Comparison of actin- and microtubule-based motility systems for application in functional nanodevices

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Abstract
Over the last 25 years, extensive progress has been made in developing a range of nanotechnological applications where cytoskeletal filaments and molecular motors are key elements. This includes novel, highly miniaturized lab on a chip systems for biosensing, nanoseparation etc but also new materials and parallel computation devices for solving otherwise intractable mathematical problems. For such approaches, both actin-based and microtubule-based cytoskeletal systems have been used. However, in accordance with their different cellular functions, actin filaments and microtubules have different properties and interaction kinetics with molecular motors. Therefore, the two systems obviously exhibit different advantages and encounter different challenges when exploited for applications. Specifically, the achievable filament velocities, the capability to guide filaments along nanopatterned tracks and the capability to attach and transport cargo differ between actin- and microtubule-based systems. Our aim here is to systematically elucidate these differences to facilitate design of new devices and optimize future developments. We first review the cellular functions and the fundamental physical and biochemical properties of actin filaments and microtubules. In this context we also consider their interaction with molecular motors and other regulatory proteins that are of relevance for applications. We then relate these properties to the advantages and challenges associated with the use of each of the motor-filament systems for different tasks. Finally, fundamental properties are considered in relation to some of the most interesting future development paths e.g. in biosensing and biocomputation.

1. Introduction

Cytoskeletal filaments in the form of actin filaments (F-actin), microtubules and intermediate filaments have a range of important roles in living cells. Whereas intermediate filaments have mainly structural and organizational functions [1, 2], F-actin and microtubules (figures 1 and 2) are involved in a wide range of dynamic processes associated with the development of force and motion in response to various cues [3, 4]. The actin filaments and microtubules have typical distributions in the cell tuned to their tasks (figure 1). This includes migration of non-muscle cells, muscle contraction, intracellular cargo transport, cytokinesis, mitosis and meiosis but also cell signalling. The actin filaments and microtubules can develop forces and produce motion by polymerization-depolymerization events coordinated by regulatory proteins, and they often interact with so called cytoskeletal molecular motors in executing their tasks. These molecular motor proteins capture the free energy stored in MgATP to produce mechanical work while interacting with their cytoskeletal tracks [5]. Cytoskeletal motors, in focus here, include the actin-based motors of the myosin superfamily and the microtubule-based motors of the kinesin and dynein superfamilies (figures 1 and 2). Other types of biological motors, such as nucleic acid-based motors [6] (e.g. DNA polymerase) and rotary motors driven by ion-flow, e.g. bacterial flagellar motors [7], eukaryotic mitochondrial ATP synthase [8]
Figure 1. Actin and microtubule cytoskeleton and related motors from the cell to nanotechnological applications. The middle panel lists important parameters to consider when exploring actin- and microtubule-based motors for nanotechnological applications.

and rotary motors underlying other transport processes [9], have also the potential to be used for nanotechnological applications but will not be considered here. In accordance with a wide variety of motor proteins with different properties of both the kinesin and myosin types, the cellular function of the actin-based and the microtubule-based systems are partly overlapping or integrated with each other. Thus both, kinesin and myosin motors, have roles in intracellular cargo-transport. Motors that are particularly well suited for this task are processive, i.e. they spend a dominant fraction of their ATP turnover cycle time attached to the respective track, taking several steps on average before dissociating. Such processive motors include kinesin-1 and myosin V that move along microtubule and actin tracks, respectively. On the other hand, non-processive motors, where each of the individual motors has only brief attachment periods to their tracks, develop force and motion by working in teams. Examples are myosin II, underlying muscle contraction, kinesin-14 with roles in assembling the mitotic spindle and axonemal dynein underlying ciliar motion. Individually, the non-processive motors usually take only one step on their track before dissociating. Notwithstanding the mentioned functional overlap, one can generally assign actin filaments and the associated motors to tasks such as cell motility including muscle contraction, development of forces for other reasons (e.g. cytokinesis, locking of components to a certain place), dynamic re-arrangement of the cell cortex and short distance cargo transportation [10]. Conversely, the microtubule-based motors are mainly responsible for long-range transport (e.g. axonal transport) [11], intracellular positioning (e.g. spindle positioning, chromosome segregation, organelle positioning) and the beating of cilia and flagella [10].

Starting in the 1990s [12–15], molecular motors and cytoskeletal filaments have been exploited with the aim to develop nanotechnological applications and novel materials. These developments, in turn, took their
Figure 2. The actin–myosin II vs the microtubule–kinesin-1 motor-filament systems. (a, top) Helical F-actin comprised of G-actin subunits, shown as molecular model as side and front view (PDB file: 6BNO) [34]. (a, middle) Schematic of three non-processive myosin-II motors, each bound to actin with one out of two heads, and acting jointly, while moving from minus-end to plus-end of the actin filament. (a, bottom) Indicated segments of the myosin II molecule can be cleaved off by limited proteolysis (see text for details). (b, top) Molecular model of α–β-tubulin dimer (PDB: 1TUB to the right [36]). (b, bottom) Schematic of kinesin-1 and cytoplasmic dynein as processive motors walking along a hollow cylindrical microtubule. Kinesin-1 walks towards the plus-end whereas dynein walks towards the minus-end. (c) Molecular model structure of actin–myosin rigor complex, showing myosin subfragment 1 interacting with an actin filament indicated by two subunits. The myosin head consists of the N-terminal fragment (∼800 amino acids) of a myosin heavy chain (brown) continuing in an α-helical neck towards the C-terminal. The latter is stabilized by an essential light chain and a regulatory light chain (blue). Structure from PDB file: 5H53 [35]. (d) Model structure of a kinesin-1 homodimer with the coiled-coils that associate via neck linkers to the motor heads. (PDB files: 2Y5W and 6IGV). (e) Schematic of the ATP turnover cycle of a myosin II head interacting with actin subdivided into strongly attached states (Att) and detached states (Det) typical for a non-processive motor with low duty ratio (see box). (f) Schematic of the ATP turnover cycle of kinesin-1 interacting with microtubules typical of a processive motor with high duty ratio.
starting point in the in vitro motility assay, originally developed by Spudich and co-workers for the actin–myosin motor system in the 1980s [16–18] and later adapted for microtubules and kinesin [19]. This assay comes in two forms, the bead assay and the gliding assay. In the bead assay, motor-coated beads move along oriented cytoskeletal filament cables. In contrast, in a gliding assay (figures 1 and 3), the filaments are propelled by motor proteins that have been adsorbed to a flat, appropriately treated surface.

The gliding assay has been most important as a basis for nanotechnological developments (bottom, figure 1). Crucial enabling technologies are capacities to selectively guide the motor propelled filaments (both actin and microtubules) along lithographically produced nano- to micrometer-sized tracks/channels and to attach a range of different cargoes to the filaments with maintained effectiveness of the motor driven transport. The proposed applications, recently summarized in a comprehensive review [20] include (examples in bottom of figure 1) imaging, nanoseparation, biosensing, mechanical force transduction and biocomputation for the solution of complex mathematical problems. In these applications, key advantages of the motor systems, compared to non-motor devices, are exploited. This includes parallel operation of a large number of agents simultaneously, very high energy efficiency and unprecedented miniaturization while circumventing the need for bulky accessory equipment such as pumps for nano-, and microfluidics.

For many types of applications, both actin-based and microtubule-based systems have been used. Reputedly, the two systems exhibit different advantages and challenges in these regards, attributed to different filament properties and different motor-filament interaction mechanisms. Whereas it is clear that the achievable filament velocities differ for actin and microtubule-based motors, there is a general perception that there are differences also in the capability for microtubules and actin filaments to be guided along nanopatterned tracks and to transport cargo. However, these differences and their mechanistic basis and implications have not yet been systematically characterized. A key aim of the current review is to overcome this limitation and to elucidate the advantages and challenges associated with the use of each of the actin- and microtubule-based motor systems for nanotechnological applications. In the process, we consider how the widely varying properties of nature’s motor and filament toolbox are most effectively used. We start from an overview of fundamental physical and biochemical properties of actin filaments and microtubules with focus on major differences. We then move on to discuss the properties of key actin- and microtubule-based molecular motors and the related challenges (middle, figure 1) that are important in development of nanodevices. Finally, we arrive at detailed considerations of the fundamental properties in relation to some of the most interesting future paths in the development of nanotechnological applications e.g. in biocomputation and biosensing.

2. The cytoskeleton

The ‘cytoskeleton’ is a historical term that does not fully appreciate the dynamic nature of the key components. The cytoskeletal elements exist in three domains of life [21] but in somewhat different forms. In eukaryotes, the key components are three types of fibrous proteins: actin filaments (F-actin), microtubules and intermediate filaments, all being polymers composed of subunits in well-defined arrangements. The cytoskeletal components have a range of varying roles. First, all components are important for the structural integrity, mechanical stability and intracellular organization of living cells (cf [22]). Second, the cytoskeletal components are dynamic to different degrees, reorganizing in response to intracellular changes and extracellular cues whether in forms of hormones or mechanical forces (see reviews in [3, 23–30]). Finally, microtubules and actin filaments are tracks for molecular motors which is not the case for intermediate filaments (cf [21]). For more on the biology of the cytoskeleton we refer the readers to recent excellent reviews of the cytoskeleton as a whole [21] as well as more specialized reviews with focus on actin [3, 23–26], microtubules [27–30] and intermediate filaments [1, 2]. Use and engineering of cytoskeletal components without involvement of the molecular motors myosin, kinesin and dynein, e.g. for the production of novel materials has been reviewed previously [20, 31, 32, 37]. Here, we focus on properties of actin filaments, microtubules and their associated molecular motors of relevance for use in applications that involve both the motors and the filaments.

3. Actin

Actin is the most abundant protein in many eukaryotes [24]. It is evolutionarily highly conserved with sequence identity of 100% between different skeletal muscle actins (α-actin) from chicken to humans, >93% identity between the three main actin isoforms in mammalian cells (α-, β- and γ-actin) and ~90% identity between mammalian and yeast actin [33]. In living cells, actin exists in either monomeric form (G-actin) or filamentous form (F-actin; figure 2(a), top) with polymerization/depolymerization,
fragmentation, bundling and branching as well as other dynamic properties regulated by a set of actin binding proteins [3, 23, 24] and also by posttranslational modifications (PTMs). Some of the actin-binding proteins (ABPs) are of potential interest in nanotechnological applications as briefly considered below. Without these proteins, G-actin monomers spontaneously polymerize into filaments by self-assembly into a polar actin filament structure with one ‘plus end’ (barbed end) where addition of monomers is faster than at the other ‘minus end’ (pointed end). The formation of nuclei of three actin monomers into trimers is rate limiting for initial filament formation [24, 25, 38]. However, once a filament is formed, it will continue to grow in length as long as the rate of monomer addition outbalances the rate of monomer loss. If the actin monomer concentration is denoted \([A]\), the second order rate constants of monomer addition at the plus and minus end, respectively, are \(k_{\text{on}}^+\) and \(k_{\text{on}}^-\), and the rate constants for monomer loss at these two ends are \(k_{\text{off}}^+\) and \(k_{\text{off}}^-\), then the conditions for maintained or growing filament length can be written as \((k_{\text{on}}^+ + k_{\text{off}}^-) [A] \geq k_{\text{off}}^+ + k_{\text{off}}^-\). Equality applies when the actin concentration is equal to what is denoted the critical concentration \([A]_{\text{crit}} = (k_{\text{off}}^+ + k_{\text{off}}^-)/(k_{\text{on}}^+ + k_{\text{on}}^-)\). The critical concentration varies with experimental conditions, e.g. \([Ca^{2+}],[Mg^{2+}]\), ionic strength and pH. Furthermore, of great functional relevance, the critical concentration is appreciably lower at the plus end [26, 39] (~0.1 \(\mu\)M) than at the minus end (0.6 \(\mu\)M). This reflects different binding affinities between subunits along F-actin whether ATP or ADP–Pi on the one hand or ADP on the other is bound to the nucleotide binding site [26, 39, 40]. In G-actin, ATP is bound to the nucleotide binding site. However, after binding of G-actin to the plus end of an existing filament, ATP is hydrolysed to ADP and Pi (inorganic phosphate), with subsequent slow Pi-release. As a consequence, ATP-actin subunits predominate at the plus end whereas the bound nucleotide is shifted to ADP towards the minus end of the filament.

For most studies involving isolated actin filaments, including nanotechnological exploitation, actin is isolated from muscle tissue, most commonly rabbit skeletal muscle. This approach is well established [41] and similar methods can be used to obtain actin from other sources, e.g. bovine cardiac muscle. The switch between these sources has negligible effects on myosin driven motility [42], consistent with an amino acid sequence difference of only four amino acids, with conservative substitutions. Striated muscle \(\alpha\)-actin is more challenging to obtain in cellular expression systems than from animal tissue but it can be expressed and engineered using the baculovirus-insect cell system [43, 44]. It is also of interest to note that actin from a range of different species can be propelled by fast skeletal muscle myosin due to high degree of sequence conservation, as for instance demonstrated for yeast actin [45]. This fact together with the insect-cell-based expression system for \(\alpha\)-actin opens for approaches to fine-tune the actin-properties both by selection of its origin and by application of genetic engineering.

