Biological molecular motors for nanodevices

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INTRODUCTION

One of the main aims of nanotechnology must be to incorporate moving parts into useful nanoscale devices. These moving parts may range from simple valves through to complex "conveyor belts" that will carry the building blocks for further construction from one place to another in an ordered and programmable manner—providing a version of the "molecular assembler", which was first described by Eric Drexler (Drexler, 1999; Drexler, 1992).

It is possible that some of the molecular motors, which will be used for some of these moving parts, will be artificial chemical constructs, or chemical molecular motors (Figure 1). One of the problems posed by many chemical motors has been the development of a simple mechanism to drive the motor away from an equilibrium position; the motor shown in Figure 1 uses a chemical system to ensure stability of each state of the motor. However, other methods have been developed such as the recently described "information ratchet" in which light is used to propel a chemical molecular machine (Serreli et al., 2007).

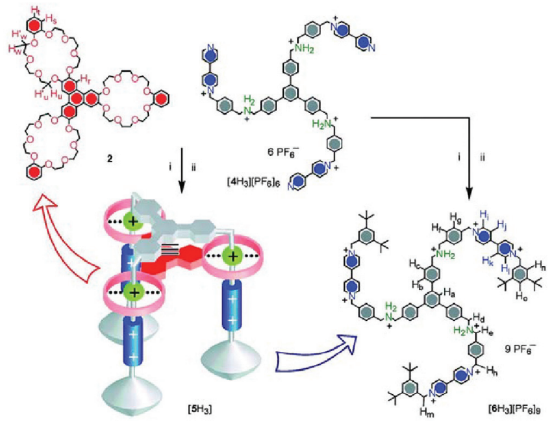

Figure 1. A molecular elevator based around chemical motors.
This chemical motor is only 3.5 nm by 2.5 nm and yet, through the integration of several chemical units is capable of generating a force of 200 pN per molecule. Importantly, the motor has an inbuilt mechanism that prevents the reverse reaction, allowing useful work to be performed at the expense of chemical energy. Reproduced with permission from Badjic et al., 2004 (see http://www.sciencemag.org/cgi/content/abstract/303/5665/1845).

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An alternative version of a chemical motor, which depends upon one of the most important biological macromolecules, is a DNA-based biochemical motor. The first description of such a motor involved a simple chemically induced transition in the DNA structure (Figure 2)—a structural flip between B- and Z-form DNA—and this movement was detected by placing fluorophores on the ends of the DNA molecules and measuring FRET[1] signals (Mao et al., 1999). This was quickly followed by a DNA-fuelled motor that could act as a simple tweezer system (Yurke et al., 2000) and used other DNA molecules as the fuel to enable motion. This motion was again detected through fluorescence output. The work has led to the idea of DNA molecules that could perform walks along a DNA "scaffold" with in-built directionality (Turberfield et al., 2003; Yin et al., 2004; Yin et al., 2005a; Yin et al., 2005b). This "DNA walker" (Figure 3) would mimic the transport system of a cell (kinesin walking along microtubules) in a truly chemical system, possibly with cargo being detached from the moving DNA by means of the "molecular scissors" of biology—DNA restriction enzymes.

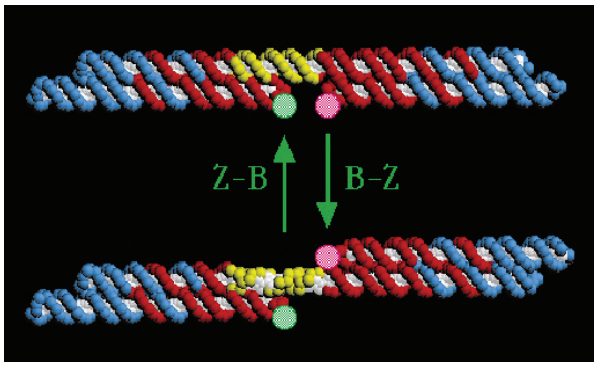

Figure 2. A DNA motor based on B to Z transitions.
This simple chemical motor depends upon the properties of DNA (Mao et al., 1999), which is capable of adopting different conformations dependent upon the buffer composition (Zhang et al., 2006). The motor action of the B-Z transition was monitored using FRET fluorescence from the two fluorophores at the end of each DNA molecule (pink and green circles). Reproduced with permission from Prof. Nadrian Seeman.

Figure 3. A DNA "walker".
This machine is also entirely built from DNA, but in this case the movement is dependent upon hybridization between complementary strands of DNA and forward, unidirectional motion is ensured through the use of ligation and cleavage reactions (Yin et al., 2004). Image reproduced with the permission of Prof. Niles A. Pierce.

[1] See the glossary of acronyms at the end.

BIOLOGICAL MOLECULAR MOTORS

Biological molecular motors abound in natural systems and many of these motors are now readily available, having been isolated and purified, and have been studied extensively at both the biochemical level (Spudich, 1994) and the single-molecule level (Wang, 1999). These motors exist as both rotary motors (e.g. ATP synthase and the bacterial flagella motor) and linear tracking motors (e.g. kinesin), which provides an opportunity for use of the motors in a wide variety of different types of devices (Kinosita, 1998). In addition, nature's machines are often optimised for efficiency (Kinosita et al., 2000), which suggests that using what is already available may be much easier than de novo design and production of equivalent machines.

Perhaps the most complex biological machine is the ribosome, which acts as the "protein factory" of the cell, continually synthesising proteins with extremely high fidelity in a complex that is no larger than 30–50 nm. This machine is seen by many as the model for future nanodevices in which a nanoscale machine can assemble atoms and molecules in a highly programmable manner (i.e. a model system on which to base the "molecular assembler"), to produce novel combinations and, consequently, new materials (Drexler, 1999). Yet such a device remains, at present, only a long term goal and is unlikely to function without the use of biological molecular motors to provide much of the movement required to transport the building blocks supplied by some programmable template and to position those building blocks for chemical synthesis of new materials (Hla & Rieder, 2003).

