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Review

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Protein Linear Molecular Motor-Powered Nanodevices

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Myosin–actin and kinesin–microtubule linear protein motor systems and their application in hybrid nanodevices are reviewed. Research during the past several decades has provided a wealth of understanding about the fundamentals of protein motors that continues to be pursued. It has also laid the foundations for a new branch of investigation that considers the application of these motors as key functional elements in laboratory-on-a-chip and other micro/nanodevices. Current models of myosin and kinesin motors are introduced and the effects of motility assay parameters, including temperature, toxicity, and in particular, surface effects on motor protein operation, are discussed. These parameters set the boundaries for gliding and bead motility assays. The review describes recent developments in assay motility confinement and unidirectional control, using micro- and nano-fabricated structures, surface patterning, microfluidic flow, electromagnetic fields, and self-assembled actin filament/microtubule tracks. Current protein motor assays are primitive devices, and the developments in governing control can lead to promising applications such as sensing, nano-mechanical drivers, and biocomputation.

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1. Introduction

Protein molecular motors are naturally evolved nanosized machines responsible for mechanical movement, which is essential for many biological functions, including cell division and movement, transport of vesicles, and muscle contraction. Protein molecular motors vary considerably in terms of their structure, complexity, motion, and biological function. The past four decades have witnessed extensive investigations into the molecular mechanisms that underpin the operation of these motors, which are central to biology. These fundamental investigations have been (i) experimental—aided by advances in molecular imaging, single molecule manipulation techniques, such as optical trapping, and development of motility assays,[1–18] and (ii) theoretical—analyzing and attempting to explain the operation of motors according to established physical, chemical, and biological principles.[19–28] The research has expanded our understanding of molecular motors, nonetheless, there remains considerable scope for elucidating molecular mechanisms and their impact on biology and medicine.[14,29]

During recent years, a new avenue of research has evolved from these fundamental inquiries that investigates motors in a more device-oriented context.[30–43] Developments during the past several years have integrated the confinement and manipulation of protein molecular motors in micro- and nano-fabricated structures with other means of motility control, for example, fluid flow and electromagnetic (EM) fields.[44–48] This research effort, therefore, focusses on design issues and characteristics of molecular motors operating in a device context rather than

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solely understanding their mechanisms per se. These devices are hybrids: consisting of naturally evolved protein motors, biochemically interfaced and housed in glass or polymeric microstructures, possibly with external (fluidic or EM) control of motility. The motivation for such devices stems from the high chemical-to-mechanical energy efficiency these motors can attain[49,50] and their naturally occurring, small-scale, bottom-up fabrication.

This short review focusses on the hybrid devices that use myosin and kinesin cytoskeletal linear motors, which are more extensively studied in comparison with other linear motors, such as dynein. In general, the synthesis and handling of dynein is not trivial and so far dynein-based nanodevices have not been reported. Similarly, actin and tubulin polymerization can yield useful mechanical work for hybrid devices[51–54] but they do not constitute molecular motors, as such. Although rotary motors[55–59] have been considered, rotary motion is currently less applicable than linear, translational motion in hybrid devices, particularly for shuttling and transportation.[60] Therefore, we have not included dynein, polymerization (actin and tubulin), and rotary motors in this short review.

The following section summarizes our current understanding of myosin–actin and kinesin–microtubule motor systems. In-vitro motility assays, extensively used for studying linear myosin and kinesin motors, are reviewed in section 3, in which device environmental effects are described including factors such as motor density, surface interface effects, toxicity, and temperature. These environmental parameters set the boundaries for successful motor operation in a hybrid device. Recent developments in assays, in terms of motility confinement and direction control using fluids and electromagnetic (EM) fields, and 'on–off' switching, is discussed in section 4. These parameters lead towards the requirements for realising a functional device, presented in section 5, which includes sensing, nano-mechanical actuation, and biocomputation. The review concludes in section 6 with perspectives on molecular-motor-powered devices in relation to other nano electro-mechanical systems (NEMS). The issues discussed include fuel supply, endurance, assay development, cargo handling, integration, external control, and monitoring.

2. Linear Protein Molecular Motors

Linear molecular motors, which work either in tandem or as a pair of proteins, essentially transform chemical energy, through the hydrolysis of ATP (adenosine triphosphate), into mechanical energy. The tandem comprises the actual motor (myosin or kinesin) and the molecular track (F-actin filament or microtubule), respectively. Linear molecular motors are actually organized into superfamilies so that myosin and kinesin motors can vary considerably in their structure and detailed operation.[14]

2.1. Myosin–Actin System

The myosin–actin system comprises a *myosin motor* and an *actin filament*, which has a cable-like structure. Myosin–actin motors are responsible for many processes, in particular, muscle contraction, which is a result of the concerted action of many myosin crossbridges pulling on actin filaments. The building block of the actin filament is the actin monomer (G-actin), which is a globular protein of molecular weight (MW) 45 kDa. The filament has a diameter of ~6 nm and can be viewed as consisting of two, right-handed helical protofilaments wrapped round each other, with a period of ~72 nm.[25] There are extensive contacts between the protofilaments. Since the actin monomers are asymmetrical, the actin filament is polar with structurally different ends. A consequence of this polarity is that the polymerization is faster at one end and slower at the other. The faster-growing end is called the plus (+) end, whereas the slower-growing end is the minus (−) end. Actin filaments range in length from 35 nm to 100 μm in cells and muscles, whereas in in-vitro experiments the filaments range up to 20 μm. Therefore, the filaments are modelled as slender rods and the mechanical properties of the filaments are estimated according to this physical model.

The myosin motor protein is a large protein with MW ≈ 500 kDa. The operation of myosin, which hydrolyzes ATP at a rate that dramatically increases upon interaction with actin, has been the subject of intense research and debate. One approach is to describe the operation of myosin through a biomolecular perspective. Figure 1A (left) illustrates a full cycle of myosin operation—taken from a comparative review.[61] It is important to emphasize that the following description is illustrative and that there are many different members of the myosin family, with very distinct behaviour. Essentially, as shown in frame (1), the myosin comprises two identical motor heads (blue: catalytic cores; yellow: lever arms in the prestroke state) anchored to a thick filament (top) by a coiled coil (gray rod extending to the upper right). In the ADP-Pi-bound state, the catalytic core binds weakly to actin. One head then docks onto an actin-binding site (green, frame (2)). Since myosin II is a non-processive motor, the two heads act (quasi)independently. In support of this independent operation, it has been demonstrated[12] that one myosin head (actually the S1 unit) is sufficient to achieve motility, albeit at lower velocities. However, there are geometrical considerations that would suggest that the myosin heads cannot operate in a fully independent manner, and indeed there are studies[62,63] that have demonstrated a certain level of cooperativity. Actin docking (3) is associated with phosphate release from the active site and the lever arm swings to the poststroke, ADP-bound state (red). This moves the actin filament by ~10 nm. After completing the stroke (4), ADP dissociates and ATP binds to the active site, which rapidly reverts the catalytic core to its weak-binding actin state. The lever arm will then re-cock back to its prestroke state, i.e., back to (1).

The second model of the myosin–actin system, which relies on thermodynamic reasoning, has been denominated as a 'thermal ratchet model'[19]—for a recent review see ref. [54]. Essentially, a thermodynamically favourable position of myosin, which is in general a result of thermally generated fluctuations, is captured by formation of the bond with actin. Subsequent relaxation of the spring provides the enthalpy necessary to perform mechanical work in the power stroke. The tight myosin–actin bond at the end of the power stroke is broken by ATP binding. In this way the binding cycle, controlled by ATP, rectifies (hence the thermal ratchet character) random thermal fluctuations into unidirectional mechanical work.

Irrespective of the precise details of the model, from a hybrid device point of view the operational parameters of the motor system are more relevant. Elaborate experiments derived from motility assays,[64,65] which involve optical traps,[4,7] microneedles,[8] or atomic force microscopy (AFM)[66] provided general engineering-relevant parameters. Apart from physical dimensions,[61] the velocity, the force generated, and ATP consumption are of prime interest. Among the myosin family, the fastest motor is myosin XI, which can achieve a velocity of 60 $\mu m\,s^{-1}$.[67] Myosin II, which is used in most experiments,

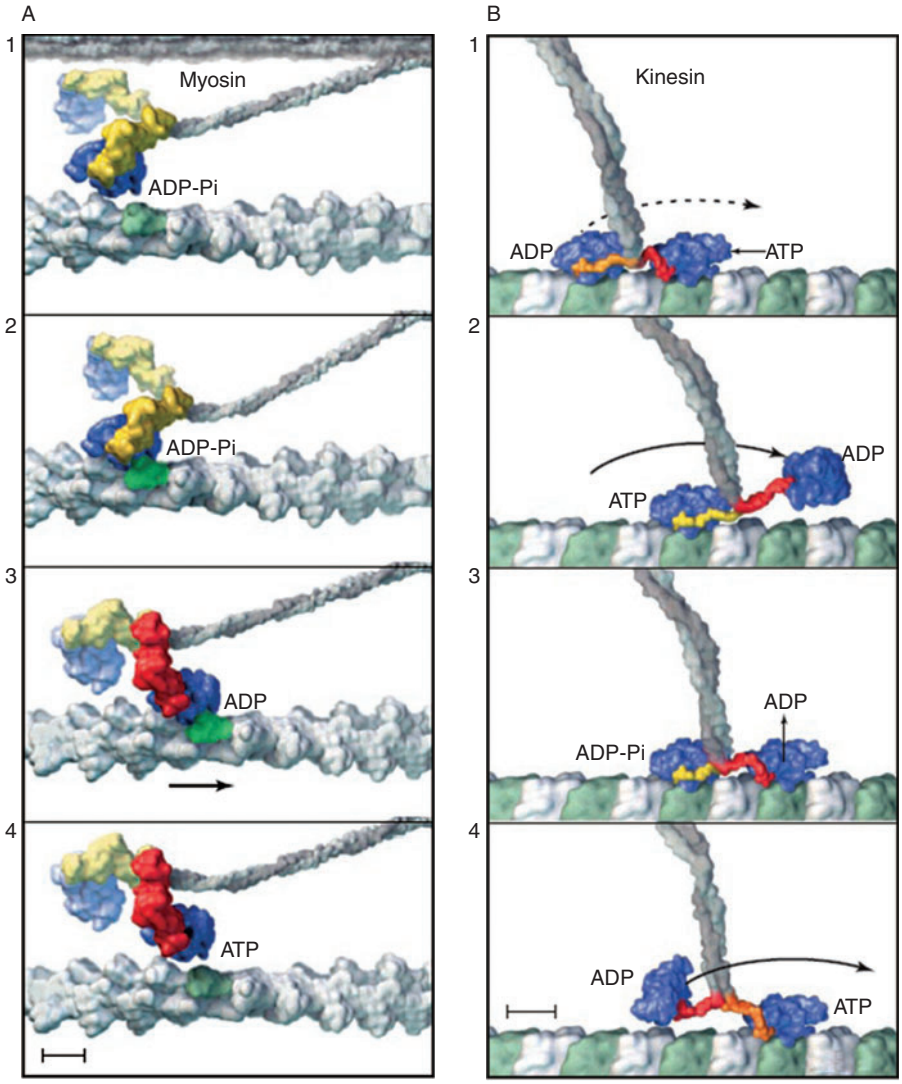

Fig. 1. Detailed cycles of the operation of non-processive myosin (A) and kinesin (B) (reprinted with permission from ref. [61]; © 2000 AAAS; see text for explanation). The scale bars are 6 nm (A) and 4 nm (B).

