9
Bionanotechnology

The interaction between nanotechnology and cell biology is an important one, which works in two directions. Nanotechnology has provided, and will no doubt continue to provide, important new tools for biology, which help biologists to unravel the way in which the nanoscale components of living systems work together to create the remarkable examples of nanomachines and devices that biology provides. In the reverse direction, a study of the mechanisms of cell biology will give us important guidelines for making synthetic nanodevices. The process of evolution has allowed nature to find highly efficient solutions to the problems of engineering at the nanoscale, and the nanotechnologist should exploit these solutions, either by directly incorporating biological systems into nanodevices, or by building synthetic systems which mimic the operating principles of their biological analogues.

9.1 NEW TOOLS FOR INVESTIGATING BIOLOGICAL SYSTEMS

Perhaps the most useful consequence of nanotechnology for biology has been the provision of powerful new tools for the investigation of biomolecular structure, function and properties. Using these new tools, it is becoming possible to make direct measurements of structural elements of cells, and molecular recognition interactions, where in the past data could only be obtained by inference from macroscopic experiments.

9.1.1 Scanning probe microscopy for biomolecular imaging

Scanning probe microscopy (SPM) has revolutionised our understanding of the structures of solid surfaces. With the publication two decades ago of the first high-resolution scanning tunnelling microscopy (STM) images revealing the arrangements of atoms on single crystal surfaces, it was clear that powerful new capabilities were at our disposal (see Section 2.5.1). Early on, there were hopes that STM might realise similar spatial

Nanoscale Science and Technology Edited by R. W. Kelsall, I. W. Hamley and M. Geoghegan

resolution in studies of biological molecules. DNA was a particular focus of early efforts. It was hoped that STM could be used to 'read' the sequence of a strand of DNA directly. Commonly, samples were prepared by depositing a solution containing the DNA sample onto a highly oriented pyrolytic graphite (HOPG) surface. Searching for plausible images proved difficult, but a number of groups published images that appeared to reveal the structure of DNA. There was much consternation, however, when Beebe and co-workers showed in 1000 that structures accurately matching the dimensions of DNA and appearing to exhibit the characteristic helical topology could be observed in images of clean graphite surfaces[1]. This raised important questions about the likely types of artefacts that might be associated with STM images, and contributed significantly to developing the maturity of the technique. Artefacts associated with substrate features have always been a problem in microscopy, and they remain a trap for the unwary today. Careful interrogation of the sample, adequate repetition of experiments and the use of complementary surface analytical techniques, such as X-ray photoelectron spectroscopy (XPS) and secondary ion mass spectrometry (SIMS) are key requirements if properly validated data are to be acquired. Under appropriate conditions, it is possible to acquire reliable images. For example, proteins have been successfully imaged. It is important to note that the force exerted on the sample by the STM tip may be substantial, leading to movement of the sample molecules, therefore care must be taken to immobilise them, usually by covalent attachment. An additional question concerns the imaging mechanism. Proteins are expected to be electrical insulators, and tunnelling is not expected to be very efficient. One suggestion is that the tip deforms the protein, modifying its electronic structure and generating new states at the Fermi level of the substrate. Another is that water, bound to the protein molecule, provides a conducting path for the flow of current from tip to substrate.

Atomic force microscopy (AFM) works by measurement of the force between a sharp tip and a sample, rather than measuring current as in the case of STM (see Section 2.5.1). Consequently, AFM can be used, in principle, on any material. AFM measurements on biomolecules may be performed in *contact mode*, in which the tip exerts a substantial force (on a molecular scale) on the sample. There is also a significant frictional interaction as the tip slides across the sample surface. The forces involved are usually more than adequate to displace biomolecules. A variety of approaches have been explored to solve these problems, including the use of covalent coupling schemes to tether biomolecules in place. Another approach is to crystallise the sample into a periodic array, and rely on the cohesive forces within the close-packed molecular assembly to counterbalance the disruptive influence of the tip. Although not all biomolecules may be crystallised, this approach has led to some spectacular successes, including insights into the molecular structure of membrane proteins. In studies of bacterial surface layers, or S-layers (the proteins that constitute the outermost layer of the cell wall), it was possible to examine the effects of enzymatic digestion with a spatial resolution better than 1 nm. The S-layers were deposited onto mica substrates and found to form bilayers or multilayers, The topmost layer exhibited a triangular structure when imaged at low force (100 pN); however, imaging at elevated loads (600 pN) led to removal of the top layer and exposure of a hexameric flower-shaped morphology.

[1] C. R. Clemmer and T. P. Beebe, Science **251** 640 (1991)

S-layers that had been enzymatically digested were found to be present as single layers that exhibited each type of surface with equal probability. In another study, AFM data on several membrane proteins were presented with a resolution of better than 0.7 nm. Importantly, in these studies, raw AFM data were presented that clearly exhibited substructural details of individual protein molecules. In contrast, electron micrographs with the best resolution typically represent averaged data from a large number of molecules. The AFM data enable the observation of crystal defects, or molecule-to-molecule variability in structure. Nevertheless, computational analysis of AFM images of large assemblies is still possible, leading to image averaging or more sophisticated analyses. One of the components of the photosynthetic apparatus of the bacterium *Rhodospirillum rubrum*, has been studied and shown to consist of a ring structure (the light-harvesting complex LH1) containing within it the reaction centre (RC). The RC receives energy from the LH1. Two-dimensional crystals of the complex were formed and deposited onto mica. Contact mode images revealed patterns of alternating bright and dark rows (Figure 9.1). These resulted from the existence of two distinct orientations for the RC–LH1 complex. On the cytoplasmic side of the complex (the cytoplasm is the content of the cell within the plasma membrane), the reaction centre protrudes, and is observed as a feature with bright contrast, whereas on the periplasmic side (the periplasm lies between the plasma membrane and the outer membrane of the cell) dark contrast is observed over the centre of the complex. A small number of crystal defects were observed, in which the LH1 complex adopted a different morphology; these would have been lost in electron microscopy investigations due to averaging. In some cases the reaction centre was observed to be missing, even at low loads, possibly attributable to its removal by the tip as it traversed the crystal. Imaging at loads of 200–300 pN was found to yield the best resolution. On the periplasmic side of the complex, an X-shaped structure was observed, which was attributed to the periplasmic face of the RC.

The development of *tapping mode* AFM has been one of the most useful developments for the imaging of biological specimens. Contact mode imaging may lead to the disruption of surface structure at elevated loads. For biological specimens, that may be composed of isolated molecules distributed on a solid substrate, and often interacting

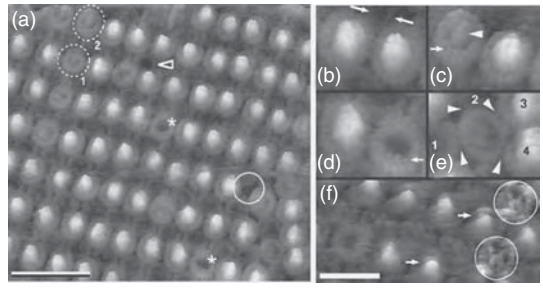

Figure 9.1 (a) High-resolution AFM image of a two-dimensional crystal of RC–LH1 complexes. The broken circle (1) and the ellipse (2) mark complexes that lack the RC–H subunit. The asterisks denote 'empty' LH1 complexes that completely lack the RC seen from the cytoplasmic side. The arrow denotes a missing RC seen from the periplasmic side. (b to e) show a variety of complexes at higher magnification. (f) shows the periplasmic side of the RC–LH1 complex imaged at higher load. The scale bars are 40 nm in (a) and 15 nm in (f). Reproduced with permission from D. Fotiadis *et al.*, J. Biol. Chem **279**, 2063 (2004)

only weakly with it, there is a real danger that the tip will move molecules around. In tapping mode, the cantilever oscillates at high frequency (100–200 kHz) and high amplitude, and only intermittently strikes the sample. This eliminates the frictional forces that contribute to damage, and reduces the rate of energy dissipation, rendering the topographical imaging of delicate materials much easier. Energy dissipation still occurs, and provides access to important additional information in the form of phase images. In *phase imaging*, the lag between the driving oscillation and the cantilever response is measured. Elastic contacts lead to a small phase lag, while contacts with viscoelastic materials, that result in a higher rate of energy dissipation, lead to a larger phase angle. Phase images reveal local variations in mechanical properties (e.g., stiffness).

Tapping mode has recently been facilitating the imaging of proteins adsorbed onto solid surfaces. In contrast to the beautiful images acquired for protein crystals, these data are typically less well resolved but do provide data for isolated molecules. For example, the von Willebrand factor (VWF), a large multimeric protein that adheres rapidly to biomaterial surfaces upon exposure to blood, has been imaged. Protein–surface interactions play a key role in regulating thrombus formation, a phenomenon of great importance when biomaterials are placed in contact with the blood because it can lead to failure of the prosthetic device. VWF adsorbed to hydrophobic monolayers of octadecyltrichlorosilane (OTS) adsorbed on glass has been compared to VWF adsorbed onto hydrophilic mica. On the OTS monolayers, VWF was found to exhibit a coiled conformation, while on mica the polypeptide chains were observed to adopt extended conformations which exhibited much larger end-to-end dimensions.

