

properties in the Weddell Sea were, however, different before and after the occurrence of the polynya (7). Explanations concerning its formation and maintenance are mostly based on meteorological and oceanographic arguments and on numerical experiments with coupled sea ice-ocean models.

The location of the Weddell Polynya in the mid-1970s and areas of low ice concentrations in later years suggest a strong influence of Maud Rise, a topographical feature on the sea floor (seamount) that modifies ocean currents and tides. In Holland's model (3), the seamount sheds cyclonic eddies when the ocean current from the northwest interacts with the seamount. Cyclonic eddies at the northeast flank of Maud Rise then apply a divergent stress to

the sea ice, increasing the amount of open water within the pack ice despite a deeper thermocline (8). In contrast, convergent motion in an anticyclonic eddy southwest of Maud Rise provides a closed ice cover despite the shallow thermocline.

Modeling studies have also shown that tidal motion modified by bottom topography may reduce sea ice concentration substantially (9). These high-resolution ocean models can identify structures in the coupled ice-ocean fields that are difficult to observe directly. They thereby help to advance our understanding of polynya-forming processes. But despite this new information, it remains a mystery why the large Weddell Polynya appeared during three consecutive winters in the 1970s but has never appeared since.

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PERSPECTIVES: CHEMICAL PHYSICS

Single-Molecule Spectroscopy Comes of Age

Anne Myers Kelley, Xavier Michalet, Shimon Weiss

In the summer of 1999, researchers working in the field of single-molecule biophysics gathered for a 1-week workshop in the French town of Tours (1). Sensing the birth of a new discipline, the participants termed the workshop "the Woodstock of single molecules," but their enthusiasm was mainly about future promise: It was clear that a considerable amount of work would be required to extend and validate methodologies originally developed for solids at cryogenic temperatures (2) to materials and biological systems (3).

In April of this year, a symposium at the American Chemical Society Meeting in San Diego (4) demonstrated that single-molecule spectroscopy is beginning to deliver on its promise. Chemists, physicists, and biologists gathered for nearly 60 talks on topics as diverse as low-temperature dynamics of single-dye molecules embedded in crystals (M. Orrit, Bordeaux I University), optical tracking of the entry of individual viruses into live cells (C. Bräuchle, Ludwig-Maximilians-University, Munich), single-photon light sources from single molecules (W. E. Moerner, Stanford University) (5), polymer conformations and dynamics (P. Barbara, University of Texas, Austin; R. Dickson, Georgia Tech) (6–8) and the mechanisms of single enzy-

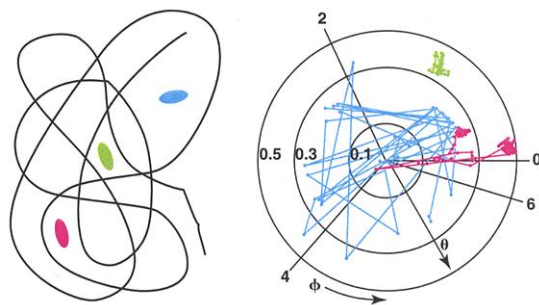
matic motors (K. Thorn, University of California, San Francisco; K. Kinoshita Jr., Keio University; T. Yanagida, Osaka University) (9, 10). The broad range of topics illustrates the power of optical approaches for studying single molecules or molecular assemblies. Very few talks emphasized methodologies but rather concentrated on the new scientific knowledge being gained, demonstrating that single-molecule spectroscopy is reaching maturity (11).

One reason for the burgeoning interest in single-molecule optical techniques is that photons may be the least perturbing probe of the state of a molecule (although in dif-

ferent power regimes, lasers can be used to exert a substantial force on atoms, molecules, and beads). Different spectroscopic signatures can be used, of which fluorescence is the most popular because of its inherent sensitivity. Fluorescence studies can be performed on biomolecules that have intrinsic fluorophores, such as photosynthetic light-harvesting complexes (J. Schmidt, Leiden University) or enzymes containing flavin cofactors (S. Xie, Harvard University). More often, however, a fluorescent moiety is attached to the species under study by genetic engineering such as tagging with green fluorescent protein (Thorn; T. Schmidt, Leiden University), by targeted chemical functionalization as in site-specific protein labeling (P. Lu, Pacific Northwest National Laboratory), or by chemical modification of DNA or RNA bases (S. Chu, Stanford; M. Ishikawa, Joint Research Center for Atom Technology, Tsukuba).

Another powerful spectroscopic tool at the single-molecule level is Raman (T. Basché, University of Mainz) or surface-enhanced Raman scattering (M. Käll, Chalmers University of Technology; S. Nie, Indiana University; L. Brus, Columbia University). Raman spectra contain more detailed information about molecular structure than fluorescence spectra and can, for the most active surface-enhanced sites, provide photon count rates competitive with fluorescence (K. Kneipp, Massachusetts Institute of Technology).

The most fundamental reason for the excitement about single-molecule spectroscopy is that ensemble studies give only an average picture of the properties exhibited by individual molecules that are not identical.



Single-molecule dynamics in polymers. Orientational trajectories of three dye molecules embedded in poly(methyl methacrylate) are probed with wide-field single-molecule optical microscopy (6). The different orientational dynamics of the different molecules illustrate the heterogeneity observed within polymer hosts even 80°C below the glass transition temperature. The cartoon shows how differences in local free volume might give rise to these differences in rotational mobility.