tends to be much slower—typically moving at velocities of $\sim 6\,\mu m\,s^{-1}$ and with an actomyosin ATPase kinetic constant of $\sim 20\,s^{-1}$.[25] A lower limit for the force generated by the myosin crossbridge has been determined to be 1 pN, but forces up to 10 pN have also been measured, with a value of 1.5 pN per crossbridge most likely for myosin II.[25]

2.2. Kinesin–Microtubule System

The kinesin–tubulin motor complex utilizes microtubules as molecular tracks. Microtubules are formed by the polymerization of the globular protein tubulin, instead of actin. The kinesin–tubulin motor performs, for example, transport of material inside cells, with vesicles being attached to the two-headed kinesin molecule as it moves along the microtubule. Microtubules have a structure that resembles a pipe with an inner diameter of ~18 nm and an outer diameter of ~25 nm.[68] The building block of the microtubule is the αβ tubulin heterodimer with a MW of 50 kDa and a length of 8 nm. The dimers associate head to tail to form a protofilament. Most microtubules have 13 protofilaments, which associate laterally to form a sheet that closes to form a cylindrical tube: the microtubule. As is the case for actin filaments, contacts exist between the protofilaments. Similar to actin filaments, microtubules have '+' and '−' ends and range in length from less than 1 μm to more than 100 μm.

The kinesin molecule[69] is rod-shaped with two globular heads (Fig. 1B, right[61]), similar to the myosin molecule. The structural similarities between myosin and kinesin suggest that nucleotide binding, hydrolysis, and release may trigger similar motions in the motor domain. Theories regarding myosin operation are equally applied to kinesin, in particular, the rotating crossbridge model.[70,71] In conventional kinesin, unlike in non-processive myosin, the two heads work in a highly coordinated manner, moving hand-over-hand,[25] or *processively* along the track as shown. Essentially, (1) the coil (gray) extends toward the top and leads up to the kinesin cargo. Each catalytic core (blue) is bound to a tubulin heterodimer (green and white) along a microtubule protofilament (13 protofilament tracks compose the microtubule). The neck linker points forward to the trailing head (orange) and rearward on the leading head (red). ATP binding to the leading head initiates neck linker docking (2), which is completed by the leading head (yellow), and throws the other head forward by 16 nm (arrow) toward the next tubulin binding site. After a random diffusional search (3), the new leading head

Table 1. Mechanical properties of actin filaments and microtubules[166]

Component	Young's modulus [$\times 10^9$ N m^{-2}]	Approximate flexural rigidity[A] [$\times 10^{-27}$ N m^2]	Persistence length[B] [μm]
Actin filaments	2.3	60	15
Microtubules	1.9	30 000	6000

[A]Flexural rigidity is a material constant, which is an analogue for the spring constant but for bending moment, not for the elastic force: the bigger the flexural rigidity, the more difficult it is to bend a slender rod (which in this case is a physical model for the filament).
[B]Persistence length has the intuitive meaning of the length over which the bending as a result of random (thermal) fluctuations becomes appreciable, or the length over which the bending of one point influences the bending of another point.

Table 2. Experimental data on myosin II and conventional kinesin[25]
For further in depth discussion see refs [70,71]

Parameter	Myosin II	Kinesin
Force/head at V = 0 μm s^{-1} [pN]	1.5	3
Speed (high ATP, no force) [μm s^{-1}]	6	0.8
Mechanical work [pN nm]	50	50
Cycle time (at V_{max}) [ms]	40	20
Distance per ATP [nm]	200–400	16
Work distance [nm]	5	8
Next binding site [nm]	36	8

docks tightly onto the binding site, completing the 8 nm motion of the attached cargo. Polymer binding also accelerates ADP release, and during this time, the trailing head hydrolyzes ATP. After ADP dissociates (4), ATP binds to the leading head and the neck linker begins to zipper onto the core (partially docked neck indicated by the orange colour). The trailing head, which has released its Pi and detached its neck linker (red) from the core, is in the process of being thrown forward.

The single-molecule experiments performed with kinesin have shown that some members of the kinesin molecular motor family can develop velocities up to 1.8 μm s^{-1}.[4] The conventional kinesin can develop velocities up to 0.8 μm s^{-1},[25] moving toward the plus end of the microtubule, like myosin. As the working distance of kinesin is 8 nm, it moves from one heterodimer to the next along the protofilament, with the binding sites being ~8 nm apart. Since kinesin is a processive motor, a single kinesin molecule is able to pull a 0.2 μm diameter glass bead for hundreds of nanometres.[2] The maximum force kinesin can work against is ~6 pN and the velocity of the movement decreases approximately linearly with the increase of the opposing force.[25] The main parameters of actin filaments and microtubules, and myosin and kinesin motors, are compared in Tables 1 and 2, respectively.

3. Methods for Studying Motors

Two important experimental techniques for understanding and characterizing molecular motors are (i) in-vitro motility assays, and (ii) single molecule visualization, manipulation, and measurement.

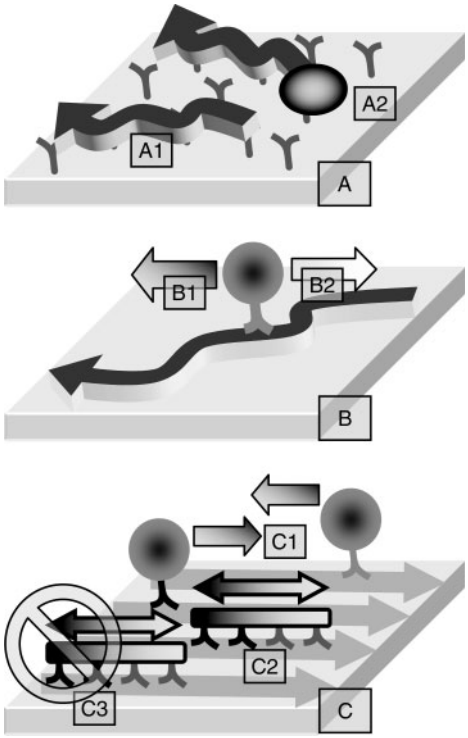

Fig. 2. Types of motility assays: A, Gliding motility assays. A1, Classical gliding motility assay in which actin filaments or microtubules slide on a surface covered with molecular motors (a protein in the myosin or kinesin family). A2, Bead tailed gliding motility assays in which a bead functionalized with gelsolin is attached at the trailing, barbed end of the filament. Small beads are more likely to be bound to a single filament, but larger beads will statistically have more than one filament attached, effectively arresting the motility. B, Bead motility assays. B1, Classical bead motility assay in which a bead is functionalized with motor protein (proteins in myosin or kinesin family, or motor fragments) and runs unidirectionally, according to motor type, on single filaments or microtubule immobilized on the surface. B2, Single molecule studies using optical traps or microneedles apply a force in the opposite direction of the movement, thus offering the possibility to measure the force generated by the motor. C, Possible bidirectionality assays. C1, Beads with functionalized with anti-parallel directionality, e.g., kinesin and Ncd, move in opposite directions on parallel tracks (microtubules). C2, Payloads functionalized with antiparallel motors move in one direction according to the result of the 'tug of war' between motors. C3, The 'tug of war' mechanism is replaced by the coordination between motors, in the sense that one motor works (right) the other is blocked (left).

3.1. Motility Assays and Single Molecule Techniques

The motility assay, with various architectures presented in Fig. 2, is based on observation of the motile element of the motor system in the presence of ATP on a surface, which is functionalized (for dual protein systems) with its complementary protein. There are two possible configurations for in-vitro motility assays of linear motors: (i) the *gliding assay*, which consists of actin filaments or microtubules sliding on myosin- or kinesin-functionalized surfaces; and (ii) the *bead assay*, which consists of objects (e.g., a microbead, typically 1 μm in diameter) functionalized with the motor protein (i.e., myosin or kinesin) sliding along actin filaments or microtubules that have been immobilized on the surface.[70,72,73] The gliding assay for both myosin–actin[64,74] and kinesin–microtubule[75] systems is generally used more frequently than the bead assay because it is simpler to set up and operate. It also offers, potentially, unlimited distances of transport.

Motility experiments occur in a flow cell, which comprises two parallel surfaces, at least one being transparent, which are separated by a thin approx. 100 μm spacer, typically grease or double sided tape. The motility flow cell is mounted on a microscope, which is used to observe the movement of the motile element, either by fluorescence if the motile element is fluorescently tagged, or using differential interference contrast (DIC) for larger microtubules, or other elaborate methods, such as, tailing the gliding actin filaments with beads.[76] The motility has been observed using AFM with a fast procedure protocol[66] and using a variety of optical microscopy methods, for example, fluorescence interference contrast (FLIC) microscopy.[18] In-vitro motility assays have also been developed for rotary motors (for example see ref. [55]) and actin and microtubule polymerization.[77,78]

Single molecule techniques, (for example see refs [4,7,12,16,17,79]), which are modifications of the in-vitro motility assays, have been used to measure the distances moved by single molecules and the forces they generate. In the filament assay, the filament or microtubule is held in a force transducer and presented to a motor that is fixed to a surface. In the bead assay, the motor is attached to a bead held in a force transducer and the bead is presented to a filament that is fixed to a surface. The force transducers, for example, cantilever rods, AFM tips, or optical tweezers, must be able to produce and monitor forces in the pN range. The motion produced when the motor interacts with the filament is measured using photodiodes capable of sensing nanometer displacements with millisecond resolution.[25]

3.2. Motility Assays: Interaction of Motors with Their Environment

Protein molecular motors are quite robust in cells but they are sensitive to environmental conditions imposed by in-vitro assays. These environmental conditions include surface material effects, concentration of motor protein, toxicity, and temperature.