Fibronectin (FN) is another protein of considerable importance in the development of prosthetic biomaterials. FN plays an important role in cellular attachment, and is recognised by integrin receptors in cell membranes, which regulate the mechanism of attachment. FN is a dimeric protein, consisting of two polypeptide chains joined by disulphide linkages. A specific region of the molecule, containing the tripeptide sequence arginine–glycine–aspartic acid (RGD), is recognised by the integrin receptors. FN undergoes surface-specific conformational changes, and these changes in conformation lead to differences in the orientation of the cell binding domain of the molecule with respect to the solid surface on which the molecule adsorbs. The characterisation of the conformations of adsorbed proteins is very challenging, and many techniques, such as infrared spectroscopy, provide only limited information. Tapping mode AFM has been used to image FN adsorbed onto the surface of mica, single FN molecules being observed. FN was exposed to heparin-functionalised gold nanoparticles. Bound nanoparticles could be resolved as bright features situated part-way along the FN polypeptide chain, enabling the binding site to be estimated. It was concluded that there were two binding sites, based on the AFM data, attributed to the Hep I and Hep II sites previously identified using biochemical means. A difference in binding affinity for the two sites was postulated, based on the observation that twice as many functionalised nanoparticles were observed to bind to Hep I than to Hep II.

Scanning near field optical microscopy (SNOM) – see Section 2.2.4.1 – provides a route to fluorescence measurements of biological molecules with a spatial resolution of around 50 nm – well below the diffraction limit. When combined with suitable collection optics (such as an avalanche photon counting system), the detection of optical data from single molecules becomes feasible. If SNOM is combined with a Raman spectrometer, it is

possible to acquire spectroscopic data with similar resolution by taking advantage of the surface-enhanced Raman effect on appropriate substrates.

Recently there has been interest in apertureless SNOM methods, in which the optical fibre is replaced with a tip. The tip is either fabricated from a noble metal, or coated with one. The tip and sample are irradiated using a laser with the tip in close proximity to the sample. A surface plasmon is excited at the tip surface, and in the region directly beneath the tip, the electrostatic field experiences a strong near-field enhancement. The field associated with a surface plasmon is spatially highly confined in the region of a noble metal asperity, leading to an intense excitation of the sample in a small defined region. Published data (including apertureless Raman microscopy) suggest a spatial resolution of 25 nm is feasible, and better resolution may well be possible.

9.1.2 Force measurement in biological systems

Force–distance measurement (also known as force spectroscopy) using the atomic force microscope has been particularly important in the investigation of biological systems. In a force–distance measurement, the AFM tip is lowered towards the sample surface. When the tip approaches very close to the surface, a mechanical instability causes it to snap into contact. Bringing the tip closer leads to a repulsive interaction, due to the quantum mechanical repulsions experienced by atoms at close proximity. The cycle is then reversed and the tip retracted from the surface. Adhesion of the tip to the surface results in hysteresis; i.e., the path followed during retraction of the tip does not exactly follow the approach path. In particular, the tip must be pulled further than the initial point of contact to separate it from the surface. Eventually the tip separates from the surface, and the adhesive load immediately prior to separation is referred to as the pull-off force or the adhesion force.

There are now a large number of illustrations of the kinds of measurement that can be made on biological systems in this way. Biotinylated bovine serum albumin (BSA) has been adsorbed to a glass microsphere attached to an AFM cantilever, and the interaction force with streptavidin distributed on a mica surface has been measured (Figure 9.2). The biotin–streptavidin lock-and-key recognition mechanism has been studied using a biotinylated agarose bean and a streptavidin-coated tip. The pull-off forces were measured and subsequently the relationship between the interaction force and enthalpy was investigated. The recognition forces between complementary strands of DNA have also been studied. The DNA strands were thiolated at their 3′ and 5′ ends[2] for attachment to alkylsilane monolayers attached to a silica probe and a planar surface. A slightly different approach is to attach bases to tip and surface and measure single base-pair interactionss and this approach has been adopted to try to develop a sensor. Peptide nucleic acids (PNAs) have been modified with cysteine to enable attachment to gold-coated AFM tips, and interaction forces with alkanethiol monolayers were probed

[2] Each of the two strands of DNA terminates in a hydroxyl (3′) at one end and a phosphate (5′) at the other. The strands are intertwined such that the double helix has a complementary 3′ and 5′ at each end.

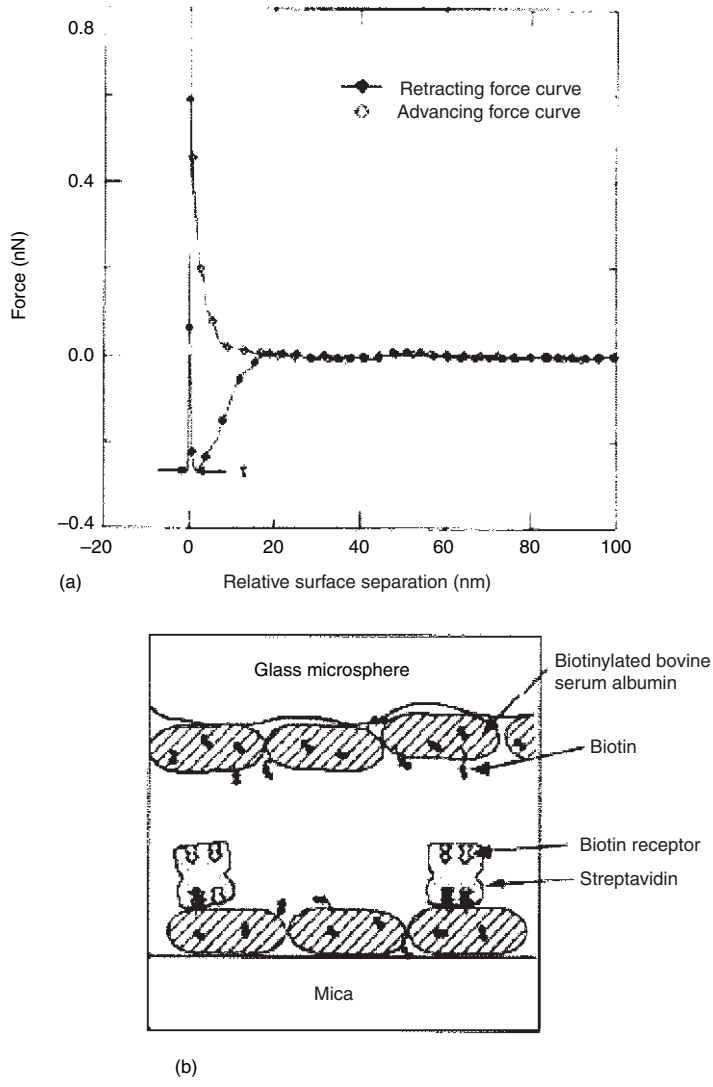

Figure 9.2 Top: measured forces between a biotinylated bovine serum albumin-coated glass microsphere attached to an AFM cantilever and a streptavidin surface in pH 7.0 phosphate buffered saline solution. Bottom: schematic diagram showing the experimental arrangement. Reproduced with permission from G. U. Lee, D. A. Kidwell and R. J. Colton, *Langmuir* **10**, 354 (1994)

before and after hybridisation with PNA or RNA. The pull-off force was reduced by hybridisation. A DNA-modified latex microparticle has been used as a probe of micron-scale patterned arrays of immobilised oligonucleotides.

These illustrations suggest the potential usefulness of techniques based on highly sensitive recognition measurements by AFM. At a fundamental level, the field of SPM-based force measurement in biology has become a very large one. Force spectroscopy has become a more or less established biophysical tool, although the interpretation of

force data remains complex, and the subject of intense academic activity. Importantly for applications in other areas, the complexity of phenomena such as protein unfolding means that practitioners of biological force microscopy have had to grapple with a variety of technical issues that while less important in simpler systems, may still be significant. One illustration is the issue of rate-dependent phenomena. It has been demonstrated that the application of an external mechanical force effectively tilts the 'energy landscape' for the unfolding process, reducing the activation energy. This means it is necessary to explore unfolding processes at a range of unloading rates in order to accurately quantify the events involved. The procedure has now been widely adopted and has facilitated quantitative investigation of a range of phenomena.

The application of AFM to the characterisation of cellular structure and function presents significant experimental challenges. In particular, cells are rather soft structures. The cell membrane is a fairly fluid structure, being composed of a bilayer of lipid molecules, and the internal contents of the cell are fluid. However, AFM offers the exciting possibility of probing cellular properties and interactions with very high spatial resolution. Recent work suggests that the rewards may be considerable. Extremely elegant investigations of cellular structure have been conducted, which have demonstrated that AFM can be utilized to probe the structures of living rat liver macrophages and chicken cardiocytes. Actin is a small protein that becomes organised to form filamentous structures as cellular attachment begins to occur. The process of stress fibre formation is linked with the formation of focal adhesions that anchor cells to the substratum on which they are cultured. AFM has been used to probe the mechanical properties of actin stress fibres, which can be readily imaged. Remarkably, it proved possible to conduct investigations on migrating cells (Figure 9.3), and it was possible to detect mechanical differences between the active and stable edges of motile fibroblasts. Other cell types have also been investigated such as microbial cells, for which surprisingly well-resolved images were obtained. It is also possible to conduct force–distance measurements on cell surfaces, and to compare the adhesiveness of microbial cell surfaces. By varying the pH of the ionic fluid medium in which the pull-off forces were measured, it is possible to investigate the surface charge distribution of the cell surface.

