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Chapter 14

Biomolecular Motors Operating in Engineered Environments

by Stefan Diez*, Jonne H. Helenius, and Joe Howard*

Max Planck Institute for Molecular Cell Biology and Genetics
Pfotenhauerstr. 108
D- 01307 Dresden
Germany

* Corresponding Authors

diez@mpi-cbg.de

howard@mpi-cbg.de

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Overview

Recent advances in understanding how biomolecular motors work has raised the possibility that they might find applications as nanomachines. For example, they could be used as molecule-sized robots that work in molecular factories where small, but intricate structures are made on tiny assembly lines; that construct networks of molecular conductors and transistors for use as electrical circuits; or that continually patrol inside "adaptive" materials and repair them when necessary. Thus biomolecular motors could form the basis of bottom-up approaches for constructing, active structuring and maintenance at the nanometer scale. We will review the current status of the operation of biomolecular motors in engineered environments, and discuss possible strategies aimed at implementing them in nanotechnological applications. We cite reviews whenever possible for the biochemical and biophysical literature, and include primary references to the nanotechnological literature.

Biomolecular motors are the active workhorses of cells (Alberts, 1998). They are complexes of two or more proteins that convert chemical energy, usually in the form of the high-energy phosphate bond of ATP, into directed motion. The most familiar motor is the protein myosin which moves along filaments, formed from the protein actin, to drive the contraction of muscle. It turns out that all cells, not just specialized muscle cells, contain motors that move cellular components such as proteins, mitochondria and chromosomes from one part of the cell to another. These motors include relatives of muscle myosin (that also move along actin filaments), as well as members of the kinesin and dynein families of proteins. The latter motors move along another type of filament called the microtubule. The reason that motors are necessary in cells is that diffusion is too slow to efficiently transport molecules from where they are made, typically near the nucleus, to where they are used, often at the periphery of the cell. For example, the passive diffusion of a small protein to the end of a 1-meter-long neuron would take approximately 1000 years, yet kinesin moves it in week. This corresponds to a speed of 1-2 $\mu\text{m/s}$, which is typical for biomolecular motors.(Howard, 2001) Actin filaments and microtubules form a network of highways within cells, and localized cues are used to target specific cargoes to specific sites in the cell (Alberts, 2002). Using filaments and motors, cells build highly complex and active structures on the molecular (nanometer) scale. Little imagination is needed to envisage employing biomolecular motors to build molecular robots (Crichton, 2002).

Biomolecular motors are unusual machines that do what no man-made machines do: they convert chemical energy to mechanical energy directly rather than via an intermediate such as heat or electrical energy. This is essential because the confinement of heat, for example, on the nanometer scale is not possible because of its high diffusivity in aqueous solutions (Howard, 2001). As energy converters, biomolecular machines are highly efficient. The chemical energy available from the hydrolysis of ATP is $100 \times 10^{-21} \text{ J} = 100 \text{ pN}\cdot\text{nm}$ (under physiological conditions where the ATP concentration is 1 mM and the concentrations of the products ADP and phosphate are 0.01 mM and 1 mM, respectively). With this energy, a

kinesin molecule is able to perform an 8 nm step against a load of 6 pN (Howard, 2001). The energy efficiency is therefore nearly 50%. For the rotary motor F_1F_0 -ATPase synthase which uses the electrochemical gradient across mitochondrial and bacterial membranes to generate ATP, the efficiency is reported to be between 80% and 100% (Kinosita et al., 1998; Soong et al., 2000). The high efficiency demonstrates that, like other biological systems, the operation of biological motors has been optimized through evolution.

High efficiency is but one feature that makes biomolecular motors attractive for nanotechnological applications. Other features are:

- 1) they are small and can therefore operate in a highly parallel manner,
- 2) they are easy to produce and can be modified through genetic engineering,
- 3) they are extremely cheap. For example, 20×10^9 kinesin motors can be acquired for one US cent from commercial suppliers (1 mg = 3.3×10^{15} motors cost \$1500, Cytoskeleton, Inc., Colorado) and the price could be significantly decreased if production were scaled up. And
- 4) a wide array of biochemical tools have been developed to manipulate these proteins outside the cell.

This review focuses on two broad categories of molecular motors. *Linear motors* generate force as they move along intracellular filaments. In addition to myosin and kinesin mentioned above, linear motors also include enzymes that move along DNA and RNA. *Rotary motors* generate torque via the rotations of a central core within a larger protein complex. They include ATP synthase, mentioned above, as well as the motor that drives bacterial motility. Representatives of both categories have been used to manipulate molecules and nanoparticles. Mechanical and structural properties of relevant filaments are contained in Table 1 and those of several associated motors in Table 2.