3.2.1. Surface Effects

Traditionally, the gliding motility assays have been run on surfaces that are covered by a mixture of motor protein and a blocking protein, usually bovine serum albumin (BSA) or casein. Regarding the motile proteins, the blocking of the sites not occupied by a motor protein will cancel the non-specific binding of the F-actin or microtubule to the surface and, hence, their surface-induced denaturation. On the motor protein side, the molecular confinement of isolated motor proteins in a blocking protein 'sea' is expected to decrease the surface-induced deactivation of the motors, which would increase with time. As the optimum ratio between motor and blocking proteins is difficult to find, there have been attempts to use engineered antibodies to 'decouple' the contact of the motor proteins from the surface[33,80,81] but these methods are far from trivial.

The motility of actin filaments powered by myosin or its fragments, such as heavy meromyosin (HMM), has been demonstrated on various surfaces, which include nitrocellulose,[72,76] glass,[33,64] poly(methyl methacrylate) (PMMA),[32] poly(*tert*-butyl methacrylate) (PtBuMA), poly(methacrylic acid) (PMAA),[34] poly(tetrafluoroethylene) (PTFE),[31] *O*-acryloyl acetophenone oxime (AAPO) copolymer,[82,83] printable crosslinkable UV-resist (MRL-6000),[84] and glass surfaces derivatized with trimethylchlorosilane (TMCS).[85,86] Similarly, the motility of microtubules powered by kinesin has also been demonstrated on surfaces, such as glass,[75,87–91] PTFE,[35] deepUV resist (SAL601),[37] silicon,[87] PMMA,[88] poly(dimethylsiloxane) (PDMS),[88] ethylene–vinyl alcohol copolymer (EVOH),[88] thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAM) grafted onto polyglycidyl methacrylate (PGMA),[92] and reconstituted microtubules.[93]

Some features worth noting are that different surfaces induce differential adsorption of proteins (i.e., BSA and motors) and small variations in experimental conditions can lead to entirely different motilities. For instance, several authors[32,34] demonstrated the motility of actin filaments on patterned PMMA, whereas others[84] used PMMA for patterns that do not support motility. The difference in experimental results, which is less evident for hard surfaces, such as glass and silicon, derives from the complex character of polymers and their surfaces. Indeed, the same polymer, but with different polymer physico-chemistry (e.g., MW), processed under different conditions (e.g., different cast solvents, baking temperatures, and durations) and exposed to different buffer conditions (e.g., duration, temperature, ionic strength) will present different surfaces to the motor proteins and thereby induce different motility behaviour. In addition, the polymer surfaces, especially for non-crosslinked polymers and polymers with a low glass transition temperature (i.e., in a rubber state) swell in contact with the buffer, which leads to the interaction of the top polymeric chains with the motor proteins. For this reason, PDMS and poly(urethane) (PU) do not support motility for myosin–actin systems[94] while the use of thermoresponsive PNIPAM[92] exploits the effect of polymer swelling to achieve spatiotemporal control of kinesin–microtubule motors. In the latter case, hydrated PNIPAM polymer chains, below a critical temperature (32–33°C) form extended structures that repel gliding microtubules, but become compact when heated, thus enabling motility. Finally, the critical failure mechanism for motility assays is the denaturation of the motor protein[34,83] and degradation of the filaments and microtubules.[88]

Since the bead motility assay is used less frequently, data regarding the surfaces that support motility are less extensive. An optimum bead motility assay would entail a compact carpet of aligned actin filaments, or microtubules, immobilized on the surface. Indeed, the first motility assays used naturally self-assembled actin filaments in the algae Nitella[72] and later artificially self-assembled F-actin paracrystals on lipid surfaces.[95] Early[4,30] and later work[90] demonstrated that microtubules adhere strongly to amine-terminated surfaces while retaining the ability to act as substrates for kinesin-coated beads. The critical issue of microtubule deactivation has also been addressed through stabilization with taxol[96] or glutaraldehyde.[97]

3.2.2. Protein–Surface Interaction: Biomolecular Considerations

Proteins often change their conformation on surfaces on which they are immobilized,[98] which in turn affects their bioactivity and performance as motors. An optimum single surface that preserves reasonable bioactivity levels when many different proteins are immobilized on it is almost a 'Holy Grail'. Although molecular motor proteins are nominally a single molecule, they are 'multiple' proteins in the sense that they present different molecular structures for each stage in the power stroke cycle—separated by time intervals on the order of nanoseconds. Figure 3A, for example, presents the structure of the molecular surface of the S1 unit of myosin in two states (structures collected from the Protein Data Base, at www.pdb.org), i.e., rigor state (2MYS, left) and flexed state (1DFK, right), as probed by a probe ball with a large radius (1 nm) using Connolly's method.[99–101]

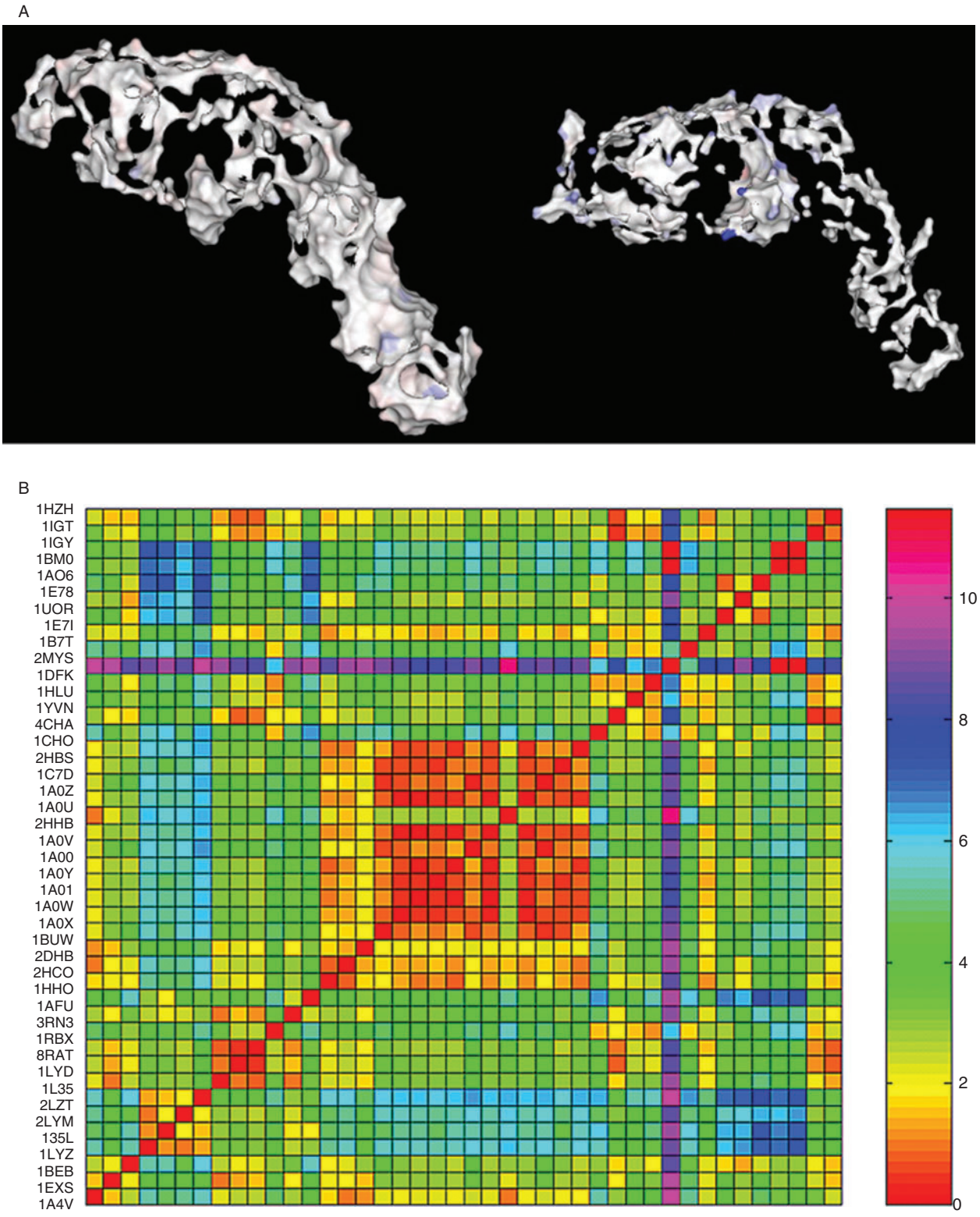

Fig. 3. Molecular motor comparisons (reprinted with permission from ref. [104]). A, Molecular surface of the S1 unit of a myosin head in the rigor and working state, left and right, respectively. B, Similarity comparison between these two S1 states (1DFK and 1MYS, respectively, from the Protein Data Base, www.pdb.org) and another 40 proteins. Perfectly similar proteins (self-similar, identical proteins, on the diagonal) in red; most dissimilar proteins in purple.

It was found[102] that with this large value of the probing ball, the properties of the protein molecular surface do not vary, as the ball is essentially a flat surface. The two structures of the same biomolecule presented in Fig. 3A[103] are starkly different. First, the S1 region of the myosin rigor state presents a much larger molecular area, particularly in the neck region. Second, the molecular surface of the S1 region of myosin in a working state presents 'hot spots' of hydrophobicity and hydrophilicity (red

and blue, respectively), particularly in the ATP pocket region. A similarly large difference can possibly be found for other motor proteins (e.g., kinesin). The method used to visualize the distribution of hydrophobicity on the molecular surface can also be used to quantify other properties (e.g., charges) in terms of mean values and densities. Figure 3B[104] compares the similarity of the molecular surfaces of 42 proteins according to a cluster analysis (nearest neighbour, simple Euclidian distance) using mean values, density (e.g., total hydrophobicity per total area), and specific density (e.g., hydrophobicity per hydrophobic area) for the following properties: hydrophobicity, hydrophilicity, and positive and negative charges. The dissimilarity between S1 myosin-rigor (2MYS) and S1 myosin-working (1DFK) is clearly very large (red rectangles on the first diagonal represent self-identical structures; white circle focusses on S1 structures). More importantly, this difference is at least as large as the difference between the former structure and all other 40 proteins, which are indeed extremely different (i.e., lysozyme, haemoglobin in the middle of the cluster, IgG, albumin, and so on).

