Molecular Motors as Components of Future Medical Devices and Engineered Materials

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A new frontier in the development of prosthetic devices is the design of nanoscale systems which replace, augment, or support individual cells. Similar to cells, such devices will require the ability to generate mechanical movement, either for transport or actuation. Here, the development of nanoscale transport systems, which integrate biomolecular motors, is reviewed. To date, close to 100 publications have explored the design of such "molecular shuttles" based on the integration of synthetic molecules, nano- and microparticles, and micropatterned structures with kinesin and myosin motors and their associated cytoskeletal filaments, microtubules, and actin filaments. Tremendous progress has been made in addressing the key challenges of guiding, loading, and controlling the shuttles, providing a foundation for the exploration of applications in medicine and engineering. [DOI: 10.1115/1.3212823]

1 Introduction

Biomedical engineering has succeeded in replacing and augmenting the function of diseased or damaged organs with prosthetic devices. The "iron lung," the dialysis machine, and the artificial heart have enabled major progress in medical practice. Due to their macroscopic dimensions, these devices interact with the biological system at the organ level. Microfabricated devices go a step further and permit the addressing of individual cells. Prominent examples are cochlear implants [1,2] and artificial retinas [3,4], which direct impulses toward specific neurons via microscale electrodes. Our increasing mastery of nanotechnology allows an even farther-reaching vision [5] which complements cell transplantation [6]: the replacement, augmentation, and support of diseased, damaged, or overstressed cells with nanoscopic devices.

The interaction with cells has so far been the province of pharmaceutical science via the delivery of small molecules and proteins, and increasingly tissue engineering, which aims to organically replace diseased and damaged cells with cultured cells derived, e.g., from stem cells. Despite the success and promise of these approaches, reasonable arguments can be made in favor of pursuing the development of prosthetic devices at the cellular scale. A key advantage of most prosthetic devices is that they confer a benefit immediately. Consequently, they can be utilized in emergency situations to give the organism time to rebuild the function of natural systems. A basic example is a titanium plate attached by an orthopedic surgeon to a broken bone to support it during the healing period. Second, devices may perform functions which are not typically performed by cells and may enable the interaction with the environment. Highly sophisticated drug delivery systems, capable of identifying and addressing specific cells and of communicating with systems outside the body would be examples of this class of devices. Finally, prosthetics are often necessitated by the limitations of pharmaceutics and tissue engineering, such as drug resistance and side effects, as well as limited success with certain tissues.

Visions of cellular scale devices [7–9]—a creative term is "pharmacytes" [10]—endow the devices with the capabilities of bacteria: active movement, energy harvesting from the environment, molecular sensing, simple information processing, basic communication, and mechanical/chemical interaction with cells. Significant progress has been made in the past 10 years in the development of components [11] and theoretical concepts [12] which could be used in such devices. However, the integration of the different elements is still an open challenge.

Due to the unsurpassed performance of biological nanomachines, these components often rely on biological nanostructures as building blocks. The thousands of different natural enzymes alone provide a wide selection of highly evolved active nanostructures. These can be further optimized to a range of environments depending on their "organism of origin." In many cases, the performance is close to the theoretical maximum, e.g., the efficiency of F1-ATPase is close to 100% [13]. In the case of motor proteins—ATPases coupling an adenosine triphosphate (ATP) hydrolysis cycle with mechanical motion—the concept of a nanomachine is particularly striking as the conversion of chemical energy into mechanical work is one of the primary tasks of macroscopic machinery.

One of the most active fields of research, a key component of cellular scale devices, and the focus of this review is the integration of kinesin and myosin motor proteins with synthetic structures toward the goal of achieving autonomous transport and actuation on the nanoscale. Starting with the first related publication in 1995 [14] and followed by close to one hundred publications, a wide range of challenges relating to the design of such hybrid microdevices has been explored. In particular the past year has brought significant advances in the design of these "molecular shuttles" or "nanotransporters," (Fig. 1) which merit a detailed discussion in this review.

Two principal design routes have been followed, the "gliding geometry" shown in Fig. 1 and the "bead geometry" [15]. In the gliding geometry, the motors are immobilized on the surface and moving cytoskeletal filaments (microtubules or actin filaments) transport the cargo. In the bead geometry, the filament is immobilized and the cargo is attached to one or several motors walking on the filaments. However, several reports have combined these approaches by utilizing the gliding geometry to orient and immobilize microtubules for subsequent use as tracks in the bead geometry [16–18].

The second key choice is the selection of a motor/filament pair. Both pairs, myosin/actin filaments and kinesin/microtubules, are utilized by several research groups and have characteristic advantages highlighted below.