Overview of types of biological molecular motors

Even the simplest organisms possess molecular motors:

- The bacteriophage (a virus that infects bacteria) possesses a rotary motor (Figure 4) used to pack DNA into the bacteriophage head (Smith et al., 2001). This motor works rather like a cork and corkscrew, where the DNA is the corkscrew (Simpson et al., 2000). Simple but elegant, the molecular motor, consisting of a 10 nm ring of proteins, compresses DNA into the phage head by reducing the volume occupied by the DNA approximately 6000 fold; the resulting internal pressure within the phage head is thought to be of the order of 60 atmospheres.
- Bacteria have rotary motors that drive the whip-like motion of their flagellae (Figure 5), which, in turn, provide the bacteria with a swimming movement (Ryu et al., 2000).

These motors provide one of the best examples of self-assembly in nature. They comprise ~40 proteins (Suzuki et al., 2004; Samatey et al., 2004) that are synthesised within the cell and then transported, through the self-assembled structure of the motor, to the appropriate site for assembly of the flagellum on the outside of the cell. This type of self-assembly is frequently observed in biological systems and provides a blueprint for the requirements of nanodevices—they will have to be able to self-assemble at precise locations to provide the required "bottom-up" approach to nanotechnology (Zhang, 2003).

- Muscle (myosin) is a typical linear motor in that it enables sliding of actin fibres along myosin fibres, although the motion at the heart of the myosin motor is in part a rotary motion that is transmitted to the actin fibre as a linear motion through a long lever-arm. This lever-arm also amplifies the motion produced by the molecular motor from a few nanometres to 10 nm (Yanagida et al., 2000). Myosins are ubiquitous in the cell, with a wide range of functions

ranging from control of balance in complex organisms such as man, through to cell division during mitosis.

- Kinesins may represent the most useful type of linear motors as nature already uses them for carrying objects around the cell (Jia et al., 2004). These motors travel along microtubules (Figure 6), which radiate around the cell in three dimensions, transporting their cargoes to various parts of it. The cargoes can be proteins, vesicles or organelles many times the size of the motor (Sheetz, 1999; Steinberg, 2000; Kamal & Goldstein, 2002; Seog et al., 2004).
- Dyneins also act as ATP-driven molecular motors that are able to generate a force relative to microtubules. They are placed in three classes, which are primarily determined by subcellular localization—inner- and outer-flagella-arm dyneins and cytoplasmic dyneins. This localization matches the cellular activities, the first being the inner- and outer-flagella-movements and the second is the movement of cellular organelles (cf. kinesins—Harrison & King, 2000).
- Another important group of motors are those that utilize DNA as their linear track (rather like kinesin and dynein use microtubules). These include polymerases such as RNA polymerase (Harada et al., 1999), DNA polymerases, helicases and topoisomerases. One key area of interest generated by these molecular motors is single molecule DNA sequencing (Meldrum, 2000).
- Finally, an unusual, but maybe an all-important group of motors that move DNA are DNA translocases (Seidel et al., 2004). Unlike the other motors mentioned above, these enzymes do not simply run along the DNA track, but rather bind the DNA and pull the rest of it through the bound complex (Figure 7). This provides for a very flexible system because the motors usually have a specific recognition sequence on the DNA (therefore, the position on the DNA can be readily defined) and they can produce useful work, because they create relative motion with respect to the surface on which the DNA is attached (they do not require the motor to be surface-attached to obtain useful work, which is the case for polymerases).

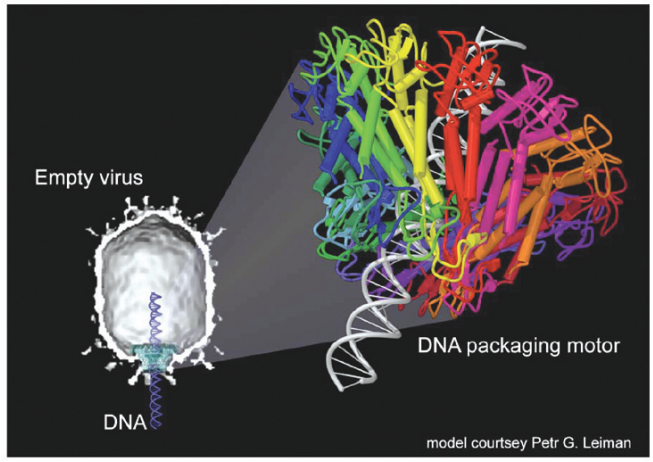

Figure 4. A DNA packaging motor from a bacteriophage.
This multisubunit motor acts as the "cork" on a DNA "corkscrew" and literally winds the DNA into the bacteriophage head (Simpson et al., 2000). Reproduced with kind permission of Prof. Michael Rossman, Purdue University.

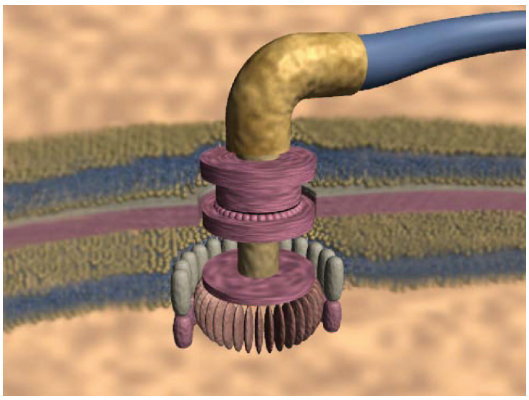

Figure 5. The rotary motor of the bacterial flagellum.
The bacterial flagellum consists of approximately 40 proteins. The proteins are produced within the cell and then self-assemble at the bacterial membrane to produce the molecular motor, which trasverses the lipid bilayer of the bacterial envelope, and through which the flagellum proteins pass to assemble the external flagellum (Berg, 2003). The motor comprises many of the components associated with motors constructed on the macroscale—bearings, a drive shaft and a stator through which energy is transmitted to drive the motor. We acknowledge Access Research Network at http://www.arn.org/docs/mm/motor.htm,, who hold the copyright for this image, and who allow reproduction of the image for non-profit use.

