nature biotechnology

PERSPECTIVE

The 'right' size in nanobiotechnology

George M Whitesides

The biological and physical sciences share a common interest in small structures (the definition of 'small' depends on the application, but can range from 1 nm to 1 mm). A vigorous trade across the borders of these areas of science is developing around new materials and tools (largely from the physical sciences) and new phenomena (largely from the biological sciences). The physical sciences offer tools for synthesis and fabrication of devices for measuring the characteristics of cells and sub-cellular components, and of materials useful in cell and molecular biology; biology offers a window into the most sophisticated collection of functional nanostructures that exists.

The current enthusiasm for things 'nano' in size has led naturally to a search for connections between these things and biology (the hottest area of science) and technology (where science pays off): if each, individually, is good (so the reasoning goes), the three together must be better. Scientific interest in this intersection of fields is based on the perception that nanotechnology offers biology new tools, and that biology offers nanotechnology access to new types of functional nanosystems—components of the cell—that are unquestionably interesting and possibly useful. Public interest in the intersection of 'nano' and 'bio' is also high, although based in significant part on liberal doses of science-fantasy—'gray goo,' 'little submarines,' 'the assembler' and self-replicating metal-biological hybrids[1–3].

There *are* important applications of nanoscience in biology and biotechnology; biology also provides unparalleled examples of functional nanostructures to excite the imagination of nanotechnologists of all persuasions. The story is, however, not entirely 'nano,' but includes structures having a wide range of sizes. When small structures are considered for biological applications, or when small biologically derived structures are determined to have remarkable properties, the size of the system can be 'nano' (which we define, with some arbitrariness, as 1–100 nm) but also 'micro' (from 100 nm, or 0.1 μm, to perhaps 1,000 μm, or 1 mm; **Fig. 1**). The range of sizes covered by these terms—nanoscale, microscale and simply 'small'—is important: structures vital to the cell have dimensions ranging from those of small molecules to those of millimeter-scale fluidic devices; which size is most important depends on what question one is asking. Enthusiasm for the potential value of 'nano' should be balanced against the established and rapidly expanding value of 'micro.'

Nanoscience and nanotechnology have grown exuberantly in a rich mixture of legitimate scientific opportunity, technological imperative and hyperbole. Much of the initial impetus for the development of nanotechnology came from its relevance to electronics. *Micro*electronics has, unarguably, changed the world through its impact on information processing and communications, and the progress in the field has been measured by its adherence to Moore's Law: as microelectronic devices have become smaller, they have become less expensive, faster and more portable. Until recently, the critical lateral dimensions in integrated circuits were greater than 100 nm. It is now clear that *nano*electronics—integrated circuits with lateral dimensions smaller than 100 nm—will also be an important technology[4].

The majority of the very small devices used in nanoelectronics will be generated—at least initially—by *evolutionary* technologies developed from the existing methods of microelectronics (that is, methods of fabrication based primarily on photolithography). It is not clear yet whether *revolutionary* technologies—technologies based, perhaps, on the remarkable electron transport properties of silicon nanorods, or on nanometer structures supporting quantum computation, or on scanning probe microscopies for ultradense information storage—will emerge as sufficiently practical to be commercialized[5–9].

New tools have galvanized *physical* nanoscience. Scanning probe microscopies make it possible to visualize individual atoms and localized electronic states[10]. New, catalytic processes for growth of materials make nanotubes and nanospheres readily available in a wide variety of compositions[11–14]. Electron beam writing has begun to move into the mainstream of fabrication of nanostructures[15], and electron microscopy is, of course, a mainstay of observational nanoscience.

The situation in *biological* science is different for several reasons. First, biological structures are relatively large compared to structures in electronics and in physical nanoscience. A mammalian cell is approximately 10 μm in diameter when rounded, and perhaps 50 μm in diameter when fully spread in attached tissue culture; these dimensions will not change. Although it is certainly important to explore the smaller, biologically vital components of the cell, the technological imperative to *make things smaller* that has dominated microelectronics for the past 50 years does not have the same urgency in biology. The ability to observe intracellular structures with high selectivity, and to follow the dynamic behavior of these structures, may be more important than the ability simply to resolve small features by some form of microscopy. Second, the scanning probe microscope has been less revolutionary in its impact on biology than on the physical sciences. Biological structures—even those on surfaces—are soft and electrically insulating, and not easily imaged[16,17]. Moreover, most of biology goes on inside the cell, where the scanning probe tip cannot reach. Third, there already exists a highly developed science concerned with biologically relevant nanostructures: this science is called 'chemistry,' and it fabricates nanostructures—biological molecules of all sizes, from low-molecular-weight drugs to DNA, with dimensions from a nanometer to hundreds

George M. Whitesides is in the Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138, USA. e-mail: gwhitesides@gmwgroup.harvard.edu