3.2.3. Motor Protein Concentration

The concentration of motor protein in the solution used for immobilization also governs the active (i.e., non-denatured) and the total number of motor molecules on the device surface. The impact of the surface density of active motors is markedly more different for processive (e.g., kinesin and myosin V) than for non-processive motors (e.g., myosin II). For instance, myosin V velocities are found not to vary over several decades of myosin density,[11] a behaviour similar to kinesin. This density independence is in contrast to the behaviour of myosin II, which exhibits a drop in velocity as the density is decreased.[3] Another study[105] demonstrated that the sliding velocity of actin filaments decreased non-linearly with reduced density of HMM from myosin II molecules. This is consistent with the unloaded filament sliding velocity being limited by the number of cycling cross-bridges so that the maximal velocity is attained at a critical, low level of actin–myosin interactions. Such data are understandable in view of myosin II having a low duty ratio (ratio of the strongly bound state time to the total actin activated ATPase cycle time) and myosin V having a high duty ratio. A high-duty ratio for myosin V correlates with the property of processivity, in which case the duty ratio for a given myosin head need be at least 0.5.

3.2.4. Toxicity

The extreme sensitivity of protein molecular motor proteins requires minimization of chemical species toxic to motility, for example, radical species produced in situ by the photobleaching of fluorophores. Some of these are removed by oxygen scavengers (e.g., amines). Other toxic chemical species, for example, small organic molecules, can ex-diffuse from the polymeric walls of the flow cell. This is especially true of polymers in an elastomeric state, and a recent comparative study[88] regarding the motility of microtubules in flow cells made of different polymers (i.e., PU, PMMA, PDMS, and EVOH) demonstrated the complex relationship between the fluid environment, building materials, and operation conditions. Essentially, without illumination, only PU had a substantial negative impact on microtubule motility, while PMMA, PDMS, and EVOH showed stabilities comparable to glass. Under the influence of light, however, the microtubules degraded rapidly on PDMS or PMMA, similarly with motility experiments in glass flow cells if oxygen scavengers were not added to the medium.[88] Strong photobleaching of the fluorophores, which occurs mainly on the polymer surface, coincided with accelerated microtubule depolymerization. Although the sensitivity of the kinesin–microtubule system can be addressed by correct operating conditions and appropriate materials or fixation with glutaraldehyde for gliding and bead motility assays, the sensitivity of the myosin–actin system is more difficult to treat. Traditionally, the use of well-controlled motility assay conditions and addition of phalloidin, a pentapeptide present in a toxic mushroom that blocks the depolymerization of actin,[106] allows for several hours of gliding motility.

Scenarios where the problem of environmental sensitivity is less relevant can also be considered. These include devices that operate in fluid environments that have very few, non-deactivating, chemical species (e.g., oligonucleotides[89]), and which operate under controlled conditions (e.g., temperature and pH). Alternatively, one can consider applications where it is precisely the deactivation of the motility by the fluid environment that constitutes the function of the device (e.g., detection by heavy metal ions[107]).

3.2.5. Temperature

Since motility is the mechanical expression of a chemical reaction, the rate of operation (motility speed) of protein motors will increase with the operating temperature. However, excessive temperature will result in a decrease of the operation rate as a result of protein denaturation. Other operational parameters (e.g., force) could, in principle, also be affected by temperature, but the processivity of the motors is an important parameter. Indeed, a study[108] using bead motility assays of kinesin (a processive motor) demonstrated that the gliding velocity of kinesin-coated beads increases with temperature following an Arrhenius law between 15 and 35°C, whereas the force generated remained essentially the same (7.3 pN). The authors concluded that the force generation could be attributed to the temperature insensitive nucleotide-binding state(s) and/or conformational change(s) of the kinesin–tubulin complex, whereas the gliding velocity is determined by the ATPase rate. Similar studies on non-processive myosin and kinesin also demonstrated that the sliding force increased moderately with temperature over the approximate range of 5 to 40°C, whereas the velocity increased by an order of magnitude.[109,110]

4. Towards Hybrid-Nanodevices: Controlling the Operation of Motors in Motility Assays

Motility assays can be viewed as primitive dynamic hybrid nanodevices, and the factors discussed above set operational boundaries. Conceivably, the success of future nanodevices based on linear molecular motors will depend on resolving at least some of the following challenges: (i) precise positioning of the motors on designed areas, (ii) confinement of the movement of motile elements exclusively on fabricated paths for gliding assays, (iii) achievement of unidirectional polarity of the movement of the motile elements, and (iv) the on–off control of their operation.

4.1. Lateral Confinement of Movement for Motile Elements

For devices using the gliding architecture, the lateral confinement of the filaments/microtubules motility can be achieved by (i) mechanical confinement in micro- or nano-fabricated conduits, for example, channels, or (ii) selective patterning of protein motors, assuming that the filaments or microtubule will preferentially stay on motor tracks, or (iii) both of these methods simultaneously.

Initial studies focussed on mechanical confinement only. For instance, it has been demonstrated that parallel nano-scratches fabricated in PTFE can effectively confine the movement of actin filaments[31] and microtubules.[35] More recent studies have demonstrated the confinement of microtubules in micro-channels fabricated in kinesin-adhesive PU and AZ5214 photoresist.[60,111] Mechanical-only confinement within deep channels and walls with retrograde slopes has been successful for microtubules[60] but less likely for small and very flexible actin filaments,[112] although the retrograde slope should in principle be effective.

The patterning of linear motors on micro- and nano-fabricated tracks, which is expected to significantly improve the confinement of the motility of motile filaments or microtubules, has been achieved by preferential adsorption of motor proteins (i) on flat, adhesive patterns in a non-adhesive background, (ii) on adhesive microfeatures elevated from a non-adhesive base, and (iii) in microchannels with non-adhesive walls. The motor patterning on flat tracks has been achieved for HMM on hydrophobic PtBuMA tracks on a less-adhesive PMMA background, i.e., unexposed areas left after deep-UV or e-beam negative tone lithography,[34] and for kinesin on adhesive glass areas on a non-adhesive poly(ethylene oxide) (PEO)-coated background.[111] The second technological choice was implemented in an early study[32] by preferential adsorption of HMM on PMMA-microfabricated features on a non-adhesive glass background. Most studies, however, couple the patterning of motor proteins with the mechanical confinement of motility in profiled microfabricated micro-channels, which has been achieved for HMM/myosin for several architectures, e.g., (i) floor: hydrophobic glass, walls: PMMA;[33] (ii) floor: hydrophobic glass, walls: BSA-coated ablated AAPO copolymer;[82,83] (iii) floor: UV-resist (MRL-6000), walls: PMMA;[84] (iv) floor: glass, walls: metal electrodes;[113] and more recently (v) floor: TMCS–SiO_2, walls: lift-off resist (LOR070A) and PMMA.[112,114] Similarly, kinesin immobilization in micro-channels has been demonstrated for (i) floor: SiO_2, walls: PEO-coated SU8 (photoresist);[111] (ii) floor: glass, walls: SAL601 (e-beam resist);[37] and (iii) floor: glass, walls: SU8.[90] Figure 4 presents alternative strategies that ensure lateral confinement of motile elements in gliding motility assays.

The challenges of confining the motility of motor-coated beads stems from the difficulty of patterning and aligning actin filaments and microtubules. Nonetheless, an early study[30] demonstrated confinement of the movement of kinesin-coated beads on microtubules that had been pre-aligned in a flow field and immobilized on aminosilane patterns. More recently, microfabricated electrodes and flow fields were used to transversally align filaments.[113] In addition, studies regarding tubulin polymerization demonstrated the transversal alignment,[78] and star-shaped features in microfabricated chambers.[115]

4.2. Control of Unidirectional Movement by External Means

The most straightforward method for the direction control of motile elements is the alignment of actin filaments and microtubules by fluid flow that occurs in microfabricated structures either intentionally (e.g., microfluidics) or unintentionally (e.g., higher evaporation rate at one end of the flow cell). For instance, microtubules[87] and actin filaments[94] have been aligned by a fluid flow when exiting a motility chamber connected with the flow current area. The control of unidirectional movement by flow can also be used for the *operation* of bead geometry assays, but the more specific unidirectional control exerted by the polarity of the actin filament or microtubule is hindered. It is, therefore, more likely that the alignment of filaments/microtubules will be useful for the *fabrication* of devices based on bead geometry.

The most popular method for achieving directional control in gliding geometry devices is based on the mechanical guidance by microfabricated 'molecular selectors'. These were initially fabricated,[116] and then demonstrated for a kinesin–microtubule system[37] using e-beam fabricated arrow-shaped channels. The channels connected two reservoirs of microtubules so as to statistically select and redirect the motility in a preferential direction, depleting one 'pool' of microtubules and enriching the other. This extremely elegant work has been followed by several fabrication improvements. These include the use of SU8 and UV lithography instead of SAL600 and e-beam lithography,[90] and the development of more efficient rectifiers with gold floors and PEG-coated SiO_2 surfaces that block unwanted motility.[44] Practical improvements for achieving unidirectional motility include the fabrication of large-area loading zones where filaments can be conveniently deposited.[112,117] Once deposited, the loading zones feed filaments to the channels that are sufficiently narrow so the filaments cannot make U-turns. The implementation of mechanically controlled unidirectionality has been also supported by extensive statistical work regarding the mechanics of microtubules colliding with microfabricated obstacles.[91,111] It is very likely that mechanically controlled unidirectionality for actin filaments will be less successful, or at least it will have to use different, more rounded shapes of the molecular selector. This is because actin filaments are much more flexible compared with microtubules so that they are capable of overcoming rigid mechanical walls when approached directly. Using rounded shapes means the filaments approach the walls at slighter angles and are subsequently guided by them. Indeed, early work regarding motility confinement of actin filaments[32] demonstrated the smooth movement of filaments on ∞-shaped features. Figure 5 presents several molecular selector geometries. More recent work has demonstrated the above points, for example, the use of circular molecular selectors[118] and the less effective directionality control for actin filaments.[86,112]

An alternative method to achieve unidirectional control uses EM fields, predominately either magnetic or electric, the latter spanning radio and optical frequencies. Motors or filaments/microtubules by themselves do not respond to magnetic fields. However, it has been recently shown[119,120] that functionalizing microtubules with cobalt ferrite nanoparticles enables them to be aligned by magnets located under the flow cell. The microtubules retain their gliding motility but the direction of their movement becomes oriented parallel to the lines of the magnetic field. While motor systems respond very little to magnetic fields, and need appropriate attachment to ferrites or the like, they are very responsive to electric fields. There are two ways in which electric fields, established by electrodes immersed in the assay buffer, orient the movement of electrically charged actin filaments or microtubules. One technique uses direct current (DC) electric fields to act on the charged actin filament or microtubule (typically negative at neutral pH[121])—the Coulombic forces cause electrophoretic (EP) movement. The electrokinetics of movement is not straightforward: the electric field also acts on the counter-ions (typically cations) that surround the microtubule and the supporting substrate, which yields electroosmotic flow that hydrodynamically acts against the moving filament or