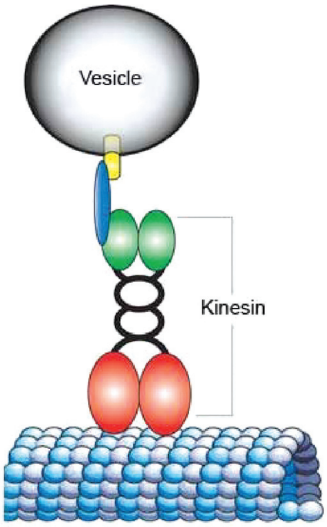

Figure 6. The kinesin molecular motor.
Kinesin motors can "walk" along the microtubule of the cell through a series of steps that involve hydrolysis of ATP (Hua et al., 1997); the directionality of the walk appears to be inbuilt into the microtubule, although different kinesin family members can move in different directions (Woehlke & Schliwa, 2000). This image is from Duncan & Goldstein (2006), an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Figure 7. DNA translocation by a DNA translocase molecular motor.
(a) The DNA-binding molecular motor binds to DNA attached to a surface, at a specific DNA sequence unique to each motor. (b) The motor pulls the DNA through the bound complex toward both the surface and the bound motor. (c) Pulling the DNA also pulls the DNA-bound magnetic bead toward the motor. (d) The motor stops at the bead and the motor subunit is released, resetting the nanoactuator. (e) After resetting, fresh motor protein will allow the nanoactuator to be reused (Seidel et al., 2004; Firman, 1999). Copyright the University of Portsmouth, Keith Firman and Bob Healey.

STATE OF THE ART IN THE USE OF MOLECULAR MOTORS

Much of the work with molecular motors originated in laboratories involved in the study of cell biology and this work, as a consequence, has centred on myosin(s) and kinesin(s). In the last few years this work has also involved increasing amounts of single molecule analysis and measurement, stimulated by the availability of scanning probe microscopy (SPM) and atomic force microscopy (AFM) (Baibyrin et al., 1999), and rapidly led to the development of tools for handling and measuring forces at the single molecule level—optical tweezers (Marx, 2001; Coirault et al., 2003) and magnetic tweezers (Zlatanova & Leuba, 2003; Hosu et al., 2003).

Atomic force microscopy and related microscopy

The first atomic force microscope, produced in 1986, was a direct descendant of the scanning tunnelling microscope (STM) (Binnig et al., 1986). The need for such a microscope resulted from the desire to image insulating materials, particularly biological molecules and polymers where electron tunnelling effects are difficult to achieve. The microscope consists of a small stylus or tip which extends in a perpendicular direction from the free end of a silicon, or silicon nitride, cantilever. The radius of curvature of the tip lies between 10–100 nm. During operation, the cantilever unit remains stationary and the underlying sample is scanned back and forth. The cantilever acts as a soft spring of known spring constant, which obeys Hooke's Law,

$$F = -kz$$

where F is the extending force acting on the cantilever, k is the cantilever spring constant and z is the vertical displacement of the cantilever. The deflexion of the cantilever is monitored by a laser beam, which is reflected from the back of the cantilever surface onto photodiodes via a feedback circuit that ensures a constant force is applied to the sample surface. When operating in this way, the AFM is said to be in "contact" mode. The forces observed during scanning are of the order of 1–10 nN.

The majority of early AFM experiments were carried out in air but a great advantage of the technique is that images can be obtained whilst the sample is immersed in liquid. This is important because it not only allows the visualization of dynamic biological events in real time in a natural environment but also because it greatly reduces the interactive forces between the tip and substrate. In liquids, capillary forces are very nearly eliminated. To minimize the forces during "contact mode" imaging that may damage, or move, the sample, AFM measurements may also be carried in "non-contact" or "tapping mode" (Camesano et al., 2000; Pang et al., 1997). This technique, often termed scanning probe microscopy (SPM), may be done in both air and liquid environments and has proven to be highly successful for gaining additional structural details about the samples under investigation, particularly biological specimens (Figure 8). Tapping mode operates by oscillating the cantilever a known distance from the sample surface whilst the sample is being scanned in a lateral direction. When the tip-sample distance varies due to the surface features, the frequency of the cantilever oscillation is forced to change and the amplitude at a given frequency is lowered. Feedback systems then lower the position of the sample stage so that the cantilever resorts to its original amplitude of oscillation; thus, the topography of the sample is recorded. Care needs to be taken when interpreting SPM images to avoid artefacts that may result from tip shape or dimensions (e.g. double images will be obtained if

there is a double tip (Beebe et al., 1989). The tip is also susceptible to contamination and so image quality may decline with scanning.

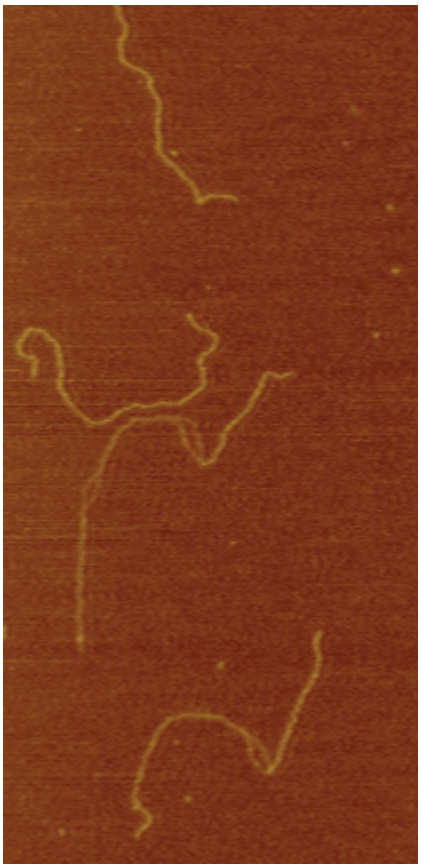

Figure 8. An image of DNA produced using SPM. DNA molecules were spread on a mica surface as described in van Noort et al. (2004). The image quality is such that local melting of the DNA can be easily observed. This image is reproduced with kind permission of Cees Dekker and John van Noort.

Optical tweezers

As the laser power in a focused beam is increased, the distribution of molecules in solution no longer follows a Poisson distribution (Chiu & Zare, 1996). The molecules no longer undergo random diffusion, but instead have a tendency to move to the point of maximum electromagnetic radiation (the focus of the laser beam, see Figure 9). This movement is due to the interaction of an induced dipole moment on the molecule and the electromagnetic field that induces the dipole. The resultant interaction is attractive and has been termed an "optical trap". Initially such optical traps were used to manipulate microscopic beads and microspheres, but at high enough laser power single molecules such as DNA can be moved (Nie & Zare, 1997; Wang, 1999). Feedback systems have been developed for optical traps allowing the direct measurement of molecular forces produced by molecular motors (Finer et al., 1994). DNA-based uses of molecular tweezers, or optical traps, usually involve attachment of beads at the ends of the DNA. These beads are held apart in the trap and molecular forces such as DNA-looping are measured through the movement of the beads (Sakata-Sogawa et al., 1998).