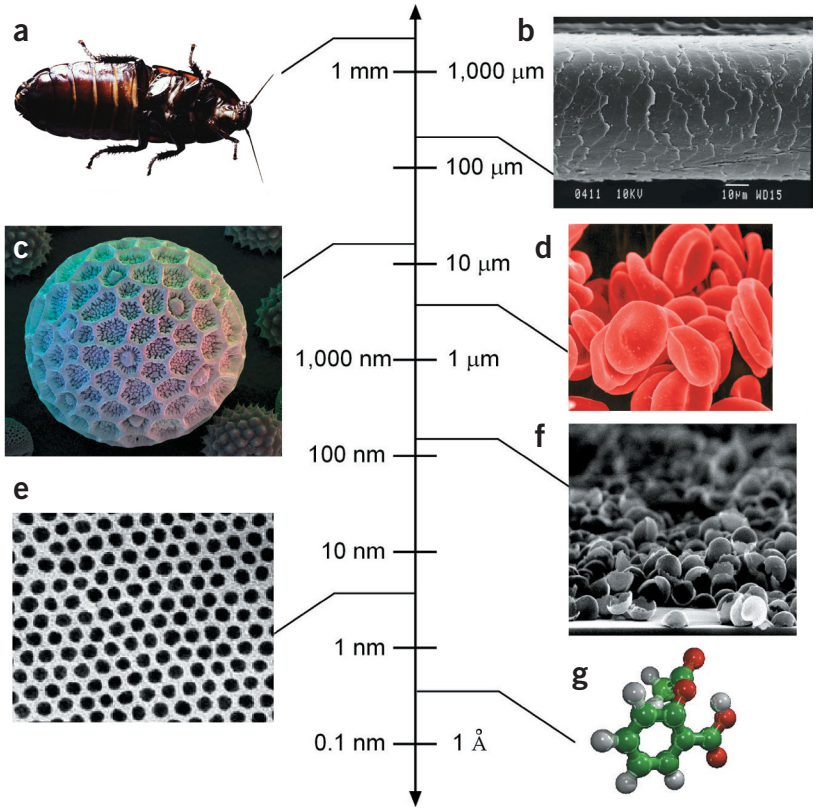

Figure 1 Sizes of representative 'small' objects. (**a**) A cockroach. (**b**) A human hair. (Image copyright Bioimaging Laboratory, University of Wales.) (**c**) *Polygonum* pollen grain. (Image copyright David Scharf.) (**d**) Red blood cells. (Image copyright Tina Weatherby-Carvalho, MicroAngela.) (**e**) Cobalt nanocrystal superlattice[83]. (**f**) An aggregate of half-shells of palladium[84]. (**g**) Aspirin molecule.

of microns—with the 0.01 nm precision in placement of atoms relative to one another made possible by synthetic procedures that form covalent chemical bonds.

The cell is the core of biology: it is the smallest unit that is alive. The cell is, in a reductionist view, a compartment in which a collection of reactions occurs. These reactions—essentially all catalyzed—modulate one another, and together form a network with the remarkable properties—self-replication, energy dissipation, adaptation—that we call 'life'[18]. The scale of sizes that characterize chemical bonds, and the molecules—even very large molecules—made of them, is familiar. The cell is, in a holistic view, an entity with phenotypic properties and behaviors: it moves, replicates or destroys itself, harvests energy from sunlight or generates it in useful form by burning glucose and dioxygen, applies force, transmits signals, senses its environment, stores and transmits information[19]. The science of biological molecules studied *ex vivo*, in dilute buffer, is highly advanced; the science that studies higher-order behaviors of cells (and of molecules in cells) is still developing[20,21].

There would, arguably, be no need to invent a new discipline of nanobiology, were there not concepts and tools that were not adequately covered by the existing disciplines. In fact, there are many unmet needs.

Molecules as nanostructures and molecules in nanostructures

Conventional molecular science (in the guise of enzymology, or analytical biochemistry, or biophysics) deals competently with molecules in solution. In the cell, however, molecules are often organized into functional aggregates, normally with nanometer-scale dimensions[22]. Visualizing and studying these structures—especially as they change dynamically during cycles of function—is one of the key challenges posed to nanoscience by biology. Progress toward these objectives is now rapid: spectroscopic studies of single molecules provide one example; biophysical studies of multiprotein aggregates are a second.

Single molecules. The single molecule is, in a sense, the ultimate nanostructure. Chemistry has, of necessity, long operated on the ergodic hypothesis: that the average behavior of a single molecule, observed over a long period of time, is the same as the behavior of a collection of molecules[23]. The sensitivity of analytical methods has been such that only their ensembles could be studied. The rapidly growing ability (based on advances in both lasers and detectors) to observe single molecules using high-resolution microscopy, and to examine the fluctuations in the behavior of these molecules over short times, is revealing a wealth of new information about the dynamics of molecules—especially catalysts—relevant to biology. A substantial effort has focused on observations of catalytic activity in enzymes, where at least some of the fluctuations are relatively slow[24–27]. It is not presently clear whether these fluctuations are biologically functional, but the possibility that they might contribute to the control of metabolic or signal-transduction pathways is a new element to consider in systems biology[28,29].