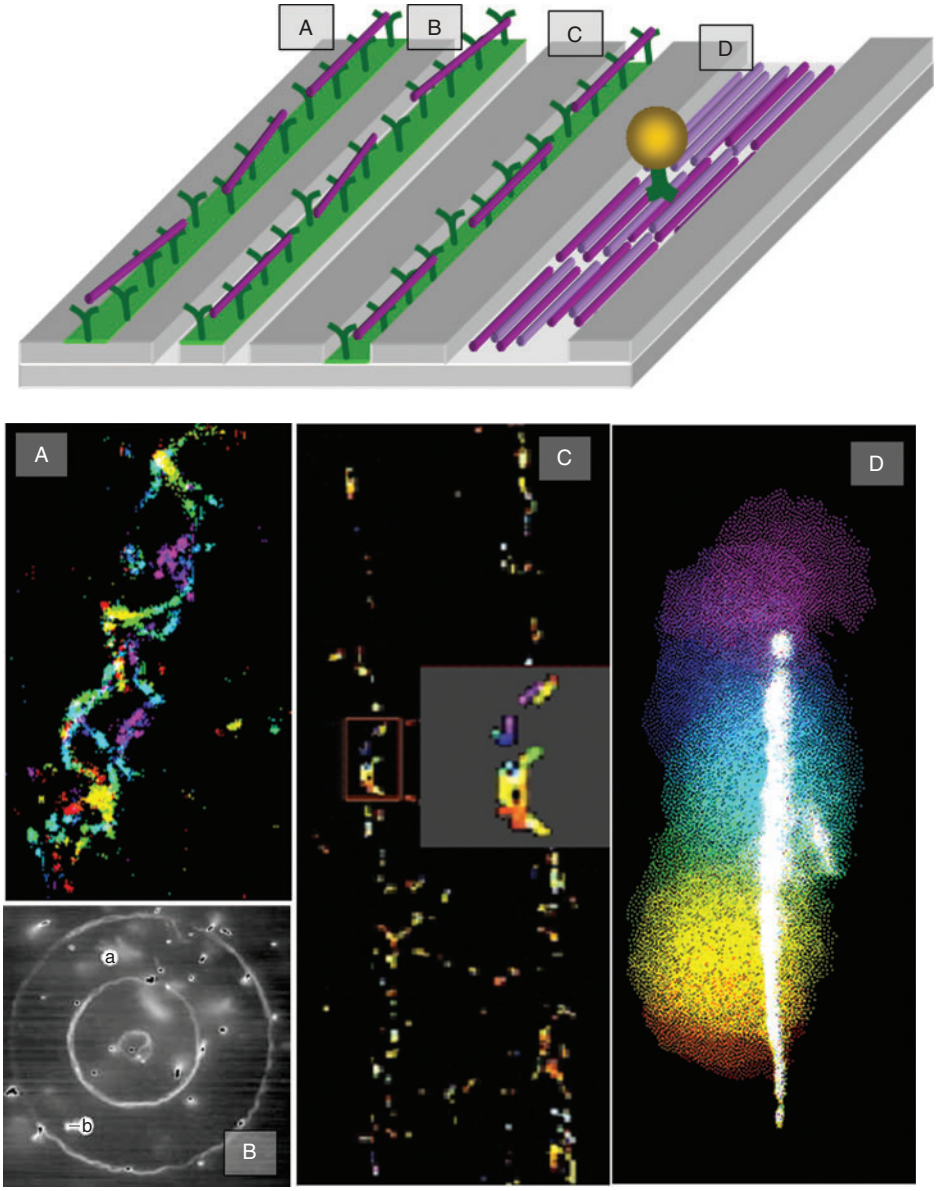

Fig. 4. Mechanisms for lateral confinement of motile elements using micro/nano-fabrication. A, Actin filaments and microtubules slide on motor-functionalized stripes (green) on flat surfaces. Colour coded trajectory of actin filaments on HMM-functionalized tracks (adapted from ref. [34]); red: beginning of the sequence; purple: end of the 4s sequence). B, The filaments (and in principle, microtubules) slide on top of motor-functionalized microstructures. The inset presents an overlap of images of actin filaments (from ref. [32]; reproduced with permission from the Biophysical Society). C, Confinement of filaments or microtubules in microchannels with a motor-functionalized floor. The inset presents the colour-coded trajectories, with an enlargement, for actin filaments sliding in 3 μm channels in a 5s sequence (adapted from ref. [83]). D, Confinement of the movement of motor-functionalized beads in micro-channels that have filaments or microtubules aligned on the floor of the channel. The filaments/microtubules are parallel aligned, but have random directionalities. The inset presents the colour-coded trajectory of a HMM-coated bead on an actin bundle self-assembled in a microchannel (adapted from ref. [50]).

microtubule. In addition, the net movement must overcome the randomizing effects of thermal (Brownian) motion. The electric field strengths required to successfully orient actin motility filaments parallel to the electric field range are 1–10 kV m^{-1}[33,122] and are higher for microtubules, 10–100 kV m^{-1}.[46,121,122]

The other electrical technique uses the *non-uniformity* of the field to induce a net dipole moment in the filaments or microtubules, which yields a force that is proportional to the gradient of the electric field squared. Consequently, alternating current (AC) electric fields can be used instead of DC, thus avoiding electrochemical reactions at the buffer–electrode interface. The resulting movement is called dielectrophoresis (DEP),[123] and typically uses radio frequencies (10 kHz–10 MHz). DEP depends on the dielectric polarizability of the filaments, or microtubules, relative to the surrounding buffer and is, therefore, frequency dependent. The polarizability of filaments and microtubules over a wide range of radio frequencies has been found to be greater than that of the buffer so that they move to regions where the non-uniformity is greatest, called *positive* DEP. Recently, a study[113] of the effect of DEP

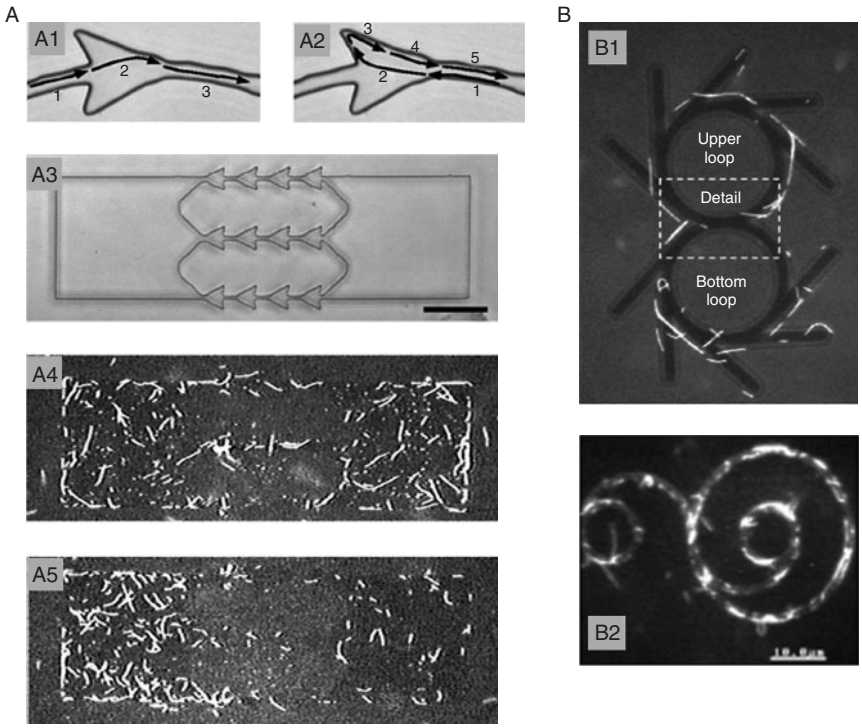

Fig. 5. Control of directionality with microfabricated structures (molecular selectors) functionalized with motor proteins. A, Microfabricated arrowheads rectify microtubule movements (from ref. [37]; reproduced with permission from the Biophysical Society). A microtubule entering the arrowhead feature from the left (A1) passes through the rectifier, but when entering from the right (A2) it is statistically biased to turn back. Two microfabricated 'pools' separated by three rectifiers (A3) are equally filled with microtubules (A4) but after a while the left pool has the majority of microtubules (A5). B, Microfabricated rounded routers. B1, The 'figure 8'-shaped track comprises two circular loops that form a tangential crossing junction and is decorated with unidirectional reflectors for microtubules (from ref. [91]; reproduced with permission of the Royal Society of Chemistry). B2, Spiral shaped router conduits for actin filaments (from ref. [118]; reproduced with permission).

forces on the motility of actin filaments, using microfabricated quadrupole planar electrodes, showed actin filaments glided in a manner that was aligned parallel to the electric field—using strengths of $1.5 \times 10^6\,\mathrm{V\,m^{-1}}$ ($7\,V_{rms}$) and a frequency range of 100 kHz–30 MHz, values that are readily generated by standard laboratory-on-a-chip type supplies. Filaments longer than 1 μm were attracted to regions where the DEP force was greatest, i.e., electrode edges, whereas other particles, such as 500 nm diameter latex spheres, were repelled (negative DEP) at the same frequency (2 MHz). This demonstrated that DEP can conveniently modulate, in real-time, the direction of actin motility.

Confined electric fields at optical frequencies, in the form of optical (laser) tweezers, can also be used to direct movement. Optical tweezers, for example, were used recently to pull kinesin motors with bead cargoes away from one microtubule rail and transfer them onto another where they continued to move towards a new, predetermined destination.[124] The demonstrated principle of macromolecular sorting again shows how combining motility with real-time, non-contact, electric-field control can yield new functionalities for hybrid devices.

4.3. Control of Unidirectional Movement by Self-Assembled Tracks

Unidirectional movement of the motile elements is an inherent feature of the bead architecture assays at the filament/microtubule level. Once the actin-filament or microtubule is immobilized on the surface, motility of the motor can occur in one direction only, but the challenge is laying down the filaments/microtubules in an organized fashion. Therefore, the challenges are not operational but fabrication, as filament/microtubules have to be patterned in stripes or in channels, perfectly aligned, parallel, and with the same polarity. The need is more critical for non-processive systems, e.g., myosin II–actin. Apart from the inherent unidirectionality, this approach has important advantages over the gliding architecture, namely (i) the possibility to engineer the motor protein to carry different payloads; and importantly (ii) the possibility to run objects in opposite directions when functionalized with motors that move with opposite polarities (e.g., kinesin and Ncd).