Optical tweezers provide an alternative means to AFM for the investigation of interaction forces in biological systems. They rely on trapping a dielectric particle in a laser beam (Figure 9.4). The extremely high electric field gradient near to a tightly focused laser beam exerts a mechanical force on a dielectric particle placed close to it, drawing it towards the centre of the beam and propelling it in the direction of propagation. Although two counterpropagating beams may be used, and the name appears to imply two pincers gripping an object, in fact a single beam is sufficient and provides the basis for a typical modern instrument. The diameter of the particle is critical, and should be about 1 μm. It is usually fabricated from polymer. It is not possible to trap individual molecules using optical tweezers, so to measure the force of a biological interaction at the molecular level it is necessary to use a functionalised bead instead. Nevertheless, the method is extraordinarily powerful.

The mechanical actions of molecules involved in the contraction of muscle have been studied. When muscle contracts, mysosin pulls on actin filaments (protein fibres formed by the association of actin molecules). It was possible to investigate this action by attaching an actin fibre between two microspheres (Figure 9.4). The filament was then placed close to a third microsphere coated with myosin. The whole assembly was

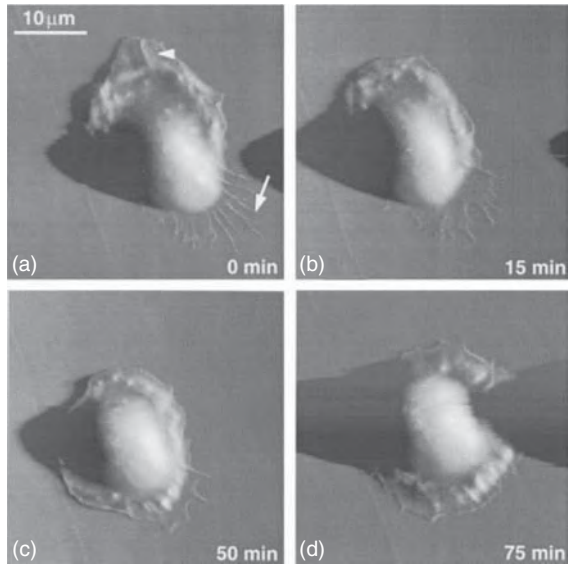

Figure 9.3 Cellular dynamics imaged in real time by AFM. Spreading is evident at the lower edge of the cell in the first frame, and by 15 min a fully developed, flat lamellipodium is evident. Simultaneously the cell's upper body is retracted and increased in height. After 50 min, all lamellipodia are retracted and the cell adopts a rounded morphology. After 75 min the cell may be observed to undergo spreading again. Reproduced with permission from C. Rotsch, F. Braet, E. Wisse and M. Radmacher, *Cell Biol. Intl.* **21**, 685 (1997)

submerged within a fluid medium containing adenosine triphosphate (ATP), which serves as a fuel supply for biological molecules. During contact between myosin and actin, an ATP molecule was digested and a tug was registered on the actin filament. The force exerted on one of the beads could be measured using optical tweezers, and the influence of the ATP concentration on the rate of the displacement process measured.

The interaction between FN and the cell cytoskeleton has been examined using laser tweezers. A short segment of FN, containing the cell binding domain, was attached to silica beads and was then allowed to interact with a cell. The bead bound to one of the integrin receptors, the $\alpha_v\beta_3$ receptor, known to recognise the part of the FN molecule containing the RGD sequence. When a bead attached to a motile lamellipodium (a part of the cell membrane that is being extended as the cell pulls itself forwards), the bead was observed to move, exerting force on the bead. The force was measured using the optical tweezers. Beads moved until the force was great enough to cleave the bond between cell and bead, at which point a sudden movement of the bead was observed, as it abruptly returned to the centre of the optical trap. The force of interaction between the functionalised bead and the cell could thus be measured. When a peptide containing the cell-binding sequence RGD was added to the culture medium, the force was observed to be reduced. The presence of talin a protein found on the interior of the cell and thought to be involved in the process of focal contact formation, linking integrin receptors to actin within the cell, is necessary to be able to measure the interaction between the cell and the bead. Remarkably, this force was estimated to be only 2 pN.

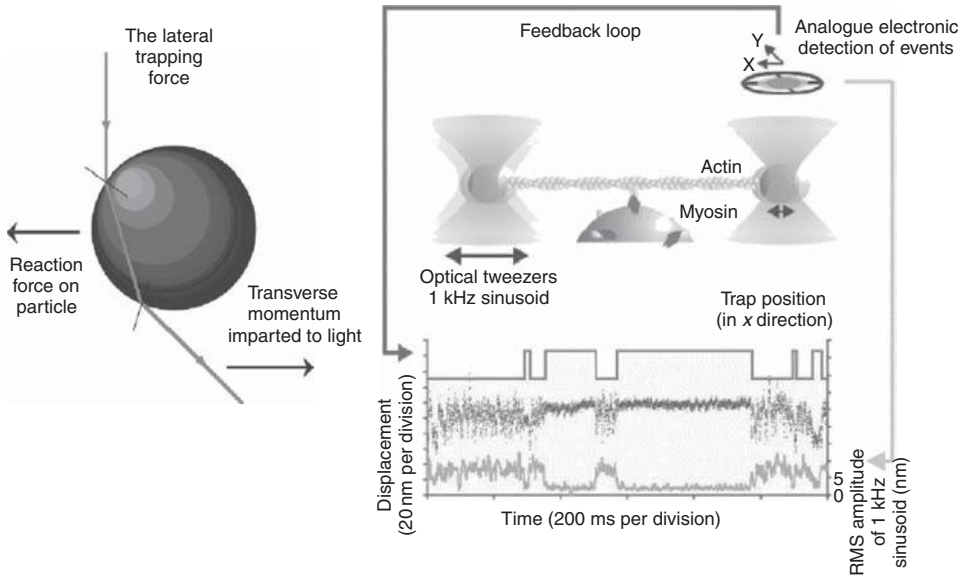

Figure 9.4 Left: schematic diagram showing the optical trapping of a dielectric particle. Right: schematic diagram showing the experimental arrangement employed by Molloy and co-workers to measure the interaction between myosin and actin. The recording shows the bead position measured in parallel with the actin filament axis versus time. To detect myosin binding with adequate time resolution, the position of one laser tweezer was oscillated at a frequency of 1 kHz and an amplitude of 35 nm RMS. The transmission of this signal to the bead in the stationary tweezer was determined from its discrete Fourier transform (grey trace) so that the position of both optical tweezers could be moved rapidly (blue trace) to apply load to the bound crossbridge. Reproduced with permission from J. E. Molloy and M. J. Padgett, *Contemp. Phys.* **43**, 241 (2002) (published by Taylor and Francis Ltd., www.tandf.co.uk/journals) and C. Viegel, J. E. Molloy, S. Schmitz and J. Kendrick-Jones, *Nature Cell Biol.* **5**, 980 (2003)

Cells respond to mechanical stresses. The process of mechanotransduction, by which cells respond to external mechanical stresses by the triggering of internal biochemical pathways, has attracted growing interest. Mechanical stress has a profound influence on the actin cytoskeleton, and it appears that physical stimuli may activate genes and signalling proteins that are also triggered by molecules that bind to specific cell surface receptors. Cells may be switched between entirely different phenotypes by alterations to the extracellular matrix structure or mechanical influences that induce changes in cell shape, independent of growth factor binding or integrin binding. These observations have stimulated a search for methods to induce cell stress in a defined fashion, and for making quantitative measurements of the mechanical behaviour of cells.

One of the best ways to induce stress in cells is to constrain their spreading by culturing them on surfaces patterned with regions that are adhesive and resistant to attachment. Much pioneering work has been carried out by the Whitesides group at Harvard. They have used self-assembled monolayers (SAMs) of alkanethiols ($HS(CH_2)_nX$) to control surface chemical structure. The SAMs may readily be patterned by microcontact printing, in which an elastomeric stamp, inked with a solution of a thiol of interest, is used to transfer it to a gold surface. The stamp is removed to leave behind a molecular pattern.

The gaps in the pattern (bare regions of gold) may be filled in by immersing the sample in a solution of a contrasting thiol in ethanol. The second thiol adsorbs at the surface, yielding a surface composed of geometrically well-defined regions with very different biological responses. If one of the thiols is terminated in an oligo(ethylene glycol) unit, it will resist the adsorption of proteins, rendering it non-adhesive to cells. However, if the other thiol is, for example, a methyl-terminated adsorbate, then proteins will adsorb and attachment will result. By defining the regions at the surface occupied by the methyl-terminated thiol, it is possible to precisely define the shapes and sizes of the areas of the surface to which cells may attach. One of the most significant results has been the observation that the area of the adhesive regions cannot be reduced arbitrarily. Eventually a point comes where the patches are so small that cell spreading is highly constrained, and the process of apoptosis, or programmed cell death, is triggered.