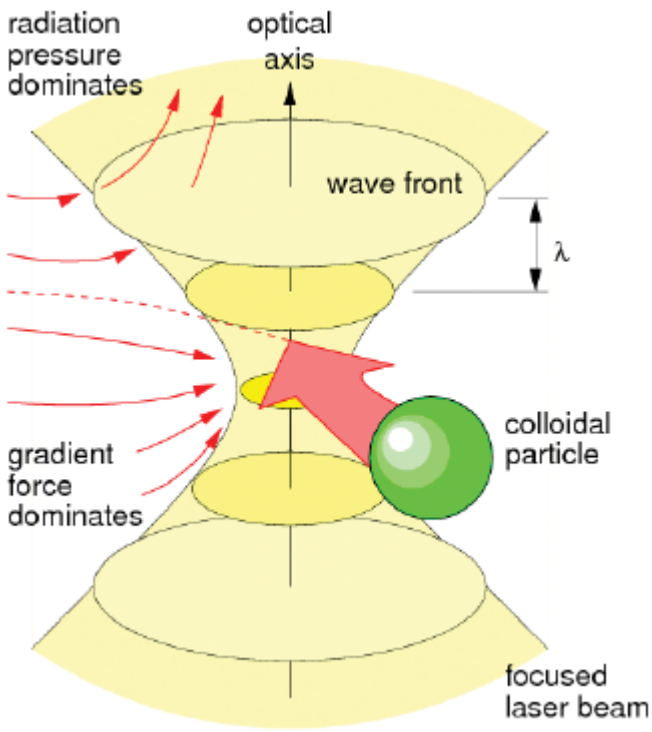

Figure 9. An optical tweezer setup.
A laser beam with a Gaussian intensity profile is used to trap a particle at the centre of the beam. The movement of this particle within the beam can be monitored through a feedback mechanism to determine forces and directional movement. Reprinted with permission from Macmillan Publishers Ltd: Nature (Grier, 2003), copyright (2003).

Magnetic tweezers

Magnetic "tweezers" are another form of a field gradient trap (Figure 10). The first magnetic tweezer setup was designed by Crick & Hughes (1950); permanent magnets were used to apply forces to ferromagnetic beads attached to the surface of cells. The original apparatus has been modified in more recent experiments to use strong electromagnets in place of permanent magnets and superparamagnetic beads in place of ferromagnetic beads (Figure 10). However, the concepts remain the same: using the electromagnets to produce a strong field for the alignment of the superparamagnetic beads and high field gradients for the actual manipulation of the beads and any attached substrate. Magnetic tweezers can be used to apply forces of up to 10 pN and allow force resolution down to below 0.1 pN, in addition to their ability to manipulate particles. Recent research using the magnetic tweezers setup has characterized the motor activity of polymerases (Maier et al., 2000), translocases (Seidel et al., 2004) and topoisomerases (Dekker et al., 2002), and insight has been gained into chromatin assembly (Leuba et al., 2003).

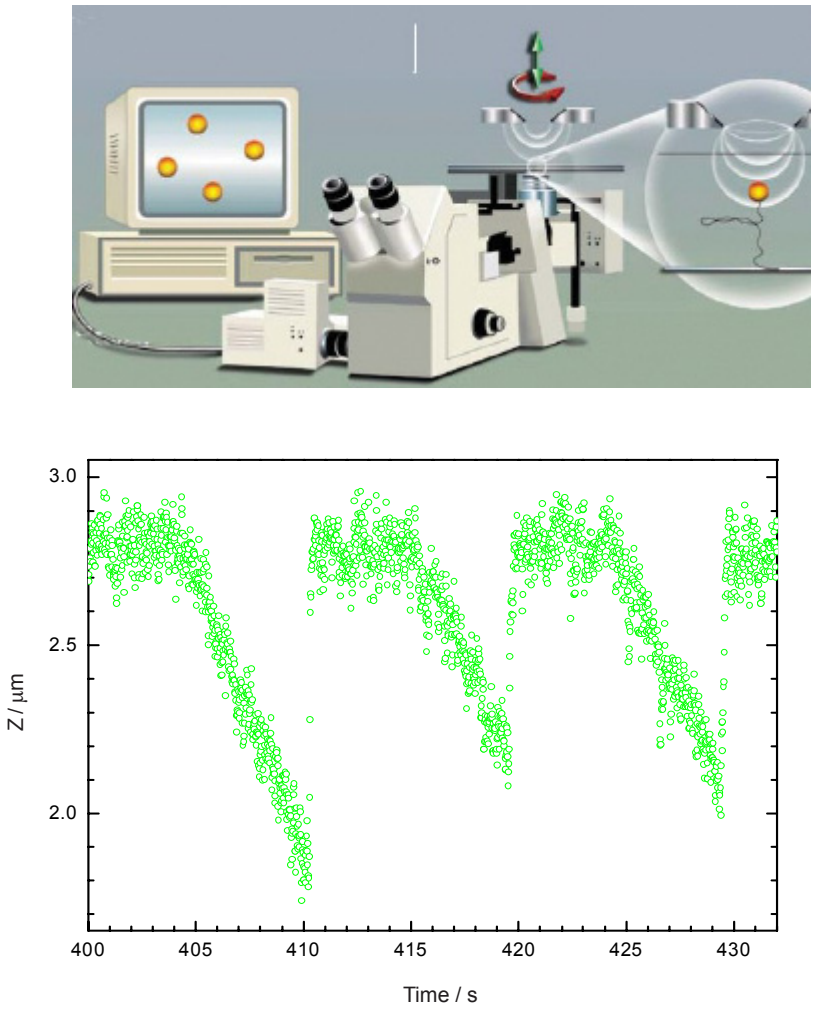

Figure 10. A magnetic tweezer setup.
The setup consists of an inverted microscope with a flowcell mounted above the objective. DNA is attached to the surface of the flowcell (inset) and a DNA-attached magnetic bead is used to stretch the DNA vertically. The height of the bead above the floor of the flowcell can be determined using the diffraction rings that surround the image of the bead. These can be plotted against time and used to determine speed of movement of the bead (green traces are images of the bead movement produced by EcoR124I). Magnetic tweezers can also be used to twist the DNA, producing positive or negative supercoils. The illustration of the magnetic tweezer setup was kindly provided by Prof. David Bensimon and the translocation data (lower image) was provided by kind permission of Cees Dekker and Ralf Seidel (Seidel et al., 2004).

