

Nanostructure science and technology: Impact and prospects for biology

H. G. Craighead^{a)}

School of Applied and Engineering Physics and The Nanobiotechnology Center, Cornell University, Ithaca, New York 14853

(Received 9 June 2003; accepted 17 June 2003; published 2 September 2003)

Life processes function by the activity of complex interacting molecular systems. Advances in nanostructure science and technology are providing powerful tools for investigating those systems. Developments such as atomic force microscopy, luminescent quantum dots, and nanofabricated probes have provided new capabilities for molecular and cell biology. The function of biomolecules, cellular components, and organelles and the interaction of living cells with their environment can now be explored in exquisite detail with nanoscale probes and devices. Materials and devices engineered at the nanometer scale are being developed and employed in biochemical analysis, medical diagnostics, and therapeutic devices. With the ability to understand, manipulate, and harness enzymes, receptors, and other biomolecules, new types of biosensors are emerging and new research approaches to biological systems are becoming possible. © 2003 American Vacuum Society. [DOI: 10.1116/1.1600444]

I. INTRODUCTION

Biological systems operate, at the nanoscale, with functional molecular systems combining to create the complex functions of macroscopic living organisms. Microscopy has already provided revolutionary advances in understanding the microstructure and function of living systems, but largely at micron size scales *in vivo* or submicron in vacuum. With the potential for *in vivo* measurement of nanostructures, nanoscience and technology could provide the methods leading to a revolution in understanding biological systems and new forms of biotechnology. New understanding of life processes at the fundamental subcellular and molecular level will have a profound impact on medicine and on understanding of ecological interactions. It could also increase our abilities to utilize biological systems for energy and materials processing. The IWGN Interagency Workshop report on Nanotechnology Research Directions,¹ for example, notes biology and medicine as significant areas of opportunity for nanoscale research. The capability to manipulate and use biological molecules could lead to new hybrid devices utilizing active molecules integrated with electronic, optical, or magnetic devices. This organic-inorganic interface is an area of active interdisciplinary research bringing together biologists, physical scientists, and engineers.^{2,3} In this article we look at some of the ongoing research activities utilizing nanoscale technology to address biological systems.

The American Vacuum Society, or now more correctly, AVS, has been a leading society in fostering interdisciplinary areas of science and technology. It has been effective over the years in bringing together scientists and engineers who have helped form the scientific understanding and the technological capabilities behind developments in microelectronics, data storage, display technology, and optical communications. It has a history in technology and research tool development. I expect I am not the only person involved in

biologically related research who, when announcing he was headed to the "Vacuum Society Meeting," has received a smirking question like, "what sort of biology exists in vacuum?" It may not be obvious to some that the AVS has been and remains a leading society in connecting those individuals developing new tools with those individuals exploring a diverse set of research and technology applications. The AVS has evolved with technology and emerging scientific applications, developing new divisions in the Biomaterials Interface and Nanometer-Scale Science and Technology. The research instrumentation, materials processing, and surface analysis techniques, fostered by the AVS, have been brought to bear effectively on biology at the nanoscale. I do not attempt in this article to present a comprehensive review of this rapidly growing and diverse field. Entirely new journals are emerging in nanobiotechnology. I only attempt to show a few examples that are utilizing the emerging capabilities in nanostructure science and technology on biological science and applications.

II. PROBES OF BIOMOLECULAR ACTIVITY

Development of imaging methods has revolutionized the understanding of biological systems.⁴ The advent of optical microscopy allowed imaging of individual cells and provided a breakthrough in probing the basic units of complex organisms. Electron microscopy was the initial vehicle for direct imaging nanometer-scale features of biological systems.⁵ Transmission electron microscopy and scanning electron microscopy remain essential tools in imaging subcellular components at resolution much better than diffraction-limited light optics. The necessity of operating electron beam instruments in a vacuum limits the types of samples that can be imaged, and specimens are not usually in a natural state. New types of nanoscale probes are being developed to image and quantify chemical activity in more natural sample.

