NANOTECHNOLOGICAL APPLICATIONS OF BIOMOLECULAR MOTOR SYSTEMS

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Biomolecular motors are the active workhorses of cells [1]. 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: it drives the contraction of a muscle by moving along a filament formed from the protein actin. However, 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 (see Figure 1a).

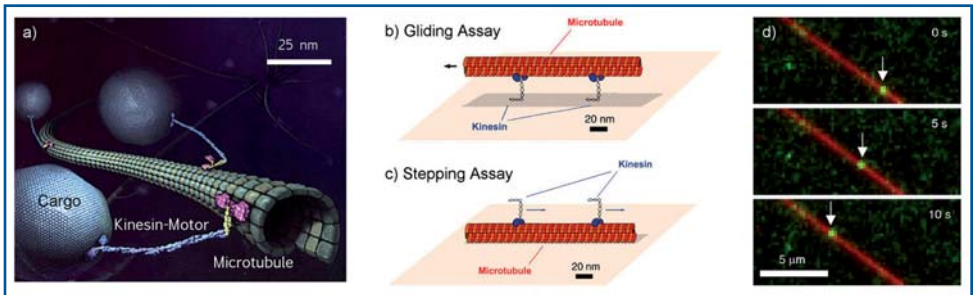

Fig. 1 Kinesin-microtubule assays. a) In a cellular environment, kinesin motors transport cargo, such as membrane-bounded vesicles, along microtubule tracks (Image courtesy of Graham Johnson of www.fivth.com and The Scripps Research Institute). The motility of molecular motors can be reconstituted in vitro in b) gliding assays (where the filaments are propelled by surface-bound motor molecules) and c) stepping assays (where the filaments are immobilized on the surface and motors walk on them). d) Sequence of fluorescent micrographs showing the movement of a kinesin motor (labeled with the green fluorescent protein) along a microtubule (red) in a single-molecule stepping assay. Images were acquired at the indicated times using total-internal-reflection fluorescence microscopy.

SUMMARY

Biological cells contain molecular machines that perform complex mechanical tasks such as intracellular transport, chromosome separation and muscle contraction. These processes are driven by biomolecular motors, proteins that convert the chemical energy of ATP directly into mechanical work. Recent advances in understanding how such motor proteins work have 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.

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 a week. This corresponds to a speed of 1 – 2 µm/s, which is typical of biomolecular motors [2].

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 [3]. 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.

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 interme-

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diate 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 [2]. As energy converters, biomolecular machines are highly efficient. The chemical energy available from the hydrolysis of ATP is 100×10^{-21} J = 100 pN•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 [2]. 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 lie between 80 % and 100 % [4]. 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 ($1 \text{ mg} = 3.3 \times 10^{15}$ 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 has been developed to manipulate these proteins outside the cell.

This report focuses on *linear motors* that 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. Another class of motors are *rotary motors* that 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.

RECONSTRUCTION OF MOTILITY SYSTEMS *IN VITRO*

The general setups for studying cytoskeletal 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 1b). In the stepping assay, the filaments are laid out on the surface where they form tracks for the motors to move along (Figure 1c, d). Both assays are performed in aqueous solution, where the environmental conditions are chosen similar to those present in cells. Movement is