Several methods have been used to measure the forces exerted by cells, for example using a bed of microneedles. The microneedles are fabricated in poly(dimethyl siloxane) (PDMS). Using photolithographic methods, an array of pillars is first formed in a silicon master. PDMS is then cast onto this master and cured, leading to the formation of a PDMS master that contains an array of wells. A second batch of PDMS is then cast onto this master, and when cured and removed it consists of an array of elastomeric needles. Cells may be cultured on the needles, and as they grow and exert mechanical forces, the needles bend. If the mechanical properties of the PDMS are known, then the force exerted by the cells may be determined from the deflection of the needles.

9.1.3 Miniaturisation and analysis

The miniaturisation of analytical systems brings a variety of advantages for the investigation of biological systems. One motivation is that it is possible to carry out large numbers of experiments rapidly in parallel. There has recently been a great deal of interest in chip-based methods for the characterisation of biological systems. One approach that has been important in the emergence of genomics, the branch of science that seeks to sequence the genome of a species and then utilize the resulting information to explain and predict behaviour. The method has been enabled by the development of array-based systems of analysis. In a *microarray*, spots are deposited onto a solid support. Each spot contains a single molecule with a known identity. Because of the specificity of biological recognition each molecule will bind a specific partner, so that each spot functions as a sensor for that partner. The array is exposed to a test solution, and afterwards it is determined which spots (i.e., which molecules) have bound their partner. Usually this is achieved by the use of a fluorescent conjugate; using an optical microscope, the array is scanned and positive results scored for spots that are found to emit fluorescence. The spots in the array may range from a few tens of micrometres to 100 μm in size and the arrays may be large, containing thousands of different molecules. In DNA analysis each spot may contain a short sequence (oligonucleotide) that is complementary to a specific gene (there are approximately 40 000 in the human genome, so an array encoding the entire genome would be very extensive). The advantage for the biologist is that in a single experiment it is possible to examine whether a very large number of genes are present in the DNA sample, or even to sequence the DNA.

There are a variety of approaches to the fabrication of such arrays. In one method, oligonucleotides are synthesised in situ on a solid substrate using photolithographic methods. Photocleavable protecting groups are attached to each sequence as it grows. By selectively exposing particular spots, they may be deprotected and a particular base attached. It is essential that the chemistry is extremely effective, because even a tiny failure rate (a few percent) in the attachment of a specific base will result in a significant probability that an error will occur during the synthesis of a lengthy sequence (say 20 bases). Other approaches involve the spotting of molecules (which may be oligonucleotides or other molecules, such as proteins) using inkjet printing technology.

Although these methods offer very high throughput, there are significant drawbacks. The interaction between the solid substrate is critical and it is necessary to optimise this in order to ensure efficient binding. However, this remains problematic and hybridisation efficiencies are low in commercial systems. The sensitivities are also limited. In the case of DNA analysis, it is necessary to use polymerase chain reaction (PCR) technology to 'amplify' samples. PCR creates multiple duplicates of a piece of DNA, increasing the sample size and thus helping to counterbalance the poor sensitivity of the microarray analysis. However, for proteins there is no equivalent. Moreover, in protein analysis, antibodies are typically bound to the solid substrate, but there are no established methods for the efficient immobilisation of antibodies such that more than a small percentage of them are able to bind to their complementary antigen. Consequently, there is a tension between the limitations of the methods, on the one hand, and their potential benefits to biologists through provision of very high throughput on the other.

The improvements in sensitivity that may potentially be realised through miniaturised systems, and the possibilities offered for high-throughput screening via large numbers of miniaturised experiments in parallel, have sparked enormous interest in the development of methods for handling small volumes of materials. Interest in microfluidic systems; i.e., miniaturised flow systems, has been growing rapidly, not just for applications in biology, but more generally across the field of analytical science. The concept of a lab-on-a-chip has now firmly taken root. The basic idea, called micro total analysis, is to undertake a complete process on a single miniaturised chip, typically fabricated from silicon or a polymer such as PDMS. The chip consists of miniaturised reactors, flow channels for manipulating samples and miniaturised valves for controlling the flow of reagents. Flow in small channels presents some interesting technological challenges. Fluid behaviour is often dominated by the interfacial free energy of the fluid–solid interface. Whereas in a macroscopic flow channel or reactor, the surface region is a small fraction of the total fluid volume, this is not true in a channel that is $\sim$10-100 μm wide. Reynolds numbers may also be very low, leading to laminar flow, in which separate streams of fluid converging into a single channel may proceed essentially unmixed (Section 8.8.2). Some mixing does occur, by diffusion, at the interface between separate streams, but this may be controlled such that the width of the interface region remains small compared to the width of the flow channel or cell. The approach has used to produce multiple laminar streams to expose different parts of a cell to different reagents. By controlling the flow velocity, channel geometry, channel wall interactions and diffusion coefficient of specific reagents, they could exert significant control over the composition of the laminar flow stream reaching a particular cell (Figure 9.5).

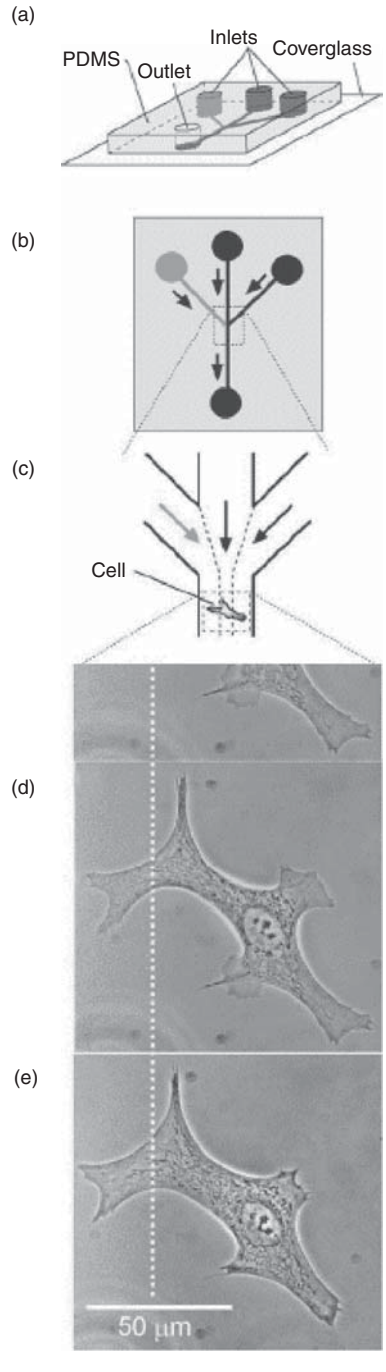

Figure 9.5 Use of microfluidic channels to generate two spatially localised populations (fluorescently labelled and unlabelled) of mitochondria within a single bovine capillary endothelial (BCE) cell. Reproduced with permission from S. Takayama, E. Ostuni, P. LeDuc, K. Naruse, D. E. Ingber and G. M. Whitesides, *Chem. and Biol.* **10**, 123 (2003)

The movement of fluids in small channels presents a variety of technological challenges. One approach is to use pressure-driven flow, by analogy with a macroscopic pumping system. Miniaturised pumps may be used. An alternative approach is to use centrifugal flow, in which the microfluidic system is spun so that fluid is forced outwards through channels. Controlling the flow of very small volumes can be problematic, and as channels become very small, larger pressure differentials are required. Electroosmotic flow provides an alternative approach. If the walls of the flow channel are charged, then the fluid in the layer close to the walls will be charged, containing a higher than average density of counterions. If an electric potential is applied parallel to the flow channel, then this fluid will move under the influence of the resulting electric field. The result is a convective motion.

A variety of separation and analytical devices may be combined with a microfluidic network to yield the micro total analysis system. Separation is a critical operation in biological analysis, where samples may often be complex and contain multiple components. Nanotechnology is providing new tools for sample handling including separation, and these tools may ultimately be integrated into micro total analysis systems. One illustration is the use of lithographic techniques to fabricate chambers containing regular arrays of silicon pillars that provide obstacles to the movement of DNA molecules when the sample is added. The structure has a regular and controllable structure, in contrast to the gels more conventionally used in biological analysis, and is thus subjectable to a rigorous physical analysis, permitting accurate and effective separation of DNA fragments by their molecular weight.

Magnetism provides a useful approach to separation of biological components. Although biological molecules and cells are strongly influenced by electric fields, they are typically non-magnetic. Attachment to magnetic nanoparticles thus provides an elegant method of moving them in a very precisely controlled fashion through a miniaturised experimental system. Superparamagnetic particles have been used to separate DNA in a microchannel device fabricated using soft lithography. Under the influence of a magnetic field, the particles may not only be caused to move but also to organise themselves (e.g., into columns). If suitable linkers are bound to the surfaces of the particles (e.g., pairs of molecules with a strong recognition, such as biotin and streptavidin) then the arrangement may be made permanent when the magnetic field is removed.

Fluorescent labelling provides a powerful and widely used approach to the investigation of cellular structure and organisation. Biochemical probes (often antibodies) functionalised with fluorescent dyes are used to highlight structural features (e.g., the actin cytoskeleton in cells is often highlighted using FITC-conjugated phalloidin). Nanoparticles offer a different approach to analysis with ultra-high sensitivity. Quantum dots made of materials such as cadmium selenide may have high fluorescence yields and offer the possibility of conducting optical analyses at high exposures without the problems of photobleaching commonly associated with fluorescent labels. However, they are also cytotoxic so there has been interest in trying to derivatise them with molecular adsorbates in order to render them inert in the cellular environment. One approach has been to use alkanethiols, which interact reasonably strongly with CdSe nanoparticles and passivate them. Other approaches exist based on gold nanoparticles, which may also be derivatised using alkanethiols and utilized as probes for cellular structure.