Atomic force microscopy (AFM) is one of the more recently developed technologies that has driven the growing

^{a)}Electronic mail: hgc1@cornell.edu



FIG. 1. AFM image of stalled elongation complexes of RNAP III with template T377. Newly created RNA strands are indicated by arrow and the long branch is the downstream (already read) DNA. Orientation of the transcript and the position of the second RNA provide insights into the RNA transcription process. Reprinted with permission from Rivetti *et al.* (Ref. 9).

interest in nanoscale phenomena. It has been a powerful new tool for biological systems, addressing molecular-scale imaging and force measurement. The near-field scanning optical microscope, scanning electrochemical probe and, of course, the scanning tunneling microscope are among the related scanning probes. Each has been employed in analysis of biological systems. Because it has the capability to probe molecular features in an aqueous environment, the AFM has been particularly useful for imaging features on cell surfaces and biomolecules in a natural state. Reviews of AFM methods for probing biological systems are presented, for example, by Hansma and Kumar.^{6,7} Nanoscale biological processes such as receptor-ligand binding, the activity of membrane proteins, and vesicular transport in cells have been studied by AFM. The use of carbon nanotubes as well-characterized and ultrafine AFM tips has advanced resolution for imaging the structure of individual protein molecules.⁸ Imaging of deoxyribose nucleic acid (DNA) and ribonucleic acid (RNA) polymerase function has provided insights into genetic activity. Rivetti *et al.*⁹ have imaged individual RNA polymerase molecules and imaged the stalled position of the RNA polymerase activity at specific sites on a DNA strand. Figure 1 from Rivetti *et al.*⁹ shows this transcription process in action. AFM images of individual regulator protein binding on DNA molecules also provides insights into gene expression at the molecular level.¹⁰ By observing the location and confirmation of the DNA in these cases, direct information about transcription and regulation can be obtained. This information could previously be obtained only by indirect biochemical approaches.

In addition to the power of direct imaging of biomolecular activity, scanned probes can be used as active elements to interrogate the forces responsible for biopolymer conformations. A chemically functionalized AFM tip can be bonded to a single molecule on a surface. By retracting the tip and measuring the resulting forces as the molecule unfolds, information can be extracted about the energetics and mechanics of the conformational changes. This type of force-distance measurement has also been applied by Bustamante *et al.* to the mechanical properties of DNA and the forces responsible for its coiling and the conformations.¹¹ A review of force spectroscopy using probe devices has been given by Gimzewski and Joachim. Figure 2 shows an example of

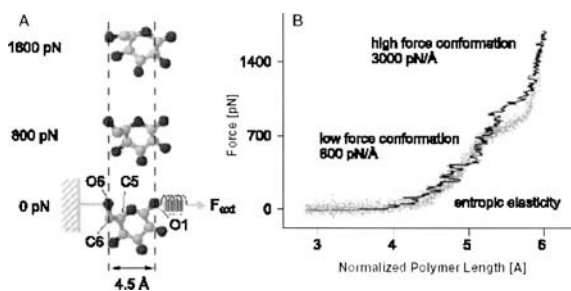


FIG. 2. Images on the left (A) result from molecular dynamics calculations of the dextran as a result of varying extension forces. Dark curve B is the measured force vs. elongation of a five-glucose unit of dextran extended in water and the gray curve is the superposition of 10 normalized curves measured on native dextran. Reprinted with permission from Ref. 12.

results from this review, using an AFM to observe bond twists in single molecules of dextran.¹² This approach has also been used to measure rupture forces of antigen-antibody and receptor-ligand binding, which are fundamental and important biological molecular interactions.

Forces on individual molecules have also been applied by fields and flow in reduced dimension structures. Chu *et al.* observed the elongation and confirmation of fluorescently labeled DNA molecules in fluid flow.¹³ As with the AFM-based force application this type of work was important for movement toward consideration of molecules as entities that can be manipulated, evaluated, and characterized as individual mechanical elements. Molecules can also be manipulated by application of electric fields and by the application of mechanical forces derived from moving a molecule relative to a mechanical nanostructure. Of course, naturally occurring nanoporous media have long been used for molecular filtration. Engineering pores at the molecular scale enables their easier incorporation in device structures and their exploitation in devices that can sort, separate and analyze molecules based on size or mechanical properties. Technologies derived from studies by researchers have yielded lithographic approaches and etching processes that can be controlled to a few nanometers. These have been used to create structures which can be used for the application of controlled entropic forces on individual charged molecules in solution.¹⁴⁻¹⁸ Nanolithography and thin film processing can be exploited in nanofluidic systems such as nanoporous arrays for which time dependent electric fields can be used to controllably elongate biopolymers and gate their entry into an isotropically restricted region. The example of such an entropic recoil device is shown schematically in Fig. 3 along with fluorescent optical micrographs of elongated DNA molecules at an entropic interface. This is an example of a molecular separation device based on time- and space-dependent fields.