This review's organization follows the three main streams of research which have emerged in this field: control of the direction of movement, control of cargo loading, and control of the activa-

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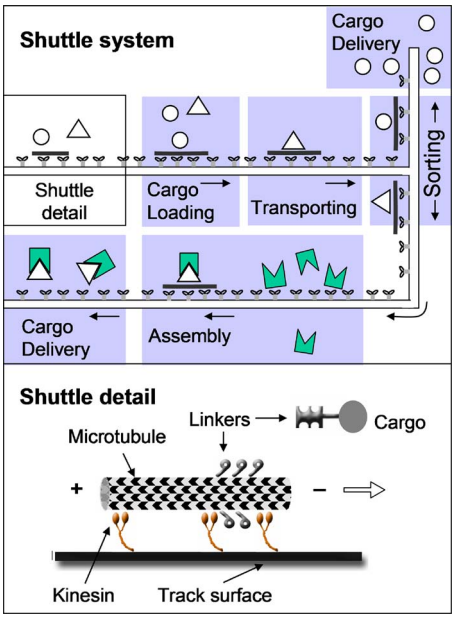

Fig. 1 Molecular shuttles are one example of hybrid microdevices utilizing biomolecular motors. The design shown here aims to achieve controlled transport of nanoscale cargo by immobilizing kinesin motors in tracks and utilizing the functionalized microtubules gliding on these motors as cargo carrying elements. Reproduced with permission from Ref. [24]. Copyright 2003, American Chemical Society.

tion of motors. Here, our focus will be on recent achievements which have not been discussed in previous reviews [19–23].

2 Controlling the Direction of Movement

In this section we review the continual efforts to guide the movement of either motor proteins in a bead assay or filament shuttles in an inverted assay. The gliding geometry has in general been the more popular choice for molecular shuttle development, especially where guiding the direction of movement is involved. In the bead geometry, only the chemical properties of the surface influence the adsorption of filament tracks whereas in the gliding geometry, guiding can be achieved by controlling the surface topography as well as surface chemistry. Recent reports have shown that these approaches are not mutually exclusive. The gliding geometry can be used to orient and immobilize microtubules which then go on to serve as tracks for the bead assays. Other guiding methods involve the use of flow fields, electric fields, and magnetic fields to direct the movement of filaments with external control in real time.

2.1 Guiding Using Surface Topography. A topographically patterned surface, e.g., possessing 1 μm deep open channels, confines filament motility through vertical barriers (Fig. 2(*a*), [24]). The barriers transform the propelling force of motor proteins into bending forces, which results in guiding along channel walls.

Initially, ridges and grooves along the shear axis of a mechanically deposited PTFE film on glass were used to direct the gliding of filaments for both, the myosin-actin [14] and the kinesin-microtubule system [25]. Tracks with arbitrary shape were then created using replica molding of 2 μm wide and 1 μm deep polyurethane channels [26]. Track designs incorporating "arrowhead" shaped rectifiers (Fig. 3(*b*)) define a preferred direction of filament movement [27,28]. Subsequent work has highlighted the physical mechanisms of guiding and the importance of the filament flexibility and track geometry for guiding efficiency [29,30].

A new and highly effective wall geometry consisting of an undercut at the bottom of the channel wall was introduced in Ref. [24]. Microtubules [31] and actin filaments [32] moving on the bottom surface are unable to climb the sidewall and remain on the bottom surface (Fig. 2(*c*)) and preferentially move in the undercut section of the channel. Different track designs (Fig. 3) have also been evaluated and optimized [28,33,34]. Developments in rectifier designs and overhang design were brought together in Ref. [18], where kinesin-propelled short microtubule seeds were oriented in an isopolar fashion in microfabricated open channels. Seeds were then grown into oriented networks of microtubules,