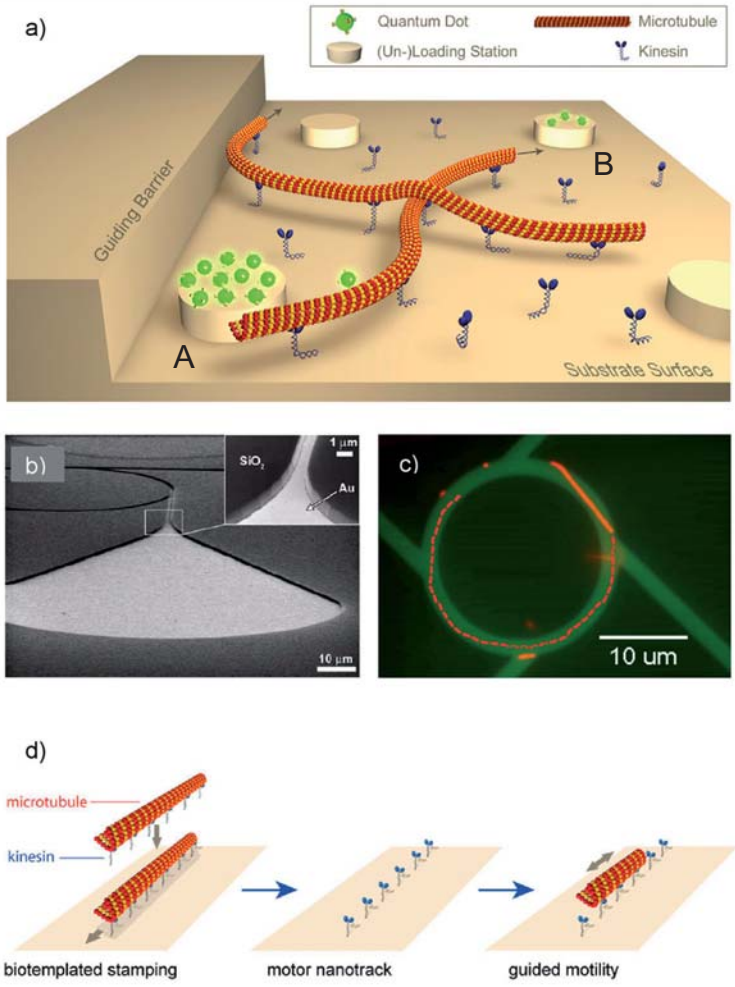

Fig. 2 Examples of a kinesin-microtubule transport system operating in a synthetic environment. a) Microtubules are propelled over the surface by immobilized kinesin motors in the presence of ATP. Cargo (e. g. quantum dots) is to be picked up at point A and moved to point B. To reliably guide the movement of gliding filaments, a combination of topographical and chemical surface modifications has proven most efficient. b) Example of a lithographically structured Si-chip where the bottoms of 300-nm deep channels are coated by gold (Au) in order to achieve a selective binding of motor proteins. The surrounding regions are blocked by polyethylene glycol which prevents protein adhesion (from Ref. [12]). c) Fluorescent image showing the kinesin-driven, unidirectional movement (red dotted line) of a rhodamine-labeled microtubule (red) along a chemically and topographically structured channel. d) Biotemplated stamping of kinesin nanotracks without topographical surface modifications. Kinesin molecules, which are bound in an oriented manner to the lattice of a template microtubule, are transferred onto the surface by a stamping process. After adsorption the 'template' microtubule is released when the deposited motor molecules propel the microtubule off the generated track in the presence of ATP. The same molecules will move and guide the 'transport' microtubules (from Ref. [13]).

observed under the light microscope using fluorescence markers or high-contrast techniques. At the Max Planck Institute for Molecular Cell Biology and Genetics Dresden (MPI-CBG) the depicted motility assays are currently applied in combination with novel optical imaging techniques to further the biophysical understanding of biomolecular motors [5–8]. 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 is the most promising setup for nanotechnological usage. 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 (see Figure 2a).

However, for use in nanotechnological applications, the movement of gliding filaments has to be controllable in space and time. A number of methods to control the path along which filaments glide, a process that we call 'guiding', have been developed. Specifically, static spatial control has been achieved using topographical features [9], chemical surface modifications [10], and a combination of both [11,12] (see Figure 2b-d). While it is possible to use chemical and topographical patterning to guide filaments, it is more difficult to control the direction of movement along the path. This difficulty arises because the orientation in which motors bind to a uniform surface is not controlled. However, because the motors bind stereospecifically to the filament, they will exert force in only one direction. Consequently, only the orientation of the filament determines its direction of motion and directionality has so far only been achieved by the design of sophisticated guiding geometries. For example, unidirectional movement of filaments can be achieved if arrow-and ratchet-based guiding structures are employed [10–12] (Figure 2c). However, the generation of topographical surface structures is costly and labor-intensive. It is therefore desirable to produce non-topographical tracks of motor proteins, leading to reliable microtubule guiding when the structural widths are small (below 1 µm, see Figure 2d) [13].

CONTROL OVER MOTOR ACTIVITY BY EXTERNAL SIGNALS

Spatial control in a dynamic manner can be achieved by the application of external forces. For example, 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 [14-16]. 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 [17]. Directional control of microtubule gliding has also been achieved using hydrodynamic flow fields [18-20].

Regarding temporal control mechanisms, 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 [20]. 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 [9], though the start-up and slow-down times were slow, in the order of minutes.