Filament	Diameter	Strands per filament	Repeat length	Persistence length	Young's modulus	Maximum length	Motors	References
Actin filament	6 nm	2	5.5 nm	10 μ m	2 GPa	100 μ m	Myosin	(Sheterline et al., 1995)
Microtubule	25 nm	~13	8 nm	5 mm	2 GPa	10 cm	Kinesin, Dynein	(Nogales, 2000)
DNA	2 nm	2	0.34 nm	50 nm	1 GPa	~100 mm	RNA Polymerase, DNA helicase, Topoisomerase	(Bustamante et al., 2003)
RNA	2 nm	2	0.34 nm	75 nm	1.5 GPa	~30 μ m	Ribosome	(Hagerman, 1997)

Table 1. Physical attributes of actin filaments, microtubules, DNA and RNA. The persistence length (L_p) is related to the flexural rigidity (EI) by: $L_p = EI / kT$. where k is the Boltzmann constant and T is absolute temperature. The Young's modulus (E) is calculated assuming that the filament is homogenous and isotropic. The repeat length describes the periodicity along a strand of the filament.

Motor	Filament	Size* (nm)	Step size (nm)	Maximum speed (nm/s)	Maximum force (pN)	Efficiency	References
Myosin II	Actin	16	5	30000 nm/s	10 pN	50%	(Howard, 2001; Ruegg et al., 2002)
Myosin V	Actin	24	36	300 nm/s	1.5 pN	50%	(Mehta, 2001)
Conventional kinesin	Microtubule	6	8	800 nm/s	6 pN	50%	(Howard, 2001; Kull, 2000)
dynein	Microtubule	24		6400 nm/s	6 pN		(Burgess et al., 2003; Shingyoji et al., 1998)
T7 DNA polymerase (exonuclease activity)	DNA		0.34	>100 bases	34 pN	NA	(Wuite et al., 2000)
RNA polymerase	DNA	15	0.34	5 nm/s	25 pN	NA	(Forde et al., 2002; Wang et al., 1998)
Topoisomerase	DNA		up to 43 nm/turn	--		NA	(Champoux, 2001; Strick et al., 2002)
Bacteriophage portal motor	DNA		0.34	100 bps	57 pN		(Smith et al., 2001)
Type IV pilus retraction motor	pilus			1000 nm/s	110 pN		(Maier et al., 2002; Merz et al., 2000)
F ₁ -ATPase	NA	8 x 14	120 degrees	8 rps	100 pN nm	80%	(Soong et al., 2000)
Flagellar motor	NA	45		300 rps	~550 pN nm		(DeRosier, 1998)

Table 2. Values characterizing the operation of several important biomolecular motors. The filaments along which the linear motors operate are indicated in Table 1. The sizes refer to the motor domains. Dynamic parameters were determined by in vitro experiments at high ATP concentration. NA – not applicable.

The general setups for studying motor proteins outside cells—the so-called motility assays—are depicted in Figure 1. In the gliding assay, the motors are immobilized on a surface and the filaments glide over the assembly (Figure 1A). In the stepping assay, the filaments are laid out on the surface where they form tracks for the motors to move along (Figure 1B). In both assays, movement is observed under the light microscope using fluorescence markers or high-contrast techniques. Variations on these assays have been used to reconstitute linear motility on the four types of filaments—actin filaments, microtubules, DNA and RNA.

The gliding motility assay has provided detailed data on the directionality, speed and force generation of purified molecular motors (Howard, 2001; Scholey, 1993). However, for use in nanotechnological applications, the movement of gliding filaments has to be controllable in space and time. For example, a simple application would be to employ a moving filament to pickup cargo at point A, move it along a user-defined path to point B, and then release it.