Single-molecule microscopy is one of a number of new optical techniques that are either circumventing the classical limits of microscopy or providing dramatically more informative images within the classical limits. Confocal microscopes, near-field optical systems, highly sensitive Raman microscopes and total internal reflection microscopes provide further examples of the rapid progress being made in characterizing nanostructures using light microscopy[30–35].

Molecular machines and organelles. The ultimate in functional nanosystems—'biological nanomachines'—populate the cell. The ribosome, Na^+/K^+ ATPase, flagellar micromotor of bacteria, linear micromotors of muscle and of the microtubules that organize and move the cell, voltage-gated ion channels, DNA replication complexes, multimeric membrane receptors, and the photosynthetic reaction center: these, and countless other structures in the cell, are astonishingly complex, nonintuitive in their operation, and instructive to contemplate. Patch clamping was one of the first techniques for monitoring the activity of single molecules and small protein complexes[36]. Information generated by X-ray crystallography has begun to clarify these nanostructures: we can see that their function depends centrally on the catalytic activities of their constituent proteins, on the modulation of these activities by changes in the conformation of the proteins induced by the environment (*e.g.*, the transmembrane potential) and by changes in the conformations reflecting catalysis[37–43]. No technique yet has the sensitivity and resolution to allow the direct observation of single molecules during complete cycles of biological activity, but combinations of crystallography carried out on intermediates in these cycles with other kinds of information are beginning to clarify *what* happens

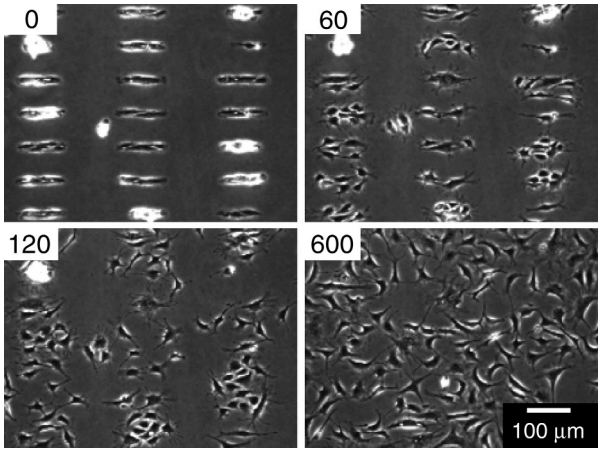

Figure 2 Selective cell release and spreading[54]. The figures show the behavior of bovine capillary endothelial cells attached to a surface patterned with ethylene glycol– and methyl-terminated thiols. Application of a cathodic voltage pulse (–1.2 V for 30 s) released the cells from the microislands. The numbers indicate the time elapsed (in minutes) after the voltage pulse.

during functional catalytic cycles or signaling events; understanding of *why* these cycles proceed as they do (in terms of the details of protein structure, organization and energetics) lies ahead[44–46].

Understanding biological nanostructures will be enormously stimulating for nanotechnology. The concept of very tiny machines has always appealed—to scientists and nonscientists alike—but the nascent field of nanotechnology originally assumed that nanomachines would probably resemble macromachines in their design[47]. It is seldom possible to prove that something cannot happen in science, and it is difficult to prove that one cannot build nanomachines that are analogous to familiar macromachines. The fact that biological structures—although functioning in familiar ways—operate using principles that are entirely unfamiliar based on everyday experience suggests that would-be designers of nanomachines have much to learn from biology[48].

An example is the rotary flagellar motor of bacteria (and the functionally related Na^+/K^+ ATPase). This motor does have a structure that serves as a shaft, and another structure that anchors the motor in the cell membrane, but beyond that, any resemblance to a macroscopic motor (whether internal combustion or electrical) stops[49,50]. The components of the machine are complex, three-dimensional structures (proteins) that self-assemble in a series of steps, starting from a linear polypeptide chain; the mechanism of rotary motion seems to involve a sequential set of changes in conformation of the proteins driven by ions moving across the cell membrane. It certainly does not involve electrical current, magnetic fields or expansion of hot gasses in a cylinder.

Micro/nanostructures as tools for biotechnology

One of the areas where biology can benefit from nanoscience is new materials and structures. There is a wide variety: surfaces patterned with self-assembled monolayers (SAMs) to guide cell attachment and growth; needles, holes and channels for biophysical tools; microstructured, three-dimensional scaffolds for tissue engineering; nanoparticles as probes; photonic band-gap structures; new optical systems for imaging. There are many such examples; they represent a wave of development of tools by physical science for application to biological science, and are a part of the *rapprochement* between the physical and biological sciences. A few examples illustrate this rapidly developing area.