In order to develop novel mechanisms for controlling microtubule motility by external signals, we measured the height at which microtubules glide over a kinesin-coated surface in the

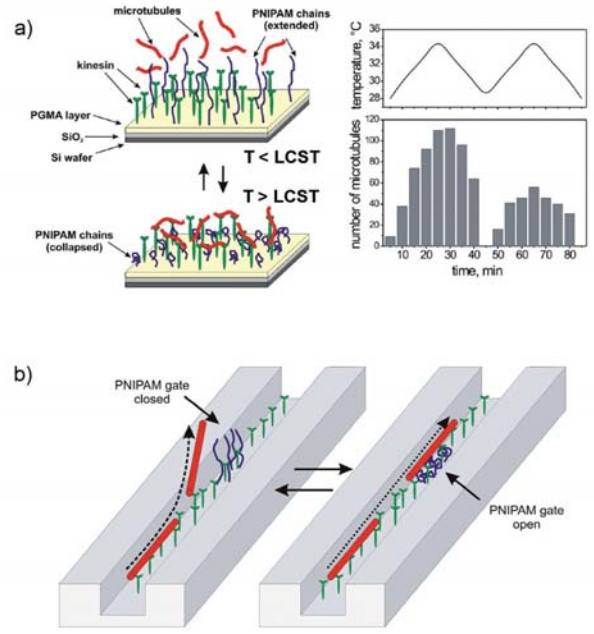

Fig. 3 Control of microtubule motility on switchable polymer surfaces. a) Kinesin motors are embedded amongst thermoresponsive PNIPAM molecules on a substrate surface. Repeated changes in the temperature result in the reversible switching of PNIPAM chains between the expanded conformation (where microtubules are repelled from the surface and cannot bind to the kinesin heads) and the collapsed conformation (where microtubules can glide unhindered on the kinesin molecules). The number of microtubules gliding on the surface thus varies as a function of temperature (LCST: lower critical solution temparature, PGMA: poly glycidyl methacrylate). b) Proposed incorporation of switchable polymers into guiding channels. Upon external signal, incoming microtubule transporters can be derailed (when the PNIPAM gate is closed) or they can continue to travel along the channel when the PNIPAM gate is open (Image courtesy of Leonid Ionov, Max Planck Institute for Molecular Cell Biology and Genetics Dresden).

presence of ATP. To perform height measurements with nanometer precision, we used fluorescence-interference contrast (FLIC) microscopy, which is based on the self-interference of fluorescent light from objects near a reflecting surface (similar to the setup shown in Figure 2a). We determined that kinesin-1 molecules elevate gliding microtubules about 20 nm above the surface [7]. While this value is significantly lower than the contour length of the motor molecule (in the order of 70 nm), it is consistent with the segmented structure of the molecule. Given the low height of filament movement above the substrate material, another possibility to influence motility is provided by dynamic switching of the surface properties. Towards this end, we embedded in collaboration with the Leibniz Institute of Polymer Research Dresden kinesin molecules within a thin layer of the thermoresponsive polymer poly(N-isopropyl acrylamide) (PNIPAM) (see Figure 3a). PNIPAM chains adopt a compact or extended conformation above or below the lower critical solution temperature (32 °C), respectively. We observed that microtubules can land and glide on a PNIPAM-kinesin surface at high temperature (when the polymer chains are compacted and the surface-attached kinesin molecules are accessible for the microtubules) or are released from the surface at low temperature [21]. This process can be repeated multiple times demonstrating the reversibility of the effect. While we were so far only able to regulate the motility on a global scale, we are currently working towards implementing smart polymer systems into more sophisticated environments (Figure 3b).

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 [22] and the inhibition of kinesin by its cargo-binding "tail" domain [23]. 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 [24]. ATP-driven rotation can be restored by the addition of metal ion chelators. Along these lines we are currently working on the genetic engineering of motors that potentially provide temporal control mechanisms switched by temperature and light.

FIRST TECHNOLOGICAL APPLICATIONS

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 [9] and virus particles [25], the sorting of macromolecular assemblies [26], and the measurement of forces in the pN range [27].