III. NANOSCIENCE OF BIOLOGICAL SYSTEMS

Nanostructures formed by lithography and etching have also been used to control molecular access to cells in implanted medical devices. Desai *et al.*¹⁹ have encapsulated insulin producing cells in protective silicon capsules with 18

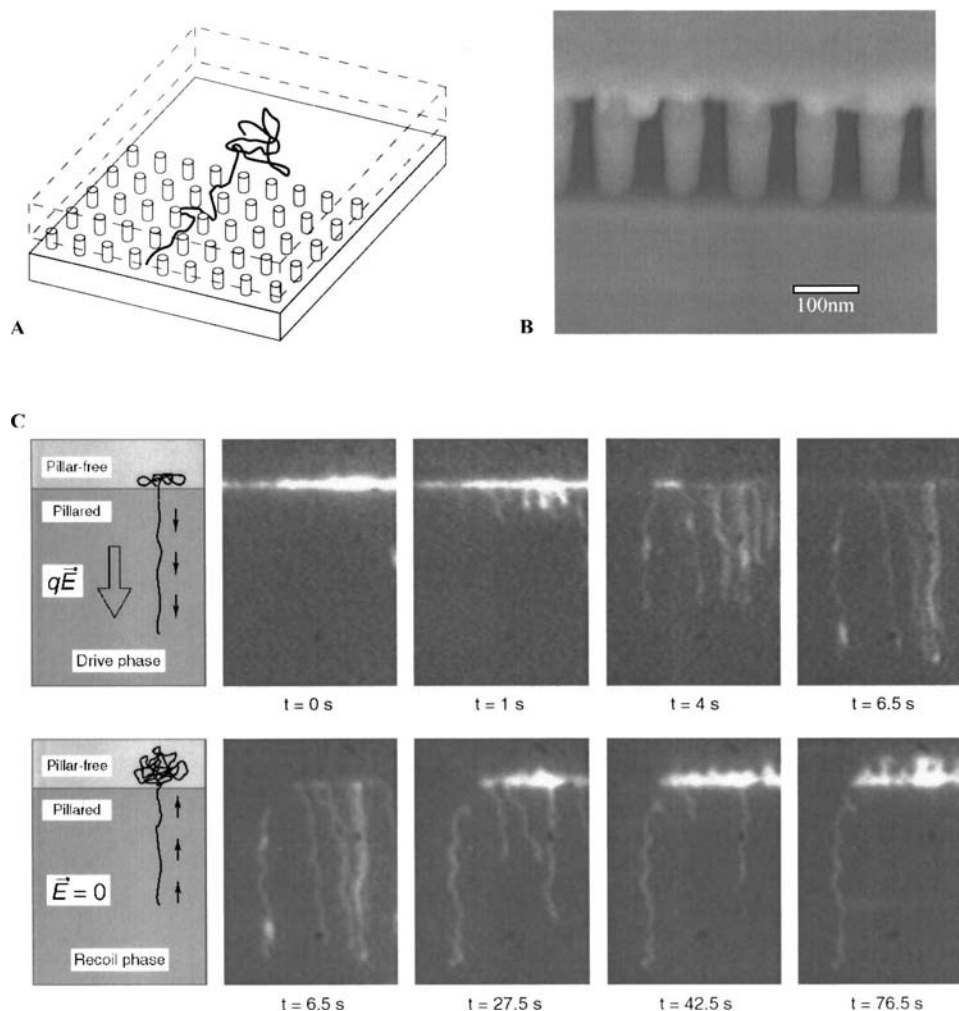


FIG. 3. (a) Schematic of a fluidic nanostructure, with features smaller than the molecular diameter, which presents an entropic barrier to motion. (b) Electron micrograph of an etched entropic barrier device. (c) Fluorescent optical micrographs of DNA being driven into the nanostructured region and the recoil from the nanostructured region during the recoil phase. From Turner *et al.* (Refs. 16–18).