9.1.4 Organisation of biomolecular structure at the nanometre scale

There has been growing interest in the manipulation of biomolecular structure on length scales approaching molecular dimensions. This is a far more challenging undertaking than the examples discussed in the preceding section, which really only explore structural effects on micron length scales. There are several motivations for seeking to manipulate molecular organisation on smaller length scales, including the possibility of fabricating highly miniaturised devices for biomolecular detection and analysis (extrapolating the gains in sensitivity and throughput realised through the adoption of miniaturised micron-scale analytical systems) and the desire to build systems for fundamental investigations of biological interactions and organisation (e.g., the fabrication of arrays of proteins molecule by molecule to challenge cells or investigate molecular recognition). There has been much more speculative discussion of bioelectronic devices too. By and large, however, it is fair to summarise the state of the art as focused on the development of new tools – working devices will require much further development.

Microcontact printing (μCP), in which an elastomeric stamp is formed by casting PDMS against a (usually) silicon master, has already been described in Section 1.4. μCP provides a rapid, straightforward and flexible method for the deposition of molecules in patterns on surfaces, and has attracted a great deal of interest as a means for organising biological molecules and cells. The earliest application of μCP was the deposition of alkanethiols onto gold. These structures were used to guide cellular attachment, or to pattern the adsorption of biological molecules. However, it is possible to stamp other molecules, including alkylsilanes onto silicon dioxide to form patterned monolayers, and polymers. The stamping of polymers is an attractive recent development, because of their stability and also because of the possibility of generating molecular relief in this way.

Although there are reports in the literature of the fabrication of structures with dimensions less than 100 nm by microcontact printing, physical limitations in the process have led researchers to explore methods based on scanning probe microscopy for the fabrication of patterns with dimensions approaching the molecular level. Dip pen nanolithography (DPN) is a method which offers much by way of analogy with microcontact printing. In DPN molecules are deposited on the surface from an atomic force microscope tip, rather than an elastomeric stamp (Figure 9.6). The tip is inked with a solution of the molecule of interest and brought into contact with the sample surface. Under normal ambient conditions, a capillary forms between an AFM tip and the surface that it contacts. In DPN this capillary functions as a liquid bridge to facilitate transfer of fluid from the tip to the surface. Control of the ambient humidity therefore has an influence on the sizes of the features formed. The first demonstrations of DPN were based on the deposition of alkanethiols onto gold surfaces. However, a variety of molecules may be deposited, including alkylsilanes, which form monolayers on silicon dioxide, and conducting polymers, by using an electrochemical AFM to polymerise 3,4-ethylenedioxythiophene during deposition. By writing simultaneously with an array of cantilevers, DPN also offers the possibility of generating multiple structures in parallel. One of the widely quoted criticisms of lithographic methods based on SPM is that, unlike conventional photolithography (a parallel process in which an entire circuit incorporating a large number of features may be fabricated in a single

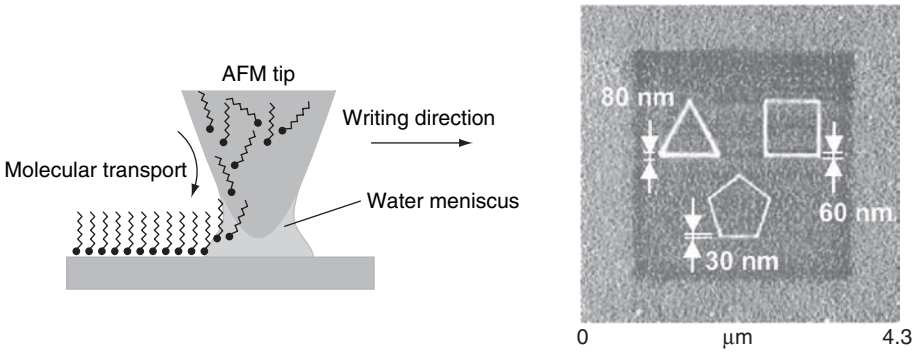

Figure 9.6 Left: schematic diagram showing the basic principle behind DPN. Right: polygons drawn by depositing mercaptohexadecanoic acid onto gold using DPN. The area around the polygons has been overwritten with a monolayer of octadecanethiol. Reproduced from S. Hong, J. Zhu and C. A. Mirkin, *Science* **286**, 523 (1999)

process), they are serial methods – features are created one after the other. Serial writing is time-consuming, and this has been an obstacle to the exploitation of electron beam lithography for electronic device manufacturing. However, the invention of the Millipede by Gerd Binnig and co-workers has provided a new paradigm for nanofabrication using SPM. The Millipede is a microelectromechanical device consisting of several thousand AFM-type cantilevers, each capable of being separately and simultaneously actuated. It thus provides the capability to implement a massive number of serial lithography functions in parallel, meaning that its capability is comparable to that of a parallel fabrication method.

A variety of biological nanofabrication processes have been demonstrated using DPN. One approach is to write thiol structures onto a surface and then attach biological molecules to these (see Figure 9.6 for an example). Patterns of mercaptohexadecanoic acid (MHA) were created on a gold substrate, then DNA was coupled to them. Gold nanoparticles functionalised with a complementary sequence of DNA were then bound to these. An alternative but equivalent approach is to deposit MHA by DPN and then use these nanopatterns as a resist for etching the underlying gold substrate. This allows gold to be removed from the sample, except where MHA is adsorbed, leading to the formation of MHA-capped gold nanostructures. After removal of the MHA by exposure to UV light, bare gold nanostructures are formed which may then be functionalised with thiol-linked DNA molecules. Gold nanoparticles derivatised with a complementary sequence may be bound to these. Thiol-linked oligonucleotides may also be deposited directly onto gold surfaces by incorporating the oligonucleotide into the ink. In this way, DNA features about 150 nm wide were written. Similar strategies have been applied to the deposition of proteins. MHA has been patterned onto gold and then bare regions between the MHA features have been filled in with an oligoethylene glycol (OEG) functionalised thiol. Proteins are extremely adhesive, and in the fabrication of any miniaturised protein structure, the inhibition of protein adsorption is a critical concern. Polyethylene glycol is a polymer that is highly resistant to the adsorption of proteins, and it has been demonstrated that OEG-terminated thiols are also highly resistant to protein adsorption and thus a powerful tool for the manipulation

of biological organisation at surfaces. MHA/OEG patterns have been exposed to immunoglobulin G (IgG) leading to adsorption only on the MHA-functionalised regions of surface. It is also possible to deposit protein directly, however. An AFM tip has been inked in a solution of IgG and written directly onto bare silicon dioxide and silicon dioxide modified with aldehyde functionalities to covalently bind the protein.

DPN is a method based on the deposition of molecules, but other methods exist that enable the selective removal of material. One approach is to physically scrape adsorbates from a solid substrate, an approach known as nanoshaving. In nanoshaving, the tip of an AFM is scanned across a region of surface at elevated load while submerged beneath a solution of a thiolated molecule. As thiols are displaced from the surface, it is refunctionalised with fresh molecules adsorbing from the solution phase. The approach may be extended to the immobilisation of DNA, by shaving the thiol layer with a thiol-linked DNA strand in the solution phase. An AFM tip has been used to scratch holes in monolayers of thiol-linked DNA molecules. The thickness of the functionalised DNA monolayer was measured from the height of the step between the top of the monolayer and the adjacent region of surface. A thiolated DNA strand of a different (longer) length was then inserted into the holes. The surface was then incubated with a still longer DNA strand capable of hybridising to immobilised sequences, and again, binding could be verified by measuring the change in height in AFM images. Nanoshaving may also be used to immobilise proteins. Lysozyme has been adsorbed to charged patches introduced to methyl and oligoethylene glycol terminated surfaces, and has been covalently bound to patterned structures created by nanoshaving. Small patches have been created in an oligoethylene glycol terminated monolayer, and three different thiols have been adsorbed to them, terminated by methyl, amine and carboxylic acid groups. When the sample was exposed to a solution of lysozyme, the protein adsorbed only to the carboxylic acid functionalised region (Figure 9.7).

Photopatterning provides a convenient way to pattern self-assembled monolayers (SAMs) of alkanethiols, and has proved a useful method for patterning biological organisation at the micron scale. On exposure to UV light in the presence of air, alkanethiolates are oxidised to weakly bound alkylsulfonates which may be readily

[Image not available in this electronic edition.]