TYPES OF BIOLOGICAL MOLECULAR MOTORS AVAILABLE

Single molecule studies have allowed workers to determine the forces generated by a wide variety of molecular motors (Table 1) and have opened the way for possible applications of these motors in nanotechnology.

Table 1. Characteristics of a variety of molecular motors.

Name of motor	Type of motion	Fuel	Forces generated	Speed
Viral head motor of Phi29 bacteriophage	Rotary	ATP	57–60 pN	100 bp sec^{-1} (0.03 μm sec^{-1})
Bacterial flagella	Rotary	Protons or cations (1000 protons per revolution)	~25 pN	1700 Hz
ATP Synthase (Kinosita et al., 2004; Kinosita, 1999)	Rotary	Proton flux	~30 pN-nm (torque)	130 Hz
Myosin(s)	Nominally linear	ATP	5–6 pN	n/a
Kinesin(s)	Linear	ATP	5–7 pN	100 steps sec^{-1}
DNA and RNA polymerase	Linear	Chemical synthesis / hydrolysis of nucleotides	7 pN	550 bp sec^{-1} (0.19 μm sec^{-1})
DNA translocases	Linear	ATP	8 pN	550–5500 bp sec^{-1} (0.19–1.9 μm sec^{-1})

Biological molecular motors offer a number of opportunities for nanotechnology, of which perhaps the most important is their ability to self-assemble. The bacterial flagellum is an excellent example of the capabilities of biological systems for self assembly.

A key aspect of bionanotechnology is to harness this ability to self-assemble, through precise pre-attachment of suitable material, onto surfaces, which will enable the self-assembly to occur at precisely determined positions in defined ways. Perhaps one key technology that might enable patterning of surfaces in a reliable manner is the "dip pen nanolithography" (DPN) device that takes the technology of nanolithography one step further by providing a simple to use interface that resembles a computer 'art' package (Ginger et al., 2004). DPN is direct-write scanning probe-based lithography in which an AFM tip is used to deliver chemical reagents directly to nanoscopic regions of a target substrate (Piner et al., 1999; Hong & Mirkin, 2000; Hong et al., 1999).

ATP Synthase

Perhaps the ATP Synthase motor lends itself to the construction of nanodevices most readily. It is a rotary motor that exists in two parts (FoF1-ATPase), one is buried in the mitochondrial membrane (F_o in Figure 11), while the other part is attached to the central spindle of the membrane motor and is spun in vivo by movement of the membrane motor (F_1). The F_o motor is driven by a proton flux across the membrane and its movement leads to rotation of the F_1 component. The lower motor then converts this mechanical energy into chemical energy that is used to synthesise ATP (which is the fuel of many other motors (Table 1)). However, both motors are reversible and consumption of ATP can produce a proton pump that will pump protons out of the cell, across the membrane (Fillingame et al., 2000; Stock et al., 1999; Yasuda et al., 2001; Sabbert et al., 1996; Gao et al., 2005).

The lower component of the motor has been purified and using modern affinity purification methods high yields have been obtained (Ishmukhametov et al., 2005). The motor has also been

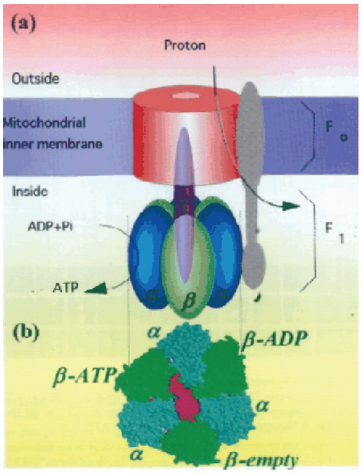

Figure 11. ATP synthase.
ATP synthase consists of a membrane-bound motor, driven by a flux of protons or sodium ions, which drives a separate motor that converts the mechanical energy into the synthesis of ATP (Stock et al., 1999; Cross, 2000; Stock et al., 2000). Reproduced with kind permission of Dr Alan E. Senior.

genetically engineered in such a way that surface attachment is relatively simple (Adachi et al., 2000), allowing useful work to be obtained from the motor, and furthermore attachment of a fluorophore to the motor allowed the motion to be directly visualized (Figure 12). In this elegant work moving images of the rotating fluorophore were captured, with the individual molecular motor behaving rather like a lighthouse. The rotating fluorophore can be readily seen in Figure 12 and a sinusoidal wave is apparent due to the rotation of the light source.

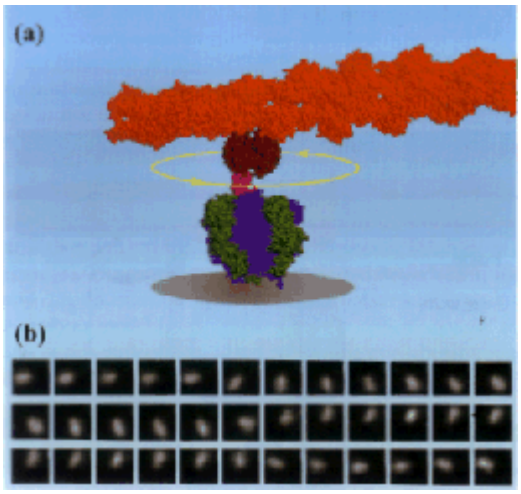

Figure 12. ATP synthase rotating an actin fibre.
An actin fibre, with attached fluorophore, is fixed to the ATP synthase molecular motor. Rotation of the fluorophore is shown in the still images captured in the figure (insets). Reproduced from Kinosita (1999), with kind permission from Kazuhiko Kinosita.