Generally, in applications, the actin filaments are stabilized by the fungal cyclic peptide phalloidin which effectively lowers the critical concentration to almost zero [46, 47]. Phalloidin binds specifically and with high affinity in a 1:1 stoichiometric ratio via binding sites on four neighbouring subunits along F-actin [48]. This compound is also useful for attaching other molecules to actin. Thus, a range of conjugates between fluorophores and phalloidin as well as between biotin and phalloidin are commercially available [31]. However, when used for cargo-attachment to actin, one should keep in mind that, despite the high affinity between phalloidin and actin, the half-time of phalloidin dissociation is approximately 30 min [49].

Whether stabilized by phalloidin or not, F-actin can be structurally characterized as a right-handed helix with two strands of subunits wound around each other with a helical half-pitch of 36–38 nm. Alternatively, it can be described as a left-handed helix with 2.75 nm half-pitch by considering subsequent sub-units in different strands. Accordingly, there are approximately 360 actin monomers along a 1 \(\mu\)m long filament. The helical structure is a key determinant of the mechanical properties of actin filaments as further considered below.

4. Microtubules

In eukaryotic cells, microtubules self-assemble from the cellular machinery known as the microtubule-organizing centre (MTOC). In animal cells the basal bodies, which are found at the base of cilia and flagella, and the centrosome, which is coordinated with cell replication and assembly of the mitotic spindle [50] are the main organelles that function as MTOC. Additionally, several microtubule-associated proteins (MAPs) act as nucleating factors and are involved in the temporal and spatial control of microtubule nucleation. In vitro, microtubules spontaneously nucleate from solutions of purified tubulin subunits in the presence of GTP. Thus, \(\alpha-\beta\)-tubulin heterodimers, 8 nm in length, assemble head-to-tail into protofilaments which then associate laterally until a hollow tube with an outer diameter of 25 nm is formed (figure 2(b)). The resulting microtubule is a polar polymer with a fast-growing plus end, where \(\beta\)-tubulin is exposed, and a slow-growing minus end, where \(\alpha\)-tubulin is exposed instead. Hence, the
pattern formed by the dimers is uniform and oriented, allowing motor proteins to transport cargos in the cell efficiently and with directionality determined by the track-motor complementarity.

Microtubules show a characteristic dynamic behaviour, known as ‘dynamic instability’. In this stochastic process, microtubules alternate between states of catastrophe and recovery, where they go from growth to fast shrinkage and from shrinkage to slow growth, respectively [27, 51]. Inside the cell, this reiterated polymerization and depolymerization of microtubules is fundamental to many of their cellular functions. The process generates pulling and pushing forces [52] that cooperate in the formation of membrane

The second moment of inertia, 

the value for the Young’s modulus,

The latter quantity is directly proportional to the flexural rigidity, also known as bending stiffness. Formally,

modulus in the range of 100–4000 MPa [79] with lower and higher values for taxol-stabilized and taxol-free microtubules, respectively. For comparison, nylon has a Young’s modulus in the range 1000–10 000 MPa [76]. The stiffness of actin filaments and microtubules can also be characterized by their persistence length.

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5. Mechanics of cytoskeletal filaments

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Thus, actin filaments have a Young’s modulus in the range of 300–1000 MPa [77, 78] which becomes about ten-fold higher after the addition of tropomyosin to the filaments [77]. Microtubules have a similar Young’s modulus in the range of 100–4000 MPa [79] with lower and higher values for taxol-stabilized and taxol-free microtubules, respectively. For comparison, nylon has a Young’s modulus in the range 1000–10 000 MPa [76]. The stiffness of actin filaments and microtubules can also be characterized by their persistence length. The latter quantity is directly proportional to the flexural rigidity, also known as bending stiffness. Formally, the value for the Young’s modulus, \( E \), together with the geometry of the filament cross-section (contained in the second moment of inertia, \( I \)), determine the bending persistence length, \( L_B = EI/k_B T \) where \( EI \) is the flexural rigidity, \( k_B \) is the Boltzmann constant and \( T \) is the absolute temperature (e.g. [80]). Thus, the
Figure 3. In vitro motility assays and difference in speed and persistence lengths between actin filaments and microtubules. (a; top) Schematic of in vitro motility assay where actin filament is propelled by surface-adsorbed motor fragments of myosin II. (a, bottom) Time series illustrating snapshots of fluorescent (bright) actin filaments (at 0, 1, 2, 3 and 4 s) and their trajectories after 5 s (image within red frame). Arrow points to one given filament imaged at different times and its trajectory. (b; top) Schematic of in vitro motility assay with microtubule propelled by surface-adsorbed kinesin-1 molecules. (b: bottom) Time series illustrating snapshots (at 0, 10, 20, 30 and 40 s) of taxol-stabilized and fluorescence labelled microtubules propelled by kinesin-1 and their trajectories after 50 s (within red frame). Note, appreciably higher speed of actin filaments than microtubules which is clear from ten-fold longer time interval between individual sub-panels at equal image size in (a) and (b). Note further, more curved paths of the actin filaments due to their ten-fold shorter persistence length. The filament trajectories in the lower right panel in (a) and (b) (in red frame) was obtained using the maximum projection function of ImageJ.

6. Actin binding proteins (except motors) and other modes of actin regulation

Actin is involved in more protein–protein interactions than any other protein [88] and the number of currently known ABPs exceeds ∼300 [89]. These ABPs are involved in controlling virtually every aspect of actin function by changing the physicochemical properties of F-actin and the kinetics of actin polymerization and depolymerization. Some ABPs also play key roles in the interactions of actin with other
cellular components such as cell membranes [90] as well as other cytoskeletal and regulatory proteins [89, 91]. Other ABPs sever filaments, nucleate filaments by oligomer formation, cap the barbed or pointed end of F-actin and, finally, crosslink and stabilize or cause branching of the actin cytoskeleton to organize it into higher order functional networks [24, 92].

Based on their different functions, ABPs have been classified into seven groups [89]: (1) monomer-binding proteins (e.g. profilin), (2) filament-depolymerizing proteins (e.g. cofilin), (3) filament capping or end-binding proteins either at the pointed end (tropomodulin) or the barbed end (CapZ, gelsolin), (4) filament severing proteins (gelsolin, ADF/cofilin), (5) cross-linking proteins (fascin), (6) stabilizing proteins (tropomyosin), and (7) motor proteins (the myosin family) [89]. Many ABPs share a common binding motif similar to the hydrophobic pocket in actin subdomain 1 that is located near the entrance of the hydrophobic cleft between the subdomains 1 and 3 of G-actin [88, 93]. Below we discuss ABPs of potential relevance in nanotechnological applications.

Actin filament severing is a critical aspect of filament turnover in the cell and may be useful in nanotechnological applications, e.g. biocomputation [94], as part of a filament multiplication process. The two main families of severing proteins are ADF/cofilin and gelsolin. ADF/cofilins are small globular proteins, preferentially binding to ADP-bound actin subunits towards the filament minus end [95]. The ADF/cofilin binds cooperatively between the longitudinal neighbouring subunits of F-actin to induce a significant change in twist of the actin filament as well as reduced flexural rigidity [96, 97]. These changes increase the susceptibility of the filament to fragmentation. Actin filaments saturated with ADF/cofilins are relatively stable while filaments only partially decorated with ADF/cofilins undergo severing [24] at the interface between the stiff bare and flexible decorated segments [24, 95].

Gelsolin (GSN) is an actin filament severing, capping, and nucleating protein which belongs to the large gelsolin superfamily of ABPs [98]. The activity of gelsolin is primarily regulated by binding of calcium [98, 99]. Gelsolin has three actin binding regions in six homologous globular domains (G1–G6) which are connected by linkers of variable lengths. They include the monomer binding calcium-independent fragment (G1), and the filament binding calcium-independent fragments (G2–3), as well as the calcium-dependent monomer binding fragment (G4–6). It is interesting to note that the N-terminal half of plasma gelsolin (G1–G3), when phosphorylated, functions independently of calcium, while the C-terminal is strictly calcium dependent [100]. Upon binding of calcium, gelsolin undergoes conformational changes which disrupt the interactions between G2 and G6, releasing a C-terminal hatch to induce large scale inter-domain rearrangements, eventually leading to activation [101]. As a sign of these structural changes, Ca\(^{2+}\) binding increases the hydrodynamic radius of gelsolin from 3.9 to 4.5 nm and appreciably increases the non-specific surface adsorption of the protein [102]. Following its activation, gelsolin severs actin filaments and forms a cap at the barbed end, which prevents further elongation by monomer binding.

Actin filament elongation, by addition of G-actin monomers to existing filaments, is an essential aspect of filament multiplication and therefore important in certain nanotechnological applications such as biocomputation. There are a number of ABPs, including formins that promote polymerization through nucleation of monomers into a trimer. These are typical nucleation proteins which trigger elongation with higher rates together with profilin. Formins exhibit three distinct functions such as: (1) nucleation of actin filament assembly, (2) processive movement on growing barbed ends of actin filaments together with prevention of barbed end capping and (3) acceleration of actin filament elongation together with profilin. Formins consist of a unique, highly conserved formin homology domain 2 (FH2), which binds to the barbed end of the actin filaments and promotes their elongation at higher rates [103]. This mainly occurs by nucleation that is simplified by flexing of the formin homology 2 (FH2) domain dimer to accommodate the processive addition of actin monomers to the barbed end of a filament.

Profiling regulates actin polymerization by binding G-actin in a 1:1 ratio with an affinity of 0.1 \(\mu\)M, thus effectively sequestering G-actin monomers and preventing their incorporation into the growing barbed ends of actin filaments. Profilin can function as both inhibitor and promoter of actin polymerization, acting as a gatekeeper by regulating the activities of actin nucleation proteins, e.g. formins [104]. Elongation of linear, unbranched actin filaments is promoted by formin-mediated activity. In this process, profilin decorates the growing filament barbed ends through an active, polymerization-coupled mechanism and provides the formins with actin monomers to add to the filaments. Both profilin and formin act synergistically by destabilizing or by interfering with each other at the growing barbed ends of actin filaments [105].

Tropomyosins are highly conserved ABPs, which bind, rather flexibly, along each of the two long-pitch helices of the actin filament [106–108] and stabilize the filament by preventing spontaneous depolymerization. Besides playing an important role in regulating the interaction of actin filament and myosin in striated muscles, tropomyosins also protect the filament against gelsolin and ADF/cofilin mediated severing in a tropomyosin isoform dependent way [109]. Tropomyosins could be useful in
nanotechnological applications by stabilizing the actin filaments and also by increasing the persistence length up to two-fold [84].

Filament cross-linking proteins such as fascins stabilize higher-order structures mainly in the filament bundles characteristic of microvilli, filopodia, and certain other networks of actin filaments. Fascin possesses a two-fold symmetry which promotes filament bundle formation by establishing physical connections between actin filaments through the two calponin-homology domains present in their actin-binding domains [24]. This ABP is potentially highly useful for nanotechnological applications because it cross-links actin filaments into bundles where all filaments have the same polarity. Therefore, the bundles are propelled by surface adsorbed myosin motors at similar velocities as individual actin filaments [110]. However, the bundles have ten-fold longer persistence length than the individual filaments [111] which would be an advantage for certain applications. The bundles also have an appreciably increased capacity, compared to actin filaments to carry protein sized, as well as micrometer-sized, cargoes with maintained myosin driven motile function [112] (see further below). Some ABPs may also be useful for cargo-transportation by acting as linkers to attach cargoes to specific points on the actin filament. Thus, actin capping proteins, such as gelsolin [113] and CapZ [114] have been used to attach cargoes of different sizes to the actin filaments at the barbed end. This corresponds to the trailing filament end of a myosin II propelled actin filament (figure 2(a)). This mode of attachment may limit motility-hindering effects of the cargo. Furthermore, attachment of fluorophores or quantum dots by this means has been used for tracking filaments with nanometer accuracy for functional studies [114]. Whereas the ability of gelsolin to alter the actomyosin function by severing actin filaments could limit its use for cargo transport, CapZ does not sever actin filaments. It may therefore be more favourable for use in this role with fewer structural changes along the filament.