Self-assembled monolayers. SAMs have provided the ability to 'synthesize' extended surfaces. Dipping a thin gold film (typically 40 nm thick, supported on glass or silicon) into a solution of an alkanethiol ($R(CH_2)_{11–18}SH$) forms SAMs. The sulfur atom chemisorbs on the gold, releases a hydrogen atom and forms a strong sulfur-gold bond. This process allows the 'synthesis' of macroscopic structures—structures comprising areas of square centimeters of ordered molecules. These structures typically contain ~5×10^{14} molecules/cm^2 and are a form of polymer: a structure containing many organic side groups (the thiols) attached covalently to a backbone (the gold film). They comprise numbers of atoms that could not be 'synthesized' in any conventional approach to organic synthesis. These surfaces are needed for applications in cell biology, as the projected area of a cell onto a surface of this type includes ~10^9–10^{10} thiolate molecules. SAMs are nanostructured materials—interfacial films with a thickness of approximately 2 nm, which allow atomic-level precision (relative to the mean plane of the surface) in the placement of functional groups. As the structures self-assemble, relatively simple synthesis of the precursors allows the formation of structures presenting complex, functional ligands[51].

An example demonstrates the versatility of SAMs in controlling the environment of cells. SAMs terminating in methyl groups are hydrophobic, and adsorb proteins from solution. SAMs terminating in oligo(ethylene glycol) moieties resist the adsorption of proteins from solution. When cells attach to a surface, they do not, in general, attach to the surface directly: they attach to proteins adsorbed on the surface. The combination of SAMs with 'printing' using elastomeric stamps (so-called 'soft lithography') allows the surface to be patterned into regions to which cells attach and regions to which they do not[52,53]. Having allowed the cells to attach and to spread to the limits of the regions to which they adhere, it is then possible to employ a useful electrochemical trick developed in surface science for other reasons: application of a brief voltage pulse to the gold layer selectively detaches the nonadsorbing thiols[54]. As soon as they have left the surface, proteins from the culture medium adsorb on the gold, and generate a surface across which cells *can* spread. **Figure 2** illustrates this process. The ability to grow cells in patterns, and then to release them from these patterns with a simple electrochemical manipulation that seems not to damage them, provides the basis for new types of bioassays that make use of observations of cell motility.

Nanotips and microspheres. The origin of nanotechnology is tools for imaging nanostructures: originally electron microscopy, but more recently, and famously, scanning probe devices. Although the direct imaging of biological structures has proved difficult, scanning probe devices have been enormously successful in allowing the application of forces directly to single molecules. The kind of information provided by these force-distance curves has, for example, made it possible to infer how stress is stored as strain in molecules by the unfolding of protein domains[55–58].

Complementary information comes from studies in which the force is applied using optical tweezers or, more recently, magnetic beads. The use of optical tweezers has provided insights into the mechanism and function of proteins involved in active transport in the cells (*e.g.*, myosin and kinesin[59,60]). Magnetic tweezers have the singular advantage that it is possible to carry out parallel measurements on large numbers of beads, and thus to improve the statistics of measurements[61–63].

Channels and pores. Microchannels are the basis of microfluidic devices. When microchannels were made in silicon using conventional photolithography, the techniques were too slow, specialized and cumbersome to be broadly useful to biologists. The development of soft-lithographic methods for microfabrication, and the realization that high-resolution printing provided adequate resolution for fabrication of

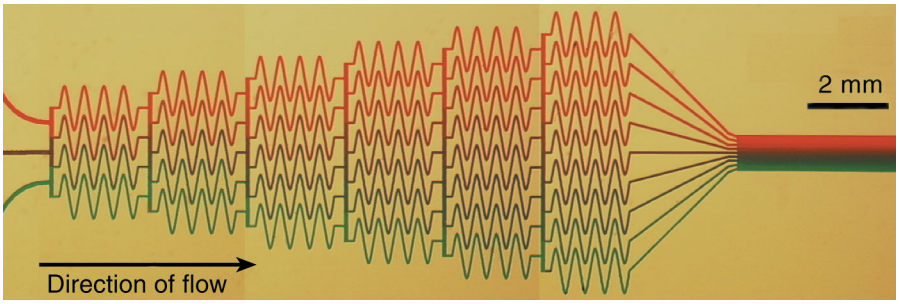

Figure 3 Generation of gradients in a microfluidic device[67]. The photograph shows a microfluidic device that generates a gradient of green and red dyes in solution. The three incoming channels (left) were connected to syringes via tubings (not visible). After the streams were combined into a single, wide channel (right), a gradient was formed across the channel, perpendicular to the direction of flow.

channels with widths from 10 μm to 1 mm, opened the door to active development of this area as a convenient and practical technique for fabricating microfluidic devices[64,65].