nm holes that allow the insulin to flow out and nutrients to reach the cell. The nanoporous capsule prevents the attack on the protected cells by antibodies of the host immune system that would destroy the implanted cells. The hope is to use such a device as a treatment for insulin-dependent diabetes, using the nanostructures as long-term implantable therapeutic devices. The area of nanostructured devices and surfaces is of relevance to a range of medical implants and prosthetic devices. A variety of etching approaches, for example, have been explored to explore cell response to surface structure feature sizes.²⁰ This area of research is benefited from the surface modification and characterization processes that have been developed and perfected over the last 50 years by those in the AVS community.

Molecules that form active pores in cell membranes have been an area of biophysical research. In addition to microscopic imaging to elucidate the structure and location of the pores, robust pores have been incorporated in artificial systems where they can be studied and used as controllable gates for ion passage. Alpha-haemolysin, for example, is a bacterial toxin that self-assembles on lipid bilayers to form a ~ 10 nm diam controllable molecular pore. Gu *et al.*²¹ demonstrated that reversible blocking of the ionic current through a single transmembrane pore could be done by bind-

ing cyclodextrin molecules to the molecular pore (Fig. 4). The transient binding of a single molecule therefore results in a significant and measurable change in ion current through the pore. This presents the possibility of single molecule detection that could be adapted to a range of molecules of biological interest.²²

In the case of the membrane pore, the ion channel was electrically isolated so the effect of modulation of the ionic transport properties could be detected by changes in electrical conduction with high signal to noise ratio. In other recent work a single active enzyme, in this case DNA polymerase, was optically isolated in a channel (or pore) in metal film that acted as a nonpropagating (zero mode) optical waveguide. The optical isolation was utilized to detect single base binding events during DNA polymerization.²³ This nanometer-scale device for ultrasubwavelength optical confinement is shown schematically in Fig. 5. The use of an artificial nanostructure, in this case a metallic nanopore, is one of a class of engineered nanostructures that can be utilized to enhance the detection sensitivity of single molecule events and to observe fundamental biochemical processes and discrete events. Lawrence and Weiss²⁴ note optical approaches to direct real-time observation of molecular-scale events to include, in addition to the zero mode waveguide,

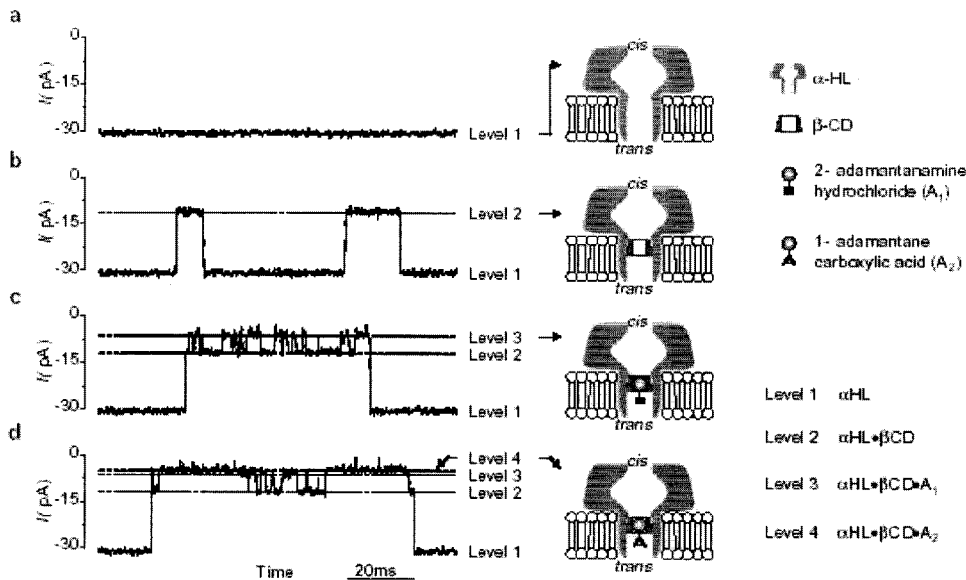


FIG. 4. Recording of ion current through a single α -haemolysin (α -HL) membrane pore with β -cyclodextrin (β -CD) and other molecules as noted in the diagrams at the right blocking the pore. Curve a is from an unblocked, continuously open pore, with the other curves showing transient blockages. Reprinted with permission from Gu *et al.* (Ref. 21).