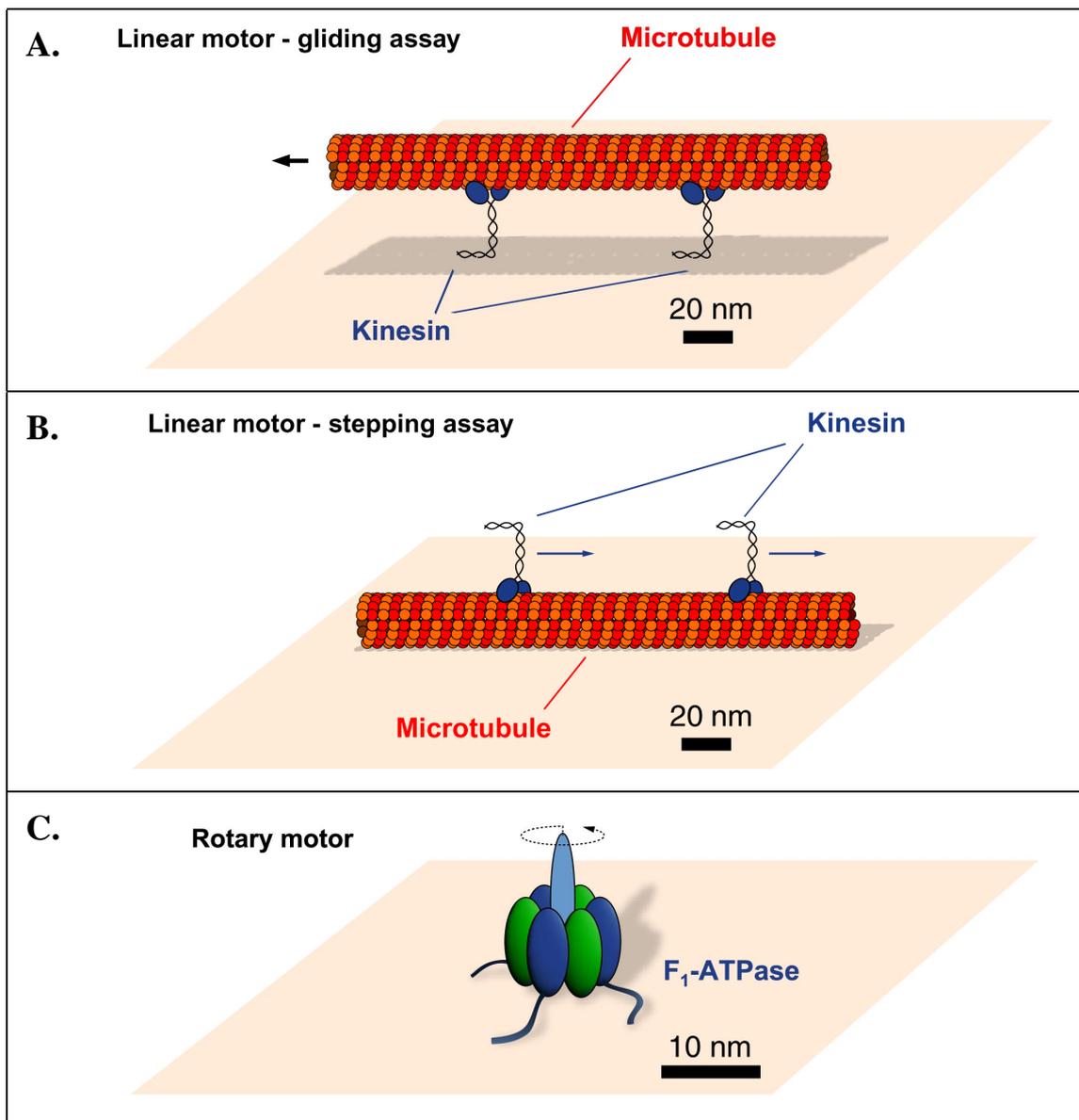


Figure 1. Biomolecular motor systems currently applicable for nanotechnological developments. **A** Linear transport of filaments by surface bound motor molecules (gliding assay). **B** Linear movement of motor proteins along filaments (stepping assay). **C** Rotation generated by a rotary motor.

A number of methods for the spatial and temporal control of filament movement have been developed. Spatial control has been achieved using topographical features (Clemmens et al., 2003; Dennis et al., 1999; Hess et al., 2002b; Hess et al., 2001), chemical surface modifications (Hess et al., 2002b; Nicolau et al., 1999; Suzuki et al., 1997; Wright et al., 2002), and a combination of both (Bunk et al., 2003; Hiratsuka et al., 2001; Mahanivong et al., 2002; Moorjani et al., 2003). Electrical fields (Asokan et al., 2003; Riveline et al., 1998; Stracke et al., 2002) and hydrodynamic flow (Prots et al., 2003; Stracke et al., 2000) have also been used to direct the motion of gliding filaments. An example from our laboratory of gliding microtubules that are guided by channels is shown in Figure 2. Temporal control has been achieved by manipulating the ATP concentration (Bohm et al., 2000a; Hess et al., 2001).