The physics of fluids flowing in microchannels provided another important component of this area of microtechnology. In microchannels, aqueous buffers almost always flow laminarly—that is, without eddies, and with only diffusive mixing of streams flowing side by side[66]. This combination of physical phenomena has begun to generate a wide variety of new devices: micro cell separators and particle counters, microsystems for cell culture, gradient generators and analytical systems[67–69]. **Figure 3** shows an example of a gradient generator.

Using more complex methods of fabrication, it is now possible to make true nanochannels[70,71]. The practical value of these systems remains to be seen—it is difficult to keep them from plugging up with particles invariably present in real systems—but they do have the advantage that their dimensions are smaller than the size of biological macromolecules, with potentially useful consequences for separations; they also have a very high ratio of surface to volume, and thus allow the study of wall effects in biological separations.

Micro- and nanofabrication techniques also offer access to pores (which are, in essence, very short channels). Nanofabricated pores with dimensions down to approximately 2 nm have been demonstrated and proposed as the basis for single-molecule DNA sequencing[72]. Although it is uncertain whether this application will ultimately succeed, pores will certainly be useful in a range of other applications[73].

Interestingly, biology is beginning to offer its own set of methods for making channels and pores with nanoscale dimensions; the ease with which some of these systems can be assembled suggests that they may, in the long run, provide systems that are at least as useful as those fabricated top-down. Lipid nanotubes and channels in lipid membranes based on pore-forming peptides and proteins are examples[74,75].

Nanoparticles. Nanoparticles are among the first nanoscale materials to be directly useful in biology: fluorescent particles labeled with antibodies[76] (as tags that do not photobleach) and superparamagnetic magnetite particles coated with dextran[77] (as image-enhancement agents in magnetic resonance imaging) are commercially available; a wide range of other fluorescent or magnetic nanoparticles will soon be available. Eventually, these small particles must be made much more useful and informative if they are to play an important role in understanding the workings of the cell, but nanotechnology has clearly identified the field of nanoparticles as one where new techniques in synthesis will make a wide range of particles available, and where these particles meet a need as labels for biological structure and function[78–80].

Conclusions

Physical and biological sciences show nanoscience in different lights. To the physical sciences, 'nano' offers quantum phenomena (size-dependent fluorescence, long ballistic electron trajectories) and remarkable physical properties (mechanical moduli, electrical conductivity)[81,82]. Biology adds incredibly sophisticated nanomachines, operating by entirely classical molecular mechanisms. To the biological sciences, 'nano' offers new tools: tools—many from the physical sciences—that will be necessary to put together a conceptual model of life, and a fresh framework on which to hang ideas about functional aggregates (*i.e.*, biological nanomachines). The road from reading the information in the genome to understanding life will be an arduous one, and reading the genome may be the easiest part. Understanding how molecules organize and function in cells will require new tools and concepts, and as the assemblies of molecules of greatest interest will have nanometer dimensions, the tools must be appropriate for the task: that is, they must be able to characterize structures 0.1–0.001 times the size of the cell. At the same time, selecting, sorting, maintaining, stimulating, herding and characterizing the cells will require tools substantially larger than the cell. Both nanometer and micrometer dimensions are relevant.

The different perspectives are not quite as disparate as those of the seven blind men with an elephant (or perhaps a flea, in this context), but the idea remains: nano- and microscience will show different aspects to different fields, and the integration of these aspects will yield some of the new concepts and techniques that will build a more complete picture of the cell.

The flagellar rotary motor again provides an example of the range of opportunities—and dimensions—facing 'nanobiotechnology'. This structure might provide an illustration of 'principles' that could be used to design a nonprotein nanomotor; it might be useful by itself, separated from the cell and employed to perform some nanoscopic task; it might be useful left *in vivo*, with the organism itself employed to do the work. Each application has possibilities and each involves different scales of sizes, and different critical dimensions. 'Small'—both 'nano' and 'micro'—must be a part of the future of biotechnology.

ACKNOWLEDGMENTS

This work was supported by the US Defense Advanced Research Projects Agency, National Science Foundation (ECS-0004030) and National Institutes of Health (GM-65364).