7. Microtubule-associated proteins (except motors) and other modes of microtubule regulation

MAPs regulate in manifold ways microtubule structure and mechanics, microtubule dynamics as well as their functional organization into higher order structures [115]. The MAPs can be classified based on their localization on the microtubule: structural MAPs (e.g. MAP2, MAP4, tau, katanin) decorate microtubules along their length whereas preferential MAPs (e.g. XMAP215, EB1, CAMSAP, stathmin) recognize and bind to specific sites on the microtubule such as the microtubule plus or minus end. Members of both classes can be further categorized according their activity as stabilizers, destabilizers, capping proteins and bundlers/cross linkers [29]. Many MAPs have been subject of biophysical investigations but have hardly been used for nanotechnological applications so far. Albeit, the features of some MAPs could prospectively be very interesting for particular tasks. Microtubule stabilizers, binding to more than one tubulin dimer, could be applied for modulating mechanical properties of microtubules. While copolymerization with tau significantly increases microtubule rigidity, the addition of MAP65-1 or Ase1 decreases the rigidity most likely related to variations in the intradimer and interdimer distances upon binding [115]. Microtubule destabilizers and, more specifically, microtubule severing proteins like katanin, spastin or fidgetin [116] seem particularly promising for amplifying the number of microtubules on-site within a device. The microtubule severase spastin, an AAA ATPase, assembles on the microtubule lattice into hexameric rings and uses the energy of ATP hydrolysis to pull out individual tubulin dimers. Continuous action of spastin eventually leads to a severing event and thus results in several, shorter microtubule pieces. Interestingly, such severing activity was also observed for taxol-stabilized microtubules [117]. Other MAPs regulate the activity of microtubule motors and may be advantageous for generating microtubule batches that could be transported to different locations. Finally, the ability of capping or end-binding proteins to recognize microtubule sub-structures and interact with the cell cortex and other subcellular structures [118] further expands the existing application possibilities of MAPs.

8. Actin-based motors

Myosins (the term coined in the 19th century [119]) are actin-based molecular motors with numerous fundamental functions in biology such as intracellular transport, cell division, cell migration (including muscle contraction), signal transduction and more. All proteins in the myosin superfamily are characterized by the presence of one or two heavy chains each with an approximately 80 kDa globular head domain at the N-terminal. The latter contains an actin-binding site approximately 4 nm from the active site, where ATP turnover is catalysed [120]. The more than 2200 sequenced myosin genes, out of which the human genome holds around 40 [121–123], are sorted into 35 classes. Among those, the earliest known and studied motors
belong to the myosin II class and includes the molecular motor behind muscle contraction. Motors in this class form filaments and are denoted as ‘conventional myosins’ because they were the only known myosins for a long time [124]. In contrast the ‘unconventional’ myosins, discovered more recently, do not form filaments.

Among the vast number of different myosin motor species, we here focus on those that have found use for nanotechnological applications or which are obvious candidates for such use. The most extensively used myosin motor for this purpose is myosin II. This motor (figure 2(a) and (c)), like myosin of most other classes, can be subdivided into three well-defined domains; head (often denoted catalytic domain or motor domain), neck (alternatively light-chain-binding domain or lever arm) and tail. The myosin head domain (figure 2(c)) contains the ATPase and actin-binding sites. The neck domain (figure 2(c)) consists of an α-helix that is stabilized by binding of one essential and one regulatory light chain to allow it to function as a lever arm that amplifies small structural changes in the motor domain to several nm displacements. The tail domain consists of a coiled coil heavy chain region which polymerizes together with other myosin molecules to form myosin filaments (figure 2(a), middle) under physiological conditions. When myosin II molecules are subject to limited proteolysis using chymotrypsin, different fragments are obtained as indicated in the bottom part of figure 2(a); LMM (light meromyosin) which contains a major part of the coiled-coil tail and HMM (heavy meromyosin) that contains part of the tail as well as the head and neck domains. By further proteolytic cleavage of HMM, using either chymotrypsin or papain, one obtains (figure 2(a), bottom): S1 (subfragment 1, head + neck; figure 2(c)) and S2 (subfragment 2, part of the coiled-coil tail) [125]. It should be mentioned that the terminology used here is not universal with regard to subdivision of subfragment 1 into head and neck [120, 126–129]. Thus, often 'subfragment 1' is taken as synonymous to 'head' which is then further subdivided into lever arm and catalytic domain or motor domain [120, 126, 127].

Among skeletal muscles from adult mammals, there exist four different myosin heavy-chain (MHC) isoforms; MHC-IIa (fast), MHC-IIb (fast), MHC-IIx (fast) and beta MHC-I (slow). Whether the myosin II isoform is classified as slow or fast is determined based on the contractile speed (unloaded shortening speed) and the ATP turnover rate [130, 131]. The different myosin II motors are responsible for the contractile behaviour of muscle cells or bundles of stress fibres in non-muscle cells, along with other functions such as cell adhesion, cell migration and cell division [132, 133].

Striated muscle myosin-II is a non-processive motor with a fast MgATP turnover rate (particularly for the fastest isoform) and with the largest fraction of the MgATP turnover cycle spent detached from actin, i.e. it has a low duty ratio (figure 2(e)). Several conformational motor states are recognized during the cycle, the most important being: a pre-hydrolysis state with MgATP in the active site, post-hydrolysis, pre-power-stroke states with MgADP (adenosine diphosphate) and inorganic phosphate (Pi) in the active site and, finally, post-power-stroke states with either MgADP or no nucleotide in the active site. The pre-hydrolysis and pre-power-stroke states are either not bound to actin or weakly bound. Upon binding to actin of the myosin motor domain with MgADP and Pi in the active site, the release of Pi is accelerated, triggering conformational changes that lead to increased actomyosin affinity along with a swing of the myosin lever arm, the so called power-stroke that tends to displace the actin filament relative to myosin. An individual myosin-II motor performs a step of size 5–10 nm during the power-stroke, while it can produce forces of 5–10 pN or greater (reviewed in [134, 135]).

In most work towards nanotechnological applications, but also in functional studies, the soluble myosin motor fragment HMM is preferred over full-length myosin II. This does not appreciably modify the actin–myosin interaction described above. However, in addition to the fact that HMM does not spontaneously form filaments, its smaller dimensions facilitate diffusion into nanoscale channels and it is straightforward to achieve selectivity in actin propulsion between different nanoscale areas based on hydrophobic–hydrophilic surface patterning when HMM is used. Thus, HMM adsorbs, via the chymotryptic cleavage point in the tail, in an actin-propelling configuration on moderately hydrophobic surfaces with the motor domains extending out from the surface [136–140]. In contrast, on negatively charged hydrophilic surfaces, HMM adsorbs via the positively charged actin binding regions, preventing interaction between the motor domains and actin [136, 138–141]. The myosin II isoform that has generally been used in applications is the fast isoform (MHC-IIx) from skeletal muscle. When working in teams, as is the case with surface adsorbed motors, the motors propel the actin filaments at a speed of about 5 μm s⁻¹ at room temperature (23 °C) with more than two-fold increase for a 10 °C increase in temperature. This myosin isoform has been used for transportation of actin filaments or actin filament bundles in devices for nanoseparation [142, 143], diagnostics [112, 143] or biocomputation [94]. Also other applications have been suggested [144].

In contrast to myosin II, myosin-V is a processive motor, capable of moving several steps while maintaining strong association with the actin filament. Its two motor domains contain appreciably longer...
lever arms than myosin II, which enables this motor to take a step of nearly 36 nm along actin with the two motor domains walking hand-over-hand. Myosin-V has two motor domains each with heavy chains and neck domains containing six light chains that continues in a coiled-coil tail ending in a cargo binding globular domain at the C-terminal [145]. In accordance with its processive nature, the duty ratio of myosin-V is appreciably higher under unloaded conditions (0.7) [145] than for myosin-II (~0.05) [146]. In the cell, myosin V is responsible for intracellular transport of cargoes like vesicles, organelles and RNA. In addition, it has also been found in the mitotic spindle pole arrangement and the centrosome [147, 148]. In a recent study myosin-V, in combination with DNA origami, was proposed as nanometric thermometer using optically trapped gold nanoparticles [149].

Myosin-VI is another processive motor with step size similar to myosin-V (30–36 nm). Interestingly, myosin-VI is the only exception among myosin motors in that it moves towards the minus end of the actin filament [150–152]. Such reverse directionality is due to the presence of additional amino acid inserts in the ‘converter’ domain close to the neck region. These inserts into the converter domain allows myosin-VI to generate 180-degrees angle switch of the lever arm compared to other myosin motors. In the cell, myosin-VI is involved in e.g. the mediation of endocytosis, cell adhesion and cell movement [153]. Myosin-VI, when cargo-free, can mimic the non-processive myosin-I motor with force anchoring on actin filaments [10, 154]. This motor has been exploited to achieve an engineered bidirectional motor with optical switching by insert of artificial lever arms containing photoactive protein domains enabling light-dependent conformational changes in the lever arm [155]. This work lends inspiration from a previous pioneering study [156] where a backwards moving myosin motor was engineered by fusing a forward-moving class I myosin motor domain to appropriate molecular components, including a directional inverter and an artificial lever arm.

In algae and other plant species, cytoplasmic streaming and organelle transport occur through transportation relying on fast, plus end class XI myosins [157, 158]. Wild type myosin-XI has been purified from Nicotiana tabacum, Arabidopsis thaliana, Chara corallina plant species with velocities of up to 50 μm s⁻¹ or more, the highest velocities observed among myosins and among the processive motor category [159]. Myosin-XI has a step size of nearly 36 nm, while the generated force is low, approximately 0.5 pN [159]. Similar to myosin-VI, myosin-XI has also been engineered as a bidirectional motor which can be switched optically while maintaining its fast speed [155]. Such engineered motors with selective optical switching holds great potential in nanotechnological applications such as biocomputation (for light-based programming of motor networks) and biosensing (e.g. for sorting applications).

Rotation around the long axis of myosin II propelled actin filaments has been observed under certain conditions in the in vitro motility assay [160, 161] and different mechanisms have been considered [162]. However, rotation is not universally observed [113], the torque is low and ‘translocation and rotation are not tightly coupled’ [113]. Such lack of tight coupling explains why single liposomes of diameter > 50 nm could be transported for 100 μm by a myosin propelled actin filament while being attached to the side of the filament [114].

In summary, the molecular motors of the myosin superfamily come in different types, from fast non-processive motors that move in teams as exemplified by striated muscle myosin II to those that are slow, processive and walk alone or in small groups such as myosin V. The myosin II motors of striated muscle are those that have been used most frequently in nanotechnological applications. This is partly due to the fact that they are readily available by purification from muscle and partly due to their high speed (>10 μm s⁻¹ at 30 °C). They have also been studied extensively and the conventional in vitro motility assay was initially developed for these myosin motors. Additionally, the key myosin II motor fragment HMM is readily immobilized with full function on moderately hydrophobic areas but with negligible actin propelling function on hydrophilic negatively charged surfaces, making selective surface immobilization in nanoscale channels straightforward by hydrophobic/hydrophilic surface patterning. Whereas other motors of the myosin superfamily have been used less frequently in efforts towards nanotechnological applications than myosin II, the processivity of some of these motors is of potential interest to use for carrying cargoes substantial distances along oriented actin cables. A problem with several myosin motors other than myosin II is that it is not so straightforward to obtain nanostructured areas with and without actin propelling function of the motors. Thus, these other myosin motors have generally been attached to surfaces either directly to a ubiquitously protein binding nitrocellulose film (that is not readily nanostructured) or via antibodies, e.g. towards an affinity tag or a fluorescent protein co-expressed with the motor at its C-terminal. Whereas selective immobilization of antibodies may be achieved, e.g. to gold areas surrounded by protein repellant polyethylene glycol coated surfaces, this increases the complexity of the assay and requires more fabrication and incubation steps. However, such developments may be worthwhile e.g. if they will allow the use in nanotechnological applications of myosin motors engineered to have new functionalities.
9. Microtubule-based motors

Until 1985, only dynein was known for binding to microtubules and performing work. Then Vale, Reese and Sheetz found a new protein translocator in the giant squid axon, which differed from dynein according to structural and enzymatic criteria [163]. They called it kinesin, after the greek verb κινεῖν (kineo), meaning to move. These two superfamilies of microtubule-based motor proteins, the dyneins and kinesins (figure 2(b)), are both involved in intracellular cargo transport, they use MgATP as fuel and are end-directed proteins. However, they are very different in structure and unrelated on an evolutionary level.

Dyneins move towards the minus end of microtubules. They are divided into two groups: cytoplasmic dyneins, responsible for organelle transport and centrosome assembly, and axonemal dynein, driving the beat of cilia and flagella. Motors of both groups are complex protein assemblies of smaller subunits out of which they share some. All dyneins thus have one to three heavy chains of >500 kDa, which consist of an N-terminal tail domain and a C-terminal motor domain. The motor domain contains six AAA ATPase units forming a ring-shaped hexameric complex as observed in other members of the AAA+ family of ATPases.