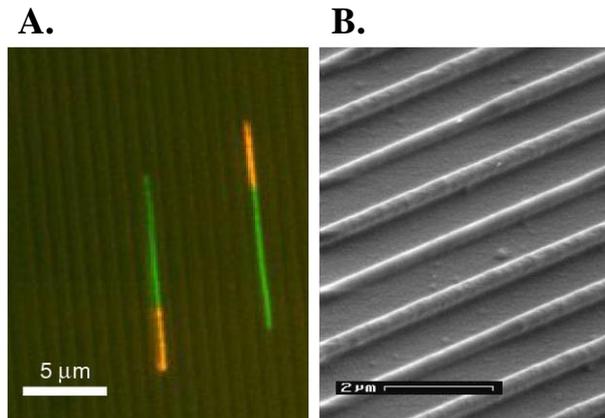


Figure 2. *A* Directed movement of gliding microtubules along microstructured channels on the surface of a coverslip. The initial positions of the microtubules are shown in orange, while the paths they traveled over the subsequent 12s are shown in green. *B* SEM image of the polyurethane channels. The channels are a replica mold of a Si-master (channel width 500nm, periodicity 1000nm, depth 300nm) produced using a PDMS stamp as an intermediate. Note, that the ridges have been "undercut". This probably aids the guiding of the microtubules in the channels. Silicon master provided by T. Pompe (Institute of Polymer Research, Dresden, Germany).

In addition to these basic techniques for controlling motion, some simple applications of the gliding assay have been demonstrated. These include the transport of streptavidin-coated beads (Hess et al., 2001), the transport and stretching of individual DNA molecules (Diez et al., 2003), the measurement of forces in the pN range (Hess et al., 2002c), and the imaging of surfaces (Hess et al., 2002a).

The stepping assay opens up additional possibilities. Initially, micrometer-sized beads were coated with motor proteins and visualized as they moved along filaments. The movement of beads can be tracked with nanometer precision to determine the speed and step size (Howard, 2001) and the use of optical tweezers allows forces to be measured (Mehta et al., 1999). In addition to beads, 10- μm -diameter glass-particles (Bohm et al., 2001) and Si-microchips (Limberis and Stewart, 2000) have been transported along filaments. In another variation, high-sensitivity fluorescence microscopy is used to visualize individual motor molecules as they step along filaments (Vale et al., 1996; Yildiz et al., 2003). An example from our laboratory of a single kinesin motor fused to the green fluorescent protein moving along a microtubule is shown in Figure 3. Despite the power of single-molecule techniques, they have yet to be exploited for nanotechnological applications.

Rotary motors can be studied *in vitro* by fixing the stator to a surface and following the movement of the rotor (Figure 1C). Rotation can be visualized under the light microscope by attaching a fluorescent label or a microscopic marker to the rotor. Both techniques have been used to investigate the stepwise rotation generated by F_1 -ATPase, which is a component of the F_1F_0 -ATP synthesis machinery (Kinosita et al., 1998; Noji et al., 1997). Individual motors have been integrated into nanoengineered environments by arraying them on a nanostructured surface and using them to rotate fluorescent microspheres (Montemagno and Bachand, 1999) or to drive Ni-nanopropellers (Soong et al., 2000).

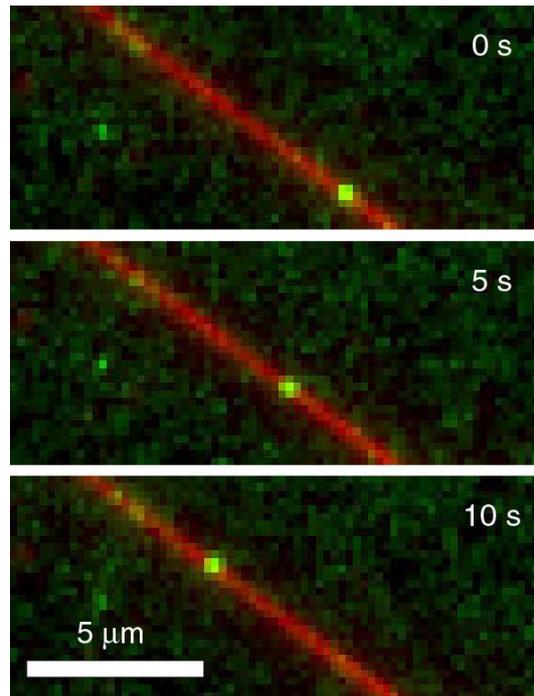


Figure 3. Movement of a single kinesin molecule (labeled with the green fluorescent protein) along a microtubule (red). Micrographs were acquired at the indicated times using total-internal-reflection fluorescence microscopy.