1. Drexler, K.E. *Engines of Creation* (Anchor Press/Doubleday, Garden City, New York, USA, 1986).
2. Stephenson, N. *The Diamond Age* (Bantam, New York, 1995).
3. Joy, B. Why the future doesn't need us. *Wired* **8**(4), 1–11 (April 2000). http://www.wired.com/wired/archive/8.04/joy_pr.html
4. Bohr, M.T. Nanotechnology goals and challenges for electronic applications. *IEEE Trans. Nanotechnol.* **1**, 56–62 (2002).
5. Wu, J.-J., Wong, T.-C. & Yu, C.-C. Growth and characterization of well-aligned nc-Si/SiOx composite nanowires. *Adv. Mater.* **14**, 1643–1646 (2002).
6. Cahen, D. & Hodes, G. Molecules and electronic materials. *Adv. Mater.* **14**, 789–798 (2002).
7. Lieberman, M. *et al.* Quantum-dot cellular automata at a molecular scale. *Ann. N.Y. Acad. Sci.* **960**, 225–239 (2002).
8. Vettiger, P. *et al.* The 'Millipede'—nanotechnology entering data storage. *IEEE Trans. Nanotechnol.* **1**, 39–55 (2002).
9. Vettiger, P. & Binnig, G. The nanodrive project. *Sci. Am.* **288**, 46–53 (2003).
10. Ouyang, M., Huang, J.L. & Lieber, C.M. Scanning tunneling microscopy studies of the one-dimensional electronic properties of single-walled carbon nanotubes. *Annu. Rev. Phys. Chem.* **53**, 201–220 (2002).
11. Wagner, R.S. & Ellis, W.C. Vapor-liquid-solid mechanism of single crystal growth. *Appl. Phys. Lett.* **4**, 89–90 (1964).