In dynein, all six ATPase units are incorporated into a single polypeptide. For force generation, the energy from ATP hydrolysis is transferred to the microtubule-binding stalk, an antiparallel 15 nm long coiled-coil alpha-helix bearing a globular end structure for microtubule association. Additionally, a diversity of intermediate, light intermediate or light chains are associated with dynein, mostly at the cargo-binding base of the molecule. Dimeric dynein molecules show processivity and the observed step size varies between 8 nm and 32 nm. Moreover, dynein can also take sidewards and backwards steps [164]. It was found that most dynein steps alternate in time but the dynein motors do not necessarily switch the leading head like in a hand-over-hand walk. If the motor heads are close together, the stepping is stochastic whereas for more separated heads it becomes coordinated through a tension-based mechanism [165, 166]. In the absence of dynactin and cargo adaptor proteins single dynein molecules remain in an inactive state [167] but when bound to a cargo or a surface, processive motility is activated, presumably mechanically. In gliding motility assays an increase of microtubule gliding speed was observed with increasing dynein surface density and microtubule length [168]. However, in vitro reconstitution of dynein motility is cumbersome [169, 170], and thus the motor has not been traditionally used for nanotechnological applications. Nevertheless, some experiments have been successfully performed: for example engineered dynein-binding gold nanoparticles were transported in the intracellular environment and across the nuclear membrane by cytoplasmic dynein [171]; bidirectional motility was studied on a surface with spatially patterned kinesin-1 and cytoplasmic dynein molecules [172]. Finally, the effect of micrometre-sized walls on the motion of microtubules propelled by axonemal dynein was characterized using isolated proteins [173].

In contrast to the minus end-directed dyneins, most kinesins move towards the plus end of the microtubule. Over the years, the number of known kinesins has grown so fast as to require a standardized nomenclature defining 14 different kinesin families [174] where each new kinesin is classified according to sequence comparison. All members belonging to the kinesin superfamily share a conserved motor domain, which is located at the carboxyl-terminus, the amino-terminus or in the intervening region. On this basis, kinesins are classified as C-kinesins (e.g. kinesin-14) and N-kinesins (e.g. kinesin-1), moving towards the minus and plus end of microtubules, respectively, or as M-kinesins (e.g. kinesin-13), depolymerizing microtubules [175]. Kinesins cover a plethora of cellular functions, including cell division, signal transduction, regulation of microtubule dynamics and intracellular transport. Furthermore, they have a large variety of structures and operate as monomers, dimers, or tetramers. The N-terminal kinesin-1 motor is a homodimer, where each monomer contains a motor head, responsible for nucleotide and microtubule binding, a neck-linker, serving as a lever arm in force generation, a long coiled-coil for dimerization, and globular cargo-binding tail domain (Figure 2(d)). In the dimer, the long coiled-coils form a central stalk. The processive kinesin-1 is primarily involved in intracellular transport of vesicles and organelles [176]. It has been shown to move as many as 100 steps before disassociating from the track [177], specializing for long-distance cargo transport e.g. in neurons [178]. Unlike dynein, kinesin-1 moves stepwise using a hand-over-hand mechanism [179–181]. One molecule of ATP is hydrolysed every time the center-of-mass of a kinesin dimer is displaced by 8 nm, which is the length of a single tubulin heterodimer. This requires that each motor head takes individual 16 nm steps along the microtubule and that their biochemical cycles are tightly coordinated, so that the one motor head stays attached to the microtubule until the other head reattaches (Figure 2(f)). When ATP binds to the attached head of the motor protein, it induces a high-affinity association with the microtubule and a conformational change. Due to this conformational change in the attached head, the detached head moves forward. Then, ATP is hydrolysed to ADP and P_i in the bound head, the second head attaches to the microtubule in front of the bound head and its ADP is released. Release of P_i from the previously attached head leads to its dissociation from the microtubule track and the cycle starts again with binding of ATP. When taking forward steps while holding a cargo, kinesin-1...
is able to produce forces up to 6 pN (the stall force), which is sufficient to transport cargo across the cell [182]. At physiological cellular concentrations of ATP, ADP and Pi the stall force produced at each step corresponds to an energy production of $49 \times 10^{-21}$ J (≈12kJ/T). The energy efficiency is almost 50%, which makes kinesin-1 and molecular motors in general promising candidates for use in nanotechnological applications [183]. Moreover, motor proteins are relatively robust as they withstand temperatures in the range of 5 °C to 45 °C [184]. The gliding velocities of kinesin-1 propelled microtubules display an Arrhenius temperature dependence and can reach values of 1 μm s⁻¹ at 35 °C [185]. Kinesin-1 expressed in insect cells has been demonstrated to be an optimal choice in terms of gliding efficiency in nanostructures and long-term stability [186].

Other kinesins, like kinesin-5, kinesin-8 and kinesin-14, mostly involved in the mitotic process, do not only move strictly in parallel with the microtubule axis but show off-axis components. By using the in vitro gliding motility assays this can be studied by following the sinusoidal side-to-side and up-and-down motion of quantum dot labelled microtubules with nanometer accuracy in three dimensions [70]. The processive kinesin-8 motor, Kip3, was found to rotate microtubules counterclockwise around their longitudinal axes with periodicities of ∼1 mm [187]. These rotations cannot only be linked to the supertwist of protofilaments as for kinesin-1 but are probably induced by a long or an asymmetric neck linker complex that indicate the switching of protofilaments by the motors with a bias towards the left. Kinesin-14, a non-processive and minus-end directed motor protein, induces a right-handed rotation of microtubules with a pitch of 300 nm due to a working stroke that comprises two sub-steps of different directions [188]. In addition to the mentioned behaviour, some of the motors can interact with two microtubules simultaneously by forming tetramers or by containing a microtubule-binding site in the motor tail. For instance, the C-terminal of the kinesin-14 motor, exhibits an ATP-independent microtubule binding site. By interaction of this binding site with one microtubule and of the motor domains with another microtubule, kinesin-14 statically crosslinks microtubules with polarities oriented in the same direction or slides microtubules with opposite polarity relative to each other [189]. The non-processivity of kinesin-14 might be useful in some nanotechnological applications to reduce the probability of undesired microtubule binding events to surface areas that non-perfectly passivated against protein binding. In contrast to kinesin-1, at least 3–4 kinesin-14 molecules are necessary to reliably propel a filament forward. This property could also decisively improve the guiding reliability within topographically structured surfaces because a single non-processive motor molecule on a passivated guiding wall would not be able to propel a microtubule off the structure into the solution. However, the effect of microtubules bundling due to the ATP-independent microtubule-binding site of kinesin-14 is undesirable for many transport applications.

In summary, molecular motors of the kinesin and dynein superfamilies exhibit a large variety of properties. Kinesin-1, one of the most extensively studied motors, has so far been a frequent choice in nanotechnological applications. The expression and purification of kinesin-1 from bacterial or insect cells is straightforward. This is important both because it makes motor molecules readily available and because it enables genetic engineering of the motors. In gliding motility assays, motors are commonly adsorbed with their tails directly to the surface (non-specifically) or to immobilized antibodies (specifically). Although other motors of the kinesin superfamily have so far been barely applied for nanotechnological applications, specific properties of some of these motors might be of potential interest.

10. Key functional differences between actin- and microtubule-based systems of relevance for applications

There are several biophysical properties of the different motor filament systems that are of particularly critical importance for applications. These properties are summarized in table 1 and considered in greater detail below.

10.1. Persistence length

The persistence length of motor propelled filaments is of central importance in nanotechnological applications. This is largely because it is a key determinant of how winding the paths of a filament get when propelled by the motors in an in vitro motility assay [85, 97, 191]; the shorter the persistence length, the more winding the paths. This characteristic, in turn, determines the tendency of filaments to move straight or turn at topographical channel junctions, of great importance e.g. in biocomputation [94]. The paths taken by the motor propelled filaments are characterized by a persistence length that a priori, is expected to be approximately equal to the persistence length of the filaments in solution [85, 195]. Accordingly, equation (1) also applies for the filament paths if the angles $\theta(0)$ and $\theta(s)$ denote tangent angles of the filament paths instead of those of thermally fluctuating filaments and $s$ is taken as the distance along the path rather than along the filament. This equality between persistence length of the filaments and that of
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Table 1. Key differences in fundamental biophysical properties of the actin–myosin II and microtubule–kinesin-1 motor systems of relevance for their use in applications.

| Property                                      | Actin–myosin II | Microtubules–kinesin-1 |
|----------------------------------------------|-----------------|------------------------|
| i. Persistence length                        | 10–20 μm [84, 85, 97] | 90–130 μm [190, 191] |
| ii. Number of subunits per μm                | 362             | ~1600*                 |
| iii. Processivity (duty ratio)               | Low (<0.05) [102, 146] | High (>0.8) [192, 193] |
| iv. Maximum unloaded speed At 25°C (related to maximum track detachment rate of each motor domain) | 5–10 μm s⁻¹ [102, 194] | 0.5–0.8 μm s⁻¹ [184, 185] |

*Effective persistence length in the in vitro motility assay.

The paths follow from the thermal search of the leading end of the filament for the next surface-adsorbed motors [85, 195]. However, interestingly, the persistence length of microtubules propelled by kinesin in an in vitro motility assay appears to be appreciably shorter than that (>1 mm) found for filaments in solution [191]. This was attributed to the length dependence of the microtubule persistence length [190]. In contrast, the actin filament persistence length, deduced from the winding path of filaments propelled by myosin II, is only slightly shorter (~<two-fold) than that of isolated filaments in solution [85, 97]. Despite this effect, however, the microtubule persistence length in the in vitro motility assay is still an order of magnitude longer than that of the motor propelled actin filaments (figure 3).

The magnitude of the persistence length is an important determinant of the probability that motor propelled filaments make U-turns along topographically defined channels [144]. Thus, filaments with shorter persistence lengths have a higher probability to make U-turns. The critical maximum channel width ($w_m$) that is feasible without U-turns was calculated for actin filaments to be 300 nm based on the formula [144]:

$$w_m = \sqrt{\frac{k_B T L_B}{2d \rho G_{AM}}}, \tag{2}$$

where $d$ is the width of a band around the filament where interaction with adsorbed motors is possible, $\rho$ is the motor surface density and $f$ is the duty ratio of the motor. Finally, $G_{AM}$ represents the free energy of binding between the motor and its cytoskeletal track. Inserting typical values for actin filaments and myosin II ($d = 30$ nm, $\rho = 5000$ μm⁻², $L_B = 20$ μm, $f = 0.02$ and $G_{AM} = 20 k_B T$) gives $w_m \approx 410$ nm. Similarly, for kinesin-1 and microtubules, setting: ($d = 20$ nm, $\rho = 200$ μm⁻², $L_B = 200$ μm, $f = 0.9$ and $G_{AM} = 20 k_B T$) into equation (2), gives $w_m \approx 1.2$ μm. Further comparisons between guiding track requirements for motor propelled actin filaments and microtubules can be found elsewhere [196].

An advantage of a short persistence length is that it reduces the risk that a filament lands in a nanoscale channel from bulk solution because it is improbable that a filament with short persistence length will both be sufficiently straight and correctly oriented along the channel for unhindered channel entrance from the bulk solution. This is important in applications where filaments are usually fed into nanoscale channels from larger motor coated areas, so called loading zones, and where the directionality of the gliding is of key significance. A filament landing in the channels from solution would be equally likely to move in either direction along the channel, which would be deleterious in some applications, e.g. biocomputation [94]. Different persistence lengths of actin filaments and microtubules are also of relevance in biosensing applications of motor propelled filaments as considered in greater detail elsewhere [197].

In view of the above arguments, the short persistence length of sliding actin filaments (10–20 μm), compared to those of microtubules (>100 μm), is one important basis for differences in performance between the motor systems. One way to eliminate the effects of this factor is to bundle actin filaments together in a unipolar fashion by the actin-bundling protein fascin. This approach produces bundles of actin filaments with a persistence length up to 150 μm but with similar velocities as myosin propelled single filaments [110, 111]. However, the bundles are instable, dynamic structures that disassemble into single filaments over time.

10.2. Number of available subunits, processivity and duty ratio

One important filament property for many applications is the number of mono/dimer subunits per filament length. As subunits provide both binding sites for motors and cargo, a high density is often advantageous. Single actin filaments possess only ~360 subunits per μm whereas microtubules have 1600 subunits per μm (for a microtubule geometry with 13 protofilaments) [198].
Besides the number of available binding sites on the filaments, the processivity of the filament-propelling molecular motors might also be of importance for the cargo carrying capacity. Whereas only few processive motors need access to their binding sites on the cytoskeletal filament, this number appreciably increases for non-processive motors that need to work in large teams to effectively hold and propel the filaments. Therefore, one would expect that more subunits can be occupied by cargoes on filaments propelled by processive motors before propulsion is inhibited. If this idea is correct, one would expect better capacity of actin filaments to transport cargoes if the filaments are propelled by processive myosin motors, e.g. myosin V, rather than by non-processive myosin II. Similarly, one would expect better cargo carrying capacity of microtubules if these are propelled by processive motors such as kinesin-1 rather than by non-processive motors such as kinesin-14 (ncd). Conversely, however, non-processive motors might be more flexible in dealing with obstacles on their filament path, whereas processive motors, particularly those that follow single microtubule protofilaments (e.g. kinesin-1), are known to stall at obstacles before either detaching or continuing to move [199]. The deceleration of cargo-laden microtubules observed in gliding assays is most likely also attributed to an obstruction of kinesin-1 paths [198].