Methods

There are many challenges in applying biomolecular motors to nanotechnology. Motility must be robust. It must be controlled both spatially and temporally. And the motors must be hitched to and unhitched from their cargoes. This section summarizes key techniques towards these ends.

General conditions for motility assays

Motility assays are performed in aqueous solutions that must fulfill a number of requirements. We will illustrate these requirements with the kinesin/microtubule system. Kinesin uses ATP as its fuel; the maximum speed is reached at ~0.5 mM, approximately equal to the cellular concentration. Other nucleotides such as GTP, TTP and CTP can substitute for ATP, but the speed is lower (Cohn et al., 1989). Motility also requires divalent cations, with magnesium preferred over calcium, and strontium and barium unable to substitute (Bohm et al., 1997). Optimal motility, assessed by gliding speed, occurs over a range of pH, between 6 and 9 (Bohm et al., 2000b; Cohn et al., 1989), and over a range of ionic strengths, between 50 mM and 300 mM (Bohm et al., 2000b). The speed increases with temperature, doubling for each 10 °C between 5 °C and 50 °C (Bohm et al., 2000a; Kawaguchi and Ishiwata, 2001); motility fails at higher temperatures. The force is independent of temperature between 15 °C and 35 °C (Kawaguchi and Ishiwata, 2000). When assays are performed in the middle of these ranges, motility is robust, and only a small drop in the mean velocities is seen after 3 hrs (Bohm et al.,

2000a; Bohm et al., 2000b). If fluorescent markers are used, an oxygen scavenging enzyme system must be present to prevent photodamage. Many experimental details, including a discussion of the densities of the motors, can be found in Scholey (1993)(Scholey, 1993).

Temporal control

Motors can be reversibly switched off and on by regulating the concentration of fuel, or by adding and removing inhibitors. The ATP concentration can be rapidly altered by flowing in a new solution. In such a setup, the kinesin-dependent movement of microtubules can be stopped within 1 s and restarted within 10 s (unpublished data from our lab). Similarly, inhibitors such as AMP-PNP, a non-hydrolysable analogue of ATP (Schnapp et al., 1990), adociasulfate-2 a small molecule isolated from sponge (Sakowicz et al., 1998) and monastrol (Mayer et al., 1999) can be perfused to stop motility.

An alternative method to control energy supply is to use photoactivatable ATP. In this method, a flash of UV light is used to release ATP from a derivatized, non-functional precursor; an ATP-consuming enzyme is also present to return the ATP concentration to low levels following release. Using such a system, microtubule movement has been repeatedly started and stopped (Hess et al., 2001), though the start-up and slow-down times were slow, on the order of minutes. The advantage of this method is that the solution in the flow cell does not have to be exchanged.

Fortuitously, many proteins possess natural regulatory mechanisms and, once understood, these might offer additional means to regulate the motors *in vitro*. Examples include the regulation of myosins by phosphorylation and calcium/calmodulin (Sellers and Goodson, 1995) and the inhibition of kinesin by its cargo-binding "tail" domain (Coy et al., 1999). Because such natural controls might not always be applicable in a synthetic environment, there is strong interest in the development of artificial control mechanisms for motor proteins. Towards this end, metal-ion binding sites have been genetically engineered into the F₁-ATPase motor. The binding of ions at the engineered site immobilizes the moving parts of the motor thus inhibiting its rotation (Liu et al., 2002). ATP driven rotation can be restored by the addition of metal ion chelators. Clever genetic engineering of motors could provide temporal control mechanisms switched by temperature, light, electrical fields or buffer composition.

Spatial control

In order to control the path along which filaments glide, a process that we call guiding, it is necessary to restrict the location of active motors to specific regions of a surface. This can be done by coating a glass or silicon surface with resist polymers such as PMMA, SU-8 or SAL601 and using UV, electron beam or soft lithography to remove resist from defined regions (Bunk et al., 2003; Hiratsuka et al., 2001; Mahanivong et al., 2002; Moorjani et al., 2003; Nicolau et al., 1999; Riveline et al., 1998; Suzuki et al., 1997; Wright et al., 2002). The motor-containing solution is then perfused across the surface. By choosing appropriate properties of this solution—the concentration of motors, salts, other blocking proteins such as

casein and BSA, and detergents such as Triton X-100—motility can be restricted to either the unexposed, resist surface or to the exposed, underlying substrate. However, the interactions of the motors with these surfaces are not well understood. For example, myosin motility is primarily restricted to the more hydrophobic resist surfaces while kinesin motility is primarily restricted to the more hydrophilic non-resist surfaces. One limitation of this approach to binding proteins to surfaces is that the motors tend to bind everywhere, so it is difficult to attain good contrast. A proven method to prevent motor binding is to coat a surface with polyethylene oxide (PEO) (deCastro et al., 1999; Hess et al., 2002b). Techniques to specifically bind motors and filaments to surfaces are summarized in the last section of the

Methods.