Attachment of an actin fibre to this motor, labelled at one end with a fluorophore (Kinosita, 1999) quickly leads to the concept of using ATP synthase as a nanodevice that spins objects in such a way so as to "do useful work". One such concept would be a rotating fibre driven by photons absorbed by the fluorophore (Yinghao et al., 2005). However, it is not clear that the individual motor is capable of sufficient reliability and flexibility of motion for such tasks in any real device (Su et al., 2006), nor how to activate this motor in a truly useful manner—the requirement is one of efficient use of the photons captured.

Recently, a more efficient system for converting light into rotary motion has been described (Su et al., 2006), which also allows self-assembly of many rotary motors onto an actin fibre (Figure 13). The outcome of this arrangement was a swimming-like motion of the actin fibre, which could ultimately be used as a means for propulsion of tiny nanomachines within a liquid environment. In effect the fibres attached to the motors are spinning like tiny propellers and this provides sufficient driving force to move the microscale actin fibre (~5–10 μm long) over significant distances (up to ~70 μm). Such microscale movement results from the cooperative action of these motors acting in parallel.

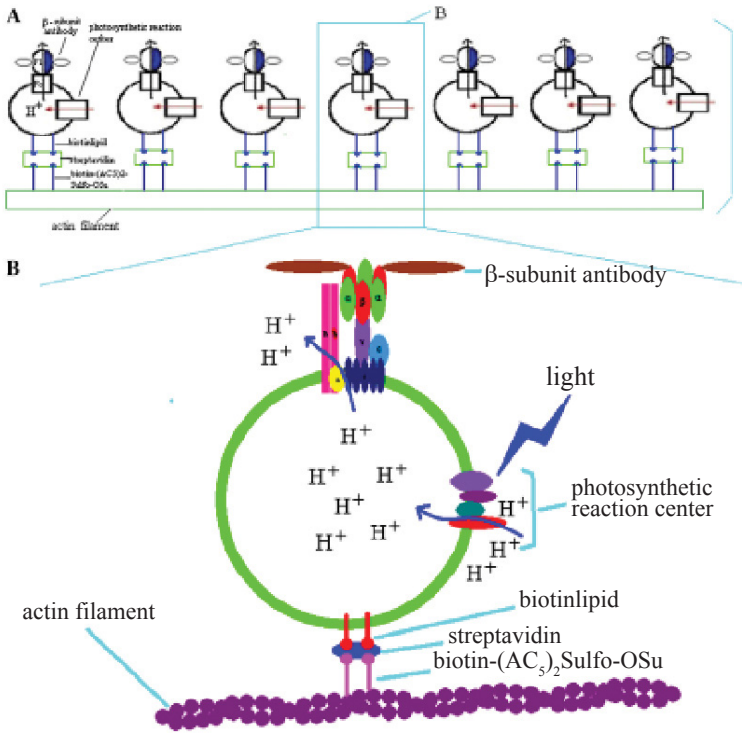

Figure 13. A nanodevice driven by light, swimming through an aqueous environment.
A nanodevice constructed from ATP synthase, which is able to use light to produce physical work in the form of a swimming motion produced through a propeller-like arrangement of the motor (Su et al., 2006). Reproduced from Su et al. (2006), with kind permission from Yue Jiachang.

Kinesin and microtubules

As mentioned previously, kinesin is the cargo system of the eukaryotic cell, carrying many different sizes of cargo along microtubules within a cell (Klumpp & Lipowsky, 2005), but the complexity of the microtubule system suggests at first that using these motors in any nanodevice may prove difficult. However, a first step toward both handling these motors and making them carry out useful work was the observation that a surface coated with kinesin would transport microtubules (Vale et al., 1985), but this motion is a random motion and it was not until surface etching was used (Hiratsuka et al., 2001) that the motion of the microtubules could be organized (Figure 14).

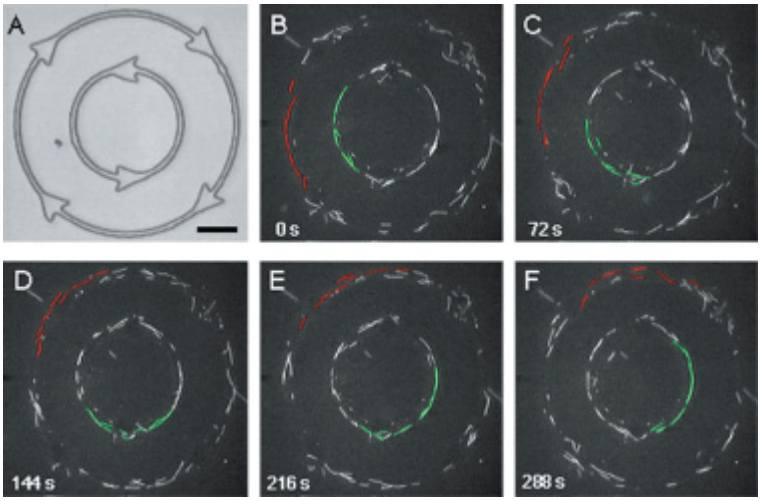

Figure 14. An etched surface used to control motion of microtubules.
Directional control of the movement of microtubules, on a surface covered with kinesin, by means of an appropriately etched surface. Reproduced with kind permission from Taro QP Uyeda, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki 305-8562, Japan.

Therefore, it is now possible to imagine custom-designed surfaces that can be used for transporting material between different regions, but in this device using microtubules as the means for transporting the cargo. This simple arrangement has been greatly improved by the use of minute forces generated by electrical steering techniques, which have been used to demonstrate sorting of microtubules at Y junctions (van den Heuvel et al., 2006), and the use of similar techniques to dock microtubules onto surfaces (van den Heuvel et al., 2005), which could provide the mechanism for loading cargo onto the system.

The main problem now remaining for such a system, in a nanodevice, is how to efficiently release cargo from the transport system. Such a release system may depend upon site-specific proteases to cleave protein fusions.

RNA and DNA polymerases

Polymerases that copy nucleic acids are also linear tracking motors. They obtain the energy for molecular motion from chemical synthesis—creation of the phosphodiester bond, a high energy covalent bond—and as a consequence they move along the DNA helix as they synthesise the new strand of DNA or RNA. One advantage of using motors that move DNA is that it is relatively easy to attach other molecules to the moving DNA. However, the problem with polymerases is that to obtain relative motion of the DNA (and the attached object) with respect to a surface, the motor itself must be surface-attached (Figure 15).