Figure 9.7 AFM topographical images showing three differently charged patches of area about 400 nm × 400 nm before (left) and after (right) exposure to lysozyme. In each case, (a) is neutral (b) is positively charged and (c) is negatively charged. Reproduced from X. Zhou, L. Wang, R. Birch, T. Rayment and C. Abell, *Langmuir* **19**, 10557 (2003)

displaced by solution-phase thiols. Exposure of the monolayer through a mask leads to spatially selective oxidation, so the subsequent immersion step results in the formation of the new chemistry in exposed regions while retaining the original chemistry in masked areas. The diffraction limit of $\lambda/2$ associated with optical techniques has generally been regarded as placing a lower limit on the sizes of features that may be created photolithographically. However, a scanning near-field optical microscope coupled to a UV laser has been used to break the diffraction limit and fabricate features as small as $\lambda/9$. This approach has been called scanning near-field photolithography (SNP). Lines were traced in fresh monolayers, leading to selective oxidation in regions as narrow as 25 nm (see Figure 8.5). Immersion of the sample in a solution of a contrasting thiol led to formation of a chemical pattern. Immersion in a solution of an etchant for the underlying gold substrate led to its selective removal, resulting in the formation of nanotrenches as narrow as 50 nm. Patterned SAMs formed in this way may be functionalised using proteins and polymer nanoparticles, opening up a variety of strategies for surface functionalisation. Finally, hydrogen-passivated silicon samples were also patterned under a layer of fluid alkene, leading to selective attachment to the surface and forming nanostructured alkylsilicon structures. The ability to pattern a wide range of materials with nanometre spatial resolution, combined with the capability to function in a fluid medium means that SNP potentially provides a route to the rapid fabrication of complex, multicomponent arrays with nanoscale features, a valuable and unique possibility.

9.2 BIOMIMETIC NANOTECHNOLOGY

Biology offers some outstandingly elegant and effective examples of the principles of self-assembly. It is also a truism that cell biology offers a compelling existence proof that a radical nanotechnology, involving sophisticated machines and mechanisms, is possible. In this section we consider some examples where mechanisms from biology can be exploited to make nanoscale devices. This is clearly an area of huge potential, and these initial efforts will surely be extensively built on in years to come.

9.2.1 DNA as a nanotechnology building block

The remarkable and very specific base-pairing mechanism that underlies the operation of DNA makes this molecule a very attractive candidate as a building block for creating complex nanostructures by self-assembly.

The key features that underly the self-assembly of DNA are as follows. A single DNA chain is a sequenced copolymer in which one of four possible bases are attached to a backbone of alternating sugar and phosphate groups. The four bases are adenine (A), guanine (G), cytosine (C) and thymine (T) and they form complementary pairs – A and T, C and G – in which strong, multiple hydrogen bonds hold the complementary pairs of bases in an edge-to-edge configuration. Two complementary strands of DNA will strongly associate to form a double helix. In nature DNA always exists as a pair of strands that are complementary to each other over their whole length; these associate to

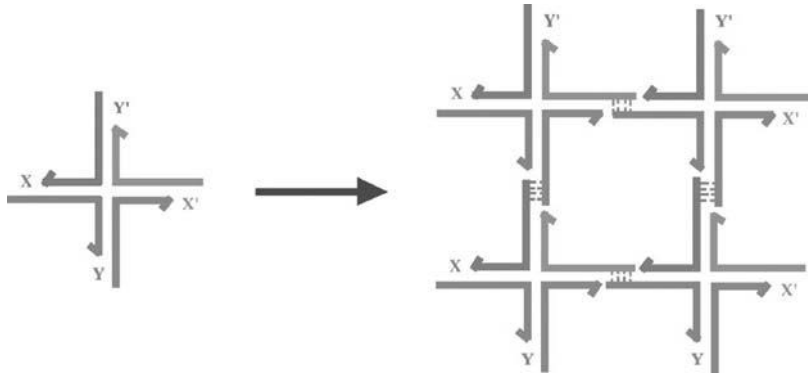

Figure 9.8 Formation of a 2D lattice from a junction with sticky ends. X and Y are sticky ends and X′ and Y′ are their complements. Four of the monomers on the left are complexed to yield the structure on the right. DNA ligase can close the gaps left in the complex, which can be extended by the addition of more monomers. From N. C. Seeman and A. M. Belcher, *Proc. Natl Acad. Sci. USA* **99**, 6451 (2002)

form a single, linear double helix. However, synthetic methodologies exist that allow one to make DNA strands with an arbitrary sequence of bases. This allows one to design more complicated topologies. Consider three strands with the following sequences. Strand 1 can be thought of as two sequences, X and Y, joined together. Strand 2 consists of X′, the complementary sequence to X, and another sequence Z. Strand 3 consists of Z′, the complementary sequence to Z, joined to Y′, the complementary sequence to Y. When these three strands are put together, they associate to form a branch point where three double helices merge. Many complex junctions can be designed in this way.

The key structural unit in building three-dimensional structures from DNA is the sticky end. Imagine a pair of complementary DNA strands, one of which has been extended by a short additional sequence. This short piece of single DNA is available for bonding with a sequence of bases on another DNA helix with the complementary sticky end. As shown in Figure 9.8, if branched DNA constructs can be prepared with properly designed sticky ends, they will self-assemble in two or even three dimensions.

This area of research provides an impressive proof-of-principle demonstration of the power of biomolecular self-assembly, but does it have any practical applications? Three possibilities stand out: firstly, as guides for the growth and interaction of nanoparticles; secondly, as templates for molecular electronics, and thirdly, to create nanomachines and motors. These are discussed in turn below.

9.2.1.1 Directed assembly using DNA

Nanoparticles will interact strongly with each other, but these interactions are typically non-specific and irreversible. It is possible, by attaching definite sequences of DNA to the surface of the nanoparticles, to program specific interactions that allow the nanoparticles to be used as building blocks to be assembled in quite precisely determined

ways. Suppose we have two different types of nanoparticles, which we call A and B, and we want to arrange them in a three dimensional structure in which the A particles and B particles alternate. This may be achieved in a very elegant manner by creating two different, non-complementary sequences of DNA. One sequence is attached to particle A, the other to particle B. For example, if the nanoparticles are gold colloids, and the DNA sequences are terminated with an alkanethiol group, the DNA can be easily end-grafted at the surface of the particles to form a polymer brush. If the two types of particle are mixed, each with their different sequences of DNA grafted on, there is no interaction. The DNA sequences, not being complementary, do not interact, and the nanoparticle suspension behaves simply as a sterically stabilised colloid. However, a DNA duplex with two sticky ends – one complementary to the sequence on the A particles, and the other complementary to that on the B particles – will act as a specific linking agent which will join A particles to B particles.

9.2.1.2 DNA as a template for molecular electronics

Can one use structures self-assembled from DNA to make electrical circuits? The work described in the previous section makes it clear that circuits of quite complex topologies can be formed by self-assembly from mixtures of DNA strands with carefully designed sequences. Unfortunately, the electrical properties of DNA molecules by themselves do not seem to permit the use of the molecules as the basis of electronic devices. It seems that DNA is an insulator. The early literature on this subject is confusing, with values of resistivity being reported that vary by ten orders of magnitude, but more recent results confirm its status as a good insulator.

Although DNA molecules cannot be used directly as molecular wires, it is possible to use them as templates to grow nanowires. In the first demonstration of this, DNA with thiol terminations at each end was attached to gold electrodes, spanning the 12–16 μm gap between them. To convert the insulating DNA strand into an electrical conductor, the first step was to exchange the sodium counterions of the negatively charged DNA chain with silver ions. These silver ions were then reduced using a basic hydroquinone solution to give a string of silver nanoparticles along the DNA backbone. The system was then exposed to an acidic solution of hydroquinone and silver ions which, when illuminated, led to the deposition of more silver on the existing nanoparticle nuclei until a continuous silver nanowire was obtained.

By combining the idea of using DNA to make nanowires with its role in directing the assembly of nanoparticles, it is possible to self-assemble a functioning molecular electronic device. A specific sequence of single-stranded DNA was complexed with a protein molecule. Self-assembly was then used to direct this protein-decorated DNA strand to a specific, complementary strand of DNA. A carbon nanotube decorated with streptavidin was then directed to this DNA strand via an antibody to the bound protein. The DNA strand was then metallised with silver; the section of DNA to which the nanotube was bound was protected against metallization by the bound protein. The result was a semiconducting nanotube well connected to two nanowires, which, when a gating voltage was applied to the substrate, behaved as a field-effect transistor.

It is not difficult to see that by combining the ability of DNA to form complex, self-assembled three-dimensional structures with these methods of selectively metallising the

DNA and controllably binding, in selected locations, semiconducting elements such as nanotubes, semiconductor nanowires or conducting polymer molecules, it may be possible to take the first steps towards creating integrated circuits based on nanoscale semiconducting elements.

9.2.1.3 DNA-based motors and nanomachines

The specificity and predictability of the base-pair interaction makes it possible to design not just sequences that produce specific structures by self-assembly, but structures that yield specified function, such as catalysis. This allows one to go beyond DNA-based structures to actual, functioning nanomachines.

Figure 9.9 shows a particularly elegant scheme that illustrates the principles. Here a catalyst molecule M has been designed which binds to the complex QL′, opening up the loop. A strand L, which is complementary to L′, then displaces the catalyst molecule M, which is then free to begin another cycle. During this cycle the catalyst molecule undergoes a cyclic conformational change from coil to rod and back to coil again; in principle this conformational change could be exploited to do work. The energy source for this work is the difference in base-pair binding energies between the complex QL′ and the duplex LL′. The key element in the design is that each of the stages results in a lowering of the free energy of the system without the need to overcome an energy barrier.