The degree of motor processivity is also of importance for effective guiding along narrow tracks/channels. Thus, very few (down to one) processive motors is sufficient to either pull filaments from the tracks into solution (see further section 11.2) or vice versa, from solution into the tracks. The latter effect is analogous to landing with random filament polarity as discussed in section 10.1.

### 10.3. Speed

Partly overlapping ranges of gliding velocities can be obtained for both myosin propelled actin filaments and kinesin propelled microtubules. As for all enzymatically powered systems the velocities are strongly temperature dependent, with a $Q_{10}$ of approximately 2 for myosin II propelled actin filaments as well as for kinesin-1 propelled microtubules. Furthermore, the velocities can be readily modified by varied substrate (MgATP) concentrations and addition of inhibitors. In the case of fast skeletal muscle myosin II, the gliding speed depends hyperbolically on [MgATP] with a maximum speed ($V_{\text{max}}$) at 30 °C of almost 15 $\mu$m s$^{-1}$ (5–10 $\mu$m s$^{-1}$ at 25 °C; table 1) and a Michaelis–Menten constant [MgATP] for half $V_{\text{max}}$ of approximately 0.3 mM.

However, as mentioned above there are different myosin and kinesin motor types with different properties including different maximum velocities. For myosin propelled actin filaments at close to saturating [MgATP] at 30 °C the mean speed ranges from around 100 nm s$^{-1}$ (e.g. myosin VI) to $>50$ $\mu$m s$^{-1}$ (certain variants of myosin XI) in gliding assays [200]. Similarly, for kinesin propelled microtubules the range of attainable velocities vary between 60 nm s$^{-1}$ (Eg 5, a kinesin-5 motor) to 2.6 $\mu$m s$^{-1}$ (Nckin, a kinesin-3 motor). Thus, generally, the maximal achievable velocities are higher for the actin–myosin system by more than ten-fold (cf [200–202]).

### 10.4. Patterned substrates for guided motility

In the first conventional in vitro motility assays, myosin and myosin motor fragments were adsorbed non-specifically to nitrocellulose [18] whereas kinesin-1 was adsorbed to casein-coated glass surfaces [203]. In these assays, the filaments were propelled across the surface with random directions. In order to control their motion in the development of nanodevices, chemical surface structuring were applied to allow adsorption of functional motors only in certain areas on a chip [12–14]. This was then generally supplemented with topographical guiding structures [204] to direct the filament movement within open narrow channels. For the actin–myosin system, nitrocellulose could not readily be nanopatterned and therefore, alternatives had to be developed. The surface treatments tested, have either relied on motility supporting polymers on SiO$_2$ (with SiO$_2$ not supporting actin–myosin motility) [12, 13, 205, 206], a combination of polymers with different properties [14, 207] or, more recently, channel floors for motility silanized with trimethylchlorosilane. The latter approach produces moderately hydrophobic channel floors (water contact angle of 70–80°) surrounded by walls of negatively charged and hydrophilic polymer resists [94, 142, 144, 208–210]. This approach, with hydrophobic–hydrophilic nanopatterning, is ideal for selective localization of motility to the nanoscale tracks using HMM motor fragments of myosin II [136, 139, 140]. However, it is not conveniently adapted to myosin motors of other classes, which generally are surface-immobilized via antibodies to a part of the myosin molecule, often to a green fluorescent protein fused to the C-terminal of the motor and co-expressed with it. For the kinesin–microtubule system, several approaches have used glass substrates with chemical patterns or topographic polymer walls [204, 211–213]. Each of these systems has its pros and cons with respect to motility and guiding performance. The choice of a hydrophobic polymer engraved with nanoscale channels with hydrophilic glass floors allows the selective modification of the surrounding parts (walls etc) of the structured polymer with a protein-repelling surfactant polyol such as Pluronic F108 or F127. This would form the basis for selective motility on the...
channel floors but no motility on surrounding areas [214]. Another system with selective motility has been achieved on gold coated silicon wafers with patterned silicon oxide [94, 215]. In this case, kinesin-1 is immobilized to the gold tracks on the channel floors whereas kinesin-1 adsorption to the surrounding SiO2 areas and channel walls is inhibited by polyethylene glycol-silane derivatization. However, the main challenge in this advanced and labor-intensive approach is accomplishing a smooth surface of the gold layer which is a main determinant of reliable microtubule transport within a structured sample.

11. General aspects of nanotechnological applications—actin vs microtubules

In this section, we focus on how different challenges related to the use of motor-filament systems in nanotechnological applications have been addressed. Particularly, we emphasize differences between the actin–myosin and microtubule–kinesin systems.

11.1. Long-term storage of functional devices

For any successful use of motor driven filaments in applications it is essential to allow long time storage after fabrication and assembly. Different methods have been tested for this purpose. Thus, for kinesin–microtubule motility assays, it was found that freezing and lyophilization or critical point-drying could extend the lifetime up to several months [216, 217] for kinesins adsorbed to glass surfaces. Similar methods have not been tested for the actin–myosin motor systems. However, the simpler approach of just placing an assembled flow cell with surface adsorbed myosin II motors and actin filaments in assay solution in a freezer at −20°C was found to preserve function [218]. Thus, after removing the assay from the freezer and again testing it at 28°C there was only negligible decay in gliding speed and the fraction of motile filaments compared to initial studies at the same temperature before freezing. The effect of freezing was tested for more than a month but it seems likely that much more prolonged storage is possible without decline in function. Storage in a refrigerator (4°C) for up to two weeks was also verified for actomyosin motility assays with gliding speed maintained at more than half the initial value. To summarize, long-term storage of nanodevices seems straightforward whether the actin–myosin II or kinesin 1–microtubule systems are used. Further studies may be required to select the best method of storage as this has not been fully explored for both systems.

11.2. Longevity of operation

When in vitro motility assays are used for functional studies of motor proteins it is usually not critical with prolonged run times; less than 10–15 min may suffice. However, for use in nanotechnological applications, particularly biocomputation that requires extensive exploration of large nano-networks, it is important to prolong motility run-times to several hours at room temperature. If a given total gliding distance of the filaments needs to be achieved, it is important to consider the longevity in relation to the filament speed. For instance, microtubules driven by kinesin-1 at a speed ∼0.6 μm s⁻¹ glide ∼2.1 mm h⁻¹ or ∼50 mm day⁻¹. For comparison, myosin II (HMM) propelled actin filaments (speed >5 μm s⁻¹) glide >18 mm h⁻¹ or >43 cm day⁻¹. Thus, if a certain sliding distance is required, the effective run-time of kinesin-propelled microtubules needs to be ~9 times longer than for myosin II propelled actin filaments. It is of interest, in this connection, to compare the current longevity status for the two motor systems. First, kinesin-1 motility run times of >24 h, without inert atmosphere chambers, have been achieved by expression of kinesin-1 in insect cells rather than in bacteria (with <5 h run-time) [186]. Even longer run-times of several days were observed using kinesin-1 expressed in E. coli if the motility assay was enclosed in a chamber with inert atmosphere. This suggests that very long run times (probably >1 week) should eventually be achievable by combining different actions. Whereas the run-time of myosin II motor fragments purified from muscle have not been systematically tested under a range of conditions, run-times close to 2 h have been observed in nanostructures [144]. However, there are approaches to increase the longevity also of myosin motor fragments, as considered further below. It therefore seems likely that run times of more than 1 day should be readily achievable also for actomyosin. Indeed results to support that view exist in preliminary form [219].

Before going further into approaches for improved longevity, it is appropriate to consider possible reasons for functional deterioration with time. These could include: (1) depletion of ATP and accumulation of ATP turnover products such as ADP and inorganic phosphate (Pi), (2) oxidation or other chemical modifications of the proteins, (3) denaturation of the proteins, (4) pharmacological effects of chemicals/toxins, (5) reduced pH and finally, (6) detachment of filaments from surfaces/nano-networks. Whereas both the filaments and the motors may show deterioration, the effects on the motors are usually most severe because they are immobilized on the chip and cannot be exchanged. Therefore, we primarily consider longevity of the motors. However, it also deserves to be mentioned that filament fragmentation as
result of covalent modifications could be problematic under certain circumstances e.g. with strong illumination (phototoxicity) and large amounts of dissolved oxygen in the assay solutions [220].

With regards to effects on the motors, depletion of ATP and accumulation of ATP hydrolysis products would reduce the sliding speed [221]. It is of interest to consider if these effects are of relevance in nanotechnological applications. Let us consider a device where we assume that 10 000 actin filaments glide continuously in nanochannels with a surface motor density of 5000 HMM μm⁻² (10 000 motor domains μm⁻²) [137, 209, 222]. We assume that the total area with HMM (for the two surfaces of a flow cell) is 1 cm² = 10⁶ μm². We further assume, that all motors (whether attached to areas with or without motility; cf [209]) have a basal myosin ATP turnover rate (in the absence of actin) of 0.05 s⁻¹. Under these assumptions the basal ATP turnover will consume 10 000 × 10⁸ × 0.05 ATP per s = 5 × 10¹⁰ ATP molecules per s ≈ 5 × 10⁻¹⁴ mol s⁻¹. An average filament of length 2 μm is expected (e.g. [222]) to interact with 10–20 motors at each give point in time. If the filament moves at 5 μm s⁻¹ and each motor stays attached for 10 nm on average, then 5000–10 000 ATP molecules are consumed per s per filament. With 10 000 such filaments, the total ATP consumption is 5 × 10⁷–10 × 10⁷ per s. Even if the number of filaments further increase 1000-fold the amount of ATP required for filament propulsion would still not be appreciably higher than that due to the basal ATP turnover. Thus, for all practical purposes, we can assume that the ATP consumption is dominated by the basal ATP turnover being equal to about 0.1 pmol s⁻¹ or <0.3 nmol h⁻¹. Now, with a typical flow cell volume of 10 μl this corresponds to complete depletion of ATP in 1 h from an assay solution with an ATP concentration of 30 μM which is >30-fold lower than the typical concentrations (~1 mM) used in nanotechnological applications. Clearly, the effects of ATP depletion would be negligible as would the accumulation of ADP giving a maximal possible concentration of the latter compound of 0.1 μM. However, if the motility run time would be increased to 24 h, ~70% of the ATP content of the motility chamber would be consumed and, with such long run-times, the spontaneous non-enzymatic hydrolysis of ATP starts to become relevant. Therefore, addition of ATP regeneration systems (creatine phosphate and creatine kinase) is required. Metabolic effects of the mentioned types would be less of an issue for kinesin-1 due to a very low basal ATP turnover and need for a much lower motor density on the surface due to processivity of the kinesin-1 motor. Thus, to summarize, there are no severe metabolic limitations on longevity provided that mM ATP concentrations are used and, in the case of actomyosin, ATP regeneration systems are included, when the run time is about a day or longer.

Covalent modifications of motor proteins can completely block motility. This is clearly illustrated by the blocking effect on motility of oxidation of key sulphydryls in myosin II [223]. To prevent such effects, as well as phototoxic effects and photobleaching of necessary fluorophores, it is important to degas solutions, keep assay solutions in sealed containers before use and to employ appropriate oxygen scavengers to remove remaining dissolved oxygen during the assay. It is also important to avoid contaminating metal ions in the assay solutions. Finally, the liberal use of reducing agents as additives is highly advisable e.g. dithiothreitol (DTT). If DTT is used, it is important to consider that the half-life in water is only a little longer than 10 h at 20 °C (pH 7). One must also make sure that the reducing agent does not have specific pharmacological actions on the motor as they are often used in concentrations of tens of mM. The importance of oxygen removal for longevity was clearly illustrated by findings that a container with inert atmosphere prolonged motility to several days with kinesin-1 propelled microtubules [224]. Covalent modifications of motors can often result in stationary filaments but it is also possible that certain modifications could cause detachment of the filaments from the surface.