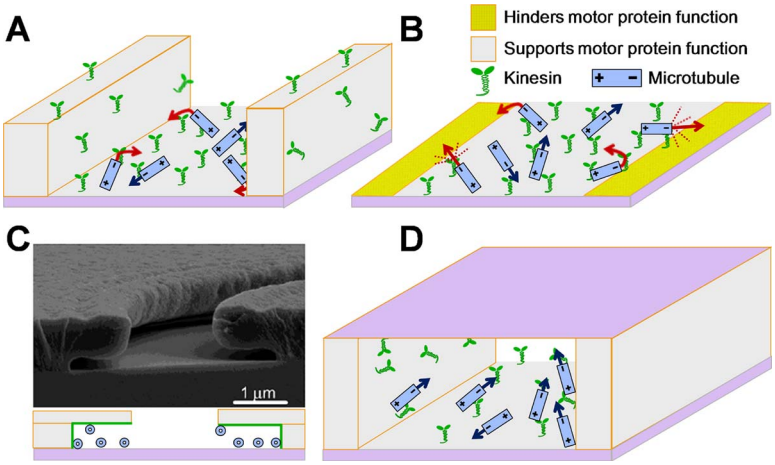

Fig. 2 Approaches to confine filament motility: (*a*) physical confinement—filaments hitting the channel wall get redirected in the direction of the channel axis. However, aligned filaments can gradually climb the wall. If the surface chemistry of walls is designed to interfere with adsorption or functioning of motors, filaments can only glide on the bottom surface (combined confinement), (*b*) chemical confinement—motility restriction is obtained by contrast in active surface motor density between adjacent surfaces, (*c*) confinement due to semi-enclosed channels—excellent guiding is achieved by the undercut regions discovered in Ref. [24], and (*d*) confinement within totally enclosed channels—introduced in Ref. [36] and improved in Ref. [37], enclosed channels do not allow any escape of filaments.

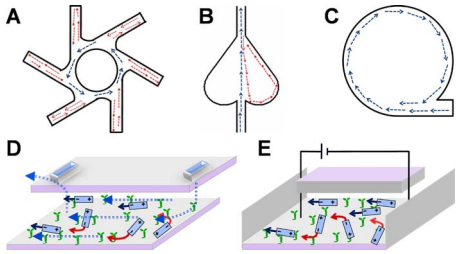

Fig. 3 Designs of track elements. (*a*) A ratchet pattern used to obtain counterclockwise motion of filaments [18,29]. (*b*) A linear rectifier, where filaments moving upwards are unaffected in their direction of motion and filaments going downwards are redirected upwards [27,34]. (*c*) A concentrator capable of trapping filaments [31]. (*d*) Alignment of gliding filaments by fluid flow [16,17,63,65,66]. (*e*) Alignment of gliding filaments by electric fields [28,38,67,69].

which supported motility of kinesin-coated nanospheres with a directional preference. In another report [35], UV light was used to immobilize microtubules which had entered guiding channels from one end (and hence were isopolar) and utilized as tracks for transport in the "bead geometry."

The logical conclusion of these experiments is the utilization of enclosed microfluidic channels [36–38] as illustrated in Fig. 2(*d*). Within enclosed channels selective adsorption of motor proteins is no longer needed and 100% confinement can be achieved.

2.2 Guiding Using Surface Chemistry. As an alternative to topographically defined tracks, tracks can also be defined by a contrast in surface chemistries (Fig. 2(*b*)). Guiding only requires sufficient contrast in the density of functional motors, which means that both approaches, preventing adsorption and modulation of motor activity, are effective in defining nontrack regions. Myosin activity was shown to be modulated strongly by the nature of the surface. In general, cationic or hydrophobic surfaces can adsorb and support myosin motor function while negatively charged surfaces are used to bring motility contrast to the system. On the other hand, kinesin activity is supported by a wide variety of surfaces; hence, development of nonfouling coatings was the method of choice for bringing contrast in microtubule motility between surfaces.

In the first example of guided transport [39], silane films were chemisorbed on polished silicon wafers or glass coverslips and patterned using a deep UV lithographic process. Selective immobilization of microtubules on these lithographically patterned silane surfaces was performed in the presence of a fluid flow field to partially align them. Adsorbed microtubules retained their ability to support the transport of kinesin-coated microspheres. Selective adsorption of microtubules was also carried out using deoxyribonucleic acid (DNA) hybridization [40]. The selective adsorption of kinesin motors to surfaces patterned with plasma-polymerized non fouling coatings were successfully used to define tracks for microtubules [41,42]. In a radically new biotemplated stamping approach [43], microtubules were coated with kinesin motors, the decorated microtubules adhered to a surface via the kinesin motors, and addition of ATP resulted in microtubule release from the chain of surface-adhered motors. Gliding of microtubules on this track of kinesin was observed.

The contrast in myosin adsorption between poly(methyl methacrylate) (PMMA) and glass has been the first and most popular choice for patterning myosin adsorption along various shapes and in varying sizes of patterns [44,45]. The surface hydrophobicity of PMMA electron-beam resist material can also be modulated by exposure to the e-beam and used to pattern myosin adsorption, and hence actin motility [46]. Screening of several materials identified trimethylchlorosilane (TMCS) and glass as a suitable combination for selective myosin binding [47,48]. Biorecognition between biotinylated myosin and a surface patterned with streptavidin and albumin is an alternative means to achieve contrast in myosin density [49]. Recently, tracks of chemically modified myosin (which bound filaments but could not exert force on them) were laid down [50]. These filament-binding tracks captured nascent filaments from solution and guided the direction of their subsequent elongation.

In general, the stiff microtubules fail to reorient on chemical tracks when crossing the boundary to the nonfouling surface and eventually detach. Chemical guiding was more successful for the flexible actin filaments, in part because actin gliding on the nonprocessive myosin motors is more motor density dependent than microtubule gliding on the processive kinesin motors. As a result, the contrast in motor adhesion between track and nontrack regions does not have to be as pronounced.