Conceptually, the simplest method to fabricate parallel, unidirectional tracks with microtubules is to use the flow-induced alignment in combination with surface immobilization. Early work demonstrated that the flow-oriented actin filaments have nearly the same high degree of alignment as the actin filaments in muscle fibres.[125] This general approach has been used to orient microtubules by end-specific immobilization with minus-end specific antibodies followed by flow-induced alignment.[126] An array of aligned microtubules has also been fabricated by the immobilization of short microtubule polymerization seeds at the beginning of microchannels followed by microtubule polymerization in flow.[127] Recently, we also used this method to fabricate bundles of actin filaments on which HMM-functionalized beads moved unidirectionally.[50]

Apart from the mechanical-based alignment methods described above, electrical fields can be used to align filaments as discussed above for motility gliding assays. This approach has also been demonstrated using microtubules,[90] again the use of AC DEP rather than DC electrophoresis avoids heating of (uncoated) gold electrodes. It is important to note that whereas DC electric fields tend to generate long-range EP forces, DEP, which is generated by the electric field non-uniformity, tends to be short range and localized near dielectric discontinuities. It is possible, therefore, that both electric forces could be used to complement each other, for example, using insulating posts to focus a pre-existing electric field thus creating DEP without the need for electrodes that require electrical supply buses.[128,129] In addition, it is not only the direct (EP and/or DEP) action of these forces that can be used, but the indirect interaction of filaments with (i) the surrounding solvent, i.e., using electrohydrodynamic movement to align filaments, and (ii) with each other, for example, dipole–dipole interactions that cause filaments to bridge with each other end-on so as to span a distance much greater than each of their separate lengths (pearl-chaining effect). These effects have been recently reported for other biological macromolecules, such as DNA.[130]

However, all of the above methods ignore an intrinsically useful property of actin filaments and microtubules, that is, their ability to self-assemble on molecularly or nano-organized surfaces. This has been achieved by deposition of actin filaments on lipid monolayers through a simplified Langmuir–Blodgett technique,[95,131] and more recently on mica and highly oriented pyrolithic graphite (HOPG).[132] It has been found that, apart from the unidirectionality of movement, the velocity of myosin-coated beads increased linearly (up to 100 $\mu m\, s^{-1}$) with bead diameter (up to 60 μm in diameter), a counterintuitive finding with important potential for future nanodevices. The most exciting technological avenue would be to design and 'write' complex tracks that comprise individual actin filaments and/or microtubules for motor-functionalized objects to run on and perform designed functions (e.g., transport, load–unload) in particular locations. Several works have demonstrated that actin filaments self-assemble in two-dimensional paracrystal circular shapes when deposited on phospholipid layers.[133–135] More recently, almost perfect circular shapes have been demonstrated when depositing F-actin from a solution on mica.[132] The self-assembly of individual microtubules in tracks with a small curvature radius would be more difficult given their rigidity and dimensions. Fig. 6 presents methods for patterning and the self-assembly of actin filaments and microtubules.[132]

4.4. On–Off Control of the Operation of Protein Molecular Motors Devices

There are several 'off-switches', but many of these will shut down the devices permanently and, therefore, are not useful unless a one-off function is envisaged, e.g., some biosensors.[89,107] The inhibition of microtubule-based kinesin motility using local anaesthetics has been demonstrated[136] and a pausing effect can be provided by electric fields that are capable of docking microtubules.[137] Microtubules have been shown to be repelled by thermoresponsive PNIPAM when the surface was cooled and re-permitted to glide when heated.[92] In that work, the temperature was transferred by a Peltier element in contact with the PNIPAM and reversible on–off switching took ~20 s to take effect. Recently, photolysis of caged inhibitor peptides, located at the kinesin C-terminus domain and which interfere with the interactions between the microtubule, has demonstrated motility cessation or switch-off.[138] In principle, on–off control can be achieved through variation of ATP concentration, ionic strength (Mg^{2+} and Ca^{2+}), and pH of the buffer solution, and studies have mapped the response of the myosin–actin[139,140] and kinesin–microtubule[110] motility systems. Microtubule motility has been turned on by the UV-induced release of caged ATP, and off through the enzymatic ATP degradation by hexokinase.[141,142] However, despite their operational importance, ATP, ionic strength, and pH are unlikely to be the most effective controls for on–off switching. ATP concentration is either practically constant (when the motors operate in relatively large volumes compared to the mass of protein) or it is regulated in nanoconfined spaces by the functioning of the motors. In addition, the ionic strength and pH are supposed to remain at an optimum constant level. Moreover, the variation of the concentration of these parameters, if desirable, will be slow controllers of the functions of the motors. Along with packaging and long-term storage,[143] the reliable, fast, 'smart', nano-localized on–off control of the molecular motor-powered devices presents many challenges, and perhaps may be solved by a combination of biochemical, electrical, and thermal techniques engineered together.

5. Applications of Protein Molecular Motors

The possible applications for future molecular motor-based nanodevices can be clustered into a few targeted areas: (i) biosensing devices; (ii) nanomechanics, ranging from simple force generation to more complex nanomanufacturing; and (iii) information processing and storage.

5.1. Sensing Devices

Biosensing devices can operate in a global, randomly 'distributed' manner, or in a spatially addressable manner. The distributed sensing devices are essentially classical motility assays with motility modulated by a biomolecular recognition event, e.g., protein–protein, antibody–antigen, or DNA–DNA interaction. Spatially addressable sensing devices rely on the same principle, but the motility is confined on purposefully designed microfabricated structures, thus enabling parallelization of the analysis.

The distributed devices for biosensing based on protein molecular motors have their first patent as early as 1998, albeit in a liberal definition of biosensing.[96] Essentially the patent describes the use of motility assays as a means to detect cytoskeletonal modulators, which are potential targets for drugs and agrochemicals, for example, through the abrupt change of motility. The same principle has been used for the detection of very small concentrations of heavy metal ions (detection limit of one ion per myosin head) by the myosin–actin system.[107] A more advanced architecture would rely on the mounting of 'detectors' (e.g., ssDNA, antibodies, etc.) on the motile elements. This principle has been demonstrated for (i) microtubules decorated with streptavidin that detect and stretch biotin-terminated λ-phase DNA molecules,[89] (ii) detection of antigens through the cessation of the motility of actin filaments decorated with antibodies,[144] which include more selective streptavidin–antibody multilayer sandwiches biotinylated to microtubules.[145] Figure 7 presents two possibilities for distributed nanobiosensing devices.

The spatially addressable devices will multiplex the functions demonstrated by simpler devices and, therefore, have not been yet developed to the same extent as distributed devices. One

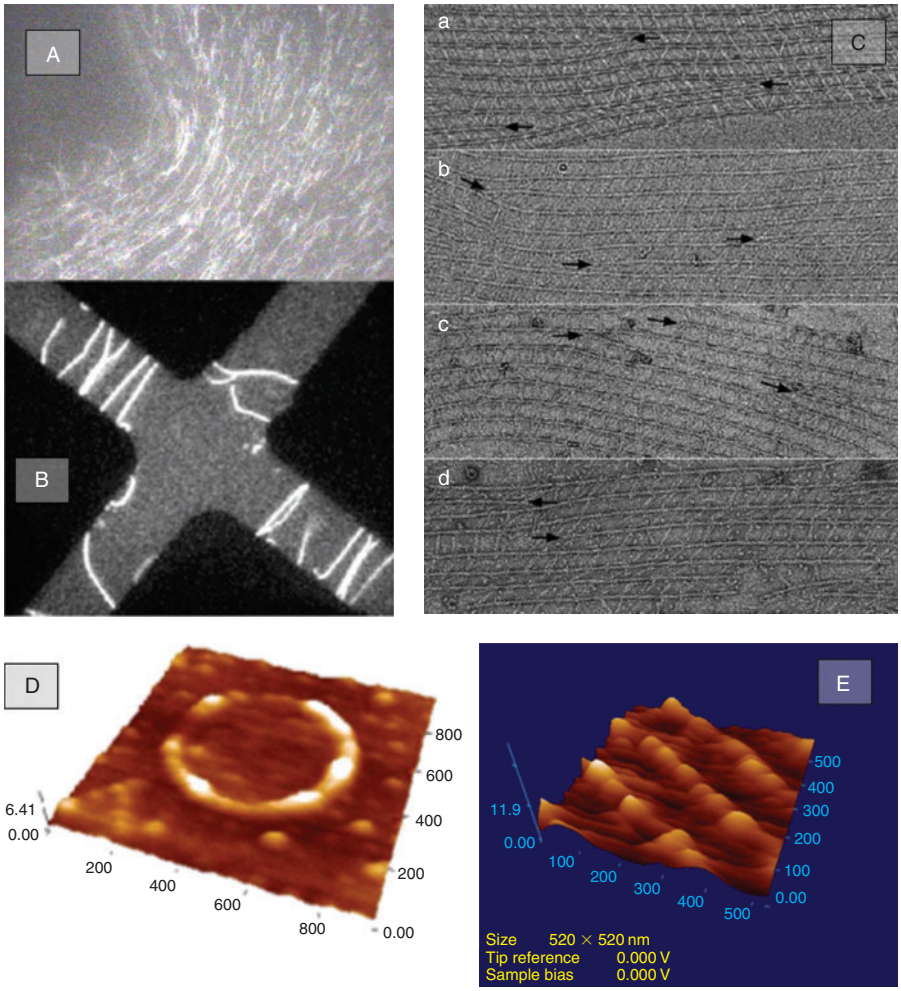

Fig. 6. Methods for patterning and self-assembly of actin filaments and microtubules. A, Alignment of actin filaments in flow conditions. B, Alignment of actin filaments between microfabricated electrodes (reprinted with permission from ref. [113]; © American Chemical Society). C, Arrays of actin filaments (from ref. [165]; reproduced with permission © Rockefeller University Press) cross-linked with a-actinin from (a) skeletal muscle, (b) smooth muscle, (c) cardiac muscle, and (d) dictyostelium discoidium, a non-muscle isoform. In all cases the morphology of the bundles is similar. D, Circular shape of actin filaments (imaged with AFM) deposited on hydrophilic mica (from ref. [132]; reproduced with permission). E, Parallel actin features on hydrophobic HOPG, aligned (~200 nm) longitudinally (from ref. [132]; reproduced with permission).

device that relies on the modulation of motility by antibody–antigen recognition and the transduction of motility in EM signals has also been proposed.[116,146]