In addition to covalent modifications, partial or complete protein unfolding (denaturation) is another mechanism that can lead to irreversible loss of motility. The tendency for unfolding increases with increased temperature and with more prolonged interactions of the motor domains with surfaces (particularly hydrophobic) or liquid-air interfaces (cf [138, 225]). It is thus important to limit the motor domain contacts with the underlying surface. For HMM motor fragments, this is effectively achieved [136, 137, 139, 209, 226] by adsorption of HMM via its C-terminal tail on trimethylchlorosilane derivatized glass or SiO₂ at high densities (~5000 μm⁻²; almost saturating) as usually employed with non-processive myosin II in nanotechnology. Under these conditions the N-terminal motor domains are held about 30–60 nm away for the surface on average [139]. With motors that are used at low densities, such as processive kinesin-1, neighbouring motors are too sparsely distributed to effectively hinder the motor domains of other motors to interact with the underlying surface. In these cases it is important to add blocking proteins, usually caseine or bovine serum albumin (cf [227]), to prevent surface based denaturation. Alternatively, one might use surface adsorbed antibodies to immobilize the motors in appropriate orientations thereby also adding a spacer against surface interactions of the motor domains. The exact approach for obtaining the motor from living cells can also be important for the stability of the native conformation. This was suggested by appreciably prolonged motility assay run-time (from <5 h to >24 h) by expressing kinesin-1 in insect cells rather than in E. coli [186].
One fascinating approach to prevent and even reverse myosin unfolding is to use a pharmacologic chaperone as demonstrated for the substance EMD 57033 [228]. This small molecular compound was demonstrated to produce refolding of partially or fully unfolded motor domains from cardiac beta-myosin II. For instance, completely blocked motility in the in vitro motility assay was rescued by addition of EMD 57033 at 10 μM and incubation for several hours. The effect was rather selective for cardiac myosin II and it is not yet clear if it also has similar effects on fast myosin II from skeletal muscle. An interesting observation in this context was that the blocked motility, before EMD 57033 mediated re-folding, was characterized by non-motile filaments that were attached to the surface rather than by filaments detaching from the surface which is the intuitively expected outcome of motor denaturation. Whether, this is a typical effect of motor unfolding or not, it is clear that compounds like EMD 57033 would be of potentially great value for improved longevity in motor driven nanodevices.

Chemicals with toxic effects on motors and/or filaments can be an issue when performing nanofabrication for motor-assisted nanotechnological applications by methods that are usually optimized for electronics rather than for biological systems in a liquid environment. This is important to be aware of as well as the possible toxicity of substances that may be released from thin tapes that are used to construct flow cells between a nanostructured chip and a glass cover-slip. These effects may go unnoticed with run-times of 10–20 min as in many conventional motility assays but may become severe problems when run-times of several hours are desirable.

One issue is reduced pH that may develop with time. It is of course important to use effective biological buffers at sufficient concentrations to keep pH in the range 7–8. However, it turns out that reduced pH with time for in vitro motility assay is usually attributed to inclusion of the enzyme, glucose oxidase in a typically used oxygen scavenger cocktail. This enzyme depletes oxygen by catalysing a reaction between oxygen and added glucose with the formation of hydrogen peroxide (depleted by added catalase) and gluconic acid. The latter compound is acidic enough to appreciably lower pH to damaging levels in the presence of standard concentrations of biological buffers. One convenient way around this is to exchange glucose oxidase for pyranose oxidase [229] in which case oxygen is depleted under the formation of a neutral product without a pH lowering effect. The pyranose oxidase + glucose + catalase system has been found to work well with the microtubule–kinesin system and enable rather prolonged motility in open microwell-based assay platforms instead of sealed flow cells [230]. More recently, its use has also been found (in preliminary studies) [219] to extensively prolong myosin II driven actin motility.

A final issue to consider in relation to longevity, is escape of filaments from nano-channels. This is expected to be more severe with processive motor (compared to non-processive motors) where as little as one single functional motor on a nano-channel wall may be sufficient to pull a filament out of the channel. In line with this idea, microtubules propelled by processive kinesin-1 are appreciably more likely to be lost from nanoscale channels with time than actin filaments propelled by myosin II motor fragments. The solution to this problem is to replace processive motors with non-processive (if possible for other reasons) or to improve the protein repellent function of the walls that surround the nanochannels.

To summarize, a set of similar strategies for improved longevity can be applied to both actin-based and microtubule-based systems. This includes measures to prevent oxidation and other covalent modifications of the proteins as well as methods to prevent unfolding and reduced pH of the assay solutions. There are also some aspects related to longevity that are specific to each motor system. First, for applications that require very long traversed filament paths by each filament it is important to achieve longer run-times for microtubules than for actin filaments due to higher velocities of the latter. Second, because kinesins can be expressed in different non-mammalian cell systems whereas the conventionally used myosin II motors must be expressed in mammalian expression systems it is more straightforward to improve kinesin longevity by modifying the method of expression. Third, there are specific features related to the processivity of kinesin-1 (or any processive motor) that can lead to terminated function due to escape of filaments from their tracks.

11.3. Issues of importance when motor systems are combined with nanostructured surfaces

In most nanotechnological applications of molecular motors it is central to spatially control the movement of motor propelled filaments, e.g. to deliver an analyte molecule to a detector or to execute specific computation tasks. Such control is achieved by localized motor tracks on the surface, i.e. based on non-topographical chemical patterning [13, 14, 144, 211] (see further above) most often combined with narrow topographical channels. A network of channels can be manufactured by UV-lithography if the minimal channel dimensions are approximately 1 μm or larger. This is advantageous because UV-lithography is a high-throughput technique where all channel structures are produced in one step by illumination of a polymer resist layer on a surface through masks that contain the desired pattern. This illumination step is followed by removal of the illuminated resist by development chemicals and other
chemical treatments to make the channels suitable for the selective motor function. In addition to the advantage of producing the entire pattern in one go, this procedure confers sustainability if many chip replicas are needed due to the fact that the mask can be reused virtually indefinitely. In contrast, if it is desirable to produce channels of dimensions appreciably smaller than 1 μm (a few 100 nm and less) electron beam lithography (EBL) is the method of choice. This is an appreciably slower process than UV-lithography where the pattern is imprinted into the polymer resist by an electron beam which serially traces out the entire channel pattern. This is followed by a step with chemical development to remove the polymer from the E-beam exposed resist areas.

Motor channel patterns produced by UV-lithography in devices are useful in some applications of kinesin propelled microtubules because the long persistence length of the microtubules make U-turns unlikely. For a similar reason, applications using myosin propelled actin filaments are not possible due to the short persistence length of the actin filaments. Thus, as shown above, the threshold channel width to fully prevent U-turns with actin filaments is 300–400 nm. This would increase to above 1 μm for microtubules, provided that they are long enough that they cannot simply rotate around a single motor attachment point within the width of a channel. Therefore, applications in nanoseparation or biosensing, where microtubules (but not actin filaments) transport analytes to a detector, could be readily produced using UV-lithography. However, there are other challenges with micrometer sized channels than the risk of motor propelled filaments making U-turns. This includes the risk that microtubules land in channels from the solution which is a serious drawback in biocomputation applications demanding the use of EBL for nanostructuring (see above).

Among the nanofabrication procedures, EBL in particular, is demanding on time, resources and energy, making sustainability of the applications an issue. Whereas this might be partly alleviated by the use of high-throughput nanofabrication such as nanoimprint lithography this technique has its own challenges and there have so far been only limited attempts to use it and then only for myosin propelled actin filaments [231, 232]. An alternative approach to improve the sustainability of nanofabrication in connection with motor-filament devices was described recently [233] when it was shown that such devices for both the actin–myosin and the microtubule–kinesin systems could be regenerated by use of detergent and proteolytic enzyme and thereby be used more than once for in vitro motility assays.

11.4. Binding and transportation of analytes/cargoes
The application of motor proteins and cytoskeletal filaments for transporting analytes has long been considered a particularly compelling idea for enhancing miniaturized detection devices [183] or customized lab-on-a-chip systems for research. Thus, the inspiration of using motor proteins for these purposes comes from nature itself, since both kinesins and myosins have been evolutionarily optimized in the cell to, among other tasks, transport nanoscale cargoes in nanoscale environments. In this cellular cargo transport the motors are immobilized to the cargoes which are then transported along appropriately oriented cytoskeletal filaments to re-position the cargoes e.g. from their site of production to their site of use. A similar approach, with motor-carrying cargoes moving along immobilized cytoskeletal filament tracks, has also been explored in synthetic environments [16, 234, 235]. However, it is less versatile in applications than the reverse approach were motors are adsorbed on the surface and the cargoes are immobilized on the filaments. Therefore, we only consider the latter motor driven transportation variant in greater detail below.

In a well-established transportation approach such as microfluidics, the limit for further miniaturization is posed by the high surface-volume ratio that increases friction and the need for external pumps that would make the technology bulky and cumbersome. The advantages of using microtubules and actin filaments as molecular shuttles go beyond their nanoscale dimensions: the energy demands are low and can be provided by ATP with no additional request of external power sources. Furthermore, functionalizing filaments with biotin/streptavidin and/or antibodies is relatively straightforward.

In a pioneering paper [236] (see also review in [237]) microtubules, gliding on a kinesin-coated surface, were functionalized with biotin for binding of a model analyte in the form of streptavidin. Additionally, transport along engineered surfaces and temporal control was achieved by the release of ATP from caged ATP by means of UV-light. This paper laid important grounds for later developments towards applications in biosensing. However, the first demonstration of cargo transport in a gliding motility assay had actually come some years before in the form of a myosin-transported bead–actin filament complex using 1 μm polystyrene beads as cargo [113].

Starting from early 2000s, gliding motility assays with biotinylated microtubules were also developed for the scope of transporting and stretching individual λ-phage DNA molecules [238]. Similar streptavidin/biotin coating strategies were soon used to attach quantum dots to myosin II driven actin filaments [239] as well as to kinesin–I propelled microtubules [240]. These streptavidin coated nanocrystals serve both as bright fluorescent tags [241, 242] and handles for cargo transport, by being coated with
several streptavidin molecules, in addition to that cross-linked via biotin to the filament. In the same time period, actin filaments were also functionalized with metallic nanowires with partly maintained motile function [243]. In the following years, the streptavidin coupling strategy was extended to the attachment of antibodies and antigen-antibody complexes to microtubules [244–246] but several different covalent conjugation strategies have later been exploited [247–252] for that purpose. The latter strategies require more steps than streptavidin-based conjugations but also have advantages such as generally reduced tendency for inter-filament cross-linking. Details of key strategies are reviewed elsewhere [31, 253] but two recent methods differ from those used in most other studies and have been applied either to the actin filaments or microtubules. Thus, Kumar et al [250] used heterobifunctional cross-linkers to attach fully functional antibodies to actin filament with labelling of up to 10% of the actin monomers in some filaments with fully maintained motile function and without inter-filament cross-linking. Later, Chaudhuri et al [251, 252], successfully investigated the application of inverse-electron-demand Diels–Alder addition as a conjugation method to covalently link microtubules to antibodies, in a biocompatible and highly specific manner enabling detection of sub-picromolar concentrations of a model analyte.

_A priori_, the processivity of kinesin-1 and the larger number of subunits per μm of microtubules than actin filaments (see above) would be expected to confer advantages to microtubules in cargo transportation. However, to the best of our knowledge, no explicit comparison exists. We therefore consider this issue. First we evaluate results with protein sized cargoes using streptavidin as a model cargo. For actin filaments, the fraction of motile myosin II propelled filaments was generally reduced by >50% if ~10% of the subunits (35 sub-units per μm) were loaded with streptavidin [114]. However, occasional actin filaments were motile even if up to 20% of the subunits (70 per μm) were loaded with streptavidin and the speed of these filaments was only slightly reduced. For microtubules, Korten et al [198] found that >110 streptavidin molecules (>6% of the subunits) could be attached per μm of a microtubule without full saturation of the loading capacity but appreciable reduction of the gliding speed. This suggests that the total loading capacity is appreciably higher for microtubules than actin filaments but with no marked difference in the maximum fraction of the sub-units that can be loaded with protein-sized cargo. Interestingly, there seems to be a qualitative difference between the motility inhibition caused by increased streptavidin loading of myosin II propelled actin filaments and kinesin-1 propelled microtubules. Whereas motility inhibition with actin was generally seen as off-switching (at least temporarily) of motility with little change in the average speed [114], the main effect of increased cargo loading of microtubules was a consistent reduction in speed [198]. A basis for this difference [114] could be the non-processivity of myosin II combined with few actin sub-units per μm as contrasted with the processivity of kinesin-1 and many sub-units per μm along microtubules. Thus, temporary blocking of single kinesin motors at ‘cargo-road-blocks’ without detachment [198] would be reflected in reduced speed of the microtubule when propelled by an ensemble of kinesin motors. In contrast, detachment of several non-processive myosin II motors when they encounter cargo-molecules may lead to complete off-switching of the motility (as the motors actually detach) and possible detachment of the entire filament from the surface.

For cargoes noticeably larger than proteins it seems that the superiority of microtubules over actin filaments in cargo carrying capacity is enhanced. Thus, the fraction of motile myosin II propelled actin filaments fell quickly for cargoes of larger size than proteins [114]. e.g. on average, only 50% of the actin filaments with 3–4 quantum dots attached and only 20% of actin filaments carrying more than 4 liposomes of >50 nm diameter were propelled by HMM compared to ~80% in the absence of cargo. However, again, as for the case with protein sized cargoes, the speed of the motile filaments was closely similar to that in the absence of cargo. In contrast, microtubules seem to be quite effective also in transporting larger cargoes without motility inhibition, e.g. viruses [254], large antigen-antibody aggregates, microvesicles and microspheres [254] of more than 1 μm diameter. Even individual red blood cells could be attached and transported in this way by kinesin-1 propelled microtubules [255]. It is of interest to note, however, that for actin filament bundles, the cargo carrying capacity seems to be considerably higher compared to single actin filaments [112, 256, 257]. This is of interest to investigate further in view of similar high speed for myosin II propelled fascin-actin bundles as for individual actin filaments.