2.3 Guiding Using a Combination of Topographically and Chemically Structured Surfaces. The disadvantage of topographical pattern is that filaments can climb the sidewalls of guiding channels due to the presence of motors on all surfaces. In contrast, tracks defined by selective adsorption of functional motors do not provide a force to redirect filaments approaching a track boundary. The logical solution, described first by Hiratsuka et al. [27], is to combine both approaches to confine motor adsorption to the bottom surface of a guiding channel. A quantitative discussion of physical, chemical, and combined guiding is found in Ref. [42]. Typically, a polymeric material is patterned on a glass surface and coated with a lipid (Triton X) or block copolymer (Pluronic F108) to suppress adsorption of functional motors [42,51,52]. Alternatively, etching of a track pattern into a SiO_2 surface and covering the bottom surface of the channels with gold prior to functionalization of SiO_2 with poly(ethylene glycol) chains creates a combined pattern with selective adsorption of kinesin motors to the gold bottom surfaces [34,53].

Similar efforts for the actin/myosin system [54,55] identified different resist polymers as myosin-adsorbing and nonadsorbing regions, spin-coated these resists to form a bilayer, and used e-beam lithography to create channels in the nonadsorbing region to expose the underlying adsorbing region. Nonadsorbing walls with 100 nm height were generated using the simpler microcontact printing process [56]. Laser ablation has also been shown to be a promising direct-write tool to create channels with a defined topography and adsorption contrast [57,58]. In an experimentally challenging but very successful approach, polymer bilayers were laid down on a SiO_2 surface [32,48]. After e-beam treatment, the bottom layer served as channel walls and top layer provided a "bottleneck" structure similar to Ref. [24]. The SiO_2 was derivatized with TMCS, which then supported robust motility and long-term trapping of actin filaments on a closed-loop track (width <250 nm) [48].

2.4 Guiding Using Flow Fields. Initially, flow based alignment of microtubules [59,60] and actin filaments [61] was pursued in the absence of motors. Later on, microtubule seeds were immobilized, and subsequent polymerization and immobilization resulted in isopolar microtubule arrays [62]. For filaments gliding on motor proteins a flow field exerts a guiding influence due to the drag force exerted on the free tip (leading end of the filament cantilevered beyond its motor protein support) of the advancing filament. As a result of this force, filaments become ordered into an isopolar array over time (Fig. 3(*d*)) [63] rather than randomly oriented parallel arrays [64]. This enhances their utility as tracks for "bead geometry" designs. The rate of microtubule alignment during shear flow of varying strength and at different motor densities on the surface has been investigated in Ref. [65] giving a detailed picture of the physical mechanism underlying guiding by flow fields [66].

2.5 Guiding Using Electrical Fields. Electric fields can exert electrophoretic forces on the negatively charged actin filaments

[67] and microtubules [68] and induce alignment along the field lines as shown in Fig. 3(*e*). However, electroosmotic flow of counter ions against the moving filament, heating of electrodes, and generation of harmful oxygen are problems associated with DC fields. These drawbacks can be mitigated by spatially separating the electrodes from the motors and filaments. Active, real-time, noninvasive control over the microtubule movement using DC fields was demonstrated in closed submicron channels forming Y junctions [38]. A detailed statistical analysis [69] of microtubule guiding by DC fields proved that the microtubule tip is deflected toward the anode while translocating until it is engaged by another kinesin and the process repeats. Hence, electric field strength, kinesin surface density, and microtubule translocation speed affect the rate of redirection of microtubules.

AC fields exert dielectrophoretic forces on moving filaments and direct them toward regions of highest nonuniformity of fields. Dielectrophoresis solves some of the above-mentioned problems with DC fields and permits the placement of electrodes into the vicinity of motors and filaments [70,71].

2.6 Guiding Using Magnetic Fields. Unlike electric fields, magnetic fields exert negligible forces on microtubules or filaments [72]. However, microtubules can be functionalized with ferrite particles, and aligned [73,74] and directed while being propelled [75] using an externally applied magnetic field.

2.7 Summary of Guiding Approaches. With contributions from many research groups, the repertoire of tools available to guide microtubules and actin filaments as they glide on kinesin and myosin is large and well-understood. Static definition of tracks, e.g., as pioneered in Ref. [27], has been augmented with external, dynamic control over alternative tracks, as demonstrated in Ref. [38]. A more detailed understanding of filament gliding trajectories [76] also enabled the simulation of track designs [77]. These advances have created the engineering foundation for the design of application-oriented devices.