5.2. Nanomechanical Devices

Most bead motility assays are primitive self-propelled nanodevices with inherent directionality control. An early study demonstrated the high speed of large beads functionalized with HMM running on paracrystal actin filaments self-assembled on a lipid monolayer mounted on glass.[95] This study, which unfortunately was not followed up, is important because it demonstrates the benefits of using self-aligned nano-tracks in terms of both directionality and amplification of force. In addition, the transport of micro-objects has been demonstrated for (i) myosin-coated (e.g., magnetic) beads walking on actin bundles of Nitella [147]; (ii) gelsolin-functionalized 40 nm gold nanoparticles attached at one end of actin filaments[148]; (iii) transportation of quantum dots using actin filaments,[15] and (iv) HMM-functionalized beads travelling on actin bundles self-assembled in microfabricated channels.[50] The kinesin–microtubule system was also used for the (i) transport, rotation, and flip-over of kinesin-powered micro-chips made of silicon along flow-aligned microtubules immobilized on the surface of a flow cell,[126] (ii) formation of membrane tubes and tubular networks by lipid giant unilamellar vesicles, decorated with kinesin-functionalized polystyrene beads,[149] (iii) capture and transport of streptavidin-coated beads by biotin-decorated microtubules,[141,150] and (iv) transport of quantum dots linked to microtubules[145] and also to kinesin for imaging single molecule movement.[43]

Bi-directional devices have not yet been demonstrated in vitro, but they have been recently examined and described in vivo. Two possible mechanisms that explain the bidirectional movement of cargo in natural processes, e.g., axonal vesicles, mitochondria, melanosomes, etc., are ‘tug of war’ and coordination.[151] In the tug of war model, opposite-polarity motors are active simultaneously (Fig. 2 C2). Net motion results when one set of motors successfully competes against the opposing motors. On the other hand, in the motor coordination model, competition is avoided because when plus-end motors are active,

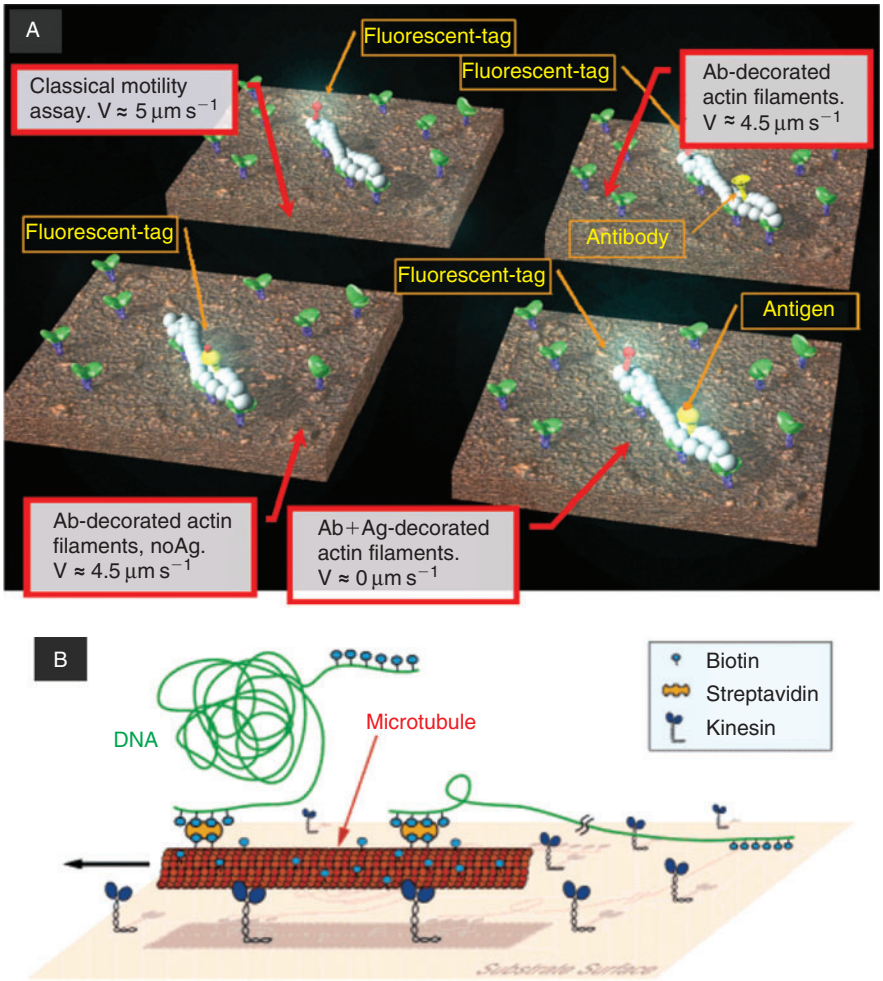

Fig. 7. Distributed bionanosensing devices powered by molecular motors. A, The motility of an antibody*-decorated actin* filament (where the symbol * refers to fluorescently tagged) is stopped by the binding of antibodies with their anti-antibodies (from ref. [144]; reproduced with permission). B, Schematic diagram of the microtubule–DNA transport and manipulation system (reprinted with permission from ref. [89]; © American Chemical Society). Microtubules decorated with biotin–streptavidin stretch DNA molecules immobilized on surfaces when they pick up the biotinylated end of a DNA molecule.

minus-end motors are turned off and vice versa (Fig. 2, see C3). For clarity, in Fig. 2 only the cargo and the motors are depicted, hypothetical molecules that allow the motors to assemble into complexes and that mediate interactions between motors are not shown.

Finally, planar devices, i.e., devices that operate on a micro/nano-fabricated area rather than on a linear dimension, are perhaps the most advanced in conceptualization, if not also in device implementation. In fact, progress in this area has reached the extent that simulations, or *in silico* design tools, are being developed that reduce the need for expensive e-beam lithographed prototypes.[42] Essentially, planar devices address the problem of continuous operation of nanodevices based on protein molecular motors. Indeed, most of the 'single dimension' devices described above (bar the natural or hypothetical bi-directional devices) cannot continuously operate as the unidirectional movement has to stop when the motile element (e.g., microtubule or bead) reaches the end of the micro/nano-fabricated pathway. Some possibilities to 'recharge' these devices do exist, e.g., the use of magnetic beads as loads[146,147,152] and the application of a directional EM field that will collect the beads at one end of the track. Such a system, however, will address the motile elements globally and, therefore, much of the functionality of individual nanodevices will be lost. An alternative is to run the motile elements in a circular fashion on micro- or nano-tracks on larger areas, perhaps with functions being sequentially performed along the way. Most molecular selectors proposed and demonstrated[32,37,58,90,116,153] are essentially planar nanodevices. More elaborate schemes have also been proposed, for instance a device that would comprise a 'bay area' for microtubules decorated with molecular linkers or 'hooks'; a 'fishing' path, where the motile elements capture different payloads; a mechanical selector that sorts and redirects the motile elements according to their cargo, and cargo delivery areas.[60] Another device that has also been proposed comprises an optical detector connected to an electrical gate (electrodes placed on the sides of a channel), which selects the motile elements according to a particular property, e.g., electric charge or mass.[90] These are illustrated in Fig. 8.

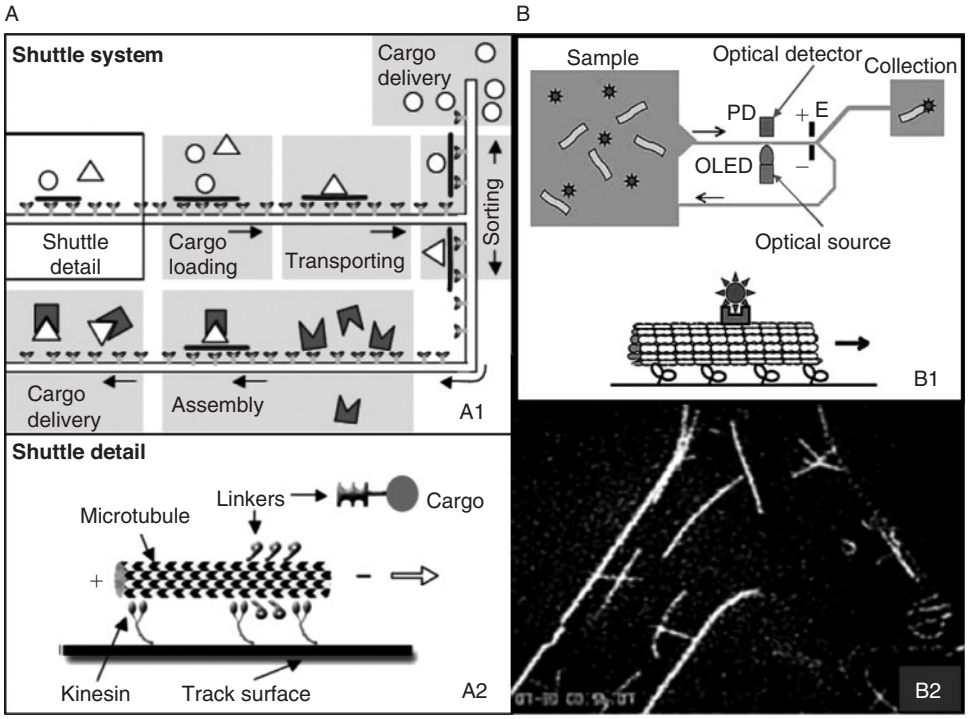

Fig. 8. Planar nanomechanical devices. A, Projected system for loading, transport, sorting, and assemble (reprinted with permission from ref. [60]; © American Chemical Society). A1, Molecular shuttle system with subsystems connected by tracks. A2, Shuttle design 'hooks' for cargo. B, Projected system for detection and purification (from ref. [90]; reproduced with kind permission of Springer Science and Business Media). B1, Integrated system, comprising sample chamber with microtubules and analyte molecules, motor-functionalized channels, organic LED exciter and photodiode (PD) detector, and electrodes (E) to direct cargo-laden microtubules to the collection chamber. B2, Image of the electrode controlled gate.

5.3. Information Storage and Processing

Molecular motor-powered devices for information storage and processing are in their infancy, despite the fact that this is one of the main functions of linear natural molecular motors. For instance, a kinesin–microtubule system is the workhorse of transporting information in neuronal systems and the dynein–microtubule system is instrumental in bio-camouflage.

Because of their very small dimensions, the motile elements can hypothetically visit and detect very small and perhaps concealed nanostructures on surfaces—a principle that can be used for very high-density information storage. A device that images microscopic surface properties has been proposed.[154] The information about surface properties such as topography is obtained by repeated acquisition of an optical signal (e.g., fluorescence) from a large number of motile elements (e.g., microtubules) moving on random paths across a micro/nanostructured surface. These self-propelled probes sample the surface in a statistical process in contrast to the deterministic, linear sampling performed by a scanning probe microscope. The spatial distribution of hydrophobicity on a nano-patterned surface, as opposed to topography, can also be mapped with very high resolution using the observation that the fluorescence of rhodamine-phaloidin-tagged actin filaments varies with the nature of the surface.[34]

More opportunities, but also more challenges, will arise in the area of biocomputation devices. One prospect would be to use the possible, unusual ferroelectric properties of microtubules and/or the formation of travelling kink solitons,[155,156] for example, for new quantum electronic devices. A more immediate application would arise from DNA-based computation[157] and from maze-solving with microfluidics devices,[158] or by amoeboid cells.[159] Essentially, it is entirely possible that the motility of actin filaments, or microtubules, can be used to explore mazes or other more elaborate micro- or nano-fabricated networks that code a mathematical problem,[160] as illustrated in Fig. 9. Many advancements in the area of nanomechanical devices, e.g., electrode-controlled gates,[90] molecular rectifiers, selectors, and sorters[37,44,46,154,161,162] will be useful modules for a molecular motor-based calculator.