the restricted evanescent fields associated with the near-field scanning optical microscope and the physical restriction of molecules in optically accessible nanofluidic channels. Xie and Lu provided a “minireview” of single-molecule enzymology as a class of studies that reveal activity of chemical kinetics obscured in the conventional measurements of ensemble averages.²⁵ I think it is clear that engineered nanostructures combined with highly functional biomolecules present a fruitful direction for research as well as an opportunity for new class of functional hybrid devices.

The study of molecular motors has been an area of research in molecular biology. These molecular machines are responsible for generating motion in the body—from macroscopic muscle contraction to motion of cellular components. The understanding of the magnificent molecular machinery

of motion has been advanced by using scanning probes and fluorescent nanoscale elements to detect and quantify the motion. A wide class of motor molecules, such as the linear motors of actomyosin to rotary enzymes such as ATPase, have benefited by nanoscale manipulation and sensing.^{26,27} The forces and distances involved in these molecular processes utilize and challenge the use of nanoscale probes such as ultrafine needles and optical traps to measure piconewton forces and nanometer displacements.^{28,29} Consideration has also been given to utilizing molecular motors as sources of motion in ultrasmall engineered devices. There has been interesting work on organizing molecular motors to move a nanoparticle or small structure in prescribed manner such as along a track of patterned molecules,³⁰ or positioned on and connected to nanofabricated structures.³¹ It is fascinating to consider and even see these small forces coordinated to perform a function. In the long term it is possible these approaches could be utilized for mechanical device function, such as actuation of nanomechanical systems. Much to the chagrin of serious researchers, research that involves mechanical motion is sometimes confused with the science fiction version of so-called nanotechnology that evokes the existence of unrealistically capable nanobots, usually bent on the destruction of humans.³²

Perhaps the simplest nanoscale structure is a small particle or quantum dot. Studies of small metal particles or colloids have been ongoing for decades. Light scattering by nanometer scale small particles was considered by Mie roughly a century ago.³³ These small objects are of interest because the optical, magnetic, electronic, mechanical, or chemical properties are different from the bulk and the properties can often be modified by controlling the size or shape. A variety of beads and particles have been used to label or manipulate molecules. Magnetic beads, chemically linked to a molecule of interest, can be used to move or sort the molecules by application of magnetic fields.³⁴ Metal particles can be used to enhance the electron scattering for observa-

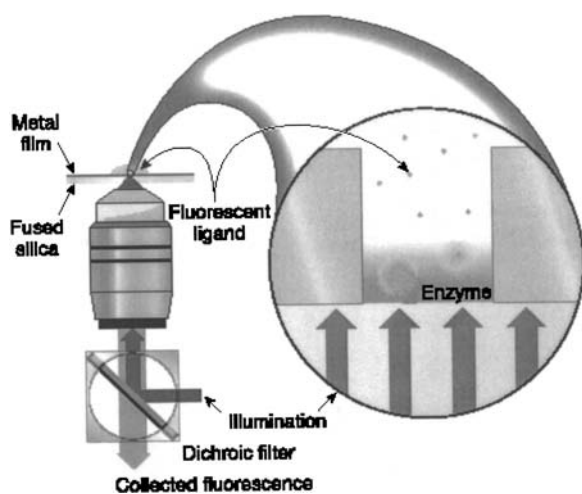


FIG. 5. Schematic of a metallic “nanopore” or zero mode waveguide containing an isolated DNA polymerase enzyme. The engineered nanostructure isolates the fluorescent excitation to nanometer-scale volume surrounding the enzyme. Transient fluorescent bursts represent incorporation events of fluorescently labeled individual bases. Reprinted with permission from Ref. 23.