While chemical patterning can restrict movement of filaments to areas with a high density of active motors, walking off the trails is not prevented. This was demonstrated by Hess et al. (Hess et al., 2002b) who showed that microtubules move straight across a boundary between high motor density (non-PEO) and low motor density (PEO) where they dissociate from the surface. The problem with a purely chemical pattern is that if a rigid filament is propelled by several motors along its length there is nothing to stop the motors at the rear from pushing the filament across a boundary into an area of low motor density. Combining chemical and topographic features, as occurs in the lithographic studies described above, leads to more efficient guiding. For example, in the study of Moorjani et al. (Moorjani et al., 2003) filaments remained at the bottom of the channels formed in the SU-8 even when they collided with the walls at angles above 80 degrees. When the leading end of the microtubule hits the wall, the motors at the rear force the microtubule to bend into the region of high motor density and in this way the motion is guided by the boundary (see Figure 4). The behavior of microtubules colliding with the walls of channels imprinted in polyurethane has been studied by Clemmens et al. (Clemmens et al., 2003). They found that the probability of a filament being guided by the walls decreased as the approach angle increased. At high incident angles, guiding was not observed and instead the microtubules climbed the walls. Taken together, these studies show that a combination of chemical and topographic patterning is necessary for efficient guiding.

While it is possible to use chemical and topographical patterning to guide filaments—that is to restrict their movement to particular paths—it is more difficult to control the direction of movement along the path. The difficulty arises because the orientation in which motors bind to a uniform surface is not controlled. Some motors will be oriented so that they propel filaments in one direction along the path, whereas others will propel filaments in the opposite direction. The reason that motors do not counteract each other is that filaments are polar structures: the orientation of the proteins that form up the filaments is maintained all along the length of the filament (see Figure 1). Because the motors bind stereospecifically to the filament, they will exert force in only one direction. Thus, the orientation of the filament determines its direction of motion; one end always leads.