3 Control of Cargo Loading

Within cells, motor proteins bind to designated cargo, such as vesicles, proteins, or RNA, via a specific protein complex [78]. The bead geometry resembles the natural configuration and has been used in studies of motors properties [79–84] and in transport system designs, which rely on ordered arrays of filaments [16–18,35,40,64,66,85,86]. Typically, motors have been attached to micro- and nanospheres by nonspecific adsorption. However, since the bead geometry requires dense overlapping filament tracks [18] and a high motor density on the cargo [83,84] for uninterrupted transport, the gliding (inverted) geometry has significant advantages provided that cargo can be linked to the gliding filaments. The exception to this argument is the transport of microscopic objects, which are large enough to connect to multiple filaments at once [64,85,87].

A variety of bioconjugation approaches has been employed to link cargo (including DNA, microspheres, quantum dots, magnetic particles, and cyclodextrin) to filament transporters. The goal is to create a specific, strong, durable, and reversible link which does not interfere with the attachment and movement of motors to the filament. In the following discussion, the tradeoffs between different published approaches are highlighted.

The first example of cargo loading was provided by the binding of streptavidin-coated superparamagnetic polystyrene microspheres to biotinylated microtubules [26]. Biotin-streptavidin has remained the most popular chemistry for linking cargo to filaments due to its high degree of specificity and strong affinity [88].

However, it has been discovered that the distribution of biotin linkages along the microtubule or actin filament has a strong influence on gliding. Biotinylated microtubules precoated uniformly with 15 nm quantum dots bind to surface-adhered kinesin to a much smaller degree and are unable to move [89]. In contrast, binding and gliding of microtubules with biotinylated segments is

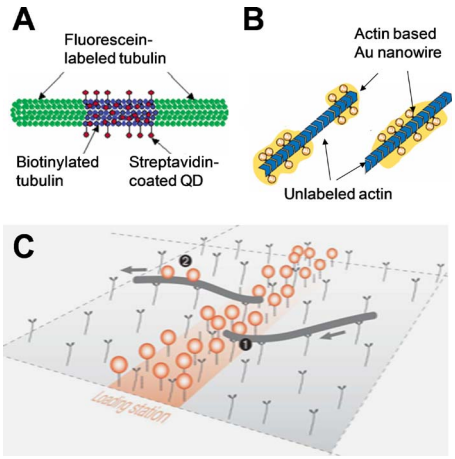

Fig. 4 Recent advances in development of schemes for loading cargo onto filaments. (*a*) Microtubules precoated with cargo in segments also support kinesin based motility. Reproduced with permission from Ref. [89]. Copyright 2004, American Chemical Society. (*b*) Au nanoparticle linked actin and pure actin were polymerized to result in a segmented actin filament. Subsequent polymerization of Au precursors resulted in actin based Au wires which supported myosin based motility. Permission requested from Ref. [90]. Copyright 2004, Nature Publishing Group. (*c*) Unladen microtubules walk into cargo rich "loading stations" to pick up cargo and transport them into cargo free regions. Reproduced with permission from Ref. [91]. Copyright 2007, The Royal Society of Chemistry.

barely affected by the presence of the quantum dots (Fig. 4(*a*), [90,91]). Similar findings were obtained in a study aiming to attach magnetic nanoparticles to microtubules [73]. It was observed that precoating biotinylated microtubules with neutravidin leads to a large reduction in gliding velocities (900 nm/s for 0%, 300 nm/s for 37%, and 25 nm/s for 85% biotinylation). Precoating biotinylated microtubules with neutravidin and then with biotinylated 15 nm magnetic particles also has a dramatic negative effect on the fraction of motile microtubules [73,74]. In agreement with Ref. [89], if microtubules are biotinylated in segments and then precoated with neutravidin and particles [75], microtubule binding to kinesin and transport is not affected.

The size and concentration of cargo particles has an effect on microtubule motility as well [92]. When biotinylated microtubules propelled by kinesin were exposed to different sizes of streptavidin-coated particles, it was discovered that motility was severely affected for 40 nm microspheres at concentrations greater than 10 pM and for 15 nm quantum dots at concentrations greater than 1 nM. It was hypothesized that nanoparticles at high concentrations bind to the "underside" of the microtubule, thus significantly inhibiting the interaction between the motor protein and tubulin dimers. However, since recent measurements indicate that kinesin molecules elevate gliding microtubules 17 ± 2 nm above the surface [93], it is difficult to develop a mechanical model supporting this hypothesis. Streptavidin, microspheres, quantum dots, and magnetic particle loaded microtubules have evoked much interest not only for the development of a nanotransport system but also to study and utilize interactions between gliding microtubules [94–96].