tion by electron microscopy or to enhance the optical scattering or absorption for optical detection or analysis. Metal particles can also be functionalized for binding specific compounds and detected by a variety of techniques including optical changes or modifications of electrical conductivity.³⁵ A great deal of recent interest has been directed to semiconductor particles that have efficient photoluminescence.^{36–38} This work has been advanced by the larger body of work on compound semiconductors, the growth and study of which has been at the center of much ongoing research of the AVS. The fluorescent semiconductor quantum dots or nanocrystals, combined with surface chemical functionality, can be used to label specific chemicals or cellular components often in viable cells. A review article on fluorescent semiconductor nanocrystals and their application to biological labeling is given by Michalet *et al.*³⁹ Fluorescent microscopy with organic dyes is a very powerful technique that has been used in biology for some time. Dyes combined with specific chemical functionality have been used to identify and localize specific chemicals. The advantages of the semiconductor particles include their resistance to fluorescent bleaching that is inherent in conventional dyes. They can also be functionalized with surface compounds to provide chemical specificity to the labels. In addition to the chemical composition of the particles the fluorescent wavelength of the particles can be controlled by quantum confinement. Such quantum dot systems are becoming more available and utilized in cellular and biochemical analysis. A variety of types of nanocrystals are becoming commercially available.

IV. CONCLUSION

Fundamental studies of biology drive experiments at the molecular and subcellular length scales. For the purposes of focusing on one aspect of the emerging impact of biologically related studies I have tried to concentrate on items that relate to designed structures and devices at the smallest dimensions. Bio-inspired approaches may have impact in new materials, which is the topic for another review. In this category are macromolecular assemblies that are being constructed with growing complexity and size.⁴⁰ I have not addressed a large body of important research involving miniaturized biological devices or microtechnology. A great deal of important work and applications exist at dimensions larger than nanometers. In fact, many of the important applications of bio-related technology are likely to be with miniaturized systems with features much larger than nanometers. It would not be wise, in the zeal to “go nano” to ignore important science and technology utilization at larger scales. Much of the technology at the nanoscale has related aspects at the micrometer or even millimeter scale. In the earlier-noted examples a recurring theme is the development of devices, instruments, materials, and approaches that originated from physics and material studies and the engineering of advanced electrical and optical devices. These trends have moved the electron microscope into the biology laboratory. The atomic force microscope and range of other nanoscale probes and devices are similarly having impact on the re-

search capabilities in the biological sciences. We may anticipate a range of new nanobased devices as minimally invasive probes for *in vivo* study of cellular processes.

In addition to this instrumentation a new class of biotechnologies, uniting active biomolecules and engineered nanoscale structures and devices is beginning to emerge. The impact of this work should be found in the coming decades in our everyday lives. There is hope for improved medical diagnosis and treatment and new types of chemical and biological sensors for environmental monitoring and defense. It is wise to consider the ethical and social impact of all technologies including those being advanced by nanometer scale science and technology. In the “nano” case, however, there may be an additional educational burden on active researchers to properly put their work in context in the environment where a science fiction version of nanotechnology involving artificial life and autonomous nanobots potentially raise public anxieties.

While much of the current activity at the nanoscale is in basic science and technology development there are prospects for developing technology for significant applications. The research may provide new methods of realizing bio-inspired and biomimetic methods for sensing and signal processing, emulating the infrared sensors of a snake, the directional hearing of an insect, or the chemical sensing capabilities of a dog’s nose. This could be realized either by understanding the mechanisms that exist in natural systems and employing those concepts in a different, perhaps inorganic architecture; or by molecular components of natural origin incorporated into an inorganic transduction element. From these approaches could emerge technologies for practical single molecule analysis, exquisitely sensitive chemical and biological sensors, less invasive or higher speed diagnostic devices. Bio-inspired approaches may have impact in new materials, which is the topic for another review.

¹Nanotechnology Research Directions: IWGN Workshop Report, International Technology Research Institute, 1999.

²See for example, the call for papers for the AVS 50th International Symposium at <http://www.avs.org/>, showing numerous sessions on the nano/bio and bio/inorganic interfaces.