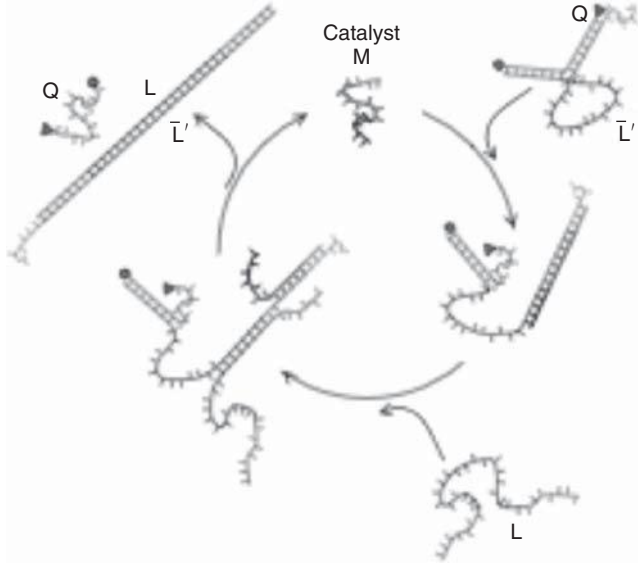

Figure 9.9 A free-running DNA nanomachine. The catalyst molecule M undergoes cyclic conformational changes when supplied with the 'fuel' – the complex QL′ and strand L, the complementary strand to L′. Free Q and the LL′ duplex represent the 'exhaust'. From A. J. Turberfield, J. C. Mitchell *et al.*, *Phys. Rev. Lett.* **90** (2003) 118102

9.2.2 Molecular motors

Some of the most striking nanoscale machines to be found in biology are molecular motors, nanoscale assemblies of proteins that convert chemical energy directly to mechanical energy, usually with remarkable efficiency. These motors are found in simple, single-celled prokaryotes such as the bacteria *E.coli* and in complex, multicellular eukaryotic organisms such as human beings, and they perform a wide variety of functions. These functions include propelling single-celled organisms, moving materials and structures around within eukaryotic cells, and providing the power for the muscles of multicellular animals. Many different molecular motors have evolved during the history of life, and here we consider only a few representative examples.

The study of biological molecular motors is clearly of central importance to molecular biology and biophysics. Their importance for nanotechnology is twofold. Firstly, they offer us some remarkable models to emulate. Biological motors have evolved to a considerable pitch of perfection, and the way they operate – exploiting distinctive features of the nanoworld such as the dominating presence of Brownian motion and the strength of surface forces – and the way they operate gives us valuable clues about the design rules we would need to follow in creating synthetic molecular motors. Secondly, they offer us working, off-the-shelf components that can be disassembled from the cells in which they are found and reassembled as part of hybrid structures combining synthetic and biological nanotechnology.

Before we discuss the operational mechanism of biological motors in detail, it is worth making some general comments. Biological motors are quite unlike the heat engines which we are familiar with on the macroscale. Like a petrol engine, a biological motor converts the chemical energy of a fuel into useful mechanical work. But the resemblance ends there. In a heat engine the chemical energy is first converted into heat, and then work is extracted from the flow of heat energy from a hot reservoir to a cold reservoir. In a biological motor, energy is converted from chemical energy to mechanical work directly in conditions of constant temperature. The mechanism of this conversion relies on the coupling of a cyclic chemical reaction – often the hydrolysis of the energy storage molecule adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and a phosphate ion – to a conformational change that occurs in the motor protein in response to this reaction.

It is worth stressing at the outset that biological motors can reach astonishingly high efficiencies, despite the apparent difficulties of the nanoscale environment in which they operate. To someone steeped in the tradition of macroscale mechanical engineering, the prospect of making a motor that works in conditions of very high dissipation, with the constant agitation of Brownian motion, with surfaces that are prone to stick together, using components which lack the rigidity that we would assume was a prerequisite for any kind of sensible design, seems very unpromising. Biological motors succeed because they exploit these special features of the nanoworld.

Moving on to specifics, we can distinguish between linear motors, in which a molecule moves along a track, and rotary motors, which generate a spinning motion. Linear motors include various types of myosin motor, including the motors that drive our muscles, and the kinesin motors that are used for transporting organelles within cells. Rotary motors include the bacterial flagellar motor, which bacteria use to swim with, and ATP synthase, a remarkable and complex enzyme that synthesises the energy storage molecule ATP.

9.2.2.1 The operation of biological motors

The detailed way in which biological motors operate is now beginning to be understood in some detail, largely thanks to single-molecule experiments of the kind introduced in Section 8.1.2, together with detailed structural studies using X-ray diffraction and high-resolution electron microscopy. A good review for the linear motor systems myosin and kinesin is provided by Vale and Milligan.

In these linear motors, there are two key elements. There is a binding site, to which the energy storage molecule ATP binds, and there is a sticky patch which reversibly attaches the motor protein to the linear track along which it runs – actin filaments in the case of myosin, microtubules in the case of kinesin. In the case of myosin, the cycle begins with the binding of an ATP molecule to the ATP binding site. With ATP bound, the association between myosin and the actin filament is at its weakest, and the myosin head becomes detached from the track. The next stage is the hydrolysis of ATP to ADP and a phosphate group. In this state the myosin head binds to an actin filament. The phosphate group then leaves the catalytic site; this triggers a substantial conformational change in the myosin molecule. The force generated by this conformational change provides the power stroke of the motor. Dissociation of the ADP molecule soon follows, after which another ATP molecule binds to the catalytic site, the myosin becomes detached from the actin filament and the cycle begins again.

This same coupling of conformational change and chemical reaction also underlies the operation of rotary motors. The smallest rotary motor, as well as the most important, is the enzyme ATP synthase. A ubiquitous feature of all life, it uses the energy stored in a gradient of hydrogen ions to synthesise the energy-containing molecule adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and a phosphate ion, and generates rotational motion in the process. Alternatively, the machine can be run backwards, using ATP as a fuel to pump hydrogen ions across a membrane. This complex assembly of proteins has two main parts. The hydrogen ion pump is called F_0; this sits in a membrane and includes the channel through which protons move. The unit that either synthesises or hydrolyses the ATP is called F_1; this consists of six subunits connected to the F_0 unit by a fixed stalk. Threaded through the middle of the six subunits of F_0 is a rotating shaft. When ATP synthase operates as a pump, conformational changes coupled to the hydrolysis of ATP in the six subunits of the F_1 component cause the shaft to rotate. The rotation of the part of the shaft that threads the F_0 component pumps hydrogen ions through the ion channel. Conversely, when the machine is synthesising ATP, it is the motion of hydrogen ions through the ion channel that causes the rotation of the shaft.

The performance of ATP synthase is astonishingly good. When it operates as a pump, each bound ATP molecule leads to a stepwise 120° rotation. The energy of hydrolysis of ATP is converted into a strain energy of about $24\,k_B T$, which is converted into mechanical energy with approaching 100% efficiency, allowing the motor to develop a torque of up to 45 pN nm^{-1}. This high efficiency underlines the most important point to be made about biological motors. They are not heat engines, so their operating efficiency is not bounded by the Carnot cycle limit of classical thermodynamics. Instead they exploit Brownian motion and molecular conformational change to achieve extremely high performance; evolution has led to designs that are finely optimised for operation in the nanoscale environment.

9.2.2.2 Biological motors as components of synthetic systems

The remarkably good performance of biological motors makes the idea of using them in synthetic nanodevices extremely attractive; currently no synthetic molecular motors exist with anything like the performance of these natural machines. The drawbacks are that these systems need to operate in something approximating physiological conditions. Progress towards integrating biological molecular motors into synthetic nanodevices is currently at the proof-of-principle stage rather than the device creation stage. Nonetheless, the important principle these demonstrations establish is that biological motors can be removed from their biological contexts and operated with the same high efficiencies attained in the cell environment.

Perhaps the best-developed scheme for using biological motor proteins in synthetic environments has emerged from techniques developed to characterise protein linear motors, known as motility assays. For example, in a gliding assay, a layer of linear motor proteins is immobilised on a surface. The most commonly used motors to date have been kinesins. Microtubules are then introduced into the solution in contact with the surface. The density of motor proteins on the surface needs to be sufficiently high, and the microtubules need to be sufficiently long, so that at least three kinesin molecules attach to each microtubule. Then if fuel in the form of ATP molecules is supplied to the solution, the microtubules will move continuously across the surface, powered by the kinesin motors.

One can imagine kinesin-coated surfaces such as this serving as the basis for molecular shuttles, moving molecules around from place to place in a nanoscale chemical plant, for example. But to achieve this goal, two problems need to be overcome. Firstly, one needs to find a way of guiding the microtubules to direct their motion to where their cargos are needed, and secondly, one needs to find a way of loading and unloading the cargo onto the microtubules.

Suggested methods for guiding the motion of microtubules include selectively adsorbing the motor proteins on predetermined 'tracks'. This could be done by writing lines on the surface that would selectively adsorb the motor proteins using electron beam lithography, photolithography or a soft lithography technique. Alternatively, topography could be used; if the motor proteins were adsorbed to the bottoms of physical channels, the motion of the microtubules could be constrained to move only in the directions defined by the channels. Another approach to steering the motion of microtubules would use an externally applied field. Electric fields could be applied by means of a patterned array of electrodes on the surfaces, while magnetic fields might be practical if the microtubules were decorated with magnetic microspheres.