Cargo loading onto gliding actin filaments has been studied as well, even though the helical structure of actin filaments poses an additional problem for oriented binding, since actin filaments rotate once per micrometer moved [97]. Bound cargo would presumably follow the filament rotation and potentially get stuck between filament and surface or affect the actin-myosin interaction. Hence it is desirable to attach large cargoes to the trailing barbed end rather than to other positions along the filaments. In the first example of cargo loading onto myosin propelled actin filaments

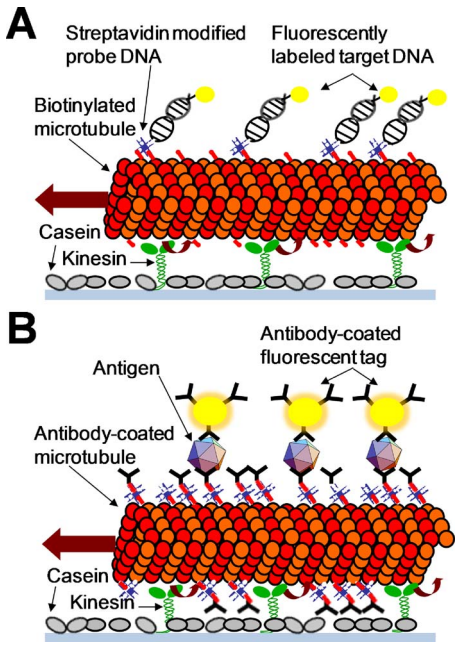

Fig. 5 Loading, transport, and detection of analytes. (*a*) Target DNA prelabeled with fluorescent tags can be selectively captured from a pool of DNA [102]. (*b*) Virus particles and proteins can be captured by microtubules and detected by antibodies linked to fluorescent spheres or quantum dots [106,107].

[98], cargo was functionalized with gelsolin, an actin binding protein. The procedure enabled actin filaments to carry micrometer-sized cargoes without significant reduction in gliding velocity [98]. A recent report shows that smaller streptavidin-coated quantum dots can be attached to arbitrary positions all along the biotinylated actin filament while preserving the actomyosin function [99]. Stretching the idea of cargo loading is the templated synthesis of gold nanowires on segments of actin filaments decorated with gold nanoparticles by Patolsky et al. (Fig. 4(*a*)) [90]. However, only about 30–40% of the segmented Au-wire actin composite polymers were motile and gliding velocities were an order of magnitude slower than those of unlabelled actin filaments.

An interesting demonstration is the manipulation of DNA molecules with motor-driven transport systems. For example, biotinylated microtubules gliding on kinesin were successfully linked to biotinylated DNA molecules via streptavidin [100]. Bifunctional λ-DNA molecules with thiol groups at one end and biotin/streptavidin at the other enabled the stretching of initially random coils of λ-DNA between a gold surface and moving biotinylated microtubules [101]. The sensitivity and selectivity of complementary DNA capture by DNA loaded microtubules was also tested and confirmed [102]. The velocity of the microtubules loaded with hybridized DNA (Fig. 5(*a*)) was equal to that of the microtubules carrying only probe DNA prior to hybridization. In a related set of experiments [103], biotinylated microtubules loaded with streptavidin linked malachite green served as probes for the detection of malachite green aptamers. It was demonstrated that these probes could select target aptamers from a transcription mixture and transport them without losing their inherent mobility. Furthermore, it was the first time that cargo had been reversibly loaded onto moving microtubules, since the aptamers could be unloaded from these probes by adding free malachite green to the solution. Similarly, it was shown that β-cyclodextrin linked via streptavidin to biotinylated microtubules can capture 1-Anlinonapthalene-8-Sulfonic Acid (1,8-ANS) from the solution [104]. While the cyclodextrin/1,8-ANS bond is in principle reversible, unloading was not yet demonstrated and transport velocities were severely reduced.

Capture and transport of engineered cargo, such as fluorescently-labeled cowpea mosaic virus (CPMV), has also been achieved using both NeutrAvidin-biotin and antibody-antigen interactions [105]. It was proposed that the virus with an average diameter of 28 nm can simultaneously serve both as an efficient fluorescent tag for biomolecules and as a portable scaffold for the transport of biomolecules as cargo on microtubules. To spatially separate cargo-pick-up and its subsequent utilization, "loading stations"—defined surface regions with a high density of cargo attached to the surface by reversible tethers—were patterned onto the surface (Fig. 4(*c*)) [91].

A significant step toward the utilization of molecular shuttles in biosensing applications was the demonstration of specific capture and transport of virus particles [106] and proteins [107] by antibody-functionalized microtubules. A recent work also reported the development of a similar sensor for ssDNA and RNA [108]. A critical advantage of antibody or DNA-based capture is that cargo does not have to be tagged with a linker prior to capture. Building on the earlier studies, antibodies were covalently attached to microtubules, or streptavidin was used to immobilize commercially available biotinylated antibodies onto the biotinylated microtubules. The captured antigens were detected by a second antibody equipped with an optical tag, which in effect created a nanoscale double-antibody-sandwich assay (Fig. 5(*b*)).