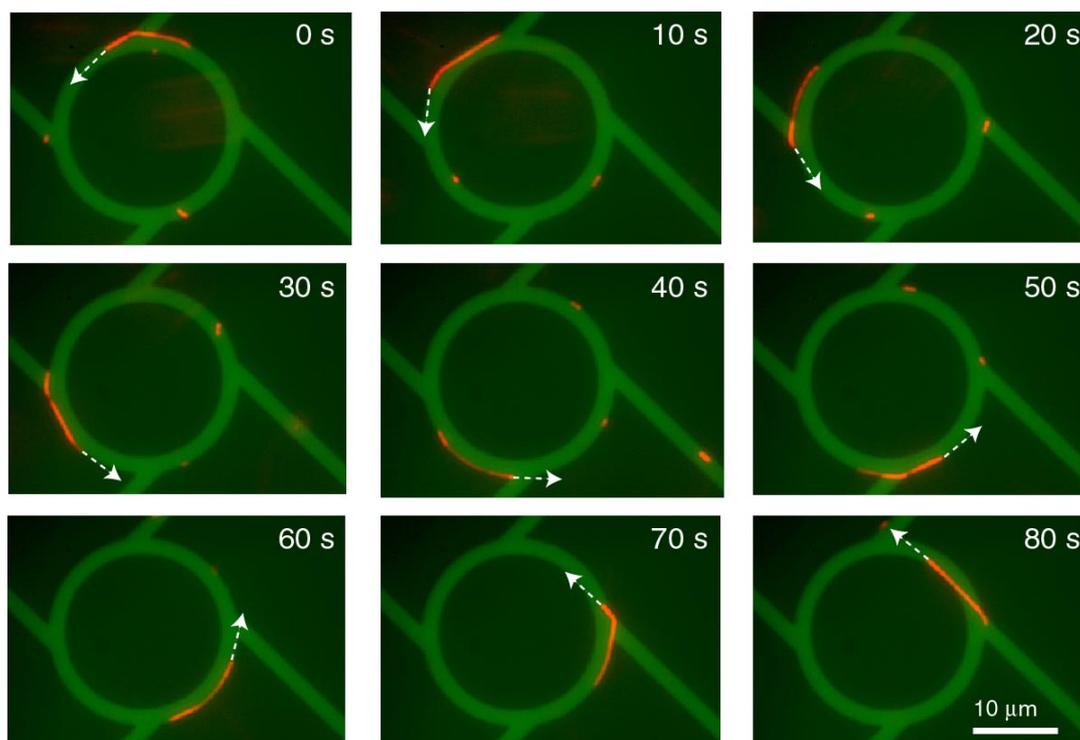


Figure 4. Sequence of fluorescent images showing the kinesin driven, unidirectional movement of a rhodamine-labeled microtubule (red) along a chemically and topographically structured Si-chip. The bottom of the channels (green), whose depths are 300 nm, is coated with kinesin. The surrounding regions are blocked by polyethylene glycol. Collaboration with R.M.M. Smeets, M.G.L. van den Heuvel, and C. Dekker (Delft University of Technology, The Netherlands).

The direction of filament gliding can be controlled by the application of external forces. Actin filaments and microtubules both possess negative net charges, and consequently, in the presence of a uniform electric field, will experience a force directed towards the positive electrode. It is possible to apply high enough electric fields to steer motor-driven filaments in a specified direction (Riveline et al., 1998; Stracke et al., 2002). Because the refractive index of protein differs from that of water, filaments become electrically polarized in the presence of an electric field, and consequently in a non-uniform field they move in the direction of highest field strength. This so-called dielectrophoretic force has been used to direct the gliding of actin filaments on a myosin coated substrate (Asokan et al., 2003). It is even possible to manipulate a microtubule using optical gradients produced by focusing a laser beam (i.e. an optical tweezers). (Felgner et al., 1997) Directional control of microtubule gliding has also been achieved using hydrodynamic flow fields (Bohm et al., 2001; Prots et al., 2003).

An alternative approach to directionality relies on more sophisticated guiding concepts. For example, unidirectional movement of filaments can be achieved if guiding geometries based on arrow and ratchet structures are employed (Hess et al., 2002b; Hiratsuka et al., 2001). An example of the unidirectional movement of a microtubule on a topographically and chemically structured silicon chip is depicted in Figure 4 (unpublished results from our laboratory).

To control the direction of motion in stepping assays, the orientation of the filaments on the surface must be controlled. Towards this end, the generation of isopolar filament arrays has been achieved by binding specific filament ends to a surface, and using hydrodynamic flow to align the filaments along the surface to which they are subsequently adhered to (Brown and Hancock, 2002; Limberis et al., 2001; Limberis and Stewart, 2000; Spudich et al., 1985). Alternatively, moving filaments can be aligned in a particular orientation by a flow field prior to fixation by glutaraldehyde (Bohm et al., 2001; Prots et al., 2003), which has been shown not to interfere with kinesin motility (Turner et al., 1996). Fluid flow has also been used to align microtubules binding to patterned silane surfaces, though the orientation of the microtubules was not controlled (Turner et al., 1995).

Connecting to cargoes

Cargoes can be attached to filaments using several different approaches. The prospective cargo can be coated with an antibody to the filament (Wada et al., 2000) or to a filament-binding protein such as gelsolin (Veigel et al., 1999). A clever refinement of this technique is to genetically fuse gelsolin with a cargo protein thereby generating a dual-functional protein (Yajima et al., 2002). Alternatively, the cargo can be coated with streptavidin which binds to filaments that have been derivatized with biotin (Gittes et al., 1996). There are many other possibilities which have not yet been realized.

Analogous methods can be used to couple motors to surfaces. For example, the motor can be fused with the bacterial biotin-binding protein (Berliner et al., 1994) and in this way bound to streptavidin-coated cargoes or surfaces. There are many peptide tags that can be fused to proteins to aid their purification (Jarvik and Telmer, 1998; Terpe, 2003). These tags can be used to couple to surfaces coated with the complementary ligand. A popular tag is the hexahistidine tag which binds Ni^{2+} and other metals that are chelated to nitriloamines (NTA). A nice approach is to couple the NTA to the terminal ethyleneoxides of triblock copolymers containing PEO. In principle, this provides specific binding of a his-tagged motor (or another protein) to a surface while the PEO groups block non-specific binding (Bearinger et al., 2003; deCastro et al., 1999).

Controlled unloading of cargo has not been demonstrated, but ought to be feasible. For example, there are biotins that can be irreversibly cleaved by light and reversibly cleaved by reducing agents, and the histidine- Ni^{2+} -NTA connection can be broken by sequestering the Ni^{2+} with EDTA.

Outlook

The first steps have been made towards the operation of biomolecular motors in engineered environments. However, many advances are necessary before these motors can be used in nanotechnological applications such as working in molecular factories and building circuits.