In a collaboration with the BioNanoStructuring group at the Max Bergmann Center of Biomaterials Dresden we used chemically modified microtubules to transport and stretch individual

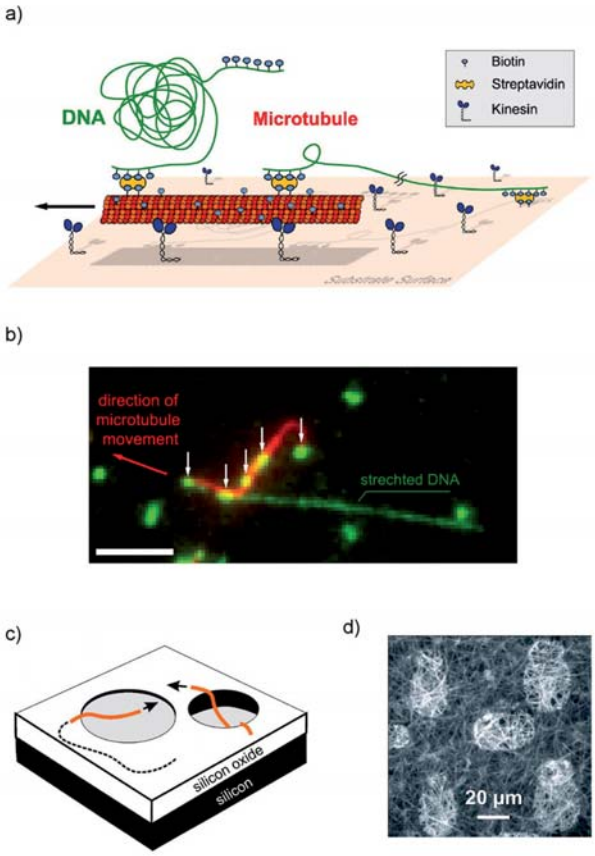

Fig. 4 First nanotechnological applications of kinesin-microtubule transport systems. a) Setup of DNA-nanocircuits: Biotinylated microtubules are used to transport and stretch individual λ-phage DNA molecules that are biotinylated at their ends and attached to the microtubule via a streptavidin linkage (from Ref. [28]). b) Fluorescence micrograph of a moving microtubule (red) transporting (white arrows) and stretching individual DNA molecules (green) (scale bar 5 µm). This technique may offer an efficient mechanism for the generation of multidimensional, DNA-based networks, which can be metallized nano electronic applications. c) Surface imaging by motile microtubules: Schematic diagram of kinesin-driven microtubule nanoprobes crossing shallow or deep pits of a transparent silicon oxide layer above a reflective silicon mirror. d) Due to interference effects, a maximum projection of the microtubule trajectories provides a topographical image of the surface with nanometer height resolution (unpublished work).

λ-phage DNA molecules across a surface [28] (see Figure 4a and b) and extended this approach to the manipulation of bifunctional DNA molecules, where individual DNA molecules were simultaneously bound to a structured gold surface (via thiolization of one DNA end) and to biotinylated microtubules (via biotinylation of the other DNA end and streptavidin) [29]. This technique, in contrast to existing nanotechnology tools, enables the parallel yet individual manipulation of many molecules and may offer an efficient mechanism for the generation of multidimensional DNA-based networks which,

after metallization, can be used for nano-electronic applications. Secondly, we employed gliding microtubules as self-propelled probes to measure local height information using FLIC-microscopy. At high motor densities, the trajectories of the flexible microtubules reproduce the topography of the surface (Figure 4c). Utilizing a patterned silicon oxide layer, kinesin-driven microtubules moved over the edges and changed their distance to the underlying silicon substrate accordingly. By projecting the maximum intensities of all microtubule trajectories onto a single image, the topography of the various patches is reproduced (Figure 4d). In this application, the microtubules can be regarded as massively parallel, extremely sensitive height probes. In combination with the above mentioned advancements in the directional control of microtubules on surfaces, this nano-probing could bridge the gap between global probing techniques such as imaging ellipsometry, and local techniques such as atomic force microscopy. Moreover, deep pits and cavities could be imaged that are inaccessible by larger probes.

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 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. Additionally, 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 increase their robustness can be envisaged. First, motors could be purified from thermophilic or halophilic bacteria some of which grow at high temperatures and high salt concentrations. 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. 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 three-dimensional nanostructures. While these features have been exploited to produce DNA-based structures [30], 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 [31,32]. While these ideas seem impracticable in the context of the living organism, they may be realizable for biomolecular motors operating in engineered environments.