3.1 Summary of Loading Approaches. While initial experiments relied on specifically tagged cargo and created links which were irreversible on the timescale of the assay, current experiments focus on the selective capture of unmodified cargo, e.g., by antibodies, and the controlled breaking of the linkage [109]. The design of the shuttle/cargo complex poses intriguing challenges with respect to its mechanical engineering since all elements are soft, flexible, and subject to thermal forces. A clear and detailed picture of the interactions between cargo, filaments, and motors will have to be developed, building on initial insights [95].

4 Control of Motor Activation

The activity of biomolecular motors, such as kinesin [110–114] and myosin [115–118], depends on a variety of parameters such as ATP concentration, Ca^{2+} and Mg^{2+} concentration, pH, and temperature. Here, we review the studies seeking control over motor activity within a nanoengineering context.

The control of motor activation is inspired by skeletal muscle, where an increase in calcium concentration leads to contraction within milliseconds and is fully reversible. Unfortunately, central elements of the mechanism are not retained in the myosin/actin in vitro system and kinesin/microtubule transport does not possess a similar regulatory mechanism. However, a similar calcium-dependent switch was added to kinesin by genetic engineering [119]. Both, kinesin-microtubule motility [120] and actomyosin motility [121], were demonstrated to be reversibly inhibited by local anesthetics in a dose-dependent manner. The inhibitory effect of mercury ions on the chemical and mechanical functions of myosin can be exploited in the design of a novel biosensor [122]. While all these approaches require fluid injection and exchange every time switching is required [123], other methods have been developed which involve an externally applied stimulus to activate/inactivate and regulate motor activity.

Thermal activation causes exponential variations in enzyme activities in general and molecular motor speeds, in particular [110–113,117]. Recent efforts aimed for the initiation of rapid and localized temperature changes and a concomitant fast and localized response of motor activation. Temperature-pulse microscopy has been utilized to control myosin [124] as well as kinesin [114] activity. Electrically controlled thermal activation was realized by MEMS fabrication [125].

Stimulus-responsive polymer coatings on which motor proteins can be adsorbed have provided an elegant alternative for motor activity control [126]. Kinesins were adsorbed onto a silicon sub-