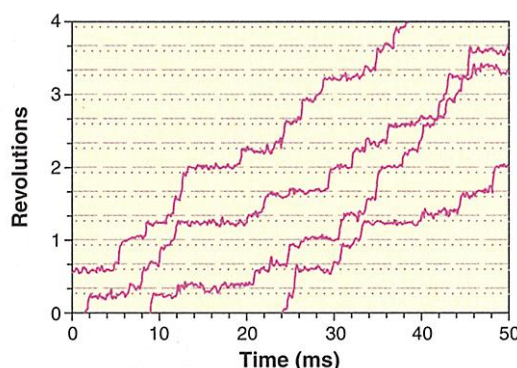
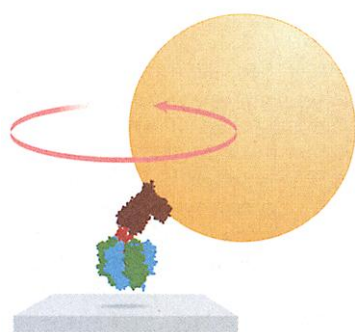
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In some cases, the heterogeneity arises from definable chemical differences such as the extent of glycosylation, as seen in the intermolecular variations in catalytic rates in mammalian alkaline phosphatase (N. Dovichi, University of Washington). But intermolecular variations often persist even when there are no chemical differences. This seems to fly in the face of the ergodic theorem, which states that a measurement of some property of an ensemble at a given time should be equivalent to the long-time average of the same property on any one member.

The key is the time scale over which the averaging is performed. Single-molecule measurements are interesting only for systems in which different molecules retain distinguishably different behavior on time scales longer than that necessary (from signal-to-noise ratio considerations) to make a measurement. It turns out that a great majority of biochemical and material systems fall in this category. Single-

versity of California, Santa Barbara), and light-emitting organic polymers (Barbara) (7). The emission spectra and diffusion constants of single embedded dyes are valuable probes of the highly heterogeneous local environments in polymer or glass matrices (Deschenes; Dickson; D. Higgins, Kansas State University) (6, 8, 13) (see the first figure).

Time-resolved measurements on single molecules also provide powerful insights into the rates and mechanisms of kinetic processes that are fundamentally stochastic: Even if all members of the ensemble could be synchronized to begin at the same time, they would rapidly run out of phase with one another, obscuring the kinetic steps. Beautiful illustrations of single-molecule dynamical studies of this type were provided by several speakers (Yanagida, Chu, Xie, and Kinosita). Kinosita, for instance, illustrated the power of a simple dark-field laser illumination to monitor the stepwise submillisecond dynamics of the F_1 -adenosine triphosphate



Single-molecule dynamics in biomolecules. When isolated, the F_1 part of ATP synthase hydrolyzes ATP, leading to rotation of its shaftlike γ subunit within an $\alpha\beta$ -trimer. The system has a threefold rotational symmetry, and the γ subunit is therefore expected to rotate by discrete 120° steps. Because of the stochastic nature of the motion, single-molecule techniques are required to resolve these individual steps. Yasuda *et al.* (10) attached a fluorescent molecule or gold bead to individual protein molecules and imaged them with a laser dark-field microscope and an ultrafast camera, allowing the 120° jumps of individual molecules to be observed on the submillisecond time scale. At low ATP concentrations, each 120° step can be resolved into substeps of 90° and 30° , which correspond to ATP binding and the release of the hydrolysis products, respectively (10).

molecule studies often reveal temporal fluctuations of the recorded quantity ("dynamic disorder"), which provide insight into fluctuation time scales hidden in bulk measurements. There may also be large variations from molecule to molecule ("static disorder"). The distinction between dynamic and static disorder depends on the time resolution of the experiment, and intermediate situations may arise.

Studies of single embedded molecules are providing a wealth of information on the local properties of complex materials such as biological and biomimetic membranes (G. Harms, Pacific Northwest National Laboratories; R. Dunn, University of Kansas) (12), inorganic semiconductors (S. Buratto, Uni-

(ATP) synthase rotary motor (10) (see the second figure).

Several papers indicated that multiparameter studies (measuring, for example, fluorescence intensity, lifetime, polarization anisotropy, and spectral peak position at the same time) are needed to develop a complete picture of the molecular state and environment of single molecules [Harms; C. Seidel, Max Planck Institute, Göttingen; M. Sauer, University of Heidelberg; C. Hübner, Eidgenössische Technische Hochschule (ETH)] (14–16). The finer characterization gained by multiparameter observation allows variations resulting from multiple states of a single molecule to be distinguished more easily from varia-

tions arising from multiple molecular species. This allows distinct species to be identified with a reduced number of detected photons, increasing the effective time resolution of single-molecule identification schemes (Sauer; Hübner).

Several speakers described optical configurations that enable simultaneous time- and wavelength-resolved measurements, which will probably become standard in future studies. The additional wealth of data will, in turn, call for new data reduction schemes. Hidden-Markov models (D. Talaga, Rutgers University), multidimensional correlation functions (J. Cao, Massachusetts Institute of Technology), and other techniques will have to be developed to go beyond mere comparison of bulk average values to single-molecule histograms (17). These and other methods will have to be imported from research fields accustomed to dealing with large, complex data sets. In addition, more elaborate physical models will be needed to interpret the increasingly detailed data that will emerge from future experiments (E. Geva, University of Michigan; T. Plakhotnik, ETH; P. Wolynes, University of California, San Diego).

Single-molecule optical studies have clearly come of age. These techniques are being applied to an increasing number of problems in chemistry, physics, and biology. In combination with single-molecule manipulation, microfluidics, and microelectromechanical systems, they will open up ever more possibilities for new discoveries.

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