The first single molecule studies of a polymerase involved RNA polymerase and these were used to determine the force and speed of these motors (see Table 1 and Wang et al., 1998). As can be seen in Figure 15, the polymerase is surface-attached and subsequently binds to the DNA, initiates RNA synthesis, which leads to the polymerase pulling the DNA toward the surface, and as a consequence pulls the bead out of the optical trap.

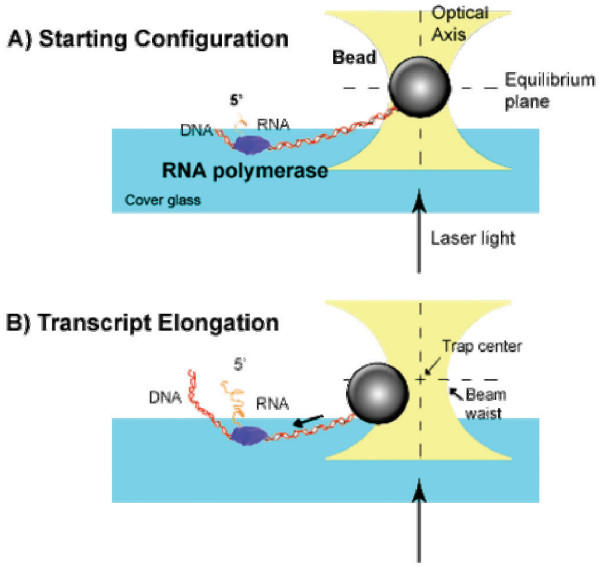

Figure 15. Measuring motion of RNA polymerase.
RNA polymerase was isolated on a coverslip and then the transcript elongation monitored using an optical tweezer setup. As the RNA polymerase transcribed the DNA template, Wang et al. (1998) were able to monitor the velocity and intensity of forces, using a feedback-controlled instrument that could optically clamp the particle in place. Copyright The University of Portsmouth, Keith Firman and James Youell.

This work was extended to show, by direct observation, that the polymerase does in fact follow the helical thread of the DNA. The polystyrene bead used in the previous experiment was replaced with a magnetic bead, which was held vertically by an external magnetic field. In addition, the magnetic bead was visualized by attachment of small fluorescent nano-sized beads to the magnetic bead. Movement by the polymerase motor during transcription of the DNA produced rotation of the magnetic bead, which was visualized as a sinusoidal wave in the fluorescence output.

More recent work with RNA polymerases has shown that the positional resolution of force-paused complexes can be resolved to 5 bp (Shundrovsky et al., 2004), and that the forward motion is that of a 'Brownian ratchet' (Guo & Sousa, 2006). This work has opened up the possibility of single-molecule DNA sequencing (Braslavsky et al., 2003).

DNA translocases

In an attempt to overcome the inherent problems associated with surface attachment of molecular motors and the problems associated with precise start points for transcription or replication, which inhibit the potential uses of polymerase motors, we have initiated a single molecule study of DNA translocases.

There is only a limited number of such enzymes, of which the most studied are the Type I Restriction-Modification (R-M) enzymes (www.typei-rm.info), but others include chromatin remodelling factors (Flaus & Owen-Hughes, 2001), Type III R-M enzymes (Reich et al., 2004), motors used in chromosome segregation (Saleh et al., 2005a) and certain fusion proteins that link motor activity and specific DNA binding.

We have studied the Type IC R-M enzyme EcoR124I in particular detail (Firman & Szczelkun, 2000), including single molecule analysis (Seidel et al., 2004). In collaboration with other groups we have also closely studied the motor activity of FtsK (an enzyme involved in chromosome segregation in bacteria), including single-molecule studies (Saleh et al., 2004). The single molecule studies have involved development of a magnetic tweezer setup (Alenghat et al., 2000—Figure 10; Zlatanova & Leuba, 2003) to study forces generated by these motors, their speed and the dynamics of DNA binding (Seidel et al., 2004; Seidel et al., 2005; Saleh et al., 2004; Saleh et al., 2005b; Saleh et al., 2005a). The magnetic tweezer setup is a particularly useful tool for studying DNA-binding motors, which also illustrates the advantages of using a DNA translocase for manipulating DNA. The main advantage these motors offer is that DNA can be readily attached to the surface and the motor will bind at a known specific location on the DNA, pulling the magnetic bead attached at the other end of the DNA toward the surface, producing a movement trace of the vertical position of the bead (Figure 10, inset).

The outcome of this type of motion is that the translocases are nanoactuators. They have a wide range of uses from simple valve-like devices through to motors for single-molecule DNA sequencing. However, perhaps their most important potential was demonstrated using EC-funding for the "Mol Switch Project" (www.nanonet.org.uk/molswitch/). In this project we have shown that the moving magnetic bead can act as a "molecular dynamo", generating electrons in a suitable sensor placed under the DNA (Figure 16). Such a device is a single-molecule

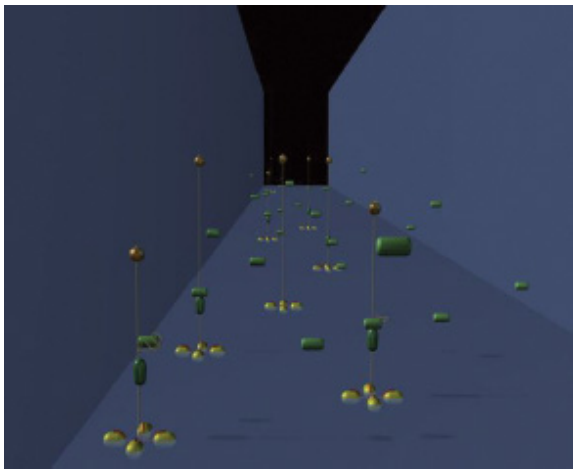

Figure 16. The "Mol Switch" device.
The proposed device is a single-molecule reporting system for use in biosensing—the Mol Switch Device. It consists of a microfluidics lab-on-a-chip device, which has Hall effect (or magnetoresistive) sensors located within the main microfluidics channel. A single-molecule of DNA is attached above each sensor (through a digoxygenin-anti-DIG interaction following incorporation of DIG into one end of the DNA using PCR). The other end of the DNA molecule has an attached magnetic bead (through a biotin-avidin interaction following introduction of biotin into a PCR product). Introduction of an external magnetic field stretches the DNA molecules vertically prior to introduction of the molecular motor proteins. The presence of the molecular motor proteins, or any linked biological process generating motor or fuel, is signalled electronically by the individual sensors (single molecule signalling) each time the motor(s) pull a magnetic bead toward the sensor. Copyright The University of Portsmouth, Keith Firman and Robert Healey.