Already now, medical applications can benefit tremendously from biomolecular motors in artificial environments. Recent studies have revealed that kinesin, dynein and myosin play significant roles in the pathogenesis of a variety of diseases. For example, kinesin deficiencies have been identified as cause for Charcot-Marie-Tooth disease and some kidney diseases. Dynein deficiencies can lead to chronic infections of the respiratory tract as cilia fail to function without dynein. Defects in muscular myosin predictably cause myopathies, whereas defects in unconventional myosin are the cause for Usher syndrome and deafness [33]. Understanding the basic principles of motor operation will thus allow to design specific drugs to enhance (or inhibit) the (mal)function of these motors [34]. Here, the *in vitro* reconstitution of particular subcellular transport systems will provide the testbeds for a high-throughput drug screening [35]. Moreover, when operated as molecular sorting devices, motor-driven filaments can be used to collect and concentrate specific reagents that are present in a test sample at extremely low concentration. When combined with micro-chip technologies, such systems might form the base for a new class of point-of-care molecular detection and diagnosis devices.

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REFERENCES

1. B. Alberts, “The cell as a collection of protein machines: preparing the next generation of molecular biologists”, *Cell*, **92**, 291–294 (1998).
2. J. Howard, “Mechanics of Motor Proteins and the Cytoskeleton”, Sunderland, MA: Sinauer Associates (2001).
3. B. Alberts, “Molecular biology of the cell, 4th edition”, New York: *Garland Science* (2002).
4. K. Kinosita, Jr., R. Yasuda, H. Noji, S. Ishiwata, M. Yoshida, “F1-ATPase: a rotary motor made of a single molecule”, *Cell,* **93**, p. 21–24 (1998).