³*Nanofabrication and Biosystems*, edited by H. Hoch, L. Jelinski, and H. G. Craighead (Cambridge University Press, Cambridge, 1996).

⁴*Handbook of Biological Confocal Microscopy*, edited by J. Pawley (Plenum, New York, 1990).

⁵*Principles and Techniques of Electron Microscopy: Biological Applications*, edited by M. Hayat (Cambridge University, Cambridge, 2000).

⁶H. G. Hansma, *Annu. Rev. Phys. Chem.* **52**, 71 (2001).

⁷S. Kumar and J. H. Hoch, *Traffic Q.* **2**, 746 (2001).

⁸J. H. Hafner *et al.*, *Prog. Biophys. Mol. Biol.* **77**, 73 (2001).

⁹C. Rivetti *et al.*, *J. Mol. Biol.* **326**, 1413 (2003).

¹⁰S. Jafri *et al.*, *J. Mol. Biol.* **288**, 811 (1999).

¹¹C. Bustamante, Z. Bryant, and S. B. Smith, *Nature (London)* **421**, 423 (2003).

¹²M. Reif *et al.*, *Science* **275**, 1295 (1997); J. K. Gimzewski and C. Joachim, *ibid.* **283**, 1683 (1999).

¹³T. T. Perkins, D. E. Smith, and S. Chu, *Science* **276**, 2106 (1977).

¹⁴J. Han and H. G. Craighead, *J. Vac. Sci. Technol. A* **17**, 2142 (1999).

¹⁵J. Han, S. W. Turner, and H. G. Craighead, *Phys. Rev. Lett.* **83**, 1688 (1999).

¹⁶S. W. Turner *et al.*, *J. Vac. Sci. Technol. B* **16**, 3835 (1988).

¹⁷S. W. Turner *et al.*, *Phys. Rev. Lett.* **88**, 128103 (2002).

¹⁸M. Cabodi *et al.*, *Anal. Chem.* **74**, 5169 (2002).

- ¹⁹T. Desai *et al.*, *Biomed. Microdevices* **1**, 131 (1999).
- ²⁰S. W. Turner *et al.*, *J. Vac. Sci. Technol. B* **15**, 2848 (1997).
- ²¹L.-Q. Gu *et al.*, *Nature (London)* **398**, 686 (1999).
- ²²D. Branton and J. Golovchenko, *Nature (London)* **398**, 660 (1999).
- ²³M. Levene *et al.*, *Science* **299**, 682 (2003).
- ²⁴T. A. Laurence and S. Weiss, *Science* **299**, 667 (2003).
- ²⁵X. S. Xie and H. P. Lu, *J. Biol. Chem.* **274**, 15967 (1999).
- ²⁶K. Kitamura *et al.*, *Nature (London)* **397**, 129 (1999).
- ²⁷H. Noji, *Science* **282**, 1844 (1998).
- ²⁸A. Kishino and T. Yanagida, *Nature (London)* **334**, 74 (1998).
- ²⁹J. T. Finer, R. M. Simmons, and J. A. Spudich, *Nature (London)* **368**, 113 (1994).
- ³⁰H. Hess *et al.*, *Appl. Phys. A: Mater. Sci. Process.* **75**, 309 (2002).
- ³¹R. K. Soong *et al.*, *Science* **290**, 1555 (2000).
- ³²M. Crichton, *Prey* (Harper Collins, New York, 2002).
- ³³G. Mie, *Ann. Phys. (Leipzig)* **25**, 377 (1908).
- ³⁴G. Lee *et al.*, *Anal. Biochem.* **287**, 261 (2000).
- ³⁵S.-J. Park *et al.*, *Science* **295**, 1503 (2002).
- ³⁶L. Brus, *J. Phys. Chem. Solids* **59**, 459 (1998).
- ³⁷L. Brus, *J. Chem. Phys.* **79**, 5566 (1983).
- ³⁸A. P. Alvisatos, *Science* **271**, 933 (1996).
- ³⁹X. Michalet *et al.*, *Single Mol.* **2**, 261 (2001).
- ⁴⁰J. D. Hartgerink, E. Beniash, and S. I. Stupp, *Science* **294**, 1684 (2001).