reporting device, outputting an electrical signal from any biological input that generates fuel or releases motor. This is likely to find uses in biosensing, and we are exploring its use in toxicity testing and drug-DNA interactions. We have also demonstrated that the EcoR124I motor can be fused to a responsive polymer (Pennadam et al., 2004a), which prevents motor activity—acting as a switch—and may also provide protection to the motor in biosensing systems involving extreme conditions (Pennadam et al., 2004b).

FUTURE PROSPECTS—AN ARTIFICIAL RIBOSOME?

It was K. Eric Drexler who first suggested the idea of a molecular assembler (an artificial ribosome capable of programmed assembly of materials other than amino acids—Drexler, 1992). He believes that the development of such a device is crucial to the future development of nanotechnology. Yet such a complex nanodevice still seems a distant dream.

There are four components required to produce such an artificial ribosome:

1. A programmable system for ordered delivery of the building blocks of the material to be synthesised;
2. A mechanism for bringing these building blocks (in the correct order) to the site for assembly;
3. A means for carrying out highly localized single-molecule chemistry at the site of assembly;
4. A mechanism for release of the built material and resetting of the machine.

DNA provides an ideal material for programming the order of delivery of the building blocks and a sensible approach would be to link the building blocks to short oligonucleotides (~12-mers), which could assemble along a stretch of single-stranded DNA in an order directed by the sequence of the DNA (which would be produced synthetically).

By making use of DNA translocases, a "conveyor belt" could easily be produced to pull the DNA and attached building blocks past an assembly site, which could be the tip of an STM (Hla & Rieder, 2003), so as to allow the synthetic chemistry that will join the building blocks together in the required order. DNA translocases such as EcoR124I are particularly useful in this area as they will bind at one end of a long DNA molecule, at a known sequence; they can pull the DNA against an external force such as a magnetic bead held by a weak magnetic force (which would stretch the DNA in one dimension, simulating a conveyor belt); and they can translocate past nicks and small gaps in the DNA (Stanley et al., 2006), which may exist on the DNA as the oligos that were attached to the building blocks pass the translocase.

Release of the building blocks from the DNA can be carried out using nicking restriction enzymes such as N.BstSEI, which would cleave the oligonucleotide carrying the building block near the site of attachment of the building block to the DNA, releasing the newly synthesis material.

The system can be reusable because the short oligonucleotides, still attached to the DNA conveyor belt and which will enable translocation of the DNA, can be subsequently washed away using salt solution.

In theory such a device could synthesise any polymeric material for which building blocks are readily available and which can be joined together by a relatively simple chemistry. However, before such devices can be constructed a great deal of improvement needs to be made

in the reliable and reproducible positioning of biomolecules and the subsequent self-assembly of the components of the nanodevice to the required design.

SYNTHETIC BIOLOGY AND SURFACE ENGINEERING

Synthetic Biology is seen by some researchers as "the new nanotechnology", but in reality this subject area is better imagined as the application of engineering approaches to biological systems. An illustration of the concept of Synthetic Biology is provided by the iGEM Project, which allows non-skilled people to construct novel genetic elements from "component parts" such as promoters, genes, transcription terminators etc. (http://parts.mit.edu/wiki/index.php/Main_Page). However, the basic concept of this type of Synthetic Biology, that biological components can be *mixed and matched* to produce a wide range of novel functional constructs is likely to be an oversimplification and many combinations will not work together. One obvious problem is protein solubility—it has been observed that different protein fusions can result in radically different solubilities for the final protein. However, the concept is a guide for what types of information would be useful for the future and a list of such components that includes a description of known results including negative results (e.g. combinations that have produced insoluble or aggregated protein) would be a powerful resource for the future.

Another aspect of Synthetic Biology will be the careful positioning of biomaterial (Leach et al., 2001), most probably through surface attachment, in microfluidics channels, which will become part of lab-on-a-chip devices (Daw & Finkelstein, 2006). Engineering of such nanoscale devices will make great demands on current metrology.

CONCLUSIONS

With the advent of single molecule sensing, in the form of optical and magnetic tweezer setups and AFM and related microscopies, better characterization of molecular motors has been achieved, allowing the first steps of nanodevice design to take place.

The incorporation of well-characterized molecular motors into simple nanodevices has already begun (e.g. The Mol Switch Project, www.nanonet.org.molswitch/; The BioNano-Switch Project, http://www.bionano-switch.info/; Su et al., 2006) and such devices have a broad range of applications, from toxin sensing to single-molecule DNA sequencing.

As more molecular motors are characterized it seems that the goal of producing a "molecular assembler", as suggested by Eric Drexler, could be achieved. It would seem probable that a production line required for such a device would incorporate several molecular motors with separate, but complementary, roles, which would be defined by their function. In addition, some form of stable "conveyor belt" mechanism would be required for the transport of component molecules, for which DNA would seem the most likely candidate because of its inbuilt ability to carry the required programming information. Surface engineering and chemistry will have to provide a stable environment for the biological components to work and will also have to be designed so that single molecules can be accurately coupled to surfaces, in order that a production line can be accurately placed. The main stumbling blocks in the development of such a system will be the design of fusion proteins and DNA constructs that will maintain the desired functions without affecting their behaviour in the novel environments of the system.

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GLOSSARY

AFM, atomic force microscope or microscopy
ATP, adenosine triphosphate
bp, base pair
DNA, deoxyribonucleic acid
FRET, fluorescence or Förster resonance energy transfer
PCR, polymerase chain reaction
SPM, scanning probe microscope or microscopy
STM, scanning tunnelling microscope or microscopy.