5. J. Helenius, G. Brouhard, Y. Kalaidzidis, S. Diez, J. Howard, "The depolymerizing kinesin MCAK uses lattice diffusion to rapidly target microtubule ends", *Nature*, **441**, 115–119 (2006).
6, V. Varga, J. Helenius, K. Tanaka, A.A. Hyman, T.U. Tanaka, J. Howard, "Yeast kinesin-8 depolymerizes microtubules in a length-dependent manner", *Nature Cell Biology*, **8**, 957–U960 (2006).
7. J. Kerssemakers, J. Howard, H. Hess, S. Diez, "The distance that kinesin-1 holds its cargo from the microtubule surface measured by fluorescence interference contrast microscopy", *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 15812–15817 (2006).
8. C. Leduc, F. Ruhnow, J. Howard, S. Diez, "Detection of fractional steps in cargo movement by the collective operation of kinesin-1 motors", *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 10847–10852 (2007).
9. H. Hess, J. Clemmens, D. Qin, J. Howard, V. Vogel, "Light-controlled molecular shuttles made from motor proteins carrying cargo on engineered surfaces", *Nano Letters*, **1**, 235–239 (2001).
10. H. Hess, J. Clemmens, C.M. Matzke, G.D. Bachand, B.C. Bunker, V. Vogel, "Ratchet patterns sort molecular shuttles", *Applied Physics a (Materials Science Processing),* **A75**, 309–313 (2002).
11. Y. Hiratsuka, T. Tada, K. Oiwa, T. Kanayama, T.Q.P. Uyeda, "Controlling the direction of kinesin-driven microtubule movements along microlithographic tracks", *Biophysical Journal*, **81**, 1555–1561 (2001).
12. M.G.L. van den Heuvel, C.T. Butcher, R.M.M. Smeets, S. Diez, C. Dekker, "High rectifying efficiencies of microtubule motility on kinesin-coated gold nanostructures", *Nano Letters*, **5**, 1117–1122, (2005).
13. C. Reuther, L. Hajdo, R. Tucker, A.A. Kasprzak, S. Diez, "Biotemplated nanopatterning of planar surfaces with molecular motors", *Nano Letters*, **6**, 2177–2183, (2006).
14. D. Riveline, A. Ott, F. Julicher, D.A. Winkelmann, O. Cardoso, J.J. Lacapere, S. Magnusdottir, J.L. Viovy, L. Gorre-Talini, J. Prost, "Acting on actin: the electric motility assay", *European Biophysics Journal with Biophysics Letters*, **27**, 403–408, (1998).
15. R. Stracke, K.J. Bohm, L. Wollweber, J.A. Tuszynski, E. Unger, "Analysis of the migration behaviour of single microtubules in electric fields", *Biochemical and Biophysical Research Communications*, **293**, 602–609, (2002).
16. M.G.L. van den Heuvel, M.P. De Graaff, C. Dekker, "Molecular sorting by electrical steering of microtubules in kinesin-coated channels", *Science*, **312**, 910–914, (2006).
17. S.B. Asokan, L. Jawerth, R.L. Carroll, R.E. Cheney, S. Washburn, R. Superfine, "Two-Dimensional Manipulation and Orientation of Actin-Myosin Systems with Dielectrophoresis", *Nano Letters*, **3**, 431–437, (2003).
18. K.J. Bohm, R. Stracke, P. Muhlig, E. Unger, "Motor protein-driven unidirectional transport of micrometer-sized cargoes across isopolar microtubule arrays", *Nanotechnology*, **12**, 238–244, (2001).
19. I. Prots, R. Stracke, E. Unger, K.J. Bohm, "Isopolar microtubule arrays as a tool to determine motor protein directionality" *Cell Biology International*, **27**, 251–253, (2003).
20. F.U. Gast, P.S. Dittrich, P. Schwille, M. Weigel, M. Mertig, J. Opitz, U. Queitsch, S. Diez, B. Lincoln, F. Wottawah, S. Schinkinger, J. Guck, J. Kas, J. Smolinski, K. Salchert, C. Werner, C. Duschl, M.S. Jager, K. Uhlig, K.; Geggier, P.; Howitz, S., "The microscopy cell (MicCell), a versatile modular flowthrough system for cell biology, biomaterial research, and nanotechnology. In: Microfluidics and Nanofluidics", **2**, 21–36, (2006).
21. L. Ionov, M. Stamm, S. Diez, "Reversible switching of microtubule motility using thermoresponsive polymer surfaces", *Nano Letters*, **6**, 1982–1987, (2006).
22. J.R. Sellers, H.V. Goodson, "Motor proteins 2: myosin", *Protein Profile*, **2**, 1323–1423, (2006).
23. D.L. Coy, W.O. Hancock, M. Wagenbach, J. Howard, "Kinesin's tail domain is an inhibitory regulator of the motor domain", *Nat Cell Biol*, **1**, 288–292, (1999).
24. H. Liu, J.J. Schmidt, G.D. Bachand, S.S. Rizk, L.L. Looger, H.W. Hellinga, C.D. Montemagno, "Control of a biomolecular motor-powered nanodevice with an engineered chemical switch", *Nat Mater*, **1**, 173–177, (2002).
25. G.D. Bachand, S.B. Rivera, A. Carroll-Portillo, H. Hess, M. Bachand, "Active capture and transport of virus particles using a biomolecular motor-driven, nanoscale antibody sandwich assay", *Small*, **2**, 381–385, (2006).
26. L. Ionov, M. Stamm, S. Diez, "Size sorting of protein assemblies using polymeric gradient surfaces", *Nano Letters*, **5**, 1910–1914, (2005).
27. H. Hess, J. Howard, V. Vogel, "A piconewton forcemeter assembled from microtubules and kinesins", *Nano Letters*, **2**, 1113–1115, (2002).
28. S. Diez, C. Reuther, C. Dinu, R. Seidel, M. Mertig, W. Pompe, J. Howard, "Stretching and Transporting DNA Molecules Using Motor Proteins", *Nano Letters*, **3**, 1251–1254, (2003).
29. C.Z. Dinu, J. Opitz, W. Pompe, J. Howard, M. Mertig, S. Diez, "Parallel manipulation of bifunctional DNA molecules on structured surfaces using kinesin-driven microtubules", *Small*, **2**, 1090–1098, (2006).
30. N.C. Seeman, "DNA in a material world", *Nature*, **421**, 427–431, (2003).
31. S.R. Hameroff, R.C. Watt, "Information processing in microtubules", *J Theor Biol*., **98**, 549–561, (1982).
32. R. Penrose, "Consciousness, the brain, and spacetime geometry: an addendum. Some new developments on the Orch OR model for consciousness", *Ann N Y Acad Sci.*, **929**, 105–110, (2001).
33. N. Hirokawa, R. Tekamura, "Biochemical and molecular characterization of diseases linked to motor proteins", *Trends in Biochemical Sciences*, **28**, 558–565, (2003).
34. R. Sakowicz, M.S. Berdelis, K. Ray, C.L. Blackburn, C. Hopmann, D.J. Faulkner, L.S.B. Goldstein, "A marine natural product inhibitor of kinesin motors", *Science*, **280**, 292–295, (1998).
35. See, for example: http://www.cytokinetics.com