12. Goia, D.V. & Matijevic, E. Preparation of monodispersed metal particles. *New J. Chem.* **22**, 1203–15 (1998).
13. Gudiksen, M.S., Wang, J. & Lieber, C.M. Synthetic control of the diameter and length of single crystal semiconductor nanowires. *J. Phys. Chem. B.* **105**, 4062–4064 (2001).
14. Hyeon, T. Chemical synthesis of magnetic nanoparticles. *Chem. Comm.* issue 8, 927–934 (2003).
15. Bates, A.K. *et al.* Review of technology for 157-nm lithography. *IBM J. Res. Dev.* **45**, 605–614 (2001).
16. Hafner, J.H., Cheung, C.-L., Woolley, A.T. & Lieber, C.M. Structural and functional imaging with carbon nanotube AFM probes. *Prog. Biophys. Mol. Bio.* **77**, 73–110 (2001).
17. Scheuring, S. *et al.* Single proteins observed by atomic force microscopy. *Single Mol.* **2**, 59–67 (2001).
18. Eschenmoser, A. & Kisakurek, M.V. Chemistry and the origin of life. *Helv. Chim. Acta* **79**, 1249–1259 (1996).
19. Gierer, A. Theoretical approaches to holistic biological features: pattern formation, neural networks and the brain-mind relation. *J. Biosci.* **27**, 195–205 (2002).
20. Deutsch, J., Desai, T.A., Motlagh, D. & Russell, B. Microfabricated *in vitro* cell culture systems for investigating cellular interactions. *Proc. Soc. Photooptical Instrum. Eng.* **3912**, 105–113 (2000).
21. Kanehisa, M. Prediction of higher order functional networks from genomic data. *Pharmacogenomics* **2**, 373–385 (2001).
22. Alberts, B. *et al.* (eds.). *Molecular Biology of the Cell* (Garland Science, Taylor & Francis Group, New York, 2002).
23. Reif, F. *Fundamentals of Statistical and Thermal Physics* (McGraw-Hill, Boston, MA, USA, 1965).
24. Xue, Q.F. & Yeung, E.S. Differences in the chemical reactivity of individual molecules of an enzyme. *Nature* **373**, 681–683 (1995).
25. Craig, D.B., Arriaga, E.A., Wong, J.C.Y., Lu, H. & Dovichi, N.J. Studies on single alkaline phosphatase molecules: reaction rate and activation energy of a reaction catalyzed by a single molecule and the effect of thermal denaturation—the death of an enzyme. *J. Am. Chem. Soc.* **118**, 5245–5253 (1996).
26. Zhuang, X. *et al.* A single-molecule study of RNA catalysis and folding. *Science* **288**, 2048–2051 (2000).
27. van Oijen, A.M. *et al.* Single-molecule kinetics of lambda exonuclease reveal base dependence and dynamic disorder. *Science* **301**, 1235–1238 (2003).
28. Murray, A.W. Whither genomics? *Genome Biol.* **1**, 003.1 (2000). http://genomebiology.com/2000/1/1/comment/003.1
29. Yanai, I. & DeLisi, C. The society of genes: networks of functional links between genes from comparative genomics. *Genome Biol.* **3**, 0064.1 (2002). http://genomebiology.com/2002/3/11/research/0064.1.
30. Nie, S.M. & Emery, S.R. Probing single molecules and single nanoparticles by surface-enhanced Raman scattering. *Science* **275**, 1102–1106 (1997).
31. Nie, S.M. & Zare, R.N. Optical detection of single molecules. *Annu. Rev. Biophys. Biomol. Struct.* **26**, 567–596 (1997).
32. Bahlmann, K., Jacobs, S. & Hell, S. W. 4Pi-confocal microscopy of live cells. *Ultramicroscopy* **87**, 155–164 (2001).
33. Subramaniam, V., Kirsch, A.K. & Jovin, T.M. Cell biological applications of scanning near-field optical microscopy (SNOM). *Cell. Mol. Biol.* **44**, 689–700 (1998).
34. Hartschuh, A., Sanchez, E.J., Xie, X.S. & Novotny, L. High-resolution near-field Raman microscopy of single-walled carbon nanotubes. *Phys. Rev. Lett.* **90**, 095503/1–095503/4 (2003).
35. Sako, Y., Minoghchi, S. & Yanagida, T. Single-molecule imaging of EGFR signalling on the surface of living cells. *Nat. Cell Biol.* **2**, 168–172 (2000).
36. Sakmann, B. & Neher, E. (eds.) *Single-Channel Recording* (Plenum Press, New York, 1995).
37. Deisenhofer, J., Epp, O., Miki, K., Huber, R. & Michel, H. Structure of the protein subunits in the photosynthetic reaction center of *Rhodopseudomonas viridis* at 3 Å resolution. *Nature* **318**, 618–624 (1985).
38. Abrahams, J.P., Leslie, A.G.W., Lutter, R. & Walker, J.E. Structure at 2.8-Angstrom resolution of F1-ATPase from bovine heart-mitochondria. *Nature* **370**, 621–628 (1994).
39. Simpson, A.A. *et al.* Structure of the bacteriophage ϕ29 DNA packaging motor. *Nature* **408**, 745–750 (2000).
40. Kikkawa, M. *et al.* Switch-based mechanism of kinesin motors. *Nature* **411**, 439–445 (2001).
41. Dutzler, R., Campbell, E.B., Cadene, M., Chait, B.T. & MacKinnon, R. X-ray structure of a ClC chloride channel at 3.0 Å reveals the molecular basis of anion selectivity. *Nature* **415**, 287–294 (2002).
42. Jiang, Y. *et al.* The open pore conformation of potassium channels. *Nature* **417**, 523–526 (2002).
43. Jiang, Y. *et al.* Crystal structure and mechanism of a calcium-gated potassium channel. *Nature* **417**, 515–522 (2002).
44. Noji, N., Yasuda, R., Yoshida, M. & Kinosita, K. Direct observation of the rotation of F-1-ATPase. *Nature* **386**, 299–302 (1997).
45. Yasuda, R., Noji, H., Yoshida, M., Kinosita, K. & Itoh, H. Resolution of distinct rotational substeps by submillisecond kinetic analysis of F1-ATPase. *Nature* **410**, 898–904 (2001).
46. Simons, K. & Ikonen, E. Functional rafts in cell membranes. *Nature* **387**, 569–572 (1997).
47. Drexler, K.E. Nanosystems: Molecular Machinery, Manufacturing, and Computation (Wiley, New York, 1992).
48. Soong, R.K. *et al.* Powering an inorganic nanodevice with a biomolecular motor. *Science* **290**, 1555–1558 (2000).
49. Sambongi, Y. *et al.* Mechanical rotation of the c subunit oligomer in ATP synthase (F_0F_1): direct observation. *Science* **286**, 1722–1724 (1999).