One further challenge for certain applications of cargo transport, e.g. for use in biocomputation for ‘barcoding’ of the filaments, is the integration of cargo loading and unloading stations as well as cargo pick up and unload by motor-propelled filaments. Brunner et al [258] proposed non-covalent interactions using both biotin–anti-biotin interaction and hybridized DNA oligonucleotides for binding cargoes to loading stations in a suitably stable manner to enable a microtubule to pick up, bind and transport cargo upon collision. Moreover, Schmidt et al [259] exploited DNA hybridization in zipping and shearing geometries for cargo pick-up and unloading by passing microtubules in an integrated device.
12. Examples of specific nanotechnological applications

A number of applications where molecular motors are used as key components or tools have been proposed as described in comprehensive reviews on the subject [20, 183, 260, 261] from different time periods. Below we give three examples of quite diverse applications where we focus on a comparison between microtubule-based and actin-based motor systems.

12.1. Nanoseparation, biosensing and diagnostics

The advantages of using motor driven filaments to capture analytes (the molecules to be detected), such as disease biomarkers, over large surface areas and then concentrate them on a detector has been analysed theoretically, particularly by Katira and Hess [262] and Nitta and Hess [197] but also to some degree in other studies [142]. Key features of this approach are illustrated in figure 4. There are also other ideas for specific detection of analytes. This includes the observation of transportation of analytes by filaments equipped with specific recognition molecules [143, 249, 254] such as antibodies or oligonucleotides and aggregation of filaments with recognition molecules in the presence of analyte [112, 251, 263]. Quite advanced proof of principle experimental studies have been published for both the microtubule [245, 246, 251] and the actin-based [112, 142, 143] systems. From first prototypes to full devices, the implemented technologies can exploit the intrinsic advantage of cargo-transporting motile filaments, i.e. the separation of the antigen capture and (fluorescence) tagging zone (‘sorter region’ in figures 4(a) and (b)) [245], where analytes bind and get tagged, from the ‘detection zone’ (‘collector region’ in figures 4(a) and (b)). This reduces the noise coming from non-specific binding. The concept was further elaborated on in a smart dust biosensor [246], using antibodies as recognition elements on the microtubules rather than biotin (as in [245]). As further extension from the study in [245], the traditional capture-wash-tag-wash-detect sequence of a double-antibody-sandwich assay was replaced by a complete capture-transport-tag-transport-detect sequence in [246] using secondary fluorescent antibodies for tagging. The moving molecular shuttles (the antibody coated microtubules) and the specific interaction between analyte and detection antibody allowed omission of washing steps. More recently, similar approaches were described for the actomyosin system, utilizing the higher speed of myosin propelled actin filaments to achieve ultrafast nanoseparation and biosensing [142].

Other recent studies give contributions that would help overcoming some of the technical limitations of the mentioned devices: for example, more precise spatio-temporal control of kinesin motor proteins can be achieved by using the photo-switching properties of azobenzene-based molecules [264]. Furthermore, label-free detection of microvesicles and proteins is now possible using antibody-conjugated microtubules or actin filaments that crosslink when analytes bind to multiple filaments [112, 251] (see also [265]). In these studies, the superior capability of kinesin propelled microtubules to carry large cargoes without motility inhibition allowed quantitative analysis of the concentration of specific leukaemia associated microvesicles. Similar quantitative analysis was not possible with the actin-based system due to problems in transporting several microvesicles on each actin filament. Another possibility to detect analytes without labelling, is offered by speed measurements of two distinct microtubule populations: one capable of binding the analyte and the other one as control. A difference in velocities indicates the presence of the analyte and the other one as control. A difference in velocities indicates the presence of the analyte and the other one as control.

Additional improvements include increased signal-to-noise ratio at detection points. This can be achieved e.g. by using perpendicular gold lines that increase the contrast at specific check points and require few pixels of CCD detectors per line [266, 267].

Predicted challenges with all the mentioned systems for actual implementation in clinical molecular diagnostics include: capability to bind and transport analyte molecules via specific recognition molecules on the cytoskeletal filaments, robust operation of the motor-filament system in the presence of body fluids, long-term storage of assembled devices and sufficient longevity of the actual operation. For devices that include artificially nanostructured chips, e.g. to achieve nanoseparation and concentration on nanoscale detector areas, additional issues are important: this includes sustainability related to nanofabrication, optimal performance of the filaments in the nanostructures with respect to speed and error-proof guiding along the nano-channels. It also includes the capacity of motor propelled filaments to carry bulky cargoes, such as antibodies, antigens, microvesicles, bacteria, viruses and even mammalian cells.

Most of the issues considered in the previous paragraph were considered above as they are of general importance for virtually any nanotechnological applications of the motor-filament systems. In contrast, an issue specific to motor-driven nanodevices for diagnostic purposes and biosensing is that the molecule or organism to be detected is usually present in a complex solution such as blood plasma, whole blood or other body fluids. Ideally it should be possible to apply the undiluted complex sample directly to the
Figure 4. A model concentration and detection device enhanced by molecular motors and cytoskeletal filaments with biotin as a model recognition element on the filaments (instead of antibodies) and streptavidin as a model analyte. The fluorescent streptavidin is captured while the microtubules are propelled by kinesin-1 on the sorter regions of the microfabricated device illustrated schematically and using scanning electron microscopy in (a). The filaments are concentrated, due to kinesin driven transport in a collector region, under a cover that prevents escape of filaments (b) and (c). The microtubule concentration (including bound model analyte) saturates in less than half an hour, at orders of magnitude higher value on the collector region compared to the situation without a microfabricated structure (d). Reprinted with permission from [245]. Copyright (2008) American Chemical Society.

motor-filament system on a chip surface. The analyte in the sample, e.g. a biomarker to be detected, should then be captured by recognition elements attached to the filaments, followed by motor driven separation and concentration of the analyte on a detector site. For this to work, however, both the filaments and the motor-filament interactions need to be functional in the presence of the foreign complex sample. To the best of our knowledge, only one study has been performed to investigate this issue. However, that study by Korten et al [255] included both, the actin–myosin II and the microtubule–kinesin-1 system. It showed that full functionality was maintained only if the body fluids (whether whole blood, serum or cell lysate) were diluted approximately 100 times or more, before applying them to a motility assay. We assumed then that the basis for this requirement was binding of body fluid components to motors and/or filaments with inhibition of motility in the absence of dilution. Clearly, the maintenance of full functionality of the motors is an important challenge that appears to be similar for both actin–myosin and kinesin–microtubule. If pre-dilution of the sample is not acceptable the only solution seems to be some form of pre-separation of the analyte before applying it to the motor-filament-based device. One approach of this type was tested by Kumar et al [143] for a model protein analyte. In this case, magnetic nanoparticles with specific recognition elements (antibodies) were first used to fish out the analyte from blood plasma followed by pelleting of the magnetic particles using magnetic forces. Then, the particle-analyte conjugates were re-suspended in suitable biological buffer and applied to an in vitro motility assay surface with myosin propelled actin filaments where the actin filaments were supplied with recognition elements against the analyte. The success
of the approach was demonstrated by evidence for cross-linking of magnetic particles to actin filaments via the analyte molecule and motor driven transport of the aggregate. Whereas this approach has only been tested for the actin–myosin system it is likely to be feasible also with microtubules and kinesin. Another approach was used in diagnostics method to detect the presence of disease-related microvesicles in the blood plasma [112, 251]. Here, a standard approach to isolate microvesicles from blood plasma was used followed by re-suspension of the microvesicles in biological buffers suitable for either the actin–myosin II [112] or microtubule–kinesin-1 [251] systems. In both cases, the formation of aggregates of filaments (actin–actin and microtubule–microtubule) was demonstrated as evidence for the presence of microvesicles with a leukemia-related biomarker.

In summary, motility assays are inhibited by complex body fluids for both motor-filament systems but use of motor-enhanced nanodevices, e.g. in diagnostics seems anyway to be possible by introduction of pre-separation steps. Further studies are, however, required to develop such processes for general usefulness with both actin- and microtubule-based motor systems.

12.2. Imaging

Fluorescent motor propelled filaments may be used for fluorescence microscopy-based topographical and chemical characterization of surface patterns from nanoscale to microscale dimensions (figure 5). This requires quite prolonged observation of the filaments and/or, for some applications, a high filament density to allow the fluorescent filaments to explore and trace out the accessible surface area [268]. The application is founded on (1) lack of motor function on certain surface chemistries or (2) exclusion of filaments from certain topographical features on a surface [268] and (3) recording of the motor propelled filament paths using a fluorescence microscope.

Characterization of topographical features on a surface that supports motor propelled filament sliding can be achieved if the fluorescent filaments are excluded from exploring a certain part of a surface solely based on topographical hindering structures (figures 5(a) and (b)). This was the first type of motility-based imaging described [268]. In this initial study, kinesin 1 propelled microtubules were used and, to the best of our knowledge, no similar study exists for myosin II propelled actin filaments. However, prevention of filament access to a raised area with steep edges, as imaged by exclusion of fluorescent filaments in [268], would seem to require rather rigid filaments, like microtubules. These seem more likely than flexible actin filaments to fail to bend sufficiently to allow them to climb the steep walls despite similar motility-supporting chemistry as the flat areas. We thus believe that kinesin propelled microtubules are likely to have advantages for imaging topographical features without chemical contrast using the described approach. An alternative approach, based on fluorescence interference contrast microscopy (cf [269, 270]), for imaging topographical features in transparent thin (<1 μm) materials on reflective surfaces (e.g. Si, gold) could potentially be used with both microtubules and actin filaments. However, this approach in which the intensity of moving fluorescent filaments changes as function of their distance to the underlying reflective surface, has so far only been described for kinesin propelled microtubules [271].

Imaging using motor-propelled filaments could also focus on characterizing patterns with different surface chemistries rather than topographical patterns. For this purpose, myosin II propelled actin filaments and kinesin-1 propelled microtubules exhibit different surface chemistry specificities. Thus, under certain assay conditions there is no actin filament motility on hydrophilic SiO2 and glass but good motility on moderately hydrophobic surfaces [136, 144, 209]. In contrast, with kinesin-1 there is good microtubule motility on glass but not on several polymer resists [204]. Therefore, due to this difference, one may select between the two motor filament systems for achieving the most effective imaging of different chemically defined surface patterns. The motility contrast between different surface chemistries allow probing of chemical differences from the nanoscale to microscale by fluorescence microscopy (figure 5(c)) based on cumulative recording of the selective motor propelled sliding [144, 209]. The spatial resolution of this light microscopy-based imaging can surpass diffraction limitation (reaching a few nm resolution) by nanometer tracking of the filament paths [272]. Due to the short actin filament persistence length, myosin II propelled actin filaments are capable of tracing out even quite curved nanometer sized chemically defined patterns without detaching from the surface [144, 209]. This is likely to be facilitated by the non-processive properties of myosin II as many functional motors must be adsorbed to the non-imaged areas having surface properties that support poor motility. Such imaging is expected to be more challenging with microtubules due to their high persistence length, particularly when processive motors such as kinesin-1 are used. Both microtubules and actin filaments would, however, trace out nanoscale chemically defined patterns with high curvatures if they are delineated by topographical barriers (walls) in addition to the chemical patterning [144, 209, 213, 273]. In such topographically and chemically defined channels the filaments have a tendency to follow the channel edges [274]. With the help of nm tracking the latter
property would allow detailed characterization of the channel geometry, including detection of defects in the chemical surface functionalization.

Finally, an imaging application that takes full advantage of the parallel exploitation of a surface by motor propelled filaments as well as the cargo carrying capacity of the filaments was reported quite recently. In this study [241], luminescent quantum dots attached on kinesin-propelled microtubules were used as parallel

**Figure 5.** Motor propelled fluorescent filaments used for imaging of surface properties. (a) Schematic illustration of a kinesin-propelled microtubule moving on a surface with a pedestal with steep walls that the stiff microtubule cannot access. (b) Sums of a large number of sequential fluorescence microscopic images of kinesin-propelled microtubules on surface with arrays of pedestals similar to those indicated in (a). (c) Sums of a large number of sequential fluorescence microscopic images of myosin II-propelled actin filaments on surface with a flat trimethylchlorosilane (TMCS derivatized rectangular area to the far right with nano-scale track (100–400 nm width) extending to the left. (d) Schematic illustrating a kinesin propelled microtubule with attached quantum dots (QDs) that explores a gold surface with nano-slit. To the right, principle for imaging using far-field optics. (e) A scanning electron micrograph illustrating a 25 nm thick gold surface with nano-slits of different widths as indicated. (f) Maximum-intensity projection of QDs transported across the nanostructured sample in (e) with the slits in the centre of regions indicated by dashed lines. Panels (a) and (b) Reprinted with permission from [268]. Copyright (2002) American Chemical Society. Panel (c) Reprinted with permission from [209]. Copyright (2006) American Chemical Society. Panels (d)–(f) Reprinted by permission from Springer Nature Customer Service Centre GmbH: Nature. Nat. Nanotech. [241] © 2018.
scanning probes to monitor near-field interactions. Specifically, the usefulness of the approach was demonstrated using near-field interactions of individual quantum dots with nano-slits engraved in a thin gold layer under constant far-field illumination in fluorescence microscope (figures 5(d)–(f)). This allowed high-resolution detection of the slit positions based on enhanced light intensities when a microtubule attached quantum dot passed the nano-slit.