Binding of cargo molecules to the microtubules could be carried out by specific protein–ligand bonding pairs. One demonstration of this principle involved treating the microtubules with biotin, and attaching a streptavidin-coated bead to the microtubule using the specific interaction between streptavidin and biotin. However, it is probably fair to say that a great deal of work remains to be done before the gliding assay can be converted into a mechanism for directed nanoscale transport in useful devices.

One other spectacular demonstration of the integration of biological motors with synthetic systems involves the rotary motor part of ATP synthase, F1-ATPase (Figure 9.10). An array of nanostructured nickel posts was made by electron beam lithography, and self-assembly was used to mount a rotary motor on each post. Finally, nickel

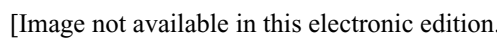

Figure 9.10 Schematic diagram of an F1-ATPase biomolecular motor-powered nanomechanical device, consisting of (A) an Ni post (height 200 nm, diameter 80 nm), (B) the F1-ATPase biomolecular motor, and (C) a nanopropeller (length 750–1400 nm, diameter 150 nm). The device (D) was assembled using sequential additions of individual components and differential attachment chemistries. From R. K. Soong, *et al*., *Science* **290**, 1555 (2000)

nano propellers were attached to each motor using the specific binding between streptavidin and biotin. In the resulting structure, the propellers turned when exposed to a solution of ATP.

9.2.3 Artificial photosynthesis

There are very few situations in nature where coherent electron transport is important, so molecular electronics does not have many biological analogues. The vital exception to this rule is in photosynthesis, where highly optimised complexes of proteins and dye groups efficiently harvest light energy and convert it into chemical energy. Considerable efforts have been made to understand this process, and on the basis of this understanding, attempts are being made to replicate the process synthetically. In some cases only the broad outline of the design philosophy is used to make novel solar cells, whereas in other cases photosynthesis is copied more directly.

Photosynthesis takes place in structures that are enclosed in membranes (Figure 9.11). In the simplest photosynthesising systems, purple bacteria, the photosynthetic membrane is located just within the cell wall and encloses the whole cell, whereas in green plants, photosynthesis takes place in the membranes of specialised organelles called chloroplasts.

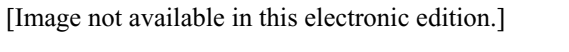

Figure 9.11 Schematic representation of an artificial photosynthetic membrane. The lipid bilayer of a liposome vesicle contains the components of a light-driven proton pump, which when illuminated leads to transport of hydrogen ions into the liposome interior. The resulting hydrogen ion gradient drives the production of ATP in ATP synthase assemblies inserted in the membrane. From D. Gust, T. A. Moore *et al.*, *Acc. Chem. Res.* **34**, 40 (2001)

At the heart of the photosynthetic system is the dye molecule chlorophyll (or bacteriochlorophyll in purple bacteria). Light energy is converted here into an electron–hole pair, an exciton. To harvest the energy of light, this exciton needs to be separated before the electron and hole have a chance to recombine. This is accomplished by the transfer of the electron through three different dye molecules, all of which are held in a well-defined spatial relationship with each other, with the chlorophyll molecules and with the membrane in a large complex of protein molecules – the photosynthetic reaction centre. The electron is finally transferred to a mobile dye molecule, quinone. This initiates a series of reactions which have the ultimate effect of oxidising a source of hydrogen and creating a higher concentration of hydrogen ions inside the the membrane than outside. This hydrogen ion gradient is then used to drive membrane-bound ATP-synthase complexes, storing the energy in the form of ATP, which can then fuel all the other biochemical processes the cell needs to carry out.

In the simplest photosynthetic bacteria, the source of the hydrogen ions is the reduction of hydrogen sulfide. But with profound consequences for the earth, evolution devised a modified scheme involving two photosynthetic systems running in tandem, whereby hydrogen ions could be obtained from water, leaving oxygen as a by-product. This is the process used by green plants.

One final refinement that should be mentioned is that the efficiency of photosynthesis is greatly increased by the use of *light-harvesting complexes*. These complexes consist of a great many dye molecules, perhaps tens or hundreds, bound together with proteins in a precise spatial relationship. The different dye molecules absorb light in different wavelength bands, and the resulting exciton is passed extremely rapidly through the complex until it reaches the chlorophyll molecules of the reaction centre. In this way, the efficiency of photosynthesis can be maximised by ensuring that all wavelengths of light are efficiently converted into electrical energy.

Photosynthesis offers a powerful model for the conversion of solar energy into electrical and chemical energy. Attempts to emulate it synthetically fall into two categories; those systems in which the mechanisms of photosynthesis are copied rather faithfully, and systems in which only the most general operating principles are emulated.

The first category includes synthetic molecules, typically derivatives of porphyrins, sometimes bound to C_{60} fullerene, which can be inserted into a lipid bilayer. Using the spatially extended conjugation of the molecules, an exciton generated by the absorption of light can be split into an electron and a hole, and the energy used to pump hydrogen ions across the membrane. If these molecules are inserted into the membrane of a lipid vesicle or liposome, then the interior of the vesicle will be steadily made more acid on exposure to light. This requires, of course, that these asymmetric molecules are all inserted into the membrane with the same sense. The hydrogen ion gradient can then be used to power ATP synthase complexes, resulting in the storage of the light energy in ATP.

This synthetic scheme follows the biological example very closely, and results in the synthesis of the biological energy-carrying molecule ATP. At the other end of the spectrum are schemes which borrow only the most general concepts from photosynthesis. The most well developed of these are the photovoltaic and photoelectrochemical devices employing titanium dioxide sensitised by the surface adsorption of dyes, invented by Grätzel. The analogy between these important devices and photosynthesis lies purely at a conceptual level. There are two important processes that must take place in a photovoltaic device. The energy of a photon must be absorbed in the creation of a bound electron–hole pair (an exciton), and then the exciton must be split into a separate electron and hole, which are then transported to their respective electrodes. In a conventional photovoltaic, both processes take place in a bulk semiconductor. As we have seen, photosynthesis relies on excitons being formed in dye molecules highly optimised for the purpose. Grätzel cells similarly separate the processes of exciton formation and charge transport; light is absorbed by a dye that is absorbed at the surface of a nanostructured wide band gap semiconductor (typically titanium dioxide). An electron is injected into the TiO_2, and the circuit is completed by a hole-carrying electrolyte or p-type semiconductor. The advantage of Grätzel cells over conventional semiconductor photovoltaic cells is that they combine reasonable efficiencies with the potential for low-cost solution-based processing.

9.3 CONCLUSIONS

Nanotechnology has provided us with a multitude of new tools to explore biological systems on small length scales. The opening up of single-cell and single-molecule phenomena to experimental investigation represents an important step forward, both conceptually and philosophically, and promises to yield important new insights into how biological systems are assembled. The availability of miniaturised systems for use in molecular separation and analysis will not only catalyse the exploration of single-molecule phenomena but will also provide important new methodologies by which the genome revolution may ultimately be translated into practical reality through an enhanced understanding of disease and through the development of new therapeutic procedures.

Cell biology, on the other hand, offers nanotechnology some remarkable exemplars of nanoscale devices and machines. These can be exploited directly in hybrid systems, or used as inspiration for synthetic devices drawing on the same physical principles.

BIBLIOGRAPHY

General

P. Ball, Natural strategies for the molecular engineer. *Nanotechnology* **13**, R15–R28 (2003).
R. A. L. Jones, *Soft Machines*, Oxford University Press, Oxford (2004).

DNA nanotechnology

E. Braun, Y. Eichen *et al*., DNA-templated assembly and electrode attachment of a conducting silver wire. *Nature* **391**, 775–778 (1998).
K. Keren, R. S. Berman *et al*., DNA-templated carbon nanotube field-effect transistor. *Science* **302**, 1380–1382 (2003).
C. A. Mirkin, Programming the assembly of two- and three-dimensional architectures with DNA and nanoscale inorganic building blocks. *Inorganic Chemistry* **39**, 2258–2272 (2000).
C. M. Niemeyer, Nanoparticles, proteins, and nucleic acids: biotechnology meets materials science. *Angewandte Chemie International Edition* **40**, 4128–4158 (2001).
C. M. Niemeyer and M. Adler, Nanomechanical devices based on DNA. *Angewandte Chemie International Edition* **41**, 3779–3783 (2002).
N. C. Seeman and A. M. Belcher, Emulating biology: building nanostructures from the bottom up. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 6451–6455 (2002).
A. J. Turberfield, J. C. Mitchell *et al*., DNA fuel for free-running nanomachines. *Physical Review Letters* **90**, 118102 (2003).

Molecular motors

H. Hess and V. Vogel, Molecular shuttles based on motor proteins: active transport in synthetic environments. *Reviews in Modern Biotechnology* **82**, 67–85 (2001).
R. K. Soong, G. D. Bachand *et al*., Powering an inorganic nanodevice with a biomolecular motor. *Science* **290**, 1555–1558 (2000).
R. D. Vale and R. A. Milligan, The way things move: looking under the hood of molecular motor proteins. *Science* **288**, 88–95 (2000).

Photosynthesis

D. Gust, T. A. Moore *et al*., Mimicking photosynthetic solar energy transduction. *Accounts of Chemical Research* **34**, 40–48 (2001).
M. Grätzel, (2001) Photoelectrochemical cells. *Nature* **414**, 338–344 (2001).