50. Stock, D., Leslie, A.G.W. & Walker, J.E. Molecular architecture of the rotary motor in ATP synthase. *Science* **286**, 1700–1705 (1999).
51. Mrksich, M. & Whitesides, G.M. Patterning self-assembled monolayers using microcontact printing—a new technology for biosensors. *Trends Biotechnol.* **13**, 228–235 (1995).
52. Whitesides, G.M., Ostuni, E., Takayama, S., Jiang, X. & Ingber, D.E. Soft lithography in biology and biochemistry. *Annu. Rev. Biomed. Eng.* **3**, 335–373 (2001).
53. Michel, B. *et al.* Printing meets lithography: soft approaches to high-resolution patterning. *Chimia* **56**, 527–542 (2003).
54. Jiang, X.Y., Ferrigno, R., Mrksich, M. & Whitesides, G.M. Electrochemical desorption of self-assembled monolayers noninvasively releases patterned cells from geometrical confinements. *J. Am. Chem. Soc.* **125**, 2366–2367 (2003).
55. Svoboda, K. & Block, S.M. Biological applications of optical forces. *Annu. Rev. Biophys. Biomol. Struct.* **23**, 247–285 (1994).
56. Engel, A., Gaub, H.E. & Muller, D.J. Atomic force microscopy: a forceful way with single molecules. *Curr. Biol.* **9**, R133–R136 (1999).
57. Bustamante, C., Macosko, J.C. & Wuite, G.J.L. Grabbing the cat by the tail: manipulating molecules one by one. *Nat. Rev. Mol. Cell Biol.* **1**, 130–136 (2000).
58. Ha, T. *et al.* Initiation and re-initiation of DNA unwinding by the *Escherichia coli* Rep helicase. *Nature* **419**, 638–641 (2002).
59. Knight, A.E., Veigel, C., Chambers, C. & Molloy, J.E. Analysis of single-molecule mechanical recordings: application to acto-myosin interactions. *Prog. Biophys. Mol. Biol.* **77**, 45–72 (2001).
60. Block, S.M., Asbury, C.L., Shaevitz, J.W. & Lang, M.J. Probing the kinesin reaction cycle with a 2D optical force clamp. *Proc. Natl. Acad. Sci. USA.* **100**, 2351–2356 (2003).
61. Evans, E. Probing the relation between force-lifetime-and chemistry in single molecular bonds. *Annu. Rev. Biophys. Biomol. Struct.* **30**, 105–128 (2001).
62. Assi, F., Jenks, R., Yang, J., Love, J.C. & Prentiss, M. Massively parallel adhesion and reactivity measurements using simple and inexpensive magnetic tweezers. *J. Appl. Phys.* **92**, 5584–5586 (2002).
63. Danilowicz, C. *et al.* DNA unzipped under a constant force exhibits multiple metastable intermediates. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1694–1699 (2003).
64. Delamarche, E. *et al.* Microfluidic networks for chemical patterning of substrates: design and application to bioassays. *J. Am. Chem. Soc.* **120**, 500–508 (1998).
65. Whitesides, G.M. & Stroock, A.D. Flexible methods for microfluidics. *Phys. Today* **54**, 42–48 (2001).
66. Hatch, A. *et al.* A rapid diffusion immunoassay in a T-sensor. *Nature Biotechnol.* **19**, 461–465 (2001).
67. Dertinger, S.K.W., Chiu, D.T., Jeon, N.L. & Whitesides, G.M. Generation of gradients having complex shapes using microfluidic networks. *Anal. Chem.* **73**, 1240–1246 (2001).
68. Dertinger, S.K.W., Jiang, X.Y., Li, Z.Y., Murthy, V.N. & Whitesides, G.M. Gradients of substrate-bound laminin orient axonal specification of neurons. *Proc. Natl. Acad. Sci. USA* **99**, 12542–12547 (2002).
69. Hong, J.W. & Quake, S.R. Integrated nanoliter systems. *Nat. Biotechnol.* **21**, 1179–1183 (2003).
70. Han, J. & Craighead, H.G. Separation of long DNA molecules in a microfabricated entropic trap array. *Science* **288**, 1026–1029 (2000).
71. Cao, H., Tegenfeldt, J.O., Austin, R.H. & Chou, S.Y. Gradient nanostructures for interfacing microfluidics and nanofluidics. *Appl. Phys. Lett.* **81**, 3058–3060 (2002).
72. Li, J. *et al.* Ion-beam sculpting at nanometre length scales. *Nature* **412**, 166–169 (2001).
73. Mayer, M., Kriebel, J.K., Tosteson, M.T. & Whitesides, G.M. Microfabricated Teflon membranes for low-noise recordings of ion channels in planar lipid bilayers. *Biophys. J.* **85**, 2684–2695 (2003).
74. Graff, A., Winterhalter, M. & Meier, W. Nanoreactors from polymer-stabilized liposomes. *Langmuir* **17**, 919–923 (2001).
75. Karlsson, M. *et al.* Formation of geometrically complex lipid nanotube-vesicle networks of higher-order topologies. *Proc. Natl. Acad. Sci. USA* **99**, 11573–11578 (2002).
76. Martin, C.R. & Mitchell, D.T. Nanomaterials in analytical chemistry. *Anal. Chem.* **70**, 322A–327A (1998).
77. Harisinghani, M.G. *et al.* Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. *New Eng. J. Med.* **348**, 2491–2499 (2003).
78. Hulteen, J.C. & Martin, C.R. A general template-based method for the preparation of nanomaterials. *J. Mater. Chem.* **7**, 1075–1087 (1997).
79. Murray, C.B., Kagan, C.R. & Bawendi, M.G. Synthesis and characterization of monodisperse nanocrystals and close-packed nanocrystal assemblies. *Annu. Rev. Mater. Sci.* **30**, 545–610 (2000).
80. Taton, T.A. Nanostructures as tailored biological probes. *Trends Biotechnol.* **20**, 277–279 (2002).
81. Alivisatos, A.P. Semiconductor clusters, nanocyrstals, and quantum dots. *Science* **271**, 933–937 (1996).
82. Liang, W., Shores, M.P., Bockrath, M., Long, J.R. & Park, H. Kondo resonance in a single-molecule transistor. *Nature* **417**, 725–729 (2002).
83. Black, C.T., Murray, C.B., Sandstrom, R.L. & Sun, S. Spin-dependent tunneling in self-assembled cobalt-nanocrystal superlattices. *Science* **290**, 1131–1134 (2000).
84. Love, J.C., Gates, B.D., Wolfe, D.B., Paul, K.E. & Whitesides, G.M. Fabrication and wetting properties of metallic half-shells with submicron diameters. *Nano Lett.* **2**, 891–894 (2002).