^3), work at extremely low concentrations (<10^{-9} mol/liter), and rigorously optimize the collection of the single-molecule emission while rejecting background signals. This field of study can appropriately be called "single-molecule nanophotonics" because exactly one molecule is optically pumped at a time, providing an exquisitely sensitive probe of the structure and dynamics of the molecule itself and of the immediate local environment. As they report on page 1074 of this issue, Vanden Bout *et al.* (*2*) have used such methods to probe a new system: a single conjugated polymer molecule.

Why is it useful to study individual molecules, each of which may be marching to a different drummer? Indeed, single-molecule measurements completely remove the normal ensemble averaging that occurs when a large number of molecules are probed at the same time. It is important to remember that workers in this field never measure only one single molecule, but many single molecules, one by one. This process allows direct observation of the true distribution of behavior, rather than just the average (mean) value of an experimental parameter. To illustrate by analogy, consider the problem of characterizing the houses in which people live: One could judge the quality of housing by noting the average square footage, the average number of rooms, and so on, or one could observe in detail many houses, one by one, and build up a comprehensive distribution of features (*3*). Clearly, the distribution contains more information than the average alone.



A sequence of 100 images of the emission from a single green fluorescent protein mutant molecule trapped in a gel. Each frame corresponds to a 100-ms exposure with a 488-nm–wavelength laser in a total–internal-reflection geometry. [Figure courtesy of R. M. Dickson; see (*6*) for details]

Surprisingly, almost all single-molecule systems studied to date have shown some form of fluctuating, flickering, or stochastic behavior. For example, in the initial work on single molecules of the aromatic hydrocarbon pentacene in a *p*-terphenyl crystal at liquid-helium temperatures (*4*), spectral shifts of the resonance frequency were observed, attributable to phonon-driven fluctuations in the local environment. This behavior has been termed "spectral diffusion," and it has also been observed for single molecules in polymers at low temperatures (*1*) and for single molecules on surfaces at room temperature (*5*). If a fixed-frequency laser is used to probe a spectrally diffusing molecule, the shifting of the absorption in and out of resonance generates amplitude

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fluctuations in the detected emission signal, which can be analyzed to gain knowledge about the underlying physical mechanism. In the work of Vanden Bout *et al.*, additional flickering effects (fluorescence amplitude jumps) have been observed for single conjugated polymer molecules (2), a recent entry into the single-molecule arena. In a similar vein, blinking and switching effects have also been reported for single molecules of a novel fluorescent protein (6).

As an example of what "blinking" means, the figure shows a sequence of 100 microscopic images of the emission from a single protein molecule. Its emission blinks on and off even though the pumping laser beam is continuously present. Such fluctuations are now becoming near-universal features of the single-molecule regime, and they provide unprecedented insight into behavior that is normally hidden by the usual ensemble averaging.

Conjugated polymers are a relatively new class of semiconducting materials that combine the electronic and optical properties of semiconductors and the processability of conventional polymers (7). The conjugation results from overlap of the π-electron orbitals along the chain of the polymer. In the work of Vanden Bout *et al.* (2), a derivatized poly(*p*-phenylene vinylene) (PPV)–poly(*p*-pyridylene vinylene) (PPyV) copolymer was chosen, because the photoluminescence and electroluminescence of this and related conjugated polymers show particular promise for light-emitting device (LED) and laser applications. In such systems, one of the more important elementary excitations is an exciton (bound electron-hole pair), which is typically delocalized over a few monomer units and produces an emitted photon when recombination occurs. Such single excitons are known to migrate in semiconductor materials until a recombination center is reached, and several years ago, these single luminescent entities were first observed in a GaAs/AlGaAs quantum-well material with a sophisticated scanning near-field optical microscopy technique at liquid-helium temperatures (8).

For the work on the PPV-PPyV copolymer at room temperature, a scanning confocal microscope allowed observation of localized emission spots arising from photogenerated excitons (2). The occurrence of localized spots showed that strong communication occurs along the polymer chain. For each bright spot, a complex flickering (fluctuations in the fluorescence intensity), which was different from spot to spot, was observed, along with eventual photobleaching. Interestingly, by using a clever two-wavelength pumping technique, the workers were able to prove that the flickering was not the result of spectral diffusion effects but rather the generation of a quenching defect at some location along the polymer chain. This conclusion should have important ramifications in the development of these materials for LED and sensor applications. In particular, it will be most important to reduce the concentration of quenching defects in order to maintain high-efficiency light emission for future LED applications.

A key feature of these studies on both single conjugated polymer molecules and on single fluorescent proteins is the observation of novel mechanisms for flickering, blinking, and switching in single quantum emitters. Such blinking and flickering effects would be almost unobservable and certainly misinterpreted in studies on large ensembles, because the various emitters contributing to the overall sum are generally uncorrelated: The mean value of the total emission would simply be smaller. These observations are clear examples of the power of single-molecule spectroscopy and microscopy in opening up a frontier of previously hidden physical effects.

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COMPUTATIONAL BIOLOGY

Biological Information Processing: Bits of Progress

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Are there principles of information processing common to all biological systems, whether simple or complex, fast or slow? A recent conference (1) that brought together researchers from a wide range of disciplines hinted that there are. Several key principles of biological information processing emerged, and new computational methods were presented for analyzing complex biological systems. There were two prominent themes of the meeting.

The first was the many ways in which biochemical reactions within cells can be used for computation. A variety of biological processes—concatenations of chemical amplifiers and switches—can perform computations such as exponentiation, differentiation, and integration. These computational cascades include metabolic processes as diverse as gene transcription, cell cycle timing, and decoding oscillations of second messengers (2). Enzymatic amplification, regulatory

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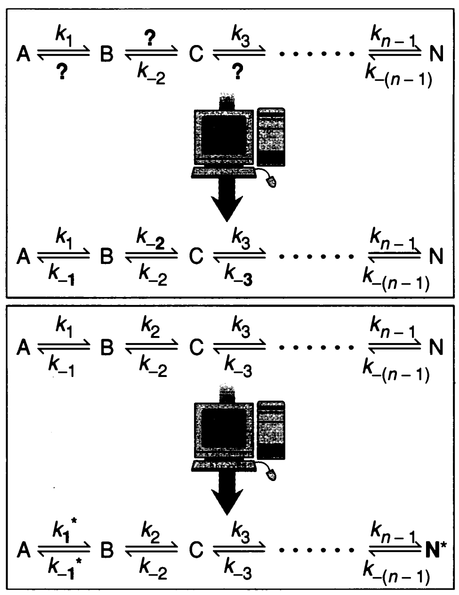

Fig. 1. Computing by chemical switches. (**Top**) Simulation of signaling or metabolic cascades allows assignment of unidentified rate constants for specific steps in the pathway generating the product N. (**Bottom**) Optimizing the cascade to achieve a different outcome (N*), which mimics an evolutionary process, identifies critical rate constants that change and focuses further investigation on specific components.