An immediate task is to improve the spatial and temporal control over the motors. By combining improved surface techniques with the application of external electric, magnetic and/or optical fields it should be possible, in the near future, to stretch and collide single molecules, to control cargo loading and unloading, and to sort and pool molecules.

A crucial longer-term goal is to control the position and orientation of motors with molecular precision. This means placing motors with an accuracy of ~10 nm on a surface and controlling their orientation within a few degrees. In this way both the location and the direction of motion of filaments can be controlled. One approach to molecular patterning is to “decorate” filaments with stereospecifically bound motors. Once aligned along the filament matrix, the motors can be transferred to another surface. This approach was taken by Spudich et al. (Spudich et al., 1985; Toyoshima et al., 1989) and should be followed up. A further development of this idea is to directly produce, perhaps by stamping a mold made with a filament, surfaces that have structures functionally similar to motor binding sites. An alternative approach is to use dip-pen lithography (see Chapter 1.2 in this book) or other AFM techniques (Jiang et al., 2003) to directly pattern motors on surfaces.

The robustness of motors must be increased. Motors operate only in aqueous solutions and under a restricted range of solute concentrations and temperatures. While it is inconceivable that protein-based motors could operate in a non-aqueous environment, two approaches to increasing their robustness can be envisaged. First, motors could be purified from thermophilic or halophilic bacteria some of which grow at temperatures up to 112 °C and salinities above 5 M. There are extreme eukaryotes that grow up to 62 °C or 5 M NaCl. This approach has already been taken for ATP synthase (Hazard and Montemagno, 2002), but not with linear motors because no obvious homologues of myosins or kinesins have been found in bacteria. Second, a genetic screening approach might reveal mutations that allow motors to operate in less restrictive or different conditions. A longer-term goal is to use the design principles learnt from the study of biomolecular motors to build purely artificial nanomotors that can operate in air or vacuum. This is a daunting prospect and it is not even clear what fuel(s) might be used. A potential way forward is to use chemical energy from a surface: for example it was demonstrated that tin particles slide across copper surfaces driven by the formation of bronze alloy (Schmid et al., 2000). This is analogous to paraffin-driven toy boats.

Besides the motor systems discussed so far, other biomechanical assemblies are good candidates for nanotechnological applications. Besides providing paths along which motors move, active biological filaments might find use in nanotechnological applications. The

pushing and pulling forces generated by the polymerization and depolymerization of actin filaments and microtubules provide an alternative method of moving molecules (Dogterom and Yurke, 1997; Howard, 2001). This ability is of particular interest because bacteria possess actin- (van den Ent et al., 2002) and microtubule-like (Lowe and Amos, 1998) filaments, and as mentioned above the proteins of extremophilic bacteria function in extreme environmental conditions. Filaments and motors can also self-organize under certain conditions (Humphrey et al., 2002; Kruse and Julicher, 2000; Nedelec et al., 1997; Surrey et al., 1998). On a side note, the flagellar filament in conjunction with the flagellar motors allow the bacteria to move in three dimensional liquid space (Ryu et al., 2000).

In addition to the motors that we have described so far, cells contain numerous biomolecular machines that can also be thought of as motors (see e.g. Alberts, 2002 (Alberts, 2002)). These machines use chemical energy to replicate DNA (DNA polymerases) and process it (recombinases, topoisomerases and endonucleases), to produce RNA (RNA polymerases) and splice it (spliceosomes), to make proteins (ribosomes) and fold them (chaperones) and move them across membranes (translocases) and finally destroy them (proteasomes). The energy is provided by another group of machines that generate the electrochemical gradients (electron transport system, bacteriorhodopsin) used by the F_1F_0 -ATP synthase to make ATP or by flagellar motors to propel bacteria. All these machines are candidates for nanotechnological applications, and a recent report of the use of chaperones to maintain nanoparticles in solution (Ishii et al., 2003) is a step in this general direction.

We finish up by pointing out that the high order and nanometer-scale periodicity of DNA, actin filaments and microtubules make them ideal scaffolds on which to erect 3-dimensional nanostructures. While these features have been exploited to make DNA-based structures (Seeman, 2003) (see Chapter III), the use of DNA motors to address specific sites (based on nucleotide sequence) has not, to our knowledge, been realized. Some years ago it was proposed that the regular lattice of microtubules might serve as substrates for molecular computing and information storage (Hameroff and Watt, 1982; Penrose, 2001). While these ideas seem crazy in the context of the living organism, they may be realizable for biomolecular motors operating in engineered environments. At the moment, anything is possible.

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