6. Perspectives

Protein molecular motors offer many promises for the design, fabrication, and operation of dynamic nanodevices. Nevertheless, many challenges for the future molecular motor-based nanodevices still remain open, in particular, to give a realistic answer to the following questions:

Competitive edge. What is the benefit, exactly, of protein molecular motors compared to other alternative devices? Molecular motor-based sensing devices offer a clear technological path for single molecule-based biomolecular recognition, but much more work has to be done to transform this possible advantage into a competitive one. It would be expected that this, potential, single molecule detection will translate to extremely sensitive and fast devices. This advantage is indeed critical to bioterrorism applications or very early detection of pandemics where speed of detection is essential, and it could be that all other issues (e.g., cost) would be less relevant. Similar analysis has to be made for other groups of applications, i.e., nanomechanical or biocomputation devices. As a general rule of thumb, high value-added

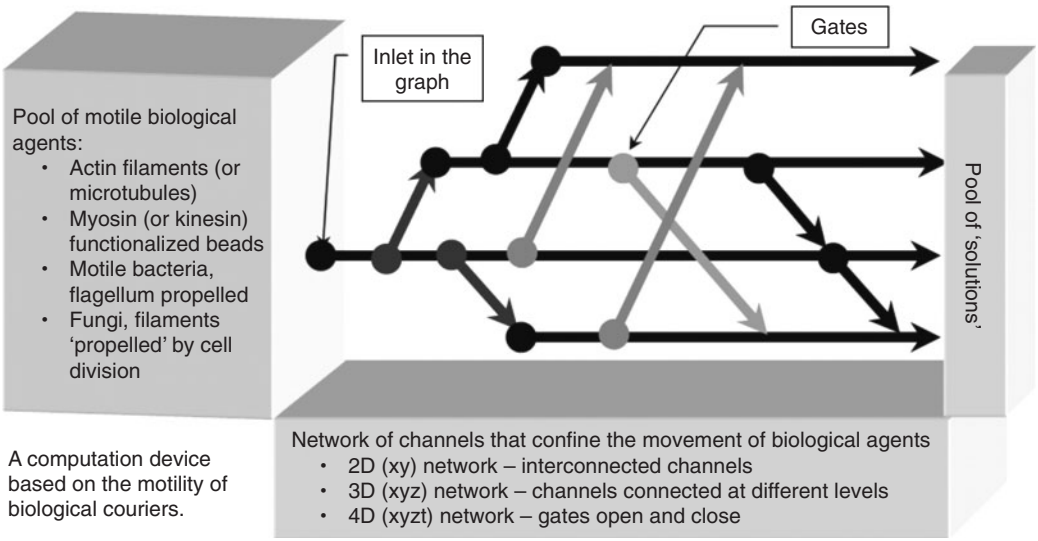

Fig. 9. A possible scheme of a biocomputation device using biological agents, e.g., actin filaments or microtubules; or even molecular motor-propelled whole organisms (bacteria or fungi). Reprinted with permission from ref. [160].

uses, where the costs are less important, are more likely to offer 'killer applications'.

Endurance. How can the endurance of protein molecular motors be dramatically improved to a level where they would become competitive in fabrication and operation costs with other, non-protein-based dynamic devices, e.g., NEMS? Would a 'quantum leap' search for inherently robust proteins, e.g., those present in living beings that exist in extreme environments (thermophiles or arctic temperatures) or motor proteins that work in extreme conditions (e.g., from sharks) yield the answer to this problem? Or would a more gradual improvement based on a better understanding of the impact of operating conditions, i.e., surfaces, temperature, etc. be more productive? Alternatively, would the use of molecular motor-based devices be better restricted to those applications that are environmentally controlled, e.g., laboratory-only applications, such as genomics or biocomputation? This is also in view of device storage where very recent demonstrations of snap freezing and lyophilization of kinesin–microtubule motor systems show that motility can be retained after thawing or rehydration.[163]

Linear motors: to bead or not to bead? Is the simplicity of implementation of the gliding geometry a good trade-off against the loss of information regarding directionality, which is built in actin filaments/microtubules, for bead geometry? In certain applications, e.g., distributed sensing devices, the gliding geometry has important advantages, but for more advanced devices, where control of directionality is essential, careful design and accurate fabrication of loading zones, selectors, and channels is needed so that the devices operate with sufficiently low error rates. On the other hand, the bead geometry, despite long-term advantages, raises important and immediate technological problems. If the inherently unidirectional tracks are to be laid in a designed manner, how would this be achieved? A solution would be to locally control the actin and tubulin polymerization on a nano-structured surface using multi-photon stereo-lithography and caged compounds that initiate polymerization. The issue then arises as to how to orient the structures on that surface in the desired manner. Alternatively, one could manipulate fluid-flow and/or local electric fields to capture filaments or microtubules in desired positions and orientations.

Linear motors: cargo blues. In most if not all devices proposed so far, a cargo, i.e., a biomolecule (antibody, streptavidin or biotin, DNA), a bead, or a rod, is transported by the motor or filament/microtubule. With the exception of devices based on rotary motors and possibly others (e.g., those for power generation or detection of movement) the cargo has to be unloaded sometime and in precise locations, in particular if a continuous mode of operation is envisaged. Although many schemes have been devised regarding the attachment of payloads, the much more difficult issue of controlled loading and unloading deserves more work. A less important, but also a less challenging issue is the use of rods instead of beads as carried objects. Rods have the advantage of being easier to functionalize differently at their ends as well as being able to improve the direction of movement.

Fuel. Where is the ATP coming from? If a continuous operation is envisaged, the fuel for the dynamic nanodevices has to be continuously provided. At the limit, these nanodevices can operate in large (relative to protein mass) volumes and, therefore, the ATP concentration would be quasi-constant. A more sustainable solution would be to synthesis ATP in a separate chamber ('ATP factory') and re-circulate ADP through a microfluidics network. ATP can be provided by bacteria,[164] in which case careful filtering has to be arranged between the ATP factory and the operational area that comprises the molecular motor-powered devices. Perhaps the most sustainable, albeit challenging, solution is to integrate other motors, such as ATPases, for fuel production and recirculation as well as for their mechanical capabilities.

Integration. Most of the primitive devices demonstrated so far use the kinesin–microtubule system, with a few important exceptions that use rotary motors, myosin–actin, and actin polymerization systems. The relative robustness and processive nature of kinesin and the rigidity and larger dimensions of microtubules assists demonstration of prototypes, but the myosin–actin filament system has advantages, in particular, higher velocities and in some applications (e.g., biocomputation) flexibility. Moreover, if continuous operation is envisaged, which will require bi-directionality, we need at least two types of motor proteins to work in the same device, possibly on the same cargo. Finally, there are applications where actin and possibly

microtubule polymerization, e.g., fabrication of tracks, is essential. It appears that future efforts have to be directed towards the integration of several types of motors on the same device. It is possible that other types of motors are used *in conjunction*, not in competition, with the 'micro' linear motors, for example, micro rotary motors, just as macro mechanical rotary and linear devices are synergistically used in explosion engines.

External control. The operation of molecular motor systems, myosin–actin and kinesin–microtubule, inside hybrid nanodevices should be capable of being externally controlled and monitored. How would this be achieved? It depends on the purpose of the hybrid nanodevice and the context in which it operates: whether it is stand alone, or part of a larger system (e.g., laboratory-on-a-chip). The most likely transmission method for rapidly communicating signals to, and from, the motors would be EM fields (radio or optical frequencies), fluid flow, or both. Control of individual motors and/or their filaments/microtubules *inside* nanodevices can be exercized in two ways: (i) directly, for example, using forces generated by EM fields (e.g., electrophoresis, DEP, magnetophoresis), fluid-flow, or mechanical (such as thermocoiling polymer) to act on the motor systems themselves, and (ii) indirectly, for example, using EM fields to signal an intermediate agent which then influences the motors (e.g., optically induced uncaging of peptides in kinesin, which switches it off).

The control can be defined in terms of its spatiotemporal distribution, i.e., either broadcast over the entire device or confined to particular regions or sub cells, and for programmed time periods. Separately addressable, micrometer or nanometer-sized electrodes, for example, could be used to achieve electrophoretic or AC DEP movement and positioning, particularly the latter, which uses electric field inhomogeneities and can generate almost point-like forces. Needless to say, these options need consideration in terms of the force strengths generated that will surely compete with the randomising effects of thermal motion and interfering viscous forces from nearby motors and other hydrodynamic sources. Control can also be individualized to motility systems with an established *identity*, for example, by attaching beads with particular electric or magnetic properties. Likewise, the monitoring of motor systems can be direct (e.g., optical detection of microtubule presence), or indirect (e.g., microtubule unloading of cargo that is then detected) with spatiotemporal definition. An example of the latter would again use nano-electrodes, but instead of generating controlling forces, they detect changes in localized electric fields, or dielectric permittivity, thus inferring the presence of nearby motor systems. As with control, monitoring will require careful technical consideration regarding signal-to-noise ratios and other unwanted effects in order to avoid, or lessen, the chances of false detection.

In order for hybrid nanodevices to be strong technology contenders, aspects such as fuel supply, endurance, motor and assay methods, cargo handling, integration, external control, and reliable monitoring will need considerable advancement and will provide ample opportunities for novel research. In addition, in parallel to nanodevice advancement, new opportunities for applications in mainstream or niche sectors will also need to be identified.

7. Conclusions

Although several aspects regarding the fundamentals of molecular motors and associated proteins are still the subject of intense study, the molecular biology and biophysics-oriented research has reached critical mass where many fundamental parameters necessary for the engineering design of molecular motor-powered nanodevices are already available. A separate line of research, focussed on molecular motor-based devices, which has developed in the last half a decade, also provides some answers regarding the possible engineering options for their design, fabrication, and operation. Many important challenges still remain to be resolved, but the primitive prototypes already demonstrated offer useful benchmarks in the development of robust and continuous-operation devices and systems for biosensing, mechanical work, and possibly biocomputation.

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