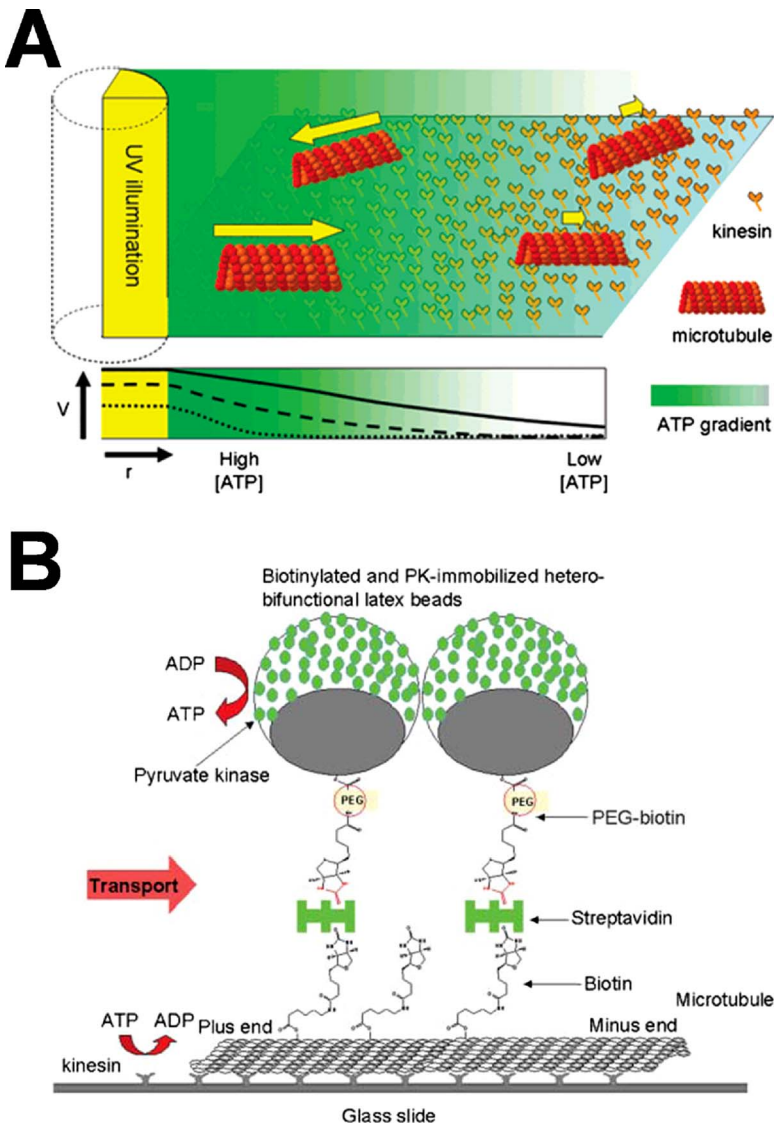

Fig. 6 Control of motor activation by ATP production. (*a*) Caged-ATP can be locally photolyzed to ATP, which is subsequently sequestered to achieve microtubule gliding with high spatial and temporal control. Reproduced with permission from Ref. [130]. Copyright 2008, American Chemical Society. (*b*) A microtubule-particle complex which produces ATP for consumption by the kinesin motors. Reproduced with permission from Ref. [131]. Copyright 2005, Royal Society of Chemistry.

strate between surface-grafted polymer chains of thermoresponsive poly(N-isopropylacrylamide). By controlling the temperature between 27°C and 35°C, conformational changes were induced in the polymer chains, which either allowed the kinesins to bind microtubules (collapsed conformation) or prevented binding (extended conformation). While the electric field surrounding a kinesin-coated electrode has been shown to accelerate microtubule binding [127], a more surprising effect was recently demonstrated using an electrically switchable polymer surface [128]: When the polymer (poly($CH_2OH-EDOT$)) was switched from its dedoped (semiconducting) state to its doped (conducting) state, the ATPase activity of the adsorbed kinesin complex reversibly decreased by 35%.

However, the classic engineering approach to throttling an engine is to control the fuel supply. Since motor speed follows ATP concentration according to Michaelis–Menten kinetics, controlling ATP concentration can specifically affect motor activity whereas changes in ionic strength and temperature have additional effects on the system. While ATP concentrations can of course be changed by replacing the solution [87], the use of caged ATP removes the need for solution exchanges [26,129]. Photolysis of caged ATP by UV light releases ATP for motor consumption (Fig. 6(*a*) [130,131]). However, the high diffusivity of ATP and the low consumption rate of ATP by motors imposes fundamental limitations with respect to temporal and spatial control of activation, which can only be partially reduced by enzymatic sequestration of ATP [130].

An exciting development is the coupling of microtubules with enzyme-coated particles which have the ability to generate ATP in situ (Fig. 6(*b*)) [131]. Unfortunately, the achieved velocities are less than 10 nm/s, due to the limited amount of enzyme present.

Finally, a "fuse" was created by adding caged inhibitor peptides to the kinesin C-terminus domain [132]. Upon uncaging, these peptides interfered with motility and reduced speeds to 20% within 20–30 s of exposure.

4.1 Summary of Activation Strategies. Three general approaches have emerged: temperature control, control of ATP concentration, or genetic engineering of a molecular switch into the motor. For most control approaches, the rapid diffusion of molecules or heat at the nanoscale limits spatial resolution. The utilization of electric pulses or light as stimuli seems generally preferable to an exchange of the buffer solution. However, the design of integrated "traffic signals" for molecular shuttles [126,128], possibly utilizing stimuli-responsive polymers, has not yet been achieved.

5 Further Applications and Conclusions

In addition to the vision of cellular scale devices outlined in the introduction and the utilization of molecular shuttles for biosensing, a number of application concepts relying on the integration of molecular motors and synthetic components have been proposed. For example, a molecular motor-powered micropump has been modeled in detail [133]. The effect of surface properties on the trajectories of gliding filaments can be applied to surface imaging [134], and the adsorption of motors followed by filament binding can be exploited for highly sensitive adsorption measurements [135]. Finally, the utilization of motors for biocomputation devices is an intriguing idea [136].

A key consideration for all hybrid devices is of courses the degradation of the biological components prior and post activation. This issue was studied in some detail for kinesin/microtubule shuttles. Taxol-stabilized microtubules were found to limit the lifetime of the device to less than a day [137], while partial chemical cross-linking of microtubules can extend the lifetime to a week without gliding [138]. Freezing, freeze-drying, and critical point drying are approaches to prepare kinesin/microtubule systems for extended storage [139–141]. Grove et al. [142] presented a degradation study for the myosin/actin system. Surprisingly, kinesin is not very susceptible to chemicals used in the processing steps of microfabrication, such as removers/solvents or developers, which may enable a close integration of biomolecular self-assembly processes and top-down fabrication [143].

The engineering of nanosystems with biomolecular motors as components has evolved in the past 10 years from the initial idea to an established subfield of bionanotechnology. Successful approaches to the key challenges were identified and are continuously refined in laboratories around the world. The central challenge at this stage is the development of convincing application concepts, which integrate the above discussed elements into a complex device delivering significant performance enhancements.

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