12.3. Biocomputation
Recently, we and our co-workers [94], reported the use of myosin II propelled actin filaments and kinesin-1 propelled microtubules to solve a proof of principle combinatorial problem. Specifically, the filaments explored a network of guiding channels that encoded a small instance of the so-called subset-sum problem (figure 6). While both motor-filament systems were able to successfully solve the problem we also identified system-specific challenges and advantages for upscaling the approach to larger combinatorial problems.

At physically crossing channels, so called pass-junctions, filaments are expected to move straight due to network encoding. The high flexural rigidity of microtubules compared to actin filaments appreciably reduces the risk at these sites for right-, or left-turns. Therefore, ten-fold lower junction error rates were found when using kinesin propelled microtubules than myosin propelled actin filaments. Such errors would be a serious problem in an upsampled network with a very large number of pass junctions. It will thus be essential to reduce this source of error for actin filaments to be able to take advantage of their approximately ten-fold higher speed, which is a major advantage of this motor system. It is not yet clear if it is possible to significantly reduce this type of error, e.g. by optimizing the network and channel designs or somehow increase the flexural rigidity of the filament. Interesting alternatives may be the use of actin filament bundles (see above), provided that these can be stabilized in the long term, as well as the implementation of 3D junctions, which spatially separate crossing channels.

However, the low flexural rigidity of the actin filaments is not only a disadvantage. For instance, this property makes it very unlikely that filaments floating in solution will temporarily straighten out to land in a non-permitted channel in a network with 200 nm channel width. Accordingly, we found no problems with calculation errors due to such landing events using the actin filaments. In contrast, for the microtubule system more landing errors were observed. However, this was not only due to their higher flexural rigidity but also due to wider channels of the applied networks. Thus, these errors due to filament landing in the channels can be reduced by narrower channels. Another way to avoid landing of filaments in channels is to use hydrodynamic focussing where the filament-containing solution is applied only to the loading zones, while the pure buffer solution is flown across the rest of the network.

A further advantage of myosin II propelled actin filaments compared to kinesin-1 propelled microtubules in the biocomputation network was that there was negligible escape of the actin filaments from the network compared to more extensive microtubule escape. This is attributed to the processivity of kinesin-1 and non-processivity of myosin II as considered above.

A key general challenge with the upscaling of biocomputation devices based on any motor-filament system traversing large networks is that the filaments get distributed over more and more channels. As a result, very few filaments will explore channels towards the end of the network as only a limited number of filaments per time can be fed into the network at its starting point. Therefore, it would be desirable that the filaments multiply during their progress through the network by repeated filament severing and elongation cycles. In this context, regulatory proteins (see above) that produce filament-severing, polymerization and crosslinking are of particular interest. Severing proteins include gelsolin (for actin; cf [102]) and spastin (for microtubules [275]). For the purpose of multiplying the filaments, elongation of the fragmented filaments is of critical importance. Otherwise the fragmented filaments may become so tiny that they detach from the surface or simply turn around in nanochannels by rotating around a single motor attachment point. The elongation is achieved in the most straightforward way by addition of G-actin monomers or tubulin dimers to the assay solution under polymerizing conditions that allow elongation of the filaments on the surface but largely prevent the nucleation of new filaments. However, the rate may be increased by taking advantage of regulatory proteins. E.g. for actin, the formins, acting together with profilins (see above), seem to be of particular interest for this purpose.

Another type of computation/information processing by molecular motors was described recently by Keya et al [276] using DNA to selectively and reversibly control swarming behaviour of kinesin-1 driven microtubules. In this approach, as pointed out by the authors, the information is not encoded in the positions of the filaments in a guiding structure, such as in network-based bicomputation, described above, but in the positions of the filaments relative to each other. A central precondition for this application was quite dense labelling of microtubules with DNA oligonucleotides without disturbed motile function. As discussed above, such dense labelling seems more challenging to achieve with actin filaments.
Figure 6. Use of myosin II propelled actin filaments and kinesin-1 propelled microtubules for biocomputation. (a) Layout of a computation network for the subset sum problem (SSP) [2, 5, 9] adapted for microtubules. Loading zones (balloon-like areas) where filaments enter the network from solution and nano-channels that are traversed by the microtubules during calculation, shown in green. Channels that are forbidden according to the 'traffic rules' of the network-encoding, shown in grey. Exit numbers corresponding to correct and incorrect results are shown in green and magenta, respectively. Insets: scanning electron micrographs depicting split- and pass junctions, the key architectural elements of the network. The layout of the device for calculations using actin filaments (not shown) is similar with regard to loading zones, allowed paths and architectural elements. The main difference is narrower channels. (b) Performance of device utilizing myosin propelled actin filaments in solving the SSP [2, 5, 9] problem. The image depicts average projections of sequences of several 100 fluorescence microscopy images of actin filaments illustrating the preferred filament paths through the network, closely corresponding to the allowed paths according to the encoding in (a). (c) Performance of device utilizing kinesin propelled microtubules. The image shows maximum projections of image sequences as for actin filaments in (b). (d) Experimental results using 2251 actin filaments (experimental run time: 26 min) error bars: counting error (n). (e) Experimental results from 179 microtubules (experimental run time: 180 min). Slight difference in performance between the microtubule and actin device is explained in the original paper [94]. Reproduced with permission from [94]. © Proceedings of the National Academy of Sciences of the United States of America. https://creativecommons.org/licenses/by-nc-nd/4.0/legalcode
Table 2. Operational properties of nano-devices relying on actin–myosin II and microtubule–kinesin-1 (MT) motor systems.

| Property                        | Actin | MT          | Related biophysical property | Comments related to use in applications |
|---------------------------------|-------|-------------|------------------------------|----------------------------------------|
| Shelf-life                      | Months| Months      | —                            | Satisfactory for both systems          |
| [218]                           | [216, 217] |           |                              |                                        |
| Longevity of operation          | Several hours | Days      | —                            | Satisfactory for both systems considering higher actin speed |
| [277]                           | [186, 224] |           |                              |                                        |
| Operation in body fluids        | Requires > 100-fold dilution [255] | Requires > 100-fold dilution [255] | — A challenge that must be solved for both systems |
| Speed of operation              | Fast  | Slow        | iv                           | High speeds generally desirable in most devices |
| Risk of landing in nanochannel  | Very low | Low-moderate | i, iii                       | Beneficial to minimize (narrower channels) |
| from solution                   | [144, 208] | [94]       |                              |                                        |
| Risk of escaping from nanochannel| Low   | Moderate    | ii and iii                   | Beneficial to minimize                 |
| [144, 208]                      | [94]  |            |                              |                                        |
| Risk of U-turns                 | Moderate | Low         | i                           | Must be avoided in large scale computation and generally beneficial to minimize |
| and wrong turns (errors) at junctions | [94, 144, 196, 208] | [94, 196] |                              |                                        |
| Transport efficiency for protein| Good  | Very good (> 100 cargoes/μm) [114] | ii and iii | Both motor-filament systems useful |
| sized cargoes                   | [114] | [198]       |                              |                                        |
| Transport efficiency for cargoes| Moderate-poor | Good | ii and iii | MTs (or possibly actin filament bundles) [112, 257] need to be used |
| >quantum dot                    | [112, 114] | [244, 251, 254] |                              |                                        |

*See table 1.

13. Conclusions and perspectives

A range of prototype devices and applications have been developed where molecular motor propelled cytoskeletal filaments are central. The field has been dominated by devices that employ microtubules and kinesin rather than actin and myosin. However, in an appreciable fraction of the applications, the actin–myosin system has also been tested. Here, we have therefore put emphasis on comparing the two motor systems to more easily assess the challenges and advantages associated with their use. A summary of this comparison is given in table 2 and further considered below.

A challenge that faces any type of molecular motor driven device is the need to store the device before use and then maintain full functionality for a sufficient time during operation, presumably at room temperature. Notably, however, similar problems have already been solved for a range of commercially available test kits that make use of antibodies and other biological components, often immobilized on surfaces. The major difference is the somewhat greater complexity of the motor driven systems. However, importantly, as described above, there are simple approaches for long-term storage of assembled nanodevices with maintained motor-filament function. Even methods to recycle the devices have been described [233]. From the studies cited above, it seems clear that the shelf-life should be no issue. This also applies to the longevity of operation of the actual devices in ‘room temperature’ (20 °C–25 °C). For commercially useful devices it will, however, be critical with strict quality control and standardization of fabrication to ensure that the performance criteria are consistently met between different batches of a device. The measures to ensure this need to take aim both on the protein preparation, the nanofabrication with surface treatments, the storage of the devices and the preparation of all assay solutions. Such industry-level standardization and dedicated quality control has not yet been applied to any motor operated device. It is therefore quite remarkable that devices with consistent shelf-life of several months and equally consistent operation for hours to days have already been developed for both actin and microtubule-based devices.

A challenge with both motor systems, if the aim is to use them in diagnostics, is the requirement for at least 100-fold dilution of body-fluids to maintain motor-filament functionality. Because such dilution would necessarily lower the sensitivity of the devices, fast, reliable and cheap pre-separation steps need to be developed to separate the biomarker of interest from the body fluids. Standardized approaches to achieve this already exists for some biomarkers such as microvesicles, a fact we made use of in recent studies [112, 251]. However, more general pre-separation methods need to be developed that also allow pre-separation of e.g. protein biomarkers. Only simple proof of principle approaches have been tested so far [143].

The above three paragraphs summarize challenges and advantages that are similar to the two motor systems. However, there are also differences, which reflect key differences in biophysical properties, primarily filament persistence length, motor speed, degree of processivity and the number of available
filament subunits per μm. A quite universal advantage of the actin–myosin system is the higher speed. The longer persistence length of the microtubules, on the other hand, is an advantage for most, but not all purposes. With regard to cargo carrying capacity, kinesin-1 propelled microtubules are more effective than myosin II propelled actin filaments in carrying large number of cargo-molecules and/or large particles and vesicles (e.g. compare [244, 251, 254, 276] to [112, 114, 250]). This is most likely primarily attributed to the higher processivity of kinesin-1 compared to myosin II. However, because the cargo-carrying capacity of actin was appreciably increased by the formation of actin filament bundles [112], it seems likely that also the larger number of subunits per length of a microtubule than a single actin filaments is important for the better cargo-carrying capacity. In summary, actin filaments have advantages for rapid diagnostics and nanoseparation because of higher speed but a disadvantage due to apparently lower cargo-carrying capacity.

For the future it will be important to select applications where the motor-filament enhanced devices could give extra benefits compared to existing systems. In this regard we see diagnostics, nanoseparation, customized lab-on-a-chip devices and biocomputation as interesting candidate applications. For use in diagnostics, a major hurdle seems to be the need to dilute body-fluids at least 100-fold and it will be important to dedicate quite extensive efforts to overcome this complication. More generally, it is important for all these applications, including biocomputation, that the filaments can effectively transport cargoes. However, it would also be of interest with high transportation velocities. This suggests the need to combine advantages of the microtubule–kinesin and actin–myosin systems. One possibility, suggested above that partly achieves this, is to use bundles of actin filaments. It is also of interest to consider the possibility of hybrids that combine components from the actin and microtubule-based systems [278, 279] with the aim to take advantage of the most desirable property of each. It is, of course, also of interest to engineer the motors and filaments of each system to introduce unique properties that are not available in naturally occurring motors. This could include remote control by light or different levels of processivity combined with high velocities [155, 280].

Finally, in order to make any commercial breakthrough, it will be essential to demonstrate major advantages of devices with motor-filament systems as key components. In diagnostics one may consider the possibility to achieve extreme sensitivity, specificity or speed compared to conventional platforms. If appreciable advantages can be demonstrated at low cost and high level of sustainability in a sub-field with sufficient market, diagnostics industries may consider to switch from their existing, potentially inferior but safe, approach to devices enhanced by molecular motors and cytoskeletal filaments. In biocomputation, it may be difficult to beat traditional computers and upcoming quantum computation in all areas. However, it should be possible to find specific sub-fields where the high energy efficiency, extensive parallelism and capability of cargo transportation (allowing filament barcoding) confer the motor filament systems with important advantages. In this context one may also consider to offer the solution of specific computational problems by motor driven devices as a cloud service operated at a distance by well-trained personnel in appropriate facilities. This is most likely an appreciably more viable alternative than selling an actual biocomputation product to be operated by laymen.

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Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

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